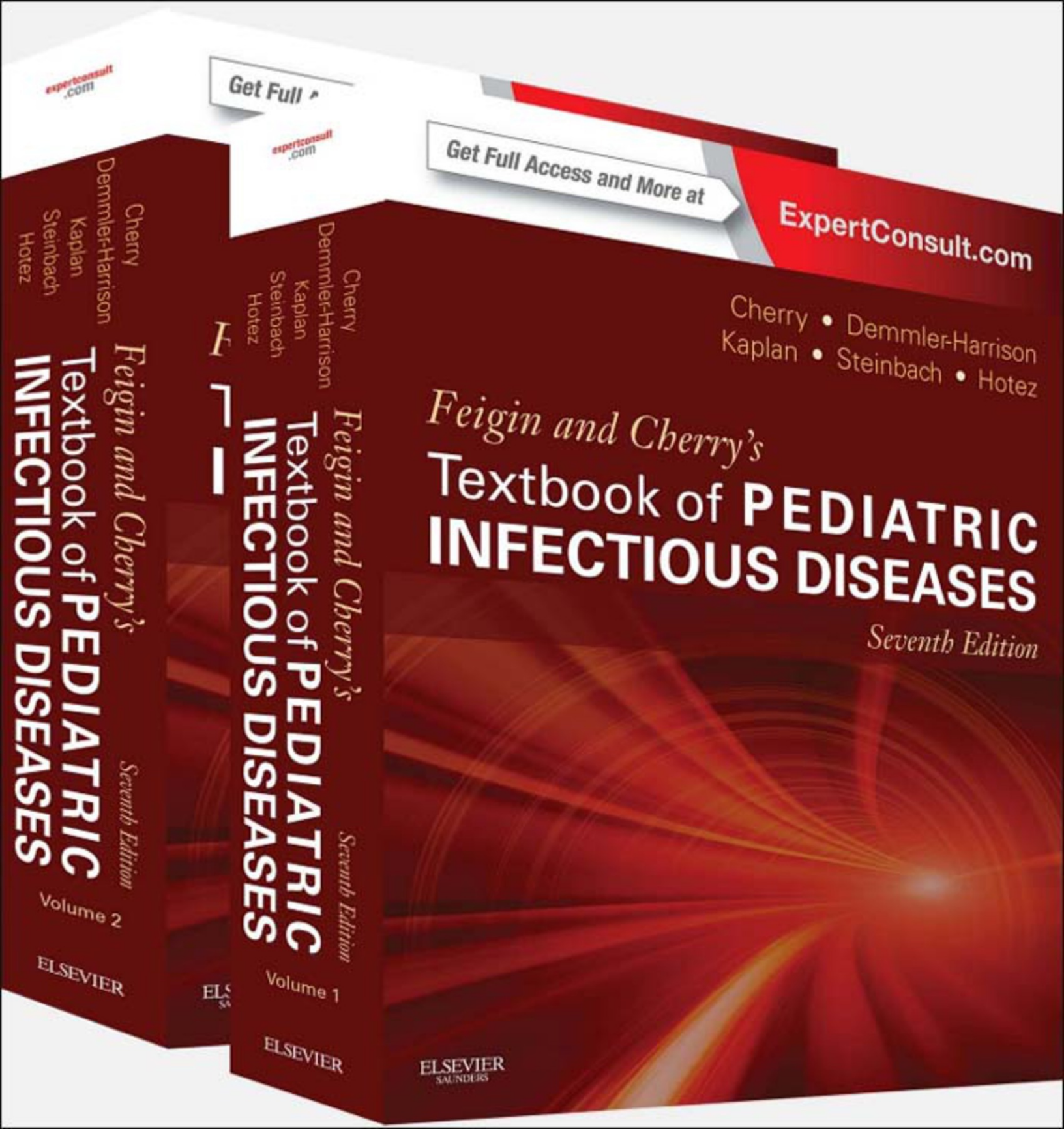
Get Full Access and More at
ExpertConsult.com
Cherry • Demmler-Harrison
Kaplan • Steinbach • Hotez
Feigin and Cherry's
Textbook of PEDIATRIC INFECTIOUS DISEASES
Seventh Edition
Volume 1
Volume 2
ELSEVIER
SAUNDERS

About the pagination of this eBook

Due to the unique page numbering scheme of this book, the electronic pagination of the eBook does not match the pagination of the printed version. To navigate the text, please use the electronic Table of Contents that appears alongside the eBook or the Search function.

For citation purposes, use the page numbers that appear in the text.

Don't Forget Your Online Access to **ExpertConsult.com**

Mobile. Searchable. Expandable.

ACCESS it on any Internet-ready device

SEARCH all Expert Consult titles you own

LINK to PubMed abstracts

ALREADY REGISTERED?

1. Log in at expertconsult.com
2. Scratch off your Activation Code below
3. Enter it into the "Add a Title" box
4. Click "Activate Now"
5. Click the title under "My Titles"

FIRST-TIME USER?

1. ***REGISTER***
   - Click "Register Now" at expertconsult.com
   - Fill in your user information and click "Continue"
2. ***ACTIVATE YOUR BOOK***
   - Scratch off your Activation Code below
   - Enter it into the "Enter Activation Code" box
   - Click "Activate Now"
   - Click the title under "My Titles"

For technical assistance:
email online.help@elsevier.com
call 800-401-9962 (inside the US)
call +1-314-995-3200 (outside the US)

Activation Code

ExpertConsult.com

FEIGIN AND CHERRY'S TEXTBOOK OF PEDIATRIC INFECTIOUS DISEASES

SEVENTH EDITION

James D. Cherry, MD, MSc
Distinguished Professor of Pediatrics
David Geffen School of Medicine at UCLA
Attending Physician
Pediatric Infectious Diseases
Mattel Children's Hospital UCLA
Los Angeles, California

Gail J. Harrison, MD
Professor
Department of Pediatrics
Section of Infectious Diseases
Baylor College of Medicine
Attending Physician
Infectious Diseases Service
Texas Children's Hospital
Houston, Texas

Sheldon L. Kaplan, MD
Professor and Vice-Chairman for Clinical Affairs
Head, Section of Infectious Diseases
Department of Pediatrics
Baylor College of Medicine
Chief, Infectious Disease Service
Texas Children's Hospital
Houston, Texas

William J. Steinbach, MD
Associate Professor of Pediatrics, Molecular Genetics and Microbiology
Department of Pediatrics
Department of Molecular Genetics and Microbiology
Attending Physician
Division of Pediatric Infectious Diseases
Duke University Medical Center
Durham, North Carolina

Peter J. Hotez, MD, PhD
Dean, National School of Tropical Medicine
Professor, Pediatrics and Molecular & Virology and Microbiology
Head, Section of Pediatric Tropical Medicine
Baylor College of Medicine
Texas Children's Hospital Endowed Chair of Tropical Pediatrics
Director, Sabin Vaccine Institute & Texas Children's Hospital Center for Vaccine Development
President, Sabin Vaccine Institute
Houston, Texas

ELSEVIER
SAUNDERS

ELSEVIER
SAUNDERS

1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

Feigin & Cherry's Textbook of Pediatric Infectious Diseases

Volume 1: Part no. 9996092720
Volume 2: Part no. 9996092844
Two-volume set ISBN: 978-1-4557-1177-2

Copyright © 2014, 2009, 2004, 1998, 1992, 1987, 1981 by Saunders, an imprint of Elsevier Inc. Chapter 146—Infant Botulism—Stephen S. Arnon—Government employee. This chapter will be in the public domain.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
Feigin & Cherry's textbook of pediatric infectious diseases.
Feigin and Cherry's textbook of pediatric infectious diseases / [edited by] James D. Cherry, Gail J. Demmler-Harrison, Sheldon L. Kaplan, Peter Hotez, William J. Steinbach.—Seventh edition.
p. ; cm.
Textbook of pediatric infectious diseases
Preceded by: Feigin & Cherry's textbook of pediatric infectious diseases / [edited by] Ralph D. Feigin—[et al.]. 6th ed. c2009.
Includes bibliographical references and index.
ISBN 978-1-4557-1177-2 (two-volume set : alk. paper)
I. Cherry, James D. (James Donald), 1930- editor of compilation. II. Demmler-Harrison, Gail J., editor of compilation. III. Kaplan, Sheldon L., editor of compilation. IV. Hotez, Peter J., editor of compilation. V. Steinbach, William J., editor of compilation. VI. Title. VII. Title: Textbook of pediatric infectious diseases.
[DNLM: 1. Communicable Diseases. 2. Child. 3. Pediatrics–methods. WC 100]
RJ401
618.92′98–dc23

2013033020

Senior Content Specialist: Stefanie Jewell-Thomas
Content Development Specialist: Lisa Barnes
Publishing Services Manager: Patricia Tannian
Senior Project Manager: Claire Kramer
Design Direction: Steven Stave

Printed in the United States of America

Last digit is the print number: 9 8 7 6 5 4 3 2 1

Working together to grow libraries in developing countries
www.elsevier.com • www.bookaid.org

To my wife, Jeanne M. Cherry
To my children, James Cherry, Jeffrey Cherry and Kass Hogan, Susan Cherry, and Kenneth and Jennifer Cherry
To my grandchildren, Ferguson, Dennis, and Siena Rose Cherry
James Cherry

To my husband, Neil Harrison
To our children, Emily Demmler Wolfe, Joshua Wolfe, Matthew Demmler, Amy Demmler, Anna Rose Demmler, Kelly Harrison Green, Daniel Green, and Haley Harrison
To our grandchild, Jensen Wolfe
To my in-laws, Elizabeth and Garth Harrison
Gail Harrison

To my wife, Marsha Kaplan
To my children, Lauren Kaplan, Mindy Kaplan Langland, and son-in-law Lance Langland
To my grandchildren, Reece and Macy Langland
Sheldon Kaplan

To my wife, Sandy Steinbach
To my children, Amelia Steinbach, Aidan Steinbach, and Conner Franzen
To my parents, Charles and Kathy Steinbach
William Steinbach

To my wife, Ann Hotez
To my children, Matthew, Emily, Rachel, and Daniel Hotez
To my parents, Ed and Jean Hotez
To my in-laws, Don and Marcia Frifield
Peter Hotez

Ralph D. Feigin, M.D.: April 3, 1938–August 14, 2008

This seventh edition of the *Textbook of Pediatric Infectious Diseases* is dedicated to Ralph D. Feigin. As everyone in pediatrics and, in particular, pediatric infectious diseases, knows, Ralph was an extraordinary individual, and his untimely death in 2008 leaves a void that will never be filled.

Ralph Feigin was born in New York City on April 3, 1938. He graduated from Columbia College in New York City in 1958 and received his M.D. from Boston University School of Medicine in 1962. He married Judith S. Zobel, a childhood friend, in 1960 while in medical school. Ralph completed his first two years of pediatric residency at Boston City Hospital and his third year at the Massachusetts General Hospital. He then fulfilled his military service requirement at the United States Army Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland. While at the United States Army Research Institute, he participated in significant studies relating to circadian periodicity and susceptibility to infections, as well as other studies that resulted in eight publications for which he was the first author. After completing his service commitment, he was Chief Resident at Massachusetts General Hospital during the 1967-68 academic year.

Ralph was recruited to Washington University in St. Louis by Phil Dodge in 1968, and soon thereafter he and one of us (JDC), who was then at St. Louis University, got together and forged an academic and personal friendship that continued until the time of his death. Over 40 years ago, Ralph and Jim recognized the need for a comprehensive book on pediatric infectious diseases, but because of their busy schedules the plan was put on hold, and in 1973 Jim moved to California. In 1976, the pediatric research meetings were held in St. Louis, and at this time Jim and Ralph met with W. B. Saunders representatives, and the book was conceived. The first edition of the textbook was published 5 years later in the fall of 1981. In comparison with this 7th edition, it was a modest effort, with 44 chapters and 124 contributors.

At Washington University and St. Louis Children's Hospital, Ralph developed one of the finest infectious diseases divisions in the country. His "Feigin Rounds" were an unparalleled learning experience and were legendary among medical students and residents. In 1977, Ralph moved to Houston, Texas, to accept the challenge of being the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital. During the ensuing 30 years, the Department grew from 43 faculty members to almost 500. One of us (SLK) came under Ralph's spell in St. Louis and moved to Houston with him. Another one of us (GJH), an intern in Houston in 1977, was waiting for Dr. Feigin when he arrived.

In Houston, Ralph served as the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital for 31 years. For 7 years of his tenure, he also served as President and CEO of Baylor College of Medicine. In addition to his commitments in Houston, Ralph served in leadership roles on more than 100 local, regional, and national committees and professional societies. His efforts in persuading government officials of all ranks helped children in Texas, the United States, and in all parts of the world. Many consider him to have been the foremost pediatrician in the world.

Not only was Dr. Feigin a powerhouse of energy, speed, and unsurpassed accomplishments, but he also was a gentleman, full of compassion, warmth, and kindness, and a man who kept people and patients first in his heart and mind. He was a loving husband to his wife, Judy, and a proud father to his three children, Susan, Debra, and Michael; doting grandfather to his six grandchildren, Rebecca, Matthew, Sarah, Rachel, Jacob, and Eli; and a mentor to so many in the field of pediatrics and pediatric infectious diseases. Ralph Feigin is missed by everyone who knew him, particularly by Judy Feigin and the family as well as by the present editors of this seventh edition of Feigin and Cherry.

A Tribute To: Caroline Breese Hall
February 4, 1939–December 10, 2012

It is with great sadness that I present this tribute to Caren Hall. She was a personal friend of mine for over 35 years, and she contributed to the chapters "Respiratory Syncytial Virus" and "Parainfluenza Viruses" in all seven editions of *Feigin and Cherry*.

Caren was a leader in the study of respiratory viral illnesses and also, more recently, in the manifestations of primary HHV-6 infections in young children.

A hallmark of all her chapters in *Feigin and Cherry* was the presentation of original poems at key points in the text. I have taken the liberty to reproduce those here:

As by one bow on varied strings, the tune is played,
By both the microbe and the host, the disease is made

A tiny thistle—
  Of coiled spine
  And outer quill …

Each breath's toll is virus spread and shed. …
Through the newly born the mother flows yet,
  Tiny proteins seeded to protect—
    though imperfect …

We view their chests ballooning, with the fears
That they're "pink puffers" of more tender years.[1]

Too minuscule for us to perceive,
But distinctive fingerprints it leaves.

Thus, if infected at this tender stage
Will they become "blue bloaters" as they age?[1]

With perseverance
And the dreamer's scanning scope,
Reality is made of distant hope …

What occult power pries loose the lid to give you water flight,
But with the lengthening light of spring gives cloak and leaden wing?

The newly born for mother shorn
With seeds unsown and shield unknown …

Though minuscule of micron measure,
It leaves its scent and prints precise;
A fleeing hare for hunt and snare …

In the future, we all will miss Caren's further contributions to the understanding and treatment of respiratory viral infections in children.

J.D.C.

[1]Reprinted with permission from Hall CB. A comment for the future. *Pediatr Res* 11:March, 1977 (Part 2).

James D. Cherry, M.D., M.Sc
Distinguished Professor of Pediatrics
David Geffen School of Medicine at UCLA
Attending Physician
Pediatric Infectious Diseases
Mattel Children's Hospital UCLA
Los Angeles, California

Gail J. Harrison, M.D.
Professor
Department of Pediatrics
Section of Infectious Diseases
Baylor College of Medicine
Attending Physician
Infectious Diseases Service
Texas Children's Hospital
Houston, Texas

Sheldon L. Kaplan, M.D.
Professor and Vice-Chairman for Clinical Affairs
Head, Section of Infectious Diseases
Department of Pediatrics
Baylor College of Medicine
Chief, Infectious Disease Service
Texas Children's Hospital
Houston, Texas

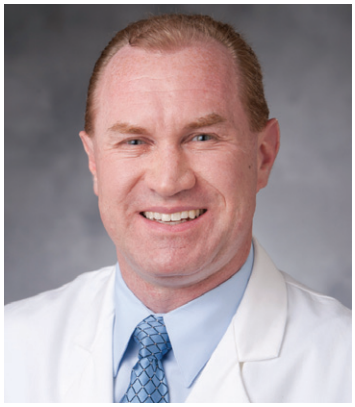

William J. Steinbach, M.D.
Associate Professor of Pediatrics, Molecular Genetics and Microbiology
Department of Pediatrics
Department of Molecular Genetics and Microbiology
Attending Physician
Division of Pediatric Infectious Diseases
Duke University Medical Center
Durham, North Carolina

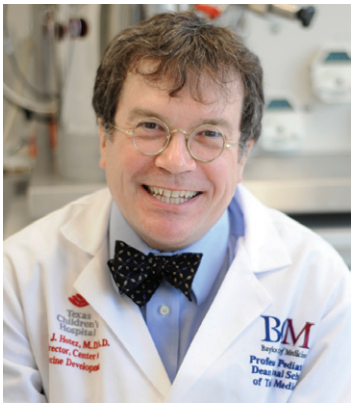

Peter J. Hotez, M.D., Ph.D.
Dean, National School of Tropical Medicine
Professor, Pediatrics and Molecular & Virology and Microbiology
Head, Section of Pediatric Tropical Medicine
Baylor College of Medicine
Texas Children's Hospital Endowed Chair of Tropical Pediatrics
Director, Sabin Vaccine Institute & Texas Children's Hospital Center for Vaccine Development
President, Sabin Vaccine Institute
Houston, Texas

Contributors

John G. Aaskov, B.Sc., Ph.D., F.R.C.Path.
W.H.O. Collaborating Centre for Arbovirus Reference and Research, Institute of Health and Biomedical Innovation. Queensland University of Technology, Brisbane, Australia
Ross River Virus Arthritis; *Murray Valley Encephalitis*

Susan M. Abdel-Rahman, Pharm.D.
Pediatric Clinical Pharmacologist, Clinical Pharmacology and Medical Toxicology, Children's Mercy Hospital and Clinics; Professor, Department of Pediatrics, University of Missouri-Kansas City School of Medicine, Kansas City, Missouri
The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics

Kristina Adachi, M.D., M.A.
Research Fellow in Pediatric Infectious Diseases, Mattel Children's Hosptial, UCLA, Los Angeles, California
Rubella Virus

Christoph Aebi, M.D.
Professor of Pediatrics and Infectious Diseases, Chairman, Department of Pediatrics, University of Bern, Bern, Switzerland
Tick-Borne Encephalitis

Kenneth A. Alexander, M.D., Ph.D.
Professor of Pediatrics, Department of Pediatrics, The University of Chicago; Chief, Section of Pediatric Infectious Diseases, Department of Pediatrics, Comer Children's Hospital, Chicago, Illinois
Human Papillomaviruses

Nicole E. Alexander-Scott, M.D., M.P.H.
Adult-Pediatric Infectious Diseases, Brown University; Assistant Professor of Internal Medicine and Pediatrics, Rhode Island Department of Health; Medical Consultant, Division of Infectious Disease and Epidemiology, Providence, Rhode Island
Active Immunizing Agents

Margot Anderson, M.D.
Pediatric Infectious Disease Fellow, Tulane University, New Orleans, Louisiana
Bacillus cereus

Marsha S. Anderson, M.D.
Associate Professor of Pediatrics, Assistant Dean for Longitudinal Curriculum, University of Colorado School of Medicine, Aurora, Colorado
Meningococcal Disease

Stephen S. Arnon, M.D.
Founder and Chief, Infant Botulism Treatment and Prevention Program, California Department of Health Services, Richmond, California
Infant Botulism

Ann M. Arvin, M.D.
Lucile Salter Packard Professor of Pediatrics and Professor of Microbiology and Immunology, Vice Provost and Dean of Research, Stanford University School of Medicine, Stanford, California
Herpes Simplex Viruses 1 and 2

Robert L. Atmar, M.D.
Professor of Medicine, Baylor College of Medicine, Houston,Texas
Rhinoviruses

Carol J. Baker, M.D.
Professor, Departments of Pediatrics and Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas
Cervical Lymphadenitis; *Group B Streptococcal Infections*

Robert S. Baltimore, M.D.
Professor, Pediatrics and Epidemiology, Yale University School of Medicine; Associate Director, Infection Control, Yale-New Haven Hospital, New Haven, Connecticut
Epidemiology and Biostatistics of Infectious Diseases

Stephen J. Barenkamp, M.D.
Professor of Pediatrics, Department of Pediatrics, St. Louis University School of Medicine; Director, Division of Pediatric Infectious Diseases, Cardinal Glennon Children's Medical Center, St. Louis, Missouri
Haemophilus influenzae; *Other Haemophilus Species (ducreyi, haemolyticus, influenzae Biogroup aegyptius, parahaemolyticus, and parainfluenzae) and Aggregatibacter (Haemophilus) aphrophilus*

Elizabeth D. Barnett, M.D.
Professor of Pediatrics, Department of Pediatrics, Boston University School of Medicine; Director, International Clinic, Department of Pediatrics, Boston Medical Center, Boston, Massachusetts
Malaria

William R. Beisel, M.D.
Adjunct Professor (Retired), W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Metabolic Response of the Host to Infections

Elizabeth C. Bellino, M.D., M.P.H.
Department of Pediatric Infectious Disease, Tulane University School of Medicine, New Orleans, Louisiana; Staff Pediatrician, Department of Pediatrics, Mutolere Hospital, Kisoro, Uganda
Metabolic Response of the Host to Infections

Gil Benard, M.D., Ph.D.
Medical Researcher, Dermatology, University of São Paulo School of Medicine; Medical Researcher, Laboratory of Medical Mycology, Tropical Medicine Institute of the University of São Paulo, São Paulo, Brazil
Paracoccidioidomycosis

David I. Bernstein, M.D., M.A.
Professor of Pediatrics, Director of the Gamble Program for Clinical Studies, Cincinnati Children's Hospital Medical Center, University of Cincinnati Department of Pediatrics, Cincinnati, Ohio
Rotavirus

Andrea A. Berry, M.D.
Assistant Professor, Department of Pediatrics and Medicine, University of Maryland School of Medicine, Baltimore, Maryland
Diarrhea-Causing and Dysentery-Causing Escherichia coli

Laurence Bichaud, Ph.D.
Emergence des Pathologies Virales, Aix-Marseille University; IRD French Institute of Research for Development; EHESP French School of Public Health; Laboratory of Virology, IHU Mediterranee Infection, AP-HM Public Hospitals of Marseille, Marseille, France
Toscana Virus

Charles D. Bluestone, M.D.
Distinguished Professor of Otolaryngology, Department of Otolaryngology, University of Pittsburgh School of Medicine; Former Director, Department of Pediatric Otolaryngology, Children's Hospital of Pittsburgh of University of Pittsburgh Medical College, Pittsburgh, Pennsylvania
Otitis Media

Jeffrey L. Blumer, Ph.D., M.D.
Professor and Chairman, Department of Pediatrics, The University of Toledo, Toledo, Ohio
Antibiotic Resistance

Robert Bortolussi, M.D., F.R.C.P.C.
Professor, Department of Pediatrics; Professor of Pediatrics and Microbiology and Immunology; Professor, Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada
Listeriosis

Kenneth M. Boyer, M.D.
Woman's Board Professor of Pediatrics, Rush Medical College; Chairman, Department of Pediatrics, Rush Children's Hospital of Rush University Medical Center, Chicago, Illinois
Toxoplasmosis

John S. Bradley, M.D.
Professor of Pediatrics, Department of Pediatrics, Division of Infectious Diseases, University of California San Diego School of Medicine; Director, Infectious Diseases, Rady Children's Hospital San Diego, San Diego, California
Pediatric Community-Acquired Pneumonia; *Retroperitoneal Infections*; *Outpatient Intravenous Antimicrobial Therapy for Serious Infections*

Michael T. Brady, M.D.
Chair, Department of Pediatrics, The Ohio State University; Physician-in-Chief, Department of Pediatrics, Nationwide Children's Hospital, Columbus, Ohio
Pseudomonas and Related Genera

William J. Britt, M.D.
Professor, Department of Pediatrics, University of Alabama, Birmingham; Department of Pediatrics, Children's Hospital of Alabama, Birmingham, Alabama
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinkeer Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia)

David E. Bronstein, M.D., M.S.
Department of Pediatrics, Southern California Permanente Medical Group, Palmdale, California
Aseptic Meningitis and Viral Meningitis; *Encephalitis and Meningoencephalitis*

David A. Bruckner, Sc.D.
Professor Emeritus, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California
Classification of Fungi; *Nomenclature for Aerobic and Anaerobic Bacteria*; *Fungal Laboratory Analysis: Specimen Collection, Direct Detection, and Culture*; *Parasitic Laboratory Diagnosis*

Kristina Bryant, M.D.
Associate Professor, Department of Pediatrics, University of Louisville, Louisville, Kentucky
Antibiotic-Associated Colitis

Steven C. Buckingham, M.D.
Associate Professor, Department of Pediatrics, University of Tennessee College of Medicine; Attending Physician, Department of Pediatrics, Le Bonheur Children's Hospital, Memphis, Tennessee
Bacteroides, Fusobacterium, and Prevotella

Carrie L. Byington, M.D.
HA and Edna Benning Presidential Professor of Pediatrics; Vice Chair Research, Department of Pediatrics; Vice Dean, Academic Affairs & Faculty Development, School of Medicine, General Pediatrics and Infectious Diseases, University of Utah School of Medicine, Salt Lake City, Utah
Pediatric Community-Acquired Pneumonia; *Spirillum minus (Rat-Bite Fever)*

Miguel M. Cabada, M.D., M.Sc.
Instructor, Internal Medicine–Infectious Disease, University of Texas Medical Branch, Galveston, Texas; Research Associate, Internal Medicine–Tropical Medicine Institute, Universidad Peruana Cayetano Heredia, Lima, Peru
Foodborne Trematodes

Audrey P. Calzada, M.D.
Chief Resident, Head and Neck Surgery, University of California, Los Angeles, Los Angeles, California
Mastoiditis

Judith R. Campbell, M.D.
Associate Professor, Department of Pediatrics, Director of Fellowship Program in Pediatric Infectious Diseases, Baylor College of Medicine; Attending Physician, Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Parotitis; *Peritonitis and Intra-Abdominal Abscess*

Maria Carrillo-Marquez, M.D.
Assistant Professor, Department of Pediatrics, University of South Dakota, Sioux Falls, South Dakota
Otitis Externa

Mariam R. Chacko, M.B.B.S.
Professor, Department of Pediatrics, Baylor College of Medicine; Medical Director, Baylor College of Medicine Teen Health Clinics; Attending Physician, Adolescent Medicine Service, Texas Children's Hospital, Houston, Texas
Genital Infections; *Calymmatobacterium granulomatis*; *Trichomonas Infections*

Lakshmi Chandramohan, Ph.D.
Research Associate, Molecular Microbiology Laboratory, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Bacterial Laboratory Diagnosis

Louisa E. Chapman, M.D., M.S.P.H.
Medical Epidemiologist, Captain US Public Health Service, Division of Notifiable Health Care Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Hantaviruses

Rémi N. Charrel, M.D., Ph.D.
Professor of Virology, Emergence des Pathologies Virales, Aix-Marseille University; IRD French Institute of Research for Development; EHESP French School of Public Health; Laboratory of Virology, IHU Mediterranee Infection, AP-HM Public Hospitals of Marseille, Marseille, France
Lymphocytic Choriomeningitis Virus; *Arenaviral Hemorrhagic Fevers*; *Toscana Virus*

Elsa Chea-Woo, M.D.
Professor, Medical Clinical Department, Faculty of Medicine, Universidad Peruana Cayetano Heredia; Chief, Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru
Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

Ira M. Cheifetz, M.D., F.C.C.M., F.A.A.R.C.
Professor of Pediatrics, Division Chief, Department of Pediatrics, Pediatric Critical Care Medicine, Duke Children's Hospital; Director, Pediatric Critical Care Services, Duke University Health System; Medical Director, Pediatric Intensive Care Unit and Pediatric Respiratory Care Services, Duke University Medical Center, Durham, North Carolina
Acute Respiratory Distress Syndrome in Children

Leon L. Chen, M.D.
Fellow, Pediatric Gastroenterology, Department of Pediatrics, Baylor College of Medicine; Fellow, Pediatric Gastroenterology, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Hepatitis

James D. Cherry, M.D., M.Sc.
Distinguished Professor of Pediatrics, David Geffen School of Medicine at UCLA; Attending Physician, Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
The Common Cold; *Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis)*; *Rhinosinusitis*; *Mastoiditis*; *Croup (Laryngitis, Laryngotracheitis, Spasmodic Croup, Laryngotracheobronchitis, Bacterial Tracheitis, and Laryngotracheobronchopneumonitis) and Epiglottitis (Supraglottitis)*; *Acute Bronchitis*; *Cutaneous Manifestations of Systemic Infections*; *Roseola Infantum (Exanthem Subitum)*; *Aeromonas*; *Pertussis and Other Bordetella Infections*; *Tetanus*; *Adenoviruses*; *Smallpox (Variola Virus)*; *Enteroviruses, Parechoviruses, and Saffold Viruses*; *Reoviruses*; *Rubella Virus*; *Measles Virus*; *Mumps Virus*; *Mycoplasma and Ureaplasma Infections*

Javier Chinen, M.D., Ph.D.
Private Practice Physician, Lake Houston Asthma Allergy and Immunology, Humble, Texas
Primary Immunodeficiencies

Natascha Ching, M.D.
Physician, Department of Pediatric Infectious Diseases, 'Specially for Children, Austin, Texas
Arcanobacterium haemolyticum

Susan E. Coffin, M.D., M.P.H.
Associate Professor of Pediatrics, Division of Infectious Diseases, Perelman School of Medicine at the University of Pennsylvania; Hospital Epidemiologist and Medical Director, Department of Infection Prevention and Control, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Health Care–Associated Infections

Armando G. Correa, M.D.
Assistant Professor, Department of Pediatrics, Baylor College of Medicine; Attending Physician, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Acinetobacter

Holly Corwin, M.P.H.
Research Assistant, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
Global Health

Andrea T. Cruz, M.D., M.P.H.
Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
Tuberculosis; *Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses*

Zev Davidovics, M.D.
Fellow, Department of Pediatric Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas
Whipple Disease

Eszter Deak, M.D., M.P.H.
Postdoctoral Fellow, Clinical Microbiology, Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, California
Classification of Fungi

Xavier de Lamballerie, M.D.
Professor, Emergence des Pathologies Virales, Aix-Marseille University; IRD French Institute of Research for Development; EHESP French School of Public Health; Laboratory of Virology, IHU Mediterranee Infection, AP-HM Public Hospitals of Marseille, Marseille, France
Lymphocytic Choriomeningitis Virus; *Arenaviral Hemorrhagic Fevers*;*Toscana Virus*

Penelope H. Dennehy, M.D.
Director, Division of Pediatric Infectious Diseases, Hasbro Children's Hospital; Professor, Department of Pediatrics, The Warren Alpert Medical School of Brown University, Providence, Rhode Island
Active Immunizing Agents

Minh L. Doan, M.D.
Chief, Pediatric Pulmonology, Department of Pediatrics, Tripler Army Medical Center, Honolulu, Hawaii
Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis

Simon R. Dobson, M.D., F.R.C.P.C.
Clinical Associate Professor, Department of Pediatric Infectious and Immunological Diseases, British Columbia Children's Hospital, Vancouver, Canada
Syphilis

Jan E. Drutz, M.D.
Professor of Pediatrics, Department of Pediatrics, Baylor College of Medicine; Professor of Pediatrics, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Arthropods

Kara Dubray, M.D.
Fellow, Department of Pediatric Infectious Diseases, Children's Hospital & Research Center, Oakland, California; Research Fellow, Communicable Diseases and Emergency Response Branch, California Department of Public Health, Richmond, California
Streptobacillus moniliformis (Rat-Bite Fever); *Spirillum minus (Rat-Bite Fever)*

Andrea Duppenthaler, M.D.
Head of Unit, Department of Pediatric Infectious Diseases, University Children's Hospital Bern, Bern, Switzerland
Tick-Borne Encephalitis

Christopher C. Dvorak, M.D.
Assistant Professor, Department of Pediatric Blood and Marrow Transplant, University of California, San Francisco, San Francisco, California
Opportunistic Infections in Hematopoietic Stem Cell Transplantation

Paul H. Edelstein, M.D.
Professor of Pathology and Laboratory Medicine, Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania; Director of Clinical Microbiology, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania
Legionnaires' Disease, Pontiac Fever, and Related Illnesses

Kathryn M. Edwards, M.D.
Sarah H. Sell Professor of Pediatrics, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee
Bartonella Infections

Morven S. Edwards, M.D.
Professor of Pediatrics, Baylor College of Medicine; Attending Physician, Department of Pediatrics, Section of Infectious Diseases, Texas Children's Hospital, Houston, Texas
Mediastinitis; *Anthrax*; *Animal and Human Bites*

B. Keith English, M.D.
Professor and Chair, Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan
Enterococcal and Viridans Streptococcal Infections

Janet A. Englund, M.D.
Professor, Department of Pediatrics, University of Washington/Seattle Children's Hospital, Seattle, Washington
Rhinoviruses; *Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome*

Leland L. Fan, M.D.
Professor, Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado
Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis

†Ralph D. Feigin, M.D.
Formerly J.S. Abercrombie Professor, Chairman, Department of Pediatrics, Distinguished Service Professor, Baylor College of Medicine; Physician-in-Chief, Texas Children's Hospital, Ben Taub General Hospital; Chief, Pediatric Service, The Methodist Hospital, Houston, Texas
Metabolic Response of the Host to Infections

Philip R. Fischer, M.D.
Professor of Pediatrics, Mayo Clinic, Rochester, Minnesota
Cestodes; *Foodborne Trematodes*; *Schistosomiasis*

Brian T. Fisher, D.O., M.P.H., M.S.C.E.
Assistant Professor of Pediatrics, Department of Pediatrics, Division of Infectious Diseases, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania
The Febrile Neutropenic Patient

Randall G. Fisher, M.D.
Associate Professor, Pediatrics, Eastern Virginia Medical School; Medical Director, Division of Pediatric Infectious Diseases, Children's Hospital of The King's Daughters, Norfolk, Virginia
Miscellaneous Gram-Positive Cocci; *Erysipelothrix rhusiopathiae*; *Corynebacterium and Rhodococcus*; *Citrobacter*; *Enterobacter*; *Klebsiella*; *Morganella morganii*; *Proteus*; *Providencia*; *Serratia*; *Miscellaneous Enterobacteriaceae*; *Vibrio vulnificus*; *Miscellaneous Non-Enterobacteriaceae Fermentative Bacilli*; *Achromobacter (Alcaligenes)*; *Eikenella corrodens*; *Elizabethkingia and Chryseobacterium Species*

Douglas S. Fishman, M.D., F.A.S.G.E.
Associate Professor, Department of Pediatrics, Baylor College of Medicine; Director, Gastrointestinal Endoscopy, Department of Pediatric Gastroenterology, Hepatology, and Nutrition, Texas Children's Hospital, Houston, Texas
Cholangitis and Cholecystitis

Anthony R. Flores, M.D., M.P.H., Ph.D.
Assistant Professor, Infectious Diseases, Department of Pediatrics, Baylor College of Medicine; Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Group A, Group C, Group G Beta Hemolytic Streptococcal Infections

Ellen M. Friedman, M.D., F.A.A.P., F.A.C.S.
Professor, Dr. Bobby R. Alford Chair in Pediatric Otolaryngology, Baylor College of Medicine; Chief of Service, Department of Otolaryngology, Texas Children's Hospital, Houston, Texas
Otitis Externa

Carla Garcia, M.D.
Assistant Professor, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas
Antibacterial Therapeutic Agents

Lynne S. Garcia, M.S., C.L.S., M.T. (A.S.C.P.), F.A.A.M.
Director, LSG & Associates, Santa Monica, California
Classification and Nomenclature of Human Parasites; *Leishmaniasis*; *Trypanosomiasis*

Gregory M. Gauthier, M.D., M.S.
Assistant Professor (CHS), Department of Medicine, Division of Infectious Diseases, University of Wisconsin, Madison, Wisconsin
Blastomycosis

Patrick J. Gavin, M.D.
Department of Pathology and Laboratory Medicine, Evanston Northwestern Heathcare, Evanston, Illinois
Naegleria, Acanthamoeba, and Balamuthia Infections

†Deceased.

Anne A. Gershon, M.D.
Professor, Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York
Varicella-Zoster Virus

Francis Gigliotti, M.D.
Professor and Chief of Infectious Diseases, Associate Chair for Academic Affairs, Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, New York
Pneumocystis Pneumonia

Mark A. Gilger, M.D.
Professor of Pediatrics, Section of Pediatric Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Baylor College of Medicine, Houston, Texas; Physician-in-Chief, Children's Hospital of San Antonio, San Antonio, Texas
Whipple Disease; *Helicobacter pylori*

Susan L. Gillespie, M.D.
Associate Professor, Section of Retrovirology and Global Health, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Oncoviruses (Human T-Cell Lymphotropic Viruses) and Lentiviruses (Human Immunodeficiency Virus Type 2)

Carol A. Glaser, D.V.M., M.P.V.M., M.D.
Chief of Encephalitis and Special Investigations Section, Communicable Disease and Emergency Response Branch, Division of Communicable Disease Control, California Department of Public Health, Richmond, California
Aseptic Meningitis and Viral Meningitis; *Encephalitis and Meningoencephalitis*; *Streptobacillus moniliformis (Rat-Bite Fever)*

Mary P. Glodé, M.D.
Professor of Pediatrics, Department of Pediatrics, University of Colorado School of Medicine; Chief, Pediatric Infectious Disease, Children's Hospital Colorado, Aurora, Colorado
Meningococcal Disease

David Goldman, M.D.
Associate Professor, Department of Pediatrics, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, New York
Cryptococcosis

Jennifer L. Goldman, M.D.
Department of Pediatrics, Children's Mercy Hospitals and Clinics, Kansas City, Missouri
The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics

Nira A. Goldstein, M.D., M.P.H.
Associate Professor, Attending Physician, Interim Director, Department of Pediatric Otolaryngology, Department of Otolaryngology, State University of New York Downstate Medical Center, Brooklyn, New York
Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses

Edmond T. Gonzales, Jr., M.D.
Professor of Urology, Scott Department of Urology, Baylor College of Medicine; Pediatric Urology Service, Texas Children's Hospital, Houston, Texas
Renal Abscess

Blanca E. Gonzalez, M.D.
Center for Pediatric Infectious Diseases, Associate Staff, Cleveland Clinic, Cleveland, Ohio
Infections of the Oral Cavity

Michael Green, M.D., M.P.H.
Professor, Department of Pediatrics, Surgery and Clinical and Translational Research, University of Pittsburgh School of Medicine; Attending Physician, Division of Infectious Diseases, Children's Hospital of Pittsburgh of University of Pittsburgh Medical College, Pittsburgh, Pennsylvania
Opportunistic Infections in Liver and Intestinal Transplantation

Andreas H. Groll, M.D.
Associate Professor of Pediatrics, Department of Pediatric Hematology and Oncology, Westphalian Wilhelm University; Attending Physician and Head, Infectious Disease Research Program, Center for Bone Marrow Transplantation and Department of Pediatric Hematology/Oncology, Children's University Hospital, Münster, Germany
Antifungal Agents

Charles Grose, M.D.
Professor, Department of Pediatrics, University of Iowa; Director of Infectious Diseases, Children's Hospital, Iowa City, Iowa
Bacterial Myositis and Pyomyositis; *Human Herpesviruses 6, 7, and 8*

Duane J. Gubler, Sc. D., F.A.A.A.S., F.I.D.S.A.
Professor, Signature Research Program in Emerging Infectious Diseases, Duke–NUS Graduate Medical School, Singapore
Yellow Fever

Javier Nieto Guevara, M.D., M.P.H.
Deputy Director, Dirección General, Instituto Conmemorativo Gorgas de Estudios de la Salud, Panamá, Republic of Panama
Parameningeal Infections

Kathleen Gutierrez, M.D.
Associate Professor, Department of Pediatrics, Division of Pediatric Infectious Disease, Stanford University School of Medicine, Stanford, California
Herpes Simplex Viruses 1 and 2

†Caroline Breese Hall, M.D.
Formerly Professor, Department of Pediatrics and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York
Parainfluenza Viruses; *Respiratory Syncytial Virus*

Scott B. Halstead, M.D.
Senior Advisor, Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Korea; Adjunct Professor, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland
Chikungunya; *Dengue, Dengue Hemorrhagic Fever, and Severe Dengue*

Shinjiro Hamano, M.D., Ph.D.
Professor, Institute of Tropical Medicine, Nagasaki University, Japan
Amebiasis

Margaret R. Hammerschlag, M.D.
Professor, Department of Pediatrics and Medicine, State University of New York Downstate Medical Center College of Medicine; Director, Division of Pediatric Infectious Diseases, State University of New York Downstate Medical Center, Brooklyn, New York
Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses; *Chlamydia Infections*

I. Celine Hanson, M.D.
Professor, Department of Pediatrics, Section of Allergy, Immunology and Rheumatology, Baylor College of Medicine; Clinic Chief, Allergy and Immunology Service, Texas Children's Hospital, Houston, Texas
Chronic Bronchitis

Nada Harik, M.D.
Assistant Professor, Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Nocardia

Kathleen H. Harriman, Ph.D., M.P.H., R.N.
Chief, Vaccine Preventable Diseases Epidemiology Section, Immunization Branch, California Department of Public Health, Richmond, California
Public Health Aspects of Infectious Disease Control

Gail J. Harrison, M.D.
Professor, Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine; Attending Physician, Infectious Diseases Service, Texas Children's Hospital, Houston, Texas
Approach to Infections in the Fetus and Newborn; *Opportunistic Infections in Kidney Transplantation*; *Human Papillomaviruses*; *Cytomegalovirus*; *Epstein-Barr Virus*; *Mimiviruses*; *Eastern Equine Encephalitis*; *Western Equine Encephalitis*; *Venezuelan Equine Encephalitis*; *Yellow Fever*; *Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers*; *Rift Valley Fever*; *Crimean-Congo Hemorrhagic Fever*; *Phlebotomus Fever (Sandfly Fever)*; *Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinker Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia)*; *Global Health*; *Antiviral Agents*; *Probiotics*

Rick E. Harrison, M.D.
Professor of Clinical Pediatrics, Pediatrics, David Geffen School of Medicine at UCLA; Medical Director, Mattel Children's Hospital UCLA, Los Angeles, California
Tetanus

C. Mary Healy, M.B., B.Ch., B.A.O., M.D.
Assistant Professor, Department of Pediatrics, Infectious Diseases Section, Baylor College of Medicine; Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Cervical Lymphadenitis

Ulrich Heininger, M.D.
Professor and Chair, Pediatric Infectious Diseases, University Children's Hospital; Member, Medical Faculty, University of Basel, Basel, Switzerland
Pertussis and Other Bordetella Infections

Sheryl L. Henderson, M.D., Ph.D.
Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, American Family Children's Hospital, Madison, Wisconsin
Infections in Child Care Environments

Gloria P. Heresi, M.D.
Professor of Pediatrics, Division Director, Infectious Diseases, Division Director, Fellowship Program, Medical Director, Donald M. Gross Pediatric Group Research, Department of Pediatrics, University of Texas–Houston Medical School, Houston, Texas
Campylobacter jejuni

Peter W. Hiatt, M.D.
Associate Professor and Section Head, Pulmonary Medicine, Department of Pediatrics , Baylor College of Medicine; Chief of Pulmonary Medicine, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Cystic Fibrosis

†Deceased.

Harry R. Hill, M.D.
Professor, Department of Pathology, Pediatrics, and Internal Medicine, University of Utah, Salt Lake City, Utah
Immunomodulating Agents

David C. Hilmers, M.D., E.E., M.P.H.
Associate Professor, Departments of Internal Medicine and Pediatrics, Center for Space Medicine, Baylor College of Medicine, Houston, Texas
Nonvenereal Treponematoses

Jill A. Hoffman, M.D.
Associate Professor, Department of Pediatrics, Keck School of Medicine of the University of Southern California; Attending Physcian, Division of Infectious Diseases, Children's Hospital Los Angeles, Los Angeles, California
Infections in Pediatric Lung Transplantation

J. Lloyd Holder, Jr., M.D., Ph.D.
Assistant Professor, Department of Pediatrics, Division of Neurology and Developmental Neuroscience, Baylor College of Medicine; Assistant Professor, Department of Pediatrics, Division of Neurology and Developmental Neuroscience, Texas Children's Hospital, Houston, Texas
Parainfectious and Postinfectious Demyelinating Disorders of the Central Nervous System; *Infection-Associated Myelitis and Myelopathies of the Spinal Cord*

Peter J. Hotez, M.D., Ph.D.
Dean, National School of Tropical Medicine, Professor, Pediatrics and Molecular & Virology and Microbiology, Head, Section of Pediatric Tropical Medicine, Baylor College of Medicine; Texas Children's Hospital Endowed Chair of Tropical Pediatrics; Director, Sabin Vaccine Institute & Texas Children's Hospital Center for Vaccine Development; President, Sabin Vaccine Institute; Houston, Texas
Blastocystis hominis and Blastocystis spp. Infection; *Entamoeba coli Infection*; *Balantidium coli Infection*; *Parasitic Nematode Infections*

Leigh M. Howard, M.D., M.P.H.
Fellow, Pediatric Infectious Diseases, Vanderbilt University Medical Center, Nashville, Tennessee
Bartonella Infections

Kristina G. Hulten, Ph.D.
Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

Romney M. Humphries, Ph.D.
Assistant Professor, Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California
Viral Laboratory Diagnosis

David A. Hunstad, M.D.
Associate Professor of Pediatrics and Molecular Microbiology, Washington University; Attending Physician, Pediatric Infectious Diseases, St. Louis Children's Hospital, St. Louis, Missouri
Molecular Determinants of Microbial Pathogenesis

W. Garrett Hunt, M.D.
Associate Professor, Section of Infectious Diseases, Department of Pediatrics, College of Medicine, The Ohio State University, Nationwide Children's Hospital, Columbus, Ohio
La Crosse Virus and Other California Serogroup Viruses

Eugene S. Hurwitz, M.D.
Associate Clinical Professor, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia
Reye Syndrome

W. Charles Huskins, M.D., M.Sc.
Assistant Professor of Pediatrics, Mayo Clinic Medical School; Consultant, Pediatric Infectious Diseases, Mayo Clinic; Chair, Quality and Safety, Department of Pediatric and Adolescent Medicine, Mayo Clinic Children's Center, Rochester, Minnesota
Health Care–Associated Infections

Mohamed Hussein, M.D.
Assistant Professor, Department of Ophthalmology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas
Ocular Infections

David Y. Hyun, M.D.
Associate Professor, Department of Pediatrics, George Washington University School of Medicine and Health Sciences; Children's National Medical Center, Center for Cancer and Immunology Research, Washington, District of Columbia
Coagulase-Negative Staphylococcal Infections; *Vibrio parahaemolyticus*

Mary Anne Jackson, M.D.
Professor, Department of Pediatrics, University of Missouri, Kansas City School of Medicine; Division Chief, Infectious Diseases, Department of Pediatrics, The Children's Mercy Hospital, Kansas City, Missouri
Bacterial Skin Infections

Michael R. Jacobs, M.B., B.Ch., Ph.D.
Professor, Department of Pathology, Case Western Reserve University; Director, Clinical Microbiology, University Hospitals Case Medical Center, Cleveland, Ohio
Pneumococcal Infections

Richard F. Jacobs, M.D., F.A.A.P.
Robert H. Fiser, Jr. MD Endowed Chair in Pediatrics, Department of Pediatrics, Arkansas Children's Hospital; Professor, Department of Pediatrics, University of Arkansas for Medical Sciences; President, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas
Complications of Pneumonia; *Fungal Meningitis*; *Other Mycobacteria*; *Nocardia*; *Aggregatibacter actinomycetemcomitans*; *Actinomycosis*

Ravi Jhaveri, M.D.
Clinical Associate Professor of Pediatrics, Division of Allergy, Immunology, Rheumatology, and Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, North Carolina
Molecular Determinants of Microbial Pathogenesis; *Hepatitis E Virus*; *Hepatitis C Virus*

Samantha H. Johnston, M.D.
Associate Physician, Division of Pediatric Infectious Diseases, Children's Hospital & Research Center Oakland, Oakland, California
Monkeypox and Other Poxviruses

Maureen Jonas, M.D.
Professor, Department of Pediatrics, Harvard Medical School; Senior Associate in Medicine, Gastroenterology, Hepatology, and Nutrition, Boston Children's Hospital, Boston, Massachusetts
Hepatitis B and D Viruses

Meena R. Julapalli, M.D.
Clinical Assistant Professor, Department of Dermatology, University of Texas, Southwestern, Dallas, Texas; Dell Children's Hospital, Department of Pediatric Dermatology, Department of Pediatric and Adolescent Dermatology, 'Specially for Children, Austin, Texas
Viral and Fungal Skin Infections

Sheldon L. Kaplan, M.D.
Professor and Vice-Chairman for Clinical Affairs, Head, Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine; Chief, Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Infectious Pericarditis; *Renal Abscess*; *Prostatitis*; *Pyogenic Liver Abscess*; *Bacteremia and Septic Shock*; *Infections in Pediatric Heart Transplantation*; *Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)*

David A. Kaufman, M.D.
Associate Professor, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Virginia
Candidiasis

Gregory L. Kearns, Pharm.D., Ph.D.
Professor and Associate Chairman, Department of Pediatrics, Children's Mercy Hospital, Kansas City, Missouri
The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics

Kwang Sik Kim, M.D.
Professor and Director, Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland
Bacterial Meningitis Beyond the Neonatal Period

Martin B. Kleiman, M.D.
Ryan White Professor of Pediatrics, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana
Histoplasmosis

Bruce S. Klein, M.D.
Gerard B. Odell Professor, Department of Pediatrics; Professor, Internal Medicine; Professor, Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin
Blastomycosis

Jerome O. Klein, M.D.
Professor, Department of Pediatrics, Boston University School of Medicine; Former Director, Division of Pediatric Infectious Diseases, Boston Medical Center, Boston, Massachusetts
Otitis Media

Mark W. Kline, M.D.
J.S. Abercrombie Professor and Chairman, Ralph D. Feigin Chair, Department of Pediatrics, Baylor College of Medicine; Physician-in-Chief, Texas Children's Hospital; President, Baylor International Pediatric AIDS Initiative, Houston, Texas
Primary Immunodeficiencies

Stephan A. Kohlhoff, M.D.
Assistant Professor, Department of Pediatrics and Medicine, State University of New York Downstate Medical Center, Brooklyn, New York
Chlamydia Infections

Tobias Kollmann, M.D., Ph.D.
Assistant Professor, Department of Pediatrics, Division of Infectious Diseases, University of British Columbia, Vancouver, British Columbia, Canada
Listeriosis

Margaret Kosek, M.D.
Assistant Professor, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; Principal Investigator, Department of Biomedical Investigation, Asociacion Benefica PRISMA, Iquitos, Loreto, Peru
Shigella

Michael P. Koster, M.D., F.A.A.P.
Clinical Assistant Professor of Pediatrics, Alpert Medical School of Brown University; Hasbro Children's Hospital, Providence, Rhode Island
Active Immunizing Agents

Peter J. Krause, M.D.
Senior Research Scientist, Yale School of Public Health, Yale School of Medicine, New Haven, Connecticut
Lyme Disease; Relapsing Fever; Babesiosis

Leonard R. Krilov, M.D.
Chief, Pediatric Infectious Diseases; Vice Chairman, Department of Pediatrics, Children's Medical Center, Winthrop University Hospital, Mineola, New York; Professor of Pediatrics, State University of New York, Stony Brook School of Medicine, Stony Brook, New York
Chronic Fatigue Syndrome

Paul Krogstad, M.D.
Professor, Departments of Pediatrics and Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA; Attending Physician, Mattel Children's Hospital UCLA, Los Angeles, California
Esophagitis; *Osteomyelitis*; *Septic Arthritis*; *Enteroviruses, Parechoviruses, and Saffold Viruses*

Damian J. Krysan, M.D., Ph.D.
Associate Professor, Department of Pediatrics and Microbiology/Immunology, University of Rochester, Rochester, New York
Fusariosis and Scedosporiosis

Thomas L. Kuhls, M.D.
Private Practice Pediatrician, Norman Pediatric Associates, Norman, Oklahoma
Appendicitis and Pelvic Abscess; *Pancreatitis*

Paul M. Lantos, M.D.
Medical Instructor, Division of Pediatric Infectious Diseases, Duke University School of Medicine, Durham, North Carolina
Rickettsial and Ehrlichial Diseases

Timothy R. La Pine, M.D.
Professor, Departments of Pediatrics and Pathology, University of Utah; Director of Neonatology, St. Mark's Hospital, Salt Lake City, Utah
Immunomodulating Agents

Matthew B. Laurens, M.D., M.P.H.
Assistant Professor, Howard Hughes Medical Institute, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland
Cholera

Charles T. Leach, M.D.
Professor, Department of Pediatrics, Division of Infectious Diseases & Immunology, University of Texas Health Sciences Center, San Antonio, Texas
Epstein-Barr Virus

Grace E. Lee, M.D.
Fellow, Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Candidiasis

Jan Hau Lee, M.B.B.S., M.R.C.P.C.H.
Pediatric Critical Care Medicine, Duke Children's Hospital, Durham, North Carolina; Children's Intensive Care Unit, KK Women's and Children's Hospital, Singapore
Acute Respiratory Distress Syndrome in Children

Robert J. Leggiadro, M.D.
Adjunct Clinical Professor, Department of Pediatrics, Hofstra North Shore–LIJ School of Medicine, Hempstead, New York; Medical Director, Vaccines, Medicines Development Group, Pfizer Incorporated, Collegeville, Pennsylvania
Bioterrorism

Pedro Legua, M.D., M.Sc. (C.T.M.)
Professor, Department of Medicine, Universidad Peruana Cayetano Heredia; Attending Physician, Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Lima, Peru
Cestodes

Deborah Lehman, M.D.
Professor, Department of Pediatrics, David Geffen School of Medicine at UCLA; Associate Director, Pediatric Infectious Diseases, Cedars-Sinai Medical Center, Los Angeles, California
Passive Immunization

Diana R. Lennon, M.B., Ch.B., F.R.A.C.P.
Professor of Population Health, Child, and Youth, Department of Paediatrics, University of Auckland; Paediatrician, Department of Paediatric Infectious Diseases, Starship Children's Hospital, Auckland, New Zealand
Acute Rheumatic Fever

Eric Leroy, M.D., D.V.M., Ph.D.
Director General, Center for International Medical Research in Franceville, Franceville, Gabon
Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers

Daniel H. Leung, M.D.
Assistant Professor, Department of Pediatrics, Section of Gastroenterology, Hepatology and Nutrition, Baylor College of Medicine; Medical Director, Viral Hepatitis Clinic, Texas Children's Hospital, Houston, Texas
Hepatitis

Moise L. Levy, M.D.
Clinical Professor, Dermatology, University of Texas Southwestern Medical School, Dallas, Texas; Clinical Professor, Dermatology and Pediatrics, Baylor College of Medicine, Houston, Texas; Chief, Pediatric Dermatology, Physician-in-Chief, Dell Children's Medical Center, Austin, Texas
Viral and Fungal Skin Infections

W. Matthew Linam, M.D., M.S.
Assistant Professor, Division of Pediatric Infectious Diseases, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, Arkansas
Other Mycobacteria

Timothy E. Lotze, M.D.
Associate Professor, Departments of Pediatrics and Neurology, Baylor College of Medicine; Medical Director, Blue Bird Circle Multiple Sclerosis, Muscular Dystrophy, and Peripheral Nerve Clinics; Texas Children's Hospital, Houston, Texas
Parainfectious and Postinfectious Demyelinating Disorders of the Central Nervous System; *Infection-Associated Myelitis and Myelopathies of the Spinal Cord*

Yalda C. Lucerno, M.D., Ph.D.
Assistant Professor, Department of Pediatrics, Faculty of Medicine, University of Chile; Pediatrician, Department of Pediatrics, Dr. Luis Calvo Mackenna Hospital, Santiago Metropolitan Region, Chile
Caliciviruses

Ruth Ann Luna, Ph.D.
Director, DNA Sequencing Core, Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, Houston, Texas
Cystic Fibrosis

Timothy Mailman, M.D., F.R.C.P.C.
Associate Professor, Departments of Pathology and Pediatrics, Dalhousie University; Staff, Division of Infectious Diseases, Department of Pediatrics, IWK Health Centre, Halifax, Nova Scotia, Canada
Listeriosis

Susan A. Maloney, M.D., M.H.Sc.
Global TB Coordinator, Center for Disease Control and Prevention, U.S. Centers for Disease Control and Prevention, Atlanta, Georgia
International Travel Issues for Children

Mario J. Marcon, Ph.D.
Clinical Professor of Pathology and Pediatrics, Ohio State University College of Medicine; Director, Clinical/Molecular Microbiology and Virology, Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio
Pseudomonas and Related Genera

Kimberly C. Martin, D.O.
Pediatric Infectious Disease Fellow, Infectious Disease, Children's National Medical Center, Washington, District of Columbia; Medical Officer, Division of Anti-Infective Products, U.S. Food and Drug Administration, Silver Spring, Maryland
St. Louis Encephalitis

Laurene Mascola, M.D., M.P.H., F.A.A.P.
Chief, Acute Communicable Disease Program, Los Angeles County Department of Public Health, Los Angeles, California
Public Health Aspects of Infectious Disease Control

Edward O. Mason, Ph.D.
Professor, Department of Pediatrics, Baylor College of Medicine; Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

Wilbert H. Mason, M.D., M.P.H.
Children's Hospital Los Angeles; Department of Pediatrics, The Keck School of Medicine at the University of Southern California, Los Angeles, California
Legionnaires' Disease, Pontiac Fever, and Related Illnesses

Aldo Maspons, M.D.
Fellow, Pediatric Gastroenterology, Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Houston, Texas
Helicobacter pylori

David O. Matson, M.D., Ph.D.
Professor of Health Professions and Pediatrics, Director, Graduate Program in Public Health, Eastern Virginia Medical School, Old Dominion University; Attending Physician in Pediatric Infectious Diseases, Children's Hospital of The King's Daughters, Sentara Norfolk General Hospital, Norfolk, Virginia
Caliciviruses

Marc A. Mazade, M.D.
Consultant, Pediatric Infectious Diseases, Medical Director of Infection Control, Division of Pediatric Infectious Diseases, Cook Children's Medical Center, Fort Worth, Texas
Infections Related to Craniofacial Surgical Procedures

James B. McAuley, M.D., M.P.H.
Professor of Pediatrics and Internal Medicine, Department of Pediatrics, Rush University Medical Center, Chicago, Illinois; Medical Epidemiologist, Center for Global Health, Centers for Disease Control and Prevention, Lusaka, Zambia
Toxoplasmosis

George H. McCracken, Jr., M.D.
The GlaxoSmthKline Distinguished Professor of Pediatric Infectious Disease, Department of Pediatrics, University of Texas Southwestern Medical Center at Dallas; Attending Physician, Children's Medical Center Dallas, Dallas, Texas
Antibacterial Therapeutic Agents

Kenneth McIntosh, M.D.
Professor, Department of Pediatrics, Harvard Medical School; Senior Physician, Medicine, Children's Hospital, Boston, Massachusetts
Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome

James E. McJunkin, M.D.
Professor of Pediatrics, West Virginia University Health Sciences Center, Charleston South Carolina
La Crosse Virus and Other California Serogroup Viruses

Kelly T. McKee, Jr., M.D., M.P.H.
Vice President and Managing Director, Public Health and Government Services, Quintiles Transnational Corporation, Durham, North Carolina
Hantaviruses

Ross McKinney, Jr., M.D.
Professor, Department of Pediatrics, Duke University School of Medicine, Durham, North Carolina
Rickettsial and Ehrlichial Diseases

Rima L. McLeod, M.D.
Departments of Pediatrics (Infectious Diseases) and Ophthalmology and Visual Sciences, Committees on Molecular Medicine and Immunology and Fellow, Institute of Systems Biology, The University of Chicago; Attending Physician, The University of Chicago Hospitals, Chicago, Illinois
Toxoplasmosis

Monica Malone McNeal, M.S.
Associate Director, Laboratory for Specialized Clinical Studies, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
Rotavirus

H. Cody Meissner, M.D.
Professor of Pediatrics, Tufts University School of Medicine; Chief, Pediatric Infectious Disease, Department of Pediatrics, Tufts Medical Center, Boston, Massachusetts
Respiratory Syncytial Virus

Maria José Soares Mendes-Giannini, Ph.D.
Full Professor, Análises Clínicas, University Estadual Paulista-UNESP; Research Vice-Rector, UNESP, Araraquara, São Paulo, Brazil
Paracoccidioidomycosis

Marian G. Michaels, M.D., M.P.H.
Professor of Pediatrics and Surgery, Department of Pediatrics, University of Pittsburgh School of Medicine; Division of Pediatric Infectious Diseases, Children's Hospital of Pittsburgh of University of Pittsburgh Medical College, Pittsburgh, Pennsylvania
Opportunistic Infections in Liver and Intestinal Transplantation

Ian C. Michelow, M.D.
Assistant Professor of Pediatrics, Warren Alpert Medical School of Brown University, Center for International Health Research, Hasbro Children's Hospital, Division of Pediatric Infectious Diseases, Providence, Rhode Island
Active Immunizing Agents

Aaron M. Miller, M.D., M.B.A.
Clinical Faculty Instructor, Ophthalmology, Baylor College of Medicine; Pediatric Ophthalmologist, Houston Eye Associates, Houston, Texas
Ocular Infections

Marjorie J. Miller, Dr.P.H.
Senior Specialist, Virology, Department of Pathology and Laboratory Medicine, University of California, Los Angeles Medical Center, Los Angeles, California
Classification and Nomenclature of Viruses; *Viral Laboratory Diagnosis*

James N. Mills, M.D.
Adjunct Faculty, Population Biology, Ecology, and Evolution, Emory University, Atlanta, Georgia; Editor-in-Chief, Journal of Wildlife Diseases, Lawrence, Kansas
Hantaviruses

Kathryn S. Moffett, M.D.
Professor of Pediatrics, Section Chief, Division of Pediatric Infectious Diseases, West Virginia University, Morgantown, West Virginia
Cystic Fibrosis

Martin Montes, M.D.
Assistant Professor, Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru; Assistant Professor, Internal Medicine—Division of Infectious Diseases, University of Texas Medical Branch, Galveston, Texas
Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis

Jagmeet Mundi, M.D.
Head and Neck Surgery, University of California, Los Angeles, Los Angeles, California
Rhinosinusitis

James R. Murphy, Ph.D.
Professor, Department of Pediatrics, Pediatric Infectious Diseases, University of Texas Medical School at Houston, Houston, Texas
Campylobacter jejuni

Santhosh Nadipuram, M.D., M. S.
Research Fellow in Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Aeromonas; *Adenoviruses*

Colleen B. Nash, M.D., M.P.H.
Fellow, Pediatric Infectious Diseases, Department of Pediatrics, Section of Pediatric Infectious Diseases, University of Chicago, Chicago, Illinois
Human Papillomaviruses

James P. Nataro, M.D., Ph.D., M.B.A.
Benjamin Armistead Shepherd Professor and Chair, Department of Pediatrics, University of Virginia School of Medicine; Pediatrician-in-Chief, University of Virginia Children's Hospital, Charlottesville, Virginia
Diarrhea-Causing and Dysentery-Causing Escherichia coli

Delma J. Nieves, M.D.
Clinical Instructor, Division of Pediatric Infectious Diseases, University of California Irvine School of Medicine, Children's Hospital of Orange County, Orange, California
Leptospirosis

Richard A. Oberhelman, M.D.
Professor, Tropical Medicine, Tulane School of Public Health and Tropical Medicine; Professor, Department of Pediatrics, Tulane School of Medicine, New Orleans, Louisiana
Bacillus cereus

Theresa J. Ochoa, M.D.
Assistant Professor, Department of Pediatrics/Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru; Assistant Professor, Epidemiology, Center for Infectious Diseases, University of Texas School of Public Health, Houston, Texas
Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning; *Shigella*; *Salmonella*; *Cryptosporidiosis*

Rosemary M. Olivero, M.D.
Fellow, Division of Pediatric Infectious Diseases, Boston Medical Center, Boston, Massachusetts
Malaria

Alina Olteanu, M.D., Ph.D.
Pediatrician, MyChildren's Medical Center, Plano, Texas
Metabolic Response of the Host to Infections

Miguel L. O'Ryan, M.D.
Professor and Research Director, Faculty of Medicine, University of Chile; Chair, Chilean National Academy of Medicine, Santiago, Chile
Caliciviruses

Gary D. Overturf, M.D.
Professor Emeritus, Pediatrics and Pathology, Department of Pediatrics, University of New Mexico School of Medicine; Medical Director, Infectious Diseases, Pathology, TriCore Reference Laboratories, Albuquerque, New Mexico
Plague (Yersinia pestis); *Clostridial Intoxication and Infection*; *Antimicrobial Prophylaxis*

Debra L. Palazzi, M.D.
Associate Professor of Pediatrics, Division of Infectious Diseases, Baylor College of Medicine; Chief, Infectious Diseases Clinic, Texas Children's Hospital, Houston, Texas
Fever Without Source and Fever of Unknown Origin

Pia S. Pannaraj, M.D., M.P.H.
Assistant Professor, Pediatric Infectious Diseases, University of Southern California, Children's Hospital Los Angeles, Los Angeles, California
Cholangitis and Cholecystitis; *Group B Streptococcal Infections*

Janak A. Patel, M.D.
Professor and Division Director, Division of Pediatric Infectious Disease and Immunology, Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas
Infections in Burn Patients

Mary E. Paul, M.D.
Associate Professor, Department of Pediatrics, Baylor College of Medicine; Chief of Service, Retrovirology and Global Health, Texas Children's Hospital, Houston, Texas
Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

Stephen I. Pelton, M.D.
Professor of Pediatrics and Epidemiology, Department of Pediatrics, Boston University Schools of Medicine and Public Health; Chief, Division of Pediatric Infectious Diseases, Department of Pediatrics, Boston Medical Center, Boston, Massachusetts
Pneumococcal Infections

John Perfect, M.D.
Professor of Medicine, Chief, Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, North Carolina
Cryptococcosis

C. J. Peters, M.D.
John Sealy Distinguished University Chair in Tropical and Emerging Virology; Professor, Departments of Microbiology & Immunology and Pathology; Director for Biodefense, Center for Biodefense & Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, Texas
Hantaviruses; *Rift Valley Fever*

William A. Petri, Jr., M.D., Ph.D.
Chief, Division of Infectious Diseases and International Health, University of Virginia Health System, Charlottesville, Virginia
Amebiasis

Francisco P. Pinheiro, M.D.
Department of Arbovirus, Instituto Evandro Chagas, FNS, Ministry of Health, Belem, Para, Brazil
Oropouche Fever

Kathy Pinkerman, M.D.
Clinical Research Specialist, Baystate Mary Lane Hospital, Ware, Massachusetts
Pasteurella multocida

Benjamin Pinsky, M.D.
Assistant Professor, Department of Pathology, Stanford University School of Medicine; Director, Clinical Virology Laboratory, Stanford Hospital and Clinics and Lucile Packard Children's Hospital; Assistant Professor, Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California
Herpes Simplex Viruses 1 and 2

Stanley A. Plotkin, M.D.
Emeritus Professor of Pediatrics, University of Pennsylvania, Emeritus Professor, Wistar Institute of Research, Emeritus Staff, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; Adjunct Professor, Johns Hopkins University, Baltimore, Maryland
Rabies Virus

Alice Pong, M.D.
Division of Pediatric Infectious Diseases, University of California San Diego, Rady Children's Hospital San Diego, San Diego, California
Retroperitoneal Infections

Eric A. Porsch, B.S.
Graduate Student, Molecular Genetics and Microbiology, Department of Pediatrics, Duke University, Durham, North Carolina
Kingella kingae

Joan S. Purcell, M.D.
Adolescent Medicine and Pediatrics, STEP Pediatrics, Woodlands, Texas
Trichomonas Infections

Natalie M. Quanquin, M.D., Ph.D.
Research Fellow in Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Mycoplasma and Ureaplasma Infections

Kevin K. Quinn, M.D.
Research Fellow, in Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Mumps Virus

Ramya Ramraj, M.D., M.B.B.S.
Affiliate Assistant Professor, Oregon Health and Sciences University; Attending Pediatrician and Pediatric Gastroenterologist, Doernbecher Children's Hospital and Kaiser NW Permanente, Portland, Oregon
Mimiviruses

Sergio E. Recuenco, M.D., M.P.H., Dr.P.H.
Surveillance, Epidemiology, and Ecology Unit Team Lead, Rabies Program, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Rabies Virus

Jack S. Remington, M.D.
Professor Emeritus, Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California; Consultant, Research Institute of the Palo Alto Medical Foundation, Palo Alto, California
Toxoplasmosis

Paula A. Revell, Ph.D.
Assistant Professor, Pathology and Immunology and Department of Pediatrics, Baylor College of Medicine; Director of Microbiology and Virology, Pathology, Texas Children's Hospital, Houston, Texas
Bacterial Laboratory Diagnosis

Anne W. Rimoin, Ph.D., M.P.H.
Associate Professor, Epidemiology, University of California, Los Angeles School of Public Health, Los Angeles, California
Monkeypox and Other Poxviruses

Ana Maria Ríos, M.D.
Pediatric Infectious Disease Specialist, Pediatric Infectious Disease, Cook Children's Medical Center, Fort Worth, Texas
Hepatitis A Virus

José R. Romero, M.D.
Horace C. Cabe Professor of Infectious Diseases, Department of Pediatrics, University of Arkansas for Medical Sciences; Director, Pediatric Infectious Diseases Section, Department of Pediatrics, Arkansas Children's Hospital; Director, Clinical Trials Research, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas
Fungal Meningitis; *St. Louis Encephalitis*; *West Nile Virus*

Lawrence A. Ross, M.D.
Professor of Pediatrics, Division of Pediatric Infectious Diseases, Children's Hospital of Los Angeles; Professor of Pediatrics, Division of Pediatric Infectious Diseases, Keck School of Medicine at the University of Southern California, Los Angeles, California
Trypanosomiasis

Charles E. Rupprecht, V.M.D., M.S., PhD.
Director of Research, The Global Alliance for Rabies Research, Manhattan, Kansas
Rabies Virus

Xavier Sáez-Llorens, M.D.
Professor of Pediatrics, Head of Infectious Diseases, Hospital del Niño, Panama City, Panama
Parameningeal Infections

Julia Shaklee Sammons, M.D., M.S.C.E.
Assistant Professor of Pediatrics, University of Pennsylvania School of Medicine; Associate Hospital Epidemiologist and Medical Director, Department of Infection Prevention and Control, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Health Care–Associated Infections

Pablo J. Sánchez, M.D.
Professor of Pediatrics, Department of Pediatrics, University of Texas Southwestern Medical Center; Attending Physician, Parkland Health & Hospital System; Attending Physician, Children's Medical Center, Dallas, Texas
Syphilis

Linette Sande, M.D.
Assistant Professor, Department of Pediatrics, Loma Linda University; Attending Physician, Division of Pediatric Infectious Diseases, Loma Linda University Children's Hospital, Loma Linda, California
Group A, Group C, Group G Beta Hemolytic Streptococcal Infections

Javier Santisteban-Ponce, M.D.
Professor of Pediatrics, Department of Pediatrics, Universidad Peruana Cayetano Heredia; Attending Physician, Department of Pediatrics, Hospital Nacional Edgardo Rebagliati–EsSalud, Lima, Peru
Salmonella

Laura A. Sass, M.D.
Assistant Professor, Department of Pediatrics, Eastern Virginia Medical School; Attending Physician, Division of Pediatric Infectious Diseases, Children's Hospital of The King's Daughters, Norfolk, Virginia
Enterobacter

Carlos A. Sattler, M.D.
Senior Director, Medical Affairs, Merck Vaccines, West Point, Pennsylvania
Stenotrophomonas (Xanthomonas) maltophilia

Robin Schmucker, M.D.
Fellow, Pediatric Infectious Diseases, University of Louisville, Louisville, Kentucky
Antibiotic-Associated Colitis

Danica J. Schulte, M.D.
Assistant Professor in Residence, Department of Pediatrics, University of California, Los Angeles; Attending Physician, Department of Pediatrics, Section of Pediatric Infectious Diseases, Immunology and Allergy, Cedars-Sinai Medical Center, Los Angeles, California
Human Parvovirus B19; *Passive Immunization*

Jennifer E. Schuster, M.D.
Fellow, Pediatric Infectious Diseases, Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, Tennessee
Human Bocaviruses; *Human Metapneumovirus*

Gordon E. Schutze, M.D.
Vice Chairman for Educational Affairs, Department of Pediatrics, Baylor College of Medicine; Vice President for International Medical Services, Baylor International Pediatric AIDS Initiative at Texas Children's Hospital, Houston, Texas
Tularemia

Carlos Seas, M.D., M.Sc.
Associate Professor, Department of Medicine, Universidad Peruana Cayetano Heredia; Vice Director, Instituto de Medicina Tropical Alexander von Humboldt; Attending Physician, Infectious and Tropical Medicine, Hospital Nacional Cayetano Heredia, Lima, Peru
Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis

Filiz O. Seeborg, M.D., M.P.H.
Assistant Professor, Department of Pediatrics, Section of Allergy, Immunology, and Rheumatology, Baylor College of Medicine; Allergy and Immunology Service, Texas Children's Hospital, Houston, Texas
Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

Patrick C. Seed, M.D., Ph.D.
Associate Professor, Pediatrics/Pediatric Infectious Diseases; Assistant Professor, Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina
Extraintestinal Pathogenic Escherichia coli

Eugene D. Shapiro, M.D.
Professor of Pediatrics, Epidemiology, and Investigative Medicine, Yale University, New Haven, Connecticut
Epidemiology and Biostatistics of Infectious Diseases

Nina L. Shapiro, M.D.
Associate Professor, Department of Head and Neck Surgery, UCLA School of Medicine; Director, Pediatric Otolaryngology, University of California, Los Angeles School of Medicine, Los Angeles, California
Rhinosinusitis; *Mastoiditis*

William T. Shearer, M.D., Ph.D.
Professor, Departments of Pediatrics and Immunology, Baylor College of Medicine; Former Chief, Allergy and Immunology and Rheumatology Service, Texas Children's Hospital, Houston, Texas
Chronic Bronchitis; *Primary Immunodeficiencies*; *Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome*

Kareem W. Shehab, M.D.
Fellow, Division of Pediatric Infectious Diseases, Stanford University, Stanford, California
Coccidioidomycosis

Ziad M. Shehab, M.D.
Professor, Departments of Pediatrics and Pathology, University of Arizona, Tucson, Arizona
Coccidioidomycosis

[†]Jerry L. Shenep, M.D.
Formerly Member, Department of Infectious Diseases, St. Jude Children's Research Hospital; Professor, Department of Pediatrics, University of Tennessee Health Science Center, Memphis, Tennessee
Enterococcal and Viridans Streptococcal Infections

W. Donald Shields, M.D.
Professor, Department of Pediatric Neurology, David Geffen School of Medicine at UCLA; Mattel Children's Hospital UCLA, Los Angeles, California
Encephalitis and Meningoencephalitis

Robyn Shimizu-Cohen
Clinical Laboratory Scientist, MT (ASCP), Department of Pathology and Laboratory Medicine, UCLA Health System, Los Angeles, California
Parasitic Laboratory Diagnosis

Debbie-Ann T. Shirley, M.B.B.S.
Fellow, Division of Infectious Diseases and Tropical Pediatrics, Department of Pediatrics, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland
Cholera

Stanford T. Shulman, M.D.
Virginia H. Rogers Professor of Pediatric Infectious Diseases, Northwestern University Feinberg School of Medicine; Chief, Division of Infectious Diseases, Department of Pediatrics, Children's Memorial Hospital, Chicago, Illinois
Kawasaki Disease

[†]Deceased.

Constantine Simos, D.M.D.
Assistant Clinical Professor, Oral and Maxillofacial Surgery, Columbia University College of Dental Medicine, New York, New York; Attending Physician, Oral and Maxillofacial Surgery, Robert Wood Johnson University Hospital; Attending Physician Oral and Maxillofacial Surgery, Saint Peter's University Hospital, New Brunswick, New Jersey
Infections of the Oral Cavity

Arnold L. Smith, M.D.
Professor of Pathobiology, University of Washington School of Public Health; Director, Microbial Pathogens Program, Seattle Biomedical Research Institute, Seattle, Washington
Meningococcal Disease

Joseph W. St. Geme III, M.D.
Chairman of Pediatrics and Physician-in-Chief, The Children's Hospital of Philadelphia; Chairman and Professor of Pediatrics and Professor of Microbiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania
Molecular Determinants of Microbial Pathogenesis; *Kingella kingae*

Mary Allen Staat, M.D., M.P.H.
Professor, Department of Pediatrics, University of Cincinnati, College of Medicine; Director, International Adoption Center, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
Genital Infections; *Rotavirus*; *Infectious Disease Considerations in International Adoptees and Refugees*

Damien Stark, B.Sc., M.Sc., Ph.D., F.A.S.M, F.A.C.T.M.
Associate, Department of Medical and Molecular Biosciences, University of Technology Sydney, Broadway, New South Wales, Australia; Senior Hospital Scientist, Microbiology, St. Vincent's Hospital, Darlinghurst, New South Wales, Australia
Dientamoeba fragilis Infections

Jeffrey R. Starke, M.D.
Professor, Department of Pediatrics, Baylor College of Medicine; Medical Director, Department of Infection Control, Texas Children's Hospital, Houston, Texas
Infective Endocarditis; *Tuberculosis*

Kimberly F. Starr, B.S., M.S.
Graduate Student, Molecular Genetics and Microbiology, Pediatrics, Duke University, Durham, North Carolina
Kingella kingae

Barbara W. Stechenberg, M.D.
Pediatric Infectious Diseases Specialist, Department of Pediatrics, Baystate Children's Hospital, Springfield, Massachusetts; Professor of Pediatrics, Department of Pediatrics, Tufts University School of Medicine, Boston, Massachusetts
Eosinophilic Meningitis; *Moraxella catarrhalis*; *Diphtheria*; *Pasteurella multocida*; *Bartonella Infections*

William J. Steinbach, M.D.
Associate Professor of Pediatrics, Molecular Genetics and Micobiology, Department of Pediatrics, Department of Molecular Genetics and Microbiology; Attending Physician, Division of Pediatric Infectious Diseases, Duke University Medical Center, Durham, North Carolina
Opportunistic Infections in Hematopoietic Stem Cell Transplantation; *Aspergillosis*; *Mucormycosis and Entomopthoramycosis (formerly Zygomycosis)*, *Fusariosis and Scedosporiosis*; *Miscellaneous Mycoses*

Paul G. Steinkuller, M.D.
Associate Professor, Departments of Ophthalmology and Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Ocular Infections

Janis Stoll, M.D.
Instructor, Department of Pediatrics, Division of Pediatric Gastroenterology, Washington University School of Medicine; Pediatric Gastroenterology and Liver Transplantation Services, Saint Louis Children's Hospital, Saint Louis, Missouri
Hepatitis B and D Viruses

Jeffrey Suen, M.D.
Clinical Physician, Division of Pediatric Infectious Diseases, David Geffen School of Medicine at UCLA; Attending Physician, Division of Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Toxic Shock Syndrome

Ciro V. Sumaya, M.D.
Professor, Health Policy and Management, Cox Endowed Chair in Medicine; Founding Dean, School of Rural Public Health, Texas A&M Health Sciences Center, College Station, Texas
Epstein-Barr Virus

Lillian Sung, M.D., Ph.D.
Associate Professor, Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada
The Febrile Neutropenic Patient

Douglas S. Swanson, M.D.
Associate Professor of Pediatrics, Pediatric Infectious Diseases, Children's Mercy Hospitals and Clinics; Associate Professor of Pediatrics, University of Missouri–Kansas City, Kansas City, Missouri
The Human Microbiome

Tina Q. Tan, M.D.
Professor of Pediatrics, Department of Pediatrics, Feinberg School of Medicine, Northwestern University; Attending Physician, Division of Infectious Diseases, Co-Director, Travel Medicine Clinic, Director, International Adoptee Clinic, Division of Pediatric Infectious Diseases, Ann and Robert H. Lurie Children's Hospital, Chicago, Illinois
Infections Related to Prosthetic or Artificial Devices; *Giardiasis*; *Naegleria, Acanthamoeba, and Balamuthia Infections*

Herbert B. Tanowitz, M.D.
Medical Director of Diagnostic Parasitology Laboratory, Albert Einstein College of Medicine, Department of Pathology, Bronx, New York
Trypanosomiasis

Robert B. Tesh, M.D.
Professor, Department of Pathology, University of Texas Medical Branch, Galveston, Texas
Crimean-Congo Hemorrhagic Fever; *Phlebotomus Fever (Sandfly Fever)*

Michael A. Tolle, M.D.
Assistant Professor, Department of Pediatrics, Retrovirology and Global Health Section, International Pediatric AIDS Initiative (BIPAI), Baylor College of Medicine Children's Foundation, Texas Children's Hospital, Houston, Texas; Tanzania Bugando Medical Centre, Mwanza, Tanzania
Oncoviruses (Human T-Cell Lymphotropic Viruses) and Lentiviruses (Human Immunodeficiency Virus Type 2)

Philip Toltzis, M.D.
Professor, Department of Pediatrics, Rainbow Babies and Children's Hospital, Cleveland, Ohio
Antibiotic Resistance

Michael F. Tosi, M.D.
Professor of Pediatrics, Division of Pediatric Infectious Diseases, Mount Sinai School of Medicine, New York, New York
Normal and Impaired Immunologic Responses to Infection

Amelia P. A. Travassos da Rosa, B.Sc.
Research Associate, Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, Texas
Oropouche Fever

Theodore F. Tsai, M.D., M.P.H.
Head, Scientific Affairs, Novartis Vaccines, Cambridge, Massachusetts
Orbiviruses, Coltiviruses, and Seadornaviruses; *Eastern Equine Encephalitis*; *Western Equine Encephalitis*; *Venezuelan Equine Encephalitis*; *Other Alphaviral Infections*; *St. Louis Encephalitis*; *Japanese Encephalitis*; *Tick-Borne Encephalitis*; *Other Flaviviral Infections*

David A. Turner, M.D.
Director, Pediatric Critical Care Fellowship Program, Assistant Professor, Department of Pediatrics, Division of Pediatric Critical Care, Duke Children's Hospital, Duke University Medical Center, Durham, North Carolina
Acute Respiratory Distress Syndrome in Children

Timothy M. Uyeki, M.D., M.P.H., M.P.P.
Deputy Chief for Science, Epidemiology and Prevention Branch, Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia; Associate Clinical Professor, Department of Pediatrics, University of California, San Francisco, San Francisco, California
Influenza Viruses

Jesus G. Vallejo, M.D.
Associate Professor, Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine; Medical Director, International Patient Services, Texas Children's Hospital, Houston, Texas
Myocarditis; *Bacteremia and Septic Shock*

John A. Vanchiere, M.D., Ph.D.
Associate Professor, Department of Pediatrics, Chief, Pediatric Infectious Diseases, Lousiana State University Health Sciences Center, Shreveport, Louisiana
Human Polyomaviruses

Pedro Fernando da C. Vasconcelos, M.D., Ph.D.
Chief, Department of Arbovirology and Hemorrhagic Fevers, Coordinator, National Reference Laboratory of Arboviruses; Director, National Institute of Science and Technology for Viral Hemorrhagic Fevers; Director, PAHO-WHO CC for Research and Diagnostic Reference on Arbovirus, Instituto Evandro Chagas, SVS/Ministry of Health, Ananindeua, Brazil
Oropouche Fever

Jorge J. Velarde, M.D., Ph.D.
Fellow, Division of Infectious Diseases, Children's Hospital Boston, Boston, Massachusetts
Diarrhea-Causing and Dysentery-Causing Escherichia coli

Robert S. Venick, M.D.
Assistant Professor, Department of Pediatrics and Surgery, David Geffen School of Medicine at UCLA; Attending Physician, Mattel Children's Hospital UCLA, Los Angeles, California
Esophagitis

Ellen R. Wald, M.D.
Professor and Chair, Department of Pediatrics, University of Wisconsin School of Medicine and Public Health; Pediatrician-in-Chief, Department of Pediatrics, American Family Children's Hospital, Madison, Wisconsin
Uvulitis; *Urethritis*; *Cystitis and Pyelonephritis*; *Infections in Child Care Environments*

Thomas J. Walsh, M.D.
Director, Transplantation-Oncology Infectious Diseases Program, Weill Cornell Medical Center of Cornell University, New York, New York
Antifungal Agents

Mark A. Ward, M.D.
Associate Professor, Department of Pediatrics; Director of Pediatric Housestaff Education, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Fever: Pathogenesis and Treatment

Michelle Weinberg, M.D., M.P.H.
Medical Epidemiologist, Immigrant, Refugee and Migrant Health Branch, Division of Global Migration and Quarantine, Centers for Disease Control and Prevention, Atlanta, Georgia
International Travel Issues for Children

Nicholas Weinberg, M.D.
Emergency Physican, CVPH Medical Center, Plattsburgh, New York
International Travel Issues for Children

Melanie Wellington, M.D.
Assistant Professor, Pediatric Infectious Diseases, University of Rochester Medical School Center Rochester, New York
Fusariosis and Scedosporiosis

Robert C. Welliver, Sr., M.D.
Professor of Pediatrics, CMRI Hobbs-Recknagel Endowed Chair in Pediatrics, Chief, Division of Pediatric Infectious Diseases, Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma
Bronchiolitis and Infectious Asthma

J. Gary Wheeler, M.D., M.P.S.
Professor of Pediatrics, Division of Infectious Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Complications of Pneumonia

A. Clinton White, Jr., M.D.
Paul R. Stalnaker Distinguished Professor, Department of Internal Medicine; Director, Division of Infectious Diseases, Internal Medicine, University of Texas Medical Branch, Galveston, Texas
Cryptosporidiosis; *Cyclosporiasis*, *Cystoisosporiasis*, *and Microsporidiosis*; *Cestodes*; *Foodborne Trematodes*; *Schistosomiasis*

Suzanne Whitworth, M.D.
Division of Pediatric Infectious Diseases, Cook Children's Healthcare System, Fort Worth, Texas
Aggregatibacter actinomycetemcomitans; *Actinomycosis*

Bernhard L. Wiedermann, M.D., M.A.
Professor, Department of Pediatrics, George Washington University School of Medicine and Health Sciences; Attending Physician, Division of Infectious Diseases, Children's National Medical Center, Washington, District of Columbia
Sporotrichosis

John V. Williams, M.D.
Associate Professor, Department of Pediatrics; Associate Professor, Departments of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, Tennesee
Human Bocaviruses; *Human Metapneumovirus*

Natalie Williams-Bouyer, M.D.
Assistant Professor, Department of Pathology; Assistant Director, Division of Clinical Microbiology, University of Texas Medical Branch, Galveston, Texas
Infections in Burn Patients

Murray Wittner, M.D., Ph.D.
Department of Pathology, Albert Einstein College of Medicine, Bronx, New York
Trypanosomiasis

Charles R. Woods, Jr., M.D., M.S.
Professor of Pediatrics (Infectious Diseases), Vice Chair for Faculty Development; Director, Child Health Services Research Unit, Director, Pediatric Infectious Diseases Laboratory, Department of Pediatrics, University of Louisville School of Medicine, Louisville, Kentucky
Genital Infections; *Gonococcal Infections*; *Other Yersinia Species*

Terry W. Wright, Ph.D.
Associate Professor, Department of Pediatrics, University of Rochester School of Medicine, Rochester, New York
Pneumocystis Pneumonia

Ram Yogev, M.D.
Director, Section of Pediatric, Adolescent, and Maternal HIV, Department of Pediatrics, Children's Memorial Hospital; Professor, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, Illinois
Infections Related to Prosthetic or Artificial Devices; *Parainfluenza Viruses*

Edward Young, A.B., M.S., M.D.
Professor, Department of Medicine, Baylor College of Medicine, Michael E DeBakey Veterans Affairs Medical Center, Houston, Texas
Brucellosis

Ramia Zakhour, M.D.
Fellow, Department of Pediatrics, Pediatric Infectious Diseases, University of Texas–Houston Medical School, Houston, Texas
Campylobacter jejuni

Mara Zambruni, M.D., M.P.H.
Fellow, Pediatric Infectious Diseases, Department of Pediatrics, University of Texas, Houston, Texas
Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

Theoklis E. Zaoutis, M.D., M.S.C.E.
Thomas Frederick McNair Scott Professor of Pediatrics and Professor of Epidemiology, Perelman School of Medicine, University of Pennsylvania; Associate Chief, Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Candidiasis; *Mucormycosis and Entomopthoramycosis (formerly Zygomycosis)*

This page intentionally left blank

Preface

Morbidity and mortality rates related to infectious diseases decreased dramatically during the first half of the 20th century in the developed world because of major improvements in public health (e.g., clean water, adequate sanitation, and vector control) and personal health. Further major reduction in morbidity and mortality rates occurred in the second half of that century following the introduction of antimicrobial therapy, as well as active and passive immunization efforts. Despite these advances in the 20th century, infectious diseases in the developed world remain the leading cause of morbidity in infants and children in the 21st century. Children continue to experience three to nine respiratory infections and one to three gastrointestinal illnesses annually, requiring visits to physicians that outnumber the visits made for the purpose of well-child care. Infectious diseases are also the most common cause of school absenteeism. In more recent years, the emergence of resistance to multiple antibiotics by a large number of bacterial microorganisms (e.g., community-associated methicillin-resistant *Staphylococcus aureus*) has contributed to the morbidity and mortality related to infectious diseases processes, as have new infectious agents (e.g., swine influenza virus, SARS coronavirus) and changes in the clinical manifestations and severity of established infectious agents (e.g., enterovirus 71, swine influenza). The developing world is confronted with the same present-day problems seen in the developed world (antimicrobial resistance and new infectious agents), and these challenges are compounded by malnutrition and lack of adequate public health services and antimicrobial agents.

The first edition of *Feigin and Cherry* was written because Drs. Feigin and Cherry and many of their colleagues were concerned that no single reference text existed that comprehensively covered infectious diseases in children and adolescents. With each subsequent edition, including this one, the goal has been to provide comprehensive coverage of all subjects pertinent to the study of infectious diseases in children. Any attempt to summarize our present understanding of infectious diseases for serious students of the subject is a formidable task. In many areas, new information continues to accrue so rapidly that material becomes dated before it can appear in a text of this magnitude. Nevertheless, in this edition the editors and their author colleagues have endeavored to provide the most comprehensive and up-to-date discussion of pediatric infectious diseases ever compiled. This new edition is available online as well as in print. Purchasers can access the online version by registering their personal identification number (PIN) (found on the inside front cover of the book) at www.expertconsult.com. Online access includes not only fully searchable text, photos, illustrations, and tables, but also references linked to PubMed.

To provide a text as comprehensive and authoritative as possible, we, the editors, have enlisted contributions from a large number of individuals whose collective expertise is responsible for whatever success we may have had in meeting our objective. We offer our most profound appreciation to the 301 fellow contributors from 80 universities or institutions in 17 countries for their professional expertise and devoted scholarship. Their cooperation and willingness to work with us leave us deeply in their debt. Of note is the fact that 18 authors (Carol Baker, Ken Boyer, James Cherry, Morven Edwards, Mary Glode, Charles Grose, Caroline Hall, Scott Halstead, Margaret Hammerschlag, Shelly Kaplan, Jerry Klein, Edward Mason, George McCracken, Stanley Plotkin, Arnold Smith, Barbara Stechenberg, Robert Tesh, and Murray Wittner) have contributed to all seven editions of *Feigin and Cherry*.

Once again, infectious diseases are discussed according to organ systems that may be affected, as well as individually by microorganisms. In all sections in which diseases related to specific agents are discussed, emphasis has been placed, to the greatest extent possible, on the specificity of clinical manifestations that may be related to the organism causing the disease. Detailed information regarding the best means to establish a diagnosis and explicit recommendations for therapy is provided. In the present era of instant information, we have noted that historical perspectives relating to disease categories, as well as specific agents, are ignored. Because history is an important teacher, we have retained relevant historical details in this seventh edition.

Throughout the 32 years and seven-edition history of the *Textbook of Pediatric Infectious Diseases*, a number of classic chapters exist (e.g., measles, rubella, enteroviruses, and mycoplasma infections). The data in these chapters are unavailable in any other single source publication.

The entire text of this seventh edition has been revised extensively. The sixth edition contained almost 4000 pages, which is close to the maximum that can be included in a two-volume book. Therefore, with this seventh edition, we were faced with a major dilemma: specifically, how to include new important material that had become available since the sixth edition but not to substantially increase the size of the book. We approached this dilemma in two ways. One problem in previous editions was redundancy, which we have addressed by reducing the number of chapters from 266 to 253. This reduction has been accomplished by combining information in some previous separate chapters into more concise single presentations. The second way to reduce pages in the printed

version of this textbook was to print only new references. The electronic version, though, contains all references.

This edition continues the format that was initiated in the fourth edition in that infections with specific microorganisms have been organized to provide appropriate emphasis on the common features that may relate specific microorganisms to one another. Thus, all gram-positive coccal organisms are presented sequentially and are followed by gram-negative cocci, gram-positive bacilli, enterobacteria, gram-negative coccobacilli, Treponemataceae, anaerobic bacteria, and so forth. In addition, special sections of the text have been devoted to discussions of each of the following: molecular determinants of microbial pathogenesis; immunologic and phagocytic responses to infection; metabolic response of the host to infections; interaction of infection and nutrition; pathogenesis and treatment of fever; the human microbiome; epidemiology and biostatistics of infectious diseases; infections of the compromised host; Kawasaki disease; chronic fatigue syndrome; international travel issues for children; infectious disease problems of international adoptees and refugees; nosocomial infections; prevention and control of infections in hospitalized children; pharmacology and pharmacokinetics of antibacterial, antiviral, antifungal, and antiparasitic agents; immunomodulating agents; active and passive immunizing agents; public health considerations; infections in day care environments; and use of the bacteriology, mycology, parasitology, virology, and serology laboratories. The section on infections in the compromised host, which includes 16 chapters, has again been expanded. This expansion has been necessitated by the large number of children, particularly posttransplantation recipients, who now serve as the source of many infectious disease problems and constitute a large part of the consulting practice of many pediatric infectious disease physicians.

With some sadness, we have retained a section on bioterrorism, which is necessitated by the current state of world affairs. The section on immunomodulating agents and their potential use in the treatment of infectious diseases has been expanded because information on this subject has become more extensive since the publication of the last edition. Human and animal bites are now combined in one chapter, and a chapter on probiotics has been added.

This project could not have been brought to fruition without the help and assistance of many people whose names do not appear in the text. No words are sufficient to adequately convey our gratitude appropriately; we hope that they know they have our heartfelt thanks.

We would like to single out certain individuals for specific mention. First and foremost, we convey our appreciation to Laura Wennstrom Sheehan (Administrative Analyst, Department of Pediatrics, David Geffen School of Medicine at UCLA, and the Division of Infectious Diseases, Mattel Children's Hospital UCLA) for the thousands of hours she devoted to this seventh edition. From our UCLA office she coordinated the overall process of moving the book forward. She tended to numerous details relating to copyediting, transcribing, references, timing, and communication between the editors and Elsevier. Her expertise in EndNote was invaluable to the authors, editors, and publishing team for the organization of countless references throughout this edition. We are extremely grateful to have her as a part of our team. We would also like to acknowledge the hard work of Jordan Mann, Matthew Sheehan, and Gayla Wennstrom, who assisted Laura throughout this process.

The following students at UCLA played a key role in processing chapters and in particular helping with references: Amabelle Aguelo, Elham Eftekhari, Abe Harn, Jewel Powe, and Anum Qureshi. Tiki Valentino (Administrative Assistant III, Department of Pediatrics, David Geffen School of Medicine at UCLA, and the Division of Infectious Diseases, Mattel Children's Hospital UCLA) coordinated the work of all the students, and her efforts deserve much praise.

We would also like to thank Nathaniel Wilder Wolf at Baylor who coordinated all the parasite chapters.

Of course this seventh edition of *Feigin and Cherry* would not have been possible without Elsevier. In planning this edition we suffered an initial setback when there was a change in key personnel at Elsevier. The continuing efforts, though, of Stefanie Jewell-Thomas (Senior Content Strategist, Global Clinical Reference), Lisa Barnes (Content Development Specialist), and Claire Kramer (Senior Project Manager) allowed the book publishing to proceed in an efficient manner. Their efforts are much appreciated.

Finally, we thank the Baylor College of Medicine and Texas Children's Hospital in Houston, Texas, the David Geffen School of Medicine at UCLA and the Mattel Children's Hospital UCLA in Los Angeles, California, and Duke University School of Medicine in Durham, North Carolina for providing an environment that is supportive of intellectual pursuits.

James D. Cherry, M.D.
Sheldon L. Kaplan, M.D.
Gail J. Harrison, M.D.
William J. Steinbach, M.D.
Peter J. Hotez, M.D

Contents

VOLUME ONE

PART I
HOST-PARASITE RELATIONSHIPS AND THE PATHOGENESIS OF INFECTIOUS DISEASES

1 Molecular Determinants of Microbial Pathogenesis, 3
David A. Hunstad • Ravi Jhaveri • Joseph W. St. Geme III

2 Normal and Impaired Immunologic Responses to Infection, 22
Michael F. Tosi

3 Metabolic Response of the Host to Infections, 65
Elizabeth C. Bellino • Alina Olteanu • Ralph D. Feigin • William R. Beisel

4 Fever: Pathogenesis and Treatment, 83
Mark A. Ward

5 The Human Microbiome, 88
Douglas S. Swanson

6 Epidemiology and Biostatistics of Infectious Diseases, 95
Eugene D. Shapiro and Robert S. Baltimore

PART II
INFECTION OF SPECIFIC ORGAN SYSTEMS

SECTION I
UPPER RESPIRATORY TRACT INFECTIONS, 131

7 The Common Cold, 132
James D. Cherry

8 Infections of the Oral Cavity, 140
Constantine Simos and Blanca E. Gonzalez

9 Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis), 156
James D. Cherry

10 Uvulitis, 165
Ellen R. Wald

11 Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses, 167
Nira A. Goldstein and Margaret R. Hammerschlag

12 Cervical Lymphadenitis, 175
C. Mary Healy and Carol J. Baker

13 Parotitis, 189
Judith R. Campbell

14 Rhinosinusitis, 193
James D. Cherry • Jagmeet Mundi • Nina L. Shapiro

15 Otitis Externa, 203
Ellen M. Friedman and Maria Carrillo-Marquez

16 Otitis Media, 209
Jerome O. Klein and Charles D. Bluestone

17 Mastoiditis, 233
James D. Cherry • Audrey P. Calzada • Nina L. Shapiro

18 Croup (Laryngitis, Laryngotracheitis, Spasmodic Croup, Laryngotracheobronchitis, Bacterial Tracheitis, and Laryngotracheobronchopneumonitis) and Epiglottitis (Supraglottitis), 241
James D. Cherry

SECTION II
LOWER RESPIRATORY TRACT INFECTIONS, 261

19 Acute Bronchitis, 262
James D. Cherry

20 Chronic Bronchitis, 265
I. Celine Hanson and William T. Shearer

21 Bronchiolitis and Infectious Asthma, 271
Robert C. Welliver, Sr.

22 Pediatric Community-Acquired Pneumonia, 283
Carrie L. Byington and John S. Bradley

23 Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis, 295
Minh L. Doan and Leland L. Fan

24 Complications of Pneumonia, 306
J. Gary Wheeler and Richard F. Jacobs

25 Cystic Fibrosis, 323
Peter W. Hiatt • Ruth Ann Luna • Kathryn S. Moffett

SECTION **III**
INFECTIONS OF THE HEART, 349

26 Infective Endocarditis, 350
Jeffrey R. Starke

27 Infectious Pericarditis, 371
Sheldon L. Kaplan

28 Myocarditis, 381
Jesus G. Vallejo

29 Acute Rheumatic Fever, 401
Diana R. Lennon

30 Mediastinitis, 419
Morven S. Edwards

SECTION **IV**
CENTRAL NERVOUS SYSTEM INFECTIONS, 424

31 Bacterial Meningitis Beyond the Neonatal Period, 425
Kwang Sik Kim

32 Parameningeal Infections, 462
Xavier Sáez-Llorens and Javier Nieto Guevara

33 Fungal Meningitis, 473
José R. Romero and Richard F. Jacobs

34 Eosinophilic Meningitis, 481
Barbara W. Stechenberg

35 Aseptic Meningitis and Viral Meningitis, 484
David E. Bronstein and Carol A. Glaser

36 Encephalitis and Meningoencephalitis, 492
David E. Bronstein • W. Donald Shields • Carol A. Glaser

37 Parainfectious and Postinfectious Disorders of the Nervous System, 513

37A ■ PARAINFECTIOUS AND POSTINFECTIOUS DEMYELINATING DISORDERS OF THE CENTRAL NERVOUS SYSTEM, 513
J. Lloyd Holder, Jr. and Timothy E. Lotze

37B ■ INFECTION-ASSOCIATED MYELITIS AND MYELOPATHIES OF THE SPINAL CORD, 520
J. Lloyd Holder, Jr. and Timothy E. Lotze

SECTION **V**
GENITOURINARY TRACT INFECTIONS, 529

38 Urethritis, 530
Ellen R. Wald

39 Cystitis and Pyelonephritis, 535
Ellen R. Wald

40 Renal Abscess, 553
Edmond T. Gonzales, Jr. and Sheldon L. Kaplan

41 Prostatitis, 558
Sheldon L. Kaplan

42 Genital Infections, 560
Mariam R. Chacko • Mary Allen Staat • Charles R. Woods, Jr.

SECTION **VI**
GASTROINTESTINAL TRACT INFECTIONS, 592

43 Esophagitis, 593
Paul Krogstad and Robert S. Venick

44 Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning, 598
Theresa J. Ochoa • Mara Zambruni • Elsa Chea-Woo

45 Antibiotic-Associated Colitis, 632
Robin Schmucker and Kristina Bryant

46 Whipple Disease, 639
Zev Davidovics and Mark A. Gilger

SECTION **VII**
LIVER DISEASES, 647

47 Hepatitis, 648
Daniel H. Leung and Leon L. Chen

48 Cholangitis and Cholecystitis, 658
Pia S. Pannaraj and Douglas S. Fishman

49 Pyogenic Liver Abscess, 672
Sheldon L. Kaplan

50 Reye Syndrome, 676
Eugene S. Hurwitz

SECTION **VIII**
OTHER INTRA-ABDOMINAL INFECTIONS, 678

51 Appendicitis and Pelvic Abscess, 679
Thomas L. Kuhls

52 Pancreatitis, 689
Thomas L. Kuhls

53 Peritonitis and Intra-Abdominal Abscess, 698
Judith R. Campbell

54 Retroperitoneal Infections, 706
Alice Pong and John S. Bradley

SECTION **IX**
MUSCULOSKELETAL INFECTIONS, 710

55 Osteomyelitis, 711
Paul Krogstad

56 Septic Arthritis, 727
Paul Krogstad

57 Bacterial Myositis and Pyomyositis, 735
Charles Grose

SECTION **X**
SKIN INFECTIONS, 740

58 Cutaneous Manifestatiions of Systemic Infections, 741
James D. Cherry

59 Roseola Infantum (Exanthem Subitum), 768
James D. Cherry

60 Skin Infections, 772

60A ■ BACTERIAL SKIN INFECTIONS, 772
Mary Anne Jackson

60B ■ VIRAL AND FUNGAL SKIN INFECTIONS, 782
Meena R. Julapalli and Moise L. Levy

SECTION **XI**
OCULAR INFECTIOUS DISEASES, 793

61 Ocular Infections, 794
Mohamed Hussein • Aaron M. Miller • Paul G. Steinkuller

SECTION **XII**
SYSTEMIC INFECTIOUS DISEASES, 823

62 Bacteremia and Septic Shock, 824
Sheldon L. Kaplan and Jesus G. Vallejo

63 Fever Without Source and Fever of Unknown Origin, 837
Debra L. Palazzi

64 Toxic Shock Syndrome, 848
Jeffrey Suen

65 Acute Respiratory Distress Syndrome in Children, 866
Jan Hau Lee • Ira M. Cheifetz • David A. Turner

SECTION **XIII**
INFECTIONS OF THE FETUS AND NEWBORN, 876

66 Approach to Infections in the Fetus and Newborn, 877
Gail J. Harrison

SECTION **XIV**
INFECTIONS OF THE COMPROMISED HOST, 902

67 Primary Immunodeficiencies, 903
Javier Chinen • Mark W. Kline • William T. Shearer

68 The Febrile Neutropenic Patient, 921
Brian T. Fisher and Lillian Sung

69 Opportunistic Infections in Hematopoietic Stem Cell Transplantation, 933
Christopher C. Dvorak and William J. Steinbach

70 Infections in Pediatric Heart Transplantation, 945
Sheldon L. Kaplan

71 Infections in Pediatric Lung Transplantation, 957
Jill A. Hoffman

72 Opportunistic Infections in Liver and Intestinal Transplantation, 989
Michael Green and Marian G. Michaels

73 Opportunistic Infections in Kidney Transplantation, 1002
Gail J. Harrison

74 Infections Related to Prosthetic or Artificial Devices, 1015
Ram Yogev and Tina Q. Tan

75 Infections Related to Craniofacial Surgical Procedures, 1043
Marc A. Mazade

76 Infections in Burn Patients, 1047
Janak A. Patel and Natalie Williams-Bouyer

SECTION **XV**
UNCLASSIFIED INFECTIOUS DISEASES, 1063

77 Kawasaki Disease, 1064
Stanford T. Shulman

78 Chronic Fatigue Syndrome, 1085
Leonard R. Krilov

PART III
INFECTIONS WITH SPECIFIC MICROORGANISMS

SECTION **XVI**
BACTERIAL INFECTIONS, 1095

79 Nomenclature for Aerobic and Anaerobic Bacteria, 1096
David A. Bruckner

SUBSECTION **1**
GRAM-POSITIVE COCCI, 1113

80 *Staphylococcus aureus* Infections (Coagulase-Positive Staphylococci), 1113
Sheldon L. Kaplan • Kristina G. Hulten • Edward O. Mason

81 Coagulase-Negative Staphylococcal Infections, 1131
David Y. Hyun

82 Group A, Group C, Group G Beta Hemolytic Streptococcal Infections, 1140
Linette Sande and Anthony R. Flores

83 Group B Streptococcal Infections, 1153
Pia S. Pannaraj and Carol J. Baker

84 Enterococcal and Viridans Streptococcal Infections, 1170
B. Keith English and Jerry L. Shenep

85 Pneumococcal Infections, 1198
Stephen I. Pelton and Michael R. Jacobs

86 Miscellaneous Gram-Positive Cocci, 1246
Randall G. Fisher

SUBSECTION **2**
GRAM NEGATIVE COCCI, 1250

87 *Moraxella catarrhalis*, 1250
Barbara W. Stechenberg

88 Meningococcal Disease, 1253
Marsha S. Anderson • Mary P. Glodé • Arnold L. Smith

89 Gonococcal Infections, 1271
Charles R. Woods, Jr.

SUBSECTION **3**
GRAM-POSITIVE BACILLI, 1301

90 Diphtheria, 1301
Barbara W. Stechenberg

91 Anthrax, 1311
Morven S. Edwards

92 *Bacillus cereus*, 1316
Margot Anderson and Richard A. Oberhelman

93 *Arcanobacterium haemolyticum*, 1322
Natascha Ching

94 Erysipelothrix rhusiopathiae, 1327
Randall G. Fisher

95 Listeriosis, 1329
Tobias Kollmann • Robert Bortolussi • Timothy Mailman

96 Tuberculosis, 1335
Andrea T. Cruz and Jeffrey R. Starke

97 Other Mycobacteria, 1381
W. Matthew Linam and Richard F. Jacobs

98 Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses, 1392
Andrea T. Cruz

99 *Nocardia*, 1418
Nada Harik and Richard F. Jacobs

100 Corynebacterium and Rhodococcus, 1423
Randall G. Fisher

SUBSECTION **4**
GRAM-NEGATIVE BACILLI, 1429

101 Citrobacter, 1429
Randall G. Fisher

102 *Enterobacter*, 1433
Laura A. Sass and Randall G. Fisher

103 Extraintestinal Pathogenic *Escherichia coli*, 1439
Patrick C. Seed

104 Diarrhea-Causing and Dysentery-Causing *Escherichia coli*, 1449
Jorge J. Velarde • Andrea A. Berry • James P. Nataro

105 Klebsiella, 1465
Randall G. Fisher

106 *Morganella morganii*, 1469
Randall G. Fisher

107 *Proteus*, 1471
Randall G. Fisher

108 *Providencia*, 1474
Randall G. Fisher

109 *Shigella*, 1476
Theresa J. Ochoa and Margaret Kosek

110 *Serratia*, 1488
Randall G. Fisher

111 Salmonella, 1491
Theresa J. Ochoa and Javier Santisteban-Ponce

112 Plague *(Yersinia pestis)*, 1509
Gary D. Overturf

113 Other *Yersinia* Species, 1514
Charles R. Woods, Jr.

114 Miscellaneous Enterobacteriaceae, 1532
Randall G. Fisher

115 Aeromonas, 1538
Santhosh Nadipuram and James D. Cherry

116 *Pasteurella multocida*, 1545
Barbara W. Stechenberg and Kathy Pinkerman

117 Cholera, 1548
Debbie-Ann T. Shirley and Matthew B. Laurens

118 Vibrio parahaemolyticus, 1556
David Y. Hyun

119 *Vibrio vulnificus*, 1560
Randall G. Fisher

120 Miscellaneous Non-Enterobacteriaceae Fermentative Bacilli, 1563
Randall G. Fisher

121 *Acinetobacter*, 1568
Armando G. Correa

122 *Achromobacter (Alcaligenes)*, 1573
Randall G. Fisher

123 *Eikenella corrodens*, 1576
Randall G. Fisher

124 *Elizabethkingia* and *Chryseobacterium* Species, 1578
Randall G. Fisher

125 *Pseudomonas* and Related Genera, 1582
Michael T. Brady and Mario J. Marcon

126 *Stenotrophomonas (Xanthomonas) maltophilia*, 1605
Carlos A. Sattler

SUBSECTION **5**
GRAM-NEGATIVE COCCOBACILLI, 1610

127 *Aggregatibacter actinomycetemcomitans*, 1610
Suzanne Whitworth and Richard F. Jacobs

128 Brucellosis, 1611
Edward Young

129 Pertussis and Other *Bordetella* Infections, 1616
James D. Cherry and Ulrich Heininger

130 *Calymmatobacterium granulomatis*, 1640
Mariam R. Chacko

131 Campylobacter jejuni, 1644
Ramia Zakhour • Gloria P. Heresi • James R. Murphy

132 Tularemia, 1657
Gordon E. Schutze

133 *Haemophilus influenzae*, 1665
Stephen J. Barenkamp

134 Other *Haemophilus* Species (*ducreyi, haemolyticus, influenzae* Biogroup aegyptius, *parahaemolyticus,* and *parainfluenzae*) and *Aggregatibacter (Haemophilus) aphrophilus*, 1685
Stephen J. Barenkamp

135 *Helicobacter pylori*, 1691
Aldo Maspons and Mark A. Gilger

136 *Kingella kingae*, 1699
Eric A. Porsch • Kimberly F. Starr • Joseph W. St. Geme III

137 Legionnaires' Disease, Pontiac Fever, and Related Illnesses, 1705
Paul H. Edelstein and Wilbert H. Mason

138 *Streptobacillus moniliformis* (Rat-Bite Fever), 1715
Kara Dubray and Carol A. Glaser

139 *Bartonella* Infections, 1720
Leigh M. Howard • Kathryn M. Edwards • Barbara Stechenberg

SUBSECTION **6**
TREPONEMATACEAE, 1729

140 Borrelia, 1729

140A ■ LYME DISEASE, 1729
Sunil K. Sood and Peter J. Krause

140B ■ RELAPSING FEVER, 1739
Peter J. Krause

141 Leptospirosis, 1743
Delma J. Nieves

142 *Spirillum minus* (Rat-Bite Fever), 1759
Kara Dubray and Carrie L. Byington

143 Syphilis, 1761
Simon R. Dobson and Pablo J. Sánchez

144 Nonvenereal Treponematoses, 1782
David C. Hilmers

SUBSECTION **7**
ANAEROBIC BACTERIA, 1791

145 Clostridial Intoxication and Infection, 1791
Gary D. Overturf

146 Infant Botulism, 1801
Stephen S. Arnon

147 Tetanus, 1809
James D. Cherry and Rick E. Harrison

148 Actinomycosis, 1820
Suzanne Whitworth and Richard F. Jacobs

149 *Bacteroides, Fusobacterium,* and *Prevotella*, 1825
Steven C. Buckingham

VOLUME TWO

SECTION **XVII**
VIRAL INFECTIONS, 1835

150 Classification and Nomenclature of Viruses, 1836
Marjorie J. Miller

DNA VIRUSES—SUBSECTION **1**
PARVOVIRIDAE, 1843

151 Human Parvovirus B19, 1843
Danica J. Schulte

152 Human Bocaviruses, 1858
Jennifer E. Schuster and John V. Williams

DNA VIRUSES—SUBSECTION **2**
POLYOMAVIRIDAE, 1863

153 Human Polyomaviruses, 1863
John A. Vanchiere

154 Human Papillomaviruses, 1871
Colleen B. Nash • Gail J. Harrison • Kenneth A. Alexander

DNA VIRUSES—SUBSECTION **3**
ADENOVIRIDAE, 1888

155 Adenoviruses, 1888
James D. Cherry and Santhosh Nadipuram

DNA VIRUSES—SUBSECTION **4**
HEPATOVIRIDAE, 1911

156 Hepatitis B and D Viruses, 1911
Maureen Jonas and Janis Stoll

DNA VIRUSES—SUBSECTION **5**
HERPESVIRIDAE, 1933

157 Herpes Simplex Viruses 1 and 2, 1933
Kathleen Gutierrez • Benjamin Pinsky • Ann M. Arvin

158 Cytomegalovirus, 1968
Gail J. Harrison

159 Epstein-Barr Virus, 1992
Charles T. Leach • Ciro V. Sumaya • Gail J. Harrison

160 Human Herpesviruses 6, 7, and 8, 2016
Charles Grose

161 Varicella-Zoster Virus, 2021
Anne A. Gershon

DNA VIRUSES—SUBSECTION **6**
POXVIRIDAE, 2033

162 Smallpox (Variola Virus), 2033
James D. Cherry

163 Monkeypox and Other Poxviruses, 2040
Samantha H. Johnston and Anne W. Rimoin

164 Mimiviruses, 2048
Ramya Ramraj and Gail J. Harrison

RNA VIRUSES—SUBSECTION **1**
PICORNAVIRIDAE, 2051

165 Enteroviruses, Parechoviruses, and Saffold Viruses, 2051
James D. Cherry and Paul Krogstad

166 Rhinoviruses, 2109
Robert L. Atmar and Janet A. Englund

167 Hepatitis A Virus, 2128
Ana María Ríos

RNA VIRUSES—SUBSECTION **2**
CALICIVIRIDAE, 2147

168 Caliciviruses, 2147
Yalda C. Lucero • Miguel L. O'Ryan • David O. Matson

169 Hepatitis E Virus, 2158
Ravi Jhaveri

RNA VIRUSES—SUBSECTION **3**
REOVIRIDAE, 2168

170 Reoviruses, 2168
James D. Cherry

171 Orbiviruses, Coltiviruses, and Seadornaviruses, 2172
Theodore F. Tsai

172 Rotavirus, 2176
Mary Allen Staat • Monica Malone McNeal • David I. Bernstein

RNA VIRUSES—SUBSECTION **4**
TOGAVIRIDAE, 2195

173 Rubella Virus, 2195
James D. Cherry and Kristina Adachi

174 Alphaviruses, 2226

174A ■ EASTERN EQUINE ENCEPHALITIS, 2226
Theodore F. Tsai and Gail J. Harrison

174B ■ WESTERN EQUINE ENCEPHALITIS, 2230
Theodore F. Tsai and Gail J. Harrison

174C ■ VENEZUELAN EQUINE ENCEPHALITIS, 2237
Theodore F. Tsai and Gail J. Harrison

174D ■ CHIKUNGUNYA, 2241
Scott B. Halstead

174E ■ ROSS RIVER VIRUS ARTHRITIS, 2248
John G. Aaskov

174F ■ OTHER ALPHAVIRAL INFECTIONS, 2251
Theodore F. Tsai

SUBSECTION **5**
FLAVIVIRIDAE, 2253

175 Flaviviruses, 2253

175A ■ ST. LOUIS ENCEPHALITIS, 2253
Kimberly C. Martin • José R. Romero • Theodore F. Tsai

175B ■ WEST NILE VIRUS, 2261
José R. Romero

175C ■ YELLOW FEVER, 2274
Duane J. Gubler and Gail J. Harrison

175D ■ DENGUE, DENGUE HEMORRHAGIC FEVER, AND SEVERE DENGUE, 2278
Scott B. Halstead

175E ■ JAPANESE ENCEPHALITIS, 2291
Theodore F. Tsai

175F ■ MURRAY VALLEY ENCEPHALITIS, 2301
John G. Aaskov

175G ■ TICK-BORNE ENCEPHALITIS, 2303
Andrea Duppenthaler • Christoph Aebi • Theodore F. Tsai

175H ■ OTHER FLAVIVIRAL INFECTIONS, 2313
Theodore F. Tsai

176 Hepatitis C Virus, 2317
Ravi Jhaveri

SUBSECTION **6**
ORTHOMYXOVIRIDAE, 2326

177 Influenza Viruses, 2326
Timothy M. Uyeki

SUBSECTION **7**
PARAMYXOVIRIDAE, 2360

178 Parainfluenza Viruses, 2360
Ram Yogev and Caroline Breese Hall

179 Measles Virus, 2373
James D. Cherry

180 Mumps Virus, 2395
James D. Cherry and Kevin K. Quinn

181 Respiratory Syncytial Virus, 2407
H. Cody Meissner and Caroline Breese Hall

182 Human Metapneumovirus, 2434
Jennifer E. Schuster and John V. Williams

SUBSECTION **8**
RHABDOVIRIDAE, 2444

183 Rabies Virus, 2444
Sergio E. Recuenco • Stanley A. Plotkin • Charles E. Rupprecht

SUBSECTION **9**
ARENAVIRIDAE AND *FILOVIRIDAE*, 2463

184 Lymphocytic Choriomeningitis Virus, 2463
Rémi N. Charrel and Xavier de Lamballerie

185 Arenaviral Hemorrhagic Fevers, 2466
Rémi N. Charrel and Xavier de Lamballerie

186 Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers, 2478
Eric Leroy and Gail J. Harrison

SUBSECTION **10**
CORONAVIRIDAE AND *TOROVIRIDAE*, 2486

187 Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome, 2486
Kenneth McIntosh and Janet A. Englund

SUBSECTION **11**
BUNYAVIRIDAE, 2496

188 Hantaviruses, 2496
Louisa E. Chapman • C. J. Peters • James N. Mills • Kelly T. McKee, Jr.

189 La Crosse Encephalitis and Other California Serogroup Viruses, 2513
W. Garrett Hunt and James E. McJunkin

190 Other Bunyaviridae, 2528

190A ■ RIFT VALLEY FEVER, 2528
C.J. Peters and Gail J. Harrison

190B ■ CRIMEAN-CONGO HEMORRHAGIC FEVER, 2531
Robert B. Tesh and Gail J. Harrison

190C ■ PHLEBOTOMUS FEVER (SANDFLY FEVER), 2535
Robert B. Tesh and Gail J. Harrison

190D ■ OROPOUCHE FEVER, 2537
Francisco P. Pinheiro • Amelia P. A. Travassos da Rosa • Pedro Fernando da C. Vasconcelos

190E ■ TOSCANA VIRUS, 2544
Rémi N. Charrel • Xavier de Lamballerie • Laurence Bichaud

SUBSECTION **12**
RETROVIRIDAE, 2552

191 Human Retroviruses, 2552

191A ■ ONCOVIRUSES (HUMAN T-CELL LYMPHOTROPIC VIRUSES) AND LENTIVIRUSES (HUMAN IMMUNODEFICIENCY VIRUS TYPE 2), 2552
Michael A. Tolle and Susan L. Gillespie

191B ■ HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME, 2591
Filiz O. Seeborg • Mary E. Paul • William T. Shearer

SUBSECTION **13**
PRION-RELATED DISEASES, 2619

192 Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinker Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia), 2619
William J. Britt and Gail J. Harrison

SECTION **XVIII**
CHLAMYDIA, 2630

193 Chlamydia Infections, 2631
Margaret R. Hammerschlag and Stephan A. Kohlhoff

SECTION **XIX**
RICKETTSIAL DISEASES, 2646

194 Rickettsial and Ehrlichial Diseases, 2647
Paul M. Lantos and Ross McKinney, Jr.

SECTION **XX**
MYCOPLASMA, 2667

195 Mycoplasma and Ureaplasma Infections, 2668
James D. Cherry and Natalie M. Quanquin

SECTION **XXI**
FUNGAL DISEASES, 2701

196 Classification of Fungi, 2702
David A. Bruckner and Eszter Deak

197 Aspergillosis, 2704
William J. Steinbach

198 Blastomycosis, 2723
Gregory M. Gauthier and Bruce S. Klein

199 Candidiasis, 2735
Grace E. Lee • David A. Kaufman • Theoklis E. Zaoutis

200 Coccidioidomycosis, 2769
Ziad M. Shehab and Kareem W. Shehab

201 Paracoccidioidomycosis, 2780
Gil Benard and Maria José Soares Mendes-Giannini

202 Cryptococcosis, 2795
David Goldman and John Perfect

203 Histoplasmosis, 2807
Martin B. Kleiman

204 Sporotrichosis, 2832
Bernhard L. Wiedermann

205 Mucormycosis and Entomopthoramycosis (Formerly Zygomycosis), 2837
Theoklis E. Zaoutis and William J. Steinbach

206 Fusariosis and Scedosporiosis, 2846
Damian J. Krysan • Melanie Wellington • William J. Steinbach

207 Miscellaneous Mycoses, 2852
William J. Steinbach

SECTION **XXII**
PARASITIC DISEASES, 2860

208 Classification and Nomenclature of Human Parasites, 2861
Lynne S. Garcia

SUBSECTION **1**
PROTOZOA 2866

209 Amebiasis, 2866
Shinjiro Hamano and William A. Petri, Jr.

210 *Blastocystis hominis* and *Blastocystis* spp. Infection, 2875
Peter J. Hotez

211 *Entamoeba coli* Infection, 2877
Peter J. Hotez

212 Giardiasis, 2878
Tina Q. Tan

213 *Dientamoeba fragilis* Infections, 2884
Damien Stark

214 Trichomonas Infections, 2887
Joan S. Purcell and Mariam R. Chacko

215 *Balantidium coli* Infection, 2894
Peter J. Hotez

216 Cryptosporidiosis, 2896
Theresa J. Ochoa and A. Clinton White, Jr.

217 Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis, 2905
A. Clinton White, Jr. • Carlos Seas • Martin Montes

218 Babesiosis, 2915
Peter J. Krause

219 Malaria, 2921
Rosemary M. Olivero and Elizabeth D. Barnett

220 Leishmaniasis, 2947
Lynne S. Garcia

221 Trypanosomiasis, 2959
Lawrence A. Ross • Lynne S. Garcia • Murray Wittner • Herbert B. Tanowitz

222 *Naegleria, Acanthamoeba,* and *Balamuthia* Infections, 2975
Patrick J. Gavin and Tina Q. Tan

223 Toxoplasmosis, 2986
James B. McAuley • Kenneth M. Boyer • Jack S. Remington • Rima L. McLeod

224 *Pneumocystis* Pneumonia, 3005
Francis Gigliotti and Terry W. Wright

SUBSECTION **2**
NEMATODES, 3012

225 Parasitic Nematode Infections, 3012
Peter J. Hotez

SUBSECTION **3**
CESTODES, 3030

226 Cestodes, 3030
A. Clinton White, Jr. • Philip R. Fischer • Pedro Legua

SUBSECTION **4**
TREMATODES, 3049

227 Foodborne Trematodes, 3049
Miguel M. Cabada • Philip R. Fischer • A. Clinton White, Jr.

228 Schistosomiasis, 3060
Philip R. Fischer and A. Clinton White, Jr.

SUBSECTION **5**
ARTHROPODS, 3070

229 Arthropods, 3070
Jan E. Drutz

SECTION **XXIII**
GLOBAL HEALTH, 3080

230 Global Health, 3081
Holly Corwin and Gail J. Harrison

231 International Travel Issues for Children, 3091
Michelle Weinberg • Nicholas Weinberg • Susan A. Maloney

232 Infectious Disease Considerations in International Adoptees and Refugees, 3121
Mary Allen Staat

PART IV
THERAPEUTICS

233 Antibiotic Resistance, 3137
Philip Toltzis and Jeffrey L. Blumer

234 The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics, 3159
Susan M. Abdel-Rahman • Jennifer L. Goldman • Gregory L. Kearns

235 Antibacterial Therapeutic Agents, 3182
Carla Garcia and George H. McCracken, Jr.

236 Antimicrobial Prophylaxis, 3242
Gary D. Overturf

237 Outpatient Intravenous Antimicrobial Therapy for Serious Infections, 3257
John S. Bradley

238 Antiviral Agents, 3265
Gail J. Harrison

239 Antifungal Agents, 3291
Andreas H. Groll and Thomas J. Walsh

240 Drugs for Parasitic Infections, 3327

241 Immunomodulating Agents, 3355
Timothy R. La Pine and Harry R. Hill

242 Probiotics, 3369
Gail J. Harrison

PART V
PREVENTION OF INFECTIOUS DISEASES

243 Health Care–Associated Infections, 3377
W. Charles Huskins • Julia Shaklee Sammons • Susan E. Coffin

244 Active Immunizing Agents, 3420
Penelope H. Dennehy • Ian C. Michelow • Michael P. Koster • Nicole E. Alexander-Scott

245 Passive Immunization, 3491
Deborah Lehman and Danica J. Schulte

SECTION **XXIV**
OTHER PREVENTIVE CONSIDERATIONS, 3537

246 Public Health Aspects of Infectious Disease Control, 3538
Kathleen H. Harriman and Laurene Mascola

247 Infections in Child Care Environments, 3550
Sheryl L. Henderson and Ellen R. Wald

248 Animal and Human Bites, 3570
Morven S. Edwards

249 Bioterrorism, 3579
Robert J. Leggiadro

PART VI
APPROACH TO THE LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES

250 Bacterial Laboratory Diagnosis, 3589
Paula A. Revell and Lakshmi Chandramohan

251 Fungal Laboratory Analysis: Specimen Collection, Direct Detection, and Culture, 3596
David A. Bruckner

252 Viral Laboratory Diagnosis, 3600
Romney M. Humphries and Marjorie J. Miller

253 Parasitic Laboratory Diagnosis, 3620
David A. Bruckner and Robyn Shimizu-Cohen

This page intentionally left blank

PART I

HOST-PARASITE RELATIONSHIPS AND THE PATHOGENESIS OF INFECTIOUS DISEASES

This page intentionally left blank

CHAPTER 1

Molecular Determinants of Microbial Pathogenesis

David A. Hunstad • Ravi Jhaveri • Joseph W. St. Geme III

Despite the availability of a wide variety of antimicrobial agents and expansion of vaccination programs, infectious diseases remain a leading cause of childhood morbidity and mortality worldwide. A number of factors contribute to the continuing importance of infectious agents: rates of antimicrobial resistance are on the rise, global travel has become routine, and the number of individuals with altered immunity has increased. Furthermore, in recent years, microorganisms have been implicated in diseases previously considered noninfectious and a variety of new, emerging, and reemerging pathogens have been recognized.

Pathogens are defined as microorganisms that are capable of causing disease. However, not all pathogens are equal with respect to their pathogenic potential (i.e., their virulence). Many pathogens are, in fact, commensal organisms that live in harmony with their host under most conditions, causing disease only when normal immune mechanisms are disrupted or absent. Other pathogens produce disease even in the setting of intact host defenses and almost always cause symptoms.

For a given microbe, pathogenic potential is determined by the genomic content and regulation of virulence-associated genes. Some bacterial species are capable of natural transformation and readily acquire fragments of DNA from other organisms, thus expanding or altering their genetic composition, occasionally with consequences related to virulence or antimicrobial resistance. A number of microorganisms carry virulence-associated genes on mobile genetic elements, including plasmids, transposons, and bacteriophages. These elements may equip the organism with genetic information that facilitates rapid adaptation to an unfavorable or changing environment. Comparison of genomes from pathogenic and nonpathogenic bacteria within a single genus or species has led to the identification of *pathogenicity islands*, which are large blocks of chromosomal DNA that are present in pathogens and absent from related nonpathogens. These blocks are flanked by insertion sequences or repeat elements and differ in nucleotide composition relative to the surrounding genome, suggesting acquisition by horizontal exchange. Pathogenicity islands in bacteria encode a variety of virulence factors, including protein secretion systems, secreted effector molecules, adhesins, and regulatory proteins. In an analogous way, some viral pathogens such as influenza virus are capable of exchanging nucleic acid segments with other viruses, leading to changes in pathogenicity and host tropism.

To be successful, a pathogen must enter the host, assume an appropriate niche, and then multiply. Sometimes the pathogen will induce damage to the host and then spread to other tissues, either nearby the initial site of infection or more distant. Often the pathogen will stop short of causing death to the host, maintaining latent infection or producing symptoms such as cough or diarrhea that facilitate its spread to another host. In this chapter we address several key steps in the pathogenic process, each illustrated with examples of pathogens and paradigms of relevance to infectious diseases in children.

COLONIZATION

Most bacterial infections begin with microbial colonization of a host surface, typically the skin, the respiratory tract, the gastrointestinal tract, or the genitourinary tract. Although colonization is not sufficient for an organism to produce disease, it is a necessary prerequisite. The process of bacterial colonization requires specialized microbial factors, called adhesins, that promote adherence to host structures and enable these organisms to overcome local mechanical defenses, such as mucociliary function, peristalsis, and urinary flow. The cognate receptors for these interactions are generally either carbohydrate or protein structures, in some cases expressed on host cells and in other instances present in mucosal secretions or in submucosal tissue.

Pilus Adhesins

Perhaps most common among bacterial adhesins are hairlike fibers called pili (also called fimbriae). Pili are polymeric protein structures whose major subunit usually ranges in size from 15 to 25 kDa. Because of their size and morphology, most pili can be visualized by negative-stain transmission electron microscopy.

The prototype example among adhesive pili is the P (or Pap) pilus, which is expressed by uropathogenic *Escherichia coli* (UPEC) and has been strongly associated with pyelonephritis. P pili recognize globoseries glycolipids, which are host molecules that are characterized by a core structure consisting of Gal-α1,4-Gal. The globoseries glycolipids are especially abundant in renal epithelium,[23] thus accounting for the predilection of P-piliated *E. coli* to adhere to kidney tissue and cause pyelonephritis. An analogous fiber also expressed by

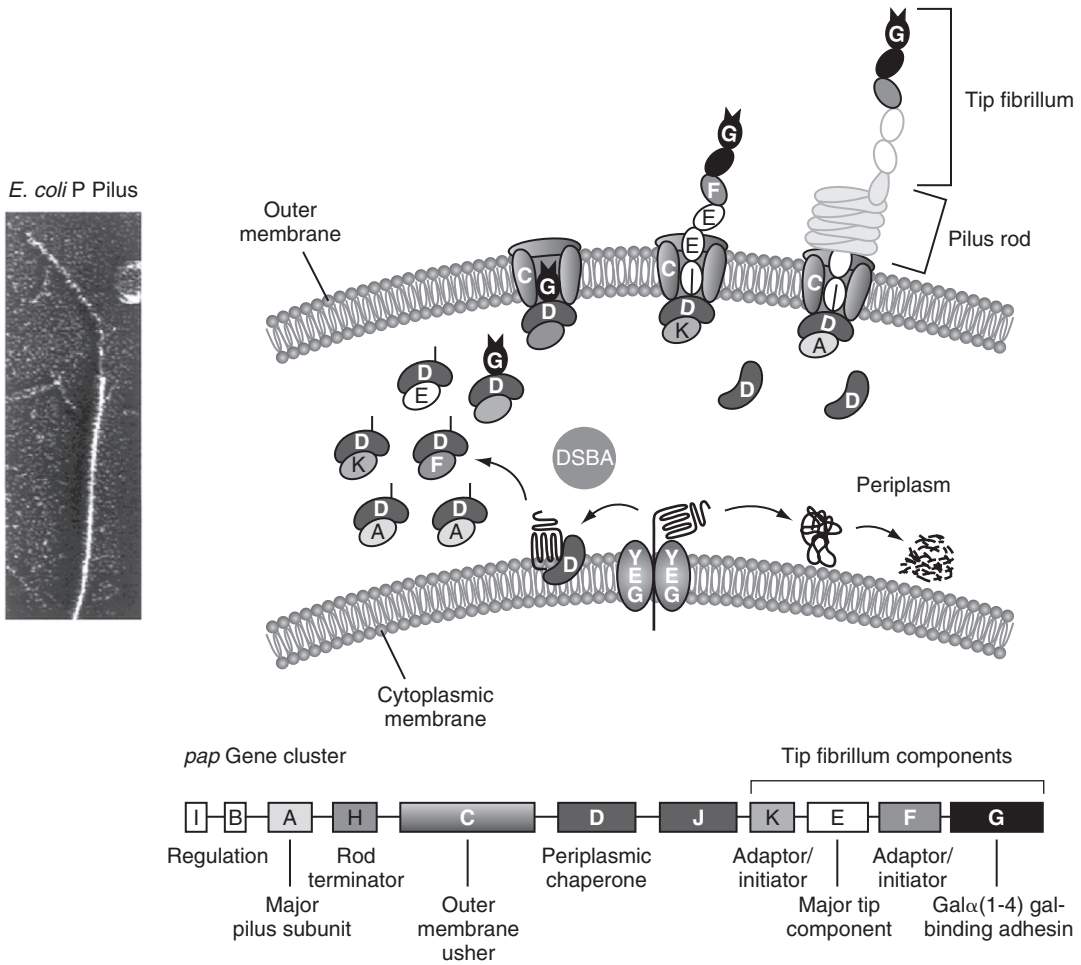

FIGURE 1-1 ■ Biogenesis and structure of *Escherichia coli* P pili. The *pap* gene cluster and the function of each of the gene products are indicated in the lower portion of the figure. Nascent pilin subunits are complexed with the PapD chaperone and added to the base of the developing pilus via the PapC usher. The mature pilus rod is composed of repeating units of PapA; the tip fibrillum contains the adhesin PapG. The ultrastructure of the pilus is shown in the electron micrograph at the left side of the figure. (Courtesy S.J. Hultgren and F.J. Sauer.)

UPEC, the type 1 pilus, binds mannosylated uroplakin proteins in the mammalian bladder to initiate cystitis.[147] As shown in Figure 1-1, P pili are composite structures and consist of two subassemblies, including a thick rod that emanates from the bacterial surface and a thin tip fibrillum that extends distally.[168,264] The pilus rod is a right-handed helical cylinder and is composed of repeating PapA subunits, whereas the tip fibrillum has an open helical configuration and contains mostly repeating PapE subunits. The two subassemblies are joined to each other by the PapK adaptor protein. PapG contains the adhesive moiety and is located at the distal end of the tip fibrillum, joined to PapE by the PapF adaptor.[144]

P pili are assembled through a canonical process termed the *chaperone-usher pathway* that involves a periplasmic chaperone (PapD) and an outer membrane usher (PapC) (see Fig. 1-1).[60,169] Subunit proteins (e.g., PapA) are translated in the bacterial cytoplasm, enter the periplasm through the inner membrane Sec machinery, and are stabilized by interaction with the chaperone, which ferries them to the usher. The crystal structures of PapD alone and PapD interacting with the PapK pilin subunit have been solved, revealing significant insights into PapD interaction with Pap subunits and the mechanism of P pilus assembly.[263] PapD consists of two immunoglobulin-like folds oriented in an "L" shape with an intervening cleft. Each pilus subunit consists of a single immunoglobulin-like fold but lacks the seventh, carboxy (C)-terminal β-strand (strand G1). The absence of this strand leaves a deep groove along the subunit surface that exposes its hydrophobic core, predisposing the subunit to aggregation and degradation. In the chaperone-subunit complex, the G1 strand of PapD occupies the subunit's groove and completes the immunoglobulin-like fold, stabilizing the subunit in a phenomenon referred to as *donor-strand complementation* (Fig. 1-2A).[263] Ultimately, PapD delivers PapK and other Pap subunits to the PapC usher, which forms a dimer with twin pores in the outer membrane.[174] As the pilus is assembled on the bacterial surface, the amino (N)-terminal strand of a neighboring subunit (the one most recently added to the pilus base) replaces the G1 strand of PapD in a process called *donor strand exchange*. Thus, in the mature pilus, each subunit completes the

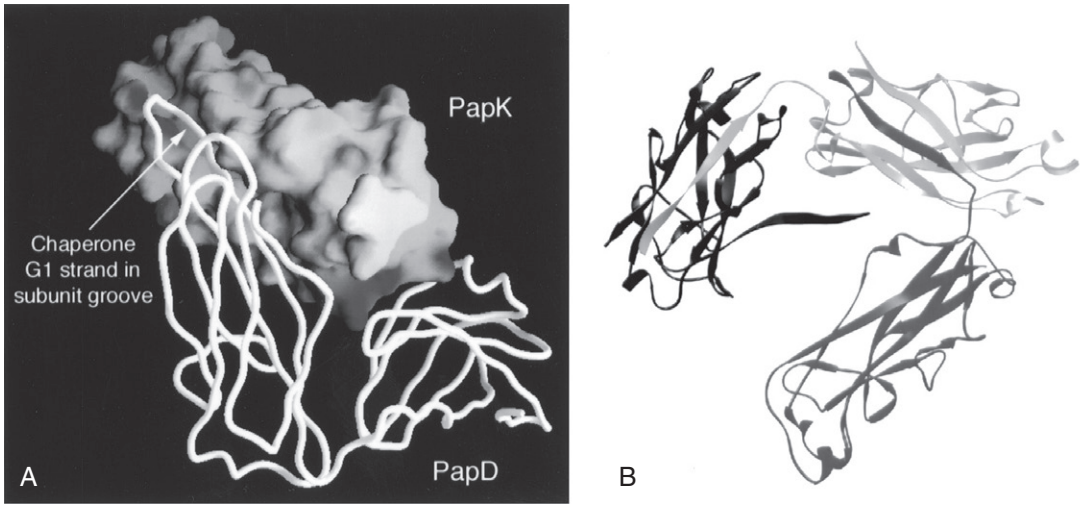

FIGURE 1-2 ■ **A,** The G1 strand of the PapD chaperone completes the immunoglobulin-like fold of the PapK pilin subunit (donor strand complementation). **B,** The mature P pilus rod comprises a helix (3.28 subunits per turn) of repeating PapA subunits; each subunit is completed by a strand donated by its neighbor. (Courtesy S.J. Hultgren and F.J. Sauer.)

immunoglobulin-like fold of its neighbor (see Fig. 1-2B).[132] The PapA major subunit employs an intramolecular "hinge" to first exit the usher vertically and then adopt its final conformation within the helical cylinder that forms the pilus fiber.[206] Extrusion of the nascent fiber is controlled by a "plug" domain in the usher pore; and because the periplasm is devoid of adenosine triphosphate (ATP), the assembly process is energetically driven by the entropically favorable final conformation of the incorporated subunits.[184,252] The final length of the fiber is determined by incorporation of the PapH subunit, a "terminator" protein whose G1 groove is tight and will not accommodate the donor strand of other subunits.[309]

More than 30 different bacterial adhesive structures are assembled via this chaperone-usher pathway, with distinct PapD-like chaperones and PapC-like ushers. These PapD-like chaperones can be divided into two distinct subfamilies based on conserved structural differences that occur near the subunit binding site.[133] One subfamily is involved in the assembly of rodlike pili similar to P pili, whereas the second subfamily participates in the biogenesis of more atypical filamentous structures, such as Caf1 of *Yersinia pestis* (the plague bacterium), which forms an amorphous "mat" on the bacterial surface. Thus, the nature of the chaperone is directly linked to the architecture of the adhesive appendage that it helps to assemble.[279]

Type 4 pili represent a second class of pili, distinguished by a methylated first amino acid (usually phenylalanine), a short positively charged leader sequence, a conserved hydrophobic N-terminal domain, and a tendency to form bundle-like structures. Type 4 pili have been identified in a number of gram-negative bacterial pathogens, including *Neisseria gonorrhoeae*, *N. meningitidis*, enteropathogenic *E. coli* (EPEC), *Vibrio cholerae*, *Pseudomonas aeruginosa*, *Kingella kingae*, *Eikenella corrodens*, and *Moraxella* species.* Although the mechanism of assembly of type 4 pili is still being elucidated, existing data suggest that the process is complex. For example, between 20 and 40 gene products are required for the assembly of *P. aeruginosa* type 4 pili and at least 15 plasmid-encoded proteins are involved in the biogenesis of EPEC type 4 pili.[121,289] Based on studies of *P. aeruginosa*, EPEC, *Neisseria*, and *V. cholerae*, the presence of an inner membrane prepilin peptidase appears to be a general prerequisite for type 4 pilus biogenesis.[155,171,220] Type 4 pili are often glycosylated, with carbohydrate decoration affecting function in at least some cases and perhaps serving to obscure antigenic epitopes.[30,185,272,310] However, despite marked differences in the assembly pathways for type 4 pili and P pili, significant structural similarities may exist. For example, gonococcal type 4 pili are composed predominantly of PilE structural subunits polymerized into a helical rod.[229] A minor phase-variable adhesive protein called PilC is displayed at the tip of the pilus and is essential for pilus-mediated binding to epithelial cells.[148,260] These observations suggest that *N. gonorrhoeae* pili may be composite structures with a tip-associated adhesin, analogous to P pili and other pili assembled by the chaperone-usher pathway.

Although adhesive pili are more prevalent in gram-negative bacteria, they are also found in some gram-positive species. One example is *Streptococcus parasanguinis*, an oral pathogen and a member of the *S. sanguinis* family. This organism binds to calcium phosphate (the primary mineral component of tooth enamel) and also to other oral bacteria, epithelial cells, platelets, and fibronectin. Several adhesins mediate these binding functions, including pili referred to as long fimbriae. Based on studies of *S. parasanguinis* strain FW213, long fimbriae are fashioned primarily from Fap1, a 200-kDa protein that includes an unusually long (50 amino acids) signal sequence and a cell-wall sorting signal typical of other gram-positive bacterial surface proteins.[329,330] Specific glycosylation of Fap1 appears critical to the adhesive function of this fimbrial protein.[25,288,328] Interestingly, similar to gram-negative bacterial pili, long fimbriae appear to have a composite structure with a pilus tip. The

*References 94, 188, 203, 244, 249, 266, 291, 314.

tip contains an additional adhesin called FimA, which in purified form is capable of blocking bacterial adherence to saliva-coated hydroxyapatite.[75,224] In work by Burnette-Curley and coworkers, disruption of the *fimA* gene resulted in a 7- to 20-fold reduction in the incidence of endocarditis after intravenous inoculation of rats.[27] Other gram-positive organisms capable of expressing pili include *Streptococcus agalactiae* (group B streptococcus; a common cause of neonatal pneumonia, sepsis, and meningitis)[68,257] and *Enterococcus faecalis* (a cause of endocarditis and urinary tract infections).[210,271]

Nonpilus Adhesins

Beyond pili, a variety of nonpilus adhesins exist. In most cases, nonpilus adhesins are proteinaceous, monomeric or oligomeric surface structures, although isolated examples of carbohydrate and lipid-containing adhesive structures have been identified. In general, these molecules are difficult to visualize by electron microscopy, even with high-resolution techniques. Similar to pili, for the most part nonpilus adhesins can be classified according to their mechanism of secretion and presentation on the bacterial surface.

Among the best-characterized bacterial nonpilus adhesins is filamentous hemagglutinin (FHA), a surface protein expressed by *Bordetella pertussis* and other *Bordetella* species. FHA is synthesized as a large precursor protein with a calculated molecular mass of 367 kDa. A cleavage event occurs to eliminate the C-terminal third of the protein and produce the mature 220-kDa species.[64] The export of FHA to the surface of the organism occurs via the so-called *two-partner secretion (TPS) pathway*, a conserved strategy in which a secreted (TpsA) protein interacts, via specific determinants within its N terminus, with a cognate outer membrane transporter (TpsB).[122] Thus, in *B. pertussis*, the TpsA-type protein FHA is transported by a TpsB-type outer membrane protein called FhaC, which has β-barrel pore-forming properties and facilitates translocation of FHA across the outer membrane.[324] Homologous TpsB proteins in other species export the hemolysins of *Serratia marcescens*, *Proteus mirabilis*, and *Haemophilus ducreyi;* the *H. influenzae* heme:hemopexin binding protein (HxuA); and the *H. influenzae* HMW1 and HMW2 adhesins, among others.* The crystal structure of FhaC reveals a 16-stranded β-barrel that is occluded by an N-terminal α-helix and an extracellular loop and a periplasmic module composed of two polypeptide-transport-associated (POTRA) domains. Functional studies have demonstrated that the N terminus of FHA interacts with the FhaC POTRA 1 domain.[35]

Examination of purified FHA by transmission electron microscopy and circular dichroism spectroscopy showed that the FHA molecule is 50 nm in length and adopts the shape of a horseshoe nail. It has a globular head, a 37-nm long shaft that averages 4 nm in width but tapers slightly from the head end, and a small flexible tail (Fig. 1-3A).[151,183] In the crystal structure of the N terminus of FHA (the so-called TPS domain that interacts with FhaC), a series of 19-residue repeat motifs forms a β-helix that is central to the overall structure of full-length FHA (see Fig. 1-3B).[36] Consistent with its large size, FHA contains at least five separate binding domains, four of which have been localized. The region involved in adherence to sulfated saccharides has been mapped to the N terminus of the FHA molecule.[201] Sulfated saccharides such as heparin and heparan sulfate are a major component of mucus and extracellular matrix in the respiratory tract and are also found on the surface of epithelial cells.[190,333] The region that recognizes lactosylceramides and promotes adherence to ciliated respiratory epithelial cells and macrophages has been localized to amino acids 1141 to 1279 (the carbohydrate recognition domain).[245] An arginine-glycine-aspartic acid (RGD) tripeptide is located at amino acids 1097 to 1099 and interacts with leukocyte response integrin (LRI), a leukocyte integrin that stimulates upregulation of complement receptor type 3 (CR3).[140] The C terminus of mature FHA has been demonstrated to interact with epithelial cells and macrophage-like cells and appears to modulate the immune response to *Bordetella* infection.[149] Finally, FHA recognizes CR3 (CD11b/CD18), allowing organisms to be ingested by macrophages without stimulating an oxidative burst.[251,327] The location of the CR3-binding domain is currently unknown.

A growing number of nonpilus adhesins belong to the so-called *autotransporter family*. These proteins are synthesized as precursor proteins with three functional domains, including an N-terminal canonical signal sequence, an internal passenger domain, and a C-terminal outer membrane domain. The signal sequence directs the protein to the Sec machinery and is cleaved after it facilitates transport of the polypeptide from the cytoplasm to the periplasm. The C-terminal domain inserts into the outer membrane and forms a β-barrel with a central hydrophilic channel. Ultimately, the passenger domain is presented on the surface of the organism and influences interaction with host molecules.[114] Recent studies have established that autotransporter proteins can be separated into two distinct groups, designated conventional autotransporters and trimeric autotransporters (Fig. 1-4).[48] In conventional autotransporters, the C-terminal outer membrane domain contains roughly 300 amino acids and is a monomeric β-barrel with a single N-terminal α-helix spanning the pore (Fig. 1-5A).[225,294] In trimeric autotransporters, the C-terminal outer membrane domain contains approximately 70 amino acids and forms heat- and detergent-resistant trimers in the outer membrane. Each trimer forms a β-barrel with four strands from each of the three subunits and with three N-terminal α-helices spanning the pore (Fig. 1-5B).[199]

One example of a conventional autotransporter adhesin is the *H. influenzae* Hap protein, which was discovered based on its ability to promote adherence and low-level invasion in assays with cultured epithelial cells.[280] Hap also promotes bacterial binding to extracellular matrix proteins and bacterial microcolony formation.[77,115] Examination of chimeric proteins and studies with purified protein have demonstrated that the adhesive activity responsible for Hap-mediated adherence, invasion, binding to extracellular matrix proteins, and

*References 8, 42, 228, 243, 281, 306.

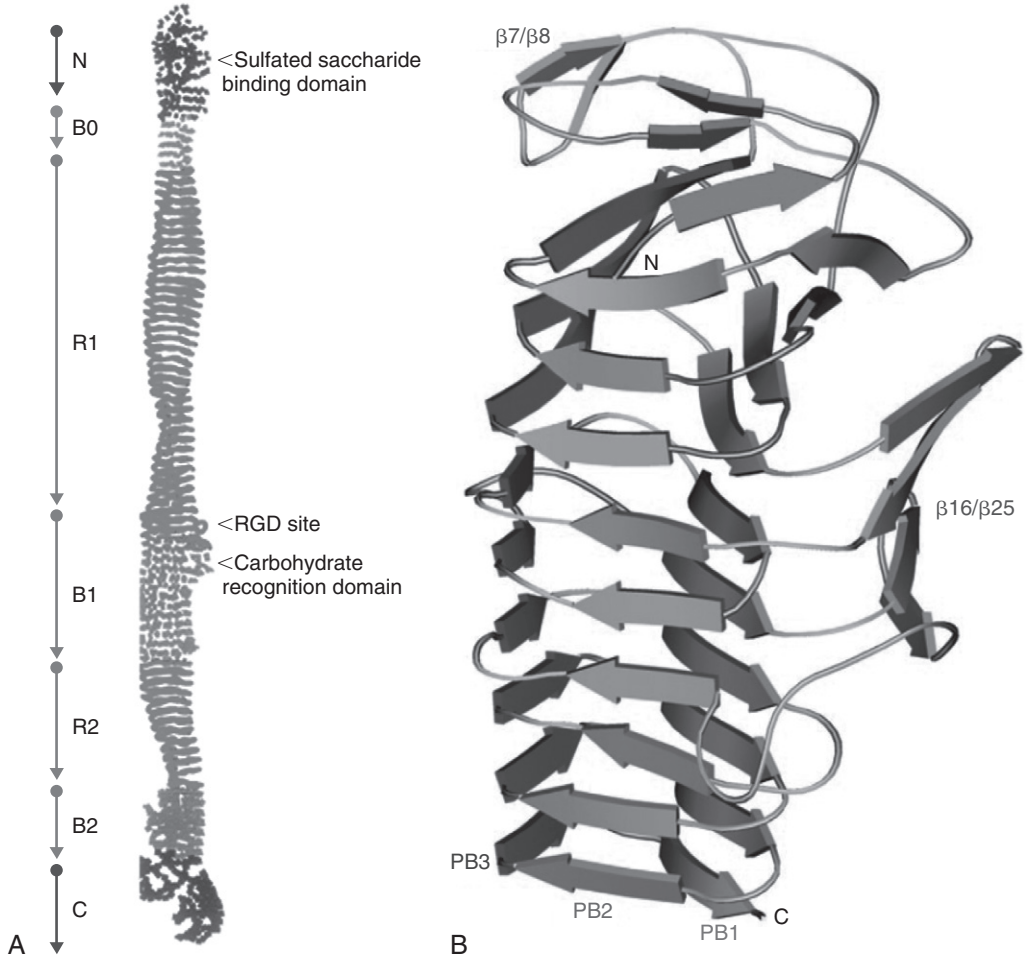

FIGURE 1-3 ■ **A,** Ribbon representation model structure of filamentous hemagglutinin from *Bordetella pertussis.* There are five regions that are assigned β-helical coils, designated B0, R1, B1, R2, and B2. The N terminus of the protein is designated with "N," and the C terminus of the protein is designated with "C." The locations of the sulfated saccharide binding domain, the carbohydrate recognition domain, and the RGD tripeptide are noted. **B,** Ribbon representation of the N-terminal secreted portion of filamentous hemagglutinin. (See companion Expert Consult web site for color version.) (**A** from Kajava AV, Cheng N, Cleaver R, et al. Beta-helix model for the filamentous haemagglutinin adhesin of *Bordetella pertussis* and related bacterial secretory proteins. *Mol Microbiol* 2001;**42**:279–92; **B** from Clantin B, Delattre AS, Rucktooa P, et al. Structure of the membrane protein FhaC: a member of the Omp85-TpsB transporter superfamily. *Science* 2007;**317**:957–61.)

microcolony formation localizes to the passenger domain, referred to as Hap_s.[77,115] More detailed characterization of Hap_s has established that the region responsible for interaction with host epithelial cells and microcolony formation resides in the C-terminal 311 amino acids and may have utility as a vaccine antigen.[52,76,178] This region folds into a triangular prism-like structure that can mediate Hap-Hap dimerization and higher degrees of multimerization, facilitating interbacterial interaction and microcolony formation.[197] A prototype member of the trimeric autotransporter subfamily is the *H. influenzae* Hia adhesin. This protein is expressed in a subset of nontypable *H. influenzae* strains and contains two homologous high-affinity trimeric binding domains, creating the potential for stable multivalent interaction with respiratory epithelial cells.[170,198,334]

Another group of nonpilus adhesins is typified by intimin, a protein expressed by enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), and the murine pathogen *Citrobacter rodentium.* Intimin contains a flexible N terminus, a central β-barrel domain that integrates into the outer membrane, and a C-terminal binding domain that interacts with the translocated intimin receptor (Tir).[303] Tir is an interesting example of a pathogen inserting its own receptor into its targeted host cell. After initial cell attachment mediated by type 4 pili, EPEC employs a type III secretion system (discussed in detail later in this chapter) to inject Tir into the host cell cytoplasm,[158,326] from where it is then inserted into the host cell membrane.[247] The subsequent interaction between intimin (on the bacterial surface) and Tir (now present on the host cell surface) triggers receptor clustering, dramatic rearrangement of the actin cytoskeleton, and formation of a distinctive pedestal referred to as an attaching and effacing (A/E) lesion (Fig. 1-6).[158,256] The bacterial genes essential for formation of A/E lesions reside within a 35-kb region of the EPEC chromosome called the locus of enterocyte effacement (LEE), an example of a pathogenicity island.[66,191] This locus is highly conserved in content and organization across all

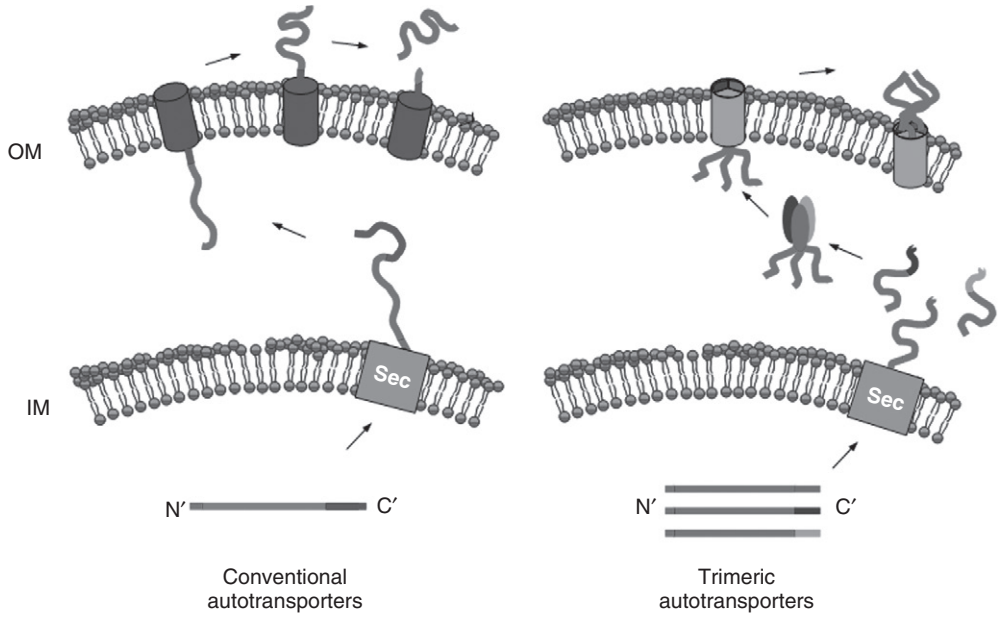

FIGURE 1-4 ■ Autotransporter protein secretion pathway. Conventional autotransporter secretion is shown on the left, and trimeric autotransporter secretion is shown on the right. Autotransporter proteins are synthesized as preproteins with three functional domains, including an N-terminal signal sequence (shown in green), an internal passenger domain (shown in red), and a C-terminal outer membrane β-barrel domain (shown in blue). IM indicates inner membrane, and OM indicates outer membrane. Protein secretion begins with export of the protein from the cytoplasm via the inner membrane Sec machinery (Sec). Most conventional autotransporters are cleaved on the bacterial surface. (See companion Expert Consult web site for color version.) (From Cotter SE, Surana NK, St Geme JW III. Trimeric autotransporters: a distinct subfamily of autotransporter proteins. *Trends Microbiol* 2005;**13**: 199–205.)

A/E pathogens and contains the genes encoding intimin, Tir, and the requisite type III secretion system. The interactions of Tir and other type III secreted effectors with host proteins influencing actin polymerization are beginning to be understood.[258,335] Tir contains domains analogous to host immunoreceptor tyrosine-based inhibition motifs (ITIM) important for regulation of eukaryotic signaling. On this basis, Tir recruits certain host proteins to regulate actin dynamics and inhibit proinflammatory signaling pathways.[56,259,276,319] Given its central role in EHEC/EPEC pathogenesis and its immunogenicity, intimin is also being examined as a target for the development of antivirulence therapeutics or vaccines in A/E diseases.

In recent years, investigators have identified a large family of nonpilus adhesins involved in adherence to host extracellular matrix proteins, including fibronectin, laminin, vitronectin, collagen, fibrinogen, and a variety of proteoglycans. These adhesins have been classified as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) and are especially prevalent among gram-positive bacteria.[232] In gram-positive organisms they are covalently anchored to the cell wall peptidoglycan and have a characteristic primary amino acid sequence. In particular, the C terminus contains a segment rich in proline and glycine residues, an LPXTG motif (involved in sorting and covalently anchoring the protein to the cell wall), a hydrophobic membrane-spanning domain, and a short positively charged segment that resides in the cytoplasm and serves as a cell wall retention signal. Adhesive functions are typically located near the N terminus.[79]

Staphylococcus aureus is a common gram-positive pathogen in children and is capable of producing a variety of MSCRAMMs, including collagen-binding protein (CNA), fibronectin-binding proteins A and B, and clumping factors A and B. Recent work indicates that although these proteins mediate typical binding interactions with host proteins, they are not monospecific, and a given MSCRAMM may bind multiple host connective tissue components or multiple motifs within a single host fiber type.[278] In addition, many are capable of provoking platelet activation. *S. aureus* strains recovered from patients with septic arthritis commonly express CNA, which mediates binding to cartilage in vitro and appears to play a key role in the pathogenesis of septic arthritis in experimental mice.[231,233,295] The current model of CNA binding suggests that the two major domains (N1 and N2) and an intervening linker polypeptide together form a spherical "hole" through which the bound collagen fiber passes.[345] Fibronectin-binding protein A (FnBPA) shares homology with *S. pyogenes* protein F and mediates binding to fibronectin and the γ chain of fibrinogen, as well as elastin and tropoelastin.[156,255,313] Accordingly, this protein is important in *S. aureus* endocarditis[240] and in infections of implanted biomaterials, which become coated with fibrinogen and fibrin soon after implantation. Clumping factor (ClfA) was named based on the observation that it mediates bacterial clumping in the presence of soluble fibrinogen.[192] Similar to FnBPA, ClfA mediates binding to fibrinogen-coated surfaces in vitro and probably contributes to infections of artificial surfaces. Reminiscent of donor-strand complementation, the binding of ClfA to the C-terminal portion of the fibrinogen γ-chain

involves a proposed "dock, lock, and latch" binding sequence that creates a favorable immunoglobulin-like β-sheet conformation composed of strands from each of the partners.[91,242]

Other Mechanisms of Adherence

Candida albicans is a common inhabitant of mucosal surfaces and an important cause of systemic disease, especially in patients with compromised immunity. *Candida* blastospores are capable of efficient adhesion to epithelial cells, leading to budding and division. In addition, germ tube formation occurs, facilitating penetration through the epithelial barrier and then dissemination to distant sites.[129] In recent years, several candidate *C. albicans* adhesins have been identified.[9,128,176,337] Of particular interest is a protein called INT1, which shares functional homology with the vertebrate integrin family. Integrins are normally expressed by cells of the human immune system (neutrophils, monocytes, macrophages) and mediate cellular binding and shape-changing functions. Each integrin is a heterodimer of an α chain and a β chain. There are a number of different α and β chains, and each combination displays a unique binding specificity. INT1 is an α integrin–like protein that recognizes the RGD sequence of the C3 fragment iC3b on epithelial cells. In in vitro assays, short peptides encompassing the RGD sequence are capable of inhibiting *C. albicans* adherence by 50%, confirming that INT1 plays a significant role as an adhesin and suggesting that other adhesins also exist.[129] Beyond promoting adherence to epithelium, INT1 disguises organisms as leukocytes, allowing evasion of phagocytosis. Of note, introduction of INT1 into *Saccharomyces cerevisiae* confers a capacity for adherence and also results in germ tube formation, indicating the importance of this protein in morphogenesis.[88,89]

The adhesive properties of *C. albicans* are closely tied to its morphologic state. For example, adherence to buccal epithelial cells is greater by organisms bearing germ tubes than by yeast forms.[163] With this information in mind, Staab and coworkers searched a germ tube cDNA library and identified a putative adhesin called hyphal wall protein 1 (Hwp1), encoded by the *hwp1* gene. Examination of the predicted amino acid sequence of Hwp1 revealed similarity to proteins that are substrates for mammalian transglutaminase enzymes.[284] These enzymes form a cornified envelope on squamous epithelial cells (including buccal epithelial cells) by cross-linking relevant substrates.[286] Interestingly, the interactions of germ tubes with buccal epithelial cells resist stresses (e.g., heating or treatment with sodium dodecylsulfate) capable of dissociating most typical microbe-host adhesive pairs, and elimination of expression of Hwp1 results in a marked reduction in adhesion to buccal epithelial cells.[21,283] Thus, Hwp1 represents a unique adhesive strategy, employing host transglutaminase enzymes to cross-link Hwp1 (via a glycosylphosphatidylinositol remnant anchor) directly to surface proteins on buccal epithelial cells.[282] More recently, Hwp1 has been shown to be important for *Candida* biofilm formation,[72,214] indicating that a similar mechanism may also support interactions between candidal cells.

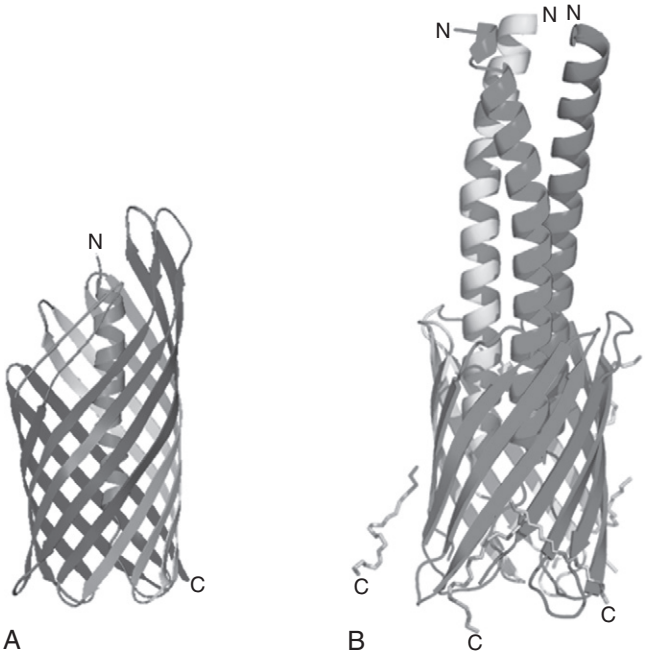

FIGURE 1-5 ■ Crystal structures of the C-terminal outer membrane β-barrel of autotransporter proteins. **A,** Crystal structure of NalP, a conventional autotransporter; β strands are shown in shades of blue, and the α-helix that crosses the channel is shown in red. **B,** Crystal structure of Hia, a trimeric autotransporter; individual subunits are shown in red, green, and yellow. (See companion Expert Consult web site for color version.) (**A** from Surana NK, Cotter SE, Yeo HJ, et al. Structural determinants of *Haemophilus influenzae* adherence to host epithelium: variations on type V secretion. In: Waksman G, Caparon MG, Hultgren SJ, editors. *Structural basis of bacterial pathogenesis*. Washington, DC: American Society for Microbiology; 2005. p. 129–148; **B** from Meng G, Surana NK, St Geme JW III, et al. Structure of the outer membrane translocator domain of the *Haemophilus influenzae* Hia trimeric autotransporter. *EMBO J* 2006;**25**:2297–304.)

FIGURE 1-6 ■ Enteropathogenic *E. coli* (EPEC) are perched on pedestals in the attaching and effacing lesion. (Courtesy B.B. Finlay; reproduced with permission from Rosenshine I, Ruschkowski S, Stein M, et al. A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation. *EMBO J* 1996;**15**:2613–24.)

Tissue Tropism

Most microorganisms demonstrate restriction in the range of hosts, tissues, and cell types that they colonize. This restriction is referred to as tropism and generally reflects the specificity of the interaction between a given microbial adhesin and its cognate receptor. Accordingly, tropism is determined by the distribution of the relevant host receptor.

P pili of uropathogenic *E. coli* serve as the platform for presentation of one of three different PapG variants, referred to as class I, class II, and class III PapG. All three variants recognize globoseries glycolipids, but each binds with a distinct specificity to the globoseries glycolipid isotypes. For example, class I PapG preferentially recognizes globotriosylceramide (GbO3, Gal-α1,4-Gal-β1,3-Glc-ceramide), class II PapG preferentially recognizes globoside (GbO4, GalNAc-β1,3-Gal-α1,4-Gal-β1,3-Glc-ceramide), and class III PapG preferentially interacts with Forssman antigen (GbO5, GalNAc-α1,3-GalNAc-β1,3-Gal-α1,4-Gal-β1,3-Glc-ceramide).[292] Globoside is the dominant globoseries glycolipid expressed in human kidney, and most human isolates of *E. coli* associated with pyelonephritis express class II PapG. In contrast, Forssman antigen is the most abundant globoseries glycolipid in dog kidney, and more than 50% of canine urinary isolates of *E. coli* express class III PapG.[332] *E. coli*–expressing P pili with class II PapG are not found as a cause of urinary tract infection in dogs. Thus, the specificity of the PapG variant at the tip of the P pilus influences host range, favoring infection of either human or dog.

The crystal structure of class II PapG bound to Gal-α1,4-Gal was solved by Dodson and coworkers, uncovering the structural basis of PapG binding specificity.[61] Of particular interest, the PapG receptor binding site is located on the side of the molecule and must be oriented with its N- to C-terminal axis parallel to the host cell membrane to allow docking to the receptor. This orientation may be facilitated by the flexibility inherent in the tip fibrillum. The PapG binding site consists of two regions. The first forms a β-barrel, and the second is composed of a central antiparallel β-sheet that is flanked on one side by two 2-stranded β-sheets and on the other side by an α-helix. When class II PapG interacts with GbO4, the arginine residue at position 170 in PapG makes contact with the GbO4 side chain. Interestingly, in class I PapG, a histidine residue occupies position 170, interfering with potential contact with the GbO4 side chain. Similarly, class II PapG and class III PapG differ in amino acids required for interaction with the GbO5 side chain.[61]

Group A streptococcus (*S. pyogenes*) is a common cause of infections of skin and soft tissue, including impetigo, cellulitis, and necrotizing fasciitis. Adherence to host cells by *S. pyogenes* is influenced by nonpilus adhesins called M protein and protein F. M protein forms a fiber and consists of a C-terminal region that anchors the protein in the cell wall, a coiled-coil rod region extending approximately 50 nm from the cell wall, and a short nonhelical domain extending more distally.[78] Protein F is a 120-kDa protein that is notable for a tandem repeat element consisting of up to six repeats of 32-44 amino acids adjacent to the C terminus.[106,227] M protein promotes adherence to human keratinocytes via interaction with the CD46 molecule (also called membrane cofactor protein or MCP), whereas protein F mediates adherence to epidermal Langerhans cells, which are located in the basal layer of the epidermis.[222,223] Thus, both M protein and protein F contribute to group A streptococcal adherence to the skin but each protein directs interaction with a different population of epidermal cells.

Early studies demonstrated that human immunodeficiency virus type 1 (HIV-1) infects $CD4^+$ cells and interacts with the CD4 molecule but that CD4 alone is not sufficient to permit infection. More recent observations have established that a number of host cell chemokine receptors, especially CCR5 and CXCR4, serve as coreceptors for HIV-1 and are required for viral entry into $CD4^+$ target cells. These coreceptors appear to influence the cellular tropism displayed by different HIV-1 variants.[57] All HIV variants are able to replicate in primary T cells, but only some can also replicate in primary macrophages or in immortalized T-cell lines. Asymptomatic HIV-infected individuals carry strains that generally use CCR5 as a coreceptor (termed *M5 strains*) and are nonsyncytium inducing in vitro. Such strains have classically been described as macrophage tropic (M-tropic), but recent experiments have demonstrated that these M5 strains can also infect $CD4^+$ T cells and peripheral blood mononuclear cells.[238] Rapid viral mutation due to the error-prone HIV polymerase and HIV reverse transcriptase leads to the production within the host of syncytium-inducing, T-cell–tropic (T-tropic) HIV-1 strains, which predominate in the circulation of patients with acquired immunodeficiency syndrome.[57] These variants are generally restricted to CXCR4 (expressed on T cells) as a coreceptor, although some primary syncytium-inducing variants can use both CCR5 and CXCR4.[67,74,270] T-tropic, syncytium-inducing strains are characterized by positively charged residues at fixed positions of the V3 loop and changes in charge and length of the V2 region of the viral envelope glycoprotein gp120, which binds to CD4 and coreceptors before viral entry into host cells.[80,81,100] Thus, cellular tropism is closely aligned, but not synonymous, with HIV coreceptor usage.

New HIV-1 infection is selectively established by M-tropic HIV-1 strains, even if the transmitting host harbors more pathogenic non–M-tropic strains as well.[307,343] CCR5 is also expressed on the surface of rectal and vaginal epithelial cells, which may be sites of initial encounter between HIV-1 and the human host.[341] The importance of CCR5 in HIV-1 binding to $CD4^+$ cells is underscored by the observation that individuals homozygous for a 32-bp deletion in CCR5 (the Δ32 allele) are resistant to infection with HIV-1.[131,179] The Δ32 heterozygous state does not necessarily protect against HIV-1 acquisition, although HIV disease in heterozygous patients may follow an attenuated course. This allele is surprisingly frequent (10% to 14%) in white populations, leading to speculation that it provided a survival advantage during one or more historical epidemics of

infectious diseases.[219] However, more recent data suggest that the Δ32 allele may actually confer immune deficiency in the presence of challenge with certain viral pathogens, such as West Nile virus.[95,96] Of note, CCR5 may have a role in controlling the development of malignancy, including lymphoma, raising some concern about developing anti-HIV pharmacologic agents that target CCR5 function.[172] Finally, co-evolution of viral determinants and host cell receptors may determine the spectrum of tissue and organ involvement within the host. For example, the chemokine receptor CCR8 may facilitate the entry of neurotropic HIV-1 strains into brain cells,[146] and envelopes derived from brain isolates of HIV are adapted to infect cells with low-level CD4/CCR5 expression, such as neuroglia and brain macrophages.[237]

Other viruses also demonstrate tropism for specific cells or tissues within the host. Hepatitis C virus (HCV) has been demonstrated to use multiple cell surface molecules in sequence to locate and gain entry into target cells. HCV is bound to low-density lipoprotein (LDL) in serum and first binds to scavenger-receptor B1 (SR-B1), which is enriched on liver cells and serves to bind lipoprotein molecules.[31] After this initial binding event, HCV E2 protein interacts with the CD81 molecule, the critical receptor for free virus.[153,312,340] Once virus has bound to CD81, this virus-receptor complex traffics to the gap junction where the virus interacts with two key gap junction proteins, claudin-1 and occludin-1, to enter cells.[73,108,165] Human occludin-1 recently has been shown to be necessary for HCV entry into mouse cells, an advance that will facilitate disease modeling in the laboratory.[241] Although the liver does not exclusively express any of these four molecules, the combination of these four, the structural organization of liver cells, and other yet-undetermined intracellular factors account for the tropism of HCV for the liver.[239]

Similar observations have been made for coxsackievirus isolates, which bind to the coxsackie-adenovirus receptor (CAR) molecule located in the tight junction for entry into cells.[10,38,49,152] Brain, heart, and muscle cells are enriched for CAR in the fetal and neonatal period, whereas adult cells from these tissues express significantly lower levels of the receptor.[125,141] This developmental difference in CAR density is the likely explanation for severe coxsackievirus infections that are disproportionately seen in infants and young children compared with adults. CAR then allows for an interaction with the tight junction that is mediated by occludin-1, which allows for viral co-opting of host trafficking pathways.[49]

Biofilms

After attachment to a particular surface, a number of pathogens are capable of forming biofilms, which can be defined as structured communities of microbial cells enclosed in a self-produced exopolysaccharide matrix. Although most studies of biofilms have involved a single species, it is likely that biofilms relevant to human infection often involve multiple species sharing the advantages of biofilm existence. Human infections associated with biofilms include dental caries, lower airway infection with *P. aeruginosa* in patients with cystic fibrosis, and foreign body infections in patients with prostheses and implanted devices. In addition, biofilm formation likely occurs during osteomyelitis and endocarditis.[45]

P. aeruginosa is a model organism for the study of biofilms and forms pillars of stationary (sessile) bacteria held together by an extracellular polysaccharide called alginate. Interposed among these pillars are channels that facilitate the flow of nutrients and provide pathways for motile (planktonic) organisms to move about (Fig. 1-7A). In experiments directed at defining the early steps of *P. aeruginosa* biofilm formation, O'Toole and Kolter established that flagella are required for initial bacterial attachment, presumably because these appendages promote movement toward the relevant surface. After attachment, type 4 pili and pilus-mediated twitching motility promote formation of microcolonies[226] in which transcription of *algC*, *algD*, and *algU* is activated, resulting in synthesis of alginate.[54] Pulmonary isolates from patients with cystic fibrosis often form highly mucoid colonies (reflecting expression of alginate) or can form tiny colonies on agar plates, the so-called small-colony variant (SCV) phenotype associated with biofilm formation and increased antibiotic resistance.[109,110,285]

Development of the complex community present within a biofilm requires intercellular communication to coordinate the metabolic and other activities of members of the community. *P. aeruginosa* employs several identified *quorum-sensing* systems, which involve the production of small molecules that are sensed by neighboring organisms and regulate gene expression in these neighbors. One well-studied system is based on the acyl-homoserine lactone called *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL).[55,230] 3OC12-HSL is synthesized in a reaction catalyzed by LasI and accumulates with increases in population density. Ultimately, 3OC12-HSL reaches a critical concentration and then interacts with LasR, serving to activate transcription of a number of genes. Host inflammatory pathways are also induced directly by accumulated 3OC12-HSL.[277] Organisms with a mutation in *lasI* are capable of attachment and microcolony formation, but the resulting microcolonies remain thin, undifferentiated, and sensitive to dispersion by detergents. Addition of the missing lactone signal to the *lasI* mutant restores development into structured, thick, biocide-resistant biofilms, analogous to those observed with wild-type organisms.[55] Moreover, mutation in *lasI* impedes establishment of pulmonary infection in mice.[235,331]

Biofilms also play a prominent role in human infections with *Candida* species, such as oral thrush and catheter-associated infections. Although several pathogenic *Candida* species can form biofilms in the host, their ultrastructure and the molecular strategies underlying biofilm formation vary. The best studied of these fungi, *C. albicans* relies on the expression of certain cell wall proteins (including Hwp1),[213] a regulated yeast-to-hyphal switch as described earlier, and quorum-sensing molecules including *E,E*-farnesol, which represses filamentation and can suppress the growth of other nearby bacterial and fungal species.[58,127,269]

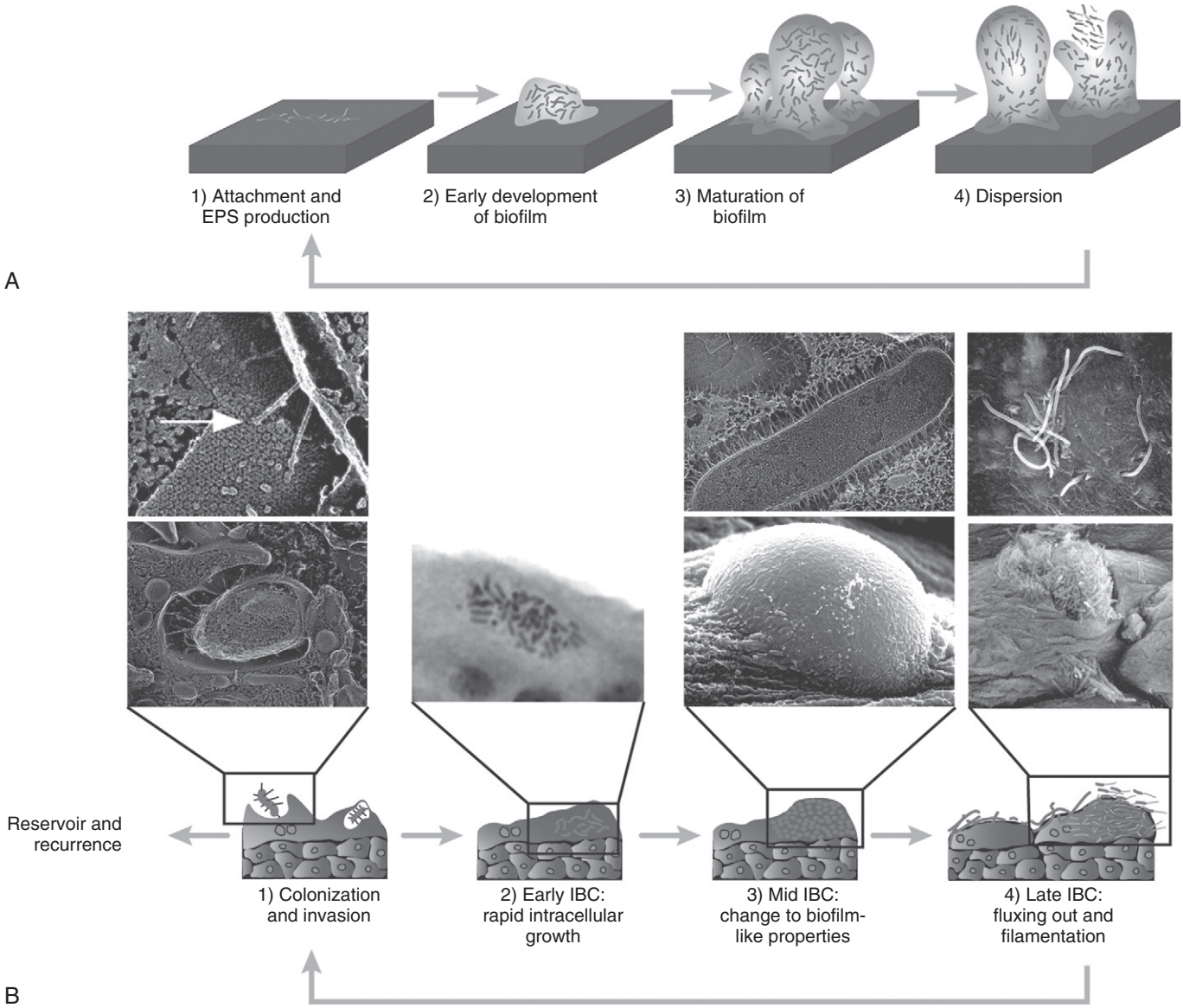

FIGURE 1-7 ■ In vitro *Pseudomonas* biofilm formation and parallel stages of formation of uropathogenic *Escherichia coli* intracellular bacterial communities (IBCs). **A,** Dynamics of *P. aeruginosa* biofilm formation on an inert surface. Keys to the formation of the biofilm include flagella-mediated attachment, type 4 pilus–based twitching motility, and a quorum sensing system. **B,** Composite representation of the stages of IBC formation and maturation. (**A** from Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science 1999;**284**:1318–22. Copyright 1999 American Association for the Advancement of Science; **B** from Kau AL, Hunstad DA, Hultgren SJ. Interaction of uropathogenic *Escherichia coli* with host uroepithelium. Curr Opin Microbiol 2005;**8**:54–9.)

Biofilms constitute a protected mode of growth that allows survival in a hostile environment, for example, in the presence of host immune mechanisms or antimicrobial agents.[45] Based on studies of *P. aeruginosa*, sessile bacteria release antigens and stimulate production of antibodies, but these antibodies are ineffective in killing organisms within biofilms.[37] Similarly, sessile *P. aeruginosa* stimulate a diminished oxidative burst and are relatively refractory to phagocytic uptake. In addition, fungi and bacteria within biofilms are resistant to the effects of a number of antimicrobial agents, in part because these agents are unable to diffuse into the biofilm and in part because these organisms may exist in a slow-growing or otherwise protected phenotypic state.[45] Biofilm-like microbial communities have also been described within host epithelial cells, as with uropathogenic *E. coli* in the mammalian bladder.[6] Recently, new approaches to antimicrobial therapy include novel natural products and other small molecules that inhibit quorum-sensing and biofilm formation.[33,90]

CELL ENTRY AND INTRACELLULAR LIFE

After adherence to a host surface, many pathogenic bacteria are able to invade and survive inside epithelial cells and other nonprofessional phagocytes (for instance, M

cells in intestinal Peyer's patches). In addition, some pathogens are able to survive inside professional phagocytes (macrophages and neutrophils). Invasion may represent a mechanism to breach host mucosal barriers and gain access to deeper or more distant tissues. Alternatively, invasion may provide the organism with a special niche (e.g., protecting it from host immune mechanisms). In the case of viruses, cell entry ensures access to the cell machinery required for viral replication.

Generally, the process of bacterial invasion involves a class of molecules called *invasins* that mediate adherence and entry. Invasion is an active event that relies on underlying host cell functions and is associated with rearrangement of the host cell cytoskeleton. Once inside the host cell, the invading or ingested organism usually is localized within a membrane-bound vacuole that contains lysosomal enzymes. In some cases, the pathogen escapes from this vacuole and enters the cytoplasm, a more permissive environment. In other cases, the pathogen remains in the vacuole and neutralizes lysosomal enzymatic activity. The processes of invasion into cells, survival within cells, cell-to-cell spread, and entry into the circulation define the extent of infection and dissemination.

Invasion

In considering the molecular mechanism of bacterial invasion, perhaps best characterized are the enteropathogenic *Yersinia* species, namely, *Y. pseudotuberculosis* and *Y. enterocolitica*. These organisms are usually acquired by ingestion of contaminated food or water and typically cause self-limited enteritis or mesenteric adenitis. In infants and other individuals with compromised immunity, they sometimes produce systemic disease. The primary determinant of *Y. pseudotuberculosis* and *Y. enterocolitica* invasion is an adhesive outer membrane protein called invasin, which is encoded by a chromosomal locus called *inv* and binds tightly to a family of β_1 integrins expressed on host cells, including $\alpha_3\beta_1$ integrin on the surface of intestinal M cells.[139] The interaction between invasin and β_1 integrins initiates a cascade of signaling steps in the host cell, resulting in actin rearrangement and formation of large complexes of cytoskeletal elements (talin, vinculin, α-actinin, and others) termed *focal adhesions.*[138] Bacterial entry into the host cell occurs via a "zipper-like" mechanism, with the plasma membrane zippering around the invading organism.

Beyond invasin, two additional proteins called YadA and Ail also influence invasion by enteropathogenic *Yersinia* species. YadA is a 45-kDa surface protein that is encoded by the 70-kb *Yersinia* virulence plasmid. It is highly expressed under environmental conditions (e.g., temperature of 37° C) in which invasin is repressed.[70] YadA reaches the bacterial surface via the autotransporter pathway and exists in a trimeric form that is essential for its adhesive activity.[47] Like invasin, YadA promotes invasion through binding to β_1 integrins on the host cell surface, but its binding occurs indirectly via extracellular matrix molecules including collagens, laminin, and fibronectin.[71] Based on studies using a mouse oral infection model, in *Y. enterocolitica* YadA is essential for survival and multiplication in Peyer's patches whereas in *Y. pseudotuberculosis* YadA is dispensable for full virulence.[20] Ail is a 17-kDa outer membrane protein that also is encoded by a chromosomal locus *(ail)* and mediates high levels of adherence and low levels of invasion in assays with cultured epithelial cells. In addition, Ail mediates resistance to complement-mediated serum killing, independent of an effect on invasion.[17]

Similar to *Y. enterocolitica* and *Y. pseudotuberculosis*, *Listeria monocytogenes* invades epithelial cells via a zipper-like mechanism. Invasion is mediated by proteins called internalin A (InlA) and internalin B (InlB), which are required for virulence in animal models. InlA interacts with E-cadherin, a host cell transmembrane protein with an intracellular domain that interacts with the cytoskeleton.[200] InlB interacts with C1q on host cells and promotes invasion by activating the PI-3 kinase pathway.[22] Uropathogenic strains of *E. coli* also invade epithelial cells via a zipper-like mechanism, mediated by the FimH adhesin expressed on the tip of type 1 pili. In experiments with cultured bladder epithelial cells, FimH is both necessary and sufficient for entry, as demonstrated by examination of a *fimH*$^-$ mutant and of latex beads coated with purified FimH.[189] In vitro experiments further suggest that FimH-mediated bacterial binding to a mannose-coated surface is actually strengthened by shear forces, such as fluid flow over the surface.[299,300] After FimH-dependent invasion into superficial epithelial cells of the murine bladder, UPEC multiply rapidly to form intracellular bacterial communities, which display some features of biofilms, including community behavior, differential gene expression, and protection from antimicrobial agents (see Fig. 1-7B).[6,150,154] A subset of successfully internalized bacteria ultimately form a quiescent bacterial reservoir within the epithelium that resists immune clearance and antibiotic therapy and may serve as a seed for recurrent infections.[134,208,209]

Salmonella enterica serovar *typhimurium (S. typhimurium)* is an example of a pathogen that invades cells by a mechanism distinct from zippering. On contact with the epithelial cell surface, *S. typhimurium* triggers a dramatic host cell response characterized by actin rearrangement, calcium and inositol phosphate fluxes, and a "splash" of membrane ruffling surrounding the point of entry. Bacterial internalization into the cell occurs rapidly, with organisms appearing in membrane-bound vacuoles within a few minutes of initial contact with the host cell. The determinants of *S. typhimurium* invasion are encoded by a pathogenicity island called SPI-1, located at centisome 63 on the bacterial chromosome.[86] Especially important is a prototypical *type III secretion system*, which forms a needle-like complex on the bacterial surface that breaches the host cell membrane and serves to translocate bacterial proteins directly into the host cell, altering the host cell cytoskeleton[39,181] and influencing immune responses. The base of the needle complex spans both the inner and outer membranes and is about 40 nm in diameter, whereas the needle itself is 8 nm in width and approximately 80 nm in length (Fig. 1-8).[41,87] The base is composed of proteins called PrgH, PrgK, and InvG, with InvG playing a key role in pore formation in the bacterial outer membrane. The needle, in turn, is

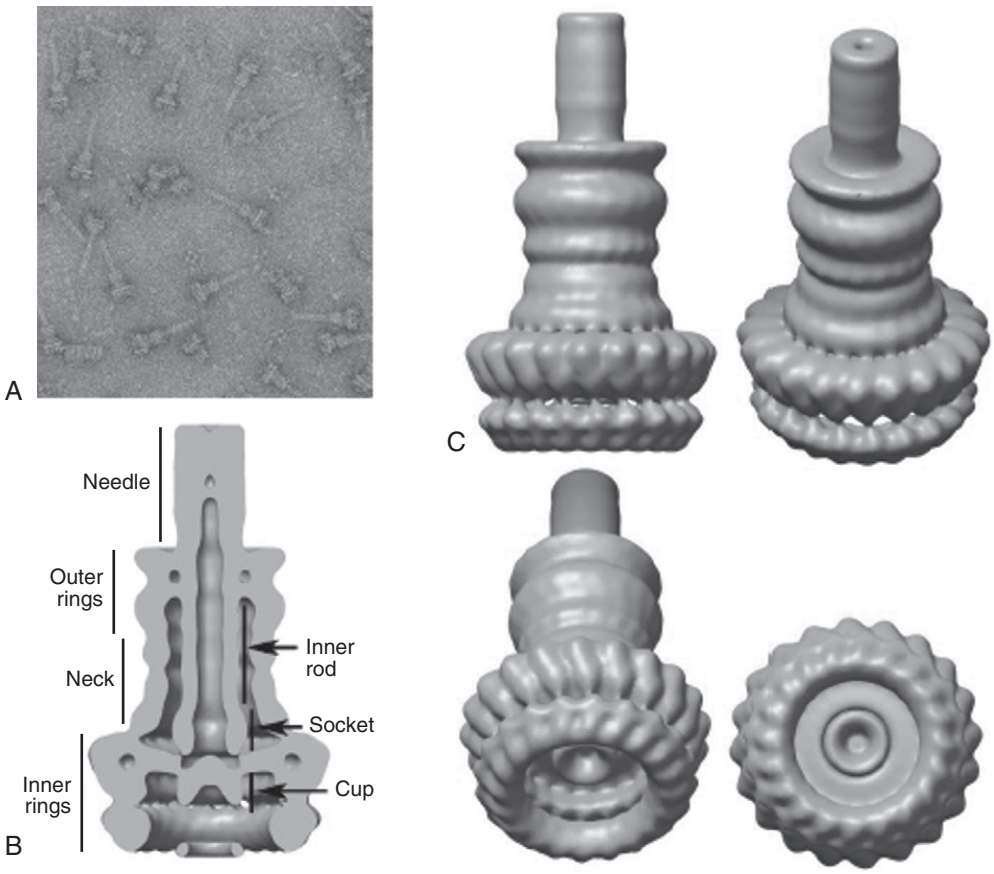

FIGURE 1-8 ■ General structure of the gram-negative type III secretion system, according to electron micrographic and other data. Represented are **A,** electron micrographs of isolated needle complexes, **B,** cross-sectional schematic of the components of the needle complex, and **C,** surface views of the assembly. **(See companion Expert Consult web site for color version.)** (Reproduced with permission from Galan JE, Wolf-Watz H. Protein delivery into eukaryotic cells by type III secretion machines. *Nature* 2006;**444**:567–73.)

composed of PrgI, and its length is influenced by a protein called InvJ.[166,167]

The proteins secreted through the *S. typhimurium* needle complex (and other type III secretion systems) and into the host cell are referred to as *effector proteins*. SopE is an effector protein that mediates the initial rearrangement of actin and ruffling of the host cell membrane. It functions as a guanyl-nucleotide exchange factor (GEF) and activates two host cell Rho GTPase proteins called Rac and Cdc42.[32,105,107] SptP is an effector protein that functions as an antagonist of SopE, mediating reversal of actin rearrangement by converting Rac and Cdc42 to the inactive forms (GDP forms). Consistent with these functions, SopE and SptP directly antagonize each other when coinjected into cells.[85] Other effector proteins secreted by the *S. typhimurium* SPI-1 type III secretion system include the inositol phosphate phosphorylase SopB, which disrupts normal host cell signaling mechanisms,[218] and AvrA, which interferes with the nuclear factor kappaB (NF-κB) signaling pathway in host cells, thereby downregulating host inflammatory responses.[40]

Important accessory and regulatory genes are also present within SPI-1. As an example, the *sicA* gene is just upstream of the *sipB* and *sipC* genes and encodes an accessory protein with chaperone activity essential for stabilization and translocation of SipB, SipC, and SopE.[304] Other chaperones encoded by SPI-1 are involved in the stabilization and translocation of other effector proteins. The genetic and environmental factors that regulate the expression of type III secretion machinery and secreted proteins represent an area of ongoing study.[5]

Intracellular Survival

Once an organism invades a nonprofessional phagocyte or is ingested by a professional phagocyte, several potential outcomes exist. Often, the organism is killed. However, some pathogens have developed strategies to survive and replicate inside host cells, in some cases within a vacuole and in others by escaping from the vacuole.

There is general agreement that *S. typhimurium* resides within a membrane-bound vacuole in both professional and nonprofessional phagocytes. However, the vacuole lacks several lysosomal markers typical of the main endocytic pathway (the mannose-6-phosphate receptor pathway) and appears to be distinct from this pathway. Insight into the molecular determinants of intravacuolar survival came in 1996, when two independent groups reported the discovery of a second *Salmonella* pathogenicity island, now called SPI-2.[116,221] This island maps to

centisome 31 and encodes another type III secretion system, including structural proteins (*ssa* locus),[116] effector proteins (*sse* locus), and accessory proteins (*ssc* locus). In addition, this region encodes a *two-component regulatory system* consisting of a membrane-located sensor kinase (SsrA) and a transcriptional regulator (SsrB).[221] Mutations in SPI-2 result in reduced survival inside macrophages, with no effect on adherence and invasion in assays with intestinal epithelial cells.[221] *Salmonella* SPI-2 mutants demonstrate reduced virulence in experimental mice (up to a 10^4-fold reduction in 50% lethal dose), suggesting that survival inside macrophages is a key factor in the pathogenesis of disease.[267] Expression of SPI-2 genes within the macrophage vacuole depends at least in part on the acidic intravacuolar environment. Inhibition of macrophage vacuolar acidification using bafilomycin A1 (an inhibitor of the vacuolar proton ATPase) results in a sharp attenuation in transcription of SPI-2 genes. This effect is not reproduced by low pH alone outside the vacuole, suggesting that other environmental effects within the vacuole play a role.[34] Recent work indicates that *Salmonella* SPI-2 transcription is activated before invasion, apparently preparing the pathogen for the hostile intracellular environment.[24] Although we will not discuss the large number of individual effector proteins here, the SPI-2 genes as a group appear to modulate host endocytic and exocytic transport mechanisms and inflammatory signaling.[1,136]

A third *Salmonella* pathogenicity island called SPI-3 also influences survival inside macrophages. This island is located at centisome 82 and was discovered by examining the *Salmonella selC* locus, a tRNA gene where pathogenicity islands reside in some strains of *E. coli*.[13,15] SPI-3 contains the *mgtBC* operon, which permits *S. typhimurium* growth in environments with low concentrations of Mg^{2+}, including macrophages. In particular, mutation of the *mgtBC* operon abolishes the ability of *S. typhimurium* to replicate in low-Mg^{2+} liquid media and in macrophages, and addition of Mg^{2+} to the medium after phagocytosis restores the ability to survive intracellularly. Homologous *mgtBC* genes have been found in other organisms with intracellular lifestyles, such as *Brucella melitensis* and *Yersinia pestis*.[14] In *Salmonella*, the *mgtBC* genes are expressed after internalization into host cells under control of the PhoP-PhoQ two-component regulatory system, a complex that directs expression of a number of virulence determinants.[186]

The ability to survive within phagocytic cells may provide *Salmonella* with a means to exploit an intrinsic host pathway and disseminate to distant sites. In particular, certain phagocytes express the β_2 integrin CD18, which mediates leukocyte migration in response to various stimuli. During *S. typhimurium* infection, CD18-expressing phagocytes transfer organisms from the intestine to the spleen. Indeed, bacterial loads in the liver and spleen are reduced after oral inoculation in CD18-deficient mice, when compared with infection in wild-type mice.[308] On the one hand this function of CD18 facilitates initiation of a systemic immune response and benefits the host. However, at the same time, it provides bacteria with a mechanism of transit from the gut to organs of the reticuloendothelial system and elsewhere.

Mycobacterium tuberculosis, another intracellular pathogen, uses an array of mechanisms to ensure intracellular survival. The *M. tuberculosis* vacuole lacks usual amounts of the vesicular proton ATPase responsible for mediating acidification and fails to acidify to normal levels.[293] In addition, *M. tuberculosis* blocks fusion of the vacuole with acidic lysosomes, further preventing acidification.[261] Similar to intracellular gram-negative bacterial pathogens, *M. tuberculosis* contains an *mgtC* gene, and mutation of this gene results in impaired virulence in cultured human macrophages and in mouse spleen and lung. Low Mg^{2+} concentration and mildly acidic pH inhibit the growth of the *mgtC* mutant, suggesting that the gene is important for survival in the phagosome, where such conditions may exist.[26] Another factor that influences *M. tuberculosis* survival within macrophages is isocitrate lyase, an enzyme of the glycolytic shunt that is essential for metabolism of fatty acids. Expression of isocitrate lyase is upregulated during infection of activated macrophages and is required for full virulence in a murine model of infection, independent of an effect on bacterial growth.[193] The structure of *M. tuberculosis* isocitrate lyase has been solved and may provide a target for new drug therapies against persistent infection, because this enzyme is absent from vertebrates.[265,273]

During the course of interaction with macrophages, *M. tuberculosis* (at a low to moderate multiplicity of infection) is capable of stimulating caspase-1 and inducing macrophage apoptosis. This process may be beneficial to the host, preventing systemic spread of infection: less virulent strains of *M. tuberculosis* are more potent inducers of apoptosis.[157] At the same time, *M. tuberculosis* possesses at least two antiapoptotic mechanisms that further influence the outcome of macrophage encounters. First, *M. tuberculosis* infection enhances host macrophage production of soluble TNFR2, a protein that binds to tumor necrosis factor-α (TNF-α) and interferes with apoptosis.[7] Second, *M. tuberculosis* infection activates production of NF-κB, a transcriptional regulator that activates antiapoptotic pathways within the host cell.[92] Interestingly, higher multiplicities of infection with virulent strains of *M. tuberculosis* can induce caspase-independent cell death in macrophages, a mechanism proposed to contribute to the formation of necrotic lesions during tuberculous disease.[173]

L. monocytogenes is an example of an organism that escapes from the phagocytic vacuole in macrophages and epithelial cells and moves into the cytoplasm. This organism causes meningitis and focal brain abscesses in humans and has a predilection for the fetoplacental unit. In pregnant women, listeriosis results in fetal loss in 30% of cases. Intravacuolar replication and escape from the vacuole are dependent on listeriolysin O, a hemolysin encoded by the *hly* gene.[11] Listeriolysin O interacts with cholesterol in host cell membranes and forms pores, leading to lysis of the phagosome.[84] Host enzymatic activities may also contribute to listerial escape from the phagosome. In human epithelial cells the contributions of a broad-range phospholipase C (called PC-PLC) and a metalloproteinase called Mpl are most important for vacuolar escape in the absence of listeriolysin O.[101,187,274]

Viral Cell Entry

Viruses have developed a specialized family of proteins that specifically function to engage host cell proteins and fuse with cell membranes and that allow for transfer of viral genetic material. The details regarding structure and function of these proteins are reviewed elsewhere.[162] There are two known classes of viral fusion proteins: class I proteins form a hairpin structure with a known α-helix domain (e.g., HIV gp41, influenza HA2), whereas class II proteins exist in β-sheets and transition from a moderately stable dimeric form to a very stable trimer (e.g., dengue E protein). These fusion proteins undergo conformational changes when transitioning from the prefusion to the postfusion state, resulting in a more stable form that favors the process of viral fusion and entry.

Cell-to-Cell Spread

Movement from one cell to another may help an organism gain a stronger foothold in host tissues. *L. monocytogenes* is one example of a pathogen capable of cell-to-cell spread. Once this organism is free in the cytoplasm, actin begins to polymerize on the bacterial surface. Eventually the condensed actin forms a polar tail or comet, which propels the organism through the cytoplasm and into adjacent cells. The rate of bacterial movement within a cell correlates with actin tail length.[297] Actin accumulation and condensation is mediated by the *L. monocytogenes* ActA protein, which is tightly anchored to the bacterial surface and is expressed asymmetrically over the length of the organism.[275,296] ActA is the sole *Listeria* factor required for actin polymerization, because actin tails form in *Xenopus* cytoplasmic extracts containing ActA-coated beads. However, in these experiments, motility occurs only when ActA is distributed asymmetrically on the beads.[28] ActA appears to interact directly with actin and also with a variety of other host cytoskeletal proteins.[84,298] Cytochalasin D is an inhibitor of actin polymerization and inhibits the cell-to-cell spread of *L. monocytogenes* in epithelial monolayers.[53,204]

On reaching the plasma membrane, bacteria protrude from the cell in filipodium-like structures (called listeriopods), which are then engulfed by neighboring cells. This engulfment may be part of a normal host process, because MDCK cells demonstrate low-level endocytosis of adjacent cell membrane fragments even in the absence of bacteria.[254] The formation of listeriopods and the engulfment of these structures by neighboring cells are independent of listeriolysin O, PI-PLC, and PC-PLC.[93] Once inside a nascently infected cell, *Listeria* escapes from the double-membrane vacuole via the action of PI-PLC, PC-PLC, and Mpl.[84] On arrival in the cytosol, bacteria can enter another cycle of actin-based motility and cell-to-cell spread, although one or two bacterial generations may be necessary to regain motility.[254]

A second pathogen capable of actin-based motility and cell-to-cell spread is *Shigella flexneri*. In *Shigella*, a single protein called IcsA is sufficient to induce formation of an actin tail, similar to that observed in *L. monocytogenes*. IcsA is an outer membrane protein that is encoded on the *Shigella* virulence plasmid and is distributed on the bacterial surface in a polarized fashion, possibly as a result of specialized machinery for autotransporter protein secretion near the poles of some gram-negative bacteria.[145] Initially, IcsA is distributed over the whole bacterial surface, with a predominance at one pole. However, over time a secreted bacterial protease called IcsP cleaves roughly half of the surface IcsA, mostly at the opposite pole, further polarizing distribution.[69,287] Elimination of expression of IcsP leads to increased quantities of IcsA and increased actin-based motility, suggesting that IcsA (rather than host factors) is rate limiting in the motility process.[268] Like ActA, IcsA is necessary and sufficient to induce polymerization of the actin tail, and the tail forms at the end where IcsA concentration is highest.[97] Despite the functional similarities between IcsA and ActA, there is no significant sequence homology between the two proteins. In contrast to ActA, no direct interaction between IcsA and actin has been demonstrated; and IcsA is found throughout the actin tail, not only at the bacterial pole-actin tail junction.

When considering cell-to-cell spread of viruses, it is helpful to return to the example of HCV. Claudin-1 and occludin-1 are the gap junction proteins that serve as the final entry point for this virus.[73,241] It has been demonstrated that HCV can infect neighboring liver cells directly via these gap junction proteins, likely bypassing the extracellular release of virus and subsequent SR-B1 and CD81 binding steps.[301] Experiments demonstrated that antibodies blocking the E2-CD81 interaction would block infection from virus-infected media but would not block infection of naïve cells when cocultured with previously HCV-infected cells.[301] It has also been shown that after infecting a liver cell, HCV promotes breakdown of typical apical to basal organization of liver cells, exposing the gap junction complexes and presumably facilitating cell-to-cell spread.[194,195]

DAMAGE TO THE HOST

Damage to host cells and host tissues represents a fundamental mechanism by which a pathogen is able to survive at a given site and then spread within a host. Generally, damage is induced by microbial toxins. Most toxins are released extracellularly and are capable of inducing damage at very low concentrations *(exotoxins)*. Microbial attachment and invasion facilitate toxin delivery to target cells and target tissues and serve to enhance toxicity.

Historically, microbial toxins have been classified according to a variety of criteria, including cellular target of action (e.g., enterotoxins, leukotoxins, neurotoxins), mechanism of action (e.g., adenosine diphosphate [ADP]–ribosylating toxins, adenylate cyclase toxins, pore-forming toxins, proteolytic toxins), and major biologic effect (e.g., hemolytic toxins, edema-producing toxins). In recent years the term *toxin* has been applied more broadly to include enzymes that mediate damaging effects via phospholipase or hyaluronidase activity.

Bordetella pertussis Toxins

Whooping cough (*B. pertussis* infection) is a classic example of a toxin-mediated disease and involves an interplay of multiple toxins.[159] The pathogenesis of whooping cough begins with *B. pertussis* colonization of the trachea, which is facilitated by a molecule called tracheal cytotoxin (TCT). TCT is a naturally occurring disaccharide-tetrapeptide fragment of peptidoglycan and belongs to the family of muramyl peptides.[98] Many gram-negative organisms produce an analogous fragment during normal turnover of cell wall components, but significant extracellular release appears to occur only in *Bordetella* species and gonococci. In most other species, an inner membrane protein called AmpG recycles this fragment back into the bacterial cell.[46] TCT is toxic to tracheal epithelial cells in vitro, stimulating nitric oxide synthase and local production of interleukin-1 and causing inhibition of ciliary motility, inhibition of DNA synthesis, and cell death.[111-113,118] During natural infection, TCT is thought to paralyze the mucociliary escalator and thereby interfere with clearance of *B. pertussis* and respiratory mucus.

Pertussis toxin is believed to be a key determinant of the clinical manifestations of whooping cough. This toxin belongs to a family of bacterial ADP-ribosyltransferase enzymes. The target of pertussis toxin is host cell G proteins, resulting in disruption of normal signaling processes. A number of biologic effects have been ascribed to pertussis toxin, including induction of lymphocytosis, stimulation of insulin release, sensitization to histamine, and disruption of phagocytic cell function; however, the specific relationship between the effects of pertussis toxin and the symptoms of whooping cough remains unclear.[119] Of note, *B. parapertussis* is closely related to *B. pertussis* and produces a similar cough illness but fails to produce pertussis toxin because of mutations in the *ptx* promoter region.[212]

B. pertussis also elaborates a toxin called adenylate cyclase toxin (CyaA), a member of the RTX (repeat-in-toxin) family of bacterial cytolysins whose prototype is the *E. coli* hemolysin HlyA.[46] These toxins cause target cell lysis by creating pores in the host cell plasma membrane, but at sublytic concentrations, many of these toxins also manipulate host enzymatic and signaling pathways within the host cell. In the case of *B. pertussis*, CyaA inhibits host adenylate cyclase, resulting in accumulation of cyclic adenosine monophosphate (cAMP); elevated levels of cAMP within phagocytic cells inhibit oxidative activity and induce apoptosis, thus disabling this arm of the immune system.[160,161,236] In respiratory epithelial cells, elevated cAMP may result in increased fluid and mucus secretion, further impairing mucociliary function.

Among other examples of these dual-function RTX toxins, the prototypic HlyA of uropathogenic *E. coli* induces the degradation of actin-associated proteins, resulting in exfoliation of the superficial epithelial layer in the bladder.[59] The α-hemolysin of contemporary community-associated *S. aureus* strains activates a host epithelial cell surface molecule called ADAM10, which cleaves E-cadherin at cell-cell junctions to permit access of the pathogen across the epithelial layer.[137,323]

Hemolytic-Uremic Syndrome and Shiga Toxins

A number of intestinal pathogens produce Shiga toxins, including *Shigella dysenteriae*, enterohemorrhagic *E. coli* (including *E. coli* O157:H7), and *Citrobacter freundii*, among others. Shiga toxins are classic A-B toxins, consisting of an A subunit that has toxic activity and five B subunits arranged in a pentameric ring-like structure that promotes binding to host cells and delivery of the A subunit. The B subunits interact with host cell globoseries glycolipids, especially the Pk trisaccharide moiety of globotriaosylceramide (GbO3). The A subunit is endocytosed by the host cell and traverses the cytoplasm in membrane-bound vesicles. Some of these vesicles travel in a retrograde fashion to the Golgi apparatus and then to the endoplasmic reticulum.[262] Shiga toxin then co-opts the function of the endoplasmic reticulum proteins HEDJ and BiP to enter the cytosol, where it enzymatically inhibits host 28S ribosomal RNA by cleaving a single adenine residue, resulting in inhibition of protein synthesis and cell death.[262,338]

In humans, *E. coli* O157:H7 is an important cause of hemorrhagic colitis and sometimes produces hemolytic-uremic syndrome. Infection begins with adherence to epithelial cells via intimin and other proteins encoded by the locus of enterocyte effacement (LEE), resulting in formation of attaching and effacing lesions analogous to those observed in EPEC infection.[191] After adherence, the organism releases Shiga toxin, which traverses the intestinal epithelial cell and enters the bloodstream.[2] Toxin circulates to distant organs and mediates damage via toxicity to endothelium. Diarrhea likely results from damage to endothelium in small mesenteric vessels, leading to ischemia and sloughing of the intestinal mucosa. The renal effects observed in human hemolytic-uremic syndrome arise from microvascular and glomerular damage with luminal occlusion by fibrin and platelets.[344] Hemolysis and thrombocytopenia likely develop as a consequence of microangiopathy.

Tissue-Degrading Toxins

A number of toxins have enzymatic activity and are capable of degrading tissue components. One example is hyaluronidase, which degrades hyaluronic acid, a repeating disaccharide glycosaminoglycan involved in cell motility, adhesion, and proliferation in normal hosts. Hyaluronic acid contains alternating *N*-acetylglucosamine and glucuronic acid moieties, connected by β linkages. It is prominent in extracellular matrix when cell turnover and tissue repair are prominent, for example in embryogenesis, wound healing, and carcinogenesis.[50] The primary host receptor for hyaluronic acid is CD44, which undergoes post-translational modification that varies according to host cell type. Interactions between hyaluronic acid and CD44 are critical to T- and B-cell stimulation, growth of certain lymphoid malignancies, and propagation of certain inflammatory responses.[202]

In *S. pyogenes*, hyaluronidase is a 96-kDa protein that is encoded by the *hylA* gene and is released extracellularly. It is proposed to promote invasion through cell layers

and tissue planes and is considered one of several *S. pyogenes* spreading factors.[135] Interestingly, *S. pyogenes* also produces a thick "capsule" of hyaluronic acid that can interact with other host cellular and extracellular matrix proteins to contribute to tissue invasion by the organism. Other pathogens that produce a hyaluronidase include *S. agalactiae* (group B streptococcus), *Treponema pallidum*, *Candida*, *Entamoeba histolytica*, and *Ancylostoma braziliense*.[50]

EVASION OF IMMUNITY

To survive and replicate within the host, a pathogen must evade the host immune system. Initially the organism must circumvent innate immune mechanisms, including mechanical forces, resident phagocytes, and complement activity. Over time the organism must overcome adaptive immunity as well, including the presence of specific antibodies.

Antiphagocytic Factors

As described earlier in this chapter, invasin-mediated entry into M cells plays an important role in the early stages of *Yersinia* infection. At the same time, evasion of phagocytosis is critical to the pathogenesis of *Yersinia* disease. The ability to avoid phagocytosis is dependent on the *Yersinia* virulence plasmid, which encodes a number of proteins called Yops.[44,290] Both YopE and YopH interfere with ingestion by macrophages and neutrophils via slightly different mechanisms. YopE shares sequence homology with the *Salmonella typhimurium* SptP protein and downregulates all three of the Rho GTPases (Rho, Cdc42, and Rac), thus inhibiting actin rearrangement and blocking formation of membrane ruffles (lamellipodia) and spikes (filopodia).[3,16] YopH is a protein tyrosine phosphorylase that appears to act on a host cell cytosolic protein called Cas, interfering with recruitment of Rho, Cdc42, and Rac and preventing formation of actin stress fibers, focal complexes, and focal adhesions.[12,16] YopJ is an acetyltransferase that covalently modifies and inactivates intermediate kinases in the mitogen-activated protein kinase and NF-κB signaling pathways, leading to host cell apoptosis.[207,336] Importantly, some Yop effectors also represent important immunogens; for example, the immunodominant epitope of YopE represents a major $CD8^+$ T-cell antigen in experimental plague and may facilitate a new direction in *Yersinia* vaccine development.[177,342]

Shigella employs another strategy to induce apoptosis in phagocytic cells. This pathogen produces hemorrhagic enterocolitis and is an important cause of bloody diarrhea in children. Infection begins with ingestion of organisms, which attach to intestinal M cells and then cross the intestinal epithelium.[348] On entry into the subepithelial space, organisms are engulfed by resident macrophages and contained in membrane-bound vacuoles. However, they quickly escape from macrophage vacuoles and move to the cytosol of the cell, where they induce apoptosis.[347] The mechanism of apoptosis involves a protein called IpaB, which is encoded by the *Shigella* virulence plasmid and is injected into host cell membranes via the *Shigella* type III secretion system.[18] Work by Zychlinsky and coworkers showed that IpaB binds to cytosolic interleukin-1β converting enzyme (caspase-1), a cysteine protease that cleaves IL-1β to its active form.[120] Of note, the *S. typhimurium* SipB protein shares homology with IpaB and also induces apoptosis by interacting with caspase-1.[117] Interestingly, recent work has shown that IpaB has contrary effects in epithelial cells, binding to the cell-cycle regulator Mad2L2 to inhibit epithelial turnover and promoting epithelial colonization with *Shigella*.[142]

Evasion of Complement Activity

S. pyogenes expresses at least three factors that interfere with host complement activity. Perhaps best known is M protein, which inhibits activation of the alternative complement pathway. This effect is mediated at least in part by the ability of M protein to bind complement factor H, a regulatory protein that inhibits assembly and accelerates decay of C3bBb. Recent studies indicate that serotype M1 and M57 strains express an extracellular protein called Sic (streptococcal inhibitor of complement-mediated lysis), which associates with human plasma proteins called clusterin and histidine-rich glycoprotein (HRG) and apparently blocks formation of the membrane attack complex (C5b-C9).[4] Studies of epidemic waves of M1 infection demonstrate that Sic undergoes significant variation over time, perhaps in response to the selective pressure associated with specific antibodies.[123,124,196] Of note, nonpolar inactivation of *sic* results in reduced mucosal colonization of mice.[180] In addition, *S. pyogenes* produces a serine protease called C5a peptidase, which cleaves and inactivates C5a.[321] C5a is a cleavage product of C5 and serves as a powerful chemoattractant for neutrophils; thus, streptococcal C5a peptidase serves to attenuate the neutrophil response to infection.

N. gonorrhoeae is a common cause of cervicitis, urethritis, and pelvic inflammatory disease and is also capable of producing disseminated disease. Recent spread of antibiotic resistance in this pathogen highlights the need for understanding its pathogenic mechanisms to develop new mitigating strategies. Resistance to complement-mediated killing is important in gonococcal pathogenesis and is due in part to sialylation of lipo-oligosaccharide (LOS), which involves addition of host-derived cytidine monophospho-*N*-acetylneuraminic acid (CMP-NANA) by a bacterial sialyltransferase. Given the requirement for CMP-NANA, subcultivation in the absence of human serum or human neutrophils is associated with loss of sialylation and loss of resistance. Sialylated LOS binds factor H, resulting in downregulation of activity of the alternative pathway C3 convertase. In addition, sialylated LOS interferes with neutrophil phagocytosis and with the normal oxidative burst in neutrophils.[253,320] A second determinant of resistance to complement-mediated killing is Por1, an outer membrane porin protein that binds both factor H and C4b binding protein (C4b BP).[248] C4b BP binds C4b and serves to inhibit assembly and accelerate decay of C4b2a, the classical pathway C3 convertase. Gonococci also produce a third factor that influences resistance to complement, namely, an outer membrane-expressed nitrite reductase called AniA.[19,29]

Evasion of Humoral Immunity

A number of pathogens have evolved mechanisms to vary surface-exposed immunogenic molecules, thus facilitating evasion of a specific antibody response. *Antigenic variation* represents one such mechanism and is characterized by the emergence of modified molecules with novel antigenic properties. *Phase variation* represents a second such mechanism and is typified by the reversible loss or gain of a given molecule or structure.

N. gonorrhoeae is capable of producing recurrent infection, reflecting the fact that the antibody response to infection fails to provide lasting immunity. In this context, it is noteworthy that *N. gonorrhoeae* pili are an important target of serum antibody and undergo frequent antigenic variation. Gonococcal pilin expression is controlled by the *pilE* locus (the expression locus), which contains an intact pilin gene along with promoter sequences. In addition to *pilE*, the gonococcal chromosome contains numerous copies of variant *pil* sequences, called *pilS* loci.[104] These loci are transcriptionally inactive because they lack a promoter and 5′ coding sequence. However, they can be introduced into the expression locus by RecA-dependent recombination, resulting in an altered structural subunit and antigenically variant pili.[130] Because *N. gonorrhoeae* is naturally transformable, horizontal exchange of species-specific DNA may also give rise to new *pil* sequences.

The African trypanosomes (including *Trypanosoma brucei*) cause sleeping sickness in sub-Saharan Africa and account for more than 50,000 deaths per year. These organisms are able to avoid humoral immunity by antigenic variation of a large family of proteins called variable surface glycoproteins (VSGs), which coat the entire surface of the trypanosome. VSGs are highly immunogenic and stimulate antibodies that lead to efficient and rapid clearing of parasites from the bloodstream. However, at any given point in time, the organism is able to express a new VSG, allowing some organisms to escape the antibody response against the previous VSG. Each parasite can express over 100 different VSGs, with variation in expression occurring spontaneously at a rate of up to 10^{-2} per cell per generation. Overall, the genome of *T. brucei* contains more than 1000 *vsg* genes, including so-called expression sites (ESs) located near telomeres on mini-chromosomes and silent loci in nontelomeric sites on large chromosomes.[234,302] In general, VSG antigenic variation occurs by two different mechanisms. The first is called in situ activation and involves the simultaneous activation of a new ES and inactivation of the old ES, occurring independent of DNA rearrangement. The second involves DNA recombination, either between the expressed *vsg* and another telomeric ES (reciprocal recombination) or between the expressed *vsg* and a silent *vsg* locus (gene conversion).[302]

In the case of *H. influenzae*, lipopolysaccharide (LPS) is likely a key factor in facilitating colonization and is also a major target of the antibody response to infection. Interestingly, *H. influenzae* LPS undergoes phase variation. LPS biosynthesis involves multiple enzymatic steps and a number of genes. Among these genes, *lic1A*, *lic2A*, *lic3A*, *lex-2*, *lgtC*, and an *oafA*-like gene contain long stretches of tandem four-base pair repeats within their 5′-coding region. In studies of the *lic* loci, Weiser and coworkers observed that the number of repeats varies spontaneously, generating translational frameshifts with different ATG start codons falling in or out of frame.[315] Such frameshifts result in synthesis of a protein with a different N terminus or eliminate protein production altogether (when no in-frame start codon exists). The mechanism of variation in repeat number is presumed to be slipped-strand mispairing, which occurs during DNA replication and involves a single repeat looping out on either the template or the replicating strand. Changes in *lic2A* and *lic3A* influence glycotransferase activity and alter reactivity with monoclonal antibodies directed against specific LPS oligosaccharide epitopes.[102] The *lic2A* gene product is responsible for the addition of a Gal-α1,4-Gal moiety, which resembles the globoseries glycolipids and protects *H. influenzae* from antibody-mediated killing, possibly by molecular mimicry.[316] *lgtC* may be involved in formation of a Gal-β1,4-Glu moiety.[126] Variation in the *lic1A* gene affects production of a choline kinase responsible for addition of phosphorylcholine (ChoP) to the LPS molecule, a physical change that enhances binding of C-reactive protein and results in susceptibility to serum bactericidal activity.[182,317,318] Expression of *lex2* results in addition of a tetrasaccharide (Gal-α1,4-Gal-β1,4-Glc-β1,4-Glc) to the proximal heptose in LPS and increases resistance to complement-mediated serum killing.[99] Similarly, expression of the *oafA*-like gene results in LPS O-acetylation, which facilitates resistance to serum killing.[82]

Encapsulation

Expression of an extracellular capsule represents a common strategy to evade phagocytosis, complement activity, and humoral immunity among pathogenic bacteria, fungi, and parasites. One example is *H. influenzae*, a common cause of childhood bacteremia and meningitis in underdeveloped countries. Among isolates of *H. influenzae*, six structurally and antigenically distinct capsular types are recognized, designated serotypes a to f. Historically, serotype b isolates have accounted for more than 95% of all *H. influenzae* invasive disease, reflecting the distinct virulence properties of the type b capsule, which is a polymer of ribose and ribitol-5-phosphate (PRP) and is encoded by the *cap*b locus.[205] In animal studies comparing derivatives of *H. influenzae* strain Rd expressing type a, b, c, d, e, or f capsule, the strain expressing the type b capsule was associated with the highest incidence of bacteremia after intranasal inoculation of infant rats. Similarly, this strain was associated with the highest magnitude of bacteremia and incidence of meningitis after intraperitoneal inoculation of experimental rats.[346]

In considering the mechanism by which the type b capsule promotes intravascular survival and invasive disease, in vitro studies using mouse peritoneal macrophages and human peripheral blood monocytes provide some insights. The type b capsule inhibits bacterial binding to macrophages in the absence of complement and a source of C3.[217] In addition, the type b capsule interferes with ingestion by macrophages when anti-PRP antibody is lacking.[216,217] Furthermore, the type b capsule

blocks complement deposition on the bacterial surface and resultant complement-mediated bacteriolysis. In almost all isolates of *H. influenzae* type b, the *cap*b locus is a tandem repeat of 18-kb *cap*b gene sequences.[215] As a consequence of this arrangement, the *cap*b locus serves as a template for further amplification of capsule gene sequences in vivo, resulting in increased capsule production. In a study by Corn and colleagues, 23 of 66 minimally passaged invasive isolates had between three and five copies of the 18-kb repeat.[43] Further analysis demonstrated that amplification of the repeat results in augmented resistance to phagocytosis and complement-mediated bacterial killing.[215] The importance of the type b capsule in disease pathogenesis was recognized in vaccine development efforts, and the routine implementation of the Hib polysaccharide-conjugate vaccine in many countries has sharply curtailed the incidence of invasive Hib disease.

Capsule production is also a critical virulence determinant for a number of disease-causing fungi, including the opportunistic pathogen *Cryptococcus neoformans*, which causes infections primarily in HIV-infected and other immunocompromised hosts. *C. neoformans* elaborates a thick polysaccharide capsule that is the basis for the "halo" appearance of the organism on India ink staining of cerebrospinal fluid in patients with cryptococcal meningitis. The capsule comprises a complex polymer with a galactose backbone modified by xylose, mannose, and glucuronic acid. The enzymes responsible for assembly have begun to be identified, suggesting new possible targets for new antifungal development.[164,250] However, major questions persist regarding the spatial organization of capsule synthesis and the basis for interstrain variation in the chemical structure and antibody reactivity of the galactoxylomannan backbone.[63]

Viral Immune Suppression and Latency

Infection with HCV, like that with other viruses, is a potent inducer of interferon-stimulated gene expression.[322] However, HCV has developed several mechanisms to evade host innate immune responses. The viral protease NS3-NS4A interferes with nuclear localization of interferon regulatory factor-3 (IRF-3) in response to interferon in HCV-infected hepatocytes.[83] This disruption of IRF-3 signaling, which prevents cells from activating genes downstream of IRF-3, results from specific cleavage of the molecule IPS-1.[175] Similar observations regarding evasion of innate immunity have been made with the influenza virus NS1 protein and with West Nile virus and HIV.[62,65,325]

Among some other viral pathogens, *latency* represents an important mechanism for persistence in the presence of host immunity, especially in the case of viruses belonging to the herpesvirus family. Herpes simplex viruses (HSV types 1 and 2) commonly establish latency after either gingivostomatitis or genital tract infection. After infection of a host cell, HSV replication begins. Eventually cell death occurs, resulting in cell lysis and release of viral particles, which can then infect adjacent cells. This so-called *lytic replication cycle* is under control of a small number of *immediate early* (IE) genes, which must be transcribed in moderate amounts to allow expression of the remainder of the viral genome. IE gene expression is activated by VP16, a viral protein that binds to a sequence common to IE gene promoters.[246] After lysis of the host cell, new virions enter local nerve termini and travel up the long axon to sensory ganglia, where latency is established within days. In the latent state, viral DNA can be detected in the neuron but infectious virions cannot be isolated. During latency (reviewed recently by Nicoll and colleagues),[211] IE genes are repressed and only one fragment of viral DNA is actively transcribed, yielding several latency-associated transcripts (LATs) via alternative splicing.[339] No protein product has been definitively attributed to the LAT; instead, recent work has demonstrated HSV-1 production of microRNAs, transcribed from LAT exons, that promote latency by inhibiting transforming growth factor-β signaling, favoring survival of infected cells, and regulating the expression of activation-associated viral genes.[51,103,305] LAT-deficient mutants are still able to establish initial latency, suggesting that IE gene expression may be under multiple controls.[311] The mechanism by which HSV is reactivated is an area of intense study and some controversy. Host cellular mechanisms may provide the inciting signals, and the actions of viral thymidine kinase and the protein ICP0 are required for a return to lytic replication.[211]

CONCLUSION

With the explosion of molecular techniques in recent years, our understanding of the specific microbial and host factors involved in the pathogenesis of a variety of infectious diseases continues to expand remarkably. As a consequence of this understanding, we have witnessed the development of a number of new vaccines and potential targets for antivirulence therapeutics. In the coming years, it is likely that advances in immunology and microbial pathogenesis will inform novel approaches for treating and preventing human infections. Examples might include inhibitors of type III protein secretion systems,[143] antagonists of periplasmic chaperones, analogs of important host cell receptors, and vaccine adjuvants that direct polarization of T-cell responses. However, given the impressive adaptability of pathogenic organisms, as new therapeutic agents become available, we must remain vigilant for new microbial strategies allowing evasion of our interventions.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Aepfelbacher M, Roppenser B, Hentschke M, et al. Activity modulation of the bacterial Rho GAP YopE: an inspiration for the investigation of mammalian Rho GAPs. *Eur J Cell Biol* 2011;**90**:951–4.
11. Birmingham CL, Canadien V, Kaniuk NA, et al. Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature* 2008;**451**:350–4.
25. Bu S, Li Y, Zhou M, et al. Interaction between two putative glycosyltransferases is required for glycosylation of a serine-rich streptococcal adhesin. *J Bacteriol* 2008;**190**:1256–66.
33. Christensen LD, van Gennip M, Jakobsen TH, et al. Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing inhibitors against *Pseudomonas*

aeruginosa in an intraperitoneal foreign-body infection mouse model. *J Antimicrob Chemother* 2012;**67**:1198–206.
56. de Groot JC, Schluter K, Carius Y, et al. Structural basis for complex formation between human IRSp53 and the translocated intimin receptor Tir of enterohemorrhagic *E. coli*. *Structure* 2011;**19**:1294–306.
58. De Sordi L, Muhlschlegel FA. Quorum sensing and fungal-bacterial interactions in *Candida albicans*: a communicative network regulating microbial coexistence and virulence. *FEMS Yeast Res* 2009;**9**:990–9.
59. Dhakal BK, Mulvey MA. The UPEC pore-forming toxin α-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways. *Cell Host Microbe* 2012;**11**:58–69.
62. Doehle BP, Hladik F, McNevin JP, et al. Human immunodeficiency virus type 1 mediates global disruption of innate antiviral signaling and immune defenses within infected cells. *J Virol* 2009;**83**:10395–405.
63. Doering TL. How sweet it is! Cell wall biogenesis and polysaccharide capsule formation in *Cryptococcus neoformans*. *Annu Rev Microbiol* 2009;**63**:223–47.
72. Ene IV, Bennett RJ. Hwp1 and related adhesins contribute to both mating and biofilm formation in *Candida albicans*. *Eukaryot Cell* 2009;**8**:1909–13.
90. Galloway WR, Hodgkinson JT, Bowden SD, et al. Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. *Chem Rev* 2011; **111**:28–67.
91. Ganesh VK, Rivera JJ, Smeds E, et al. A structural model of the *Staphylococcus aureus* ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. *PLoS Pathog* 2008;**4**:e1000226.
108. Harris HJ, Davis C, Mullins JG, et al. Claudin association with CD81 defines hepatitis C virus entry. *J Biol Chem* 2010; **285**:21092–102.
109. Haussler S. Multicellular signalling and growth of *Pseudomonas aeruginosa*. *Int J Med Microbiol* 2010;**300**:544–8.
134. Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. *Annu Rev Microbiol* 2010;**64**:203–21.
136. Ibarra JA, Steele-Mortimer O. *Salmonella*—the ultimate insider: *Salmonella* virulence factors that modulate intracellular survival. *Cell Microbiol* 2009;**11**:1579–86.
137. Inoshima I, Inoshima N, Wilke GA, et al. A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. *Nat Med* 2011;**17**:1310–14.
143. Izore T, Job V, Dessen A. Biogenesis, regulation, and targeting of the type III secretion system. *Structure* 2011;**19**:603–12.
149. Julio SM, Inatsuka CS, Mazar J, et al. Natural-host animal models indicate functional interchangeability between the filamentous haemagglutinins of *Bordetella pertussis* and *Bordetella bronchiseptica* and reveal a role for the mature C-terminal domain, but not the RGD motif, during infection. *Mol Microbiol* 2009; **71**:1574–90.
152. Kallewaard NL, Zhang L, Chen JW, et al. Tissue-specific deletion of the coxsackievirus and adenovirus receptor protects mice from virus-induced pancreatitis and myocarditis. *Cell Host Microbe* 2009;**6**:91–8.
164. Klutts JS, Doering TL. Cryptococcal xylosyltransferase 1 (Cxt1p) from *Cryptococcus neoformans* plays a direct role in the synthesis of capsule polysaccharides. *J Biol Chem* 2008;**283**:14327–34.
165. Krieger SE, Zeisel MB, Davis C, et al. Inhibition of hepatitis C virus infection by anti-claudin-1 antibodies is mediated by neutralization of E2-CD81-claudin-1 associations. *Hepatology* 2010;**51**:1144–57.
177. Lin JS, Szaba FM, Kummer LW, et al. *Yersinia pestis* YopE contains a dominant CD8 T cell epitope that confers protection in a mouse model of pneumonic plague. *J Immunol* 2011;**187**: 897–904.
184. Mapingire OS, Henderson NS, Duret G, et al. Modulating effects of the plug, helix, and N- and C-terminal domains on channel properties of the PapC usher. *J Biol Chem* 2009;**284**: 36324–33.
194. Mee CJ, Farquhar MJ, Harris HJ, et al. Hepatitis C virus infection reduces hepatocellular polarity in a vascular endothelial growth factor–dependent manner. *Gastroenterology* 2010;**138**:1134–42.
195. Mee CJ, Harris HJ, Farquhar MJ, et al. Polarization restricts hepatitis C virus entry into HepG2 hepatoma cells. *J Virol* 2009;**83**:6211–21.
197. Meng G, Spahich N, Kenjale R, et al. Crystal structure of the *Haemophilus influenzae* Hap adhesin reveals an intercellular oligomerization mechanism for bacterial aggregation. *EMBO J* 2011;**30**:3864–74.
198. Meng G, St Geme JW III, Waksman G. Repetitive architecture of the *Haemophilus influenzae* Hia trimeric autotransporter. *J Mol Biol* 2008;**384**:824–36.
211. Nicoll MP, Proenca JT, Efstathiou S. The molecular basis of herpes simplex virus latency. *FEMS Microbiol Rev* 2012;**36**: 684–705.
239. Pietschmann T. Virology: final entry key for hepatitis C. *Nature* 2009;**457**:797–8.
240. Piroth L, Que YA, Widmer E, et al. The fibrinogen- and fibronectin-binding domains of *Staphylococcus aureus* fibronectin-binding protein A synergistically promote endothelial invasion and experimental endocarditis. *Infect Immun* 2008;**76**:3824–31.
241. Ploss A, Evans MJ, Gaysinskaya VA, et al. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009;**457**:882–6.
250. Reilly MC, Levery SB, Castle SA, et al. A novel xylosylphosphotransferase activity discovered in *Cryptococcus neoformans*. *J Biol Chem* 2009;**284**:36118–27.
252. Remaut H, Tang C, Henderson NS, et al. Fiber formation across the bacterial outer membrane by the chaperone/usher pathway. *Cell* 2008;**133**:640–52.
259. Ruchaud-Sparagano MH, Muhlen S, Dean P, et al. The enteropathogenic *E. coli* (EPEC) Tir effector inhibits NF-κB activity by targeting TNFα receptor–associated factors. *PLoS Pathog* 2011;**7**:e1002414.
269. Silva S, Negri M, Henriques M, et al. Adherence and biofilm formation of non-*Candida albicans Candida* species. *Trends Microbiol* 2011;**19**:241–7.
276. Smith K, Humphreys D, Hume PJ, et al. Enteropathogenic *Escherichia coli* recruits the cellular inositol phosphatase SHIP2 to regulate actin-pedestal formation. *Cell Host Microbe* 2010;**7**: 13–24.
278. Speziale P, Pietrocola G, Rindi S, et al. Structural and functional role of *Staphylococcus aureus* surface components recognizing adhesive matrix molecules of the host. *Future Microbiol* 2009;**4**: 1337–52.
281. St Geme JW III, Yeo HJ. A prototype two-partner secretion pathway: the *Haemophilus influenzae* HMW1 and HMW2 adhesin systems. *Trends Microbiol* 2009;**17**:355–60.
285. Starkey M, Hickman JH, Ma L, et al. *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. *J Bacteriol* 2009;**191**: 3492–503.
301. Timpe JM, Stamataki Z, Jennings A, et al. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 2008;**47**:17–24.
305. Umbach JL, Kramer MF, Jurak I, et al. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature* 2008;**454**:780–3.
319. Weiss SM, Ladwein M, Schmidt D, et al. IRSp53 links the enterohemorrhagic *E. coli* effectors Tir and EspFU for actin pedestal formation. *Cell Host Microbe* 2009;**5**:244–58.
323. Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* α-hemolysin-mediated cellular injury. *Proc Natl Acad Sci U S A* 2010;**107**:13473–8.
325. Wilson JR, de Sessions PF, Leon MA, et al. West Nile virus nonstructural protein 1 inhibits TLR3 signal transduction. *J Virol* 2008;**82**:8262–71.
335. Yi Y, Ma Y, Gao F, et al. Crystal structure of EHEC intimin: insights into the complementarity between EPEC and EHEC. *PLoS One* 2010;**5**:e15285.
342. Zhang Y, Mena P, Romanov G, et al. A protective epitope in type III effector YopE is a major CD8 T cell antigen during primary infection with *Yersinia pseudotuberculosis*. *Infect Immun* 2012;**80**:206–14.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abrahams GL, Hensel M. Manipulating cellular transport and immune responses: dynamic interactions between intracellular *Salmonella enterica* and its host cells. *Cell Microbiol* 2006;**8**: 728–37.
2. Acheson DW, Moore R, De Breucker S, et al. Translocation of Shiga toxin across polarized intestinal cells in tissue culture. *Infect Immun* 1996;**64**:3294–300.
3. Aepfelbacher M, Roppenser B, Hentschke M, et al. Activity modulation of the bacterial Rho GAP YopE: an inspiration for the investigation of mammalian Rho GAPs. *Eur J Cell Biol* 2011;**90**:951–4.
4. Åkesson P, Sjoholm AG, Bjorck L. Protein SIC, a novel extracellular protein of *Streptococcus pyogenes* interfering with complement function. *J Biol Chem* 1996;**271**:1081–8.
5. Altier C. Genetic and environmental control of *Salmonella* invasion. *J Microbiol* 2005;**43 Spec No**:85–92.
6. Anderson GG, Palermo JJ, Schilling JD, et al. Intracellular bacterial biofilm-like pods in urinary tract infections. *Science* 2003;**301**:105–7.
7. Balcewicz-Sablinska MK, Keane J, Kornfeld H, et al. Pathogenic *Mycobacterium tuberculosis* evades apoptosis of host macrophages by release of TNF-R2, resulting in inactivation of TNF-α. *J Immunol* 1998;**161**:2636–41.
8. Barenkamp SJ, St Geme JW III. Genes encoding high-molecular-weight adhesion proteins of nontypeable *Haemophilus influenzae* are part of gene clusters. *Infect Immun* 1994;**62**:3320–8.
9. Barki M, Koltin Y, Yanko M, et al. Isolation of a *Candida albicans* DNA sequence conferring adhesion and aggregation on *Saccharomyces cerevisiae*. *J Bacteriol* 1993;**175**:5683–9.
10. Bergelson JM, Cunningham JA, Droguett G, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997;**275**:1320–3.
11. Birmingham CL, Canadien V, Kaniuk NA, et al. Listeriolysin O allows *Listeria monocytogenes* replication in macrophage vacuoles. *Nature* 2008;**451**:350–4.
12. Black DS, Bliska JB. Identification of p130Cas as a substrate of *Yersinia* YopH (Yop51), a bacterial protein tyrosine phosphatase that translocates into mammalian cells and targets focal adhesions. *EMBO J* 1997;**16**:2730–44.
13. Blanc-Potard AB, Groisman EA. The *Salmonella selC* locus contains a pathogenicity island mediating intramacrophage survival. *EMBO J* 1997;**16**:5376–85.
14. Blanc-Potard AB, Lafay B. MgtC as a horizontally-acquired virulence factor of intracellular bacterial pathogens: evidence from molecular phylogeny and comparative genomics. *J Mol Evol* 2003;**57**:479–86.
15. Blanc-Potard AB, Solomon F, Kayser J, et al. The SPI-3 pathogenicity island of *Salmonella enterica*. *J Bacteriol* 1999; **181**:998–1004.
16. Bliska JB. Yop effectors of *Yersinia* spp. and actin rearrangements. *Trends Microbiol* 2000;**8**:205–8.
17. Bliska JB, Falkow S. Bacterial resistance to complement killing mediated by the Ail protein of *Yersinia enterocolitica*. *Proc Natl Acad Sci U S A* 1992;**89**:3561–5.
18. Blocker A, Gounon P, Larquet E, et al. The tripartite type III secreton of *Shigella flexneri* inserts IpaB and IpaC into host membranes. *J Cell Biol* 1999;**147**:683–93.
19. Boulanger MJ, Murphy ME. Crystal structure of the soluble domain of the major anaerobically induced outer membrane protein (AniA) from pathogenic *Neisseria*: a new class of copper-containing nitrite reductases. *J Mol Biol* 2002;**315**:1111–27.
20. Boyd AP, Cornelis GR. Yersinia. In: Groisman EA, editor. *Principles of bacterial pathogenesis*. San Diego: Academic Press; 2001. p. 227–64.
21. Bradway SD, Levine MJ. Do proline-rich proteins modulate a transglutaminase catalyzed mechanism of candidal adhesion? *Crit Rev Oral Biol Med* 1993;**4**:293–9.
22. Braun L, Nato F, Payrastre B, et al. The 213-amino-acid leucine-rich repeat region of the *Listeria monocytogenes* InlB protein is sufficient for entry into mammalian cells, stimulation of PI 3-kinase and membrane ruffling. *Mol Microbiol* 1999;**34**:10–23.
23. Breimer ME, Karlsson KA. Chemical and immunological identification of glycolipid-based blood group ABH and Lewis antigens in human kidney. *Biochim Biophys Acta* 1983;**755**:170–7.
24. Brown NF, Vallance BA, Coombes BK, et al. *Salmonella* pathogenicity island 2 is expressed prior to penetrating the intestine. *PLoS Pathog* 2005;**1**:e32.
25. Bu S, Li Y, Zhou M, et al. Interaction between two putative glycosyltransferases is required for glycosylation of a serine-rich streptococcal adhesin. *J Bacteriol* 2008;**190**:1256–66.
26. Buchmeier N, Blanc-Potard A, Ehrt S, et al. A parallel intraphagosomal survival strategy shared by *Mycobacterium tuberculosis* and *Salmonella enterica*. *Mol Microbiol* 2000;**35**:1375–82.
27. Burnette-Curley D, Wells V, Viscount H, et al. FimA, a major virulence factor associated with *Streptococcus parasanguis* endocarditis. *Infect Immun* 1995;**63**:4669–74.
28. Cameron LA, Footer MJ, van Oudenaarden A, et al. Motility of ActA protein-coated microspheres driven by actin polymerization. *Proc Natl Acad Sci U S A* 1999;**96**:4908–13.
29. Cardinale JA, Clark VL. Expression of AniA, the major anaerobically induced outer membrane protein of *Neisseria gonorrhoeae*, provides protection against killing by normal human sera. *Infect Immun* 2000;**68**:4368–9.
30. Castric P, Cassels FJ, Carlson RW. Structural characterization of the *Pseudomonas aeruginosa* 1244 pilin glycan. *J Biol Chem* 2001;**276**:26479–85.
31. Catanese MT, Graziani R, von Hahn T, et al. High-avidity monoclonal antibodies against the human scavenger class B type I receptor efficiently block hepatitis C virus infection in the presence of high-density lipoprotein. *J Virol* 2007;**81**:8063–71.
32. Chen LM, Hobbie S, Galan JE. Requirement of Cdc42 for *Salmonella*-induced cytoskeletal and nuclear responses. *Science* 1996;**274**:2115–18.
33. Christensen LD, van Gennip M, Jakobsen TH, et al. Synergistic antibacterial efficacy of early combination treatment with tobramycin and quorum-sensing inhibitors against *Pseudomonas aeruginosa* in an intraperitoneal foreign-body infection mouse model. *J Antimicrob Chemother* 2012;**67**:1198–206.
34. Cirillo DM, Valdivia RH, Monack DM, et al. Macrophage-dependent induction of the *Salmonella* pathogenicity island 2 type III secretion system and its role in intracellular survival. *Mol Microbiol* 1998;**30**:175–88.
35. Clantin B, Delattre AS, Rucktooa P, et al. Structure of the membrane protein FhaC: a member of the Omp85-TpsB transporter superfamily. *Science* 2007;**317**:957–61.
36. Clantin B, Hodak H, Willery E, et al. The crystal structure of filamentous hemagglutinin secretion domain and its implications for the two-partner secretion pathway. *Proc Natl Acad Sci U S A* 2004;**101**:6194–9.
37. Cochrane DM, Brown MR, Anwar H, et al. Antibody response to *Pseudomonas aeruginosa* surface protein antigens in a rat model of chronic lung infection. *J Med Microbiol* 1988;**27**:255–61.
38. Cohen CJ, Shieh JT, Pickles RJ, et al. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci U S A* 2001;**98**:15191–6.
39. Collazo CM, Galan JE. The invasion-associated type III system of *Salmonella typhimurium* directs the translocation of Sip proteins into the host cell. *Mol Microbiol* 1997;**24**:747–56.
40. Collier-Hyams LS, Zeng H, Sun J, et al. Cutting edge: *Salmonella* AvrA effector inhibits the key proinflammatory, anti-apoptotic NF-κB pathway. *J Immunol* 2002;**169**:2846–50.
41. Coombes BK, Finlay BB. Insertion of the bacterial type III translocon: not your average needle stick. *Trends Microbiol* 2005;**13**:92–5.
42. Cope LD, Yogev R, Muller-Eberhard U, et al. A gene cluster involved in the utilization of both free heme and heme:hemopexin by *Haemophilus influenzae* type b. *J Bacteriol* 1995;**177**:2644–53.
43. Corn PG, Anders J, Takala AK, et al. Genes involved in *Haemophilus influenzae* type b capsule expression are frequently amplified. *J Infect Dis* 1993;**167**:356–64.
44. Cornelis GR, Biot T, Lambert de Rouvroit C, et al. The *Yersinia yop* regulon. *Mol Microbiol* 1989;**3**:1455–9.
45. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;**284**:1318–22.
46. Cotter PA, Miller JF. Bordetella. In: Groisman EA, editor. *Principles of bacterial pathogenesis*. San Diego: Academic Press; 2001. p. 619–74.
47. Cotter SE, Surana NK, Grass S, et al. Trimeric autotransporters require trimerization of the passenger domain for stability and adhesive activity. *J Bacteriol* 2006;**188**:5400–7.

48. Cotter SE, Surana NK, St Geme JW III. Trimeric autotransporters: a distinct subfamily of autotransporter proteins. *Trends Microbiol* 2005;**13**:199–205.
49. Coyne CB, Shen L, Turner JR, et al. Coxsackievirus entry across epithelial tight junctions requires occludin and the small GTPases Rab34 and Rab5. *Cell Host Microbe* 2007;**2**:181–92.
50. Csoka TB, Frost GI, Stern R. Hyaluronidases in tissue invasion. *Invasion Metastasis* 1997;**17**:297–311.
51. Cui C, Griffiths A, Li G, et al. Prediction and identification of herpes simplex virus 1-encoded microRNAs. *J Virol* 2006;**80**: 5499–508.
52. Cutter D, Mason KW, Howell AP, et al. Immunization with *Haemophilus influenzae* Hap adhesin protects against nasopharyngeal colonization in experimental mice. *J Infect Dis* 2002;**186**: 1115–21.
53. Dabiri GA, Sanger JM, Portnoy DA, et al. *Listeria monocytogenes* moves rapidly through the host-cell cytoplasm by inducing directional actin assembly. *Proc Natl Acad Sci U S A* 1990;**87**:6068–72.
54. Davies DG, Geesey GG. Regulation of the alginate biosynthesis gene *algC* in *Pseudomonas aeruginosa* during biofilm development in continuous culture. *Appl Environ Microbiol* 1995;**61**:860–7.
55. Davies DG, Parsek MR, Pearson JP, et al. The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* 1998;**280**:295–8.
56. de Groot JC, Schluter K, Carius Y, et al. Structural basis for complex formation between human IRSp53 and the translocated intimin receptor Tir of enterohemorrhagic *E. coli*. *Structure* 2011;**19**:1294–306.
57. de Roda Husman AM, Schuitemaker H. Chemokine receptors and the clinical course of HIV-1 infection. *Trends Microbiol* 1998;**6**:244–9.
58. De Sordi L, Muhlschlegel FA. Quorum sensing and fungal-bacterial interactions in *Candida albicans*: a communicative network regulating microbial coexistence and virulence. *FEMS Yeast Res* 2009;**9**:990–9.
59. Dhakal BK, Mulvey MA. The UPEC pore-forming toxin α-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways. *Cell Host Microbe* 2012;**11**:58–69.
60. Dodson KW, Jacob-Dubuisson F, Striker RT, et al. Outer-membrane PapC molecular usher discriminately recognizes periplasmic chaperone-pilus subunit complexes. *Proc Natl Acad Sci U S A* 1993;**90**:3670–4.
61. Dodson KW, Pinkner JS, Rose T, et al. Structural basis of the interaction of the pyelonephritic *E. coli* adhesin to its human kidney receptor. *Cell* 2001;**105**:733–43.
62. Doehle BP, Hladik F, McNevin JP, et al. Human immunodeficiency virus type 1 mediates global disruption of innate antiviral signaling and immune defenses within infected cells. *J Virol* 2009;**83**:10395–405.
63. Doering TL. How sweet it is! Cell wall biogenesis and polysaccharide capsule formation in *Cryptococcus neoformans*. *Annu Rev Microbiol* 2009;**63**:223–47.
64. Domenighini M, Relman D, Capiau C, et al. Genetic characterization of *Bordetella pertussis* filamentous haemagglutinin: a protein processed from an unusually large precursor. *Mol Microbiol* 1990;**4**:787–800.
65. Donelan NR, Dauber B, Wang X, et al. The N- and C-terminal domains of the NS1 protein of influenza B virus can independently inhibit IRF-3 and β-interferon promoter activation. *J Virol* 2004;**78**:11574–82.
66. Donnenberg MS, Kaper JB, Finlay BB. Interactions between enteropathogenic *Escherichia coli* and host epithelial cells. *Trends Microbiol* 1997;**5**:109–14.
67. Doranz BJ, Rucker J, Yi Y, et al. A dual-tropic primary HIV-1 isolate that uses fusin and the beta-chemokine receptors CKR-5, CKR-3, and CKR-2b as fusion cofactors. *Cell* 1996;**85**:1149–58.
68. Dramsi S, Caliot E, Bonne I, et al. Assembly and role of pili in group B streptococci. *Mol Microbiol* 2006;**60**:1401–13.
69. Egile C, d'Hauteville H, Parsot C, et al. SopA, the outer membrane protease responsible for polar localization of IcsA in *Shigella flexneri*. *Mol Microbiol* 1997;**23**:1063–73.
70. Eitel J, Dersch P. The YadA protein of *Yersinia pseudotuberculosis* mediates high-efficiency uptake into human cells under environmental conditions in which invasin is repressed. *Infect Immun* 2002;**70**:4880–91.
71. El Tahir Y, Skurnik M. YadA, the multifaceted *Yersinia* adhesin. *Int J Med Microbiol* 2001;**291**:209–18.
72. Ene IV, Bennett RJ. Hwp1 and related adhesins contribute to both mating and biofilm formation in *Candida albicans*. *Eukaryot Cell* 2009;**8**:1909–13.
73. Evans MJ, von Hahn T, Tscherne DM, et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007;**446**:801–5.
74. Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein–coupled receptor. *Science* 1996;**272**:872–7.
75. Fenno JC, Shaikh A, Spatafora G, et al. The *fimA* locus of *Streptococcus parasanguis* encodes an ATP-binding membrane transport system. *Mol Microbiol* 1995;**15**:849–63.
76. Fink DL, Buscher AZ, Green B, et al. The *Haemophilus influenzae* Hap autotransporter mediates microcolony formation and adherence to epithelial cells and extracellular matrix via binding regions in the C-terminal end of the passenger domain. *Cell Microbiol* 2003;**5**:175–86.
77. Fink DL, Green BA, St Geme JW III. The *Haemophilus influenzae* Hap autotransporter binds to fibronectin, laminin, and collagen IV. *Infect Immun* 2002;**70**:4902–7.
78. Fischetti VA. Streptococcal M protein: molecular design and biological behavior. *Clin Microbiol Rev* 1989;**2**:285–314.
79. Foster TJ, Höök M. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol* 1998;**6**:484–8.
80. Fouchier RA, Broersen SM, Brouwer M, et al. Temporal relationship between elongation of the HIV type 1 glycoprotein 120 V2 domain and the conversion toward a syncytium-inducing phenotype. *AIDS Res Hum Retroviruses* 1995;**11**:1473–8.
81. Fouchier RA, Groenink M, Kootstra NA, et al. Phenotype-associated sequence variation in the third variable domain of the human immunodeficiency virus type 1 gp120 molecule. *J Virol* 1992;**66**:3183–7.
82. Fox KL, Yildirim HH, Deadman ME, et al. Novel lipopolysaccharide biosynthetic genes containing tetranucleotide repeats in *Haemophilus influenzae*, identification of a gene for adding O-acetyl groups. *Mol Microbiol* 2005;**58**:207–16.
83. Foy E, Li K, Wang C, et al. Regulation of interferon regulatory factor-3 by the hepatitis C virus serine protease. *Science* 2003; **300**:1145–8.
84. Fsihi H, Steffen P, Cossart P. Listeria monocytogenes. In: Groisman EA, editor. *Principles of bacterial pathogenesis*. San Diego: Academic Press; 2001. p. 751–803.
85. Fu Y, Galan JE. A *Salmonella* protein antagonizes Rac-1 and Cdc42 to mediate host-cell recovery after bacterial invasion. *Nature* 1999;**401**:293–7.
86. Galan JE. Molecular genetic bases of *Salmonella* entry into host cells. *Mol Microbiol* 1996;**20**:263–71.
87. Galan JE, Wolf-Watz H. Protein delivery into eukaryotic cells by type III secretion machines. *Nature* 2006;**444**:567–73.
88. Gale C, Finkel D, Tao N, et al. Cloning and expression of a gene encoding an integrin-like protein in *Candida albicans*. *Proc Natl Acad Sci U S A* 1996;**93**:357–61.
89. Gale CA, Bendel CM, McClellan M, et al. Linkage of adhesion, filamentous growth, and virulence in *Candida albicans* to a single gene, *INT1*. *Science* 1998;**279**:1355–8.
90. Galloway WR, Hodgkinson JT, Bowden SD, et al. Quorum sensing in Gram-negative bacteria: small-molecule modulation of AHL and AI-2 quorum sensing pathways. *Chem Rev* 2011; **111**:28–67.
91. Ganesh VK, Rivera JJ, Smeds E, et al. A structural model of the *Staphylococcus aureus* ClfA-fibrinogen interaction opens new avenues for the design of anti-staphylococcal therapeutics. *PLoS Pathog* 2008;**4**:e1000226.
92. Gao L, Abu Kwaik Y. Hijacking of apoptotic pathways by bacterial pathogens. *Microbes Infect* 2000;**2**:1705–19.
93. Gedde MM, Higgins DE, Tilney LG, et al. Role of listeriolysin O in cell-to-cell spread of *Listeria monocytogenes*. *Infect Immun* 2000;**68**:999–1003.
94. Giron JA, Ho AS, Schoolnik GK. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. *Science* 1991;**254**: 710–13.
95. Glass WG, Lim JK, Cholera R, et al. Chemokine receptor CCR5 promotes leukocyte trafficking to the brain and survival in West Nile virus infection. *J Exp Med* 2005;**202**:1087–98.

96. Glass WG, McDermott DH, Lim JK, et al. CCR5 deficiency increases risk of symptomatic West Nile virus infection. *J Exp Med* 2006;**203**:35–40.
97. Goldberg MB, Barzu O, Parsot C, et al. Unipolar localization and ATPase activity of IcsA, a *Shigella flexneri* protein involved in intracellular movement. *Infect Agents Dis* 1993;**2**:210–11.
98. Goldman WE, Klapper DG, Baseman JB. Detection, isolation, and analysis of a released *Bordetella pertussis* product toxic to cultured tracheal cells. *Infect Immun* 1982;**36**:782–94.
99. Griffin R, Cox AD, Makepeace K, et al. Elucidation of the monoclonal antibody 5G8-reactive, virulence-associated lipopolysaccharide epitope of *Haemophilus influenzae* and its role in bacterial resistance to complement-mediated killing. *Infect Immun* 2005;**73**:2213–21.
100. Groenink M, Fouchier RA, Broersen S, et al. Relation of phenotype evolution of HIV-1 to envelope V2 configuration. *Science* 1993;**260**:1513–16.
101. Grundling A, Gonzalez MD, Higgins DE. Requirement of the *Listeria monocytogenes* broad-range phospholipase PC-PLC during infection of human epithelial cells. *J Bacteriol* 2003;**185**: 6295–307.
102. Gulig PA, Patrick CC, Hermanstorfer L, et al. Conservation of epitopes in the oligosaccharide portion of the lipooligosaccharide of *Haemophilus influenzae* type b. *Infect Immun* 1987;**55**: 513–20.
103. Gupta A, Gartner JJ, Sethupathy P, et al. Anti-apoptotic function of a microRNA encoded by the HSV-1 latency-associated transcript. *Nature* 2006;**442**:82–5.
104. Hagblom P, Segal E, Billyard E, et al. Intragenic recombination leads to pilus antigenic variation in *Neisseria gonorrhoeae*. *Nature* 1985;**315**:156–8.
105. Hall A. Rho GTPases and the actin cytoskeleton. *Science* 1998;**279**:509–14.
106. Hanski E, Caparon M. Protein F, a fibronectin-binding protein, is an adhesin of the group A streptococcus *Streptococcus pyogenes*. *Proc Natl Acad Sci U S A* 1992;**89**:6172–6.
107. Hardt WD, Chen LM, Schuebel KE, et al. *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* 1998;**93**:815–26.
108. Harris HJ, Davis C, Mullins JG, et al. Claudin association with CD81 defines hepatitis C virus entry. *J Biol Chem* 2010;**285**:21092–102.
109. Haussler S. Multicellular signalling and growth of *Pseudomonas aeruginosa*. *Int J Med Microbiol* 2010;**300**:544–8.
110. Haussler S, Ziegler I, Lottel A, et al. Highly adherent small-colony variants of *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *J Med Microbiol* 2003;**52**:295–301.
111. Heiss LN, Flak TA, Lancaster JR Jr, et al. Nitric oxide mediates *Bordetella pertussis* tracheal cytotoxin damage to the respiratory epithelium. *Infect Agents Dis* 1993;**2**:173–7.
112. Heiss LN, Lancaster JR Jr, Corbett JA, et al. Epithelial autotoxicity of nitric oxide: role in the respiratory cytopathology of pertussis. *Proc Natl Acad Sci U S A* 1994;**91**:267–70.
113. Heiss LN, Moser SA, Unanue ER, et al. Interleukin-1 is linked to the respiratory epithelial cytopathology of pertussis. *Infect Immun* 1993;**61**:3123–8.
114. Henderson IR, Nataro JP. Virulence functions of autotransporter proteins. *Infect Immun* 2001;**69**:1231–43.
115. Hendrixson DR, St Geme JW III. The *Haemophilus influenzae* Hap serine protease promotes adherence and microcolony formation, potentiated by a soluble host protein. *Mol Cell* 1998;**2**: 841–50.
116. Hensel M, Shea JE, Raupach B, et al. Functional analysis of *ssaJ* and the *ssaK/U* operon, 13 genes encoding components of the type III secretion apparatus of *Salmonella* pathogenicity island 2. *Mol Microbiol* 1997;**24**:155–67.
117. Hersh D, Monack DM, Smith MR, et al. The *Salmonella* invasin SipB induces macrophage apoptosis by binding to caspase-1. *Proc Natl Acad Sci U S A* 1999;**96**:2396–401.
118. Hewlett EL. Pertussis: current concepts of pathogenesis and prevention. *Pediatr Infect Dis J* 1997;**16**:S78–84.
119. Hewlett EL. A commentary on the pathogenesis of pertussis. *Clin Infect Dis* 1999;**28**:S94–98.
120. Hilbi H, Moss JE, Hersh D, et al. *Shigella*-induced apoptosis is dependent on caspase-1 which binds to IpaB. *J Biol Chem* 1998;**273**:32895–900.
121. Hobbs M, Collie ES, Free PD, et al. PilS and PilR, a two-component transcriptional regulatory system controlling expression of type 4 fimbriae in *Pseudomonas aeruginosa*. *Mol Microbiol* 1993;**7**:669–82.
122. Hodak H, Clantin B, Willery E, et al. Secretion signal of the filamentous haemagglutinin, a model two-partner secretion substrate. *Mol Microbiol* 2006;**61**:368–82.
123. Hoe NP, Kordari P, Cole R, et al. Human immune response to streptococcal inhibitor of complement, a serotype M1 group A *Streptococcus* extracellular protein involved in epidemics. *J Infect Dis* 2000;**182**:1425–36.
124. Hoe NP, Nakashima K, Lukomski S, et al. Rapid selection of complement-inhibiting protein variants in group A *Streptococcus* epidemic waves. *Nat Med* 1999;**5**:924–9.
125. Honda T, Saitoh H, Masuko M, et al. The coxsackievirus-adenovirus receptor protein as a cell adhesion molecule in the developing mouse brain. *Brain Res Mol Brain Res* 2000;**77**:19–28.
126. Hood DW, Deadman ME, Allen T, et al. Use of the complete genome sequence information of *Haemophilus influenzae* strain Rd to investigate lipopolysaccharide biosynthesis. *Mol Microbiol* 1996;**22**:951–65.
127. Hornby JM, Jensen EC, Lisec AD, et al. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. *Appl Environ Microbiol* 2001;**67**:2982–92.
128. Hostetter MK. Adhesins and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. *Clin Microbiol Rev* 1994;**7**:29–42.
129. Hostetter MK. Linkage of adhesion, morphogenesis, and virulence in *Candida albicans*. *J Lab Clin Med* 1998;**132**:258–63.
130. Howell-Adams B, Seifert HS. Molecular models accounting for the gene conversion reactions mediating gonococcal pilin antigenic variation. *Mol Microbiol* 2000;**37**:1146–58.
131. Huang Y, Paxton WA, Wolinsky SM, et al. The role of a mutant CCR5 allele in HIV-1 transmission and disease progression. *Nat Med* 1996;**2**:1240–3.
132. Hung DL, Hultgren SJ. Pilus biogenesis via the chaperone/usher pathway: an integration of structure and function. *J Struct Biol* 1998;**124**:201–20.
133. Hung DL, Knight SD, Woods RM, et al. Molecular basis of two subfamilies of immunoglobulin-like chaperones. *EMBO J* 1996;**15**:3792–805.
134. Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. *Annu Rev Microbiol* 2010;**64**:203–21.
135. Hynes WL, Dixon AR, Walton SL, et al. The extracellular hyaluronidase gene (*hylA*) of *Streptococcus pyogenes*. *FEMS Microbiol Lett* 2000;**184**:109–12.
136. Ibarra JA, Steele-Mortimer O. *Salmonella*—the ultimate insider. *Salmonella* virulence factors that modulate intracellular survival. *Cell Microbiol* 2009;**11**:1579–86.
137. Inoshima I, Inoshima N, Wilke GA, et al. A *Staphylococcus aureus* pore-forming toxin subverts the activity of ADAM10 to cause lethal infection in mice. *Nat Med* 2011;**17**:1310–14.
138. Isberg RR. Uptake of enteropathogenic *Yersinia* by mammalian cells. *Curr Top Microbiol Immunol* 1996;**209**:1–24.
139. Isberg RR, Leong JM. Multiple β1 chain integrins are receptors for invasin, a protein that promotes bacterial penetration into mammalian cells. *Cell* 1990;**60**:861–71.
140. Ishibashi Y, Claus S, Relman DA. *Bordetella pertussis* filamentous hemagglutinin interacts with a leukocyte signal transduction complex and stimulates bacterial adherence to monocyte CR3 (CD11b/CD18). *J Exp Med* 1994;**180**:1225–33.
141. Ito M, Kodama M, Masuko M, et al. Expression of coxsackievirus and adenovirus receptor in hearts of rats with experimental autoimmune myocarditis. *Circ Res* 2000;**86**:275–80.
142. Iwai H, Kim M, Yoshikawa Y, et al. A bacterial effector targets Mad2L2, an APC inhibitor, to modulate host cell cycling. *Cell* 2007;**130**:611–23.
143. Izore T, Job V, Dessen A. Biogenesis, regulation, and targeting of the type III secretion system. *Structure* 2011;**19**:603–12.
144. Jacob-Dubuisson F, Heuser J, Dodson K, et al. Initiation of assembly and association of the structural elements of a bacterial pilus depend on two specialized tip proteins. *EMBO J* 1993;**12**:837–47.
145. Jain S, van Ulsen P, Benz I, et al. Polar localization of the autotransporter family of large bacterial virulence proteins. *J Bacteriol* 2006;**188**:4841–50.

146. Jinno A, Shimizu N, Soda Y, et al. Identification of the chemokine receptor TER1/CCR8 expressed in brain-derived cells and T cells as a new coreceptor for HIV-1 infection. *Biochem Biophys Res Commun* 1998;**243**:497–502.
147. Jones CH, Pinkner JS, Roth R, et al. FimH adhesin of type 1 pili is assembled into a fibrillar tip structure in the Enterobacteriaceae. *Proc Natl Acad Sci U S A* 1995;**92**:2081–5.
148. Jonsson AB, Nyberg G, Normark S. Phase variation of gonococcal pili by frameshift mutation in *pilC*, a novel gene for pilus assembly. *EMBO J* 1991;**10**:477–88.
149. Julio SM, Inatsuka CS, Mazar J, et al. Natural-host animal models indicate functional interchangeability between the filamentous haemagglutinins of *Bordetella pertussis* and *Bordetella bronchiseptica* and reveal a role for the mature C-terminal domain, but not the RGD motif, during infection. *Mol Microbiol* 2009;**71**:1574–90.
150. Justice SS, Hung C, Theriot JA, et al. Differentiation and developmental pathways of uropathogenic *Escherichia coli* in urinary tract pathogenesis. *Proc Natl Acad Sci U S A* 2004;**101**:1333–8.
151. Kajava AV, Cheng N, Cleaver R, et al. Beta-helix model for the filamentous haemagglutinin adhesin of *Bordetella pertussis* and related bacterial secretory proteins. *Mol Microbiol* 2001;**42**: 279–92.
152. Kallewaard NL, Zhang L, Chen JW, et al. Tissue-specific deletion of the coxsackievirus and adenovirus receptor protects mice from virus-induced pancreatitis and myocarditis. *Cell Host Microbe* 2009;**6**:91–8.
153. Kapadia SB, Barth H, Baumert T, et al. Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. *J Virol* 2007;**81**: 374–83.
154. Kau AL, Hunstad DA, Hultgren SJ. Interaction of uropathogenic *Escherichia coli* with host uroepithelium. *Curr Opin Microbiol* 2005;**8**:54–9.
155. Kaufman MR, Seyer JM, Taylor RK. Processing of TCP pilin by TcpJ typifies a common step intrinsic to a newly recognized pathway of extracellular protein secretion by gram-negative bacteria. *Genes Dev* 1991;**5**:1834–46.
156. Keane FM, Clarke AW, Foster TJ, et al. The N-terminal A domain of *Staphylococcus aureus* fibronectin-binding protein A binds to tropoelastin. *Biochemistry* 2007;**46**:7226–32.
157. Keane J, Balcewicz-Sablinska MK, Remold HG, et al. Infection by *Mycobacterium tuberculosis* promotes human alveolar macrophage apoptosis. *Infect Immun* 1997;**65**:298–304.
158. Kenny B, DeVinney R, Stein M, et al. Enteropathogenic *E. coli* (EPEC) transfers its receptor for intimate adherence into mammalian cells. *Cell* 1997;**91**:511–20.
159. Kerr JR, Matthews RC. *Bordetella pertussis* infection: pathogenesis, diagnosis, management, and the role of protective immunity. *Eur J Clin Microbiol Infect Dis* 2000;**19**:77–88.
160. Khelef N, Guiso N. Induction of macrophage apoptosis by *Bordetella pertussis* adenylate cyclase-hemolysin. *FEMS Microbiol Lett* 1995;**134**:27–32.
161. Khelef N, Zychlinsky A, Guiso N. *Bordetella pertussis* induces apoptosis in macrophages: role of adenylate cyclase-hemolysin. *Infect Immun* 1993;**61**:4064–71.
162. Kielian M, Rey FA. Virus membrane-fusion proteins: more than one way to make a hairpin. *Nat Rev Microbiol* 2006;**4**:67–76.
163. Kimura LH, Pearsall NN. Relationship between germination of *Candida albicans* and increased adherence to human buccal epithelial cells. *Infect Immun* 1980;**28**:464–8.
164. Klutts JS, Doering TL. Cryptococcal xylosyltransferase 1 (Cxt1p) from *Cryptococcus neoformans* plays a direct role in the synthesis of capsule polysaccharides. *J Biol Chem* 2008;**283**:14327–34.
165. Krieger SE, Zeisel MB, Davis C, et al. Inhibition of hepatitis C virus infection by anti-claudin-1 antibodies is mediated by neutralization of E2-CD81-claudin-1 associations. *Hepatology* 2010;**51**:1144–57.
166. Kubori T, Matsushima Y, Nakamura D, et al. Supramolecular structure of the *Salmonella typhimurium* type III protein secretion system. *Science* 1998;**280**:602–5.
167. Kubori T, Sukhan A, Aizawa SI, et al. Molecular characterization and assembly of the needle complex of the *Salmonella typhimurium* type III protein secretion system. *Proc Natl Acad Sci U S A* 2000;**97**:10225–30.
168. Kuehn MJ, Heuser J, Normark S, et al. P pili in uropathogenic *E. coli* are composite fibres with distinct fibrillar adhesive tips. *Nature* 1992;**356**:252–5.
169. Kuehn MJ, Normark S, Hultgren SJ. Immunoglobulin-like PapD chaperone caps and uncaps interactive surfaces of nascently translocated pilus subunits. *Proc Natl Acad Sci U S A* 1991;**88**: 10586–90.
170. Laarmann S, Cutter D, Juehne T, et al. The *Haemophilus influenzae* Hia autotransporter harbours two adhesive pockets that reside in the passenger domain and recognize the same host cell receptor. *Mol Microbiol* 2002;**46**:731–43.
171. Lauer P, Albertson NH, Koomey M. Conservation of genes encoding components of a type IV pilus assembly/two-step protein export pathway in *Neisseria gonorrhoeae*. *Mol Microbiol* 1993;**8**:357–68.
172. Lederman MM, Penn-Nicholson A, Cho M, et al. Biology of CCR5 and its role in HIV infection and treatment. *JAMA* 2006;**296**:815–26.
173. Lee J, Remold HG, Ieong MH, et al. Macrophage apoptosis in response to high intracellular burden of *Mycobacterium tuberculosis* is mediated by a novel caspase-independent pathway. *J Immunol* 2006;**176**:4267–74.
174. Li H, Qian L, Chen Z, et al. The outer membrane usher forms a twin-pore secretion complex. *J Mol Biol* 2004;**344**:1397–407.
175. Li K, Foy E, Ferreon JC, et al. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc Natl Acad Sci U S A* 2005;**102**: 2992–7.
176. Li RK, Cutler JE. Chemical definition of an epitope/adhesin molecule on *Candida albicans*. *J Biol Chem* 1993;**268**:18293–9.
177. Lin JS, Szaba FM, Kummer LW, et al. *Yersinia pestis* YopE contains a dominant CD8 T cell epitope that confers protection in a mouse model of pneumonic plague. *J Immunol* 2011;**187**:897–904.
178. Liu DF, Mason KW, Mastri M, et al. The C-terminal fragment of the internal 110-kilodalton passenger domain of the Hap protein of nontypeable *Haemophilus influenzae* is a potential vaccine candidate. *Infect Immun* 2004;**72**:6961–8.
179. Liu R, Paxton WA, Choe S, et al. Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* 1996;**86**:367–77.
180. Lukomski S, Hoe NP, Abdi I, et al. Nonpolar inactivation of the hypervariable streptococcal inhibitor of complement gene (*sic*) in serotype M1 *Streptococcus pyogenes* significantly decreases mouse mucosal colonization. *Infect Immun* 2000;**68**:535–42.
181. Ly KT, Casanova JE. Mechanisms of *Salmonella* entry into host cells. *Cell Microbiol* 2007;**9**:2103–11.
182. Lysenko E, Richards JC, Cox AD, et al. The position of phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* affects binding and sensitivity to C-reactive protein-mediated killing. *Mol Microbiol* 2000;**35**:234–45.
183. Makhov AM, Hannah JH, Brennan MJ, et al. Filamentous hemagglutinin of *Bordetella pertussis:* a bacterial adhesin formed as a 50-nm monomeric rigid rod based on a 19-residue repeat motif rich in beta strands and turns. *J Mol Biol* 1994;**241**:110–24.
184. Mapingire OS, Henderson NS, Duret G, et al. Modulating effects of the plug, helix, and N- and C-terminal domains on channel properties of the PapC usher. *J Biol Chem* 2009;**284**:36324–33.
185. Marceau M, Nassif X. Role of glycosylation at Ser63 in production of soluble pilin in pathogenic *Neisseria*. *J Bacteriol* 1999;**181**: 656–61.
186. Marcus SL, Brumell JH, Pfeifer CG, et al. *Salmonella* pathogenicity islands: big virulence in small packages. *Microbes Infect* 2000;**2**:145–56.
187. Marquis H, Doshi V, Portnoy DA. The broad-range phospholipase C and a metalloprotease mediate listeriolysin O–independent escape of *Listeria monocytogenes* from a primary vacuole in human epithelial cells. *Infect Immun* 1995;**63**:4531–4.
188. Marrs CF, Schoolnik G, Koomey JM, et al. Cloning and sequencing of a *Moraxella bovis* pilin gene. *J Bacteriol* 1985;**163**:132–9.
189. Martinez JJ, Mulvey MA, Schilling JD, et al. Type 1 pilus-mediated bacterial invasion of bladder epithelial cells. *EMBO J* 2000;**19**: 2803–12.
190. Mawhinney TP, Adelstein E, Morris DA, et al. Structure determination of five sulfated oligosaccharides derived from tracheobronchial mucus glycoproteins. *J Biol Chem* 1987;**262**:2994–3001.

191. McDaniel TK, Jarvis KG, Donnenberg MS, et al. A genetic locus of enterocyte effacement conserved among diverse enterobacterial pathogens. *Proc Natl Acad Sci U S A* 1995;**92**:1664–8.
192. McDevitt D, Francois P, Vaudaux P, et al. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. *Mol Microbiol* 1994;**11**:237–48.
193. McKinney JD, Höner zu Bentrup K, Munoz-Elias EJ, et al. Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* 2000;**406**:735–8.
194. Mee CJ, Farquhar MJ, Harris HJ, et al. Hepatitis C virus infection reduces hepatocellular polarity in a vascular endothelial growth factor-dependent manner. *Gastroenterology* 2010;**138**:1134–42.
195. Mee CJ, Harris HJ, Farquhar MJ, et al. Polarization restricts hepatitis C virus entry into HepG2 hepatoma cells. *J Virol* 2009;**83**:6211–21.
196. Mejia LM, Stockbauer KE, Pan X, et al. Characterization of group A *Streptococcus* strains recovered from Mexican children with pharyngitis by automated DNA sequencing of virulence-related genes: unexpectedly large variation in the gene (*sic*) encoding a complement-inhibiting protein. *J Clin Microbiol* 1997;**35**: 3220–4.
197. Meng G, Spahich N, Kenjale R, et al. Crystal structure of the *Haemophilus influenzae* Hap adhesin reveals an intercellular oligomerization mechanism for bacterial aggregation. *EMBO J* 2011;**30**:3864–74.
198. Meng G, St Geme JW III, Waksman G. Repetitive architecture of the *Haemophilus influenzae* Hia trimeric autotransporter. *J Mol Biol* 2008;**384**:824–36.
199. Meng G, Surana NK, St Geme JW III, et al. Structure of the outer membrane translocator domain of the *Haemophilus influenzae* Hia trimeric autotransporter. *EMBO J* 2006;**25**:2297–304.
200. Mengaud J, Ohayon H, Gounon P, et al. E-cadherin is the receptor for internalin, a surface protein required for entry of *L. monocytogenes* into epithelial cells. *Cell* 1996;**84**:923–32.
201. Menozzi FD, Mutombo R, Renauld G, et al. Heparin-inhibitable lectin activity of the filamentous hemagglutinin adhesin of *Bordetella pertussis*. *Infect Immun* 1994;**62**:769–78.
202. Menzel EJ, Farr C. Hyaluronidase and its substrate hyaluronan: biochemistry, biological activities and therapeutic uses. *Cancer Lett* 1998;**131**:3–11.
203. Meyer TF, Billyard E, Haas R, et al. Pilus genes of *Neisseria gonorrhoeae*: chromosomal organization and DNA sequence. *Proc Natl Acad Sci U S A* 1984;**81**:6110–14.
204. Mounier J, Ryter A, Coquis-Rondon M, et al. Intracellular and cell-to-cell spread of *Listeria monocytogenes* involves interaction with F-actin in the enterocyte-like cell line Caco-2. *Infect Immun* 1990;**58**:1048–58.
205. Moxon ER, Kroll JS. The role of bacterial polysaccharide capsules as virulence factors. *Curr Top Microbiol Immunol* 1990;**150**:65–85.
206. Mu XQ, Bullitt E. Structure and assembly of P pili: a protruding hinge region used for assembly of a bacterial adhesion filament. *Proc Natl Acad Sci U S A* 2006;**103**:9861–6.
207. Mukherjee S, Keitany G, Li Y, et al. *Yersinia* YopJ acetylates and inhibits kinase activation by blocking phosphorylation. *Science* 2006;**312**:1211–14.
208. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect Immun* 2001;**69**:4572–9.
209. Mysorekar IU, Hultgren SJ. Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract. *Proc Natl Acad Sci U S A* 2006;**103**:14170–5.
210. Nallapareddy SR, Singh KV, Sillanpaa J, et al. Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. *J Clin Invest* 2006;**116**:2799–807.
211. Nicoll MP, Proenca JT, Efstathiou S. The molecular basis of herpes simplex virus latency. *FEMS Microbiol Rev* 2012;**36**: 684–705.
212. Nicosia A, Rappuoli R. Promoter of the pertussis toxin operon and production of pertussis toxin. *J Bacteriol* 1987;**169**:2843–6.
213. Nobile CJ, Mitchell AP. Genetics and genomics of *Candida albicans* biofilm formation. *Cell Microbiol* 2006;**8**:1382–91.
214. Nobile CJ, Nett JE, Andes DR, et al. Function of *Candida albicans* adhesin Hwp1 in biofilm formation. *Eukaryot Cell* 2006;**5**:1604–10.
215. Noel GJ, Brittingham A, Granato AA, et al. Effect of amplification of the *Cap* b locus on complement-mediated bacteriolysis and opsonization of type b *Haemophilus influenzae*. *Infect Immun* 1996;**64**:4769–75.
216. Noel GJ, Hoiseth SK, Edelson PJ. Type b capsule inhibits ingestion of *Haemophilus influenzae* by murine macrophages: studies with isogenic encapsulated and unencapsulated strains. *J Infect Dis* 1992;**166**:178–82.
217. Noel GJ, Mosser DM, Edelson PJ. Role of complement in mouse macrophage binding of *Haemophilus influenzae* type b. *J Clin Invest* 1990;**85**:208–18.
218. Norris FA, Wilson MP, Wallis TS, et al. SopB, a protein required for virulence of *Salmonella dublin*, is an inositol phosphate phosphatase. *Proc Natl Acad Sci U S A* 1998;**95**:14057–9.
219. Novembre J, Galvani AP, Slatkin M. The geographic spread of the CCR5 Delta32 HIV-resistance allele. *PLoS Biol* 2005;**3**:e339.
220. Nunn DN, Lory S. Product of the *Pseudomonas aeruginosa* gene *pilD* is a prepilin leader peptidase. *Proc Natl Acad Sci U S A* 1991;**88**:3281–5.
221. Ochman H, Soncini FC, Solomon F, et al. Identification of a pathogenicity island required for *Salmonella* survival in host cells. *Proc Natl Acad Sci U S A* 1996;**93**:7800–4.
222. Okada N, Liszewski MK, Atkinson JP, et al. Membrane cofactor protein (CD46) is a keratinocyte receptor for the M protein of the group A streptococcus. *Proc Natl Acad Sci U S A* 1995;**92**:2489–93.
223. Okada N, Pentland AP, Falk P, et al. M protein and protein F act as important determinants of cell-specific tropism of *Streptococcus pyogenes* in skin tissue. *J Clin Invest* 1994;**94**:965–77.
224. Oligino L, Fives-Taylor P. Overexpression and purification of a fimbria-associated adhesin of *Streptococcus parasanguis*. *Infect Immun* 1993;**61**:1016–22.
225. Oomen CJ, van Ulsen P, van Gelder P, et al. Structure of the translocator domain of a bacterial autotransporter. *EMBO J* 2004;**23**:1257–66.
226. O'Toole GA, Kolter R. Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol Microbiol* 1998;**30**:295–304.
227. Ozeri V, Tovi A, Burstein I, et al. A two-domain mechanism for group A streptococcal adherence through protein F to the extracellular matrix. *EMBO J* 1996;**15**:989–98.
228. Palmer KL, Munson RS, Jr. Cloning and characterization of the genes encoding the hemolysin of *Haemophilus ducreyi*. *Mol Microbiol* 1995;**18**:821–30.
229. Parge HE, Forest KT, Hickey MJ, et al. Structure of the fibre-forming protein pilin at 2.6 Å resolution. *Nature* 1995; **378**:32–8.
230. Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci U S A* 2000;**97**:8789–93.
231. Patti JM, Bremell T, Krajewska-Pietrasik D, et al. The *Staphylococcus aureus* collagen adhesin is a virulence determinant in experimental septic arthritis. *Infect Immun* 1994;**62**:152–61.
232. Patti JM, Höök M. Microbial adhesins recognizing extracellular matrix macromolecules. *Curr Opin Cell Biol* 1994;**6**:752–8.
233. Patti JM, Jonsson H, Guss B, et al. Molecular characterization and expression of a gene encoding a *Staphylococcus aureus* collagen adhesin. *J Biol Chem* 1992;**267**:4766–72.
234. Pays E, Nolan DP. Expression and function of surface proteins in *Trypanosoma brucei*. *Mol Biochem Parasitol* 1998;**91**:3–36.
235. Pearson JP, Feldman M, Iglewski BH, et al. *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect Immun* 2000;**68**:4331–4.
236. Pearson RD, Symes P, Conboy M, et al. Inhibition of monocyte oxidative responses by *Bordetella pertussis* adenylate cyclase toxin. *J Immunol* 1987;**139**:2749–54.
237. Peters PJ, Bhattacharya J, Hibbitts S, et al. Biological analysis of human immunodeficiency virus type 1 R5 envelopes amplified from brain and lymph node tissues of AIDS patients with neuropathology reveals two distinct tropism phenotypes and identifies envelopes in the brain that confer an enhanced tropism and fusigenicity for macrophages. *J Virol* 2004;**78**:6915–26.
238. Peters PJ, Sullivan WM, Duenas-Decamp MJ, et al. Non–macrophage-tropic human immunodeficiency virus type 1 R5

envelopes predominate in blood, lymph nodes, and semen: implications for transmission and pathogenesis. *J Virol* 2006;**80**:6324–32.
239. Pietschmann T. Virology: final entry key for hepatitis C. *Nature* 2009;**457**:797–8.
240. Piroth L, Que YA, Widmer E, et al. The fibrinogen- and fibronectin-binding domains of *Staphylococcus aureus* fibronectin-binding protein A synergistically promote endothelial invasion and experimental endocarditis. *Infect Immun* 2008;**76**:3824–31.
241. Ploss A, Evans MJ, Gaysinskaya VA, et al. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009;**457**:882–6.
242. Ponnuraj K, Bowden MG, Davis S, et al. A "dock, lock, and latch" structural model for a staphylococcal adhesin binding to fibrinogen. *Cell* 2003;**115**:217–28.
243. Poole K, Schiebel E, Braun V. Molecular characterization of the hemolysin determinant of *Serratia marcescens*. *J Bacteriol* 1988;**170**:3177–88.
244. Potts WJ, Saunders JR. Nucleotide sequence of the structural gene for class I pilin from *Neisseria meningitidis:* homologies with the pilE locus of *Neisseria gonorrhoeae*. *Mol Microbiol* 1988;**2**:647–53.
245. Prasad SM, Yin Y, Rodzinski E, et al. Identification of a carbohydrate recognition domain in filamentous hemagglutinin from *Bordetella pertussis*. *Infect Immun* 1993;**61**:2780–5.
246. Preston CM. Repression of viral transcription during herpes simplex virus latency. *J Gen Virol* 2000;**81**:1–19.
247. Race PR, Lakey JH, Banfield MJ. Insertion of the enteropathogenic *Escherichia coli* Tir virulence protein into membranes in vitro. *J Biol Chem* 2006;**281**:7842–9.
248. Ram S, Mackinnon FG, Gulati S, et al. The contrasting mechanisms of serum resistance of *Neisseria gonorrhoeae* and group B *Neisseria meningitidis*. *Mol Immunol* 1999;**36**:915–28.
249. Rao VK, Progulske-Fox A. Cloning and sequencing of two type 4 (N-methylphenylalanine) pilin genes from *Eikenella corrodens*. *J Gen Microbiol* 1993;**139**:651–60.
250. Reilly MC, Levery SB, Castle SA, et al. A novel xylosylphosphotransferase activity discovered in *Cryptococcus neoformans*. *J Biol Chem* 2009;**284**:36118–27.
251. Relman D, Tuomanen E, Falkow S, et al. Recognition of a bacterial adhesion by an integrin: macrophage CR3 (alpha M beta 2, CD11b/CD18) binds filamentous hemagglutinin of *Bordetella pertussis*. *Cell* 1990;**61**:1375–82.
252. Remaut H, Tang C, Henderson NS, et al. Fiber formation across the bacterial outer membrane by the chaperone/usher pathway. *Cell* 2008;**133**:640–52.
253. Rest RF, Frangipane JV. Growth of *Neisseria gonorrhoeae* in CMP-N-acetylneuraminic acid inhibits nonopsonic (opacity-associated outer membrane protein-mediated) interactions with human neutrophils. *Infect Immun* 1992;**60**:989–97.
254. Robbins JR, Barth AI, Marquis H, et al. *Listeria monocytogenes* exploits normal host cell processes to spread from cell to cell. *J Cell Biol* 1999;**146**:1333–50.
255. Roche FM, Downer R, Keane F, et al. The N-terminal A domain of fibronectin-binding proteins A and B promotes adhesion of *Staphylococcus aureus* to elastin. *J Biol Chem* 2004;**279**:38433–40.
256. Rosenshine I, Ruschkowski S, Stein M, et al. A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation. *EMBO J* 1996;**15**:2613–24.
257. Rosini R, Rinaudo CD, Soriani M, et al. Identification of novel genomic islands coding for antigenic pilus-like structures in *Streptococcus agalactiae*. *Mol Microbiol* 2006;**61**:126–41.
258. Ross NT, Miller BL. Characterization of the binding surface of the translocated intimin receptor, an essential protein for EPEC and EHEC cell adhesion. *Protein Sci* 2007;**16**:2677–83.
259. Ruchaud-Sparagano MH, Muhlen S, Dean P, et al. The enteropathogenic *E. coli* (EPEC) Tir effector inhibits NF-κB activity by targeting TNFα receptor-associated factors. *PLoS Pathog* 2011;**7**:e1002414.
260. Rudel T, Scheurerpflug I, Meyer TF. *Neisseria* PilC protein identified as type 4 pilus tip-located adhesin. *Nature* 1995;**373**:357–9.
261. Russell DG. What does "inhibition of phagosome-lysosome fusion" really mean? *Trends Microbiol* 1998;**6**:212–14.
262. Sandvig K, van Deurs B. Entry of ricin and Shiga toxin into cells: molecular mechanisms and medical perspectives. *EMBO J* 2000;**19**:5943–50.
263. Sauer FG, Fütterer K, Pinkner JS, et al. Structural basis of chaperone function and pilus biogenesis. *Science* 1999;**285**:1058–61.
264. Sauer FG, Knight SD, Waksman, et al. PapD-like chaperones and pilus biogenesis. *Semin Cell Dev Biol* 2000;**11**:27–34.
265. Sharma V, Sharma S, Höner zu Bentrup K, et al. Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. *Nat Struct Biol* 2000;**7**:663–8.
266. Shaw CE, Taylor RK. *Vibrio cholerae* O395 *tcpA* pilin gene sequence and comparison of predicted protein structural features to those of type 4 pilins. *Infect Immun* 1990;**58**:3042–9.
267. Shea JE, Hensel M, Gleeson C, et al. Identification of a virulence locus encoding a second type III secretion system in *Salmonella typhimurium*. *Proc Natl Acad Sci U S A* 1996;**93**:2593–7.
268. Shere KD, Sallustio S, Manessis A, et al. Disruption of IcsP, the major *Shigella* protease that cleaves IcsA, accelerates actin-based motility. *Mol Microbiol* 1997;**25**:451–62.
269. Silva S, Negri M, Henriques M, et al. Adherence and biofilm formation of non-*Candida albicans Candida* species. *Trends Microbiol* 2011;**19**:241–7.
270. Simmons G, Wilkinson D, Reeves JD, et al. Primary, syncytium-inducing human immunodeficiency virus type 1 isolates are dual-tropic and most can use either Lestr or CCR5 as coreceptors for virus entry. *J Virol* 1996;**70**:8355–60.
271. Singh KV, Nallapareddy SR, Murray BE. Importance of the *ebp* (endocarditis- and biofilm-associated pilus) locus in the pathogenesis of *Enterococcus faecalis* ascending urinary tract infection. *J Infect Dis* 2007;**195**:1671–7.
272. Smedley JG III, Jewell E, Roguskie J, et al. Influence of pilin glycosylation on *Pseudomonas aeruginosa* 1244 pilus function. *Infect Immun* 2005;**73**:7922–31.
273. Smith CV, Sharma V, Sacchettini JC. TB drug discovery: addressing issues of persistence and resistance. *Tuberculosis (Edinb)* 2004;**84**:45–55.
274. Smith GA, Marquis H, Jones S, et al. The two distinct phospholipases C of *Listeria monocytogenes* have overlapping roles in escape from a vacuole and cell-to-cell spread. *Infect Immun* 1995;**63**:4231–7.
275. Smith GA, Portnoy DA. How the *Listeria monocytogenes* ActA protein converts actin polymerization into a motile force. *Trends Microbiol* 1997;**5**:272–6.
276. Smith K, Humphreys D, Hume PJ, et al. Enteropathogenic *Escherichia coli* recruits the cellular inositol phosphatase SHIP2 to regulate actin-pedestal formation. *Cell Host Microbe* 2010;**7**:13–24.
277. Smith RS, Harris SG, Phipps R, et al. The *Pseudomonas aeruginosa* quorum-sensing molecule N-(3-oxododecanoyl)homoserine lactone contributes to virulence and induces inflammation in vivo. *J Bacteriol* 2002;**184**:1132–9.
278. Speziale P, Pietrocola G, Rindi S, et al. Structural and functional role of *Staphylococcus aureus* surface components recognizing adhesive matrix molecules of the host. *Future Microbiol* 2009;**4**:1337–52.
279. St Geme JW III. Bacterial adhesins: determinants of microbial colonization and pathogenicity. *Adv Pediatr* 1997;**44**:43–72.
280. St Geme JW III, de la Morena ML, Falkow S. A *Haemophilus influenzae* IgA protease-like protein promotes intimate interaction with human epithelial cells. *Mol Microbiol* 1994;**14**:217–33.
281. St Geme JW III, Yeo HJ. A prototype two-partner secretion pathway: the *Haemophilus influenzae* HMW1 and HMW2 adhesin systems. *Trends Microbiol* 2009;**17**:355–60.
282. Staab JF, Bahn YS, Tai CH, et al. Expression of transglutaminase substrate activity on *Candida albicans* germ tubes through a coiled, disulfide-bonded N-terminal domain of Hwp1 requires C-terminal glycosylphosphatidylinositol modification. *J Biol Chem* 2004;**279**:40737–47.
283. Staab JF, Bradway SD, Fidel PL, et al. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* 1999;**283**:1535–8.
284. Staab JF, Ferrer CA, Sundstrom P. Developmental expression of a tandemly repeated, proline- and glutamine-rich amino acid motif on hyphal surfaces on *Candida albicans*. *J Biol Chem* 1996;**271**:6298–305.

285. Starkey M, Hickman JH, Ma L, et al. *Pseudomonas aeruginosa* rugose small-colony variants have adaptations that likely promote persistence in the cystic fibrosis lung. *J Bacteriol* 2009;**191**: 3492–503.
286. Steinert PM, Candi E, Kartasova T, et al. Small proline-rich proteins are cross-bridging proteins in the cornified cell envelopes of stratified squamous epithelia. *J Struct Biol* 1998;**122**:76–85.
287. Steinhauer J, Agha R, Pham T, et al. The unipolar *Shigella* surface protein IcsA is targeted directly to the bacterial old pole: IcsP cleavage of IcsA occurs over the entire bacterial surface. *Mol Microbiol* 1999;**32**:367–77.
288. Stephenson AE, Wu H, Novak J, et al. The Fap1 fimbrial adhesin is a glycoprotein: antibodies specific for the glycan moiety block the adhesion of *Streptococcus parasanguis* in an in vitro tooth model. *Mol Microbiol* 2002;**43**:147–57.
289. Stone KD, Zhang HZ, Carlson LK, et al. A cluster of fourteen genes from enteropathogenic *Escherichia coli* is sufficient for the biogenesis of a type IV pilus. *Mol Microbiol* 1996;**20**:325–37.
290. Straley SC, Skrzypek E, Plano GV, et al. Yops of *Yersinia* spp. pathogenic for humans. *Infect Immun* 1993;**61**:3105–10.
291. Strom MS, Lory S. Cloning and expression of the pilin gene of *Pseudomonas aeruginosa* PAK in *Escherichia coli*. *J Bacteriol* 1986;**165**:367–72.
292. Stromberg N, Marklund BI, Lund B, et al. Host-specificity of uropathogenic *Escherichia coli* depends on differences in binding specificity to Gal alpha 1-4Gal-containing isoreceptors. *EMBO J* 1990;**9**:2001–10.
293. Sturgill-Koszycki S, Schlesinger PH, Chakraborty P, et al. Lack of acidification in *Mycobacterium* phagosomes produced by exclusion of the vesicular proton-ATPase. *Science* 1994;**263**: 678–81.
294. Surana NK, Cotter SE, Yeo HJ, et al. Structural determinants of *Haemophilus influenzae* adherence to host epithelium: variations on type V secretion. In: Waksman G, Caparon MG, Hultgren SJ, editors. *Structural basis of bacterial pathogenesis*. Washington, DC: American Society for Microbiology; 2005. p. 129–48.
295. Switalski LM, Patti JM, Butcher W, et al. A collagen receptor on *Staphylococcus aureus* strains isolated from patients with septic arthritis mediates adhesion to cartilage. *Mol Microbiol* 1993;**7**: 99–107.
296. Theriot JA. The cell biology of infection by intracellular bacterial pathogens. *Annu Rev Cell Dev Biol* 1995;**11**:213–39.
297. Theriot JA, Mitchison TJ, Tilney LG, et al. The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization. *Nature* 1992;**357**:257–60.
298. Theriot JA, Rosenblatt J, Portnoy DA, et al. Involvement of profilin in the actin-based motility of *L. monocytogenes* in cells and in cell-free extracts. *Cell* 1994;**76**:505–17.
299. Thomas WE, Nilsson LM, Forero M, et al. Shear-dependent "stick-and-roll" adhesion of type 1 fimbriated *Escherichia coli*. *Mol Microbiol* 2004;**53**:1545–57.
300. Thomas WE, Trintchina E, Forero M, et al. Bacterial adhesion to target cells enhanced by shear force. *Cell* 2002;**109**:913–23.
301. Timpe JM, Stamataki Z, Jennings A, et al. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 2008;**47**:17–24.
302. Tomlinson S, Raper J. The lysis of *Trypanosoma brucei brucei* by human serum. *Nat Biotechnol* 1996;**14**:717–21.
303. Touze T, Hayward RD, Eswaran J, et al. Self-association of EPEC intimin mediated by the β-barrel–containing anchor domain: a role in clustering of the Tir receptor. *Mol Microbiol* 2004;**51**:73–87.
304. Tucker SC, Galan JE. Complex function for SicA, a *Salmonella enterica* serovar typhimurium type III secretion-associated chaperone. *J Bacteriol* 2000;**182**:2262–8.
305. Umbach JL, Kramer MF, Jurak I, et al. MicroRNAs expressed by herpes simplex virus 1 during latent infection regulate viral mRNAs. *Nature* 2008;**454**:780–3.
306. Uphoff TS, Welch RA. Nucleotide sequencing of the *Proteus mirabilis* calcium-independent hemolysin genes (*hpmA* and *hpmB*) reveals sequence similarity with the *Serratia marcescens* hemolysin genes (*shlA* and *shlB*). *J Bacteriol* 1990;**172**:1206–16.
307. van't Wout AB, Kootstra NA, Mulder-Kampinga GA, et al. Macrophage-tropic variants initiate human immunodeficiency virus type 1 infection after sexual, parenteral, and vertical transmission. *J Clin Invest* 1994;**94**:2060–7.
308. Vazquez-Torres A, Jones-Carson J, Baumler AJ, et al. Extraintestinal dissemination of *Salmonella* by CD18-expressing phagocytes. *Nature* 1999;**401**:804–8.
309. Verger D, Miller E, Remaut H, et al. Molecular mechanism of P pilus termination in uropathogenic *Escherichia coli*. *EMBO Rep* 2006;**7**:1228–32.
310. Virji M, Stimson E, Makepeace K, et al. Posttranslational modifications of meningococcal pili: identification of a common trisaccharide substitution on variant pilins of strain C311. *Ann N Y Acad Sci* 1996;**797**:53–64.
311. Wagner EK, Bloom DC. Experimental investigation of herpes simplex virus latency. *Clin Microbiol Rev* 1997;**10**:419–43.
312. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005;**11**:791–6.
313. Wann ER, Gurusiddappa S, Höök M. The fibronectin-binding MSCRAMM FnbpA of *Staphylococcus aureus* is a bifunctional protein that also binds to fibrinogen. *J Biol Chem* 2000;**275**: 13863–71.
314. Weir S, Marrs CF. Identification of type 4 pili in *Kingella denitrificans*. *Infect Immun* 1992;**60**:3437–41.
315. Weiser JN, Maskell DJ, Butler PD, et al. Characterization of repetitive sequences controlling phase variation of *Haemophilus influenzae* lipopolysaccharide. *J Bacteriol* 1990;**172**:3304–9.
316. Weiser JN, Pan N. Adaptation of *Haemophilus influenzae* to acquired and innate humoral immunity based on phase variation of lipopolysaccharide. *Mol Microbiol* 1998;**30**:767–75.
317. Weiser JN, Pan N, McGowan KL, et al. Phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. *J Exp Med* 1998;**187**: 631–40.
318. Weiser JN, Shchepetov M, Chong ST. Decoration of lipopolysaccharide with phosphorylcholine: a phase-variable characteristic of *Haemophilus influenzae*. *Infect Immun* 1997;**65**:943–50.
319. Weiss SM, Ladwein M, Schmidt D, et al. IRSp53 links the enterohemorrhagic *E. coli* effectors Tir and EspFU for actin pedestal formation. *Cell Host Microbe* 2009;**5**:244–58.
320. Wetzler LM, Barry K, Blake MS, et al. Gonococcal lipooligosaccharide sialylation prevents complement-dependent killing by immune sera. *Infect Immun* 1992;**60**:39–43.
321. Wexler DE, Chenoweth DE, Cleary PP. Mechanism of action of the group A streptococcal C5a inactivator. *Proc Natl Acad Sci U S A* 1985;**82**:8144–8.
322. Wieland SF, Chisari FV. Stealth and cunning: hepatitis B and hepatitis C viruses. *J Virol* 2005;**79**:9369–80.
323. Wilke GA, Bubeck Wardenburg J. Role of a disintegrin and metalloprotease 10 in *Staphylococcus aureus* α-hemolysin-mediated cellular injury. *Proc Natl Acad Sci U S A* 2010;**107**:13473–8.
324. Willems RJ, Geuijen C, van der Heide HG, et al. Mutational analysis of the *Bordetella pertussis fim/fha* gene cluster: identification of a gene with sequence similarities to haemolysin accessory genes involved in export of FHA. *Mol Microbiol* 1994;**11**:337–47.
325. Wilson JR, de Sessions PF, Leon MA, et al. West Nile virus nonstructural protein 1 inhibits TLR3 signal transduction. *J Virol* 2008;**82**:8262–71.
326. Wolff C, Nisan I, Hanski E, et al. Protein translocation into host epithelial cells by infecting enteropathogenic *Escherichia coli*. *Mol Microbiol* 1998;**28**:143–55.
327. Wright SD, Silverstein SC. Receptors for C3b and C3bi promote phagocytosis but not the release of toxic oxygen from human phagocytes. *J Exp Med* 1983;**158**:2016–23.
328. Wu H, Bu S, Newell P, et al. Two gene determinants are differentially involved in the biogenesis of Fap1 precursors in *Streptococcus parasanguis*. *J Bacteriol* 2007;**189**:1390–8.
329. Wu H, Fives-Taylor PM. Identification of dipeptide repeats and a cell wall sorting signal in the fimbriae-associated adhesin, Fap1, of *Streptococcus parasanguis*. *Mol Microbiol* 1999;**34**:1070–81.
330. Wu H, Mintz KP, Ladha M, et al. Isolation and characterization of Fap1, a fimbriae-associated adhesin of *Streptococcus parasanguis* FW213. *Mol Microbiol* 1998;**28**:487–500.
331. Wu H, Song Z, Givskov M, et al. *Pseudomonas aeruginosa* mutations in *lasI* and *rhlI* quorum sensing systems result in milder chronic lung infection. *Microbiology* 2001;**147**:1105–13.
332. Xu H, Storch T, Yu M, et al. Characterization of the human Forssman synthetase gene: an evolving association between glycolipid

synthesis and host-microbial interactions. *J Biol Chem* 1999;**274**: 29390–8.
333. Yanagishita M, Hascall VC. Cell surface heparan sulfate proteoglycans. *J Biol Chem* 1992;**267**:9451–4.
334. Yeo HJ, Cotter SE, Laarmann S, et al. Structural basis for host recognition by the *Haemophilus influenzae* Hia autotransporter. *EMBO J* 2004;**23**:1245–56.
335. Yi Y, Ma Y, Gao F, et al. Crystal structure of EHEC intimin: insights into the complementarity between EPEC and EHEC. *PLoS One* 2010;**5**:e15285.
336. Yoon S, Liu Z, Eyobo Y, et al. *Yersinia* effector YopJ inhibits yeast MAPK signaling pathways by an evolutionarily conserved mechanism. *J Biol Chem* 2003;**278**:2131–5.
337. Yu L, Lee KK, Sheth HB, et al. Fimbria-mediated adherence of *Candida albicans* to glycosphingolipid receptors on human buccal epithelial cells. *Infect Immun* 1994;**62**:2843–8.
338. Yu M, Haslam DB. Shiga toxin is transported from the endoplasmic reticulum following interaction with the luminal chaperone HEDJ/ERdj3. *Infect Immun* 2005;**73**:2524–32.
339. Zabolotny JM, Krummenacher C, Fraser NW. The herpes simplex virus type 1 2.0-kilobase latency-associated transcript is a stable intron which branches at a guanosine. *J Virol* 1997;**71**:4199–208.
340. Zhang J, Randall G, Higginbottom A, et al. CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. *J Virol* 2004;**78**:1448–55.
341. Zhang L, He T, Talal A, et al. In vivo distribution of the human immunodeficiency virus/simian immunodeficiency virus coreceptors: CXCR4, CCR3, and CCR5. *J Virol* 1998;**72**:5035–45.
342. Zhang Y, Mena P, Romanov G, et al. A protective epitope in type III effector YopE is a major CD8 T cell antigen during primary infection with *Yersinia pseudotuberculosis*. *Infect Immun* 2012;**80**: 206–14.
343. Zhu T, Mo H, Wang N, et al. Genotypic and phenotypic characterization of HIV-1 patients with primary infection. *Science* 1993;**261**:1179–81.
344. Zoja A, Remuzzi G. The pivotal role of the endothelial cell in the pathogenesis of HUS. In: Kaplan BS, Trompeter RS, Moake JL, editors. *Hemolytic uremic syndrome and thrombotic thrombocytopenic purpura*. New York: Marcel Dekker; 1992. p. 389–404.
345. Zong Y, Xu Y, Liang X, et al. A "collagen hug" model for *Staphylococcus aureus* CNA binding to collagen. *EMBO J* 2005;**24**: 4224–36.
346. Zwahlen A, Kroll JS, Rubin LG, et al. The molecular basis of pathogenicity in *Haemophilus influenzae*: comparative virulence of genetically-related capsular transformants and correlation with changes at the capsulation locus *cap*. *Microb Pathog* 1989;**7**:225–35.
347. Zychlinsky A, Prevost MC, Sansonetti PJ. *Shigella flexneri* induces apoptosis in infected macrophages. *Nature* 1992;**358**:167–9.
348. Zychlinsky A, Sansonetti PJ. Apoptosis as a proinflammatory event: what can we learn from bacteria-induced cell death? *Trends Microbiol* 1997;**5**:201–4.

CHAPTER 2

Normal and Impaired Immunologic Responses to Infection

Michael F. Tosi

This chapter provides an overview of immunologic responses to infection and considers host interactions with different classes of pathogens, normal innate and adaptive immune mechanisms, the developing host responses of neonates, specific primary and secondary immunodeficiencies, and approaches to the evaluation of children suspected of having impaired immunity. Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) are not considered here because they are addressed fully in Chapter 191B. This chapter is intended to supply sufficient information for the development of a basic understanding of mechanisms involved in normal host responses to infection, an appreciation of the underlying basis and clinical presentation of important immunodeficiencies, and familiarity with general principles of evaluation and management of patients with suspected or documented disorders of immunity. For greater depth and detail regarding specific topics, readers will be referred to excellent reviews.

HOST–PARASITE INTERACTIONS

General Features of Host–Parasite Interactions

Humans are constantly exposed to a daunting number and diversity of microorganisms that can cause infection. Many organisms that usually coexist harmoniously with the human host on the skin or on mucous membranes of the oral cavity, upper airways, or lower gastrointestinal tract may invade and become pathogens only if the balance of the commensal relationship is disrupted. Other organisms are more virulent, and they overtly challenge the host's normal surface barriers and internal defense mechanisms. The human host has evolved a complex array of protective mechanisms designed to defend itself against these continuous microbial challenges.[626] To understand the pathogenesis, pathology, and natural history of infectious diseases, familiarity with the features of infectious agents that confer virulence is necessary; these topics are addressed elsewhere in this book. However, it is equally important to understand the elements of the host's response that contribute to containment, elimination, and protection against subsequent infection with these agents. Furthermore, it is important to recognize that host responses to infections also may contribute to the pathophysiology of infectious diseases and may injure the host in other ways.

The characteristic features of specific infectious diseases are determined by the interactions of structural components and released products of microbial pathogens with host tissue, cells, and their products. Virulence tactics commonly employed by organisms include adherence to host cell surfaces, internalization within or invasion of host cells, production of toxins, elaboration of surface barriers such as bacterial polysaccharide capsules, usurpation of host synthetic mechanisms, and direct inhibition of specific defense mechanisms within host cells. The successful evolution of host strategies to protect against microbial attack has resulted in defenses designed to interfere with or to counteract many of these modes of microbial virulence.[626] In recent decades, some of humanity's oldest microbial adversaries (e.g., smallpox, poliomyelitis, measles) systematically have been, or are being, eradicated with aggressive implementation of immunization programs. In the meantime, previously unrecognized human pathogens such as human immunodeficiency virus–1 (HIV-1) and Ebola virus have emerged as new adversaries. However, many of our oldest nemeses (e.g., tuberculosis, malaria) continue to elude our efforts to bring them under control, and they remain serious problems worldwide. Continued research at the interface between microbial pathogenesis and immunologic mechanisms is essential for the development of innovative approaches that can support and augment human immune responses to both old and new infectious diseases.

Main Features of Host Responses to Specific Classes of Infectious Agents

Viruses

Viruses are obligate intracellular parasites that consist of genetic material in the form of either DNA or RNA that usually is surrounded by a protein coat and may or may not be bound by a lipid envelope.[395] Diseases caused by viruses are remarkably diverse, ranging from mild and merely inconvenient to rapidly fatal and from acute or brief to chronic or lifelong. However, certain features are common to the pathogenesis of most viral infections. First, viruses must enter host cells to replicate. Viral entry ordinarily is initiated by attachment of a viral surface protein to a specific receptor molecule on the host cell.

The specific viral ligands or their corresponding host cell receptors have been identified for some viruses. For example, rhinovirus has evolved a capsid protein that binds to human intercellular adhesion molecule–1 (ICAM-1) on respiratory epithelium[255]; the envelope glycoproteins of HIV-1 interact with CD4 on T lymphocytes and distinct chemokine receptors on lymphocytes or macrophages[163,327,623]; and internalization of adenoviruses depends on interaction between a specific peptide sequence in the penton base complex of the viral capsid and α_V integrins on host cell surfaces.[618] After the virus has entered the host cell, the cellular synthetic machinery is redirected to the synthesis of viral components. As with many native proteins synthesized by the host cell, a portion of newly synthesized viral protein is processed into peptides and presented on the infected cell surface by major histocompatibility complex (MHC) class I molecules (see later discussion). The host mechanisms most important in defense against the majority of viral pathogens include the production of specific neutralizing antibodies against viral surface proteins, the development of specific $CD8^+$ cytotoxic T-cell responses that eliminate infected cells, and the production by different immune cells of type 1 interferons (IFNs) that disrupt viral replication.[40,367,369,504,566] Other host defenses also may exhibit antiviral activity, although the importance of some of these mechanisms in protection against viral infection in humans has not been established as firmly. Natural killer (NK) cells appear to mediate the destruction of some virus-infected host cells,[126,543] and antibody-dependent cellular cytotoxicity (ADCC) may ensue after immunoglobulin (Ig)G antibodies bind to viral antigens on the infected cell, permitting subsequent attachment of either NK cells or cytotoxic T cells via IgG Fc receptors.[209] IFNs and other cytokines may enhance NK and ADCC activity, and cytokines such as tumor necrosis factor-α (TNF-α) may exert cytotoxic actions on cells infected with certain viruses.[369] Additionally, opsonic complement components bound to viral surfaces can interfere with cell attachment and the complement-derived membrane attack complex can lyse enveloped viruses.[63]

Bacteria

The human host is colonized with a large variety of bacteria at skin and mucous membrane surfaces.[113,428] The integrity of these mechanical barriers ordinarily prevents systemic invasion of local commensal bacteria.[93] The epithelial cells that constitute these barriers, on recognition of an organism as a pathogen, also can release defensins and other microbicidal molecules.[235] In healthy hosts, circulating polymorphonuclear leukocytes (PMNs) help keep the resident flora in check by leaving the bloodstream at the mucosal sites containing the highest bacterial burdens, such as the lower intestine and the gingival crevices of the oral cavity.[30] This phenomenon helps account for the increased risk for local and systemic infection caused by oral and intestinal organisms in patients with severe neutropenia, including those who receive prolonged chemotherapy for malignancies, and in patients with phagocyte migration disorders, such as leukocyte adhesion deficiency syndromes.[30] Important host defenses against most bacteria that invade the human host systemically include the complement system, specific antibodies that promote both the opsonic and the bacteriolytic functions of complement, and phagocytes.[3,30,63,310]

Fungi

Host mechanisms critical for defense against fungi are less well understood than those directed at bacteria and viruses, but phagocytes and cell-mediated immunity appear to be most important.[197,224] The relative importance of these factors appears to depend on the specific organisms involved, as is demonstrated by clinical observations in patients with isolated defects of one or the other. Severe mucosal infections caused by *Candida* spp are common in patients with acquired or primary cell-mediated immune deficits, such as HIV infection, thymic aplasia (see later discussion), chronic mucocutaneous candidiasis, and some forms of severe combined immunodeficiency, as well as in patients with disorders of leukocyte migration.[30,197] Disseminated candidiasis more often is attributed to iatrogenic factors, such as prolonged antimicrobial therapy and indwelling vascular catheters. Patients with malignancies, complicated postsurgical courses, and burns also appear to be at increased risk. Although neutrophils from patients with myeloperoxidase deficiency kill *Candida* organisms more slowly than do those from normal persons, these patients usually do not develop *Candida* infections, suggesting that this aspect of neutrophil function is not critical.[30,197] In contrast to *Candida*, *Aspergillus* infections are not as great a problem for patients with cell-mediated immune defects as they are for patients with defects in phagocytic host defenses, such as neutropenia associated with cancer chemotherapy or stem cell transplantation, or genetic defects in phagocyte killing such as chronic granulomatous disease.[61,622] Fungi such as *Histoplasma* and *Cryptococcus*, like *Candida*, tend to cause severe infections in patients with defects in cell-mediated immunity, although phagocytes clearly are required for optimal clearance of these organisms.[180,307,616] The main role of antibodies and complement in protection from fungi probably is to provide opsonic activity to enhance phagocyte function.[181]

Parasites

Parasites such as protozoa and helminths comprise such a widely varying group of pathogenic organisms that it is difficult to generalize about mechanisms of immunity to these organisms as a group. However, the importance of specific host mechanisms in defense against certain parasites may be appreciated by considering the characteristic host responses mobilized by parasitic infection or infestations, as well as examples of special susceptibilities to certain parasites among patients with impairments of different components of immunity. Some helminths induce production by host cells of chemokines that recruit eosinophils and stimulate their production. This suggests a likely role for these cells in antiparasitic defenses, and eosinophils have been shown to be important in protection against helminths such as *Strongyloides*

and other parasites in this group that can invade tissues. IgE, among the immunoglobulins, appears to play a special role, often in concert with eosinophils, in anthelmintic defenses. IgG also may be important, based on the susceptibility of individuals with hypogammaglobulinemia to hyperinfection with *Strongyloides*. Patients with hypogammaglobulinemia also are at risk for chronic or severe infestations with the flagellate intestinal parasite *Giardia lamblia*, suggesting a role for some degree of antibody-mediated protection in normal hosts. Patients with primary or acquired disorders of cell-mediated immunity are prone to development of serious central nervous system and ocular manifestations of infection with the protozoan *Toxoplasma gondii*, an obligate intracellular parasite, as well as hyperinfection with *Strongyloides*.[318,454]

FEATURES OF NORMAL IMMUNE FUNCTION

The organization of the immune system often has been viewed as consisting of several separate arms or compartments, such as complement, phagocytes, cell-mediated immunity, and humoral immunity. A more current approach often considers two broader categories, innate immunity and adaptive immunity, The former encompasses the more rapid and phylogenetically primitive, nonspecific responses to infection, such as surface defenses, cytokine elaboration, complement activation, and phagocytic responses. The latter involves more slowly developing, persistent, and highly evolved antigen-specific responses, such as cell-mediated immunity and antibody production that exhibit extraordinarily diverse ranges of specificities. Although the individual components of innate and adaptive immunity will be addressed separately, the various arms of the immune system engage in a wide range of interactions that may enhance or regulate functions of other components of immunity, adding to the already remarkable complexity of the human immune response, and numerous examples of such interactions will be provided.

Innate Immune Responses

Epithelia, Defensins, and Other Antimicrobial Peptides

The epithelium of skin and mucosal tissue functions as a mechanical barrier to the invasion of microbial pathogens. In recent decades, it has become clear that epithelial cells also are a major source of antimicrobial peptides that play important roles in local host defense.[50,233,234,446] Studies of their structure, sources, expression, and actions also have revealed an unexpected range of immunologic activities for these molecules, whose functions once were considered mainly antimicrobial in nature.[2,35]

Epithelial cells of mucous membranes of the airways and intestines, as well as keratinocytes, express the human β-defensins (HBD)-1, HBD-2, HBD-3, and HBD-4. These small cationic peptides are similar to the α-defensins stored in the azurophilic granules of neutrophils, and they display antimicrobial activity against a broad range of bacteria, fungi, chlamydiae, and enveloped viruses.[50,233,235,446] Their production by epithelial cells may be constitutive, as for HBD-1, or inducible as for HBD-2, HBD-3, and HBD-4. For example, recent evidence indicates that epithelial cells of the airway or intestine can produce HBD-2 in response to activation by bacterial products via the Toll-like receptors TLR2 or TLR4 (see later discussion) on the epithelial cells.[275,602,608] Stimulation of epithelium by cytokines, including interleukin (IL)-1 or TNF-α also can induce defensin production.[50,235] Defensins have been reported to exert their antimicrobial action either by the creation of membrane pores or by membrane disruption resulting from electrostatic interaction with the polar head groups of membrane lipids, with more evidence now favoring the latter mechanism.[50,288] Some microorganisms have evolved mechanisms for evading the action of defensins. For example, bacterial polysaccharide capsules may limit access of microbial peptides to the cell membrane,[118] and an exoprotein of *Staphylococcus aureus*, staphylokinase, neutralizes the microbicidal action of neutrophil α-defensins.[304]

Several immunoregulatory properties of defensins and related peptides, distinct from their antimicrobial actions, have been documented.[233] Several such peptides have been shown to facilitate posttranslational processing of IL-1β.[464] Some of the β defensins have been shown to function as chemoattractants for neutrophils, memory T cells, and immature dendritic cells by binding to the chemokine receptor CCR-6.[287,426,446] Separately, HBD-2 has been shown to act, via a mechanism that requires TLR4, to activate immature dendritic cells and promote their maturation.[72,625] The β-defensins also act as chemoattractants for mast cells and can induce mast cell degranulation.[427] HBD-2 and several other antimicrobial peptides can interfere with binding between bacterial lipopolysaccharide (LPS) and LPS-binding protein (LBP), a process important in activating inflammatory cells via TLR4 (see later discussion).[522]

Additional antimicrobial peptides of epithelial cells include lysozyme and cathelicidin. Lysozyme, an antimicrobial peptide also found in neutrophil granules, attacks the peptidoglycan cell walls of bacteria and may be released from cells by mechanisms that involve TLR activation.[456] Cathelicidin, or LL37, like lysozyme, is released from both neutrophils and epithelial cells. It exhibits broad antimicrobial activity and can inhibit lentiviral replication.[287,556] Cathelicidin also exhibits chemotactic activity for neutrophils, monocytes, and T lymphocytes. This activity is mediated via a formyl peptide receptor–like molecule (FPRL-1), rather than the chemokine receptor (CCR)6 bound by β-defensins.[624]

The release of defensins in response to activation of TLRs and the various actions of these peptides, including their direct antimicrobial activities, their chemoattractant actions for a wide range of immune cells, and their activation of dendritic cell maturation, already suggest a highly complex and regulatory role in the development of host defense and immunity. Genomic evidence for the possible existence of many additional human defensins that have not yet been characterized suggests that current

knowledge describes but a small sample of the overall contribution of these peptides to immune responses.[50,519]

Toll-Like Receptors

Mononuclear phagocytes, including circulating monocytes and tissue macrophages, other phagocytic cells, and many epithelial cells express a family of receptors that is highly homologous to the *Drosophila* receptor called Toll.[99,275,393,602,608] These receptors mediate a phylogenetically primitive, nonclonal mechanism of pathogen recognition based on binding, not to specific antigens, but to structurally conserved pathogen-associated molecular patterns.[9,442,443,629] At least 10 human TLRs with a range of microbial ligands have been identified, such as gram-negative bacterial LPS, bacterial lipoproteins, lipoteichoic acids of gram-positive bacteria, bacterial cell wall peptidoglycans, cell wall components of yeast and mycobacteria, unmethylated CpG dinucleotide motifs in bacterial DNA, some viral particles, and viral RNA.[9,442,443,629] Gram-positive cell wall components bind mainly to TLR2, and TLR2 also can bind components of herpes simplex virus.[345,568] TLR2 forms dimers with either TLR1 or TLR6 when bound jointly by their ligands.[304,364] Gram-negative LPS activates TLR4 indirectly by first binding to LBP, which transfers the LPS to the host accessory protein CD14 at the cell surface. The bound CD14 has no transmembrane domain, but associates directly with an extracellular domain of TLR4.[443,568] MD-2, an additional accessory protein associated with TLR4, also plays a role in binding LPS.[459] TLR5 has been identified as the receptor for bacterial flagellin, TLR9 recognizes CpG motifs of bacterial and viral DNA, and TLR3 has been shown to bind synthetic and viral double-stranded RNA.[59,265,341,345] A listing of known human TLRs, with their major ligands and cellular distribution, is summarized in Table 2-1.

Signaling by TLRs occurs via a well-described pathway in which receptor binding generates a signal via an adaptor molecule, myeloid differentiation factor 88 (MyD88), that leads to intracellular association with IL-1 receptor–associated kinase (IRAK). In turn, this leads to activation of TNF receptor–associated factor–6 (TRAF-6), which results in nuclear translocation of nuclear factor-κB (NF-κB).[140] NF-κB is an important transcription factor that activates the promoters of the genes for a broad range of cytokines and other proinflammatory products, such as TNF-α, IL-1, IL-6, and IL-8. This signaling pathway, based on studies with TLR4, is similar, but not identical, to the signaling pathways activated by other TLRs.[140] The activation of cytokine production by TLRs plays an important role in recruiting other components of innate host defense against bacterial pathogens. However, with large-scale cytokine release, the deleterious effects of sepsis or other forms of the systemic inflammatory response syndrome demonstrate that these pathways have both beneficial and potentially harmful effects for the host.[140] Genetic polymorphisms in TLRs may play a role in determining the balance of these effects in certain individuals responding to the challenge of systemic infection.[140,373,374]

In addition to their "first responder" roles in generating an inflammatory response to invading pathogens, TLRs may network with other components of innate and adaptive immunity. TLR4 function is suppressed by activation of cells via the chemokine receptor CXCR4.[326] Activation of some TLRs also can induce expression of the costimulatory molecule B7 on antigen-presenting cells, which is required for activation of naïve T cells.[393]

Cytokines

A heterogeneous group of soluble small polypeptide or glycoprotein mediators, often collectively called *cytokines*, form part of a complex network that helps regulate immune and inflammatory responses. Included in this group of mediators, whose molecular weights range from about 8 to about 45 kDa, are the ILs, IFNs, growth factors, and chemokines (see separate discussions later). Most cells of the immune system and many other host cell types release cytokines, respond to cytokines via specific cytokine receptors, or both. The range of sources and effects of cytokines and their actions and interrelationships are of such complexity that they cannot be addressed here in detail. A list of cytokines and related molecules that play a role in immune function, with selected characteristics, is provided in Table 2-2.[344,445,468] Excellent general reviews are available,[343,344,369,445,468] and

TABLE 2-1 **Human Toll-like Receptors: Their Ligands and Cellular Distribution**

TLR	Ligands	Cellular Distribution
TLR1 (+TLR2) TLR2 (+TLR6)	Mycobacterial lipoarabinomannans, and bacterial lipoproteins, bacterial lipoteichoic acids, bacterial and fungal β-glucans	Mo, DC, MC, Eos, Bas, AEC
TLR3	Viral double-stranded RNA	NK cell
TLR4 (+CD14, MD-2)	Bacterial lipopolysaccharide	MΦ, DC, MC, Eos, AEC
TLR5	Bacterial flagellin	AEC, IEC
TLR7	Viral single-stranded RNA	PDC, NK, Eos, BL
TLR8	Viral single-stranded RNA	NK cell
TLR9	Unmethylated CpG dinucleotides	PDC, Eos, BL, Bas (bacteria, herpesvirus)
TLR10	Unknown ligands	PDC, Eos, BL, Bas

AEC, Airway epithelial cell; *Bas,* basophil; *BL,* B lymphocyte; *DC,* dendritic cell; *Eos,* eosinophil; *IEC,* intestinal epithelial cell; *MΦ,* macrophage; *MC,* mast cell; *Mo,* monocyte; *PDC,* plasmacytoid dendritic cell; *TLR,* Toll-like receptor.

TABLE 2-2 **Features of Selected Human Cytokines and Growth Factors**

Cytokines and Growth Factors	Main Cellular Sources	Biologic Effects
IL-1	Mo, TL, BL, NK, PMN, others	Broad range of cellular activation in inflammatory and immune responses
IL-2	TL, BL, NK	TL, BL proliferation and activation; enhances TL and NK cytotoxicity
IL-3	TL	General stimulation of hematopoiesis
IL-4	TL, BL, Mast, Mo	TL, BL proliferation; BL isotype switching; stimulates IgE synthesis; enhances MHC class II expression
IL-5	TL	Stimulation of Eos production
IL-6	TL, BL, Mo	Broad inflammatory activity; stimulates BL differentiation and megakaryocyte production
IL-7	Marrow and thymus stromal cells	TL, BL growth and differentiation
IL-8	Mac, Mo, Endo, Epi, PMN, Eos	Activation and chemotaxis of PMN, Eos
IL-9	TL	Mast growth and differentiation; growth of activated TL
IL-10	TL, BL, Mast, Mac	Broad anti-inflammatory actions; inhibits synthesis of several other cytokines (TNF, IL-2, IL-3, IFN-γ)
IL-11	Marrow stromal cells	General stimulation of hematopoiesis; BL growth and differentiation
IL-12	BL, Mo	Stimulation of TL growth; induction of IFN-γ production; enhancement of TL and NK cytotoxicity
IL-13	TL	BL proliferation and isotype switching; enhances MHC class II expression; inhibits production of cytokines by Mac
IL-14	TL, malignant BL	Induces BL growth
IL-15	Epi, Endo, Mo, Mac, marrow stromal cells	Enhances NK growth, development, function; enhances TL growth and migration
IL-17	TL	Enhances TL growth; induces Mac cytokine release
IL-18	Kupffer cells, Epi, spleen, Mac	Promotes TL, BL, NK cytokine release; promotes TL, BL cytotoxicity
IL-21	TL	Promotes BL, TL proliferation; NK cytoxicity
IL-23	Dendritic cells, Mac	Similar to IL-12
IL-25	TL (T_H2), Mast	TL, Mac T_H2 cytokine secretion
IL-27	Dendritic cells, Mac	TL responsiveness to IL-12
IFN-α	Mo, TL	Interference with viral replication; increases MHC class I expression
IFN-β	Epi, Fibro	Similar to IFN-α
IFN-γ	TL, NK	Similar to IFN-α, IFN-β; stimulates Mac inflammatory functions
TNF-α	Mo, Mac, TL, NK	Broad inflammatory effects; fever; cachexia; stimulates catabolism; activation of leukocytes and Endo
GM-CSF	TL, BL, Mo, PMN, Eos, Fibro, Mast, Endo	Growth of PMN, Eos, Mo, and Mac precursors; enhances leukocyte function
G-CSF	Mo, Epi, Fibro	Enhances production and function of granulocytes
M-CSF	Mo, TL, BL, Endo, Fibro	Promotes Mo production; stimulates Mo and Mac function

BL, B lymphocyte; *Endo,* endothelial cell; *Eos,* eosinophil; *Epi,* epithelial cell; *Fibro,* fibroblast; *G-CSF,* granulocyte colony-stimulating factor; *GM-CSF,* granulocyte-macrophage colony-stimulating factor; *IFN,* interferon; *IL,* interleukin; *Mac,* macrophage; *Mast,* mast cell; *M-CSF,* macrophage colony-stimulating factor; *MHC,* major histocompatibility complex; *Mo,* monocyte; *NK,* natural killer cell; *PMN,* polymorphonuclear leukocyte; *TL,* T lymphocyte; *TNF,* tumor necrosis factor.

the use of cytokines as immunomodulating agents is discussed in Chapter 241. Some cytokines are considered in following sections because they play important roles in the functions of various immune cells. However, two cytokines, IL-1 and TNF-α, are of such fundamental importance in acute host responses to infection that they warrant specific attention here.

IL-1 and TNF-α are small polypeptides, each with a molecular weight of approximately 17 kDa, that exhibit a broad range of effects on immunologic responses, inflammation, metabolism, and hematopoiesis.[69,445] IL-1 originally was described as "endogenous pyrogen," referring to its ability to produce fever in experimental animals, and TNF-α, which produces some of the same effects produced by IL-1, was originally named "cachectin" after the wasting syndrome it produced when injected chronically in mice.[69,445] Many of the physiologic changes associated with gram-negative sepsis can be reproduced by injecting experimental animals with these cytokines in the absence of microorganisms. Depending on the doses injected, these effects may include fever, hypotension, and either neutrophilia or leukopenia.[69,445] In the development of endotoxic shock resulting from gram-negative sepsis, IL-1 and TNF-α are produced by mononuclear phagocytes in response to activation of TLRs by bacterial LPS. They in turn activate the production of

other cytokines and chemokines, lipid mediators such as platelet-activating factor and prostaglandins, and reactive oxygen species. They also induce expression of adhesion molecules of both endothelial cells and leukocytes, stimulating recruitment of leukocytes by inducing release of the chemokine IL-8 and activating neutrophils for phagocytosis, degranulation, and oxidative burst activity.[69,140] These are all important, usually beneficial host responses to infection. However, at very high levels of activation, pathologic effects of this proinflammatory cascade may occur, including vascular instability, decreased myocardial contractility, capillary leak, tissue hypoperfusion, coagulopathy, and multiple organ failure.[140,603] For some systemic actions, notably the production of hemodynamic shock, IL-1 and TNF-α are synergistic. Both IL-1 and TNF-α also induce production of IL-6, a somewhat less potent cytokine that exhibits some of the actions of IL-1 and TNF-α.[445] The human host produces several soluble antagonists of IL-1 and TNF-α that can modulate their effects, including IL-1 receptor antagonist (IL-1ra), soluble TNF-α receptor (sTNF-αR) and anti-inflammatory cytokines, especially IL-10.[140]

The importance of effects mediated by IL-1 and TNF-α in the pathophysiology of septic shock has prompted much active research aimed at blocking their effects to reduce morbidity and mortality. Monoclonal antibodies against TNF-α and other inhibitors of TNF-α or IL-1 showed early promise in vitro and in animal models of septic shock.[31,45,140,222,445] However, they have been far more effective at preventing the effects of cytokines than reversing them. More recent attempts to address the issue of the timing of intervention have been directed at the intracellular signaling mechanisms activated through the TNF-α receptor or at mediators that appear later than TNF-α. Lipophilic inhibitors of protein tyrosine kinases, enzymes that propagate the cellular signals via TNF-α receptors, have been found to enhance survival in experimental animals, even when administered 2 hours after systemic injection with endotoxin.[594] Additionally, monoclonal antibodies against a cytokine-like nonhistone nucleoprotein product of macrophages, high-mobility group B1 (HMGB1), which appears much later than TNF-α or IL-1 after LPS stimulation, were found to rescue mice from endotoxin shock, when given 2 hours after an otherwise lethal dose of LPS.[606] Initial clinical trials with activated protein C, a regulatory protein in the coagulation cascade, demonstrated beneficial effects in selected patients with septic shock by mechanisms that may involve inhibition of NFκB activation, but subsequent studies did not demonstrate improved survival of patients with sepsis.[100,482] To date, despite progress, clinical strategies to interfere with the cytokine-induced cascade that leads to endotoxin shock have continued, overall, to meet with limited success.

Chemokines

A specialized group of small cytokine-like polypeptides, chemokines, which all share the feature of being ligands for G-protein coupled, seven-transmembrane-segment receptors, play an increasingly complex role in the immune response as cellular activators that induce directed cell migration, mainly of immune and inflammatory cells.* The chemokines and their receptors have been classified into four families, based on the motif displayed by the first two cysteine residues of the respective chemokine peptide sequence. Each of at least 16 CXC chemokines binds to one or more of the CXCRs, CXCR1-6. Examples of CXC chemokines include IL-8 and Gro-α. Similarly, at least 28 CC chemokines, such as macrophage inflammatory protein (MIP)-1α; regulated and normal T cell expressed and secreted (RANTES); and eotaxin-1, -2, and -3 bind to one or more of the CCRs, CCR1 to 10. The sole CX3C chemokine, fractalkine, or neurotaxin, binds to CX3CR1, currently the only receptor in its family. The two XC chemokines, including lymphotaxin, bind to the sole receptor in this family, XCR1. A chemokine nomenclature currently designates each of the chemokines as a numbered ligand for its respective receptor family. In this system, Gro-α is CXC ligand (L)-1 (or CXCL-1) and IL-8 now becomes CXCL-8. Similarly, RANTES becomes CCL-5, fractalkine is CX3CL-1, and lymphotactin is XCL-1.[300,500] A review of this nomenclature system tabulates the members of each family with their respective ligands and receptors, as well as the traditional names in both human and murine systems.[300]

Virtually every cell type of the immune system expresses receptors for one or more of the chemokines. The cells of virtually any inflamed tissue can release a range of chemokines, and tissues infected with different bacteria or viruses release chemokines that recruit characteristic sets of immune cells.[244,323] For example, whereas rhinoviruses induce the release of chemokines that result mainly in recruitment of neutrophils (early in the course of infection), Epstein-Barr virus induces a set of chemokines that result in recruitment of B cells, NK cells, and both $CD4^+$ and $CD8^+$ T cells.[244] It is of interest that almost mutually exclusive sets of chemokines are induced by cytokines associated with T_H1 (IFN-γ) versus T_H2 (IL-4, IL-13) versus T_H17 (IL-17) immune responses (see later discussion), indicating a tight interplay between cytokines and chemokines in determining the type of immune response to specific infectious challenges generated under differing conditions.[75] The specificity of such responses is strongly influenced by the type of chemokines released by specific tissues, the vascular adhesion molecules expressed in those tissues, the chemokine receptors expressed by different leukocyte populations, and the specific adhesion molecules expressed by leukocytes.[75,244,323]

Modulation of chemokine functions may occur by several mechanisms. Chemokines themselves may be potentiated or inactivated by tissue proteases, including tissue peptidases and matrix metalloproteases.[392,444] Heparin sulfate–related proteoglycans on endothelial cell surfaces tether chemokines locally, where they can most efficiently activate circulating leukocytes for adhesion (see later discussion). However, similar proteoglycans free in the extracellular environment may act to bind and

*References 46, 300, 323, 386, 419, 500.

sequester chemokines, keeping them from interacting with their cellular receptors.[145,346] Finally, in addition to the well-described use of chemokine receptors as coreceptors for viral entry by HIV-1, other viruses, especially members of the herpesvirus family, encode soluble decoy receptors that compete with native host receptors for chemokine binding, thereby disrupting normal host responses.[145,497]

Natural Killer Cells

NK cells are an important cellular component of innate immunity. They are lymphoid cells that do not express clonally distributed receptors, such as T-cell receptors or surface immunoglobulin, for specific antigens.[411,412,543] They respond in an antigen-independent manner to help contain viral infections before the development of adaptive immune responses, and they aid in the control of malignant tumors.[542,543] NK cells are found in the peripheral circulation, spleen, and bone marrow. Like many other leukocytes, they can be recruited to sites of inflammation by chemokines and other chemoattractants. They appear to be important for the control of tumors in vivo and serve a critical function in host defense against viral infections, especially those caused by members of the herpesvirus family.[126,542] Activated NK cells also are an important source of IFN-γ, which limits tumor angiogenesis and promotes the development of specific protective immune responses.[411,412,542,543]

Regulation of NK cell activity involves a complex balance between activating and inhibitory signals. Several cytokines can activate NK cell proliferation, cytotoxicity, or IFN-γ production, including IL-12, IL-15, IL-18, IL-21, and IFN-αβ.[542] Activating signals via other receptors on NK cells, such as NKG2D, may lead either to cytotoxicity or cytokine production, or both, depending on the receptor's association with distinct intracellular adaptor proteins that signal via different kinases.[542,598] Other molecules on NK cells may act as either costimulatory or adhesion receptors, including CD27, CD28, CD154 (CD40 ligand), and lymphocyte function–associated (LFA)-1 (CD11a/CD18).[53,542] Additionally, FcγRIII (CD16) can contribute to NK cell cytotoxic activity by mechanisms that include antibody-dependent cell cytotoxicity.[209,412] NK cells are able to distinguish normal cells of self-origin via receptors that recognize specific MHC class I molecules. Activation of such receptors provides an inhibitory signal that protects healthy host cells from NK cell–mediated lysis. Virus-infected cells and malignant cells may express MHC class I molecules at reduced levels and thus may be less able to generate inhibitory NK cell signals, rendering them more susceptible to attack by NK cells.[126,543] NK cell inhibitory receptors, some of which have been characterized, appear to contain intracytoplasmic tyrosine-based inhibition motifs and antagonize NK cell activation pathways via protein tyrosine phosphatases.[480,542] Thus the regulation of the phosphorylation state of specific tyrosine residues by activating kinases and the relative effect of this phosphorylation on inhibitory phosphatases appear to be pivotal determinants of NK cell activation.

NK cells kill virus-infected or malignant cells by the release of perforin and granzymes from granular storage compartments and by binding of the death receptors Fas and TRAIL-R on target cells via their respective NK cell ligands.[513,542,543] The mechanisms by which perforin and granzymes mediate target cell death are not fully understood. The best available evidence suggests that perforin and one or more of five human granzymes, released along with perforin from cytotoxic granules of NK cells, associate with the cell membranes of target cells, either by binding via the mannose 6-phosphate receptor or by another mechanism that remains to be defined. One or more of the granzymes appear to activate intracellular pathways leading to target cell apoptosis via pathways that involve the mitochondria or caspases or both.[319,584] Separately, binding of the death receptors also activates caspases, causing target cell apoptosis.[542] It is notable that, although some tumor cells do not express Fas, NK cells can induce Fas expression on these targets by releasing IFN-γ then proceed to kill them by binding to the newly expressed Fas.[523]

NK cells engage in several kinds of interactions with other cells of the immune system, including dendritic cells and other antigen-presenting cells. Dendritic cells can influence the proliferation and activation of NK cells both by release of cytokines, including IL-12, and by cell surface interactions, including CD40/CD40L, LFA-1/ICAM-1, and CD27/CD70.[173] In return, NK cells can provide signals that result in either dendritic cell maturation or apoptosis.[126,543]

Complement System

The complement system consists of more than 30 different free and membrane-bound activation and regulatory proteins. It has multiple key roles in the clearance of invading microbes, including opsonization, recruitment of phagocytic cells, and lytic destruction of pathogens.* Complement and antibody often act synergistically in host defense against infection. Activation of the alternative or mannan-binding lectin (MBL) pathways of the complement system during the host's initial encounter with an organism occurs before specific antibody can be produced. Later, when specific antibody is available, it serves to activate complement more efficiently (via the classical pathway) and to direct complement binding to locations on the microbial surface that support the optimal execution of its effector functions, such as opsonization and killing.

Approximately 90 percent of complement proteins are synthesized in the liver, but some components can be produced locally at sites of infection by tissue mononuclear phagocytes and fibroblasts.[143,463] In healthy persons, the majority of complement is found in the circulation; less than 10 percent is in mucosal secretions, and little is detectable in cerebrospinal fluid. Circulating complement levels vary over time, particularly in the presence of inflammation. The inflammatory response may lead to increases in levels of those complement components such as C3 that are acute-phase reactants or to decreases in

*References 62, 177, 178, 196, 198, 217, 306, 307, 309, 416.

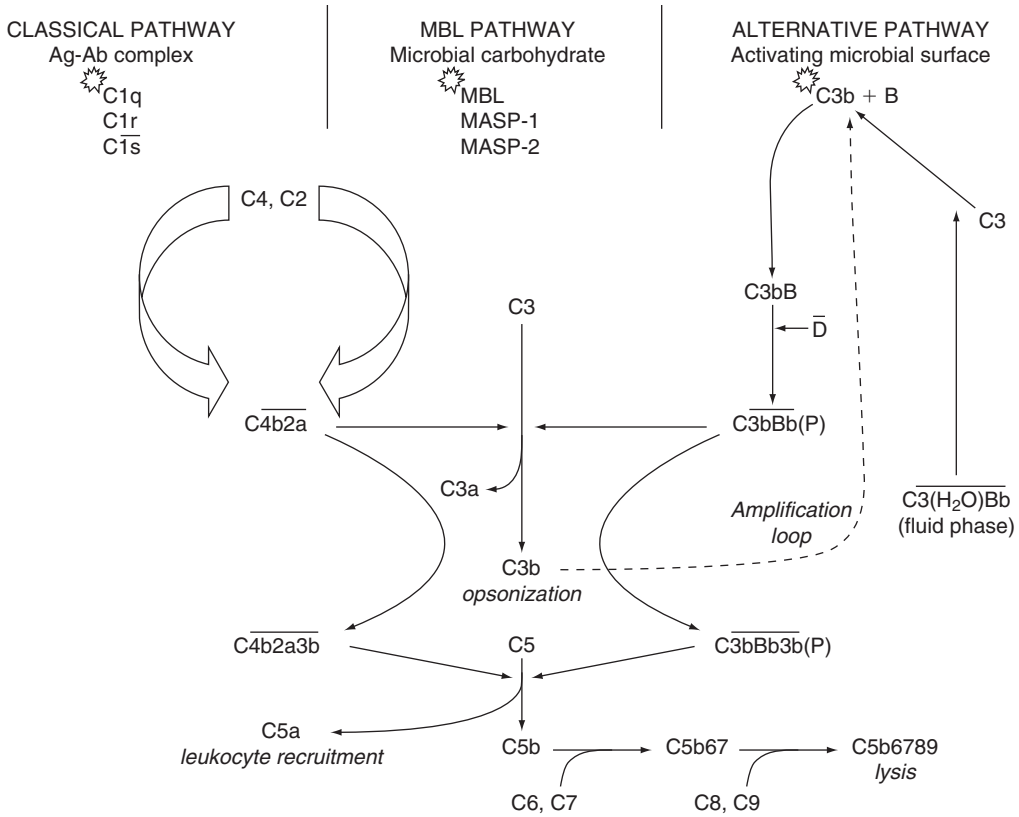

FIGURE 2-1 ■ The complement cascade. The initial binding events of the classical, mannan-binding lectin *(MBL),* and alternative pathways are indicated by a *small starburst.* These pathways intersect at the conversion of *C3* to *C3b.* This is followed by activation of the terminal components, beginning with the binding and cleavage of *C5,* releasing *C5a* and leaving bound *C5b* to initiate assembly of the remaining components to form the membrane attack complex *(C5b6789).* Enzymatically active proteases of the classical and alternative pathways that cleave and activate subsequent components, are, by convention, shown with an *overbar.* The alternative pathway *C3* and *C5* convertases are shown associated with properdin (P), which increases their stability. *B,* Complement factor B; *D,* complement factor D; *MASP,* MBL-associated serine proteases.

individual components and total complement activity as a result of consumption.

The importance of normal complement component levels and activity in host defense has been well established and is based primarily on the increased susceptibility of patients with specific complement component deficiencies to recurrent or severe bacterial infections.[176,177,196,306,307] Although the complement response to infection usually is beneficial to the host, it also may be associated with adverse clinical manifestations, such as septic shock and acute respiratory distress syndrome.[165,212,610]

Complement Activation. Complement proteins are activated in a specific sequence or "cascade" via one of at least three pathways, the classical pathway, the alternative pathway, and the more recently described MBL pathway, as shown in Figure 2-1. These pathways converge at C3, and the complement cascade downstream from C3 proceeds identically, irrespective of the pathway by which activation occurs. The C3 convertases, C4b2a for the classical and MBL pathways, and C3bBb for the alternative pathway, cleave the C3 molecule at exactly the same location, producing C3b, which binds to the target surface, and C3a, which is released into the fluid phase. Cleavage and activation of C3 lead to a conformational change in C3b that transiently renders its reactive thioester group capable of forming covalent ester or amide bonds with acceptor molecules on the target surface.[291,353] If the acceptor molecules are situated on the surface of a microorganism, the bound C3b can act as an opsonin to promote phagocytosis or it can bind with the classical and alternative pathway C3 convertases to form the C5 convertases, C4b2a3b and C3bBb3b, respectively.[478] C5 convertases bind and then cleave C5, with release of the C5a fragment into the fluid phase. The bound C5b fragment then can initiate formation of the membrane attack complex by the sequential incorporation of the remaining terminal components, C6, C7, C8, and multiple molecules of C9. The membrane attack complex can insert into the outer membrane of target cells, such as erythrocytes or gram-negative bacteria, and cause cell lysis and death.[309]

The Classical Pathway. Ordinarily, the classical pathway is activated by IgM or IgG bound to microbial antigenic targets or by other kinds of antigen–antibody complexes.[178] IgM activates complement more efficiently than IgG because only one molecule of polymeric IgM is required, in contrast to at least two molecules of IgG.[151] Activation is initiated when C1q binds directly to an immunoglobulin molecule on the surface of an organism or, less often, to a surface molecule of the organism itself. C1r and C1s are activated and bound to C1q sequentially,

forming C1qrs. The enzymatic activity of this complex, which resides in the C1s molecule, can cleave multiple molecules of C4 and C2 into two fragments each. The C4a and C2b fragments are released into the environment, whereas C4b and C2a remain bound to each other on the target surface to form the classical pathway C3 convertase, C4b2a. C4bC2a can cleave and activate C3 and localize C3b binding to nearby sites on the target surface. As noted earlier, some C3b binds with C4b2a to form the classical pathway C5 convertase, C4b2a3b. Classical pathway activation ordinarily is not initiated by complexes of antigens with IgG4, IgA, IgD, or IgE.

The Alternative Pathway. Whereas the classical pathway requires specific antibody, and thus contributes to host defense in immune individuals, the alternative pathway is more important in protection of nonimmune hosts, such as premature infants who have low levels of transplacentally acquired antibody and older infants and young children whose maternal antibody has waned but who have not yet produced their own specific antibodies. It can be initiated by microbial surface macromolecules (e.g., polysaccharide, lipopolysaccharide, and teichoic acid), although, as noted earlier, in some circumstances specific antibody increases alternative pathway efficiency and may direct the location of C3b binding.[309]

A spontaneous low level of hydrolysis of the thioester of C3 in the fluid phase results in an activated form of C3, C3(H_2O). This activated form of C3 can bind factor B, and the latter is then cleaved by factor D to form the fluid phase C3 convertase C3(H_2O)Bb. The constitutive presence of small amounts of this convertase in the fluid phase ensures that a small amount of C3b always is available to bind to microbial surfaces and initiate the alternative pathway.[457] The alternative pathway protein factor B has structural and functional similarities to those of C2, including the ability to bind to surface-bound C3b. Once bound to C3b, factor B undergoes proteolytic cleavage by factor D to release a small soluble fragment, Ba, leaving the larger fragment, Bb, associated with C3b. C3bBb, the alternative pathway C3 convertase, is analogous to the classical pathway C3 convertase, C4b2a. Properdin stabilizes the C3 convertase C3bBb, permitting more efficient activation of C3 to form more C3b, creating the C3 amplification loop (see Fig. 2-1).[211,214] Alternative pathway activation of C3 by this mechanism is several times less efficient than activation via the classical pathway, but it is vital to host defense because it is the principal means by which a nonimmune host can activate the effector functions of complement until a specific antibody response can be mounted.[175,214]

The most important factor in determining whether a specific microbial pathogen will activate the alternative pathway is the biochemical nature of its surface. There is only a partial understanding of the surface biochemical features of a microorganism that characterize it as an activator. However, it is well known that microorganisms that bear large amounts of surface sialic acid are usually nonactivators. In fact, the expression of surface sialic acid is one means by which mammalian host cells are protected from complement-mediated lysis in vivo.[347] On surfaces rich in sialic acid, bound C3b is less able to bind factor B, because another molecule, factor H, has a strong competitive advantage over factor B under these conditions. When bound by factor H, C3b becomes highly susceptible to further cleavage by factor I (C3b inactivator) resulting in C3bi (or iC3b). Although C3bi can function as an opsonin, it cannot bind factor B. Thus, no alternative pathway convertases can be formed and no amplification loop is established.[62,416] Organisms whose surfaces do not support activation of the alternative pathway are some of the most successful pathogens in infants and young children, who often lack specific humoral immunity. They include K1 *Escherichia coli*, groups A and B streptococci, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae* type b, and some salmonellae.[122,309] Development of specific IgG or IgM antibody against these organisms permits efficient activation of the classical pathway of complement at their surfaces and correlates with protection.

The Mannan-Binding Lectin Pathway. The most recently described complement activation pathway is the MBL pathway. It is similar to the classical pathway but does not involve antibodies. MBL is a serum protein of the collectin family that has structural and functional similarities to those of C1q. However, it does not require antigen–antibody complexes to initiate its complement-activating function. MBL binds to mannose-containing carbohydrates on microbial surfaces, leading to its association at the microbial surface with activated MBL-associated serine proteases (MASP-1 and -2). These latter proteases have structural and functional similarities to C1r and C1s, respectively, and result in activation of C4, with sequential binding of C4b and C2a and formation of C4b2a, the C3 convertase of the classical pathway. C3 is activated, and the cascade proceeds as described. A more detailed characterization of the MBL pathway and its role in immune responses to infection may be found in an excellent review.[465]

Effector Functions of Complement in Host Defense. All complement effector functions in host defense against infection require activation of the complement cascade through at least C3. The cascade needs to be activated only through C3 for effective opsonization, stimulation of leukocytosis, and immune regulation. Activation through C5 is required to produce a normal inflammatory response, including recruitment of phagocytes to sites of inflammation, and activation through C8 is needed for formation of the membrane attack complex. Complement may be activated and bound to cell surfaces but not able to carry out its effector functions if it is bound at a disadvantageous location.[97,250,290] For example, C3b bound to pneumococcal cell wall underneath a thick polysaccharide capsule is not accessible to CR1 on phagocytes and therefore does not promote effective opsonophagocytosis. Similarly, complement-mediated killing of some strains of *Salmonella* is prevented when the membrane attack complex is bound to long lipopolysaccharide molecules distant from the organism's cell membrane.[309]

Opsonic Activity. Complement opsonic activity is essential for effective removal of organisms from the circulation by macrophages in the liver and spleen and from other sites by neutrophils and tissue macrophages.[82] Opsonins facilitate recognition, binding, ingestion, and

killing of microorganisms by phagocytes. Opsonization particularly is important for protection against gram-positive bacteria and fungi because their thick cell walls prevent them from being killed by the membrane attack complex.

As noted earlier, activation of C3 leads to a conformational change in C3b that permits its reactive thioester to bind covalently with acceptor molecules on microbial surfaces, where it can serve as an opsonin.[121] C3b also can be cleaved by inactivators such as factor I and CR1 to form iC3b, which may be a more efficient opsonin than C3b.[250,291] Some organisms, including certain serotypes of pneumococci, have surfaces that support degradation of differing amounts of surface-bound C3b to iC3b.[290] Surface-bound C3b and iC3b permit microbes to be recognized by circulating and tissue phagocytes by interacting with the phagocyte surface complement receptors, CR1 (CD35) and CR3 (CD11b/CD18), respectively. These interactions lead to binding, ingestion, and intracellular killing of the organisms.[250,291,353]

Antibodies, especially of the IgG class, are important opsonins in their own right, but they also facilitate more rapid complement activation and more effective localization of C3b binding to the surface of the organism. In the absence of specific antibody to direct complement binding, complement deposition on the surface of the target will be determined principally by the nature of the surface, and effector functions such as opsonization may not be carried out as successfully. As noted earlier, this is of particular importance for encapsulated organisms such as pneumococci, because in the absence of specific anticapsular antibody, most C3b will be deposited at the cell wall, where it is inaccessible to phagocyte receptors.[97,290,311]

Inflammation. The cleavage products of several complement proteins contribute to the development of an inflammatory response. C3a stimulates an increase in the number of circulating granulocytes, and C5a serves as a potent stimulus for monocyte, neutrophil, and eosinophil migration toward the source of C5a gradients being produced at infected tissue sites. C5a also upregulates phagocyte expression of CR1 and CR3 and stimulates them to release stored enzymes and other granular contents that also are important mediators of inflammation, aggregation, and production of microbicidal oxidants. C5a-induced neutrophil aggregation and stasis in the pulmonary circulation can be an important feature of the respiratory distress syndrome associated with sepsis.[610]

The anaphylatoxins, C4a, C3a, and especially C5a, induce release of histamine from mast cells and basophils, causing increased vascular dilation and permeability, which, in turn, permit local influx of other inflammatory mediators.[294,610] In this way, they help produce the hallmark clinical manifestations of inflammation, swelling, and erythema. When large quantities of anaphylatoxins are released rapidly, they can contribute to septic shock.[212]

Microbicidal Activity. Complement can act directly on certain bacteria to kill them. As noted earlier, C5b and the terminal complement proteins C6, C7, C8, and C9 form the membrane attack complex, which can kill and lyse target cells such as gram-negative bacteria by penetrating their outer membranes.[309] The C5b-C8 complex serves as a polymerization site for several molecules of C9.[71] Although C9 is not essential to membrane penetration, its presence as poly-C9 allows it to proceed more efficiently.[569] Electron microscopy of the membrane attack complex demonstrates that it is composed of a ringlike structure at the outer surface of the target cell membrane and a perpendicular cylindrical component that penetrates the cell membrane. As has been noted, the membrane attack complex cannot penetrate the thick cell walls of gram-positive bacteria and fungi and therefore cannot kill these organisms directly. The membrane attack complex can lyse some virus-infected host cells and some enveloped viruses themselves.[152]

Immune Regulation. Complement components and fragments can modulate several features of the immune response, both directly by binding to CR1, CR2, and CR3 on the surfaces of T cells, B cells, and other cells involved in antigen recognition and indirectly by stimulating the synthesis and release of cytokines.[204] For example, the C3b cleavage product, C3dg, when covalently bound to antigen, brings the antigen close to B cells by binding to B-cell CR2 (CD21).[73,88,119] Complement decreases the amount of antigen required to induce an immune response and increases the efficiency of the process by facilitating antigen presentation and localization. C3 influences antigenic localization within germinal centers, and it is involved in anamnestic responses and isotype switching. The importance of complement in regulating the adaptive immune response has been suggested by the finding that C1-, C2-, C4-, and C3-deficient animals have decreased antibody responses that can be restored by providing the missing protein.[73,88,119] Deficiency of early complement components, such as C2, in humans also has been associated with antibody deficiencies.[17,119]

Phagocytes

The first recognized cellular mechanism of host defense was the accumulation of phagocytic host cells around a foreign body in starfish observed by Metchnikoff.[397] PMNs, the most abundant circulating phagocytes in the human host, will serve as a model for discussing phagocyte functions. These cells constitute a major line of defense against invading bacteria and fungi. The proliferation of myeloid marrow progenitors and their differentiation into mature progeny are regulated by specific growth factors and cytokines.[47,367,368,577] The normal half-life of circulating PMNs is approximately 8 to 12 hours.[388,604] In the absence of active infection, most PMNs leave the circulation via the gingival crevices and the lower gastrointestinal tract, where the resident flora stimulate ongoing local extravasation of PMNs, a process that helps maintain the integrity of these tissues.[31] In response to invasive bacterial infection, circulating PMNs engage in three major functions: (1) migration to the site of infection, (2) recognition and ingestion of invading microorganisms, and (3) killing and digestion of these organisms.

Phagocyte Recruitment to Infected Sites. Activation of endothelial cells that line the microvessels of acutely

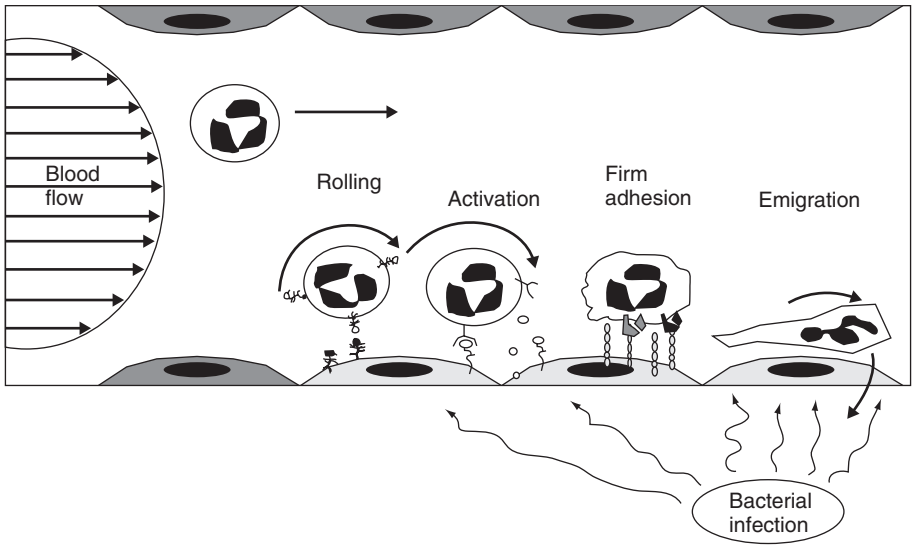

FIGURE 2-2 ■ Events during leukocyte (polymorphonuclear leukocytes [PMNs]) recruitment to infected sites. Interactions between microorganisms in infected tissue and host cells and proteins result in elaboration of mediators that diffuse to the local microcirculation and stimulate the endothelial cells. This induces new surface expression of P-selectin and E-selectin, release of interleukin-8 and other chemokines, and new surface expression of intercellular adhesion molecule 1 (ICAM-1). The endothelial selectins bind to constitutively expressed carbohydrate ligands on circulating PMNs and slow the passage of the PMNs through the microvessels. As the PMNs slow further, they become activated by interaction with chemokines bound to complex glycopeptides on the endothelial surface. This activation of PMNs increases their expression and binding activity of the β_2 (CD11/CD18) integrins, Mac-1 and lymphocyte function–associated antigen–1 (LFA-1). Interactions between these integrins and ICAM-1 (and ICAM-2 in the case of LFA-1) lead to tight adhesion and spreading on the endothelial surface. These latter adhesive interactions also are used for migration between endothelial cells and through the subjacent extracellular matrix in response to the gradient of chemoattractants, such as C5a, chemokines, and bacterial peptides, released at the infected site. Homophilic interactions between PECAM-1 on the PMNs and endothelial cells (not diagrammed) also appear to contribute to transendothelial migration. (Courtesy Scott Seo, MD.)

infected tissue occurs via locally produced cytokines, eicosanoid compounds, and microbial products.[115,534] As a result, the endothelial cells rapidly upregulate their surface expression of P-selectin from preformed intracellular storage pools, and subsequently of E-selectin by new synthesis.[366,537] These selectins engage in lectin-like interactions with the fucosylated tetrasaccharide moiety sialyl Lewis X, which is presented on constitutively expressed glycoproteins on PMNs, including L-selectin and P-selectin glycoprotein ligand–1 (PSGL-1).[350,366,631] These early interactions slow the PMNs in this first adhesive phase of leukocyte recruitment, sometimes described as "slow rolling."[70,115,534] Within several hours, newly synthesized ICAM-1 is expressed at the endothelial surface.[115,534,536] The slowly rolling PMNs are activated by transient selectin-mediated interactions and locally produced mediators, especially endothelium-derived chemokines, such as IL-8.[365] These chemokines are most effective in activating the PMNs when they are bound by complex proteoglycans at the endothelial cell surface.[346,607] The activated PMNs then signal the conformational activation of binding function of their surface β_2 integrins LFA-1 and Mac-1,[179,595] as well as translocating an additional large quantity of Mac-1 from intracellular storage pools to the cell surface.[63,84,86] This newly translocated Mac-1 also may undergo conformational activation as the PMN is exposed to increasing concentrations of activating mediators.[179,282] These activated β_2 integrins interact with the endothelial cell ICAM-1 in this second, firm adhesion phase mediated by integrin-ICAM interactions, which is ultimately necessary for transendothelial migration of the PMNs.* Other chemoattractants, such as C5a, N-formyl bacterial oligopeptides, and leukotrienes (e.g., LTB_4) that diffuse from the site of infection, further activate PMNs and provide a chemotactic gradient for PMN migration into tissue.[186,241,419] The receptors for these chemoattractants, like the chemokine receptors, are G-protein coupled, and have a seven transmembrane–domain structure.[241,419] They constitute important sensory mechanisms of the PMNs for activating adhesion, directional orientation, and the contractile protein-dependent lateral movement of adhesion sites in the PMN membrane necessary for locomotion.[26,241,419,561] A scheme for PMN recruitment from the microcirculation into infected tissue is presented in Figure 2-2, and a diagram depicting more specifically the interactions among the surface molecules that mediate PMN–endothelial adhesion is provided in Figure 2-3. Although the specific stimuli and adhesion molecules may vary, this general scheme applies to the local recruitment of virtually all circulating cells of the immune system.[75,244,323]

Phagocytosis. After PMNs reach the site of infection, they must recognize and ingest, or phagocytose, the invading bacteria. Opsonization, especially with IgG and fragments of C3, greatly enhances phagocytosis.[290,310] Although nonopsonic phagocytosis may occur, only opsonin-mediated phagocytosis is considered here.[511,580]

*References 63, 115, 180, 365, 525, 534, 536.

CR1 and CR3 are the main phagocytic receptors for opsonic C3b and iC3b, respectively.[63-65,213] When PMNs are activated by chemoattractants or other stimuli, CR1 and CR3 are rapidly translocated to the cell surface from intracellular storage compartments, increasing surface expression up to 10-fold.[63,213] Note that CR3 is identical to the adhesion-mediating integrin Mac-1.[36,63] CR1 and CR3 act synergistically with receptors for the Fc portion of antibodies, especially IgG.[37,310] Phagocytic cells may express up to three different types of IgG Fc receptors, or FcγRs, all of which can mediate phagocytosis.[209,587] FcγRI (CD64) is a high-affinity receptor that is expressed mainly on mononuclear phagocytes.[587] The two FcγRs ordinarily expressed on circulating PMN are FcγRII (CD32) and FcγRIII (CD16).[577,587] FcγRII is conventionally anchored in the cell membrane, exhibits polymorphisms that determine preferences for binding of certain IgG subclasses, and can directly activate PMN oxidative burst activity.[577,587,588] FcγRIII is expressed on PMNs as a glycolipid-anchored protein, although it is anchored conventionally on NK cells and macrophages.[510,579,587] Most phagocytes also express IgA receptors. The best-characterized FcαR, CD89, binds monomeric IgA and promotes phagocytosis and killing of IgA-opsonized bacteria.[292,409]

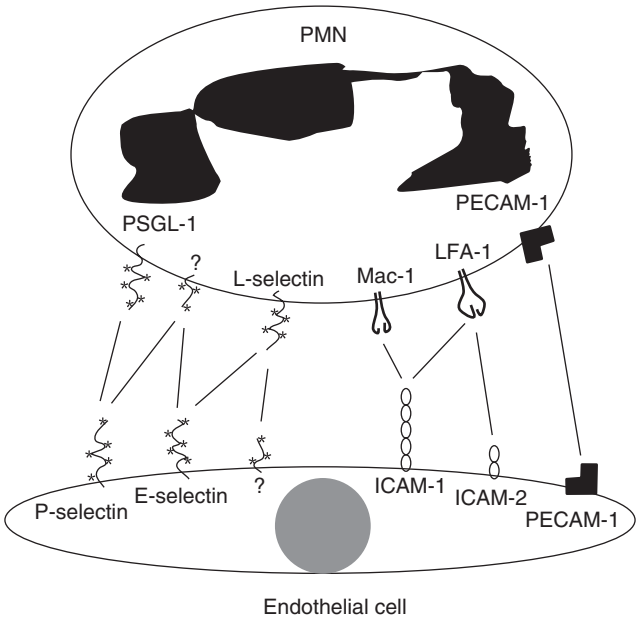

FIGURE 2-3 ■ Molecular interactions that mediate polymorphonuclear leukocyte *(PMN)*-endothelial adhesion. The selectins, P-selectin and E-selectin on endothelium and L-selectin on leukocytes, bind to oligosaccharide moieties, especially sialyl Lewis X (sLeX, represented by asterisks [*]), that decorate the selectins and other glycoproteins on the opposite cell, including P-selectin glycoprotein ligand–1 *(PSGL-1)* on leukocytes. These interactions mediate the early adhesive phase of leukocyte recruitment. The β_2 integrins macrophage antigen 1 *(Mac-1)* (CD11b/CD18) and lymphocyte function–associated antigen–1 *(LFA-1)* (CD11a/CD18) on *PMNs* both bind intercellular adhesion molecule 1 *(ICAM-1)* on endothelial cells. LFA-1 also can bind *ICAM-2,* which is expressed constitutively on endothelium. Platelet-endothelial cell adhesion molecule-1 *(PECAM-1)* molecules interact with each other and are expressed on both PMNs and endothelial cells.

The engagement of phagocyte receptors with microbial opsonins on microbes locally activates cytoskeletal contractile elements, leading to invagination of the membrane at the site of initial engagement and the extension of pseudopods around the microbe. The ligation of additional opsonin-receptor pairs typically leads to engulfment of the microbe within a sealed phagosome,[560] as depicted in Figure 2-4. This is followed by fusion of the phagosome with lysosomal compartments containing the phagocyte's array of microbicidal products.

Phagocyte Microbicidal Mechanisms. The microbicidal mechanisms of PMNs can be categorized as either oxygen-dependent or oxygen-independent.[492] Intracellular killing usually takes place after the phagosome fuses with one or more types of lysosomal granules that contain many of the cell's microbicidal weapons. The contents of the three major populations of intracellular granules of PMN, which contain many of the PMN's microbicidal molecules, either free within the granules or anchored in the granule membrane, are shown in Table 2-3.[84,85,87,235]

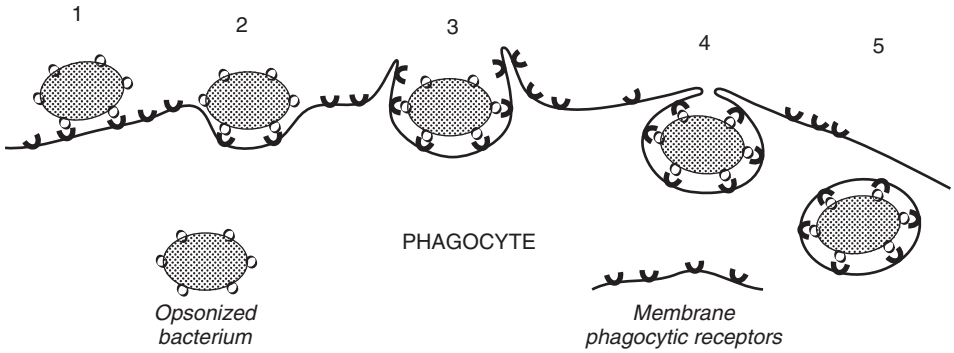

FIGURE 2-4 ■ Opsonin/receptor-mediated phagocytosis. Phagocytes such as polymorphonuclear leukocytes (PMNs) that reach the site of bacterial infection are activated for enhanced recognition, attachment, and ingestion of bacteria that have been coated with opsonic C3 fragments, IgG, or both via specific receptors on the PMN surface. Binding of opsonized bacteria to opsonin receptors *(1),* activates contractile elements of the cytoskeleton to produce an invagination at the initial site of attachment *(2),* with subsequent extension of membrane pseudopods around the organism *(3).* This in turn allows engagement of additional opsonin-receptor pairs, as the organism becomes engulfed *(4).* Finally, the plasma membrane fuses completely around the bacteria to create the phagosome *(5),* which soon will fuse with lysosomal granules, exposing the bacterium to the microbicidal components of the phagocyte.

TABLE 2-3 **Stored Contents of Neutrophil Granules and Vesicles**

Primary (Azurophilic) Granules	Secondary (Specific) Granules*	Tertiary Granules†	Secretory Vesicles‡
Elastase	Lactoferrin	Gelatinase	Alkaline phosphatase
Cathepsin G	Vitamin B_{12}–binding protein		
Myeloperoxidase	Lysozyme		
Defensins	Gelatinase		
Bactericidal/permeability-increasing protein			
"p15s"			
Cathelicidin (LL37)			
Lysozyme			

Selected membrane-bound proteins in intracellular granules and vesicles:
*,†,‡f-met-leu-phe receptor.
‡Type 1 complement receptor (CR1).
*,†,‡CD11b.

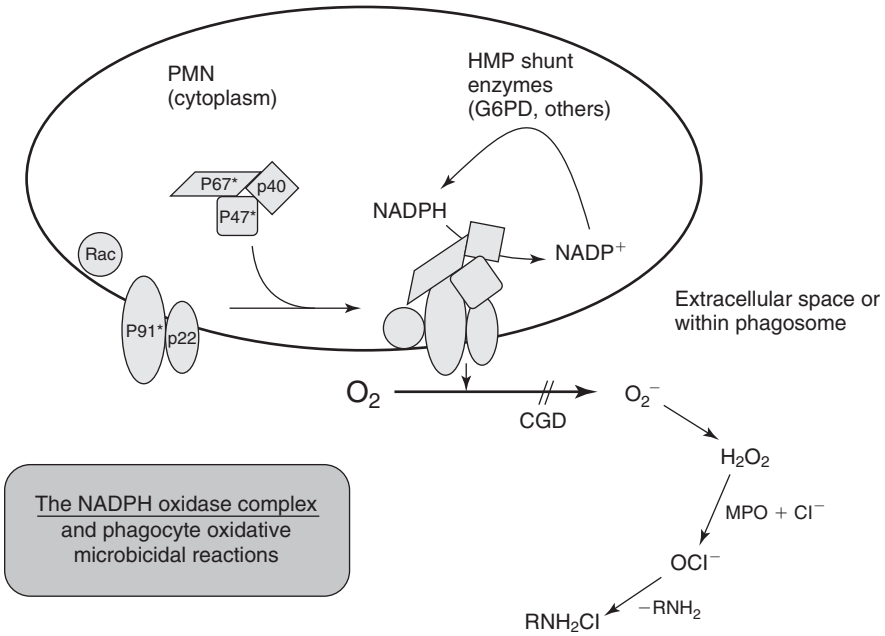

FIGURE 2-5 ■ The phagocyte reduced nicotinamide adenine dinucleotide phosphate *(NADPH)*-oxidase enzyme complex and the major reactions in the evolution of oxygen-dependent PMN microbicidal activity. The diagram depicts the six main components of the NADPH oxidase complex: the 91-kDa and 22-kDa subunits of the membrane-bound cytochrome, the 40-kDa, 47-kDa, and 67-kDa cytosolic components, and a Rac-1 signaling molecule. Components designated by an asterisk (*) are those for which a mutation is known to result in chronic granulomatous disease (CGD, see later). After assembly at the plasma or phagolysosome membrane, the enzyme catalyzes the conversion of molecular oxygen (O_2) to superoxide anion (O_2^-), the initial step in the sequence of production of oxidant antimicrobial products. This reaction requires a supply of NADPH, most of which is derived from activity of enzymes of the hexose monophosphate shunt (not shown). Shown in sequence are subsequent reactions for the spontaneous formation of hydrogen peroxide (H_2O_2), the myeloperoxidase-catalyzed formation of hypochlorite (OCl^-), and formation of chloramines (RNH_2Cl).

The oxygen-dependent microbicidal mechanisms of phagocytes depend on a complex enzyme, reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which catalyzes the conversion of molecular oxygen (O_2) to superoxide anion (O_2^-), the reaction that is deficient in chronic granulomatous disease (see later discussion).[41,42,135] As the name suggests, the reaction catalyzed by this enzyme requires a supply of NADPH, which is supplied in turn by reactions of enzymes of the hexose monophosphate shunt. The NADPH oxidase is assembled at the activated cell membrane from six known components that include a cytochrome (α- and β-subunits, designated gp91^{phox} and p22^{phox}, respectively), and at least three cytosolic proteins, p40^{phox}, p47^{phox} and p67^{phox} ("phox" refers to phagocyte oxidase), along with a Rac-1 GTPase that assemble with the membrane-associated components to form the active enzyme complex.[42,84,135] Each of the main oxidant products derived from these reactions exhibits microbicidal activity, including the earliest products, O_2^- and H_2O_2, and the more potent downstream products hypochlorite (OCl^-) and chloramines (NH_3Cl, RNH_2Cl), chloramines being the most stable.[258,492] Figure 2-5 depicts assembly of the enzyme complex at the plasma or phagosomal membrane and the sequence of the main

oxidative reactions of PMN starting with the formation of superoxide anion.

The oxygen-independent microbicidal activity of PMNs resides mainly in a group of proteins and peptides stored within their primary (azurophilic) granules. Lysozyme is contained in both the primary and the secondary (specific) granules of PMN.[551] It cleaves important linkages in the peptidoglycan of bacterial cell walls and is most effective when it can act in concert with the complement MAC.[310] The primary granules contain several cationic proteins with important microbicidal activity. A 59-kDa protein, bactericidal/permeability-increasing protein, is active against only gram-negative bacteria.[612] Smaller arginine- and cysteine-rich peptides, the α-defensins, similar to the β-defensins of epithelial cells, are active against a range of bacteria, fungi, chlamydiae, and enveloped viruses, and other related molecules include cathelicidin and a group of peptides called p15s.[230,234,235,359,362] The mechanisms of action of these peptides is not fully understood, although this is addressed earlier in the section on antimicrobial peptides of epithelial cells. Some of these PMN proteins and peptides interact with each other synergistically to enhance overall antimicrobial activity.[363]

Important Interactions Among Innate Immune Mechanisms

A schematic overview of many of the main features of innate immunity discussed earlier, along with some of their important interactions, is diagrammed in Figure 2-6. Several levels of interactions are depicted, from initial host–pathogen contact, through a variety of activating signals, to the attack by host effector mechanisms on their respective pathogenic targets.[576]

Adaptive Immune Responses. Adaptive immunity involves the host's antigen-specific responses to infectious challenges that can provide specific protection against subsequent challenges by the same infectious agent. The major steps in the development of adaptive immunity include the processing and presentation of specific antigens to T lymphocytes (T cells) by antigen-presenting cells; the activation and differentiation of T cells for specific cytotoxic T-cell activity, T-cell cytokine production, and T-cell help in activating antigen-specific B cells; and the differentiation of activated B cells into plasma cells for the production of specific antibodies. However, the innate immune responses described earlier

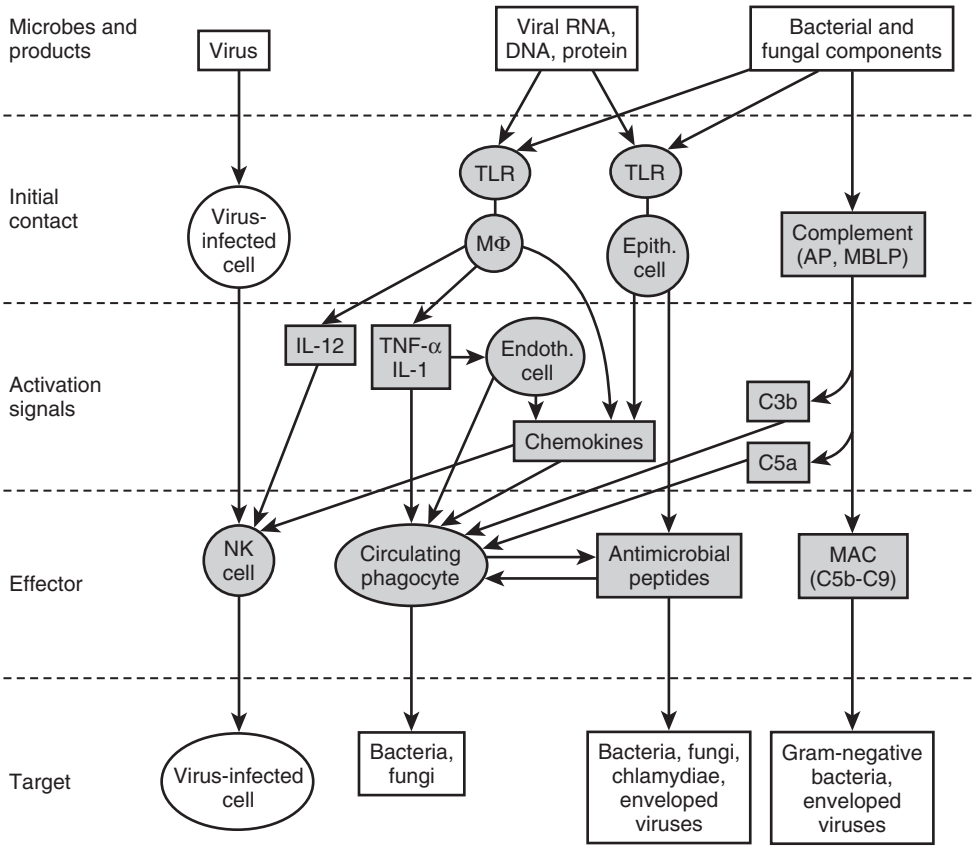

FIGURE 2-6 ■ Innate immunity: First contact, intermediate signals, and effector mechanisms. Diagrammed are important host responses to infection that are independent of specific cell-mediated immunity or antibodies. Initial contact between the host and microbes or their products may result in viral infection of cells, activation of Toll-like receptors *(TLR)* on macrophages *(MΦ)* and epithelial cells, and activation of the alternative pathway *(AP)* or mannose-binding lectin pathway *(MBLP)* of complement. The resulting activation signals, including cytokines (e.g., interleukin *[IL]-12,* tumor necrosis factor α *[TNF-α], IL-1*), chemokines, and products of the complement cascade mobilize both cellular (natural killer *[NK]* cells, phagocytes) and humoral (antimicrobial peptides, membrane attack complex *[MAC]*) effectors that attack their respective microbial targets. (From Tosi MF. Innate immune responses to infection. *J Allergy Clin Immunol* 2005;**116**:241–9.)

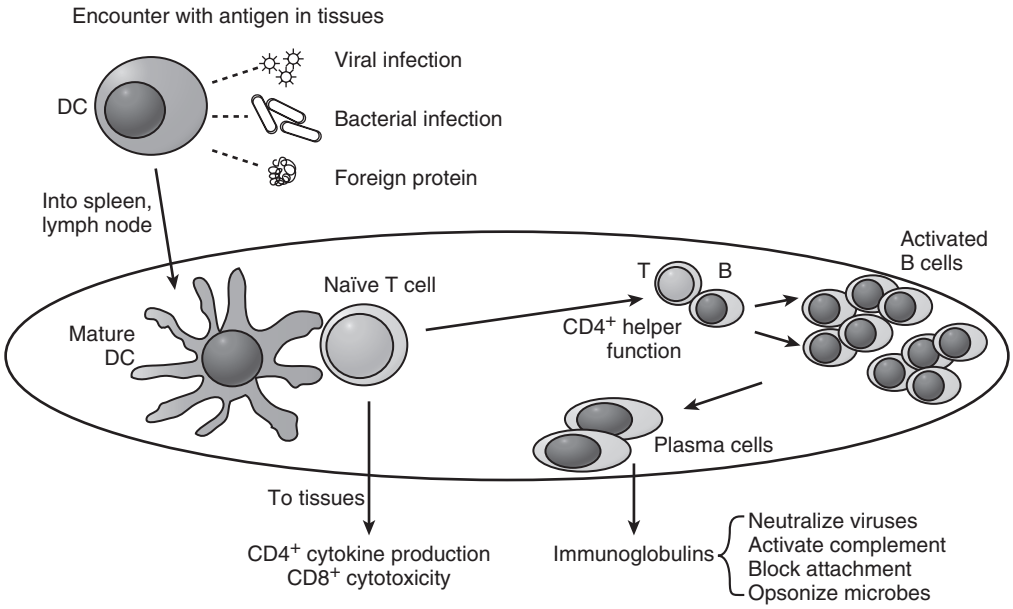

FIGURE 2-7 ■ A simplified scheme of major events in the development of adaptive immune responses. When antigen-presenting cells (APCs), such as dendritic cells *(DCs)*, encounter and internalize microbes or their protein antigens in peripheral tissues, they process the microbial proteins and present the resulting antigenic peptides on either class I or class II MHC molecules. The activated APCs migrate to lymphoid tissue, where they undergo maturation. When mature dendritic cells encounter $CD4^+$ or $CD8^+$ T cells expressing T-cell receptors specific for the peptides presented in the appropriate major histocompatibility complex (MHC) context (CD4/MHC-II; CD8/MHC-I), binding between the cells occurs via TCR-peptide, MHC-CD4/8, and other pairs of accessory molecules, all necessary for stimulating the T cells to become effector cells. Cytotoxic effector $CD8^+$ T cells migrate into the periphery and kill virus-infected cells that present viral peptides via MHC class I. Effector $CD4^+$ cells either migrate to the periphery where they produce cytokines and otherwise regulate immune responses or remain in the lymphoid tissue to provide help to antigen-specific B cells, promoting their proliferation, differentiation, and eventual production by their progeny plasma cells of specific antibodies that can neutralize viruses, prevent microbial attachment, opsonize microorganisms, or activate complement.

often occur in a matter of minutes to hours and may activate early cellular responses that are essential for the development of adaptive immunity, the full development of most adaptive immune responses requires days to weeks. Once developed, however, the latter often can provide durable protection. A summary of the major events in the adaptive immune response to infection is diagrammed in Figure 2-7.

Antigen Presentation and Specific Cell-Mediated Immunity

Specific cell-mediated immunity provides T-cell help for antibody production by B cells, cytokine production for the stimulation and regulation of a range of immune responses, and cytotoxic T-cell activity against host cells infected with viruses.[184,415,461] The development of cell-mediated immunity requires complex interactions between T cells and antigen-presenting cells via several types of surface molecules on the respective cell surfaces. These include binding of an antigen-specific T-cell receptor on the T lymphocyte to a peptide antigen presented on the class I or II MHC by the antigen-presenting cells, with concurrent binding of the class I or class II MHC by CD8 or CD4, respectively,[153,574] as represented in Figure 2-8. Other respective pairs of accessory cell-surface molecules that enhance interactions between T cells and antigen-presenting cells include CD40 ligand/CD40, LFA-1/ICAM-1, and CD28/B7. An additional molecule, cytotoxic T lymphocyte antigen–4 (CTLA-4), expressed on activated T cells, also can bind to B7 molecules on APCs to generate a suppressive signal that may terminate T cell activation.[574] The sustained physical interface between T cells and antigen-presenting cells at which these molecular interactions take place has been characterized as the "immunologic synapse."[34,96,252]

Class I Major Histocompatibility Complex. Virtually all human cells, except neurons, express class I MHC.[161,162] The class I MHC molecule presents antigenic peptides to $CD8^+$ cytotoxic T lymphocytes.[70,431] It consists of a heavy chain that contains both the peptide-binding domain and a transmembrane domain and a smaller extracellular subunit, β_2-microglobulin.[77,488] The three major types of class I MHC heavy chains in humans, human leukocyte antigen (HLA)-A, HLA-B, and HLA-C, have at least 22, 31, and 12 different alleles, respectively.[630] This polymorphism permits a great diversity in the peptide-binding repertoire in individuals and within populations. A restricted degree of MHC genetic polymorphism has been invoked as a possible explanation for the predisposition of certain populations to develop infections.[78] Class I MHC molecules within the cell ordinarily bind peptides derived from recently synthesized proteins, either of self-origin or of infecting viruses.[207,208] A portion of newly synthesized proteins is processed into peptides at a cytoplasmic site, the proteasome.[245] These peptides are actively transported into the endoplasmic reticulum, where they are bound in the peptide-binding cleft of MHC class I. Suitable peptides usually are

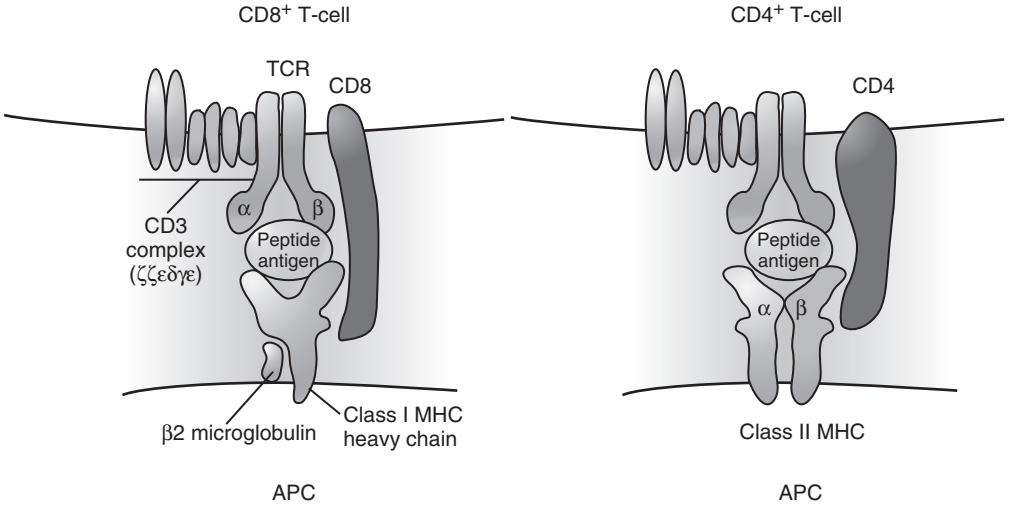

FIGURE 2-8 ■ Principal cell surface interactions between CD8 and CD4 T lymphocytes and peptide antigens complexed with major histocompatibility complex *(MHC)* class I and class II molecules, respectively. CD3 (composed of six subunits, *ζ, ζ, ε, δ, γ, ε)* is associated closely with the T-cell receptor *(TCR),* which recognizes a specific peptide presented on MHC molecules. Class I and class II MHC determinants are recognized by CD8 and CD4, respectively. Additional or accessory interactions are discussed in the text. *APC,* Antigen-presenting cell. (Modified from Lewis DB, Wilson CB. Developmental immunology and role of host defenses in neonatal susceptibility to infection. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* 6th ed. Philadelphia: WB Saunders; 2006. p. 92)

restricted to 8 to 10 amino acids in length, and they must contain certain amino acids at specific "anchor" positions on the peptide to bind.[295] Allelic variants of MHC class I may require different amino acids at these anchor positions.[227] The other amino acids of the peptide constitute the antigenic determinant recognized, after trafficking of the MHC-peptide to the cell surface, by a specific T-cell receptor on CD8+ cytotoxic T cells, the latter concurrently binding the heavy chain of MHC class I via CD8.[70,227,295,582]

Class II Major Histocompatibility Complex. Mononuclear phagocytes, B lymphocytes, and dendritic cells, including specialized tissue-specific dendritic cells, such as the Langerhans cells of the skin, serve the immune system as "professional" antigen-presenting cells.[597] Dendritic cells, the most efficient antigen-presenting cells for primary activation of naïve T cells, are macrophage-like cells of a distinct lineage that take up and process antigens in tissues and migrate to local lymph nodes or to the spleen, where they are likely to encounter T cells specific for the presented antigens.[191,192,263,489] A defining feature of these professional antigen-presenting cells is their expression of class II MHC molecules in addition to class I MHC.[162] Class II MHC molecules comprise an α and a β chain, which together form a peptide-binding cleft.[98,503] Class II MHC molecules present peptides, 13 to 17 amino acids in length, derived from proteins that are internalized by endocytosis or during phagocytosis of microorganisms.[261,296,503] The three major types of class II MHC alpha and beta chains are HLA-DR, HLA-DP, and HLA-DQ, each exhibiting a high degree of polymorphism.[486] MHC class II, bound to a separate smaller molecule known as the "invariant chain," traffics via the Golgi to endosomal/lysosomal compartments, where it must dissociate from the invariant chain to bind antigenic peptides derived from internalized proteins.[487,572,573] The class II MHC–peptide complexes then move to the cell surface, where the peptides are bound by T-cell receptors of CD4+ T cells, which concurrently bind class II MHC via CD4.[192,372]

Figure 2-9 depicts the essential features of the conventional antigen presentation pathways that involve class I and class II MHC molecules, as described earlier. Alternative mechanisms have been documented by which class I MHC can present peptides derived from internalized exogenous proteins and class II MHC may present peptides derived from newly synthesized proteins. The importance of these unconventional pathways of antigen presentation in the immune response is not fully understood, but evidence indicates that such "cross-presentation" may be important for generation of CD8+ cytotoxic T-cell response against some viruses or fungi taken up via endocytosis by antigen-presenting cells.[38,313]

CD1 Family of Antigen-Presenting Molecules. The CD1 family comprises proteins with significant homology and structural similarity to that of the MHC class I heavy chain but that present lipid and glycolipid antigens. All mammalian species express one or more members of the CD1 family, principally on professional antigen-presenting cells. Four human CD1 proteins, CD1a, CD1b, CD1c, and CD1d have been identified, each tightly associated with a β_2-microglobulin subunit. Mycolic acid, lipoarabinomannans, and other related components of mycobacteria are the best documented foreign antigens presented by CD1 molecules, and both internalized antigens and antigens synthesized within the antigen-presenting cells by ingested mycobacteria may be presented via distinct trafficking patterns of the CD1-antigen complexes. It has been hypothesized that other antigens, such as the lipoteichoic acids of gram-positive bacteria, the complex capsular polysaccharides of *H. influenzae* type b, and *N. meningitidis*, and the

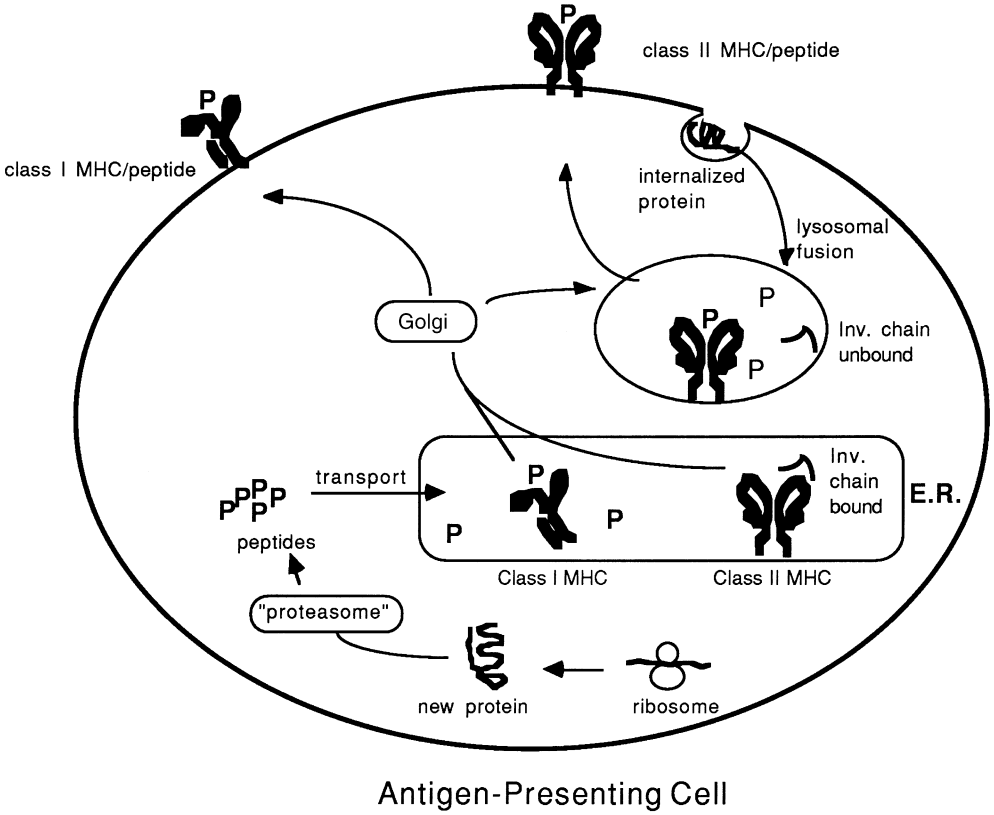

FIGURE 2-9 ■ Conventional pathways for peptide antigen presentation by class I and class II major histocompatibility complex *(MHC)* molecules. In the antigen-presenting cell, a proportion of newly synthesized proteins, whether of viral or host origin, undergoes proteolysis into peptides by enzymes that constitute the "proteasome." The peptides actively are transported into the endoplasmic reticulum *(E.R.),* where those with the appropriate length and sequence bind to MHC class I molecules. MHC class II cannot bind peptides in the E.R. because of interference by the associated "invariant *(inv.)* chain." The class I MHC/peptide complex is transported via the Golgi to the cell surface, where it may be recognized by $CD8^+$ lymphocytes. Class II MHC molecules pass via the Golgi to a lysosomal compartment, where conditions favor the release of the invariant chain. This permits class II MHC to bind peptides derived from internalized proteins that have entered the lysosomal compartment via fusion of endosomes or phagosomes with the lysosome. The lysosome translocates to the cell surface, where the class II MHC/peptide complex may be recognized by $CD4^+$ lymphocytes.

glycophosphoinositol (GPI) components of GPI-linked proteins of malarial parasites and trypanosomes also could be presented by CD1 molecules. Antigens presented on antigen-presenting cells by CD1 molecules are recognized by a specialized subset of CD1-restricted T cells that usually lack CD4 and CD8, known as NK T cells. These cells share characteristics of both NK cells and T cells, and exhibit a limited range of T-cell receptor specificity. They represent a sizeable fraction of the T-cell compartment, although their function in the immune response is incompletely understood. Greater detail regarding the structure, function, phylogeny, trafficking, expression, and T-cell interactions for members of the CD1 family may be found in an extensive recent review.[470]

Plasmacytoid Dendritic Cells. A specialized class of dendritic cells known as "plasmacytoid" dendritic cells plays a multifactorial role in both innate and adaptive immune responses. These cells are early responders to viral infections by virtue of their expression of TLR7/9 and their ability to produce large amounts of type 1 interferons that disrupt viral replication.[40,566] Additionally, they can play an auxiliary role in the adaptive immune response by providing help to conventional dendritic cells during antigen presentation, apparently by helping to sustain IL-12 production by the latter in response to IFN-γ released by interacting T cells.[40,566]

T Lymphocytes

The development of T lymphocytes, or T cells, begins when prothymocytes leave the marrow and enter the subcapsular region of the thymus.[190] By mechanisms that are poorly understood, the thymic environment induces the rearrangement of T-cell receptor V (variable), D (diversity), and J (joining) gene segments with the eventual expression of mature α-β T-cell receptors complexed with CD3. The T cells, now coexpressing CD4 and CD8, migrate to the thymic cortex, where they undergo screening for T-cell receptor specificity both to optimize the repertoire for distinguishing self from nonself and to eliminate T-cell receptor rearrangements that result in undesirably high self-reactivity. The mechanism by which this screening takes place is the subject of intense investigation and theoretical controversy, and several interesting reviews of the subject are cited here.*

*References 141, 249, 317, 387, 396, 601.

Thymocytes that do not pass this dual screening procedure receive signals that induce programmed cell death (apoptosis).[396,418,600] Only about 5 percent of the original thymocytes pass this screening, after which they express either CD4 or CD8, but not both.[396,418,484,600] Mature thymocytes are released into the periphery, where the $CD4^+$ cells serve as the main source of IL-2 and provide help for B-cell antibody production, and the $CD8^+$ cells engage in specific cytotoxic activity.[240,415] This discussion of T cells and T-cell receptors specifically relates to T cells that express T-cell receptors composed of α and β chains, or α-β T cells. T cells of a distinct type, γ-δ T cells, are far less numerous in most tissues (intestinal epithelium is a notable exception), exhibit much less T-cell receptor diversity than do α-β T cells, may not require an intact thymus for development, and play a role in host responses to certain intracellular bacterial pathogens, including *Listeria* and mycobacteria.[110,264]

Antigen specificity of α-β T cells resides in their T-cell receptors, which are integral membrane proteins that exhibit structural homology with immunoglobulins. T-cell receptor diversity results from a rearrangement of V, (D), and J segments.[236] There are up to 100 different V segments, one (D) segment, and as many as 100 different J segments in the complete germline configuration of the T-cell receptor genes. Rearrangement of these gene segments into a mature VDJ sequence occurs by the action of a recombinase enzyme complex formed by two proteins, RAG-1 and RAG-2.[439,516] T-cell receptor diversity is generated by several factors, including the range of possible combinations of V, (D), and J segments; the imprecise action of the recombinase complex; the variability in the number of nucleotides deleted during rearrangement; and the action of another enzyme, terminal deoxytransferase, which appears to add nucleotides at random to extend segments during rearrangement.[215,533] The actions of Artemis and DNA ligase IV, two enzymes critical for the processing and joining of DNA ends, introduce additional sources of variability.[103,200,376,620] It has been estimated that as many as 10^{15} different T-cell receptor specificities theoretically could result from the several mechanisms involved in T-cell receptor segment rearrangement.[168]

Stimulation of naïve $CD4^+$ or $CD8^+$ T cells occurs as they circulate through peripheral lymphoid tissue and encounter dendritic cells and other professional antigen presenting cells. Localized T-cell migration is highly regulated by specific chemokines and adhesive interactions with local endothelium and involves mechanisms similar to those discussed earlier for circulating phagocytes.[194,342] When T cells engage antigen-presenting cells presenting specific peptide antigens on the appropriate MHC molecules, they are activated via their T-cell receptor and several costimulatory molecules, especially CD28, to produce IL-2 and proliferate and differentiate into effector T cells.[134,299] Effector $CD4^+$ T cells may be of the T_H1 or T_H2 type, and this type is influenced by several factors, including the specific cytokines elicited by a particular microbial pathogen.[417] Naïve T cells activated in the presence of IL-12 and IFN-γ are likely to develop into T_H1 cells, whereas IL-4 and IL-6 tend to drive development in the direction of T_H2 cells.[413,417,421] Preferential development of T_H1 effector cells leads mainly to macrophage activation and cell-mediated immunity, whereas T_H2 effector cells help drive certain aspects of humoral immunity, including immunoglobulin class switching to IgE in allergic responses.[417] Until recently, with the identification of the T_{FH} subset (see later discussion), T_H2 cells were thought to be the principal cell in providing T-cell help for B-cell antibody production.

A third major subset of effector CD4 T cells are T_H17 cells, whose main function appears to involve protection against extracellular bacteria and fungi by stimulating phagocytic cell responses to these pathogens.[285,370,406] Their development is favored by the presence of IL-6 and TGF-β and by the absence of IL-4 and IL-12. They are distinguished by their ability to produce IL-17 cytokines, which in turn stimulate local tissues to produce chemokines, such as IL-8, that recruit neutrophils and other phagocytic cells to tissue sites.[285,370,406] Development of T_H17 cells involves production of IL-21, which acts in an autocrine fashion to activate signal transducer and activator of transcription 3 (STAT3), a transcription factor that drives T_H17 cell development.[285,406] Of interest, recently, mutations in STAT3 have been found to account for hyper-IgE syndrome (see later discussion).

In contrast to T_H1, T_H2, and T_H17 CD4 T cells, which exert their main effector functions in the periphery, a fourth T-cell subset, T follicular helper cells, or T_{FH} cells, appears to account for most of the CD4 T cells that provide help to B cells in the lymphoid follicles for antibody production.[325,435] T_{FH} cells are characterized by their location in lymphoid follicles, expression of the CXCR5 chemokine receptor, and their ability to secrete cytokines typical of both T_H1 and T_H2 cells.[325,435] The developmental origins of these cells in humans and their relationships and interactions with the other T-cell subsets is a subject of current research.

Activation of naïve $CD8^+$ T cells by antigen binding, costimulation by accessory binding molecules on antigen-presenting cells, and exposure to cytokines, including IL-2, all lead to clonal proliferation of specific $CD8^+$ cells and their differentiation into cytotoxic effector cells. Effector $CD4^+$ T cells bound in common to an antigen-presenting cell may play a role in activating naïve $CD8^+$ T cells, either by releasing IL-2 or by activating the antigen-presenting cell to provide greater costimulation to the $CD8^+$ T cell to make its own IL-2.[33] Antigenically experienced effector $CD8^+$ T cells respond to specific antigen and costimulatory molecules on infected host cells by activating cytotoxic mechanisms similar to those described earlier for NK cells, including the release of both perforin and granzymes and generation of receptor-mediated signals for target cell apoptosis.[367,504,583]

Regulatory T Cells. The existence of T suppressor cells was long a subject of debate among immunologists. Only within the last decade has solid evidence been developed to support the existence of suppressor T cells, now referred to as regulatory T cells, or T-regs. These cells were discovered when thymectomized mice were noted to develop autoimmune disease. Transfer of T cells that expressed CD25, the α chain of the IL-2

receptor, from normal adult mice to thymectomized mice prevented autoimmune disease. This population of $CD4^+CD25^+$ T-regs can suppress the activity of other immune cells and has been shown to prevent graft-versus-host disease and allograft rejection.[476] The mechanism of suppression by T-regs is uncertain, but may involve direct contact with other cells or secretion of inhibitory cytokines, including IL-10.[370,512] These inhibitory cytokines can interfere with T-cell proliferation and inhibit the ability of antigen-presenting dendritic cells to promote T-cell activation.[370,512] The role of T-regs in immunity to infection is only beginning to be studied, but some current evidence suggests that the action of T-regs with specificity for microbial antigens may suppress protective immune responses to some infections but may also suppress excessive or injurious host responses.[476]

T-Cell Memory. Some proportion of activated $CD4^+$ and $CD8^+$ T cells become endowed with the capacity for long-term antigenic memory and can rapidly become effectors on re-exposure to specific antigen. Whether these cells develop directly from naïve T cells or previously have been effector cells, or both, is uncertain, and the mechanisms by which they become memory T cells are poorly understood. Among the features of memory T cells include high-level expression of CD45RO, the ability to suppress activation of naïve T cells of the same specificity, and a homeostatic level of ongoing proliferation in bone marrow and peripheral lymphoid organs.[314,378,509,632]

T-Cell Activation by Superantigens. The term *superantigen* describes a class of protein antigens, mainly microbial exotoxins, including most staphylococcal enterotoxins, staphylococcal toxic shock syndrome toxin–1 (TSST-1), and related streptococcal TSST-1–like toxins. These bacterial toxins are potent pyrogens, can induce a potentially lethal toxic shock syndrome, and share binding domains for both T-cell receptor V regions and MHC class II molecules. Superantigens bypass normal antigen processing and presentation pathways by binding directly to class II MHC molecules on antigen-presenting cells and to specific variable regions on the β-chain of the T-cell antigen receptor. Through these interactions, superantigens induce a polyclonal activation of T cells at orders of magnitude above levels induced by antigen-specific activation, resulting in massive release of cytokines from T cells and antigen-presenting cells, including TNF-α and TNF-β, IL-1, IL-2, and IFN-γ that are believed to be responsible for the most severe features of toxic shock syndromes.[16,475]

B Lymphocytes and Immunoglobulins

B Lymphocytes. B lymphocytes (B cells) are the source of humoral immunity in the form of specific immunoglobulin. The earliest recognizable marrow precursors of B cells are pro-B cells, whose surfaces bear the pan-B marker CD19. Further differentiation produces pre-B cells and then mature B cells, the latter expressing cell-surface immunoglobulin by which they recognize and bind antigen. B lymphocytes constitute approximately 20 percent of the lymphocytes in the circulation and peripheral lymphoid tissues, including the lymph nodes, spleen, bone marrow, tonsils, and intestines, and they are identified by the presence of surface immunoglobulin and the pan-B differentiation markers CD19 and CD20.[114,394]

B-cell activation is initiated by recognition and binding of specific antigens to B-cell surface immunoglobulins. Early activation leads to increased expression of receptors that either bind cytokines (e.g., IL-2, IL-4, and IL-6) or interact with T cells,[349] leading in turn to clonal proliferation and differentiation into memory B cells and plasma cells in the germinal centers of peripheral lymphoid tissue.[321] Some data suggest that B-cell differentiation into memory B cells is favored by exposure to the CD40 ligand on dendritic cells in lymphoid organs, whereas differentiation into plasma cells is favored by exposure to CD23, IL-1a, IL-6, and IL-10.[321,571] The plasma cells, later found in bone marrow and liver, as well as peripheral lymphoid tissue, are responsible for most free immunoglobulin production.[571]

The B-cell response to protein antigens depends on T-cell help. B cells can process and present antigen to $CD4^+$ T cells they encounter in the lymph nodes and spleen.[320,321,571] In the typical sequence of events, B-cell surface immunoglobulin binds to a protein antigen, which is internalized, processed, and presented to the T cell via class II MHC molecules. B cell–mediated activation of T cells during antigen presentation is much more effective for memory T cells, whereas naïve T cells are more likely to be turned off or rendered tolerant.[206,229] T-cell help is provided for B cell proliferation and production of antibody against the specific protein antigen. This is mediated by signaling via CD40-ligand interactions with CD40 on the B cell and by the release of cytokines, which also can induce isotype switching.[172,429,555] Most B-lymphocyte responses to polysaccharide antigens proceed largely without formal T-cell help, although antibody responses to some such antigens may be enhanced in the presence of T cells.[408]

Immunoglobulin. Immunoglobulin molecules may be bound at the surface of B cells or free in the circulation, mucosal secretions, or tissues. Free immunoglobulins function in host defense against infection by binding to microbial surfaces to prevent microbial attachment, activating complement via the classical pathway, neutralizing viruses and toxins, and participating in the formation of immune complexes.[134]

Ig molecules are composed of two identical heavy and two identical light chains, as diagrammed in Figure 2-10.[195,455] The carboxyl terminus of the immunoglobulin molecule is the heavy chain constant, or Fc, region. The amino acid sequence of this region determines the immunoglobulin isotype. The heavy chain is encoded by V, (D), J, and constant (C) regions on chromosome 14.[66,605] Each immunoglobulin molecule has a pair of either κ or λ light chains, defined by distinct constant regions. The variable region of the immunoglobulin molecule contains the antigen binding site. Like the T-cell receptor, the Fab region consists of two identical heavy and light chain pairs; similarly, broadly diverse antigen specificity results from the variable nature of recombinase-mediated DNA rearrangements of the three hypervariable, or complementarity-determining, regions (CDR1, CDR2,

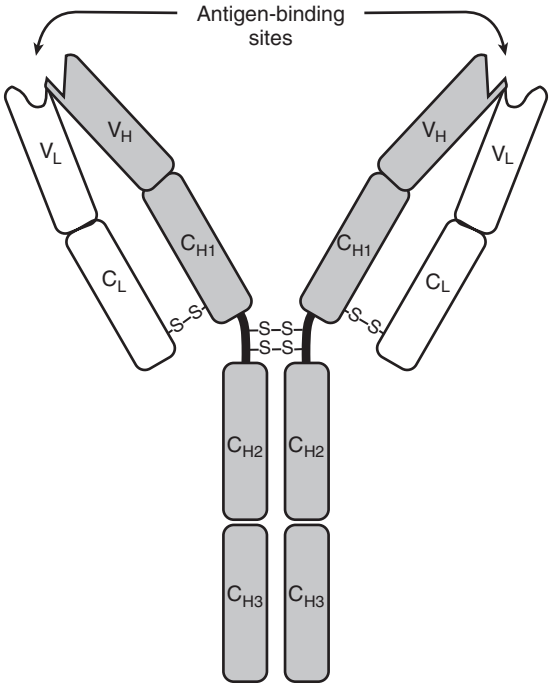

FIGURE 2-10 ■ Structure of an immunoglobulin molecule. The schematic structure of immunoglobulin G (IgG) is shown, depicting the variable *(V)* and constant *(C)* regions of both the heavy *(H)* and light *(L)* chains, the disulfide bonds that link the two heavy chains at the hinge region and the *CL* region with CH_1, and the antigen-binding sites formed by the complementarity-determining regions of *VH* and *VL*.

and CDR3) and the four framework regions during B cell development.[210,356,381] The imprecision inherent in this rearrangement, involving mechanisms similar to those described for the T-cell receptor, leads to the generation of more than 10^{12} potential antigenic specificities. Somatic hypermutation of variable regions after gene rearrangement adds to the repertoire, and further diversity results from differences in approximation of the three CDRs in relation to each other, affecting the three-dimensional structure of the antigen recognition site.[432,458] Thus, unlike most T-cell receptors, which recognize specific peptide sequences, the antigen-binding domain of an immunoglobulin molecule recognizes the three-dimensional structures of its respective antigen.[166,458]

All immunoglobulin is derived from B cells expressing surface IgM. B cells may change immunoglobulin isotype when they differentiate into plasma cells, which produce only one class or subclass of immunoglobulin each. Isotypes other than IgM are the result of isotype switching by replacing a part of the constant region of the immunoglobulin heavy chain with another isotype-specific segment.[473,555] As already noted, isotype switching primarily depends on specific B-cell interactions with cytokines and T cells. The variable region remains unchanged during isotype switching; thus, there is no change in antigen specificity. However, important features of immunoglobulins, including half-life, localization in tissues, ability to activate complement, and interactions with cellular IgG receptors, are directly determined by isotype.[95,303,587]

Immunoglobulin Isotypes. IgG accounts for about 80 percent of circulating immunoglobulin and includes the subclasses IgG1, IgG2, IgG3, and IgG4.[67] The half-life of IgG ordinarily is about 21 days (7 days for IgG3).[303] Initial exposure to most microbial protein antigens first induces IgM and then an IgG response consisting of IgG1 and IgG3. IgG2 and IgG4 usually are produced during the secondary immune response. IgG1 usually is made in response to protein antigens.[303] In adults, the main antibody response to polysaccharides is IgG2, whereas in infants, IgG1 predominates.[7,19] The functions of IgG in host defense include blocking microbial attachment, opsonization, complement activation, toxin and virus neutralization, and promoting antibody-dependent cell cytotoxicity. IgG1, IgG2, and IgG3, but not IgG4, can trigger complement activation via the classical pathway by binding to C1q.[95]

Free IgM usually exists as an immunoglobulin pentamer that has a molecular weight of approximately 950,000 and is stabilized by a single J chain.[195,336,398,458] Present mainly in the circulation, its half-life is approximately 8 to 10 days. The IgM response is the earliest of the isotype responses, appearing within the first few days of infection, but it is transient. The formation of an IgM response in the absence of an IgG response to infection is not associated with the formation of memory B cells. The main function of IgM in host defense is the activation of complement to promote opsonization and lysis of microorganisms.[398]

IgA exists in monomeric circulating and polymeric secretory forms and has a half-life of about 7 days.[303] Both forms are produced mainly by plasma cells that have migrated to mucosal sites. Secretory IgA is made up of two or three IgA molecules joined by a stabilizing J segment that is secreted by plasma cells and a secretory component produced by mucosal epithelial cells.[322,336] The secretory component permits delivery of IgA to mucosal surfaces.[430] There are two subclasses, IgA1 and IgA2, that differ in the composition of their heavy chains. Most IgA in the circulation is IgA1, whereas most IgA in secretions is IgA2. IgA1, but not IgA2, may be cleaved at mucosal sites by bacterial proteases.[335] IgA neutralizes viruses at mucosal sites, may block bacterial adhesion, and can act directly as an opsonin to promote phagocytosis and killing of microbes via Fcα receptors.[292,322]

The IgE molecule has a molecular weight of 200,000 and a half-life of only 2.3 days.[303] Most IgE is produced by plasma cells in lymphoid tissue near gastrointestinal and respiratory mucosal surfaces and released into the circulation.[301,564] IgE acts via Fcε receptors to trigger activation and degranulation of mast cells and basophils, leading to immediate hypersensitivity reactions.[564] Persons with intestinal metazoan parasites often have elevated serum levels of IgE, and IgE may have a role in protecting against parasitic disease by stimulating mediator release from mast cells that can recruit eosinophils and cause intestinal smooth muscle contraction and expulsion of parasites.[564]

IgD has a molecular weight of approximately 180,000 and a half-life of 3 days.[303] It is expressed along with IgM on surfaces of naïve B cells but is present in normal adult serum and secretions in very low concentrations. Some antigenic specificity for IgD has been

demonstrated, and although its function in host defense is unclear, it may serve as a secondary antigen receptor on B cells, where it may regulate the development of B-cell antibody responses.[80]

CLINICAL CONDITIONS ASSOCIATED WITH DEFICIENT HOST RESPONSES TO INFECTION

Immature Host Responses of the Newborn Infant

A mild febrile respiratory illness in a 10-month-old infant might prompt little more than gentle reassurance over the telephone from the child's pediatrician. However, if the patient is an infant in the first few weeks of life, the physician's response is likely to include an evaluation for systemic bacterial infection and administration of parenteral antibiotics in the hospital until a serious infection can be ruled out. Similarly, the appearance of a few cutaneous perioral vesicles characteristic of herpes simplex virus in an older infant usually evokes little concern and no specific treatment. In contrast, the same condition in the first 3 weeks of life is likely to lead to a prolonged hospitalization for antiviral therapy because of the risk for developing serious central nervous system or disseminated infection.[617] It is well recognized that newborn infants are much more susceptible to serious infections from many types of organisms than are older children and adults. This predisposition to infection is even more profound in infants born prematurely.[364] The basis for this special vulnerability of the neonate is complex and encompasses all arms of the immune system.[354] It is of such importance that the clinical approach to possible infections in infants during the first month of life usually is far more aggressive than in older children.

Cell-Mediated Immunity

Antigen presentation per se, via the mechanisms discussed earlier, appears to be relatively intact in the newborn infant. Expression of class I and II MHC molecules has been documented in a broad range of fetal tissues by 12 weeks' gestation,[284,441] and levels of expression are sufficient to mediate normal MHC class II–restricted antigen presentation by neonatal monocytes to maternal or paternal $CD4^+$ T cells, as well as to induce vigorous rejection of allogeneic fetal tissue by $CD8^+$ cytotoxic T cells.[269,283]

By about 20 weeks' gestation, the fetal repertoire of diversity of T-cell receptors has developed fully.[593] At the time of birth, although most basic functions of cell-mediated immunity are present, a high proportion of immature T cells are in the peripheral circulation, which can be identified by their coexpression of CD4 and CD8.[364] This phenotype typifies type II thymocytes, which usually are not found in the periphery in older persons.

Neonatal T cells appear to be relatively deficient in most of their major functions, including $CD8^+$ T cell–mediated cytotoxicity, delayed hypersensitivity, and T-cell help for B-cell differentiation. Diminished cytokine production by neonatal T cells likely accounts for much of this deficiency. In turn, the relatively naïve status of most neonatal T cells may account for reduced cytokine production because memory T cells are much more efficient in all of these functions.[267,364]

B Cells and Antibody

B Cells. Pre-B cells are found in the fetal liver and omentum by 8 weeks' gestation and in the fetal bone marrow by 13 weeks' gestation.[237,364,545] Pre-B cells with surface IgM have been detected as early as 10 weeks' gestation. After 30 weeks' gestation and delivery, pre-B cells are seen only in the bone marrow. Mature B cells are present in the circulation by the eleventh week and have reached adult levels in the bone marrow, blood, and spleen by the twenty-second week of gestation.[170,237,545]

Fetal B cells express only IgM, whereas most adult B cells express both IgM and IgD. Neonatal B cells may express three immunoglobulin isotypes (e.g., different combinations of IgG, IgA, IgM, and IgD) on their surfaces.[237,260] Data from experiments in mice suggest that exposing B cells with surface IgM, but not IgD, to antigens leads to anergy, or B-cell inactivation. It has been speculated that the absence of surface IgD on fetal B cells contributes to the induction of tolerance to self and, possibly, maternal antigens in utero. In addition, the fetus has a higher proportion of the functionally immature $CD5^+$, or B1, cells than adults. These cells produce autoantibodies and, thus, may play a role in the development of tolerance to self-antigens, maternal antigens, or both.

Although germinal centers are not present in lymphoid tissue at birth, they begin to develop in the first few months of life concomitant with the infant's exposure to antigens.[575] Despite conflicting in vitro data, neonatal T-cell help for B cells probably is comparable to that of adult T cells, as is reflected by the excellent T-dependent antibody response of the newborn to immunization with protein antigens.[174] However, neonatal T-cell help is associated with secretion of IgM alone, not other isotypes, possibly because neonatal T cells have diminished production of cytokines critical for promoting isotype switching. Addition of cytokines such as IL-2, IL-4, and IL-6 helps overcome neonatal B-cell dysfunction in vitro.[364] In contrast to B cells of older individuals, B cells of neonates and young infants cannot respond to polysaccharides without T-cell help. The recruitment of T-cell help for the immature response to polysaccharides has been achieved with the advent of protein–polysaccharide conjugate vaccines. Such vaccines elicit help from T cells specific for peptides derived from the protein component as presented by polysaccharide-specific B cells that have internalized the protein–polysaccharide complex, allowing peptide-specific T cells to activate polysaccharide-specific B cells.[79]

Antibody. Maternal IgG accounts for the great majority of the newborn's circulating immunoglobulin, because almost none is made by the healthy fetus, and IgG is the

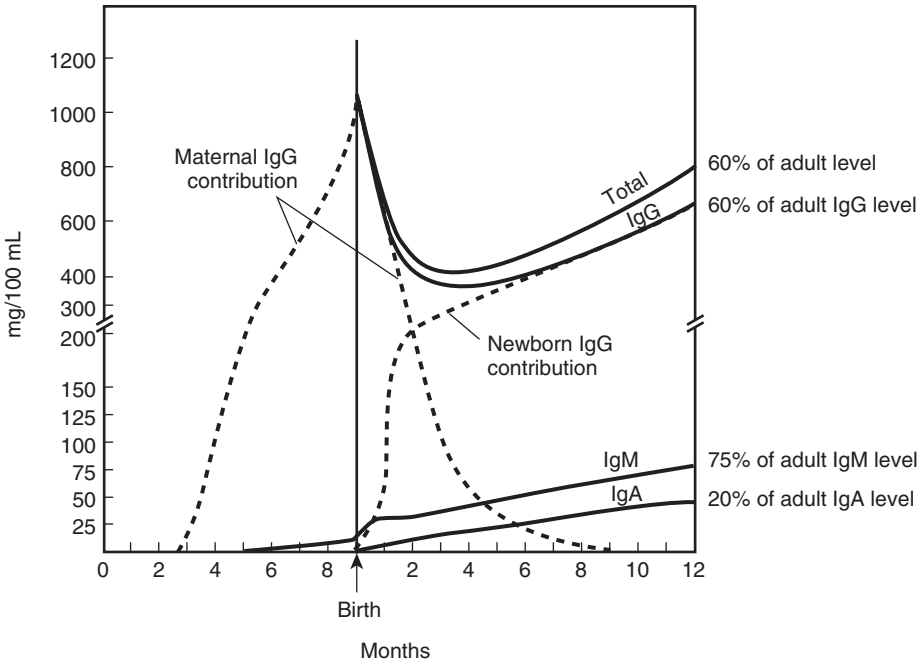

FIGURE 2-11 ■ Immunoglobulin (*IgG, IgM,* and *IgA*) levels in the fetus and infant in the first year of life. The IgG of the fetus and newborn infant solely is of maternal origin. The maternal IgG disappears by 9 months of age, by which time endogenous synthesis of IgG by the infant is well established. The IgM and IgA of the neonate are synthesized entirely endogenously, for maternal IgM and IgA do not cross the placenta. (From Braun J, Stiehm ER. The B-lymphocyte system. In: Stiehm ER, editor. *Immunologic disorders in infants and children.* 4th ed. Philadelphia: W. B. Saunders; 1996. p. 67.)

TABLE 2-4 **Levels of Immunoglobulins in Sera of Normal Subjects, by Age**

Age (mo)	IgG (mg/dL)	IgG (mg/dL)	IgG (mg/dL)	Total Immunoglobulin (mg/dL)
Newborn	1031 ± 200*	11 ± 5	2 ± 3	1044 ± 201
1-3	430 ± 119	30 ± 11	21 ± 13	481 ± 127
4-6	427 ± 186	43 ± 17	28 ± 18	498 ± 204
7-12	661 ± 219	54 ± 23	37 ± 18	752 ± 242
13-14	762 ± 209	58 ± 23	50 ± 24	870 ± 258
25-36	892 ± 183	61 ± 19	71 ± 37	1024 ± 205

Modified from Stiehm ER, Fudenberg HH. Serum levels of immune globulins in health and disease: a survey. *Pediatrics* 1996;**37**:715–27. Copyright American Academy of Pediatrics, 1966.
*Values were derived from measurements made in 296 children and 30 adults. Levels were determined by the radial diffusion plate method using specific rabbit antisera to human immunoglobulins. Values are ± 1 standard deviation.

only isotype of maternal immunoglobulin that crosses the placenta.[333,384] Maternal transport of IgG can be detected as early as 8 weeks' gestation, and the newborn's IgG level is directly proportional to gestational age, reaching 100 mg/dL by 17 to 20 weeks' gestation and 50 percent of the maternal level by 30 weeks' gestation (Figure 2-11).[49,94,116] Maternal IgG is transported both passively and actively via trophoblast Fc receptors. Trophoblast Fc receptors have higher affinity for IgG1 and IgG3 than for IgG2 and IgG4, and thus more of those subclasses are transported from the mother.[199,358] Therefore, newborns may have higher proportions of IgG1 and IgG3 than IgG2 and IgG4 than adults, and term newborns' IgG1 levels may exceed maternal levels.

By about 2 months of chronologic age, approximately half of the term infant's quantitative IgG is of maternal and half is of infant origin. The physiologic nadir of IgG in all infants is about 3 to 4 months of age and ranges from less than 100 mg/dL in preterm infants with very low birth-weight to about 400 mg/dL in term infants (see Figure 2-11, Table 2-4).[49,627] Maternal IgG usually has waned completely by about 12 months of age, at which time infant levels are approximately 60 percent of adult levels. Production of IgG1 and IgG3 matures more rapidly than that of IgG2 and IgG4, reaching adult levels by approximately 8 years of age, versus 10 and 12 years of age, respectively.[437]

Little IgM, IgA, IgE, or IgD normally is produced by the fetus, and none is transported from the mother.[333,384] The presence of total IgM levels greater than 20 mg/dL at birth suggests an intrauterine infection, and documentation at birth of specific serum IgM or IgA against relevant organisms, such as *T. gondii* and others, would be diagnostic.[201,422,440] Serum IgA levels at birth in both preterm and term infants usually are less than 5 mg/dL and consist of both IgA1 and IgA2. Secretory IgA is not

detectable until after birth but usually is present within the first few weeks of life. IgM and IgA reach approximately 60 and 20 percent of adult levels by 1 year of age, respectively (see Fig. 2-9). Secretory IgA reaches adult levels by 6 to 8 years of age.[364]

The IgG transferred from the mother to the fetus has been demonstrated to protect the newborn from many infectious agents, including viruses such as varicella, polio, measles, mumps, and rubella and bacteria such as tetanus, diphtheria, *H. influenzae* type b, and group B streptococcus. However, the infant is not protected by the mother if she does not have specific IgG antibody, even if she is immune to a given organism. For example, the mother may have low or absent levels of circulating IgG antibody but good memory B cells capable of mounting a rapid recall response. In this case, the mother is protected, but she cannot transfer protection to her infant.[364]

The concept of passive transfer of protective IgG is the basis for development of vaccines for maternal immunization before or during pregnancy so that passive transfer of vaccine-induced antibody will result in protection during the neonatal period. Examples of organisms for which such strategies have been investigated include group B streptococcus, *H. influenzae* type b, meningococcus, pneumococcus, rotavirus, and respiratory syncytial virus.[48,203,297]

It has been documented that the fetus can respond to antigenic stimulation in the form of maternal immunization with tetanus toxoid vaccine and be primed for a secondary antibody response to repeat immunization after birth.[242,243] Some fetuses near term also can make IgM and IgA antibody to organisms such as toxoplasmosis, rubella, cytomegalovirus, or herpes.[201,422] The amount of fetal antibody produced in response to intrauterine antigenic stimulation is proportional to gestational age.[171,557]

Maternal antibody inhibits the infants' ability to respond to vaccines, especially live-virus vaccines against certain organisms, such as measles, but it does not prevent them from mounting protective immune responses to most childhood vaccine antigens, such as tetanus, diphtheria, polio, hepatitis B, and protein-conjugated polysaccharide vaccines.[15] In general, neonates have protective responses to T-dependent antigens, even though they may produce less antibody to some antigens than do older infants and adults.[13,164,174,218,540,541]

The newborn infant's response to TI-1 antigens is slightly decreased and to TI-2 antigens is poor.[246] The antibody response to most TI-2 antigens, including the polysaccharide capsules of group B streptococci, pneumococci, and *H. influenzae* type b, is not mature until 18 to 24 months of age, although infants as young as 3 months of age can respond to group A meningococcal polysaccharide.[538] In contrast, in the first few weeks of life, infants mount excellent antibody responses to T-independent polysaccharide antigens that have been rendered T-dependent by covalent conjugation of the polysaccharide to a protein carrier, as noted earlier.[79]

It has been proposed that immunologic tolerance may be induced in infants by early exposure to some antigens. The subject remains controversial, but some data suggest that immunization to pertussis in the newborn period results in lower levels of antibody to subsequent doses of vaccine than does initially immunizing at 1 month of age.[47,52] However, no evidence indicates that tolerance is induced by administration of tetanus, diphtheria, or oral polio vaccines in the newborn period.[174,518]

The response of premature infants to most routine childhood vaccines by 2 months of age, including diphtheria, tetanus, pertussis, and oral and inactivated polio, is comparable to that of 2-month-old term infants.[8,68,123,541] However, premature infants may not respond as well to hepatitis B vaccine for reasons that are unclear.[147,352]

Complement

Complement proteins do not cross the placenta, but studies of mothers with congenital complement component deficiencies and mother–infant pairs discordant for variants of individual components, as well as studies of fetal tissues, have provided evidence for fetal synthesis of complement beginning as early as 5½ weeks' gestation. Most complement proteins are present by 10 weeks' gestation.[144,333] Levels of complement activity and of individual complement components vary significantly among infants, but, in general, classical pathway hemolytic activity of term neonates ranges from 50 to 80 percent of maternal values (Table 2-5). Because serum complement levels are modestly elevated during pregnancy, term neonates' levels of most complement components are about 60 to 90 percent of normal adult values.[197,628] Alternative pathway hemolytic activity is decreased more consistently than classical pathway activity and is approximately 50 to 70 percent of normal adult values at term (see Table

TABLE 2-5 **Summary of Published Complement Levels in Neonates**

	Mean Percentage of Adult Levels	
Complement Component	**Term Neonate (No. of Studies)**	**Preterm Neonate (No. of Studies)**
CH_{50}	56-90 (4)	45-71 (3)
AP50	49-65 (3)	40-51 (2)
C1q	65-90 (3)	27-58 (2)
C4	60-100 (4)	42-91 (3)
C2	76-100 (2)	96 (1)
C3	60-100 (5)	39-78 (4)
C5	75 (1)	—
C6	47 (1)	—
C7	67 (1)	—
C8	20 (1)	—
C9	<20 (2)	—
B	35-59 (8)	36-50 (3)
P	33-71 (5)	13-65 (2)
H	61 (1)	—
C3bi	55 (1)	—

Data from Lewis DB, Wilson CB. Developmental immunology and role of host defenses in neonatal susceptibility to infection. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* 4th ed. Philadelphia: W. B. Saunders; 1995. p. 65.

2-5).[6,193,433,531] Complement activity usually is lower in premature than term infants, with hemolytic activity of both pathways, as well as serum levels of most complement proteins, usually corresponding directly with gestational age.[193] Infants who are small for gestational age (SGA) have complement levels comparable to those of babies of the same gestational age who are not SGA.[433,531]

Hemolytic activity of both the classical and the alternative pathway rises rapidly and reaches adult levels by 3 to 6 months of age and by approximately 6 to 18 months of age, respectively. In addition to hemolytic activity, complement-mediated opsonic and bactericidal activity is decreased in newborn sera and generally correlates with C3 and factor B levels.[197] Studies of opsonic and bactericidal activity of newborn sera have been reviewed in detail elsewhere.[197,306] Levels of individual complement proteins do not always correlate with their functional activity.[238,290] Zach and Hostetter[628] reported not only that total C3 levels were decreased as measured by enzyme-linked immunosorbent assay but also that C3 thioester reactivity was decreased and that it correlated with gestational age. Because the opsonic function of C3 is mediated by its thioester, such a defect might contribute to the newborn's deficiency in functional complement activity. Although complement levels and activity are decreased significantly in most newborn infants, these abnormalities are relatively mild in contrast to those seen in hereditary complement deficiencies in which the level of an individual component usually is nil. Despite studies in vitro that suggest increased risk for infection, the extent to which lower neonatal complement levels predispose to infection in vivo is uncertain.

Phagocytes

The newborn infant exhibits both quantitative and qualitative deficits in phagocytic defenses. Although the number of circulating PMNs usually does not differ greatly from that in older children and adults, under conditions of stress, including systemic infection, the availability of marrow reserves of PMNs is impaired markedly.[131] In fact, whereas the ratio of marrow neutrophil reserves to circulating cells in older persons is nearly 15 : 1, in the newborn infant this ratio is more often between 2 : 1 and 3 : 1.[131] Thus, neutropenia is more likely during severe systemic infections in the newborn than in older children and adults.[131] The resulting deficiency in PMNs available for delivery to infected sites under conditions of stress is a serious disadvantage for the neonate in containing bacterial and fungal infections. Distinct from this quantitative deficiency in marrow reserves of PMNs, functional impairments of PMNs also are important in understanding neonatal phagocytic defenses.

The most important and best-documented functional impairments of neonatal PMNs are related to defective adhesion and migration.* Specific structural, functional, and biochemical abnormalities have been documented, any or all of which may contribute to the overall impairment in adhesion and migration of these cells.[280] Impaired adhesion of neonatal PMNs to endothelial cells and other biologic substrates has been linked with deficiencies in the expression or function of the β_2 integrins Mac-1 (CD11b/CD18) and LFA-1 (CD11a/CD18).[4,29,99,312,390] Perhaps the best documented of these is the diminished level of surface expression of Mac-1 on activated neonatal PMNs, although expression on resting PMNs is similar to that of adults.[99,312] Although a neonatal PMN defect in translocation of stored Mac-1 to the cell surface cannot be ruled out, the deficiency in stimulated Mac-1 surface expression can be explained sufficiently by the observation that the total cell content of Mac-1 in PMN from term neonates is only about 60 percent of that in adult PMNs.[4] The total cell content of Mac-1 in PMN at the time of birth is related directly to gestational age, and cell lysates of PMNs from very early premature infants (less than 30 weeks' gestation) have been found to contain less than 20 percent of the Mac-1 content of an equal number of adult PMNs.[390] The PMN content of LFA-1, which is normal at term, appears to be reduced in infants born before 35 weeks' gestation.[390] In addition to reduced integrin expression, reduced adhesive function of the β_2 integrin molecules themselves at the surface of activated PMN has been documented.[29] Several other defects of neonatal relative to adult PMNs that might influence chemotaxis have been documented. These include defective redistribution of surface adhesion sites,[26] impaired uropod formation during stimulated shape change,[28] reduced cell deformability,[310] impaired microtubule assembly,[28] deficient F-actin polymerization,[262,506] reduced lactoferrin content and release,[27] reduced ability to effect membrane depolarization and intracellular calcium ion flux,[507] and impaired uptake of glucose during stimulation by chemoattractants.[5] The relative importance of each of these individual impairments to the overall deficiency in chemotaxis by neonatal PMNs has not been determined.

Evidence suggests that the number and binding efficiencies of neonatal PMN receptors for chemoattractants, including C5a and synthetic bacterial peptides such as the formyl peptide N-formylmethionine leucylphenoalanine (N-f-met-leu-phe), are normal.[26,507,562] Phagocytosis and microbicidal activity of neonatal PMNs have been found in several studies to be similar to those of adult PMNs.[399,401] However, in some studies in which assay conditions are designed to expose a potential defect in these functions (e.g., limiting concentrations of opsonins and high bacterial inocula), defects in phagocytosis and killing have been demonstrated.[399,401] It is not clear whether these potential deficiencies play a role in impaired neonatal phagocyte defenses in vivo.

Primary and Heritable Immunologic Deficiencies

Physicians who care for children often are faced with the problem of a child who has a history of recurrent infections. Most of these children are normal infants or toddlers who have been exposed to a succession of common respiratory infections when they entered daycare

*References 4, 5, 26, 27-29, 339, 390, 400, 401.

or a similar setting for the first time.[117] Fortunately, for most of these children, repeated exposure elicits relative immunity to many or most of the infectious agents. It is an important challenge for the physician to distinguish those infants and children who do not fit into this common pattern but who have an unusual susceptibility to specific types of infection with respect to frequency, severity, causative agents, and response to appropriate treatment that could suggest a primary immunodeficiency. This challenge usually falls to the pediatrician or family physician because, with a few exceptions, the primary immunodeficiencies appear during infancy and early childhood.[149]

As suggested earlier, the infant or toddler who experiences even six to eight presumed viral upper respiratory tract infections during the course of a winter season, without other complications, ordinarily would not be considered likely to have an immunodeficiency. In contrast, a child who had experienced five episodes of acute otitis media in the previous 4 months, several episodes accompanied by sinusitis or pneumonia, has displayed reasonable cause to suspect a humoral immunodeficiency.[149] For certain organisms, infection in the healthy host is so decidedly uncommon that even a single episode should prompt a high suspicion of impaired host defenses. *Pneumocystis jiroveci* pneumonia strongly suggests a severe defect of T-cell number or function.[149] Similarly, lymphadenitis or osteomyelitis caused by gram-negative enteric bacilli suggests a defect of phagocytic killing, such as chronic granulomatous disease.[308,474] The following discussion of specific immunologic defects, their genetic basis, if known, and their infectious consequences focuses on well-characterized prototypic disorders within each class of defects but will address other related disorders.

Antibody Deficiencies

Humoral immunity is provided by antibody and plays an important role in host defense against most pathogens, as is illustrated by the finding that patients with significant antibody deficiencies develop recurrent and sometimes life-threatening infections.[148,156] They characteristically are prone to recurrent otitis media, sinusitis, pneumonia, and, less often, sepsis and meningitis. Antibodies participate in complement-dependent and complement-independent opsonization, bactericidal activity, virus and toxin neutralization, and the formation of immune complexes that can be cleared by the reticuloendothelial system.[134,270] The role of immunoglobulins globally and of distinct isotypes and subclasses in humoral immunity is elucidated by the nature of infections found in patients with the specific deficiencies addressed in the following section.

X-Linked Agammaglobulinemia. X-linked agammaglobulinemia, first described by Bruton, is a primary immunodeficiency disorder of the B-cell lineage and is the most serious disorder of humoral immunity.[102,357,493] It is characterized by absent or severely decreased numbers of circulating B lymphocytes and absent or extremely low levels of all classes of circulating immunoglobulins. It is caused by several different mutations in the gene encoding for a B cell–specific tyrosine kinase, *Btk*, which maps to the long arm of the X chromosome at Xq22.[585,596] This abnormality in kinase activity results in an arrest in the development of B cells, usually at the pre-B stage, and thus few B cells or their progeny (e.g., plasma cells) are in the circulation or lymphoid tissues.[248]

Most persons with X-linked agammaglobulinemia develop chronic or recurrent pyogenic bacterial respiratory or gastrointestinal tract infections, and some may have recurrent skin infections.[102,357] Systemic disease, such as sepsis, and serious focal infections resulting from bacteremia, such as meningitis, osteomyelitis, and septic arthritis, do not occur as frequently as respiratory and gastrointestinal tract infections but are more common and more severe than in normal hosts. The causative agents of most of these infections are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*, but *Staphylococcus aureus* and *Pseudomonas aeruginosa*, as well as other gram-negative organisms, may be implicated. The most troublesome gastrointestinal tract infections in X-linked agammaglobulinemia are caused by *Salmonella*, *Campylobacter*, and chronic infestation with *G. lamblia*. Although these patients do not have increased susceptibility to most viruses, they have been found to have unusually severe or chronic enterovirus infections that can be manifested by chronic arthritis, meningoencephalitis, dermatomyositis, hepatitis, or a combination thereof, and several patients with X-linked agammaglobulinemia have developed vaccine-related paralytic poliomyelitis after receiving the live oral polio vaccine.[357]

The only typical abnormality on physical examination that is not related directly to infections is the absence or a paucity of normal B cell–containing lymphoid tissues, such as tonsils, adenoids, and peripheral lymph nodes. Patients who are diagnosed and treated early in life have normal physical examination, growth, and development.

Persons with X-linked agammaglobulinemia have very low serum levels of IgG, IgM, IgA, and IgE. The diagnosis can be confirmed by studying lymphocyte markers. There is a lack of circulating cells that stain for surface immunoglobulin or with B cell–specific monoclonal antibodies against CD19, CD20, or both. The number and function of T lymphocytes are normal in X-linked agammaglobulinemia. It may be difficult to establish the diagnosis based on immunoglobulin levels in the newborn period because of the presence of maternally derived IgG. However, if specific reasons to suspect this diagnosis exist, such as a newborn male with a documented or suspicious family history, the diagnosis can be made by documenting a paucity of circulating B cells by flow cytometry.

Advances in genetic techniques have enabled detection of maternal carriers of X-linked agammaglobulinemia.[148] In contrast to carriers of some other X-linked diseases examined later (see discussion of chronic granulomatous disease), circulating B lymphocytes of X-linked agammaglobulinemia carriers express only one population of B cells, those with the normal allele on the X chromosome, presumably because B cells with the mutant allele are at a selective disadvantage and do not

develop. Prenatal diagnosis can be made by genetic studies of amniotic fluid cells or quantitation of fetal circulating B cells.[148]

The prognosis for patients with X-linked agammaglobulinemia has improved markedly with earlier diagnosis, high-dose intravenous immunoglobulin (IVIG) therapy, and aggressive use of antibiotics. Before the availability of IVIG, most patients who survived to the third decade of life had chronic lung disease from recurrent pulmonary infections and hearing loss from recurrent otitis media.[357]

IgG Subclass Deficiency. Persons with IgG subclass deficiencies have levels of one or more IgG subclass that are more than two standard deviations below normal for age, normal to slightly decreased total IgG, normal levels of other immunoglobulin isotypes, and, often, a poor antibody response to certain antigens.* Patients with IgG subclass deficiency who also have IgM and IgA deficiency may have another immunodeficiency disorder such as common variable immunodeficiency (CVID).

Patients with individual or combined deficiencies of IgG1, IgG2, and IgG3 may be at increased risk for respiratory bacterial infection, particularly if the deficiency is associated with an abnormal antibody response to antigenic stimulation. The most common kinds of infections in patients with any clinically significant IgG subclass deficiency include otitis media, sinusitis, and pneumonia. Ordinarily, these patients do not have life-threatening systemic infections.

Deficiency of IgG1 is likely to be associated with subnormal levels of total IgG, because this subclass accounts for about 60 percent of total IgG, and it often is associated with other subclass deficiencies.[18,19,520,527-529]

IgG2 deficiency usually is associated with normal total serum IgG levels and is more likely to be clinically significant if accompanied by IgG4 or IgA deficiency. Patients with IgG2 deficiency typically have poor antibody responses to polysaccharide antigens but normal responses to protein antigens. Like most patients with deficiencies of humoral immunity, their infections primarily are due to encapsulated bacteria and are localized to the respiratory tract, although some patients have had recurrent meningococcal meningitis or disseminated pneumococcal disease.[527,529]

IgG3 deficiency has been associated with low total levels of serum IgG and recurrent respiratory infections, which also may lead to chronic pulmonary disease.[438]

IgG4 deficiency is difficult to diagnose because many normal persons have low serum levels of IgG4 and most normal infants have no detectable IgG4.[437] IgG4 deficiency appears to be of clinical significance, however, if it is associated with IgG2 and IgA deficiency.

The treatment for children with IgG subclass deficiency typically is individualized according to the frequency and severity of symptoms. Noninvasive infections usually can be treated successfully with appropriate antibiotics. Patients with more severe presentations may benefit from regular IVIG therapy, but those who also are completely IgA-deficient should be treated only with IgA-depleted IVIG preparations.

IgA Deficiency. IgA deficiency is the most common immunodeficiency, occurring as frequently as 1/400. This disorder appears to occur sporadically, but familial cases have been described.[24,156] Most of the functions of serum IgA can be performed by IgG and IgM.[156,437] Thus, although deficiencies of secretory IgA may lead to recurrent respiratory or gastrointestinal tract infections, deficiency of serum IgA alone usually is not associated with increased susceptibility to systemic infections.[24] IgA deficiency has been associated with many other conditions, including recurrent infections, IgG2 deficiency, autoimmune disorders, and malignancy.[23] Recurrent infections are most likely to occur in the subset of IgA-deficient patients who also have IgG2 deficiency.[156,450] The infections usually are relatively mild and involve the upper respiratory and gastrointestinal tracts. Chronic gastrointestinal tract disease in these patients can be caused by *G. lamblia* infestations, nodular lymphoid hyperplasia, lactose intolerance, malabsorption, or inflammatory bowel disease. Other autoimmune diseases associated with IgA deficiency include rheumatoid arthritis, systemic lupus erythematosus, thyroiditis, myasthenia gravis, and vitiligo.[156] About 20 percent of IgA-deficient patients have allergy, and many have elevated levels of IgE.[156] Food allergy is common and may be the result of abnormal processing of antigen at mucosal surfaces.

Serum IgA levels less than 5 mg/dL can be clinically significant because patients with levels this low who receive transfusions may make antibody against donor IgA and have severe reactions when transfused again.[517] IVIG reactions also may occur because IVIG preparations contain varying amounts of IgA. IgA-depleted preparations are available and usually are well tolerated.[159]

Transient Hypogammaglobulinemia of Infancy. Hypogammaglobulinemia is a normal physiologic phenomenon occurring in all infants beginning about 3 to 4 months of age when maternal antibody wanes and infant synthesis of immunoglobulin has not compensated yet.[437] The syndrome of transient hypogammaglobulinemia of infancy can be differentiated from this physiologic hypogammaglobulinemia because immunoglobulin levels of normal infants begin to rise by about 6 months of age, whereas those of infants with transient hypogammaglobulinemia of infancy do not begin to increase until between 18 and 36 months of age.[437] Infants suspected of having this syndrome should be evaluated for X-linked agammaglobulinemia and CVID (see later discussion) and followed closely until their immunoglobulin levels normalize for age.

Antibody Deficiency with Normal or Elevated Levels of Immunoglobulins. Some persons with normal levels of all circulating immunoglobulin isotypes are at increased risk for infections similar to those seen in specific deficiencies of immunoglobulin levels described earlier.[18,20,437] The cause of this disorder remains poorly defined but may be related to an inability to respond to specific antigens or the induction of tolerance by exposure to certain

*References 272-274, 410, 437, 450, 451, 520, 521, 526-530.

antigens very early in development. As in other forms of humoral immunodeficiency, the most common infections in these patients are recurrent bacterial respiratory tract infections, although a few patients have developed pneumococcal sepsis.[20] Such persons can be identified by their inability to make antibody in response to stimulation with specific antigens. They have normal total serum immunoglobulin classes and subclasses and overall normal B-cell and T-cell quantity and function. They can respond to some, but not all, antigenic stimulation. Therefore, it is important to test them with a variety of stimuli. A good way to test for this syndrome is to immunize with protein antigens, such as tetanus and diphtheria toxoids, and with polysaccharide antigens, such as pneumococcal and *H. influenzae* type b capsular polysaccharide vaccines. Patients who can respond to protein but not to polysaccharide antigens usually will respond to protein-polysaccharide conjugates. Treatment with IVIG may help prevent recurrent infections in these patients, although their normal overall levels of immunoglobulin could pose difficulties in determining the appropriate doses of IVIG and intervals between infusions.

Defects of Cell-Mediated Immunity: DiGeorge Syndrome

The prototypic pure T-cell defect, DiGeorge syndrome, is characterized clinically by congenital heart disease (usually involving the aortic arch), hypocalcemic tetany, unusual facial features, and recurrent infections.[182] The classical, or complete, form of this disorder has absence or hypoplasia of the thymus and parathyroid glands, cardiac or aortic arch deformities, and a stereotypical constellation of abnormal facial features, most notably micrognathia and hypertelorism.[182,340,567] Although the condition usually is considered to be associated with immunodeficiency because of the thymic hypoplasia, only about 25 percent of patients actually exhibit an immunologic defect.[58] The term *partial DiGeorge syndrome* sometimes has been used to describe patients with the typical constellation of anatomic findings but without immunodeficiency or similar patients with mild immunologic impairment.[286] Some sources designate this disorder as an "anomaly" or "sequence" rather than a syndrome, because of confusion about its relationship to 22q11 deletion syndrome (del22q11), or the more recently defined microdeletion, del22p11.2, a deletion also associated with velocardiofacial syndrome.[101] Robin and Shprintzen[485] hold that the findings in DiGeorge sequence, although often associated with del22q11.2, are etiologically heterogeneous and have been associated with other chromosomal deletions such as del10p and del17p or del10q13. Moreover, some individuals with del22p11.2 exhibit abnormalities quite distinct from those of the DiGeorge sequence.* This genetic and phenotypic heterogeneity should be considered when evaluating patients with the classic facial and cardiac findings that have so often been associated with the serious T-cell defect. One candidate gene, *TBX1*, encoding a T-box transcription factor and located in 22q.11.2, has been a recent focus of research into the underlying genetic defect in DiGeorge syndrome. Although mice with mutations in *TBX1* exhibit some features consistent with DiGeorge syndrome, data remain insufficient to confirm the precise role of *TBX1* in human patients with this disorder.[259]

DiGeorge syndrome often is recognized in the newborn period by the presence of the unusual but characteristic facial features, hypocalcemic tetany in the first 2 days of life, or the presence of serious cardiovascular manifestations, most commonly associated with an interrupted aortic arch or truncus arteriosus.[286,371] Because of the serious nature of the cardiovascular defect, many patients with DiGeorge syndrome in earlier decades have not survived long enough for the immune defect to become a clinical problem.[286] However, with improvements in aggressive surgical treatment of the heart defects, more of these infants now survive long enough to display manifestations of the immunodeficiency that results in an increased frequency or severity of viral and fungal infections, as well as *Pneumocystis* pneumonia. In such patients, management often has included prophylaxis against *Pneumocystis*, avoidance of live virus vaccines, and, because antibody production is poor as a result of lack of T-cell help, periodic intravenous immunoglobulin infusions.[286] HLA-matched bone marrow transplantation has been successful in some cases.[87,247,286] Earlier work with transplantation of fetal or postnatal thymic tissue provided some long-term success in correcting the immunologic defect.[247,286] Recently, a highly promising large series of cases of transplantation with postnatal cultured thymic tissue in patients with complete DiGeorge syndrome yielded immune reconstitution with 73 percent survival at 2 years.[383]

Combined Defects of Cellular and Humoral Immunity

Severe Combined Immunodeficiency Disease. Severe combined immunodeficiency (SCID) describes a heterogeneous group of heritable immunodeficiencies that involve serious impairments of both cellular and humoral immunity, thus leading to recurrent severe infections by a wide range of viral, bacterial, and fungal organisms, similar in scope and severity to those seen in patients with X-linked agammaglobulinemia and DiGeorge syndrome combined. SCID has multiple forms, which have been reviewed in greater detail elsewhere.[106,108,501] At this writing, at least 10 genes have been identified with abnormalities known to result in SCID. X-linked SCID, the most common form, is due to a mutation in the common γ chain of the receptor for IL-2 and several other cytokines (γ_c).[108,361] The other known forms of SCID are either known or presumed to be autosomal recessive. These include a deficiency of adenosine deaminase, a purine salvage pathway enzyme; a deficiency in Janus kinase 3 (Jak3), a cytokine receptor signaling molecule; and a defect in the α chain of the IL-7 receptor, IL-7Rα.[108,315,586] Mutation of one of at least six different genes whose products play a role in T-cell receptor or immunoglobulin gene recombination or T-cell receptor signaling, including RAG1, RAG2, Artemis, DNA ligase

*References 253, 254, 286, 348, 462, 485.

IV, CD3-delta, and CD3-epsilon, also results in SCID.* Additionally, SCID is caused by a mutation in CD45, a phosphatase that regulates signaling thresholds in immune cells.[315] Flow cytometry analysis of lymphocyte markers reveals very low to absent B cell and T cell numbers in patients with most forms of SCID.

Long-term management of patients with SCID involves modalities employed in both T-cell and B-cell disorders, including prophylaxis against *P. jiroveci* pneumonia, avoidance of live viral vaccines, and immunoglobulin replacement therapy.[108] Bone marrow transplantation from HLA-matched siblings successfully has corrected the defect in some cases and is considered the current treatment of choice.[105] Considerable effort has been made toward development of gene therapy to treat SCID.[361] Adenosine deaminase deficiency is of historical interest in that it is the first heritable disorder for which gene therapy was attempted, although early success was limited.[105] Approaches using retroviral-based gene therapy for X-linked SCID appeared, initially, to be successful. However, at least three patients developed lymphoproliferative disorders similar to lymphocytic leukemia, with malignant cells demonstrating insertion of the vector into the promoter or first intron of a proto-oncogene, *LMO2*.[83,106,124,220,472]

Because the earliest possible treatment with stem cell transplantation often is the key to meaningful survival in patients with SCID, newborn screening programs for SCID based on assays of bloodspots have been introduced in several states and in some countries. These assays are based on quantitation of T-cell receptor excision circles—circular fragments of DNA that are a by-product of T-cell receptor rearrangement during fetal development.[48,127,471]

Common Variable Immunodeficiency. CVID is a heterogeneous group of combined immunodeficiencies that differ from most other primary immunodeficiencies in that they often present in the second or third decade of life, although they may present at any age.[155,157] Some sources suggest that CVID may be the most common symptomatic primary immunodeficiency. Patients with CVID characteristically have normal or only modestly decreased numbers of circulating B cells; low, but not absent, levels of IgG, IgM, and IgA; poor responsiveness to antigens; and abnormal T-lymphocyte function.[158]

Although both T-cell and B-cell abnormalities often can be demonstrated, the clinical presentation usually is comparable with that in patients with humoral or B-cell defects (i.e., recurrent bacterial otitis media and sinopulmonary infections).[155,271,493] Occasionally, however, in addition to the organisms causing infections in patients with antibody deficiencies, these patients also have infections with organisms more common in persons with T-lymphocyte abnormalities, such as *Pneumocystis*, *Mycoplasma pneumoniae*, recurrent herpes simplex virus, and herpes zoster virus infections. Chronic gastrointestinal problems may be due to *G. lamblia* or other intestinal pathogens. CVID patients are prone to nodular lymphoid hyperplasia, autoimmune diseases, and malignancies. CVID occasionally has been reported to be familial, and it has been described in families with IgA deficiency.

Several gene mutations have been found in patients with CVID. These include the genes encoding for CD19, transmembrane activator and CAML interactor (TACI), receptor for B-cell activating factor of the TNF receptor family (BAFF-R), and inducible costimulatory molecule (ICOS).[120,514,592]

Patients with CVID usually can benefit from therapy with IVIG, which reduces the incidence of acute infections. However, most patients with CVID, even some of those who undergo long-term treatment with IVIG, develop chronic sinopulmonary disease.[157,449]

Hyper–Immunoglobulin M Syndrome. Immunoglobulin deficiency with increased IgM is characterized by low levels of IgG, IgA, and IgE but normal to increased levels of IgM in the circulation and normal numbers of circulating B cells.[15,39,167] The disorder is caused by an intrinsic T-cell abnormality that alters switching from IgM to other isotypes. The basis for the various genetic forms of this defect is in one of several possible defects that involve interactions between CD40 on B cells and CD40 ligand (CD40L) on T cells. In normal persons, CD40 on the surface of B cells interacts with CD40 ligand on activated T cells to cause B-cell differentiation into memory B cells and switching from IgM to other isotypes. B cells from patients with the hyper-IgM syndrome only make IgM antibody, and their B cells only express surface IgM and IgD. The originally described and most common form of the defect is X-linked recessive and results from a mutation in the gene encoding CD40L, a protein expressed transiently on activated T cells.[15,39,167] More recently described autosomal recessive forms of this disorder involve mutations either in CD40 itself[167,216] or in a CD40-activated RNA editing enzyme, activation-induced cytidine deaminase.[481] Other cells have surface CD40, and therefore immunologic abnormalities such as the neutropenia and the increased incidence of infections caused by *Pneumocystis* and of malignancies in patients with hyper-IgM syndrome also may be the result of impaired cell interactions via CD40.

Clinically, hyper-IgM syndrome is manifested by recurrent bacterial infections, especially of the respiratory tract, beginning after maternal immunoglobulin levels subside over the first few months of life.[167] Such persons are susceptible to the same kinds of recurrent pyogenic infections associated with other immunoglobulin deficiencies, as well as to infections with organisms more commonly encountered in patients with T-cell defects (e.g., *P. jiroveci)*.[51] Some patients with this syndrome have recurrent diarrhea as a result of *G. lamblia* and *Cryptosporidium* infection that is severe enough to require parenteral nutrition. Life-threatening peritonsillar and peritracheal soft tissue infections also have been observed. About half of patients have persistent or recurrent neutropenia. Those with autoantibodies may have thrombocytopenia, hemolytic anemia, nephritis, hypothyroidism, or arthritis, as noted earlier.

*References 108, 200, 219, 315, 377, 483.

Physical examination usually is normal except for the sequelae of infections and lymphoid hyperplasia, which probably is caused by constant antigenic stimulation. Arthritis and arthralgia may be the result of chronic infection or the production of autoantibodies.

The diagnosis of X-linked hyper-IgM syndrome may be established by using immunofluorescence to document absent expression of CD40L on activated T cells, absent CD40 expression on B cells, or demonstrating a mutation in one of the genes encoding CD40, CD40L, or the enzyme activation-induced cytidine deaminase.

Treatment of patients with the hyper-IgM syndrome with IVIG usually results in marked clinical improvement.[51] It also may be necessary to treat those who have neutropenia with granulocyte colony-stimulating factor and those with arthritis or other autoimmune symptoms with steroids. Hyper-IgM patients usually do not do as well as those with X-linked agammaglobulinemia, because, in addition to their immunoglobulin deficiency, they have an increased incidence of neutropenia, autoimmune disease, and malignancy.[167,216]

Wiskott-Aldrich Syndrome. Wiskott-Aldrich syndrome is a rare X-linked disorder characterized by thrombocytopenia, small platelets, eczema, recurrent infections, autoimmune disease, and hematologic malignancy. Although Wiskott-Aldrich syndrome affects both T-cell and B-cell compartments, it presents phenotypically more like a B-cell or humoral deficiency, with recurrent otitis media, sinusitis, and pneumonia. The defect involves a mutation in the gene encoding a signaling molecule, Wiskott-Aldrich syndrome protein, which regulates "immune synapse" formation and IL-2 production. T cells from patients with Wiskott-Aldrich syndrome are markedly deficient in their ability to polymerize actin, secrete IL-2, and proliferate on T-cell receptor ligation.[436,438]

Ataxia-Telangiectasia. Ataxia-telangiectasia is a rare autosomal recessive disorder characterized by progressive cerebellar dysfunction with ataxia, oculocutaneous telangiectasias, recurrent bacterial respiratory infections such as those seen in humoral deficiencies, and a predilection to hematologic malignancy and breast cancer. Serum immunoglobulins often are low, as are lymphocyte counts. The disorder is due to a mutation in the *ATM* gene, located on chromosome 11. This gene encodes a serine-threonine kinase that is important in cell cycle regulation and double-stranded DNA repair, and it influences expression of *BRCA1* genes associated with breast cancer. The risk for breast cancer in patients with ataxia-telangiectasia is increased 15- to 20-fold over that in the general population. Heterozygotes for missense mutations of the *ATM* gene can be affected, because copies of the abnormal protein can interfere with function of the wild-type protein.[391,434]

Defects of the Interferon-Gamma (IFN-γ) and Interleukin-12 (IL-12) Pathways

Macrophages infected by intracellular pathogens, especially *Mycobacterium* or *Salmonella* spp, are stimulated via a TLR4-dependent mechanism to release IL-12, along with IL-18, IL-23, and IL-27. These cytokines stimulate T cells and NK cells to produce IFN-γ, via interactions with cellular receptors for the aforementioned cytokines. The released IFN-γ, in turn, further stimulates the macrophage to release more IL-12 and to activate killing. The mechanism by which this intracellular killing occurs is unknown. This cycle of mutual activation is essential for normal defense against mycobacterial pathogens, and some genetic defects of these cytokines, their cellular receptors, or related molecules critical for receptor-mediated signaling have been associated with increased susceptibility to mycobacterial infections. Deficiencies in this system have resulted from mutations in either of the two receptors for IFN-γ, IFN-γR1, and IFN-γR2, as well as mutations in STAT1, a molecule critical for transducing signals from both IFN-γ receptors. Mutations also have been described in the 40-kDa subunit of IL-12, IL-12p40, and the IL-12 receptor, IL-12Rβ1. These disorders are rather uncommon, all involving fewer than 100 known patients.[448,498]

Mutation of either of the IFN-γRs is associated with increased risk for mycobacterial infections. Deficiencies of IFN-γR1 may be either autosomal recessive or dominant and either complete or partial. Most recessive defects are complete and result in absent IFN-γ responsiveness. Dominant IFN-γR1 deficiency is associated with mutations that result in heterozygous truncations of the cytoplasmic domain of the receptor with excessive accumulation of nonfunctional receptor at the cell surface, leading to reduced but not absent responsiveness to IFN-γ. Patients with the recessive complete form of this deficiency have a much more severe clinical phenotype than those with the dominant partial form, although the latter have a fivefold greater frequency of nontuberculous mycobacterial osteomyelitis. Defects in IFN-γR2, much less common, also may be recessive or dominant in inheritance and either complete or partial. Rare deficiencies in the receptor signaling molecule STAT1 have led to increased mycobacterial infections in a partial deficiency or, in two patients with a recessive complete form, to postvaccination disseminated bacille Calmette-Guérin (BCG) disease followed later by death from severe viral infections. The latter probably relates to the additional role of STAT1 in development of IFN-α/β-mediated antiviral activity.[498]

Deficiencies of IL-12p40 or its receptor IL-12Rβ1 are associated with disseminated nontuberculous mycobacterial infections, tuberculosis, and *Salmonella* infections. The receptor deficiency results in unresponsiveness to IL-12. This defect is apparently autosomal recessive with variable clinical penetrance. Deficiency of IL-12p40 also is variable in its clinical phenotype and has resulted in deaths from severe mycobacterial infections.[453,498,554]

Diagnosis of defects of the IFN-γ pathway usually first involves immunofluorescence flow cytometry to examine cells for either deficient or abnormally excessive expression of IFN-γBCG disease R1. If these studies are normal, studies of STAT1 phosphorylation in response to IFN-γ stimulation may reveal a defect in IFN-γR2 function or expression. For evaluation of the IL-12 pathway, IL-12p40 can be measured in cell supernatants

with an enzyme immunoassay and IL-12Rβ1 expression function can be assessed, first with flow cytometry and, if necessary, by measuring STAT4 phosphorylation after stimulation with IL-12. These studies ordinarily should be done only in a highly specialized reference laboratory.[498]

Complement Deficiencies

Approximately 0.03 percent of the general population have complement deficiencies resulting from acquired or congenital abnormalities of single or multiple complement components or regulatory proteins. Excellent reviews of complement deficiencies are available elsewhere.*

The most common complement deficiencies are acquired and transient. These include the relative complement deficiencies in infants (see earlier discussion) and those that result from complement consumption in various inflammatory states, such as connective tissue disorders and acute or chronic infections.[175,176,178,306] Acquired complement deficiencies usually are associated with low levels of more than one complement component.[176] These deficiencies generally are not complete and are of doubtful clinical significance in host defense against infection.

In contrast, congenital or hereditary deficiencies more often are manifested by abnormality or complete absence of a single complement protein, and most of these have been well documented to predispose to potentially life-threatening infections. Most primary complement abnormalities (C1q dysfunction and C1rs, C4, C2, C3, C5, C6, C7, C8, and C9 deficiencies) are inherited as autosomal codominant traits.[176]

Patients with homozygous or heterozygous deficiency of the early classical pathway proteins, C1, C2, and C4, are more prone to develop autoimmune disease than infections, for reasons that are not fully understood. However, approximately 20 percent of patients with homozygous deficiency of early components have problems with recurrent or severe infections that are similar to those seen in C3 deficiency.[176,217,499] Ordinarily, these patients do not have as serious or as frequent problems with infections as patients with alternative pathway deficiencies, because they can derive some protection via the alternative pathway regardless of whether they have specific antibody to the infecting organism. Their predilection for autoimmune disease probably is due, at least in part, to abnormal solubilization and removal of immune complexes. Reports have shown that deficiency of one of the isotypes of C4 (C4B) is associated with increased susceptibility to infection caused by encapsulated organisms; however, subsequent studies have not confirmed this finding.[74,122] C2 deficiency has been associated with antibody deficiencies in individuals with recurrent infections.[17,119]

Deficiencies of alternative pathway proteins predispose to serious, often fatal, infections because of the lack of ability to respond promptly to organisms not previously encountered.[178] Although the classical pathway is intact, patients with alternative pathway deficiencies often succumb before they have the opportunity to make the specific antibody required for activation of the classical pathway. No homozygous factor B–deficient patients have been reported. Properdin deficiency, the only X-linked complement deficiency, has been associated with fulminant, usually fatal, meningococcal infection.[178] Factor D deficiency is rare and appears to predispose to recurrent neisserial infection.[330]

Mutations or variants in the gene for MBL, the initiator of the MBL pathway (see earlier discussion), have been associated with an increased risk for recurrent infections.[185,277] In particular, homozygosity for such mutations or variants was found to be associated with an increased risk for systemic meningococcal disease.[223]

Because all three complement activation pathways converge at the activation of C3, patients who are deficient in C3 are unable to mobilize any of the three main effector functions of complement in host defense—opsonization, phagocyte recruitment, or bacteriolysis. Thus, it is not surprising that the most serious complement deficiency state is the total absence of C3.[176] Congenital C3 deficiency is rare and results from decreased production of C3.[217,499] Patients with deficiencies of factors H and I have low, but detectable, levels of C3, because absence of either of these regulatory factors allows continuous activation of the alternative pathway and uncontrolled C3 consumption. Patients with C3 deficiency caused by any of these mechanisms have increased susceptibility to infections caused by encapsulated bacteria, such as *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b. Many of these infections involve the respiratory tract (otitis, sinusitis, bronchitis, and pneumonia), but C3-deficient patients also are predisposed to sepsis and meningitis.[176,217,499] In addition, some C3-deficient persons may develop any of several different autoimmune diseases.[176,217,499]

Deficiencies of terminal complement proteins C5, C6, C7, and C8 greatly increase the risk for developing systemic infections with *N. meningitidis* or *Neisseria gonorrheae*.[76] Because C9 is not absolutely required for bacteriolysis—although it makes this process more efficient—C9 deficiency increases the risk for infection to a lesser degree than do deficiencies of other terminal components. C9 deficiency was found to be present in approximately 0.1 percent of the population in Japan, and whereas the risk for meningococcal disease was increased 5000-fold in C7-deficient persons, it was increased 700-fold in C9-deficient persons.[420]

The risk for infection is higher in patients with C5 deficiency than in those with deficiencies of other terminal proteins because, in addition to the role of C5 in initiating assembly of the membrane attack complex, the free C5 fragment, C5a, is an important chemoattractant and thus is critical for leukocyte recruitment to sites of microbial invasion and for bactericidal activity.[17,56] Although a rare patient with C5 deficiency had recurrent subcutaneous skin infections, as might characterize a disorder of chemotaxis, most patients with this disorder are at increased risk for meningococcal and disseminated gonococcal infections.

*References 175, 176, 178, 185, 277, 306, 499, 614.

At least one episode of meningococcal disease occurs in approximately 60 percent of persons who have been identified as having C5, C6, C7, C8, or properdin deficiency, and 75 to 85 percent of documented bacterial infections in complement-deficient persons are meningococcal.[176,217,499] Conversely, approximately 14 percent of patients presenting with sporadic meningococcal diseases may be expected to have a defect in one of the late complement components, and this percentage rises to about one third among individuals with two or more meningococcal infections. Differences are seen in the features of meningococcal disease between persons with complement component deficiencies and normal hosts. First, meningococcal disease occurs in individuals who are older (mean, 17 vs. 3 years), and a higher proportion of disease is caused by groups Y, W135, and X in complement-deficient persons than in normal persons.[176] Additionally, the mortality rate in meningococcal infection is much lower than in normal persons, probably because many patients with one of these deficiencies have developed antibodies to meningococci that can activate the classical pathway leading to normal opsonization and phagocyte activation, functions upstream in the cascade from the membrane attack complex. In contrast, individuals who develop meningococcal infection with normal complement levels usually do so because they do not have specific antibodies with which to activate the classical pathway, and meningococci are poor activators of the alternative pathway.

Currently no specific treatment exists for patients with hereditary complement deficiencies. Replacement of missing complement proteins has been attempted with fresh frozen plasma but is not practical because of the short half-life of most of the components.[55,351,502] Immunization of complement-deficient patients and their close household contacts against encapsulated organisms, especially *N. meningitidis*, may reduce the risk for infections from these organisms.

Disorders of Phagocyte Function

General Features of Phagocyte Disorders. The most frequently encountered reminder of the importance of an adequate supply of well-functioning phagocytes comes from patients who develop neutropenia after chemotherapy for malignancies. The high risk for bacterial and fungal infections in these patients mainly is the result of a lack of circulating neutrophils available for delivery to infected tissue.[467] The qualitative disorders of phagocyte function discussed in this section result in similar susceptibilities to these infections, either because the circulating cells are unable to migrate to an infected site or because, once having migrated to the infected tissue, they are unable to effect normal microbicidal activity. There is some overlap among the types of infectious complications associated with disorders of migration versus killing. However, as a rule, defects of neutrophil migration tend to be associated with infections at skin, subcutaneous tissue, and mucous membrane sites. In contrast, killing defects are more likely to result in infections of deeper soft tissues and internal organs, although skin infections are not uncommon.

Intrinsic Disorders of Cell Migration

Type 1 Leukocyte Adhesion Deficiency. In the late 1970s and the first half of the ensuing decade, several reports described patients with recurrent bacterial infections, diminished neutrophil motility, and delayed separation of the umbilical cord.* The neutrophils of these patients were discovered to be markedly deficient in adherence to both natural and artificial surfaces, response to complement-opsonized particles, and expression of surface glycoproteins in the molecular weight range of 150 to 180 kDa. The deficient glycoproteins were found to be members of a family of heterodimeric glycoproteins, LFA-1, Mac-1, and pl50,95, each defined by its own unique α subunit, CD11a, CD11b, and CD11c, respectively, but sharing a common 95-kDa β subunit designated CD18.[25,31,36,37,552] A fourth α subunit, CD11d, whose importance remains poorly understood, has been described more recently. These proteins, also called the β_2 leukocyte integrins, were identified as critical determinants of adhesion-dependent functions on neutrophils and other phagocytic cells, and their absence appeared to be directly responsible for the striking adherence-dependent defects that characterized the function of leukocytes from patients with this disorder.[25,30,31,36,37,552] Variously called Mac-1 deficiency, MO1 deficiency, LFA-1 deficiency, CD11/CD18 deficiency, or CR3 deficiency, this disorder, now usually called type 1 leukocyte adhesion deficiency (LAD-1), is an autosomal recessive disorder with one of numerous mutations in the β_2 integrin subunit, CD18, localized to chromosome 21.[25,36,552,553] It has been identified in more than 150 persons worldwide and encompasses a broad ethnic diversity.[25,36] Patients may exhibit a moderate or severe phenotype, depending on the extent of the defect in protein expression.[25,31] The documented mutations of the β_2 subunit (CD18) that result in LAD-1 have been diverse, leading to abnormalities ranging from complete absence of the protein to extensions of the molecule, truncations of the extracellular portion or of the cytoplasmic domain of the molecule, small deletions, and point mutations.[33,36,282]

Patients with LAD-1 develop recurrent necrotic skin and soft tissue infections with poor or absent pus formation, and they exhibit poor wound healing.[25,31] They develop a severe generalized form of gingivitis or periodontitis, often losing most or all of their primary and secondary dentition along with some of their alveolar bone.[25,31,589] They also may develop enterocolitis much like that seen in neutropenic patients.[31,32] Delayed separation of the umbilical cord, presumably resulting from an impaired inflammatory response, is a common feature of the more severe phenotype of this disorder,[25,31] but this finding alone in infants without infectious complications or other characteristic features is of doubtful significance.[619] Pronounced leukocytosis is a common feature of LAD-1, even in the absence of active infection.[25,31] The reason for this is not completely understood. The fact that LAD-1 neutrophils are incapable of normal egress from the circulation via the oral cavity or lower intestinal tract was long believed to provide the principal

*References 3, 25, 30, 31, 37, 89, 154, 221, 268.

explanation for the high circulating neutrophil counts. However, recent studies in CD18-null LAD-1 mice reveal abnormally high circulating granulocyte colony-stimulating factor (G-CSF) levels and suggest that a more likely explanation involves the absence of a negative feedback mechanism on G-CSF production that occurs during normal transendothelial migration of leukocytes and involves IL-17. Absent ongoing transendothelial migration results in failure of this putative feedback mechanism, resulting in elevated G-CSF levels and higher circulating granulocyte counts.[225]

Functional studies of neutrophils from patients with LAD-1 reveal a marked impairment of adherence-dependent functions that require the β_2 integrins, including attachment to various surfaces, orientation in a chemotactic gradient, chemotaxis through nitrocellulose filters or under agarose gels, aggregation, phagocytosis of iC3b-opsonized particles, degranulation or activation of the oxidative metabolic burst in response to such particles, and recruitment of PMNs in vivo to Rebuck skin windows or dermal suction blisters.* In contrast, neutrophil functions that are independent of CD11/CD18-mediated interactions, including degranulation or oxidative burst activation in response to soluble stimuli or polarized shape change in suspension in response to chemoattractants, are normal.[25,30,31] PMNs and NK cells from patients with LAD-1 exhibit impaired ADCC for virus-infected target cells, suggesting that CD11/CD18-mediated cell–cell adhesion is essential for normal killing of virus-infected cells by this mechanism[331] and that the increased severity of viral infections in a few of the most severely affected patients could be related to defective ADCC. Currently the specific diagnosis of LAD usually is made by demonstrating absent or markedly deficient expression of the CD11/CD18 family of glycoproteins on circulating leukocytes by immunofluorescence flow cytometry, although various other methods have been used.[25,30,31,36]

Careful attention to skin and oral hygiene, aggressive management of infections, and meticulous local care of wound sites are important in the care of patients with LAD-1 or any serious disorder of neutrophil migration. Prophylactic antibiotics, usually trimethoprim-sulfamethoxazole, have been used in many of these patients, but their efficacy has not been well established. Granulocyte transfusions have been employed with some success to treat severe infections in a few patients with LAD-1.[32,89] Bone marrow transplantation with HLA-matched allogeneic marrow has had mixed results, from complete correction of the phagocytic defect to death 9 months after transplantation from graft-versus-host disease.[25,221] The human CD18 gene has been cloned and sequenced, and human LAD-1 cells have been corrected successfully in vitro with the normal CD18 complementary DNA carried by retrovirus vectors, hinting at the future promise of gene therapy for patients with LAD-1.[25,32] The development of genetic knockout mice deficient in CD18 may provide a useful model for studying gene therapy in vivo, as well as for helping to elucidate features of the underlying deficiency itself.[25,338]

*References 25, 30, 31, 112, 232, 282.

Type 2 Leukocyte Adhesion Deficiency. In 1992, two unrelated patients were reported, both products of consanguineous matings, who exhibited clinical characteristics virtually identical to those described for LAD-1.[228] However, expression of the β_2 (CD18) integrins on leukocytes was normal. In addition to defects in neutrophil motility, these children exhibited short stature, psychomotor retardation, and the Bombay (hh) erythrocyte phenotype (homozygous for absence of the H antigen). Phagocytosis by PMNs was normal. Recently, it has been documented that this defect is due to one or more mutations of a specific guanosine diphosphate (GDP)-fucose transporter,[375] resulting in the absence of fucosyl residues on sialyl Lewis X, the tetrasaccharide moiety that serves as the principal ligand for members of the selectin family of adhesion molecules.[228,350,600] In vivo and in vitro studies comparing the adhesive functions of PMNs from LAD-1 and this new disorder, now called LAD-2, provided elegant validation of the distinct roles of selectins and integrins in the recruitment of leukocytes in vivo, with the initial selectin-mediated "rolling" stage (deficient in LAD-2) required first for the second integrin-mediated "firm adhesion and extravasation" stage (deficient in LAD-1) to occur (see Fig. 2-2).[600] A deficiency in either mechanism results in defective delivery of PMNs to infected sites and is manifested clinically as a form of LAD. The other somatic and neurologic features of LAD-2 may be related to more widespread consequences of the generalized defect in fucosylation of glycoproteins.[375] Because of the generalized nature of this deficiency, it has been proposed by some to designate this disorder "type IIc congenital disorder of glycosylation," or CDG-IIc.[375]

Type 3 Leukocyte Adhesion Deficiency (Integrin Activation Defect). During the past decade, several patients have been reported who have a clinical phenotype that includes features of both type 1 LAD and Glanzmann thrombasthenia, a bleeding disorder associated with mutations in the $\alpha_{IIb}\beta_3$ integrin on platelets. Laboratory studies of these patients revealed markedly deficient integrin-mediated adhesive functions of both leukocytes and platelets despite normal surface expression of both leukocyte and platelet integrins. Further studies led to the conclusion that this defect in integrin function was the result of defective "inside-out" signaling pathways that normally lead to integrin activation.[205,324,389] It has been reported that cells from one patient with this disorder exhibit deficient activation of a small GTPase, Rap1, an important regulator of inside-out integrin activation.[324] More recent data on several kindreds with this disorder, studied in different laboratories, confirmed the presence of mutations in the gene encoding Kindlin-3, a molecule that, during cell activation, forms a critical bridge between the actin cytoskeleton and the cytoplasmic domain of the β subunit of multiple classes of integrins expressed by cells of hematopoietic lineages.[279,380,414,565] This bridging by Kindlin-3 is essential for normal integrin activation in both leukocytes and platelets. Some investigators in the field have suggested that this defect of integrin activation be termed *type 3 leukocyte adhesion deficiency (LAD-3)*, although this name does not make reference to the defect in platelet

function.[205,324,565] For this reason, others have preferred the term *integrin activation deficiency disorder*.[380]

Specific Granule Deficiency. Rare patients with hereditary specific granule deficiency have been reported, beginning with Spitznagel's original description in 1972.[91,231,550] These persons exhibited recurrent and severe infections, primarily of the skin and mucous membranes, sometimes involving the lung and, in one patient, the mastoid. Normal human neutrophils contain both azurophilic (primary) granules and specific (secondary) granules, whose contents have been summarized previously. Neutrophils from patients with this disorder exhibit bilobed nuclei and absent specific granules on Wright-stained blood smears. Lactoferrin released from specific granules reduces the negative surface charge of the plasma membrane, contributing to nonspecific adhesiveness of the cell.[231] The specific granule membrane also contains some of the intracellular store of the important adhesion molecule Mac-1 (CD11b/CD18) that is mobilized to the plasma membrane on stimulation by chemoattractants or other stimuli that induce granule secretion.[63,85] Thus, specific granule deficiency results in marked impairment of adhesion and migration of neutrophils, probably on the basis of both diminished intracellular pools of adhesion molecules and the inability to effect the change in surface charge caused by lactoferrin. This, in turn, leads to the recurrent skin and mucous membrane infections resulting from *S. aureus*, gram-negative bacilli, and *Candida* spp that characterize the natural history of patients with this disorder.[91,231,550] Neutrophils in this disorder also exhibit diminished microbicidal activity, presumably because of diminished amounts of the cytochrome b558 component of NADPH oxidase that are stored in the membrane of specific granules. Although the disorder is probably too rare to make such generalizations, specific granule deficiency likely is autosomal recessive in its mode of inheritance because both males and females are represented equally. The recent documentation of a specific granule deficiency phenotype in mice rendered genetically null for an important myeloid cell transcription factor known as CCAAT/enhancer binding protein epsilon (C/EBPε) led to studies in a few patients with specific granule deficiency that confirmed a deletion in the *C/EBPε* gene with absent expression of this transcription factor, although not all patients with this disorder have a mutation of this gene.[183,360] Specific granule deficiency may be diagnosed in a person with recurrent skin and mucous membrane infections whose neutrophils exhibit the characteristic absence of specific granules on Wright stain, as well as a marked impairment of chemotaxis in vivo and in vitro.

Chédiak-Higashi Syndrome. Chédiak-Higashi syndrome is a complex, rare autosomal recessive disorder characterized by partial oculocutaneous albinism, recurrent pyogenic infections, peripheral neuropathy, and neutropenia.[81] The illness also may involve an accelerated lymphoproliferative phase.[81] Granular cells, including neutrophils, contain giant lysosomal granules that are the apparent result of spontaneous intracellular fusion of azurophilic granules and, to a lesser extent, specific granules.[81] Corresponding disorders of intracellular pigment granules and vesicle trafficking in axons account for the albinism and other manifestations of this disease.[81] Similar disorders have been described in Aleutian mink, beige mice, albino Hereford cattle, and albino whales.[81] The genetic basis of the defect is now known to involve either a nonsense or a frameshift mutation in the gene encoding a large protein called the *lysosomal trafficking regulator (LYST)*, homologous to the "beige" gene in mice, with all mutations studied so far resulting in a truncated protein.[54,125] Patients with Chédiak-Higashi syndrome develop recurrent skin and mucosal infections, most often caused by *S. aureus*, that are characteristic of those observed in defects of phagocyte migration.[25,81] A consistent in vitro defect in cell migration occurs that appears to be related to abnormal regulation of microtubule polymerization on stimulation by chemoattractant agents.[81] The possible role of intracellular levels of cyclic adenosine monophosphate and guanylic acid in this microtubule abnormality has been suggested,[93] but the relationship between cyclic nucleotides and the microtubule dysfunction in Chédiak-Higashi syndrome has not been established. Ascorbic acid has been shown in at least one study to normalize both the elevated levels of cyclic adenosine monophosphate and the number of microtubules present within the cell.[93] Studies of two brothers with Chédiak-Higashi syndrome demonstrated abnormally increased tyrosinylation of the α subunit of tubulin.[81,423] Phagocytosis is normal, but killing of ingested bacteria is defective or delayed. The reason for this deficient or delayed killing is uncertain but may involve defective phagolysosomal fusion or abnormalities in levels of microbicidal defensins, which also are stored in primary granules.[235] The diagnosis of Chédiak-Higashi syndrome usually is suspected clinically on the basis of partial oculocutaneous albinism and recurrent pyogenic infections. A Wright stain demonstrating giant lysosomal granules and laboratory studies showing defective cell migration are confirmatory.

Neutrophil Actin Dysfunction. Filamentous actin constitutes the main contractile mechanism of neutrophils for migration and phagocytosis.[560] An extremely rare, and apparently heterogeneous disorder, neutrophil actin dysfunction has been characterized by recurrent skin infections caused by *S. aureus* and *Candida albicans*. Biopsies of infected skin lesions in one child demonstrated necrotic tissue with a notable absence of neutrophils. In vivo and in vitro studies revealed severely impaired neutrophil chemotaxis and phagocytosis.[90] The capacity for polymerization of actin from cell extracts also was diminished markedly. It is of interest that PMNs from family members of this patient also were found to be variably deficient in the CD11/CD18 family of glycoproteins that are the basis of LAD-1.[547] The nature and significance of this association are uncertain. Another infant reported to have recurrent skin and mucosal infections and defective neutrophil chemotaxis was found to have abnormally high levels of a 47-kDa protein, now identified as *lymphocyte-specific protein–1 (LSP-1)*, which exhibits actin-binding activity.[138]

Glycogen Storage Disease Type 1B. Beaudet and colleagues[60] first reported the association of recurrent infection, neutropenia, and impaired neutrophil migration with glycogen storage disease type 1B, a metabolic

disorder characterized by defective microsomal transport of glucose-6-phosphate. In 1985, Ambruso and coworkers[21] reviewed the features of 21 patients with glycogen storage disease type 1B, 15 of whom suffered from frequent infections, especially of the skin and subcutaneous tissues. Osteomyelitis, pneumonitis, sinusitis, and septicemia also were reported. Of these 21 patients, 17 were found to have serum inhibitors of myeloid stem cell proliferation, which were presumed to account for their chronic neutropenia. Impaired neutrophil motility was found in 8 of 11 patients in whom this was evaluated. Assays of neutrophil microbicidal capacity generally were normal. A specific relationship between the underlying metabolic defect in glycogen storage disease type 1B and the mechanism of impaired cell motility has not been established. However, exogenous glucose is an important energy source for chemotaxis,[611] and it is interesting to note that the uptake of glucose by PMNs in response to chemoattractant stimulation is impaired in glycogen storage disease type 1B, as well as in neonates, both examples of patients with impaired PMN migration.[5,56]

Extrinsic or Secondary Defects of Polymorphonuclear Leukocyte Migration

Defective Neutrophil Chemotaxis Associated with Serum Inhibitors of Cell Function. Investigators have reported the presence of inhibitors of PMN chemotaxis in the serum of patients with recurrent infection.[337,535,546,590,609] In most cases, the pathophysiologic mechanisms of these inhibitors are unknown. In many of the patients described, other associated immunologic disorders could account for at least part of the increased susceptibility to infection. However, in each case, the patient's neutrophils exhibited diminished chemotaxis in the presence of autologous serum or plasma, whereas identical assays in the presence of control serum or plasma resulted in a normal chemotactic response. Most such inhibitors appear to be immunoglobulins or immunoglobulin-like molecules.

Hyper–Immunoglobulin E Syndrome In 1966, Davis and colleagues[169] described two young girls with coarse facial features, reddish hair, fair skin, severe eczema, dystrophic nails, staphylococcal skin abscesses, and recurrent sinopulmonary infections. The absence of classic signs of inflammation accompanying the staphylococcal abscesses led to their being characterized as cold abscesses. The term *Job syndrome* was suggested, referring to the similar biblical affliction. Additional patients were described with a similar disorder, first associated by Buckley and associates[109] with very high serum IgE levels, including a patient who exhibited a defect in neutrophil chemotaxis reported in 1973 by Clark and associates.[137] Subsequent reports of similar patients have demonstrated that certain features are common to all of the patients with the disease now termed *hyper-IgE syndrome.* These consistent features include a history of staphylococcal infections of the skin and sinopulmonary tract beginning in infancy or early childhood and serum levels of IgE that are greater than 2000 IU/mL.[104,109,188] Other characteristic, but variable, features of this disorder include coarse facies, cold abscesses of the skin and subcutaneous tissues, a chronic eczematoid rash, eosinophilia, and mucocutaneous candidiasis.[104,188] Comprehensive reviews have provided detailed characterizations of the abnormalities of patients with this multisystem disorder.[104,188,226] Consistent abnormalities of cell-mediated immune functions in patients with hyper-IgE syndrome suggest that the pathogenic basis involves a defect of T-cell regulation.[239] Documented abnormalities include diminished reactivity to *Candida* and tetanus toxoid in delayed hypersensitivity skin testing, decreased in vitro lymphocyte proliferative responses to these antigens, and reduced numbers of T cells with the CD45RO memory T-cell phenotype.[107,132,188] An extensive study of 19 kindreds revealed autosomal dominant inheritance with a genetic locus for hyper-IgE syndrome on chromosome 4, in the proximal 4q region.[256,257] In 2007, it ultimately was determined that this disorder can be attributed to dominant negative mutations in STAT3, a factor critical for signal transduction by at least 10 different cytokines, some with proinflammatory and others with anti-inflammatory functions.[285,405,548] The immunologic defect is characterized further by a paucity of T_H17 lymphocytes, which normally promote protective leukocyte responses to bacteria and fungi.[406] Recently, a rare and clinically distinct autosomal recessive form of hyper-IgE syndrome has been described, resulting from a mutation in *DOCK8*, a member of a family of atypical guanine nucleoside exchange factors highly expressed in lymphocytes.[563] Patients with this form of hyper IgE syndrome have developed chronic viral infections, severe allergies, and early-onset malignancies.[83,256,548,563]

Although hyper-IgE syndrome might more properly belong in discussions of defective T-cell regulation, some patients with hyper-IgE syndrome may have a defect in neutrophil chemotaxis.[188] The defect sometimes has been intermittent, and in several cases, the presence of a serum inhibitor of chemotaxis has been recognized.[188] Donabedian and Gallin[187] demonstrated an inhibitor of granulocyte chemotaxis in supernatants from cultured peripheral blood monocytes from patients with hyper-IgE syndrome. More recent data suggest that keratinocytes and other epithelial cells from patients with hyper-IgE syndrome may produce reduced amounts of neutrophil-attracting chemokines, and neutrophils of these patients exhibit reduced expression of chemoattractant receptors.[406,407]

Impaired Generation of Serum-Derived Chemotaxins. A deficiency in the host's ability to produce chemotaxins derived from serum components may have profound consequences for the recruitment of PMNs to an infected site. The most important serum-derived chemotaxin is the fragment of the fifth component of complement, C5a, and its des-arg form. Several kindreds have been described with either absent or defective C5.[496] The chemoattractant activity measured in activated normal serum virtually is absent in C5-deficient serum.[495] The risk for developing systemic *Neisseria* infections as a result of deficient activation of the lytic terminal complement sequence appears far more significant than any phagocytic recruitment defect caused by impaired production of chemotaxins.[62,217] Patients with C3 deficiency also have impaired chemotaxigenesis because C5 cannot be

activated. Host impairment usually is more severe because of the importance of C3 in opsonization, as well as its role in the activation of the remainder of the complement cascade.[499]

Other Secondary or Poorly Defined Disorders of Polymorphonuclear Leukocyte Migration. Patients with protein-calorie malnutrition have defective PMN chemotaxis that appears to be based on systemic preactivation of circulating cells resulting from chronic low-level endotoxemia from impaired intestinal mucosal integrity.[128,512] Shwachman-Diamond syndrome, in addition to pancreatic insufficiency, neutropenia, and growth retardation, also is associated with defective PMN migration.[11] Two kindreds with congenital ichthyosis and an associated defect of PMN migration have been described.[404] Patients with severe thermal injuries develop an acquired form of specific granule deficiency with impaired PMN migration beginning about 14 days after injury.[231] In some children with juvenile periodontitis, reduced PMN chemotaxis has been associated with gingival overgrowth of *Capnocytophaga*, an anaerobic gram-negative organism that can elaborate factors that markedly impair PMN migration in vitro. However, in one such patient the ultimate diagnosis was LAD-1, suggesting that overgrowth of *Capnocytophaga* is more likely a consequence than a cause of such a disorder.[282] Several reports have been published of a poorly defined disorder of neutrophil migration referred to as *lazy leukocyte syndrome*,[10,402,403] marked by recurrent staphylococcal skin infections, rhinitis, gingivitis, stomatitis, neutropenia despite adequate marrow precursors, and diminished in vivo and in vitro migration of neutrophils.

Defects in Phagocyte Microbicidal Activity. As described earlier, the broad array of available phagocyte microbicidal mechanisms may be divided into oxygen-dependent and oxygen-independent mechanisms. To date, no specific deficiency of any oxygen-independent microbicidal mechanism has been described. Thus, this section is concerned mainly with the known deficiencies of oxygen-dependent microbicidal mechanisms of phagocytes, especially chronic granulomatous disease, the prototypical defect in this group. PMN migration usually is normal in these killing defects. Monocytes and the fixed phagocytes of the reticuloendothelial system generally share in the deficient microbicidal activity.

Chronic Granulomatous Disease. Chronic granulomatous disease was one of the earliest syndromes of phagocyte dysfunction to be characterized[474] and probably is the most extensively studied among individual phagocyte defects. It is recognized now to be a family of biochemically and genetically heterogeneous disorders of distinct components of the phagocyte NADPH oxidase complex.[128,183] Thus, chronic granulomatous disease results in the inability of phagocytes to generate superoxide anion and other reactive oxygen species.[183] Organisms that produce catalase pose a special problem for patients with this disease.[183,189,308,355] This encompasses a broad range of pathogens, including staphylococci, gram-negative enteric bacteria, *Pseudomonas* spp, yeast, fungi, *Nocardia* spp, and numerous other pathogenic species.[189,306,355,622] Most microorganisms produce H_2O_2, which might be used, even by the chronic granulomatous disease phagocyte, as an effective microbicidal weapon because it feeds into the sequence of oxidant reactions downstream from the defective oxidase enzyme (see Fig. 2-5).[491] Organisms that produce catalase are able to survive within these deficient cells, because catalase is an enzyme that degrades H_2O_2 to oxygen and water.[308,492] Infections with catalase-negative bacteria, such as *Streptococcus*, *Haemophilus*, and *Neisseria* spp, do not occur with increased frequency in CGD patients,[622] and these organisms are killed normally in vitro by chronic granulomatous disease phagocytes. Phagocyte functions not directly related to oxidative mechanisms of intracellular killing, including adherence, chemotaxis, phagocytosis, and degranulation, usually are normal in patients with chronic granulomatous disease.[44,405,474,544]

The genetic defect in chronic granulomatous disease may be inherited by either X-linked recessive or autosomal recessive mechanisms.[87,136,160] In the report of a registry of 368 patients with chronic granulomatous disease in the United States,[622] more than two thirds of the patients had the X-linked recessive form with absent $gp91^{phox}$, the larger subunit of the cytochrome b_{558}; about 12 percent had an autosomal recessive form with absent cytosolic $p47^{phox}$; fewer than 5 percent each had autosomal recessive disease with absent cysolic $p67^{phox}$ or absence of $p22^{phox}$, the smaller subunit of the cytochrome b_{558}. Approximately 12 percent had an unknown genetic form of the disease. About 5 percent of patients with chronic granulomatous disease have normal levels of an abnormal protein that is inactive, and at least 410 different mutations have been reported to result in chronic granulomatous disease.[276] These genetically diverse defects all result in defective function of the oxidase and the characteristic chronic granulomatous disease phenotype. In the female obligate carriers of X-linked chronic granulomatous disease, the proportion of cells that express the defect usually is between 35 percent and 65 percent, depending on the proportion of cells in which random inactivation of the normal versus the affected X chromosome occurs.[376,622] Included in the previously mentioned registry were two rare adult women whose sons had X-linked disease and who exhibited clinical signs of the chronic granulomatous disease phenotype because of dramatically skewed X-chromosome inactivation with 5 percent or fewer of their phagocytes demonstrating oxidase activity. Overall, patients with X-linked disease have more severe courses and experience higher yearly death rates than patients with autosomal recessive forms of the disease.[622]

Patients with chronic granulomatous disease experience recurrent serious bacterial and fungal infections, usually beginning in the first few months of life. *S. aureus* and gram-negative bacilli, especially *Serratia marcescens* and *Burkholderia cepacia*, are the most common causes of infection in patients with chronic granulomatous disease. Fungi, especially *Aspergillus* spp, also are prominent etiologic agents,[355,479,622] and infections caused by *Aspergillus* are the most common cause of death in these patients.[622]

Lymphadenopathy associated with lymphadenitis and chronic suppuration with poor healing is a common presenting feature. Granuloma formation at infected sites is one of the histologic hallmarks of this disorder.[307,479] Pulmonary infections and their complications have been the reported cause of death in up to 50 percent of these patients in some series, and *Aspergillus* predominates.[622] These infections often are protracted and respond slowly to appropriate antibiotic therapy.[308,479] Progression to lung abscess, empyema, or both occurs in about 20 percent of patients with chronic granulomatous disease with pneumonia.[308] Liver abscesses occur in about half of patients and may be recurrent.[62,133] The hepatosplenomegaly common in chronic granulomatous disease may result from these infections but more likely results from chronic infections at various sites with systemic lymphoid hyperplasia.[47,308] Osteomyelitis occurs in about one third of patients.[308,479,622] In contrast to normal children, in whom this infection usually involves the metaphyseal area of long bones, patients with chronic granulomatous disease more often develop infections of the small bones of the hands and feet. In normal children, *S. aureus* is the most common etiologic agent and causes a significant proportion of cases in chronic granulomatous disease. However, gram-negative bacilli and *Aspergillus* appear to be the predominant etiologies, and other agents, including *Nocardia*, also may be important etiologic agents of bone infection in chronic granulomatous disease.[462,622] Skin infections in this disorder may include pyoderma, purulent dermatitis, and cutaneous or subcutaneous abscesses and often are preceded by a chronic eczematoid skin rash.[479] However, skin infections are somewhat less of a problem in these patients than in those with leukocyte migration defects.

Although localized infections are the rule in patients with chronic granulomatous disease, these patients also may develop septicemia.[308,479,622] The most common cause of septicemia in most series has been *Salmonella*, but other gram-negative enteric bacilli also have been prominent.[307,355] Of note, *S. aureus*, the single most common etiologic agent of localized infections in chronic granulomatous disease, is a proportionally less common cause of septicemia in these patients.[479,622] Other infections sometimes seen in these patients include recurrent urinary tract infections in about 6 to 8 percent of patients; ocular infections with conjunctivitis, blepharitis, or both in about 20 percent of patients; and, rarely, chorioretinitis.[385,479]

Granuloma formation adjacent to hollow viscera in patients with chronic granulomatous disease has been found to produce clinically significant obstruction. Reported examples of this problem include obstruction of the gastric outlet, esophagus, small intestine, and ureters.[22,129,189] This complication usually responds to treatment with corticosteroids.[129]

Chronic granulomatous disease should be suspected in patients with a history of recurrent indolent infections caused by catalase-positive organisms such as those described earlier, especially if granulomas are found in biopsy specimens of lymph nodes or other tissues. Confirmation of the diagnosis usually rests on the demonstration of an absent or nearly absent oxidative metabolic burst in the patient's phagocytes. This can be detected classically by the slide NBT test or by other measurements of oxidative burst activity, such as cytochrome reduction, lucigenin- or luminol-enhanced chemiluminescence, oxygen consumption, hydrogen peroxide release, and more recently flow cytometry of PMNs loaded with oxidant-sensitive fluorescent dyes.[14,42,57,491,578] Figure 2-12A is an example of the slide NBT test for an X-linked carrier; Figure 2-12B is an idealized set of flow cytometry histograms with typical patterns for a normal control, a patient with chronic granulomatous disease, and both X-linked and autosomal recessive carriers. Prenatal diagnosis has been achieved by the use of the slide NBT test with blood from placental vessels obtained at fetoscopy.[425]

The management of patients with chronic granulomatous disease traditionally has relied on antibiotic prophylaxis, usually with trimethoprim-sulfamethoxazole or an oral antistaphylococcal penicillin, and an aggressive approach to the specific diagnosis and treatment of acute infections.[479] The additional use of antifungal prophylaxis with itraconazole or voriconazole that has activity against *Aspergillus*, has become standard in most centers. Granulocyte transfusions have been reported to be at least partially beneficial in a few cases, although this is rarely performed.[111,142] Bone marrow transplantation met with limited success in the late 1970s and early 1980s, with only one successful long-term engraftment reported among four separate attempts in different patients.[251,316,615] However, allogeneic peripheral blood stem cell transplantation from HLA-identical sibling donors, with prior myeloablative conditioning, has been considerably more promising, as suggested by the European experience from 1985 to 2000, with 22 of 23 patients "cured," with median follow-up of 12 years.[524]

An important development in the treatment of patients with chronic granulomatous disease is the administration of IFN-γ. A multicenter study initially demonstrated that daily subcutaneous injections of this agent reduced the requirement for hospitalization of these patients for serious infections by about two thirds.[298] IFN-γ does not increase oxidase activity in neutrophils, and the mechanism by which IFN-γ exerts its beneficial effect in chronic granulomatous disease has not been determined. Long term follow-up of patients being treated with IFN-γ has indicated that it continues to reduce the rate of serious infections over many years and is generally well tolerated.[382] This treatment carries some mild systemic side effects, such as fever, fatigue, and myalgia,[298] but it has been used in the management of the majority of patients with this disorder.[622]

The definition of the molecular basis for chronic granulomatous disease and the cloning of the genes responsible for the various forms of this defect have led to correction of some forms of the defect in cultured cells and the development of animal models in mice,[183,301,469,599] both of which are crucial steps in developing gene therapy for patients with this disorder in the future. Preclinical studies of gene therapy in chronic granulomatous disease mice have shown promise when bone

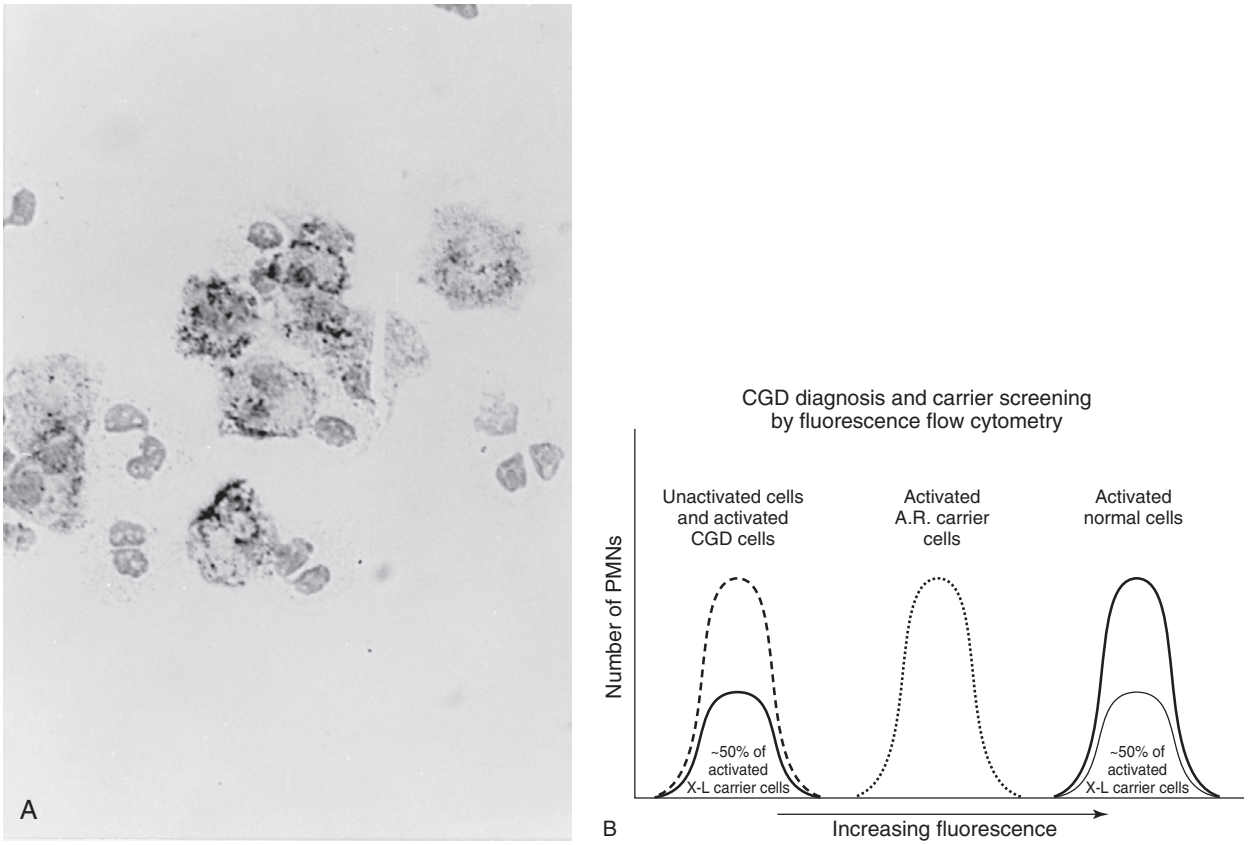

FIGURE 2-12 ■ **A,** Photomicrograph of a slide nitroblue tetrazolium (NBT) test of polymorphonuclear leukocytes (PMNs) isolated from the blood of a maternal carrier of X-linked recessive chronic granulomatous disease (CGD). Because of random inactivation of either the normal or the affected X chromosome in maternal carriers of this disorder, approximately half of the PMNs exhibit the granular blue-black staining characteristic of the oxidative reduction of NBT by normal PMNs. In contrast, the remaining PMNs, which express the reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase defect of CGD, are visible only by their nuclear counterstain. **B,** Flow cytometry in CGD diagnosis and carrier testing. These idealized flow cytometry histograms demonstrate the oxidative burst activity of populations of PMNs that have been loaded with an agent such as dihydrorhodamine that becomes fluorescent when it reacts with products formed during the oxidative burst. The taller curve on the *far left* represents the fluorescence typical of both inactivated normal PMNs and activated PMNs from a patient with CGD with an absent oxidative burst. The taller histogram on the *far right* demonstrates the increased level of fluorescence exhibited by activated normal PMNs. The two smaller curves on the *left* and the *right* represent the two populations of activated PMNs from a typical maternal carrier of X-linked CGD, indicating about 50 percent normal PMNs and 50 percent expressing the defect. The taller histogram in the *middle* represents typical results for the cells of a carrier of autosomal recessive CGD, with all cells exhibiting an intermediate level of fluorescence.

marrow cells are transduced using retroviral vectors ex vivo and transplanted into irradiated syngeneic recipients.[183] Fifty to eighty percent of circulating neutrophils were oxidase positive 12 to 14 weeks after transplantation, and protection from respiratory challenge with *Aspergillus* was achieved.[183] Early human phase I trials with both autosomal recessive (p47^{phox})and X-linked chronic granulomatous disease, using autologous peripheral blood stem cells retrovirally transduced ex vivo, then reinfused without prior marrow conditioning, have yielded a maximum of 0.2 percent oxidase-corrected neutrophils in the circulation,[379] far short of what probably must be achieved to produce a clinically meaningful correction.[183,447]

Deficiencies of Glucose-6-Phosphate Dehydrogenase and Glutathione Peroxidase. The normal activity of the NADPH oxidase enzyme complex depends on the continued availability of NADPH to reduce molecular oxygen to form superoxide anion.[135,491] The primary source of NADPH for this enzyme is the hexose monophosphate shunt, and this pathway is provided with the hexose substrate, 6-phosphoglucose, by the enzyme glucose-6-phosphate dehydrogenase (G6PD), which also generates NADPH in a coupled reaction.[492] The reactions of the hexose monophosphate shunt itself are coupled to two other enzymes, glutathione reductase and glutathione peroxidase, which recycle oxidized and reduced glutathione.[491] The absence of any of these three enzymes results in a lack of available NADPH to drive the NADPH oxidase. Thus, deficiencies in any of these other three enzymes may result in a phagocyte killing defect similar to chronic granulomatous disease. Patients with deficiencies of G6PD dehydrogenase or glutathione peroxidase have been described with functional oxidative metabolic defects and presentations that are clinically indistinguishable from chronic granulomatous disease, caused by defects in the NADPH oxidase itself.[43,505] G6PD deficiency usually involves erythrocytes and is associated with hemolytic anemia, especially in conjunction with the administration of

sulfonamides.[150] Only when the defect also involves myeloid cells and is severe or complete (<5 percent of normal enzyme levels) is the chronic granulomatous disease–like disorder manifested.[43,505] A partial deficiency of glutathione reductase has been reported, with hemolytic anemia and early cataracts, but no increased incidence of infection was noted.[490]

Glutathione Synthetase Deficiency. Glutathione, along with glutathione peroxidase and glutathione reductase, the two enzymes involved in its recycling between oxidized and reduced forms, constitutes a protective mechanism in PMNs against membrane damage mediated by reactive oxygen intermediates formed during PMN activation.[490] Thus, the synthesis of an adequate supply of glutathione is critical to these cells. Two brothers with glutathione synthetase deficiency who presented with neutropenia, hemolytic anemia, acidosis, 5-oxyprolinuria, and recurrent infection were reported by Spielberg and colleagues[549] and Boxer and associates.[92] The PMNs from these patients exhibited elevated cytosolic hydrogen peroxide levels, diminished oxidative microbicidal activity, and impaired microtubule assembly. Antioxidant therapy with vitamin E normalized the in vitro abnormalities of the patients' PMNs, with no further difficulty with recurrent infections.[92] This suggested that vitamin E protected the cell membranes by scavenging excess hydrogen peroxide produced during PMN activation and thus prevented or minimized oxidant-induced membrane damage.

Myeloperoxidase Deficiency. Congenital deficiency of neutrophil myeloperoxidase, once thought to be a relatively rare disorder, has come to be recognized as the single most common heritable disorder of neutrophil function. However, its clinical significance remains in doubt. Population surveys made possible with the advent of automated flow cytochemical techniques have indicated an incidence of myeloperoxidase deficiency of about 1/2000 persons.[424,460] Approximately half of these patients have complete absence of this neutrophil enzyme, and the remainder have a partial deficiency. The precise mode of inheritance of this defect has not been established, but myeloperoxidase is known to be a product of a single gene on chromosome 17.[591] The reaction of MPO with hydrogen peroxide and chloride, causing the formation of hypochlorite, is one of the most effective microbicidal mechanisms of neutrophils, and cells from some patients with myeloperoxidase deficiency have been found to exhibit delayed killing of *C. albicans* and *S. aureus*. However, myeloperoxidase deficiency rarely has been associated with unusual infectious complications.

Important Examples of Secondary Immunodeficiency (Excluding Human Immunodeficiency Virus Infection)

Asplenia

Fulminant infections can occur in patients who have anatomic or functional asplenia.[82] The mortality rate from these infections in asplenic persons ranges from 40 to 80 percent.[82,621] The most common pathogens are encapsulated bacteria, including *S. pneumoniae* (50 to 70 percent), *H. influenzae*, and *N. meningitidis*.[621] Other streptococci, *S. aureus*, *Salmonella*, and other gram-negative bacilli are important but less common pathogens. All can cause fulminant, often fatal, disease characterized by rapid onset of shock. Malaria and babesiosis also are more severe in asplenic persons. Infections can occur at any time but are most common within the first 2 years after splenectomy.

Both the liver and the spleen are important in phagocytic clearance of bacteria from the circulation, and the spleen is an important site for antibody production. The spleen is relatively more important than the liver in processing antigen in the naïve host. The younger the person is when splenic function is lost, the higher the risk for serious infection. Thus, young children who become asplenic are much more susceptible to fulminant infection than are adults, because adults are more likely to have encountered antigens before splenectomy than are children. Persons whose indication for splenectomy is thalassemia or Hodgkin disease are at higher risk for dying of overwhelming infection than are those who have functional asplenia from sickle-cell disease. Patients who undergo spleen removal for spherocytosis or idiopathic thrombocytopenia have a lower risk for infection. The lowest-risk group consists of individuals whose spleens are removed surgically after trauma.[82,146]

Congenital asplenia usually is associated with complex congenital cardiac disease and occasionally with structural abnormalities of the gastrointestinal or genitourinary tracts. Thus, asplenia should be suspected in any patient with congenital heart disease and sepsis resulting from encapsulated organisms. Anatomic or functional asplenia also should be suspected in patients with erythrocytes that contain Howell-Jolly bodies, because these typically are removed from the circulation by the normally functioning spleen. Ultrasound or radionuclide liver and spleen scan usually can confirm asplenia or significant hyposplenia.[82]

Elective splenectomy for conditions such as hereditary spherocytosis should be delayed as long as possible, and splenic repair or subtotal splenectomy should be performed whenever possible after trauma. Asplenic persons are managed using prophylactic antibiotics, usually penicillin V or amoxicillin until at least 5 years of age.[146] They also should be immunized against encapsulated organisms at the appropriate ages (e.g., *H. influenzae* type b and pneumococcal conjugate vaccines beginning at 2 months and meningococcal conjugate vaccine as early as 9 months).[146] Patients should be warned about their increased risk for serious infections from malaria and babesiosis.

Sickle-Cell Disease

Immunodeficiency in sickle-cell disease patients is due, in large part, to their functional asplenia.[512] Part of the risk for infection stems from local infarction and tissue necrosis resulting from sickling, which causes sludging and resultant tissue hypoxia. The reticuloendothelial system also may be obstructed by having to deal with

chronic hemolysis. Patients with sickle-cell disease are protected partially from *Plasmodium falciparum* malaria but have a high incidence of fulminant sepsis and meningitis caused by encapsulated organisms (e.g., *S. pneumoniae*, *H. influenzae* type b, *N. meningitidis*) and *Salmonella*.[224] The relative risk for pneumococcal meningitis in children with sickle-cell disease is approximately 500 times that of normal children. *Salmonella* infections often are associated with osteomyelitis or meningitis.[202]

Patients with sickle-cell disease appear to have normal antibody response to most antigens, including age-appropriate responses to vaccines. There have been rare reports of patients with sickle-cell disease who have deficiencies of factor B, but sickle-cell disease patients have normal CH_{50} and normal levels of properdin, C3, and factor I.[306]

Patients with sickle-cell disease should be managed with prophylactic antibiotics until at least 5 years of age and should be immunized against *H. influenzae* type b, pneumococci, and meningococci at the appropriate ages.[146]

Cystic Fibrosis

Cystic fibrosis is an autosomal recessive disorder caused by mutations in both alleles of the gene encoding the protein called cystic fibrosis transmembrane conductance regulator (CFTR), a cyclic AMP–regulated chloride channel expressed at the luminal surface of airway epithelial cells.[613] Most patients with cystic fibrosis develop chronic endobronchial infection with *P. aeruginosa* of the mucoid phenotype. This infection is accompanied by an intense chronic airway inflammation with an exuberant influx of neutrophils that leads to bronchiectasis and destruction and fibrosis of lung and airway tissue and early death.[130,334] No systemic disorder of immunity has been documented in cystic fibrosis, but local factors in the airway inflammatory milieu, including neutrophil-derived proteases such as elastase, contribute to secondary impairments in opsonic and phagocytic host defenses by cleaving opsonic antibody and complement fragments, as well as important phagocytic receptors for these opsonins.[581] Impaired mucociliary clearance of bacteria from the cystic fibrosis airway is impaired because of viscous, dehydrated epithelial lining fluid, and large quantities of DNA from dying neutrophils impair clearance of secretions by markedly enhancing their viscosity. Although the early pathogenesis of the unique chronic endobronchial infection in cystic fibrosis and its relationship to the underlying genetic defect have remained obscure, several possible explanations have been offered. It has been suggested that CFTR on airway epithelial cells may function as a receptor for *Pseudomonas* that the cells normally use to internalize the organisms before being sloughed and cleared by mucociliary action and that this internalization may be deficient in cystic fibrosis epithelial cells.[466] Additionally, evidence suggests that microbicidal peptides of or related to the defensin family, released from airway epithelial cells as a local defense mechanism, may be inactivated by abnormal salt concentrations at the airway epithelial surface, thereby thwarting an important first line of local antibacterial defense.[539] Neither of these explanations has proved satisfactory. A reduction in cell surface sialic acid on cystic fibrosis epithelial cells unmasks the glycoprotein asialo GM1, which appears to function as an epithelial receptor for adhesion by *P. aeruginosa* and may promote early airway colonization.[508] More recently, it has been proposed that reduced expression of inducible nitric oxide synthase by cystic fibrosis airway epithelial cells may impair airway host defenses against early small inocula of bacteria. The unresolved question of the relationship between the cystic fibrosis genetic defect and *Pseudomonas* chronic lung infection continues to stimulate active research.[130] Management of patients typically includes nutritional support and pancreatic enzyme replacement, frequent aggressive chest physiotherapy to mobilize secretions, intermittent courses of aerosolized tobramycin, oral azithromycin for its anti-inflammatory effects, aggressive management of pulmonary exacerbations, and recombinant aerosolized DNAse to help liquefy secretions. Gene therapy has been explored but has not resulted in correction of the defect at the clinical level. Recently a novel and promising trial of pharmacotherapy targeted to the basic defect in cystic fibrosis, the drug ivacaftor, an orally administered potentiator of CFTR chloride channel function, in cystic fibrosis patients with at least one G551D-CFTR mutation resulted in sustained improvement of lung function through 48 weeks of treatment; ivacaftor is now licensed for cystic fibrosis patients with this mutation.[477]

Ciliary Dyskinesia

Primary ciliary dyskinesia is a rare disorder of deranged ciliary motility that predisposes patients to recurrent respiratory infections such as otitis media, sinusitis, bronchitis, and pneumonia, as a result of poor clearance of secretions.[559] It is due to a mutation in one of several genes encoding for a component of either the dynein arms or radial spokes of the cilia that provide the energy, movement, and structural integrity of the specific tubulin structures that make up the normal cilia of respiratory epithelium.[559] Approximately 50 percent of these patients may have dextrocardia and situs inversus. In the absence of these features, this disorder should be suspected in patients who have recurrent infections, as noted earlier, but who have undergone a comprehensive evaluation of humoral immunity that has not detected an abnormality. Diagnosis ordinarily requires a brush biopsy of nasal epithelial cells, best evaluated by transmission electron microscopy, which usually will reveal an abnormality of the inner or outer dynein arm or of the radial spokes of the cilia.[532,559] Patients in whom this diagnosis is suspected should be referred to clinicians with expertise at centers with the capability to perform the necessary diagnostic studies. Long-term management can be challenging and often involves antimicrobial prophylaxis and attempts to assist in mobilization of secretions. Many of these patients develop bronchiectasis as a result of recurrent and chronic airway and lung infection. There is no specific treatment for the ciliary dysfunction.[559]

EVALUATION FOR IMMUNODEFICIENCY IN THE CHILD WITH RECURRENT OR SEVERE INFECTIONS

Most immunodeficiency disorders can be diagnosed readily by employing a methodical process that begins with careful analysis of the child's presenting history and physical examination.[149,156,178,512,515] This information serves as the foundation, when indicated, for a rational laboratory evaluation. It should be borne in mind that it is normal for children to have several infections every year. It is not unusual for normal children who are exposed to other children, particularly older school age siblings or classmates, to develop approximately one infection per month, especially from late fall to early spring, when respiratory viruses are most active. The overwhelming majority of infections in immunocompetent children are characterized by being mild and localized to the gastrointestinal or upper respiratory tracts, and they either are self-limited or respond rapidly to conventional therapy. Immunocompromised hosts tend to have more frequent, severe, and unusual infections that may not respond readily to appropriate therapy.

History

A detailed history alone often is sufficient to determine whether an immunologic evaluation should be pursued in many children who have recurrent or severe infections. If tests for immunity are indicated, the history also serves as a guide to the types of studies that should be performed initially. Table 2-6 provides a list of historical information that is valuable in assessing the likelihood of immunodeficiency. Whenever possible, the child's complete medical records (including growth charts) should be obtained, particularly if several physicians have provided care, because the history often is complicated and incomplete or inaccurate information may be misleading.

The age of onset of suspicious infections usually helps in defining the underlying problem. For example, children with isolated immunoglobulin deficiencies tend to do well during the first few months of life because they are protected by maternal antibody. They usually start developing serious infections later in the first year of life. Those with cell-mediated or phagocytic disorders may begin developing infections in the newborn period (see earlier discussion). In contrast, healthy children who have been cared for at home by their mothers and who have no siblings often have relatively few infections in the first few years of life but may be brought to the physician for immunologic evaluation when they develop recurrent respiratory tract infections beginning the first few weeks after entering daycare, nursery school, or kindergarten, because of new exposure to various respiratory viral pathogens.

The number, nature, etiology, and severity of infections help in determining how aggressively to pursue an immunologic evaluation. Certain clinical presentations of disease and causative organisms are associated with a high likelihood of an immunodeficiency. Antibody or complement deficiencies or functional asplenia should be suspected in children with recurrent or life-threatening infections such as sepsis and meningitis caused by encapsulated organisms (e.g., *S. pneumoniae*, *H. influenzae* type b).[102,178,306,357,494] Complement deficiencies should be considered in persons with recurrent or severe neisserial disease.[175] Chronic granulomatous disease should be suspected in the presence of recurrent or unusual deep tissue infections, such as liver abscess, lymphadenopathy, pneumonia, or osteomyelitis, especially when caused by unusual gram-negative bacteria, such as *Serratia marcescens*, or by *Aspergillus* species.[622] Bacterial skin infections in patients who have never formed pus at infected sites may suggest a leukocyte adhesion deficiency. Pneumonia caused by *Pneumocystis* suggests a T-cell deficiency, either hereditary or as a result of HIV infection. In contrast, recurrent or even severe infections with group A streptococcus have not been associated with immunodeficiency, and recurrent urinary tract infections usually are associated with anatomic abnormalities of the urinary tract rather than immunodeficiency.

Documentation of the child's growth pattern is essential, because children who are thriving are much less

TABLE 2-6 **History in the Evaluation of a Child with Recurrent or Severe Infections**

Age at onset of infections
Number, frequency, and periodicity of infections
Nature of infections
Location on body
Organism(s)
Severity
Duration
Nature and duration of therapy
Response to therapy
Hospitalizations
Surgery
Growth pattern
Periodontal disease
Allergies
Immunizations
Exposure history
Contagious diseases in family, school, or community
Number and ages of siblings
Parents' occupations
Babysitting or daycare arrangements
Foreign travel
Parental smoking
Wood furnaces
Family history (especially in males)
Immunodeficiency
Recurrent, severe, or unusual infections
Cause of early deaths
Autoimmune disease
Allergy
Consanguinity
Days of school missed (and why)

likely to have serious immune disorders than are those with failure to thrive.

The immunization history should be documented carefully because it may prove to be useful in evaluating the child's ability to mount an antibody response to specific vaccine antigens. The history of recent live viral immunization should be obtained in children who have clinical presentations compatible with polio or measles, because infection caused by vaccine strains of these viruses may be the first indication of immunodeficiency in some children.

Children who attend daycare facilities or preschools are constantly being exposed to common infections. Familiarity with community patterns of disease such as prevalent clinical manifestations of enterovirus infection or the beginning of croup, respiratory syncytial virus, influenza, or rotavirus seasons can be used to reassure families of normal children with frequent mild infections.

A detailed family history should be obtained that includes questions about the presence of immunodeficiency, recurrent or severe infections, contributing factors to any early deaths, the gender of affected persons, and consanguinity. A history of recurrent or severe infections in maternal male relatives should be given special attention because of the prominence of X-linked disorders among the primary immunodeficiencies. Autoimmune diseases might suggest a familial disorder of complement or cell-mediated immunity.[175]

Physical Examination

Physical examination may provide valuable clues to the nature of an immune disorder. In certain cases, such as some patients with the hyper-IgE syndrome[104,281] and the DiGeorge syndrome,[182] the characteristic facies may suggest the diagnosis.

As noted earlier, one of the most obvious signs that a child may have a serious underlying medical problem is failure to thrive. Every immunologic evaluation must include documentation of current growth parameters and comparison with past growth.

Many immunodeficiency disorders have dermatologic manifestations. Eczematoid rashes are seen in patients with the hyper-IgE syndrome and chronic granulomatous disease.[104,281] Chronic granulomatous disease and leukocyte adhesion deficiencies also are characterized by slow wound healing and abnormal scarring patterns.[622] Patients with Chédiak-Higashi syndrome have partial albinism.[81] Severe gingival disease and early loss of teeth are prominent clinical features in disorders of neutrophil migration, such as LAD.[31,35]

The chest should be evaluated for physical signs of active disease, such as rales and rhonchi, as well as evidence of chronic infection, such as an increased anterior-posterior diameter. Pneumonia, bronchitis, bronchiectasis, and scarring can occur with many immunodeficiencies but are associated most frequently with immunoglobulin deficiencies.[102,271]

Cardiac abnormalities may be associated with primary asplenia or immunodeficiency disorders such as DiGeorge syndrome,[182] and dextrocardia with situs inversus should suggest ciliary dyskinesia as a cause of recurrent infections.[149]

Although hepatosplenomegaly may be found in many types of primary immunodeficiency disease, it is more common in patients with disorders of phagocyte function, especially chronic granulomatous disease.[622]

Laboratory Studies

The laboratory evaluation should be guided by the history and physical findings. Relatively simple and inexpensive screening tests often can help narrow the differential diagnosis and streamline the evaluation. One of the first tests that should be performed is a complete blood count with differential and evaluation of the blood smear. This simple test can detect several immunologic abnormalities, including neutropenia, lymphopenia associated with HIV-1 or forms of SCID, the abnormal neutrophil granules associated with the Chédiak-Higashi syndrome, Howell-Jolly bodies found with asplenia, and some malignancies. Chest radiographs should be examined for thymic tissue, mediastinal lymphadenopathy, pneumonia, bronchiectasis, and other evidence of pulmonary infections. Consideration also should be given to evaluating patients with chronic pulmonary disease for cystic fibrosis with a sweat chloride test.

In most patients, the specific clinical features of infections and their etiologies provide an optimal guide to choosing the most relevant and specific laboratory tests for evaluating for immunodeficiency, when this is indicated. All too frequently, a comprehensive, and expensive, evaluation of every major aspect of immunity is undertaken for a patient whose clinical presentation suggests a more carefully considered and targeted approach.

Children with a clear history of recurrent bacterial otitis media, sinusitis, or pneumonia are candidates for evaluation of humoral immunity. Quantitative measurement of Ig levels provides a useful screening test, but it must be remembered that IgG2 deficiency often is not reflected in the total IgG level because it makes up such a relatively small proportion of total IgG. Thus, patients with suspected humoral immunodeficiency always should be tested for IgG subclass levels, as well as quantitative total IgG, IgA, and IgM. Enumeration of B cells and marker studies by immunofluorescence flow cytometry should be considered if more severe forms of humoral deficiency, such as X-linked agammaglobulinemia or some forms of CVID, are suspected. Very high IgE levels may be helpful in establishing the diagnosis of hyper-IgE syndrome, and it should be noted that many laboratories do not include IgE levels in the standard Ig quantitation, and this must be ordered separately. Children who have normal quantitative Ig and IgG subclass levels but who continue to have frequent sinopulmonary infections that do not respond well to appropriate medical and surgical management (e.g., ventilation tubes) also can be evaluated by measuring antibody responses to specific antigens such as tetanus and diphtheria toxoids, *H. influenzae* type b, and meningococcal and pneumococcal capsular polysaccharides. Antibody levels can be measured before immunization and approximately 1 to 2 months after

immunization to evaluate the child's ability to respond to different kinds of antigens, including T cell–dependent antigens, such as diphtheria and tetanus toxoid, or T cell–independent antigens such as unconjugated pneumococcal capsular polysaccharide vaccine. In a patient with recurrent bacterial respiratory infections in whom no antibody-related abnormality can be identified after a comprehensive evaluation, the possibility of ciliary dyskinesia should be considered and a brush biopsy of nasal epithelial cells performed for electron microscopic studies (see earlier discussion).

The complement system should be evaluated in persons with recurrent or life-threatening neisserial disease, including systemic gonococcal infections and sporadic meningococcal disease. The best screening test for hemolytic complement is the CH_{50}. A normal CH_{50} reflects a normal quantity and function of classical pathway proteins (C1, C4, C2), C3, and terminal components through C8, as noted earlier.[143] Alternative pathway deficiencies are extremely rare; therefore, demonstration of a normal CH_{50} generally is a sufficient indicator of normal complement activity. An abnormal CH_{50} should be confirmed, being careful that the specimen is handled correctly. Complement abnormalities may be quantitative or qualitative. Thus, if a very low CH_{50} is confirmed, it is important to determine both the serum levels of individual complement proteins and their functional activity in consultation with a reference laboratory.

In patients with infections suggesting a T-cell defect, such as *Pneumocystis*, *Cryptococcus*, or recurrent candidiasis, delayed hypersensitivity skin testing with antigens such as *Candida* or mumps antigens may be useful to assess cell-mediated immunity, but results are not always reliable. T-lymphocyte subset quantitation may be helpful in diagnosing such conditions as SCID, but more sophisticated testing of lymphocyte function such as mitogen and antigen stimulation should be performed in patients with recurrent or severe fungal infection or in whom CVID is suspected. Such studies ideally should be directed by a clinical immunologist.

Similarly, suspected phagocyte function disorders should be evaluated in consultation with experts in phagocyte function because lack of proper standardization and expertise often leads to misleading results from commercial laboratories. Phagocyte function studies should be directed toward defects of adhesion and migration in patients with recurrent skin and mucosal infections, poor or absent pus formation, and persistent leukocytosis suggestive of a leukocyte adhesion defect. Tests of oxidative metabolic activity and killing should be performed in patients with recurrent staphylococcal or unusual gram-negative or fungal tissue infections suggestive of chronic granulomatous disease.

When clinical suspicion of an inherited immunodeficiency is confirmed by appropriate functional laboratory studies, it may be desirable to arrange for specific genetic testing to determine the precise nature of a patient's genetic mutation or variation, when such testing is available. This may assist greatly in subsequent genetic counseling and will add important information to the clinical database on patients with the specific disorder.

MANAGEMENT OF IMMUNODEFICIENCY DISORDERS

In many of the earlier sections, approaches to management of specific disorders have been considered. The remainder of discussion here is focused on more general aspects of management of patients whose immunodeficiencies have been determined.

Education and Counseling

After thorough characterization of the immunologic abnormality, the first step in management is to educate the family and, when he or she is old enough, the patient about environmental risks, all necessary medications and how to take them, and precisely when and where to seek medical care in the event of an acute illness or change in clinical status. Families of patients with inherited disorders should receive specific genetic counseling and be offered the option of future prenatal screening if it is available for the disease in question.

Evaluation and Treatment for Infection

Patients with known immunodeficiency disorders should be evaluated promptly and thoroughly for unexplained fevers or any other indication of infection. Immunodeficient persons are susceptible to a wide variety of pathogens, their responses to appropriate therapy may be slow, and they often require prolonged treatment. Thus, an effort should be made when possible to identify the infecting organism so that treatment can be specific. Unless the pathogen and its antimicrobial susceptibility are known, extended courses of empiric broad-spectrum coverage may be required, and this may lead to emergence of multiply resistant pathogens.

Treatment of Infections

Many aspects of disease-specific therapy already have been discussed. In general, patients with immunodeficiency who are susceptible to bacterial infections should be treated empirically and aggressively with antibiotics at the first indication of infection. Antifungal therapy should be added empirically in patients with increased risk for fungal infection (e.g., patients with cell-mediated immune and neutrophil disorders) if a prompt response to antibacterial therapy does not occur. Once a definitive etiology has been established, treatment should be tailored to the pathogen. The duration of therapy must be individualized, but, in general, patients with abnormal immunity require longer courses of treatment than normal hosts who have similar infections.

Prevention of Infection

Patients and household members should be immunized with appropriate vaccines as soon and as fully as possible after a diagnosis of a family member immunodeficiency.[146] Although many immunodeficient patients, such as those with X-linked agammaglobulinemia or SCID, cannot

respond normally to vaccines, immunization of household members and other close contacts may reduce the patients' risk for infection. Patients with complement deficiencies, asplenia, and sickle-cell disease should be immunized with vaccines directed against encapsulated organisms, such as meningococci, pneumococci, and *H. influenzae* type b. However, it should be remembered that these persons may not have normal responses to immunization, and therefore, if possible, their antibody responses to these vaccines should be measured; if responses are poor, they may benefit from additional doses of the vaccines. Live viral vaccines should not be administered to most patients with primary defects of cell-mediated immunity.

Patients with disorders characterized by recurrent or severe bacterial or fungal infections may require prophylactic antibacterial or antifungal therapy. Although this is an essential component of management for many of the more severe immunodeficiencies, such as DiGeorge syndrome, SCID, and chronic granulomatous disease, the benefit of this approach has not been documented for all immune disorders. For many other disorders the uncertainty of the benefits of long-term antimicrobial prophylaxis must be weighed carefully along with the risk for emergence of antimicrobial resistance.

Prospects for Correction of Serious Primary Immunodeficiencies

Bone marrow or stem cell transplants have been successful in a few patients with specific immunologic disorders, including SCID,[108,124] Wiskott-Aldrich syndrome,[452] LAD, and chronic granulomatous disease.[25,221,615] Current protocols for thymus transplantation are showing great promise in treatment of patients with DiGeorge syndrome.[383] Gene therapy has raised new possibilities for correcting certain immunologic defects, having demonstrated early promise for some forms of SCID, and early success in animal models of chronic granulomatous disease.[183,472] The development of malignancies associated with retroviral gene therapy in some cases has raised serious concerns about the ultimate feasibility of this approach,[106,472] but the means of overcoming these and other potential obstacles to gene therapy continue to be sought.

NEW REFERENCES SINCE THE SIXTH EDITION

48. Baker MW, Laessig RH, Katcher ML, et al. Implementing routine testing for severe combined immunodeficiency within Wisconsin's newborn screening program. *Public Health Rep* 2010;**125**(suppl 2):88–95.
79. Blanchard-Rohner G, Pollard AJ. Long-term protection after immunization with protein-polysaccharide conjugate vaccines in infancy. *Expert Rev Vaccines* 2011;**10**:673–84.
87. Borregaard N, Sørensen OE, Theilgaard-Mönch K. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol* 2007;**28**:340–5.
146. Committee on Infectious Diseases. *Red book: Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 84–6.
167. Davies EG, Thrasher AJ. Update on the hyper immunoglobulin M syndromes. *Br J Haematol* 2010;**149**:167–80.
259. Guo T, McDonald-McGinn D, Blonska A, et al. Genotype and cardiovascular phenotype correlations with *TBX1* in 1,022 velo-cardio-facial/DiGeorge/22q11.2 deletion syndrome patients. *Hum Mutat* 2011;**32**:1278–89.
279. Hidalgo A, Frenette PS. When integrins fail to integrate. *Nat Med* 2009;**15**:249–50.
285. Holland SM, DeLeo FR, Elloumi HZ, et al. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med* 2007;**357**:1608–19.
304. Jin MS, Kim SE, Heo JY, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated peptide. *Cell* 2007;**130**:1071–82.
325. King C. New insights into the differentiation and function of T follicular helper cells. *Nat Rev Immunol* 2009;**9**:757–66.
370. Littman DH, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* 2010;**140**:845–58.
380. Malinin NL, Zhang L, Choi J, et al. A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. *Nat Med* 2009;**15**:313–18.
383. Markert ML, Devlin BH, McCarthy EA. Thymus transplantation. *Clin Immunol* 2010;**135**:236–46.
391. McKinnon PJ. ATM and the molecular pathogenesis of ataxia-telangiectasia. *Annu Rev Pathol* 2012;**7**:303–21.
405. Minegishi Y, Saito M, Nagasawa M, et al. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. *J Exp Med* 2009;**206**:1291–301.
406. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 2007;**448**:1058–62.
407. Mintz R, Garty BZ, Meshel T, et al. Reduced expression of chemoattractant receptors by polymorphonuclear leukocytes in hyper-IgE syndrome patients. *Immunol Lett* 2010;**130**:97–106.
436. Ochs HD. Mutations of the Wiskott–Aldrich syndrome protein affect protein expression and dictate the clinical phenotypes. *Immunol Res* 2009;**44**:84–8.
438. Ochs HD, Filipovich AH, Veys P, et al. Wiskott–Aldrich syndrome: diagnosis, clinical and laboratory manifestations, and treatment. *Biol Blood Marrow Transplant* 2009;**15**(1 suppl):84–90.
440. Olariu TR, Remington JS, McLeod R, Alam A, Montoya JG. Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. *Pediatr Infect Dis J* 2011;**30**:1056–61.
459. Park BS, Song DH, Kim HM, et al. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 2009;**458**:1191–5.
471. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. *J Allergy Clin Immunol* 2012;**129**:607–16.
512. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010;**10**:170–81.
532. Shoemark A, Dixon M, Corrin B, Dewar A. Twenty-year review of quantitative transmission electron microscopy for the diagnosis of primary ciliary dyskinesia. *J Clin Pathol* 2012;**65**:267–71.
548. Sowerwine KJ, Holland SM, Freeman AF. Hyper-IgE syndrome update. *Ann NY Acad Sci* 2012;**1250**:25–32.
559. Stillwell PC, Wartchow EP, Sagel SD. Primary ciliary dyskinesia in children: a review for pediatricians, allergists, and pediatric pulmonologists. *Pediatr Allergy Immunol Pulmonol* 2011;**24**:191–6.
563. Su HC, Jing H, Zhang Q. DOCK8 deficiency. *Ann NY Acad Sci* 2011;**1246**:26–33.
565. Svensson L, Howarth K, McDowall A, et al. Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nat Med* 2009;**15**:306–12.
566. Swiecki M, Colonna M. Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity and tolerance. *Immunol Rev* 2010;**234**:142–62.
632. Zinkernagel RM. Immunological memory does not equal protective immunity. *Cell Mol Life Sci* 2012;**69**:1635–40.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abraham E, Wunderink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor-α in patients with sepsis syndrome. *JAMA* 1995;**273**:934–41.
2. Abramson JS, Wheeler JG, Quie PG. The polymorphonuclear leukocyte system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 94–112.
3. Abramson JS, Mills EL, Sayer MK, et al. Recurrent infections and delayed separation of the umbilical cord in an infant with abnormal phagocytic cell locomotion and oxidative response during opsonized particle phagocytosis. *J Pediatr* 1981;**99**:887–94.
4. Abughali N, Berger M, Tosi MF. Deficient total cell content of CR3 (CD11b) in neonatal neutrophils. *Blood* 1994;**83**:1086–92.
5. Abughali N, Dubyak G, Tosi MF. Impairment of chemoattractant-stimulated hexose uptake in neonatal neutrophils. *Blood* 1993;**82**: 2182–7.
6. Adamkin D, Stitzel A, Urmson J, et al. Activity of the alternative pathway of complement in the newborn infant. *J Pediatr* 1978;**93**:604–8.
7. Adderson EE, Schackelford PG, Quinn A, et al. Restricted Ig H chain V gene usage in the human antibody response to *Haemophilus influenzae* type b capsular polysaccharide. *J Immunol* 1991;**147**:1667–74.
8. Adenyi-Jones SCA, Faden H, Ferdon MB, et al. Systemic and local immune responses to enhanced-potency inactivated poliovirus vaccine in premature and term infants. *J Pediatr* 1992; **120**:686–9.
9. Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. *Nature* 2000;**406**:782–7.
10. Aggarwal J, Khan AJ, Diamond S, et al. Lazy leukocyte syndrome in a black infant. *J Natl Med Assoc* 1985;**77**:928–31.
11. Aggett PJ, Harries JT, Harvey BAM, et al. An inherited defect of neutrophil mobility in Shwachman syndrome. *J Pediatr* 1979; **94**:391–4.
12. Albelda SM, Muller WA, Buck CA, et al. Molecular and cellular properties of PECAM-1 (endoCAM/CD31): a novel vascular cell-cell adhesion molecule. *J Cell Biol* 1991;**114**:1059–68.
13. Alford CA, Stagno S, Reynold DW. Diagnosis of chronic perinatal infections. *Am J Dis Child* 1975;**129**:455–63.
14. Allen RC, Loose LD. Phagocytic activation of a luminol-dependent chemiluminescence in rabbit alveolar and peritoneal macrophages. *Biochem Biophys Res* 1976;**69**:245–52.
15. Allen RC, Armitage RJ, Conley ME, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993;**259**:990–3.
16. Alouf JE, Muller-Alouf H. Staphylococcal and streptococcal superantigens: molecular, biological and clinical aspects. *Int J Med Microbiol* 2003;**292**:429–40.
17. Alper CA, Xu J, Cosmopoulos K, et al. Immunoglobulin deficiencies and susceptibility to infection among homozygotes and heterozygotes for C2 deficiency. *J Clin Immunol* 2003;**23**:297–305.
18. Ambrosino DM, Siber GR, Chilmonczyk BA, et al. An immunodeficiency characterized by impaired antibody responses to polysaccharides. *N Engl J Med* 1987;**316**:790–3.
19. Ambrosino DM, Sood SK, Lee MC, et al. IgG1, IgG2, and IgM responses to two *Haemophilus influenzae* type b conjugate vaccines in young infants. *Pediatr Infect Dis J* 1992;**11**:855–9.
20. Ambrosino DM, Umetsu DT, Siber GR, et al. Selective defect in the antibody response to *Haemophilus influenzae* type b in children with recurrent infections and normal serum IgG subclass levels. *J Allergy Clin Immunol* 1988;**81**:1175–9.
21. Ambruso DR, Edward RB, McCabe MD, et al. Infectious and bleeding complications in patients with glycogenosis 1b: relationship to neutrophil and platelet function. *Am J Dis Child* 1985; **139**:691–7.
22. Ament ME, Ochs HD, Davis SD. Structure and function of the gastrointestinal tract in primary immunodeficiency syndromes: a study of 39 patients. *Medicine* 1973;**52**:227–48.
23. Ammann AJ, Hong R. Selective IgA deficiency and autoimmunity. *Clin Exp Immunol* 1970;**7**:833–8.
24. Ammann AJ, Hong R. Selective IgA deficiency: presentation of 30 cases and a review of the literature. *Medicine* 1971;**60**:223–36.
25. Anderson DC, Smith CW. Leukocyte adhesion deficiencies and other disorders of leukocyte motility. In: Scriver CR, Beaudet AL, Sly WS, et al, editors. *The metabolic basis of inherited disease*, 8th ed. New York: McGraw-Hill; 2001. p. 4829–56.
26. Anderson DC, Hughes BJ, Smith CW. Abnormal mobility of neonatal polymorphonuclear leukocytes: relationship to impaired redistribution of surface adhesion sites by chemotactic factor or colchicine. *J Clin Invest* 1981;**68**:863–74.
27. Anderson DC, Freeman KB, Hughes BJ, et al. Secretory determinants of impaired adherence and mobility of neonatal PMNs. *Pediatr Res* 1985;**19**:257A.
28. Anderson DC, Hughes BJ, Wible LJ, et al. Impaired motility of neonatal PMN leukocytes: relationship to abnormalities of cell orientation and assembly of microtubules in chemotactic gradients. *J Leukocyte Biol* 1984;**36**:1–15.
29. Anderson DC, Rothlein R, Martin SD, et al. Impaired transendothelial migration of neonatal neutrophils: abnormalities of MAC-I (CD11/CD18) dependent adherence reactions. *Blood* 1990;**76**: 2613–21.
30. Anderson DC, Schmalstieg FC, Arnaout MA, et al. Abnormalities of polymorphonuclear leukocyte function associated with a heritable deficiency of high molecular weight surface glycoproteins (gp 138): common relationship to diminished cell adherence. *J Clin Invest* 1984;**74**:536–51.
31. Anderson DC, Schmalsteig F, Finegold MJ, et al. The severe and moderate phenotypes of heritable MAC-1, LFA-1, P150,95 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features. *J Infect Dis* 1985;**152**:668–89.
32. Anderson DC, Wibble LJ, Hughes BJ, et al. Cytoplasmic microtubules in polymorphonuclear leukocytes: effects of chemotactic stimulation and colchicine. *Cell* 1982;**31**:719–29.
33. Andreasen SO, Christensen JE, Marker O, Thomsen AR. Role of CD40 ligand and CD28 in induction and maintenance of antiviral CD8+ effector T cell responses. *J Immunol* 2000;**164**: 3689–97.
34. Appleman LJ, Berezovskaya A, Grass I, Boussiotis VA. CD28 costimulation mediates T cell expansion via IL-2-independent and IL-2-dependent regulation of cell cycle progression. *J Immunol* 2000;**164**:144–51.
35. Arai K-I, Lee F, Miyajima A, et al. Cytokines: coordinators of immune and inflammatory responses. *Annu Rev Biochem* 1990; **59**:783–836.
36. Arnaout MA. Structure and function of the leukocyte adhesion molecules CD11/CD18. *Blood* 1990;**75**:1037–50.
37. Arnaout MA, Pitt J, Cohen HJ, et al. Deficiency of a granulocyte membrane glycoprotein (gp 150) in a boy with recurrent bacterial infections. *N Engl J Med* 1982;**306**:693–9.
38. Arrode F, Boccaccio C, Abastado JP, Davrinche C. Cross-presentation of human cytomegalovirus pp565 (UL83) to CD8+ T cells is regulated by virus-induced soluble-mediator-dependent maturation of dendritic cells. *J Virol* 2002;**76**:142–50.
39. Aruffo A, Farrington M, Hollenbaugh D, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* 1993;**72**:291–300.
40. Asselin-Paturel C, Trinchieri G. Production of type 1 interferons: plasmacytoid dendritic cels and beyond. *J Exp Med* 2005;**202**: 461–5.
41. Babior BM, Kipnes RS, Curnutte JT. Biological defense mechanisms: the production by leukocytes of superoxide, a potential bactericidal agent. *J Clin Invest* 1973;**52**:741–4.
42. Babior BM, Rosin RE, McMurrich BJ, et al. Arrangement of the respiratory burst oxidase in the plasma membrane of the neutrophil. *J Clin Invest* 1981;**67**:1724–30.
43. Baehner RL, Johnston RB, Nathan DG. Comparative study of the metabolic and bactericidal characteristics of severely glucose-6-phosphate dehydrogenase-deficient polymorphonuclear leukocytes and leukocytes from children with chronic granulomatous disease. *J Reticul Soc* 1972;**12**:150–60.
44. Baehner RL, Karnovsky MJ, Karnovsky ML. Degranulation of leukocytes in chronic granulomatous disease. *J Clin Invest* 1969;**48**:187–92.
45. Bagby GJ, Plessala KJ, Wilson LA, et al. Divergent efficacy of antibody to tumor necrosis factor-α in intravascular and peritonitis models of sepsis. *J Infect Dis* 1991;**163**:83–8.
46. Baggiolini M. Chemokines in pathology and medicine. *J Intern Med* 2001;**250**:91–104.
47. Bainton DF. Developmental biology of neutrophils and eosinophils. In: Gallin JI, Goldstein IM, Snyderman R, editors.

Inflammation: Basic principles and clinical correlates. 2nd ed. New York: Raven Press; 1992. p. 303–24.
48. Baker MW, Laessig RH, Katcher ML, et al. Implementing routine testing for severe combined immunodeficiency within Wisconsin's newborn screening program. *Public Health Rep* 2010;**125**(suppl 2):88–95.
49. Ballow M, Cates KL, Rowe JC, et al. Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infections. *Pediatr Res* 1986;**20**:899–904.
50. Bals R. Epithelial antimicrobial peptides in host defense against infection. *Respir Res* 2000;**1**:141–50.
51. Banatvala N, Davies J, Kanariou M, et al. Hypogammaglobulinaemia associated with normal or increased IgM (the hyper IgM syndrome): a case series review. *Arch Dis Child* 1994;**71**:150–2.
52. Baraff LJ, Leake RD, Burstyn DG, et al. Immunologic response to early and routine DTP immunization in infants. *Pediatrics* 1984;**73**:37–42.
53. Barber DF, Faure M, Long EO. LFA-1 contributes an early signal for NK cell cytotoxicity. *J Immunol* 2004;**173**:3653–9.
54. Barbosa MD, Nguyen QA, Tchernev VT, et al. Identification of the homologous beige and Chédiak-Higashi syndrome genes. *Nature* 1996;**382**:262–5.
55. Barrett DJ, Boyle MDP. Restoration of complement function in vivo by plasma infusion in factor I (C3b inactivator) deficiency. *J Pediatr* 1984;**104**:76–81.
56. Bashan N, Potashnik R, Hagay Y, et al. Impaired glucose transport in polymorphonuclear leukocytes in glycogen storage disease 1b. *Inherit Metab Dis* 1987;**10**:234–9.
57. Bass DA, Parce JW, Dechatelet LR, et al. Flow cytometric studies of oxidative product formation by neutrophils: a graded response to membrane stimulation. *J Immunol* 1983;**130**:1910–17.
58. Bastian J, Law S, Vogler L, et al. Prediction of persistent immunodeficiency in the DiGeorge anomaly. *J Pediatr* 1989;**115**: 391–6.
59. Bauer S, Kirschning CJ, Hacker H, et al. Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci U S A* 2001;**98**:9237–42.
60. Beaudet AL, Anderson DC, Michels VV, et al. Neutropenia and impaired neutrophil migration in type 1B glycogen storage disease. *J Pediatr* 1980;**97**:906–10.
61. Bennett JE. *Aspergillus* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 2306–11.
62. Berger M, Frank MM. The serum complement system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 133–58.
63. Berger M, O'Shea J, Cross AS, et al. Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. *J Clin Invest* 1984;**74**:1566–71.
64. Berger M, Wetzler E, August JT, et al. Internalization of type 1 complement receptors and de novo multivesicular body formation during chemoattractant-induced endocytosis in human neutrophils. *J Clin Invest* 1994;**94**:1113–25.
65. Berger M, Wetzler EM, Wallis RS. Tumor necrosis factor is the major monocyte product that increases complement receptor expression on mature human neutrophils. *Blood* 1988;**71**:151–8.
66. Berman JE, Mellis SJ, Pollock R, et al. Content and organization of the human Ig V_H locus: definition of three new V_H families and linkage to the Ig C_H locus. *EMBO J* 1988;**7**:727–38.
67. Berman S, Lee B, Nuss R, et al. Immunoglobulin G, total and subclass, in children with or without recurrent otitis media. *J Pediatr* 1992;**121**:249–51.
68. Bernbaum JC, Daft A, Anolik R, et al. Response of preterm infants to diphtheria-tetanus-pertussis immunizations. *J Pediatr* 1985;**107**:148–8.
69. Beutler B, Cerami A. The biology of cachectin/TNFα primary mediator of the host response. *Annu Rev Immunol* 1989;**7**: 625–55.
70. Bevan MJ. Helping the CD8+ T cell response. *Nat Rev Immunol* 2004;**4**:595–602.
71. Bhakdi S, Tranum-Jensen J. Complement lysis: a hole is a hole. *Immunol Today* 1991;**12**:318–20.
72. Biragyn A, Ruffini PA, Leifer CA, et al. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 2002;**298**:1025–9.
73. Bird P, Lachman PJ. The regulation of IgG subclass production in man: low serum IgG4 in inherited deficiencies of the classical pathway of C3 activation. *J Immunol* 1988;**18**:1217–22.
74. Bishof NA, Welch TR, Beischel LS. C4B deficiency: a risk factor for bacteremia with encapsulated organisms. *J Infect Dis* 1990;**162**:248–50.
75. Bisset LR, Schmid-Grendelmeier P. Chemokines and their receptors in the pathogenesis of allergic asthma: progress and perspective. *Curr Opin Pulm Med* 2005;**11**:35–42.
76. Bjorkman PJ, Saper MA, Samraoui B, et al. Structure of the human class I histocompatibility antigen, HLA-A2. *Nature* 1987; **329**:506–12.
77. Bjorkman PJ, Saper MA, Samraoui B, et al. The foreign antigen binding site and T cell recognition regions of class I histocompatibility antigens. *Nature* 1987;**329**:512–18.
78. Black FL. Why did they die? *Science* 1992;**258**:1739–40.
79. Blanchard-Rohner G, Pollard AJ. Long-term protection after immunization with protein-polysaccharide conjugate vaccines in infancy. *Expert Rev Vaccines* 2011;**10**:673–84.
80. Blattner FR, Tucker PW. The molecular biology of immunoglobulin D. *Nature* 1984;**307**:417–22.
81. Blume RS, Wolff SM. The Chédiak-Higashi syndrome: studies in four patients and a review of the literature. *Medicine* 1972;**51**: 247–80.
82. Bohnsack JF, Brown EJ. The role of the spleen in resistance to infection. *Annu Rev Med* 1986;**37**:49–59.
83. Bonilla FA, Geha RS. Update on primary immunodeficiency diseases. *J Allergy Clin Immunol* 2006;**117**:S435–41.
84. Borregaard N, Heiple JM, Simons ER, et al. Subcellular localization of the b-cytochrome component of the human neutrophil microbicidal oxidase: translocation during activation. *J Cell Biol* 1983;**97**:52–61.
85. Borregaard N, Kjeldsen L, Lollike K, et al. Granules and vesicles of human neutrophils: the role of endomembranes as source of plasma membrane proteins. *Eur J Haematol* 1993;**51**:318–22.
86. Borregaard N, Kjeldsen L, Sengelov H, et al. Changes in subcellular localization and surface expression of L-selectin, alkaline phosphatase, and Mac-1 in human neutrophils during stimulation with inflammatory mediators. *J Leukoc Biol* 1994;**56**:80–7.
87. Borregaard N, Sørensen OE, Theilgaard-Mönch K. Neutrophil granules: a library of innate immunity proteins. *Trends Immunol* 2007;**28**:340–5.
88. Bottger EC, Bitter-Suermann D. Complement and the regulation of humoral immune responses. *Immunol Today* 1987;**8**:261–4.
89. Bowens TS, Ochs HD, Altman LC, et al. Severe recurrent bacterial infections associated with defective adherence and chemotaxis in two patients with neutrophils deficient in cell-associated glycoproteins. *J Pediatr* 1982;**101**:932–40.
90. Boxer LA, Hedley-Whyte ET, Stossel TP. Neutrophil actin dysfunction and abnormal neutrophil behavior. *N Engl J Med* 1974;**29**:1093–9.
91. Boxer LA, Coates TD, Haak RA, et al. Lactoferrin deficiency associated with altered granulocyte function. *N Engl J Med* 1982;**307**:404–10.
92. Boxer LA, Oliver JM, Spielberg SP, et al. Protection of granulocytes by vitamin E in glutathione synthetase deficiency. *N Engl J Med* 1979;**301**:901–5.
93. Boxer LA, Watanabe AM, Rister M, et al. Correction of leukocyte function in Chédiak-Higashi syndrome by ascorbate. *N Engl J Med* 1976;**295**:1041–5.
94. Braun J, Stiehm ER. The B-lymphocyte system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 35–74.
95. Bredius RG, Van de Winkel JG, Weening RS, Out TA. Effector functions of IgG subclass antibodies. *Immunodeficiency* 1993;**4**: 51–3.
96. Bromley SK, Burack WR, Johnson KG, et al. The immunological synapse. *Annu Rev Immunol* 2001;**19**:375–96.
97. Brown EJ. Interaction of gram-positive microorganisms with complement. *Curr Top Microbiol Immunol* 1985;**121**:159–97.
98. Brown JH, Jardetzky T, Saper MA, et al. A hypothetical model of the foreign antigen binding site of class II histocompatibility molecules. *Nature* 1988;**353**:845–50.
99. Bruce MC, Baley JE, Medvik KA, et al. Impaired surface membrane expression of C3bi but not C3b receptors on neonatal neutrophils. *Pediatr Res* 1987;**21**:306–11.

100. Brueckmann M, Hoffmann U, Dvortsak E, et al. Drotrecogin alfa (activated) inhibits NF-kappa B activation and MIP-1-alpha release from isolated mononuclear cells of patients with severe sepsis. *Inflamm Res* 2004;**53**:528–33.
101. Brunet A, Gabau E, Perich RM, et al. Microdeletion and microduplication 22q11.2 screening in 295 patients with features of DiGeorge/velocardiofacial syndrome. *Am J Med Senet* 2006; **140**:2426–32.
102. Bruton OC. Agammaglobulinemia. *Pediatrics* 1952;**9**:722–8.
103. Buck D, Moshous D, de Chasseval R, et al. Severe combined immunodeficiency and microcephaly in siblings with hypomorphic mutations in DNA ligase IV. *Eur J Immunol* 2006; **36**:224–35.
104. Buckley RH. Disorders of the IgE system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 409–22.
105. Buckley RH. Molecular defects in human severe combined immunodeficiency and approaches to immune reconstitution. *Annu Rev Immunol* 2004;**22**:625–55.
106. Buckley RH. The multiple causes of human SCID. *J Clin Invest* 2004;**114**:1409–11.
107. Buckley R, Schiff RI, Schiff SE, et al. Human severe combined immunodeficiency: genetic, phenotypic, and functional diversity in one hundred eight infants. *J Pediatr* 1997;**130**:378–87.
108. Buckley RH, Schiff SE, Hayward AR. Reduced frequency of $CD45RO^+$ T lymphocytes in blood of hyper IgE syndrome patients. *J Allergy Clin Immunol* 1991;**87**:313–21.
109. Buckley RH, Wray BB, Belmaker EZ. Extreme hyperimmunoglobulinemia E and undue susceptibility to infection. *Pediatr* 1972;**49**:59–70.
110. Bucy RP, Chan C-L, Cooper MD. Tissue localization and CD8 accessory molecule expression of T gamma delta cells in humans. *J Immunol* 1989;**142**:3045–9.
111. Buescher ES, Gallin JI. Leukocyte transfusions in chronic granulomatous disease. *N Engl J Med* 1982;**307**:800–3.
112. Buescher ES, Gaither T, Nath J, et al. Abnormal adherence-related function of neutrophils, monocytes, and EB virus-transformed B cells in a patient with C3bi receptor deficiency. *Blood* 1985;**65**:1382–90.
113. Burnett GW, Scherp HW. *Oral microbiology and infectious disease*. Baltimore: Williams & Wilkins; 1968.
114. Burrows PD, Cooper MD. B cell development and differentiation. *Curr Opin Immunol* 1997;**9**:239–44.
115. Butcher EC. Leukocyte-endothelial cell recognition: three (or more) steps to specificity and diversity. *Cell* 1991;**67**:1033–6.
116. Cabau N, Levy FM, Zivy D, et al. Evolution of titre of serum IgG in newborn. *Med Microbiol Immunol* 1974;**162**:251–8.
117. Campbell AD, Long MW, Wicha MS. Haemonectin: a bone marrow adhesion protein specific for cells of granulocyte lineage. *Nature* 1987;**329**:744.
118. Campos MA, Vargas MA, Regueiro V, et al. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. *Infect Immun* 2004;**72**:7107–14.
119. Carroll MC. The complement system in B cell regulation. *Mol Immunol* 2004;**41**:141–6.
120. Castigli E, Wilson SA, Garibyan L, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nat Genet* 2005;**37**:829–34.
121. Cates KL, Levine RP. C3 binding to bacterial surfaces. In: Cabello F, Pruzzo C, editors. *Bacteria, complement, and the phagocytic cell*. NATO ASI SERIES. New York: Springer-Verlag; 1988. p. 109–28.
122. Cates KL, Densen P, Lockman JD, et al. C4B deficiency is not associated with meningitis or bacteremia with encapsulated bacteria. *J Infect Dis* 1992;**165**:942–4.
123. Cates KL, Goetz C, Rosenberg N, et al. Longitudinal development of specific and functional antibody in very low birth weight premature infants. *Pediatr Res* 1988;**23**:14–22.
124. Cavazzana-Calvo M, Lagresie C, Hacein-Bey-Abina S, Fischer A. Gene therapy for severe combined immunodeficiency. *Annu Rev Med* 2005;**56**:585–602.
125. Certain S, Barrat F, Pastural E, et al. Protein truncation test of LYST reveals heterogeneous mutations in patients with Chédiak Higashi syndrome. *Blood* 2000;**95**:979–83.
126. Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. *Nat Rev Immunol* 2001;**1**:41–9.
127. Chan K, Puck J. Development of population-based newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2005;**115**:391–8.
128. Chandra RK. Fetal malnutrition and postnatal immunocompetence. *Am J Dis Child* 1975;**29**:450–4.
129. Chin TW, Stiehm ER, Faloon J, Gallin JI. Corticosteroids in treatment of obstructive lesions of chronic granulomatous disease. *J Pediatr* 1987;**111**:349–52.
130. Chmiel J, Davis P. State of the art: why do the lungs of patients with cystic fibrosis become infected and why can't they clear the infection? *Resp Res* 2003;**4**:8–19.
131. Christensen RD. Hematopoiesis in the fetus and neonate. *Pediatr Res* 1989;**26**:531–5.
132. Church JA, Frenkel LD, Wright DG. T lymphocyte dysfunction, hyperimmunoglobulinemia E, recurrent bacterial infections, and defective neutrophil chemotaxis in a negro child. *J Pediatr* 1976;**88**:982–4.
133. Chusid MJ. Pyogenic hepatic abscess in infancy and childhood. *Pediatrics* 1978;**62**:554–9.
134. Clark MR. IgG effector mechanisms. *Chem Immunol* 1997;**65**: 88–110.
135. Clark RA, Leidal KG, Pearson DW, Nauseef WM. NADPH oxidase of human neutrophils: subcellular localization and characterization of an arachidonate-activatable superoxide-generating system. *J Biol Chem* 1987;**262**:4065–74.
136. Clark RA, Malech HL, Gallin JI, et al. Genetic variants of chronic granulomatous disease: prevalence of deficiencies of two cytosolic components of the NADPH oxidase system. *N Engl J Med* 1989;**321**:647–52.
137. Clark RA, Root RK, Kimball HR, et al. Defective neutrophil chemotaxis and cellular immunity in a child with recurrent infection. *Ann Intern Med* 1973;**78**:515–19.
138. Coates TD, Torkildson JC, Torres M, et al. An inherited defect of neutrophil motility and microfilamentous cytoskeleton associated with abnormalities in 47-Kd and 89-Kd proteins. *Blood* 1991;**78**:1338–46.
139. Reference deleted in proofs.
140. Cohen J. The immunopathogenesis of sepsis. *Nature* 2002; **420**:885–91.
141. Cohn M. The a priori principles which govern immune responsiveness. In: *Cellular basis of immune modulation*. New York: Liss; 1989. p. 11–44.
142. Cohen MS, Isturiz RE, Malech HL. Fungal infection in chronic granulomatous disease: the importance of the phagocyte in defense against fungi. *Am J Med* 1981;**71**:59–66.
143. Colten HR. Complement biosynthesis. *Clin Immunol Allergy* 1985;**5**:287–300.
144. Colten HR, Goldberger G. Ontogeny of serum complement proteins. *Pediatrics* 1979;**64**(suppl.):775–80.
145. Comerford I, Nibbs RJ. Post-translational control of chemokines: a role for decoy receptors? *Immunol Lett* 2005;**96**:163–74.
146. Committee on Infectious Diseases. *Red book: Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 84–6.
147. Committee on Infectious Diseases. Update on timing of hepatitis B vaccination for premature infants and for children with lapsed immunization. *Pediatrics* 1994;**94**:403–4.
148. Conley ME, Puck JM. Carrier detection in typical and atypical X-linked agammaglobulinemia. *J Pediatr* 1988;**112**:688–94.
149. Conley ME, Stiehm ER. Immunodeficiency disorders: General considerations. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 201–52.
150. Cooper MR, DeChatelet LR, McCall CE. Complete deficiency of leukocyte glucose-6-phosphate dehydrogenase with defective bactericidal activity. *J Clin Invest* 1972;**51**:769–78.
151. Cooper NR. The classical complement pathway: activation and regulation of the first complement component. *Adv Immunol* 1985;**37**:151–216.
152. Cooper NR, Nemerow GR. Complement-dependent mechanisms of virus neutralization. In: Ross GD, editor. *Immunobiology of the complement system*. Orlando: Academic Press; 1986. p. 139–62.
153. Croft M, Duncan DD, Swain SL. Response of naive antigen-specific $CD4^+$ T cells *in vitro*: characteristics and antigen-presenting cell requirements. *J Exp Med* 1992;**176**:1431–7.

154. Crowley CA, Curnutte JT, Roskin RE, et al. An inherited abnormality of neutrophil adhesion: its genetic transmission and its association with a missing protein. *N Engl J Med* 1980;**302**: 1163–8.
155. Cunningham-Rundles C. Clinical and immunologic analyses of 103 patients with common variable immunodeficiency. *J Clin Immunol* 1989;**9**:22–33.
156. Cunningham-Rundles C. Disorders of the IgA system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 423–42.
157. Cunningham-Rundles C. Common variable immunodeficiency. *Curr Allergy Asthma Rep* 2001;**1**:421–9.
158. Cunningham-Rundles S, Cunningham-Rundles C, Ma DI, et al. Impaired proliferative response to B lymphocyte activators in common variable immunodeficiency. *J Clin Immunol* 1981;**1**: 65–72.
159. Cunningham-Rundles C, Zhou Z, Mankarious S, et al. Long-term use of IgA depleted intravenous immunoglobulin in immunodeficient subjects with anti-IgA antibodies. *J Clin Immunol* 1993; **13**:272–8.
160. Curnutte JT. Classification of chronic granulomatous disease. *Hematol Oncol Clin North Am* 1988;**2**:241–52.
161. Daar AS, Fuggle SV, Fabre JW, et al. The detailed distribution of HLA-A, B, C antigens in normal human organs. *Transplantation* 1984;**38**:287–92.
162. Daar AS, Fuggle SV, Fabre JW, et al. The detailed distribution of MHC class II antigens in normal human organs. *Transplantation* 1984;**38**:293–8.
163. Dalgleish A, Beverly P, Clapham P, et al. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* 1984;**312**:763–7.
164. Dancis J, Osburn JJ, Kunz HW. Studies of immunology of the newborn infant. IV. Antibody formation in the premature infant. *Pediatrics* 1953;**12**:151–6.
165. Davis AE, III: C1 inhibitor and hereditary angioneurotic edema. *Annu Rev Immunol* 1988;**6**:595–628.
166. Davies DR, Cohen GH. Interactions of protein antigens with antibodies. *Proc Natl Acad Sci U S A* 1996;**93**:7–12.
167. Davies EG, Thrasher AJ. Update on the hyper immunoglobulin M syndromes. *Br J Haematol* 2010;**149**:167–80.
168. Davis MD, Bjorkman PJ. T-cell antigen receptor genes and T-cell recognition. *Nature* 1988;**334**:395–402.
169. Davis SD, Schaller J, Wedgewood RJ. Job's syndrome: recurrent "cold" staphylococcal abscesses. *Lancet* 1966;**1**:1013–15.
170. DeBiagi M, Andreani M, Centis F. Immune characterization of human fetal tissues with monoclonal antibodies. *Prog Clin Biol Res* 1985;**193**:89–94.
171. Decoster A, Darcy F, Caron A, et al. Anti-P30 IgA antibodies as prenatal markers of congenital toxoplasma infection. *Clin Exp Immunol* 1992;**87**:310–15.
172. Defrance T, Vanbervliet F, Briere F, et al. Interleukin 10 and transforming growth factor-β cooperate to induce anti-CD40-activated naive human B cells to secrete immunoglobulin A. *J Exp Med* 1992;**175**:671–82.
173. Degli-Esposti MA, Smyth MJ. Close encounters of different kinds: dendritic cells and NK cells take centre stage. *Nat Rev Immunol* 2005;**5**:112–24.
174. Dengrove J, Lee EJ, Heiner DC, et al. IgG and IgG subclass specific antibody responses to diphtheria and tetanus toxoids in newborns and infants given DTP immunization. *Pediatr Res* 1986;**20**:735–9.
175. Densen P. Human complement deficiency states and infection. In: Whaley K, Loos M, Weiler JM, editors. *Complement in health and disease*. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1993. p. 173–97.
176. Densen P. Complement. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 58–78.
177. Densen P, McRill C, Ross SC. The contribution of the alternative and classical complement pathways to gonococcal killing and C3 fixation. In: Poolman JT, Zanen HC, Meyer TF, et al, editors. *Gonococci and meningococci*. Dordrecht, The Netherlands: Kluwer Academic Publishers; 1988. p. 693–7.
178. Densen P, Weiler JM, Griffiss JM, et al. Familial properdin deficiency and fatal meningococcemia: correction of the bactericidal defect by vaccination. *N Engl J Med* 1987;**316**:922–6.
179. Diamond MS, Springer TA. A subpopulation of Mac-1 (CD11b/CD18) molecules mediates neutrophil adhesion to ICAM-1 and fibrinogen. *J Cell Biol* 1993;**120**:545–56.
180. Diamond RD. Cryptococcus neoformans. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 2331–40.
181. Diamond RD, Root RK, Bennett JE. Factors influencing killing of *Cryptococcus neoformans* by human leukocytes in vitro. *J Infect Dis* 1972;**163**:1108–13.
182. DiGeorge AM. A new concept of the cellular basis of immunity (discussion). *J Pediatr* 1965;**67**:907–8.
183. Dinauer MC, Lekstrom-Himes JA, Dale DC. Inherited neutrophil disorders: molecular basis and new therapies. *Hematology (Am Soc Hematol Educ Program)* 2000;**1**:303–18.
184. Doherty PC, Allan W, Eichelberger M. Roles of alpha beta and gamma delta T cell subsets in viral immunity. *Annu Rev Immunol* 1992;**10**:123–51.
185. Dommett RM, Klein N, Turner MW. Mannose-binding lectin in innate immunity: past, present and future. *Tissue Antigens* 2006;**68**:193–209.
186. Donabedian H, Gallin JI. Deactivation of human neutrophil chemotaxis by chemoattractants: effect on receptors for the chemotactic factor f-met-leu-phe. *J Immunol* 1981;**127**:839–44.
187. Donabedian H, Gallin JI. Mononuclear cells from patients with the hyperimmunoglobulin E-recurrent infection syndrome produce an inhibitor of leukocyte chemotaxis. *J Clin Invest* 1982;**69**:1155–63.
188. Donabedian H, Gallin JI. The hyperimmunoglobulin E recurrent infection (Job's) syndrome: a review of the NIH experience and the literature. *Medicine* 1983;**62**:195–207.
189. Donowitz GR, Mandell GL. Clinical presentation and unusual infections in chronic granulomatous disease. In: Gallin JI, Fauci AS, editors. *Advances in host defense mechanisms*. New York: Raven Press; 1983. p. 55–75.
190. Donskoy E, Goldschneider I. Thymocytopoiesis is maintained by blood-borne precursors throughout postnatal life: a study of parabiotic mice. *J Immunol* 1992;**148**:1604–12.
191. Douglas SD, Yoder MC. The mononuclear phagocyte and dendritic cell systems. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 113–32.
192. Doyle C, Stominger JL. Interaction between CD4 and class II MHC molecules mediates cell adhesion. *Nature* 1987;**330**: 256–9.
193. Drew JH, Arroyave CM. The complement system of the newborn infant. *Biol Neonate* 1980;**37**:209–17.
194. Ebnet K, Kaldjian EP, Anderson AO, Shaw S. Orchestrated information transfer underlying leukocyte endothelial interactions. *Annu Rev Immunol* 1996;**14**:155–77.
195. Edelman GM. Antibody structure and molecular immunology. *Scand J Immunol* 1991;**34**:1–22.
196. Edwards JE. *Candida* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 2289–306.
197. Edwards MS. Complement in neonatal infections: an overview. *Pediatr Infect Dis* 1986;**5**:S168–170.
198. Eichenfield LF, Johnston RB, Jr. Secondary disorders of the complement system. *Am J Dis Child* 1989;**143**:595–602.
199. Einhorn MS, Granoff DM, Nahm MH, et al. Concentrations of antibodies in paired maternal and infant sera: relationship to IgG subclass. *J Pediatr* 1987;**111**:783–8.
200. Enders A, Fisch P, Schwartz K, et al. A severe form of human combined immunodeficiency due to mutations in DNA ligase IV. *J Immunol* 2006;**176**:5060–8.
201. Enders G. Serologic test combinations for safe detection of rubella infections. *Rev Infect Dis* 1985;**7**:S113–22.
202. Engh CA, Hughes JL, Abrams RC, et al. Osteomyelitis in the patient with sickle-cell disease. *J Bone Joint Surg Am* 1971;**53**:1–15.
203. Englund JA, Glezen WP, Turner C, et al. Transplacental antibody transfer following maternal immunization with polysaccharide and conjugate *Haemophilus influenzae* type b vaccines. *J Infect Dis* 1995;**171**:99–105.
204. Erdei A, Fust G, Gergely J. The role of C3 in the immune response. *Immunol Today* 1991;**12**:332–7.

205. Etzione A, Alon R. Leukocyte adhesion deficiency III: a group of integrin activation defects in hematopoietic lineage cells. *Curr Opin Allergy Clin Immunol* 2004;**4**:485–90.
206. Eynon EE, Parker DC. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. *J Exp Med* 1992;**175**:131–8.
207. Falk K, Rotzschke O, Deres K, et al. Identification of naturally processed viral nonapeptides allows their quantification in infected cells and suggests an allele-specific T cell epitope forecast. *J Exp Med* 1991;**174**:425–34.
208. Falk K, Rotzschke O, Stevanovic S, et al. Allele-specific motifs revealed by sequencing of self-peptides eluted from MHC molecules. *Nature* 1991;**351**:290–6.
209. Fanger M, Shen L, Graziano R, Guyre P. Cytotoxicity mediated by human Fc receptors for IgG. *Immunol Today* 1989;**10**:92–9.
210. Fanning LJ, Connor AM, Wu GE. Development of the immunoglobulin repertoire. *Clin Immunol Immunopathol* 1996;**79**:1–14.
211. Fearon DT, Austen KF. Properdin: binding to C3b and stabilization of the C3b-dependent C3 convertase. *J Exp Med* 1975; **142**:856–63.
212. Fearon DT, Austen KF. The alternative pathway of complement: a system for host resistance to microbial infection. *N Engl J Med* 1980;**303**:259–63.
213. Fearon DT, Collins LA. Increased expression of C3b receptors on polymorphonuclear leukocytes induced by chemotactic factors and by purification procedures. *J Immunol* 1983;**130**:370–5.
214. Fearon DT, Ruddy S, Schur PH, et al. Activation of the properdin pathway of complement in patients with gram-negative bacteremia. *N Engl J Med* 1975;**292**:937–40.
215. Ferguson SE, Thompson CBA. A new break in V(D)J recombination. *Curr Biol* 1993;**3**:51–3.
216. Ferrari S, Giliani S, Insalaco A, et al. Mutations of CD40 gene cause an autosomal recessive form of immunodeficiency with hyper-IgM. *Proc Natl Acad Sci U S A* 2001;**98**:12614–19.
217. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. *Clin Microbiol Rev* 1991;**4**:359–95.
218. Fink CW, Miller WE, Dorward B, et al. The formation of macroglobulin antibodies. II. Studies on neonatal infants and older children. *J Clin Invest* 1962;**41**:1422–8.
219. Fischer A, de Saint Basile G, Le Deist F. CD3 deficiencies. *Curr Opin Allergy Clin Immunol* 2005;**5**:491–5.
220. Fischer A, Hacein-Bey S, Le Deist F, et al. Gene therapy of severe combined immunodeficiencies. *Immunol Rev* 2000;**178**: 13–20.
221. Fisher A, Trung PH, Descamps-Latsdra B, et al. Bone marrow-transplantation for inborn error of phagocytic cells associated with defective adherence, chemotaxis, and oxidative response during opsonized particle phagocytosis. *Lancet* 1983;**2**:473–5.
222. Fisher CJ Jr, Dhainaut JF, Opal SM, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. *JAMA* 1994;**271**:1836–43.
223. Fleishmann J, Lehrer RI. Phagocytic mechanism in host response. In: Howard DH, editors. *Fungi pathogenic for humans and animals*. Part B2. New York: Marcel Dekker; 1985. p. 123–49.
224. Fleming AF, Storey J, Molineaux L, et al. Abnormal haemoglobins in the Sudan savanna of Nigeria. I. Prevalence of haemoglobins and relationships between sickle cell trait, malaria, and survival. *Ann Trop Med Parasitol* 1979;**73**:161–72.
225. Forlow SB, Schurr JR, Kolls JK, et al. Increased granulopoiesis through interleukin-17 and granulocyte colony-stimulating factor in leukocyte adhesion deficient mice. *Blood* 2001;**98**:3309–14.
226. Freeman AF, Kleiner DE, Nadiminti H, et al. Causes of death in hyper-IgE syndrome. *J Allergy Clin Immunol* 2007;**119**: 1234–40.
227. Fremont DH, Matsumura M, Stura EA, et al. Crystal structures of two viral peptides in complex with murine MHC class I H-2Kb. *Science* 1992;**257**:919–27.
228. Frydman M, Etzioni A, Eidlitz-Markus T, et al. Rambam-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility, and Bombay phenotype. *Am J Med Genet* 1992;**44**:297–302.
229. Fuchs EJ, Matzinger PB. B cells turn off virgin but not memory T cells. *Science* 1992;**258**:1156–9.
230. Gabbay JE, Almeida RP. Antibiotic peptides and serine protease homologs in human polymorphonuclear leukocytes: defensins and azurocidin. *Curr Opin Immunol* 1993;**5**:97–102.
231. Gallin JI. Neutrophil specific granule deficiency. *Annu Rev Med* 1985;**36**:263–74.
232. Garibyan L, Lobito AA, Siegel RM, et al. Dominant-negative effect of the heterozygous C104R TACI mutation in common variable immunodeficiency (CVID). *J Clin Invest* 2007;**117**: 1550–7.
233. Ganz T. Defensins: antimicrobial peptides of innate immunity. *Nature Rev Immunol* 2003;**3**:710–20.
234. Ganz T, Lehrer RI. Antimicrobial peptides of vertebrates. *Curr Opin Immunol* 1998;**10**:41–4.
235. Ganz T, Weiss J. Antimicrobial peptides of phagocytes and epithelia. *Semin Hematol* 1997;**34**:343–54.
236. Garcia KC, Teyton L, Wilson IA. Structural basis of T cell recognition. *Annu Rev Immunol* 1999;**17**:369–97.
237. Gathings WE, Lawton AR, Cooper MD. Immunofluorescent studies of the development of pre-B cells, B lymphocytes and immunoglobulin isotype diversity in humans. *Eur J Immunol* 1977;**7**:804–10.
238. Geelan SPM, Bazemer AC, Gerards LJ, et al. Deficiencies in opsonic defense to pneumococci in the human newborn despite adequate levels of complement and specific IgG antibodies. *Pediatr Res* 1990;**27**:514–18.
239. Geha RS, Reinherz E, Leung D, et al. Deficiency of suppressor T cells in the hyperimmunoglobulin E syndrome. *J Clin Invest* 1981;**68**:783–91.
240. Gelfand EW, Finkel TH. The T-lymphocyte system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 14–34.
241. Gerard C, Gerard N. C5a anaphylatoxin and its seven transmembrane-segment receptor. *Annu Rev Immunol* 1994;**12**: 775–808.
242. Gill TJ, Repetti CF, Metlay LA, et al. Transplacental immunization of the human fetus to tetanus by immunization of the mother. *J Clin Invest* 1983;**72**:987–96.
243. Gill TJ, III, Karasic RB, Antoncic J, Rabbin BS. Long-term follow-up of children born to women immunized with tetanus toxoid during pregnancy. *Am J Reprod Immunol* 1991;**25**:69–71.
244. Glass WG, Rosenberg HF, Murphy PM. Chemokine regulation of inflammation during acute viral infection. *Curr Opin Allergy Clin Immunol* 2003;**3**:467–73.
245. Goldberg AL, Rock KL. Proteolysis, proteasomes and antigen presentation. *Nature* 1992;**357**:375–9.
246. Golding B, Muchmore AV, Blaese RM. Newborn and Wiskott-Aldrich patient B cells can be activated by TNP–*Brucella abortus:* evidence that TNP–*Brucella abortus* behaves as a T-independent type 1 antigen in humans. *J Immunol* 1984;**133**:2966–71.
247. Goldsobel AB, Haas A, Stiehm ER. Bone marrow transplantation in DiGeorge syndrome. *J Pediatr* 1987;**111**:40–4.
248. Good RA. Studies on agammaglobulinemia: II. Failure of plasma cell formation in the bone marrow and lymph nodes of patients with agammaglobulinemia. *J Lab Clin Med* 1955;**46**: 167–81.
249. Goodnow CC. Balancing immunity, autoimmunity, and self-tolerance. *Ann NY Acad Sci* 1997;**815**:55–66.
250. Gordon DL, Hostetter MK. Complement and host defense against microorganisms. *Pathology* 1986;**18**:365–75.
251. Goudemand J, Anssens R, Delmas-Marsalet Y, et al. Essai de traitement d'un cas de granulomatose familiale chronique par greffe de moelle osseuse allogenique. *Arch Fr Pediatr* 1976;**33**: 121–9.
252. Grakoui A, Bromley SK, Sumen C, et al. The immunological synapse: a molecular machine controlling T cell activation. *Science* 2003;**285**:221–7.
253. Greenberg F, Elder FFB, Haffner P, et al. Cytogenic findings in a prospective series of patients with DiGeorge anomaly. *Am J Hum Genet* 1988;**43**:605–11.
254. Greenberg F, Crowder WE, Paschall V, et al. Familial DiGeorge syndrome and associated partial monosomy of chromosome 22. *Hum Genet* 1984;**65**:317–19.
255. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell* 1989;**56**:839–47.
256. Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. *Immunol Rev* 2005;**203**:244–50.
257. Grimbacher B, Schaffer AA, Holland SM, et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. *Am J Hum Genet* 1999;**65**:735–44.

258. Grisham MB, Jefferson MM, Melton DF, Thomas EL. Chlorination of endogenous amines by isolated neutrophils: ammonia-dependent bactericidal, cytotoxic, and cytolytic activities of the chloramines. *J Biol Chem* 1984;**259**:10404–13.
259. Guo T, McDonald-McGinn D, Blonska A, et al. Genotype and cardiovascular phenotype correlations with *TBX1* in 1,022 velo-cardio-facial/DiGeorge/22q11.2 deletion syndrome patients. *Hum Mutat* 2011;**32**:1278–89.
260. Gupta S, Rahwa R, O'Reilly R, et al. Ontogeny of lymphocyte subpopulations in human fetal liver. *Proc Natl Acad Sci U S A* 1976;**73**:919–22.
261. Harding CV, Collins DS, Slot JW, et al. Liposome-encapsulated antigens are processed in lysosomes, recycled, and presented to T cells. *Cell* 1991;**64**:393–401.
262. Harris BH, Shalit M, Southwick FS. Diminished actin polymerization by neutrophils from newborn infants. *Pediatr Res* 1992; **33**:27–31.
263. Hart DNJ. Dendritic cells: unique leukocyte populations which control the primary immune response. *Blood* 1997;**90**:3245–87.
264. Havran WL, Boismenu R. Activation and function of gamma delta T cells. *Curr Opin Immunol* 1994;**6**:442–6.
265. Hayashi F, Smith KD, Ozinsky A, et al. The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001;**410**:1099–103.
266. Hayashi SI, Kundisada T, Ogawa M, et al. Stepwise progress of B lineage differentiation supported by interleukin 7 and other stromal cell molecules. *J Exp Med* 1990;**171**:1683–95.
267. Hayward AR. Development of lymphocyte responses and interactions in the human fetus and newborn. *Immunol Rev* 1981;**57**: 39–60.
268. Hayward AR, Leonard J, Wood CBS, et al. Delayed separation of the umbilical cord, widespread infections and defective neutrophil mobility. *Lancet* 1979;**1**:1099–101.
269. Heath WR, Kane KP, Mescher MF, et al. Alloreactive T cells discriminate among a diverse set of endogenous peptides. *Proc Natl Acad Sci U S A* 1991;**88**:5101–05.
270. Heinzel F. Antibodies. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 36–57.
271. Hermaszewski RA, Webster ADB. Primary hypogammaglobulinaemia: a survey of clinical manifestations and complications. *Q J Med* 1993;**86**:31–42.
272. Herrod HG. Clinical significance of IgG subclasses. *Curr Opin Pediatr* 1993;**5**:696–9.
273. Herrod HG. Management of the patient with IgG subclass deficiency and/or selective antibody deficiency. *Ann Allergy* 1993;**70**:3–11.
274. Herrod HG, Gross S, Insel R. Selective antibody deficiency to *Haemophilus influenzae* type b capsular polysaccharide vaccination in children with recurrent respiratory tract infection. *J Clin Immunol* 1989;**9**:429–34.
275. Hertz CJ, Wu Q, Porter EM, et al. Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. *J Immunol* 2003;**171**: 6820–6.
276. Heyworth PG, Cross AR, Curnutte JT. Chronic granulomatous disease. *Curr Opin Clin Immunol* 2003;**15**:578–84.
277. Hibberd ML, Sumiya M, Summerfield JA, et al. Association of variants of the gene for mannose-binding lectin with susceptibility to meningococcal disease. *Lancet* 1999;**353**:1049–53.
278. Hibbs ML, Wardlaw AJ, Stacker SA, et al. Transfection of cells from patients with leukocyte adhesion deficiency with an integrin B subunit (CD18) restores lymphocyte function-associated antigen-1 expression and function. *J Clin Invest* 1990;**85**:674–81.
279. Hidalgo A, Frenette PS. When integrins fail to integrate. *Nat Med* 2009;**15**:249–50.
280. Hill HR. Biochemical, structural, and functional abnormalities of polymorphonuclear leukocytes in the neonate. *Pediatr Res* 1987;**22**:375–82.
281. Hill HR, Quie PG. Raised serum IgE levels and defective neutrophil chemotaxis in three children with eczema and recurrent bacterial infections. *Lancet* 1974;**1**:183–7.
282. Hixson P, Smith CW, Shurin SB, Tosi MF. Unique CD18 mutations involving a deletion in the extracellular stalk region and a major truncation of the cytoplasmic domain in a patient with leukocyte adhesion deficiency type 1. *Blood* 2004;**103**:1105–13.
283. Hoffman AA, Hayward AR, Kurnick JT, et al. Presentation of antigen by human newborn monocytes to maternal tetanus toxoid-specific T-cell blasts. *J Clin Immunol* 1981;**1**:217–21.
284. Hofman FM, Danilovs JA, Taylor CR. HLA-DR (Ia)-positive dendritic-like cells in human fetal nonlymphoid tissues. *Transplantation* 1984;**27**:590–4.
285. Holland SM, DeLeo FR, Elloumi HZ, et al. STAT3 mutations in the hyper-IgE syndrome. *N Engl J Med* 2007;**357**: 1608–19.
286. Hong R. Disorders of the T-cell system. In: Stiehm ER, editors. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 339–408.
287. Hoover DM, Boulegue C, Yang D, et al. The structure of human macrophage inflammatory protein-3alpha CCL20. Linking antimicrobial and CC chemokine receptor-6-binding activities with human beta-defensins. *J Biol Chem* 2002;**277**:37647–54.
288. Hoover DM, Rajashankar KR, Blumenthal R, et al. The structure of human beta-defensin-2 shows evidence of higher order oligomerization. *J Biol Chem* 2000;**275**:32911–18.
289. Horwitz MA. Interactions between *Legionella pneumophilia* and human mononuclear phagocytes. In: Thornsberry C, Balows A, Feeley JC, et al, editors. *Legionella*. Proceedings of the 2nd International Symposium. Washington, DC: American Society for Microbiology; 1984. p. 159–66.
290. Hostetter MK, Kreuger RA, Schmeling DJ. The biochemistry of opsonization: central role of the reactive thiolester of the third component of complement. *J Infect Dis* 1984;**150**:653–61.
291. Hostetter MK. Serotypic variations among virulent pneumococci in deposition and degradation of covalently bound C3b: Implications for phagocytosis and antibody production. *J Infect Dis* 1986;**153**:682–93.
292. Hostoffer RW, Krukovets I, Berger M. Enhancement by tumor necrosis factor-a of Fca receptor expression and IgA-mediated superoxide generation and killing of *Pseudomonas aeruginosa* by polymorphonuclear leukocytes. *J Infect Dis* 1994;**170**:82–7.
293. Hudson AW, Ploegh HL. The cell biology of antigen presentation. *Exp Cell Res* 2002;**272**:1–7.
294. Hugli TE. Structure and function of the anaphylatoxins. *Springer Semin Immunopathol* 1984;**7**:193–220.
295. Hunt DF, Henderson RA, Shababowitz J, et al. Characterization of peptides bound to the class I MHC molecule HLA-A2.1 by mass spectrometry. *Science* 1992;**255**:1261–3.
296. Hunt DF, Michel H, Dickinson TA, et al. Peptides presented to the immune system by the murine class II major histocompatibility complex molecule I-A^{d}. *Science* 1992;**256**:1817–20.
297. Insel RA, Amstey M, Woodin K, et al. Maternal immunization to prevent infectious diseases in the neonate or infant. *Int J Technol Assess Health Care* 1994;**10**:143–53.
298. International Chronic Granulomatous Disease Cooperative Study Group. A controlled trial of interferon gamma to prevent infection in chronic granulomatous diseases. *N Engl J Med* 1991;**324**:509–16.
299. Itano AA, Jenkins MK. Antigen presentation to naive CD4 T cells in the lymph node. *Nat Immunol* 2003;**4**:733–9.
300. IUIS/WHO Subcommittee on Chemokine Nomenclature. Chemokine/chemokine receptor nomenclature. *Cytokine* 2003; **21**:48–9.
301. Jackson SH, Gallin JI, Holland SM. The p47phox mouse knockout model of chronic granulomatous disease. *J Exp Med* 1995;**182**:751–8.
302. Janeway CA, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002;**20**:197–216.
303. Janeway CA Jr, Travers P, Walport M, Schlomchik MJ. The generation of lymphocyte antigen receptors. In: Janeway CA Jr, Travers P, Walport M, Schlomchik MJ, editors. *Immunobiology: The immune system in health and disease*. 6th ed. New York: Garland Science; 2005. p. 135–68.
304. Jin MS, Kim SE, Heo JY, et al. Crystal structure of the TLR1-TLR2 heterodimer induced by binding of a tri-acylated peptide. *Cell* 2007;**130**:1071–82.
305. Jin T, Bokarewa M, Foster T, et al. *Staphylococcus aureus* resists human defensins by production of staphylokinase, a novel bacterial evasion mechanism. *J Immunol* 2004;**172**:1169–76.
306. Johnston RB, Jr. Disorders of the complement system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 133–58.

307. Johnston RB, Baehner RL. Chronic granulomatous disease: correlation between pathogenesis and clinical findings. *Pediatrics* 1971;**48**:730–7.
308. Johnston RB, Newman SL. Chronic granulomatous disease. *Pediatr Clin North Am* 1977;**24**:365–76.
309. Joiner KA. Complement evasion by bacteria and parasites. *Ann Rev Microbiol* 1988;**42**:201–30.
310. Joiner KA, Brown EJ, Frank MM. Complement and bacteria: chemistry and biology in host defense. *Ann Rev Immunol* 1984;**2**:461–91.
311. Joiner KA, Fries LF, Frank MM. Studies of antibody and complement function in host defense against bacterial infection. *Immunol Lett* 1987;**14**:197–202.
312. Jones DH, Schmalsteig FC, Dempsey K, et al. Subcellular distribution and mobilization of Mac-1 (CD11b/CD18) in neonatal neutrophils. *Blood* 1990;**75**:488–98.
313. Lin JS, Yang CW, Wang DW, Wu-Hsieh BA. Dendritic cells cross-present exogenous fungal antigens to stimulate a protective CD8 T cell response to infection by *Histoplasma capsulatum*. *J Immunol* 2005;**174**:6282–91.
314. Kaech SM, Hemby S, Kersh E, Ahmed R. Molecular and functional profiling of memory CD8 T cell differentiation. *Cell* 2002;**111**:837–51.
315. Kalman L, Lindergren ML, Koibrynski L, et al. Mutations in genes required for T-cell development: IL7R, CD45, IL2RG, JAK3, RAG1, RAG2, ARTEMIS, and ADA and severe combined immunodeficiency: HuGE review. *Genet Med* 2004;**6**:16–26.
316. Kamani N, August CS, Douglas SD, et al. Transplantation in chronic granulomatous disease. *J Pediatr* 1984;**105**:42–6.
317. Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. *Cell* 1987;**49**:273–80.
318. Keiser PB, Nutman TB. *Strongyloides stercoralis* in the immunocompromised population. *Clin Microbiol Rev* 2004;**17**:208–17.
319. Kelly JM, Waterhouse NJ, Cretney E, et al. Granzyme M mediates a novel form of perforin-dependent cell death. *J Biol Chem* 2004;**279**:22236–42.
320. Kelsoe G. The germinal center: a crucible for lymphocyte selection. *Semin Immunol* 1996;**8**:179–84.
321. Kelsoe GF, Zheng B. Sites of B cell activation in vivo. *Curr Opin Immunol* 1993;**5**:418–22.
322. Kerr MA. The structure and function of human IgA. *Biochem J* 1990;**271**:285–96.
323. Kim CH. Chemokine-chemokine receptor network in immune cell trafficking. *Curr Drug Targets Immune Endocr Metabol Disord* 2004;**4**:343–61.
324. Kinashi T, Aker M, Sokolovsky-Eisenberg M, et al. LAD-III, a leukocyte adhesion deficiency syndrome associated with defective Rap1 activation and impaired stabilization of integrin bonds. *Blood* 2004;**103**:1033–6.
325. King C. New insights into the differentiation and function of T follicular helper cells. *Nat Rev Immunol* 2009;**9**:757–66.
326. Kishore SP, Bungum MK, Platt JL, Brunn GJ. Selective suppression of Toll-like receptor 4 activation by chemokine receptor 4. *FEBS Lett* 2005;**579**:699–704.
327. Klatzman D, Champagne E, Chamaret S, et al. T-lymphocyte T4 molecule behaves as a receptor for human retrovirus LAV. *Nature* 1984;**312**:767–70.
328. Klein JO, Marcy SM. Bacterial sepsis and meningitis. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 4th ed. Philadelphia: W. B. Saunders; 1995. p. 835–90.
329. Klein RB, Fisher TJ, Gard SE, et al. Decreased mononuclear and polymorphonuclear chemotaxis in human newborn infants and young children. *Pediatrics* 1977;**60**:467–72.
330. Kluin-Nelemans HC, van Velzen-Blad H, van Helden HPT, et al. Functional deficiency of complement factor D in a monozygous twin. *Clin Exp Immunol* 1984;**58**:724–30.
331. Kohl S, Springer TA, Schmalstieg FC, et al. Defective natural killer cytotoxicity and polymorphonuclear leukocyte antibody-dependent cellular cytotoxicity in patients with LFA-1/OKM-1 deficiency. *J Immunol* 1984;**133**:2972–8.
332. Kohler PF. Maturation of the human complement system. I. Onset of the time and site of fetal C1q, C4, C3 and C5 synthesis. *J Clin Invest* 1973;**52**:671–7.
333. Kohler PF, Farr RS. Elevation of cord over maternal IgG immunoglobulin: evidence for an active placental IgG transport. *Nature* 1966;**210**:1070–1.
334. Konstan M, Berger M. Infection and inflammation in the lung in cystic fibrosis. In: Davis P, editor. *Cystic fibrosis*. New York: Marcel Dekker; 1993. p. 221–76.
335. Kornfeld SJ, Plaut AG. Secretory immunity and the bacterial IgA proteases. *Rev Infect Dis* 1981;**3**:521–34.
336. Koshland ME. The coming of age of the immunoglobulin J chain. *Annu Rev Immunol* 1985;**3**:425–53.
337. Kramer N, Perez HD, Goldstein IM. An immunoglobulin (IgG) inhibitor of polymorphonuclear leukocyte motility in a patient with recurrent infection. *N Engl J Med* 1980;**303**:1253–8.
338. Kraus JC, Mayo-Bond L, Rogers CE, et al. An in vivo animal model of gene therapy for leukocyte adhesion deficiency. *J Clin Invest* 1991;**88**:1412–17.
339. Krause PJ, Maderazo EG, Scroggs M. Abnormalities of neutrophil adherence in newborns. *Pediatrics* 1982;**69**:184–87.
340. Kretschmer R, Say B, Brown D, et al. Congenital aplasia of the thymus gland (DiGeorge's syndrome). *N Engl J Med* 1968; **279**:1295–301.
341. Kulka M, Alexopoulou L, Flavell RA, Metcalfe DD. Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. *J Allergy Clin Immunol* 2004;**114**: 174–82.
342. Kunkel EJ, Butcher EC. Chemokines and the tissue-specific migration of lymphocytes. *Immunity* 2002;**16**:1–4.
343. Kunkel S, Standiford T, Chensue SW, et al. Cellular and molecular mechanisms of cytokine networking. *Agents Actions Suppl* 1991; **32**:205–18.
344. Kunkel SL, Strieter RM. Cytokine networking in lung inflammation. *Hosp Pract* 1990;**25**:63–76.
345. Kurt-Jones EA, Belko J, Yu C, et al. The role of toll-like receptors in herpes simplex infection in neonates. *J Infect Dis* 2005;**191**: 746–8.
346. Kuschert GS, Coulin F, Power CA, et al. Glycosaminoglycans interact selectively with chemokines and modulate receptor binding and cellular responses. *Biochemistry* 1999;**38**:12959–68.
347. Lachmann PJ. The control of homologous lysis. *Immunol Today* 1991;**12**:312–15.
348. Lammer EJ, Opitz JM. The DiGeorge anomaly as a developmental field defect. *Am J Med Genet* 1986;**2**(suppl.):113–27.
349. Lane P, Traunecker A, Hubele S, et al. Activated human T cells express a ligand for the human B cell-associated antigen CD40 which participates in T cell-dependent activation of B lymphocytes. *Eur J Immunol* 1992;**22**:2573–8.
350. Lasky LA. Selectins: interpreters of cell-specific carbohydrate information during inflammation. *Science* 1992;**258**:964–9.
351. Lassiter HA, Wilson JL, Feldhoff RC, et al. Supplemental complement component C9 enhances the capacity of neonatal serum to kill multiple isolates of pathogenic *Escherichia coli*. *Pediatr Res* 1994;**35**:389–96.
352. Lau Y-L, Tam AYC, Ng KW. Clinical laboratory observations: response of preterm infants to hepatitis B vaccine. *J Pediatr* 1992;**121**:962–5.
353. Law SK, Lichtenberg NA, Levine RP. Covalent binding an hemolytic activity of complement proteins. *Proc Natl Acad Sci U S A* 1980;**77**:7194–8.
354. Lawton AR, Cooper MD. Development and function of the immune system. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 1–13.
355. Lazarus GM, Neu HC. Agents responsible for infection in chronic granulomatous disease of childhood. *J Pediatr* 1975;**86**: 415–17.
356. Lebecque SG, Gearhart PJ. Boundaries of somatic mutation in rearranged immunoglobulin genes: 5′ boundary is near the promoter, and 3′ boundary is > 1 kb from V(D)J gene. *J Exp Med* 1990;**172**:1717–27.
357. Lederman HM, Winkelstein JA. X-linked agammaglobulinemia: an analysis of 96 patients. *Medicine* 1985;**64**:145–56.
358. Lee SI, Heiner DC, Wara D. Development of serum IgG subclass levels in children. *Monogr Allergy* 1986;**19**:108–21.
359. Lehrer RI, Ganz T. Antimicrobial polypeptides of human neutrophils. *Blood* 1990;**76**:2169–81.
360. Lekstrom-Himes JA, Dorman SE, Kopar P, et al. Neutrophil specific granule deficiency results from a novel mutation with loss of function of the transcription factor CCAAT/enhancer binding protein epsilon. *J Exp Med* 1999;**189**:1847–52.

361. Leonard WJ. X-linked severe combined immunodeficiency: from molecular cause to gene therapy within seven years. *Mol Med Today* 2000;**6**:403–7.
362. Levy O, Weiss J, Zarember K, Ooi CE, Elsbach P. Antibacterial 15-kDa protein isoforms (p15s) are members of a novel family of leukocyte proteins. *J Biol Chem* 1993;**268**:6058–63.
363. Levy O, Ooi CE, Weiss J, et al. Individual and synergistic effects of rabbit granulocyte proteins on *Escherichia coli*. *J Clin Invest* 1994;**94**:672–82.
364. Lewis DB, Wilson CB. Developmental immunology and role of host defenses in fetal and neonatal susceptibility to infection. In: Remington JS, Klein JO, editors. *Infectious Diseases of the Fetus and Newborn Infant*. 7th ed. Philadelphia: W. B. Saunders; 2011. p. 80–190.
365. Ley K. Integration of inflammatory signals by rolling neutrophils. *Immunol Rev* 2002;**186**:8–18.
366. Ley K. The role of selectins in inflammation and disease. *Trends Mol Med* 2003;**9**:263–8.
367. Lieberman J. The ABCs of granule-mediated cytotoxicity: new weapons in the arsenal. *Nat Rev Immunol* 2003;**3**:361–70.
368. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. I and II. *N Engl J Med* 1992;**327**:28–35, 99–106.
369. Liles WC, Van Voorhis WC. Review: Nomenclature and biologic significance of cytokines involved in inflammation and the host immune response. *J Infect Dis* 1995;**172**:1573–80.
370. Littman DH, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* 2010;**140**:845–58.
371. Lodewyk HS, Van Mierop MD, Kutsche LM. Cardiovascular anomalies in DiGeorge syndrome and importance of neural crest as a possible pathogenic factor. *Am J Cardiol* 1986;**58**: 133–7.
372. Long EO. Antigen processing for presentation to $CD4^+$ T cells. *New Biol* 1992;**4**:274–82.
373. Lorenz E, Mira JP, Frees KL, Schwartz DA. Relevance of mutations in the TLR4 receptor in patients with gram-negative septic shock. *Arch Intern Med* 2002;**162**:1028–32.
374. Lorenz E, Mira JP, Cornish KL, et al. A novel polymorphism in the toll-like receptor 2 gene and its potential association with staphylococcal infection. *Infect Immun* 2000;**68**:6398–401.
375. Lubke T, Marquardt T, Etzioni A, et al. Complementation cloning identifies CDG-IIc, a new type of congenital disorders of glycosylation, as a GDP-fucose transporter deficiency. *Nat Genet* 2001;**28**:73–6.
376. Lyon MF. Some milestones in the history of X-chromosome inactivation. *Annu Rev Genet* 1992;**26**:17–28.
377. Ma Y, Pannicke U, Schwartz K, Lieber MR. Hairpin opening and overhang processing by an Artemis/DNA-dependent protein kinase complex in nonhomologous end joining and V(D)J recombination. *Cell* 2002;**108**:781–94.
378. Macallan DC, Wallace D, Zhang Y, et al. Rapid turnover of effector-memory CD4(+) T cells in healthy humans. *J Exp Med* 2004;**200**:255–60.
379. Malech H, Horwitz M, Linton G, et al. Prolonged production of NADPH oxidase-corrected granulocytes after gene therapy of chronic granulomatous disease. *Proc Natl Acad Sci U S A* 1997;**94**:12133–8.
380. Malinin NL, Zhang L, Choi J, et al. A point mutation in KINDLIN3 ablates activation of three integrin subfamilies in humans. *Nat Med* 2009;**15**:313–18.
381. Marchalonis JJ, Jensen I, Schluter SF. Structural, antigenic and evolutionary analyses of immunoglobulins and T cell receptors. *J Mol Recognit* 2002;**15**:260–71.
382. Marciano BE, Wesley R, De Carlo ES, et al. Long-term interferon-γ therapy for patients with chronic granulomatous disease. *Clin Infect Dis* 2004;**39**:692–9.
383. Markert ML, Devlin BH, McCarthy EA. Thymus transplantation. *Clin Immunol* 2010;**135**:236–46.
384. Martensson L, Fudenberg HH. Gm genes and gamma G-globulin synthesis in the human fetus. *J Immunol* 1965;**94**:514–20.
385. Martyn LJ, Lischner HW, Pileggi AJ. Chorioretinal lesions in familial chronic granulomatous disease of childhood. *Trans Am Ophthalmol Soc* 1971;**69**:84–112.
386. Matsukawa A, Hogaboam CM, Lukacs NW, Kunkel SL. Chemokines and innate immunity. *Rev Immunogenet* 2000;**2**:339–58.
387. Matzinger P. Tolerance, danger and the extended family. *Annu Rev Immunol* 1994;**12**:991–1045.
388. Mauer AM, Athens JW, Ashenbrucker H, et al. Leukokinetic studies. II. A method for labeling granulocytes in vitro with radioactive di-isopropylfluorophosphate (DFP32). *J Clin Invest* 1960;**39**:1482–9.
389. McDowall A, Inwald D, Leitinger B, et al. A novel form of integrin dysfunction involving β1, β2, and β3 integrins. *J Clin Invest* 2003;**111**:51–60.
390. McEvoy LT, Zakem-Cloud H, Tosi MF. Total cell content of CR3 (CD11b/CD18) and LFA-1 (CD11a/CD18) in neonatal neutrophils: relationship to gestational age. *Blood* 1996;**87**:3929–33.
391. McKinnon PJ. ATM and the molecular pathogenesis of ataxia-telangiectasia. *Annu Rev Pathol* 2012;**7**:303–21.
392. McQuibban GA, Butler GS, Gong JH, et al. Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *J Biol Chem* 2001;**276**:43503–8.
393. Medzhitov R, Preston-Hurlburt P, Janeway CA, Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997;**388**:394–7.
394. Melchers F, ten Boekel E, Seidl T, et al. Repertoire selection by pre-B-cell receptors and B-cell receptors, and genetic control of B-cell development from immature to mature B cells. *Immunol Rev* 2000;**175**:33–46.
395. Melnick JL. Nomenclature and classification of viruses. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 3rd ed. Philadelphia: W. B. Saunders; 1992. p. 1374–89.
396. Merkenschlager M, Graf D, Lovatt M, et al. How many thymocytes audition for selection? *J Exp Med* 1997;**186**:1149–58.
397. Metchnikoff E. *Immunity in infectious diseases*. Binnie FG, trans. London: Cambridge University Press; 1905.
398. Metzger H. Structure and function of gamma M macroglobulins. *Adv Immunol* 1970;**12**:57–116.
399. Miller ME. Phagocytosis in the newborn infant: humoral and cellular factors. *J Pediatr* 1969;**74**:255–9.
400. Miller ME. Chemotactic function in the human neonate: humoral and cellular aspects. *Pediatr Res* 1971;**5**:487–92.
401. Miller ME. Phagocytic function in the neonate: selected aspects. *Pediatrics* 1979;**64**:S709–12.
402. Miller ME, Oski FA, Harris MB. Lazy leukocyte syndrome: a new disorder of neutrophil function. *Lancet* 1971;**1**:665–9.
403. Miller ME, Norman ME, Koblenzer PJ, et al. A new familial defect of neutrophil movement. *J Lab Clin Med* 1973;**82**:1–8.
404. Mills EL, Quie PG. Congenital disorders of the functions of polymorphonuclear neutrophils. *Rev Infect Dis* 1980;**2**:505–17.
405. Minegishi Y, Saito M, Nagasawa M, et al. Molecular explanation for the contradiction between systemic Th17 defect and localized bacterial infection in hyper-IgE syndrome. *J Exp Med* 2009;**206**: 1291–301.
406. Minegishi Y, Saito M, Tsuchiya S, et al. Dominant-negative mutations in the DNA-binding domain of STAT3 cause hyper-IgE syndrome. *Nature* 2007;**448**:1058–62.
407. Mintz R, Garty BZ, Meshel T, et al. Reduced expression of chemoattractant receptors by polymorphonuclear leukocytes in hyper-IgE syndrome patients. *Immunol Lett* 2010;**130**:97–106.
408. Mond JJ, Vos Q, Lees A, Snapper CM. T cell independent antigens. *Curr Opin Immunol* 1995;**7**:349–54.
409. Monteiro RC, Cooper MD, Kubagawa H. Molecular heterogeneity of Fcα receptors detected by receptor-specific monoclonal antibodies. *J Immunol* 1992;**148**:1764–71.
410. Morell A, Skvaril F, Hitzig WH. IgG subclass: development of the serum concentrations in "normal" infants and children. *J Pediatr* 1972;**80**:960–4.
411. Moretta L, Bottino C, Pende D, et al. Human natural killer cells: their origin, receptors and function. *Eur J Immunol* 2002;**32**: 1205–11.
412. Moretta A, Bottino C, Mingari MC, et al. What is a natural killer cell? *Nat Immunol* 2002;**3**:6–8.
413. Moser M, Murphy KM. Dendritic cell regulation of T_H1-T_H2 development. *Nat Immunol* 2000;**1**:199–205.
414. Moser M, Bauer M, Schmid S, et al. Kindlin-3 is required for beta2 integrin–mediated leukocyte adhesion to endothelial cells. *Nat Med* 2009;**15**:300–5.
415. Mosmann TR, Li L, Hengartner H, et al. Differentiation and functions of T cell subsets. *Ciba Found Symp* 1997;**204**:148–54.

416. Muller-Eberhard HJ. Complement: Chemistry and pathways. In: Gallin JI, Snyderman R, editors. *Inflammation: Basic principles and clinical correlates*. 2nd ed. New York: Raven Press; 1992. p. 33–61.
417. Murphy KM, Reiner SL. The lineage decisions of helper T cells. *Nat Rev Immunol* 2002;**2**:933–44.
418. Murphy KM, Heimberger AB, Loh DY. Induction by antigen of intrathymic apoptosis of $CD4^+$ $CD8^+$ TCR^{lo} thymocytes *in vivo*. *Science* 1990;**250**:1720–3.
419. Murphy PM. The molecular biology of leukocyte chemoattractant receptors. *Annu Rev Immunol* 1994;**12**:593–633.
420. Nagata M, Hara T, Aoki T, et al. Inherited deficiency of ninth component of complement: an increased risk of meningococcal meningitis. *J Pediatr* 1989;**114**:260–4.
421. Nakamura T, Kamogawa Y, Bottomly K, Flavell RA. Polarization of IL-4-and IFN-gamma-producing CD4+ T cells following activation of naive CD4+ T cells. *J Immunol* 1997;**158**:1085–94.
422. Naot YD, Desmonts G, Remington JS. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital *Toxoplasma* infection. *J Pediatr* 1981;**98**:32–6.
423. Nath J, Flavin M, Gallin JI. Tubulin tyrosinolation in human polymorphonuclear leukocytes: studies in normal subjects and in patients with Chédiak-Higashi syndrome. *J Cell Biol* 1982; **95**:519–26.
424. Nauseef WM, Root RK, Malech HL. Biochemical and immunologic analysis of hereditary myeloperoxidase deficiency. *J Clin Invest* 1983;**71**:1297–307.
425. Newburger PE, Cohen HJ, Rothchild SB, et al. Prenatal diagnosis of chronic granulomatous disease. *N Engl J Med* 1979;**300**: 178–81.
426. Niyonsaba F, Ogawa H, Nagaoka I. Human beta-defensin-2 functions as a chemotactic agent for tumour necrosis factor-alpha-treated human neutrophils. *Immunology* 2004;**111**:273–81.
427. Niyonsaba F, Iwabuchi K, Matsuda H, et al. Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. *Int Immunol* 2002;**14**:421–6.
428. Noble WC. Skin microbiology: coming of age. *J Med Microbiol* 1984;**17**:1–12.
429. Noelle RJ, Ledbetter JA, Aruffo A. CD40 and its ligand, an essential ligand-receptor pair for thymus-dependent B-cell activation. *Immunol Today* 1992;**13**:431–3.
430. Norderhaug IN, Johansen FE, Schjerven H, Brandtzaeg P. Regulation of the formation and external transport of secretory immunoglobulins. *Crit Rev Immunol* 1999;**19**:481–508.
431. Norment AM, Salter RD, Parham P, et al. Cell-cell adhesion mediated by CD8 and MHC class I molecules. *Nature* 1988;**336**: 79–81.
432. Nossal GJV. The molecular and cellular basis of affinity maturation in the antibody response. *Cell* 1992;**68**:1–2.
433. Notarangelo LD, Chirico G, Chiara A, et al. Activity of classical and alternative pathways of complement in preterm and small for gestational age infants. *Pediatr Res* 1984;**18**:281–5.
434. Nowak-Wegrzyn A, Crawford TO, Winkelstein JA, Carson KA, Lederman HM. Immunodeficiency and infections in ataxia-telangiectasia. *J Pediatr* 2004;**144**:505–11.
435. Nurieva RI, Chung Y. Understanding the development and function of T follicular helper cells. *Cell Mol Immunol* 2010;**7**: 190–7.
436. Ochs HD. Mutations of the Wiskott–Aldrich syndrome protein affect protein expression and dictate the clinical phenotypes. *Immunol Res* 2009;**44**:84–8.
437. Ochs HD, Wedgwood RJ. IgG subclass deficiencies. *Annu Rev Med* 1987;**38**:325–40.
438. Ochs HD, Filipovich AH, Veys P, et al. Wiskott–Aldrich syndrome: diagnosis, clinical and laboratory manifestations, and treatment. *Biol Blood Marrow Transplant* 2009;**15**(1 suppl):84–90.
439. Oettinger MA, Schatz DG, Gorka C, et al. RAG-1 and RAG-2, adjacent genes that synergistically activate V(D)J recombination. *Science* 1990;**248**:1517–23.
440. Olariu TR, Remington JS, McLeod R, et al. Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. *Pediatr Infect Dis J* 2011;**30**:1056–61.
441. Oliver AM, Sewell HF, Abramovich DR, et al. The distribution and differential expression of MHC class II antigens (HLA-DR, DP, and DQ) in human fetal adrenal, pancreas, thyroid and gut. *Transplant Proc* 1989;**21**:651–2.
442. O'Neill LA, Greene C. Signal transduction pathways activated by the IL-1 receptor family: ancient signaling machinery in mammals, insects, and plants. *J Leukoc Biol* 1998;**63**:650–7.
443. O'Neill LAJ. TLRs: Professor Mechnikov, sit on your hat. *Trends Immunol* 2004;**25**:687–93.
444. Opdenakker G, Van den Steen PE, Dubois B, et al. Gelatinase B functions as regulator and effector in leukocyte biology. *J Leukoc Biol* 2001;**69**:851–9.
445. Oppenheim JJ, Feldman M. Introduction to the Role of Cytokines in Innate Host Defense and Adaptive Immunity. In: Oppenheim JJ, Feldman M, Durum SK, et al., editors. *Cytokine reference*. San Diego: Academic Press; 2001. p. 3–20.
446. Oppenheim JJ, Biragyn A, Kwak LW, Yang D. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. *Ann Rheum Dis* 2003;**62**(Suppl 2):17–21.
447. Ott MG, Schmidt M, Schwartzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16, or SETBP1. *Nat Med* 2006;**12**:401–9.
448. Otternhoff TH, De Boer T, van Dissel JT, Verreck FA. Human deficiencies in type 1 cytokine receptors reveal the essential role of type 1 cytokines in immunity to intracellular bacteria. *Adv Exp Med Biol* 2003;**531**:279–94.
449. Ouinti I, Soresinsa A, Spadaro G, et al. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. *J Clin Immunol* 2007;**27**:308–16.
450. Oxelius V-A. IgG subclass levels in infancy and childhood. *Acta Paediatr Scand* 1979;**68**:23–7.
451. Oxelius V-A, Laurell AB, Linquist B, et al. IgG subclasses in selective IgA deficiency. *N Engl J Med* 1981;**304**:1476–7.
452. Ozsahin H, Le Deist F, Benkerrou M, et al. Bone marrow transplantation in 26 patients with Wiskott-Aldrich syndrome from a single center. *J Pediatr* 1996;**129**:238–44.
453. Ozen M, Mehmet C, Ozden S, et al. Recurrent Salmonella bacteremia in interleukin-12 receptor β1 deficiency. *J Trop Pediatr* 2006;**52**:296–8.
454. Padigel UM, Lee JJ, Nolan TJ, et al. Eosinophils can function as antigen-presenting cells to induce primary and secondary immune responses to *Strongyloides stercoralis*. *Infect Immun* 2006;**74**:3232–8.
455. Padlan EA. Anatomy of the antibody molecule. *Mol Immunol* 1994;**31**:169–217.
456. Palaniyar N, Nadesalingam J, Reid KB. Pulmonary innate immune proteins and receptors that interact with gram-positive bacterial ligands. *Immunobiology* 2002;**205**:575–94.
457. Pangburn MK. The alternative pathway. In: Ross GD, editor. *Immunobiology of the complement system*. Orlando: Academic Press; 1986. p. 45–62.
458. Papavasiliou FN, Schatz DG. Somatic hypermutation of immunoglobulin genes: merging mechanisms for genetic diversity. *Cell* 2002;**109**(suppl):S35–44.
459. Park BS, Song DH, Kim HM, et al. The structural basis of lipopolysaccharide recognition by the TLR4-MD-2 complex. *Nature* 2009;**458**:1191–5.
460. Parry MF, Root RK, Metcalf JA, et al. Myeloperoxidase deficiency: prevalence and clinical significance. *Ann Intern Med* 1981;**95**: 293–301.
461. Paul WE. Pleiotropy and redundancy: T cell–derived lymphokines in the immune response. *Cell* 1989;**57**:521–4.
462. Perez E, Sullivan KE. Chromosome 22q11.2 deletion syndrome (DiGeorge and velocardiofacial syndromes. *Curr Opin Pediatr* 2002;**14**:678–83.
463. Perlmuter DH, Colten HR. Molecular immunobiology of complement biosynthesis: a model of single-cell control of effector-inhibitor balance. *Annu Rev Immunol* 1986;**4**:231–51.
464. Perregaux DG, Bhavsar K, Contillo L, et al. Antimicrobial peptides initiate IL-1 beta posttranslational processing: a novel role beyond innate immunity. *J Immunol* 2002;**168**:3024–32.
465. Peterson SV, Thiel S, Jensenius JC. The mannan-binding lectin pathway of complement activation: biology and disease association. *Mol Immunol* 2001;**38**:133–49.
466. Pier GB, Grout M, Zaidi TS, et al. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science* 1996;**271**:64–7.

467. Pizzo PA, Robechaud KJ, Gill FA, et al. Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am J Med* 1982;**72**:101–11.
468. Plaeger SF. Principal human cytokines. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 1063–5.
469. Pollack JD, Williams DA, Gifford MA, et al. Mouse model of X-linked chronic granulomatous disease, an inherited defect in phagocyte superoxide production. *Nat Genet* 1995;**9**:202–9.
470. Porcelli SA, Modlin RL. The CD1 system: antigen-presenting molecules for T cell recognition of lipids and glycolipids. *Annu Rev Immunol* 1999;**17**:297–329.
471. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. *J Allergy Clin Immunol* 2012;**129**:607–16.
472. Puck JM, Malech HL. Gene therapy for immune disorders: good news tempered by bad news. *J Allergy Clin Immunol* 2006; **117**:865–9.
473. Purkerson J, Isakson P. A two-signal model for regulation of immunoglobulin isotype switching. *FASEB J* 1992;**6**:3245–52.
474. Quie PG, White JG, Holmes B, et al. In vitro bactericidal capacity of human polymorphonuclear leukocytes: diminished activity in chronic granulomatous disease of childhood. *J Clin Invest* 1967; **46**:668–79.
475. Rago JV, Schlievert PM. Mechanisms of pathogenesis of staphylococcal superantigens. *Curr Top Microbiol Immunol* 1998; **225**:81–97.
476. Raghavan S, Holmgren J. CD4+CD25+ suppressor T cells regulate pathogen induced inflammation and disease. *FEMS Immunol Med Microbiol* 2004;**44**:121–7.
477. Ramsey BW, Davies J, McElvaney NG, et al. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med* 2011;**365**:1663–72.
478. Rawal N, Pangburn MK. Structure/function of C5 convertases of complement. *Int Immunopharmacol* 2001;**1**:415–22.
479. Regelmann W, Hays N, Quie PG. Chronic granulomatous disease: Historical perspective and clinical experience at the University of Minnesota Hospitals. In: Gallin JI, Fauci AS, editors. Advances in host defense mechanisms. Vol. 3. New York: Raven Press; 1983. p. 3–23.
480. Regunathan J, Chen Y, Wang D, Malarkannan S. NKG2D receptor-mediated NK cell function is regulated by inhibitory Ly49 receptors. *Blood* 2005;**105**:233–40.
481. Revy P, Muto T, Levy Y, et al. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the hyper-IgM syndrome (HIGM2). *Cell* 2000;**102**:565–75.
482. Rice TW, Bernard GR. Therapeutic intervention and targets for sepsis. *Annu Rev Med* 2005;**56**:225–48.
483. Rieux-Laucat F, Hivroz C, Lim A, et al. Inherited and somatic CD3zeta mutations in a patient with T-cell deficiency. *N Engl J Med* 2006;**354**:1913–21.
484. Robey EA, Fowlkes BJ, Gordon JW, et al. Thymic selection in CD8 transgenic mice supports an instructive model for commitment to a CD4 or CD8 lineage. *Cell* 1991;**64**:99–107.
485. Robin NH, Shprintzen RJ. Defining the clinical spectrum of deletion 22q11.2. *J Pediatr* 2005;**147**:90–6.
486. Robinson J, Marsh SG. HLA informatics: Accessing HLA sequences from sequence databases. *Meth Mol Biol* 2003; **210**:3–21.
487. Roche PA, Cresswell P. Invariant chain association with HLA-DR molecules inhibits immunogenic peptide binding. *Nature* 1990;**345**:615–18.
488. Rock KL, Gamble S, Rothstein L, et al. Dissociation of β_2-microglobulin leads to the accumulation of a substantial pool of inactive class I MHC heavy chains on the cell surface. *Cell* 1991;**85**:611–20.
489. Romani N, Koide S, Crowley M, et al. Presentation of exogenous protein antigens by dendritic cells to T cell clones: intact protein is presented best by immature, epidermal Langerhans cells. *J Exp Med* 1989;**169**:1169–78.
490. Roos D, Weening RS, Voteman AA, et al. Protection of phagocytic leukocytes by endogenous glutathione: studies in a family with glutathione reductase deficiency. *Blood* 1979;**53**:851–7.
491. Root RK, Cohen MS. The microbicidal mechanisms of human neutrophils and eosinophils. *Rev Infect Dis* 1981;**3**:565–98.
492. Root RK, Metcalf J, Oshino N, et al. H_2O_2 release from human granulocytes during phagocytosis. I. Documentation, quantitation, and some regulating factors. *J Clin Invest* 1975;**55**:945–55.
493. Rosen FS, Janeway CA. The gamma globulins, III. The antibody deficiency syndromes. *N Engl J Med* 1966;**275**:769–75.
494. Rosen FS, Cooper MD, Wedgwood RJ. The primary immunodeficiencies. *N Engl J Med* 1984;**311**:235–42.
495. Rosenfield SI, Kelly ME, Leddy JP. Hereditary deficiency of the fifth component of complement in man. I. Clinical, immunochemical, and family studies. *J Clin Invest* 1976;**57**:1626–34.
496. Rosenfield SI, Baum J, Steigbigel RT, et al. Hereditary deficiency of the fifth component of complement in man. II. Biological properties of C5-deficient human serum. *J Clin Invest* 1976; **57**:1635–43.
497. Rosenkilde MM. Virus-encoded chemokine receptors: putative novel antiviral drug targets. *Neuropharmacology* 2005;**48**:1–13.
498. Rosenzweig SD, Holland SM. Defects in the interferon-γ and interleukin-12 pathways. *Immunol Rev* 2005;**203**:38–47.
499. Ross SC, Densen P. Complement deficiency states and infection: epidemiology, pathogenesis, and consequences of neisserial and other infections in an immune deficiency. *Medicine* 1984;**63**: 243–73.
500. Rossi D, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000;**18**:217–42.
501. Rudd CE. Disabled receptor signaling and new primary immunodeficiency disorders. *N Engl J Med* 2006;**354**:1874–7.
502. Ruddy S, Carpenter CB, Chin KW, et al. Human complement metabolism: an analysis of 144 studies. *Medicine* 1975;**54**:165–78.
503. Rudensky AY, Preston-Hurlburt P, Hong SC, et al. Sequence analysis of peptides bound to MHC class II molecules. *Nature* 1991;**353**:622–7.
504. Russel JH, Ley TJ. Lymphocyte-mediated cytotoxicity. *Annu Rev Immunol* 2002;**20**:323–70.
505. Rutenberg WD, Yang MC, Doberstyn B, et al. Multiple leukocyte abnormalities in chronic granulomatous disease: a familial study. *Pediatr Res* 1977;**11**:158–63.
506. Sacchi F, Hill HR. Defective membrane potential changes in neutrophils from human neonates. *J Exp Med* 1984;**160**: 1247–52.
507. Sacchi F, Augustine NH, Hill HR. Abnormality in actin polymerization associated with defective chemotaxis in neutrophils from neonates. *Int Arch Allergy Appl Immunol* 1987;**84**:32–9.
508. Saiman L, Prince A. *Pseudomonas aeruginosa* pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. *J Clin Invest* 1993;**92**:1875–80.
509. Sallusto F, Lenig D, Forster R, et al. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature* 1999;**401**:708–12.
510. Salmon JE, Edberg JC, Kimberly RP. Fc gamma receptor III on human neutrophils: allelic variants have functionally distinct capacities. *J Clin Invest* 1990;**85**:1287–95.
511. Salmon JE, Kapur S, Kimberly RP. Opsonin-independent ligation of Fc gamma receptors; the 3G8-bearing receptors on neutrophils mediate the phagocytosis of concanavalin A–treated erythrocytes and non-opsonized *E. coli*. *J Exp Med* 1987;**166**:1798–813.
512. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010;**10**:170–81.
513. Sato K, Hida S, Takayanagi H, et al. Antiviral response by natural killer cells through TRAIL gene induction by IFN-alpha/beta. *Eur J Immunol* 2001;**31**:3138–46.
514. Schaffer AA, Salzer U, Hammarstrom L, Grimbacher B. Deconstructing common variable immunodeficiency by genetic analysis. *Curr Opin Genet Dev* 2007;**17**:201–12.
515. Schaffer FM, Ballow M. Immunodeficiency: the office work-up. *J Respir Dis* 1995;**16**:523–46.
516. Schatz DG, Oettinger MA, Schlissel MS. V (D) J recombination: molecular biology and regulation. *Annu Rev Immunol* 1992;**10**: 359–84.
517. Schmidt AP, Taswell HF, Gleich GJ. Anaphylactic transfusion reaction associated with anti-IgA antibody. *N Engl J Med* 1969;**280**:188–93.
518. Schoub BD, Johnson S, McAnerney J, et al. Monovalent neonatal polio immunization strategy for the developing world. *J Infect Dis* 1988;**147**:836–9.
519. Schur PH. IgG subclasses: a historical perspective. *Monogr Allergy* 1988;**23**:1–11.

520. Schutte BC, Mitros JP, Bartlett JA,et al. Discovery of five conserved beta-defensin gene clusters using a computational search strategy. *Proc Natl Acad Sci USA* 2002;**99**:2129–33.
521. Schur PH, Rosen F, Norman ME. Immunoglobulin subclasses in normal children. *Pediatr Res* 1979;**13**:181–3.
522. Scott MG, Vreugdenhil AC, Buurman WA, Hancock RE, Gold MR. Cutting edge: cationic antimicrobial peptides block the binding of lipopolysaccharide (LPS) to LPS binding protein. *J Immunol* 2000;**164**:549–53.
523. Screpanti V, Wallin RP, Ljunggren HG, Grandien A. A central role for death receptor-mediated apoptosis in the rejection of tumors by NK cells. *J Immunol* 2001;**167**:2068–73.
524. Seger RA, Gungor T, Belohradsky BH, et al. Treatment of chronic granulomatous disease with myeloablative conditioning and an unmodified hemopoietic allograft: a survey of the European experience. *Blood* 2002;**100**:4344–50.
525. Seo SM, McIntire LV, Smith CW. Effects of IL-8, Gro-alpha, and LTB(4) on the adhesive kinetics of LFA-1 and Mac-1 on human neutrophils. *Am J Physiol Cell Physiol* 2001;**281**:C1568–78.
526. Shackelford PG. IgG subclasses: Importance in pediatric practice. *Pediatr Rev* 1993;**14**:291–6.
527. Shackelford PG, Granoff DM. IgG subclass composition of the antibody response of healthy adults, and normal or IgG2-deficient children and to immunization with *H. influenzae* type b polysaccharide vaccine or Hib PS-protein conjugate vaccines. *Monogr Allergy* 1988;**23**:269–81.
528. Shackelford PG, Granoff DM, Madassery JV, et al. Clinical and immunologic characteristics of healthy children with subnormal serum concentrations of IgG2. *Pediatr Res* 1990;**27**:16–21.
529. Shackelford PG, Granoff DM, Polmar SH, et al. Subnormal serum concentrations of IgG2 in children with frequent infections associated with varied patterns of immunologic dysfunction. *J Pediatr* 1990;**116**:529–38.
530. Shackelford PG, Polmar SH, Mayus JL, et al. Spectrum of IgG2 subclass deficiency in children with recurrent infections: prospective study. *J Pediatr* 1986;**108**:647–53.
531. Shapiro R, Beatty DW, Woods LI, et al. Serum complement and immunoglobulin values in small-for-gestational-age infants. *J Pediatr* 1981;**99**:139–41.
532. Shoemark A, Dixon M, Corrin B, Dewar A. Twenty-year review of quantitative transmission electron microscopy for the diagnosis of primary ciliary dyskinesia. *J Clin Pathol* 2012;**65**:267–71.
533. Siu G, Kronenberg M, Strauss E, et al. The structure, rearrangement and expression of D_β gene segments of the murine T-cell antigen receptor. *Nature* 1984;**311**:344–50.
534. Smith CW. Molecular determinants of neutrophil adhesion. *Am J Respir Cell Mol Biol* 1990;**2**:487–9.
535. Smith CW, Hollers JC, Dupree E, et al. A serum inhibitor of leukotaxis in a child with recurrent infections. *J Lab Clin Med* 1972;**79**:878–83.
536. Smith CW, Marlin SD, Rothlein R, et al. Cooperative interaction of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence and transendothelial migration of human neutrophils *in vitro*. *J Clin Invest* 1989;**83**:2008–17.
537. Smith CW, Rothlein R, Hughes BJ, et al. Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transendothelial migration. *J Clin Invest* 1988; **82**:1746–56.
538. Smith DH, Peter G, Ingram DL, et al. Responses of children immunized with the capsular polysaccharide of *Haemophilus influenzae*. *Pediatrics* 1973;**52**:637–44.
539. Smith JJ, Travis SM, Greenberg EP, Welsh MJ. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996;**86**:1–20.
540. Smith RT, Eitzman DV, Catlin ME, et al. The development of the immune response. *Pediatrics* 1964;**33**:163–83.
541. Smolen P, Bland R, Heiligenstein E, et al. Antibody response to oral polio vaccine in premature infants. *J Pediatr* 1983;**103**: 917–20.
542. Smyth MJ, Hayakawa Y, Takeda K, Yagita H. New aspects of natural-killer-cell surveillance and therapy of cancer. *Nat Rev Cancer* 2002;**2**:850–61.
543. Smyth MJ, Cretney E, Kelly JM, et al. Activation of NK cell cytotoxicity. *Mol Immunol* 2005;**42**:501–10.
544. Snyderman R, Pike MC, Altman LC. Abnormalities of leukocyte chemotaxis in human disease. *Ann N Y Acad Sci* 1975;**256**:386–8.
545. Solvason N, Kearney JF. The human fetal omentum: a site of B cell generation. *J Exp Med* 1992;**175**:397–404.
546. Soriano RB, South MA, Goldman AS, d Smith CW. Defect of neutrophil motility in a child with recurrent bacterial infections and disseminated cytomegalovirus infection. *J Pediatr* 1973;**83**: 951–5.
547. Southwick FS, Howard TH, Holbrook T, et al. The relationship between CR3 deficiency and neutrophil actin assembly. *Blood* 1989;**73**:1973–9.
548. Sowerwine KJ, Holland SM, Freeman AF. Hyper-IgE syndrome update. *Ann NY Acad Sci* 2012;**1250**:25–32.
549. Spielberg SP, Boxer LA, Oliver JM, et al. Oxidative damage to neutrophils in glutathione synthetase deficiency. *Br J Haematol* 1979;**42**:215–23.
550. Spitznagel JK, Cooper MR, McCall AE, et al. Selective deficiency of granules associated with lysozyme and lactoferrin in human polymorphs with reduced microbicidal capacity. *J Clin Invest* 1972;**51**:93A.
551. Spitznagel JK, Dalldorf FG, Leffell MS, et al. Character of azurophil and specific granules purified from human polymorphonuclear leukocytes. *Lab Invest* 1974;**30**:774–85.
552. Springer TA, Anderson DC. The importance of adherence, chemotaxis, and migration into inflammatory sites: Insights from an experiment of nature. In: Evered D, Nugent J, O'Connor M, editors. *Biochemistry of macrophages*. London, Pittman: Ciba Foundation Symposium; 1986. p. 102–26.
553. Springer TA, Thompson WS, Miller J, et al. Inherited deficiency of the Mac-1, LFA-1, P150,95 glycoprotein family and its molecular basis. *J Exp Med* 1984;**160**:1901–18.
554. Staretz-Haram O, Melamed R, Lifshitz M. Interleukin-12 receptor β1 deficiency presenting as recurrent *Salmonella* infection. *Clin Infect Dis* 2003;**37**:137–40.
555. Stavnezer J. Immunoglobulin class switching. *Curr Opin Immunol* 1996;**8**:199–205.
556. Steinstraesser L, Tippler B, Mertens J, et al. Inhibition of early steps in the lentiviral replication cycle by cathelicidin host defense peptides. *Retrovirology* 2005;**2**:2–13.
557. Stepick-Biek P, Thulliez P, Araujo FG, et al. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. *J Infect Dis* 1990;**162**:270–3.
558. Stevens R, Dichek D, Keld B, et al. IgG_1 is the predominant subclass of *in vivo*- and *in vitro*-produced anti-tetanus toxoid antibodies and also serves as the membrane IgG molecule for delivering inhibitory signals to anti-tetanus toxoid antibody-producing B cells. *J Clin Immunol* 1983;**3**:65–9.
559. Stillwell PC, Wartchow EP, Sagel SD. Primary ciliary dyskinesia in children: a review for pediatricians, allergists, and pediatric pulmonologists. *Pediatr Allergy Immunol Pulmonol* 2011;**24**:191–6.
560. Stossel TP. Phagocytosis: recognition and ingestion. *Semin Hematol* 1975;**12**:83.
561. Stossel TP. The mechanical responses of white blood cells. In: Gallin JI, Goldstein IM, Snyderman R, editors. *Basic principles and clinical correlates*. New York: Raven Press; 1992. p. 459–75.
562. Strauss RG, Snyder EL. Chemotactic peptide binding by intact neutrophils from human neonates. *Pediatr Res* 1984;**18**:63–6.
563. Su HC, Jing H, Zhang Q. DOCK8 deficiency. *Ann NY Acad Sci* 2011;**1246**:26–33.
564. Sutton BJ, Gould HJ. The human IgE network. *Nature* 1993;**366**:421–8.
565. Svensson L, Howarth K, McDowall A, et al. Leukocyte adhesion deficiency-III is caused by mutations in KINDLIN3 affecting integrin activation. *Nat Med* 2009;**15**:306–12.
566. Swiecki M, Colonna M. Unraveling the functions of plasmacytoid dendritic cells during viral infections, autoimmunity and tolerance. *Immunol Rev* 2010;**234**:142–62.
567. Taitz LS, Zarate-Salvador C, Schwartz E. Congenital absence of the parathyroid and thymus glands in an infant (III and IV pharyngeal pouch syndrome). *Pediatrics* 1966;**38**:412–18.
568. Takeuchi O, Hoshino K, Kawai T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram positive bacterial cell wall components. *Immunity* 1999;**11**:443–51.
569. Taylor PW. Bactericidal and bacteriolytic activity of serum against gram-negative bacteria. *Microbiol Rev* 1983;**47**:46–83.
570. Tenen DG, Hromas R, Licht JD, Zhang DE. Transcription factors, normal myeloid development, and leukemia. *Blood* 1997; **90**:489–519.

571. Tew JG, DiLosa RM, Burton GF, et al. Germinal centers and antibody production in bone marrow. *Immunol Rev* 1992;**126**: 99–112.
572. Teyton L, Peterson PA. Assembly and transport of MHC class II molecules. *New Biol* 1992;**4**:441–7.
573. Teyton L, O'Sullivan D, Dickson PW, et al. Invariant chain distinguishes between the exogenous and endogenous antigen presentation pathways. *Nature* 1990;**348**:39–44.
574. Thery C, Amigorena S. The cell biology of antigen presentation in dendritic cells. *Curr Opin Immunol* 2001;**13**:45–51.
575. Timens W, Boes A, Rozeboom-Uiterwijk T, Poppema S. Immaturity of the human splenic marginal zone in infancy: possible contribution to the deficiency infant immune response. *J Immunol* 1989;**143**:3200–6.
576. Tosi MF. Innate immune responses to infection. *J Allergy Clin Immunol* 2005;**116**:241–9.
577. Tosi M, Berger M. Functional differences between the 40 kDa and 50 to 70 IgG Fc receptors on human neutrophils revealed by elastase treatment and antireceptor antibodies. *J Immunol* 1988;**141**:2097–103.
578. Tosi MF, Hamedani A. A rapid, specific assay for superoxide release from phagocytes in small volumes of whole blood. *Am J Clin Pathol* 1992;**97**:566–73.
579. Tosi MF, Zakem H. Surface expression of Fcγ receptor III (CD16) on chemoattractant-stimulated neutrophils is determined by both surface shedding and translocation from intracellular storage compartments. *J Clin Invest* 1990;**90**:462–70.
580. Tosi MF, Anderson DC, Barrish J, et al. Effect of piliation on interactions of *Haemophilus influenzae* type b with human polymorphonuclear leukocytes. *Infect Immun* 1985;**47**:780–5.
581. Tosi MF, Zakem H, Berger M. Neutrophil elastase cleaves C3bi on opsonized *Pseudomonas* as well as CR1 on neutrophils to create a functionally important opsonin-receptor mismatch. *J Clin Invest* 1990;**86**:300–8.
582. Townsend A, Bodmer H. Antigen recognition by class I-restricted T lymphocytes. *Annu Rev Immunol* 1989;**7**:601–24.
583. Trambas CM, Griffiths GM. Delivering the kiss of death. *Nature Immunol* 2003;**4**:399–403.
584. Trapani JA, Smyth MJ. Functional significance of the perforin/granzyme cell death pathway. *Nat Rev Immunol* 2002;**2**:735–47.
585. Tsukada S, Saffran DC, Rawlings DJ, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 1993;**72**:279–90.
586. Uckun FM, Dibirdik I, Smith R, et al. Interleukin 7 receptor ligation stimulates tyrosine phosphorylation, inositol phospholipid turnover, and clonal proliferation of human B-cell precursors. *Proc Natl Acad Sci U S A* 1991;**88**:3589–3593.
587. Unkeless JC, Shen Z, Lin CW, DeBeus E. Function of human Fc gamma RIIA and Fc gamma RIIIB. *Semin Immunol* 1995; **7**:37–44.
588. van der Pol W-L, van de Winkel JGJ. IgG receptor polymorphisms: risk factors for disease. *Immunogenetics* 1998;**48**:222–32.
589. Van Dyke TE, Horoszewicz HU, Genco RJ. The polymorphonuclear leukocyte (PMNL) locomotor defect in juvenile periodontitis: study of random migration, chemokinesis and chemotaxis. *J Peridontol* 1982;**53**:682–7.
590. Van Epps D, Palmer DL, Williams RC. Characterization of serum inhibitors of neutrophil chemotaxis is associated with anergy. *J Immunol* 1974;**113**:189–200.
591. van Tuinen P, Johnson KR, Ledbetter S, et al. Localization of myeloperoxidase to the long arm of human chromosome 17: relationship to the 15:17 translocation of acute promyelocytic leukemia. *Oncogene* 1987;**1**:319–26.
592. Zelm MC, Reisli I, vander Burg M, et al. An antibody deficiency syndrome due to mutations in the CD19 gene. *N Engl J Med* 2006;**354**:1901–12.
593. Vanderkerckhove BAE, Baccala R, Jones D, et al. Thymic selection of the human T-cell receptor Vβ repertoire in SCID-hu mice. *J Exp Med* 1992;**176**:1619–24.
594. Vanichkin A, Patya M, Gazit A, et al. Late administration of lipophilic tyrosine kinase inhibitor prevents lipopolysaccharide and *Escherichia coli*-induced lethal toxicity. *J Infect Dis* 1996; **173**:927–33.
595. Vedder NB, Harlan JM. Increased surface expression of CD11b/CD18 (Mac-1) is not required for stimulated neutrophil adherence to cultured endothelium. *J Clin Invest* 1988;**81**:676–82.
596. Vetrie D, Vorechovsky I, Sideras F, et al. The gene involved in X-linked agammaglobulinemia is a member of the *src* family of protein-tyrosine kinases. *Nature* 1993;**361**:226–33.
597. Villadangos JA. Presentation of antigens by MHC class II molecules: getting the most out of them. *Mol Immunol* 2001; **38**:329–46.
598. Vivier E, Nunes JA, Vely F. Natural killer cell signaling pathways. *Science* 2004;**306**:1517–19.
599. Volpp BD, Nauseef WM, Donelson JE, et al. Cloning of the cDNA and functional expression of the 47 kilodalton cytosolic component of the human neutrophil respiratory burst oxidase. *Proc Natl Acad Sci* 1989;**86**:7195–9.
600. von Andrian UH, Berger EM, Ramezani L, et al. In vivo behavior of neutrophils from two patients with distinct inherited leukocyte adhesion deficiency syndromes. *J Clin Invest* 1993;**91**: 2893–7.
601. von Boehmer H, Teh HS, Kisielow P. The thymus selects the useful, neglects the useless and destroys the harmful. *Immunol Today* 1989;**10**:57–61.
602. Vora P, Youdim A, Thomas LS, Fukata M, Tesfay SY, Lukasek K, et al. Beta-defensin-2 expression is regulated by TLR signaling in intestinal epithelial cells. *J Immunol* 2004;**173**:5398–405.
603. Waage A, Halstensen A, Espevik T. Association between tumor necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet* 1987;**1**:355–7.
604. Walker RI, Willemze R. Neutrophil kinetics and the regulation of granulopoiesis. *Rev Infect Dis* 1980;**2**:282–92.
605. Walter MA, Surti U, Hofker MH, et al. The physical organization of the human immunoglobulin heavy chain gene complex. *EMBO J* 1990;**9**:3303–13.
606. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;**285**:248–51.
607. Wang L, Fuster, Sriramarao P, Esko JD. Endothelial heparan sulfate deficiency impairs L-selectin- and chemokine-mediated neutrophil trafficking during inflammatory responses. *Nat Immunol* 2005;**6**:902–10.
608. Wang X, Zhang Z, Louboutin JP, Moser C, Weiner DJ, Wilson JM. Airway epithelia regulate expression of human beta-defensin 2 through Toll-like receptor 2. *FASEB J* 2003;**17**:1727–9.
609. Ward PA, Schlegel RJ. Impaired leukotactic responsiveness in a child with recurrent infection. *Lancet* 1969;**2**:344–7.
610. Weaver LJ, Craddock PR, Jacob HS. Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome: pathophysiological relevance and possible prognostic value. *Lancet* 1980;**1**:947–9.
611. Weisdorf DJ, Craddock PR, Jacob HS. Granulocytes utilize different energy sources for movement and phagocytosis. *Inflammation* 1982;**6**:245–51.
612. Weiss J, Victor M, Elsbach P. Role of charge and hydrophobic interactions in the action of the bactericidal/permeability-increasing protein of neutrophils on gram-negative bacteria. *J Clin Invest* 1983;**71**:540–9.
613. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993;**73**:1251–4.
614. Wen J, Atkinson JP, Giclas PC. Clinical and laboratory evaluation of complement deficiency. *J Allergy Clin Immunol* 2004;**113**: 585–93.
615. Westminster Hospitals Bone-Marrow Transplant Team. Bone marrow transplant from an unrelated donor for chronic granulomatous disease. *Lancet* 1977;**1**:210–13.
616. Wheat LJ, Connolly-Stringfield PA, Baker RL. Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment, and review of the literature. *Medicine* 1990;**69**:361–74.
617. Whitley RJ, Arvin AM. Herpes simplex virus infections. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn Infant*. 4th ed. Philadelphia: W. B. Saunders; 1995. p. 354–76.
618. Wickham TJ, Mathias P, Cheresh DA, Nemerow GR. Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ promote adenovirus internalization but not virus attachment. *Cell* 1993;**73**:309–19.
619. Wilson CB, Ochs HD, Almquiest J, et al. When is umbilical cord separation delayed? *J Pediatr* 1985;**107**:292–3.
620. Wilson RK, Lai E, Concannon P, et al. Structure, organization and polymorphism of murine and human T-cell receptors α/β chain gene families. *Immunol Rev* 1988;**101**:149–72.

621. Winkelstein JA, Lambert GH, Swift A. Pneumococcal serum opsonizing activity in splenectomized children. *J Pediatr* 1975; **87**:430–3.
622. Winkelstein JA, Marino MC, Johnston RB, et al. Chronic granulomatous disease: report on a national registry of 368 patients. *Medicine* 2000;**79**:155–69.
623. Wu L, Gerard NP, Wyatt R, et al. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. *Nature* 1996;**384**:179–83.
624. Yang D, Chen Q, Schmidt AP, et al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. *J Exp Med* 2000;**192**:1069–74.
625. Yang D, Chertov O, Bykovskaia SN, et al. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 1999;**286**:525–8.
626. Yang KD, Hill HR. Immune responses to infectious diseases: an evolutionary perspective. *Pediatr Infect Dis J* 1996;**15**:355–64.
627. Yeung CY, Hobbs JR. Serum-gamma G-globulin levels in normal, premature, post-mature and "small-for-dates" newborn babies. *Lancet* 1968;**1**:1167–70.
628. Zach TL, Hostetter MK. Biochemical abnormalities of the third component of complement in neonates. *Pediatr Res* 1989; **26**:116–20.
629. Zarember KA, Godowski PJ. Tissue expression of human toll-like receptors and differential regulation of toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J Immunol* 2002;**168**:554–61.
630. Zemmour J, Parham P. HLA class I nucleotide sequences. *Hum Immunol* 1991;**31**:195–206.
631. Zimmerman GA. Two by two: the pairings of P-selectin and P-selectin glycoprotein ligand-1. *Proc Natl Acad Sci USA* 2001; **98**:10023–4.
632. Zinkernagel RM. Immunological memory does not equal protective immunity. *Cell Mol Life Sci* 2012;**69**:1635–40.

CHAPTER 3

Metabolic Response of the Host to Infections

Elizabeth C. Bellino • Alina Olteanu • Ralph D. Feigin • William R. Beisel

Although infectious microorganisms constitute a continuing threat at all ages of life, they are particularly dangerous in neonates, infants, and young children. Because children are the most vulnerable to infectious diseases, infectious microorganisms are now the world's biggest killer of children and young adults and account for more than 13 million deaths per year. Despite the availability of modern sanitation, public health measures, vaccines, and antibiotics, most children have many discrete episodes of acute infection before reaching adulthood. Depending on their severity and duration, infectious illnesses can interrupt normal growth patterns. More importantly, if closely spaced in time, a series of infections can initiate a downhill health spiral, leading to malnutrition, chronic debilitation, immune system dysfunction, and death.[16,18,93,94,138] This possibility is of greatest concern in newborns and weaning children, especially in Third World areas.

The function of the immune system is to protect the host from invasion of foreign organisms by distinguishing "self" from "nonself." The immune system consists of both specific and nonspecific components, which have distinct yet overlapping functions. Once a microorganism enters a host tissue, a complement system and coagulation cascade are activated, which induces the release of chemical mediators of the inflammatory response. The human host normally protects itself against invading microorganisms by maintaining a broad array of general defensive mechanisms and immunologic responses. This array includes the initiation of acute-phase reactions triggered by the release of proinflammatory cytokines from macrophages, monocytes, and other cells.[10,16,18,47] These proinflammatory cytokines mediate natural immunity, regulate lymphocyte grown and activation, activate inflammatory cells, affect leukocyte movements, and stimulate hematopoiesis. To cause disease, all microorganisms must encounter the host, gain entry into the host, multiply, spread from the site of entry, and injure the host cell. These cytokines cause injury directly by producing cytotoxins or indirectly by producing an inflammatory response. The resulting nonspecific defensive measures typically include fever and anorexia, slow-wave sleep, accelerated production of phagocytic cells, hormonal responses, and participation of many biochemical pathways and molecular mechanisms within body cells.[10,11,14-16,18,181] The acute-phase reaction produces stereotyped patterns of transient metabolic sequelae (diagrammed in Fig. 3-1) that accompany and follow most acute, generalized infectious illnesses and some localized ones.[66] A pediatrician should anticipate these metabolic changes to recognize dangerous complications, such as hypoglycemia and electrolyte imbalance, that may occur during an acute infection.

Inflammatory responses vary, whether they are mild to moderate in intensity or severe. An elevated body temperature accentuates lymphocyte responses; inflammatory hyperemia optimizes delivery of phagocytes to the sites of infection. This results in decreased availability of iron, which inhibits the growth of microorganisms that require iron as a nutrient. However, in an extreme or acute injury, the inflammatory response differs and extreme tissue damage occurs, as in the case of sepsis. The array of metabolic changes depicted in Figure 3-1 typically is shared as a group of common responses during all generalized acute infectious diseases.[9-11,13,16,18,151] Similar changes are seen during other types of disease or trauma when accompanied by fever or inflammatory reactions.[83] These common acute-phase responses are composed of a hypermetabolic admixture of anabolic and catabolic components. Each 1°C of fever causes basal cellular oxygen consumption to increase approximately 13 percent.[8] The resultant increase in cellular energy expenditure comes at a time when food intake and intestinal absorption are diminished by anorexia and sometimes by vomiting and diarrhea. In the absence of an adequate intake of nutrients, body energy needs are met mostly by the oxidation of metabolic substrates derived from nutrient stores already contained within body tissues. The hypermetabolic effects of acute fever are fueled primarily by carbohydrates (some of which are derived from the metabolism of amino acids),[12] but if infectious illnesses become subacute or chronic, body fats become the important sustaining fuel.

Generalized acute-phase metabolic responses may be modified by numerous factors, such as the severity of an infectious process, its duration, and its possible progression to a subacute or chronic disease.[10-12,14-16,18,74] The age and sex of the patient, the presence of genetic resistance (or susceptibility) factors or partial immunity, the adequacy of nonspecific defensive mechanisms and de novo immune responsiveness, the preexisting nutritional status, and the presence or absence of other diseases combine to modify host metabolic responses through their diverse influences on the infectious process.

Superimposed on this general array of common host metabolic responses are additional metabolic changes

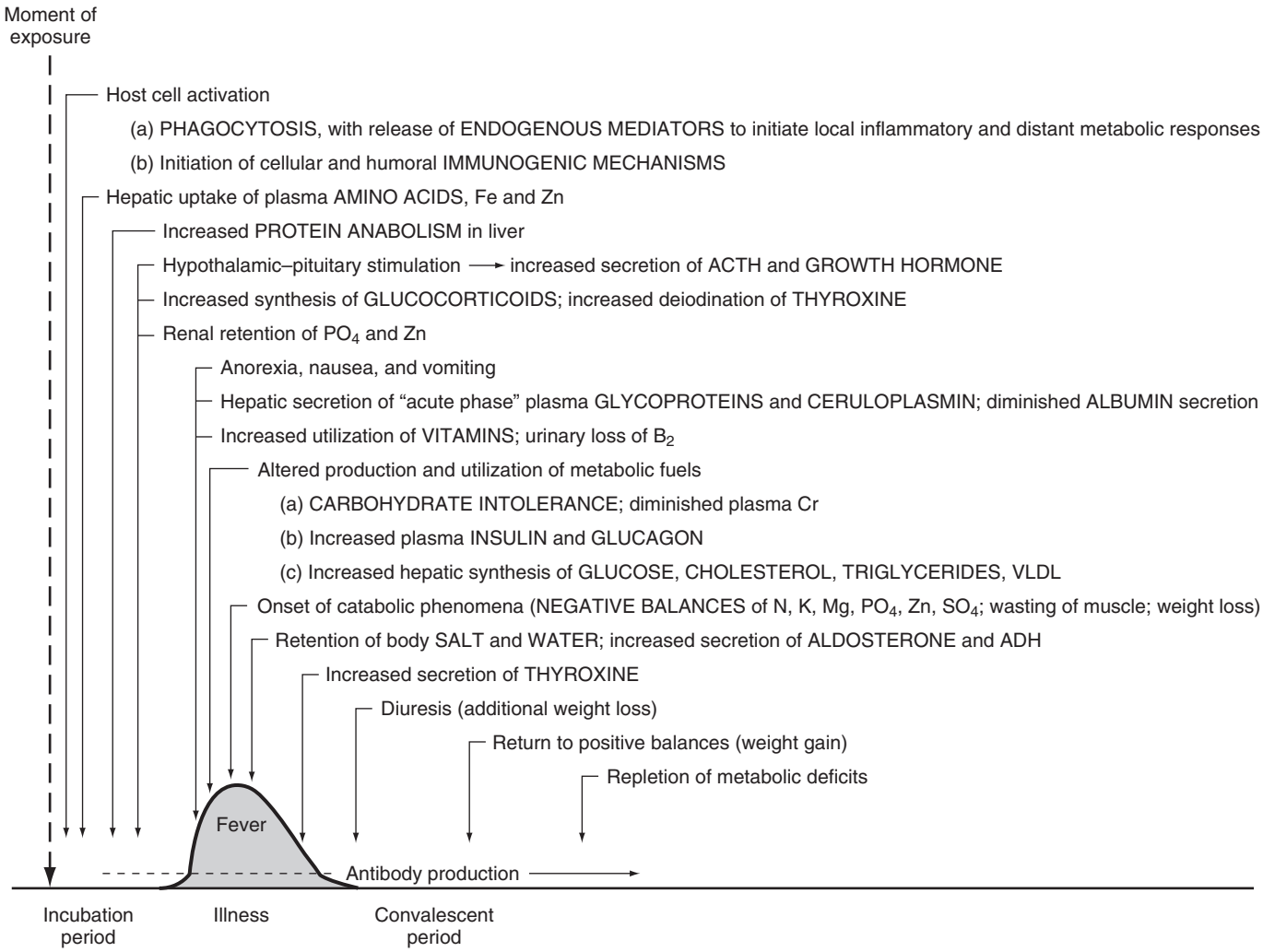

FIGURE 3-1 ■ Onset time of various host metabolic responses in relation to the sequential phases of a "model" acute, self-limited, generalized infectious illness. (From McKigney JI, Munro HN, editors. *Nutrient requirements in adolescence*. Cambridge, Mass: MIT Press; 1976. p. 250.)

that occur when an infectious process becomes localized within certain anatomic sites or organ systems. Diarrhea that develops during gastrointestinal infections can lead to depletion of fluids and electrolytes, hepatic infections can lead to derangements of carbohydrate and amino acid metabolism, and infections of the central nervous system that cause neuronal destruction are accompanied by muscle paralysis and atrophy. Furthermore, meningitis involves a sequence of events in which virulent microorganisms overcome the host's defense mechanism and begin a cascade of metabolic changes both locally and in other organ systems. The inflammatory response occurs in the subarachnoid space, which is mediated by interleukins and tumor necrosis factor (TNF), resulting in cerebral edema, vasculitis, and infarction. The cascade of events then leads to decreased cerebrospinal fluid outflow, hydrocephalus, worsening cerebral edema, increased intracranial pressure, and decreased cerebral blood flow. Other infections localized within the cranial vault often produce an inappropriate secretion of antidiuretic hormone, leading to development of dangerous overhydration in children.[14] The development of shock syndromes during infectious diseases imposes additional metabolic derangements because of progressive stagnant hypoxia.[14,144]

In recent years, the role of genetic variations in host genes important in the immune response to infections has been studied. Wide variations in individual response to several microorganisms, such as *Chlamydia trachomatis*[44] and *Candida albicans*, have been documented.[158]

The total number of discrete metabolic responses known to occur during acute and chronic infectious illnesses continues to expand.[14] The most widely recognized of these multiple responses are grouped for discussion into major categories, including changes in nitrogen, amino acid, carbohydrate, lipid, electrolyte, vitamin, and trace element metabolism. Another important mechanism involved in the host response to infection is oxidative stress, which plays a major role in the systemic inflammatory response in bacterial[164] and viral infections.[157] The important initiating and control mechanisms provided by cytokines, antioxidants, and the endocrine system also are discussed.

Each of the many individual metabolic changes that occur during the course of an infection must be interpreted in a manner that reflects its longitudinal development and progression over time and its relationship to the evolving phases of the infectious process.[10] Some metabolic changes may be detected during the incubation period, many other responses occur at the onset of fever, and still other phenomena develop during the recovery phase of illness or later during convalescence.

NITROGEN METABOLISM

Nitrogen requires compounds needed for a range of functions, including hormones, neurotransmitters, and immune competence. Nitrogen homeostasis involves a series of changes, including amino acid oxidation, protein turnover, urea production, and nitrogen excretion, during the fasting and fed periods of the day. Therefore, the importance of nitrogen has a significant role in body maintenance requirements and is affected by acute and chronic disease processes. The role of nitrogen metabolism in infectious diseases has been apparent and recognized for many years. Fever and its accompanying hypermetabolic state, as induced by acute-phase responses during infectious illnesses, trigger a complex assortment of changes in protein, amino acid, and nitrogen metabolism.[12] Many acute infectious illnesses have been shown to be accompanied by a negative nitrogen balance, which begins after the onset of the febrile response.[8] The human body experiences nitrogen losses during an acute infection, regardless of whether a normal or increased quantity of caloric intake and dietary protein is present.[133] Although the catabolic destruction of skeletal muscle protein is most obvious clinically, important anabolic events occur simultaneously. These events involve the synthesis of new proteins and cells that are important in host defense mechanisms. Increases in whole-body protein turnover involve catabolic and anabolic events in chronic infections also, as shown by leucine kinetic studies in patients with human immunodeficiency virus (HIV) infections.[62,89] Using amino acid isotope tracing techniques to measure muscle protein synthesis, researchers also showed that high plasma HIV RNA interferes with muscle amino acid synthesis and muscle proteolysis.[185]

Nitrogen Balance Studies

Because the ability of body cells to synthesize new proteins is a fundamental necessity for maintaining all known host defensive mechanisms, including the immunologic ones, understanding the changes that occur in the nitrogen metabolism of an infected individual is important.[117] When the availability of free amino acids within body pools is restricted by diet or disease, the catabolism of certain existing body proteins (chiefly the contractile proteins of skeletal muscle) generates the free amino acids required to synthesize new body proteins with higher priorities in terms of the many new proteins needed for defensive purposes. Information about these metabolic responses has been gained through quantitative analyses of proteins and other nitrogen-containing compounds in tissues, body fluids, and excretions and through kinetic studies with tagged molecules.[89,106,114,129,169]

Many attempts have been made using different approaches to quantify the amount of nitrogen needed to defend the human host from disease. One useful approach has been the measurement of nitrogen balance throughout the sequential course of an infectious process.[12,14,117] Daily measurements of nitrogen intake and all nitrogen losses that occur via different routes are obtained to determine whether the body is losing or retaining nitrogen. Other investigative techniques are needed to provide specific information about the molecular mechanisms involved in producing any observed changes in nitrogen balance. Nonetheless, the use of balance techniques has provided information concerning the typical losses of body nitrogen and muscle mass during acute generalized infections (Fig. 3-2). Quantitation of these catabolic losses provides an important framework of reference for more detailed studies of changes in nitrogen metabolism.

A comprehensive series of nitrogen balance studies was obtained in young adult male volunteers subjected to different kinds of experimentally induced infections during the course of studies to test vaccine efficacy.[12] Extensive normal baseline data were obtained on each volunteer before the time of his or her inoculation with or exposure to infectious bacteria, viruses, or rickettsiae. Longitudinal serial measurements made throughout the course of the infectious process allowed comprehensive, prospective evaluation of metabolic balance changes in nitrogen and other elements.[12]

Nitrogen balances did not change from baseline values during the incubation periods of the infectious diseases studied (see Fig. 3-2). The body began to lose nitrogen only after symptoms and fever had begun. These losses persisted for a period encompassing the acute illness. In convalescence, the subjects began to retain nitrogen so that gradually, over the course of several weeks, body nitrogen losses were regained. Similar nitrogen balance studies were conducted in healthy control volunteers who were not infected.[12,18,20] Instead, these controls were subjected to (1) partial food deprivation to mimic the anorexia-induced reduction in dietary intake measured in the patients with infection, (2) a 24-hour exposure to artificially induced high environmental temperatures to produce an increase in body temperature comparable to that seen in patients with infection, (3) treatment with antibiotic therapy in courses identical to those given to patients with infections, or (4) treatment with oral hydrocortisone using sequentially changing daily doses to mimic the measured adrenal responses of infected subjects.

These balance studies showed that the major losses in body nitrogen resulted from the combined effects of a reduced intake of food plus a continued (or increased) loss of nitrogen via the urine.[12] In contrast, simple starvation produced a prompt decrease in the daily losses of nitrogen via the urine. A patient with an acute infectious illness differed markedly from someone subjected to only simple dietary deprivation. Urinary nitrogen losses did not decline appreciably, or they increased in the presence

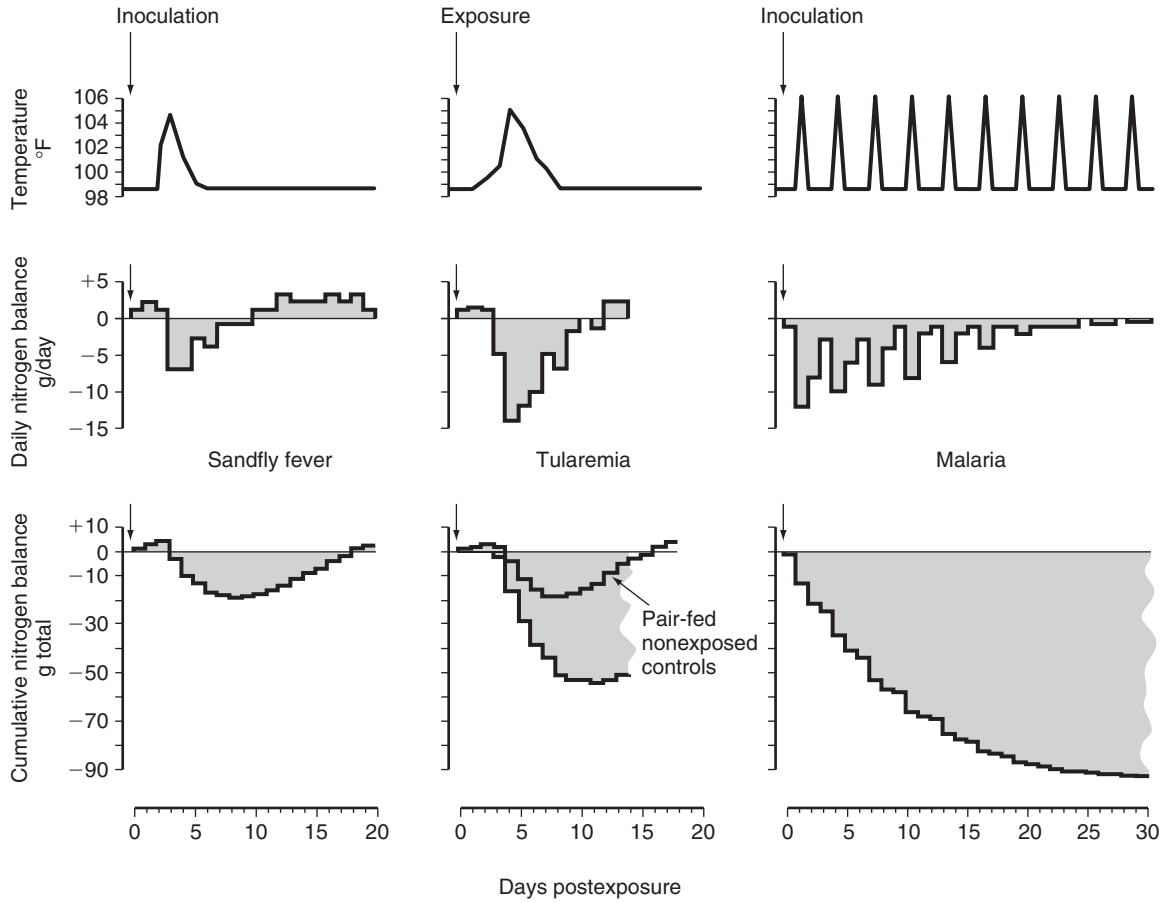

FIGURE 3-2 ■ Comparisons between the occurrence of fever *(top)* and changes in daily nitrogen balance *(middle)* and cumulative nitrogen *(bottom)* in patients with viral, bacterial, and generalized parasitic infections. Cumulative balance values for pair-fed healthy control subjects show the amounts of body nitrogen loss that can be ascribed to diminished food intake during infection. (From McKigney JI, Munro HN, editors. *Nutrient requirements in adolescence.* Cambridge, Mass: MIT Press; 1976. p. 261.)

of fever, whether the fever was caused by an active infection or an artificial increase in environmental temperatures.[120] The control studies also showed that the negative nitrogen balances that occurred during infection were not caused by adrenal glucocorticoid hormones or the antibiotics used in therapy.[10,18] Rather, by terminating infection quickly, antibiotics helped to reverse the loss of body nitrogen, allowing a more rapid restoration of nitrogen stores.

Because the loss of sizable quantities of nitrogen has important nutritional consequences, the same balance data were used to determine patterns of cumulative loss of body nitrogen.[10,12,14] As shown in Figure 3-2, total losses of body nitrogen grew progressively larger as the acute febrile phase of illness continued; the cumulative total loss of body nitrogen persisted throughout early convalescence. When nitrogen balances finally became positive, nitrogen was recovered over the course of weeks as body stores were accumulated slowly.

Studies performed in adult patients with infections such as tuberculosis and malaria suggested that the depletion of body stores of protein nitrogen did not continue unabated during subacute or chronic infection.[73] Rather, a new state of relative nitrogen equilibrium became established as chronically ill patients lapsed into a cachectic state. Total body nitrogen in such chronically ill patients was neither gained nor lost. Vital body processes continued to function for extended periods, despite the presence of cachexia and markedly depleted body nitrogen stores.[11] This state was hazardous at best and was comparable to the threat faced by infants and children with severe protein-energy malnutrition, who constantly face the additional threat of developing dangerous new or superimposed infectious diseases.[93,94,111,138]

Only limited nitrogen balance data have been collected in children with acute febrile infections. These data tend to reflect closely the patterns of change described in adults, with one major exception. Adults normally are in a state of nitrogen equilibrium, with neither a net gain nor a net loss over time, whereas healthy infants and children are consistently in positive balance because they must retain nitrogen to meet their needs for normal growth.

Few prospective measurements are available to document the changes in nitrogen balance throughout an entire infectious process in children. A study was conducted based on the nitrogen balance in children aged 12 to 24 months recovering from severe cholera. Daily nitrogen balance, cumulative nitrogen balance, and daily stool output were measured. With the introduction of milk, nitrogen balance was achieved and was retained in the majority of patients. This study showed that nitrogen

balance promptly improved with feeding even before diarrhea was controlled and the negative nitrogen balance that occurred appeared to be primarily due to reduced intake.[81]

Acute nondiarrheal infections typically do not cause an increased loss of fecal nitrogen; however, if diarrhea is present during an illness, fecal losses of nitrogen and other nutrients can occur.[103,125] Nitrogen losses also can occur through sweat, exudates, blood loss (from illness or from laboratory tests), sputum, gaseous exchanges, or sites of surgical drainage.[9,15]

In 1926, Beck[9] in Germany reported a series of metabolic balance studies conducted in healthy infants inoculated with vaccinia virus. One study was begun in a child exposed to varicella 15 days before the onset of pustules; another study was begun in an infant exposed to measles virus 14 days before the onset of clinical illness. The grouped nitrogen balance data from the vaccinated infants are shown in Figure 3-3. Despite the development of a short-lived fever in this mild infection, the infants maintained their usual food intake. The vaccinated infants did not go into negative nitrogen balance, and their rate of growth barely was slowed.[6] In measles or varicella, illness was of greater severity and a transient period of negative nitrogen balance was recorded.

Similarly, Viteri and Behar[167] measured nitrogen balance during childhood infections or after administration of live vaccines. Nitrogen loss varied with the severity of the illness. Wilson and colleagues[183] reported changes in nitrogen balances in hospitalized children who were convalescing from kwashiorkor when they developed varicella. A reduction in nitrogen retention occurred, and some children developed negative balances. Despite efforts to maintain a constant dietary intake, the children consumed less food during the course of the infection.

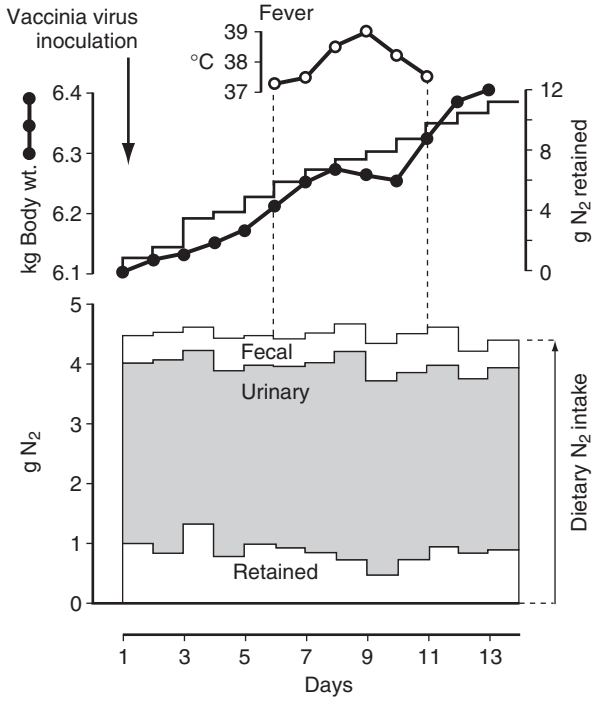

FIGURE 3-3 ■ Sequential measurements of body weight and cumulative nitrogen balance *(top)* and daily nitrogen balance *(bottom)* in a group of five healthy infants inoculated with vaccinia virus. Nitrogen intake values (plotted upward from baseline) and fecal and urinary losses (plotted downward from intake) reveal that the growing infants continued to retain nitrogen despite having brief periods of fever. (Drawn from numerical data published by Beck.[9])

The absolute loss of body nitrogen, as measured by metabolic balance techniques during infection, also can be used as a guide for estimating the absolute losses from the body of other intracellular elements, such as potassium, magnesium, phosphate, and, to a lesser degree, sulfur and zinc.[12,14] By using the ratios present in normal skeletal muscle of nitrogen to potassium and of nitrogen to magnesium, losses of body nitrogen during infection can be measured to estimate concomitant losses of potassium and magnesium from the body. Similarly, a ratio of nitrogen to phosphorus in skeletal muscle can be used to estimate inorganic phosphate losses that occur during infection, provided corrections are made first to account for any phosphate losses originating from demineralized bone. Calculations such as these suggest that the absolute losses of body nitrogen during infection are derived primarily from intracellular sources because they correlate in timing and magnitude with losses of the other principal intracellular elements.[12]

In contrast to infection-induced losses of intracellular elements, the losses of extracellular ions, sodium and chloride, follow an independent course during infection. Calcium losses are minimal in acute infection in the absence of long-term bed rest or paralysis with body immobilization.[12,179]

A newer method to measure total protein metabolism involves the use of a single oral dose of [^{15}N] glycine. This method was used to study children with septic shock as a result of meningococcemia and showed that the protein metabolism of these patients is increased significantly in contrast to that in healthy children.[161] A similar method was used previously in children with malaria, HIV, and measles.[161]

Urinary Excretion of Nitrogen-Containing Compounds

The onset of fever typically is accompanied by an increased urinary loss of creatinine. This loss usually is followed within 1 day by increased losses of urea and ammonia.[12] Although α-amino nitrogen losses may increase transiently at the onset of fever in some infections, this excretion tends to be lower than baseline measurements during the remainder of the illness and throughout convalescence. When measured prospectively, excretion of urinary α-amino nitrogen or most individual free amino acids did not change appreciably during sandfly fever, a mild viral infection.[157,170] In contrast, urinary losses of 3-methylhistidine and phenylalanine increased. The increased loss of phenylalanine seemed to reflect its increased concentrations in plasma.[158] Studies using radioactive compounds in rats suggested that most of the excess urea excreted during an acute infection was generated by the deamination of endogenous amino acids derived principally from skeletal muscle.[14]

Increased excretion of one of the amino acids, 3-methylhistidine, indicates the extent of skeletal muscle catabolism. This amino acid cannot be reused after its release from contractile proteins.[86,189]

Uric acid excretion may increase during febrile periods, but the greatest changes have been reported during infectious mononucleosis.[109] Uric acid losses are ascribed to an accelerated turnover of nucleic acids and enhanced purine degradation.

An increased excretion of urinary diazo reactants commonly occurs during most acute infectious illnesses, especially typhoid fever. These reactants include metabolites, such as kynurenine, 3-hydroxykynurenine, *p*-aminohippuric acid, xanthurenic acid, and anthranilic acid, which are created during the accelerated hepatic metabolism of tryptophan via the kynurenine pathway.[125]

A sudden, dramatic increase in urinary nitrate excretion occurs during acute infections. This increase is a reflection of the cytokine-stimulated production of nitric oxide as a host defensive measure.[49,154,158,168]

METABOLISM OF FREE AMINO ACIDS

The distribution, use, and metabolism of free amino acids undergo profound alterations during the course of acute infectious illness. Measurements in patients show patterns of change that can be explained by studies performed in experimental laboratory animals.[51,117,119,172] As some proteins are produced, others are degraded, and many of the released amino acids are redistributed throughout the body before they are reused. The majority of plasma amino acids shows a decline early in the infectious process and continues to decline through the onset of fever.[7] However, plasma phenylalanine and tryptophan levels have been shown to rise in an infectious process.[7,124,138]

A diminution in the concentration of most free individual amino acids in plasma may begin before the onset of symptoms. Hypoaminoacidemia generally persists throughout the febrile period of an illness, the largest decreases occurring in the branched-chain amino acids, glutamine, leucine, isoleucine, threonine, and valine.[106] The decline in branched-chain amino acids is caused primarily by an accelerated uptake of free amino acids by the liver. This increased flux from plasma to liver has been shown to occur in several species of animals during a variety of infections.[98] The amino acids that enter the liver are used for a variety of purposes. Amino acids may be metabolized to other compounds, oxidized, or reused for the synthesis of new proteins. Interleukin-6 (IL-6) seems to trigger many of the alterations in hepatic protein and amino acid metabolism that occur during an acute infection.[16,142]

Amino acids become important sources of energy during the hypermetabolic phase of acute infections. Some amino acids are oxidized directly in muscle, whereas others are transformed progressively into glucose and glucagon. Gluconeogenic amino acids, such as alanine, are deaminated, their carbon skeletons are used as the principal substrate for producing glucose, and their nitrogen is used to create urea for excretion. Serum and the total body pool of certain amino acids are depleted in various infections, presumably to diminish the substrate that some microorganisms may use to replicate. Interferon (IFN)-γ secretion causes a depletion of the body pool of tryptophan to retard the progression of *Chlamydia* infection.[123] A complete spectrum of amino acids is required for the synthesis of new body proteins generated during infection, including hepatic enzymes,[126] such as tryptophan oxygenase and tyrosine transaminase, and the lipoproteins and glycoproteins released into the plasma.[14,117,118] This group of glycoproteins constitutes the acute-phase reactants, which include α_1-antitrypsin, amyloid, α_1-acid glycoprotein, haptoglobin, C-reactive protein, fibrinogen (the third component of complement), and ceruloplasmin.

These acute-phase reactant proteins are produced in excess by the liver during infections, even in protein-deficient children who exhibit acute kwashiorkor.[115] The acute-phase proteins have a high phenylalanine and tryptophan concentration. Some of the potentially beneficial functions attributed to acute-phase reactants include an amplification of humoral and cell-mediated immunity, antiproteinase effects that could limit or contain harmful enzymes liberated during inflammatory reactions, oxidase activities, and the detoxification of free hemoglobin.[117] These changes in plasma amino acid levels continue throughout the infectious process and are also associated with a cytokine-stimulated anabolic response by the liver.[68]

In addition to the accelerated synthesis of proteins within the liver, infection increases the production of phagocytic and lymphoid cells. A need to produce increased quantities of such diverse proteins as the cytokines, complement and kinin components, fibronectin, and the several classes of immunoglobulins also exists. All of these requirements involve the need for free amino acids.

Proinflammatory cytokines and IFN-γ stimulate the production of nitric oxide from arginine, its sole precursor.[49,168] Among the many important biologic effects of nitric oxide are the effects resulting from its being a highly potent microbicidal, parasiticidal, and tumoricidal agent,[147] and from having major immunomodulatory functions.[91] Research also has shown that nitric oxide may act as an antiviral agent against adenoviruses and other similar viruses.[30]

A study on tuberculosis relating its dependence on the immune system response to protein concentration demonstrates how some infections can proliferate. In the study, mice were fed a 2 percent protein diet or a 20 percent protein diet and were exposed to tuberculosis. The mice fed the 2 percent protein succumbed to infection with *Mycobacterium tuberculosis* and died quickly.[55] The malnourished mice fed the 2 percent protein showed a decrease in the IFN-γ and TFN-α factors in the lungs. This study suggests that malnutrition created susceptibility to infection by the decrease of nitrous oxide and other cytokines, specifically in the lungs.[49]

The importance of nitric oxide in these roles may rival that of free oxygen radicals in potency and effectiveness.[154] This newly recognized host defense agent is generated when cytokine-induced nitric oxide synthase

initiates the oxygenation of one of arginine's guanido nitrogen groups to produce citrulline and nitric oxide.[149] During sepsis, arginine balance becomes negative as consumption of arginine exceeds the rate of synthesis. Because arginine is the sole source of nitric oxide, it becomes an essential amino acid in critically ill children.[4] Arginine also plays an important role in wound healing. In patients with infected chronic wounds, total plasma arginine is decreased and wound healing is impaired secondary to defective usage of arginine.[42]

Amino Acid Availability

Specific amino acid requirements of the body depend on the infection and the use of the amino acid. A pattern of amino acid requirement occurs in infection, as seen in an increase in hepatic acute-phase protein synthesis, as well as an increase in amino acids from skeletal muscle as a result of the protein breakdown. In the presence of anorexia and a diminished intake of food nutrients, the body in large measure must use endogenous sources to supply its increased amino acid needs. The principal sources of potentially available endogenous amino acids are the proteins of somatic tissues, including skeletal muscle and skin. The free amino acid pool contains the equivalent of approximately 12.5 g of protein in a normal man, an amount that represents only 0.1 percent of total body protein.[169] Because the normal turnover of protein in a healthy adult is approximately 200 to 250 g each day, the free amino acid pool must be resupplied continually from endogenous and exogenous sources. During infections, the absorption of individual amino acids from the intestine may be depressed and delayed[100] or increased,[39] depending on the infection.

Protein synthesis is not stopped entirely within body somatic tissues during an infection, but it is slowed markedly. At the same time, degradation rates of somatic proteins are accelerated, so that a net loss of muscle, skin, and other somatic proteins occurs through catabolic wasting. Catabolic degradation of skeletal muscle protein can be initiated by IL-1,[5,11,37,47] which triggers muscular proteases via the intracellular production of prostaglandin E_2. The body apparently sacrifices contractile proteins of skeletal muscle to obtain the free amino acids needed for higher priority requirements.

Some of the amino acids liberated during muscle protein catabolism are lost directly to the plasma. Others, such as the branched-chain group, are used in situ as sources of energy for muscle fibers; after oxidation, their amino groups are used to manufacture new glutamine and alanine, which can be used elsewhere for gluconeogenesis.[86,148] Because the branched-chain amino acids can be oxidized within skeletal muscle, their rate of release from muscle is decreased, which exaggerates the decline in their concentrations in plasma.[14,106,169] Branched-chain amino acids also play a major role in protein synthesis for lymphocytes and in immunity.[28] Some of the free amino acids liberated within muscle are retained within contractile cells and reused for the slowed but continuing process of muscle protein anabolism.

This catabolic breakdown of somatic protein to supply amino acids for use in other parts of the body is a highly visible clinical phenomenon. In this regard, the proteins of skin, skeletal muscle, and other somatic structures seem to constitute the principal body pool of "labile" nitrogen, which is called on to maintain protein homeostasis and the physiologic functions of visceral tissues during periods of infectious illness or other stresses. Because infants and small children have very little muscle protein (and a very small "bank" of readily available amino acids), the infection-induced need for free amino acids puts them at a special disadvantage.

To study the complex interrelationships involved in amino acid metabolism, workers have used isotopes to measure the simultaneous rates of anabolism and catabolism in the protein of skeletal muscle and other tissues of experimental animals during the course of infection.[129] In addition, clinically useful estimates of skeletal muscle degradation can be obtained in patients through the quantitative daily measurements of 3-methylhistidine excretion into urine. More than 90 percent of the total body content of 3-methylhistidine is associated with the peptide chains of actin in all muscle and of myosin in white muscle fibers.[189] This unique amino acid is formed in situ through the transmethylation of histidine, but only after the histidine first has been incorporated into the amino acid structure of myofibrillar proteins. When released, 3-methylhistidine cannot be reused by the body and instead is lost via the urine.

The increase in excretion of 3-methylhistidine during acute infections is in keeping with other estimates of skeletal muscle wasting. The extent to which proteolysis occurs during periods of severe surgical sepsis also has been quantified by measuring the differences among concentrations of glucose, lactate, free fatty acids, ketones, and alanine in femoral arterial and venous blood.[113] Neither free fatty acid nor ketone use was increased during occurrences of acute fevers, but an increased oxidation of glucose and of amino acids derived from protein was used to fuel cellular hypermetabolism. Although infection stimulates a sizable increase in the rates of body protein synthesis, the acceleration of protein catabolism is increased even further and the body experiences a net loss of somatic proteins and even some visceral ones.[169]

Phenylalanine is one of the amino acids released by the accelerated catabolism of somatic proteins. Only limited amounts of phenylalanine can be used, however, for the synthesis of new proteins or for its conversion into tyrosine.[169,171] Phenylalanine concentrations in the circulating free amino acid pool tend to increase rather than decrease during periods of fever. These increases occur coincidentally with the typical declines in plasma values for tyrosine and most other free amino acids. The exaggerated phenylalanine-to-tyrosine ratio can be useful for evaluating these opposing changes during febrile illnesses. An increase in this ratio is a useful clinical index of the severity of infection-induced alterations in amino acid metabolism.[169] During occurrences of infections of great severity and infections with hepatocellular dysfunction, hypoaminoacidemia may be replaced by an excessive accumulation of many other free amino acids in plasma.[14,144,181]

Free tryptophan, also liberated from degrading protein, undergoes an accelerated metabolism via several

different metabolic pathways in the liver.[14,103,104,120] In response to the increased hepatic availability of tryptophan, the increased synthesis of tryptophan oxygenase, or both, excess tryptophan enters the kynurenine pathways, where it is converted into different diazo-reactant compounds and pyrimidine nucleotides.[120] At the same time, tryptophan enters other pathways, leading possibly to increased synthesis of serotonin or indoleacetic acid.[125]

CARBOHYDRATE METABOLISM

During the febrile phase of infectious illnesses, carbohydrate metabolism provides much of the additional energy needed by body cells. An increased demand for metabolizable energy is met by a resetting of the endocrine control mechanisms to permit a marked acceleration of the synthesis and release of glucose from the liver.[41,60,182] Although some glucose is derived from hepatocellular glycogen, most additional requirements for glucose during acute infections are met by accelerated hepatic gluconeogenic mechanisms. Recycled lactate and amino acids, especially amino acids with gluconeogenic potential, such as alanine, are the principal substrates used by the liver to increase its output of newly synthesized glucose; glycerol (derived from the metabolism of triglyceride lipids) and pyruvate also are used.[86]

Early in acute infectious illnesses, basal blood glucose concentrations tend to become elevated. Diabetic patients who require insulin often develop glycosuria at the onset of febrile infections, and more insulin must be given to maintain their control. The tendency for acute infections to be accompanied by an early modest hyperglycemia also is seen in laboratory animals. Modest hyperglycemia often is noted in animals after they have been injected with bacterial endotoxin.[25,96]

Although an early hyperglycemic response seems to be the initial change in carbohydrate metabolism, the ability of the body to sustain an accelerated production of glucose may be lost. Hypoglycemia may emerge as an important clinical problem. Hypoglycemia generally results from one of two major pathogenic mechanisms: a diminished availability of substrate molecules required for gluconeogenesis or a failure of the metabolic mechanisms needed to produce hepatic glucose. Carbohydrate pathway defects at the molecular level have not been found in overwhelming bacterial infections in laboratory animals.[67,110] Carbohydrate depletion during overwhelming or terminal infections can be ascribed to nonavailability of substrate. If hepatic cells are injured during an infectious disease, such as viral hepatitis, hypoglycemia can result from a failure of gluconeogenic processes.[14,52,135]

The depletion of substrate for adequate gluconeogenesis occurs primarily as a dangerous clinical complication of neonatal sepsis. Infants are born with only minimal amounts of skeletal muscle protein. Because muscle contains the principal labile nitrogen pool for supplying amino acids that can be converted into glucose, neonatal infants lack a sufficient quantity of potential substrate molecules for sustaining long-term gluconeogenesis. Newborns especially are prone to develop severe hypoglycemia when a septic process becomes established.[187] Hypoglycemia that results from a breakdown of hepatic gluconeogenic mechanisms occurs primarily when liver cells are damaged by an infectious or toxemic process. Hypoglycemia can become an important complicating factor in severe viral hepatitis, yellow fever, or endotoxemia during gram-negative sepsis.[52,96] These conditions can cause hepatocellular damage and a breakdown of enzyme-synthesizing mechanisms within the liver.[96,159]

A change in glucose tolerance can be detected within hours after the onset of a febrile infection.[128,141] Baseline blood glucose values may be elevated, and disappearance of glucose is slowed moderately as a component aspect of a febrile illness. Under these circumstances, the pancreatic islets produce insulin in excess quantities. Despite an increase in plasma insulin concentrations, glucose disappearance rates are slowed; these combined metabolic changes resemble the changes caused by mild insulin resistance. In low-birth-weight neonates, hyperglycemia could be an ominous sign and suggests the presence of systemic fungal infections.[92]

Bacterial and viral infections also cause increased secretion of pancreatic glucagon,[128,131] which, together with release of catecholamines accompanying infection,[64,67,182] provides hormonal stimuli for accelerating gluconeogenesis within the liver. These hormonal stimuli initiate their hepatic effects during infection by activating adenylate cyclase.[35] The unusual simultaneous increase in basal concentrations of insulin and glucagon in plasma results from direct cytokine stimulation of pancreatic islet cells.[11,60,186] Because of these hormonal changes and high plasma glucocorticoid values in patients with severe bacterial sepsis, infusions of glucose may fail to suppress hepatic gluconeogenesis.[86,88] The resultant increase in glucose pool size helps explain the apparent slowing of glucose disappearance and the hyperinsulinemia that occur during infections.

Certain viral infections in children seem prone to initiate the acute juvenile type of insulin-requiring diabetes mellitus. Although the evidence for a viral cause of diabetes has not been established fully in humans, epidemiologic findings indicate that juvenile diabetes may develop several months after onset of certain viral illnesses, especially mumps, coxsackievirus B (type 4) infection, rubella, or cytomegalovirus infection.[108] Additional epidemiologic evidence is based on a tendency for new cases of the juvenile type of diabetes to appear in clusters.

Susceptibility to juvenile diabetes also may be influenced by genetic makeup. A high incidence of human leukocyte antigen types B8 and Bw15 has been detected in juvenile diabetics.[136] Studies in laboratory animals suggest that genetically susceptible species may have virus receptor sites on their insulin-producing beta cells. These receptors in species with high-risk haplotypes allow viruses to be absorbed by the pancreatic beta cells, with subsequent destruction of insulin-producing cells.

Malaria changes the glucose tolerance and life span of an erythrocyte by changing the carbohydrate metabolism and makeup of the cell it infects. The normal, mature human erythrocyte has a low level of glucose metabolism and lactate production because it is a metabolically

inactive cell; however, the malarial parasite *Plasmodium falciparum* has a need for increased glucose consumption.[32] The metabolism of glucose in *P. falciparum* infected human red blood cells is increased up to 100-fold during infection to accommodate the needs of the parasite, which must synthesize RNA, protein, and DNA and undergo cell division every 48 hours, as opposed to the lifespan of the healthy erythrocyte of 120 days. The glycolytic enzymes hexokinase, enolase, and pyruvate kinase, found within the parasite, increase glucose metabolism to lactic acid. The increase in hexokinase is proportionate to the parasitemia. Red cells exposed to the parasite showed a decrease in the concentration of 2,3-diphoshoglycerate (2,3-DPG), which functions as an inhibitor for certain essential enzyme pathways.

The cytokine granulocyte colony-stimulating factor influences the uptake of glucose by phagocytic cells and their mobilization during inflammatory reactions.[84,132] The availability of glucose contributes directly to the respiratory burst that accompanies phagocytosis and to the production of the myeloperoxidase enzymes needed to kill engulfed organisms.

LIPID METABOLISM

As Table 3-1 shows, numerous changes in lipid metabolism have been documented during infection. In contrast to the stereotyped patterns of change in nitrogen and amino acid metabolism and in the accelerated production of glucose, responses involving body lipids vary more from infection to infection. Plasma lipid changes also may be biphasic during the course of a single infection. This complexity results partly from the multiple factors that control the metabolism of lipids during infectious illnesses,[14,144] including the need for hepatic synthesis of serum lipoproteins, variations in the production and use of free fatty acids and ketones as sources of cellular energy, and variable changes in rates of uptake or release of lipids from body fat depots.[14,144]

Lipids have another function crucial during acute-phase reactions that involves the cytokine-stimulated use of polyunsaturated fatty acids localized within cell walls to serve as precursor molecules for various families of eicosanoids (prostaglandins, prostacyclins, thromboxanes, and lipoxins). These eicosanoid lipids trigger many of the diverse cellular responses seen during infection, inflammation, and immune responses.[80,128]

Plasma Lipids and Lipoproteins

Because the concentrations of individual lipid moieties in plasma depend on the algebraic summation of input and removal rates, the mechanisms that control the release or uptake of individual lipids must be studied throughout the course of various kinds of infectious illnesses. In the cholesterol metabolic pathway a disruption exists between the innate immune system and the signaling and regulation of sterol and fatty acid metabolism.[32] Concentrations of cholesterol have been reported to increase, decrease, or remain unchanged.[13] Infection and inflammation are associated with an increase in the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase in cholesterol synthesis in the liver. Yet, a decrease occurs in enzyme expression in the mevalonate pathway, and, as a result, only a moderate increase occurs in cholesterol synthesis in the liver in some infections. In severe meningococcal sepsis, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) levels are low, and the severity of total hypocholesterolemia correlates with the severity of disease.[163] Other studies have shown that hypocholesterolemia in sepsis may have prognostic value. Decrease of cholesterol parallels increase of C-reactive protein, fibrinogen, and white blood cells in septic patients.[34]

Mild viral infections often are associated with a transient decline in serum cholesterol values. When longitudinal studies were conducted during the course of

TABLE 3-1 **Basic Mechanisms Leading to Observed Effects of Infection on Lipid Metabolism of the Host**

Effects Associated with the Presence of Invading Microorganisms

1. Direct effects
   a. Microorganism use of host lipids for replication
   b. Disruption of host cell metabolism by intracellular microorganisms
   c. Localized destruction of fat cells by infectious process
2. Indirect effects
   a. Alterations in host metabolism caused by bacterial exotoxins, endotoxins, or bacterial enzymes
   b. Activation of lipases and other lipid-affecting enzymes within host phagocytes

Effects Secondary to Development of Generalized Illness from Infection

1. Decreased dietary fat intake
2. Interference with intestinal digestion and absorption of lipids
3. Alterations in lipid transport
   a. Changing concentrations of lipid transport proteins
   b. Decreased lipoprotein lipase activity (caused by tumor necrosis factor), which allows triglycerides to accumulate in plasma
4. Altered lipid metabolism within host cells caused by proinflammatory cytokines
   a. Activation of cell wall phospholipases by proinflammatory cytokines
   b. Creation, from cell wall polyunsaturated fatty acids, of intracellular arachidonic or eicosapentaenoic acids or both
   c. Transformation of arachidonic and eicosapentaenoic acids into various eicosanoids (prostaglandins, prostacyclin, thromboxanes, and lipoxins)
5. Other alterations of lipid metabolism within host cells
   a. Altered rates of hormone-mediated lipolysis within fat depots
   b. Accelerated fatty acid synthesis within the liver
   c. Depressed hepatic ketogenesis
   d. Altered rates of lipid uptake and use by peripheral tissues
6. Participation of newly formed eicosanoids in inflammatory and immunologic responses, and coagulation mechanisms
7. Effects related to prior nutritional status of the patient
8. Terminal pathologic hyperlipidemic effects associated with gram-negative sepsis and hypotensive shock

experimentally induced sandfly fever in young adult male volunteers, plasma values for total and esterified cholesterol, phospholipids, and free fatty acids declined in conjunction with, or immediately before, the onset of fever.[85] This decline in concentration included the cholesterol and the protein components of the low-density β lipoproteins. In contrast, the plasma triglyceride values showed a biphasic pattern of change—an initial decline was followed by an increase above baseline control concentrations during the early convalescent period. Depressions of total serum cholesterol values also have been reported to occur during pneumococcal pneumonia, cholera, tuberculosis, and malaria.

When studied in rhesus monkeys experimentally infected with *Streptococcus pneumoniae* or *Salmonella typhimurium*, rates of [^{3}H] mevalonic acid incorporation into free cholesterol were accelerated,[53] and no evidence of an inhibition of squalene synthesis or its conversion to cholesterol was found. In contrast, the synthesis of cholesterol was blocked partially when monkeys with diet-induced hypercholesterolemia were subjected to a pneumococcal infection.[53]

A study involving mouse cytomegalovirus infection links the interference in viral replication and the downregulation of cholesterol biosynthesis. When cytomegalovirus (CMV) is infected into bone marrow–derived cells, the immune system responds with production of interferon, which downregulates certain enzyme levels in the sterol biosynthesis cascade. The downregulation in sterol lipid metabolism is in a gradual, temporal decline starting from 6 hours after infection. What results is a specific alteration of sterol biosynthesis, in which levels of free cholesterol, as well as its precursors, zymosterol, 14-demethyl-lanosterol, and 7-dehydrocholesterol, were reduced at 24 and 48 hours after infection.[26]

Marked increases in the concentrations of total serum lipids and triglycerides are found consistently in patients with infections caused by gram-negative bacilli,[14,76,144] but this response is minimized or fails to occur in patients with viral or gram-positive coccal infections. A similar difference has been noted in monkeys with either *S. pneumoniae* or *S. typhimurium* infection.[77] TNF, a proinflammatory cytokine with a unique additional function that inhibits the enzyme lipoprotein lipase, seems to be of central importance in accounting for these differences.[76]

Although a release of TNF from activated macrophages may occur in many infections, its release is stimulated markedly by bacterial endotoxins released during gram-negative infections.[14,99] Hypertriglyceridemia occurring during periods of *S. typhimurium* infection in monkeys was generated by TNF-induced mechanisms that reduced the clearance of lipids from plasma and the activity of heparin-sensitive plasma lipoprotein lipase. Hypertriglyceridemia that accompanied pneumococcal infections was far less severe than that observed with gram-negative infections. After endotoxin was administered,[76] hypertriglyceridemia developed chiefly as the result of an impaired lipid disposal mechanism, secondary to release of TNF from macrophages.[11] An acceleration in the rate of hepatic production of triglycerides from free fatty acid precursors also occurred. This hypertriglyceridemic effect occurs within 2 hours of activation of the endotoxin and was sustained for at least 24 hours.[32] Other cytokines involved in the increase of hypertriglyceride include IL-1, IL-2, IL-4, and IL-6.

A marked decrease in serum levels of HDL, apoA-I, lecithin cholesterol acetyltransferase (LCAT), hepatic lipase, and transferrin occurs in infection.[33] ApoA-I and LCAT impair cholesterol removal from cells, and hepatic lipase and transferring decreases the ability of HDL to protect against LDL oxidation. During infection, HDL is known as acute-phase HDL and has different characteristics than normal, healthy HDL. Acute-phase HDL is larger and is depleted in cholesteryl ester but has more free cholesterol, triglycerides, phospholipid content, and free fatty acids.[33] Another role of HDL is to protect LDL against oxidation by means of HDL protein, which has antioxidant activity. During infection, HDL loses these proteins by inactivation and thus HDL loses its antioxidant capacity and becomes pro-oxidant.

In addition to the decrease in HDL, a decrease in LDL occurs during infection. The lipid and protein component of LDL particles is altered during infection, which increases its susceptibility to oxidation. C-reactive protein is an acute-phase protein that is associated with very-low-density lipoprotein (VLDL) and LDL and is also present in atherosclerotic lesions. During an infection, when LDL becomes oxidized, CRP binds oxidized LDL and oxidized phospholipids, which enhances their uptake by macrophages. Because the physical change in the LDL structure and function are changed in infection, LDL becomes proatherogenic.

Schistosomiasis is a parasitic infection in which the human body's resistance to the parasite is mediated by lipoproteins. In rats infected with *Schistosoma* spp., an increase occurs in serum levels of CRP, which is associated with VDRL and LDL. LDL and oxidized LDL bind to the surface of the schistosomula, which then activates monocytes, allowing endocytosis of the oxidized LDL into the monocytes. Once these bound LDLs are removed by endocytosis, the schistosomula is exposed to attack by monocytes and other immune-mediated cells.

Lipids and Energy Metabolism During Infection

Requirements for cellular energy during periods of chronic infections in laboratory animals seem to be met largely through the catabolism of stored body fat.[89] In contrast, fat wasting does not seem to be as prominent during rapidly lethal bacterial infections. Ketogenesis also seems to be blunted during periods of acute infections.[110] Concentrations of free fatty acid in plasma normally are increased by the actions of catecholamines, growth hormone, and glucagon, which are increased during the course of infection.[14] Concomitantly high concentrations of plasma insulin apparently serve to inhibit lipolysis and ketogenesis.[14,110] The contribution of free fatty acids to energy production during an infection is influenced by the interplay of these hormones, the availability of glucose, and the functional capacity of the liver to take up fatty acids and convert them to triglycerides.[53,54,59,60]

Finally, brown fat is thought to make an important contribution to heat production in newborns via sympathetic nerve activation of this tissue. Because numerous viruses are known to proliferate in brown fat,[145] fever that occurs with neonatal viral infections may involve brown fat thermogenesis.

Acute infectious illnesses produce few effects on the intestinal absorption of fat, although it may be reduced with intestinal infections and parasitemic or enterotoxemic illnesses.[116,135] Lipid precursors needed for the replication of microorganisms within host tissues must be supplied from metabolic pools within the host. Malarial parasites obtain their structural lipids from red blood cell precursors; lipid-containing viruses apparently obtain the lipid components needed for their assembly from lipid-rich membranes already present within host cells.

Lipid Metabolism and Host Defensive Measures

Rapid metabolic responses by cell wall lipids play a major role in initiating a panoply of intracellular events during acute-phase reactions. The attachment of proinflammatory cytokines to cell wall receptors triggers the conversion of polyunsaturated fatty acid in cell wall membranes to a variety of biologically active eicosanoids. Lipids of various eicosanoid families become the messenger molecules responsible for many of the acute-phase metabolic and cellular responses[11,47] that occur with infection. These short-lived eicosanoids are biopotent at nanomolar to picomolar concentrations.

In addition to their importance within lymphocytes of the immune system, eicosanoid lipids transmit stimulatory or inhibitory signals between other cells and tissues in health and disease.[80,130] Although they do not initiate disease processes, various eicosanoid lipids (i.e., prostaglandins, leukotrienes, prostacyclin, lipoxins, and thromboxanes) are important components of the pathogenic progression of infectious diseases.[80,130]

Eicosanoids also have important effects on other white blood cells and platelets and on such diverse organs as the brain, lungs, stomach, and kidneys. These eicosanoids seem to be components of complex, often overlapping, regulatory mechanisms that interconnect the immune system, central nervous system, endocrine glands, and functions of many other organs and tissues. These homeostatic (and at times pathogenic) mechanisms are characterized by numerous duplications, amplifications, checks and balances, and feedback control loops.

Biochemical details now are well established concerning the intracellular synthesis of individual eicosanoids from *n*-3 and *n*-6 polyunsaturated fatty acid precursors.[80,130] After cytokines interact with cell wall receptors, they activate cell membrane phospholipase enzymes. These enzymes initiate the release (into the cell interior) of arachidonic acid from cell wall (omega-6) polyunsaturated fatty acid or eicosapentaenoic acid from (omega-3) polyunsaturated fatty acid. These first steps of eicosanoid synthesis can be blocked by adrenocorticoid hormones. In target cells having cyclooxygenase enzymes, arachidonic acid is oxygenated into 4-series leukotrienes, 2-series prostaglandins, or thromboxanes and prostacyclin. If a cell possesses lipo-oxygenase enzymes, arachidonic acid is oxygenated into the sometimes less potent 5-series leukotrienes, 3-series prostaglandins, or lipoxins.[130] The highly active eicosanoid molecules are degraded rapidly. Importantly, synthesis of prostaglandins of the 2-series and the 4-series can be blocked by certain nonsteroidal anti-inflammatory drugs, such as aspirin, indomethacin, and ibuprofen.[80,130]

Other fatty acids may participate in defense mechanisms of the host and occasionally even may be involved in pathogenic events of a harmful nature. An increase in the fat content of vital organs is a common occurrence in patients dying of severe bacterial infections; fatty metamorphosis especially is prominent in the liver, kidney, and heart.[72] Hyperlipemia in gram-negative septicemia may be accompanied by fat embolization to the lungs. Other deleterious actions of body lipids involve the liberation of phospholipids from platelets and their subsequent participation in the activation of the blood-clotting cascade, leading to disseminated intravascular coagulation. Chronic *Chlamydia pneumoniae* pneumonia infection leads to oxidation of low-density lipoprotein, which promotes atherogenesis.[48]

ELECTROLYTE AND ACID-BASE METABOLISM

Many life-threatening medical emergencies faced by infants and children with acute infectious diseases involve problems in the areas of salt and water or acid-base balance. Some infections cause severe overhydration, and others produce dehydration with hypovolemic shock. Pathogenic mechanisms active during various infections can lead to metabolic alkalosis or acidosis, respiratory alkalosis or acidosis, or complex admixtures of these pathophysiologic perturbations.[11,22]

The onset of fever typically is accompanied by tachypnea and accelerated respiratory gas exchange, which lead to an exaggerated loss of dissolved carbon dioxide from blood and a state of uncompensated respiratory alkalosis.[14] Alkalosis may persist as long as febrile tachypnea lasts and gas exchange within the alveoli remains unimpeded.

Conversely, infections that produce extensive pulmonary consolidation can impair carbon dioxide exchange and cause respiratory acidosis. Respiratory acidosis also is a complication in patients whose pulmonary musculature no longer can function effectively, such as patients with poliomyelitis, tetanus, botulism, or respiratory distress syndrome.

Metabolic acidosis generally develops whenever an infectious disease process becomes severe. With the hypotension, vascular stasis, and cellular anoxia seen during gram-negative sepsis, the generation of excessive lactic acid and other acidic metabolic products exceeds the capacity of the body's buffering systems.[14,144]

Falciparum malaria can cause a high-anion-gap metabolic acidosis, which has been noted to be the strongest single predictor of fatality in children with malaria. Once the parasites accumulate in various tissues, this compromises blood flow, thus increasing local tissue glucose

usage and forcing tissues into an anaerobic metabolic state. The cause of this metabolic derangement can be attributed to impaired renal function accounting for the rise of urea and creatinine, which in turn results in a reduced acid excretion. Hyperlactatemia also can accompany the metabolic acidosis. The increased anaerobic metabolism caused by tissue hypoxia results in an increased production of lactate by anaerobic glycolysis. Hypovolemia may contribute to the acidosis if dehydration is present, which often occurs in children with delayed diagnosis of malaria. This would be demonstrated by hyperosmolality. Another possible contributing factor to hypovolemia can be from ketoacids, which can be detected in the urine in children with malaria. Another contributing factor to the acidotic state seen in many children with *Falciparum* malaria is the accumulation of lactic acid and 3-hydroxybutyric acid.

Because anemia often can accompany malaria in children, the delivery of oxygen to the tissues can be further impaired, which also increases the symptoms of acidosis. Severe anemia contributes to the development of acidosis. Metabolic acidosis caused by *Falciparum* malaria is easily treated and reversible once the patient has received intravenous fluids and antimalarials, which makes prompt treatment of severe malaria of the utmost importance.

Diarrheal diseases can be accompanied by two other forms of metabolic acid-base derangement.[22,135,174] Toxigenic diarrhea characterized by high-volume stool loss, such as that seen in Asiatic cholera or *Escherichia coli* enterotoxemia, causes excessive loss of fecal bicarbonate and an alkaline stool, with a resultant decline in blood pH. Bicarbonate is secreted actively in the lower ileum and cannot be reabsorbed completely by the colonic mucosa if high-volume losses of watery stool occur.[22]

In contrast, diarrhea accompanied by only low-volume stool losses, as in rotavirus infections,[135] tends to be associated with an acid stool and exaggerated loss of fecal potassium rather than bicarbonate. If fecal potassium losses persist chronically for a long time or occur rapidly as part of the massive fluid loss of acute secretory diarrhea, such as in pediatric cholera, body potassium can be depleted severely. The loss of cellular potassium can produce metabolic alkalosis, cardiac arrhythmia, paralytic ileus, and weakness in children and the occurrence of hypokalemic vacuolization in cells of the myocardium and renal tubular epithelium. Even if the balance of fluid is restored promptly in these patients, a prolonged state of metabolic alkalosis can persist until body potassium stores are replenished.

The importance of diarrheal diseases in infants and small children is great, with an estimated 2 to 3 million deaths occurring worldwide each year.[111] Life-threatening dehydration can result from the loss of body water and electrolytes during an episode of high-volume diarrhea. Because massive diarrhea produces an iso-osmotic loss of body water and electrolytes, the fluid losses come from the extracellular rather than the intracellular space.[174] The circulating blood becomes thick and viscous because of a relative increase in hematocrit values and a progressive concentration of serum proteins, which can increase to more than twice their normal values. Despite serious dehydration, concentrations of plasma sodium remain normal, when expressed in terms of plasma water.[174] This type of acute, massive diarrhea can lead to the rapid onset of hypovolemic shock, renal failure, and death.

Dehydration usually does not become a problem in infectious diseases that lack protracted vomiting, diarrhea, or prominent sweat loss. Instead, severe generalized infections in children may be accompanied by some retention of body water and salt. Soon after the onset of a febrile illness, the adrenal secretion of aldosterone increases,[12,17] and this mineralocorticoid stimulates the renal retention of sodium and chloride. These electrolytes may virtually disappear from the urine during a severe febrile illness. Retention of salt tends to be accompanied by a retention of body water throughout the illness.[14] Generalized edema is an infection-induced manifestation of kwashiorkor and can be seen during severe infections in well-nourished children. Accumulations of excess water and salt typically are excreted after the acute phase of illness by a transient period of diuresis during the early convalescent period.

Some infections, particularly infections that become localized within the central nervous system, also are complicated by an inappropriate secretion of antidiuretic hormone from the posterior pituitary.[50,180] The ensuing retention of body water may dilute the sodium and chloride in plasma. Redistribution of sodium also may contribute to the development of hyponatremia during infections. Sodium may begin to accumulate within body cells, apparently because the sodium-pumping mechanisms in extracellular membranes may fail to maintain internal electrolyte homeostasis.[14] This form of sodium sequestration is evidence of severe illness. It is not reversed easily and may be a major complication in patients with severe infections, such as meningococcemia or Rocky Mountain spotted fever.

VITAMIN METABOLISM

Although few measurement data are available concerning infection-induced changes in vitamin metabolism, the consensus is that the use or metabolism of most vitamins is accelerated.[21,166] Scattered reports suggest that infectious diseases in humans may be followed by classic scurvy, beriberi, pellagra, or xerophthalmia.[138]

More recent attention has focused on vitamin A, which previously was termed the *anti-infection vitamin*. Declining concentrations of plasma vitamin A during episodes of childhood infections are accompanied or perhaps caused by a marked urinary loss of this vitamin.[1,152] Not only does the heightened vitamin A deficiency induced by infection contribute to the subsequent development of ocular and conjunctival pathologic conditions but also subclinical deficiencies of vitamin A and their associated immunologic dysfunctions can heighten the mortality associated with childhood infections,[129,139] as shown most dramatically in measles.[147] Depressed plasma concentrations of several other vitamins have been reported.[13] In addition to the urinary losses of vitamin A,[1,152] increased excretion of urinary riboflavin and vitamin C may occur in conjunction with negative nitrogen balance.[20]

Vitamins are known to participate in metabolic processes activated during host defensive mechanisms.[11] The rapid synthesis of steroid hormones by the adrenal cortex is accompanied by a decline in the adrenal content of vitamin C. The B group vitamins, vitamin C, and folate all participate in the metabolism of activated phagocytic cells.

Controversy continues to exist over whether massive daily doses of vitamin C can suppress or prevent the common cold and other viral respiratory infections. More than 2 decades ago, the American Academy of Pediatrics Committee on Drugs[2] failed to find sufficient scientific evidence to support Pauling's claim, but new data subsequently were introduced.[69] Concentrations of vitamin C in neutrophils decline during infectious diseases. Vitamin C is recognized for its importance in the locomotive activity of phagocytic cells and its contributions to the immune system.[3]

Intestinal parasites, such as tapeworms, may take up sufficient vitamin B_{12} from the succus entericus to diminish vitamin B_{12} absorption and lead to the development of megaloblastic anemia. The intestinal absorption of fat-soluble vitamins and folate also may be impaired for a time in children with enteric infections or parasitic diseases.[90]

The antioxidant role of several vitamins (A, C, and E) has been recognized more recently. Antioxidants act to reduce oxidative stress within the body by serving as scavengers of singlet oxygen radicals. The antioxidant functions of several other provitamin-A carotenoids are of still greater importance. They include beta carotene, lycopene (the red pigment found in tomatoes and other brightly colored fruits), lutein (from spinach), and zeoxanthin (from kale and other dark green collard greens). Lycopene is more abundant in plasma than is beta carotene and twice as powerful in quenching free oxygen radicals. Lutein and zeoxanthin are retinal pigments, with lutein being found throughout the retina and zeoxanthin being concentrated in the macula. Antioxidants are thought to protect cell membranes, DNA, and arteries to augment the immune system and the function of natural killer cells and help prevent heart attacks, macular degeneration, and various cancers, especially prostate cancer. Little is known about the probable role of antioxidants during episodes of infectious diseases of children.

More recent studies on a small sample of adults suggest that vitamin D is decreased in patients with HIV infection and that vitamin D levels correlate directly with the CD4 count and inversely with mortality in HIV-infected patients.[165] Vitamin C seems to be decreased in patients with HIV infection, whereas vitamin E concentrations are similar to those in healthy subjects.[153] Oxidative damage is known to play an important role in inflammation, but the exact mechanism by which vitamin C is involved in the antioxidant host defense is unclear. One study that looked at *Klebsiella pneumoniae* infection showed that vitamin C does not prevent lipid and protein oxidation. Another study suggests that vitamin C is protective against oxidative stress, at least partly by decreasing lipid peroxidation.[79] Vitamin C seems to protect against infections in mouse models.[58]

TRACE ELEMENT METABOLISM

As Table 3-2 shows, infectious illnesses often are accompanied by changes in the concentration of several of the trace elements in plasma. The most consistent responses

TABLE 3-2 **Infection-Related Changes in Trace Elements**

Trace Element	Observed Change During Infection	Suggested Pathophysiologic Mechanisms
Iron	Hyposideremia (common)	Flux of iron into liver and reticuloendothelial system cells
		Increased synthesis of ferritin
		Sequestration of iron in tissue stores
		Diminished iron absorption
	Anemia	Accelerated red blood cell destruction
		Direct blood loss
		Inhibition of erythropoiesis
	Reduced serum iron-binding capacity	Reduced synthesis of transferrin
	Hypersideremia (selective)	Hepatocyte damage during hepatitis
Zinc	Hypozincemia	Accelerated flux of zinc into liver
		Hepatic synthesis of additional metallothioneins
		Negative body balance of zinc
	Hyperzincuria	Hepatitis-induced inhibition of zinc binding to plasma proteins
Copper	Hypercupremia	Accelerated hepatic synthesis and release of ceruloplasmin
Chromium	Hypochromia	Unknown
Manganese	Hypermagnesemia	Hepatocyte damage during hepatitis
Cobalt	Hypercobaltemia	Hepatocyte damage during hepatitis
	Macrocytic anemia	Intestinal parasitic competition for available vitamin B_{12}
Gallium	Hypergallemia	Hepatocyte damage during hepatitis
	Accumulation in sites of localized infection	Unknown
Iodine	Accelerated deiodination of thyroid hormones	Increased cellular metabolic rates
		Increased iodine availability for cellular bactericidal functions

include a decrease in the concentrations of plasma iron and zinc and an increase in plasma copper.[14] This triad of trace-element changes has been reported in bacterial, viral, rickettsial, and parasitic infections. In acute viral hepatitis, serum iron values tend to increase, however, in the second and third weeks of illness. Hepatitis also is associated with an unusual change in the binding of zinc to various ligands.[71] Soon after the acute onset of jaundice, plasma zinc is found to be bound almost entirely as microligands to small molecules, such as the amino acids. This phenomenon contrasts with the normal propensity for approximately 95 percent of plasma zinc to bind predominantly as a macroligand with either albumin or α_2-macroglobulin. In any event, plasma zinc is sequestered rapidly in the liver, where it becomes tightly bound during acute infections to newly synthesized metallothioneins.[146]

Changes in Iron Metabolism

An abrupt depression in serum iron concentrations has been observed in virtually all infections in which iron values have been measured except acute viral hepatitis, with pyogenic infections causing the greatest effects. This hypoferremia has been ascribed primarily to an accelerated flux of iron from plasma into the liver, where it becomes localized in reticuloendothelial cells and hepatocytes. There the iron becomes sequestered as intracellular hemosiderin molecules or as a complex with ferritin.[11,177] TNF and IL-1 seem to trigger this sequestration of iron by inducing the formation of ferritin,[29] some of which may be found in plasma. Plasma ferritin values provide a valuable clue concerning the quantities of body iron available in storage depots. When iron has become sequestered, it is not released readily until the infection is terminated.

Hepcidin is a peptide involved in major iron metabolic pathways. Secretion of hepcidin in the liver is induced during infections, likely by cytokines such as IL-6. Increased production of hepcidin leads to decreased plasma iron, which can be detrimental to the host but nonetheless acts as a host defense mechanism by reducing the iron available to microorganisms.[57] Despite a low pool of plasma iron, some microorganisms, such as mycobacteria, have developed survival mechanisms by using the iron stored intracellularly in macrophages.[137]

During acute and chronic infections, the normal mechanisms that allow for the continuing release from tissue stores of the iron needed for use in erythropoiesis seem to be inhibited. If an infection persists for a prolonged period, anemia may develop. Although the anemia of infection resembles iron-deficiency anemia in its peripheral manifestations, it develops in the presence of adequate quantities of iron in storage sites and cannot be reversed by the therapeutic administration of iron.[11]

Other factors that influence iron metabolism during episodes of infection include accelerated destruction of red blood cells and their direct loss in diseases in which hemolysis or bleeding is a factor. Excessive laboratory testing of blood samples can occur. In malaria, serum iron values tend to decline, despite the unusually large amounts of hemoglobin released from parasitized red blood cells. The released hemoglobin rapidly complexes with haptoglobin and is taken up by reticuloendothelial cells.[47] As in other forms of hemolytic anemia, a depression of serum haptoglobin values in malaria can serve as an index of the severity of red blood cell destruction.

Inhibition of iron absorption in the intestinal tract also can contribute to an infection-induced depression of serum iron values.[177] Studies using radioactive iron showed that a febrile illness or the febrile response of infants or young children to immunization could depress intestinal iron absorption for several days.[24]

The abrupt decrease in serum iron concentration, which can reach virtually undetectable values, occurs without any appreciable change in total serum iron-binding capacity. Although concentrations of serum transferrin decline along with concentrations of albumin during states of severe protein deficiency, they decline only slowly, if at all, during acute infections. The combination of a normal iron-binding capacity and a markedly depressed concentration of serum iron in previously well-nourished children results in an increase in the concentration of serum unsaturated transferrin.[11] Unsaturated transferrin may play an important host defensive role by competing with siderophores of bacteria, which need to acquire iron for growth and replication.[176] Because of its high affinity constant for iron, unsaturated transferrin is an important potential mechanism for inhibiting bacterial replication.

Increased concentrations of serum iron have been reported during bacillary dysentery, typhoidal infections, and, most commonly, acute viral hepatitis. The increase in concentrations of serum iron is delayed in hepatitis until several weeks after the initial appearance of jaundice; this increase may be large enough to virtually saturate the iron-binding capacity of serum. An impairment of mechanisms accounting for the normal daily hepatic removal of iron from serum has been thought to explain the hyperferremia noted during infections associated with liver cell dysfunction. An alternative hypothesis suggests that hepatitis-induced hyperferremia is caused by an escape of iron from the damaged hepatocytes. The latter explanation also is used to explain the reported increase in concentrations of serum manganese, gallium, and cobalt during acute hepatitis.[14]

Changes in Zinc Metabolism

Concentrations of zinc decline during episodes of various infectious diseases, as they do with a large variety of other diseases characterized by the presence of an inflammatory process.[14,118] However, zinc values rarely decrease as much as iron values. This difference apparently is because of the large amount of plasma zinc that is bound tightly to alpha$_2$-macroglobulin. This plasma protein remains constant during periods of infection.[13]

Similar to iron, zinc seems to move into the liver at an accelerated rate during episodes of acute infections. This increased flux of zinc from serum to the liver apparently is triggered by the action of proinflammatory cytokines released from activated monocytes and macrophages.[11,14] The hepatic uptake and sequestration of zinc

are associated with an increased synthesis of zinc-binding metallothioneins within the hepatic cells.[146] The reason for this flux of zinc from serum to the liver has not been explained in terms of host defense.

Body balances of zinc are thought to become negative during episodes of infectious diseases because of a combination of factors, including a reduction in dietary zinc intake, diminished absorption from the intestinal tract, and concomitant continuation (or increase) of zinc losses via the urine and possibly via the feces and sweat. During periods of acute infectious hepatitis, a marked increase in the urinary loss of zinc occurs. This loss is caused by enhanced glomerular filtration and excretion of zinc microligands.[71] The increased formation of zinc microligands with amino acids, such as histidine and cysteine, permits plasma zinc to be excreted from the body via the urine. Whether an increased binding of zinc to amino acid microligands occurs with infections other than acute hepatitis is unknown; this phenomenon was not detected with sandfly fever. Zinc deficiency is important, particularly in developing countries, where zinc deficiency is widespread and plays a significant role in diarrhea, pneumonia, tuberculosis, and malaria.[40]

Changes in Copper Metabolism

Concentrations of plasma copper increase in virtually all infections. This increase apparently accompanies the accelerated synthesis and release from the liver of ceruloplasmin, the copper-binding protein of plasma. The increase in plasma ceruloplasmin resembles that of other acute-phase reactant proteins produced by the liver during inflammatory states when triggered by IL-6 and other proinflammatory cytokines.[11] Increases in ceruloplasmin and copper occur later than the abrupt depressions in serum iron and zinc. The increase in copper values tends to persist longer than the changes in zinc and iron, apparently as a result of the long in vivo half-disappearance time of ceruloplasmin from plasma. Body balances of copper have not been studied during an episode of an infectious illness.

Changes in Selenium Metabolism

Selenium is an essential trace mineral required for the proper function of the immune system. Selenium behaves both as an antioxidant and anti-inflammatory agent. As an antioxidant, selenium is a component of the glutathione peroxidase enzyme, which can reduce hydrogen peroxide and lipid and phospholipid hydroperoxides, thereby decreasing the release of free radicals. As an anti-inflammatory, it reduces hydroperoxide intermediates in the cyclo-oxygenase and lipoxygenase pathways, diminishing the production of inflammatory prostaglandins and leukotrienes.[1]

Some studies suggest a deficiency of selenium is accompanied by loss of immune complexes because it is found in significant amounts in immune tissues, liver, spleen, and lymph nodes. Selenium deficiency is known to affect the virulence of HIV, coxsackievirus, and influenza viruses. Most studies have focused on in vivo experiments using animals fed selenium deficient diets. However, the in vitro studies suggest a cell-specific response occurs in regard to selenium deficiencies.

One example has been found in HIV-infected patients, in whom low plasma selenium levels are associated with the development of severe cardiomyopathy.[6] Moreover, point mutations in coxsackievirus B3 (CVB3/0) and influenza A viruses (H3N2) have been associated with increased disease severity in selenium-deficient mice and an increase in reactive oxygen species was demonstrated to enhance HIV replication in T-lymphocytic and monocytic cells.[6] Thus, selenium deficiency leads to increased virulence.

PROINFLAMMATORY CYTOKINES

The generalized metabolic and physiologic responses to febrile infections are initiated and sustained by a unique control mechanism. This mechanism involves the secretion by various body cells of hormone-like cytokines (mainly monokines and lymphokines).[42] The cytokines include ILs, interferons, colony-stimulating factors, and TNF. Cytokines also function during localized infections.[116]

Complex interactions of cytokines that occur during illness only now are being unraveled.[8,42] Cytokines are not classified as hormones, because they are produced by a variety of different cells located throughout the body, rather than by anatomically distinct glands; because cytokines are effective at far lower concentrations than hormones; and because their interacting array of checks and balances is far more complex than that of hormones. Although cytokines are not components of either the central nervous or endocrine systems, they interact with and trigger responses by both of these major regulatory control systems.[8,110]

Proinflammatory cytokines (which include IL-1, IL-6, and IL-8, and TNF) initiate and orchestrate the highly complex but stereotyped admixture of concomitant anabolic and catabolic events that make up the generalized host response to febrile infections. Individual components of this generalized response form distinct patterns. Some metabolic responses begin during the incubation period, some at the onset of fever, some late in the course of fever, and some during convalescence.[11,14-16,19,25] No matter which type of microorganism causes an acute, generalized, febrile infection, the onset of most metabolic, biochemical, or physiologic responses seems to occur at consistent, predictable times. These many responses can be categorized temporally by their relationships to the time of onset of clinical symptoms and fever.[11] Virtually every metabolic or biochemical process is influenced in some manner by the body's response to an acute infection. The duration and magnitude of individual components of the generalized response show some variability from infection to infection. More must be learned about how the cytokines influence this variability in host metabolic responses.

In today's terminology, this overall response is termed an *acute-phase response*, an *acute-phase reaction*, or the *systemic inflammatory response syndrome*. This acute-phase response may accompany other severe medical and

surgical problems, in addition to infection.[82] This complex response, which also activates complement and stimulates the immune system, seems to help defend the body.[11,82] On the negative side, acute-phase responses generate important nutritional costs,[8,13,124] which can produce severe, life-threatening malnutrition. In addition, acute-phase responses that become excessive or overly prolonged can lead to hypotensive shock, multiorgan dysfunction, and death.[47,86,113,134]

Acute-phase responses are initiated and controlled by the proinflammatory cytokines.* The cytokine IFN-α also can contribute to the wasting syndrome of acute infections.[38] The acute-phase reaction may be modified by bacterial endotoxins, which uniquely stimulate an exaggerated release of TNF, a cytokine that inhibits lipoprotein lipase enzymes, to account for the high concentrations of plasma triglycerides that develop during gram-negative sepsis.[11,76]

The primary control mechanisms that initiate acute-phase responses normally exist in a standby mode but can be turned on whenever necessary by the activation of macrophages, blood monocytes, or other body cells and the subsequent rapid release of proinflammatory cytokines.[11,47,105] The many diverse types of stimuli that can activate these cells include the phagocytosis of microorganisms, tissue debris, or other particulate matter; the effects of polynucleotides, certain drugs and chemicals, antigen-antibody complexes, and bacterial toxins; and the actions of other cytokines. During infectious illnesses, proinflammatory cytokines can be detected in body fluids, body secretions, urine, and stools.[61,95,124,127]

After release from producing cells, proinflammatory cytokines circulate via the plasma or diffuse through tissue fluids to stimulate cell populations in many locations throughout the body. These cytokines have multiple, often overlapping actions, and they stimulate the release of companion cytokines. IL-1 previously was termed *endogenous pyrogen*, *leukocytic endogenous mediator*, and *lymphocyte-activating factor*;[47] and TNF previously was termed *cachectin*. Proinflammatory cytokines stimulate the hypothalamic temperature-regulating center to initiate fever,[11,47] endocrine glands to secrete hormones, the liver to take up amino acids and trace elements and synthesize many proteins,[11,118,143] the pancreatic islets to release insulin and glucagon,[59,186] and the muscle cells to catabolize contractile proteins.[5,37] These cytokines also stimulate the bone marrow to produce and release neutrophils[110] and synovial cells and fibroblasts to activate collagenase.[47] Proinflammatory cytokines also may affect growth during certain viral infections by exerting a direct effect on bone remodeling.[151] Marrow stimulation involves the effect of IL-1 on progenitor cells[97] and its ability to trigger the release of various colony-stimulating cytokines.[11,72,105] In this regard, IL-1 has been found to be identical with hematopoietin.[105] The proinflammatory cytokines also activate the immune system and cause certain subsets of T lymphocytes to secrete IL-2, IL-3, IL-4, and IL-5.[11,47]

Proinflammatory cytokines are thought to function, after attachment to their specific cell wall receptors, by activating phospholipase A_2 within the cell walls, leading to the intracellular release of arachidonic acid, eicosapentaenoic acid, or both, from cell wall polyunsaturated fatty acid phospholipids.[47] Subsequent responses by the cytokine-stimulated cell are determined by the intracellular enzymes that can metabolize these acids to one of many possible eicosanoids.

CONTROL MECHANISMS FOR PROINFLAMMATORY CYTOKINE ACTIONS

Similar to the action of hormones, proinflammatory cytokine actions are regulated by numerous checks and balances. To act on a cell, a cytokine first must attach itself to a protein receptor on an exterior cell wall. Cells produce other similar receptor proteins that are released to float free in plasma.[99,130] Freely circulating receptors can intercept and inactivate their matching cytokine.[56,62] Other unique plasma protein molecules—receptor antagonists—also are produced.[184] They can attach to and block the cellular receptors for specific cytokines.[91,122] Cytokine inhibitor proteins have been identified.[33,160] Other cytokines, such as IL-4 and IL-10, can suppress the cellular production of proinflammatory cytokines.[45,63,83,122,155] Cytokine stimulation of release of cortisol has complex feedback effects because cortisol can block the intracellular formation of eicosanoids stimulated by the cytokines. Cortisol and epinephrine also can stimulate the production of cytokines.[87]

Cytokine Detection in Biologic Fluids

Individual components of this complex cytokine system of checks and balances now can be measured in body fluids, and their relationships can be studied throughout the course of an acute-phase reaction.* These cytokines and their free receptors can be found in mucosal fluids and plasma.[112,121,127] Cytokine measurements may have diagnostic and prognostic value.[31,143] Identification of IL-8 in amniotic cord sera was reported to be a specific marker for preterm chorioamnionitis.[143] Raqib and colleagues[127] described longitudinal measurements of cytokines and receptor antagonists in the plasma and stools of patients with acute shigellosis. Concentrations of TNF-α, IL-1β, IL-6, IL-8, and IL-1 receptor antagonist in stools, were quite high when patients with shigellosis first were seen and gradually returned to normal values during the next 2 weeks.[143] IL-6 and IL-8 can be used as markers for neonatal sepsis with coagulase-negative staphylococci and to differentiate between bacterial and enteroviral infections.[162] Proinflammatory cytokines also are responsible for the cachexia associated with acute or chronic inflammatory diseases.[43] The pathogenic or physiologic effects of these interacting molecules in either systemic or localized diseases still are not fully understood.

*References 47, 68, 70, 78, 86, 143, 152, 178.

*References 27, 31, 47, 78, 83, 152, 160.

HORMONAL RESPONSES

Infectious illnesses are accompanied by a variety of endocrine responses that include increased secretion of the hormones that regulate carbohydrate and energy metabolism and the hormones that influence salt and water retention. These hormonal responses are secondary to and often initiated by the primary release of proinflammatory cytokines from activated cells.

In addition to their participation in physiologic responses, endocrine functions also may be affected by pathologic complications of an infectious process that results in the direct destruction or dysfunction of hormone-producing cells. The adrenal glands may be destroyed if tubercle bacilli localize in these glands or if hemorrhagic necrosis becomes a complication of infections such as acute meningococcemia. Pancreatic islet cells may be destroyed during episodes of viral infections of experimental animals, and the same pathogenic possibility may initiate insulin-requiring diabetes in children. Thyroid function may be impaired after occurrence of a viral infection that triggers an autoimmune thyroiditis, and gonadal tissues may be the site of localization and destruction by the mumps virus.

Pituitary Gland Functions

Secretion of certain of the trophic hormones produced by the anterior pituitary is increased during infection. An increase in adrenocorticotropic hormone production (stimulated by proinflammatory cytokines) triggers an increased secretion of several adrenocorticoids. Concentrations of plasma growth hormone generally are increased in acute infections in which this hormone has been measured,[23,128] but the increases do not seem to correlate directly in timing or magnitude with the presence or severity of fever.[10,14] This increase in plasma growth hormone may be due partly to its production by mononuclear leukocytes.[175] Concentrations of plasma growth hormone increase rapidly in a paradoxical manner if an intravenous infusion of glucose is given to a patient or a laboratory animal with an acute infection.[128] Thyroid-stimulating hormone does not respond during the early phases of an acute febrile illness but may increase during the convalescent period. No increases in the gonadotropic hormones have been documented during an episode of an infection. A release of several anterior pituitary hormones can be stimulated by fever-producing doses of bacterial endotoxin. Such responses have been used in the clinical testing of anterior pituitary function.[81,102]

As described in the earlier discussion of salt and water imbalances, inappropriate secretion of antidiuretic hormone may occur in patients with severe infectious diseases.[50] This phenomenon also seems to be a characteristic response in patients whose infectious process becomes localized within the cranial vault.

Adrenal Functions

Although the adrenocortical hormones are known to influence the ability of a patient to respond to stressful situations, few data define the duration and magnitude of the adrenal response or characterize the spectrum of specific steroid hormones produced in excess.[21,101] Available data suggest that acute generalized infections typically are accompanied by a transient increase in the adrenal output of glucocorticoid hormones that is of short duration. Although these responses generally are limited in magnitude, they serve to maintain a constant concentration of cortisol in plasma throughout the early periods of fever.[21]

The usual diurnal decline in plasma 17-hydroxycorticosteroid values fails to occur during the afternoon and evening hours if fever is present. Cortisol-binding proteins have not been observed to change in plasma during periods of acute infection.[14] The plasma concentrations of unbound, physiologically active cortisol are maintained at or higher than the normal peak early morning values throughout the initial period of a febrile illness.[14,21] The total increase in glucocorticoid secretion rates during early illness ranges from 2 to 5 times normal values in infections that have been studied. Lesser degrees of increase have been noted in the adrenocortical output of pregnanetriol and the weak ketosteroid androgens.[21] If an infectious illness becomes chronic, adrenal output generally returns to or even falls below baseline values and diminished adrenal responsiveness to adrenocorticotropic hormone may occur.

Extremely high concentrations of plasma 17-hydroxycorticosteroids may develop during gram-negative septicemia or before death in patients with other severe acute infections.[98,101] These high terminal values may be ascribed to failures in the hepatic clearance of cortisol from plasma and the metabolic pathways for converting cortisol to water-soluble metabolites; high plasma values are not the result of an extraordinary increase in adrenal secretion rates.[98] In a detailed study of adrenal function in children with meningitis, Migeon and coworkers[101] reported a maximum average increase in cortisol secretion rate of approximately threefold during uncomplicated aseptic or bacterial meningitis. This increase was accompanied by a threefold increase in the excretion of urinary 17-hydroxycorticosteroids during the first few days of illness.[65] On admission to the hospital, these children had concentrations of plasma cortisol that ranged from high normal to twice normal values. Severely ill patients who were dying of adrenal hemorrhage generally had values that were depressed or absent.

Mechanisms by which small increases in adrenal glucocorticoid secretion might protect the host have not been defined. However, the ability of the liver to produce some of its proteins during periods of infection is known to depend on the permissive presence of glucocorticoid hormones.[21]

The increase in production of aldosterone seems to lag behind the increase in production of cortisol during periods of acute infections and then persists longer. Increases in aldosterone stimulate the intense retention of sodium and chloride by the kidney during episodes of acute infections.[17]

The adrenomedullary secretion of catecholamines may increase in severe infectious diseases.[66,182] High plasma epinephrine and norepinephrine values develop

in patients with gram-negative sepsis and bacterial meningitis. The catecholamine response contributes significantly to the acceleration of gluconeogenesis during periods of infection.

Carbohydrate-Regulating Hormones

Hormones that serve in the normal regulation of carbohydrate metabolism also participate in the host response to infection. In addition to the heightened secretion of the glucocorticoids, catecholamines, and growth hormone, the major pancreatic hormones insulin and glucagon circulate in increased concentrations in the plasma of patients with acute infectious diseases.[182] The combined effect of these hormonal actions is to accelerate the production of glucose within the liver and stimulate the release of glucose from stored glycogen. These actions cause the glucose pool size to increase twofold or threefold to provide an important source of metabolizable energy during the early febrile periods of acute infections.

Intravenous glucose tolerance tests performed early in the course of febrile infections in young adults[128,141] led to an exaggerated increase of concentrations of plasma insulin in magnitude and duration, an appropriate decline in elevated fasting concentrations of plasma glucagon, and a paradoxical stimulation of release of growth hormone. Extreme hyperinsulinism also was observed after glucose infusions were given to dogs with endotoxic shock.[25,186]

The modest increase in concentrations of fasting plasma insulin that occurs during periods of infection seems to account for an inhibition of ketogenesis and fat depot lipolysis, two responses that would be expected to occur because of concomitant starvation or semistarvation. The simultaneous combination of high fasting plasma glucagon and insulin values and their effects on the liver and peripheral fat depots and somatic tissues helps explain the observed differences between the anorexia-induced semistarvation associated with infectious illnesses and the starvation caused by food deprivation.

Thyroid Hormones

Thyroid hormones do not seem to initiate or sustain the hypermetabolic response to fever. Nonetheless, thyroxine (T_4) and triiodothyronine (T_3) are deiodinated at accelerated rates within the body tissues, especially in the liver, during the early phases of infections studied in humans and experimental animals.[46,173,184] This acceleration in the metabolism of peripheral thyroid hormones accounts for an early decline in serum protein-bound iodine values.[140] Only after an infectious illness has progressed for several days does the thyroid gland hormone output seem to increase. This increase in thyroid secretion persists into early convalescence so that for a time the production of thyroid hormones exceeds apparent body requirements. As a result, protein-bound iodine values in serum are increased in the convalescent period. This sequence of events produces a biphasic response pattern with an initial decrease and a late increase in concentrations of thyroid hormones. Serum concentrations of "reverse T_3" increase during febrile illnesses.[35] Because reverse T_3 has an apparent role in regulating the peripheral cellular actions of T_4 and T_3, its function during acute infectious illness remains to be elucidated.

In studies of patients with falciparum malaria infection, serum T_3 values declined abruptly, whereas serum T_4 values were stable or increased slightly.[173] The decline in serum T_3 concentrations was accompanied by reciprocal increases in reverse T_3. The slowing of T_4 turnover during periods of malaria may be caused by an impaired ability of hepatocytes to metabolize or deiodinate T_4. Hypothalamic suppression in early stages of malaria seems to result in a decreased release of thyroid-stimulating hormone from the anterior pituitary and a secondary decrease in T_4 and T_3 secretion from the thyroid gland. In patients with sepsis and septic shock, thyroid hormones (total and free T_3, T_4, and thyroid-stimulating hormone) are decreased and are associated with poor prognosis.[188]

Procalcitonin

Procalcitonin, discovered in the early 1960s, is a prohormone, the precursor of the hormone calcitonin. Multiple studies have shown that procalcitonin is involved in the pathogenesis of infections, and that it can be a useful diagnostic marker for infections such as bacterial pneumonia,[75] bacterial sepsis and septic shock, meningitis, infectious endocarditis, pancreatitis, and urinary tract infections.[36,107,156]

The role of calcitonin is unclear; most likely, it had an evolutionary role, and it currently is nonessential in humans.[36] Serum concentrations of procalcitonin and other precursors of mature calcitonin are elevated significantly during episodes of sepsis and other infectious and noninfectious causes of inflammation. Serum concentration of procalcitonin also is elevated in patients with malaria. The serum concentration of procalcitonin usually correlates with the severity of infection and with mortality.[36]

Secretion of procalcitonin is stimulated by IL-1β and TNF-α in patients with bacterial infections and by IFN-γ in patients with viral infections. The target tissues are lung, liver, kidney, adipose tissue, and muscle.

Several studies have suggested procalcitonin could be used as a marker for bacterial sepsis and to differentiate infectious from noninfectious causes of inflammation. Although procalcitonin has been shown to possibly have greater specificity and sensitivity than C-reactive protein as a marker for bacterial infections, it should not be used without evaluation of other clinical and laboratory data in making therapeutic decisions, because serum procalcitonin concentration may be normal in patients with sepsis or increased in some individuals with no clinical symptoms.[36]

NEW REFERENCES SINCE THE SIXTH EDITION

5. Bai J, Wu S, Ge KY, et al. The combined effect of selenium deficiency and viral infection on the myocardium of mice. *Acta Acad Med Sin* 1980;**2**:29–31.

7. Beck MA. Increased virulence of coxsackievirus B3 in mice due to vitamin E or selenium deficiency. *J Nutr* 1997;**127**(Suppl. 5): 966S–70S.
8. Beck MA, Nelson HK, Shi Q, et al. Selenium deficiency increases the pathology of influenza virus infection. *FASEB J* 2001;**15**: 1481–3.
13. Beisel WR. Metabolic response to infection. *Annu Rev Med* 1975;**26**:9–20.
26. Blanc M, Hsieh WY, Robertson KA, et al. Host defense against viral infection involves interferon mediated down-regulation of sterol biosynthesis. *PLoS Biol* 2011;**9**:e1000598. doi:10.1371/journal.pbio.1000598.
32. Castrillo A, Joseph SB, Baidya S, et al. Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. *Mol Cell* 2003;**12**:805–16.
55. Flynn JL, Goldstein MM, Chan J, et al. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 1995;**2**:561–72.
75. Jennings G, Bourgeois C, Elia M. The magnitude of the acute phase protein response is attenuated by protein deficiency in rats. *J Nutr* 1992;**122**:1325–31.
89. Mahalanabis D. Nitrogen balance during recovery from secretory diarrhea of cholera in children. *Am J Clin Nutr* 1981;**34**: 1548–51.
132. Roth E, Funcovics J, Muhlbacher F, et al. Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. *Clin Nutr* 1982;**1**:25–42.
133. Roth EF. Malarial parasite hexokinase and hexokinase-dependent glutathione reduction in the *Plasmodium falciparum*-infected human erythrocyte. *J Biol Chem* 1987;**262**:15678–82.
148. Souba WW, Klimberg VS, Plumley DA. The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. *J Surg Res* 1990;**48**:383–91.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 4

Fever: Pathogenesis and Treatment

Mark A. Ward

Fever is the thermoregulated increase in body temperature above normal as the result of a coordinated response to a pathologic insult. The two essential features of fever are that it represents an abnormal elevation in body temperature and that it results from a coordinated physiologic response. The first feature differentiates fever from normal regulated elevations in body temperature (e.g., elevations associated with the circadian rhythm), whereas the latter distinguishes it from conditions in which the regulatory mechanisms are overwhelmed or dysfunctional (e.g., heat stroke). In children, the pathologic insult most likely to result in fever is infection. A variety of other conditions, including malignancies and autoimmune diseases, also may result in this phenomenon, however.

NORMAL BODY TEMPERATURE

Although the general public and physicians alike often refer to "*the*" body temperature, the implication that a single number can represent the thermal state of the entire body is inaccurate. Depending on the site of measurement, body temperature may vary by 1°C or more.[35] These regional variations in temperature do not have a fixed relationship to each other. Although axillary temperatures are consistently lower than rectal temperatures, the absolute difference between the two varies greatly.[4] In addition, even in the healthy state, body temperature is not constant; it varies depending on numerous factors, such as time of day, level of activity, and phase of the menstrual cycle. Generally, clinicians have been most interested in the core body temperature, defined as the temperature of the internal organs of the trunk and head. Under normal circumstances, core temperature is higher than the temperature of more superficial tissues such as skin. Even within these two anatomic regions, temperature gradients exist, however.

The most widely accepted definition of normal body temperature is 37°C (98.6°F).[46] This number is derived from studies performed in the 19th century by Wunderlich.[87] He reportedly arrived at this figure based on the result of several million measurements conducted in approximately 25,000 individuals. Other, more recent studies have found slightly lower mean temperatures in healthy individuals, despite the fact that these more recent studies are based on oral or rectal temperatures whereas Wunderlich's studies relied on axillary temperatures.[29] Mackowiak and colleagues[47] determined the mean oral temperature in adults to be 36.8°C (98.2°F), with the upper limit of normal ranging from 37.2°C (98.9°F) at 6:00 AM to 37.7°C (99.9°F) at 4:00 PM. Given the limitations imposed by the technology available at the time, however, perhaps the most surprising aspect of

REFERENCES

1. Alvarez JO, Salazar-Lindo E, Kohatsu J, et al. Urinary excretion of retinol in children with acute diarrhea. *Am J Clin Nutr* 1995;**61**:1273–6.
2. American Academy of Pediatrics Committee on Drugs. Vitamin C and the common cold. *Nutr Rev* 1974(Suppl):39–40.
3. Anderson R, Smit MJ, Joone GK, Van Staden AM. Vitamin C and cellular immune functions: Protection against hypochlorous acid-mediated inactivation of glyceraldehyde-3-phosphate dehydrogenase and ATP generation in human leukocytes as a possible mechanism of ascorbate-mediated immunostimulation. *Ann N Y Acad Sci* 1990;**587**:34–48.
4. Argaman Z, Young VR, Noviski N, et al. Arginine and nitric oxide metabolism in critically ill septic pediatric patients. *Crit Care Med* 2003;**31**:591–7.
5. Bai J, Wu S, Ge KY, et al. The combined effect of selenium deficiency and viral infection on the myocardium of mice. *Acta Acad Med Sin* 1980;**2**:29–31.
6. Baracos V, Rodemann HP, Dinarello CA, et al. Stimulation of muscle protein degradation and prostaglandin E2 release by leukocytic pyrogen (interleukin-1): A mechanism for the increased degradation of muscle proteins during fever. *N Engl J Med* 1983;**308**:553–8.
7. Beck MA. Increased virulence of coxsackievirus B3 in mice due to vitamin E or selenium deficiency. *J Nutr* 1997;**127**(Suppl. 5): 966S–70S.
8. Beck MA, Nelson HK, Shi Q, et al. Selenium deficiency increases the pathology of an influenza virus infection. *FASEB J* 2001;**15**: 1481–83.
9. Beck O. Weitere untersuchungen zub fieberstoffwechsel des sauglings: Die qualitativen Veranderungen des Stickstoffwechsel im Fiber. *Jahrb Kinderh* 1926;**112**:184–216.
10. Beisel W. The influence of infection or injury on nutritional requirements during adolescence. In: McKingey JI, Munro HN, editors. *Nutrient requirements in adolescents*. Cambridge, Mass: MIT Press; 1976.
11. Beisel W. Nutrition and infection. In: Linder MC, editors. *Nutritional biochemistry and metabolism*. 2nd ed. New York: Elsevier Science; 1991. p. 508–42.
12. Beisel W, Sawyer WD, Ryell ED, et al. Metabolic effects of intracellular infections in man. *Ann Intern Med* 1967;**67**:755–79.
13. Beisel WR. Metabolic response to infection. *Annu Rev Med* 1975;**26**:9–20.
14. Beisel WR. Metabolic effects of infection. *Progr Food Nutr Sci* 1984;**8**:43–75.
15. Beisel WR. Nutrition, infection, specific immune response, and nonspecific host defenses: A complex interaction. In: Watson R, editor. *Nutrition, disease resistance and immune function*. New York: Marcel Dekker; 1984. p. 3–34.
16. Beisel WR. Herman Award Lecture, 1995. Infection-induced malnutrition: from cholera to cytokines. *Am J Clin Nutr* 1995; **62**:813–9.
17. Beisel WR, Rapoport MI. Inter-relation between adrenocortical functions and infectious illness. *N Engl J Med* 1969;**280**: 569–604.
18. Beisel WR, Bruton J, Anderson KD, et al. Adrenocortical responses during tularemia in human subjects. *J Clin Endocrinol Metab* 1967;**27**:61–9.
19. Beisel WR, Feigin RD, et al. Proceedings of a workshop: Impact of infection on nutritional status of the host. *Am J Clin Nutr* 1977;**30**:1203–1371, 1439–1566.
20. Beisel WR, Goldman RF, Joy RJT. Metabolic balance studies during induced hyperthermia in man. *J Appl Physiol* 1968;**24**: 1–10.
21. Beisel, WR, Herman YF, Sauberlich HE, et al. Experimentally induced sandfly fever and vitamin metabolism in man. *Am J Clin Nutr* 1972;**25**:1165–73.
22. Beisel WR, Watten RH, Blackewell Q, et al. The role of bicarbonate pathophysiology and therapy in Asiatic cholera. *Am J Med* 1963;**35**:58–66.
23. Beisel WR, Woeber KA, Bartelloni. PJ, et al. Growth hormone response during sandfly fever. *J Clin Endocrinol Metab* 1968;**28**: 1220–3.
24. Beresford CH, Neale RJ, Brooks OG. Iron absorption and pyrexia. *Lancet* 1971;**1**:568–75.
25. Blackard WG, Anderson Jr JH, Spitzer JJ. Hyperinsulinism in endotoxin shock dogs. *Metabolism* 1976;**25**:675–84.
26. Blanc M, Hsieh WY, Robertson KA, et al. Host defense against viral infection involves interferon mediated down-regulation of sterol biosynthesis. *PLoS Biol* 2011;**9**:e1000598. doi:10.1371/journal.pbio.1000598.
27. Brown CC, Poli G, Lubaki N, et al. Elevated levels of tumor necrosis factor-α in Zairian neonate plasmas: Implications for perinatal infection with the human immunodeficiency virus. *J Infect Dis* 1994;**169**:975–80.
28. Calder PC. Branched-chain amino acids and immunity. *J Nutr* 2006;**136**:288S–93S.
29. Campbell CH, Solgonick RM, Linder MC. Translational regulation of ferritin synthesis in rat spleen: Effects of iron and inflammation. *Biochem Biophys Res Commun* 1989;**160**:453–9.
30. Cao W, Baniecki ML, McGrath WJ, et al. Nitric oxide inhibits the adenovirus proteinase in vitro and viral infectivity in vivo. *FASEB J* 2003;**17**:2345–6.
31. Casey LC, Balk RA, Bone RC. Plasma cytokine and endotoxin levels correlate with survival in patients with sepsis syndrome. *Ann Intern Med* 1993;**119**:771–8.
32. Castrillo A, Joseph SB, Baidya S, et al. Crosstalk between LXR and toll-like receptor signaling mediates bacterial and viral antagonism of cholesterol metabolism. *Mol Cell* 2003;**12**:805–16.
33. Chang DM, Shaio MF. Production of interleukin-1 (IL-1) and IL-1 inhibitor by human monocytes exposed to dengue virus. *J Infect Dis* 1994;**170**:811–7.
34. Chiarla C, Giovannini I, Siegel JH. The relationship between plasma cholesterol, amino acids and acute phase proteins in sepsis. *Amino Acids* 2004;**27**:97–100.
35. Chopra IJ, Chopra U, Smith SR, et al. Reciprocal changes in serum concentrations of 3,3N5N-triiodothyronine (reverse T3) and 3,3N5-triiodothyronine (T3) in systemic illness. *J Clin Endocrinol Metab* 1975;**41**:1043–9.
36. Christ-Crain M, Muller B. Procalcitonin in bacterial infections: hype, hope, more or less? *Swiss Med Wkly* 2005;**135**:451–60.
37. Clowes Jr GH, George BC, Villee Jr CA, Saravis CA. Muscle proteolysis induced by a circulating peptide in patients with sepsis or trauma. 1983. *Nutrition (Burbank, Los Angeles County, Calif)* 1995;**11**:775; discussion 774, 776–7.
38. Constans J, Pellegrin I, Pellegrin JL, et al. Plasma interferon alpha and the wasting syndrome in patients with human deficiency virus. *Clin Infect Dis* 1995;**20**:1069–70.
39. Cook GC. Increased glycine absorption rate associated with acute bacterial infections in man. *Br J Nutr* 1973;**29**:377–86.
40. Cuevas LE, Koyanagi A. Zinc and infection: A review. *Ann Trop Paediatr* 2005;**25**:149–60.
41. Curnow RT, Rayfield EJ, George DT, et al. Altered hepatic glycogen metabolism and glucoregulatory hormones during sepsis. *Am J Physiol* 1973;**230**:1296–301.
42. Debats IB, Booi D, Deutz NE, et al. Infected chronic wounds show different local and systemic arginine conversion compared with acute wounds. *J Surg Res* 2006;**134**:205–14.
43. Delano MJ, Moldawer LL. The origins of cachexia in acute and chronic inflammatory diseases. *Nutr Clin Pract* 2006;**21**: 68–81.
44. den Hartog JE, Ouburg S, Land JA, et al. Do host genetic traits in the bacterial sensing system play a role in the development of *Chlamydia trachomatis*-associated tubal pathology in subfertile women? *BMC Infect Dis* 2006;**6**:122.
45. Derkx B, Marchant A, Goldman M, et al. High levels of interleukin-10 during the initial phase of fulminant meningococcal septic shock. *J Infect Dis* 1995;**171**:229–32.
46. DeRubertis FR, Woeber KA. Accelerated cellular uptake and metabolism of L-thyroxine during acute *Salmonella typhimurium* sepsis. *J Clin Invest* 1973;**52**:78–87.
47. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. *J Infect Dis* 1991;**163**:1177–4.
48. Dittrich R, Dragonas C, Mueller A, et al. Endothelial *Chlamydia pneumoniae* infection promotes oxidation of LDL. *Biochem Biophys Res Commun* 2004;**319**:501–05.
49. Drapier JC, Wietzerbim J, Hibbs Jr JB. Interferon-gamma and tumor necrosis factor induct the L-arginine-dependent cytotoxic effector mechanism in murine macrophages. *J Immunol* 1988;**18**: 1587–92.

50. Feigin RD, Kaplan S. Inappropriate secretion of antidiuretic hormone (ADH) in children with bacterial meningitis. *Am J Clin Nutr* 1977;**30**:1482–4.
51. Feigin RD, Middelkamp JN, Reed CA. Murine myocarditis due to coxsackie B 3 virus: Blood amino acid, virologic, and histopathologic correlates. *J Infect Dis* 1972;**126**:574–84.
52. Felig P, Brown W, Levine RA, et al. Glucose homeostasis in viral hepatitis. *N Engl J Med* 1970;**283**:1436–40.
53. Fiser RH, Denniston JC, Beisel WR. Infection with *Diplococcus pneumoniae* and *Salmonella typhimurium* in monkeys: Changes in plasma lipids and lipoproteins. *J Infect Dis* 1972;**125**:54–60.
54. Fiser RH, Denniston JC, Kastello MD, et al. Cholesterogenesis during acute infection in chronically hypercholesterolemic rhesus monkeys. *Proc Soc Exp Biol Med* 1972;**140**:314–8.
55. Flynn JL, Goldstein MM, Chan J, et al. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 1995;**2**:561–72.
56. Frieling JTM, van Deuren M, Wijdens J, et al. Circulating interleukin-6 receptor in patients with sepsis syndrome. *J Infect Dis* 1995;**171**:469–72.
57. Ganz T. Hepcidin: a peptide hormone at the interface of innate immunity and iron metabolism. *Curr Top Microbiol Immunol* 2006;**306**:183–98.
58. Gaut JP, Belaaouaj A, Byun J, et al. Vitamin C fails to protect amino acids and lipids from oxidation during acute inflammation. *Free Rad Biol Med* 2006;**40**:1494–501.
59. George DT, Rayfield EJ, Wannemacher Jr RW. Altered glucoregulatory hormones during acute pneumococcal sepsis in the rhesus monkey. *Diabetes* 1974;**23**:544–9.
60. George DT, Abels FB, Mapes CA, et al. Effect of leukocytic endogenous mediators on endocrine pancreas secretory responses. *Am J Physiol* 1977;**233**:E240–5.
61. Girardin E, Grau GE, Dayer JM, et al. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med* 1988;**319**:397–400.
62. Godfried MH, van der Poll T, Weverling GJ, et al. Soluble receptors for tumor necrosis factor as predictors of progression to AIDS in asymptomatic human deficiency virus type 1 infection. *J Infect Dis* 1994;**169**:739–45.
63. Gomez-Jimenez J, Martin MC, Sauri R, et al. Interleukin-10 and the monocyte/macrophage-induced inflammatory response in septic shock. *J Infect Dis* 1995;**171**:472–5.
64. Griffiths J, Groves AC, Leung FY. Hypertriglyceridemia and hypoglycemia in gram-negative sepsis in the dog. *Surg Gynecol Obstet* 1973;**136**:897–903.
65. Groofman JE, Molina JM, Scadden DT. Hematopoietic growth factors. *N Engl J Med* 1989;**321**:1449–59.
66. Groves AC, Griffiths J, Leung F, et al. Plasma catecholamines in patients with serious postoperative infection. *Ann Surg* 1973; **178**:102–7.
67. Guckian JC. Role of metabolism in pathogenesis of bacteremia due to *Diplococcus pneumoniae* in rabbits. *Infect Dis* 1973;**127**:1–8.
68. Halstensen A, Ceska M, Brandtzaeg P, et al. Interleukin-8 in serum and cerebrospinal fluid from patients with meningococcal disease. *J Infect Dis* 1993;**167**:471–5.
69. Hemila H, Herman ZS. Vitamin C and the common cold: A retrospective analysis of Chalmers' review. *J Am Coll Nutr* 1995;**14**:116–23.
70. Heney D, Lewis IJ, Evans SW, et al. Interleukin-6 and its relationship to C-reactive protein and fever in children with febrile neutropenia. *J Infect Dis* 1992;**165**:886–90.
71. Henkin RI, Smith FR. Zinc and copper metabolism in acute viral hepatitis. *Am J Med Sci* 1972;**264**:401–9.
72. Hirsch RL, McKay DG, Travers RI, et al. Hyperlipidemia, fatty liver, and bromsulfophthalein retention in rabbits injected intravenously with bacterial endotoxins. *J Lipid Res* 1964;**5**: 563–8.
73. Howard JE, Bighan Jr RS, Mason RE. Studies on convalescence. V. Observations on the altered protein metabolism during induced malarial infections. *Trans Assoc Am Physicians* 1946;**59**:242–7.
74. Jennings G, Bourgeois C, Elia M. The magnitude of the acute phase protein response is attenuated by protein deficiency in rats. *J Nutr* 1992;**122**:1325–31.
75. Jereb M, Kotar T. Usefulness of procalcitonin to differentiate typical from atypical community-acquired pneumonia. *Wien Klin Wochenschr* 2006;**118**:170–4.
76. Kauffmann RL, Matson CF, Beisel WR. Hypertriglyceridemia produced by endotoxin: Role of impaired triglyceride disposal mechanism. *J Infect Dis* 1976;**133**:548–55.
77. Kauffmann RL, Matson CF, Rowberg AH, et al. Defective lipid disposal mechanisms during bacterial infection in rhesus monkeys. *Metabolism* 1976;**25**:615–24.
78. Keuter M, Dharmana E, Gasem MH, et al. Patterns of proinflammatory cytokines and inhibitors during typhoid fever. *J Infect Dis* 1994;**169**:1306–11.
79. Kim JY, Lee SM. Vitamins C and E protect hepatic cytochrome P450 dysfunction induced by polymicrobial sepsis. *Eur J Pharm* 2006;**534**:202–9.
80. Kinsella JE, Lokesh B, Broughton S, Whelan J. Dietary polyunsaturated fatty acids and eicosanoids: Potential effects on the modulation of inflammatory and immune cells—An overview. *Nutrition (Burbank, Los Angeles County, Calif)* 1990;**6**:24–44; discussion 59–62.
81. Kohler PO, O'Malley BW, Rayford PL, et al. Effect of pyrogen on blood levels of pituitary trophic hormones: Observations of the usefulness of the growth hormone response in the detection of pituitary disease. *J Clin Endocrinol Metab* 1967;**27**:219–26.
82. Koj A. The role of interleukin-6 as the hepatocyte stimulating factor in the network of inflammatory cytokines. *Ann N Y Acad Sci* 1989;**557**:1–8.
83. Lang CH, Bagby GJ, Dobrescu C. Effect of granulocyte colony-stimulating factor on sepsis-induced changes in neutrophil accumulation and organ glucose uptake. *J Infect Dis* 1992; **166**:336–42.
84. Lees RS, Fiser RH, Beisel Jr WR, et al. Effects of an experimental viral infection on plasma lipid and lipoprotein metabolism. *Metabolism* 1972;**21**:825–33.
85. Liao J, Keiser JA, Scales WE, et al. Role of epinephrine in TNF and IL-6 production from isolated perfused rat liver. *Am J Physiol* 1995;**268**:R896–901.
86. Long CL, Kinney JM, Geiger JW. Nonsuppressibility of gluconeogenesis by glucose in septic patients. *Metabolism* 1976;**25**: 193–201.
87. Long CL, Haverber LN, Young VR, et al. Metabolism of 3-methylhistidine in man. *Metabolism* 1974;**24**:929–35.
88. Macallan DC, McNurlan MA, Milne E, et al. Whole-body protein turnover from leucine kinetics and the response to nutrition in human immunodeficiency virus infection. *Am J Clin Nutr* 1995;**61**:818–26.
89. Mahalanabis D. Nitrogen balance during recovery from secretory diarrhea of cholera in children. *Am J Clin Nutr* 1981;**34**: 1548–51.
90. Mahalanabis D, Jalan KN, Maitra TK, et al. Vitamin A absorption in ascariasis. *Am J Clin Nutr* 1976;**29**:1372–5.
91. Mannick JB. Immunoregulatory and antimicrobial effects of nitrogen oxides. *Proc Am Thor Soc* 2006;**3**:161–5.
92. Manzoni P, Castagnola E, Mostert M, et al. Hyperglycaemia as a possible marker of invasive fungal infection in preterm neonates. *Acta Paediatr* 2006;**95**:486–93.
93. Mata L. Malnutrition-infection interaction in the tropics. *Am J Trop Med Hyg* 1975;**24**:564–74.
94. Mata LJ, Kronmal RA, Urrutia JJ, et al. Antenatal events and postnatal growth and survival of children: Prospective observation in a rural Guatemalan village. In: White PL, editor. *Proceedings of the Western Hemisphere Nutrition Congress IV*. Acton, Mass: Publishing Sciences Group; 1975.
95. Maury CPJ, Salo E, Pelekonen P. Circulating interleukin-1 beta in patients with Kawasaki disease. *N Engl J Med* 1988;**319**: 1670–1.
96. McCallum RE, Seale TW, Stith RD. Influence of endotoxin treatment on dexamethasone induction of hepatic phosphoenolpyruvate carboxykinase. *Infect Immun* 1983;**39**:213–9.
97. McKigney JI, Munro HN, editors. *Nutrient requirements in adolescence*. Cambridge, Mass: MIT Press; 1976.
98. Melby JC, Spink WW. Comparative studies on adrenal cortical function and cortisol metabolism in healthy adults and in patients with shock due to infection. *J Clin Invest* 1958;**37**:1791–8.
99. Michie HR, Manogue KR, Sprigs DR, et al. Detection of circulating tumor necrosis factor after endotoxic administration. *N Engl J Med* 1988;**318**:1481–86.
100. Migasena P, Maegraith BG. Intestinal absorption in malaria. I. The absorption of an amino acid (AIB-i-14C) across the gut

membrane in normal and in *Plasmodium knowlesi*-infected monkeys. *Ann Trop Med Parasitol* 1969;**63**:439–48.
101. Migeon CJ, Kenny FM, Hung W, et al. Study of adrenal function in children with meningitis. *Pediatrics* 1967;**40**:163–83.
102. Moberg GP. Site of action endotoxins on hypothalamic-pituitary-adrenal axis. *Am J Physiol* 1971;**220**:397–400.
103. Moon RJ, Tremblay ES, Morris KM. Distribution and metabolism of 14C-tryptophan in normal and endotoxic-poisoned mice. *Infect Immun* 1973;**8**:604–11.
104. Morris KM, Moon RJ. Quantitative analysis of serotonin biosynthesis in endotoxemia. *Infect Immun* 1974;**10**:340–6.
105. Morrissey PJ, Mochizuki DY. Interleukin-1 is identical to hematopoietin. I. Studies on its therapeutic effects of myelopoiesis and lymphopoiesis. *Biotherapy* 1989;**1**:281–91.
106. Moyer ED. Amino acid metabolism during infectious illness. In: Powanda M, editors. *Infection: The physiologic and metabolic responses of the host*. Amsterdam: Elsevier/North Holland; 1981.
107. Muller B, Becker KL. Procalcitonin: How hormone became a marker and mediator of sepsis. *Swiss Med Wkly* 2001;**131**: 595–602.
108. Nelson PG, Pyke DA, Gamble DR. Viruses and the aetiology of diabetes: A study in identical twins. *B M J* 1975;**4**:249–51.
109. Nessan VJ, Geerken RC, Ulvilla J. Uric acid excretion in infectious mononucleosis: A function of increased purine turnover. *J Clin Endocrinol Metab* 1974;**38**:652–4.
110. Neufeld HA, Pace JA, Kaminski MY, et al. A probable endocrine basis for the depression of ketone bodies during infections or inflammatory state in rats. *Endocrinology* 1980;**107**:596–601.
111. Nichols BL, Soriano HA. A critique of oral therapy of dehydration due to diarrheal syndromes. *Am J Clin Nutr* 1977;**30**:1457–72.
112. Noah TL, Henderson FW, Wortman IA, et al. Nasal cytokine production in viral acute upper respiratory infection of childhood. *J Infect Dis* 1995;**171**:584–92.
113. O'Donnel TF, Clowes Jr GH, Blackburn GL, et al. Proteolysis associated with a deficit of peripheral energy fuel substrates in septic man. *Surgery* 1976;**80**:192–200.
114. Paris MM, Friedland IR, Ehrett S, et al. Effect of interleukin-1 receptor antagonist and soluble tumor necrosis factor receptor in animal models of infection. *J Infect Dis* 1995;**171**:161–9.
115. Patwardhan VN, Maghrabi RH, Mousa W, et al. Serum glycoproteins in protein-calorie deficiency disease. *Am J Clin Nutr* 1971; **24**:906–12.
116. Pawlowski ZS. Implications of parasite-nutrition interactions from a world perspective. *Fed Proc* 1984;**43**:256–60.
117. Powanda M. Change in body balance of nitrogen and other key nutrients: Description and underlying mechanism. *Am J Clin Nutr* 1977;**30**:1254–68.
118. Powanda MC, Beisel WR. Hypothesis: Leukocyte endogenous mediator/endogenous pyrogen/lymphocyte-activating factor modulates the development of nonspecific immunity and affects nutritional status. *Am J Clin Nutr* 1982;**35**:762–8.
119. Powanda MC, Wannemacher Jr RW, Cockerell GL. Nitrogen metabolism and protein synthesis during pneumococcal sepsis in rats. *Infect Immun* 1972;**6**:266–71.
120. Powanda MC, Dinterman R, Wannemacher Jr RW, et al. Tryptophan metabolism in relation to amino acid alterations during typhoid fever. *Acta Vitaminol Enzymol* 1975;**29**:164–8.
121. Proud D, Gwaltney Jr JM, Hendley JO, et al. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinovirus colds. *J Infect Dis* 1994;**169**: 1007–13.
122. Puccetti P, Mencacci A, Cenci E, et al. Cure of murine candidiasis by recombinant soluble interleukin-4 receptor. *J Infect Dis* 1994; **169**:1325–31.
123. Rahman MA, Azuma Y, Fukunaga H, et al. Serotonin and melatonin, neurohormones for homeostasis, as novel inhibitors of infections by the intracellular parasite chlamydia. *J Antimicrob Chemother* 2005;**56**:861–8.
124. Ramsey KH, Schneider H, Cross AS, et al. Inflammatory cytokines produced in response to experimental human gonorrhea. *J Infect Dis* 1995;**172**:186–91.
125. Rapoport MI, Beisel WR. Studies of tryptophan metabolism in experimental animals and man during infectious illness. *Am J Clin Nutr* 1971;**24**:807–14.
126. Rapoport MI, Lust G, Beisel WR. Host enzyme induction of bacterial infection. *Arch Intern Med* 1968;**121**:11–6.
127. Raqib R, Wretlind B, Anderson J, et al. Cytokine secretion in acute shigellosis is correlated to disease activity and directed more to stool than to plasma. *J Infect Dis* 1995;**171**:376–84.
128. Rayfield EJ, Curnow RT, George DT, et al. Impaired carbohydrate metabolism during a mild viral illness. *N Engl J Med* 1973;**289**:618–21.
129. Reiss E. Protein metabolism in infection: Change in certain visceral proteins studies with glycin-N^{15}. *Metabolism* 1959;**8**: 151–9.
130. Robinson DR. Lipid mediators of inflammation. *Rheum Dis Clin North Am* 1987;**13**:385–405.
131. Rocha DM, Santeusanio F, Faloona GR, et al. Abnormal pancreatic alpha-cell function in bacterial infections. *N Engl J Med* 1973;**288**:700–3.
132. Roth EF. Malarial parasite hexokinase and hexokinase-dependent glutathione reduction in the *Plasmodium falciparum*-infected human erythrocyte. *J Biol Chem* 1987;**262**:15678–82.
133. Roth E, Funcovics J, Muhlbacher F, et al. Metabolic disorders in severe abdominal sepsis: glutamine deficiency in skeletal muscle. *Clin Nutr* 1982;**1**:25–42.
134. Roubenoff R, Roubenoff RA, Cannon JG, et al. Rheumatoid cachexia: Cytokine-driven hypermetabolism accompanying reduced body cell mass in chronic inflammation. *J Clin Invest* 1994;**93**:2379–86.
135. Sack D, Rohads M, Molla A, et al. Carbohydrate malabsorption in infants with rotavirus diarrhea. *Am J Clin Nutr* 1982;**36**: 1112–18.
136. Sasazuki T, McDevitt HO, Grumet FC. The association between genes in the major histocompatibility complex and disease susceptibility. *J Clin Nutr* 1977;**28**:425–52.
137. Schaible UE, Kaufmann SH. Iron and microbial infection. *Nat Rev* 2004;**2**:946–53.
138. Scrimshaw NS, Gordon JE. *Interaction of nutrition and infection*. Geneva: World Health Organization; 1968.
139. Semba RD. Vitamin A, immunity, and infection. *Clin Infect Dis* 1994;**19**:489–99.
140. Shambaugh III GE, Beisel WR. Early alterations in thyroid hormone physiology during acute infections in man. *J Clin Endocrinol Metab* 1967;**27**:1667–73.
141. Shambaugh III GE, Beisel WR. Insulin response during tularemia in man. *Diabetes* 1967;**16**:369–76.
142. Shaw AR. Molecular biology of cytokines: An introduction. In: Thompson AW, editors. *The cytokine handbook*. New York: Academic Press; 1991. p. 19–42.
143. Shimoya K, Matsuzaki N, Taniguchi T, et al. Interleukin-8 in cord sera: A sensitive and specific marker for the detection of preterm chorioamnionitis. *J Infect Dis* 1992;**165**:957–60.
144. Siegel JH, Cerra FB, Coleman B, et al. Physiological and metabolic correlations in human sepsis: Invited commentary. *Surgery* 1979;**86**:163–93.
145. Smith RE, Horwitz BA. Brown fat and thermogenesis. *Physiol Rev* 1969;**49**:330–425.
146. Sobocinski PZ, Canterbury Jr WJ, Mapes CA, Dinterman RE. Involvement of hepatic metallothioneins in hypozincemia associated with bacterial infection. *Am J Physiol* 1978;**234**:E399–406.
147. Sommer A. Vitamin A, infectious disease, and childhood mortality: A 2c solution? *J Infect Dis* 1993;**167**:1003–7.
148. Souba WW, Klimberg VS, Plumley DA. The role of glutamine in maintaining a healthy gut and supporting the metabolic response to injury and infection. *J Surg Res* 1990;**48**:383–91.
149. Stamler JS, Singel DJ, Loscalzo J. Biochemistry of nitric oxide and its redox-activated forms. *Science* 1992;**258**:1891–902.
150. Reference deleted in proofs.
151. Stephensen CB. Burden of infection on growth failure. *J Nutr* 1999;**129**:534S–8S.
152. Stephensen CB, Alvarez JO, Kohatsu J, et al. Vitamin A is excreted in the urine during acute infection. *Am J Clin Nutr* 1994;**60**: 388–92.
153. Stephensen CB, Marquis GS, Jacob RA, et al. Vitamins C and E in adolescents and young adults with HIV infection. *Am J Clin Nutr* 2006;**83**:870–79.
154. Stuehr DJ, Nathan CF. Nitric oxide A macrophage product responsible for cytostasis and respiratory inhibition of tumor target cells. *J Exp Med* 1989;**169**:1543–55.

155. te Vede M, Huijbens RJF, Heije K, et al. Interleukin-4 (IL-4) inhibits secretion of IL-1 beta, tumor necrosis factor alpha, and IL-6 by human monocytes. *Blood* 1990;**76**:1392–7.
156. Uzzan B, Cohen R, Nicolas P, et al. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: A systematic review and meta-analysis. *Crit Care Med* 2006;**34**: 1996–2003.
157. Valyi-Nagy T, Dermody TS. Role of oxidative damage in the pathogenesis of viral infections of the nervous system. *Histol Histopathol* 2005;**20**:957–67.
158. Van der Graaf CA, Netea MG, Morre SA, et al. Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for *Candida* bloodstream infection. *Eur Cytokine Network* 2006;**17**: 29–34.
159. van der Pool T, van Deventer SJH, ten Cate H, et al. Tumor necrosis factor is involved in the appearance of interleukin-1 receptor antagonist in endotoxemia. *J Infect Dis* 1994;**169**:665–7.
160. van Deuren M, van der Ven-Jongerkrijg J, Demacker PMN. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. *J Infect Dis* 1993;**169**:157–61.
161. van Waardenburg DA, Deutz NE, Hoos MB, et al. Assessment of whole body protein metabolism in critically ill children: Can we use the [15N]glycine single oral dose method? *Clin Nutr* 2004;**23**:153–60.
162. Verboon-Maciolek MA, Thijsen SF, Hemels MA, et al. Inflammatory mediators for the diagnosis and treatment of sepsis in early infancy. *Pediatr Res* 2006;**59**:457–61.
163. Vermont CL, den Brinker M, Kakeci N, et al. Serum lipids and disease severity in children with severe meningococcal sepsis. *Crit Care Med* 2005;**33**:1610–15.
164. Victor VM, Rocha M, Esplugues JV, De la Fuente M. Role of free radicals in sepsis: Antioxidant therapy. *Curr Pharmaceut Design* 2005;**11**:3141–58.
165. Villamor E. A potential role for vitamin D on HIV infection? *Nutr Rev* 2006;**64**:226–33.
166. Vitale JJ. The impact of infection on vitamin metabolism: An unexplored area. *Am J Clin Nutr* 1977;**30**:1473–77.
167. Viteri FE, Behar M. Efectos de diversas infecctiones sobre la nutricion del prescholar especialmente el sarampi. *Bole Ofic Sani Panam* 1975;**78**:226–40.
168. Wagner DA, Tannenbaum SR. Enhancement of nitrate biosynthesis by *Escherichia coli* lipopolysaccharides. In: McGee PN, editor. *Nitrosamines and human cancer*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratories; 1982. p. 437–41.
169. Wannemacher Jr RW. Key role of individual amino acids in host response to infection. *Am J Clin Nutr* 1977;**30**:1269–80.
170. Wannemacher Jr RW, Dinterman RE, Pekarek RS, et al. Urinary amino acid excretion during experimentally induced sandfly fever in man. *Am J Clin Nutr* 1975;**28**:110–8.
171. Wannemacher Jr RW, Klainer AS, Dinterman RE, et al. The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection. *Am J Clin Nutr* 1976;**29**: 997–1006.
172. Wannemacher Jr RW, Powanda MC, Dinterman RE. Amino acid flux and protein synthesis after exposure of rats to either *Diplococcus pneumoniae* or *Salmonella typhimurium*. *Infect Immun* 1974;**10**:60–5.
173. Wartofsky L, Burman KD, Dimond RC, et al. Studies on the nature of thyroidal suppression during acute falciparum malaria: Integrity of pituitary response to TRH and alterations in serum T_3 and reverse T_3. *J Clin Endocrinol Metab* 1977;**14**:85–90.
174. Watten RH, Morgan FM, Songkhla YN, et al. Water and electrolyte studies in cholera. *J Clin Invest* 1959;**38**:1879–89.
175. Weigent DA, Baxter JB, Wear WE, et al. Production of immunoreactive growth hormone by mononuclear leukocytes. *FASEB J* 1988;**2**:2812–18.
176. Weinberg ED. Iron and susceptibility to infectious disease. *Science* 1974;**184**:952–6.
177. West HD, Jackson AH, Elliott RR, et al. The utilization of ingested iron in disease. *South Med J* 1952;**45**:629–33.
178. Westerdorp RGJ, Langermans JAM, de Bel CE, et al. Release of tumor necrosis factor: An innate host characteristic that may contribute to the outcome of meningococcal disease. *J Infect Dis* 1995;**171**:1057–60.
179. Whedon GD, Shorr E. Metabolic studies in paralytic acute anterior poliomyelitis. II. Alterations in calcium and phosphorus metabolism. *J Clin Invest* 1957;**36**:966–81.
180. White MG, Carter NW, Rector FC, et al. Pathophysiology of epidemic St. Louis encephalitis. *Ann Intern Med* 1969;**71**: 691–702.
181. Wiles JB, Cerra FB, Siegel JH, Border JR. The systemic septic response: Does the organism matter? *Crit Care Med* 1980;**8**: 55–60.
182. Wilmore DW, Long JM, Mason Jr AD, et al. Catecholamines: Mediator of the hypermetabolic response to thermal injury. *Ann Surg* 1974;**180**:653–69.
183. Wilson D, Bressani R, Scrimshaw NS. Infection and nutritional status. I. The effect of chicken pox on nitrogen metabolism in children. *Am J Clin Nutr* 1961;**9**:154–8.
184. Woeber KA. Alterations in thyroid hormone economy during acute infection with *Diplococcus pneumoniae* in the rhesus monkey. *J Clin Invest* 1971;**50**:378–87.
185. Yarasheski KE, Smith SR, Powderly WG. Reducing plasma HIV RNA improves muscle amino acid metabolism. *Am J Physiol* 2005;**288**:E278–84.
186. Yelish CY, Filkins JP. Mechanism of hyperinsulinism in endotoxicosis. *Am J Physiol* 1980;**239**:E156–61.
187. Yeung CY. Hypoglycemia in neonatal sepsis. *J Pediatr* 1970; **77**:812–7.
188. Yildizdas D, Onenli-Mungan N, Yapicioglu H, et al. Thyroid hormone levels and their relationship to survival in children with bacterial sepsis and septic shock. *J Pediatr Endocrinol Metab* 2004;**17**:1435–42.
189. Young VR, Alexis SD, Maliga BS, Yapicioglu H, et al. Metabolism of administered 3-methylhistidine: Lack of muscle transfer ribonucleic acid charging and quantitative excretion as 3-methyhistidine and its *N*-acetyl derivative. *J Biol Chem* 1972;**247**:3592–600.

the value reported by Wunderlich is how closely it approximates these more recent determinations.

As noted previously, body temperature fluctuates depending on numerous normal physiologic factors. Core temperature shows a diurnal variation of 1°C, the nadir occurring in the early morning hours and the peak in the late afternoon.[37,47] After exercise and in the postprandial state, body temperature increases.[52] In addition, variations in the normal body temperature of women associated with the menstrual cycle are well described, with increases in baseline temperature occurring after ovulation.[15]

THERMOREGULATION

Humans, similar to other mammals, are homeothermic, indicating that they regulate body temperature within a narrow range despite wide variations in the ambient temperature. Regulation of temperature is mediated by a variety of physiologic (e.g., vasoconstriction, sweating) and behavioral (e.g., moving to a warmer environment, putting on additional clothing) responses.

The principal thermoregulatory area is located within the brain in the preoptic area and anterior hypothalamus. Although it frequently is conceptualized as a single center, no single neuronal structure seems to control all aspects of temperature regulation.[10] Rather, a complex interplay occurs among a variety of neural pathways, with the final result being the maintenance of the body's temperature within a narrow range. Regardless of the precise nature of central regulation, body temperature ultimately is a function of the balance between heat gain and heat loss.

Heat energy is a by-product of the inefficiency of the body's normal metabolic processes. It is this "waste" heat that renders the homeothermic state possible. During exercise, the increased metabolic activity in muscle tissue results in increased production of heat, leading to an increase in the body's temperature.[66] Shivering, an involuntary form of muscle activity, is the primary means by which the body generates additional heat under conditions of cold stress. Heat also may be generated by a process known as *nonshivering thermogenesis*. Originally described in rats, nonshivering thermogenesis has been found to occur in a variety of mammals, including humans. It seems to be of greater importance in neonates than in adults. Although this process has been shown to occur in a variety of tissues, brown adipose tissue seems to be the most important site for this phenomenon. Under the control of the adrenergic system, production of free fatty acid is increased, resulting in an uncoupling of oxidative phosphorylation and the production of large amounts of heat.[9,28]

Four mechanisms are responsible for heat transfer: radiation, conduction, convection, and evaporation. Heat loss owing to radiation occurs when heat is transferred directly between two objects not in direct contact. Conduction involves the transfer of heat energy between two objects in contact with each other. Convection is the result of the movement of a fluid or gas across the surface of the body (e.g., as the result of fanning). Evaporative heat loss occurs in association with the energy required to convert liquid to gas form.

Under normal conditions, radiation accounts for most of the body's heat loss. By contrast, conductive losses are smaller under normal circumstances. Conductive losses may become substantial, however, under conditions in which a substantial portion of the individual's body surface is in direct contact with a cooler object (e.g., an unclothed infant in an unheated bassinet). Convective losses are proportional to the amount of air moving over the body surface; these losses are greatest in windy conditions. Conductive and convective heat losses are particularly important in infants and children because of their relatively greater body surface area compared with that of adults. Evaporative losses occur when fluids such as sweat evaporate from the skin's surface. In addition, substantial evaporative losses are associated with respiration.

PATHOGENESIS OF FEVER

In the classic model of fever pathogenesis, exogenous pyrogens stimulate the release of circulating endogenous pyrogens, which act via prostaglandins to increase the set point of the hypothalamic thermoregulatory center.[17] In this model, exogenous pyrogens are substances extrinsic to the body, primarily various bacterial microorganisms or the products of those microorganisms. Conversely, endogenous pyrogens are a varied group of proteins produced within the human body that share the intrinsic ability to induce fever. The first endogenous pyrogen was described originally more than 60 years ago and was derived from leukocytes, primarily granulocytes, and now is known as interleukin-1.[18] Numerous other cytokines that qualify as endogenous pyrogens, including tumor necrosis factor, interferon-α, interferon-γ, and interleukin-6, have been identified.[45,88]

A variety of alternative and complementary theories of fever pathogenesis have been proposed.[53] The actual mechanisms involved likely are more varied and complex than just described. A variety of intrinsically produced substances (e.g., antigen-antibody complexes) may act as "exogenous" pyrogens.[64] The classic model provides a reasonable framework, however, for understanding most of the observed phenomena associated with the febrile response.

Regardless of the precise pathogenesis, the height of fever seems to be limited. Retrospective studies of hyperpyrexia in children have found that it is unusual for the body temperature to rise above 41.1°C (106°F), and it rarely rises above 41.7°C (107°F).[58,80] Children with temperatures exceeding this range almost always have an element of heat illness.

EFFECTS OF FEVER

Attempts to treat fever often are predicated on the assumption that fever has harmful effects and that reduction in temperature would abrogate such harm. The evidence with regard to these premises is mixed, however.

Adverse Effects

Some animal studies have found that high fever may impair certain immunologic responses, including phagocytosis of staphylococci by polymorphonuclear leukocytes[5,21,23] and lymphocyte transformation in response to mitogens.[63] Whether these isolated in vitro phenomena observed in animal models are relevant to human infection is unknown.

Fever may cause seizures, a phenomenon observed most frequently in young children. The onset generally occurs in infants 6 to 30 months of age. Recurrence is common, occurring in approximately one third of children experiencing an initial febrile seizure. The primary adverse consequences of febrile seizures are the emotional distress experienced by patients and their families and the need for medical evaluation, which may involve invasive testing and substantial expense. Febrile seizures do not cause brain injury and are not associated with subsequent intellectual or neurologic deficits.[20,82] Furthermore, numerous studies have failed to demonstrate that use of antipyretics is effective in preventing febrile seizures.[68,76]

Beneficial Effects

Fever may be beneficial—by enhancing the host response to infection and by directly inhibiting the infecting agent. Several studies have demonstrated that the immune system responds to mildly elevated body temperature by increasing migration of leukocytes, production of interferon, and lymphocyte transformation and phagocytosis.[2,7,63,65] Studies of bacterial infection in reptiles and fish have shown an increased survival rate in groups maintained at approximately 4°C (reptiles) and 2.5°C (fish) above baseline.[14,38] Kluger and Vaughn[40] showed that rabbits infected with *Pasteurella multocida* had improved survival rates at body temperatures of approximately 4.5°C above normal. Although these data are impressive, their clinical significance with regard to humans has yet to be determined.

Fever also may inhibit the growth and survival of some infectious agents. One potential mechanism for this inhibition is the decrease in serum iron and increase in ferritin that are associated with fever, coupled with the increased iron requirement of many bacteria at higher temperatures.[5,39] Fever therapy was used historically to treat neurosyphilis and gonococcal urethritis, correlating with more recent studies that show that certain gonococci and *Treponema* are eradicated at temperatures of 40°C (104°F) and greater.[12,36] Finally, growth of some pneumococci and viruses seems to be impaired at higher temperatures.[27,74,84]

Several studies have found that the treatment of fever with antipyretics is associated with adverse consequences, providing indirect evidence of a beneficial effect of fever. Ahmady and Samadi[2] reported that the use of aspirin in children with measles prolonged the duration of the illness and was associated with an increase in prevalence of respiratory complications and diarrhea. Other investigators have reported that the length of time to total scabbing in varicella was significantly longer in children treated with acetaminophen compared with children treated with placebo.[19] Several studies in animal models and in humans have found prolongation of viral shedding, depressed neutralizing antibody response, and increased nasal symptoms in association with the use of antipyretics.[25,32,79] Although these studies establish that an association exists between reduction of fever and adverse outcomes, they do not prove a causal relationship. The adverse effects possibly are mediated by some direct physiologic effects of antipyretics, rather than indirectly by their impact on fever.

CLINICAL THERMOMETRY

Types of Thermometers

For many years, glass thermometers containing mercury were the most common type of thermometer used to measure body temperature. This type of thermometer is reasonably accurate for most clinical purposes. Although they are still available, use of mercury-containing thermometers has diminished greatly because of environmental concerns about mercury exposure from broken or discarded thermometers. These thermometers have been replaced largely by digital thermometers or glass thermometers containing liquids other than mercury.

Electronic thermometers (often referred to as *digital thermometers*) previously were used primarily in the hospital and office setting. As their cost has decreased, they are used more frequently in the home as well. Electronic thermometers have the advantage over mercury thermometers of requiring a significantly shorter dwell time, that is, the time they must remain in situ to obtain an accurate reading. Hospital-grade electronic thermometers typically have two modes: monitor and predictive. In the monitor mode, these thermometers function similarly to mercury thermometers in that they must remain in place until equilibration occurs, a process that may require several minutes. In the predictive mode, a complex algorithm is used to estimate the final temperature based on measurements made during the first few seconds. Because the predictive mode produces a temperature reading within seconds, it is the mode used most often in clinical settings. Determinations of temperatures using these two modes have been found to correlate well.[23,54]

Infrared thermometers are a more recent addition to the clinician's armamentarium. Devices that determine the temperature by detecting infrared radiation emitted from the ear drum are used most frequently. Tympanic temperature should provide an accurate estimation of the core temperature because its blood supply is derived from the carotid artery. Additional advantages of this type of thermometer are its speed, acceptance by patients, and decreased risk of cross contamination compared with oral or rectal thermometers. Studies of the accuracy of tympanic thermometers have yielded mixed results, with numerous studies finding tympanic thermometers inaccurate compared with mercury in glass or electronic thermometers.[13,50] Discrepancies seem to be particularly common in infants who are in the first few months of

life.[71] Tympanic thermometers should not be used in young infants because of the importance of fever in making management decisions in these patients.[16]

Even more recently, the temporal artery thermometer has been introduced. These thermometers use an infrared sensor to determine skin temperature as the device is passed across the forehead and temporal area. The site of highest measured temperature is assumed to represent that of the temporal artery. An algorithm is applied to the measured temperature to estimate the core temperature. Studies to date suggest that temporal artery thermometer temperatures correlate significantly better with rectal and core temperatures than with temperatures determined by tympanic thermometers. The initial data also suggest that these thermometers are more sensitive at detecting fever in children than in adults. Temporal artery thermometers do not seem to correlate well enough with rectal or core temperature measurements, however, to replace rectal thermometry in clinical situations in which accurate measurement of fever is crucial for making decisions about management. The accuracy of temporal artery thermometer readings is adversely affected by sweating and may be affected by vascular constriction or dilation. In addition, data comparing temporal artery and axillary thermometry are lacking.[26,77]

Several other types of thermometers have been developed. Among them are electronic pacifier thermometers, which are used for obtaining an oral temperature in infants. Although they are appealing in theory, this type of thermometer has the disadvantage of requiring a prolonged dwell time and has not been found to be sufficiently accurate to recommend its use.[59] Another approach to measuring temperature is the use of liquid crystal thermometers that are applied to the skin of the forehead. Results generally have been disappointing when these thermometers have been compared with more standard techniques.[41,69]

Measurement Site

The most common locations for measuring body temperature are the mouth, rectum, axilla, and tympanic membrane. Because of the previously noted regional variations in body temperature, each of these sites has its own range of normal temperatures. The oral cavity historically has been the preferred site for measuring temperature in older children and adults. When taking a temperature orally, one should place the thermometer in the sublingual space because the blood supply for structures in this region is derived from branches of the carotid arteries and should reflect the core temperature accurately. Younger children usually are unable to cooperate adequately to permit the use of oral thermometers. In addition, the oral temperature may be affected by recent ingestion of hot or cold liquids, and it may be altered by tachypnea.

Rectal temperatures are used frequently in younger children. The rectal temperature correlates well with the core body temperature. The rectal temperature may exceed the core temperature (using the pulmonary artery temperature as the reference standard), however, possibly because of the effects of bacterial activity in the rectum. The use of rectal thermometry has the disadvantage of causing the patient discomfort and is contraindicated in patients with neutropenia because of the risks of causing invasive infection via trauma to the rectal mucosa.

Axillary temperatures are appealing because of the ready accessibility of the axillae. Considerable variability occurs in the readings obtained, however, particularly in younger children. One should not rely on axillary temperatures, particularly in neonates and young children.

TREATMENT

As noted previously, fever may have numerous beneficial effects, convincing evidence of harm owing to fever is lacking, and treatment of fever may be associated with undesirable effects. Routine intervention to reduce fever is not warranted. Rather, physicians should individualize the decision to treat fever and the specific method chosen to do so. This approach is in accordance with the recommendations of the American Academy of Pediatrics.[78]

Indications

Antipyretic therapy often is considered for children who have an increased risk of having febrile seizures, either because of age or because of a history of febrile seizures. Although treatment seems rational in this situation, studies of antipyretic therapy to prevent febrile seizures have failed to show its efficacy.[11,68,76,81] Even this indication should be considered relative rather than absolute.

Antipyretic therapy also should be considered for children with poorly compensated underlying cardiac or pulmonary disease, significant neurologic impairment, or sepsis and for children with significant alterations of fluid and electrolyte balance. Definitive controlled trials to support these indications are lacking. Rather, the recommendations are based on the metabolic consequences of fever and their potential adverse impact on the underlying disease.

Perhaps the most frequent indication for the use of antipyretics is to improve the patient's comfort. Despite the absence of definitive studies to support this practice and the potential adverse consequences of using antipyresis, such an approach is reasonable in the absence of definitive evidence to the contrary. In some circumstances, improving the patient's comfort may enhance the ability to assess the seriousness of the patient's illness accurately.[6]

Antipyretics

A wide variety of antipyretic agents is available. In the United States, the drugs used most frequently for treatment of fever in children are acetaminophen and ibuprofen. Previously, aspirin was the antipyretic used most frequently. Aspirin has fallen into disuse for the management of fever in children, however, primarily because of its association with Reye syndrome, particularly when used in managing children with varicella or influenza.[31] In addition, aspirin has a variety of other adverse effects, including inhibition of platelet function, gastritis and

gastrointestinal bleeding, and provocation of asthma exacerbations, although this third complication occurs more frequently in adults.[33,62,70] Aspirin has greater toxicity in situations of overdose than do acetaminophen and ibuprofen.

Each of these agents acts to restore normal body temperature by reducing the set point of the temperature regulatory center in the hypothalamus. The specific mechanism of action seems to be interference with prostaglandin synthesis in the preoptic anterior hypothalamus (PAOH). When selecting among the available antipyretic agents, one should consider efficacy and potential toxicity.

Similar to aspirin, ibuprofen inhibits prostaglandin synthesis in a variety of tissues outside the central nervous system. It shares many of the toxicities associated with aspirin. One exception is that ibuprofen lacks the association with Reye syndrome.[60] Ibuprofen inhibits platelet function because of its effect on prostaglandin synthesis, but this effect is reversible with discontinuation of the drug, and platelet dysfunction is short lived compared with the effect of aspirin.[55] Because prostaglandins are important to the integrity of the gastrointestinal mucosa, inhibition by ibuprofen may result in gastrointestinal upset and bleeding. Although ibuprofen has been associated with exacerbations of asthma in some children, the risk seems to be small and may not be greater than that associated with the use of acetaminophen.[42,43]

Acetaminophen has a lengthy track record of safety. When used in the usual therapeutic doses, it has few adverse effects. Acetaminophen inhibits prostaglandin synthase activity, but this action is inhibited by peroxide. Because peroxide is generated at sites of inflammation, acetaminophen has little anti-inflammatory activity. It also lacks the adverse gastrointestinal and platelet effects of aspirin and ibuprofen. A growing body of evidence suggests that acetaminophen use is associated with an increased risk of subsequent asthma.[22] Despite the fact that studies have consistently found this association, evidence of a causal relationship has yet to be conclusively established. Recommended dosing of ibuprofen is 5 to 10 mg/kg every 6 hours as needed. Acetaminophen is administered at a dose of 10 to 15 mg/kg every 4 hours, but no more frequently than five times per day. An important note for individuals administering these agents is that a variety of over-the-counter combination medications contain one or the other of these agents. Coadministration may result in inadvertent overdosing.

In addition, recognition that ibuprofen and acetaminophen come in a variety of formulations is important.[73] Acetaminophen, once available as infant drops (concentration 10 mg/mL) and children's liquid (concentration 32 mg/mL) is now only available in a single concentration (32 mg/mL). The voluntary removal from the market of the more concentrated formulation was prompted by reports of inadvertent overdose from substitution of infant drops for the children's liquid without adjusting the volume administered to reflect the difference in concentration. Acetaminophen also is available in suppository form. Absorption varies, however, and is delayed compared with oral administration. In addition, the medication is not distributed uniformly throughout the suppository, resulting in potential dosing errors if the suppositories are divided before use.[8] The use of acetaminophen in suppository form should be discouraged. Ibuprofen continues to be available in two concentrations: a children's liquid (20 mg/mL) and infant drops (40 mg/mL). The dual concentrations create the potential for incorrect dosing seen previously with acetaminophen.

The antipyretic efficacy of acetaminophen and ibuprofen has been the subject of numerous clinical trials.[34,83,85,86] These trials have shown uniformly that both are effective antipyretic agents. Ibuprofen seems to result in a greater decrease in temperature than does acetaminophen, however. In addition, the antipyretic effect of ibuprofen is more prolonged, not surprising in light of ibuprofen's longer half-life compared with acetaminophen. These observations were confirmed in a meta-analysis of trials comparing ibuprofen and acetaminophen.[57]

Acetaminophen and ibuprofen frequently are used in combination.[44] Use of alternating doses often is advocated by practicing physicians.[49] The pathways for metabolism of these drugs are distinct, and, theoretically, metabolism of one should not affect the metabolism of the other. Controlled trials documenting safety and efficacy of combination or alternating use are sparse, however. In one study, using alternate doses of acetaminophen (12.5 mg/kg per dose) and ibuprofen (5 mg/kg per dose) every 4 hours was found to be associated with a more rapid reduction in fever, lower mean temperature, and fewer caregiver days absent from work and infant days absent from daycare compared with use of either agent alone.[67] However, the groups assigned to a single agent received dosing that was either on the low end or infrequent, compared with usual practice in the United States. In a more recent study utilizing full therapeutic doses and comparing ibuprofen alone with alternating and combined schedules of ibuprofen and acetaminophen, the alternating and combined schedules were found to provide greater antipyresis.[56] A 2011 systematic review of combined- versus single-agent treatment with acetaminophen and ibuprofen concluded that the antipyretic effect of combined therapy was statistically superior but the clinical relevance was marginal at best. Furthermore, the studies found no evidence of increased toxicity with combined treatment but were not designed to definitively address the issue.[61]

Another approach to antipyretic treatment frequently employed is the use of a second antipyretic when the initial agent is judged to have resulted in an inadequate response. Theoretically, some individuals may have a better antipyretic response to one agent than another. Although the premise is reasonable, sequential use of acetaminophen and ibuprofen remains unproven in terms of efficacy and safety.

Acetaminophen and ibuprofen have been proven to be remarkably safe when used in the recommended doses. Although ibuprofen may be slightly more efficacious in producing and sustaining fever reduction, acetaminophen remains the antipyretic of choice because of its longer track record and more favorable side effects profile. Because acetaminophen lacks significant anti-inflammatory activity, ibuprofen or another nonsteroidal

anti-inflammatory drug may be preferred in febrile conditions for which anti-inflammatory activity is desired (e.g., juvenile arthritis). Limited data suggest that use of acetaminophen and ibuprofen in combination may be safe and more efficacious than is either agent alone. Prudence suggests, however, that combined therapy seldom is warranted for this generally benign condition. Perhaps most important, patients and their parents should be educated to the benign nature of fever and the lack of evidence to indicate that routine treatment, particularly complete suppression, is either necessary or beneficial.

External Cooling

The use of external cooling in the management of fever has a long history. Compared with standard antipyretics, sponging with tepid water is inferior in fever reduction at 2 to 3 hours, although sponging was found to reduce the temperature more quickly than did antipyretics in one trial.[1,3] External cooling without concomitant administration of antipyretics makes little sense from a physiologic standpoint, however. In a febrile patient whose temperature regulatory center set point has not been reset by administration of an antipyretic agent, external cooling inevitably results in an increase in the body's heat-production mechanisms.

When used in conjunction with an antipyretic agent, the usual goal of external cooling is to reduce the body's temperature more rapidly or to a greater degree. Several studies have compared the use of external cooling combined with antipyretics with antipyretics alone. Results have been mixed; some studies showed no difference in efficacy of the two approaches, whereas others found combination therapy to be superior.[24,30,48,72,75] Even in the studies in which a difference was found, the superiority of combination therapy was shown primarily in the very early phase of treatment. In addition, the use of external cooling usually is uncomfortable for the patient. When external cooling is to be used, sponging with tepid water is preferred. Alcohol and solutions containing alcohol should not be used for this purpose. Absorption of alcohol vapors via the lungs may occur in sufficient quantities to produce toxicity and even death.[51]

NEW REFERENCES SINCE THE SIXTH EDITION

22. Etminan M, Mohsen S, Jafair S, et al. Acetaminophen use and the risk of asthma in children and adults: a systematic review and meta-analysis. *Chest* 2009;**136**:1316–23.
56. Paul IM, Sturgis SA, Yang C, et al. Efficacy of standard doses of ibuprofen alone, alternating, and combined with acetaminophen for the treatment of febrile children. *Clin Ther* 2010;**32**:2433–40.
61. Purssell E. Systematic review of studies comparing combined treatment with paracetamol and ibuprofen, with either drug alone. *Arch Dis Child* 2011;**96**:1175–9.
76. Strengell T, Uhari M, Tarkka R, et al. Antipyretic agents for preventing recurrences of febrile seizures. *Arch Pediatr Adolesc Med* 2009;**163**:799–804.
78. Sullivan JE, Farrar HC, and the Section on Clinical Pharmacology and Therapeutics, and Committee on Drugs. Clinical report—fever and antipyretic use in children. *Pediatrics* 2011;**127**:580–7.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 5

The Human Microbiome

Douglas S. Swanson

The terms *indigenous flora* and *normal flora* describe the microorganisms that colonize the internal and external surfaces of healthy individuals.[131] These microorganisms (also known as the *microbiota*) are estimated to outnumber human cells by 10-fold.[196] Molecular methods using DNA technologies that are culture-independent have expanded our understanding of the human indigenous flora, such that the combination of microbial genomes colonizing the human body is referred to as the *human microbiome*. Colonization is the presence of replicating microorganisms on or within a host, without evidence of the development of disease. The microbial agents that compose the normal flora are called *commensal*, *colonizing*, or *endogenous* organisms. They usually do not cause injury to the host and can be divided into categories of resident or transient flora.[31,184] Resident flora are organisms present routinely in a specified anatomic location. *Staphylococcus epidermidis* is a resident organism of the skin, and *Escherichia coli* is a resident organism of the gastrointestinal tract. Transient flora are organisms present only temporarily in a certain anatomic location, such as *Staphylococcus aureus* on the skin or *Pseudomonas aeruginosa* in the gastrointestinal tract. Blood, cerebrospinal fluid, urine, bile, and synovial fluid are considered sterile fluids and have no normal flora.

REFERENCES

1. Agbolosu NB, Cuevas LE, Milligan P, et al. Efficacy of tepid sponging versus paracetamol in reducing temperature in febrile children. *Ann Trop Paediatr* 1997;**17**:283–8.
2. Ahmady AS, Samadi AR. The adverse effects of antipyretics in measles. *Indian Pediatr* 1981;**18**:49–52.
3. Aksoylar S, Aksit S, Caglayan S, et al. Evaluation of sponging and anti-pyretic medication to reduce body temperature in febrile children. *Acta Paediatr Jpn* 1997;**39**:215–7.
4. Anagnostakis D, Matsaniotis N, Grafakos S, et al. Rectal-axillary temperature difference in febrile and afebrile infants and children. *Clin Pediatr* 1993;**32**:268–72.
5. Austin TW, Truant G. Hyperthermia, antipyretics and function of polymorphonuclear leukocytes. *Can Med Assoc J* 1978;**118**:493–5.
6. Baker RC, Tiller T, Bellet PS, et al. Severity of disease correlated with fever reduction in febrile infants. *Pediatrics* 1989;**83**:1016–9.
7. Bernheim HA, Bodel PT, Askenase PW, et al. Effects of fever on host defense mechanisms after infection in the lizard, *Dipsosaurus dorsalis*. *Br J Exp Pathol* 1978;**59**:76–84.
8. Birmingham PK, Tobin MJ, Henthorn TK, et al. Twenty-four-hour pharmacokinetics of rectal acetaminophen in children: an old drug with new recommendations. *Anesthesiology* 1997;**87**:244–52.
9. Boulant JA. Thermoregulation. In: Mackowiak PA, editor. *Fever—basic mechanisms and management*. 2nd ed. Philadelphia: Lippincott-Raven; 1997. p. 38–58.
10. Boulant JA. Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin Infect Dis* 2000;**31**:S157–61.
11. Camfield PR, Camfield CS, Shapiro SH, et al. The first febrile seizure: antipyretic instruction plus either phenobarbital or placebo to prevent recurrence. *J Pediatr* 1980;**97**:16–21.
12. Carmichael LE, Barnes FD, Percy DH. Temperature as a factor in resistance of young puppies to canine herpesvirus. *J Infect Dis* 1969;**120**:669–78.
13. Chamberlain JM, Grandner J, Rubinoff JL, et al. Comparison of a tympanic thermometer to rectal and oral thermometers in a pediatric emergency department. *Clin Pediatr* 1991;**30**:24–9.
14. Covert JB, Reynolds WW. Survival value of fever in fish. *Nature* 1977;**267**:43–5.
15. Coyne MD, Kesick CM, Doherty TJ. Circadian rhythm in core temperature over the menstrual cycle: method for noninvasive monitoring. *Am J Physiol Regul Integr Comp Physiol* 2000;**279**:1316–20.
16. Craig JV, Lancaster GA, Taylor S, et al. Infrared ear thermometry compared with rectal thermometry in children: a systematic review. *Lancet* 2002;**360**:603–9.
17. Dinarello CA. Thermoregulation and the pathogenesis of fever. *Infect Dis Clin North Am* 1996;**10**:433–49.
18. Dinarello CA. Cytokines as endogenous pyrogens. In: Mackowiak PA, editor. *Fever—basic mechanisms and management*. 2nd ed. Philadelphia: Lippincott-Raven; 1997. p. 87–116.
19. Doran TF, De Angelis C, Baumgardner RA, et al. Acetaminophen: more harm than good for chickenpox? *J Pediatr* 1989;**114**:1045–8.
20. Ellenberg JH, Nelson KB. Febrile seizures and later intellectual performance. *Arch Neurol* 1978;**35**:17–21.
21. Ellingson HV, Clark PF. The influence of artificial fever on mechanisms of resistance. *J Immunol* 1942;**43**:65–83.
22. Etminan M, Mohsen S, Jafair S, et al. Acetaminophen use and the risk of asthma in children and adults: a systematic review and meta-analysis. *Chest* 2009;**136**:1316–23.
23. Fallis WM, Christiani P. Neonatal axillary temperature measurements: a comparison of electronic thermometer predictive and monitor modes. *Obstet Gynecol Neonatal Nurs* 1999;**28**:389–94.
24. Friedman AD, Barton LL, and the Sponging Study Group: Efficacy of sponging vs. acetaminophen for reduction of fever. *Pediatr Emerg Care* 1990;**6**:6–7.
25. Graham NM, Burrell CJ, Douglas RM, et al. Adverse effects of aspirin, acetaminophen, and ibuprofen on immune function, viral shedding, and clinical status in rhinovirus-infected volunteers. *J Infect Dis* 1990;**162**:1277–82.
26. Greenes DS, Fleisher GR. Accuracy of a noninvasive temporal artery thermometer for use in infants. *Arch Pediatr Adolesc Med* 2001;**155**:376–81.
27. Grieger TA, Kluger MJ. Fever and survival: the role of serum iron. *J Physiol* 1978;**279**:187–96.
28. Himms-Hagen J. Nonshivering thermogenesis. *Brain Res Bull* 1984;**12**:151–60.
29. Horvath SM, Menduke H, Perso GM. Oral and rectal temperatures of man. *JAMA* 1950;**144**:1562–5.
30. Hunter J. Study of antipyretic therapy in current use. *Arch Dis Child* 1973;**48**:313–5.
31. Hurwitz ES, Barrett MJ, Bregman D, et al. Public Health Service study of Reye's syndrome and medications: report of the main study. *JAMA* 1987;**257**:1905–11.
32. Husseini RH, Sweet C, Collie MH, Smith H. Elevation of nasal viral levels by suppression of fever in ferrets infected with influenza viruses of differing virulence. *J Infect Dis* 1982;**145**:520–4.
33. Jenkins C, Costello J, Hodge L. Systematic review of prevalence of aspirin induced asthma and its implications for clinical practice. *BMJ* 2004;**328**:434.
34. Kauffman RE, Sawyer LA, Scheinbaum ML. Antipyretic efficacy of ibuprofen vs acetaminophen. *Am J Dis Child* 1992;**146**:622–5.
35. Kelly G. Body temperature variability, part 1: A review of the history of body temperature and its variability due to site selection, biological rhythms, fitness, and aging. *Altern Med Rev* 2006;**11**:278–93.
36. Kendell HW. *Fever therapy*. Springfield, Ill. Charles C Thomas; 1935. p. 67–83.
37. Kleitman N, Titelbaum S, Hoffmann H. The establishment of the diurnal temperature cycle. *Am J Physiol* 1937;**119**:48–54.
38. Kluger MJ, Ringler DH, Anver MR. Fever and survival. *Science* 1975;**188**:166–8.
39. Kluger MJ, Rothenberg BA. Fever and reduced iron: their interaction as a host defense response to bacterial infection. *Science* 1979;**203**:374–6.
40. Kluger MJ, Vaughn LK. Fever and survival in rabbits infected with *Pasteurella multocida*. *J Physiol* 1978;**282**:243–51.
41. Kongpanichkul A, Bunjongpak S. A comparative study on accuracy of liquid crystal forehead, digital electronic axillary, infrared tympanic with glass-mercury rectal thermometer in infants and young children. *J Med Assoc Thai* 2000;**83**:1068–76.
42. Lesko SM, Mitchell AA. An assessment of the safety of pediatric ibuprofen: a practitioner-based randomized clinical trial. *JAMA* 1995;**273**:929–33.
43. Lesko SM, Mitchell AA. The safety of acetaminophen and ibuprofen among children younger than two years old. *Pediatrics* 1999;**104**:e39.
44. Li SF, Lacher B, Crain EF. Acetaminophen and ibuprofen dosing by parents. *Pediatr Emerg Care* 2000;**16**:394–7.
45. Luheshi F, Rothwell N. Cytokines and fever. *Int Arch Allergy Immunol* 1996;**109**:301–7.
46. Mackowiak PA, Wasserman SS. Physicians' perceptions regarding body temperature in health and disease. *South Med J* 1995;**88**:934–8.
47. Mackowiak PA, Wasserman SS, Levine MM. A critical appraisal of 37°C (98.6°F), the upper limit of the normal body temperature, and other legacies of Carl Reinhold August Wunderlich. *JAMA* 1992;**268**:1578–80.
48. Mahar AFT, Allen SJ, Milligan P, et al. Tepid sponging to reduce temperature in febrile children in a tropical climate. *Clin Pediatr* 1994;**33**:227–31.
49. Mayoral CE, Marino RV, Rosenfeld W, et al. Alternating antipyretics: is this an alternative? *J Pediatr* 2000;**105**:1009–12.
50. Modell JG, Katholi CR, Kumaramangalam SM, et al. Unreliability of the infrared tympanic thermometer in clinical practice: a comparative study with oral mercury and oral electronic thermometers. *South Med J* 1998;**91**:649–54.
51. Moss MH. Alcohol-induced hypoglycemia and coma caused by alcohol sponging. *Pediatrics* 1970;**46**:445–7.
52. Nagai N, Sakane N, Hamada T, et al. The effect of a high-carbohydrate meal on postprandial thermogenesis and sympathetic nervous system activity in boys with recent onset of obesity. *Metabolism* 2005;**54**:430–8.
53. Netea MG, Kullberg BJ, Van der Meer JW. Circulating cytokines as mediators of fever. *Clin Infect Dis* 2000;**31**:S178–84.
54. Nuckton TJ, Goldreich D, Wendt FC, et al. A comparison of 2 methods of measuring rectal temperatures with digital thermometers. *Am J Crit Care* 2001;**10**:146–50.
55. Parks WM, Hoak JC, Czervionke RL. Comparative effect of ibuprofen on endothelial and platelet prostaglandin synthesis. *J Pharmacol Exp Ther* 1981;**219**:415–9.

56. Paul IM, Sturgis SA, Yang C, et al. Efficacy of standard doses of ibuprofen alone, alternating, and combined with acetaminophen for the treatment of febrile children. *Clin Ther* 2010;**32**:2433–40.
57. Perrott DA, Piira T, Goodenough B, Champion D. Efficacy and safety of acetaminophen vs ibuprofen for treating children's pain or fever. *Arch Pediatr Adolesc Med* 2004;**158**:521–6.
58. Press S. Association of hyperpyrexia with serious disease in children. *Clin Pediatr* 1994;**33**:19–25.
59. Press S, Quinn BJ. The pacifier thermometer: comparison of supralingual with rectal temperatures in infants and young children. *Arch Pediatr Adolesc Med* 1997;**151**:551–4.
60. Prior MJ, Nelson EB, Temple AR. Pediatric ibuprofen use increases while incidence of Reye's syndrome continues to decline. *Clin Pediatr* 2000;**39**:245–7.
61. Purssell E. Systematic review of studies comparing combined treatment with paracetamol and ibuprofen, with either drug alone. *Arch Dis Child* 2011;**96**:1175–9.
62. Rachelefsky GS, Coulson A, Siegel SC, et al. Aspirin intolerance in chronic childhood asthma: detected by oral challenge. *Pediatrics* 1975;**56**:443–8.
63. Roberts NJ, Steigbigel RT. Hyperthermia and human leukocyte function: effects on response of lymphocytes to mitogens and antigen and bactericidal capacity of monocytes and neutrophils. *Infect Immunol* 1977;**18**:673–9.
64. Root RK, Wolff SM. Pathogenetic mechanisms in experimental immune fever. *J Exp Med* 1968;**128**:309–23.
65. Ruiz-Gomez J, Isaacs A. Interferon production by different viruses. *Virology* 1963;**19**:8–12.
66. Saltin B, Hermansen L. Esophageal, rectal, and muscle temperature during exercise. *J Appl Physiol* 1966;**21**:1757–62.
67. Sarrell ME, Wielunsky EW, Cohen HA. Antipyretic treatment in young children with fever: acetaminophen, ibuprofen, or both alternating in a randomized, double-blind study. *Arch Pediatr Adolesc Med* 2006;**160**:197–202.
68. Schnaiderman D, Lahat E, Sheefer T, et al. Antipyretic effectiveness of acetaminophen in febrile seizures: ongoing prophylaxis versus sporadic use. *Eur J Pediatr* 1993;**152**:747–9.
69. Scholefield JM, Gerber MA, Dwyer P. Liquid crystal forehead temperature strips: a clinical appraisal. *Am J Dis Child* 1982;**136**: 198–201.
70. Schuhl JF, Pereyra JG. Oral acetylsalicylic acid (aspirin) challenge in asthmatic children. *Clin Allergy* 1979;**9**:83–8.
71. Selfridge J, Shea SS. The accuracy of the tympanic membrane thermometer in detecting fever in infants aged 3 months and younger in the emergency department setting. *J Emerg Nurs* 1993;**19**:127–30.
72. Sharber J. The efficacy of tepid sponge bathing to reduce fever in young children. *Am J Emerg Med* 1997;**15**:188–92.
73. Sinha Y, Cranswick NE. Prescribing safely for children. *J Paediatr Child Health* 2007;**43**:112–6.
74. Small PM, Tauber MG, Hackbarth CJ, et al. Influence of body temperature on bacterial growth rates in experimental pneumococcal meningitis in rabbits. *Infect Immunol* 1986;**52**:484–7.
75. Steele RW, Tanaka PT, Lara RP, Bass JW. Evaluation of sponging and of oral antipyretic therapy to reduce fever. *J Pediatr* 1970;**77**: 824–9.
76. Strengell T, Uhari M, Tarkka R, et al. Antipyretic agents for preventing recurrences of febrile seizures. *Arch Pediatr Adolesc Med* 2009;**163**:799–804.
77. Suleman MI, Doufas AG, Akca O, et al. Insufficiency in a new temporal-artery thermometer for adult and pediatric patients. *Anesth Analg* 2002;**95**:67–71.
78. Sullivan JE, Farrar HC, and the Section on Clinical Pharmacology and Therapeutics, and Committee on Drugs. Clinical report—fever and antipyretic use in children. *Pediatrics* 2011;**127**:580–7.
79. Toms GL, Davies JA, Woodward CG, et al. The relation of pyrexia and nasal inflammatory response to virus levels in nasal washings of ferrets infected with influenza viruses of differing virulence. *Br J Exp Pathol* 1977;**58**:444–58.
80. Trautner BW, Caviness AC, Gerlacher GR, et al. Prospective evaluation of the risk of serious bacterial infection in children who present to the emergency department with hyperpyrexia (temperature of 106°F or higher). *Pediatrics* 2006;**118**:34–40.
81. Uhari M, Rantala H, Vainionpaa L, et al. Effect of acetaminophen and of low intermittent doses of diazepam on prevention of recurrences of febrile seizures. *J Pediatr* 1995;**126**:991–5.
82. Verity CM, Greenwood R, Golding J. Long-term intellectual and behavioral outcomes of children with febrile convulsions. *N Engl J Med* 1998;**338**:1723–8.
83. Walson PD, Galletta G, Chomilo F, et al. Comparison of multidose ibuprofen and acetaminophen therapy in febrile children. *Am J Dis Child* 1992;**146**:626–32.
84. Walter DL, Boring WD. Factors influencing host-virus interactions, part III: Further studies on the alteration of Coxsackie virus infection in adult mice by environmental temperature. *J Immunol* 1958;**80**:39–44.
85. Wilson JT, Brown RD, Kearns GL, et al. Single-dose, placebo-controlled comparative study of ibuprofen and acetaminophen antipyresis in children. *J Pediatr* 1991;**119**:803–11.
86. Wong A, Sibbald A, Ferrero F, et al. Antipyretic effects of dipyrone versus ibuprofen versus acetaminophen in children. *Clin Pediatr* 2001;**40**:313–24.
87. Wunderlich C, Seguin E. *Medical thermometry and human temperature*. New York: W. Wood & Co.; 1871.
88. Zetterstrom M, Lundkvist J, Malinowsky D, et al. Interleukin-1–mediated febrile responses in mice and interleukin-1b activation of NFkB in mouse primary astrocytes, involves the interleukin-1 receptor accessory protein. *Eur Cytokine Netw* 1998;**9**:131–8.

Environmental factors, host characteristics, and microbial properties all can influence the composition of the human microbiome. Disruption of the balance among these various factors can have a profound impact on the normal flora and their subsequent potential for pathogenicity. Pathogens are microorganisms that are able to cause disease in the host. Strict pathogens, such as *Neisseria gonorrhoeae* and the rabies virus, are always associated with disease and are not considered part of the normal flora. Organisms such as *P. aeruginosa*, *S. epidermidis*, and *Serratia marcescens* are opportunistic pathogens and do not cause disease except in immunocompromised hosts or in special circumstances that support their dissemination. Central venous catheters can become contaminated with *S. epidermidis*, resulting in a bloodstream infection. Similarly, after a dental procedure, transient bacteremia can cause viridans streptococcal endocarditis in a patient with an abnormal heart valve.

Facultative pathogens fall somewhere between strict and opportunistic pathogens. They have the capacity to cause disease in healthy individuals and compose much of the indigenous flora found in the body (e.g., *Streptococcus pneumoniae*, *E. coli*, and *S. aureus*).[173,184]

It is useful for clinicians to have a general understanding of the normal human microbiota. First, familiarity with the organisms that compose the normal flora may help with the interpretation of culture reports, such as the isolation of diphtheroids from a peripheral blood culture. Second, when an infectious disease is present, knowledge of the indigenous flora can assist in determining the likely causative agents and provide a basis for determining the initial antimicrobial therapy. This knowledge is especially important because of the increasing number of immunocompromised individuals who are vulnerable to endogenous infections. Third, an understanding of the normal flora may assist in making the decision to withhold antimicrobial therapy when a culture report identifies an organism considered to be a pathogen but is not for that patient's particular clinical presentation. The isolation of *S. pneumoniae* from a throat culture of a patient with pharyngitis can be disregarded if it is recognized that this organism is a normal part of the nasopharyngeal microbial environment and an unlikely cause of pharyngitis. Fourth, clinicians may prescribe broad-spectrum antimicrobial agents more judiciously if they have an appreciation for the role that indigenous flora play as part of the host's defense system.[160] Finally, the human microbiota probably contributes to host nutrition, immune system development, and susceptibility to pathogenic organisms.[196]

ACQUISITION OF INDIGENOUS FLORA BY THE NEWBORN

The fetus normally is in a sterile environment in the uterus. The newborn begins to acquire indigenous microbial flora during delivery. Maternal flora provide the initial source of colonizing organisms. Exposure to organisms from other people and environmental sources contributes to the formation of the neonate's eventual normal flora.[161]

The gestational age, mode of delivery, and type of feeding can affect the formation of a newborn's indigenous flora. Premature infants requiring prolonged hospitalization have a delay in bacterial colonization and are more frequently colonized with hospital flora, especially *Klebsiella*, *Enterobacter*, and *Citrobacter* spp., compared with healthy term newborns.[56,65,72] Infant-to-infant transmission by the hands of health care workers is an important factor contributing to gram-negative colonization of premature neonates.[65] Infants born by cesarean delivery have delayed intestinal colonization with anaerobic bacteria, and gut colonization can remain altered for 6 months after birth.[22,79,124,167] The intestinal flora also are influenced by the newborn's diet. *Bifidobacterium* spp. and a few other anaerobic bacteria dominate the intestinal flora of breast-fed newborns, whereas infants fed formula have a more complex intestinal microbial flora.* As the infant gets older, the composition of the indigenous flora begins to more closely resemble that of an adult. However, although bacterial communities have predictable biogeographic patterns, even adult human body habitats display considerable microbial diversity among individuals.[41]

MECHANISMS OF COLONIZATION

The normal flora differ substantially among the various anatomic sites because of local barriers to colonization. Environmental conditions, such as moisture, pH, oxygen tension, and nutritional supply, influence the ability of microorganisms to establish residence.[64,131] In certain anatomic regions, mucociliary clearance, epithelial cell turnover, and the flow of secretions restrict colonization to specific species of microbes.[131] Additional barriers include specific and nonspecific immune factors, the production of lysozyme, local attachment-blocking proteins, and microbial competition.[40]

The primary mechanisms of bacterial colonization are adherence to epithelial cells, colonization of mucus, and attachment to other colonizing organisms.[40] Adherence of bacteria to epithelial cells occurs primarily by specific binding between microbial surface antigens (adhesins) and epithelial receptor molecules.[13,83,111] Oral bacteria colonize specific sites within the mouth. *Streptococcus salivarius* binds to the epithelial cells of the tongue and buccal mucosa, whereas *Streptococcus mutans* and *Streptococcus sanguis* bind to tooth enamel.[160,209] Normal flora also can be established by the colonization of the mucous layer without bacterial attachment to host cells, as shown in the intestinal tract.[40] Finally, microbes can attach to colonizing organisms through adhesin-receptor binding. This means is established most clearly in the formation of dental plaque.[209]

EXOGENOUS INFLUENCES ON THE HUMAN MICROBIOME

An individual's normal flora tends to remain stable and consistent but can be affected by a variety of exogenous

*References 7, 14, 45, 127, 181, 213.

factors. Antibacterial soaps, topical antiseptics, and deodorants can temporarily suppress the skin flora.[15,86,109] Similarly, brushing teeth with fluoride toothpaste reduces dental microflora. Dietary patterns appear to influence the composition of the intestinal microbiome[211]; some microbial communities are more frequently associated with protein and animal fat diets, and others are associated with carbohydrate diets. Furthermore, changing the content of fat and fiber in the diet can modify the intestinal microbiome within a 24-hour period.[211] Eating fresh fruits and vegetables may provide a source of transient intestinal colonization with *P. aeruginosa*.[112,155] Cigarette smoke has been found to alter the normal flora and increase the number of potential pathogens in the nasopharynx.[28,29] Viral infections can disrupt the normal flora and predispose the patient to development of bacterial superinfections.* Influenza virus infection increases susceptibility to bacterial pneumonia, otitis media, and bacteremia partly by facilitating the adherence of pathogenic bacteria to respiratory epithelial cells.[70,169,214]

Medications can influence the composition of the normal flora. Acid reduction therapy may permit bacterial overgrowth within the stomach and small intestine, resulting in deconjugation of bile salts and malabsorption.† Gastric acid suppression also may reduce the number of ingested pathogens needed to cause enteric disease.[210] Antimicrobial agents can significantly alter the indigenous flora and promote colonization with potential pathogens by eliminating susceptible commensals.‡ Such changes can lead to superinfections with overgrowing organisms.[6,25,126,215] Antibiotics can reduce the barrier effect of the normal intestinal flora, permitting *Clostridium difficile* to propagate and produce toxins, resulting in pseudomembranous colitis.[10,33] Antimicrobial effects on the indigenous flora depend on the type of antibiotic, route, dose, and duration of administration.

Severe or chronic illnesses alone can cause changes in an individual's indigenous flora.[92,105,106,120] Hospitalized patients with severe illness are much more likely to develop pharyngeal colonization with gram-negative bacilli than are hospitalized patients who are physiologically normal.[105,106] Medical devices may alter the host's normal flora. Thomas and colleagues[191] showed that patients requiring placement of nasogastric tubes had significantly greater nasopharyngeal colonization with aerobic gram-negative bacilli in contrast to patients in a control group.

BACTERIAL COMPOSITION AT SPECIFIC LOCATIONS

Skin

Because of the presence of moisture and sebum, most skin flora are associated with sweat glands (Table 5-1). Bacterial concentrations are highest on the face, neck, finger and toe webs, and axillae and groin.[133,134] The organisms occupy the most superficial layers of the epidermis and are found primarily around hair follicles, although some are located deeper within follicles.[12,133,140] Although soaps and other skin cleansers substantially reduce the number of most surface bacteria, organisms within hair follicles and sweat glands quickly reestablish the normal flora.[86,109]

The normal skin flora are primarily of coagulase-negative *Staphylococcus* spp. (especially *S. epidermidis*), *Propionibacterium acnes*, *Corynebacterium* spp. (diphtheroids), and *Micrococcus* spp. *P. acnes* emerges at the onset of puberty, occupies hair follicles and sebaceous glands, and is a major contributing cause of acne. *S. aureus* and group A streptococci are not usual residents of the skin but may cause transient colonization.[177] *Acinetobacter* spp. are a common resident of the toe webs. Other gram-negative organisms, such as *Klebsiella*, *Proteus*, and *Enterobacter* spp. and *E. coli*, are uncommon findings and transient residents of the skin. The fungi *Pityrosporum* (*ovale* and *orbiculare*) and *Malassezia* spp. normally inhabit the skin,[78,157,158] but *Candida* spp. do not.[37] Many other microorganisms come in contact with the skin, but because of the skin's dryness and presence of organic fatty acids, they are present only briefly and do not propagate. Molecular genomic techniques have identified additional skin microbiota, such as the phyla Proteobacteria, which was not reported from culture-based methods.[78]

Newborns

Within hours, a normal full-term infant's skin becomes colonized with microbes. In addition to typical normal skin flora, potential pathogens (group B streptococci, *E. coli*, and *Klebsiella* spp.) frequently are recovered from cultures of the external ear canal.[128,144] Obtaining routine surveillance cultures in an attempt to identify infants at risk for acquiring invasive disease is not recommended, however, because their predictive value is limited.[102,128,144]

Conjunctivae

The mechanical actions of the eyelids and the washing effect of tears containing antimicrobial substances inhibit microbial colonization of the eye; however, normal flora do exist. The conjunctival flora probably originate from the eyelids and the nasolacrimal ducts. The organisms most commonly isolated from bacterial culture are coagulase-negative staphylococci, *Corynebacterium*, *Micrococcus*, and *Propionibacterium* spp. Other isolates include *S. aureus*, *Haemophilus* spp., viridans streptococci, *S. pneumoniae*, and *Bacillus* spp.[119,150,160,206] A DNA sequencing–based investigation of bacterial conjunctival colonization in four healthy adult males found a more diverse microbiota.[53] The 10 most prevalent genera identified were *Pseudomonas* (20%), *Propionibacterium* (20%), *Bradyrhizobium* (16%), *Corynebacteria* (15%), *Acinetobacter* (12%), *Brevundimonas* (5%), *Staphylococcus* (4%), *Aquabacterium* (2%), *Sphingomonas* (1%), and *Streptococcus* (1%).

*References 35, 57, 59, 70, 81, 82, 125, 153, 187.
†References 46, 77, 108, 164, 190, 192, 210.
‡References 11, 44, 49, 69, 89, 95, 101, 103, 135, 141, 142.

TABLE 5-1 **Common Indigenous Flora at Various Anatomic Sites**

Body Site and Type of Pathogen	Resident Flora	Transient Flora
Skin		
Opportunistic pathogens	*Corynebacterium, Staphylococcus epidermidis, Micrococcus, Peptococcus, Brevibacterium, Acinetobacter, Demodex folliculorum, Malassezia*	Viridans streptococci, *Enterococcus*
Facultative pathogens	*Propionibacterium acnes, Pityrosporum*	*Staphylococcus aureus, Enterobacter, Escherichia coli, Klebsiella, Proteus,* group A streptococci, *Candida, Trichophyton*
Eye		
Opportunistic pathogens	Coagulase-negative staphylococci, *Corynebacterium, Micrococcus*	*Bacillus,* viridans streptococci, *Propionibacterium*
Facultative pathogens	*Haemophilus*	*S. aureus, Streptococcus pneumoniae*
Mouth and Oropharynx		
Opportunistic pathogens	Viridans streptococci, coagulase-negative staphylococci, *Haemophilus,* non–group A β-hemolytic streptococci, *Treponema, Veillonella, Porphyromonas, Prevotella, Lactobacillus, Peptostreptococcus, Bacteroides,* nonmeningococcal *Neisseria, Corynebacterium, Gemella, Granulicatella*	*Eikenella corrodens*
Facultative pathogens	*Fusobacterium, Streptococcus mutans,* Actinomyces**	Group A streptococci, *Lactobacillus,* Neisseria meningitidis, Kingella, S. pneumoniae, Moraxella, Candida,* cytomegalovirus, herpes simplex virus
Nose and Nasopharynx		
Opportunistic pathogens	Coagulase-negative staphylococci, viridans streptococci, *Corynebacterium*	Nonmeningococcal *Neisseria*
Facultative pathogens	—	*S. aureus, N. meningitidis, S. pneumoniae, Moraxella*
Stomach		
Opportunistic pathogens	—	*Streptococcus, Lactobacillus*
Facultative pathogens	—	*Helicobacter pylori*
Small Intestine		
Opportunistic pathogens	*Lactobacillus, Streptococcus, Veillonella, Prevotella, Porphyromonas, Bifidobacterium*	*Candida, Entamoeba coli, Endolimax nana, Iodamoeba bütschlii, Trichomonas hominis, Chilomastix mesnili*
Facultative pathogens	*Bacteroides,* Enterobacteriaceae, *Clostridium*	*Blastocystis hominis*
Large Intestine		
Opportunistic pathogens	*Bifidobacterium, Peptostreptococcus, Lactobacillus, Veillonella, Eubacterium, Fusobacterium, Prevotella, Porphyromonas, Enterococcus, Peptococcus, Ruminococcus*	*Candida, Corynebacterium, Pseudomonas, Mycobacterium avium* complex, *E. coli, E. nana, I. bütschlii, T. hominis, C. mesnili*
Facultative pathogens	Bacteroides, Clostridium, Enterobacteriaceae	*Aeromonas, B. hominis,* enterovirus
Anterior Urethra		
Opportunistic pathogens	*Corynebacterium,* coagulase-negative staphylococci, viridans streptococci, *Lactobacillus* (women)	*Mycobacterium smegmatis, Bacteroides, Fusobacterium*
Facultative pathogens	—	Enterobacteriaceae, *Enterococcus, Ureaplasma, Mycoplasma*
Vagina		
Opportunistic pathogens	*Lactobacillus, Streptococcus,* coagulase-negative staphylococci	*Enterococcus*
Facultative pathogens	*Gardnerella vaginalis, Mobiluncus, Prevotella, Actinomyces*	Group B streptococci, *Candida, Trichomonas vaginalis*

*Contribute to dental caries.

Respiratory Tract

The mouth and oropharynx contain several unique microbial habitats. Viridans (α-hemolytic and nonhemolytic) streptococci are the most prominent commensals and include *S. mutans*, *S. sanguis*, *S. salivarius*, *Streptococcus anginosis* (formerly *S. milleri*), and *Streptococcus mitis*.[113,160,184] The flora of the gingival crevice include a diverse collection of facultative and anaerobic organisms. More common isolates include *S. mitis*, *S. mutans*, and *Actinomyces*, *Fusobacterium*, *Treponema*, *Veillonella*, and *Peptostreptococcus* spp. *Bacteroides* spp., *S. sanguis*, and *S. mitis* are the initial colonizers of the tooth surface. Dental plaque forms when *S. mutans* and *Actinomyces*, *Fusobacterium*, *Treponema*, and *Veillonella* spp., along with other organisms, attach to the initial bacterial cell layer and each other.[147,209] The buccal mucosa, tongue, and saliva are colonized most heavily by *S. salivarius*, *S. mitis*, and *Veillonella*, *Gemella*, *Granulicatella*, and *Lactobacillus* spp.[1] Asymptomatic pharyngeal carrier rates of group A streptococci in children range from 3 to 50 percent, with the highest prevalence rates associated with school outbreaks of pharyngitis.*

When evaluated by bacterial culture, the microbial flora of the nose and nasopharynx are less diverse, in contrast to flora of the mouth and oropharynx. Coagulase-negative staphylococci, viridans streptococci, *Corynebacterium* spp., and *S. aureus* are the most prevalent strains isolated. Nasal carriage rates of *S. aureus* average 20 to 35 percent, and the prevalence of methicillin-resistant *S. aureus* nasal colonization has been increasing, with estimates ranging from 0.2 to 22 percent, depending on demographic characteristics.† Colonization of children with *Neisseria*, *Haemophilus*, *Moraxella* spp., and *S. pneumoniae* is a common occurrence. Using metagenomic analysis, the nasopharyngeal microbiota of 96 children were found to be highly diverse, with strong seasonal variability.[24] The interindividual variability was too high for a "core" microbiome to be determined.

Pneumococcal colonization of the nasopharynx generally is transient, averaging in duration from 1 to 4 months.[58,76] Increased colonization is seen with younger age, overcrowding, attendance at a daycare center, winter season, and exposure to tobacco smoke. Nasopharyngeal carriage rates of *S. pneumoniae* average 40 to 50 percent in children and 20 to 30 percent in adults.[68] Nasopharyngeal colonization occurs even with the implementation of pneumococcal protein conjugate vaccine; several studies indicate a change in serotype distribution, with an increased presence of drug-resistant strains.[62,96,99] Despite the presence of potential pathogens in the nasopharynx, no convincing evidence supports the use of nasopharyngeal cultures to predict the etiology of acute otitis media, pneumonia, or sinusitis.[60,67,186,202]

The sinuses and lower respiratory tract generally are assumed to be sterile. In actuality, bacteria from the mouth and nose probably reach these regions daily but are cleared promptly by local defense mechanisms.[160]

Newborns

During the first week of life, the newborn's oropharynx usually is colonized by maternal vaginal flora, primarily *Lactobacillus* spp. and *Streptococcus viridans*.[30,137] These bacteria gradually are replaced by mouth flora from the mother and caretakers. *S. salivarius* and *S. mitis* predominate. Anaerobes, *S. mutans*, and *S. sanguinis* are uncommon findings until teeth erupt.[113] *Ureaplasma* and *Mycoplasma* spp. from the maternal vagina readily colonize the newborn's respiratory tract.[185] They are a suspected, yet still undetermined, cause of chronic lung disease in premature infants.[88,143,204] Enteric bacilli can be recovered from the throats of more than half of normal infants older than 2 months of age.[8]

Gastrointestinal Tract

The gastrointestinal tract consists of the esophagus, stomach, small intestine, and colon. The esophagus usually is sterile, and the stomach generally harbors only a few bacteria. The digestive enzymes and acid in the stomach destroy most swallowed organisms, or they pass promptly into the small intestine. Among the microflora of the stomach are acid-tolerant organisms, such as lactobacilli and streptococci,[171] and *Helicobacter pylori*, which survives within the mucous layer overlying the gastric mucosal epithelium.[23,66,107] The worldwide prevalence of *H. pylori* colonization in children ranges from 10 to 90 percent.[188] Factors associated with colonization include lower socioeconomic status, household crowding, ethnicity, and household contact with carriers.[75,148,194] When the gastric pH is increased, nasopharyngeal and the fecal type of flora may colonize the stomach.[210]

Low concentrations of *Streptococcus*, *Lactobacillus*, *Veillonella*, and rare *Bacteroides* spp. can be found in the upper small intestine.[18,32,50] Higher concentrations of the same bacteria plus *Bifidobacterium* spp., *Clostridium* spp., and Enterobacteriaceae are present in the lower small intestine.[19,132] Small bowel bacterial overgrowth can occur under conditions of gastric achlorhydria, a blind loop, or dysmotility. This syndrome may result in malabsorption and diarrhea.[110]

The colon contains a large, diverse population of microorganisms.[73,175] More microbes occur in this location than anywhere else in the body. For many years, because of inadequate techniques available for culturing strict anaerobic bacteria, *E. coli* was thought to be the principal resident of the large intestine. It has become apparent that the strict anaerobes outnumber the facultative microbes by 1000 to 1, with hundreds of different anaerobic species being isolated from the colon.[171] Molecular analysis of fecal flora has expanded understanding of the microbial diversity found in the gastrointestinal tract.[55,205] *Bacteroides* and *Bifidobacterium* spp. constitute the largest percentage of fecal flora. Other major organisms in the colon include Enterobacteriaceae and *Peptostreptococcus*, *Peptococcus*, *Ruminococcus*,

*References 38, 51, 61, 91, 151, 179, 182, 207, 212.
†References 2, 4, 34, 90, 98, 114, 174, 183, 199.

Enterococcus, *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Clostridium*, *Lactobacillus*, and *Eubacterium* spp.[87]

Newborns

The newborn's intestinal flora usually are derived from organisms within the mother's birth canal, mother's gut, breast milk, parental skin, and the newborn's environment.[3] Diet can influence the composition of the infant's intestinal flora.[40,45,124,129] Facultative bacteria (Enterobacteriaceae, *Enterococcus*, and *Streptococcus*) colonize the neonatal intestine in the first days of life.[145,197] However, after the first week, anaerobic organisms become prominent. The medical literature reports variable results regarding the impact of either breast-feeding or formula-feeding on the neonatal intestinal microbiota. In general, full-term, breast-fed infants are colonized predominantly with *Bifidobacterium* spp. and *Lactobacillus* spp., microbes considered to be beneficial.[85,145,197] Formula-fed infants have a more complex intestinal flora in which *Bifidobacterium*, *Bacteroides*, *Lactobacillus*, *Enterobacter*, *Clostridia*, and *Enterococcus* spp. are prevalent.* The mode of delivery can affect the infant's indigenous microbial flora. Infants delivered vaginally are more likely to have intestinal colonization with anaerobic organisms in contrast to those delivered by cesarean section. Infants delivered by cesarean section have a 6-month delay in colonization with anaerobic organisms.[22,79,124] At 12 months of age, after the introduction of solid foods, most infants have intestinal flora that more closely resemble those of an adult.[45,197]

Intestinal colonization of very-low-birth-weight infants is delayed, and the development of anaerobic flora and microbial diversity are diminished.[100,162] Anaerobes, especially *Bifidobacterium* and *Lactobacillus* spp., are thought to protect the host against invasion by pathogens. Paucity of these organisms permits bacterial overgrowth with potential pathogens, especially *C. difficile*, *E. coli*, and *Klebsiella*, *Enterobacter*, and *Citrobacter* spp.[100,162] Researchers suspect that this aberrant intestinal colonization of premature infants contributes to the pathogenesis of necrotizing enterocolitis.[36,47,118,146]

Genitourinary Tract

The endogenous flora of the genitourinary tract normally are confined to the distal portion of the urethra and the vaginal mucosa. The anterior urethra in men and women commonly is colonized with skin flora (coagulase-negative staphylococci, *Corynebacterium* spp., and streptococci) and may contain Enterobacteriaceae, *Mycobacterium smegmatis*, and *Bacteroides*, *Fusobacterium*, and *Enterococcus* spp. The female anterior urethra also contains *Lactobacillus* spp. Asymptomatic colonization with *Ureaplasma urealyticum* and, to a lesser extent, with *Mycoplasma hominis*, is a common event in sexually active individuals.[138,189]

The microbial flora of the vagina is influenced profoundly by hormonal factors.[116,117] During the first few weeks of life, estrogen from the maternal circulation creates in the newborn girl a vaginal environment conducive for the growth of *Lactobacillus* spp. This event is followed by development of a scant vaginal flora, containing mostly coagulase-negative staphylococci, *Corynebacterium* spp., and occasionally Enterobacteriaceae and *Streptococcus* spp. With the onset of puberty, lactobacilli again become the predominant organisms isolated. Coagulase-negative staphylococci and *Corynebacterium* spp. and various anaerobic streptococci also are common findings.[9,84,154] Enterobacteriaceae and *Enterococcus* and *Candida* spp. are found less frequently.[9] *U. urealyticum* is present in approximately half of premenopausal women, and *M. hominis* is found in fewer numbers.[138,189] The vaginal colonization rate of group B streptococci in pregnant women is 5 to 35 percent.[5] Heavy colonization with *Gardnerella vaginalis*, *Trichomonas vaginalis*, *Atopobium vaginae*, and *Mobiluncus* and *Prevotella* spp. is associated with bacterial vaginosis.[115,178]

BENEFICIAL EFFECTS OF THE HUMAN MICROBIOME

It is generally recognized that pathogens first need to colonize the host before causing disease. The indigenous flora help protect the host against infections through colonization resistance, also called *bacterial interference*.* Colonization resistance is accomplished by several mechanisms, including competition for nutrients, competition for epithelial cell receptors, production of toxins and bacteriocins, and stimulation of the immune response.[20,26,27,195,208] Suppression of the normal flora with antimicrobial therapy correlates with an increased susceptibility to candidiasis,[172] *C. difficile* colitis,[10] and *Salmonella* spp. infection.[94] Use of probiotic agents, live microbial food supplements (e.g., *Lactobacillus* and *Bifidobacterium* spp.), helps restore the normal intestinal microflora during antibiotic therapy and inhibits the growth of potential pathogens.[39,80,156,166] Several studies have demonstrated a decreased incidence of necrotizing enterocolitis in preterm infants fed breast milk and probiotics.[48,123,139]

The endogenous intestinal flora seem to stimulate the cellular and humoral mucosal immune system.[16,152,193,203] This relationship influences the development of the newborn's immune system. Germ-free animals have underdeveloped, poorly differentiated lymphoidal tissues and low serum immunoglobulin concentrations.[17] These animals have increased susceptibility to experimental challenges with pathogenic microbes.[54] The intestinal microflora also may affect the development of atopic disease and allergy.[21,149]

The normal flora provide some nutritional supplement for the host. The intestinal microflora ferment

*References 7, 14, 45, 127, 145, 181, 197, 213.

*References 27, 42, 93, 104, 122, 136, 168, 180, 200, 201.

undigested carbohydrates into short-chain fatty acids. The short-chain fatty acids affect colonic epithelial cell transport and serve as an energy source for colonic epithelial cells, the liver, and muscle.[43,159,163,165] The intestinal flora also participate in the enterohepatic recirculation of biliary metabolites and the degradation of toxins and carcinogens.[71,132] In addition, normal flora microbes produce essential vitamins, such as vitamins K and B_{12}. Whether humans use these vitamins in any substantial manner is uncertain.[52]

ADVERSE EFFECTS OF THE HUMAN MICROBIOME

The human microbiome can have unpleasant or even harmful effects on the host and is the source of intestinal gas and body odor. Oral flora are associated with dental caries and periodontal disease. In special circumstances, bacterial overgrowth of normal flora in the small intestine can result in malabsorption, diarrhea, and weight loss.[97,110] Other clinical conditions can create the potential for bacterial translocation, the invasion of indigenous bowel flora across the intestinal mucosa.[16,121] Intestinal toxins from endogenous bacteria may contribute to the encephalopathy, renal failure, and coagulopathy seen in hepatic failure.[37] Injury to the host from endotoxic shock may be caused to some extent by a hypersensitivity response induced by endotoxin from intestinal flora because germ-free animals are highly resistant to the effects of injected endotoxin.[184] Intestinal flora are speculated to cause cancer by metabolically activating carcinogens or making carcinogenic products.[130] Finally, the intestinal normal flora may have a causative role in atopic diseases and autoinflammatory disorders.[63,74,170,176,198]

SUMMARY

The human microbe is unique to specific anatomic locations. It is influenced by intrinsic microbial properties, host characteristics, and exogenous factors. Indigenous flora provide clear benefits to the host and potential adverse affects. Familiarity with the normal flora should help clinicians provide improved care for their patients.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Alderberth I, Wold AE. Establishment of the gut microbiota in Western infants. *Acta Pediatr* 2009;**98**:229–38.
33. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile* associated diarrhea. *J Infect Dis* 2008;**197**:435–8.
41. Costello EK, Lauber CL, Hamady M, et al. Bacterial community variation in human body habitats across space and time. *Science* 2009;**326**:1694–97.
48. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics* 2010;**125**:921–30.
49. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A* 2011;**108**(suppl. 1):4554–61.
53. Dong Q, Brulc JM, Iovieno A, et al. Diversity of bacteria at healthy human conjunctiva. *Invest Ophthalmol Vis Sci* 2011;**52**:5408–13.
74. Gore C, Munro K, Lay C, et al. *Bifidobacterium pseudocatenulatum* is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants. *J Allergy Clin Immunol* 2008;**121**:135–40.
78. Grice EA, Julia A, Segre JA. The skin microbiome. *Nat Rev Microbiol* 2011;**9**:244–53.
87. Hattori M, Taylor TD. The human intestinal microbiome: a new frontier of human biology. *DNA Res* 2009;**16**: 1–12.
100. Jacquot A, Neveu D, Aujoulat F, et al. Dynamics and clinical evolution of bacterial gut microflora in extremely premature patients. *J Pediatr* 2011;**158**:390–6.
101. Jakobsson HE, Jernberg C, Andersson AF, et al. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS ONE* 2010;**5**:e9836.
103. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* 2010;**156**(pt 11):3216–23.
115. Lamont R, Sobel J, Akins R, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. *BJOG* 2011;**118**:533–49.
123. Lin HC, Hsu CH, Chen HL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics* 2008;**122**: 693–700.
139. Meinzen-Derr J, Poindexter B, Wrage L, et al. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. *J Perinatol* 2009;**29**:57–62.
162. Rougé C, Goldenberg O, Ferraris L, et al. Investigation of the intestinal microbiota in preterm infants using different methods. *Anaerobe* 2010;**16**:362–70.
170. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008;**134**:577–94.
176. Sjögren YM, Jenmalm MC, Bottcher MF, et al. Altered early infant gut microbiota in children developing allergy up to 5 years of age. *Clin Exp Allergy* 2009;**39**:518–26.
197. Vael C, Desager K. The importance of the development of the intestinal microbiota in infancy. *Curr Opin Pediatr* 2009;**21**: 794–800.
198. Vael C, Nelson V, Verhulst SL, et al. Early intestinal *Bacteroides fragilis* colonization and development of asthma. *BMC Pulm Med* 2008;**8**:19.
211. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;**334**:105–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aas JA, Paster BJ, Stokes LN, et al. Defining the normal bacterial flora of the oral cavity. *J Clin Microbiol* 2005;**43**:5721–32.
2. Abudu L, Blair I, Fraise A, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA): A community-based prevalence survey. *Epidemiol Infect* 2001;**126**:351–6.
3. Alderberth I, Wold AE. Establishment of the gut microbiota in Western infants. *Acta Pediatr* 2009;**98**:229–38.
4. Alfaro C, Mascher-Denen M, Fergie J, Purcell K. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in patients admitted to Driscoll Children's Hospital. *Pediatr Infect Dis J* 2006;**25**:459–61.
5. American Academy of Pediatrics. Group B streptococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *2009 Red Book: report of the committee on infectious diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2000. p. 628–34.
6. Asay LD, Koch R. *Pseudomonas* infections in infants and children. *N Engl J Med* 1960;**262**:1062–6.
7. Balmer SE, Wharton BA. Diet and faecal flora in the newborn: Breast milk and infant formula. *Arch Dis Child* 1989;**64**: 1672–7.
8. Baltimore RS, Duncan RL, Shapiro ED, et al. Epidemiology of pharyngeal colonization of infants with aerobic gram-negative rod bacteria. *J Clin Microbiol* 1989;**27**:91–5.
9. Bartlett JG, Polk BF. Bacterial flora of the vagina: Quantitative study. *Rev Infect Dis* 1984;**6**(Suppl 1):S67–72.
10. Bartlett JG, Chang TW, Gurwith M, et al. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978;**298**:531–4.
11. Barza M, Giuliano M, Jacobus NV, et al. Effect of broad-spectrum parenteral antibiotics on "colonization resistance" of intestinal microflora of humans. *Antimicrob. Agents Chemother* 1987;**31**: 723–7.
12. Baxby D, Woodroffe RCS. The location of bacteria in the skin. *J Appl Bacteriol* 1965;**28**:316–21.
13. Beachey EH. Bacterial adherences: Adhesion-receptor interactions mediating the attachment of bacteria to mucosal surfaces. *J Infect Dis* 1981;**143**:325–45.
14. Benno Y, Sawada K, Mitsuoka T. The intestinal microflora of infants: Composition of fecal flora in breast-fed infants and bottle-fed infants. *Microbiol Immunol* 1984;**28**:975–86.
15. Benohanian A. Antiperspirants and deodorants. *Clin Dermatol* 2001;**19**:398–405.
16. Berg RD. Bacterial translocation from the gastrointestinal tract. *Trends Microbiol* 1995;**3**:149–54.
17. Berg RD, Savage DC. Immune responses of specific pathogen-free and gnotobiotic mice to antigens of indigenous and nonindigenous microorganisms. *Infect Immun* 1975;**11**:320–9.
18. Bernhardt H, Knoke M. Recent studies on the microbial ecology of the upper gastrointestinal tract. *Infection* 1989;**17**:259–63.
19. Bhat P, Albert MJ, Rajan D, et al. Bacterial flora of the jejunum: A comparison of luminal aspirate and mucosal biopsy. *J Med Microbiol* 1980;**13**:247–56.
20. Bibel DJ, Aly R, Bayles C, et al. Competitive adherence as a mechanism of bacterial interference. *Can J Microbiol* 1983;**29**:700–3.
21. Bjorksten B, Sepp E, Julge K, et al. Allergy development and the intestinal microflora during the first year of life. *J Allergy Clin Immunol* 2001;**108**:516–20.
22. Blakey JL, Lubitz L, Barnes GL, et al. Development of gut colonisation in pre-term neonates. *J Med Microbiol* 1982;**15**: 519–29.
23. Blaser MJ. Hypothesis: The changing relationships of *Helicobacter pylori* and humans: Implications for health and disease. *J Infect Dis* 1999;**179**:1523–30.
24. Bogaert D, Keijser B, Huse S, et al. Variability and diversity of nasopharyngeal microbiota in children: a metagenomic analysis. *PLoS ONE* 2011;**6**:e17035.
25. Bonhogg M, Drake BL, Miller CP. Effect of streptomycin on susceptibility of intestinal tracts to experimental *Salmonella* infection. *Proc Soc Exp Biol Med* 1954;**86**:132–7.
26. Borriello SP. The influence of the normal flora on *Clostridium difficile* colonisation of the gut. *Ann Med* 1990;**22**:61–7.
27. Brook I. Bacterial interference. *Crit Rev Microbiol* 1999;**25**: 155–72.
28. Brook I, Gober AE. Recovery of potential pathogens and interfering bacteria in the nasopharynx of smokers and nonsmokers. *Chest* 2005;**127**:2072–5.
29. Brook I, Gober AE. Effect of smoking cessation on the microbial flora. *Arch Otolaryngol Head Neck Surg* 2007;**133**:135–8.
30. Carlsson J, Grahnen H, Jonsson G, et al. Early establishment of *Streptococcus salivarius* in the mouths of infants. *J Dent Res* 1970;**49**:415–8.
31. Casadevall A, Pirofski LA. Host-pathogen interactions: Basic concepts of microbial commensalism, colonization, infection, and disease. *Infect Immun* 2000;**68**:6511–8.
32. Challacombe DN, Richardson JM, Anderson CM. Bacterial microflora of the upper gastrointestinal tract in infants without diarrhea. *Arch Dis Child* 1974;**49**:264–9.
33. Chang JY, Antonopoulos DA, Kalra A, et al. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile* associated diarrhea. *J Infect Dis* 2008;**197**:435–8.
34. Charlebois ED, Bangsberg DR, Moss NJ, et al. Population-based community prevalence of methicillin-resistant *Staphylococcus aureus* in the urban poor of San Francisco. *Clin Infect Dis* 2002;**34**:425–33.
35. Chonmaitree T, Heikkinen T. Viruses and acute otitis media. *Pediatr Infect Dis J* 2000;**19**:1005–7.
36. Claud EC, Walker WA. Hypothesis: Inappropriate colonization of the premature intestine can cause neonatal necrotizing enterocolitis. *FASEB J* 2001;**15**:1398–1403.
37. Clayton YM, Noble WC. Observations on the epidemiology of *Candida albicans*. *J Clin Pathol* 1966;**19**:76–8.
38. Cockerill FR, MacDonald KL, Thompson RL, et al. An outbreak of invasive group A streptococcal disease associated with high carriage rates of the invasive clone among school-aged children. *JAMA* 1997;**277**:38–43.
39. Collins MD, Gibson GR. Probiotics, prebiotics, and symbiotics: Approaches for modulating the microbial ecology of the gut. *Am J Clin Nutr* 1999;**69**:1052S–7S.
40. Cooperstock MS. Indigenous flora in host economy and pathogenesis. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 3rd ed. Philadelphia: W. B. Saunders; 1992. p. 91–119.
41. Costello EK, Lauber CL, Hamady M, et al. Bacterial community variation in human body habitats across space and time. *Science* 2009;**326**:1694–7.
42. Crowe CC, Sanders Jr WE, Longley S. Bacterial interference. II. Role of the normal throat flora in prevention of colonization by group A *Streptococcus*. *J Infect Dis* 1973;**128**:527–32.
43. Cummings JH, Macfarlane GT. Role of intestinal bacteria in nutrient metabolism. *J Parenter Enteral Nutr* 1997;**21**:357–65.
44. Dagan R, Leibovitz E, Cheletz G, et al. Antibiotic treatment in acute otitis media promotes superinfection with resistant *Streptococcus pneumoniae* carried before initiation of treatment. *J Infect Dis* 2001;**183**:880–6.
45. Dai D, Walker WA. Protective nutrients and bacterial colonization in the immature human gut. *Adv Pediatr* 1999;**46**:353–82.
46. Deane S, Youngs D, Poxon V, et al. Cimetidine and the gastric microflora. *Br J Surg* 1980;**67**:371.
47. Deitch EA. Role of bacterial translocation in necrotizing enterocolitis. *Acta Paediatr Suppl* 1994;**396**:33–6.
48. Deshpande G, Rao S, Patole S, Bulsara M. Updated meta-analysis of probiotics for preventing necrotizing enterocolitis in preterm neonates. *Pediatrics* 2010;**125**:921–30.
49. Dethlefsen L, Relman DA. Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. *Proc Natl Acad Sci U S A* 2011;**108**(suppl 1):4554–61.
50. Dickman MD, Chappelka AR, Schaedler RW. The microbial ecology of the upper small bowel. *Am J Gastroenterol* 1976;**65**: 57–62.
51. Dierksen KP, Inglis M, Tagg JR. High pharyngeal carriage rates of *Streptococcus pyogenes* in Dunedin school children with a low incidence of rheumatic fever. *N Z Med J* 2000;**113**:496–9.
52. Donaldson Jr RM. Normal bacterial populations of the intestine and their relation to intestinal function. *N Engl J Med* 1964; **270**:938–46, 994-1000, 1050-6.

53. Dong Q, Brulc JM, Iovieno A, et al. Diversity of bacteria at healthy human conjunctiva. *Invest Ophthalmol Vis Sci* 2011;**52**: 5408–13.
54. Dubos RJ, Schaedler RW. The effect of the intestinal flora on the growth rate of mice, and on their susceptibility to experimental infections. *J Exp Med* 1960;**111**:407–17.
55. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science* 2005;**308**:1635–8.
56. Ehrenkranz NJ. Bacterial colonization of newborn infants and subsequent acquisition of hospital bacteria. *J Pediatr* 1970;**76**: 839–47.
57. Eichenwald HF, Kotsevalov O, Fasso LA. Some effects of viral infection on aerial dissemination of staphylococci and on susceptibility to bacterial colonization. *Bacteriol Rev* 1961;**25**:274–81.
58. Ekdahl K, Ahlinder I, Hansson HB, et al. Duration of NP carriage of PRP: Experiences from the South Swedish Pneumococcal Intervention Project. *Clin Infect Dis* 1997;**25**:1113–7.
59. Elahmer OR, Raza MW, Ogilvie MM, et al. The effect of respiratory virus infection on expression of cell surface antigens associated with binding of potentially pathogenic bacteria. *Adv Exp Med Biol* 1996;**408**:169–77.
60. Eldan M, Leibovitz E, Piglansky L, et al. Predictive value of pneumococcal nasopharyngeal cultures for the assessment of nonresponsive acute otitis media in children. *Pediatr Infect Dis J* 2000;**19**:298–303.
61. Engelgau MM, Woernle CH, Schwartz B, et al. Invasive group A streptococcus carriage in a child care centre after a fatal case. *Arch Dis Child* 1994;**71**:318–22.
62. Farrell DJ, Klugman KP, Pichichero M. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *Pediatr Infect Dis J* 2007;**26**:123–8.
63. Frank DN, St. Amand AL, Feldman RA, et al. Molecular phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* 2007;**104**:13780–5.
64. Freter R. Interactions between mechanisms controlling the intestinal microflora. *Am J Clin Nutr* 1974;**27**:1409–16.
65. Fryklund B, Tullus K, Berglund B, et al. Importance of the environment and the faecal flora of infants, nursing staff, and parents as sources of gram-negative bacteria colonizing newborns in three neonatal wards. *Infection* 1992;**20**:253–7.
66. Ganga-Zandzou PS, Michaud L, Vincent P, et al. Natural outcome of *Helicobacter pylori* infection in asymptomatic children: A two-year follow-up study. *Pediatrics* 1999;**104**:216–21.
67. Gehanno P, Lenoir G, Barry B, et al. Evaluation of nasopharyngeal cultures for bacteriologic assessment of acute otitis media in children. *Pediatr Infect Dis J* 1996;**15**:329–32.
68. Ghaffar F, Friedland IR, McCracken GH. Dynamics of nasopharyngeal colonization by *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 1999;**18**:638–46.
69. Giuliano M, Barza M, Jacobus NV, et al. Effect of broad-spectrum parenteral antibiotics on composition of intestinal microflora of humans. *Antimicrob Agents Chemother* 1987;**31**:202–6.
70. Glezen WP. Prevention of acute otitis media by prophylaxis and treatment of influenza virus infections. *Vaccine* 2000;**19** (Suppl 1):S56–8.
71. Goldin BR. Intestinal microflora: Metabolism of drugs and carcinogens. *Ann Med* 1990;**22**:43–8.
72. Goldmann DA, Leclair J, Macone A. Bacterial colonization of neonates admitted to an intensive care environment. *J Pediatr* 1978;**93**:288–93.
73. Gorbach SL. Intestinal microflora. *Gastroenterology* 1971;**60**: 1110–29.
74. Gore C, Munro K, Lay C, et al. *Bifidobacterium pseudocatenulatum* is associated with atopic eczema: a nested case-control study investigating the fecal microbiota of infants. *J Allergy Clin Immunol* 2008;**121**:135–40.
75. Graham DY, Malaty HM, Evans DJ, et al. Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States: Effect of age, race, and socioeconomic status. *Gastroenterology* 1991;**100**:1495–501.
76. Gray BM, Converse GM III, Dillon Jr HC. Epidemiologic studies of *Streptococcus pneumoniae* in infants: Acquisition, carriage, and infection during the first 24 months of life. *J Infect Dis* 1980;**142**:923–33.
77. Gray JD, Shiner M. Influence of gastric pH on gastric and jejunal flora. *Gut* 1967;**8**:574–81.
78. Grice EA, Segre JA. The skin microbiome. *Nat Rev Microbiol* 2011;**9**:244–53.
79. Gronlund MM, Lehtonen OP, Eerola E, et al. Fecal microflora in healthy infants born by different methods of delivery: Permanent changes in intestinal flora after cesarean delivery. *J Pediatr Gastroenterol Nutr* 1999;**28**:19–25.
80. Guarner F, Malagelada JR. Gut flora in health and disease. *Lancet* 2003;**361**:512–9.
81. Gwaltney Jr JM, Sande MA, Austrian R, et al. Spread of *Streptococcus pneumoniae* in families. II. Relation of transfer of *S. pneumoniae* to incidence of colds and serum antibody. *J Infect Dis* 1975;**132**:62–8.
82. Hakansson A, Kidd A, Wadell G, et al. Adenovirus infection enhances in vitro adherence of *Streptococcus pneumoniae*. *Infect Immun* 1994;**62**:2707–14.
83. Hamada S, Amano A, Kimura S, et al. The importance of fimbriae in the virulence and ecology of some oral bacteria. *Oral Microbiol Immunol* 1998;**13**:129–38.
84. Hammerschlag MR, Alpert S, Onderdonk A, et al. Anaerobic microflora of the vagina in children. *Am J Obstet Gynecol* 1978;**131**:853–6.
85. Harmsen HJ, Wildeboer-Veloo AC, Raangs GC, et al. Analysis of intestinal flora development in breast-fed and formula-fed infants by using molecular identification and detection methods. *J Pediatr Gastroenterol Nutr* 2000;**30**:61–7.
86. Hartmann AA. Daily bath and its effect on the normal human skin flora: Quantitative and qualitative investigations of the aerobic skin flora. *Arch Dermatol Res* 1979;**265**:153–64.
87. Hattori M, Taylor TD. The human intestinal microbiome: a new frontier of human biology. *DNA Res* 2009;**16**: 1–12.
88. Heggie AD, Bar-Shain D, Boxerbaum B, et al. Identification and quantification of ureaplasma colonizing the respiratory tract and assessment of their role in the development of chronic lung disease in preterm infants. *Pediatr Infect Dis J* 2001;**20**:854–9.
89. Heikkinen T, Saeed KA, McCormick DP, et al. A single intramuscular dose of ceftriaxone changes nasopharyngeal bacterial flora in children with acute otitis media. *Acta Paediatr* 2000; **89**:1316–21.
90. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998;**279**:593–8.
91. Hoffmann S. The throat carrier rate of group A and other beta hemolytic streptococci among patients in general practice. *Acta Pathol Microbiol Immunol Scand* 1985;**93**:347–51.
92. Holdeman LV, Good IJ, Moore WEC. Human fecal flora: Variation in bacterial composition within individuals and a possible effect of emotional stress. *Appl Environ Microbiol* 1976; **31**:359–75.
93. Holm SE, Grahn E. Bacterial interference in streptococcal tonsillitis. *Scand J Infect Dis Suppl* 1983;**39**:73–8.
94. Holmberg SD, Osterholm MT, Senger KA, et al. Drug-resistant *Salmonella* from animals fed antimicrobials. *N Engl J Med* 1984;**311**:617–22.
95. Hooker KD, Di Piro JT. Effect of antimicrobial therapy on bowel flora. *Clin Pharm* 1988;**7**:878–88.
96. Huang SS, Platt R, Rifas-Shiman SL, et al. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. *Pediatrics* 2005;**116**:e408–13.
97. Husebye E. Gastrointestinal motility disorders and bacterial overgrowth. *J Intern Med* 1995;**237**:419–27.
98. Hussain FM, Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus* colonization in healthy children attending an outpatient pediatric clinic. *Pediatr Infect Dis J* 2001;**20**:763–7.
99. Jacobs MR, Good CE, Sellner T, et al. Nasopharyngeal carriage of respiratory pathogens in children undergoing pressure equalization tube placement in the era of pneumococcal protein conjugate vaccine use. *Laryngoscope* 2007;**117**:295–8.
100. Jacquot A, Neveu D, Aujoulat F, et al. Dynamics and clinical evolution of bacterial gut microflora in extremely premature patients. *J Pediatr* 2011;**158**:390–6.

101. Jakobsson HE, Jernberg C, Andersson AF, et al. Short-term antibiotic treatment has differing long-term impacts on the human throat and gut microbiome. *PLoS ONE* 2010;**5**:e9836.
102. Jarvis WR. The epidemiology of colonization. *Infect Control Hosp Epidemiol* 1996;**17**:47–52.
103. Jernberg C, Löfmark S, Edlund C, Jansson JK. Long-term impacts of antibiotic exposure on the human intestinal microbiota. *Microbiology* 2010;**156**(pt 11):3216–23.
104. Johanson Jr WG, Blackstock R, Pierce AK, et al. The role of bacterial antagonism in pneumococcal colonization of the human pharynx. *J Lab Clin Med* 1970;**75**:946–52.
105. Johanson Jr WG, Pierce AK, Sanford JP, et al. Nosocomial respiratory infections with gram-negative bacilli: The significance of colonization of the respiratory tract. *Ann Intern Med* 1972;**77**:701–6.
106. Johanson WG, Pierce AK, Sanford JP. Changing pharyngeal bacterial flora of hospitalized patients: Emergence of gram-negative bacilli. *N Engl J Med* 1969;**281**:1137–40.
107. Karlsson KA. The human gastric colonizer *Helicobacter pylori*: A challenge for host-parasite glycobiology. *Glycobiology* 2000;**10**: 761–71.
108. Karmeli Y, Stalnikowitz R, Eliakim R, et al. Conventional dose of omeprazole alters gastric flora. *Dig Dis Sci* 1995;**40**: 2070–3.
109. Keswick BH, Berge CA, Bartolo RG, et al. Antimicrobial soaps: Their role in personal hygiene. In: Aly R, Beutner KR, Maibach H, editors. *Cutaneous infection and therapy*. New York: Marcel Dekker; 1997. p. 49–82.
110. Kirsch M. Bacterial overgrowth. *Am J Gastroenterol* 1990; **85**:231–7.
111. Klemm P, Schembri MA. Bacterial adhesins: Function and structure. *Int J Med Microbiol* 2000;**290**:27–35.
112. Kominos SD, Copeland CE, Grosiak B, et al. Introduction of *Pseudomonas aeruginosa* into a hospital via vegetables. *Appl Microbiol* 1972;**24**:567–70.
113. Kononen E: Development of oral bacterial flora in young children. *Ann Med* 2000;**32**:107–12.
114. Kuehnert MJ, Kruszon-Moran D, Hill HA, et al. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001-2002. *J Infect Dis* 2006;**193**:172–9.
115. Lamont R, Sobel J, Akins R, et al. The vaginal microbiome: new information about genital tract flora using molecular based techniques. *BJOG* 2011;**118**:533–49.
116. Larsen B, Galask RP. Vaginal microbial flora: Composition and influences of host physiology. *Ann Intern Med* 1982;**96**: 926–30.
117. Larsen B, Monif GR. Understanding the bacterial flora of the female genital tract. *Clin Infect Dis* 2001;**32**:69–77.
118. Lawrence G, Bates J, Gaul A. Pathogenesis of neonatal necrotizing enterocolitis. *Lancet* 1982;**1**:137–9.
119. Levine J, Snyder RW. Practical ophthalmic microbiology. *J Ophthalmic Nurs Technol* 1999;**18**:50–9.
120. Li L, Wu Z, Ma W, et al. Changes in intestinal microflora in patients with chronic severe hepatitis. *Chin Med J* 2001; **114**:869–72.
121. Lichtman SM. Bacterial translocation in humans. *J Pediatr Gastroenterol Nutr* 2001;**33**:1–10.
122. Liljemark WF, Gibbons RJ. Suppression of *Candida albicans* by human oral streptococci in gnotobiotic mice. *Infect Immun* 1973;**8**:846–9.
123. Lin HC, Hsu CH, Chen HL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter, randomized, controlled trial. *Pediatrics* 2008;**122**: 693–700.
124. Long SS, Swenson RM. Development of anaerobic fecal flora in healthy newborn infants. *J Pediatr* 1977;**91**:298–301.
125. Loukides S, Panagou P, Kolokouris D, et al. Bacterial pneumonia as a suprainfection in young adults with measles. *Eur Respir J* 1999;**13**:356–60.
126. Louria DB, Kaminski T. The effects of four antimicrobial drug regimens on sputum superinfection in hospitalized patients. *Am Rev Respir Dis* 1962;**85**:649–65.
127. Lundquist B, Nord CE, Winberg J. The composition of the faecal microflora in breast fed and bottle fed infants from birth to eight weeks. *Acta Paediatr Scand* 1985;**74**:45–51.
128. MacGregor RR, Tunnessen WW. The incidence of pathogenic organisms in the normal flora of the neonate's external ear and nasopharynx. *Clin Pediatr* 1973;**12**:697–700.
129. Mackie RI, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999;**69**(Suppl):1035S–45S.
130. Mackowiak PA. Microbial oncogenesis. *Am J Med* 1987;**82**: 79–97.
131. Mackowiak PA. The normal microbial flora. *N Engl J Med* 1982;**307**:83–93.
132. Mallory A, Kern F, Smith J, et al. Patterns of bile acids and microflora in the human small intestine. I. Bile acids. *Gastroenterology* 1973;**64**:26–33.
133. Marples MJ. The normal flora of the human skin. *Br J Dermatol* 1969;**81**(Suppl 1):2–13.
134. Marples R. The normal flora of different sites in the young adult. *Curr Med Res Opin* 1982;7(Suppl 2): 67–70.
135. Marples RR, Kligman AM. Ecological effects of oral antibiotics on the microflora of human skin. *Arch Dermatol* 1971;**103**: 148–53.
136. Martin RR, White A. The reacquisition of staphylococci by treated carriers: A demonstration of bacterial interference. *J Lab Clin Med* 1968;**71**:791–7.
137. McCarthy C, Snyder ML, Parker RB. The indigenous oral flora of man, I: The newborn to one year old infant. *Arch Oral Biol* 1965;**10**:61–70.
138. McCormack WM, Rosner B, Alpert S, et al. Vaginal colonization with *Mycoplasma hominis* and *Ureaplasma urealyticum*. *Sex Transm Dis* 1986;**13**:67–70.
139. Meinzen-Derr J, Poindexter B, Wrage L, et al. Role of human milk in extremely low birth weight infants' risk of necrotizing enterocolitis or death. *J Perinatol* 2009;**29**:57–62.
140. Montes LF, Wilborn WH. Location of bacterial skin flora. *Br J Dermatol* 1969;**81**(Suppl I):23–6.
141. Nord CE, Heimdahl A, Kager L. Antimicrobial induced alterations of the human oropharyngeal and intestinal microflora. *Scand J Infect Dis Suppl* 1986;**49**:64–72.
142. Norrby SR. Ecological consequences of broad spectrum versus narrow spectrum antibacterial therapy. *Scand J Infect Dis Suppl* 1986;**49**:189–95.
143. Ollikainen J, Heiskanen-Kosma T, Korppi M, et al. Clinical relevance of *Ureaplasma urealyticum* colonization in preterm infants. *Acta Paediatr* 1998;**87**:1075–8.
144. Ostfeld E, Segal J, Segal A, et al. Bacterial colonization of the nose and external ear canal in newborn infants. *Isr J Med Sci* 1983;**19**:1046–9.
145. Palmer C, Bik EM, DiGiulio DB, et al. Development of the human infant intestinal microbiota. *PLoS Biol* 2007;**5**:e177.
146. Panigrahi P. Necrotizing enterocolitis: A practical guide to its prevention and management. *Pediatr Drugs* 2006;**8**:151–65.
147. Paster BJ, Boches SK, Galvin JL, et al. Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001;**183**:3770–83.
148. Peek RM. The biological impact of *Helicobacter pylori* colonization. *Semin Gastrointest Dis* 2001;**12**:151–66.
149. Penders J, Stobberingh EE, van den Brandt PA, Thijs C. The role of the intestinal microbiota in the development of atopic disorders. *Allergy* 2007;**62**:1223–36.
150. Perkins RE, Kundsin RB, Pratt MV, et al. Bacteriology of normal and infected conjunctiva. *J Clin Microbiol* 1975;**1**: 147–9.
151. Pichichero ME, Marsocci SM, Murphy ML, et al. Incidence of streptococcal carriers in private pediatric practice. *Arch Pediatr Adolesc Med* 1999;**153**:624–8.
152. Pickard KM, Bremner AR, Gordon JN, MacDonald TT. Microbial-gut interactions in health and disease: Immune responses. *Best Pract Res Clin Gastroenterol* 2004;**18**:271–85.
153. Ramirez-Ronda CH, Fuxench-Lopez Z, Nevarez M. Increased pharyngeal bacterial colonization during viral illness. *Arch Intern Med* 1981;**141**:1599–603.
154. Redondo-Lopez V, Cook RL, Sobel JD. Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis* 1990;**12**:856–72.
155. Remington JS, Schimpff SC. Occasional notes: Please don't eat the salads. *N Engl J Med* 1981;**304**:433–5.

156. Resta-Lenert S, Barrett KE. Live probiotics protect intestinal epithelial cells from the effects of infection with enteroinvasive Escherichia coli (EIEC). *Gut* 2003;**52**:988–97.
157. Roberts SO. *Pityrosporum orbiculare*: Incidence and distribution on clinically normal skin. *Br J Dermatol* 1969;**81**:264–9.
158. Roberts SO. The mycology of the clinically normal scalp. *Br J Dermatol* 1969;**81**:626–8.
159. Roediger WE. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* 1980;**21**:793–8.
160. Roscoe DL, Chow AW. Normal flora and mucosal immunity of the head and neck. *Infect Dis Clin N Am* 1988;**2**:1–19.
161. Rotimi VO, Duerden BI. The development of the bacterial flora in normal neonates. *J Med Microbiol* 1981;**14**:51–62.
162. Rougé C, Goldenberg O, Ferraris L, et al. Investigation of the intestinal microbiota in preterm infants using different methods. *Anaerobe* 2010;**16**:362–70.
163. Royall DR, Wolever TM, Jeejeebhoy KN. Clinical significance of colonic fermentation. *Am J Gastroenterol* 1990;**80**:1307–12.
164. Ruddell WS, Axon AT, Findlay JM, et al. Effect of cimetidine on the gastric bacterial flora. *Lancet* 1980;**1**:672–4.
165. Ruppin H, Bar-Meir S, Soergel KH, et al. Absorption of short chain fatty acids by the colon. *Gastroenterology* 1980;**78**:1500–7.
166. Saavedra JM. Clinical applications of probiotic agents. *Am J Clin Nutr* 2001;**73**:1147S–51S.
167. Sakata H, Yoshioka H, Fujita K. Development of the intestinal flora in very low birth weight infants compared to normal full-term infants. *Eur J Pediatr* 1985;**144**:186–90.
168. Sanders E. Bacterial interference. I. Its occurrence among the respiratory tract flora and characterization of inhibition of group A streptococci by viridans streptococci. *J Infect Dis* 1969;**120**: 698–707.
169. Sanford BA, Shelokov A, Ramsay MA. Bacterial adherence to virus-infected cells: A cell culture model of bacterial superinfection. *J Infect Dis* 1978;**137**:176–81.
170. Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008;**134**:577–94.
171. Savage DS. Microbial ecology of the gastrointestinal tract. *Annu Rev Microbiol* 1977;**31**:107–33.
172. Seelig MS. The role of antibiotics in the pathogenesis of *Candida* infections. *Am J Med* 1966;**40**:887–917.
173. Sharp SE. Commensal and pathogenic microorganisms of humans. In: Murray PA, Baron EJ, Pfaller MA, et al, editors. *Manual of clinical microbiology*. 7th ed. Washington D.C. ASM Press; 1999. p. 23–32.
174. Shopsin B, Mathema B, Martinez J, et al. Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the community. *J Infect Dis* 2000;**182**:359–62.
175. Simon GL, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 1984;**86**:174–93.
176. Sjögren YM, Jenmalm MC, Bottcher MF, et al. Altered early infant gut microbiota in children developing allergy up to 5 years of age. *Clin Exp Allergy* 2009;**39**:518–26.
177. Somerville DA. The normal flora of the skin in different age groups. *Br J Dermatol* 1969;**81**:248–58.
178. Spiegel CA. Bacterial vaginosis. *Clin Microbiol Rev* 1991;**4**: 485–502.
179. Spitzer J, Hennessy E, Neville L. High group A streptococcal carriage in the Orthodox Jewish community of North Hackney. *Br J Gen Pract* 2001;**51**:101–5.
180. Sprunt K, Redman W. Evidence suggesting importance of role in interbacterial inhibition in maintaining balance of normal flora. *Ann Intern Med* 1968;**68**:579–90.
181. Stark PL, Lee A. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J Med Microbiol* 1982;**15**:189–203.
182. Stromberg A, Schwan A, Cars O. Throat carrier rates of beta-hemolytic streptococci among healthy adults and children. *Scand J Infect Dis* 1988;**20**:411–7.
183. Suggs AH, Maranan MC, Boyle-Vavra S, et al. Methicillin-resistant and borderline methicillin-resistant asymptomatic *Staphylococcus aureus* colonization in children without identifiable risk factors. *Pediatr Infect Dis J* 1999;**18**:410–4.
184. Swartz MN, Gibbons R, Socransky S. Indigenous bacteria: Oral microbiology. In: Davis BD, Dulbecco R, Eisen HN, et al, editors. *Microbiology*. 4th ed. Philadelphia: J. B. Lippincott; 1990. p. 727–36.
185. Syringiannopoulos GA, Kapatais-Zoumbos K, Decavalas GO, et al. *Ureaplasma urealyticum* colonization of full term infants: Perinatal acquisition and persistence during early infancy. *Pediatr Infect Dis J* 1990;**9**:236–40.
186. Syrjänen RK, Herva EE, Mäkelä PH, et al. The value of nasopharyngeal culture in predicting the etiology of acute otitis media in children less than two years of age. *Pediatr Infect Dis J* 2006;**25**:1032–6.
187. Takase H, Nitanai H, Yamamura E, et al. Facilitated expansion of pneumococcal colonization from the nose to the lower respiratory tract in mice preinfected with influenza virus. *Microbiol Immunol* 1999;**43**:905–7.
188. Taylor DN, Blaser MJ. The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev* 1991;**13**:42–59.
189. Taylor-Robinson D, McCormack WM. The genital mycoplasmas. *N Engl J Med* 1980;**302**:1003–10, 1063-7.
190. Theisen J, Nehra D, Citron D, et al. Suppression of gastric acid secretion in patients with gastroesophageal reflux disease results in gastric bacterial overgrowth and deconjugation of bile acids. *J Gastrointest Surg* 2000;**4**:50–4.
191. Thomas S, Raman R, Idikula J, et al. Alterations in oropharyngeal flora in patients with a nasogastric tube: A cohort study. *Crit Care Med* 1992;**20**:1677–80.
192. Thorens J, Froehlich F, Schwizer W, et al. Bacterial overgrowth during treatment with omeprazole compared with cimetidine: A prospective randomised double blind study. *Gut* 1996;**39**: 54–9.
193. Tlaskalova-Hogenova H, Sterzl J, Stepankova R, et al. Development of immunological capacity under germfree and conventional conditions. *Ann N Y Acad Sci* 1983;**409**:96–113.
194. Torres J, Perez-Perez G, Goodman KJ, et al. A comprehensive review of the natural history of *Helicobacter pylori* infection in children. *Arch Med Res* 2000;**31**:431–69.
195. Tramont EC, Hoover DL. Innate (general or nonspecific) host defense mechanisms. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 31–8.
196. Turnbaugh PJ, Ley RE, Hamady M, et al. The human microbiome project. *Nature* 2007;**449**:804–10.
197. Vael C, Desager K. The importance of the development of the intestinal microbiota in infancy. *Curr Opin Pediatr* 2009;**21**: 794–800.
198. Vael C, Nelson V, Verhulst SL, et al. Early intestinal *Bacteroides fragilis* colonization and development of asthma. *BMC Pulm Med* 2008;**8**:19.
199. VandenBergh MF, Yzerman EP, van Belkum A, et al. Follow-up of *Staphylococcus aureus* nasal carriage after 8 years: Redefining the persistent carrier state. *J Clin Microbiol* 1999;**37**:3133–40.
200. van der Waaij D, Berghuis-de Vries JM, Lekkerkerk van der Wees JEC. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. *J Hyg* 1971;**69**: 405–11.
201. Vollaard EJ, Clasener HA. Colonization resistance. *Antimicrob Agents Chemother* 1994;**38**:409–14.
202. Wald ER, Milmoe GJ, Bowen A, et al. Acute maxillary sinusitis in children. *N Engl J Med* 1981;**304**:749–54.
203. Walker WA. Role of nutrients and bacterial colonization in the development of intestinal host defense. *J Pediatr Gastroenterol Nutr* 2000;**30**(Suppl 2):S2–7.
204. Wang EE, Ohlsson A, Kellner JD. Association of *Ureaplasma urealyticum* colonization with chronic lung disease of prematurity: Results of a metaanalysis. *J Pediatr* 1995;**127**:40–4.
205. Wang M, Ahrne S, Antonsson M, Molin G. T-RFLP combined with principal component analysis and 16S rRNA gene sequencing: an effective strategy for comparison of fecal microbiota in infants of different ages. *J Microbiol Methods* 2004;**59**:53–69.
206. Weiss A, Brinser JH, Nazar-Stewart V. Acute conjunctivitis in childhood. *J Pediatr* 1993;**122**:10–4.
207. Weiss K, Laverdiere M, Lovgren M, et al. Group A *Streptococcus* carriage among close contacts of patients with invasive infections. *Am J Epidemiol* 1999;**149**:863–8.
208. Wells CL, Maddaus MA, Jechorek RP, et al. Role of intestinal anaerobic flora in colonization resistance. *Eur J Clin Microbiol Infect Dis* 1988;**7**:107–13.
209. Whittaker CJ, Klier CM, Kolenbrander PE. Mechanisms of adhesion by oral bacteria. *Annu Rev Microbiol* 1996;**50**:513–52.

210. Williams C. Occurrence and significance of gastric colonization during acid-inhibitory therapy. *Best Pract Res Clin Gastroenterol* 2001;**15**:511–21.
211. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011; **334**:105–8.
212. Yagupsky P, Landau D, Beck A, et al. Carriage of *Streptococcus pyogenes* among infants and toddlers attending day-care facilities in closed communities in southern Israel. *Eur J Clin Microbiol Infect Dis* 1995;**14**:54–8.
213. Yoshioka H, Iseki K, Fujita K. Development and differences of intestinal flora in the neonatal period in breast-fed and bottle-fed infants. *Pediatrics* 1983;**72**:317–21.
214. Young LS, LaForce FM, Head JJ, et al. A simultaneous outbreak of meningococcal and influenza infections. *N Engl J Med* 1972;**287**:5–9.
215. Yow M. Development of *Proteus* and *Pseudomonas* infection during antimicrobial therapy. *JAMA* 1952;**149**:1184–8.

CHAPTER 6

Epidemiology and Biostatistics of Infectious Diseases

Eugene D. Shapiro • Robert S. Baltimore

Clinicians care for individual patients, each of whom has certain unique problems. Clinicians base their decisions on a panoply of factors that include features of the acute and chronic medical problems of the patients as well as features of both their personalities (e.g., How likely are they to comply with a certain therapeutic regimen?) and their private lives (e.g., Can they afford a certain medication? Are they planning to travel out of the country in the next few days?).

In contrast, epidemiologists study groups of people. They draw conclusions from studies of large numbers of patients and usually base conclusions on probabilities and biostatistical analyses of average (mean) outcomes.

Fortunately, these two approaches—the individualistic approach of the clinician and the probabilistic approach of the classic epidemiologist—are not incompatible. Indeed, each can illuminate the other. So it is that a chapter on epidemiology and biostatistics is included in a clinical textbook of pediatric infectious diseases. The goal of this chapter is not to make the reader an expert in these fields—courses and textbooks are designed for that purpose.* Instead, the goal is to summarize how selected key aspects of epidemiology and biostatistics can be applied to understand studies that will improve our ability to evaluate and treat children with infectious diseases. Readers concerned with the epidemiology of a particular infection should consult the appropriate chapter.

EPIDEMIOLOGIC STUDIES

Design of Studies

Overview

Epidemiologic studies generally are designed to be either descriptive or analytic. In *descriptive* studies, the goal is to describe a population (e.g., the clinical manifestations and prognosis of children with tuberculosis). In *analytic* studies, the goal is to assess associations between two or more variables (e.g., whether children who receive an experimental vaccine are less likely to develop the infection that the vaccine is intended to prevent than are controls). Indeed, analytic studies usually are designed to assess whether a causal association exists between the variables (e.g., Is a vaccine efficacious? Do children who attend daycare centers have a higher risk of acquiring infections caused by certain bacteria or viruses?). Of course, categorizing a study so easily may not be possible. In a study that is primarily descriptive, causal associations within subgroups may be assessed. For instance, in a study of the clinical epidemiology of tuberculosis in children, the investigators may compare the mortality rates of younger children with those of older children or the mortality rates of children with tuberculous meningitis with those of children who have infection at other sites.

Most studies, whether descriptive or analytic, seek to reach conclusions about the particular group that is being assessed (e.g., children with tuberculosis). However, because studying all persons with the condition of interest is not possible, investigators inevitably study a *sample* of persons and hope that the conclusions drawn from studying the sample apply to the entire population of interest (the *target population*). The *validity* of a study refers to the extent to which the results are true (accurate). The extent to which the conclusions drawn from the study sample (e.g., children with tuberculosis at Bellevue Hospital in New York City) are valid for the target population (e.g., all children with tuberculosis in New York City) is a measure of the study's *internal validity*. The extent to which the conclusions drawn from the study sample are valid for a less restrictively defined, larger population (e.g., all children with tuberculosis in the United States) is a measure of the study's *external validity*, which also is called its *generalizability*. To ensure that a study is both valid and generalizable, investigators must take steps to protect against a variety of potential biases (discussed later) that may distort the results of a study.

Elements of an Analytic Study

Epidemiologists refer to the major elements of an analytic study as the *exposure* and the *outcome*. The *exposure* is the factor that the investigator hypothesizes is related causally to the outcome of interest. In this context, the term *exposure* is not used in the classic sense of potential contact with an infectious agent; rather, it is any factor (e.g., living at a certain altitude, race, receipt of either a vaccine or a medication, smoking cigarettes) that may be associated causally with the outcome. The *outcome* is the effect (e.g., disease caused by a particular bacterium) that putatively is related causally to the exposure. The

*References 15, 26, 28, 30, 31, 53, 54, 57.

putative association may be one in which the exposure either causes or prevents the outcome of interest.

Before the specific type of study is defined, several additional elements of a study must be considered. One is how the *sample* for the study is selected. In general, investigators select a sample of the population either on the basis of exposure (e.g., patients who either received or did not receive a vaccine or who either attend or do not attend group daycare) or on outcome (e.g., patients who either had or did not have pneumococcal bacteremia or patients who either died or survived). Another key element is the *timing* of the study in relation to the timing of exposure and outcome. In studies in which the timing is *historical*, both the exposure and the outcome occurred before the study was initiated (e.g., a case-control study of a vaccine's efficacy in which cases with disease are identified from historical logbooks and antecedent receipt of the vaccine is determined from medical records). Timing may be *concurrent*—that is, both the exposure and the outcome occur after the study is initiated (e.g., a randomized clinical trial of the efficacy of a new antibiotic). Timing also may be *mixed*, a mixture of historical and concurrent (e.g., a study of the current intelligence quotient of children who previously had aseptic meningitis).

A final important element of a study is its *direction*. Direction is used to describe the order in which outcome and exposure are assessed. It may be done in a *forward* direction, from exposure to outcome (e.g., in a clinical trial of a vaccine's efficacy, the exposure [receipt or nonreceipt of the experimental vaccine] occurs first and the occurrence of the outcome [the infection that the vaccine is designed to prevent] is determined subsequently). Alternatively, a study's direction may be *backward*, from outcome to exposure (e.g., in a case-control study, the outcome [e.g., pneumococcal bacteremia] is determined first, and exposure [e.g., previous receipt of pneumococcal vaccine] is determined subsequently). Alternatively, exposure and outcome may be determined simultaneously (e.g., a survey in which determination of whether subjects have sickle-cell disease and whether they are taking penicillin occurs at the same time). A study's direction can have important implications for inferences about causal associations; for example, even though a strong statistical association may exist between sickle-cell disease and the use of penicillin, concluding that the penicillin caused the sickle-cell disease would be erroneous. Although such an inference obviously is not plausible in this instance, the dangers of making erroneous inferences about causality are very real when exposures and outcomes that are less well understood are studied.

Although the terms *prospective* and *retrospective* are used widely, substantial variability exists in how they are applied. The term *prospective* is used to describe studies in which selection of the sample is based on the exposure, in which the timing is concurrent, or in which the direction of the study is forward (from exposure to outcome). The term *retrospective* is used to describe studies in which selection of the sample is based on the outcome, in which the timing is historical, or in which the direction of the study is backward (from outcome to exposure). Referring to the specific elements of the study is preferable to using the terms *prospective* and *retrospective*, which are applied so imprecisely.

Types of Studies

Epidemiologic studies may be classified as either experimental or observational. In *experimental studies*, the exposure is assigned by the investigators (e.g., in a clinical trial of an experimental vaccine, the investigator assigns subjects to receive either the experimental vaccine or a placebo). In *observational studies*, the exposure occurs naturally (e.g., rainfall in a study that assesses the effect of the amount of rainfall on rates of mosquito-borne infections), is selected by the subjects or their parents (e.g., attendance at group daycare in a study that assesses the frequency of infectious illnesses in children who attend and others who do not attend group daycare), or is assigned in the course of regular medical care (e.g., receipt of an approved vaccine in a case-control study of the effectiveness of the vaccine). Observational studies also are called *surveys*.

Experimental Studies

The paradigm for an experimental study is the *randomized clinical trial*, which is a special type of *longitudinal cohort study* in which the exposure is assigned randomly to the subjects by the investigators. In randomized trials, the direction of the study always is forward, selection (or categorization) of subjects always is based on exposure (i.e., exposure is determined at the time of randomization), and timing always is concurrent (both exposure and outcome are determined during the real period of the study). Randomized trials are the *sine qua non* for evaluating the effect of new agents designed either to prevent (e.g., vaccines, prophylactic drugs) or to treat (e.g., antimicrobial agents) diseases. By randomly allocating subjects to either receive or not receive the agent that is being tested, potential bias is minimized. Theoretically, if the size of the sample is adequate, the only difference between the groups is whether they received the experimental agent. Consequently, if the study is conducted properly, inferring that statistically significant differences in outcomes between the groups were related causally to the experimental agent is reasonable. For this reason, in most instances, the efficacy of new therapeutic agents or new vaccines must be demonstrated in clinical trials before the U.S. Food and Drug Administration (FDA) will approve them (although in some instances new products are approved if criteria for safety and for some surrogate end point, such as a serologic correlate of immunity, are met).

The advantages and disadvantages of randomized trials are summarized in Table 6-1. Although clinical trials are the gold standard for investigators who wish to design a scientifically valid study, they do have numerous limitations. A major problem is that clinical trials usually are expensive. Subjects need to be selected, enrolled, and monitored longitudinally to detect the outcomes. When the outcome is a disease that is relatively rare (e.g., pneumococcal bacteremia), large samples are necessary to provide adequate statistical power. Because sponsors of

TABLE 6-1 **Randomized Clinical Trials**

Advantages

Gold standard for scientific validity

Randomization ensures unbiased allocation of the exposure (e.g., a new drug or an experimental vaccine)

Blinding ensures unbiased assessment of outcomes

Disadvantages

Poor statistical power for rare diseases

Requires large samples

Requires longitudinal follow-up

Logistically complex and expensive

Impaired generalizability when the study population differs from the ultimate target population

Ethical issues

a. Requires informed consent

b. Difficult to use to evaluate approved (presumably efficacious) products

clinical trials usually want to test a new drug or an experimental vaccine under conditions that will maximize the chance that it will be found to be efficacious, patients with comorbid conditions (e.g., sickle-cell disease, asplenia, metastatic cancer) that might affect their response to the new agent often are excluded from the study. If the subjects who are excluded are an important part of the target population for the new agent (e.g., the patients with comorbid conditions are likely to be at high risk for serious complications of the disease and thus potentially could benefit greatly from an effective new intervention), the generalizability of the results of the clinical trial may be impaired.

Most randomized clinical trials are conducted in a double-blind manner; that is, neither the investigators nor the subjects know whether they received the experimental intervention (e.g., a new drug) or the comparison agent (e.g., either a placebo or an agent used in the standard manner). Double-blinding helps ensure lack of bias in ascertainment of the outcome because neither the subject nor the investigator can be influenced either to (or not to) seek medical care or undergo diagnostic tests on the basis of which of the interventions was received.

Randomized clinical trials may pose difficult ethical problems because the new (and potentially efficacious) agent is not given to the controls. By the time that clinical trials are begun, usually some evidence suggests that the agent is efficacious (e.g., preliminary studies showing that a new vaccine induces antibodies). Accordingly, some patients and patient advocates might suggest that withholding a potentially efficacious therapeutic agent or a vaccine from persons at risk is not ethical; consequently, it may be difficult to have persons agree to be potential controls in studies of a promising new therapy.

Problems also may arise when approval of a product for the target population is based on studies that are conducted in a different population. For example, polyvalent pneumococcal polysaccharide vaccine was approved in the United States for the elderly and for adults with chronic conditions, such as chronic obstructive pulmonary disease and congestive heart failure, that put them at increased risk of acquiring serious pneumococcal infections. However, the data on which approval was based were derived from clinical trials conducted among young gold miners in South Africa who were at risk largely not because of their age or underlying illnesses but because of the conditions in which they lived and worked. Reports of vaccine failure and poor antibody response to the vaccine in the target population in the United States led to questions about the vaccine's efficacy. However, once the vaccine was approved, conducting a randomized clinical trial in the target population was difficult ethically because it would mean withholding, on a random basis, an approved (and presumably efficacious) vaccine from patients at risk. Consequently, all but one of the postlicensure studies of this vaccine's efficacy were observational studies.

Conducting an experimental study in which the exposure is not assigned randomly is possible. For example, one might conduct an experimental cohort study in which volunteers receive a certain intervention (e.g., a new drug) while controls receive no intervention. Both groups would be monitored forward in time while undergoing surveillance for the outcome event. Although such a study incorporates some of the features of a randomized clinical trial, because the intervention is not assigned randomly, such studies are subject to significant biases.

There are also situations in which special kinds of clinical trials are needed. For example, cluster randomized clinical trials are now common. In this type of experimental study, the unit of randomization is not the individual but rather a group or "cluster" (e.g., randomization by community). This can be important in trials of, for example, experimental vaccines or other interventions that may have strong herd immunity effects so that unimmunized individuals in a community with vaccinees may be at lower risk than unimmunized individuals in the general population. However, this type of study also presents special challenges in its design and statistical analysis.[8] Another special kind of clinical trial is a noninferiority or an equivalence trial, designed to show that the effectiveness of a new agent is not inferior to a standard agent (by a specified amount) or that its effect is equivalent (with specified parameters). These kinds of trials also present special problems in design and analysis.[45,55,63]

Observational Studies

Cohort Studies

The direction of cohort studies always is forward, and the selection (or categorization) of subjects always is based on exposure; however, the timing may be concurrent, historical, or mixed. Thus, a cohort study may identify a cohort of subjects at a point (or at several points) in time in the recent or remote past, categorize them regarding their status with respect to the exposure (e.g., receipt of either a vaccine or a drug), and then monitor them forward in time for the occurrence of the outcome event (e.g., an infection) until a certain point in time, which could be in the past, present, or future. As in a clinical trial, subjects must not have the outcome at the time of enrollment in the study.

Cohort studies share many of the disadvantages of randomized clinical trials (see Table 6-1) but have the additional disadvantage that because they are not experimental they are subject to many potential biases. On the other hand, cohort studies have many practical advantages (e.g., the timing can be historical, so conducting a 30-year follow-up study in just months is possible), and because they are observational studies, they usually have fewer potential ethical problems than does a randomized trial. In addition, for rare exposures, a critical factor is to be able to base selection of subjects on exposure.

Case-Control Studies

In both clinical trials and observational cohort studies, the selection of subjects is based on their exposures and they are monitored in a forward direction until the outcome is determined. In *case-control studies*, the process is reversed. Subjects are selected on the basis of outcomes. The cases have the outcome, usually a disease; the controls do not have the outcome. The direction of the study is backward—the previous exposure is ascertained after the subjects are selected. The timing of case-control studies usually is historical, but it may be mixed; the timing of the exposure always is historical, but the timing of the outcome may be either historical or concurrent. For example, an investigator may conduct a case-control study of a vaccine's effectiveness in which persons who are infected (cases) are identified concurrently through active surveillance of a microbiology laboratory. Because case-control studies are nonexperimental and the exposure (and, sometimes, the outcome) occurred in the past, the potential for bias is great, both in selection of the sample and in ascertainment of both the exposure and the outcome. The advantages and disadvantages of case-control studies are shown in Table 6-2. Recently, use of "sham" outcomes and/or exposures in addition to the true outcomes or exposures of interest has been suggested as a means of assessing the internal validity of case-control studies.[60]

Cross-Sectional Studies

Unlike cohort studies (both experimental and observational), in which subjects are monitored forward from exposure to outcome, and case-control studies, in which subjects are monitored backward from outcome to exposure, in cross-sectional studies, outcome and exposure are determined at the same time. In cohort studies, causal inference is made from cause to effect, whereas in case-control studies, causal inference is made from effect to cause. In cross-sectional studies, determining whether the exposure preceded the outcome may not be possible. Consequently, making valid causal inferences from a cross-sectional study also may not be possible.

Selection of subjects for a cross-sectional study may be based on outcome, exposure, or neither. However, because cross-sectional studies include only persons with prevalent outcomes, they are particularly problematic for studies of infectious diseases because patients whose illnesses (outcomes) either resolved or resulted in death before the study is conducted are not counted as having the outcome. Consequently, cross-sectional studies are more suitable to the study of chronic conditions and rarely are used in studies of infectious diseases.

TABLE 6-2 **Case-Control Studies**

Advantages

Statistically powerful method to assess outcomes that are rare or delayed

Logistically easier and more efficient than large experimental or observational cohort studies

No longitudinal follow-up, so it can be completed relatively quickly and inexpensively

Ethically acceptable because it is an observational study

Disadvantage

Subject to many potential biases

Analysis of Epidemiologic Studies

The results of epidemiologic studies with dichotomous exposures and outcomes often are displayed in a *2 × 2 contingency table*. However, the way that the results are analyzed statistically depends on the type of study.

Cohort Studies

Analysis of longitudinal cohort studies (both experimental trials and observational studies) is shown in Table 6-3. The measure of association between the exposure and the outcome is the *relative risk* (sometimes called the *risk ratio*), which is an expression of the *magnitude* of this association in the study sample and represents an *estimate* of the association in the population from which the study sample was drawn. A relative risk of 1 indicates that no association exists between the exposure and the outcome, a relative risk greater than 1 indicates that the exposure is associated with an increased risk of the outcome, and a relative risk less than 1 indicates that the exposure is associated with a decreased risk of the outcome. The *attributable risk* (the risk of the outcome that is attributable to the exposure) is calculated as the risk in exposed subjects minus the risk in unexposed subjects. Of course, testing the statistical significance of any association is necessary because the relative risk could be greater than 1 or less than 1 by chance (see later sections on stochastic statistics and confidence intervals).

TABLE 6-3 **Analysis of Experimental or Observational Cohort Studies**

	Outcome Present	Outcome Absent	Total
Exposed	a	b	a + b
Unexposed	c	d	c + d
Total		a + c	b + d

Risk in exposed subjects: a/(a + b).
Risk in unexposed subjects: c/(c + d).
Relative risk: (a/a + b) ÷ (c/c + d).
Attributable risk: (a/a + b) − (c/c + d).

These analyses assume that no attrition exists among the subjects in the study. However, because of death, migration out of the study area, and loss to follow-up, as well as variation in the time of enrollment (enrollment in either a clinical trial or an observational cohort study may occur over a prolonged period or, indeed, throughout a study), variation virtually always exists among subjects in the duration of time that they are at risk for the outcome. If the average time at risk among the exposed and the unexposed subjects is equal, the analysis shown in Table 6-3 is likely to be valid. However, if a great deal of irregular attrition or new enrollment occurs during the study, the denominator is expressed better as person-time at risk (e.g., person-months, person-years) rather than as the number of persons in the group. When this method is used, the rate then is called the *incidence density rate* and the index of comparison is called the *incidence density ratio.*

In studies of infectious diseases, the outcome event often is the occurrence of an infection. Frequently, persons who become infected recover and may remain at risk for the outcome event again. Nonetheless, a subject usually should be censored (i.e., removed from the study) once the outcome event occurs. The fact that the outcome occurred may indicate that the subject has an increased risk for the outcome, independent of the exposure that is being assessed. For example, consider a study of the protective efficacy of a conjugate pneumococcal vaccine in which one of the subjects (whether the subject is a vaccinee or a control does not matter) experiences three or four episodes of invasive infection caused by pneumococci (perhaps because the subject has a previously unrecognized underlying condition, such as acquired immunodeficiency syndrome or an immunoglobulin deficiency). The estimate of the vaccine's efficacy would be distorted substantially if each of the outcome events were counted. Certainly, in a primary analysis, only the initial event should be counted. Another reason to censor the subject is that in some instances the outcome event may be fatal. If some subjects die of the outcome (and, therefore, must be censored), not censoring subjects with the outcome who survive is, in effect, incorporating an assessment of the exposure's effect on prognosis and not just on the risk of the outcome event.

Using a different method to adjust for unequal durations of follow-up in a study may be necessary, particularly when a long latent period exists between exposure and outcome (e.g., a condition that often is met when the study involves the effect of a potential carcinogen). In such instances, simply to sum the total person-times of exposure may be misleading because the outcome is more likely to occur many years after exposure. For example, although the total person-time at risk would be the same, the risk of an outcome developing clearly may be substantially different for 500 persons, each of whom is monitored for 2 years after exposure, than it may be for 40 persons, each of whom is monitored for 25 years. In such situations, the analyses must be adjusted for variation in the length of follow-up, which can be done with the use of *survival analysis* (also known as *life-table analysis*). The two basic methods of survival analysis, the *actuarial* method and the *Kaplan-Meier (product-limit)* method, are described in detail elsewhere.[34]

TABLE 6-4 **Analysis of Case-Control Studies**

	Cases (Outcome Present)	Controls (Outcome Present)
Exposed	a	b
Unexposed	c	d

Proportion of exposed cases: a/(a + c).
Proportion of exposed controls: b/(b + d).
Odds of exposure among cases: a/c.
Odds of exposure among controls: b/d.
Odds ratio: (a/c) ÷ (b/d) or (ad) ÷ (bc).

Case-Control and Cross-Sectional Studies

Analysis of a case-control study (with dichotomous outcomes and exposures) is shown in Table 6-4. Because selection of subjects is based on their outcomes, one cannot calculate the risk of development of the outcome, as one would in a longitudinal study (such as a clinical trial). Instead, one calculates the proportion of each group (cases and controls) that is exposed. The measure of association in a case-control study is the *odds ratio.* The *odds* of some occurrence means the probability that it will occur divided by the probability that it will not occur. In a case-control study, we are interested in the odds of exposure. The odds ratio is the ratio of the odds of exposure among cases (a/c) divided by the odds of exposure among controls (b/d). For rare events, the odds ratio closely approximates the relative risk of exposure that would be found in a longitudinal study of the same association. Use of a method known as *risk set sampling* allows odds ratios from case-control studies to approximate a risk ratio even if the outcome is not rare.[4] Cross-sectional studies are analyzed either like a case-control study (if the selection of subjects was based on their outcomes) or like a cohort study (if the selection of subjects was based on their exposures).

Summary Statistics

Perhaps the most basic use of statistical analysis is to summarize data. This type of statistical analysis often is called either *summary* or *descriptive* statistics. The specific summary measures that are used depend, in part, on whether the variables being summarized are *continuous* (variables with equal distance between intervals) or *categorical* (variables with two or more discrete categories). Age, weight, height, temperature, concentration of creatinine in serum, and the number of hours spent in group daycare all are examples of continuous variables (sometimes called *dimensional* or *quantitative* variables). Race, sex, country of residence, and whether a patient is being ventilated artificially are examples of categorical variables (sometimes called *discrete* variables). Of course, continuous variables can be analyzed categorically (e.g., age can be divided into either a *dichotomous* variable [<45 years or

≥45 years] or a *polychotomous* variable [20 to 44 years, 45 to 59 years, and ≥ 60 years]).

Continuous Variables

A *frequency distribution* is classification of the values of a sample of continuous variables into successive categories (e.g., for a sample of different ages, one might place each value into different 5-year [birth to 4 years, 5 to 9 years, etc.], 3-year [birth to 2 years, 3 to 5 years, etc.], or 1-year [0 year, 1 year, 2 years, 3 years, etc.] categories) and expression of the frequency of values within each category.

A sample of values of a continuous variable also can be summarized mathematically by describing its central tendency, shape, and spread. The *central tendency* can be expressed as a *mean* (or *average*), a *median* (the middle value of the sample), or a *mode* (the most frequent value in the sample). The mean value is calculated by summing all the individual values in the sample and dividing by the number of individual values. Thus, for a sample that is composed of subjects aged 1, 1, 2, 2, 2, 3, 3, 4, 10, and 12 years, the mean age is 4 years (40/10), the median age (the age for which half the subjects are older than and half are younger than its value—if the number of subjects is even, the median is the mean of the two middle values) is 2.5 years [(2 + 3)/2], and the mode is 2 years. These summary measures provide only a limited view of the data. For example, the mean of a distribution can be shifted in the direction of extreme outlying values; in the example, the mean is 4 years even though 70 percent of the subjects are younger than 4 years. Likewise, from the median value alone, one would not know whether outlying values existed. Consequently, describing the spread of a distribution is important.

The *spread* of a sample may be expressed by its *range* and by its *standard deviation*. The range of a sample is the interval between the highest and the lowest value in its distribution. In the example, the range is from 1 to 12 years. Another useful way of mathematically summarizing the spread of a distribution is to express the interval between each individual value and the mean value of the sample. To summarize these values, one cannot simply sum all the differences because the sum always equals zero (the sum of the positive differences equals the sum of the negative differences). To avoid this problem, the standard deviation of a sample is used. This parameter is calculated by taking the square root of the *variance* of the sample. The variance is the sum of the squares of the differences from the mean of each individual value, divided by the number of *degrees of freedom* of the sample (which is equal to the number of values in the sample minus one, or $N - 1$). The sum of the squares is divided by the number of degrees of freedom of the sample ($N - 1$), rather than by N (the actual number in the sample), because this calculation is thought to represent more accurately the true variance of the population from which a sample is taken.

To calculate the standard deviation of the sample in the example, one must determine the variance, which is the sum of the squares of the difference of each individual value from the mean of the sample divided by the degrees of freedom (number of values minus one):

$$(2[4-1]^2 + 3[4-2]^2 + 2[4-3]^2 + [4-4]^2 + [4-10]^2 + [4-12]^2) \div (10-1)$$

which equals 132 ÷ 9, or 14.6667 years. The standard deviation simply is the square root of the variance, which is 3.8297 years.

In the medical literature, investigators often use the *standard error of the mean* (which is the square root of the variance divided by the square root of the number in the sample) to express the spread of the frequency distribution of a sample. However, the standard error actually is designed to be a measure of the standard deviation of the means of repeated samples from a single source population. Because the standard error always is smaller than the standard deviation of a single sample, it gives the erroneous impression that the spread of a sample is smaller than it actually is. A large sample with a large spread (and a large standard deviation) may have a small standard error. The standard error should not be used to describe the spread of a single sample.

The general shape of the frequency distribution can be inferred from the parameters given earlier. The characteristics of the most important distribution in statistics, the familiar bell-shaped curve of the normal distribution, are shown in Figure 6-1 and are discussed in a later section about diagnostic tests. The peak value of any distribution is the mode. A distribution may be bimodal, trimodal, and so on if it has two or more modes. A

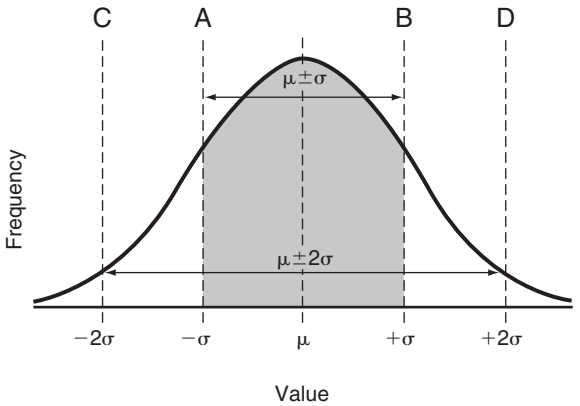

FIGURE 6-1 ■ Theoretic normal (gaussian) distribution showing where 1 and 2 standard deviations (SD) above and below the mean would fall. The Greek letter mu (μ) stands for the mean in the theoretic distribution, and the Greek letter sigma (σ) stands for the standard deviation in the theoretic population. In this figure, the area under the curve represents all the observations in the distribution. One standard deviation above and below the mean, shown in dark gray and represented by the distance from point A to point B, is equivalent to 68 percent of the area under the curve, and therefore 68 percent of the observations in a normal distribution fall within this range. Two standard deviations above and below the mean, represented by the area under the curve between lines C and D, are equivalent to 95.4 percent of the area of the curve or 95.4 percent of the observations in a normal distribution. (From Jekel JF, Elmore JG, Katz DL. *Epidemiology, biostatistics, and preventive medicine.* Philadelphia: WB Saunders; 1996. p. 118.)

frequency distribution may be asymmetric, or *skewed*. If the mean is greater than the median (as in the example), the distribution is skewed to the right; conversely, if the mean is less than the median, the distribution is skewed to the left.

An investigator may want to summarize the relationship between two different continuous variables, which can be done with the use of linear regression, in which a straight line is constructed to represent the relationship between a continuous *dependent variable* (y) and a continuous *independent variable* (x). An equation ($y = a + bx$) can be created that represents the best "fit" to describe the relationship between the actual values of y and x, in which a (the *intercept*) is the value of y when x equals zero and b (the *regression coefficient*) is the slope of the line. Such an equation allows us to summarize the relationship between these variables and to extrapolate the value of each variable for conditions that may not have been observed in the sample. For example, one can calculate the incremental value of y for each interval change in the value of x.

Categorical Variables

The measure generally used to summarize categorical data is called a *rate* or a *proportion*. Rates consist of a numerator and a denominator. The numerator represents the actual number of persons in the sample who have the characteristic of interest (e.g., those with fever, those with tuberculosis, those who received a vaccine), and the denominator is the total number of persons in the sample. The persons in the numerator must be included in the group that the denominator represents, and the persons in the denominator must be able to have the characteristic that the numerator represents. For example, if one were interested in the rate of testicular cancer in the population, women should not be included in the denominator because they cannot have testicular cancer. Some experts believe that rates, by definition, should include a time factor (e.g., incident cases per person-months or per year). However, the term is used widely to refer to a proportion of a sample, as described earlier.

One of the most important rates that is calculated in epidemiologic studies is the *incidence rate* or *attack rate*, which is the number of persons in whom a certain outcome develops divided by the number of persons in whom the outcome could have developed during a specified period. For example, a comparison may be made of the incidence rates of an infection over time in two groups in which the interventions given to prevent an infection differed (e.g., vaccine, placebo).

Another important rate, the *prevalence rate*, is the number of persons in whom a certain outcome develops divided by the total number of persons in the group at a specific point in time. In calculating the incidence rate, persons who at *zero time* (before the study begins) already have the outcome (e.g., prevalent cases) are excluded from both the numerator and the denominator. At time zero, the numerator of the incidence rate is zero. Thus, if one were interested in the incidence rate of lung cancer, persons who had lung cancer at the beginning of the study period would be excluded. The prevalence rate, by contrast, is measured at one point in time, and the numerator includes all persons with the outcome at that time.

Certain features of many infectious diseases affect both the prevalence rate and the incidence rate as measures of the occurrence of disease. The prevalence rate of any condition is related to its chronicity and the mortality rate associated with it; conditions that are common and chronic but are not associated necessarily with immediate mortality (e.g., atherosclerotic heart disease) have a relatively high prevalence rate. On the other hand, the prevalence rates are relatively low for conditions that either have a low mortality rate and resolve completely (e.g., many infectious illnesses) or have a high short-term mortality rate. To get a reliable estimate of the importance of such conditions, one must determine their incidence rates.

To describe variations in occurrence of infections over limited periods within a single population, such as a city or a state, a simple *numerical frequency* (number of cases or infections) often is used—daily or weekly (during an epidemic), monthly (to reflect seasonal patterns), or annually (to compare successive years). Comparisons among different populations or subgroups within a population or at widely separated times in the same population require use of the *incidence rate*, or *attack rate*.

For infections transmitted by contact, the frequency with which infection or disease occurs among exposed susceptible individuals provides a measure of the infectivity of the agent. This frequency, called the *secondary attack rate*, is defined as the number of contacts who become infected (or who develop disease) within the maximal incubation period of the infectious agent divided by the number of susceptible persons in the exposed group or population in question. The secondary attack rate is applied most usefully only to closed groups, households, or classrooms, where exposure safely can be presumed for all members. The first, or primary, case is the presumed source of exposure; other initial cases that occur within the minimal incubation period or since presumed exposure of the primary cases are called *co-primary cases*. In calculating the secondary attack rate, primary and co-primary cases are excluded from the numerator and the denominator. Cases that occur subsequently within the maximal incubation period constitute the secondary cases. Cases that develop later are excluded because it is presumed they occurred from either a different exposure or from tertiary spread. The exclusion of immune individuals from the denominator usually is feasible only for diseases sufficiently characteristic clinically that the history serves to identify them (e.g., measles, chickenpox). Although immune individuals are not readily identifiable in the case of common respiratory diseases, the secondary attack rate based on all exposed members of the group still may be a useful measure. Its utility decreases, however, when the period of communicability of the primary case (as with *Mycoplasma pneumonia* infections) is longer than the incubation period because distinguishing between secondary and tertiary cases becomes difficult.

Finally, the occurrence of death caused by a specific disease typically is expressed in two different ways. One,

the *cause-specific mortality rate*, is defined as the number of deaths from the disease during a specified period of time divided by the total number of persons in the population. It is a measure of the consequences of the disease in the population. The effect of the disease among infected individuals is reflected by the *case-fatality rate*, which is defined as the number of deaths due to the infection in a specified period of time divided by the number of persons infected during the same period.

Bias

Bias occurs when the estimate of the association between the exposure and the outcome in the sample differs systematically from the true value in the population. Bias in the estimate of the association is different from bias in the measurement of individual variables. The latter bias (which may occur, for example, if an instrument such as a thermometer is calibrated incorrectly) may or may not affect the estimate of association in an epidemiologic study. Biased measurements usually affect the exposed and the unexposed groups equally. By contrast, *analytic bias* is the effect of *differential error (nonrandom error)* on the assessment of the relationship between exposure and outcome. Analytic bias can occur because of *information bias* (as a result of differential error in ascertainment of either the exposure or the outcome), *sample distortion bias* (because the joint distribution of the exposure and the outcome in the sample chosen for the study is not representative of the distribution of these factors in the target population), and *confounding bias* (because the joint distribution of one or more variables that independently are related both to the exposure and to the outcome is unequal in the groups that are being compared). Ensuring that bias does not affect a study is critical to its validity. Furthermore, although using statistical methods to adjust for certain sources of bias (particularly for confounding bias) in analysis of the results of a study sometimes is possible, adjustment for bias after the fact may be impossible.

An example of information bias is *detection bias*, which occurs when there is differential detection of the outcome in the exposed and the unexposed groups. For example, numerous studies in which clinical scales to assess whether children with fever have a serious illness have been developed.[38] Often, however, the "serious illness" may include certain abnormal laboratory test results (e.g., an abnormal radiograph of the chest or a low concentration of sodium in serum). Because children with high scores on these scales were more likely to undergo diagnostic tests, detection bias may have occurred because similar abnormalities (e.g., a "silent" pneumonia or a mildly depressed concentration of sodium in serum) might go undetected in the "unexposed" group (children with lower scores on the clinical scale), a much smaller proportion of whom underwent a diagnostic test to detect a possible outcome. The best way to avoid having information bias is to use standardized, consistent methods to ascertain both the exposure and the outcome and to blind subjects and investigators to ensure that no differential ascertainment of either the outcome (in longitudinal studies) or the exposure (in case-control studies) occurs.

Sample distortion bias occurs when the sample in a study is not representative of the target population. However, a nonrepresentative sample does not lead to bias necessarily; bias in an assessment of the association between exposure and outcome occurs only if differential distribution of the exposure (or differential risk of the outcome) occurs in the subjects who are selected for the study versus the overall target population. This distribution can be the result of *selection bias*. For example, consider a case-control study of the effectiveness of an approved vaccine in which the control subjects (the uninfected patients) were chosen from private practices in affluent suburbs, whereas the case subjects (the infected patients) were any patients in whom a serious infection developed and who were hospitalized. The association between antecedent vaccination and infection probably is biased because the controls are not a representative sample of the population from which the cases emerged and because the proportion of them likely to have been vaccinated is higher than in the group of all uninfected persons in the population. Another possible source of sample distortion bias is differential loss to follow-up in a longitudinal study. The potential for sample distortion bias can be minimized by maximizing the probability that a representative sample will be selected, ideally by random sampling (or some other kind of unbiased sampling), and by minimizing loss to follow-up in longitudinal studies.

Confounding bias occurs when the association between exposure and outcome is distorted by a variable (a *confounder*) that is distributed unequally between the groups, is associated independently with both the exposure and the outcome, and is not in the causal pathway from exposure to outcome. One example of confounding bias is *susceptibility bias*, which occurs when the risk of the subjects (to development of the outcome) differs in the exposed and unexposed groups, independent of the exposure. For example, imagine a longitudinal study of the protective efficacy of a vaccine against *Haemophilus influenzae* type b in infants. Ensuring that an equal proportion of subjects in the vaccinated and the unvaccinated groups attended group daycare is important because attendance at group daycare is associated with an increased risk of developing infection with *H. influenzae* type b. If a substantially higher proportion of vaccinees than of controls attended group daycare, their attendance might confound assessment of the vaccine's efficacy and result in a biased (lower) estimate of the effect of the vaccine. By contrast, if a disproportionate number of controls attended group daycare, the estimate of the vaccine's efficacy might be biased in the opposite direction (it would be erroneously high because controls would have a disproportionately higher risk of acquiring infection with *H. influenzae* type b than vaccinees would).

In the previous example, adjusting for the effect of the confounder might be possible by either stratification or multivariable analysis (see later). However, identifying confounders may not always be possible. Random allocation of subjects probably is the best strategy to protect against confounding, especially if potential confounders cannot be identified.

CAUSES OF DISEASE

Historical Perspectives

Although the previously discussed principles apply to all epidemiologic studies, in studies of infectious diseases three additional factors contribute uniquely to who is and who is not affected: (1) the cause is a specific external agent (the infecting organism); (2) transmission of the organism to the host is required; and (3) certain host factors, such as immunity to infection or disease, may affect the outcome. Recognition of these factors (the infecting agent, transmission, and immunity) evolved gradually over many years.[22]

The discipline of epidemiology evolved from the study of great epidemic diseases such as plague, cholera, and smallpox. The periodic waves of these diseases, which were associated with high mortality rates, stimulated the first serious efforts to explain the occurrence of disease on the basis of factors other than supernatural or divine forces.

Fundamental to such explanations was the concept of contagion. This factor long had been implicit in attitudes toward victims of leprosy, as exemplified by such early Christian practices as conducting antemortem funerals for lepers, who then were given a bell and cup and forbidden further human contact or, more drastically, were buried alive or burned at the stake.[52] The English physician Thomas Sydenham (1624-1689) introduced laudanum (derived from opium) as a painkiller; recognized the efficacy of Peruvian bark (quinine) in malaria; and revived the Hippocratic idea of "epidemic constitutions" (of atmospheric nature), which by grafting onto existing illness, gave all concurrent illnesses the character reflecting the then prevailing "constitution." These views persisted in colonial America, where they were expounded by such eminent individuals as Noah Webster (of dictionary fame) and Dr. Benjamin Rush of Philadelphia.[71]

Nonetheless, by the mid-18th century, the theory of contagion had gained acceptance for particular diseases, including measles, syphilis, and smallpox. The theory is alleged to have been exploited in an early act of biologic warfare: Massachusetts colonists reportedly presented the blankets of smallpox victims as gifts to the Indians, who then suffered a decimating epidemic.[18]

The true origin of the concept of immunity is uncertain, but it was applied first in relation to smallpox. Variolation (inoculation of young people with lesion material expected to induce modified, but immunizing, disease) was practiced in China in the 11th or 12th century and in England and the American colonies in the early 18th century. Also popular in rural England at this time was the theory that cowpox, the minor disease acquired from afflicted cattle, induced immunity to smallpox. This theory was verified by Edward Jenner (reported in 1798) and resulted years later in general acceptance of cowpox vaccine (vaccinia) to protect against smallpox.

The germ theory of disease was stated explicitly in 1855 by John Snow, an English anesthesiologist who took up cholera epidemiology as an avocation. Snow argued that the causative agent of cholera was a living cell that multiplied with great rapidity but was too small to be seen under the microscopes then in use.[65] Louis Pasteur (1822-1895) formally validated the germ theory by showing that the microorganisms responsible for fermentation were not generated spontaneously but came from the air.[44] On this basis, Joseph Lord Lister revolutionized surgery by using carbolic acid to combat atmospheric germs and minimize "putrification" in surgical procedures.[62]

In Pasteur's wake, bacteria were cultured with great frequency from ill individuals and often were identified erroneously as causal agents. Robert Koch (1843-1910), who first isolated the bacterial causes of tuberculosis and cholera, also was the first to introduce scientific rigor into the proof of primary causation. His famed "postulates," to be satisfied before a causal relationship between a bacterium and a disease could be accepted, required that (1) the presence of the agent be shown in every case by its recovery in pure culture; (2) the agent not be found in cases of other disease; (3) the agent, when isolated, be capable of reproducing the disease in experimental animals; and (4) the agent be recovered in pure culture from such experimental disease.[21]

Koch's postulates since have been modified, largely to meet problems posed by viruses. As obligate intracellular parasites, viruses cannot be "cultivated in pure culture." In addition, they often are host specific and do not produce disease in an animal model. Other considerations that were invoked as elements of proof included the significance of recovery of the agent from diseased tissues, the demonstration of an increase in titer of specific antibody in temporal relation to the disease, and, most conclusive, the specific preventive effect of vaccines containing the viral antigen.[25] One further situation not recognized by Koch is that infections with true pathogens do not always cause disease. We now recognize pathogenicity (defined as the proportion of infections that result in disease) as an important characteristic of infectious disease agents.

General Concepts

Causation of infectious diseases is defined in terms of the *primary cause* and *contributing factors* (or secondary causes). The former is the specific microorganism (the agent of disease) without which the particular disease cannot occur. Contributing factors affect the likelihood that infection will occur and help determine that disease will result, given infection. Identification of the causative agent may lead to the development of effective means for providing specific protective immunization (e.g., diphtheria and tetanus toxoids, vaccines against polio and measles). Finding the cause of a disease also may lead to other means of control. An example is the discovery of *Legionella pneumophila* as the cause of legionnaires' disease. The discovery of the organism led to the understanding of how the disease is transmitted—via aerosols from cooling towers, which serve as reservoirs for the bacteria.[14,39] Disinfection of these towers has helped to control legionnaires' disease.

Infection and disease are not synonymous, although infection is necessary for an infectious disease to occur. *Infection* denotes replication of organisms in the tissues of a host, usually with an associated immune response;

infection may be either subclinical (asymptomatic) or, if it results in development of overt clinical manifestations, it is denoted as *disease*. There are many examples of infections, such as poliomyelitis and mumps, that may be either asymptomatic or the cause of serious disease in the host. When disease occurs, it may vary in severity among infected individuals. Some infections produce full-blown disease in all infected susceptible individuals; measles is an example. Simple *colonization*, in contrast to infection and disease, is a state in which the organism parasitizes the host at an appropriate given site, replicates, and may persist, but it does not proceed further to cause infection, disease, or induction of immunity. The *carrier state*, in which the organism persists over time and can be infective for others, may occur after colonization, infection, or disease. Examples of organisms that behave in this way include group A streptococcus and *Neisseria meningitidis*.

Many contributing factors, largely related to the host and to the conditions of exposure, determine whether colonization occurs and whether the subsequent processes of infection and disease occur. These contributing or risk factors are many and varied, and from the standpoint of the host may include, but are not limited to, age, sex, race, immune status, genetic constitution, and general state of health, including underlying diseases. Similarly, contributing factors unrelated to the host may include climate, the presence of vectors, quality of sanitation, intimacy of exposure, and socioeconomic conditions. These contributing factors vary among infectious diseases and are discussed in chapters about specific infectious agents.

Factors Related to the Infectious Agent

What characteristics of infectious agents are significant epidemiologically? Properties directly important to the occurrence of disease are properties that relate to perpetuation of the agent as a species, properties that govern the type of contact required to infect humans, and properties that determine the production of disease. Also important are characteristics useful in classification and specific identification of agents. Some important characteristics are *intrinsic*, in that they can be described after appropriate direct examination of the agent. Others can be described only on the basis of the behavior of the agents in the host; they are *host related*.

Intrinsic Properties

Precise classification and identification of agents are basic to the specific recognition of infections and related disease. Both depend on intrinsic properties, including morphology (which alone provides the basis for identifying most higher organisms), chemical composition (the type of nucleic acid being important in viral classification), and antigenic character. The last is central to specific identification of agent isolates and antibodies induced by infection. Requirements for growth or replication provide keys to the identification of some bacteria (e.g., sugar fermentation) and many viruses that replicate optimally or only in cultures of certain types of cells incubated at specified temperatures. Rhinoviruses replicate best in human diploid cells incubated at 33° C.

In recent years, rapid development of species detection and classification by genome identification has occurred. The use of whole-genome analysis, molecular analysis of gene fragments, and various methods of polymerase chain reaction has resulted in reclassification of some microorganisms and of rapid identification of pathogens, including microorganisms that cannot be cultivated by conventional means. Developments in this field are shifting the clinical microbiology laboratory from morphologic and biochemical tests to molecular tests.

Several intrinsic properties relate to transmission and long-term survival of infectious agents. Persistence in the free state outside the host depends on requirements for replication (viruses replicate only within the cells of their host, whereas the nutrient requirements of bacteria often exist in food or milk) and on viability under natural conditions of temperature, moisture, and radiation. The ability of agents to persist determines whether transmission requires direct contact, as with influenza viruses, or can involve indirect mechanisms operating over longer periods. Examples include polioviruses, typhoid bacilli, and the bacterial cause of legionnaires' disease.

The spectrum of animals and arthropods that an agent can infect (the host range) helps determine the possibilities for successful links in the transmission and reservoir mechanisms. The broader the range, the greater the possibilities. Agents that use arthropod vectors include St. Louis encephalitis virus and *Borrelia burgdorferi*, the cause of Lyme disease. The former can be transmitted by a wide variety of mosquitoes, whereas the latter is transmitted by a limited number of species of ticks. Among agents that do not require a vector, many infect only humans (diphtheria bacillus, the meningococcus, and measles virus), whereas others have multiple natural hosts (rabies virus, most of the *Salmonella* group of bacteria).

Elaboration of exotoxins is an intrinsic attribute of many bacteria and contributes in varying degrees to disease pathogenesis and indirectly to immunity in many infections. Another attribute, which can operate in two opposing ways, is susceptibility to chemotherapeutic agents or antibiotics. Successful treatment may shorten the period of communicability, as in group A streptococcal and *Bordetella pertussis* infections, but it may lead to relaxed vigilance for infection; syphilis and gonorrhea are notable examples.

The instability of some intrinsic characteristics as a result of the emergence of genetically different populations because of mutations, selective pressure, gene or plasmid transfer between bacteria, or genetic recombination can be important. One example is the resistance to chemotherapeutic or antibiotic agents that may result from selective pressure (the probable explanation for the rapid acquisition of multiple antibiotic resistance by gonococci and in human immunodeficiency virus [HIV] or plasmid transfer of resistance to antibiotics among enteric bacteria). Antibiotic resistance is of increasing importance, as exemplified by the appearance of multidrug-resistant *Mycobacterium tuberculosis*, penicillin-resistant pneumococci, and methicillin-resistant staphylococci.

Change in antigenic character can diminish the effectiveness of immunity and complicate specific recognition of infection. Influenza A virus is the classic example, with periodic major changes (shift) occurring in either or both crucial surface antigens, hemagglutinin and neuraminidase, associated with pandemic disease and progressive minor changes in hemagglutinin in the interpandemic period. Finally, the emergence of new diseases, such as St. Louis encephalitis, which first affected humans in Paris, Illinois, in 1932, or the appearance of a known disease in a new reservoir, possibly exemplified by the emergence of West Nile virus in North America, can be the result of adaptation of the agent to a new host.[3]

Epidemiologic Properties Relating to the Host

Some epidemiologically important properties of infectious agents can be defined only with reference to specific hosts. Such properties include infectivity, pathogenicity, virulence, and immunogenicity.

Infectivity. Infectivity (ability to invade and multiply in a host) is measured conceptually in terms of the minimal number of infective particles required to establish an infection. This number, which can vary from one host to another and within the same host, depending on the portal of entry, host age, and, in some cases, medications, can be determined only experimentally. Except for benign agents with which challenge of human volunteers is permissible, the infectivity of agents for humans must be inferred from the facility with which they spread in populations or, more directly, from the frequency with which infection develops in exposed susceptible individuals within a reasonable incubation period *(secondary attack rate)*. Some researchers have used prisoners to study infectivity of *Salmonella* and *Shigella*, but the ethics of such studies has been questioned.[35] Measles, varicella, and polioviruses are highly infective because they require few infective particles to cause infection; rubella, mumps, and rhinoviruses have intermediate infectivity; and salmonellae and tubercle bacilli have low infectivity. Infectivity and pathogenicity may vary among strains of the same organism. The infectivity of group A streptococci is related directly to the amount of M protein in the cell wall, and strains of *Staphylococcus aureus* that appear identical in the laboratory may differ strikingly in infectivity and virulence. Additionally, some evidence indicates that strains of influenza A may vary in infectivity and virulence independent of preexisting immunity in the host. Contemporary studies show that variation in certain genes that are not ordinarily expressed in the clinical diagnostic laboratory are responsible for these variations in infectivity.

Pathogenicity. Pathogenicity (ability to induce disease) is measured in terms of the proportion of infections that result in disease. It ordinarily can be determined readily by studies of the incidence and outcome of naturally occurring infections in humans. This proportion may be affected by the size of the infecting dose and numerous host factors, including age. Highly pathogenic agents include typhoid bacilli, rabies, measles, varicella, and rhinoviruses. Agents of intermediate pathogenicity include rubella, mumps, and adenoviruses; polioviruses and the tubercle bacillus have low pathogenicity.

Virulence. Virulence may sometimes be a synonym for *pathogenicity* but is defined more usefully as a measure of the severity of the disease that does occur. Various criteria may be used: days confined to bed, hospitalization rate, serious sequelae such as persisting paralysis, and death. The measure of virulence is the number of severe cases over the total number of cases, which when death is the criterion becomes the familiar *case-fatality rate*. With this as our measure, the viral agents previously mentioned fall into a different gradient from that based on pathogenicity. Rabies virus (with a case-fatality rate of nearly 100 percent) qualifies as highly virulent, and poliovirus (with a case-fatality rate of 7 to 10 percent for paralytic disease) can be classed as moderately virulent. Measles, with an occasional death from encephalitis or pneumonia, is far down the scale but is still ahead of mumps, varicella, nonfetal rubella, and rhinoviruses, for which the case-fatality rates are very low. Outcomes of severity short of death such as days of intensive care utilization or of requirement for supplemental oxygen can be used in a similar fashion.

Immunogenicity. Immunogenicity (ability to induce specific immunity) is measured best functionally in terms of the degree and duration of resistance conferred by infection. Although agents may differ with respect to the immunogenicity of their intrinsic "protective antigens," more important factors are the sites of primary infection and disease and the amount of antigen formed during infection to stimulate a host response. Superficial sites, such as the respiratory mucosa, are guarded chiefly by secretory antibody, which is poorly persistent; agents such as rhinoviruses, which replicate only at such sites, are ineffective stimulants of the systemic immune response. The amounts of the respective toxins released during clinical tetanus and diphtheria usually do not induce satisfactory immunity. In contrast, systemic viral infections, such as with measles and yellow fever viruses, induce solid and long-lasting immunity.

Factors Related to Relationship Between Infectious Agent and Host

The infected host provides a shelter in which the agent can multiply and from which it may spread. Key questions involve how long the agent can persist in the host and over what period and by what avenues it can escape. The time relationships and descriptive terms of different phases of infection are suggested schematically in Figure 6-2.

When the agent is not readily recoverable but perhaps is hidden within host cells or at some other site, infection is termed *latent*. Conversely, when the agent is being shed, as in feces or respiratory secretions, or can be recovered from blood or tissues, infection is said to be *patent*. Infections are necessarily latent at first (the *latent period*) and become patent when the agent has multiplied sufficiently for shedding to begin. The *period of*

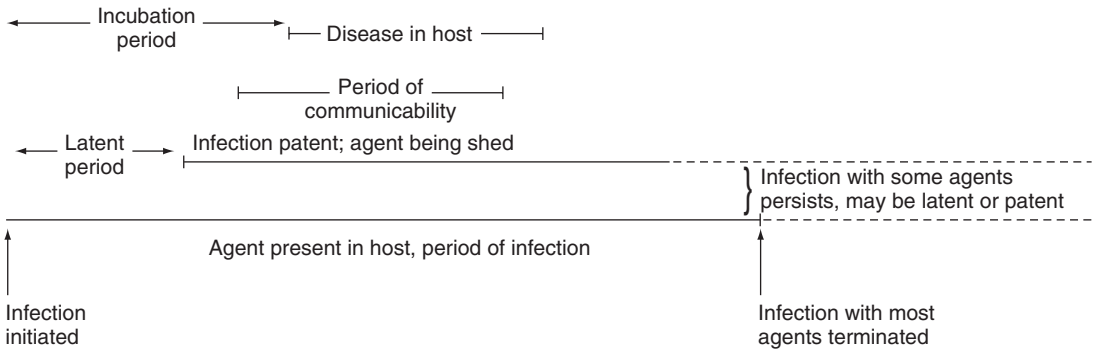

FIGURE 6-2 ■ Important phases of infection in vertebrate hosts.

communicability commonly starts soon after initial shedding begins and continues as long as the level of shedding is sufficient for transmission. The rapidity with which disease is spread is related to the length of the latent period, which almost always is shorter (sometimes much shorter) than the better known *incubation period* (time until disease develops). The period of communicability has no consistent relationship to either the occurrence or the duration of disease.

Persistence in the host is important to the agent for as long as escape remains possible. The period of persistence (see Fig. 6-2) varies widely among agents. Infection terminates completely within 2 to 3 weeks with many agents, such as most respiratory viruses, and after a few months with some agents, such as polioviruses or adenoviruses. Truly persistent lifelong infections may become permanently latent as with some herpesvirus group (Epstein-Barr virus, cytomegalovirus) infections. Infection may remain permanently patent (approximately 3 percent of typhoid cases, numerous hepatitis B virus infections), may be intermittently patent (herpes simplex virus infections [HSV]), or after years of latency may recrudesce with patency and associated disease (tuberculosis, Brill disease caused by *Rickettsia prowazekii*, varicella-zoster virus [herpes zoster]).

Reservoirs of Infectious Agents. *Reservoir* is defined as any entity (living [e.g., animal or plant] or not [e.g., soil]) in which the infectious agent can survive. The reservoir may also serve as the site from which the agent can be transmitted to living hosts or to vectors. The reservoir may be a continuing chain of transmission from one host to another (including vertebrate and invertebrate species). Chains with long links requiring infrequent transmission are especially favorable to survival of agent species.

Among agents for which humans are the only natural vertebrate host, many contrasting patterns exist. The most common is exemplified by infections with most respiratory viruses, which are characterized by short latent periods (1 to several days) and short periods of communicability (rarely > 1 week). The links are short, and frequent transmission is necessary. At the other extreme are long-persisting infections associated with continuous (typhoid carriers, hepatitis B virus, HIV) or intermittent (HSV) *patency* or shedding. The links in this case may be as long as the postinfection life of the host and render generation-to-generation transmission possible. Such transmission also may occur via congenital infection, as in mice infected with lymphocytic choriomeningitis virus. Examples in humans include cytomegalovirus and hepatitis B virus infections. Long links also occur in persistent infections that after many years of latency recrudesce to cause disease and renewed shedding (varicella-zoster virus and *R. prowazekii*).

When infection of invertebrate hosts (vectors) is a link in the chain of transmission, a wide range of reservoir patterns is possible. The simplest involves agents for which humans are the only natural vertebrate hosts (malarial parasites, *R. prowazekii*, dengue virus), with the chain consisting of alternating links of human and vector infection. More commonly, the basic reservoir is a similar alternating chain primarily involving lower vertebrate hosts, with humans being an opportunistic and usually blind-end host. Examples include murine typhus rickettsiae and plague bacillus (both cycling primarily in rats and rat fleas), Lyme disease, and various arboviruses (yellow fever, St. Louis encephalitis). In the case of the latter, the broad vertebrate (numerous avian and mammalian species) and invertebrate (various mosquito species) host range results in very complex patterns that in a given area are defined by the prevalent susceptible host species. The links in these chains are defined temporally by the persistence of patent infection in the vertebrate host and the short life span of the invertebrate host.

Several aspects of infection of the invertebrate (vector) host are important. Typically, infection is acquired in a blood meal and endures for (and does not influence) the life span of the arthropod. Hibernating arthropods may be the long link in the chain by which the agent survives the winter. In at least two instances, infection kills the vector: *R. prowazekii* in the body louse and plague bacilli in the rat flea. As with malaria, infection of the arthropod also may permit completion of an essential stage in the developmental cycle of the agent. Finally, transmission of infection from arthropod to arthropod may be alternate or necessary links in the chain. Transovarial transmission of *Rickettsia tsutsugamushi* (scrub typhus) in mites is essential because the individual mite feeds only once, during the larval stage, on vertebrate hosts. Transovarial transmission also occurs in ticks infected with *Rickettsia rickettsii* (Rocky Mountain spotted fever) and in *Aedes triseriatus* mosquitoes infected with La Crosse virus (California encephalitis), in both cases affording an

TABLE 6-5 **Typical Modes of Transmission from Host, Conveyance, and Portal of Entry**

Agent Shed via	How Conveyed	Portal of Entry	Disease Example
Respiratory secretions	Airborne droplets, fomites, or direct contact	Respiratory	Common cold, influenza, respiratory syncytial virus
Feces	Food, fomites, water, flies	Oral	Poliomyelitis, typhoid, rotavirus
Blood	Arthropod vector, transfusion, needle stick	Skin—via insect bite, intravenous device	Typhus, dengue, malaria, human immunodeficiency virus, hepatitis B and C
Lesion exudate	Direct contact, sexual intercourse, fomites, flies	Skin, genital or ocular mucous membrane	Syphilis, trachoma, staphylococcal abscess
Aerosols	Droplet nuclei, direct inhalation	Respiratory	Tuberculosis, varicella

overwintering mechanism. Venereal transmission of La Crosse virus between mosquitoes has also been shown.[67]

Finally, the inanimate environment can play a role in the reservoir mechanism. Examples are bacteria that can multiply in the free state (salmonellae and staphylococci in food) and agents endowed with unusual survival capacity (tetanus bacillus and *Histoplasma capsulatum*, both of which form highly resistant spores). It also occurs when a brief sojourn under proper environmental conditions is required for a necessary stage in the life cycle (e.g., hookworm eggs from human feces must hatch into larvae to become infectious).

Mechanisms of Transmission. *Transmission*, in this context, is defined as the transport of an agent from one vertebrate host to another. It involves escape from the source host and conveyance to and entry into the recipient host. The basic interdependence of these sequential steps is illustrated in Table 6-5, which also presents specific examples of diseases. Although humans are the usual or only source for most agents of human disease, lower vertebrates serve as the major or only (rabies virus) source for some pathogens.

Fomites are intimate personal articles, such as handkerchiefs, playthings, and eating utensils. *Direct contact* includes not only physical contact (shaking hands, kissing, sexual intercourse) but also, in practice, short-range (within 3 ft), airborne transmission by large droplets (>5 μm) containing hundreds or thousands of organisms that descend rapidly to the ground or floor. These heavy droplets are the primary route of transmission of group A streptococcal pharyngitis,[68] pertussis, and influenza.

Indirect transmission for respiratory and some other infections includes the acquisition of organisms from dust (e.g., tubercle bacilli), from fomites (inanimate objects in the environment such as bedding[47]), and from airborne droplet nuclei (<5 μm) containing only one or a few organisms that promptly dry, float in the air for long periods, and may be wafted for moderately long distances, and even between rooms or floors in a hospital. Transmission by airborne droplet nuclei is limited to highly infectious agents such as varicella virus. Because respiratory colonization with group A streptococci requires a large inoculum, airborne droplet nuclei play no role in their transmission. Other forms of indirect transmission include inanimate vectors such as food, milk, and water, which are frequent vehicles for spread, particularly of intestinal infections. Another source of indirect transmission is animate vectors, which either may function as a vehicle for transport (as with flies that carry organisms from feces to food) or may be infected. In the latter case, multiplication and transformation in the vector are required for transmission, as with African trypanosomiasis and the tsetse fly.

Conveyance ends when the agent reaches a portal of entry, which to be effective must provide ready access to a tissue in which the pathogen can lodge and multiply. For a given agent, a particular portal (nasal, genital, or oral mucosa) often is obligatory or usual, but alternative portals may be possible. Rhinoviruses replicate only in nasal mucosa, whereas typhus rickettsiae typically enter through skin broken by a louse bite but also can infect via ocular or respiratory mucous membranes.

Factors Related to the Host

Many biologic and behavioral characteristics of the human host influence the occurrence of infection and subsequent disease. Although the host characteristics to be considered are of widely differing nature, they operate by influencing one or more of the following: degree of exposure, innate susceptibility to infection, and the likelihood of specific immunity. Although much of the following discussion focuses on the individual human host, factors that influence individuals also affect whole human populations. A key principle to be emphasized is the usual existence of a gradient of response to exposure to an infectious agent. Because of this gradient, the occurrence of characteristic overt disease is a notably unreliable measure of the extent of activity of a disease agent. Given a particular exposure, infection may not occur, disease may not result, or the consequences may range from trivial to the fully developed syndrome "characteristic" of the agent. Accumulating data indicate that specific host genes may be responsible for differences in host susceptibility.

An inadequate challenge dose, an unsuitable portal of entry, or specific host immunity also may explain failure of infection to develop. Whether infection causes disease and the extent, nature, and outcome of the resulting disease are determined partly by host-related properties of the agent (pathogenicity and virulence) and partly by

host defense mechanisms, a variety of which confront an infectious agent that has reached a site of primary infection. Bacteria and other extracellular parasites stimulate an inflammatory response at the site in an effort to localize the invaders by a retaining fibrin network, and the invaders are destroyed by congregating numerous phagocytic cells. Invader organisms may meet sinusoidal passages lined by phagocytic cells in regional (lymph nodes) and bloodstream (bone marrow, spleen, and liver) filters. In addition, clinicians are beginning to understand the importance of so-called innate immunity. This complex network of receptors and responders prevents invader microorganisms from gaining access to tissues. In addition to physical barriers already mentioned, the innate immune system includes a family of receptors known as the Toll-like receptors. These recognition molecules signal the host to activate numerous protective mechanisms. Discussion of this system is beyond the scope of this chapter.

Several aspects of the outcome of infection are important, the first being survival of the host. Death is an unsatisfactory outcome not only for the host but also for the many agents for which survival as a species depends on continued transmission by the host. The remaining aspects relate to the surviving host. Was recovery from disease complete, or were permanent sequelae present? If the latter, were they stationary (as in paralysis resulting from polioviruses) or potentially progressive (rheumatic heart disease resulting from streptococcal infection, pulmonary tuberculosis)? Another aspect, persistence of the agent, was discussed previously (see Factors Related to Relationship Between Infectious Agent and Host). The final aspect is the state of postinfection resistance. If it is incomplete, the recovered host may experience reinfection, with or without disease, and again become a source of infection for others.

Biologic Factors

Biologic factors include characteristics such as age, sex, and race (ethnic group), which are so important and easily ascertained that determining their relationship to occurrence of disease is a usual first step in an epidemiologic description. Biologic factors also include genetic makeup, general health status, and specific immunity.

Age

The influence of age is illustrated best by patterns of common diseases such as varicella, measles, and mumps before the advent of vaccines. All three diseases occur predominantly in young children, who are affected because of their usual lack of immunity and their high risk of exposure to other children, among whom most infections occur. Older individuals are likely to be immune and, unless they are parents of young children, are unlikely to be exposed to infected individuals. Vaccines that provide only temporary immunity could cause a shift to an older age at presentation in the population.

Age is also often related to the outcome of infections in nonimmune individuals. Demonstration of the influence of age requires that all infections be recognized and classified according to the severity of the resulting disease (if any). When many or most infections are subclinical (as with polioviruses and other enteroviruses), the increase in the case-fatality rate with age is apparent immediately, but special studies to document asymptomatic or mild infections are required to show that the proportion of infections that resulted in clinical disease also increased with age. In contrast, the case-fatality rate for pertussis is highest in young infants. With measles and varicella-zoster virus, initial infection at any age usually results in characteristic disease, but the frequency of serious disease increases with age.

Sex, Race, and Ethnicity

That sex may be a factor is illustrated by the fact that most infectious diseases typically occur more frequently in males than females. The question, with respect to any particular disease, is whether these differences between sexes reflect innate differences in susceptibility to disease or can be attributed to sex-associated differences in behavior, occupation, or stress. The increased rate and poorer outcome of infection in neonates suggests a true biologic difference.

The incidence of many diseases varies greatly between groups defined by race or ethnic group. This variation may be explained by differences in behavior or environment for which socioeconomic status is a marker. Ethnic groups share many genetically determined traits, however, which may include either heightened susceptibility or increased resistance to specific infectious agents. Selective evolutionary pressure may be invoked to explain the greater resistance of whites to tuberculosis and the heightened resistance of blacks to malaria.[1] Differences in host susceptibility based on race, familial clustering, and ethnicity are being investigated at the genomic level now that the code for the whole human genome has been elucidated. Current investigations are directed at discovering polymorphisms of genes that involve the immune response.

General Health Status

General health status includes the physiologic state, nutritional status, presence of intercurrent disease, and stress. The importance of such factors is commonly accepted in many cases but rarely documented by well-controlled studies. Infancy, during which immune mechanisms are immature, is a period of special vulnerability to many infectious diseases. HSV, enteroviruses, and group B streptococci manifest as disseminated infections in neonates, in marked contrast to their presentation in older individuals. Puberty, associated with rapid growth and change in endocrine balance, is a period of vulnerability to acne and tuberculosis. Pregnancy predisposes to more severe influenza, tuberculosis, paralytic poliomyelitis, and varicella.

Gross protein malnutrition causes definite impairment in the cell-mediated immune response,[29] a correspondingly increased susceptibility to bacterial and parasitic infections, and increased morbidity and mortality in many viral infections. Viral infections may depress

cell-mediated immunity (and increase susceptibility further) to other concurrent infections. In the developing world, where malnutrition may be epidemic, the consequences of diarrhea, measles, and other respiratory virus infections are many times more devastating. Diarrheal diseases also may be the cause and the consequence of malnutrition. Diabetics are especially vulnerable to bacterial infections; measles and pertussis may reactivate quiescent tuberculosis; and, perhaps of greatest importance, otherwise benign respiratory viral infections, notably influenza, may pave the way for development of serious bacterial pneumonia. Infection with HIV enhances the susceptibility to and severity of tuberculosis, toxoplasmosis, and many other infections.

Finally, stress induced by widely divergent stimuli (including strong emotions, physical exertion, trauma, or excessive heat or cold), according to Selye,[58] may operate through a pituitary-adrenocortical hormonal path to decrease resistance to infections. Widely accepted examples include physical exertion, child bearing, and rearing of children as factors predisposing to paralytic poliomyelitis and pregnancy and rapid growth during puberty as causes of reactivation of quiescent tuberculosis. The living conditions and stresses of military training are associated with an increased rate of respiratory tract infections, including invasive meningococcal disease.

Immunity and Immune Response

Immunity and immune response to viruses warrant special attention. Although important in recovery from bacterial infection, antibody response is of questionable significance in viral infections because viruses within cells are inaccessible to antibody, and by the time antibody appears, many or most susceptible cells have been infected already. Nonetheless, the importance of antibody in viral infections is suggested by the great vulnerability of immunodeficient children to vaccine strains of poliovirus, other enteroviruses, and varicella and by the sometimes beneficial effect of passive immunoprophylaxis in preventing measles and hepatitis B. The benefits of previous experience with viruses can be seen with the decreased severity of rotavirus infections following the first bout of rotavirus and the protection afforded by varicella-zoster immunoglobulin in highly vulnerable children.

At its maximum (exemplified by postinfection immunity to measles), protection against infection is virtually absolute. At the other extreme (exemplified by many respiratory viral infections), susceptibility to infection persists or wanes as with pertussis, although the severity of related disease may be reduced. In most instances, protection seems to be mediated by antibody, but cell-mediated immunity also may be important. Children with defects in cell-mediated immunity are at particular risk for acquiring infection with varicella, *Pneumocystis carinii*, and otherwise noninvasive fungi.

To the extent that immunity protects the individual against infection or acts to minimize shedding of the agent when infection occurs, an immune host can play little or no part in the spread of an infectious agent in the population. If a sufficient proportion of a population is immune, an agent transmitted via contact cannot spread, and nonimmune members of the population would be spared exposure. The concept of *herd immunity* assumes that a nonimmune individual is protected from a communicable infection by being surrounded by immune individuals. What proportion must be immune to achieve effective *herd immunity* usually is unknown.[19] This concept is valid only in homogeneous, randomly mixing populations in which all possible pairs of individuals have the same probability of making effective contact. Although measles vaccine has been used extensively in the United States, outbreaks of measles continue to occur in segments of the population who group together in small communities and fail to accept vaccine; these groups are often defined by beliefs, race, economic status, and social behavior.

Human Behavior

Governed largely by habits of the individual and the customs and culture of groups, human behavior greatly influences exposure to and modes of transmission of infectious agents. Cultural factors also underlie attitudes toward preventive and curative practices.

Water is a potential vehicle for many agents. When commonly imbibed without boiling, as in the United States, community water systems constitute potential channels for transmission that usually are well guarded. Occasional operational failures occur, as exemplified by failure of a water quality–monitoring device in a filtration plant that resulted in an outbreak of *Cryptosporidium* diarrhea affecting an estimated 403,000 individuals in Milwaukee in 1993.[36] Foods and milk, especially items that are consumed raw or after minimal cooking, likewise are excellent vehicles for transmission of disease. Well-known examples include trichinosis from undercooked pork; bacterial enteritis from unpasteurized milk; fish tapeworm from raw fish; and various forms of food poisoning caused by bacterial contamination during handling, poor refrigeration, and inadequate cooking. In recent years, outbreaks of hemorrhagic diarrhea caused by *Escherichia coli* O157:H7, sometimes associated with hemolytic-uremic syndrome, have occurred as a result of improperly prepared products of bovine origin, particularly ground beef, but also vegetables and fruits.[48]

Closely related to water and foods is the disposal of human excreta. Casual defecation near habitations or in or near running water leads to dissemination of enteric pathogens by filth flies and by water. The use of human feces (night soil) to fertilize crops commonly eaten raw, such as strawberries and lettuce, has an obvious similar potential.

Many more individual types of behavior also are important. Infrequent bathing and laundering of clothes favors infestation with body lice. Inadequate clothing increases exposure to arthropod vectors and, as in young children lightly clad for summer weather, facilitates the exchange of feces. Going barefoot provides exposure to hookworm larvae. Hand washing minimizes the role of hands in indirect transmission of enteric (fecal) and respiratory (nasal secretion) pathogens. Rhinovirus infections result from inserting contaminated fingers into the nose and eyes.[24] Intimate personal contact such as hand

shaking, kissing, and play among young children fosters the spread of a wide variety of agents. Even recreation, such as travel, picnics, and camping, may lead to unusual exposure to infectious agents. Sexual behavior is associated with the transmission of numerous infections, including HIV, syphilis, gonorrhea, group B streptococci, and hepatitis B.

Factors Related to the Environment

The environment in concept embraces all that is external to the individual human host. There are three broad environmental areas: *physical*, which includes geologic and climatologic or meteorologic features; *biologic*, which consists of all flora and fauna and all living microbial pathogens; and *socioeconomic*, which encompasses the interrelationships of humans. Environmental factors often act through indirect paths, and some have the potential to affect the agent, the host, and the agent-host relationship. Solar radiation is lethal for many pathogens in the free state, helps humans synthesize vitamin D, and can provoke recrudescence of a latent HSV infection and result in recurrent fever blisters. The capacity of humans to modify adverse environmental conditions beneficially is another important factor.

Geographic and Geologic Factors

Spread of infectious agents on a global scale requires their transport, which is influenced by distance alone and by geographic features—mountain ranges, oceans, rivers—that assist or impede travel. The importance of these factors declines as the extent and speed of travel increase, but it remains substantial, especially in developing countries. The minimal effect of geographic barriers in containing highly infective agents is overcome by modern travel, and rapid spread by airline travel is exemplified by epidemics of severe acute respiratory syndrome, which spread from China throughout the world in a matter of weeks.[46]

The natural paths of travel (including waterways), natural harbors, and the location of mineral deposits help determine where populations concentrate. Water supply, dependent in part on geologic formations, is a factor limiting population size and, together with fossil fuels and mineral deposits, influences the type, extent, and location of industrial development. Soil types vary greatly in their ability to hold and purify water and in their capacity to support vegetation, which influences the type and abundance of animal life. Soil is a determinant of the type and importance of agriculture and a major factor influencing the biologic environment.

Climate

The term *climate* describes the typical annual pattern, along with its seasonal variation, of weather conditions in a specified region. Such conditions (climatologic factors) include solar radiation, temperature, humidity, barometric pressure, winds, precipitation (and drought), and lightning. These factors can affect infectious disease agents directly. Many microbial agents in the free-living state are vulnerable to excessive heat and radiation and uncontrolled drying. The life cycles and reservoir mechanisms of many pathogens, including higher parasites, depend on appropriate temperature and humidity. Maturation and hatching of hookworm larvae from ova deposited in the soil require warmth and reasonable humidity, and the multiplication of malarial parasites and arboviruses in their mosquito vectors and the abundance of the vectors are favored by warm temperatures.

The usual seasonal variation in the incidence of specific infectious diseases suggests important influences of climatologic factors, but how they operate may be hard to determine. Overall, respiratory tract infections occur more frequently in colder months, but within this period (roughly October through mid-May in the Northern Hemisphere) the prevalence of the many respiratory pathogens varies greatly. Rhinoviruses peak in the early fall and spring, and influenza viruses are most active in midwinter. Parainfluenza virus type 1 and 2 infections usually peak in the fall. Increased congregation of people indoors facilitates transmission, and fluctuations in temperature and humidity not only affect the viability of agents in airborne droplet nuclei or on fingers or fomites but also may affect host susceptibility to infection.

Enterically transmitted infections occur most frequently in the warmer months, presumably largely because of season-related changes in host behavior. The outdoor play of scantily clad children facilitates the spread of skin infection and fecally shed agents such as enteroviruses. Rotavirus infections are an exception in that they occur most frequently in colder months. More completely understood are the seasonal patterns of infections spread by arthropod vectors; these patterns reflect seasonal variations in the abundance and activity of the vectors and the various lower vertebrate host species, which together constitute the reservoir mechanisms of the specific infectious agents. Climate overall, as a major determinant of the biologic environment, helps determine the abundance and the particular species of flora and fauna in a given area.

Longer-term changes in climate have been associated with changes in patterns of infection. The hantavirus outbreak in the southwestern United States in 1993 has been attributed to unusually heavy precipitation in the spring of 1993 after 6 years of drought. This precipitation resulted in marked proliferation of the deer mouse population, the reservoir of the virus.[69]

Socioeconomic Conditions

Socioeconomic factors depend on the density and distribution of populations; the available natural resources; the level of social, political, cultural, and scientific development; and, most importantly, the interrelationships of people. These factors typically affect health by indirect means, and, because they often are interrelated closely, evaluating the impact of individual factors is very difficult.

The relationship of population distribution and density to the occurrence of infectious diseases is substantial. Increasing density favors the spread of infectious agents to humans from human and nonhuman sources and the

occurrence of related disease and development of immunity. In large and dense populations, agents such as measles virus typically infect in early childhood and persist because a sufficient number of new susceptible individuals are added continuously by birth. In smaller populations, the agents are unable to persist and are reintroduced at unpredictable intervals; as a result, manifestations of "childhood" diseases may be delayed for long periods. Populations of urban and rural areas differ not only in relative density but also in other important ways. Exposure to zoonotic agents, especially agents prevalent in wildlife and livestock, is greater in rural areas, although rats and stray dogs may abound in city slums. Environmental sanitation (protection of water and milk supplies, safe disposal of sewage) often is a personal problem for rural residents but is handled by cooperative effort in wealthier urban populations. In addition, the importance of schools and school buses in facilitating exchange of infectious agents is greater in rural areas, where isolation of farm residents otherwise restricts contact between young children.

The basic population unit is *the household*, membership in which has similar implications for health in rural and urban areas. Family members are similar genetically; share a common diet and economic status; are subject to the same cultural, religious, and educational influences; and are exposed to a common local physical and biologic environment. Most important for contact-transmitted diseases, intrafamilial contact is prolonged and increases in intimacy with household crowding. In the winter, many homes are insulated and the windows are closed so that air exchange is poor and exposure to airborne organisms is great. Prolonged contact is especially important for persisting infections such as HSV infection and tuberculosis. Family size, regardless of the degree of crowding, is particularly important for acute infections because it determines the number of potential introducers who bring home infections acquired elsewhere. The likelihood of exposure in early childhood increases with family size. Except for early infancy, a period of special vulnerability to some agents (e.g., respiratory syncytial virus, pertussis), early exposure is beneficial because most resulting infections are less likely to have serious consequences.

A population with a highly developed *social and political structure*, through its capacity for cooperative action, enjoys many advantages that directly or indirectly benefit health, including provision of preventive and curative health services, effective environmental sanitation, and well-developed educational facilities. Education closely relates to personal health practices that are based on understanding what individuals should do to minimize disease hazards. Schools, where the educational process begins, have been identified already as important factors in the exchange of infectious agents among children, especially agents spread by contact and airborne droplet nuclei. This matter is offset partly by the benefits derived from school-based immunization programs.

Economic status affects the occurrence of diseases indirectly through its relationship to adequacy of housing, nutrition, level of education, sanitation, and availability and use of health services. It is also related closely to occupation, which may be associated with exposure to specific infections, such as Rocky Mountain spotted fever (forest workers and hikers in the south Atlantic coast states) and ornithosis (workers in poultry-processing plants).

Occurrence of Disease in Populations

Patterns of occurrence of disease that are not random, but instead reflect the influence of underlying causes (risk factors), not only help predict future occurrence of disease but also provide important clues to understanding causation. Describing the pattern of occurrence of disease begins with definition and classification of disease in the individual so that cases can be identified and counted reliably. The occurrence of disease in defined populations can be expressed quantitatively.

Infection and Disease in the Individual

Epidemiologic interest focuses on the specific etiologic identification of *infection* and *disease*, terms that are not synonyms. Technically, any deviation from normal function or state constitutes *disease*. Because virtually all infections cause at least some deviation from the normal state (e.g., a change in the white blood cell pattern and mobilization of such cells at the site of infection), they cause disease. In practice, many infections result in no clinical evidence of disease and are important to the individual only for the reason that they induce immunity. Because subclinical infections help define the overall pattern of occurrence of infection and often play a significant role in its spread, their recognition is important epidemiologically. Subclinical infections go unrecognized except when healthy individuals are observed for infection in longitudinal or case-control studies.

Only infections resulting in disease usually come to the attention of the medical community. To the extent that they are recognized etiologically, they provide the earliest and most available indicator of the pattern of infection in the population.

With few exceptions, such as measles and chickenpox, typical clinical syndromes are not pathognomonic and require the clinician to develop a differential diagnosis. Basically, clinical manifestations depend more on the site or sites of disease than on the infecting agent. Because the number of possible disease targets in the body is small and the number of potential agents is large, reasonably distinct clinical entities may be caused by any of several agents. Notable examples include "common colds," approximately 40 percent of which are caused by rhinoviruses and 60 percent by any of many other viruses, and aseptic meningitis, which may be caused by such agents as mumps virus, many enteroviruses, or *B. burgdorferi*.

Infection with a specific agent may have several possible clinical outcomes. Infection with polioviruses usually is (perhaps 80%) subclinical but can result in brief febrile illness (approximately 15%), aseptic meningitis (4% to 5%), or classic paralytic disease (<1%). The response to agents with multiple potential targets varies even more widely. Group B coxsackieviruses can cause such disparate entities as acute upper respiratory tract disease,

aseptic meningitis, polio-like paralytic disease, myocarditis, and epidemic pleurodynia (Bornholm disease).

Knowledge of the agents active in the community when a given illness occurs helps narrow the differential diagnosis, but confirming etiologic diagnosis requires laboratory assistance: by culturing the agent, direct visualization in specimens, antigen detection, genome identification using molecular techniques, or demonstration of a specific antibody response in tests of "acute" and "convalescent" serum pairs.[2] The diagnosis is most secure when both approaches suggest infection with the same agent. Although demonstration of the agent in relation to the disease site carries special weight (e.g., in a pharyngeal swab specimen from a respiratory illness), its presence could be the result of preexisting persisting infection unrelated to the current illness. Antibody response indicates a newly acquired infection.

Infection and Disease in Populations

Sources of Information. Many sources provide data regarding the incidence of infectious diseases, including U.S. Vital Statistics, which tabulates only fatal cases; the Centers for Disease Control and Prevention (CDC), which receives reports of specific notifiable diseases from state health departments and summarizes them in the *Morbidity and Mortality Weekly Report;* and state and local health departments. In the absence of a focused surveillance project, these data vary in their completeness by source and by disease because of underreporting, subjects who are not seen, and errors in diagnosis.

Among the different states, reporting requirements vary, and adherence to these requirements by providers also varies. Reporting is most complete for uncommon but characteristic disorders of unusual interest, particularly if they are severe or fatal and require hospitalization, such as rabies, anthrax, trichinosis, plague, and diphtheria. Reporting is enhanced by outbreaks, as with measles, mumps, and classic pertussis in recent years. Some notifiable infections, such as leptospirosis and atypical pertussis in partially immune individuals, often are unrecognized and are underreported. Reporting requirements may change from year to year based on CDC or state interests (neonatal sepsis and West Nile infection) or concerns about bioterrorism. Another source of data, often useful in certain areas, is state health department laboratories, which perform specific microbiologic or serologic tests for providers.

Infectious diseases that are not notifiable by law pose a difficult problem. Although necessarily limited in scale, longitudinal studies of defined populations of families have yielded valuable information. Finally, researchers now can use well-designed serosurveys to make reliable estimates of rates of previous infection with agents that induce long-persisting antibody.

International data on the incidence of infectious diseases are less precise except in well-developed countries. For the developing world, the World Health Organization (WHO) and the United Nations International Children's Emergency Fund provide estimates of the incidence of morbidity and mortality from various infectious diseases in different nations based on local reports, which are not always collected systematically. Continuing collection of such data is important for monitoring the effects of the Expanded Program of Immunization, which is directed at controlling the major vaccine-preventable diseases of childhood. More difficult to develop are definitive data about the incidence and causation of the respiratory and diarrheal diseases, which are estimated to kill 6 million children annually in the developing world (approximately 5% of the yearly birth cohort).

For developing these kinds of statistics, it is important to have a definition of disease that is as useful as possible, which means that the sensitivity and specificity of the definition should be so balanced that as many cases of the disease as possible are identified, while avoiding the confusion that occurs when other disorders with overlapping manifestations meet criteria that are too nonspecific. A well-known example of a useful definition of disease is the Jones criteria for the diagnosis of rheumatic fever, established in 1944 at the request of the National Research Council in an effort to bring order out of chaos at a time when the disease was a major problem in the civilian and military populations.[27] These criteria subsequently have been modified to enhance their specificity by making evidence of a previous group A streptococcal infection a sine qua non for the reason that too many cases of polyarthritis of other causes met the original criteria.[16] Similarly, the criteria for the diagnosis of Kawasaki disease were revised more recently to avoid missing some episodes in infants and missing the opportunity to prevent coronary artery aneurysms by using intravenous immunoglobulin.[41]

Recognizing the importance of standardized diagnostic criteria for surveillance of infectious diseases of public health importance, the CDC published in 1990 case definitions for reportable infections.[70] Optimal use of these criteria and reporting of confirmed and probable cases to the proper authorities are of particular importance currently, when, for a variety of reasons, some formerly well-controlled contagious diseases are becoming recrudescent (e.g., mumps, pertussis). All states mandate reporting contagious diseases of major public health importance, particularly diseases affecting children.

Relating Infection and Disease to Personal Characteristics. Multiple characteristics may serve to distinguish one individual from another. Some factors are determined at conception—age, sex, ethnicity, genetic makeup, and birth order. Others, far more numerous, are acquired subsequently. They may be biologic (specific immunity, nutritional state), behavioral (smoking, dietary, recreational habits), or socioeconomic (occupation, educational level, marital status). Many of these characteristics relate to exposure to infectious agents or to susceptibility or resistance to the effects of such agents and to the occurrence and severity of disease.

Relative Usefulness and Importance of Characteristics. Although almost any potentially relevant characteristic of an individual patient can be identified, it is not useful for purposes of description unless we can estimate how many people in the population also possess the characteristic. From census data or other accessible records,

numbers of people in groups defined by age, sex, race, occupation, or marital status can be estimated easily. Special surveys would need to be conducted to estimate the prevalence of specific immunity or possibly significant exposure, such as to household pets.

The importance of personal characteristics to the description of a disease varies in two ways. One is in the degree of association that exists between a characteristic and a specific disease. Age is associated strongly with disease caused by prevalent contagious agents, whereas sex usually is not. The second way is in the independence or relative interdependence of characteristics as variables. Inherent characteristics, such as age, sex, and ethnic origin, are independent of one another, whereas acquired characteristics rarely are. The nature of interpersonal contacts, degree of personal hygiene, and usual forms of recreation are associated closely with age or sex or both. The common interdependence of characteristics means that before making an inference from a particular association, one should explore association with other, possibly correlated, characteristics.

Age Patterns. The occurrence of infection and disease generally is related so strongly to age that until possible differences in age distribution are taken into account, differences in occurrence among population subgroups defined by other characteristics cannot be interpreted meaningfully. Age as a characteristic is ascertained easily and reliably for affected individuals and the total membership of the relevant population. Description of the age pattern involves computing a series of *age-specific rates* for sequential age groups, usually defined in intervals of 5 years or multiples thereof (e.g., birth to 4, 5 to 9, 10 to 19). For conditions of pediatric concern, the use of single-year intervals (<1, 1, 2, 3, and 4 years) to cover early childhood may be more informative. In some cases, especially in the first 2 years of life, a breakdown by months of age would be informative.

Affected individuals in an age group form the numerator, and all individuals in the population in that age group serve as the denominator. Rates so computed describe the age profile of immunity at a specified point in time (age-specific antibody prevalence rates), the age profile of new infections or disease (age-specific incidence rates), or the age profile of deaths caused by a disease (disease-specific and age-specific mortality rates). Age-specific incidence rates for acute infectious diseases indicate the risk of disease occurring in each age group and, depending on the disease agent, more or less accurately reflect the underlying age-specific infection rates.

Age Adjustment of Rates. The need to take age distribution into account when comparing disease in different populations is indicated when (1) the rates vary with age and (2) the distribution of the populations by age differs substantially. From published U.S. mortality data for 1983 and 1984, one can compare pneumonia and influenza mortality rates for Alaska and Florida. During those years, 89 deaths were recorded among 986,000 Alaskans at risk, for an annual mortality rate of 9/100,000. In contrast, in Florida, 4,703 deaths occurred from pneumonia and influenza among the 21,792,000 residents at risk for those 2 years, for a rate of 21.6/100,000, nearly 2.5 times that of Alaska. These rates are called *crude mortality rates.* In this instance, these rates are misleading for the reasons that the likelihood of death occurring from pneumonia and influenza increases with age and the age distributions of the populations of these two states differ markedly. National death rates from these infections are nearly 10-fold greater in people 65 to 74 years of age than in people 55 to 64 years of age. For those years, 17.5 percent and 3 percent of the Florida and Alaska populations, respectively, were 65 years or older.

To make a valid comparison of the pneumonia and influenza mortality rates for these two states, one must perform an age adjustment; this is a simple process that is not detailed here because the method can be found in available texts of biostatistics and epidemiology. Briefly, one determines mortality rates for specific age groups (usually categorized within 5 or 10 years) for the two populations and calculates the deaths that would be expected in a common (or standard) population for the same age groupings by using the age-specific rates of the populations being compared, in this instance those of Alaska and of Florida. Summation of these expected deaths permits calculation of the rates that would have occurred in the standard population if the age-specific rates of Alaska applied and if the age-specific rates of Florida applied. In this example, the age-adjusted mortality rate for pneumonia and influenza for Alaska is 35.2/100,000, and that for Florida is 21.8/100,000, nearly the reverse of the crude rates. (The combined population of the two states was used as the standard.)

These age-adjusted rates are not true rates; they are used for comparison. Although often applicable to infectious diseases, age-adjusted rates almost always are required for comparisons of morbidity and mortality from chronic diseases.

Sex Patterns. Because sex is a readily ascertained characteristic of the membership of populations, the occurrence of infections and disease in relation to sex is described easily. Its simplest form is the *sex ratio*, or the ratio of cases in males to cases in females. This ratio is meaningful only when, as in childhood, the population is divided approximately equally by sex. Although the number of males exceed the number of females at birth (106:100), the death rate for males exceeds that for females at all ages (average 1.5:1). From approximately age 20 years on, females outnumber males, the difference increasing with age, which means that when comparing sex-specific rates, age adjustment must be made, or, better yet, the age profiles for the sexes should be compared directly so that important differences in the contour can be seen.

Ethnic or Racial Patterns. A third characteristic by which members of the population can be grouped in describing occurrence of disease is race or ethnic origin, the usefulness of which has decreased with the increasing frequency of mixed marriages. The U.S. census classification is based on information collected regarding race and native origin. Individuals of mixed racial parentage are classified by the race of the nonwhite parent or, if both

are nonwhite, by that of the father. People of foreign birth are classified by country. Native-born children of foreign-born parents are identified as "foreign stock" and grouped according to parental origin. Census data provide estimates of population subgroups belonging to several "races" (white, black, Native American, Chinese, Japanese) or "foreign stocks" (including foreign born and first generation).

Although controversial currently, this method yields differences in the occurrence of many infections and other diseases among such population subgroups. Knowledge of such differences is useful in case finding and organizing the application of specific preventive measures. Subgroups defined by ethnicity possess some similarity in genetic constitution that may determine susceptibility or resistance to specific agents. They also may be affected distinctively by environmental factors because of voluntary or involuntary differences in behavior and patterns of living.

Disease Patterns in Kinships. Genetically determined susceptibility and resistance to specific infectious agents have not yet been associated clearly with recognized genetic markers that could serve as a basis for defining population subgroups. Most efforts to look for genetic influences have been studies of occurrence of diseases in individuals of differing degrees of relationship within kinships or in the total memberships of different kinships. With rapid advancement in the database of the human genome, considerable interest has been generated in polymorphisms at loci that may define susceptibility to infectious diseases on a genetic basis. Populations with a high prevalence of certain alleles and known to have an increased risk of certain infections could be studied to determine whether the risk is due to abnormal function of the specified gene.

Family Episodes of Infection and Disease. With respect to contact-transmitted infectious diseases, the family is more important as the basic epidemiologic subgroup of a population than for its shared genes. Observation of family units for episodes of infection and related illnesses has contributed significantly to knowledge of the epidemiology of widely prevalent infectious agents. The situation in all family studies begins with one member's infection, acquired from outside the house. That member then exposes the other family members. The introductory infection and any infections in family members who are exposed constitute a family episode, which is described basically in terms of the times of onset of the related infections and the identities (age, sex, position in the family) of the introducer and the infected and uninfected contacts.

Analysis of cumulated episodes of common respiratory illness, observed in the early studies, identified children as the most frequent introducers (important in community spread). Analysis also yielded estimates of the risk of cross-infection occurring within the family, expressed in terms of secondary attack rates among specified members (e.g., younger children) exposed to specified introducers (e.g., a schoolchild or a parent). Generally, the risk in contacts was related inversely to age overall, as a result of the influence of immunity, and to intimacy of within-family contact (ready exchange between spouses and between children nearest in age). Finally, the relationship of time between the onset of illness in the introducer and onset in family members exposed serves to define the range of incubation periods.

Analysis of the episodes can yield additional information concerning such crucial aspects as mode and duration of the agent's shedding; the spectrum of clinical response to infection, including the proportion that is subclinical; and the significance of previous immunity in the presence of close exposure, as measured by the frequency and clinical consequences of the reinfections that result. The results of the analysis of adenovirus episodes in the Virus Watch program are illustrative.[20] Virus appears regularly in the feces and less often (approximately 50%) in the pharynx, and shedding may be abortive (a few days only) or continue intermittently for many months. Overall, 50 percent of infections are subclinical, and illness, typically febrile and respiratory, occurs more commonly with pharyngeal excretion (65%) than with only fecal shedding (31%). Immunity is 85 percent protective against infection; reinfections that do occur usually are subclinical. Young children and especially infants younger than 2 years of age are the usual introducers, and within-family spread depends more on duration than on the mode of virus excretion by the introducer.

Socioeconomic Patterns. *Socioeconomic status* covers a complex of characteristics, including levels of education and income and, less tangibly, "social standing." The problem is to discover a useful single indicator. One possibility is area of residence as classified by median income or measures that reflect housing standards, such as type of plumbing and average number of individuals per room. Relevant data are available for census tracts, which have proved useful when the tracts are homogeneous.

Occupation of the head of a household seems to be the one attribute most closely reflecting socioeconomic status. On this basis, the British have defined five broad social classes that directly apply to employed adults and can be extended to cover their dependents. These classes, in descending order, are professional, intermediate, skilled, partly skilled, and unskilled occupations. For use in the United States, based on census-recorded occupations, these terms have been translated as follows:

- Professional workers
- Non-farm technical, administrative, and managerial workers
- Clerical, sales, and skilled workers
- Semiskilled workers
- Non-farm laborers
- Farm workers of whatever level are included in a sixth group as agricultural workers

Relating Infection and Disease to Place

Place is of interest, epidemiologically, when occupied by humans and, unless indicated as relating to work, recreation, or travel, refers here to residence. Place usually is classified geographically (hemisphere, continent, nation) but also can be classified usefully by environmental

characteristics such as climate, altitude, stage of economic development, population density, and urban or rural nature. Variations in occurrence of disease with place reflect parallel variations in the operation of causative factors and raise the question of whether these factors are to be found in the characteristics of the physical and biologic environment inherent to the place or in the characteristics of the inhabitants. The former is suggested when the age-adjusted risk of disease increases for immigrants and decreases for emigrants, when risk does not vary among the ethnic groups present, and when similar ethnic groups in other places enjoy a lower risk. One also must consider possible differences in reliability and completeness of recognition and reporting of disease.

Global Variation. On the global scale, the WHO collects and publishes information concerning the occurrences of diseases derived from statistics compiled routinely within nations for morbidity from notifiable infectious diseases and for causes of death. The great variations among nations in the quality and availability of medical care and other health services result in corresponding variations in the reliability and completeness of the data collected by the WHO. Generally, basic demographic data and the quality and availability of health services are equally good in well-developed countries, so specific disease rates can be compared. In less-developed countries, demographic data may be inaccurate and medical services may be inconsistent in quality and concentrated in urban populations, within which their availability varies with economic status. Many illnesses and deaths are unattended medically, especially in rural areas, and births commonly are attended by midwives. Because infant deaths are reported more completely than births are, infant mortality rates may be unreliable.

With respect to infectious and parasitic diseases, knowledge of the frequency of disease is less important than is qualitative knowledge of the distribution and spread of disease. Such knowledge guides the application and enforcement of international control measures and is the basis for advice given by physicians to prospective foreign travelers. Important diseases, such as yellow fever, plague, and cholera, because of their high case-fatality rate and characteristic clinical picture, are almost certain to come to attention when substantial numbers of cases occur. Such knowledge may not be publicized, however, or may not be promptly available. Some countries, in the hope that a new outbreak (perhaps of cholera) will be controlled soon, may withhold information to avoid discouraging economically important tourists.

Two additional considerations are relevant to evaluating the disease hazards of foreign travel. One is the fact that recognized occurrence of disease in the indigenous population may be an inaccurate index of risk to a newcomer. Particular agents, such as polioviruses in the past and hepatitis A virus and Epstein-Barr virus at present, may be so prevalent that infections in natives occur so early in life that they usually are subclinical. The second consideration is the nature of the proposed travel. The usual tourist or business traveler visits chiefly larger population centers and popular tourist attractions, where the most important hazards are pathogens transmitted by food or water. Travelers whose activities bring them into more intimate contact with the people and the biologic environment (Peace Corps workers, global health researchers, military personnel) may encounter additional hazards, such as rabies and the locally prevalent arthropod-transmitted pathogens.

As suggested in considering geographic influences on occurrence of disease, the distribution of many diseases is influenced by relevant environmental factors, rather than political boundaries. In depicting (or predicting) the global distribution of a particular disease, identifying regions defined by the presence of factors thought to be important to disease occurrence is useful.

For most agents pathogenic for humans, the chief environmental requisite is a susceptible human population, and most such agents already exist wherever the size and density of the population are sufficient for them to persist. Concern about global spread is limited to a few important pathogens, such as the cholera vibrio and the influenza virus. Cholera is a special case in that its spread also depends on poor sanitation. Recent experience in Haiti demonstrated that transmission of this agent can occur by having disaster aid workers from foreign countries introduce cholera which can then spread because sanitation is poor.[12] Neither persistence nor even limited spread should occur after its introduction into highly developed areas. Influenza A virus continues to be a major, and so far unstoppable, threat by virtue of its ability to emerge at irregular intervals in a new antigenic coat, which largely negates the preexisting widespread immunity.

Local Patterns of Infection and Disease. "Local" units of population for which demographic data are readily available in the United States include "large" units, such as counties, metropolitan areas, and large cities, which contain smaller units (smaller cities and towns [within counties] and census tracts [within metropolitan areas and large cities]). The smaller units, including unincorporated areas within counties, often can be characterized by variables (urban or rural nature, population density, socioeconomic status, racial or ethnic group) that may help explain observed differences in the occurrence of specific infections and related diseases.

Particularly in relation to outbreaks of acute infectious diseases, spot maps commonly are used to show the local distribution of individual cases. Placing new pins (a different color each week) to mark the residences of newly reported cases serves to visualize the outbreak's geographic progression. The final distribution of the pins may help identify a major source of infection. A classic example is the 1854 outbreak of cholera in the Golden Square district of London, in which the clustering of residences of fatal cases helped Snow incriminate the Broad Street pump as the source.[65] Sometimes, place of work is a better guide to the source of infection than place of residence. In another classic study, that of endemic typhus in Montgomery, Alabama, in the early 1920s, the residences of cases (Fig. 6-3) were scattered widely whereas the workplaces (Fig. 6-4) were concentrated in relation to feed stores and food-handling businesses, all heavily rat infested. This finding led Maxcy[37] to perform

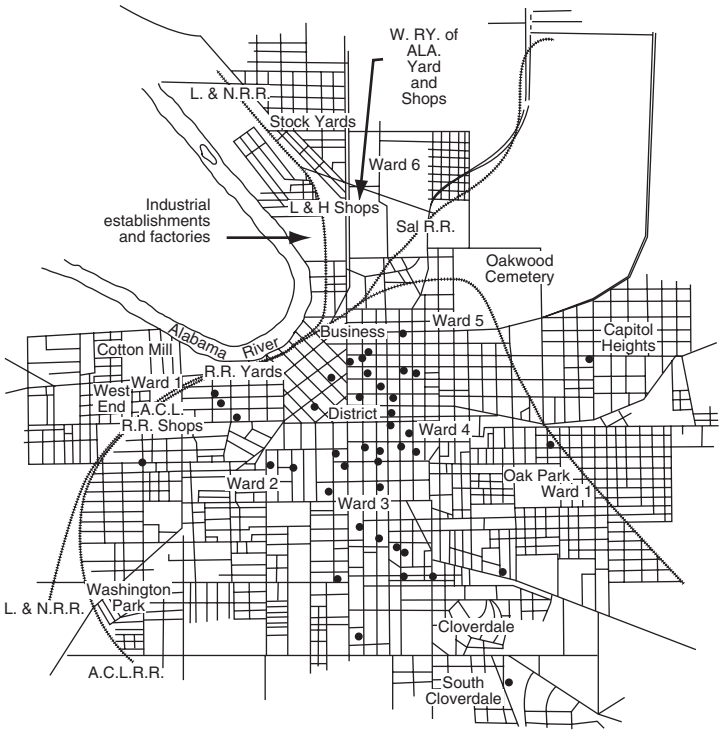

FIGURE 6-3 ■ Cases of mild typhus (Brill disease) in Montgomery, Alabama, 1922 to 1925, spotted according to residence. (From Maxcy KF. An epidemiological study of endemic typhus [Brill disease] in the southeastern United States. *Public Health Rep* 1926;**41**:2967–95.)

FIGURE 6-4 ■ Cases of mild typhus (Brill disease) in Montgomery, Alabama, 1922 to 1925, spotted according to place of employment or, if unemployed, according to place of residence. (From Maxcy KF. An epidemiological study of endemic typhus [Brill disease] in the southeastern United States. *Public Health Rep* 1926;**41**:2967–95.)

studies showing the basic role of rats and rat fleas in this disease.

Temporal Patterns of Infection and Disease

Definitions. The unit of time used can vary from hours to decades to centuries. In describing acute outbreaks, the units are short—hours for food poisoning, days or weeks for most infectious diseases—whereas long-term time trends are described in longer units of years or decades. Comparisons extending over 1 or more decades may be complicated by changes in diagnostic standards and reporting.

Finally, the meanings of two words commonly used to describe the occurrence of disease over time in a given area should be defined clearly. *Endemic* refers to diseases

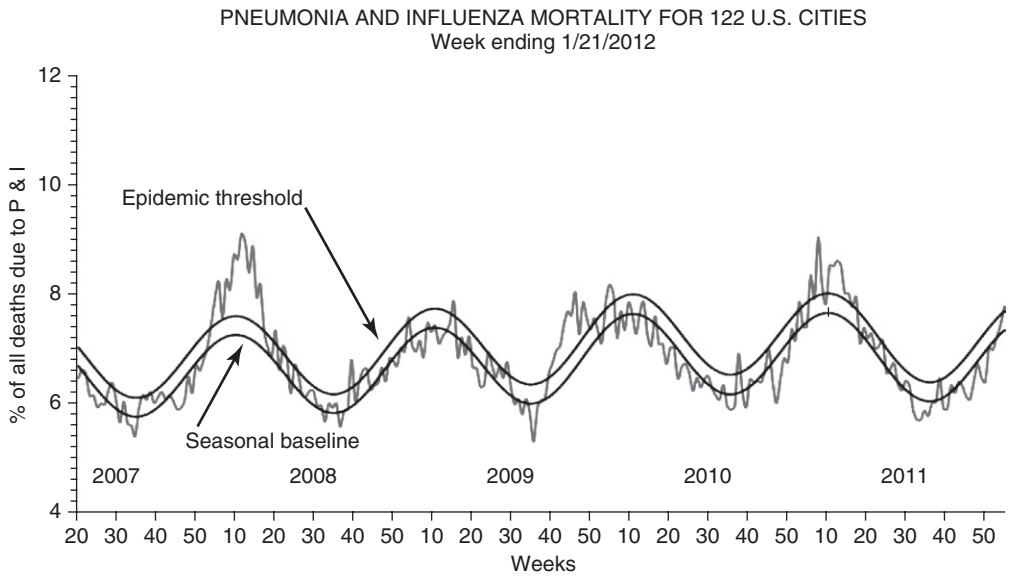

FIGURE 6-5 ■ Percentage of all deaths attributed to pneumonia and influenza reported by the 122 Cities Mortality Reporting System, by week and year—United States, week ending January 21, 2012. (http://www.cdc.gov/flu/weekly/; [accessed 1/31/12])

regularly present. The usual frequency, including expected seasonal variations, is called the *endemic level.* The term *epidemic* applies to any number of cases, small or large, that is a significant increase over the usual, or endemic, level (although just what constitutes a "significant increase" is not clearly defined).

This principle underlies the monitoring of many infectious diseases, deaths, and pneumonia caused by influenza by the CDC. Such monitoring is illustrated in Figure 6-5, in which the weekly ratios of deaths caused by pneumonia and influenza to the total deaths observed from 2007 to 2011 are compared with the ratios expected from use of the time series method[13] and "epidemic threshold," which depicts the upper 95 percent confidence limit. When the observed value exceeds the expected one (an increase of 1.645 standard deviations from the seasonal baseline) for 2 successive weeks, an influenza epidemic is indicated. On this basis, epidemics began in approximately the week 4 of 2008 and week 40 of 2009, which was unusually early and was due to the H1N1 swine strain.

In practice, especially at the local level, health authorities use the term *outbreak*, rather than *epidemic* (unless the number of cases is very large), to minimize public alarm. The term *pandemic* is used to describe excess disease occurring in many countries, as influenza did in 1957, 1968, and 2009.

Time Clusters. Time clusters relate to the recognition and interpretation of events (infections and disease) that occur with some increased frequency within a limited period or clustering in time. An important determinant of clustering of infections is the incubation period. Infection resulting from a known exposure is manifested within a predictable range of time by the initiation of shedding of the agent and the onset of disease (if it occurs). Although the average incubation period of a given disease is remarkably constant, the usual range broadens as the incubation period increases. This range can be estimated by accumulating cases for which the time of exposure is known precisely or approximately and, with this point as day 0 on the time axis, plotting the day of onset of each subsequent case. The width of the resulting cluster provides an estimate of the range of the incubation period. The best estimate is obtained by using only cases with single, clearly timed exposure, such as when contact with the source case occurred only once, as during a playmate's birthday party.

A more readily obtained but less precise estimate is that derived from the cumulative analysis of family episodes of a disease based on the assumption that the initial or primary case is the source of subsequent disease in family contacts. Because the primary case is infectious for several days, on any of which effective exposure may occur, the onset of secondary cases would cluster over a range that theoretically may reflect the true range plus the period of infectivity of the primary case.

Knowledge of the range of incubation periods has several practical implications. For contagious diseases, it can be used to distinguish true secondary cases that occur in families from others arising as a result of extrafamilial sources (cases with onset too soon after that of the primary case are called *co-primary cases*) or representing a second generation of spread within the family (i.e., *tertiary cases*). Knowledge of the range of incubation periods also determines the length of time after known exposure that contacts should be observed or possibly held in quarantine for the subsequent development of disease. For poorly contagious diseases, such as typhoid fever, or for noncontagious diseases, such as food poisoning, the distribution of onset in an outbreak can be used to distinguish between "point" epidemics (common time and place of exposure) and outbreaks reflecting exposure to a possibly continuing source. In the former case, onset would fall within the usual range of incubation periods. When the point source is not obvious, investigation can be focused on the interval defined by subtracting the shortest incubation period from the date of onset of the

first case and the longest period from the date of onset of the last case.

Short-Term Patterns

Epidemics. "Point" epidemics have a duration limited by the range of the period of incubation of the particular disease because no secondary spread of the agents occurs. Much more commonly, outbreaks or epidemics extend over longer periods. On the basis of the causative agents and the mechanisms of transmission, three distinct types can be recognized.

The first type typically consists of outbreaks of poorly contagious or noncontagious diseases; such outbreaks reflect the new but persisting activity of a source that must be identified and terminated quickly. One example of such a "continuing source" type of outbreak was a community-wide outbreak of salmonellosis in Madera, California, in 1965, which was traced quickly to the water supply.[51] Unrecognized typhoid carriers working as food handlers and shellfish harvested from sewage-polluted waters contaminated with hepatitis A virus are other examples of outbreaks requiring prompt epidemiologic investigation to identify the source. One of the major causes of point epidemics is food contaminated with *E. coli* O157:H7. More recent outbreaks, such as the contamination of spinach at the growing source, have been reported by the CDC.[9] Often the CDC posts interim reports of investigations on the CDC website (www.cdc.gov) before publication in *Morbidity and Mortality Weekly Reports.*

The element of contagion distinguishes the second type of epidemic, in which disease spreads from person to person. Such epidemics generally are self-limited, and the curve describing them often resembles the bell-shaped curve of a normal distribution. Modification of this curve, as in an abrupt decline from the peak, is the expected result of successful efforts to control the epidemic.[40] Epidemics of contagious diseases occur when the population in which the agent persists or is newly introduced contains a sufficient number of susceptible individuals with contact with one another that is adequate to permit transfer of infection from each new case to, on average, more than one susceptible individual. When this average falls below 1, the curve declines, and when the probability of successful transfer of infection approaches zero, the epidemic stops (typically well before the supply of susceptible individuals has been exhausted).

Reasons that underlie this decline and termination of transmission include seasonal changes in environmental factors that affect viability of the agent, such as temperature and humidity; changes in the behavior of hosts that affect the intimacy of contact, such as indoor school versus outdoor play; and the progressive conversion of individuals from susceptible to immune. Institution of appropriate hygienic precautions likely was an important facet of control and termination of the worldwide outbreak of severe acute respiratory syndrome in 2003.[10] Institution of an intensive vaccination program may also reduce transmission and abort an outbreak. If one assumes constant units on the time axis, the slope of the ascending limb of the epidemic curve is determined by the incubation period (interval between successive cases) and by factors that influence transmission (infectivity of the agent, frequency of adequate contact between susceptible individuals). In the absence of effective control measures, the duration of epidemics of a particular disease depends on the size of the susceptible population and the persistence of favorable environmental factors.

Epidemics of "diseases in nature" constitute the third type. Because susceptible individuals usually are abundant in the human population, these epidemics typically reflect increases in the number of sources of infection in nature. With zoonoses such as arbovirus encephalitis, the initiation, slope of the curve, and duration of the epidemic are determined by the number of susceptible lower vertebrate hosts, the seasonally determined abundance of the vector mosquitoes, and the length of the extrinsic incubation period in the vector. The occurrence of West Nile virus in the United States, which began in August 1999 and has continued with outbreaks in the late summer of each succeeding year in mosquitoes and humans, is an example.[3,11]

Seasonal and Cyclic Variations. Predictable periodic variations in disease take two forms, seasonal and cyclic, neither of which is understood completely in the case of agents infecting only humans. *Seasonal* variations presumably reflect the influence of changes in temperature, precipitation, and length of daylight on the activity of the agent. This variation is readily apparent with respect to diseases, such as Lyme disease (the peak incidence of which typically occurs in the summer months), which depend on lower vertebrate hosts and arthropod vectors, the abundance and activity of which are determined seasonally. Similarly, the increased occurrence of enteric bacterial infections (spread by indirect means) in the warmer months is explainable largely by more rapid bacterial growth in unrefrigerated milk and food, the increase in abundance of flies, and the lack of sanitary precautions associated with summer recreational activities.

The mode of transmission is only a partial determinant of the seasonal pattern. Although the enteroviruses (including poliovirus), the rotaviruses that cause acute gastroenteritis, and hepatitis A virus all are spread chiefly by fecal-oral mechanisms (direct and indirect), the seasonal patterns are distinctly different (late summer and fall for enteroviruses and late winter for rotaviruses and hepatitis A virus). Similarly, although infections with agents present in respiratory secretions and spread by either airborne mechanisms or contaminated fomites occur infrequently in the summer, their seasonal peaks occur at significantly different times. The season of respiratory diseases coincides roughly with the school year. Infections with rhinoviruses occur most frequently in the early fall and late spring, and infections with parainfluenza virus types 1 and 2 occur most frequently in the fall. The annual peaks of RSV vary among fall, winter, and spring. Mumps peaks in late fall, influenza in late winter or early spring, and measles typically in the spring.

Plotting variation over a series of years reveals a roughly regular cyclic variation for some diseases. In larger metropolitan areas, the usual biennial measles epidemic appears as an enlarged annual wave. Before 1950, deaths caused by meningococcal meningitis occurred on a nationwide basis in cycles of 7 to 9 years, a phenomenon

presumably terminated by the advent of prophylactic antibiotics. Pandemic influenza A, which provides the most dramatic example of cycling, is explainable by a major change in the antigenic character of the agent.

Long-Term Trends. Except for poliomyelitis during the first half of the 20th century, clearly defined long-term trends in the occurrence of infectious diseases in the United States usually have been downward, and in no case has description or understanding of the mechanisms posed a major difficulty. In the case of poliomyelitis, the increase in the rate of paralytic disease that began about 1880 is attributable to numerous factors, including improved sanitation, which resulted in an expanding proportion of infection occurring among older children and young adults without previous exposure to the viruses. In young infants, most poliovirus infections are benign but produce long-lasting immunity; the older an individual is when infected, the more likely that individual will have paralysis. The frequency of paralytic poliomyelitis is related inversely to the level of sanitation. In the United States, the increase in paralytic disease with epidemics of polio continued unabated (with an apparent sharp increase around 1940 because of changes in reporting criteria) until the widespread use of polio vaccines.

The post-1955 decline in poliomyelitis; the much earlier decline in pertussis and diphtheria; the disappearance of smallpox; and the more recent marked decline in measles, rubella, *H. influenzae* type b, and varicella all are attributable chiefly to widespread use of effective specific vaccines. Similarly, the disappearance of indigenous malaria and the decline of rabies to a negligible level in humans in certain areas reflect the application of a variety of effective control measures. Unfortunately, with the exception of smallpox, these measures are not permanent and loss of infrastructure for delivery of vaccines or of environmental controls will cause a recrudescence of disease.

Emerging Infections. Although in the 1980s one might have said that most infectious disorders had been explored thoroughly epidemiologically and that little was left to do, this clearly is not the case. Infectious disease epidemiology has become increasingly challenging, and in many ways the problems are more complex and necessitate greater cooperation with additional disciplines, such as microbiology and molecular biology.

In part, this increased complexity is due to so-called *emerging infections*, a term that has come into vogue in recent years and refers to two different groups of disorders. The first is associated with the appearance of previously unrecognized or possibly heretofore nonexistent infections in humans. Well-known examples are legionellosis, hantavirus pulmonary disease, acquired immunodeficiency syndrome, Lyme disease, hemorrhagic colitis caused by *E. coli* O157:H7, and Ebola virus infection. The other group includes previously recognized human infections that exhibit changes in epidemiologic behavior or biologic characteristics that enhance their transmission or virulence. These changes usually can be attributed to external influences, such as altered demographics including increasing population and rural-urban migration, international travel, new technology or technologic failure, changes in land use, adaptation of infecting organisms to various influences, and inadequate or underused public health measures.[33] To this list should be added changes in host factors such as immune defenses.

No clear dividing line exists between new infections and old ones that exhibit new behavior. Examples of some with new behavior include multidrug-resistant tuberculosis, penicillin-resistant pneumococcal infections, invasive group A streptococcal (popularly known as flesh-eating bacteria) infections, staphylococcal toxic shock syndrome, community-acquired methicillin-resistant staphylococcal infections, H5N1 influenza ("bird flu"), cryptosporidiosis, and numerous infections that are fostered by immunosuppression or various therapeutic measures such as antibiotics and catheters.

These emerging infections are not minor threats. Although in some instances small populations have been affected to date, the potential for widespread disease of epidemic or even of pandemic proportions exists. This potential can be expected to increase because the demographic and other conditions that have predisposed to the emergence of these infections continue to grow and intensify. Maximal efforts must be expended to reverse this process. To achieve this goal requires worldwide collaborative effort among various disciplines, including epidemiology, microbiology, entomology, immunology, clinical medicine, demography, nutrition, sanitation, and even political science.

The responsibilities of epidemiologists regarding emerging infections may be described in three categories. The first category is surveillance, including recognition of the appearance of a previously unrecognized infection or a new variant of an existing disease. Optimal surveillance requires systematic observations and specific diagnostic criteria to ensure precision. An important part of surveillance is to determine who, when, and where: who is affected (e.g., age and other personal characteristics, contact with others who are ill), when the disorder occurs (e.g., year-to-year variations, season, temporal course of the outbreak), and where it occurs (e.g., geographic locations, urban or rural, local ecology). Second, an important task is to use surveillance and other data to develop an understanding of the epidemiology of the infection, which often provides clues to its etiology and pathogenesis and to approaches to controlling the disease. The third role of epidemiologists is that of monitoring the effects of various control measures, including assessment of the safety and efficacy of new vaccines, and their effects on microbial ecology. A description of how the efficacy of vaccines is determined is provided at the end of this chapter.

The increasing magnitude and speed of international travel enhance the likelihood of global spread of disease and complicate approaches to prevent or contain epidemic infections. Many of the emerging infections are not limited geographically by their ecologic requirements. The nations of the world increasingly depend on each other for surveillance, a task that is not accomplished easily given the logistics; costs (in the presence of other needs and priorities, particularly of many developing countries); and the required standardization,

collaboration, communication, coordination, and centralized resource for assembly and analysis of surveillance. Although the WHO, the Pan American Health Organization, the U.S. Military and Public Health Service, and various other organizations maintain surveillance systems and laboratories in various parts of the world, these efforts at present are considered to be inadequate except for some infections and in some areas. The development of comprehensive national and worldwide surveillance systems has been urgently recommended.[33]

BIOSTATISTICS

Statistical Significance

Stochastic statistics deals with probability. Most readers are familiar with the use of "*p* values" to assess the *statistical significance* of a finding from a study. Usually, the threshold used to separate "significant" from "nonsignificant" results is a probability (*p* value) of less than 0.05, which means that (arbitrarily, by convention) if a result is likely to be observed by chance less than 1 in 20 times, the finding is considered to be "statistically significant." It must be emphasized, however, that statistical significance and clinical significance are very different. A statistically significant result means that the result is considered unlikely to be attributable to chance alone. As we shall see, the *p* value is related not only to the magnitude of the difference between the things that are being compared (e.g., a characteristic of either the exposed and the unexposed groups in a longitudinal study or the cases and the controls in a case-control study) but also to the size of the sample. Consequently, differences that are clinically unimportant (e.g., a difference in mean weight of 0.2 kg between two groups, a difference of 4% in the distribution of whites between two groups) can be statistically significant if the size of the sample is large. Of importance is to remember that a statistically significant finding may be of no clinical significance.

By the same token, the "magic" *p* value of 0.05 itself may be misleading because it is an arbitrary threshold; in fact, usually little difference exists in the results associated with a *p* value of 0.04 and one associated with a *p* value of 0.06. If the size of a sample is small, a large, clinically important difference may not be statistically significant. One should not automatically dismiss the importance of a finding for which the *p* value is 0.05 or higher.

Hypothesis Testing

In most epidemiologic studies, stochastic tests are used to check the validity of a hypothesis. By convention, one tests the validity of the *null hypothesis* that no difference exists in either the mean value of the characteristic (for continuous variables) or the frequency distribution of the characteristic (for categorical values) between the groups being compared. In essence, one is testing the probability that the two samples being compared (exposed subjects versus unexposed subjects or cases versus controls) emerged from the same population—that is, whether a difference between the groups could have been the result of chance if the two samples were selected randomly from the same population or whether the difference is so great that it probably (<1 chance in 20, or a *p* value of <0.05) did not occur by chance; that is, the samples probably actually represent a true difference in the populations of either exposed subjects versus unexposed subjects or cases versus controls.

Hypotheses can be either *bidirectional (nondirectional)* or *unidirectional (directional)*. A nondirectional hypothesis asks whether an association exists between exposed and unexposed subjects, but it does not assume in which direction the association will occur. For example, one might ask whether an association exists between the height of fever and the outcomes of children with typhoid fever without specifying whether the association would be in the direction of better or worse outcomes (perhaps high fever indicates a more severe infection, or perhaps it indicates a more vigorous immune response). In such instances, the associated *p* value should be interpreted in a bidirectional, or *two-tailed*, manner because the distribution of the test statistic contains two "tails," one at either end of the curve; the *p* value represents the sum of the area under the two tails of the curve. In effect, one is specifying that for a result to be statistically significant, the probability of the result being in a positive direction is less than 2.5 percent and the probability of the result being in a negative direction is less than 2.5 percent. Most tables of *p* values provide results that are to be interpreted in a two-tailed manner (because this is the most conservative approach to testing a hypothesis), so the *p* value for a given test statistic can be read directly from the table.

In some instances, however, one might have good reason to suspect that an exposure is associated with an outcome in a unidirectional manner (e.g., that possession of a handgun is associated with an increased risk of sustaining a gunshot wound). If, in advance, the investigator decides to test this hypothesis in a unidirectional manner (the null hypothesis for which would be that ownership of a gun is not associated with an increased risk of incurring a gunshot wound), interpreting the results of a test statistic in a unidirectional, or *one-tailed*, manner might be appropriate. To derive a one-tailed *p* value, the two-tailed value is divided by 2. Thus, if the two-tailed *p* value is 0.06, the one-tailed value for the same result is 0.03. Clearly, obtaining statistically significant results by testing hypotheses in a unidirectional manner is easier. However, of importance is to specify before the study is conducted the *a priori* plan for how the results of statistical tests will be interpreted. Furthermore, although many hypotheses might seem to lend themselves to unidirectional analyses (e.g., one might think that an experimental vaccine that has been shown to be immunogenic could only be beneficial), in fact, unidirectional analyses are used only rarely. First, the possibility that an exposure may have an effect opposite to that expected usually cannot be ruled out in advance. For example, researchers found that persons who were immunized with a vaccine composed of inactivated respiratory syncytial virus had an increased risk for developing symptomatic infection with the virus. In addition, because two-tailed interpretation of data is more conservative,

many authorities (e.g., editors of journals and such licensing agencies as the FDA) demand two-tailed analyses.

Type I Error, Type II Error, and Statistical Power

Two types of errors can be made when testing the validity of a null hypothesis. The first occurs when the null hypothesis is true but is rejected erroneously, which leads to an erroneous conclusion that a difference exists between the exposed and unexposed subjects; this kind of mistake is termed a *type I error*; or *alpha error*. If a test statistic with a *p* value of less than 0.05 is accepted as statistically significant (and the null hypothesis is rejected on that basis), approximately 5 percent of the time a type I error is made because if no difference actually exists between the groups that result will occur by chance 1 in 20 times. By contrast, if the *p* value is less than 0.001, a type I error will occur less than 1 in 1000 times if the null hypothesis is rejected.

The other kind of error, *type II error*; or *beta error*; occurs when the null hypothesis erroneously is not rejected even though a difference between the two groups truly exists. If the *p* value associated with the result is 0.05 or greater, the probability that the null hypothesis is false is not sufficiently low to reject it (even though the probability that the observed result could have occurred by chance may be only 6%).

Type I and type II errors are mutually exclusive. If one rejects the null hypothesis, one risks committing a type I error, the probability of which is equal to the *p* value. In such instances, a type II error cannot occur. If one accepts (i.e., fails to reject) the null hypothesis, one may commit a type II error (in which case a type I error cannot occur). The probability of committing a type II error is not related directly to the observed *p* value. Instead, one can estimate the probability of committing a type II error (beta error) before the study begins on the basis of an assumed (clinically important) degree of association between the exposure and the outcome. Certain assumptions also must be made about the magnitude of the effect among the exposed subjects and the frequency of the outcome in the unexposed subjects. One is calculating a unidirectional probability that, by chance, the study will fail to detect an association equal to or greater than the magnitude that is specified for what is, in effect, an *alternative hypothesis* (i.e., that an association truly exists between the exposure and the outcome). The *statistical power* of a study (the unidirectional probability that the null hypothesis will be rejected given a specified degree of type I error—usually <5%) is equal to 1 minus beta.

In designing a study, adequate statistical power is critical to ensure that a true association between the exposure and the outcome is not missed erroneously. The probability of a type II error depends on the size of the sample, the degree of variance within the sample, and the magnitude of the association that one wants to be able to detect: the smaller the sample, the larger the variance, and the smaller the magnitude of the association that one wants to detect, the larger the beta error (type II error) and the lower the statistical power of the study to detect an association. Of these factors, the investigator usually has control over only the size of the sample (because in the population, the variance generally is a relatively fixed attribute of the characteristic being measured and the magnitude of association that is chosen usually is the minimal association that is clinically meaningful; for example, to design a study of an experimental vaccine so that it could detect as little as 30 percent efficacy would not make sense because such a product would not be approved in most instances unless the magnitude of its effect was much greater).

Exactly what constitutes adequate power is debatable. Because enrolling a large number of subjects in a study often is expensive and time consuming, frequently the power that a study is designed to have depends on both the financial resources and the time available to conduct the study. A statistical power of 80 percent often is chosen as a reasonable compromise between ensuring that the study has a sufficient number of subjects to answer the research hypothesis and the realities of logistic and financial exigencies. On the other hand, if a pharmaceutical company has invested many years and millions of dollars to develop a new drug or a new vaccine, it may demand that a pivotal clinical trial of the product have at least 90 percent power to detect a clinically meaningful effect in an attempt to ensure that the product will be approved if it is efficacious.

Multiple Comparisons

Another more subtle problem in interpreting results arises when more than one hypothesis is assessed in a single study. If multiple tests of statistical significance are performed in a study, the probability that by chance alone the *p* value associated with any one variable will be less than 0.05 is substantially greater than 5 percent. In fact, the probability that by chance at least one test will be associated with a *p* value less than 0.05 is equal to $1 - (0.95)^k$, with k equal to the number of independent tests of statistical significance that are performed. Thus, if 10 different hypotheses are tested, a 40 percent probability exists that at least one of them will be statistically significant (i.e., that at least one of the tests will have a *p* value of <0.05). Of course, this matter violates the arbitrary rule that we consider a finding to be statistically significant only if it is likely to have occurred by chance less than 5 percent of the time. If multiple different associations in a study have *p* values less than 0.05, determining which occurred by chance and which truly are statistically significant is difficult.

On the other hand, studies often are expensive, time consuming, and logistically difficult to conduct. Therefore, sometimes a reasonable approach is to try to increase efficiency by assessing more than one hypothesis in a single study. To address this problem, one can specify in advance the *primary hypothesis* and the *secondary* and *tertiary hypotheses*. One then might give most credence to the statistical test of the primary hypothesis. Another approach is to lower the threshold used to define statistical significance by dividing 0.05 by the number of different tests that are performed. Thus, if five independent hypotheses are tested, any one would have to be

associated with a *p* value of less than 0.01 before the null hypothesis would be rejected. However, this approach might be too stringent because the different hypotheses that are being tested often are not truly independent, and the probability that two or more associations will be statistically significant may be greater than the product of their individual probabilities. For example, one might assess whether infection is associated both with diarrhea and with vomiting; if it is associated with one of these symptoms, usually a higher than chance probability exists that it also is associated with the other. At the least, an investigator should acknowledge this potential problem and its consequences before drawing conclusions from the study.

Tests of Statistical Significance

Going into detail about the many different stochastic tests that are available is beyond the scope of this chapter. However, several general comments, as well as descriptions of a few commonly used tests, are in order.

Tests of statistical significance are either parametric or nonparametric. The test statistic of a *parametric* test is based on the assumption that the frequency distribution of the characteristic being assessed in the source population follows certain parameters. For example, for certain tests, one assumes that the characteristic in the population has a normal distribution. If the assumption is violated (e.g., the characteristic is not distributed normally), the statistical test will not be valid. In such instances, an appropriate approach might be to use a test based on a different, skewed distribution, such as the *Poisson distribution* for rare events, which might reflect more accurately the distribution of the characteristic in the population.

Alternative approaches to assess the statistical significance of differences between groups, if the distribution of the characteristic in the population is known to be highly skewed, include transforming the data so that they are normalized, usually by converting the values to their logarithmic equivalent, or using a *nonparametric* test.[61] Nonparametric tests, such as the Mann-Whitney *U* test and the Wilcoxon rank sum test, do not depend directly on the numerical values of the data; instead, the individual values are ranked in order of their values and the statistical analyses are based on the relative ranks of the values in the different groups rather than on an assumed distribution in the population or in the sample.

For large samples, parametric tests usually are valid and often are easier to calculate than are nonparametric tests. For smaller samples and for samples with highly skewed distributions, nonparametric tests or tests that use log-transformed data may be preferable.

Continuous Variables

To test the statistical significance of differences between groups in continuous variables (e.g., degree of fever, intelligence quotient, age, or a score on a standardized questionnaire), the mean values of the different groups can be compared.[64] The tests of statistical significance use the magnitude of the difference of the means and the variance in the sample to assess the probability that the difference could occur by chance. If more than two groups are being compared for a single variable, the procedure is called *one-way analysis of variance*. The null hypothesis is that the mean values from each group are the same (i.e., that the different groups are random samples from the same population). The total variance in all the subjects is separated into the amount that is attributable to differences between the groups of subjects (the *intergroup variance*) and the amount that is attributable to differences among the subjects within each group (the *intragroup variance*). The greater the quotient of the intergroup variance divided by the intragroup variance (the *F ratio*), the more likely that differences between the groups are statistically significant (i.e., that they are not due to chance variation). The statistical significance of the F ratio can be determined from the *F test* table of *p* values. When this type of test is performed to assess the statistical significance of the simultaneous effects of two factors (by stratifying into additional groups), the test is called *two-way analysis of variance*.

The familiar t *test* is just a special form of one-way analysis of variance in which the means of just two groups are compared. A *one-sample* t *test* assesses whether the mean value of the study sample is statistically significantly different from the mean value in the source population. It assumes that the mean value in the source population is known and is calculated by dividing the difference of the mean of the sample minus the mean of the population by the quotient of the variance of the sample divided by the square root of the number in the sample.

A more common use of the t *test* is to assess the statistical significance of the difference between the mean values of either a characteristic or an outcome of subjects in two different samples (e.g., of the exposed and the unexposed groups in a study). The null hypothesis of this *two-sample* t *test* is that the mean values of the two samples are not statistically significantly different (i.e., that they could be random samples from the same population). The *t* test statistic is equal to the difference in the mean values of the two samples divided by the square root of the pooled variance of the samples (the standard error of the difference of the means of the two groups). The *p* value of the result *(t)* is read from the table of the *t* distribution, with the degrees of freedom equal to the sum of the number of subjects in each sample minus 2.

Categorical Values

The chi-square statistic (χ^2) commonly is used to assess the statistical significance of differences in categorical values between groups.[6] Most often, proportions of two different groups (e.g., the proportions of the exposed and of the unexposed groups in which the outcome developed) are compared. Typically, the data are displayed in a *2 × 2 contingency table* (Table 6-6). The null hypothesis is that no association exists between the exposure and the outcome (or whatever the rows and columns represent), that is, that the two groups could be random samples from the same population. This hypothesis is tested statistically by comparing the number of subjects who would be expected to be in a given cell by chance with the actual

TABLE 6-6 **2 × 2 Contingency Table**

	Outcome Present	Outcome Absent	Total
Exposed	a	b	a + b
Unexposed	c	d	c + d
Total	a + c	b + d	N

$\chi^2 = [(ad - bc)^2 N] \div [(a + b)(a + c)(b + d)(c + d)]$

frequency (the observed frequency). The expected frequency can be calculated from the total number of subjects in the rows and the columns (the marginal totals) and is equal to the product of the number of subjects in the row times the number of subjects in the column divided by the total number of subjects in the sample.

The value of χ^2 (with N equal to the total number of subjects) is shown in Table 6-6. The statistical significance of χ^2 can be determined by reading the *p* values from a table of the χ^2 distribution. The larger the value of χ^2, the less likely that the observed proportions could have occurred by chance. Unfortunately, assuming that the probability that the observed proportions of a random sample of subjects will follow the χ^2 distribution is not necessarily accurate if the expected frequency of any cell is small. Consequently, many experts use a *continuity correction* (sometimes called the *Yates correction*) to adjust for the deviation from the smooth, continuous theoretic distribution of χ^2 when the discrete values of small numbers of expected subjects disrupt the validity of the assumed distribution. The equation for χ^2 with the continuity correction is

$$\chi_c^2 = [(ad - bc) - (N/2)]^2 N \div [(a + b)(a + c)(b + d)(c + d)]$$

If the expected frequency in any cell is very small (often defined as <5), the χ^2 test, even with the continuity correction, is not a valid estimate of the probability that the observed values could have occurred by chance. In such instances, the *Fisher exact test*, which is based on a hypergeometric distribution, should be used to assess the statistical significance of differences in categorical values.

Confidence Intervals

Although the validity of a null hypothesis typically is tested by stochastic tests and the use of *p* values, construction of a *confidence interval* (usually a 95% confidence interval [CI]) has the advantage of providing information about both the precision of the estimate of the association (the narrower the confidence interval, the more precise the estimate) and its statistical significance.[7] CIs have become standard components of reports of the results of epidemiologic studies.

Analyses of studies are concerned with both estimation (of the "true" value of the association between the exposure and the outcome in the population) and hypothesis testing (i.e., what probability that any association that is observed could have occurred by chance alone). A sample of the population is enrolled in any study. The association observed in the study is hoped to be an accurate estimate of the true value of the association in the entire population. However, if an unbiased study were repeated many times, by chance alone the results would vary (because of random sampling error). A CI is a range of values, based on the estimate and the spread of the data from a single study, within which the true value of the association in the population is likely to lie with a specified probability (i.e., 95% of the time for a 95% CI, 99% of the time for a 99% CI). CIs provide information about both the magnitude of associations (so that the clinical significance of the estimate can be assessed) and their statistical significance. If the CI is calculated for a single outcome and if the 95 percent CI for the difference between two groups does not include zero, the outcome is statistically significant (i.e., <5% probability that the null hypothesis of no difference existing between the groups is true). If the CI is for a risk ratio or an odds ratio, the association will be statistically significant if the 95 percent CI for the association does not include 1 (i.e., <5% probability that the null hypothesis that no association exists is true). CIs can be calculated with both continuous and categorical data, as well as for estimates of a single proportion and for differences (or associations) between two groups.

Adjustment for Potential Confounding Variables

The effect of confounding variables on the associations between exposure and outcome may be controlled in either the design or the analysis of a study. Random allocation of subjects to the exposed and the unexposed groups is a common strategy to avoid the effect of confounding in longitudinal studies. Although randomization actually ensures only lack of bias if a confounding variable is distributed unevenly between the groups (because an uneven distribution is as likely to favor the exposed group as the unexposed group), randomization is likely to be effective in preventing confounding if the size of the sample is large (which renders substantial inequalities between groups in the distribution of an independent variable unlikely to occur). Nonetheless, an investigator must check to ensure that confounding does not occur, even in a randomized clinical trial.

Another way to ensure that a potential confounding variable does not affect the results of a study is to *match* on that variable. Subjects in the exposed and the unexposed groups (or, in a case-control study, the cases and the controls) can be matched on certain variables that are known to be associated independently with both the exposure and the outcome. Such matching can be achieved either by matching subjects individually or by *frequency matching* (i.e., ensuring that the overall frequency distribution of the potential confounder is the same among the cases and the controls). Matching can ensure that the matched variable (e.g., race, age) does not affect the observed association between exposure and outcome.

One disadvantage of matching is that the effect of the matched variable on the association between exposure and outcome cannot be assessed as it might be if either stratification or multivariable analysis were used to control for confounding. If matching is used in the

TABLE 6-7 **Analysis of a Matched Case-Control Study**

	Cases	
Controls	**Exposed**	**Unexposed**
Exposed	a	b
Unexposed	c	d

Matched odds ratio: b/c.
McNemar χ^2: $(b - c)^2/(b + c)$.
McNemar χ^2 (with continuity correction): $(b - c - 1)^2/(b + c)$.

design of the study, analyzing the data with special tests for matched designs is necessary. For example, in a matched-pairs case-control study, only discordant pairs (matched pairs in which the cases and controls differ in their exposure status) provide information. Calculation of the matched odds ratio and the associated test of statistical significance (McNemar χ^2) is shown in Table 6-7.

Adjusting for the effect of potential confounders in the analyses also is possible. One way to accomplish this adjustment is with *stratification*, which is performed by dividing the data into different groups, or *strata*, according to the potential confounder. For example, a study may indicate that children whose parents both work were three times more likely to become febrile during the course of a study than were children whose parents both do not work. We may suspect, however, that it is not having both parents at work, per se, that results in this increased risk of fever; rather, another factor probably is related independently to the risk for fever and to the probability that both parents work. Attendance at group daycare is such a factor. If we stratified the results of the study by whether the children attended group daycare, we most likely would find that a disproportionate number of children whose parents both work also attended group daycare. When the risk ratios in the two different strata are combined (by using the Mantel-Haenszel technique), we probably would find that the apparent association between having both parents work and the risk of fever disappears when the effect of attendance of group daycare is controlled by stratification.

Advantages of stratification are that it can be understood easily and it can be performed without changing the design of the study after it is completed. One disadvantage is that it requires being able to identify and to measure accurately the potential confounders (which is not necessary if one allocates subjects to different groups randomly). In addition, if several different potential confounders exist, performing the calculations may be difficult and time consuming, and the sizes of the individual strata may be so small that the ability to make statistical inferences is poor.

Finally, one can adjust for confounding with the use of one of many multivariable statistical techniques, such as *multiple linear regression* (if both the dependent and the independent variables are continuous) or *logistic regression* (if the dependent variable is dichotomous and the independent variables are either categorical or continuous). Providing a detailed description of multivariable techniques is beyond the scope of this chapter. However, in general, these techniques involve creating a mathematical model that fits the data and then using that model to analyze various associations.* Although multivariable techniques are a powerful tool for both assessing the effects of and controlling for potential confounding variables, they depend on mathematical assumptions that may not always be valid and use techniques that are not intuitively obvious.

Meta-analysis

The term *meta-analysis* refers to a number of different types of analysis. The common feature of all meta-analyses is that they summarize the results of two or more different studies. The term often is used to refer to a quantitative technique in which the results of different studies are combined after they are weighted in the calculations according to the sizes of their samples and the variances of their results. The term also sometimes refers to a qualitative analysis of a group of studies, including critical analyses that rate the methodological soundness of different studies but do not combine their results quantitatively. For example, one might review a group of studies that reached different conclusions about a topic. If the methodologically rigorous studies had similar results that differed from those of the weaker studies, one might conclude that the results of the more rigorous studies were more likely to be valid.

The concept of producing a single, statistically valid result by combining the results of different, often contradictory, studies has great appeal. However, quantitative meta-analysis has many shortcomings. Perhaps chief among them is that no distinction is made between studies that are sound methodologically and those that are not. Thus, the results of a meta-analysis may be dominated by a large, but methodologically questionable, study, whereas a superbly performed, but smaller study may fail to override the impact of the larger study in the meta-analysis. Another major problem is the selection of studies to include in a meta-analysis. Should only published, peer-reviewed studies be included? Clearly a bias exists that studies with positive results are more likely to be published than studies with negative results. Imagine that many small studies with poor statistical power are conducted and submitted to journals for publication. If the study found no association with the putative risk factor, it likely would not be published, on the grounds that the statistical power of the study is poor (i.e., the risk of making a type 2 error and erroneously concluding there is no association when such an association truly exists is great). On the other hand, if the study did find a statistically significant association with the risk factor, it might be published and could be included in a meta-analysis. If many such small studies that found an association were published, but even more small studies that did not find an association were not published, this kind of *publication bias* could lead to erroneous conclusions in a meta-analysis. Should the analysis be limited to only those studies that meet certain basic

*References 5, 6, 15, 17, 28, 31, 57.

criteria for methodological rigor (and who should decide on the criteria and whether they are met), or should only randomized clinical trials be included? What formula should be used to weight the different studies? Is combining the results of methodologically different studies valid? Results of meta-analyses should be interpreted with great circumspection.[66]

DIAGNOSTIC TESTS

Although clinicians typically order diagnostic tests many times each day, misinterpretation and misuse of diagnostic tests are extremely common occurrences.[50] The widespread practice of interpreting continuous values (e.g., the total white blood cell count) in a dichotomous manner (so that the result is either "normal" or "abnormal") and failure to appreciate fully the difference between the accuracy of a diagnostic test (i.e., its sensitivity and specificity) and its predictive value are major factors in the misuse of diagnostic tests.

What is Normal?

In most instances the "cutoff" for an abnormal test of a continuous variable, such as the total white blood cell count or the concentration of antibodies against an infectious agent, is based on the distribution of the results of the test in the population. One usually assumes that the distribution of most such results in the population will follow a gaussian (or "normal") distribution, which is illustrated by the bell-shaped curve in Figure 6-1. Gauss and other statisticians used the term *normal* to refer to the shape of this curve. However, in medicine, the term *normal* long has been used to mean something very different—the dichotomy between a state of health *(normal)* and one of disease *(abnormal)*. Unfortunately, the different meanings of these terms often have been used interchangeably, which has added to confusion in the interpretation of diagnostic tests.

In a normal distribution, the mode (the most frequent value) is the mean value, and 68.3 percent, 95.4 percent, and 99.7 percent of the values lie within (plus or minus) 1, 2, and 3 standard deviations (SD) from the mean value, respectively. The cutoff for an abnormal test result often is defined arbitrarily as any value that is more than 2 SD from the mean of a normally distributed population; thus, 5 percent (actually 4.6%) of the population would be categorized as abnormal. Half the people with abnormal results will be 2 SD below the mean, and half will be 2 SD above the mean. Thus, 2.5 percent (actually 2.3%) of the population will have abnormally high values. In some instances, 3 SD (or some other arbitrary parameter) is used to determine the cutoff. However, of importance is to realize that these definitions of normal and abnormal are merely statistical models that should not necessarily be translated to mean that 2.5 percent of the population is "diseased." As noted earlier, with this kind of statistical logic, the diagnosis of disease may be related to the number of tests performed because if multiple tests are conducted, the likelihood that the result of any one will be abnormal is increased. If 15 independent tests are performed on a patient, the probability that any one of the test results will be abnormal by chance is 54 percent.

Other statistical models might be more appropriate for assessing a diagnostic test. For example, interpreting diagnostic tests would be relatively easy if two separate, nonoverlapping normal distributions of diseased and disease-free patients existed. Unfortunately, although this model may apply for some rare conditions (e.g., genetic disorders in which affected persons are missing an enzyme that metabolizes certain substances, which leads to uniquely high concentrations of the substance in persons with the disorder), the model rarely is applicable. A model that is more appropriate for most infectious illnesses is shown in Figure 6-6. In this model (the example of which uses levels of serum calcium), the diseased and nondiseased populations have separate, partially overlapping distributions. The specificity of a diagnostic test is related directly to the degree to which the two distributions overlap.

Accuracy of a Diagnostic Test

Two important characteristics of a diagnostic test—its *reproducibility* and its *validity*—are the key components of its accuracy. The reproducibility (sometimes called *reliability* or *precision*) of a test simply is the degree to which retesting yields the same result. A test that provides results that are not reproducible is of little diagnostic value. The validity of the results of a diagnostic test may be divided into two components: *sensitivity* and *specificity* (Table 6-8). The *sensitivity* of a test is the proportion of persons with disease who are identified accurately by the test as having disease (true positives/[true positives + false negatives]). The *specificity* of a test is the proportion of persons without disease who are identified accurately by the test as not having disease (true negatives/[true negatives + false positives]). The ideal test has both a specificity and a sensitivity of 1. Of course, no diagnostic tests are that accurate. Indeed, a tradeoff exists between sensitivity and specificity—usually, the more sensitive a test is, the less specific it is, and vice versa.

Although the sensitivity and specificity of a given test often are considered to be absolute characteristics of a test, in fact, these characteristics do depend on the types of patients included in the studies in which the indices of the test were developed. Indeed, tests that appear to be both highly sensitive and highly specific in preliminary studies commonly prove to be much less so when used in actual clinical practice because the preliminary studies that establish a test's sensitivity and specificity frequently are affected by problems of spectrum and bias.[49] If the patients enrolled in studies used to assess the test (both those with disease and those without disease) have a relatively narrow spectrum of clinical manifestations, both the sensitivity and the specificity of a diagnostic test may appear to be better than they actually are. For example, if one were to assess the sensitivity of a differential heterophile antibody test to diagnose infection with Epstein-Barr virus (EBV), its sensitivity would appear to be far better if all the subjects infected with EBV were teenagers with severe pharyngitis (a high proportion of whom will have a positive heterophile test result) than if it included

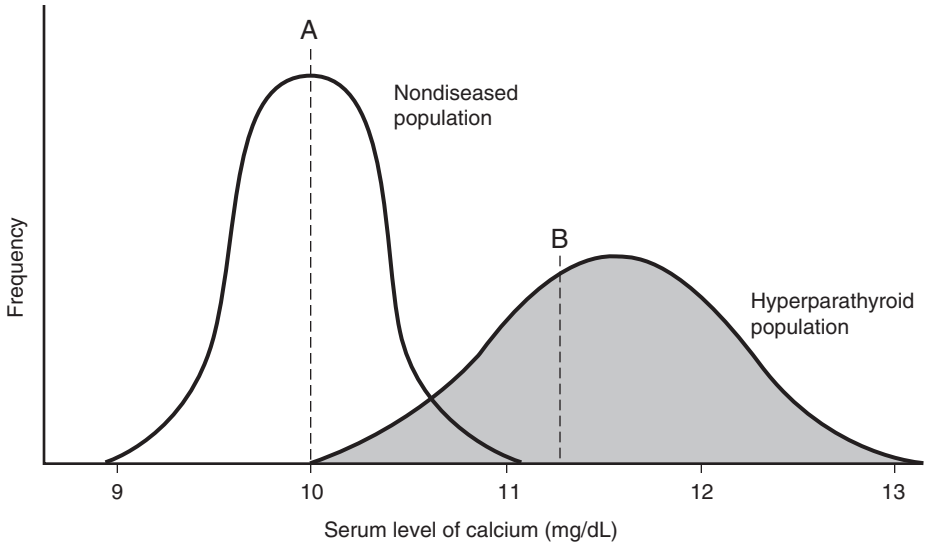

FIGURE 6-6 ■ Overlap in values of randomly performed tests in a population in which most of the people are healthy (curve on the *left*) but some of the people are diseased (curve on the *right*). A person with a level of calcium below point A would be unlikely to have hyperparathyroidism. A person with a level of calcium above point B would be likely to have an abnormality in calcium metabolism, possibly hyperparathyroidism. A person with a level of calcium between point A and point B may or may not have an abnormality in calcium metabolism. (*Note:* The normal range of calcium depends on the method used in a specific laboratory. In some laboratories, the range is from 8.5 to 10.5 mg/dL. In others, as in this illustration, it is from 9 to 11 mg/dL.) (From Jekel JF, Elmore JG, Katz DL. *Epidemiology, biostatistics, and preventive medicine.* Philadelphia: WB Saunders; 1996. p. 88.)

subjects with a broader spectrum of manifestations of EBV infection (e.g., toddlers with symptoms of an infection of the upper respiratory tract, who are much less likely to have a positive heterophile test result when infected with EBV). Likewise, the specificity of a diagnostic test may appear to be better when only asymptomatic, healthy persons are used as nondiseased subjects instead of including patients with other illnesses that produce symptoms similar to those of the target illness. The effects of spectrum of the illness on sensitivities and specificities of a diagnostic test also have been reported for dipstick urinalyses as a diagnostic test for urinary tract infections.[32]

In addition, of importance is to realize that the cutoffs for classifying results as either normal or abnormal usually are somewhat arbitrary and may depend on how the test is used, as well as the nature of the disease that one is trying to detect. If a diagnostic test is being used to screen a population for a relatively mild disorder with a low prevalence, one would want to use a cutoff that results in high specificity to avoid having a large number of false-positive results, even at the expense of lower sensitivity. On the other hand, if the diagnostic test is for a serious disorder or is used not for screening but for diagnosis in a targeted population with at least a moderate prevalence of the disorder (e.g., a white blood cell count in cerebrospinal fluid to detect possible bacterial meningitis in febrile children), one might want to err on the side of overdiagnosis and, therefore, use a cutoff with a high sensitivity at the expense of lower specificity.

Predictive Value of a Diagnostic Test

Sensitivity and specificity are important characteristics of a diagnostic test, but to calculate them, one must know whether the patients do or do not have the disease; clearly, if a physician already has this knowledge, the test does not need to be performed in the first place. From the perspective of a clinician who is caring for an individual patient, the key characteristic of a diagnostic test is its *predictive value* (see Table 6-8). That is, if a test result is positive, what is the probability that the patient has the disease *(positive predictive value)*? Conversely, if a test result is negative, what is the probability that a patient does not have the disease *(negative predictive value)*? However, unlike sensitivity and specificity, the predictive value of a test depends critically on the prevalence of the disease among the subjects who are tested.

TABLE 6-8 **Statistical Indices of a Diagnostic Test**

Result of Test	Disease	No Disease
Positive	a	b
Negative	c	d
True positives: a	Sensitivity = a/(a + c)	
False positives: b	Specificity = d/(b + d)	
True negatives: d	Positive predictive value = a/(a + b)	
False negatives: c	Negative predictive value = d/(c + d)	

This concern is illustrated by the examples in Table 6-9. In example *a*, the prevalence of the disease in the sample is 1 percent and the positive predictive value of a positive test result is only 8.8 percent. By contrast, as shown in examples *b* and *c*, when the prevalence of disease in the sample rises to 10 percent and to 50 percent, respectively, the positive predictive value of a positive test

TABLE 6-9 **Predictive Value of a Diagnostic Test with 95% Sensitivity and 90% Specificity with Different Prevalence of Disease in the Sample**

	Test	Disease	No Disease	Total
a*	Positive	95	990	1,085
	Negative	5	8,910	8,915
	Total	100	9,900	10,000
	Positive predictive value = 8.8%	Negative predictive value = 99.9%		
b†	Positive	950	900	1,850
	Negative	50	8,100	8,150
	Total	1,000	9,000	10,000
	Positive predictive value = 51.4%	Negative predictive value = 99.4%		
c‡	Positive	4,750	500	5,250
	Negative	250	4,500	4,750
	Total	5,000	5,000	10,000
	Positive predictive value = 90.5%	Negative predictive value = 94.7%		

*Prevalence of disease: 1%.
†Prevalence of disease: 10%.
‡Prevalence of disease: 50%.

result rises to 51.4 percent and to 90.5 percent, respectively, even though the sensitivity and the specificity of the test are constant. By the same token, as the prevalence of disease in the sample that is tested rises, the predictive value of a negative test result falls, although the negative predictive value remains reasonably good until the prevalence of disease becomes high.

Of importance for clinicians is to be aware of these epidemiologic truths when ordering diagnostic tests for patients. Sometimes a physician orders tests to "rule out" the possibility that a patient's symptoms are caused by a disease that, based on the patient's history, physical examination, and previous laboratory tests, is unlikely to be the cause of the problem (i.e., the "prior probability" that the patient has the disease is low). Unless the specificity of such tests approaches 100 percent (which is unlikely for most serologic tests), in such situations the great majority of positive test results will be falsely positive; the physician then will be in the unfortunate position of ignoring the result, ordering additional diagnostic tests, or treating the patient for a disease that probably is not the cause of the symptoms.[56] Consequently, clinicians should be selective in ordering diagnostic tests.

TABLE 6-10 **Calculation of Protective Efficacy (PE) of Pneumococcal Vaccine**

Clinical Trial or Cohort Study	Pneumococcal Infection	No Pneumococcal Infection	Total
Vaccinated	a	b	a + b
Not vaccinated	c	d	c + d

PE = [(c/c + d) − (a/(a + b)] ÷ (c/c + d) = 1 − [(a/(a + b) ÷ (c/c + d)]*

Case-Control Study	Pneumococcal Infection (Cases)	No Pneumococcal Infection (Controls)
Vaccinated	a	b
Not vaccinated	c	d

PE = 1 − [(ad) ÷ (bc)]†

*The expression [(a/(a +b) ÷ (c/c +d)] is also known as the relative risk of infection in vaccinated versus unvaccinated persons.
†The expression [(ad) ÷ (bc)] is the odds ratio relating vaccination to infection. In a case-control study, the odds ratio approximates the relative risk.

ASSESSMENT OF THE PROTECTIVE EFFICACY OF A VACCINE (OR OF ANY INTERVENTION)

Assessment of the protective efficacy of a vaccine is discussed in this section, but the same methods (with different outcomes specified, of course) can be applied to any intervention, such as a drug or physical therapy. The protective efficacy (PE) of a vaccine may be assessed with either experimental or observational studies. The classic way to assess a vaccine's efficacy is in an experimental, randomized, double-blind clinical trial. The major observational designs available are cohort studies and case-control studies—although a large variety of hybrid designs such as household exposure studies and indirect cohort studies are available—but complete descriptions of them are beyond the scope of this chapter.[42,43] Table 6-10 illustrates calculation of the PE of a pneumococcal vaccine with data from clinical trials, cohort studies, and case-control studies.

Clinical Trials

In a randomized clinical trial, subjects at risk of acquiring the infection in question would be assigned randomly either to receive the vaccine (vaccinees) or not to receive the vaccine (controls). Subsequently, all subjects would be observed for occurrence of the infection during an appropriate period of follow-up. At the conclusion of the

study, the *protective efficacy* of the vaccine, an index devised by Greenwood and Yule[23] to indicate the proportionate reduction in the frequency of disease attributable to the vaccine, would be calculated. PE is defined as follows:

$$\text{PE} = (\text{Risk of infection in controls} - \text{Risk of infection in vaccinees}) \div \text{Risk of infection in controls}$$

A PE of 100 percent indicates complete protection against infection, a PE of 0 percent indicates no protection, and negative values indicate that a greater risk of acquiring infection existed among vaccinees than among controls. By rearranging terms, PE equals 1 minus the risk of infection in vaccinees divided by the risk of infection in controls. Because the ratio in this equation is the relative risk of infection developing in vaccinees versus controls, PE is, by definition, equal to 1 minus the relative risk.

Observational Cohort Studies

In a cohort study, subjects at risk would be selected on the basis of whether they had been vaccinated or had been left unvaccinated during routine clinical care. The vaccinated and unvaccinated groups then would be monitored longitudinally to assess frequencies of infection. The analysis of a vaccine's PE for cohort studies is similar to that for clinical trials, but because the study is nonexperimental, the results are more subject to bias than are those of a well-conducted, randomized clinical trial.

Case-Control Studies

In a case-control study, patients with antecedent conditions that place them at high risk for pneumococcal infection would be eligible to be subjects. Patients with serious pneumococcal infection would become subjects in the case group. The control group would consist of subjects with similar high-risk conditions but without pneumococcal infection. The two groups then would be compared for the frequency of antecedent vaccination with pneumococcal vaccine.[59]

In a case-control study, the strength of the relationship between vaccination and subsequent infection is measured by an odds ratio (the ratio of the odds of vaccination in the case subjects to the odds of vaccination in the controls). Because the odds ratio in a case-control study closely approximates the relative risk of pneumococcal infection in a longitudinal study and because PE is defined as 1 minus the relative risk, the value (1 – odds ratio) closely approximates the PE that would be calculated from a longitudinal study.

NEW REFERENCES SINCE THE SIXTH EDITION

8. Campbell MK, Elbourne DR, Altman DG. Consort statement: extension to cluster randomized trials. *BMJ* 2004;**328**;702–8.
11. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States, 2010. *M M W R Morb Mortal Wkly Rep* 2011;**60**:1009–13.
12. Chin C-S, Sorenson J, Harris JB, et al. The origin of the Haitian cholera outbreak. *N Engl J Med* 2011;**364**:33–42.
26. Hulley SB, Cummings SR, Browner WS, et al. *Designing clinical research*. 3rd ed. Baltimore: Lippincott Williams & Wilkins; 2006.
45. Piaggio G, Elbourne DR, Altman DG, et al. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. *JAMA* 2006;**295**:1152–60.
46. Peiris JSM, Yuen KY, Osterhaus ADME, et al. The severe acute respiratory syndrome. *N Engl J Med* 2003;**349**:2431–41.
48. Rangel JM, Sparling PH, Crowe C, et al. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 2005;**11**:603–9.
55. Scott IA. Non-inferiority trials: determining whether alternative treatments are good enough. *Med J Aust* 2009;**190**:326–30.
63. Snappin SM. Noninferiority trials. *Curr Control Trials Cardiovasc Med* 2000;**1**:19–21.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Allison AC. Protection afforded by sickle-cell trait against subtertian malarial infection. *BMJ* 1954;**1**:290–4.
2. Anderson P, Johnston RB, Smith DH. Human serum activities against *Hemophilus influenzae*, type b. *J Clin Invest* 1972;**51**:31–8.
3. Asnis DS, Conetta R, Teixeira AA, et al. The West Nile virus outbreak of 1999 in New York: the Flushing Hospital experience. *Clin Infect Dis* 2000;**30**:413–8.
4. Benichou J, Wacholder S. A comparison of three approaches to estimate exposure-specific incidence rates from population-based case-control data. *Stat Med* 1994;**13**:651–61.
5. Breslow NE, Day NE, Davis W, editors. Statistical methods in cancer research. Vol. I. The analysis of case-control studies. Lyon: International Agency for Research on Cancer; 1980.
6. Breslow NE, Day NE, Heseltine E, editors. Statistical methods in cancer research. Vol. II. The design and analysis of cohort studies. Lyon: International Agency for Research on Cancer; 1987.
7. Bulpitt CJ. Confidence intervals. *Lancet* 1987;**1**:494–7.
8. Campbell MK, Elbourne DR, Altman DG. Consort statement: extension to cluster randomized trials. *BMJ* 2004;**328**;702–8.
9. Centers for Disease Control and Prevention. Ongoing multistate outbreak of *Escherichia coli* type O157:H7 infections associated with consumption of fresh spinach—United States. *M M W R Morb Mortal Wkly Rep* 2006;**55**:1045–6.
10. Centers for Disease Control and Prevention. *Public Health guidance for community-level preparedness and response to severe acute respiratory syndrome (SARS). Supplement D: Community containment measures, including non-hospital isolation and quarantine*. January 8, 2004. Available at http://www.cdc/ncidod/sars/guidance/D/pdf/d.pdf. Accessed January 1, 2007.
11. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States, 2010. *M M W R Morb Mortal Wkly Rep* 2011;**60**:1009–13.
12. Chin C-S, Sorenson J, Harris JB, et al. The origin of the Haitian cholera outbreak. *N Engl J Med* 2011;**364**: 33–42.
13. Choi K, Thacker SB. An evaluation of influenza mortality surveillance, 1962-1979, I: Time series forecasts of expected pneumonia and influenza deaths, and II: Percentage of pneumonia and influenza as an indicator of influenza activity. *Am J Epidemiol* 1981; **113**:216–26 and 227–35.
14. Donsero TJ, Rendtorff RC, Mallison GF, et al. An outbreak of Legionnaires' disease associated with a contaminated air-conditioning cooling tower. *N Engl J Med* 1980;**302**:365–70.
15. Feinstein AR. *Clinical epidemiology: the architecture of clinical research*. Philadelphia: W. B. Saunders; 1985.
16. Ferrieri P, for the Jones Criteria Working Group. AHA Scientific Statement. Proceedings of the Jones criteria workshop. *Circulation* 2002;**106**:2521–3.
17. Fleiss JL, Levin B, Cho-Paik M, et al. *Statistical methods for rates and proportions*. 3rd ed. New York: John Wiley & Sons; 2003.
18. Fothergill LC. Biological warfare and its defense. *Public Health Rep* 1957;**72**:865–71.
19. Fox JP, Elveback LR. Herd immunity: changing concepts. In: Notkins AL, editor. *Viral immunology and immunopathology*. New York: Academic Press; 1975. p. 273–90.
20. Fox JP, Hall CE, Cooney MK. The Seattle Virus Watch. VII: Observations of adenovirus infections. *Am J Epidemiol* 1977;**105**: 362–86.
21. Frobisher M. *Fundamentals of microbiology*. 7th ed. Philadelphia: W. B. Saunders; 1962. p. 354.
22. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus, I: The role of humoral antibodies. *J Exp Med* 1969;**129**:1307–26.
23. Greenwood M, Yule UG. The statistics of anti-typhoid and anti-cholera inoculations, and the interpretation of such statistics in general. *Proc R Soc Med* 1915;**8**:113–94.
24. Gwaltney Jr JM, Hendley JO. Rhinovirus transmission: One if by air, two if by hand. *Am J Epidemiol* 1978;**107**:357–61.
25. Huebner RJ. The virologist's dilemma. *Ann N Y Acad Sci* 1957; **67**:430–8.
26. Hulley SB, Cummings SR, Browner WS, et al. *Designing clinical research*. 3rd ed. Baltimore: Lippincott Williams & Wilkins; 2006.
27. Jones TD. Diagnosis of rheumatic fever. *JAMA* 1944;**126**: 481–4.
28. Kahn HA, Sempos CT. *Statistical methods in epidemiology. Vol. 12. Monographs in epidemiology and biostatistics*. New York: Oxford University Press; 1989.
29. Katz M, Stiehm ER. Host defenses in malnutrition. *Pediatrics* 1977;**59**:490–5.
30. Kelsey JL, Thompson WD, Evans AS. *Methods in observational epidemiology. Vol. 10. Monographs in epidemiology and biostatistics*. New York: Oxford University Press; 1986.
31. Kleinbaum DG, Kupper LL, Morgenstern H. *Epidemiologic research: principles and quantitative methods*. Belmont, CA: Lifetime Learning Publications Division, Wadsworth; 1982.
32. Lachs MS, Nachamkin I, Edelstein PH, et al. Spectrum bias in the evaluation of diagnostic tests: lessons from the rapid dipstick test for urinary tract infection. *Ann Intern Med* 1992;**117**:135–40.
33. Lederberg J, Shope RE, Oaks Jr SC, editors. *Emerging infections: microbial threats to health in the United States*. Washington, DC: National Academy Press; 1992.
34. Lee ET. *Statistical methods for survival data analysis*. Belmont, CA: Lifetime Learning Publications Division, Wadsworth; 1980.
35. Levine MM, DuPont HL, Formal SB, et al. Pathogenesis of *Shigella dysenteriae* 1 (Shiga) dysentery. *J Infect Dis* 1973;**127**:261–70.
36. MacKenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med* 1994;**331**:161–7.
37. Maxcy KF. An epidemiological study of endemic typhus (Brill's disease) in the southeastern United States. *Public Health Rep* 1926;**41**:2967–95.
38. McCarthy PL, Sharpe MR, Spiesel SZ, et al. Observation scales to identify serious illness in febrile children. *Pediatrics* 1982; **70**:802–9.
39. McDade JE, Shepard CC, Fraser DW, et al. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. *N Engl J Med* 1977;**297**:1197–203.
40. Mortimer Jr EA. Hospital staphylococcal infections: interruption of transmission as a means of control. *Med Clin North Am* 1963;**47**:1247–56.
41. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease: a statement for health professionals from the Committee on Rheumatic Fever, Endocarditis and Kawasaki Disease, Council on Cardiovascular Disease in the Young, American Heart Association. *Circulation* 2004;**110**:2747–71.
42. Orenstein WA, Bernier RH, Dondero TJ, et al. Field evaluation of vaccine efficacy. *Bull WHO* 1985;**63**:1055–68.
43. Orenstein WA, Bernier RH, Hinman AR. Assessing vaccine efficacy in the field: further observations. *Epidemiol Rev* 1988;**10**:212–41.
44. Pasteur L. The physiological theory of fermentation. In: Eliot C, editor. *Scientific papers*. New York: P. F. Collier & Sons; 1910. p. 289–381.
45. Piaggio G, Elbourne DR, Altman DG, et al. Reporting of noninferiority and equivalence randomized trials: an extension of the CONSORT statement. *JAMA* 2006;**295**:1152–60.
46. Peiris JSM, Yuen KY, Osterhaus AD, Stöhr K. The severe acute respiratory syndrome. *N Engl J Med* 2003;**349**:2431–41.
47. Perry WD, Siegel AC, Rammelkamp Jr CH, et al. Transmission of group A streptococci, I: The role of contaminated bedding. *Am J Hyg* 1957;**66**:85–101.
48. Rangel JM, Sparling PH, Crowe C, et al. Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 2005;**11**:603–9.
49. Ransahoff DF, Feinstein AR. Problems of spectrum and bias in evaluating the efficacy of diagnostic tests. *N Engl J Med* 1978;**299**:926–30.
50. Riegelman RK. *Studying a study and testing a test: how to read the medical evidence*. 5th ed. Philadelphia: Lippincott, Williams & Wilkins; 2004.
51. Renteln HA, Hinman AR. A waterborne epidemic of gastroenteritis in Madera, California. *Am J Epidemiol* 1967;**86**:1–10.
52. Rosen G. *A history of public health*. New York: M.D. Publications; 1958. p. 62–4.
53. Sackett DL, Haynes RB, Guyatt GH, et al. *Clinical epidemiology: a basic science for clinical medicine*. 2nd ed. Boston: Little, Brown; 1991.
54. Schlesselman JJ. *Case-control studies: design, conduct, analysis (Monographs in epidemiology and biostatistics)*. New York: Oxford University Press; 1982.
55. Scott IA. Non-inferiority trials: determining whether alternative treatments are good enough. *Med J Aust* 2009;**190**:326–30.

56. Seltzer EG, Shapiro ED. Misdiagnosis of Lyme disease: when not to order serologic tests. *Pediatr Infect Dis J* 1996;**15**:762–3.
57. Selvin S. Statistical analysis of epidemiologic data. In: Kelsey JL, Marmot MG, Stolley PD, et al, editors. Monographs in epidemiology and biostatistics. Vol. 17. New York: Oxford University Press; 1991.
58. Selye H. *The physiology and pathology of exposure to stress*. Montreal: Acta; 1950.
59. Shapiro ED, Berg AT, Austrian R, et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *N Engl J Med* 1991;**325**:1453–60.
60. Shapiro ED. Case-control studies of the effectiveness of vaccines: validity and assessment of bias. *Pediatr Infect Dis J* 2004;**23**:127–31.
61. Siegel S, Castellan Jr NJ, *Nonparametric statistics for the behavioral sciences*. New York: McGraw-Hill; 1988.
62. Sigerist HE. *The great doctors*. New York: W. W. Norton; 1933. p. 100–8, 375–9.
63. Snappin SM. Noninferiority trials. *Curr Control Trials Cardiovasc Med* 2000;**1**:19–21.
64. Snedecor GW, Cochran WG. *Statistical methods*. 8th ed. Ames: Iowa State University Press; 1989.
65. Snow on Cholera, being a reprint of two papers by John Snow, M. D., together with a biographical memoir by B. W. Richardson and an introduction by Wade Hampton Frost. New York: Commonwealth Fund; 1936.
66. Thompson SG, Pocock SJ. Can meta-analyses be trusted? *Lancet* 1991;**338**:1127–30.
67. Thompson WH, Beaty BJ. Venereal transmission of La Crosse (California encephalitis) arbovirus in *Aedes triseriatus* mosquitoes. *Science* 1977;**196**:530–1.
68. Wannamaker LW. The epidemiology of streptococcal infections. In: McCarty M, editor. *Streptococcal infections*. New York: Columbia University Press; 1954. p. 157.
69. Wenzel RP. A new hantavirus infection in North America. *N Engl J Med* 1994;**330**:1004–5.
70. Wharton M, Chorba TL, Vogt RL, et al. Case definitions for public health surveillance. *M M W R Recomm Rep* 1990;**39**(RR-13): 1–43.
71. Winslow CEA. The colonial era and the first years of the republic (1606-1799): the pestilence that walketh in darkness. In: Top FH, editor. *The history of American epidemiology*. St. Louis: CV Mosby; 1954. p. 31–44.

PART II

INFECTION OF SPECIFIC ORGAN SYSTEMS

This page intentionally left blank

SECTION I

UPPER RESPIRATORY TRACT INFECTIONS

CHAPTER 7

The Common Cold

James D. Cherry

The common cold is an acute, communicable, viral disease characterized by nasal stuffiness, sneezing, coryza, throat irritation, and no or minimal fever. Although the terms *upper respiratory tract infection (URI)* and *nasopharyngitis* are used frequently as synonyms for the common cold by physicians and other health care workers, the practice should be discouraged; the term *URI* is too broad, and pharyngitis is not present in most colds. To add to the confusion regarding terminology, "a cold" frequently has an even more inclusive connotation to the layperson.

HISTORY

Although the common cold undoubtedly has had an impact on the events of history, the specific symptom complex in ancient times was overshadowed by more severe contagious problems (influenza, plague, smallpox) and by septic diseases (otitis, sinusitis, mastoiditis, pneumonia) that were complications of upper respiratory tract viral infections. The name *common cold* most certainly arose from the fact that the onset of symptoms included the feeling of chilliness on exposure to cold. This association was perceived as a cause-and-effect relationship. More than 200 years ago, Benjamin Franklin pointed out that colds were caught from other people rather than by exposure to cold.[158]

In 1914, Kruse[125] showed that colds could be transmitted by the nasal instillation in healthy adults of Berkefeld-filtered nasal washings from ill individuals and that the causative agent was smaller than common bacteria. These findings were confirmed clearly in 1930 by Dochez and associates.[54] The way was paved for more extensive study of respiratory viral infections in 1933, when Smith and colleagues[184] reported the isolation and cultivation of an influenza A virus from a human.

The greatest contribution to the present understanding of the common cold has been the use of human volunteers under carefully controlled conditions. The Common Cold Research Unit at Salisbury, England, which was established in 1946,[5,179] and volunteer studies at this institution and studies done in the United States* are responsible for much of the present understanding of colds in adults.

Although studies of respiratory illness in children also have been extensive, controlled virus challenge trials have not been done. Pediatric studies have been most useful in delineating the spectrum of clinical manifestations by age group and seasonal prevalence rates of the respiratory viruses.

During the last decade the use of molecular methods has increased our understanding of the contributions of both newly discovered and established viruses in the etiology of the common cold and other respiratory illnesses.[71,135,173]

ETIOLOGIC AGENTS

Initial investigations into the etiology of the common cold were based on the hypothesis that only one etiologic agent needed to be discovered. Subsequent studies revealed that many different groups of viruses were involved etiologically and that many types frequently existed within each group. Table 7-1 lists the agents associated with colds. Each of these agents is covered more fully in other chapters; this chapter presents an overview. As a group, rhinoviruses are the most common cause of colds in children and adults. Also of major importance in the etiology of colds are reinfections with parainfluenza viruses and respiratory syncytial virus (RSV). Although the quantitative contribution of coronaviruses to colds in children is less well known, they are significant contributors.* More recent studies also indicate that human metapneumovirus and human bocavirus cause colds in children.†

Enteroviruses and adenoviruses have been implicated frequently in upper respiratory tract illnesses, but in most instances the illnesses do not conform to strictly defined colds. Reoviruses cause colds, but their contribution to the overall incidence is unknown. *Mycoplasma pneumoniae*, *Chlamydophila* (formerly *Chlamydia*) *pneumoniae*, *Streptococcus pneumoniae*, and *Haemophilus influenzae* infections were identified serologically in one study involving young adults with colds, but in most instances, concomitant viral infections also were noted.[136] Other agents, such as *Coccidioides immitis*, *Histoplasma capsulatum*, *Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella bronchiseptica*, *Bordetella holmesii*, *Chlamydophila psittaci*, and *Coxiella burnetii*, also have been associated with illnesses with initial coldlike symptoms.

*References 1, 27, 43, 46, 49-52, 88-91, 94-97, 111, 112, 118, 140, 143, 198, 200, 202, 204, 205.

*References 55, 71, 80, 166, 173, 190.
†References 8, 14, 39, 69-71, 100, 165, 173, 214, 215.

EPIDEMIOLOGY

The common cold is an exceedingly frequent illness in childhood. For rhinoviruses alone, more than 100 serologically different viral types can cause this illness. Nonetheless, a general predictability of incidence and seasonal occurrence exists for the common cold. Numerous epidemiologic studies have been conducted on the occurrence of respiratory tract illnesses, but calculating a precise incidence of the common cold from these studies is difficult because criteria of disease classification have been different. The findings in three carefully performed studies are presented in Table 7-2. Two of these studies were done approximately 60 years ago, and the other was done more than 40 years ago. These studies involved a wide range of family life settings, and the average number of colds per year in children varied from three to eight.[10-13,24,76,136] Adults had on the average approximately half the number of colds as their children.

In more recent times and in the era of the common use of daycare facilities, studies of the scope and magnitude of those presented in Table 7-2 have not been done. In one study published in the 1980s, the incidence of acute respiratory tract infections in daycare attendees peaked at 10 per child-year during the second 6 months of life.[49] A study by Wald and associates[211] found that children in home care had 4.7 illnesses per year, whereas the illness rate was 7.1 per year in children in daycare. Of the illnesses, 89 percent in daycare attendees were respiratory tract infections.

Recently, Grüber and associates[87] reported the history of respiratory infections in a cohort of German children. The children in this cohort were seen at ages 3, 6, 12, 18, and 24 months and then annually until age 12 years. The common cold illness rates per year were infancy 3.1, year two 3.2, year three 2.1, year four 2.3, year five 1.8, year six 1.3, years seven to nine 1.1, and years ten to twelve 1.0. The reason for these overall decreased rates in contrast to previous studies is not apparent, but less complete data ascertainment seems likely. In addition, smaller family size and less use of daycare could be factors.

In a study in Arizona, researchers found that 52 percent of 2-year-old children who attended large daycare centers had six to nine colds per year.[16] The conventional spread of colds has its initial focus in the school.[10,13,24] School-age children become infected and introduce secondary infections into the home. Under these conditions, the secondary attack rate was highest in other school-age and preschool-age children. Generally, the secondary attack rate in adult family members was approximately half that of the children. The introduction of infection into the family by adults is unusual. In crowded settings, such as a university, infection is common and can be associated with substantial morbidity. In a cohort study of more than 3000 college students, colds and influenza-like illness were common findings and were associated with missed classes, poor performance on class assignments, missed extracurricular activities, and increases in use of medical care.[147]

As noted previously, the present trend toward the use of daycare centers and preschool programs has increased the number of primary infections in younger children and has made them the source from which secondary family infections frequently occur.[145] In a study that involved 606 children who received care at home or in a daycare program during the first 3 years of life, researchers found that children who attended a large daycare facility (six or more unrelated children) had more

TABLE 7-1 **Infectious Agents Associated with the Common Cold**

Category	Agents
Common viruses that usually cause the common cold	Rhinoviruses Parainfluenza viruses Respiratory syncytial virus Coronaviruses Human metapneumovirus
Common infectious agents that occasionally cause illness with common cold symptoms	Adenoviruses Enteroviruses Influenza viruses Reoviruses
Illnesses with initial symptoms suggestive of the common cold	Human bocavirus *Coccidioides immitis* *Histoplasma capsulatum* *Bordetella pertussis, Bordetella parapertussis, Bordetella bronchiseptica* *Chlamydophila psittaci* *Coxiella burnetii*

TABLE 7-2 **Comparative Incidence of Upper Respiratory Infection by Age: London, 1952-1953; Seattle, 1965-1969; and Cleveland, 1948-1950**

London		*Seattle*		*Cleveland*	
Age (yr)	**Illnesses/Person/Yr**	**Age (Yr)**	**Illnesses/Person/Yr**	**Age (yr)**	**Illnesses/Person/Yr**
0-4	5	<1	3.5	<1	6.9
5-9	3.6	1	3.8	1	8.3
10-16	4.1	2-5	3.7	2-5	8.3
Adult	2.9	6-9	2.7	6-9	6.1
		10-19	1.6	10->11	5.5
		Adult	2.9	Adult	4.8

Data modified from Brimblecombe et al.,[24] Fox et al.,[76] and Badger et al.[10]

frequent colds at year 2 and less frequent colds at years 6 through 11 than did the children with home care only.[16]

Close personal contact between children is necessary for the transmission of viruses that cause colds. In the typical pediatric practice office setting, no increased risk for the acquisition of respiratory tract illnesses by well infants has been shown.[133] Among children, boys tend to have more colds than do girls.[10,24] In the conventional family setting, mothers tend to have at least one more cold per year than their spouses have.[10,24,76,77] The usual incubation period of colds is 1 to 5 days.

In all nonisolated populations, colds occur more frequently during the winter months than during the summer.[53,78,136,206] This seasonal discrepancy in incidence is as apparent in areas of high wintertime mean temperatures as it is in locations with extremely low temperatures. In the tropics, colds are more prevalent during the rainy season. Colds occur throughout the world. In isolated populations in which the number of people is few (e.g., members of Antarctic exploration teams and isolated island communities), colds do not occur unless introduced by a visiting person.[105]

Although colds can be produced regularly in volunteers, the method of transmission of viruses that results in colds under natural circumstances is unclear.* In individuals infected with respiratory viruses that cause colds, the greatest concentration of virus is in the nasal secretions. Children tend to have greater concentrations of virus than do adults, and they tend to shed virus for longer periods. Secretions related to coughing or saliva do not contain appreciable amounts of virus, and both are unlikely sources of contagion. During the process of talking, little virus is disseminated into the air. The greatest amount of virus from an infected individual is contributed to the surrounding environment by sneezing, nose blowing, and the general contamination of external surfaces (including the person's hands) with nasal secretions.

The route of acquisition of virus is by the nose and possibly the conjunctiva. With these facts in mind, one can easily see that a susceptible individual can become infected by inhalation of virus in droplet nuclei (small particles) resulting from a sneeze, by the direct nasal hit of virus containing large droplets from a sneeze, by nose blowing, or by the inoculation of virus (usually by the fingers of the recipient) from nasal secretions from disseminators that have been transmitted directly or indirectly. In children, most likely the spread either involves close contact with large droplets of nasal secretions containing virus that are applied to the nose from the hands of the future host or occurs by close-range airborne acquisition.

Considerable folklore is related to the catching of a cold. However, all available evidence to date indicates that cold weather per se, chilling, wet feet, and drafts neither cause nor increase the susceptibility of individuals to colds caused by viral infections.[56,111] A study of 180 subjects showed that acute cooling of the feet was associated with the onset of common cold symptoms.[117] On day 4, after the cooling of the feet or the control procedure, significantly more subjects in the chilled group thought they were suffering from a cold (14.4%) compared with the control group (5.6%). The subjects who thought they were suffering from a cold had a history of more colds each year in contrast to subjects who did not develop cold symptoms. In this study, no virologic studies were done, so no evidence exists that the cold symptoms observed were caused by viral infections.

In a study of respiratory tract infections in Finnish military recruits, it was found that cold temperature and low humidity were associated with an increased occurrence of respiratory infections; a decrease in temperature and humidity preceded the onset of the infections.[137] However, no virologic data support the findings in this study.

In contrast, in controlled studies in which virologic studies were performed, no increased susceptibility to colds after exposure to chilling conditions was found.[5,56,60] In adults, risk factors for increased susceptibility to colds include stress, smoking, high basal levels of catecholamines, infrequent exercise, low intake of vitamin C, low sleep efficiencies, and lack of diverse social networks.[42,189] Physical stress, as opposed to mental stress, does not seem to be associated with increased susceptibility to colds. In a study of nine healthy men who were competitive cyclists who underwent intensive interval training, the men experienced enhanced performance but had no change in white blood cells, cytokine levels, or the frequency of upper respiratory tract infections.[61]

A fascinating volunteer study in adults by Cohen and colleagues[41] has recently been reported. In this study the subjects were given rhinovirus 39 or influenza A/Texas/36/91 intranasally. It was found that subjects with increased subjective socioeconomic status were less susceptible to upper respiratory tract infections than subjects with decreased subjective socioeconomic status. In contrast, no relationship was found between objective socioeconomic status and susceptibility to the two challenge viruses.

PATHOPHYSIOLOGY

The pathophysiology of human infections with viruses that cause colds is presented in the sections of this book covering the individual infectious agents. A general overview is presented here. Few studies on the pathophysiology of respiratory tract infections have been done in children; the material presented in this section has been derived mainly from studies in adults.* The clinical syndrome of the common cold can occur in association with more than 100 different viral types and in many instances can occur with either a primary infection or a reinfection with a particular viral type.

Although the primary site of virus inoculation in some colds may be on the conjunctival surface, most result from inhalation or self-inoculation of virus onto the nasal mucosa. After acquisition of a virus has occurred,

*References 25, 28, 43, 46, 47, 52, 88, 90, 94, 103, 111, 116, 140, 170, 205, 206.

*References 9, 37, 57, 120, 148, 152, 168, 180, 195, 205, 206, 217, 221.

infection of the cells of the local respiratory epithelium develops. This infection varies in the degree of cytopathology on the basis of the viral agent. The infection spreads locally, resulting in an increase in nasal secretions with an increased protein content. Symptoms (nasal stuffiness and throat irritation followed by sneezing) begin on the second or third day and are caused by cellular damage and irritation. Virus shedding is at its maximum in 2 to 7 days, although some shedding may continue for another 2 weeks.

Hilding[104] examined biopsy specimens, scrapings, and smears of nasal secretions and noted that submucosal edema occurred initially, followed by shedding of the ciliated epithelial cells. By the fifth day, the epithelial damage had reached its maximum, with regeneration occurring during the next 10 days. Winther and associates[218] performed similar studies and noted the sloughing of epithelial cells, but they found that the epithelial lining remained continuous with normal cell borders. On the second day of disease, an increase in the number of neutrophils in the epithelium and lamina propria occurred. Epithelial mast cells were not involved in the inflammation. The nasal discharge during the second to the seventh day is mucopurulent, owing to its content of desquamated epithelial cells and polymorphonuclear leukocytes.

In experimental rhinovirus colds in adults, little or no discernible damage to the nasal epithelium has been shown.[89,195,216,217] Arruda and associates[9] noted in adults infected with rhinovirus types 14 and 39 that virus replicates in ciliated and nonciliated cells in the nasopharynx and that only a very small proportion of the cells were infected.

Because damage to the nasal epithelium is not noted in rhinovirus colds, apparently cell death is not the cause of symptoms. Naclerio and colleagues[144] and Proud and associates[167] found that kinins are generated locally and their concentrations correlate with the severity of symptoms. The cause-and-effect relationship between kinins and symptoms is questionable, however, because treatment with a bradykinin antagonist failed to lessen symptoms.[203] In addition, steroid therapy was found to reduce the concentration of kinins in nasal wash fluid, but it did not affect clinical symptoms.

More recently, researchers noted that interleukin-1 (IL-1) may contribute to the pathogenesis of rhinovirus infections.[168] Ongoing molecular and biochemical research has provided information on methods by which rhinoviruses interfere with the host response, induce epithelial expression of IL-6 and IL-8, and mediate infection via activation of kinases and receptor binding activity.[63,122,141,157] Similarly, research into the other viruses of the common cold has helped in the understanding of their similarities and differences, such as the cell-mediated immune response to human metapneumovirus and RSV.[59]

In studies in children with acute upper respiratory tract infections, IL-1β, IL-8, IL-6, and tumor necrosis factor (TNF)-α were found to be elevated markedly in nasal lavage fluid.[148] Pacifico and associates[152] found that IL-8 concentrations and white blood cell and neutrophil counts were significantly greater in children with rhinovirus colds than in well children. In an adult volunteer study, Turner and colleagues[205] noted a significant rank correlation between nasal obstruction severity, rhinorrhea severity, and nasal-wash albumin concentrations and the increase in IL-8 concentration from baseline to days 2 to 4 after virus challenge. In a study of 285 children with a parental history of asthma, other respiratory allergy, or both, blood specimens were obtained at birth and at 1 year of age.[81] The cytokine responses of mononuclear cells when incubated with phytohemagglutinin, RSV, and a rhinovirus were analyzed. Vigorous IL-13 and interferon (IFN)-γ responses to phytohemagglutinin and a marked secretion of IFN-γ in response to the viruses were associated with a reduced risk for developing wheezing with viral infections in the first year of life.

Pedersen and colleagues[156] studied nasal mucociliary transport in naturally acquired colds, noting that transport was reduced markedly during the acute illness and that slight impairment remained for approximately 1 month. They point out that because some children have four to six colds during a winter season, these children may have constantly impaired mucociliary transport.

Although viremia has been noted during infections with some of the viruses that cause colds, viremia is not known to occur during the typical common cold. The infection is restricted to the epithelial surfaces of the upper respiratory tract air passages, including the sinuses and eustachian tubes. With infection, local IFN is produced and presumably has a major role in controlling the infection.[36] Serum antibody and secretory antibody regularly result from infection. The roles of cell-mediated factors in immunity and disease pathogenesis of colds are unknown.

Levandowski and associates[129] noted in rhinovirus-challenged volunteers that total T cells, and particularly helper T cells, were depressed. The magnitude of this finding correlated with progression of infection and symptoms. In a study in which volunteers received rhinovirus type 39, Skoner and associates[180] found a slight increase in helper ($CD4^+$) and suppressor ($CD8^+$) T cells during illness. Noting that asthma exacerbations and rhinovirus infections are associated with decreased pH and ammonium levels in exhaled breath condensates, Carraro and colleagues[31] studied whether a direct rhinovirus infection, the host immune response to the infection, or both decreased the airway epithelial cell surface pH in vitro. They found that airway epithelial cell pH is affected partly by T-helper type 1 cytokines. This decrease in pH can provide an innate host defense, inhibiting viral replication in the lower airways. By mechanisms not well understood, within already infected cells, low pH is thought to trigger uncoating of rhinoviral RNA from within the endosome, enabling viral replication.[23]

The role of antibody (serum and secretory) in the protection against reinfection and clinical colds is complicated. High levels of secretory and serum antibodies seem to be protective against reinfections.* Clinically abortive colds probably are reinfection colds with early antibody recall. Fleet and colleagues[74] demonstrated a

*References 34, 35, 74, 109, 110, 112, 113, 128, 181, 194.

short-lived heterologous resistance to rhinovirus colds that probably is not caused by interferon or antibody.

Unexplained constitutional factors also seem to control the clinical manifestations of colds.[174] Although studies have shown genetic disease susceptibility patterns related to tissue types, no studies relating to common respiratory tract infections have been done.[38] In experimental coronavirus infections in adults, clinical severity correlated with detectable immunoglobulin (Ig)E in nasal secretions.[29] This finding suggests that atopy may be related to symptoms in colds caused by coronaviruses. Although clinical symptoms and virologic data suggest that colds are upper respiratory tract diseases, some studies of pulmonary function also have indicated occult lower respiratory tract involvement.[6,35,160]

CLINICAL PRESENTATION

Because the common cold is caused by more than 100 different viral types, considerable variation in the clinical manifestations occurs. As indicated in the beginning of this chapter, the limits of illness to be considered under the diagnosis of the common cold have been set arbitrarily but rigidly. A disappointing note is that although many comprehensive studies of respiratory viral illnesses of children have been done, little attention has been given to the details of upper respiratory tract infections.

Illness in children must be considered under two categories—infants and older children. The latter is similar to illness in adults. In studies involving 100 young adults, Jackson and colleagues[109] noted that virtually all patients complained of nasal discharge, nasal obstruction, and sore throat; approximately 80 percent had malaise, postnasal discharge, headache, and cough; slightly fewer than 50 percent reported a feverish feeling and chilliness; and approximately 25 percent noted burning eyes and nasal membranes and muscle aching. In older children, the onset of illness is heralded by dryness and irritation in the nose and a scratchy feeling in the throat.[146] The initial symptoms are followed within a few hours by sneezing and watery nasal discharge; chilly feelings and occasionally muscular aches also are noted. Other complaints include headache, general malaise, anorexia, and low-grade fever.

After a variable period of 1 to 3 days, the illness changes; the nasal secretions become thicker and frequently develop a purulent appearance. Persistent nasal discharge, associated with the trauma of repeated blowing of the nose, leads to excoriation around the nose. Nasal obstruction leads to mouth breathing, which causes drying of the throat, increasing the discomfort in the throat. The usual duration of illness is approximately 7 days, but lingering cough and nasal discharge may persist for 2 weeks or more.[58]

In infants, the manifestations of illness may be more varied. The onset of illness in infants is associated more often with fever (38° to 39° C [100.4° to 102.2° F]) than it is in older children. Nasal manifestations in infants are similar to manifestations in older children, but the only other manifestations are irritability and restlessness. Occasionally, coryza is the only symptom. Nasal obstruction may interfere significantly with feeding and sleeping. Vomiting and diarrhea also may occur.

DIFFERENTIAL DIAGNOSIS

Because the clinical entity of the common cold is an arbitrary grouping of signs and symptoms limited to anatomic boundaries and is caused by many different viral types, the approach to the differential diagnosis must consider clinical and etiologic criteria. Many upper respiratory tract illnesses are caused by numerous infectious agents that should not be confused with colds. A diagnosis of the common cold should not be considered if objective pharyngitis, other enanthema, or evidence of obstructive airway disease is present. Because the common cold is an acute, self-limited disease, the diagnosis should not be considered in a child who has persistent nasal signs or symptoms. Subacute or chronic illness should suggest the possibility of adenoiditis or sinusitis.

The most important differential diagnostic considerations are clinical entities of noninfectious etiology. Allergic rhinitis is a particularly important possibility in a child with "recurrent colds." Careful attention to family history, a search for allergies, the presence or absence of nasal eosinophilia, and the serum IgE value help confirm or exclude this consideration.

Although not reported particularly in pediatric patients, mental stress can lead to vasomotor responses and rhinitis in some susceptible patients. Chemical irritants can cause coldlike symptoms; the clinical response varies greatly across individuals. Early symptoms of many illnesses, such as pertussis, croup, epiglottitis, measles, and diphtheria, are those of a cold, but in a short time, the more serious nature of the actual illness becomes apparent.

SPECIFIC DIAGNOSIS

The epidemiologic history is the most important aspect of specific diagnosis. In children, if exposure history is requested, a contact usually is uncovered. If strict attention to clinical criteria of the common cold has been adopted, routine laboratory study is unnecessary. Frequently, the physician has an urge to take a throat culture to rule out the possibility of group A streptococcal infection. Usually, one is unnecessary because nasal symptoms are not characteristic of acute streptococcal illness except in infancy, and pharyngitis is not within the limits of the diagnosis of the common cold. The white blood cell count also is of little use.

A specific diagnosis can be made by the isolation of virus from nasopharyngeal secretions. It is performed best by a nasal-wash technique[93] or a nasopharyngeal swab or aspirate. With laboratory techniques of diagnostic virologic facilities such as those in many university hospitals, parainfluenza viruses, RSV, and most rhinoviruses and influenza viruses are recovered. Direct antigen-detection techniques can be used to identify infections caused by RSV, parainfluenza viruses, adenoviruses, and influenza viruses. Today, nucleic acid techniques for the

determination of all known causes of the common cold are available in many reference laboratories.* (See Chapter 252.)

TREATMENT

Although literally hundreds of cold remedies are available, few offer benefit to the pediatric patient and many may be harmful.† No clinically available antiviral agents have been shown effective in the treatment of colds.

In the approach to a child with a cold, the best assumption is that no therapy is indicated in most cases. On occasion, specific symptomatic care can be added in the individual case when it appears to be needed. Many children and adults feel miserable when they have a cold, and therapy with an analgesic often is used. Because aspirin is a risk factor for Reye syndrome in children, the use of acetaminophen rather than aspirin is prudent. The dose per single administration of acetaminophen by year of age is as follows: younger than 1 year, 60 mg; 1 to 3 years, 60 to 120 mg; 3 to 6 years, 120 mg; 6 to 12 years, 150 to 300 mg; older than 12 years, 325 to 650 mg. Administration may be repeated every 6 to 8 hours in young children and every 4 hours in older children. Acetaminophen rarely should be given to infants younger than 6 months of age.

In adult volunteers with rhinovirus infections, acetaminophen was found to be associated with suppression of the serum neutralizing antibody response, and an increase in nasal symptoms was noted in contrast to subjects who received a placebo.[85] In another adult volunteer study, administration of naproxen resulted in a reduction in headache, malaise, myalgia, and cough in contrast to placebo.[187]

Over-the-counter (OTC) cough and cold medications that contain antihistamines, antitussives, expectorants, decongestants, and antipyretics/analgesics in various combinations are readily available for children.[153] Results of the Slone Survey noted that in a 1-week period 10.1% of children in the United States received an OTC cough/cold medication.[208] Adverse events and deaths in children younger than 6 years of age have been observed.‡ In 2007, a U.S. Food and Drug Administration (FDA) advisory group recommended that OTC cough and cold medications not be used in children younger than 2 years of age. The American Academy of Pediatrics in 2008 recommended that OTC cough and cold medications should not be used in children younger than 6 years of age.

Relief of nasal obstruction is the most important therapeutic consideration in young children. Locally applied or orally administered, systemically active decongestants are used frequently, but neither their true efficacy nor their adverse effects have been evaluated carefully. Excessive use of sprays and drops with vasoconstrictive drugs can lead to rebound obstruction, which prolongs the illness. The associated drying effect of vasoconstrictive drugs administered orally can be expected to be deleterious to normal mechanisms of clearance. In young infants, sympathomimetic-antihistamine mixtures in oral drop dosage forms particularly are dangerous because respiratory depression may occur.[83] In addition, in a controlled trial, brompheniramine maleate–phenylpropanolamine hydrochloride was found to be no better than a placebo in relieving cold symptoms in children 6 months to 5 years of age.[40] If vasoactive drugs are used, their use should be restricted to times when maximum benefit would occur (i.e., bedtime), and they should be discontinued within 3 days.

The use of isotonic saline drops and gentle aspiration may be effective in the temporary relief of nasal obstruction in an infant. However, a Cochrane Collaboration review noted that available data was not sufficient to demonstrate efficacy of saline nasal irrigation.[119] It also noted that 40% of babies did not tolerate nasal saline drops. The general humidification of room air may be useful because this moisture tends to dilute tenacious nasal mucus and facilitate its elimination. In a Cochrane Collaboration review, steam inhalation was not shown to offer any consistent benefits in the treatment of the common cold.[178]

Antibiotics have no place in the routine therapy of common colds.* In children, persistent cough occasionally is a problem of such magnitude that it disturbs sleep. For cough, codeine and dextromethorphan often are used.[3] No well-controlled studies support either the efficacy or the safety of codeine or dextromethorphan as an antitussive in children, however. Eccles[66] suggested that the apparent efficacy of liquid cough syrups may be due to the sweetness of the products rather than their active ingredients. The proposed mechanism is that a sweet taste may affect cough at the level of the nucleus tractus solitarius by stimulating the production of endogenous opioids.

In the past, antihistamines were often given to children with colds, but efficacy had not been demonstrated.[213] In more recent years, first-generation antihistamines, but not second-generation products, have been shown to lessen rhinorrhea in adults with colds.[142,143,195,196,203] Doxylamine succinate, clemastine fumarate, chlorpheniramine maleate, and brompheniramine have been shown to offer benefit in controlled trials. The effect of these antihistamines is due to their anticholinergic properties. To date, no studies in children have been reported.

A major controversy relates to the efficacy of vitamin C in the common cold prophylactically and therapeutically. In two carefully controlled volunteer studies, the administration of 3 g of ascorbic acid per day did not prevent or alter the symptoms of experimental colds.[175,212] In addition, several large controlled trials in which vitamin C and placebo preparations were used to prevent and to treat colds were conducted.† Benefit was reported in some of these studies, whereas in others, no efficacy was noted. The reported benefits probably are a result of

*References 14, 55, 69, 70, 80, 100, 135, 173, 190.
†References 2, 7, 15, 18, 20-22, 26, 32, 33, 48, 50, 62, 64, 65, 79, 82, 83, 92, 102, 106, 108, 119, 124, 126, 131, 132, 138, 139, 149, 151, 153-155, 159, 162, 164, 171, 177-179, 182, 183, 188, 192, 197, 208-211, 220.
‡References 48, 126, 153, 171, 177, 183, 208, 209.

*References 84, 107, 115, 123, 127, 130, 150, 172, 185.
†References 4, 30, 44, 45, 67, 118, 161.

statistical artifacts and placebo effect owing to poor study design, rather than specific pharmacologic drug effects. The antihistaminic action of vitamin C[207,222] probably afforded relief to some patients with allergic rhinitis, who thought that their illnesses were colds. Because of the many toxic effects of ascorbic acid,[17] and because its use in treating respiratory illnesses is questionable at best, giving children vitamin C in excess of normal daily requirements seems unwarranted.

For many years, efforts have been made to develop effective rhinovirus chemotherapy, but showing convincing efficacy in natural infection has been a challenge.[154] IFN alfa-2b administered intranasally has been shown to have some efficacy in the prevention of rhinovirus colds in controlled clinical trials.[186] The effect is variable, however, and adverse effects of the medication are frequent occurrences.[191] Intranasal IFN alfa-2b was ineffective in the treatment of naturally occurring colds but showed some benefit in experimental coronavirus colds.[98,200] Studies in adults using an antiviral anti-inflammatory combination for treatment of the common cold showed some benefit in contrast to either agent used alone.[92] However, the irritation caused by intranasal IFN alfa-2b and the drowsiness associated with first-generation antihistamines render these drug combinations suboptimal.

Zinc lozenges have been used to treat the common cold; the internet and lay literature are full of claims of efficacy, although in well-done, controlled studies, efficacy has not been shown.* However, a recent Cochrane Collaboration review noted that zinc administered within 24 hours of onset of symptoms reduced the duration and severity of the common cold in healthy people.[179] It also noted that prophylactic use reduced the incidence of colds and decreased school absenteeism and antibiotic use in children. The use of intranasal zinc preparations also is not well validated and raises some concern because of the possible development of the zinc-induced anosmia syndrome.[2,21] In a double-blind, placebo-controlled trial, the use of intranasal corticosteroid (fluticasone propionate) offered no clinical benefit in young adults with colds and induced prolonged shedding of rhinovirus.[169]

In a controlled trial in adults, a soluble intercellular adhesion molecule-1 product (tremacamra) reduced the severity of rhinovirus colds.[204] The antiviral pleconaril induced an early reduction in severity of symptoms in adults with colds caused by rhinovirus.[99] Pleconaril is an antipicornavirus drug that interacts directly with viral capsid proteins. It blocks attachment of virus to cells through intercellular adhesion molecule-1 and subsequent uncoating and release of viral RNA.

In another randomized, double-blind, placebo-controlled trial, the symptomatic efficacy of pleconaril was linked to its in vivo antiviral effects and to the drug susceptibility of the infecting virus.[158] In March 2002, the Antiviral Drugs Advisory Committee of the U.S. Food and Drug Administration voted against recommending pleconaril for approval of its use in the treatment of the common cold in adults. This decision was based on drug interactions, poor risk-to-benefit ratio, and concerns of development of resistant virus.[154]

Clarithromycin, a macrolide antibiotic, enhances mucosal immunity in mice by increasing levels of IL-12, IgA, and IgG.[121] In a controlled trial, however, clarithromycin had no effect on the severity of cold symptoms. The intranasal administration of nedocromil sodium has been observed to have a beneficial effect on rhinoviral infections in adult volunteers.[19] Mucolytics have emerged as potential therapeutics for modulating the function of airway epithelial cells and altering the course of viral infections.[8,220] Leukoprotease inhibitors and pulmonary surfactant have been shown to be upregulated by ambroxol—a mucolytic and antioxidant agent.[7,149]

In one study, adults who took sauna baths once or twice per week were found to have fewer colds than did members of a non–sauna-bathing control group.[68] In two studies, volunteers with colds did not benefit from inhaling heated vapor.[75,151]

The list of available and proposed complementary and alternative medicines thought to be useful for the common cold is long.* Blinding subjects in placebo-controlled trials can be difficult, and the placebo effect seems to play a significant role in the popularity of all complementary and alternative medicines.† Fluid extracts of *Echinacea* spp. are popular for the prevention and the treatment of colds.[18,86,195,200] Because several species of *Echinacea* have been used in prevention and treatment studies, criticism of the negative results in some studies has been expressed by *Echinacea* advocates.‡[22,33,82,124,176,188,192,197]

In a controlled trial, Taylor and associates[193] evaluated *Echinacea* for the treatment of upper respiratory tract infections in children aged 2 to 11 years. No benefit was noted, and the *Echinacea*-treated children had an increased occurrence of rash. Preparations of North American ginseng have been reported to decrease the frequency of colds in adults.[15,139,164] In a study of North American ginseng extract involving children 3 to 12 years of age, no treatment benefit was noted.[210]

In a recent study in children 2 to 11 years of age, Paul and associates[155] found that the application of Vicks VapoRub to the child's neck and chest at bedtime gave symptomatic relief from nocturnal cough, congestion, and sleep difficulty caused by an upper respiratory tract infection. This was a controlled study that compared the children treated with Vicks VapoRub with those who had received petrolatum to the chest and those who had no treatment.

PROGNOSIS

The prognosis of common colds in children is excellent. Secondary complications do occur, however, and frequently require careful and prolonged therapy. The most common complications are otitis media, sinusitis, bacterial adenoiditis, bacterial pharyngitis, and lower respiratory tract bacterial infections. The primary viral pathogens

*References 64, 65, 72, 114, 134, 163, 199, 211.

*References 18, 22, 26, 62, 132, 210, 219.
†References 20, 22, 32, 50, 102, 197.
‡References 22, 33, 82, 124, 176, 188, 192, 197.

identified in hospitalized children with lower respiratory tract infections include RSV, influenza A and B viruses, human metapneumovirus, parainfluenza viruses, adenoviruses, rhinoviruses, and enteroviruses.[123] Infections with multiple agents also occur.

PREVENTION

Studies in isolated populations have shown that when a particular respiratory viral infection has run through the entire group, no further respiratory viral illnesses can occur until a new infected individual enters the population. This type of evidence indicates that quarantine or isolation practices could prevent colds. However, today's average urban society is so complex that prevention through isolation procedures is impractical. Efforts to control the spread of respiratory virus should be minimal and practical. For children with undue susceptibility to complications, contact with crowds or infected children and adults should be avoided.

A double-blind, placebo-controlled study over a 6-month period in children 3 to 5 years of age found that the children receiving a probiotic twice daily had a decreased incidence of fever, coughing, and rhinorrhea with cold and influenza-like symptoms than placebo recipients.[131] The children who received the probiotic had a significant reduction in days absent from group childcare and a reduced incidence of antibiotic use than the placebo recipients.

The use of virucidal nasal tissues has been shown to reduce markedly the spread of rhinovirus colds in human volunteers and to reduce modestly colds in the family setting.[51,73] Heikkinen and associates[101] found that intranasal administration of an immunoglobin preparation by nasal sprays twice per day significantly reduced the occurrence of rhinitis in children attending daycare centers. If confirmed, this form of prophylaxis might be useful for selected children. A study showed that organic acids commonly used in OTC skin care and cosmetic products had substantial virucidal activity against rhinoviruses. The amount of acid applied to the hands correlated directly with the prevention of infection in the deliberate infection model.[198,201]

NEW REFERENCES SINCE THE SIXTH EDITION

14. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008;**167**:63–9.
18. Barrett B, Brown R, Rakel D, et al. Echinacea for treating the common cold: a randomized trial. *Ann Intern Med* 2010;**153**:769–77.
41. Cohen S, Alper CM, Doyle WJ, et al. Objective and subjective socioeconomic status and susceptibility to the common cold. *Health Psychol* 2008;**27**:268–74.
48. Dart RC, Paul IM, Bond GR, et al. Pediatric fatalities associated with over the counter (nonprescription) cough and cold medications. *Ann Emerg Med* 2009;**53**:411–7.
55. Dominguez SR, Robinson CC, Holmes KV. Detection of four human coronaviruses in respiratory infections in children: a one-year study in Colorado. *J Med Virol* 2009;**81**:1597–604.
69. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**:1337–42.
70. Fabbiani M, Terrosi C, Martorelli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol* 2009;**81**:750–6.
71. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
80. Gaunt ER, Hardie A, Claas EC, et al. Epidemiology and clinical presentations of the four human coronaviruses 229E,HKU1,NL63,and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol* 2010;**48**:2940–7.
87. Grüber C, Keil T, Kulig M, et al. History of respiratory infections in the first 12 yr among children from a birth cohort. *Pediatr Allergy Immunol* 2008;**19**:505–12.
100. Heikkinen T, Osterback R, Peltola V, et al. Human metapneumovirus infections in children. *Emerg Infect Dis* 2008;**14**:101–6.
119. Kassel JC, King D, Spurling GK. Saline nasal irrigation for acute upper respiratory tract infections. *Cochrane Database Syst Rev* CD006821, 2010.
126. Kuehn BM. Debate continues over the safety of cold and cough medicines for children. *JAMA* 2008;**300**:2354–6.
131. Leyer GJ, Li S, Mubasher ME, et al. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* 2009;**124**:e172–9.
132. Lissiman E, Bhasale AL, Cohen M. Garlic for the common cold. *Cochrane Database Syst Rev* CD006206, 2009.
135. Mahony JB. Detection of respiratory viruses by molecular methods. *Clin Microbiol Rev* 2008;**21**:716–47.
137. Makinen TM, Juvonen R, Jokelainen J, et al. Cold temperature and low humidity are associated with increased occurrence of respiratory tract infections. *Respir Med* 2009;**103**:456–62.
151. Pach D, Knochel B, Ludtke R, et al. Visiting a sauna: does inhaling hot dry air reduce common cold symptoms? A randomised controlled trial. *Med J Aust* 2010;**193**:730–4.
153. Pappas DE, Hendley JO. The common cold and decongestant therapy. *Pediatr Rev* 2011;**32**:47–54; quiz 5.
155. Paul IM, Beiler JS, King TS, et al. Vapor rub, petrolatum, and no treatment for children with nocturnal cough and cold symptoms. *Pediatrics* 2010;**126**:1092–9.
162. Prasad AS, Beck FW, Bao B, et al. Duration and severity of symptoms and levels of plasma interleukin-1 receptor antagonist, soluble tumor necrosis factor receptor, and adhesion molecules in patients with common cold treated with zinc acetate. *J Infect Dis* 2008;**197**:795–802.
166. Principi N, Bosis S, Esposito S. Effects of coronavirus infections in children. *Emerg Infect Dis* 2010;**16**:183–8.
171. Rimsza ME, Newberry S. Unexpected infant deaths associated with use of cough and cold medications. *Pediatrics* 2008;**122**:e318–22.
173. Ruohola A, Waris M, Allander T, et al. Viral etiology of common cold in children, Finland. *Emerg Infect Dis* 2009;**15**:344–6.
177. Shehab N, Schaefer MK, Kegler SR, et al. Adverse events from cough and cold medications after a market withdrawal of products labeled for infants. *Pediatrics* 2010;**126**:1100–7.
178. Singh M. Heated, humidified air for the common cold. *Cochrane Database Syst Rev* CD001728, 2011.
179. Singh M, Das RR. Zinc for the common cold. *Cochrane Database Syst Rev* CD001364, 2011.
183. Smith SM, Schroeder K, Fahey T. Over-the-counter medications for acute cough in children and adults in ambulatory settings. *Cochrane Database Syst Rev* CD001831, 2008.
190. Talbot HK, Shepherd BE, Crowe JE, Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J* 2009;**28**:682–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abisheganaden JA, Avila PC, Kishiyama JL, et al. Effect of clarithromycin on experimental rhinovirus-16 colds: a randomized, double-blind, controlled trial. *Am J Med* 2000;**108**:453–9.
2. Alexander TH, Davidson TM. Intranasal zinc and anosmia: the zinc-induced anosmia syndrome. *Laryngoscope* 2006;**116**:217–20.
3. American Academy of Pediatrics. Committee on Drugs. Use of codeine- and dextromethorphan-containing cough remedies in children. *Pediatrics* 1997;**99**:918–20.
4. Anderson TW, Beaton GH, Corey P, et al. Winter illness and vitamin C: the effect of relatively low doses. *Can Med Assoc J* 1975;**112**:823–6.
5. Andrewes CH. The natural history of the common cold. *Lancet* 1949;**1**:71–5.
6. Aquilina AT, Hall WJ, Douglas RG, Jr, et al. Airway reactivity in subjects with viral upper respiratory tract infections: the effects of exercise and cold air. *Am Rev Respir Dis* 1980;**122**:3–10.
7. Arnold JC, Singh KK, Spector SA, et al. Human bocavirus: prevalence and clinical spectrum at a children's hospital. *Clin Infect Dis* 2006;**43**:283–8.
8. Arroll B. Non-antibiotic treatments for upper-respiratory tract infections (common cold). *Respir Med* 2005;**99**:1477–84.
9. Arruda E, Boyle TR, Winther B, et al. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. *J Infect Dis* 1995;**171**:1329–33.
10. Badger GF, Dingle JH, Feller AE, et al. A study of illness in a group of Cleveland families. II. Incidence of the common respiratory diseases. *Am J Hyg* 1953;**58**:31–40.
11. Badger GF, Dingle JH, Feller AE, et al. A study of illness in a group of Cleveland families. III. Introduction of respiratory infections into families. *Am J Hyg* 1953;**58**:41–6.
12. Badger GF, Dingle JH, Feller AE, et al. A study of illness in a group of Cleveland families. IV. The spread of respiratory infections within the home. *Am J Hyg* 1953;**58**:174–8.
13. Badger GF, Dingle JH, Feller AE, et al. A study of illness in a group of Cleveland families. V. Introductions and secondary attack rates as indices of exposure to common respiratory diseases in the community. *Am J Hyg* 1953;**58**:179–82.
14. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008;**167**:63–9.
15. Bakalar N. Ginseng may reduce number and severity of colds. *The New York Times*, November 1, 2005.
16. Ball TM, Holberg CJ, Aldous MB, et al. Influence of attendance at day care on the common cold from birth through 13 years of age. *Arch Pediatr Adolesc Med* 2002;**156**:121–6.
17. Barness LA. Safety considerations with high ascorbic acid dosage. *Ann N Y Acad Sci* 1975;**258**:523–8.
18. Barrett B, Brown R, Rakel D, et al. Echinacea for treating the common cold: a randomized trial. *Ann Intern Med* 2010;**153**:769–77.
19. Barrow GI, Higgins PG, Al-Nakib W, et al. The effect of intranasal nedocromil sodium on viral upper respiratory tract infections in human volunteers. *Clin Exp Allergy* 1989;**20**:45–51.
20. Barton R. Efficacy of echinilin for the common cold. *Clin Infect Dis* 2005;**41**:761–2; author reply 3–4.
21. Belongia EA, Berg R, Liu K. A randomized trial of zinc nasal spray for the treatment of upper respiratory illness in adults. *Am J Med* 2001;**111**:103–8.
22. Berman JD, Straus SE. Complementary and alternative medicines for infectious diseases. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 6th ed. Philadelphia: W. B. Saunders; 2005. p. 603–11.
23. Blacklow SC. Catching the common cold. *Nat Struct Mol Biol* 2004;**11**:388–90.
24. Brimblecombe FS, Cruickshank R, Masters PL, et al. Family studies of respiratory infections. *Br Med J* 1958;**1**:119–28.
25. Buckland FE, Tyrrell DA. Experiments on the Spread of Colds. I. Laboratory studies on the dispersal of nasal secretion. *J Hyg (Lond)* 1964;**62**:365–77.
26. Buechi S, Vogelin R, von Eiff MM, et al. Open trial to assess aspects of safety and efficacy of a combined herbal cough syrup with ivy and thyme. *Forsch Komplementarmed Klass Naturheilkd* 2005;**12**:328–32.
27. Bush RK, Busse W, Flaherty D, et al. Effects of experimental rhinovirus 16 infection on airways and leukocyte function in normal subjects. *J Allergy Clin Immunol* 1978;**61**:80–7.
28. Bynoe ML. The common cold. *Practitioner* 1966;**197**:739–46.
29. Callow KA, Tyrrell DA, Shaw RJ, et al. Influence of atopy on the clinical manifestations of coronavirus infection in adult volunteers. *Clin Allergy* 1988;**18**:119–29.
30. Carr AB, Einstein R, Lai LY, et al. Vitamin C and the common cold: using identical twins as controls. *Med J Aust* 1981;**2**:411–2.
31. Carraro S, Doherty J, Zaman K, et al. S-nitrosothiols regulate cell-surface pH buffering by airway epithelial cells during the human immune response to rhinovirus. *Am J Physiol Lung Cell Mol Physiol* 2006;**290**:L827–32.
32. Caruso TJ, Gwaltney JM, Jr. Reply to Barton and Hemila. *Correspondence Clin Infect Dis* 2005;**41**:763–4.
33. Caruso TJ, Gwaltney JM, Jr. Treatment of the common cold with echinacea: a structured review. *Clin Infect Dis* 2005;**40**:807–10.
34. Cate TR, Couch RB, Johnson KM. Studies with rhinoviruses in volunteers: production of illness, effect of naturally acquired antibody, and demonstration of a protective effect not associated with serum antibody. *J Clin Invest* 1964;**43**:56–67.
35. Cate TR, Douglas RG, Jr, Couch RB. Interferon and resistance to upper respiratory virus illness. *Proc Soc Exp Biol Med* 1969;**131**:631–6.
36. Cate TR, Rossen RD, Douglas RG, Jr, et al. The role of nasal secretion and serum antibody in the rhinovirus common cold. *Am J Epidemiol* 1966;**84**:352–63.
37. Cherry JD. Newer respiratory viruses: their role in respiratory illness of children. *Adv Pediatr* 1973;**20**:225–90.
38. Cherry JD. Respiratory virus diseases. Comments. *Pediatr Res* 1977;**11**:250–1.
39. Ciancio B. *Understanding the socioeconomic burden of human metapneumovirus in childhood: Italian study recommends further research*. Eurosurveillance v10, Feb 10, 2005.
40. Clemens CJ, Taylor JA, Almquist JR, et al. Is an antihistamine-decongestant combination effective in temporarily relieving symptoms of the common cold in preschool children? *J Pediatr* 1997;**130**:463–6.
41. Cohen S, Alper CM, Doyle WJ, et al. Objective and subjective socioeconomic status and susceptibility to the common cold. *Health Psychol* 2008;**27**:268–74.
42. Cohen S, Doyle WJ, Skoner DP, et al. Social ties and susceptibility to the common cold. *JAMA* 1997;**277**:1940–4.
43. Couch RB, Cate TR, Douglas RG, Jr, et al. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol Rev* 1966;**30**:517–29.
44. Coulehan JL. Ascorbic acid and the common cold: reviewing the evidence. *Postgrad Med* 1979;**66**:153–5, 157–8, 160.
45. Coulehan JL, Eberhard S, Kapner L, et al. Vitamin C and acute illness in Navajo school children. *N Engl J Med* 1976;**295**:973–7.
46. D'Alessio DJ, Meschievitz CK, Peterson JA, et al. Short-duration exposure and the transmission of rhinoviral colds. *J Infect Dis* 1984;**150**:189–94.
47. D'Alessio DJ, Peterson JA, Dick CR, et al. Transmission of experimental rhinovirus colds in volunteer married couples. *J Infect Dis* 1976;**133**:28–36.
48. Dart RC, Paul IM, Bond GR, et al. Pediatric fatalities associated with over the counter (nonprescription) cough and cold medications. *Ann Emerg Med* 2009;**53**:411–7.
49. Denny FW, Collier AM, Henderson FW. Acute respiratory infections in day care. *Rev Infect Dis* 1986;**8**:527–32.
50. Desbiens NA. Lessons learned from attempts to establish the blind in placebo-controlled trials of zinc for the common cold. *Ann Intern Med* 2000;**133**:302–3.
51. Dick EC, Hossain SU, Mink KA, et al. Interruption of transmission of rhinovirus colds among human volunteers using virucidal paper handkerchiefs. *J Infect Dis* 1986;**153**:352–6.
52. Dick EC, Jennings LC, Mink KA, et al. Aerosol transmission of rhinovirus colds. *J Infect Dis* 1987;**156**:442–8.
53. Dingle JH, Badger GF, Feller AE, et al. A study of illness in a group of Cleveland families. I. Plan of study and certain general observations. *Am J Hyg* 1953;**58**:16–30.

54. Dochez AR, Shibley GS, Mills KC. Studies in the common cold: IV. Experimental transmission of the common cold to anthropoid apes and human beings by means of a filtrable agent. *J Exp Med* 1930;**52**:701–16.
55. Dominguez SR, Robinson CC, Holmes KV. Detection of four human coronaviruses in respiratory infections in children: a one-year study in Colorado. *J Med Virol* 2009;**81**:1597–604.
56. Douglas RG, Jr. Pathogenesis of rhinovirus common colds in human volunteers. *Ann Otol Rhinol Laryngol* 1970;**79**:563–71.
57. Douglas RG, Jr, Lindgren KM, Couch RB. Exposure to cold environment and rhinovirus common cold: Failure to demonstrate effect. *N Engl J Med* 1968;**279**:742–7.
58. Doust J, Del Mar C. Diagnosing coughs and colds. *Editorials Br J Gen Pract* 2004;**54**:5–6.
59. Douville RN, Bastien N, Li Y, et al. Human metapneumovirus elicits weak IFN-gamma memory responses compared with respiratory syncytial virus. *J Immunol* 2006;**176**:5848–55.
60. Dowling HF, Jackson GG, Spiesman IG, et al. Transmission of the common cold to volunteers under controlled conditions. III. The effect of chilling of the subjects upon susceptibility. *Am J Hyg* 1958;**68**:59–65.
61. Dressendorfer RH, Petersen SR, Moss Lovshin SE, et al. Performance enhancement with maintenance of resting immune status after intensified cycle training. *Clin J Sport Med* 2002;**12**:301–7.
62. Duenwald M. Keeping colds at bay: Or maybe not. The consumer. *The New York Times*, February 1, 2005.
63. Dumitru CA, Dreschers S, Gulbins E. Rhinoviral infections activate p38MAP-kinases via membrane rafts and RhoA. *Cell Physiol Biochem* 2006;**17**:159–66.
64. Eby GA. Elimination of efficacy by additives in zinc acetate lozenges for common colds. *Clin Infect Dis* 2001;**32**:1520.
65. Eby GA, Halcomb WW. Ineffectiveness of zinc gluconate nasal spray and zinc orotate lozenges in common-cold treatment: a double-blind, placebo-controlled clinical trial. *Altern Ther Health Med* 2006;**12**:34–8.
66. Eccles R. Mechanisms of the placebo effect of sweet cough syrups. *Respir Physiol Neurobiol* 2006;**152**:340–8.
67. Elwood PC, Hughes SJ, Leger AS. A randomized controlled trial of the therapeutic effect of vitamin C in the common cold. *Practitioner* 1977;**218**:133–7.
68. Ernst E, Pecho E, Wirz P, et al. Regular sauna bathing and the incidence of common colds. *Ann Med* 1990;**22**:225–7.
69. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**: 1337–42.
70. Fabbiani M, Terrosi C, Martorelli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol* 2009;**81**:750–6.
71. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
72. Farr BM, Conner EM, Betts RF, et al. Two randomized controlled trials of zinc gluconate lozenge therapy of experimentally induced rhinovirus colds. *Antimicrob Agents Chemother* 1987;**31**:1183–7.
73. Farr BM, Hendley JO, Kaiser DL, et al. Two randomized controlled trials of virucidal nasal tissues in the prevention of natural upper respiratory infections. *Am J Epidemiol* 1988;**128**:1162–72.
74. Fleet WF, Couch RB, Cate TR, et al. Homologous and heterologous resistance to rhinovirus common cold. *Am J Epidemiol* 1965;**82**:185–96.
75. Forstall GJ, Macknin ML, Yen-Lieberman BR, et al. Effect of inhaling heated vapor on symptoms of the common cold. *JAMA* 1994;**271**:1109–11.
76. Fox JP, Hall CE, Cooney MK, et al. The Seattle virus watch. II. Objectives, study population and its observation, data processing and summary of illnesses. *Am J Epidemiol* 1972;**96**:270–85.
77. Foy HM, Cooney MK, Hall C, et al. Case-to-case intervals of rhinovirus and influenza virus infections in households. *J Infect Dis* 1988;**157**:180–2.
78. Frost WH, Gover M. The incidence and time distribution of common colds in several groups kept under continuous observation. In: Maxcy KF, editor. *Papers of Wade Hampton Frost*. New York: Commonwealth Fund; 1941. p. 359–92.
79. Gadomski A, Horton L. The need for rational therapeutics in the use of cough and cold medicine in infants. *Pediatrics* 1992;**89**: 774–6.
80. Gaunt ER, Hardie A, Claas EC, et al. Epidemiology and clinical presentations of the four human coronaviruses 229E,HKU1,NL63,and OC43 detected over 3 years using a novel multiplex real-time PCR method. *J Clin Microbiol* 2010;**48**: 2940–7.
81. Gern JE, Brooks GD, Meyer P, et al. Bidirectional interactions between viral respiratory illnesses and cytokine responses in the first year of life. *J Allergy Clin Immunol* 2006;**117**:72–8.
82. Goel V, Lovlin R, Chang C, et al. A proprietary extract from the echinacea plant *(Echinacea purpurea)* enhances systemic immune response during a common cold. *Phytother Res* 2005;**19**: 689–94.
83. Goldbloom RB. Nasopharyngitis (the common cold). In: Gellis SS, Kagan BM, editors. *Current pediatric therapy*. 11th ed. Philadelphia: W. B. Saunders; 1984. p. 93.
84. Gordon M, Lovell S, Dugdale AE. The value of antibiotics in minor respiratory illness in children: A controlled trial. *Med J Aust* 1974;**1**:304–6.
85. Graham NM, Burrell CJ, Douglas RM, et al. Adverse effects of aspirin, acetaminophen, and ibuprofen on immune function, viral shedding, and clinical status in rhinovirus-infected volunteers. *J Infect Dis* 1990;**162**:1277–82.
86. Grimm W, Muller HH. A randomized controlled trial of the effect of fluid extract of *Echinacea purpurea* on the incidence and severity of colds and respiratory infections. *Am J Med* 1999;**106**:138–43.
87. Grüber C, Keil T, Kulig M, et al. History of respiratory infections in the first 12 yr among children from a birth cohort. *Pediatr Allergy Immunol* 2008;**19**:505–12.
88. Gwaltney JM, Jr, Hendley JO. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol* 1982;**116**:828–33.
89. Gwaltney JM, Jr, Hendley JO, Simon G, et al. Rhinovirus infections in an industrial population. I. The occurrence of illness. *N Engl J Med* 1966;**275**:1261–8.
90. Gwaltney JM, Jr, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Ann Intern Med* 1978;**88**:463–7.
91. Gwaltney JM, Jr, Moskalski PB, Hendley JO. Interruption of experimental rhinovirus transmission. *J Infect Dis* 1980;**142**: 811–5.
92. Gwaltney JM, Jr, Winther B, Patrie JT, et al. Combined antiviral-antimediator treatment for the common cold. *J Infect Dis* 2002; **186**:147–54.
93. Hall CB, Douglas RG, Jr. Clinically useful method for the isolation of respiratory syncytial virus. *J Infect Dis* 1975;**131**:1–5.
94. Hall CB, Douglas RG, Jr, Schnabel KC, et al. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infect Immun* 1981;**33**:779–83.
95. Harris JM, 2nd, Gwaltney JM, Jr. Incubation periods of experimental rhinovirus infection and illness. *Clin Infect Dis* 1996; **23**:1287–90.
96. Hayden FG, Gwaltney JM, Jr. Intranasal interferon alpha 2 for prevention of rhinovirus infection and illness. *J Infect Dis* 1983;**148**:543–50.
97. Hayden FG, Gwaltney JM, Jr. Intranasal interferon-alpha 2 treatment of experimental rhinoviral colds. *J Infect Dis* 1984;**150**: 174–80.
98. Hayden FG, Kaiser DL, Albrecht JK. Intranasal recombinant alfa-2b interferon treatment of naturally occurring common colds. *Antimicrob Agents Chemother* 1988;**32**:224–30.
99. Hayden FG, Kim K, Hudson SA, et al. *Pleconaril treatment provides early reduction of symptom severity in viral respiratory infection due to picornaviruses. Abstract 414*. In: Program and Abstracts 39th Annual Meeting of the Infectious Diseases Society of America, San Francisco. October 25–8, 2001.
100. Heikkinen T, Osterback R, Peltola V, et al. Human metapneumovirus infections in children. *Emerg Infect Dis* 2008;**14**:101–6.
101. Heikkinen T, Ruohola A, Ruuskanen O, et al. Intranasally administered immunoglobulin for the prevention of rhinitis in children. *Pediatr Infect Dis J* 1998;**17**:367–72.
102. Hemila H. Echinacea, vitamin C, the common cold, and blinding. *Clin Infect Dis* 2005;**41**:762–3; author reply 3–4.
103. Hendley JO, Wenzel RP, Gwaltney JM, Jr. Transmission of rhinovirus colds by self-inoculation. *N Engl J Med* 1973;**288**: 1361–4.
104. Hilding A. The common cold. *Arch Otolaryngol* 1930;**12**:133–50.

105. Holmes MJ, Allen TR. Viral respiratory diseases in isolated communities: A review. *Br Antarct Surv Bull* 1973;**35**:23–31.
106. Hrastinger A, Dietz B, Bauer R, et al. Is there clinical evidence supporting the use of botanical dietary supplements in children? *J Pediatr* 2005;**146**:311–7.
107. Huang N, Morlock L, Lee CH, et al. Antibiotic prescribing for children with nasopharyngitis (common colds), upper respiratory infections, and bronchitis who have health-professional parents. *Pediatrics* 2005;**116**:826–32.
108. Hutton N, Wilson MH, Mellits ED, et al. Effectiveness of an antihistamine-decongestant combination for young children with the common cold: a randomized, controlled clinical trial. *J Pediatr* 1991;**118**:125–30.
109. Jackson GG. Understanding of viral respiratory illnesses provided by experiments in volunteers. *Bacteriol Rev* 1964;**28**:423–30.
110. Jackson GG, Dowling HF, Akers LW, et al. Immunity to the common cold from protective serum antibody. Time of appearance, persistence and relation to reinfection. *N Engl J Med* 1962;**26**:791–6.
111. Jackson GG, Dowling HF, Anderson TO. Neutralization of common cold agents in volunteers by pooled human globulin. *Science* 1958;**128**:27–8.
112. Jackson GG, Dowling HF, Anderson TO, et al. Susceptibility and immunity to common upper respiratory viral infections: the common cold. *Ann Intern Med* 1960;**53**:719–38.
113. Jackson GG, Dowling HF, Muldoon RL. Present concepts of the common cold. *Am J Public Health* 1962;**52**:940–5.
114. Jackson JL, Peterson C, Lesho E. A meta-analysis of zinc salts lozenges and the common cold. *Arch Intern Med* 1997;**157**: 2373–6.
115. Jacobs RF. Judicious use of antibiotics for common pediatric respiratory infections. *Pediatr Infect Dis J* 2000;**19**:938–43.
116. Jennings LC, Dick EC, Mink KA, et al. Near disappearance of rhinovirus along a fomite transmission chain. *J Infect Dis* 1988;**158**:888–92.
117. Johnson C, Eccles R. Acute cooling of the feet and the onset of common cold symptoms. *Fam Pract* 2005;**22**:608–13.
118. Karlowski TR, Chalmers TC, Frenkel LD, et al. Ascorbic acid for the common cold: A prophylactic and therapeutic trial. *JAMA* 1975;**231**:1038–42.
119. Kassel JC, King D, Spurling GK. Saline nasal irrigation for acute upper respiratory tract infections. *Cochrane Database Syst Rev* CD006821, 2010.
120. Kaul P, Singh I, Turner RB. Effect of nitric oxide on rhinovirus replication and virus-induced interleukin-8 elaboration. *Am J Respir Crit Care Med* 1999;**159**:1193–8.
121. Kido H, Okumura Y, Yamada H, et al. Secretory leukoprotease inhibitor and pulmonary surfactant serve as principal defenses against influenza A virus infection in the airway and chemical agents up-regulating their levels may have therapeutic potential. *Biol Chem* 2004;**385**:1029–34.
122. Kim J, Sanders SP, Siekierski ES, et al. Role of NF-kappa B in cytokine production induced from human airway epithelial cells by rhinovirus infection. *J Immunol* 2000;**165**:3384–92.
123. Klig JE. Office pediatrics: current perspectives on the outpatient evaluation and management of lower respiratory infections in children. *Curr Opin Pediatr* 2006;**18**:71–6.
124. Knight V. Echinacea treatment for the common cold. *Clin Infect Dis* 2005;**40**:811–2.
125. Kruse W. Die erreger von husten und schnupfen. *München Med Wochenschr* 1914;**61**:1547.
126. Kuehn BM. Debate continues over the safety of cold and cough medicines for children. *JAMA* 2008;**300**:2354–6.
127. Kuyvenhoven M, van Essen G, Schellevis F, et al. Management of upper respiratory tract infections in Dutch general practice; antibiotic prescribing rates and incidences in 1987 and 2001. *Fam Pract* 2006;**23**:175–9.
128. Lefkowitz LB, Jr, Jackson GG, Dowling HF. The role of immunity in the common cold and related viral respiratory infections. *Med Clin North Am* 1963;**47**:1171–84.
129. Levandowski RA, Ou DW, Jackson GG. Acute-phase decrease of T lymphocyte subsets in rhinovirus infection. *J Infect Dis* 1986;**153**:743–8.
130. Lexomboon U, Duangmani C, Kusalasai V, et al. Evaluation of orally administered antibiotics for treatment of upper respiratory infections in Thai children. *J Pediatr* 1971;**78**:771–8.
131. Leyer GJ, Li S, Mubasher ME, et al. Probiotic effects on cold and influenza-like symptom incidence and duration in children. *Pediatrics* 2009;**124**:e172–9.
132. Lissiman E, Bhasale AL, Cohen M. Garlic for the common cold. *Cochrane Database Syst Rev* CD006206, 2009.
133. Lobovits AM, Freeman J, Goldmann DA, et al. Risk of illness after exposure to a pediatric office. *N Engl J Med* 1985;**313**: 425–8.
134. Macknin ML, Piedmonte M, Calendine C, et al. Zinc gluconate lozenges for treating the common cold in children: a randomized controlled trial. *JAMA* 1998;**279**:1962–7.
135. Mahony JB. Detection of respiratory viruses by molecular methods. *Clin Microbiol Rev* 2008;**21**:716–47.
136. Makela MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 1998;**36**: 539–42.
137. Makinen TM, Juvonen R, Jokelainen J, et al. Cold temperature and low humidity are associated with increased occurrence of respiratory tract infections. *Respir Med* 2009;**103**:456–62.
138. Marinetti L, Lehman L, Casto B, et al. Over-the-counter cold medications-postmortem findings in infants and the relationship to cause of death. *J Anal Toxicol* 2005;**29**:738–43.
139. McElhaney JE, Goel V, Toane B, et al. Efficacy of COLD-fX in the prevention of respiratory symptoms in community-dwelling adults: a randomized, double-blinded, placebo controlled trial. *J Altern Complement Med* 2006;**12**:153–7.
140. Meschievitz CK, Schultz SB, Dick EC. A model for obtaining predictable natural transmission of rhinoviruses in human volunteers. *J Infect Dis* 1984;**150**:195–201.
141. Moser R, Snyers L, Wruss J, et al. Neutralization of a common cold virus by concatemers of the third ligand binding module of the VLDL-receptor strongly depends on the number of modules. *Virology* 2005;**338**:259–69.
142. Mossad SB. Treatment of the common cold. *BMJ* 1998;**317**: 33–6.
143. Muether PS, Gwaltney JM, Jr. Variant effect of first- and second-generation antihistamines as clues to their mechanism of action on the sneeze reflex in the common cold. *Clin Infect Dis* 2001;**33**:1483–8.
144. Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinovirus colds. *J Infect Dis* 1988;**157**:133–42.
145. Nafstad P, Hagen JA, Oie L, et al. Day care centers and respiratory health. *Pediatrics* 1999;**103**:753–8.
146. Nelson WE. Infections of the upper respiratory tract. In: Nelson WE, editor. *Textbook of pediatrics*. 6th ed. Philadelphia: W. B. Saunders; 1954. p. 770–86.
147. Nichol KL, D'Heilly S, Ehlinger E. Colds and influenza-like illnesses in university students: impact on health, academic and work performance, and health care use. *Clin Infect Dis* 2005; **40**:1263–70.
148. Noah TL, Henderson FW, Wortman IA, et al. Nasal cytokine production in viral acute upper respiratory infection of childhood. *J Infect Dis* 1995;**171**:584–92.
149. Nobata K, Fujimura M, Ishiura Y, et al. Ambroxol for the prevention of acute upper respiratory disease. *Clin Exp Med* 2006;**6**: 79–83.
150. Nyquist AC, Gonzales R, Steiner JF, et al. Antibiotic prescribing for children with colds, upper respiratory tract infections, and bronchitis. *JAMA* 1998;**279**:875–7.
151. Pach D, Knochel B, Ludtke R, et al. Visiting a sauna: does inhaling hot dry air reduce common cold symptoms? A randomised controlled trial. *Med J Aust* 2010;**193**:730–4.
152. Pacifico L, Iacobini M, Viola F, et al. Chemokine concentrations in nasal washings of infants with rhinovirus illnesses. *Clin Infect Dis* 2000;**31**:834–8.
153. Pappas DE, Hendley JO. The common cold and decongestant therapy. *Pediatr Rev* 2011;**32**:47–54; quiz 5.
154. Patick AK. Rhinovirus chemotherapy. *Antivir Res* 2006;**71**:391–6.
155. Paul IM, Beiler JS, King TS, et al. Vapor rub, petrolatum, and no treatment for children with nocturnal cough and cold symptoms. *Pediatrics* 2010;**126**:1092–9.
156. Pedersen M, Sakakura Y, Winther B, et al. Nasal mucociliary transport, number of ciliated cells, and beating pattern in naturally acquired common colds. *Eur J Respir Dis Suppl* 1983;**128**(Pt 1):355–65.

157. Peng T, Kotla S, Bumgarner RE, et al. Human rhinovirus attenuates the type I interferon response by disrupting activation of interferon regulatory factor 3. *J Virol* 2006;**80**:5021–31.
158. Pepper W. *The medical side of Benjamin Franklin*. Philadelphia: W. J. Campbell; 1911. p. 50–1, 60–5, 72–3.
159. Pevear DC, Hayden FG, Demenczuk TM, et al. Relationship of pleconaril susceptibility and clinical outcomes in treatment of common colds caused by rhinoviruses. *Antimicrob Agents Chemother* 2005;**49**:4492–9.
160. Picken JJ, Niewoehner DE, Chester EH. Prolonged effects of viral infections of the upper respiratory tract upon small airways. *Am J Med* 1972;**52**:738–46.
161. Pitt HA, Costrini AM. Vitamin C prophylaxis in marine recruits. *JAMA* 1979;**241**:908–11.
162. Prasad AS, Beck FW, Bao B, et al. Duration and severity of symptoms and levels of plasma interleukin-1 receptor antagonist, soluble tumor necrosis factor receptor, and adhesion molecules in patients with common cold treated with zinc acetate. *J Infect Dis* 2008;**197**:795–802.
163. Prasad AS, Fitzgerald JT, Bao B, et al. Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate: A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2000;**133**:245–52.
164. Predy GN, Goel V, Lovlin R, et al. Efficacy of an extract of North American ginseng containing poly-furanosyl-pyranosyl-saccharides for preventing upper respiratory tract infections: a randomized controlled trial. *CMAJ* 2005;**173**:1043–8.
165. Principi N, Bosis S, Esposito S. Human metapneumovirus in paediatric patients. *Clin Microbiol Infect* 2006;**12**:301–8.
166. Principi N, Bosis S, Esposito S. Effects of coronavirus infections in children. *Emerg Infect Dis* 2010;**16**:183–8.
167. Proud D, Gwaltney JM, Jr, Hendley JO, et al. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinovirus colds. *J Infect Dis* 1994;**169**: 1007–13.
168. Proud D, Naclerio RM, Gwaltney JM, et al. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 1990;**161**:120–3.
169. Puhakka T, Makela MJ, Malmstrom K, et al. The common cold: effects of intranasal fluticasone propionate treatment. *J Allergy Clin Immunol* 1998;**101**:726–31.
170. Reed SE. An investigation of the possible transmission of Rhinovirus colds through indirect contact. *J Hyg (Lond)* 1975;**75**: 249–58.
171. Rimsza ME, Newberry S. Unexpected infant deaths associated with use of cough and cold medications. *Pediatrics* 2008;**122**: e318–22.
172. Rosenstein N, Phillips WR, Gerber M, et al. The common cold: principles of judicious use of antimicrobial agents. *Pediatrics* 1998;**101**:181–4.
173. Ruohola A, Waris M, Allander T, et al. Viral etiology of common cold in children, Finland. *Emerg Infect Dis* 2009;**15**:344–6.
174. Sargent F, 2nd, Lombard OM, Sargent VW. Further studies on stability of resistance to the common cold; the importance of constitution. *Am J Hyg* 1947;**45**:29–32.
175. Schwartz AR, Togo Y, Hornick RB, et al. Evaluation of the efficacy of ascorbic acid in prophylaxis of induced rhinovirus 44 infection in man. *J Infect Dis* 1973;**128**:500–5.
176. Sharma M, Arnason JT, Burt A, et al. Echinacea extracts modulate the pattern of chemokine and cytokine secretion in rhinovirus-infected and uninfected epithelial cells. *Phytother Res* 2006;**20**: 147–52.
177. Shehab N, Schaefer MK, Kegler SR, et al. Adverse events from cough and cold medications after a market withdrawal of products labeled for infants. *Pediatrics* 2010;**126**:1100–7.
178. Singh M. Heated, humidified air for the common cold. *Cochrane Database Syst Rev* CD001728, 2011.
179. Singh M, Das RR. Zinc for the common cold. *Cochrane Database Syst Rev* CD001364, 2011.
180. Skoner DP, Whiteside TL, Wilson JW, et al. Effect of rhinovirus 39 infection on cellular immune parameters in allergic and nonallergic subjects. *J Allergy Clin Immunol* 1993;**92**:732–43.
181. Smith CB, Purcell RH, Bellanti JA, et al. Protective effect of antibody to parainfluenza type 1 virus. *N Engl J Med* 1966; **275**:1145–52.
182. Smith MB, Feldman W. Over-the-counter cold medications: A critical review of clinical trials between 1950 and 1991. *JAMA* 1993;**269**:2258–63.
183. Smith SM, Schroeder K, Fahey T. Over-the-counter medications for acute cough in children and adults in ambulatory settings. *Cochrane Database Syst Rev* CD001831, 2008.
184. Smith W, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. *Lancet* 1933;**2**:66–8.
185. Soyka LF, Robinson DS, Lachant N, et al. The misuse of antibiotics for treatment of upper respiratory tract infections in children. *Pediatrics* 1975;**55**:552–6.
186. Sperber SJ, Hayden FG. Chemotherapy of rhinovirus colds. *Antimicrob Agents Chemother* 1988;**32**:409–19.
187. Sperber SJ, Hendley JO, Hayden FG, et al. Effects of naproxen on experimental rhinovirus colds: A randomized, double-blind, controlled trial. *Ann Intern Med* 1992;**117**:37–41.
188. Sperber SJ, Shah LP, Gilbert RD, et al. *Echinacea purpurea* for prevention of experimental rhinovirus colds. *Clin Infect Dis* 2004;**38**:1367–71.
189. Takkouche B, Regueira C, Gestal-Otero JJ. A cohort study of stress and the common cold. *Epidemiology* 2001;**12**:345–9.
190. Talbot HK, Shepherd BE, Crowe JE, Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J* 2009;**28**:682–7.
191. Tannock GA, Gillett SM, Gillett RS, et al. A study of intranasally administered interferon A (rIFN-alpha 2A) for the seasonal prophylaxis of natural viral infections of the upper respiratory tract in healthy volunteers. *Epidemiol Infect* 1988;**101**:611–21.
192. Taylor JA, Weber W, Calabrese C. Echinacea for treating colds in children. Letters. *JAMA* 2004;**291**:1323–4.
193. Taylor JA, Weber W, Standish L, et al. Efficacy and safety of echinacea in treating upper respiratory tract infections in children: a randomized controlled trial. *JAMA* 2003;**290**:2824–30.
194. Tremonti LP, Lin JS, Jackson GG. Neutralizing activity in nasal secretions and serum in resistance of volunteers to parainfluenza virus type 2. *J Immunol* 1968;**101**:572–7.
195. Turner RB. The common cold. *Pediatr Ann* 1998;**27**:790–5.
196. Turner RB. The treatment of rhinovirus infections: progress and potential. *Antiviral Res* 2001;**49**:1–14.
197. Turner RB. Studies of "natural" remedies for the common cold: pitfalls and pratfalls. *CMAJ* 2005;**173**:1051–2.
198. Turner RB, Cetnarowski WE. Effect of treatment with zinc gluconate or zinc acetate on experimental and natural colds. *Clin Infect Dis* 2000;**31**:1202–8.
199. Turner RB, Hendley JO. Virucidal hand treatments for prevention of rhinovirus infection. *J Antimicrob Chemother* 2005;**56**:805–7.
200. Turner RB, Riker DK, Gangemi JD. Ineffectiveness of echinacea for prevention of experimental rhinovirus colds. *Antimicrob Agents Chemother* 2000;**44**:1708–9.
201. Turner RB, Biedermann KA, Morgan JM, et al. Efficacy of organic acids in hand cleansers for prevention of rhinovirus infections. *Antimicrob Agents Chemother* 2004;**48**:2595–8.
202. Turner RB, Felton A, Kosak K, et al. Prevention of experimental coronavirus colds with intranasal alpha-2b interferon. *J Infect Dis* 1986;**154**:443–7.
203. Turner RB, Sperber SJ, Sorrentino JV, et al. Effectiveness of clemastine fumarate for treatment of rhinorrhea and sneezing associated with the common cold. *Clin Infect Dis* 1997;**25**: 824–30.
204. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. *JAMA* 1999;**281**: 1797–804.
205. Turner RB, Weingand KW, Yeh CH, et al. Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. *Clin Infect Dis* 1998;**26**:840–6.
206. Tyrrell DAJ. *Common colds and related diseases*. Baltimore: Williams & Wilkins; 1965.
207. Valic F, Zuskin E. Pharmacological prevention of acute ventilatory capacity reduction in flax dust exposure. *Br J Ind Med* 1973; **30**:381–4.
208. Vernacchio L, Kelly JP, Kaufman DW, et al. Cough and cold medication use by US children, 1999-2006: results from the slone survey. *Pediatrics* 2008;**122**:e323–9.

209. Vernacchio L, Kelly JP, Kaufman DW, et al. Pseudoephedrine use among US children, 1999-2006: results from the Slone survey. *Pediatrics* 2008;**122**:1299–304.
210. Vohra S, Johnston BC, Laycock KL, et al. Safety and tolerability of North American ginseng extract in the treatment of pediatric upper respiratory tract infection: a phase II randomized, controlled trial of 2 dosing schedules. *Pediatrics* 2008;**122**:e402–10.
211. Wald ER, Dashefsky B, Byers C, et al. Frequency and severity of infections in day care. *J Pediatr* 1988;**112**:540–6.
212. Walker GH, Bynoe ML, Tyrrell DA. Trial of ascorbic acid in prevention of colds. *Br Med J* 1967;**1**:603–6.
213. West S, Brandon B, Stolley P, et al. A review of antihistamines and the common cold. *Pediatrics* 1975;**56**:100–7.
214. Wilkesmann A, Schildgen O, Eis-Hubinger AM, et al. Human metapneumovirus infections cause similar symptoms and clinical severity as respiratory syncytial virus infections. *Eur J Pediatr* 2006;**165**:467–75.
215. Wilkesmann A, Schildgen O, Eis-Hubinger AM, et al. [Human metapneumovirus in hospitalized children: a review]. *Klin Padiatr* 2007;**219**:58–65.
216. Winther B. Effects on the nasal mucosa of upper respiratory viruses (common cold). *Dan Med Bull* 1994;**41**:193–204.
217. Winther B, Brofeldt S, Christensen B, et al. Light and scanning electron microscopy of nasal biopsy material from patients with naturally acquired common colds. *Acta Otolaryngol* 1984;**97**:309–18.
218. Winther B, Farr B, Turner RB, et al. Histopathologic examination and enumeration of polymorphonuclear leukocytes in the nasal mucosa during experimental rhinovirus colds. *Acta Otolaryngol Suppl* 1984;**413**:19–24.
219. Xie Y, Lu W, Cao S, et al. Preparation of bupleurum nasal spray and evaluation on its safety and efficacy. *Chem Pharm Bull (Tokyo)* 2006;**54**:48–53.
220. Yasuda H, Yamaya M, Sasaki T, et al. Carbocisteine reduces frequency of common colds and exacerbations in patients with chronic obstructive pulmonary disease. *J Am Geriatr Soc* 2006;**54**:378–80.
221. Zhu Z, Tang W, Gwaltney JM, Jr, et al. Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappaB. *Am J Physiol* 1997;**273**:L814–24.
222. Zuskin E, Lewis AJ, Bouhuys A. Inhibition of histamine-induced airway constriction by ascorbic acid. *J Allergy Clin Immunol* 1973;**51**:218–26.

CHAPTER 8

Infections of the Oral Cavity

Constantine Simos • Blanca E. Gonzalez

Although most infections of the oral cavity in children are odontogenic and may be treated simply with local measures, the occasional spread of these infections to adjacent or distant fascial spaces or to the maxilla and mandible may result in life-threatening complications. Consequently, careful attention, including liberal use of the dental consultation, should be given to such infections.[24,63]

MICROBIOLOGIC CONSIDERATIONS IN DENTAL INFECTIONS

Normal Flora

That the oral cavity provides an environment favorable to the growth of microorganisms is substantiated by reports of bacterial counts of 10^8 to 10^{11} organisms/mL of saliva.[4,9] More than 30 species of bacteria normally can be identified in saliva in varying proportions, depending on a dynamic interaction of microbial ecosystems, including the tongue, the gingival crevice, and the presence of plaque.[59,71] Age, anatomic relationships, eruption of teeth, presence of decayed teeth, diet, oral hygiene, antibiotic therapy, systemic disease, cancer chemotherapy,[61] and hospitalization can modify the microbial population. In the older literature, emphasis was placed on the role of *Streptococcus* and *Staphylococcus* spp. in producing odontogenic infections, to the exclusion of most anaerobic bacteria. This emphasis probably was the result of failure to culture satisfactorily for anaerobic organisms, and it is now well known that the ratio of anaerobic to aerobic organisms ranges from 3:1 to 10:1.[4,14]

The nomenclature of the oral flora is changing rapidly as a result of the improved understanding of the genetic makeup of these bacteria provided by molecular biology techniques. Table 8-1 summarizes changes in nomenclature among selected members of the oral flora.[15,77,78] Molecular methods based on the polymerase chain reaction allow direct identification of bacterial species to be made from the oral flora and odontogenic infections by isolation of their DNA, RNA, or both. These methods have led to appreciation of the true oral flora, for which 60 percent of species are unculturable. In recent years, many new species and phylotypes have been identified in the normal and pathologic oral flora.

The flora of children is similar to that of adults, with several exceptions. At birth, the oral cavity is sterile, but colonization with *Streptococcus salivarius* occurs rapidly. This organism has been found in 80 percent of cultures taken from 1-day-old infants.[80] The percentage of *Streptococcus* spp. decreases from 98 percent at day 1 to 70 percent at 4 months[57] as other organisms become established. *Staphylococcus*, *Neisseria*, *Veillonella*, *Actinomyces*, *Nocardia*, *Fusobacterium*, *Bacteroides*, *Corynebacterium*, and *Candida* spp. and a variety of coliforms gradually become established by the time the child reaches 1 year of age. As the deciduous dentition erupts, anaerobic organisms become well established in the gingival crevice, yet the spirochetes, *Bacteroides* and *Prevotella* spp., and related oral anaerobes, which commonly are associated with the gingival crevice in adults, seem to be present in fewer numbers in patients younger than 13 to 16 years.[9,80] Eruption of deciduous teeth also is associated with the establishment of *Streptococcus mutans* and *Streptococcus sanguinis*, which adhere to the enamel surface.

Pathogenic Organisms

Not all residents of the oral flora are pathogens. In the odontogenic infections caries and periodontal disease, a progression from initiating infections caused by oral streptococci toward a predominance of oral anaerobes in the more severe and long-standing infections apparently occurs. Caries is initiated primarily by *S. mutans*, a member of the α-hemolytic *Streptococcus viridans* group. As tooth decay progresses toward the dental pulp, *Lactobacillus* and *Actinomyces* spp. join the carious milieu. Severe pulpal infections generally are caused by a combination of these same oral facultative streptococci plus obligate anaerobes, such as *Porphyromonas endodontalis*, formerly classified as *Bacteroides endodontalis*.[83]

Periodontal infections also are polymicrobial; gram-positive aerobes, primarily streptococci, predominate in gingivitis, and the gram-negative anaerobic rods predominate in bone-destroying periodontitis. Juvenile periodontitis (formerly called *periodontosis*), a particularly aggressive periodontal infection in children and adolescents, shows a predominance of *Aggregatibacter actinomycetemcomitans* (formerly known as *Actinobacillus actinomycetemcomitans*) in its cultivable flora.

Orofacial odontogenic infections that spread beyond the teeth and alveolar processes are polymicrobial, yielding on average four to six isolates per case.[8,42,55] With the use of molecular methods, even greater numbers of species can be identified in these infections, ranging from 5 to 18 species per case.[22] Severe orofacial infections have been associated statistically with *Fusobacterium nucleatum*.[33] The concept of the progression from aerobic streptococci to anaerobic gram-negative rods in orofacial infections is supported further by studies that have found a predominance of streptococci in early infections (in the first 3 days of symptoms) and a predominance of

TABLE 8-1 Terminology Changes for Selected Oral Pathogens

Older Terminology	Current Terminology
Streptococcus viridans	*Streptococcus anginosus* *Streptococcus intermedius* *Streptococcus constellatus* *Streptococcus mutans* *Streptococcus sanguinis* *Streptococcus mitis* *Streptococcus salivarius* *Streptococcus vestibularis*
Streptococcus milleri	*Streptococcus anginosus* *Streptococcus intermedius* *Streptococcus constellatus*
Bacteroides melaninogenicus	*Prevotella melaninogenica* *Prevotella intermedia* *Prevotella oralis* *Prevotella buccae* *Prevotella denticola* *Prevotella nigrescens* *Porphyromonas asaccharolytica* *Porphyromonas gingivalis* *Porphyromonas endodontalis* *Porphyromonas salivosa* *Porphyromonas circumdentaria*
Streptococcus faecalis *Streptococcus faecium*	*Enterococcus faecalis* *Enterococcus faecium*
Peptococcus species	*Peptostreptococcus* spp. (main oral pathogen is *P. micros*)

TABLE 8-2 Most Frequent Pathogens Isolated from Orofacial Infections in Two Studies

	Percentage of Cases	
Microorganism	Lewis et al.*	Sakamoto et al.†
Streptococcus milleri	50	65
Peptostreptococcus spp.	64	65
Other anaerobic streptococci	8	9
Bacteroides (Prevotella) oralis	40	74
Bacteroides (Prevotella) gingivalis	28	‡
Bacteroides (Porphyromonas) melaninogenicus	24	17
Fusobacterium spp.	14	52

*Data from Lewis MAO, MacFarlane TW, McGowan DA. Quantitative bacteriology of acute dentoalveolar abscesses. *J Med Microbiol* 1986;**21**:101–4.
†Data from Sakamoto H, Kato H, Sato T, Sasaki J. Semiquantitative bacteriology of closed odontogenic abscesses. *Bull Tokyo Dent Coll* 1998;**39**:103–7.
‡This organism was not reported in this study.

anaerobes in late infections.[42] Table 8-2 lists the frequency with which the major pathogens in orofacial infections were isolated in two studies.[34,47,74] Although the majority of bacterial identification studies are done with adult subjects, research indicates that odontogenic infections in children are caused by similar bacteria. In a recent study, *S. viridans* and *Neisseria* and *Eikenella* spp. were the most frequently isolated aerobic and facultative organisms. *Prevotella* and *Peptostreptococcus* spp. predominated among anaerobes.[73]

Infections originating from nonodontogenic causes (facial trauma, surgical manipulation, tonsillitis) are included in most studies of soft tissue and fascial space infections, and contamination from the skin or oropharynx might allow aerobic organisms, such as *Staphylococcus aureus* and aerobic *Streptococcus* spp., to become established.[9] In contrast, infections originating solely from the dental periapical tissues are much more likely to be predominantly anaerobic.

A pitfall in the identification of organisms as described in the older literature was the failure to culture satisfactorily for anaerobic organisms. The more current literature recognizes this fact.[46,58] The preponderance of anaerobic organisms in odontogenic infections mandates the use of anaerobic and aerobic culturing techniques in situations in which cultures are indicated.

ANATOMIC CONSIDERATIONS

Most severe orofacial infections develop consequent to dental infection—periapical, periodontal, or pericoronal. Spread occurs along anatomic pathways of least resistance.[4,14,35,45,82] Periodontal and pericoronal infections rarely have major sequelae because they generally drain from the gingival sulcus along the surface of the tooth into the oral cavity. Infections associated with the root apices generally are confined within the bony alveolar process (Fig. 8-1). Should spontaneous intraoral drainage occur through either the periodontium or the pulp chamber, further spread through the marrow spaces is unlikely. If such drainage does not occur, spread through bone (osteomyelitis) or perforation of the cortical plate of the affected jaw may occur. Infections associated with root apices close to the buccal cortical plate generally spread buccally, whereas infections close to the lingual or palatal cortical plate or maxillary sinus spread in those directions (Fig. 8-2). When penetration of the cortical plate occurs, infection involves the adjacent soft tissues and may manifest as cellulitis or a soft tissue abscess, which eventually may perforate the mucous membrane or skin as a sinus tract (Fig. 8-3).

Perforation of periapical infections through bone follows a typical pattern that results from the position of the root apices in relation to the bony cortex and to muscle attachments (Fig. 8-4). Infections involving maxillary anterior teeth and buccal roots of maxillary posterior teeth generally perforate labially or buccally, whereas infections involving palatal roots of posterior teeth perforate palatally or rarely into the maxillary sinus. The presence of the buccinator muscle attachment superior to the root apices usually confines these infections and fistulas to the oral cavity. In children, maxillary root apices often are superior to the buccinator, however, and infections may spread to the buccal or infraorbital space or to the periorbital tissues. They eventually may drain through the skin.

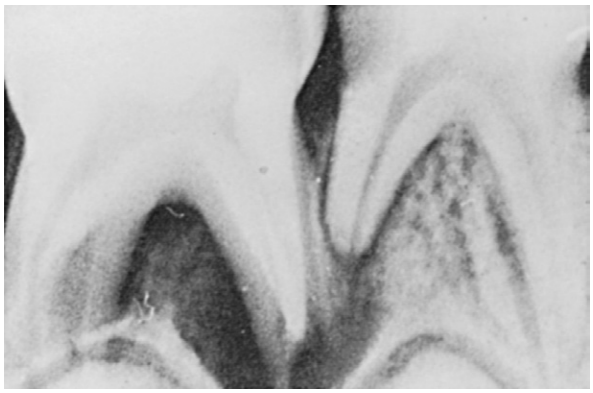

FIGURE 8-1 ■ Radiolucency representing a chronic periapical abscess involving the mesial root of the deciduous second molar and the distal root of the deciduous first molar. The developing mandibular bicuspids are seen inferior to the deciduous roots. The cause of the abscess is the deep carious lesions in both teeth, which appear to have penetrated the pulp chambers.

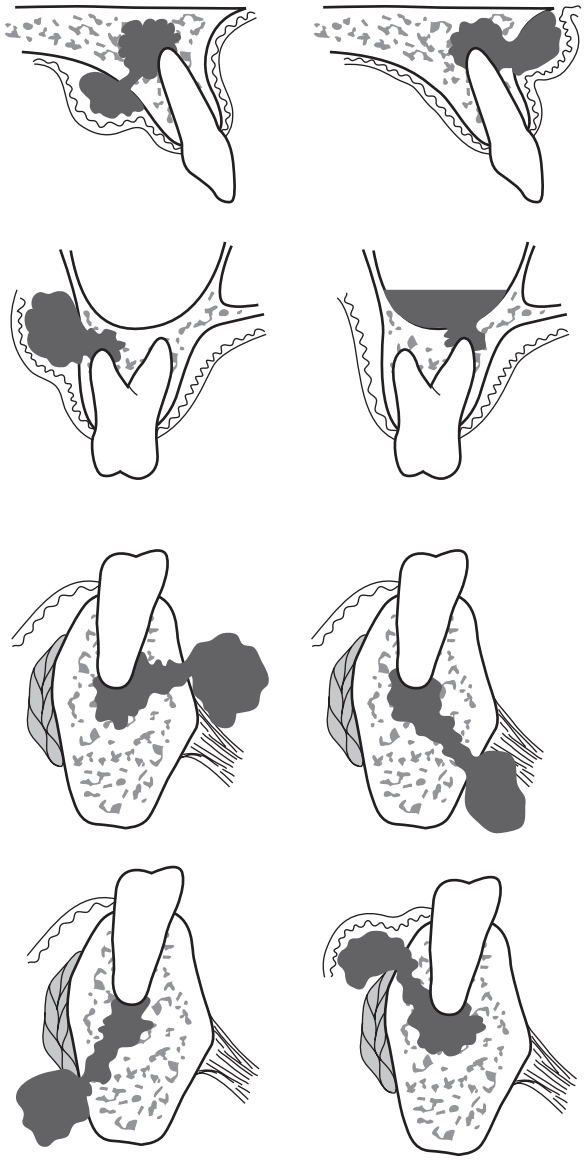

FIGURE 8-2 ■ Possible pathways of spread of periapical infection. (From Shafer WG, Hine MK, Levy BM. *Textbook of oral pathology*. 2nd ed. Philadelphia: W. B. Saunders; 1963.)

Infections of the mandibular incisor or canine tooth may spread either labially or lingually because the alveolar process is thin in this area. Labial perforation, which occurs more commonly, may be confined intraorally if the root apices are superior to the origin of the mentalis muscle but may spread extraorally if the apices are inferior to the mentalis attachment (Fig. 8-5). Infections of the mandibular premolar and first molar often perforate buccally, whereas the second and third molars perforate lingually.

When spread of mandibular infections occurs medially, the relationship of the tooth apices to the mylohyoid muscle origin is significant (Fig. 8-6). From the first molar forward, the dental root apices are superior to the mylohyoid, and these infections localize intraorally in the floor of the mouth (sublingual space). The apices of the second and third molars generally are inferior to the mylohyoid, and so the submandibular space is involved, with an extraoral presentation. As in maxillary infections, the relationship of the buccinator muscle to the root apices determines whether the infection spreads intraorally or extraorally.

Two fascial spaces commonly associated with odontogenic infections are the submandibular and masticator spaces.[28,29,45] The submandibular space is formed within the superficial layer of deep cervical fascia inferior to the mylohyoid muscle and inferomedial to the mandible. Anteriorly and posteriorly, it is limited by the bellies of the digastric muscle. Within this space lies the submandibular gland and portions of the facial artery and anterior facial vein. This space is closely approximated to the sublingual and masticator spaces. Infections of the submandibular space may originate in these adjacent spaces and in mandibular posterior teeth.

The masticator space also is formed within the superficial layer of deep cervical fascia. Its name is appropriate because its contents include the masseter, internal and external pterygoid, and temporalis muscles, as well as the mandibular ramus and the inferior alveolar neurovascular bundle. The submandibular, lateral pharyngeal, and retropharyngeal spaces are adjacent. Infections of the masticator space may originate in adjacent spaces or spread to it from periapical or pericoronal infections of the mandibular second and third molars and maxillary third molar.

TREATMENT OF ODONTOGENIC INFECTIONS

Patients with odontogenic infections may present with symptoms ranging from minor to life-threatening. Too often, a patient may be given a thorough systemic and extraoral head and neck evaluation while the intraoral search for the etiologic agent is overlooked.

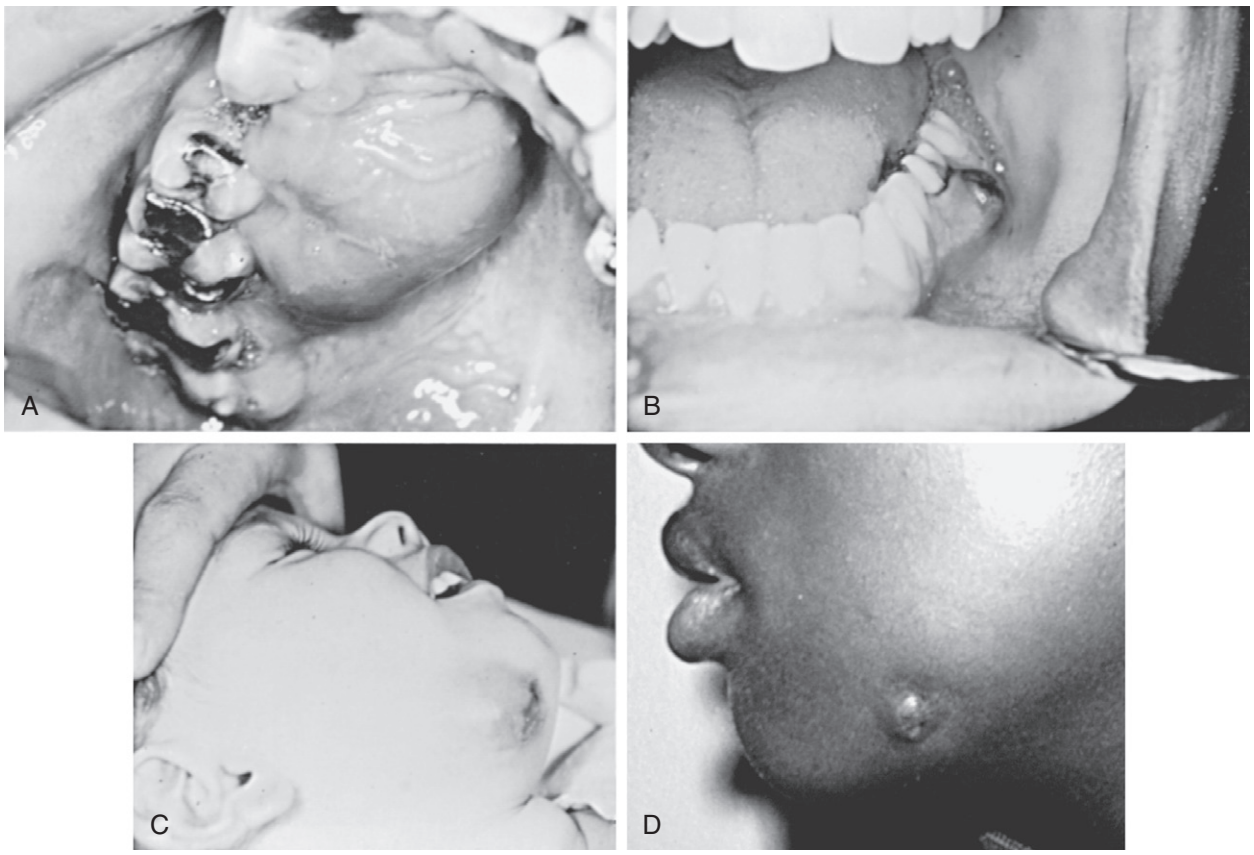

FIGURE 8-3 ■ Spread of odontogenic infection. **A,** Palatal abscess resulting from infected first premolar. **B,** Intraoral mucosal fistula from periapical abscess of mandibular left first molar. **C,** Soft tissue infection secondary to periapical abscess. **D,** Draining cutaneous sinus tract from a chronically infected lower molar in an adolescent girl. (**A** from Piecuch J. Odontogenic infections. Dent Clin North Am 1982;26:129-45. **D** from Flynn TR, Topazian RG. Infections of the oral cavity. In: Waite DE, editor. *Textbook of practical oral and maxillofacial surgery*. Philadelphia: Lea & Febiger; 1987.)

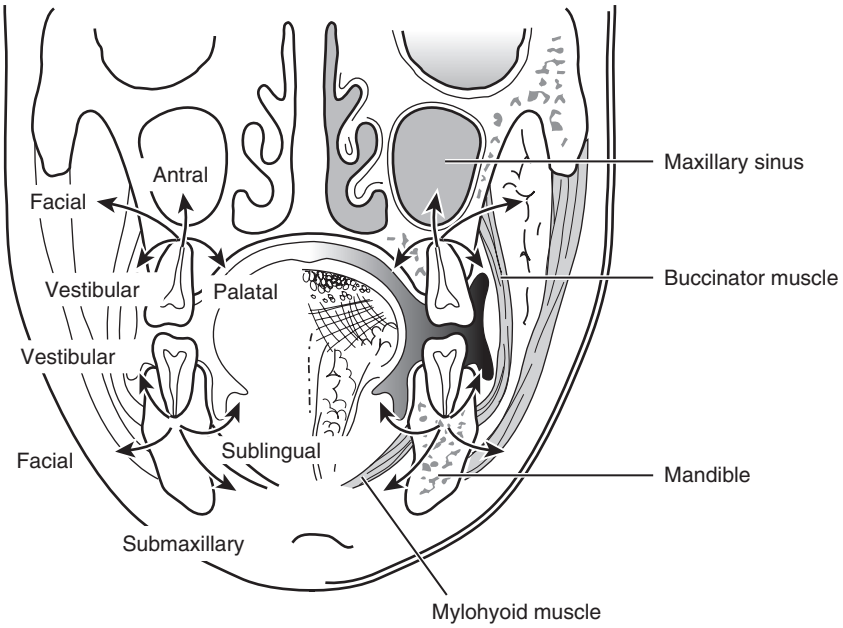

FIGURE 8-4 ■ Common pathways of spread of periapical infection. (From Kruger G. *Textbook of oral surgery*. 4th ed. St. Louis: C. V. Mosby; 1980.)

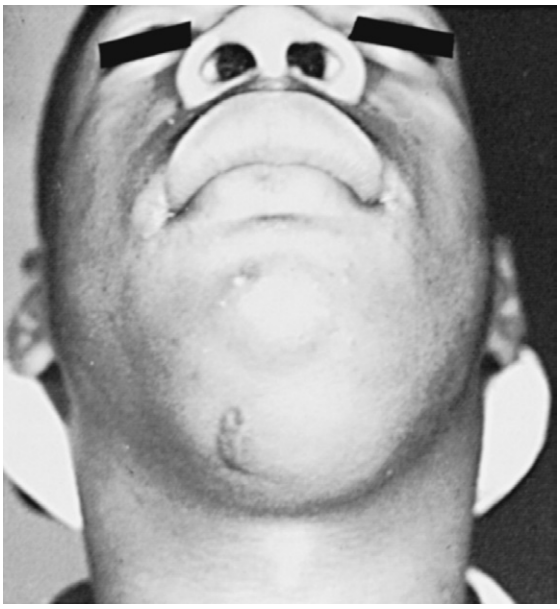

FIGURE 8-5 ■ Submental space abscess.

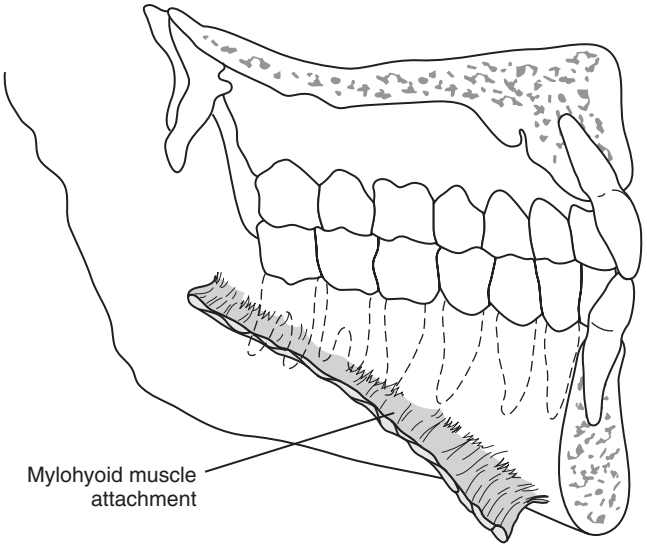

FIGURE 8-6 ■ Relation of tooth apices to the origin of mylohyoid muscle. (From Waite D. *Textbook of practical oral surgery*. Philadelphia: Lea & Febiger; 1978.)

A thorough oral examination begins with an evaluation of the degree of mandibular opening. Interincisal distance on wide opening extends 40 mm or more, even in young children. Painful limitation of the oral opening, or trismus, is associated with inflammation of the muscles of mastication and indicates spread of the infection to the masticator space. In association with a high fever, it can represent a serious turn of events. Teeth are inspected visually for caries, by percussion for tenderness, and by electric sensitivity or hot and cold stimulation for the pulpal pain response. Gingival tissues are probed for periodontal defects, and salivary glands are palpated for tenderness and milked to observe for purulent discharge from the duct orifices.

General Therapeutic Principles

As with infections elsewhere in the body, the principles of treatment of oral infections involve surgical drainage and antibiotics. Surgical drainage may comprise standard incision and drainage of an orofacial swelling or, in the case of localized periapical infection, endodontic drainage through the pulp or extraction of the offending tooth.

Surgical treatment of odontogenic infections is primary. In a systematic review of the literature, Flynn concluded that once an abscess was drained, the usual antibiotics used for treatment of odontogenic infections worked.[20,21] Dodson and colleagues,[17] in a review of head and neck infections requiring hospitalization of children, found that facial infections of the regions at or above the level of the upper lip and teeth most frequently were upper respiratory tract or sinus related and that lower face infections primarily were odontogenic. Infections of the upper face resolved without surgery in 65 percent of cases, whereas infections of the lower face resolved without surgery in only 25 percent of cases. Odontogenic infections almost always required some sort of surgical intervention. This finding may be due to the fact that the portal of entry in respiratory tract infections is through the surface mucosa, whereas the tooth roots carry the invading bacterial pathogens deep into the bone of the jaw, through which the surrounding deep fascial spaces become infected.

Respiratory pathogens frequently are viral, and odontogenic infections almost uniformly are bacterial, which may explain the propensity of odontogenic infections to form abscesses that need to be drained. Odontogenic infections treated with antibiotics only almost always recur in worse form than their previous manifestation. The indications for treating with antibiotics in addition to appropriate dental surgical therapy are fever, trismus, lymphadenopathy, osteomyelitis, and compromise of the immune system. Minor infections localized to the alveolar processes can be treated by tooth extraction, gingival curettage, or root canal therapy, with or without intraoral incision and drainage, without the use of antibiotics in the nonimmunocompromised individual.

Approximately 10% of all antimicrobials prescribed in the community are for the treatment of an oral process.[13] Misuse and overuse of antimicrobials have led to increasing resistance among many bacterial species. Oral pathogens have not been the exception. In an effort to promote the judicious use of antibiotics, the American Academy of Pediatric Dentistry (AAPD) and American Dental Association have developed guidelines intended to help reduce the inappropriate use of antimicrobial therapy in oral infections.[1] Despite the guidelines, the adherence to the recommendations continue to be low, as demonstrated in a recent survey of 154 dentists revealing that adherence to guidelines was between 10% and 40%.[13] In these guidelines, consideration for treatment with antibiotics involves the assessment of certain factors, such as the type of infection, location and extent of infection, risk for bacterial contamination (e.g., deep crown fractures), trauma, and immunologic status of the patient.

Selecting the appropriate antibiotic should follow the basic principles of therapeutics, which include choosing the narrowest spectrum based on cultures and susceptibilities, selecting an agent that concentrates adequately in the desired tissues and that has a good safety profile, convenient dosing, and the fewest side effects. In addition, it is important to inquire about the patient's previous antimicrobial exposures, which is a known risk factor for harboring more resistant organisms, as well as documentation of any drug allergies. In the adolescent population, asking about oral contraceptives is important because certain antibiotics used to treat dental infections, such as penicillins and tetracycline derivatives, can interfere with the efficacy of contraceptives.[1]

Antibiotic selection for odontogenic infections, although ultimately based on Gram stain and aerobic and anaerobic cultures, generally is begun empirically before culture results are available. Odontogenic infections are usually polymicrobial, with anaerobes playing an important role.[8] Penicillin and amoxicillin have been the first choice for outpatient infections on the basis of a good safety profile, bactericidal nature, and sensitivity of most streptococci and oral anaerobes to penicillin. However, there have been more reports of penicillin and amoxicillin resistance among oral pathogens that produce β-lactamases. Moreover, penicillin offers little or no coverage against *Neisseria* spp., a pathogen frequently found in odontogenic infiltrates.[73] A study of hospitalized patients with odontogenic infections found penicillin-resistant organisms in 54 percent of cases and therapeutic failure of empiric penicillin in 21 percent of cases.[21] The duration of previous therapy with β-lactam antibiotics also has been correlated with increased numbers of β-lactamase–producing bacteria in persisting infections. Patients with persisting infection after 3 days of treatment with a β-lactam antibiotic had a 50 percent incidence of β-lactamase–producing bacteria in the infection.[32,39] β-Lactamase inhibitors used in combination with β-lactam antibiotics may improve their effectiveness against resistant anaerobes, and using higher doses of the amoxicillin component may overcome resistance of some oral streptococci.[5]

Antibiotic susceptibility studies indicate that the oral anaerobes (especially *Fusobacterium*) and *Streptococcus* spp. now are largely resistant to erythromycin.[27,62,64] Based on these data, erythromycin should not be used as first choice in patients with odontogenic infections who are allergic to penicillin. Clindamycin, on the other hand, has an excellent track record in the treatment of odontogenic infections and can be used in patients allergic to penicillin. It concentrates well in saliva and bone and has enhanced activity against anaerobes, including bacteroides. However, resistance of odontogenic flora to this lincosamide derivative is also on the rise. Poeschl and colleagues[65] tested 73 streptococci spp. isolated from patients with odontogenic deep space infections and found 16% clindamycin resistance among *Streptococcus* spp. and 11% resistance among oral anaerobes (*Prevotella*, *Bacteroides*, and *Peptostreptococcus* spp.). *Eikenella corrodens*, an occasional pathogen in odontogenic infections, is uniformly resistant to clindamycin, which may explain the lack of effectiveness of clindamycin in some cases. A feared complication of the use of clindamycin is the development of *Clostridium difficile* colitis. This entity was first described after the use of this antibiotic and has been reported after use of clindamycin as prophylaxis for dental infections.[7] However, more cases are reported yearly of *C. difficile* after the use of penicillin and cephalosporins than with clindamycin.[8] Penetration of this drug through the blood-brain barrier is poor and should not be used to treat central nervous system abscesses of odontogenic origin.

Moxifloxacin, a fluoroquinolone with a spectrum of activity similar to that of amoxicillin-clavulanate, has been studied for the treatment of odontogenic infections with very good outcomes. A double-blind, phase 2 randomized trial comparing moxifloxacin with clindamycin in odontogenic infections found that moxifloxacin has excellent activity against *Streptococcus* spp. and oral anaerobes and was very effective in treatment of inflammatory odontogenic infections and abscesses.[10] However, this trial did not include children. Fluoroquinolones, especially ciprofloxacin and levofloxacin, have been used more often in children in recent years, especially in those with cystic fibrosis. The concern of musculoskeletal adverse events (e.g., tendinitis or adverse cartilage effects) has always been the limiting factor for their use in children. Ciprofloxacin and levofloxacin are less active against oral pathogens, and no pediatric dosing recommendations are available for moxifloxacin. Clinical trials are under way to determine the safety, tolerability, and pharmacokinetics of a single dose of moxifloxacin in children.

The second-line antibiotics in odontogenic infections are the cephalosporins, which, with the exception of the cephamycins (i.e., cefoxitin, cefotetan), have poor activity against oral anaerobes. Cephalosporins can be combined with metronidazole to enhance anaerobic coverage. Metronidazole has excellent activity against anaerobic bacteria but no activity against aerobic organisms. Moreover, it has no activity against organisms such as *Actinomyces* and microaerophilic streptococci, limiting their use as monotherapy for odontogenic infections.[43]

Tetracycline is incorporated permanently into newly formed dentin, causing permanent disfiguring discoloration of the dentition. It should not be used in children until they are at least 9 years old, when all but the third molar teeth would have full crown formation.

Penetration into the blood-brain barrier is important when treating odontogenic infections that spread beyond the oral cavity, approaching the cranial cavity. Penicillin will penetrate the barrier when the meninges are inflamed, whereas first- and second-generation cephalosporins will not. Clindamycin has poor penetration into the central nervous system and should not be used in brain abscesses. Conversely, metronidazole has excellent central nervous system penetration but needs to be combined with treatment for aerobic bacteria.[43]

The aforementioned considerations suggest that the empiric antibiotics of choice are penicillin in mild odontogenic infections and clindamycin for severe cases or in a patient with penicillin allergy. Table 8-3 lists our recommendations for empiric antibiotic therapy in odontogenic infections.

TABLE 8-3 **Empiric Antibiotics of Choice for Odontogenic Infections***

Type of Infection	Antibiotic of Choice
Outpatient infections	Penicillin Clindamycin Azithromycin
Penicillin allergy	Clindamycin Azithromycin
Inpatient infections	Clindamycin Ampicillin + metronidazole Ampicillin + sulbactam
Penicillin allergy	Clindamycin Third-generation cephalosporin IV (if the penicillin allergy was not the anaphylactoid type—use caution)

*Empiric antibiotic therapy is used before culture and sensitivity reports are available. Cultures should be taken in severe infections that threaten vital structures.

TABLE 8-4 **Indications for Antibiotic Therapy in Odontogenic Infections**

Antibiotic therapy is necessary
Acute-onset facial or oral swelling
Swelling inferior to the mandible
Trismus
Dysphagia
Lymphadenopathy
Fever >38.3°C (>101°F)
Pericoronitis
Osteomyelitis
Antibiotic therapy is *not* necessary*
Asymptomatic periapical abscess
Parulis (draining sinus tract)
Dry socket (alveolar osteitis)
Periodontal disease
Dental extractions
Root canal therapy

*With coexisting immune system compromise, antibiotic therapy may be indicated in some of these conditions.

Nursing Bottle Caries

Nursing bottle caries is a pattern of tooth decay affecting mainly the primary upper incisors and frequently the upper and lower primary molars in children of bottle-feeding age. It is caused by a practice of putting the child to bed with a nursing bottle filled with a sugar-containing drink, such as milk, fruit juice, or a soft drink. The child sucks on the bottle intermittently during sleep, when salivary secretion is low, and the sugar-containing liquid stays in the mouth for extended periods. This situation provides an excellent environment for the growth of caries-producing organisms, such as *S. mutans*. Nursing bottle caries can destroy virtually the entire primary dentition of a child as it erupts. Pediatric physicians and dentists should instruct parents to avoid putting their children to bed with nursing bottles or, if they must do so, to use water only in the bedtime drink.

Periapical Abscess

Extension of microorganisms through the root apex leads to the formation of an abscess. Early in this process, the acute abscess is indistinguishable clinically and radiographically from an acute pulpitis, particularly because radiographic evidence of bone destruction may take 7 to 14 days or more to develop. Sensitivity to heat stimulus (relieved by cold), exquisite sensitivity to percussion, and tenderness to finger pressure on the alveolar process are indications that the tooth has become abscessed. Electric pulp testing may be diagnostic if the tooth shows no response to the electric stimulus, but a positive pain response may be equivocal in multirooted teeth. Chronic abscesses are diagnosed more easily by looseness of the tooth, suppuration from draining sinuses or gingival crevice (see Fig. 8-3), and radiolucency on the radiographs (see Fig. 8-1). Depending on the path of least resistance, fluctuant areas may be noted in the buccal or lingual mucosa. Spread through the tissues, or cellulitis, may lead to the classic presentation of swollen face, pain, elevated temperature, and malaise.

In 1951, Krogh[39] showed a 3 percent complication rate when 2626 infected teeth were removed at the time of initial presentation. In 1975, Martis and Karakasis[54] published a similar study in which they treated 1376 acute dentoalveolar abscesses by immediate extraction. A 3 percent complication rate was found in this study, as well. A complication was defined as further extension of the infection, requiring additional treatment. Hall and associates[31] published a report in 1968 in which 350 patients with odontogenic cellulitis were divided randomly into two groups. The first group had extractions performed on the day of initial presentation, whereas the second group waited (with antibiotics) until the fourth day for surgical treatment to be performed after "localization" had occurred. The investigators' observations showed that extraction did not spread the cellulitis in either group. Patients with earlier extractions recovered more rapidly, whereas patients with delayed treatment had a greater need for incision and drainage, which was twice as likely to be extraoral than intraoral. In 1978, Martis and colleagues[54] showed in a series of more than 2000 patients that extraction without antibiotics in the presence of periapical infection led to the same complication rate as did extraction of noninfected teeth. Current literature validates these earlier findings, indicating that infections in patients with poor oral health and a lack of preceding dental treatment produce a stronger systemic response. Extraction of involved teeth when indicated and other dental treatments lead to a less severe course of infection.[76]

Considering the prospect of early relief of symptoms and a 97 percent chance that extraction (or occasionally root canal treatment) will cure the infection, early surgical intervention is mandatory. The use of antibiotics must be determined on an individual basis according to principles outlined previously (Table 8-4).

Periodontal Infections

Surrounding the teeth is a distinctive, pink keratinized mucosa, the gingiva (Fig. 8-7A). Normal gingiva is

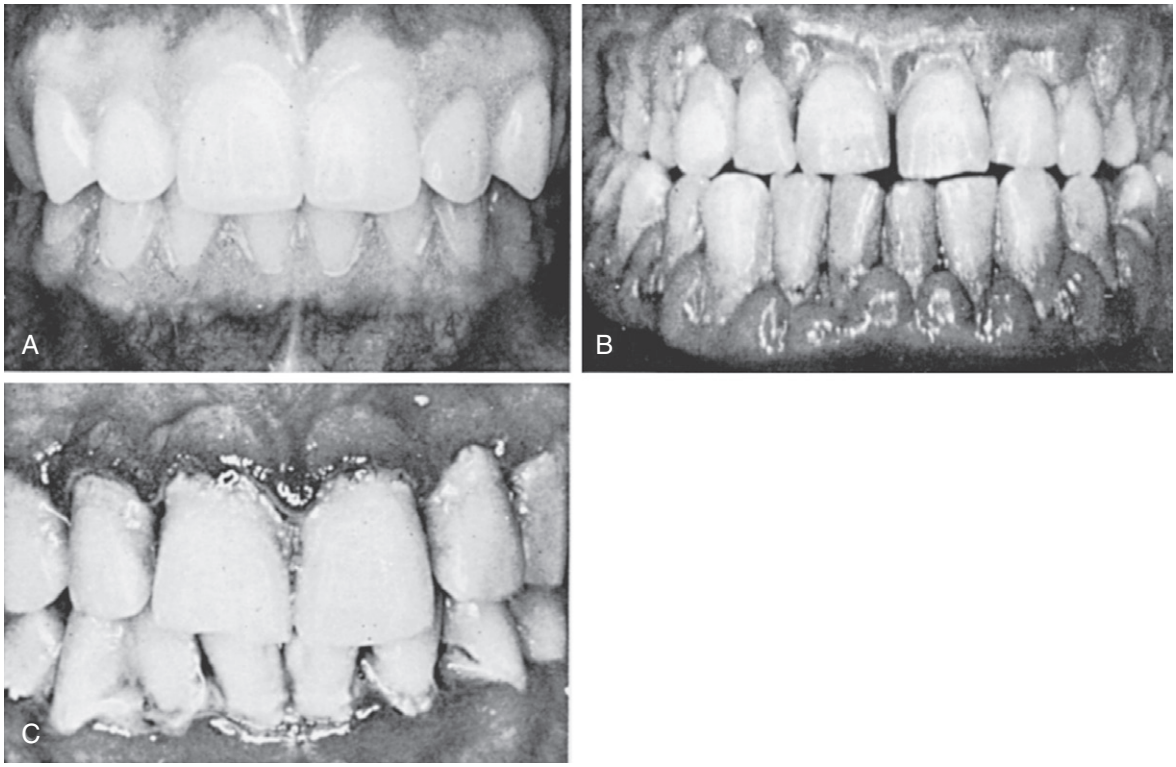

FIGURE 8-7 ■ **A,** Normal gingivae. **B,** Severe gingivitis. The maxillary gingivae exhibit mild inflammation; the mandibular interdental papillae are distorted grossly in form. Accumulations of plaque are prominent adjacent to the mandibular incisors. **C,** Acute necrotizing ulcerative gingivitis.

attached firmly to the alveolar bone and extends between the teeth as the interdental papilla. A thin cuff of free (nonattached) gingiva surrounds each tooth, and the resulting crevice between the free gingiva and the tooth normally is 1 to 3 mm in depth. It is represented by a thin roll of tissue along each tooth in Figure 8-7A.

Accumulation of food deposits and bacteria in the gingival crevice may result in gingivitis, a localized inflammation of the free gingiva that manifests as an erythematous, nonpainful swelling of the interdental papillae. In severe cases (see Fig. 8-7B), the gingival architecture may become distorted, and accumulations of plaque are evident. Although gingivitis is prevalent at all ages, affecting more than 50 percent of children[56] and almost all adults to some degree, it often is most severe in compromised hosts, including patients with diabetes and immunosuppressed patients. Poor oral hygiene is the usual precipitating factor for development of gingivitis, and this condition generally responds to dental scaling and improved oral hygiene.

In adolescents and adults, gingivitis may progress to periodontitis, a progressively severe infection characterized by hypertrophied gingivae, tooth mobility caused by irreversible resorption of alveolar bone, and a purulent exudate. This insidious condition usually is painless and may progress for years before being recognized. Localized periodontal treatment and meticulous oral hygiene may arrest the condition.

A rare variant, juvenile periodontitis,[42] usually is localized to the molar and incisor regions of younger, otherwise healthy children. Deep gingival pocketing and severe bone resorption are characteristic of this process and may result in loss of the dentition in these areas. The etiology is thought to involve a gram-negative anaerobe, *A. actinomycetemcomitans*, and localized bacterial inhibition of leukocyte function. Tetracycline in older patients has been useful in combination with periodontal surgery and meticulous home care.

Acute necrotizing ulcerative gingivitis (see Fig. 8-7C) is a specific infection caused by fusiform bacilli and spirochetes. Synonyms include trench mouth and Vincent infection. Erythema at the tips of the interdental papillae soon is supplanted by frank ulceration and foci of spontaneous bleeding. A pseudomembranous necrotic exudate forms along the marginal gingivae and the interdental papillae. The papillae later become blunted. Acute necrotizing ulcerative gingivitis is characterized by pain, foul breath and taste, thick ropy saliva, malaise, and occasionally fever. Theories suggest a concomitant viral etiology. Treatment consists initially of penicillin therapy followed within a few days by localized gingival curettage and oral rinses with 0.5 percent hydrogen peroxide or 0.12 percent chlorhexidine.[49]

Pericoronitis

Impaction of microorganisms and debris under the soft tissue overlying the crown of a tooth, often a mandibular third molar, or any erupting permanent tooth leads to the development of inflammation. Drainage usually occurs spontaneously from under the flap, localizing the problem. Blockage of natural drainage may lead to spread

FIGURE 8-8 ■ Pericoronitis.

of infection to adjacent soft tissues and fascial spaces (Fig. 8-8).

Pericoronitis is a polymicrobial infection; the periodontal pathogens *Prevotella* and *Porphyromonas* spp. and oral spirochetes, such as *Treponema denticola*, usually are the causative organisms. The *Streptococcus milleri* group bacteria also have been found to have a significant role in acute pericoronitis.[34] These organisms usually are sensitive to penicillin or penicillin combined with metronidazole. Pericoronitis most frequently occurs around the posterior portion of the crown of the lower third molar because it erupts during adolescence. In most cases, partial eruption of the third molar is caused by insufficient length of the horizontal ramus of the mandible to house all of the teeth. Part of the third molar is trapped under the oral mucosa covering the buccinator muscle and the superior pharyngeal constrictor because they form the most anterior portion of the oropharynx. In cases in which room is insufficient for the eruption of the third molar, the pericoronitis becomes recurrent or chronic and the impacted third molar should be removed.

Lower third molars lie in proximity to the pterygomandibular space, a portion of the masticator space. When these infections spread to involve this space, trismus results, which obscures the infection to clinical examination. The presence of trismus with a history of pain in the third molar region is an ominous sign of infection involving the masticator space, which, although not manifested by external facial swelling, may begin to involve the deeper parapharyngeal spaces. These infections may become life-threatening. The lower third molar is the most frequent offending tooth in severe odontogenic infections requiring hospitalization, and these infections occur most frequently in adolescents and young adults.[33]

Various treatment modalities, including local incision and drainage and extraction of the tooth, are applicable to pericoronitis.[53,72] Antibiotic therapy is used if fever, trismus, or lymphadenopathy is present (see Table 8-4). Resolution of symptoms should occur in less than 1 week.

Oral Manifestations of Human Immunodeficiency Virus Infection in Children

The most common oral lesions in children with human immunodeficiency virus (HIV) disease are oral candidiasis, herpes simplex virus infections, linear gingival erythema, parotid salivary gland enlargement, and recurrent aphthous ulcerations. In contradistinction to adult HIV infection, HIV-associated periodontitis and gingivitis are much less common. Neoplastic oral manifestations of HIV infection, such as Kaposi sarcoma, non-Hodgkin lymphoma, and hairy leukoplakia, continue to be rare findings. In contrast to adults, children infected with HIV have a greater susceptibility to bacterial infections, especially with encapsulated organisms, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Septicemia from an oral focus of infection can become a life-threatening problem in an HIV-infected child, and optimal oral health must be established and vigorously maintained in these children.[41] Routine use of chlorhexidine gluconate 0.12 percent mouth rinse may be helpful in minimizing gingivitis, candidiasis, and bacterial superinfections of the oral cavity, although its safety and effectiveness have not been shown in children.

Oral candidiasis usually is of the pseudomembranous type, which is seen in the oral cavity as a creamy white plaque that is rubbed off easily, leaving a reddened surface mucosa exposed. Because oral candidiasis is rare in normal children older than 6 months, persistence of oral candidiasis for 2 months or more in a child older than 6 months who has not received antibiotic therapy in the past 2 weeks is suggestive of HIV infection. Persistent oral candidiasis indicates acquired immunodeficiency category P-2D3 (symptomatic infection with secondary infectious diseases) in the Centers for Disease Control and Prevention classification of HIV infection in children younger than 12 years.[38]

Oral candidiasis was associated with a decreased survival time in a study of 99 children with perinatally acquired HIV infection. The median time from birth to the manifestation of the first lesion of oral candidiasis was 2.4 years, and the median time from the appearance of lesions to death was 3.4 years, with a relative hazard rate of 14.2.[37]

Treating oral candidiasis lesions is difficult because of frequent recurrence of oral fungal infection with resistant biotypes of *Candida albicans* or colonization by related, but more resistant, species, such as *Candida krusei*, *Candida*

parapsilosis, and *Candida guilliermondii*. Treatment regimens may progress from nystatin to clotrimazole, fluconazole, and amphotericin B, depending on the extent of disease, clinical response, and culture and sensitivity results.[51] Sudden onset of rampant dental caries has been associated with prolonged oral use of sucrose-containing antifungal antibiotic preparations.[85]

Linear gingival erythema, formerly referred to as HIV gingivitis, is the most common form of periodontal disease seen in children with HIV infection. It is described as a fiery red, 2- to 3-mm-wide linear band of inflammation of the gingiva. Pain is not associated with the lesion, but the gingivae are likely to bleed during tooth brushing or even spontaneously. The microbiology of this lesion is unclear, but *Candida* spp. may be a possible cause.

Parotid salivary gland enlargement, which may be painful and become secondarily infected, has been reported in 14 to 30 percent of HIV-infected children. The enlargement apparently is caused by infiltration of the glands by T8 lymphocytes and has been associated with increased survival time. The median time was 4.6 years from birth to development of parotid enlargement and 5.4 years from development of lesions to death, with a relative hazard rate of 0.38.[37]

Herpes simplex virus infections, although common occurrences in normal children, seem to be particularly severe and recur more often in HIV-infected children. The lesions appear first as multiple clustered vesicles on the lips or keratinized oral mucosa, which soon rupture to leave painful irregular oral ulcers or crusted labial ulcers. Fever and dysphagia may warrant hospital admission for hydration, nutrition, and therapy with parenteral acyclovir. Less severe cases may be treated with oral acyclovir.

Dental caries is increased in pediatric HIV cohorts. The cause of this finding is unclear. It may be due to xerostomia secondary to parotid enlargement in some cases, prolonged use of sucrose-containing antifungal agents in others, and nursing bottle caries in still others. The association of nursing bottle caries with pediatric HIV infection may be due to their common increased prevalence in urban dwellers with limited economic resources, although nursing bottle caries also is found frequently in children with other chronic diseases.[85]

COMPLICATIONS OF ODONTOGENIC INFECTIONS

Fascial Space Infections

Spread of infection to the fascial spaces may result in dramatic facial swelling, high fever, and, if untreated, respiratory embarrassment. The characteristics of the more common fascial space infections related to odontogenic infection are described in this section.

Infraorbital space infections generally are related to maxillary anterior teeth and are well localized to the infraorbital fossa by the levator labii superioris and levator anguli oris muscles. Facial swelling lateral to the nose is prominent, as is decreased mobility of the upper lip caused by inflammation of these muscles. If the area is fluctuant, intraoral incision and drainage with placement of a small Penrose drain for 1 or 2 days generally is sufficient treatment. Antibiotics are indicated for all infections of the fascial spaces.

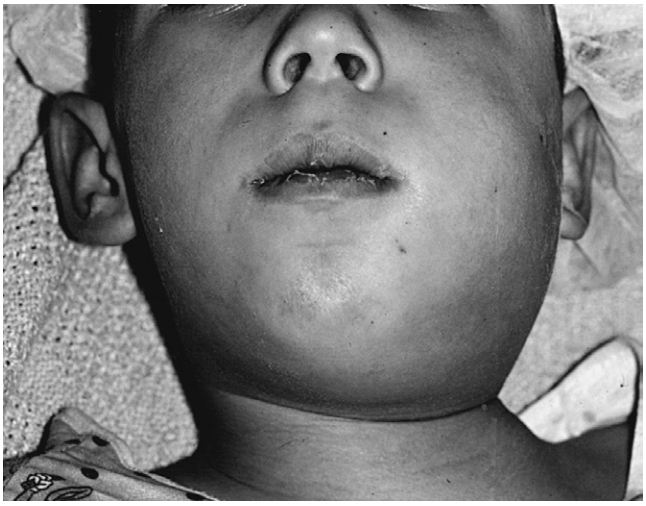

FIGURE 8-9 ■ Lateral pharyngeal space abscess in a 6-year-old boy. Note the swelling above the hyoid bone and anterior to the sternocleidomastoid muscle. Swelling also occurs in the buccal and submandibular spaces.

Trismus is the hallmark of infection of the masticator space. Trismus is caused by spasm in the muscles of mastication, which define this large potential space. The resulting inability to open the mouth hinders access to the airway for endotracheal intubation. In addition, abscesses of the masticator space may rupture into the oropharynx, causing aspiration of pus, or they may pass easily around the medial pterygoid muscle to involve the lateral pharyngeal and retropharyngeal spaces. Figure 8-9 shows a 6-year-old boy whose lower primary molar abscesses spread to involve the buccal, pterygomandibular, and lateral pharyngeal spaces. Extraoral and intraoral drainage, prolonged intubation, and extraction of the offending teeth were required.

Infections of the submandibular space (Fig. 8-10) may be localized unilaterally or may involve bilateral structures. Treatment of submandibular space infection is via extraoral incision and drainage.

First described in 1836, Ludwig angina consists of infection of the sublingual and submandibular spaces bilaterally and is characterized by hard, brawny swelling and a minimum of suppuration. The tongue often is edematous and raised to the roof of the mouth, with little mobility (Fig. 8-11). Airway obstruction should be considered imminent; the greatest cause of death in Ludwig angina is blockage of the airway by soft tissue swelling, pus, or blood, which occurred in more than 50 percent of its victims in the preantibiotic era.[30] In 1940, Williams[89] published a series of 37 cases of Ludwig angina that reported a 54 percent mortality rate. The airway management policy at that time was emergency tracheotomy if necessary. Three years later, Williams and Guralnick[89,90] published a series of 20 cases of Ludwig angina treated with a new policy of immediate establishment of airway security by intubation or tracheotomy and

FIGURE 8-10 ■ Submandibular space abscess.

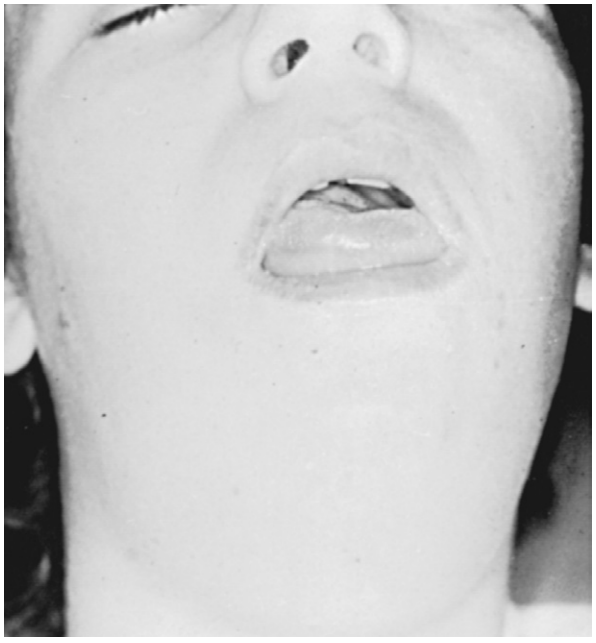

FIGURE 8-11 ■ Ludwig angina.

aggressive incision and drainage of all anatomic spaces affected by the infection. By these measures, they were able to reduce the mortality rate of this dreaded infection to 20 percent only 3 years later. Antibiotics were unavailable during the course of these two studies. These studies underscore the importance of airway security and aggressive surgical management in the treatment of severe odontogenic infections.

Today, death rarely occurs from severe odontogenic infections, although the need for tracheotomy or prolonged endotracheal intubation is common. The cause of this infection often is odontogenic infection but may include laceration of the floor of the mouth and mandibular fracture. Usually a disease of middle-aged adults, it is a rare occurrence in children but may occur in greater frequency in immunologically compromised children.[26] Surgical drainage of all four spaces, accompanied by vigorous antibiotic therapy, is indicated.

Necrotizing Fasciitis

Necrotizing fasciitis, which causes a frightening loss of skin and underlying tissues, has received considerable notoriety in the press. Cervicofacial necrotizing fasciitis often is odontogenic and typically causes a superficial spreading of cellulitis that follows the platysma muscle from the cheek down the entire neck to the anterior chest wall (Fig. 8-12A). Figure 8-12B illustrates such a swelling in an 8-year-old boy. The presumptive cause was odontogenic infection of the primary molars, which caused a high fever and a rapidly progressive cellulitis extending from the cheek to the chest. The cause of these infections often is group A streptococci, but a wide variety of microorganisms may be involved.

Wide-spectrum antibiotic therapy is indicated empirically, along with hydration, transfusions if necessary, and support of electrolyte balance, especially with calcium, which may be sequestered by necrotic fat molecules.[3] Timely surgery is important in the management of necrotizing fasciitis. Incision and drainage of the involved spaces, débridement of necrotic tissue, and fasciotomy often are performed as soon as possible after the patient's admission.[88] Hyperbaric oxygen therapy has been advocated as an adjunct in the treatment of necrotizing fasciitis. A significant reduction in the mortality rate and length of hospitalization associated with necrotizing fasciitis has been shown in adults.[88] Necrotizing fasciitis is cited as an indication for hyperbaric oxygen therapy in infants and children and should be considered if available.[87]

Odontogenic Sinusitis

A significant percentage of cases of sinusitis are odontogenic, especially in adults, because the maxillary sinus follows the erupting permanent tooth roots into the alveolar process. This pneumatization of the alveolar process progresses throughout life and is accelerated by loss of the upper posterior teeth. Dental infections of the periapical upper posterior teeth occasionally rupture through the maxillary sinus floor to involve the paranasal sinuses. Dental infection should be eliminated in the complete treatment of severe recurrent sinusitis in children.

Figure 8-13 illustrates a case in a 9-year-old boy of left pansinusitis, including ethmoiditis and a subperiosteal orbital abscess associated with infected upper primary molars. His treatment involved a team approach of the dental and otolaryngology services for tooth extraction, incision and drainage of the buccal and infraorbital

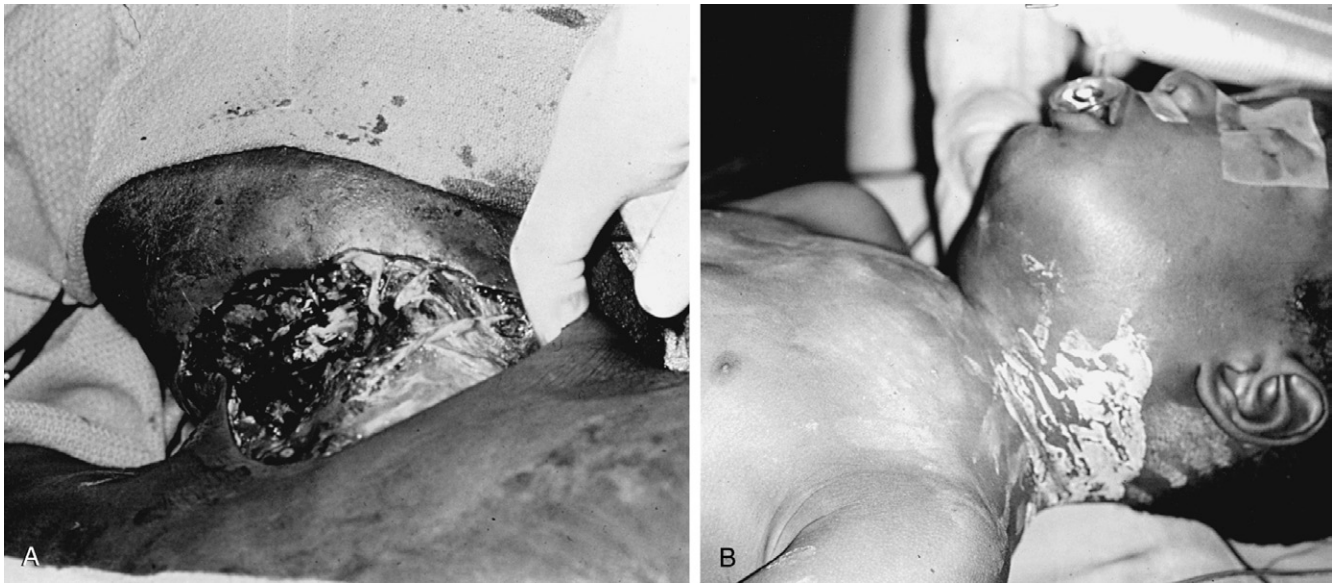

FIGURE 8-12 ■ **A,** Surgical débridement of necrotic skin and platysma muscle of the left neck of a diabetic female patient with necrotizing fasciitis. Note how easily blunt finger dissection can undermine the skin in the plane of the necrotic platysma muscle. **B,** Necrotizing fasciitis in an 8-year-old boy. Note the swelling extending from the buccal space down the neck and onto the anterior chest wall, following the extent of the platysma muscle. The chalky material on the posterior neck is calamine lotion placed by the patient's mother for vesicles that resembled poison ivy.

spaces, endoscopic sinus surgery, and external drainage of the orbital abscess.

Buccal and Periorbital Cellulitis

A child occasionally presents with an acute buccal or periorbital space swelling and cellulitis with no clinically apparent odontogenic cause. These infections tend to occur in young children, usually younger than 36 months. They usually have a history of recent upper respiratory infection or sinusitis. *H. influenzae* type b and *S. pneumoniae* have been implicated as pathogens in these conditions, although the widespread use of conjugated vaccines has reduced drastically the incidence of invasive disease from *H. influenzae*.[25] A possible mechanism for the inoculation of the soft tissues is migration of organisms through emissary veins piercing the thin cortical bone overlying the lateral surface of the maxillary sinus, in cases with existing sinusitis. Unless the infection is severe, incision and drainage usually are unnecessary, and treatment with antibiotics is successful. Blood cultures frequently are positive in more severe cases. The role of lumbar puncture remains controversial in the overall management of infants and young children. The incidence of this infection has declined significantly as a result of the widespread use of *H. influenzae* vaccine.

Orbital and Intracranial Complications

Odontogenic infections that spread to involve the orbit and the brain are rare. Orbital and intracranial abscesses may have an odontogenic origin, however, and the dental condition of patients with these conditions should be evaluated by a dentist. Probably no more than 5 to 10 percent of orbital cellulitis is odontogenic in origin.[36,91] This infection generally is unilateral and is characterized by proptosis, chemosis, lid edema, and restriction of extraocular motion secondary to edema.[67] No nerve palsies or visual changes are present. Treatment includes surgical drainage, antibiotics, and elimination of the dental infection.

Cavernous sinus thrombosis, which may be difficult to differentiate clinically from orbital cellulitis, is considerably more serious because microorganisms proliferate intracranially. The risk for death is high. Characteristics include bilateral involvement with rapid progression from one eye to the other, proptosis, chemosis, and lid edema. Extraocular movements are limited because of inflammation of the third, fourth, and sixth cranial nerves. Systemic signs of meningeal irritation and ophthalmoscopic evidence of obstruction of the retinal veins also are present.[14,67,82] Treatment includes high doses of parenteral antibiotics, elimination of the causative dental pathosis, and incision and drainage of infected fascial spaces. Odontogenic cavernous sinus thrombosis seems to be propagated by an ascending thrombophlebitis.

Brain abscess and subdural empyema are rare findings today compared with several decades ago. Dental sources of infection have been implicated in brain abscesses from less than 1 percent to 8.5 percent of cases reported.[11,66,70,79] Odontogenic brain abscesses seem to occur by direct extension through the paranasal sinuses, usually to the frontal lobe through the frontal sinuses. Typically a pansinusitis intervenes between the dental infection and the brain; however, hematogenous and contiguous anatomic routes of spread are possible.[40,50,75] The literature supports the fact that oral flora are found in brain abscesses, but a correlating culture from the supposed oral source is rarely found, often making brain abscess secondary to dental infection a diagnosis of exclusion.[40]

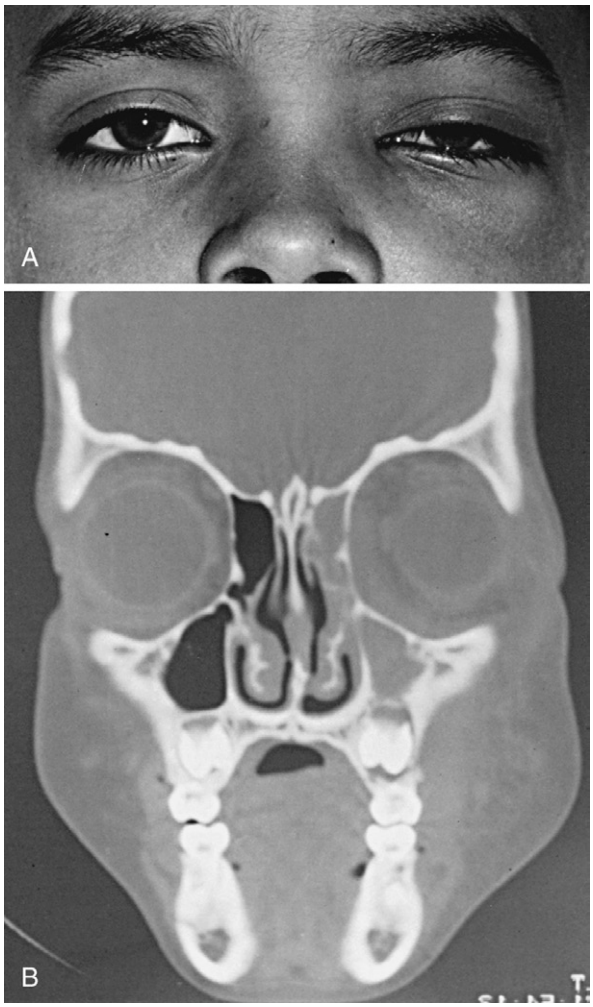

FIGURE 8-13 ■ **A,** A 9-year-old boy with a left pansinusitis and an abscess of the upper left first primary molar. Note the left periorbital discoloration and swelling, with partial ptosis and displacement of the globe laterally. **B,** Computed tomography scan of the same patient. Note the left maxillary sinus opacification close to the infected tooth, opacification of the ethmoid sinuses, elevation of the periosteum away from the medial orbital wall, and displacement of the globe laterally within the orbit.

OSTEOMYELITIS OF THE JAWS IN CHILDREN

Osteomyelitis of the jaws in children usually results from periodontal or, more commonly, periapical infection. Open fracture of the jaws with delayed treatment also is a significant cause of osteomyelitis. Extension from contiguous infections, such as otitis, parotitis, and mastoiditis, occurs much less often.

Osteomyelitis of the jaws occurring in children must be viewed with great concern because it may result in the following problems: (1) loss of primary and permanent teeth; (2) sequestration of segments of the jaws; (3) growth defects, such as mandibular hypoplasia, asymmetry, and ankylosis[19]; (4) disfiguring facial scars and cutaneous fistulas; and (5) lesions suggestive of malignancy, which require open biopsy. For these reasons, osteomyelitis of the jaws in children should be diagnosed rapidly and treated aggressively. Table 8-5 presents a useful classification of this disease.[84]

TABLE 8-5 Osteomyelitis of the Jaws

Suppurative	Nonsuppurative
Acute suppurative	Chronic sclerosing
Chronic suppurative	Facial sclerosing
Primary	Diffuse sclerosing
Secondary	Garré sclerosing
Infantile	Actinomycotic
	Radiation osteomyelitis and necrosis

Predisposing Factors

Preexisting systemic disease with accompanying alteration of host resistance plays a major role in the initiation of osteomyelitis of the jaws. It includes conditions such as uncontrolled diabetes, agranulocytosis, leukemia, sickle-cell disease, and febrile illnesses. Conditions that alter the vascularity of bone and the ability to combat infections, including bone tumors, fibrous dysplasia, Paget disease, and radiation to the jaws, also are important predisposing conditions. Major maxillofacial injuries resulting in open fractures of the jaws, especially fractures that are not treated immediately, are an important cause of osteomyelitis.

Microbiology

Because the etiology of osteomyelitis of the jaws includes causes other than purely odontogenic infections, the bacterial spectrum is broad. Most instances of osteomyelitis of the jaws are caused by aerobic streptococci (α-hemolytic streptococci and *S. viridans* group), anaerobic streptococci, and other anaerobes, particularly *Peptostreptococcus*, *Fusobacterium*, and *Bacteroides* and related genera.[62] Only occasional cases are caused by *S. aureus*, with entry through the skin being the probable route. Other bacteria involved include oral anaerobes, aerobic and microaerophilic cocci, and gram-negative organisms. Specific forms of osteomyelitis are caused by *Actinomyces israelii*, *Treponema pallidum*, and *Mycobacterium tuberculosis*. *Salmonella* organisms have been associated with osteomyelitis of the jaw in patients with sickle-cell anemia.[16]

Clinical Findings

Osteomyelitis involves the mandible far more frequently than the maxilla because the poor blood supply to the mandible comes primarily from one major vessel and the periosteal blood supply. Four major forms of the disease, which may be distinguished clinically, are (1) acute suppurative; (2) secondary chronic, which begins as an acute osteomyelitis and becomes chronic; (3) primary chronic, which has no acute phase and always has seemed to be a low-grade infection; and (4) nonsuppurative osteomyelitis. The forms most often seen in children are the acute

suppurative, the secondary chronic, and one nonsuppurative form, Garré sclerosing osteomyelitis. These conditions are described in some detail.

Suppurative Osteomyelitis

Suppurative osteomyelitis usually begins with deep and intense pain in the jaws, high intermittent fever, and an obvious cause, most often a deeply carious or discolored tooth. In the early stages, mental nerve paresthesia occasionally is present. During the course of several days, facial swelling develops, and in 10 to 14 days, teeth begin to loosen, pus exudes around the gingival sulcus, and multiple mucosal or cutaneous sinus tracts form. In addition to the draining sinuses, a firm cellulitis is present in the soft tissues, accompanied by trismus and cervical lymphadenopathy. A leukocytosis, ranging typically from 8000 to 15,000 cells/mm^3, is present, although it does not ordinarily reach the levels seen in acute osteomyelitis of the long bones. After 10 days to 2 weeks, radiographs may show scattered areas of bone destruction suggestive of a moth-eaten appearance (Fig. 8-14), and periosteal reaction characterized by the laying down of new bone commonly is seen. Smears of specimens and cultures, including cultures of bone sequestra, should be taken whenever possible. Interpretation of cultures must be made with caution because of the possibility of skin and oral contaminants in the specimen.

Initially, antibiotic doses adjusted for age may be given empirically, but the selection of the antibiotic is determined by culture and sensitivity testing of specimens taken directly from the infected site.[23] The antibiotics of choice in mandibular osteomyelitis include amoxicillin-clavulanate, clindamycin, and fluoroquinolones. Fluoroquinolones are excellent choices because of their complete absorption from the gastrointestinal tract and high penetration of bone, but these drugs generally should be avoided in children because they may be chondrotoxic during growth. As results from smears and culture are obtained, antibiotics may be changed unless the infection is responding favorably, in which case no change is made. The involved tooth is removed as early as possible to allow drainage and provide material for culture.

Antibiotic therapy is continued for at least 2 to 4 weeks after all symptoms subside. If the infection persists, repeated cultures are obtained and the antibiotic is changed if necessary. The greater vascularity of the jaws may explain their more rapid response to antibiotic therapy and surgery in contrast to that of the long bones. The duration of intravenous antibiotic therapy in osteomyelitis of the jaws may not need to be as prolonged as in that of the long bones. Consideration should be given to sequestrectomy, saucerization, or the placement of closed-wound irrigation and suction. Saucerization involves the removal of teeth in the immediate area and removal of the overlying buccal plate of bone, allowing access to the medullary portion and sequestra that may be present. Placement of catheters through an extraoral approach occasionally is necessary for closed irrigation and suction. It permits instillation of antibiotics, allowing direct contact with the bone. Hyperbaric oxygen treatment may be considered in chronic cases refractory to antibiotic treatment.[56]

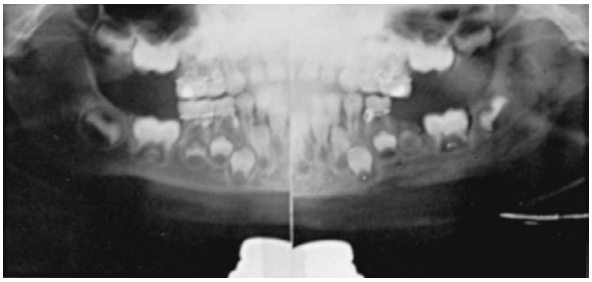

FIGURE 8-14 ■ Radiograph of the jaws of a 4-year-old girl with suppurative osteomyelitis of the left mandible. The film shows marked destruction of the body and ramus of the mandible.

Infantile Osteomyelitis

Osteomyelitis of the jaws in a newborn is an uncommon occurrence but warrants special mention because of its serious sequelae. It occurs most often a few weeks after birth and usually involves the maxilla. It is not odontogenic in origin, but is thought to arise from neonatal trauma to oral tissues, hematogenous spread (from skin, middle ear, mastoid, or tonsils), or an infected nipple.[69] The patient has facial cellulitis centered around the orbit (Fig. 8-15). Irritability and malaise precede development of cellulitis and are followed by marked elevation in temperature, anorexia, and dehydration. Extraorally, inner canthal swelling, palpebral edema with closure of the eye, conjunctivitis, and proptosis may be seen, together with a purulent discharge from the nose or inner canthus. Oral examination shows swelling of the maxilla on the affected

FIGURE 8-15 ■ Characteristic clinical picture of a 3-week-old child with infantile osteomyelitis. (Courtesy Dr. M. Michael Cohen, Sr.)

side extending to the buccal and the palatal regions, with fluctuation often present with multiple sinus tracts. *S. aureus* is the organism usually found.

Aggressive, prompt treatment must be undertaken to prevent permanent optic damage, neurologic complications, loss of tooth buds and bone, and extension to the dural sinuses. Intravenous penicillin and a penicillinase-resistant penicillin are given simultaneously with surgical drainage of all fluctuant areas, repeated Gram smears, and culture and sensitivity testing. Antibiotics are continued orally for 2 to 4 weeks after all signs of the infection have disappeared. If sequestra form, they should be removed conservatively. Tooth buds may be lost, and surviving teeth may be deformed or discolored after eruption.

Garré Sclerosing Osteomyelitis

Garré sclerosing osteomyelitis, also known as chronic nonsuppurative sclerosing osteomyelitis and proliferative osteomyelitis of Garré,[6] is notable because of the similarity of some of its characteristics to those of other neoperiostoses. It is characterized by a localized, hard, nontender swelling of the mandible (Fig. 8-16). Lymphadenopathy, hyperpyrexia, and leukocytosis are not present. Garré osteomyelitis is associated commonly with a carious tooth, usually the lower first molar (Fig. 8-17), and a history of a past toothache. It also may be associated with a recent dental extraction or an infected flap of tissue over an erupting tooth.[48] Radiographs are impressive, showing a focal area of well-calcified, smooth bone proliferation that often has a laminated or onion-peel appearance (Fig. 8-18).

Garré osteomyelitis is thought to be a response to a low-grade stimulus, such as a dental infection, that influences the potentially active periosteum of young individuals. Its appearance resembles that of infantile cortical hyperostosis (Caffey disease), osteosarcoma, and Ewing sarcoma, and it must be distinguished from these conditions.[18] Treatment consists of extraction or endodontic treatment of the involved tooth, with continued clinical and radiographic follow-up of the patient to ensure that the new bone formation does not progress. Ordinarily, remodeling occurs over the course of time, but biopsy should be done to rule out neoplasm if the lesion does not regress. No antibiotic therapy is necessary.

HERPES SIMPLEX VIRUS INFECTIONS

Herpes simplex virus type 1 infections commonly are manifested as herpetic gingivostomatitis.[81] Five stages of infection have been identified: (1) primary mucocutaneous infection, (2) acute infection of ganglia, (3) establishment of latency, (4) reactivation, and (5) recurrent infection.

FIGURE 8-16 ■ Enlargement of the right side of the mandible in a 12-year-old patient with Garré sclerosing osteomyelitis. The swelling is hard and nontender.

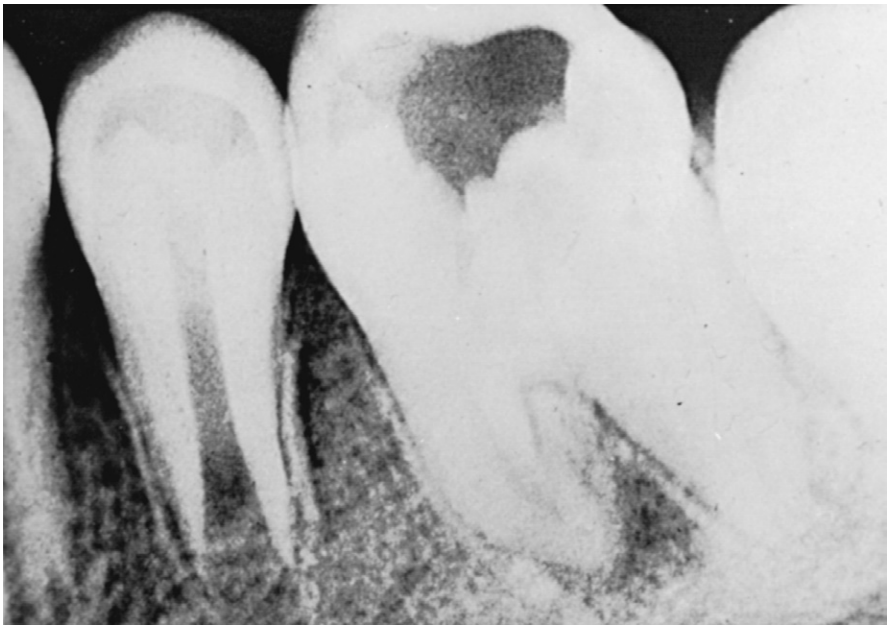

FIGURE 8-17 ■ Radiograph of a deeply carious lower first molar tooth with periapical spread of infection. It is the usual cause of Garré osteomyelitis.

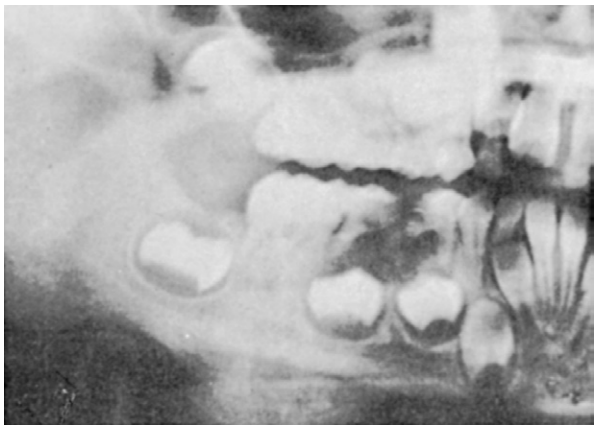

FIGURE 8-18 ■ Characteristic radiograph of Garré osteomyelitis showing the laminated or onion-peel appearance of the mass. (Courtesy Dr. Larry J. Peterson.)

Primary infection is established by direct contact with individuals who have draining lesions or asymptomatic carriers who may continue to shed the virus despite the lack of symptoms. The highest incidence of primary infection appears to be in children 2 to 4 years old. Infants are protected by maternal antibodies, which may explain why this infection is not found in infants younger than 6 months. It appears to have no seasonal variation or male or female difference in incidence.

The incubation period is thought to be approximately 6 days, followed by the development of small vesicles that may coalesce to form larger lesions or ulcers. In severe cases, the lips, gingivae, oral mucosa, and pharynx may be involved. Many patients with primary herpes labialis may be asymptomatic, however, and symptoms may not develop. Healing occurs in 1 to 2 weeks, with gradual crusting of the lesions followed by re-epithelialization.

Latency is thought to continue throughout life, with reactivation occurring at various times, possibly triggered by actinic radiation and emotional or physical stress. Recurrent disease is manifested by vesicles at the mucocutaneous border, which are painful for about 2 days, followed by crusting and complete healing in 7 to 8 days.

Fifty percent of the adult population in industrialized countries and a higher percentage in less-developed countries may have recurrent herpes labialis. Many, if not most, adults who have recurrent "cold sores" are not aware that they can transmit the disease and should be counseled in this regard. Likewise, medical, dental, and nursing personnel should be advised that occurrence of cutaneous lesions (herpetic whitlow) is possible after direct contact of the practitioner's fingers with lesions during the physical examination.

In treating recurrent herpes labialis in nonimmunocompromised adults, acyclovir has been shown to decrease the duration of symptoms by 12 to 24 hours, at a cost of approximately $75.00 per day. Frequent use of acyclovir also may promote the spread of resistant viral strains. The use of acyclovir for treatment of recurrent herpes labialis in nonimmunocompromised individuals seems to offer little benefit at significant expense and risk.[68]

INTRAORAL AND PERIORAL PIERCINGS

Tattoos and piercings are not uncommon in the adolescent population. Forty-six percent of teenagers will receive their first piercing between the ages of 11 and 16 years.[12] The mouth and surrounding tissues are commonly pierced sites, with the tongue being the most frequently pierced.[44,60] Moreover, a lack of awareness exists among these age groups on the risks associated with these body modification practices.[60] Many times these piercings are performed by nonregulated piercing parlors and unlicensed personnel.[92]

Complications from piercing have ranged from simple local complications, such as bleeding, pain, and local infections, to severe, life-threatening infection.[86,92] Among the most severe complications, bacterial endocarditis and brain abscess have been reported. Yu and associates,[92] in a review on bacterial complications of oral piercings, found eight cases of bacterial endocarditis after oral piercings reported between 2001 and 2009, of which two were teenagers. Oral organisms such as *S. viridans*, *S. aureus*, and *Gemella* and *Neisseria* spp. were reported. Ludwig angina, glossal abscesses, and lymphadenitis, among other conditions, also were included in their report. Martinello and Cooney[52] reported a case of a solitary brain abscess in a patient 1 month after a tongue piercing. The abscess was polymicrobial with *S. viridans*, *Peptostreptococcus micros*, and *Actinomyces* and *Eikenella* spp. isolated.

Because of the risks associated with the use of oral jewelry and piercing, the AAPD has issued a statement strongly opposing the practice of piercing intraoral and perioral tissues and use of jewelry on these tissues.[2]

NEW REFERENCES SINCE THE SIXTH EDITION

11. Carpenter J, Stapleton S, Holliman R. Retrospective analysis of 49 cases of brain abscess and review of the literature. *Eur J Clin Microbiol Infect Dis* 2007;**26**:1–11.
40. Lazow SK, Izzo SR, Vazquez D. Do dental infections really cause central nervous system infections? *Oral Maxillofac Surg Clin North Am* 2011;**23**:569–78, vii.
66. Prasad KN, Mishra AM, Gupta D, et al. Analysis of microbial etiology and mortality in patients with brain abscess. *J Infect* 2006;**53**:221–7.
70. Roche M, Humphreys H, Smyth E, et al. A twelve-year review of central nervous system bacterial abscesses; presentation and aetiology. *Clin Microbiol Infect* 2003;**9**:803–9.
73. Rush DE, Abdel-Haq N, Zhu JF, et al. Clindamycin versus Unasyn in the treatment of facial cellulitis of odontogenic origin in children. *Clin Pediatr (Phila)* 2007;**46**:154–9.
76. Seppanen L, Lemberg KK, Lauhio A, et al. Is dental treatment of an infected tooth a risk factor for locally invasive spread of infection? *J Oral Maxillofac Surg* 2011;**69**:986–93.
79. Sichizya K, Fieggen G, Taylor A, Peter J. Brain abscesses: the Groote Schuur experience, 1993-2003. *S Afr J Surg* 2005;**43**:79–82.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. American Academy of Pediatric Dentistry. Guideline on appropriate use of antibiotic therapy for pediatric dental patients. *Pediatr Dent* 2008;**30**(7 suppl):212–4.
2. American Academy of Pediatric Dentistry. Policy on intraoral and perioral piercing. *Pediatr Dent* 2008;**30**(7 suppl):56–7.
3. Balcerak RJ, Sisto JM, Bosack RC. Cervicofacial necrotizing fasciitis: report of three cases and literature review. *J Oral Maxillofac Surg* 1988;**46**:450–9.
4. Bartlett JG, Gorbach SL. Anaerobic infections of the head and neck. *Otolaryngol Clin North Am* 1976;**9**(3):655–78.
5. Bascones Martinez A, Aguirre Urizar JM, Bermejo Fenoll A, et al. Consensus statement on antimicrobial treatment of odontogenic bacterial infections. *Med Oral Patol Oral Cir Bucal* 2004;**9**:369–76; 3–9.
6. Benca PG, Mostofi R, Kuo PC. Proliferative periostitis (Garre's osteomyelitis). *Oral Surg Oral Med Oral Pathol* 1987;**63**:258–60.
7. Bombassaro AM, Wetmore SJ, John MA. *Clostridium difficile* colitis following antibiotic prophylaxis for dental procedures. *J Can Dent Assoc* 2001;**67**:20–2.
8. Brook I, Frazier EH, Gher ME. Aerobic and anaerobic microbiology of periapical abscess. *Oral Microbiol Immunol* 1991;**6**:123–5.
9. Busch DF. Anaerobes in infections of the head and neck and ear, nose, and throat. *Rev Infect Dis* 1984;**6**(suppl 1):S115–22.
10. Cachovan G, Boger RH, Giersdorf I, et al. Comparative efficacy and safety of moxifloxacin and clindamycin in the treatment of odontogenic abscesses and inflammatory infiltrates: a phase II, double-blind, randomized trial. *Antimicrob Agents Chemother* 2011; **55**:1142–7.
11. Carpenter J, Stapleton S, Holliman R. Retrospective analysis of 49 cases of brain abscess and review of the literature. *Eur J Clin Microbiol Infect Dis* 2007;**26**:1–11.
12. Carroll ST, Riffenburgh RH, Roberts TA, Myhre EB. Tattoos and body piercings as indicators of adolescent risk-taking behaviors. *Pediatrics* 2002;**109**:1021–7.
13. Cherry WR, Lee JY, Shugars DA, White RP, Jr, Vann WF, Jr. Antibiotic use for treating dental infections in children: a survey of dentists' prescribing practices. *J Am Dent Assoc* 2012;**143**:31–8.
14. Chow AW, Roser SM, Brady FA. Orofacial odontogenic infections. *Ann Intern Med* 1978;**88**:392–402.
15. Coykendall AL. Classification and identification of the viridans streptococci. *Clin Microbiol Rev* 1989;**2**:315–28.
16. Daramola JO. Massive osteomyelitis of the mandible complicating sickle cell disease: report of case. *J Oral Surg* 1981;**39**:144–6.
17. Dodson TB, Perrott DH, Kaban LB. Pediatric maxillofacial infections: a retrospective study of 113 patients. *J Oral Maxillofac Surg* 1989;**47**:327–30.
18. Eversole LR, Leider AS, Corwin JO, Karian BK. Proliferative periostitis of Garre: its differentiation from other neoperiostoses. *J Oral Surg* 1979;**37**:725–31.
19. Fisher AD. Osteomyelitis of the mandible in a child. *J Oral Surg* 1977;**35**:60–3.
20. Flynn TR. What are the antibiotics of choice for odontogenic infections, and how long should the treatment course last? *Oral Maxillofac Surg Clin North Am* 2011;**23**:519–36, v–vi.
21. Flynn TR, Shanti RM, Levi MH, et al. Severe odontogenic infections. I. Prospective report. *J Oral Maxillofac Surg* 2006;**64**: 1093–103.
22. Flynn TR, Stokes LM, Lee AM. Molecular microbiology of orofacial infections. *J Oral Maxillofac Surg* 2002;**60**(suppl 1):72–3.
23. Gilbert DN, Moellering RC, Eliopoulos GM, Sanle MA. *The Sanford guide to antimicrobial therapy*. Sperryville, Va: B.I. Publications; 2006.
24. Gilmore WC, Jacobus NV, Gorbach SL, et al. A prospective double-blind evaluation of penicillin versus clindamycin in the treatment of odontogenic infections. *J Oral Maxillofac Surg* 1988; **46**:1065–70.
25. Givner LB, Mason EO, Jr, Barson WJ, et al. Pneumococcal facial cellulitis in children. *Pediatrics* 2000;**106**:E61.
26. Goldberg MH, Topazian RG. Odontogenic infection and deep fascial space infection of the dental origin. In: Topazian RG, Goldberg MH, editors. *Oral and Maxillofacial Infections*, 3rd ed. Philadelphia: W.B. Saunders Co.; 1994.
27. Gomes BP, Jacinto RC, Montagner F, et al. Analysis of the antimicrobial susceptibility of anaerobic bacteria isolated from endodontic infections in Brazil during a period of nine years. *J Endod* 2011;**37**:1058–62.
28. Granite EL. Anatomic considerations in infections of the face and neck: review of the literature. *J Oral Surg* 1976;**34**:34–44.
29. Grodinsky M, Holyoke EA. The fasciae and fascial spaces of the head, neck and adjacent regions. *Am J Anat* 1938;**63**:367–408.
30. Gross SJ, Nieburg PI. Ludwig angina in childhood. *Am J Dis Child* 1977;**131**:291–2.
31. Hall HD, Gunter JW, Jr, Jamison HC, McCallum CA, Jr. Effect of time of extraction on resolution of odontogenic cellulitis. *J Am Dent Assoc* 1968;**77**:626–31.
32. Handal T, Olsen I. Antimicrobial resistance with focus on oral beta-lactamases. *Eur J Oral Sci* 2000;**108**:163–74.
33. Haug RH, Hoffman MJ, Indresano AT. An epidemiologic and anatomic survey of odontogenic infections. *J Oral Maxillofac Surg* 1991;**49**:976–80.
34. Heimdahl A, von Konow L, Satoh T, Nord CE. Clinical appearance of orofacial infections of odontogenic origin in relation to microbiological findings. *J Clin Microbiol* 1985;**22**:299–302.
35. Howe G. Orofacial infections and their management. In: Howe G, editor. *Minor Oral Surgery*. Bristol, John Wright & Sons; 1966.
36. Kaban LB, McGill T. Orbital cellulitis of dental origin: differential diagnosis and the use of computed tomography as a diagnostic aid. *J Oral Surg* 1980;**38**:682–5.
37. Katz MH, Mastrucci MT, Leggott PJ, et al. Prognostic significance of oral lesions in children with perinatally acquired human immunodeficiency virus infection. *Am J Dis Child* 1993;**147**:45–8.
38. Ketchem L, Berkowitz RJ, McIlveen L, et al. Oral findings in HIV-seropositive children. *Pediatr Dent* 1990;**12**:143–6.
39. Krogh HW. Extraction of teeth in the presence of acute infections. *J Oral Surg (Chic)* 1951;**9**:136–51.
40. Lazow SK, Izzo SR, Vazquez D. Do dental infections really cause central nervous system infections? *Oral Maxillofac Surg Clin North Am* 2011;**23**:569–78, vii.
41. Leggott PJ. Oral manifestations of HIV infection in children. *Oral Surg Oral Med Oral Pathol* 1992;**73**:187–92.
42. Lesco BA, Brownstein MP. Recognition of periodontal disease in children. *Pediatr Clin North Am* 1982;**29**:457–74.
43. Levi ME, Eusterman VD. Oral infections and antibiotic therapy. *Otolaryngol Clin North Am* 2011;**44**:57–78, v.
44. Levin L, Zadik Y. Oral piercing: complications and side effects. *Am J Dent* 2007;**20**:340–4.
45. Levitt GW. Cervical fascia and deep neck infections. *Otolaryngol Clin North Am* 1976;**9**:703–16.
46. Lewis MA, MacFarlane TW, McGowan DA. Quantitative bacteriology of acute dento-alveolar abscesses. *J Med Microbiol* 1986;**21**:101–4.
47. Lewis MA, Carmichael F, MacFarlane TW, Milligan SG. A randomised trial of co-amoxiclav (Augmentin) versus penicillin V in the treatment of acute dentoalveolar abscess. *Br Dent J* 1993;**175**: 169–74.
48. Lichty G, Langlais RP, Aufdemorte T. Garre's osteomyelitis: literature review and case report. *Oral Surg Oral Med Oral Pathol* 1980;**50**:309–13.
49. Magnusson BO, Matsson L, Modeen T. Gingivitis and periodontal disease in children. In: Magnusson BO, Koch B, Poulsen S, editors. *Periodontics: A Systematic Approach*. Copenhagen, Munksgaard: 1981.
50. Maniglia AJ, Goodwin WJ, Arnold JE, Ganz E. Intracranial abscesses secondary to nasal, sinus, and orbital infections in adults and children. *Arch Otolaryngol Head Neck Surg* 1989;**115**:1424–9.
51. Marchisio P, Principi N. Treatment of oropharyngeal candidiasis in HIV-infected children with oral fluconazole. Multicentre Study Group [corrected]. *Eur J Clin Microbiol Infect Dis* 1994;**13**:338–40.
52. Martinello RA, Cooney EL. Cerebellar brain abscess associated with tongue piercing. *Clin Infect Dis* 2003;**36**:e32–4.
53. Martis C, Karabouta I, Lazaridis N. Extractions of impacted mandibular wisdom teeth in the presence of acute infection. *Int J Oral Surg* 1978;**7**:541–8.
54. Martis CS, Karakasis DT. Extractions in the presence of acute infections. *J Dent Res* 1975;**54**:59–61.
55. Marx RE, Johnson RP, Kline SN. Prevention of osteoradionecrosis: a randomized prospective clinical trial of hyperbaric oxygen versus penicillin. *J Am Dent Assoc* 1985;**111**:49–54.
56. Massler M, Cohen A, Schour I. Epidemiology of gingivitis in children. *J Am Dent Assoc* 1952;**45**:319–24.

57. McCarthy C, Snyder ML, Parker RB. The indigenous oral flora of man. I. The newborn to the 1-year-old infant. *Arch Oral Biol* 1965;**10**:61–70.
58. Moenning JE, Nelson CL, Kohler RB. The microbiology and chemotherapy of odontogenic infections. *J Oral Maxillofac Surg* 1989;**47**:976–85.
59. Morhart RE, Fitzgerald RJ. Nutritional determinants of the ecology of the oral flora. *Dent Clin North Am* 1976;**20**:473–89.
60. Oberholzer TG, George R. Awareness of complications of oral piercing in a group of adolescents and young South African adults. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; **110**:744–7.
61. O'Sullivan EA, Duggal MS, Bailey CC, et al. Changes in the oral microflora during cytotoxic chemotherapy in children being treated for acute leukemia. *Oral Surg Oral Med Oral Pathol* 1993; **76**:161–8.
62. Peterson LJ. Microbiology of head and neck infections. *Oral Maxillofac Clin N Am* 1991;**3**:247–57.
63. Piecuch JE. Odontogenic infections. *Dent Clin North Am* 1982;**26**:129–45.
64. Poeschl PW, Crepaz V, Russmueller G, et al. Endodontic pathogens causing deep neck space infections: clinical impact of different sampling techniques and antibiotic susceptibility. *J Endod* 2011;**37**:1201–5.
65. Poeschl PW, Spusta L, Russmueller G, et al. Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; **110**:151–6.
66. Prasad KN, Mishra AM, Gupta D, et al. Analysis of microbial etiology and mortality in patients with brain abscess. *J Infect* 2006;**53**: 221–7.
67. Price CD, Hameroff SB, Richards RD. Cavernous sinus thrombosis and orbital cellulitis. *South Med J* 1971;**64**:1243–7.
68. Raborn GW, McGaw WT, Grace M, et al. Oral acyclovir and herpes labialis: a randomized, double-blind, placebo-controlled study. *J Am Dent Assoc* 1987;**115**:38–42.
69. Ramon Y, Oberman M, Horovitz I, Freedman A. Osteomyelitis of the maxilla in the newborn. *Int J Oral Surg* 1977;**6**:90–4.
70. Roche M, Humphreys H, Smyth E, et al. A twelve-year review of central nervous system bacterial abscesses; presentation and aetiology. *Clin Microbiol Infect* 2003;**9**:803–9.
71. Rogers AH. The oral cavity as a source of potential pathogens in focal infection. *Oral Surg Oral Med Oral Pathol* 1976;**42**:245–8.
72. Rud J. Removal of impacted lower third molars with acute pericoronitis and necrotising gingivitis. *Br J Oral Surg* 1970;**7**: 153–60.
73. Rush DE, Abdel-Haq N, Zhu JF, et al. Clindamycin versus Unasyn in the treatment of facial cellulitis of odontogenic origin in children. *Clin Pediatr (Phila)* 2007;**46**:154–9.
74. Sakamoto H, Kato H, Sato T, Sasaki J. Semiquantitative bacteriology of closed odontogenic abscesses. *Bull Tokyo Dent Coll* 1998;**39**: 103–7.
75. Schwaber MK, Pensak ML, Bartels LJ. The early signs and symptoms of neurotologic complications of chronic suppurative otitis media. *Laryngoscope* 1989;**99**:373–5.
76. Seppanen L, Lemberg KK, Lauhio A, et al. Is dental treatment of an infected tooth a risk factor for locally invasive spread of infection? *J Oral Maxillofac Surg* 2011;**69**:986–93.
77. Shah HN, Collins MD. Proposal for Reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, *and Bacteroides endodentalis* in a new genus, porphyromanas. *Int J Syst Bacteriol* 1988;**38**:128–31.
78. Shah HN, Collins DM. Prevotella, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int J Syst Bacteriol* 1990;**40**:205–8.
79. Sichizya K, Fieggen G, Taylor A, Peter J. Brain abscesses: the Groote Schuur experience, 1993-2003. *S Afr J Surg* 2005;**43**: 79–82.
80. Socransky SS, Manganiello SD. The oral microbiota of man from birth to senility. *J Periodontol* 1971;**42**:485–96.
81. Straus SE, Rooney JF, Sever JL, et al; National Institutes of Health Conference. Herpes simplex virus infection: biology, treatment, and prevention. *Ann Intern Med* 1985;**103**:404–19.
82. Summers GW. The diagnosis and management of dental infections. *Otolaryngol Clin North Am* 1976;**9**:717–28.
83. Sundqvist G. Taxonomy, ecology, and pathogenicity of the root canal flora. *Oral Surg Oral Med Oral Pathol* 1994;**78**:522–30.
84. Topazian RG. Uncommon infections of the oral and maxillofacial regions. In: Topazian RG, Goldberg MH, editors. *Oral and Maxillofacial Infections*, 3rd ed. Philadelphia: W.B. Saunders; 1994.
85. Valdez IH, Pizzo PA, Atkinson JC. Oral health of pediatric AIDS patients: a hospital-based study. *ASDC J Dent Child* 1994; **61**:114–8.
86. Vieira EP, Ribeiro AL, Pinheiro Jde J, Alves Sde M, Jr. Oral piercings: immediate and late complications. *J Oral Maxillofac Surg* 2011;**69**:3032–7.
87. Waisman D, Shupak A, Weisz G, Melamed Y. Hyperbaric oxygen therapy in the pediatric patient: the experience of the Israel Naval Medical Institute. *Pediatrics* 1998;**102**:E53.
88. Whitesides L, Cotto-Cumba C, Myers RA. Cervical necrotizing fasciitis of odontogenic origin: a case report and review of 12 cases. *J Oral Maxillofac Surg* 2000;**58**:144–51; discussion 52.
89. Williams AC. Ludwig's angina. *Surg Gynecol Obstet* 1940;**70**: 140–9.
90. Williams AC, Guralnick WC. The diagnosis and treatment of Ludwig's angina. *N Engl J Med* 1943;**228**:443–50.
91. Woods R. Pyogenic dental infections: a ten year review. *Aust Dent J* 1978;**23**:107–11.
92. Yu CH, Minnema BJ, Gold WL. Bacterial infections complicating tongue piercing. *Can J Infect Dis Med Microbiol* 2010;**21**:e70–4.

CHAPTER 9

Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis)

James D. Cherry

Pharyngitis is an inflammatory illness of the mucous membranes and underlying structures of the throat. The clinical diagnostic category includes tonsillitis, tonsillopharyngitis, and nasopharyngitis; inflammation also frequently involves the uvula and soft palate. Illness usually is acute but also may be subacute or chronic. Establishing the diagnosis of pharyngitis requires objective evidence of inflammation (erythema, exudate, or ulceration). The symptom of sore throat invariably accompanies pharyngitis, but it should not be used as the sole criterion; sore throat is a common complaint of children with colds in whom no objective evidence of pharyngeal inflammation is present.

Although the clinical finding of pharyngitis suggests an almost exclusive group A streptococcal cause to many physicians, etiologic considerations should include a multitude of viruses, bacteria, and other infectious and noninfectious agents. Etiologically, pharyngitis is subdivided into two categories: illness with nasal symptoms (nasopharyngitis) and illness without nasal involvement (pharyngitis or tonsillopharyngitis). In acute illness, nasopharyngitis nearly always is of viral origin whereas pharyngitis without nasal signs has diverse etiologic possibilities, including bacteria, viruses, fungi, and other unidentified causes. In this chapter, nasopharyngitis and pharyngitis without nasal involvement are considered separately.

HISTORY

Although throat inflammation undoubtedly has been a physical finding of disease throughout human existence, only in more recent years has attention been given to pharyngitis as a primary complaint. The throat findings of diphtheria were mentioned in the 3rd century AD,[188] and Vincent angina was noted in the military before the Christian era,[58] but group A streptococcal infection and pharyngitis were not clearly associated until World War II.[39,163] Although Glover and Griffith[76] mentioned streptococcal tonsillitis in 1931, the major reference to streptococci in the preantibiotic era was in association with scarlet fever, erysipelas, and suppurative processes.[31]

NASOPHARYNGITIS

Etiologic Agents

Etiologic agents of nasopharyngitis, categorized by type of lesion, frequency, season of occurrence, and duration of illness, are listed in Table 9-1. The relative importance of nasal and pharyngeal manifestations also is presented. Although Table 9-1 shows three bacterial agents and one rickettsia, most occurrences of nasopharyngitis are caused by viral infections. The specific infectious agents are discussed fully in their respective sections of this book; only an overview is presented here.

Adenoviruses are the most common cause of nasopharyngitis; types 1, 2, 3, 4, 5, 6, 7, 7a, 9, 14, and 15 account for most illnesses.* Nasopharyngitis also commonly occurs with influenza and parainfluenza viral infections.† Although rhinoviral, respiratory syncytial viral, human metapneumovirus, and human bocavirus infections are common occurrences in children and all always have nasal manifestations (coryza), the occurrence of objective pharyngeal manifestations is less common.‡ Respiratory symptoms with cough, nasal discharge, and pharyngitis frequently occur in children with rotaviral gastroenteritis.[83,115,165]

Epidemiology

Nasopharyngitis is a common illness of childhood. It tends to be most prevalent in young children, in association with primary infections with respiratory viruses. Nasal symptoms with enteroviruses occur less frequently in school-aged children than in preschool-age children. In contrast, older children rarely have pharyngitis with respiratory syncytial viral, parainfluenza viral, and rhinoviral infections. Nasopharyngitis caused by adenoviral infection is a particularly frequent occurrence in adolescents and young adults in military training.[42,174,194]

Nasopharyngitis occurs more commonly during the cold weather months (see Table 9-1). No apparent sex

*References 16, 29, 42, 44, 101, 135, 153, 174, 179, 194.
†References 11, 85, 94, 95, 101, 102, 141, 149-151, 160, 161, 169, 183.
‡References 7, 28, 52, 62, 89, 92-95, 101, 130, 149, 152, 172.

TABLE 9-1 **Etiologic Agents of Nasopharyngitis**

Etiologic Agent	*Type of Pharyngeal Lesion**			*Relative Importance of Nasal and Pharyngeal Symptoms†*		Frequency of Pharyngitis‡	Main Season	Duration of Pharyngitis
	Erythematous	Follicular	Exudative	Nasal	Pharyngeal			
Bacteria								
Corynebacterium diphtheriae[208]	+++		++++	+	+++	+	Fall, winter, spring	Acute, subacute
Haemophilus influenzae[198]	++			++	++	+	Fall, winter, spring	Acute, subacute
Neisseria meningitidis[175]	++			+	+++	+	Fall, winter, spring	Acute, subacute
Viruses								
Adenoviruses[16,29,42,44,49,135,153,174,179,194]	++++	++++	++	+	+++	++++	All seasons	Acute
Enteroviruses (poliovirus, coxsackieviruses A and B, echovirus)[32,41,105,106,135,170,171,199]	+++		+	+	+++	+++	Summer, fall	Acute
Influenza A and B[105,150,160,169,183]	+++			+	+++	++	Fall, winter	Acute
Parainfluenza[2,4,5,8,11,85,94,95,102,141,161,168,171,173,183]	++		+	+++	+	++	Fall, winter, spring	Acute
Respiratory syncytial[28,92,95,130,149]	++			+++	+	+	Fall, winter, spring	Acute
Rhinoviruses[89,105,152]	+			+++	+	+	Fall, winter, spring	Acute
Human metapneumovirus[7,172]	++			+++	+	+	Fall, winter, spring	Acute
Human bocavirus[52,62]	++			+++	+	+	Fall, winter, spring	Acute
Rotaviruses[84,115,130,165]	++			++	++	++	Fall, winter, spring	Acute
Rickettsia								
Coxiella burnetii[100]	++			++	++	+	All seasons	Acute

*Plus signs indicate the relative degree and severity of the lesion (++++, most marked; +, minimal).
†Each +, 25%.
‡++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; +, 1 to 25 percent.

predilection has been found. The method of transmission is similar to that of other respiratory viral infections (see Chapter 7).

Pathophysiology

The pathophysiology of nasopharyngitis is discussed in Chapter 7, in the pharyngitis section of this chapter, and in the chapters discussing the individual viral agents. In nasopharyngitis associated with *Haemophilus influenzae* and *Neisseria meningitidis*, the nasal symptoms may result from a concomitant respiratory viral infection.[30]

Clinical Presentation

Because nasopharyngitis is caused by many different etiologic agents, a reasonable expectation is varied clinical manifestations. These differences are highlighted in Table 9-1. Fever occurs in nearly all cases of nasopharyngitis. With adenoviral and influenza viral disease, the pharyngeal findings are most prominent; with the other respiratory viruses, coryza is more notable than are pharyngeal complaints. In adenoviral infections, follicular pharyngitis is the rule, and exudate is a common manifestation. In contrast, patients with the other respiratory viral infections usually present with pharyngeal erythema only. Nasopharyngitis of a viral etiology most often is an acute, self-limited disease lasting 4 to 10 days. Generally, adenoviral illnesses tend to be more prolonged than are illnesses resulting from the other respiratory viruses. Other symptoms in nasopharyngitis are related to the causative virus. Parainfluenza and respiratory syncytial viral infections also might have lower respiratory tract findings (laryngotracheitis, pneumonia, or bronchiolitis), and influenza might be associated with more severe, generalized complaints.

Although respiratory symptoms in association with rotaviral gastroenteritis have been noted frequently, little careful clinical study of the respiratory manifestations has been performed. Lewis and associates,[115] in a careful study, observed a statistically significant occurrence of nasal discharge, cough, and red throat in children with rotaviral diarrhea compared with children with diarrhea caused by other agents.

Nasopharyngitis with *H. influenzae* or *N. meningitidis* infections has been noted mainly in patients with septicemia and meningitis. The nasal symptoms (coryza) usually preceded the pharyngitis and severe systemic disease by a few to several days. In Q fever, the predominant finding is pneumonia. With diphtheria, the exudative pharyngitis and constitutional symptoms are most prominent.

PHARYNGITIS, TONSILLITIS, AND TONSILLOPHARYNGITIS

Etiologic Agents

Etiologic agents of pharyngitis categorized by type of lesion, frequency of occurrence, and duration of illness are presented in Table 9-2. Numerous diverse possibilities exist for the differential diagnosis of pharyngitis. The specific agents or factors are presented in their respective sections of this book, and only an overview is given here.

As with all infectious diseases, etiologic prevalence depends on multiple factors (status of the host, age, season, environment, exposure, and type of lesion) that must be considered in each individual case. In otherwise healthy children, the following infectious agents account for more than 90 percent of acute infections with pharyngeal involvement: *Streptococcus pyogenes;* adenoviruses; influenza viruses A and B; parainfluenza viruses 1, 2, and 3; Epstein-Barr virus; enteroviruses; and *Mycoplasma pneumoniae.*[*] Although group A streptococci are suggested frequently[33] as the only worthy bacterial consideration in the etiology of pharyngitis, the data in Table 9-2 indicate broader possibilities. When streptococci with beta-hemolysis recovered from children and adolescents with pharyngitis are typed, group B, C, and G strains occasionally are found.[†] Turner and associates[190] found that of the group C streptococci, only *Streptococcus equisimilis* caused pharyngitis; *Streptococcus anginosus* (formerly *S. milleri*) was part of the normal oropharyngeal flora.

Laboratory accidents have provided evidence that *H. influenzae* can cause pharyngitis,[148,197] and children with systemic illnesses caused by *H. influenzae* and *N. meningitidis* frequently have an associated marked pharyngitis.[173,198,203] *Arcanobacterium haemolyticum* and *Corynebacterium ulcerans* occasionally cause an illness mimicking diphtheria.[‡] *A. haemolyticum* also causes an illness that has been confused with streptococcal scarlet fever.[57,104,121,125]

Because anaerobic microorganisms are universal constituents of the normal throat flora, assigning etiologic significance to these agents in throat infections frequently is difficult. Vincent stomatitis and angina seem to result from mixed infections with anaerobes.[59,77,124,193] Brook and Gober[19] noted a significant association between encapsulated organisms of the *Bacteroides melaninogenicus* group *(Prevotella melaninogenica)* and acute tonsillitis in children. My impression is that acute and subacute infections with anaerobes account for numerous pharyngeal infections in adolescents in which cultures do not reveal group A streptococci and infectious mononucleosis test results are negative. Gonococcal and treponemal infections should be considered in sexually active or known exposed teenagers and other children.[25,40,60,98,202]

Adenoviral types 1, 2, 3, 4, 5, 6, 7, 7a, 9, 11, 14, 15, and 16 are the most common causes of pharyngitis in young children, and they are prominent etiologic agents in older children and adolescents.[§] Pharyngeal involvement frequently is overshadowed by other respiratory symptoms (e.g., cough, coryza) in parainfluenza viral infections and by systemic complaints (e.g., fever, exanthem, meningitis) in enteroviral infections.[¶] An

*References 2, 11, 16, 17, 32, 33, 41, 42, 46, 53, 75, 80, 85, 91, 94, 95, 102, 105, 106, 123, 134-136, 141, 149-151, 160-162, 168-171, 174, 177, 179, 180, 194, 195, 199, 201.
†References 9, 34, 36, 71, 90, 190, 191.
‡References 23, 82, 104, 108, 125, 154.
§References 16, 26, 42, 45, 135, 138, 153, 174, 179, 194.
¶References 2, 11, 32, 41, 85, 94, 95, 102, 106, 135, 141, 149, 151, 161, 168, 171, 177, 183, 199.

TABLE 9-2 **Etiologic Agents of Pharyngitis**

Etiologic Agent or Factor	*Type of Lesion**					Frequency of Occurrence†	Duration of Pharyngitis
	Erythematous	Follicular	Exudative	Ulcerative	Petechial		
Bacteria							
Streptococcus pyogenes[17,46,55,75,134,136,162,180,201]	++++	++	+++		+++	++++	Acute
Other streptococci (groups B, C, and G)[4,34-36,64,90,119,190,191]	+++	+	++			++	Acute
Brucella sp.[206]	+				+	+	Subacute
Corynebacterium diphtheriae[131,162,208]	+++		++++			+	Acute
Corynebacterium pyogenes[196]	++++		++++			+	Acute
Corynebacterium ulcerans[121,154,187]	++++		+++			+	Acute
Arcanobacterium haemolyticum[8,23,57,77,104,108,125]	++++	++	+++			+	Acute
Mixed anaerobes (*Prevotella* spp., *Peptostreptococcus, Fusobacterium* spp.)[18,19,24,53,77,124,193]	++++		+	++++		++	Subacute
Actinomyces spp.[59]	+			+		+	Chronic
Helicobacter pylori[3,103]	+					+	Chronic
Francisella tularensis[20,99,192,204]	++++		+++			+	Acute, subacute
Haemophilus influenzae[148,182,197,198,203]	++					++	Acute, subacute
Legionella pneumophila[139]	++++					+	Acute
Neisseria meningitidis[173]	++		+			++	Acute
Neisseria gonorrhoeae[98,202]	++		+			+	Acute, subacute, chronic
Leptospira spp.[96,155]	++++					+	Acute
Treponema pallidum[25,40,60,84]	+	+		+		+	Subacute
Borrelia spp.[112]	++++					+	Acute
Streptobacillus moniliformis[158]	+					+	Acute
Yersinia enterocolitica[164,184]	++++		++			+	Acute
Yersinia pseudotuberculosis[167]	+					+	Acute
Streptococcus pneumoniae[128]	+			+		+	Acute
Salmonella typhi[5,140]	+					+	Acute
Rothia dentocariosa[143]	+		+			+	Acute
Mycobacterium tuberculosis[37]				+		+	Chronic
Chlamydia							
Chlamydophila pneumoniae[51,81]	++++					++	Acute
Chlamydia trachomatis[142]	++	+	+			+	Acute, recurrent
Viruses							
Adenoviruses[16,44,135,138,153,174,194]	++++	++++	++			++++	Acute
Influenza A and B[150,160,169,183]	+++					+++	Acute
Parainfluenza[2,4,5,8,11,85,94,95,141,149,151,161,168,183]	++					+++	Acute

Continued on following page

TABLE 9-2 **Etiologic Agents of Pharyngitis** (Continued)

Etiologic Agent or Factor	*Type of Lesion**					Frequency of Occurrence†	Duration of Pharyngitis
	Erythematous	Follicular	Exudative	Ulcerative	Petechial		
Respiratory syncytial[28,92-95,130,149]	++					+	Acute
Enteroviruses (polio, coxsackieviruses A and B, echovirus)[2,32,97,106,170,171,177,199,200]	+++		+	++		+++	Acute
Epstein-Barr virus[91,123,195]	+++	+	++++		++	+++	Acute, subacute
Reoviruses[114,207]	++					+	Acute
Cytomegalovirus[15,110]	+					+	Acute
Herpes simplex[27,31,53,135,200]	++		++	++++		++	Acute
Measles[30]	+++				+	++	Acute
Rubella[30,61]					++	+	Acute
Rhinoviruses[89]	+					+	Acute
HIV[10]	++						Acute
Mycoplasma							
Mycoplasma pneumoniae[33,51,75,80,95]	++	+	+			++	Acute
Mycoplasma hominis[137]	+		+			+	Acute
Rickettsia							
Coxiella burnetii[49,100,145]	++					+	Acute
Fungi							
Candida spp.[113,175]	+		++++			+++	Acute, subacute, chronic
Parasites							
Toxoplasma gondii[111]	+					+	Acute
Recognized Illnesses of Uncertain Etiology							
Aphthous stomatitis[78,165]	+			++++		++	Acute, recurrent
PFAPA[55,64,97,99,122,129,144,186]	++		++	++		+	Acute, recurrent
Behçet syndrome[127]	+			++++		+	Chronic, recurrent
Kawasaki disease[87,133]	++	+				+	Acute
Stevens-Johnson syndrome[30]	+		+	++++		+	Acute
Illness in which Host Factors or Therapeutic Agents are Primary Causes							
Neutropenia, other immunodeficiencies, cancer, chemotherapeutic agents, generalized neoplastic disease[30]	+			++++		++	Chronic

*Plus signs indicate the relative degree and severity of the lesion (++++, most marked; +, minimal).
†++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; +, 1 to 25 percent.
HIV, human immunodeficiency virus; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, and adenitis (syndrome).

enteroviral etiology should be suspected when small ulcerative lesions are noted that involve the soft palate and uvula and the posterior pharyngeal wall (see Chapter 165). Infection with Epstein-Barr virus causes infectious mononucleosis with pharyngeal involvement similar to that resulting from group A streptococcal infection.[91,195] The clinical manifestations of Epstein-Barr virus are age-related; young children rarely have marked pharyngeal involvement.

Acquired cytomegalovirus infection causes an infectious mononucleosis–like syndrome, but pharyngitis occurs less commonly than it does in Epstein-Barr virus mononucleosis.[110] Primary and recurrent herpes simplex virus infections occasionally have pharyngeal manifestations.[27,31,53,135] Many illnesses that are ascribed clinically to herpes simplex virus actually are misidentified instances of aphthous stomatitis, however. Virtually all instances of herpes simplex virus infections with pharyngitis also reveal lesions in the anterior mouth and externally around the mouth. Although Koplik spots are known universally as the enanthem of measles, many physicians are unaware of the diffuse nature of the associated measles pharyngitis.[30] Primary infection with human immunodeficiency virus may cause the acute retroviral syndrome with fever, nonexudative pharyngitis, lymphadenopathy, exanthem, arthralgia, myalgia, and lethargy.[10]

The role of *Chlamydia* in the cause of pharyngitis is unclear. Grayston and associates[79,81] noted the occurrence of pharyngitis in adolescents and young adults infected with *Chlamydophila pneumoniae* (formerly *Chlamydia pneumoniae*). In a study in children, IgM antibody to *Chlamydia trachomatis* was found in children with pharyngitis.[86] Because cross-reactivity between *C. trachomatis* and *C. pneumoniae* exists, the illnesses studied were likely to be caused by *C. pneumoniae*, rather than by *C. trachomatis*. Ogawa and colleagues[142] noted prolonged and recurrent tonsillitis in association with sexually transmitted *Chlamydia trachomatis*.

Mild pharyngitis occurs in young children with *M. pneumoniae* infections; in older children, pharyngeal involvement is more pronounced.[33,75,80,95] In young adult volunteers, *Mycoplasma hominis* was noted to cause pharyngitis.[120] Although lower respiratory tract findings and systemic complaints are most marked in Q fever, moderate subjective and mild objective evidence of pharyngitis also is noted.[50,100,145] Exudative pharyngeal involvement with *Candida* spp. commonly occurs.[113,175] *Candida* infection most commonly occurs in children whose normal throat flora has been disrupted and in children who have a compromised immunologic response.

Recurrent aphthous stomatitis usually involves the anterior oral cavity, but occasionally is noted with extensive pharyngeal and soft palate lesions.[78,113,166,175] Although L-forms (spheroplasts) of *Streptococcus sanguinis* can be recovered consistently from the lesions of this disease,[78,113,166,175] their role in the etiology is unclear.

In Behçet syndrome, the ulcerative lesions are subacute or chronic and usually are not associated with surrounding pharyngeal inflammation.[127] In Kawasaki disease, the pharyngeal mucosa is deeply erythematous.[133]

Pharyngeal involvement is a common occurrence in noninfectious illnesses in which host resistance has been altered. The lesions usually are ulcerative, but secondary bacterial or fungal overgrowth can lead to marked erythematous and exudative findings.

Epidemiology

Pharyngitis is a common occurrence in children and, as noted in Table 9-2, is the result of many different infectious agents. Generally, bacterial pharyngitis occurs more commonly in the cold weather seasons; an enteroviral etiology is most common in the summer and fall. In younger children, viral pharyngitis tends to occur more frequently than does bacterial disease.[45] It has no apparent sex predilection.

Most diseases associated with pharyngitis require close person-to-person contact for spread. Pathogens are transmitted directly by close-range airborne dissemination or indirectly by the hands of the future host. Several food-borne outbreaks of streptococcal pharyngitis have been reported.[54,63] Prechewing of food by adults also may be a cause of streptococcal pharyngitis in infants.[178]

Pathophysiology

The pathology and pathophysiology of diseases in which pharyngitis is prominent are presented in the chapters of this book on specific infectious agents (in particular, see Chapters 82, 155, and 165).

Clinical Presentation

General

The clinical findings in pharyngitis are highlighted in Table 9-2. Manifestations related to individual pathogens are detailed in the chapters of this book dealing with the specific agents. The onset of pharyngitis usually is sudden and accompanied by fever and the complaint of sore throat. Frequently, parents observe that the child's breath is not normal and that the throat and particularly the tonsils are red. Other initial complaints include headache, nausea, vomiting, and sometimes abdominal pain. Anorexia is the rule, as is some degree of lessened activity. Parents also report frequently that the child's cervical lymph nodes are enlarged and tender.

Physical examination usually substantiates the parents' observations—the child is febrile with moderate to severe pharyngeal erythema and some degree of cervical adenitis. As noted in Table 9-2, the pharyngeal response varies. With acute common infections, the basic lesion is erythema. Associated with erythema can be follicular, ulcerative, and petechial lesions and generalized or circumscribed exudative areas. Follicular lesions are most characteristic of adenoviral infections, whereas exudative lesions occur most commonly in group A streptococcal infections and in infectious mononucleosis. Meland and associates[132] noted that the absence of cough and the presence of swollen lymph nodes had the highest specificity in predicting a streptococcal cause of pharyngitis.

Ulcerative lesions are observed most frequently in enteroviral infections (see Chapter 165). Petechiae on the soft palate frequently are seen in group A streptococcal infections, but also commonly occur in infectious mononucleosis, measles, and rubella.

Occurrences of pharyngitis in children almost always are acute, self-limited diseases. Pharyngitis of viral origin lasts 4 to 10 days, and pharyngitis caused by group A streptococci, if untreated, lasts slightly longer. Subacute and chronic pharyngeal disease is an uncommon occurrence in children, but the etiologic possibilities are numerous (see Table 9-2).

Periodic Fever, Aphthous Stomatitis, Pharyngitis, and Adenitis (PFAPA)

In 1987, Marshall and colleagues[129] described a new syndrome, periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA), and it subsequently has been reported on by many others.* In 1999, Thomas and associates[186] presented an analysis of data in their PFAPA registry of 94 cases. The male-to-female ratio was 1.8:1, and the mean age of PFAPA onset was 2.8 years (95% confidence interval [CI] 2.4 to 3.3). The children had an average 11.5 febrile episodes per year, and the average symptom-free interval between episodes was 28.2 days (99% CI 26 to 30.4 days). The average duration of an episode was 4.8 days, with the temperature being greater than 38.3° C (101 °F) for 3.8 days. The average maximum temperature per episode was 40.5° C (104.9 °F) (95% CI 40.4 to 40.6° C [104.7 to 105 °F]). Along with the periodic fevers, the children also experienced aphthous stomatitis (67%), pharyngitis (65%), cervical lymphadenopathy (77%), chills (80%), headache (65%), abdominal pain (45%), nausea (52%), diarrhea (30%), cough (20%), coryza (18%), and rash (15%). In addition to fever, 97 percent had one or more of the following: aphthous stomatitis, pharyngitis, or adenopathy. The mean leukocyte count was 13,000 with 62 percent polymorphonuclear leukocytes, and the mean erythrocyte sedimentation rate was 41 mm/hr.

More recently, Feder and Salazar[56] reported their review of 105 patients that they saw between 1998 and 2007. In this group there were 62% males. The median age at onset was 30 months (range 3 to 144 months); the mean duration of febrile episodes was 4.1 days (range 2 to 7 days); and the mean interval between episodes was 29.8 days (range 14 to 50 days). It should be noted that with any single episode of recurring fever that the patients often do not have all three of the syndrome manifestations (i.e., aphthous stomatitis, pharyngitis, and cervical adenitis).

In the Feder and Salazar[56] series the maximum temperatures ranged from 39.2° (102.5 °F) to 42.1°C (107.8 °F) and other symptoms included headache, 44%; vomiting, 27%; and mild abdominal pain, 41%. The patients were said to be listless but not toxic. The parents of 46% of the children reported that their children with PFAPA were less likely to get respiratory and gastrointestinal viral illnesses that were circulating in their families and communities. Sixty-two percent of the children had a prodrome (fatigue, headache, abdominal pain, or irritability) that began 20 hours (mean) before the onset of fever. Patients in this series had normal white blood cell counts and erythrocyte sedimentation rates when afebrile. With illness the white blood cell count tended to be slightly elevated with a preponderance of neutrophils. At the onset of illness the C-reactive protein value was elevated.

Cochard and colleagues[38] studied the familial relationships in PFAPA syndrome. They noted a positive family history for recurrent fever in 38 of 84 (45%) of children with PFAPA. The affected family member was a sibling or parent in 29 of 38 (76%) of the cases.

The etiology of the PFAPA syndrome is unknown. In extensive studies by Stojanov and associates[181] the following were noted in PFAPA attacks: complement (C1QB, C2, SERPIN GI), interleukin-1 (IL)–related (IL-1B, IL-1RN, CASP1, IL18RAP), and interferon-induced (AIM2, IP-10/CXCL 10) genes were overexpressed, and T-cell–associated transcripts (CD3, CD8B) were downregulated. During attacks there were increased serum levels of chemokines for activated T cells (IP-10/CXCL 10, MIG/CXCL 9), granulocyte colony-stimulating factor, and proinflammatory cytokines (IL-18, IL-6). A relative lymphopenia was noted.

In another study, Brown and colleagues[20] noted increased monocytes and decreased eosinophils during attacks and increased thrombocytes during the afebrile period.

Wurster and associates[205] carried out a long-term follow-up study of 59 patients in a PFAPA registry. The follow-up time ranged between 12 and 21 years. Of this group 50 (85%) had complete resolution. The mean duration of the syndrome was 6.3 years (95% CI, 5.4 to 7.3) and none of these patients had sequelae.

Differential Diagnosis

As noted in Table 9-2, the differential diagnoses in pharyngitis are numerous, as in nasopharyngitis (see Table 9-1). Although considerable overlap exists in the spectrum of illness in pharyngeal infections, many clues help in ruling in or out certain diagnostic possibilities. The diagnosis of pharyngitis requires carefully eliciting epidemiologic and other historical data (i.e., exposure, season, incubation period, age of patient, associated clinical findings), in addition to observing pharyngeal physical findings.

Most acute instances of nasopharyngitis are of viral origin (see Table 9-1), with adenoviruses accounting for the greatest number of cases. Nasopharyngitis also occurs during epidemic influenza A and B and parainfluenza 1 and 2 infections and with sporadic parainfluenza 3 infections. In all cases, proper epidemiologic and historical data should be elicited so that early diphtheria and other unusual but treatable illnesses are not diagnosed incorrectly. Retrospective study has indicated that nasopharyngitis occasionally occurs in severe infections from *H. influenzae* and meningococci, so the possible presence of

*References 1, 20, 22, 55, 56, 64, 67, 97, 99, 118, 122, 144, 181, 186, 205.

these infections should be considered when the epidemiology suggests it.

Although most cases of pharyngitis without nasal symptoms are caused by viral infections, numerous other etiologic possibilities exist. The approach taken frequently by many physicians is to consider all pharyngitis as being of bacterial origin and to treat it with antibiotics. The initial consideration in a child with pharyngitis should be the duration of illness. Subacute, chronic, and recurrent illnesses (see Table 9-2) generally suggest more unusual problems and require a more deliberate approach. In all instances of acute pharyngitis, ruling out streptococcal disease is mandatory.

PFAPA syndrome must be differentiated from monogenic periodic fevers, which are caused by mutations of genes involved in regulation of the inflammatory response.[67] These illnesses include familial Mediterranean fever, tumor necrosis factor receptor–associated periodic syndrome, and mevalonate kinase deficiency.

Specific Diagnosis

The epidemiologic history and careful clinical categorization are the most important aspects of specific diagnosis. The most pressing diagnostic need in upper respiratory tract infections in everyday pediatric practice is distinguishing bacterial from viral disease—who and who not to treat with antibiotics.

A child with the common cold, herpangina, or pharyngoconjunctival fever has a viral disease and does not need therapy with antibiotics. In most instances, when these clinical diagnoses are apparent, performing throat cultures for bacterial pathogens is unnecessary. A child with severe acute pharyngitis with exudate and fever and cervical lymphadenitis also is treated easily because most of these children have disease resulting from group A streptococci.

Because young children usually cry when examined by the physician, the clinical differentiation between pharyngitis/tonsillitis without nasal symptoms and nasopharyngitis is difficult. Therefore, on clinical grounds, there is no certain way to make an etiologic diagnosis and specifically to rule out infection caused by *S. pyogenes*. In the past, the usually recommended approach to management of pharyngitis and nasopharyngitis was to obtain a throat culture to determine whether group A streptococci were present. Because several rapid tests (rapid antigen detection tests) for the detection of group A streptococci are available, establishing the immediate diagnosis and providing treatment of streptococcal pharyngitis are possible in the practice setting.* Generally, these rapid tests have high specificity, but sensitivity is suboptimal. If early treatment is desirable today, a reasonable approach to establishing the diagnosis of streptococcal pharyngitis is to do a rapid test. If the test result is positive, specific therapy is instituted. In the case of a negative rapid test result, a routine throat culture is done, and therapy is withheld pending the culture results.[13,48,156]

A common approach is to obtain two throat swabs at the time the child is examined and send both to the laboratory. The laboratory can then run the RADT and only carry out the culture if the rapid test is negative for group A streptococci.

A question exists regarding whether early treatment of streptococcal pharyngitis is desirable.[49,69,70,156,157] In the public clinic, where following up children with positive throat cultures often is difficult, providing immediate diagnosis and treatment is important. As Pichichero and associates[156,157] and El-Daher and colleagues[49] have shown, early treatment may result in a decreased desirable antibody response, allowing reinfection with type-specific organisms to occur. In settings in which the communication between physicians and parents is satisfactory, an advisable approach in many instances is to withhold the institution of treatment for 1 or 2 days. Gerber and associates[49,69,70] argued against this approach because providing immediate therapy can reduce the risk of transmission of infection, and they challenged the interpretation of the findings of Pichichero and associates[72,156,157] and El-Daher and colleagues.[49]

Cultures for other pathogens should be reserved for unusual situations, such as persistent symptoms, indicative epidemiology, or other pertinent historical data. Because *H. influenzae* and *Streptococcus pneumoniae* frequently are part of the normal flora, their isolation is not always etiologically significant. If cultures reveal predominant growths of either *H. influenzae* or *S. pneumoniae*, however, they are likely contributing to the disease process, and antibiotic therapy directed at the specific pathogen would benefit the patient.[31,128,203] When the possibility of disease because of anaerobic agents exists, a Gram-stained smear from an exudative area may be rewarding. When the pharyngeal findings are unique, or when many cases of a similar illness are observed, a viral culture from the throat is indicated.

Treatment

The specific treatments of diseases with nasopharyngitis and pharyngitis are discussed in chapters of this book that deal with the individual pathogens. That antimicrobial agents are prescribed inappropriately in children with sore throat and pharyngitis is common knowledge. Based on the etiologic agents noted in Tables 9-1 and 9-2, it is apparent that many infectious agents are associated with nasopharyngitis and pharyngitis, which can be treated effectively with antimicrobial agents. Except in rare specific circumstances, the only common pathogens that require treatment are group A streptococci. The treatment of streptococcal pharyngitis is discussed in detail in Chapter 82. A study noted that physicians prescribed antibiotics to 53 percent of children with sore throat, which is far greater than the 15 to 36 percent likelihood of a group A streptococcal infection.[120] This study also noted that this rate of antimicrobial usage is an improvement from 1995, when the prescribing rate was 66 percent.

Although a multitude of proprietary remedies are available for respiratory tract infections, and sore throat specifically, none has a place in the care of pediatric

*References 6, 10, 12, 13, 43, 47, 48, 65, 68, 72-74, 93, 109, 117, 126, 157, 159, 176, 185, 189, 201.

patients. Particularly to be condemned are throat lozenges that contain numerous useless ingredients, many potentially harmful. Antibiotic-containing lozenges particularly are to be condemned because they may allow streptococcal disease to go unrecognized. Antiseptic mouthwashes have no value, and decongestants and antihistamines have no proven efficacy and frequently lead to troublesome side effects.

Corticosteroids have been used for pain relief in acute pharyngitis, and although they appear to be modestly effective, I believe that this is a bad practice and therefore should be discouraged.[88,107,206]

Because children with pharyngitis frequently feel ill, therapy with an analgesic is reasonable. Formerly, aspirin was the analgesic usually recommended. However, because aspirin is an etiologic factor in influenza-associated Reye syndrome and because differentiating influenza viral infections from other respiratory viral infections is difficult clinically, using acetaminophen rather than aspirin is prudent. The dose per single administration of acetaminophen by year of age is as follows: younger than 1 year, 60 mg; 1 to 3 years, 60 to 120 mg; 3 to 6 years, 120 mg; 6 to 12 years, 150 to 300 mg; older than 12 years, 325 to 650 mg. Administration may be repeated three or four times daily in young children and every 4 hours in older children. Acetaminophen rarely should be given to infants younger than 6 months old. In young children, careful attention to adequate hydration is particularly necessary.

Children with PFAPA syndrome have been treated with corticosteroids, cimetidine, tonsillectomy, and colchicine.* Most often used is prednisolone 1 mg/kg in a single dose.[56] Some patients received one or two additional doses at 12-hour intervals. This treatment is highly effective in reducing the duration of the febrile episode. However, this treatment tends to shorten the interval between febrile episodes by 7 to 14 days. Feder and Salazar[56] note that in their experience with prednisolone treatment the initially shortened cycles lengthened over time.

Complications

The main bacterial complications associated with tonsillitis/pharyngitis are retropharyngeal and peritonsillar abscesses and Lemierre syndrome (see Chapter 11). Nonbacterial complications include rheumatic fever, acute glomerulonephritis, and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (see Chapters 29 and 82).

Prognosis

Almost all occurrences of nasopharyngitis and pharyngitis are self-limited, and the overall prognosis is excellent. Keeping a constant vigil for streptococcal and other more serious diseases is necessary, however. Failure to diagnose and to treat group A streptococcal infections, syphilis, and other more unusual infections can lead to serious short-term and long-term difficulties.

*References 21, 56, 66, 88, 107, 116, 129, 186, 206.

Prevention

Because pharyngitis and nasopharyngitis are caused by infections with many different respiratory pathogens, no practical specific approach to prevention exists. Occasionally, streptococcal disease can be prevented by the judicious use of prophylactic penicillin. For young children or others with undue susceptibility to serious disease with common respiratory pathogens, reducing contact situations (e.g., daycare centers) is prudent. In the 1980s, Paradise and colleagues[146] studied the effect of tonsillectomy on the rate of recurrent throat infections in severely affected children. In this controlled study, children needed to have a history of seven or more episodes of tonsillitis in the preceding year, or five or more episodes in each of 2 preceding years, or three or more episodes in the 3 preceding years. The findings in this study indicated that children in the surgical group had significantly fewer throat infections than occurred in the nonsurgical control group during the ensuing 2 years of follow-up. In a more recent but similar study with less stringent criterion for surgery, Paradise and colleagues[147] found that tonsillectomy or adenotonsillectomy conferred a modest benefit. They concluded, however, that the modest benefit of tonsillectomy or adenotonsillectomy in children with recurrent throat infections does not justify the risks, morbidity, and cost of the operations.

Blakley and Magit[14] reviewed four randomized controlled trials in which tonsillectomy was favored over medical therapy in reducing pharyngitis and found in only one of these trials that the result was statistically significant. Their meta-analysis suggested that tonsillectomy resulted in a 43% reduction in the incidence of pharyngitis.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Adachi M, Watanabe A, Nishiyama A, et al. Familial cases of periodic fever with aphthous stomatitis, pharyngitis, and cervical adenitis syndrome. *J Pediatr* 2011;**158**:155–9.
3. Aladag I, Bulut Y, Guven M, et al. Seroprevalence of *Helicobacter pylori* infection in patients with chronic nonspecific pharyngitis: preliminary study. *J Laryngol Otol* 2008;**122**:61–4.
6. Ayanruoh S, Waseem M, Quee F, et al. Impact of rapid streptococcal test on antibiotic use in a pediatric emergency department. *Pediatr Emerg Care* 2009;**25**:748–50.
7. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008;**167**:63–9.
14. Blakley BW, Magit AE. The role of tonsillectomy in reducing recurrent pharyngitis: a systematic review. *Otolaryngol Head Neck Surg* 2009;**140**:291–7.
20. Brown KL, Wekell P, Osla V, et al. Profile of blood cells and inflammatory mediators in periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome. *BMC Pediatr* 2010;**10**:65.
21. Burton MJ, Pollard AJ, Ramsden JD. Tonsillectomy for periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPA). *Cochrane Database Syst Rev* CD008669, 2010.
22. Caorsi R, Pelagatti MA, Federici S, et al. Periodic fever, apthous stomatitis, pharyngitis and adenitis syndrome. *Curr Opin Rheumatol* 2010;**22**:579–84.
38. Cochard M, Clet J, Le L, et al. PFAPA syndrome is not a sporadic disease. *Rheumatology (Oxford)* 2010;**49**:1984–7.
52. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**:1337–42.

56. Feder HM, Salazar JC. A clinical review of 105 patients with PFAPA (a periodic fever syndrome). *Acta Paediatr* 2010;**99**:178–84.
66. Garavello W, Pignataro L, Gaini L, et al. Tonsillectomy in children with periodic fever with aphthous stomatitis, pharyngitis, and adenitis syndrome. *J Pediatr* 2011;**159**:138–42.
67. Gattorno M, Caorsi R, Meini A, et al. Differentiating PFAPA syndrome from monogenic periodic fevers. *Pediatrics* 2009;**124**:e721–8.
74. Giraldez-Garcia C, Rubio B, Gallegos-Braun JF, et al. Diagnosis and management of acute pharyngitis in a paediatric population: a cost-effectiveness analysis. *Eur J Pediatr* 2011;**170**:1059–67.
88. Hayward G, Thompson M, Heneghan C, et al. Corticosteroids for pain relief in sore throat: systematic review and meta-analysis. *BMJ* 2009;**339**:b2976.
103. Kaptan ZK, Emir H, Uzunkulaoglu H, et al. Determination of *Helicobacter pylori* in patients with chronic nonspecific pharyngitis. *Laryngoscope* 2009;**119**:1479–83.
107. Korb K, Scherer M, Chenot JF. Steroids as adjuvant therapy for acute pharyngitis in ambulatory patients: a systematic review. *Ann Fam Med* 2010;**8**:58–63.
116. Licameli G, Jeffrey J, Luz J, et al. Effect of adenotonsillectomy in PFAPA syndrome. *Arch Otolaryngol Head Neck Surg* 2008;**134**:136–40.
118. Lin CM, Wang CC, Lai CC, et al. Genital ulcers as an unusual sign of periodic fever, aphthous stomatitis, pharyngotonsillitis, cervical adenopathy syndrome: a novel symptom? *Pediatr Dermatol* 2011;**28**:290–4.
123. Luzuriaga K, Sullivan JL. Infectious mononucleosis. *N Engl J Med* 2010;**362**:1993–2000.
126. Maltezou HC, Tsagris V, Antoniadou A, et al. Evaluation of a rapid antigen detection test in the diagnosis of streptococcal pharyngitis in children and its impact on antibiotic prescription. *J Antimicrob Chemother* 2008;**62**:1407–12.
152. Peltola V, Jartti T, Putto-Laurila A, et al. Rhinovirus infections in children: a retrospective and prospective hospital-based study. *J Med Virol* 2009;**81**:1831–8.
159. Pockett CR, Thompson GC. Adherence of families to a group A streptococcal pharyngitis protocol used in a pediatric emergency department. *Pediatr Emerg Care* 2011;**27**:374–8.
176. Spellerberg B, Brandt C. Streptococcus. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology, vol. 1. 10th ed.* Washington, DC: ASM Press; 2011. p. 331–49.
181. Stojanov S, Lapidus S, Chitkara P, et al. Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and Th1 activation responsive to IL-1 blockade. *Proc Natl Acad Sci U S A* 2011;**108**:7148–53.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 10

Uvulitis

Ellen R. Wald

Infections of the uvula have been reported infrequently in the medical literature. When the uvula is the most inflamed structure in the posterior pharynx of a febrile child, acute infection should be suspected. Other causes of uvulitis include trauma (from instrumentation), inhalant irritation (from cannabis use), vasculitis, and allergy.[1,3-5,9,13]

ETIOLOGY

The main bacterial agents that cause uvulitis in children include *Haemophilus influenzae* type b and *Streptococcus pyogenes*.[8] Uvulitis caused by *H. influenzae* may occur concurrently with epiglottitis or as an isolated infection.[10,16] Uvulitis caused by *S. pyogenes* seems always to occur in concert with pharyngitis. Brook[2] reported two cases of uvulitis caused by anaerobic bacteria (*Fusobacterium nucleatum* and *Prevotella intermedia*). No search for viral agents has been conducted. Several cases of uvulitis caused by *Candida albicans* have been described in immunocompetent toddlers.[9] In adults, *Streptococcus pneumoniae* and *H. influenzae* have been reported to cause uvulitis.[6,15] In many patients, an associated epiglottitis has been present.[12,15]

EPIDEMIOLOGY

The epidemiology of uvulitis is the epidemiology of its two etiologic agents, *S. pyogenes* and *H. influenzae* type b. It occurs in school-aged children 5 to 15 years old (the so-called streptococcal age group) in association with pharyngitis. Similarly, it can be seen in the *H. influenzae* age group (3 months to 5 years) if a child has not received the now routine and universally recommended conjugate vaccine to prevent infections caused by *H. influenzae* type b. Cases of uvulitis in association with epiglottitis have been reported in the United States and in England.[2,8,14] Infections caused by *S. pyogenes* and *H. influenzae* occur

REFERENCES

1. Adachi M, Watanabe A, Nishiyama A, et al. Familial cases of periodic fever with aphthous stomatitis, pharyngitis, and cervical adenitis syndrome. *J Pediatr* 2011;**158**:155–9.
2. Ager EA, Felsenstein WC, Alexander ER, et al. An epidemic of illness due to coxsackie virus group B, type 2. *JAMA* 1964;**187**: 251–6.
3. Aladag I, Bulut Y, Guven M, et al. Seroprevalence of *Helicobacter pylori* infection in patients with chronic nonspecific pharyngitis: preliminary study. *J Laryngol Otol* 2008;**122**:61–4.
4. Arditi M, Shulman ST, Davis AT, et al. Group C beta-hemolytic streptococcal infections in children: nine pediatric cases and review. *Rev Infect Dis* 1989;**11**:34–45.
5. Ash I, McKendrick GD, Robertson MH, et al. Outbreak of typhoid fever connected with corned beef. *BMJ* 1964;**1**:1474–8.
6. Ayanruoh S, Waseem M, Quee F, et al. Impact of rapid streptococcal test on antibiotic use in a pediatric emergency department. *Pediatr Emerg Care* 2009;**25**:748–50.
7. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008;**167**:63–9.
8. Banck G, Nyman M. Tonsillitis and rash associated with *Corynebacterium haemolyticum*. *J Infect Dis* 1986;**154**:1037–40.
9. Benjamin JT, Perriello VA, Jr. Pharyngitis due to group C hemolytic streptococci in children. *J Pediatr* 1976;**89**:254–6.
10. Bisno AL. Acute pharyngitis. *N Engl J Med* 2001;**344**:205–11.
11. Bisno AL, Barratt NP, Swanston WH, et al. An outbreak of acute respiratory disease in Trinidad associated with para-influenza viruses. *Am J Epidemiol* 1970;**91**:68–77.
12. Bisno AL, Gerber MA, Gwaltney JM, Jr, et al. Diagnosis and management of group A streptococcal pharyngitis: a practice guideline. Infectious Diseases Society of America. *Clin Infect Dis* 1997;**25**:574–83.
13. Bisno AL, Gerber MA, Gwaltney JM, Jr, et al. Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. Infectious Diseases Society of America. *Clin Infect Dis* 2005;**35**:113–25.
14. Blakley BW, Magit AE. The role of tonsillectomy in reducing recurrent pharyngitis: a systematic review. *Otolaryngol Head Neck Surg* 2009;**140**:291–7.
15. Bonkowsky HL, Lee RV, Klatskin G. Acute granulomatous hepatitis: occurrence in cytomegalovirus mononucleosis. *JAMA* 1975;**233**:1284–8.
16. Brandt CD, Kim HW, Vargosko AJ, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am J Epidemiol* 1969;**90**:484–500.
17. Breese BB. Streptococcal pharyngitis and scarlet fever. *Am J Dis Child* 1978;**132**:612–6.
18. Brook I. The role of anaerobic bacteria in tonsillitis. *Int J Pediatr Otorhinolaryngol* 2005;**69**:9–19.
19. Brook I, Gober AE. *Bacteroides melaninogenicus:* its recovery from tonsils of children with acute tonsillitis. *Arch Otolaryngol* 1983;**109**:818–20.
20. Brown KL, Wekell P, Osla V, et al. Profile of blood cells and inflammatory mediators in periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA) syndrome. *BMC Pediatr* 2010; **10**:65.
21. Burton MJ, Pollard AJ, Ramsden JD. Tonsillectomy for periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome (PFAPA). *Cochrane Database Syst Rev* CD008669, 2010.
22. Caorsi R, Pelagatti MA, Federici S, et al. Periodic fever, apthous stomatitis, pharyngitis and adenitis syndrome. *Curr Opin Rheumatol* 2010;**22**:579–84.
23. Carlson P, Kontianinen S, Renkonen OV, et al. *Arcanobacterium haemolyticum* and streptococcal pharyngitis in army conscripts. *Scand J Infect Dis* 1995;**27**:17–8.
24. Carrie S, Fenton PA. Necrobacillosis—an unusual case of pharyngotonsillitis. *J Laryngol Otol* 1994;**108**:1097–8.
25. Catalano PM, Schragger AH. Early and latent syphilis: current management. *Arch Dermatol* 1965;**92**:433–5.
26. Centers for Disease Control. Adenovirus type 16-Long Island, New York. *MMWR Morb Mortal Wkly Rep* 1979;**28**:530–2.
27. Cesario TC, Poland JD, Wulff H, et al. Six years experience with herpes simplex virus in a children's home. *Am J Epidemiol* 1969;**90**:416–22.
28. Chanock RM, Parrott RH, Vargosko AJ, et al. Acute respiratory diseases of viral etiology. IV. Respiratory syncytial virus. *Am J Public Health Nations Health* 1962;**52**:918–25.
29. Chen HL, Chiou SS, Hsiao HP, et al. Respiratory adenoviral infections in children: a study of hospitalized cases in southern Taiwan in 2001-2002. *J Trop Pediatr* 2004;**50**:279–84.
30. Cherry JD. Personal observations, 1964, 1971.
31. Cherry JD. Newer respiratory viruses: their role in respiratory illness of children. *Adv Pediatr* 1973;**20**:225–90.
32. Cherry JD, Jahn CL. Herpangina: the etiologic spectrum. *Pediatrics* 1965;**36**:632–4.
33. Cherry JD, Welliver RC. *Mycoplasma pneumoniae* infections of adults and children. *West J Med* 1976;**125**:47–55.
34. Chretien JH, McGinniss CG, Thompson J, et al. Group B beta-hemolytic streptococci causing pharyngitis. *J Clin Microbiol* 1979;**10**:263–6.
35. Cimolai N, Elford RW, Bryan L, et al. Do the beta-hemolytic non-group A streptococci cause pharyngitis? *Rev Infect Dis* 1988;**10**:587–601.
36. Cimolai N, Morrison BJ, MacCulloch L, et al. Beta-haemolytic non-group A streptococci and pharyngitis: a case-control study. *Eur J Pediatr* 1991;**150**:776–9.
37. Cinar U, Seven H, Vural C, et al. Tuberculosis tonsillitis. *Otolaryngol Head Neck Surg* 2002;**126**:448–9.
38. Cochard M, Clet J, Le L, et al. PFAPA syndrome is not a sporadic disease. *Rheumatology (Oxford)* 2010;**49**:1984–7.
39. Commission on Acute Respiratory Diseases. Endemic exudative pharyngitis and tonsillitis: etiology and clinical characteristics. *JAMA* 1944;**125**:1163–9.
40. Conant MA, Lane B. Secondary syphilis misdiagnosed as infectious mononucleosis. *Calif Med* 1968;**109**:462–4.
41. Cramblett HG, Moffet HL, Black JP, et al. Coxsackie virus infections: clinical and laboratory studies. *J Pediatr* 1964;**64**: 406–14.
42. Dascomb HE, Hilleman MR. Clinical and laboratory studies in patients with respiratory disease caused by adenoviruses (RI-APC-ARD agents). *Am J Med* 1956;**21**:161–74.
43. Denny FW. Current problems in managing streptococcal pharyngitis. *J Pediatr* 1987;**111**:797–806.
44. Dominguez O, Rojo P, de Las Heras S, et al. Clinical presentation and characteristics of pharyngeal adenovirus infections. *Pediatr Infect Dis J* 2005;**24**:733–4.
45. Douglas RM, Miles H, Hansman D, et al. Acute tonsillitis in children: microbial pathogens in relation to age. *Pathology* 1984;**16**:79–82.
46. Dyment PG, Klink LB, Jackson DW. Hoarseness and palatal petechiae as clues in identifying streptococcal throat infections. *Pediatrics* 1968;**41**:822–3.
47. Ebell MH, Smith MA, Barry HC, et al. The rational clinical examination: does this patient have strep throat? *JAMA* 2000; **284**:2912–8.
48. Edmonson MB, Farwell KR. Relationship between the clinical likelihood of group A streptococcal pharyngitis and the sensitivity of a rapid antigen-detection test in a pediatric practice. *Pediatrics* 2005;**115**:280–5.
49. El-Daher NT, Hijazi SS, Rawashdeh NM, et al. Immediate vs. delayed treatment of group A beta-hemolytic streptococcal pharyngitis with penicillin V. *Pediatr Infect Dis J* 1991;**10**:126–30.
50. Eshchar J, Waron M, Alkan WJ. Syndromes of Q fever. *JAMA* 1966;**195**:146–9.
51. Esposito S, Bosis S, Begliatti E, et al. Acute tonsillopharyngitis associated with atypical bacterial infection in children: natural history and impact of macrolide therapy. *Clin Infect Dis* 2006;**43**: 206–9.
52. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**: 1337–42.
53. Evans AS, Dick EC. Acute pharyngitis and tonsillitis in University of Wisconsin Students. *JAMA* 1964;**190**:699–708.
54. Farley TA, Wilson SA, Mahoney F, et al. Direct inoculation of food as the cause of an outbreak of group A streptococcal pharyngitis. *J Infect Dis* 1993;**167**:1232–5.

55. Feder HM, Jr. Periodic fever, aphthous stomatitis, pharyngitis, adenitis: a clinical review of a new syndrome. *Curr Opin Pediatr* 2000;**12**:253–6.
56. Feder HM, Salazar JC. A clinical review of 105 patients with PFAPA (a periodic fever syndrome). *Acta Paediatr* 2010;**99**: 178–84.
57. Fell HW, Nagington J, Naylor GR, et al. *Corynebacterium haemolyticum* infections in Cambridgeshire. *J Hyg (Lond)* 1977; **79**:269–74.
58. Finegold SM, Bartlett JG, Chow AW, et al. Management of anaerobic infections. *Ann Intern Med* 1975;**83**:375–89.
59. Finegold SM, Rosenblatt JE. Practical aspects of anaerobic sepsis. *Medicine (Baltimore)* 1973;**52**:311–22.
60. Fiumara NJ, Berg M. Primary syphilis in the oral cavity. *Br J Vener Dis* 1974;**50**:463–4.
61. Forchheimer F. The enanthem of German measles. *Phila Med J* 1898;**II**:15–7.
62. Foulongne V, Olejnik Y, Perez V, et al. Human bocavirus in French children. *Emerg Infect Dis* 2006;**12**:1251–3.
63. Fries SM. Diagnosis of group A streptococcal pharyngitis in a private clinic: comparative evaluation of an optical immunoassay method and culture. *J Pediatr* 1995;**126**:933–6.
64. Galanakis E, Papadakis CE, Giannoussi E, et al. PFAPA syndrome in children evaluated for tonsillectomy. *Arch Dis Child* 2002; **86**:434–5.
65. Gallo G, Berzero R, Cattai N, et al. An outbreak of group A foodborne streptococcal pharyngitis. *Eur J Epidemiol* 1992;**8**:292–7.
66. Garavello W, Pignataro L, Gaini L, et al. Tonsillectomy in children with periodic fever with aphthous stomatitis, pharyngitis, and adenitis syndrome. *J Pediatr* 2011;**159**:138–42.
67. Gattorno M, Caorsi R, Meini A, et al. Differentiating PFAPA syndrome from monogenic periodic fevers. *Pediatrics* 2009;**124**: e721–728.
68. Gerber MA. Comparison of throat cultures and rapid strep tests for diagnosis of streptococcal pharyngitis. *Pediatr Infect Dis J* 1989;**8**:820–4.
69. Gerber MA. Effect of early antibiotic therapy on recurrence rates of streptococcal pharyngitis. *Pediatr Infect Dis J* 1991;**10**: S56–60.
70. Gerber MA, Randolph MF, DeMeo KK, et al. Lack of impact of early antibiotic therapy for streptococcal pharyngitis on recurrence rates. *J Pediatr* 1990;**117**:853–8.
71. Gerber MA, Randolph MF, Martin NJ, et al. Community-wide outbreak of group G streptococcal pharyngitis. *Pediatrics* 1991;**87**: 598–603.
72. Gerber MA, Spadaccini LJ, Wright LL, et al. Latex agglutination tests for rapid identification of group A streptococci directly from throat swabs. *J Pediatr* 1984;**105**:702–5.
73. Gerber MA, Tanz RR, Kabat W, et al. Optical immunoassay test for group A beta-hemolytic streptococcal pharyngitis: an office-based, multicenter investigation. *JAMA* 1997;**277**:899–903.
74. Giraldez-Garcia C, Rubio B, Gallegos-Braun JF, et al. Diagnosis and management of acute pharyngitis in a paediatric population: a cost-effectiveness analysis. *Eur J Pediatr* 2011;**170**:1059–67.
75. Glezen WP, Clyde WA, Jr, Senior RJ, et al. Group A streptococci, mycoplasmas, and viruses associated with acute pharyngitis. *JAMA* 1967;**202**:455–60.
76. Glover JA, Griffith F. Acute tonsillitis and some of its sequels: epidemiological and bacteriological observations. *BMJ* 1931;**2**: 521–7.
77. Gorbach SL, Bartlett JG. Anaerobic infections (third of three parts). *N Engl J Med* 1974;**290**:1289–94.
78. Graykowski EA, Barile MF, Lee WB, et al. Recurrent aphthous stomatitis: clinical, therapeutic, histopathologic, and hypersensitivity aspects. *JAMA* 1966;**196**:637–44.
79. Grayston JT. *Chlamydia pneumoniae* (TWAR) infections in children. *Pediatr Infect Dis J* 1994;**13**:675–84; quiz 685.
80. Grayston JT, Alexander ER, Kenny GE, et al. *Mycoplasma pneumoniae* infections: clinical and epidemiologic studies. *JAMA* 1965; **191**:369–74.
81. Grayston JT, Campbell LA, Kuo CC, et al. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. *J Infect Dis* 1990;**161**:618–25.
82. Green SL, LaPeter KS. Pseudodiphtheritic membranous pharyngitis caused by *Corynebacterium hemolyticum*. *JAMA* 1981;**245**: 2330–1.
83. Gurwith M, Wenman W, Hinde D, et al. A prospective study of rotavirus infection in infants and young children. *J Infect Dis* 1981;**144**:218–24.
84. Hamlyn E, Marriott D, Gallagher RM. Secondary syphilis presenting as tonsillitis in three patients. *J Laryngol Otol* 2006;**120**:602–4.
85. Harris DJ, Wulff H, Ray CG, et al. Viruses and disease. II. An outbreak of parainfluenza type 2 in a children's home. *Am J Epidemiol* 1968;**87**:419–25.
86. Harrison HR, Magder LS, Boyce WT, et al. Acute *Chlamydia trachomatis* respiratory infection in childhood: serologic evidence. *Am J Dis Child* 1986;**140**:1068–71.
87. Hathursinghe HR, Patel S, Uppal HS, et al. Acute tonsillitis: an unusual presentation of Kawasaki syndrome: a case report and review of the literature. *Eur Arch Otorhinolaryngol* 2006; **263**:336–8.
88. Hayward G, Thompson M, Heneghan C, et al. Corticosteroids for pain relief in sore throat: systematic review and meta-analysis. *BMJ* 2009;**339**:b2976.
89. Higgins PG, Ellis EM, Woolley DA. Viruses associated with acute respiratory infections in Royal Air Force personnel. *J Hyg (Lond)* 1970;**68**:647–54.
90. Hill HR, Caldwell GG, Wilson E, et al. Epidemic of pharyngitis due to streptococci of Lancefield group G. *Lancet* 1969;**2**: 371–4.
91. Hoagland RJ. Clinical manifestations of infectious mononucleosis: a report of two hundred cases. *Am J Med Sci* 1960; **240**:21–8.
92. Hoekstra RE, Herrmann EC, Jr, O'Connell EJ. Virus infections in children: clinical comparison of overlapping outbreaks of influenza A2-Hong Kong-68 and respiratory syncytial virus infections. *Am J Dis Child* 1970;**120**:14–6.
93. Hofer C, Binns HJ, Tanz RR. Strategies for managing group A streptococcal pharyngitis: A survey of board-certified pediatricians. *Arch Pediatr Adolesc Med* 1997;**151**:824–9.
94. Holzel A, Parker L, Patterson WH, et al. Virus isolations from throats of children admitted to hospital with respiratory and other diseases, Manchester 1962-4. *BMJ* 1965;**1**:614–9.
95. Horn ME, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967–1972. *J Hyg (Lond)* 1975;**74**:157–68.
96. Humphry T, Sanders S, Stadius M. Leptospirosis mimicking MLNS. *J Pediatr* 1977;**91**:853–4.
97. Isaacs D, May M. Recurrent episodes of fever with tonsillitis, mouth ulcers and adenopathy. *J Paediatr Child Health* 2003;**39**: 627–8.
98. Jamsky RJ. Gonococcal tonsillitis: report of a case. *Oral Surg Oral Med Oral Pathol* 1977;**44**:197–200.
99. John CC, Gilsdorf JR. Recurrent fever in children. *Pediatr Infect Dis J* 2002;**21**:1071–7.
100. Johnson JE, Kadull PJ. Laboratory-acquired Q fever: a report of fifty cases. *Am J Med* 1966;**41**:391–403.
101. Jordan WS, Jr. Acute respiratory diseases of viral etiology. I. Ecology of respiratory viruses-1961. *Am J Public Health Nations Health* 1962;**52**:897–902.
102. Kapikian AZ, Chanock RM, Reichelderfer TE, et al. Inoculation of human volunteers with parainfluenza virus type 3. *JAMA* 1961;**178**:537–41.
103. Kaptan ZK, Emir H, Uzunkulaoglu H, et al. Determination of *Helicobacter pylori* in patients with chronic nonspecific pharyngitis. *Laryngoscope* 2009;**119**:1479–83.
104. Karpathios T, Drakonaki S, Zervoudaki A, et al. *Arcanobacterium haemolyticum* in children with presumed streptococcal pharyngotonsillitis or scarlet fever. *J Pediatr* 1992;**121**:735–7.
105. Kellner G, Popow-Kraupp T, Kundi M, et al. Contribution of rhinoviruses to respiratory viral infections in childhood: a prospective study in a mainly hospitalized infant population. *J Med Virol* 1988;**25**:455–69.
106. Kibrick S. Current status of coxsackie and echo viruses in human disease. *Prog Med Virol* 1964;**6**:27–70.
107. Korb K, Scherer M, Chenot JF. Steroids as adjuvant therapy for acute pharyngitis in ambulatory patients: a systematic review. *Ann Fam Med* 2010;**8**:58–63.
108. Kovatch AL, Schuit KE, Michaels RH. *Corynebacterium hemolyticum* peritonsillar abscess mimicking diphtheria. *JAMA* 1983; **249**:1757–8.

109. Kurtz B, Kurtz M, Roe M, et al. Importance of inoculum size and sampling effect in rapid antigen detection for diagnosis of *Streptococcus pyogenes* pharyngitis. *J Clin Microbiol* 2000;**38**: 279–81.
110. Lajo A, Borque C, Del Castillo F, et al. Mononucleosis caused by Epstein-Barr virus and cytomegalovirus in children: a comparative study of 124 cases. *Pediatr Infect Dis J* 1994;**13**:56–60.
111. Lascari AD, Bapat VR. Syndromes of infectious mononucleosis. *Clin Pediatr (Phila)* 1970;**9**:300–5.
112. Le CT. Tick-borne relapsing fever in children. *Pediatrics* 1980;**66**:963–6.
113. Lehner T. Oral thrush, or acute pseudomembranous candidiasis; a clinicopathologic study of forty-four cases. *Oral Surg Oral Med Oral Pathol* 1964;**18**:27–37.
114. Lerner AM, Cherry JD, Klein JO, et al. Infections with reoviruses. *N Engl J Med* 1962;**267**:947–52.
115. Lewis HM, Parry JV, Davies HA, et al. A year's experience of the rotavirus syndrome and its association with respiratory illness. *Arch Dis Child* 1979;**54**:339–46.
116. Licameli G, Jeffrey J, Luz J, et al. Effect of adenotonsillectomy in PFAPA syndrome. *Arch Otolaryngol Head Neck Surg* 2008; **134**:136–40.
117. Lieu TA, Fleisher GR, Schwartz JS. Cost-effectiveness of rapid latex agglutination testing and throat culture for streptococcal pharyngitis. *Pediatrics* 1990;**85**:246–56.
118. Lin CM, Wang CC, Lai CC, et al. Genital ulcers as an unusual sign of periodic fever, aphthous stomatitis, pharyngotonsillitis, cervical adenopathy syndrome: a novel symptom? *Pediatr Dermatol* 2011;**28**:290–4.
119. Lindbaek M, Hoiby EA, Lermark G, et al. Clinical symptoms and signs in sore throat patients with large colony variant beta-haemolytic streptococci groups C or G versus group A. *Br J Gen Pract* 2005;**55**:615–9.
120. Linder JA, Bates DW, Lee GM, et al. Antibiotic treatment of children with sore throat. *JAMA* 2005;**294**:2315–22.
121. Lipsky BA, Goldberger AC, Tompkins LS, et al. Infections caused by nondiphtheria corynebacteria. *Rev Infect Dis* 1982;**4**: 1220–35.
122. Long SS. Syndrome of periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA)—what it isn't. What is it? *J Pediatr* 1999;**135**:1–5.
123. Luzuriaga K, Sullivan JL. Infectious mononucleosis. *N Engl J Med* 2010;**362**:1993–2000.
124. Macdonald JB, Socransky SS, Gibbons RJ. Aspects of the pathogenesis of mixed anaerobic infections of mucous membranes. *J Dent Res* 1963;**42**:529–44.
125. Mackenzie A, Fuite LA, Chan FT, et al. Incidence and pathogenicity of *Arcanobacterium haemolyticum* during a 2-year study in Ottawa. *Clin Infect Dis* 1995;**21**:177–81.
126. Maltezou HC, Tsagris V, Antoniadou A, et al. Evaluation of a rapid antigen detection test in the diagnosis of streptococcal pharyngitis in children and its impact on antibiotic prescription. *J Antimicrob Chemother* 2008;**62**:1407–12.
127. Mamo JG, Baghdassarian A. Behcet's disease: a report of 28 cases. *Arch Ophthalmol* 1964;**71**:4–14.
128. Markowitz M. Cultures of the respiratory tract in pediatric practice. *Am J Dis Child* 1963;**105**:12–8.
129. Marshall GS, Edwards KM, Butler J, et al. Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. *J Pediatr* 1987;**110**:43–6.
130. Maynard JE, Feltz ET, Wulff H, et al. Surveillance of respiratory virus infections among Alaskan Eskimo children. *JAMA* 1967; **200**:927–31.
131. McCloskey RV, Eller JJ, Green M, et al. The 1970 epidemic of diphtheria in San Antonio. *Ann Intern Med* 1971;**75**:495–503.
132. Meland E, Digranes A, Skjaerven R. Assessment of clinical features predicting streptococcal pharyngitis. *Scand J Infect Dis* 1993;**25**:177–83.
133. Melish ME, Hicks RM, Larson EJ. Mucocutaneous lymph node syndrome in the United States. *Am J Dis Child* 1976;**130**: 599–607.
134. Moffet HL, Cramblett HG, Smith A. Group A streptococcal infections in a children's home. II. Clinical and epidemiologic patterns of illness. *Pediatrics* 1964;**33**:11–7.
135. Moffet HL, Siegel AC, Doyle HK. Nonstreptococcal pharyngitis. *J Pediatr* 1968;**73**:51–60.
136. Mortimer EA, Jr, Boxerbaum B. Diagnosis and treatment: group A streptococcal infections. *Pediatrics* 1965;**36**:930–2.
137. Mufson MA. *Mycoplasma hominis* I in respiratory tract infections. *Ann N Y Acad Sci* 1970;**174**:798–808.
138. Nakayama M, Miyazaki C, Ueda K, et al. Pharyngoconjunctival fever caused by adenovirus type 11. *Pediatr Infect Dis J* 1992; **11**:6–9.
139. Nigro G, Pastoris MC, Fantasia MM, et al. Acute cerebellar ataxia in pediatric legionellosis. *Pediatrics* 1983;**72**:847–9.
140. Nourmand A, Ziai M. Typhoid and paratyphoid fever in children: review of symptoms and therapy in 165 cases. *Clin Pediatr (Phila)* 1969;**8**:235–8.
141. Numazaki Y, Yano N, Shigeta S, et al. Studies on parainfluenza virus infections among infants and children in Sendai. II. Serologic and epidemiologic investigation. *Jpn J Microbiol* 1968;**12**: 343–51.
142. Ogawa H, Hashiguchi K, Kazuyama Y. Prolonged and recurrent tonsillitis associated with sexually transmitted *Chlamydia trachomatis*. *J Laryngol Otol* 1993;**107**:27–9.
143. Ohashi M, Yoshikawa T, Akimoto S, et al. Severe acute tonsillitis caused by Rothia dentocariosa in a healthy child. *Pediatr Infect Dis J* 2005;**24**:466–7.
144. Padeh S, Brezniak N, Zemer D, et al. Periodic fever, aphthous stomatitis, pharyngitis, and adenopathy syndrome: clinical characteristics and outcome. *J Pediatr* 1999;**135**:98–101.
145. Pappas G, Giannoutsos C, Christou L, et al. *Coxiella burnetii:* an unusual ENT pathogen. *Am J Otolaryngol* 2004;**25**:263–5.
146. Paradise JL, Bluestone CD, Bachman RZ, et al. Efficacy of tonsillectomy for recurrent throat infection in severely affected children: results of parallel randomized and nonrandomized clinical trials. *N Engl J Med* 1984;**310**:674–83.
147. Paradise JL, Bluestone CD, Colborn DK, et al. Tonsillectomy and adenotonsillectomy for recurrent throat infection in moderately affected children. *Pediatrics* 2002;**110**:7–15.
148. Park WH, Cooper GV. Accidental inoculation of influenza bacilli on the mucous membranes of healthy persons with development of infection in at least one: persistence of type characteristics of the bacilli. *J Immunol* 1921;**6**:81–5.
149. Parrott RH. Viral respiratory tract illnesses in children. *Bull N Y Acad Med* 1963;**39**:629–48.
150. Parrott RH, Kim HW, Vargosko AJ, et al. Serious respiratory tract illness as a result of Asian influenza and influenza B infections in children. *J Pediatr* 1962;**61**:205–13.
151. Parrott RH, Vargosko A, Luckey A, et al. Clinical features of infection with hemadsorption viruses. *N Engl J Med* 1959;**260**: 731–8.
152. Peltola V, Jartti T, Putto-Laurila A, et al. Rhinovirus infections in children: a retrospective and prospective hospital-based study. *J Med Virol* 2009;**81**:1831–8.
153. Pereira MS. Adenovirus infections. *Postgrad Med J* 1973;**49**: 798–801.
154. Pers C. Infection due to "*Corynebacterium ulcerans*", producing diphtheria toxin—a case report from Denmark. *Acta Pathol Microbiol Immunol Scand B* 1987;**95**:361–2.
155. Peter G. Leptospirosis: a zoonosis of protean manifestations. *Pediatr Infect Dis* 1982;**1**:282–8.
156. Pichichero ME, Disney FA, Green JL, et al. Comparative reliability of clinical, culture, and antigen detection methods for the diagnosis of group A beta-hemolytic streptococcal tonsillopharyngitis. *Pediatr Ann* 1992;**21**:798–805.
157. Pichichero ME, Disney FA, Talpey WB, et al. Adverse and beneficial effects of immediate treatment of group A beta-hemolytic streptococcal pharyngitis with penicillin. *Pediatr Infect Dis J* 1987;**6**:635–43.
158. Place EH, Sutton LE. Erythema arthriticum epidemicum (Haverhill fever). *Arch Intern Med* 1934;**54**:659–84.
159. Pockett CR, Thompson GC. Adherence of families to a group a streptococcal pharyngitis protocol used in a pediatric emergency department. *Pediatr Emerg Care* 2011;**27**:374–8.
160. Podosin RL, Felton WL, 2nd. The clinical picture of Far East influenza occurring at the Fourth National Boy Scout Jamboree; report of 616 cases. *N Engl J Med* 1958;**258**:778–82.
161. Poland JD, Wulff H, Welton ER, et al. Viruses and disease: studies in a children's home. *Am J Epidemiol* 1966;**84**:92–102.
162. Rammelkamp CH, Jr, Schmidt WC. Bacterial infections of the nasopharynx, with particular reference to the prevention of

rheumatic fever and glomerulonephritis. *Pediatr Clin North Am* 1957;**Feb**:139–54.
163. Rantz LA, Boisvert PJ, Spink WW. Hemolytic streptococcic and nonstreptococcic diseases of the respiratory tract; a comparative clinical study. *Arch Intern Med (Chic)* 1946;**78**:369–86.
164. Rodriguez WJ, Controni G, Cohen GJ. Yersinia enterocolitica enteritis in children. *JAMA* 1979;**242**:1978–80.
165. Rodriguez WJ, Kim HW, Arrobio JO, et al. Clinical features of acute gastroenteritis associated with human reovirus-like agent in infants and young children. *J Pediatr* 1977;**91**:188–93.
166. Rogers RSI. Recurrent aphthous stomatitis: clinical characteristics and evidence for an immunopathogenesis. *J Invest Dermatol* 1977;**69**:499–509.
167. Saari TN, Triplett DA. *Yersinia pseudotuberculosis* mesenteric adenitis. *J Pediatr* 1974;**85**:656–9.
168. Saliba GS, Glezen WP, Chin TD. Etiologic studies of acute respiratory illness among children attending public schools. *Am Rev Respir Dis* 1967;**95**:592–602.
169. Schmidt JP, Metcalf TG, Mil Tenerger FW. An epidemic of Asian influenza in children at Ladd Air Force Base, Alaska, 1960. *J Pediatr* 1962;**61**:214–20.
170. Scott TF. Clinical syndromes associated with enterovirus and REO virus infections. *Adv Virus Res* 1961;**8**:165–97.
171. Siegel W, Spencer FJ, Smith DJ, et al. Two new variants of infection with coxsackievirus group B, type 5, in young children: a syndrome of lymphadenopathy, pharyngitis and hepatomegaly or splenomegaly, or both, and one of pneumonia. *N Engl J Med* 1963;**268**:1210–6.
172. Sloots TP, Mackay IM, Bialasiewicz S, et al. Human metapneumovirus, Australia, 2001-2004. *Emerg Infect Dis* 2006;**12**:1263–6.
173. Smith HW, Thomas L, Dingle JH, et al. Meningococcic infections: report of 43 cases of meningococcic meningitis and 8 cases of meningococcemia. *Ann Intern Med* 1944;**20**:12–32.
174. Sohier R, Chardonnet Y, Prunieras M. Adenoviruses: status of current knowledge. *Prog Med Virol* 1965;**7**:253–325.
175. Solomon P. Oral moniliasis complicating combined broad-spectrum-antibiotic and antifungal therapy. *N Engl J Med* 1961;**265**:847–8.
176. Spellerberg B, Brandt C. Streptococcus. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology, vol. 1. 10th ed*. Washington, DC: ASM Press; 2011. p. 331–49.
177. Steigman AJ, Lipton MM, Braspennickx H. Acute lymphonodular pharyngitis: a newly described condition due to coxsackie A virus. *J Pediatr* 1962;**61**:331–6.
178. Steinkuller JS, Chan K, Rinehouse SE. Prechewing of food by adults and streptococcal pharyngitis in infants. *J Pediatr* 1992;**120**:563–4.
179. Sterner G. Adenovirus infection in childhood: an epidemiological and clinical survey among Swedish children. *Acta Paediatr* 1962;**142**:5–30.
180. Stillerman M, Bernstein SH. Streptococcal pharyngitis: evaluation of clinical syndromes in diagnosis. *Am J Dis Child* 1961;**101**: 476–89.
181. Stojanov S, Lapidus S, Chitkara P, et al. Periodic fever, aphthous stomatitis, pharyngitis, and adenitis (PFAPA) is a disorder of innate immunity and Th1 activation responsive to IL-1 blockade. *Proc Natl Acad Sci U S A* 2011;**108**:7148–53.
182. Stollerman GH. Sore throat—a diagnostic and therapeutic dilemma. *JAMA* 1964;**189**:145–6.
183. Sutton RN. Respiratory viruses in a residential nursery. *J Hyg (Lond)* 1962;**60**:51–67.
184. Tacket CO, Davis BR, Carter GP, et al. *Yersinia enterocolitica* pharyngitis. *Ann Intern Med* 1983;**99**:40–2.
185. Tanz RR, Gerber MA, Kabat W, et al. Performance of a rapid antigen-detection test and throat culture in community pediatric offices: implications for management of pharyngitis. *Pediatrics* 2009;**123**:437–44.
186. Thomas KT, Feder HM, Jr, Lawton AR, et al. Periodic fever syndrome in children. *J Pediatr* 1999;**135**:15–21.
187. Tomlinson AJH. Human pathogenic coryneform bacteria: their differentiation and significance in public health today. *J Appl Bacteriol* 1966;**29**:131–7.
188. Top FHS. Diphtheria. In: Top FH, Sr., Wehrle PF, editors. *Communicable and infectious diseases*. St. Louis: C. V. Mosby; 1972, p. 190–207.
189. Tsevat J, Kotagal UR. Management of sore throats in children: a cost-effectiveness analysis. *Arch Pediatr Adolesc Med* 1999;**153**: 681–8.
190. Turner JC, Fox A, Fox K, et al. Role of group C beta-hemolytic streptococci in pharyngitis: epidemiologic study of clinical features associated with isolation of group C streptococci. *J Clin Microbiol* 1993;**31**:808–11.
191. Turner JC, Hayden GF, Kiselica D, et al. Association of group C beta-hemolytic streptococci with endemic pharyngitis among college students. *JAMA* 1990;**264**:2644–7.
192. Tyson HK. Tularemia: an unappreciated cause of exudative pharyngitis. *Pediatrics* 1976;**58**:864–6.
193. Uohara GI, Knapp MJ. Oral fusospirochetosis and associated lesions. *Oral Surg Oral Med Oral Pathol* 1967;**24**:113–23.
194. Van Der Veen J. The role of adenoviruses in respiratory disease. *Am Rev Respir Dis* 1963;**88**(Suppl):167–80.
195. Veltri RW, Sprinkle PM, McClung JE. Epstein-Barr virus associated with episodes of recurrent tonsillitis. *Arch Otolaryngol* 1975;**101**:552–6.
196. Von Graevenitz A. Which bacterial species should be isolated from throat cultures? *Eur J Clin Microbiol* 1983;**2**:1–3.
197. Walker JE. Infection of laboratory worker with bacillus influenzae. *J Infect Dis* 1928;**43**:300–5.
198. Walker SH. The respiratory manifestations of systemic *Hemophilus influenzae* infection. *J Pediatr* 1963;**62**:386–92.
199. Ward R. Poliomyelitis. *Pediatr Clin North Am* 1960;**7**:947–63.
200. Wat PJ, Strickler JG, Myers JL, et al. Herpes simplex infection causing acute necrotizing tonsillitis. *Mayo Clin Proc* 1994;**69**: 269–71.
201. Wessels MR. Clinical practice: streptococcal pharyngitis. *N Engl J Med* 2011;**364**:648–55.
202. Wiesner PJ, Tronca E, Bonin P, et al. Clinical spectrum of pharyngeal gonococcal infection. *N Engl J Med* 1973;**288**: 181–5.
203. Willard CY, Hansen E. Bacterial flora of the nasopharynx in children: influence of respiratory infections and previous antimicrobial therapy. *AMA J Dis Child* 1959;**97**:318–25.
204. Wills PI, Gedosh EA, Nichols DR. Head and neck manifestations of tularemia. *Laryngoscope* 1982;**92**:770–3.
205. Wurster VM, Carlucci JG, Feder HM, Jr, et al. Long-term follow-up of children with periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis syndrome. *J Pediatr* 2011;**159**: 958–64.
206. Zachou K, Papamichalis PA, Dalekos GN. Severe pharyngitis in stockbreeders: an unusual presentation of brucellosis. *Occup Med (Lond)* 2008;**58**:305–7.
207. Zalan E, Leers WD, Labzoffsky NA. Occurrence of reovirus infection in Ontario. *Can Med Assoc J* 1962;**87**:714–5.
208. Zalma VM, Older JJ, Brooks GF. The Austin, Texas, diphtheria outbreak: clinical and epidemiological aspects. *JAMA* 1970;**211**: 2125–9.

primarily in the winter and spring, but both types can occur throughout the year.

PATHOGENESIS

Uvulitis is an acute cellulitis characterized by dramatic swelling and erythema. Infection of the uvula probably arises from direct invasion by *S. pyogenes* or *H. influenzae* type b; both are recognized as normal nasopharyngeal flora. In the latter case, epiglottitis also may arise by direct extension and the bacteremia may result secondarily from either the uvula or the epiglottis as a primary site of infection.

Uvulitis that is noninfectious may result from injury, chemical irritation, or allergic inflammation. An occurrence in a child who was ultimately diagnosed to have Kawasaki disease also has been reported.[7]

CLINICAL MANIFESTATIONS

In a review of five patients with streptococcal uvulitis, all had associated pharyngitis.[8] The patients presented with low-grade fever and sore throat. Three of the five patients experienced a choking or gagging sensation in the pharynx that induced coughing and spitting; one of these patients also presented with drooling. Although pharyngitis was noted on physical examination, the swelling and erythema of the uvula were most dramatic (Fig. 10-1). None of the patients had evidence of respiratory distress.

In most children with uvulitis and epiglottitis, the presentation usually is typical for epiglottitis, with sudden onset of high fever, dysphagia, and increasing respiratory distress. Rapkin,[14] however, reported a case of uvulitis and epiglottitis in a child in which the epiglottitis initially was unsuspected. The same observation has been made in some adults with uvulitis and epiglottitis.[6,12] Lateral neck radiography (performed in one case to evaluate the possibility of a retropharyngeal abscess) belatedly alerted the clinicians to the correct diagnosis.

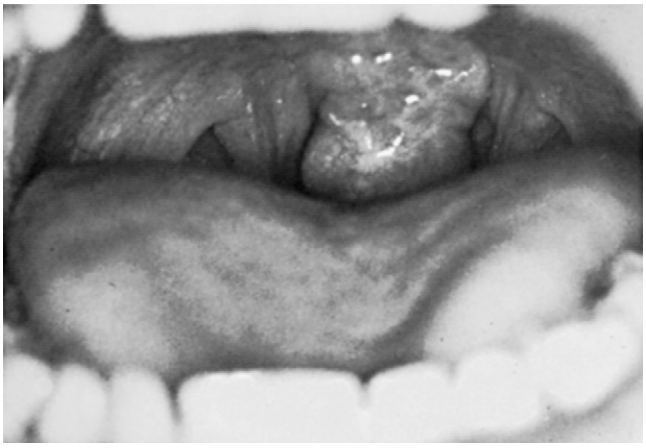

FIGURE 10-1 ■ Swollen (two to three times normal size) and erythematous uvula in a patient without epiglottitis or pharyngitis.

In patients with uvulitis and no epiglottitis, the presentation may be similar to that of epiglottitis, with acute onset of fever, odynophagia, and drooling, or, less specific, with fever and irritability or decreased appetite.[10,11,16] The diagnosis in these cases is provided by physical examination of the oropharynx, which shows a swollen and erythematous uvula (see Fig. 10-1).

DIAGNOSIS

The diagnosis of streptococcal uvulitis is suspected when a school-aged child presents with low-grade fever, pharyngitis, and uvulitis. The diagnosis is confirmed by the recovery of *S. pyogenes* from a surface culture of the throat or uvula or both.

The diagnosis of uvulitis caused by *H. influenzae* is suspected in a highly febrile infant or preschool-aged child who has uvular inflammation on physical examination. Lateral neck radiography must be performed to evaluate the possibility of epiglottitis, unless obvious signs of upper respiratory tract obstruction are present, in which case immediate endoscopy is warranted. If epiglottitis is discovered, the airway must be secured and appropriate parenteral antimicrobial agents must be initiated after blood and surface culture specimens are obtained. Any surface culture specimen obtained to search for *H. influenzae* must be plated onto chocolate agar. After appropriate culture specimens are obtained, parenteral antimicrobial agents should be initiated, as in other infections associated with bacteremia caused by *H. influenzae*.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of a patient with acute onset of fever, dysphagia, and drooling includes herpes simplex gingivostomatitis, uvulitis, epiglottitis, severe pharyngitis, and peritonsillar or retropharyngeal abscess. Although being extremely cautious in examining the pharynx of any patient with suspected epiglottitis is appropriate, some children tolerate attempted visualization of the oral cavity without becoming unduly upset. Instrumentation with a tongue blade should be avoided. If the examination does not show gingivostomatitis or peritonsillar abscess, lateral neck radiography should be performed. If epiglottitis or retropharyngeal abscess is confirmed, management of the airway and administration of antimicrobial agents is indicated for epiglottitis and incision and drainage and administration of antimicrobial agents is indicated for retropharyngeal abscess. If the lateral neck is normal and the uvula is inflamed, uvulitis with or without pharyngitis is confirmed.

TREATMENT

Management of uvulitis is guided primarily by the associated pharyngitis or epiglottitis, if either is present. In the case of streptococcal pharyngitis, penicillin therapy for 10 days is most appropriate. These patients usually can

be treated orally with penicillin V 25 to 50 mg/kg/day administered in two divided doses.

In the case of uvulitis and epiglottitis, management of the airway is most important and can be accomplished by performing nasotracheal intubation or tracheotomy. Appropriate parenteral antibiotic therapy usually is initiated.

In the case of uvulitis without epiglottitis in an infant or preschool-aged child, antimicrobial therapy should be planned for possible *H. influenzae* type b bacteremia. As many as 50 percent of respiratory isolates of *H. influenzae* produce β-lactamase. Administration of an advanced-generation cephalosporin, such as cefotaxime 200 mg/kg/day in four divided doses or ceftriaxone 100 mg/kg/day in one dose or two divided doses, is appropriate. In a patient with serious penicillin hypersensitivity, aztreonam 100 mg/kg/day in three divided doses and ciprofloxacin 30 mg/kg/day in two divided doses are also satisfactory regimens. After the patient has defervesced and has improved clinically, an oral antimicrobial agent can be substituted. The results of blood and surface cultures now can guide therapy. For an ampicillin-sensitive *H. influenzae* infection, amoxicillin 45 mg/kg/day in two divided doses should be prescribed to complete a 7- to 10-day course of treatment. For β-lactamase–producing *H. influenzae*, a variety of oral agents, including cefixime 10 mg/kg in a single daily dose, cefuroxime 30 mg/kg/day in two divided doses, or amoxicillin-potassium clavulanate 45 mg/kg/day of amoxicillin in two divided doses, can be prescribed.

Resolution was prompt in the two cases of uvulitis allegedly caused by *C. albicans*. One child was treated with topical nystatin, and the other had spontaneous improvement.[9]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Gilmore T, Mirin M. Traumatic uvulitis from a suction catheter. *J Emerg Med* 2012;**43**:e479.
5. Gunn A. An unusual sore throat. *Aust Fam Physician* 2007;**36**:163.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 11

Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses

Nira A. Goldstein • Margaret R. Hammerschlag

A deep neck abscess is a collection of pus in a potential space bounded by fascia.[6] These potential spaces are areas of least resistance to the spread of infection. An infection may begin with a minimal area of cellulitis and progress to a deep neck abscess, which may extend to invade adjacent potential spaces; these spaces frequently encompass vital structures in the neck. Destruction and dysfunction of these structures represent the major complications of deep neck infections.[54]

EPIDEMIOLOGY OF HEAD AND NECK SPACE INFECTIONS IN CHILDREN

Data concerning the frequency of head and neck space infections in children are limited. A survey of members of the American Academy of Otolaryngology found the incidence of peritonsillar abscesses to be approximately 30 per 100,000 person-years, or approximately 45,000 cases annually in the United States and Puerto Rico,[38] whereas a 3-year study in Sweden in a well-defined catchment area of 179,200 inhabitants reported an incidence of 37 per 100,000 person-years.[72] Data from the 2003 Kids' Inpatient Database (KID) reported 1321 hospital admissions for treatment of retropharyngeal abscesses in patients younger than 21 years of age sampled from 3438 hospitals distributed across 36 states.[51] The incidence of retropharyngeal abscesses appears to be increasing. Kirse and Roberson[48] reported 73 cases of retropharyngeal space infection from 1989 to 1998, with a twofold to fivefold increase in the number of patients treated in the last 4 years of the study compared with the first 6 years. Elsherif and associates[26] described 130 cases of retropharyngeal abscess between 2002 and 2007, and the incidence significantly increased over the study period from 13 cases in 2002 to 44 in 2007. Wright and colleagues[95]

REFERENCES

1. Boyce SH, Quigley MA. Uvulitis and partial upper airway obstruction following cannabis inhalation. *Emerg Med* 2002;**14**:106–8.
2. Brook I. Uvulitis caused by anaerobic bacteria. *Pediatr Emerg Care* 1997;**13**:221.
3. Butterton JR, Clawson-Simons J. Hymenoptera uvulitis. *N Engl J Med* 1987;**317**:1291.
4. Gilmore T, Mirin M. Traumatic uvulitis from a suction catheter. *J Emerg Med* 2012;**43**:e479.
5. Gunn A. An unusual sore throat. *Aust Fam Physician* 2007;**36**:163.
6. Jerrard DA, Olshaker J. Simultaneous uvulitis and epiglottitis without fever or leukocytosis. *Am J Emerg Med* 1996;**14**:551–2.
7. Kazi A, Gauthier M, Lebel MH, et al. Uvulitis and supraglottitis: early manifestations of Kawasaki disease. *J Pediatr* 1992;**120**:564–7.
8. Kotloff KL, Wald ER. Uvulitis in children. *Pediatr Infect Dis* 1983;**2**:392–3.
9. Krober MS, Weir MR. Acute uvulitis apparently caused by *Candida albicans*. *Pediatr Infect Dis J* 1991;**10**:73.
10. Li KI, Kiernan S, Wald ER. Isolated uvulitis due to *Hemophilus influenzae* type b. *Pediatrics* 1984;**74**:1054–7.
11. McNamara R. Clinical characteristics of acute uvulitis. *Am J Emerg Med* 1994;**12**:51–2.
12. McNamara R, Koobatian T. Simultaneous uvulitis and epiglottitis in adults. *Am J Emerg Med* 1997;**15**:161–3.
13. Peghini PL, Salcedo JA, Al-Kavas FH. Traumatic uvulitis: a rare complication of upper GI endoscopy. *Gastrointest Endosc* 2001;**53**:818–20.
14. Rapkin RH. Simultaneous uvulitis and epiglottitis. *JAMA* 1980;**43**:1843.
15. Westerman EL, Hutton JP. Acute uvulitis associated with epiglottitis. *Arch Otolaryngol Head Neck Surg* 1986;**112**:448–9.
16. Wynder SG, Lampe RM, Shoemaker ME. Uvulitis and *Hemophilus influenzae* bacteremia. *Pediatr Emerg Care* 1986;**2**:23–5.

also found a doubling in the number of cases of retropharyngeal space infections from 1997 to 2001 versus 2001 to 2005. In contrast, Segal and associates[78] found no increase in the number of peritonsillar infections from 2004 to 2007.

Peritonsillar abscesses rarely occur in young children but most frequently occur in patients in their teens and early 20s. In a 5-year study of 1010 incident head and neck infections, the median (interquartile range) age of peritonsillar abscesses was 13 (9 to 17) years, of retropharyngeal abscesses it was 2 (1 to 6) years, and of parapharyngeal abscesses it was 6 (5 to 7) years.[77] Retropharyngeal and parapharyngeal abscesses are more common in boys,[26,33,44,51] whereas peritonsillar abscesses occur equally among boys and girls.[72] Retropharyngeal abscesses most commonly occur in the winter and spring,[33,44,51,77] whereas peritonsillar abscesses have been reported to be more common in the autumn and spring[78] and the spring and summer.[77]

PERITONSILLAR ABSCESS (QUINSY)

A peritonsillar abscess (quinsy) is circumscribed medially by the fibrous wall of the tonsil capsule and laterally by the superior constrictor muscle. Pus may be localized in the superior pole, midpoint, or inferior pole or rarely may be dispersed, with multiple loculations in the peritonsillar space. The superior pole is the most common location, with a frequency range of 41.2 to 70 percent; the remaining inferior locations account for the balance.[8,11,57]

The etiology of peritonsillar abscesses is not constant. The abscesses may occur after any "virulent" tonsillitis, with extension through the fibrous tonsil capsule. Results of a meta-analysis of 15 previously reported series of patients with peritonsillar abscess reported by Herzon[38] found prior tonsillar infection rates ranging from 11 to 56 percent, with an overall rate of 36 percent. Group A β-hemolytic streptococcus has a moderate role in the development of peritonsillar abscesses because throat cultures are only positive in 18 percent of patients,[84] rapid antigen tests are only positive in 20 to 30 percent of patients,[73] and abscess cultures demonstrate group A β-hemolytic streptococcus in 46 percent.[28]

Clinical Manifestations

The patient's recent history may include a sore throat with occasional unilateral ear pain, malaise, low-grade pyrexia, chills, diaphoresis, dysphagia, reduced oral intake, trismus, and a muffled "hot potato voice." Trismus results from irritation and reflex spasm of the internal pterygoid muscle. Impaired palatal motion from edema contributes to the muffled voice.

Physical examination reveals minimal to moderate toxicity, dehydration, and drooling. Inspection of the oropharynx may be compromised by trismus. The soft palate is displaced toward the unaffected side, swollen and red, and frequently palpably fluctuant. The edematous uvula is pushed across the midline (Fig. 11-1). The displaced tonsil and its crypts rarely are coated with exudate. The breath is fetid, and ipsilateral, tender, cervical adenopathy is present. The white blood cell count is elevated, with a predominance of polymorphonuclear leukocytes.

Brodsky and associates[12] attempted to identify the clinical signs that might distinguish peritonsillar abscess from peritonsillar cellulitis in a group of 21 children admitted to the Children's Hospital of Buffalo from 1985 through 1987. No significant difference in age, duration of sore throat, fever, or white blood cell count was noted, although a greater degree of pharyngotonsillar bulge and muffled voice was found in the patients with abscess. Patients with peritonsillar cellulitis improved after receiving 24 hours of intravenous antibiotics, whereas patients

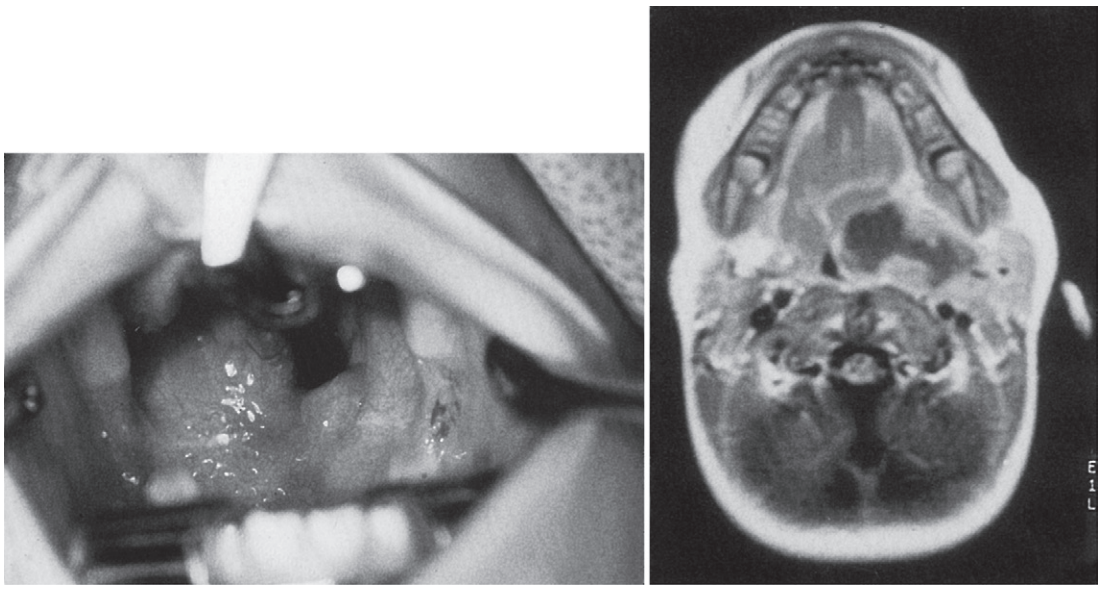

FIGURE 11-1 ■ **Left peritonsillar abscess in an 18-month-old child.** (From Wiatrak BJ, Woolley AL. Pharyngitis and adenotonsillar disease. In: Cummings CW, Flint PW, Harker LA, et al., editors. *Otolaryngology: head and neck surgery*. 4th ed. Philadelphia: Elsevier; 2005. p. 4144.)

with peritonsillar abscess had no change or worsening of symptoms. Blotter and colleagues[9] confirmed these findings in 102 patients admitted to Children's Hospital of Columbus, Ohio, between 1995 and 1998.

In uncomplicated cases, computed tomography (CT) has not been as useful as clinical assessment and follow-up evaluation in the management of peritonsillar abscess. CT is useful in young children with suspected peritonsillar abscess who are not cooperative with examination or children with other suspected deep neck infections.[30]

Treatment

Traditionally, management of peritonsillar abscess in children involved hospital admission for intravenous hydration, antibiotic therapy and analgesia, and either intraoral incision and drainage of the abscess or "acute quinsy tonsillectomy" with removal of the medial wall of the abscess. Although older children may tolerate incision and drainage under local anesthetic, the procedure is not well tolerated in young children and carries the risk of potential injury to adjacent vascular structures. The administration of a general anesthetic is required for tonsillectomy in all age groups and often is required for incision and drainage in young children. Acute tonsillectomy often was done to prevent future recurrence of the peritonsillar abscess.

Although it is a commonly accepted clinical observation, a high recurrence rate of peritonsillar abscess has not been well documented. A meta-analysis reported by Herzon[38] of 19 studies from the United States, Europe, and Israel involving 1399 patients found recurrence rates of 10 to 15 percent for peritonsillar abscess. The rates of recurrence seem to be lower in the United States (0% to 17%) than in the series reported from Europe and Israel (3% to 22%). A retrospective analysis of 290 patients treated for peritonsillar abscess found that patients who had a history of recurrent tonsillitis before development of the abscess had a fourfold greater rate of recurrence than patients with no history (40% vs. 9.6%).[50] The authors recommended that patients with a history of recurrent tonsillitis before admission be treated with tonsillectomy.[50]

The risks of acute tonsillectomy are not greater than the risks associated with delayed tonsillectomy.[56,62,94] In addition, the morbidity caused by two hospitalizations involving two procedures is reduced by acute tonsillectomy.[5,10,11] An additional benefit of acute tonsillectomy is the ability to evacuate inferior pole abscesses that technically are difficult to drain by needle aspiration or incision and drainage.[57]

Studies have suggested that many peritonsillar abscesses can be managed by simple needle aspiration combined with antibiotic therapy on an outpatient basis.* An extensive meta-analysis of 10 studies conducted from 1961 through 1994 involving 496 patients with peritonsillar abscesses found that needle aspiration had an overall success rate of 94 percent (range, 85% to 100%).[38] This success rate compares favorably with that reported for incision and drainage. Weinberg and associates[91] successfully performed needle aspiration in 41 of 43 children 7 to 18 years old (mean age, 13.9 years). All were admitted for intravenous antibiotic therapy, 2 (5%) required repeated aspiration for resolution, and 5 (12%) did not respond and required acute tonsillectomy. A pooled analysis of three prospective studies comparing needle aspiration versus incision and drainage showed no significant difference in resolution rates (93.7% for incision and drainage and 91.6% for needle aspiration), but the power was low because of the small sample size.[43] Other studies, which have included adults and children with peritonsillar abscesses, have reported that 0 to 48 percent of patients require hospitalization.[38,62,69,83]

Younger children often require admission to correct dehydration.[30] Younger children also are more likely than are older children to respond to intravenous antibiotics alone and to have negative findings at surgical drainage.[9] The use of conscious sedation has been reported to be a safe and effective approach for the drainage of peritonsillar abscesses in children.[59,85]

In a 10-year retrospective review of 83 children with peritonsillar abscesses by Schraff and colleagues,[76] 65 percent were treated by incision and drainage (51% knife vs. 14% needle aspiration), 31 percent had quinsy tonsillectomy, and 4 percent were admitted and treated with intravenous antibiotics alone. Of patients treated surgically, 51 percent of the procedures were done in the emergency department and 49 percent were done in the operating room. Forty-eight percent of the children required admission, and the average length of stay was 0.9 days (standard deviation 1.37).

A suggested approach to the management of children with peritonsillar abscess is as follows.[39] Cooperative children should undergo needle aspiration of the abscess and treatment with antibiotics. Children who can tolerate liquids orally may be managed as outpatients, and the remainder should be admitted for hydration and administration of intravenous antibiotics. Approximately 4 percent of children require a repeated aspiration for resolution.[38] Children who remain symptomatic after undergoing needle aspiration require incision and drainage or acute quinsy tonsillectomy, depending on the prior history of recurrent tonsillitis. Children who cannot tolerate needle aspiration on initial presentation are admitted for administration of intravenous antibiotics. If no response occurs within 24 hours, incision and drainage or acute tonsillectomy is done, depending on the prior history of recurrent tonsillitis. Delayed tonsillectomy is reserved for children who recover from the peritonsillar abscess without general anesthesia but have a history of recurrent tonsillitis or prior peritonsillar abscess.

There are new data suggesting that corticosteroids may be beneficial for patients with peritonsillar abscesses. Ozbek and associates[70] randomized patients aged 16 to 65 years to antibiotics with a single dose of intravenous corticosteroid or antibiotics with a single dose of placebo after needle aspiration. The patients who received corticosteroids demonstrated significant improvements in throat pain, fever, trismus, and hospitalization time compared with control patients. Additional studies in children are needed to support their routine use.

*References 12, 38, 69, 75, 76, 83, 89, 91.

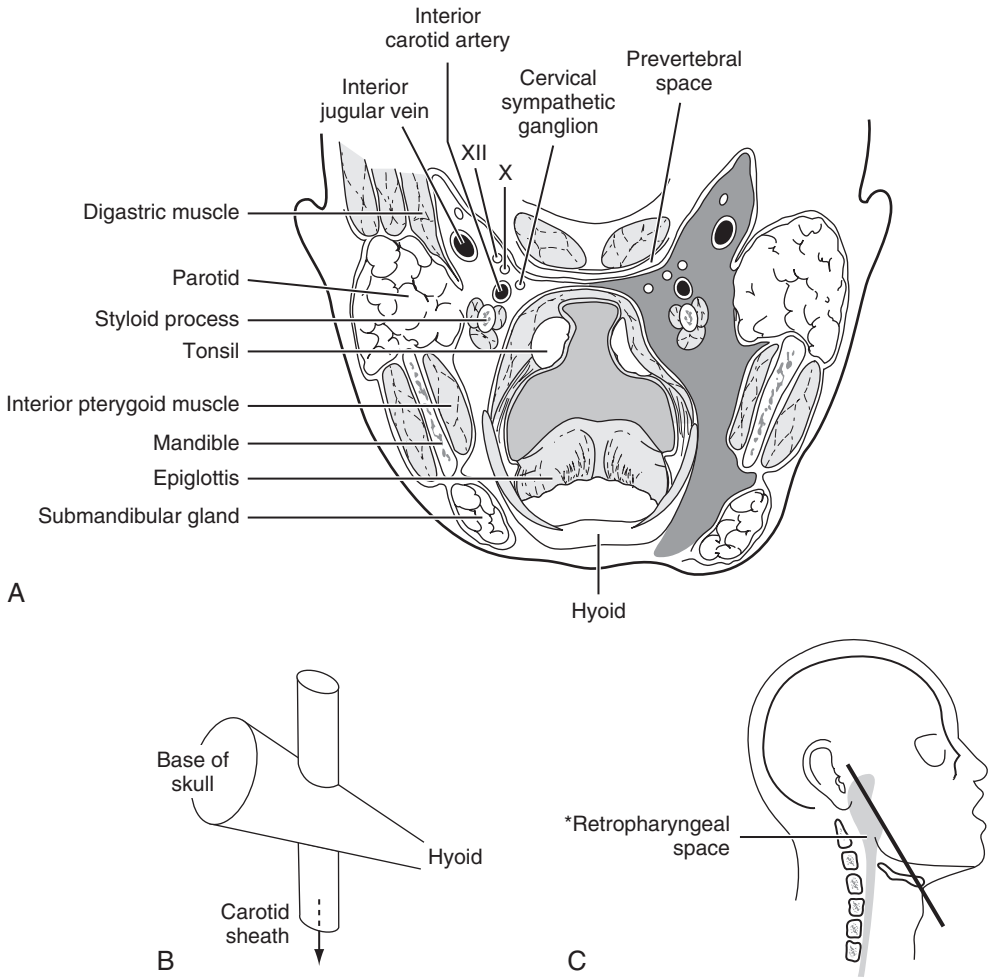

FIGURE 11-2 ■ **A,** Oblique transverse section of the oropharynx posterosuperiorly and the hypopharynx anteroinferiorly. Depicted are a peritonsillar abscess on the right and a pterygomaxillary space abscess on the left. **B,** The "cone" of the potential pterygomaxillary space with its carotid sheath. **C,** The vertical dimensions of the retropharyngeal space *(asterisk).* The *black oblique line* represents the level of the drawing in **A**.

Untreated peritonsillar abscess may point, with spontaneous rupture, or extend to the pterygomaxillary space, with potentially fatal complications. Upper airway obstruction, septicemia, and vascular catastrophe may occur. Necrotizing fasciitis also has been reported in adults with peritonsillar abscess.[32,92]

RETROPHARYNGEAL ABSCESS (POSTERIOR VISCERAL SPACE, RETROVISCERAL SPACE, AND RETROESOPHAGEAL SPACE ABSCESSES)

The anterior wall of the retropharyngeal space is the middle layer of the deep cervical fascia that abuts the posterior esophageal wall (the superior pharyngeal constrictor muscle). The deep layer of the deep cervical fascia circumscribes the posterior wall of this potential space. Inferiorly, these two fasciae fuse to limit the depth of this pocket at a level between the first and second thoracic vertebrae. A retropharyngeal abscess can erode inferiorly through the junction of these fasciae to extend posteriorly into the prevertebral space (Fig. 11-2). Subsequently, pus in the prevertebral space can descend inferiorly below the diaphragm to the psoas muscles.

The retropharyngeal space contains two paramedial chains of lymph nodes that receive drainage from the nasopharynx, adenoids, posterior paranasal sinuses, middle ear, and eustachian tube. These structures are prominent in early childhood and atrophy at puberty.[34] Retropharyngeal abscesses are common occurrences in young children and are thought to be secondary to suppurative adenitis of these retropharyngeal nodes.[4] Other sources of infection are penetrating foreign bodies, endoscopy, trauma, pharyngitis, vertebral body osteomyelitis, petrositis, dental procedures,[89] and branchial cleft anomalies.[42] In one series of 17 cases of retropharyngeal abscesses at the Children's Hospital, Denver, Colorado, 7 children (41%), including two neonates (most likely associated with attempts at intubation), had perforations of the hypopharynx or esophagus.[64] In a series of 117 children with head and neck space infections treated at the Children's Hospital of Pittsburgh, 63 percent of the children with retropharyngeal abscesses had antecedent tonsillitis, pharyngitis, or viral upper respiratory tract infection.[89] Two children had previous trauma; however,

no details on the type of trauma were given. In adults, tuberculosis and syphilis were common causes of retropharyngeal abscesses in the preantibiotic era.[67]

Clinical Manifestations

The symptoms of retropharyngeal abscess frequently begin insidiously after mild antecedent infection. Signs and symptoms include sore throat, fever, limitation of neck motion, torticollis, neck pain, neck mass, drooling, odynophagia, poor oral intake, and lethargy. Airway stridor or respiratory distress from edema, cellulitis, or an obstructing mass occasionally occurs.[19,20,22,90] Limited neck motion or torticollis is reported in 50 to 80 percent of patients.[20,33,71] In adults, the symptoms may be milder. Complaints of chest pain may reflect mediastinal extension.

Early in the course, midline or unilateral swelling of the posterior pharynx occurs. Later, gentle palpation may show a large fluctuant mass in the posterior pharynx. Vigorous palpation should be avoided because it may cause the abscess to rupture into the upper airway. As with other abscesses, the white blood cell count is increased, with a predominance of granulocytes.

Plain films of the neck may be performed initially but must be obtained with the patient in a true lateral position, with the neck in extension, and on inspiration, or the child's retropharyngeal soft tissues may appear abnormally thickened. Widening of the prevertebral soft tissues exceeding the anteroposterior diameter of the contiguous vertebral bodies or thickening of the retropharyngeal space greater than 7 mm at C2 in children and adults or 14 mm at C6 in children or 22 mm at C6 in adults suggests retropharyngeal inflammation. Rarely, a prevertebral soft tissue mass, air–fluid level, or gas may be seen. The normal cervical lordosis may be lost or reversed secondary to muscle spasm or local inflammation.

CT has rendered the diagnosis and management of deep neck space infections more precise.* In contrast to conventional radiologic studies, CT distinguishes cellulitis of the neck, which usually does not require surgical treatment, from a deep neck abscess, which does require surgical drainage. With its ability to define differences in tissue density, CT permits an accurate determination of the extent of the abscess and its extension and involvement of adjacent spaces to be made. An abscess is distinguished from cellulitis by a low-attenuation homogeneous area surrounded by a ring enhancement of contrast material (Fig. 11-3). Kirse and Roberson[48] reported scalloping of the abscess wall to be a more useful predictor of the presence of pus than ring enhancement. Freling and colleagues[29] reported that the presence of air within or adjacent to the fluid collection indicated an abscess in all cases. When more than one space is involved, accurate assessment of these spaces may ensure sufficient surgical drainage. Vascular structures can be identified, as can potential complications such as venous thrombosis.

A 10-year retrospective study from the Massachusetts Eye and Ear Infirmary compared preoperative CT scans with intraoperative findings in 38 patients who underwent surgical exploration of the parapharyngeal or retropharyngeal space within 48 hours after the scans were performed. Overall, the intraoperative findings confirmed the CT scan interpretation in 76.3 percent of the patients.[53] Of the 38 patients, 5 (13.2%) had CT scans indicative of abscesses that were not confirmed at surgery. Exploration of the parapharyngeal or retropharyngeal space revealed cellulitis. The false-negative rate was 10.5 percent. The sensitivity of CT for detection of parapharyngeal or retropharyngeal space abscess was 87.9 percent. Additional studies have reported that the accuracy of CT in predicting surgical findings was 63 percent, 69 percent, 73 percent, 76 percent, 76 percent, and 92 percent.*

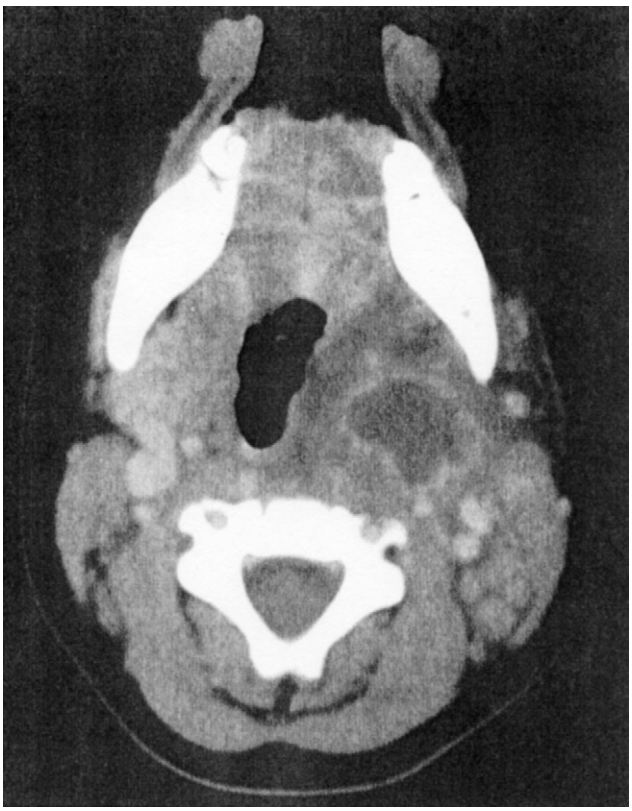

FIGURE 11-3 ■ Appearance on CT of a focal, ring-enhancing retropharyngeal abscess with a scalloped contour to the abscess wall. (From Kirse DJ, Roberson DW. Surgical management of retropharyngeal space infections in children. *Laryngoscope* 2001; **111**:1416.)

Treatment

The treatment of choice is administration of intravenous antibiotics and incision and drainage. If the mass is small, a peroral incision with the patient in the Rose position (supine with the neck hyperextended) may provide some drainage, but a slight risk of aspiration exists. If the mass is large and extends lateral to the great vessels, or if fever persists after peroral drainage is performed, an external incision is preferred. A tracheotomy may be required if risk of compromising the airway exists. Children may need to be kept intubated postoperatively if there is significant airway obstruction.

*References 13, 25, 48, 53, 87, 90, 93.

*References 25, 33, 71, 82, 90, 93.

Posterior mediastinitis can result from the spread of infection from the retropharyngeal area into the prevertebral space. The development of mediastinitis has been found to be associated with methicillin-resistant *Staphylococcus aureus* infection.[3,95] Other complications may be seen when the abscess extends to the parapharyngeal space and involves the great vessels and cranial nerves.

Reports have documented that patients with small retropharyngeal abscesses may respond to treatment with intravenous antibiotics alone. Broughton[15] described the experience at the University of Kentucky Medical Center, where 8 of 14 patients with deep neck infections seen during a 9-year period were treated successfully by antibiotics alone. All were reported to have small abscesses on CT. Possibly some had only cellulitis, however. Johnston and associates[44] treated 22 of 32 children with retropharyngeal or parapharyngeal abscesses with a trial of at least 24 hours of intravenous antibiotics. Although 13 (59%) ultimately required drainage, 9 (41%) were successfully managed medically. There were no significant differences in age or white blood cell count on admission between the two groups, but children who required drainage had significantly larger abscesses (5.38 cm^2 vs. 1.53 cm^2). Close clinical follow-up is mandatory for children treated with intravenous antibiotics alone. Children who do not improve within 24 to 48 hours require surgical drainage.

PARAPHARYNGEAL ABSCESS (PTERYGOMAXILLARY, PHARYNGOMAXILLARY, LATERAL, AND PHARYNGEAL SPACE ABSCESSES)

The potential parapharyngeal or pterygomaxillary space is an inverted conical cavity (see Fig. 11-2B) lying along an oblique axis roughly parallel to the ramus of the mandible (see Fig. 11-2C). The base of the skull at the jugular foramen forms the base of the "cone," and its apex is at the hyoid bone (see Fig. 11-2B). The buccopharyngeal fascia, lateral to the superior pharyngeal constrictor, delineates the medial boundary, and the parotid gland and its partially dehiscent deep layer of the superficial cervical fascia form the lateral wall of this cone. The internal pterygoid muscle and mandible demarcate the cone on its anterolateral aspect. The parapharyngeal space is contiguous with the peritonsillar, submandibular, and retropharyngeal spaces, all of which are potential avenues of egress for an extending parapharyngeal space abscess (see Fig. 11-2A).

The posterior portion of the cone contains the contents of the carotid sheath (carotid artery and internal jugular vein), cranial nerves IX through XII, and the cervical sympathetic chain. The internal pterygoid muscle and fatty connective tissue are anterior.

Involvement of these structures determines the clinical manifestations and complications of the parapharyngeal space abscess. An abscess in the posterior compartment may show medial displacement of the lateral pharyngeal wall and parotid space induration and swelling, with variable overlying facial nerve weakness, carotid artery erosion, and hemorrhage,[52] internal jugular vein thrombosis, decreased gag reflex and dysphagia, ipsilateral vocal cord paralysis, weakness of the ipsilateral trapezius muscle, ipsilateral lingual deviation, and Horner syndrome from cervical sympathetic chain involvement.[18]

Extension of the abscess into the anterior compartment causes trismus from irritation of the internal pterygoid muscle. Induration at the angle of the jaw and medial displacement of the tonsil and pharyngeal wall also occur with an anterior compartment abscess.

By the time a patient with an abscess seeks medical attention, the source of the parapharyngeal space infection may be unclear. Reports indicate variable causes, including incompletely or inadequately treated bacterial pharyngitis, tonsillitis,[4,60,71] peritonsillar abscess, dental infections, bacterial parotitis, otitis, mastoiditis (Bezold abscess from a mastoid tip infection traveling along the digastric muscles), petrositis, cervical adenitis with suppuration, cervical vertebral tubercular adenitis,[7,65] foreign bodies,[18,21] trauma, intravenous drug abuse,[37] branchial cleft anomalies,[86] and cat-scratch disease.[96] Parapharyngeal abscesses seem to be less common than are peritonsillar and retropharyngeal abscesses in children. Only three cases of parapharyngeal abscess and cellulitis were reported in the Pittsburgh series,[89] and only three cases were reported in a 15-year review of pediatric neck abscesses from Rainbow Babies and Children's Hospital in Cleveland, Ohio.[23] Amar and Manoukian[1] reported on 25 children with parapharyngeal abscesses during an 11-year period at Montreal Children's Hospital.

Clinical Manifestations

In addition to the symptoms noted in the preceding description, tender cervical swelling, induration and erythema of the side of the neck, torticollis, sore throat, dysphagia, trismus, hoarseness, malaise, chills, and diaphoresis may occur. Variable low-grade pyrexia with occasional temperature spikes occurs. Examination discloses variable toxicity, respiratory tract distress, laryngeal edema, medial displacement of the lateral pharyngeal wall and inferior tonsil pole, trismus, and, infrequently, drooling. Palpation of the neck reveals a tender, high cervical mass, initially diffuse and later fluctuant. Pharyngeal blood clots may presage erosion of the carotid artery. The complications of parapharyngeal abscesses are related to the structures involved: involvement of the carotid artery can produce hemiplegia from emboli; involvement of the internal jugular vein can lead to internal jugular vein thrombosis; and cephalad extension of the thrombosis may lead to cavernous sinus thrombosis. Internal jugular vein thrombosis is characterized by spiking temperature, toxicity with intense diaphoresis, headaches, and increased intracranial pressure. Septic pulmonary emboli occasionally are present.

Extension into the retropharyngeal region by a parapharyngeal abscess may lead to a posterior mediastinitis. Airway obstruction occurring secondary to laryngeal edema and aspiration pneumonia from suppuration of the abscess into the pharynx have been reported. Initially, the parapharyngeal abscess may be difficult to differentiate

from a peritonsillar abscess, but the latter usually is less toxic and has a distinct, soft palatal fluctuation.

As described for the diagnosis of retropharyngeal abscess, CT is an extremely useful tool for distinguishing parapharyngeal abscess from cellulitis and for localizing the abscess for surgical planning. In a review of 47 children who presented with deep neck infections to the Children's Hospital of Buffalo during a 5.5-year period, CT showed that 34 (77%) of 44 patients who underwent CT had involvement of the parapharyngeal and retropharyngeal spaces.[66] The involvement of both spaces had implications for the approach to surgical drainage.

Treatment

Intravenous antibiotic therapy with incision and drainage is the primary treatment. An otolaryngologic consultation should be obtained for this potentially complex surgery of the neck. The conventional method of approaching this abscess is an external incision with sufficient exposure to provide immediate access to the common carotid artery for ligation, should erosion of the carotid artery be present.[58] Intraoperative ultrasonography has been described as a useful modality to assist with external drainage of difficult abscesses located medial to the great vessels and close to the skull base.[24]

An intraoral drainage procedure traditionally has been condemned because rapid access to the vital structures of the neck is impossible with this approach. Nagy and associates[66] used an intraoral approach, however, to treat successfully 21 of 22 children with either parapharyngeal or combined parapharyngeal and retropharyngeal abscesses. The authors emphasized that CT with intravenous contrast enhancement showed that all of the abscesses were located medial to the great vessels and were adjacent to the pharyngeal wall. Amar and Manoukian[1] retrospectively compared 15 children with parapharyngeal abscesses who underwent intraoral drainage with 10 patients who underwent external neck drainage and found no complications or recurrences in either group. The children who underwent intraoral drainage had significantly reduced anesthesia time. The duration of intravenous antibiotics and duration of hospital stay also were shorter in the children who underwent intraoral drainage, but the differences between groups were not statistically significant. Cable and coworkers[16] described 12 children presenting to three centers with superior parapharyngeal space abscesses medial to the great vessels who were treated successfully by intraoral drainage using CT-guided systems to assist with localization of the abscess cavity. Marques and colleagues[60] successfully drained all 11 children presenting with parapharyngeal abscesses over a 5-year period via an intraoral approach with tonsillectomy. A transnasal endoscopic approach had also been used to drain an abscess located near the skull base in a 3-year-old boy.[55]

The use of CT has made it possible for some patients with parapharyngeal abscesses to be managed with intravenous antibiotics. The number of reported cases is small, however, and usually analyzed with cases of retropharyngeal abscess.[15,66] Nagy and associates[66] used intravenous antibiotics alone to treat 3 (13%) of 24 children with small parapharyngeal abscesses. Sichel and associates[80] used intravenous antibiotics alone to successfully treat 12 children with infection limited to the parapharyngeal space. Close clinical follow-up is necessary for children with parapharyngeal cellulitis or small parapharyngeal abscesses that are treated conservatively with intravenous antibiotics. Surgical drainage should be performed in children whose infection does not improve within 24 to 48 hours.

In another report, Sichel and colleagues[79] reviewed the CT scans of 29 patients with infections of the parapharyngeal space and divided the patients into two groups: 22 patients with posterior parapharyngeal space infections and 7 patients with anterior parapharyngeal space infections. The patients with posterior infections generally were children who responded well to intravenous antibiotics and did not develop complications. The patients with anterior infections of the parapharyngeal space more commonly were adults who required external drainage in addition to intravenous antibiotics and developed complications, including septic shock, respiratory distress, mediastinitis, pericarditis, and lung empyema. The authors suggested that the location of the infection in the fat of the anterior parapharyngeal space resulted in liquefaction of the fat, with formation of pus and rapid spread of the infection to other anatomic spaces.

MICROBIOLOGY OF DEEP NECK ABSCESSES

Group A streptococci *(Streptococcus pyogenes)*, viridans streptococci (including *Streptococcus anginosus* [formerly *S. milleri*]), anaerobes and *S. aureus* are the organisms most frequently associated with pharyngeal space infections. These data have been consistent for more than 30 years.* Anaerobes and group A streptococci are responsible for the gas seen on lateral neck radiographs. This finding is not surprising because the main portals of entry for pharyngeal space infections are the nasopharynx, oropharynx, paranasal sinuses, mastoid, and lower molars, all areas that are colonized with anaerobes.

The most complete microbiologic data available are from studies of peritonsillar abscesses. Flödstrom and Hallander,[28] in 1976, reported the results of bacterial cultures on aspirates of pus from 37 patients with peritonsillar abscesses. The ages of the patients were not given. Group A streptococci were isolated from 17 of these patients, whereas 15 had an increase in their antistreptolysin-O or anti-DNAase titers. Anaerobes were found in 28 of the cultures, including those of 8 patients that also disclosed the presence of streptococci. The most common anaerobic species isolated were fusobacteria (13), peptostreptococci (16), and *Bacteroides* spp. (18). Among the aerobic organisms, *S. aureus* was isolated four times and *Haemophilus influenzae* was isolated twice. No isolates of aerobic gram-negative enteric organisms were present. Jokipii and colleagues[45] performed semiquantitative cultures of aspirated pus from 42

*References 2, 13, 17, 28, 31, 35, 45, 46, 81, 84, 89.

peritonsillar abscesses and found similar results. Group A streptococci, the aerobic bacteria isolated most frequently, were isolated in pure culture 4 of 10 times. Anaerobes were more abundant than were aerobes; the most important species, in frequency and quantitatively, were *Bacteroides*, *Peptostreptococcus*, and *Fusobacterium*. Most of the infections were polymicrobial, with two to seven bacteria in 83 percent of the specimens. Subsequent studies from the United States and Finland have reported similar findings.[14,46,75,81]

Kieff and associates[47] found that streptococci were the most frequent isolates; group A streptococci and other non–group A β-hemolytic streptococci were present in more than 30 percent of patients, and α-hemolytic streptococci accounted for another 27.2 percent. The pathogenic role of anaerobic bacteria in peritonsillar abscesses has been reinforced by reports of complications caused by fusobacterial infection in children.[68,74] *Fusobacterium* and *Bacteroides* spp. have been associated with septic thrombophlebitis and pulmonary emboli from the jugular veins. Recent data from Aarhus, Denmark have found *Fusobacterium necrophorum* to be the most prevalent pathogen in peritonsillar abscesses, being isolated from 41% of cultures.[49] Of the 191 *F. necrophorum* isolates detected, 81% grew as pure culture. However, patients from whom *F. necrophorum* was isolated did not have a more severe presentation or outcome but tended to be younger and did have higher neutrophil counts and C-reactive protein levels than did children with other bacteria, including group A streptococci. Recent studies from other countries including Israel, Taiwan, and Japan have not found *F. necrophorum* to be an important pathogen in peritonsillar abscesses.[17,31,40]

Data on the microbiology of retropharyngeal and parapharyngeal abscesses in children are more limited. The organisms isolated are similar to those found in peritonsillar abscesses, but with a higher number of anaerobic species. Brook[13] examined aspirated pus from 14 children, 1 to 6 years old (median age, 3 years, 2 months), with retropharyngeal abscesses. Anaerobes were isolated from all patients; they were the only organisms isolated in 2 patients (14%) and were mixed with aerobes in the remainder (86%). The predominant anaerobic species were *Bacteroides*, *Peptostreptococcus*, and *Fusobacterium*. The predominant aerobic species were α-hemolytic and γ-hemolytic streptococci, *S. aureus*, *Haemophilus* spp., and group A β-hemolytic streptococci. Seventy-one percent of the isolates were β-lactamase–positive and included all isolates of the *S. aureus* group, 6 of 18 of the *Bacteroides melaninogenicus* group (33%), and 2 of 3 of the *Bacteroides oralis* group.

Dodds and Maniglia[23] reported the results of cultures from nine retropharyngeal and three parapharyngeal abscesses from children and adolescents. The organisms isolated were similar to those reported by Brook,[13,14] but the microbiology was not as complete because the study was retrospective and not all specimens may have been processed for anaerobic culture. Streptococcal species were the isolates that occurred most frequently, followed by *S. aureus* and *H. influenzae*. One isolate each of *Fusobacterium necrophorum*, *Escherichia coli*, and *Klebsiella pneumoniae* was found. Asmar[2] performed cultures on material from 17 children with retropharyngeal abscesses; viridans streptococci were isolated from 11 of the abscesses, *S. aureus* from 8, and group A streptococci from 6. The most frequently identified anaerobes were *Peptostreptococcus* spp. Overall, 45 aerobic and 18 anaerobic species were identified.

Rarely, retropharyngeal abscess may result from anterior extension from cervical osteomyelitis. This condition has been described with tuberculosis[67] and atypical mycobacteria causing a retropharyngeal abscess in a similar clinical setting. Barratt and colleagues[4] reported one case of retropharyngeal abscess caused by *Coccidioides immitis* in a 24-year-old woman with Hodgkin disease. The infection also was secondary to cervical vertebral osteomyelitis. Other rare and unusual causes of deep neck abscesses include cat-scratch disease (from *Bartonella*),[96] *Streptococcus pneumoniae*,[63] and Kawasaki disease.[41]

Because a large variety of organisms can be found in pharyngeal space infections, obtaining adequate culture specimens is crucial. The optimal material for culture is an aspirate of the pus obtained at operation. Throat swabs or swabs of the abscess obtained after drainage usually are inadequate because of contamination with normal oropharyngeal flora. The pus, when obtained, can be transported in a capped syringe if anaerobic transport media are unavailable. Most pathogenic obligate anaerobes can survive in a purulent exudate, despite extended periods of exposure to air.[27] A Gram stain of the exudate would provide important clues to the bacterial etiology. A Gram stain showing a mixture of organisms suggests a mixed aerobic-anaerobic infection.

Although use of a β-lactamase–resistant antibiotic may be necessary in the treatment of deep neck abscesses because of the presence of β-lactamase–producing bacteria, including *S. aureus* and *Bacteroides* spp.,[13,14,89] results of two studies suggest that penicillin alone was equivalent to broad-spectrum antibiotics for treatment of peritonsillar abscesses.[47,97] Yilmaz and colleagues[97] compared procaine penicillin with intramuscular administration of ampicillin-sulbactam in outpatient treatment of 40 patients with peritonsillar abscesses that were drained perorally. No statistical difference in duration of symptoms and clinical recovery was found between the two groups. Kieff and associates[47] retrospectively evaluated 103 patients with peritonsillar abscesses who were treated with incision and drainage. Fifty-eight patients were treated with broad-spectrum antibiotics, including ampicillin-sulbactam, clindamycin, cephalosporins, and metronidazole, alone and in combination; 45 patients were treated with penicillin alone. All patients were hospitalized after drainage, and the clinical outcomes, including duration of hospitalization and fever, did not differ significantly between the two groups. No significant difference in the organisms isolated was found, and failure and complication rates did not differ.

No comparative treatment studies for retropharyngeal or parapharyngeal abscesses have been reported. Treatment in these cases should be based on the results of cultures, as stated before. Drugs that may be effective include penicillin–β-lactamase inhibitor combinations such as ampicillin-sulbactam, ticarcillin-clavulanic acid, and piperacillin-tazobactam; expanded-spectrum

cephalosporins such as ceftriaxone; or penicillinase-resistant penicillins, including oxacillin or nafcillin. Cephalosporins, oxacillin, or nafcillin should be used in combination with clindamycin or metronidazole for adequate anaerobic coverage. Erythromycin and other macrolides (azithromycin, clarithromycin) are less satisfactory because of increasing macrolide resistance in group A streptococci and less activity against *Bacteroides fragilis* and *Fusobacterium*.

A study by Hanna and colleagues[36] reported macrolide resistance in 26 percent of group A streptococcal isolates obtained from peritonsillar abscesses seen between August 2001 and July 2002. The routine use of aminoglycoside antibiotics is not indicated because aerobic gram-negative enteric rods rarely are found in these infections. More recently, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has emerged as a pathogen in deep neck abscesses in children, especially in the United States.[35,88] However, there can be significant geographic variation in prevalence of MRSA.[61] In areas where CA-MRSA is highly prevalent, including vancomycin as initial therapy may be indicated. Clindamycin may be an alternative, but resistance of CA-MRSA to clindamycin, including inducible resistance, has been increasing in some geographic areas in the United States.[61] Antibiotic therapy is effective only in conjunction with adequate surgical drainage.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Baldassari CN, Howell R, Amorn M, et al. Complications in pediatric neck space abscesses. *Otolaryngol Head Neck Surg* 2011;**144**:592–5.
17. Chang L, Chi H, Chiu N-C, et al. Deep neck infections in different age groups of children. *J Microbiol Immunol Infect* 2010;**43**:47–52.
26. Elsherif AM, Park AH, Alder SC, et al. Indicators of a more complicated clinical course for pediatric patients with retropharyngeal space abscess. *Int J Pediatr Otorhinolaryngol* 2010;**74**:198–201.
29. Freling N, Roele E, Schaefer-Prokop C, et al. Prediction of deep neck space abscesses by contrast-enhanced computerized tomography in 76 clinically suspect consecutive patients. *Laryngoscope* 2009;**119**:1745–52.
31. Gavriel H, Lazarovitch T, Pomortsev A, et al. Variations in the microbiology of peritonsillar abscess. *Eur J Clin Microbiol Infect Dis* 2009;**28**:27–31.
33. Grisaru-Soen G, Komisar O, Aizenstein O, et al. Retropharyngeal and parapharyngeal abscess in children—epidemiology, clinical features and treatment. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1016–20.
44. Johnston D, Schmidt R, Barth P. Parapharyngeal and retropharyngeal infections in children: argument for a trial of medical therapy and intraoral drainage for medical treatment failures. *Int J Pediatr Otorhinolaryngol* 2009;**73**:761–5.
49. Klug TE, Rusan M, Fuursted K, et al. *Fusobacterium necrophorum:* most prevalent pathogen in peritonsillar abscess in Denmark. *Clin Infect Dis* 2009;**49**:1467–72.
51. Lander L, Lu S, Shah RK. Pediatric retropharyngeal abscesses: a national perspective. *Int J Pediatr Otorhinolaryngol* 2008;**72**:1837–43.
55. Lee C-H, Lee T-J, Chen C-W. Transnasal endoscopic approach for drainage of pediatric parapharyngeal space abscess. *Otolaryngol Head Neck Surg* 2010;**143**:467–8.
60. Marques PM, Spratley JE, Leal LM, et al. Parapharyngeal abscess in children—five year retrospective study. *Braz J Otorhinolaryngol* 2009;**75**:826–30.
71. Page NC, Bauer EM, Lieu JEC. Clinical features and treatment of retropharyngeal abscess in children. *Otolaryngol Head Neck Surg* 2008;**138**:300–6.
72. Risberg S, Engfeldt P. Hugosson S. Incidence of peritonsillar abscess and relationship to age and gender: retrospective study. *Scand J Infect Dis* 2008;**40**:792–6.
73. Risberg S, Engfeldt P, Hugosson S. Peritonsillar abscess and cellulitis and their relation to a positive antigen detection test for streptococcal infection. *Scand J Infect Dis* 2010;**42**:747–51.
78. Segal N, El-Saied S, Puterman M. Peritonsillar abscess in children in the southern district of Israel. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1148–50.
84. Sunnergren O, Swanberg J, Mölstad S. Incidence, microbiology and clinical history of peritonsillar abscess. *Scand J Infect Dis* 2008;**40**:752–5.
95. Wright CT, Stocks RMS, Armstrong DL, et al. Pediatric mediastinitis as a complication of methicillin-resistant *Staphylococcus aureus* retropharyngeal abscess. *Arch Otolaryngol Head Neck Surg* 2008;**134**:408–13.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 12

Cervical Lymphadenitis

C. Mary Healy • Carol J. Baker

Cervical lymphadenopathy is enlargement of the lymph nodes in the neck. *Cervical lymphadenitis* implies one or more nodes are inflamed. The inflammatory response by the host is triggered by some form of injury or invasion proximal to the involved lymph node or nodes. The nodes become affected secondarily by drainage through connecting afferent lymphatic channels. The injury may be acute or chronic, infectious or noninfectious. Proper anatomic definition of the inflamed node or nodes,[87] combined with knowledge of the structures of the head

REFERENCES

1. Amar YG, Manoukian JJ. Intraoral drainage: recommended as the initial approach for the treatment of parapharyngeal abscesses. *Otolaryngol Head Neck Surg* 2004;**130**:676–80.
2. Asmar BI. Bacteriology of retropharyngeal abscess in children. *Pediatr Infect Dis J* 1990;**9**:595–6.
3. Baldassari CN, Howell R, Amorn M, et al. Complications in pediatric neck space abscesses. *Otolaryngol Head Neck Surg* 2011; **144**:592–5.
4. Barratt GE, Koopmann CF, Coulthard SW. Retropharyngeal abscess: a ten-year experience. *Laryngoscope* 1984;**94**:455–63.
5. Bateman GH, Kodicek J. Primary quinsy tonsillectomy. *Ann Otol Rhinol Laryngol* 1959;**68**:315–21.
6. Beck AL. Deep neck infection. *Ann Otol Rhinol Laryngol* 1947; **56**:439–81.
7. Beck AL. Deep neck infections. *Ann Otol Rhinol Laryngol* 1947; **56**:722–65.
8. Beeden AG, Evans JNG. Quinsy tonsillectomy: a further report. *J Laryngol Otol* 1970;**84**:443–8.
9. Blotter JW, Yin L, Glynn M, Wiet GJ. Otolaryngology consultation for peritonsillar abscess in the pediatric population. *Laryngoscope* 2000;**110**:1698–701.
10. Bonding P. Tonsillectomy à chaud. *J Laryngol Otol* 1973;**81**: 1171–82.
11. Brandon EC Jr. Immediate tonsillectomy for peritonsillar abscess. *Trans Am Acad Ophthalmol Otolaryngol* 1973;**77**:412–16.
12. Brodsky L, Sobie SR, Korwin D, et al. A clinical prospective study of peritonsillar abscess in children. *Laryngology* 1988;**98**: 780–3.
13. Brook I. Microbiology of retropharyngeal abscesses in children. *Am J Dis Child* 1987;**141**:202–4.
14. Brook I. Microbiology and management of peritonsillar, retropharyngeal, and parapharyngeal abscesses. *J Oral Maxillofac Surg* 2004;**62**:1545–50.
15. Broughton RA. Nonsurgical management of deep neck infections in children. *Pediatr Infect Dis J* 1992;**11**:14–18.
16. Cable BE, Brenner P, Bauman NB, Mair EA. Image-guided surgical drainage of medial parapharyngeal abscesses in children: a novel adjuvant to a difficult approach. *Ann Otol Rhinol Laryngol* 2004; **113**:115–20.
17. Chang L, Chi H, Chiu N-C, et al. Deep neck infections in different age groups of children. *J Microbiol Immunol Infect* 2010;**43**:47–52.
18. Chassaignae E. *Traite pratique de la suppuration et du drainage chirurgal*. **vol. II**. Paris: Masson; 1859.
19. Coticchia JM, Getnick GS, Yun RD, Arnold JE. Age-, site-, and time-specific differences in pediatric deep neck abscesses. *Arch Otolaryngol Head Neck Surg* 2004;**130**:201–7.
20. Craig FW, Schunk JE. Retropharyngeal abscess in children: clinical presentation, utility of imaging, and current management. *Pediatrics* 2003;**111**:1394–8.
21. Danforth HB, Brown AK Jr. A foreign body etiology of pterygomaxillary space abscess. *Laryngoscope* 1963;**73**:1485.
22. Daya H, Lo S, Papsin BC, et al. Retropharyngeal and parapharyngeal infections in children: the Toronto experience. *Int J Pediatr Otorhinolaryngol* 2005;**69**:81–6.
23. Dodds B, Maniglia AJ. Peritonsillar and neck abscesses in the pediatric age group. *Laryngoscope* 1988;**98**:956–9.
24. Duque CS, Guerra L, Roy S. Use of intraoperative ultrasound for localizing difficult parapharyngeal space abscesses in children. *Int J Pediatr Otorhinolaryngol* 2007;**71**:375–8.
25. Elden LM, Grundfast KM, Vezina G. Accuracy and usefulness of radiographic assessment of cervical neck infections in children. *J Otolaryngol* 2001;**30**:82–9.
26. Elsherif AM, Park AH, Alder SC, et al. Indicators of a more complicated clinical course for pediatric patients with retropharyngeal space abscess. *Int J Pediatr Otorhinolaryngol* 2010;**74**: 198–201.
27. Finegold SM. *Anaerobic bacteria in human disease*. New York: Academic Press; 1977. p. 129–41.
28. Flödstrom A, Hallander HO. Microbiological aspects of peritonsillar abscesses. *Scand J Infect Dis* 1976;**8**:157–60.
29. Freling N, Roele E, Schaefer-Prokop C, et al. Prediction of deep neck space abscesses by contrast-enhanced computerized tomography in 76 clinically suspect consecutive patients. *Laryngoscope* 2009;**119**:1745–52.
30. Friedman NR, Mitchell RB, Pereira KD, et al. Peritonsillar abscess in early childhood: presentation and management. *Arch Otolaryngol Head Neck Surg* 1997;**123**:630–2.
31. Gavriel H, Lazarovitch T, Pomortsev A, et al. Variations in the microbiology of peritonsillar abscess. *Eur J Clin Microbiol Infect Dis* 2009;**28**:27–31.
32. Greinwald JH, Wilson JF, Haggerty PG. Peritonsillar abscess: an unlikely cause of necrotizing fasciitis. *Ann Otol Rhinol Laryngol* 1995;**104**:133–7.
33. Grisaru-Soen G, Komisar O, Aizenstein O, et al. Retropharyngeal and parapharyngeal abscess in children—epidemiology, clinical features and treatment. *Int J Pediatr Otorhinolaryngol* 2010;**74**: 1016–20.
34. Grodinsky M. Retropharyngeal and lateral pharyngeal abscesses: an anatomic and clinical study. *Ann Surg* 1939;**110**:177–99.
35. Guss J, Kazahaya K. Antibiotic-resistant *Staphylococcus aureus* in community-acquired pediatric neck abscesses. *Int J Pediatr Otorhinolaryngol* 2007;**71**:943–8.
36. Hanna BC, McMullan R, Gallagher G, Hedderwick S. The epidemiology of peritonsillar abscess disease in Northern Ireland. *J Infect* 2006;**52**:247–53.
37. Har-El G, Aroesty JH, Shaha A, Lucente FE. Changing trends in deep neck abscess: a retrospective study of 110 patients. *Oral Surg Oral Med Oral Pathol* 1994;**77**:446–50.
38. Herzon FS. Peritonsillar abscess: incidence, current management practices, and a proposal for treatment guidelines. *Laryngoscope* 1995;**105**:1–17.
39. Herzon FS, Nicklaus P. Pediatric peritonsillar abscess: management guidelines. *Curr Probl Pediatr* 1996;**26**:270–8.
40. Hidaka H, Kuriyama S, Yano H, et al. Precipitating factors in the pathogenesis of peritonsillar abscess and bacteriological significance of the *Streptococcus milleri* group. *Eur J Clin Microbiol Infect Dis* 2011;**30**:527–32.
41. Homicz MR, Carvalho D, Kearns DB, et al. An atypical presentation of Kawasaki disease resembling a retropharyngeal abscess. *Int J Pediatr Otolaryngol* 2000;**54**:45–9.
42. Huang RY, Damrose EJ, Alavi S, et al. Third branchial cleft anomaly presenting as a retropharyngeal abscess. *Int J Pediatr Otolaryngol* 2000;**54**:167–72.
43. Johnson RF, Stewart MG, Wright CC. An evidence-based review of the treatment of peritonsillar abscess. *Otolaryngol Head Neck Surg* 2003;**128**:332–43.
44. Johnston D, Schmidt R, Barth P. Parapharyngeal and retropharyngeal infections in children: argument for a trial of medical therapy and intraoral drainage for medical treatment failures. *Int J Pediatr Otorhinolaryngol* 2009;**73**:761–5.
45. Jokipii AM, Jokipii L, Sipilä P, et al. Semiquantitative culture results and pathogenic significance of obligate anaerobes in peritonsillar abscesses. *J Clin Microbiol* 1988;**26**:957–61.
46. Jousimies-Somer H, Savolainen S, Makitie A, et al. Bacteriologic findings in peritonsillar abscesses in young adults. *Clin Infect Dis* 1993;**16**:S292–8.
47. Kieff DA, Bhattacharyya N, Siegel NS, Salman SD. Selection of antibiotics after incision and drainage of peritonsillar abscesses. *Otolaryngol Head Neck Surg* 1999;**120**:57–61.
48. Kirse DJ, Roberson DW. Surgical management of retropharyngeal space infections in children. *Laryngoscope* 2001;**111**:1413–22.
49. Klug TE, Rusan M, Fuursted K, et al. *Fusobacterium necrophorum*: most prevalent pathogen in peritonsillar abscess in Denmark. *Clin Infect Dis* 2009;**49**:1467–72.
50. Kronenberg J, Wolf M, Leventon G. Peritonsillar abscess: recurrence rate and the indication for tonsillectomy. *Am J Otolaryngol* 1987;**8**:82–4.
51. Lander L, Lu S, Shah RK. Pediatric retropharyngeal abscesses: a national perspective. *Int J Pediatr Otorhinolaryngol* 2008;**72**: 1837–43.
52. Langenbrunner DJ, Dajani S. Pharyngomaxillary space abscess with carotid artery erosion. *Arch Otolaryngol* 1971;**94**:447–57.
53. Lazor JB, Cunningham J, Eavey RD, et al. Comparison of computed tomography and surgical findings in deep neck infections. *Otolaryngol Head Neck Surg* 1994;**111**:746–50.
54. Leavitt GW. Cervical fascia and deep neck infections. *Otolaryngol Clin North Am* 1976;**9**:703–16.
55. Lee C-H, Lee T-J, Chen C-W. Transnasal endoscopic approach for drainage of pediatric parapharyngeal space abscess. *Otolaryngol Head Neck Surg* 2010;**143**:467–8.

56. Lee KJ, Traxler JH, Smith AW, et al. Tonsillectomy: treatment of peritonsillar abscess. *Trans Am Acad Ophthalmol Otolaryngol* 1973; **77**:417–21.
57. Licameli GR, Grillone GA. Inferior pole peritonsillar abscess. *Otolaryngol Head Neck Surg* 1998;**118**:95–9.
58. Liston RL. On a variety of false aneurysm. *Br Foreign Med Rev* 1843;**15**:155–61.
59. Luhmann JD, Kennedy RM, McAllister JD, Jaffe DM. Sedation for peritonsillar abscess drainage in the pediatric emergency department. *Pediatr Emerg Care* 2002;**18**:1–3.
60. Marques PM, Spratley JE, Leal LM, et al. Parapharyngeal abscess in children—five year retrospective study. *Braz J Otorhinolaryngol* 2009;**75**:826–30.
61. Marzouk HA, Nathawad R, Hammerschlag MR, et al. Methicillin resistant *Staphylococcus aureus* colonization in otitis-prone children. *Arch Otolaryngol Head Neck Surg* 2011;**137**:1217–22.
62. McCurdy JA Jr. Peritonsillar abscess. *Arch Otol* 1977;**103**:414–15.
63. Medina M, Goldfarb J, Traquina D, et al. Cervical adenitis and deep neck infection caused by *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 1997;**16**:823–4.
64. Morrison JE Jr, Pashley NR. Retropharyngeal abscess in children: a 10-year review. *Pediatr Emerg Care* 1988;**4**:9–11.
65. Mosher HP. The submaxillary fossa approach to deep pus in the neck. *Trans Am Acad Ophthalmol Otolaryngol* 1926;**34**:19–36.
66. Nagy M, Pizzuto M, Backstrom J, Brodsky L. Deep neck infections in children: a new approach to diagnosis and treatment. *Laryngoscope* 1997;**107**:1627–34.
67. Neumann JL, Schlueter DP. Retropharyngeal abscess as the presenting feature of tuberculosis of the cervical spine. *Am Rev Respir Dis* 1974;**110**:508–11.
68. Oleske JM, Starr SE, Nahmias AJ. Complications of peritonsillar abscess due to *Fusobacterium necrophorum*. *Pediatrics* 1976;**57**: 570–1.
69. Ophir D, Bawnik J, Porat M, et al. Peritonsillar abscess: a prospective evaluation of outpatient management by needle aspiration. *Arch Otolaryngol* 1988;**114**:661–3.
70. Ozbek C, Aygenc E, Tuna EU. Use of steroids in the treatment of peritonsillar abscess. *J Laryngol Otol* 2004;**118**:439–42.
71. Page NC, Bauer EM, Lieu JEC. Clinical features and treatment of retropharyngeal abscess in children. *Otolaryngol Head Neck Surg* 2008;**138**:300–6.
72. Risberg S, Engfeldt P. Hugosson S. Incidence of peritonsillar abscess and relationship to age and gender: retrospective study. *Scand J Infect Dis* 2008;**40**:792–6.
73. Risberg S, Engfeldt P, Hugosson S. Peritonsillar abscess and cellulitis and their relation to a positive antigen detection test for streptococcal infection. *Scand J Infect Dis* 2010;**42**:747–51.
74. Rubinstein E, Onderdonk AB, Rahal JJ. Peritonsillar infection and bacteremia caused by *Fusobacterium gonidiaformans*. *J Pediatr* 1974;**85**:673.
75. Savolainen S, Jousimies-Somer HR, Makitie AA, et al. Peritonsillar abscess. *Arch Otolaryngol Head Neck Surg* 1993;**119**:521–4.
76. Schraff S, McGinn JD, Derkay CS. Peritonsillar abscess in children: a 10-year review of diagnosis and management. *Int J Pediatr Otorhinolaryngol* 2001;**57**:213–18.
77. Schweinfurth HM. Demographics of pediatric head and neck infections in a tertiary care hospital. *Laryngoscope* 2006;**116**:887–9.
78. Segal N, El-Saied S, Puterman M. Peritonsillar abscess in children in the southern district of Israel. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1148–50.
79. Sichel J-Y, Attal P, Hocwald E, Eliashar R. Redefining parapharyngeal space infections. *Ann Otol Rhinol Laryngol* 2006;**115**:117–23.
80. Sichel J-Y, Dano I, Hocwald E, et al. Nonsurgical management of parapharyngeal space infections: a prospective study. *Laryngoscope* 2002;**112**:906–10.
81. Sprinkle PM, Veltri RW, Kantor CM. Abscesses of the head and neck. *Laryngoscope* 1974;**84**:1142–8.
82. Stone ME, Walner DL, Koch BL, et al. Correlation between computed tomography and surgical findings in retropharyngeal inflammatory processes in children. *Int J Pediatr Otorhinolaryngol* 1999;**49**:121–5.
83. Stringer SP, Schaefer SD, Close LG. A randomized trial for outpatient management of peritonsillar abscess. *Arch Otolaryngol* 1988;**114**:278–98.
84. Sunnergren O, Swanberg J, Mölstad S. Incidence, microbiology and clinical history of peritonsillar abscess. *Scand J Infect Dis* 2008; **40**:752–5.
85. Suskind DL, Park J, Piccirillo JF, et al. Conscious sedation: a new approach for peritonsillar abscess drainage in the pediatric population. *Arch Otolaryngol Head Neck Surg* 1999;**125**:1197–200.
86. Takimoto T, Itoh M. Parapharyngeal abscess associated with the second pharyngeal pouch. *J Laryngol Otol* 1993;**107**:456–7.
87. Thawley SE, Godo M, Fuller TR. Computerized tomography in the evaluation of head and neck lesions. *Laryngoscope* 1978;**88**: 451–9.
88. Thomason TS, Brenski A, McClay J, et al. The rising incidence of methicillin-resistant *Staphylococcus aureus* in pediatric neck abscesses. *Otolaryngol Head Neck Surg* 2007;**137**:459–64.
89. Ungkanont K, Yellon RF, Weissman JL, et al. Head and neck space infections in infants and children. *Otolaryngol Head Neck Surg* 1995;**112**:375–82.
90. Vural C, Gungor A, Comerci S. Accuracy of computerized tomography in deep neck infections in the pediatric population. *Am J Otolaryngol* 2003;**24**:143–8.
91. Weinberg E, Brodsky L, Stanievich J, et al. Needle aspiration of peritonsillar abscess in children. *Arch Otolaryngol Head Neck Surg* 1993;**119**:169–72.
92. Wenig BL, Shikowitz MJ, Abramson AL. Necrotizing fasciitis as a lethal complication of peritonsillar abscess. *Laryngoscope* 1984;**94**: 1576–9.
93. Wetmore RF, Mahboubi S, Soyupak SK. Computed tomography in the evaluation of pediatric neck infections. *Otolaryngol Head Neck Surg* 1998;**119**:624–7.
94. Windfuhr JP, Chen Y-S. Immediate abscess tonsillectomy—a safe procedure? *Auris Nasus Larynx* 2001;**28**:323–7.
95. Wright CT, Stocks RMS, Armstrong DL, et al. Pediatric mediastinitis as a complication of methicillin-resistant *Staphylococcus aureus* retropharyngeal abscess. *Arch Otolaryngol Head Neck Surg* 2008;**134**:408–13.
96. Yeh SH, Zangwill KM, Hall B, et al. Parapharyngeal abscess due to cat-scratch disease. *Clin Infect Dis* 2000;**30**:599–601.
97. Yilmaz T, Ünal ÖF, Figen G, et al. A comparison of procaine penicillin with sulbactam-ampicillin in the treatment of peritonsillar abscesses. *Eur Arch Otolaryngol* 1998;**255**:163–5.

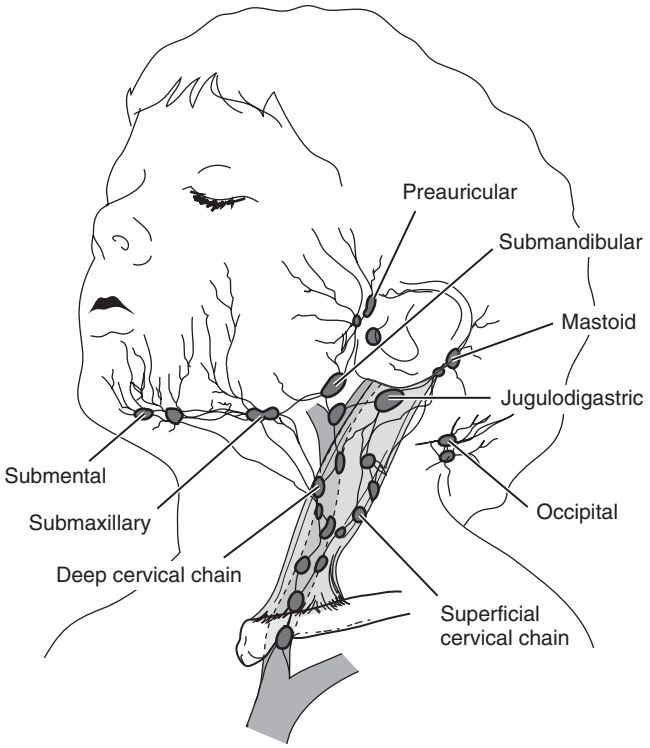

FIGURE 12-1 ■ The lymphatic drainage and lymph nodes involved in infants and children with cervical lymphadenitis.

and neck drained by them, may allow identification of a portal of entry for infectious agents, the most common cause of cervical lymphadenitis in infants and children.

Figure 12-1 illustrates the regional lymph nodes commonly affected in infants and children with cervical lymphadenitis. The superficial cervical lymph nodes lie on top of the sternocleidomastoid muscle along the course of the external jugular vein. They receive afferents from the superficial tissues of the neck, mastoid, superficial parotid (preauricular) nodes, and submaxillary glands. Their efferents terminate in the upper deep cervical lymph nodes. The mastoid lymph nodes overlie the mastoid process of the temporal bone and receive drainage from the parietal scalp and inner surface of the pinna. The occipital lymph nodes lie on the upper part of the trapezius and receive afferents from the occipital scalp and superficial portions of the upper posterior neck. Their efferents terminate in the deep cervical glands, as do the efferents from the mastoid nodes.

The deep cervical lymph nodes lie deep to the sternomastoid muscle along the whole length of the internal jugular vein and are divided into upper and lower groups. The jugulodigastric gland, a member of the upper group, lies at the angle of the jaw below the posterior belly of the digastric muscle. The lymphoid tissue of the palatine tonsil is drained into this gland; it frequently becomes enlarged in patients with "tonsillitis" or with tuberculous infection originating from the tonsils. The larynx, trachea, thyroid gland, and esophagus drain into the lower deep cervical glands. The submental lymph nodes, which lie between the digastric muscles below the myohyoid, receive superficial and deep drainage from the anterior tongue, lower lip, and chin, from both sides of the midline. They send efferents to the submandibular and upper deep cervical glands. The submandibular lymph nodes lie adjacent to the submandibular salivary gland and receive wide, superficial drainage from the lateral aspect of the lower lip, the vestibule of the nose, the cheeks, the medial parts of the eyelids, and the forehead. Deep drainage to these nodes arises from the posterior part of the mouth, gums, teeth, and tongue and from superficial and submental lymph nodes.

Because most of the lymphatic drainage of the head and neck goes to the submaxillary and deep cervical nodes, these glands are involved in more than 80 percent of cases of cervical adenitis in young children. Submental and superficial cervical lymphadenitis is observed less frequently.

EPIDEMIOLOGY

The epidemiology of infectious cervical adenitis is that of its infectious agents. Although cervical lymphadenitis can be a manifestation of focal viral infections of the oropharynx or respiratory tract, often it is part of a more generalized reticuloendothelial response to systemic infection. Viruses commonly associated with prominent cervical adenitis include Epstein-Barr virus, cytomegalovirus, and human immunodeficiency virus (HIV). In HIV-infected children, cervical adenopathy may either herald or be a part of the more generalized lymphadenopathy associated with this infection. Although human herpesvirus–6 (HHV-6), the cause of roseola in infants (exanthem subitum), is associated with the development of a mononucleosis syndrome and cervical adenopathy in adults,[3] lymphadenitis is not a prominent feature in children with primary infection.[60] Adenoviral and enteroviral infections are causes of generalized lymphadenopathy, rather than of cervical adenitis alone. The epidemiology of cervical adenitis varies with age, geographic location, and socioeconomic status. Generally, lower socioeconomic status is associated with a higher incidence of infection in younger children.

When bacterial in origin, with the exception of group A streptococci and *Mycobacterium tuberculosis*, the agents isolated from these glands are the normal inhabitants of the nose, mouth, pharynx, and skin—*Staphylococcus aureus*, anaerobes, nontuberculous mycobacteria, *Actinomyces* spp.—and person-to-person transmission does not occur. In contrast, group A streptococci and *M. tuberculosis* infection of cervical lymph nodes results from contact with human infection by way of airborne droplets. Except in neonates, for whom male dominance has been reported in cases caused by group B streptococci,[11] infectious lymphadenitis has no sex or seasonal predilection.[13,37]

Any age group can be affected by cervical lymphadenitis. In neonates, *S. aureus* and group B streptococci are the most common pathogens. Suppurative cervical lymphadenitis caused by *Staphylococcus epidermidis* in an otherwise healthy infant has been reported.[134] Nonetheless, despite the high frequency of nasal colonization by *S. epidermidis*, it remains a rare etiologic agent. There are rare reports of *Streptococcus pneumoniae* causing

suppurative adenitis in older children.[103] Some studies indicate that *S. aureus* has the leading role in infants, whereas in children, either group A streptococci or *S. aureus* is equally likely to be pathogenic.[37,151,170] Other reports have varied regarding the relationship between age and probable etiologic agent.[13,24]

Overall, *S. aureus* and *Streptococcus pyogenes* accounted for 65 to 89 percent of consecutive cases in prospectively evaluated series.[13,37,170] Methicillin-resistant *S. aureus* (MRSA) must be strongly considered as a possible etiology because of the increased incidence of health care–acquired MRSA and community-associated MRSA (CA-MRSA) infections in the United States and elsewhere* and recognition that the highest rates of CA-MRSA colonization and disease are found in children.[44,108] Many studies have shown that CA-MRSA strains predominantly cause skin and soft tissue infections, but a wide spectrum of illness is reported and invasive CA-MRSA has been associated with a variety of clinical manifestations, including severe illness and death.[50,64,66,68,110] Retrospective and prospective case series report an increasing incidence of CA-MRSA infections manifesting as adenitis and neck infections.[25,40,61,68,108] One single-center study found that MRSA was the infectious etiology of no head and neck abscesses from July 1999 through December 2001 but accounted for 34 percent of cases from January 2002 through June 2004.[117] In another report of 185 children with neck abscess, MRSA was the etiologic agent in 42 percent, and was more likely to be found in abscesses located laterally, in black patients and in children younger than age 16 months.[40]

The epidemiology of bacterial lymphadenitis varies by geographic location. Resurgence of infection with *Yersinia pestis* in the southwestern United States means that, in areas where it is endemic, it also must be considered in the differential diagnosis. Epidemic diphtheria, reported in the Russian Federation in 1990, subsequently spread to several newly independent states in the European region, with the number of reported cases (50,425) peaking in 1995. After the implementation of diphtheria control measures, the number of diphtheria cases declined by more than 95% during 2000 through 2009.[163] However, because circulation continues in some countries in Eastern Europe and sporadic cases occur elsewhere, diphtheria must be included as a possible cause of cervical lymphadenitis in those regions of the world.

The distinctive epidemiologic features of mycobacterial infection are summarized in Table 12-1. Scrofula caused by *M. tuberculosis* is a rare disease. When it does occur, it usually affects adults and older children. In contrast, children with atypical mycobacterial infection almost always are 1 to 6 years of age, live in suburban or rural communities, and have no history of contact with *M. tuberculosis*.[147] Although *M. tuberculosis* is an infection acquired primarily by inhalation, the gastrointestinal tract or respiratory tract may be the primary portal of entry for nontuberculous mycobacteria.[51,113,123,128] There seems to be an ethnic predilection for nontuberculous mycobacterial infection to occur in whites and for tuberculous infections to occur in blacks, Hispanics, Asians, and the Australian Aboriginal population.[112,149]

TABLE 12-1 Differentiation of *Mycobacterium tuberculosis* and Nontuberculous Mycobacterial Cervical Adenitis

	NTM CA	*M. tuberculosis*
Age	1-6 yr	All ages
Ethnicity	White	Black, Asian, Hispanic
Exposure to tuberculosis	Absent	Present
Abnormal chest radiographs	Rare	Often
Residence	Suburban	Urban
TST >15 mm	Uncommon	Often
Positive IGRA	Unlikely*	Yes
Bilateral involvement	Rare	Not uncommon

CA, Cervical adenitis; *IGRA,* interferon-γ release assay; *NTM,* nontuberculous mycobacteria; *TST,* tuberculin skin test.
*Except for infection with *M. kansasii, M. szulgai, M. marinum, M. gordonae.*

The advent of the HIV pandemic had a major impact on the nature and frequency of mycobacterial infections. A marked increase in the incidence of tuberculous infection occurred in HIV-infected adults and children, with annualized case rates for tuberculosis in HIV-infected children increasing from 58/100,000 during the years 1981 through 1985 to 478/100,000 during the years 1990 through 1992.[56] The availability of highly active antiretroviral therapy (HAART) resulted in significant decreases in the incidence of opportunistic infections, including tuberculous and nontuberculous mycobacterial infections, in HIV-infected children.[52]

The presence of tuberculosis infection in the community means that all children are at risk for exposure to an infectious adult.[20] The annual tuberculosis rate in the United States decreased steadily during the years 1993 through 2009, but the annual decline has decelerated.[33] Drug-resistant tuberculosis also is increasingly detected in industrialized and developing countries. During the years 2000 through 2004, 33 percent of tuberculous isolates from industrialized countries were multidrug-resistant (e.g., resistant to isoniazid and rifampin, first-line drugs in the treatment of tuberculosis), and 6 percent were classified as extensively drug-resistant (resistant to isoniazid, rifampin, and three of the six classes of second-line antituberculosis drugs).[32]

The incidence of nontuberculous mycobacterial infections has increased since the 1980s, although some of this apparent increase most likely has resulted from improvements in diagnostic methods. Nontuberculous mycobacteria are ubiquitous and are found in food, water, animals, and soil. An association between cervical adenitis caused by atypical mycobacteria with cold weather is prompted by observations that 68 percent of cases occur in the winter and spring months[92,121,166] and the disease exhibits a steady incidence throughout the year in cold climates.[51]

*References 21, 44, 50, 53, 64, 66, 68, 108.

A 10-year Canadian retrospective study reported that 70 percent of cases occurred in girls; however, most reports suggest no gender difference.[121]

Until the 1970s, *Mycobacterium scrofulaceum* was the usual etiologic agent isolated from children, followed by *Mycobacterium avium-intracellulare*.[166] This trend has reversed, with *M. avium-intracellulare* now accounting for 50 to 98 percent of culture-proven cases.* Previously uncommon species, such as *Mycobacterium kansasii*, *Mycobacterium malmoense*, *Mycobacterium fortuitum*, *Mycobacterium haemophilum*, and *Mycobacterium bohemicum*, also are being detected more frequently.[7,10,120] Some of these uncommon species probably are responsible for many cases of culture-negative mycobacterial lymphadenitis because of their fastidious growth requirements. New diagnostic methods have led to case reports of cervical adenitis secondary to slow-growing species, such as *Mycobacterium lentiflavum* and *Mycobacterium interjectum*.[39,58,62,125,145] Cervical lymphadenitis caused by *Mycobacterium chelonae* is rare, usually involves the submandibular glands, and typically occurs in patients with an antecedent history of dental pathology.[5]

Cervical lymphadenopathy may be the direct result of infection with HIV per se. However, the development of acute, tender adenitis in an HIV-infected child should provoke a search for another etiology. Although the typical childhood pathogens remain the most common pathogens in this setting, as in other immunocompromised children, opportunists also should be sought.[56] Patients with HIV infection beginning therapy with potent antiretroviral agents can develop new-onset mycobacterial lymphadenitis (tuberculous and nontuberculous mycobacteria). When this lymphadenitis occurs, it is more localized, is associated with more sinus formation, and is more often caused by nontuberculous mycobacteria (*M. avium-intracellulare*) than by *M. tuberculosis*.[124]

Cat-scratch disease is a common cause of lymphadenitis in children and young adults.[23] In 1988, English and associates[42] first isolated a pleomorphic gram-negative bacillus, later identified as *Afipia felis*, from lymph nodes of patients with cat-scratch disease. *Bartonella henselae* (formerly named *Rochalimaea henselae*), a morphologically similar but genetically distinct pleomorphic gram-negative bacillus, now is recognized as the cause of most cat-scratch disease.[1]

The cervical nodes are the second most common site of cat-scratch disease involvement. Although unusual and severe manifestations of this infection have been described, it remains mostly a mild, self-limited infection in children and adolescents, with no ethnic predilection. Seasonal variation with an increased incidence in fall, winter, and early spring does occur in temperate zones. A history of animal contact with cats usually can be elicited.[27] The importance of bites and scratches by kittens in transmitting this disease has been well defined.[100,173] However, the absence of a history of traumatic contact with cats in a substantial number of cases supports the hypothesis of an alternative mode of transmission. The detection of *Bartonella* DNA by polymerase chain reaction assay in collections of fleas from cats owned by two infected patients suggests that fleas or other arthropods may serve as vectors, but their role in human transmission is not well established.[6,172]

*References 43, 45, 63, 88, 92, 111, 112, 152, 166.

PATHOPHYSIOLOGY

Although cervical lymphadenitis is a common entity in pediatric clinical practice, little information exists regarding its pathogenesis. Viral cervical adenitis may be part of either a local response to viruses invading the oropharynx or respiratory tract (e.g., adenoviruses or coxsackieviruses) or a more generalized reticuloendothelial response to systemic viral infection (e.g., Epstein-Barr virus, cytomegalovirus, HHV-6, or HIV). Infection attributed to group A streptococci and *S. aureus* is presumed to enter the cervical lymphatics from the oropharynx (group A streptococci) or anterior nares (*S. aureus*). In a patient with group A streptococcal pharyngitis or tonsillitis, whether infection remains localized at the pharyngotonsillar tissues or spreads to cervical lymph nodes and results in suppuration primarily is a function of host response. Although peak attack rates for group A streptococcal pharyngitis are observed among school-age children, suppurative cervical adenitis is an uncommon occurrence. In contrast, infants and children younger than 3 years of age rarely have group A streptococci isolated from throat cultures, but this age group more commonly has suppurative cervical lymphadenitis.[127]

In infections attributed to *S. aureus*, colonization of the anterior nares is thought to be a prerequisite for cervical lymphadenitis. Brook and Winter[22,23] arrived at this conclusion because organisms of identical phage types were isolated from the anterior nares and the cervical abscesses of their patients. An investigation of children in St. Louis found no such correlation between isolates from nasal and cervical node cultures.[13] The role of *S. aureus* as a primary pathogen has been the subject of some debate. In most series, 30 percent of aspirates yield mixed cultures of *S. aureus* and group A streptococci, and frequently significant elevations of antistreptolysin O titer are found in the sera of patients whose lymph nodes yielded a pure culture of *S. aureus*.

In a California study, 65 percent of patients had node aspirates yielding a pure culture of *S. aureus*, and 41 percent exhibited an immune response to one or more of the extracellular antigens of group A streptococci.[170] Similarly, the finding that many children improve with penicillin or ampicillin treatment, despite the high prevalence of penicillin resistance among *S. aureus*, suggests that, although streptococci and staphylococci may coexist in these nodes, staphylococci may play a subsidiary role as secondary invaders. However, most children with isolates of *S. aureus* from suppurative lymph nodes show no evidence of coexistent streptococcal infection or viral upper respiratory tract infection. In addition, the resurgence of *S. aureus* (often MRSA) as a single pathogen in more recent case series argues that this organism has a strong capacity to be the primary invader.[40]

Recovery of anaerobic bacteria from cervical nodes suggests invasion of the lymphatics by mouth flora, often

as a result of local tissue destruction by periodontal disease.[24] The delineation of the pathophysiology of cervical lymphadenitis of diverse bacterial etiology requires an understanding of the interaction between a given microorganism (e.g., inoculum size, elaboration of extracellular enzymes, and ability to adhere to epithelium) and the host (e.g., humoral and surface immune capacity and degree of trauma).

Tuberculous cervical lymphadenopathy occurs within months of the initial exposure, through pulmonary infection and involvement of the regional and then more distant lymph nodes. It is a rapid process; chest radiographic evidence of active pulmonary disease often is seen. Nontuberculous mycobacteria are ubiquitous in the environment. Oropharyngeal acquisition and local infection lead to lymph node involvement. Most children with nontuberculous mycobacterial cervical lymphadenitis are immunocompetent, although some studies suggest that children who develop necrotic nodes may have deficient production of interferon(IFN)-γ,[111] and disseminated infections are almost always associated with impaired T cell function.[7] Despite *M. avium* skin test positivity being linked with pet birds, no clear relationship with lymphadenitis has been shown.[80] Discontinuation of childhood bacille Calmette-Guérin vaccination has been associated with an increase in atypical mycobacterial infection in many countries, suggesting that this vaccine may have a protective effect.[160] Progressive cervical adenitis developing after bacille Calmette-Guérin vaccination also has been reported.[114]

CLINICAL PRESENTATION

The clinical manifestations of cervical lymphadenitis vary considerably but are consistent with the diverse etiologies associated with cervical node enlargement in infants and children. Categorizing the mode of presentation as acute, subacute, or chronic is useful because, although the boundaries are ill-defined and much overlap exists, common etiologies tend to fall consistently within one of the categories. Cervical lymphadenitis of acute onset may be categorized further as bilateral or unilateral. In most situations, acute, bilateral cervical adenitis is either part of a generalized reticuloendothelial response to a systemic infection or a localized reaction to acute pharyngitis. The presence or absence of associated features (e.g., pharyngitis, enanthems or exanthems, generalized adenopathy, and hepatosplenomegaly) aids in making the differentiation.

Acute unilateral cervical lymphadenitis is caused by streptococcal or staphylococcal infection in 53 to 89 percent of cases.* In newborns, *S. aureus* is the most common cause and clinical features are similar to those seen in older children. Group B streptococci have been described as causative in a "cellulitis-adenitis" syndrome in infancy.[11] These infants differ from infants with staphylococcal adenitis in that they are younger, are more often male, and have a greater incidence of systemic symptoms, irritability, and anorexia; 94 percent have associated bacteremia. The typical patient has fever, facial or submandibular cellulitis, and ipsilateral otitis media.[11] Isolated cervical adenitis caused by group B streptococci also has been described.[46]

Patients with disease attributed to *S. aureus* or group A streptococci can become ill at any age but typically are 1 to 4 years old (70%-80% of cases), and the male-to-female ratio is equal. Clinically, little differentiates streptococcal from staphylococcal infections. Cervical adenitis can occur as part of the "streptococcosis" syndrome of infancy, with an onset heralded by coryza, an irregular low-grade fever, nasal discharge with excoriation and crusting around the nares, vomiting, and loss of appetite. Lymph node enlargement occurs within a few days of onset and resolves, as do other symptoms, without treatment within 6 to 8 weeks.[127] Suppuration of cervical glands can occur at any time during this interval but seldom does so if antimicrobial therapy is given early in the illness. Group A streptococci also should be suspected as a cause of cervical adenitis in a patient with typical vesiculopustular or crusted lesions of impetigo involving the face or scalp.

Systemic symptoms in children with staphylococcal or streptococcal cervical adenitis usually are minimal or absent unless associated with cellulitis, metastatic foci of infection, or bacteremia. The primary site of lymph node involvement by frequency is submandibular (50%-60%), upper cervical (25%-30%), submental (5%-8%), occipital (3%-5%), and lower cervical (2%-5%).[13,37,167] Involved nodes generally vary in size from 2 to 6 cm in diameter, and one fourth to one third suppurate. Patients with lymphadenitis caused by *S. aureus* are more likely to develop suppuration and a longer duration of symptoms and signs before diagnosis than are patients with disease caused by other bacterial agents (Fig. 12-2).[13,151] Among patients who develop suppurative adenitis, 86 percent do so within 2 weeks of onset.[167]

Approximately one third of patients in one study had concomitant lymphadenopathy at other anatomic sites.[13]

FIGURE 12-2 ■ A 2-year-old boy with fever and unilateral inflammation of the cervical lymph nodes of 2 days' duration. Needle aspirate culture of this nonfluctuant node grew *Staphylococcus aureus*. Antistaphylococcal therapy resulted in complete resolution of adenitis without surgical drainage.

*References 13, 37, 40, 67, 159, 170.

A history of recent upper respiratory tract symptoms, including sore throat (40%), earache or coryza (16%), and impetigo (32%), is a frequent finding, as are signs of pharyngitis, tonsillitis, or otitis media.[13,37] These factors do not help delineate the etiology, however. Hepatomegaly or splenomegaly is a rare occurrence and, if present, should suggest bacteremia or generalized disease processes (e.g., infectious mononucleosis, reticuloendotheliosis, tuberculosis, or HIV infection).

Kawasaki disease may manifest as a febrile illness associated with bilateral or unilateral cervical lymphadenopathy and may be confused with more common acute pyogenic infections.[162] Other features (e.g., conjunctivitis, oral manifestations, changes in the peripheral extremities, and polymorphic erythematous rash) are required criteria for the diagnosis.[104] Although originally termed *mucocutaneous lymph node syndrome*, unilateral lymph node enlargement of at least 1.5 cm is the most inconsistent feature.[15,104] Lymphadenopathy usually subsides when the fever subsides, although in some cases it may follow a more chronic course.

The rapid development of painful lymphadenitis, quickly succeeding the sudden onset of fever, chills, weakness, and headache, is a classic presentation of infection caused by *Y. pestis* (bubonic plague). The groin is the site most often involved. Other locations, including the cervical area, may be affected, however. Establishing the diagnosis and providing treatment quickly are crucial because infection can be fulminant.

In cases of diphtheria, cervical adenopathy develops secondary to infection of the posterior structures of the mouth and proximal pharynx. A whitish gray membrane covers the mucosal surfaces. In severe cases, the cervical adenopathy, which typically is bilateral, can result in a "bull neck" appearance.

Careful physical examination of the head and neck, particularly areas drained by affected lymph nodes, may yield important clues about etiology. The presence of periodontal disease is associated with a higher incidence of anaerobic organisms causing adenitis,[24] the history or presence of tick bites suggests the possibility of tularemia,[144] and the presence of papular or pustular lesions suggesting an inoculation site raises the possibility of other causes of infection, including *Nocardia* and *Bartonella* spp., actinomycosis, sporotrichosis, plague, and cutaneous diphtheria.

Mycobacterial and *Bartonella* spp. infections and toxoplasmosis are more common entities presenting as subacute or chronic lymphadenitis. The epidemiologic and clinical features that aid in the differentiation of tuberculous and nontuberculous mycobacterial infections are summarized in Table 12-1. The clinical manifestations virtually are identical (Fig. 12-3).[19,31,106] Typically, a child presents with a history of painless (so-called cold) cervical node swelling. The submandibular cervical nodes usually are involved in nontuberculous mycobacterial infection, whereas other cervical nodes are involved more frequently with *M. tuberculosis*.[4,19,70,99,136] As the infection progresses, the skin overlying the node may develop a pinkish or violaceous discoloration caused by increased vascularity, although the skin temperature is not increased. This finding may be followed by adherence of the skin to the underlying mass. If left untreated, fluctuance and spontaneously draining sinus tracts may develop.

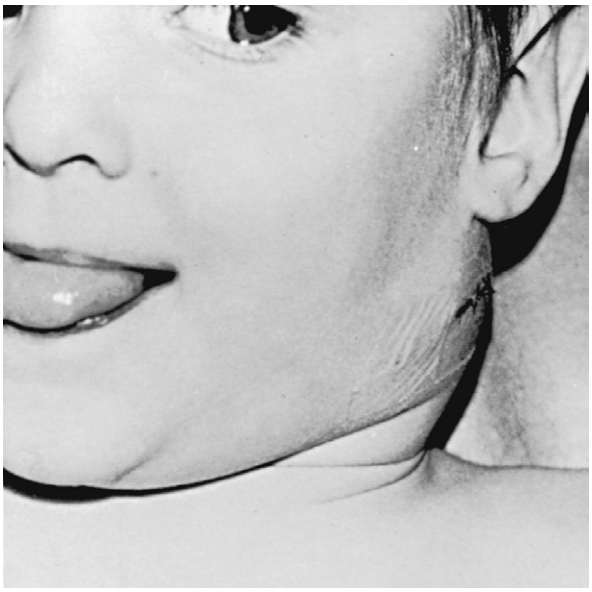

FIGURE 12-3 ■ A 4-year-old boy who had bilateral nontender enlargement of lymph nodes of 6 weeks duration, without other symptoms. Placement of a tuberculin skin test resulted in 18-mm induration, excisional biopsy acid-fast stain was positive, and cultures grew *Mycobacterium tuberculosis*.

A patient with *M. tuberculosis* is more likely than one with atypical mycobacterial disease to be older than 4 years of age and to have generalized lymphadenopathy (10%-20% of cases), bilateral node enlargement (10% of cases), a history of exposure to tuberculosis (93% of cases), and an urban residence.[19,74,99,116] No differences have been noted, however, with regard to duration of adenopathy, fever, and presence or absence of constitutional symptoms. An abnormal chest radiograph has been noted in 28 to 71 percent of cases caused by *M. tuberculosis*,[136,137,148,159] in contrast to the 98 to 100 percent of normal chest radiographs found in patients with nontuberculous mycobacterial adenitis.[51,135,136,152,158]

In a summary of 447 reported childhood cases of nontuberculous mycobacterial infections from 15 countries, Lincoln and Gilbert[79] detected only 6 cases of bilateral cervical node involvement, 4 with abnormal chest radiographs, and none with nodal enlargement other than cervical. Similar findings have been reported by other investigators.[135,158] Intradermal tuberculin skin testing with purified protein derivative uncommonly produces more than 15 mm of induration at 48 hours in a child with nontuberculous mycobacterial infection, but reactions between 5 and 15 mm are common.[84,95] Reactions of 10 to 20 mm can occur with *Mycobacterium marinum* and *M. fortuitum* infection.[123] Tuberculin skin test positivity may persist, even when children are retested many years after infection.[166] Unlike the findings with tuberculosis, IFN-γ release assays are generally negative with nontuberculous mycobacterial infections, unless the infecting organism is *M. kansasii*, *Mycobacterium szulgai*, *M. marinum*, or *Mycobacterium gordonae*.[7]

Bartonella infection may manifest days to weeks after the initial inoculation. Characteristically, a history of contact with a cat or kitten or a scratch is present.[173] Later, when the primary lesion may have healed, tender regional adenopathy appears. Although axillary nodes most frequently are affected, 25 percent of children have isolated cervical node involvement. Middle cervical and parotid nodes are involved more often than submandibular nodes.[131] Constitutional symptoms, present early in the course of the illness, usually are mild and may have resolved by the time the adenitis appears. Fever is observed in one fourth of patients and, if present, has a mean duration of 5 to 7 days.[94] Fever prolonged beyond this time should prompt evaluation for characteristic hepatosplenic granulomatous lesions defined by high-resolution ultrasonography with disseminated cat-scratch disease. Nodes suppurate in one tenth to one third of patients.[29,30] Rare manifestations include Parinaud oculoglandular syndrome,[27] encephalopathy,[91] exanthems[29] (usually of the erythema nodosum type), and osteolytic lesions.[28]

Acquired toxoplasmosis may manifest as regional lymphadenopathy, frequently with posterior cervical node involvement.[102,130,156] Most children exhibit few, if any, constitutional symptoms. If present, fatigue and generalized myalgia are prominent. The characteristic location combined with a history of exposure to cats or of eating undercooked meats should raise this diagnostic possibility, and the diagnosis can be confirmed by serologic testing. It is an uncommon etiology for cervical adenopathy in children living in the United States.

Chronic, recurrent cervical adenitis forms part of the periodic fever, aphthous ulcers, pharyngitis, cervical adenitis (PFAPA) syndrome, a chronic syndrome first described in 1987.[101] It is characterized by periodic episodes of high fever, greater than 39° C (102.2° F), lasting 3 to 6 days and recurring every 3 to 8 weeks in association with aphthous ulcers, pharyngitis, and cervical adenitis. Symptoms such as abdominal pain, nausea, diarrhea, and headache also are described in 20 to 73 percent of children,[157] but if any of these are the dominant features, other hereditary fever syndromes should be excluded.[49] In most children with PFAPA, the onset of disease occurs before they reach 5 years of age, the syndrome is self-limited, and recovery without long-term sequelae is the rule.[169] Oral corticosteroids are effective in aborting an attack.[77,118,157]

Kikuchi-Fujimoto disease, also called *subacute necrotizing lymphadenitis*, is an uncommon disorder of uncertain etiology that also may manifest as cervical adenopathy, with or without fever. This disorder was described first in 1972 and seems to have a predilection for Asian women 25 to 30 years of age, who generally have a benign course with spontaneous resolution over 3 to 4 months.[48,161] Kikuchi-Fujimoto disease also has been reported in children, typically adolescents, although cases in children 2 years old are documented.[36,76,78,164]

In contrast to the 4:1 female predominance documented in adult series, pediatric Kikuchi-Fujimoto disease occurs more commonly in boys (male-to-female ratio ranges from 1.2:1 to 1.9:1). The most common presentation is unilateral or bilateral lymphadenopathy of multiple nodes. In most cases, posterior cervical chain nodes are involved and affected nodes can be painful and tender. Fever is an inconsistent symptom. Extranodal manifestations include malaise, night sweats, weight loss, maculopapular skin rashes, gastrointestinal symptoms, hepatosplenomagaly, arthritis, and aseptic meningitis. Associated laboratory findings include leukopenia (33%-100% of cases with prolonged fever), elevated erythrocyte sedimentation rate, mild anemia, elevated C-reactive protein, and elevated liver enzymes.

Diagnosis is established by biopsy of the lymph nodes and not by fine-needle aspiration cytology, showing lymphadenitis with focal proliferation of reticular cells accompanied by histiocytes and extensive nuclear debris. Lymph node biopsy also has been associated with prompt resolution of fever.[36] Treatment generally is supportive, although steroids are reported to provide more rapid resolution of symptoms. The prognosis is favorable; most cases of Kikuchi-Fujimoto disease resolve within 6 months, although a recurrence rate of 3 to 4 percent is recorded. Some children subsequently develop autoimmune disorders, most commonly systemic lupus erythematosus; thus regular clinical follow-up looking for signs of evolving autoimmune disorder is recommended.[35,109,141,164] Whether Kikuchi-Fujimoto disease is infectious or genetic, perhaps the result of infection with a single novel agent or a nonspecific host response to any of a variety of agents, remains to be determined.

Another rare but important cause of cervical lymphadenitis in association with generalized lymphadenopathy or hepatosplenomegaly is hemophagocytic lymphohistiocytosis, also known as hemophagocytic syndrome. This diagnosis should be considered if the aforementioned features occur with prolonged fever, cytopenia in at least two cell lines, low fibrinogen, and high ferritin and triglyceride serum levels.[119] Other features include rash, respiratory distress, hypotension, and coagulopathy. The diagnosis is confirmed by showing hemophagocytosis in bone marrow or lymph node biopsy specimens. Finally, when presented with a history of subacute or chronic lymphadenitis, careful physical examination should be undertaken to exclude obvious local causes (e.g., seborrhea, head lice, tinea capitis, and chronic otitis media) before an extensive diagnostic evaluation is initiated.

DIFFERENTIAL DIAGNOSIS

Cervical swellings are encountered frequently in pediatric practice, and most of them represent lymph nodes. When considering the diagnostic possibilities in patients with cervical lymphadenitis, whether the pathologic process involves a lymph node first must be ascertained, then whether its cause is infectious, and, if infectious, the likely etiologic agent. The duration of the cervical swelling aids in the differential diagnosis because most tumors or developmental anomalies have been noted for weeks. Rapid enlargement may occur in the latter entities, but usually as a result of secondary infection. Location is a helpful clue because midline masses rarely represent lymph nodes and the most common neck masses of congenital origin (thyroglossal duct cyst, branchial cleft

cyst, and cystic hygromas) have characteristic anatomic locations.

Of the midline masses, thyroglossal duct cysts are the most common.[71,126] These cysts occur from the foramen cecum to the thyroid, are midline, and move on protrusion of the tongue. They may have an associated sinus tract, midline or just lateral to it, from which cloudy mucus sometimes can be expressed. They can become infected secondarily, but, in the noninfected state, these cysts are nontender, smooth, and round, with well-defined margins. Thyroglossal duct cysts must be differentiated from other midline masses, including epidermoid cysts, lipomas, thyroid tumors, and the rare midline lymph node.

The second most common benign congenital neck mass is the branchial cleft cyst. It usually arises from the second branchial cleft and lies at the anterior border of the sternocleidomastoid muscle. Although such cysts usually manifest as skin dimples, they may become infected secondarily and manifest as inflammatory swellings or draining sinus tracts. A careful examination should detect a sinus tract. Branchial cleft cysts can occur in individuals of any age, but usually occur in school-aged children.

Cystic hygromas, considerably less common than thyroglossal duct or branchial cleft cysts, are the third most frequent cause of congenital neck masses. These arise from lymphatics derived from the jugular vein or the mesenchymal tissue. They can occur elsewhere but usually are found posterior to the sternocleidomastoid muscle in the supraclavicular fossa. Most cystic hygromas appear in the first 2 years of life, many being noted at birth or soon thereafter. They are soft, compressible tumors that transilluminate well, and, although benign in themselves, they may cause symptoms through pressure exerted on surrounding structures. Confusion may arise when cystic hygromas increase in size in association with an upper respiratory tract infection. The latter process causes increased lymph flow so that the hygroma persists while other lymph nodes decrease in size after resolution of the infection. In most circumstances, palpation and transillumination readily distinguish these congenital malformations.

These four cervical masses—thyroglossal duct cysts, thyroid tumors, branchial cleft cysts, and cystic hygromas—accounted for 63.7 percent of lesions in children with persistent cervical masses reported by Moussatos and Baffes.[106] Other lesions included neurogenic tumors, parotid tumors, and miscellaneous benign tumors (12.3%). The remainder of masses represented lymph nodes. As a rule, masses located completely anterior to the sternocleidomastoid muscle are benign. The exception is the thyroid tumor.[106] Malignancies that mimic cervical lymph nodes usually are located in the posterior triangle or are multiple masses extending across the anterior and the posterior triangles. In contrast, approximately 50 percent of masses in the posterior triangle represent malignancies, most of which are of lymphoid origin. Although most cysts and tumors manifest as solitary, unilateral, nontender masses, lymph nodes of noninfectious etiology frequently are multiple and bilateral and may be mildly tender.

TABLE 12-2 **Noninfectious Etiology of Cervical Adenitis**

	Isolated Cervical	Cervical Associated with Generalized Adenopathy
Malignancy		
Hodgkin disease	+	+
Non-Hodgkin lymphomas	+	+
Rhabdomyosarcoma	+	–
Neuroblastoma	+	+
Leukemia	+	+
Metastatic carcinoma	+	–
Thyroid tumors	+	–
Drugs		
Isoniazid	–	+
Phenytoin (Dilantin)	–	+
Serum Sickness	–	+
Collagen Vascular Disease		
Juvenile rheumatoid arthritis	–	+
Systemic lupus erythematosus	–	+
Miscellaneous		
Sarcoidosis	–	+
Reticuloendotheliosis	–	+
Sinus histiocytosis with massive lymphadenopathy	+	+
Histiocytosis X	–	+
Postvaccinial	+	–
Storage disorders	–	+
Kawasaki disease	+	+
Hemophagocytic syndrome	–	+
PFAPA syndrome	+	–
Kikuchi-Fujimoto disease	+	+
Masses Simulating Adenopathy		
Cystic hygroma	+	–
Branchial cleft cyst	+	–
Thyroglossal duct cyst	+	–
Epidermoid cyst	+	–
Sternocleidomastoid tumor	+	–

PFAPA, Periodic fever, aphthous ulcers, pharyngitis, cervical adenitis.

Noninfectious chronic inflammatory involvement of cervical lymph nodes may represent a variety of uncommon, usually benign, but sometimes malignant entities (Table 12-2). Fifty percent of malignant neck masses in children are caused by Hodgkin and non-Hodgkin lymphomas. Neuroblastoma is the second most common malignancy, accounting for 15 percent. The likelihood of a given diagnosis is age-dependent, with neuroblastoma being more common than Hodgkin disease in younger age groups.[67] Thyroid tumors are the third most frequent

TABLE 12-3 **Infectious Etiology of Cervical Adenitis**

	Isolated Cervical	Cervical Associated with Generalized Adenopathy
Bacterial		
Staphylococcus aureus	+	–
Group A streptococci	+	+
Mycobacterium tuberculosis	+	+
Nontuberculous mycobacteria	+	–
Bartonella henselae	+	–
Gram-negative enterics	+	–
Anaerobes	+	–
Haemophilus influenzae	+	–
Yersinia pestis	–	+
Actinomyces israelii	+	–
Diphtheria	+	–
Tularemia	+	–
Brucellosis	–	+
Syphilis	+	+
Viral		
Measles	+	+
Rubella	+	+
Epstein-Barr virus	+	+
Herpes simplex	+	–
Human herpesvirus–6	+	+
Cytomegalovirus	+	+
Mumps	+	–
Varicella	+	+
HIV	+	+
Fungal		
Histoplasmosis	+	+
Cryptococcus	+	–
Aspergillosis	+	–
Candida	+	–
Sporotrichosis	+	–
Parasitic		
Toxoplasma gondii	+	+

HIV, Human immunodeficiency virus.

neck malignancies. Other entities to be included in the differential diagnosis include leukemia,[26] metastatic carcinoma,[106] phenytoin-induced pseudolymphoma,[26] serum sickness,[26] storage disorders (Gaucher disease and Niemann-Pick disease), collagen vascular disease,[26] sarcoidosis,[69] sinus histiocytosis with massive lymphadenopathy,[12,133] and reticuloendotheliosis or histiocytosis X. Except for malignancies, these disease entities almost always are associated with lymphadenopathy that is not limited to the cervical region and have a variety of clinical and laboratory findings that allow the correct diagnosis to be made.

Numerous infectious agents have been reported in association with cervical adenitis in infants and children (Table 12-3). Among patients evaluated prospectively

FIGURE 12-4 ■ A 9-year-old boy who developed high fever and markedly tender submental lymph node inflammation after a tooth extraction. Cultures from this fluctuant mass grew three anaerobes and viridans streptococci.

with needle aspirate cultures or cultures from incision and drainage specimens of affected lymph nodes, *S. aureus* or group A streptococci are the organisms most frequently isolated.* No significant difference has been reported that distinguishes between patients with adenitis caused by streptococci or staphylococci with respect to sex, dental problems, symptoms, presence of fever, or site or size of lymph nodes. In patients from whom *S. aureus* is isolated, a longer duration of disease before diagnosis is established and a larger percentage of fluctuant lymph nodes[13,151] and tendency toward slower resolution often are found.[36] Duggal and associates[40] reported that *S. aureus* was 10-fold more common in infants 16 months of age and younger than in older children with neck abscesses and also noted a predominance of CA-MRSA among black children. Most patients with bacterial cervical lymphadenitis, including patients with mycobacterial infection, are 1 to 6 years of age. Older children are more likely to have negative lymph node aspirate cultures.[13,143,151]

In early studies, anaerobes rarely were associated with cervical adenitis.[13,37] Proper bacteriologic techniques for the isolation of these fastidious organisms allowed Brook[24] to report anaerobes alone in 18 percent and mixed anaerobic and aerobic bacteria in 20 percent of patients, suggesting that anaerobic organisms play a more significant role in the etiology of cervical lymphadenitis than recognized previously. An older child with "negative" cultures, especially a child with poor dental hygiene or periodontal disease, may have anaerobic infection, as did the 9-year-old boy in Figure 12-4. Needle aspiration of this cervical mass yielded *Peptococcus* and *Peptostreptococcus* spp., *Bacteroides fragilis*, and viridans streptococci. Resolution of the lymphadenitis occurred promptly after incision and drainage and penicillin therapy.

*References 13, 24, 37, 40, 151, 170.

Dental disease or manipulation also should suggest the possibility of cervicofacial actinomycosis (lumpy jaw), an uncommon entity in children. These patients have a chronic submandibular mass and frequently a fistula from the skin to the oral cavity.[16] Less frequently occurring bacteria,[75] viruses, fungi, and parasites can cause cervical lymphadenitis in children, but these patients usually have less evidence of acute inflammation, with or without adenopathy at additional sites, and historical and physical findings that suggest unusual causes of cervical lymph gland enlargement.

SPECIFIC DIAGNOSIS

A detailed history to ascertain preceding dental problems, presence of skin lesions, animal exposure (including exposure to fleas and ticks), duration of illness, presence of associated symptoms, contact with tuberculosis, presence of risk factors for HIV infection, drug usage (especially phenytoin) or other unusual ingestions (e.g., undercooked meat or unpasteurized dairy products), recent travel outside the geographic region of residence, and sites of occult infection drained by the affected node may yield important diagnostic clues in a patient with cervical lymphadenitis. Physical examination should include careful inspection for the presence of dental disease, noncervical lymphadenopathy, hepatosplenomegaly, and oropharyngeal or skin lesions.

Radiologic evaluation of adenitis is unnecessary in most mild to moderate cases. Ultrasonography often is performed when the presenting neck mass is very large, is increasing in size, or has not responded to initial antibiotic therapy. It is useful in diagnosing suppuration and expediting incision and drainage. High-resolution and color Doppler ultrasonography (with or without contrast enhancement) defining longitudinal to transverse nodal ratio and vascularity patterns has had some success in differentiating benign and malignant lymph nodes in adults.[2,105,107,168,171]

In children in whom most adenopathy is infectious or reactive in etiology, ultrasonography is less discriminating. In one study of 35 children, ultrasonography showed significant differences in lymph nodes in 22 children with a diagnosis of Kawasaki disease in contrast to 8 children with bacterial lymphadenitis, but findings were similar to those in children with lymphadenitis secondary to Epstein-Barr virus infection.[154]

In another study of 146 children, unilateral lymph nodes and cystic necrosis were found only in lymphadenitis caused by cat-scratch disease or bacterial or tuberculous infection.[122] Individual sonographic findings were nonspecific for diagnosis, although these findings combined with clinical signs were helpful. No patients with nontuberculous mycobacterial adenitis were evaluated in this study. Nodal calcifications and spread of nodal masses into the subcutaneous tissues by ultrasonography and characteristic low-density, ring-enhancing lesions with minimal or absent inflammatory stranding of subcutaneous fat seen on computed tomography and magnetic resonance imaging have been reported with nontuberculous mycobacterial adenitis in children.[59,63,86,132] These findings may be helpful in differentiating this etiology from other bacterial causes when the diagnosis is not suspected clinically or early in the course. Differences in clinical presentation between the two entities should obviate the need for such imaging, however, in all but the most complicated of cases.

In the acute stage of cervical lymphadenitis, needle aspiration of the affected node is a valuable diagnostic tool. Of patients with acute cervical lymphadenitis subjected to needle aspiration of the affected node for bacterial and mycobacterial culture, 50 to 88 percent have an etiologic agent recovered.[13,24,70,147,170] Only inflamed nodes should be aspirated, but they need not be fluctuant. No serious complications of this procedure have been recorded.

The largest or most fluctuant node should be selected, and the skin should be cleansed and anesthetized. Skin anesthesia can be induced effectively using a topical anesthetic cream (e.g., lidocaine-prilocaine [EMLA]) placed on the selected aspiration site under an occlusive dressing 30 to 45 minutes before the procedure. An 18- or 20-gauge needle attached to a 20-mL syringe is used. If no material is aspirated, sterile *nonbacteriostatic* saline 1 to 2 mL is injected into the node and reaspirated. The aspirate should be inoculated directly from the syringe onto aerobic (including chocolate agar) and anaerobic media, onto Sabouraud agar (fungi), and into a broth medium suitable for the early detection of mycobacteria, such as the Bactec radiometric assay. In the latter system, the release of labeled carbon dioxide in an automated ion chamber system can detect mycobacteria 12 to 17 days after inoculation of the broth.[142] When mycobacterial infection is suspected, consultation with the laboratory is helpful to ensure that cultures are handled correctly because some species, such as *M. haemophilum*, require that the culture be kept at a temperature of 25°C (77°F).[8] Gram and acid-fast stains are mandatory and serve as a guide to initial antimicrobial therapy.

Polymerase chain reaction assays have successfully confirmed the presence of tuberculous and nontuberculous mycobacteria in gastric aspirates and specimens obtained from lymph nodes by aspiration or biopsy.* This technique shows great promise in rapidly providing a specific diagnosis, but it is not yet routinely available.

Thioglycolate broth and anaerobically incubated blood agar plates are incapable of providing optimal conditions for the isolation of many anaerobic bacteria.[13] Optimal methods for cultivation of fastidious anaerobes should be employed because anaerobic organisms may be recovered in 20 percent of cases.[24] Cultures of infected skin lesions and exudates on tonsils also should be done, but not to the exclusion of needle aspiration.

Isolation of group A streptococci from the throat or skin cultures of a patient with lymphadenitis does *not* confirm the etiology of the lymph node inflammation. Patients have been noted to have isolation of group A streptococci from throat and of *S. aureus* from lymph node aspirate cultures.[13,127] Tuberculin skin tests should

*References 41, 47, 57, 58, 140, 145.

be performed. Induration of 15 mm or greater suggests infection with *M. tuberculosis*, whereas reactions of 5 to 14 mm may be caused by either a tuberculous or a nontuberculous mycobacterial infection, because the purified protein derivative preparation shares some antigens with nontuberculous species.[7,95]

In an attempt to develop a rational approach to the use and interpretation of differential skin testing, Huebner and associates,[65] using tuberculin skin tests and nontuberculous mycobacterial antigens, studied 144 children with chronic cervical adenopathy, of whom 123 had mycobacterial culture results available. The low incidence of tuberculosis within the study population (four cases) precluded making an interpretation regarding the utility of these antigens in distinguishing disease caused by *M. tuberculosis* from that caused by other mycobacteria. Children with culture-confirmed mycobacterial lymphadenopathy had significantly larger reactions to nontuberculous mycobacterial antigens than did children with microscopy-negative and culture-negative results. The study was terminated prematurely because of an unacceptably high incidence of a blistering skin reaction to nontuberculous mycobacterial antigens. IFN-γ release assays may be useful pending definitive cultures because these assays are positive with tuberculosis but negative with nontuberculous agents, with the exception of mycobacterial species noted in Table 12-1.

Intradermal skin testing, using a crude extract from affected nodes, historically has been used to establish the diagnosis of cat-scratch disease.[29] A diagnosis usually is reached, however, based on the presence of regional adenopathy, a history of cat exposure (particularly if the patient has a history of a scratch or a primary skin lesion), and negative laboratory studies for other causes of lymphadenopathy. Serologic methods for the detection of immunoglobulin (Ig)G antibodies to *Bartonella* spp. are available[1,6,172] and should be considered the gold standard for diagnosis. However, sensitivity varies among laboratories. Polymerase chain reaction assays are available in some commercial and research laboratories and at the Centers for Disease Control and Prevention.[6] In a few cases, a lymph node biopsy should be undertaken to exclude other, more serious pathologies.

If, after the aforementioned evaluation, the etiology of adenitis remains uncertain or lymphadenopathy has persisted with no detectable response to antimicrobial therapy, a more intense diagnostic evaluation is indicated. Studies may include a complete blood count; serology for Epstein-Barr virus, cytomegalovirus, HHV-6, HIV, histoplasmosis, coccidioidomycosis, toxoplasmosis, tularemia, *B. henselae*, and *Brucella;* and a radiograph of the chest. If the diagnosis remains in doubt, and the node persists, enlarges, is hard, or is fixed to the adjacent structures, biopsy *should* be performed. Biopsy material should be submitted for the studies outlined earlier for lymph node aspirate cultures and routine histology; Giemsa, periodic acid–Schiff, and methenamine silver stains; and, in select cases only, viral cultures. If the histologic examination reveals noncaseating granulomas and the child has a history of cat exposure, the most likely diagnosis is cat-scratch disease.[94,96] Sarcoidosis involving lymph nodes would have a similar histology but is rare in children and a condition in which isolated cervical node involvement has not been observed.[69,139]

Older children are more likely to have negative cultures of lymph node aspirates[13,151] and to be more frequent candidates for excisional lymph node biopsy. They also are more likely to have lymphomas. It is important that appropriate tissue be excised, especially from adolescents, so that precise diagnostic interpretation can be done. This interpretation can be facilitated by the proper selection of a lymph node for a biopsy to be performed; intact removal of the node chosen; and proper fixation, cutting, and staining of the specimen. If only one node or one anatomic group of nodes is enlarged, the largest node should be excised. If several groups of lymph nodes are involved, the site for biopsy should be selected according to the likelihood of diagnostic yield. Biopsy specimens from the lower neck and supraclavicular area have the highest yields.[72] Other areas, including the upper cervical, submandibular, axillary, and parotid lymph nodes, are much more likely to be affected by reactive hyperplasia, which may or may not be related to the underlying disease process. If lymphoma is suspected, needle biopsies or frozen sections are contraindicated.[18,26]

Even under optimal conditions, many reactive processes, including rheumatoid arthritis, toxoplasmosis, phenytoin-induced adenopathy, dermatopathic adenitis, and infectious mononucleosis, have been noted to simulate lymphoma.[26] Obtaining a thorough history and performing appropriate serologic studies should provide sufficient information for the physician to exclude reactive processes known to simulate lymphoma.

TREATMENT

Optimal management of a child with cervical lymphadenitis depends on an accurate assessment of the underlying etiology. Because almost all cases are associated with infectious agents, every effort should be made to ascertain the etiologic agent so that specific therapy can be initiated. Aspiration of the affected lymph node for Gram and acid-fast stains is a guide for initial therapy, and culture and antimicrobial susceptibility form the basis for prescribing specific treatment in patients with bacterial lymphadenitis.[13,37] When the patient has findings typical of acute bacterial lymphadenitis, however, empiric therapy may be undertaken without prior needle aspiration. In this situation, close follow-up is essential because failure to show some clinical response after 48 hours of therapy is an indication for further diagnostic procedures to be done.

Acute suppurative cervical lymphadenitis most frequently is caused by infection with *S. aureus* or group A streptococci.* In nodes that progress to abscess formation, *S. aureus* is the most frequent agent isolated,[13,37,40,138] and drainage is mandatory. Because of the frequency of infection caused by *S. aureus* or group A streptococci, empiric antimicrobial therapy should be directed against

*References 13, 22, 37, 138, 151, 170.

these two agents. Penicillinase-resistant penicillins should be used. If the patient requires parenteral therapy and CA-MRSA is uncommon in the geographic area, oxacillin or nafcillin 150 mg/kg/day or cefazolin 75 to 100 mg/kg/day may be used.

When oral therapy is deemed adequate, cloxacillin 50 mg/kg/day, dicloxacillin 25 mg/kg/day, or cephalexin 25 to 50 mg/kg/day is recommended. Augmentin, the fixed combination of amoxicillin and clavulanic acid, provides good activity against methicillin-susceptible staphylococci and streptococci and has an expanded spectrum of activity against the oral anaerobic organisms. These features, combined with its palatability, render it an attractive alternative to the traditional penicillinase-resistant penicillins. Clavulanate-associated diarrhea can be problematic in some children. In penicillin-allergic patients, cephalosporins can be used. Ceftriaxone 50 to 100 mg/kg/day is an attractive and effective alternative to the parenteral antibiotics that require more frequent administration.

In areas where CA-MRSA is prevalent and clindamycin resistance is low, clindamycin 30 mg/kg/day for parenteral or oral use is appropriate for empiric or alternative therapy. In addition to its good activity against anaerobes and methicillin-susceptible *S. aureus*, clindamycin is effective against most CA-MRSA isolates. However, clindamycin resistance is increasing among CA-MRSA isolates, so close clinical follow-up to ensure a therapeutic response has been achieved is mandatory when this option is chosen.[44,64,66,68,108] In a severely ill child needing hospitalization or with signs of airway compromise, vancomycin 60 mg/kg/day divided every 6 hours is appropriate in combination with another agent until culture results are obtained. Trimethoprim-sulfamethoxazole 10 mg/kg/day of the trimethoprim component or doxycycline 2 to 4 mg/kg/day if the child is aged 8 years or older are alternative choices for oral therapy of CA-MRSA infections[44,64,66,108] but should not be used initially because they are not active against group A streptococci. Linezolid is active against MRSA and group A streptococci, but because of concerns regarding cost and bacterial resistance, use should be reserved for cases in which a microbiological need has been confirmed or clinical failure of first-line therapies has occurred.

Antibiotic therapy may need to be modified if an obvious primary focus of infection suggests a different etiologic agent. In a patient with periodontal or dental disease, adequate anaerobic activity is mandatory, and therapy with penicillin V 50 mg/kg/day, amoxicillin-clavulanate 40 mg/kg/day, or clindamycin 30 mg/kg/day should be initiated, pending results of cultures.

Patients with marked lymph node enlargement, moderate to severe systemic symptoms, or concomitant cellulitis frequently require parenteral therapy for the first few days. This therapy allows for a high concentration of the antimicrobial agent within the inflamed tissue and may promote more rapid localization, especially in patients with staphylococcal adenitis. Although the use of parenteral drugs has to be individualized, most infants and children with staphylococcal or streptococcal lymphadenitis respond to orally administered antimicrobials.

Adenitis caused by group A streptococci should be treated with penicillin G 100,000 IU/kg/day or penicillin V 50 mg/kg/day for a total of 10 days. In a child with penicillin allergy, erythromycin ethyl succinate 40 mg/kg/day; azithromycin 10 mg/kg/day on day 1, then 5 mg/kg/day on days 2 through 5; or cephalexin 25 to 50 mg/kg/day can be used. These drugs have been shown effective in the treatment of cervical lymphadenitis.[13,22] Treatment should be continued for at least 10 days or approximately 5 days after signs of local inflammation and systemic toxicity have disappeared, whichever is longer. If required, analgesics should be given and not overlooked in infants and children too young to verbalize their discomfort. The average duration of antibiotic therapy is 10 days, unless abscess formation occurs late in the first or early in the second week of treatment.[13] In this situation, incision and drainage is indicated,[13,23,37] and therapy should be continued until resolution of the acute process occurs, usually within another 5 to 7 days.

Some clinical improvement is to be expected within 48 hours after initiation of therapy and is manifested by a decrease in inflammation and tenderness of the lymph node and a decrease in the maximum daily temperature. The size of the lymph node may not show evidence of regression at this stage, and total resolution of fever should not be expected. It is important to record accurate measurements of the node at the time of presentation because a subjective evaluation is an unreliable indicator of lymph node evolution during therapy. If no clinical improvement is noted by 48 hours, needle aspiration is recommended. The history and physical examination should be reassessed, and a more detailed laboratory evaluation should be initiated.

In a study of 284 children admitted to the hospital with acute cervical adenitis, age younger than 1 year and node involvement for more than 48 hours before admission predicted the need for node aspiration.[90] Regression of the size of a lymph node is slow, usually requiring 4 to 6 weeks or more. Persistence of significant enlargement beyond 6 to 8 weeks, even in the face of good initial response to antimicrobial therapy, demands that an underlying disorder be excluded. When signs of acute inflammation have resolved, prolonged antimicrobial therapy is of little value because penetration of antimicrobials through the fibrous capsule of the node is poor.[22] Spontaneous regression occurs in most patients, although it may require several weeks. Uncommonly, reactivation of inflammation may occur, and a meticulous search for an untreated primary source of bacterial infection, such as secondarily infected dermatitis, infestation, foreign body, or dental abscess, should be undertaken. Re-treatment should include specific measures to eliminate the predisposing condition.

If Gram stain of the lymph node aspirate suggests a microorganism other than *S. aureus* or group A streptococci, initial antimicrobial therapy should include therapy for the most likely agent until culture results are known. Because attempts to perform careful Gram stains and careful anaerobic cultures of lymph node aspirates in most reported series have been limited, the large number of infants and children with sterile aspirates may be attributed partly to a failure to isolate fastidious

anaerobes indigenous to the mouth. These microorganisms should respond to penicillin G therapy. For penicillin-resistant organisms, clindamycin is a useful alternative drug.

For infants in the first 2 months of life, group B streptococci and *S. aureus* are important pathogens to consider in selecting initial therapy. Penicillinase-resistant penicillins are active against both agents, unless the occurrence of CA-MRSA is frequent, in which case clindamycin or vancomycin should be considered for initial therapy. If group B streptococci are isolated, penicillin G can be substituted. Final bacteriologic identification and antimicrobial susceptibility tests should be the ultimate guide to selecting specific antimicrobial therapy in all patients. Treatment of cervical lymph node infections associated with rarely encountered bacteria, fungi, and parasites listed in Table 12-3 is discussed under those specific disease entities.

Although controversy exists as to whether cervical adenitis associated with *M. tuberculosis* in a child is truly a localized process, only rarely do patients have disseminated infection.[38,56,115] When infection is not localized, pulmonary or hilar lymph node involvement is a common finding.[38,70,116,170] A 2-month regimen of isoniazid 10 mg/kg/day, rifampin 10 to 20 mg/kg/day, pyrazinamide 30 mg/kg/day, and ethambutol 20 mg/kg/day is recommended for the treatment of uncomplicated pulmonary tuberculosis or isolated cervical lymphadenitis in children. This therapy is given for 2 months, daily for at least the first 2 weeks, and then 2 to 3 times per week, after which isoniazid and rifampin are administered 2 to 3 times per week for the ensuing 4 months.[8]

In areas where multidrug resistance in *M. tuberculosis* is prevalent, streptomycin 20 to 40 mg/kg/day or another aminoglycoside (kanamycin, amikacin, or capreomycin) is added for initial treatment until drug susceptibilities are known.[8] The addition of a fifth drug should occur after consultation with an expert in the field because these drugs may have toxic effects and careful assessment of the risks and benefits is warranted. Detailed discussion of the treatment of tuberculosis in children is provided in Chapter 96. Response to antituberculous therapy is usual, with rapid resolution of symptoms and marked regression of lymph nodes within 3 months. Nodes remain palpable for months, however, because scarring and fibrosis are regular accompaniments to resolution of disease. Draining sinuses, a common complication of lymph node aspiration or incision, and drainage before the advent of effective antituberculosis chemotherapy no longer develop.[107]

Cervical lymphadenitis attributed to nontuberculous mycobacteria is much more common in a young child than that caused by *M. tuberculosis.* These microorganisms exhibit in vitro resistance to commonly employed antituberculosis drugs. Resistance particularly is common among *M. scrofulaceum* and *M. intracellulare*. Surgical excision remains the treatment of choice for nontuberculous mycobacterial lymphadenitis, and total removal of all the visibly affected nodes is recommended.* Early (within 1 month of onset) removal of affected nodes was associated significantly with better aesthetic results.[92] Thorough curettage has been found effective,[82,93,115] but results in delayed healing, higher relapse rates, and less favorable aesthetic result.[45,82]

Antimicrobial therapy alone is less effective.[83,99] The macrolides clarithromycin and azithromycin, rifampin and its analogue rifabutin, ethambutol, amikacin, and cefoxitin show activity against nontuberculous mycobacteria.[7,55,153] Retrospective case series with small cohorts of patients showed that clarithromycin monotherapy and combination therapy with clarithromycin and ethambutol or rifampin (or rifabutin) had some success in the treatment of nontuberculous lymphadenitis and a relatively low rate of recurrence.* The regimens used generally were well tolerated. Rifabutin resulted in adverse effects in four of seven patients treated for 6 months in one study (neutropenia and yellow skin pigmentation), which disappeared after dose reduction.[88] In HIV-infected children receiving protease inhibitors, rifabutin generally is contraindicated, but it can be given at a much reduced dosage if deemed necessary.[7]

In one randomized controlled clinical trial, 100 children (median age 45.5 months, range 9 to 168 months) with microbiologically proven nontuberculous lymphadenitis received either surgical resection or clarithromycin 15 mg/kg/day and rifabutin 5 mg/kg/day orally for 12 weeks.[83] There was a 30% higher cure rate (96% vs. 66%) for patients treated surgically in contrast to those who received antibiotics alone. The cure rate was not influenced by lymph node stage of infection or infecting mycobacterial species. Postoperative wound infection and facial nerve weakness occurred in 12% and 14% of children, respectively, and, in one child, facial nerve weakness was permanent. In the two patients in whom surgical resection was not curative, the lesions responded to 3 months of the antibiotic regimen. In a follow-up study of this cohort 1 year later, aesthetic outcome also was significantly better in the surgical group.[85] In another study of 50 patients with advanced nontuberculous mycobacterial adenitis who received either the previously mentioned antibiotic regimen or no treatment, the median time to resolution was only 4 weeks earlier (36 vs. 40 weeks) in the treated group.[81]

The conclusion from studies to date is that regimens of a macrolide (clarithromycin or azithromycin) plus either ethambutol and/or rifampin (or rifabutin) are options for the treatment of nontuberculous mycobacterial adenitis for which complete excision of the affected node would endanger the facial nerve or its branches, if a reduction in size of the swelling would facilitate a complete and aesthetic excision at a later stage, or if surgery is refused.[7] Macrolide monotherapy is not recommended because of the risk for inducing resistant organisms. The optimal duration of therapy is unknown, but regimens of 4 to 6 months or longer are usual. Expert opinion should be sought, especially as new mycobacterial species are described.

Cat-scratch disease usually is a benign, self-limited disorder requiring no specific therapy, but the use of

*References 4, 7, 31, 43, 61, 82, 83, 97, 113, 121, 129, 135, 147, 166.

*References 17, 54, 63, 88, 89, 93, 155.

antimicrobials may relieve symptoms and hasten recovery. Rifampin, trimethoprim-sulfamethoxazole, azithromycin, ciprofloxacin, and parenteral gentamicin may be useful in promoting fever defervescence and in clinical resolution of systemic cat-scratch disease.[6,9,14,98] If the lymph node progresses to fluctuance, needle aspiration may hasten resolution and relieve discomfort. Surgical excision may be required in a few patients who have persistent problems despite having needle aspiration or who develop draining sinuses.

PROGNOSIS

With effective antimicrobial therapy, complete resolution of cervical lymphadenitis caused by *S. aureus*, group A streptococci, and *M. tuberculosis* is the rule. Delay in establishing the diagnosis or initiating therapy can prolong the clinical course and result in complications or sequelae, such as sinus tracts (mycobacteria),[19,34,116] abscess formation,[23,40] cellulitis or bacteremia (*S. aureus* and *S. pyogenes*),[13] acute glomerulonephritis (group A streptococci),[37] disseminated disease (*M. tuberculosis*),[70] or mycotic carotid artery aneurysm.[165] Except for abscess formation, these complications are rare events. Although lymph node infection caused by *S. aureus* is more likely to result in abscess formation, at least one study has noted a significantly greater duration of infection before treatment in patients in whom *S. aureus* was isolated from the abscess cavity cultures.[13] The extracellular products of this organism (e.g., coagulase, fibrinolysin, and hyaluronidase) partly explain its propensity for abscess formation, which occurs in 50 to 70 percent of patients.[23,37,167]

Even in patients whose course is complicated by suppuration, appropriate drainage in conjunction with specific antimicrobial therapy results in prompt resolution of signs and symptoms and relapse occurs only rarely. Today, surgical excision of affected nodes seldom is recommended except when the disease is caused by nontuberculous mycobacteria, in which case surgical excision remains the treatment of choice. Antimicrobial therapy has been responsible for the disappearance of the events commonly associated with cervical adenitis historically, including thrombosis of the internal jugular vein, rupture of the carotid artery, generalized septic embolic phenomena, mediastinal abscess, purulent pericarditis, and even death.[73,150,167]

With the advent of effective antituberculous agents, the prognosis for tuberculous cervical adenitis also is excellent. When surgical excision is performed early in the course of lymphadenitis caused by nontuberculous mycobacterial infection, resolution can be anticipated.* Persistent and recurrent disease is the most frequent complication encountered.[166] Macrolide monotherapy and combination therapy are useful in ameliorating these complications or in cases for which surgery is not feasible.[7,123,146] Cat-scratch disease usually is a benign, self-limited disorder in which therapeutic intervention, such as needle aspiration, to relieve pain is uncommonly needed, but antibiotic therapy can hasten recovery.

*References 45, 92, 121, 129, 135, 146.

PREVENTION

Providing appropriate medical and, occasionally, surgical therapy of predisposing conditions (e.g., dental caries, abscess, group A streptococcal pharyngitis or nasopharyngitis, purulent otitis media, impetigo, other infections involving the face and scalp) and minimizing the exposure of infants and children to adults with active tuberculosis should reduce the incidence of cervical lymphadenitis. Some authors suggest that decreased exposure to animals may result in fewer infections,[13] especially for adenitis attributed to toxoplasmosis or *Bartonella*.[30,130]

NEW REFERENCES SINCE THE SIXTH EDITION

6. American Academy of Pediatrics. Cat-scratch disease. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 269–71.
7. American Academy of Pediatrics. Diseases caused by nontuberculous mycobacteria. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 759–67.
8. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 736–59.
25. Brook I. The increased risk of community-acquired methicillin-resistant *Staphylococcus aureus* in neck infections in young children. *Curr Infect Dis Rep* 2012;**14**:119–20.
35. Cheng CY, Sheng WH, Lo YC, et al. Clinical presentations, laboratory results and outcomes of patients with Kikuchi's disease: emphasis on the association between recurrent Kikuchi's disease and autoimmune diseases. *J Microbiol Immunol Infect* 2010;**43**:366–71.
40. Duggal P, Naseri I, Sobol SE. The increased risk of community-acquired methicillin-resistant *Staphylococcus aureus* in young children. *Laryngoscope* 2012;**121**:51–5.
49. Gattorno M, Caorsi R, Meini A, et al. Differentiating PFAPA syndrome from monogenic periodic fevers. *Pediatrics* 2009;**124**: e721–8.
50. Gerber JS, Coffin SE, Smathers SA, Zaoutis TE. Trend in the incidence of methicillin-resistant *Staphylococcus aureus* infections in children's hospitals in the United States. *Clin Infect Dis* 2009;**49**:65–71.
55. Griffith DS, Aksamit T, Brown-Ellitt BA, et al. An official ATS/IDSA statement: diagnosis, treatment and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
81. Lindeboom JA. Conservative wait-and-see therapy versus antibiotic treatment for nontuberculous cervicofacial lymphadenitis in children. *Clin Infect Dis* 2011;**52**:180–4.
82. Lindeboom JA. Surgical treatment for nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis in children. *J Oral Maxillofac Surg* 2012;**70**:345–8.
83. Lindeboom JA, Kuijper EJ, Bruijnestein van Coppenraet ES, et al. Surgical excision versus antibiotic treatment for nontuberculous mycobacterial cervicofacial lymphadenitis in children: a multicenter, randomized, controlled trial. *Clin Infect Dis* 2007; **44**:1057–64.
84. Lindeboom JA, Kuijper EJ, Prins JM, et al. Tuberculin skin testing is useful in the screening for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Clin Infect Dis* 2006;**43**: 1547–51.

85. Lindeboom JA, Lindeboom R, Bruijnestein van Coppenraet ES, et al. Esthetic outcome of surgical excision versus antibiotic therapy for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Pediatr Infect Dis J* 2009;**28**:1028–30.
147. Starke JR. The natural history of nontuberculous mycobacterial cervical adenitis. *Pediatr Infect Dis J* 2008;**27**:923–4.
163. Wagner KS, White JM, Lucenko I, et al. Diphtheria in the post-epidemic period, Europe, 2000-2009. *Emerg Infect Dis* 2012;**18**:217–25.
170. Wurster WM, Carlucci JG, Feder HM, Edwards KM. Long-term follow-up of children with periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome. *J Pediatr* 2011;**159**: 958–64.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 13

PAROTITIS

Judith R. Campbell

Parotitis, inflammation of the parotid gland, is caused by a variety of infectious agents and noninfectious systemic illnesses. Several terms are used to describe the clinical presentations and etiologic processes that lead to parotid gland swelling and inflammation. *Suppurative parotitis*, first described in the 1800s, is a serious bacterial infection in neonates and postsurgical patients.[50] *Epidemic parotitis*, particularly prevalent in the prevaccine era, was caused primarily by mumps virus infection.[57] In the postvaccine era, this form of parotitis also is caused by other viral pathogens and is referred to as *viral parotitis*. Rarely, a more indolent, slowly progressive, granulomatous infection may occur that is referred to as *granulomatous parotitis*. *Recurrent parotitis of childhood* is a unique illness characterized by multiple episodes of acute and subacute parotid gland swelling. The histologic findings in this disease include architectural changes in the ducts and chronic inflammation. Many noninfectious systemic illnesses cause persistent or recurrent parotid gland swelling and inflammation, which is referred to as *chronic parotitis*. Bilateral parotid enlargement is a frequent presentation of human immunodeficiency virus (HIV) infection as part of the *diffuse infiltrative lymphocytosis syndrome*.

PATHOPHYSIOLOGY

Despite the various agents that cause parotitis, involvement of the gland occurs mainly by three mechanisms. The most common mechanism is a localized infection limited to the gland and surrounding structures. Parotitis may be a manifestation of a systemic infection, as in mumps, or rarely may develop secondary to hematogenous seeding during periods of transient bacteremia. Several common contributing factors and pathophysiologic mechanisms lead to swelling of the gland. The parotid is well encapsulated and consists of superficial and deep lobes separated by the facial nerve. The parotid duct (Stensen duct) traverses the buccal soft tissue anteriorly and exits opposite the second upper molar. Thin, watery secretions from the parotid gland cleanse the ductal system and have some bacteriostatic properties, preventing accumulation of bacteria and debris.[37] Factors that predispose individuals to the development of parotitis include side effects of certain drugs and diseases that lead to dehydration, xerostomia, or ductal obstruction (Table 13-1).[37,50] Decreased salivary flow allows retrograde migration of bacteria. Stasis in the ductal system, caused by ductal ectasia, inflammation, calculi, or strictures, allows proliferation of bacteria and inflammation within the gland.

ETIOLOGY

Infectious parotitis may be caused by aerobes, anaerobes, mycobacteria, and viruses (Table 13-2). In all age groups, *Staphylococcus aureus* is the organism most commonly associated with suppurative parotitis.[48,50] Gram-negative pathogens (e.g., *Escherichia coli* and *Klebsiella* and *Pseudomonas* spp.) also may cause suppurative parotitis, particularly in neonates and debilitated or hospitalized patients.[11,32,49,50] The role of anaerobic organisms in this infection has become apparent, especially when poor oral hygiene and oral pathology are associated features.[4,5,40] In cases of recurrent parotitis of childhood, *Streptococcus* spp. are the bacteria most commonly

REFERENCES

1. Adal KA, Cockerell CJ, Petri WA Jr. Cat scratch disease, bacillary angiomatosis, and other infections due to *Rochalimaea*. *N Engl J Med* 1994;**330**:1509–15.
2. Ahuja AT, Ying M, Ho SS, Metreweli C. Distribution of intranodal vessels in differentiating benign from metastatic neck nodes. *Clin Radiol* 2001;**56**:197–201.
3. Akashi K, Eizuru Y, Sumiyoshi Y, et al. Brief report: severe infectious mononucleosis-like syndrome and primary human herpes 6 infection in an adult. *N Engl J Med* 1993;**329**:168–71.
4. Altman RP, Margileth AM. Cervical lymphadenopathy from atypical mycobacteria: diagnosis and surgical treatment. *J Pediatr Surg* 1975;**10**:419–22.
5. Alvi A, Myssiorek D. *Mycobacterium chelonae* causing recurrent neck abscess. *Pediatr Infect Dis J* 1993;**12**:617–18.
6. American Academy of Pediatrics. Cat-scratch disease. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 269–71.
7. American Academy of Pediatrics. Diseases caused by nontuberculous mycobacteria. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 759–67.
8. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 736–59.
9. Arisoy ES, Correa AG, Wagner ML, et al. Hepatosplenic cat-scratch disease in children: Selected clinical features and treatment. *Clin Infect Dis* 1999;**28**:778–84.
10. Armstrong KL, James RW, Dawson DJ, et al. *Mycobacterium haemophilum* causing perihilar or cervical lymphadenitis in healthy children. *J Pediatr* 1992;**121**:202–5.
11. Baker CJ. Group B streptococcal cellulitis-adenitis in infants. *Am J Dis Child* 1982;**136**:631–3.
12. Bankaci M, Morris RF, Stool SE, et al. Sinus histiocytosis with massive lymphadenopathy: report of its occurrence in two siblings with retropharyngeal involvement in both. *Ann Otol Rhinol Laryngol* 1978;**87**:327–31.
13. Barton LL, Feigin RD. Childhood cervical lymphadenitis: a reappraisal. *J Pediatr* 1974;**84**:846–52.
14. Bass JW, Freitas BC, Freitas AD, et al. Prospective randomized double blind placebo-controlled evaluation of azithromycin for treatment of cat-scratch disease. *Pediatr Infect Dis J* 1998;**17**: 447–52.
15. Bell DM, Morens DM, Holman RC, et al. Kawasaki syndrome in the United States. *Am J Dis Child* 1983;**137**:211–14.
16. Bennhoff DF. Actinomycosis: diagnostic and therapeutic considerations and a review of 32 cases. *Laryngoscope* 1984;**94**: 1198–217.
17. Berger C, Pfyffer GE, Nadal D. Treatment of nontuberculous mycobacterial lymphadenitis with clarithromycin plus rifabutin. *J Pediatr* 1996;**128**:383–6.
18. Betsill WL Jr, Hajdu SI. Percutaneous aspiration biopsy of lymph nodes. *Am J Clin Pathol* 1980;**73**:471–9.
19. Black BG, Chapman JS. Cervical adenitis in children due to human and unclassified mycobacteria. *Pediatrics* 1984;**33**:887–93.
20. Braun MM, Cauthen G. Relationship of the human immunodeficiency virus epidemic to pediatric tuberculosis and bacillus Calmette-Guérin immunization. *Pediatr Infect Dis J* 1992;**11**: 220–7.
21. Bronzwaer SL, Buchholz U, Kool JL, et al. EARSS activities and results: Update. *Eur Surveill* 2001;**6**:2–5.
22. Brook AH, Winter GB. Cervico-facial suppurative lymphadenitis due to staphylococcal infection in childhood. *Br J Oral Surg* 1971;**8**:257–63.
23. Brook AH, Winter GB. Staphylococcal cervico-facial lymphadenitis in children. *Lancet* 1972;**2**:660–1.
24. Brook I. Aerobic and anaerobic bacteriology of cervical adenitis in children. *Clin Pediatr* 1980;**19**:693–6.
25. Brook I. The increased risk of community-acquired methicillin-resistant *Staphylococcus aureus* in neck infections in young children. *Curr Infect Dis Rep* 2012;**14**:119–20.
26. Butler JJ. Non-neoplastic lesions of lymph nodes of man to be differentiated from lymphomas. *Natl Cancer Inst Monogr* 1969;**32**: 233–49.
27. Carithers HA. Oculoglandular disease of Parinaud: a manifestation of cat-scratch disease. *Am J Dis Child* 1978;**132**:1195–200.
28. Carithers HA. Cat-scratch disease associated with an osteolytic lesion. *Am J Dis Child* 1983;**137**:968–70.
29. Carithers HA. Cat-scratch disease: an overview based on a study of 1200 patients. *Am J Dis Child* 1985;**139**:1124–33.
30. Carithers HA, Carithers CM, Edwards RO Jr. Cat-scratch disease: its natural history. *J A M A* 1969;**207**:312–16.
31. Castro DJ, Hoover L, Castro DJ, et al. Cervical mycobacterial lymphadenitis. *Arch Otolaryngol* 1985;**111**:816–19.
32. Centers for Disease Control and Prevention. Emergence of *Mycobacterium tuberculosis* with extensive resistance to second-line drugs: worldwide, 2000-2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:301–5.
33. Centers for Disease Control and Prevention. Trends in tuberculosis: United States, 2005. *MMWR Morb Mortal Wkly Rep* 2006; **55**:305–8.
34. Chapman JS, Guy LR. Scrofula caused by atypical mycobacteria. *Pediatrics* 1959;**23**:323–31.
35. Cheng CY, Sheng WH, Lo YC, et al. Clinical presentations, laboratory results and outcomes of patients with Kikuchi's disease: emphasis on the association between recurrent Kikuchi's disease and autoimmune diseases. *J Microbiol Immunol Infect* 2010;**43**: 366–71.
36. Chuang CH, Yan DC, Chiu CH, et al. Clinical and laboratory manifestations of Kikuchi's disease in children and differences between patients with and without prolonged fever. *Pediatr Infect Dis J* 2005;**24**:551–4.
37. Dajani AS, Garcia RE, Wolinsky E. Etiology of cervical lymphadenitis in children. *N Engl J Med* 1963;**268**:1329–33.
38. Davis SD, Comstock GW. Mycobacterial cervical adenitis in children. *J Pediatr* 1961;**58**:771–8.
39. De Baere T, Moerman M, Rigouts L, et al. *Mycobacterium interjectum* as causative agent of cervical lymphadenitis. *J Clin Microbiol* 2001;**39**:725–7.
40. Duggal P, Naseri I, Sobol SE. The increased risk of community-acquired methicillin-resistant *Staphylococcus aureus* in young children. *Laryngoscope* 2012;**121**:51–5.
41. El Amin NM, Hanson HS, Petterson B, et al. Identification of non-tuberculous mycobacteria: 16S rRNA gene sequence analysis vs. conventional methods. *Scand J Infect Dis* 2000;**32**:47–50.
42. English CK, Wear DJ, Margileth AM, et al. Cat-scratch disease: isolation and culture of the bacterial agent. *J A M A* 1988;**259**: 1347–52.
43. Evans AK, Cunningham MJ. Atypical mycobacterial cervicofacial lymphadenitis in children: a disease as old as mankind, yet a persistent challenge. *Am J Otolaryngol* 2005;**26**:337–43.
44. Fergie JE, Purcell K. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in South Texas children. *Pediatr Infect Dis J* 2001;**20**:860–3.
45. Flint D, Mahadevan M, Barber C, et al. Cervical lymphadenitis due to non-tuberculous mycobacteria: surgical treatment and review. *Int J Pediatr Otorhinolaryngol* 2000;**53**:187–94.
46. Fluegge K, Greiner P, Berner R. Late onset group B streptococcal disease manifested by isolated cervical lymphadenitis. *Arch Dis Child* 2003;**88**:1019–20.
47. Frevel T, Schafer KL, Totsch M, et al. PCR based detection of mycobacteria in paraffin wax embedded material routinely processed for morphologic examination. *J Clin Pathol* 1999;**52**: 283–8.
48. Fujimori T, Shioda K, Sussman EB. Subacute necrotising lymphadenitis: a clinicopathologic study. *Acta Pathol Jpn* 1981;**31**: 791–7.
49. Gattorno M, Caorsi R, Meini A, et al. Differentiating PFAPA syndrome from monogenic periodic fevers. *Pediatrics* 2009;**124**: e721–8.
50. Gerber JS Coffin SE, Smathers SA, Zaoutis TE. Trend in the incidence of methicillin-resistant *Staphylococcus aureus* infections in children's hospitals in the United States. *Clin Infect Dis* 2009;**49**: 65–71.
51. Gill MJ, Fanning EA, Chomyc S. Childhood lymphadenitis in a harsh northern climate due to atypical mycobacteria. *Scand J Infect Dis* 1987;**19**:77–83.

52. Gona P, Van Dyle RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *J A M A* 2006;**296**:292–300.
53. Gorak EJ, Yamada SM, Brown JD. Community-acquired methicillin-resistant *Staphylococcus aureus* in hospitalized adults and children without known risk factors. *Clin Infect Dis* 1999; **29**:797–800.
54. Green PA, von Reyn CF, Smith RP Jr. *Mycobacterium avium* complex parotid lymphadenitis: successful therapy with clarithromycin and ethambutol. *Pediatr Infect Dis J* 1993;**12**:615–17.
55. Griffith DS, Aksamit T, Brown-Ellitt BA, et al. An official ATS/IDSA statement: diagnosis, treatment and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
56. Gutman LT, Moye J, Zimmer B, et al. Tuberculosis in human immunodeficiency virus-exposed or -infected United States children. *Pediatr Infect Dis J* 1994;**13**:963–8.
57. Haas WH, Amthor B, Engelmann G, et al. Preoperative diagnosis of *Mycobacterium avium* lymphadenitis in two immunocompetent children by polymerase chain reaction of gastric aspirates. *Pediatr Infect Dis J* 1998;**17**:1016–20.
58. Haase G, Kentrup H, Skopnik H, et al. *Mycobacterium lentiflavum*: an etiologic agent of cervical lymphadenitis. *Clin Infect Dis* 1997;**25**:1245–6.
59. Haber HP, Warmann SW, Fuchs J. Cervical atypical mycobacterial lymphadenitis in childhood: findings on sonography. *Ultraschall Med* 2006;**27**:462–6.
60. Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infection in children. *N Engl J Med* 1994;**331**:432–8.
61. Harris BH, Webb W, Wilkinson AH, et al. Mycobacterial lymphadenitis. *J Pediatr Surg* 1982;**17**:589–90.
62. Hazra R, Floyd MM, Sloutsky A, et al. Novel mycobacterium related to *Mycobacterium triplex* as a cause of cervical lymphadenitis. *J Clin Microbiol* 2001;**39**:1227–30.
63. Hazra R, Robson CD, Perez-Atayde AR, et al. Lymphadenitis due to nontuberculous mycobacteria in children: presentation and response to therapy. *Clin Infect Dis* 1999;**28**:123–9.
64. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *J A M A* 1998;**279**:593–8.
65. Huebner RE, Schein MF, Cauthen GM, et al. Usefulness of skin testing with mycobacterial antigens in children with cervical lymphadenopathy. *Pediatr Infect Dis J* 1992;**11**:450–6.
66. Hulten KG, Kaplan SL, Gonzalez BE, et al. Three-year surveillance of community onset health care-associated *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2006;**25**: 349–53.
67. Jaffe BF. Pediatric head and neck tumors: a study of 178 cases. *Laryngoscope* 1973;**83**:1644–51.
68. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
69. Kendig EL Jr. The clinical picture of sarcoidosis in children. *Pediatrics* 1974;**54**:289–92.
70. Kent DC. Tuberculous lymphadenitis: not a localized disease process. *Am J Med Sci* 1967;**254**:866–73.
71. Knight PJ, Hamoudi AB, Vassy LE. The diagnosis and treatment of midline neck masses in children. *Surgery* 1983; **93**:603–11.
72. Knight PJ, Mulne AF, Vassy LE. When is lymph node biopsy indicated in children with enlarged peripheral nodes? *Pediatrics* 1982;**69**:391–6.
73. Kratz RC, Stine FA, Grover JW, et al. Suppurations of the neck. *Arch Otolaryngol* 1959;**70**:692–5.
74. Lai KK, Stottmeier KD, Sherman IH, et al. Mycobacterial cervical lymphadenopathy: relation of etiologic agents to age. *J A M A* 1984;**251**:1286–8.
75. Lampe RM, Baker CJ, Septimus EJ, et al. Cervicofacial nocardiosis in children. *J Pediatr* 1981;**99**:593–5.
76. Lee KY, Yeon YH, Lee BC. Kikuchi-Fujimoto disease with prolonged fever in children. *Pediatrics* 2004;**114**:e752–6.
77. Leong SCL, Karkos PD, Apostolidou MT. Is there a role for the otolaryngologist in PFAPA syndrome? A systematic review. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1841–5.
78. Lin HC, Su CY, Huang SC. Kikuchi's disease in Asian children. *Pediatrics* 2005;**115**:e92–6.
79. Lincoln EM, Gilbert LA. Disease in children due to mycobacteria other than *Mycobacterium tuberculosis*. *Am Rev Respir Dis* 1972; **105**:683–714.
80. Lind A, Larsson LO, Bentzon MW, et al. Sensitivity to sensitins and tuberculin in Swedish children. I. A study of school-children in an urban area. *Tubercle* 1991;**72**:29–36.
81. Lindeboom JA. Conservative wait-and-see therapy versus antibiotic treatment for nontuberculous cervicofacial lymphadenitis in children. *Clin Infect Dis* 2011;**52**:180–4.
82. Lindeboom JA. Surgical treatment for nontuberculous mycobacterial (NTM) cervicofacial lymphadenitis in children. *J Oral Maxillofac Surg* 2012;**70**:345–8.
83. Lindeboom JA, Kuijper EJ, Bruijnestein van Coppenraet ES, et al. Surgical excision versus antibiotic treatment for nontuberculous mycobacterial cervicofacial lymphadenitis in children: a multicenter, randomized, controlled trial. *Clin Infect Dis* 2007; **44**:1057–64.
84. Lindeboom JA, Kuijper EJ, Prins JM, et al. Tuberculin skin testing is useful in the screening for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Clin Infect Dis* 2006;**43**: 1547–51.
85. Lindeboom JA, Lindeboom R, Bruijnestein van Coppenraet ES, et al. Esthetic outcome of surgical excision versus antibiotic therapy for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Pediatr Infect Dis J* 2009;**28**:1028–30.
86. Lindeboom JA, Smets AM, Kuijper EJ, et al. The sonographic characteristics of nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Pediatr Radiol* 2006;**36**:1063–7.
87. Lockhart RD, Hamilton GF, Fyfe FW. *Anatomy of the human body*. Philadelphia: J. B. Lippincott; 1959. p. 662–4.
88. Losurdo G, Castagnola E, Cristino E, et al. Cervical lymphadenitis caused by nontuberculous mycobacteria in immunocompetent children: clinical and therapeutic experience. *Head Neck* 1998; **20**:245–9.
89. Luong A, McClay JE, Hasan SJ, et al. Antibiotic therapy for nontuberculous mycobacterial cervicofacial lymphadenitis. *Laryngoscope* 2005;**115**:1746–51.
90. Luu TM, Chevalier I, Gauthier M, et al. Acute adenitis in children: clinical course and factors predictive of surgical drainage. *J Paediatr Child Health* 2005;**41**:273–7.
91. Lyon LW. Neurologic manifestations of cat-scratch disease: report of a case and review of the literature. *Arch Neurol* 1971;**25**:23–7.
92. Maltezou HC, Spyridis P, Kafetzis D. Nontuberculous mycobacterial lymphadenitis in children. *Pediatr Infect Dis J* 1999;**18**: 968–70.
93. Mandell DL, Wald ER, Michaels MG, et al. Management of nontuberculous mycobacterial cervical lymphadenitis. *Arch Otolaryngol Head Neck Surg* 2003;**129**:341–4.
94. Margileth AM. Cat scratch disease: nonbacterial regional lymphadenitis—the study of 145 patients and a review of the literature. *Pediatrics* 1968;**42**:803–18.
95. Margileth AM. The use of purified protein derivative mycobacterial skin test antigens in children and adolescents: purified protein derivative skin test results correlated with mycobacterial isolates. *Pediatr Infect Dis J* 1983;**2**:225–31.
96. Margileth AM. Cat-scratch disease update. *Am J Dis Child* 1984;**138**:711–3.
97. Margileth AM. Management of nontuberculous (atypical) mycobacterial infections in children and adolescents. *Pediatr Infect Dis J* 1985;**4**:119–21.
98. Margileth AM. Antibiotic therapy for cat-scratch disease: clinical study of therapeutic outcome in 268 patients and a review of the literature. *Pediatr Infect Dis J* 1992;**11**:474–8.
99. Margileth AM, Chandra R, Altman RP. Chronic lymphadenopathy due to mycobacterial infection: clinical features, diagnosis, histopathology, and management. *Am J Dis Child* 1984;**138**: 917–21.
100. Margileth AM, Wear DJ, Hadfield TL, et al. Cat-scratch disease: bacteria in skin at the primary inoculation site. *J A M A* 1984;**252**:928–31.
101. Marshall GS, Edwards KM, Butler J, et al. Syndrome of periodic fever, pharyngitis, and aphthous stomatitis. *J Pediatr* 1987;**110**: 43–6.
102. McCabe RE, Brooks RG, Dorfman RF, et al. Clinical spectrum in 107 cases of toxoplasmic lymphadenopathy. *Rev Infect Dis* 1987;**9**:754–74.

103. Medina M, Goldfarb J, Traquinna D, et al. Cervical adenitis and deep neck infection caused by *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 1997;**16**:823–4.
104. Melish ME, Hicks RV, Reddy R. Kawasaki syndrome: an update. *Hosp Pract* 1982;**17**:99–105.
105. Moritz JD, Ludwig A, Oestmann JW. Contrast-enhanced color Doppler sonography for evaluation of enlarged cervical lymph nodes in head and neck tumors. *AJR Am J Roentgenol* 2000; **174**:1279–84.
106. Moussatos GH, Baffes TG. Cervical masses in infants and children. *Pediatrics* 1963;**32**:251–6.
107. Na DG, Lim HK, Byun HS, et al. Differential diagnosis of cervical lymphadenopathy: usefulness of color Doppler sonography. *AJR Am J Roentgenol* 1997;**168**:1311–16.
108. Naimi TS, LeDell KH, Boxrud DJ, et al. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996-1998. *Clin Infect Dis* 2001;**33**:990–6.
109. Nambiar S, Chandra RS, Schwartz RH, et al. Seven-year-old Indian girl with fever and cervical lymphadenitis: Kikuchi-Fujimoto disease. *Pediatr Infect Dis J* 2001;**20**:464–5, 469.
110. Naidu SI, Donepudi SK, Stocks RMS, et al. Methicillin-resistant *Staphylococcus aureus* as a pathogen in deep neck abscesses: a pediatric case series. *Int J Pediatr Otorhinolaryngol* 2005;**69**:1367–71.
111. Nylen O, Berg-Kelly K, Andersson B. Cervical lymph node infections with non-tuberculous mycobacteria in preschool children: interferon gamma deficiency as a possible cause of clinical infection. *Acta Paediatr* 2000;**89**:1322–5.
112. O'Brien DP, Currie BJ, Krause VL. Nontuberculous mycobacterial disease in Northern Australia: a case series and review of the literature. *Clin Infect Dis* 2000;**31**:958–68.
113. O'Brien RJ, Geiter LJ, Snider DE, Jr. The epidemiology of nontuberculous mycobacterial diseases in the United States. *Am Rev Respir Dis* 1987;**135**:1007–14.
114. Oguz F, Mujgan S, Alper G, et al. Treatment of bacillus Calmette Guérin associated lymphadenitis. *Pediatr Infect Dis J* 1992;**11**:87.
115. Olson NR. Nontuberculous mycobacterial infections of the face and neck: practical considerations. *Laryngoscope* 1981;**91**: 1714–26.
116. Ord RJ, Matz GJ. Tuberculous cervical lymphadenitis. *Arch Otolaryngol* 1974;**99**:327–9.
117. Ossowski K, Chun RH, Suskind D, et al. Increased isolation of methicillin-resistant *Staphylococcus aureus* in pediatric head and neck abscesses. *Arch Otolaryngol Head Neck Surg* 2006;**132**: 1176–81.
118. Padeh S, Brezniak N, Zemer D, et al. Periodic fever, aphthous stomatitis, pharyngitis, and adenopathy syndrome: clinical characteristics and outcome. *J Pediatr* 1999;**135**:98–101.
119. Palazzi DL, McClain KL, Kaplan SL. Hemophagocytic syndrome in children: an important diagnostic consideration in fever of unknown origin. *Clin Infect Dis* 2003;**36**:306–12.
120. Palca A, Aebi C, Weimann R, et al. *Mycobacterium bohemicum* cervical lymphadenitis. *Pediatr Infect Dis J* 2002;**21**:982–4.
121. Panesar J, Higgins K, Daya H, et al. Nontuberculous mycobacterial cervical adenitis: a ten-year retrospective review. *Laryngoscope* 2003;**113**:149–54.
122. Papakonstantinou O, Bakantaki A, Paspalaki P, et al. High-resolution and color Doppler ultrasonography of cervical lymphadenopathy in children. *Acta Radiol* 2001;**42**:470–6.
123. Perlman DC, D'Amico R, Salomon N. Mycobacterial infections of the head and neck. *Curr Infect Dis Rep* 2001;**3**:233–41.
124. Phillips P, Kwiatkowski MB, Copland M, et al. Mycobacterial lymphadenitis associated with the initiation of combination antiretroviral therapy. *J AIDS* 1999;**20**:122–8.
125. Piersimoni C, Goteri G, Nista D, et al. *Mycobacterium lentiflavum* as an emerging causative agent of cervical lymphadenitis. *J Clin Microbiol* 2004;**42**:3894–7.
126. Pounds LA. Neck masses of congenital origin. *Pediatr Clin North Am* 1981;**28**:841–4.
127. Powers GF, Boisvert PL. Age as a factor in streptococcosis. *J Pediatr* 1944;**25**:481–504.
128. Prince DS, Peterson DD, Steiner RM, et al. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. *N Engl J Med* 1989;**321**:863–8.
129. Pumberger W, Hallwirth U, Pawlowsky J, et al. Cervicofacial lymphadenitis due to atypical mycobacteria: a surgical disease. *Pediatr Dermatol* 2004;**21**:24–9.
130. Rafaty FM. Cervical adenopathy secondary to toxoplasmosis. *Arch Otolaryngol* 1977;**103**:547–9.
131. Ridder GJ, Richter B, Disko U, et al. Gray-scale sonographic evaluation of cervical lymphadenopathy in cat-scratch disease. *J Clin Ultrasound* 2001;**29**:140–5.
132. Robson CD, Hazra R, Barnes PD, et al. Nontuberculous mycobacterial infection of the head and neck in immunocompetent children: CT and MR findings. *AJNR Am J Neuroradiol* 1999;**20**:1829–35.
133. Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy: a newly recognized benign clinicopathological entity. *Arch Pathol* 1969;**87**:63–70.
134. Ryan-Poirier K, Patrick CC. Cervical adenitis caused by *Staphylococcus epidermidis*. *J Clin Microbiol* 1993;**31**:426–7.
135. Schaad UB, Votteler TP, McCracken GH, et al. Management of atypical mycobacterial lymphadenitis in childhood: a review based on 380 cases. *J Pediatr* 1979;**95**:356–60.
136. Schroder KE, Elverland HH, Mair IWS, et al. Granulomatous cervical lymphadenitis. *J Otolaryngol* 1979;**8**:127–31.
137. Schuit KE, Powell DA. Mycobacterial lymphadenitis in childhood. *Am J Dis Child* 1978;**132**:675–7.
138. Scobie WG. Acute suppurative adenitis in children: review of 964 cases. *Scott Med J* 1969;**14**:352–4.
139. Siltzbach LE, Greenberg GM. Childhood sarcoidosis: a study of 18 patients. *N Engl J Med* 1968;**279**:1239–44.
140. Singh K, Muralidar M, Kumar A, et al. Comparison of in house polymerase chain reaction techniques for the detection of *Mycobacterium tuberculosis* DNA in granulomatous lymphadenopathy. *J Clin Pathol* 2000;**53**:355–61.
141. Smith HL, II. Necrotizing lymphadenitis (Kikuchi's disease). *Pediatrics* 1993;**91**:152.
142. Sommers HM, Good RC. Mycobacterium. In: Lennette EH, Balows A, Hausler WJ Jr, et al., editors. *Manual of clinical microbiology*. 4th ed. Washington, D.C.: American Society for Microbiology; 1985. p. 216–40.
143. Spark RP, Fried ML, Bean CK, et al. Nontuberculous mycobacterial adenitis of childhood. *Am J Dis Child* 1988;**142**:106–8.
144. Speert DP, Britt WJ, Kaplan EL. Tick-borne tularemia presenting as ulcerative lymphadenitis. *Clin Pediatr* 1979;**18**:239–41.
145. Springer B, Kirschner P, Rost-Meyer G, et al. *Mycobacterium interjectum*, a new species isolated from a patient with chronic lymphadenitis. *J Clin Microbiol* 1993;**31**:3083–9.
146. Starke JR. Management of nontuberculous mycobacterial cervical adenitis. *Pediatr Infect Dis J* 2000;**19**:674–5.
147. Starke JR. The natural history of nontuberculous mycobacterial cervical adenitis. *Pediatr Infect Dis J* 2008;**27**:923–4.
148. Starke JR, Taylor-Watts KT. Tuberculosis in the pediatric population of Houston, Texas. *Pediatrics* 1989;**84**:28–35.
149. Stout JE, Saharia KK, Nageswaran S, et al. Racial and ethnic disparities in pediatric tuberculosis in North Carolina. *Arch Pediatr Adolesc Med* 2006;**160**:631–7.
150. Stuteville OH. Otorhinolaryngologic surgery: The spread of infections in the head and neck. *J Int Coll Surg* 1958;**29**:750–4.
151. Sundaresh HP, Kumar A, Hokanson JT, et al. Etiology of cervical lymphadenitis in children. *Am Fam Physician* 1981;**24**: 147–51.
152. Suskind DL, Handler SD, Tom LWC, et al. Nontuberculous mycobacterial cervical adenitis. *Clin Pediatr (Phila)* 1997;**36**: 403–9.
153. Tartaglione T. Treatment of nontuberculous mycobacterial infections: role of clarithromycin and azithromycin. *Clin Ther* 1997;**19**:626–38.
154. Tashiro N, Matsurba T, Uchida M, et al. Ultrasonographic evaluation of cervical lymph nodes in Kawasaki disease. *Pediatrics* 2002;**109**:E77.
155. Tessier M-H, Amoric JC, Mechinaud F, et al. Clarithromycin for atypical mycobacterial lymphadenitis in nonimmunocompromised children. *Lancet* 1994;**344**:1778.
156. Thomaidis T, Anastassea-Vlachou K, Mandalenaki-Lambrou C, et al. Chronic lymphoglandular enlargement and toxoplasmosis in children. *Arch Dis Child* 1977;**52**:403–7.
157. Thomas KT, Feder HM Jr, Lawton AR, et al. Periodic fever syndrome in children. *J Pediatr* 1999;**135**:15–21.
158. Thompson JN, Watanabe MJ, Greene GR, et al. Atypical mycobacterial cervical adenitis: clinical presentation. *Laryngoscope* 1980;**90**:287–94.

159. Tomblin JL, Roberts FJ. Tuberculous cervical lymphadenitis. *Can Med Assoc J* 1979;**121**:324–30.
160. Trinka L, Dankova D, Svandova E. Six years experience with the discontinuation of the BCG vaccination. IV. Protective effect of BCG vaccination against *Mycobacterium avium intra-cellulare* complex. *Tubercle Lung Dis* 1994;**75**:348–52.
161. Turner RR, Martin J, Dorfman RF. Necrotizing lymphadenitis: a study of 30 cases. *Am J Surg Pathol* 1983;**7**:115–23.
162. Waggoner-Fountain LA, Hayden GF, Hendley JO. Kawasaki syndrome masquerading as bacterial lymphadenitis. *Clin Pediatr* 1995;**34**:185–9.
163. Wagner KS, White JM, Lucenko I, et al. Diphtheria in the post-epidemic period, Europe, 2000-2009. *Emerg Infect Dis* 2012;**18**: 217–25.
164. Wang TJ, Yang YH, Lin YT, et al. Kikuchi-Fujimoto disease in children: clinical features and disease course. *J Microbiol Immunol Infect* 2004;**37**:219–24.
165. Wells RG, Sty JR. Cervical lymphadenitis complicated by mycotic carotid artery aneurysm. *Pediatr Radiol* 1991;**21**:402–3.
166. Wolinsky E. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. *Clin Infect Dis* 1995;**20**:954–63.
167. World Health Organization. Vaccine-preventable diseases and immunization: diphtheria control. Available at: http://www.euro.who.int/vaccine/20030724_3.
168. Wright NL. Cervical infections. *Am J Surg* 1967;**113**:379–86.
169. Wu CH, Lee MM, Huang KC, et al. A probability prediction rule for malignant cervical lymphadenopathy using sonography. *Head Neck* 2000;**22**:223–8.
170. Wurster WM, Carlucci JG, Feder HM, Edwards KM. Long-term follow-up of children with periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis syndrome. *J Pediatr* 2011;**159**: 958–64.
171. Yamauchi T, Ferrieri P, Anthony BF. The aetiology of acute cervical adenitis in children: serological and bacteriological studies. *J Med Microbiol* 1980;**13**:37–43.
172. Ying M, Ahuja A, Brook F. Accuracy of sonographic vascular features in differentiating different causes of cervical lymphadenopathy. *Ultrasound Med Biol* 2004;**30**:441–7.
173. Zangwill KM, Hamilton DH, Perkins BA, et al. Cat scratch disease in Connecticut: epidemiology—risk factors, and evaluation of a new diagnostic test. *N Engl J Med* 1993;**329**:8–13.

TABLE 13-1 **Predisposing Factors for Parotitis**

Drug-Induced Xerostomia
Anticholinergics
Antihistamines
Antidepressants
Phenothiazines
β blockers
Diuretics
General anesthesia
Disease-Related Xerostomia
Sjögren syndrome
Diabetes mellitus
Chronic liver disease
Cystic fibrosis
Obstruction
Dental appliances
Oral tumors
Radiation therapy
Trauma

TABLE 13-2 **Reported Infectious Etiologies of Parotitis**

Aerobic Bacteria
Staphylococcus aureus
Streptococcus pneumoniae
Streptococcus pyogenes
Viridans streptococci
Francisella tularensis
Haemophilus spp.
Moraxella catarrhalis
Pseudomonas aeruginosa
Escherichia coli
Proteus spp.
Salmonella spp.
Klebsiella spp.
Brucella spp.
Anaerobic Bacteria
Peptostreptococcus spp.
Prevotella spp.
Fusobacterium spp.
Actinomyces spp.
Mycobacteria
Mycobacterium tuberculosis
Mycobacterium avium-intracellulare
Other mycobacteria
Viruses
Mumps
Coxsackieviruses A and B
Echoviruses
Epstein-Barr virus
Influenza A
Parainfluenza viruses 1 and 3
Cytomegalovirus
Herpes simplex virus type 1
Human herpesvirus–6
Lymphocytic choriomeningitis virus
Human immunodeficiency virus

isolated.[22,43,47] Granulomatous parotitis most often is caused by *Mycobacterium tuberculosis* and may occur in the absence of systemic or disseminated tuberculous disease.[37,45,53] Other causes of granulomatous parotitis include *Mycobacterium avium-intracellulare*, *Actinomyces* spp., and gram-negative intracellular organisms (*Francisella tularensis* and *Brucella* spp.).[26,37,60]

In the postvaccine era, the most common viral cause of parotitis still is the paramyxovirus mumps virus. coxsackieviruses, Epstein-Barr virus, influenza A virus, parainfluenza viruses, adenovirus, human herpesvirus–6, herpes simplex virus, cytomegalovirus, and lymphocytic choriomeningitis virus all have been implicated in cases of parotitis.*

CLINICAL PRESENTATION AND DIAGNOSIS

A detailed history and physical examination are crucial in assisting the clinician in determining the most likely etiology of parotid gland swelling. One should determine the onset and duration of symptoms, their periodicity, and the character of salivary secretions. In addition, the presence of a systemic disease must be excluded. Examination of the parotid gland is achieved best by simultaneous palpation of the intraoral and extraoral salivary structures. Gentle external pressure should be applied to the gland, and the parotid duct should be examined for evidence of purulent secretions or surrounding erythema.

Suppurative parotitis occurs most commonly in neonates or patients with dehydration, poor oral hygiene, malnutrition, immunosuppression, oral trauma, sepsis, or any medication or disease that decreases salivary secretions.[19] Usually, the disease is unilateral; however, bilateral suppurative parotitis may occur in 17 percent of cases.[43] The disease is characterized by acute onset of pain, swelling, warmth, and induration of the involved gland and purulent discharge from the Stensen duct. Associated physical findings include fever, trismus, malaise, and cervical adenitis. In suppurative parotitis, Gram stain and culture (aerobic and anaerobic) of purulent material from the duct can provide a specific microbiologic diagnosis. In addition, elevation of the white blood cell count with a neutrophil predominance may help differentiate this form of parotitis from viral parotitis and parotid disease of a noninfectious etiology.

Mumps is the most common form of viral parotitis and is characterized by a prodrome of fever, malaise, anorexia, and headache. Usually, the following day, unilateral or bilateral earache and parotid tenderness develop. The gland or glands enlarge during the subsequent 2 to 3 days, and the orifice of the Stensen duct is erythematous and swollen, yet secretions from the duct are clear. At the point of maximal swelling, the angle of the jaw is

*References 1, 2, 16, 29, 31, 33, 36, 37.

obliterated and the earlobe is lifted upward and outward. The other salivary glands are involved in 10 percent of cases.[43] Rare systemic manifestations of mumps infection include epididymo-orchitis, meningitis, meningoencephalitis, and oophoritis. More recent outbreaks of confirmed and probable mumps emphasize that this infection may occur in highly immunized populations.[6,17] In 2006, the United States had the largest mumps epidemic in two decades, involving more than 6000 patients. Most cases occurred in Midwestern states (Iowa, Kansas, Wisconsin, Illinois, Nebraska, and South Dakota). The highest incidence was among college-aged students, most of whom had received two doses of measles-mumps-rubella (MMR) vaccine.[6,17] Other viral agents may produce similar clinical manifestations and can be differentiated from mumps only by culture and hemagglutination inhibition, complement fixation, or enzyme-linked immunosorbent assay serology.[16] In viral parotitis, the white blood cell count may be normal, slightly elevated, or depressed, with a lymphocytic predominance.

Granulomatous parotitis typically manifests as a painless, slowly enlarging mass without surrounding inflammation. It may be misdiagnosed as a slow-growing tumor until the correct diagnosis is made by biopsy and culture. *M. tuberculosis* and *M. avium-intracellulare* may cause infection in the parenchyma of the gland or in intraglandular or periglandular lymph nodes.[44,60] Clinical evidence of systemic tuberculous disease usually is absent. Parotitis has been observed as an extension of nontuberculous cervical adenitis.[60] Actinomycosis of the parotid gland causes a slowly enlarging, nodular, nontender gland; associated oral or cervicofacial infection usually is present. Fistulas draining yellow or white material with sulfur granules are common findings.[26]

Recurrent parotitis of childhood is rare, with onset typically occurring before the child reaches 10 years of age and a peak incidence at approximately 6 years of age.[18,43] Some authors hypothesize that an underlying congenital abnormality, such as sialectasis, is a common predisposing feature.[34,44] Others suggest that selective IgA deficiency may be a contributing variable.[20] Clinically, these children experience repeated episodes of fever, pain, and unilateral swelling of the parotid gland. Purulent material often can be expressed from the Stensen duct and, when cultured, often yields streptococcal organisms. Sialography and ultrasound reveal multiple areas of sialectasis throughout the parotid glands bilaterally, even if only one side is symptomatic. The frequency of attacks varies, and each episode of parotitis may last 2 weeks, when it resolves spontaneously.[10] Several authors have noted that recurrences become less frequent with increasing age and that the disease tends to cease at the onset of puberty or early adulthood.[18,43]

HUMAN IMMUNODEFICIENCY VIRUS AND PAROTID ENLARGEMENT

Salivary gland enlargement has been recognized as a common finding in children infected with HIV since before the era of highly active antiretroviral therapy (HAART).[35] The prevalence of this manifestation in HIV-infected individuals is 0 to 58 percent.[47] More recently, in a retrospective report of oral lesions in a cohort of HIV-infected children from Brazil, Miziara and colleagues[41] noted the prevalence of parotid gland enlargement to be 7.6 percent. Although parotid enlargement occurred more commonly in children older than 5 years of age, the prevalence did not differ between children who were receiving antiretroviral therapy without protease inhibitors and children who were receiving HAART.

The exact pathophysiology is unknown, but proposed mechanisms include lymphoepithelial cysts, lymph node enlargement within the gland, cytomegalovirus or Epstein-Barr virus infection, and diffuse infiltrative lymphocytosis syndrome of the gland.[47] This entity in HIV-positive children possibly is associated with the HLA-DR5 and HLA-DR11 phenotypes, but the significance of this finding is unclear.[28,47,56] Bilateral parotid enlargement frequently is seen as part of diffuse infiltrative lymphocytosis syndrome, which is characterized by proliferation of $CD8^+$ lymphocytes within the circulation and is of unclear etiology. Growth of the parotid is secondary to infiltration of CD8 lymphocytes into the gland, follicular hyperplasia of intraparotid lymphoid tissue (as occurs in lymph nodes throughout the body in HIV infection), and development of diffuse intraparotid, lymphoepithelial cysts.[8,38] Epstein-Barr virus has been proposed as an impetus for CD8 lymphoproliferation because the virus has been isolated from parotid tissue of some affected patients.[9] The absence of positive serology for the virus renders it an unlikely causative agent, however. HIV, which may be the inciting agent, has been detected in dendritic cells, macrophages, and lymphocytes isolated from the parotid glands of affected patients.[9]

Parotid enlargement may be present in 20 to 50 percent of children with HIV infection and acquired immunodeficiency syndrome (AIDS).[30,34,55,58] The median time from birth to development of parotid enlargement is 4.6 years, and often it is the first manifestation of HIV infection acquired during the perinatal period in an otherwise healthy older child.[28,30,35] HIV-positive children with enlarged parotid glands tend to have a slower progression to death than do HIV-positive children with oral herpes or candidiasis.[30] Usually, both parotid glands are involved, and an affected patient presents with enlarged, tender parotid glands, xerostomia, and increased serum amylase level.[7] Severity of pain and size of the gland tend to fluctuate without apparent cause. This manifestation of HIV infection and AIDS is still commonly seen in developing countries where access to antiretroviral therapy is limited.

The differential diagnoses for parotid enlargement in an HIV-positive patient include viral and bacterial parotitis. Some patients have preexisting xerostomia that may increase their susceptibility to parotitis. In addition, the immunocompromised state of patients with AIDS may predispose them to development of infection of the parotid gland with other agents, such as cytomegalovirus, Epstein-Barr virus, bacteria, mycobacteria, and fungi.[24,52,59,62] Noninfectious etiologies, such as non-Hodgkin lymphoma or Kaposi sarcoma, also should be included in the differential diagnosis of parotid gland

enlargement in an HIV-infected patient, although these manifestations usually are observed in adults.

DIFFERENTIAL DIAGNOSIS

Parotitis most often is diagnosed based on clinical presentation, microbiology, serology, and response to empiric therapy. Ultrasound may be useful as a screening tool to prompt more sensitive modalities if the ultrasound scan is abnormal.[42] Computed tomography is most useful in the presence of anatomic defects, radiolucent calculi, or abscess formation in the parotid gland.[51] X-ray sialography is the gold standard in examining the parotid gland ducts; however, sialography is contraindicated in the setting of acute infection. Magnetic resonance sialography is a promising alternative and has several advantages. In contrast to x-ray sialography, magnetic resonance sialography is not contraindicated during acute parotitis and does not require injection of contrast material or involve manipulation of the Stensen duct.[21] Nonetheless, experience with magnetic resonance sialography is limited and this mode alone may not be sufficiently sensitive to detect tertiary salivary ductules or calculus disease.[21,63]

Noninfectious causes of parotid swelling and inflammation include collagen vascular diseases (Sjögren syndrome and systemic lupus erythematosus), metabolic disorders (hepatic disease, hyperlipoproteinemia, and hyperuricemia), endocrine disorders (diabetes mellitus and hypothyroidism), tumors, leukemic infiltration, drugs (antineoplastic chemotherapy), and poisons (iodine).[37,43,48] Sjögren syndrome, the most common cause of noninfectious parotitis, is caused by lymphocyte-mediated destruction of the exocrine glands.[25,48] Patients with this disease have diminished or absent glandular secretions and mucosal dryness; xerostomia and keratoconjunctivitis sicca are prominent clinical features. In addition, the parotid glands are enlarged bilaterally, are firm, and have an irregular contour. Sialography reveals sialectasia, and saliva from these patients has unique biochemical characteristics. Antibodies to nuclear antigens SS-A and SS-B can be detected in the sera of patients with Sjögren syndrome.[48] Patients with chronic noninfectious parotitis have changes in the ductular architecture or strictures that can predispose them to episodes of infectious parotitis.

TREATMENT

Treatment of parotitis includes rehydration, parotid massage, discontinuation of any medications that diminish salivary flow, and sialalogues (e.g., lemon drops, hard candy, and chewing gum), which increase salivary flow.[3,37,48,50] In cases of suspected suppurative parotitis, a broad-spectrum antibiotic regimen that is effective against *S. aureus*, *Streptococcus* spp., gram-negative organisms, and anaerobes should be administered empirically, pending specific culture results. Antibiotic regimens frequently employed include penicillinase-resistant penicillins, first-generation cephalosporins, and clindamycin in combination with an aminoglycoside.[3] Vancomycin should be used if methicillin-resistant *S. aureus* (MRSA) is the likely pathogen. If the patient has been hospitalized for a prolonged period, or if the predominant organisms on Gram stain of the purulent discharge are gram-negative, ceftazidime should be considered as initial empiric therapy.[48,50]

Surgical incision and drainage of purulent fluid are indicated if there is slow or no response to medical therapy or if fluctuance increases.[54] The treatment of viral parotitis consists of antipyretics, analgesia, and hydration. In cases of mycobacterial infection, excision of the gland may be required, in addition to administration of specific antimycobacterial therapy.[45,53] Reports have described successful treatment with clarithromycin and azithromycin of parotitis caused by atypical mycobacteria.[23] In contrast, actinomycosis of the parotid gland is managed medically with penicillin G.[26] Children with recurrent parotitis should be treated with antibiotics during acute episodes, but chronic suppressive antimicrobial therapy is not recommended.

Tympanic neurectomy involves severing the parasympathetic secretomotor fibers of the tympanic plexus to the parotid. This procedure attenuates secretion from the gland and relieves sialectasis and further episodes of parotitis in more than 70 percent of patients.[15,46] Only 10 to 20 percent of these patients require parotidectomy for persistence of symptoms beyond puberty.[13] Although it is the optimal treatment for complete resolution of recurrent parotitis, parotidectomy carries a risk for facial nerve injury. Nahlieli and colleagues[44] described the use of endoscopy to diagnose and endoscopic irrigation to treat children with recurrent parotitis.

COMPLICATIONS

With improved fluid management of postsurgical patients and the use of broad-spectrum antimicrobial agents, complications secondary to infectious parotitis now are rare events. In neonates or immunocompromised patients, sepsis may be a severe complication of this infection. Abscess formation may result from delayed or ineffective therapy. Compromise of the facial nerve may occur and can resolve with successful treatment of the infected gland.[39] The most serious and rare complication is extension to other structures of the head and neck and along fascial planes to the face, external auditory canal, jugular vein, mandible, and mediastinum.

PREVENTION

Suppurative parotitis can be prevented in postsurgical patients by maintaining adequate hydration and good oral hygiene. The most common form of viral parotitis, mumps, can be prevented by appropriate vaccination. Between 1968 and 1993, a 99 percent reduction in the incidence of new cases of mumps occurred.[61] In the mid-1980s and more recently in 2006, a resurgence of the incidence of mumps in previously vaccinated populations was noted.[6,12,17,27] The most recent outbreaks occurred among college students and young adults in several states,

raising concern of waning immunity in this highly vaccinated population.[6,17] The current recommendation is to provide two doses of live mumps vaccine for school-age children (i.e., grades kindergarten through 12) with MMR vaccine and to ensure that students in college or other post–high school educational institutions also have received two doses of MMR vaccine.[6,14,17]

NEW REFERENCE SINCE THE SIXTH EDITION

17. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. *N Engl J Med* 2008;**358**: 1580–9.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 14

Rhinosinusitis

James D. Cherry • Jagmeet Mundi • Nina L. Shapiro

Rhinosinusitis (usually referred to as "sinusitis") is an inflammation of the mucosal lining of one or more of the paranasal sinuses. Although this inflammation of sinus mucosa most probably occurs to some degree with every upper respiratory tract infection that produces rhinitis, most of these episodes apparently have a spontaneous resolution.[99] Studies during the past 2 decades estimate that 5 to 10 percent of upper respiratory tract infections are complicated by acute sinusitis.[2,190,208] This rate range represents a significant increase from earlier reports,[40] possibly because of a greater awareness of the illness and improved imaging techniques. The growing number of children in daycare has led to an actual increase in the incidence of upper respiratory tract infections.[207,208] In addition, recognition that sinus infection can have a negative effect on the health of children with chronic pulmonary disease has increased the interest in this disease.[114]

When considering a diagnosis of sinusitis in a child, the major problem is to distinguish simple upper respiratory tract infection or allergic inflammation from secondary bacterial infection of the sinuses. Unless complications such as periorbital cellulitis or cavernous sinus thrombosis render the diagnosis obvious, the clinician has no reliable way to establish a diagnosis of acute sinusitis in the office setting. Sometimes, symptoms and signs of sinusitis occur simultaneously with rhinitis, but most often they occur after an episode of rhinitis. Infection in the sinuses usually persists after the preceding rhinitis has resolved. Sinusitis is classified by the duration of clinical symptoms: acute (≤3 weeks), subacute (3 to 10 weeks), and chronic (>10 weeks). Available data comparing acute and subacute sinusitis are sparse; both entities may have a similar etiology, diagnosis, and prognosis; the distinction seems to be arbitrary and to have no clinical significance.

HISTORY

Purulent sinusitis and its relationship to orbital inflammation have been known for more than 2000 years.[75] Highmore, a 17th-century English physician and anatomist, is given credit for the separation of dental and antral disease.[143] Hunter indicated the importance of surgical drainage in purulent sinusitis and suggested perforating the partition between the maxillary antrum and the nose.[143] During the first half of the 20th century, sinusitis was responsible for considerable morbidity and mortality, and surgical care of sinusitis frequently was lifesaving. Since the advent of antibiotics, sinusitis has had a lower medical profile. Interest in this topic has increased, however. Some factors involved in this increased interest include the advent of the newer surgical techniques of functional endoscopic sinus surgery that yield comparable results to sinus puncture and aspiration,[187] which now have been applied to children; improved diagnostic imaging studies; and the greater social importance of upper respiratory tract infections for parents who must be absent from work to seek treatment for their children.[114]

ANATOMY

All the paranasal sinuses develop as outpouchings of the nasal cavity. Three shelflike structures—the inferior, middle, and superior turbinates—are on the lateral nasal wall. The superior turbinate is not well developed in the first year of life.[219] Beneath each turbinate is the corresponding meatus into which the sinuses open; that is, the frontal, maxillary, and anterior ethmoidal sinuses open into the middle meatus. The sphenoidal and posterior

REFERENCES

1. Arditi M, Langman CB, Christensen M, et al. Probable herpes simplex virus type 1-related acute parotitis, nephritis and erythema multiforme. *Pediatr Infect Dis J* 1988;**7**:427–9.
2. Brill SJ, Gilfillan R. Acute parotitis associated with influenza type A: a report of twelve cases. *N Engl J Med* 1977;**296**:1391–2.
3. Brook I. Diagnosis and management of parotitis. *Arch Otolaryngol Head Neck Surg* 1992;**118**:469–71.
4. Brook I, Finegold SM. Acute suppurative parotitis caused by anaerobic bacteria: report of two cases. *Pediatrics* 1978;**62**:1019–20.
5. Brook I, Frazier EH, Thompson DH. Aerobic and anaerobic microbiology of acute suppurative parotitis. *Laryngoscope* 1991; **101**:170–2.
6. Centers for Disease Control and Prevention. Update: mumps activity—United States, January 1-October 7, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1152–3.
7. Chanock SJ, McIntosh K. Pediatric infection with the human immunodeficiency virus: Issues for the otorhinolaryngologist. *Otolaryngol Clin North Am* 1989;**22**:637–60.
8. Chapnik JS, Noyek AM, Berris B, et al. Parotid gland enlargement in HIV infection: clinical/imaging findings. *J Otolaryngol* 1990;**19**: 189–94.
9. Chetty R, Vaithilingum M, Thejpal R. Epstein-Barr virus status and the histopathological changes of parotid gland lymphoid infiltrates in HIV-positive children. *Pathology* 1999;**31**:413–7.
10. Chitre VV, Premchandra DJ. Recurrent parotitis. *Arch Dis Child* 1997;**77**:359–63.
11. Chiu C, Lin T. Clinical and microbiological analysis of six children with acute suppurative parotitis. *Acta Paediatr* 1996;**85**:106–8.
12. Cochi SL, Preblud SR, Orenstein WA. Perspectives on the relative resurgence of mumps in the United States. *Am J Dis Child* 1988; **142**:499–507.
13. Cohen HA, Gross S, Nussinovitch M, et al. Recurrent parotitis. *Arch Dis Child* 1992;**67**:1036–37.
14. Committee on Infectious Diseases, American Academy of Pediatrics. *2009 Red book: Report of the Committee on the Control of Infectious Diseases*. 28th ed. Evanston, Ill: American Academy of Pediatrics; 2009.
15. Daud AS, Pahor AL. Tympanic neurectomy in the management of parotid sialectasis. *J Laryngol Otol* 1995;**109**:1155–8.
16. Davidkin I, Jokinen S, Leinikki P, Peltola H. Etiology of mumps-like illnesses in children and adolescents vaccinated for measles, mumps and rubella. *J Infect Dis* 2005;**191**:719–23.
17. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. *N Engl J Med* 2008;**358**:1580–9.
18. Ericson S, Zetterlund B, Ohman J. Recurrent parotitis and sialectasis in childhood: Clinical, radiologic, immunologic, bacteriologic, and histologic study. *Ann Otol Rhinol Laryngol* 1991;**100**:527–35.
19. Fathalla B, Collins D, Ezhuthachan S. Acute suppurative parotitis: uncommon presentation in a premature infant. *J Perinatol* 2000;**1**: 57–9.
20. Fazekas T, Wiesbauer P, Schroth B, et al. Selective IgA deficiency in children with recurrent parotitis of childhood. *Pediatr Infect Dis J* 2005;**24**:461–2.
21. Fischbach R, Kugel H, Ernst S, et al. MR sialography: initial experience using a T2-weighted fast SE sequence. *J Comput Assist Tomogr* 1997;**21**:826–30.
22. Giglio MS, Landaeta M, Pinto ME. Microbiology of recurrent parotitis. *Pediatr Infect Dis J* 1997;**16**:386–90.
23. Green PA, von Reyn CF, Smith RP Jr. *Mycobacterium avium* complex parotid lymphadenitis: successful therapy with clarithromycin and ethambutol. *Pediatr Infect Dis J* 1993;**12**:615–7.
24. Hanekom WA, Chadwick EG, Yogev R. Pneumococcal parotitis in a human immunodeficiency virus-infected child. *Pediatr Infect Dis J* 1995;**14**:1113–4.
25. Hearth-Holmes M, Baethge BA, Abreo F, et al. Autoimmune exocrinopathy presenting as recurrent parotitis of childhood. *Arch Otolaryngol Head Neck Surg* 1993;**119**:347–9.
26. Hensher R, Bowerman J. Actinomycosis of the parotid gland. *Br J Oral Maxillofac Surg* 1995;**23**:128–34.
27. Hersh BS, Fine PEM, Kent WK, et al. Mumps outbreak in a highly vaccinated population. *J Pediatr* 1991;**119**:187–93.
28. Itescu S. Diffuse infiltrative lymphocytosis syndrome in children and adults infected with HIV-1: a model of rheumatic illness caused by acquired viral infection. *Am J Reprod Immunol* 1992;**28**: 247–50.
29. Jantausch BA, Wiedermann BL, Jeffries B. Parainfluenza virus type 2 meningitis and parotitis in an 11-year-old child. *South Med J* 1995;**88**:230–1.
30. Katz MH, Mastrucci MT, Leggot PJ, et al. Prognostic significance of oral lesions in children with perinatally acquired human immunodeficiency virus infection. *Am J Dis Child* 1993;**147**: 45–8.
31. Krilov LR, Swenson P. Acute parotitis associated with influenza A infection. *J Infect Dis* 1985;**152**:853.
32. Leake D, Leake R. Neonatal suppurative parotitis. *Pediatrics* 1970;**46**:203–7.
33. Lee ACW, Lim WL, So KT. Epstein-Barr virus associated parotitis. *J Paediatr Child Health* 1997;**33**:177–8.
34. Leerdam CM, Martin HCO, Isaacs D. Recurrent parotitis of childhood. *J Paediatr Child Health* 2005;**41**:631–4.
35. Lepage P, Van de Perre P, Van Vliet G, et al. Clinical and endocrinologic manifestations in perinatally human immunodeficiency virus type 1-infected children aged 5 years or older. *Am J Dis Child* 1991;**145**:1248–51.
36. Lewis JM, Utz JP. Orchitis, parotitis and meningoencephalitis due to lymphocytic-choriomeningitis virus. *N Engl J Med* 1961; **265**:776–80.
37. Loughran DH, Smith LG. Review: Infectious disorders of the parotid gland. *N J Med* 1988;**85**:311–4.
38. Mandel L, Kim D, Uy C. Parotid gland swelling in HIV diffuse infiltrative CD8 lymphocytosis syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1998;**85**:565–8.
39. Mathur NB, Goyal RK, Khalil A. Neonatal suppurative parotitis with facial palsy. *Indian Pediatr* 1988;**25**:806–7.
40. Matlow A, Korentager R, Keystone E, et al. Parotitis due to anaerobic bacteria. *Rev Infect Dis* 1988;**10**:420–3.
41. Miziara ID, Araujo Filho BC, Weber R. Oral lesions in Brazilian HIV-infected children undergoing HAART. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1089–96.
42. Murray ME, Buckenham TM, Joseph AEA. The role of ultrasound in screening patients referred for sialography: a possible protocol. *Clin Otolaryngol* 1996;**21**:21–3.
43. Myer C, Cotton RT. Salivary gland disease in children: a review. I. Acquired non-neoplastic disease. *Clin Pediatr* 1986;**25**:314–22.
44. Nahlieli O, Shacham R, Shlesinger M, Eliav E. Juvenile recurrent parotitis: a new method of diagnosis and treatment. *Pediatrics* 2004;**114**:9–12.
45. O'Connell JE, George MK, Speculand B, et al. Mycobacterial infection of the parotid gland: an unusual case of parotid swelling. *J Laryngol Otol* 1993;**107**:561–4.
46. Perera AM, Kumar BN, Pahor AL. Long-term results of tympanic neurectomy for chronic parotid sialectasis. *Rev Laryngol Otol Rhinol* 2000;**121**:95–8.
47. Pinto A, DeRossi SS. Salivary gland disease in pediatric HIV patients: an update. *J Dent Child* 2004;**71**:33–7.
48. Pou AM, Johnson JT, Weissman J. Management decisions in parotitis. *Compr Ther* 1995;**21**:85–92.
49. Pruett TL, Simmons RL. Nosocomial gram-negative bacillary parotitis. *JAMA* 1984;**251**:252–3.
50. Raad II, Sabbagh MF, Caranasos GJ. Acute bacterial sialadenitis: a study of 29 cases and review. *Rev Infect Dis* 1990;**12**:591–601.
51. Rabinov JD. Imaging of salivary gland pathology. *Radiol Clin North Am* 2000;**38**:1047–57.
52. Redleaf MI, Bauer CA, Robinson RA. Fine-needle detection of cytomegalovirus parotitis in a patient with acquired immunodeficiency syndrome. *Arch Otolaryngol Head Neck Surg* 1994;**120**: 414–6.
53. Rowe-Jones JM, Vowles R, Leighton SEJ, et al. Diffuse tuberculous parotitis. *J Laryngol Otol* 1992;**106**:1094–5.
54. Sabatino G, Verrotti A, de Martino M, et al. Neonatal suppurative parotitis: a study of five cases. *Eur J Pediatr* 1999;**158**:312–4.
55. Schuval SJ, Bonagura VR, Ilowite NT. Rheumatologic manifestations of pediatric human immunodeficiency virus infection. *J Rheumatol* 1993;**20**:1578–82.
56. Schuval SJ, O'Reilly ME, Bonagura VR. Increased frequency of HLA-DR11 in pediatric human immunodeficiency virus-associated parotid gland enlargement. *Clin Diagn Lab Immunol* 1997;**4**: 258–60.

57. Simpson REH. Infectiousness of communicable diseases in the household (measles, chickenpox, and mumps). *Lancet* 1952;**2**: 549–54.
58. Sperling NM, Lin P. Parotid disease associated with human immunodeficiency virus infection. *Ear Nose Throat J* 1990;**69**:475–7.
59. Stellbrink H, Albrecht H, Greten H. Pneumococcal parotitis and cervical lymph node abscesses in an HIV-infected patient. *Clin Invest* 1994;**72**:1037–40.
60. Tunkel DE. Atypical mycobacterial adenitis presenting as a parotid abscess. *Am J Otolaryngol* 1995;**16**:428–32.
61. van Loon FP, Holmes SJ, Sirotkin BI, et al. Mumps surveillance—United States, 1988-1993. *MMWR Morb Mortal Wkly Rep* 1995;**44**:1–14.
62. Vargas PA, Mauad T, Bohm GM, Saldiva PHN. Parotid gland involvement in advanced AIDS. *Oral Dis* 2003;**9**:55–61.
63. Varghese JC, Thornton F, Lucey BC, et al. A prospective comparative study of MR sialography and conventional sialography of salivary disease. *AJR Am J Roentgenol* 1999;**173**:1497–503.

ethmoidal cells open high in the nasal vault into the superior meatus.[198]

The maxillary sinuses develop early in the second trimester of fetal life as lateral outpouchings in the posterior aspect of the middle meatus. They are present at birth,[7,122,161,219] with floors being barely below the attachment of the inferior turbinates.[198] They expand rapidly by the time the child is 4 years of age.[219] Ultimately, at full size, the lateral borders of the maxillary sinuses reach the lateral orbital rims. The position of the floors of the sinuses is determined by the eruption of the dentition.[198] The ostia of the maxillary sinuses are located high on the medial walls of the sinuses, which impedes gravitational drainage of secretions; ciliary activity is required to move secretions from the body of the maxillary sinuses through the ostia into the nose.[198]

The ethmoidal sinuses develop in the fourth month of gestation[198] and are present at birth.[122,161,219] They are not a single large cavity but a grouping of cells, 3 to 15 in number, each with its own opening or ostium. They have a honeycombed radiographic appearance and are small anteriorly and large posteriorly. The walls of the ethmoidal labyrinth, especially the lateral walls bordering on the orbits, are thin and referred to as the *lamina papyracea*.[198]

Development of the frontal sinuses is variable. In adults, 80 percent have bilateral frontal sinuses, 1 to 4 percent have agenesis of the frontal sinuses, and the remainder have unilateral hypoplasia. The position of the frontal sinuses is supraorbital after the child reaches 4 years of age, but they are not distinguished radiographically from the ethmoidal sinuses until the child is 6 to 8 years old. The frontal sinuses do not reach adult size for another 8 to 10 years.[198]

The onset of development of the sphenoidal sinuses occurs during the child's first 2 years of life, but they remain rudimentary until the child is approximately 6 years of age. They have reached their permanent size, although not their permanent shape, by the time the child is 12 years old.[219]

Although the full development of the sinuses may take 20 years, by the time the child is 12 years old the nasal cavity and the paranasal sinuses nearly have completed their development and have reached adult proportions.[219] Sinus disease in postpubertal adolescents is similar to that in adults.

The mucosal lining of all the paranasal sinuses is composed of ciliated columnar epithelium and goblet cells.[164] It is continuous and similar to the lining of the nasal cavity except that the mucosa in the nose is thicker and contains more glands. The epithelium of all the paranasal sinuses and nasal cavity is covered in part by a blanket of mucus.

PATHOPHYSIOLOGY

The pathogenesis of sinus infection undoubtedly is similar to that of otitis media.[204] The middle ear, with its extension, the eustachian tube, and the paranasal sinuses normally are sterile, but their contiguous areas (nasopharynx and nose) have a dynamic microbial flora. Under normal conditions, ciliary function with mucus flow can be expected to keep the sinuses clear of pathogens. The cilia within the sinuses propel the mucus toward their respective ostia and, from there, nasal ciliary action moves the mucus blanket posteriorly toward the pharynx. Insults that damage the ciliary epithelium and affect the morphology, number, and function of cilia and insults that alter the production or viscosity of the mucus blanket lead to obstruction of the flow of mucus, however, which allows the inoculation of numerous microorganisms into the sinuses that can lead to infection. In a study in adults in whom sneezing, coughing, and nose blowing were stimulated or initiated voluntarily, intranasal pressures were measured and the deposition of contrast medium (which before the initiation of the event had been inoculated into the nasopharynx) was determined by computed tomography (CT).[70] Results of this study showed that nose blowing introduced viscous fluid into the maxillary sinuses, whereas coughing or sneezing did not generate enough pressure to propel fluid into the sinuses. When instituted, sinus infection is complicated further by inflammatory obstruction of the ostium leading to the nose.

The most important factor leading to purulent sinus infection in children and in adults is upper respiratory tract viral infection.[24,47,114,208] Wald and colleagues[208] in a large prospective study involving children younger than 3 years of age showed a doubling of the rate of sinusitis (defined as upper respiratory tract symptoms persisting >15 days) among children in a daycare setting compared with children not in daycare. The differences presumably were due to increased exposure to viral respiratory illnesses. Radiographic studies in children with acute colds regularly indicate abnormalities of the maxillary sinuses, suggesting that the infection involves these areas.[117] These asymptomatic sinus opacifications may persist for 2 weeks after the symptoms of the upper respiratory tract illness have resolved.[44,97,198] Viral infection that involves the sinuses rarely is differentiated from its primary manifestations, such as the common cold, nasopharyngitis, and influenza; and recovery is the rule. If the effect of the viral infection on the mucosal surface is severe, and is associated with the inoculation of one or more pathogenic bacterial agents and obstruction of an ostium, disease occurs.

The mechanisms by which upper respiratory tract viral infections set the stage for secondary bacterial infection in the sinuses are complex. Using in situ hybridization, rhinovirus RNA was shown inside epithelial cells of maxillary sinus in 50 percent of a small number of adults with acute sinusitis.[140] This finding is remarkable because in experimental rhinovirus infections, only a small percentage of nasal epithelial cells were noted to contain rhinovirus RNA.[8] These differences may reflect only differences in inoculum between experimental and natural infection, but they may indicate heavier infection in the sinuses than in the nose. Symptoms in upper respiratory tract viral infections are not caused by extensive damage to ciliated nasal epithelium but rather to aspects of the host response (see Chapter 7).*

*References 8, 69, 129, 131, 138, 144, 145, 218.

Other irritants can set the stage for sinus infection. Swimming in ocean, lake, or chlorinated pool water can lead to sinus involvement. Drying of the nasal mucosa, which occurs commonly during the winter in cold climates, may be a precipitating factor.

Dental infections or extractions also can lead to maxillary sinusitis if the tooth root is adjacent to the maxillary sinus floor.[35] Sudden change in pressure, as with diving or during descent in an airplane, physically can overcome local mucociliary defense mechanisms and lead to the sudden onset of acute sinusitis.[128]

A number of host factors and perhaps microbiologic factors are associated with recurrent and chronic sinusitis.* Recurrent chronic sinusitis implies a problem with local mucociliary defense, a defect in systemic immunity, or a fixed anatomic sinus obstruction. Often, the predisposing factors work in tandem, as in a child with a septal deformity and a viral illness.[114] In chronic sinusitis, the mucosa is thickened and marked edema, vessel dilation, and infiltration of inflammatory cells are present.[188] Goblet cells are decreased in density, and seromucous glands are increased in density compared with their presence in normal sinuses.

Children with respiratory allergies are prone to sinusitis,[55,105,148-150] and allergy probably is the second most prevalent predisposing factor in childhood sinusitis, acting through mucosa congestion and perhaps depressing local and systemic immune responses.[114,169] The treatment of respiratory allergies may contribute to sinusitis because ciliary damage occurring after the administration of nasal decongestants has been shown in organ culture and animal studies.[44,45,124] Richards and colleagues[156] reported a diagnosis of atopy in 62 percent of a selected cohort of pediatric patients who had documented recurrent sinusitis and were referred to allergy clinics in Los Angeles.

Defects of ciliary function, such as those occurring in the immotile cilia syndrome and Kartagener syndrome, predispose a child to chronic sinusitis.† Refractory sinusitis also occurs commonly in children with primary and acquired immunodeficiency diseases.‡ A growing population of immunocompromised children undergoing treatment for malignancies and organ transplantations constitute another population with a potential for developing sinusitis that is difficult to manage. Finally, anatomic obstruction caused by septal deformities, craniofacial anomalies, foreign bodies, adenoidal hypertrophy, or nasal masses or polyps predisposes children to sinusitis. Nasal polyps in young children usually are not caused by allergies and should be an indication for evaluation for cystic fibrosis.[114] Biofilms play an important role in chronic sinusitis.[136,184]

Immunologic mechanisms are important in the pathogenesis of sinus infections, as indicated by the high prevalence of chronic sinus infections in children with immunodeficiencies.§ Sinus and nasal mucus contains IgA, IgG, and IgM, and lysozymes.[27,160] Secretory IgA, which is produced locally, is the predominant immunoglobulin in nasal mucus.[73] IgG antibodies in nasal mucus result from passive leakage from plasma cells in the epithelium and submucosa and from the serum.[19] Generally, with the patient's increasing age and as a result of previous exposures, these immunoglobulins develop species-specific and type-specific antibodies that block epithelial colonization by specific microorganisms.

Shapiro and associates[170] studied 61 children with refractory sinusitis and found that 34 had abnormal immunologic studies. Abnormal findings included poor response to pneumococcal type 7 antigen after immunization, IgG3 subclass deficiency, low serum IgA or IgG values, and elevated serum IgE values.

ETIOLOGY

Table 14-1 lists etiologic agents of sinusitis by age of patient and type of illness. In all age groups and in acute, subacute, and chronic disease, *Haemophilus influenzae* and *Streptococcus pneumoniae* are the principal pathogens in most cases. Also, a large number of different bacterial species have been recovered from the sinuses of affected patients. In young children, more than 90 percent of all cases of sinusitis are caused by five organisms: *H. influenzae*, *S. pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Streptococcus pyogenes*. Concern has been raised as to whether *S. aureus* is a cause of sinusitis in children.[205] This concern is based on the fact that *S. aureus* is common in the noses of all children and therefore there is likely contamination at the time of maxillary sinus puncture or endoscopic surgery. Similar concern should also be raised regarding α-hemolytic streptococci, coagulase-negative *Staphylococcus*, and various anaerobes because they also are part of the nasal flora. In adolescents, the same organisms, plus largely penicillin-sensitive anaerobes, account for most cases. As noted in Table 14-1, a variety of gram-negative enteric and other bacilli have been recovered from patients with sinusitis, in most instances from patients who have had various forms of antibiotic therapy before culture. Organisms previously considered to be nonpathogens, such as *Staphylococcus epidermidis*, have been implicated etiologically.

Although clinically recognized sinusitis has occurred rarely in patients with *Mycoplasma pneumoniae* infection, Griffin and Klein[64] noted radiographic evidence of sinusitis in approximately two thirds of a group of U.S. Navy recruits with *M. pneumoniae* pneumonia. In adults with chronic suppurative maxillary sinusitis, mycoplasmas have been sought but not recovered.[16,62,182] Bhattacharyya and colleagues[16] noted L-forms in 21 percent of all sinuses in patients with chronic disease.

In a study of 25 adults with chronic rhinosinusitis, *Chlamydophila pneumoniae* was recovered from nasopharyngeal samples in 2 patients but not from any of 10 healthy controls.[48] In addition, the patients were more likely than control subjects (20 percent) to have serum IgG antibody titers to *C. pneumoniae* of 1:64 or greater (72%). IgA antibody titers to *C. pneumoniae* of 1:32 or greater also were more prevalent in the patients (48%) than in the controls (10%). In a study involving 20

*References 13, 31, 32, 44, 45, 49, 55, 83, 94, 105, 114, 124, 126, 137, 148, 150, 156, 161, 162, 169, 170, 173, 175, 184, 188, 191, 201, 223.
†References 49, 83, 94, 150, 161, 175.
‡References 31, 114, 126, 162, 170, 191, 201.
§References 31, 32, 114, 126, 162, 170, 173, 191.

TABLE 14-1 **Etiologic Agents in Sinusitis Analyzed by Patient Age and Type of Illness**

	Frequency				Age Group (yr)		
	Overall	Acute	Subacute	Chronic	≤5	6-12	>12
Aerobic Bacteria							
Haemophilus influenzae	++++	++++	++++	++++	++++	++++	++++
Streptococcus pneumoniae	++++	++++	++++	++++	++++	++++	++++
Moraxella catarrhalis	+++	+++	++	+	+++	+	++
Staphylococcus aureus	++	+	+	++	++	++	++
Streptococcus pyogenes	++	++	++	++	+	++	++
α-Hemolytic and nonhemolytic streptococci	+		+	+			++
Staphylococcus epidermidis	+		+	+		+	++
Alcaligenes spp.	+			+			++
Escherichia coli	+			+			++
Klebsiella pneumoniae	+			+			++
Pseudomonas aeruginosa	+			+			++
Other*	+			+			++
Anaerobic Bacteria							
Peptostreptococcus spp.	++	+	+	+++		+	++
Prevotella and *Porphyromonas* spp.	++			++		+	++
Fusobacterium spp.	++			++			
Propionibacterium spp.	++			++			
Bifidobacterium spp.	+			+			
Bacteroides fragilis	+	+	+	+			+
Veillonella spp.	+	+	+	+			+
Fungi							
Scopulariopsis spp.	+			+			+
Aspergillus spp.	+	+		+	+	++	++
Alternaria spp.	+	+		+	+	+	+
Penicillium spp.	+			+	+	+	+
Curvularia spp.	+		+	+	+	+	+
Drechslera spp.	+			+	+	+	+
Bipolaris spp.	+			+	+	+	+
Mucor spp. and other Zygomycetes	+	+			+	+	+
Candida spp.	+			+			+
Mycoplasma and Chlamydophila							
Mycoplasma pneumoniae	+	+					+
Chlamydophila pneumoniae†				+	+	+	+
Other							
L-forms	+			+			++
Mixed aerobes and anaerobes	++	+	+	++			++
Mixed *Haemophilus influenzae* with other organisms	++	+	+	++		+	++
Rhinovirus, adenovirus, cytomegalovirus	+	+		+		+	+

* *Serratia* spp., diphtheroids, *Enterococcus* spp., *Neisseria* spp., *Haemophilus* spp., *Proteus* spp., *Acinetobacter* spp., *Citrobacter* spp., *Eikenella corrodens, Arcanobacterium haemolyticum.*

†Formerly *Chlamydia pneumoniae.*

Data from references 9, 16, 21-23, 26, 29, 31, 34, 46, 48, 52, 53, 61, 62, 84, 90, 91, 95, 98, 107, 108, 113, 123, 135, 150, 152, 194, 196, 197, 206, 209, 210, 216.

children with chronic sinusitis, Cultrara and colleagues[34] cultured material from 13 bilateral endoscopic ethmoidectomies with maxillary antrostomies, 10 adenoidectomies, and 3 bilateral maxillary sinus lavages. They isolated *C. pneumoniae* from a nasopharyngeal swab and adenoid tissue from a 6-year-old child.

Fungal diseases of the sinuses have been well described in adults.[212] Acute fulminate fungal sinusitis is seen in immunosuppressed individuals and is associated with high morbidity and mortality rates.[37,179] *Aspergillus* spp. are the most common fungal causes of sinusitis. Many cases of chronic sinusitis from which a microorganism is not recovered have been thought to be caused by *Aspergillus* spp. infections.[93] The presence of eosinophils, Charcot-Leyden crystals, and hyphae found retrospectively, and not noted on the original examination, in

mucus recovered from sinuses suggests that some cases of chronic sinusitis may represent *Aspergillus* hypersensitivity. This allergic aspergillosis in the sinuses is similar to allergic bronchopulmonary aspergillosis. In a series of six patients who were 8 to 16 years of age and had allergic aspergillosis sinusitis, all presented with nasal polyposis and facial deformity, indicating advanced disease.[116] Mucormycosis, an infection caused by Zygomycetes (formerly Phycomycetes), is seen in immunocompromised children and adults.[89] *Drechslera* spp., *Bipolaris* spp., *Scopulariopsis* spp., *Candida* spp., and *Curvalaria lunata* can cause sinusitis in children.*

Although sinusitis has been reported as a complication of Epstein-Barr virus infection, the sinus infections seem to be a complication of corticosteroid treatment and not specifically the viral infection.[58] *Nocardia* spp. have been reported as a cause of acute sinusitis in an adult transplant recipient[157] and of chronic sinusitis in immunocompetent and immunocompromised individuals.[193]

EPIDEMIOLOGY

Although sinus involvement occurs commonly with viral infection of the respiratory tract, sinusitis seldom is identified as a specific illness in previously healthy children. In a survey of all office visits, totaling 2613, Breese and colleagues[20] noted only 6 children (0.23%) in whom the initial diagnosis was sinusitis. The true incidence of sinusitis in childhood is unknown. In 1989, Wald and colleagues[206] estimated that 0.5 to 5 percent of upper respiratory tract infections are complicated by acute sinusitis. More recent estimates by the same authors have been 10 percent.[208] The most recent estimates of greater incidence could be related to a heightened awareness and concern for lost work days by working parents, a possible correlation between pulmonary problems in an increasing number of children with chronic lung disease, better imaging techniques, increased interest in endoscopic sinus surgery,[187] more disease because of more exposure as a result of more children being in daycare,[114] and an increased recognition or perhaps incidence of allergy-related illness.[155,156] Seasonal prevalence has not been studied, but a reasonable assumption is that disease would increase during the cold weather months because it is the time of greatest respiratory viral activity. Cases in older children also can be expected to occur more frequently in association with swimming.

Shapiro and colleagues[168] used data from the National Ambulatory Medical Case Survey and National Hospital Ambulatory Medical Case Survey between 1998 and 2007 and found that the annual visit rate for acute sinusitis for children younger than 18 years of age ranged between 11 to 14 visits per 1000.

Although it is not well documented, sinusitis seems to be more of a problem in geographic areas where marked temperature changes occur. In children, sinusitis seems to occur more commonly in boys than in girls.[76,122] Ueda and Yoto[190] found abnormal findings in the maxillary sinuses in 135 (6.7%) of 2013 children who presented to an outpatient department with upper respiratory symptoms; of this group, 65 percent were boys, and 35 percent were girls. Manning and associates[115] found similar distribution in a group of 60 children diagnosed by CT or magnetic resonance imaging (MRI). Host factors are important in sinusitis because the illness occurs more commonly in allergic children; in children with chronic ear infections; and in patients with cystic fibrosis, primary humoral immunodeficiencies, and Kartagener syndrome.[25,83,130,161] Although an association between sinus disease and asthma seems to exist, controversy continues regarding whether sinusitis and other upper airway stimuli can induce asthma.[54,156,166,221] A review of hospital admissions of patients with status asthmaticus at the Children's Hospital of Los Angeles showed a marked increase in admissions, and sinusitis was diagnosed in 23 percent.[155] Children with various immunologic defects frequently have sinusitis.

Sinusitis is noncontagious from person to person, but point-source outbreaks are possible from swimming in heavily contaminated water. A cluster of seven cases of invasive nosocomial fungal sinusitis in severely neutropenic patients has been described. It was caused by the release of airborne fungal spores from soil reservoirs that were distributed during hospital construction during a 2-year period.[109]

CLINICAL PRESENTATION

The clinical symptoms of sinusitis vary by age. Older children and adolescents have localized complaints similar to those of adults, whereas in young children the findings are related less clearly to the sinuses.[199] The overall frequencies of symptoms, signs, and laboratory findings for acute, subacute, and chronic disease are presented in Table 14-2.

In young children, disease involves only the ethmoidal and maxillary sinuses. In these children, illness frequently has its onset after they have had an upper respiratory tract viral infection. A period of general improvement may occur, however, between the acute respiratory illness and the onset of symptoms related to sinus infection. The most prominent symptom in all children, and particularly in children younger than 10 years of age, is persistent rhinorrhea. The discharge frequently is purulent, but it occasionally can be serous or watery. Associated with rhinorrhea is cough, which becomes more prominent with increasing duration of disease. The cough particularly is troublesome at night because it is caused by the stimulation of the sinus drainage as it traverses the pharyngeal wall. The posterior drainage also occasionally causes vomiting. Fever is of variable occurrence in sinusitis and generally is related inversely to age and duration of illness. Malodorous breath often is reported by parents. The first evidence of illness in some children is fever and periorbital swelling. In most instances, periorbital cellulitis is a manifestation of ethmoidal sinusitis.

Although facial pain and headache are frequent complaints of sinus disease in adults, they have been noted in only approximately one third of the cases in children and

*References 15, 26, 53, 61, 108, 178.

TABLE 14-2 **Clinical Findings in Acute, Subacute, and Chronic Sinusitis of Children**

	Occurrence (%)	
	Acute and Subacute Sinusitis	Chronic Sinusitis
Symptoms		
Fever	50	20
Rhinorrhea	80	80
Cough (persistent and evening)	50	90
Pain/headache	30	30
Sore throat	20	20
Periorbital swelling	30	0
Vomiting	20	10
Allergic history	20	40
Malodorous breath	20	20
Signs		
Rhinorrhea	80	80
Temperature ≥38.3° C (≥101° F)	20	0
Sinus tenderness	20	10
Otitis media	40	60
Posterior pharyngeal pus	0	10
Transillumination positive	30	10
Periorbital swelling	30	0
Malodorous breath	20	20
Laboratory		
Abnormal radiographs	100	100
Maxillary	90	90
Ethmoidal	40	40
Frontal and sphenoidal	10	10
Unilateral	70	10
Bilateral	30	90
Erythrocyte sedimentation rate elevation	50	10
White blood cell count elevation with an increased percentage of band form neutrophils	40	10

Data from references 3, 11, 76, 79, 88, 94, 96, 122, 130, 156, 161, 181, 202, 211.

are unusual occurrences in young children. The main symptom in older children and adolescents is rhinorrhea. In older patients with more chronic disease, the nasal symptoms may be minimal or absent. Troublesome postnasal drip is a frequent complaint.

Acute isolated sphenoidal sinusitis in children is rare but often misdiagnosed because the symptoms are vague and there are no specific physical findings.[118] Findings include fever, headache, and neurologic symptoms. Swimming and diving are possible predisposing factors.

Physical signs in sinusitis also differ by age. Nasal discharge is the most frequent finding in all age groups. Young children are more likely to have a serous or watery discharge, however, than are adolescents. Elevation of temperature occurs more commonly in acute disease and in association with orbital cellulitis. Sinus tenderness, a common finding in older patients, is noted only rarely in children. Particularly significant is tenderness with percussion of the upper molars. Examination of the throat frequently reveals free exudate. Occasionally, the breath is malodorous.

The ears are abnormal in almost half of all patients with sinusitis. In acute disease in young children, it can be acute otitis media, but usually the findings are more suggestive of serous disease. Acute sinusitis frequently is unilateral, whereas chronic disease more often is bilateral.

Children with chronic sinusitis frequently have only minimal complaints. The parent notes that the child does not feel well and frequently reports that the child has had a persistent respiratory tract infection for months. In a series of children with chronic (>3 months) upper respiratory tract complaints who were referred to allergy clinics, 60 percent had sinusitis.[130] In this study, the combination of moderate to severe rhinorrhea and cough with minimal sneezing was reported to have a specificity of 95 percent and a sensitivity of 38 percent in predicting the presence of chronic sinusitis. In the referred children in this study, sinusitis was found in 63 percent of atopic children and in 75 percent of nonatopic children.

Laboratory studies other than cultures and radiography are not useful in the evaluation of a child with sinusitis. Herz and Gfeller[79] noted that in their study erythrocyte sedimentation rates were elevated in only approximately half of the patients and leukocytosis occurred in only one third. Generally, younger children with orbital cellulitis and ethmoidal sinusitis are more likely to have elevated sedimentation rates and white blood cell counts. The American Academy of Pediatrics (AAP) Subcommittee of Sinusitis and Committee on Quality Improvement has recommended that the diagnosis of acute bacterial sinusitis be based on clinical criteria in children who present with upper respiratory symptoms that are either persistent (nasal or postnasal discharge of any quality with or without daytime cough for >10 to 14 days) or severe (temperature >39° C [102.2 °F] and purulent nasal discharge present concurrently for at least 3 or 4 consecutive days in a child who appears ill).[5]

COMPLICATIONS

Serious complications, including meningitis, osteomyelitis, cavernous sinus thrombosis, and epidural, subdural, brain, and orbital abscesses, occur in untreated sinusitis.* Signs and symptoms of neurologic involvement in sinusitis frequently call for aggressive surgical management of the sinusitis and the intracranial and paracranial lesions. Osteomyelitis of the frontal bone may be a complication of frontal sinusitis and may manifest as a Pott puffy tumor if a subperiosteal abscess also is present.

*References 1, 17, 36, 57, 58, 71, 80, 101, 119, 125, 133, 134, 146, 163, 180, 189, 213, 215.

DIFFERENTIAL DIAGNOSIS

Differential considerations in sinusitis are few and are more concerned with whether sinus involvement in a particular child is the primary event or a secondary problem related to a more general host defect. Children with recurrent and chronic sinusitis should be evaluated for respiratory allergy, cystic fibrosis, immunologic deficiency, and Kartagener and other immotile cilia syndromes.

Foreign bodies in the nose can be mistaken for sinusitis, as can cysts in the maxillary antra. Nasal structural defects (congenital and acquired), such as palatal clefts, unilateral choanal atresia, nasal polyps, and septal deviation, can be confused with sinusitis, but more commonly these problems are predisposing factors in sinus infections.

Dental infections frequently are mistaken for maxillary sinus disease. Dental infections can lead by direct extension to sinus involvement. Primary infections in the region of the eye also occur without sinus disease. In young children, a chronic infection of the adenoids can be confused clinically with sinusitis. Infections with *Bordetella pertussis* can be confused with subacute sinusitis.

SPECIFIC DIAGNOSIS

Although persistent nasal symptoms and the presence of other clinical findings as listed in Table 14-2 indicate a diagnosis of sinusitis, the only certain way to make the diagnosis is by obtaining radiographs and cultures reflecting sinus content. Although it has been suggested that maxillary sinus radiographs frequently are abnormal for normal children,[117,174] other data indicate that normal children older than 1 year of age seldom have abnormal radiographs.[97] During infancy, the maxillary sinuses are so small that minimal mucosal edema may "opacify" a sinus on a radiograph. Radiographs in acute upper respiratory viral infections as a rule are abnormal; these radiographs are not false-positive ones but are the result of viral infections. From a therapeutic point of view, sinus radiographs usually should not be obtained unless nasal symptoms in an upper respiratory tract illness have not shown signs of improvement after 5 to 7 days.[6]

Plain film radiographic examination has been supplanted mainly by CT and MRI for the diagnosis of sinusitis. Many endoscopic sinus surgeons consider CT to be a mandatory part of the preoperative evaluation. MRI is useful in cases that may be complicated by orbital or intracranial extension. The high prevalence of incidental sinus opacification in asymptomatic infants and children noted radiographically has been confirmed by CT studies.[39,59,81] Since the advent of MRI, researchers have realized that many incidental sinus abnormalities also occur in adults. These findings in children and adults may be from subclinical or resolving respiratory infections or may be due to unrecognized allergies.[39]

CT has been recognized widely as the standard for the diagnosis of paranasal sinus disease.[14] In particular, coronal thin-section images offer excellent delineation of lesions in the osteomeatal complex.[222] Obtaining axial images is useful for evaluating periorbital and intraorbital complications.[51] In some institutions, a so-called screening CT of the sinuses is performed with a limited number of slices.[67] It can be offered at a cost and radiation exposure that are similar to those associated with plain film studies but with much greater accuracy. Some young children and infants require sedation for CT, which limits its suitability.

The AAP Subcommittee of Sinusitis and Committee on Quality Improvement has recommended that imaging studies are not necessary to confirm a diagnosis of clinical sinusitis in children 6 years of age or younger.[5] The need for radiographic evidence as a confirmatory test in children older than 6 years with severe symptoms is controversial. The American College of Radiology considers that the diagnosis of acute uncomplicated sinusitis should be made only on clinical grounds.[120]

Which radiographic technique (plain radiography, CT, or MRI) is selected for evaluation of a child with presumed sinusitis should be determined by availability of techniques and the expertise of the radiologist and by clinical symptoms. Radiography is indicated for children with continuing symptoms of sinusitis after extensive medical therapy or for children with possible complications of sinusitis. CT is optimal.[6] A limited CT scan with axial cuts may be obtained. It allows for excellent visualization of sinus anatomy and pathology while limiting the radiation exposure to the child. A limited sinus CT scan has radiation similar to that of a sinus plain film series. In the absence of low-cost screening CT, plain radiography should be the initial imaging study in most children who have symptoms of sinus disease.[38] Children who have periorbital swelling or proptosis should undergo immediate contrast-enhanced CT studies in axial and coronal planes. If symptoms or CT findings suggest intracranial extension, MRI should be performed.[6,38]

Although ultrasonography would seem to offer an alternative to sinus radiography, some question remains about its dependability unless one normal air-filled maxillary sinus or one opacified maxillary sinus is present for comparison.[197,204] The hallmark of specific diagnosis in sinusitis is similar to that of other infectious diseases—the culture of infected material.

The definitive method for culture requires puncture of the sinus cavity. As noted in the section on etiology, false-positive findings may occur because of contamination by the nasal flora. Nevertheless, sinus puncture and drainage is imperative in immunocompromised patients who may have fungal or other life-threatening infections. Sinus puncture for culture is not necessary for children who have uncomplicated sinus infections. However, evidence of etiology can be obtained by bacterial culture from the nose at the level of the maxillary ostium in the middle meatus. Although many experts do not believe in the usefulness of this procedure, there is considerable evidence to support the culture findings and etiology. Although the normal flora of the nasopharynx often contains the most common causes of sinusitis (i.e., *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *S. pyogenes*), the

anterior nose does not.* Therefore, we believe that properly performed nasal cultures will reveal the causative organism in many instances. This is particularly useful in the recovery of *S. pneumoniae*, which may be resistant to usual antimicrobial treatment.

Nasal culture should be taken from the region of the maxillary ostium in the middle meatus. Culture specimens should be obtained from this area and not from the nasopharynx. Best results are obtained when a vasoconstrictor, such as 0.25 percent phenylephrine hydrochloride, is administered first and the culture specimen is obtained with a cotton-tipped wire swab under direct vision. With this technique, material frequently can be obtained as it comes from the sinus ostium. Bilateral cultures always should be obtained. In serious cases, such as in children with neurologic complications or in treatment failures, performing antral puncture for culture can be lifesaving. Anaerobic and aerobic cultures should be performed on any material recovered by antral puncture.

TREATMENT

Acute and Subacute Sinusitis

The successful treatment of acute and subacute sinusitis in children depends primarily on the administration of an appropriate antibiotic in adequate dosage for a sufficient period.[5,203] In most instances, therapy should be instituted before obtaining the results of cultures. Antibiotic selection in this situation is not a great problem in children because the etiologic agent is *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, or *S. pyogenes* in more than 90 percent of acute cases.

Initial selection of an antibiotic should be based on the severity of the clinical illness and must take into consideration the antibiotic resistance patterns of the common causative organisms and the cost and ease of administration of the treatment regimen. Today, approximately 50 percent of nontypeable *H. influenzae* and 100 percent of *M. catarrhalis* strains produce β-lactamases and are resistant to amoxicillin.[41,42] In addition, 15 to 38 percent of *S. pneumoniae* strains have either intermediate (7% to 19%) or complete (5% to 19%) penicillin resistance.[12,28,43]

The AAP Subcommittee of Sinusitis and Committee on Quality Improvement, in their "Clinical Practice Guideline: Management of Sinusitis," recommended amoxicillin as first-line therapy for children younger than 2 years of age suspected of having acute bacterial sinusitis of mild to moderate severity who do not attend daycare and who have not been treated recently with an antimicrobial agent.[5] They recommend an amoxicillin dose of either 45 mg/kg/day in two divided doses or 90 mg/kg/day in two divided doses. They recommend cefdinir 14 mg/kg/day in one or two doses, cefuroxime 30 mg/kg/day in two divided doses, or cefpodoxime 10 mg/kg/day once daily for the child who is "allergic" to amoxicillin if the past allergic reaction was not a type 1 hypersensitivity reaction. If the past allergic reaction was of a type 1 nature, they recommend clarithromycin 15 mg/kg/day in two divided doses or azithromycin 10 mg/kg/day on day 1 followed by 5 mg/kg/day as a single dose for 4 days. They suggest clindamycin 30 to 40 mg/kg/day in three divided doses as an alternative therapy for the penicillin-allergic patient who is known to be infected with a penicillin-resistant pneumococcal strain. Their recommendation is based on the safety, tolerability, low cost, and narrow spectrum of amoxicillin and a calculated success rate based on data regarding treatment of acute otitis media.

The AAP committees further recommended that children who did not improve while receiving the lower amoxicillin dose, children with more severe illness, and children who attend daycare should be treated with high-dose amoxicillin-clavulanate 80 to 90 mg/kg/day of amoxicillin component in two divided doses. For children with vomiting, a single dose of ceftriaxone 50 mg/kg/day given either intravenously or intramuscularly is suggested. After this regimen, the child's therapy is switched to an oral regimen.

These suggestions of the committees applied only to children younger than 2 years of age; however, because the spectrum of etiologic agents is similar to that for older children, it should also apply for those older than 2 years of age.

In contrast to the AAP committees' recommendations, we consider that a more appropriate approach is to use amoxicillin-clavulanate 90 mg/kg/day in two divided doses. The duration of treatment in the outpatient setting has not been studied adequately. Wald[202] suggests that therapy should be continued for 7 days after the child becomes symptom free.

A seriously ill child should be hospitalized, and therapy for β-lactamase–producing staphylococci and highly resistant pneumococci should be implemented, in addition to coverage for amoxicillin-resistant *H. influenzae* and *M. catarrhalis*. This coverage is achieved with vancomycin 40 mg/kg per 24 hours every 6 hours and cefotaxime 100 to 200 mg/kg per 24 hours every 6 hours or ceftriaxone 100 mg/kg per 24 hours every 12 hours. Therapy should be adjusted on the basis of clinical response and culture results. The dosage and duration of antimicrobial therapy in sinusitis are crucial considerations. Penicillins penetrate the sinuses poorly.[10,50,68,89,110] The duration of therapy should be a minimum of 10 days.

The relief of obstruction at the sinus ostia and the establishment of drainage are time-honored principles of therapy. To achieve these goals, locally applied and systemically active vasoconstrictive drugs are used. To date, no evidence supports their therapeutic effectiveness, however.[167] The beneficial effects of oral, systemically active, vasoconstrictive drugs are hampered by the fact that their drying effect may be deleterious to the mucus blanket. Topical vasoconstrictor drugs (e.g., phenylephrine hydrochloride and oxymetazoline) are plagued by rebound vasodilation. We consider that these drugs should be used rarely in acute disease; their main use is to relieve pain caused by obstruction, and they should be used only for 2 to 3 days.

*References 18, 47, 56, 63, 72, 74, 75, 77, 82, 83, 86, 87, 91, 113, 122, 132, 171, 194, 196, 197, 206, 209, 210, 214, 220.

Chronic and Recurrent Sinusitis

Allergic disorders are common in chronic and recurrent sinusitis.[114,200] Children should be evaluated for allergy, and, when identified, specific treatment should be employed. Specific allergens and irritants should be avoided (e.g., through air filtering, removal of pets, avoidance of tobacco smoke), and pharmacologic management should be implemented.

Nasal saline washes (twice daily in each nostril) are useful because they liquefy secretions and enhance mucociliary transport, which improves sinus drainage and ventilation. Antihistamines may be useful if allergic rhinitis is a contributing factor to the chronic sinus infection. Anti-inflammatory agents also may be useful. In selected cases, use of either topically applied corticosteroids or cromolyn sodium may be beneficial. Corticosteroids should be used carefully because their use occasionally can lead to superinfection in the sinuses with *Pseudomonas* spp., other highly resistant gram-negative bacilli, or fungi. For effective corticosteroid use, Wald[200] suggests using a topical decongestant first so that the corticosteroid preparation can reach the affected areas better.

In chronic or recurrent disease, antimicrobial treatment should be based on culture and sensitivity data. Specific antimicrobial agents are the same as the agents used in acute and subacute disease, but treatment should be prolonged for 3 weeks or more and for 7 days after the resolution of symptoms. In 2004, Kuhn and Swain[100] defined acute fungal sinusitis in adults by 5 major criteria: (1) type I IgE-mediated hypersensitivity, (2) nasal polyps, (3) characteristic CT imaging, (4) allergic mucin within the sinuses, and (5) positive fungal smear. McClay and colleagues[121] and Campbell and associates[26] have further characterized acute fungal sinusitis in the pediatric population. It typically presents in atopic children with recurrent sinusitis and nasal polyps. Children, when compared with adults with acute fungal sinusitis, typically present with more facial abnormalities, including proptosis, and they present more frequently with unilateral disease or asymmetric disease. Surgical intervention is a mainstay of treatment for acute fungal sinusitis, with nearly half the patients requiring multiple procedures.[26] Acute fungal sinusitis is typically refractory to medical management until the fungal allergic mucin is first débrided and the sinus cavities are marsupialized for postoperative irrigation.

Aspergillus and *Bipolaris* spp. and other fungal infections require prolonged therapy with an antifungal agent to which the specific agent is susceptible. Itraconazole, ketoconazole, fluconazole, and amphotericin B all have been effective in selected cases. Allergic fungal sinusitis can be treated with endoscopic sinus débridement of all fungal and polypoid disease, followed by topical and systemic corticosteroids and close follow-up, including frequent endoscopic cleaning.[33,101,102,147] Allergic aspergillosis of the sinuses can be managed with topical corticosteroids without specific antifungal therapy (see Chapter 23).

In the past, surgical therapy for sinusitis in children was of questionable benefit. Surgical therapy included diagnostic and therapeutic irrigation; permanent drainage procedures in children with complications of sinusitis and in children who had immune defects; and such procedures as adenoidectomy,[104,192] septoplasty, and turbinectomy to relieve anatomic obstructions to improve nasal and sinus ventilation. In one uncontrolled study of children with otitis media with effusion and sinusitis, the sinusitis was improved 6 months after adenoidectomy in 56 percent of children whereas only 24 percent of similar children who did not undergo surgery had similar improvement.[185]

Historically, creating nasoantral windows was the most common major surgical procedure for treating chronic sinusitis in children.[65,127] Long-term success with this procedure was poor, however, because of the high rate of closure of the windows. A new interest in sinus surgery has resulted from the introduction of endoscopic techniques. Several studies have found endoscopic surgery to be safe and effective.[66,103,112,141,159] Pediatric endoscopic sinus surgery now is recognized as a viable option for children with chronic recurrent sinusitis refractory to medical therapy.[30,106,151] A meta-analysis of 832 children who underwent endoscopic sinus surgery from 1986 to 1996 revealed an overall 88.4 percent positive outcome after surgery.[78]

The goal of functional endoscopic sinus surgery is to remove obstruction at the osteomeatal complex where the mucociliary flow from the frontal, maxillary, and ethmoidal sinuses converges.[103,159] This removal results in improved drainage and restoration of normal physiologic function of the frontal, maxillary, and ethmoidal sinuses. Surgery involves an anterior ethmoidectomy and enlargement of the natural ostium of the maxillary sinus. Follow-up surgery performed 2 to 3 weeks after the initial surgery sometimes is necessary to remove crusts, blood clots, any stenting material, granulative tissue, and adhesions.[211]

In a study of 210 children with a history of chronic sinusitis for 3 months or longer, functional endoscopic sinus surgery resulted in successful outcomes in 165 (79%).[103] The follow-up period was 3 to 36 months (mean 18 months), and all of the infections in these children had failed to respond to prior extensive medical management. In this series, no major complications occurred.

Functional endoscopic sinus surgery should be considered for children with chronic or recurrent sinusitis that has failed extensive, prolonged, and adequate medical management.[106] There have been recent advancements in image guidance for pediatric sinus surgery. Image-guided surgery was first introduced for neurosurgical procedures and was quickly adapted by otolaryngologists in the 1990s for revision sinus surgery. The indications for image-guided surgery were expanded by the American Academy of Otolaryngology–Head and Neck Surgery (AAO-HNS) to include patients with any distorted sinus anatomy, those with extensive sinonasal polyposis, pathologic processes in the frontal, posterior ethmoidal, or sphenoidal sinuses, and patients undergoing skull base surgery.[4,92,165] Image-guided surgery has not been as well studied in the pediatric population as it has in the adult population[111]; however, the largest series to date by Postec

and coworkers,[142] suggests a similar utility in the pediatric population to that of the adult population. A recent literature review by Parikh and associates[136] showed that image-guided surgery can be safely utilized in children with chronic sinusitis, in those undergoing revision surgeries, and in any patient in whom the anatomy may be distorted, without increasing the rate of intraoperative complications.

Balloon catheter sinuplasty is another recent advancement in the treatment of sinus disease. This technology was first introduced in 2006 and provides a less invasive technique for enlargement of the sinus ostium. It involves the placement of a guide wire under fluoroscopic or fiberoptic light confirmation into a sinus cavity and the inflation of an oblong balloon to dilate the natural ostium by approximately 5 mm.[183] The main advantage of this technology is that, unlike traditional functional endoscopic sinus surgery, the balloon catheter sinuplasty provides a mucosal sparing technique without the removal of tissue. To date, there are no published prospective trials comparing functional endoscopic sinus surgery to balloon catheter sinuplasty in the pediatric population. The major limitation of balloon catheter sinuplasty is the inability to address other anatomic abnormalities such as a malpositioned uncinate or anterior ethmoidal disease. A limited number of studies demonstrate that balloon catheter sinuplasty is safe and efficacious in reducing sinus symptoms up to 1 year post procedure.[152-154] However, Ramadan and Terrell[154] also showed that the same reduction in symptoms was also accomplished with standard maxillary sinus irrigation plus adenoidectomy performed in children with chronic or recurrent sinusitis.

Many pediatric otolaryngologists have resumed performing adenoidectomy as a surgical intervention before proceeding with functional endoscopic sinus surgery.[177] It is thought that adenoidectomy removes a reservoir of bacteria that contributes to chronic or recurrent sinus infections.[172,176] Recently, there has been a renewed interest in evaluating both endoscopic sinus surgery and adenoidectomy and clarifying indications for each. Ramadan, in 2004,[151] performed a prospective nonrandomized study evaluating endoscopic sinus surgery and adenoidectomy. In this study, 202 children were divided into three groups, with group 1 receiving endoscopic sinus surgery with an adenoidectomy, group 2 receiving endoscopic sinus surgery alone, and group 3 receiving only an adenoidectomy. After 1 year follow-up, it was found that group 1 had 87 percent improvement in symptoms, compared with 75 percent and 52 percent in groups 2 and 3, respectively. After multivariate analysis of risk factors such as asthma, smoke exposure, and age, Ramadan concluded that adenoidectomy alone is recommended for children younger than 6 years old without asthma. Children with asthma at any age or children older than 6 years old with significant disease on the CT scans benefit from endoscopic sinus surgery at the time of the adenoidectomy. Other studies examining the efficacy of adenoidectomy alone in alleviating symptoms of chronic or recurrent sinusitis have demonstrated a range from 50 to 70 percent.[186,195] Meanwhile, a meta-analysis of pediatric patients treated with endoscopic sinus surgery found symptomatic improvement in nearly 90 percent of patients.[74]

In conjunction with endoscopic sinus surgery, specific antimicrobial therapy for organisms identified by culture is carried out. In addition, other contributing conditions, such as cystic fibrosis, asthma, or immunologic disorders need to be addressed.

Orbital and intracranial abscesses and cavernous sinus thrombosis secondary to sinus infection require emergency surgery, which often is lifesaving.[158,163,213,215] Cellulitis, osteomyelitis, and meningitis also frequently require surgery if they do not respond to antimicrobial therapy.[158] Surgery in these cases involves drainage of the sinuses and abscesses. Endoscopic techniques may allow for intranasal drainage and avoidance of development of facial scars.[7] If an intracranial complication involves an intracranial abscess, concomitant craniotomy along with functional endoscopic sinus surgery may be required.[60] Surgical procedures also may be indicated for a child with acute or chronic disease resulting from an identified underlying problem, such as an immunologic deficiency.[217]

PROGNOSIS

The prognosis of identified and adequately treated sinusitis in otherwise normal children is excellent. Frequently, children have subnormal health because sinusitis goes unrecognized; it may be treated only partially because of other clinical impressions, which contributes to the chronicity of the problem. Sinusitis is likely to be recurrent in children with a history of previous chronic disease and in children with repeated adverse exposure, such as swimming in contaminated or irritating water. Children with allergic respiratory disease also are likely to have frequent recurrences. Sinusitis in an immunocompromised child frequently is resistant to cure; long-term continuous therapy can be beneficial in such patients. Signs and symptoms of neurologic involvement in sinusitis frequently call for aggressive surgical management of the sinusitis and the intracranial and paracranial lesions.

Paranasal sinusitis also has been noted occasionally to be associated with bronchial asthma.[139,149] Its successful treatment has resulted in clearing of the asthma.[85,166]

PREVENTION

Sinusitis is not preventable in most instances. In some individuals, change of lifestyle can do much to improve the situation, however. Sinusitis in some children is related to their swimming habits and can be controlled by elimination of swimming or perhaps by the use of nose plugs. Good allergic management, including intranasal corticosteroid or cromolyn therapy, prevents sinus disease in certain atopic children. Relief of nasal airway obstruction caused by allergic rhinitis, enlarged adenoids, or other anatomic problems also should help to prevent sinusitis. Early attention given to persistent nasal discharge also can be expected to lessen the damage associated with sinus infection.

NEW REFERENCES SINCE THE SIXTH EDITION

13. Berger G, Kogan T, Paker M, et al. Pediatric chronic rhinosinusitis histopathology: differences and similarities with the adult form. *Otolaryngol Head Neck Surg* 2011;**144**:85–90.
17. Blumfield E, Misra M. Pott's puffy tumor, intracranial, and orbital complications as the initial presentation of sinusitis in healthy adolescents, a case series. *Emerg Radiol* 2011;**18**:203–10.
29. Chan KH, Kraai TL, Richter GT, et al. Toxic shock syndrome and rhinosinusitis in children. *Arch Otolaryngol Head Neck Surg* 2009;**135**:538–42.
32. Coffinet L, Chan KH, Abzug MJ, et al. Immunopathology of chronic rhinosinusitis in young children. *J Pediatr* 2009;**154**: 754–8.
36. DeMuri GP, Wald ER. Complications of acute bacterial sinusitis in children. *Pediatr Infect Dis J* 2011;**30**:701–2.
61. Gluck O, Segal N, Yariv F, et al. Pediatric invasive sinonasal *Scopulariopsis brevicaulis*—a case report and literature review. *Int J Pediatr Otorhinolaryngol* 2011;**75**:891–3.
80. Hicks CW, Weber JG, Reid JR, et al. Identifying and managing intracranial complications of sinusitis in children: a retrospective series. *Pediatr Infect Dis J* 2011;**30**:222–6.
84. Hsin CH, Su MC, Tsao CH, et al. Bacteriology and antimicrobial susceptibility of pediatric chronic rhinosinusitis: a 6-year result of maxillary sinus punctures. *Am J Otolaryngol* 2010;**31**:145–9.
108. Lin JJ, Wu CT, Hsia SH, et al. Pneumocephalus: a rare presentation of *Candida* sphenoid sinusitis. *Pediatr Neurol* 2009;**40**: 398–400.
123. McNeil JC, Hulten KG, Mason Jr EO, et al. Serotype 19A is the most common *Streptococcus pneumoniae* isolate in children with chronic sinusitis. *Pediatr Infect Dis J* 2009;**28**:766–8.
125. Mittal MK, Zimmerman RA. Meningitis and epidural abscess related to pansinusitis. *Pediatr Emerg Care* 2009;**25**:267–8.
133. Osman Farah J, Kandasamy J, May P, et al. Subdural empyema secondary to sinus infection in children. *Childs Nerv Syst* 2009;**25**:199–205.
136. Parikh SR, Cuellar H, Sadoughi B, et al. Indications for image-guidance in pediatric sinonasal surgery. *Int J Pediatr Otorhinolaryngol* 2009;**73**:351–6.
137. Payne SC, Borish L, Steinke JW. Genetics and phenotyping in chronic sinusitis. *J Allergy Clin Immunol* 2011;**128**:710–20; quiz 721-2.
152. Ramadan HH. Safety and feasibility of balloon sinuplasty for treatment of chronic rhinosinusitis in children. *Ann Otol Rhinol Laryngol* 2009;**118**:161–5.
153. Ramadan HH, McLaughlin K, Josephson G, et al. Balloon catheter sinuplasty in young children. *Am J Rhinol Allergy* 2010;**24**:e54–56.
154. Ramadan HH, Terrell AM. Balloon catheter sinuplasty and adenoidectomy in children with chronic rhinosinusitis. *Ann Otol Rhinol Laryngol* 2010;**119**:578–82.
167. Shaikh N, Wald ER, Pi M. Decongestants, antihistamines and nasal irrigation for acute sinusitis in children. *Cochrane Database Syst Rev* CD007909, 2010.
168. Shapiro DJ, Gonzales R, Cabana MD, et al. National trends in visit rates and antibiotic prescribing for children with acute sinusitis. *Pediatrics* 2011;**127**:28–34.
172. Shin KS, Cho SH, Kim KR, et al. The role of adenoids in pediatric rhinosinusitis. *Int J Pediatr Otorhinolaryngol* 2008;**72**:1643–50.
173. Shin SY, Choi GS, Park HS, et al. Immunological investigation in the adenoid tissues from children with chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 2009;**141**:91–6.
176. Silviu-Dan F. Pediatric chronic rhinosinusitis: the old, the new, and the reasonable. *Pediatr Ann* 2011;**40**:213–20.
180. Soon VT. Pediatric subperiosteal orbital abscess secondary to acute sinusitis: a 5-year review. *Am J Otolaryngol* 2011;**32**:62–8.
183. Stewart AE, Vaughan WC. Balloon sinuplasty versus surgical management of chronic rhinosinusitis. *Curr Allergy Asthma Rep* 2010;**10**:181–7.
184. Suh JD, Ramakrishnan V, Palmer JN. Biofilms. *Otolaryngol Clin North Am* 2010;**43**:521–30, viii.
189. Tsai BY, Lin KL, Lin TY, et al. Pott's puffy tumor in children. *Childs Nerv Syst* 2010;**26**:53–60.
204. Wald ER. Acute otitis media and acute bacterial sinusitis. *Clin Infect Dis* 2011;**52**(Suppl 4):S277–83.
205. Wald ER. *Staphylococcus aureus:* is it a pathogen of acute bacterial sinusitis in children and adults? *Clin Infect Dis* 2012;**54**:826–31.
216. Whitby CR, Kaplan SL, Mason Jr EO, et al. *Staphylococcus aureus* sinus infections in children. *Int J Pediatr Otorhinolaryngol* 2011;**75**:118–21.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 15

Otitis Externa

Ellen M. Friedman • Maria Carrillo-Marquez

Otitis externa is the inflammation of the external ear canal commonly caused by infection. It constitutes one of the most common otolaryngologic encounters in the emergency department. This condition is addressed commonly as *swimmer's ear* or *tropical ear* because it often occurs after a history of repeated water exposure and occurs more commonly in warm and humid environments. This condition became of significant interest during World War II because of its high incidence among the troops in the South Pacific. During World War II, otitis externa accounted for 50 to 70 percent of the caseload for otolaryngologists in the South Pacific.[28]

Today, otitis externa accounts for 7.5 million annual ototopical prescriptions in the United States and is a significant cause of ear pain and conductive hearing loss.[56] This chapter discusses the basic anatomy of the external

REFERENCES

1. Adame N, Hedlund G, Byington CL. Sinogenic intracranial empyema in children. *Pediatrics* 2005;**116**:e461–7.
2. Aitken M, Taylor JA. Prevalence of clinical sinusitis in young children followed up by primary care pediatricians. *Arch Pediatr Adolesc Med* 1998;**152**:244–8.
3. Alfaro VR. Nasal sinus disease in children. *Pediatr Clin North Am* 1962;**9**:1061–72.
4. American Academy of Otolaryngology—Head and Neck Surgery. Policy on intra-operative use of computer-aided surgery. http://www.entlink.net/practice/rules/image-guiding.cfm, 2002.
5. American Academy of Pediatrics Subcommittee on Management of Sinusitis and Committee on Quality Improvement: Clinical Practice Guideline: Management of Sinusitis—Subcommittee on Management of Sinusitis and Committee on Quality Improvement. *Pediatrics* 2001;**108**:798.
6. April MM, Zinreich SJ, Baroody FM, et al. Coronal CT scan abnormalities in children with chronic sinusitis. *Laryngoscope* 1993;**103**:985–90.
7. Arjmand EM, Lusk RP, Muntz HR. Pediatric sinusitis and subperiosteal orbital abscess formation: diagnosis and treatment. *Otolaryngol Head Neck Surg* 1993;**109**:886–94.
8. Arruda E, Boyle TR, Winther B, et al. Localization of human rhinovirus replication in the upper respiratory tract by in situ hybridization. *J Infect Dis* 1995;**171**:1329–33.
9. Axelsson A, Brorson JE. The correlation between bacteriological findings in the nose and maxillary sinus in acute maxillary sinusitis. *Laryngoscope* 1973;**83**:2003–11.
10. Axelsson A, Grebelius N, Jensen C, et al. Treatment of acute maxillary sinusitis. IV. Ampicillin, cephradine and erythromycin estolate with and without irrigation. *Acta Otolaryngol* 1975;**79**:466–72.
11. Axelsson A, Runze U. Symptoms and signs of acute maxillary sinusitis. *ORL J Otorhinolaryngol Relat Spec* 1976;**38**:298–308.
12. Baquero F, Loza E. Antibiotic resistance of microorganisms involved in ear, nose and throat infections. *Pediatr Infect Dis J* 1994;**13**:S9–14; discussion S20–2.
13. Berger G, Kogan T, Paker M, et al. Pediatric chronic rhinosinusitis histopathology: differences and similarities with the adult form. *Otolaryngol Head Neck Surg* 2011;**144**:85–90.
14. Berry AJ, Kerkering TM, Giordano AM, et al. Phaeohyphomycotic sinusitis. *Pediatr Infect Dis* 1984;**3**:150–2.
15. Bhattacharyya N, Jones DT, Hill M, et al. The diagnostic accuracy of computed tomography in pediatric chronic rhinosinusitis. *Arch Otolaryngol Head Neck Surg* 2004;**130**:1029–32.
16. Bhattacharyya TK, Mehra YN, Agarwal SC. Incidence of bacteria, L-form and mycoplasma in chronic sinusitis. *Acta Otolaryngol* 1972;**74**:293–6.
17. Blumfield E, Misra M. Pott's puffy tumor, intracranial, and orbital complications as the initial presentation of sinusitis in healthy adolescents, a case series. *Emerg Radiol* 2011;**18**:203–10.
18. Box QT, Cleveland RT, Willard CY. Bacterial flora of the upper respiratory tract: I. Comparative evaluation by anterior nasal, oropharyngeal, and nasopharyngeal swabs. *Arch Pediatr Adolesc Med* 1961;**102**:293–301.
19. Brandtzaeg P. Mucosal immunology—with special reference to specific immune defence of the upper respiratory tract. *ORL* 1988;**50**:225–35.
20. Breese BB, Disney FA, Talpey W. The nature of a small pediatric group practice. *Pediatrics* 1966;**38**:264–77.
21. Brook I. Bacteriologic features of chronic sinusitis in children. *JAMA* 1981;**246**:967–9.
22. Brook I. Beta-lactamase–producing bacteria in head and neck infection. *Laryngoscope* 1988;**98**:428–31.
23. Brook I. The role of anaerobic bacteria in sinusitis. *Anaerobe* 2006;**12**:5–12.
24. Brook I, Yocum P, Shah K. Aerobic and anaerobic bacteriology of concurrent chronic otitis media with effusion and chronic sinusitis in children. *Arch Otolaryngol Head Neck Surg* 2000;**126**:174–6.
25. Buehring I, Friedrich B, Schaaf J, et al. Chronic sinusitis refractory to standard management in patients with humoral immunodeficiencies. *Clin Exp Immunol* 1997;**109**:468–72.
26. Campbell JM, Graham M, Gray HC, et al. Allergic fungal sinusitis in children. *Ann Allergy Asthma Immunol* 2006;**96**:286–90.
27. Carenfelt C, Lundberg C, Karlen K. Immunoglobulins in maxillary sinus secretion. *Acta Otolaryngol* 1976;**82**:123–30.
28. Centers for Disease Control and Prevention. Geographic variation in penicillin resistance in *Streptococcus pneumoniae*—selected sites, United States, 1997. *MMWR Morbid Mortal Wkly Rep* 1999;**48**:656–61.
29. Chan KH, Kraai TL, Richter GT, et al. Toxic shock syndrome and rhinosinusitis in children. *Arch Otolaryngol Head Neck Surg* 2009;**135**:538–42.
30. Chan KH, Winslow CP, Levin MJ, et al. Clinical practice guidelines for the management of chronic sinusitis in children. *Otolaryngol Head Neck Surg* 1999;**120**:328–34.
31. Cherry JD. Infection in the compromised host. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 975–1013.
32. Coffinet L, Chan KH, Abzug MJ, et al. Immunopathology of chronic rhinosinusitis in young children. *J Pediatr* 2009;**154**:754–8.
33. Corey JP, Delsupehe KG, Ferguson BJ. Allergic fungal sinusitis: allergic, infectious, or both? *Otolaryngol Head Neck Surg* 1995;**113**:110–9.
34. Cultrara A, Goldstein NA, Ovchinsky A, et al. The role of *Chlamydia pneumoniae* infection in children with chronic sinusitis. *Arch Otolaryngol Head Neck Surg* 2003;**129**:1094–7.
35. Dawes JD. Diagnosis and treatment of sinusitis. *BMJ* 1966;**1**:843–5.
36. DeMuri GP, Wald ER. Complications of acute bacterial sinusitis in children. *Pediatr Infect Dis J* 2011;**30**:701–2.
37. Deshazo RD, O'Brien M, Chapin K, et al. A new classification and diagnostic criteria for invasive fungal sinusitis. *Arch Otolaryngol Head Neck Surg* 1997;**123**:1181–8.
38. Diament MJ. The diagnosis of sinusitis in infants and children: x-ray, computed tomography, and magnetic resonance imaging: diagnostic imaging of pediatric sinusitis. *J Allergy Clin Immunol* 1992;**90**:442–4.
39. Diament MJ, Senac Jr MO, Gilsanz V, et al. Prevalence of incidental paranasal sinuses opacification in pediatric patients: a CT study. *J Comput Assist Tomogr* 1987;**11**:426–31.
40. Dingle JH, Badjer DF, Jordan Jr WS. *Patterns of illness: illness in the home*. Cleveland, Ohio: Western Reserve University; 1964.
41. Doern GV, Brueggemann AB, Pierce G, et al. Antibiotic resistance among clinical isolates of *Haemophilus influenzae* in the United States in 1994 and 1995 and detection of β-lactamase–positive strains resistant to amoxicillin-clavulanate: results of a National Multicenter Surveillance study. *Antimicrob Agents Chemother* 1997;**41**:292–7.
42. Doern GV, Jones RN, Pfaller MA, et al. *Haemophilus influenzae* and *Moraxella catarrhalis* from patients with community-acquired respiratory tract infections: antimicrobial susceptibility patterns from the SENTRY antimicrobial Surveillance Program (United States and Canada, 1997). *Antimicrob Agents Chemother* 1999;**43**:385–9.
43. Dowell SF, Butler JC, Giebink GS, et al. Acute otitis media: management and surveillance in an era of pneumococcal resistance—a report from the Drug-resistant *Streptococcus pneumoniae* Therapeutic Working Group. *Pediatr Infect Dis J* 1999;**18**:1–9.
44. Dudley JP, Cherry JD. The effect of mucolytic agents and topical decongestants on the ciliary activity of chicken tracheal organ cultures. *Pediatr Res* 1977;**11**:904–6.
45. Dudley JP, Cherry JD. Effects of topical nasal decongestants on the cilia of a chicken embryo tracheal organ culture system. *Laryngoscope* 1978;**88**:110–6.
46. Dudley JP, Goldstein EJC, George WL. Sinus infection due to *Eikenella corrodens*. *Arch Otolaryngol* 1978;**104**:462–3.
47. Dunlap MB, Harvey HS. Host influence on upper respiratory flora. *N Engl J Med* 1956;**255**:640–6.
48. Edvinsson M, Asplund MS, Hjelm E, et al. *Chlamydophila pneumoniae* in chronic rhinosinusitis. *Acta Otolaryngol* 2006;**126**:952–7.
49. Eliasson R, Mossberg B, Camner P, et al. The immotile-cilia syndrome: a congenital ciliary abnormality as an etiologic factor in chronic airway infections and male sterility. *N Engl J Med* 1977;**297**:1–6.
50. Eneroth CM, Lundberg C, Wretlind B. Antibiotic concentrations in maxillary sinus secretions and in the sinus mucosa. *Chemotherapy* 1975;**21**(Suppl 1):1–7.

51. Fernbach SK, Naidich TP. CT diagnosis of orbital inflammation in children. *Neuroradiology* 1981;**22**:7–13.
52. Frederick J, Braude AI. Anaerobic infection of the paranasal sinuses. *N Engl J Med* 1974;**290**:135–7.
53. Frenkel L, Kuhls TL, Nitta K, et al. Recurrent *Bipolaris* sinusitis following surgical and antifungal therapy. *Pediatr Infect Dis J* 1987;**6**:1130–2.
54. Friday Jr GA, Fireman P. Sinusitis and asthma: clinical and pathogenetic relationships. *Clin Chest Med* 1988;**9**:557–65.
55. Friedman R, Ackerman M, Wald E, et al. Asthma and bacterial sinusitis in children. *J Allergy Clin Immunol* 1984;**74**:185–9.
56. Gellady AM, Shulman ST, Ayoub EM. Periorbital and orbital cellulitis in children. *Pediatrics* 1978;**61**:272–7.
57. Germiller JA, Monin DL, Sparano AM, et al. Intracranial complications of sinusitis in children and adolescents and their outcomes. *Arch Otolaryngol Head Neck Surg* 2006;**132**:969–76.
58. Givner LB, McGehee D, Taber LH, et al. Sinusitis, orbital cellulitis and polymicrobial bacteremia in a patient with primary Epstein-Barr virus infection. *Pediatr Infect Dis* 1984;**3**:254–6.
59. Glasier CM, Ascher DP, Williams KD. Incidental paranasal sinus abnormalities on CT of children: clinical correlation. *AJNR Am J Neuroradiol* 1986;**7**:861–4.
60. Glickstein JS, Chandra RK, Thompson JW. Intracranial complications of pediatric sinusitis. *Otolaryngol Head Neck Surg* 2006; **134**:733–6.
61. Gluck O, Segal N, Yariv F, et al. Pediatric invasive sinonasal *Scopulariopsis brevicaulis*—a case report and literature review. *Int J Pediatr Otorhinolaryngol* 2011;**75**:891–3.
62. Gnarpe H, Lundberg C. Preliminary report. L-phase organisms in maxillary sinus secretions. *Scand J Infect Dis* 1971;**3**:257–9.
63. Gordts F, Abu Nasser I, Clement PA, et al. Bacteriology of the middle meatus in children. *Int J Pediatr Otorhinolaryngol* 1999;**48**:163–7.
64. Griffin JP, Klein EW. Role of sinusitis in primary atypical pneumonia. *Clin Med* 1971;**78**:23–7.
65. Gross CW. The diagnosis and management of sinusitis in children. Surgical management: an otolaryngologist's perspective. *Pediatr Infect Dis* 1985;**4**:S67–72.
66. Gross CW, Gurucharri MJ, Lazar RH, et al. Functional endonasal sinus surgery (FESS) in the pediatric age group. *Laryngoscope* 1989;**99**:272–5.
67. Gross GW, McGeady SJ, Kerut T, et al. Limited-slice CT in the evaluation of paranasal sinus disease in children. *AJR Am J Roentgenol* 1991;**156**:367–9.
68. Gullers K, Lundberg C, Malmborg AS. Penicillin in paranasal sinus secretions. *Chemotherapy* 1969;**14**:303–7.
69. Gwaltney Jr JM, Hendley JO, Simon G, et al. Rhinovirus infections in an industrial population. I. The occurrence of illness. *N Engl J Med* 1966;**275**:1261–8.
70. Gwaltney Jr JM, Hendley JO, Phillips CD, et al. Nose blowing propels nasal fluid into the paranasal sinuses. *Clin Infect Dis* 2000;**30**:387–91.
71. Hakim HE, Malik AC, Aronyk K, et al. The prevalence of intracranial complications in pediatric frontal sinusitis. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1383–7.
72. Hamory BH, Sande MA, Sydnor Jr A, et al. Etiology and antimicrobial therapy of acute maxillary sinusitis. *J Infect Dis* 1979; **139**:197–202.
73. Hansson LA, Ahlstedt S, Andersson B, et al. Mucosal immunity. *Ann N Y Acad Sci* 1983;**409**:1–21.
74. Harvey HS, Dunlap MB. Seasonal prevalence of upper respiratory pathogens. *N Engl J Med* 1961;**264**:684–6.
75. Hawkins DB, Clark RW. Orbital involvement in acute sinusitis: lessons from 24 childhood patients. *Clin Pediatr (Phila)* 1977;**16**: 464–71.
76. Haynes RE, Cramblett HG. Acute ethmoiditis: its relationship to orbital cellulitis. *Am J Dis Child* 1967;**114**:261–7.
77. Hays GC, Mullard JE. Can nasal bacterial flora be predicted from clinical findings? *Pediatrics* 1972;**49**:596–9.
78. Hebert 2nd RL, Bent 3rd JP. Meta-analysis of outcomes of pediatric functional endoscopic sinus surgery. *Laryngoscope* 1998; **108**:796–9.
79. Herz G, Gfeller J. Sinusitis in paediatrics. *Chemotherapy* 1977; **23**:50–7.
80. Hicks CW, Weber JG, Reid JR, et al. Identifying and managing intracranial complications of sinusitis in children: a retrospective series. *Pediatr Infect Dis J* 2011;**30**:222–6.
81. Hill M, Bhattacharyya N, Hall TR, et al. Incidental paranasal sinus imaging abnormalities and the normal Lund score in children. *Otolaryngol Head Neck Surg* 2004;**130**:171–5.
82. Holdaway MD, Turk DC. Capsulated *Haemophilus influenzae* and respiratory-tract disease. *Lancet* 1967;**1**:358–60.
83. Hoshaw TC, Nickman NJ. Sinusitis and otitis in children. *Arch Otolaryngol* 1974;**100**:194–5.
84. Hsin CH, Su MC, Tsao CH, et al. Bacteriology and antimicrobial susceptibility of pediatric chronic rhinosinusitis: a 6-year result of maxillary sinus punctures. *Am J Otolaryngol* 2010;**31**:145–9.
85. Ikeda K, Tanno N, Tamura G, et al. Endoscopic sinus surgery improves pulmonary function in patients with asthma associated with chronic sinusitis. *Ann Otol Rhinol Laryngol* 1999;**108**:355–9.
86. Ilki A, Ulger N, Inanli S, et al. Microbiology of sinusitis and the predictive value of throat culture for the aetiology of sinusitis. *Clin Microbiol Infect* 2005;**11**:407–10.
87. Jacobson LO, Dick GF. Normal And Abnormal Bacterial Flora Of The Nose. *JAMA* 1941;**117**:2222–5.
88. Jaffe BF. Chronic sinusitis in children: Comments on pathogenesis and management. *Clin Pediatr (Phila)* 1974;**13**:944–8.
89. Jeppesen F, Illum P. Concentration of ampicillin in antral mucosa following administration of ampicillin sodium and pivampicillin. *Acta Otolaryngol* 1972;**73**:428–32.
90. Jousimies-Somer HR, Savolainen S, Ylikoski JS. Bacteriological findings of acute maxillary sinusitis in young adults. *J Clin Microbiol* 1988;**26**:1919–25.
91. Jousimies-Somer HR, Savolainen S, Ylikoski JS. Comparison of the nasal bacterial floras in two groups of healthy subjects and in patients with acute maxillary sinusitis. *J Clin Microbiol* 1989;**27**:2736–43.
92. Kacker A, Tabaee A, Anand V. Computer-assisted surgical navigation in revision endoscopic sinus surgery. *Otolaryngol Clin North Am* 2005;**38**:473–82, vi.
93. Katzenstein AL, Sale SR, Greenberger PA. Pathologic findings in allergic *Aspergillus* sinusitis: a newly recognized form of sinusitis. *Am J Surg Pathol* 1983;**7**:439–43.
94. Kern EB. Sinusitis. *J Allergy Clin Immunol* 1984;**73**:25–31.
95. Kim HJ, Lee K, Yoo JB, et al. Bacteriological findings and antimicrobial susceptibility in chronic sinusitis with nasal polyp. *Acta Otolaryngol* 2006;**126**:489–97.
96. Kogutt MS, Swischuk LE. Diagnosis of sinusitis in infants and children. *Pediatrics* 1973;**52**:121–4.
97. Kovatch AL, Wald ER, Ledesma-Medina J, et al. Maxillary sinus radiographs in children with nonrespiratory complaints. *Pediatrics* 1984;**73**:306–8.
98. Krajina Z, Koskovic F, Babic I. The bacteriology of the respiratory tract in various pathological conditions. *Acta Otolaryngol* 1969;**67**:453–9.
99. Kristo A, Uhari M, Luotonen J, et al. Paranasal sinus findings in children during respiratory infection evaluated with magnetic resonance imaging. *Pediatrics* 2003;**111**:e586–9.
100. Kuhn FA, Swain Jr R. Allergic fungal sinusitis: diagnosis and treatment. *Curr Opin Otolaryngol Head Neck Surg* 2003;**11**:1–5.
101. Kupferberg SB, Bent JP. Allergic fungal sinusitis in the pediatric population. *Arch Otolaryngol Head Neck Surg* 1996;**122**:1381–4.
102. Kupferberg SB, Bent 3rd JP, Kuhn FA. Prognosis for allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1997;**117**:35–41.
103. Lazar RH, Younis RT, Gross CW. Pediatric functional endonasal sinus surgery: review of 210 cases. *Head Neck* 1992;**14**:92–8.
104. Lee D, Rosenfeld RM. Adenoid bacteriology and sinonasal symptoms in children. *Otolaryngol Head Neck Surg* 1997;**116**:301–7.
105. Lehtinen P, Jartti T, Virkki R, et al. Bacterial coinfections in children with viral wheezing. *Eur J Clin Microbiol Infect Dis* 2006;**25**:463–9.
106. Lieser JD, Derkay CS. Pediatric sinusitis: when do we operate? *Curr Opin Otolaryngology Head Neck Surg* 2005;**13**:60–6.
107. Limjoco-Antonio AD, Janda WM, Schreckenberger PC. *Arcanobacterium haemolyticum* sinusitis and orbital cellulitis. *Pediatr Infect Dis J* 2003;**22**:465–7.
108. Lin JJ, Wu CT, Hsia SH, et al. Pneumocephalus: a rare presentation of *Candida* sphenoid sinusitis. *Pediatr Neurol* 2009;**40**: 398–400.
109. Lueg EA, Ballagh RH, Forte V. Analysis of the recent cluster of invasive fungal sinusitis at the Toronto Hospital for Sick Children. *J Otolaryngol* 1996;**25**:366–70.
110. Lundberg C, Malmburg AS. Studies of antibiotics in sinus secretions. *Rhinology* 1971;**9**:166–8.

111. Lusk R. Computer-assisted functional endoscopic sinus surgery in children. *Otolaryngol Clin North Am* 2005;**38**:505–13, vii.
112. Lusk RP, Muntz HR. Endoscopic sinus surgery in children with chronic sinusitis: a pilot study. *Laryngoscope* 1990;**100**: 654–8.
113. Lystad A, Berdal P, Lund-Iversen L. The bacterial flora of sinusitis with an in vitro study of the bacterial resistance to antibiotics. *Acta Otolaryngol Suppl* 1964;**188**.
114. Manning SC. Pediatric sinusitis. *Otolaryngol Clin North Am* 1993;**26**:623–38.
115. Manning SC, Biavati MJ, Phillips DL. Correlation of clinical sinusitis signs and symptoms to imaging findings in pediatric patients. *Int J Pediatr Otorhinolaryngol* 1996;**37**:65–74.
116. Manning SC, Vuitch F, Weinberg AG, et al. Allergic aspergillosis: a newly recognized form of sinusitis in the pediatric population. *Laryngoscope* 1989;**99**:681–5.
117. Maresh MM, Washburn AH. Paranasal sinuses from birth to late adolescence: II. Clinical and roentgenographic evidence of infection. *Arch Pediatr Adolesc Med* 1940;**60**:841–61.
118. Marseglia GL, Pagella F, Licari A, et al. Acute isolated sphenoid sinusitis in children. *Int J Pediatr Otorhinolaryngol* 2006;**70**: 2027–31.
119. May ML. Severe consequences of sinusitis. *J Paediatr Child Health* 2004;**40**:311–4.
120. McAlister WH, Parker BR, Kushner DC, et al. Sinusitis in the pediatric population. American College of Radiology. ACR Appropriateness Criteria. *Radiology* 2000;**215**(Suppl):811–18.
121. McClay JE, Marple B, Kapadia L, et al. Clinical presentation of allergic fungal sinusitis in children. *Laryngoscope* 2002;**112**: 565–9.
122. McLean DC. Sinusitis in children: lessons from twenty-five patients. *Clin Pediatr (Phila)* 1970;**9**:342–5.
123. McNeil JC, Hulten KG, Mason Jr EO, et al. Serotype 19A is the most common *Streptococcus pneumoniae* isolate in children with chronic sinusitis. *Pediatr Infect Dis J* 2009;**28**:766–8.
124. Min YG, Kim HS, Suh SH, et al. Paranasal sinusitis after long-term use of topical nasal decongestants. *Acta Otolaryngol* 1996; **116**:465–71.
125. Mittal MK, Zimmerman RA. Meningitis and epidural abscess related to pansinusitis. *Pediatr Emerg Care* 2009;**25**:267–8.
126. Mofenson LM, Korelitz J, Pelton S, et al. Sinusitis in children infected with human immunodeficiency virus: clinical characteristics, risk factors, and prophylaxis. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *Clin Infect Dis* 1995;**21**:1175–81.
127. Muntz HR, Lusk RP. Nasal antral windows in children: a retrospective study. *Laryngoscope* 1990;**100**:643–6.
128. Muntz HR, Lusk RP. Bacteriology of the ethmoid bullae in children with chronic sinusitis. *Arch Otolaryngol Head Neck Surg* 1991;**117**:179–81.
129. Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinovirus colds. *J Infect Dis* 1988;**157**:133–42.
130. Nguyen KL, Corbett ML, Garcia DP, et al. Chronic sinusitis among pediatric patients with chronic respiratory complaints. *J Allergy Clin Immunol* 1993;**92**:824–30.
131. Noah TL, Henderson FW, Wortman IA, et al. Nasal cytokine production in viral acute upper respiratory infection of childhood. *J Infect Dis* 1995;**171**:584–92.
132. Orobello Jr PW, Park RI, Belcher LJ, et al. Microbiology of chronic sinusitis in children. *Arch Otolaryngol Head Neck Surg* 1991;**117**:980–3.
133. Osman Farah J, Kandasamy J, May P, et al. Subdural empyema secondary to sinus infection in children. *Childs Nerv Syst* 2009;**25**:199–205.
134. Oxford LE, McClay J. Complications of acute sinusitis in children. *Otolaryngol Head Neck Surg* 2005;**133**:32–7.
135. Palva T, Gronroos JA, Palva A. Bacteriology and pathology of chronic maxillary sinusitis. *Acta Otolaryngol* 1962;**54**:159–75.
136. Parikh SR, Cuellar H, Sadoughi B, et al. Indications for image-guidance in pediatric sinonasal surgery. *Int J Pediatr Otorhinolaryngol* 2009;**73**:351–6.
137. Payne SC, Borish L, Steinke JW. Genetics and phenotyping in chronic sinusitis. *J Allergy Clin Immunol* 2011;**128**:710–20; quiz 721–2.
138. Pedersen M, Sakakura Y, Winther B, et al. Nasal mucociliary transport, number of ciliated cells, and beating pattern in naturally acquired common colds. *Eur J Respir Dis Suppl* 1983;**128** (Pt 1):355–65.
139. Phipatankul CS, Slavin RG. Bronchial asthma produced by paranasal sinusitis. *Arch Otolaryngol* 1974;**100**:109–12.
140. Pitkaranta A, Starck M, Savolainen S, et al. Rhinovirus RNA in the maxillary sinus epithelium of adult patients with acute sinusitis. *Clin Infect Dis* 2001;**33**:909–11.
141. Poole MD. Pediatric sinusitis is not a surgical disease. *Ear Nose Throat J* 1992;**71**:622–3.
142. Postec F, Bossard D, Disant F, et al. Computer-assisted navigation system in pediatric intranasal surgery. *Arch Otolaryngol Head Neck Surg* 2002;**128**:797–800.
143. Proctor DF. The historical background of modern otolaryngology. In: Ravitch MM, editor. *The nose, paranasal sinuses and ears in childhood*. Springfield, Ill: Charles C Thomas; 1963. p. 3–19.
144. Proud D, Gwaltney Jr JM, Hendley JO, et al. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinovirus colds. *J Infect Dis* 1994;**169**:1007–13.
145. Proud D, Naclerio RM, Gwaltney JM, et al. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 1990;**161**:120–3.
146. Quraishi H, Zevallos JP. Subdural empyema as a complication of sinusitis in the pediatric population. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1581–6.
147. Quraishi HA, Ramadan HH. Endoscopic treatment of allergic fungal sinusitis. *Otolaryngol Head Neck Surg* 1997;**117**:29–34.
148. Rachelefsky GS, Katz RM, Siegel SC. Chronic sinusitis in children with respiratory allergy: the role of antimicrobials. *J Allergy Clin Immunol* 1982;**69**:382–7.
149. Rachelefsky GS, Katz RM, Siegel SC. Chronic sinus disease with associated reactive airway disease in children. *Pediatrics* 1984;**73**:526–9.
150. Rachelefsky GS, Katz RM, Siegel SC. Chronic sinusitis in the allergic child. *Pediatr Clin North Am* 1988;**35**:1091–101.
151. Ramadan HH. Surgical management of chronic sinusitis in children. *Laryngoscope* 2004;**114**:2103–9.
152. Ramadan HH. Safety and feasibility of balloon sinuplasty for treatment of chronic rhinosinusitis in children. *Ann Otol Rhinol Laryngol* 2009;**118**:161–5.
153. Ramadan HH, McLaughlin K, Josephson G, et al. Balloon catheter sinuplasty in young children. *Am J Rhinol Allergy* 2010;**24**:e54–6.
154. Ramadan HH, Terrell AM. Balloon catheter sinuplasty and adenoidectomy in children with chronic rhinosinusitis. *Ann Otol Rhinol Laryngol* 2010;**119**:578–82.
155. Richards W. Hospitalization of children with status asthmaticus: a review. *Pediatrics* 1989;**84**:111–8.
156. Richards W, Roth RM, Church JA. Underdiagnosis and undertreatment of chronic sinusitis in children. *Clin Pediatr (Phila)* 1991;**30**:88–92.
157. Roberts SA, Bartley J, Braatvedt G, et al. *Nocardia asteroides* as a cause of sphenoidal sinusitis: case report. *Clin Infect Dis* 1995;**21**:1041–2.
158. Rosenfeld EA, Rowley AH. Infectious intracranial complications of sinusitis, other than meningitis, in children: 12-year review. *Clin Infect Dis* 1994;**18**:750–4.
159. Rosenfeld RM. Pilot study of outcomes in pediatric rhinosinusitis. *Arch Otolaryngol Head Neck Surg* 1995;**121**:729–36.
160. Rossen RD, Butler WT, Cate TR, et al. Protein composition of nasal secretion during respiratory virus infection. *Proc Soc Exp Biol Med* 1965;**119**:1169–76.
161. Rulon JT. Sinusitis in children. *Postgrad Med* 1970;**48**:107–12.
162. Rynnel-Dagoo B, Forsgren J, Freijd A, et al. Rationale for antibiotic therapy in pediatric ear, nose and throat infections: immunologic issues. *Pediatr Infect Dis J* 1994;**13**:S15–20; discussion S20–2.
163. Sable NS, Hengerer A, Powell KR. Acute frontal sinusitis with intracranial complications. *Pediatr Infect Dis* 1984;**3**:58–61.
164. Schenck NL, Rauchbach E. Frontal sinus disease. IV. Cellular response to experimentally-induced infection. *Laryngoscope* 1976;**86**:1726–33.
165. Schlondorff G, Mosges R, Meyer-Ebrecht D, et al. CAS (computer assisted surgery). A new procedure in head and neck surgery. *HNO* 1989;**37**:187–90.
166. Senior BA, Kennedy DW, Tanabodee J, et al. Long-term impact of functional endoscopic sinus surgery on asthma. *Otolaryngol Head Neck Surg* 1999;**121**:66–8.

167. Shaikh N, Wald ER, Pi M. Decongestants, antihistamines and nasal irrigation for acute sinusitis in children. *Cochrane Database Syst Rev* CD007909, 2010.
168. Shapiro DJ, Gonzales R, Cabana MD, et al. National trends in visit rates and antibiotic prescribing for children with acute sinusitis. *Pediatrics* 2011;**127**:28–34.
169. Shapiro GG. The role of nasal airway obstruction in sinus disease and facial development. *J Allergy Clin Immunol* 1988;**82**: 935–40.
170. Shapiro GG, Virant FS, Furukawa CT, et al. Immunologic defects in patients with refractory sinusitis. *Pediatrics* 1991;**87**:311–6.
171. Shibley GS, Hanger FM, Dochez AR. Studies in the common cold: I. Observations of the normal bacterial flora of nose and throat with variations occurring during colds. *J Exp Med* 1926;**43**:415–31.
172. Shin KS, Cho SH, Kim KR, et al. The role of adenoids in pediatric rhinosinusitis. *Int J Pediatr Otorhinolaryngol* 2008;**72**:1643–50.
173. Shin SY, Choi GS, Park HS, et al. Immunological investigation in the adenoid tissues from children with chronic rhinosinusitis. *Otolaryngol Head Neck Surg* 2009;**141**:91–6.
174. Shopfner CE, Rossi JO. Roentgen evaluation of the paranasal sinuses in children. *AJR Am J Roentgentol* 1973;**118**:176–86.
175. Shurin PA. Etiology and antimicrobial therapy of paranasal sinusitis in children. *Ann Otol Rhinol Laryngol Suppl* 1981;**90**:72–4.
176. Silviu-Dan F. Pediatric chronic rhinosinusitis: the old, the new, and the reasonable. *Pediatr Ann* 2011;**40**:213–20.
177. Sobol SE, Samadi DS, Kazahaya K, et al. Trends in the management of pediatric chronic sinusitis: survey of the American Society of Pediatric Otolaryngology. *Laryngoscope* 2005;**115**:78–80.
178. Sobol SM, Love RG, Stutman HR, et al. Phaeohyphomycosis of the maxilloethmoid sinus caused by *Drechslera spicifera:* a new fungal pathogen. *Laryngoscope* 1984;**94**:620–7.
179. Sohail MA, Al Khabori M, Hyder J, et al. Acute fulminant fungal sinusitis: clinical presentation, radiological findings and treatment. *Acta Trop* 2001;**80**:177–85.
180. Soon VT. Pediatric subperiosteal orbital abscess secondary to acute sinusitis: a 5-year review. *Am J Otolaryngol* 2011;**32**:62–8.
181. Sparrevohn UR, Buch A. The bacteriology of maxillary sinusitis, I: Technique. *Acta Otol* 1946;**34**:425–36.
182. Sprinkle PM. Current status of mycoplasmatales and bacterial variants in chronic otolaryngic disease. *Laryngoscope* 1972;**82**: 737–47.
183. Stewart AE, Vaughan WC. Balloon sinuplasty versus surgical management of chronic rhinosinusitis. *Curr Allergy Asthma Rep* 2010;**10**:181–7.
184. Suh JD, Ramakrishnan V, Palmer JN. Biofilms. *Otolaryngol Clin North Am* 2010;**43**:521–30, viii.
185. Takahashi H, Fujita A, Honjo I. Effect of adenoidectomy on otitis media with effusion, tubal function, and sinusitis. *Am J Otolaryngol* 1989;**10**:208–13.
186. Takahashi H, Honjo I, Fujita A, et al. Effects of adenoidectomy on sinusitis. *Acta Otorhinolaryngol Belg* 1997;**51**:85–7.
187. Talbot GH, Kennedy DW, Scheld WM, et al. Rigid nasal endoscopy versus sinus puncture and aspiration for microbiologic documentation of acute bacterial maxillary sinusitis. *Clin Infect Dis* 2001;**33**:1668–75.
188. Tos M, Mogensen C. Mucus production in chronic maxillary sinusitis: a quantitative histopathological study. *Acta Otolaryngol* 1984;**97**:151–9.
189. Tsai BY, Lin KL, Lin TY, et al. Pott's puffy tumor in children. *Childs Nerv Syst* 2010;**26**:53–60.
190. Ueda D, Yoto Y. The ten-day mark as a practical diagnostic approach for acute paranasal sinusitis in children. *Pediatr Infect Dis J* 1996;**15**:576–9.
191. Umetsu DT, Ambrosino DM, Quinti I, et al. Recurrent sinopulmonary infection and impaired antibody response to bacterial capsular polysaccharide antigen in children with selective IgG-subclass deficiency. *N Engl J Med* 1985;**313**:1247–51.
192. Ungkanont K, Damrongsak S. Effect of adenoidectomy in children with complex problems of rhinosinusitis and associated diseases. *Int J Pediatr Otorhinolaryngol* 2004;**68**:447–51.
193. Unzaga MJ, Crovetto MA, Santamaria JM, et al. Maxillary sinusitis caused by *Nocardia nova*. *Clin Infect Dis* 1996;**23**:184–5.
194. Van Cauwenberge P, Verschraegen G, Van Renterghem L. Bacteriological findings in sinusitis (1963-1975). *Scand J Infect Dis Suppl* 1976;72–7.
195. Vandenberg SJ, Heatley DG. Efficacy of adenoidectomy in relieving symptoms of chronic sinusitis in children. *Arch Otolaryngol Head Neck Surg* 1997;**123**:675–8.
196. Wald ER. Epidemiology, pathophysiology and etiology of sinusitis. *Pediatr Infect Dis* 1985;**4**:S51–4.
197. Wald ER. Sinusitis in children. *Pediatr Infect Dis J* 1988;**7**: S150–3.
198. Wald ER. Rhinitis and acute and chronic sinusitis. In: Bluestone CD, Stool SE, Scheetz MD, editors. *Pediatric otorhinolaryngology*. 2nd ed. Philadelphia: W. B. Saunders; 1990. p. 729–944.
199. Wald ER. Sinusitis in infants and children. *Ann Otol Rhinol Laryngol Suppl* 1992;**155**:37–41.
200. Wald ER. Chronic sinusitis in children. *J Pediatr* 1995;**127**: 339–47.
201. Wald ER. Microbiology of acute and chronic sinusitis in children and adults. *Am J Med Sci* 1998;**316**:13–20.
202. Wald ER. Sinusitis. *Pediatr Ann* 1998;**27**:811–8.
203. Wald ER. Beginning antibiotics for acute rhinosinusitis and choosing the right treatment. *Clin Rev Allergy Immunol* 2006; **30**:143–52.
204. Wald ER. Acute otitis media and acute bacterial sinusitis. *Clin Infect Dis* 2011;**52**(Suppl 4):S277–83.
205. Wald ER. *Staphylococcus aureus:* is it a pathogen of acute bacterial sinusitis in children and adults? *Clin Infect Dis* 2012;**54**:826–31.
206. Wald ER, Byers C, Guerra N, et al. Subacute sinusitis in children. *J Pediatr* 1989;**115**:28–32.
207. Wald ER, Dashefsky B, Byers C, et al. Frequency and severity of infections in day care. *J Pediatr* 1988;**112**:540–6.
208. Wald ER, Guerra N, Byers C. Upper respiratory tract infections in young children: duration of and frequency of complications. *Pediatrics* 1991;**87**:129–33.
209. Wald ER, Milmoe GJ, Bowen A, et al. Acute maxillary sinusitis in children. *N Engl J Med* 1981;**304**:749–54.
210. Wald ER, Reilly JS, Casselbrant M, et al. Treatment of acute maxillary sinusitis in childhood: a comparative study of amoxicillin and cefaclor. *J Pediatr* 1984;**104**:297–302.
211. Walner DL, Falciglia M, Willging JP, et al. The role of second-look nasal endoscopy after pediatric functional endoscopic sinus surgery. *Arch Otolaryngol Head Neck Surg* 1998;**124**:425–8.
212. Washburn RG, Kennedy DW, Begley MG, et al. Chronic fungal sinusitis in apparently normal hosts. *Medicine (Baltimore)* 1988;**67**:231–47.
213. Wassermann D. Acute paranasal sinusitis and cavernous sinus thrombosis. *Arch Otolaryngol* 1967;**86**:205–9.
214. Watters EC, Wallar PH, Hiles DA, et al. Acute orbital cellulitis. *Arch Ophthalmol* 1976;**94**:785–8.
215. Whitaker CW. Intracranial complications of ear, nose, and throat infections. *Laryngoscope* 1971;**81**:1375–80.
216. Whitby CR, Kaplan SL, Mason Jr EO, et al. *Staphyloccus aureus* sinus infections in children. *Int J Pediatr Otorhinolaryngol* 2011;**75**:118–21.
217. Wiatrak BJ, Willging P, Myer CM 3rd, Cotton RT. Functional endoscopic sinus surgery in the immunocompromised child. *Otolaryngol Head Neck Surg* 1991;**105**:818–25.
218. Winther B. Effects on the nasal mucosa of upper respiratory viruses (common cold). *Dan Med Bull* 1994;**41**:193–204.
219. Wolf G, Anderhuber W, Kuhn F. Development of the paranasal sinuses in children: implications for paranasal sinus surgery. *Ann Otol Rhinol Laryngol* 1993;**102**:705–11.
220. Yang HS. Nasal flora of the children in a day nursery. *Arch Pediatr Adolesc Med* 1941;**61**:262–72.
221. Zimmerman B, Stringer D, Feanny S. Prevalence of abnormalities found by sinus x-rays in childhood asthma: lack of relation to severity of asthma. *J Allergy Clin Immunol* 1987;**80**:268–73.
222. Zinreich SJ, Kennedy DW, Rosenbaum AE, et al. Paranasal sinuses: CT imaging requirements for endoscopic surgery. *Radiology* 1987;**163**:769–75.
223. Zurak K, Vagic D, Drvis P, et al. Bacterial colonization and granulocyte activation in chronic maxillary sinusitis in asthmatics and non-asthmatics. *J Med Microbiol* 2009;**58**:1231–5.

canal, different etiologies of otitis externa, and treatment and prevention that are needed by the pediatric practitioner managing this condition.

EPIDEMIOLOGY

According to the CDC, in 2007, the burden of acute otitis externa in the United States was 2.4 million health care visits (8.1 visits/1000 population), an estimated 1/123 persons affected during that year. Estimated annual rates of ambulatory care visits for acute otitis externa between 2003 and 2007 were highest among children 5 to 9 (18.6%) and 10 to 14 years of age (15.8%). Approximately 3 percent of emergency department visits for acute otitis externa in 2007 led to hospitalization. Incidence peaked during summer months, and the regional rate was highest in the South. Direct health care costs were approximately $0.5 billion annually, and nearly 600,000 hours annually of clinician's time.[14]

Otitis externa was the most common cause of outpatient visits for waterborne disease between 2005 and 2006.[21] Outbreaks of otitis externa have been identified in competitive swimmers in association with insufficiently chlorinated pools,[49] in divers,[3] and in association with swimming in recreational fresh water lakes.[71]

NORMAL ANATOMY

The external ear is composed of the auricle and external auditory canal. The auricle of the ear is a skin-covered cartilaginous structure on the temporal region of the head and is an extension of the external auditory canal. The external auditory canal is divided into two regions—a lateral cartilaginous portion and a medial bony portion that ends at the tympanic membrane.

The cartilaginous portion of the external auditory canal makes up approximately 40 percent of its 2.5 cm total length and typically is directed slightly upward and backward. This shape and the presence of cerumen help to prevent water and foreign objects from entering the canal.[36] In the anterior portion of the cartilaginous canal are the fissures of Santorini, which provide a route for the spread of infection from the canal into the adjacent parotid and surrounding tissues. The bony canal makes up the remainder of the canal length and is directed slightly downward and anterior. The isthmus, which is the narrowest portion of the canal, corresponds to the bony–cartilaginous junction.

The sensory innervation of the external auditory canal is complex and includes contributions from cranial nerves V, VII, IX, and X. This robust sensory innervation is responsible for the exquisite pain associated with otitis externa.[36] The skin of the entire external auditory canal consists of keratinizing stratified squamous epithelium. It is the only keratinizing epithelium that lacks eccrine sweat glands.

Differences exist in the canal skin of the cartilaginous canal and the bony canal. The cartilaginous canal skin is thicker and contains rete pegs, dermis, dermal papillae, hair follicles, and sebaceous and cerumen glands, which are absent in the bony canal. The thinner skin of the bony canal lacks dermal papillae and rete pegs. The lack of subcutaneous tissue in the bony canal allows the tight attachment of the skin to the underlying periosteum, rendering the bony canal wall more vulnerable to trauma.

PROTECTIVE MECHANISMS OF THE EXTERNAL EAR

Three major defense mechanisms protect the external auditory canal and lateral surface of the tympanic membrane—the tragus and antitragus, the skin with its cerumen coat, and the isthmus of the canal. The tragus and antitragus provide a partial barrier to the entrance of the foreign bodies into the external auditory canal.

The skin of the cartilaginous canal contains many hair cells and sebaceous and apocrine glands, such as cerumen glands. Together, these three adnexal structures are termed the *pilosebaceous unit* and provide a protective function in the external auditory canal. Migration of the skin of the external auditory canal also helps keep the canal free of debris. The pattern of migration is from the tympanic membrane laterally and radially away from the umbo.[2] Glandular secretions combine with sloughed squamous epithelium to form an acidic coat of cerumen, one of the primary barriers to infection of the canal. Cerumen is composed of lipids that are hydrophobic, and its major function is to waterproof the canal. Racial and sex differences that exist in the characteristics of cerumen do not seem to have major clinical significance. Whites and blacks produce cerumen with higher levels of lipids in contrast to Asians, who produce cerumen with higher levels of proteins. Cerumen in males also has been shown to have a higher pH level than that in females.[23] The acidic nature of cerumen has been shown to inhibit bacterial and fungal growth.[15,23,33] This acidic pH also helps maintain the cohesion and integrity of the stratum corneum.[29]

The canal normally is a self-protecting and self-cleansing structure. The cerumen coat gradually works its way to the lateral part of the canal and sloughs externally. Instrumentation and excessive cleansing of the canal can alter this primary protective barrier and may lead to infection (Table 15-1).

NORMAL BACTERIAL FLORA

The normal bacterial flora of the external auditory canal is a combination of aerobic (80%) and anaerobic (20%)

TABLE 15-1 **Predisposing Factors for Otitis Externa**

Hot and humid environment
Water exposure
Instrumentation of ear canal
Previous radiation therapy
Eczema
Draining ear
Contact dermatitis

organisms.[11] Aerobic bacteria include *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, α-hemolytic streptococci, and diphtheroids.

The bacteriology of the cerumen and the external auditory canal are essentially the same. In a study by Stroman and colleagues[70] of 291 bacteria isolated from cerumen and 302 bacteria isolated from the canal, 99 and 96 percent, respectively, were gram-positive. Staphylococci accounted for 63 percent of bacteria. *Staphylococcus auricularis* is the isolate found most frequently (23% in cerumen and 21% in canal), followed by *S. epidermidis* (14% from cerumen and 17% from the canal, respectively). After staphylococci, the coryneform bacteria (diphtheroids) are the organisms most frequently isolated. The third most frequently recovered bacteria belong to the streptococci and enterococci groups. *Alloiococcus otitidis* was isolated with the greatest frequency (>95%).[70]

In addition, seven species of *Bacillus* were isolated. From the Micrococcaceae family, *Micrococcus luteus* was isolated most frequently. *Turicella otitidis* was the primary coryneform recovered—58 percent from cerumen and 65 percent from the canal. *Corynebacterium auris* was the second most frequently isolated coryneform, accounting for approximately 12 percent of bacteria. Twenty-one other species of coryneforms (including 10 previously undefined species of *Corynebacterium* and 4 of *Microbacterium*) were isolated as well. Coryneforms represented approximately 20 percent of the bacteria isolated. *Propionibacterium acnes* and a variety of *Peptococcus* spp. make up the anaerobic flora.[70]

ACUTE OTITIS EXTERNA

Acute otitis externa is an infection of the external auditory canal that often is the end result of a combination of factors. Bacterial and fungal infections of the external auditory canal occur when the natural defenses break down, resulting in a significant reduction in the amount of cerumen, an injury to the skin of the canal, or a shift of the normal canal flora. Humidity, heat, and maceration all produce itching, which often leads to manipulation and instrumentation of the canal, which in turn leads to additional trauma. Otitis externa may be caused by insults that result in the removal of the protective lipid film from the canal, allowing the entrance of organism to the apopilosebaceous unit. Rapid proliferation of bacteria occurs as a result of the warm, dark, and moist canal environment.

Inflammation and infection can cause canal edema or complete obstruction of the canal in severe cases, with purulent discharge. If the infectious process goes untreated, it leads to cellulitis of the auricle and surrounding area.

Symptoms of otitis externa, in addition to pruritus and drainage, include pain and tenderness on palpation or manipulation of the external ear. Pain arises as the soft tissues and skin of the canal distract the periosteal lining of the bony canal. Pain is severe enough to interfere with daily activities and is the major reason for medical consultation.

History and Physical Examination

Otitis externa is a clinical diagnosis. The evaluation often reveals exposure to water, previous auricular instrumentation, or trauma. The physician should inquire about predisposing factors, such as diabetes, immunosuppression, history of eczema, or previous radiation therapy, all of which may predict a more complicated course.

On physical examination, the pinna may appear swollen or erythematous or may be protruding. Primary care practitioners and emergency department personnel frequently establish the diagnosis of mastoiditis based on auricular protrusion. On manipulation of the auricle, the patient often experiences severe pain. Tragal tenderness is another key feature of this disease. A handheld otoscope often suffices to establish the diagnosis, but the microscope is recommended for full cleaning of the ear canal. The pinna may appear erythematous, with edema and eczematization (Fig. 15-1). The canal appears swollen, with various grades of patency, depending on the severity of the infection. Purulent discharge combined with keratin debris usually fills the canal. It is important to attempt to visualize the tympanic membrane if the canal is not completely obstructed. Absence of drainage from the middle ear confirms the diagnosis of otitis externa.

Pathogens in Acute Otitis Externa

Common bacterial pathogens that cause otitis externa include *P. aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Proteus* spp. (Table 15-2). *Pseudomonas* spp. have been found to be the predominant organism in various studies.[7,10,52,63] Historically, *Pseudomonas* spp. have accounted for 50 to 80 percent of bacteria isolated from cases of chronic otitis externa.[6,46,48,52,63] In a study by Roland and Stroman,[52] gram-negative bacteria accounted for 53 percent of recovered organisms; 45.3 percent were gram-positive. In this study, *P. aeruginosa* accounted for 37.7 percent of the total number of isolates, whereas *Pseudomonas otitidis* accounted for 2.3 percent of recovered organisms.

FIGURE 15-1 ■ Patient with acute otitis externa, with purulent drainage from the ear canal and mild edema and erythema of the pinna. (See companion Expert Consult website for color version.)

TABLE 15-2 **Common Pathogens in Otitis Externa**

Gram-Negative Organisms
Pseudomonas aeruginosa
Pseudomonas spp. Nov. "otitidis"
Proteus mirabilis
Serratia marcescens
Gram-Positive Organisms
Staphylococcus aureus
Staphylococcus epidermidis
Corynebacterium auris
Enterococcus faecalis
Fungi and Yeasts
Aspergillus fumigatus
Candida albicans
Candida parapsilosis

Staphylococci are the most common gram-positive organisms recovered in cases of otitis externa, accounting for 25 percent of the cases. *S. epidermidis* is the most common staphylococcal species recovered, followed by *S. aureus*. Coryneforms are the second largest group of gram-positive bacteria isolated from cases of otitis externa.[52] Reports of methicillin-resistant *S. aureus* as an increasing pathogen isolated from otorrhea resulting from otitis externa showed variable prevalence: 0.3,[52] 5.3,[44] 6.3,[73] and up to 9.8 percent of cases.[32]

Other organisms identified as pathogens include *Enterobacter*, *Klebsiella*, *Serratia*, and *Proteus* spp. and *E. coli*. Although *Microbacterium* spp. previously were considered normal flora, more recently they have been identified at a 10 times higher rate in infected ears in contrast to normal controls. *Microbacterium* also has been the single recovered isolate in treatment failures and reinfections.[52]

Management of Acute Otitis Externa

No consensus exists about the most effective treatment for otitis externa. Treatment usually includes thorough cleaning and suctioning of the purulent debris and application of topical agents. Topical treatment has been shown effective and is the mainstay of treatment. A wick may be placed in the canal to provide adequate delivery of the topical solution when significant swelling of the external auditory canal occurs. Although no clinical trials have assessed the effectiveness of aural toilet alone,[35] without the wick and adequate cleaning of the canal, the topical drops may not achieve adequate penetration and are ineffective. When topical therapy is prescribed, various options, including multiple antibiotics, antiseptics, steroids, and combination agents, are available.[12,24,54,56] Topical antimicrobial therapy increased absolute clinical cure rates of acute otitis externa by 46 percent and bacteriologic cure rates by 61 percent in contrast to placebo.[56] Freedman[25] compared topical neomycin/colistin/hydrocortisone with topical placebo and found less severe edema and itching by day 3 and less severe edema, itching, redness, scaling, and weeping by day 7.[56] Without treatment, only 15 percent of patients with acute otitis externa have clinical cure within 10 days; however, the cure rate increases to 65 to 85 percent when topical antimicrobial therapy is administered.[25,56]

Rosenfeld and colleagues[56] performed a meta-analysis to assess topical antiseptics and antibiotics and found comparable clinical cures at 7 to 14 days. The most common antiseptics used in the treatment of otitis externa include acetic acid, boric acid/ethyl alcohol and aluminum acetate, and *N*-chlorotaurine. Acetic acid and aluminum acetate solution at pH 3.0, commonly known as modified Burow solution, has been found to inactivate in vitro *Pseudomonas* spp., gram-positive organisms (including MRSA, but excluding *Enterococcus*), and *Candida albicans* within 5 minutes and all gram-negative bacteria within 20 minutes.[34]

Topical antibiotic preparations usually are divided into two groups—quinolones and nonquinolone antibiotics. Nonquinolone antibiotics, such as polymyxin, neomycin, gentamicin, tobramycin, and oxytetracycline, have been the mainstay of treatment for otitis externa for several decades. Most of these agents have some degree of ototoxicity, however, which renders them undesirable in the case of a tympanic membrane perforation or a patent pressure equalizing tube.[39,50] The introduction of quinolones in the late 1980s for management of otitis externa and necrotizing external otitis provided a nonototoxic alternative treatment. Topical antibiotic therapy allows for the administration of high concentrations of antibiotics, which can overcome organisms with high minimal inhibitory concentrations. Rosenfeld and colleagues[56] reported comparable clinical cure rates for topical quinolone antibiotics compared with nonquinolones at 3 to 4 days, 7 to 10 days, and 14 to 28 days. This same study found no differences in adverse effects between the two antibiotic groups.

Of importance, pseudomonal resistance to fluoroquinolones has been reported. Berenholz and colleagues[8] reported 33 percent ciprofloxacin resistance in cases of necrotizing otitis externa.[54] Other studies have shown strains of *S. aureus* and *S. epidermidis* that developed quinolone resistance.[54] One of the most common mistakes in the treatment of otitis externa is not identifying a tympanic membrane perforation and draining middle ear as the source of the infection, which may have treatment implications because of the ototoxicity of some compounds.

Typical duration of topical treatment is 7 to 10 days. Patients with more severe infections may require 10 to 14 days of treatment. It is commonly recommended that drops be given for 3 days beyond the cessation of symptoms.[61]

Oral antibiotics are not routinely indicated for the treatment of otitis externa. A clinical trial by Roland and colleagues[53] comparing oral antibiotic plus antibiotic/steroid drop versus antibiotic/steroid drop showed that a single topical agent is equivalent to combination of topical and oral antibiotic treatment for otitis externa and has similar clinical outcomes.

Indications for systemic antibiotics in acute otitis externa are few and include complicated infection by associated cellulitis of the surrounding skin or other

underlying conditions (e.g., diabetes or immunosuppression).[35] Nevertheless, up to 40 percent of patients are prescribed systemic antibiotics in addition to topical therapy, many of which are not active against *P. aeruginosa* or *S. aureus*, the most common pathogens associated with otitis externa.[30,58] Use of systemic antibiotics can be only partially explained by concomitant diagnosis of otitis media or regional spread of infection. Oral antibiotics, when not appropriately prescribed, increase treatment cost, potential side effects, and likelihood of noncompliance and are more likely to contribute to emergence of antimicrobial resistance than is topical antimicrobial therapy. An evidence-based review concluded that no significant topical antibiotic resistance develops from the use of ototopical antibiotic treatment alone.[74]

CHRONIC OTITIS EXTERNA

Chronic otitis externa is an inflammatory condition of the ear canal skin, with symptoms lasting from 6 weeks to more than 3 months. This condition generally is caused by the loss of the protective coating of cerumen and oils secondary to constant mechanical débridement, such as with cotton applicators or repeated water exposure. Associated pruritus leading to self-cleaning perpetuates the vicious cycle. The ear canal with chronic otitis externa often is more vulnerable to bacterial superinfection.[51] *S. aureus* and *P. aeruginosa* have been shown to secrete proteases that may continue to establish pathologic skin conditions in the external auditory canal in cases of chronic otitis externa.[43] In chronic otitis externa, biofilm formation has been postulated as responsible for the pathogenesis and difficulty eradicating infection.[26] A shift in external ear canal pH from acidic to alkaline has been noted in patients with chronic otitis externa.[41] A subtype of chronic otitis externa is reported in 6 to 40 percent of patients secondary to ear canal maceration as a result of middle ear drainage after insertion of pressure equalization tubes.[38,40,55,74]

Treatment of chronic otitis externa involves débridement and application of topical anti-inflammatory agents, such as corticosteroids. Cessation of habitual canal manipulation is necessary for achieving a positive response. For recalcitrant cases, surgical removal of the canal skin and replacement with skin grafts may be required. Chemical peeling of canal skin also has been used in refractory cases.[26]

OTOMYCOSIS

Otomycosis is a fungal infection of the skin of the external canal. Otomycosis often is the result of superinfection of chronic bacterial infection of the external canal or middle ear. It is often found after the use of multiple oral and ototopical antibacterial medications. All fungi have three basic growth requirements—moisture, warmth, and darkness—all of which commonly are present in the external auditory canal. The most common fungus is *Aspergillus* spp., although *Candida* spp., *Actinomyces* spp., and Zygomycetes also have been reported.[10] Pruritus is the primary clinical manifestation. Patients also complain of feeling moisture in their ears. Physical examination commonly shows a white, black, or dotted gray membrane in the external auditory canal. Diagnosis often is confirmed with a fungal culture.

Thorough cleaning with removal of the matted fungal debris and topical application of an acidifying solution, such as aluminum sulfate–calcium acetate (Domeboro), or a drying powder, such as boric acid, often is adequate treatment. Antifungals such as clotrimazole cream or solution (Lotrimin) also may be used. It is important to assess for perforation of the tympanic membrane, because antifungals can be ototoxic.[39] Gentian violet usually is well tolerated in patients with mastoid cavities, but it permanently stains skin and clothing. Many patients with refractory otomycotic infections have had previous canal wall mastoid surgery and require a hearing aid with a closed mold. Because the patient relies on the hearing aid virtually all day, trauma associated with placing and removing the hearing aid throughout the day can cause a significant problem. Ointments are not recommended for patients with closed hearing aids because they may promote fungal growth secondary to the accumulation of moisture.

NECROTIZING OTITIS EXTERNA

Necrotizing otitis externa is an infection of the external auditory canal that spreads to the surrounding subcutaneous tissues and can lead to osteomyelitis of the skull base.[19] This condition was described first by Chandler[16,18] and referred to as *malignant otitis externa;* although the disease is actually benign, aggressive extension occurs and historically it often has a poor outcome. Survival rates have improved as a result of increased awareness, allowing earlier diagnosis and treatment.[19,20] Patients often are diabetic, immunocompromised, or undergoing postradiation therapy.[7,60,64] The infection often begins as any other acute otitis externa involving the bony–cartilaginous junction and may extend through the fissures of Santorini to involve the parotid region. Elevation of inflammatory markers is common, in particular the erythrocyte sedimentation rate.[59] Facial paralysis can occur as a complication of this disease and does so more commonly in children than in adults.[47] Facial nerve paralysis in necrotizing otitis externa often is permanent[17] but not associated with poor prognosis.[66] Other complications include cranial nerve palsy (X and XI),[1] subperiosteal abscess formation, mastoiditis, meningitis, and sinus thrombosis.[42]

P. aeruginosa is the most common pathogen in necrotizing otitis externa. *Proteus mirabilis* also has been reported as a causal agent in some cases.[19,20] Necrotizing otitis externa should be suspected in the presence of a pseudomonal infection that does not resolve and may be associated with facial nerve paralysis. The presence of granulation in the external canal at the bony–cartilaginous junction also should raise suspicion of necrotizing otitis externa.

Diagnostic imaging, including computed tomography or magnetic resonance imaging, of the temporal region often is required.[27] The most common finding of extension beyond the ear canal is retrocondylar fat

infiltration.[37] A gallium-67 scan often is diagnostic in the early course of the disease and provides a good way to monitor treatment efficacy. A positive technetium-99m scan is diagnostic for acute osteolytic osteomyelitis because it measures osteoclastic activity, but it may remain positive long after the infection has subsided. For this reason, a ^{99m}Tc scan is not ideal for monitoring treatment response.[69]

Treatment of necrotizing otitis externa often involves multiple surgical débridements combined with topical and intravenous antibiotics. Transition to oral ciprofloxacin for infections with susceptible organisms has been reported with clinical improvement.[31] Duration of therapy is usually prolonged for at least 6 weeks and until clinical and radiographic improvement has been achieved. Hyperbaric oxygen also has been tried, with some success.[22] In diabetic or immunocompromised patients, coadjuvant treatment to control the primary condition is crucial and should be initiated immediately.

DIFFERENTIAL DIAGNOSIS

Not every condition that manifests with pain and purulent discharge from the external auditory canal is otitis externa. Multiple other entities that may manifest with one or more similar symptoms include relapsing polychondritis, suppurative otitis media, and herpetic infections.

Relapsing polychondritis is a progressive inflammatory disorder affecting cartilage. When it involves the ear, it usually involves the pinna and the cartilaginous portion of the external auditory canal, manifesting as swelling and erythema.[5] This condition can affect any cartilaginous structure, including the trachea, nasal septum, and larynx. Destruction of the cartilage by inflammatory infiltrates is followed by granulation, fibrosis, and calcifications. It often is accompanied by arthralgias involving one or more joints and rarely affects only one ear.

Herpes simplex and herpes zoster also can affect the external auditory canal. Both conditions manifest with painful vesicles along the canal (Fig. 15-2) and can be accompanied by facial nerve paralysis (Ramsay Hunt syndrome or herpes zoster oticus).[4,72] Treatment is with antivirals, such as acyclovir.

Dermatologic conditions can give the appearance of otitis externa. Allergic and contact dermatitis can manifest with erythema, weeping areas, and itching. Allergic dermatitis is a delayed hypersensitivity reaction resulting from substances such as poison ivy, rubber, and nickel compounds.[45,68] Type IV cell-mediated hypersensitivity reaction to components of ototopical agents used to treat acute otitis externa has been described.[65] Treatment is administration of topical steroids and removal of the causative agent. Dermatophytid eruptions can manifest in the ear canal in response to hematogenously spread fungal by-products from a primary focus (e.g., nails, scalp, or vagina).[13,61,62,65]

Furunculosis is a localized form of otitis externa, usually involving the outer third of the ear canal. It is characterized by abscess development in a hair follicle, with spreading of the infection to the surrounding skin. The most common causative agent is *S. aureus*.

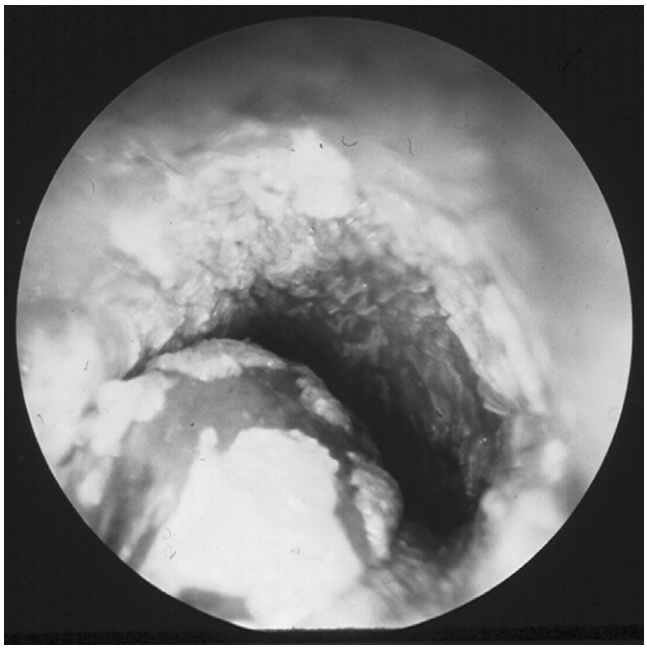

FIGURE 15-2 ■ Ear canal with multiple vesicles from herpes zoster oticus. (See companion Expert Consult website for color version.)

Treatment includes incision and drainage of pointing, fluctuant abscesses and application of topical antistaphylococcal antibiotics.[9]

Ear pain can be secondary to referred pain from the pharynx, tonsils, larynx, and teeth. In patients with otalgia and normal otoscopic examination, additional attention should extend to these areas.

PREVENTION

Avoiding ear trauma and instrumentation, minimizing exposure of the ears to water (e.g., using ear plugs or swim caps, drying ears thoroughly after water exposure, and using alcohol-based ear drying solutions after water exposure for persons with recurring episodes of acute otitis externa), and avoiding insertion of solid objects into the ear canal are the mainstay of prevention of otitis externa.[35,57,67] Additionally, pool operators can help prevent transmission of *Pseudomonas* spp. and other common causes of infectious acute otitis externa in treated recreational water venues (e.g., pools, interactive fountains, and water parks) by maintaining proper chlorine and pH levels.[75]

Groups at high risk for infection, particularly competitive swimmers, should be the target for educational interventions. More information on preventing swimmer's ear is available at http://www.cdc.gov/healthywater/swimming/rwi/illnesses/swimmers-ear-prevention-guidelines.html.

CONCLUSION

Understanding the anatomy and physiology of the external ear allows clinicians to better comprehend the natural

history of the diseases that affect this region. Despite improvements in diagnostic imaging techniques, no substitute exists for a good history and physical examination to differentiate among various conditions with a similar presentation. Treatment of otitis externa should focus on achieving relief of the acute condition; at the same time, clinicians should try to help prevent recurrences in patients with higher risk factors.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Al-Noury K, Lotfy A. Computed tomography and magnetic resonance imaging findings before and after treatment of patients with malignant external otitis. *Eur Arch Otorhinolaryngol* 2011;**268**:1727–34.
3. Alcock SR. Acute otitis externa in divers working in the North Sea: a microbiological survey of seven saturation dives. *J Hyg (Lond)* 1977;**78**:395–409.
9. Bernius M, Perlin D. Pediatric ear, nose, and throat emergencies. [Review]. *Pediatr Clin North Am* 2006;**53**:195–214.
13. Busch RF. Dermatophytid reaction and chronic otitis externa. *Otolaryngol Head Neck Surg* 1998;**118**(3 Pt 1):420.
14. Centers for Disease Control and Prevention. Estimated burden of acute otitis externa: United States, 2003-2007. *MMWR Morb Mortal Wkly Rep* 2011;**60**:605–9.
21. Collier SA, Stockman LJ, Hicks LA, et al. Direct healthcare costs of selected diseases primarily or partially transmitted by water. *Epidemiol Infect* 2012;**140**:2003–13.
26. Fusconi M, Petrozza V, Taddei AR, et al. Is biofilm the cause of chronic otitis externa? *Laryngoscope* 2011;**121**:2626–33.
29. Hachem JP, Crumrine D, Fluhr J, et al. pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 2003;**121**:345–53.
30. Halpern MT, Palmer CS, Seidlin M. Treatment patterns for otitis externa. *J Am Board Fam Pract* 1999;**12**:1–7.
31. Hickey SA, Ford GR, O'Connor AF, et al. Treating malignant otitis with oral ciprofloxacin. *BMJ* 1989;**299**:550–1.
32. Hwang JH, Tsai HY, Liu TC. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in discharging ears. *Acta Otolaryngol* 2002;**122**:827–30.
40. Martin TJ, Kerschner JE, Flanary VA. Fungal causes of otitis externa and tympanostomy tube otorrhea. *Int J Pediatr Otorhinolaryngol* 2005;**69**:1503–8.
41. Martinez Devesa P, Willis CM, Capper JW. External auditory canal pH in chronic otitis externa. *Clin Otolaryngol Allied Sci* 2003;**28**:320–4.
42. Mehrotra P, Elbadawey MR, Zammit-Maempel I. Spectrum of radiological appearances of necrotising external otitis: a pictorial review. [Review]. *J Laryngol Otol* 2011;**125**:1109–15.
43. Miedzobrodzki J, Kaszycki P, Bialecka A, Kasprowicz A. Proteolytic activity of *Staphylococcus aureus* strains isolated from the colonized skin of patients with acute-phase atopic dermatitis. *Eur J Clin Microbiol Infect Dis* 2002;**21**:269–76.
44. Mukassabi K. Bacteriology of discharging ears. [Letter]. *Ir Med J* 2007;**100**:379–80.
58. Rowlands S, Devalia H, Smith C, et al. Otitis externa in UK general practice: a survey using the UK General Practice Research Database. *Br J Gen Pract* 2001;**51**:533–8.
61. Sander R. Otitis externa: a practical guide to treatment and prevention. [Review]. *Am Fam Physician* 2001;**63**:927–36, 941–2.
62. Schapowal A. Otitis externa: a clinical overview. [Review]. *Ear Nose Throat J* 2002;**81**(suppl 1):21–2.
65. Sood S, Strachan DR, Tsikoudas A, Stables GI. Allergic otitis externa. [Review]. *Clin Otolaryngol Allied Sci* 2002;**27**:233–6.
66. Soudry E, Joshua BZ, Sulkes J, Nageris BI. Characteristics and prognosis of malignant external otitis with facial paralysis. *Arch Otolaryngol Head Neck Surg* 2007;**133**:1002–4.
67. Springer GL, Shapiro ED. Fresh water swimming as a risk factor for otitis externa: a case-control study. *Arch Environ Health* 1985;**40**:202–6.
71. van Asperen IA, de Rover CM, Schijven JF, et al. Risk of otitis externa after swimming in recreational fresh water lakes containing *Pseudomonas aeruginosa*. *BMJ* 1995;**311**:1407–10.
73. Walshe P, Rowley H, Timon C. A worrying development in the microbiology of otitis externa. *Clin Otolaryngol Allied Sci* 2001;**26**:218–20.
74. Weber PC, Roland PS, Hannley M, et al. The development of antibiotic resistant organisms with the use of ototopical medications. [Review]. *Otolaryngol Head Neck Surg* 2004;**130**(3 suppl):S89–94.
75. Yoder JS, Hlavsa MC, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events: United States, 2005-2006. *MMWR Surveill Summ* 2008;**57**:1–29.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Al-Noury K, Lotfy A. Computed tomography and magnetic resonance imaging findings before and after treatment of patients with malignant external otitis. *Eur Arch Otorhinolaryngol* 2011; **268**:1727–34.
2. Alberti PW. Epithelial migration on the tympanic membrane. *J Laryngol Otol* 1964;**78**:808–30.
3. Alcock SR. Acute otitis externa in divers working in the North Sea: a microbiological survey of seven saturation dives. *J Hyg (Lond)* 1977;**78**:395–409.
4. Aleksic SN, Budzilovich GN, Lieberman AN. Herpes zoster oticus and facial paralysis (Ramsay Hunt syndrome): clinico-pathologic study and review of literature. *J Neurol Sci* 1973;**20**:149–59.
5. Bachor E, Blevins NH, Karmody C, Kuhnel T. Otologic manifestations of relapsing polychondritis: review of literature and report of nine cases. *Auris Nasus Larynx* 2006;**33**:135–41.
6. Beers SL, Abramo TJ. Otitis externa review. *Pediatr Emerg Care* 2004;**20**:250–6.
7. Benuck I, Traisman HS. Malignant external otitis in a diabetic adolescent. *J Adolesc Health Care* 1986;**7**:57–9.
8. Berenholz L, Katzenell U, Harell M. Evolving resistant pseudomonas to ciprofloxacin in malignant otitis externa. *Laryngoscope* 2002; **112**:1619–22.
9. Bernius M, Perlin D. Pediatric ear, nose, and throat emergencies. [Review]. *Pediatr Clin North Am* 2006;**53**:195–214.
10. Bojrab DI, Bruderly T, Abdulrazzak Y. Otitis externa. [Review]. *Otolaryngol Clin North Am* 1996;**29**:761–82.
11. Brook I. Microbiological studies of the bacterial flora of the external auditory canal in children. *Acta Otolaryngol* 1981;**91**: 285–7.
12. Brook I. Treatment of otitis externa in children. [Review]. *Paediatr Drugs* 1999;**1**:283–9.
13. Busch RF. Dermatophytid reaction and chronic otitis externa. *Otolaryngol Head Neck Surg* 1998;**118**(3 Pt 1):420.
14. Centers for Disease Control and Prevention. Estimated burden of acute otitis externa: United States, 2003-2007. *MMWR Morb Mortal Wkly Rep* 2011;**60**:605–9.
15. Chai TJ, Chai TC. Bactericidal activity of cerumen. *Antimicrob Agents Chemother* 1980;**18**:638–41.
16. Chandler JR. Malignant external otitis. *Laryngoscope* 1968;**78**: 1257–94.
17. Chandler JR. Malignant external otitis and facial paralysis. [Review]. *Otolaryngol Clin North Am* 1974;**7**:375–83.
18. Chandler JR. Malignant external otitis: further considerations. *Ann Otol Rhinol Laryngol* 1977;**86**(4 Pt 1):417–28.
19. Chandler JR. Malignant external otitis and osteomyelitis of the base of the skull. *Am J Otol* 1989;**10**:108–10.
20. Chandler JR, Grobman L, Quencer R, Serafini A. Osteomyelitis of the base of the skull. *Laryngoscope* 1986;**96**:245–51.
21. Collier SA, Stockman LJ, Hicks LA, et al. Direct healthcare costs of selected diseases primarily or partially transmitted by water. *Epidemiol Infect* 2012;**140**:2003–13.
22. Davis JC, Gates GA, Lerner C, et al. Adjuvant hyperbaric oxygen in malignant external otitis. [Case Reports]. *Arch Otolaryngol Head Neck Surg* 1992;**118**:89–93.
23. Fabricant ND, Kolb LW. External auditory canal and lobule temperature. *Arch Otolaryngol* 1961;**74**:340–1.
24. Fairbanks DN. Otic topical agents. *Ear Nose Throat J* 1981;**60**: 239–42.
25. Freedman R. Versus placebo in treatment of acute otitis externa. *Ear Nose Throat J* 1978;**57**:198–204.
26. Fusconi M, Petrozza V, Taddei AR, et al. Is biofilm the cause of chronic otitis externa? *Laryngoscope* 2011;**121**:2626–33.
27. Gherini SG, Brackmann DE, Bradley WG. Magnetic resonance imaging and computerized tomography in malignant external otitis. *Laryngoscope* 1986;**96**:542–8.
28. Gordon AT. Otitis externa. *Bull U S Army Med Dep* 1948;**8**:245.
29. Hachem JP, Crumrine D, Fluhr J, et al. pH directly regulates epidermal permeability barrier homeostasis, and stratum corneum integrity/cohesion. *J Invest Dermatol* 2003;**121**:345–53.
30. Halpern MT, Palmer CS, Seidlin M. Treatment patterns for otitis externa. *J Am Board Fam Pract* 1999;**12**:1–7.
31. Hickey SA, Ford GR, O'Connor AF, et al. Treating malignant otitis with oral ciprofloxacin. *BMJ* 1989;**299**:550–1.
32. Hwang JH, Tsai HY, Liu TC. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in discharging ears. *Acta Otolaryngol* 2002;**122**:827–30.
33. Hyslop Jr NE. Ear wax and host defense. *N Engl J Med* 1971;**284**:1099–100.
34. Jinnouchi O, Kuwahara T, Ishida S, et al. Anti-microbial and therapeutic effects of modified Burow's solution on refractory otorrhea. *Auris Nasus Larynx* 2012;**39**:374–7.
35. Kaushik V, Malik T, Saeed SR. Interventions for acute otitis externa. *Cochrane Database Syst Rev* 2010;(1):CD004740.
36. Kelly KE, Mohs DC. The external auditory canal: anatomy and physiology. [Review]. *Otolaryngol Clin North Am* 1996;**29**: 725–39.
37. Kwon BJ, Han MH, Oh SH, et al. MRI findings and spreading patterns of necrotizing external otitis: is a poor outcome predictable? *Clin Radiol* 2006;**61**:495–504.
38. Manolidis S, Friedman R, Hannley M, et al. Comparative efficacy of aminoglycoside versus fluoroquinolone topical antibiotic drops. *Otolaryngol Head Neck Surg* 2004;**130**(3 suppl):S83–8.
39. Marsh RR, Tom LW. Ototoxicity of antimycotics. *Otolaryngol Head Neck Surg* 1989;**100**:134–6.
40. Martin TJ, Kerschner JE, Flanary VA. Fungal causes of otitis externa and tympanostomy tube otorrhea. *Int J Pediatr Otorhinolaryngol* 2005;**69**:1503–8.
41. Martinez Devesa P, Willis CM, Capper JW. External auditory canal pH in chronic otitis externa. *Clin Otolaryngol Allied Sci* 2003;**28**:320–4.
42. Mehrotra P, Elbadawey MR, Zammit-Maempel I. Spectrum of radiological appearances of necrotising external otitis: a pictorial review. [Review]. *J Laryngol Otol* 2011;**125**:1109–15.
43. Miedzobrodzki J, Kaszycki P, Bialecka A, Kasprowicz A. Proteolytic activity of *Staphylococcus aureus* strains isolated from the colonized skin of patients with acute-phase atopic dermatitis. *Eur J Clin Microbiol Infect Dis* 2002;**21**:269–76.
44. Mukassabi K. Bacteriology of discharging ears. [Letter]. *Ir Med J* 2007;**100**:379–80.
45. Nakada T, Iijima M, Nakayama H, Maibach HI. Role of ear piercing in metal allergic contact dermatitis. *Contact Dermatitis* 1997;**36**:233–6.
46. Nichols AC, Perry ET. Studies on the growth of bacteria in the human ear canal. *J Invest Dermatol* 1956;**27**:165–70.
47. Nir D, Nir T, Danino J, Joachims HZ. Malignant external otitis in an infant. *J Laryngol Otol* 1990;**104**:488–90.
48. Perry ET. A practical approach to external otitis. *J Am Med Assoc* 1957;**163**:161–4.
49. Reid TM, Porter IA. An outbreak of otitis externa in competitive swimmers due to *Pseudomonas aeruginosa*. *J Hyg (Lond)* 1981;**86**: 357–62.
50. Roland PS. Clinical ototoxicity of topical antibiotic drops. [Review]. *Otolaryngol Head Neck Surg* 1994;**110**:598–602.
51. Roland PS. Chronic external otitis. [Review]. *Ear Nose Throat J* 2001;**80**(6 suppl):12–6.
52. Roland PS, Stroman DW. Microbiology of acute otitis externa. *Laryngoscope* 2002;**112**(7 Pt 1):1166–77.
53. Roland PS, Belcher BP, Bettis R, et al. A single topical agent is clinically equivalent to the combination of topical and oral antibiotic treatment for otitis externa. *Am J Otolaryngol* 2008;**29**: 255–61.
54. Roland PS, Pien FD, Schultz CC, et al. Efficacy and safety of topical ciprofloxacin/dexamethasone versus neomycin/polymyxin B/hydrocortisone for otitis externa. *Curr Med Res Opin* 2004;**20**: 1175–83.
55. Roland PS, Stewart MG, Hannley M, et al. Consensus panel on role of potentially ototoxic antibiotics for topical middle ear use: introduction, methodology, and recommendations. *Otolaryngol Head Neck Surg* 2004;**130**(3 suppl):S51–6.
56. Rosenfeld RM, Brown L, Cannon CR, et al. Clinical practice guideline: acute otitis externa. *Otolaryngol Head Neck Surg* 2006; **134**(4 suppl):S4–23.
57. Rosenfeld RM, Singer M, Wasserman JM, Stinnett SS. Systematic review of topical antimicrobial therapy for acute otitis externa. [Review]. *Otolaryngol Head Neck Surg* 2006;**134**(4 suppl):S24–48.
58. Rowlands S, Devalia H, Smith C, et al. Otitis externa in UK general practice: a survey using the UK General Practice Research Database. *Br J Gen Pract* 2001;**51**:533–8.

59. Rubin J, Yu VL. Malignant external otitis: insights into pathogenesis, clinical manifestations, diagnosis, and therapy. [Review]. *Am J Med* 1988;**85**:391–8.
60. Rubin J, Yu VL, Stool SE. Malignant external otitis in children. [Review]. *J Pediatr* 1988;**113**:965–70.
61. Sander R. Otitis externa: a practical guide to treatment and prevention. [Review]. *Am Fam Physician* 2001;**63**:927–36, 941–2.
62. Schapowal A. Otitis externa: a clinical overview. [Review]. *Ear Nose Throat J* 2002;**81**(8 suppl 1):21–2.
63. Senturia BH. Diffuse external otitis: pathogenesis and treatment. II. *Laryngoscope* 1955;**65**:313–21.
64. Sobie S, Brodsky L, Stanievich JF. Necrotizing external otitis in children: report of two cases and review of the literature. *Laryngoscope* 1987;**97**:598–601.
65. Sood S, Strachan DR, Tsikoudas A, Stables GI. Allergic otitis externa. [Review]. *Clin Otolaryngol Allied Sci* 2002;**27**:233–6.
66. Soudry E, Joshua BZ, Sulkes J, Nageris BI. Characteristics and prognosis of malignant external otitis with facial paralysis. *Arch Otolaryngol Head Neck Surg* 2007;**133**:1002–4.
67. Springer GL, Shapiro ED. Fresh water swimming as a risk factor for otitis externa: a case-control study. *Arch Environ Health* 1985;**40**:202–6.
68. Stevenson OE, Finch TM. Allergic contact dermatitis from rectified camphor oil in Earex ear drops. *Contact Dermatitis* 2003;**49**:51.
69. Stokkel MP, Takes RP, van Eck-Smit BL, Baatenburg de Jong RJ. The value of quantitative gallium-67 single-photon emission tomography in the clinical management of malignant external otitis. *Eur J Nucl Med* 1997;**24**:1429–32.
70. Stroman DW, Roland PS, Dohar J, Burt W. Microbiology of normal external auditory canal. *Laryngoscope* 2001;**111**(11 Pt 1):2054–9.
71. van Asperen IA, de Rover CM, Schijven JF, et al. Risk of otitis externa after swimming in recreational fresh water lakes containing *Pseudomonas aeruginosa*. *BMJ* 1995;**311**:1407–10.
72. Vest C, Munneke JA, Smith R. Herpes zoster oticus: uncommon but recognizable cause of facial paralysis. *Postgrad Med* 1979;**65**:143–50.
73. Walshe P, Rowley H, Timon C. A worrying development in the microbiology of otitis externa. *Clin Otolaryngol Allied Sci* 2001;**26**:218–20.
74. Weber PC, Roland PS, Hannley M, et al. The development of antibiotic resistant organisms with the use of ototopical medications. [Review]. *Otolaryngol Head Neck Surg* 2004;**130**(3 suppl):S89–94.
75. Yoder JS, Hlavsa MC, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events: United States, 2005-2006. *MMWR Surveill Summ* 2008;**57**:1–29.

combination of these forms. Fluctuating or persisting loss of hearing is present in most patients who have middle ear effusion; hearing impairment is the most frequent complication of AOM or otitis media with effusion. Suppurative complications of otitis media occur when inflammation and infection extend beyond the mucoperiosteal lining of the middle ear (e.g., mastoiditis, epidural abscess). An extended review of otitis media in infants and children was written by the authors and published in 2007.[33]

INCIDENCE AND EPIDEMIOLOGY OF ACUTE OTITIS MEDIA

AOM is one of the most common infectious diseases of childhood. A survey of diagnoses made in office practices in the United States in 1990 identified 24.5 million visits for which the principal diagnosis was otitis media; the number of diagnoses of otitis media had increased from 9.91 million visits recorded in 1975.[193] In Boston, Teele and associates[217] found that about 33 percent of pediatric office visits for illness of any kind were attributable to AOM or otitis media with effusion. The same group of investigators reported that 62 percent of children had had at least one episode of AOM by the time they reached 1 year of age and 17 percent had experienced three or more episodes.[215] By 3 years of age, more than 80 percent of children had experienced at least one episode of AOM and 46 percent had had three or more episodes. A similar preponderance of cases of AOM during the first or second year of life with a decline in incidence rate thereafter has been reported by investigators from locations as diverse geographically as Finland[179,204]; Sweden[104]; Cleveland, Ohio[139]; Huntsville, Alabama[101]; and Galveston, Texas.[20] The results of these studies suggest that by the time children reach 3 years of age they may be categorized into three groups of about equal size relative to acute infections of the middle ear: one group is free of ear infections; a second group may have occasional episodes of otitis media, usually associated with infections of the respiratory tract; and a third group is otitis prone, subject to repeated (three or more) episodes of acute infection.

A prospective study beginning in 1991 of more than 2000 children in Pittsburgh, Pennsylvania, surveyed during the first 2 years of life provided insight into the prevalence of otitis media, the amount of antibiotic used, and the proportion of children who required surgery for severe and recurrent disease.[166] Middle ear effusion was present 20.4 percent and 16.6 percent of the time during the first and second years of life, respectively; mean number of days of antimicrobial therapy for otitis media was 41.9 and 48.6 in the first and second years of life, respectively (>90% of all antibiotic treatment in the first 2 years of life was for otitis media); and 1.8 percent and 4.2 percent of the children had myringotomy and insertion of tympanostomy tubes in the first and second years of life, respectively.

Three factors are likely to alter the incidence and epidemiology of AOM in the United States: (1) the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) in 2000 and the 13-valent pneumococcal conjugate vaccine (PCV13) in 2010; (2) the publication of management guidelines by the American Academy of Pediatrics (AAP) and the American Academy of Family Physicians (AAFP) in 2004, which included criteria for diagnosis of AOM and an option of "observation" rather than immediate use of antimicrobial drugs; and (3) the educational campaigns of the Centers for Disease Control and Prevention (CDC) and other professional groups to influence parents and physicians to avoid inappropriate uses of antimicrobial agents for trivial, usually viral, respiratory tract infections because of concern for development of multidrug bacterial resistance. Any one or all of these factors may play a role in decreasing the number of diagnoses of AOM, altering the microbiology of AOM, reducing the incidence of severe and recurrent AOM, decreasing the number of surgical procedures for severe and recurrent otitis media, and reducing the volume of antimicrobial agents used in infants and children. A large-scale survey of infants younger than 2 years of age indicated that a reduction in diagnoses of AOM and antibiotic prescriptions attributable to AOM had taken place by 2004; analyses of databases of more than 40,000 infants compared a baseline from 1997 to 1999 with findings in 2004; ambulatory visits and antibiotic prescriptions attributable to AOM decreased by 42.7% and 42.9%, respectively, from the baseline to 2004.[233]

Host Risk Factors

The risk factors for developing severe and recurrent AOM are shown in Table 16-1. The peak age-specific attack rate occurs between 6 and 18 months of age. The frequent occurrence of otitis media in otherwise healthy infants is partly a reflection of the fact that the eustachian tube of the young child is shorter, floppier, straighter, and more horizontal than the eustachian tube of the older child. Organisms from the nasopharynx reach the middle ear more readily than they do in older children. By 3 years of age, the incidence of AOM decreases because of changes in the child's anatomy and physiology and

TABLE 16-1 **Risk Factors for Severe and Recurrent Acute Otitis Media**

Male gender
Familial aggregation—disease in siblings and parents
Very low birth weight (<1500 g) and gestational age <33 wk
Early onset of disease
Race—Native American, Alaskan Eskimo, Australian Aborigine
Poverty—crowded living conditions, poor sanitation, lack of access to medical care
Prone sleeping position
Use of pacifier
Not breast-fed
Group daycare
Exposure to smoke and environmental antigens
Congenital or acquired immunodeficiency

maturing immune mechanisms. Children who have had little or no experience with otitis media by the time they reach age 3 years are unlikely to develop problems with middle ear infections, unless some predisposing factor, such as tumor or fracture of the base of the skull or a facial bone or acquired immunodeficiency, occurs.

AOM, similar to most bacterial infections in children, seems to occur more often in boys than in girls.[215] A genetic predisposition to AOM has been shown by a study in twins.[49] Histories of severe and recurrent ear infections in siblings and parents are found frequently in families with an otitis-prone child.[76,215] A genetic susceptibility to recurrent and severe otitis media is an active area of investigation.[103] Proinflammatory cytokine gene polymorphisms and polymorphisms in immune response genes were associated with recurrent AOM.[65,168] Although prematurity has not been associated previously with predisposition to middle ear infection, a study from the Netherlands suggests that a gestational age of younger than 33 weeks and very low birth weight (<1500 g) are risk factors for developing recurrent otitis media.[66] Age at first episode of AOM is associated significantly with recurrent episodes.[215]

Race and ethnicity as predisposing factors may be difficult to separate from poor social and economic conditions. Particularly high rates of the disease have been observed among Alaska natives,[110,180,192] American Indians,[165] and Australian Aboriginal children.[147] Factors of poverty predisposing children to development of respiratory infections and otitis media include crowded living conditions, poor sanitation, and limited access to medical care.[105,142]

Although most children with recurrent and severe otitis media have no obvious predisposing factor, a few have altered host defenses, including anatomic changes (e.g., cleft palate or uvula, submucous cleft), alterations of normal physiologic defenses (e.g., patulous eustachian tube, barotrauma), and congenital or acquired immunologic deficiencies (e.g., immunoglobulin deficiency, chronic granulomatous disease, malignancy). Active middle ear disease is a constant event in children with cleft palate.[138,159,160] Children with acquired immunodeficiency syndrome have a higher age-specific incidence of otitis media beginning at 6 months of age compared with uninfected children.[11] Nasotracheal intubation has been identified as a factor in the development of AOM and otitis media with effusion in neonates and older children.[18,62,170]

Environmental Risk Factors

An increased incidence of respiratory tract infections, including otitis media, among children in group daycare compared with children receiving home care has been documented in the United States,[92,150,195] Sweden,[94] and Finland.[4,203] A survey of children in Memphis, Tennessee, found that children in daycare experienced more episodes of otitis media and were more likely to have placement of ventilation tubes.[12] By the second year of life, 21 percent of children in Pittsburgh observed from birth who were in group daycare (seven children or more) had surgical procedures for middle ear disease (almost all were myringotomy and placement of tubes) compared with only 3 percent of children in home care.[229] The increase in incidence of otitis media from 9.91 million office visits in 1975 to 24.5 million office visits in 1990 is associated with increased usage of group daycare for young infants.[193]

Placing infants in daycare usually is necessitated by the professional needs of one or both parents. Paid maternal leave, as is fostered in some European countries, results in an increased proportion of mothers who are breast-feeding and in delayed entry of infants into out-of-home daycare. In the Czech Republic, paid maternal leave is available to all women for 9 months after giving birth and is optional to the child's third birthday. Paid parental leave now is reaching a stage of discussion and experimental programs in the United States. The Federal Family and Leave Act guarantees 6 weeks of unpaid leave, but more generous programs are available in Massachusetts, Vermont, Maryland, and Washington.

Breast-fed infants have fewer incidents of ear disease than bottle-fed infants. In a Boston, Massachusetts, study,[215] breast-feeding for 3 months or more was associated with decreased risk of developing AOM in the first year of life. Although bottle-fed infants are placed in a reclining or horizontal position and breast-fed infants are held in a vertical position, the data suggest that a constituent of breast milk is the important factor and not position during feeding. Of children with cleft palate who were provided breast milk or formula in a similar container, children who received breast milk had fewer cases of middle ear effusion.[163]

Sleep position and use of a pacifier have been identified as risk factors for recurrent episodes of AOM. More episodes of AOM were identified in infants who slept prone (compared with infants who slept supine) in an investigation of 14,000 infants in Bristol, England.[77] Use of a pacifier increased the risk for development of recurrent AOM in Finnish children attending daycare centers.[152] More than three episodes of AOM occurred in 29.5 percent of children younger than 2 years of age using pacifiers compared with 20.6 percent of children not using pacifiers; in children 2 to 3 years old, the incidences of recurrent AOM were 30.6 percent and 13.2 percent, respectively.

Allergy to environmental antigens plays a role in congestion of the mucosa of the eustachian tube. Exposure to smoke can result in goblet cell hyperplasia, mucus hypersecretion, ciliostasis, and decreased mucociliary transport.[223] The availability of a biochemical marker, cotinine, in saliva, serum, or urine has rendered documentation of passive exposure to tobacco smoke more reliable than that provided by history alone. High concentrations of serum cotinine were associated by Etzel and colleagues[68] with increased incidence of AOM and increased duration of middle ear effusion. Kim and colleagues[116] in Houston, Texas, documented the association of invasive pneumococcal infections in children and adults with increased environmental exposures to sulfur dioxide (a marker for air pollution) and higher counts of ragweed pollen.

Studies in the United Kingdom and the United States show seasonal variation in the occurrence of AOM. The

pattern within a period of 1 year is sinusoidal, with the peak incidence in December through March and lowest incidence in July through September.[3,102] These findings do not correlate with general climatic conditions because the U.S. studies were performed in Texas and Washington, D.C., and the United Kingdom study was done in northern England. The incidence coincides, however, with the peak incidence of respiratory tract infections in both countries.

Cost Analyses

Analyses of cost of management of otitis media have provided additional insights into the epidemiology of otitis media. Direct costs of an episode of otitis media include health care visits, cost of drugs, consultations, surgical procedures, audiometry and remedial speech and language visits, and hospitalizations related to severe AOM or its complications. Indirect costs include transportation, child care, and lost time from work. The average total cost of treating an episode of otitis media was estimated to be $116[109] to $131 in 1997 and 2000.[43] In 1996, the combined cost of AOM and its sequelae was estimated to be more than $5 billion every year. Mean costs of surgical procedures for severe and recurrent AOM in a managed care population in northern California for 1997 included myringotomy and placement of tympanostomy tube at $1400, mastoidectomy at $5062, and adenoidectomy at $1500.[43]

ETIOLOGIC AGENTS

The microbiology of otitis media has been documented by cultures of middle ear effusions obtained by needle aspiration (Table 16-2). In 1906, German investigators studied the bacteriology of AOM by aspiration of middle ear fluids. Many microbiologic studies were done, most in European otolaryngologic centers, before the introduction of antimicrobial agents. In the 1960s, investigative studies of the bacteriology of AOM were done in pediatric clinics and offices. Group A streptococcus was the dominant pathogen throughout the early studies but diminished in importance as more cases of mild and moderate disease were studied in pediatric programs, in contrast to the more severe disease referred to otolaryngologic centers. *Streptococcus pneumoniae*, which had been second to group A streptococci in frequency in the studies before 1960, now became the pathogen most frequently identified, followed by *Haemophilus influenzae* and *Moraxella catarrhalis*. More recent studies suggest the extensive use of the conjugate pneumococcal vaccine may have resulted in a decrease in the number of episodes of AOM caused by *S. pneumoniae* and a proportional increase in the incidence of disease caused by *H. influenzae* and *M. catarrhalis*.[25,46]

TABLE 16-2 **Bacterial Pathogens Isolated from Middle Ear Aspirates in Infants and Children with Acute Otitis Media (Percentage of Children with Pathogen, 1985-1992)***

Pathogen	Mean	Range
Streptococcus pneumoniae	38	27-52
Haemophilus influenzae	27	16-52
Moraxella catarrhalis	10	2-15
Group A streptococcus	3	0-11
Staphylococcus aureus	2	0-16
Miscellaneous bacteria	8	0-24
None or nonpathogens	28	12-35

*Percentage total >100 because of more than one pathogen per middle ear effusion.
From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007.

Bacteria may be isolated from middle ear fluid in approximately two thirds of patients with AOM. The proportion of patients with isolation of bacteria from middle ear fluids is likely to depend on criteria for selection of patients for tympanocentesis. The finding that approximately one third of patients have sterile cultures may reflect limitations of bacterial culture methods because antigen detection tests often indicate the presence of pneumococcal capsular polysaccharide in sterile middle ear fluids.[125,132] In addition, polymerase chain reaction for bacterial genome sequences has identified DNA of *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* in patients with otitis media with effusion whose cultures were negative for these bacterial species.[176] Concomitant isolation of two or more organisms in the same effusion occurs in 7 percent of cases. Disparate results of cultures in children with bilateral AOM occur in approximately 20 percent of cases.[169] The detection of bacterial biofilms in the middle ear mucosa of children with chronic otitis media raises the possibility of metabolically active bacteria in culture-negative middle ear effusions. Laser scanning microscopic images were taken from middle ear mucosa biopsy specimens in children undergoing placement of tympanostomy tubes; mucosal biofilms were identified in 46 of 50 specimens from children with otitis media with effusion and recurrent AOM.[86,176]

Several clinical situations warrant special consideration: (1) the occurrence of purulent conjunctivitis in association with AOM (conjunctivitis-otitis syndrome) usually is attributable to nontypeable *H. influenzae*[36,37]; (2) AOM occurs commonly among children hospitalized in intensive care units, and the bacteriology may be reflective of the hospital environment[62]; (3) renewed signs of AOM within 14 days usually represent relapse, whereas signs occurring 21 or more days after a prior infection are likely to indicate a new infection or recurrence[44]; and (4) children with tympanostomy tubes may develop AOM caused by organisms associated with otitis externa and AOM (e.g., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *S. pneumoniae*, and *H. influenzae*).[194]

The bacteriology of otitis media with effusion mimics that of AOM.* In contrast, the etiologic agents of chronic suppurative otitis media with persistent perforation include *P. aeruginosa*, *S. aureus*, anaerobic bacteria, and

*References 79, 80, 145, 182, 200, 209.

TABLE 16-3 **Distribution of Pneumococcal Serotypes in Children with Acute Otitis Media (%)**

	Year of Study, City/Country						
	1948-1951 Turku Finland	**1970-1977 U.S.**	**1985-1989 Birmingham**	**1994-2000 Multinational**	**1995-1999 Finland**	**1996-1999 U.S.**	**2000-2002 Czech Republic and Slovakia**
No. Strains	239	958	228	5520	414	500	189
Serotype							
1	10*	2.7	1.8	1.6	—	—	—
3	33.9	6.3	6.1	4	3.1	9.6	9
4	1.7	3.5	0.9	0.7	1	0.4	1.6
5	3.3	0.2	—	1	—	0.4	—
6	5.9	—	14	—			
6A	—	4.9	—	7.3	10.9	9.6	5.8
6B	—	7.3	—	10.1	13.5	10.8	12.7
7	3.8	2.2	0.4	0.8	—	0.4	—
8	2.9	1.9	—	—	—		—
9	—	—	4.4	—	—		—
9N	—	0.8	—	—	1.9		2.1
9V	—	7.1	—	4.6	—	5.2	—
10	—	1.2	—	—	—	—	—
11	1.3	1.2	3.1	1.3	5.8		—
14	1.7	10.2	10.9	13.1	6.3	15.8	11.6
15	1.3	4	—	2.1	5.6		—
18	3.8	5.3	2.2	—	0.2	1.4	3.7
19	10	—	33.2				
19A		5.5		6.6		5.4	1.6
19F		15.7		16.1	14	23.8	22.8
23	5	12.5	14	—			
23A		0.1	—	—	1	0.4	1.1
23F	—	11.4	—	14.9	19.8	10	9.5
27	3.3	0.1	—	—			
35	—	0.1	—	—	2.4		—
Author	Lahikainen, 1953[123a]	Austrian et al., 1977[6]	Orange and Gray, 1993[156a]	Hausdorff et al., 2002[89a]	Eskola et al., 2001[67]	Joloba et al., 2001[105a]	Prymula et al., 2006[178]

*Four most frequent serotypes are shaded.

enteric gram-negative bacilli.[40,114,158] *Mycobacterium tuberculosis* is a rare but important cause of chronic suppurative otitis media with persistent perforation.[232]

Bacteria found in middle ear aspirates usually are present in the nasopharynx of children with AOM, but multiple pathogens may be present in the nasopharynx that are not present in the middle ear.[69] Although not useful for specific microbiologic diagnosis of AOM, nasopharyngeal cultures are valuable for monitoring antibiotic susceptibility patterns of bacterial pathogens associated with AOM. Several investigators have noted quantitative differences in the nasopharyngeal flora of patients with and without otitis media, and these differences may play a role in the pathogenesis of middle ear disease. Long and colleagues[129] described a significant association between the recovery of abundant *H. influenzae* (at least 50% total colony count) from the nasopharynx and bacteriologically confirmed otitis media. An additional finding was that a semiquantitative nasopharyngeal culture was sensitive and specific in predicting the middle ear pathogen. Similar nasopharyngeal colonization rates for *S. pneumoniae* occur in ill and healthy children.[93,128,129] Gray and coworkers[84] have correlated the occurrence of AOM with nasopharyngeal acquisition of new serotypes of *S. pneumoniae*.

S. pneumoniae is the most frequent cause of severe AOM and suppurative complications. Of the 90 antigenically distinct pneumococcal serotypes, only a few serotypes are responsible for most cases of otitis media. In more recent surveys, the most common types responsible for AOM in order of decreasing frequency were types 19F, 23, 14, 6B, and 3 (Table 16-3). All of these types except serotype 3 were included in the conjugate vaccine, PCV7. The serotypes responsible for sequential episodes of AOM were described by Austrian and colleagues.[6] Of interest is the constancy of the four most frequently isolated types in recurrent episodes. Other features of interest in the study are (1) simultaneous infection of the middle ear fluid by two pneumococcal types, (2) isolation of the same type in consecutive episodes, and (3) isolation of the same type after an intercurrent episode caused by another type. Introduction of

PCV7 resulted in a decrease in nasopharyngeal carriage and AOM due to vaccine serotypes.[45] Serotype 19A was not included in PCV7 and emerged as the most frequent pneumococcal serotype isolated from middle ear fluids.[59,173] Because PCV13 includes 19A it is likely that serotype 19A will no longer be an important cause of AOM. Monitoring of pneumococcal serotypes responsible for AOM continues to be important in determining the durability of efficacy of PCV13 and determining whether replacement by nonvaccine serotypes will again emerge as causes of AOM.[22]

Otitis media caused by *H. influenzae* almost always is associated with nontypeable strains.[9,10,99,100,207] The clinical presentation of AOM caused by nontypeable *H. influenzae* usually is less severe than acute infection caused by *S. pneumoniae*, as measured by temperature elevation, ear pain, suppurative complications, and perforation of the tympanic membrane. Before the introduction of the conjugate *H. influenzae* type b vaccine, approximately 10 percent of children with *Haemophilus* spp. otitis had disease caused by type b and approximately one fourth of these children had concurrent or subsequent bacteremia or meningitis.[88] *H. influenzae* causes AOM in all age groups, including older children and adolescents.[196,197] Of *H. influenzae* strains isolated from middle ear fluids, 30 to 60 percent produce β-lactamase.[123,202,226]

Although *S. pneumoniae* and *H. influenzae* are responsible for most cases of bacterial otitis media, *M. catarrhalis* and group A streptococci are responsible for some cases and should be considered in choosing appropriate antimicrobial agents. The incidence of AOM caused by *M. catarrhalis* in most studies is less than 10 percent; an Israeli study of 12,799 episodes of AOM from 1999 to 2006 revealed *M. catarrhalis* in 4.8% of middle ear fluids[39]; but a higher incidence was noted elsewhere, including 22 percent and 27 percent in 1983 reports from Pittsburgh[123] and Cleveland,[202] respectively, and 18.4 percent in a study of Finnish children observed between 1995 and 1997.[115] Most strains of *M. catarrhalis* isolated from middle ear fluids produce β-lactamase, and some patients fail to improve if they are treated with a β-lactamase–susceptible drug. Episodes of AOM due to *M. catarrhalis* had few complications.[39] *M. catarrhalis* DNA is detected more frequently from children with otitis media with effusion than children with AOM.[149]

During the preantibiotic era, otitis media caused by group A streptococci frequently was associated with scarlet fever and often was of a severe and destructive form. In recent years, group A streptococci have been isolated frequently in some studies from Scandinavia but have been found infrequently in most studies from the United States. A survey of Israeli children identified group A streptococci in 350 of 11,311 episodes (3.1%) of AOM during 1999 to 2003. The incidence of mastoiditis was higher for patients with streptococcal AOM than for patients with other pathogens.[199]

Tuberculous otitis was an occasional cause of severe middle ear disease at the turn of the 20th century in the United States and western Europe and still occurs in developing countries. Otitis caused by *M. tuberculosis* is characterized by a painless, watery otorrhea through single or multiple perforations of the tympanic membrane.[205] Other bacteria, including *S. aureus* (which occurs infrequently in the United States but is the etiologic agent in 10% of cases in Japan[7]), gram-negative enteric bacilli (responsible for approximately 20% of otitis media cases in neonates, but a rare finding in older infants), anaerobic bacteria, *Clostridium tetani*, and *Corynebacterium diphtheriae*, are responsible for occasional cases of AOM.

Fusobacterium necrophorum is associated with Lemierre syndrome (pharyngitis with septic thrombosis of the internal jugular veins) but is also an uncommon cause of AOM.[124] *Alloiococcus otitidis* has been identified by culture of middle ear fluids in rare cases of AOM but recent studies using multiplex polymerase chain reaction (PCR) suggest that it is responsible for up to 9% of cases of AOM.[113] *Chlamydia trachomatis*[219] has been identified in infants 6 months of age and younger, often associated with pneumonia. The role of *Chlamydophila pneumoniae* (formerly *Chlamydia pneumoniae*) in AOM is uncertain.[155,211]

The clinical history suggests that viral infection serves as a frequent initiating event of AOM by producing congestion of the mucosa of the upper respiratory tract. Changes in the appearance of the tympanic membrane can be seen early in the course of respiratory tract viral infection.[108] Epidemiologic data support an association between viral respiratory infection and the occurrence of AOM.[91] Upper respiratory tract infection with respiratory syncytial virus, influenza viruses, and adenoviruses was associated with a greater risk for developing otitis media than was infection with other viruses. Early studies identified a low viral isolation rate from middle ear fluid in patients with otitis media. A virus was isolated from only 29 of 663 (4.4%) specimens obtained by tympanocentesis and reviewed by Klein and Teele in 1976.[122] A higher virus identification rate in middle ear fluid using culture and antigen detection has been reported.[53,117,191]

Pitkaranta and colleagues[175] used reverse transcriptase polymerase chain reaction to identify viruses in middle ear fluids of children with AOM and otitis media with effusion; evidence of rhinovirus was found in 22 percent and 19 percent, respectively, and respiratory syncytial virus was found in 18 percent and 8 percent, respectively. Ruuskanen and colleagues[189] summarized eight studies published between 1982 and 1990 using immunoassay or isolation; virus was identified in middle ear fluids in 17 percent of the samples—as a single agent in 6 percent and in combination with a bacterial pathogen in 11 percent. Viruses identified in middle ear fluids have included respiratory syncytial virus, influenza viruses, adenoviruses, parainfluenza viruses, enteroviruses, and rhinoviruses.

Concomitant isolation of viral and bacterial pathogens from middle ear fluid seems to be a common finding.[53,172,191] A survey of the microbiology of new-onset otorrhea in 79 children with tympanostomy tubes identified bacteria in 92 percent, viruses in 70 percent, and bacteria and viruses in 66 percent.[188] In an accompanying commentary, Chonmaitree[52] noted that AOM no longer should be considered a pure bacterial disease and that antibacterial treatment may not result in optimal outcome in some cases because of viral coinfection.

Etiology in Neonates

Clinical investigators have performed needle tympanocentesis to isolate bacterial pathogens causing otitis media in the first 6 weeks of life. Four studies included 169 infants.[17,24,201,218] Bacteria were isolated from middle ear fluid in 68 percent of cases. As in older children, *S. pneumoniae* and *H. influenzae* were the organisms most frequently isolated. Other than the more frequent occurrence of disease caused by gram-negative enteric organisms (approximately 20% of cases) and the occasional isolation of other neonatal pathogens (e.g., group B streptococci), the bacteriology of otitis media in this age group was similar to that in older children.

PATHOGENESIS

The pathogenesis of otitis media is likely to follow a similar sequence of events in most children. The patient has an antecedent event (usually caused by an upper respiratory viral infection) that results in congestion of the respiratory mucosa throughout the respiratory tract, including the nose, nasopharynx, eustachian tube, and middle ear; congestion of the mucosa in the eustachian tube results in obstruction of the narrowest portion of the tube, the isthmus. The obstruction results in negative pressure in the middle ear and then development of middle ear effusion. The secretions of the mucosa of the middle ear, which usually drain through the eustachian tube, now have no egress and accumulate in the middle ear. The effusion may be asymptomatic (i.e., lacking the signs and symptoms of acute infection) and is termed *otitis media with effusion*. If pathogenic bacteria or viruses that colonize the nasopharynx are present in the middle ear after obstruction of the eustachian tube has occurred, the organisms multiply, resulting in an acute suppurative infection, with an abscess, and characterized by signs and symptoms of acute infection such as fever and otalgia.[32,33,81,190] For children with recurrent episodes of AOM or otitis media with effusion, anatomic or physiologic abnormalities of the eustachian tube seem to be predisposing factors.

It also is possible that subtle changes in immune response occur that predispose a child to frequent episodes of otitis media. Experimental studies provide evidence that virus-induced impairments in neutrophil migration and bacterial killing also may be important in the pathogenesis of AOM.[1]

With growth of the skull and change in the position, length, and width of the eustachian tube over the course of time, the predilection to otitis media in accompanying acute infections of the upper respiratory tract in the first 3 years of life diminishes, and the patient has fewer episodes of AOM. Children younger than 3 years of age with similar respiratory infections are predisposed to the complication of acute infection of the middle ear, whereas older children challenged by the same microorganism have signs of upper respiratory tract infection but need not have a complicating ear infection.

The pathogenesis of persistent middle ear effusion or otitis media with effusion is uncertain. An effective antimicrobial agent sterilizes the acute bacterial infection of AOM. The middle ear effusion, now sterile, may persist for weeks to months. The median duration of middle ear effusion after AOM is approximately 23 days (Fig. 16-1). The type of antibacterial drug used does not seem to alter the duration of fluid in the middle ear after acute infection occurs.

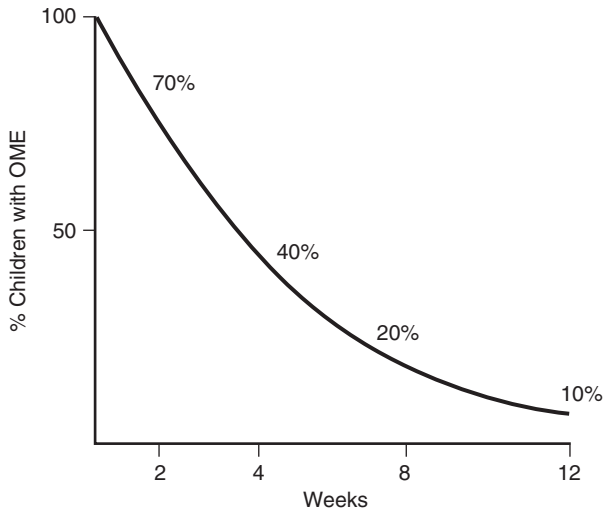

FIGURE 16-1 ■ Persistence of middle ear effusion after onset of acute otitis media. OME, otitis media with effusions. (Modified from Teele DW, Klein JO, Rosner BA. Epidemiology of otitis media in children. *Ann Otol Rhinol Laryngol* 1980;**89**:5–6; in Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 80.)

Most recently, Bluestone and Swarts[35] have posited that otitis media is most likely limited to humans, because if animals in the wild would have developed it the associated hearing loss would have reduced the fitness of affected individuals as the result of predation. The impact of human evolution on the palate and the paratubal muscles has resulted in relatively poor eustachian tube function in humans when compared with the monkey.

PATHOPHYSIOLOGY

Tympanic Membrane

In the presence of otitis media, changes in the tympanic membrane occur rapidly. The presence of congested blood vessels, edema (which obscures normal landmarks), and bulging or sagging of the Shrapnell membrane indicate not only a myringitis (inflammation of the tympanic membrane) but also the presence of fluid in the middle ear space. Blebs that appear on the surface epithelium are a consequence of AOM or edema or hydropic degeneration of the membrane.

Inflammation may occur on outer epithelial or inner mucosal sides of the fibrous layer (middle layer) of the eardrum. In severe cases, infection may involve the fibrous layer itself. The membrane thickens as a result of edema and infiltration of polymorphonuclear leukocytes. All three layers of the eardrum may undergo dissolution, owing to pressure necrosis resulting from the expanding

middle ear abscess or thrombophlebitis of tympanic veins, with resulting perforation. With evacuation of the contents of the middle ear abscess, healing may be rapid, and the perforation usually seals within a few days. In the process of healing, metaplasia of the epithelium, hyaline degeneration, calcium deposition, and scar formation may occur.

Occasionally, when a perforation is close to the margin of the annulus or occurs in the Shrapnell membrane, the skin of the external auditory canal and the surface squamous epithelium of the tympanic membrane may grow through the aperture and invade the middle ear. This event may lead to formation of a cholesteatoma (epidermal inclusion cyst). Even if the perforation heals, a differential in gas pressure across the tympanic membrane caused by malfunction of the eustachian tube may result in resorption of gas in the middle ear cavity and negative pressure in the middle ear, which causes retraction of the Shrapnell membrane or an atrophic scar into the middle ear or mastoid attic.

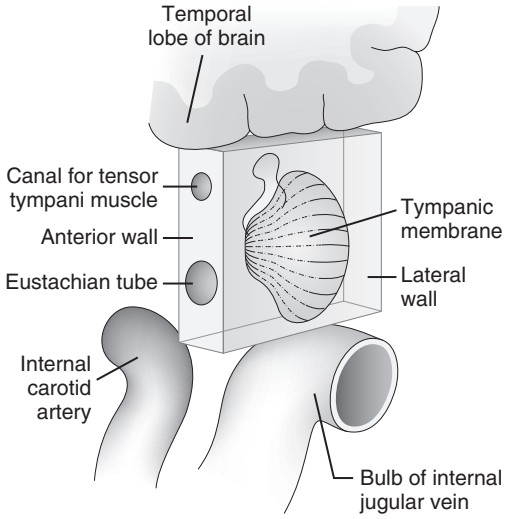

FIGURE 16-2 ■ The middle ear.

Eustachian Tube

The eustachian tube is approximately 3.8 cm long in an adult. It opens in the fossa of Rosenmüller and extends upward, backward, and laterally to open in the upper anterior wall of the tympanic cavity (protympanum). At birth, the eustachian tube is immature, that is, it is shorter and floppier than at 1 year of age.[30] It is composed of two portions: the cartilaginous portion extending into the nasopharynx and the bony portion originating in the middle ear. The upper third of the tube is bony; the middle ear opening is the widest, and the medial end (the part joining the cartilaginous eustachian tube), or isthmus, is the narrowest. Pneumatic peritubal air cells arising from the middle ear cavity surround it and can extend to the petrous apex. The internal carotid artery lies anteromedial to this region (Fig. 16-2).

The lower two thirds of the eustachian tube is a narrow, slitlike, fibrocartilaginous passage. It makes a 160-degree angle with the bony portion at its junction. The cross section of the tube looks like a shepherd's crook, with a cartilaginous superior and medial surface and a fibrous lateral surface.

Three muscles are associated with the eustachian tube. The tensor tympani muscle lies on top of it; the levator veli palatini muscle lies under it; and the tensor veli palatini muscle arises on the tube, scaphoid fossa, and spine of the sphenoid and then courses around the hook of the hamulus and forms an aponeurosis with its mate (from the opposite side) in the soft palate. This muscle is the only one that acts directly on the eustachian tube.[26]

The eustachian tube area, protympanum, and hypotympanum are lined by ciliated columnar epithelium with goblet cells or secretory cells. The epithelium is continuous with the upper airway system and paranasal sinuses. This area also contains a well-defined subepithelial connective tissue layer, which thins out and may be absent nearing the antrum and mastoid air cell system. The movement of the cilia and mucus blanket always is toward the eustachian tube and nasopharynx. The tube is surrounded by a plexus of lymphoid channels. It has an arterial supply from a branch of the middle meningeal or accessory meningeal artery and from branches of the artery of the pterygoid canal. The nerve supply is from the tympanic plexus (ninth cranial nerve) (sensory) and sphenopalatine ganglion (sympathetics and parasympathetic palatine fiber).

The bony portion is rigid and patulous; the medial two thirds normally is held closed by elastic recoil of the fibrocartilaginous tissue. Contraction of the tensor palatini muscle that inserts in the anterolateral wall opens the tube on swallowing. On average, an adult swallows once per minute while awake and once every 5 minutes while asleep. Suckling infants usually swallow five times per minute.

Mucus and ciliary action flow from the middle ear to the eustachian tube. The eustachian tube acts as a unidirectional valve that favors outflow from the middle ear to the pharynx. Reverse flow can be induced by an increase in pressure in the nasopharynx (Valsalva, barotrauma). During occlusion of the eustachian tube, oxygen and carbon dioxide (and other gases) are absorbed from the middle ear by diffusion into the rich vasculature, and a negative pressure is created. A patent eustachian tube is a crucial prerequisite for subsidence of middle ear disease.

DIAGNOSIS

To establish uniformity of diagnosis, the clinical practice guideline published by the AAP and the AAFP proposed criteria for diagnosis of AOM.[173] The diagnosis required (1) a history of acute onset of signs and symptoms, (2) the presence of middle ear effusion, and (3) signs and symptoms of middle ear inflammation. The presence of middle ear effusion was identified by any of the following: (1) bulging of the tympanic membrane, (2) limited or absent mobility of the tympanic membrane, (3) air-fluid

level behind the tympanic membrane, or (4) otorrhea. Signs or symptoms of middle ear inflammation were identified by either erythema of the tympanic membrane or otalgia that results in interference with or precludes normal activity or sleep.

CLINICAL PRESENTATION

Children with AOM may have nonspecific signs and symptoms, including fever, irritability, headache, apathy, anorexia, vomiting, and diarrhea. Signs of viral respiratory tract infection, including cough and coryza, usually are present before the specific signs of ear infection occur. Fever occurs in approximately one third[198] to two thirds[148] of children with otitis media.

Specific signs and symptoms associated with otitis media and its complications and sequelae include the following:

1. Otalgia, or ear pain, is the most common complaint of infants and children with AOM. The symptom is suggested in young infants who are pulling at the ear or excessively irritable. Some infants do not have earache; Hayden and Schwartz[91] identified absence of ear pain in approximately one fifth of 335 consecutively diagnosed episodes of otitis media, usually among children older than 2 years of age.
2. Otorrhea is discharge from the middle ear through a perforation in the tympanic membrane or from the external auditory canal when inflamed. The acute perforation usually is central in the membrane. Relief of the pressure on the tympanic membrane results in immediate pain relief and usually a decrease in temperature. Because the tympanic membrane has a dense network of blood vessels, rapid repair of the membrane occurs, and the perforation usually is unapparent within 24 to 72 hours. If the tympanic membrane seals and mucous membrane infection still is present, fluid may reaccumulate with renewed acute signs of otitis media.
3. Hearing loss occurs whenever fluid fills the middle ear space, whether the fluid is associated with acute infection or with otitis media with effusion. When fluid fills the middle ear space, the median hearing loss is 25 dB (the equivalent of having plugs in the ear canals).[74]
4. Vertigo occurs but is not a common complaint of children with otitis media. Vertigo occurs more commonly in unilateral than bilateral disease and may be caused by labyrinthitis. Older children describe a feeling of spinning, whereas younger children may not be able to verbalize these symptoms but manifest disequilibrium by falling or stumbling.
5. Tinnitus is an uncommon complaint in children, but when it does occur, the symptom often is caused by otitis media and eustachian tube dysfunction.
6. Swelling around the ear, especially in the postauricular area, may be a sign of mastoiditis.
7. Facial paralysis in children occurs as a complication of AOM or chronic otitis media with perforation of the tympanic membrane or as a result of an enlarging cholesteatoma.
8. Conjunctivitis has been associated with AOM caused by nontypeable strains of *H. influenzae*. The conjunctivae are injected, with tearing or purulent discharge.[37]
9. Craniofacial anomalies, such as cleft palate, mandibulofacial dysostosis, and those associated with Down syndrome, may predispose to frequent ear disease. Hypernasal speech suggests velopharyngeal insufficiency.

EXAMINATION OF THE EAR

Otoscopy

Examination of the ear should begin with observation of the auricle and the external auditory meatus. Palpation of the periauricular areas should be done to indicate presence of periostitis or diffuse external otitis. The ear canal should be examined for inflammation or cerumen that obstructs vision of the tympanic membrane.

For proper assessment of the tympanic membrane and its mobility, a pneumatic otoscope in which the diagnostic head has a secure seal should be used. The speculum should have the largest lumen that can fit comfortably into the child's cartilaginous external auditory meatus. The important landmarks of the tympanic membrane that can be visualized with the otoscope are indicated in Figure 16-3. The otoscopic examination should include observation of the following conditions of the tympanic membrane:

- *Position:* Normal is slightly convex; bulging indicates increased pressure from positive air pressure or fluid; a retracted eardrum indicates negative pressure with or without effusion; fullness of the tympanic membrane is apparent initially in the posterosuperior portion of the pars tensa and the pars flaccida because these two areas are the most highly compliant parts of the membrane.
- *Appearance and color:* The normal color is pearly gray and translucent; any congestion of the mucous

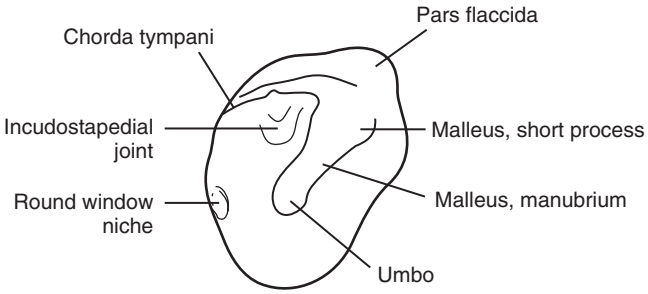

FIGURE 16-3 ■ Important landmarks of the tympanic membrane that usually can be visualized with the otoscope. (From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 156.)

membrane of the middle ear would be reflected in congestion of the vessels of the tympanic membrane and appear pink; a blue discoloration suggests blood in the middle ear associated sometimes with basal skull fracture; the inflamed middle ear mucosa usually is reflected in a bright red tympanic membrane.

- *Integrity of the membrane:* All four quadrants of the tympanic membrane should be inspected for presence or absence of perforation, retraction pockets, or cholesteatoma.
- *Mobility:* Application of positive and negative pressures by the pneumatic otoscope enables the viewer to determine the presence of an air-filled space (rapid excursion of the membrane on positive and negative pressures) or a fluid-filled space (limited or no excursion of the membrane); a middle ear with negative pressure does not respond to otoscopic negative pressure, and a middle ear with high positive pressure does not respond to otoscopic positive pressure (Fig. 16-4).

Tympanometry

Tympanometry measures the compliance of the tympanic membrane (Fig. 16-5).* Under normal circumstances, the pressure in the middle ear virtually is the same as the external ambient pressure; the eustachian tube functions to equate middle ear pressure to atmospheric pressure. If for any reason a pressure differential occurs across the tympanic membrane, stress is applied to the drum. Middle ear compliance varies as a function of the pressure differential across the tympanic membrane. When blockage of the eustachian tube with no fluid in the middle ear occurs, the tympanometry curve has a shape similar to that observed on a normal tympanogram, but the point of maximal compliance is shifted to the negative pressure side because maximal compliance is reached when the tympanic membrane reaches a peak of compliance (i.e., when external auditory canal pressure is reduced to the same level as in the middle ear).

If ossicular discontinuity or a flaccid or atrophic tympanic membrane is present, the eardrum is highly compliant and a highly peaked tympanogram is obtained. Abnormal tympanograms obtained in the presence of fluid in the middle ear are characterized by the following:

1. Reduced height of the curve (i.e., the middle ear has reduced compliance)
2. Shift to negative pressure sides of the curve and point of maximal compliance (i.e., eustachian tube blockage)
3. A flat curve without definite peak, the most characteristic feature

Although tympanometry with the electroacoustic impedance bridge has proved to be a satisfactory method for detecting the presence of fluid in the middle ear cavity, technical difficulties exist in obtaining accurate readings in infants and children, including the requirement for a secure seal of the probe in the ear canal, the need for a period of quiet to obtain an accurate reading, and decreased accuracy in infants younger than 7 months of age (because of the highly compliant external auditory canal in infants <7 months).[167] In addition, only a few of many instruments currently on the market in the United States have data about sensitivity and specificity based on tympanometric patterns of otoscopic results before myringotomy.[72,153] Less persuasive data are available from studies correlating tympanometry and otoscopy findings.[41,141,177]A recent study concluded that pneumatic otoscopy, even performed by an inexperienced examiner, is more sensitive and specific than tympanometry.[183]

Acoustic Reflectometry

The acoustic otoscope, or reflectometer (EarCheck, Innovia Medical, Omaha, Nebraska), is a handheld instrument that uses a microphone in the probe tip placed in the opening of the child's external ear canal. A professional and a consumer model were developed but, at present, only the consumer model is available. The tip measures the level of transmitted and reflected sound from a less than 90-dB sound source that varies from 1800 to 4400 Hz in a 750-msec period. Acoustic energy is reflected back toward the probe tip from the ear canal and eardrum. The operating principle is based on the fact that a sound wave in a closed tube would be reflected when it strikes the end of the tube. Sound reflectivity is measured in units that indicate the status of a fluid-filled or air-filled middle ear.[57] Babonis and associates[8] found that tympanometry and reflectometry had comparable accuracy in predicting middle ear effusion documented by myringotomy. Acoustic reflectometry has some technical advantages compared with tympanometry: accurate readings can be obtained in crying children, and a secure seal of the probe tip in the ear canal is not required.

Audiometric Tests

Audiometric testing may be employed to measure auditory acuity and evaluate conductive hearing losses, but assessment of hearing is an inaccurate method for identifying middle ear effusion. Hearing loss is the most prevalent complication of otitis media and is present uniformly whenever fluid fills the middle ear. The audiogram usually reveals a mild to moderate conductive hearing loss (median, 25 dB).[74] Obstruction of the eustachian tube early in the clinical course of otitis media results in absorption of gases from the middle ear and eardrum retraction. The reduced compliance of the eardrum results in increased stiffness in the ossicular chain system. The audiogram reveals a low-frequency conductive hearing loss. As serous effusion appears and the middle ear fills with serum and pus, the ossicular system has an increased mass applied to it, and the audiogram flattens out, resulting in a high-frequency conductive hearing loss.

Tympanocentesis and Myringotomy

Tympanocentesis, a needle aspiration of the middle ear effusion, is used primarily for establishing the presence

*References 41, 58, 141, 177, 181, 220.

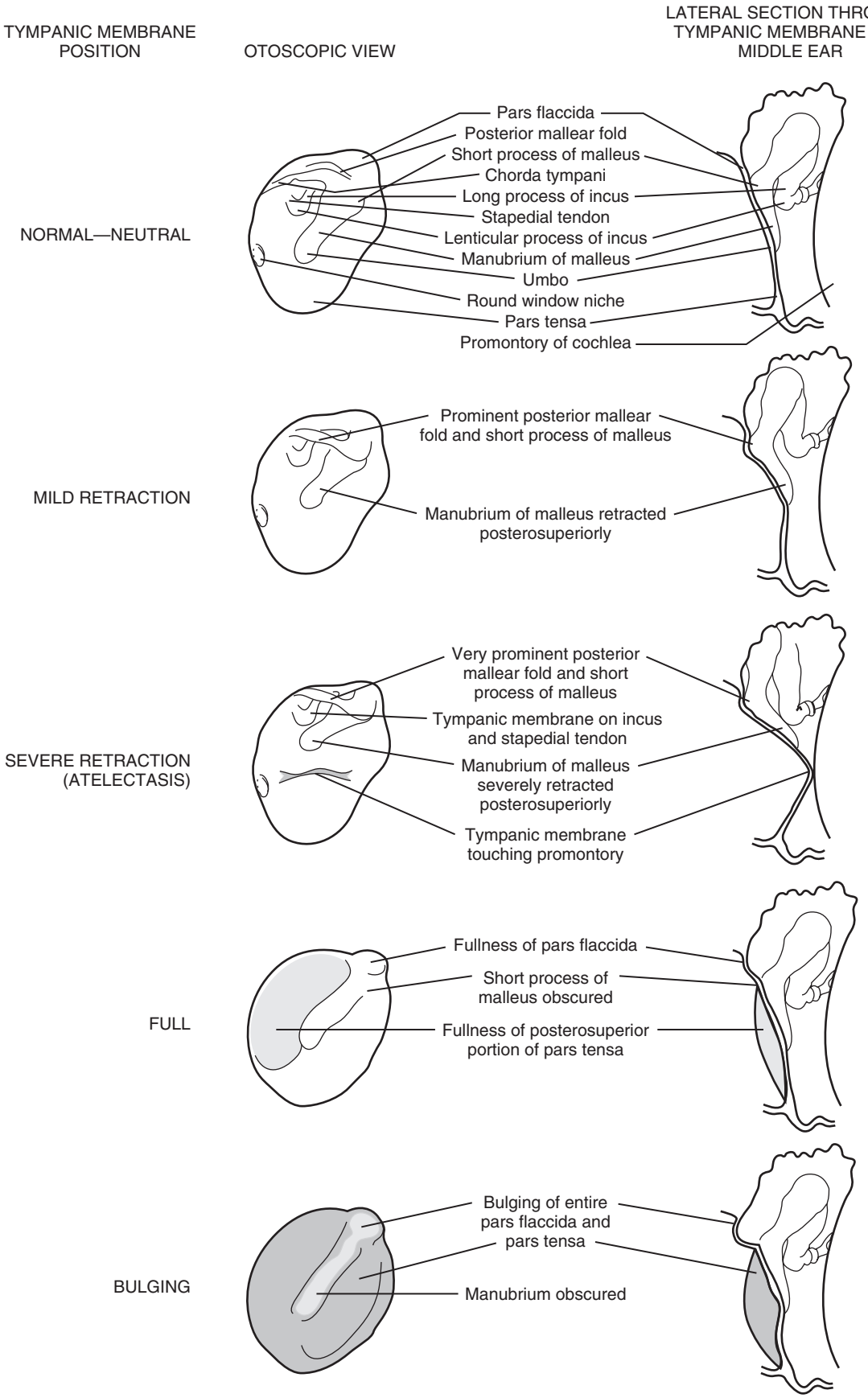

FIGURE 16-4 ■ Otoscopic views and corresponding lateral sections through the tympanic membranes and middle ear show the various positions of the eardrum with their respective anatomic landmarks. (From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 153.)

	TYMPANOGRAM TYPES	COMMON VARIANTS	PRESUMPTIVE DIAGNOSIS OF TYMPANIC MEMBRANE MIDDLE EAR CONDITION
1.	Normal	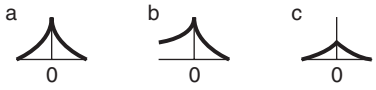	Normal
2.	High compliance (normal pressure)		Flaccid tympanic membrane or ossicular discontinuity
3.	Negative pressure (normal compliance)	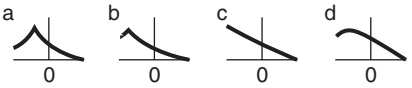	High negative pressure with or without middle ear effusion
4.	High negative pressure and high compliance		Flaccid tympanic membrane and high negative pressure (or ossicular discontinuity and high negative pressure)
5.	High positive pressure		High positive pressure with or without middle ear effusion
6.	Low compliance	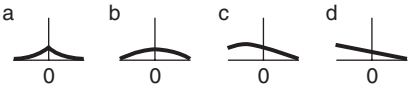	Middle ear effusion and/or thickened tympanic membrane and/or ossicular fixation and/or adhesive otitis media

FIGURE 16-5 ■ Tympanogram types related to presumptive conditions of the middle ear. (From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 171.)

or absence of an effusion and for microbiologic study. Because cultures of the upper respiratory tract are of limited value in providing specific microbiologic diagnosis of otitis media, only materials obtained by aspiration of the middle ear abscess can be considered a true reflection of the etiology of AOM. Myringotomy is an incision in the anterior lower quadrant of the tympanic membrane for therapeutic drainage. Tympanocentesis or myringotomy should be considered in patients who at onset appear toxic or are seriously ill, in patients who are toxic after initiation of antimicrobial therapy, in the presence of suppurative complications (including mastoiditis and meningitis), and in immunologically deficient patients in whom an unusual organism may be present.

Radiography

Radiographic evaluation of the temporal bone is indicated when complications or sequelae of otitis media are suspected or present. Plain radiographs are of limited value in the diagnosis of osteitis of the mastoid or cholesteatoma; computed tomography and magnetic resonance imaging are more precise and should be obtained if a suppurative intratemporal or intracranial complication is suspected.

DIFFERENTIAL DIAGNOSIS

Inflammation or foreign body in the external ear canal may produce ear pain simulating that of AOM. When external otitis or furunculosis of the external auditory canal is present, the patient often has severe itching in the ear canal and pain elicited by manipulation of the pinna. The canal may be narrowed and so tender that performing an otoscopic examination is impossible.

An erythematous tympanic membrane may be caused by an upper respiratory tract infection with congestion of the mucosa lining the entire respiratory tract, including the middle ear. A "red drum" also may be produced by trauma or aggressive examination of the external auditory canal and may appear suddenly in a crying child.

Otalgia may be associated with infections of the tonsils, adenoids, teeth, nasopharynx, hypopharynx, or larynx. Tumors in those regions can refer pain to the ipsilateral ear along the tenth cranial nerve. Lymphomas, leukemias, and rhabdomyosarcomas involving the palate, nasopharynx, or base of the skull eventually occlude one or both eustachian tubes, producing serous effusions and otalgia.

AOM must be differentiated from an acute exacerbation of an unrelated disease in a patient with persistent middle ear effusion. Because fluid persists for weeks to months after each episode of AOM, an intercurrent infection, not associated with middle ear disease, may be misdiagnosed as AOM because of the presence of acute systemic signs of an infectious illness plus a middle ear effusion. This event may be one of the reasons for overdiagnosis of AOM. At present, no technique, other than tympanocentesis, is readily available to distinguish a relapse of AOM from a new and recurrent episode of AOM or from an intercurrent and unrelated infection associated with persistent middle ear effusion.

OTITIS MEDIA WITH EFFUSION

The presence of an asymptomatic middle ear effusion has many synonyms, such as secretory, nonsuppurative, and serous otitis media, but the most acceptable term is *otitis media with effusion*. After every episode of AOM, fluid persists in the middle ear for weeks to months (see Fig. 16-1).[216] In a study of children with AOM in Boston, 70 percent of the patients still had effusion at 2 weeks, 40 percent had effusion at 1 month, 20 percent had effusion at 2 months, and 10 percent had effusion at 3 months. Similar results of persistent middle ear effusion after an episode of AOM have been noted in all other clinical studies of AOM. The incidence or prevalence of otitis media with effusion that is unrecognized by parents and not brought to medical attention has been studied extensively.[47,71,174] The prevalence of effusion varied with age and the time of year. Incidence of otitis media with effusion peaked during the second year of life and was more prevalent in winter than in summer.[71,174,210] In some children, the duration of otitis media with effusion may be only 1 or several days.[21] Because hearing loss is present whenever fluid fills the middle ear space, physicians are concerned about the many children with prolonged time spent with effusion; the accompanying hearing loss; and possible adverse effects on speech, language, and cognitive development.

Bacteria can be recovered from one third to one half of specimens obtained at the time of myringotomy or tympanostomy tube insertion.[79,80,145,182,200] The bacteriology in such cases has mimicked closely the bacteriology of AOM, with *S. pneumoniae* and *H. influenzae* being the predominant organisms isolated. The significance of this finding at present is unknown. The bacteria merely may colonize middle ear fluid without producing inflammation, or they may play a role in the production or persistence of middle ear fluid. In addition to live bacteria, nonviable bacteria, pneumococcal capsular polysaccharide, and endotoxin have been found in chronic middle ear effusions.[61,79,125]

Much attention has been given to the nature and composition of the middle ear effusion. The presence of biologic mediators of inflammation in the middle ear fluid has been shown[19,126,206]; they include chemotactic factors, macrophage-inhibiting factors, activated complement, histamine, prostaglandins, leukotrienes,[106,107,146] and immune complexes.[228] Elevated levels of IgA, IgE, IgM, and IgG also have been noted in serous effusions.

Clinical evaluation depends on otologic examination and audiologic and tympanometric testing. Symptoms of this disease include conductive deafness, which usually is fluctuant and may be position dependent. The patient may have a dull earache or a sensation of fullness in the ear. The eardrum usually is dull with a poor light reflex and may be retracted. It may be pale pink or have a ground-glass appearance.

COMPLICATIONS AND SEQUELAE

Intracranial suppurative complications of otitis media, including meningitis, brain abscess, and lateral sinus thrombosis, are uncommon occurrences today in developed countries (Fig. 16-6). Intratemporal complications that occur within the aural cavity and adjacent structures of the temporal bone are seen more commonly. They include acute and chronic perforation of the tympanic membrane, chronic suppurative otitis media, mastoiditis,

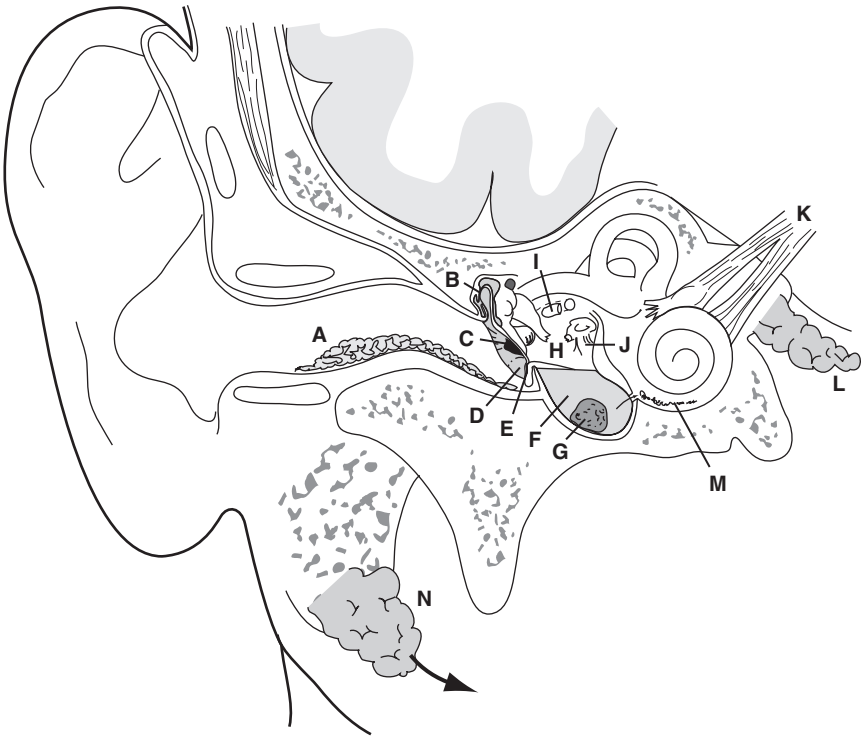

FIGURE 16-6 ■ Intratemporal complications and sequelae of otitis media include the following: A, infectious eczematoid dermatitis; B, cholesteatoma; C, retraction pocket of tympanic membrane; D, tympanosclerosis; E, perforation of tympanic membrane; F, chronic suppurative otitis media; G, cholesterol granuloma; H, ossicular discontinuity; I, facial paralysis; J, adhesive otitis media with fixation of the ossicles; K, hearing loss; L, petrositis; M, labyrinthitis; and N, mastoiditis with extension into the neck (Bezold abscess). (From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 329.)

cholesteatoma and retraction pocket, adhesive otitis media, tympanosclerosis, and ossicular discontinuity and fixation. The most frequent complication is hearing loss that occurs whenever the middle ear cavity is filled with fluid.

Hearing Loss

Fluctuating or persisting hearing loss is present in most children who have middle ear effusion; impairment of hearing is the most prevalent complication of otitis media with effusion. Audiograms of children with middle ear effusion usually reveal a mild to moderate conductive loss of 15 to 40 dB.[74] With such deficits, the softer speech sounds and voiceless consonants may be missed. The hearing loss is not influenced by the quality of fluid in the middle ear; ears with thin fluids are impaired to the same degree as are ears with fluids of gluelike consistency.[42,231] The hearing impairment usually is reversed with resolution of the effusion. Uncommonly, permanent conductive hearing loss occurs because of irreversible changes from the inflammatory reaction, resulting in adhesive otitis media or ossicular discontinuity. High negative pressure in the middle ear or atelectasis in the absence of effusion also may cause conductive loss. The presence of middle-ear effusion has been reported in neonates who failed newborn screening.[38]

Sensorineural hearing loss after a case of AOM may occur as a result of increased tension and stiffness of the round-window membrane and is reversible. A permanent sensorineural loss may occur as a result of spread of infection or products of inflammation through the round-window membrane.[131]

Vestibular Dysfunction

Middle-ear effusion can cause disequilibrium and even vertigo, apparently secondary to products of inflammation crossing the round window membrane or pressure on the oval or round windows. Tympanostomy tube placement can relieve the symptoms.[56]

Effects of Otitis Media on Development of the Child

Children with severe or recurrent otitis media have prolonged time spent with middle ear effusion. Hearing impairment accompanies the effusion in most children. If the hearing impairment occurs at a time of rapid intellectual growth, the result may be impaired development of speech, language, and cognitive abilities (Fig. 16-7). Because language acquisition is dynamic during infancy, any problems in receiving or interpreting sound signals might have a significant effect on development of speech and language. Softer speech sounds and voiceless consonants, in particular, may be missed or confused when effusion is present in the middle ear.

Although many studies have been done and are reviewed in a clinical practice guideline (i.e., *Otitis Media with Effusion in Young Children*) published by the Agency for Health Care Policy and Research of the U.S. Department of Health and Human Services,[210] the limitations

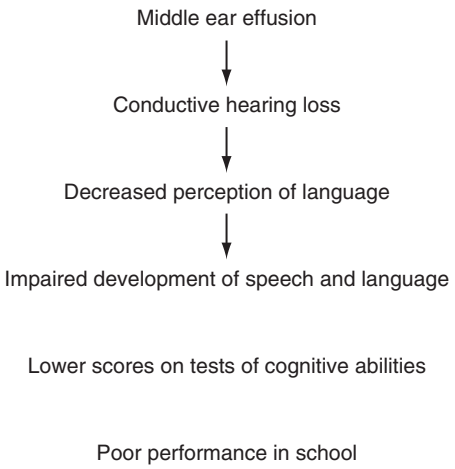

FIGURE 16-7 ■ **Long-term sequelae of middle ear effusion.** (From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 333.)

of design of many of the studies and inconsistencies of the results limit the ability to make conclusions about the effect of otitis media on development. The interested reader should consult the guideline for a valuable review and extensive bibliography. A study by Paradise and colleagues[164] indicates that early placement of ventilating tubes in children with prolonged time spent with middle ear fluid did not measurably improve developmental outcomes at age 3 years. Nonetheless, some infants and young children with recurrent otitis media and prolonged time spent with middle ear effusion possibly have substantive loss in potential for development of speech, language, and cognitive abilities. Selected references about long-term outcomes of otitis media include studies by Gravel and Wallace,[83] Teele and colleagues,[214] and Friel-Patti and associates[75] and reviews by Berman[15] and Klein.[119]

Child Behavior and Quality-of-Life Outcomes

Disturbances in the child's behavior associated with otitis media have been reported to include restlessness, frequent disobedience, impaired task orientation in the classroom, short attention span and distractibility, attention deficits, and restricted social interaction. Only selected children may be most affected. Paradise and colleagues[165] found that stress in the parent–child relationship and behavior problems were highest among children from the most socioeconomically disadvantaged homes.

A working parent who has spent a sleepless night attending to a child who is fretful because of ear pain may have work-related and home-related stress. Chase[51] described the response of parents of 1-year-old children to structured interaction tasks; parents of a child who had one or more episodes of otitis media were less effective in gaining the child's attention, less able to respond effectively when the child was distracted from the task, and less able to help the child understand and perform the task.

Perforation of the Tympanic Membrane and Chronic Suppurative Otitis Media

Acute perforation (not caused by trauma) usually is secondary to AOM but also may occur during the course of otitis media with effusion. The perforation occurs because of pressure of the expanding middle ear contents on the membrane, resulting in local ischemia and tissue damage, usually in the central portion of the membrane. With rupture, the middle ear contents are discharged into the external ear canal, with instant relief of pain and defervescence in acute infection. Because the membrane is highly vascular, the perforation may seal quickly and not be evident within hours to days. If the mucous membrane of the middle ear remains inflamed, fluid may reaccumulate behind the resealed tympanic membrane.

Chronic perforation may occur after an acute episode, spontaneous extrusion, or removal of a tympanostomy tube. If squamous epithelium grows at the edges of the perforation, healing may be prevented, and the perforation persists. The term *chronic suppurative otitis media* is limited to a stage of ear disease in which chronic inflammation of the middle ear and mastoid occurs and in which a nonintact tympanic membrane (caused by perforation or tympanostomy tube) and otorrhea are present. Mastoiditis usually is present, and a cholesteatoma may have formed.[27]

Cholesteatoma

A cholesteatoma usually is a cystic structure lined by squamous epithelium resting on a fibrous strand. The contents of the cyst are the products of desquamation, keratinization, and pus formation. A cholesteatoma may invade, causing local bone erosion and destruction of the ossicular chain. Aural cholesteatomas can be classified as congenital or acquired.

A congenital cholesteatoma is a congenital rest of epithelial tissue and appears as a white cystlike structure within the middle ear or temporal bone. Acquired cholesteatoma may be secondary to implantation of epithelial tissue or may be a sequela of otitis media or a retraction pocket, or both. Implantation cholesteatoma may develop either from epithelium that has migrated through a perforation of the tympanic membrane or from intra-aural epithelium remaining after middle ear or mastoid surgery. A recent histologic study concluded that cholesteatoma can result from stimulated migration of squamous epithelium from the tympanic membrane.[112]

The prevalence of cholesteatoma is currently low. A large longitudinal cohort study of approximately 7000, whose tympanic membranes were periodically recorded by video-otoscopy, observed from birth to age 10, revealed only 15 overt or suspected cases of cholesteatoma.[140]

Infection caused by such organisms as *S. aureus*, *P. aeruginosa*, *Proteus* spp., nonhemolytic streptococci, and *Aspergillus* spp. may be present. The process of alternating infection and healing causes the advancement of squamous epithelium into the middle ear and antrum.[70,127,187] The persistent infection also stimulates proliferation of the mucoperiosteum of the attic region, creating an accelerated tissue growth (increased production of collagenase) and a destructive and expansive process. It is characterized by a foul smell, pus, squames, and bone destruction.

Management of cholesteatoma is surgical removal of the entire cyst. Antimicrobial therapy may be necessary if secondary infection is present.

Adhesive Otitis Media

Adhesive otitis media is a result of healing after chronic inflammation of the middle ear and mastoid. Fibrous tissue proliferates in the mucosal lining and may impair movement of the ossicles and result in conductive hearing loss. Adhesive changes may bind the eardrum to the ossicles and surrounding middle ear structures and cause resorption of the ossicles.

Tympanosclerosis

Tympanosclerosis, or scarring of the tympanic membrane, may be a sequela of chronic middle ear inflammation or trauma. White plaques are present in the tympanic membrane, with nodular deposits in the submucosal layers. The histopathology is marked by hyaline degeneration resulting from a healing reaction characterized by fibroblastic invasion of the submucosa followed by thickening and fusion of the fibers.

Ossicular Discontinuity and Fixation

Ossicular chain abnormalities, including osteitis, may be secondary to chronic inflammation in the middle ear or the presence of a retraction pocket or cholesteatoma. The long process of the incus most commonly is involved. Erosion of the blood supply, cholesteatoma, or adhesive otitis media may be the cause of the bone erosion and disarticulation. Conductive hearing loss is present to a varying degree. Diagnosis may be assisted by computed tomography or magnetic resonance imaging.

Mastoiditis

Reports from Denmark,[171] Italy,[133] and the United States[111] indicate that mastoiditis continues to be an important complication of AOM in developed countries, although at a low rate. In developing countries, untreated otitis media may lead to persistent perforation of the tympanic membrane, dysarticulation of ossicles, and mastoiditis. Berman[14] estimated that mastoiditis occurred as a complication of otitis media in developing countries in 0.74 percent of cases of otitis, based on community and school surveys, and in 1.7 to 18 percent, based on hospital clinical record reviews. In a survey of eight children's hospitals in the United States between 1993 and 1998 for pneumococcal diseases, Kaplan and colleagues[111] identified 34 children with pneumococcal mastoiditis; serogroup 19 accounted for 57 percent of the isolates. The cases occurred primarily in children younger than 2 years of age. At Texas Children's Hospital, the number of cases of pneumococcal mastoiditis increased after introduction of PCV7 due in large part to disease

cases by the nonvaccine serotype 19A (which is now included in PCV13).[156]

At birth, the mastoid consists of a single cell, the antrum, connected to the middle ear by a small channel, the aditus ad antrum. Pneumatization of the mastoid bone occurs soon after birth and usually is extensive by the time the child reaches 2 years of age. Likely, whenever AOM occurs, some degree of mastoiditis is present. With healing of the middle ear infection, healing of the mastoid also occurs. In a few cases, mastoid disease progresses with hyperemia and edema of the mucosal lining of the pneumatized cells, accumulation of serous and then purulent exudates in the cells, demineralization of the cellular walls and necrosis of bone, and formation of abscess cavities caused by coalescence of adjacent cells after destruction of the cell walls. Pus may escape into contiguous areas, including the posterior cranial fossa, middle cranial fossa, sigmoid and lateral sinuses, canal of the facial nerve, semicircular canals, and petrous tip of the temporal bone.

Signs of acute mastoiditis with periostitis include fever, otalgia, postauricular erythema, tenderness, and slight swelling. The pinna may be displaced inferiorly and anteriorly.

Initial management of acute mastoiditis includes administration of parenteral antibiotics and myringotomy to provide drainage of the middle ear and mastoid contents. Surgical drainage of the mastoid should be performed if the symptoms of the acute infection, including fever and otalgia, persist. If the infection progresses, causing destruction of the bony trabeculae, and a mastoid empyema results, mastoidectomy should be performed to prevent spread of the infection to adjacent structures.

Petrositis

Petrositis occurs when suppurative infection extends from the middle ear and mastoid into the petrous portion of the temporal bone. Signs of petrositis include pain behind the eye, deep ear pain, persistent ear discharge, and sixth nerve palsy.[28] The triad of pain behind the eye, aural discharge, and sixth nerve palsy is termed *Gradenigo syndrome*. Management is similar to that described earlier for mastoiditis.

Labyrinthitis

Spread of AOM into the cochlear and vestibular apparatus through the round (less commonly, the oval) window results in inflammation of the labyrinth. The signs of labyrinthitis include sudden, progressive, or fluctuating sensorineural hearing loss or vertigo in association with otitis media or mastoiditis. Signs of suppurative labyrinthitis (in the absence of meningitis) warrant performing aggressive otologic surgery and administering parenteral antimicrobial therapy.[31]

Meningitis

Meningitis may be associated with middle ear infections in three circumstances:

1. *Direct invasion:* A suppurative focus in the middle ear or mastoid spreads through the dura, extends to the pia-arachnoid, and causes generalized meningitis.
2. *Inflammation in an adjacent area:* The meninges may become inflamed if there is suppuration in an adjacent area, such as the mastoid air cells.
3. *Concurrent infection:* Otitis media arises by spread of bacteria from the upper respiratory tract, and meningitis concurrently invades the blood from the upper respiratory focus.

Children with cochlear implants are at risk for development of meningitis. Management of children who are to receive cochlear implants is reviewed in a recent policy statement by the American Academy of Pediatrics: children should receive age-appropriate doses of PCV13 and *H. influenzae* type b conjugate vaccine and annual influenza virus vaccines. Imaging of the temporal bone and inner ear should be performed before cochlear implantation to identify inner ear malformations or cerebrospinal fluid fistulas or ossification of the cochlea that might increase risk of bacterial meningitis after surgery.[186] Although the implant has been implicated in the subsequent development of meningitis, some children likely had an underlying congenital inner or middle ear malformation that provided a pathway for the bacterium to enter the cerebrospinal fluid.[29]

Facial Paralysis

Facial paralysis may occur as a sequela of AOM because of exposure of the facial nerve in the middle ear cleft caused by a bony dehiscence. The palsy usually is unilateral. The paralysis usually resolves with medical therapy for AOM, but if paralysis of the facial nerve persists, decompression may be necessary.

Other Suppurative Complications

The middle ear and mastoid air cells are adjacent to the dura of the posterior and middle cranial fossa, the sigmoid venous sinus of the brain, and the inner ear (Fig. 16-8). Suppuration in the middle ear or mastoid may spread to these structures, producing suppurative complications, such as meningitis, extradural abscess, subdural empyema, focal encephalitis, brain abscess, and lateral sinus thrombosis. Intracranial complications should be suspected when a child with acute or chronic otitis media develops persistent and severe headache, severe otalgia, and change in affect or level of responsiveness. Conversely, children with diagnosed intracranial infection, such as meningitis, should have middle ear or mastoid disease assessed as the origin of the central nervous system disease.

Intracranial extension of infection from the middle ear into the intracranial area may occur because of any of the following:

1. Progressive thrombophlebitis, permitting infection to spread through the intact bone
2. Erosion of the bony walls of the middle ear or mastoid

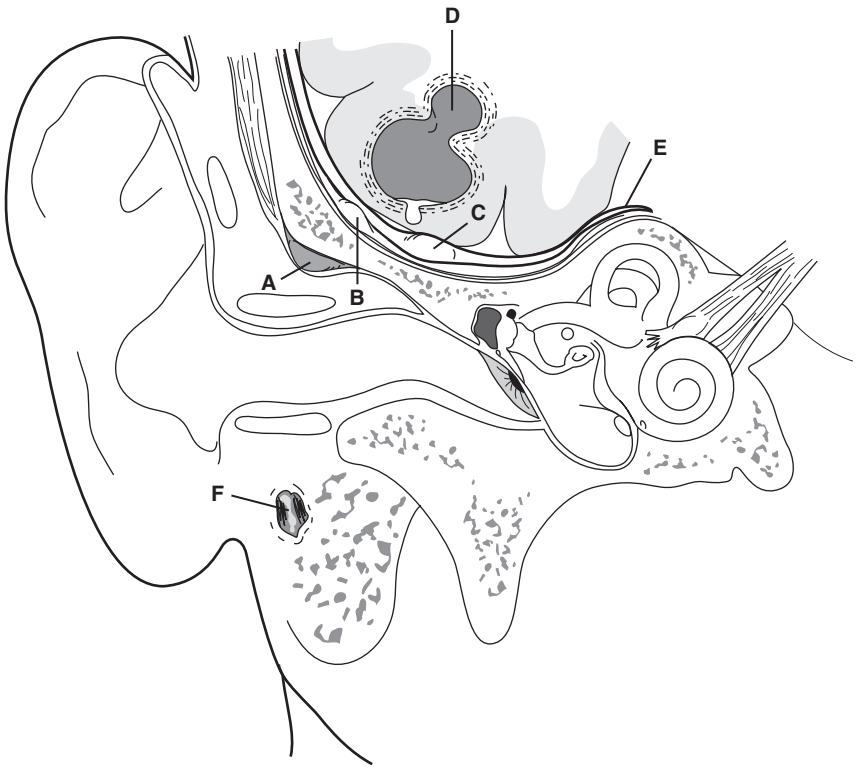

FIGURE 16-8 ■ Suppurative complications of otitis media and mastoiditis: A, subperiosteal abscess; B, extradural abscess; C, subdural empyema; D, brain abscess; E, meningitis; and F, lateral sinus thrombosis. (From Bluestone CD, Klein JO. Otitis media in infants and children. 4th ed. Hamilton, Ontario: BC Decker; 2007, p. 434.)

3. Extension along preformed pathways, such as the round window, dehiscent sutures, skull fractures, and congenital or surgically acquired bony dehiscences.

The microbiology, pathogenesis, diagnosis, and management of intracranial complications of otitis media and mastoiditis are discussed extensively elsewhere (see Chapters 17, 31, 32, and 36).

MANAGEMENT OF ACUTE OTITIS MEDIA

Management of AOM focuses on the choice of an appropriate antimicrobial agent or an option to observe initially rather than treat. Decongestants and antihistamines may provide some comfort for a patient who has congestion of the upper respiratory tract, but they provide no benefit in terms of earlier resolution of the middle ear infection. The antimicrobial agent should have a spectrum of activity that includes *S. pneumoniae* and *H. influenzae* and have documented clinical and microbiologic efficacy, limited side effects, availability in a convenient dosage schedule, palatability when provided in suspension, and reasonable cost. When treated with appropriate antimicrobial therapy, a patient should have substantial resolution of signs and symptoms within 72 hours and absence of signs of relapse, recurrence, or suppurative sequelae.

Table 16-4 includes the daily dosage schedules of 15 oral or parenteral antimicrobial agents approved for the indication of therapy of AOM. In addition, ofloxacin otic

TABLE 16-4 **Daily Dosage Schedule for Antimicrobial Agents Useful in Acute Otitis Media**

Agent	24-Hr Dosage
Amoxicillin	40-80 mg/kg in 2-3 doses
Amoxicillin-clavulanate	40-80 mg/kg in 2 doses (90 mg/kg in 2 doses for Augmentin ES-600)
Cefprozil	30 mg/kg in 2 doses
Cefpodoxime	10 mg/kg in 2 doses
Cefaclor	40 mg/kg in 2-3 doses
Cefixime	8 mg/kg in 1 dose
Cefuroxime axetil	30 mg/kg in 2 doses
Loracarbef	30 mg/kg in 2 doses
Ceftriaxone	50 mg/kg in 1 dose* (1-3 days)
Ceftibuten	9 mg/kg in 1 dose
Cefdinir	14 mg/kg in 1-2 doses
Erythromycin-sulfisoxazole	50 mg/kg erythromycin, 150 mg/kg sulfisoxazole in 4 doses
Clarithromycin	15 mg in 2 doses
Azithromycin	30 mg/kg in 1 dose (1 day)
	10 mg/kg in 1 dose (3 days)
	10 mg/kg in 1 dose (day 1); 5 mg/kg in 1 dose (days 2-5)
Trimethoprim-sulfamethoxazole	8 mg trimethoprim, 40 mg sulfamethoxazole in 2 doses

*Intramuscular route.

and ciprofloxacin (combined with dexamethasone) otic have been approved by the U.S. Food and Drug Administration (FDA) for treatment of AOM in children with tympanostomy tubes in place.

Clinical Implications of Antibiotic Resistance

Increased resistance of bacterial pathogens to available antimicrobial agents has been a constant concern since the introduction of antimicrobial agents. Multidrug-resistant pneumococci add complexity to the choice of optimal antimicrobial agents for AOM. At present, the incidence of strains of pneumococci that are nonsusceptible to penicillin averages approximately 25 percent in the United States but is more than twice that in southeastern and southwestern states. Reasons for the regional differences are unknown. The major risk factors for presence of multidrug-resistant pneumococci are recent (within 30 days) administration of an antimicrobial agent and attendance in daycare. In addition, the increasing proportion of β-lactamase–producing strains of *H. influenzae* and *M. catarrhalis*, now 30 to 75 percent in the United States, warrants consideration in choice of optimal agents.

Diffusion of Antimicrobial Agents into Middle Ear Fluids

Most antimicrobial agents of value for treatment of AOM achieve significant concentrations in middle ear fluid. The concentrations are generally parallel to, although lower than, concentrations of drug in serum. Purulent fluids have higher concentrations of drug than do mucoid or serous fluids. Penicillins and cephalosporins achieve concentrations in middle ear fluids that are approximately one fifth to one third the levels present in serum. Sulfonamides and erythromycin achieved middle ear concentrations that were approximately 50 percent of serum concentrations. An extensive review of concentrations achieved in middle ear fluids is provided in the textbook *Pediatric Otolaryngology*, edited by Bluestone, Stool, and Kenna.[34]

Sterilization of Middle Ear Fluids by Antimicrobial Agents

To define the ability of antimicrobial agents to eradicate bacterial pathogens from middle ear fluids of children with AOM, investigators have used serial aspirates of the infected fluids.[60,97,98,138,143] The initial aspirate identifies the bacterial pathogen of the acute middle ear infection; the second aspirate, obtained days after initiation of therapy, defines the ability of the drug to eradicate the infection. The results of these tests generally are consistent with data available from in vitro assays of the drugs against the major bacterial pathogens and the concentrations of drug achieved in the middle ear fluids.[120] Penicillin-susceptible pneumococcal infections were sterilized by most penicillins, cephalosporins, and macrolides; failure rates of 10 percent or more were identified only in infections treated with cefaclor, cefixime, and cefpodoxime; sulfonamides alone were ineffective, but trimethoprim-sulfamethoxazole was effective.

Dagan[60] reviewed sterilization of middle ear fluids by antimicrobial agents for pneumococci that were penicillin susceptible and penicillin nonsusceptible. The sterilization of middle ear fluids infected with susceptible strains was consistent with prior data. Nonsusceptible strains were less readily eradicated from the middle ear fluid, and the failure rates were twofold or more than those for penicillin-susceptible strains. The failure rates for penicillin-nonsusceptible versus penicillin-susceptible strains were 20 percent versus 10 percent for amoxicillin, 53 percent versus 0 percent for ceftriaxone administered as one dose, 9 percent versus 0 percent for ceftriaxone administered as three consecutive daily doses, 62 percent versus 10 percent for cefaclor, 92 percent versus 5 percent for azithromycin, and 79 percent versus 0 percent for trimethoprim-sulfamethoxazole.

Sterilization of middle ear fluids infected with *H. influenzae* was influenced by the strain differences—β-lactamase–positive or β-lactamase–negative for β-lactam drugs. Failure rates for amoxicillin were 21 percent when the strain was β-lactamase negative but were similar to placebo (60%) when the strain produced β-lactamase. Ceftriaxone eradicated all isolates of *H. influenzae* regardless of whether they produced β-lactamase. Cefuroxime axetil was more effective (15% failures) compared with cefaclor (40% failures). Failures for azithromycin were comparable to placebo (57% vs. 52%). Although similar data based on dual ear aspirates are unavailable for middle ear infections caused by *M. catarrhalis*, the high proportion of β-lactamase–producing strains suggests microbiologic results similar to those of *H. influenzae*.

Sterilization of Middle Ear Fluids Without Antibacterial Agents

Using the same technique of dual aspirates to identify the microbiologic efficacy of antibacterial drugs, Howie[96] identified sterilization of infected middle ear fluids without drugs. A placebo replaced active therapy in the dual aspirate study: 2 to 7 days after the initial aspirate identified the presence of pneumococci or *H. influenzae*, 19 percent of the pneumococci and 48 percent of the *Haemophilus* strains no longer were present. The differential clearing of the bacteria with persistence of most pneumococci but resolution of one half of the infections caused by nontypeable *H. influenzae* likely is associated with some immune or bacteriostatic factor in the middle ear inflammatory exudate that acts to inhibit growth of these organisms. These data of spontaneous resolution need to be considered in evaluating the efficacy of new and old antibacterial drugs.

Initial Observation Rather Than Antimicrobial Agent Therapy

Before the introduction of sulfonamides in 1936, management of AOM included watchful waiting or, when the suppurative process produced severe clinical signs, use of myringotomy to drain the middle ear abscess. Spread of infection to the mastoid, meninges, or other intracranial

foci was a feared complication of otitis media. Early therapeutic trials identified the value of using antimicrobial agents for resolution of clinical signs and decreased incidence of suppurative complications. Most children with AOM respond clinically without use of antimicrobial agents. Children who improve without antimicrobial drugs include one third with AOM who have a bacteriologically sterile effusion and are presumed to have a viral infection and children who have bacterial infections that clear without antimicrobial agents (19% of pneumococcal infections, 48% of infections caused by nontypeable strains of *H. influenzae*, and approximately 75% of AOM caused by *M. catarrhalis*).[120] Because of the increased incidence of bacterial pathogens resistant to available antimicrobial agents, and the data associating extensive use of the drugs with development of resistant strains, limiting the use of antimicrobial agents for children with AOM has been suggested. The option of observation and symptomatic treatment rather than initial antibiotic treatment has been advocated in Western Europe based on studies by van Buchem and colleagues.[224,225] A meta-analysis of children who were observed rather than treated with antibiotics found that risk features for a prolonged illness included children 2 years of age and younger and those with bilateral AOM.[185] In the United States, interest in this management increased with concerns for multidrug resistance resulting from the extensive use of antimicrobial agents, but the recommendations of the CDC and the AAP in 1998 focused on increasing the accuracy of diagnosis, avoiding use of antimicrobial agents for otitis media with effusion and continuing use of these drugs for treatment of AOM.[64]

The AAP and the AAFP in 2004 altered their positions and for the first time provided a guideline for an observation option for selected patients.[5] The guideline recommended that observation without use of antimicrobial agents could be considered in a child older than 24 months of age with uncomplicated AOM with nonsevere signs (mild otalgia, temperature <39° C [102.2 °F] orally in the past 24 hours) when follow-up was ensured, and antibacterial agents could be started if symptoms persisted or worsened. The options considered in the guidelines are listed in Table 16-5. The AAP guidelines may have resulted in reduction of AOM by establishing precise criteria for diagnosis, but a recent study indicates that there is no evidence that the pattern of prescribing antibiotics for AOM has been altered.[55] Meropol[144] provided decision analysis to survey the observation option for management of AOM and concluded the parent demand for antibiotics may be rational when driven by the value of parent time. Because of the limitations of the clinical trials that supported the strategy of watchful waiting, investigators in Pittsburgh, Pennsylvania, and Turku, Finland, each performed a randomized blinded trial of amoxicillin-clavulanate compared with placebo in infants younger than 2 and 3 years, respectively. The results of each study showed a significant benefit among children who received the antibiotic with respect to duration of acute signs of illness.[95,212] A recent study from Sweden evaluated children who presented with a spontaneously draining ear due to acute otitis media and concluded prompt antibiotic treatment is indicated.[151] In another recent study from Sweden, there was no apparent increase in the number of cases of mastoiditis in children between 1993 and 2007 following new guidelines that recommended selective administration of antibiotics for acute otitis media, although there was an increase in infants after 2000 despite the recommended treatment with antibiotics for all patients in this age group.[85]

TABLE 16-5 Guidelines for Management of Acute Otitis Media from the American Academy of Pediatrics and American Academy of Family Physicians

Age (mo)	Certain Diagnosis*	Uncertain Diagnosis
<6	Antibiotics	Antibiotics
6-24	Antibiotics	Antibiotics if severe; observe if not severe†
>24	Antibiotics if severe; observe if not severe†	Observe

*Certain diagnosis = middle ear effusion, rapid onset, symptoms of middle ear inflammation.
†Not severe = mild otalgia; temperature <39°C (102.2 °F) orally or <39.5°C (103.1 °F) rectally in past 24 hr.
From American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.

Choice of Antimicrobial Agents

Oral amoxicillin remains the first-line antimicrobial agent for treating AOM because of the expected low failure rates. The Drug-resistant *Streptococcus pneumoniae* Therapeutic Working Group of the CDC[63] and the 2004 guidelines of the AAP and the AAFP[5] suggested that an increase in dosage used of amoxicillin for empirical treatment from 40 to 45 mg/kg/day to 80 to 90 mg/kg/day would be effective for more nonsusceptible strains of *S. pneumoniae*. The 2004 guidelines for initial treatment and treatment failures are listed in Table 16-6.

The guidelines differentiate treatment choice of drug by severity of signs. Children with nonsevere disease may be treated initially with high-dose amoxicillin, but children with severe signs should be treated with amoxicillin-clavulanate. The use of high-dose amoxicillin-clavulanate responds to concern about failure of amoxicillin in cases of AOM caused by β-lactamase–producing *H. influenzae*. If failure occurred because of a high-level resistant strain of *S. pneumoniae*, however, the same dosage of amoxicillin in the combination would not be advantageous. Cefuroxime axetil, cefpodoxime, and cefdinir have equivalent profiles in vitro against *S. pneumoniae* and *H. influenzae*. Because of bitter taste, cefuroxime and cefpodoxime are not well accepted by young patients, and the better taste of cefdinir increased acceptability and compliance. Intramuscular ceftriaxone was most effective in eradicating nonsusceptible pneumococci when provided in three daily doses, but an alternative regimen is to provide a

TABLE 16-6 **Recommended Antibacterial Agents for Patients Who Are Being Treated Initially with Antibacterial Agents or Who Have Failed Initial Management with Antibacterial Agents**

*At Diagnosis for Patients Being Treated Initially with Antibacterial Agents**			*Clinically Defined Treatment Failure at 48-72 Hr After Initial Management with Antibacterial Agents*	
Temperature ≥39° C (102.2 °F) and/or Severe Otalgia	**Recommended**	**Alternative for Penicillin Allergy**	**Recommended**	**Alternative for Penicillin Allergy**
No	Amoxicillin 80-90 mg/kg/day	Non–type I: cefdinir, cefuroxime, cefpodoxime Type I: azithromycin, clarithromycin	Amoxicillin-clavulanate (90 mg/kg/day of amoxicillin component, with 6.4 mg/kg/day of clavulanate)	Non–type I: ceftriaxone—3 days Type I: clindamycin
Yes	Amoxicillin-clavulanate (90 mg/kg/day of amoxicillin with 6.4 mg/kg/day of clavulanate)	Ceftriaxone—1 or 3 days	Ceftriaxone—3 days	Tympanocentesis, clindamycin

*Choices are the same for patients who were initially managed with observation but require therapy at 48 to 72 hours.
From American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.

single dose and, if signs do not resolve at 48 hours, to proceed to a second or third dose.[118]

Dosage Schedules

Dosage schedules of the antimicrobial agents of value for therapy of AOM have been determined on the basis of studies of the pharmacokinetics and results of clinical trials (see Table 16-4).

Duration of Therapy

Duration of therapy is based on clinical trials and tradition. Most clinical trials and standard pediatric practice include a 10-day course of an antimicrobial agent. The FDA approved a 5-day schedule of once-a-day azithromycin administered orally based on studies comparing the clinical efficacy of 5-day azithromycin with 10-day amoxicillin-clavulanate courses. These data suggest that short courses of therapy may be appropriate for many children with AOM, although some children (likely children with severe and recurrent disease) would require more prolonged schedules.

Clinical Course After Initiation of Therapy

The clinical course of a child who receives appropriate antimicrobial therapy includes significant resolution of acute signs within 48 to 72 hours. Instructions to the parent should indicate the need to contact the physician if the signs or symptoms worsen at any time or are unimproved at 72 hours. Persistent ear pain or systemic signs, such as fever, signal the need for reevaluation to examine for other foci of infections, to determine the need for another antimicrobial agent, or to perform tympanocentesis or myringotomy to incise and drain the middle ear abscess and culture the fluid to identify the pathogen. If a new antibiotic is needed, one with β-lactamase stability and activity against penicillin-resistant pneumococci (if such information is available from local surveillance studies) should be chosen.

Follow-up visits should be made to determine that the child has recovered from the acute infection and to diagnose persistent middle ear effusion if it is present. The utility of the traditional 10- to 14-day visit was reassessed by Hathaway and coworkers[89] and Mandel and colleagues[135]; these investigators concluded that the follow-up visit can be extended to 4 to 6 weeks after onset of treatment in children whose parents thought the disease had resolved at 10 to 14 days. For children who still had signs or symptoms of disease (other than persistent middle ear effusion), the 10- to 14-day visit was recommended. Visits at 4 to 6 weeks and repeated at 1-month intervals if effusion is present are valuable in determining the duration of middle ear effusion after the acute episode and identifying children who may be candidates for placement of tympanostomy tubes.

Symptomatic Therapy

Administration of antipyretics and analgesics and application of local heat usually are helpful in treating a child with an acute painful and febrile episode. Acetaminophen or ibuprofen is recommended for children with mild to moderate pain. Topical agents such as benzocaine may provide brief benefit in patients older than 5 years of age. Narcotic analgesia with codeine or analogues should be reserved for patients with moderate or severe pain. An oral decongestant, such as pseudoephedrine hydrochloride, may relieve nasal congestion, and antihistamines may help patients with known or suspected nasal allergy. The efficacy of antihistamines and decongestants for resolution of middle ear effusion is unproven.

Management of Acute Otitis Media in a Child with Tympanostomy Tubes

Children with tympanostomy tubes also experience AOM. Because the tube permits drainage of the middle ear fluid and an abscess does not develop, the major pathologic process is inflammation of the mucous lining of the middle ear and the dominant clinical sign is otorrhea. The bacterial pathogens include those responsible for AOM (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*) and bacteria that may invade the middle ear from the external auditory canal (*S. aureus* and *P. aeruginosa*). Although amoxicillin or amoxicillin-clavulanate is the usual choice for treatment of AOM in a child with tympanostomy tubes, ofloxacin otic and ciprofloxacin-dexamethasone otic were effective for children who presented with acute otorrhea. The efficacy of the otic suspensions for AOM in children with tympanostomy tubes indicates that high concentrations of drug reach the middle ear mucosa and result in eradication of bacterial pathogens. Additional advantages of otic versus systemic antimicrobial administration include lesser systemic adverse events (e.g., diarrhea) and absence of effect on the upper respiratory flora and absence of selection of resistant bacteria.[82,184,230]

Management of Otitis Media with Effusion

The following options have been investigated for management of a child with prolonged middle ear fluid or with otitis media with effusion:

1. Another 10-day course of a broad-spectrum antimicrobial agent that has activity against β-lactamase–producing organisms has been recommended because bacterial pathogens are found in approximately one fourth of patients with otitis media with effusion; a meta-analysis of blinded studies identified resolution of effusion in 14 percent of cases.[210]
2. Myringotomy or myringotomy and tympanostomy tubes drain the middle ear fluid, aerate the middle ear space, and permit the middle ear mucosa to return to normal.[137]
3. Adenoidectomy with or without tonsillectomy for children who have recurrences after an initial placement of tympanostomy tubes is controversial.[78] A recent clinical trial from Pittsburgh did not find a significant advantage of the addition of an adenoidectomy with tympanostomy tube placement versus tympanostomy tube insertion alone in 2- and 3-year-old children who had chronic otitis media with effusion.[50] A Finnish study concluded that adenoidectomy did not reduce the incidence of AOM in otitis-prone children younger than 4 years of age who received tympanostomy tubes.[87]
4. Corticosteroid therapy alone or with an antibiotic has been shown to be effective in some children with otitis media with effusion. Berman[16] recommended a regimen of prednisone, 1 mg/kg/day (given orally in two doses) for 7 days, with an antibiotic for 14 to 21 days. Mandel and coworkers[136] failed to find a significant difference for systemic corticosteroid therapy in children with chronic otitis media with effusion. Children without a history of varicella infection who have been exposed to the virus in the month before receiving treatment should not be given prednisone because of the risk for developing disseminated disease. The guidelines published by the Agency for Health Care Policy and Research concluded that the data were insufficient in sample size or duration of observation to recommend use of corticosteroids for otitis media with effusion.[210]

Topical or systemic nasal decongestants, antihistamines, and anti-inflammatory agents,[2,227] alone or in combination, have been found to be of limited or no value in the management of otitis media with effusion.

We favor an initial course of a broad-spectrum antibiotic for children with otitis media with effusion for 3 or more months. If the effusion does not resolve, use of tympanostomy tubes is the most efficient method for managing prolonged middle ear effusion. The use of tympanostomy tubes first was suggested more than 100 years ago by Politzer, but the procedure did not become readily available until it was reintroduced by Armstrong in 1954. Myringotomy and placement of ventilating tubes result in the following immediate benefits to the patient with otitis media with effusion:

- The effusion is drained, and the fluid-filled space is aerated.
- The middle ear secretions, which constantly are being formed by the secretory cells of the mucosa, are drained.
- The chronically diseased mucosa (characterized by hypertrophic secretory cells) returns to normal.
- The hearing impairment caused by the middle ear fluid disappears.
- Concern for effects of hearing loss on development of speech and cognitive abilities is diminished.
- The child who was not responsive or attentive (a condition often unrecognized as hearing impairment by the parent) becomes more social and more involved with siblings, parents, and playmates.

The procedure may be disadvantageous for the following reasons:

- General anesthesia is required.
- The cost is significant.
- Although uncommon, sequelae, such as persistent otorrhea, permanent perforation, scarring of the membrane, and cholesteatoma, may occur.

In addition, developmental outcomes may not be improved after early rather than delayed insertion of tympanostomy tubes.[162]

Current recommendations for pediatric otitis media with effusion include follow-up visits for 1 to 2 months after the acute episode. When the effusion persists for 3 or more months, the child should receive medical treatment with a course of antibiotics (2 to 3 weeks) or in conjunction with a 7-day regimen of prednisone. If the effusion fails to resolve with medical management, the child should be referred to an otolaryngologist for consideration of placement of tympanostomy tubes with or without adenoidectomy.[137,161]

PREVENTION

Advising Parents

Parents of children who have severe and recurrent otitis media or risk factors for developing middle ear infections should be advised of measures that may reduce the incidence of infection, such as breast-feeding; enrolling children in small, rather than large, group daycare centers; and reducing exposure to tobacco smoke. In addition, data about the risks for developing recurrent otitis media associated with the prone sleeping position and use of a pacifier, although requiring corroboration, may be added to the discussion with the parent. Physicians also may advise parents that the seasonal incidence of otitis media suggests that their child's condition is expected to improve in late spring and summer and that aggressive measures of management, including chemoprophylaxis and surgery, may be postponed until the course of disease has been determined in the next respiratory season.

Pneumococcal Vaccines

A pneumococcal polysaccharide vaccine was introduced in the United States in the 1970s, but it had limited efficacy in infants 2 years of age and younger and only a modest effect in prevention of AOM. In February 2000, the FDA approved a safe and effective 7-valent conjugate pneumococcal vaccine (PCV7) that was immunogenic in infants beginning at 2 months of age. PCV7 was been effective in decreasing the incidence of invasive disease and, to a lesser extent, the incidence of AOM. The results of two large PCV7 clinical trials have been published: (1) an evaluation of the efficacy of the vaccine for prevention of invasive bacterial disease, pneumonia, and otitis media in approximately 38,000 Northern California infants[23,73] and (2) a clinical and microbiologic study of the efficacy of PCV7 for prevention of AOM in 1662 Finnish infants.[67] A randomized controlled trial of efficacy of PCV7 against AOM was conducted among Navajo and White Mountain Apache infants. These infants are known to have high rates of acute and severe AOM. Although the vaccine failed to demonstrate a benefit preventing AOM there was a modest benefit (5.1% efficacy) against severe episodes and a decrease in placement of tympanostomy tubes.[154] A clinical trial of an 11-valent polysaccharide vaccine conjugated with an outer membrane protein of nontypeable *H. influenzae* was found to be effective in infants in the Czech Republic and Slovakia against AOM caused by *S. pneumoniae* and *H. influenzae*.[178] PCV7 was replaced by PCV13 in the spring of 2010, and the 13-valent vaccine is now the only pneumococcal conjugate vaccine available in the United States. The serotypes included in the various pneumococcal vaccines are listed in Table 16-7.

In the Northern California study, the efficacy of PCV7 for prevention of episodes of AOM was based on clinical criteria. The vaccine was 7.8 percent effective in preventing episodes of AOM, antibiotic prescriptions decreased by 5.8 percent, and 24.9 percent fewer surgical procedures (most were placement of ventilating tubes) were performed in children immunized with PCV7 compared with children who received the control vaccine (conjugate polysaccharide meningococcal group C).

TABLE 16-7 **Serotypes in Pneumococcal Vaccines—2012**

		Polysaccharide Conjugates		
Serotype	Polysaccharide 23 Type (Merck)	7 Type (Pfizer)	13 Type (Pfizer)	10 Type* (GSK)
1	+		+	+
2	+			
3	+		+	
4	+	+	+	+
5	+		+	+
6A	–		+	
6B	+	+	+	+
7F	+			+
7V	–		+	
8	+			
9N	+			
9V	+	+	+	+
10A	+			
11A	+			
12F	+			
14	+	+	+	+
15B	+			
17F	+			
18C	+	+	+	+
19A	+		+	
19F	+	+	+	+
20	+			
22F	+			
23F	+	+	+	+
33F	+			

*Not approved by the FDA for use in the United States as of February 2013.

The Finnish trial provided microbiologic data based on aspirates of middle ear fluids in children who had AOM. Children who received PCV7 had 6 percent fewer episodes of AOM; culture-confirmed pneumococcal episodes were reduced by 34 percent. Children who received PCV7 experienced a 57 percent reduction in episodes caused by pneumococcal serotypes included in the vaccine and a 51 percent reduction in episodes caused by cross-reactive pneumococcal serotypes. A 33 percent increase in episodes of AOM caused by nonvaccine pneumococcal serotypes and an 11 percent increase in episodes caused by *H. influenzae* occurred (Table 16-8).

The 11-valent pneumococcal polysaccharide vaccine conjugated with an outer membrane protein of nontypeable *H. influenzae* was found to be effective against AOM caused by *S. pneumoniae* and *H. influenzae*.[178] The vaccine prevented 52 percent of episodes of AOM caused by pneumococcal vaccine serotypes and 35.3 percent of episodes caused by nontypeable *H. influenzae*. The vaccine is now available in Europe, Canada, South America, and other regions but not in the United States as a 10-valent

TABLE 16-8 **Efficacy of PCV7 in Finnish Children with Acute Otitis Media (AOM)**

End Point	AOM Episodes: PCV7 (N = 831)	AOM Episodes: Control (N = 831)*	Vaccine Efficacy Point Estimate
Any AOM	1251	1345	6
Pneumococcal AOM	271	414	34
Vaccine serotypes	107	250	57
Cross-reactive serotypes	41	84	51
Nonvaccine serotypes	125	95	–33
Haemophilus influenzae AOM	315	287	–11
Moraxella catarrhalis AOM	379	381	–1

*Hepatitis B vaccine.
Adapted from data published in Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 2001;**344**:403–9.

conjugate vaccine (Synflorix, GSK); the type 3 antigen was eliminated because of lack of efficacy in the clinical trial.

The 23-type polysaccharide vaccine currently available produces an independent antibody response in children 2 years of age or older and in adults. Studies of polysaccharide vaccines in Finland and the United States have indicated that the vaccines were effective in preventing type-specific pneumococcal otitis media if an adequate immune response occurred, but the number of types producing an adequate response in children younger than 2 years was limited.[134,208,213] Administration of the polysaccharide vaccine in older children may provide protection against types not included in PCV13 and has been recommended by the AAP and the Advisory Committee on Immunization Practices of the Surgeon General for high-risk children ages 2 years and older who have received PCV13. For children 2 years old and older who continue to have severe and recurrent AOM, use of the polysaccharide vaccine after administration of PCV13 may be valuable for protection against additional pneumococcal serotypes.

Influenza Virus Vaccines

Trivalent inactivated influenza virus vaccines have resulted in a reduction in cases of influenza-associated AOM in children attending daycare centers.[54,157] A similar reduction in episodes of influenza-associated AOM was reported in children after administration of a live, attenuated cold-adapted intranasal vaccine.[13,26] Annual administration of influenza virus vaccines should be part of the strategy for reducing the incidence of AOM for children with recurrent and severe disease.

Chemoprophylaxis

Use of chemoprophylaxis has succeeded in reducing the number of new symptomatic episodes of AOM in children who have a history of recurrent infections. Children at risk for developing severe and recurrent disease should be considered for chemoprophylaxis despite the concern for development of resistant strains of bacterial pathogens after use of the regimen of a modified dosage of an antimicrobial agent. Results of controlled clinical trials of modified courses of antimicrobial agents compared with trials in which placebo or historical controls were used have been reviewed.[121] Most of these studies used a sulfonamide or a broad-spectrum penicillin. Most reports indicated benefit to the enrollees in reduction of new episodes when they were compared with controls: amoxicillin efficacy was 44 to 67 percent, and sulfonamide efficacy was 40 to 88 percent (although the efficacy of sulfonamides was reported as only 8% in one study).

We recommend the following protocol based on the results of these studies. Criteria for enrollment should include three documented episodes of AOM in 6 months or four episodes in 12 months. Because children who have episodes of acute infection early in life or have siblings with severe and recurrent ear infections are prone to develop otitis media, prophylaxis also should be considered for children who have one episode in the first 6 months of life plus a family history of ear infections or who have two episodes in the first year of life.

A sulfonamide or amoxicillin is the agent used most often and provides the advantages of demonstrated efficacy, safety, and low cost. The drug is administered at half the therapeutic dose (administered once a day). Amoxicillin is given at a dose of 40 mg/kg, and sulfisoxazole is given at a dose of 50 mg/kg. Chemoprophylaxis should be provided during the fall, winter, and early spring (when respiratory tract infections are most frequent) for 6 months.

Children, when free of signs of acute infection, should be examined at approximately 2-month intervals to determine whether middle ear effusion is present. Management of prolonged middle ear effusion should be considered separately from prevention of recurrences of acute infection.

Acute infections are expected to occur, although at a lower rate, during the course of prophylaxis. The infection should be treated with an alternative regimen. Amoxicillin-clavulanate or intramuscular ceftriaxone would be a suitable alternative, regardless of the prophylactic agent used.

Xylitol is a sugar alcohol found in various fruits, such as raspberries and plums that inhibits growth of *Streptococcus mutans* and *S. pneumoniae*. Studies of a xylitol chewing gum administered five times a day found a reduction in the incidence of AOM,[221,222] but no protection from AOM was provided by a thrice-daily dosage schedule.[90] Although xylitol is a promising mode of prevention of AOM, further data are needed to corroborate the results of the early studies of a five-times-a-day dosage schedule.

Surgical Options

When nonsurgical methods of prevention fail to prevent recurrent otitis media, surgery is a reasonable option. Among the options that have been shown to be effective is insertion of tympanostomy tubes, with or without adenoidectomy. Casselbrant and colleagues[48] randomly assigned children into treatment groups receiving amoxicillin, tympanostomy tube placement, or placebo and found that amoxicillin and insertion of the tympanostomy tube were effective. A recent review of the effect of tympanostomy tubes also concluded that long-term antibiotic treatment or tympanostomy tubes are effective in prevention of recurrent acute otitis media.[130] With the current concern about the association of low-dose, long-term antimicrobial prophylaxis and emergence of resistant otitis pathogens,[63] myringotomy and placement of tympanostomy tubes may be more desirable than antimicrobial prophylaxis. When tympanostomy tubes extrude and the child continues to have recurrent episodes of otitis media, replacement of the tympanostomy tubes in conjunction with adenoidectomy has been shown to be effective.[161] As shown by Paradise and coworkers,[160] neither adenoidectomy nor adenotonsillectomy is effective in children not treated previously with tympanostomy tubes when prevention of otitis media is the only indication.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Barkai G, Belmaker I, Givon-Lavi N, et al. The effect of universal toddlers-only hepatitis A virus vaccination program on seropositivity rate in unvaccinated toddlers: evidence for reduced virus circulation in the community. *Pediatr Infect Dis J* 2009;**28**:391–3.
10. Barkai G, Leibovitz E, Givon-Lavi N, et al. Potential contribution by nontypable *Haemophilus influenzae* in protracted and recurrent acute otitis media. *Pediatr Infect Dis J* 2009;**28**:466–71.
22. Black S. The volatile nature of pneumococcal serotype epidemiology—potential for misinterpretation. *Pediatr Infect Dis J* 2009;**29**:3.
26. Block SL, Heikkinen T, Toback SL, et al. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. *Pediatr Infect Dis J* 2011;**30**:203–7.
35. Bluestone CD, Swarts JD. Human evolutionary history: consequences for the pathogenesis of otitis media. *Otolaryngol Head Neck Surg* 2010;**143**:739–44.
38. Boudewyns A, Declau F, Van den Ende J, et al. Otitis media with effusion: an underestimated cause of hearing loss in infants. *Otol Neurotol* 2011;**32**:799–804.
39. Broides A, Dagan R, Greenberg D, et al. Acute otitis media caused by *Moraxella catarrhalis*: epidemiologic and clinical characteristics. *Clin Infect Dis* 2009;**49**:1641–7.
45. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2010;**29**:304–9.
50. Casselbrant ML, Mandel EM, Rockette HE, et al. Adenoidectomy for otitis media with effusion in 2-3-year-old children. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1718–24.
55. Coco A, Vernacchio L, Horst M, et al. Management of acute otitis media after publication of the 2004 AAP and AAFP clinical practice guideline. *Pediatrics* 2010;**125**:214–20.
56. Cohen MS, Mandel EM, Furman JM, et al. Tympanostomy tube placement and vestibular function in children. *Otolaryngol Head Neck Surg* 2011;**145**:666–72.
59. Couloigner V, Levy C, François M, et al. Pathogens implicated in acute otitis media failures after 7-valent pneumococcal conjugate vaccine implementation in France: distribution, serotypes, and resistance levels. *Pediatr Infect Dis J* 2012;**31**:154–8.
85. Groth A, Enoksson F, Hermansson A, et al. Acute mastoiditis in children in Sweden 1993-2007—no increase after new guidelines. *Int J Pediatr Otorhinolaryngol* 2011;**75**:1496–501.
95. Hoberman A, Paradise JL, Rockette HE, et al. Treatment of acute otitis media in children under 2 years of age. *N Engl J Med* 2011;**364**:105–15.
103. Ilia S, Goulielmos GN, Samonis G, Galanakis E. Host's response in otitis media: understanding genetic susceptibility. *Pediatr Infect Dis J* 2008;**27**:929–33.
105. Jacoby P, Carville KS, Hall G, et al. Crowding and other strong predictors of upper respiratory tract carriage of otitis media-related bacteria in Australian Aboriginal and non-Aboriginal children. *Pediatr Infect Dis J* 2011;**30**:480–5.
108. Kalu SU, Ataya RS, McCormick DP, et al. Clinical spectrum of acute otitis media complicating upper respiratory tract viral infection. *Pediatr Infect Dis J* 2011;**30**:95–9.
112. Karmody CS, Northrop C. The pathogenesis of acquired cholesteatoma of the human middle ear: support for the migration hypothesis. *Otol Neurotol* 2012;**33**:42–7.
113. Kaur R, Adlowitz DG, Casey JR, et al. Simultaneous assay for four bacterial species including *Alloiococcus otitidis* using multiplex-PCR in children with culture negative acute otitis media. *Pediatr Infect Dis J* 2010;**29**:741–5.
124. Le Monnier A, Jamet A, Carbonnelle E, et al. *Fusobacterium necrophorum* middle ear infections in children and related complications: report of 25 cases and literature review. *Pediatr Infect Dis J* 2008;**27**:613–7.
130. Lous J, Ryborg CT, Thomsen JL. A systematic review of the effect of tympanostomy tubes in children with recurrent acute otitis media. *Int J Pediatr Otorhinolaryngol* 2011;**75**:1058–61.
140. Maw AR, Hall AJ, Pothier DD, et al. The prevalence of tympanic membrane and related middle ear pathology in children: a large longitudinal cohort study followed from birth to age ten. *Otol Neurotol* 2011;**32**:1256–61.
144. Meropol SB. Valuing reduced antibiotic use for pediatric acute otitis media. *Pediatrics* 2008;**121**:669–73.
149. Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. *Clin Infect Dis* 2009;**49**:124–31.
151. Neumark T, Ekblom M, Brudin L, et al. Spontaneously draining acute otitis media in children: an observational study of clinical findings, microbiology and clinical course. *Scand J Infect Dis* 2011;**43**:891–8.
154. O'Brien KL, David AB, Chandran A, et al. Randomized, controlled trial efficacy of pneumococcal conjugate vaccine against otitis media among Navajo and White Mountain Apache infants. *Pediatr Infect Dis J* 2008;**27**:71–3.
156. Ongkasuwan J, Valdez TA, Hulten KG, et al. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics* 2008;**122**:34–9.
172. Pettigrew MM, Gent JF, Pyles RB, et al. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011;**49**:3750–5.
183. Rogers DJ, Boseley ME, Adams MT, et al. Prospective comparison of handheld pneumatic otoscopy, binocular microscopy, and tympanometry in identifying middle ear effusions in children. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1140–3.
186. Rubin LG, Papsin B, and the Committee on Infectious Diseases and Section on Otolaryngology–Head and Neck Surgery. Policy Statement—Cochlear implants in children: surgical site infections and prevention and treatment of acute otitis media and meningitis. *Pediatrics* 2010;**126**:11.
212. Tahtinen PA, Laine MK, Huovinen P, et al. A placebo-controlled trial of antimicrobial treatment for acute otitis media. *N Engl J Med* 2011;**364**:116–26.
230. Wall GM, Stroman DW, Roland PS, et al. Ciprofloxacin 0.3%/dexamethasone 0.1% sterile otic suspension for the topical treatment of ear infections: a review of the literature. *Pediatr Infect Dis J* 2009;**28**:141–4.
233. Zhou F, Shefer A, Kong Y, Nuorti JP. Trends in acute otitis media-related health care utilization by privately insured young children in the United States, 1997-2004. *Pediatrics* 2008;**121**:8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abramovich S, O'Grady J, Fuller A, et al. Naproxen in otitis media with effusion. *J Laryngol Otol* 1986;**100**:263–6.
2. Abramson JS, Giebink GS, Mills EL, et al. Polymorphonuclear leukocyte dysfunction during influenza virus infection in chinchillas. *J Infect Dis* 1981;**143**:836–45.
3. Acute otitis media in general practice. Medical Research Council Working Party Report. *Lancet* 1957;**2**:510–4.
4. Alho OP, Koivu M, Sorri M, et al. Risk factors for recurrent acute otitis media and respiratory infection in infancy. *Int J Pediatr Otorhinolaryngol* 1990;**19**:151–61.
5. American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.
6. Austrian R, Howie VM, Ploussard JH. The bacteriology of pneumococcal otitis media. *Johns Hopkins Med J* 1977;**141**: 104–11.
7. Baba S. Recent aspects of clinical bacteriology in otitis media. Presented at Presymposium on Management of Otitis Media. Kyoto; January 12, 1985.
8. Babonis TR, Weir MR, Kelly PC. Impedance tympanometry and acoustic reflectometry at myringotomy. *Pediatrics* 1991;**87**: 475–80.
9. Barkai G, Belmaker I, Givon-Lavi N, et al. The effect of universal toddlers-only hepatitis A virus vaccination program on seropositivity rate in unvaccinated toddlers: evidence for reduced virus circulation in the community. *Pediatr Infect Dis J* 2009;**28**:391–3.
10. Barkai G, Leibovitz E, Givon-Lavi N, et al. Potential contribution by nontypable *Haemophilus influenzae* in protracted and recurrent acute otitis media. *Pediatr Infect Dis J* 2009;**28**:466–71.
11. Barnett ED, Klein JO, Pelton SI, et al. Otitis media in children born to human immunodeficiency virus–infected mothers. *Pediatr Infect Dis J* 1992;**11**:360–4.
12. Bell DM, Gleiber DW, Mercer AA, et al. Illness associated with child day care: a study of incidence and cost. *Am J Public Health* 1989;**79**:479–84.
13. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted trivalent, intranasal influenza virus vaccine in children. *N Engl J Med* 1998;**338**:1459–61.
14. Berman S. Otitis media in children. *N Engl J Med* 1995; **332**:1560–5.
15. Berman S. Otitis media in developing countries. *Pediatrics* 1995;**96**:126–31.
16. Berman S. Management of otitis media and functional outcomes related to language, behavior, and attention: is it time to change our approach? *Pediatrics* 2001;**107**:1175–6.
17. Berman SA, Balkany TJ, Simmons MA. Otitis media in infants less than 12 weeks of age: differing bacteriology among in-patients and out-patients. *J Pediatr* 1978;**93**:453–4.
18. Berman SA, Balkany TJ, Simmons MA. Otitis media in the neonatal intensive care unit. *Pediatrics* 1978;**62**:168.
19. Bernstein JM. Biological mediators of inflammation im middle ear effusions. *Ann Otol Rhinol Laryngol* 1976;**85**:90–6.
20. Biles RW, Buffler PA, O'Donell AA. Epidemiology of otitis media: a community study. *Am J Public Health* 1980;**70**:593–8.
21. Birch L, Elbrond O. Daily impedance audiometric screening of children in a day-care institution: changes through one month. *Scand Audiol* 1985;**14**:5–8.
22. Black S. The volatile nature of pneumococcal serotype epidemiology—potential for misinterpretation. *Pediatr Infect Dis J* 2009;**29**:3.
23. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* 2000;**19**:187–95.
24. Bland RD. Otitis media in the first six weeks of life: diagnosis, bacteriology, and management. *Pediatrics* 1972;**49**:187–97.
25. Block SL, Hedrick J, Harrison CJ, et al. Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. *Pediatr Infect Dis J* 2004;**23**:829–33.
26. Block SL, Heikkinen T, Toback SL, et al. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. *Pediatr Infect Dis J* 2011;**30**:203–7.
27. Bluestone CD. Epidemiology and pathogenesis of chronic suppurative otitis media: implications for prevention and treatment. *Int J Pediatr Otorhinolaryngol* 1998;**42**:207–23.
28. Bluestone CD. Prevention of meningitis: cochlear implants and inner ear abnormalities. *Arch Otolaryngol Head Neck Surg* 2003;**129**:279–81.
29. Bluestone CD. Labyrinthitis. In: Bluestone CD, Alper CM, Casselbrant ML, et al, editors. *Advanced therapy of otitis media*. Hamilton, Ontario: B. C. Decker; 2004.
30. Bluestone CD. Petrositis. In: Bluestone CD, Alper CM, Casselbrant ML, et al, editors. *Advanced therapy of otitis media*. Hamilton, Ontario: B. C. Decker; 2004. p. 362–4.
31. Bluestone CD. *Eustachian tube: structure, function, and role in otitis media*. Hamilton, Ontario: B. C. Decker; 2005.
32. Bluestone CD. Humans are born too soon: impact on pediatric otolaryngology. *Int J Pediatr Otorhinolaryngol* 2005;**69**:8.
33. Bluestone CD, Klein JO. *Otitis media in infants and children*. 4th ed. Hamilton, Ontario: B. C. Decker; 2007.
34. Bluestone CD, Stool SE, Kenna MA. *Pediatric otolaryngology*. 3rd ed. vol. 1. Philadelphia: W. B. Saunders; 2003. p. 603–4.
35. Bluestone CD, Swarts JD. Human evolutionary history: consequences for the pathogenesis of otitis media. *Otolaryngol Head Neck Surg* 2010;**143**:739–44.
36. Bodor FF. Systemic antibiotics for treatment of the conjunctivitis-otitis media syndrome. *Pediatr Infect Dis J* 1989;**8**:287–90.
37. Bodor FF, Marchant CD, Shurin PA, et al. Bacterial etiology of conjunctivitis-otitis media syndrome. *Pediatrics* 1985;**76**:26–8.
38. Boudewyns A, Declau F, Van den Ende J, et al. Otitis media with effusion: an underestimated cause of hearing loss in infants. *Otol Neurotol* 2011;**32**:799–804.
39. Broides A, Dagan R, Greenberg D, et al. Acute otitis media caused by *Moraxella catarrhalis:* epidemiologic and clinical characteristics. *Clin Infect Dis* 2009;**49**:1641–7.
40. Brook I. Prevalence of beta-lactamase–producing bacteria in chronic suppurative otitis media. *Am J Dis Child* 1985;**139**: 280–3.
41. Brooks DN. Hearing screening: a comparative study of an impedance method and pure tone testing. *Scand Audiol* 1973;**2**:67–72.
42. Brown DT, Marsh RR, Potsic WP. Hearing loss induced by viscous fluids in the middle ear. *Int J Pediatr Otorhinolaryngol* 1983;**5**:39–46.
43. Capra AM, Lieu TA, Black SB, et al. Costs of otitis media in a managed care population. *Pediatr Infect Dis J* 2000;**19**:354–5.
44. Carlin SA, Marchant CD, Shurin PA, et al. Early recurrences of otitis media: reinfection or relapse? *J Pediatr* 1987;**110**:20–5.
45. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2010;**29**:304–9.
46. Casey JR, Pichichero ME. Changes in frequency and pathogens causing acute otitis media in 1995-2003. *Pediatr Infect Dis J* 2004;**23**:824–8.
47. Casselbrant ML, Brostoff LM, Cantekin EI, et al. Otitis media with effusion in preschool children. *Laryngoscope* 1985;**95**: 428–36.
48. Casselbrant ML, Kaleida PH, Rockette HE, et al. Efficacy of antimicrobial prophylaxis and of tympanostomy tube insertion for prevention of recurrent acute otitis media: results of a randomized clinical trial. *Pediatr Infect Dis J* 1992;**11**:278–86.
49. Casselbrant ML, Mandel EM, Fall PA, et al. The heritability of otitis media: a twin and triplet study. *JAMA* 1999;**282**:2125–30.
50. Casselbrant ML, Mandel EM, Rockette HE, et al. Adenoidectomy for otitis media with effusion in 2-3-year-old children. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1718–24.
51. Chase C. Hearing loss and development: a neuropsychologic perspective. In: Eavey RD, Klein JO, editors. *Hearing loss in childhood: a primer. Report of the 102nd Ross Conference on Pediatric Research*. Columbus, OH: Ross Laboratories; 1992. p. 88–94.
52. Chonmaitree T. Acute otitis media is not a pure bacterial disease. *Clin Infect Dis* 2006;**43**:1423–5.
53. Chonmaitree T, Howie VM, Truant AL. Presence of respiratory viruses in middle ear fluids and nasal wash specimens from children with acute otitis media. *Pediatrics* 1986;**77**:698–702.
54. Clements DA, Langdon L, Bland C, et al. Influenza A vaccine decreased the incidence of otitis media in 6- to 30-month old children in day care. *Arch Pediatr Adolesc Med* 1997;**149**:1113–7.

55. Coco A, Vernacchio L, Horst M, et al. Management of acute otitis media after publication of the 2004 AAP and AAFP clinical practice guideline. *Pediatrics* 2010;**125**:214–20.
56. Cohen MS, Mandel EM, Furman JM, et al. Tympanostomy tube placement and vestibular function in children. *Otolaryngol Head Neck Surg* 2011;**145**:666–72.
57. Combs JT. Two useful tools for exploring the middle ear. *Contemp Pediatr* 1993;**10**:60–75.
58. Cooper Jr JE, Gates GA, Owen JH, et al. An abbreviated impedance bridge technique for school screening. *J Speech Hear Disord* 1975;**40**:260–9.
59. Couloigner V, Levy C, François M, et al. Pathogens implicated in acute otitis media failures after 7-valent pneumococcal conjugate vaccine implementation in France: distribution, serotypes, and resistance levels. *Pediatr Infect Dis J* 2012;**31**:154–8.
60. Dagan R. Treatment of acute otitis media: challenges in the era of antibiotic resistance. *Vaccine* 2001;**19**:S9–S16.
61. DeMaria TF, Prior RB, Briggs BR, et al. Endotoxin in middle ear effusions from patients with chronic otitis media with effusion. In: Lim DJ, Bluestone CD, Klein JO, et al, editors. *Recent advances in otitis media with effusion*. Philadelphia: B. C. Decker; 1984. p. 123–5.
62. Derkay CS, Bluestone CD, Thompson AE, et al. Otitis media in the pediatric intensive care unit: a prospective study. *Otolaryngol Head Neck Surg* 1989;**100**:292–9.
63. Dowell SF, Butler JC, Giebink GS, et al. Acute otitis media: management and surveillance in an era of pneumococcal resistance—a report from the Drug-resistant *Streptococcus pneumoniae* Therapeutic Working Group. *Pediatr Infect Dis J* 1999; **18**:1–9.
64. Dowell SF, Marcy SM, Phillips WR, et al. Principles of judicious use of antimicrobial agents for pediatric upper respiratory tract infections. *Pediatrics* 1998;**101**:163–5.
65. Emonts M, Veenhoven RH, Wiertsema SP, et al. Genetic polymorphisms in immunoresponse genes TNFA, IL6, IL10, and TLR4 are associated with recurrent acute otitis media. *Pediatrics* 2007;**120**:814–23.
66. Engel JAM, Anteunis LJC, Hendriks JJT, et al. Epidemiological aspects of otitis media with effusion in infancy. In: Abstracts of the sixth international symposium on recent advances in otitis media. Fort Lauderdale, Fla; June 4-8, 1995. p. 15.
67. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 2001;**344**:403–9.
68. Etzel RA, Pattishall EN, Haley NJ, et al. Passive smoking and middle ear effusion among children in day care. *Pediatrics* 1992;**90**:228–32.
69. Faden H, Stanievich J, Brodsky L, et al. Changes in nasopharyngeal flora during otitis media of childhood. *Pediatr Infect Dis J* 1990;**9**:623–6.
70. Fernandez C, Lindsay JR, Moskowitz M. Some observations on the pathogenesis of middle-ear cholesteatoma. *AMA Arch Otolaryngol* 1959;**69**:537–46.
71. Fiellau-Nikolajsen M. Tympanometry in three-year-old children, part II. Seasonal influence on tympanometric results in nonselected groups of three-year-old children. *Scand Audiol* 1979;**8**: 181–5.
72. Finitzo T, Friel-Patti S, Chinn K, et al. Tympanometry and otoscopy prior to myringotomy: issues in diagnosis of otitis media. *Int J Pediatr Otorhinolaryngol* 1992;**24**:101–10.
73. Fireman B, Black SB, Shinefield HR, et al. Impact of the pneumococcal conjugate vaccine on otitis media. *Pediatr Infect Dis J* 2003;**22**:10–6.
74. Fria TJ, Cantekin EI, Eichler JA. Hearing acuity of children with otitis media with effusion. *Arch Otolaryngol* 1985;**111**: 10–6.
75. Friel-Patti S, Finitzo-Hieber T, Conti G, et al. Language delay in infants associated with middle ear disease and mild, fluctuating hearing impairment. *Pediatr Infect Dis* 1982;**1**:104–9.
76. Fry J, Dillane JB, McNab Jones RF, et al. The outcome of acute otitis media. (A report to the Medical Research Council). *Br J Prev Soc Med* 1969;**23**:205–9.
77. Gannon MM, Haggard MP, Golding J, et al. Sleeping position: a new environmental risk factor for otitis media? In: Abstracts of the Sixth International Symposium on Recent Advances in Otitis Media. Fort Lauderdale, Fla; June 4-8, 1995. p. 24.
78. Gates GA, Avery CA, Prihoda TJ, et al. Effectiveness of adenoidectomy and tympanostomy tubes in the treatment of chronic otitis media with effusion. *N Engl J Med* 1987;**317**:1444–51.
79. Giebink GS, Juhn SK, Weber ML, et al. The bacteriology and cytology of chronic otitis media with effusion. *Pediatr Infect Dis* 1982;**1**:98–103.
80. Giebink GS, Mills EL, Huff JS, et al. The microbiology of serous and mucoid otitis media. *Pediatrics* 1979;**63**:915–9.
81. Giebink GS, Quie PG. Otitis media: the spectrum of middle ear inflammation. *Annu Rev Med* 1978;**29**:285–306.
82. Goldblatt EL, Dohar J, Nozza RJ, et al. Topical ofloxacin versus systemic amoxicillin/clavulanate in purulent otorrhea in children with tympanostomy tubes. *Int J Pediatr Otorhinolaryngol* 1998; **46**:91–101.
83. Gravel JS, Wallace IF. Listening and language at 4 years of age: effects of early otitis media. *J Speech Hear Res* 1992;**35**:588–95.
84. Gray BM, Converse 3rd GM, Dillon Jr HC. Serotypes of *Streptococcus pneumoniae* causing disease. *J Infect Dis* 1979;**140**: 979–83.
85. Groth A, Enoksson F, Hermansson A, et al. Acute mastoiditis in children in Sweden 1993-2007—no increase after new guidelines. *Int J Pediatr Otorhinolaryngol* 2011;**75**:1496–501.
86. Hall-Stoodley L, Hu FZ, Gieseke A, et al. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. *JAMA* 2006;**296**:202–11.
87. Hammaren-Malmi S, Saxen H, Tarkkanen J, et al. Adenoidectomy does not significantly reduce the incidence of otitis media in conjunction with the insertion of tympanostomy tubes in children who are younger than 4 years: a randomized trial. *Pediatrics* 2005;**116**:185–9.
88. Harding AL, Anderson P, Howie VM, et al. *Hemophilus influenzae* isolated from children with otitis media. In: Sell SH, Karzon DT, editors. *Hemophilus influenzae*. Nashville: Vanderbilt University Press; 1973.
89. Hathaway TJ, Katz HP, Dershewitz RA, et al. Acute otitis media: who needs posttreatment follow-up? *Pediatrics* 1994;**94**:143–7.
89a. Hausdorff WP, Yothers G, Dagan R, et al. Multinational study of pneumococcal serotypes causing acute otitis media in children. *Pediatr Infect Dis J* 2002;**21**:1008–16.
90. Hautalahti O, Renko M, Tapiainen T, et al. Failure of xylitol given three times a day for preventing acute otitis media. *Pediatr Infect Dis J* 2007;**26**:423–7.
91. Hayden GF, Schwartz RH. Characteristics of earache among children with acute otitis media. *Am J Dis Child* 1985;**139**:721–3.
92. Henderson FW, Collier AM, Sanyal MA, et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N Engl J Med* 1982;**306**:1377–83.
93. Hendley JO, Sande MA, Stewart PM, et al. Spread of *Streptococcus pneumoniae* in families, part I. Carriage rates and distribution of types. *J Infect Dis* 1975;**132**:55–61.
94. Hesselvik L. Respiratory infections among children in day nurseries. *Acta Paediatr Scand* 1949;**74**:33–103.
95. Hoberman A, Paradise JL, Rockette HE, et al. Treatment of acute otitis media in children under 2 years of age. *N Engl J Med* 2011;**364**:105–15.
96. Howie VM. Eradication of bacterial pathogens from middle ear infections. *Clin Infect Dis* 1992;**14**:209–10.
97. Howie VM. Otitis media. *Pediatr Rev* 1993;**14**:320–3.
98. Howie VM, Ploussard JH. The "in vivo sensitivity test"—bacteriology of middle ear exudate, during antimicrobial therapy in otitis media. *Pediatrics* 1969;**44**:940–4.
99. Howie VM, Ploussard JH. Bacterial etiology and antimicrobial treatment of exudative otitis media: relation of antibiotic therapy to relapses. *South Med J* 1971;**64**:233–9.
100. Howie VM, Ploussard JH, Lester Jr RL. Otitis media: a clinical and bacteriological correlation. *Pediatrics* 1970;**45**:29–35.
101. Howie VM, Ploussard JH, Sloyer J. The "otitis-prone" condition. *Am J Dis Child* 1975;**129**:676–8.
102. Howie VM, Schwartz RH. Acute otitis media: one year in general pediatric practice. *Am J Dis Child* 1983;**137**:155–8.
103. Ilia S, Goulielmos GN, Samonis G, Galanakis E. Host's response in otitis media: understanding genetic susceptibility. *Pediatr Infect Dis J* 2008;**27**:929–33.
104. Ingvarsson L, Lundgren A, Oloffson B. Epidemiology of acute otitis media in children: a cohort study in an urban population. In: Lim DJ, Bluestone CD, Klein JO, et al, editors. *Recent advances*

in otitis media with effusion. Philadelphia: B.C. Decker; 1984. p. 19–22.
105. Jacoby P, Carville KS, Hall G, et al. Crowding and other strong predictors of upper respiratory tract carriage of otitis media-related bacteria in Australian Aboriginal and non-Aboriginal children. *Pediatr Infect Dis J* 2011;**30**:480–5.
105a. Joloba ML, Windau A, Bajaksouzian S, et al. Pneumococcal conjugate vaccine serotypes of *Streptococcus pneumoniae* isolates and the antimicrobial susceptibility of such isolates in children with otitis media. *Clin Infect Dis* 2001;**33**:1489–94.
106. Jung TT, Juhn SK, Michael AF. Localization of prostaglandin-forming cyclooxygenase in middle ear and external canal tissue. *Otolaryngol Head Neck Surg* 1983;**91**:187–92.
107. Jung TT, Smith DM, Juhn SK, et al. Prostaglandins and otitis media: studies in the chinchilla. *Otolaryngol Head Neck Surg* 1980;**88**:316–23.
108. Kalu SU, Ataya RS, McCormick DP, et al. Clinical spectrum of acute otitis media complicating upper respiratory tract viral infection. *Pediatr Infect Dis J* 2011;**30**:95–9.
109. Kaplan B, Wandstrat TL, Cunningham JR. Overall cost in the treatment of otitis media. *Pediatr Infect Dis J* 1997;**16**:S9–11.
110. Kaplan GJ, Fleshman JK, Bender TR, et al. Long-term effects of otitis media: a ten-year cohort study of Alaskan Eskimo children. *Pediatrics* 1973;**52**:577–85.
111. Kaplan SL, Mason Jr EO, Wald ER, et al. Pneumococcal mastoiditis in children. *Pediatrics* 2000;**106**:695–9.
112. Karmody CS, Northrop C. The pathogenesis of acquired cholesteatoma of the human middle ear: support for the migration hypothesis. *Otol Neurotol* 2012;**33**:42–7.
113. Kaur R, Adlowitz DG, Casey JR, et al. Simultaneous assay for four bacterial species including *Alloiococcus otitidis* using multiplex-PCR in children with culture negative acute otitis media. *Pediatr Infect Dis J* 2010;**29**:741–5.
114. Kenna MA. Intravenous antibiotics for otorrhea. In: Alper CM, Bluestone CD, Casselbrant ML, et al, editors. *Advanced therapy of otitis media*. Hamilton, Ontario: B. C. Decker; 2004.
115. Kilpi T, Herva E, Kaijalainen T, et al. Bacteriology of acute otitis media in a cohort of Finnish children followed for the first two years of life. *Pediatr Infect Dis J* 2001;**20**:654–62.
116. Kim PE, Musher DM, Glezen WP, et al. Association of invasive pneumococcal disease with season, atmospheric conditions, air pollution, and the isolation of respiratory viruses. *Clin Infect Dis* 1996;**22**:100–6.
117. Klein BS, Dollete FR, Yolken RH. The role of respiratory syncytial virus and other viral pathogens in acute otitis media. *J Pediatr* 1982;**101**:16–20.
118. Klein JO. Microbiologic efficacy of antibacterial drugs for acute otitis media. *Pediatr Infect Dis J* 1993;**12**:973–5.
119. Klein JO. Preventing recurrent otitis: what role for antibiotics? *Contemp Pediatr* 1994;**11**:44–60.
120. Klein JO. Review of consensus reports on management of acute otitis media. *Pediatr Infect Dis J* 1999;**18**:1150–3.
121. Klein JO. The burden of otitis media. *Vaccine* 2001;**19**:S2–8.
122. Klein JO, Teele DW. Isolation of viruses and mycoplasmas from middle ear effusions: a review. *Ann Otol Rhinol Laryngol* 1976;**85**:140–4.
123. Kovatch AL, Wald ER, Michaels RH. β-Lactamase–producing *Branhamella catarrhalis* causing otitis media in children. *J Pediatr* 1983;**102**:261–4.
123a. Lahikainen EA. Clinico-bacteriologic studies on acute otitis media aspiration of tympanum as diagnostic and therapeutic method. *Acta Otolaryngol Suppl* 1953;**107**:7–82.
124. Le Monnier A, Jamet A, Carbonnelle E, et al. *Fusobacterium necrophorum* middle ear infections in children and related complications: report of 25 cases and literature review. *Pediatr Infect Dis J* 2008;**27**:613–7.
125. Leinonen MK. Detection of pneumococcal capsular polysaccharide antigens by latex agglutination, counterimmunoelectrophoresis, and radioimmunoassay in middle ear exudates in acute otitis media. *J Clin Microbiol* 1980;**11**:135–40.
126. Lim DJ, Bluestone CD, Saunders WH, et al. Report of research committee on middle ear effusions. *Ann Otol Rhinol Laryngol* 1976;**85**:1–295.
127. Lim DJ, Saunders WH. Acquired cholesteatoma: light and electron microscopic observations. *Ann Otol Rhinol Laryngol* 1972;**81**:1–11.
128. Loda FA, Collier AM, Glezen WP, et al. Occurrence of *Diplococcus pneumoniae* in the upper respiratory tract of children. *J Pediatr* 1975;**87**:1087–93.
129. Long SS, Henretig FM, Teter MJ, et al. Nasopharyngeal flora and acute otitis media. *Infect Immun* 1983;**41**:987–91.
130. Lous J, Ryborg CT, Thomsen JL. A systematic review of the effect of tympanostomy tubes in children with recurrent acute otitis media. *Int J Pediatr Otorhinolaryngol* 2011;**75**:1058–61.
131. Lundman L, Juhn SK, Bagger-Sjoback D, et al. Permeability of the normal round window membrane to *Haemophilus influenzae* type b endotoxin. *Acta Otolaryngol* 1992;**112**:524–9.
132. Luotonen J, Herva E, Karma P, et al. The bacteriology of acute otitis media in children with special reference to *Streptococcus pneumoniae* as studied by bacteriological and antigen detection methods. *Scand J Infect Dis* 1981;**13**:177–83.
133. Magliulo G, Vingolo GM, Petti R, et al. Acute mastoiditis in pediatric age. *Int J Pediatr Otorhinolaryngol* 1995;**31**:147–51.
134. Makela PH, Leinonen M, Pukander J, et al. A study of the pneumococcal vaccine in prevention of clinically acute attacks of recurrent otitis media. *Rev Infect Dis* 1981;**3**:124–33.
135. Mandel EM, Casselbrant ML, Rockette HE, et al. Efficacy of 20- versus 10-day antimicrobial treatment for acute otitis media. *Pediatrics* 1995;**96**:5–13.
136. Mandel EM, Casselbrant ML, Rockette HE, et al. Systemic steroid for chronic otitis media with effusion in children. *Pediatrics* 2002;**110**:1071–80.
137. Mandel EM, Rockette HE, Bluestone CD, et al. Myringotomy with and without tympanostomy tubes for chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg* 1989;**115**:1217–24.
138. Marchant CD, Shurin PA, Turcyzk VA, et al. A randomized controlled trial of cefaclor compared with trimethoprim-sulfamethoxazole for treatment of acute otitis media. *J Pediatr* 1984;**105**:633–8.
139. Marchant CD, Shurin PA, Turczyk VA, et al. Course and outcome of otitis media in early infancy: a prospective study. *J Pediatr* 1984;**104**:826–31.
140. Maw AR, Hall AJ, Pothier DD, et al. The prevalence of tympanic membrane and related middle ear pathology in children: a large longitudinal cohort study followed from birth to age ten. *Otol Neurotol* 2011;**32**:1256–61.
141. McCandless GA, Thomas GK. Impedance audiometry as a screening procedure for middle ear disease. *Trans Am Acad Ophthalmol Otolaryngol* 1974;**78**:98–102.
142. McEldowney D, Kessner DM. Review of the literature: epidemiology of otitis media. In: Gloric A, Gerwin KS, editors. *Otitis media*. Springfield, Ill: Charles C Thomas; 1972.
143. McLinn SE. Cefaclor in treatment of otitis media and pharyngitis in children. *Am J Dis Child* 1980;**134**:560–3.
144. Meropol SB. Valuing reduced antibiotic use for pediatric acute otitis media. *Pediatrics* 2008;**121**:669–73.
145. Meyerhoff WL, Giebink GS. Pathology and microbiology of otitis media. *Laryngoscope* 1982;**92**:273–7.
146. Mogi G. Secretory IgA and antibody activities in middle ear effusions. *Ann Otol Rhinol Laryngol* 1976;**85**:97–102.
147. Morris PS. A systematic review of otitis media in Australian Aboriginal children. In: Abstracts of the Sixth International Symposium on Recent Advances in Otitis Media. Fort Lauderdale, Fla, June 4-8, 1995. p 11.
148. Mortimer Jr EA, Watterson Jr RL. A bacteriologic investigation of otitis media in infancy. *Pediatrics* 1956;**17**:359–67.
149. Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. *Clin Infect Dis* 2009;**49**:124–31.
150. Murwitz ES, Gunn WJ, Pinsky PF, et al. Risk of respiratory illness associated with day-care attendance: a nationwide study. *Pediatrics* 1991;**87**:62–9.
151. Neumark T, Ekblom M, Brudin L, et al. Spontaneously draining acute otitis media in children: an observational study of clinical findings, microbiology and clinical course. *Scand J Infect Dis* 2011;**43**:891–8.
152. Niemela M, Uhari M, Mottonen M. A pacifier increases the risk of recurrent acute otitis media in children in day care centers. *Pediatrics* 1995;**96**:884–8.
153. Nozza RJ, Bluestone CD, Kardatzke D, et al. Towards the validation of aural acoustic immittance measures for diagnosis of middle ear effusion in children. *Ear Hear* 1992;**13**:442–53.

154. O'Brien KL, David AB, Chandran A, et al. Randomized, controlled trial efficacy of pneumococcal conjugate vaccine against otitis media among Navajo and White Mountain Apache infants. *Pediatr Infect Dis J* 2008;**27**:71–3.
155. Ogawa H, Fujisawa T, Kazuyama Y. Isolation of *Chlamydia pneumoniae* from middle ear aspirates of otitis media with effusion: a case report. *J Infect Dis* 1990;**162**:1000–1.
156. Ongkasuwan J, Valdez TA, Hulten KG, et al. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics* 2008;**122**:34–9.
156a. Orange M, Gray BM. Pneumococcal serotypes causing disease in children in Alabama. *Pediatr Infect Dis J* 1993;**12**:244–6.
157. Ozgur SK, Beyazova U, Kemaloglu YK, et al. Effectiveness of inactivated influenza vaccine for prevention of otitis media in children. *Pediatr Infect Dis J* 2006;**25**:401–4.
158. Papastavros T, Giamarellou H, Varlejides S. Role of aerobic and anaerobic microorganisms in chronic suppurative otitis media. *Laryngoscope* 1986;**96**:438–42.
159. Paradise JL, Bluestone CD. Early treatment of the universal otitis media of infants with cleft palate. *Pediatrics* 1974;**53**:48–54.
160. Paradise JL, Bluestone CD, Felder H. The universality of otitis media in 50 infants with cleft palate. *Pediatrics* 1969;**44**:35–42.
161. Paradise JL, Bluestone CD, Rogers KD, et al. Efficacy of adenoidectomy for recurrent otitis media in children previously treated with tympanostomy-tube placement: results of parallel randomized and nonrandomized trials. *JAMA* 1990;**263**:2066–73.
162. Paradise JL, Campbell TF, Dollaghan CA, et al. Developmental outcomes after early or delayed insertion of tympanostomy tubes. *N Engl J Med* 2005;**353**:576–86.
163. Paradise JL, Elster BA, Tan L. Evidence in infants with cleft palate that breast milk protects against otitis media. *Pediatrics* 1994;**94**:853–60.
164. Paradise JL, Feldman HM, Campbell TF, et al. Effect of early or delayed insertion of tympanostomy tubes for persistent otitis media on developmental outcomes at the age of three years. *N Engl J Med* 2001;**344**:1179–87.
165. Paradise JL, Feldman HM, Colborn DK, et al. Parental stress and parent-rated child behavior in relation to otitis media in the first three years of life. *Pediatrics* 1999;**104**:1264–73.
166. Paradise JL, Rockette HE, Colborn DK, et al. Otitis media in 2253 Pittsburgh-area infants: prevalence and risk factors during the first two years of life. *Pediatrics* 1997;**99**:318–33.
167. Paradise JL, Smith CG, Bluestone CD. Tympanometric detection of middle ear effusion in infants and young children. *Pediatrics* 1976;**58**:198–210.
168. Patel JA, Nair S, Revai K, et al. Association of proinflammatory cytokine gene polymorphisms with susceptibility to otitis media. *Pediatrics* 2006;**118**:2273–9.
169. Pelton SI, Teele DW, Shurin PA, et al. Disparate cultures of middle ear fluids: results from children with bilateral otitis media. *Am J Dis Child* 1980;**134**:951–3.
170. Persico M, Barker GA, Mitchell DP. Purulent otitis media—a "silent" source of sepsis in the pediatric intensive care unit. *Otolaryngol Head Neck Surg* 1985;**93**:330–4.
171. Petersen CG, Ovesen T, Pedersen CB. Acute mastoidectomy in a Danish county from 1977 to 1996 with focus on the bacteriology. *Int J Pediatr Otorhinolaryngol* 1998;**45**:21–9.
172. Pettigrew MM, Gent JF, Pyles RB, et al. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011;**49**:3750–5.
173. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *JAMA* 2007; **298**:1772–8.
174. Pitkaranta A, Jero J, Arruda E, et al. Polymerase chain reaction–based detection of rhinovirus, respiratory syncytial virus, and coronavirus in otitis media with effusion. *J Pediatr* 1998;**133**: 390–4.
175. Pitkaranta A, Virolainen A, Jero J, et al. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. *Pediatrics* 1998;**102**:291–5.
176. Post JC, Preston RA, Aul JJ, et al. Molecular analysis of bacterial pathogens in otitis media with effusion. *JAMA* 1995; **273**:1598–604.
177. Poulsen G, Tos M. Screening tympanometry in newborn infants and during the first six months of life. *Scand Audiol* 1978;**7**:159–66.
178. Prymula R, Peeters P, Chrobok V, et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and nontypable *Haemophilus influenzae:* a randomised double-blind efficacy study. *Lancet* 2006;**367**:740–8.
179. Pukander J, Luotonen J, Sipila M, et al. Incidence of acute otitis media. *Acta Otolaryngol* 1982;**93**:447–53.
180. Reed D, Brody J. Otitis media in urban Alaska. *Alaska Med* 1966;**8**:64–7.
181. Renvall U, Holmquist J. Tympanometry revealing middle ear pathology. *Ann Otol Rhinol Laryngol* 1976;**85**:209–15.
182. Riding KH, Bluestone CD, Michaels RH, et al. Microbiology of recurrent and chronic otitis media with effusion. *J Pediatr* 1978;**93**:739–43.
183. Rogers DJ, Boseley ME, Adams MT, et al. Prospective comparison of handheld pneumatic otoscopy, binocular microscopy, and tympanometry in identifying middle ear effusions in children. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1140–3.
184. Roland PS, Kreisler LS, Reese B, et al. Topical ciprofloxacin/dexamethasone otic suspension is superior to ofloxacin otic solution in the treatment of children with acute otitis media with otorrhea through tympanostomy tubes. *Pediatrics* 2004;**113**: e40–6.
185. Rovers MM, Glasziou P, Appelman CL, et al. Predictors of pain and/or fever at 3 to 7 days for children with acute otitis media not treated initially with antibiotics: a meta-analysis of individual patient data. *Pediatrics* 2007;**119**:579–85.
186. Rubin LG, Papsin B, and the Committee on Infectious Diseases and Section on Otolaryngology–Head and Neck Surgery. Policy Statement—Cochlear implants in children: surgical site infections and prevention and treatment of acute otitis media and meningitis. *Pediatrics* 2010;**126**:11.
187. Ruedi L. Cholesteatoma formation in the middle ear in animal experiments. *Acta Otolaryngol* 1959;**50**:233–40; discussion 240–2.
188. Ruohola A, Meurman O, Nikkari S, et al. Microbiology of acute otitis media in children with tympanostomy tubes: prevalences of bacteria and viruses. *Clin Infect Dis* 2006;**43**:1417–22.
189. Ruuskanen O, Arola M, Heikkinen T, et al. Viruses in acute otitis media: increasing evidence for clinical significance. *Pediatr Infect Dis J* 1991;**10**:425–7.
190. Sanyal MA, Henderson FW, Stempel EC, et al. Effect of upper respiratory tract infection on eustachian tube ventilatory function in the preschool child. *J Pediatr* 1980;**97**:11–5.
191. Sarkkinen H, Ruuskanen O, Meurman O, et al. Identification of respiratory virus antigens in middle ear fluids of children with acute otitis media. *J Infect Dis* 1985;**151**:444–8.
192. Schaefer O. Otitis media and bottle-feeding: an epidemiological study of infant feeding habits and incidence of recurrent and chronic middle ear disease in Canadian Eskimos. *Can J Public Health* 1971;**62**:478–89.
193. Schappert SM. Office visits for otitis media: United States, 1975-90. *Adv Data* 1992;1–19.
194. Schneider ML. Bacteriology of otorrhea from tympanostomy tubes. *Arch Otolaryngol Head Neck Surg* 1989;**115**:1225–6.
195. Schwartz B, Giebink GS, Henderson FW, et al. Respiratory infections in day care. *Pediatrics* 1994;**94**:1018–20.
196. Schwartz R, Rodriguez WJ, Khan WN, et al. Acute purulent otitis media in children older than 5 years: incidence of *Haemophilus* as a causative organism. *JAMA* 1977;**238**:1032–3.
197. Schwartz RH, Rodriguez WJ. Acute otitis media in children eight years old and older: a reappraisal of the role of *Hemophilus influenzae*. *Am J Otolaryngol* 1981;**2**:19–21.
198. Schwartz RH, Rodriguez WJ, Brook I, et al. The febrile response in acute otitis media. *JAMA* 1981;**245**:2057–8.
199. Segal N, Givon-Lavi N, Leibovitz E, et al. Acute otitis media caused by *Streptococcus pyogenes* in children. *Clin Infect Dis* 2005;**41**:35–41.
200. Senturia BH. Classification of middle ear effusions. *Ann Otol Rhinol Laryngol* 1970;**79**:358–70.
201. Shurin PA, Howie VM, Pelton SI, et al. Bacterial etiology of otitis media during the first six weeks of life. *J Pediatr* 1978;**92**:893–6.

202. Shurin PA, Marchant CD, Kim CH, et al. Emergence of beta-lactamase–producing strains of *Branhamella catarrhalis* as important agents of acute otitis media. *Pediatr Infect Dis* 1983;**2**:34–8.
203. Sipila M, Karma P, Pukander J, et al. The Bayesian approach to the evaluation of risk factors in acute and recurrent acute otitis media. *Acta Otolaryngol* 1988;**106**:94–101.
204. Sipila M, Pukander J, Karma P. Incidence of acute otitis media up to the age of 1 1/2 years in urban infants. *Acta Otolaryngol* 1987;**104**:138–45.
205. Skolnik PR, Nadol Jr JB, Baker AS. Tuberculosis of the middle ear: review of the literature with an instructive case report. *Rev Infect Dis* 1986;**8**:403–10.
206. Skoner DP, Stillwagon PK, Casselbrandt ML, et al. Inflammatory mediators in chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg* 1988;**114**:1131–3.
207. Sloyer Jr JL, Cate CC, Howie VM, et al. The immune response to acute otitis media in children, part II: Serum and middle ear fluid antibody in otitis media due to *Haemophilus* influenza. *J Infect Dis* 1975;**132**:685–8.
208. Sloyer Jr JL, Ploussard JH, Howie VM. Efficacy of pneumococcal polysaccharide vaccine in preventing acute otitis media in infants in Huntsville, Alabama. *Rev Infect Dis* 1981;**3**:119–23.
209. Sriwardhana KB, Howard AJ, Dunkin KT. Bacteriology of otitis media with effusion. *J Laryngol Otol* 1989;**103**:253–6.
210. Stool SE, Berg AO, Berman S, et al. *Otitis media with effusion in young children: Clinical Practice Guideline. Number 12. AHCPR publication No. 94-0622*. Rockville, Md: Agency for Health Care Policy and Research, Public Health Service, U.S. Department of Health and Human Services; July 1994.
211. Storgaard M, Ostergaard L, Jensen JS, et al. *Chlamydia pneumoniae* in children with otitis media. *Clin Infect Dis* 1997;**25**:1090–3.
212. Tahtinen PA, Laine MK, Huovinen P, et al. A placebo-controlled trial of antimicrobial treatment for acute otitis media. *N Engl J Med* 2011;**364**:116–26.
213. Teele DW, Klein JO, Bratton L, et al, for the Greater Boston Collaborative Otitis Media Study Group. Use of pneumococcal vaccine for prevention of recurrent acute otitis media in infants in Boston. *Rev Infect Dis* 1981;**3**:113–8.
214. Teele DW, Klein JO, Chase C, et al. Otitis media in infancy and intellectual ability, school achievement, speech, and language at age 7 years. Greater Boston Otitis Media Study Group. *J Infect Dis* 1990;**162**:685–94.
215. Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. *J Infect Dis* 1989;**160**:83–94.
216. Teele DW, Klein JO, Rosner BA. Epidemiology of otitis media in children. *Ann Otol Rhinol Laryngol Suppl* 1980;**89**:5–6.
217. Teele DW, Klein JO, Rosner B, et al. Middle ear disease and the practice of pediatrics: burden during the first five years of life. *JAMA* 1983;**249**:1026–9.
218. Tetzlaff TR, Ashworth C, Nelson JD. Otitis media in children less than 12 weeks of age. *Pediatrics* 1977;**59**:827–32.
219. Tipple MA, Beem MO, Saxon EM. Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. *Pediatrics* 1979; **63**:192–7.
220. Tos M, Poulsen G, Hancke AB. Screening tympanometry during the first year of life. *Acta Otolaryngol* 1979;**88**:388–94.
221. Uhari M, Kontiokari T, Koskela M, et al. Xylitol chewing gum in prevention of acute otitis media: double blind randomised trial. *BMJ* 1996;**313**:1180–4.
222. Uhari M, Kontiokari T, Niemela M. A novel use of xylitol sugar in preventing acute otitis media. *Pediatrics* 1998;**102**:879–84.
223. U.S. Department of Health and Human Services. *The health consequences of smoking: a report from the Surgeon General. Department of Health and Human Services publication (DHS)84-50205*. Rockville, Md: Office on Smoking and Health; 1984. p. 292.
224. van Buchem FL, Dunk JH, van't Hof MA. Therapy of acute otitis media: myringotomy, antibiotics, or neither? A double-blind study in children. *Lancet* 1981;**2**:883–7.
225. van Buchem FL, Peeters MF, van't Hof MA. Acute otitis media: a new treatment strategy. *BMJ (Clin Res Ed)* 1985;**290**:1033–7.
226. Van Hare GF, Shurin PA, Marchant CD, et al. Acute otitis media caused by *Branhamella catarrhalis:* biology and therapy. *Rev Infect Dis* 1987;**9**:16–27.
227. Varsano IB, Volovitz BM, Grossman JE. Effect of naproxen, a prostaglandin inhibitor, on acute otitis media and persistence of middle ear effusion in children. *Ann Otol Rhinol Laryngol* 1989;**98**:389–92.
228. Veltri RW, Sprinkle PM. Secretory otitis media: an immune complex disease. *Ann Otol Rhinol Laryngol* 1976;**85**:135–9.
229. Wald ER, Dashefsky B, Byers C, et al. Frequency and severity of infections in day care. *J Pediatr* 1988;**112**:540–6.
230. Wall GM, Stroman DW, Roland PS, et al. Ciprofloxacin 0.3%/dexamethasone 0.1% sterile otic suspension for the topical treatment of ear infections: a review of the literature. *Pediatr Infect Dis J* 2009;**28**:141–4.
231. Wiederhold ML, Zajtchuk JT, Vap JG, et al. Hearing loss in relation to physical properties of middle ear effusions. *Ann Otol Rhinol Laryngol Suppl* 1980;**89**:185–9.
232. Yaniv E. Tuberculous otitis: an underdiagnosed disease. *Am J Otolaryngol* 1987;**8**:356–60.
233. Zhou F, Shefer A, Kong Y, Nuorti JP. Trends in acute otitis media-related health care utilization by privately insured young children in the United States, 1997-2004. *Pediatrics* 2008;**121**:8.

CHAPTER 17

Mastoiditis

James D. Cherry • Audrey P. Calzada • Nina L. Shapiro

Mastoiditis, a suppurative infection of the mastoid air cells, is a potential complication of all cases of otitis media caused by the continuity of the mucoperiosteal lining of the mastoid with that of the middle ear.[25] The spectrum of disease in mastoiditis ranges from asymptomatic cases with apparent spontaneous resolution to progressive disease with life-threatening complications.[11] Since the advent of antibiotic therapy, mastoiditis is seen much less frequently, but the complications remain similar.[21,24,26,98] With mastoiditis occurring less commonly, physicians are less likely to consider the diagnosis, especially when the clinical picture has been masked by antibiotic therapy or when the process is chronic and of low grade. Appropriate antibiotic therapy, often accompanied by surgical drainage, can halt and prevent serious complications if mastoiditis is diagnosed early.

HISTORY

Before the advent of antibiotics, mastoiditis was a frequent complication of otitis media that could be treated only by expectant waiting or surgery.[38,42] The first surgical opening of the mastoid cavity was performed in 1736 by Petit using a trepanation system. The trepanation system gave way to the chisel and gouge, which was then replaced by the electric drill, which was introduced at the end of the 19th century.[56] When surgery was used, many patients with mastoiditis were cured by simple mastoid drainage alone, with a mortality rate quoted at 2 percent.[42] Intracranial complications of mastoiditis carried a very grave prognosis, however. In the preantibiotic era, between 1928 and 1933, 25 of every 1000 deaths at Los Angeles County Hospital in California were caused by intracranial complications of otitis media, such as meningitis, venous sinus thrombosis, and brain abscess. In contrast, between 1949 and 1954, only 2.5 per 1000 deaths at the same hospital were caused by complications of otitis or mastoiditis. The use of antibiotics in treating mastoiditis initially led to a marked decrease in the surgical approach to treatment of this illness.[11,98] The realization that infection can persist and that complications of mastoiditis can occur even while the patient is receiving antibiotic therapy has resulted in the present-day approach of combined antibiotics and surgery, necessitating collaboration between the pediatrician and otolaryngologist.*

*References 26, 27, 30, 46, 53, 59, 64, 87, 96.

ANATOMY AND PATHOPHYSIOLOGY

The mastoid process comprises the posterior part of the temporal bone and, as such, is adjacent to many important structures. Within the mastoid is an interconnecting system of air cells divided by bony septa that drain superiorly into the middle ear via a narrow aditus.[11,25] Only the superior portion of the mastoid airspace, the antrum, is present at birth; pneumatization of the mastoid starts soon after birth and usually is completed by the time the child is 2 years of age.[4,42] Structures lying anteromedial to the mastoid process include the middle ear and ossicles, the facial nerve, the posterior bony wall of the external auditory canal, the jugular vein, and the internal carotid artery. Posteromedially, the mastoid borders the posterior cranial fossa and the sigmoidal sinus. Superiorly, the mastoid borders the middle cranial fossa. Medially, the mastoid cortex encases the cochlea and semicircular canals. The soft tissues and muscles of the lateral neck are located inferiorly. Any or all of these adjacent structures can be affected by extension of a suppurative process in the mastoid.

A certain amount of mastoid inflammation accompanies all cases of otitis media because the mastoid airspaces are continuous with the middle ear cavity and both are lined by a continuous mucoperiosteum.[4,25] The first stage of an ear and mastoid infection is associated with hyperemia of the middle ear and the mastoid air cell mucosa. If the infection persists, an exudative stage develops, with serum, fibrin, polymorphonuclear cells, and red blood cells accumulating in the middle ear and mastoid. The accumulation of purulent exudate increases the middle ear pressure, eventually resulting in perforation of the tympanic membrane, followed by drainage of mucopurulent matter from the middle ear and mastoid air cells. Some children also have such marked mucoperiosteal swelling that the drainage of pus from the mastoid is blocked. The pus under pressure creates an environment of local acidosis, hypoxia, and ischemia, causing decalcification and resorption of the bony septa. The term *coalescent mastoiditis* is applied to this process because, with the destruction of the bony septa, the mastoid air cells coalesce into large cavities. Osteomyelitis of the adjacent bone may develop, with subsequent bony erosion and eventual extension of the infection into surrounding structures.[4,25]

Congenital cholesteatomas usually manifest as a "squamous pearl" in the anterosuperior quadrant of the middle ear, abutting the tympanic membrane. They may be associated with recurrent otitis media.[50] Acquired

cholesteatomas often are a result of chronic infections and tympanic membrane perforation. The perforation allows for squamous material from the external auditory canal to enter the middle ear space.[68] This tissue contains osteolytic enzymes, leading to bony erosion or mastoid air cell obstruction.[60] Cholesteatomas also may cause slow, insidious erosion of underlying bone, predisposing the patient to extramastoid spread of infection months or years later.[25,73]

MICROBIOLOGY

The bacteriologic findings in 10 studies of acute mastoiditis and one study of chronic mastoiditis are presented in Table 17-1. These studies, which were all carried out in the antibiotic era, span a 53-year time period and occurred in six countries.* In all studies the leading causative agent in acute mastoiditis is *Streptococcus pneumoniae;* surprisingly the second organism of importance is *Streptococcus pyogenes.*† Other organisms of importance in acute mastoiditis are *Staphylococcus aureus*, *Haemophilus influenzae*, and *Pseudomonas aeruginosa*. Because nontypable *H. influenzae* is a very common cause of otitis media it is surprising that this agent is not more commonly recovered in acute mastoiditis. Similarly, *Moraxella catarrhalis*, another common cause of otitis media, is rarely found in association with acute mastoiditis.[41]

Also, because neither *S. aureus* nor *P. aeruginosa* is a cause of acute otitis media it is likely that these agents, when isolated in acute mastoiditis, are secondary rather than primary pathogens. The same is likely for coagulase-negative staphylococci and various anaerobic organisms. However, in contrast to *S. aureus* and *P. aeruginosa*, if coagulase-negative staphylococci and anaerobic organisms are present they may be contaminates and may not contribute to the infectious process.

An increased incidence of penicillin-resistant *S. pneumoniae* infections has occurred during the past 2 decades, leading to a higher likelihood of mastoiditis being a complication of otitis media.[10,63,65,97] The percentage of pneumococcal mastoiditis caused by penicillin-resistant strains increased from 25 to 44 percent between 1994 and 1998, without an increase in the total number of cases of pneumococcal mastoiditis.[36] Pneumococcal conjugate vaccine (PCV7) was introduced into the pediatric immunization schedule in 2000 and over the ensuing decade invasive pneumococcal disease decreased dramatically (see Chapter 85). PCV7 contains the following serotypes: 14, 6B, 19F, 18C, 23F, 4, and 9V. Surprisingly, in a large study performed by Choi and Lander,[10] the number of admissions for mastoiditis at an urban tertiary care children's hospital did not decrease in the PCV7 vaccine era. In fact, the post-PCV7 patients had more severe disease compared with patients seen between 1996 and 2002.

Ongkasuwan and associates[65] studied 41 cases of pneumococcal mastoiditis between 1995 and 2007. In the pre-PCV7 era (1995-1999) there were 12 cases and none was caused by the nonvaccine serotype 19A. Between April 2000 and October 2005, there were 15 cases of pneumococcal mastoiditis and 5 of these cases were due to pneumococcal serotype 19A. In their last time period (November 2006 to June 2007), there were 14 cases and all were due to serotype 19A. Sixty-eight percent of the serotype 19A isolates had multidrug antibiotic resistance.

The bacteriologic spectrum of chronic mastoiditis differs from that of acute mastoiditis. Aerobic cultures of chronic mastoiditis and chronic otitis media show predominantly *S. aureus* and gram-negative bacilli, especially *P. aeruginosa*.[6,16,73] In addition, a wide variety of anaerobic organisms can be isolated from an infected mastoid and middle ear.[6,16] Brook[6] studied the aerobic and anaerobic bacteriology of chronic otitis media (of ≥3 months' duration) in 24 children. Anaerobic isolates alone were found in 17 percent, aerobic organisms alone were found in 4 percent, and mixed aerobic and anaerobic infections were found in 79 percent. All cases had from two to seven different bacterial isolates. *Peptococcus* spp., *Actinomyces* spp., and *Bacteroides melaninogenicus (Prevotella melaninogenica)* were the most commonly isolated anaerobic organisms. Seventeen patients were infected with β-lactamase–producing organisms (i.e., *S. aureus* or *P. melaninogenica*, *Bacteroides fragilis*, or other *Bacteroides* spp. that were resistant to ampicillin).

Mycobacterium tuberculosis currently is an uncommon cause of mastoiditis in the United States but continues to be a cause of chronically draining ears in lower socioeconomic groups and immigrants from endemic areas.[7,54,57] Case reports of mastoiditis implicate such organisms as nontuberculous mycobacteria,[48,62] *Turicella otitidis*,[76,81] *Aspergillus fumigatus*,[28] *Paragonimus*-like trematodes,[66] *Nocardia asteroides*,[52] *Actinomyces* spp.,[86] *Blastomyces dermatitidis*,[36] and *Histoplasma capsulatum*.[52] *Pneumocystis jiroveci* otitis media and mastoiditis have occurred as the first manifestation of acquired immunodeficiency syndrome.[22]

CLINICAL PRESENTATION

The classic presentation of acute mastoiditis is a febrile child with ear pain, postauricular swelling, and postauricular tenderness developing days to weeks after the beginning of development of acute otitis media (Fig. 17-1).[21,24,59,64,97] If antibiotics were used to treat acute otitis media, the child may have seemed to improve, only to become ill again while still receiving therapy or after the antibiotics were stopped; conversely, the inflammation may not have responded to the antibiotics at all. Examination of the tympanic membrane in acute mastoiditis usually shows that it is abnormal.[24,30,59] Early in the course of illness, periosteal inflammation produces swelling and tenderness and sometimes redness over the mastoid process.[5,24] Palpable postauricular fluctuance occurs later, when pus from the mastoid air cells breaks through the underlying bony cortex and forms a subperiosteal abscess.[29] In children older than 1 year of age, the most common area where fluctuance is felt lies behind the ear, where it pushes the earlobe up and out; however, in children younger than 1 year, the fluctuance often may occur above the ear, pushing the pinna down and out.[24]

*References 2, 6, 21, 24, 33, 59, 64, 70, 75, 82, 97.
†References 2, 21, 24, 30, 33, 41, 59, 64, 70, 75, 82, 97.

TABLE 17-1 **Summary of Bacterial Isolates from Middle Ear, Subperiosteal Abscess, or Mastoid of Children with Mastoiditis in 11 Studies**

	Acute Mastoiditis										***Chronic Mastoiditis***
Isolates	**Ginsburg et al., 1955-1979[24]**	**Hoppe et al., 1975-1992[33]**	**Ogle and Lauer, 1973-1984[64]**	**Nadal et al., 1971-1988[59]**	**Ghaffar et al., 1983-1999[21]**	**Zapalac et al., 1993-2000[97]**	**Bakhos et al., 1994-2008[2]**	**Quesnel et al., 2001-2008[75]**	**Stenfeldt and Hermansson, 1996-2005[82]**	**Pang et al, 1996-2006[70]**	**Brook, 1976-1980[6]**
Streptococcus pneumoniae	14	13	5	9	20	15	19	87	19	16	1
Streptococcus pyogenes	8	4	3	4	4	10	4	20	4	3	2
Staphylococcus aureus	8	2	1	4	5	3	—	6	5	4	8
Staphylococcus spp. coagulase negative	1	2	2	6	7	12	1	13	—	—	—
Other aerobic gram-positive cocci	3	—	1	2	2	—	—	3	—	—	4
Haemophilus influenzae	1	—	2	1	—	1	—	8	1	3	—
Pseudomonas aeruginosa	2	—	—	3	5	—	—	8	5	11	7
Other aerobic gram-negative rods	1	1	1	1	4	—	—	7	—	—	7
Anaerobic cocci	1	—	—	1	—	—	—	—	—	—	23
Anaerobic gram-positive bacilli	—	—	—	1	—	—	—	—	—	—	14
Anaerobic gram-negative bacilli	1	—	1	—	—	—	1	11	—	—	24
Other	1	—	4	—	—	10	—	8	—	5	—
Total patients with cultures	49	28	30	54	49	64	31	158	42	56	24

FIGURE 17-1 ■ An 18-month-old child with right-sided mastoiditis and a proptotic right auricle.

Chronic mastoiditis is a much more indolent disease process than acute mastoiditis. It develops when long-standing middle ear disease, usually having a duration of months to years, has been present.[73] Fever and postauricular swelling may or may not be present. Persistent or intermittent drainage of mucopurulent matter from a previously perforated eardrum suggests chronic mastoiditis. Hearing loss and ear pain also may accompany chronic mastoiditis.[73] All these symptoms can be mild enough to be ignored until serious intracranial suppuration occurs. Persistent ear drainage, persistent ear pain, or an otitis media nonresponsive to antibiotics should prompt a search for mastoiditis.

COMPLICATIONS

Complications of mastoiditis include subperiosteal abscess,[29] Bezold abscess,[18,51,78] facial nerve paralysis,[15,90] meningitis,[11,24] brain abscess,[9,11,24,35,79] cerebellar abscess,[4] epidural abscess,[4] subdural empyema,[5] labyrinthitis,[25] venous sinus thrombophlebitis,* bacteremia,[11,53] benign intracranial hypertension,[5] osteomyelitis of the temporal bone with occasional extension to adjacent bones,[4,25] hearing loss,[69] septic pulmonary emboli,[34] and cerebrospinal fluid otorrhea.[25] Subperiosteal abscesses appear as a postauricular fluctuant mass that obscures the postauricular sulcus. They occur when pus in the mastoid breaks through the bony cortex or extends along vascular channels and dissects under the overlying periosteum.[29]

A Bezold abscess develops when a mastoid infection erodes through the bony cortex of the inferior aspect of the mastoid tip and dissects down the tissue planes to form a deep neck abscess. Fluctuance over the mastoid is not felt. Rather, swelling and tenderness are present below the mastoid process and under the sternocleidomastoid muscle.[18]

The facial nerve runs close to the mastoid and the middle ear, rendering it vulnerable to injury when extension of mastoid or middle ear infection occurs.[25] Pressure on and inflammation of the facial nerve from symptomatic or asymptomatic mastoiditis can lead to transient or permanent facial nerve paralysis that usually is unilateral, although bilateral facial palsy from mastoiditis can occur.[4,90]

Because the temporal bone that houses the mastoid air cells constitutes the floor of the middle and posterior cranial fossae, bony erosion from osteomyelitis, preexisting bony defects, or spread of infection along vascular channels can allow for intracranial spread of mastoid infections into the middle and posterior cranial fossae. The infection may remain confined to the extradural space as an extradural abscess, or it may penetrate the dura and produce a subdural empyema, a brain abscess, a cerebellar abscess, or meningitis.[4,25]

Invasion of infection into the bony labyrinth through the oval or round window triggers labyrinthitis. Initial tinnitus, hearing loss, nausea, and dizziness progress to severe vertigo, ear pain, vomiting, nystagmus, and difficulties with balance.[25]

Intracranial venous sinus thrombophlebitis is a rare but potentially fatal complication of mastoiditis.* The lateral aspect of the sigmoid sinus is formed by the temporal bone. Venous sinus thrombophlebitis results when an underlying mastoiditis extends through the temporal bone in close proximity to the lateral or sigmoid venous sinus. A perisinus abscess initially is formed, followed by formation of a mural thrombus in the sinus wall. The thrombus eventually may occlude the entire sinus, or it may suppurate and spread along the sinus, resulting in septicemia, increased intracranial pressure, septic emboli, and extension of infection to other intracranial structures.[14,19] The classic findings of septic thrombosis of the lateral sinus are spiking fevers, shaking chills, and tenderness along the jugular vein associated with acute or chronic otitis. A palpable "cord" at the jugular vein (indicating a jugular vein thrombus) also may be present. When only a perisinus abscess is present, or if the patient is being treated partially with antibiotics, the only features may be a low-grade fever and headache.[27,88]

*References 3, 14, 23, 39, 67, 72, 77, 92.

*References 3, 5, 23, 25, 39, 67, 72, 77, 88, 92.

Benign intracranial hypertension can be seen in association with lateral sinus obstruction secondary to mastoiditis and is termed *otitic hydrocephalus*. Rarely, otitic hydrocephalus can be seen with mastoiditis in the absence of lateral sinus thrombosis. The decreased venous drainage caused by the venous sinus obstruction results in increased intracranial pressure, headache, papilledema, and sixth nerve palsy without enlarged ventricles or a space-occupying lesion.[25,27]

Permanent conductive hearing loss occurs when the middle ear mastoid infection is severe enough to damage or destroy the ossicles. Tuberculous mastoiditis classically manifests as marked conductive hearing loss that often is irreversible.[47]

Osteomyelitis secondary to mastoiditis can spread to adjacent bones. Involvement of the petrous portion of the temporal bone produces a syndrome, described by Gradenigo in 1907, with a triad of abducens paralysis or paresis, severe pain in the distribution of the trigeminal nerve, and suppurative otitis media; additional cranial nerve deficits also may occur.[8,46,75] Antibiotics may mask the classic signs of petrositis and allow progression to severe intracranial complications, such as meningitis and epidural abscess. Petrositis may be suspected only when antibiotic and surgical management for mastoiditis fails to control chronic ear drainage.[25]

M. tuberculosis mastoiditis is an uncommon finding but should be considered in children who have chronic ear discharge despite having received antibiotic therapy.[47,54] Children can go for months or years with chronically draining ears before the diagnosis of tuberculous mastoiditis is considered.[7,57] Children from lower socioeconomic homes, immigrants from endemic areas, and children with tuberculosis contacts in the family are at risk. The classic presentation in the preantibiotic era was an afebrile young child with painless persistent watery ear drainage, an enlarged preauricular lymph node, a history of contact with a person with tuberculosis, and often facial nerve paralysis.[80,91]

Tuberculous mastoiditis not always is painless.[95] Sometimes, the diagnosis initially is suspected only when a mastoidectomy wound does not heal.[44,73] Early in the course of the disease, physical examination may reveal small yellow spots (caseating granulomas) on a thickened and hyperemic tympanic membrane. These spots coalesce early and produce tympanic membrane perforations.[44] The discharge through these perforations initially is watery, but later it becomes purulent.[43] Pale, avascular granulation tissue is abundant throughout the middle ear and mastoid and often is seen in the external auditory canal and around the tympanic membrane perforation.[7,80,95] Preauricular and postauricular nontender, enlarged lymph nodes may be present,[43,52] and early and severe hearing loss is characteristic.[95] Often, there is evidence of tuberculosis elsewhere in the body.[95] A tuberculin skin test usually, but not always, is positive.[57]

DIFFERENTIAL DIAGNOSIS

Postauricular swelling, a chronically draining ear, or radiographic evidence of mastoid abnormalities also can appear in other disease entities. Postauricular lymphadenopathy can occur secondary to a scalp infection, causing postauricular swelling. The swelling would be discrete, would not displace the pinna, and would not obliterate the postauricular sulcus.[24] Severe otitis externa may lead to periauricular cellulitis, with postauricular swelling, erythema, and tenderness.[32] Mumps can cause parotid swelling, pushing the earlobe up and out, but the swelling is over the parotid gland, rather than located postauricularly. Histiocytosis,[49] acute lymphocytic leukemia,[58] acute myelogenous leukemia,[89] Burkitt lymphoma,[93] aneurysmal bone cysts,[13] and other benign and malignant tumors of the mastoid bone[12] also can manifest as symptoms clinically suggestive of mastoiditis. Kawasaki disease may mimic acute mastoiditis with postauricular lymph node swelling and ear pain.[74] Children with severe and recurrent ear infections may have an underlying congenital or acquired immunodeficiency.

SPECIFIC DIAGNOSIS

The diagnosis of mastoiditis can be made on clinical grounds alone when a child has an acute episode of fever, otitis media, and posterior auricular tenderness and fluctuance. Temporal bone computed tomography (CT) always is recommended to confirm the diagnosis. Mastoiditis is much less likely when swelling and tenderness over the mastoid process are absent, such as when an infection has been masked by antibiotic treatment or when it has extended to an area other than over the mastoid process. Mastoiditis needs to be considered in all cases of otitis media not responding to antibiotics and in all intracranial suppurative diseases that do not have an apparent focus.

Obtaining an aspirate from the middle ear is an important part of properly diagnosing and managing mastoiditis. Gram stains of aspirates from the middle ear are quite accurate and, as such, can help in the initial selection of antibiotic therapy for chronic mastoiditis. Brook[6] found that in 24 children with tympanocentesis, half of the Gram stains showed a complete correlation with subsequent culture results and the other half showed a partial correlation (one bacterial species was not seen). Leukocytes were seen on all the Gram stains. In addition, cultures from the middle ear accurately reflect mastoid disease.[24,53]

Ginsburg and associates[24] compared the results of cultures of middle ear aspirates and mastoid cultures in 16 patients with acute mastoiditis and found that the same bacterial species was isolated from both sites. A sterile aspiration through an intact tympanic membrane gives the most accurate culture information. If the tympanic membrane is perforated, the purulent drainage may be contaminated by colonizing ear canal flora. An aspirate for culture generally should be obtained from the ear drainage, preferably from as close to the perforation as possible. Aspiration of postauricular fluctuance also is useful in identifying the responsible organisms.[29,64] In addition, specimens should be obtained directly from the mastoid at surgery. All of them should be sent for aerobic and anaerobic cultures with proper anaerobic transport

technique. If the child has had a chronic ear infection, or if the child is in a high-risk population for acquiring tuberculosis, mycobacterial stains and cultures also should be obtained and a tuberculin skin test should be placed or a serum immunologic-based tuberculosis test should be performed.

A lumbar puncture should be performed if the clinical presentation suggests meningeal irritation. A CT scan should be obtained before performing the lumbar puncture if papilledema or a suggestion of focal intracranial extension is present. Lymphocytosis of the cerebrospinal fluid suggests a parameningeal focus of infection. An immunologic evaluation should be considered if a child has had recurrent episodes of otitis media leading to mastoiditis.

Peripheral white blood cell counts in mastoiditis may be normal or elevated, often with an increase in band-form neutrophils.[30,59] The erythrocyte sedimentation rate often is elevated in acute mastoiditis, but it usually is normal in chronic mastoiditis.[69]

Mastoiditis often can be diagnosed on clinical findings alone. When a patient is not improving on medical therapy, radiologic imaging of the temporal bone is needed. Changes in the temporal bone are seen best on a CT scan.[84] Early in the course of mastoiditis, nonspecific clouding of the middle ear and mastoid is seen. With time, necrosis and coalescence of the bony septa occur. Other CT findings in mastoiditis include hypoaeration of the mastoid and adjacent bony destruction. A CT scan of a child with unilateral mastoiditis complicated by lateral sinus thrombosis and elevated intracranial pressure is shown in Figure 17-2. Magnetic resonance imaging is valuable to search for suppurative intracranial complications of mastoiditis.[83,84] In addition, sometimes magnetic resonance angiography or venography (or both) is indicated to diagnose sigmoidal sinus thrombosis.[12]

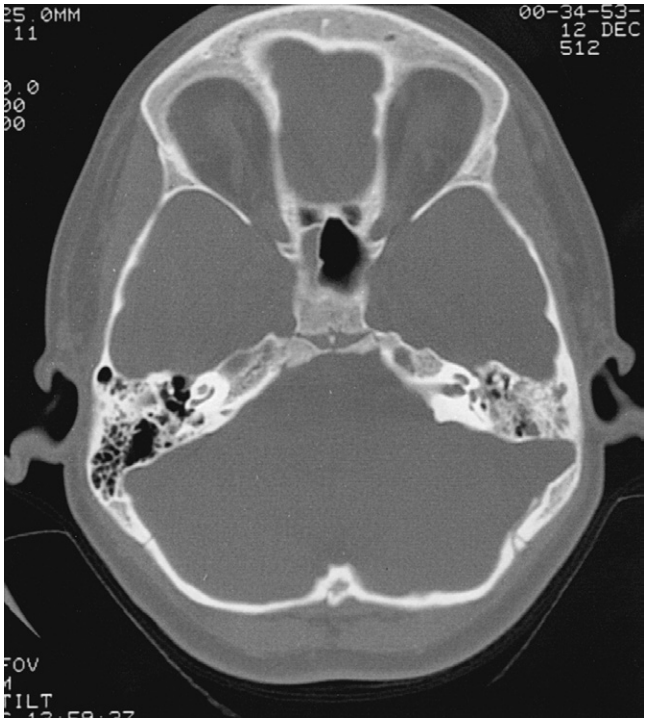

FIGURE 17-2 ■ Computed tomographic scan of the head of a 12-year-old boy with left lateral sinus thrombosis and increased intracranial pressure complicating left mastoiditis. The left mastoid air cells are opacified with marked loss of the fine bony septa, in contrast to the right mastoid air cells.

TREATMENT

Medical and surgical treatments differ for acute versus chronic mastoiditis. For acute mastoiditis, the mainstay of treatment is antimicrobial therapy. The initial choice of antibiotics is made empirically after appropriate cultures have been obtained. To address the most common causes of acute mastoiditis (*S. pneumoniae*, *S. pyogenes*, *S. aureus*, and *H. influenzae*), oxacillin 150 mg/kg/day given every 6 hours or cefazolin 100 mg/kg/day given every 8 hours and cefotaxime 200 mg/kg/day divided every 6 hours or ceftriaxone 100 mg mg/kg/day given every 12 hours are reasonable initial choices. When intracranial complications are suspected, substituting vancomycin 60 mg/kg/day intravenously every 6 hours for oxacillin or cefazolin is recommended to cover resistant pneumococci or staphylococci. Antibiotic therapy for acute bacterial mastoiditis should conform to the principles of therapy for osteomyelitis in that treatment is begun with intravenous antibiotics with continuation of long-term intravenous therapy only in those cases with intracranial extension with an organism for which no effective oral antibiotic exists (e.g., *P. aeruginosa*). Otherwise, after several days to a week, most patients respond well clinically and can be transferred to high-dose oral antibiotic therapy.[55] Total duration of therapy should be based on clinical response and the normalization of the white blood cell count, sedimentation rate, and the C-reactive protein value. Usually, minimum therapy will be 3 weeks, but it may possibly last longer depending on severity of infection, complications, and what organism is isolated. Tailored antibiotic therapy to a specific cultured organism is ideal but not always possible, especially because many children with acute mastoiditis will have already undergone some form of outpatient antibiotic therapy.

All children with acute mastoiditis may potentially require surgical intervention in the form of myringotomy with pressure equalization tube placement and/or mastoidectomy; therefore, it is imperative that pediatricians and otolaryngologists work together in treating mastoiditis.[24,30,59,64,97] The timing and degree of surgical intervention for acute mastoiditis varies by center. Traditionally, the mainstay of treatment of acute mastoiditis in children has been myringotomy with or without placement of ventilation tubes, based on several large series.[20,45] This procedure is necessary for obtaining middle ear fluid cultures, for relieving pain, and for evacuating pus from the middle ear cavity.[85]

Mastoidectomy refers to removing the infected and inflamed mastoid air cells, thereby eliminating the further multiplication and spread of infection. In acute mastoiditis, indications for more complete surgical intervention in the form of a mastoidectomy include postauricular

fluctuance; facial nerve palsy; nausea, vomiting, and vertigo suggestive of labyrinthitis; meningitis; brain abscess; venous sinus thrombosis with or without intracranial hypertension; epidural or subdural empyema; and petrositis. In addition, patients with acute mastoiditis treated initially with parenteral antibiotics who have progression of postauricular swelling, fever, otalgia, or purulent otorrhea should also undergo mastoidectomy.* There is a growing trend toward upfront conservative management of pediatric mastoiditis with intravenous antibiotics and myringotomy and tube placement alone, reserving mastoidectomy for cases with complications or failure to respond to conservative treatment.[85] One pediatric center in France recently published an algorithm for treatment of acute mastoiditis based on their series of 188 children treated between 2001 and 2008. Clinical acute mastoiditis at that center is treated initially with tympanocentesis or postauricular puncture under local anesthesia and parenteral broad-spectrum antibiotics with clinical reassessment at 48 hours. Mastoidectomy is performed only in patients who fail to improve on intravenous antibiotics alone, which in this series was necessary in only 33 percent of cases.[75]

Recently, retroauricular puncture with tympanostomy tube placement has been advocated in lieu of a formal mastoidectomy, especially in cases of subperiosteal abscesses, because it has been shown to be effective in limiting the number of mastoidectomies performed and shortening the hospital stay.[2] Surgical treatment should always include culture of the middle ear or mastoid, which is becoming increasingly important as penicillin-resistant pneumococci and methicillin-resistant staphylococci have become more widespread.† In addition, during the mastoidectomy, inflammatory tissue should always be sent for histologic evaluation to exclude the rare case of eosinophilic granuloma or rhabdomyosarcoma, which can present similarly to acute mastoiditis.

In children with acute mastoiditis secondary to a cholesteatoma, modified radical mastoidectomy with removal of the cholesteatoma should be performed. In most cases, a "second-look" operation 6 to 9 months later is performed to ensure complete eradication of squamous epithelium from the middle ear cleft.

Preoperative audiograms should also be obtained in all children with mastoiditis to assess for conductive versus sensorineural hearing loss, both of which can occur with acute and chronic mastoiditis.

The antimicrobial and surgical treatment in chronic mastoiditis differs from acute mastoiditis. By definition, symptoms have been present for longer than 1 month in children with chronic mastoiditis. In these children, *S. aureus*, gram negative bacilli (especially *P. aeruginosa*), and anaerobes are seen more frequently (see Table 17-1). Therefore, combination broad-spectrum intravenous antibiotics are recommended. An aminoglycoside such as gentamicin 7.5 mg/kg/day divided every 8 hours can be administered for gram-negative bacillary coverage and a semisynthetic penicillin such as piperacillin-tazobactam 200 to 300 mg/kg/day divided every 6 hours can be used to cover *P. aeruginosa* (synergistic with gentamicin), anaerobes, and methicillin-sensitive *S. aureus*. As with cases of acute mastoiditis, antibiotic therapy is tailored according to culture results from the middle ear or mastoid. In cases of intracranial extension caused by *P. aeruginosa*, cefepime 150 mg/kg/day divided every 8 hours is recommended because of its improved penetration of cerebrospinal fluid.

Indications for surgical intervention in patients with chronic mastoiditis include all of the indications for acute mastoiditis, with the addition of patients who have persistent chronic otorrhea with bony changes on radiographs. Complete mastoidectomy is advocated in these children because of the duration and therefore likely extent of diseased mastoid and middle ear mucosa. The presence of a middle ear cholesteatoma should always be suspected, especially in patients with chronic unremitting otorrhea.

In the unusual case of *M. tuberculosis* mastoiditis, antituberculous chemotherapy should be started in a manner similar to treatment of bony tuberculosis (see Chapter 96). It is also important to remember the possibility of underlying immunodeficiency in children with mastoiditis who demonstrate a slow response to medical and surgical therapy.

The treatment of complications of acute and chronic mastoiditis needs to be tailored to the specific complication. Treatment of subperiosteal abscesses includes postauricular needle aspiration under local anesthesia, incision and drainage under local anesthesia with placement of a surgical drain, and mastoidectomy. More recent series have advocated the more minimally invasive treatments under local anesthesia, citing a 92 percent improvement rate without the complications of mastoidectomy.[85] Treatment of a Bezold abscess requires surgical drainage in addition to intravenous antibiotics.

When facial nerve paralysis occurs, treatment consists of myringotomy with pressure equalization tube placement along with parenteral antibiotics. In cases of complete facial paralysis, a mastoidectomy is thought to be beneficial.[15]

Otogenic meningitis is treated similarly to other forms of meningitis (see Chapter 31). Mastoidectomy is theoretically beneficial, although not urgent, and is infrequently performed.

In intracranial abscesses, in addition to intravenous antibiotics, surgical treatment should consist of a complete mastoidectomy because the confinement of the infection in the middle ear and mastoid allows for the disease to spread beyond the confines of the mastoid bone. Intracranial drainage either from the mastoid route or via a formal craniotomy should also be done.[40] Small, multifocal, or inaccessible intracranial lesions can be treated with antibiotics alone with a mastoidectomy[35] (see also Chapter 32).

Treatment of lateral sinus thrombosis includes long-term antibiotics, pressure equalization tube placement, and mastoidectomy to remove the infective focus with either needle aspiration of the sinus or thrombectomy. The use of anticoagulation for otogenic lateral sinus thrombosis is controversial and determined on an individual basis. Children who develop lateral sinus

*References 14, 25, 29, 30, 59, 64, 87.
†References 1, 17, 31, 37, 61, 71, 94.

thrombosis should be evaluated for an underlying hypercoagulable state. Although rare, it is essential to consider lateral sinus thrombosis in any child with a recent history of otomastoiditis presenting with headaches and/or neurologic deficits.

PROGNOSIS

The prognosis of mastoiditis depends on the extent of the infection and the causative organism. Mastoiditis that is treated adequately early in the course of illness before the onset of intracranial extension has a very good prognosis. Facial nerve paralysis is reversible early on,[80,90] and benign intracranial hypertension resolves with treatment of the mastoiditis.[25] Permanent neurologic deficits and death may occur if mastoiditis extends to cause meningitis, brain abscesses, epidural abscess, subdural empyema, or venous sinus thrombophlebitis. Symptoms of mastoiditis may recur if antibiotic therapy is of insufficient duration or if surgical débridement of an infected bone or of an infected cholesteatoma is inadequate. Sensorineural and conductive hearing deficits are reversible early on in mastoiditis; however, chronic infection may produce irreversible hearing loss.

PREVENTION

Early adequate antibiotic treatment of otitis media reduces significantly a child's risk for developing mastoiditis. In addition, rapid treatment of known mastoiditis along with early investigation of persistent ear drainage, persistent ear pain, or an otitis media that is not responding to antibiotic management decreases the risk for developing suppurative complications associated with mastoiditis.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Bakhos D, Trijolet JP, Moriniere S, et al. Conservative management of acute mastoiditis in children. *Arch Otolaryngol Head Neck Surg* 2011;**137**:346–50.
3. Bales CB, Sobol S, Wetmore R, et al. Lateral sinus thrombosis as a complication of otitis media: 10-year experience at the children's hospital of Philadelphia. *Pediatrics* 2009;**123**:709–13.
10. Choi SS, Lander L. Pediatric acute mastoiditis in the post-pneumococcal conjugate vaccine era. *Laryngoscope* 2011;**121**:1072–80.
20. Geva A, Oestreicher-Kedem Y, Fishman G, et al. Conservative management of acute mastoiditis in children. *Int J Pediatr Otorhinolaryngol* 2008;**72**:629–34.
23. Ghosh PS, Ghosh D, Goldfarb J, et al. Lateral sinus thrombosis associated with mastoiditis and otitis media in children: a retrospective chart review and review of the literature. *J Child Neurol* 2011;**26**:1000–4.
35. Isaacson B, Mirabal C, Kutz Jr JW, et al. Pediatric otogenic intracranial abscesses. *Otolaryngol Head Neck Surg* 2010;**142**:434–7.
48. McAvoy MJ, Carron MA, Poulik J, et al. Sequelae of rapid growing mycobacteria otomastoiditis in a child. *Arch Otolaryngol Head Neck Surg* 2009;**135**:602–4.
51. McMullan B. Bezold's abscess: a serious complication of otitis media. *J Paediatr Child Health* 2009;**45**:616–8.
56. Mudry A. History of instruments used for mastoidectomy. *J Laryngol Otol* 2009;**123**:583–9.
65. Ongkasuwan J, Valdez TA, Hulten KG, et al. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics* 2008;**122**:34–9.
70. Pang LH, Barakate MS, Havas TE. Mastoiditis in a paediatric population: a review of 11 years experience in management. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1520–4.
72. Porcher FK, Losek JD, Jackson BF. Cerebral sinovenous thrombosis associated with mastoiditis secondary to otitis media. *J Pediatr Health Care* 2010;**24**:326–32.
75. Quesnel S, Nguyen M, Pierrot S, et al. Acute mastoiditis in children: a retrospective study of 188 patients. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1388–92.
77. Scardapane A, Del Torto M, Nozzi M, et al. Gradenigo's syndrome with lateral venous sinus thrombosis: successful conservative treatment. *Eur J Pediatr* 2010;**169**:437–40.
81. Stahelin-Massik J, Podvinec M, Jakscha J, et al. Mastoiditis in children: a prospective, observational study comparing clinical presentation, microbiology, computed tomography, surgical findings and histology. *Eur J Pediatr* 2008;**167**:541–8.
82. Stenfeldt K, Hermansson A. Acute mastoiditis in southern Sweden: a study of occurrence and clinical course of acute mastoiditis before and after introduction of new treatment recommendations for AOM. *Eur Arch Otorhinolaryngol* 2010;**267**:1855–61.
85. Tamir S, Shwartz Y, Peleg U, et al. Shifting trends: mastoiditis from a surgical to a medical disease. *Am J Otolaryngol* 2010;**31**:467–71.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Antonelli PJ, Dhanani N, Giannoni CM, et al. Impact of resistant pneumococcus on rates of acute mastoiditis. *Otolaryngol Head Neck Surg* 1999;**121**:190–4.
2. Bakhos D, Trijolet JP, Moriniere S, et al. Conservative management of acute mastoiditis in children. *Arch Otolaryngol Head Neck Surg* 2011;**137**:346–50.
3. Bales CB, Sobol S, Wetmore R, et al. Lateral sinus thrombosis as a complication of otitis media: 10-year experience at the children's hospital of Philadelphia. *Pediatrics* 2009;**123**:709–13.
4. Bluestone CD, Klein JO. *Otitis media in infants and children*. Philadelphia: Saunders; 1988. p. 233–44.
5. Bluestone CD, Klein JO. Intracranial suppurative complications of otitis media and mastoiditis. In: Bluestone CD, Stool SE, Kenna MA, editors. *Pediatric otolaryngology*. Philadelphia, Saunders, 1996. p. 636–48.
6. Brook I. Aerobic and anaerobic bacteriology of chronic mastoiditis in children. *Am J Dis Child* 1981;**135**:478–9.
7. Buchanan G, Rainer EH. Tuberculous mastoiditis. *J Laryngol Otol* 1988;**102**:440–6.
8. Burston BJ, Pretorius PM, Ramsden JD. Gradenigo's syndrome: successful conservative treatment in adult and paediatric patients. *J Laryngol Otol* 2005;**119**:325–9.
9. Carpenter J, Stapleton S, Holliman R. Retrospective analysis of 49 cases of brain abscess and review of the literature. *Eur J Clin Microbiol Infect Dis* 2007;**26**:1–11.
10. Choi SS, Lander L. Pediatric acute mastoiditis in the post-pneumococcal conjugate vaccine era. *Laryngoscope* 2011;**121**: 1072–80.
11. Davison FW. Otitis media—then and now. *Laryngoscope* 1955;**65**: 142–51.
12. Davison SP, Facer GW, McGough PF, et al. Use of magnetic resonance imaging and magnetic resonance angiography in diagnosis of sigmoid sinus thrombosis. *Ear Nose Throat J* 1997;**76**:436–41.
13. deVries EJ, Kamerer DB, Rafalko D. Aneurysmal bone cyst masquerading as acute mastoiditis. *Otolaryngol Head Neck Surg* 1989;**100**:613–6.
14. Doyle KJ, Jackler RK. Otogenic cavernous sinus thrombosis. *Otolaryngol Head Neck Surg* 1991;**104**:873–7.
15. Elliott CA, Zalzal GH, Gottlieb WR. Acute otitis media and facial paralysis in children. *Ann Otol Rhinol Laryngol* 1996;**105**:58–62.
16. Finegold SM. Anaerobic infections in otolaryngology. *Ann Otol Rhinol Laryngol Suppl* 1981;**90**(Suppl 84):13–6.
17. Fridkin SK, Hageman JC, Morrison M, et al. Methicillin-resistant Staphylococcus aureus disease in three communities. *N Engl J Med* 2005;**352**:1436–44.
18. Gaffney RJ, O'Dwyer TP, Maguire AJ. Bezold's abscess. *J Laryngol Otol* 1991;**105**:765–6.
19. Garcia RD, Baker AS, Cunningham MJ, et al. Lateral sinus thrombosis associated with otitis media and mastoiditis in children. *Pediatr Infect Dis J* 1995;**14**:617–23.
20. Geva A, Oestreicher-Kedem Y, Fishman G, et al. Conservative management of acute mastoiditis in children. *Int J Pediatr Otorhinolaryngol* 2008;**72**:629–34.
21. Ghaffar FA, Wordemann M, McCracken Jr GH. Acute mastoiditis in children: a seventeen-year experience in Dallas, Texas. *Pediatr Infect Dis J* 2001;**20**:376–80.
22. Gherman CR, Ward RR, Bassis ML. *Pneumocystis carinii* otitis media and mastoiditis as the initial manifestation of the acquired immunodeficiency syndrome. *Am J Med* 1988;**85**:250–2.
23. Ghosh PS, Ghosh D, Goldfarb J, et al. Lateral sinus thrombosis associated with mastoiditis and otitis media in children: a retrospective chart review and review of the literature. *J Child Neurol* 2011;**26**:1000–4.
24. Ginsburg CM, Rudoy R, Nelson JD. Acute mastoiditis in infants and children. *Clin Pediatr (Phila)* 1980;**19**:549–53.
25. Glasscock ME, Shambaugh GE, Johnson GD. *Surgery of the ear*. Philadelphia: Saunders; 1990.
26. Goldstein NA, Casselbrant ML, Bluestone CD, et al. Intratemporal complications of acute otitis media in infants and children. *Otolaryngol Head Neck Surg* 1998;**119**:444–54.
27. Goycoolea MV, Jung TTK. Complications of suppurative otitis media. In: Paparella MM, Shumrick DA, Gluckman JL, et al, editors. *Otolaryngology*. 3rd ed. Philadelphia: W. B. Saunders; 1991. p. 1381–404.
28. Hall PJ, Farrior JB. *Aspergillus* mastoiditis. *Otolaryngol Head Neck Surg* 1993;**108**:167–70.
29. Hawkins DB, Dru D. Mastoid subperiosteal abscess. *Arch Otolaryngol* 1983;**109**:369–71.
30. Hawkins DB, Dru D, House JW, et al. Acute mastoiditis in children: a review of 54 cases. *Laryngoscope* 1983;**93**:568–72.
31. Hofmann J, Cetron MS, Farley MM, et al. The prevalence of drug-resistant *Streptococcus pneumoniae* in Atlanta. *N Engl J Med* 1995;**333**:481–6.
32. Hopkin RJ, Bergeson PS, Pinckard KC, et al. Otitis externa posing as mastoiditis. *Arch Pediatr Adolesc Med* 1994;**148**:1346–9.
33. Hoppe JE, Koster S, Bootz F, et al. Acute mastoiditis—relevant once again. *Infection* 1994;**22**:178–82.
34. Hughes CE, Spear RK, Shinabarger CE, et al. Septic pulmonary emboli complicating mastoiditis: Lemierre's syndrome revisited. *Clin Infect Dis* 1994;**18**:633–5.
35. Isaacson B, Mirabal C, Kutz Jr JW, et al. Pediatric otogenic intracranial abscesses. *Otolaryngol Head Neck Surg* 2010;**142**:434–7.
36. Istorico LJ, Sanders M, Jacobs RF, et al. Otitis media due to blastomycosis: report of two cases. *Clin Infect Dis* 1992;**14**:355–8.
37. Kaplan SL, Mason Jr EO, Wald ER, et al. Pneumococcal mastoiditis in children. *Pediatrics* 2000;**106**:695–9.
38. Keeler JC. *Modern otology*. Philadelphia: F. A. Davis; 1930. p. 417–81.
39. Koitschev A, Simon C, Lowenheim H, et al. Delayed otogenic hydrocephalus after acute otitis media in pediatric patients: the changing presentation of a serious otologic complication. *Acta Otolaryngol* 2005;**125**:1230–5.
40. Kurien M, Job A, Mathew J, et al. Otogenic intracranial abscess: concurrent craniotomy and mastoidectomy—changing trends in a developing country. *Arch Otolaryngol Head Neck Surg* 1998;**124**: 1353–6.
41. Leskinen K, Jero J. Acute mastoiditis caused by *Moraxella catarrhalis*. *Int J Pediatr Otorhinolaryngol* 2003;**67**:31–3.
42. Levine M. *Practical otology*. Philadelphia: Lea & Febiger; 1938.
43. Lincoln EH, Sewell EM. *Tuberculosis in children*. New York: McGraw-Hill; 1963.
44. Lucente FE, Tobias GW, Parisier SC, et al. Tuberculous otitis media. *Laryngoscope* 1978;**88**:1107–16.
45. Luntz M, Brodsky A, Nusem S, et al. Acute mastoiditis—the antibiotic era: a multicenter study. *Int J Pediatr Otorhinolaryngol* 2001;**57**:1–9.
46. Lutter SA, Kerschner JE, Chusid MJ. Gradenigo syndrome: a rare but serious complication of otitis media. *Pediatr Emerg Care* 2005;**21**:384–6.
47. MacAdam AM, Rubio T. Tuberculous otomastoiditis in children. *Am J Dis Child* 1977;**131**:152–6.
48. McAvoy MJ, Carron MA, Poulik J, et al. Sequelae of rapid growing mycobacteria otomastoiditis in a child. *Arch Otolaryngol Head Neck Surg* 2009;**135**:602–4.
49. McCaffrey TV, McDonald TJ. Histiocytosis X of the ear and temporal bone: review of 22 cases. *Laryngoscope* 1979;**89**:1735–42.
50. McGill TJ, Merchant S, Healy GB, et al. Congenital cholesteatoma of the middle ear in children: a clinical and histopathological report. *Laryngoscope* 1991;**101**:606–13.
51. McMullan B. Bezold's abscess: a serious complication of otitis media. *J Paediatr Child Health* 2009;**45**:616–8.
52. Meyerhoff WL. Granulomas and other specific diseases of the ear and temporal bone. In: Paparella MM, Shumrick DA, editors. *Otolaryngology*. Philadelphia: Saunders; 1980. p. 1548–75.
53. Moloy PJ. Anaerobic mastoiditis: a report of two cases with complications. *Laryngoscope* 1982;**92**:1311–5.
54. Mongkolrattanothai K, Oram R, Redleaf M, et al. Tuberculous otitis media with mastoiditis and central nervous system involvement. *Pediatr Infect Dis J* 2003;**22**:453–6.
55. Moore JA, Wei JL, Smith HJ, et al. Treatment of pediatric suppurative mastoiditis: is peripherally inserted central catheter (PICC) antibiotic therapy necessary? *Otolaryngol Head Neck Surg* 2006;**135**: 106–10.
56. Mudry A. History of instruments used for mastoidectomy. *J Laryngol Otol* 2009;**123**:583–9.
57. Mumtaz MA, Schwartz RH, Grundfast KM, et al. Tuberculosis of the middle ear and mastoid. *Pediatr Infect Dis* 1983;**2**:234–6.
58. Nabors MW, Narayan RK, Poplack DG. Intracranial and otological presentation of acute lymphocytic leukemia. *Neurosurgery* 1985;**17**:309–12.

59. Nadal D, Herrmann P, Baumann A, et al. Acute mastoiditis: clinical, microbiological, and therapeutic aspects. *Eur J Pediatr* 1990;**149**:560–4.
60. Nadol JB. Chronic otitis media. In: Nadol JB, Schuknecht HF, editors. *Surgery of the ear and temporal bone*. New York: Raven Press; 1993. p. 155–70.
61. Nelson CT, Mason Jr EO, Kaplan SL. Activity of oral antibiotics in middle ear and sinus infections caused by penicillin-resistant *Streptococcus pneumoniae:* implications for treatment. *Pediatr Infect Dis J* 1994;**13**:585–9.
62. Nylen O, Alestig K, Fasth A, et al. Infections of the ear with non-tuberculous mycobacteria in three children. *Pediatr Infect Dis J* 1994;**13**:653–6.
63. Oestreicher-Kedem Y, Raveh E, Kornreich L, et al. Complications of mastoiditis in children at the onset of a new millennium. *Ann Otol Rhinol Laryngol* 2005;**114**:147–52.
64. Ogle JW, Lauer BA. Acute mastoiditis: diagnosis and complications. *Am J Dis Child* 1986;**140**:1178–82.
65. Ongkasuwan J, Valdez TA, Hulten KG, et al. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics* 2008;**122**:34–9.
66. Oyediran AB, Fajemisin AA, Abioye AA, et al. Infection of the mastoid bone with a *Paragonimus*-like trematode. *Am J Trop Med Hyg* 1975;**24**:268–73.
67. Ozdemir D, Cakmakci H, Ikiz AO, et al. Sigmoid sinus thrombosis following mastoiditis: early diagnosis enhances good prognosis. *Pediatr Emerg Care* 2005;**21**:606–9.
68. Palva A, Karma P, Karja J. Cholesteatoma in children. *Arch Otolaryngol* 1977;**103**:74–7.
69. Palva T, Pulkkinen K. Mastoiditis. *J Laryngol Otol* 1959;**73**: 573–88.
70. Pang LH, Barakate MS, Havas TE. Mastoiditis in a paediatric population: a review of 11 years experience in management. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1520–4.
71. Pikis A, Akram S, Donkersloot JA, et al. Penicillin-resistant pneumococci from pediatric patients in the Washington, DC, area. *Arch Pediatr Adolesc Med* 1995;**149**:30–5.
72. Porcher FK, Losek JD, Jackson BF. Cerebral sinovenous thrombosis associated with mastoiditis secondary to otitis media. *J Pediatr Health Care* 2010;**24**:326–32.
73. Procter B. Chronic otitis media and mastoiditis. In: Paparella MM, Shumrick DA, editors. *Otolaryngology*. Philadelphia: W. B. Saunders; 1980. p. 1455–89.
74. Puczynski MS, Stankiewicz JA, Ow PE. Mucocutaneous lymph node syndrome mimicking acute coalescent mastoiditis. *Am J Otol* 1986;**7**:71–3.
75. Quesnel S, Nguyen M, Pierrot S, et al. Acute mastoiditis in children: a retrospective study of 188 patients. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1388–92.
76. Reynolds SJ, Behr M, McDonald J. *Turicella otitidis* as an unusual agent causing a posterior auricular abscess. *J Clin Microbiol* 2001; **39**:1672–3.
77. Scardapane A, Del Torto M, Nozzi M, et al. Gradenigo's syndrome with lateral venous sinus thrombosis: successful conservative treatment. *Eur J Pediatr* 2010;**169**:437–40.
78. Schondorf HJ, Roth B, Streppel M. Bezold's abscess following chronic mastoiditis in a newborn. *Ann Otol Rhinol Laryngol* 2004;**113**:843–5.
79. Sennaroglu L, Sozeri B. Otogenic brain abscess: review of 41 cases. *Otolaryngol Head Neck Surg* 2000;**123**:751–5.
80. Singh B. Role of surgery in tuberculous mastoiditis. *J Laryngol Otol* 1991;**105**:907–15.
81. Stahelin-Massik J, Podvinec M, Jakscha J, et al. Mastoiditis in children: a prospective, observational study comparing clinical presentation, microbiology, computed tomography, surgical findings and histology. *Eur J Pediatr* 2008;**167**:541–8.
82. Stenfeldt K, Hermansson A. Acute mastoiditis in southern Sweden: a study of occurrence and clinical course of acute mastoiditis before and after introduction of new treatment recommendations for AOM. *Eur Arch Otorhinolaryngol* 2010;**267**:1855–61.
83. Swartz JD. Temporal bone inflammatory disease. In: Som PM, Curtin HD, editors. *Head and neck imaging*. St. Louis: Mosby; 1996. p. 1391–431.
84. Swartz JD, Harnsberger HR. *Imaging of the temporal bone*. 2nd ed. New York, N.Y.: Thieme Medical Publishers; 1992.
85. Tamir S, Shwartz Y, Peleg U, et al. Shifting trends: mastoiditis from a surgical to a medical disease. *Am J Otolaryngol* 2010;**31**:467–71.
86. Tarabichi M, Schloss M. Actinomycosis otomastoiditis. *Arch Otolaryngol Head Neck Surg* 1993;**119**:561–2.
87. Taylor MF, Berkowitz RG. Indications for mastoidectomy in acute mastoiditis in children. *Ann Otol Rhinol Laryngol* 2004;**113**:69–72.
88. Teichgraeber JF, Per-Lee JH, Turner Jr JS. Lateral sinus thrombosis: a modern perspective. *Laryngoscope* 1982;**92**:744–51.
89. Todd Jr NW, Bowman CA. Acute myelogenous leukemia presenting as atypical mastoiditis with facial paralysis. *Int J Pediatr Otorhinolaryngol* 1984;**7**:173–7.
90. Tovi F, Leiberman A. Silent mastoiditis and bilateral simultaneous facial palsy. *Int J Pediatr Otorhinolaryngol* 1983;**5**:303–7.
91. Turner AL. Tuberculosis of the middle ear cleft in children. *J Laryngol Rhinol Otol* 1915;**30**:209–47.
92. van den Bosch MA, Vos JA, de Letter MA, et al. MRI findings in a child with sigmoid sinus thrombosis following mastoiditis. *Pediatr Radiol* 2003;**33**:877–9.
93. Welling DB, McCabe BF. American Burkitt's lymphoma of the mastoid. *Laryngoscope* 1987;**97**:1038–42.
94. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000;**343**:1917–24.
95. Windle-Taylor PC, Bailey CM. Tuberculous otitis media: a series of 22 patients. *Laryngoscope* 1980;**90**:1039–44.
96. Zanetti D, Nassif N. Indications for surgery in acute mastoiditis and their complications in children. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1175–82.
97. Zapalac JS, Billings KR, Schwade ND, Roland PS. Suppurative complications of acute otitis media in the era of antibiotic resistance. *Arch Otolaryngol Head Neck Surg* 2002;**128**:660–3.
98. Zoller H. Acute mastoiditis and its complications: a changing trend. *South Med J* 1972;**65**:477–80.

CHAPTER 18

Croup (Laryngitis, Laryngotracheitis, Spasmodic Croup, Laryngotracheobronchitis, Bacterial Tracheitis, and Laryngotracheobronchopneumonitis) and Epiglottitis (Supraglottitis)

James D. Cherry

The term *croup* is used to identify several different respiratory illnesses characterized by varying degrees of inspiratory stridor, cough, and hoarseness resulting from obstruction in the region of the larynx. The etiology of croup syndromes is diverse, and the consideration of noninfectious possibilities in the differential diagnosis is of major importance. Table 18-1 classifies etiologic considerations in supraglottic, laryngeal, and infraglottic acute obstructions.

Croup is discussed under the subheadings of laryngitis, laryngotracheitis, spasmodic croup, laryngotracheobronchitis, bacterial tracheitis, and laryngotracheobronchopneumonitis. Diphtheric croup is presented in Chapter 90.

Epiglottitis (supraglottitis) is an illness characterized by inflammation and edema of the epiglottis and frequently also of the arytenoepiglottic folds and ventricular bands at the base of the epiglottis.[80] Until the present *Haemophilus influenzae* type b vaccine era this disorder was usually caused by *H. influenzae* type b and was mainly a disease of children. The illness is characterized by rapid onset and progression, and, without treatment, death caused by obstruction of the airway occurs. Epiglottitis in children is a pediatric otolaryngologic emergency.

HISTORICAL ASPECTS

The word *croup* is derived from the Anglo-Saxon word *kropan*, "to cry aloud."[81] Until the 20th century, most croup-like illnesses were thought to be diphtheria. Diphtheritic croup is an ancient disease that has been traced to the time of Homer. The historical trail of diphtheria disappeared in the 5th century and did not reappear until 1100 years later. In the 16th century, epidemics were noted in Europe. Top[411] credits Bretonneau for differentiating diphtheritic croup from spasmodic croup in 1826. In the middle third of the 20th century, the history of croup was marked by three important events: (1) the rapid decline in incidence of diphtheria associated with the use of toxoid, (2) the introduction and widespread use of antibiotics, and (3) the advent of tissue culture techniques, resulting in the establishment of viruses as etiologic agents. After these three events occurred, a prevalent academic view was that all croup was of viral etiology, and bacteria generally were dismissed as causative agents.[82,94,127,325] However, a careful review of many publications from the first half of the 20th century indicates a causative role for several bacteria, in addition to *Corynebacterium diphtheriae*, in croup.* Bacterial croup (bacterial tracheitis) was rediscovered in 1979.†

In the 1940s, Davison[107] separated spasmodic croup from other, more severe forms of croup. The clinical and pathologic aspects of this entity were poorly defined, and today it often is not separated clinically from more severe forms of croup.

The early history of epiglottitis is obscure, probably because of the importance of diphtheritic croup.[81] In 1887, Baron[19] described in detail a 30-year-old woman with epiglottitis who recovered after treatment with hot poultices and steam with tincture of benzoin. In 1900, Theisen[410] described three cases in the United States. Not until the early 1940s did acute epiglottitis become recognized as a definite clinical entity caused by *H. influenzae* type b.[4,30,371] In 1948, Rabe,[324] in a study of 347 children with "infectious croup," presented evidence for the division of the clinical illness into three etiologic categories—diphtheritic croup, viral croup, and *H. influenzae* type b croup (acute epiglottitis). Since the early

*References 27, 54, 56, 105, 107, 131, 158, 195, 209, 280, 299, 311, 331, 332.

†References 79, 80, 90, 113, 118, 121, 125, 128, 130, 163, 173, 180, 188, 213, 220, 258, 259, 278, 286, 298, 302, 383, 396, 397, 404, 431, 451.

TABLE 18-1 **Clinical Considerations in Acute Supraglottic, Laryngeal, and Infraglottic Obstructions**

Infectious
Acute epiglottitis
Laryngitis
Laryngeal diphtheria
Laryngotracheitis
Laryngotracheobronchitis
Laryngotracheobronchopneumonitis
Bacterial tracheitis
Spasmodic croup
Mechanical
Foreign body
Secondary to trauma resulting from intubation
Extrinsic or intrinsic mass
Allergic
Acute angioneurotic edema

Data from references 78, 84, 94, 135, 142, 146, 301, 324, 457.

1990s, a dramatic decrease in the number of cases of epiglottitis has occurred in developed countries because of the widespread use of *H. influenzae* type b conjugate vaccines.*

TERMINOLOGY

The terminology and classification of infectious illnesses involving the larynx and supraglottic and infraglottic regions have evolved over time. Classifications often have mixed etiologic systems with anatomic systems and have led to confusion. Croup often has been presented in articles under the heading of *laryngotracheobronchitis* when the authors actually were discussing laryngotracheitis and spasmodic croup.† The term *membranous croup* has been used as the title for articles dealing with bacterial croup.[113,173] This use is confusing because membranous croup historically was diphtheria. Many articles dealing with bacterial croup also have been titled *bacterial tracheitis*.‡ This term seems inappropriate because most cases of bacterial croup seen today have lower respiratory tract involvement in addition to tracheal findings. Table 18-2 lists the classifications and definitions used in this chapter.

ETIOLOGY OF CROUP SYNDROMES

The etiologic agents in laryngitis, laryngotracheitis, spasmodic croup, laryngotracheobronchitis, and laryngotracheobronchopneumonitis are presented by frequency and severity of illness in Table 18-3. Laryngitis is a common manifestation of infection with many respiratory viruses in older children, adolescents, and adults. Outbreaks of laryngitis in closed population groups (e.g., boarding schools and military training camps) most frequently are caused by adenovirus types 4 and 7, and community outbreaks most often are noted in association with epidemic influenza. Sporadic instances of laryngitis usually are caused by adenoviral infections. Laryngitis also has been reported in association with group A streptococcal infections; the incidence of this association has varied from 2 to 40 percent.[51,288,420] Recently Somenek and associates[387] described an 11-month-old boy with severe membranous laryngitis caused by methicillin-resistant *Staphylococcus aureus* (MRSA).

Generally accepted today is that acute laryngotracheitis and spasmodic croup, which rarely are differentiated clinically, are caused by infection with many different viruses. Although numerous studies of respiratory viral infection exist, almost no attempt has been made to delineate the differences in etiologic spectrum by severity of illness.

Parainfluenza virus type 1 is the most common cause of acute laryngotracheitis and is responsible for frequent and clearly delineated fall and winter epidemics. Croup with parainfluenza type 2 virus seldom is severe but occasionally is related to small outbreaks. Parainfluenza virus type 3 is a frequent cause of sporadic but severe illness.

The most severe laryngotracheitis has been noted in association with influenza A viral infections. Respiratory syncytial virus and several different adenoviruses frequently are isolated in croup. Generally, these illnesses are not severe, but lower respiratory tract involvement occasionally is a problem. Laryngeal, tracheal, and bronchial involvement commonly occurs in measles.[82] Although rhinoviruses, *Mycoplasma pneumoniae*, enteroviruses, herpes simplex virus, and reoviruses have been associated with croup, they generally cause only minimal distress. More recently, croup has been noted in association with infection with the novel coronavirus NL63, human bocavirus, and human metapneumovirus.* Recurrent croup may be due to infection with a human papillomavirus.[457]

Bacteria, other than *H. influenzae* in epiglottitis and *C. diphtheriae* in membranous croup, generally were dismissed as causative agents in croup until more recently.[94,127,325] Many publications on laryngotracheobronchitis from the first half of the 20th century indicate a role for several common bacterial pathogens.† In 1979, bacterial croup was rediscovered,[213] and numerous reports of this illness have been published since then.‡

In the reports from the preantibiotic era, *Streptococcus pyogenes* was the pathogen implicated most frequently. Since 1979, *S. aureus* has been the agent implicated most commonly. Other important bacteria are *Streptococcus pneumoniae* and *H. influenzae*. More recently, *Moraxella*

*References 5, 59, 132, 143, 160, 164, 257, 281, 284, 318, 421, 433, 452.
†References 84, 215, 260, 279, 319, 342, 373, 405.
‡References 90, 118, 119, 125, 128, 130, 188, 213, 220, 258, 259, 298, 383, 431, 451.

*References 15, 24, 85, 336, 394, 423.
†References 27, 54, 56, 90, 106, 118, 128, 131, 156, 163, 195, 209, 280, 299, 311, 331, 396, 404, 431.
‡References 34, 113, 121, 125, 130, 173, 180, 188, 220, 258, 259, 278, 286, 298, 301, 383, 397, 451.

TABLE 18-2 Classification and Definition of Infectious Illnesses Involving the Larynx and Supraglottic and Infraglottic Regions

Category	Other Terms	Definitions
Supraglottitis	Epiglottitis	Infection of the epiglottis, and/or arytenoepiglottic folds and ventricular bands of the base of the epiglottis resulting in swelling and upper airway obstruction
Laryngitis		Inflammation of larynx resulting in hoarseness; usually occurs in older children and adults in association with common upper respiratory tract viral infection
Laryngeal diphtheria	Membranous croup, true croup, diphtheritic croup	Infection involving larynx and other areas of upper and lower airway caused by *Corynebacterium diphtheriae,* resulting in gradually progressive obstruction of airway and associated inspiratory stridor
Laryngotracheitis	False croup, virus croup, acute obstructive subglottic laryngitis	Inflammation of larynx and trachea usually caused by infection with parainfluenza and influenza viruses
Laryngotracheobronchitis and laryngotracheo-bronchopneumonitis	Membranous laryngotracheobronchitis, pseudomembranous croup	Inflammation of larynx, trachea, and bronchi or lung, or both; usually similar in onset to laryngotracheitis, but more severe illness; bacterial infection frequently has causative role
Bacterial croup	Bacterial tracheitis, membranous croup, membranous tracheitis, membranous laryngotracheobronchitis, pseudomembranous croup	Severe form of laryngotracheitis, laryngotracheobronchitis, or laryngotracheobron-chopneumonitis resulting from bacterial infection
Spasmodic croup	Spasmodic laryngitis, catarrhal spasm of the larynx, subglottic allergic edema	Illness characterized by sudden onset at night of inspiratory stridor; associated with mild upper respiratory tract infection without inflammation or fever but with edema in subglottic region

TABLE 18-3 Etiologic Agents in Laryngitis, Spasmodic Croup, Laryngotracheitis, Laryngotracheobronchitis, and Laryngotracheobronchopneumonitis Presented by Frequency and Severity of Illness

Category	Etiologic Agents	Frequency*	Associated with Outbreaks	Severity†	References
Laryngitis	Adenoviruses				
	Types 4 and 7	++++	Yes	+ to +++	100,187,420
	Types 2, 3, 5, 8, 11, 14, and 21	+++	No	+ to +++	
	Influenza viruses Types a and b	++++	Yes	+ to ++++	16,187,313,420
	Parainfluenza viruses				187,420
	Type 1	++	Yes	+ to +++	
	Types 2 and 3	+	Yes	+ to ++	
	Coronavirus	++	Yes	++	23
	Rhinoviruses and respiratory syncytial virus	++	No	+ to ++	162,288,302,376
	Enteroviruses	+	No	+	187,420
	Streptococcus pyogenes	+ to +++	Yes	+ to ++	51,288
Laryngotracheitis and spasmodic croup	Parainfluenza viruses	++++		+ to +++	37,74-76,82,111,113,150,152,157, 181,186,189,193,235,249,261, 262,267,292,313,316,317,320, 336,424,440
	Type 1	++++	Yes		
	Type 2	++	Yes		
	Type 3	++	No		
	Influenza viruses	++			60,74,75,78,111,112,129,141,150, 157,189,191,193,261,267,292, 313,316,317,320,336,424,440
	Type a	+++	Yes	+ to ++++	
	Type b	+	Yes	+ to ++	

Continued on following page

TABLE 18-3 **Etiologic Agents in Laryngitis, Spasmodic Croup, Laryngotracheitis, Laryngotracheobronchitis, and Laryngotracheobronchopneumonitis Presented by Frequency and Severity of Illness** (Continued)

Category	Etiologic Agents	Frequency*	Associated with Outbreaks	Severity†	References
	Respiratory syncytial virus	++	No	+ to ++	73-76,82,111,113,149,150,157,189,235,262,287,316,317,319,413,430,445,453
	Human metapneumovirus	++	Yes	+	15
	Coronavirus	++	Yes	++	85,336,394,423
	Human bocavirus	+	No	+	24,336
	Measles virus	++	Yes	+ to +++	81
	Adenoviruses	++	No	+ to ++	49,73,74,76,78,110,157,186,189,235,249,261,262,292,316,317,319,336,390,413,424,430
	Unspecified types and types 1, 2, 3, 5, 6, and 7				
	Rhinoviruses	+	No	+	78,150,215,261,292,336
	Mycoplasma pneumoniae	+	No	+	73,76,78,110-112,157,189,262,336
	Enteroviruses	+	No	+	74,82,95,149,150,157,189,203,261,287,336,391,413,445
	Coxsackievirus type A9	+	No	+	
	Coxsackievirus types B4 and B5	+	No	+	
	Echoviruses types 4, 11, and 21	+	No	+	
	Herpes simplex viruses	+	No	+	189,197,236,292,391
	Reoviruses	+	No	+	453
	Human papillomavirus	+	No	+ to +++	457
Laryngotracheobronchitis and laryngotracheo-bronchopneumonitis	Parainfluenza viruses types 1, 2, and 3	+	No	+++	54,149,158,186,262,280,315,317
	Influenza viruses types a and b	+	No	++++	129,141,189,316
	Staphylococcus aureus, Staphylococcus pyogenes, Streptococcus pneumoniae, Haemophilus Influenzae, and *Moraxella catarrhalis*	++	No	++++	27,34,54,56,106,107,110,121,125,130,131,156,173,180,188,195,209,213,220,258,259,278,279,299,301,311,331,332,383,451
	Other bacteria	±	No	++++	130,173,185,220,258,286,451
	Cryptosporidium	—	No	++	175

*++++, Most frequent; +++, frequent; ++, occasional; +, rare; —, questionable.
†++++, Most severe; +++, severe; ++, not severe; +, minimal distress.

catarrhalis has been found to be the causative agent in several cases.[34,130,220,451] In most instances, bacterial croup is likely to be the result of bacterial superinfection in viral disease.* *Cryptosporidium* also has been recovered from the trachea of an infant with a subacute illness.[175]

ETIOLOGY OF SUPRAGLOTTITIS

Acute supraglottitis in children in the prevaccine era almost always was caused by *H. influenzae* type b.† In 34 pediatric series in which blood cultures were performed in 1570 children, *H. influenzae* type b was recovered in 76 percent. Supraglottitis caused by other bacteria was rare. In the 1570 children with blood cultures, the following other organisms were recovered: *S. pneumoniae*, 3; *S. aureus*, 3; *Haemophilus parainfluenzae*, 2; *H. influenzae* type a, 1; *H. influenzae* nontypable, 2; and *Bacillus* sp. 1.

In countries where *H. influenzae* type b immunization is routine, supraglottitis is a rare illness in children. Nevertheless, cases still occur in association with some infectious agents. The following pathogens have been implicated in this regard: *S. pneumoniae; S. aureus; H. parainfluenzae;* groups A, B, C, and G streptococci; *Pseudomonas aeruginosa* (in a patient with severe combined immunodeficiency syndrome); untypable *H. influenzae;* and *Bacillus* spp.* *Candida tropicalis* was isolated from the blood of a 3½-year-old girl with supraglottitis who had

*References 64, 90, 125, 173, 209, 259, 278, 297, 298, 301, 306.
†References 22, 25, 26, 30, 31, 42, 46, 50, 53, 55, 57, 61, 65, 87, 88, 120, 133, 154, 164, 184, 208, 263, 270, 271, 276, 282, 285, 289, 324, 371, 380, 382, 427, 428.

*References 18, 29, 33, 122, 198, 244, 246, 256, 263, 341, 374, 379, 444, 454.

been the recent recipient of an autologous bone marrow transplant.[432]

Candida albicans was noted in a case in a newborn whose mother had vaginal candidiasis and in a 6-year-old boy with chronic mucocutaneous candidiasis.[3] *Candida* spp. epiglottitis was noted in two children infected with human immunodeficiency virus (HIV),[296,369] and *C. albicans* epiglottitis was observed recently in a 2-year-old girl who was immunocompromised as a result of receiving chemotherapy for a primitive neuroectodermal tumor.[273]

Necrotizing epiglottitis in a 5-year-old boy with hemophagocytic lymphohistiocytosis associated with a mixed infection (*Enterococcus faecalis*, *Eikenella corrodens*, *Prevotella melaninogenica*, and *Neisseria* spp.) has been described.[233] Reed and colleagues[329] described a 17-year-old boy with an aryepiglottic abscess associated with a mixed infection (*Capnocytophaga ochraceae*, *Bacteroides stercoris*, and *P. melaninogenica*).

The possibility that supraglottitis could be caused by a virus has been noted: a 16-month-old child with type 1 herpes simplex virus stomatitis complicated by stridor and respiratory distress had an epiglottis and arytenoepiglottic folds that were edematous and covered with vesicular lesions resembling those in the oral mucosa.[44] An 18-year-old girl had supraglottitis that also was caused by herpes simplex virus.[284] In addition, parainfluenza type 3 and influenza type b viruses were isolated from the nasopharynx of two children with supraglottic inflammation.[167] *Haemophilus paraphrophilus*[214] was recovered from the epiglottic surface of a single patient, as was *M. catarrhalis* in another patient.[426]

In adults, *H. influenzae* type b also has been the major cause of epiglottitis, but other organisms occur more commonly in adults than in children.* In 1992, Daum and Smith[101] reviewed 474 published cases of epiglottitis in adults, 293 of whom had blood cultures performed; 79 of those cultures (27%) yielded *H. influenzae*. Of these positive cultures, 43 were *H. influenzae* type b; 35 isolates were not typed, and 1 isolate was not *H. influenzae* type b. Trollfors and associates[417] in Sweden found that of blood cultures obtained from 185 of 356 (52%) adult patients, *H. influenzae* was isolated from 53 percent. Of these, 53 were *H. influenzae* type b and the type of the remaining 45 was not known.

S. pneumoniae was reported to be isolated from the blood of 18 adults with supraglottitis, 10 of whom were receiving immunosuppressive therapy or were infected with HIV-1,† and *H. parainfluenzae* was isolated from the blood of 5 patients.[86,140,268,357,436] In Denmark, between 1995 and 2002, *H. influenzae* type f was recovered from 13 cases of epiglottitis in adults.[62] Numerous other infectious agents have been implicated in case reports of adults with supraglottitis.‡ These agents include *S. pneumoniae*, *H. influenzae* type b, *Aeromonas hydrophila*, *Pasteurella multocida*, *Kingella kingae*, *Klebsiella pneumoniae*, group A and B streptococci, *Bacteroides* spp., *Fusobacterium necrophorum*, *Vibrio vulnificus*, *Serratia marcescens*, *S. aureus*, *Neisseria meningitidis*, *Aspergillus flavus*, herpes simplex virus, and parainfluenza virus type 2. Epiglottitis has been reported as a complication of infectious mononucleosis caused by Epstein-Barr virus infection.[66]

Epiglottitis also can result from noninfectious causes. Hot foods and water can cause thermal epiglottitis, as can poisoning with corrosive agents, including cocaine alkaloid.[28,225,241,247] Hereditary angioedema may manifest with findings typical of epiglottitis.[308]

EPIDEMIOLOGY OF CROUP

Croup accounts for approximately 15 percent of lower respiratory tract disease seen in pediatric practice. In a large 11-year study in a pediatric practice in Chapel Hill, North Carolina, Denny and associates[112] noted the incidence of croup by age and sex. The highest attack rate occurred in children 7 to 36 months of age. Few cases occurred after the sixth birthday. Hoekelman[185] studied the occurrence of illness prospectively in 246 full-term, first-born, well infants during their first year of life. Three infants (1.2%) had croup during the study year. The analysis of a pediatric practice with approximately 3000 active records and approximately 10,000 yearly visits of children younger than 5 years old disclosed five cases of croup in a group of 50 consecutive hospitalized patients.[52]

Although croup occurs occasionally in older children, most cases occur within the first 3 years of life.[112,127,135,342,364]

Croup occurs more commonly in boys than in girls.* In a 6-year study in Alberta, Canada involving 20,079 emergency department visits, 61.3 percent were boys.[344] In the Chapel Hill studies, an increase was noted in the number of croup cases beginning in September, with a peak in October and November, and then a decrease during the next 7-month period.[112] In a 2-year emergency department study in Toronto involving 1700 cases, the peak month of visits and hospital admissions was found to be October.[364] Marx and associates[272] reviewed the National Hospital Discharge Survey data for hospitalizations for croup between 1979 and 1993. They also examined Centers for Disease Control and Prevention laboratory-based surveillance data and published reports with virus isolation studies. Major peaks in hospitalizations for croup occurred in October of odd-number years at the time of peak parainfluenza virus type 1 activity. Minor peaks in hospitalizations for croup occurred each year in February when influenza A, influenza B, and respiratory syncytial viral infections were common occurrences. Epidemic peaks of acute laryngotracheitis reflect community-wide activity with parainfluenza 1 and 2 viruses or influenza A or B outbreaks.[112,158,272]

In the Alberta, Canada study in 1990 to 2000 and 2004 to 2005, biennial trends were apparent.[344,345] Peak emergency department visits for croup occurred in November

*References 19, 48, 50, 80, 86, 99, 134, 144, 145, 155, 159, 176, 178, 204, 205, 211, 226, 238, 274, 300, 303, 339, 360, 370, 378, 392, 401, 417, 418.

†References 41, 50, 200, 224, 226, 250, 324, 343, 346, 367.

‡References 12, 45, 48, 72, 114, 145, 155, 159, 183, 199, 204, 205, 210, 222, 255, 264, 269, 282, 295, 300, 303, 309, 333-335, 349, 360, 375, 389, 429, 455.

*References 32, 112, 237, 343, 345, 346.

for odd years and in February for the other years. For the odd years, the overall rate increased from 43.4/1000 in 1999 to 2000 to 49.6/1000 in 2003 to 2004. A similar increase was noted in the even years (30.9/1,000 in 2000 to 2001 to 34.1/1000 in 2004 to 2005).

In the Toronto study, the time of the visit to the emergency department was analyzed.[364] The peak number of visits occurred between 10 PM and 4 AM. During this period, approximately 17 percent of the children seen were admitted to the hospital. In contrast, of children seen between noon and 6 PM, approximately 50 percent were admitted to the hospital. Quite similar findings were noted in Alberta in the 6-year period between 1999 and 2005.[344,345] A study of croup hospitalizations in Ontario over 14 years from 1988 to 2002 noted a biennial mid-autumn peak and an annual summer trough throughout the entire study period.[363] A striking finding in this study was a marked decrease in hospitalizations for croup after the winter of 1993 to 1994. This decrease continued in linear fashion for the remainder of the study duration. The authors suggest that the decreasing trend in croup hospitalizations was likely due to the increasing use of corticosteroid treatment in patients who presented to emergency departments with croup.

Because croup is caused by the same viruses that cause other respiratory illnesses, the method of spread probably is similar for all (see discussion of the common cold in Chapter 7). In children, most spread involves close person-to-person contact, with large droplets of virus-containing nasal secretions being applied to the nose from the hands of the future host or by close-range airborne acquisition. Parainfluenza viruses are common causes of colds in adults, so older individuals with trivial illnesses may be the source of more severe childhood croup.

EPIDEMIOLOGY OF SUPRAGLOTTITIS

In the prevaccine era, the epidemiology of invasive *H. influenzae* type b disease varied markedly among population groups.[433,434] Epiglottitis also differed by population group, but this variation was not related necessarily to the rate of overall *H. influenzae* type b invasive disease in the population. For example, among Alaskan Eskimos and Navajo Native Americans, whose risk for acquiring invasive *H. influenzae* type b disease was 4 to 10 times that of most other American populations, epiglottitis was not recognized among 295 patients with invasive *H. influenzae* type b illness.[93,434]

Of *H. influenzae* type b invasive disease, the percentage of cases of epiglottitis varied markedly among localities. For example, in Israel, only 0.3 percent of invasive *H. influenzae* type b infections were epiglottitis, whereas in Ireland, Wales, northeast England, Australia (Sydney), and Denmark, the percentage of epiglottitis cases varied between 16 and 32 percent.* In Minnesota and Dallas County, Texas, only 6 and 3 percent, respectively, of invasive *H. influenzae* type b infections were epiglottitis.[293] In contrast with these findings in the United States, Europe, and Australia, the most common manifestation of invasive *H. influenzae* type b infection in Sweden was epiglottitis.[87,416] In Sweden, the incidence of epiglottitis in children 14 years of age or younger in 1981 to 1983 was 10/100,000 per year. The annual incidence in Minnesota and Dallas County, Texas, in children 5 years of age or younger in 1982 to 1984 was 5.4 and 4.4 cases, respectively, per 100,000 per year.[294]

In children in the United States in the prevaccine era, the peak occurrence of epiglottitis was during the third year of life and 72 percent of all cases occurred in children 1 to 5 years of age.* The disease occurred more commonly in boys than in girls; in 8 studies with 611 cases, 58 percent of cases were in boys.†

In specific geographic areas, marked differences in the yearly percentage of cases of epiglottitis occurred, but no intercity, national, or international cycles of illness were demonstrated.‡ Seasonal prevalence varied by locality but was not marked. The greatest number of cases in three studies occurred in the winter and spring,[33,208,289] whereas Baxter[28] observed more cases during the summer and in November, and Cohen and Chai[88] found no seasonal pattern.

Epiglottitis is a disease that occurs most commonly in temperate climates.[136,212] In several U.S. military hospitals, a wide geographic variation in incidence was noted; no cases were found among 4625 admissions at Gorgas Hospital in Panama, whereas 1/600 admissions to Elmendorf Hospital in Alaska was for epiglottitis.[22] In the past, acute epiglottitis had not been reported in either Taiwan or Hong Kong.[206]

Epiglottitis also occurs in adults but less commonly than in children.[144,274,401,419] Annual rates in Rhode Island, Denmark, Finland, and northern California were 1.0, 0.9, 0.2, and 1.8/100,000, respectively. In both Ireland and Israel, rates of reported epiglottitis in adults appear to be increasing.[31,171] Whether these increases are true increases or are the result of increased awareness is not apparent.

In the present *H. influenzae* type b conjugate vaccine era, the incidence of epiglottitis has fallen dramatically in all countries employing routine immunization, as has the incidence of all invasive disease caused by *H. influenzae*.§ In northern Finland, the incidence in children 4 years of age or younger fell from 7.6/100,000 before 1988 to 0/100,000 after 1988.[5] In Sweden, in the 10-year period from 1986 to 1996, the rate of epiglottitis in children younger than 14 years fell from 8.2/100,000 to less than 1/100,000.[151] At the Children's Hospital of Philadelphia, the average annual incidence of epiglottitis declined from 10.9/10,000 admissions before 1990 to 1.8/10,000 admissions from 1990 through 1992.[164] In this study, investigators also noted that the median age of patients increased from 35.5 months before 1990 to 80.5 months in the post-1989 period.

It should be noted that sporadic cases of epiglottitis mostly caused by non–*H. influenzae* type b viruses

*References 98, 139, 190, 239, 277, 322.

*References 22, 28, 33, 88, 133, 208.
†References 22, 28-30, 33, 88, 133, 208, 289.
‡References 22, 28, 88, 133, 208, 289.
§References 5, 59, 132, 147, 164, 169, 257, 281, 284, 317, 421, 433, 452.

continue to occur in the United States.[366] In 2006, 3305 cases were reported.

In 2006, an increase in incidence of invasive *H. influenzae* type b disease, including acute epiglottitis, was reported in England.[281] Many of the cases occurred in children who had received *H. influenzae* type b conjugate vaccine at 2, 3, and 4 months of age but not a booster dose in the second year of life.

PATHOLOGY AND PATHOGENESIS OF CROUP

Laryngoscopic studies in acute laryngotracheitis reveal redness and swelling of the lateral walls of the trachea, just below the vocal cords.[105,107,399] Because the subglottic trachea is surrounded by a firm cartilaginous ring, the inflammatory swelling can occur only by encroaching on the patency of the airway; the subglottic space often is reduced to a slit 1 to 2 mm wide. As the disease progresses, the tracheal lumen becomes obstructed further by a fibrinous exudate, and its surface is covered by pseudomembranes composed of the exudative material. The vocal cords frequently are swollen, and their mobility is impaired.

Histologic study of postmortem material from the larynx and trachea reveals marked edema and cellular infiltration in the lamina propria, submucosa, and adventitia. The cellular infiltrate includes histiocytes, lymphocytes, plasma cells, and polymorphonuclear leukocytes.[54,56,291,311,332] The cotton rat model of laryngotracheitis caused by human parainfluenza virus type 3 reveals pathologic findings similar to those noted in human postmortem studies.[312] This model also indicates the time course of events. Early in the course of an infection (first 2 to 4 days), moderate mucosal and submucosal inflammatory infiltrates were noted in the subglottic and proximal tracheal regions. The infiltrates initially contained lymphocytes and neutrophils, and subsequently a mononuclear infiltrate with lymphocytes and macrophages in the submucosa was noted. Cell injury was most marked on days 6 to 8 after infection. The ciliated epithelial cells were blunted, with loss of cilia in large patches. By 12 days after infection, the region reverted to a nearly normal appearance.

The older literature indicates that classic laryngotracheobronchitis and the same disease with pneumonia represent extension of disease from the trachea to the bronchi and alveoli. The progressive obstructive disease with exudate and pseudomembrane obstruction at the bronchial and bronchiolar levels usually is the result of secondary bacterial involvement. In bacterial croup, the tracheal wall is infiltrated with inflammatory cells, and ulceration, pseudomembranes, and microabscess formation occur.* In addition to the findings in laryngotracheitis of viral origin, thick pus is present within the lumen of the trachea and lower air passages.[180,220,258,278] Spasmodic croup is an enigma because it occurs in association with respiratory viral infections similar to those that cause more severe laryngotracheitis. Using direct laryngoscopy, Davison[105] noted that the subglottic tissues in spasmodic croup showed noninflammatory edema.

Although the eventual site of clinically important pathologic change in laryngotracheitis is within the larynx and trachea, the initial acquisition of infection is similar to that of other respiratory viral infections and occurs within the upper air passages, including the nasal and pharyngeal epithelial surfaces. After acquisition of virus, infection of the cells of the local respiratory epithelium develops and spreads locally to involve the larynx and trachea. The initial symptoms of nasal stuffiness and throat irritation reflect the primary sites of involvement. Studies in organ culture systems and in the cotton rat model have shown that several respiratory viruses inhibit tracheal ciliary function and eventually lead to marked destruction of the epithelium and evidence of viral infection in the lamina propria.[232,312,330] In uncomplicated croup, failure of gas exchange within the lung, in addition to hypoxia resulting from subglottic tracheal obstruction, may occur.[302,405]

Parainfluenza viruses types 1, 2, and 3 all are significant causes of respiratory infections in children (see Chapter 178). Infection with parainfluenza virus type 3 occurs in most infants, whereas infections with types 1 and 2 generally occur more frequently in older children. Only a third of children have antibodies against these two viral types by the time they reach 4 years of age.

Because parainfluenza viral infections are common occurrences in young children, and because only a few get croup, host factors probably are important in the pathogenesis. In contrast to the numerous studies of the pathogenesis of bronchiolitis and respiratory syncytial virus infection, few similar studies relating to parainfluenza viruses and croup have been reported.[83]

These pathologic data suggest that the findings in laryngotracheitis are directly virus-related (parainfluenza viruses types 1, 2, and 3 and influenza virus types A and B) because of the direct cytopathic effect of a virus, are related to the concomitant host response, or perhaps both.[83] In the cotton rat model, the use of topical steroid therapy led to a significant reduction in the degree of inflammatory infiltrates and cell injury, which suggests that the host response contributes to the pathologic findings.[312] The pathogenesis of laryngotracheobronchitis and laryngotracheobronchopneumonitis is similar to that described previously, but with the extension of infection to the lower respiratory tract and usually the occurrence of secondary bacterial infection.

Many studies suggest that allergic factors play a role in recurrent croup.* Welliver and associates[443] found that children with croup caused by parainfluenza viral infections had titers of IgE-specific antibody in their nasopharyngeal secretions that were 3.6-fold higher than titers of similarly infected children with only upper respiratory tract infections. The children with croup had a cell-mediated immune response to parainfluenza virus that was 1.6-fold greater than that of the children with only upper respiratory tract infections.

*References 6, 77, 80, 81, 84, 90, 113, 118, 121, 124, 130, 163, 180, 188, 213, 220, 258-260, 301, 306, 383, 397.

*References 68, 182, 305, 422, 441-443, 450, 456.

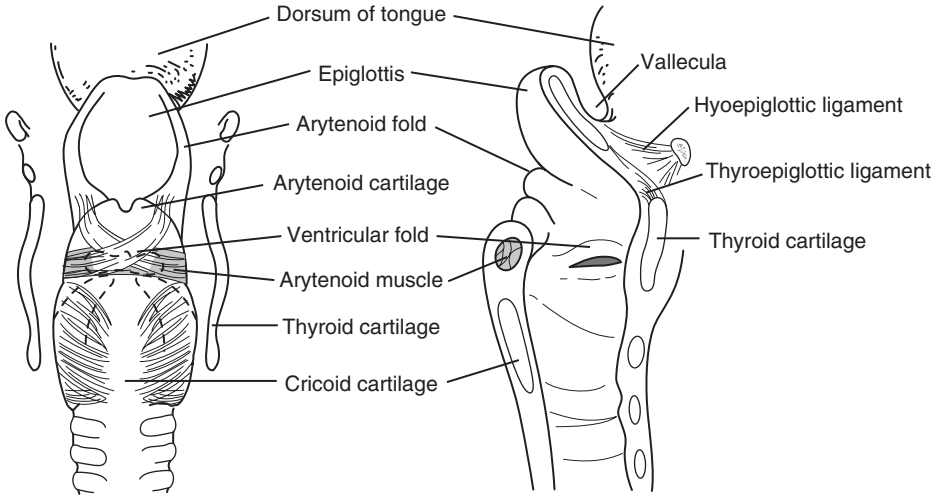

FIGURE 18-1 ■ Anatomic relationships of the supraglottic larynx. Posterior view *(left)* and sagittal view *(right).*

In a subsequent review, investigators noted that an atopic disposition might be associated with the development of croup.[440] Children with croup caused by a parainfluenza virus had specific IgE antibodies and released histamine into the airway more frequently than did control patients with parainfluenzal viral upper respiratory tract infections. They also noted that children with recurrent croup had several atopic features, such as positive skin tests to environmental allergens, and were more likely to develop asthma when they grew older. Also, some children with a history of recurrent croup develop stridor with histamine challenge.

The above-mentioned studies by Welliver and associates[441,442] and other reports make it easy to accept a role of atopy in recurrent croup. What is difficult to explain, however, is why many apparent primary infections result in only spasmodic croup. As I have suggested previously, initial sensitization may be parainfluenza virus group–specific and not type-specific.[37] This proposal suggests that early infection (primary infection) with parainfluenza virus type 3 would set the stage for spasmodic croup with parainfluenza viral types 1 and 2. The primary infection itself could have been mild owing to transplacentally acquired antibody.

ANATOMY AND PATHOPHYSIOLOGY OF SUPRAGLOTTITIS

The thin, elastic, leaflike epiglottic cartilage is attached to the anterior surface of the thyroid cartilage by the thyroepiglottic ligament (Fig. 18-1). The hyoepiglottic ligament also provides support and anchors the epiglottis to the hyoid bone. The superior aspect of the epiglottis arches slightly posteriorly. Stratified squamous epithelium covers the anterior surface of the epiglottis and the superior third of the posterior portion; respiratory epithelium covers the remaining posterior surface. The stratified squamous epithelium is loosely adherent and creates a large potential space for the accumulation of inflammatory cells and edema fluid.

The arytenoepiglottic folds arise from the epiglottis and terminate posteriorly near the paired arytenoid cartilages. These structures commonly are involved in the supraglottic infection and occasionally are the site of serious disease without epiglottitis per se.[28] Immediately anterior to the epiglottis are the valleculae epiglotticae, where saliva pools before deglutition.

Supraglottic cellulitis with marked edema involving the epiglottis, arytenoepiglottic folds, ventricular bands, and arytenoids is the hallmark of this illness. As the edema increases, the epiglottis curls posteriorly and inferiorly. Inspiration tends to draw the inflamed supraglottic ring into the laryngeal inlet, whereas expiration is unopposed.[363] This "ball-valve" mechanism is thought to produce slight hypoxia without hypercapnia.[352] Diffuse infiltration with polymorphonuclear leukocytes, hemorrhage, edema, and fibrin deposition can be seen microscopically; this infiltration can progress to microabscesses, with *H. influenzae* type b occasionally seen in the tissue.[212,339] Frank abscess formation has been documented in adults.* Infection of the supraglottic larynx may spread inferiorly to involve the paraglottic space,[177] but as a rule, neither upward extension into the laryngeal lymphatics nor downward extension into the subglottic region occurs.[30,212]

Infection of the supraglottic structures probably arises from direct invasion by *H. influenzae* type b, with subsequent development of bacteremia. The bacteremia appears to be relatively short in duration and of low concentration, as suggested by several observations: (1) the serum concentration of the capsular polysaccharide is directly proportional to the concentration and duration of bacteremia,[353] (2) children with epiglottitis have less *H. influenzae* type b capsular polysaccharide in their sera than do patients with meningitis,[435] and (3) the density of *H. influenzae* type b in blood is significantly lower in patients with epiglottitis than in patients with meningitis.[243,350,393] In 23 patients with epiglottitis, the geometric mean number of organisms was 123

*References 31, 255, 269, 335, 349, 389, 437, 448.

colony-forming units (CFUs)/mL, whereas the geometric mean number in 43 patients with meningitis was 2203 CFU/mL ($p < .001$).[243]

What predisposes the epiglottis to infection is unknown. Possibly, mild trauma to the epiglottis occurs during food intake. This trauma could result in damage to the mucosal surface, which, in turn, could allow the invasion of organisms that already were present in the upper respiratory tract. Also possible is that a viral infection damages the mucosal surface and thus predisposes the patient to secondary bacterial infection.

Acute-phase sera in most children with epiglottitis lack specific *H. influenzae* type b bactericidal and hemagglutinating antibody. Seroconversion regularly occurs after infection.[50,270]

Conflicting evidence exists relating to a possible genetic difference between patients with *H. influenzae* type b epiglottitis and other individuals.[7,166,406,449] Whisnant and associates[448] surveyed human leukocyte antigens (HLAs) and erythrocytic antigens among 30 children with epiglottitis and 20 patients with meningitis. HLA-A11 was found in 3 and 17 percent of the patients with epiglottitis and meningitis, respectively ($p < .01$). HLA-B5 occurred in 3 and 13 percent of patients with meningitis and epiglottitis, respectively ($p < .05$), whereas HLA-B40 occurred more often (23% vs. 10%) in patients with meningitis than in those with epiglottitis ($p < .05$). Moreover, the frequency of HLA-A28 and HLA-B17 was higher among the patients with epiglottitis than in uninfected control subjects.[448] However, the results of another study did not confirm these observations.[166]

The distribution and frequency of MNS erythrocyte antigens in patients with epiglottitis may differ from those observed in others. For example, the NNSS genotype occurred in 6.4 percent of patients with epiglottitis and in 0.5 percent of healthy control subjects ($p < .0002$),[448] but this difference was not confirmed.[166] However, the results of two studies suggest that the MNS genotype occurs less often in patients with *H. influenzae* type b meningitis than in patients with epiglottitis.[166,448] In one study, white children with *H. influenzae* type b meningitis lacked G2m(n), an allotype antigen of immunoglobulin (Ig)G2 subclass heavy chains, more frequently than did control subjects.[7] In another study in a white population in Finland, however, this difference was not noted.[402] The frequency of the Km(1) immunoglobulin allotype in children with epiglottitis did not differ significantly from the prevalence of that marker in blacks or whites. However, in blacks with *H. influenzae* type b meningitis, the Km(1) marker occurred less frequently in patients than in control subjects.[166] The identification of one outer-membrane protein subtype of *H. influenzae* type b that was associated relatively infrequently with epiglottitis suggests that isotype-specific differences in the propensity of *H. influenzae* type b to cause epiglottitis may exist.[403]

CLINICAL PRESENTATION

Acute Laryngitis

Table 18-4 summarizes clinical characteristics of laryngitis. Laryngitis is mainly a disease of older children, adolescents, and adults that is disturbing but self-limited. The specific clinical manifestation is hoarseness. Other symptoms depend on the causative infectious agent. Adenoviruses and influenza viruses cause the most severe instances of laryngitis. With these viruses, fever usually occurs, and sore throat, headache, muscle aches and pains, and prostration are common symptoms. In contrast, patients with laryngitis resulting from rhinoviral, parainfluenza viral, or respiratory syncytial viral infections have minimal or no fever and few systemic complaints. They usually have pronounced nasal symptoms (coryza and stuffiness), however. Occasionally, hoarseness may persist, which may be a result of secondary bacterial infection of the upper respiratory tract.

Acute Laryngotracheitis

Although the clinical spectrum of acute laryngotracheitis varies considerably, its manifestations usually are significantly different from the manifestations of the other acute diseases with obstruction in the region of the larynx (see Table 18-4). Onset of illness usually is not alarming and suggests the onset of a cold. Initial symptoms are nasal complaints and include dryness, irritation, and coryza. Ordinary cough and the complaint of sore throat occur frequently. Fever is a usual occurrence within the first 24 hours, which is not true of the common cold. After a period as short as a few hours, but usually after 12 to 48 hours, upper airway obstructive signs and symptoms are seen. The cough first becomes "croupy" (sounding like a barking seal), and then evidence of respiratory stridor (difficulty associated with inspiration) gradually increases. Examination at this time reveals a child with a hoarse voice, coryza, a normal or minimally inflamed pharynx, and a slightly increased respiratory rate with a prolonged inspiratory phase. Temperature nearly always is between 37.8°C and 40.5°C (100°F and 105°F).

The speed of progression and final degree of upper airway obstruction vary. Some children have hoarseness and barking cough but no other evidence of obstruction; in these cases, the symptoms last approximately 3 to 7 days, with a gradual return to normal. In other cases, the obstruction is progressive and leads to severe respiratory distress with supraclavicular and infraclavicular and sternal retractions, cyanosis of varying degrees, and apprehension. With hypoxia, the cardiac rate increases and the child becomes restless. Without intervention, asphyxial death occurs rapidly in some children. In others, the problem of hypoxia is more prolonged, and respiratory fatigue may lead to the patient's demise. The duration of illness in a severely affected child, regardless of therapy, is rarely less than 7 days and frequently is 14 days.

Laboratory study in acute laryngotracheitis is of only minimal value. The white blood cell count frequently is greater than 10,000 cells/mm^3, and polymorphonuclear cells predominate.[78,304] Very high white blood cell counts (>20,000/mm^3) with numerous band-form neutrophils should suggest bacterial superinfection or the possibility of acute epiglottitis. The posteroanterior chest radiograph reveals the subglottic narrowing (steeple sign), and a lateral neck radiograph indicates the size of the epiglottis.

TABLE 18-4 **Differential Diagnosis of Acute Obstruction in the Region of the Larynx**

Category	Acute Epiglottitis	Laryngeal Diphtheria	Laryngitis	Acute Laryngotracheitis	Laryngotracheobronchitis and Laryngotracheobronchopneumonitis (Including Bacterial Tracheitis)	Spasmodic Croup	Foreign Body	Acute Angioneurotic Edema
Common age of occurrence	1-8 yr	All ages	Older children and adults	3 mo to 3 yr	3 mo to 3 yr	3 mo to 3 yr	All ages	All ages
Past and family history	Not contributory	No or inadequate immunization	Not contributory	Family history of croup	May be family history of croup	Family history of croup; perhaps previous attack	Occasional history of ingestion	Allergic history; perhaps previous attack
Prodrome	Occasionally coryza	Usually pharyngitis	Usually stuffy nose or coryza	Usually coryza	Usually coryza	Minimal coryza	None	Occasionally cutaneous allergic manifestations
Onset (time to full-blown disease)	Rapid; 4-12 hr	Slowly for 2- to 3-day period	Variable; 12 hr to 4 days	Moderate but variable; 12-48 hr	Usually gradually progressive; 12 hr to 7 days	Sudden; always at night	Usually sudden	Rapid
Symptoms on presentation Fever	Yes; usually 39.5°C (103°F)	Yes; usually 37.8°C to 38.5°C (100°F to 101°F)	Yes; 37.8°C to 39.4°C (100°F to 103°F) with adenoviral and influenza viral infections; usually minimal with other viruses	Yes; variable, 37.8°C to 40.5°C (100°F to 105°F)	Yes; variable, 37.8°C to 40.5°C (100°F to 105°F)	No	No, unless secondary infection	No
Hoarseness and barking cough	No	Yes	Yes	Yes	Yes	Yes	Usually no	No
Dysphagia	Yes; usually severe	Usually yes	No	No	No	No	Frequently yes	Yes
Inspiratory stridor	Yes; moderate to severe	Yes; minimal to severe	No	Yes; minimal to severe	Yes; usually severe	Yes; moderate	Variable	Yes
Toxic appearance	Severe	Usually no	No	Usually minimal	Usually moderate; may be severe	No	No	No

TABLE 18-4 **Differential Diagnosis of Acute Obstruction in the Region of the Larynx** (Continued)

Category	Acute Epiglottitis	Laryngeal Diphtheria	Laryngitis	Acute Laryngotracheitis	Laryngotracheobronchitis and Laryngotracheobronchopneumonitis (Including Bacterial Tracheitis)	Spasmodic Croup	Foreign Body	Acute Angioneurotic Edema
Signs on presentation								
Oral cavity	Pharyngitis and excessive salivation	Membranous pharyngitis	Normal or mild to moderate pharyngitis	Usually minimal pharyngitis	Usually minimal pharyngitis	Normal	Normal	Pale appearance
Epiglottis	Cherry-red and swollen	Usually normal; may contain membrane	Normal	Normal	Normal	Normal	Normal	Swollen and pale
Radiographs	Swollen epiglottis on lateral film	Not useful	Not useful	Subglottic narrowing on PA film	Subglottic narrowing on PA film; irregular soft tissue densities within trachea on lateral film	Not useful	May reveal foreign body	Swollen epiglottis on lateral film
Laboratory								
Leukocyte count	Usually markedly elevated with increased percentage of band forms	Usually elevated with increased percentage of band forms	Usually normal	Mildly elevated with >70% polymorphonuclear cells	Variable; usually mildly elevated with 70% polymorphonuclear cells; may be increased band count	Normal	Normal, unless secondary infection	Normal; sometimes eosinophilia
Bacteriology	Throat and blood cultures yield *Haemophilus influenzae* type b	Smear and culture from membrane reveal organism	Usually normal flora in throat; occasionally *Streptococcus pyogenes* in throat	Only important if secondary infection suspected	Normal throat flora; tracheal culture often yields *S. pyogenes, Staphylococcus aureus, Streptococcus pneumoniae,* or *H. influenzae*	Normal flora	Only important if secondary infection suspected	Normal flora
Clinical course	Rapidly progressive; cardiorespiratory arrest occurs within hours if not treated	Slowly progressive obstruction of airway	Hoarseness persists at a constant degree about 4-7 days; occasionally persists 2-3 wk	Variable speed of progression of obstruction; usually does not require surgical intervention	Degree of obstruction usually severe; persists 7-14 days; frequently requires surgical intervention	Symptoms of short duration with treatment; repeated attacks common	Variable depending on size and substance of foreign body	Variable; sometimes leads to rapid asphyxia without therapy

Data from references 105, 107, 110, 125, 137, 148, 180, 213, 258, 259, 283, 407.
PA, Posteroanterior.

Acute Laryngotracheobronchitis and Laryngotracheobronchopneumonitis (Bacterial Tracheitis)

Laryngotracheobronchitis and laryngotracheobronchopneumonitis are far less common occurrences than laryngotracheitis and spasmodic croup; however, these illnesses occur more commonly than generally realized.* These entities may be considered an extension of acute laryngotracheitis, as numerous descriptions in the literature suggest.† The severity of the illness is due to secondary bacterial infection. Initial signs and symptoms are similar to those of laryngotracheitis (see Table 18-4). An afflicted child usually has mild to moderately severe illness for 2 to 7 days and then suddenly becomes markedly worse. Occasionally, upper and lower airway obstructions seem to occur simultaneously. In many children, the distress from tracheal obstruction is of such magnitude that the signs and symptoms of lower respiratory tract involvement go unnoticed. Signs and symptoms associated with extension of disease to the bronchi, bronchioles, and lung substance include rales, air trapping, wheezing, and a further increase in the respiratory rate. Obstruction in these illnesses usually is of such a degree that either intubation or tracheostomy is necessary.

Several instances of laryngotracheobronchopneumonitis with toxic shock syndrome have been observed.[58,77,306,386,397] Generally, children with these staphylococcal infections initially have the onset of croup, then the more severe manifestations of bacterial tracheitis develop, and finally the exanthem and other manifestations of toxic shock syndrome develop. An infant with tracheitis and supraglottitis caused by *M. catarrhalis* has been described.[6] Other findings in laryngotracheobronchitis and laryngotracheobronchopneumonitis are presented in Table 18-4.

Spasmodic Croup

In recent years, the clinical entity of spasmodic croup has been incorporated by many physicians into the overall diagnosis of croup. Although distinguishing mild cases of laryngotracheitis from spasmodic croup is difficult at the onset in some instances, the delineation of the two entities is important from prognostic and therapeutic perspectives (see Table 18-4).

Spasmodic croup occurs in children 3 months to 3 years of age. The onset always is at night, and the characteristic presentation occurs in a child who previously was thought to be well or to have had a mild cold with coryza as the only symptom. The child awakens at night with sudden dyspnea, croupy cough, and inspiratory stridor, but no fever. The symptoms apparently are the result of sudden subglottic edema; relief is achieved easily by general reassurance and administration of moist air. The occurrence of spasmodic croup tends to run in families, with repeated attacks occurring in some children. After one attack, the child is likely to have another attack the same evening and on three or four successive evenings. These attacks can be prevented by employing mild sedation at bedtime and ensuring that the bedroom air is adequately humidified.

Supraglottitis

The classic onset of epiglottitis in children is abrupt, and progression of disease is rapid; careful history on occasion reveals the occurrence of a trivial antecedent upper respiratory tract infection.* The total duration of illness before hospitalization usually is less than 24 hours and occasionally is as short as 2 hours. In one study in which 142 medical records of children with epiglottitis were reviewed, the duration of illness before tracheotomy was performed was found to be 12 hours or less in 73 percent and more than 24 hours in only four patients.[88]

The most common presentation of acute epiglottitis in children includes the sudden onset of fever, severe sore throat, dysphagia, and drooling. Airway obstruction always occurs and is rapidly progressive. It is manifested by distress on inspiration, a choking sensation, irritability, restlessness, and anxiety. The speech is muffled or thick sounding, but hoarseness usually does not occur. The child usually insists on sitting up in a characteristic posture with the arms back, the trunk leaning forward, the neck hyperextended, and the chin pushed forward. This posture increases the diameter of the obstructed airway.

In contrast to acute laryngotracheitis, in which marked inspiratory stridor occurs, the degree of observed stridor in epiglottitis often is not severe. This apparent lack of respiratory distress often leads the unwary physician to underestimate the severity of the child's illness. With progression, the air exchange becomes progressively worse, and hypoxia, hypercapnia, and acidosis develop. These developments cause increased irritability, restlessness, and disorientation, and if an artificial airway is not established, the child will experience sudden cardiorespiratory arrest.

Fever occurs in virtually all children; most temperatures are between 38.8°C and 40.0°C (101.8°F and 104°F). Blood leukocyte counts almost always are elevated; the mean total count was approximately 20,000 cells/mm^3 in five studies.[5,33,88,208,439] The differential cell count reveals an increased percentage of neutrophils and band forms; most patients have absolute band counts that are more than 500 cells/mm^3.

The clinical picture of epiglottitis in adults is more indolent than that in children.[31,176,385,459] Berger and associates[33] reviewed 118 cases of acute epiglottitis seen between 1986 and 2000. Of these patients, 33 percent were admitted to the hospital within 1 day of the onset of symptoms and 77 percent were admitted within 3 days of onset. Symptoms on admission were as follows: sore throat, 85 percent; odynophagia, 83 percent; temperature elevation, 38 percent; respiratory difficulties, 34 percent; muffled voice, 25 percent; and drooling, 7 percent. In two

*References 34, 80, 90, 113, 118, 121, 125, 130, 163, 173, 180, 188, 213, 220, 258, 259, 278, 286, 298, 397, 451.

†References 27, 54, 56, 106, 107, 131, 156, 195, 220, 280, 311, 323, 326, 331, 332.

*References 22, 25, 30, 33, 50, 57, 88, 132, 176, 208, 211, 254, 263, 270, 271, 289, 310, 325, 372, 428, 447.

other studies, an average of 1 to 3 days elapsed before medical aid was sought. The mean temperature was 38.2°C (100.7°F), and some patients were afebrile; the temperature range was 36.6°C to 40.0°C (98.0°F to 104°F). Blood leukocyte counts averaged 17,000/mm^3 (range, 8000 to 32,000/mm^3).[176,385] Sore throat and dysphagia were universal occurrences. Zwahlen and Regamy[459] reviewed the clinical features of 100 reported adult cases of epiglottitis. Of these patients, 78 percent had dyspnea, 49 percent had dysphonia, 41 percent had cyanosis, and 38 percent had stridor. Forty-six percent had edema of the neck. A precordial purring or fluttering sensation was described by one adult patient.[354] In three cases in adults, Ehara[126] noted on physical examination that all had tenderness of the anterior neck over the hyoid bone.

DIFFERENTIAL DIAGNOSIS

The therapeutic approaches to the various acute obstructions in the region of the larynx vary markedly. Establishing a correct diagnosis is essential and frequently lifesaving. Table 18-4 lists the differential points of eight conditions with symptoms and signs of acute upper airway obstruction.

The most frequent serious differential diagnostic problem is the recognition of acute epiglottitis and its separation from the less fulminant laryngotracheitis. In epiglottitis (a rare disease in children today because of universal immunization with *H. influenzae* type b conjugate vaccines), the important differential points are lack of a croupy cough; the presence of a swollen, cherry-red epiglottis; the sitting posture of the child with the chin pushed forward and a reluctance or refusal to lie down; and the relatively greater apprehension and anxiety of the patient than the degree of chest retraction suggests. In contrast, a child with acute laryngotracheitis has a normal epiglottis on examination, always has a typical barking cough, is comfortable in a supine position, and frequently appears to have only minimal apprehension, despite retractions in which the sternum appears to be indenting 2 inches or more.

Early in the course of epiglottitis, the diagnosis can be confirmed only by the observation of the epiglottis, which can be done without difficulty.[234] Later in the disease, the posture of the child and the history of rapidly progressing disease render the differential from laryngotracheitis readily apparent, so examination of the epiglottis directly (a dangerous procedure if the child is forced to lie down) or indirectly by a lateral neck radiograph rarely is indicated and usually is contraindicated.

Laryngotracheobronchitis and laryngotracheobronchopneumonitis can be recognized by signs of lower respiratory involvement (rales, air trapping, wheezing, and pulmonary infiltrates on the radiograph). Bacterial disease should be suspected in laryngotracheobronchitis and laryngotracheobronchopneumonitis and when symptoms and signs become worse in laryngotracheitis. A lateral radiograph can be useful in the evaluation because it may reveal soft tissue densities within the trachea. Lateral neck and chest radiographs are regarded by many physicians as definitive tests to determine whether to rule out epiglottitis and laryngotracheitis. In a careful study by Stankiewicz and Bowes,[388] the sensitivity and specificity of both radiographs were low, however.

Although a rare occurrence today, laryngeal diphtheria always should be considered and ruled out in croup. Recently, Hsia and associates[192] noted a child with Guillain-Barré syndrome who initially presented with a crouplike cough.

Spasmodic croup rarely should be confused with acute laryngotracheitis, but a perusal of the literature indicates that the two entities most commonly are considered laryngotracheitis, which is unfortunate because prognostic considerations for the two entities are different. Spasmodic croup always is of sudden onset at night, occurs without fever, and is relieved by simple therapeutic modalities.

The possibility of a foreign body and angioneurotic edema always must be considered in upper airway obstructive disease. Differential points are presented in Table 18-4. Rarely, acute upper airway obstruction occurs in adolescents as a result of psychogenic and emotional factors.[153,227,351]

SPECIFIC DIAGNOSIS IN CROUP SYNDROMES

The epidemiologic history frequently is an important factor in establishing a specific diagnosis. Obtaining bacterial culture specimens from the throat, laryngeal region, and blood is helpful in diagnosing epiglottitis and important in identifying laryngotracheitis, laryngotracheobronchitis, and laryngotracheobronchopneumonitis when secondary infection is suspected. The white blood cell count should be obtained because it can be helpful when secondary bacterial infection is considered.

A specific etiologic diagnosis can be made by the isolation of virus or its identification by a direct antigen test from a nasopharyngeal specimen. The diagnostic virologic facilities of many medical centers enable rapid identification of parainfluenza viruses, respiratory syncytial viruses, adenoviruses, most rhinoviruses, and influenza viruses to be established.

SPECIFIC DIAGNOSIS IN SUPRAGLOTTITIS

The clinical picture of sore throat, dysphagia, drooling, anxiety, and inspiratory distress without significant stridor and the characteristic sitting position should suggest the presumptive diagnosis in most cases. The definitive anatomic diagnosis is made by visualization of the epiglottis, and the etiologic diagnosis is confirmed by culture of an organism from the blood or the surface of the epiglottis.

In the typical case, the epiglottis is fiery red and greatly swollen. In children, the epiglottis can be seen by simple depression of the tongue with a tongue blade. In older children and adults, indirect or direct laryngoscopy usually is necessary to confirm the diagnosis. On

occasion, the obstruction is caused by swelling of the ventricular bands and the arytenoepiglottic folds so that the epiglottis may appear relatively normal.

Major controversy exists concerning the safety of using a tongue depressor to examine a child with suspected epiglottitis because sudden cardiorespiratory arrest has been noted to occur. However, most instances of cardiorespiratory arrest that I am aware of occurred after the child was forced into a supine position rather than because of the examination itself (Cherry, unpublished data, 1973). In many instances, patients with presumptive epiglottitis can be examined, while they are in an upright position, by using a tongue blade or indirect laryngoscopy.

Case management should be individualized. In the child with moderate or advanced disease, the clinical diagnosis should be apparent without having to do an intraoral examination. In this situation, intraoral examination should not be performed, but the child should be prepared for the establishment of an airway. This preparation should be rapid but controlled so that intubation can be performed in an operating room.

The diagnosis of epiglottitis can be established by the classic appearance on a lateral neck radiograph (Fig. 18-2).[327,439] However, my opinion is that this radiographic procedure rarely is necessary; all too often, it leads to a delay in providing the necessary definitive therapy.[88,212,285] The use of the lateral neck radiograph should be reserved for subacute cases in which the specific diagnosis after completion of a clinical examination is not clear.

FIGURE 18-2 ■ Acute epiglottitis. A lateral neck radiograph from a child with acute epiglottitis showing the swollen epiglottis (thumb sign) encroaching on the airway. (Courtesy Dr. Ines Boechat.)

The lateral film of the neck, delineating the soft tissues and taken with the patient upright, gives the best view of the upper airway anatomy (see Fig. 18-2). The hypopharynx is dilated; normal cervical lordosis may be replaced by a straight or kyphotic contour. The valleculae are narrowed and may be obliterated. A thickened mass of tissue stretching from the valleculae to the arytenoids emphasizes the appropriateness of the term *supraglottitis*. In adults with epiglottitis, the widths of the epiglottis and arytenoepiglottic folds uniformly exceed 8 and 7 mm, respectively.[359]

When performed, radiography of the neck in the anteroposterior projection usually reveals that tracheal narrowing is absent. However, some children with acute supraglottitis have localized subglottic narrowing indistinguishable from that found in acute laryngotracheitis.[365,379]

All patients with suspected epiglottitis should have a blood culture, and a culture specimen should be obtained from the surface of the epiglottis when an artificial airway is established. Today, cultures are of increasing importance because of the change in epidemiology of *H. influenzae* type b infection and the resulting increased likelihood that a different organism may be causing the illness. A white blood cell count with a differential also may provide useful information. In children who have received antimicrobial treatment before cultures were obtained, performing a direct antigen test for *H. influenzae* type b on the blood and urine is worthwhile.

TREATMENT OF CROUP

During the last 70 years, the treatment of croup has created considerable controversy: tracheostomy versus no tracheostomy, intubation versus tracheostomy, warm versus cold humidification, antibiotics versus no antibiotics, corticosteroids versus no corticosteroids, sedation versus no sedation, and racemic epinephrine therapy versus mist therapy alone. Few of these controversies have been resolved scientifically, but the passage of time has lessened the importance of some of the discrepant opinions.

Of most importance in the evaluation of therapeutic modalities and the specific approach to therapy in croup is making an accurate differential diagnosis. A look at the most important controversy (the use of steroids) indicates that in most instances, cases of spasmodic croup, in which a favorable outcome invariably can be expected, were not separated clinically from cases of laryngotracheitis, in which the outcome is less predictable.

Acute Laryngotracheitis and Spasmodic Croup

In managing acute laryngotracheitis and spasmodic croup, each case must be treated individually; one child may need minimal therapy, whereas another may require

consideration of all modalities. In all children with acute laryngotracheitis, attention should be given to the anxiety and apprehension of the patient and parents. The parents should be reassured immediately, and it is important that the child is not separated from them. Physical examination should be done rapidly by one physician, and all but absolutely necessary procedures should be deferred.

In the past, mist therapy was the cornerstone of management of croup. In more recent years, the value of this therapy has been questioned.[47,253,290,361] It had been my opinion, as expressed in previous editions of this book, that mist therapy in croup was useful.

Oxygen should be administered to a child who is hypoxemic from respiratory distress. The studies of Newth and associates[302] and Taussig and colleagues[405] indicate that mild hypoxemia occurs more commonly than is realized clinically. The drying effect of oxygen is counterproductive to the removal of tracheal exudate, so it should not be used routinely.

Since 1952, numerous communications in the English literature have described the use of corticosteroids in croup. Nine double-blind, controlled studies were conducted before 1989.* In 1964, Eden and Larkin[123] noted no difference between control and methylprednisolone therapy in a study of 50 children with acute croup. In 1967, Eden and colleagues[124] studied another 50 patients and could find no benefit from dexamethasone in contrast to a control preparation. Sussman and colleagues[398] also could not show any benefit from dexamethasone therapy.

In contrast to these three studies, Skowron and coworkers[377] noted a slight benefit regarding duration of stridor, retractions, fever, and days in the hospital in a group receiving dexamethasone. They suggested, however, that steroids not be used routinely for laryngotracheitis because the overall benefits were minimal and a potential risk exists in administration of steroids. In 1969, James[203] noted that dexamethasone-treated patients recovered from obstructive symptoms more quickly than did the control group. In another study involving 30 children, Leipzig and associates[252] concluded that dexamethasone in an adequate dose (0.3 mg/kg initially and repeated in 2 hours) given intramuscularly hastens the recovery from uncomplicated croup. This study and its predecessors have major inadequacies in design.[84,418] In a study with 72 children, Koren and colleagues[234] noted that dexamethasone did not offer any benefit to patients with laryngotracheitis but did decrease significantly the respiratory rate in children with spasmodic croup in contrast to placebo-treated control subjects. Although these findings were statistically significant, the benefits were not clinically significant.

Two additional modest studies of the use of dexamethasone in croup were published in 1988 and 1989, as was a meta-analysis of the evidence from the various randomized trials and a set of related editorials and a review.† Kuusela and Vesikari[242] concluded that dexamethasone was beneficial in acute spasmodic croup, and Super and associates[395] concluded that dexamethasone is beneficial in reducing the overall severity of moderate to severe acute laryngotracheitis during the first day of treatment.

The 1989 meta-analysis suggested that the use of steroids in children hospitalized with croup resulted in a significantly increased number with clinical improvement 12 hours and 24 hours after treatment and a reduced incidence of endotracheal intubation than occurred in the control subjects.[219] In this analysis, improvement at 12 hours was noted to be greater in the children who received higher initial doses of steroid (≥125 mg of cortisone) than in children who received lower doses.

In the early 1990s, nebulized steroids were evaluated in the treatment of croup, which led to another round of testimonials, controlled studies, and treatment articles supporting the efficacy of steroids.* Since the 1989 meta-analysis, more than 15 additional controlled steroid treatment trials have been performed, and another meta-analysis was published in 1999.[14] Finally, a large controlled trial of oral dexamethasone for mild croup was published in 2004, and a comprehensive meta-analysis was published in 2005.[40,348] Also published more recently are many articles comparing doses, types, and methods of administration of steroids and a further round of commentaries.† On the basis of the three meta-analyses and the specific studies analyzed, most reviews on the management of croup recommend the routine use of steroids (administered orally, intramuscularly, or by nebulization) in the treatment of croup.‡

In the past, when I reviewed the data on the use of steroids in croup, what concerned me was that the specific clinical entity that was being treated was poorly defined and that no evidence indicated that steroids worked in any illness other than spasmodic croup.[79] Of most concern to me today is that the risks of steroid use have not been evaluated and that, because of small sample sizes in all but two of the available studies, they cannot be evaluated by meta-analysis.

I have seen lower respiratory tract complications develop in three children receiving steroid treatment for croup. In one case, an adenoviral pneumonia worsened, and in the other cases, bacterial tracheitis with pneumonia occurred. In these three cases, they all received corticosteroid treatment over the course of several days and not single-dose treatment. In the study of Super and colleagues,[395] two steroid-treated patients developed pneumonia during therapy; none of the control subjects developed pneumonia. In a more recent trial in which 28 children received nebulized dexamethasone, two children with neutropenia developed bacterial tracheitis.[207] Burton and associates[64] reported the occurrence of *Candida* laryngotracheitis as a complication of corticosteroid and antibiotic treatment in a child with croup, and Myers and colleagues[297] reported an infant who developed multiple pulmonary abscesses caused by *Legionella pneumophila* after receiving prolonged corticosteroid treatment of severe croup.

*References 123, 124, 203, 234, 242, 252, 377, 395, 398.
†References 11, 218, 242, 376, 381, 395.

*References 14, 97, 162, 168, 194, 202, 207, 217, 228-231, 337, 340, 376, 441.
†References 39, 71, 83, 117, 137, 138, 152, 227, 266, 337, 409.
‡References 9, 38, 39, 96, 108, 109, 137, 138, 162, 168, 196, 202, 203, 227-229, 231, 337, 340, 409.

Today, the standard of care for the initial treatment of laryngotracheitis and spasmodic croup is the use of corticosteroids administered orally, intramuscularly, or by nebulization.[9,39,137] Recommended therapy is a single dose of intramuscular or oral dexamethasone. The limited use of nebulized budesonide also is effective. In the most definitive study, the treatment dose was dexamethasone 0.6 mg/kg given orally once.[40] In a subsequent review, Bjornson and Johnson[39] noted that the standard dose of dexamethasone is 0.6 mg/kg (oral or intramuscular); they suggested that oral is preferable because absorption is excellent, and peak serum concentrations are achieved as rapidly as with intramuscular administration. Recently Dobrovoljac and Geelhoed[116] reported on the effectiveness of a single 0.15 mg/kg dose of dexamethasone. They noted that this lower dose was as effective as the 0.6 mg/kg dose that they had previously used.

As noted earlier, I and others have observed bacterial and fungal infections as complications in children who have received multiple doses of steroids in croup. It is disturbing to me to note that in pediatric practice many children are receiving 3- to 5-day treatment courses rather than the recommended single-dose schedule. The data from the cotton rat model of human parainfluenza virus type 3 laryngotracheitis provides strong evidence supporting the limited use of steroids in croup.[312] In this model, topical steroid therapy significantly reduced the degree of inflammatory infiltrates and cell damage. The steroid therapy increased the virus load but did not prolong virus shedding.

I believe the most dramatic evidence supporting the use of steroid therapy in laryngotracheitis and spasmodic croup is the marked decrease in the number of hospitalizations for croup in Ontario, which coincided with the 1992 recommendation by the Canadian Pediatrics Society to use dexamethasone for treatment.[196,363] Steroids should not be used in laryngotracheobronchitis, laryngotracheobronchopneumonitis, or epiglottitis.

Because laryngotracheitis is a disease of viral etiology, what seems apparent is that antibiotic therapy would not be indicated and consequently not employed. In our analysis of more than 200 hospitalized children with laryngotracheitis, we noted that antibiotics had been administered to 85 percent (Unpublished data, Gardner HG, Powell KR, Cherry JD, 1973).

A review of the records in many instances revealed that the physician had given antibiotic therapy because the possibility of epiglottitis had not been ruled out adequately. In several instances, the epiglottis had been observed and thought to be reddened or questionably enlarged.

A second consideration with regard to antibiotic therapy in croup is that the most dramatic reduction in mortality rates coincides with the introduction and widespread use of antibiotics. In my opinion, many of the deaths attributed to croup in the preantibiotic era were caused by secondary bacterial infections. Although use of antibiotics contributed to the reduction in the number of deaths caused by croup, other factors may be important. At about the same time that antibiotics were introduced, disease caused by *S. pyogenes* decreased in incidence and severity. Reasons for the decreased frequency of streptococcal disease were not clear.

Most patients with laryngotracheitis today do not need antibiotic therapy. In severe cases in which bacterial sepsis cannot be ruled out, however, antibiotic therapy should be employed. The pathogens to consider include pneumococci, group A streptococci, *S. aureus*, and *H. influenzae*. In patients with laryngotracheitis in whom fever persists or signs change, secondary infection should be considered. In these instances, appropriate culture specimens should be obtained before therapy is initiated (see the section on laryngotracheobronchitis for specific antibiotic therapy).

The use of nebulized racemic epinephrine, which was introduced by Jordan[215] in 1966 and popularized by Jordan and other members of the Utah group,[1,216] has been adopted widely throughout the United States and elsewhere.* The usual method of nebulization of racemic epinephrine is by intermittent positive-pressure breathing. This form of therapy was associated with a marked reduction in tracheostomies in several series. In a recent review it was found that nebulized racemic epinephrine was no better than routine L-epinephrine and that intermittent positive-pressure breathing offered no advantage over simple nebulization.[38]

In 1973, Gardner and associates[148] performed the first double-blind, controlled study in which racemic epinephrine was nebulized by a compressor without intermittent positive-pressure breathing. They found that saline-treated patients responded as well to therapy, as did the racemic epinephrine recipients. In retrospect, this study can be criticized because the investigators failed to differentiate spasmodic croup from laryngotracheitis. In 1975, Taussig and associates[405] reported a small but carefully conducted study with intermittent positive-pressure breathing and racemic epinephrine in which they noted acute improvement in all cases, recurrence of symptoms in 2 hours, and no change in partial pressure of oxygen with clinical improvement; 24 to 36 hours after therapy, treated and untreated children were clinically similar. In another significant study that involved children hospitalized with severe croup without improvement after admission to a high-humidity mist room, Westley and colleagues[446] showed that racemic epinephrine therapy caused definite short-term improvement in children compared with saline treatment. This study is particularly important because a parainfluenza viral etiology was documented in more than 65 percent of the study's subjects, and all cases were clearly laryngotracheitis and not spasmodic croup.

The following points can be made about the use of racemic epinephrine in the treatment of croup: (1) Many children with croup respond to moist air alone; (2) significant rebound occurs after racemic epinephrine therapy, so it frequently needs to be repeated many times; (3) in a hospitalized child with severe acute laryngotracheitis, racemic epinephrine should be used. Tracheotomy or endotracheal intubation can be prevented in some cases. The most important issue today regarding the use of racemic epinephrine is whether it

*References 13, 38, 223, 240, 260, 283, 347, 373.

can be used safely for outpatient therapy.[91,220,321] In the past, most experts advised against the use of racemic epinephrine in the outpatient setting because of the known rebound that occurs. Some data suggest that with careful observation for a sufficient period (at least 2 hours) after administration, patients can be managed safely, and the number of hospitalizations can be decreased.[91,221,320] In a small study, investigators found that the administration of a helium-oxygen mixture (heliox) resulted in improvement in children with croup similar to that with racemic epinephrine treatment.[439]

The establishment of a mechanical airway seldom is necessary today in patients with laryngotracheitis. The planned procedure has a better outcome than does the procedure performed under emergency conditions. Traditionally, tracheostomy was the preferred method when a mechanical airway was needed.[17,146,458] When careful attention is given to tube size and other aspects of placement and maintenance, however, nasotracheal intubation compares favorably with tracheostomy.[17,458] The management of a child with a mechanical airway requires trained pediatric intensive care physicians and the facilities of an adequately staffed intensive care unit. A helium-oxygen mixture (heliox) has been used to treat croup.[438] However, this treatment is no better than racemic epinephrine.

Five antiviral drugs have activity against viruses that cause laryngotracheitis.[172,201,275,400,449] Amantadine and rimantadine are approved for use in treating influenza A viral infections, and ribavirin is active against parainfluenza viruses, influenza A and B viruses, and respiratory syncytial virus (see Chapters 177 and 238). The neuraminidase inhibitors, zanamivir and oseltamivir, are active against influenza a and b types.[70,92] At present, consideration of therapy for severe croup that occurs during documented epidemics caused by influenza A or B viruses would seem reasonable. Because of high levels of resistance of influenza A strains to amantadine and rimantadine, treatment with the neuraminidase inhibitors is recommended.[70]

Laryngotracheobronchitis and Laryngotracheobronchopneumonitis (Bacterial Tracheitis)

Generally, all the treatment considerations discussed for laryngotracheitis except corticosteroids and racemic epinephrine by aerosol apply to laryngotracheobronchitis and laryngotracheobronchopneumonitis. Most important, however, because most patients have bacterial disease, antibiotics should be administered to all patients after appropriate culture specimens are obtained. Empiric therapy should be directed against *S. aureus*, *S. pyogenes*, *S. pneumoniae*, and *H. influenzae*. At present, initial treatment with vancomycin 40 mg/kg/day intravenously every 6 hours and a third-generation cephalosporin, such as cefotaxime 150 mg/kg/day every 6 hours intravenously, is reasonable. If methicillin-sensitive *S. aureus* is isolated, treatment should be changed to oxacillin 150 mg/kg/day every 6 hours intravenously or a similar agent.

Most children with advanced laryngotracheobronchitis or laryngotracheobronchopneumonitis need the placement of a mechanical airway. Whenever possible, this procedure should be done electively rather than as an emergency.

Laryngitis

Patients with laryngitis should rest their voices as much as possible. Increased fluid intake and perhaps the use of a vaporizer may help liquefy secretions and might provide symptomatic relief. Because group A streptococcal infection is a cause of laryngitis, culture should be performed. If it is positive, penicillin or a suitable alternative antimicrobial agent should be administered. In children and adolescents with prolonged hoarseness, sinusitis should be considered. Radiographs of the sinuses and a quantitative culture from the nose should be performed in search of a predominant abnormal bacterial flora. If either is positive, therapy with appropriate antibiotics is indicated. If laryngeal symptoms are persistent, the child should undergo laryngoscopic examination and other appropriate studies to exclude tumor, foreign body, and other chronic diseases.

TREATMENT OF SUPRAGLOTTITIS

The treatment of acute epiglottitis should be relatively simple in that it involves only two main aspects of therapy: an airway must be established, and an appropriate antimicrobial agent needs to be administered. However, in the past, the mortality rate from epiglottitis varied from 0 to 32 percent,[88,176] a finding suggesting major differences in the implementation of treatment.

Most deaths occur in transit to the hospital or within the first few hours after arrival. Once the diagnosis is suspected, the patient should be attended constantly by individuals skilled in resuscitation with appropriate equipment for airway stabilization and ventilatory support. Delays of 2 or 3 hours have proved fatal; every effort should be made to reduce the time needed to secure a patient's airway and to initiate antibiotic therapy, before which unnecessary stress should be avoided. In most cases, radiographic confirmation should be omitted. Blood tests, extensive history taking, and delay in transport should be eliminated.

Medical centers and pediatric services that have planned protocols for the diagnostic investigation and treatment of patients with suspected acute epiglottitis generally have better morbidity and mortality statistics than do services that do not have such protocols in place. Pediatricians, radiologists, otolaryngologists, and anesthetists may contribute to the assessment and management of a case; defining roles and responsibilities of each service in advance minimizes confusion and renders the institution of care easier.

Securing the Airway

In general, the cornerstone of all management plans is the establishment of an airway in all children in whom the diagnosis of epiglottitis is made.[81] In 1938, Sinclair[371] recognized that tracheostomy was lifesaving. Berenberg

and Kevy[31] advocated hospitalization for all patients with epiglottitis but tracheostomy only "if necessary." However, Bass and associates[22] presented a compelling argument for performing routine tracheostomy. Among 83 patients with documented epiglottitis, 11 of whom were adults, these authors noted that 16 (19%) had life-threatening obstruction when they were first seen. An additional 14 (17%) progressed to this point within 6 hours of admission; 9 of the 83 (11%) required emergency tracheostomy while they were hospitalized. Of these 9, 2 died and 1 suffered anoxic brain damage. All 9 were being monitored carefully, with bedside tracheostomy equipment available and trained personnel nearby. Six of the adults required tracheostomy for survival.[22] Margolis and colleagues[271] noted that elective tracheostomy, performed at the time of diagnosis, eliminated fatalities in 15 consecutive patients. This observation was in contrast to 4 deaths in 20 patients observed until tracheostomy "was required."

A large body of literature attests to the safety and efficacy of nasotracheal intubation as a replacement for tracheostomy,* which has a complication rate of 50 percent.[408,447] Biologically inert tubes, with their decreased risk for complicating subglottic stenosis,[368] and the recollection that endotracheal tube insertion was a universal approach before tracheostomy[285] was an accepted procedure led to the routine use of nasotracheal intubation in this disease. Nasotracheal intubation requires a shorter duration of airway maintenance: 32 patients with epiglottitis managed with tracheostomy had a mean duration of intubation of 7.5 days and a mean hospitalization of 8.8 days. In contrast, a nasotracheal tube was used for a mean of 38 hours with a 6.5-day hospitalization in 5 patients.[285] Diaz and Lockhart[115] managed 104 patients with nasotracheal intubation. The mean intubation time was 53 ± 14.9 hours; 7 patients (6.8%) extubated themselves, and 2 required reintubation. Laryngeal edema occurred in 3 patients (2.9%) who had been intubated. In 2 patients, subglottic granulations required excision.

My position, as well as that of Daum and Smith,[101] is that the argument for performing elective tracheostomy or, preferably, intubation in all children with supraglottitis is compelling, and the procedure should be performed immediately after the diagnosis is established. Whether this "stimulus-response" approach is necessary for adults with epiglottitis is controversial.† MayoSmith and associates[274] compared selected clinical features in adults with epiglottitis at the time of diagnosis who died or who received an artificial airway with those of adults who recovered without airway intervention. Few differences emerged. Patients who died or who were managed with airway intervention had respiratory distress and bacteremia more often than did those who recovered without airway intervention. Obviously, the presence of bacteremia was not known on presentation. Moreover, the mortality rate among all adults managed expectantly was 4.6 percent, a figure comparable to the mortality rate (6.1%) of children in a series reported before recommendations were made for routine securing of the airway at diagnosis.[67,274] The preponderance of evidence until very recently has suggested that securing the airway in all adults with supraglottitis by nasotracheal intubation should reduce mortality rates.* However, Frantz and associates[144] reported an analysis of 129 cases of acute epiglottitis in adults in which no deaths occurred in the cohort and 85 percent of these patients were managed without airway intervention.

In the large series reported by Berger and colleagues[33] involving 118 cases, 19 had airways established on admission (16 endotracheal intubation, 2 tracheotomy, 1 cricothyrotomy), and 6 other patients subsequently required either endotracheal intubation or tracheotomy. The remaining 93 patients were managed successfully without the need for airway intervention. Fifty-eight of the patients were treated with intravenous corticosteroids in addition to antibiotics, and these patients tended to have more prolonged hospital stays than those who did not receive steroids (5.7 ± 4.1 days vs. 4.7 ± 4.3 days; $p = .2$).

To aid in intubation and reduce long-term sequelae, most investigators have advocated use of a nasotracheal tube 0.5 to 1.0 mm smaller than that predicted by the patient's age.[179,285,356] Published criteria for extubation (summarized in reference 14) include those based on duration of therapy and those based on daily examination of the epiglottis and supraglottic structures by direct laryngoscopy[104] or fiberoptic bronchoscopy.[307,425]

Long-term complications of nasotracheal intubation are rare. Thirty-three children with epiglottitis who were managed with nasotracheal intubation (mean duration of intubation, 55 hours) were evaluated 1 to 8 years later. By history and measurement of peak expiratory flow rates, no complications were found.[356] Although additional long-term data are necessary to ensure absence of residua, elective nasotracheal intubation appears to be the procedure of choice.

Several reports documented the recognition of idiopathic pulmonary edema before[251] or, more commonly, after† insertion of an endotracheal tube to relieve laryngeal obstruction caused by epiglottitis. One hypothesis to explain this phenomenon is that airway obstruction produces markedly negative intrapleural pressure with increased venous return to the right side of the heart, with decreased left ventricular output and increased pulmonary blood volume.[248] These changes increase the pulmonary microvascular pressure and produce pulmonary hyperemia and edema. Endotoxemia may play a role in altering vascular permeability, but it is not necessary for recognition of this complication of airway obstruction because abrupt onset of pulmonary edema was described when airway obstruction caused by croup, foreign body, and malignant neoplasm was relieved acutely.[63] The frequency of pulmonary edema as a complication of intubation in supraglottitis was approximately 9 percent,[379] and it has occurred in an adult.[338] Continuous positive airway pressure in all intubated patients with epiglottitis probably will provide prophylaxis against this complication,[115,147] but controlled data are lacking.

*References 25, 89, 179, 245, 271, 310, 328, 356, 358, 368, 408, 412.
†References 21, 143, 176, 314, 362, 370, 385, 450.

*References 10, 20-22, 35, 36, 99, 226, 274.
†References 43, 104, 119, 147, 219, 384, 414.

Antibiotics

The mainstay of antibiotic therapy for acute epiglottitis in the recent past has been ceftriaxone 50 to 100 mg/kg/day given every 12 hours intravenously or cefotaxime 100 to 200 mg/kg/day given every 6 hours intravenously, because virtually all cases in both children and adults were caused by *H. influenzae* type b. However, in the present conjugate vaccine era, the incidence of all invasive *H. influenzae* type b disease has decreased dramatically. Therefore, in children previously vaccinated, the cause is likely to be another organism. Culture results today have added significance because one possible cause that would require a change in therapy is *S. aureus*.

No controlled data exist about the duration of antimicrobial administration, but a course of 7 to 10 days seems appropriate. In the event that group A streptococci are isolated from the airway, penicillin is the drug of choice. A first-generation semisynthetic penicillinase-resistant penicillin or vancomycin (if MRSA is suspected) should be used for *S. aureus*.

Other Supportive Measures

Expert respiratory nursing care is essential. Inadvertent extubation must be avoided, particularly in the first 24 hours. Judicious use of sedatives that do not appreciably depress respiration may be appropriate.

PROGNOSIS

The prognosis of acute laryngotracheitis has improved markedly during the last 55 years. Today, a child with croup only rarely requires a mechanical airway, and virtually all deaths should be preventable. The child should be observed for the following complications: hypoxemia and cardiorespiratory failure, pulmonary edema, pneumothorax and pneumomediastinum, mechanical problems caused by tracheotomies and nasotracheal tubes, and secondary bacterial infections. Children with a history of croup have a higher prevalence of increased bronchial reactivity than children without such history.[170,265,441]

The prognosis in epiglottitis after the successful establishment of an airway and the administration of specific antimicrobial agents is generally good.

Extraepiglottic complications are not common occurrences in children with acute epiglottitis. In a study involving 72 children with epiglottitis, investigators noted that 25 percent had pneumonia, 25 percent had cervical adenitis, 8 percent had tonsillitis, and 5 percent had otitis media.[289] The other common invasive manifestations of *H. influenzae* type b infections (meningitis, arthritis, and cellulitis) rarely are found in conjunction with epiglottitis.[289,355]

PREVENTION OF CROUP

At present, acute laryngotracheitis is not preventable. The widespread use of influenza vaccines could reduce the incidence of croup caused by influenza A and B viruses.

Prevention of Epiglottitis Caused by *Haemophilus influenzae* Type b

The universal use of *H. influenzae* type b vaccine in infancy with an additional dose in the second year of life has had a profound effect on the occurrence of invasive *H. influenzae* type b diseases.

In the pre–conjugate vaccine era, household contacts of patients with *H. influenzae* type b infection were at increased risk for acquiring the infection.[165] Whether an increased risk occurred in daycare contacts was unresolved.[103] Although contacts of patients with *H. influenzae* type b epiglottitis younger than 5 years of age are colonized less frequently than contacts of patients with other *H. influenzae* type b invasive infections,[102] secondary disease was described in household contacts when an index patient had epiglottitis.[2,160,161,415] Secondary *H. influenzae* type b epiglottitis was described in a child[2] and two adults who were household contacts of a patient with *H. influenzae* type b meningitis.[160,161] *H. influenzae* type b epiglottitis occurred in two siblings who presented with the condition within 1 day.[174]

Rifampin prophylaxis with 20 mg/kg/day (600 mg/dose maximum) for 4 days, is recommended for all unvaccinated members of a patient's contact group when the index patient has *H. influenzae* type b epiglottitis and at least one contact in the group is 4 years of age or younger.[8,69]

The recognition that adults occasionally may acquire secondary infection, particularly epiglottitis, on exposure to children with invasive *H. influenzae* type b infection prompted some experts to extend prophylaxis to all of a patient's contact groups, regardless of the presence of one or more contacts who are 4 years of age or younger. All experts, however, recommended that adults and older children be made aware of the signs and symptoms of *H. influenzae* type b disease, particularly when the patient's contact group would not receive prophylaxis under current guidelines.

NEW REFERENCES SINCE THE SIXTH EDITION

12. Apisarnthanarak A, Pheerapiboon P, Apisarnthanarak P, et al. Fulminant epiglottitis with evolution to necrotizing soft tissue infections and fasciitis due to *Aeromonas hydrophila*. *Infection* 2008;**36**:94–5.
13. Argent AC, Hatherill M, Newth CJ, et al. The effect of epinephrine by nebulization on measures of airway obstruction in patients with acute severe croup. *Intensive Care Med* 2008;**34**:138–47.
38. Bjornson C, Russell KF, Vandermeer B, et al. Nebulized epinephrine for croup in children. *Cochrane Database Syst Rev* 2011;(2):CD006619.
72. Chalmers C. Necrotising fasciitis complicating *Haemophilus influenzae* type b epiglottitis in an adult. *J Laryngol Otol* 2010;**124**:807–9.
84. Cherry JD. Clinical practice. Croup. *N Engl J Med* 2008; **358**:384–91.
116. Dobrovoljac M, Geelhoed GC. 27 years of croup: an update highlighting the effectiveness of 0.15 mg/kg of dexamethasone. *Emerg Med Australas* 2009;**21**:309–14.

169. Guldfred LA, Lyhne D, Becker BC. Acute epiglottitis: epidemiology, clinical presentation, management and outcome. *J Laryngol Otol* 2008;**122**:818–23.
183. Hirshoren N, Eliashar R, Weinberger JM. Candida epiglottitis: a rare emergent condition—appearance, treatment and pitfalls. *Eur J Intern Med* 2008;**19**:e84–5.
192. Hsia SH, Lin JJ, Wu CT, et al. Guillain-Barré syndrome presenting as mimicking croup. *Am J Emerg Med* 2010;**28**:749.e1–3.
193. Hsieh YJ, Chin H, Chiu NC, et al. Hospitalized pediatric parainfluenza virus infections in a medical center. *J Microbiol Immunol Infect* 2010;**43**:360–5.
199. Isakson M, Hugosson S. Acute epiglottitis: epidemiology and *Streptococcus pneumoniae* serotype distribution in adults. *J Laryngol Otol* 2011;**125**:390–3.
233. Kong MS, Engel SH, Zalzal GH, et al. Necrotizing epiglottitis and hemophagocytic lymphohistiocytosis. *Int J Pediatr Otorhinolaryngol* 2009;**73**:119–25.
264. Lotfizadeh A, Chhetri DK. Viral supraglottitis in an adult. *Ear Nose Throat J* 2009;**88**:768–70.
309. O'Connell K, Fennell J, Callaghan J, et al. A fatal case of *Pasteurella multocida* epiglottitis. *Ir J Med Sci* 2009;**178**:541–2.
329. Reed J, Shah RK, Jantausch B, et al. Aryepiglottic abscess manifesting as epiglottitis. *Arch Otolaryngol Head Neck Surg* 2009;**135**:953–5.
333. Richardson DK, Helderman T, Lovett P. Meningococcal epiglottitis in a diabetic adult patient: a case report. *J Emerg Med* 2012;**43**:634–6.
336. Rihkanen H, Ronkko E, Nieminen T, et al. Respiratory viruses in laryngeal croup of young children. *J Pediatr* 2008;**152**:661–5.
341. Rogers DJ, Sie KC, Manning SC. Epiglottitis due to nontypeable *Haemophilus influenzae* in a vaccinated child. *Int J Pediatr Otorhinolaryngol* 2010;**74**:218–20.
344. Rosychuk RJ, Klassen TP, Metes D, et al. Croup presentations to emergency departments in Alberta, Canada: a large population-based study. *Pediatr Pulmonol* 2010;**45**:83–91.
345. Rosychuk RJ, Klassen TP, Voaklander DC, et al. Seasonality patterns in croup presentations to emergency departments in Alberta, Canada: a time series analysis. *Pediatr Emerg Care* 2011;**27**:256–60.
366. Shah RK, Stocks C. Epiglottitis in the United States: national trends, variances, prognosis, and management. *Laryngoscope* 2010;**120**:1256–62.
374. Sivakumar S, Latifi SQ. Varicella with stridor: think group A streptococcal epiglottitis. *J Paediatr Child Health* 2008;**44**:149–51.
387. Somenek M, Le M, Walner DL. Membranous laryngitis in a child. *Int J Pediatr Otorhinolaryngol* 2010;**74**:704–6.
394. Sung JY, Lee HJ, Eun BW, et al. Role of human coronavirus NL63 in hospitalized children with croup. *Pediatr Infect Dis J* 2010;**29**:822–6.
429. Vigil KJ, Mulanovich VE, Chemaly RF, et al. Severe parainfluenza virus type 2 supraglottitis in an immunocompetent adult host: an unusual cause of a paramyxoviridae viral infection. *J Intern Med* 2009;**265**:397–400.
440. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. *J Pediatr* 2009;**154**:694–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adair JC, Ring WH, Jordan WS, et al. Ten-year experience with IPPB in the treatment of acute laryngotracheobronchitis. *Anesth Analg* 1971;**50**:649–55.
2. Addy MG, Ellis PD, Turk DC. Haemophilus epiglottitis: nine recent cases in Oxford. *Br Med J* 1972;**1**:40–2.
3. Alba D, Perna C, Torres E, et al. Isolated candidal epiglottitis: report of a fatal case. *Clin Infect Dis* 1996;**22**:732–3.
4. Alexander HE, Ellis C, Leidy C. Treatment of type-specific *H. influenzae* infections in infancy and childhood. *J Pediatr* 1942;**20**:673–98.
5. Alho OP, Jokinen K, Pirila T, et al. Acute epiglottitis and infant conjugate *Haemophilus influenzae* type b vaccination in northern Finland. *Arch Otolaryngol Head Neck Surg* 1995;**121**:898–902.
6. Alligood GA, Kenny JF. Tracheitis and supraglottis associated with *Branhamella catarrhalis* and respiratory syncytial virus. *Pediatr Infect Dis J* 1989;**8**:190–1.
7. Ambrosino DM, Schiffman G, Gotschlich EC, et al. Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. *J Clin Invest* 1985;**75**:1935–42.
8. American Academy of Pediatrics. *Haemophilus influenzae* infections. In: Pickering LK, editor. *2000 red book: report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2000. p. 203–17.
9. American Academy of Pediatrics. Parainfluenza viral infections. In Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 479–81.
10. Andreassen UK, Husum B, Tos M, et al. Acute epiglottitis in adults: a management protocol based on a 17-year material. *Acta Anaesthesiol Scand* 1984;**28**:155–7.
11. Anonymous. Steroids and croup. *Lancet* 1989;**2**:1134–6.
12. Apisarnthanarak A, Pheerapiboon P, Apisarnthanarak P, et al. Fulminant epiglottitis with evolution to necrotizing soft tissue infections and fasciitis due to *Aeromonas hydrophila*. *Infection* 2008;**36**:94–5.
13. Argent AC, Hatherill M, Newth CJ, et al. The effect of epinephrine by nebulization on measures of airway obstruction in patients with acute severe croup. *Intensive Care Med* 2008;**34**:138–47.
14. Ausejo M, Saenz A, Pham B, et al. The effectiveness of glucocorticoids in treating croup: meta-analysis. *BMJ* 1999;**319**:595–600.
15. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008;**167**:63–9.
16. Banatvala JE, Reiss BB, Nitkin BC, et al. Asian influenza in 1963 in two general practices in Cambridge, England. *Can Med Assoc J* 1965;**93**:593–7.
17. Barker GA. Current management of croup and epiglottitis. *Pediatr Clin North Am* 1979;**26**:565–79.
18. Barnham M, Kerby J, Chandler RS, et al. Group C streptococci in human infection: a study of 308 isolates with clinical correlations. *Epidemiol Infect* 1989;**102**:379–90.
19. Baron BJ. Comments on a case of extremely acute oedematous laryngitis. *Br Med J* 1887;**2**:1328.
20. Bass JW. Routine tracheotomy for epiglottitis: what are the odds? *J Pediatr* 1973;**83**:510–11.
21. Bass JW. Response to CPC. *N Engl J Med* 1978;**298**:342–3.
22. Bass JW, Steele RW, Wiebe RA. Acute epiglottitis: a surgical emergency. *JAMA* 1974;**229**:671–5.
23. Bastien N, Anderson K, Hart L, et al. Human coronavirus NL63 infection in Canada. *J Infect Dis* 2005;**191**:503–6.
24. Bastien N, Chui N, Robinson JL, et al. Detection of human bocavirus in Canadian children in a 1-year study. *J Clin Microbiol* 2007;**45**:610–3.
25. Battaglia JD, Lockhart CH. Management of acute epiglottitis by nasotracheal intubation. *Am J Dis Child* 1975;**129**:334–6.
26. Baugh R, Baker SR. Epiglottitis in children: review of 24 cases. *Otolaryngol Head Neck Surg* 1982;**90**:157–62.
27. Baum HL. Acute laryngotracheobronchitis. *JAMA* 1928;**91**: 1097–102.
28. Baxter JD. Acute epiglottitis in children. *Laryngoscope* 1967; **77**:1358–67.
29. Belfer RA. Group A beta-hemolytic streptococcal epiglottitis as a complication of varicella infection. *Pediatr Emerg Care* 1996;**12**:203–4.
30. Benjamin B, O'Reilly B. Acute epiglottitis in infants and children. *Ann Otol Rhinol Laryngol* 1976;**85**:565–72.
31. Berenberg W, Kevy S. Acute epiglottitis in childhood; a serious emergency, readily recognized at the bedside. *N Engl J Med* 1958;**258**:870–4.
32. Berg RB. Weight and sex of children hospitalized with infectious croup: an analysis of 850 cases. *Pediatrics* 1963;**31**(1)Pt 1:18–21.
33. Berger G, Landau T, Berger S, et al. The rising incidence of adult acute epiglottitis and epiglottic abscess. *Am J Otolaryngol* 2003;**24**:374–83.
34. Bernstein T, Brilli R, Jacobs B. Is bacterial tracheitis changing? A 14-month experience in a pediatric intensive care unit. *Clin Infect Dis* 1998;**27**:458–62.
35. Bishop MJ. Epiglottitis in the adult. *Anesthesiology* 1981;**55**: 701–2.
36. Bishop MJ, Weymuller Jr EA. Adult epiglottitis revisited. *Anesthesiology* 1982;**57**:545–6.
37. Bisno AL, Barratt NP, Swanston WH, et al. An outbreak of acute respiratory disease in Trinidad associated with para-influenza viruses. *Am J Epidemiol* 1970;**91**:68–77.
38. Bjornson C, Russell KF, Vandermeer B, et al. Nebulized epinephrine for croup in children. *Cochrane Database Syst Rev* 2011;(2): CD006619.
39. Bjornson CL, Johnson DW. Croup-treatment update. *Pediatr Emerg Care* 2005;**21**:863–70; quiz 871–3.
40. Bjornson CL, Klassen TP, Williamson J, et al. A randomized trial of a single dose of oral dexamethasone for mild croup. *N Engl J Med* 2004;**351**:1306–13.
41. Black MJ, Harbour J, Remsen KA, et al. Acute epiglottitis in adults. *J Otolaryngol* 1981;**10**:23–7.
42. Blackstock D, Adderley RJ, Steward DJ. Epiglottitis in young infants. *Anesthesiology* 1987;**67**:97–100.
43. Blankson VN. Pulmonary edema complicating epiglottitis. *J Med Soc N J* 1983;**80**:939–41.
44. Bogger-Goren S. Acute epiglottitis caused by herpes simplex virus. *Pediatr Infect Dis J* 1987;**6**:1133–4.
45. Bolivar R, Gomez LG, Luna M, et al. Aspergillus epiglottitis. *Cancer* 1983;**51**:367–70.
46. Bottenfield GW, Arcinue EL, Sarnaik A, et al. Diagnosis and management of acute epiglottitis: report of 90 consecutive cases. *Laryngoscope* 1980;**90**:822–5.
47. Bourchier D, Dawson KP, Fergusson DM. Humidification in viral croup: a controlled trial. *Aust Paediatr J* 1984;**20**:289–91.
48. Bower CM, Suen JY. Adult acute epiglottitis caused by *Serratia marcescens*. *Otolaryngol Head Neck Surg* 1996;**115**:156–9.
49. Brandt CD, Kim HW, Vargosko AJ, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am J Epidemiol* 1969;**90**:484–500.
50. Branefors-Helander P, Jeppsson PH. Acute epiglottitis; a clinical, bacteriological and serological study. *Scand J Infect Dis* 1975;**7**:103–11.
51. Breese BB. Diagnosis of streptococcal pharyngitis. In: Breese BB, Hall CB, editors. *Beta hemolytic streptococcal diseases*. Boston: Houghton Mifflin; 1978. p. 79–96.
52. Breese BB, Disney FA, Talpey W. The nature of a small pediatric group practice. I. *Pediatrics* 1966;**38**:264–77.
53. Breivik H, Klaastad O. Acute epiglottitis in children: review of 27 patients. *Br J Anaesth* 1978;**50**:505–10.
54. Brennemann J, Clifton WM, Frank A, et al. Acute laryngotracheobronchitis. *Am J Dis Child* 1938;**55**:667–95.
55. Briggs WH, Altenau MM. Acute epiglottitis in children. *Otolaryngol Head Neck Surg* 1980;**88**:665–9.
56. Brighton GR. Laryngotracheobronchitis. *Ann Otol Rhinol Laryngol* 1940;**49**:1070–82.
57. Brilli RJ, Benzing 3rd G, Cotcamp DH. Epiglottitis in infants less than two years of age. *Pediatr Emerg Care* 1989;**5**:16–21.
58. Britto J, Habibi P, Walters S, et al. Systemic complications associated with bacterial tracheitis. *Arch Dis Child* 1996;**74**: 249–50.
59. Broadhurst LE, Erickson RL, Kelley PW. Decreases in invasive *Haemophilus influenzae* diseases in US Army children, 1984 through 1991. *JAMA* 1993;**269**:227–31.

60. Brocklebank JT, Court SD, McQuillin J, et al. Influenza-A infection in children. *Lancet* 1972;**2**:497–500.
61. Broughton SJ, Warren RE. A review of *Haemophilus influenzae* infections in Cambridge 1975-1981. *J Infect* 1984;**9**:30–42.
62. Bruun B, Gahrn-Hansen B, Westh H, et al. Clonal relationship of recent invasive *Haemophilus influenzae* serotype f isolates from Denmark and the United States. *J Med Microbiol* 2004;**53**: 1161–5.
63. Burtner DD, Goodman M. Anesthetic and operative management of potential upper airway obstruction. *Arch Otolaryngol* 1978;**104**:657–61.
64. Burton DM, Seid AB, Kearns DB, et al. Candida laryngotracheitis: a complication of combined steroid and antibiotic usage in croup. *Int J Pediatr Otorhinolaryngol* 1992;**23**:171–5.
65. Butt W, Shann F, Walker C, et al. Acute epiglottitis: a different approach to management. *Crit Care Med* 1988;**16**:43–7.
66. Caballero M, Sabater F, Traserra J, et al. Epiglottitis and necrotizing fasciitis: a life-threatening complication of infectious mononucleosis. *Acta Otolaryngol* 2005;**125**:1130–3.
67. Cantrell RW, Bell RA, Morioka WT. Acute epiglottitis: intubation versus tracheostomy. *Laryngoscope* 1978;**88**:994–1005.
68. Castro-Rodriguez JA, Holberg CJ, Morgan WJ, et al. Relation of two different subtypes of croup before age three to wheezing, atopy, and pulmonary function during childhood: a prospective study. *Pediatrics* 2001;**107**:512–8.
69. Centers for Disease Control and Prevention. Immunization Practices Advisory Committee: Haemophilus b conjugate vaccine for prevention of *Haemophilus influenzae* type b disease among infants and children 2 months of age and older. *MMWR Morb Mortal Wkly Rep* **40**:1–7, 1991.
70. Centers for Disease Control and Prevention. Prevention and control of influenza. *MMWR Morb Mortal Wkly Rep* 2006; **55**:1–42.
71. Cetinkaya F, Tufekci BS, Kutluk G. A comparison of nebulized budesonide, and intramuscular, and oral dexamethasone for treatment of croup. *Int J Pediatr Otorhinolaryngol* 2004;**68**:453–6.
72. Chalmers C. Necrotising fasciitis complicating *Haemophilus influenzae* type b epiglottitis in an adult. *J Laryngol Otol* 2010;**124**:807–9.
73. Chanock R, Chambon L, Chang W, et al. WHO respiratory disease survey in children: a serological study. *Bull World Health Organ* 1967;**37**:363–9.
74. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. *Pediatrics* 1965;**36**:21–39.
75. Chanock RM, Parrott RH, Johnson KM, et al. Myxoviruses: parainfluenza. *Am Rev Respir Dis* 1962;**88**:152–66.
76. Chapman RS, Henderson FW, Clyde Jr WA, et al. The epidemiology of tracheobronchitis in pediatric practice. *Am J Epidemiol* 1981;**114**:786–97.
77. Chenaud M, Leclerc F, Martinot A. Bacterial croup and toxic shock syndrome. *Eur J Pediatr* 1986;**145**:306–7.
78. Cherry JD. Newer respiratory viruses: their role in respiratory illnesses of children. In: Schulman I, editor. *Advances in pediatrics*. vol. 20. Chicago: Year Book; 1973. p. 225–89.
79. Cherry JD. The treatment of croup: continued controversy due to failure of recognition of historic, ecologic, etiologic and clinical perspectives. *J Pediatr* 1979;**94**:352–4.
80. Cherry JD. Acute epiglottitis, laryngitis, and croup. In: Remington JS, Swartz MN, editors. *Current clinical topics in infectious diseases*. New York: McGraw-Hill; 1981. p. 1–30.
81. Cherry JD. Croup. In: Kiple KF, editor. *The Cambridge world history of human disease*. Cambridge: Cambridge University Press; 1993. p. 654–7.
82. Cherry JD. Measles. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 4th ed. Philadelphia: W. B. Saunders; 1998.
83. Cherry JD. State of the evidence for standard-of-care treatments for croup: are we where we need to be? *Pediatr Infect Dis J* 2005;**24**:S198–202; discussion S201.
84. Cherry JD. Clinical practice. Croup. *N Engl J Med* 2008;**358**: 384–91.
85. Chiu SS, Chan KH, Chu KW, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. *Clin Infect Dis* 2005;**40**:1721–9.
86. Chow AW, Bushkell LL, Yoshikawa TT, et al. Case report: *Haemophilus parainfluenzae* epiglottitis with meningitis and bacteremia in an adult. *Am J Med Sci* 1974;**267**:365–8.
87. Claesson B, Trollfors B, Ekstrom-Jodal B, et al. Incidence and prognosis of acute epiglottitis in children in a Swedish region. *Pediatr Infect Dis* 1984;**3**:534–8.
88. Cohen SR, Chai J. Epiglottitis: twenty-year study with tracheotomy. *Ann Otol Rhinol Laryngol* 1978;**87**:461–7.
89. Coker SB, Scherz RG. Safe alternative to tracheostomy in acute epiglottitis. [Letter]. *Am J Dis Child* 1975;**129**:136.
90. Conley SF, Beste DJ, Hoffmann RG. Measles-associated bacterial tracheitis. *Pediatr Infect Dis J* 1993;**12**:414–5.
91. Corneli HM, Bolte RG. Outpatient use of racemic epinephrine in croup. *Am Fam Physician* 1992;**46**:683–4.
92. Couch RB. Prevention and treatment of influenza. *N Engl J Med* 2000;**343**:1778–87.
93. Coulehan JL, Michaels RH, Hallowell C, et al. Epidemiology of *Haemophilus influenzae* type B disease among Navajo Indians. *Public Health Rep* 1984;**99**:404–9.
94. Cramblett HG. Croup-present day concept. *Pediatrics* 1960;**25**: 1071–6.
95. Cramblett HG, Moffet HL, Black JP, et al. Coxsackie virus infections: clinical and laboratory studies. *J Pediatr* 1964;**64**: 406–14.
96. Cressman WR, Myer 3rd CM. Diagnosis and management of croup and epiglottitis. *Pediatr Clin North Am* 1994;**41**:265–76.
97. Cruz MN, Stewart G, Rosenberg N. Use of dexamethasone in the outpatient management of acute laryngotracheitis. *Pediatrics* 1995;**96**:220–3.
98. Dagan R. A two-year prospective, nationwide study to determine the epidemiology and impact of invasive childhood *Haemophilus influenzae* type b infection in Israel. The Israeli Pediatric Bacteremia and Meningitis Group. *Clin Infect Dis* 1992;**15**:720–5.
99. Darnell JC. Acute epiglottitis in adults: report of a case and review of the literature. *J Indiana State Med Assoc* 1976;**69**:21–3.
100. Dascomb HE, Hilleman MR. Clinical and laboratory studies in patients with respiratory disease caused by adenoviruses (RI-APC-ARD agents). *Am J Med* 1956;**21**:161–74.
101. Daum RS, Smith AL. Epiglottitis (supraglottitis). In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 3rd ed. Philadelphia: W. B. Saunders; 1992. p. 197–209.
102. Daum RS, Glode MP, Goldmann DA, et al. Rifampin chemoprophylaxis for household contacts of patients with invasive infections due to *Haemophilus influenzae* type b. *J Pediatr* 1981;**98**:485–91.
103. Daum RS, Granoff DM, Gilsdorf J, et al. *Haemophilus influenzae* type b infections in day care attendees: implications for management. *Rev Infect Dis* 1986;**8**:46–55.
104. Davis HW, Gartner JC, Galvis AG, et al. Acute upper airway obstruction: croup and epiglottitis. *Pediatr Clin North Am* 1981;**28**:859–80.
105. Davison FW. Acute laryngeal obstruction in children. *JAMA* 1959;**171**:1301–5.
106. Davison FW. Acute laryngotracheobronchitis; further studies on treatment. *Arch Otolaryngol* 1948;**47**:455–64.
107. Davison FW. Acute obstructive laryngitis in children. *Penn Med J* 1950;**53**:250–4.
108. Dawson K, Cooper D, Cooper P, et al. The management of acute laryngo-tracheo-bronchitis (croup): a consensus view. *J Paediatr Child Health* 1992;**28**:223–4.
109. De Boeck K. Croup: a review. *Eur J Pediatr* 1995;**154**:432–6.
110. Denny FW, Clyde Jr WA. Acute lower respiratory tract infections in nonhospitalized children. *J Pediatr* 1986;**108**:635–46.
111. Denny FW, Clyde Jr WA, Glezen WP. Mycoplasma pneumoniae disease: clinical spectrum, pathophysiology, epidemiology, and control. *J Infect Dis* 1971;**123**:74–92.
112. Denny FW, Murphy TF, Clyde Jr WA, et al. Croup: an 11-year study in a pediatric practice. *Pediatrics* 1983;**71**:871–6.
113. Denneny 3rd JC, Handler SD. Membranous laryngotracheobronchitis. *Pediatrics* 1982;**70**:705–7.
114. Devita MA, Wagner IJ. Acute epiglottitis in the adult. *Crit Care Med* 1986;**14**:1082–3.
115. Diaz JH, Lockhart CH. Early diagnosis and airway management of acute epiglottitis in children. *South Med J* 1982;**75**:399–403.
116. Dobrovoljac M, Geelhoed GC. 27 years of croup: an update highlighting the effectiveness of 0.15 mg/kg of dexamethasone. *Emerg Med Australas* 2009;**21**:309–14.

117. Donaldson D, Poleski D, Knipple E, et al. Intramuscular versus oral dexamethasone for the treatment of moderate-to-severe croup: a randomized, double-blind trial. *Acad Emerg Med* 2003;**10**: 16–21.
118. Donnelly BW, McMillan JA, Weiner LB. Bacterial tracheitis: report of eight new cases and review. *Rev Infect Dis* 1990;**12**: 729–35.
119. Donnelly J, Overton JH, Mellis CM. Pulmonary oedema following relief of epiglottitis. *Anaesth Intensive Care* 1981;**9**:290–1.
120. Drake-Lee AB, Broughton SJ, Grace A. Children with epiglottitis. *Br J Clin Pract* 1984;**38**:218–20, 242.
121. Dudin AA, Thalji A, Rambaud-Cousson A. Bacterial tracheitis among children hospitalized for severe obstructive dyspnea. *Pediatr Infect Dis J* 1990;**9**:293–5.
122. Dudley JP. Supraglottitis and *Hemophilus parainfluenzae:* pathogenic potential of the organism. *Ann Otol Rhinol Laryngol* 1987;**96**:400–2.
123. Eden AN, Larkin VD. Corticosteroid treatment of croup. *Pediatrics* 1964;**33**:768–9.
124. Eden AN, Kaufman A, Yu R. Corticosteroids and croup: controlled double-blind study. *JAMA* 1967;**200**:403–4.
125. Edwards KM, Dundon MC, Altemeier WA. Bacterial tracheitis as a complication of viral croup. *Pediatr Infect Dis* 1983;**2**:390–1.
126. Ehara H. Tenderness over the hyoid bone can indicate epiglottitis in adults. *J Am Board Fam Med* 2006;**19**:517–20.
127. Eichenwald HF. Respiratory infections in children. *Hosp Pract* 1976;**11**:81–90.
128. Eid NS, Jones VF. Bacterial tracheitis as a complication of tonsillectomy and adenoidectomy. *J Pediatr* 1994;**125**:401–2.
129. Eller JJ, Fulginiti VA, Plunket DC, et al. Attack rates for hospitalized croup in children in a military population: importance of A2 influenza infection. *Pediatr Res* 1972;**6**:386.
130. Ernst TN, Philp M. Bacterial tracheitis caused by *Branhamella catarrhalis*. *Pediatr Infect Dis J* 1987;**6**:574.
131. Everett AR. Acute laryngotracheobronchitis: an analysis of 1,475 cases with 98 tracheotomies. *Laryngoscope* 1951;**61**:113–23.
132. Faden H. The dramatic change in the epidemiology of pediatric epiglottitis. *Pediatr Emerg Care* 2006;**22**:443–4.
133. Faden HS. Treatment of *Haemophilus influenzae* type b epiglottitis. *Pediatrics* 1979;**63**:402–7.
134. Farley MM, Stephens DS, Brachman Jr PS, et al. Invasive *Haemophilus influenzae* disease in adults: a prospective, population-based surveillance. CDC Meningitis Surveillance Group. *Ann Intern Med* 1992;**116**:806–12.
135. Fearon B. Acute obstructive laryngitis in infants and children. *Hosp Med* 1968;**4**:51–67.
136. Fearon BW, Bell RD. Acute epiglottitis: a potential killer. *Can Med Assoc J* 1975;**112**:760–1, 764, 766.
137. Fitzgerald DA. The assessment and management of croup. *Paediatr Respir Rev* 2006;**7**:73–81.
138. Fitzgerald DA, Kilham HA. Croup: assessment and evidence-based management. *Med J Aust* 2003;**179**:372–7.
139. Fogarty J, Moloney AC, Newell JB. The epidemiology of *Haemophilus influenzae* type b disease in the Republic of Ireland. *Epidemiol Infect* 1995;**114**:451–63.
140. Fontanarosa PB, Polsky SS, Goldman GE. Adult epiglottitis. *J Emerg Med* 1989;**7**:223–31.
141. Forbes JA. Severe effects of influenza virus infection. *Med J Aust* 1958;**2**:75–9.
142. Foskey Jr G, Singer J. Artificial nail aspiration masquerading as refractory croup. *Pediatr Emerg Care* 2005;**21**:523–6.
143. Frantz TD, Rasgon BM. Acute epiglottitis: changing epidemiologic patterns. *Otolaryngol Head Neck Surg* 1993;**109**:457–60.
144. Frantz TD, Rasgon BM, Quesenberry Jr CP. Acute epiglottitis in adults: analysis of 129 cases. *JAMA* 1994;**272**:1358–60.
145. Freeman L, Wolford R. Acute epiglottitis caused by methicillin-resistant *Staphylococcus aureus* in adults. *Clin Infect Dis* 1998;**26**: 1240–1.
146. Fried MP. Controversies in the management of supraglottitis and croup. *Pediatr Clin North Am* 1979;**26**:931–42.
147. Galvis AG, Stool SE, Bluestone CD. Pulmonary edema following relief of acute upper airway obstruction. *Ann Otol Rhinol Laryngol* 1980;**89**:124–8.
148. Gardner HG, Powell KR, Roden VJ, et al. The evaluation of racemic epinephrine in the treatment of infectious croup. *Pediatrics* 1973;**52**:68–71.
149. Gardner PS. Virus infections and respiratory disease of childhood. *Arch Dis Child* 1968;**43**:629–45.
150. Gardner PS, McQuillin J, McGuckin R, et al. Observations on clinical and immunofluorescent diagnosis of parainfluenza virus infections. *Br Med J* 1971;**2**:7–12.
151. Garpenholt O, Hugosson S, Fredlund H, et al. Epiglottitis in Sweden before and after introduction of vaccination against *Haemophilus influenzae* type b. *Pediatr Infect Dis J* 1998;**18**: 490–3.
152. Geelhoed GC. Budesonide offers no advantage when added to oral dexamethasone in the treatment of croup. *Pediatr Emerg Care* 2005;**21**:359–62.
153. Geist R, Tallett SE. Diagnosis and management of psychogenic stridor caused by a conversion disorder. *Pediatrics* 1990;**86**: 315–7.
154. Gerber AC, Pfenninger J. Acute epiglottitis: management by short duration of intubation and hospitalisation. *Intensive Care Med* 1986;**12**:407–11.
155. Giani G, Quirino F, Sacrini A, et al. Supraglottitis due to herpes simplex virus type 1 in an adult. *Clin Infect Dis* 1996;**22**:382–3.
156. Gittens TR. XXXIII: laryngitis and tracheobronchitis in children: special reference to nondiphtheritic infections. *Ann Otol Rhinol Laryngol* 1932;**41**:422–38.
157. Glezen WP, Loda FA, Denny FW. The parainfluenza viruses. In: Evans AS, editor. *Viral infections of humans: epidemiology and control*. New York: Plenum; 1976. p. 337–49.
158. Glezen WP, Loda FA, Clyde Jr WA, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
159. Glickman M, Klein RS. Acute epiglottitis due to *Pasteurella multocida* in an adult without animal exposure. *Emerg Infect Dis* 1997;**3**:408–9.
160. Glode MP. Post exposure prophylaxis for bacterial meningitis. In: Sande MA, Smith AL, Root RK, editors. *Bacterial meningitis*. New York: Churchill Livingstone; 1985.
161. Glode MP, Halsey NA, Murray M, et al. Epiglottitis in adults: association with *Haemophilus influenzae* type b colonization and disease in children. *Pediatr Infect Dis* 1984;**3**:548–51.
162. Godden CW, Campbell MJ, Hussey M, et al. Double blind placebo controlled trial of nebulised budesonide for croup. *Arch Dis Child* 1997;**76**:155–8.
163. Gold SM, Shott SR, Myer 3rd CM. Radiological case of the month: membranous laryngotracheobronchitis. *Arch Pediatr Adolesc Med* 1996;**150**:97–8.
164. Gorelick MH, Baker MD. Epiglottitis in children, 1979 through 1992: effects of *Haemophilus influenzae* type b immunization. *Arch Pediatr Adolesc Med* 1994;**148**:47–50.
165. Granoff DM, Daum RS. Spread of *Haemophilus influenzae* type b: recent epidemiologic and therapeutic considerations. *J Pediatr* 1980;**97**:854–60.
166. Granoff DM, Pandey JP, Boies E, et al. Response to immunization with *Haemophilus influenzae* type b polysaccharide-pertussis vaccine and risk of *Haemophilus* meningitis in children with the Km(1) immunoglobulin allotype. *J Clin Invest* 1984;**74**: 1708–14.
167. Grattan-Smith T, Forer M, Kilham H, et al. Viral supraglottitis. *J Pediatr* 1987;**110**:434–5.
168. Griffin S, Ellis S, Fitzgerald-Barron A, et al. Nebulised steroid in the treatment of croup: a systematic review of randomised controlled trials. *Br J Gen Pract* 2000;**50**:135–41.
169. Guldfred LA, Lyhne D, Becker BC. Acute epiglottitis: epidemiology, clinical presentation, management and outcome. *J Laryngol Otol* 2008;**122**:818–23.
170. Gurwitz D, Corey M, Levison H. Pulmonary function and bronchial reactivity in children after croup. *Am Rev Respir Dis* 1980;**122**:95–9.
171. Hafidh MA, Sheahan P, Keogh I, et al. Acute epiglottitis in adults: a recent experience with 10 cases. *J Laryngol Otol* 2005;**120**:310–3.
172. Hall CB, McBride JT, Walsh EE, et al. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection: a randomized double-blind study. *N Engl J Med* 1983;**308**: 1443–7.
173. Han BK, Dunbar JS, Striker TW. Membranous laryngotracheobronchitis (membranous croup). *AJR Am J Roentgenol* 1979;**133**: 53–8.

174. Handler SD, Plotkin SA, Potsic WP, et al. *Hemophilus influenzae* epiglottitis occurring concurrently in two siblings. *Clin Pediatr (Phila)* 1982;**21**:634–5.
175. Harari MD, West B, Dwyer B. Cryptosporidium as cause of laryngotracheitis in an infant. *Lancet* 1986;**1**:1207.
176. Hawkins DB, Miller AH, Sachs GB, et al. Acute epiglottitis in adults. *Laryngoscope* 1973;**83**:1211–20.
177. Healy GB, Hyams VJ, Tucker Jr GF. Paraglottic laryngitis in association with epiglottitis. *Ann Otol Rhinol Laryngol* 1985;**94**: 618–21.
178. Hebert PC, Ducic Y, Boisvert D, et al. Adult epiglottitis in a Canadian setting. *Laryngoscope* 1998;**108**:64–9.
179. Heldtander P, Lee P. Treatment of acute epiglottitis in children by long-term intubation. *Acta Otolaryngol* 1973;**75**:379–81.
180. Henry RL, Mellis CM, Benjamin B. Pseudomembranous croup. *Arch Dis Child* 1983;**58**:180–3.
181. Herrmann Jr EC, Hable KA. Experiences in laboratory diagnosis of parainfluenza viruses in routine medical practice. *Mayo Clin Proc* 1970;**45**:177–88.
182. Hide DW, Guyer BM. Recurrent croup. *Arch Dis Child* 1985;**60**:585–6.
183. Hirshoren N, Eliashar R, Weinberger JM. Candida epiglottitis: a rare emergent condition—appearance, treatment and pitfalls. *Eur J Intern Med* 2008;**19**:e84–5.
184. Hodge KM, Ganzel TM. Diagnostic and therapeutic efficiency in croup and epiglottitis. *Laryngoscope* 1987;**97**:621–5.
185. Hoekelman RA. Infectious illness during the first year of life. *Pediatrics* 1977;**59**:119–21.
186. Holzel A, Parker L, Patterson WH, et al. Virus isolations from throats of children admitted to hospital with respiratory and other diseases, Manchester 1962-4. *Br Med J* 1965;**1**:614–9.
187. Hope-Simpson RE, Higgins PG. A respiratory virus study in Great Britain: review and evaluation. *Prog Med Virol* 1969;**11**: 354–407.
188. Hopkins A, Lahiri T, Salerno R, et al. Changing epidemiology of life-threatening upper airway infections: the reemergence of bacterial tracheitis. *Pediatrics* 2006;**118**:1418–21.
189. Horn ME, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967-1972. *J Hyg (Lond)* 1975;**74**:157–68.
190. Howard AJ, Dunkin KT, Musser JM, et al. Epidemiology of *Haemophilus influenzae* type b invasive disease in Wales. *BMJ* 1991;**303**:441–5.
191. Howard JB, McCracken Jr GH, Luby JP. Influenza A 2 virus as a cause of croup requiring tracheotomy. *J Pediatr* 1972;**81**: 1148–50.
192. Hsia SH, Lin JJ, Wu CT, et al. Guillain-Barré syndrome presenting as mimicking croup. *Am J Emerg Med* 2010;**28**:749.e1–3.
193. Hsieh YJ, Chin H, Chiu NC, et al. Hospitalized pediatric parainfluenza virus infections in a medical center. *J Microbiol Immunol Infect* 2010;**43**:360–5.
194. Husby S, Agertoft L, Mortensen S, et al. Treatment of croup with nebulised steroid (budesonide): a double blind, placebo controlled study. *Arch Dis Child* 1993;**68**:352–5.
195. Hyde CI, Ruchman J. Acute infectious edematous laryngitis in which recovery followed tracheotomy. *Arch Pediatr* 1931; **48**:124–9.
196. Infectious Diseases and Immunization Committee, C. P. S. Steroid therapy for croup in children admitted to hospital. *Can Med Assoc J* 1992;**147**:429–30.
197. Inglis Jr AF. Herpes simplex virus infection: a rare cause of prolonged croup. *Arch Otolaryngol Head Neck Surg* 1993;**119**: 551–2.
198. Isaacson G, Isaacson DM. Pediatric epiglottitis caused by group G beta-hemolytic streptococcus. *Pediatr Infect Dis J* 2003;**22**: 846–7.
199. Isakson M, Hugosson S. Acute epiglottitis: epidemiology and *Streptococcus pneumoniae* serotype distribution in adults. *J Laryngol Otol* 2011;**125**:390–3.
200. Isenberg DA, Lipkin DP, Mowbray JF, et al. Fatal pneumococcal epiglottitis in lupus overlap syndrome. *Clin Rheumatol* 1984;**3**: 529–32.
201. Jackson GG, Stanley ED. Prevention and control of influenza by chemoprophylaxis and chemotherapy; prospects from examination of recent experience. *JAMA* 1976;**235**:2739–42.
202. Jaffe DM. The treatment of croup with glucocorticoids. *N Engl J Med* 1998;**339**:553–5.
203. James JA. Dexamethasone in croup: a controlled study. *Am J Dis Child* 1969;**117**:511–6.
204. Jenkins WA. *Pasteurella multocida* as a cause of acute adult epiglottitis. *Am J Emerg Med* 1997;**15**:323.
205. Jerrard DA, Olshaker J. Simultaneous uvulitis and epiglottitis without fever or leukocytosis. *Am J Emerg Med* 1996;**14**:551–2.
206. Jiang JH, Chiu NC, Lin YY, et al. Acute epiglottitis caused by *Haemophilus influenzae* type b: a case report. *J Microbiol Immunol Infect* 2003;**36**:69–71.
207. Johnson DW, Schuh S, Koren G, et al. Outpatient treatment of croup with nebulized dexamethasone. *Arch Pediatr Adolesc Med* 1996;**150**:349–55.
208. Johnson GK, Sullivan JL, Bishop LA. Acute epiglottitis: review of 55 cases and suggested protocol. *Arch Otolaryngol* 1974;**100**: 333–7.
209. Johnson MC. Acute laryngotracheobronchitis in infants: report of three cases. *Arch Otolaryngol* 1933;**17**:230–4.
210. Johnson RH, Rumans LW. Unusual infections caused by *Pasteurella multocida*. *JAMA* 1977;**237**:146–7.
211. Johnstone JM, Lawy HS. Acute epiglottitis in adults due to infection with *Haemophilus influenzae* type b. *Lancet* 1967;**2**:134–6.
212. Jones HM. Acute epiglottitis: a personal study over twenty years. *Proc R Soc Med* 1970;**63**:706–12.
213. Jones R, Santos JI, Overall Jr JC. Bacterial tracheitis. *JAMA* 1979;**242**:721–6.
214. Jones RN, Slepack J, Bigelow J. Ampicillin-resistant *Haemophilus paraphrophilus* laryngo-epiglottitis. *J Clin Microbiol* 1976;**4**:405–7.
215. Jordan WS. Laryngotracheobronchitis: evaluation of new therapeutic approaches. *Rocky Mt Med J* 1966;**63**:69.
216. Jordan WS, Graves CL, Elwyn RA. New therapy for postintubation laryngeal edema and tracheitis in children. *JAMA* 1970; **212**:585–8.
217. Kaditis AG, Wald ER. Viral croup: current diagnosis and treatment. *Pediatr Infect Dis J* 1998;**17**:827–34.
218. Kairys SW, Olmstead EM, O'Connor GT. Steroid treatment of laryngotracheitis: a meta-analysis of the evidence from randomized trials. *Pediatrics* 1989;**83**:683–93.
219. Kanter RK, Watchko JF. Pulmonary edema associated with upper airway obstruction. *Am J Dis Child* 1984;**138**:356–8.
220. Kasian GF, Bingham WT, Steinberg J, et al. Bacterial tracheitis in children. *CMAJ* 1989;**140**:46–50.
221. Kelley PB, Simon JE. Racemic epinephrine use in croup and disposition. *Am J Emerg Med* 1992;**10**:181–3.
222. Kennedy CA, Rosen H. *Kingella kingae* bacteremia and adult epiglottitis in a granulocytopenic host. *Am J Med* 1988;**85**: 701–2.
223. Kepes ER, Martinez LR, Andrews IC, et al. Racemic epinephrine in postintubation laryngeal edema. *N Y State J Med* 1972;**72**: 583–4.
224. Kessler HA, Schade R, Trenholme GM, et al. Acute pneumococcal epiglottitis in immunocompromised adults. *Scand J Infect Dis* 1980;**12**:207–10.
225. Kharasch S, Vinci R, Reece R. Esophagitis, epiglottitis, and cocaine alkaloid ("crack"): "accidental" poisoning or child abuse? *Pediatrics* 1990;**86**:117–9.
226. Khilanani U, Khatib R. Acute epiglottitis in adults. *Am J Med Sci* 1984;**287**:65–70.
227. Klass P. Croup: the bark is worse than the bite. *N Engl J Med* 2004;**351**:1283–4.
228. Klassen TP. Croup: a current perspective. *Pediatr Clin North Am* 1999;**46**:1167–78.
229. Klassen TP, Craig WR, Moher D, et al. Nebulized budesonide and oral dexamethasone for treatment of croup: a randomized controlled trial. *JAMA* 1998;**279**:1629–32.
230. Klassen TP, Feldman ME, Watters LK, et al. Nebulized budesonide for children with mild-to-moderate croup. *N Engl J Med* 1994;**331**:285–9.
231. Klassen TP, Watters LK, Feldman ME, et al. The efficacy of nebulized budesonide in dexamethasone-treated outpatients with croup. *Pediatrics* 1996;**97**:463–6.
232. Klein JD, Collier AM. Pathogenesis of human parainfluenza type 3 virus infection in hamster tracheal organ culture. *Infect Immun* 1974;**10**:883–8.

233. Kong MS, Engel SH, Zalzal GH, et al. Necrotizing epiglottitis and hemophagocytic lymphohistiocytosis. *Int J Pediatr Otorhinolaryngol* 2009;**73**:119–25.
234. Koren G, Frand M, Barzilay Z, et al. Corticosteroid treatment of laryngotracheitis v spasmodic croup in children. *Am J Dis Child* 1983;**137**:941–4.
235. Korppi M, Halonen P, Kleemola M, et al. The role of parainfluenza viruses in inspiratory difficulties in children. *Acta Paediatr Scand* 1988;**77**:105–11.
236. Krause I, Schonfeld T, Ben-Ari J, et al. Prolonged croup due to herpes simplex virus infection. *Eur J Pediatr* 1998;**157**:567–9.
237. Kravitz H. Sex distribution of hospitalized children with acute respiratory diseases, gastroenteritis and meningitis. *Clin Pediatr (Phila)* 1965;**4**:484–91.
238. Kristensen K. *Haemophilus influenzae* type b infections in adults. *Scand J Infect Dis* 1989;**21**:651–3.
239. Kristensen K, Kaaber K, Ronne T, et al. Epidemiology of *Haemophilus influenzae* type b infections among children in Denmark in 1985 and 1986. *Acta Paediatr Scand* 1990;**79**:587–92.
240. Kristjansson S, Berg-Kelly K, Winso E. Inhalation of racemic adrenaline in the treatment of mild and moderately severe croup: clinical symptom score and oxygen saturation measurements for evaluation of treatment effects. *Acta Paediatr* 1994;**83**:1156–60.
241. Kulick RM, Selbst SM, Baker MD, et al. Thermal epiglottitis after swallowing hot beverages. *Pediatrics* 1988;**81**:441–4.
242. Kuusela AL, Vesikari T. A randomized double-blind, placebo-controlled trial of dexamethasone and racemic epinephrine in the treatment of croup. *Acta Paediatr Scand* 1988;**77**:99–104.
243. La Scolea Jr LJ, Rosales SV, Welliver RC, et al. Mechanisms underlying the development of meningitis or epiglottitis in children after *Haemophilus influenzae* type b bacteremia. *J Infect Dis* 1985;**151**:1162–5.
244. Lacroix J, Ahronheim G, Arcand P, et al. Group A streptococcal supraglottitis. *J Pediatr* 1986;**109**:20–4.
245. Lacroix J, Blanc VF, Weber M, et al. [100 consecutive cases of acute epiglottitis]. *Union Med Can* 1982;**111**:774–9.
246. Lacroix J, Gauthier M, Lapointe N, et al. *Pseudomonas aeruginosa* supraglottitis in a six-month-old child with severe combined immunodeficiency syndrome. *Pediatr Infect Dis J* 1988;**7**:739–41.
247. Lai SH, Wong KS, Liao SL, et al. Non-infectious epiglottitis in children: two cases report. *Int J Pediatr Otorhinolaryngol* 2000;**55**:57–60.
248. Lang SA, Duncan PG, Shephard DA, et al. Pulmonary oedema associated with airway obstruction. *Can J Anaesth* 1990;**37**:210–8.
249. Laxdal OE, Robertson HE, Braaten V, et al. Acute respiratory infections in children. I. An intensive study of etiology in an open community. *Can Med Assoc J* 1963;**88**:1049–54.
250. Lederman MM, Lowder J, Lerner PI. Bacteremic pneumococcal epiglottitis in adults with malignancy. *Am Rev Respir Dis* 1982;**125**:117–8.
251. Lee SC, Meislin H, Iserson KV. Epiglottitis presenting as acute pulmonary edema. *Ann Emerg Med* 1985;**14**:60–3.
252. Leipzig B, Oski FA, Cummings CW, et al. A prospective randomized study to determine the efficacy of steroids in treatment of croup. *J Pediatr* 1979;**94**:194–6.
253. Lenney W, Milner AD. Treatment of acute viral croup. *Arch Dis Child* 1978;**53**:704–6.
254. Lewis JK, Gartner JC, Galvis AG. A protocol for management of acute epiglottitis: successful experiences with 27 consecutive instances treated by nasotracheal intubation. *Clin Pediatr (Phila)* 1978;**17**:494–6.
255. Lindquist JR, Franzen RE, Ossoff RH. Acute infectious supraglottitis in adults. *Ann Emerg Med* 1980;**9**:256–9.
256. Lipson A, Kronick JB, Tewfik L, et al. Group B streptococcal supraglottitis in a 3-month-old infant. *Am J Dis Child* 1986;**140**:411–2.
257. Liptak GS, McConnochie KM, Roghmann KJ, et al. Decline of pediatric admissions with *Haemophilus influenzae* type b in New York State, 1982 through 1993: relation to immunizations. *J Pediatr* 1997;**130**:923–30.
258. Liston SL, Gehrz RC, Jarvis CW. Bacterial tracheitis. *Arch Otolaryngol* 1981;**107**:561–4.
259. Liston SL, Gehrz RC, Siegel LG, et al. Bacterial tracheitis. *Am J Dis Child* 1983;**137**:764–7.
260. Lockhart CH, Battaglia JD. Croup [laryngotracheal bronchitis] and epiglottitis. *Pediatr Ann* 1977;**6**:262–9.
261. Loda FA, Clyde Jr WA, Glezen WP, et al. Studies on the role of viruses, bacteria, and *M. pneumoniae* as causes of lower respiratory tract infections in children. *J Pediatr* 1968;**72**:161–76.
262. Loda FA, Glezen WP, Clyde Jr WA. Respiratory disease in group day care. *Pediatrics* 1972;**49**:428–37.
263. Losek JD, Dewitz-Zink BA, Melzer-Lange M, et al. Epiglottitis: comparison of signs and symptoms in children less than 2 years old and older. *Ann Emerg Med* 1990;**19**:55–8.
264. Lotfizadeh A, Chhetri DK. Viral supraglottitis in an adult. *Ear Nose Throat J* 2009;**88**:768–70.
265. Loughlin GM, Taussig LM. Pulmonary function in children with a history of laryngotracheobronchitis. *J Pediatr* 1979;**94**:365–9.
266. Luria JW, Gonzalez-del-Rey JA, DiGiulio GA, et al. Effectiveness of oral or nebulized dexamethasone for children with mild croup. *Arch Pediatr Adolesc Med* 2001;**155**:1340–5.
267. Macasaet FF, Kidd PA, Bolano CR, et al. The etiology of acute respiratory infections. III. The role of viruses and bacteria. *J Pediatr* 1968;**72**:829–39.
268. Mace SE. Acute epiglottitis in adults. *Am J Emerg Med* 1985;**3**:543–50.
269. Macneil A, Campbell AM, Clark LJ. Adult acute epiglottitis in association with infection of an epiglottic cyst. *Anaesth Intensive Care* 1989;**17**:211–2.
270. Margolis CZ, Colletti RB, Grundy G. *Hemophilus influenzae* type b: the etiologic agent in epiglottitis. [Letter]. *J Pediatr* 1975;**87**:322–3.
271. Margolis CZ, Ingram DL, Meyer JH. Routine tracheotomy in *Hemophilus influenzae* type b epiglottitis. *J Pediatr* 1972;**81**:1150–3.
272. Marx A, Torok TJ, Holman RC, et al. Pediatric hospitalizations for croup (laryngotracheobronchitis): biennial increases associated with human parainfluenza virus 1 epidemics. *J Infect Dis* 1997;**176**:1423–7.
273. Mathur KK, Mortelliti AJ. Candida epiglottitis. *Ear Nose Throat J* 2004;**83**:13.
274. MayoSmith MF, Hirsch PJ, Wodzinski SF, et al. Acute epiglottitis in adults: an eight-year experience in the state of Rhode Island. *N Engl J Med* 1986;**314**:1133–9.
275. McClung HW, Knight V, Gilbert BE, et al. Ribavirin aerosol treatment of influenza B virus infection. *JAMA* 1983;**249**:2671–4.
276. McGregor AR, Dawson KP, Abbott GD. Acute epiglottitis in childhood, Christchurch 1970-84. *N Z Med J* 1985;**98**:1011–3.
277. McIntyre PB, Leeder SR, Irwig LM. Invasive *Haemophilus influenzae* type b disease in Sydney children 1985-1987: a population-based study. *Med J Aust* 1991;**154**:832–7.
278. McKenzie M, Norman MG, Anderson JD, et al. Upper respiratory tract infection in a 3-year-old girl. *J Pediatr* 1984;**105**:129–33.
279. McLean DM, Roy TE, O'Brien MJ, et al. Para-influenza viruses in association with acute laryngotracheobronchitis, Toronto, 1960-61. *Can Med Assoc J* 1961;**85**:290–4.
280. McNab JCG. Acute streptococcal infection of the trachea in an infant, aged fifteen months. *J Laryngol* 1915;**30**:337–8.
281. McVernon J, Slack MP, Ramsay ME. Changes in the epidemiology of epiglottitis following introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines in England: a comparison of two data sources. *Epidemiol Infect* 2006;**134**:570–2.
282. Mehtar S, Bangham L, Kalmanovitch D, et al. Adult epiglottitis due to *Vibrio vulnificus*. *Br Med J (Clin Res Ed)* 1988;**296**:827–8.
283. Melnick A, Berger R, Green G. Spasmodic croup in children: personal experiences with intermittent positive pressure breathing in therapy. *Clin Pediatr (Phila)* 1972;**11**:615–7.
284. Midwinter KI, Hodgson D, Yardley M. Paediatric epiglottitis: the influence of the *Haemophilus influenzae* b vaccine, a ten-year review in the Sheffield region. *Clin Otolaryngol Allied Sci* 1999;**24**:447–8.
285. Milko DA, Marshak G, Striker TW. Nasotracheal intubation in the treatment of acute epiglottitis. *Pediatrics* 1974;**53**:674–7.
286. Miller BR, Arthur JD, Parry WH, et al. Atypical croup and *Chlamydia trachomatis*. *Lancet* 1982;**1**:1022.
287. Miller DG, Gabrielson MO, Horstmann DM. Clinical virology and viral surveillance in a pediatric group practice: the use of

double-seeded tissue culture tubes for primary virus isolation. *Am J Epidemiol* 1968;**88**:245–56.
288. Mogabgab WJ. Beta-hemolytic streptococcal and concurrent infections in adults and children with respiratory disease, 1958 to 1969. *Am Rev Respir Dis* 1970;**102**:23–34.
289. Molteni RA. Epiglottitis: incidence of extraepiglottic infection: report of 72 cases and review of the literature. *Pediatrics* 1976;**58**:526–31.
290. Moore M, Little P. Humidified air inhalation for treating croup. *Cochrane Database Syst Rev* 2006;**3**:1–16.
291. Morgan EA, Wishart DE. Laryngotracheo-bronchitis (a statistical review of 549 cases). *Can Med Assoc J* 1947;**56**:8–15.
292. Mufson MA, Krause HE, Mocega HE, et al. Viruses, *Mycoplasma pneumoniae* and bacteria associated with lower respiratory tract disease among infants. *Am J Epidemiol* 1970;**91**:192–202.
293. Murphy TV, Granoff DM, Pierson LM, et al. Invasive *Haemophilus influenzae* type b disease in children less than 5 years of age in Minnesota and in Dallas County, Texas, 1983-1984. *J Infect Dis* 1992;**165**(Suppl 1):S7–10.
294. Murphy TV, Osterholm MT, Pierson LM, et al. Prospective surveillance of *Haemophilus influenzae* type b disease in Dallas County, Texas, and in Minnesota. *Pediatrics* 1987;**79**:173–80.
295. Musharrafieh UM, Araj GF, Fuleihan NS. Viral supraglottitis in an adult: a case presentation and literature update. *J Infect* 1999;**39**:157–60.
296. Myer 3rd CM. Candida epiglottitis: clinical implications. *Am J Otolaryngol* 1997;**18**:428–30.
297. Myers C, Corbelli R, Schrenzel J, et al. Multiple pulmonary abscesses caused by *Legionella pneumophila* infection in an infant with croup. *Pediatr Infect Dis J* 2006;**25**:753–4.
298. Naqvi SH, Dunkle LM. Bacterial tracheitis and viral croup. *Pediatr Infect Dis* 1984;**3**:282–3.
299. Neffson AH. Acute laryngotracheobronchitis: a 25-year review. *Am J Med Sci* 1944;**208**:524–47.
300. Nelson K, Watanakunakorn C, Watkins DA. Acute epiglottitis due to serogroup Y *Neisseria meningitidis* in in an audit. *Clin Infect Dis* 1996;**23**:1192–3.
301. Nelson WE. Bacterial croup: a historical perspective. *J Pediatr* 1984;**105**:52–5.
302. Newth CJ, Levison H, Bryan AC. The respiratory status of children with croup. *J Pediatr* 1972;**81**:1068–73.
303. Nguyen R, Leclerc J. Cervical necrotizing fasciitis as a complication of acute epiglottitis. *J Otolaryngol* 1997;**26**:129–31.
304. Nichol KP, Cherry JD. Bacterial-viral interrelations in respiratory infections of children. *N Engl J Med* 1967;**277**:667–72.
305. Nicolai T, Mutius EV. Risk of asthma in children with a history of croup. *Acta Paediatr* 1996;**85**:1295–9.
306. Nijssen-Jordan C, Donaldson JD, Halperin SA. Bacterial tracheitis associated with respiratory syncytial virus infection and toxic shock syndrome. *CMAJ* 1990;**142**:233–4.
307. Nussbaum E. Fiberoptic laryngoscopy as a guide to tracheal extubation in acute epiglottitis. *J Pediatr* 1983;**102**:269–70.
308. O'Bier A, Muniz AE, Foster RL. Hereditary angioedema presenting as epiglottitis. *Pediatr Emerg Care* 2005;**21**:27–30.
309. O'Connell K, Fennell J, Callaghan J, et al. A fatal case of *Pasteurella multocida* epiglottitis. *Ir J Med Sci* 2009;**178**:541–2.
310. Oh TH, Motoyama EK. Comparison of nasotracheal intubation and tracheostomy in management of acute epiglottitis. *Anesthesiology* 1977;**46**:214–6.
311. Orton HB, Smith EL, Bell HO, et al. Acute laryngotracheobronchitis: analysis of sixty-two cases with report of autopsies in eight cases. *Arch Otolaryngol* 1941;**33**:926–60.
312. Ottolini MG, Porter DD, Blanco JC, et al. A cotton rat model of human parainfluenza 3 laryngotracheitis: virus growth, pathology, and therapy. *J Infect Dis* 2002;**186**:1713–7.
313. Paisley JW, Bruhn FW, Lauer BA, et al. Type A2 influenza viral infections in children. *Am J Dis Child* 1978;**132**:34–6.
314. Park KW, Darvish A, Lowenstein E. Airway management for adult patients with acute epiglottitis: a 12-year experience at an academic medical center (1984-1995). *Anesthesiology* 1998;**88**:254–61.
315. Parrott RH. Viral respiratory tract illnesses in children. *Bull N Y Acad Med* 1963;**39**:629–48.
316. Parrott RH, Vargosko AJ, Kim HW, et al. Acute respiratory diseases of viral etiology. III. parainfluenza. Myxoviruses. *Am J Public Health Nations Health* 1962;**52**:907–17.
317. Parrott RH, Vargosko AJ, Kim HW, et al. Clinical syndromes among children. *Am Rev Respir Dis* 1962;**88**:73–6.
318. Peltola H. *Haemophilus influenzae* type b disease and vaccination in Europe: lessons learned. *Pediatr Infect Dis J* 1998;**17**:S126–32.
319. Plachtova-Pecenkova I, Bruckova M, Fedova D, et al. Note on the aetiology of acute laryngotracheobronchitis in children: findings in the autumn of 1965 and the spring of 1966. *J Hyg Epidemiol Microbiol Immunol* 1968;**12**:227–37.
320. Poland JD, Welton ER, Chin TD. Influenza virus B as cause of acute croup syndrome. *Am J Dis Child* 1964;**107**:54–7.
321. Prendergast M, Jones JS, Hartman D. Racemic epinephrine in the treatment of laryngotracheitis: can we identify children for outpatient therapy? *Am J Emerg Med* 1994;**12**:613–6.
322. Quigley C, Kaczmarski EB, Jones DM, et al. *Haemophilus influenzae* type b disease in north-west England. *J Infect* 1993;**26**:215–20.
323. Rabe EF. Infectious croup: etiology. *Pediatrics* 1948;**2**:255–65.
324. Rabe EF. Infectious croup: *Hemophilus influenzae* type B croup. *Pediatrics* 1948;**2**:559–66.
325. Rabe EF. Infectious croup: virus croup. *Pediatrics* 1948;**2**:415–27.
326. Rabe EF. Acute inflammatory disorders of the larynx and laryngotracheal area. *Pediatr Clin North Am* 1957;169–82.
327. Rapkin RH. The diagnosis of epiglottitis: simplicity and reliability of radiographs of the neck in the differential diagnosis of the croup syndrome. *J Pediatr* 1972;**80**:96–8.
328. Rapkin RH. Nasotracheal intubation in epiglottitis. *Pediatrics* 1975;**56**:110–2.
329. Reed J, Shah RK, Jantausch B, et al. Aryepiglottic abscess manifesting as epiglottitis. *Arch Otolaryngol Head Neck Surg* 2009;**135**:953–5.
330. Reed SE, Boyde A. Organ cultures of respiratory epithelium infected with rhinovirus or parainfluenza virus studied in a scanning electron microscope. *Infect Immun* 1972;**6**:68–76.
331. Richards L. Fulminating laryngo-tracheo-bronchitis. *Ann Otol Rhinol Laryngol* 1933;**42**:1014–40.
332. Richards L. A further study of the pathology of acute laryngotracheo-bronchitis in children. *Ann Otol Rhinol Laryngol* 1938;**47**:326–41.
333. Richardson DK, Helderman T, Lovett P. Meningococcal epiglottitis in a diabetic adult patient: a case report. *J Emerg Med* 2012;**43**:634–6.
334. Richens J, Montgomery J. Acute epiglottitis (supraglottitis) in the puerperium caused by infection with group A streptococcus. *PNG Med J* 1988;**31**:293–4.
335. Ridgeway NA, Perlman PE, Verghese A, et al. Epiglottic abscess due to group B streptococcus. [Communication]. *Ann Otol Rhinol Laryngol* 1984;**93**:277–8.
336. Rihkanen H, Ronkko E, Nieminen T, et al. Respiratory viruses in laryngeal croup of young children. *J Pediatr* 2008;**152**:661–5.
337. Rittichier KK, Ledwith CA. Outpatient treatment of moderate croup with dexamethasone: intramuscular versus oral dosing. *Pediatrics* 2000;**106**:1344–8.
338. Rivera M, Hadlock FP, O'Meara ME. Pulmonary edema secondary to acute epiglottitis. *AJR Am J Roentgenol* 1979;**132**:991–2.
339. Robbins JP, Fitz-Hugh GS. Epiglottitis in the adult. *Laryngoscope* 1971;**81**:700–6.
340. Roberts GW, Master VV, Staugas RE, et al. Repeated dose inhaled budesonide versus placebo in the treatment of croup. *J Paediatr Child Health* 1999;**35**:170–4.
341. Rogers DJ, Sie KC, Manning SC. Epiglottitis due to nontypeable *Haemophilus influenzae* in a vaccinated child. *Int J Pediatr Otorhinolaryngol* 2010;**74**:218–20.
342. Rosales JK, Davenport HT. Acute laryngotracheobronchitis and epiglottitis. *Can Anaesth Soc J* 1962;**9**:467–78.
343. Rose FB, Garman RF, Falkenberg KJ, et al. Adult epiglottitis, cellulitis, and *Streptococcus pneumoniae* bacteremia. *Scand J Infect Dis* 1982;**14**:301–3.
344. Rosychuk RJ, Klassen TP, Metes D, et al. Croup presentations to emergency departments in Alberta, Canada: a large population-based study. *Pediatr Pulmonol* 2010;**45**:83–91.
345. Rosychuk RJ, Klassen TP, Voaklander DC, et al. Seasonality patterns in croup presentations to emergency departments in Alberta, Canada: a time series analysis. *Pediatr Emerg Care* 2011;**27**:256–60.
346. Rothstein SG, Persky MS, Edelman BA, et al. Epiglottitis in AIDS patients. *Laryngoscope* 1989;**99**:389–92.

347. Rull J, Hargitai R. [Treatment of subglottic laryngitis by inhalation of micronephrin under high pressure]. *Orv Hetil* 1974;**115**: 2727–31.
348. Russel K, Wiebe N, Saenz A, et al. Glucocorticoids for croup. *Cochrane Database Syst Rev* 2004;(1):CD001955.
349. Russell GA, Gresham GA, Wight DG. Acute epiglottitis in adults not due to haemophilus. *J Laryngol Otol* 1985;**99**:1035–8.
350. Santosham M, Moxon ER. Detection and quantitation of bacteremia in childhood. *J Pediatr* 1977;**91**:719–21.
351. Schalen L, Andersson K. Differential diagnosis and treatment of psychogenic voice disorder. *Clin Otolaryngol Allied Sci* 1992;**17**: 225–30.
352. Scheidemandel HH, Page Jr RS. Special considerations in epiglottitis in children. *Laryngoscope* 1975;**85**:1738–45.
353. Scheifele DW, Daum RS, Syriopoulou VP, et al. Comparison of two antigen detection techniques in a primate model of *Haemophilus influenzae* type b infection. *Infect Immun* 1979;**26**:827–31.
354. Schiffman FJ, Lichtman HC. Paroxysmal precordial purring sign in epiglottitis. *Lancet* 1990;**335**:609.
355. Schuh S, Huang A, Fallis JC. Atypical epiglottitis. *Ann Emerg Med* 1988;**17**:168–70.
356. Schuller DE, Birck HG. The safety of intubation in croup and epiglottitis: an eight-year follow-up. *Laryngoscope* 1975;**85**:33–46.
357. Schultes A, Agia GA. Acute *Hemophilus parainfluenzae* epiglottitis in an adult. *Postgrad Med* 1984;**75**:207–11.
358. Schultz RL, Morrison WV. Short-term intubation in children with acute epiglottitis. *South Med J* 1982;**75**:158–60.
359. Schumaker HM, Doris PE, Birnbaum G. Radiographic parameters in adult epiglottitis. *Ann Emerg Med* 1984;**13**:588–90.
360. Schwam E, Cox J. Fulminant meningococcal supraglottitis: an emerging infectious syndrome? *Emerg Infect Dis* 1999;**5**:464–7.
361. Scolnik D, Coates AL, Stephens D, et al. Controlled delivery of high vs low humidity vs mist therapy for croup in emergency departments: a randomized controlled trial. *JAMA* 2006;**295**: 1274–80.
362. Scully RE, Galdabini JJ, McNeely BU. Presentation of case. *N Engl J Med* 1977;**297**:878–83.
363. Segal AO, Crighton EJ, Moineddin R, et al. Croup hospitalizations in Ontario: a 14-year time-series analysis. *Pediatrics* 2005; **116**:51–5.
364. Sendi K, Crysdale WS, Yoo J. Tracheitis: outcome of 1,700 cases presenting to the emergency department during two years. *J Otolaryngol* 1992;**21**:20–4.
365. Shackelford GD, Siegel MJ, McAlister WH. Subglottic edema in acute epiglottitis in children. *AJR Am J Roentgenol* 1978;**131**: 603–5.
366. Shah RK, Stocks C. Epiglottitis in the United States: national trends, variances, prognosis, and management. *Laryngoscope* 2010;**120**:1256–62.
367. Shalit M, Gross DJ, Levo Y. Pneumococcal epiglottitis in systemic lupus erythematosus in high-dose corticosteroids. *Ann Rheum Dis* 1982;**41**:615–6.
368. Shann FA, Phelan PD, Stocks JG, et al. Prolonged nasotracheal intubation or tracheostomy in acute laryngotracheobronchitis and epiglottitis? *Aust Paediatr J* 1975;**11**:212–7.
369. Sharma N, Berman DM, Scott GB, et al. Candida epiglottitis in an adolescent with acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 2005;**24**:91–2.
370. Shih L, Hawkins DB, Stanley Jr RB. Acute epiglottitis in adults: a review of 48 cases. *Ann Otol Rhinol Laryngol* 1988;**97**:527–9.
371. Sinclair SE. *Haemophilus influenzae* type b in acute laryngitis with bacteremia. *JAMA* 1941;**117**:170–3.
372. Singer JI, McCabe JB. Epiglottitis at the extremes of age. *Am J Emerg Med* 1988;**6**:228–31.
373. Singer OP, Wilson WJ. Laryngotracheobronchitis: 2 years' experience with racemic epinephrine. *Can Med Assoc J* 1976;**115**: 132–4.
374. Sivakumar S, Latifi SQ. Varicella with stridor: think group A streptococcal epiglottitis. *J Paediatr Child Health* 2008;**44**:149–51.
375. Sivalingam P, Tully AM. Acute meningococcal epiglottitis and septicaemia in a 65-y-old man. *Scand J Infect Dis* 1998;**30**:196–8.
376. Skolnik NS. Treatment of croup: a critical review. *Am J Dis Child* 1989;**143**:1045–9.
377. Skowron PN, Turner JA, McNaughton GA. The use of corticosteroid (dexamethasone) in the treatment of acute laryngotracheitis. *Can Med Assoc J* 1966;**94**:528–31.
378. Slack CL, Allen GC, Morrison JE, et al. Post-varicella epiglottitis and necrotizing fasciitis. *Pediatrics* 2000;**105**:e13.
379. Slovis TL, Arcinue E. Subglottic edema in acute epiglottitis in children. *AJR Am J Roentgenol* 1979;**132**:500.
380. Sly PD, Landau LI, Wagener JS. Acute epiglottitis in childhood: report of an increased incidence in Victoria. *Aust N Z J Med* 1984;**14**:131–4.
381. Smith DS. Corticosteroids in croup: a chink in the ivory tower? *J Pediatr* 1989;**115**:256–7.
382. Smith EW, Ingram DL. Counterimmunoelectrophoresis in *Hemophilus influenzae* type b epiglottitis and pericarditis. *J Pediatr* 1975;**86**:571–3.
383. Sofer S, Chernick V. Increased need for tracheal intubation for croup in relation to bacterial tracheitis. *Can Med Assoc J* 1983;**128**:160–1.
384. Soliman MG, Richer P. Epiglottitis and pulmonary oedema in children. *Can Anaesth Soc J* 1978;**25**:270–5.
385. Solomon P, Weisbrod M, Irish JC, et al. Adult epiglottitis: the Toronto Hospital experience. *J Otolaryngol* 1998;**27**:332–6.
386. Solomon R, Truman T, Murray DL. Toxic shock syndrome as a complication of bacterial tracheitis. *Pediatr Infect Dis* 1985; **4**:298–9.
387. Somenek M, Le M, Walner DL. Membranous laryngitis in a child. *Int J Pediatr Otorhinolaryngol* 2010;**74**:704–6.
388. Stankiewicz JA, Bowes AK. Croup and epiglottitis: a radiologic study. *Laryngoscope* 1985;**95**:1159–60.
389. Stanley RE, Liang TS. Acute epiglottitis in adults (the Singapore experience). *J Laryngol Otol* 1988;**102**:1017–21.
390. Sterner G, Gerzen P, Ohlson M, et al. Acute respiratory illness and gastroenteritis in association with adenovirus type 7 infections. *Acta Paediatr* 1961;**50**:457–68.
391. Stott EJ, Bell EJ, Eadie MB, et al. A comparative virological study of children in hospital with respiratory and diarrhoeal illnesses. *J Hyg (Lond)* 1967;**65**:9–23.
392. Stuart MJ, Hodgetts TJ. Adult epiglottitis: prompt diagnosis saves lives. *BMJ* 1994;**308**:329–30.
393. Sullivan TD, La Scotea LJ, Neter E. Relationship between the magnitude of bacteremia and the clinical disease. *Pediatrics* 1982;**69**:669–702.
394. Sung JY, Lee HJ, Eun BW, et al. Role of human coronavirus NL63 in hospitalized children with croup. *Pediatr Infect Dis J* 2010;**29**:822–6.
395. Super DM, Cartelli NA, Brooks LJ, et al. A prospective randomized double-blind study to evaluate the effect of dexamethasone in acute laryngotracheitis. *J Pediatr* 1989;**115**:323–9.
396. Suresh GK, Dhawan A, Kohli V. Tracheal diphtheria mimicking bacterial tracheitis. *Pediatr Infect Dis J* 1992;**11**:502.
397. Surh L, Read SE. Staphylococcal tracheitis and toxic shock syndrome in a young child. *J Pediatr* 1984;**105**:585–7.
398. Sussman S, Grossman M, Magoffin R, et al. Dexamethasone (16-alpha-methyl, 9-alpha-fluoroprednisolone) in obstructive respiratory tract infections in children. A controlled study. *Pediatrics* 1964;**34**:851–5.
399. Szpunar J, Glowacki J, Laskowski A, et al. Fibrinous laryngotracheobronchitis in children. *Arch Otolaryngol* 1971;**93**:173–8.
400. Taber LH, Knight V, Gilbert BE, et al. Ribavirin aerosol treatment of bronchiolitis associated with respiratory syncytial virus infection in infants. *Pediatrics* 1983;**72**:613–8.
401. Takala AK, Eskola J, van Alphen L. Spectrum of invasive *Haemophilus influenzae* type b disease in adults. *Arch Intern Med* 1990;**150**:2573–6.
402. Takala AK, Sarvas H, Kela E, et al. Susceptibility to invasive *Haemophilus influenzae* type b disease and the immunoglobulin G2m(n) allotype. *J Infect Dis* 1991;**163**:637–9.
403. Takala AK, van Alphen L, Eskola J, et al. *Haemophilus influenzae* type b strains of outer membrane subtypes 1 and 1c cause different types of invasive disease. *Lancet* 1987;**2**:647–50.
404. Tan AK, Manoukian JJ. Hospitalized croup (bacterial and viral): the role of rigid endoscopy. *J Otolaryngol* 1992;**21**:48–53.
405. Taussig LM, Castro O, Beaudry PH, et al. Treatment of laryngotracheobronchitis (croup): use of intermittent positive-pressure breathing and racemic epinephrine. *Am J Dis Child* 1975;**129**: 790–3.
406. Tejani A, Mahadevan R, Dobias B, et al. Occurrence of HLA types in H. influenzae type B disease. *Tissue Antigens* 1981; **17**:205–11.

407. Temple AR. Recent advances in diagnosis and management of croup. *J Fam Pract* 1975;**2**:85–9.
408. Templer JW. Trauma to the larynx and cervical trachea. In: English GM, editor. *Otolaryngology*. Hagerstown, Md: Harper & Row; 1976.
409. Tenenbein M. The steroid odyssey in croup. *Pediatrics* 2005; **116**:230–1.
410. Theisen DF. Angina epiglottidea anterior: report of three cases. *Albany Med Ann* 1900;**21**:395–405.
411. Top Sr FH. Diphtheria. In Top Sr FH, Wehrle PF, editors. *Communicable and infectious diseases*. St. Louis: C. V. Mosby; 1972. p. 190–207.
412. Tos M. Nasotracheal intubation instead of tracheotomy in acute epiglottitis in children. *Acta Otolaryngol* 1973;**75**:382–3.
413. Toth M, Major V. Virological investigation of hospitalized cases of pseudocroup and acute laryngotracheobronchitis. *Acta Microbiol* 1965;**12**:189–200.
414. Travis KW, Todres ID, Shannon DC. Pulmonary edema associated with croup and epiglottitis. *Pediatrics* 1977;**59**: 695–8.
415. Trollfors B. Invasive *Haemophilus influenzae* infections in household contacts of patients with *Haemophilus influenzae* meningitis and epiglottitis. *Acta Paediatr Scand* 1991;**80**:795–7.
416. Trollfors B, Nylen O, Strangert K. Acute epiglottitis in children and adults in Sweden 1981-3. *Arch Dis Child* 1990;**65**:491–4.
417. Trollfors B, Nylen O, Carenfelt C, et al. Aetiology of acute epiglottitis in adults. *Scand J Infect Dis* 1998;**30**:49–51.
418. Tunnessen Jr WW, Feinstein AR. The steroid-croup controversy: an analytic review of methodologic problems. *J Pediatr* 1980;**96**: 751–6.
419. Tveteras K, Kristensen S. Acute epiglottitis in adults: bacteriology and therapeutic principles. *Clin Otolaryngol Allied Sci* 1987;**12**: 337–43.
420. Tyrrell DAJ. *Common colds and related diseases*. Baltimore: Williams & Wilkins; 1965.
421. Valdepena HG, Wald ER, Rose E, et al. Epiglottitis and *Haemophilus influenzae* immunization: the Pittsburgh experience: a five-year review. *Pediatrics* 1995;**96**:424–7.
422. Van Bever HP, Wieringa MH, Weyler JJ, et al. Croup and recurrent croup: their association with asthma and allergy: an epidemiological study on 5-8-year-old children. *Eur J Pediatr* 1999; **158**:253–7.
423. Van der Hoek L, Sure K, Ihorst G, et al. Croup is associated with the novel coronavirus NL63. *PLoS Med* 2005;**2**:e240.
424. Vargosko AJ, Chanock RM, Huebner RJ, et al. Association of type 2 hemadsorption (parainfluenza 1) virus and Asian influenza A virus with infections croup. *N Engl J Med* 1959;**261**:1–9.
425. Vauthy PA, Reddy R. Acute upper airway obstruction in infants and children: evaluation by the fiberoptic bronchoscope. *Ann Otol Rhinol Laryngol* 1980;**89**:417–8.
426. Vernham GA, Crowther JA. Acute myeloid leukaemia presenting with acute *Branhamella catarrhalis* epiglottitis. *J Infect* 1993;**26**: 93–5.
427. Vernon DD, Sarnaik AP. Acute epiglottitis in children: a conservative approach to diagnosis and management. *Crit Care Med* 1986;**14**:23–5.
428. Vetto RR. Epiglottitis: report of thirty-seven cases. *JAMA* 1960;**173**:990–4.
429. Vigil KJ, Mulanovich VE, Chemaly RF, et al. Severe parainfluenza virus type 2 supraglottitis in an immunocompetent adult host: an unusual cause of a paramyxoviridae viral infection. *J Intern Med* 2009;**265**:397–400.
430. Vihma L. Surveillance of acute viral respiratory diseases in children. *Acta Paediatr Scand* 1969;**192**:8–53.
431. Walker P, Crysdale WS. Croup, epiglottitis, retropharyngeal abscess, and bacterial tracheitis: evolving patterns of occurrence and care. *Int Anesthesiol Clin* 1992;**30**:57–70.
432. Walsh TJ, Gray WC. Candida epiglottitis in immunocompromised patients. *Chest* 1987;**91**:482–5.
433. Ward J. Haemophilus influenzae. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 4th ed. Philadelphia: W. B. Saunders; 1998.
434. Ward JI, Margolis HS, Lum MK, et al. *Haemophilus influenzae* disease in Alaskan Eskimos: characteristics of a population with an unusual incidence of invasive disease. *Lancet* 1981;**1**:1281–5.
435. Ward JI, Siber GR, Scheifele DW, et al. Rapid diagnosis of *Hemophilus influenzae* type b infections by latex particle agglutination and counterimmunoelectrophoresis. *J Pediatr* 1978; **93**:37–42.
436. Warner JA, Finlay WE. Fulminating epiglottitis in adults: report of three cases and review of the literature. *Anaesthesia* 1985;**40**:348–52.
437. Warshawski J, Havas TE, McShane DP, et al. Adult epiglottitis. *J Otolaryngol* 1986;**15**:362–5.
438. Weber JE, Chudnofsky CR, Younger JG, et al. A randomized comparison of helium-oxygen mixture (Heliox) and racemic epinephrine for the treatment of moderate to severe croup. *Pediatrics* 2001;**107**:E96.
439. Weber ML, Desjardins R, Perreault G, et al. Acute epiglottitis in children: treatment with nasotracheal intubation: report of 14 consecutive cases. *Pediatrics* 1976;**57**:152–5.
440. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. *J Pediatr* 2009;**154**:694–9.
441. Welliver RC. Croup: continuing controversy. *Semin Pediatr Infect Dis* 1995;**6**:90–5.
442. Welliver RC, Sun M, Rinaldo D. Defective regulation of immune responses in croup due to parainfluenza virus. *Pediatr Res* 1985;**19**:716–20.
443. Welliver RC, Wong DT, Middleton Jr E, et al. Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. *J Pediatr* 1982;**101**:889–96.
444. Wenger JK. Supraglottitis and group A streptococcus. *Pediatr Infect Dis J* 1997;**16**:1005–7.
445. Wenner HA, Christodoulopoulou G, Weston J, et al. The etiology of respiratory illnesses occurring in infancy and childhood. *Pediatrics* 1963;**31**:4–17.
446. Westley CR, Cotton EK, Brooks JG. Nebulized racemic epinephrine by IPPB for the treatment of croup: a double-blind study. *Am J Dis Child* 1978;**132**:484–7.
447. Wetmore RF, Handler SD. Epiglottitis: evolution in management during the last decade. *Ann Otol Rhinol Laryngol* 1979;**88**: 822–6.
448. Whisnant JK, Rogentine GN, Gralnick MA, et al. Host factors and antibody response *Haemophilus influenza* type b meningitis and epiglottitis. *J Infect Dis* 1976;**133**:448–55.
449. Wilson SZ, Gilbert BE, Quarles JM, et al. Treatment of influenza A (H1N1) virus infection with ribavirin aerosol. *Antimicrob Agents Chemother* 1984;**26**:200–3.
450. Wolf M, Strauss B, Kronenberg J, et al. Conservative management of adult epiglottitis. *Laryngoscope* 1990;**100**:183–5.
451. Wong VK, Mason WH. *Branhamella catarrhalis* as a cause of bacterial tracheitis. *Pediatr Infect Dis J* 1987;**6**:945–6.
452. Wood N, Menzies R, McIntyre P. Epiglottitis in Sydney before and after the introduction of vaccination against *Haemophilus influenzae* type b disease. *Intern Med J* 2005;**35**:530–5.
453. Wulff H, Kidd P, Wenner HA. Etiology of respiratory infections: further studies during infancy and childhood. *Pediatrics* 1964; **33**:30–44.
454. Young N, Finn A, Powell C. Group B streptococcal epiglottitis. *Pediatr Infect Dis J* 1996;**15**:95–6.
455. Yousuf K, Lui B, Lemckert R, et al. Recurrent adult epiglottitis: contiguous spread from group A streptococcus lingual tonsillitis. *J Otolaryngol* 2006;**35**:65–7.
456. Zach MS. Airway reactivity in recurrent croup. *Eur J Respir Dis Suppl* 1983;**128**(Pt 1):81–8.
457. Zacharisen MC, Conley SF. Recurrent respiratory papillomatosis in children: masquerader of common respiratory diseases. *Pediatrics* 2006;**118**:1925–31.
458. Zulliger JJ, Schuller DE, Beach TP, et al. Assessment of intubation in croup and epiglottitis. *Ann Otol Rhinol Laryngol* 1982;**91**: 403–6.
459. Zwahlen A, Regamy C. Les épiglottites aiguës de l'adulte. *Schweiz Med Wochenschr* 1978;**108**:447–82.

SECTION **II**

LOWER RESPIRATORY TRACT INFECTIONS

CHAPTER 19

Acute Bronchitis

James D. Cherry

Bronchitis is a common diagnosis in pediatric practice, although little unanimity exists among physicians regarding its exact clinical constellation and in the true pathologic sense it probably never occurs as an isolated entity. Acute bronchitis is a febrile illness with cough, rhonchi, and referred breath sounds.[13] Asthmatic bronchitis (infectious asthma), similar to acute bronchitis but with associated wheezing and expiratory distress, is discussed in Chapter 21. On pathologic examination, the clinical illness of acute bronchitis reflects acute inflammatory disease of the larger air passages, including the trachea and the large and medium-sized bronchi.[22]

ETIOLOGY

Table 19-1 lists various infectious agents associated with acute bronchitis. Infections with adenoviruses, influenza viruses, parainfluenza viruses, respiratory syncytial virus, and *Mycoplasma pneumoniae* account for most cases of acute bronchitis in children. These agents, plus many rhinoviruses, a few enteroviruses, and perhaps human metapneumovirus, human bocavirus, and the newer human coronaviruses, account for virtually all cases in the United States today.

Of the adenoviruses, type 7 has been associated most commonly with acute bronchitis in children. In military recruits, including adolescents, adenovirus types 4 and 7 cause epidemic acute respiratory disease, in which bronchitis is a usual occurrence.[19,62]

Influenza A virus infection is a common cause of severe acute bronchitis, particularly at the time of an antigenic shift of influenza A virus subtype and pandemic disease. Acute bronchitis caused by influenza A virus also is a regular occurrence between pandemics in new susceptible individuals (young children) in the population. Influenza B virus also is an important cause of bronchitis, and it was a more common causative agent than was influenza A virus in one large longitudinal study.[12]

All cases of measles involve the bronchi, but measles has been an uncommon occurrence since the advent of widespread use of vaccines. Of the parainfluenza viruses, type 3 is associated most commonly with acute bronchitis. Respiratory syncytial virus is a common cause of acute bronchitis, particularly in very young children. The more recently identified human metapneumovirus and human bocavirus are also causes of acute bronchitis.[16,54,60,69,70]

Of the bacterial agents listed in Table 19-1, only *Haemophilus influenzae* clearly can be incriminated. *Bordetella pertussis* infection involves the trachea and bronchi, but fever is an uncommon event and the illness is outside the definition of acute bronchitis. When sought, *M. pneumoniae* is a common cause of bronchitis. *Chlamydia pneumoniae* has been found to be the cause of bronchitis in adolescents and young adults.[29]

EPIDEMIOLOGY

The epidemiology of the common viruses associated with bronchitis is presented in Section XVII. Chapman and associates[12] published the results of a study of acute bronchitis in a single private group pediatric practice in Chapel Hill, North Carolina. The study occurred during a 104-month period, during which 5489 episodes of lower respiratory tract illness occurred. Of these illnesses, 40.1 percent were acute bronchitis. The bronchitis attack rate was highest in children in the second year of life (6.71%), and then it decreased gradually to approximately 2 percent in teenagers. In contrast to the age-specific attack rates, the ratio of bronchitis cases to all lower respiratory illness cases increased with age. In the first year of life, the ratio was 0.29; in children 12 years old or older, it was 0.69.

During the first 6 years of life, respiratory syncytial virus and parainfluenza virus type 3 were the most common etiologic agents noted in the Chapel Hill study. During the first 2 years of life, adenoviruses also commonly were associated with bronchitis. In patients older than 6 years, *M. pneumoniae* and influenza A and B viruses were the most common etiologic agents. In a study of cough illnesses of 6 days' duration or longer in university students, investigators found that 15 of 31 students with laboratory evidence of *Bordetella* spp. infection were considered by their primary care providers to have bronchitis.[42]

The incidence of acute bronchitis peaks in the winter months, declines to midsummer, and increases again through the fall. Attack rates generally are higher in boys than in girls.[12,36,63] A sex difference is most pronounced during the first 6 years of life.

PATHOPHYSIOLOGY AND PATHOLOGY

Because acute bronchitis is an illness characterized by clinical features and one not usually associated with death, knowledge of its pathophysiology and pathology

TABLE 19-1 **Infectious Agents Associated with Acute Bronchitis**

Agent	Importance in Causation*	References
Viruses		
Adenovirus types 1-7, 12	+++	1,2,4,9-11,20,24,25,32,33,39,52,56,59,63,64,68
Enterovirus	+	27,32,33,56
Coxsackieviruses B	+	11
Echoviruses 8, 12, 14	+	61
Polioviruses	+	25,61
Herpes simplex	+	25,56,61
Influenza	+++	1,5,7,9-12,14,20,24,27,58,61
A	++	5,9,11,12,27,32,33,58,61
B	++	9,11,12,27,32
C	+	1
Measles	+	14,22,50
Mumps	+	61
Parainfluenza	+++	1,5,9-12,14,24-27,30,32,39,56,58,61,64
1	++	9-12,20,27,30
2	++	5,9-11,27,30
3	+++	1,2,5,9-12,20,27,30,33,58
4	+	26
Respiratory syncytial	+++	1-3,7,9-12,14,20,24,25,27,31-33,35,42,53,56,57,59,61,64
Human metapneumovirus	+	54
Human bocavirus	+	16,34,69,70
Human coronavirus	+	4,15,23
Rhinoviruses	++	9,11,14,24,25,27,32,33,49,55,56†
Bacteria		
Bordetella pertussis	+	22,43
Bordetella parapertussis	–	
Haemophilus influenzae	–	39,66
Moraxella catarrhalis	–	18,28
Streptococcus pneumoniae	–	39
Streptococcus pyogenes	–	39,48,56
Other		
Chlamydia psittaci	+	11
Chlamydia pneumoniae	+	29
Mycoplasma pneumoniae	+++	9-12,14,20,27,32,38,51,65

*+++, very common; ++, common; +, rare; –, of questionable etiologic significance

is meager. The general pathophysiology of human infections with viruses and *M. pneumoniae* that cause acute bronchitis is presented more completely in the sections of this book related to the individual infectious agents.

In virtually all cases of acute bronchitis, evidence of upper respiratory tract viral infection (pharyngitis, rhinitis) also is present. Tracheal and bronchial infection apparently is the result of distal spread. In bronchitis, the clinical features result from damage to the ciliated epithelium of the lower trachea and the large and medium-sized bronchi.[22] Although the cytopathologies of the various infectious agents is different,[71] the resulting obstruction of the air passages leads to similar symptoms. The duration of symptoms depends to some extent on the specific initial infectious agent and, in cases of prolonged illness, on secondary bacterial infection.

In acute bronchitis, the larynx and subglottic trachea are not involved prominently. Conversely, today, bronchial involvement is seen only occasionally in croup.

CLINICAL PRESENTATION

Initial manifestations of acute bronchitis occur in the upper respiratory tract and, depending on the etiologic agent, are predominantly nasal, as in the common cold, or show additional objective evidence of pharyngitis, as in nasopharyngitis. Fever usually is present, and temperatures vary from 37.8°C to 39°C (100°F to 102.2°F) on most occasions. Cough is always present, and its onset can be insidious or abrupt. Initially, the cough is dry and harsh and often brassy in younger children. As the illness progresses, the cough becomes looser. In older children, purulent sputum is raised and expectorated. In younger children, the swallowing of often tenacious sputum frequently leads to gagging and vomiting. Older children may complain of chest pain resulting from coughing.

On initial physical examination, a variable degree of rhinitis usually is present; many patients have diffuse pharyngeal erythema. As the disease progresses, these upper

respiratory tract signs generally decrease. Examination of the chest reveals rhonchi and referred breath sounds. Coarse, changing rales are noted frequently.

In the usual case of acute bronchitis, the illness can be separated into three phases: (1) a 1- to 2-day prodromal period when fever and upper respiratory tract symptoms predominate, (2) a 4- to 6-day period of marked tracheobronchial symptoms with some fever and general discomfort, and (3) a recovery period that may last 1 or 2 weeks and is characterized by cough and expectoration. Occasionally, the recovery period is particularly distressing and is associated with a low-grade fever, suggesting secondary bacterial infection. Bronchitis caused by *C. pneumoniae* often is insidious in onset and frequently associated with or preceded by pharyngitis.[29,37] Illness persists for several weeks but responds to appropriate antibiotic therapy.

Laboratory study in acute bronchitis is of limited use. Children in whom throat cultures reveal pathogenic bacteria in predominant growth tend to have more severe illness than do children with only viral infections.[14,44] The white blood cell count usually is greater than 10,000 cells/mm^3, and approximately one third of the cases have a predominance of neutrophils.[44] The chest radiograph is normal unless associated pulmonary involvement is present.

DIFFERENTIAL DIAGNOSIS AND SPECIFIC DIAGNOSIS

Because acute bronchitis is a clinical entity caused by multiple etiologic agents, the most difficult differential aspect of diagnosis is the selection of the specific infectious cause. Also important is the separation of acute, self-limited bronchitis from chronic, more serious problems, such as cystic fibrosis, allergic respiratory disease, and sinusitis.

An epidemiologic history frequently can help in assigning a particular virus, *M. pneumoniae*, *C. pneumoniae*, or *B. pertussis*, as the presumptive etiologic agent. If epidemic bronchiolitis is occurring in the community, respiratory syncytial virus would be a likely cause. Similarly, predictions of causation by influenza virus, parainfluenza virus, adenoviruses, *M. pneumoniae*, *C. pneumoniae*, or *B. pertussis* can be made through clinical epidemiologic observations. Specific etiologic diagnosis can be established through the isolation of an organism or its identification by a direct antigen test or polymerase chain reaction from the nasopharyngeal secretions. Serologic study on paired sera may be useful for the diagnosis of *M. pneumoniae*, *C. pneumoniae*, and *B. pertussis*. Single serum IgM studies are useful in the diagnosis of *M. pneumoniae* and *C. pneumoniae*. Single serum study is also useful in the diagnosis of *B. pertussis* infection; specifically the demonstration of significant immunoglobulin (Ig)G or IgA values to pertussis toxin.

Children with protracted illnesses or febrile exacerbations should be examined by culture, radiograph, and perhaps further imaging studies for secondary bacterial infection of the tracheobronchial tree or the lungs or both. Children with chronic recurrent illnesses should be tested for cystic fibrosis, allergic conditions, and anatomic problems, such as gastroesophageal reflux and tracheoesophageal fistula.

TREATMENT

Treatment of acute bronchitis is distinguished more by what *not* to do than by specific modalities. In most mild cases, no specific therapy is indicated.

For children who feel miserable during the initial phases of acute bronchitis, analgesic therapy may be useful. Formerly, aspirin was the recommended analgesic. Because aspirin is an etiologic factor in influenza-associated Reye syndrome and because differentiating influenza viral infections from other respiratory viral infections is difficult, it is prudent to use acetaminophen rather than aspirin. The dose per single administration of acetaminophen by year of age is as follows: younger than 1 year, 60 mg; 1 to 3 years, 60 to 120 mg; 3 to 6 years, 120 mg; 6 to 12 years, 150 to 300 mg; older than 12 years, 325 to 650 mg. Administration may be repeated three or four times daily in young children and every 4 hours in older children. Acetaminophen rarely should be given to infants younger than 6 months of age.

As a result of widespread advertising, a common practice of individuals with acute bronchitis is to use an array of cold remedies, which contain various combinations of antihistamines, decongestants, and antitussives. None has been shown to be useful in acute bronchitis, and in certain stages of illness, they may aggravate the recovery process.

Intake of fluids should be encouraged to prevent overall dehydration and decrease the viscosity of new secretions. Use of mist therapy also may help in thinning the exudate-containing respiratory secretions.[21,47]

In severe cases of acute bronchitis, treatment with specific antiviral agents should be considered. When influenza A virus is the likely etiologic agent, zanamivir or oseltamivir therapy may be beneficial.[8]

As noted in Table 19-1, most cases of acute bronchitis are caused by viruses, so antibiotic therapy would not be indicated.[45,67] In cases in which fever returns or no trend toward recovery is seen by the seventh day of illness, the possibility of a secondary bacterial infection should be considered. The association of sinusitis or a throat culture with predominant growth of a respiratory pathogen (*Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Moraxella catarrhalis*, *H. influenzae*) is an indication for therapy. Infection with *M. pneumoniae* also should be treated, but in contrast to treatment of pneumonia, the therapy usually does not show an impressive response. Bronchitis caused by *C. pneumoniae* should be treated with erythromycin or another macrolide.[29,46]

PROGNOSIS

The prognosis in acute bronchitis usually is excellent. Although the duration of cough can be disturbing to the parent and the child, full recovery is the rule. Several studies suggest that lower respiratory tract illness in the first few years of life may be associated with persistent

respiratory symptoms and with abnormalities in lung function in later life.[6,17,40,41] Although none of these studies has followed children with acute bronchitis specifically, the findings in other illnesses (bronchiolitis and croup) indicate a need to observe children with episodes of acute bronchitis carefully, as well.

PREVENTION

At present, no practical method of prevention of acute bronchitis in children exists. Because most cases result from infections with common respiratory viruses, the development of vaccines could be expected to be helpful.

NEW REFERENCES SINCE THE SIXTH EDITION

16. Chuang CY, Kao CL, Huang LM, et al. Human bocavirus as an important cause of respiratory tract infection in Taiwanese children. *J Microbiol Immunol Infect* 2011;**44**:323–7.
46. Panpanich R, Lerttrakarnnon P, Laopaiboon M. Azithromycin for acute lower respiratory tract infections. *Cochrane Database Syst Rev* 2008;(1):CD001954.
60. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev* 2010;**23**:74–98.
69. Zeng M, Zhu QR, Wang XH, et al. Human bocavirus in children with respiratory tract infection in Shanghai: a retrospective study. *World J Pediatr* 2010;**6**:65–70.
70. Zhang LL, Tang LY, Xie ZD, et al. Human bocavirus in children suffering from acute lower respiratory tract infection in Beijing Children's Hospital. *Chin Med J (Engl)* 2008;**121**:1607–10.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 20

Chronic Bronchitis

I. Celine Hanson • William T. Shearer

Chronic bronchitis is a serious and costly health problem in adults.[14] The World Health Organization estimates 23.6 million adults have chronic obstructive pulmonary disease, representing more than 15 percent of the adult population, and many of these cases meet the definition for chronic bronchitis.[14] A diagnosis of chronic bronchitis without chronic obstructive pulmonary disease was present in 3.5 percent of 10,000 adults in a French study cohort.[9] In the adult literature, the accepted definition of chronic bronchitis includes daily excessive production of sputum with manifestation of cough present on most days for 3 months in a year for not less than 2 successive years.[43]

For most pediatricians, the clinical entity of chronic bronchitis is ill-defined. Chronic cough complex can be associated with other, more common respiratory or cardiac diseases that must be excluded before a diagnosis of chronic bronchitis is considered.[6] Chronic bronchitis usually is described in the literature as chronic productive cough and is synonymous with asthmatic bronchitis.[39] The lack of uniform or standardized definitions of pediatric chronic bronchitis leads to wide discrepancies in reported childhood prevalence (Table 20-1). In affected patients, causal relationships and acute exacerbations have been linked to exposure to a noxious inhaled agent (e.g., environmental or industrial pollution, cigarette smoke), specific host factors (e.g., genetic predisposition), and infectious respiratory pathogens. The pathology of the disease entity is unclear. Bronchoscopy of pediatric patients with chronic bronchitis has revealed findings similar to those noted in children with asthma (granulocyte and mononuclear cell predominance at lavage and biopsy), which reflects the inclusion of asthma in the spectrum of the chronic bronchitis complex.[20,39]

DIFFERENTIAL DIAGNOSIS

Because chronic bronchitis is accepted by most physicians as being a complex of symptoms characterized by persistent cough with or without wheezing, it is imperative that the physician evaluate the patient for diseases that include chronic bronchitis within their spectrum of signs and symptoms. Figure 20-1 includes disorders that have clinical manifestations of chronic cough for longer than 3 months and provides guidelines for diagnostic

REFERENCES

1. Aitken CJ, Moffat MA, Sutherland JA. Respiratory illness and viral infection in an Edinburgh nursery. *J Hyg (Lond)* 1967;**65**:25–36.
2. Avila MM, Carballal G, Rovaletti H, et al. Viral etiology in acute lower respiratory infections in children from a closed community. *Am Rev Resp Dis* 1989;**140**:634–7.
3. Berglund B, Strahlmann CH. Respiratory syncytial virus infections in hospitalized children: evaluation of the virus isolation and complement-fixation techniques in the virological diagnosis—clinical and epidemiological characteristics. *Acta Paediatr Scand* 1967;**56**:1–10.
4. Boivin G, Baz M, Cote S, et al. Infections by human coronavirus-NL in hospitalized children. *Pediatr Infect Dis J* 2005;**24**:1045–8.
5. Brocklebank JT, Court SD, McQuillin J, et al. Influenza-A infection in children. *Lancet* 1972;**2**:497–500.
6. Burrows B, Knudson RJ, Lebowitz MD. The relationship of childhood respiratory illness to adult obstructive airway disease. *Am Rev Resp Dis* 1977;**115**:751–60.
7. Caul EO, Waller DK, Clarke SK. A comparison of influenza and respiratory syncytial virus infections among infants admitted to hospital with acute respiratory infections. *J Hyg (Lond)* 1976; **77**:383–92.
8. Centers for Disease Control and Prevention. Prevention and control of influenza. *MMWR Morb Mortal Wkly Rep* 2006;**55**: 1–31.
9. Chanock R, Chambon L, Chang W, et al. WHO respiratory disease survey in children: a serological study. *Bull World Health Organ* 1967;**37**:363–9.
10. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. *Pediatrics* 1965;**36**:21–39.
11. Chanock RM, Mufson MA, Johnson KM. Comparative biology and ecology of human virus and Mycoplasma respiratory pathogens: progress in medical virology. *Prog Med Virol* 1965;**7**:208–52.
12. Chapman RS, Henderson FW, Clyde Jr WA, et al. The epidemiology of tracheobronchitis in pediatric practice. *Am J Epidemiol* 1981;**114**:786–97.
13. Cherry JD. Newer respiratory viruses: their role in respiratory illnesses of children. In: Schulman I, editor. Advances in pediatrics, Vol. 20. Chicago: Year Book Medical Publishers; 1973. p. 225–90.
14. Cherry JD, Diddams JA, Dick EC. Rhinovirus infections in hospitalized children: provocative bacterial interrelationships. *Arch Environ Health* 1967;**14**:390–6.
15. Chiu SS, Chan KH, Chu KW, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. *Clin Infect Dis* 2005;**40**:1721–9.
16. Chuang CY, Kao CL, Huang LM, et al. Human bocavirus as an important cause of respiratory tract infection in Taiwanese children. *J Microbiol Immunol Infect* 2011;**44**:323–7.
17. Colley JR, Douglas JW, Reid DD. Respiratory disease in young adults: influence of early childhood lower respiratory tract illness, social class, air pollution, and smoking. *Brit Med J* 1973;**3**:195–8.
18. Darelid J, Lofgren S, Malmvall BE. Erythromycin treatment is beneficial for longstanding *Moraxella catarrhalis* associated cough in children. *Scand J Infect Dis* 1993;**25**:323–9.
19. Dascomb HE, Hilleman MR. Clinical and laboratory studies in patients with respiratory disease caused by adenoviruses (RI-APC-ARD agents). *Am J Med* 1956;**21**:161–74.
20. Denny FW, Clyde Jr WA. Acute lower respiratory tract infections in nonhospitalized children. *J Pediatr* 1986;**108**:635–46.
21. Dulfano MJ, Adler K, Wooten O. Physical properties of sputum. IV. Effects of 100 per cent humidity and water mist. *Am Rev Resp Dis* 1973;**107**:130–2.
22. Edwards G. Acute bronchitis-aetiology, diagnosis, and management. *Brit Med J* 1966;**1**:963–6.
23. Esper F, Weibel C, Ferguson D, et al. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. *J Infect Dis* 2005;**191**:492–8.
24. Gardner PS. How etiologic, pathologic, and clinical diagnoses can be made in a correlated fashion. *Pediatr Res* 1977;**11**:254–61.
25. Gardner PS. Virus infections and respiratory disease of childhood. *Arch Dis Child* 1968;**43**:629–45.
26. Gardner SD. The isolation of parainfluenza 4 subtypes A and B in England and serological studies of their prevalence. *J Hyg (Lond)* 1969;**67**:545–50.
27. Glezen WP, Loda FA, Clyde Jr WA, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
28. Gottfarb P, Brauner A. Children with persistent cough: outcome with treatment and role of *Moraxella catarrhalis*? *Scand J Infect Dis* 1994;**26**:545–51.
29. Grayston JT, Campbell LA, Kuo CC, et al. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. *J Infect Dis* 1990;**161**:618–25.
30. Herrmann Jr EC, Hable KA. Experiences in laboratory diagnosis of parainfluenza viruses in routine medical practice. *Mayo Clinic Proc* 1970;**45**:177–88.
31. Hilleman MR. Respiratory syncytial virus. *Am Rev Resp Dis* 1963;**88**(suppl):181–97.
32. Horn ME, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967–1972. *J Hyg (Lond)* 1975;**74**:157–68.
33. Kellner G, Popow-Kraupp T, Kundi M, et al. Contribution of rhinoviruses to respiratory viral infections in childhood: a prospective study in a mainly hospitalized infant population. *J Med Vir* 1988;**25**:455–69.
34. Kesebir D, Vazquez M, Weibel C, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis* 2006;**194**:1276–82.
35. Kim HW, Arrobio JO, Brandt CD, et al. Epidemiology of respiratory syncytial virus infection in Washington, D.C. I. Importance of the virus in different respiratory tract disease syndromes and temporal distribution of infection. *Am J Epidemiol* 1973;**98**:216–25.
36. Kravitz H. Sex distribution of hospitalized children with acute respiratory diseases, gastroenteritis and meningitis. *Clin Pediatr* 1965;**4**:484–91.
37. Kuo CC, Jackson LA, Campbell LA, et al. Chlamydia pneumoniae (TWAR). *Clin Microbiol Rev* 1995;**8**:451–61.
38. Kuroki H, Morozumi M, Chiba N, et al. Characterization of children with *Mycoplasma pneumoniae* infection detected by rapid polymerase chain reaction technique. *J Infect Chem* 2004;**10**:65–7.
39. Laxdal OE, Robertson HE, Braaten V, et al. Acute respiratory infections in children. I. An intensive study of etiology in an open community. *Can Med Assoc J* 1963;**88**:1049–54.
40. Lebowitz MD, Burrows B. The relationship of acute respiratory illness history to the prevalence and incidence of obstructive lung disorders. *Am J Epidemiol* 1977;**105**:544–54.
41. Leeder SR, Woolcock AJ, Blackburn CR. Prevalence and natural history of lung disease in New South Wales schoolchildren. *Int J Epidemiol* 1974;**3**:15–23.
42. McClelland L, Hilleman MR, Hamparian VV, et al. Studies of acute respiratory illnesses caused by respiratory syncytial virus. II. Epidemiology and assessment of importance. *New Engl J Med* 1961;**264**:1169–75.
43. Mink CM, Cherry JD, Christenson P, et al. A search for *Bordetella pertussis* infection in university students. *Clin Infect Dis* 1992;**14**: 464–71.
44. Nichol KP, Cherry JD. Bacterial-viral interrelations in respiratory infections of children. *New Engl J Med* 1967;**277**:667–72.
45. O'Brien KL, Dowell SF, Schwartz B, et al. Cough illness/bronchitis: principles of judicious use of antimicrobial agents. *Pediatrics* 1998;**101**:178–81.
46. Panpanich R, Lerttrakarnnon P, Laopaiboon M. Azithromycin for acute lower respiratory tract infections. *Cochrane Database Syst Rev* 2008;(1):CD001954.
47. Parks CR. Mist therapy: rationale and practice. *J Pediatr* 1970;**76**:305–13.
48. Pereira MS. Adenovirus infections. *Postgrad Med J* 1973;**49**: 798–801.
49. Person DA, Herrmann Jr EC. Experiences in laboratory diagnosis of rhinovirus infections in routine medical practice. *Mayo Clin Proc* 1970;**45**:517–26.
50. Robbins FC. Measles: clinical features: pathogenesis, pathology and complications. *Am J Dis Child* 1962;**103**:266–73.
51. Saliba GS, Glezen WP, Chin TD. Mycoplasma pneumoniae infection in a resident boys' home. *Am J Epidemiol* 1967;**86**:408–18.

52. Simila S, Jouppila R, Salmi A, et al. Encephaloningitis in children associated with an adenovirus type 7 epidemic. *Acta Paediatr Scand* 1970;**59**:310–6.
53. Spence L, Barratt N. Respiratory syncytial virus associated with acute respiratory infections in Trinidadian patients. *Am J Epidemiol* 1968;**88**:257–66.
54. Stockton J, Stephenson I, Fleming D, et al. Human metapneumovirus as a cause of community-acquired respiratory illness. *Emerg Infect Dis* 2002;**8**:897–901.
55. Stott EJ, Eadie MB, Grist NR. Rhinovirus infections of children in hospital: isolation of three possibly new rhinovirus serotypes. *Am J Epidemiol* 1969;**90**:45–52.
56. Stuart-Harris CH. The present status of the respiratory viruses and acute respiratory disease in man. *Isr J Med Sci* 1966;**2**:255–68.
57. Suto T, Yano N, Ikeda M, et al. Respiratory syncytial virus infection and its serologic epidemiology. *Am J Epidemiol* 1965;**82**:211–24.
58. Sutton RN. Respiratory viruses in a residential nursery. *J Hyg (Lond)* 1962;**60**:51–67.
59. Toth M, Barna M, Voltay B. Aetiology of acute respiratory diseases in infants and children. *Acta Paediatr Hung* 1965;**6**:367–74.
60. Tregoning JS, Schwarze J. Respiratory viral infections in infants: causes, clinical symptoms, virology, and immunology. *Clin Microbiol Rev* 2010;**23**:74–98.
61. Urquhart GE, Moffat MA, Calder MA, et al. An aetiological study of respiratory infection in children, Edinburgh City Hospital, 1961–1963. *J Hyg (Lond)* 1965;**63**:187–99.
62. Van Der Veen J. The role of adenoviruses in respiratory disease. *Am Rev Resp Dis* 1963;**88**(suppl):167–80.
63. Van Lierde S, Corbeel L, Eggermont E. Clinical and laboratory findings in children with adenovirus infections. *Eur J Pediatr* 1989;**148**:423–5.
64. Vihma L. Surveillance of acute viral respiratory diseases in children. *Acta Paediatr Scand Suppl* 1969;**192**:7–53.
65. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. *Clin Microbiol Rev* 2004;**17**:697–728.
66. Walker SH. The respiratory manifestations of systemic *Hemophilus influenzae* infection. *J Pediatr* 1963;**62**:386–92.
67. Watson RL, Dowell SF, Jayaraman M, et al. Antimicrobial use for pediatric upper respiratory infections: reported practice, actual practice, and parent beliefs. *Pediatrics* 1999;**104**:1251–7.
68. Yodfat Y, Nishmi M. Successive overlapping outbreaks of febrile pharyngitis and pharyngoconjunctival fever associated with adenovirus types 2 and 7, in a Kibbutz. *Isr J Medical Sci* 1974;**10**: 1505–9.
69. Zeng M, Zhu QR, Wang XH, et al. Human bocavirus in children with respiratory tract infection in Shanghai: a retrospective study. *World J Pediatr* 2010;**6**:65–70.
70. Zhang LL, Tang LY, Xie ZD, et al. Human bocavirus in children suffering from acute lower respiratory tract infection in Beijing Children's Hospital. *Chin Med J (Engl)* 2008;**121**:1607–10.
71. Zinserling A. Pecularities of lesions in viral and mycoplasma infections of the respiratory tract. *Virchows Arch A Pathol* 1972;**356**: 259–73.

TABLE 20-1 **Prevalence of Childhood Bronchitis**

Author	Year	Study Subjects	Prevalence (%)	Acute or Chronic Bronchitis
Bland et al.	1974	Kent schoolchildren	5.5	Acute/chronic
Burrows and Lebowitz	1975	Arizona children	7.1	Chronic
Burrows et al.	1977	Arizona retrospective	46.4	Chronic
Kubo et al.	1978	Japanese children	1.4	Chronic
Peat et al.	1980	Sydney schoolchildren	20	Acute/chronic

Modified from Morgan WT, Taussig LM. The chronic bronchitis complex in childhood. *Pediatr Clin North Am* 1984;31:851–64.

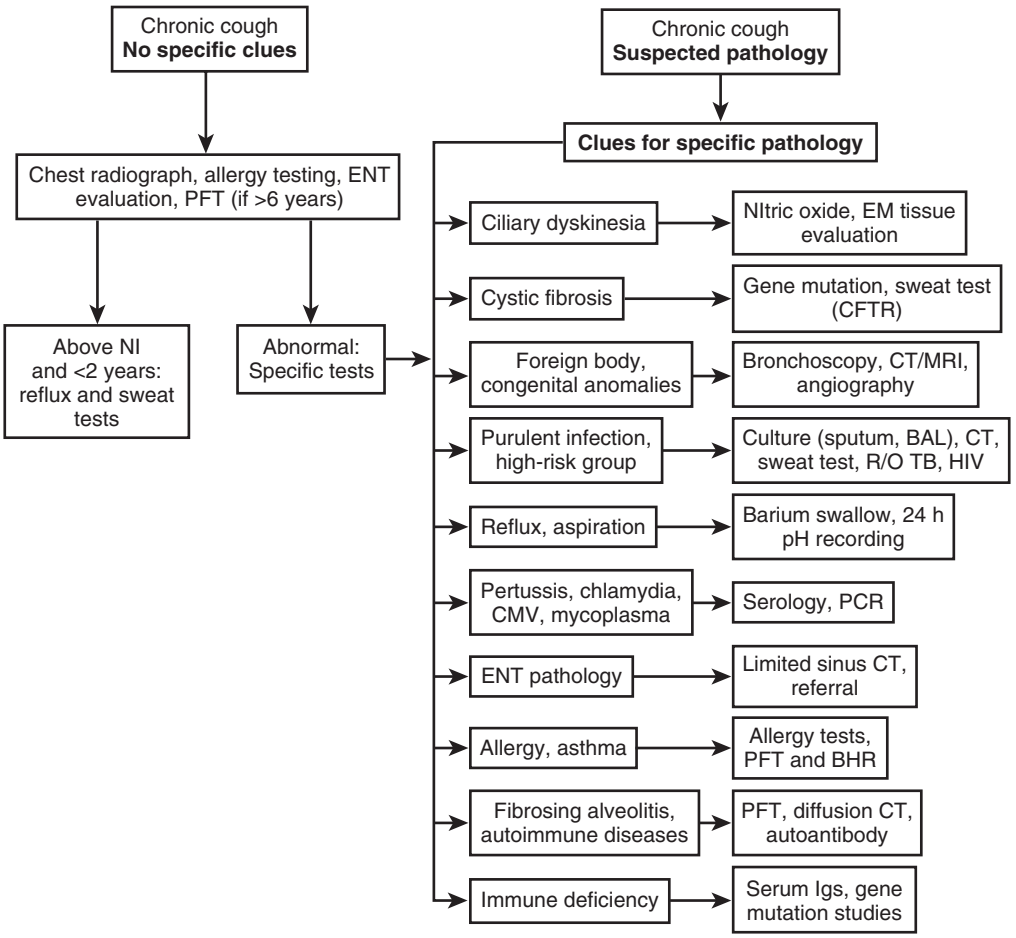

FIGURE 20-1 ■ Diagnostic evaluation of children with chronic cough. *BAL,* bronchoalveolar lavage; *BHR,* bronchial hyperreactivity; *CFTR,* cystic fibrosis transmembrane conductance regulator; *CMV,* cytomegalovirus; *CT,* computed tomography; *EM,* electron microscopy; *ENT,* ear, nose, throat; *HIV,* human immunodeficiency virus; *MRI,* magnetic resonance imaging; *NI,* no information; *PCR,* polymerase chain reaction; *PFT,* pulmonary function test; *TB,* tuberculosis. (Modified from Morice AJ, Fontana GA, Sovijarvi AR, et al. Diagnoses and management of chronic cough. *Eur Respir J* 2004;24:487.)

evaluation of children with chronic cough.[12] Many of these disorders are accompanied with abnormal chest radiographs, including evidence of bronchiectasis.

Heading the differential list is asthma, defined as reversible obstructive airways disease with a significant inflammatory component leading to increased edema and production of mucus. In the United States in 2006, more than 6.8 million children younger than 18 years had asthma and 4.1 million reported an asthma attack in that year.[21] Asthma can be distinguished from chronic bronchitis by pulmonary function evaluation documenting reversal of airway obstruction after delivery of pulmonary bronchodilators. Recurrent episodes of acute bronchitis often can be interpreted as chronic bronchitis, although the intermittent nature of these episodes and absence of a persistent cough usually distinguish this group of patients clinically.[2]

Specific viral infections (e.g., rhinovirus, respiratory syncytial virus) in children with or without allergic rhinitis may provoke airway hyperreactivity and late onset

of asthma, which may be confused symptomatically with chronic bronchitis.[13] Persistent lower respiratory tract infections (i.e., *Chlamydia* spp., pertussis, *Mycoplasma* spp., and *Mycobacterium* spp.) frequently manifest with the complex of symptoms described or chronic wet cough and are evaluated best with chest radiographs in a search for enlarged hilar nodes or interstitial lung infiltrates.[44] Respiratory tract secretions for appropriate bacterial and viral culture and serum for determinations of antibacterial antibodies should be obtained. In the case of tuberculosis, a delayed hypersensitivity skin test for *Mycobacterium tuberculosis* antigen should be applied.

Cystic fibrosis is a recessively inherited illness occurring in approximately 1 in 3700 live births, with clinical manifestations of failure to thrive, steatorrhea, nasal polyps, and recurrent lower respiratory tract symptoms. The disorder is associated with mutation of the cystic fibrosis transmembrane conductance regulator *(CFTR)* gene.[32] Cystic fibrosis can be diagnosed by newborn screening methodologies, including identifying typical *CFTR* gene mutation patterns in DNA extracted from collected blood specimens on Guthrie cards at or after birth or by detecting abnormally elevated chloride levels (>60 mEq/L) as measured by the sweat iontophoresis test.[35] In clinical practice, one or both modalities may be used for establishing the diagnosis, especially for patients with genetic variations of the *CFTR* gene not typical of the classic cystic fibrosis phenotype or for patients with uninterpretable sweat test outcomes.

Primary ciliary dyskinesia encompasses the immotile cilia disorders and Kartagener syndrome (rhinosinusitis, bronchitis or bronchiectasis, situs inversus) and affects 1 in 16,000 births in the United States.[1] Primary ciliary dyskinesia typically is inherited as an autosomal recessive disorder and is associated with defects in mucociliary transport in the respiratory tree. The symptom complex also has been associated with polycystic kidneys, hepatic disease, and central nervous system symptoms. Screening tests for primary ciliary dyskinesia include nasal nitric oxide and in vivo tests of ciliary motility, but these tests are not universally available, and standardization is problematic.[23] Genetic testing is commercially available and can detect disease-causing mutations in autonomic genes coding for outer dynein arms (present in 60% of affected individuals), radial spoke, and cytoplasm proteins involved in dynein arm assembly.[24]

The primary immune disorders associated most frequently with recurrent sinopulmonary infections include selective immunoglobulin (Ig)A deficiency, functional antibody deficiencies, hypogammaglobulinemia, and ataxia telangiectasia.[22] Selective IgA deficiency is the primary immune disorder encountered most commonly, with an incidence of 1/500. It may be asymptomatic or accompanied by a propensity for atopy and an increase in associated autoimmune disease (most often rheumatoid arthritis and systemic lupus erythematosus). The diagnosis is made readily by evaluation of quantitative serum immunoglobulins defining IgA levels of less than 10 mg/dL. IgA deficiency may be associated with IgG subclass deficiencies (IgG2 deficiency was identified in 85% in a series of >150) with or without clinical symptoms. Functional antibody deficiencies have been detected and include inability to respond serologically to vaccination with polysaccharide antigens such as *Streptococcus pneumoniae* or *Haemophilus influenzae* type b.[7]

Patients with hypogammaglobulinemia represent a diverse group having primary immune disorders that include X-linked agammaglobulinemia, common variable immune deficiency (CVID), and hyper-IgM syndrome. X-linked agammaglobulinemia typically affects boys, who experience recurrent sinopulmonary infections with onset occurring postnatally as maternal antibody wanes. X-linked agammaglobulinemia is associated with deficiency of a B-cell maturation enzyme, Bruton tyrosine kinase, and is characterized by the absence of circulating mature B lymphocytes. CVID is a disorder of young adults with immune findings of agammaglobulinemia and variable T-cell defects. Clinical presentation includes lower respiratory tract and sinus disease. Inherited CVID disorders have been described and include defects in inducible T-cell costimulator gene and transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI).[22] Patients with TACI defect and CVID may lack circulating mature B lymphocytes. More often, patients with CVID normally express B-cell markers on circulating lymphocytes. Hyper-IgM syndrome is a rare disorder associated with elevated IgM and low IgG and IgA and recurrent infections, including sinopulmonary and hepatic infections. Different gene defects have been identified in patients with hyper-IgM syndrome; patients with defects in nucleotide-modifying enzymes located only in B cells have no associated T-cell defect.

Patients with ataxia telangiectasia have depressed serum IgA concentrations and marked dysfunction of the swallowing mechanism, leading to recurrent lower respiratory tract infections probably secondary to recurrent aspiration. These patients are identified by telangiectasias of the skin and conjunctivae in association with aberrant and progressively deteriorating neurologic symptoms and immunodeficiency (depressed IgA and IgE and aberrant T-cell–mediated immunity). Some patients have depressed IgG subclasses (IgG2, IgG4) as well. The gene mutation in ataxia telangiectasia has been identified as disruption of the ataxia telangiectasia molecules responsible for detection and implementation of DNA repair dependent on phosphatidylinositol-3-kinase signal transduction pathways.

Primary immune disorders with hypogammaglobulinemia can be diagnosed through evaluation of serum immunoglobulin levels. Further characterization includes assessment of antibody production after vaccine or neoantigen challenge, evaluation of circulating lymphocyte cell surface markers, and in vitro proliferative responses to plant lectins or specific antigens. Evaluation of associated gene defects is important for determining corrective interventions (e.g., transplantation, gene therapy) and genetic counseling.

Graft-versus-host disease affecting the lungs has been described in immunocompromised patients after they have undergone bone marrow transplantation. The lesion is caused by chronic pulmonary lymphocytic infiltrates and pulmonary fibrosis mimicking the symptom complex of chronic bronchitis and often is indistinguishable

radiographically or clinically from infections that characteristically are pathogenic (i.e., *Pneumocystis jiroveci*, *Candida albicans*, *Aspergillus* spp.).

Secondary immune disorders (prematurity and pediatric human immunodeficiency virus [HIV] infection) also may be associated with significant sinopulmonary infections. Premature infants with attendant severe respiratory distress syndrome requiring positive-pressure ventilation may develop bronchopulmonary dysplasia. Diagnostic criteria include hypoxia requiring oxygen supplementation, characteristic diffuse interstitial markings on chest radiograph, and clinical signs of pulmonary disease (i.e., tachypnea, intercostal retractions). In one series, more than 60 percent of surviving children with bronchopulmonary dysplasia were documented to have significant pulmonary disease and associated morbidity (i.e., increased hospitalizations).[28] Continued improved care for premature infants and resultant decreased incidence of mortality may increase the population of infants with bronchopulmonary dysplasia.

Pediatric HIV infection has declined and is documented to affect approximately 2 percent of infants born to HIV-infected mothers in the United States and Puerto Rico.[41] This reduced infection rate can be attributed to (1) availability and use of highly active antiretroviral therapy (HAART) in pregnant women and their newborn infants, (2) U.S. practices that minimize exposure to breast milk, and (3) obstetric practices that minimize exposure to infected blood products (cesarean delivery and practices that decrease time from membrane rupture to delivery). Continued maternal-to-child infection is associated with maternal factors of late testing, breastfeeding, substance abuse, and lower CD4 cell counts.[41] HIV-infected infants report serious lower respiratory tract illness.

In previous years, lymphoid interstitial pneumonitis/hyperplasia, reported in 25 percent of all children with acquired immunodeficiency syndrome, was associated with chronic cough, but this HIV-associated outcome rarely is reported with the advent of improved antiviral medications to suppress replication of HIV. Despite restoration of immunocompetence with HAART, lower respiratory tract infections (viral, bacterial, fungal) are still common occurrences in children infected with HIV.[18] An unexpected consequence of efficacious HIV-directed therapy in affected children may lead to increased risk for developing asthma through an immune reconstitution mechanism.[33] The diagnosis of HIV infection is established by viral diagnostic assays (polymerase chain reaction) for perinatally exposed infants younger than 18 months of age.[26] HIV antibody testing (enzyme-linked immunosorbent assay and confirmation assays [Western blot analysis, indirect fluorescent antibody assay]) can confirm infection in perinatally exposed infants 18 months or older. HIV serologic assays are not appropriate for HIV infection detection in children 18 months of age or younger because passively acquired maternal HIV antibodies may persist and confuse interpretation of a positive test in this age group.

Anatomic lesions that lead to pulmonary obstructive airway disease can simulate the complex of chronic bronchitis. An infant with chronic cough, poor feeding habits, and failure to thrive should undergo evaluation for gastroesophageal reflux and tracheoesophageal fistula, which are identified most easily by barium swallow, pH probe monitoring, and nuclear scan. Mediastinal tumors can produce extrinsic obstruction, leading to recurrent cough and wheezing. Congenital heart disease should be considered in this patient group and can be evaluated with chest radiography, electrocardiography, and echocardiography.

Respiratory tract irritants have been implicated as a cause of chronic cough, as documented in adult populations of industrial European nations.[40] An assessment of the public health impact of pollution in Austria, France, and Switzerland concluded that air pollution caused 6 percent of all mortality, or 40,000 attributable cases per year.[16] Nonindustrial, rural communities, such as the forest zone of Nigeria, report virtually no chronic bronchitis, whereas increased risk for development of respiratory disease (i.e., persistent cough, persistent phlegm, asthma) is noted in children residing in six cities in Northern China with the highest ambient air levels of particulate air pollution.[25] After exposure to urban air particulate matter, animal models with induced chronic bronchitis (exposure to 200 ppm of sulfur dioxide for 6 weeks) have shown pathologic changes consistent with exacerbation of chronic bronchitis—changes in ventilatory capacity and marked pulmonary inflammation.[4]

A correlation between tobacco smoking and reduced ventilatory capacity in adults has been reported by many investigators. Peat and associates[29] described teenagers in Sydney, Australia, with recurrent episodes of bronchitis with worsened lung function when coupled with tobacco smoking. A meta-analysis of 21 relevant publications on relationships between exposure to environmental tobacco smoke and lower respiratory tract infection in infancy and early childhood concluded that exposure to environmental smoke resulted in adverse childhood respiratory outcomes (hospitalizations).[17] In a study of approximately 65,000 nonsmoking Canadians 12 years or older, vehicular environmental tobacco smoke was associated with chronic bronchitis for children between 12 and 19 with odds ratios of 2.3 and 2.25 for children and older teens.[8] Tashkin and colleagues[38] further found in 300 individuals followed for 10 years that both tobacco and marijuana smoking appeared to be causally associated with the development of chronic bronchitis. These data suggest that it is not only imperative to prevent smoking in individual youth to prevent respiratory disease but also to gather a smoking history (tobacco and drugs) for household members and apprise them of the impact of their smoking on their children.

Other noxious agents associated with outdoor pollution have been associated with poor control of chronic cough complex diseases such as asthma. In Los Angeles, adults with asthma were more poorly controlled if they resided near areas of heavy traffic.[19] Similar findings of poorly controlled lung disease associated with traffic pollution have been documented in adult and pediatric patients in Lima, Peru.[3] Occupational exposures long have been cited for exacerbating pulmonary diseases and may be responsible for about 15 percent of chronic bronchitis and chronic obstructive pulmonary disease. Classic

examples of occupational exposures leading to increased risk for developing chronic cough are described in coal dust for miners in Great Britain and recently smoking and welding fumes in Northern European welders.[10,45]

EPIDEMIOLOGY AND ETIOLOGY

Differentiating the impact of clinical, social, and environmental factors on lower respiratory tract disease, including chronic bronchitis, has been problematic and has led to conflicting outcomes in epidemiologic assessments.[5,37] In addition, the lack of a standardized definition of chronic bronchitis in the pediatric literature leads to confusing interpretation of data when attempting to appreciate the prevalence or etiology of the disease complex. In Table 20-1, the data from Kubo and associates[15] separate acute recurrent bronchitis, asthmatic bronchitis, and chronic bronchitis, leading to a decrease in the prevalence from a proposed 46.4 percent (in the Arizona questionnaire) to 1.4 percent.

Considerable overlap also exists in evaluating etiologic agents for chronic bronchitis. The same viral agents proposed as the exacerbating factors of asthmatic bronchitis are implicated in exacerbations of chronic bronchitis,[11] including rhinoviruses, parainfluenza viruses, respiratory syncytial virus, influenza A and B viruses, adenoviruses, and enteroviruses. Persistent adenovirus infection has been implicated as a cause of childhood chronic bronchitis. In a study of 11 children with chronic bronchitis, transbronchial biopsy specimens revealed no evidence of persistent adenovirus infection (culture or polymerase chain reaction),[30] suggesting that viral infections may precipitate cough, but their roles in the pathologic findings associated with chronic bronchitis should be questioned.

Table 20-2 lists predominant bacterial pathogens isolated from sputum for a group of 40 pediatric patients with chronic bronchitis and exacerbations of cough and fever; these pathogens include *H. influenzae*, *S. pneumoniae*, and *Staphylococcus aureus*.[15] These bacteria also are implicated as etiologic agents in the triggering of asthmatic bronchitis. Neither *Mycoplasma* nor human metapneumovirus was included in this listing, but this simply may reflect the time frame (late 1970s) when the Kubo study was conducted.[27,42] Treatment of exacerbations of chronic bronchitis with antibiotic therapy usually is effective in reducing the volume of sputum and purulence during the acute infection but shows no parallel elimination of the cultured microorganisms.

TABLE 20-2 **Predominant Pathogens in Washed Sputum of Chronic Bronchitis (40 Cases)**

Pathogen	No. Cases
Haemophilus influenzae and *Streptococcus pneumoniae*	21 (52.5%)
H. influenzae	17 (42.5%)
Staphylococcus aureus	2 (5%)
Superinfection with gram-negative rods	
Pseudomonas aeruginosa	4
Klebsiella pneumoniae	2
Escherichia coli	1
Enterobacter cloacae	1

From Kubo S, Funabashi S, Uehara S, et al. Clinical aspects of "asthmatic bronchitis" and chronic bronchitis in infants and children. *J Asthma Res* 1978;15:99–132.

TREATMENT

When a specific diagnosis is found in association with chronic cough or wheezing, therapy is directed toward the primary disease entity and the clinical presentation of cough. Bronchodilators (β-adrenergic agents), anti-inflammatory agents (corticosteroids), and anticholinergic agents are used, when appropriate, for the treatment of chronic cough associated with asthma or acute exacerbations of chronic obstructive pulmonary disease.[36] Appropriate positioning techniques (prone 30 degrees), feeding schedules, and medications (e.g., gastrointestinal motility enhancers, proton pump inhibitors, or cholinergics) are indicated in the approach to infants with gastroesophageal reflux. Patients with hypogammaglobulinemia can be helped with supplemental intravenous immunoglobulin preparations currently available commercially (400 to 600 mg/kg per dose every 3 to 4 weeks) in an attempt to decrease the incidence of infections.

It is imperative that patients with chronic pulmonary diseases such as cystic fibrosis, asthma, or ciliary dyskinesias understand the pulmonary irritant effect of tobacco smoking, dust exposure, and air pollution. A change of occupation may be essential for their well-being. It also is imperative to stress the irritant effect of parental smoking on the already compromised pulmonary function of the child.

Management of chronic cough in children 14 years of age or younger is outlined in Figure 20-2. In the evidence-based guidelines for chronic cough, normal chest radiography and, as developmentally appropriate, normal spirometry suggest a "watch, wait, and review" approach to clinical management. Cough will spontaneously resolve for many children with normal chest radiographic examinations. The use of cough suppressants or over-the-counter cough medicine is discouraged, especially in very young infants who may experience significant drug-associated morbidity. Persistent cough should be reassessed clinically. For children with persistent dry cough, a trial of inhaled corticosteroids is recommended in this management algorithm and antibiotics for those with persistent wet cough.

When considered, antibiotic therapy for chronic bronchitis usually consists of amoxicillin 45 to 90 mg/kg every 24 hours, erythromycin 40 mg/kg every 24 hours, or, in adolescents and adults, tetracycline 25 to 50 mg/kg every 24 hours or quinolones for 5 to 10 days.[34] Methylxanthine therapy is used less frequently in chronic bronchitis care. When it is used, certain antibiotics, such as erythromycin, lead to elevated serum concentrations of theophylline, which renders toxicity more likely to occur.

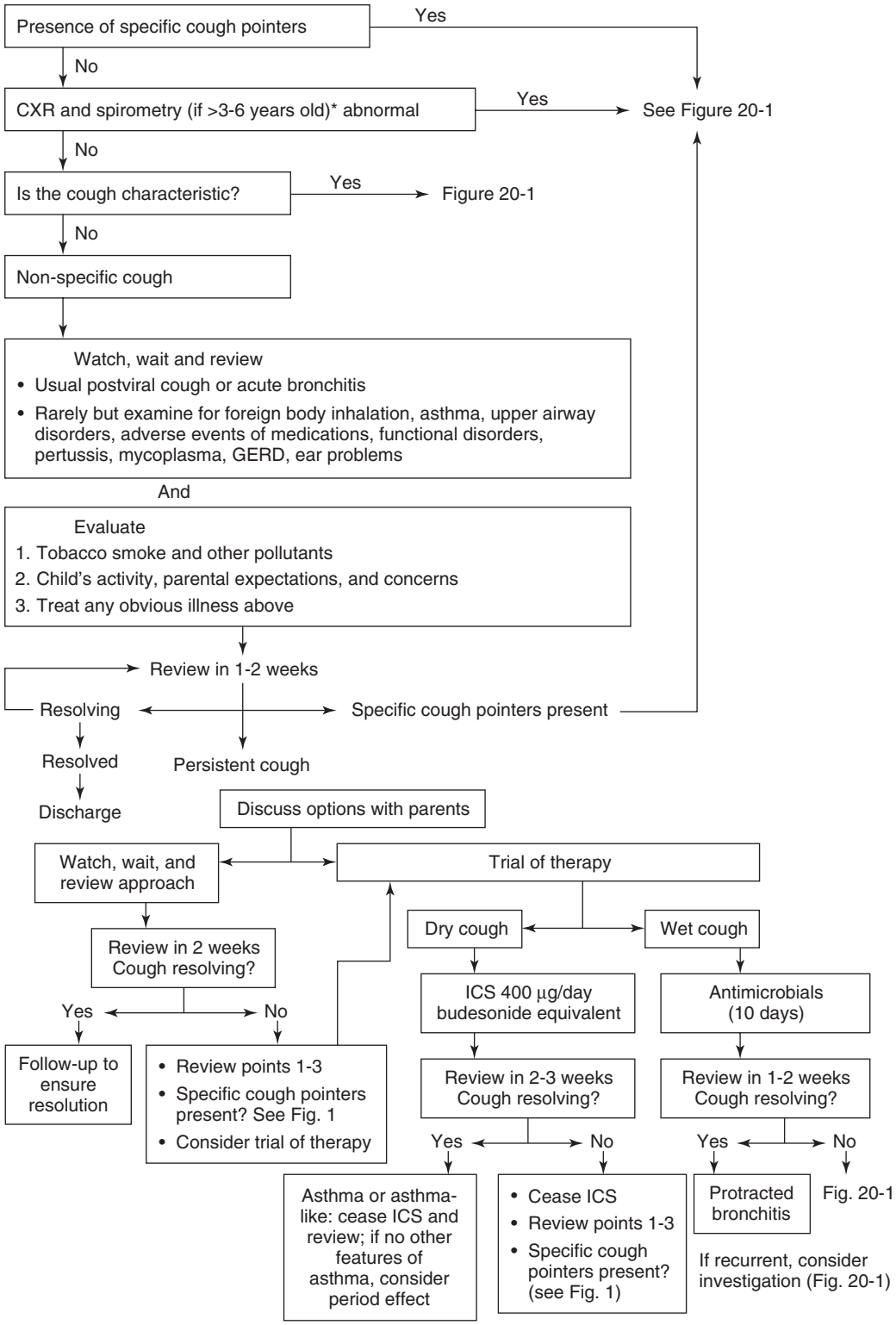

FIGURE 20-2 ■ Approach to a child younger than 15 years of age with chronic cough. *CXR,* Chest radiograph; GERD, gastroesophageal reflux disease; *ICS,* inhaled corticosteroids. (Modified from Irwin RS, Baumann MH, Blaser DC, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest* 2006;129:15–23S.)

Treatment with mucolytic agents in chronic pulmonary disease has not proved to have a significant impact except in adults with already advanced disease with multiple hospitalizations, and no evidence suggests that they are beneficial in children.[31]

As with patients with obstructive lung disease, attention must focus on careful and sequential monitoring of pulmonary function. The prognosis for chronic bronchitis varies and depends on the specific etiology of this syndrome.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Becker-Heck A, Zohn IE, Okabe N, et al. The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. *Nat Genet* 2011;**43**:79–84.
6. Donnelly D, Critchlow A, Everard ML. Outcomes in children treated for chronic bronchitis. *Thorax* 2007;**62**:80–4.
8. Evans J, Chen Y. The association between home and vehicle environmental tobacco smoke (ETS) and chronic bronchitis in a Canadian population: the Canadian Community Health Survey, 2005. *Inhal Toxicol* 2009;**21**:244–9.
9. Ferre A, Fuhrman C, Zureik M, et al. Chronic bronchitis in the general population: influence of age, gender and socio-economic conditions. *Respir Med* 2012;**106**:467–71.
13. Jackson DJ, Lemanske Jr RF. The role of respiratory virus infections in childhood asthma. *Immunol Allergy Clin North Am* 2010;**30**:513–22.
21. National Institutes of Health. National Heart, Lung and Blood Institute. Disease and conditions index: acute respiratory distress syndrome (ARDS)—treatment. November 2007. Available at http://www.nhlbi.nih.gov/health/dci/Disease/Ards/ARds_Treatments.html. Accessed March 2012.
22. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* 2010;**125**(2):S182–94.
25. Pan G, Zhang S, Feng Y, et al. Air pollution and children's respiratory symptoms in six cities of Northern China. *Respir Med* 2010;**104**:1903–11.
26. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection, August 11, 2011: p. 1–268. Available at http://aidsinfo.nih.gov/contentfiles/Ivguideliens/PediatricGuidelines.pdf. Accessed 3/20/12, p. 12.
33. Siberry GK, Leister E, Jacobson DL, et al. Increased risk of asthma and atopic dermatitis in perinatally HIV-infected children and adolescents. *Clin Immunol* 2012;**142**:201–8.
38. Tashkin DP, Simmons MS, Tseng CH. Impact of changes in regular use of marijuana and/or tobacco on chronic bronchitis. *COPD* 2012;**9**:367–74.
41. Whitmore SK, Taylor AW, Espinoza L, et al. Correlates of mother-to-child transmission of HIV in the United States and Puerto Rico. *Pediatrics* 2012;**129**:e74–81.
44. Zgherea D, Pagala S, Mediratta M, et al. Bronchoscopic findings in children with chronic wet cough. *Pediatrics* 2012;**129**:e364–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Becker-Heck A, Zohn IE, Okabe N, et al. The coiled-coil domain containing protein CCDC40 is essential for motile cilia function and left-right axis formation. *Nat Genet* 2011;**43**:79–84.
2. Boule M, Gaultier C, Tournier G, et al. Lung function in children with recurrent bronchitis. *Respiration* 1979;**38**:127–34.
3. Carbajal-Arroyo L, Barraza-Villarreal A, Durand-Pardo R, et al. Impact of traffic flow on the asthma prevalence among school children in Lima. *Peru J Asthma* 2007;**44**:197–202.
4. Clarke RW, Catalano PJ, Koutrakis P, et al. Urban air particulate inhalation alters pulmonary function and induces pulmonary inflammation in a rodent model of chronic bronchitis. *Inhal Toxicol* 1999;**11**:637–56.
5. Dodge R, Burrows B, Lebowitz MD, et al. Antecedent features of children in whom asthma develops during the second decade of life. *J Allergy Clin Immunol* 1993;**92**:744–9.
6. Donnelly D, Critchlow A, Everard ML. Outcomes in children treated for chronic bronchitis. *Thorax* 2007;**62**:80–4.
7. Edwards E, Razvi S, Cunningham-Rundles C. IgA deficiency: clinical correlates and responses to pneumococcal vaccine. *Clin Immunol* 2004;**111**:93–77.
8. Evans J, Chen Y. The association between home and vehicle environmental tobacco smoke (ETS) and chronic bronchitis in a Canadian population: the Canadian Community Health Survey, 2005. *Inhal Toxicol* 2009;**21**:244–9.
9. Ferre A, Fuhrman C, Zureik M, et al. Chronic bronchitis in the general population: influence of age, gender and socio-economic conditions. *Respir Med* 2012;**106**:467–71.
10. Holm M, Kim JL, Lillienberg L, et al. Incidence and prevalence of chronic bronchitis: impact of smoking and welding. The RHINE study. *Int J Tuberc Lung Dis* 2012;**16**:553–7.
11. Horn MEC, Gregg I. Role of viral infection and host factors in acute episodes of asthma and chronic bronchitis. *Chest* 1973;**63**: 44–85.
12. Irwin RS, Baumann MH, Blaser DC, et al. Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest* 2006;**129**:15–23S.
13. Jackson DJ, Lemanske Jr RF. The role of respiratory virus infections in childhood asthma. *Immunol Allergy Clin North Am* 2010; **30**:513–22.
14. Kim N, Leeper Jr KV. Epidemiology of chronic bronchitis and acute infective exacerbations of chronic bronchitis. *Semin Respir Crit Care Med* 2000;**21**:73–8.
15. Kubo S, Funabashi S, Uehara S, et al. Clinical aspects of "asthmatic bronchitis" and chronic bronchitis in infants and children. *J Asthma Res* 1978;**15**:99–132.
16. Kunzli N, Kaiser R, Medina S, et al. Public-health impact of outdoor and traffic-related air pollution: a European assessment. *Lancet* 2000;**356**:795–801.
17. Li JS, Peat JK, Zuan W, et al. Meta-analysis on the association between environmental tobacco smoke (ETS) exposure and the prevalence of lower respiratory tract infection in early childhood. *Pediatr Pulmonol* 1999;**27**:5–13.
18. Mato SP, Van Dyke RB. Pulmonary infections in children with HIV infection. *Semin Respir Infect* 2002;**17**:33–46.
19. Meng YY, Wilhelm M, Rull RP, et al. Traffic and outdoor air pollution levels near residences and poorly controlled asthma in adults. *Ann Allergy Asthma Immunol* 2007;**98**:455–63.
20. Morgan WT, Taussig LM. The chronic bronchitis complex in childhood. *Pediatr Clin North Am* 1984;**31**:851–64.
21. National Institutes of Health. National Heart, Lung and Blood Institute. Disease and conditions index: acute respiratory distress syndrome (ARDS)—treatment. November 2007. Available at http://www.nhlbi.nih.gov/health/dci/Disease/Ards/ARds_Treatments.html. Accessed March 2012.
22. Notarangelo LD. Primary immunodeficiencies. *J Allergy Clin Immunol* 2010;**125**:S182–94.
23. O'Callaghan C, Chilvers M, Hogg C, et al. Diagnosing primary ciliary dyskinesia. *Thorax* 2007;**62**:656–7.
24. Olbrich H, Haffner K, Kispert A, et al. Mutations in DNAH5 cause primary ciliary duskiness and randomization of left-right asymmetry. *Nat Genet* 2002;**30**:143–4.
25. Pan G, Zhang S, Feng Y, et al. Air pollution and children's respiratory symptoms in six cities of Northern China. *Respir Med* 2010;**104**:1903–11.
26. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection, August 11, 2011. Available at http://aidsinfo.nih.gov/contentfiles/Ivguideliens/PediatricGuidelines.pdf. Accessed 3/20/12, p. 12.
27. Park Y. Asthma and *Mycoplasma* pneumonia infection. *J Allergy Clin Immunol* 2007;**119**:S24.
28. Payne NR, LaCorte M, Karna P, et al. Reduction of bronchopulmonary dysplasia after participating in the Breathsavers group of the Vermont Oxford Network Neonatal Intensive Care quality improvement collaboration. *Pediatrics* 2006;**118**:S73–7.
29. Peat JK, Woolcock AJ, Leider SR, et al. Asthma and bronchitis in Sydney school children. I. Prevalence during a six-year study. *Am J Epidemiol* 1980;**111**:721–7.
30. Pichler M, Herrmann G, Schmidt H, et al. Persistent adenoviral infection and chronic obstructive bronchitis in children: is there a link? *Pediatr Pulmonol* 2001;**32**:367–71.
31. Poole PJ, Black PN. Mucolytic agents for chronic bronchitis and chronic obstructive pulmonary disease. *Cochrane Database Syst* 2006;(Rev. 19):CD001287.
32. Rich DP, Anderson MP, Gregory RJ, et al. Expression of cystic fibrosis transmembrane regulator corrects defective chloride channel regulations in cystic fibrosis airway epithelial cells. *Nature* 1990;**347**:358–63.
33. Siberry GK, Leister E, Jacobson DL, et al. Increased risk of asthma and atopic dermatitis in perinatally HIV-infected children and adolescents. *Clinical Immunol* 2012;**142**:201–8.
34. Siempos II, Dimopoulos G, Korbila IP, et al. Macrolides, quinolones and amoxicillin/clavulanate for chronic bronchitis: a meta-analysis. *Eur Respir J* 2007;**29**:1127–37.
35. Southern KW, Peckham D. Establishing a diagnosis of cystic fibrosis. *Chron Respir Dis* 2004;**1**:205–10.
36. Stoller JK. Acute exacerbations of chronic obstructive pulmonary disease. *N Engl J Med* 2002;**346**:988–94.
37. Strachan DP, Seagrott V, Cook DG. Chest illness in infancy and chronic respiratory disease in later life: an analysis by month of birth. *Int J Epidemiol* 1994;**23**:1060–8.
38. Tashkin DP, Simmons MS, Tseng CH. Impact of changes in regular use of marijuana and/or tobacco on chronic bronchitis. *COPD* 2012;**9**:367–74.
39. Taussig LM, Smith SM, Blumenfeld R. Chronic bronchitis in childhood: what is it? *Pediatrics* 1981;**67**:1–5.
40. Wanner HU. Effects of atmospheric pollution on human health. *Experientia* 1993;**49**:754–8.
41. Whitmore SK, Taylor AW, Espinoza L, et al. Correlates of mother-to-child transmission of HIV in the United States and Puerto Rico. *Pediatrics* 2012;**129**:e74–81.
42. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004;**350**:443–50.
43. Wilson R, Tillotson G, Ball P. Clinical studies in chronic bronchitis: a need for better definition and classification of severity. *J Antimicrob Chemother* 1996;**37**:205–8.
44. Zgherea D, Pagala S, Mediratta M, et al. Bronchoscopic findings in children with chronic wet cough. *Pediatrics* 2012;**129**:e364–9.
45. Zock JP, Sunyer J, Kogevinas M, et al. Occupation, chronic bronchitis, and lung function in young adults: an international study. *Am J Respir Crit Care Med* 2001;**163**:1572–7.

HISTORY

Although all practicing pediatricians today are familiar with the term *bronchiolitis* and associate it with an acute clinical illness with the signs and symptoms of airway obstruction, it has been recognized in the medical literature for only a relatively brief period. In the early years of the 20th century, the term *capillary bronchitis* was used to describe an inflammatory illness of the smallest alveoli.[60] The condition could not be distinguished clinically from pneumonia, however, and whether the pathologic entity (i.e., bronchiolitis) ever occurred separately from pneumonia was doubted. In the 11th edition of *Holt's Diseases of Infancy and Childhood*,[77] published in 1940, capillary bronchitis is discussed only briefly and is considered under pneumococcal pneumonia. Acute bronchiolitis finally was listed as a heading in the sixth edition of the *Textbook of Pediatrics*,[131] published in 1954; even at that time, bronchiolitis was associated with interstitial pneumonitis.

In contrast to the delayed textbook recognition of bronchiolitis, good clinical descriptions were presented in journals. In 1941, Hubble and Osborn[79] published "Acute Bronchiolitis in Children," in which they described an epidemic of bronchiolitis involving 50 hospitalized children. Pratt,[146] in 1944, and Nelson and Smith,[132] in 1945, published excellent clinical articles. Studies in the late 1950s and early 1960s established the etiologic association of RSV and other viruses with acute bronchiolitis.[9,25,26,160]

ETIOLOGIC AGENTS

RSV is the major cause of bronchiolitis in infancy and virtually the only important etiologic consideration when major epidemics are occurring. Other agents cause smaller annual outbreaks. The frequency of infectious agents in the overall cause of bronchiolitis is shown in Table 21-1. These data were compiled from 12 reports in which respiratory illness in children was observed over an extended period, and in most instances numerous clinical illness categories were being studied. During major epidemics in the colder months in temperate climates, virologic and serologic studies indicate RSV as the cause in 80 percent or more of the cases, especially in the more severe ones. In nonepidemic situations, more than 50 percent of the isolates or instances of serologic evidence of infection are infectious agents other than RSV. Sporadic disease rarely is associated with more than a 50 percent association of infectious agent and illness, despite the fact that infections undoubtedly are the cause of sporadic and epidemic bronchiolitis.[120]

TABLE 21-1 **Infectious Agents Associated with Acute Bronchiolitis**

Infectious Agent	Relative Frequency (%)
Respiratory syncytial virus	50
Parainfluenza viruses	25
Type 1	8
Type 2	2
Type 3	15
Adenoviruses	5
Mycoplasma pneumoniae	5
Rhinoviruses	5-15
Influenza viruses	5
Type A	3
Type B	2
Metapneumovirus	5-10
Enteroviruses	2
Herpes simplex virus	2
Mumps virus	<1

Children surviving infantile bronchiolitis often have recurrent episodes of wheezing that, on clinical grounds, seem to be precipitated by upper respiratory tract infections. Studies designed to determine the infectious agents responsible for these repeated wheezing episodes have yielded variable results, presumably because they involve patients of different ages observed during different seasons of the year.[12,106,122-125] A comprehensive study based on more than a decade of observation of children from birth through 15 years of age[71] revealed that viruses and *Mycoplasma pneumoniae* are the most common etiologic agents (Table 21-2).[128] RSV is the most common cause of recurrent, wheezing-associated respiratory illness occurring in young children, including children who previously experienced bronchiolitis, which strongly suggests the identical nature of infection-related wheezing episodes occurring in children of different ages. With increasing patient age, rhinoviruses and *M. pneumoniae* account for most infection-induced wheezing episodes. Parainfluenza viruses and influenza viruses are important causes of such wheezing episodes throughout childhood, whereas coronaviruses and adenoviruses are less common causes in infancy. Human metapneumovirus and bocaviruses are more recently identified causes of bronchiolitis, and their relative importance as etiologic agents is being determined. RSV remains an important cause of wheezing in adolescents[71] and can cause airway obstruction in middle-aged and elderly individuals.[45]

The role of bacteria as primary agents, synergistically active participants, or secondary invaders in bronchiolitis has interested investigators for many years. No evidence has been found of a primary role for bacteria in bronchiolitis of infants.[9,105] The data on synergistic viral-bacterial infections also are unconvincing. Wood and colleagues[205] in 1954 and Sell[165] in 1960 reported results of studies in which bacteriologic and serologic evidence tended to associate *Haemophilus influenzae* infections with bronchiolitis. Some of the children from these studies later were shown serologically to have had RSV infection.[28] Later studies showed evidence of mixed viral-bacterial infections in bronchiolitis[108,134] and other lower respiratory tract illnesses.[134]

These serologically defined mixed infections were found no more commonly among patients with bronchiolitis than among patients with mild upper respiratory tract disease, whereas RSV was recovered 10 times as often from patients with lower respiratory tract disease as from patients with upper respiratory tract disease alone.[108] Although debate continues about whether the

TABLE 21-2 **Principal Agents Recovered from Children of Different Ages with Wheezing Precipitated by Infection**

	Frequency of Isolation of Each Age Group†			
Agent*	0-2 YR	2-5 YR	5-9 YR	9-15 YR
Respiratory syncytial virus	++++	+++	++	++
Adenovirus	++	++	+	0
Parainfluenza viruses	++	++	++	++
Rhinoviruses	+	++ to +++	++ to +++	+++
Metapneumovirus	++	+	+	0
Mycoplasma pneumoniae	+	++	+++	++++

*Other agents that more rarely precipitate wheezing include enteroviruses, herpes simplex virus, cytomegalovirus, coronaviruses, influenza viruses, mumps virus, varicella-zoster virus, *Bordetella pertussis,* and *Coxiella burnetii.*
†++++, very common; +++, common; ++, occasional; +, uncommon; 0, unknown.
Data from references 8, 43, 49, 55, 71, 78, 83, 105, 108, 120, 124, and 136.

isolation of *H. influenzae* from the airway of infants with bronchiolitis represents secondary infection, most data indicate that a significant role for mixed viral-bacterial infection in bronchiolitis of infancy is unlikely to exist. Bacterial superinfection is an uncommon occurrence in bronchiolitis (at least in developed countries) and is discussed later in the section on complications and prognosis.

The association of asthma and infectious illnesses was appreciated throughout the 20th century. Initial reports tended to relate bacterial agents to wheezing; in 1909, Carmalt-Jones[21] reported improvement of patients with asthma in association with injections of a bacterial vaccine. Throughout the first half of the 20th century, "bacterial allergy" was the main consideration in infection-related wheezing, and controversy raged about the therapeutic merits of bacterial vaccines. Despite widespread use of bacterial vaccines in the treatment of infectious asthma, no evidence exists that bacterial organisms of the normal flora precipitate asthmatic attacks.[68,78,127] More recent, large retrospective reviews have shown the rarity with which bacterial infection complicates bronchiolitis, even in severe cases.[148,151] Generally, after a diagnosis of RSV, metapneumovirus, rhinovirus, or parainfluenza virus infection has been established, antibiotic courses started empirically can be safely stopped.

EPIDEMIOLOGY

Bronchiolitis predominantly is a disease of infancy, although an identical disease occurring in older children arbitrarily may be referred to as another diagnostic entity. In a study involving 1148 children, the peak age of incidence was 2 to 6 months, with more than 80 percent of the cases occurring during the first year of life.[141] In a study of families in or near Houston, Texas, rates of RSV infection were 68.8 per 100 children in the first year of life and 82.6 per 100 children in the second year, including many reinfections. Lower respiratory tract illness was caused by RSV in 22.4 of 100 children in the first year of life. Of all RSV infections occurring in children younger than 12 months of age, one third were accompanied by lower respiratory tract illness. Although the attack rate of RSV decreased with age, the frequency with which lower respiratory tract disease occurred among individuals infected remained constant (Table 21-3), at least until they reached 4 years of age.[56,57] In Tucson, Arizona, a study of 1179 children enrolled in a health maintenance organization found that the rate of lower respiratory tract illness in the first year of life was 32.9 episodes per 100 children; 60 percent of these episodes were diagnosed as bronchiolitis.[208]

TABLE 21-3 **Attack Rates of Respiratory Syncytial Virus**

Age in Months [per Welliver] (n)	Infections per 100 Child-Years	LRTI per 100 Child-Years	LRTI per 100 Infections
0-12 (125)	68.8	22.4	32.6
13-24 (92)	82.6	13	15.8
25-36 (65)	46.2	10.8	23.3
37-48 (39)	33.3	7.7	23.1

LRTI, lower respiratory tract infection.
Data from reference 57.

Studies in the Washington, D.C., area[92] and in North Carolina[71] estimated the risk for hospitalization for bronchiolitis among infants up to 1 year old to be 10 per 1000. A combined study involving 10 centers in Great Britain[154] concluded that the frequency of hospitalization for lower respiratory tract disease (predominantly wheezing) caused by RSV was 1 per 114 infants younger than 1 year of age and 1 per 476 children younger than 5 years of age. Peak rates of hospitalization were observed for children 1 to 3 months old (1 in 56).

More recent studies suggest that the rate of hospitalization for bronchiolitis may be higher currently than in the just-mentioned older studies. A study of the Tennessee Medicaid population found that the frequency of

hospitalization for bronchiolitis was 4.4 and 1.5 per 100 child-years of observation for infants infected in the first and second 6 months of life, respectively.[15] Approximately 123,000 infants younger than 1 year of age are hospitalized annually with bronchiolitis, with RSV infection accounting for 51,000 to 82,000 of these hospitalizations. Including cases diagnosed as pneumonia, RSV infection results in 70,000 to 120,000 hospitalizations.[167]

Between 1979 and 1997, an average of 95 bronchiolitis-associated deaths occurred annually, with 77 percent occurring during the RSV epidemic season. Including fatal cases of bronchiolitis or pneumonia, RSV infection might have resulted in 171 to 510 deaths annually.[168] The yearly incidence of hospitalization for bronchiolitis increased from 12.9 per 1000 infants in 1980 to 31.2 per 1000 in 1996, suggesting an increased severity of illness developing during this interval.[167] More frequent hospitalization may also be a result of an increased use of pulse oximetry to determine the need for hospitalization.[111] In concert with this, a subsequent study showed that the rate of RSV hospitalizations was stable from 1997-2006, at a rate of 132,000 to 172,000 hospitalizations annually in children younger than 5 years old in the United States.[178]

Epidemic bronchiolitis caused by RSV is markedly seasonal in temperate climates, with peak activity occurring from January to April annually and with virtually no activity seen from August to October.[92] Sporadic bronchiolitis cases caused by other agents are seen throughout the year. In their study of 1179 cases, Kim and associates[92] found that 77 percent of the cases of RSV infection occurred between December and June. The lowest incidence occurred in August (2%). Regional differences can be striking. In Miami, RSV activity occurs year round, with the most cases in September and October. Epidemics usually begin in October in other southern states, although still earlier than in the Northeast. Further north (Winnipeg, Canada, and Alaska), the duration of the RSV season again becomes longer and cases occur essentially year round. Near the equator, temperature and humidity are positively associated with the number of bronchiolitis cases. In contrast, further from the equator (in either direction), temperature and humidity are inversely associated with bronchiolitis cases. Ultraviolet radiation is negatively associated with bronchiolitis in several regions.[210] In contrast to yearly epidemics of RSV infection in the United States and United Kingdom, with identical peak months of occurrence each year, RSV occurs in biennial epidemic cycles in Croatia,[126] Switzerland,[39] Sweden,[155] Finland,[194] and Germany,[184] with peaks alternating between December and January in 1 year and March and April in alternate years. No explanation has been established for the biennial occurrence of these epidemics.

Bronchiolitis occurs more frequently in boys; the male-to-female ratio is approximately 1.5 : 1.[49] In older children, wheezing attributable to viral and mycoplasmal infections also occurs more frequently in boys (male-to-female ratio 1.35 : 1) until they reach 9 years of age, when the incidence becomes equal for the sexes.[71] Crowding may be a major determinant of hospitalization rates for lower respiratory tract illness caused by RSV, with the incidence of hospitalization for infants 1 to 3 months old residing in rural, urban, and heavily industrialized areas of Great Britain being 1 in 80, 1 in 60, and 1 in 40, respectively. The figures for all children younger than 5 years of age were 1 in 714, 1 in 588, and 1 in 227, respectively.[154] Studies from Tucson, Arizona, indicate that many socioeconomic factors, including absence of breast-feeding, low level of maternal education, and exposure to cigarette smoke, are associated with an increased risk for developing lower respiratory tract infection at the time of infection with RSV. The greatest risk was conferred by sharing sleeping quarters with two or more individuals.[208]

CLINICAL PRESENTATION

Acute bronchiolitis occurs most commonly in infants 1 to 12 months of age. In most instances, the patient's history reveals exposure to an adult or older child with a common cold or other trivial respiratory tract infection. Occasionally, as in the daycare setting, the child is exposed to other children with more marked respiratory illness. After exposure, the incubation period is 5 to 7 days. The initial signs include copious nasal discharge (often serous in the early stage), cough, irritability, poor feeding, and vomiting in some cases. Slightly more than 50 percent of infants have fever, with rectal temperatures ranging from normal to 40.6° C (105.4° F) (mean, 39° C [102° F]).[153] Nasal congestion with tenacious secretions and progressive cough and dyspnea dominate the clinical picture.

Symptoms of upper respiratory tract infection persist for a few days, and the onset of lower respiratory tract infection usually is precipitous, with the time of the onset of wheezing often being recognizable from the caretaker's description of the illness. The maximum severity of illness generally is attained within 24 to 36 hours of the first signs of lower respiratory tract illness.[16] Apnea may occur and may be sufficiently severe to require mechanical ventilation.[17]

At the time of hospital admission, all patients have cough and evidence of respiratory distress. The pulse is rapid, and the respiratory rate usually is 40 to 80 breaths per minute. The breathing is labored, with flaring of the alae nasae; grunting; abdominal breathing; and supraclavicular, subcostal, and intercostal retractions. The degree of retraction of the lower chest wall may be an accurate indicator of the severity of the illness. Wheezing is best heard by auscultation over the upper anterior chest but often is audible without the use of a stethoscope, and the chest is full. Hyperresonance may be detected on percussion, and auscultation generally reveals harsh rhonchi, high-pitched or low-pitched expiratory wheezes, or fine inspiratory rales. Occasionally, wheezing is not audible despite other evidence of airway obstruction. A prolonged expiratory phase of breathing may occur, suggesting the presence of a more severe degree of illness. Cyanosis also occurs in severe cases.

Other findings include a mild conjunctivitis in one third of cases, pharyngitis of varied severity in approximately half of affected infants, and otitis media in 5 to 15 percent of cases. The abdomen frequently appears distended, and the liver and spleen usually are palpable; the organs are not enlarged but are pushed down because of hyperexpansion of the lung.[1]

The duration of the hospital course of bronchiolitis varies. Significant improvement occurred in half of the cases within 2 days in one large study.[139] In the same study, approximately one third of the cases had a gradual course without evidence of clear-cut improvement at any one time; 71 percent of the patients were afebrile by the third hospital day. In another study done more than 25 years later,[58] the average duration of hospitalization was 3.4 days. Longer stays were required for infants with initial oxygen saturations of less than 90 percent and infants younger than 6 weeks at the onset of illness. Reasonable criteria for admission in otherwise healthy infants seem to include hypoxia (i.e., oxygen saturation of <90% to 92%), age younger than 6 weeks, and a degree of respiratory distress sufficient to reduce fluid intake to inadequate levels. Other criteria include apnea, immunodeficiency, premature birth, and the presence of significant underlying heart or lung disease. Most patients can be discharged from the hospital within 2 to 3 days after admission, although mild wheezing still may be present for several days or even weeks thereafter.[13]

In 121 hospitalized patients, the total white blood cell count was less than 12,500/mm^3 in 74 percent and in 15 determinations had more than 60 percent neutrophils.[2] In another study, a mean leukocyte count of 16,000/mm^3 was determined for children with lower respiratory tract disease, including bronchiolitis, as was an increased percentage of band form neutrophils compared with that of a control group.[153] As in most infections, eosinophil counts in peripheral blood are reduced at the time of acute RSV infection.[52] Nonetheless, some patients maintain detectable eosinophilia in peripheral blood; these infants may be more likely to develop childhood asthma.[40]

Although impaired oxygenation is usually present in infants with respiratory distress, cyanosis may not be evident, even in the presence of markedly reduced oxygenation.[31,169] The respiratory rate is related inversely to the degree of oxygenation except when respiratory failure is imminent. In mild to moderate cases, carbon dioxide retention does not occur because the alveoli that are functioning can compensate for alveoli that are not ventilated. In severe disease, the blood pH is low and the $Paco_2$ is elevated.[31,169] The technique of pulse oximetry has obviated the need for arterial blood gas sampling except perhaps in severe cases in which hypercarbia is a concern.

In certain patients, clinical findings (e.g., degree of chest wall retractions, wheezing) often are out of proportion to the degree of hypoxia as measured by pulse oximetry.[129,193] Infants with marked dyspnea must be evaluated carefully because respiratory failure may occur precipitously, despite their reassuring oximetry readings. Once infants are hospitalized and stabilized, subsequent deterioration to the point of requiring intensive care is unusual, occurring in less than 2 percent of cases. Respiratory rates of 63 breaths per minute and oxygen saturations less than or equal to 88 percent on admission were weakly associated with such eventual deterioration in one study.[16]

The radiographic appearance of the chest in bronchiolitis varies considerably.[96] Anteroposterior radiographs may be normal in mild cases or may reveal hyperinflation in the absence of hypoxia. In moderate to severe illness, radiographs often appear exceptionally clear because of hyperinflation. The diaphragms often are flattened or depressed. The costophrenic angle is less acute, and the hilar vascular shadows are stretched. Frequently, areas of atelectasis may give the appearance of pneumonitis, although true consolidation is a rare occurrence. The heart usually appears small. On the lateral radiograph, the diaphragm is depressed markedly and reversal of the normal convexity frequently may be seen. The anteroposterior diameter of the chest is increased.

With recurrent wheezing episodes, the prodrome may be considerably shorter in duration with little or no fever and minimal coryza. In these children, differentiation of infection-induced wheezing from more conventional asthma becomes essentially impossible clinically.[202]

PATHOPHYSIOLOGY

The pathophysiology of bronchiolitis deservedly has been the focus of numerous investigations. Several original theories can be discounted reliably, whereas others warrant further study. Pathologic examinations of the lung in bronchiolitis or human airways in organ culture[72] reveal necrosis of the respiratory epithelium with destruction of the ciliated layer; mononuclear cell invasion of the peribronchial tissues and alveolar interstitium; edema of the submucosa and adventitia; and obstruction of small airways with dense plugs consisting of dying epithelial cells, fibrin, and inflammatory cells. In contrast to asthma, mucin (periodic acid–Schiff positive material) usually is absent in fatal bronchiolitis.[200] An associated interstitial pneumonitis often is present, and patchy areas of atelectasis frequently are noted.[203]

The predominant cell types present in the lung of fatal cases are macrophages and neutrophils. Lymphocytes bearing CD4 (helper cells), CD8 (cytotoxic cells), and CD56 (natural killer cells) antigens are almost absent in fatal bronchiolitis cases.[200] In contrast to the findings in fatal cases, CD8 lymphocytes were present in the lung tissue obtained from a nonhospitalized child with a mild lower respiratory tract infection caused by RSV who died in an automobile accident.[84] Even in surviving infants, CD8 lymphocyte counts are less than 1 percent of the total cells in bronchoalveolar lavage fluids[44] and only appear in respiratory tract secretions of subjects with severe forms of disease after illness has largely resolved.[69,107] These findings suggest that CD8 lymphocyte responses are necessary for early control of RSV infection and that severe disease is perhaps related to an absence of CD8 responses. Cytokines characteristically released by CD4 and CD8 cells (interleukin [IL]-2, IL-4, IL-5, IL-13, and

interferon-γ) are present only in low concentrations in nasopharyngeal and tracheal secretions of infants with severe lower respiratory tract infections caused by RSV.[14,54,200] Staining for markers of apoptosis (caspase 3 and Fas) is positive in infected epithelial cells,[200] suggesting that recovery from primary infection depends on the antiviral activity of phagocytic cells, induction of apoptosis, and perhaps the activity of other innate immune mechanisms. Strong innate immune responses occurring early in the course of infection may limit the eventual degree of illness.[11] Rhinovirus bronchiolitis appears to occur more frequently in subjects with preexisting atopic dermatitis and eosinophilia, suggesting that allergic pathways may contribute to bronchiolitis caused by this virus.[98,150]

Recovery from bronchiolitis apparently is complete histologically,[130,203] although plugging of the airway still was prominent in an infant 5 weeks after having an acute episode of bronchiolitis. This infant had experienced an apparent full clinical recovery before eventually dying of acute pneumococcal pneumonia.[130]

Infants may be particularly prone to the development of severe illness as a result of infection of the small airways for many reasons, including the small diameters of their airways. The infant lung is deficient in collateral alveolar ventilation through the pores of Kohn, which develop only in later life.[109] Atelectatic areas therefore cannot be re-expanded readily. Other studies have shown that the small airways in children younger than age 5 years contribute fivefold to sevenfold more to total airway resistance than do small airways of adults.[76] Viral infections involving small airways in young children are more likely to present as more serious clinical manifestations than similar infections in adults. Nonetheless, these abnormalities exist in all infants, whereas most infants infected with RSV do not develop lower respiratory tract illness. This suggests that host or environmental factors may be involved in determining the pathogenesis of RSV infection. Environmental factors are listed in Table 21-4, and potentially important host factors are described later.

One factor that may predispose to the development of lower respiratory tract illness after RSV infection is the infecting dose of virus. Infants with greater quantities of RSV in their nasopharyngeal secretions are more likely to exhibit severe illness.[18] In fatal cases, abundant virus is evident in epithelial cells plugging the airway lumen by immunohistochemical staining.[200] The fact that crowding is associated with greater risk for developing lower respiratory tract infection also suggests the importance of the initial inoculum in causing disease.[207,208]

Another risk factor for the development of lower respiratory tract infection may be the relative diameter (or degree of intrinsic constriction) of the airway. When pulmonary function testing is completed on healthy infants before lower respiratory tract infection occurs, certain infants have lower air flows in smaller airways (and presumably more narrow airways) than do other infants. When observed prospectively, these infants are more likely to develop wheezing early in life than are infants with better airflow.[113,115] These abnormalities of lung function no longer are associated with an increased risk for wheezing after the child reaches 3 years of age. Instead, evidence of atopy becomes the principal risk factor for recurrent wheezing.[115]

Long-term follow-up studies of infants who had bronchiolitis reveals reduced airflow in small airways in later childhood. Studies of adolescents reveal reversibility of this restriction of airflow after receiving bronchodilator treatment, indicating the airways were constricted, but not stenotic, in early life.[176] Several studies have shown that the airways of infants are intrinsically more reactive to bronchospastic stimuli than are airways of older children,[104,183] particularly children from families with asthma.[209] How long this increased reactivity persists is unknown, and no study has shown that infants with greater degrees of hereditary airway hyperreactivity are more likely to develop bronchiolitis or recurrent virus-induced wheezing. In later childhood, repeated occurrences of viral infections are necessary to sustain this increased reactivity.[191]

In addition to the hereditary airway hyperreactivity, viruses themselves may induce increases in reactivity. Viral infections that clinically appear to be restricted to the upper respiratory tract in adults and children

TABLE 21-4 **Factors Associated with an Increased Risk and Severity of Bronchiolitis and of Postbronchiolitic Morbidity**

Factor	Increase in Frequency	Increase in Severity	Increase in Later Morbidity
Crowding	+++	+++	?
Passive smoking	+++	+++	++
Male gender	+	++	++
Absence of breast-feeding	+	+	?
Family history of asthma	±	±	±
Personal atopy	–	–	+++
Congenitally small airways	++	?	–
Airway hyperreactivity	–	+	++
RSV-specific IgE responses	++	++	++

+++, implies strong relationship; ++, implies moderate relationship; +, implies weak relationship; ±, implies controversial relationship; –, implies no relationship; *?*, implies unknown relationship.
RSV, respiratory syncytial virus.
Data from references 19, 56, 64, 71, 101, 113-115, 117, 154, 170, 180, 195, 196, 199, 201, 207-209, and 211.

nonetheless result in transient, increased constrictive responses of the airway to a variety of stimuli, including histamine, irritants, and other agents, and small airway dysfunction.[2,4,30,42] Whether these minor changes contribute to the airway obstruction observed in bronchiolitis is doubtful, in that they are of much lesser magnitude than is the markedly increased reactivity that is observed in asthmatic individuals after exposure to allergens. These virus-induced changes were observed in all infected individuals, including subjects who did not experience wheezing at the time of the virus infection. If airways already are hyperreactive in infants, on a hereditary basis or as a result of a preceding viral infection, the subsequent stimulus of RSV infection may be sufficient to cause airway obstruction.

Immunologic deficits may be expected to lead to more severe forms of virus-induced respiratory illness. Studies of the antibody response to RSV infection in serum and in respiratory secretions show, however, that the nature of responses generally is similar among patients with bronchiolitis or simple upper respiratory tract illness alone caused by this agent.[89,197] Antibody-directed cellular cytotoxicity expressed against tissue culture cells infected with RSV also is similar in patients with all forms of illness caused by RSV.[88,163] Although investigators have shown that cells infected with RSV can activate complement through classic and alternative pathways,[175] studies suggest that in vivo activation of the complement cascade occurs with equal frequency among patients with all forms of illness caused by the virus.[88] Repeated RSV infections in early life may occur because antibody formed after primary RSV exposure has a low ability to bind to neutralizing epitopes on the virus.[34,50]

The concept that serum IgG antibody to RSV, acquired by vaccination or transplacentally, might sensitize the host has been dispelled by results of studies showing that severe bronchiolitis may occur in the absence of circulating antibody[141] and that titers of maternal antibody correlate with protection against infection caused by the virus.[56,135]

Lymphocyte hypersensitivity has been suggested to play a role in the development of severe bronchiolitis. Original field trials showed that a formalin-inactivated RSV (FI-RSV) vaccine induced humoral and cell-mediated immune responses to the virus. Nonetheless, vaccinated subjects manifested more severe forms of illness than did unvaccinated controls when subjects in each group subsequently were infected naturally.[93] Cell-mediated immune responses to viral antigen were greater among recipients of this FI-RSV vaccine compared with control subjects who previously had experienced natural infection.[94] The resulting disease in vaccine recipients was similar in form to bronchiolitis, and the idea that natural RSV bronchiolitis is a consequence of lymphocyte hypersensitivity to the virus has persisted.

Nonetheless, it seems unlikely that the illness that followed FI-RSV vaccination is mediated in the same way as that following natural bronchiolitis. The two FI-RSV vaccine recipients who died after having subsequent natural infection had numerous lymphocytes and eosinophils present in the lung, which is not the case after natural RSV infection.[84,93,130,200] Infants surviving RSV infection after receiving the FI-RSV vaccine had high eosinophil counts in peripheral blood, which also is unusual in natural RSV infection.[52] In addition, cytotoxic T-lymphocyte activity is not detected easily in infants after bronchiolitis,[6] and more recent autopsy studies have shown the virtual absence of CD4 and CD8 antigen-positive cells in the lung.[200] Although the development of cell-mediated cytotoxic antiviral responses may be protecting individuals with milder disease, little reason exists to contend that lymphocyte hyperresponsiveness contributes to severe bronchiolitis.

Immediate hypersensitivity to viral antigens has received much consideration as a potential contributing factor in bronchiolitis. The association of a family history of asthma with the development of bronchiolitis in infancy remains controversial.[101,171] Production of virus-specific IgE and subsequent release of mediators of bronchoconstriction have been documented, however, in infants hospitalized with bronchiolitis caused by RSV and the parainfluenza viruses.[19,196,198,199] In bronchiolitis caused by RSV, the quantities of virus-specific IgE produced and histamine present in respiratory secretions correlate with the severity of illness, as measured by degree of Pao_2.[199] Leukotriene C_4 is a product of mast cells and eosinophils primarily, although also epithelial cells and macrophages, and is a potent stimulant of airway smooth muscle constriction and mucus secretion. This mediator is released into the airway in acute bronchiolitis,[189] as is the bronchoconstrictive agent histamine.[172,199] In addition, prostaglandins and metabolites can be detected in secretions and blood after bronchiolitis.[153] Thus, several mediators of airway obstruction that are released in response to the interaction of IgE with antigens are released in subjects with bronchiolitis.

A possible role for eosinophils in the pathogenesis of bronchiolitis is supported by the finding of increased concentrations of eosinophil cationic protein in secretions of infants with bronchiolitis and (among high responders) an overall correlation of concentrations of this protein with the degree of hypoxia.[52,53] Peripheral blood eosinophil counts are depressed during the acute phase of most infectious diseases, including RSV infection. Nonetheless, eosinophil counts in peripheral blood are higher in infants with bronchiolitis than in infants with upper respiratory tract illness only, particularly in boys.[52] This finding is notable because boys generally have more severe forms of bronchiolitis. A contrasting viewpoint is that eosinophils contribute to the clearance of viruses after infection. This view is supported by the fact that eosinophils contain enzymes with ribonuclease activity, which inactivates RSV.[36,37] IgE-dependent and eosinophil-dependent mechanisms may be important in recovery from viral infections, much as they are in recovery from parasitic infections.

The airways of asthmatic individuals are infiltrated by T lymphocytes (predominantly T-helper lymphocytes) and eosinophils. T-helper cells can be classified as type 1 (Th1), which produce primarily interferon-γ and IL-2, or type 2 (Th2), which produce IL-4, IL-5, IL-13, and others. This finding is important because IL-4, IL-5, and IL-13 are important factors in promoting IgE synthesis and eosinophil migration. Asthma is thought to be

induced by a Th2 bias in the airway. An attractive hypothesis is that RSV infection induces airway obstruction and wheezing by inciting the same type of Th2 responses as observed in asthma. However, although interferon-γ and IL-4 are found in respiratory secretions of infants with RSV bronchiolitis, IL-5 and IL-13 rarely are detectable in serum or secretions,[54,189] suggesting that bronchiolitis is not characterized by a Th2 lymphocyte bias. RSV infection in infancy does not induce a long-term Th2 lymphocyte cytokine bias lasting into later life.[23]

Chemokines are proteins released by airway epithelial cells, inflammatory cells, fibroblasts, and other cell types that are chemotactic for leukocytes. A failure to initiate strong chemokine responses at the onset of RSV infection has been linked to more severe disease outcomes, possibly because innate immune mechanisms are not activated in a timely fashion.[11] In contrast, excessive production of the chemokine MIP-1α has been linked to the development of greater degrees of hypoxia during RSV infection.[11,54] Abnormal chemokine responses, rather than Th2 cytokine responses, may underlie the pathogenesis of bronchiolitis and thus may represent therapeutic targets.

DIFFERENTIAL DIAGNOSIS

A tendency exists to attribute all infantile respiratory distress occurring after the immediate neonatal period to bronchiolitis. The list of causes of infantile dyspnea consists of conditions that are associated with upper and lower airway obstruction, however. Recognition of upper airway obstructive disease should cause little difficulty because the problem is one of distress with inspiration rather than air trapping. Illnesses that cause lower airway obstructive disease and diseases that suggest this problem are listed in Table 21-5.

The differential diagnosis of allergic disease causes the most difficulty. Generally, the first episode of allergic respiratory disease, when associated with infection, cannot be separated from bronchiolitis by any objective measures. Anatomic defects such as vascular rings can cause obstruction of the airway at many locations; inspiratory or expiratory distress, or a combination, can occur. Frequently, a child with an anatomic defect does not have any detectable difficulty until a trivial respiratory tract infection occurs, which complicates the diagnostic picture.

Foreign bodies should be considered even in very young infants. Gastroesophageal reflux disease has become recognized as a frequent cause of wheezing in young infants. Because of its obvious therapeutic implications, bacterial pneumonia is the most important differential consideration, although wheezing occurs rarely in association with bacterial pneumonia.[108,118]

TABLE 21-5 **Differential Diagnostic Considerations in Acute Bronchiolitis and Infectious Asthma**

Allergy
Asthma
Allergic pneumonias (e.g., allergic aspergillosis)
Anatomic cause
Vascular ring, lung cysts, lobar emphysema
Pneumothorax, hydrothorax, chylothorax
Foreign body
Circulatory failure
Congenital and acquired heart disease
Anemia
Nephritis
Infections
Viral, chlamydial, rickettsial, mycoplasmal, bacterial, and fungal pneumonias
Migrating parasites
Irritants
Inhalation of toxic substances (e.g., chlorine gas)
Aspiration pneumonia
Gastroesophageal reflux
Metabolic cause
Poisons (e.g., salicylate)
Acidosis

DIAGNOSIS

Because bronchiolitis and infectious asthma are clinical diseases with arbitrary boundaries and multiple etiologic agents, outlining a method for establishing specific diagnosis is difficult. When illness is epidemic, RSV usually is the cause. In nonepidemic situations, a careful history and appropriate laboratory studies and radiographs should be considered to exclude the other differential diagnostic possibilities that are listed in Table 21-5.

A specific etiologic diagnosis can be made by the isolation of virus from the nasopharynx. The diagnostic virologic facilities of many medical centers enable the isolation in tissue culture of RSV, parainfluenza viruses, influenza viruses, adenoviruses, rhinoviruses, enteroviruses, and herpesviruses. The use of the shell vial technique has been applied to RSV infection with success.[174] Until recently, rapid detection of viral antigen of RSV directly in nasopharyngeal secretions by commercially available (e.g., enzyme-linked immunosorbent assay) or fluorescent antibody techniques has been the method of choice in most laboratories.[185] The accuracy of these techniques often is superior to that of standard cell culture (at least in infants) because antigens remain stable under transport conditions that inactivate live virus. Gradually, the technique of amplifying small quantities of RNA of respiratory viruses simultaneously from clinical specimens is replacing earlier methods. Currently, polymerase chain reaction techniques can test for as many as 15 respiratory viruses simultaneously in respiratory secretions.[136]

TREATMENT

The cornerstone of therapy for bronchiolitis is the administration of oxygen and correction of fluid deficits.

Most patients hospitalized for bronchiolitis can be managed successfully and discharged within 48 to 72 hours when given no further therapy than supplemental oxygen and careful fluid rehydration.[35,142,192] Oxygen saturation values obtained at the time of hospitalization have been used to predict the duration of hospitalization[166,192] and eventual deterioration and need for care in a pediatric intensive care unit after hospitalization.[16] Oxygen saturation is usually maintained at 92 percent or higher, although it has been suggested that an overreliance on oxygen saturation values as evidence for the need for hospitalization may lead to unnecessary admissions or needlessly prolonged stays.[161] Blood gas determinations should be obtained when indicated by clinical findings (e.g., cyanosis, agitation), because pulse oximetry is less sensitive for detecting hypoxia than is arterial blood gas sampling. Oximetry cannot detect increases in Pco_2 that may indicate impending respiratory failure.

A common practice has been to place children with bronchiolitis in tents and administer mist vigorously. Although oxygen should be humidified, the use of mists and aerosols except to deliver specific antiviral therapy is discouraged; mist can act as an irritant, causing reflex bronchoconstriction, and the water usually does not reach the lower airways.[7] Although dehydration is a potential problem in children with bronchiolitis because of reduced oral intake and vomiting, care must be taken not to overhydrate such patients. Edema is an important part of the pathology of bronchiolitis, so administration of excessive amounts of intravenous fluids may contribute to obstruction of the airway.

β-Adrenergic bronchodilators have been used frequently in bronchiolitis. Slight improvements in airflow, in oxygenation, and in clinical illness scores have been reported,[67,95,103,159] but these effects are not meaningful clinically; hospitalization is not prevented, and hospital stays are not shortened.[35,47] Improvements observed after the administration of the first dose of these compounds usually are not sustained after administration of subsequent doses. In one study, oral and aerosolized β-adrenergic agents were compared with inactive substances given by the corresponding route. The degree of improvement was the same in all four groups, arguing against a benefit of β-adrenergic agents in bronchiolitis.[51] This is not surprising, given the absence of evidence that bronchoconstriction occurs in bronchiolitis. Older individuals with infectious asthma may be more likely to respond to these agents, but viral infections remain the most common precipitant of childhood wheezing either managed on an outpatient basis or requiring hospitalization.[83,85] This suggests that children of any age with virus-induced wheezing do not respond dramatically to medications commonly used to treat asthma.

In the past, epinephrine often was administered subcutaneously to older infants in an attempt to differentiate allergic disease from true bronchiolitis. This approach rarely was successful in differentiating one illness from the other or in improving the condition of the patient. Some studies have suggested, however, that use of aerosolized, racemic epinephrine produces greater improvement than the use of β-adrenergic aerosols alone.[159] Compounds containing epinephrine could be more effective in reversing airway edema than are β-adrenergic agents. Generally, however, the minor improvements in pulmonary function are not meaningful clinically, because the length of stay in the intensive care unit or in the hospital is not reduced by the use of epinephrine.

Aerosols of ipratropium also have been administered in bronchiolitis without observable benefit.[164] Possible explanations for poor responses to bronchodilators include a paucity of β-adrenergic receptors in infancy[157]; decreased amounts of smooth muscle capable of responding to bronchodilators surrounding the terminal airways[152]; the nature of the airway obstruction itself (intense plugging with cellular debris)[200,204]; and, presumably, persistence of virus in the airway, stimulating continued inflammation or release of bronchoconstrictive mediators or both.

Trials of α- or β-adrenergic aerosols may be attempted in seriously ill patients, although persistence with these approaches in the absence of an initial response may prove harmful.[80,138] A child treated for croup with multiple doses of racemic epinephrine developed ventricular tachycardia and a small myocardial infarction,[20] emphasizing the need to avoid the careless use of these compounds in viral respiratory tract infections.

Corticosteroids have been employed repeatedly in treating bronchiolitis. Controlled studies have failed to reveal any benefit in terms of prevention of hospitalization, reduced duration of hospitalization, reduced need for intubation, or reduced frequency of recurrent wheezing episodes after bronchiolitis.* In a child with nonrespiratory indications for the administration of corticosteroids, these drugs need not be withheld because of fear of complications, although the duration of viral shedding may be extended in individuals treated with corticosteroids. Combinations of salbutamol and dexamethasone also have been evaluated but usually show minimal benefit.[182] One study suggested some benefit for this combination, but differences observed were short of statistical significance.[144]

Corticosteroids have also been used to prevent recurrent wheezing in preschool-aged children. They have been more effective in those children with atopic eczema and who were known to be sensitive to aeroallergens.[5,62] Corticosteroids have been essentially ineffective in preschool-aged children with virus-induced wheezing without atopic sensitization whose episodes are less frequent.[137,140] Long-term, sustained corticosteroid therapy has unfortunately had no effect on the course of asthma once the therapy is discontinued.[62]

Early experience with the antiviral substance ribavirin in bronchiolitis suggested that mild subjective benefits and improvement in oxygenation occurred after near-continuous aerosol administration of this compound.[65,173] The degree of improvement in patients treated with ribavirin was minimal and probably occurred because the placebo group received aerosolized water (an irritant to the airway) rather than saline.[127] No study has shown whether the administration of ribavirin could prevent deaths, avoid the need for mechanical ventilation, or

*References 12, 31-33, 48, 102, 156.

shorten the duration of hospital stays.[65,127,173] Ribavirin is essentially not used for RSV infection currently.

Other approaches to therapy for RSV infection include the use of interferon-α,[29] DNase,[119] and nitric oxide.[143] The results of these studies demonstrated no substantial benefit of any of these approaches. Infants with low serum levels of vitamin A[133,149] and vitamin D[10] are more likely to develop RSV lower respiratory tract infection than infants with normal serum levels of these vitamins. However, there is currently no evidence that supplementation with these vitamins during infection has any effect on the outcome of disease. Surfactant[90,187] has been used in very small studies of bronchiolitis or virus-induced wheezing, with positive effects that require confirmation. Several recent studies have evaluated the effect of 3 percent and 5 percent nebulized saline solutions in bronchiolitis. Some studies have demonstrated reduced length of hospitalization in subjects receiving hypertonic saline,[100,112] whereas other studies have not shown any positive effects.[59,81]

RSV-infected infants have elevated levels of cysteinyl leukotrienes in respiratory tract secretions.[189,190] Although initial studies of leukotriene receptor antagonists in RSV infection suggested that the use of these antagonists could reduce the magnitude of respiratory symptoms after RSV bronchiolitis,[3,91] subsequent studies demonstrated no differences in long-term complications.[13] Preparations containing a very high titer of neutralizing antibody against RSV have been tested in the prevention and treatment of RSV infection in high-risk populations. The results of the prophylaxis studies were positive (see later discussion under Prevention). A significant therapeutic effect of these compounds once RSV infection was established could not be identified, however.[110]

Because bronchiolitis is a viral disease, antibiotics are not useful or necessary. In many instances, the radiographic picture suggests pneumonia and the blood leukocyte count is elevated. In these instances, a physician may feel compelled to administer antibiotics. However, bacterial infection of the lung is rare in bronchiolitis even when infiltrates are identified on chest radiographs. In one large study, secondary bacterial infection occurred in no more than 7 (1.2%) of 565 children with RSV infection.[66] In developed countries, bacterial pneumonia does not commonly complicate RSV infection and may be overdiagnosed when bacterial flora are recovered in cultures of tracheal aspirates.[151] For the vast majority of infants, antibiotics need not be prescribed once RSV infection has been diagnosed or can be stopped immediately thereafter. Institution of antibiotics should be considered during the course of therapy when a change in clinical findings suggests the possibility of secondary bacterial infection.

A child with bronchiolitis generally is more comfortable in the supine position with the head end of the crib slightly elevated. Infant seats are used frequently but are not optimal because the child's head tends to fall to the side or forward, which constricts the upper airway. The sitting position also causes a possibly deleterious upward pressure on the diaphragm. The onset of respiratory failure is usually recognized by virtue of reduced inspiratory breath sounds, severe inspiratory retractions, inability to maintain an oxygen saturation of more than 90 percent in 40 percent ambient oxygen, cyanosis in 40 percent oxygen, decreased or absent response to painful stimuli, and a $Paco_2$ rising to 65 mm Hg or higher. Nasotracheal intubation and positive-pressure ventilation, often with neuromuscular blockade, are indicated in these circumstances.[38,138]

PREVENTION

The development of a method to prevent RSV infection is a high priority. The initial adverse experience with formalin-inactivated vaccine (described earlier) unfortunately has nearly prevented further investigation of inactivated vaccines.[93,188] Live, temperature-sensitive vaccines have been developed by adapting RSV to grow at reduced temperatures in cell culture. This attenuated vaccine was designed to grow at the lower temperatures of the upper respiratory tract but be inactive at the higher temperatures in the lung. In initial field trials, the vaccine strain caused febrile respiratory illnesses in seronegative vaccinees but did not replicate adequately in seropositive subjects.[27] Further trials of temperature-sensitive mutant RSV strains as vaccines showed improved immunogenicity and some protection, at least against rechallenge with the vaccine strain. Nasal congestion was noted in some recipients, however, and there is concern that this degree of nasal obstruction could result in apnea in infants.[86] Numerous other vaccine candidates, including RSV nucleic acid vaccines, bovine RSV strains, parainfluenza virus strains bearing RSV antigens, and human RSV strains with gene deletions or given with immunologically active adjuvants, have been developed. Many of them are in early clinical trials, but development of a successful vaccine is not imminent.[24]

In contrast to the largely negative experience with vaccine development, protection against serious illness caused by RSV infection was achieved using a pooled preparation of human serum obtained from donors with very high titers of neutralizing antibody against RSV.[61] This compound, when administered during the RSV season on a monthly basis to infants and young children with a history of birth at less than 32 weeks' gestation or with bronchopulmonary dysplasia, caused a marked reduction in the rate of hospitalization related to RSV infection.

These trials have been repeated successfully using a humanized mouse monoclonal antibody against the RSV fusion protein.[139] The antibody is reconstructed so that it has more than 95 percent of the protein structure of a human antibody. This compound is approximately 50 percent effective in preventing RSV-related hospitalization when administered to high-risk infants, and it has received approval for use in infants born prematurely with or without lung disease of premature birth. Separate trials in infants with hemodynamically significant congenital heart disease have shown a similar

reduction in the rate of hospitalization for RSV-related illness, and the compound is now approved for use in these infants as well.[46]

COMPLICATIONS AND PROGNOSIS

Virtually all cases of bronchiolitis in healthy children resolve without acute complications. Secondary bacterial infection in bronchiolitis now is a rare occurrence, at least in developed countries. Scott and colleagues[162] identified minor electrocardiographic abnormalities in 2 percent of 188 children with bronchiolitis. Involvement of other organs apparently does not occur.

The overall mortality rate in bronchiolitis is low. Before the modern era of improved ventilatory support, mortality rates ranged from 2.0 to 5.5 percent.[33,70,75] A multicenter study in Great Britain published in 1978[154] estimated the mortality rate owing to RSV infection in infancy at 0.5 percent. Deaths should occur rarely except among infants with severe underlying cardiac or pulmonary disease; even in these cases, the mortality rate should not exceed 1 percent of cases.[46] Between 1979 and 1997, an average of 95 bronchiolitis-associated deaths occurred annually in the United States, with 20 percent occurring in infants with underlying heart disease, lung disease, or premature birth.[168]

The possible association of bronchiolitis with the subsequent development of asthma has been debated for decades. Fifty percent of patients with bronchiolitis have recurrent episodes of wheezing, although this figure decreases to approximately 10 percent by adolescence and may not be above that of the general population by this time.[116,117,178] Whether this recurrent wheezing is caused by RSV infection or, alternatively, suggests that RSV infection in early life is an indicator of a tendency toward airway obstruction is still being investigated. Recurrent wheezing after having a case of bronchiolitis could reflect an inherited asthmatic trait. Some studies find a strong correlation between atopic family history and postbronchiolitic wheezing,[41,158,211] whereas others, particularly studies from Great Britain,[147,170,171] do not. Studies of twins born in Denmark suggest that a hereditary predisposition to asthma is more likely to explain the development of bronchiolitis than bronchiolitis is likely to explain the development of asthma.[186] In other studies, titers of total serum IgE[145,177] and peripheral blood eosinophil counts[40,114,211] have some predictive value for the development of recurrent wheezing in infants having had bronchiolitis. Two studies showed that peripheral blood eosinophilia during viral infections, particularly RSV bronchiolitis, predicts the development of recurrent wheezing in school-aged children.[40,114] It has become apparent that rhinovirus-induced bronchiolitis has particular implications for recurrent wheezing in childhood. First, rhinovirus-induced bronchiolitis appears to be more common in infants with atopic dermatitis and eosinophilia than in other infants.[97,99,121] In addition, the occurrence of rhinovirus-induced bronchiolitis in infancy bears a much stronger relationship to the development of wheezing beyond 3 years of age than RSV bronchiolitis, probably because rhinovirus bronchiolitis occurs more frequently in infants predisposed to develop childhood asthma.[82] Atopy seems to explain much, although not all, of the recurrent wheezing that occurs after infantile bronchiolitis.

RSV infection in early infancy could also damage the developing airway, rendering the airway more prone to obstruction in later life. Pulmonary function tests performed in former patients with bronchiolitis 12 years after an episode of bronchiolitis reveal an increased frequency of airway hyperreactivity in response to challenge with exercise or chemical agents, increased ratios of residual volume to total lung capacity, and reduced expiratory airflow at low lung volumes.* Although these abnormalities are observed commonly in individuals with asthma, they could not be explained in several of the previous studies simply by the presence of a personal or family history of atopy.[65,147,180] Some of these retrospective studies showed, however, that a single episode of RSV bronchiolitis was not associated with long-term lung dysfunction; abnormal lung function was observed only if at least two episodes of lower respiratory tract illness had occurred before the patient had reached 2 years of age.[73,191] These studies generally suggest that atopy may explain more of the recurrent wheezing after infantile bronchiolitis than airway damage from the infection itself.

Prospective studies show that recurrent wheezing in children up to age 3 years after bronchiolitis is related to abnormalities of lung function that existed before RSV infection occurred.[113] Wheezing persisting beyond age 3 years was related, at least partially, to the atopic status of the host, as well as a history of asthma in the mother.[22,82,99] No study has shown a relationship between the severity of the initial bronchiolitis episode and the degree of abnormality of long-term lung function.[63,64,147,170] Personal atopy is responsible for many of the long-term manifestations proposed to be sequelae of bronchiolitis. Some factor other than atopy may determine in part the apparent lung abnormalities seen after a case of bronchiolitis,[116,195,207,208] but no direct evidence indicates that this other factor is the initial episode of RSV infection itself.

RSV infection could also account for recurrent wheezing by inducing persistent airway hyperreactivity. Retrospective studies have shown that airway reactivity is, in fact, greater in individuals 1 decade after having an episode of infantile bronchiolitis than in control populations without a history of bronchiolitis.[63,147] Airway reactivity in all children (even children never experiencing bronchiolitis) is greater than that in adults, however, and it is greatest in children of atopic families and in children exposed to cigarette smoke.[209] Airway hyperreactivity occurring after bronchiolitis is, therefore, not necessarily a reflection of the RSV infection itself. One prospective study found no relationship between RSV bronchiolitis and the degree of airway reactivity at age 2 years.[2]

*References 63, 87, 147, 170, 179, 180, 183, 204, 206.

RSV infection possibly can promote sensitization to allergens. In animal models, critically timed RSV infection can enhance temporarily the degree of airway reactivity induced after sensitization to an allergen. Whether this phenomenon occurs in humans is unknown. The absence of Th2-like cytokine responses at the time of having RSV bronchiolitis[54] suggests, however, that RSV infection in infancy likely does not promote a persistent atopic state in the host. The results of a recent, large European study (ALSPAC) showed that hospitalization in infancy for RSV bronchiolitis was associated with subsequent childhood wheezing and asthma but not atopy.[74]

RSV infection more likely results in the release of mediators of airway obstruction, such as histamine and leukotrienes, through a mechanism other than Th2 cytokine responses. Chemokines such as MIP-1α, which cause mast cell and basophil degranulation, are possible candidates. Release of these mediators at the time of having RSV infection may result in wheezing in susceptible individuals. These same individuals may develop wheezing again at the time of having allergen exposure in childhood (particularly if they are atopic), but the relationship of bronchiolitis in infancy and such subsequent childhood wheezing would not be causal. Rather the association would be based on the underlying susceptibility of the airway to obstruction.

The overall outlook for infantile bronchiolitis generally is excellent. In a follow-up study conducted at the Women & Children's Hospital of Buffalo,[195] severe lung disease was not observed in former bronchiolitis patients. All oxygen saturation levels were greater than 95 percent, and at least some of the airflow obstruction present in patients aged 7 to 9 years was reversible with a single bronchodilator treatment, as was confirmed later.[176] Single episodes of infantile bronchiolitis (in the absence of passive smoke exposure and without recurrent wheezing episodes) have not been associated with abnormalities of lung function or airway hyperreactivity in later childhood.[87,180,191]

The natural history of postbronchiolitic wheezing in childhood is for episodes of wheezing to become progressively milder,[63,117,147,170] and the frequency of postbronchiolitic wheezing apparently eventually decreases to essentially the same rate as that of children who did not experience bronchiolitis in infancy.[73,117,176] Nonetheless, the overall prognosis is not entirely benign, and exposure to noxious environmental elements may result in an accelerated deterioration of lung function in later life.[29,202,206] The combination of respiratory tract illness in early life and subsequent cigarette smoking especially may be harmful.[181] Individuals who develop bronchiolitis in infancy should avoid smoking in later life and occupations that are associated with exposure to respiratory irritants.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Amirav I, Luder AS, Kruger N, et al. A double-blind, placebo-controlled, randomized trial of montelukast for acute bronchiolitis. *Pediatrics* 2008;**122**:e1249–55.
5. Bacharier LB, Guilbert TW, Zeiger RS, et al. Patient characteristics associated with improved outcomes with use of an inhaled corticosteroid in preschool children at risk for asthma. *J Allergy Clin Immunol* 2009;**123**:1077–82, 1082.e1–5.
10. Belderbos ME, Houben ML, Wilbrink B, et al. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics* 2011;**127**:e1513–20.
13. Bisgaard H, Flores-Nunez A, Goh A, et al. Study of montelukast for the treatment of respiratory symptoms of post–respiratory syncytial virus bronchiolitis in children. *Am J Respir Crit Care* 2008;**178**:854–60.
22. Carroll KN, Gebretsadik T, Minton P, et al. Influence of maternal asthma on the cause and severity of infant acute respiratory tract infections. *J Allergy Clin Immunol* 2012;**129**:1236–42.
23. Castro M, Schweiger T, Yin-Declue H, et al. Cytokine response after severe respiratory syncytial virus bronchiolitis in early life. *J Allergy Clin Immunol* 2008;**122**:726–33.e3.
24. Chang J. Current progress on development of respiratory syncytial virus vaccine. *BMB Rep* 2011;**44**:232–7.
34. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009;**15**:34–41.
50. Freitas GR, Silva DA, Yokosawa J, et al. Antibody response and avidity of respiratory syncytial virus-specific total IgG, IgG1, and IgG3 in young children. *J Med Virol* 2011;**83**:1826–33.
59. Grewal S, Ali S, McConnell DW, et al. A randomized trial of nebulized 3% hypertonic saline with epinephrine in the treatment of acute bronchiolitis in the emergency department. *Arch Pediatr Adolesc Med* 2009;**163**:1007–12.
81. Ipek IO, Yalcin EU, Sezer RG, Bozaykut A. The efficacy of nebulized salbutamol, hypertonic saline and salbutamol/hypertonic saline combination in moderate bronchiolitis. *Pulm Pharmacol Ther* 2011;**24**:633–7.
82. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care* 2008;**178**:667–72.
91. Kim CK, Choi J, Kim HB, et al. A randomized intervention of montelukast for post-bronchiolitis: effect on eosinophil degranulation. *J Pediatr* 2010;**156**:749–54.
126. Mlinaric-Galinovic G, Welliver RC, Vilibic-Cavlek T, et al. The biennial cycle of respiratory syncytial virus outbreaks in Croatia. *Virol J* 2008;**5**:18.
136. Olofsson S, Brittain-Long R, Andersson LM, et al. PCR for detection of respiratory viruses: seasonal variations of virus infections. *Expert Rev Anti Infect Ther* 2011;**9**:615–26.
140. Panickar J, Lakhanpaul M, Lambert PC, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. *N Engl J Med* 2009;**360**:329–38.
144. Plint AC, Johnson DW, Patel H, et al. Epinephrine and dexamethasone in children with bronchiolitis. *N Engl J Med* 2009;**360**:2079–89.
178. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus–associated hospitalizations among infants and young children in the United States, 1997-2006. *Pediatr Infect Dis J* 2012;**31**:5–9.
186. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med* 2009;**179**:1091–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ackerman BD. Acute bronchiolitis: a study of 207 cases. *Clinical Pediatr* 1962;**1**:75–81.
2. Adler A, Ngo L, Tosta P, Tager IB. Association of tobacco smoke exposure and respiratory syncitial virus infection with airways reactivity in early childhood. *Pediatr Pulmonol* 2001;**32**:418–27.
3. Amirav I, Luder AS, Kruger N, et al. A double-blind, placebo-controlled, randomized trial of montelukast for acute bronchiolitis. *Pediatrics* 2008;**122**:e1249–55.
4. Aquilina AT, Hall WJ, Douglas Jr RG, Utell MJ. Airway reactivity in subjects with viral upper respiratory tract infections: the effects of exercise and cold air. *Am Rev Respir Dis* 1980;**122**:3–10.
5. Bacharier LB, Guilbert TW, Zeiger RS, et al. Patient characteristics associated with improved outcomes with use of an inhaled corticosteroid in preschool children at risk for asthma. *J Allergy Clin Immunol* 2009;**123**:1077–82, 1082.e1–5.
6. Bangham CR, Cannon MJ, Karzon DT, Askonas BA. Cytotoxic T-cell response to respiratory syncytial virus in mice. *J Virol* 1985;**56**:55–9.
7. Bau SK, Aspin N, Wood DE, Levison H. The measurement of fluid deposition in humans following mist tent therapy. *Pediatrics* 1971;**48**:605–12.
8. Beem M, Wright FH, Hamre D, et al. Association of the chimpanzee coryza agent with acute respiratory disease in children. *New Engl J Med* 1960;**263**:523–30.
9. Beem M, Wright FH, Fasan DM, et al. Observations on the etiology of acute bronchiolitis in infants. *J Pediatr* 1962;**61**:864–9.
10. Belderbos ME, Houben ML, Wilbrink B, et al. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics* 2011;**127**:e1513–20.
11. Bennett BL, Garofalo RP, Cron SG, et al. Immunopathogenesis of respiratory syncytial virus bronchiolitis. *J Infect Dis* 2007;**195**:1532–40.
12. Berger I, Argaman Z, Schwartz SB, et al. Efficacy of corticosteroids in acute bronchiolitis: short-term and long-term follow-up. *Pediatr Pulmonol* 1998;**26**:162–6.
13. Bisgaard H, Flores-Nunez A, Goh A, et al. Study of montelukast for the treatment of respiratory symptoms of post-respiratory syncytial virus bronchiolitis in children. *Am J Respir Crit Care Med* 2008;**178**:854–60.
14. Bont L, Heijnen CJ, Kavelaars A, et al. Local interferon-γ levels during respiratory syncytial virus lower respiratory tract infection are associated with disease severity. *J Infect Dis* 2001;**184**:355–8.
15. Boyce TG, Mellen BG, Mitchel Jr EF, et al. Rates of hospitalization for respiratory syncytial virus infection among children in medicaid. *J Pediatr* 2000;**137**:865–70.
16. Brooks AM, McBride JT, McConnochie KM, et al. Predicting deterioration in previously healthy infants hospitalized with respiratory syncytial virus infection. *Pediatrics* 1999;**104**:463–7.
17. Bruhn FW, Mokrohisky ST, McIntosh K. Apnea associated with respiratory syncytial virus infection in young infants. *J Pediatr* 1977;**90**:382–6.
18. Buckingham SC, Bush AJ, Devincenzo JP. Nasal quantity of respiratory syncytical virus correlates with disease severity in hospitalized infants. *Pediatr Infect Dis J* 2000;**19**:113–7.
19. Bui RH, Molinaro GA, Kettering JD, et al. Virus-specific IgE and IgG4 antibodies in serum of children infected with respiratory syncytial virus. *J Pediatr* 1987;**110**:87–90.
20. Butte MJ, Nguyen BX, Hutchison TJ, et al. Pediatric myocardial infarction after racemic epinephrine administration. *Pediatrics* 1999;**104**:e9.
21. Carmalt-Jones DW. The treatment of bronchial asthma by a vaccine. *BMJ* 1909;**2**:1049–50.
22. Carroll KN, Gebretsadik T, Minton P, et al. Influence of maternal asthma on the cause and severity of infant acute respiratory tract infections. *J Allergy Clin Immunol* 2012;**129**:1236–42.
23. Castro M, Schweiger T, Yin-Declue H, et al. Cytokine response after severe respiratory syncytial virus bronchiolitis in early life. *J Allergy Clin Immunol* 2008;**122**:726–33.e3.
24. Chang J. Current progress on development of respiratory syncytial virus vaccine. *BMB Rep* 2011;**44**:232–7.
25. Chanock RM, Kim HW, Vargosko AJ, et al. Respiratory syncytial virus, part I: virus recovery and other observations during 1960 outbreak of bronchiolitis, pneumonia, and minor respiratory diseases in children. *JAMA* 1961;**176**:647–53.
26. Chanock RM, Mufson MA, Johnson KM. Comparative biology and ecology of human virus and *Mycoplasma* respiratory pathogens. *Prog Med Virol* 1965;**7**:208–52.
27. Chanock RM, Murphy BR. Use of temperature-sensitive and cold-adapted mutant viruses in immunoprophylaxis of acute respiratory tract disease. *Rev Infect Dis* 1980;**2**:421–32.
28. Cherry JD. Newer respiratory viruses: their role in respiratory illness of children. *Adv Pediatr* 1973;**20**:225–90.
29. Chipps BE, Sullivan WF, Portnoy JM. Alpha-2A-interferon for treatment of bronchiolitis caused by respiratory syncytial virus. *Pediatr Infect Dis J* 1993;**12**:653–8.
30. Collier AM, Pimmel RL, Hasselblad V, et al. Spirometric changes in normal children with upper respiratory infections. *Am Rev Respir Dis* 1978;**117**:47–53.
31. Connolly JH, Field CM, Glasgow JF, et al. A double-blind trial of prednisolone in epidemic bronchiolitis due to respiratory syncytial virus. *Acta Paediatr Scand* 1969;**58**:116–20.
32. Corneli HM, Zorc JJ, Mahajan P, et al. A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. *N Engl J Med* 2007;**357**:331–9.
33. Dabbous IA, Tkachyk JS, Stamm SJ. A double-blind study on the effects of corticosteroids in the treatment of bronchiolitis. *Pediatrics* 1966;**37**:477–84.
34. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009;**15**:34–41.
35. Dobson JV, Stephens-Groff SM, McMahon SR, et al. The use of albuterol in hospitalized infants with bronchiolitis. *Pediatrics* 1998;**101**:361–8.
36. Domachowske JB, Bonville CA, Dyer KD, Rosenberg HF. Evolution of antiviral activity in the ribonuclease A gene superfamily: evidence for a specific interaction between eosinophil-derived neurotoxin (EDN/RNase 2) and respiratory syncytial virus. *Nucl Acids Res* 1998;**26**:5327–32.
37. Domachowske JB, Dyer KD, Bonville CA, Rosenberg HF. Recombinant human eosinophil-derived neurotoxin/RNase 2 functions as an effective antiviral agent against respiratory syncytial virus. *J Infect Dis* 1998;**177**:1458–64.
38. Downes JJ, Wood DW, Striker TW, Haddad C. Acute respiratory failure in infants with bronchiolitis. *Anesthesiology* 1968;**29**:426–34.
39. Duppenthaler A, Gorgievski-Hrisoho M, Frey U, Aebi C. Two-year periodicity of respiratory syncytial virus epidemics in Switzerland. *Infection* 2003;**31**:75–80.
40. Ehlenfield DR, Cameron K, Welliver RC. Eosinophilia at the time of respiratory syncytial virus bronchiolitis predicts childhood reactive airway disease. *Pediatrics* 2000;**105**:79–83.
41. Eisen AH, Bacal HL. The relationship of acute bronchiolitis to bronchial asthma—a 4-to-14-year follow-up. *Pediatrics* 1963;**31**:859–61.
42. Empey DW, Laitinen LA, Jacobs L, et al. Mechanisms of bronchial hyperreactivity in normal subjects after upper respiratory tract infection. *Am Rev Respir Dis* 1976;**113**:131–9.
43. Esper F, Martinello RA, Boucher D, et al. A 1-year experience with human metapneumovirus in children aged <5 years. *J Infect Dis* 2004;**189**:1388–96.
44. Everard ML, Swarbrick A, Wrightham M, et al. Analysis of cells obtained by bronchial lavage of infants with respiratory syncytial virus infection. *Arch Dis Child* 1994;**71**:428–32.
45. Falsey AR, Walsh EE. Respiratory syncytial virus infection in adults. *Clin Microbiol Rev* 2000;**13**:371–84.
46. Feltes TF, Cabalka AK, Meissner HC, et al. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. *J Pediatr* 2003;**143**:532–40.
47. Flores G, Horwitz RI. Efficacy of beta2-agonists in bronchiolitis: a reappraisal and meta-analysis. *Pediatrics* 1997;**100**:233–9.
48. Fox GF, Everard ML, Marsh MJ, Milner AD. Randomised controlled trial of budesonide for the prevention of post-bronchiolitis wheezing. *Arch Dis Child* 1999;**80**:343–7.
49. Foy HM, Cooney MK, Maletzky AJ, Grayston JT. Incidence and etiology of pneumonia, croup and bronchiolitis in preschool

children belonging to a prepaid medical care group over a four-year period. *Am J Epidemiol* 1973;**97**:80–92.
50. Freitas GR, Silva DA, Yokosawa J, et al. Antibody response and avidity of respiratory syncytial virus-specific total IgG, IgG1, and IgG3 in young children. *J Med Virol* 2011;**83**:1826–33.
51. Gadomski AM, Aref GH, el Din OB, et al. Oral versus nebulized albuterol in the management of bronchiolitis in Egypt. *J Pediatr* 1994;**124**:131–8.
52. Garofalo R, Dorris A, Ahlstedt S, Welliver RC. Peripheral blood eosinophil counts and eosinophil cationic protein content of respiratory secretions in bronchiolitis: relationship to severity of disease. *Pediatr Allergy Immunol* 1994;**5**:111–7.
53. Garofalo R, Kimpen JL, Welliver RC, Ogra PL. Eosinophil degranulation in the respiratory tract during naturally acquired respiratory syncytial virus infection. *J Pediatr* 1992;**120**:28–32.
54. Garofalo RP, Patti J, Hintz KA, et al. Macrophage inflammatory protein-1α (not t helper type 2 cytokines) is associated with severe forms of respiratory syncytial virus bronchiolitis. *J Infect Dis* 2001;**184**:393–9.
55. Glezen WP, Loda FA, Clyde Jr WA, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
56. Glezen WP, Paredes A, Allison JE, et al. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *J Pediatr* 1981;**98**:708–15.
57. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986;**140**:543–6.
58. Green M, Brayer AF, Schenkman KA, Wald ER. Duration of hospitalization in previously well infants with respiratory syncytial virus infection. *Pediatr Infect Dis J* 1989;**8**:601–5.
59. Grewal S, Ali S, McConnell DW, et al. A randomized trial of nebulized 3% hypertonic saline with epinephrine in the treatment of acute bronchiolitis in the emergency department. *Arch Pediatr Adolesc Med* 2009;**163**:1007–12.
60. Griffith JPC, Mitchell AG. *The diseases of infants and children*. 2nd ed. Philadelphia: WB Saunders; 1927.
61. Groothuis JR, Simoes EA, Levin MJ, et al. Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children. The Respiratory Syncytial Virus Immune Globulin Study Group. *N Engl J Med* 1993;**329**:1524–30.
62. Guilbert TW, Morgan WJ, Zeiger RS, et al. Long-term inhaled corticosteroids in preschool children at high risk for asthma. *N Engl J Med* 2006;**354**:1985–97.
63. Gurwitz D, Mindorff C, Levison H. Increased incidence of bronchial reactivity in children with a history of bronchiolitis. *J Pediatr* 1981;**98**:551–5.
64. Hall CB, Hall WJ, Gala CL, et al. Long-term prospective study in children after respiratory syncytial virus infection. *J Pediatr* 1984;**105**:358–64.
65. Hall CB, McBride JT, Walsh EE, et al. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection: a randomized double-blind study. *N Engl J Med* 1983;**308**:1443–7.
66. Hall CB, Powell KR, Schnabel KC, et al. Risk of secondary bacterial infection in infants hospitalized with respiratory syncytial viral infection. *J Pediatr* 1988;**113**:266–71.
67. Hammer J, Numa A, Newth CJ. Albuterol responsiveness in infants with respiratory failure caused by respiratory syncytial virus infection. *J Pediatr* 1995;**127**:485–90.
68. Hampton SF, Johnson MC, Galakatos E. Studies of bacterial hypersensitivity in asthma, part I: the preparation of antigens of *Neisseria catarrhalis*, the induction of asthma by aerosols, the performance of skin and passive transfer tests. *J Allergy* 1963;**34**:63–95.
69. Heidema J, Lukens MV, van Maren WWC, et al. CD8+ T cell responses in bronchoalveolar lavage fluid and peripheral blood mononuclear cells of infants with severe primary respiratory syncytial virus infections. *J Immunol* 2007;**179**:8410–7.
70. Henderson AT, Rosenzweig S. Bronchiolitis in infancy: clinical study with special emphasis on the cardiac complications. *U S Armed Forces Med J* 1951;**2**:943–52.
71. Henderson FW, Clyde Jr WA, Collier AM, et al. The etiologic and epidemiologic spectrum of bronchiolitis in pediatric practice. *J Pediatr* 1979;**95**:183–90.
72. Henderson FW, Hu SC, Collier AM. Pathogenesis of respiratory syncytial virus infection in ferret and fetal human tracheas in organ culture. *Am Rev Respir Dis* 1978;**118**:29–37.
73. Henderson FW, Stewart PW, Burchinal MR, et al. Respiratory allergy and the relationship between early childhood lower respiratory illness and subsequent lung function. *Am Rev Respir Dis* 1992;**145**:283–90.
74. Henderson J, Hilliard TN, Sherriff A, et al. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol* 2005;**16**:386–92.
75. Heycock JB, Noble TC. 1,230 cases of acute bronchiolitis in infancy. *BMJ* 1962;**2**:879–81.
76. Hogg JC, Williams J, Richardson JB, et al. Age as a factor in the distribution of lower-airway conductance and in the pathologic anatomy of obstructive lung disease. *N Engl J Med* 1970;**282**:1283–7.
77. Holt LE, Howland J. Diseases of the lungs: peculiarities of the thorax in children. In: *Holt's diseases of infancy and childhood*. 11th ed. New York: D. Appleton-Century; 1940. p. 498–513.
78. Horn MEC, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967–1972. *J Hyg* 1975;**74**:157–68.
79. Hubble D, Osborn GR. Acute bronchiolitis in children. *BMJ* 1941;**1**:107–10.
80. Hughes DM, Lesouef PN, Landau LI. Effect of salbutamol on respiratory mechanics in bronchiolitis. *Pediatr Res* 1987;**22**:83–6.
81. Ipek IO, Yalcin EU, Sezer RG, Bozaykut A. The efficacy of nebulized salbutamol, hypertonic saline and salbutamol/hypertonic saline combination in moderate bronchiolitis. *Pulm Pharmacol Ther* 2011;**24**:633–7.
82. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Resp Crit Care* 2008;**178**:667–72.
83. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004;**10**:1095–101.
84. Johnson JE, Gonzales RA, Olson SJ, et al. The histopathology of fatal untreated human respiratory syncytial virus infection. *Mod Pathol* 2006;**20**:108–19.
85. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;**310**:1225–9.
86. Karron RA, Wright PF, Belshe RB, et al. Identification of a recombinant live attenuated respiratory syncytial virus vaccine candidate that is highly attenuated in infants. *J Infect Dis* 2005;**191**:1093–104.
87. Kattan M, Keens TG, Lapierre J-G, et al. Pulmonary function abnormalities in symptom-free children after bronchiolitis. *Pediatrics* 1977;**59**:683–8.
88. Kaul TN, Welliver RC, Ogra PL. Appearance of complement components and immunoglobulins on nasopharyngeal epithelial cells following naturally acquired infection with respiratory syncytial virus. *J Med Virol* 1982;**9**:149–58.
89. Kaul TN, Welliver RC, Wong DT, et al. Secretory antibody response to respiratory syncytial virus infection. *Am J Dis Child* 1981;**135**:1013–6.
90. Kerr MH, Paton JY. Surfactant protein levels in severe respiratory syncytial virus infection. *Am J Respir Crit Care Med* 1999;**159**:1115–8.
91. Kim CK, Choi J, Kim HB, et al. A randomized intervention of montelukast for post-bronchiolitis: effect on eosinophil degranulation. *J Pediatr* 2010;**156**:749–54.
92. Kim HW, Arrobio JO, Brandt CD, et al. Epidemiology of respiratory syncytial virus infection in Washington, D.C. I. Importance of the virus in different respiratory tract disease syndromes and temporal distribution of infection. *Am J Epidemiol* 1973;**98**:216–25.
93. Kim HW, Canchola JG, Brandt CD, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969;**89**:422–34.
94. Kim HW, Leikin SL, Arrobio J, et al. Cell-mediated immunity to respiratory syncytial virus induced by inactivated vaccine or by infection. *Pediatr Res* 1976;**10**:75–8.
95. Klassen TP, Rowe PC, Sutcliffe T, et al. Randomized trial of salbutamol in acute bronchiolitis. *J Pediatr* 1991;**118**:807–11.

96. Koch DA. Roentgenologic considerations of capillary bronchiolitis. *Am J Roentgenol Radium Ther Nucl Med* 1959;**82**:433–6.
97. Korppi M, Kotaniemi-Syrjanen A, Waris M, et al. Rhinovirus-associated wheezing in infancy: comparison with respiratory syncytial virus bronchiolitis. *Pediatr Infect Dis J* 2004;**23**:995–9.
98. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, et al. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? *J Allergy Clin Immunol* 2003;**111**:66–71.
99. Kusel MMH, de Klerk NH, Kebadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol* 2007;**119**:1105–10.
100. Kuzik BA, Al-Qadhi SA, Kent S, et al. Nebulized hypertonic saline in the treatment of viral bronchiolitis in infants. *J Pediatr* 2007;**151**:266–70, 270.e1.
101. Laing I, Reidel F, Yap PL, Simpson H. Atopy predisposing to acute bronchiolitis during an epidemic of respiratory syncytial virus. *BMJ* 1982;**284**:1070–2.
102. Leer Jr JA, Green JL, Heimlich EM, et al. Corticosteroid treatment in bronchiolitis: a controlled, collaborative study in 297 infants and children. *Am J Dis Child* 1969;**117**:495–503.
103. Lenney W, Milner AD. Alpha and beta adrenergic stimulants in bronchiolitis and wheezy bronchitis in children under 18 months of age. *Arch Dis Child* 1978;**53**:707–9.
104. Lesouef PN, Geelhoed GC, Turner DJ, et al. Response of normal infants to inhaled histamine. *Am Rev Respir Dis* 1989;**139**:62–6.
105. Loda FA, Clyde Jr WA, Glezen WP, et al. Studies on the role of viruses, bacteria, and *M. pneumoniae* as causes of lower respiratory tract infections in children. *J Pediatr* 1968;**72**:161–76.
106. Loda FA, Glezen WP, Clyde Jr WA. Respiratory disease in group day care. *Pediatrics* 1972;**49**:428–37.
107. Lukens MV, van de Pol AC, Coenjaerts FEJ, et al. A systemic neutrophil response precedes robust CD8+ T-cell activation during natural respiratory syncytial virus infection in infants. *J Virol* 2010;**84**:2374–83.
108. Macasaet FF, Kidd PA, Bolano CR, et al. The etiology of acute respiratory infections, part III: the role of viruses and bacteria. *J Pediatr* 1968;**72**:829–39.
109. Macklin CC. Alveolar pores and their significance in the human lung. *Arch Pathol* 1936;**21**:202.
110. Malley R, DeVincenzo J, Ramilo O, et al. Reduction of respiratory syncytial virus (RSV) in tracheal aspirates in intubated infants by use of humanized monoclonal antibody to RSV F protein. *J Infect Dis* 1998;**178**:1555–61.
111. Mallory MD, Shay DK, Garrett J, Bordley WC. Bronchiolitis management preferences and the influence of pulse oximetry and respiratory rate on the decision to admit. *Pediatrics* 2003;**111**:e45–51.
112. Mandelberg A, Tal G, Witzling M, et al. Nebulized 3% hypertonic saline solution treatment in hospitalized infants with viral bronchiolitis. *Chest* 2003;**123**:481–7.
113. Martinez FD, Morgan WJ, Wright AL, et al. Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. *N Engl J Med* 1988;**319**:1112–7.
114. Martinez FD, Stern DA, Wright AL, et al. Differential immune responses to acute lower respiratory illness in early life and subsequent development of persistent wheezing and asthma. *J Allergy Clin Immunol* 1998;**102**:915–20.
115. Martinez FD, Wright AL, Taussig LM, et al. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995;**332**:133–8.
116. McConnochie KM, Roghmann KJ. Bronchiolitis as a possible cause of wheezing in childhood: new evidence. *Pediatrics* 1984;**74**:1–10.
117. McConnochie KM, Roghmann KJ. Wheezing at 8 and 13 years: changing importance of bronchiolitis and passive smoking. *Pediatr Pulmonol* 1989;**6**:138–46.
118. McGeorge M. Severe obstructive bronchiolitis in infancy: treatment with hydrocortisone. *Clin Pediatr* 1964;**3**:11–8.
119. Merkus PJ, de Hoog M, van Gent R, de Jongste JC. DNase treatment for atelectasis in infants with severe respiratory syncytial virus bronchiolitis. *Eur Respir J* 2001;**18**:734–7.
120. Miller DG, Gabrielson MO, Horstmann DM. Clinical virology and viral surveillance in a pediatric group practice: the use of double-seeded tissue culture tubes for primary virus isolation. *Am J Epidemiol* 1968;**88**:245–56.
121. Miller EK, Lu X, Erdman DD, et al. Rhinovirus-associated hospitalizations in young children. *J Infect Dis* 2007;**195**:773–81.
122. Minor TE, Baker JW, Dick EC, et al. Greater frequency of viral respiratory infections in asthmatic children as compared with their nonasthmatic siblings. *J Pediatr* 1974;**85**:472–7.
123. Minor TE, Dick EC, Baker JW, et al. Rhinovirus and influenza type A infections as precipitants of asthma. *Am Rev Respir Dis* 1976;**113**:149–53.
124. Minor TE, Dick EC, DeMeo AN, et al. Viruses as precipitants of asthmatic attacks in children. *JAMA* 1974;**227**:292–8.
125. Mitchell I, Inglis JM, Simpson H. Viral infection as a precipitant of wheeze in children: combined home and hospital study. *Arch Dis Child* 1978;**53**:106–11.
126. Mlinaric-Galinovic G, Welliver RC, Vilibic-Cavlek T, et al. The biennial cycle of respiratory syncytial virus outbreaks in Croatia. *Virol J* 2008;**5**:18.
127. Moler FW, Bandy KP, Custer JR. Ribavirin therapy for acute bronchiolitis: need for appropriate controls. *J Pediatr* 1991;**119**:509–10.
128. Mufson MA, Krause HE, Mocega HE, Dawson FW. Viruses, *Mycoplasma pneumoniae* and bacteria associated with lower respiratory tract disease among infants. *Am J Epidemiol* 1970;**91**:192–202.
129. Mulholland EK, Olinsky A, Shann FA. Clinical findings and severity of acute bronchiolitis. *Lancet* 1990;**335**:1259–61.
130. Neilson KA, Yunis EJ. Demonstration of respiratory syncytial virus in an autopsy series. *Pediatr Pathol* 1990;**10**:491–502.
131. Nelson WE. Viral or probable viral infections. In: Nelson WE, editor. *Pediatrics*. 6th ed. Philadelphia: WB Saunders; 1954. p. 823–8, 1438–46.
132. Nelson WE, Smith LW. Generalized obstructive emphysema in infants. *J Pediatr* 1945;**26**:36–55.
133. Neuzil KM, Gruber WC, Chytil F, et al. Safety and pharmacokinetics of vitamin A therapy for infants with respiratory syncytial virus infections. *Antimicrob Agents Chemother* 1995;**39**:1191–3.
134. Nichol KP, Cherry JD. Bacterial-viral interrelations in respiratory infections of children. *N Engl J Med* 1967;**277**:667–72.
135. Ogilvie MM, Vathenen AS, Radford M, et al. Maternal antibody and respiratory syncytial virus infection in infancy. *J Med Virol* 1981;**7**:263–71.
136. Olofsson S, Brittain-Long R, Andersson LM, et al. PCR for detection of respiratory viruses: seasonal variations of virus infections. *Expert Rev Anti Infect Ther* 2011;**9**:615–26.
137. Oommen A, Lambert PC, Grigg J. Efficacy of a short course of parent-initiated oral prednisolone for viral wheeze in children aged 1-5 years: randomised controlled trial. *Lancet* 2003;**362**:1433–8.
138. Outwater KM, Crone RK. Management of respiratory failure in infants with acute viral bronchiolitis. *Am J Dis Child* 1984;**138**:1071–5.
139. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. The IMpact-RSV Study Group. *Pediatrics* 1998;**102**:531–7.
140. Panickar J, Lakhanpaul M, Lambert PC, et al. Oral prednisolone for preschool children with acute virus-induced wheezing. *N Engl J Med* 2009;**360**:329–38.
141. Parrott RH, Kim HW, Arrobio JO, et al. Epidemiology of respiratory syncytial virus infection in Washington, D.C., part II: infection and disease with respect to age, immunologic status, race and sex. *Am J Epidemiol* 1973;**98**:289–300.
142. Patel H, Platt RW, Pekeles GS, Ducharme FM. A randomized, controlled trial of the effectiveness of nebulized therapy with epinephrine compared with albuterol and saline in infants hospitalized for acute viral bronchiolitis. *J Pediatr* 2002;**141**:818–24.
143. Patel NR, Hammer J, Nichani S, et al. Effect of inhaled nitric oxide on respiratory mechanics in ventilated infants with RSV bronchiolitis. *Intensive Care Med* 1999;**25**:81–7.
144. Plint AC, Johnson DW, Patel H, et al. Epinephrine and dexamethasone in children with bronchiolitis. *N Engl J Med* 2009;**360**:2079–89.
145. Polmar SH, Robinson Jr LD. Minnefor AB: immunoglobulin E in bronchiolitis. *Pediatrics* 1972;**50**:279–84.
146. Pratt EL. Acute bronchiolitis in infants. *Med Clin North Am* 1944:1098–107.

147. Pullan CR, Hey EN. Wheezing, asthma, and pulmonary dysfunction 10 years after infection with respiratory syncytial virus in infancy. *BMJ* 1982;**284**:1665–9.
148. Purcell K, Fergie J. Concurrent serious bacterial infections in 912 infants and children hospitalized for treatment of respiratory syncytial virus lower respiratory tract infection. *Pediatr Infect Dis J* 2004;**23**:267–9.
149. Quinlan KP, Hayani KC. Vitamin A and respiratory syncytial virus infection: serum levels and supplementation trial. *Arch Pediatr Adolesc Med* 1996;**150**:25–30.
150. Rakes GP, Arruda E, Ingram JM, et al. Rhinovirus and respiratory syncytial virus in wheezing children requiring emergency care: IgE and eosinophil analyses. *Am J Respir Crit Care Med* 1999; **159**:785–90.
151. Randolph AG, Reder L, Englund JA. Risk of bacterial infection in previously healthy respiratory syncytial virus-infected young children admitted to the intensive care unit. *Pediatr Infect Dis J* 2004;**23**:990–4.
152. Reid L. Influence of the pattern of structural growth of lung on susceptibility to specific infectious diseases in infants and children. *Pediatr Res* 1977;**11**:210–5.
153. Reilly CM, Stokes Jr J, McClelland L, et al. Studies of acute respiratory illnesses caused by respiratory syncytial virus, part 3: clinical and laboratory findings. *N Engl J Med* 1961;**264**: 1176–82.
154. Respiratory syncytial virus infection: admissions to hospital in industrial, urban, and rural areas: report to the Medical Research Council Subcommittee on Respiratory Syncytial Virus Vaccines. *BMJ* 1978;**2**:796–8.
155. Reyes M, Eriksson M, Bennet R, et al. Regular pattern of respiratory syncytial virus and rotavirus infections and relation to weather in Stockholm, 1984–1993. *Clin Microbiol Infect* 1997;**3**: 640–6.
156. Richter H, Seddon P. Early nebulized budesonide in the treatment of bronchiolitis and the prevention of postbronchiolitic wheezing. *J Pediatr* 1998;**132**:849–53.
157. Roan Y, Galant SP. Decreased neutrophil beta-adrenergic receptors in the neonate. *Pediatr Res* 1982;**16**:591–3.
158. Rooney JC, Williams HE. The relationship between proved viral bronchiolitis and subsequent wheezing. *J Pediatr* 1971;**79**: 744–7.
159. Sanchez I, De Koster J, Powell RE, et al. Effect of racemic epinephrine and salbutamol on clinical score and pulmonary mechanics in infants with bronchiolitis. *J Pediatr* 1993;**122**: 145–51.
160. Sandiford BR, Spencer B. Respiratory syncytial virus in epidemic bronchiolitis of infants. *BMJ* 1962;**2**:881–2.
161. Schroeder AR, Marmor AK, Pantell RH, Newman TB. Impact of pulse oximetry and oxygen therapy on length of stay in bronchiolitis hospitalizations. *Arch Pediatr Adolesc Med* 2004;**158**: 527–30.
162. Scott LP 3rd, Gutelius MF, Parrott RH. Children with acute respiratory tract infections: an electrocardiographic survey. *Am J Dis Child* 1970;**119**:111–3.
163. Scott R, de Landazuri MO, Gardner PS, Owen JJ. Human antibody-dependent cell-mediated cytotoxicity against target cells infected with respiratory syncytial virus. *Clin Exp Immunol* 1977; **28**:19–26.
164. Seidenberg J, Masters IB, Hudson I, et al. Effect of ipratropium bromide on respiratory mechanics in infants with acute bronchiolitis. *Aust Paediatr J* 1987;**23**:169–72.
165. Sell SH. Some observations on acute bronchiolitis in infants. *Am J Dis Child* 1960;**100**:7–15.
166. Shaw KN, Bell LM, Sherman NH. Outpatient assessment of infants with bronchiolitis. *Am J Dis Child* 1991;**145**:151–5.
167. Shay DK, Holman RC, Newman RD, et al. Bronchiolitis-associated hospitalizations among US children, 1980–1996. *JAMA* 1999;**282**:1440–6.
168. Shay DK, Holman RC, Roosevelt GE, et al. Bronchiolitis-associated mortality and estimates of respiratory syncytial virus–associated deaths among US children, 1979–1997. *J Infect Dis* 2001;**183**:16–22.
169. Simpson H, Matthew DJ, Inglis JM, George EL. Virological findings and blood gas tensions in acute lower respiratory tract infections in children. *BMJ* 1974;**2**:629–32.
170. Sims DG, Downham MA, Gardner PS, et al. Study of 8-year-old children with a history of respiratory syncytial virus bronchiolitis in infancy. *BMJ* 1978;**1**:11–4.
171. Sims DG, Gardner PS, Weightman D, et al. Atopy does not predispose to RSV bronchiolitis or postbronchiolitic wheezing. *BMJ* 1981;**282**:2086–8.
172. Skoner DP, Fireman P, Caliguiri L, Davis H. Plasma elevations of histamine and a prostaglandin metabolite in acute bronchiolitis. *Am Rev Respir Dis* 1990;**142**:359–64.
173. Smith DW, Frankel LR, Mathers LH, et al. A controlled trial of aerosolized ribavirin in infants receiving mechanical ventilation for severe respiratory syncytial virus infection. *N Engl J Med* 1991;**325**:24–9.
174. Smith MC, Creutz C, Huang YT. Detection of respiratory syncytial virus in nasopharyngeal secretions by shell vial technique. *J Clin Microbiol* 1991;**29**:463–5.
175. Smith TF, McIntosh K, Fishaut M, Henson PM. Activation of complement by cells infected with respiratory syncytial virus. *Infect Immun* 1981;**33**:43–8.
176. Stein RT, Sherrill D, Morgan WJ, et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999;**354**:541–5.
177. Stempel DA, Clyde Jr WA, Henderson FW, Collier AM. Serum IgE levels and the clinical expression of respiratory illnesses. *J Pediatr* 1980;**97**:185–90.
178. Stockman LJ, Curns AT, Anderson LJ, Fischer-Langley G. Respiratory syncytial virus–associated hospitalizations among infants and young children in the United States, 1997-2006. *Pediatr Infect Dis J* 2012;**31**:5–9.
179. Stokes GM, Milner AD, Hodges IG, Groggins RC. Lung function abnormalities after acute bronchiolitis. *J Pediatr* 1981;**98**:871–4.
180. Strope GL, Stewart PW, Henderson FW, et al. Lung function in school-age children who had mild lower respiratory illnesses in early childhood. *Am Rev Respir Dis* 1991;**144**:655–62.
181. Tager IB, Weiss ST, Munoz A, et al. Longitudinal study of the effects of maternal smoking on pulmonary function in children. *N Engl J Med* 1983;**309**:699–703.
182. Tal A, Bavilski C, Yohai D, et al. Dexamethasone and salbutamol in the treatment of acute wheezing in infants. *Pediatrics* 1983;**71**: 13–8.
183. Tepper RS, Rosenberg D, Eigen H. Airway responsiveness in infants following bronchiolitis. *Pediatr Pulmonol* 1992;**13**:6–10.
184. Terletskaia-Ladwig E, Enders G, Schalasta G, Enders M, et al. Defining the timing of respiratory syncytial virus (RSV) outbreaks: an epidemiological study. *BMC Infect Dis* 2005;**5**:20.
185. Thomas EE, Book LE. Comparison of two rapid methods for detection of respiratory syncytial virus (RSV) (Testpack RSV and ortho RSV ELISA) with direct immunofluorescence and virus isolation for the diagnosis of pediatric RSV infection. *J Clin Microbiol* 1991;**29**:632–5.
186. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med* 2009;**179**:1091–7.
187. Tibby SM, Hatherill M, Wright SM, et al. Exogenous surfactant supplementation in infants with respiratory syncytial virus bronchiolitis. *Am J Respir Crit Care Med* 2000;**162**:1251–6.
188. Tristram DA, Welliver RC, Mohar CK, et al. Immunogenicity and safety of respiratory syncytial virus subunit vaccine in seropositive children 18-36 months old. *J Infect Dis* 1993;**167**:191–5.
189. van Schaik SM, Tristram DA, Nagpal IS, et al. Increased production of IFN-gamma and cysteinyl leukotrienes in virus-induced wheezing. *J Allergy Clin Immunol* 1999;**103**:630–6.
190. Volovitz B, Welliver RC, De Castro G, et al. The release of leukotrienes in the respiratory tract during infection with respiratory syncytial virus: role in obstructive airway disease. *Pediatr Res* 1988;**24**:504–7.
191. Voter KZ, Henry MM, Stewart PW, Henderson FW. Lower respiratory illness in early childhood and lung function and bronchial reactivity in adolescent males. *Am Rev Respir Dis* 1988;**137**: 302–7.
192. Wainwright C, Altamirano L, Cheney M, et al. A multicenter, randomized, double-blind, controlled trial of nebulized epinephrine in infants with acute bronchiolitis. *N Engl J Med* 2003;**349**: 27–35.

193. Wang EE, Milner RA, Navas L, Maj H. Observer agreement for respiratory signs and oximetry in infants hospitalized with lower respiratory infections. *Am Rev Respir Dis* 1992;**145**:106–9.
194. Waris M. Pattern of respiratory syncytial virus epidemics in Finland: two-year cycles with alternating prevalence of groups A and B. *J Infect Dis* 1991;**163**:464–9.
195. Welliver RC, Duffy L. The relationship of RSV-specific immunoglobulin E antibody responses in infancy, recurrent wheezing, and pulmonary function at age 7-8 years. *Pediatr Pulmonol* 1993; **15**:19–27.
196. Welliver RC, Kaul TN, Ogra PL. The appearance of cell-bound IgE in respiratory-tract epithelium after respiratory-syncytial-virus infection. *N Engl J Med* 1980;**303**:1198–202.
197. Welliver RC, Kaul TN, Putnam TI, et al. The antibody response to primary and secondary infection with respiratory syncytial virus: kinetics of class-specific responses. *J Pediatr* 1980;**96**: 808–13.
198. Welliver RC, Wong DT, Middleton Jr E, et al. Role of parainfluenza virus-specific IgE in pathogenesis of croup and wheezing subsequent to infection. *J Pediatr* 1982;**101**:889–96.
199. Welliver RC, Wong DT, Sun M, et al. The development of respiratory syncytial virus–specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N Engl J Med* 1981; **305**:841–6.
200. Welliver TP, Garofalo RP, Hosakote Y, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis* 2007;**195**:1126–36.
201. Williams H, McNicol KN. Prevalence, natural history, and relationship of wheezy bronchitis and asthma in children: an epidemiological study. *BMJ* 1969;**4**:321–5.
202. Wittig HJ, Glaser J. The relationship between bronchiolitis and childhood asthma; a follow-up study of 100 cases of bronchiolitis. *J Allergy* 1959;**30**:19–23.
203. Wohl ME, Chernick V. State of the art: bronchiolitis. *Am Rev Respir Dis* 1978;**118**:759–81.
204. Wohl ME, Stigol LC, Mead J. Resistance of the total respiratory system in healthy infants and infants with bronchiolitis. *Pediatrics* 1969;**43**:495–509.
205. Wood SH, Buddingh GJ, Abberger Jr BF. An inquiry into the etiology of acute bronchiolitis of infants. *Pediatrics* 1954;**13**: 363–72.
206. Woolcock AJ, Leeder SR, Peat JK, Blackburn CR. The influence of lower respiratory illness in infancy and childhood and subsequent cigarette smoking on lung function in Sydney schoolchildren. *Am Rev Respir Dis* 1979;**120**:5–14.
207. Wright AL, Holberg C, Martinez FD, Taussig LM. Relationship of parental smoking to wheezing and nonwheezing lower respiratory tract illnesses in infancy. Group Health Medical Associates. *J Pediatr* 1991;**118**:207–14.
208. Wright AL, Taussig LM, Ray CG, et al. The Tucson Children's Respiratory Study, part II: lower respiratory tract illness in the first year of life. *Am J Epidemiol* 1989;**129**:1232–46.
209. Young S, Le Souef PN, Geelhoed GC, et al. The influence of a family history of asthma and parental smoking on airway responsiveness in early infancy. *N Engl J Med* 1991;**324**:1168–73.
210. Yusuf S, Piedimonte G, Auais A, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. *Epidemiol Infect* 2007;**135**:1077–90.
211. Zweiman B, Schoenwetter WF, Pappano Jr JE, et al. Patterns of allergic respiratory disease in children with a past history of bronchiolitis. *J Allergy Clin Immunol* 1971;**48**:283–9.

CHAPTER 22

Pediatric Community-Acquired Pneumonia

Carrie L. Byington • John S. Bradley

Community-acquired pneumonia (CAP) is the most important cause of death in children worldwide.[157] The World Health Organization estimates 1.4 million deaths in children younger than 5 years occur each year, accounting for almost 1 in every 5 deaths in this age group.[167] This number of deaths exceeds that of acquired immunodeficiency syndrome (AIDS), malaria, and tuberculosis combined. In the developed world, CAP results in hundreds of thousands of medical encounters and hospitalizations. In the United States, influenza and pneumonia remain an important cause of death, accounting for more than 50,000 deaths in 2010, including several hundred deaths in children.[125]

CAP is an acute lung infection that results most commonly from viral or bacterial pathogens. Less typical pathogens, including mycobacteria and fungi, also may cause pneumonia, especially in immunocompromised hosts. These infections are discussed in detail in Chapter 97 and the chapters discussing fungal infections.

In the United States, the Pediatric Infectious Diseases Society (PIDS) in collaboration with the Infectious Disease Society of America (IDSA), published guidelines in 2011 for management of CAP in infants and children older than 3 months.[26] These guidelines define CAP as the presence of signs and symptoms of pneumonia in a previously healthy child caused by an infection acquired outside of the hospital.[26] The diagnostic, management, and prevention strategies discussed in this chapter are aligned with these published guidelines.

ETIOLOGY

Because of the difficulty of documenting the microbiology of pneumonia in infants and young children, accurate data concerning the incidence and specific agents of bacterial and viral pneumonia in children are lacking. Although up to a third of CAP is thought to be due to bacterial pathogens, nonbacterial pneumonias remain the most frequent pulmonary infections encountered in pediatrics. Etiologic studies conducted between the 1970s and 1990s indicated that two bacterial pathogens were responsible for the majority of fatal cases of childhood pneumonia—*Streptococcus pneumoniae* and *Haemophilus influenzae* type b.[64,102] In the intervening years, effective protein conjugate vaccines (PCVs) that protect against *H. influenzae* type b and *S. pneumoniae* have been introduced into the vaccine programs of many countries, including the United States. In addition, diagnostic technology has advanced and molecular testing for bacterial and viral pathogens is changing our understanding of the etiology of childhood pneumonia.

Two large studies are currently underway to better document the etiology of childhood pneumonia. In the United States, the Centers for Disease Control and Prevention (CDC) is conducting the Etiology of Pneumonia in the Community (EPIC) study. It is a multicenter study with enrollment of children in Tennessee and Utah. The study participants are children younger than 18 years who are hospitalized with pneumonia and are evaluated using conventional microbiologic methods, serology, and molecular diagnostics for viral and bacterial pathogens. Preliminary data indicate that viral pathogens are the most common pathogens in children with hospitalized CAP, identified in approximately 75 percent of cases.[81] Of viral pathogens, rhinovirus is most commonly detected, identified in 37 percent of children as a single pathogen or copathogen. Children with CAP and rhinovirus have higher viral loads than healthy children with rhinovirus, although the interpretation of a positive rhinovirus test in children with CAP is evolving.[146] Respiratory syncytial virus (RSV) is the next most commonly detected, identified in 25 percent of children, usually as a sole pathogen. Other important viruses include adenovirus detected in approximately 15 percent of hospitalized cases, human metapneumovirus (hMPV) and parainfluenza detected in about 10 percent of cases, and coronaviruses and influenza viruses, detected in around 5 percent of children with hospitalized CAP.[81] Bacterial infections are less common, identified in 12 percent of children hospitalized with CAP.[81] When bacterial pathogens are recovered through culture or polymerase chain reaction (PCR) of blood or pleural fluid, *S. pneumoniae* and group A streptococcus are important bacterial causes of CAP in children, particularly CAP complicated by empyema.[9] *H. influenzae* type b was only rarely identified, although the majority of children were immunized. In addition, viral pathogens including rhinovirus, RSV, adenovirus, hMPV, coronaviruses, and influenza A and B are often identified in combination with bacteria in children hospitalized with CAP.[9]

Internationally, the Bill and Melinda Gates Foundation is sponsoring the Pneumonia Etiology Research for Child Health (PERCH) project. This case-control study involves seven countries in two geographic regions, Africa and Asia, where most cases of fatal childhood pneumonia

occur. PERCH sites include Bangladesh, Gambia, Kenya, Mali, South Africa, Thailand, and Zambia.[102] The study evaluated children 1 to 59 months and uses conventional and molecular methods to identify an etiology for pneumonia. Preliminary data from Kenya demonstrate an etiology in more than 75 percent of cases, with bacteria identified in 9 percent (most commonly *S. pneumoniae*), viruses in 53 percent, and mixed viral and bacterial infection in 15 percent.[74] An additional pilot study conducted in New Caledonia as part of the PERCH trial identified a pathogen in 89 percent of 108 hospitalized cases, with viruses representing more than 80 percent of the pathogens detected, similar to the findings of the EPIC study in the United States.[117]

Viral Pathogen

Of the viral pathogens, RSV generally is accepted as the agent found most frequently in pediatric pneumonias, particularly those associated with bronchiolitis. Although infection with this virus is quite common in all age groups, lower respiratory tract involvement is especially prominent in infancy.

hMPV, a newly identified paramyxovirus, was first described in children with upper and lower respiratory tract infection in the Netherlands in 2001.[153] Nearly 100 percent of children have serologic evidence of hMPV infection by age 5 years approximately 5 to 10 percent of children with CAP.[103,163,165] The clinical symptoms in infected children resemble those caused by RSV and influenza and may result in severe pneumonia.[153] A study conducted in Utah from 2006 to 2011 noted that the incidence of hospitalization for hMPV was highest in infants younger than 1 year of age (238/100,000) and lowest among children 5 to 17 years of age (6/100,000); 14 percent required intensive care admission, and 5 percent required mechanical ventilation.[47]

The three parainfluenza viruses (types 1, 2, and 3) are also commonly identified as pathogens in lower respiratory tract disease in infants and younger children. Parainfluenza virus type 3 is the agent most frequently found in pneumonia[67,68] and has been reported as an important pathogen with significant mortality in pediatric cancer patients with lower respiratory tract infection.[108]

Influenza A and B viruses are not as prevalent overall as RSV and parainfluenza viruses, but during periods of epidemic or pandemic spread, they may become predominant isolates in children with lower respiratory tract disease.[14,57] Infection with influenza viruses predisposes children to bacterial pneumonia and empyema, particularly as a result of *S. pneumoniae* and *Staphylococcus aureus*.* The threat of a pandemic of virulent influenza A, secondary to a recombination event resulting in a "human-adapted" avian or swine flu virus, keeps influenza in the forefront of concern for global public health agencies.[66]

Adenoviruses commonly are detected in children with CAP, particularly those who are hospitalized.[27,81,95] The overall impact of these viruses in the origin of nonbacterial pneumonia in children probably is somewhat less than that of the aforementioned agents; however, many fatal illnesses have been reported. Their common asymptomatic carriage and potential for endogenous activation by unrelated illnesses can render causation difficult to prove.[62] Of the 51 known adenoviruses, types 1, 2, 3, 4, 5, 7, 14, 21, and 35 clearly have been associated with pneumonia.[27,87] In certain populations, such as the Maori, Native Americans, and Inuit, adenoviruses commonly produce severe infection.[78,91] Adenovirus infections also have been reported in military recruits, some with fatal outcomes.[31,41,152,155]

Evidence to support the role of rhinoviruses in pneumonia is increasing, although upper respiratory tract infection with the multiple serotypes of these organisms is common. Some degree of lower respiratory tract involvement by rhinoviruses is indicated by the documented role of these viruses in bronchiolitis and exacerbations of asthma.[82] Rhinovirus C has been implicated in severe pneumonia in children and neonates.[30,38,92]

Coronaviruses HCo-OC43 and HCo-229E have been implicated as causes of pneumonia since the 1960s, but until recently, this family of viruses was considered a rare cause of human disease.[86,115] The worldwide epidemic of severe acute respiratory syndrome (SARS) that occurred in 2002 to 2004 focused new interest on these pathogens and led to a new appreciation of their reservoirs in domestic animals and their potential to cause severe pneumonia and respiratory failure.* Two other strains of coronavirus, HCo-NL63[154] and HCo-HKU1,[166] have been discovered more recently. They appear to be among the less common causes of lower respiratory tract infections, but their clinical manifestations are similar to those caused by the other common respiratory viruses.[55,135]

The respiratory virus most recently described that can cause pneumonia is HBoV.[4] It is a parvovirus closely related to bovine parvovirus and canine parvovirus. (The "bo" in "bocavirus" derives from "bovine" and the "ca" from canine.) It was identified using a novel technique based on amplification of nonspecific viral nucleotide sequences, a method that holds promise for identification of other previously uncultivated human viral pathogens. Because only a few population-based studies have been performed, the contribution of HBoV to the overall epidemiology of pediatric pneumonia remains uncertain. HBoV has been identified in respiratory specimens, including sputum and bronchoalveolar lavage in children with acute respiratory tract infection,[19,26,76,123] and was found to be a relatively common (19%) finding in a Finnish study of children with asthma exacerbations.[3] However, with bocavirus being detected far more often with another viral pathogen, rather than as a sole agent,[55] with documentation of asymptomatic respiratory tract "colonization" with bocavirus,[111] and prolonged excretion even after symptoms have resolved, it is difficult to accurately assess bocavirus-attributable lower respiratory tract morbidity.

Bacterial Pathogens

S. pneumoniae remains an important cause of bacterial CAP in children beyond the neonatal period in both

*References 10, 11, 33, 43, 61, 70, 84, 85, 136.

*References 52, 89, 98, 134, 154, 166.

TABLE 22-1 **Etiology of Community-Acquired Pneumonia by Age**

<3 Months	3 Months–5 Years	>5 Years
Viral Pathogens		
RSV	RSV	Influenza
Influenza	Influenza	Adenovirus
Parainfluenza	Parainfluenza	Human metapneumovirus
Human metapneumovirus	Human metapneumovirus	
Bacterial Pathogens		
Group B streptococcus	*Streptococcus pneumoniae*	*S. pneumoniae*
Gram-negative bacilli	*Mycoplasma pneumoniae*	*M. pneumoniae*
S. pneumoniae	*Staphylococcus aureus*	*S. aureus*
Bordetella pertussis	Group A streptococcus	Group A streptococcus
Chlamydia trachomatis	*Haemophilus influenzae* type b*	*H. influenzae* type b*

* In unimmunized infants.

immunized and unimmunized populations. In immunized populations, the serotype distribution changed significantly after the introduction of pneumococcal PCVs. After the introduction of the PCV7 in the United States, serotypes 3, 7F, and 19A emerged as important causes of CAP and complicated pneumonia.* Worldwide, serotype 1 also is often associated with complicated pneumonia.[20,36,54,129,131] These serotypes are included in the PCV13 formulation, and in 2010, the first year after introduction of PCV13 in the United States, further decreases in invasive pneumococcal disease, including pneumonia, have been reported.[42,44]

The availability of molecular diagnostics is changing our understanding of CAP and demonstrating the presence of both viral and bacterial pathogens in children with pneumonia is common.[23,72] Further, the developing immune system from the newborn to adolescent and immunization status of children also influences the pathogens responsible for CAP in different age groups. Table 22-1 provides information regarding common pathogens seen in CAP in different age groups. Knowledge of regional data regarding pneumonia etiology, particularly the likelihood of drug-resistant pneumococci and methicillin-resistant *S. aureus* (MRSA), is vital for decision making regarding diagnostic testing and treatment.

EPIDEMIOLOGY

In the developed world, CAP remains a common cause of pediatric hospitalization and ambulatory medical visits. Age is an important determinant of disease rates, with younger children more likely to have CAP and be hospitalized. In the United States, hospitalization rates for CAP ranged from a high of 1169/100,000 for infants younger than 1 year to a low of 44.7/100,000 for children 13 to 18 years of age.[94] Children 1 to 5 years had rates of 383/100,000 and those 6 to 12 years had rates of 69/100,000.[94] Race is also an important factor in CAP. Black children have higher rates of hospitalization for CAP in contrast to white children; however, the difference has decreased since the introduction of pneumococcal conjugate vaccines.[94] Immune status is also relevant when identifying risk for CAP. Children with congenital and acquired immune deficiency, including human immunodeficiency virus (HIV) infection, are at increased risk for lower respiratory tract infection caused by both common[7,127] and uncommon respiratory pathogens, which are described in more detail in the chapters on immunocompromised hosts.

The epidemiology of CAP in the United States changed significantly after the licensure of the *H. influenzae* type b and pneumococcal conjugate vaccines. Overall, rates of invasive pneumonia have decreased in children younger than 5 years, the age group targeted for pneumococcal conjugate vaccines.[132] Decreases also have been observed in adults and suggest a likely benefit from herd immunity.[132]

PATHOGENESIS

The pathogenesis and host immune response differs significantly among viral, bacterial, and mycoplasma pathogens.[65,138,156] Detailed information regarding pathogen-specific disease can be found in chapters describing each pathogen.

For viral pneumonia, the pathological processes associated with infection by RSV, adenoviruses, and influenza virus A have been studied most extensively in humans, aided by animal models for each pathogen.[49] For most viral lower respiratory tract pathogens, inoculation of the child starts with the upper respiratory tract after exposure to infectious secretions from others through sneezing or coughing or from fomites. Once in the lower respiratory tract, viral agents proliferate and spread by contiguity to involve the lower and more distal portions of the respiratory tract. Infected epithelium loses ciliary function, rounds up as a result of injury, and sloughs into the air passages, with subsequent stasis of mucus and accumulation of cellular and inflammatory debris. When infection

*References 17, 35, 36, 46, 69, 79.

extends to the terminal airways, alveolar lining cells themselves will lose structural integrity and function. As a result, surfactant production may be lost, hyaline membranes form, and pulmonary edema may develop. Mononuclear cells infiltrate submucosal and interstitial structures, further contributing to tissue edema, narrowing of air passages, and alveolar-capillary block of gas exchange.[83] With certain pathogens, particularly RSV, bronchiolar spasm in the context of accumulating airway debris gives rise to ball-valve–mediated air trapping with each inspiration, resulting in hyperinflation. Complete obstruction of an airway by debris may result in distal atelectasis. Ventilation-perfusion mismatch may add to hypoxemia that results from alveolar edema.

Histopathologic descriptions of the viral pathology can include acute bronchiolitis, necrotizing bronchiolitis, interstitial pneumonia, alveolar pneumonia, and hemorrhagic bronchopneumonia. Acute bronchiolitis is characterized by relatively superficial and reversible destruction of ciliated respiratory epithelium, along with accompanying mononuclear infiltration in submucosal tissues. Necrotizing bronchiolitis extends to the deeper submucosal layers lining the respiratory tract and may not be as readily reversible. This destructive pathology is associated particularly with adenoviral pneumonia.[130]

Interstitial pneumonia is a diffuse process in which the inflammatory mononuclear response predominantly involves the peribronchial alveolar septa. In alveolar pneumonia, the alveoli are filled with degenerating lining cells and mononuclear or polymorphonuclear inflammatory cells with or without hyaline membranes and may reflect bacterial superinfection, acute respiratory distress syndrome, or nonspecific changes associated with mechanical ventilation and oxygen toxicity. Hemorrhagic bronchopneumonia has been described in a fatal pediatric case of hMPV lower respiratory tract infection.[51] When hyaline membranes are present, the process is described as diffuse alveolar damage, the histopathologic hallmark of acute respiratory distress syndrome. Acute bronchiolitis and interstitial pneumonia have classically been observed in most cases of fatal viral pneumonia, regardless of the infecting pathogen.[1]

Both viral replication and age-specific immune responses contribute to the severity of RSV disease in infants. It appears that the severity of RSV in infants relates to a vigorous innate immune response and inadequate adaptive immune response to viral replication and destruction of the respiratory tract epithelium. RSV infection initiates tremendous innate inflammation through both chemokines and cytokines, after stimulation of airway epithelial cells and phagocytic cells, as reviewed recently.[161,162]

Three important factors that influence the pathologic expression of nonbacterial pneumonia in children are anatomy, preexisting pulmonary disease, and immunity. In young infants, the small caliber of the terminal airways and the absence of interconnections between alveolar spaces (pores of Kohn) contribute to the development of wheezing and lobular atelectasis.[164] Preexisting pulmonary disease (e.g., bronchopulmonary dysplasia) and an inability to clear the excessive secretions triggered by infection in patients with bronchopulmonary dysplasia also may lead to bronchospasm, atelectasis, and respiratory failure.

The most important factors in the pathogenesis of bacterial pneumonias are the virulence of the pathogen, the absence of specific humoral immunity, and the presence of a preceding viral respiratory tract infection. Most bacterial pneumonias are a result of colonization of the nasopharynx, followed by either bacteremia or aspiration of organisms. The lung is protected from bacterial infection by a variety of mechanisms, including entrapment and removal of organisms deposited on airways by ciliated epithelium and mucus, ingestion and killing of airway bacteria by alveolar macrophages, by reticuloendothelial system function for organisms gaining access to the lung via the bloodstream, by neutralization of invading bacteria by local and systemic nonspecific innate and specific immune substances (i.e., specific antibody, complement, opsonins), and by removal of invading organisms from the lung by lymphatic drainage. Pulmonary infection may occur when one or more of these barriers are altered, inhibited, or overwhelmed.

Animal models document that the inflammatory responses in the lung may be caused by various bacterial cell wall components, including peptidoglycans, lipoteichoic acid (of gram-positive organisms), and lipopolysaccharides (endotoxin) of gram-negative bacteria. Bacterial components stimulate a profound innate inflammatory response, initiated in part by activation of pattern-recognition receptors (most notably Toll-like receptors [TLRs]), followed by an intense inflammatory cascade mediated by cytokines and chemokines that results in upregulation of cell surface adhesion molecules in addition to multiple intracellular pathways.[37,65]

As classically described, pneumonia caused by *S. pneumoniae* begins with acute inflammation and hyperemia of the lower respiratory tract mucosa, exudation of edema fluid, deposition of fibrin, and infiltration of alveoli by polymorphonuclear leukocytes (i.e., "red hepatization"), followed by predominance of fibrin deposition and macrophage activity (i.e., "white hepatization"). Resolution subsequently occurs with absorption of exudates and return of lung morphology and physiology to normal. In contrast, when the pneumonia is caused by organisms capable of inciting even more profound tissue inflammation (e.g., *S. aureus*, gram-negative enteric bacilli or pseudomonads), destruction of tissue, and formation of abscesses frequently occur.

Recent animal data provide a scientific basis for the observation that signs and symptoms of a viral respiratory tract infection frequently precede development of bacterial pneumonia. The respiratory virus may act by both destruction of respiratory epithelium and upregulation of functional bacterial cell surface antigen receptor molecules.[112,113] Staphylococcal or pneumococcal pneumonia may occur during or shortly after infection caused by influenza virus. Severe pneumococcal pneumonia has been associated with outbreaks of influenza, and increased mortality rates from pneumonia occur during epidemics of influenza, as documented from new postmortem studies of fatalities during the influenza pandemic of 1918.[119,143] As indirect proof of the role of preceding viral infection, the use of conjugate pneumococcal vaccines

appears to have decreased some of the lower respiratory tract morbidity seen with influenza infection.[88] Further, the conjugate vaccines were shown to reduce chest radiograph–defined alveolar consolidation associated with RSV by 12 percent, with parainfluenza types 1 to 3 by 44 percent, and with hMPV by 40 percent, suggesting that concurrent pneumococcal infection was frequent in virus-associated pneumonias. By documenting the decrease of bacterial pneumonia in immunized patients, the conjugate pneumococcal vaccine has been valuable in identifying the role of viral and pneumococcal coinfection.[106,107] Immune compromise, anatomic defects, and neurologic comorbidities may predispose children to recurrent lower respiratory tract infection, but a detailed discussion of these risk factors is beyond the scope of this chapter.

Mycoplasma is primarily a mucosal pathogen, attaching extracellularly to the epithelial cells of the host airways. Attachment leads to the generation of an inflammatory response with production of cytokines with subsequent recruitment of lymphocytes and neutrophils to the airway mucosa, leading to the production of inflammatory infiltrates within the airways. *Mycoplasma* attaches to airway cells by means of unique attachment organelles, resulting in cytopathic effects on ciliated epithelium of bronchi and bronchioles that can include loss of cilia, vacuolization, loss of metabolic function, and ultimately cell death. Edema with bronchiolar and alveolar inflammatory infiltrates containing macrophages, lymphocytes, neutrophils, plasma cells have been described. Diffuse alveolar damage also has been reported.[16]

The nonpermanent immunity that develops with natural infection provides a vigorous cell-mediated immune response that, on reexposure to *Mycoplasma*, may lead to more severe clinical illness and pulmonary injury. This concept of immune-mediated lung disease provides the basis for increasing pulmonary disease severity with increasing age, which correlates with observed, specific cell-mediated immunity that also increases with age.[12,96]

CLINICAL MANIFESTATIONS

The clinical presentation of a child with pneumonia is most often based on symptoms directly related to decreased oxygenation of blood (hypoxemia), as well as symptoms directly related to lung inflammation, characteristically caused by a pathogen in a particular age group (from very young infants to adolescents). A benign viral lower respiratory tract infection caused by rhinovirus may produce only a cough, with no tachypnea, or clinical toxicity. In contrast, a bacterial pneumonia caused by MRSA, may result in tachypnea, dyspnea with grunting respirations and retractions, cyanosis, high fever, hypotension, and altered mental status. Detailed, pathogen-specific clinical presentations are provided within the chapters discussing each pathogen.

Of importance is the recent recognition of the high frequency of coinfections caused by both a virus and bacteria or caused by multiple viruses. In these situations, the clinical presentation and progression of disease may be an amalgamation of two or more pathogens. The association of influenza virus infection with subsequent bacterial pneumonia is perhaps the best studied. In these situations, the first signs of infection are those of viral disease, with coryza and sore throat, but subsequently develop into respiratory failure that is associated with consolidative pneumonia or necrotizing pneumonia that is characteristic of *S. pneumoniae* or *S. aureus*.

Clinical manifestations of hypoxemia in children may occur after infection by either viruses or bacteria. Although these clinical presentations have been best studied in the developing world, the pathogens responsible for lower respiratory tract infection, particularly bacterial pathogens, are seldom identified in these studies, making pathogen-specific descriptions of disease presentation or clinical course difficult. Regardless of pathogen, however, those with more severe hypoxemia will have various degrees of respiratory distress (Table 22-2).

Whereas the older child and adolescent can verbalize the sensation of dyspnea, the younger child or infant may present with hypoxemia with increased respiratory effort and decreased level of attentiveness, decreased consolability, poor color, and decreased spontaneous movement, which has been best described in young infants with bronchiolitis.[110] In resource-poor areas of the world, cyanosis has been documented to be associated with severe hypoxia. A systematic review of published studies, primarily in the developing world, found that cyanosis and use of accessory muscles associated with the head tilting downward with each inspiration had a higher specificity for predicting hypoxemia in children than other signs, but were not sufficiently sensitive to be able to be used in the clinical diagnosis of hypoxemia.[13]

Tachypnea is a nonspecific clinical sign that may be a result of fever, anxiety, metabolic acidosis, or the hypoxemia that accompanies respiratory failure. Whereas in the developing world, rapid breathing as perceived by the mother, as well as chest retractions, nasal flaring, and crepitations were statistically associated with hypoxemia, the sensitivity and specificity of tachypnea in diagnosing hypoxemia were both only about 70 to 75 percent in contrast to that of pulse oximetry.[105] Likewise, in a recent study from a pediatric emergency department in Boston of children younger than 5 years of age undergoing chest radiography for possible pneumonia, the respiratory rates

TABLE 22-2 **Criteria for Respiratory Distress**

Signs of Respiratory Distress in Children with Pneumonia

1. Tachypnea*
   0-2 mo: RR >60
   2-12 mo: RR >50
   1-5 yr: RR >40
   >5 yr: RR >20
2. Dyspnea
3. Retractions (suprasternal, intercostals, or subcostal)
4. Grunting
5. Nasal flaring
6. Apnea
7. Altered mental status
8. Pulse oximetry <90% on room air

*Adapted from World Health Organization criteria.

for those with documented pneumonia by chest radiograph were not statistically higher than those whose chest radiographs were normal.[140] However, in the developing world, a high, age-specific respiratory rate (tachypnea), particularly when measured at 24 hours into antimicrobial therapy (amoxicillin or penicillin G), has been linked to clinical treatment failure in children with severe pneumonia.[109]

In the developed world, realizing that clinical presentation of pneumonia is neither sensitive nor specific for the diagnosis, pulse oximetry is widely used as the basis for management decisions, to confirm clinical suspicions of pneumonia with hypoxemia. Pulse oximetry technology is widely available, accurate in most settings, and simple to perform and has the ability to quantitate the severity of lung disease. Hypoxemia is a risk factor for poor outcome in both adults and children with lower respiratory tract infection. Although pulse oximetry is used together with clinical assessment, recommendations have been made to hospitalize a child whose Spo_2 is less than 90 percent in room air, although prospectively collected data to assess the risks and benefits of this particular value have not been studied in the developed world.[26,63] Given the lack of scientific data to support specific Spo_2-based recommendations, it is understandable that some practice variation exists.[32]

Viral pathogens are far more commonly associated with lower respiratory tract infection, particularly for preschool-aged children. The clinical presentation generally follows a short upper respiratory tract infection prodrome (coryza, pharyngitis, mild fever) by a few days, with a gradual onset of cough, usually nonproductive. Clinical toxicity is usually mild for most viral pathogens. Some degree of bronchiolitis may occur with lower respiratory tract infection, leading to wheezing with difficult air entry and prolonged expiration with air trapping within the distal airways (primarily caused by RSV, hMPV, and rhinovirus). Lower respiratory tract disease is most often bilateral, affecting all lobes, in contrast to the more common presentation of focal, unilateral pneumonia caused by bacteria. Disease is usually benign, lasting only 3 to 5 days and resolving spontaneously. With young infants under 1 year of age, particularly those infected by RSV, increasing lung parenchymal inflammation can lead to decreasing oxygenation and respiratory failure. Hypoxemia and significant reactive airway disease may occur, not uncommonly leading to hospitalization. For the most common viral pathogens responsible for clinically significant lower respiratory tract disease, respiratory failure that leads to intubation is extremely unusual but may occur with influenza A or B viruses, particularly in children with comorbid medical conditions. Very uncommon viral pathogens, including SARS coronavirus and influenza H5N1 (avian influenza) may cause rapidly progressive respiratory failure with a high mortality rate.

In contrast, bacterial pneumonia may have a more rapid progression from cough to dyspnea, and is more often associated with signs and symptoms of systemic toxicity, including high fever, hypotension, myalgias, malaise, headache, gastrointestinal complaints, restlessness, apprehension, and, on occasion, manifestations of secondary sites of infection. As with viral disease, signs and symptoms of bacterial pneumonia vary with the bacterial pathogen and the age of the child. Pleural fluid collections, which may be extensive, can become infected, resulting in bacterial empyema that is a well-recognized complication of bacterial CAP. Some bacteria are associated with a specific pattern of disease, such as the lobar pneumonia of *S. pneumoniae* and the empyema, abscess, and pneumatocele formation caused by *S. aureus;* however, any of these manifestations may result from infection caused by any of the bacterial pathogens. In general, children with bacterial pneumonia have cough, tachypnea, and, with progressive disease, symptoms that are characteristic of hypoxemia, including dyspnea, shallow or grunting respirations, and flaring of the alae nasae. For lower lobe pneumonia, inflammation may be associated with gastrointestinal symptoms, including abdominal pain that may mimic that of appendicitis. Signs of pneumonia may be subtle in young infants. Percussion usually is not valuable in an infant or older child if distribution of the pneumonia is patchy. Dullness to percussion is associated more often in young children with the presence of pleural fluid than with the involvement of the parenchyma of the lung. Auscultatory findings classically include rales, or, for the child with consolidation, "tubular" breath sounds not associated with rales, lacking the normal breath sounds associated with gradual aeration and expansion of the lung with each inspiration. Abnormal findings in older children include dullness to percussion, decreased tactile and vocal fremitus on palpation, and the presence of egophony. Intercostal retraction indicates recruitment of accessory muscles, often necessary to assist respiration when hypoxemia is present.

Irritation of the pleura and accumulation of pleural fluid is accompanied by chest pain that may be severe and may limit chest movement. As the effusion enlarges, dyspnea may increase, but pleuritic pain may diminish and become a dull ache. The pain of pleural irritation may be present at the site of inflammation or may be referred. Pleural irritation over the right upper lobe may elicit meningismus, a sign of meningeal irritation.

Atypical pneumonia in children is most often caused by *Mycoplasma pneumoniae;* disease caused by either *Legionella pneumophila* or *Chlamydophila pneumoniae* is quite rare.[26] Unfortunately, no prospective natural history studies that involve routine screening of both outpatients and inpatients for *Mycoplasma* spp. infection in all pediatric age groups have been performed. Most studies of CAP in children that collected clinical information on *Mycoplasma* were retrospective, including some reporting outbreaks. Some reported data in children are derived from prospective antimicrobial therapy studies for adults, with insufficient numbers of evaluable children to allow a basic understanding of the disease in children.[122,145] *Mycoplasma* spp. clinical presentation is indistinguishable from that of viral pneumonia, prompting recommendations to clinicians to test for *Mycoplasma* before starting antimicrobial therapy. Presenting complaints are generally related to slowly progressive systemic symptoms over 3 to 7 days, with malaise, pharyngitis, and headache, followed by cough.[12,156] Cough usually is irritative and nonproductive, reflecting tracheobronchitis, a manifestation

of cell injury and host response to lung inflammation, which rarely progresses in children to hypoxemia requiring hospitalization and supplemental oxygen. Cough may last for 2 to 4 weeks or longer and is associated with clinical findings of rales, rhonchi, and wheezes that may be so prominent in the context of a child who does not appear ill as to merit the common name of "walking pneumonia." Similar to certain viral pathogens, *Mycoplasma* may be associated with increased wheezing in children with asthma, particularly younger infants. The clinical presentation in preschool children is generally less severe than in older children, presumably as a result of less immune-mediated inflammation. Those under 5 years of age are less likely to have disease with a lobar pattern on chest radiography.[96]

DIAGNOSIS

Diagnostic testing for CAP is summarized in this section and reviewed in detail in the 2011 IDSA and PIDS guidelines.[26] In children who are immunized against common bacterial causes of CAP, including *H. influenzae* type b and pneumococcus, and who do not require admission, limited diagnostic testing is recommended. Pulse oximetry is recommended to identify children with hypoxemia who may require additional testing or admission. In children who do not have hypoxemia, history and physical examination are sufficient to identify signs and symptoms of pneumonia and confirmation by chest radiography is not required at the initial visit. In children who are younger than 5 years, the majority of CAP will be due to viral pathogens and no antibiotic treatment is indicated. In children who have a worsening course with or without antibiotic therapy, a chest radiograph to evaluate the extent of infiltrate and to identify the presence or absence of pleural fluid should be obtained. Similarly, blood culture and complete blood count rarely change the treatment course in children who are managed as outpatients and are not recommended.[19,22,77,141] Testing for viral pathogens in the outpatient setting is increasingly possible. Viral testing may identify children with RSV who are at low risk for secondary bacterial pneumonia and may identify children with influenza who would benefit from antiviral therapy. Antiviral therapy for influenza should be administered to all children with influenza-like illness who are also at high risk for complications during periods of influenza circulation in the community, even if rapid antigen-based influenza testing is negative, because these tests lack high sensitivity.[6,8]

For children who require hospitalization, blood cultures are positive in approximately 1 to 11 percent of children and are recommended. When positive, blood culture results can guide definitive antibiotic treatment. Complete blood count may be useful in hospitalized children to identify anemia and thrombocytopenia associated with hemolytic uremic syndrome, a rare complication of bacterial pneumonia.[15,28,46,159] Sputum for Gram stain and culture is recommended in adults, but rarely performed in children. For older children and adolescents with severe CAP, sputum testing should be attempted. Urinary antigen tests for pneumococcus are not recommended for children with CAP, because false positive results are common.[126] The use of pneumococcal antigen testing for pleural fluid specimens, however, has been demonstrated to be reliable and improves the identification of a specific etiology for empyema (see Chapter 24).[133]

M. pneumoniae is a common cause of CAP, particularly in children aged 10 to 17 years. The role of diagnostic testing for *M. pneumoniae* is not well defined because limited studies have been conducted on the natural history of *M. pneumoniae* infection and the impact of antibiotic treatment, particularly in young children.[26] Testing may be most useful in school-aged children to guide antibiotic therapy. A variety of diagnostic tests are available. Traditional culture for *M. pneumoniae* is impractical for most clinical laboratories, given the slow growth and complex nutritional requirements of the organism. Cold-agglutinin titers greater than 1:64 are common in adults, but it is unclear how often this occurs in children. In addition, infections with other organisms may cause increases in cold-agglutinin titers, limiting the utility of this diagnostic test. Serologic diagnosis is most often used by clinical laboratories. Several serologic tests are available, including a rapid test for *M. pneumoniae* immunoglobulin (IgM), with sensitivity in published studies ranging from 74 to 96 percent and specificities ranging from 85 to 98 percent.[2,53,149,158] PCR technology is currently used in research settings, but is not widely available for clinical use. Interpretation of positive results, including the duration of positivity, from a variety of specimens such as nasal wash fluid, throat swab, sputum, and pleural fluid will all require further validation before clinical implementation of PCR can be realized.[150,169]

Pathogen-specific diagnoses for bacterial CAP remain challenging, although considerable progress has been made in the identification of pathogens through molecular testing of respiratory specimens. Both the PERCH and EPIC studies described previously use conventional and molecular diagnostics for viral and bacterial pathogens. A combination of these techniques identifies a viral or bacterial pathogen in 75 to 80 percent of hospitalized cases. Nucleic acid amplification also has been useful in identifying pathogens in culture-negative empyema.[20,54,93,124,128] The available methods and reagents are expanding rapidly and include fluorescent antibody techniques, enzyme-linked immunosorbent assay, direct DNA probes, and PCR. Clinical specimens may be tested directly or after preincubation in tissue culture systems.

MANAGEMENT

Management of children with pneumonia in clinics, hospital wards, or intensive care units is based on the severity of the disease. Although severity is clearly associated with certain pathogens, significant overlap exists between the disease caused by bacterial, viral, and atypical pathogens, leading to initial management based on clinical toxicity and degree of respiratory compromise. For this discussion, management is based on access to resources available in the developed world. Significant morbidity and

mortality occur in the developing world; the clinical manifestations of disease may reflect comorbidities and access to health care, and management decisions may depend on the resources available. The history, presentation, and examination of the child determine the severity of the illness and the appropriate level of care with respect to outpatient or inpatient management. The clinician's assessment of the child's respiratory status at the time of the medical encounter and the anticipated clinical course on antimicrobial therapy should determine whether hospitalization is necessary. Decision making should be based on clinical examination and pulse oximetry, supported by chest radiography and laboratory studies.

Any infant or child with respiratory distress (see Table 22-2) will demonstrate some degree of clinical toxicity, and most experts and professional societies recommend hospitalization for the "toxic" child. Although hospitalization in this setting is reasonable and represents the standard of care in the developed world, no prospective, controlled studies have been published that have quantified the degree of improved clinical outcome that results from hospitalization over management in the home.

Clinical decision rules based on initial scoring systems that incorporate a combination of clinical, laboratory, and radiographic findings have not been well defined for children as they have been for adults.[59,121] As previously noted, pulse oximetry represents a direct, simple, and widely available assessment for evaluating the degree of compromise of lower respiratory tract function. Clinical scores for critically ill children that currently exist may help determine whether a child should be hospitalized on a pediatric ward or be admitted to a pediatric intensive care unit. Severity of illness scores built on multiple logistic regression models, such as the Pediatric Logistic Organ Dysfunction (PELOD) score,[100] the Pediatric Risk of Mortality (PRISM) score,[137] and the Pediatric Index of Mortality (PIM),[90] predict the risk for death for children admitted to a pediatric intensive care unit, including those admitted with pneumonia.

Young age (<6 months) presents additional concerns, with some degree of immunocompromise compared with school-aged children and a more compliant chest wall that impairs deep breathing and coughing as mechanisms to help clear pulmonary secretions. In the developed world, very young infants (up to 3 months of age) with pneumonia are generally admitted to the hospital for initial management. Given the increased risks for morbidity for children under 1 year of age, recommendations for hospitalization of infants up to 6 months of age with suspected bacterial pneumonia also have been made.[80,139]

The presence of important comorbidities, including reactive airway disease, genetic syndromes, neurocognitive disorders, immune compromise, and chronic cardiac and pulmonary conditions are also risk factors for the pneumonia requiring hospitalization.[118,147] Children with a comorbid condition and influenza infection are more likely to require hospitalization than otherwise healthy children.[16,48]

Empirical antimicrobial therapy for the hospitalized child with pneumonia depends on the suspected pathogens and their presumed susceptibilities. For the vast majority of infants with pneumonia, viral pathogens are responsible and require only supportive therapy.[138] Rapid viral diagnostic tests can assist in the diagnosis, but some children with viral disease will have a bacterial coinfection that requires antimicrobial therapy. Perhaps best documented with influenza, infection may begin with rather benign upper respiratory tract symptoms that progress to a severity of illness that is unexpected or the infection may demonstrate a biphasic illness pattern with stable disease followed by subsequent deterioration, well documented during the 1918 influenza pandemic and in the 2009 H1N1 pandemic.[15]

Antiviral therapy exists for influenza virus. Most influenza A strains can be treated with neuraminidase inhibitors; in the past, adamantanes were effective treatment, but for the past several years, virtually all strains have been resistant. Most currently isolated strains of influenza B are susceptible to neuraminidase inhibitors. However, because substantial genetic variation occurs in influenza from year to year, resistance of influenza virus strains to any class of antiviral agents may develop and spread quickly. Globally, the World Health Organization tracks and reports resistance as strains are analyzed during the influenza season. In the United States, the CDC sequences a certain proportion of all strains isolated during the season to allow rapid detection of resistance to antiviral agents. Dosages of neuraminidase inhibitor agents that are currently recommended for influenza in children were evaluated in clinical trials that mandated treatment within 48 hours of onset of symptoms. Although earlier treatment will result in the most benefit in children,[75] children with serious illness that extends beyond 48 hours are still likely to benefit from therapy and should be treated.[8,56,97,114,144]

Oseltamivir is an orally available neuraminidase inhibitor in both tablet and suspension formulations. Zanamivir is available as inhalational therapy for older children who are capable of taking a deep breath on command. Dosing guidelines are available from both the American Academy of Pediatrics (AAP)[8] and the CDC.[40] Investigational neuroaminidase inhibitors that can be administered intravenously (peramivir, oseltamivir, and zanamivir), used for treatment of documented H1N1 infections during the H1N1 pandemic, are still available for compassionate investigational use, but are not yet approved by the U.S. Food and Drug Administration for general use.

For RSV pneumonia, controversy exists regarding the efficacy of inhaled ribavirin. Ribavirin has in vitro activity against RSV, but use of this drug for RSV infection is not routinely recommended in the management of lower respiratory tract disease because of marginal efficacy, cost, and potential toxicities to health care providers. New antivirals for RSV are under investigation.[160] Similarly, antivirals are under investigation for some less common, but potentially destructive viral pathogens causing pneumonia in immunocompromised hosts.

The etiology of bacterial pneumonia, as noted earlier, has been dramatically altered by effective PCVs for *H. influenzae* type b and *S. pneumoniae*. Disease caused by *H. influenzae* type b has been virtually eliminated in the developed world, and the susceptibility of *S. pneumoniae* to β-lactam agents such as penicillin G has improved dramatically. The increasing incidence of *S. aureus*, particularly methicillin-resistant strains, requires a more

TABLE 22-3 **Empirical Therapy of Suspected Community-Acquired Bacterial Pneumonia in Healthy Children**

Disease	Fully Immunized	Incompletely Immunized
Uncomplicated pneumonia (lobar or bronchopneumonia, without significant pleural effusion)	Ampicillin	Ceftriaxone or cefotaxime
Complicated pneumonia (with significant pleural fluid, empyema, abscess, pneumatocele)	Ampicillin plus Vancomycin*	Ceftriaxone plus Vancomycin*
Life-threatening, complicated pneumonia (with abscesses, pneumatoceles)	Ceftriaxone plus vancomycin plus nafcillin	

*Clindamycin may be substituted for vancomycin if local *Staphylococcus aureus* susceptibility data document rare resistance (<5%-10%).

aggressive approach to diagnosis of complicated pneumonia and consideration of the addition of empirical therapy active against all strains of *S. aureus*, in addition to that for *S. pneumoniae*. Empirical therapy is provided as the diagnostic evaluation for viral, bacterial, and atypical bacterial pathogens is in progress. Once a diagnosis is made, the most narrow-spectrum agent that is safe and well tolerated should be used.

Ampicillin or penicillin G intravenously is currently recommended for empirical therapy of acute bacterial CAP in fully immunized children (Table 22-3).[26] The use of antibiotics that are more active against penicillin-resistant pneumococci, such as ceftriaxone or vancomycin, is no longer routinely required with current low levels of β-lactam resistance. However, for children with incomplete immunization for pneumococcus, ceftriaxone or cefotaxime remain the preferred antimicrobials. The role of non–type b strains of *H. influenzae* in pneumonia in otherwise healthy children is difficult to define, because many reports of hospitalized children with pneumonia from whom non–type b strains of *H. influenzae* are recovered have underlying comorbidities, raising the possibility that these organisms may not represent a high risk to healthy, normal children. The use of ampicillin as empirical therapy will provide activity against the majority of non–type b strains as further work clarifies the role of this organism in pediatric CAP.

More difficult is the consideration of empirical therapy for CAP caused by *S. aureus*, particularly methicillin-resistant strains. Very little distinguishes between the clinical, laboratory, or radiographic characteristics of bacterial pneumonia caused by *S. aureus* in contrast to that caused by *S. pneumoniae*. Both can produce progressive disease associated with lung parenchymal destruction with empyema and signs and symptoms of systemic toxicity. Necrotizing pneumonia, particularly in the context of influenza coinfection, appears to be more characteristic of *S. aureus*, particularly with strains that are methicillin resistant.[39] Lung abscess formation with resulting complications may be more common with *S. aureus*.[39] With ampicillin as initial therapy of CAP, no antimicrobial activity is present against *S. aureus*, for either methicillin-susceptible or methicillin-resistant strains. Therefore, a high index of suspicion should be maintained for infection caused by *S. aureus* in children who do not respond to initial empirical therapy and children who present with complicated pneumonia, including pleural effusions, empyema, abscesses, and pneumatoceles. For these children with serious infection, the addition of vancomycin (or clindamycin if local susceptibility data document lack of resistance) to ampicillin while diagnostic studies are being pursued will provide adequate activity for either methicillin-susceptible or methicillin-resistant strains.[104] With β-lactam antibiotics thought to be preferred over vancomycin for susceptible strains of *S. aureus*, the addition of nafcillin to the regimen for optimal coverage of methicillin-susceptible strains is also recommended (see Table 22-3). For children who are diagnosed with MRSA pneumonia and do not respond clinically to vancomycin, linezolid is a reasonable alternative. In prospective studies in adults, linezolid therapy of nosocomial pneumonia caused by MRSA demonstrated outcomes that were at least as good as, if not better, than those with vancomycin.[168]

Definitive therapy, in contrast to *empirical* therapy, may be based on susceptibilities of the organisms to antimicrobials, using the narrowest spectrum, best tolerated, and most cost-effective agent. Recent recommendations have been made for specific antimicrobial agents, by pathogen, for both first-line and alternative agents, as well as agents for children who may be allergic to preferred agents.[26] For pneumococcus, intravenous penicillin G (or ampicillin) is preferred and for oral therapy, amoxicillin (or phenoxymethyl penicillin). For methicillin-resistant strains of *S. aureus*, intravenous vancomycin (or clindamycin) is preferred and for oral therapy, clindamycin (if susceptible) or linezolid. Insufficient prospective data are available to recommend trimethoprim-sulfamethoxazole therapy for MRSA pneumonia. For methicillin-susceptible strains, nafcillin or cefazolin should provide optimal therapy, with oral cephalexin for step-down therapy.

The duration of antimicrobial therapy in children in the developed world has primarily been studied in the context of antibiotic registration trials, comparing newer agents used for 10 days with those having previously been studied for a treatment course of 10 days. A notable exception is the azalide azithromycin, which displays unusual tissue-site pharmacokinetics, with drug exposure after 3 or 5 days of therapy comparable to 10 days of β-lactam therapy. Short-course (3 day) therapy with β-lactam amoxicillin or trimethoprim-sulfamethoxazole has been successful in the developing world for clinically defined pneumonia, but bacterial pathogen identification is infrequent in these studies, precluding conclusions about a pathogen-specific effective treatment course.[71]

Although the total course of therapy is usually 7 to 10 days for uncomplicated pneumonia, longer courses of 2 to 3 weeks may be required for more severe disease, particularly associated with pleural empyema or pulmonary abscesses. All therapy does not necessarily need to occur in a hospital setting if the child's clinical condition is improved, drainage procedures are no longer a concern, supplemental oxygen is no longer needed, and skilled nursing care is no longer required. Transition from intravenous inpatient therapy to daily intramuscular or intravenous outpatient therapy with prolonged half-life antibiotics, particularly β-lactams such as ceftriaxone or ertapenem, provides another option for the hospitalized child who still requires parenteral therapy based on issues of drug exposure within infected lung or pleural space or of issues of compliance.[151]

Depending on the response to therapy, oral step-down therapy often has been used to allow discharge from an inpatient setting. Although no prospective, controlled data are available to document the risks and benefits of this practice, clinical experience, often retrospectively documented, over several decades suggests that this practice is effective.[24] The duration of parenteral therapy before transition to oral therapy is based on the severity of the initial presentation and the rapidity of improvement. Improvement in fever, cough, tachypnea, and oxygen requirement, with improved activity and appetite, supported by a reduction in peripheral leukocyte counts or C-reactive protein or other acute-phase reactants are often used to help justify the transition to oral therapy.

Treatment for *M. pneumoniae* infection is less well studied in children than in adults.[12,96] Data extrapolated from adult studies suggest a modest benefit of therapy with tetracyclines and macrolides for illness of mild to moderate severity, with the assumption that children, particularly school-aged and adolescents, will respond similarly. Retrospective data from hospitalized children suggests that those who are treated have shorter inpatient admissions than those who do not receive therapy.[142] It is likely that children with moderate to severe disease will benefit from treatment with macrolides or tetracyclines (for children older than 7 years), prompting recommendations to test and treat for *Mycoplasma* spp. Azithromycin provides the shortest treatment course of the macrolides and is well tolerated, in contrast to erythromycin. Therapy with the respiratory fluoroquinolones has demonstrated treatment outcomes for adults not inferior to those with macrolides and tetracyclines, but fluoroquinolones are not considered agents of choice for children when other effective therapy exists. Of note, in a prospective, randomized study of preschool children with *Mycoplasma* lower respiratory tract infection documented serologically, amoxicillin-clavulanate demonstrated clinical outcomes equivalent to those of levofloxacin when evaluated by criteria for clinical cure at the end of therapy.[25] These results suggest the possibility of a high rate of spontaneous clinical resolution in this younger age group or that improvement may have occurred at an earlier time in the treatment course, not documented by the study design used.

Chlamydia trachomatis has been identified as an uncommon cause of afebrile pneumonia in very young infants, 2 to 12 weeks of age, after birth to a mother with genital infection. *C. pneumoniae* has been documented to cause atypical pneumonia in school-aged children and adolescents.[34,73] However, the clinical impact of antimicrobial therapy of *C. pneumoniae* lower respiratory tract infection in older children has been difficult to define, probably because of the difficulties in making an accurate diagnosis of active infection, as well as the relatively benign and self-resolving nature of infection.[21]

PREVENTION

Over the last two decades, great strides have been made in the prevention of CAP. The development of PCVs against both *H. influenzae* type b and *S. pneumoniae* has resulted in significant declines in cases of bacterial pneumonia in countries that have introduced these vaccines.[101,120,132] Both vaccines are provided as part of the routine childhood immunization series for all children in resource-rich areas of the world and should also be made available for all children in the developing world. Children can be protected against CAP caused by viral pathogens. Influenza vaccine is recommended for all children 6 months and older and prevents CAP resulting from influenza.[8] In addition, increasing evidence shows that bacterial CAP may occur concomitantly with influenza or as a secondary infection after influenza.* The risk for hospitalization with CAP resulting from RSV, the most common pathogen identified in infants, can be modified by the administration of palivizumab to infants with high-risk conditions.[58,148] Given the expense of this intervention, guidelines for use in high-risk infants should be followed to maximize cost-effectiveness.[5,116] Finally, the PIDS/IDSA CAP Guidelines, the CDC, and the AAP support the immunization of parents and other caregivers against influenza, to protect children who are too young to be immunized[29,99]; the AAP also recommends immunizing parents against other vaccine-preventable infections, including CAP.[29,99]

NEW REFERENCES SINCE THE SIXTH EDITION

5. American Academy of Pediatrics. Policy statements: modified recommendations for use of palivizumab for prevention of respiratory syncytial virus infections. *Pediatrics* 2009;**124**:1694–701.
6. American Academy of Pediatrics. Recommendations for prevention and control of influenza in children, 2010-2011. *Pediatrics* 2010;**126**:816–26.
7. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for the prevention of Streptococcus pneumoniae infections in infants and children: use of 13-valent pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). *Pediatrics* 2010;**126**:186–90.
8. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for prevention and control of influenza in children, 2011-2012. *Pediatrics* 2011;**128**:813–25.
9. Ampofo K. Pediatric respiratory infections in the vaccine era. Mini-Lecture. Presented at the Annual Meeting of the Infectious Diseases Society of America. October 22, 2011; Boston, Mass.
10. Ampofo K, Bender J, Sheng X, et al. Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics* 2008;**122**:229–37.
11. Ampofo K, Herbener A, Blaschke AJ, et al. Association of 2009 pandemic influenza A (H1N1) infection and increased

*References 15, 18, 37, 45, 50, 60, 72.

hospitalization with parapneumonic empyema in children in Utah. *Pediatr Infect Dis J* 2010;**29**:905–9.
12. Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of *Mycoplasma pneumoniae* infections. *FEMS Microbiol Rev* 2008;**32**:956–73.
14. Baird JS, Buet A, Hymes SR, et al. Comparing the clinical severity of the first versus second wave of 2009 influenza A (H1N1) in a New York City pediatric healthcare facility. *Pediatr Crit Care Med* 2012;**13**:375–80.
15. Bender JM, Ampofo K, Byington CL, et al. Epidemiology of *Streptococcus pneumoniae:* induced hemolytic uremic syndrome in Utah children. *Pediatr Infect Dis J* 2010;**29**:712–6.
16. Bender JM, Ampofo K, Gesteland P, et al. Development and validation of a risk score for predicting hospitalization in children with influenza virus infection. *Pediatr Emerg Care* 2009;**25**: 369–75.
17. Bender JM, Ampofo K, Korgenski K, et al. Pneumococcal necrotizing pneumonia in Utah: does serotype matter? *Clin Infect Dis* 2008;**46**:1346–52.
20. Blaschke AJ, Heyrend C, Byington CL, et al. Molecular analysis improves pathogen identification and epidemiologic study of pediatric parapneumonic empyema. *Pediatr Infect Dis J* 2011;**30**: 289–94.
23. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
26. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.
29. Brieu N, Guyon G, Rodiere M, et al. Human bocavirus infection in children with respiratory tract disease. *Pediatr Infect Dis J* 2008;**27**:969–73.
30. Broberg E, Niemela J, Lahti E, et al. Human rhinovirus C–associated severe pneumonia in a neonate. *J Clin Virol* 2011; **51**:79–82.
34. Burillo A, Bouza E. Chlamydophila pneumoniae. *Infect Dis Clin North Am* 2010;**24**:61–71.
36. Byington CL, Hulten KG, Ampofo K, et al. Molecular epidemiology of pediatric pneumococcal empyema from 2001 to 2007 in Utah. *J Clin Microbiol* 2010;**48**:520–5.
37. Calbo E, Garau J. Of mice and men: innate immunity in pneumococcal pneumonia. *Int J Antimicrob Agents* 2010;**35**:107–13.
38. Calvo C, Casas I, Garcia-Garcia ML, et al. Role of rhinovirus C respiratory infections in sick and healthy children in Spain. *Pediatr Infect Dis J* 2010;**29**:717–20.
39. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
40. Centers for Disease Control and Prevention. *Antiviral Drugs: Recommendations of the Advisory Committee on Immunization Practices (ACIP): Information for health care professionals*. http://www.cdc.gov/flu/professionals/antivirals/. Accessed February 28, 2012.
42. Centers for Disease Control and Prevention. *Active bacterial core surveillance report: Emerging Infections Program network*—Streptococcus pneumoniae, *2009*, 2010. http://www.cdc.gov/abcs/reports-findings/survreports/spneu09.html. Accessed April 18, 2012.
43. Centers for Disease Control and Prevention. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1): United States, May-August 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1071–4.
44. Centers for Disease Control and Prevention. *Active bacterial core surveillance report: Emerging Infections Program network*—Streptococcus pneumoniae, *2010*. 2012. http://www.cdc.gov/abcs/reports-findings/survreports/spneu10.html. Accessed April 18, 2012.
45. Chien YW, Klugman KP, Morens DM. Bacterial pathogens and death during the 1918 influenza pandemic. *N Engl J Med* 2009;**361**:2582–3.
46. Copelovitch L, Kaplan BS. *Streptococcus pneumoniae*–associated hemolytic uremic syndrome: classification and the emergence of serotype 19A. *Pediatrics* 2010;**125**:e174–82.
47. Davis C, Stockmann C, Korgenski K, et al. Population-based incidence and costs of hospitalization in children with human metapneumovirus infection. Paper presented at the Annual Meeting of the Pediatric Academic Societies, Boston, Mass, Abstract 3645.5. April 28-May 1, 2012.
48. Dawood FS, Fiore A, Kamimoto L, et al. Influenza-associated pneumonia in children hospitalized with laboratory-confirmed influenza, 2003-2008. *Pediatr Infect Dis J* 2010;**29**:585–90.
49. do Carmo Debur M, Raboni SM, Flizikowski FB, et al. Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal non-pandemic seasonal respiratory infections. *J Clin Pathol* 2010;**63**:930–4.
50. Don M, Korppi M, Valent F, et al. Human metapneumovirus pneumonia in children: results of an Italian study and mini-review. *Scand J Infect Dis* 2008;**40**:821–6.
51. Donoso AF, Leon JA, Camacho JF, et al. Fatal hemorrhagic pneumonia caused by human metapneumovirus in an immunocompetent child. *Pediatr Int* 2008;**50**:589–91.
56. Farias JA, Fernandez A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. *Intensive Care Med* 2010;**36**:1015–22.
57. Fawkner-Corbett DW, Docarmo M, Rose K, et al. The Impact of the H1N1 influenza pandemic on clinical presentations and viral epidemiology of acute respiratory infection in pre-school children in Brazil. *Pediatr Infect Dis J* 2012;**31**:653–5.
60. Finelli L, Fiore A, Dhara R, et al. Influenza-associated pediatric mortality in the United States: increase of Staphylococcus aureus coinfection. *Pediatrics* 2008;**122**:805–11.
64. Gilani Z, Kwong YD, Levine OS, et al. A literature review and survey of childhood pneumonia etiology studies: 2000-2010. *Clin Infect Dis* 2012;**54**(suppl 2):S102–8.
65. Gill S, Wight TN, Frevert CW. Proteoglycans: key regulators of pulmonary inflammation and the innate immune response to lung infection. *Anat Rec (Hoboken)* 2010;**293**:968–81.
69. Grijalva CG, Pelton SI. A second-generation pneumococcal conjugate vaccine for prevention of pneumococcal diseases in children. *Curr Opin Pediatr* 2011;**23**:98–104.
71. Haider BA, Saeed MA, Bhutta ZA. Short-course versus long-course antibiotic therapy for non-severe community-acquired pneumonia in children aged 2 months to 59 months. *Cochrane Database Syst Rev* 2008;(2):CD005976.
72. Hamano-Hasegawa K, Morozumi M, Nakayama E, et al. Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *J Infect Chemother* 2008;**14**:424–32.
74. Hammitt LL, Kazungu S, Morpeth SC, et al. A preliminary study of pneumonia etiology among hospitalized children in Kenya. *Clin Infect Dis* 2012;**54**(suppl 2):S190–9.
75. Heinonen S, Silvennoinen H, Lehtinen P, et al. Early oseltamivir treatment of influenza in children 1-3 years of age: a randomized controlled trial. *Clin Infect Dis* 2010;**51**:887–94.
78. Honkinen M, Lahti E, Osterback R, et al. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. *Clin Microbiol Infect* 2012;**18**:300–7.
79. Hsu KK, Shea KM, Stevenson AE, Pelton SI. Changing serotypes causing childhood invasive pneumococcal disease: Massachusetts, 2001-2007. *Pediatr Infect Dis J* 2010;**29**:289–93.
81. Jain S, Ampofo K, Arnold S, et al. Etiology of community-acquired pneumonia among hospitalized children in the United States: preliminary data from the CDC Etiology of Pneumonia in the Community (EPIC) Study. Paper presented at the Annual Meeting of the Infectious Diseases Society of America, Boston, Mass, October 20-24, 2011.
82. Jartti T, Korppi M. Rhinovirus-induced bronchiolitis and asthma development. *Pediatr Allergy Immunol* 2011;**22**:350–5.
84. Jones-Nazar C, Robertson D, Eavey J, et al. Severe methicillin-resistant *Staphylococcus aureus* community-acquired pneumonia associated with influenza: Louisiana and Georgia, December 2006-January 2007. *MMWR Morb Mortal Wkly Rep* 2007;**56**: 325–9.
85. Kallen AJ, Brunkard J, Moore Z, et al. *Staphylococcus aureus* community-acquired pneumonia during the 2006 to 2007 influenza season. *Ann Emerg Med* 2009;**53**:358–65.
88. Klugman KP, Chien YW, Madhi SA. Pneumococcal pneumonia and influenza: a deadly combination. *Vaccine* 2009;**27**(suppl 3): C9–C14.

92. Lau S, Yip C, Woo P, Yuen KY. Human rhinovirus C: a newly discovered human rhinovirus species. *Emerg Health Threats J* 2010;**3**:e2.
94. Lee GE, Lorch SA, Sheffler-Collins S, et al. National hospitalization trends for pediatric pneumonia and associated complications. *Pediatrics* 2010;**126**:204–13.
95. Lee JH, Chun JK, Kim DS, et al. Identification of adenovirus, influenza virus, parainfluenza virus, and respiratory syncytial virus by two kinds of multiplex polymerase chain reaction (PCR) and a shell vial culture in pediatric patients with viral pneumonia. *Yonsei Med J* 2010;**51**:761–7.
96. Lee KY. Pediatric respiratory infections by *Mycoplasma pneumoniae*. *Expert Rev Anti Infect Ther* 2008;**6**:509–21.
97. Lee N, Choi KW, Chan PK, et al. Outcomes of adults hospitalised with severe influenza. *Thorax* 2010;**65**:510–5.
99. Lessin HR, Edwards KM. Immunizing parents and other close family contacts in the pediatric office setting. *Pediatrics* 2012; **129**:e247–53.
100. Leteurtre S, Duhamel A, Grandbastien B, et al. Daily estimation of the severity of multiple organ dysfunction syndrome in critically ill children. *CMAJ* 2010;**182**:1181–7.
102. Levine OS, O'Brien KL, Deloria-Knoll M, et al. The pneumonia etiology research for child health project: a 21st century childhood pneumonia etiology study. *Clin Infect Dis* 2012;**54**(suppl 2): S93–S101.
104. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:e18–55.
108. Maeng SH, Yoo HS, Choi SH, et al. Impact of parainfluenza virus infection in pediatric cancer patients. *Pediatr Blood Cancer* 2012;**59**:708–10.
109. Mamtani M, Patel A, Hibberd PL, et al. A clinical tool to predict failed response to therapy in children with severe pneumonia. *Pediatr Pulmonol* 2009;**44**:379–86.
111. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis* 2010;**201**:1625–32.
116. Meissner HC, Bocchini Jr JA, Brady MT, et al. The role of immunoprophylaxis in the reduction of disease attributable to respiratory syncytial virus. *Pediatrics* 2009;**124**:1676–9.
117. Mermond S, Zurawski V, D'Ortenzio E, et al. Lower respiratory infections among hospitalized children in New Caledonia: a pilot study for the Pneumonia Etiology Research for Child Health project. *Clin Infect Dis* 2012;**54**(suppl 2):S180–9.
119. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 2008;**198**:962–70.
120. Morris SK, Moss WJ, Halsey N. *Haemophilus influenzae* type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008;**8**:435–43.
122. Mulholland S, Gavranich JB, Chang AB. Antibiotics for community-acquired lower respiratory tract infections secondary to *Mycoplasma pneumoniae* in children. *Cochrane Database Syst Rev* 2010;(7):CD004875.
123. Muller A, Klinkenberg D, Vehreschild J, et al. Low prevalence of human metapneumovirus and human bocavirus in adult immunocompromised high risk patients suspected to suffer from Pneumocystis pneumonia. *J Infect* 2009;**58**:227–31.
124. Munoz-Almagro C, Gala S, Selva L, et al. DNA bacterial load in children and adolescents with pneumococcal pneumonia and empyema. *Eur J Clin Microbiol Infect Dis* 2011;**30**:327–35.
125. Murphy S, Xu J, Kochanek K. *National vital statistics reports: Deaths—Preliminary data for 2010*. Atlanta, Ga: National Center for Vital Statistics; 2012.
127. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children: use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine—recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-11): 1–18.
128. Obando I, Camacho-Lovillo MS, Porras A, et al. Sustained high prevalence of pneumococcal serotype 1 in paediatric parapneumonic empyema in southern Spain from 2005 to 2009. *Clin Microbiol Infect* 2012;**18**:763–8.
129. Obando I, Munoz-Almagro C, Arroyo LA, et al. Pediatric parapneumonic empyema: Spain. *Emerg Infect Dis* 2008;**14**: 1390–7.
130. Ou ZY, Zeng QY, Wang FH, et al. Retrospective study of adenovirus in autopsied pulmonary tissue of pediatric fatal pneumonia in South China. *BMC Infect Dis* 2008;**8**:122.
131. Picazo J, Ruiz-Contreras J, Casado-Flores J, et al. Laboratory-based, 2-year surveillance of pediatric parapneumonic pneumococcal empyema following heptavalent pneumococcal conjugate vaccine universal vaccination in Madrid. *Pediatr Infect Dis J* 2011;**30**:471–4.
132. Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.
136. Rothberg MB, Haessler SD. Complications of seasonal and pandemic influenza. *Crit Care Med* 2010;**38**(4 suppl):e91–7.
138. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. *Lancet* 2011;**377**:1264–75.
139. Sebastian R, Skowronski DM, Chong M, et al. Age-related trends in the timeliness and prediction of medical visits, hospitalizations and deaths due to pneumonia and influenza: British Columbia, Canada, 1998-2004. *Vaccine* 2008;**26**:1397–403.
140. Shah S, Bachur R, Kim D, Neuman MI. Lack of predictive value of tachypnea in the diagnosis of pneumonia in children. *Pediatr Infect Dis J* 2010;**29**:406–9.
142. Shah SS, Test M, Sheffler-Collins S, et al. Macrolide therapy and outcomes in a multicenter cohort of children hospitalized with Mycoplasma pneumoniae pneumonia. *J Hosp Med* 2012;**7**: 311–7.
143. Sheng ZM, Chertow DS, Ambroggio X, et al. Autopsy series of 68 cases dying before and during the 1918 influenza pandemic peak. *Proc Natl Acad Sci U S A* 2011;**108**:16416–21.
144. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. *JAMA* 2010;**303**:1517–25.
146. Stockmann C, Pavia AT, Hymas W, et al. Quantification of human rhinovirus viral load and disease severity among hospitalized children with pneumonia enrolled in the CDC Etiology of Pneumonia in the Community (EPIC) Study. Paper presented at the Annual Meeting of the Infectious Diseases Society of America; Abstract 80. Oct. 9-12, 2012; San Diego, Calif.
149. Thurman KA, Walter ND, Schwartz SB, et al. Comparison of laboratory diagnostic procedures for detection of Mycoplasma pneumoniae in community outbreaks. *Clin Infect Dis* 2009;**48**: 1244–9.
150. Thurman KA, Warner AK, Cowart KC, et al. Detection of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* spp. in clinical specimens using a single-tube multiplex real-time PCR assay. *Diagn Microbiol Infect Dis* 2011;**70**:1–9.
152. Trei JS, Johns NM, Garner JL, et al. Spread of adenovirus to geographically dispersed military installations, May-October 2007. *Emerg Infect Dis* 2010;**16**:769–75.
155. Vento TJ, Prakash V, Murray CK, et al. Pneumonia in military trainees: a comparison study based on adenovirus serotype 14 infection. *J Infect Dis* 2011;**203**:1388–95.
160. Welliver RC. Pharmacotherapy of respiratory syncytial virus infection. *Curr Opin Pharmacol* 2010;**10**:289–93.
161. Welliver RC Sr. The immune response to respiratory syncytial virus infection: friend or foe? *Clin Rev Allergy Immunol* 2008; **34**:163–73.
165. Wolf DG, Greenberg D, Shemer-Avni Y, et al. Association of human metapneumovirus with radiologically diagnosed community-acquired alveolar pneumonia in young children. *J Pediatr* 2010;**156**:115–20.
167. World Health Organization. *Pneumonia. Fact sheet No 331*. Geneva: World Health Organization; 2011.
168. Wunderink RG, Niederman MS, Kollef MH, et al. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis* 2012;**54**:621–9.
169. Zhang L, Zong ZY, Liu YB, et al. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review & meta-analysis. *Indian J Med Res* 2011;**134**:270–80.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aherne W, Bird T, Court SD, et al. Pathological changes in virus infections of the lower respiratory tract in children. *J Clin Pathol* 1970;**23**:7–18.
2. Alexander TS, Gray LD, Kraft JA, et al. Performance of Meridian ImmunoCard Mycoplasma test in a multicenter clinical trial. *J Clin Microbiol* 1996;**34**:1180–3.
3. Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007;**44**:904–10.
4. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A* 2005;**102**:12891–6.
5. American Academy of Pediatrics. Policy statements: modified recommendations for use of palivizumab for prevention of respiratory syncytial virus infections. *Pediatrics* 2009;**124**:1694–701.
6. American Academy of Pediatrics. Recommendations for prevention and control of influenza in children, 2010-2011. *Pediatrics* 2010;**126**:816–26.
7. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for the prevention of *Streptococcus pneumoniae* infections in infants and children: use of 13-valent pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). *Pediatrics* 2010;**126**:186–90.
8. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for prevention and control of influenza in children, 2011-2012. *Pediatrics* 2011;**128**:813–25.
9. Ampofo K. Pediatric respiratory infections in the vaccine era. Mini-Lecture. Presented at the Annual Meeting of the Infectious Diseases Society of America. October 22, 2011; Boston, Mass.
10. Ampofo K, Bender J, Sheng X, et al. Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics* 2008;**122**:229–37.
11. Ampofo K, Herbener A, Blaschke AJ, et al. Association of 2009 pandemic influenza A (H1N1) infection and increased hospitalization with parapneumonic empyema in children in Utah. *Pediatr Infect Dis J* 2010;**29**:905–9.
12. Atkinson TP, Balish MF, Waites KB. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of *Mycoplasma pneumoniae* infections. *FEMS Microbiol Rev* 2008;**32**: 956–73.
13. Ayieko P, English M. In children aged 2-59 months with pneumonia, which clinical signs best predict hypoxaemia? *J Trop Pediatr* 2006;**52**:307–10.
14. Baird JS, Buet A, Hymes SR, et al. Comparing the clinical severity of the first versus second wave of 2009 Influenza A (H1N1) in a New York City pediatric healthcare facility. *Pediatr Crit Care Med* 2012;**13**:375–80.
15. Bender JM, Ampofo K, Byington CL, et al. Epidemiology of *Streptococcus pneumoniae*-induced hemolytic uremic syndrome in Utah children. *Pediatr Infect Dis J* 2010;**29**:712–6.
16. Bender JM, Ampofo K, Gesteland P, et al. Development and validation of a risk score for predicting hospitalization in children with influenza virus infection. *Pediatr Emerg Care* 2009;**25**: 369–75.
17. Bender JM, Ampofo K, Korgenski K, et al. Pneumococcal necrotizing pneumonia in Utah: does serotype matter? *Clin Infect Dis* 2008;**46**:1346–52.
18. Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003-2004. *N Engl J Med* 2005;**353**:2559–67.
19. Black SB, Shinefield HR, Ling S, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *Pediatr Infect Dis J* 2002;**21**:810–5.
20. Blaschke AJ, Heyrend C, Byington CL, et al. Molecular analysis improves pathogen identification and epidemiologic study of pediatric parapneumonic empyema. *Pediatr Infect Dis J* 2011; **30**:289–94.
21. Block S, Hedrick J, Hammerschlag MR, Cassell GH, Craft JC. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. *Pediatr Infect Dis J* 1995;**14**:471–7.
22. Bonadio WA. Bacteremia in febrile children with lobar pneumonia and leukocytosis. *Pediatr Emerg Care* 1988;**4**:241–2.
23. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
24. Bradley JS, Ching DK, Hart CL. Invasive bacterial disease in childhood: efficacy of oral antibiotic therapy following short course parenteral therapy in non-central nervous system infections. *Pediatr Infect Dis J* 1987;**6**:821–5.
25. Bradley JS, Arguedas A, Blumer JL, et al. Comparative study of levofloxacin in the treatment of children with community-acquired pneumonia. *Pediatr Infect Dis J* 2007;**26**:868–78.
26. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.
27. Brandt CD, Kim HW, Vargosko AJ, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am J Epidemiol* 1969;**90**:484–500.
28. Brandt J, Wong C, Mihm S, et al. Invasive pneumococcal disease and hemolytic uremic syndrome. *Pediatrics* 2002;**110**(2 Pt 1): 371–6.
29. Brieu N, Guyon G, Rodiere M, et al. Human bocavirus infection in children with respiratory tract disease. *Pediatr Infect Dis J* 2008;**27**:969–73.
30. Broberg E, Niemela J, Lahti E, et al. Human rhinovirus C–associated severe pneumonia in a neonate. *J Clin Virol* 2011; **51**:79–82.
31. Brosch L, Tchandja J, Marconi V, et al. Adenovirus serotype 14 pneumonia at a basic military training site in the United States, spring 2007: a case series. *Mil Med* 2009;**174**:1295–9.
32. Brown L, Dannenberg B. Pulse oximetry in discharge decision-making: a survey of emergency physicians. *CJEM* 2002;**4**: 388–93.
33. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infect Dis* 2006;**6**:303–12.
34. Burillo A, Bouza E. Chlamydophila pneumoniae. *Infect Dis Clin North Am* 2010;**24**:61–71.
35. Byington CL, Korgenski K, Daly J, et al. Impact of the pneumococcal conjugate vaccine on pneumococcal parapneumonic empyema. *Pediatr Infect Dis J* 2006;**25**:250–4.
36. Byington CL, Hulten KG, Ampofo K, et al. Molecular epidemiology of pediatric pneumococcal empyema from 2001 to 2007 in Utah. *J Clin Microbiol* 2010;**48**:520–5.
37. Calbo E, Garau J. Of mice and men: innate immunity in pneumococcal pneumonia. *Int J Antimicrob Agents* 2010;**35**:107–13.
38. Calvo C, Casas I, Garcia-Garcia ML, et al. Role of rhinovirus C respiratory infections in sick and healthy children in Spain. *Pediatr Infect Dis J* 2010;**29**:717–20.
39. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
40. Centers for Disease Control and Prevention. *Antiviral drugs: recommendations of the Advisory Committee on Immunization Practices (ACIP): Information for Health Care Professionals*. http://www.cdc.gov/flu/professionals/antivirals/. Accessed February 28, 2012.
41. Centers for Disease Control and Prevention. Two fatal cases of adenovirus-related illness in previously healthy young adults: Illinois, 2000. *MMWR Morb Mortal Wkly Rep* 2001;**50**:553–5.
42. Centers for Disease Control and Prevention. *Active bacterial core surveillance report: Emerging Infections Program Network*—Streptococcus pneumoniae, *2009*. 2010. http://www.cdc.gov/abcs/reports-findings/survreports/spneu09.html. Accessed April 18, 2012.
43. Centers for Disease Control and Prevention. Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1): United States, May-August 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1071–4.
44. Centers for Disease Control and Prevention. *Active bacterial core surveillance report: Emerging Infections Program Network*—Streptococcus pneumoniae, *2010*. 2012. http://www.cdc.gov/abcs/reports-findings/survreports/spneu10.html. Accessed April 18, 2012.

45. Chien YW, Klugman KP, Morens DM. Bacterial pathogens and death during the 1918 influenza pandemic. *N Engl J Med* 2009;**361**:2582–3.
46. Copelovitch L, Kaplan BS. *Streptococcus pneumoniae*–associated hemolytic uremic syndrome: classification and the emergence of serotype 19A. *Pediatrics* 2010;**125**:e174–82.
47. Davis C, Stockmann C, Korgenski K, et al. Population-based incidence and costs of hospitalization in children with human metapneumovirus infection. Paper presented at the Annual Meeting of the Pediatric Academic Societies, Boston, Mass, Abstract 3645.5. April 28-May 1, 2012.
48. Dawood FS, Fiore A, Kamimoto L, et al. Influenza-associated pneumonia in children hospitalized with laboratory-confirmed influenza, 2003-2008. *Pediatr Infect Dis J* 2010;**29**:585–90.
49. do Carmo Debur M, Raboni SM, Flizikowski FB, et al. Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal non-pandemic seasonal respiratory infections. *J Clin Pathol* 2010;**63**:930–4.
50. Don M, Korppi M, Valent F, et al. Human metapneumovirus pneumonia in children: results of an Italian study and mini-review. *Scand J Infect Dis* 2008;**40**:821–6.
51. Donoso AF, Leon JA, Camacho JF, et al. Fatal hemorrhagic pneumonia caused by human metapneumovirus in an immunocompetent child. *Pediatr Int* 2008;**50**:589–91.
52. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003;**348**:1967–76.
53. Dunn JJ, Malan AK, Evans J, Litwin CM. Rapid detection of *Mycoplasma pneumoniae* IgM antibodies in pediatric patients using ImmunoCard Mycoplasma compared to conventional enzyme immunoassays. *Eur J Clin Microbiol Infect Dis* 2004;**23**: 412–4.
54. Eltringham G, Kearns A, Freeman R, et al. Culture-negative childhood empyema is usually due to penicillin-sensitive *Streptococcus pneumoniae* capsular serotype 1. *J Clin Microbiol* 2003;**41**: 521–2.
55. Esper F, Weibel C, Ferguson D, et al. Coronavirus HKU1 infection in the United States. *Emerg Infect Dis* 2006;**12**:775–9.
56. Farias JA, Fernandez A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. *Intensive Care Med* 2010;**36**:1015–22.
57. Fawkner-Corbett DW, Docarmo M, Rose K, et al. The impact of the H1N1 influenza pandemic on clinical presentations and viral epidemiology of acute respiratory infection in pre-school children in Brazil. *Pediatr Infect Dis J* 2012;**31**:653–5.
58. Feltes TF, Cabalka AK, Meissner HC, et al. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. *J Pediatr* 2003;**143**:532–40.
59. Fine MJ, Stone RA, Singer DE, et al. Processes and outcomes of care for patients with community-acquired pneumonia: results from the Pneumonia Patient Outcomes Research Team (PORT) cohort study. *Arch Intern Med* 1999;**159**:970–80.
60. Finelli L, Fiore A, Dhara R, et al. Influenza-associated pediatric mortality in the United States: increase of *Staphylococcus aureus* coinfection. *Pediatrics* 2008;**122**:805–11.
61. Finland M, Peterson OL, Strauss E. Staphylococcic pneumonia occurring during an epidemic of influenza. *Arch Intern Med* 1942;**70**:183–205.
62. Fox JP, Brandt CD, Wassermann FE, et al. The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. VI. Observations of adenovirus infections: virus excretion patterns, antibody response, efficiency of surveillance, patterns of infections, and relation to illness. *Am J Epidemiol* 1969;**89**:25–50.
63. Fu LY, Ruthazer R, Wilson I, et al. Brief hospitalization and pulse oximetry for predicting amoxicillin treatment failure in children with severe pneumonia. *Pediatrics* 2006;**118**:e1822–30.
64. Gilani Z, Kwong YD, Levine OS, et al. A literature review and survey of childhood pneumonia etiology studies: 2000-2010. *Clin Infect Dis* 2012;**54**(suppl 2):S102–8.
65. Gill S, Wight TN, Frevert CW. Proteoglycans: key regulators of pulmonary inflammation and the innate immune response to lung infection. *Anat Rec (Hoboken)* 2010;**293**:968–81.
66. Gillim-Ross L, Subbarao K. Emerging respiratory viruses: challenges and vaccine strategies. *Clin Microbiol Rev* 2006;**19**:614–36.
67. Glezen WP, Denny FW. The parainfluenza viruses. In: Evans AS, editor. *Viral infections of humans: Epidemiology and control*. New York: Plenum; 1997. p. 551–67.
68. Glezen WP, Frank AL, Taber LH, Kasel JA. Parainfluenza virus type 3: seasonality and risk of infection and reinfection in young children. *J Infect Dis* 1984;**150**:851–7.
69. Grijalva CG, Pelton SI. A second-generation pneumococcal conjugate vaccine for prevention of pneumococcal diseases in children. *Curr Opin Pediatr* 2011;**23**:98–104.
70. Hageman JC, Uyeki TM, Francis JS, et al. Severe community-acquired pneumonia due to *Staphylococcus aureus*, 2003-04 influenza season. *Emerg Infect Dis* 2006;**12**:894–9.
71. Haider BA, Saeed MA, Bhutta ZA. Short-course versus long-course antibiotic therapy for non-severe community-acquired pneumonia in children aged 2 months to 59 months. *Cochrane Database Syst Rev* 2008;(2):CD005976.
72. Hamano-Hasegawa K, Morozumi M, Nakayama E, et al. Comprehensive detection of causative pathogens using real-time PCR to diagnose pediatric community-acquired pneumonia. *J Infect Chemother* 2008;**14**:424–32.
73. Hammerschlag MR. Pneumonia due to *Chlamydia pneumoniae* in children: epidemiology, diagnosis, and treatment. *Pediatr Pulmonol* 2003;**36**:384–90.
74. Hammitt LL, Kazungu S, Morpeth SC, et al. A preliminary study of pneumonia etiology among hospitalized children in Kenya. *Clin Infect Dis* 2012;**54**(suppl 2):S190–9.
75. Heinonen S, Silvennoinen H, Lehtinen P, et al. Early oseltamivir treatment of influenza in children 1-3 years of age: a randomized controlled trial. *Clin Infect Dis* 2010;**51**:887–94.
76. Herbert FA, Wilkinson D, Burchak E, Morgante O. Adenovirus type 3 pneumonia causing lung damage in childhood. *Can Med Assoc J* 1977;**116**:274–6.
77. Hickey RW, Bowman MJ, Smith GA. Utility of blood cultures in pediatric patients found to have pneumonia in the emergency department. *Ann Emerg Med* 1996;**27**:721–5.
78. Honkinen M, Lahti E, Osterback R, et al. Viruses and bacteria in sputum samples of children with community-acquired pneumonia. *Clin Microbiol Infect* 2012;**18**:300–7.
79. Hsu KK, Shea KM, Stevenson AE, Pelton SI. Changing serotypes causing childhood invasive pneumococcal disease: Massachusetts, 2001-2007. *Pediatr Infect Dis J* 2010;**29**:289–93.
80. Jadavji T, Law B, Lebel MH, et al. A practical guide for the diagnosis and treatment of pediatric pneumonia. *CMAJ* 1997;**156**(5): S703–11.
81. Jain S, Ampofo K, Arnold S, et al. Etiology of Community-acquired Pneumonia among Hospitalized Children in the United States: Preliminary Data from the CDC Etiology of Pneumonia in the Community (EPIC) Study. Paper presented at the Annual Meeting of the Infectious Diseases Society of America; October 2011; Boston, MA.
82. Jartti T, Korppi M. Rhinovirus-induced bronchiolitis and asthma development. *Pediatr Allergy Immunol* 2011;**22**:350–5.
83. Johnson JE, Gonzales RA, Olson SJ, et al. The histopathology of fatal untreated human respiratory syncytial virus infection. *Mod Pathol* 2007;**20**:108–19.
84. Jones-Nazar C, Robertson D, Eavey J, et al. Severe methicillin-resistant *Staphylococcus aureus* community-acquired pneumonia associated with influenza: Louisiana and Georgia, December 2006-January 2007. *MMWR Morb Mortal Wkly Rep* 2007;**56**: 325–9.
85. Kallen AJ, Brunkard J, Moore Z, et al. *Staphylococcus aureus* community-acquired pneumonia during the 2006 to 2007 influenza season. *Ann Emerg Med* 2009;**53**:358–65.
86. Kaye HS, Marsh HB, Dowdle WR. Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. *Am J Epidemiol* 1971;**94**:43–9.
87. Kim KS, Gohd RS. Fatal pneumonia caused by adenovirus type 35. *Am J Dis Child* 1981;**135**:473–5.
88. Klugman KP, Chien YW, Madhi SA. Pneumococcal pneumonia and influenza: a deadly combination. *Vaccine* 2009;**27**(suppl 3): C9–C14.
89. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;**348**:1953–66.

90. Lacroix J, Cotting J. Severity of illness and organ dysfunction scoring in children. *Pediatr Crit Care Med* 2005;**6**(3 suppl): S126–34.
91. Lang WR, Howden CW, Laws J, Burton JF. Bronchopneumonia with serious sequelae in children with evidence of adenovirus type 21 infection. *Br Med J* 1969;**1**:73–9.
92. Lau S, Yip C, Woo P, Yuen KY. Human rhinovirus C: a newly discovered human rhinovirus species. *Emerg Health Threats J* 2010;**3**:e2.
93. Le Monnier A, Carbonnelle E, Zahar JR, et al. Microbiological diagnosis of empyema in children: comparative evaluations by culture, polymerase chain reaction, and pneumococcal antigen detection in pleural fluids. *Clin Infect Dis* 2006;**42**:1135–40.
94. Lee GE, Lorch SA, Sheffler-Collins S, et al. National hospitalization trends for pediatric pneumonia and associated complications. *Pediatrics* 2010;**126**:204–13.
95. Lee JH, Chun JK, Kim DS, et al. Identification of adenovirus, influenza virus, parainfluenza virus, and respiratory syncytial virus by two kinds of multiplex polymerase chain reaction (PCR) and a shell vial culture in pediatric patients with viral pneumonia. *Yonsei Med J* 2010;**51**:761–7.
96. Lee KY. Pediatric respiratory infections by *Mycoplasma pneumoniae*. *Expert Rev Anti Infect Ther* 2008;**6**:509–21.
97. Lee N, Choi KW, Chan PK, et al. Outcomes of adults hospitalised with severe influenza. *Thorax* 2010;**65**:510–5.
98. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2003;**348**: 1986–94.
99. Lessin HR, Edwards KM. Immunizing parents and other close family contacts in the pediatric office setting. *Pediatrics* 2012; **129**:e247–53.
100. Leteurtre S, Duhamel A, Grandbastien B, et al. Daily estimation of the severity of multiple organ dysfunction syndrome in critically ill children. *CMAJ* 2010;**182**:1181–7.
101. Levine OS, Lagos R, Munoz A, et al. Defining the burden of pneumonia in children preventable by vaccination against *Haemophilus influenzae* type b. *Pediatr Infect Dis J* 1999;**18**: 1060–4.
102. Levine OS, O'Brien KL, Deloria-Knoll M, et al. The pneumonia etiology research for child health project: a 21st century childhood pneumonia etiology study. *Clin Infect Dis* 2012;**54**(suppl 2): S93–S101.
103. Lin PY, Lin TY, Huang YC, et al. Human metapneumovirus and community-acquired pneumonia in children. *Chang Gung Med J* 2005;**28**:683–8.
104. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:e18–55.
105. Lozano JM, Steinhoff M, Ruiz JG, et al. Clinical predictors of acute radiological pneumonia and hypoxaemia at high altitude. *Arch Dis Child* 1994;**71**:323–7.
106. Madhi SA, Klugman KP. A role for *Streptococcus pneumoniae* in virus-associated pneumonia. *Nat Med* 2004;**10**:811–3.
107. Madhi SA, Ludewick H, Kuwanda L, et al. Pneumococcal coinfection with human metapneumovirus. *J Infect Dis* 2006;**193**: 1236–43.
108. Maeng SH, Yoo HS, Choi SH, et al. Impact of parainfluenza virus infection in pediatric cancer patients. *Pediatr Blood Cancer* 2012; **59**:708–10.
109. Mamtani M, Patel A, Hibberd PL, et al. A clinical tool to predict failed response to therapy in children with severe pneumonia. *Pediatr Pulmonol* 2009;**44**:379–86.
110. Margolis PA, Ferkol TW, Marsocci S, et al. Accuracy of the clinical examination in detecting hypoxemia in infants with respiratory illness. *J Pediatr* 1994;**124**:552–60.
111. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis* 2010;**201**:1625–32.
112. McCullers JA, Bartmess KC. Role of neuraminidase in lethal synergism between influenza virus and *Streptococcus pneumoniae*. *J Infect Dis* 2003;**187**:1000–9.
113. McCullers JA, Rehg JE. Lethal synergism between influenza virus and *Streptococcus pneumoniae:* characterization of a mouse model and the role of platelet-activating factor receptor. *J Infect Dis* 2002;**186**:341–50.
114. McGeer A, Green KA, Plevneshi A, et al. Antiviral therapy and outcomes of influenza requiring hospitalization in Ontario, Canada. *Clin Infect Dis* 2007;**45**:1568–75.
115. McIntosh K, Chao RK, Krause HE, et al. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis* 1974;**130**:502–7.
116. Meissner HC, Bocchini Jr JA, Brady MT, et al. The role of immunoprophylaxis in the reduction of disease attributable to respiratory syncytial virus. *Pediatrics* 2009;**124**:1676–9.
117. Mermond S, Zurawski V, D'Ortenzio E, et al. Lower respiratory infections among hospitalized children in New Caledonia: a pilot study for the Pneumonia Etiology Research for Child Health project. *Clin Infect Dis* 2012;**54**(suppl 2):S180–9.
118. Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 2004;**113**:701–7.
119. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 2008;**198**:962–70.
120. Morris SK, Moss WJ, Halsey N. *Haemophilus influenzae* type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008;**8**: 435–43.
121. Mortensen EM, Coley CM, Singer DE, et al. Causes of death for patients with community-acquired pneumonia: results from the Pneumonia Patient Outcomes Research Team cohort study. *Arch Intern Med* 2002;**162**:1059–64.
122. Mulholland S, Gavranich JB, Chang AB. Antibiotics for community-acquired lower respiratory tract infections secondary to *Mycoplasma pneumoniae* in children. *Cochrane Database Syst Rev* 2010;(7):CD004875.
123. Muller A, Klinkenberg D, Vehreschild J, et al. Low prevalence of human metapneumovirus and human bocavirus in adult immunocompromised high risk patients suspected to suffer from Pneumocystis pneumonia. *J Infect* 2009;**58**:227–31.
124. Munoz-Almagro C, Gala S, Selva L, et al. DNA bacterial load in children and adolescents with pneumococcal pneumonia and empyema. *Eur J Clin Microbiol Infect Dis* 2011;**30**:327–35.
125. Murphy S, Xu J, Kochanek K. *National vital statistics reports: Deaths—Preliminary data for 2010*. Atlanta, Ga: National Center for Vital Statistics; 2012.
126. Neuman MI, Harper MB. Evaluation of a rapid urine antigen assay for the detection of invasive pneumococcal disease in children. *Pediatrics* 2003;**112**(6 Pt 1):1279–82.
127. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children: use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine—recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-11): 1–18.
128. Obando I, Camacho-Lovillo MS, Porras A, et al. Sustained high prevalence of pneumococcal serotype 1 in paediatric parapneumonic empyema in southern Spain from 2005 to 2009. *Clin Microbiol Infect* 2011;**18**:763–8.
129. Obando I, Munoz-Almagro C, Arroyo LA, et al. Pediatric parapneumonic empyema, Spain. *Emerg Infect Dis* 2008;**14**:1390–7.
130. Ou ZY, Zeng QY, Wang FH, et al. Retrospective study of adenovirus in autopsied pulmonary tissue of pediatric fatal pneumonia in South China. *BMC Infect Dis* 2008;**8**:122.
131. Picazo J, Ruiz-Contreras J, Casado-Flores J, et al. Laboratory-based, 2-year surveillance of pediatric parapneumonic pneumococcal empyema following heptavalent pneumococcal conjugate vaccine universal vaccination in Madrid. *Pediatr Infect Dis J* 2011;**30**:471–4.
132. Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.
133. Ploton C, Freydiere AM, Benito Y, et al. *Streptococcus pneumoniae* thoracic empyema in children: rapid diagnosis by using the Binax NOW immunochromatographic membrane test in pleural fluids. *Pathol Biol (Paris)* 2006;**54**:498–501.
134. Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. *N Engl J Med* 2003; **348**:1995–2005.
135. Pyrc K, Berkhout B, van der Hoek L. The novel human coronaviruses NL63 and HKU1. *J Virol* 2007;**81**:3051–7.

136. Rothberg MB, Haessler SD. Complications of seasonal and pandemic influenza. *Crit Care Med* 2010;**38**(4 suppl):e91–7.
137. Ruttimann UE, Pollack MM. Objective assessment of changing mortality risks in pediatric intensive care unit patients. *Crit Care Med* 1991;**19**:474–83.
138. Ruuskanen O, Lahti E, Jennings LC, Murdoch DR. Viral pneumonia. *Lancet* 2011;**377**:1264–75.
139. Sebastian R, Skowronski DM, Chong M, et al. Age-related trends in the timeliness and prediction of medical visits, hospitalizations and deaths due to pneumonia and influenza, British Columbia, Canada, 1998-2004. *Vaccine* 2008;**26**:1397–403.
140. Shah S, Bachur R, Kim D, Neuman MI. Lack of predictive value of tachypnea in the diagnosis of pneumonia in children. *Pediatr Infect Dis J* 2010;**29**:406–9.
141. Shah SS, Alpern ER, Zwerling L, McGowan KL, Bell LM. Risk of bacteremia in young children with pneumonia treated as outpatients. *Arch Pediatr Adolesc Med* 2003;**157**:389–92.
142. Shah SS, Test M, Sheffler-Collins S, et al. Macrolide therapy and outcomes in a multicenter cohort of children hospitalized with Mycoplasma pneumoniae pneumonia. *J Hosp Med* 2012;**7**: 311–7.
143. Sheng ZM, Chertow DS, Ambroggio X, et al. Autopsy series of 68 cases dying before and during the 1918 influenza pandemic peak. *Proc Natl Acad Sci U S A* 2011;**108**:16416–21.
144. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. *JAMA* 2010;**303**:1517–25.
145. Stevens D, Swift PG, Johnston PG, et al. *Mycoplasma pneumoniae* infections in children. *Arch Dis Child* 1978;**53**:38–42.
146. Stockmann C, Pavia AT, Hymas W, et al. Quantification of human rhinovirus viral load and disease severity among hospitalized children with pneumonia enrolled in the CDC Etiology of Pneumonia in the Community (EPIC) Study. Paper presented at the Annual Meeting of the Infectious Diseases Society of America; Abstract 80. San Diego, Calif, Oct. 9-12, 2012.
147. Tan TQ, Mason Jr EO, Barson WJ, et al. Clinical characteristics and outcome of children with pneumonia attributable to penicillin-susceptible and penicillin-nonsusceptible *Streptococcus pneumoniae*. *Pediatrics* 1998;**102**:1369–75.
148. The IMpact RSV Study Group. Palivizumab: a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 1998;**102**:531–7.
149. Thurman KA, Walter ND, Schwartz SB, et al. Comparison of laboratory diagnostic procedures for detection of *Mycoplasma pneumoniae* in community outbreaks. *Clin Infect Dis* 2009;**48**: 1244–9.
150. Thurman KA, Warner AK, Cowart KC, et al. Detection of *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella* spp. in clinical specimens using a single-tube multiplex real-time PCR assay. *Diagn Microbiol Infect Dis* 2011;**70**:1–9.
151. Tice AD, Rehm SJ, Dalovisio JR, et al. Practice guidelines for outpatient parenteral antimicrobial therapy. IDSA guidelines. *Clin Infect Dis* 2004;**38**:1651–72.
152. Trei JS, Johns NM, Garner JL, et al. Spread of adenovirus to geographically dispersed military installations, May-October 2007. *Emerg Infect Dis* 2010;**16**:769–75.
153. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;**7**:719–24.
154. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. *Nat Med* 2004;**10**:368–73.
155. Vento TJ, Prakash V, Murray CK, et al. Pneumonia in military trainees: a comparison study based on adenovirus serotype 14 infection. *J Infect Dis* 2011;**203**:1388–95.
156. Waites KB, Talkington DF. *Mycoplasma pneumoniae* and its role as a human pathogen. *Clin Microbiol Rev* 2004;**17**:697–728.
157. Wardlaw T, Salama P, Johansson EW, Mason E. Pneumonia: the leading killer of children. *Lancet* 2006;**368**:1048–50.
158. Waris ME, Toikka P, Saarinen T, et al. Diagnosis of *Mycoplasma pneumoniae* pneumonia in children. *J Clin Microbiol* 1998;**36**: 3155–9.
159. Waters AM, Kerecuk L, Luk D, et al. Hemolytic uremic syndrome associated with invasive pneumococcal disease: the United Kingdom experience. *J Pediatr* 2007;**151**:140–4.
160. Welliver RC. Pharmacotherapy of respiratory syncytial virus infection. *Curr Opin Pharmacol* 2010;**10**:289–93.
161. Welliver RC Sr. The immune response to respiratory syncytial virus infection: friend or foe? *Clin Rev Allergy Immunol* 2008; **34**:163–73.
162. Welliver TP, Garofalo RP, Hosakote Y, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis* 2007;**195**: 1126–36.
163. Werno AM, Anderson TP, Jennings LC, et al. Human metapneumovirus in children with bronchiolitis or pneumonia in New Zealand. *J Paediatr Child Health* 2004;**40**:549–51.
164. Wohl MEB. Developmental physiology of the respiratory system. In: Chernick V, Boat TF, Wilmott RW, Bush A, editors. *Kendig's disorders of the respiratory tract in children*. 7th ed. Philadelphia: Saunders; 2006. p. 23–8.
165. Wolf DG, Greenberg D, Shemer-Avni Y, et al. Association of human metapneumovirus with radiologically diagnosed community-acquired alveolar pneumonia in young children. *J Pediatr* 2010;**156**:115–20.
166. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005;**79**:884–95.
167. World Health Organization. *Pneumonia. Fact sheet No 331*. Geneva: World Health Organization; 2011.
168. Wunderink RG, Niederman MS, Kollef MH, et al. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis* 2012;**54**: 621–9.
169. Zhang L, Zong ZY, Liu YB, et al. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review & meta-analysis. *Indian J Med Res* 2011;**134**:270–80.

CHAPTER 23

Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis

Minh L. Doan • Leland L. Fan

Children's interstitial lung disease (ILD) encompasses a heterogeneous group of uncommon, diffuse lung disorders with varying clinical presentations and morbidity.[45] In many of these disorders, injury to the alveolar wall gives rise to an inflammatory response with subsequent repair that potentially can lead to pulmonary fibrosis. Infections of the lung, either chronic or acute with postinfectious sequelae, form the largest category of children's ILD in the immunocompetent and immunocompromised host. The discussion in this chapter is focused on the entities that are caused by or associated with infection and the entities that mimic community-acquired pneumonia.

CLASSIFICATION

An updated classification of the conditions that encompass children's ILD is presented in Table 23-1. Although a complete description of each disorder is beyond the scope of this chapter, certain conditions warrant brief mention because major differences exist between adult ILD and children's ILD in terms of types and distribution of disease. Usual interstitial pneumonitis, a progressive and fatal disorder, is the most common form of adult idiopathic ILD, but it probably does not occur in children. Also, hypersensitivity pneumonitis (HP) in adults is caused by a variety of occupational exposures but in children usually is caused by exposures to an avian antigen. In addition, several entities, such as neuroendocrine cell hyperplasia of infancy and pulmonary interstitial glycogenosis, are unique to infants and young children. Finally, the genetic basis for certain types of familial lung diseases has been linked to mutations in genes involved in surfactant metabolism.[69,121,122,160] With the exception of surfactant protein C and thyroid transcription factor–1 *(TTF-1)* mutations, because of high mortality rates, these disorders have been found mostly in children.

Disorders Associated with Infection

The role of infectious agents, such as adenovirus,[187] influenza,[99] *Mycoplasma*,[168] and *Chlamydia*,[6] in the development of chronic lung disease in children has been well documented. Virtually any organism that infects the lower respiratory tract is capable of producing chronic, diffuse lung disease if the injury is severe enough, although most infected children have acute, self-limited disease that resolves completely.

Bronchiolitis Obliterans

Probably the best example of postinfectious chronic lung disease is found in children who develop bronchiolitis obliterans after having severe adenoviral pneumonia.[187] Bronchiolitis obliterans is characterized by a fibrosing process of the small airways that results in severe, irreversible obstruction of the airways. Clinically, patients present with tachypnea, crackles, wheezing, and a productive cough that persist for more than 8 weeks after the initial illness. Chest radiographs commonly show a mixed pattern of hyperinflation, persistent atelectasis, and peribronchial thickening; on high-resolution computed tomography (HRCT) in which both inspiratory and expiratory images are obtained, a combination of mosaic perfusion due to air trapping, central bronchiectasis, and vascular attenuation is highly specific for bronchiolitis obliterans, although not entirely sensitive.[25,118] Occasionally, severe involvement of one lung leads to the development of a unilateral, small, hyperlucent lung, known as Swyer-James syndrome.[187] Pulmonary function testing demonstrates an obstructive pattern characterized by a disproportionate reduction in the midexpiratory flow (FEF_{25-75}) that is usually fixed, although a small response to bronchodilator may be seen.[118]

Patients with severe adenovirus pneumonia have been shown to have immune complexes containing adenovirus antigen in the lung and increased serum levels of interleukin-6, interleukin-8, and tumor necrosis factor–α.[116,117] These studies suggest that abnormal or excessive host immunologic and inflammatory responses may be important in the development of chronic lung disease from adenovirus in infants and young children. Although lower respiratory tract infection with *Mycoplasma*,[27] respiratory syncytial virus, parainfluenza, influenza, measles, varicella,[189] and pertussis[29] also can result in bronchiolitis obliterans, adenovirus is the most common etiologic agent. The need for mechanical ventilation during the initial illness is a strong independent

TABLE 23-1 Classification of Children's Interstitial Lung Diseases

Disorders Unique to Infancy

Diffuse developmental disorders
- Acinar dysplasia
- Congenital alveolar dysplasia
- Alveolar capillary dysplasia with misalignment of pulmonary veins

Growth abnormalities
- Chronic neonatal lung disease (in premature and term infants)
- Secondary pulmonary hypoplasia
- Chromosomal abnormalities (i.e., trisomy 21)
- Associated with congenital heart disease

Surfactant dysfunction disorders
- Surfactant protein B mutations
- Surfactant protein C mutations
- *ABCA3* mutations
- *TTF-1* mutations
- Histology consistent with surfactant dysfunction disorder without recognized genetic disorder

Neuroendocrine cell hyperplasia of infancy

Pulmonary interstitial glycogenosis

Primary Lung Disease

Idiopathic interstitial pneumonias
- Nonspecific interstitial pneumonia
- Cryptogenic organizing pneumonia
- Acute interstitial pneumonia
- Desquamative interstitial pneumonia
- Lymphocytic interstitial pneumonia

Alveolar hemorrhage syndromes
- Includes syndromes with and without pulmonary capillaritis

Pulmonary alveolar proteinosis
- Includes congenital, primary, and secondary causes

Pulmonary microlithiasis

Pulmonary vascular disorders

Pulmonary lymphatic disorders
- Lymphangiomatosis
- Lymphangiectasis

Pulmonary infiltrates with eosinophilia
- Includes primary and secondary causes (drugs, parasites, infections, toxins)

Systemic Disorders with Pulmonary Involvement

Connective tissue disease
- Includes systemic lupus erythematosus, systemic sclerosis, Sjögren syndrome

Malignancies

Histiocytosis

Sarcoidosis

ILD of Specific (Known) Cause

Storage disorders

Aspiration syndromes

Postinfectious bronchiolitis obliterans

Drug-induced lung disease

Hypersensitivity pneumonitis

risk factor for the subsequent development of this disease.[35]

Lymphocytic Interstitial Pneumonitis

Well recognized because it is an acquired immunodeficiency syndrome (AIDS)–defining condition in children infected with human immunodeficiency virus (HIV),[22] lymphocytic interstitial pneumonitis (LIP) also occurs in association with other immunodeficiencies, such as common variable immunodeficiency,[178] autoimmune disorders such as juvenile rheumatoid arthritis, and Sjögren syndrome,[44,104,177] and in idiopathic and familial forms.[123] LIP is not a true interstitial pneumonitis but rather is a form of pulmonary lymphoproliferative disease. It is characterized histologically by a diffuse infiltrate of mature lymphocytes, along with smaller numbers of plasma cells and histiocytes, into the pulmonary interstitium, alveolar wall, and along lymphatic pathways.[93,94] Generally, the lymphocytes are polyclonal, with B cells and a variety of T cells being identified.[135] The etiology of LIP remains unknown, but it is thought that coinfection with Epstein-Barr virus (EBV) and HIV may produce the lymphoproliferative response.[5,57,89] EBV has also been implicated in LIP in non–HIV-infected patients.[107]

LIP occurs in 30 percent of children infected perinatally with HIV and typically presents between the second and third years of life as an insidious onset of cough, tachypnea, dyspnea, and hypoxemia.* Bilateral nontender parotid enlargement is frequently present and may help to differentiate LIP from other pulmonary complications of HIV infection.[158,161] Chest radiographs characteristically reveal a diffuse, symmetric reticulonodular or nodular pattern, occasionally with mediastinal or hilar adenopathy,[108] although HRCT is more sensitive and allows for the identification of the subpleural and perilymphatic distribution of the micronodules.[10] Among HIV-infected children, the incidence of acute lower respiratory tract infection is higher in children with LIP,[158] and these patients ultimately may develop bronchiectasis.[159]

Other Conditions

Other pediatric chronic lung diseases have been associated with various infectious agents. The role of perinatal infection or colonization with *Ureaplasma urealyticum* in inducing pulmonary inflammation and the subsequent development of bronchopulmonary dysplasia in premature neonates remains controversial.[11,86,154] Parvovirus has been linked to autoimmune disease associated with ILD and other organ involvement.[13] Finally, attempts have been made to link *Stachybotrys chartarum* and its mycotoxins to acute and recurrent pulmonary hemorrhage in infants, although a causal relationship has not been proven.[23]

In a prospective study of immunocompetent children with chronic diffuse infiltrates, Fan and coworkers[60] found an infectious agent as the underlying cause in 10 (20%) of 51 children with ILD. Identified agents included adenovirus alone in 4 children, adenovirus and cytomegalovirus in 2, varicella in 1, EBV in 1, *Chlamydia* in 1, and *Toxocara* in one. Chronic infections or long-term sequelae from acute infections account for many cases of ILD of known etiology in children.

*References 87, 135, 147, 148, 158, 161.

Entities That May Mimic Community-Acquired Pneumonia

Acute Eosinophilic Pneumonia

Acute eosinophilic pneumonia is the most severe form of the idiopathic eosinophilic pneumonias. It is characterized histologically by very large numbers of eosinophils infiltrating the alveoli and interstitium, with resultant acute respiratory failure. Acute eosinophilic pneumonia can be idiopathic or result from inciting triggers such as drugs (minocycline, sertraline) and inhalational exposures (recent-onset cigarette smoking). Patients present with acute onset of fever, dyspnea, and cough; pleuritic chest pain; and myalgias. On examination, crackles are present in 80 percent of patients; wheezing is a rare manifestation. Hypoxemia is uniformly present, and patients can progress rapidly to respiratory failure with a clinical picture of acute respiratory distress syndrome. In contrast to patients with other eosinophilic lung diseases, patients with acute eosinophilic pneumonia generally do not have significant peripheral eosinophilia (>350 cells/mm^3) on presentation as a diagnostic clue. Chest radiographs and CT scans show bilateral infiltrates with a variable mix of interstitial and alveolar patterns; pleural effusions are present in 50 percent of cases. Flexible bronchoscopy to obtain a true bronchoalveolar lavage (BAL) is necessary for the diagnosis, and fluid analysis shows marked eosinophilia (>20%) in most patients. Although pleural fluid also has increased eosinophils, this finding in isolation is not specific for acute eosinophilic pneumonia. Treatment with corticosteroids (i.e., methylprednisolone 2 to 4 mg/kg/day) usually results in a rapid and complete resolution, and recurrences have not been reported.[3,143]

Pulmonary Vasculitis

In children, the spectrum of pulmonary vasculitides includes granulomatosis with polyangiitis (Wegener), Churg-Strauss syndrome, microscopic polyangiitis, and pulmonary capillaritis. These disorders can present as diffuse alveolar hemorrhage, with fever, dyspnea, hemoptysis (may not present in a third of patients), anemia, and patchy alveolar infiltrates on chest radiographs. Other patients may have fever and cavitary or nodular lesions on imaging.[80] Diffuse alveolar hemorrhage can be diagnosed with BAL, when sequentially recovered aliquots of fluid show persistently bloody return. If hemosiderin-laden macrophages are seen on BAL cytology, then this suggests that the blood has been present in the alveolar spaces for at least 3 days and is not the result of the bronchoscopy itself.[54] Renal involvement may sometimes be present, with microscopic hematuria and red blood cell casts seen on urinalysis. A positive serum antineutrophil cytoplasmic antibody (ANCA) may be seen, with antiproteinase-3 antibody resulting in a cytoplasmic (c-ANCA) staining pattern being associated with Wegener granulomatosis and with antimyeloperoxidase antibody giving a perinuclear (p-ANCA) pattern seen in Churg-Strauss syndrome and microscopic polyangiitis.[37] The presence of significant peripheral and pulmonary eosinophilia would support the diagnosis of Churg-Strauss syndrome.[15] Pulmonary capillaritis is associated with a variety of conditions and requires a surgical lung biopsy for diagnosis because it may present as an ANCA-negative, hematuria-negative diffuse alveolar hemorrhage syndrome.[68] Therapeutic options for pulmonary vasculitis include systemic corticosteroids, cyclophosphamide, intravenous immunoglobulin, and rituximab[21]; aggressive treatment is required in severe cases.

Connective Tissue Disorders

Pulmonary manifestations can precede other systemic findings in children with systemic lupus erythematosus,[34] juvenile rheumatoid arthritis,[144] and Behçet disease,[178] and these patients may present with acute respiratory symptoms associated with fever. In acute lupus pneumonitis, signs and symptoms include high fevers, dyspnea, tachypnea, crackles, and cyanosis. Chest radiographs may show areas of consolidation and pleural effusions or a pattern of interstitial infiltrates with elevation of the hemidiaphragms.[110] Other pulmonary manifestations of systemic lupus erythematosus include pulmonary hemorrhage, pulmonary hypertension, ILD, pneumothorax, and shrinking lung syndrome. Multisystem involvement, including renal and skin, may provide clues to the underlying diagnosis. Treatment usually requires corticosteroids and other immunosuppressive agents.[34]

Hypersensitivity Pneumonitis

Acute episodes of HP may present as fever, cough, dyspnea, and pulmonary infiltrates (see later section for a more detailed discussion).

CLINICAL PRESENTATION

Most children with ILD have insidious symptoms that may go unrecognized for years. Some children have been misdiagnosed as having asthma and have been treated with bronchodilators and inhaled corticosteroids.[64] Although a history of wheezing can be elicited in half of patients, it can be documented by physical examination in only approximately 20 percent of cases. Clinical suspicion for children's ILD should arise when patients meet at least three of the four following criteria: (1) presenting symptoms of dyspnea, tachypnea, retractions, cough, exercise intolerance, or respiratory failure; (2) presenting signs of crackles, failure to thrive, clubbing, or respiratory failure; (3) hypoxemia; and (4) diffuse abnormality on chest radiographs or CT not attributable to other known processes.[60]

A careful history should be taken to assess the severity of the disease and to obtain information that may contribute to establishing a diagnosis. A search for precipitating factors should include a history of feeding difficulties that may suggest aspiration; any prior acute or severe respiratory tract infections; and environmental exposures, especially to birds or molds. Hemoptysis may indicate a pulmonary vascular disorder or hemosiderosis. Joint disease or rash may indicate a systemic process, such

as a connective tissue disease. A family history of relatives or siblings with similar respiratory conditions may be clues to genetic or familial lung diseases, such as inborn errors of surfactant metabolism.

On physical examination, tachypnea and retractions often are observed, and crackles commonly are heard, particularly at the bases. In severe cases, cyanosis, clubbing, an accentuated pulmonic component of the second heart sound, and evidence of growth failure are seen. Oxygen saturation usually is normal under all conditions in most patients with mild disease, but desaturation may occur with exercise or during sleep as the disease progresses and ventilation-perfusion mismatch ensues. Patients with more advanced disease are hypoxemic at rest.

DIAGNOSTIC EVALUATION

A systematic approach to children's ILD is essential to physicians confronted with such a large differential of rare conditions. Diagnostic studies can be divided into studies used to assess the extent and severity of disease, to identify disorders that predispose to ILD, and to identify the primary ILD (Table 23-2). In adults with ILD, Raghu[137] suggested a diagnostic process that employs a thorough history and physical examination first, noninvasive tests next, and then invasive studies, including BAL and transbronchial biopsy followed by open lung biopsy if the previous less invasive studies do not provide a specific diagnosis. Based on experience from a retrospective chart review of 48 children with ILD, Fan and colleagues[64] independently developed an algorithm remarkably similar to that of Raghu's and used it prospectively in the evaluation of 51 children presenting with ILD.[60] In that study, a specific diagnosis was established by history and physical examination alone in 1 patient; noninvasive studies alone in 8 others; and invasive studies, including lung biopsy, in another 26. Of the remaining patients, 8 had a suggestive diagnosis and 8 had no specific diagnosis. This study suggests that a systematic approach to the diagnosis of children's ILD is useful and that some patients can be diagnosed with noninvasive studies, but most patients require invasive studies, including lung biopsy.

TABLE 23-2 **Diagnostic Studies for Pediatric Interstitial Lung Disease**

To Assess Extent and Severity of Disease
Chest radiography, high-resolution computed tomography
Pulmonary function studies: spirometry, pulse oximetry and arterial blood gases (resting, sleeping, and with exercise), diffusion, pressure-volume curve, infant studies
Electrocardiography, echocardiography
To Identify Primary Disorders That Predispose to Interstitial Lung Disease
HIV test
Immune studies: immunoglobulins including IgE, skin tests for delayed hypersensitivity, response to immunizations, T- and B-cell subsets, complement, others as indicated
Barium swallow, pH/impedance probe
DNA analyses for mutations in the surfactant protein B, surfactant protein C, and *ABCA3* and *TTF-1* genes
To Identify Primary Interstitial Lung Disease
Antinuclear antibody
Angiotensin-converting enzyme
Antineutrophil cytoplasmic antibody
Antiglomerular basement membrane antibody
Hypersensitivity screen
Infectious disease evaluation—cultures, titers, skin tests
Cardiac catheterization (in selected cases)
Bronchoalveolar lavage and transbronchial biopsy
Transthoracic biopsy

HIV, human immunodeficiency virus.
From Fan LL. Pediatric interstitial lung disease. In: Schwarz MI, King TE, editors. *Interstitial lung disease.* 4th ed. Hamilton, Ontario: BC Decker; 2003. p. 134–51.

Pulmonary Function Tests

In children who are old enough to undergo pulmonary function tests, standard spirometry typically shows a pattern of restrictive lung disease, with reduced forced vital capacity (FVC), forced expiratory volume in 1 second (FEV_1), and a normal or elevated FEV_1/FVC ratio.[64] On measurement of lung volumes, although the total lung capacity (TLC) often is low, the residual volume (RV) may be normal or elevated, resulting in an increased RV/TLC ratio that suggests air trapping and a mixed restrictive/obstructive pattern. These findings also can be documented in infants and young children with the use of infant pulmonary function testing techniques.

High-Resolution Computed Tomography

In the evaluation of children's ILD, HRCT is an important diagnostic modality used to provide precise detail about the extent and distribution of the lung disease, to select favorable sites for surgical lung biopsy, and, when classic patterns of abnormalities are seen, to either make a confident diagnosis for some conditions or to guide specific but less-invasive testing (genetic testing, autoantibody levels, bronchoscopy) for other conditions.[181] However, although a chest CT can be readily ordered, it is often not performed in the manner necessary to optimally evaluate for diffuse lung diseases in children. It is therefore critical that adequate consideration be given for the technique requested, so as to avoid an unhelpful exposure to ionizing radiation to the patient.

With HRCT, the thin (usually 1.25 mm or 0.625 mm in thickness) sections or reformatted scans obtained avoid the volume averaging that occurs with 2.5- to 5-mm sections, which obscures the fine parenchymal detail and airway abnormalities. The conventional noncontiguous axial HRCT technique results in lower radiation exposure but is more technically demanding, has more motion artifact, risks missing abnormal findings, and does not allow for a complete evaluation of the central airways and mediastinum. On the other hand, the multidetector CT scanners now widely available can rapidly acquire a

volumetric data set of the entire thorax, from which thin reformatted images can be shown in a contiguous fashion or at a specified interval, depending on the number of images desired for review. On some scanners, the bone reconstruction algorithm gives better lung image resolution than does the lung reconstruction algorithm. If appropriate pediatric protocols are used, a volumetric pediatric chest CT scan with high-resolution reformatted images can be performed with radiation doses of less than 1.5 mSv (equivalent to 6 months of natural background radiation exposure).[74]

Obtaining high-quality CT images of the lungs requires the avoidance of respiratory motion artifact. In younger children and infants in whom cooperation for breath-holding is impossible and in whom rapid respiratory rates especially predispose to motion artifact, a sedated controlled-ventilation CT technique can be used.[103] Giving several assisted deep breaths using mask ventilation to these sedated young children results in a short period of apnea during which the lungs can be fully inflated to obtain the routine inspiratory images. Expiratory images should also be considered to assess for air trapping and can be acquired after allowing for passive deflation of the lungs or with voluntary end-expiratory breath-holding. General anesthesia produces similar results, although frequent large sigh breaths are necessary to prevent dependent atelectasis, which occurs within minutes of intubation.[74]

In studies that have investigated the diagnostic accuracy of HRCT in pediatric diffuse lung disease, the first-choice diagnosis was proven correct in 38 to 61 percent of cases by subsequent surgical lung biopsy.[36,105,183] CT of pediatric diffuse lung diseases detailing the classic findings seen with certain of these conditions has been recently reviewed.[74]

Bronchoalveolar Lavage

BAL via flexible bronchoscopy allows for sampling of the alveolar lining fluid to assess its gross appearance and to send for measurement of cell count and differential, cytologic examination, microbial cultures, as well as molecular and biochemical analyses, all of which may be helpful in the evaluation of certain cases of children's ILD. A milky appearance of the BAL fluid with a layering of sediment strongly suggests pulmonary alveolar proteinosis, whereas the progressively bloody appearance of the fluid in sequential aliquots indicates the presence of an alveolar hemorrhage syndrome. Normal indices for pediatric BAL fluid have been described, against which abnormal results can be compared.[56] Finding significant eosinophilia or lymphocytosis in the BAL would substantially narrow the differential diagnoses, and detection of lipid-laden or hemosiderin-laden macrophages can serve as sensitive, albeit not specific, markers for aspiration or alveolar hemorrhage syndromes. Cytologic examination can also be used to diagnose pulmonary alveolar proteinosis, lysosomal storage disorders, and histiocytosis.[63]

The most common indication for pediatric BAL has been to detect infection in the immunocompromised host. The use of quantitative bacterial cultures may help to differentiate whether recovered organisms represent true infection, colonization, or contamination, whereas polymerase chain reaction (PCR) techniques allow for increased sensitivity in detecting *Pneumocystis jiroveci* pneumonia in non–HIV-immunocompromised patients[128] and for the rapid detection of *Mycobacterium tuberculosis*.[131] Cytologic examination may identify cases of aspergillosis not otherwise detected by culture alone,[4] and measurement of galactomannan levels in BAL has also been used for early diagnosis of invasive aspergillosis.[46]

When BAL should be performed may be important, because investigators have shown in a murine model that hemosiderin-laden macrophages first appear at 3 days, peak at 1 week, and persist in small numbers for 2 months after a single episode of hemorrhage.[54] In adult hematopoietic stem cell transplantation recipients with new pulmonary infiltrates, the diagnostic yield of BAL was highest when performed within 24 hours of presentation.[157]

Finally, while bronchial washings obtained via a suction catheter introduced through an endotracheal tube have proven useful in the evaluation of ventilator-associated pneumonia,[149] they do not adequately sample the alveolar lining fluid and therefore give a different cellular profile than that obtained by bronchoscopic BAL.[92] This discrepancy may result in missed detection of conditions such as acute eosinophilic pneumonia if only the former is performed.

Lung Biopsy

As in adult ILD, lung biopsy is the gold standard for establishing the diagnosis of children's ILD because most diseases are classified in terms of previously defined histopathologic patterns. Although transbronchial or percutaneous needle biopsy may be helpful in certain conditions, a transthoracic approach, usually by video-assisted thoracoscopic surgery (VATS), remains the gold standard for obtaining tissue adequate for diagnosis. Technical advances and now widespread experience with VATS, including in infants,[146] make it the method of choice, especially in light of research demonstrating comparable diagnostic yield in the evaluation of children with ILD (54% with VATS, 57% with open-lung biopsy).[61] Selection of biopsy sites should be determined by findings on HRCT, which may, in selected circumstances, actually obviate the need for lung biopsy to establish the diagnosis.

Lung biopsy material must be processed in a consistent manner to ensure optimal interpretation, and a protocol for such handling was published based on the recommendations of the Children's Interstitial Lung Disease (chILD) Pathology Group.[98] A general scheme for division of the biopsy specimen is as follows: (1) microbiology cultures, 35 percent; (2) snap-frozen for PCR or other molecular studies, 10 percent; (3) snap-frozen in cryomatrix for immunofluorescent, laser capture, or other studies requiring frozen sections, 10 percent; (4) fixed in glutaraldehyde for electron microscopy, less than 5 percent; (5) imprints for cytologic examination or rapid identification of organisms, 0 percent; and (6) expanded and fixed in formalin (methods previously described[48]) for light microscopy, 40 percent. It is

crucial that the biopsy material be interpreted by a pathologist with considerable expertise in pediatric lung disease because the normal lung of an infant differs greatly from that of an older child or adolescent and any pathologic finding needs to be interpreted in light of the normal age-dependent variations of lung architecture.

TREATMENT

Supportive care is important in the management of children's ILD. Good nutrition is paramount, to the extent that gastrostomy feedings should be considered in selected patients who have poor weight gain despite maximal conventional treatment.[181] Nocturnal or continuous oxygen therapy should be provided in children who are hypoxemic to decrease the risk for the development of pulmonary hypertension. Annual influenza vaccination through the injectable route should be given. Avoiding inhalant hazards such as tobacco smoke should be stressed. Patients with underlying systemic disorders need primary treatment for that disorder, such as intravenous gamma globulin for hypogammaglobulinemia. Specific therapy for primary ILD should be used when possible, such as aggressive management of identified causes of aspiration syndromes, lung lavage and inhalational granulocyte-macrophage colony-stimulating factor for certain types of pulmonary alveolar proteinosis,[142,191] and interferon-α for pulmonary hemangiomatosis.[130] When environmental agents such as bird antigens are causative, avoiding contact with them is crucial (see later section on HP).

Generally, corticosteroids remain the treatment of choice for most patients with ILD on the presumption that suppression of inflammation may reduce the risk for developing fibrosis.[62] Although controlled clinical studies are lacking,[101] corticosteroids have been used to treat such diverse types of diffuse lung diseases as desquamative interstitial pneumonitis, nonspecific interstitial pneumonitis, acute interstitial pneumonia, cryptogenic organizing pneumonia, lymphocytic interstitial pneumonia, HP, eosinophilic pneumonia, diffuse alveolar hemorrhage, ILD associated with connective tissue diseases, and surfactant protein C mutations.[173,180] In a retrospective study of pediatric ILD by Fan and coworkers,[64] corticosteroids were judged to be effective in 40 percent (12 of 30) of treated children in terms of improved clinical status, decreased oxygen requirements, and improved pulmonary function. A trial of prednisone or equivalent corticosteroid in a dose of 1 to 2 mg/kg/day for at least 6 to 8 weeks probably is warranted, although it may be preferable to instead use intravenous pulses of methylprednisolone 30 mg/kg, with a maximum dose of 1 g, given for either 3 consecutive days every month or 1 day every week, based on experience that pulse intravenous therapy is at least as effective as oral therapy and has fewer side effects.[181]

Alternative immunomodulatory agents that have also been used include hydroxychloroquine, azathioprine, cyclophosphamide, methotrexate, cyclosporine, intravenous gamma globulin, and mycophenylate mofetil.[150] Of these, hydroxychloroquine probably has been used most frequently.[7,64,158,164] The precise mechanism of action is unknown, but chloroquine and hydroxychloroquine have shown immunosuppressive effects with the ability to inhibit the functional capabilities of monocytes and the generation of antibody-forming cells. Hydroxychloroquine is preferred over chloroquine because the former has less retinal toxicity. The recommended dose in children for the treatment of ILD is 10 mg/kg/day. Additionally, there has been a case report describing the successful use of infliximab, an anti–tumor necrosis factor–α monoclonal antibody, in reversing bronchiolitis obliterans in a hematopoietic stem cell transplant recipient.[67] Finally, treatment regimens for ILD associated with systemic sclerosis,[184] Langerhan cell histiocytosis,[115] and Wegener granulomatosis[18] have been reviewed. The fact that many alternative pharmacologic approaches are considered for children and adults with ILD implies that conventional therapy often is ineffective. New strategies are being developed based on animal models of pulmonary fibrosis and more recent advances in the cellular and molecular biology of inflammatory reactions. Such therapies would be directed against the action of certain cytokines, oxidants, and growth factors that may be involved in the fibrotic process. One such potential agent is pirfenidone, which may have some clinical benefit in the treatment of idiopathic pulmonary fibrosis in adults.[170] The potential to deliver specific inflammatory inhibitors or inhibitors of collagen biosynthesis directly to the lung via aerosolization suggests that disease processes in the lung may be more amenable to novel therapies than are disease processes in other internal organs.

Lung transplantation can be used as a final therapeutic modality for some forms of children's ILD that progress to irreversible respiratory failure secondary to pulmonary fibrosis. Although the overall 5-year survival after lung transplantation is still disappointing at approximately 50 percent,[12] it appears that outcomes for children with diffuse lung disease are comparable to those undergoing transplantation for cystic fibrosis or pulmonary hypertension.[138] In certain systemic conditions, such as hereditary pulmonary alveolar proteinosis, however, transplantation may not be appropriate because the primary disease may recur in the transplanted lungs.[109,153]

OUTCOME

The prognosis of children with ILD varies. Infants with neuroendocrine cell hyperplasia of infancy generally do well, although they may be symptomatic and require oxygen for years.[49] At the other end of the spectrum, children with growth failure, pulmonary hypertension, and severe fibrosis do poorly. A survey that likely included many different types of pediatric interstitial lung diseases found an overall mortality rate of 15 percent.[47]

Fan and Kozinetz[59] reviewed the outcome of 99 children with chronic ILD seen in Denver, Colorado, over 15 years (1980-1994). As expected, a wide variety of disorders were encountered, and 15 recorded deaths occurred, with a probability that a patient would survive to 24 months, 48 months, and 60 months after onset of symptoms of 83 percent, 72 percent, and 64 percent,

respectively. Of the clinical features present at the time of initial evaluation, weight less than the fifth percentile, crackles, clubbing, family history of ILD, and symptom duration were not associated with decreased survival rates. A severity of illness score, based on increasing levels of hypoxemia and the presence or absence of pulmonary hypertension, was related significantly to survival, with an increasing score associated with a higher probability of decreased survival rates. A simple scoring system seems to be a useful measure of outcome in children with ILD.

HYPERSENSITIVITY PNEUMONITIS

HP, also known as *extrinsic allergic alveolitis*, is a form of immune-mediated ILD that develops in response to repeated inhalation of finely dispersed organic antigens.[40] HP should be considered in the differential diagnosis of a child who presents with acute or chronic respiratory symptoms associated with fever,[16] and obtaining an environmental exposure history is essential to arrive at the proper diagnosis. A wide variety of organic particles, including mammalian and avian proteins, fungi, thermophilic bacteria, and certain low-molecular-weight volatile and nonvolatile chemical compounds, are known to induce HP in susceptible individuals.[2] Certain systemic medications, such as ciprofloxacin,[165] dapsone,[174] and methotrexate,[41] also have been reported as triggers.

In general, three forms of HP have been described: (1) acute (or episodic), with improvement between attacks; (2) insidious (gradual onset and progressive course) with superimposed acute episodes; and (3) insidious without acute attacks.[79] Although exposure to antigens capable of provoking HP occurs commonly in the home and work environment, the overall incidence of the condition in the general population is low.[16,163] It is estimated that only 5 to 15 percent of individuals exposed to high levels of a specific organic antigen develop clinical disease.[65,184]

Pathology and Pathogenesis

Pathologically, HP is characterized by a diffuse, lymphoplasmacytic cell infiltration of the small airways and pulmonary parenchyma, often with associated multinucleated giant cells containing cholesterol clefts and poorly formed, non-necrotizing granulomas.[70] Foamy macrophages are seen commonly in the airspaces. With advanced disease, interstitial and intra-alveolar fibrosis develops that is indistinguishable from other causes of pulmonary fibrosis.

The mechanisms by which organic dusts induce these characteristic pathologic features of the disease are not completely understood, but it is thought that both type III (immune complex–mediated) and type IV (delayed) hypersensitivity mechanisms are involved.[133] A type III reaction is suggested by the presence of precipitating antibody to the offending antigen, immune complex deposition, and activation of complement. A type IV reaction is suggested by an increased percentage of T lymphocytes in BAL fluid, with a strong predominance of $CD8^+$ subsets and a low CD4/CD8 ratio, and the presence of granulomas on lung biopsy specimen. Considering the small proportion of exposed individuals who develop clinical symptoms, complex interactions among the nature of the antigen, the intensity and duration of the exposure, and the host response in affected individuals most likely are involved. Additionally, genetic factors[19] and possibly associated viral infections[43] appear to contribute to susceptibility, whereas cigarette smoking (nicotine) seems to confer a protective effect.[84] Familial cases of HP have been identified.[50]

Etiology

As shown in Table 23-3, HP in adults is caused by a wide variety of occupational and environmental exposures.[155] In contrast, in children, HP is caused mainly by exposure to an array of domestic birds (69%) and fungi (29%), based on a review of 164 reported pediatric cases. In HP due to bird exposure (bird fancier's lung), bird droppings and feathers are the major sources of the offending antigens.[112] Although exposure to pet birds is usually the cause,[119] even low levels of environmental exposure to wild birds in the yard or birds raised by a neighbor may be enough to induce clinical HP,[97,152] as may contact with feather-filled household items such as feather pillows, down comforters, and feather duvets.[16,83,119] The onset of clinical disease typically follows months to years of continuous or intermittent exposure to the offending antigen,[79] and it has been demonstrated that bird antigens can persist for months in the home environment even after removal of the birds.[39] Possible sources of mold exposure are varied and can include contaminated air humidifiers,[134] indoor hydroponics,[53] organic compost in a play area,[1] moist hay,[172] and even wind instruments,[113] in addition to overt mold contamination in areas of water damage or in poorly ventilated spaces exposed to moisture.[81,171]

Clinical Presentation

Although a wide variety of antigens can induce HP, the immunologic response and clinical presentation are similar. In the reported pediatric cases referenced previously, the mean age (± standard deviation) was 9.7 (± 4.0) years. The youngest reported patient with HP developed symptoms at 10 weeks of age.[172]

Acute episodes of HP mimic flulike illnesses, manifesting as high fevers, chills, dry cough, dyspnea, myalgias, and malaise. These symptoms begin several hours after exposure and diminish during the next 12 to 24 hours, provided that no additional exposure to the antigen occurs. Physical examination reveals a dyspneic, ill-appearing child, often with bibasilar crackles. Transient hypoxemia and nodular pulmonary infiltrates often are present. Hospitalization for presumed pneumonia often leads to resolution of symptoms due to removal from the causative environment.

More commonly, children present with more insidious and progressive symptoms, which may or may not be punctuated by intermittent acute manifestations. In the reported pediatric cases in which the following specific symptoms were recorded, exercise intolerance was

TABLE 23-3 **Etiologic Agents of Hypersensitivity Pneumonitis**

Disease	Antigen	Source
Fungal and Bacterial		
Farmer's lung	*Faeni rectivirgula*	Moldy hay, grain, silage
Ventilation pneumonitis; humidifier lung; air conditioner lung	*Thermoactinomyces vulgaris, Thermoactinomyces sacchari, Thermoactinomyces candidus, Klebsiella oxytoca*	Contaminated forced air systems, water reservoirs
Bagassosis	*T. vulgaris*	Moldy sugarcane (i.e., bagasse)
Mushroom worker's lung	*T. sacchari*	Moldy mushroom compost
Suberosis	*Thermoactinomyces viridis, Penicillium glabrum*	Moldy cork
Detergent lung; washing powder lung	*Bacillus subtilis* enzymes	Detergents (during processing or use)
Malt worker's lung	*Aspergillus fumigatus, Aspergillus clavatus*	Moldy barley
Sequoiosis	*Graphium, Pullularia,* and *Trichoderma* spp.; *Aureobasidium pullulans*	Moldy wood dust
Maple bark stripper's lung	*Cryptostroma corticale*	Moldy maple bark
Cheese washer's lung	*Penicillium casei, A. clavatus*	Moldy cheese
Woodworker's lung	*Alternaria* spp., wood dust	Oak, cedar, and mahogany dust; pine and spruce pulp
Paprika slicer's lung	*Mucor stolonifer*	Moldy paprika pods
Sauna taker's lung	*Aureobasidium* spp., other sources	Contaminated sauna water
Familial HP	*B. subtilis*	Contaminated wood dust in walls
Wood trimmer's lung	*Rhizopus* spp., *Mucor* spp.	Contaminated wood trimmings
Composter's lung	*T. vulgaris, Aspergillus* spp.	Compost
Basement shower HP	*Epicoccum nigrum*	Mold on unventilated shower
Hot-tub lung	*Cladosporium* spp.	Hot-tub mists, mold on ceiling
Wine maker's lung	*Botrytis cinerea*	Mold on grapes
Woodsman's disease	*Penicillium* spp.	Oak and maple trees
Thatched-roof lung	*Saccharomonospora viridis*	Dead grasses and leaves
Tobacco grower's lung	*Aspergillus* spp.	Tobacco plants
Potato riddler's lung	*Thermophilic actinomycetes, F. rectivirgula, T. vulgaris, Aspergillus* spp.	Moldy hay around potatoes
Summer-type pneumonia	*Trichosporon cutaneum*	Contaminated old houses
Dry rot lung	*Merulius lacrymans*	Rotten wood
Stipatosis	*A. fumigatus, T. actinomycetes*	Esparto dust
Machine operator's lung	*Pseudomonas fluorescens*	Aerosolized metal-working fluid
Amebae		
Humidifier lung	*Naegleria gruberi, Acanthamoeba polyphaga, Acanthamoeba castellani*	Contaminated water
Animal Proteins		
Pigeon breeder's or pigeon fancier's disease	Avian droppings, feathers, serum	Parakeets, budgerigars, pigeons, chickens, turkeys
Pituitary snuff taker's lung	Pituitary snuff	Bovine and porcine pituitary proteins
Fish meal worker's lung	Fish meal	Fish meal dust
Bat lung	Bat serum protein	Bat droppings
Furrier's lung	Animal fur dust	Animal pelts
Animal handler's lung; laboratory worker's lung	Rats, gerbils	Urine, serum, pelts, proteins
Insect Proteins		
Miller's lung	*Sitophilus granarius*	Dust-contaminated grain (i.e., wheat weevil)
Lycoperdonosis	Puffball spores	*Lycoperdon* puffballs
Chemical		
Pauli's reagent alveolitis	Sodium diazobenzene sulfate	Laboratory reagent
Chemical worker's lung	Isocyanates, trimellitic anhydride	Polyurethane foams, spray paints, elastomers, special glues
Vineyard sprayer's lung	Copper sulfate	Bordeaux mixture
Pyrethrum HP	Pyrethrum	Pesticide

TABLE 23-3 **Etiologic Agents of Hypersensitivity Pneumonitis** (Continued)

Disease	Antigen	Source
Epoxy resin lung	Phthalic anhydride	Heated epoxy resin
Dental technician's lung	Methyl methacrylate	Dental prosthesis
Unknown		
Bible printer's lung		Moldy typesetting water
Coptic lung (mummy handler's lung)		Cloth wrappings of mummies
Grain measurer's lung		Cereal grain
Coffee worker's lung		Coffee bean dust
Tap water lung		Contaminated tap water
Tea grower's lung		Tea plants
Mollusk-shell HP		Sea-snail shell
Swimming pool worker's lung		Aerosolized endotoxin from pool water, sprays, and fountains

From Selman M. Hypersensitivity pneumonitis. In: Schwarz MI, King TE, editors. *Interstitial lung disease*. 4th ed. Hamilton, Ontario: BC Decker; 2003. p. 452–84.

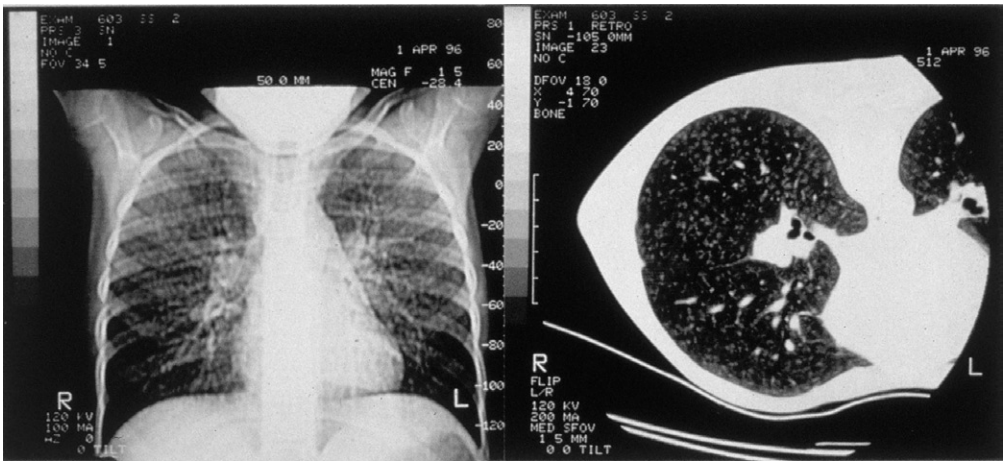

FIGURE 23-1 ■ Hypersensitivity pneumonitis from cockatiel antigens in an adolescent. Chest radiograph shows bilateral reticulonodular infiltrates. HRCT shows diffuse, multiple fine nodules. (Courtesy Robin Deterding, MD, University of Colorado, Denver, CO. From Fan LL. Pediatric interstitial lung disease. In: Schwarz MI, King TE, editors. *Interstitial lung disease*. 4th ed. Hamilton, Ontario: BC Decker; 2003. p. 134–51.)

present in 96 percent (101 of 105), cough was present in 89 percent (102 of 115), weight loss was present in 76 percent (58 of 76), and fever was present in 46 percent (38 of 82). On physical examination, crackles were present in 65 percent (66 of 101) and clubbing was present in 28 percent (19 of 68) of cases. Cough can be productive of thick, clear mucus, and wheezing and rhonchi are sometimes noted on examination, leading to a misdiagnosis of asthma or bronchitis, especially because there may be a partial response to a bronchodilator and temporary clearance of symptoms with systemic corticosteroids.[85]

Diagnosis

Establishing a diagnosis of HP requires maintaining a high index of suspicion, especially when patients have insidious chronic symptoms without episodic febrile illnesses. It is not uncommon that children who are eventually diagnosed with HP will have been treated with inhaled β_2 agonists and corticosteroids before referral.[16] Therefore, consistently taking a careful environmental history is critical to detecting the presence of potential antigens. Compatible findings on pulmonary function testing, HRCT (Fig. 23-1), BAL, and environmental challenge then can aid in establishing the diagnosis. Surgical lung biopsy is usually reserved for atypical or advanced (fibrotic) cases.

Pulmonary function tests typically show a restrictive defect, sometimes with an obstructive component. In reported pediatric cases, the mean (±standard deviation) FEV_1 and FVC in the children tested were 54.3 percent (±19.4) and 52.7 percent (±20.0) predicted. The pressure-volume curve is shifted down and to the right, consistent with decreased compliance. Low lung volumes and diminished diffusion capacity also are present.[32] Although resting room air oxygen saturation may be normal, desaturation with exercise or sleep may occur. With long-standing disease, resting oxygen desaturation can be seen.

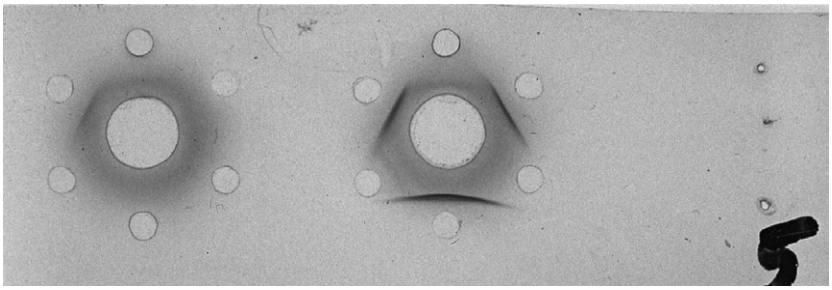

FIGURE 23-2 ■ Serum-precipitating antibodies against cockatiel antigens in the patient shown in Figure 23-1. (Courtesy Robin Deterding, MD, University of Colorado, Denver, CO.)

In the pediatric cases in which oxygenation was documented, 67 percent (64 of 95) had hypoxemia at rest. Pulmonary hypertension may be present with advanced disease, but in contrast to other forms of children's ILD, it may reverse completely with successful treatment.

Classic features of acute HP evident on chest radiography and HRCT include poorly defined centrilobular micronodules, with predominance in the upper and middle lung zones (see Fig. 23-1). On HRCT, widespread ground-glass attenuation and air trapping (especially prominent on expiratory scans) are also characteristic features.[119,162] A small percentage of patients also may have lung cysts.[66] In the more chronic phase, diffuse interstitial infiltrates may predominate, with progression to fibrosis and honeycombing that are indistinguishable from usual interstitial pneumonia.[106] HRCT is much more sensitive for detecting these abnormalities, with the initial plain chest radiographs reported as being normal in up to 37 percent of cases[16] whereas the CT was normal in only up to 2 percent of cases in reported series.[76,119] In the reported pediatric cases of HP, chest radiographs were abnormal at some point in 83 percent (130 of 156).

BAL fluid, obtained by flexible fiberoptic bronchoscopy, typically shows a significant lymphocytosis (>25%),[26] although this is not always present in patients who have insidious chronic disease without superimposed acute episodes.[125,166] In adults, a low CD4/CD8 ratio frequently accompanies the lymphocytosis. Children with HP also have marked lymphocytosis in the BAL compared with healthy pediatric controls (80% vs. 12%), although a low CD4/CD8 ratio may not be as helpful because a low ratio (0.6) is already seen in healthy children without lung disease.[140] Clinicians should be mindful, however, that the presence of BAL lymphocytosis in exposed individuals who are not symptomatic does not predict the development of HP.[38] Induced sputum does not accurately reflect the lymphocytosis seen in BAL and thus cannot be used as an alternative diagnostic step.[48] During an acute episode of HP due to avian antigen, mild BAL neutrophilia and eosinophilia may also be seen in conjunction with the lymphocytosis.[125]

Environmental challenges, in which the patient is returned to the suspected causative environment after having achieved clinical remission (usually through hospitalization or systemic corticosteroid treatment), can be helpful in establishing the exposure basis behind the condition. Signs and symptoms, spirometry, chest radiograph, and inflammatory markers are then followed for decrement. The patient's atopic status must be considered, and these challenges may be difficult to perform in patients without episodic acute manifestations.[85] Provocation challenge performed in controlled laboratory conditions using a specific suspected antigen has been used,[139] but owing to the lack of standardized inhalational antigen preparations, this technique is not routinely recommended.[26] Similarly, measurement of antigen-induced lymphocyte proliferation using lymphocytes gathered from BAL or blood can be a sensitive technique to confirm reaction to a specific antigen, but this test is not widely available.[125,126]

Detecting precipitating IgG antibodies to the suspected offending antigen can be useful in confirming the diagnosis in a patient with documented exposure and typical clinical features (Fig. 23-2). However, most commercial laboratory panels include only certain specific antibodies, so providers must be aware of which are tested before ordering such a panel. Ideally, a sample of the suspected antigen collected directly from the original source is used to test against the patient's serum, but few laboratories can perform this technique. Precipitating antibodies should be sent only in patients who have clinical disease, because 50 percent of individuals who are exposed to a particular antigen develop precipitating antibodies but only a small percentage of these develop HP.[40] Long-term follow-up has shown that the simple presence of precipitins does not increase the likelihood of developing the condition.[38] Conversely, not all individuals with symptomatic HP have positive precipitins for the tested antigens. In reported pediatric cases, positive precipitins were found in 93 percent (134 of 144) of the children tested.

Lung biopsy is typically not necessary for making the diagnosis of HP in the setting of an identified antigen exposure, typical HRCT findings, and positive serum precipitins. However, when clinical picture is atypical or the patient is found to have an undefined ILD on CT, then lung biopsy can be useful in making the diagnosis.[175] Although transbronchial biopsies may sometimes be able to confirm the diagnosis if positive, because of the high potential for sampling error, transthoracic biopsy is usually necessary to obtain tissue to look for the characteristic histologic features described previously (Fig. 23-3). Of the 164 reported pediatric cases of HP, 21 biopsy specimens were obtained and all showed typical histologic changes.

Treatment and Outcome

The mainstay of treatment is eliminating exposure to the offending antigen. However, there can be practical

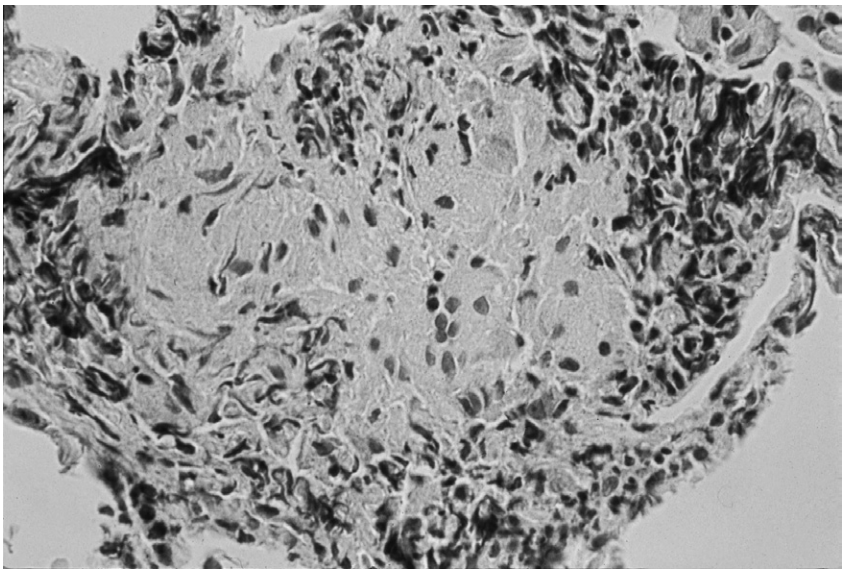

FIGURE 23-3 ■ Transbronchial biopsy specimen from the patient in Figure 23-1 shows a poorly formed granuloma consistent with hypersensitivity pneumonitis. (Courtesy Robin Deterding, MD, University of Colorado, Denver, CO.)

challenges to achieving this goal, especially because even small amounts of exposure can perpetuate the immunologic reaction and, as previously stated, bird antigen in the home environment can persist long after removal of the bird. The presence of markers of poor prognosis, such as traction bronchiectasis and honeycombing on HRCT[185] or fibrosis seen microscopically on a pathologic specimen,[33] would dictate much more aggressive avoidance measures. In the literature, exposure was eliminated in 98 percent of the pediatric cases (106 of 108), with improvement achieved in all but one fatal case.

The use of corticosteroids often results in rapid improvement of symptoms and reversal of radiographic and lung function abnormalities unless irreversible changes in the lung have occurred. Corticosteroids were used in 75 percent (98 of 130) of the reported pediatric cases, with a positive response occurring in all but the one fatal case. Generally, prednisone was given at 0.5 to 2 mg/kg daily for 2 to 4 weeks, followed by tapering of the dosage, although specific regimens have not been studied scientifically. Pulsed methylprednisolone also has been used.[16,30]

The outcome of children with HP, when properly diagnosed and treated, is excellent. In the 130 pediatric cases of HP with reported outcomes, 122 improved or became asymptomatic, 6 patients were still undergoing treatment (including 2 who had experienced relapse), 1 patient's condition remained unchanged, and 1 patient died. In this last case, an 11-year-old girl developed classic features after several years of exposure to budgerigars and other birds.[182] Despite removal of the offending antigens and treatment with corticosteroids and penicillamine, she died of respiratory failure 13 months later. The overall prognosis for children with HP is excellent, provided that a prompt diagnosis is made and appropriate treatment consisting of antigen removal and the judicious use of corticosteroids is instituted.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Andronikou S, Goussard P, Pie RP. Not all children with nodular interstitial lung patterns in South Africa have TB—a rare case of paediatric "bird fanciers' disease". *Pediatr Pulmonol* 2011;**46**:1134–6.
11. Beeton ML, Maxwell NC, Davies PL, et al. Role of pulmonary infection in the development of chronic lung disease of prematurity. *Eur Respir J* 2011;**37**:1424–30.
12. Benden C, Aurora P, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth Pediatric Lung and Heart-Lung Transplantation Report—2011. *J Heart Lung Transplant* 2011;**30**:1123–32.
16. Buchvald F, Petersen BL, Damgaard K, et al. Frequency, treatment, and functional outcome in children with hypersensitivity pneumonitis. *Pediatr Pulmonol* 2011;**46**:1098–107.
18. Cabral DA, Uribe AG, Benseler S, et al. Classification, presentation, and initial treatment of Wegener's granulomatosis in childhood. *Arthritis Rheum* 2009;**60**:3413–24.
21. Cartin-Ceba R, Fervenza FC, Specks U. Treatment of antineutrophilic cytoplasmic antibody-associated vasculitis with rituximab. *Curr Opin Rheumatol* 2012;**24**:15–23.
25. Champs NS, Lasmar LM, Camargos PA, et al. Post-infectious bronchiolitis obliterans in children. *J Pediatr (Rio J)* 2011;**87**:187–98.
26. Chan AL, Juarez MM, Leslie KO, et al. Bird fancier's lung: a state of the art review. *Clinic Rev Allerg Immunol* 2012;**43**:69–83.
33. Churg A, Sin DD, Everett D, et al. Pathologic patterns and survival in chronic hypersensitivity pneumonitis. *Am J Surg Pathol* 2009;**33**:1765–70.
36. Copley SJ, Coren M, Nicholson AG, et al. Diagnostic accuracy of thin-section CT and chest radiography of pediatric interstitial lung disease. *A J R Am J Roentgenol* 2000;**174**:549–54.
37. Cordier JF, Cottin V. Alveolar hemorrhage in vasculitis: primary and secondary. *Semin Respir Crit Care Med* 2011;**32**:310–21.
45. Das S, Langston C, Fan LL. Interstitial lung disease in children. *Curr Opin Pediatr* 2011;**23**:325–31.
46. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009;**28**:283–6.
49. Doan ML, Elidemir O, Dishop MK, et al. Serum KL-6 differentiates neuroendocrine cell hyperplasia of infancy from the inborn errors of surfactant metabolism. *Thorax* 2009;**64**:677–81.
53. Engelhart S, Rietschel E, Exner M, Lange L. Childhood hypersensitivity pneumonitis associated with fungal contamination of indoor hydroponics. *Int J Hyg Environ Health* 2009;**212**:18–20.
69. Galambos C, Levy H, Cannon CL, et al. Pulmonary pathology in thyroid transcription factor-1 deficiency syndrome. *Am J Respir Crit Care Med* 2010;**182**:549–54.
70. Girald M, Lacasse Y, Cormier Y. Hypersensitivity pneumonitis. *Allergy* 2009;**64**:322–34.
97. Kuramochi J, Inase N, Takayama K, et al. Detection of indoor and outdoor avian antigen in management of bird-related hypersensitivity pneumonitis. *Allergol Int* 2010;**59**:223–8.

101. Lenney W, Boner AL, Bont L, et al. Medicines used in respiratory diseases only seen in children. *Eur Respir J* 2009;**34**:513–51.
109. Martinez-Moczygemba M, Doan ML, Elidemir O, et al. Pulmonary alveolar proteinosis caused by deletion of the GM-CSFRalpha gene in the X chromosome pseudoautosomal region 1. *J Exp Med* 2008;**205**:2711–6.
113. Metzger F, Haccuria A, Reboux G, et al. Hypersensitivity pneumonitis due to molds in a saxophone player. *Chest* 2010;**138**: 724–6.
115. Minkov M. Multisystem Langerhans cell histiocytosis in children: current treatment and future directions. *Paediatr Drugs* 2011; **13**:75–86.
118. Moonnumakal SP, Fan LL. Bronchiolitis obliterans in children. *Curr Opin Pediatr* 2008;**20**:272–8.
119. Morell F, Roger A, Reyes L, et al. Bird fancier's lung: a series of 86 patients. *Medicine* 2008;**87**:110–30.
128. Oren I, Hardak E, Finkelstein R, et al. Polymerase chain reaction–based detection of *Pneumocystis jirovecii* in bronchoalveolar lavage fluid for the diagnosis of pneumocystis pneumonia. *Am J Med Sci* 2011;**342**:182–5.
131. Papaventsis D, Ioannidis P, Karabela S, et al. Impact of the Gen-Probe Amplified MTD Test on tuberculosis diagnosis in children. *Int J Tuberc Lung Dis* 2012;**16**:384–90.
138. Rama JA, Fan LL, Faro A, et al. Lung transplantation for childhood interstitial lung disease. *Chest* 2009;**136**:65S.
142. Reiter K, Schoen C, Griese M, et al. Whole-lung lavage in infants and children with pulmonary alveolar proteinosis. *Paediatr Anaesth* 2010;**20**:1118–23.
149. Sachdev A, Chugh K, Sethi M, et al. Diagnosis of ventilator-associated pneumonia in children in resource-limited setting: a comparative study of bronchoscopic and nonbronchoscopic methods. *Pediatr Crit Care Med* 2010;**11**:258–66.
150. Saketkoo LA, Espinoza L. Experience of mycophenolate mofetil in 10 patients with autoimmune-related interstitial lung disease demonstrates promising effects. *Am J Med Sci* 2009;**337**:329–35.
157. Shannon VR, Andersson BS, Lei X, et al. Utility of early versus late fiberoptic bronchoscopy in the evaluation of new pulmonary infiltrates following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2010;**45**:647–55.
170. Taniguchi H, Kondoh Y, Ebina M, et al. The clinical significance of 5% change in vital capacity in patients with idiopathic pulmonary fibrosis: extended analysis of the pirfenidone trial. *Respir Res* 2011;**12**:93.
173. Thouvenin G, Abou Taam R, Flamein F, et al. Characteristics of disorders associated with genetic mutations of surfactant protein C. *Arch Dis Child* 2010;**95**:449–54.
175. Trahan S, Hanak V, Ryu JH, Myers JL. Role of surgical lung biopsy in separating chronic hypersensitivity pneumonia from usual interstitial pneumonia/idiopathic pulmonary fibrosis: analysis of 31 biopsies from 15 patients. *Chest* 2008;**134**:126–32.
179. van de Ven AA, de Jong PA, Hyotema van Konijnenburg DP, et al. Airway and interstitial lung disease are distinct entities in paediatric common variable immunodeficiency. *Clin Exp Immunol* 2011;**165**:235–42.
180. Vece TJ, Fan LL. Interstitial lung disease in children older than 2 years. *Pediatr Allergy Immunol Pulmonol* 2010;**23**:33–41.
181. Vece TJ, Fan LL. Diagnosis and management of diffuse lung disease in children. *Paediatr Respir Rev* 2011;**12**:238–42.
183. Vrielynck S, Mamou-Mani T, Edmond S, et al. Diagnostic value of high-resolution CT in the evaluation of chronic infiltrative lung disease in children. *A J R Am J Roentgenol* 2008;**191**: 914–20.
184. Walker KM, Pope J, participating members of the Scleroderma Clinical Trials Consortium (SCTC), and Canadian Scleroderma Research Group (CSRG). Treatment of systemic sclerosis complications: what to do when first-line treatment fails-a consensus of systemic sclerosis experts. *Semin Arthritis Rheum* 2012;**42**: 42–55.
185. Walsh SL, Sverzellati N, Devaraj A, et al. Chronic hypersensitivity pneumonitis: high resolution computed tomography patterns and pulmonary function indices of prognostic determinants. *Eur Radiol* 2012;**22**:1672–9.
186. Warren CP. Extrinsic allergic alveolitis: a disease commoner in non-smokers. *Thorax* 1977;**32**:567–9.
191. Yamamoto H, Yamaguchi E, Agata H, et al. A combination therapy of whole lung lavage and GM-CSF inhalation in pulmonary alveolar proteinosis. *Pediatr Pulmonol* 2008;**43**:828–30.
193. Yu T, Ahmed R, Yap E, Kumar S. Dyspnoea in a 17-year-old swim instructor: a diagnosis of hot tube lung. *N Z Med J* 2008;**121**: 78–80.
195. Zar HJ. Chronic lung disease in human immunodeficiency virus (HIV) infected children. *Pediatr Pulmonol* 2008;**43**:1–10.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 24

Complications of Pneumonia

J. Gary Wheeler • Richard F. Jacobs

Most episodes of pneumonia resolve spontaneously or with limited medical therapy. Certain groups, those with underlying diseases, those with anatomic or functional abnormalities, or immunocompromised patients, are more prone to complications, but the normal host is not spared. The most concerning are those who require surgical intervention or prolonged therapy. Guideline development has provided effective management options.[15] This chapter is divided into discussions of empyema lung abscess and other complications (Table 24-1).

EMPYEMA

Collections of fluid in the pleural space have been described in the literature as transudates, pleural

REFERENCES

1. Aebischer CC, Frey U, Schoni MH. Hypersensitivity pneumonitis in a five-year-old boy: an unusual antigen source. *Pediatr Pulmonol* 2002;**33**:77–8.
2. Allen DH, Basten A, Williams GV, Woolcock AJ. Familial hypersensitivity pneumonitis. *Am J Med* 1975;**59**:505–14.
3. Allen J. Acute eosinophilic pneumonia. *Semin Respir Crit Care Med* 2006;**27**:142–7.
4. Al-Za'abi AM, MacDonald S, Geddie W, et al. Cytologic examination of bronchoalveolar lavage fluid from immunosuppressed patients. *Diagn Cytopathol* 2007;**35**:710–4.
5. Andiman WA, Eastman R, Martin K, et al. Opportunistic lymphoproliferations associated with Epstein-Barr viral DNA in infants and children with AIDS. *Lancet* 1985;**2**:1390–3.
6. Andronikou S, Goussard P, Pie RP. Not all children with nodular interstitial lung patterns in South Africa have TB—a rare case of paediatric "bird fanciers' disease." *Pediatr Pulmonol* 2011;**46**: 1134–6.
7. Avital A, Godfrey S, Maayan C, et al. Chloroquine treatment of interstitial lung disease in children. *Pediatr Pulmonol* 1994;**18**: 356–60.
8. Balasubramaniam SK, O'Connell EJ, Yunginger JW, et al. Hypersensitivity pneumonitis due to dove antigens in an adolescent. *Clin Pediatr* 1987;**26**:174–6.
9. Barker PM, Warner JO. "Atypical pneumonia" due to parakeet sensitivity: bird fancier's lung in a 10-year-old girl. *Br J Dis Chest* 1984;**78**:404–7.
10. Becciolini V, Gudinchet F, Schnyder P. Lymphocytic interstitial pneumonia in children with AIDS: high-resolution CT fidings. *Eur Radiol* 2001;**11**:1015–20.
11. Beeton ML, Maxwell NC, Davies PL, et al. Role of pulmonary infection in the development of chronic lung disease of prematurity. *Eur Respir J* 2011;**37**:1424–30.
12. Benden C, Aurora P, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth Pediatric Lung and Heart-Lung Transplantation Report—2011. *J Heart Lung Transplant* 2011;**30**:1123–32.
13. Bousvaros A, Sundel R, Thorne GM, et al. Parvovirus B19–associated interstitial lung disease, hepatitis, and myositis. *Pediatr Pulmonol* 1998;**26**:265–369.
14. Brody AS, Guillerman RP, Hay TC, et al. Neuroendocrine cell hyperplasia of infancy: diagnosis with high-resolution CT. *A J R Am J Roentgenol* 2010;**194**:238–44.
15. Brown KK. Pulmonary vasculitis. *Proc Am Thorac Soc* 2006;**3**: 48–57.
16. Buchvald F, Petersen BL, Damgaard K, et al. Frequency, treatment, and functional outcome in children with hypersensitivity pneumonitis. *Pediatr Pulmonol* 2011;**46**:1098–107.
17. Bureau MA, Fecteau C, Patriquin H, et al. Farmer's lung in early childhood. *Am Rev Respir Dis* 1979;**119**:671–5.
18. Cabral DA, Uribe AG, Benseler S, et al. Classification, presentation, and initial treatment of Wegener's granulomatosis in childhood. *Arthritis Rheum* 2009;**60**:3413–24.
19. Camarena A, Juarez A, Mejia M, et al. Major histocompatibility complex and tumor necrosis factor–alpha polymorphisms in pigeon breeder's disease. *Am J Respir Crit Care Med* 2001;**163**: 1528–33.
20. Carlsen KH, Leegaard J, Lund OD, Skjaervik H. Allergic alveolitis in a 12-year-old boy: treatment with budesonide nebulizing solution. *Pediatr Pulmonol* 1992;**12**:257–9.
21. Cartin-Ceba R, Fervenza FC, Specks U. Treatment of antineutrophilic cytoplasmic antibody-associated vasculitis with rituximab. *Curr Opin Rheumatol* 2012;**24**:15–23.
22. Centers for Disease Control and Prevention. 1994 revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *M M W R Morb Mortal Wkly Rep Recomm Rep* 1994;**43**(RR-12):1–10.
23. Centers for Disease Control and Prevention. Update: Pulmonary hemorrhage/hemosiderosis among infants—Cleveland, Ohio, 1993-1996. *M M W R Morb Mortal Wkly Rep Recomm Rep* 2000;**49**:180–4.
24. Ceviz N, Kaynar H, Olgun H, et al. Pigeon breeder's lung in childhood: is family screening necessary? *Pediatr Pulmonol* 2006;**41**:279–82.
25. Champs NS, Lasmar LM, Camargos PA, et al. Post-infectious bronchiolitis obliterans in children. *J Pediatr (Rio J)* 2011;**87**: 187–98.
26. Chan AL, Juarez MM, Leslie KO, et al. Bird fancier's lung: a state of the art review. *Clinic Rev Allerg Immunol* 2012;**43**:69–83.
27. Chan PW, Muridan R, Debruyne JA. Bronchiolitis obliterans in children: clinical profile and diagnosis. *Respirology* 2000;**5**:369–75.
28. Chandra S, Jones HE. Pigeon fancier's lung in children. *Arch Dis Child* 1972;**47**:716–8.
29. Chang AB, Masel JP, Masters B. Post-infectious bronchiolitis obliterans: clinical, radiological and pulmonary function sequelae. *Pediatr Radiol* 1998;**28**:23–9.
30. Chen C, Kleinau I, Niggemann B, et al. Treatment of allergic alveolitis with methylprednisolone pulse therapy. *Pediatr Allergy Immunol* 2003;**14**:66–70.
31. Chetty A, Bhuyan UN, Mitra DK, et al. Cryptogenic fibrosing alveolitis in children. *Ann Allergy* 1987;**58**:336–40.
32. Chiron C, Gaultier C, Boule M, et al. Lung function in children with hypersensitivity pneumonitis. *Eur J Respir Dis* 1984;**65**: 79–91.
33. Churg A, Sin DD, Everett D, et al. Pathologic patterns and survival in chronic hypersensitivity pneumonitis. *Am J Surg Pathol* 2009;**33**:1765–70.
34. Ciftci E, Yalcinkaya F, Ince E, et al. Pulmonary involvement in childhood-onset systemic lupus erythematosus: a report of five cases. *Rheumatology* 2004;**43**:587–91.
35. Colom AJ, Teper AM, Vollmer WM, Diette GB. Risk factors for the development of bronchiolitis obliterans in children with bronchiolitis. *Thorax* 2006;**61**:503–6.
36. Copley SJ, Coren M, Nicholson AG, et al. Diagnostic accuracy of thin-section CT and chest radiography of pediatric interstitial lung disease. *A J R Am J Roentgenol* 2000;**174**:549–54.
37. Cordier JF, Cottin V. Alveolar hemorrhage in vasculitis: primary and secondary. *Semin Respir Crit Care Med* 2011;**32**:310–21.
38. Cormier Y, Letourneau L, Racine G. Significance of precipitins and asymptomatic lymphocytic alveolitis: a 20-yr follow-up. *Eur Respir J* 2004;**23**:523–5.
39. Craig TJ, Hershey J, Engler RJ, et al. Bird antigen persistence in the home environment after removal of the bird. *Ann Allergy* 1992;**69**:510–2.
40. Craig TJ, Richerson HB. Update on hypersensitivity pneumonitis. *Comp Ther* 1996;**22**:559–64.
41. Cron RQ, Sherry DD, Wallace CA. Methotrexate-induced hypersensitivity pneumonitis in a child with juvenile rheumatoid arthritis. *J Pediatr* 1998;**132**:901–2.
42. Cunningham AS, Fink JN, Schlueter DP. Childhood hypersensitivity pneumonitis due to dove antigens. *Pediatrics* 1976;**58**: 436–42.
43. Dakhama A, Hegele RG, Laflamme G, et al. Common respiratory viruses in lower airways of patients with acute hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 1999;**159**:1316–22.
44. Dalvi V, Gonzalez EB, Lovett L. Lymphocytic interstitial pneumonitis (LIP) in Sjogren's syndrome: a case report and a review of the literature. *Clin Rheumatol* 2007;**26**:1339–43.
45. Das S, Langston C, Fan LL. Interstitial lung disease in children. *Curr Opin Pediatr* 2011;**23**:325–31.
46. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009;**28**:283–6.
47. Dinwiddie R, Sharief N, Crawford O. Idiopathic interstitial pneumonitis in children: a national survey in the United Kingdom and Ireland. *Pediatr Pulmonol* 2002;**34**:23–9.
48. D'Ippolito R, Chetta A, Foresi A, et al. Induced sputum and bronchoalveolar lavage from patients with hypersensitivity pneumonitis. *Respir Med* 2004;**98**:977–83.
49. Doan ML, Elidemir O, Dishop MK, et al. Serum KL-6 differentiates neuroendocrine cell hyperplasia of infancy from the inborn errors of surfactant metabolism. *Thorax* 2009;**64**:677–81.
50. du Marchie Sarvaas GJ, Merkus PJ, de Jongste JC. A family with extrinsic allergic alveolitis caused by wild city pigeons: a case report. *Pediatrics* 2000;**105**:E62.
51. Eisenberg JD, Montanero A, Lee RG. Hypersensitivity pneumonitis in an infant. *Pediatr Pulmonol* 1992;**12**:186–90.
52. El-Hefny A, Ekladious EM, El-Sharkawy S, et al. Extrinsic allergic bronchiolo-alveolitis in children. *Clin Allergy* 1980;**10**:651–8.

53. Engelhart S, Rietschel E, Exner M, Lange L. Childhood hypersensitivity pneumonitis associated with fungal contamination of indoor hydroponics. *Int J Hyg Environ Health* 2009;**212**:18–20.
54. Epstein CE, Elidemir O, Colasurdo GN, Fan LL. Time course of hemosiderin production by alveolar macrophages in a murine model. *Chest* 2001;**120**:2013–20.
55. Ettlin MS, Pache JC, Renevey F, et al. Bird breeder's disease: A rare diagnosis in young children. *Eur J Pediatr* 2006;**165**: 55–61.
56. European Respiratory Society. Bronchoalveolar lavage in children. ERS Task Force on Bronchoalveolar Lavage in Children. *Eur Respir J* 2000;**15**:217–31.
57. Fackler JC, Nagel JE, Adler WH, et al. Epstein-Barr virus infection in a child with acquired immunodeficiency syndrome. *Am J Dis Child* 1985;**139**:1000–4.
58. Fan LL, Deterding RR. Surfactant dysfunction mutations in children's interstitial lung disease and beyond. *Am J Respir Crit Care Med* 2005;**172**:940–1.
59. Fan LL, Kozinetz CA. Factors influencing survival in children with chronic interstitial lung disease. *Am J Respir Crit Care Med* 1997;**156**:939–42.
60. Fan LL, Kozinetz CA, Deterding RR, Brugman SM. Evaluation of a diagnostic approach to pediatric interstitial lung disease. *Pediatrics* 1998;**101**:82–5.
61. Fan LL, Kozinetz CA, Wojtczak HA, et al. Diagnostic value of transbronchial, thoracoscopic, and open lung biopsy in immunocompetent children with chronic interstitial lung disease. *J Pediatr* 1997;**131**:565–9.
62. Fan LL, Langston C. Chronic interstitial lung disease in children. *Pediatr Pulmonol* 1993;**16**:184–96.
63. Fan LL, Lung MC, Wagener JS. The diagnostic value of bronchoalveolar lavage in immunocompetent children with chronic diffuse pulmonary infiltrates. *Pediatr Pulmonol* 1997;**23**:8–13.
64. Fan LL, Mullen AL, Brugman SM, et al. Clinical spectrum of chronic interstitial lung disease in children. *J Pediatr* 1992;**121**: 867–72.
65. Fink JN. Hypersensitivity pneumonitis. *Clin Chest Med* 1992; **13**:303–9.
66. Franquet T, Hansell DM, Senbanjo T, et al. Lung cysts in subacute hypersensitivity pneumonitis. *J Comput Assist Tomogr* 2003; **27**:475–8.
67. Fullmer JJ, Fan LL, Dishop MK, et al. Successful treatment of bronchiolitis obliterans in a bone marrow transplant patient with tumor necrosis factor-alpha blockade. *Pediatrics* 2005;**116**: 767–70.
68. Fullmer JJ, Langston C, Dishop DK, Fan LL. Pulmonary capillaritis in children: a review of eight cases with comparison to other alveolar hemorrhage syndromes. *J Pediatr* 2005;**146**:376–81.
69. Galambos C, Levy H, Cannon CL, et al. Pulmonary pathology in thyroid transcription factor–1 deficiency syndrome. *Am J Respir Crit Care Med* 2010;**182**:549–54.
70. Girald M, Lacasse Y, Cormier Y. Hypersensitivity pneumonitis. *Allergy* 2009;**64**:322–34.
71. Grammer LC. Occupational allergic alveolitis. *Ann Allergy Asthma Immunol* 1999;**83**:602–6.
72. Grammer LC, Roberts M, Lerner C, Patterson R. Clinical and serologic follow-up of four children and five adults with bird-fancier's lung. *J Allergy Clin Immunol* 1990;**85**:655–60.
73. Grech V, Vella C, Lenicker H. Pigeon breeder's lung in childhood: varied clinical picture at presentation. *Pediatr Pulmonol* 2000;**30**: 145–8.
74. Guillerman RP, Brody AS. Contemporary perpectives on pediatric diffuse lung disease. *Radiol Clin North Am* 2011;**49**:847–68.
75. Harrison HR, Taussig LM, Fulginiti VA. *Chlamydia trachomatis* and chronic lung disease in childhood. *Pediatr Infect Dis* 1982;**1**: 29–33.
76. Hartman TE. The HRCT features of extrinsic allergic alveolitis. *Semin Respir Crit Care Med* 2003;**24**:419–26.
77. Heersma JR, Emanuel DA, Wenzel FJ, Gray RL. Farmer's lung in a 10-year-old girl. *J Pediatr* 1969;**75**:704–6.
78. Highland KB, Flume PA. A 12-year-old girl with dyspnea and a normal chest radiographic finding: hypersensitivity pneumonitis. *Chest* 2001;**120**:1372–6.
79. Hirschmann JV, Pipavath SN, Godwin JD. Hypersensitivity pneumonitis: a historical, clinical, and radiologic review. *RadioGraphics* 2009;**29**:1921–38.
80. Hoffman GS, Kerr GS, Leavitt RY, et al. Wegener granulomatosis: an analysis of 158 patients. *Ann Intern Med* 1992;**116**: 488–98.
81. Hogan MB, Patterson R, Pore RS, et al. Basement shower hypersensitivity pneumonitis secondary to *Epicoccum nigrum*. *Chest* 1996;**110**:854–6.
82. Hughes WF, Mattimore JM, Arbesman CE. Farmer's lung in an adolescent boy. *Am J Dis Child* 1969;**118**:777–80.
83. Inase N, Ohtani Y, Sumi Y, et al. A clinical study of hypersensitivity pneumonitis presumably caused by feather duvets. *Ann Allergy Asthma Immunol* 2006;**96**:98–104.
84. Israel-Assayag E, Dakhama A, Lavigne S, et al. Expression of costimulatory molecules on alveolar macrophages in hypersensitivity pneumonitis. *Am J Respir Crit Care Med* 1999;**159**:1830–4.
85. Jacobs RL, Andrews CP, Coalson JJ. Hypersensitivity pneumonitis: beyond classic occupational disease—changing concepts of diagnosis and management. *Ann Allergy Asthma Immunol* 2005; **95**:115–28.
86. Jobe AH, Bancalari E. Bronchopulmonary dysplasia. *Am J Respir Crit Care Med* 2001;**163**:1723–9.
87. Joshi VV, Oleske JM, Minnefor AB, et al. Pathologic pulmonary findings in children with the acquired immunodeficiency syndrome: a study of ten cases. *Hum Pathol* 1985;**16**:241–6.
88. Karakurum M, Doraswamy B, Bennuri SS. Index of suspicion: case 1. Hypersensitivity pneumonitis. *Pediatr Rev* 1999;**20**:53–4.
89. Katz BZ, Berkman AB, Shapiro ED. Serologic evidence of active Epstein-Barr virus infection in Epstein-Barr virus–associated lymphoproliferative disorders of children with acquired immunodeficiency syndrome. *J Pediatr* 1992;**120**:228–32.
90. Katz RM, Kniker WT. Infantile hypersensitivity pneumonitis as a reaction to organic antigens. *N Engl J Med* 1973;**288**:233–7.
91. Keith HH, Holsclaw DS, Dunsky EH. Pigeon breeder's disease in children: a family study. *Chest* 1981;**79**:107–10.
92. Kirby JG, Hargreave FE, Gleich GJ, et al. Bronchoalveolar cell profiles of asthmatic and nonasthmatic subjects. *Am Rev Respir Dis* 1987;**136**:379–83.
93. Koss MN. Pulmonary lymphoid disorders. *Semin Diagn Pathol* 1995;**12**:158–71.
94. Kradin RL, Mark EJ. Benign lymphoid disorders of the lung, with a theory regarding their development. *Hum Pathol* 1983;**14**: 857–67.
95. Krasnick J, Patterson R, Stillwell PC, et al. Potentially fatal hypersensitivity pneumonitis in a child. *Clin Pediatr* 1995;**34**:388–91.
96. Kristiansen JD, Lahoz AX. Riding-school lung? Allergic alveolitis in an 11-year-old girl. *Acta Pediatr Scand* 1991;**80**:386–8.
97. Kuramochi J, Inase N, Takayama K, et al. Detection of indoor and outdoor avian antigen in management of bird-related hypersensitivity pneumonitis. *Allergol Int* 2010;**59**:223–8.
98. Langston C, Patterson K, Dishop MK, et al. A protocol for the handling of tissue obtained by operative lung biopsy: recommendations of the chILD Pathology Co-operative Group. *Pediatr Dev Pathol* 2006;**9**:173–80.
99. Laray-Cuasay LR, DeForest A, Huff D, et al. Chronic pulmonary complications of early influenza virus infection in children. *Am Rev Respir Dis* 1977;**116**:617–25.
100. Lee SK, Kim SS, Nahm DH, et al. Hypersensitivity pneumonitis caused by *Fusarium napiforme* in a home environment. *Allergy* 2000;**55**:1190–3.
101. Lenney W, Boner AL, Bont L, et al. Medicines used in respiratory diseases only seen in children. *Eur Respir J* 2009;**34**:513–51.
102. Levenson T, Patterson R. Chronic cough in a child. *Ann Allergy Asthma Immunol* 1996;**76**:311–6.
103. Long FR, Castile RG. Technique and clinical applications of full-inflation and end-exhalation controlled-ventilation chest CT in infants and young children. *Pediatr Radiol* 2001;**31**:413–22.
104. Lovell D, Lindsley C, Langston C. Lymphoid interstitial pneumonia in juvenile rheumatoid arthritis. *J Pediatr* 1984;**105**:947–50.
105. Lynch DA, Hay T, Newell JD, et al. Pediatric diffuse lung disease: diagnosis and classification using high-resolution CT. *A J R Am J Roentgenol* 1999;**173**:713–8.
106. Lynch DA, Newell JD, Logan PM, et al. Can CT distinguish hypersensitivity pneumonitis from idiopathic pulmonary fibrosis? *A J R Am J Roentgenol* 1995;**165**:807–11.
107. Malamou-Mitsi V, Tsai MM, Gal AA, et al. Lymphoid interstitial pneumonia not associated with HIV infection: role of Epstein-Barr virus. *Mod Pathol* 1992;**5**:487–91.

108. Marks MJ, Haney PJ, McDermott MP, et al. Thoracic disease in children with AIDS. *RadioGraphics* 1996;**17**:1349–62.
109. Martinez-Moczygemba M, Doan ML, Elidemir O, et al. Pulmonary alveolar proteinosis caused by deletion of the GM-CSFRalpha gene in the X chromosome pseudoautosomal region 1. *J Exp Med* 2008;**205**:2711–6.
110. Matthay RA, Schwartz MI, Petty TL, et al. Pulmonary manifestations of systemic lupus erythematosus: review of 12 cases of acute lupus pneumonitis. *Medicine (Baltimore)* 1975;**54**:397–409.
111. McClellan JS, Albers GM, Noyes BE, et al. B-lymphocyte aggregates in alveoli from a child with hypersensitivity pneumonitis (bird breeders lung). *Ann Allergy Asthma Immunol* 1999;**83**: 357–60.
112. McSharry C, Anderson K, Boyd G. A review of antigen diversity causing lung disease among pigeon breeders. *Clin Exp Allergy* 2000;**30**:1221–9.
113. Metzger F, Haccuria A, Reboux G, et al. Hypersensitivity pneumonitis due to molds in a saxophone player. *Chest* 2010;**138**: 724–6.
114. Miller MM, Patterson R, Fink JN, Roberts M. Chronic hypersensitivity lung disease with recurrent episodes of hypersensitivity pneumonitis due to a contaminated central humidifer. *Clin Allergy* 1976;**6**:451–62.
115. Minkov M. Multisystem Langerhans cell histiocytosis in children: current treatment and future directions. *Paediatr Drugs* 2011;**13**: 75–86.
116. Mistchenko AS, Diez RA, Mariani AL, et al. Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor alpha levels with clinical outcome. *J Pediatr* 1994;**124**:714–20.
117. Mistchenko AS, Lenzi HL, Thompson FM, et al. Participation of immune complexes in adenovirus infection. *Acta Paediatr* 1992; **81**:983–8.
118. Moonnumakal SP, Fan LL. Bronchiolitis obliterans in children. *Curr Opin Pediatr* 2008;**20**:272–8.
119. Morell F, Roger A, Reyes L, et al. Bird fancier's lung: a series of 86 patients. *Medicine* 2008;**87**:110–30.
120. Nacar N, Kiper N, Yalcin E, et al. Hypersensitivity pneumonitis in children: pigeon breeder's disease. *Ann Trop Paediatr* 2004;**24**: 349–55.
121. Nogee LM, deMello DE, Dehner LP, Colten HR. Deficiency of pulmonary surfactant protein B in congenital alveolar proteinosis. *N Engl J Med* 1993;**328**:406–10.
122. Nogee LM, Dunbar AE, Wert SE, et al. A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 2001;**344**:573–9.
123. O'Brodovich HM, Moser MM, Lu L. Familial lymphoid interstitial pneumonia: a long-term follow-up. *Pediatrics* 1980;**65**: 523–8.
124. O'Connell EJ, Zora JA, Gillespie DN, Rosenow EC. Childhood hypersensitivity pneumonitis (farmer's lung): four cases in siblings with long-term follow-up. *J Pediatr* 1989;**114**:995–7.
125. Ohtani Y, Kojima K, Sumi Y, et al. Inhalation provocation tests in chronic bird fancier's lung. *Chest* 2000;**118**:1382–9.
126. Ohtani Y, Saiki S, Sumi Y, et al. Clinical features of recurrent and insidious chronic bird fancier's lung. *Ann Allergy Asthma Immunol* 2003;**90**:604–10.
127. Olesen HV, Thelle T, Moller JC. Childhood hypersensitivity pneumonitis probably caused by cat hair. *Acta Pediatr* 1998; **87**:811–3.
128. Oren I, Hardak E, Finkelstein R, et al. Polymerase chain reaction–based detection of *Pneumocystis jirovecii* in bronchoalveolar lavage fluid for the diagnosis of pneumocystis pneumonia. *Am J Med Sci* 2011;**342**:182–5.
129. Ostergaard JR. Reversible pulmonary arterial hypertension in a 6-year-old girl with extrinsic allergic alveolitis. *Acta Pediatr Scand* 1989;**78**:145–8.
130. Ozeki M, Funato M, Kanda K, et al. Clinical improvement of diffuse lymphangiomatosis with pegylated interferon alfa-2b therapy: case report and review of the literature. *Pediatr Hematol Oncol* 2007;**24**:513–24.
131. Papaventsis D, Ioannidis P, Karabela S, et al. Impact of the Gen-Probe Amplified MTD Test on tuberculosis diagnosis in children. *Int J Tuberc Lung Dis* 2012;**16**:384–90.
132. Park SM, Tremper L. Hypersensitivity pneumonitis in pediatric patients. *Immunol Allergy Pract* 1987;**9**:420–3.
133. Patel AM, Ryu JH, Reed CE. Hypersensitivity pneumonitis: current concepts and future questions. *J Allergy Clin Immunol* 2001;**108**:661–70.
134. Patterson R, Mazur N, Roberts M, et al. Hypersensitivity pneumonitis due to humidifier disease: seek and ye shall find. *Chest* 1998;**114**:931–3.
135. Pitt J. Lymphocytic interstitial pneumonia. *Pediatr Clin North Am* 1991;**38**:89–95.
136. Purtilo DT, Brem J, Ceccaci L, et al. A family study of pigeon breeders' disease. *J Pediatr* 1975;**86**:569–71.
137. Raghu G. Interstitial lung disease: a diagnostic approach: are CT scan and lung biopsy indicated in every patient? *Am J Respir Crit Care Med* 1995;**151**:909–14.
138. Rama JA, Fan LL, Faro A, et al. Lung transplantation for childhood interstitial lung disease. *Chest* 2009;**136**:65S.
139. Ramirez-Venegas A, Sansores RH, Perez-Padilla R, et al. Utility of a provocation test for diagnosis of chronic pigeon breeder's disease. *Am J Respir Crit Care Med* 1998;**158**:862–9.
140. Ratjen F, Costabel U, Griese M, Paul K. Bronchoalveolar lavage fluid findings in children with hypersensitivity pneumonitis. *Eur Respir J* 2003;**21**:144–8.
141. Reiss JS, Weiss NS, Payette KM, Strimas J. Childhood pigeon breeder's disease. *Ann Allergy* 1974;**32**:208–12.
142. Reiter K, Schoen C, Griese M, et al. Whole-lung lavage in infants and children with pulmonary alveolar proteinosis. *Paediatr Anaesth* 2010;**20**:1118–23.
143. Ribeiro JD, Fischer GB. Eosinophilic lung diseases. *Paediatr Respir Rev* 2002;**3**:278–84.
144. Rochat I, Sayegh Y, Gervaix A, et al. Acute hypoxic respiratory failure as the first manifestation of systemic-onset juvenile rheumatoid arthritis in a child. *Pediatr Pulmonol* 2004;**38**:483–7.
145. Rosal-Sanchez M, Alvarez J, Torres MJ, et al. Pigeon fancier's lung after low exposure. *Allergy* 2002;**57**:649.
146. Rothenberg SS, Wagner JS, Chang JH, Fan LL. The safety and efficacy of thoracoscopic lung biopsy for diagnosis and treatment in infants and children. *J Pediatr Surg* 1996;**31**:100–3.
147. Rubinstein A, Bernstein LJ, Charytan M, et al. Corticosteroid treatment for pulmonary lymphoid hyperplasia in children with the acquired immune deficiency syndrome. *Pediatr Pulmonol* 1988;**4**:13–7.
148. Rubinstein A, Morecki R, Silverman B, et al. Pulmonary disease in children with acquired immune deficiency syndrome and AIDS-related complex. *J Pediatr* 1986;**108**:498–503.
149. Sachdev A, Chugh K, Sethi M, et al. Diagnosis of ventilator-associated pneumonia in children in resource-limited setting: a comparative study of bronchoscopic and nonbronchoscopic methods. *Pediatr Crit Care Med* 2010;**11**:258–66.
150. Saketkoo LA, Espinoza L. Experience of mycophenolate mofetil in 10 patients with autoimmune-related interstitial lung disease demonstrates promising effects. *Am J Med Sci* 2009;**337**:329–35.
151. Saltos N, Saunders NA, Bhagwandeen SB, Jarvie B. Hypersensitivity pneumonitis in a mouldy house. *Med J Aust* 1982;**2**: 244–6.
152. Saltoun CA, Harris KE, Mathisen TL, Patterson R. Hypersensitivity pneumonitis resulting from community exposure to Canada goose droppings: when an external environmental antigen becomes an indoor environmental antigen. *Ann Allergy Asthma Immunol* 2000;**84**:84–6.
153. Santamaria F, Brancaccio G, Parenti G, et al. Recurrent fatal pulmonary alveolar proteinosis after heart-lung transplantation in a child with lysinuric protein intolerance. *J Pediatr* 2004; **145**:268–72.
154. Schelonka RL, Katz B, Waites KB, Benjamin DK. Critical appraisal of the role of *Ureaplasma* in the development of bronchopulmonary dysplasia with metaanalytic techniques. *Pediatr Infect Dis J* 2005;**24**:1033–9.
155. Selman M. Hypersensitivity pneumonitis. In: Schwarz MI, King TE, editors. *Interstitial lung disease*. 4th ed. Hamilton, Ontario: B. C. Decker; 2003. p. 452–84.
156. Shannon DC, Andrews JL, Recavarren S, Kazemi H. Pigeon breeder's lung disease and interstitial pulmonary fibrosis. *Am J Dis Child* 1969;**117**:504–10.
157. Shannon VR, Andersson BS, Lei X, et al. Utility of early versus late fiberoptic bronchoscopy in the evaluation of new pulmonary infiltrates following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2010;**45**:647–55.

158. Sharland M, Gibb DM, Holland F. Respiratory morbidity from lymphocytic interstitial pneumonitis (LIP) in vertically acquired HIV infection. *Arch Dis Child* 1997;**76**:334–6.
159. Sheikh S, Madiraju K, Steiner P, Rao M. Bronchiectasis in pediatric AIDS. *Chest* 1997;**112**:1202–7.
160. Shulenin S, Nogee LM, Annilo T, et al. *ABCA3* gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med* 2004;**350**:1296–303.
161. Simmank K, Meyers T, Galpin J, et al. Clinical features and T-cell subsets in HIV-infected children with and without lymphocytic interstitial pneumonitis. *Ann Trop Paediatr* 2001; **21**:195–201.
162. Small JH, Flower CD, Traill ZC, Gleeson FV. Air-trapping in extrinsic allergic alveolitis on computed tomography. *Clin Radiol* 1996;**51**:684–8.
163. Solaymani-Dodaran M, West J, Smith C, Hubbard R. Extrinsic allergic alveolitis: incidence and mortality in the general population. *Q J Med* 2007;**100**:233–7.
164. Springer C, Maayan C, Katzir Z, et al. Chloroquine treatment in desquamative interstitial pneumonia. *Arch Dis Child* 1987;**62**: 76–7.
165. Steiger D, Bubendorf L, Oberholzer M, et al. Ciprofloxacin-induced acute interstitial pneumonitis. *Eur Respir J* 2004;**23**: 172–4.
166. Sterclova M, Vasakova M, Dutka J, Kalanin J. Extrinsic allergic alveolitis: comparative study of the bronchoalveolar lavage profiles and radiological presentation. *Postgrad Med J* 2006;**82**: 598–601.
167. Stiehm ER, Reed CE, Tooley WH. Pigeon breeder's lung in children. *Pediatrics* 1967;**39**:904–15.
168. Stokes D, Sigler A, Khouri NF, Talamo RC. Unilateral hyperlucent lung (Swyer-James syndrome) after severe *Mycoplasma pneumoniae* infection. *Am Rev Respir Dis* 1978;**117**:145–52.
169. Swingler GH. Summer-type hypersensitivity pneumonitis in southern Africa: a report of 5 cases in one family. *S Afr Med J* 1990;**77**:104–7.
170. Taniguchi H, Kondoh Y, Ebina M, et al. The clinical significance of 5% change in vital capacity in patients with idiopathic pulmonary fibrosis: extended analysis of the pirfenidone trial. *Respir Res* 2011;**12**:93.
171. Temprano J, Becker RA, Hutcheson PS, et al. Hypersensitivity pneumonitis secondary to residential exposure to *Aureobasidium pullulans* in 2 siblings. *Ann Allergy Asthma Immunol* 2007;**99**: 562–6.
172. Thorshauge H, Fallesen I, Ostergaard PA. Farmer's lung in infants and small children. *Allergy* 1989;**44**:152–5.
173. Thouvenin G, Abou Taam R, Flamein F, et al. Characteristics of disorders associated with genetic mutations of surfactant protein C. *Arch Dis Child* 2010;**95**:449–54.
174. Tobin-D'Angelo MJ, Hoteit MA, Brown KV, et al. Dapsone-induced hypersensitivity pneumonitis mimicking *Pneumocystis carinii* pneumonia in a patient with AIDS. *Am J Med Sci* 2004;**327**:163–5.
175. Trahan S, Hanak V, Ryu JH, Myers JL. Role of surgical lung biopsy in separating chronic hypersensitivity pneumonia from usual interstitial pneumonia/idiopathic pulmonary fibrosis: analysis of 31 biopsies from 15 patients. *Chest* 2008;**134**:126–32.
176. Tsai E, Couture D, Hughes DM. A pediatric case of pigeon breeder's disease in Nova Scotia. *Can Respir J* 1998;**5**:507–10.
177. Uziel Y, Hen B, Cordoba M, Wolach B. Lymphocytic interstitial pneumonitis preceding polyarticular juvenile rheumatoid arthritis. *Clin Exp Rheumatol* 1998;**16**:617–9.
178. Uzun O, Akpolat T, Erkan L. Pulmonary vasculitis in Behçet disease: a cumulative analysis. *Chest* 2005;**127**:2243–53.
179. van de Ven AA, de Jong PA, Hyotema van Konijnenburg DP, et al. Airway and interstitial lung disease are distinct entities in paediatric common variable immunodeficiency. *Clin Exp Immunol* 2011;**165**:235–42.
180. Vece TJ, Fan LL. Interstitial lung disease in children older than 2 years. *Pediatr Allergy Immunol Pulmonol* 2010;**23**:33–41.
181. Vece TJ, Fan LL. Diagnosis and management of diffuse lung disease in children. *Paediatr Respir Rev* 2011;**12**:238–42.
182. Vergesslich KA, Gotz M, Kraft D. [Bird breeder's lung with conversion to fatal fibrosing alveolitis]. *Dtsch Med Wochenschr* 1983;**108**:1238–42.
183. Vrielynck S, Mamou-Mani T, Edmond S, et al. Diagnostic value of high-resolution CT in the evaluation of chronic infiltrative lung disease in children. *A J R Am J Roentgenol* 2008;**191**:914–20.
184. Walker KM, Pope J, participating members of the Scleroderma Clinical Trials Consortium (SCTC), and Canadian Scleroderma Research Group (CSRG). Treatment of systemic sclerosis complications: what to do when first-line treatment fails-a consensus of systemic sclerosis experts. *Semin Arthritis Rheum* 2012;**42**: 42–55.
185. Walsh SL, Sverzellati N, Devaraj A, et al. Chronic hypersensitivity pneumonitis: high resolution computed tomography patterns and pulmonary function indices of prognostic determinants. *Eur Radiol* 2012;**22**:1672–9.
186. Warren CP. Extrinsic allergic alveolitis: a disease commoner in non-smokers. *Thorax* 1977;**32**:567–9.
187. Wohl MEB, Chernic V. Bronchiolitis. *Am Rev Respir Dis* 1978;**118**:759–81.
188. Wolf SJ, Stillerman A, Weinberger M, Smith W. Chronic interstitial pneumonitis in a 3-year-old child with hypersensitivity to dove antigens. *Pediatrics* 1987;**79**:1027–9.
189. Yalcin E, Dogru D, Haliloglu M, et al. Postinfectious bronchiolitis obliterans in children: clinical and radiological profile and prognostic factors. *Respiration* 2003;**70**:371–5.
190. Yalcin E, Kiper N, Gocmen A, et al. Pigeon-breeder's disease in a child with selective IgA deficiency. *Pediatr Int* 2003;**45**:216–8.
191. Yamamoto H, Yamaguchi E, Agata H, et al. A combination therapy of whole lung lavage and GM-CSF inhalation in pulmonary alveolar proteinosis. *Pediatr Pulmonol* 2008;**43**:828–30.
192. Yee WF, Castile RG, Cooper A, et al. Diagnosing bird fancier's disease in children. *Pediatrics* 1990;**85**:848–52.
193. Yu T, Ahmed R, Yap E, Kumar S. Dyspnoea in a 17-year-old swim instructor: a diagnosis of hot tube lung. *N Z Med J* 2008; **121**:78–80.
194. Zacharisen MC, Schlueter DP, Kurup VP, Fink JN. The long-term outcome in acute, subacute, and chronic forms of pigeon breeder's disease hypersensitivity pneumonitis. *Ann Allergy Asthma Immunol* 2002;**88**:175–82.
195. Zar HJ. Chronic lung disease in human immunodeficiency virus (HIV) infected children. *Pediatr Pulmonol* 2008;**43**:1–10.

TABLE 24-1 **Major Complications of Pneumonia**

Abscess
Apnea or respiratory failure
Acute respiratory distress syndrome
Atelectasis
Bacteremia and sepsis
Dissemination (skin, solid organs, joints, central nervous system)
Empyema
Hilar adenopathy
Infarction
Necrotizing pneumonia
Pericardial effusion
Pleural effusion
Pneumothorax
Pneumatocoele
Recurrent pneumonia
Pneumothorax
Pericardial effusion

effusions, exudates, purulent pleurisy, parapneumonic effusions, empyema, and complicated empyema. A great deal of inexactitude exists in the use of these terms, and comparing methods of diagnosis and management from one study to another is difficult. The term *transudative pleural effusion* refers to fluid in the pleural space that is a nonpurulent effusion and typically nonpneumonic in origin. The term *purulent effusions* refers to effusions that are more cellular (exudative) and typically pneumonic in origin. The term *empyema* describes purulent effusions with chemical or microbial evidence of a severe process requiring drainage. *Complicated empyema* describes the processes associated with loculations or a fibropurulent rind requiring aggressive manipulations for cure. *Parapneumonic effusion* is a general term referring to any pleural exudative process resulting from an inflammatory process in the lung. Although these definitions are arbitrary and the literature is inconsistent in the use of these terms, a standard approach to the classification of these four types of effusions and their management has evolved.

The first description of parapneumonic infection is attributed to Hippocrates, who in the 4th century BCE advocated incision and drainage of empyema 2 weeks after the onset of symptoms. Since then, the physiology and microbiology of effusions have been described and parapneumonic diseases and the management of fluid collections of the pleural space have been defined. In most cases, the directions of Hippocrates still are relevant: "set him upon a stool, which is not wobbly; someone should hold his hands, then shake him by the shoulders and listen to see on which side a noise is heard. And right at this place—preferably on the left—make an incision, and then it produces death more rarely."[89]

This description of open drainage to normal atmospheric pressures was recognized to be associated with significant mortality rates from hemodynamic instability in 1918, when the Empyema Commission of the United States Army recommended that the practice be abandoned[88]; thereafter, closed-tube drainage was introduced and mortality rates decreased. Developments in radiology, antimicrobials, and surgery resulted in further improvements in care. Today, the major cause of mortality is related to the underlying disease, because the management of parapneumonic effusions in children is largely successful and without residual morbidity. The physician must understand the risks for development of pleural effusion and empyema and indications for thoracentesis, the implications of the results of pleural fluid studies, and the optimal medical and surgical management of the effusions and empyema.

Epidemiology

Parapneumonic effusions are known and expected complications in children with respiratory tract infections. The frequency of effusions may be up to 20 percent of patients with viral or mycoplasmal pneumonia[40,47,124] and 75 percent of patients with proven *Staphylococcus aureus* pneumonia.[8] Empyema has been reported to occur in 6.3 to 23/1000 admissions of children.[97,129] Recent reports suggest rates are increasing.[12,21,122,129]

Pathophysiology

The pleurae are mesodermally derived tissues approximately 30 to 40 μm thick and permeable to liquid and gas.[11,76] The parietal pleurae, which adhere to the chest wall, are fed from the intrathoracic and superior phrenic arteries and have sensory innervation. The visceral pleurae are splanchnic in origin, with blood flow from the pulmonic and pericardiophrenic arteries and no sensory innervation.

Parietal lymphatics absorb most of the excess fluids in pathologic situations and play an important role in normal physiology as well, removing 250 to 500 mL/day in adults.[137] They are the only mechanism for absorbing cells and other debris from the pleura.

The *raison d'être* of the pleural space is unknown. Some mammals do not have a pleural space.[149] In the normal situation, pleural fluid in small amounts is a necessary requirement for optimal lubrication of the pleural space and for mechanical coupling of the lung and chest wall.[76] The accumulation of excess fluid (i.e., effusion) occurs in a limited set of circumstances, through excess production or deficient absorption. Increased production occurs when vessels are leaky (e.g., in septic shock) or active secretion of fluid with mesothelial inflammation (e.g., pleural infection) is present. Decreased absorption occurs with decreased oncotic pressure (e.g., nephrosis), increased pulmonary hydrostatic pressure (e.g., congestive heart failure), or lymphatic obstruction (e.g., malignancy).[152]

The mechanisms behind pleural effusions may vary among different infectious diseases. Effusion can be a "sympathetic" pleural response to a bacterial infection in the lung associated with inflammatory cytokines and altered venous or lymphatic drainage because of local edema. Direct or hematogenous extension of a bacterial process can occur in the pleura. *Mycoplasma* is particularly pathogenic in patients with sickle-cell disease,

TABLE 24-2 Characteristics of Pleural Effusions

Characteristics	Transudative	Purulent Effusion	Empyema	Complicated Empyema
Appearance	Serous	Thin exudate	Turbid	Thick pus
Mean WBC	1000	5300	25,500	55,000
PMN (%)	50	>90	>95	>95
Protein (fluid/serum ratio)	<0.5	>0.5	>0.5	>0.5
LDH (fluid/serum ratio)	<0.6	>0.6	>0.6	>0.6
LDH (IU/L)		>200	>200	>1000
Glucose (mg/dL)	>60	<60	<60	<40
pH*	7.4-7.5	7.35-7.45	7.2-7.35	<7.2
Imaging	Fluid	Fluid	Fluid	Loculations, thick peel, scoliosis

*Should be examined immediately or stored at 0°C (32°F).
LDH, Lactate dehydrogenase; *PMN,* polymorphonuclear neutrophils; *WBC,* white blood cell count.

presumably because of pulmonary sludging, which increases pulmonic venous drainage pressures and results in accumulation of effusions. In pneumococcal disease, effusions often develop several days after the acute infection, when bacteria no longer can be recovered. These effusions may be related to immune complex disease.[18] In patients with tuberculosis, the most common cause of pleural effusions is thought to be the rupture of an old granuloma into the pleural space, with a hypersensitivity response similar to the skin test response,[19] which partly explains the low yield in cultures.

After an inflammatory process is initiated, it tends to progress through three classic stages. The first, defined as a *purulent effusion*, is the acute exudative stage, with a thin pleural exudate characterized by normal glucose, lactate dehydrogenase (LDH), and pH. The second transitional fibropurulent stage, categorized as *empyema*, is characterized by turbid fluid, decreased glucose concentration (<60 mg/dL) and pH (7.2 to 7.35), and elevated LDH (>200 U/L). The third chronic organizing stage is notable for a very low pH (<7.2) and glucose level (<40 mg/dL), LDH concentration greater than 1000 U/L, and development of loculations and peel. This fluid is found in patients with *complicated empyema*. Intervening early (<4 days) before the organizational stages of disease has been proposed to lead to a better prognosis.[64]

Analysis of the pleural fluid is most helpful when the underlying disease is unknown or when a primary pulmonic process is suspected. When patients have effusions caused by hydrostatic imbalance, the effusion is a transudate. Its protein and cell count do not exceed the range of normal pleural fluid (5000 cells/mm^3 and <2 g of protein).[8,137] Patients who have an active inflammatory process may have an exudate, defined by excess protein and cells. In children, the most common cause of exudative pleural processes is pneumonia. In adults, most pleural effusions are related to congestive heart failure or malignancy,[83] but pneumonia is the most common cause of empyema.[110] Table 24-2 summarizes the general differences among pleural effusions.

Table 24-3 lists causes of effusions. Some of these causes, particularly iatrogenic causes, such as invasive procedures and drugs, are important to consider in the differential diagnosis of a difficult case. Others are associated with specific syndromes, such as acute respiratory distress syndrome[140] and yellow nail lymphedema syndrome.[143] Motor vehicle accidents have been identified as a common cause of serosanguineous effusions when disruption of normal mechanical lung function and hematoma occur.[120]

TABLE 24-3 Causes of Pleural Effusion

- Capillary leak
- Sepsis syndrome
  - Vasculitis associated with immune complex disease
    - Connective tissue diseases
    - Inflammatory bowel disease
  - Malignancy (lymphoreticular, sarcoma, neuroblastoma)
  - Toxins (e.g., TSST-1)
  - Drugs (amiodarone, bleomycin, isoniazid, methotrexate, nitrofurantoin, phenytoin)
  - Myxedema
  - Trauma
- Increased hydrostatic pressure
  - Congestive heart failure
  - Sickle-cell disease
  - Pulmonary venous hypertension
  - Superior vena cava syndrome
  - Pregnancy
- Decreased oncotic pressure
  - Nephrosis
  - Cirrhosis
  - Protein malnutrition
- Obstructed lymphatics
  - Congenital lymphangiectasia
  - Yellow nail syndrome
  - Radiation injury
  - Neoplasia (metastatic disease)
- Pleural inflammation
  - Pneumonia
  - Lung abscess with pleural fistula
  - Pleural infection (e.g., tuberculosis)
  - Esophageal rupture
  - Pancreatitis
- Iatrogenic
  - Drugs
  - Central line misplacement

TSST-1, Toxic shock syndrome toxin–1.

Microbiology

Among children with parapneumonic effusions, no prospective study has established firmly the frequency with which effusions occur and how many are associated with particular microbes. Although respiratory viruses infrequently cause symptomatic effusions, the sheer number of cases and the presence of asymptomatic cases likely would implicate viral infection as the most common cause. Definite viral disease has been associated with cytomegalovirus, Epstein-Barr virus, measles, and adenovirus.[42,43,66,96] Other pathogens, such as *Mycoplasma* and *Chlamydia*, are difficult to diagnose but may account for a significant number of pneumonic infections in older children and adolescents.[40,43] Viral, mycoplasmal, and chlamydial organisms rarely are identified in patients with effusions requiring intervention.

The bacteriology of empyema is constantly evolving. Several past articles have established the role of various bacterial pathogens in childhood effusions. A study of 227 children by Freij and colleagues[42] published in 1984 found *S. aureus* (29%), *Streptococcus pneumoniae* (22%), and *Haemophilus influenzae* (18%) to be the three most frequent causes of parapneumonic effusions (see Table 24-3). Subsequent studies show that the frequency of these pathogens has been affected by vaccine[1,20] and antibiotic use. Pathogen frequency in different eras is shown in Table 24-4. *H. influenzae* and *S. pneumoniae* vaccines have led to a decreased incidence of vaccine-specific strains as a cause of empyema, whereas methicillin-resistant *S. aureus* (MRSA) has emerged as a frequent cause of community-acquired disease, with rates in Texas doubling from 2001 to 2009.[24,129] Among cases of *S. pneumoniae* infections, nonvaccine strains are increasing in number and severity.[20,35] Recent studies using a U.S. national hospital discharge database suggest that *S. pneumoniae* remains the most common etiology of bacterial empyema.[46] Certain groups of children (e.g., neonates,[42] immunocompromised hosts, patients with preexisting chest tubes that become infected with nosocomial pathogens, patients with a ruptured viscus, and patients with foreign body aspiration) are at higher risk for acquiring gram-negative infections.[12]

Administration of antibiotics before the diagnosis of empyema is made influences the recovery of organisms. In one report, the incidence of prethoracentesis antibiotics was 71 percent in culture-negative effusions and only 41 percent in culture-positive effusions.[57] Pretreatment with antibiotics may be associated with a decrease in the number of positive blood cultures and in the number of patients from whom *S. pneumoniae* isolates are recovered.[110] Freij and associates[42] reported the frequency of parapneumonic effusions occurring in children with pneumonia caused by specific pathogens. The rates of effusion by organism were as follows: group A streptococcus, 86 to 91 percent; *S. aureus*, 72 to 76 percent; *S. pneumoniae*, 57 percent; *H. influenzae*, 49 to 75 percent; *Mycoplasma*, 21 percent; and adenoviruses, 11 to 33 percent.

Anaerobes were sought carefully by Brook and Frazier,[17] who found them infrequently in patients younger than 6 years of age. The anaerobes rarely were found in patients with primary pneumonia, occurring most often in patients with lung abscess and aspiration pneumonia.[17] In older patients (7 to 17 years old), anaerobes were recovered as isolated pathogens in 44 percent of cases.[17] Virtually every bacterial organism has been associated with pleural effusion at one time or another. *Brucella*,[69] *Francisella tularensis*,[121] and *Yersinia enterocolitica*[65] may be associated with the development of pleural effusions. The diagnosis in such cases often is suggested by a unique history in the patient.

Mycobacterial effusions are uncommon in U.S. children but are well described. In four published reviews, only two patients (from Nigeria) were reported to have *Mycobacterium tuberculosis*.[42,57,90,96] In a series of 303 children younger than 2 years of age with tuberculosis, 3.3 percent had an effusion.[54] In adolescents with tuberculosis, the incidence of effusion likely approximates that of adult disease. In one series of adult patients with primary tuberculous disease, pleural effusion occurred in 29 percent of cases.[29] In another adult series, primarily of reactivation disease, pleural effusion occurred in 1 percent of the patients.[48] Whether coinfection with human immunodeficiency virus (HIV) is increasing the incidence of effusion is controversial.[39]

Histoplasmosis has been associated with pleural effusion in 0 to 6 percent of childhood histoplasmosis cases.[112] Blastomycosis has been associated with pleural effusions in 0 to 40 percent of cases.[111,137] Effusions resulting from other fungi (e.g., *Coccidioides*, *Aspergillus*) have been described.[88] Parasitic diseases manifesting with effusions

TABLE 24-4 **Percentage of Pathogens Recovered in Purulent Effusions from Children**

Site/Years (No. Patients)	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Haemophilus influenzae*	Other Pathogens	Sterile	Reference
Dallas/1964-1982 (227)	29	22	18	8	24	42
Nashville/1977-1989 (61)	11	34	3	11	39	57
Washington, DC/1973-1985 (33)	15	12	21	52	Not reported	70
Israel/1972-1981 (37)	14	41	Not reported	35	11	97
Dallas/1992-1998 (135)	8	32	1	13	46	34
Houston/2001-2002 (47)	19	9	Not reported	4	68	129
Australia/2007-2009* (145)	9	51	3	2	35	138

*Polymerase chain reaction results only.

are uncommon but are found in patients with *Entamoeba histolytica* disease, most often from rupture of a hepatic abscess into the pleural space.[88] Echinococcal disease also has been reported.[41]

Pleural effusion associated with adult HIV infection has been reported in 14.6 percent of hospital admissions in one series in which 67 of 160 cases were infectious. Of those cases, 50 were associated with bacterial pneumonia, 10 with tuberculosis, and 5 with *Pneumocystis jiroveci* pneumonia.[2] Another report on patients infected with HIV suggested that empyema was seen primarily in patients with intravenous drug abuse.[56] We have not seen empyema in our pediatric HIV-infected patients, perhaps because of the more recent use of more effective antiretroviral therapy.

Drug resistance in community-acquired pneumonia complicates the management of parapneumonic effusions. Intermediate or fully resistant *S. pneumoniae* was found in 12.8 percent and 10.1 percent of isolates in a study from multiple pediatric centers from 1993 to 2000; 7.5 percent were cephalosporin-resistant.[142] MRSA is a growing concern in empyema. Reports since 2000 have shown that 22 to 78 percent of isolates of *S. aureus* were methicillin-resistant, with many being community acquired.[61]

Diagnosis

Clinical Presentation

Symptoms most specific for parapneumonic processes are dyspnea and pleuritic pain. Dyspnea occurs when the volume of the effusion mechanically interferes with breathing or when pain prevents adequate gas exchange. Pain occurs with irritation of the parietal pleura and on inspiration (i.e., pleurisy). Fever is generated by the inflammatory response and pathogen-specific components (e.g., lipopolysaccharide, toxins). With an acute bacterial process, the fever can be high and hectic, mimicking the fever that occurs with an abscess. Patients in the chronic organizational phase generally have less fever. Cough and malaise are secondary symptoms. Hemoptysis and purulent sputum also may occur. The onset of symptoms of a purulent effusion may be delayed in time and distinct from the symptoms found at the onset of the pneumonia in older children; infants usually have no symptom-free period[19]; in the early phases of effusions, the patient may have no symptoms.

The physical examination usually is revealing. The child is tachypneic in more than 70 percent of cases, but breathing is shallow as a result of the child's attempt to minimize pain. Fever and cough usually occur in more than 90 percent of patients with purulent effusions.[90] The patient may appear toxic, with acute infection. Patients often posture toward the affected side. Classically, auscultation reveals a decrease in breath sounds and occasionally detects a pleural rub, but pleural rubs often are absent in a very young child. Rales from an associated pneumonia may be heard. Depending on the stage of the process, percussion may reveal a level of dullness associated with free-flowing effusion. As the process organizes, it may be less evident. Empyemas can erode through the chest wall into the subcutaneous tissue (i.e., empyema necessitatis) or into a bronchus (i.e., bronchopleural fistula).

Imaging

The diagnosis most often is made by radiographic examination of the chest. Consolidation of a lobe of the lung is present, with an effusion obscuring the diaphragm. A standard posteroanterior standing view reveals blunting of the costal diaphragmatic gutter. As fluid tracks along the lateral and posterior chest wall, a meniscus configuration is seen. Distinguishing it from pleural thickening may be difficult, and in such cases, a decubitus or cross-table view of the chest allows free-flowing fluid to layer out on the dependent chest wall. In older children and adults, a decubitus layer of fluid of more than 10 mm is considered a sufficient volume of fluid to attempt to extract by thoracentesis.[15,82] With large volumes of fluid (>1000 mL),[123] compression of the lung and shift of the trachea away from the effusion (Figure 24-1) may occur. As an empyema develops and organizes, discrete pockets of fluid (i.e., loculations) may form within the pleural cavity (Figure 24-1C). Occasionally, loculations are confused with lung abscess. Scoliosis also is well defined by the chest radiograph and occasionally is used as an indication for surgery.[57] The observation of an air-fluid level in the pleural space signifies that air has been generated in the pleural space by gas-forming organisms or has entered through a pneumothorax, perforated viscus, or bronchopleural fistula.

Ultrasonography has shown great utility in providing better guidance for thoracentesis of pleural fluid. It is noninvasive, without ionizing radiation, allows empyema to be defined by showing internal echoes and septations (Figure 24-2),[130,153] and is the imaging mode of choice for most pleural effusions.[22,103] Transudates uniformly are anechoic, although approximately one third of exudates also are anechoic.[153] Ultrasonography is not as precise as computed tomography (CT) in differentiating a lung abscess from an empyema. CT and magnetic resonance imaging occasionally are required to distinguish parenchymal from pleural disease, identify a nonopaque foreign body, or locate a fistula.[139] CT particularly is useful in a patient whose chest radiograph shows total opacification of the lung, and thus is considered by some physicians the study of choice in this situation.[57] Ultrasound can have false-negative results, and CT can have false-positive findings on examination of pleural effusions. One study of adults showed that neither ultrasound nor CT effectively predicted the stage of the effusion or predicted the surgical outcome.[67]

Pleural Fluid Analysis

Thoracentesis can play an important role in the management of parapneumonic effusions. Fluid should be obtained from the pleural cavity if the fluid is adequate in volume and anatomically accessible, if a microbial diagnosis has not been made or presumed, and if antibiotic therapy is intended or if pulmonary function is compromised by the effusion and imaging does not reveal

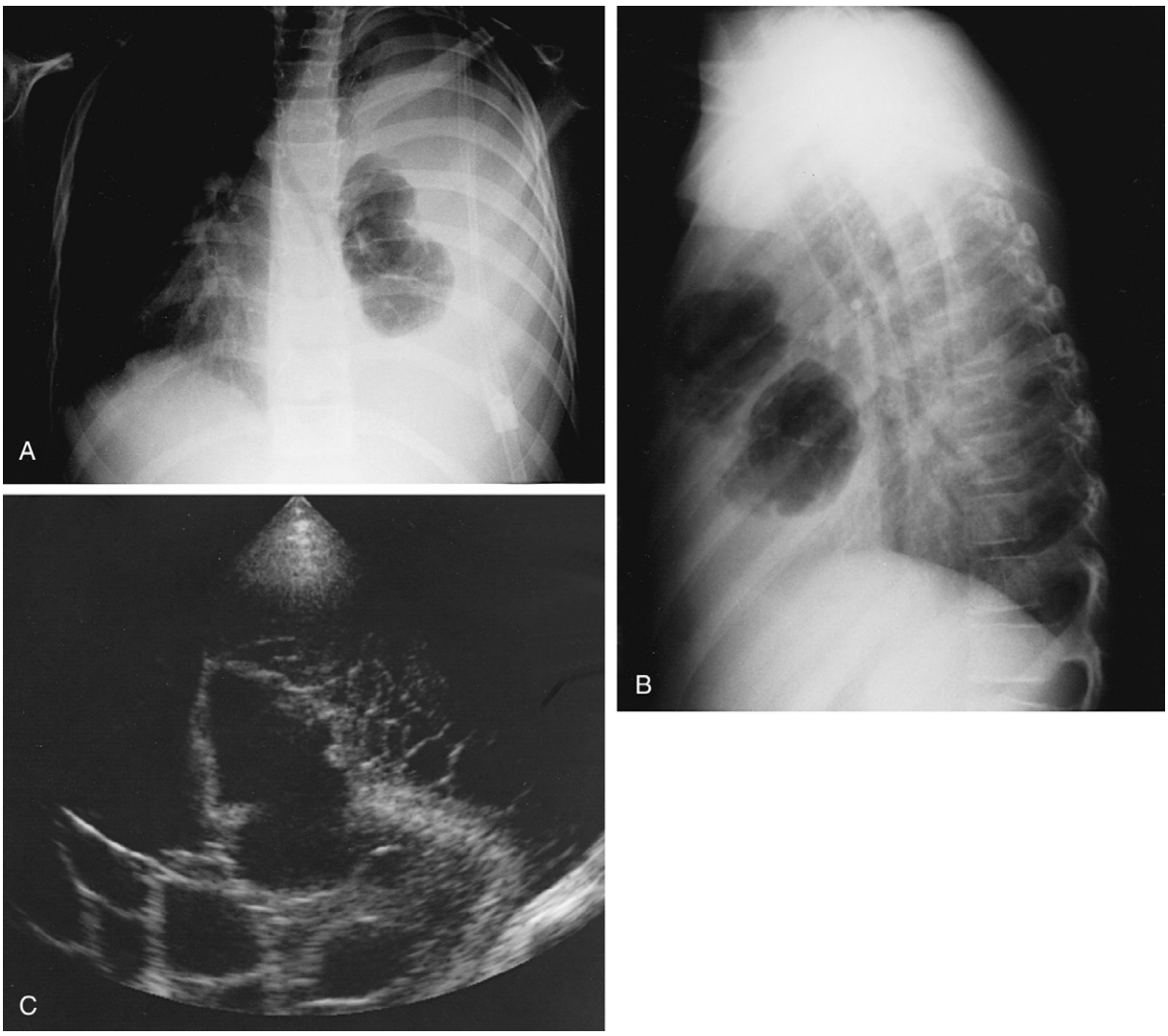

FIGURE 24-1 ■ A-C, Chest radiograph and ultrasound examination images from a 7-year-old boy with a 5-day history of vomiting, diarrhea, and low-grade fever. He had a history of varicella 2 weeks earlier. The patient was hospitalized for dehydration and developed left-sided chest pain and a mild cough. Intravenous nafcillin was begun, but his condition deteriorated over the next 4 days, with an enlarging effusion and tracheal shift. Loculations were found on ultrasound **(C)**. He had a thoracotomy performed on day 6 with decortication and removal of a fibrinous rind. The patient was afebrile within 24 hours and received 1 week of intravenous antibiotics and 1 week of oral antibiotics. He was well on follow-up examination after discharge.

evidence of organization to determine whether further intervention is necessary.

The volume and location of the fluid can be determined precisely by ultrasound examination. When a healthy child has a small pleural effusion, thoracentesis usually is not required because small effusions generally can resorb. Thoracentesis for microbiological diagnosis can often be avoided, because a 10 to 50 percent chance of recovering the etiologic organism from blood cultures exists in empyema.[6,42] We suggest, however, that when the clinical presentation is atypical or a moderately sized effusion exists, thoracentesis usually is indicated to define the microbial process. Atypical situations include a history of trauma, foreign body aspiration, prolonged or chronic disease, and underlying systemic diseases (e.g., congestive heart failure, malignancy).

In a classic article published in 1972, Light and associates[83] established the methodology by which transudates could be differentiated from exudates. Such criteria are valuable in determining whether antibiotic treatment is indicated, particularly in patients with underlying diseases that predispose them to sterile effusions but who may have a comorbid infectious condition. An exudate was defined by any of the following criteria: a fluid-to-serum protein ratio greater than 0.5, an LDH fluid-to-serum ratio greater than 0.6, a glucose concentration less than 50 mg/dL, or a pH less than 7.2. This study was based on the results from 150 adult patients, 103 of whom had exudates.[83] Modifications of Light's criteria have been made by reducing the LDH ratio threshold to 0.45.[55] A two-step process has been recommended to separate transudates from exudates using only the protein and LDH serum-to-fluid ratios in the initial evaluation. If a patient had an apparent exudate, additional studies, including cultures, stains, pH, and glucose, are indicated.[107]

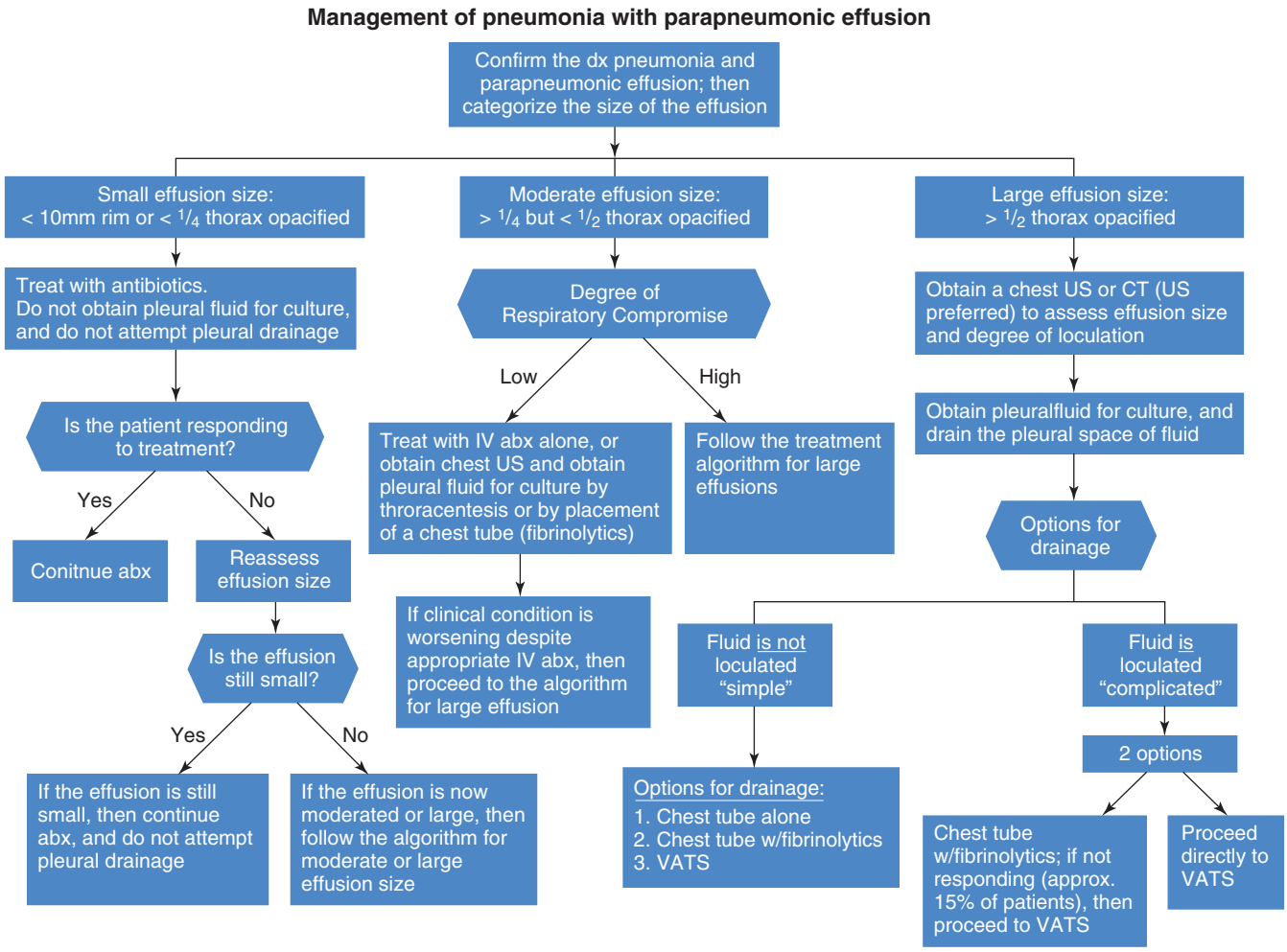

FIGURE 24-2 ■ Algorithm for the management of pneumonia with parapneumonic effusion from the IDSA guidelines.[15] *CT,* Computed tomography; *US,* ultrasound; *VATS,* video-assisted thoracic surgery. (See companion website on Expert Consult for a color version of this figure.) (From: Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.)

The application of these criteria to children has received limited study. In one series of 61 children, patients who required chest tubes or decortication had a mean pleural fluid pH of 7.24 or 7.10 in contrast to children who were treated with antibiotics only (pH 7.35). The mean pleural fluid glucose concentration was 74 g/L in the group treated with antibiotics, 10 g/L in the group treated with chest tubes, and 24 g/L in the group treated by decortication.[57] Another, more recent study confirmed that a pH less than 7.2 and low glucose concentration were predictors of reintervention.[98] The role of this analysis has been questioned and new Infectious Diseases Society of America (IDSA) guidelines for children do not recommend glucose, LDH, and pH studies because they rarely modify patient management.[15]

Standard Gram stain and bacterial culture (aerobic and anaerobic) are indicated when thoracentesis is performed in patients in whom diagnosis of infection is considered. The Gram stain often is positive in patients with bacterial infections and may be used to direct empirical therapy until culture or polymerase chain reaction (PCR) results are known. In one study of children, 12 of 54 sterile effusions from patients with negative blood cultures had a positive Gram stain.[42] Some experts consider that a positive Gram stain indicates a more severe process and that such patients are more likely to require more invasive surgical procedures.[19]

The total white blood cell count in empyema fluid can vary from 5000 to 625,000 cells/mm^3, with median values ranging from 5000 to 55,000 cells/mm^3.[42,57] Virtually all cells are neutrophils in bacterial infections. Marked eosinophilia may be seen in parasitic, fungal, tuberculous, or hypersensitivity disease and when blood is found in the pleural space.[19] Numerous small lymphocytes suggest malignancy, tularemia, or tuberculosis.[38,51,107,153] Other studies are required when the history suggests another underlying process. When tuberculosis is suspected by history, specific mycobacterial stains and cultures should be obtained. Identification of mycobacteria by stain and culture may be equivalent to the rate of identification of the disease process by pleural biopsy (approximately 25%).[102] Specific mycobacterial and fungal stains also should be obtained using a Ziehl-Neelsen/auramine stain and potassium hydroxide. Application of newer methods

for diagnosis, such as PCR and tuberculostearic acid by mass spectroscopy, may be indicated.

When malignancy or metastases are suspected, cytology is necessary.[51] Most effusions in children that prove to be malignant are of lymphoreticular origin. Amylase sometimes is measured and is elevated in cases of esophageal rupture, acute hemorrhagic pancreatitis, or pulmonary infarction.[81,123]

Molecular diagnostics is bringing promising advances to the bacteriologic diagnosis of empyema. PCR technology has been compared with routine culture and bacterial antigen and has shown increased sensitivity and good specificity for the diagnosis of *S. pneumoniae* with 16S rDNA or MRSA using the mec-300 gene probe.[35,122] In recently reported studies,[12,100] the sensitivity of PCR has been greater than 80 percent. Turnaround times can be quite rapid, exceeding traditional culture methods. The sensitivity of PCR for tuberculous effusions ranges from 20 to 81 percent, and specificity ranges from 78 to 100 percent.[39] New commercially available PCR cocktails for respiratory viruses are also available but because of current expense are best reserved for difficult or critically ill patients.

Additional Diagnostic Studies

An intradermal test should be applied to any child with a parapneumonic effusion to evaluate tuberculosis as a possible cause. One third of patients with tuberculous effusions have a negative purified protein derivative skin test result.[10] Early morning gastric aspirates have been recommended if tuberculosis is suspected but tuberculosis-specific interferon-γ release assays are now approved for children down to 5 years of age. Sensitivity and specificity remain problematic, however.[87,145] Bacterial blood cultures also are indicated because one third of patients can have positive blood cultures and negative pleural fluid Gram stain and culture results.[42] Sputum is a less reliable source from which to determine the microbial cause of an effusion but may be helpful in an older patient with purulent sputum and a single predominant organism. Sputum can be diagnostic in older children with reactivation or cavitary tuberculosis and in cases of blastomycosis and histoplasmosis. Cold agglutinins or *Mycoplasma* serology may confirm the cause of a pleural effusion, although the nonspecificity of cold agglutinins and the delay in the increase in antibody titers render these data of marginal use in the acute management of the patient. Viral cultures are useful in only the more unusual cases and generally provide information that is not helpful in the initial management of the patient. Rapid diagnostic antigen assays, such as the rapid tests for influenza A and B and other respiratory viruses, may be useful in defining the primary cause of respiratory disease but do not help exclude secondary bacterial pathogens causing pneumonia and a parapneumonic effusion. When other diseases, such as Wegener granulomatosis[7] and systemic lupus erythematosus, are suspected, disease-specific tests such as antineutrophil cytoplasmic antibody and antinucleic acid antibody are indicated.

Management

If a patient has an underlying disease process associated with pleural effusion and thoracentesis has excluded bacterial infection (e.g., normal protein and LDH fluid-to-serum ratio, normal glucose level, negative cultures, and Gram stain results), no further treatment is indicated other than treatment of the underlying disease. These patients continue to be at risk for developing infection of the effusion and may require repeat examination of pleural fluid later if infection is suggested clinically.

If a purulent effusion is suggested by imaging, thoracentesis, or surgical findings, empirical antibiotic therapy is indicated. Pathogens of most importance are *S. pneumoniae*, *S. aureus*, and *Streptococcus pyogenes*. Empirical therapy choices are changing as drug resistance patterns continue to change, immunization against *H. influenzae* type b and *S. pneumoniae* has become widespread, and the ability to make a rapid diagnosis (e.g., PCR) improves. For mildly ill patients, amoxicillin orally or penicillin or ampicillin intravenously can be used. For patients who are moderately ill, unimmunized, or allergic to penicillin or in whom *S. aureus* is suspected (because of past disease or presence of pneumatocoele), clindamycin in combination with cefotaxime is used. Vancomycin is substituted for clindamycin in critically ill patients.

Ticarcillin-clavulanate, imipenem-cilastatin, meropenem, and piperacillin-tazobactam are alternatives in nosocomial infections, gram-negative risk (neonates and postsurgical cases) or if anaerobes are considered, particularly if aspiration is thought to play a role, such as in patients with abnormal swallowing. Aminoglycosides are alternatives for proven gram-negative infections, and cefepime is helpful in nosocomial pneumonia and empyema because of its current efficacy for *Enterobacter* spp. In patients with renal failure or cephalosporin hypersensitivity, aztreonam is effective therapy for gram-negative infections. Ceftazidime or cefepime, broad-spectrum β-lactams with or without a β-lactamase inhibitor (i.e., ticarcillin–clavulanic acid, imipenem cilastatin, meropenem, or piperacillin-tazobactam), or aminoglycosides are indicated for *Pseudomonas* infection.

Surgical drainage is considered a crucial factor in resolving anaerobic infection. Clindamycin and metronidazole are effective, particularly when postsurgical infection or a ruptured gastrointestinal viscus is present. Upper respiratory tract anaerobes may be resistant to penicillins because of β-lactamase–producing oral flora (particularly *Prevotella* and *Porphyromonas* spp.).[17] In these situations, penicillin sensitivity should be documented before a penicillin is used as primary therapy.

Once a pathogen is isolated and sensitivity patterns are established, therapy may need to be modified. Past studies have suggested that resistant isolates of pneumococcus were treated effectively with parenteral penicillin in high doses.[16,17,77,105] Further, in 2008, the Clinical Laboratory Standards Institute (CLSI) issued new standards for resistance. The net effect was to reduce the number of isolates in the community with any resistance from approximately 25 percent to 7 percent.[25] New guidelines for pediatrics now recommend amoxicillin orally or penicillin or

ampicillin intravenously for *S. pneumoniae*–associated community-acquired pneumonia with a minimum inhibitory concentration (MIC) 2 μg/mL or less. When the pneumococcus is highly resistant to penicillin (>4 μg/mL), ceftriaxone is preferred, with levofloxacin, linezolid, clindamycin, and vancomycin considered alternatives.[15] New guidelines for management of MRSA were published by the IDSA in 2011.[84] These suggest that vancomycin is still the preferred treatment. The guidelines for adults suggest a nonvancomycin regimen for disease associated with an MIC greater than 2.0 μg/mL. A recent report notes, however, that a poor clinical outcome may be independent of antibiotic choice and directly related to an MIC greater than 1.5 μg/mL.[59] Clindamycin or linezolid may be considered for treatment of MRSA but is discouraged as monotherapy if the patient is bacteremic. Clindamycin sensitivity should be determined, because many MRSA and methicillin-susceptible *S. aureus* isolates are resistant to clindamycin.[128] Susceptibility should be confirmed with performance of a "D" test. Intravenous clindamycin can be switched to oral clindamycin later in the clinical course, offering a management advantage over vancomycin. New drugs such as linezolid are in limited use at this time because of high cost and some side effects, such as neuropathy and visual changes, that may be hard to monitor in small children. Daptomycin is specifically avoided in the lung because of its inactivation by surfactant.

With appropriate antibiotic therapy, the duration of fever in uncomplicated cases of purulent effusions usually is less than 48 to 72 hours.[101] When fever persists beyond 72 hours, surgical drainage may be required. The duration of antibiotic therapy is based on the response of the patient to the medical and surgical therapy provided. In one series of pediatric patients, the total duration of antibiotic therapy for patients with severe pleural infections who did or did not have surgical drainage was comparable (approximately every 12 days).[57] The duration of combined intravenous and oral therapy was 12 to 24 days in the study reported by Freij and associates,[42] with patients with *S. pneumoniae* infection receiving the shortest courses of antibiotic therapy and patients infected with *S. aureus* being treated for longer periods. A prudent standard is to continue treatment for a minimum of 1 week beyond the last febrile day.

Drainage of the pleural space has been the standard treatment of parapneumonic effusions in four classes of patients: (1) patients in whom thick, purulent material is found at thoracentesis; (2) patients with a pleural fluid pH level less than 7.35 and glucose level less than 60 mg/dL; (3) patients for whom antibiotic therapy has not been associated with a timely clinical response (72 hours); and (4) patients in whom pulmonary function is compromised, as shown by severe hypoxemia or hypercapnia. The chest tube is placed until minimal drainage is noted (usually <50 mL/day).[36]

When closed-chest-tube drainage is not associated with clinical improvement and defervescence, if the lung parenchyma is trapped by the fibrinopurulent peel, or fever persists, video-assisted thoracoscopic surgery (VATS) or decortication often is required. If the pleural involvement is limited, a small incision (mini-thoracotomy) can be employed.[113] Ultrasonography and CT are required to define these conditions. Decortication is rarely performed now but has shown comparable hospital length of stay to that with chest tube drainage with minimal morbidity.[44,57] Decortication seems to have an advantage in advanced disease in which fibrosis in the pleural cavity has resulted in a large peel.

Although general anesthesia is required for VATS, only two small incisions are needed—one through the existing chest tube tract for a telescope and the second through which operating instruments are passed. This procedure allows adhesiolysis and débridement and should be performed before a thick peel develops. Proponents of VATS argue that a brief operative procedure and the attendant risks of anesthesia outweigh the child's discomfort during thoracentesis (and chest tube placement) and postoperatively. More importantly, a definitive procedure is done, rather than running the risk of having to perform subsequent chest tube placements and thoracotomy. The hospital stay is shortened. VATS is more commonly performed than closed-tube drainage at this time, with some evidence for shorter length of stay.[68,70,127] Doski and colleagues[34] reported a series of 139 children who were studied from 1992 to 1998. By comparing historical cohorts, they showed a shorter length of stay (7 days vs. 11 and 12 days) for children who underwent VATS than for groups of children who had thoracentesis, chest tube drainage, or fibrinolytic therapy and rescue VATS for failure. Of 98 patients who received traditional therapy, 12 required thoracotomy, in contrast to none in the primary VATS group. A small, randomized study of 20 patients was conducted from 1994 to 1996 and showed that VATS was superior to chest tube drainage with fibrinolytic therapy. Compared with chest-tube drainage with fibrinolytic therapy, VATS resulted in a higher primary success rate (91% vs. 44%), fewer hospital days (8.7 vs. 12.8 days), and lower costs (approximately $16,000 vs. $24,000).[147] Another randomized study in 18 children showed VATS superior to chest tube drainage,[75] whereas a larger randomized study of 60 children showed VATS comparable to chest tube drainage with urokinase.[136] Other studies have shown similar mixed results.[45,67,95,115]

The use of radiologist-directed thoracentesis with pigtail catheters (smaller bore than standard chest tubes) for drainage with fibrinolytic therapy has been promoted as a way to reduce the number of operative procedures and achieve shorter hospital stays; it has produced good results in children.[3,119] This therapy may have value in patients with organizing pleural inflammation and inadequate drainage caused by loculations of pleural fluid without a peel.

The use of streptokinase in children with pleural effusion was reported in 1993.[119] The occasional side effects caused by streptokinase reported in adults have generated concern about its safety instead,[13] and its utility has been challenged in adults in a randomized study showing no benefit.[91] Urokinase is less expensive than streptokinase and has been used in children with minimal adverse effects.[53,71,118,139] In adults and children, these different agents seem to be equivalent,[6,14] although commercial shortages of the products have occurred. The tissue-type

plasminogen activator alteplase is the fibrinolytic therapy most commonly used at this time. It is less likely to promote allergic reactions and has a low risk for bleeding complications.[9] Reports have questioned the benefits of interventional radiologist-placed catheters with fibrinolytics for empyema.[117] A meta-analysis of eight evaluable reports from 2000 to 2004 of operative and nonoperative approaches to empyema suggested that the failure of primary therapy was intermediate for fibrinolytic therapy (9.4%), in contrast to simple chest tube drainage (23.6%) and either VATS (2.8%) or thoracotomy (3.1%).[5] A newer review identified only two randomized, controlled trials comparing intrapleural fibrinolytic therapy with VATS and showed no difference.[73] The British Thoracic Society published clinical guidelines[6] for management and concluded that fibrinolytic therapy shortened the hospital stay, but that failure should prompt early consideration of thoracic surgery. Rarely are full thoracotomy and pneumonectomy required for severe pneumonic and parapneumonic disease.

The IDSA put forth consensus guidelines for clinicians for the management of pneumonia and effusions in 2011.[15] Figure 24-2 is an algorithm from the guideline incorporating alternatives for the management of pleural collections. Other comparable guidelines exist,[62,108] but all emphasize the importance of local expertise and the emphasis on the individual circumstance of the patient. A large retrospective review with multivariable analysis of 40 pediatric hospitals using the Pediatric Health Information Systems showed no difference in VATS, CT with fibrinolysis, CT without fibrinolysis, or thoracotomy for length of stay and only marginal differences in cost. VATS was associated with fewer additional procedures.[132] Some emphasize the importance of early versus late operative strategies[62] and another structured review determined that early operative procedures did shorten hospital stay.[126]

In cases of chronic empyema, other approaches are used. A closed tube can be converted into an open drainage tube. This conversion is accomplished safely a minimum of 10 to 14 days into the course of an empyema when the visceral and parietal pleurae fuse, and a pneumothorax can be avoided safely.[88] Other options include open drainage by rib resection and creation of a pleural window. The window ultimately closes with lung expansion and granulation, with disappearance of the pleural space.

One frequent cause of bloody pleural effusions is motor vehicle accidents.[88,120] In one series of 100 children, 56 percent had pleural effusions associated with pulmonary contusions. They were treated with closed-chest-tube drainage; no antibiotics were used, and no infectious complications occurred.[120] Management of pleural hematoma secondary to trauma occasionally is complicated by infection because the bloody pleural fluid is an excellent growth medium. One study suggested that empyema is less common in posttraumatic effusion with closed-chest-tube drainage than in repeated thoracentesis.[146]

Prevention of empyema is theoretically improved by the use of vaccines against *S. pneumoniae*, the most common associated agent. One comprehensive study using national data showed an actual increase of 70 percent in empyema hospitalizations from 1997 to 2006, when the 7-valent pneumococcal vaccine was implemented.[79] At the same time, invasive pneumococcal disease rates decreased. The impact of the new 13-valent pneumococcal vaccine is not yet known.

Prognosis and Long-Term Outcome

The long-term outcome of patients with effusions depends on the underlying cause. Patients with empyema or necrotizing pneumonia who previously were well recover satisfactorily in most cases.[125] Complications from closed-chest-tube drainage include bleeding, infection of the exit wound, bronchopleural fistula, and laceration of the lung. Because of these rare complications, chest tube placement, performed in the past by the pediatrician, now is delegated more frequently to the surgeon. Other rare complications, such as temporary paralysis of the diaphragm, have been reported.[97] In three retrospective reviews, the percentage of patients who required closed-chest-tube drainage or other surgical procedures ranged from 62 to 80 percent.[42,57,97] The rate of decortication ranged from 4 to 43 percent.[42,57] The immediate mortality rate for children in recent years has been 0 to 10.8 percent.[42,57,90,97] In one of these studies, the mortality rate was highest for children younger than 1 year old.[42] Studies conducted to evaluate long-term, specific pulmonary disability using pulmonary function tests and lung volumes have shown normalization of these study results over time.[42]

LUNG ABSCESS

A lung abscess is distinguished by liquefaction and destruction of parenchymal tissue, organization, and cavitation. Necrotizing pneumonia, which on imaging lacks the "rim" representing the organization of the abscess, has a different clinical course and management.[27] A lung abscess may erupt and form an adjacent empyema. Empyema is defined strictly by involvement of the pleural tissues, however.

In adults, lung abscess has been described as a disease of male alcoholics that was managed with surgery.[52,99] Lung abscess in children was recognized early on as having a different etiology and course than lung abscess in adults.[81] As is true of many infectious diseases, it continues to change as the human host and the microbial environment change. The incidence of lung abscess has declined precipitously in the modern era. Smith[135] reported lung abscesses in 0.33 percent of pediatric admissions in 1934, and Emanuel and Shulman[37] reported a rate of 0.012 percent from 1985 to 1990. In major pediatric referral centers in Chicago, Houston, Dallas, and Montreal, the incidence of lung abscesses has ranged in the last 2 decades from 1.5 to 4.7 cases per year.[5,18,40,68] Many of these cases developed in compromised hosts.

Improvements in pediatric diagnosis and care have resulted in a decrease in the number of cases related to underlying diseases. The morbidity and mortality rates also have decreased with use of antibiotic therapy and

modern critical care medicine. In 1920, Wessler and Schwarz reported a 33-percent mortality rate, with "invalidism" and hemiplegia occurring in another 27 percent.[131,150] The mortality rates in modern U.S. reports of lung abscess were 11 percent (1982 to 1993)[18] and 4 percent (1985 to 1990),[69] and 18.5 percent mortality was reported in Taiwan (1987 to 2003).[56]

Although lung abscesses occur in children of all ages, two studies have suggested that the trend is away from children younger than 5 years of age to an older population.[18,47] In other studies, the median age ranged from 7 to 9.5 years.* No consistent racial or sexual predisposition to this condition in children has been identified. In the 1950s, one series found boys to be more at risk than girls.[35] Specific risk factors for development of lung abscess in pediatric patients include predisposition to aspiration, hematogenous spread, or compromised immunity.

Pathophysiology

Two main mechanisms explain formation of a lung abscess. The first mechanism is the introduction of pathogens directly into the air spaces, which typically results in solitary abscesses. The abscess typically follows aspiration, with a resultant neutrophilic reaction and necrosis. Lung abscesses complicated tonsillectomies in one third of cases in a 1920 report.[82] The abscesses were theorized to result from aspiration during the operative procedure[67] and characteristically developed 13 to 14 days after the procedure.[82] Great improvements in modern pediatric anesthesia with careful efforts to prevent aspiration have rendered this complication uncommon today. Most lung abscesses related to aspiration are polymicrobial and include anaerobes. The prevalence of fluoride and the low incidence of dental disease in children may be additional factors for the reduced incidence of lung abscess in children, although aspiration pneumonia does occur in the absence of dental disease. Aspiration of a foreign body is a rarer trigger of abscess formation.[80]

The second mechanism in the formation of lung abscess is hematogenous spread. Hematogenous seeding of the lung can lead to an initial pneumonia that develops into an abscess with further organization and cavitation. Primary pneumonia rarely progresses to necrosis and abscess in modern times; this phenomenon is explained largely by the ready accessibility of antibiotics. Emboli from the venous circulation (i.e., septic thrombophlebitis) and right side of the heart (i.e., endocarditis) can cause single or multiple lung abscesses, which often are subpleural. Infection in the head and neck area also is a risk factor for vascular spread to the lung, with resultant lung abscess.[61] Abscesses are found more often with sensitive tools, such as CT. Modern reviews of children suggest that single abscesses are found more frequently than multiple abscesses.[8,18,69]

A lung abscess tends to have irregular margins, with occasional bullae, and can dissect into adjacent tissues, such as the mediastinum, bronchi, and pleural space. If the abscess ruptures into a bronchus, air enters and an air–fluid level can be seen radiographically. Dissection into the pleural space creates a purulent effusion, with air noted only if an anaerobic process is present. Dissection into the mediastinum causes a widening of the mediastinum. Air is detected if communication occurs with a bronchus or in the presence of anaerobes.

Microscopically, a lung abscess is definable by a collection of necrotic material (i.e., highly neutrophilic inflammation); a surrounding irregular, fibrotic wall; and microvascular infarcts. Lymphocytes often are present and seem to play a regulatory role in the formation of the abscess.[65]

The role of preceding viral infection in undermining phagocytic host defenses is supported by the observations of preceding respiratory symptoms in patients with lung abscesses that manifest primarily in cold weather[5,18] and typically after well-defined viral illnesses, such as varicella, measles, and influenza.[35,38,77] The impact of chemotherapy on phagocytes also may explain the increased numbers of lung abscesses in patients with leukemia and other cancers.

On a macroscopic scale, lung abscesses tend to develop in all parts of the lung. If associated with aspiration, the anatomic site depends on whether the patient was supine or erect at the time of aspiration. Supine patients develop abscesses in the posterior upper and lower lobes, and erect patients develop infection in the middle and basilar lower lobes. Generally, the tendency is for aspiration-related abscesses to develop more on the right side than on the left, presumably because of the vertical anatomy of the right stem bronchus.[18,34]

Physicians have grouped lung abscesses into primary and secondary categories, presuming that primary and secondary abscesses have different microbiologic factors, management approaches, and outcomes. The arbitrary nature of this distinction is apparent when appreciating how much the microbiology, clinical course, management, and outcome of both conditions overlap.[44] Primary abscesses occur in previously normal hosts without a history of trauma or aspiration of a foreign body. Secondary abscesses occur in the setting of underlying medical illnesses predisposing to infection, airway obstruction, embolization, or aspiration. In an earlier series, secondary abscesses were found more often in children younger than 1 year of age, but this difference was not corroborated in a later study.[18] Primary abscesses were found in 64 percent of patients in Chicago,[18] 33 percent in Houston,[69] 45 percent in Little Rock (1989 to 1994) (unpublished data), 30 percent in Toronto (1956 to 1965),[38] and 30 percent in Taiwan (1987 to 2003).[56]

In the modern era, lung abscess should trigger a search to exclude underlying factors that may have prognostic value and lead to treatment of the underlying disease. In children, a classic presentation would be any child with altered mental status, associated swallowing dysfunction, or both conditions. Foreign bodies can obstruct normal clearance of pathogens and precipitate the development of a lung abscess.[18,39] Obstruction predisposes to lung abscess rarely in children with metastatic disorders. Ineffective cough in patients with neurodegenerative or myopathic disorders is another risk factor

*References 5, 8, 18, 27, 56, 69.

for developing lung abscess, similar to that in an adult alcoholic. Patients with leukemia and patients who are receiving chemotherapy also are at increased risk. Occasionally, a bronchogenic cyst can become infected and mimic a lung abscess. Tricuspid or pulmonary valve endocarditis in children with complicated congenital heart disease places them at risk for developing lung abscesses.

Immunodeficiency is another risk factor for development of a lung abscess. Patients with chronic granulomatous disease and hyper-immunoglobulin (Ig)E syndrome typically are found to have lung abscesses. Patients with hypogammaglobulinemia may develop abscesses, although bronchiectasis is the more characteristic finding. The same is true for patients with immotile cilia syndromes and cystic fibrosis, although in the latter group, large abscesses are uncommon.[56] Among pediatric patients, HIV-1 infection has not been reported as a risk factor in series from Chicago and Houston.[18,69] Studies by these authors and others of 46 reported cases of secondary lung abscesses showed associations as follows: neuropsychiatric cases (16), hematologic and oncologic disorders (11), primary pulmonary disease (8), immunodeficiency (4), congenital heart disease (2), and 1 each of solvent aspiration, foreign body aspiration, prematurity, chromosomal disorder, and endocrinopathy.[53,133]

TABLE 24-5 **Symptoms and Signs in Patients with Lung Abscess**[142]

Symptom	No. Cases	Percentage
Fever	38	84
Cough	24	53
Dyspnea	17	38
Chest pain	11	24
Anorexia	9	20
Purulent sputum	8	18
Rhinorrhea	7	16
Malaise/lethargy	5	11
Hemoptysis	4	9
Diarrhea	4	9
Nausea/vomiting	3	7
Irritability	3	7
Otitis media	2	4
Seizures	2	4
Weight loss	1	2
Sore throat	1	2
Lymphadenopathy	1	2

Microbiology

The microbiology of lung abscesses is evolving as patients and antibiotics change.[52] In the preantibiotic era, streptococci and *M. tuberculosis* were the most commonly reported causes of lung abscesses. After penicillin use began and tuberculosis skin testing, treatment, and control programs became widespread, staphylococci most frequently were recovered from lung abscesses.[53] Development of better culture techniques also increased the identification of anaerobes in lung abscess material; in past eras, these organisms probably were present but not recovered. Anaerobes were suspected by the fetid odor of abscesses, the time course of postoperative aspiration infections, and Gram stains of tissue and pus, which showed fusobacteria and spirochetes.[67,81] Table 24-5 summarizes the microbiology of pediatric lung abscesses reported from 1976 to 2006.

The primary role of anaerobes in lung abscesses has been assumed in aspiration pneumonias. Anaerobes are prominent in the oral cavity and have been recovered from the abscesses of patients with dental disease.[7] Successful growth occurs when specimens are transported in a closed syringe, with culture inoculation beginning in less than 10 minutes.[8,69] In one study of developmentally delayed children with seizure disorders, poor dental care, and suspected aspiration, transtracheal polymicrobial infections with aerobes were found in 9 of 10 samples. An average of 6.2 isolates was recovered per patient. *Peptostreptococcus* and *Bacteroides* spp. were the anaerobes recovered most frequently.[8] In a larger group of 45 children, 15 of whom had primary abscesses, 14 had polymicrobial infections.[69] Older children with neurologic disorders were the primary patients with anaerobes in both studies. Anaerobes, *S. pneumoniae*, nontypeable *H. influenzae*, and *S. aureus* have been recovered from normal patients.[24,69]

The role of tissue lysins and toxins depends on the pathogen and is thought to be crucial for the development of lung abscesses. In mixed infections, synergy probably occurs among the pathogens as proposed by Smith in 1934.[67] Nosocomial pathogens are becoming frequent causes of lung abscesses because of the increased numbers of patients with extended hospitalizations and use of advanced-generation antibiotics. The widespread use of third-generation cephalosporins has resulted in resistant *Enterobacter* spp. and other gram-negative organisms being recovered in secondary lung abscesses. In the report by Tan and associates,[142] fungal abscesses occurred in 6 of 34 cases and always were associated with debilitated, chronically hospitalized patients. Immunosuppression no doubt contributes to the recovery of other unsuspected organisms (e.g., *Neisseria mucosa*, *S. pneumoniae*, and *Legionella* and *Citrobacter* spp.)[16,17,28,54,60] and underscores the value of obtaining specimens in chronically hospitalized patients, atypical cases, and patients not responding to empirical treatment.

Among otherwise normal hosts, tuberculosis can be a cause of single and multiple abscesses. In patients with an international travel history, unusual pathogens such as parasites (e.g., hydatid cysts)[19] and regional bacteria (e.g., *Pseudomonas pseudomallei*) should be considered.

Rarely is sputum useful in defining the pathogens in a lung abscess because of three factors: (1) sputum typically is contaminated with abundant mouth flora; (2) if the lung abscess is not ruptured, no direct communication of the pathogens in the abscess occurs with the airway; and (3) sputum is difficult to obtain in preadolescent children. Obtaining cough cultures, performed by gagging a young child and culturing the coughed sputum collected on a swab before it can be swallowed, frequently

is unsuccessful. A skilled clinician occasionally can acquire useful information, however.

Bronchoscopy is effective in recovering relevant organisms if the abscess has ruptured and has therapeutic value because it may assist in clearing secretions from the airway. It has been performed infrequently in pediatric practice in the past because of a lack of skilled personnel and pediatric equipment and fear of rupture of a large volume of purulent material into the airway. Rigid bronchoscopy rarely is used to drain the abscess, but, when performed, highly informative microbiologic information may be obtained. Transtracheal aspirates, likewise performed in few pediatric patients, have similar value. The upper airway frequently is colonized in debilitated patients, and microbiologic information obtained must be interpreted with care. Direct aspiration of the abscess, typically under CT or ultrasound guidance,[70] is the most common way to provide microbiologic data and plan antimicrobial therapy. Aspiration may have therapeutic value in decompressing the abscess.

Clinical Features

Most patients with lung abscesses have had symptoms for 1 to 3 weeks before being hospitalized.[18] Fever is reported to be associated with 100 percent of primary abscesses,[18,38] and 84 percent of a mixed group of primary and secondary abscesses.[69] All patients with secondary abscesses in a small series had fever.[8] Cough occurs in 53 percent to 67 percent of cases and initially may be nonproductive, becoming purulent when rupture into a bronchus occurs. With necrosis, hemoptysis can occur. Ipsilateral chest or shoulder pain also has been described in some patients, particularly older children.[18,39] Weight loss may be present if the abscess is of more than a few days' duration. Other symptoms are listed in Table 24-5.[69]

Some differences in presentation by age exist. Neonates and young infants typically are febrile, without localizing symptoms. Older children also are febrile but may have more cough or tachypnea and focal pain.

The clinical features of a lung abscess vary with the causative organisms and patient risk factors. Patients with bacterial pneumonia can present with dramatic onset of fever and overwhelming respiratory failure, such as in staphylococcal pneumonia. In these cases, the patient often has a recent history of influenza or varicella infection. Staphylococcal abscesses may not be noticed on chest radiographs until the patient already is on ventilatory support because of the time required for an abscess to organize. Similar presentations are typical of group A β-hemolytic streptococcus and *S. pneumoniae* infections. Often, a patient has received antibiotics and a temporary defervescence occurs before the hectic fevers of an abscess reemerge. In the latter situation, the respiratory symptoms may be less notable but virtually always are present.[18] This biphasic presentation was described more than 70 years ago in postoperative aspiration[67] and continues to be typical of many lung abscesses.

Subacute presentations are typical in patients with tuberculosis or fungal abscesses and usually are associated with other chronic systemic symptoms, such as anorexia, weight loss, and malaise. Cough may be prominent. Aspiration pneumonia may take an indolent or acute course, depending primarily on the organisms in the abscess, the volume of aspirated material, and the status of the host.

The physical findings in lung abscess are limited. Children usually have fever.[38,69] Tachypnea is a variable finding. Typically, auscultation is unrevealing except in cases of very large abscesses, in which loss of normal breath sounds is perceived. Adults and older children seem to have more discrete physical findings, with rales and decreased breath sounds in approximately one third of adult cases,[52] but they are uncommon findings in young children.

An abscess can rupture into the bronchus, the mediastinum, or the pleura. In most cases, these complications are substantial. In children, all organisms seem capable of causing these complications, but polymicrobial and anaerobic infections are suspected most often. Rupture into the bronchus may not be harmful if the volume of the abscess cavity does not overwhelm the host's ability to cough and clear the material. In an immunocompromised host, rupture may lead to disseminated pneumonia and further abscesses or death. Among adult patients who died of lung abscess, 22 percent were found to have died of aspiration of the abscess contents.[20]

In an otherwise healthy individual, rupture into the bronchus can be beneficial because it decompresses the abscess and allows the affected tissues to heal more rapidly. It is associated with the sudden production of foul-smelling, abundant, and sometimes bloodstained sputum. Frank hemoptysis is uncommon. Rupture into the mediastinum can be life-threatening, can be associated with chest pain and cardiac compromise, and requires surgery to drain the resulting mediastinitis. Rupture into the pleural space results in pleuritic pain, enhancement of symptoms on inspiration, and often a more toxic presentation and may require drainage.

Routine laboratory information is of limited help. The white blood cell count and erythrocyte sedimentation rate are elevated nonspecifically, and a left shift of the white blood cell differential count typically occurs.[18] Certain laboratory tests, such as the purified protein derivative tuberculosis skin test, HIV serology, or sweat chloride test, may be helpful in revealing an underlying cause. Except for the tuberculosis skin test, such studies should not be performed routinely and should be directed by a family history or other findings, such as chronic diarrhea or lymphadenopathy. Blood cultures are helpful but are positive in fewer than 10 percent of cases.[18,69]

Differential Diagnosis

The major differential diagnoses in the management of a lung abscess are anatomic. A lung abscess must be differentiated from pneumonia, necrotizing pneumonia, pneumatocele, loculated empyema, and a purulent pleural effusion with a bronchopleural fistula. CT or ultrasound may confirm an abscess by documenting central cavitation and differentiating pleural from parenchymal tissues. An abscess may be confused with a congenital cyst, pseudocyst, hydatid cyst, saccular bronchiectasis, pneumatocele, or sequestration. Chest CT allows definition of

these entities in many cases by identifying the associated structures, such as the vascular supply and pleural borders.

Apart from the anatomic and infectious causes of lung abscess, the other rare cause is cancer. Unrecognized metastatic disease from Ewing sarcoma or osteosarcomas with associated central necrosis can mimic an abscess or, by obstructing a bronchus, can promote abscess formation.

Diagnosis

The diagnosis of lung abscess usually is made by imaging the lung. In most cases, the plain radiograph is adequate to define a lung abscess (Figure 24-3), showing a thickened cavity with an air–fluid level that can be accentuated by placing the patient in the lateral decubitus or erect position. Atelectasis often occurs as an expanding abscess compressing adjacent tissues. Pleural thickening may occur if the abscess is subpleural. Hilar adenopathy occurs in subacute situations.

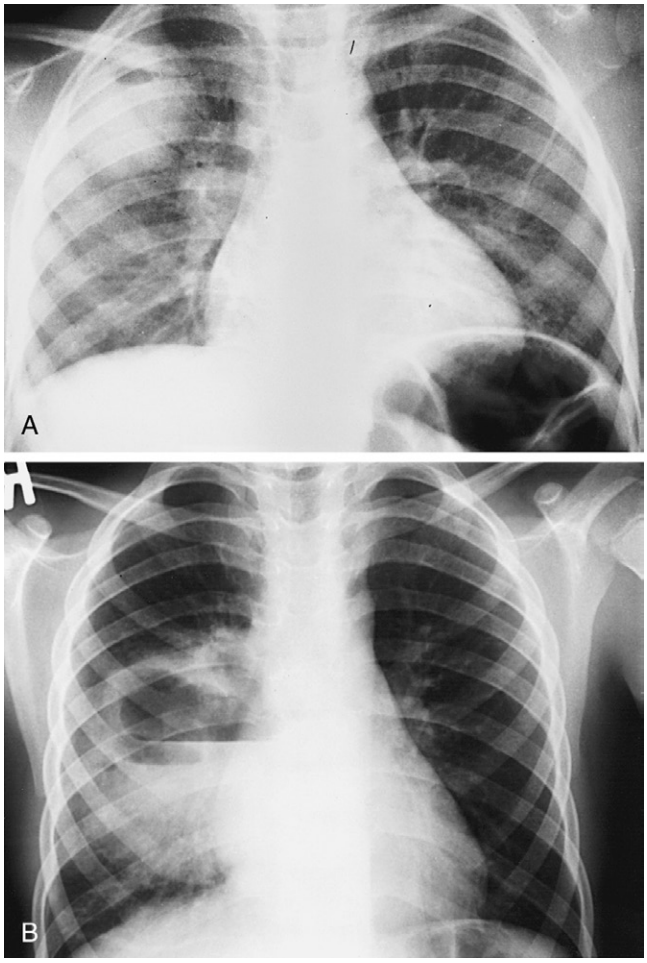

FIGURE 24-3 ■ **A,** Plain chest film of a 4-year-old boy with symptoms including cough and fever of 40° C (104° F) for 4 days. He was treated initially with oral cefaclor and re-presented with this radiograph showing an air–fluid level in the right upper lobe. On intravenous cefuroxime, he was afebrile in 48 hours and went home on oral cefuroxime axetil after 4 days. **B,** Plain chest film of a 6-year-old boy who presented after 5 days of symptoms with high fever, productive cough, dyspnea, and abdominal pain. The plain chest film reveals multiple air–fluid levels. Nafcillin and cefotaxime were begun. On day 4, an ultrasound-guided diagnostic aspiration recovered thick purulent material, but Gram stain and all cultures, including anaerobic and fungal, were negative. He was afebrile in 7 days and went home on amoxicillin-clavulanic acid at 10 days.

CT is optimal in its ability to identify smaller or multiple abscesses, document the impact of the abscess on adjacent tissues, identify cystic processes mimicking an abscess, and define an abscess for which an organized pneumonia obscures an air–fluid level on plain film.[29] CT scan of a lung abscess reveals an air–fluid level with an active rim, and the abscess is distinguished from necrotizing pneumonia, which lacks enhancement on contrast studies and lacks a distinct air–fluid level.[27] Nuclear imaging is described[13] but used rarely and adds little to the information obtained by CT.

After a presumed abscess is defined by imaging, needle aspiration of the abscess or bronchoscopic recovery of abscess fluid should allow confirmation and identification of the infectious cause of the process. Based on available pediatric studies, whether either procedure hastens recovery or reveals the microbiologic cause in pretreated individuals cannot be predicted. In a case report in which an infant had abscesses in both lungs, the time to recovery was equal in the abscess that was drained and the other abscesses that were treated medically.[41] An adult study using thin-needle aspiration showed positive cultures in 92 percent of patients not pretreated with antibiotics and in 70 percent of patients pretreated.[51]

Directly aspirating a lung abscess may be difficult or unsuccessful if the abscess is not large or peripheral, and complications such as lung laceration and sterile pleural effusions are real risks. Two groups[27,69] had satisfactory pediatric experience using direct aspiration under CT guidance (Figure 24-4). Bronchoscopy particularly is valuable if foreign bodies are suspected or pus can be recovered. When material is obtained, a putrid quality is a clue that anaerobic organisms are present. Typically, the abscess contains pure neutrophils. Occasionally, PCR or other antigen detection systems may assist in the microbiologic diagnosis when cultures are negative.[20]

Treatment

Although surgery has a role in specific situations, the treatment of small to moderate size lung abscesses in children is often successful when antibiotics alone are used.[53] In most cases, the need for surgery is limited to instances of failed antibiotic therapy or to an abscess complicated by rupture into adjacent tissues.

The initial choice of antibiotics usually is presumptive because abscess material may be unavailable. For primary lung abscesses in which no risk factors are identified and in the absence of positive blood cultures, the recommended approach is to begin therapy with a regimen that covers *S. aureus*, *S. pneumoniae*, and the anaerobic microorganisms that normally are found in the upper respiratory tract. Clindamycin, ampicillin plus sulbactam, and ticarcillin plus clavulanate frequently are used in this setting.

For patients at risk for aspiration or who are immunocompromised, gram-negative pathogens also must be

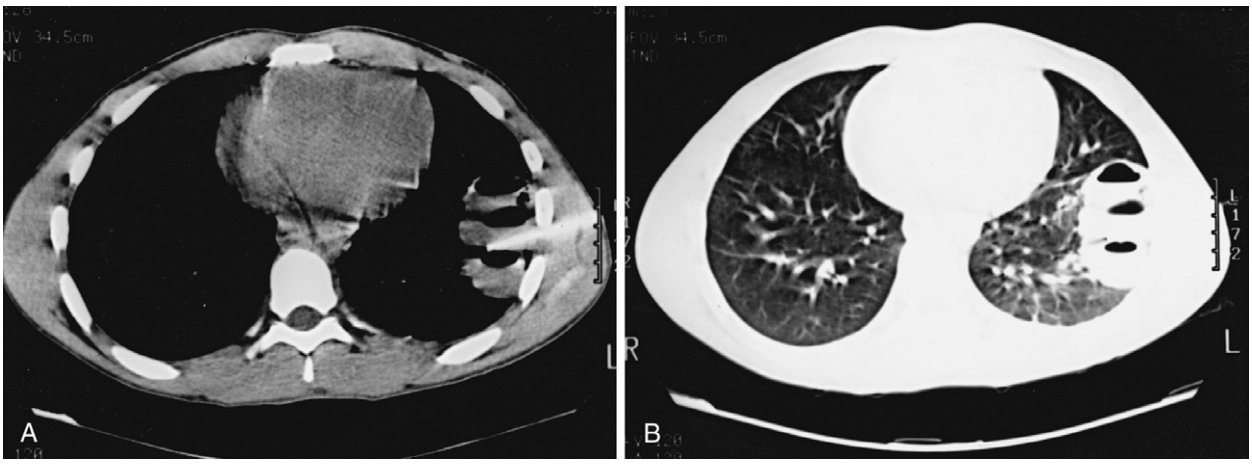

FIGURE 24-4 ■ **A** and **B,** Computed tomography (CT) scans of a 15-year-old boy who, 4 days before admission, reported a mild aspiration during fresh-water swimming. He awoke the next day with chest and back pain. He then developed low-grade fever. A plain chest film showed a large, thick-walled abscess in the left lower lobe. CT scans show insertion of a 20-gauge needle into the abscess on the first day of admission **(A)** and detail of the wall thickness and cavitation **(B)**. *Haemophilus influenzae* grew from the aspirate. Ampicillin-sulbactam was initiated and continued for 14 days.

considered. This spectrum of pathogens can be addressed with one of several drug regimens—clindamycin and cefotaxime (or an aminoglycoside); ticarcillin plus clavulanate or piperacillin plus tazobactam; or nafcillin (or cefazolin), gentamicin, and metronidazole. The possible emergence of more penicillin-resistant species, such as the milleri group of streptococci and *Klebsiella*, in community-acquired disease of adults and children supports these broad-spectrum regimens in children.[30,57] Patients with cystic fibrosis are particularly vulnerable to *Pseudomonas* spp. and should receive an aminoglycoside plus an additional antipseudomonal penicillin or cephalosporin. Carbapenem therapy would be an alternative. When endocarditis is present, treatment (e.g., vancomycin plus gentamicin with or without anaerobic coverage) should be provided for staphylococci, streptococci, and enterococci while awaiting results of blood cultures.

S. pneumoniae has been isolated infrequently in lung abscesses (Table 24-6). Most patients with isolates with reduced susceptibility are still successfully treated because achievable levels of penicillins exceed the penicillin G MICs of 0.12 to 2.0.[48] Drug resistance to *S. aureus* is a much more concerning issue in lung abscess, and empirical therapy with vancomycin is required if MRSA is suspected while waiting for confirmation, especially if the patient is critically ill.

If culture information is available, therapy is directed specifically at the pathogens isolated. In most patients, oral or intravenous antibiotic therapy has been administered before culture results are available and may affect which organisms are recovered.[16,17] For this reason, coverage should be extended to include organisms that are likely to be present but are not recovered, such as anaerobes, in a setting of aspiration.

All bacterial lung abscesses should be treated with intravenous therapy until the patient is stable and no longer toxic. In approximately two thirds to four fifths of

TABLE 24-6 **Microbiology of Pediatric Lung Abscess**

Organism	No.
Staphylococcus aureus	22
Coagulase-negative staphylococci	2
Streptococcus pyogenes	5
Streptococcus pneumoniae	15
α-Hemolytic streptococcus	17
Other aerobic streptococci	7
Enterococci	2
Moraxella catarrhalis	3
Escherichia coli	10
Klebsiella	8
Pseudomonas	13
Serratia	1
Eikenella	1
Haemophilus	7
Other gram-negative organisms	2
Bacteroides	19
Peptostreptococcus	12
Other anaerobes	26
Candida	4
Aspergillus	5
Mucor	3
Mycobacterium tuberculosis	1
Total	185

Data from references 4, 16, 23, 32, 37, 63, 72, 78, 85, 92, 93, 104, 106, 134, 141, 144, 148.

These data were reported in the literature from 1976 to 2006. The isolates were recovered by direct aspiration of abscess contents, bronchoscopic aspiration, transtracheal aspiration, blood culture, or culture of surgical specimens. Data from case reports focused on procedures are included, whereas data from case reports focused on the organism recovered are not.

patients, this condition occurs within 3 to 7 days of instituting intravenous therapy.[18,40] After the patient has been afebrile for 48 to 72 hours, initiation of oral therapy may be considered. Certain oral drugs, such as amoxicillin plus clavulanate and clindamycin, can achieve therapeutic serum levels and are effective against the spectrum of organisms in lung abscesses.

The length of total therapy for a lung abscess should be 2 to 3 weeks. Complicated infections should be treated intravenously until fever has disappeared and no evidence of continuing inflammation exists. An additional 2 to 3 weeks of oral treatment should follow. Radiographic resolution of a lung abscess that is not drained occurs over the course of weeks. Even with drainage, resolution may not occur more rapidly. Chest radiography should be repeated every 1 to 2 weeks until complete resolution is documented.

Tuberculous lung abscesses may rupture spontaneously into the pleura. Treatment is not directed at the immediate abscess or pleuritis but at preventing spread if it erupts into a bronchus. Preventing reactivation several years later is another goal of therapy.

Clinical failure is defined by persistent fever and toxicity. The length of time treatment with intravenous antibiotics should continue before declaring therapy a failure is not clearly defined. Suggestions in the literature range from 1 to 3 weeks.[2,5,53] When clinical failure occurs, several options are available, including drainage of the abscess, which permits identification of the organisms causing the disease process.

Needle aspiration (one or more) also is possible under fluoroscopy, ultrasonography, or CT guidance.[27,35,36] Simple CT-guided needle aspiration leads to relief of symptoms in approximately two thirds of cases within 48 hours.[27] The risk for complications seems to be low.[27,36] Percutaneous transthoracic tube drainage has been used in pediatric lung abscesses.[14,39,50,71,76] The risk is lowest if the abscess is peripheral, and adhesion of the opposing pleural surfaces is a consequence of the inflammatory process. When this adhesion occurs, the needle or catheter does not traverse the pleural space. Catheter drainage may be attempted in abscesses larger than 4 cm in diameter,[2,27] using 8- to 10-French pigtail catheters. Smaller abscesses are technically difficult to manage.

Chest tube thoracostomy previously was the standard therapy of lung abscesses[43,45]; it is now recommended only when conservative therapy fails and may play a role in patients with large abscesses if the abscess abuts the parietal pleura and provides a direct path from the exterior surface to the abscess.[42] A 25-year review of the literature found that significant complications, such as hemothorax, pneumothorax, bronchopleural fistula, empyema, and catheter occlusion, occurred in 9.7 percent of cases, with an overall mortality rate of 4.8 percent.[71]

Surgery for lung abscess is not well described in the pediatric literature. One report that combines surgery for lung abscess and necrotizing pneumonia had no mortality in the postsurgery period and a reversible complication rate of 10 percent.[68] Indications for surgery included associated pyopneumothorax, size of the abscess, and tension pneumatocele. Another possible surgical indication is proximity of a lung abscess to the mediastinum. In the face of antibiotic failure, an open lung procedure and wedge resection may be required, depending on the location of the abscess.[6] The procedure typically is successful and often obviates the need to perform a lobectomy.[31] In a complicated case, such as with a gangrenous lung, lobar resection may be necessary. Managing an infant with a lung abscess has required surgery more often than in older children.[66,77]

Prognosis

The outcome for pediatric patients usually is good when lung abscesses are uncomplicated, and recovery is more rapid than in adults.[44] Most patients have complete symptomatic and radiographic resolution in 3 to 6 weeks, with normal pulmonary function tests at follow-up.[5,18] Complicated disease associated with thoracostomy tubes and empyema more likely leads to residual symptoms (e.g., pleurisy) and persistent effusions or pleural thickening on radiographs. In reports published since 1982, half of patients with lung abscess have required surgical intervention, such as thoracentesis to drain an empyema, lobectomy, or decortication, and 20 percent have required lobectomy or decortication.[5,8,18,69] Lung abscess associated with underlying disorders has a higher mortality rate.[26] If patients require lung resection, they may experience immediate surgical complications; long-term exercise tolerance may be limited, and other problems, such as scoliosis, may develop. One study found normal pulmonary functions in patients studied after undergoing lobectomy, however.[47]

Rarely, a residual cavity may develop and become superinfected. The mortality rate is 4 to 11 percent for patients with primary and secondary lung abscesses.[18,69] Higher risk for morbidity and mortality occurs in patients with secondary abscesses who have complications caused by either a decrease in host resistance or underlying disease.[18,52,68,69]

Additional Complications

Necrotizing pneumonia is a complication of lung infection. In contrast to simple pneumonia more destruction of tissue occurs with limited purulence caused either by toxins of the pathogen or by infarction of tissue. The necrotizing process can be limited, requiring no special intervention or extensive surgery and having a more prolonged clinical course. The necrotizing process can lead to fistulas, pneumothorax, and pneumatocele.[58] Sawicki and colleagues[125] have reported an increased incidence of necrotizing pneumonia in children in the new millennium with an increased frequency of *S. aureus*. This has been noted in children of all ages, including infants.[131] However, necrotizing pneumonia is also described for diverse organisms, including *S. pneumoniae* (particularly nonvaccine strains), group A streptococcus, *Clostridium perfringens*, *M. pneumoniae*, and adenovirus.

The formation of a bronchopleural fistula can occur as a complication of the underlying pneumonia or as

a complication of treatment with a chest tube or postoperatively from débridement. Rates of 55 percent to 67 percent for fistula formation have been reported associated with necrotizing pneumonia.[49,86] An increased risk was noted in the United Kingdom in 2008 to 2009 (1%) in contrast to 2002 to 2007 (33%).[6,94] Thin-section CT may be required for a radiologic diagnosis.[151]

Pneumatoceles form as a result of tissue necrosis. The development occurs as an acute or delayed part of the pathologic process, with resolution occurring in some cases depending on the size of the defect. A Brazilian study reported pneumatoceles in 8.3 percent of 394 pediatric pneumonia cases and a spontaneous resolution rate of 85 percent.[74] *S. aureus* is most typically identified with this complication, although a pneumatocele represents a potential common pathway for pathologic resolution of a pneumonia and has been reported in pneumonia caused by multiple pathogens. Ramphul and colleagues[114] reported a 20 percent incidence of cavitary disease in patients with empyema, mostly associated with *S. pneumoniae*.

Pneumothorax is an uncommon complication of pneumonia but can occur in any setting in which lung damage creates a ball-valve effect trapping air in the pleural space. Necrotizing pneumonia is one predisposing factor. *P. jiroveci* in HIV disease has been reported as a specific cause of pneumothorax.[109] Other complications occur rarely secondary to pneumonia. Many of these represent local extension of an infected lung or pleural tissue into adjacent tissues including the airway, blood vessels (hemoptysis), bone, and nervous tissues (recurrent laryngeal nerve–Horner's syndrome with apical pneumonia). Pericardial effusions are surprisingly common in patients with parapneumonic effusions. Roberts and associates reported a rate of 54.2 percent although a single patient in their series of 48 had hemodynamic compromise.[116] Other complications result from the hematogenous spread of pathogens causing the pneumonia including sepsis, meningitis, osteomyelitis, other solid organ abscesses, and endocarditis. Finally, complications can arise from the immune response to pneumonia.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Ben-Or S, Feins RH, Veeramachaneni NK, Haithcock BE. Effectiveness and risks associated with intrapleural alteplase by means of tube thoracostomy. *Ann Thorac Surg* 2011;**91**:860–3.
12. Blaschke AJ, Heyrend C, Byington CL, et al. Molecular analysis improves pathogen identification and epidemiologic study of pediatric parapneumonic empyema. *Pediatr Infect Dis J* 2011; **30**:289–94.
15. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.
22. Calder A, Owens CM. Imaging of parapneumonic pleural effusions and empyema in children. *Pediatr Radiol* 2009;**39**:527–37.
24. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
46. Grijalva CG, Zhu Y, Nuorti JP, Griffin MR. Emergence of parapneumonic empyema in the USA. *Thorax* 2011;**66**:663–8.
59. Holmes NE, Turnidge JD, Munckhof WJ, et al. Antibiotic choice may not explain poorer outcomes in patients with *Staphylococcus aureus* bacteremia and high vancomycin minimum inhibitory concentrations. *J Infect Dis* 2011;**204**:340–7.
62. Janahi IA, Fakhoury K. Management and prognosis of parapneumonic effusion and empyema in children. 2011 [3/26/2012]; Available from: http://www.uptodate.com/contents/management-and-prognosis-of-parapneumonic-effusion-and-empyema-in-children?source=see_link.
73. Krenke K, Peradzynska J, Lange J, et al. Local treatment of empyema in children: a systematic review of randomized controlled trials. *Acta Paediatr* 2010;**99**:1449–53.
79. Li ST, Tancredi DJ. Empyema hospitalizations increased in US children despite pneumococcal conjugate vaccine. *Pediatrics* 2010;**125**:26–33.
80. Li Y, Wu W, Yang X, Li J. Treatment of 38 cases of foreign body aspiration in children causing life-threatening complications. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1624–9.
84. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011;**52**:285–92.
86. Macedo M, Meyer KF, Oliveira TC. Necrotizing pneumonia in children submitted to thoracoscopy due to pleural empyema: incidence, treatment and clinical evolution. *J Bras Pneumol* 2010; **36**:301–5.
87. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. *Pediatr Infect Dis J* 2011;**30**:694–700.
94. McKee AJ, Ives A, Balfour-Lynn IM. Increased incidence of bronchopulmonary fistulas complicating pediatric pneumonia. *Pediatr Pulmonol* 2011;**46**:717–21.
100. Munoz-Almagro C, Gala S, Selva L, et al. DNA bacterial load in children and adolescents with pneumococcal pneumonia and empyema. *Eur J Clin Microbiol Infect Dis* 2011;**30**:327–35.
103. Newman B. Ultrasound body applications in children. *Pediatr Radiol* 2011;**41**(suppl 2):555–61.
108. Pillai D, Song X, Pastor W, et al. Implementation and impact of a consensus diagnostic and management algorithm for complicated pneumonia in children. *J Investig Med* 2011;**59**:1221–7.
126. Scarci M, Zahid I, Bille A, Routledge T. Is video-assisted thoracoscopic surgery the best treatment for paediatric pleural empyema? *Interact Cardiovas Thorac Surg* 2011;**13**:70–6.
131. Schwartz KL, Nourse C. Panton-Valentine leukocidin-associated *Staphylococcus aureus* necrotizing pneumonia in infants: a report of four cases and review of the literature. *Eur J Pediatr* 2012; **171**:711–7.
132. Shah SS, Hall M, Newland JG, et al. Comparative effectiveness of pleural drainage procedures for the treatment of complicated pneumonia in childhood. *J Hosp Med* 2011;**6**:256–63.
138. Strachan RE, Cornelius A, Gilbert GL, et al. Bacterial causes of empyema in children, Australia, 2007-2009. *Emerg Infect Dis* 2011;**17**:1839–45.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adams WG, Deaver KA, Cochi SL, et al. Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. *JAMA* 1993;**269**:221–6.
2. Afessa B. Pleural effusion and pneumothorax in hospitalized patients with HIV infection: the Pulmonary Complications, ICU support, and Prognostic Factors of Hospitalized Patients with HIV (PIP) Study. *Chest* 2000;**117**:1031–7.
3. Akhan O, Ozkan O, Akinci D, et al. Image-guided catheter drainage of infected pleural effusions. *Diagn Interv Radiol* 2007;**13**: 204–9.
4. Asher MI, Spier S, Beland M, et al. Primary lung abscess in childhood: the long-term outcome of conservative management. *Am J Dis Child* 1982;**136**:491–4.
5. Avansino JR, Goldman B, Sawin RS, Flum DR. Primary operative versus nonoperative therapy for pediatric empyema: a meta-analysis. *Pediatrics* 2005;**115**:1652–9.
6. Balfour-Lynn IM, Abrahamson E, Cohen G, et al. BTS guidelines for the management of pleural infection in children. *Thorax* 2005;**60**(suppl 1):i1–21.
7. Bambery P, Sakhuja V, Behera D, Deodhar SD. Pleural effusions in Wegener's granulomatosis: report of five patients and a brief review of the literature. *Scand J Rheumatol* 1991;**20**:445–7.
8. Baum GL. Diseases of the pleura. *Hosp Med* 1969;**5**:6–25.
9. Ben-Or S, Feins RH, Veeramachaneni NK, Haithcock BE. Effectiveness and risks associated with intrapleural alteplase by means of tube thoracostomy. *Ann Thorac Surg* 2011;**91**:860–3.
10. Berger HW, Mejia E. Tuberculous pleurisy. *Chest* 1973;**63**: 88–92.
11. Black LF. The pleural space and pleural fluid. *Mayo Clinic Proc* 1972;**47**:493–506.
12. Blaschke AJ, Heyrend C, Byington CL, et al. Molecular analysis improves pathogen identification and epidemiologic study of pediatric parapneumonic empyema. *Pediatr Infect Dis J* 2011;**30**: 289–94.
13. Bouros D, Schiza S, Panagou P, et al. Role of streptokinase in the treatment of acute loculated parapneumonic pleural effusions and empyema. *Thorax* 1994;**49**:852–5.
14. Bouros D, Schiza S, Patsourakis G, et al. Intrapleural streptokinase versus urokinase in the treatment of complicated parapneumonic effusions: a prospective, double-blind study. *Am J Respir Crit Care Med* 1997;**155**:291–5.
15. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.
16. Brook I, Finegold SM. Bacteriology and therapy of lung abscess in children. *J Pediatr* 1979;**94**:10–2.
17. Brook I, Frazier EH. Aerobic and anaerobic microbiology of empyema: a retrospective review in two military hospitals. *Chest* 1993;**103**:1502–7.
18. Brown RB, Weinstein L. Pleural effusions. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 3rd ed. Philadelphia: W. B. Saunders; 1992. p. 309–15.
19. Brusch JL, Weinstein L. Pleural empyema. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 3rd ed. Philadelphia: W. B. Saunders; 1992. p. 315–20.
20. Byington CL, Korgenski K, Daly J, et al. Impact of the pneumococcal conjugate vaccine on pneumococcal parapneumonic empyema. *Pediatr Infect Dis J* 2006;**25**:250–4.
21. Byington CL, Samore MH, Stoddard GJ, et al. Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. *Clin Infect Dis* 2005;**41**:21–9.
22. Calder A, Owens CM. Imaging of parapneumonic pleural effusions and empyema in children. *Pediatr Radiol* 2009;**39**:527–37.
23. Canny GJ, Marcotte JE, Levison H. Lung abscess in cystic fibrosis. *Thorax* 1986;**41**:221–2.
24. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
25. Centers for Disease Control and Prevention. Effects of new penicillin susceptibility breakpoints for *Streptococcus pneumoniae:* United States, 2006-2007. *MMWR Morb Mortal Wkly Rep* 2008; **57**:1353–5.
26. Chan PC, Huang LM, Wu PS, et al. Clinical management and outcome of childhood lung abscess: a 16-year experience. *J Microbiol Immunol Infect* 2005;**38**:183–8.
27. Chartrand SA, McCracken GH Jr. Staphylococcal pneumonia in infants and children. *Pediatr Infect Dis* 1982;**1**:19–23.
28. Chonmaitree T, Powell KR. Parapneumonic pleural effusion and empyema in children: review of a 19-year experience, 1962-1980. *Clin Pediatr* 1983;**22**:414–9.
29. Choyke PL, Sostman HD, Curtis AM, et al. Adult-onset pulmonary tuberculosis. *Radiology* 1983;**148**:357–62.
30. Colice GL, Curtis A, Deslauriers J, et al. Medical and surgical treatment of parapneumonic effusions: an evidence-based guideline. *Chest* 2000;**118**:1158–71.
31. Collins TR, Sahn SA. Thoracocentesis: clinical value, complications, technical problems, and patient experience. *Chest* 1987;**91**: 817–22.
32. Cuestas RA, Kienzle GD, Armstrong JD 2nd. Percutaneous drainage of lung abscesses in infants. *Pediatr Infect Dis J* 1989;**8**: 390–2.
33. Dobranowski J, Stringer DA. Diagnosis of Legionella lung abscess by percutaneous needle aspiration. *Can Assoc Radiol J* 1989;**40**:43–4.
34. Doski JJ, Lou D, Hicks BA, et al. Management of parapneumonic collections in infants and children. *J Pediatr Surg* 2000;**35**: 265–8.
35. Eastham KM, Freeman R, Kearns AM, et al. Clinical features, aetiology and outcome of empyema in children in the north east of England. *Thorax* 2004;**59**:522–55.
36. Efrati O, Barak A. Pleural effusions in the pediatric population. *Pediatr Rev* 2002;**23**:417–26.
37. Emanuel B, Shulman ST. Lung abscess in infants and children. *Clin Pediatr* 1995;**34**:2–6.
38. Epstein DM, Kline LR, Albelda SM, Miller WT. Tuberculous pleural effusions. *Chest* 1987;**91**:106–9.
39. Ferrer J. Tuberculous pleural effusion and tuberculous empyema. *Semin Respir Crit Care Med* 2001;**22**:637–46.
40. Fine NL, Smith LR, Sheedy PF. Frequency of pleural effusions in mycoplasma and viral pneumonias. *New Engl J Med* 1970;**283**: 790–3.
41. Fitzgerald D, Harvey J, Isaacs D, Kilham H. The case of the persistent pleural effusion. *Pediatr Infect Dis J* 1991;**10**:475, 479–80.
42. Freij BJ, Kusmiesz H, Nelson JD, McCracken GH Jr. Parapneumonic effusions and empyema in hospitalized children: a retrospective review of 227 cases. *Pediatr Infect Dis* 1984;**3**:578–91.
43. Gathof BS, Kamilli I, Keller C, Zollner N. Pleural effusions in acute mononucleosis. *Bildgebung* 1991;**58**:218–20.
44. Golladay ES, Wagner CW. Management of empyema in children. *Am J Surg* 1989;**158**:618–21.
45. Grewal H, Jackson RJ, Wagner CW, Smith SD. Early video-assisted thoracic surgery in the management of empyema. *Pediatrics* 1999;**103**:e63.
46. Grijalva CG, Zhu Y, Nuorti JP, Griffin MR. Emergence of parapneumonic empyema in the USA. *Thorax* 2011;**66**:663–8.
47. Grix A, Giammona ST. Pneumonitis with pleural effusion in children due to *Mycoplasma pneumoniae*. *Am Rev Respir Dis* 1974; **109**:665–71.
48. Groff DB, Randolph JG, Blades B. Empyema in childhood. *JAMA* 1966;**195**:572–4.
49. Hacimustafaoglu M, Celebi S, Sarimehmet H, et al. Necrotizing pneumonia in children. *Acta Paediatr* 2004;**93**:1172–7.
50. Hadlock FP, Park SK, Awe RJ, Rivera M. Unusual radiographic findings in adult pulmonary tuberculosis. *AJR Am J Roentgenol* 1980;**134**:1015–8.
51. Hallman JR, Geisinger KR. Cytology of fluids from pleural, peritoneal and pericardial cavities in children: a comprehensive survey. *Acta Cytol* 1994;**38**:209–17.
52. Hamm H, Brohan U, Bohmer R, Missmahl HP. Cholesterol in pleural effusions: a diagnostic aid. *Chest* 1987;**92**:296–302.
53. Handman HP, Reuman PD. The use of urokinase for loculated thoracic empyema in children: a case report and review of the literature. *Pediatr Infect Dis J* 1993;**12**:958–9.
54. Hardy JB, Kendy EL Jr. Tuberculous pleurisy with effusion in infancy. *J Pediatr* 1945;**26**:138.

55. Heffner JE, Brown LK, Barbieri CA. Diagnostic value of tests that discriminate between exudative and transudative pleural effusions. Primary Study Investigators. *Chest* 1997;**111**:970–80.
56. Hernández Borge J, Alfageme Michavila I, Muñoz Mández J, et al. Thoracic empyema in HIV-infected patients: microbiology, management, and outcome. *Chest* 1998;**113**:732–8.
57. Hoff SJ, Neblett WW, Edwards KM, et al. Parapneumonic empyema in children: decortication hastens recovery in patients with severe pleural infections. *Pediatr Infect Dis J* 1991;**10**:194–9.
58. Hoffer FA, Bloom DA, Colin AA, Fishman SJ. Lung abscess versus necrotizing pneumonia: implications for interventional therapy. *Pediatr Radiol* 1999;**29**:87–91.
59. Holmes NE, Turnidge JD, Munckhof WJ, et al. Antibiotic choice may not explain poorer outcomes in patients with *Staphylococcus aureus* bacteremia and high vancomycin minimum inhibitory concentrations. *J Infect Dis* 2011;**204**:340–7.
60. Hughson WG, Friedman PJ, Feigin DS, et al. Postpartum pleural effusion: a common radiologic finding. *Ann Intern Med* 1982;**97**: 856–8.
61. Hussain FM, Boyle-Vavra S, Bethel CD, Daum RS. Current trends in community-acquired methicillin-resistant *Staphylococcus aureus* at a tertiary care pediatric facility. *Pediatr Infect Dis J* 2000;**19**:1163–6.
62. Janahi IA, Fakhoury K. Management and prognosis of parapneumonic effusion and empyema in children. [2011]; Available from: http://www.uptodate.com/contents/management-and-prognosis-of-parapneumonic-effusion-and-empyema-in-children?source=see_link. Accessed 3/26/2012.
63. Johnson JF, Shiels WE, White CB, Williams BD. Concealed pulmonary abscess: diagnosis by computed tomography. *Pediatrics* 1986;**78**:283–6.
64. Kalfa N, Allal H, Lopez M, et al. Thoracoscopy in pediatric pleural empyema: a prospective study of prognostic factors. *J Pediatr Surg* 2006;**41**:1732–7.
65. Kane DR, Reuman DD. *Yersinia enterocolitica* causing pneumonia and empyema in a child and a review of the literature. *Pediatr Infect Dis J* 1992;**11**:591–3.
66. Kato Y, Miyata I, Sakuma S, et al. A case of cytomegalovirus mononucleosis associated with pleural effusion. *Acta Paediatr Jpn* 1994;**36**:280–3.
67. Kearney SE, Davies CW, Davies RJ, Gleeson FV. Computed tomography and ultrasound in parapneumonic effusions and empyema. *Clin Radiol* 2000;**55**:542–7.
68. Kercher KW, Attorri RJ, Hoover JD, Morton D Jr. Thoracoscopic decortication as first-line therapy for pediatric parapneumonic empyema: a case series. *Chest* 2000;**118**:24–7.
69. Kerem E, Diav O, Navon P, Branski D. Pleural fluid characteristics in pulmonary brucellosis. *Thorax* 1994;**49**:89–90.
70. Kern JA, Rodgers BM. Thoracoscopy in the management of empyema in children. *J Pediatr Surg* 1993;**28**:1128–32.
71. Kornecki A, Sivan Y. Treatment of loculated pleural effusion with intrapleural urokinase in children. *J Pediatr Surg* 1997;**32**: 1473–5.
72. Kosloske AM, Ball WS Jr, Butler C, Musemeche CA. Drainage of pediatric lung abscess by cough, catheter, or complete resection. *J Pediatr Surg* 1986;**21**:596–600.
73. Krenke K, Peradzynska J, Lange J, et al. Local treatment of empyema in children: a systematic review of randomized controlled trials. *Acta Paediatr* 2010;**99**:1449–53.
74. Kunyoshi V, Cataneo DC, Cataneo AJ. Complicated pneumonias with empyema and/or pneumatocele in children. *Pediatr Surg Int* 2006;**22**:186–90.
75. Kurt BA, Winterhalter KM, Connors RH, et al. Therapy of parapneumonic effusions in children: video-assisted thoracoscopic surgery versus conventional thoracostomy drainage. *Pediatrics* 2006;**118**:e547–553.
76. Lee KF, Olak J. Anatomy and physiology of the pleural space. *Chest Surg Clin N Am* 1994;**4**:391–403.
77. Leggiadro RJ, Davis Y, Tenover FC. Outpatient drug-resistant pneumococcal bacteremia. *Pediatr Infect Dis J* 1994;**13**:1144–6.
78. Levine MM, Ashman R, Heald F. Anaerobic (putrid) lung abscess in adolescence. *Am J Dis Child* 1976;**130**:77–81.
79. Li ST, Tancredi DJ. Empyema hospitalizations increased in US children despite pneumococcal conjugate vaccine. *Pediatrics* 2010;**125**:26–33.
80. Li Y, Wu W, Yang X, Li J. Treatment of 38 cases of foreign body aspiration in children causing life-threatening complications. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1624–9.
81. Light RW. Pleural effusions. *Med Clin N Am* 1977;**61**:1339–52.
82. Light RW. *Pleural diseases*. Philadelphia: Lea & Febiger; 1983.
83. Light RW, Macgregor MI, Luchsinger PC, Ball WC Jr. Pleural effusions: the diagnostic separation of transudates and exudates. *Ann Intern Med* 1972;**77**:507–13.
84. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011;**52**:285–92.
85. Lorenzo RL, Bradford BF, Black J, Smith CD. Lung abscesses in children: diagnostic and therapeutic needle aspiration. *Radiology* 1985;**157**:79–80.
86. Macedo M, Meyer KF, Oliveira TC. Necrotizing pneumonia in children submitted to thoracoscopy due to pleural empyema: incidence, treatment and clinical evolution. *J Bras Pneumol* 2010;**36**:301–5.
87. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children: a systematic review and meta-analysis. *Pediatr Infect Dis J* 2011;**30**:694–700.
88. Magovern CJ, Rusch VW. Parapneumonic and post-traumatic pleural space infections. *Chest Surg Clin N Am* 1994;**4**:561–82.
89. Major RH. *Classic descriptions of disease*. 2nd ed. Springfield, Ill: Charles C. Thomas; 1939.
90. Mangete ED, Kombo BB, Legg-Jack TE. Thoracic empyema: a study of 56 patients. *Arch Dis Child* 1993;**69**:587–8.
91. Maskell NA, Davies CW, Nunn AJ, et al. U.K. Controlled trial of intrapleural streptokinase for pleural infection. *New Engl J Med* 2005;**352**:865–74.
92. Mayer T, Matlak ME, Condon V, et al. Computed tomographic findings of neonatal lung abscess. *Am J Dis Child* 1982;**136**: 39–41.
93. McCracken GH, Jr. Lung abscess in childhood. *Hosp Pract* 1978;**13**:35–6.
94. McKee AJ, Ives A, Balfour-Lynn IM. Increased incidence of bronchopulmonary fistulas complicating pediatric pneumonia. *Pediatr Pulmonol* 2011;**46**:717–21.
95. Meier AH, Smith B, Raghavan A, et al. Rational treatment of empyema in children. *Arch Surg* 2000;**135**:907–12.
96. Meyer K, Girgis N, McGravey V. Adenovirus associated with congenital pleural effusion. *J Pediatr* 1985;**107**:433–5.
97. Meyerovitch J, Shohet I, Rubinstein E. Analysis of thirty-seven cases of pleural empyema. *Eur J Clin Microbiol* 1985;**4**:337–9.
98. Mitri RK, Brown SD, Zurakowski D, et al. Outcomes of primary image-guided drainage of parapneumonic effusions in children. *Pediatrics* 2002;**110**:e37.
99. Moreira Jda S, Camargo Jde J, Felicetti JC, et al. Lung abscess: analysis of 252 consecutive cases diagnosed between 1968 and 2004. *J Bras Pneumol* 2006;**32**:136–43.
100. Munoz-Almagro C, Gala S, Selva L, et al. DNA bacterial load in children and adolescents with pneumococcal pneumonia and empyema. *Eur J Clin Microbiol Infect Dis* 2011;**30**:327–35.
101. Murphy D, Lockhart CH, Todd JK. Pneumococcal empyema: outcome of medical management. *Am J Dis Child* 1980;**134**: 659–62.
102. Nance KV, Shermer RW, Askin FB. Diagnostic efficacy of pleural biopsy as compared with that of pleural fluid examination. *Mod Pathol* 1991;**4**:320–4.
103. Newman B. Ultrasound body applications in children. *Pediatr Radiol* 2011;**41**(suppl 2):555–61.
104. Nonoyama A, Tanaka K, Osako T, Kotani S, Kagawa T. Surgical treatment of pulmonary abscess in children under ten years of age. *Chest* 1984;**85**:358–62.
105. Pallares R, Linares J, Vadillo M, et al. Resistance to penicillin and cephalosporin and mortality from severe pneumococcal pneumonia in Barcelona, Spain. *New Engl J Med* 1995;**333**: 474–80.
106. Parker LA, Melton JW, Delany DJ, Yankaskas BC. Percutaneous small bore catheter drainage in the management of lung abscesses. *Chest* 1987;**92**:213–8.
107. Peterman TA, Speicher CE. Evaluating pleural effusions: a two-stage laboratory approach. *JAMA* 1984;**252**:1051–3.

108. Pillai D, Song X, Pastor W, et al. Implementation and impact of a consensus diagnostic and management algorithm for complicated pneumonia in children. *J Invest Med* 2011;**59**:1221–7.
109. Pitcher RD, Zar HJ. Radiographic features of paediatric pneumocystis pneumonia: a historical perspective. *Clin Radiol* 2008;**63**: 666–72.
110. Pothula V, Krellenstein DJ. Early aggressive surgical management of parapneumonic empyemas. *Chest* 1994;**105**:832–6.
111. Powell DA, Schuit KE. Acute pulmonary blastomycosis in children: clinical course and follow-up. *Pediatrics* 1979;**63**:736–40.
112. Quasney MW, Leggiadro RJ. Pleural effusion associated with histoplasmosis. *Pediatr Infect Dis J* 1993;**12**:415–8.
113. Raffensperger JG, Luck SR, Shkolnik A, Ricketts RR. Minithoracotomy and chest tube insertion for children with empyema. *J Thorac Cardiovasc Surg* 1982;**84**:497–504.
114. Ramphul N, Eastham KM, Freeman R, et al. Cavitatory lung disease complicating empyema in children. *Pediatr Pulmonol* 2006;**41**:750–3.
115. Rescorla FJ, West KW, Gingalewski CA, et al. Efficacy of primary and secondary video-assisted thoracic surgery in children. *J Pediatr Surg* 2000;**35**:134–8.
116. Roberts JE, Bezack BJ, Winger DI, et al. Association between parapneumonic effusion and pericardial effusion in a pediatric cohort. *Pediatrics* 2008;**122**:e1231–1235.
117. Roberts JS, Bratton SL, Brogan TV. Efficacy and complications of percutaneous pigtail catheters for thoracostomy in pediatric patients. *Chest* 1998;**114**:1116–21.
118. Robinson LA, Moulton AL, Fleming WH, et al. Intrapleural fibrinolytic treatment of multiloculated thoracic empyemas. *Ann Thorac Surg* 1994;**57**:803–13.
119. Rosen H, Nadkarni V, Theroux M, et al. Intrapleural streptokinase as adjunctive treatment for persistent empyema in pediatric patients. *Chest* 1993;**103**:1190–3.
120. Roux P, Fisher RM. Chest injuries in children: an analysis of 100 cases of blunt chest trauma from motor vehicle accidents. *J Pediatr Surg* 1992;**27**:551–5.
121. Rubin SA. Radiographic spectrum of pleuropulmonary tularemia. *AJR Am J Roentgenol* 1978;**131**:277–81.
122. Saglani S, Harris KA, Wallis C, Hartley JC. Empyema: the use of broad range 16S rDNA PCR for pathogen detection. *Arch Dis Child* 2005;**90**:70–3.
123. Sahn SA. The differential diagnosis of pleural effusions. *West J Med* 1982;**137**:99–108.
124. Sahn SA. Pleural effusions in the atypical pneumonias. *Semin Respir Infect* 1988;**3**:322–34.
125. Sawicki GS, Lu FL, Valim C, et al. Necrotising pneumonia is an increasingly detected complication of pneumonia in children. *Eur Respir J* 2008;**31**:1285–91.
126. Scarci M, Zahid I, Bille A, Routledge T. Is video-assisted thoracoscopic surgery the best treatment for paediatric pleural empyema? *Interact Cardiovasc Thorac Surg* 2011;**13**:70–6.
127. Schneider CR, Gauderer MW, Blackhurst D, et al. Video-assisted thoracoscopic surgery as a primary intervention in pediatric parapneumonic effusion and empyema. *Am Surg* 2010;**76**:957–61.
128. Schreckenberger PC, Ilendo E, Ristow KL. Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J Clin Microbiol* 2004;**42**:2777–9.
129. Schultz KD, Fan LL, Pinsky J, et al. The changing face of pleural empyemas in children: epidemiology and management. *Pediatrics* 2004;**113**:1735–40.
130. Schutze GE, Hickerson SL, Fortin EM, et al. Blastomycosis in children. *Clin Infect Dis* 1996;**22**:496–502.
131. Schwartz KL, Nourse C. Panton-Valentine leukocidin-associated *Staphylococcus aureus* necrotizing pneumonia in infants: a report of four cases and review of the literature. *Eur J Pediatr* 2012; **171**:711–7.
132. Shah SS, Hall M, Newland JG, et al. Comparative effectiveness of pleural drainage procedures for the treatment of complicated pneumonia in childhood. *J Hosp Med* 2011;**6**:256–63.
133. Shapiro ME, Kasper DL, Zaleznik DF, et al. Cellular control of abscess formation: role of T cells in the regulation of abscesses formed in response to Bacteroides fragilis. *J Immunol* 1986;**137**: 341–6.
134. Siegel JD, McCracken GH Jr. Neonatal lung abscess: a report of six cases. *Am J Dis Child* 1979;**133**:947–9.
135. Smith DT. The diagnosis and treatment of pulmonary abscess in children. *JAMA* 1934;**103**:971–4.
136. Sonnappa S, Cohen G, Owens CM, et al. Comparison of urokinase and video-assisted thoracoscopic surgery for treatment of childhood empyema. *Am J Respir Crit Care Med* 2006;**174**:221–7.
137. Stewart PB. The rate of formation and lymphatic removal of fluid in pleural effusions. *J Clin Invest* 1963;**42**:258–62.
138. Strachan RE, Cornelius A, Gilbert GL, et al. Bacterial causes of empyema in children, Australia, 2007-2009. *Emerg Infect Dis* 2011;**17**:1839–45.
139. Stringel G, Hartman AR. Intrapleural instillation of urokinase in the treatment of loculated pleural effusions in children. *J Pediatr Surg* 1994;**29**:1539–40.
140. Tagliabue M, Casella TC, Zincone GE, et al. CT and chest radiography in the evaluation of adult respiratory distress syndrome. *Acta Radiol* 1994;**35**:230–4.
141. Tan TQ, Seilheimer DK, Kaplan SL. Pediatric lung abscess: clinical management and outcome. *Pediatr Infect Dis J* 1995;**14**:51–5.
142. Tan TQ, Mason EO Jr, Wald ER, et al. Clinical characteristics of children with complicated pneumonia caused by *Streptococcus pneumoniae*. *Pediatrics* 2002;**110**(1 Pt 1):1–6.
143. Tita JA, Wiedemann HP, Weinstein CE. Pleural effusions and abnormal nails. *Hosp Pract* 1986;**21**:65.
144. Tseng YL, Wu MH, Lin MY, et al. Surgery for lung abscess in immunocompetent and immunocompromised children. *J Pediatr Surg* 2001;**36**:470–3.
145. Vallejo JG, Ong LT, Starke JR. Clinical features, diagnosis, and treatment of tuberculosis in infants. *Pediatrics* 1994;**94**:1–7.
146. Varkey B, Rose HD, Kutty CP, Politis J. Empyema thoracis during a ten-year period: analysis of 72 cases and comparison to a previous study (1952 to 1967). *Arch Intern Med* 1981;**141**: 1771–6.
147. Wait MA, Sharma S, Hohn J, Dal Nogare A. A randomized trial of empyema therapy. *Chest* 1997;**111**:1548–51.
148. Weber TR, Vane DW, Krishna G, et al. Neonatal lung abscess: resection using one-lung anesthesia. *Ann Thorac Surg* 1983;**36**: 464–7.
149. Weissberg D, Ben-Zeev I. Talc pleurodesis: experience with 360 patients. *J Thorac Cardiol Surg* 1993;**106**:689–95.
150. Wessler H, Schwarz H. Abscess of the lung in infants and children. *Am J Dis Child* 1920;**19**:137–40.
151. Westcott JL, Volpe JP. Peripheral bronchopleural fistula: CT evaluation in 20 patients with pneumonia, empyema, or postoperative air leak. *Radiology* 1995;**196**:175–81.
152. Wiener-Kronish JP, Broaddus VC. Interrelationship of pleural and pulmonary interstitial liquid. *Ann Rev Physiol* 1993;**55**: 209–26.
153. Yang PC, Luh KT, Chang DB, et al. Value of sonography in determining the nature of pleural effusion: analysis of 320 cases. *AJR Am J Roentgenol* 1992;**159**:29–33.

CHAPTER 25

Cystic Fibrosis

Peter W. Hiatt • Ruth Ann Luna • Kathryn S. Moffett

Cystic fibrosis (CF) is the most common inherited lethal disease of whites. It occurs primarily among individuals of Central and Western European origin and affects more than 30,000 Americans and 60,000 people worldwide.[439] The estimated incidence in the United States is 1:2000 to 1:2600 white,[452] 1:19,000 black, 1:11,500 Hispanic, and 1:25,000 Asian-American live births.[270] CF has an autosomal recessive mode of inheritance. Affected individuals are phenotypic homozygotes, and both parents usually are heterozygotes or carriers. The carrier frequency in whites in the United States is approximately 1/25, with full siblings of children with CF having a one in four chance of being affected.

Mutations in a single gene located on the long arm of chromosome 7 account for the defective protein in CF.[439] A range of different mutations at the DNA level account for the abnormal protein. The most common mutation, p.Phe508del, is the absence of three sequential nucleotides, which leads to the deletion of phenylalanine at the 508 position on the CF transmembrane conductance regulator protein (CFTR). Approximately 70 percent of individuals with CF have this mutation, and half of CF patients are homozygous for p.Phe508del. To date, more than 1800 mutations in the *CFTR* gene have been identified,[84,85] but fewer than 25 occur with a frequency of more than 0.1 percent.[147] Certain populations have higher frequencies of specific mutations, such as W128X in Ashkenazi Jews[270] or G551D in French Canadians.[339]

Five classes of mutations have been proposed to account for reduced CFTR chloride channel function.[166,339,438,467] Class 1 mutations are mutations in which stop codons or frame shift mutations cause early termination of mRNA translation and minimal to no protein production. In class 2 mutations (p.Phe508del), protein fails to mature, resulting in little expression of CFTR at the cell membrane. Class 3 and class 4 mutations are associated with defective regulation and decreased conductance of chloride at the cell membrane. Protein production and transit to the cell surface occur, but altered chloride conductance is present or chloride conductance is nonexistent. Class 5 mutations are splice site mutations, which affect the amount of CFTR produced.

Disease expression in CF is affected by CFTR mutations, genetic modifiers, and the environment.[263] The presence of more than 1800 mutations for CFTR has prompted investigators to evaluate the association of genotype with clinical disease. The Cystic Fibrosis Phenotype Genotype Consortium has shown that certain mutations from classes 3 to 5 are associated with pancreatic sufficiency and lower sweat chloride levels in contrast to pancreatic insufficiency[90,446]; however, minimal correlation of CF genotype with the severity of pulmonary disease has been observed. Measurement of lung function is highly variable in patients with the same CFTR mutation (e.g., p.Phe508del), suggesting other genetic and nongenetic factors determine the severity of lung disease. In a study of monozygotic and dizygotic twins, equal contribution of genetic and nongenetic factors contributed to the variance observed in pulmonary function; that is, approximately 50 to 60 percent of the variance observed in lung function was secondary to genetic factors.[72,445] Several modifier genes have been identified that affect disease severity of different organ systems in CF.[71,93,108,205,385] The wide variation in pulmonary disease observed in CF reflect the effects of environment, patient compliance, polymorphisms in CFTR, and modifier genes that affect the function of CFTR.

CFTR is a glycoprotein expressed at low levels by surface epithelial cells in the lung, sweat glands, pancreas, liver, large intestine, and testes.[327] Higher levels of expression have been reported in submucosal glands. CFTR protein is a member of a group of membrane transport proteins known as the *ABC-transporter superfamily*.[185] Researchers have confirmed that CFTR functions as an apical chloride channel mediated by cyclic adenosine monophosphate containing 1480 amino acids. It is composed of two membrane-spanning domains, two nucleotide-binding domains that interact with adenosine triphosphate, and a regulatory domain.[6] Activation of the channel is regulated by protein kinase A, which serves as a target for phosphorylation. CFTR in epithelial cell membranes may influence the expression of other proteins important in regulating inflammation, ion transport, and cell signaling, altering the CF phenotype.[338] CFTR regulates the activity of separate calcium-activated chloride channels and downregulates sodium transport, balancing the rates of chloride secretion and sodium absorption.[139,204,217]

CFTR is responsible for the proper hydration of secretions in the airway, pancreas, and other tissues. An inability to secrete chloride and excessive absorption of sodium and water contribute to altered luminal secretions in patients with CF. In the lung, this alteration leads to decreased airway surface liquid, decreased mucociliary clearance, and a predisposition to chronic bacterial infections.

Clinical Manifestations

Individuals with CF have exocrine gland dysfunction, which results in progressive suppurative obstructive lung

TABLE 25-1 Clinical Features of Cystic Fibrosis at Diagnosis

0-2 Years
Meconium ileus
Obstructive jaundice
Hypoproteinemia and anemia
Bleeding diathesis
Heat prostration and hyponatremia
Failure to thrive
Steatorrhea
Rectal prolapse
Bronchitis/bronchiolitis
Staphylococcal pneumonia
2-12 Years
Malabsorption
Recurrent pneumonia and bronchitis
Nasal polyps
Intussusception
>13 Years
Chronic pulmonary disease
Clubbing
Abnormal glucose tolerance
Diabetes mellitus
Chronic intestinal obstruction
Recurrent pancreatitis
Focal biliary cirrhosis
Portal hypertension
Gallstones
Aspermia

From Maclusky I, Levison H. *Kendig's disorders of the respiratory tract in children.* 5th ed. Philadelphia: W. B. Saunders; 1990, p. 701.

disease, pancreatic insufficiency (85% to 90%), elevated sweat electrolytes, male infertility (>95 percent), and female infertility. Less common manifestations include hepatobiliary disease, osteoarthropathy, diabetes mellitus, nasal polyposis, and meconium ileus (Table 25-1).[63,86,87]

The potential relationship between genotype (the genetic constitution of an individual) and phenotype (the physical expression of that genotype) in CF is an area of active clinical investigation. To date, the correlation of genotype to phenotype has not led to many clear associations between severity or course of pulmonary disease and type of genetic mutations.[50,202] A family-based study of CF involving monozygotic twins, dizygotic twins, and siblings reported that a significant portion of the variability in pulmonary function was attributable to heritability, independent of CFTR genotype.[424] This finding suggests that modifier genes account for a significant amount of the variance observed in CF lung disease. Numerous candidate modifier genes have been investigated to account for the variance in CF pulmonary disease, with more recent reports identifying transforming growth factor-β1, mannose binding lectin, interferon (IFN)-related developmental regulator 1 gene, and interleukin (IL)-8 gene as candidates.[109,251] These gene modifiers affect response to infection and inflammation.[173,424] In addition to modifier genes, environmental factors, including passive smoke exposure, infection, and socioeconomic status, have been associated with reduced lung function.[77,200,280,340,355] Multiple mutations, incomplete understanding of the physiologic function of CFTR, and the role of modifier genes have delayed understanding of the genotypic influence on phenotype in CF lung disease.

A strong association between pancreatic function and genotype has been reported for individuals homozygous for p.Phe508del.[31] Most patients homozygous for p.Phe508del have pancreatic insufficiency.[83] Obstruction of the pancreatic duct begins in utero, resulting in fibrosis and loss of exocrine pancreatic function. Pancreatic fluid from patients with CF is low in enzyme and bicarbonate concentrations, resulting in maldigestion of fat and protein. Clinically, children commonly present with steatorrhea, protein-calorie malnutrition, muscle wasting, and progressive failure to thrive. A voracious appetite is characteristic, and stools are described as bulky, greasy, and foul-smelling. Approximately 10 to 15 percent of patients have enough preservation of pancreatic function to allow for normal digestion of food (pancreatic-sufficient).[101] At least five mutations from classes 3 to 5 described earlier are associated with pancreatic sufficiency, whereas almost all patients homozygous for p.Phe508del have pancreatic insufficiency.[83]

Failure to thrive is a common complication observed at the time of diagnosis; however, with the implementation of newborn screening, this complication is less common. If malnutrition is severe, hypoproteinemia and edema are observed. In addition, malabsorption can lead to vitamin deficiency, especially of fat-soluble vitamins A, D, E, and K. These problems can be reversed with pancreatic enzyme replacement therapy, oral nutritional supplements, and routine vitamin supplements. Infants diagnosed by newborn screening may not have overt malabsorption for several months after birth even with a severe mutation such as p.Phe508del. However, with time, pancreatic function is lost and symptoms of malabsorption develop.

Glucose metabolism often becomes impaired with age, because fibrosis of the pancreas occurs in patients with exocrine pancreatic insufficiency. In the 2010 CF registry, 31 percent of adults were reported to have impaired glucose tolerance or CF-related diabetes with and without fasting hyperglycemia. Decreased secretion of insulin and reductions in peripheral glucose use and hepatic insulin sensitivity are observed in patients with impaired glucose tolerance.[260] CF-related diabetes has features of type 1 and 2 diabetes. The prevalence increases with age and is associated with increased morbidity and mortality disproportionately in women.[256,259] Progressive fibrosis of the pancreas causes destruction of insulin-producing β cells within the islets of Langerhans. In addition destruction of islet α cells impairs glucagon secretion therefore ketoacidosis is rare. Microvascular complications have been reported in up to 20 percent of patients with CF-related diabetes.[8] Diabetes in CF is common and strongly determined by genetic modifiers.[27]

Liver disease in CF is associated with pancreatic insufficiency.[297] Approximately 25 percent of patients with CF develop focal biliary cirrhosis, but fewer than 5 percent progress to multilobar biliary cirrhosis and portal hypertension. In the absence of CFTR, bile becomes inspissated and associated with periductal inflammation and

fibrosis. Liver function tests frequently are abnormal, as is a small, poorly functioning gallbladder. Cholelithiasis has been reported in 12 percent of patients and may be related to loss of bile acids in the stool.[75,127,130] Meconium ileus, the thick inspissated meconium that mechanically obstructs the distal ileum, occurs in 8 to 20 percent of newborns with CF. It also is associated with pancreatic insufficiency.[201] Both CFTR and modifier genes are thought to play an important role in the development of meconium ileus.[26,56] A similar syndrome (distal intestinal obstructive syndrome) mimicking meconium ileus can occur in older children and young adults with CF. Complete or incomplete obstruction can occur in the terminal ileum or proximal colon. Higher rates are observed in patients with pancreatic insufficiency, positive history of meconium ileus, and previous episodes of distal intestinal syndrome.[74]

Absence of the vas deferens with secondary aspermia renders 98 percent of men with CF infertile.[103] Sexual potency is normal, and with microsurgical techniques for sperm aspiration, affected men can become biologic fathers.[247] Men with congenital absence of the vas deferens can have abnormal CF alleles with little clinical expression of disease other than the reproductive system. Fertility in women with CF may be decreased secondary to amenorrhea (malnutrition) and dehydrated cervical mucus.[391] Pregnancy in women with mild-to-moderate disease appears to be well tolerated and not associated with an increased risk for death.[112,150,158] The live birth rate is 1.9/100.[88]

Pulmonary disease is the primary cause of morbidity and mortality in patients with CF.[451] Expression of CFTR has been localized to the airways and submucosal glands of the lung.[410] Clinical studies in young children with CF have found significant inflammatory changes in the airways in bacterial-positive and bacterial-negative patients.* Imaging studies using high-resolution computed tomography (CT) in infants with CF describe the presence of thickened airway walls and nonhomogeneous air trapping.[94,232,242] The lungs are morphologically normal at birth; within weeks, they begin showing evidence of small airway abnormalities and inflammation. Small and medium-sized airways become obstructed, and neutrophils are the inflammatory cells primarily recovered from bronchoalveolar lavage (BAL) fluid. An intense neutrophilic response leads to the release of proteases that cause chronic injury to the respiratory epithelium and supporting airway structure. The massive numbers of neutrophils subsequently release elastase, which overwhelms the antiproteases in the airway, contributing to enhanced destruction of tissue. Large amounts of neutrophil-derived DNA and cytosol proteins are released into the airway lumen, increasing sputum viscosity and worsening airway obstruction.[91]

Progressive bronchiectasis develops with time, leading to advanced destruction of the airways and parenchyma (Figs. 25-1 and 25-2).[128] Bronchiectatic cysts are prominent, especially in the upper lobes. Death eventually occurs from respiratory failure. Progressive deterioration of pulmonary function occurs despite the routine use of anti-inflammatory therapy, mucolytics, airway clearance, and antimicrobial agents. Progressive destruction of the airway and increasing obstruction lead to air trapping, hyperinflation, hemoptysis, and spontaneous pneumothorax.

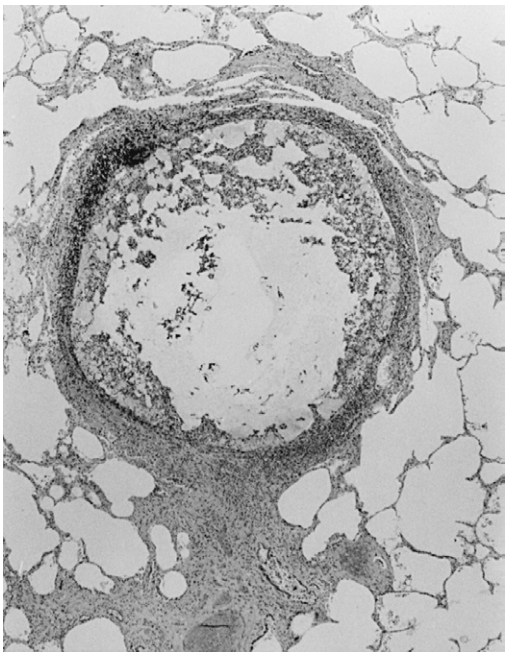

FIGURE 25-1 ■ Early stages of lung disease in cystic fibrosis are shown in this lung specimen. Airway inflammation and bronchiectasis are present. The surrounding lung parenchyma is normal.

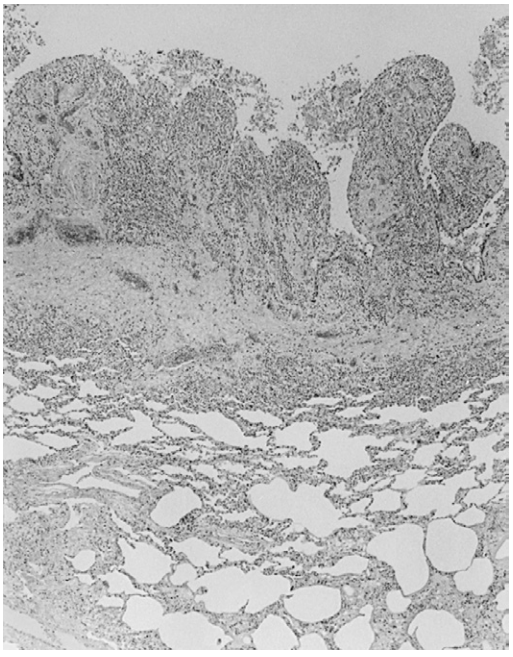

FIGURE 25-2 ■ Late stages of pulmonary disease. Epithelial ulceration of the airway, loss of smooth muscle from the airway wall, inflammation, and bronchiectasis are present in the large airway at the *top* of the photomicrograph. Compression of the surrounding lung parenchyma occurs as bronchiectasis increases.

*References 14, 91, 165, 254, 334, 335.

Many children with CF present during infancy with recurrent wheezing or persistent bronchiolitis. Most infants are asymptomatic at birth; however, many develop tachypnea, wheezing, hypoxia, and hyperinflation after having a respiratory viral infection.[171] These findings often resolve with therapy. As mucopurulent secretions increase, chronic cough develops.[63] Digital clubbing occurs gradually and correlates with severity of lung disease. Cough is the earliest symptom in infants and precedes persistent sputum production, crackles, or clubbing. It is present in half the infants in a prospective study of CF infants diagnosed by newborn screening.[127,248] Predictive risk factors included the presence of pancreatic insufficiency, infection with *Pseudomonas aeruginosa*, socioeconomic status, and ethnicity.[248] On examination, evidence of crackles and decreased breath sounds secondary to mucopurulent secretions is seen. Acute exacerbations may develop, requiring intravenous antibiotic therapy and frequent hospitalization. Approximately a third of patients with CF in the 2005 U.S. CF registry[87] experienced a pulmonary exacerbation requiring intravenous antibiotics. As lung disease progresses, tolerance for exercise is reduced, dyspnea increases, and respiratory failure develops. Marked heterogeneity occurs in the rate of progression of pulmonary disease. Some patients live to the sixth decade of life, whereas others die as a result of respiratory failure before their 10th birthday.

In 2010, the median predicted survival for individuals with CF was 38.3 years. Survival for children born in 2005 is expected to increase, with the median age approaching the fifth decade of life. In the United States, 48 percent of people with CF are 18 years old or older,[88] and within 5 years, half are expected to be older than 18 years. As life expectancy has increased, CF has become a disease of children and adults.

DIAGNOSIS

The diagnosis of CF has changed over the past 10 years. Newborn screening for CF has been adopted by the United States, Europe, Australia, New Zealand, and many Latin American countries. The CF newborn screen program identifies patients at risk for the disease by measuring values of immunoreactive trypsinogen in dried blood spots, Trypsinogen is produced in the pancreas and is released into the serum secondary to pancreatic duct dysfunction. After an abnormal immunoreactive trypsinogen value is identified, many programs perform DNA testing to identify known CFTR mutations. Other programs repeat the immunoreactive trypsinogen testing 2 weeks after the initial test. Both programs report 90 to 95 percent sensitivity and identify infants with varying disease severity.[126,432] After a positive screen, infants are referred for diagnostic testing (sweat test or molecular genetic testing). For most patients the sweat test remains the best diagnostic test to establish the diagnosis of CF. If the sweat test results are in the intermediate range, then DNA analysis may help in the diagnosis. The clinical significance of all 1800 CF mutations is unknown. Many are sequence variants without known clinical disease. Others are known to cause loss of CFTR function and associated with clinical abnormalities in one or more organ systems. Sequence analysis of the exons, introns, and promoter regions and detection of deletions and duplications identifies 98 percent of CFTR mutations.[388] Infants are generally asymptomatic at the time of diagnosis, although some may be underweight. Data from the 2010 U.S. CF registry reported that newborn screening accounted for more than 50 percent of newly diagnosed patients.[87] In the absence of newborn screening, an in utero diagnosis, or a family history of CF, a strong clinical suspicion is required for early recognition. Most children present with a history of recurrent lower respiratory tract disease and symptoms secondary to malabsorption. Approximately 15 to 20 percent of children have meconium ileus at birth, a family history of CF, or both. A consensus panel convened by the U.S. Cystic Fibrosis panel recommended a combination of phenotypic features, family history of CF, or positive newborn screen and one or more laboratory tests to diagnose CF.[129,337] Laboratory tests include identification of known CF mutations, abnormal bioelectric transepithelial membrane properties, and elevated concentrations of sweat chloride. The World Health Organization adopted similar recommendations.[453]

The quantitative pilocarpine iontophoresis sweat test is the primary test to establish a diagnosis of CF.[337] A sweat chloride concentration greater than 60 mEq/L is consistent with a diagnosis of CF. Values between 40 and 60 mEq/L are considered borderline, and values less than 40 mEq/L are normal. Infants with a positive newborn screen and sweat chloride values between 30 and 59 mEq/L under 6 months of age are considered to have values in the intermediate range, Molecular genetic testing should be pursued in these infants with indeterminate sweat chloride values. In the presence of two CF-causing mutations, a diagnosis of CF can be made. Infants with intermediate sweat test values and 1 CF-causing mutation cannot be diagnosed definitively with CF and will need to be followed longitudinally. If the sweat test is positive, it should be repeated on two separate occasions. Identification of two CF mutations by genotype is highly specific but less sensitive. Mutational analysis can be done by several different techniques, with commercial laboratories testing for the most common 30 to 87 mutations. These laboratories identify approximately 90 percent of CF mutations but leave more than 1000 mutations unidentified. Extensive screening for the remaining 10 percent of mutations is expensive. For rare mutations, gene sequencing is available.[336,337] The p.Phe508del mutation is found in 89 percent of patients with CF in the United States and a similar number of CF patients in the United Kingdom. Because each patient has two chromosomes, however, only 50 percent of patients are homozygous for p.Phe508del.

Nasal potential difference measurements assess the transepithelial electric potential difference that exists across nasal epithelium. Different patterns of potential difference are found in patients with CF.[161] Abnormalities in chloride transport and sodium absorption alters the transepithelial electric potential difference in CF in contrast to normal epithelia. The test can be useful in individuals with mild or atypical phenotypic features of CF,

TABLE 25-2 **Diagnosis of Cystic Fibrosis**

One or More Phenotypic Features
Chronic sinopulmonary disease
Gastrointestinal and nutritional abnormalities
Salt loss syndromes: acute salt depletion
Chronic metabolic alkalosis
Male urogenital abnormalities resulting in obstructive azoospermia
Plus Laboratory Evidence of CFTR Abnormality (One or More)
Elevated sweat chloride concentrations
Identification of two CFTR mutations
In vivo evidence of abnormal ion transport across nasal epithelium

CFTR, Cystic fibrosis transmembrane conductance regulator.

but it is not readily available and it requires experienced personnel to perform.[336,337]

Newborn screening programs are associated with long-term benefits in children with CF diagnosed shortly after birth. Improved nutritional status as a result of newborn screening has been reported in a controlled, randomized trial in Wisconsin,[126] as well as in countries other than the United States.[351] Ninety percent of screened infants in the Wisconsin study diagnosed with CF at birth maintained their weight greater than the 10th percentile in contrast to only 60 percent of unscreened controls. Children in the screened group were less likely to fall below the 10th percentile for weight and height from early childhood through 16 years of age.[49,126] In addition, cognitive function improved significantly in the screened group.[212,213] No long-term improvements in pulmonary status were observed in the Wisconsin study; however, several observational studies have reported improved pulmonary outcomes, less colonization with *P. aeruginosa*, decreased hospitalization for complications, and improved nutrition in children diagnosed by newborn screening.*

In addition to improving nutritional outcomes, newborn screening may result in improved survival rates for children. A systematic literature review of mortality in children with CF reported a survival benefit for patients diagnosed by newborn screening.[77,163] A survival effect also was shown in a study from Wales.[106] Without screening, approximately 60 percent of patients are diagnosed by the time they reach 1 year of age and almost 90 percent by the time they are 5 years. Early diagnosis through neonatal screening seems to improve nutritional outcome, with increasing evidence that these programs are associated with improved pulmonary outcomes and improved long-term survival.

The diagnosis of CF should be based on the presence of one or more clinical features (Table 25-2), a positive newborn screening test, and laboratory evidence of abnormal CFTR function. Laboratory tests include elevated sweat chloride concentration, two identifiable CF mutations, or abnormal in vivo nasal potential difference measurements made across the nasal epithelium.

*References 4, 127, 210, 218, 330, 335, 462.

PATHOGENESIS

The CFTR protein resides in the plasma membrane of epithelial cells and has several functions. In the sweat duct, it acts to absorb chloride; in the lung, it secretes chloride; and in the pancreas, it secretes chloride in exchange for bicarbonate.[403] Organs that are affected by the disease have abnormal chloride and fluid secretion, which impairs fluid movement and leads to ductular obstruction and organ damage. Five classes of CF mutations result in different amounts of CFTR expression and have been observed to produce varying clinical disease. The most common mutation, p.Phe508del, is a class 2 mutation. The CF protein is misfolded and degraded before reaching the cell membrane.[61,326]

Other classes of mutations produce CFTR that cannot be activated, is decreased in abundance, or has altered conductance. Patients with at least one "mild" allele have some low level of CFTR expression. They retain some degree of pancreatic function, have less severe lung disease, and have sweat chloride concentrations that are borderline normal.[77,94,446] Male patients with congenital bilateral absence of the vas deferens are pancreatic sufficient, exhibit little or no lung disease, can have normal sweat chloride concentrations, and are thought to have CFTR expression that is 10 percent of normal.[10] Carriers for CFTR who are heterozygotes expressing half the expected amount of CFTR are at increased risk for developing pancreatitis, allergic bronchopulmonary aspergillosis (ABPA), and sinusitis.[60,70,111,311]

The range of severity of disease is related to the production of functioning CFTR. The amount of expression of CFTR is important in the pathogenesis of CF. Variation in the severity of disease in individuals with the same genotype indicates other factors, including the environment, genetic modifiers, and medical therapy, are important in long-term outcomes, however.

The exact mechanism by which altered salt and water transport leads to abnormal secretions in the respiratory tract, pancreas, gastrointestinal tract, sweat glands, and other exocrine glands remains under intense investigation. The isotonic, low-volume model has been proposed to account for the pathogenesis of CF.[35,37] In the lung, abnormal respiratory tract secretions leading to decreased airway surface liquid seem to decrease mucociliary clearance and impair defenses to inhaled particulate matter and various microbial pathogens. The airway surface liquid is crucial for lung defense and is composed of a gel and sol layer. The gel or mucous layer is composed of high-molecular-weight mucins with carbohydrate side chains that bind inhaled particulate matter and pathogens. The sol or periciliary liquid layer consists of low-viscosity fluid to hydrate mucins and allows ciliary movement to occur. The periciliary liquid layer is 7 μm in height, allowing cilia to beat freely.

Airway surface liquid volume is regulated by the respiratory epithelium through ion transport processes. Salt concentrations can be changed to regulate the hydration of the airway lining fluid to maintain optimal mucociliary function. The absence of CFTR in the apical cellular membrane decreases the ability of cells to secrete

chloride into the periciliary fluid. CFTR inhibits the epithelial sodium channel; in its absence, excessive absorption of sodium occurs. Other channels are available for secretion of chloride (i.e., calcium-activated chloride channel) in the respiratory epithelium; however, they cannot compensate for the loss of CFTR. Chloride also can enter the cell through a chloride transporter. The net effect is increased absorption of sodium, chloride, and water, which reduces the periciliary volume, alters the composition of mucins, and decreases mucociliary clearance. The reduction in the periciliary fluid impairs ciliary movement, decreases mucus transport, markedly alters clearance, and sets the stage for airway obstruction, infection, inflammation, and progressive lung destruction.

Altered chloride channel function and fluid secretion help to explain the presence of disease in the sweat gland, intestine, pancreas, and male genital tract. The epithelial cells affected by CFTR mutations in various organs represent different channel and regulatory activities of CFTR but result in deficient secretion of fluids. This deficiency causes accumulation of mucus, obstruction, and various degrees of organ damage. Plugging of pancreatic ducts leads to chronic fibrosis, pancreatic atrophy, and loss of digestive enzymes and islet cells. Similar obstruction in the biliary tract can cause inflammation and focal biliary cirrhosis. Glandular obstruction of the vas deferens causes involution of the Wolffian duct and infertility in more than 95 percent of men with CF. Women with CF produce abnormally tenacious cervical mucus, with reported rates of infertility of 20 percent.

Dehydration of airway surfaces and defective mucociliary transport has been termed the *low-volume model*.[37] Obstruction of small terminal airways and submucosal glands with thickened mucopurulent secretions are the first signs of early disease in infants with CF. Ductular dilation, neutrophil infiltration, glandular hyperplasia, and peribronchiolar inflammation are classic findings of the disease. Mucus adherent to airway surfaces serves as a site for chronic infection. Human cell culture models of normal and CF epithelia have shown the airway surface liquid is decreased in CF,[244,404] and regulation of airway surface liquid volume depends on the regulation of sodium absorption and chloride secretion. Transgenic mouse models overexpressing epithelial sodium channels to mimic CF produce airway obstruction with mucus, neutrophilic inflammation, bacterial infection, and goblet cell hyperplasia.[238]

These findings are similar to observations in humans. Mucociliary clearance is decreased in CF.[35,206,328] Submucosal glands have high expression of CFTR.[121,164] Loss of CFTR function alters the composition of mucins produced by the submucosal glands,[190,206,429] leading to ductular dilation with mucus and obstruction. Mucus is tightly adhered to the respiratory epithelium and increases airway obstruction. Failure to clear mucous plugs, continued mucin secretion, and adherent mucus to the airway surface provides the focus for infection.

The respiratory airways of patients with CF are infected with several bacterial and viral pathogens that occur in an age-dependent order. Chronic infection with *P. aeruginosa* develops in 80 percent of affected individuals by the time they reach 25 years of age.[335] *Pseudomonas* infection is associated with a more rapid decline in lung function and increased rates of mortality[120,137] and becomes the dominant organism colonizing the CF airway. The prevalence of *Pseudomonas* infection may be decreasing.[228,320] Initial isolates from CF infants and toddlers are unique and suggest an environmental acquisition.[283,381,382] Over time one strain dominates and changes to a mucoid phenotype.[160] Evidence of person-to-person transmission from chronically colonized CF patients to younger CF children, siblings, and individuals in close contact has been documented.[13,16,60,98,214]

Multiple hypotheses have been proposed to define the mechanism of airway colonization with *P. aeruginosa*. Although several studies have assessed this problem, the exact mechanism of infection and colonization remains investigational. The primary hypothesis for airway infection in CF is altered mucociliary clearance secondary to abnormal salt and water transport. As the normal function of CFTR becomes defined, additional hypotheses have been put forward to explain the mechanism of early airway infection and inflammation. They involve altered epithelial cell receptors for bacteria; abnormal mucins that enhance bacterial binding, impairing airway clearance; and inactivation of epithelial-derived bactericidal activity.

The majority of evidence indicates that the hypothesis of low volume of airway surface liquid hypothesis explains the pathogenic mechanism of lung disease in CF. Dehydration of airway surface liquid and adhesion of mucus to the airway epithelium provide an environment conducive to chronic *Pseudomonas* infection (Fig. 25-3).[454] Depletion of periciliary water decreases ciliary activity, concentrates mucins, and increases adhesion of the mucous layer to the airway surface. Secretion of mucins continues from submucosal glands and goblet cells, producing plugging and airway obstruction. These mucous plaques are poorly cleared from the airway and become the sites of initial infection. Pili extending from the surface of the bacterium are able to bind to mucin.[367] An anaerobic gradient develops within the thickened mucous plugs. *Pseudomonas*, after binding to mucin, is able to penetrate the thickened mucus and grow in an anaerobic environment. *P. aeruginosa* is able to grow in anaerobic conditions because of the production of nitrate reductase, which allows it to cleave oxygen from nitrate.[360] When it is in the anaerobic environment, an alginate polysaccharide is formed. Biofilm-containing macrocolonies of *Pseudomonas* are then established. The established macrocolonies remain within the airway lumen. These macrocolonies are very resistant to antibiotics and host defense and allow chronic infection, inflammation, and airway destruction to occur.[177,296,454]

P. aeruginosa infection of the airway is associated with progressive respiratory impairment and death in most patients with CF. Bacterial colonization and infection of the lower respiratory tract occur early in infants with CF (Fig. 25-4), and airway inflammation often is established at the time the disease becomes clinically manifest.[15] Genotype functional class is an important risk factor in age at initial *Pseudomonas* infection.[333] *P. aeruginosa* infection is intermittent before chronic colonization develops.[137,164,334] Initial isolates generally are antibiotic-sensitive

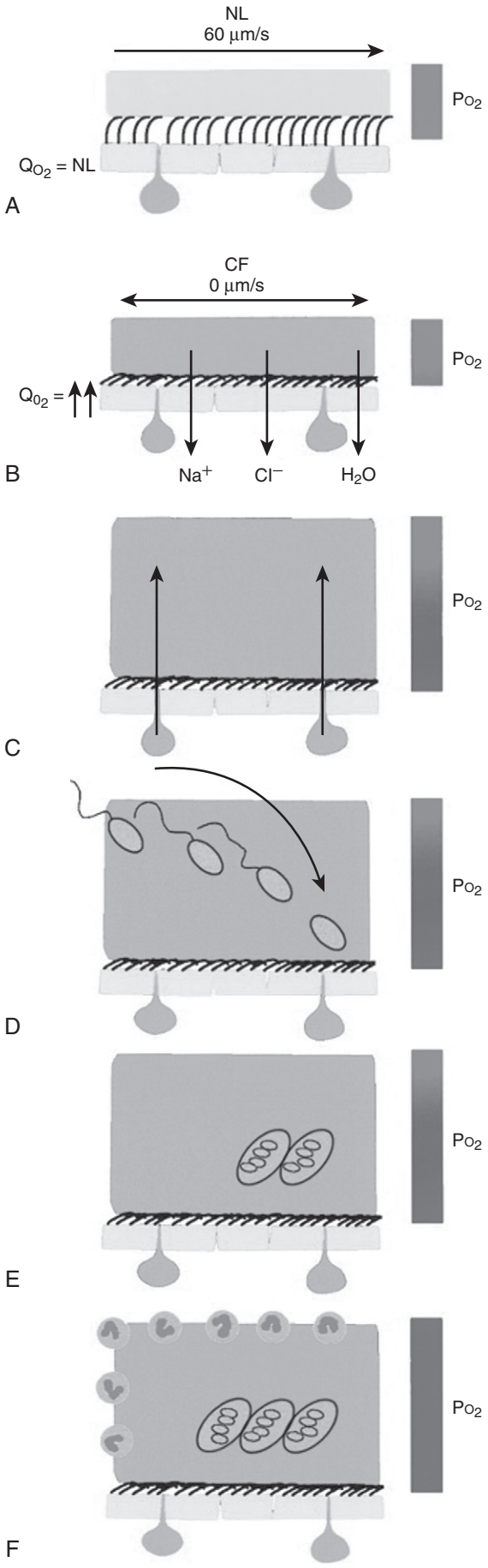

FIGURE 25-3 ■ Schematic model of the pathogenic events hypothesized to lead to chronic *Pseudomonas aeruginosa* infection in airways of patients with cystic fibrosis (CF). **A,** On normal airway epithelia, a thin mucous layer *(light gray)* resides atop the PCL *(clear).* The presence of the low-viscosity PCL facilitates efficient mucociliary clearance (denoted by *vector*). A normal rate of epithelial oxygen (O_2) consumption (QO_2; *left*) produces no O_2 gradients within this thin ASL. **B** through **F,** CF airway epithelia. **B,** Excessive CF volume depletion (denoted by v*ertical arrows*) removes the PCL, mucus becomes adherent to epithelial surfaces, and mucus transport slows or stops (*bidirectional vector*). The increased O_2 consumption *(left)* associated with accelerated CF ion transport does not generate gradients in thin films of ASL. **C,** Persistent mucus hypersecretion (denoted as mucus secretory gland/goblet cell units; *dark gray*) with time increases the height of luminal mucus masses and plugs. The increased CF epithelial QO_2 generates steep hypoxic gradients in thickened mucus masses. **D,** *P. aeruginosa* bacteria deposited on mucus surfaces penetrate actively or passively or both (as a result of mucus turbulence) into hypoxic zones within the mucus masses. **E,** *P. aeruginosa* adapts to hypoxic niches within mucus masses with increased alginate formation and the creation of macrocolonies. **F,** Macrocolonies resist secondary defenses, including neutrophils, setting the stage for chronic infection. The presence of increased macrocolony density and, to a lesser extent, neutrophils renders the now mucopurulent mass hypoxic. (From Worlitzsch D, Tarran R, Ulrich M, et al. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;109:317-25.)

and nonmucoid.[42] In contrast, isolates from patients chronically colonized usually are highly antibiotic-resistant, mucoid, and increased in density.[123,384] As lung disease progresses, the phenotype changes and the bacteria adopt a mucoid biofilm mode of growth.[316] As the organism changes from a nonmucoid to mucoid state, differences in the outer membrane proteins are expressed.[168] In addition, hypermutable strains of *Pseudomonas* are isolated and show greater antibiotic resistance.[285] As *P. aeruginosa* converts from a nonmucoid to mucoid phenotype, several bacterial lipoproteins are produced that induce inflammation.[133]

Local production of cytokines and other proteins in response to bacterial infection may play an important role in modulating chronic airway inflammation.[363] Airway inflammation in CF is characterized by marked neutrophilic infiltration, with release of bioactive lipids, oxygen metabolites, myeloperoxidase, and lysozyme.[131] Proteases (e.g., elastase) derived from neutrophils and bacteria directly damage respiratory epithelium and cleave proteins important in host defense.[394,411] Chronic infection, with retention of the by-products of inflammation, ultimately leads to the severe bronchiectatic changes and derangements of gas exchange characteristic of end-stage CF.

Several in vivo studies have assessed the location of bacterial adherence within the lung of CF patients and the attachment of bacteria to CF and non-CF cells in vitro.[20,415] *P. aeruginosa* is found within the lumen of airways of patients with CF obtained from autopsy specimens.[20] These organisms are observed within the

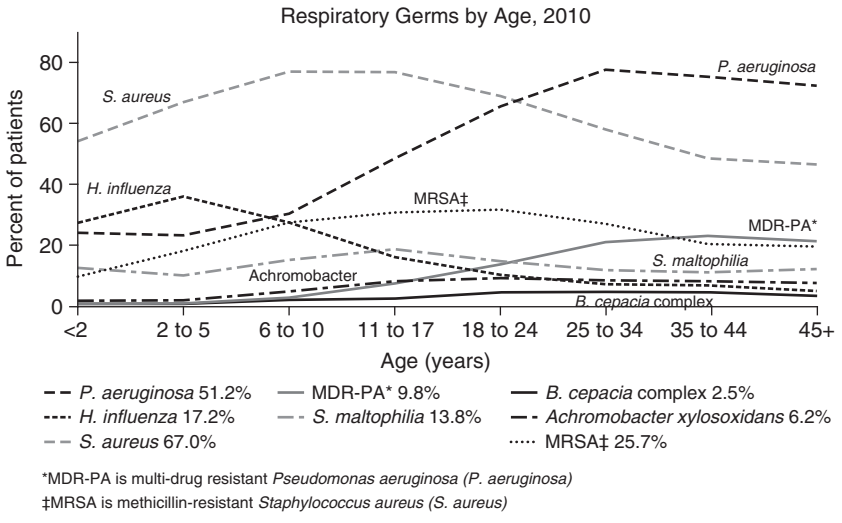

FIGURE 25-4 ■ Age-specific prevalence of pathogens in patients with cystic fibrosis. (From Cystic Fibrosis National Patient Registry, 2000.)

inflammatory exudates of the airway and not within epithelial cells lining the lung or in alveolar spaces. In vitro studies have shown adherence of *P. aeruginosa* in areas of epithelial cell destruction.[303,309,415] No adherence to the apical membrane of intact epithelial cells has been noted, however. In contrast, evidence in cell culture models indicates that defects in CFTR enhance bacterial binding to immortalized airway epithelial cells.[346] A tetrasaccharide (asialo GM_1) is expressed more on CF than on non-CF cells and promotes *P. aeruginosa* binding to the epithelial membrane. Pseudomonal exoproducts such as neuraminidase increase the amount of asialo GM_1 available for bacterial binding and facilitate bacterial adherence to airway epithelial cells.[47,342,348] CFTR itself can act as a receptor for *P. aeruginosa* and initiate an effective innate immune response in the initial stages of infection to clear *Pseudomonas* from the airway.[51] CFTR binding to *P. aeruginosa* induces release of IL-1β, nuclear translocation of nuclear factor-κB (NF-κB), and neutrophil influx. Endocytosis of *Pseudomonas* by the epithelial cells and subsequent clearance of infected epithelial cells by desquamation has been reported.[307,308] In the absence of CFTR, clearance of infection with *Pseudomonas* is impaired.

Mucins also are important in binding bacteria within the airway. Sialylated and neutral forms of mucins bind *P. aeruginosa*.[312] Removal of sialylic acid from mucin by neuraminidase reduces adherence of *P. aeruginosa*. Mucus dehydration can lead to increased concentration of mucins, decreased pH, and a reduction in glutathione, which decreases mucus viscoelasticity.[340] Decreased bicarbonate secretion results in mucin cross linking by calcium.[125] Polymeric macromolecules within the gel matrix reduce pore size from 500 nm to 150 nm, immobilizing bacteria within the mucus and inhibiting neutrophil migration and clearance.[76] The concentrated mucus impairs neutrophil migration, promotes an anaerobic environment, and reduces mucociliary transport. Mucin, a component of mucus, is decreased in CF, and its decreased content may promote development of infection.[341]

In contrast to the hypothesis of low volume of airway surface liquid discussed in detail earlier, the "high salt" theory proposes that CFTR maintains low concentration of salt in airway lining fluid. In the absence of CFTR, high concentrations of salt inactivate the secreted antimicrobial substances on airway surfaces.[458] Airway epithelial cells are vital in maintaining a pathogen-free environment within the airway. Smith and colleagues[370] showed that a monolayer of airway epithelial cells was able to kill bacteria when placed on their apical surface. In contrast, CF epithelia were unable to clear the bacterial load. Surface fluid washed from the normal cells showed bactericidal activity; however, fluid from the CF monolayer lacked bacterial killing properties. Goldman and colleagues[155] duplicated the results of this study using a different model. They implicated a human defensin, hBD-1, as the primary agent responsible for bacterial killing. The defensins are low-molecular-weight proteins produced by epithelial cells cytotoxic for bacteria and thought to be salt-sensitive. Many studies have not supported these early observations[36] but rather favor the high viscosity of the airway surface fluid, which hinders mucociliary clearance.

Oxidant stress plays a role in tissue injury observed in CF. Defective CFTR produces a redox imbalance in respiratory epithelial cells and extracellular fluid. Reactive oxygen species are important in killing bacteria and are generated by phagocytic cells and the respiratory epithelium. Oxidants are produced from activated phagocytes, metabolic pathways, inhaled oxidants, and bacteria.[54] Increased oxidant production alters ion transport and increases secretion of mucins.* Excessive oxidant production can cause tissue injury. Increased oxidant production by neutrophils has been proposed as a mechanism of airway injury. Markers of reactive oxygen species increase during acute pulmonary exacerbations and improve with treatment but do not normalize.[250,409] The continued presence of reactive oxygen species after successful treatment with antibiotics, supports the ongoing

*References 52, 79, 194, 275, 399, 401.

production of reactive oxygen species and chronic inflammation within the airways. Reactive oxygen species can further effect NF-κB transcription of proinflammatory genes, increasing the inflammatory response.[428] Neutrophils migrating into the airway lumen release large amounts of reactive oxygen species, including superoxide anion, hydrogen peroxide, and hydroxyl free radicals. These reactive species are generated by activation of nicotinamide adenine dinucleotide phosphate (NADPH). Continued interactions with bacterial products and inability to engulf bacteria lead to exaggerated production of or pro-oxidative species in the airway lumen, airway injury, and amplification of the inflammatory response.[141] One study has examined the activity of NADPH and myeloperoxidase with production of chloramines between CF and non-CF subjects.[448] Significant differences between groups were observed for myeloperoxidase activity and chloramine release. Myeloperoxidase activity and chloramine release were increased in subjects with CF. Treatment of CF neutrophils with amiloride reduced oxidant generation, suggesting that altered intracellular ion concentrations or pH may regulate myeloperoxidase activity. Other investigators have suggested alternatively that the circulating neutrophils may be primed to respond to a phagocytic stimulus and that these differences in oxidant generation represent increased neutrophil activity.[163,322]

An intracellular imbalance in oxidant metabolism has been proposed for airway epithelial cells in CF.[358] Airway surface liquid in CF is characterized by lower levels of glutathione and nitric oxide. Antioxidants are produced by enzymatic and nonenzymatic pathways.[54] Mucins, reduced glutathione, α-tocopherol, and metal-binding proteins function in the airway as antioxidants. The cysteine residues and carbohydrates of mucin account for its antioxidant properties.[82] Secretion of mucins is increased with oxidative stress; however, dehydration of mucins may impair its ability to scavenge reactive oxidative metabolites. Glutathione is important in regulating inflammation related to oxidative stress. Glutathione is reduced in airway surface liquid either from increased consumption or secondary to CFTR dysfunction.[224] Glutathione is found in large amounts in airway lung fluid from normal patients[53]; however, it is reduced in patients with CF.[391] CFTR may alter glutathione transport, impairing defense to oxidant injury. Low levels of glutathione are observed in CF knockout mice, supporting observations in humans.[426] Reduced intracellular concentrations of glutathione affect hydrogen peroxide content of airway cells and increase NF-κB production, contributing to airway inflammation. Thus, increased production of reactive oxygen species from neutrophils and impaired antioxidant biosynthesis associated with mutant CFTR creates an environment within the airway for excessive inflammation and cellular injury. Production of cell adherence molecules and inflammatory cytokines is intact in patients with CF.[104,203] Defects in bacterial opsonization, reduction in anti-inflammatory cytokines, and proinflammatory effects of bacterial DNA have been reported. Impaired phagolysosomal function and decreased respiratory burst have been found in neutrophils in CF with upregulation of Toll-like receptor (TLR)4 and downregulation in TLR2. These abnormalities would be expected to reduce the ability of neutrophils to clear bacteria.[24,262] Inflammation and infection can increase intracellular Ca^{2+} and affect the immune response mediated by airway epithelial cells and T-helper cells. Increased intracellular Ca^{2+} in CF mice helper T-cells affect gene expression and favor a Th2 differentiation rather than the Th1 needed for bacterial clearance.[268] Altered T-helper lymphocytes impair macrophage activation for bacterial killing. Humoral antibody responses are normal; however, increased elastase in the CF airway can alter opsonic receptors, impairing phagocytosis. Large amounts of elastase are present in lavage fluid from airways of patients with CF, overwhelming the normal antiprotease activity. This condition can lead to local impairment in phagocytosis of bacteria within the airway lumen, contributing to bacterial persistence.

Proinflammatory mediators are elevated in BAL fluid from young patients with CF, as are macrophages expressing intracellular cytokines.[198,269,459] Increased numbers of neutrophils and IL-8 in BAL fluid are seen in bacterial culture–negative and bacterial culture–positive infants with CF in contrast to controls.[181,444] Proinflammatory mediators IL-1, IL-2, tumor necrosis factor-α, and IL-8 are markedly elevated in children with advanced disease.[169,181,444] In contrast, some anti-inflammatory cytokines (e.g., IL-10) are found in reduced amounts in BAL from patients with CF. These observations have prompted some investigators to assess production of IL-10 from bronchial epithelial cells in normal subjects and subjects with CF.[30] Bronchial epithelial cells from normal individuals produce significantly greater amounts of IL-10 than cells of subjects with CF. NF-κB is a transcription factor for several proinflammatory mediators and is activated persistently in CF. Inhibitors to NF-κB, including IL-10, are downregulated.[102,357,427,437]

Enhanced intracellular Ca^{2+} signaling in response to infection and inflammation in both airway epithelial and immunomodular cells may account for the hyperinflammatory response noted in CF.[319,325] Both phases of Ca^{2+} release, either by Ca^{2+} influx or endoplasmic reticulum release, can upregulate NF-κB and production of proinflammatory cytokines. Chronic airway inflammation and infection increase endoplasmic reticulum Ca^{2+} stores, enhancing the inflammatory response. Therefore the CF gene defect may result in an inability to control airway inflammation after exposure to repeated infections altering the innate and immune responses to specific pathogens. Although the understanding of this disease pathogenesis is incomplete, airway damage that occurs from excessive inflammation and chronic infection remains a target for new therapeutic modalities.

MICROBIOLOGY

Epidemiology of Pathogens

Most commonly, patients with CF are infected with classic pathogens, including *Staphylococcus aureus*, nontypeable *Haemophilus influenzae*, and *P. aeruginosa*.[8,46,149,253] A few patients may be infected with *Burkholderia cepacia*

complex, which can be particularly virulent and, in some patients, associated with increased morbidity and mortality.[77,159,331] More recently, potentially emerging pathogens, such as methicillin-resistant *S. aureus* (MRSA), *Stenotrophomonas maltophilia*, *Achromobacter xylosoxidans*, nontuberculous mycobacteria (NTM), mold species (primarily *Aspergillus* spp.), and, more recently, *Scedosporium* spp., are being reported. Additional potential pathogens include *Ralstonia* and *Pandoraea* spp.[68,227] As described in more detail subsequently, the link between these pathogens and lung disease is not fully elucidated.

The etiologies behind these emerging pathogens are multifactorial. The average life expectancy of patients with CF has increased significantly during the past 2 decades from 27 years in 1986 to 38.3 years in 2010.[88] Patients with CF are treated frequently with prolonged courses of antimicrobial agents, including oral, intravenous, and aerosolized agents, placing selective pressure on their flora and leading to increased resistance.[41,43,87] Evidence of health care–associated acquisition of some pathogens, including *B. cepacia* complex, *P. aeruginosa*, and MRSA, is described subsequently. Finally, during the past 2 decades, detection of many potential pathogens has improved as clinical microbiology laboratories processing CF specimens use several selective media and fully speciate non–lactose-fermenting, gram-negative bacilli as described subsequently.[149,170,398,466]

Age-Specific Prevalence of Pathogens

The U.S. Cystic Fibrosis Foundation has maintained an annual Patient Registry since 1962.[134] The age-specific prevalence for CF pathogens in the United States as reported to this registry in 2010 is shown in Figure 25-4.[88] During the first two decades of life, *S. aureus* is the most common pathogen and is harbored by approximately 70 percent of children and adolescents. Nontypeable *H. influenzae* is a less prevalent pathogen and is cultured from the respiratory tracts of 15 percent of children with the highest prevalence in children 2 to 5 years old. Over time, *P. aeruginosa* becomes the most prevalent pathogen, but it can be recovered from 30 percent of infants. Approximately 70 percent of American patients with CF are infected with *P. aeruginosa* by the time they reach 18 years of age, at which point *P. aeruginosa* becomes more prevalent than *S. aureus*. Overall, *Burkholderia* spp.[225] are recovered from approximately 3 percent of patients with CF. During the late 1990s, the Cystic Fibrosis Foundation began to collect data on the prevalence of MRSA, *S. maltophilia*, and *A. xylosoxidans*. As shown in Figure 25-4, these organisms are more common than *B. cepacia* complex and are harbored primarily by adolescent and adult patients. As noted in patients without CF, MRSA has become increasingly common.[323]

ROLE OF THE CLINICAL MICROBIOLOGY LABORATORY

Ongoing communication between the clinical microbiology laboratory and the CF care team is crucial to ensure that appropriate methodologies are in place to provide appropriate care, to support effective infection control, and to further understanding of the epidemiology of pathogens in CF.[350] Every 5 years, the executive committee of the U.S. Cystic Fibrosis Foundation conducts site visits of accredited CF care centers and meets with the director of the clinical microbiology laboratory in efforts to ensure that standardized practices are in place.[349] In addition, a survey determined that most clinical laboratories processing CF specimens were using selective media and speciating all gram-negative bacilli,[466] which may include molecular identification strategies.

Specimen Processing

Respiratory tract specimens from patients with CF, including specimens obtained after lung transplantation, should be physically and electronically indicated as "CF specimens" to ensure proper processing by the clinical laboratory. Timely transport optimizes recovery of pathogens; ideally, specimens should be received by the laboratory within 3 to 4 hours of collection or stored at 4° C (39.2° F) and processed within 24 hours. Sputum is the ideal specimen, and at least 1 mL should be collected to ensure an adequate volume is available for plating on selective media. Sputum can be Gram stained to determine if a specimen contains bacteria and neutrophils rather than epithelial cells. Oropharyngeal cultures often are obtained in children too young to expectorate, but these cultures may not be concordant with specimens obtained from the lower airway.[332] BAL specimens generally are reserved for research protocols, lung transplant recipients, or patients with atypical courses who cannot produce sputum. More recently, induced sputum has been promoted to assess the lower respiratory tract flora of patients with CF who cannot expectorate.[175,290]

Selective Media

Selective media are needed to isolate specific pathogens, although the use of these media is costly and time-consuming. Current recommendations for selective media are shown in Table 25-3. *S. aureus* strains from patients with CF may be thymidine-deficient and require mannitol salt agar for growth.[349] Detection of *B. cepacia* complex is markedly enhanced by the use of oxidative fermentative bacitracin polymyxin B lactose (OFBPL) agar or *B. cepacia* selective media.[149,170] Multicenter studies conducted by the Centers for Disease Control and Prevention in the 1980s showed that the use of selective media improved the yield of *Burkholderia* spp. from patients with CF.[396] In addition, specimens can be plated on mycosel agar to identify *Aspergillus* spp., although this mold grows well on blood agar as well as OFBPL. Oropharyngeal swabs from nonexpectorating patients also should be plated on selective media.[365] If NTM are suspected, sputum should be stained for acid-fast bacilli and processed using an additional decontamination step to prevent overgrowth by *P. aeruginosa*.[440] This decontamination step may kill NTM present in low concentrations, however.[21] Quantitative cultures of specimens from sputum are reserved for research purposes.

TABLE 25-3 **Recommended Media and Processing for Recovery of Cystic Fibrosis Pathogens**

Organism	Recommended Media or Processing*,†
Staphylococcus aureus	Mannitol salt agar
	Columbia/colistin–nalidixic acid agar
Haemophilus influenzae	Horse blood or chocolate agar (supplemented or not with 300 mg/L bacitracin) incubated anaerobically
Pseudomonas aeruginosa	MacConkey agar
Burkholderia cepacia complex	OFPBL agar, PC agar, BCSA
Stenotrophomonas maltophilia	MacConkey agar, VIA agar
	DNase agar confirmatory media or biochemical or molecular identification
Achromobacter xylosoxidans	MacConkey agar
	Biochemical identification assay
Mycobacterial spp.	NALC-NaOH and oxalic acid decontamination step
Yeast	Mycosel agar‡
Other gram-positive organisms	Sheep blood agar supplemented with neomycin and gentamicin (streptococcal selective agar)
Other gram-negative organisms	MacConkey agar

*Detection of some pathogens may be enhanced by prolonging incubation for 4 days to allow slow-growing colonies to become apparent.
†All media are commercially available.
‡Aspergillus spp. and other molds do not grow well on Mycosel, but do grow well (although not selectively) on many of the media used for cystic fibrosis specimens, especially OFPBL. Standard biochemical and phenotypic methods should be used for confirmation of the identification of molds.

Antimicrobial Susceptibility Testing

The optimal methodology for antimicrobial susceptibility testing for mucoid and nonmucoid strains of *P. aeruginosa* has been elucidated. In studies of 500 multidrug-resistant strains of *P. aeruginosa* from patients with CF, five different antimicrobial susceptibility testing methods were compared: a reference microbroth dilution assay, Kirby Bauer disks, E-tests, and the automated commercial systems Vitek and Microscan. The agar-based diffusion methods (i.e., Kirby Bauer disks and the E-test) proved most accurate in contrast to the reference methods.[44,345] Vitek and Microscan, the commercial systems, had unacceptably high rates of very major (i.e., false-susceptible) and major (i.e., false-resistant) errors.[45] The Clinical Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) endorses the use of antibiotic-impregnated disks or reference broth microdilution assays to determine the susceptibility of multidrug-resistant strains of *P. aeruginosa* isolated from patients with CF.[272]

An intriguing concept in susceptibility testing is the potential role of susceptibility testing of bacteria grown in biofilms. As described in more detail subsequently, chronic lung infection in CF is thought to reflect a biofilm mode of growth containing bacteria in stationary phase that do not express the antibiotic targets present in log phase bacteria.[78] In vitro studies have shown that the minimal inhibitory concentration (MIC) of log phase bacteria tested in conventional susceptibility testing assays can be very different from the MIC of bacteria growing in stationary phase in biofilms.[172] Log phase–grown *P. aeruginosa* are resistant to macrolide antibiotics (MIC ≥128 μg/mL), whereas *P. aeruginosa* grown in biofilms are susceptible to these agents (MIC_{50} 2 μg/mL).[265] Studies are ongoing to compare the outcomes of pulmonary exacerbations treated using the results of conventional susceptibility testing versus the results from biofilm susceptibility testing.

Molecular Methods

Molecular diagnostics remain an important component in the accurate identification of pathogenic bacteria in respiratory specimens of patients with CF. When conventional culture methods fail to yield a definitive result, molecular testing performed on a cultured isolate is a rapid and cost-effective strategy for confirmation of suspected organisms. The most common molecular microbiology methods for bacterial identification use sequencing of the 16S rRNA gene.[25,233] Real-time PCR has also been used to detect and quantify known pathogens of interest in CF.[461] The role of microarrays and next-generation sequencing in the clinical molecular microbiology laboratory setting is still developing, but recent studies in the CF population suggest significant information regarding organisms that are not routinely cultured could be obtained using these methods, including more thorough fungal characterization.[81,96,366]

SPECIFIC CYSTIC FIBROSIS PATHOGENS

Viral Pathogens

Numerous studies have evaluated the role of nonbacterial infections with pulmonary exacerbations in CF. A clear correlation between respiratory viral infection and exacerbations of lung disease has been shown in most of these reports.* Viral lower respiratory tract infections occur more often in younger children and are associated with increased pulmonary disease in 40 percent of cases in CF.[2,419] Respiratory syncytial virus (RSV) has been identified most often with CF pulmonary exacerbations; however, influenza, adenovirus, parainfluenza virus, rhinovirus, picornavirus, and human metapneumoviruses also have been reported.[419] Respiratory viruses are isolated from patients with CF requiring

*References 2, 114, 142, 179, 284, 289, 302, 310, 314, 389, 435, 455.

hospitalization, and infection is associated with increased morbidity rates. Infants with CF infected with RSV can experience prolonged hospitalizations, mechanical ventilation, and supplemental oxygen at hospital discharge.[2] Severe viral infections in infants with CF were reported in 31/80 children diagnosed by newborn screening. Half of the children hospitalized had a respiratory virus identified at the time of hospitalization, and RSV infection accounted for hospitalization in 7 of the 31 children.[12]

Two studies have reported prolonged bronchiolitis-like syndromes in infants younger than 6 months of age.[145,231] These infants required intensive respiratory therapy, including bronchodilators, chest physiotherapy, and mechanical ventilation. Increased hospitalization was observed for children with CF who were infected with RSV in another study of children younger than 3 years of age.[171] Although prolonged hypoxia and respiratory failure were not observed, pulmonary function was markedly decreased after hospitalization and persisted for several months after infection.[171] Studies in older children with CF have reported reduction in pulmonary function, clinical scores, and radiographic scores and increased hospitalization after viral infection.[73,289,374,435,447] These studies show that infection with respiratory viruses results in clinical deterioration, hospitalization, and decreases in lung function.

Mechanisms to explain the enhanced disease observed with respiratory viral infections are being investigated. Normal airway epithelia use an adenosine-regulated pathway to maintain periciliary fluid volume by regulation of sodium absorption and chloride secretion. In a CF model, this regulation can be normalized by phasic motion simulating movement of the lung. Periciliary volume is restored to normal by release of adenosine triphosphate into the periciliary liquid and activation of alternative chloride channels. Viral infections, such as RSV, upregulate the extracellular adenosine triphosphatase activity, reducing periciliary fluid volume. Reduction in periciliary liquid volume promotes mucous stasis and plugging.[404] Other investigators have shown impaired innate defense manifested by reduced production of nitric oxide and impaired IFN-γ signaling pathway.[463,464]

The interrelationship between the acquisition of *P. aeruginosa* and viral respiratory infection is unclear. Increased colonization with *Pseudomonas* has been observed during the respiratory viral season,[193] and infection with *Pseudomonas* has been associated with hospitalization for viral respiratory illness. Antipseudomonal antibodies have been reported to increase after having RSV infection, and new infection with *Pseudomonas* has been observed during an acute respiratory illness.[73,302] Other investigators have not observed any change in bacterial infection or change in colonization associated with viral respiratory infection.[284] In cell culture models, RSV acts as a coupling agent between *P. aeruginosa* and enhances attachment to respiratory epithelial cells.[420] Petersen and colleagues[302] and Ong and associates[289] reported more severe pulmonary disease with viral infection in the presence of *P. aeruginosa* colonization, but Przyklenk and coworkers[310] found no correlation. Synergism between bacteria and respiratory virus infection was suggested by Przyklenk and coworkers[310] after an increase in the number of bacterial colony-forming units was found in sputum of subjects with CF. Hordvik and associates[179] and Efthimiou and coworkers[114] noted that patients with CF and severe pulmonary disease recovered slowly from viral infection. The underlying severity of lung disease was crucial in predicting response to viral respiratory infection.

Nontypeable *Haemophilus influenzae*

H. influenzae frequently is the earliest pathogen isolated from patients with CF, and it is most prevalent in the CF population during the first 5 years of life. The advent of the *H. influenzae* vaccine has not had an impact on the prevalence of this organism because patients with CF harbor nontypeable strains. Generally, *H. influenzae* is susceptible to a wide variety of agents and is amenable to treatment. However, recent studies suggest that more than 20 percent of young patients with CF are infected with *H. influenzae*, with some antibiotic resistance detected in current strains.[55] Despite the frequent recovery of this organism from infants and children, the impact of this organism on the clinical course of CF has been difficult to assess because of the frequent occurrence of coinfection with other pathogens.

Staphylococcus aureus

Methicillin-susceptible *S. aureus* (MSSA) and MRSA are increasing in prevalence. Patients generally retain the same clone of MSSA for their lifetime.[38,356] Data from the Cystic Fibrosis Patient Registry showed that the overall prevalence of MSSA increased from 37 percent in 1995 to 67 percent in 2010.[88] Similarly, MRSA increased from 0.1 percent in 1995 to 25.7 percent in 2010, although the prevalence varied substantially from center to center (range per center, 5% to 50%). The causes for this variability are unclear but could reflect different laboratory practices, different clinical practices, or regional differences in community-acquired MRSA. Although MRSA generally infects older patients, it usually is persistent[323] and can be associated with deterioration in both pediatric and adult patients.[255,407] A more recent report compared the outcomes of patients with MSSA versus MRSA and showed that children (<18 years of age) and adults infected with MRSA had worse lung function, more hospitalizations, and more antibiotic use.[323]

Recent hospitalization has been shown to be a risk factor for MRSA, suggesting nosocomial acquisition,[152] but the relative importance of community-acquired MRSA strains (mecA type IV and V) has not been fully elucidated. A more recent case series has suggested that community-acquired MRSA strains expressing Panton-Valentine leukocidin can be associated with increased morbidity, including focal pulmonary infiltrates with cavitary lesions and a greater decline in lung function.[116]

Pseudomonas aeruginosa

P. aeruginosa is the most common and important pathogen in patients with CF. Initial strains are nonmucoid, antibiotic-susceptible strains that express pili, flagella, and more highly acylated lipid A component of lipopolysaccharide.[161] *P. aeruginosa* also produces virulence factors, including exotoxin A, exoenzyme S, leukocidin, phospholipase C, elastase, and alkaline protease, which contribute to the pathogenesis of sepsis, acute lung infections, and bacteremia.[57,377,402] These factors may be chemotactic stimuli for neutrophils, and exotoxins may increase the viscosity of secretions and impair ciliary clearance and cause small airway obstruction and ultimately lung destruction.

Over time, *P. aeruginosa* adapts to the CF lung and undergoes genetic and phenotypic alterations. *P. aeruginosa* initially attaches to solid surfaces (e.g., mucin or respiratory epithelial cells), using flagella and type IV pili.[78] Attachment activates genes that synthesize extracellular polysaccharide (alginate),[99] which confers the mucoid phenotype of *P. aeruginosa* unique to chronic infections in CF.[160] Strains associated with chronic infections lack pili, lack flagella, and undergo structural changes in lipopolysaccharide. These changes may render *P. aeruginosa* more resistant to host defenses, including defensins and the innate inflammatory response. The *lasR-lasI* system (quorum-sensing genes) promotes the initial formation of microcolonies, which differentiate into alginate-encased mature biofilms.[78] Bacteria in biofilms avoid ciliary clearance, evade phagocytosis, and are antibiotic-resistant.[368] Biofilms are the proposed mechanism by which *P. aeruginosa* is able to infect the CF airway chronically and avoid eradication by host defenses and by antimicrobial agents. The CF lung can harbor very high concentrations of *P. aeruginosa*—10^8 to 10^9 organisms per gram of sputum may be present.[450]

In 2010, the CF Registry reported that 35.3 percent of patients with CF younger than 18 years of age were infected with *P. aeruginosa*.[88] Infection with *P. aeruginosa* is associated with increased morbidity and mortality rates caused by recurrent pulmonary exacerbations and a gradual deterioration in lung function.[202,293] Children who are infected with *P. aeruginosa* are more likely to have cough and lower chest radiograph scores than are uninfected children.[183] Investigators have shown that infants younger than 2 years of age infected with *S. aureus* and *P. aeruginosa* have worse pulmonary function, chest radiograph scores, and 10-year survival rates than do uninfected children.[3] The mucoid phenotype of *P. aeruginosa* is associated with a more rapid decline in lung function.[294] Multidrug-resistant *P. aeruginosa* (MRPA) was identified in 19 percent of all patients with CF in 2010. MRPA isolates are defined as resistant to all antibiotics evaluated in two or more of the following groups: aminoglycosides, fluoroquinolones, and β-lactams.[221] Infection with MRPA as opposed to *P. aeruginosa* has been shown to cause more rapid decline in pulmonary function[220] and increasing the likelihood of death or lung transplantation.[223] Additional studies have linked poor patient prognosis to a specific MRPA strain, as in the cases of the Liverpool epidemic strain and the Australian epidemic strains.[5,113,277,279]

Burkholderia cepacia Complex

Infection with *B. cepacia* complex and the associated "cepacia syndrome" was reported first in 1979 among adolescent Canadian patients with CF.[188,397] The cepacia syndrome is characterized by a virulent course, high fevers, bacteremia, rapid deterioration in lung function, and early death. Generally, patients with CF do not have bacteremia caused by other pathogens. Different clinical courses can be associated with *B. cepacia* complex, however, including transient colonization, a gradual decline in lung function, or the virulent cepacia syndrome.

Through a series of genetic and phenotypic studies, researchers have discovered that *B. cepacia* is actually a complex of several different species, previously called *genomovars*, which are indistinguishable phenotypically but distinguishable genotypically.[69] The genomovars of the *B. cepacia* complex are shown in Table 25-4, but additional genomovars are likely to be described.[69,422,423,430] Several international investigators have described the epidemiology and clinical courses associated with different genomovars, although these studies usually involved a single strain and might not be generalized to all strains of a given genomovar.[226,235,383] Most CF isolates are *Burkholderia cenocepacia* (genomovar III) or *Burkholderia multivorans* (genomovar II). The former may be associated with a more rapidly progressive clinical course,[230] whereas *B. multivorans* is more likely to be associated with transient colonization.[235] Patient-to-patient transmission and clinical deterioration can be associated with *B. multivorans*, however.[226] Most recently, an outbreak of *Burkholderia dolosa* (genomovar VI) occurred, and this newly described genomovar was associated with increased morbidity and mortality rates.[196]

Virulence factors for *Burkholderia* spp. include multidrug resistance, the ability to form biofilms, the ability to reside intracellularly, and the ability to spread from patient to patient potentially via the cable pilus[393] or the *B. cepacia* epidemiologic strain marker.[235] Many outbreak

TABLE 25-4 **Members of the *Burkholderia cepacia* Complex**

Species (Genomovar)	Binomial Designation
I	*B. cepacia*
II	*B. multivorans*
III	*B. cenocepacia*
IV	*B. stabilis*
V	*B. vietnamiensis*
VI	*B. dolosa*
VII	*B. ambifaria*
VIII	*B. anthina*
IX	*B. pyrrocinia*

strains do not express these transmission factors, however. Studies are ongoing to unravel potential environmental reservoirs of *Burkholderia* spp.

Stenotrophomonas Maltophilia

S. maltophilia, an intrinsically multidrug-resistant, gram-negative bacillus, is a well-known hospital-acquired pathogen in non-CF patients and is isolated with increasing frequency from the respiratory tract of patients with CF. The overall prevalence of this organism in patients with CF is 13.8 percent (range 0 to 27% in individual centers).[88,97] In a multicenter, randomized, placebo-controlled study of aerosolized tobramycin conducted in 520 patients 6 years of age and older with CF, the baseline prevalence of *S. maltophilia* determined by a core laboratory was 10.7 percent,[46] whereas the national rate among comparably aged patients reported to the Cystic Fibrosis Patient Registry was 2.9 to 3.9 percent, suggesting that many clinical laboratories were failing to identify this potential pathogen. Transient colonization also seems to be a common occurrence; Demko and colleagues[97] reported that 50 percent of patients with CF at their center had only a single positive culture for this microorganism. Increased use of antibiotics has been shown to be a potential risk factor for acquisition of *S. maltophilia*.[241,400]

The role of this organism as a pathogen in CF is still being investigated. Case-control studies have not shown that *S. maltophilia* has a significant impact on lung function or mortality.[156,241] In contrast, Demko and colleagues[97] found that the 5-year survival of patients with *S. maltophilia* ($n = 211$) with severe lung function at initial isolation of this organism was 40 percent in contrast to patients without *S. maltophilia* ($n = 471$), whose 5-year survival was 72 percent.

Achromobacter xylosoxidans

The clinical significance of *A. xylosoxidans* in CF also is unclear. In 1996, the first year this organism was reported to the Cystic Fibrosis Patient Registry, 2.7 percent of patients harbored this multidrug-resistant, gram-negative bacillus.[87] In 2010, *A. xylosoxidans* was found in 6.2 percent of patients.[88] As described for *S. maltophilia*, however, the nationally reported prevalence most likely is underestimated; 8.7 percent of participants in the aerosolized tobramycin trial had positive cultures for *A. xylosoxidans*.[46] Further complicating the epidemiology of this potentially emerging pathogen are the observations that *A. xylosoxidans* may be misidentified as some other non–lactose-fermenting, gram-negative bacilli, and that *P. aeruginosa*, *B. cepacia* complex, and *S. maltophilia* may be misidentified as *A. xylosoxidans*.[347] In addition, the impact of this organism is difficult to assess fully because *A. xylosoxidans* generally is cultured from patients concomitantly infected with other CF pathogens, especially *P. aeruginosa*,[219] but an association has been found between *A. xylosoxidans* and pulmonary exacerbations.[110,124]

Aspergillus Species and *Scedosporium* Species

Patients with CF frequently are colonized with *Aspergillus* spp.; 24.5 percent of participants in the inhaled tobramycin trial indicated they had positive cultures for this mold.[42] Oral and aerosolized antimicrobial agents are risk factors for colonization with *Aspergillus* spp.[22,315] Recent findings also confirmed the formation of biofilms and increased antifungal drug resistance by *A. fumigatus*.[362]

Of patients with CF, 2 to 10 percent develop APBA, which can be associated with a dramatic loss of lung function.[117,146,243,267] The diagnosis of ABPA can be difficult to establish because of variability in the application of standardized diagnostic criteria, confusion regarding these criteria, and limited recognition by physicians. This immunologically mediated syndrome is marked by a brisk IgE response, specific antibody to *Aspergillus fumigatus*, peripheral eosinophilia, and symptoms of reactive airway disease. Short-lived pulmonary infiltrates may be noted on chest radiograph. Although *Aspergillus* spp. do not normally invade the parenchyma, the airways can become impacted with mucus-containing fibrin, eosinophils, and mononuclear cells, which can cause airway obstruction and bronchiectasis.[117]

A workshop sponsored by the Cystic Fibrosis Foundation sought to further understanding of the diagnostic criteria needed for ABPA in CF.[387] This committee established minimum criteria for the diagnosis, which included (1) new clinical deterioration not attributable to another cause; (2) IgE greater than 500 IU/mL; (3) immediate skin reactivity to *Aspergillus* antigen or IgE antibody to *A. fumigatus*; and (4) precipitants or IgG against *A. fumigatus*, new chest radiograph, or new chest CT findings.

Scedosporium spp. can be isolated from the lungs of patients with CF, but the clinical significance of this microorganism is unknown. In the aerosolized tobramycin trial, 2.4 percent of patients harbored saprophytic fungi,[46] and in a single center, 8.6 percent of 128 patients followed for more than 5 years were colonized or infected with *Scedosporium apiospermum*.[65] Although previous studies implicated *S. apiospermum* in ABPA-like reactions,[65] additional selective media beyond routine culture methods would be necessary to recover most *Scedosporium* spp. and obtain more accurate infection rates.[29]

Nontuberculous Mycobacteria

Since the early 1990s, appreciation has been increasing that NTM may be pathogens in patients with CF. Olivier and colleagues[288] conducted a natural history study of NTM in patients with CF in 21 CF centers from 1993 to 1997. Using standardized mycobacteriologic methods,[441] these investigators found that the overall prevalence of colonization and infection with NTM was 13 percent. *Mycobacterium avium* complex (MAC) and *Mycobacterium abscessus* were most common, representing 72 percent and 16 percent of isolates, respectively. Patients with NTM were older and had better lung function, a lower prevalence of *P. aeruginosa*, and a higher prevalence of *S. aureus* than patients without NTM,

suggestive of a "healthy survivor effect." Risk factors for NTM may include geographic proximity to large bodies of water,[288] ABPA, and steroid therapy.[271]

In an individual patient, determining if NTM is colonizing the lung or causing disease can be difficult. Signs and symptoms of mycobacterial disease are nonspecific and may be consistent with CF pulmonary exacerbations. Radiographic signs generally are nonspecific, but small nodules in the lung periphery or progressive "tree and bud" lesions may be noted on high-resolution CT scan.[286] Patients with positive acid-fast bacillus smears and two or more positive cultures are more likely to be infected than colonized with NTM.[7] Patients harboring *M. abscessus* are more likely to fulfill the American Thoracic Society diagnostic criteria for NTM disease than are patients harboring MAC.[162,287] In a study to assess the clinical impact of NTM, 60 incident-case patients with positive NTM cultures were followed for 15 months and did not have increased morbidity in contrast to control patients with negative cultures.[287] Because this was a short duration of follow-up, further studies are needed to determine if NTM causes progressive deterioration in lung function and, if so, the optimal therapy. More recent studies focusing on *M. abscessus* have also failed to identify risk factors for infection.[431]

TREATMENT OF PATHOGENS IN CYSTIC FIBROSIS PATIENTS

A cornerstone of CF care is the aggressive use of oral, intravenous, and aerosolized antibiotics. The increasing longevity of patients with CF has paralleled the development of effective antibiotics. Antibiotics may be used during several stages of CF lung disease: (1) to prevent acquisition of pathogens, most commonly MSSA; (2) to eradicate initial acquisition of pathogens, most commonly *P. aeruginosa*, in efforts to prevent or delay chronic infection; (3) to treat pulmonary exacerbations caused by classic CF pathogens; (4) to treat patients infected with *P. aeruginosa*, as long-term suppressive therapy; and (5) to treat emerging multidrug-resistant pathogens.

Prophylaxis to Prevent Acquisition of *Staphylococcus Aureus* and *Pseudomonas Aeruginosa*

Few studies have been conducted on antibiotic prophylaxis in patients with CF, and these studies have focused mostly on *S. aureus*. The rationale for this strategy is to prevent *S. aureus* infection and to delay acquisition of *P. aeruginosa*.[371,372] From 1985 to 1992, British investigators studied 42 newly diagnosed infants randomly assigned to 12 months of flucloxacillin versus standard care.[23,436] Infants treated with flucloxacillin had fewer infections with *S. aureus* and fewer hospitalizations, but both groups had similar pulmonary function. In a placebo-controlled trial conducted in the United States, 119 patients newly diagnosed with CF (mean age, 16 months) were randomly assigned to receive cephalexin or placebo for 5 to 7 years.[390] No significant differences were found in pulmonary function, the number of pulmonary exacerbations, nutritional status, or chest radiograph scores in the two groups. In contrast, patients treated with cephalexin had decreased incidence of infection with *S. aureus* but increased incidence of infection with *P. aeruginosa*. Similarly, an analysis of the German national database showed that patients receiving antistaphylococcal agents had increased acquisition of *P. aeruginosa* and no improvement in lung function.[317]

Antistaphylococcal prophylaxis, although practiced in the United Kingdom in infants, has not been widely endorsed in the United States because of concerns about the emergence of resistance, the increased risk for acquiring *P. aeruginosa*, and the lack of impact on lung function. The different findings in these studies may reflect the different agents studied because cephalexin has a broader spectrum of activity than flucloxacillin, different durations of therapy, or both. In an era of increasing prevalence of MRSA, strategies to prevent MSSA may prove less useful.

A 3-year study in the Netherlands was undertaken to establish prospectively the effect of cycled prophylactic treatment of 3-monthly 3-week treatments with oral ciprofloxacin and inhaled colistin or placebo on prevention of initial *P. aeruginosa* infection in 65 children with CF. No difference was found in acquisition of *P. aeruginosa* infection between the control and treatment groups (annual incidence, 14% vs. 11%). Chronic infection was observed in 19 percent of controls and 12 percent of treated patients. Decline in pulmonary function and other clinical outcomes did not differ between the two groups. Anti-*Pseudomonas* antibodies emerged earlier in the control group, but this difference had disappeared after 3 years. In the treatment group, significantly fewer gram-positive bacteria but more non-*P. aeruginosa* nonfermentative gram-negative bacteria were isolated, suggesting caution and judicious use of antimicrobial agents. At present, no recommendation exists on use of anti-*Pseudomonas* prophylaxis in children with CF.[412]

Aerosolized recombinant human deoxyribonuclease (rhDNase I [Pulmozyme]) has been shown to increase pulmonary function and reduce exacerbations of respiratory symptoms in CF patients with moderate-to-severe reduction in pulmonary function. A study in Denmark analyzed whether aerosolized rhDNase could reduce the number of bacterial infections in the lower respiratory airways of CF patients without chronic pulmonary infection over 1 year. Overall, the number of positive cultures was significantly higher in the untreated group (82%) than the treated group (72%) The most striking difference was found for *S. aureus*, with a prevalence of 30 percent in the untreated group in contrast to 16 percent in the treated group. Whether the mechanism for decreased infection rate is from an anti-inflammatory affect, improved mucociliary clearance of sputum, or other unknown mechanism has not been elucidated.[138]

Early Eradication of *Staphylococcus aureus* and *Pseudomonas Aeruginosa*

An increasingly practiced therapeutic strategy is to use antibiotics to eradicate initial acquisition of *P. aeruginosa*

and prevent or delay chronic infection. This strategy was described first in Europe at the Danish Cystic Fibrosis Center.[136,191,395,416,425] The rationale for this approach is that antibiotics may be effective at eradicating initial infection and colonization with *P. aeruginosa* because the organism burden is low, organisms are largely susceptible, and a biofilm has not yet been established.

In some CF centers in Europe, colistin and ciprofloxacin are administered every 3 months after the initial isolation of *P. aeruginosa* has occurred.[136] In contrast to historic controls, patients treated with this approach had improved lung function, improved survival rates, decreased prevalence of *P. aeruginosa*, and increased resistance to the therapeutic regimen.[395] In Australia, investigators used intravenous antibiotics followed by ciprofloxacin or aerosolized agents and found that 6/24 children no longer had *P. aeruginosa* isolated for 12 months or longer.[14] In contrast to children treated with placebo, children treated with inhaled tobramycin within 7 to 12 weeks of initial infection and colonization with *P. aeruginosa* had shorter time to conversion from a positive to negative culture.[443] All 8 patients treated with inhaled tobramycin compared with 1/13 patients treated with placebo had successful eradication of *P. aeruginosa*.[148]

Despite the concerns about the potential emergence of antimicrobial resistance and lack of long-term studies that show the durability of eradication or an improvement in lung function, the growing consensus has been that early eradication for *P. aeruginosa* has merit. Two large, placebo-controlled studies have assessed the efficacy of different eradication regimens using different durations of tobramycin inhaled solution (TIS) with and without oral ciprofloxacin. Neither of these trials compared an active regimen with placebo—evidence that early eradication has been endorsed by CF experts. The EarLy Inhaled Tobramycin for Eradication (ELITE) study was designed to assess the efficacy and safety of two regimens (28 and 56 days) of TIS twice daily for treatment of early-onset *P. aeruginosa* infection in 88 children ages 6 months of age and older with CF. The median time to recurrence of *P. aeruginosa* (any strain) was similar between the two groups. In total, 93 and 92 percent of the patients were free of *P. aeruginosa* infection 1 month after the end of treatment and 66 and 69 percent remained free at the final visit in the 28-day and 56-day groups, respectively.[318]

A second study called Early Pseudomonas Infection Control (EPIC) was performed between 2004 and 2009 in 304 children, ages 1 to 12 years, within 6 months of first culture positivity to *P. aeruginosa*. This complex study randomized participants to one of four antibiotic regimens for 18 months (six 12-week quarters). Participants who were randomized to cycled therapy received TIS for 28 days, with oral ciprofloxacin or oral placebo for 14 days every quarter, and participants randomized to culture-based therapy received the same treatments only during quarters with positive *P. aeruginosa* cultures. There were no statistically significant differences in exacerbation rates between cycled and culture-based groups or ciprofloxacin and placebo. Treatment-emergent resistance to tobramycin or ciprofloxacin in *P. aeruginosa* was very infrequent (<1% of isolates).[413]

Despite wide use of oropharyngeal cultures to identify pulmonary infection, concerns remain over their diagnostic accuracy. The Australasian Cystic Fibrosis Bronchoalveolar Lavage (ACFBAL) randomized controlled trial recruited 170 infants diagnosed with cystic fibrosis through newborn screening programs in eight Australasian cystic fibrosis centers to study whether BAL-directed therapy for pulmonary exacerbations during the first 5 years of life provides better outcomes than current standard practice relying on clinical features and oropharyngeal cultures. The BAL-directed therapy group underwent BAL before 6 months of age when well; when hospitalized for pulmonary exacerbations, if *P. aeruginosa* was detected in oropharyngeal specimens; and after *P. aeruginosa* eradication therapy. Treatment was prescribed according to BAL or oropharyngeal culture results. The primary outcomes at 5 years of age were prevalence of *P. aeruginosa* on BAL cultures and total cystic fibrosis CT (CF-CT) score (as a percentage of the maximum score) on high-resolution chest CT scan. At 5 years of age, 8/79 children (10%) in the BAL-directed therapy group and 9/76 (12%) in the standard therapy group had *P. aeruginosa* in final BAL cultures. There was no difference in the mean total CF-CT score for the BAL-directed therapy and standard therapy groups (3.0% and 2.8%, respectively). In children with cystic fibrosis, BAL-obtained cultures did not offer any advantage in directing therapy nor result in a lower prevalence of *P. aeruginosa* infection or lower total CF-CT score in contrast to standard therapy at 5 years of age.[434]

Finally, patients with cystic fibrosis are becoming increasingly infected with MRSA as opposed to MSSA. There is some accumulating evidence that patients with MRSA show a more rapid deterioration in their degree of obstructive disease as measured by forced expiratory volume in 1 second (FEV_1) and that MRSA infection is independently associated with worse outcomes in cystic fibrosis.[80,92,157,364]

More longitudinal data are needed to understand how MRSA colonization and infection affect the course of disease in CF and whether eradication is possible. Currently few clinical data exist regarding approaches to MRSA eradication. Several investigators in Europe have published small case series of various regimens for eradication of MRSA that have included oral agents (e.g., rifampin and fusidic acid) and inhaled agents (e.g., vancomycin).[144,234,237,376] Randomized treatment trials must be performed to establish the safety and efficacy of these strategies.

Close microbiologic monitoring of airway cultures is recommended based on current evidence that oropharyngeal cultures remain the optimal screening method in infants and young children, with routine BAL-obtained cultures unnecessary. Early treatment to eradicate *P. aeruginosa* is a well-accepted treatment strategy, with strong evidence that a 28-day course of therapy of judicious use of tobramycin inhalation solution is recommended; addition of ciprofloxacin provides no benefit.

Treatment of Pulmonary Exacerbations

Pseudomonas aeruginosa

Much effort has been put into standardizing and validating the definition of a pulmonary exacerbation in CF. However, no currently accepted definition has been adopted universally for use clinically, in quality improvement initiatives, or in clinical research. A combination of clinical signs and symptoms is used to define a pulmonary exacerbation in research trials, and similar factors are used in the clinical setting.

Mild exacerbations often are treated with oral ciprofloxacin[64,176] with or without an inhaled agent such as aztreonam, tobramycin, or colistin. Treatment trials supporting the use of inhaled antibiotics for management of an exacerbation are lacking, however.

Several pivotal trials have led to widely accepted principles for intravenous treatment of more severe pulmonary exacerbations. During the 1970s and early 1980s, placebo-controlled trials showed that participants in the placebo group had increased morbidity and mortality rates in contrast to participants treated with intravenous antibiotics.[153,184,442] Hospitalized participants treated with bronchodilators and chest physiotherapy alone had less improvement in lung function and less reduction in bacterial density in contrast to patients treated with these interventions plus a β-lactam and an aminoglycoside agent.[321] Treatment with a β-lactam (azlocillin) and an aminoglycoside (tobramycin) agent leads to a significant reduction in bacterial density and a longer time to readmission for a new exacerbation in contrast to treatment with azlocillin alone.[369]

Studies also compared have various agents, singly or in combination. Most studies enrolled small numbers of patients and concluded that the comparative treatment regimens were equivalent in efficacy, but the studies were insufficiently powered to detect differences.* Many studies showed the emergence of resistance to study drug at the completion of therapy, which did not correlate with clinical response to treatment.[192,292] A larger multicenter trial randomly assigned patients undergoing a pulmonary exacerbation to ceftazidime and tobramycin (n = 52) or meropenem and tobramycin (n = 50).[28] Participants in the meropenem-treated group had a greater improvement in lung function than participants in the ceftazidime-treated group mean FEV_1 percent predicted (38.8% vs. 29.4%). An additional study in 118 patients in Germany compared ceftazidime and tobramycin to meropenem and tobramycin as treatment of a pulmonary exacerbation. Both treatment groups improved lung function measures and demonstrated reductions in bacterial sputum burden and acute phase reactant levels; no differences between treatment groups were observed.[222]

The advantage of alternative dosing schedules of time-dependent antimicrobials, by extended or continuous infusions, have theoretical advantages in the chronic treatment of *P. aeruginosa* in the CF airway. Certain antibiotics, such as β-lactams (i.e., penicillins, cephalosporins, carbapenems, and monobactams), clindamycin, macrolides (i.e., erythromycin and clarithromycin), and oxazolidinones (i.e., linezolid), can be effective because of the extensive amount of time the antibiotic binds to the microorganism. Few studies have been performed in the CF population to determine the safety and efficacy of extended or continuous infusion. A large multicenter, randomized cross-over study in France compared infusions of thrice-daily short infusions or daily continuous infusion of ceftazidime in 70 patients with CF and chronic *P. aeruginosa* infection; all patients were also treated with intravenous tobramycin. The primary end point was the variation in the FEV_1 during the course of antibiotic treatment. The improvement in FEV_1 at the end of therapy was not statistically different between the two treatment procedures (+7.6% after continuous infusion and +5.5% after short infusions) but was better after continuous ceftazidime treatment in patients harboring resistant isolates. The interval between the courses of antibiotic treatments was longer after the continuous infusion than after the short infusion of ceftazidime. The susceptibility profiles of the *P. aeruginosa* isolates remained unchanged and were similar for both regimens. Quality-of-life scores were similar whatever the treatment procedure, but 82 percent of the patients preferred the continuous-infusion regimen. Further studies are needed to demonstrate the safety and efficacy of continuous infusion of other antimicrobial agents.[182]

Finally, examination of the safety and efficacy of single daily dosing of tobramycin versus multiple daily dosing during a pulmonary exacerbation showed that single daily dosing was associated with the same efficacy and reduced nephrotoxicity; in children, the mean percentage change in creatinine in the single daily dosing group was less than the mean change in the thrice-daily dosing group (4.5% vs. 3.7%).[373]

Accepted treatment of a pulmonary exacerbation caused by *P. aeruginosa* consists of two parenteral agents from different antibiotic classes to provide synergy and delay the emergence of resistance.[147,266,313,349] Most commonly, a β-lactam agent with activity against *P. aeruginosa*, such as piperacillin or ceftazidime, is combined with an aminoglycoside agent, generally tobramycin. If a patient is coinfected with another pathogen, such as *S. aureus*, additional agents are added. Treatment trials for pulmonary exacerbations caused by other CF pathogens are unavailable, however.

Antibiotic dosages must be higher, more frequent, or both in patients with CF because the volume of distribution and clearance are increased in CF.[95] Treatment is administered for 10 to 21 days. Treatment outcomes include improved lung function; improved well-being, improved quality of life, or both; and a reduction in organism burden. Eradication of pathogens does not occur, however.[291] The Epidemiologic Study of Cystic Fibrosis analyzed the relationship between pulmonary function and treatment strategies for pulmonary exacerbations; centers with patients in the upper quartile of pulmonary function treated pulmonary exacerbations

*References 32, 89, 154, 189, 215, 252, 298, 354, 361.

more frequently than did centers in the lower quartile.[261]

Inevitably, *P. aeruginosa* develops increasing resistance to antibiotics. Molecular studies have confirmed that resistance generally develops in the infecting strains, rather than the acquisition of more resistant strains,[282] although superinfection with multidrug-resistant *P. aeruginosa* can occur as described subsequently.[245] Much interest has been generated in optimizing the treatment of pulmonary exacerbations caused by multidrug-resistant *P. aeruginosa* using in vitro synergy testing. Most commonly, checkerboard broth microdilution assays and multiple combination bactericidal testing have been studied.[221,349]

The activity of clinically achievable concentrations of pairs of antimicrobial agents with different mechanisms of action (e.g., a β-lactam agent paired with an aminoglycoside) have been tested.[349] To determine if a combination of agents is synergistic, a fractional inhibitory concentration can be calculated; synergy is defined as a fourfold reduction in the MIC of agents alone in contrast to agents in combination.[240] Most multidrug-resistant strains, defined as resistance to all agents in two or more classes of antibiotics,[343] can be inhibited by one or more combinations of agents, but the efficacy of this assay has not been validated in clinical trials. In contrast, a pivotal randomized trial to study the clinical efficacy of multiple combination bactericidal testing synergy studies was performed in Canada and Australian which patients undergoing a pulmonary exacerbation were randomly assigned to treatment guided by multiple significant differences in the time that elapsed until the next pulmonary exacerbation or improvements in lung function between the two treatment strategies were noted.

Recent studies using culture-independent molecular techniques, such as sequencing, high-throughput pyrosequencing, and phylogenetic microarray analysis, and anaerobic cultures have broadened our view of CF airway bacterial communities by comprehensively examining the airway microbiome in CF. The CF airway bacterial communities are highly complex, with anaerobes frequently present, and important evidence demonstrates that loss of community diversity and richness is associated with older age and decreased lung function in CF. Bacterial communities are also likely influenced by antibiotic use, chronic *P. aeruginosa* infection, host genetic background (del508F CFTR mutation), and geographic variations. Quantitative anaerobic cultures also detect high quantities of anaerobes from CF airway samples, including during pulmonary exacerbations. The effect of antimicrobial therapy on the airway microbiome needs further investigation. In addition, probiotic approaches have been recently studied; whether probiotics act by altering microbial communities or by modulating host inflammatory response is unknown. Complex bacterial communities, including traditional CF-associated pathogens and anaerobic bacteria, are common in CF airways. Novel therapeutic approaches aimed at modulating airway bacterial communities may lead to improved treatment of CF lung disease.[460]

Conventional antibiotic susceptibility often results correlate poorly with clinical outcomes in patients with CF. A multicenter randomized pilot trial was conducted to assess the efficacy and safety of using biofilm susceptibility testing *of P. aeruginosa* isolates to guide antibiotic regimens for chronic airway infections in clinically stable adolescent and adult CF patients. Thirty-nine participants were randomized to biofilm or conventional treatment groups; 14-day courses of two antibiotics were selected according to an activity-based algorithm using the corresponding susceptibility results. Meropenem was most active against biofilm-grown bacteria and was included in regimens for about half of each study group. Microbiologic and clinical responses in patients with biofilm testing did not differ significantly from patient regimens based on conventional testing. The predictive value of biofilm testing may nonetheless warrant evaluation in an adequately powered clinical trial in younger CF patients or those experiencing acute pulmonary exacerbation.[264]

CF experts have not arrived yet at a consensus regarding the efficacy of inpatient versus outpatient management of pulmonary exacerbations. Outpatient management has advantages; it is less costly, is less disruptive to patients and their families, and involves less risk for acquiring nosocomial pathogens. Patients treated at home were shown, however, to have longer treatment courses and less improvement in lung function.[273] Patients may improve more with hospitalization, as a result of better compliance with antibiotics, bed rest, chest physiotherapy, and bronchodilator treatments.[34]

Staphylococcus aureus *and Methicillin-Resistant* Staphylococcus aureus

The clinical utility of antistaphyloccocal agents is best shown by early studies of infants with CF treated with penicillin before the nearly universal acquisition of β-lactamases by *S. aureus*.[9,253] Infants treated during the antibiotic era had markedly improved survival rates. Experts advocate the use of a first-generation cephalosporin (e.g., cephazolin) or a semisynthetic penicillin (e.g., oxacillin) for treatment of a pulmonary exacerbation associated with MSSA.[147] Treatment trials justifying this approach are lacking, however.

Vancomycin generally is advocated for treatment of MRSA when this organism is considered a pathogen. Although no treatment trials in CF patients using other anti-MRSA antimicrobial agents (i.e., clindamycin, trimethoprim-cotrimoxazole, rifampin, or linezolid) have been conducted, a case series showed that adults with CF required 600 mg of linezolid every 8 hours to provide desired pharmacokinetics.[33,353] Linezolid-resistant MRSA did emerge in a child with CF treated for approximately 3 months.[140] A small study of eight adults with CF was randomized to receive intravenous and oral linezolid for nine doses in a cross-over design with a 9-day washout. Population pharmacokinetic analyses were performed, and at the steady state, the twice-daily dose produced 93.0 and 87.2 percent probabilities of obtaining the target pharmacodynamic exposure against the MRSA isolates for the intravenous and oral formulations, respectively. Thrice-daily dosing increased the probabilities to 97.0 and 95.6 percent, respectively. This study concluded that

standard intravenous and oral dosing regimens should be sufficient to reliably attain pharmacodynamic targets against most MRSA isolates; however, more frequent dosing may be required for isolates with MICs of 2 μg/mL or greater.[199]

Because linezolid is available orally and intravenously, the potential for "abuse" exists if clinicians do not use this agent judiciously.

Burkholderia cepacia *Complex*

Management of *B. cepacia* complex is more problematic because of higher levels of intrinsic antibiotic resistance and a paucity of clinical trials for this pathogen. Initial isolates may be susceptible to ciprofloxacin, β-lactam antibiotics, chloramphenicol, trimethoprim-sulfamethoxazole, meropenem, and minocycline, but with the exception of *Burkholderia gladioli*, all *Burkholderia* spp. are resistant to aminoglycosides. Temocillin has been used in Europe to treat pulmonary exacerbation caused by *B. cepacia*.[406] Resistance can develop, however, which severely limits therapeutic options. As described for the management of *P. aeruginosa*, combinations of two or three agents are used to treat a pulmonary exacerbation. Even more prolonged courses may be required to improve lung function. Meropenem and minocycline were noted to have the most in vitro activity against 652 multidrug-resistant *Burkholderia* strains isolated from patients with CF and inhibited 28 percent and 33 percent of strains.[232] Treatment may be guided by two or more drug combinations with an evident improvement in pulmonary function.[1,465]

Stenotrophomonas maltophilia *and* Achromobacter xylosoxidans

No published treatment trials have been conducted for patients with CF who are harboring *S. maltophilia* or *A. xylosoxidans* and are undergoing an exacerbation. Although definitive data confirming that these organisms are pathogens in CF are unavailable, clinicians target these multidrug-resistant, gram-negative bacilli if they are consistently recovered from the respiratory tract of an individual patient. At present, the Clinical Laboratory Standards Institute recommends testing ticarcillin-clavulanate, ceftazidime, minocycline, chloramphenicol, trimethoprim-sulfamethoxazole, and levofloxacin against *S. maltophilia*,[67] but CF strains frequently are resistant to these agents.[352]

Nonetheless, similar strategies are used to treat these organisms as described for *P. aeruginosa* or *B. cepacia* complex; two or more parenteral agents are chosen based on susceptibility testing and given for 2 to 3 weeks. In a survey of 263 isolates of *S. maltophilia* from 218 patients with CF isolated from 1995 to 1998, doxycycline, ticarcillin-clavulanate, piperacillin-tazobactam, and trimethoprim-sulfamethoxazole were most active and inhibited 78 percent, 39 percent, 18 percent, and 13 percent of isolates, respectively.[352,465] A survey of 94 *A. xylosoxidans* isolates from 77 patients showed that meropenem and imipenem, or piperacillin with or without tazobactam, were most active.[347]

Nontuberculous Mycobacteria

Treatment of NTM in patients with CF is challenging and guided by the clinical presentation and the mycobacterial species. Before therapy for NTM is initiated, patients should be treated aggressively for the classic CF pathogens they harbor to determine if they clinically improve without the need for specific NTM treatment. If NTM treatment is initiated, a careful history, physical examination, and pulmonary function tests should be done to determine the baseline status of the patient in efforts to monitor response to therapy. Susceptibility testing for NTM is not done routinely for patients who do not have CF. Given the increased antibiotic exposure in CF and the potential for the emergence of resistance, NTM strains isolated from patients with CF should undergo susceptibility testing at a reference laboratory. Treatment can be guided by the initial susceptibility profile.

No treatment trials have been published for NTM in CF, and generally regimens are comparable to those used in patients without CF. Treatment of MAC is straightforward and includes a macrolide agent, rifabutin, and ethambutol. Treatment of *M. abscessus* is more complex. Many experts have a lower threshold for treating *M. abscessus*, given the greater apparent virulence of this species in CF, including after lung transplant.[59] Some experts recommend oral therapy, including a macrolide and linezolid because both have excellent in vitro activity, for stable patients.[40] Some experts add vitamin B_6 to prevent peripheral neuropathy, but the pathogenesis of peripheral neuropathy from linezolid is unknown. For more symptomatic patients, intravenous therapy, including cefoxitin or a carbapenem, depending on susceptibilities and amikacin, is recommended. Such patients need to have their hearing assessed regularly. Because of the potential for different pharmacokinetic properties in CF and the fact that oral agents may not be well absorbed by patients with CF, serum levels for antimycobacterial agents should be strongly considered.[151,301]

Patients should be followed closely and have monthly sputum testing done for acid-fast bacillus smear and culture. The response to treatment generally is good for patients infected with MAC. Although the optimal duration of treatment has not been studied, experts recommend continuing therapy for 1 year after negative sputum cultures have been obtained. Cure for *M. abscessus* is less likely to occur, and the therapeutic goal may be long-term suppression. After clinical and mycobacterial improvement (i.e., a reduction in organism burden) has been achieved, intravenous antibiotics can be stopped and prolonged oral therapy with a macrolide can be initiated.

Allergic Bronchopulmonary Aspergillosis

Treatment of ABPA also can be very challenging. Although steroids are the treatment of choice because ABPA is immunologically mediated, the response to steroids varies and may have the undesirable consequence of diabetes in this vulnerable patient population. The oral antifungal therapies voriconazole[174] and itraconazole[274]

have been used with reported response and toxicity, but no controlled trials have been performed.[266] Monitoring serum levels of itraconazole is desirable because this agent may be malabsorbed if the gastric pH is nonacidic.

Long-Term Suppressive Therapy

Inhaled Antibiotics. Long-term suppressive antibiotic therapy has been used increasingly to treat patients with CF and infected with *P. aeruginosa* to prolong the time between pulmonary exacerbations and slow the progression of lung deterioration. Numerous oral agents that are not cidal for *P. aeruginosa* (e.g., amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, or tetracycline agents) are used empirically, sometimes in rotation, with anecdotal support coming from patients and physicians. The use of oral antibiotics is widespread; during a 6-month period, 90 percent of patients received at least one course of oral antibiotics.[46] No clinical trials support this practice, however, and it may contribute to antibiotic resistance.

For decades, inhaled agents such as tobramycin, colistin, gentamicin, amikacin, and various β-lactam antibiotics, including carbenicillin and cephaloridine, also have been used for management of chronic infections.[48] Definitive data supporting the use of inhaled agents were derived from a multicenter phase III, double-blind, placebo-controlled, randomized treatment trial of inhaled tobramycin (TOBI) in patients with CF and chronic *P. aeruginosa* infection.[315] Patients received 300 mg of tobramycin twice daily every other month for 6 months. The rationale for administering therapy every other month was to delay the emergence of resistance, to reduce cost, to increase compliance, and potentially to exploit the postantibiotic effect of aminoglycosides. Subjects randomly assigned to inhaled tobramycin had a 10 percent improvement in FEV_1 in contrast to subjects who received placebo, who experienced a 2 percent decline in FEV_1. Treated patients also had a reduction in bacterial density, fewer days of hospitalization, and fewer days of intravenous antibiotics. In subset analyses, adolescents had the greatest improvement in pulmonary function. During treatment, patients receiving tobramycin had increased tinnitus that was unassociated with hearing loss. Minimal systemic absorption of tobramycin occurred because serum levels of tobramycin generally were less than 1 μg/mL.

Although this study confirmed the efficacy of inhaled tobramycin in CF patients with chronic *P. aeruginosa* infection, several questions remain. In routine antimicrobial susceptibility testing, the breakpoint for resistance to tobramycin is MIC of 16 μg/mL or greater. The aerosol route delivers 100-fold higher concentrations of tobramycin without toxicity, however; the median concentration of tobramycin in the phase III trial was 1200 μg/g of sputum.[315] Although the conventional breakpoint for resistance to tobramycin is irrelevant for drug delivered by inhalation, the breakpoint for inhaled tobramycin is unknown. Commercially available susceptibility tests do not measure MICs greater than 8 μg/milk During the 6-month trial, resistance to tobramycin did occur; a trend was found toward higher MICs in the treatment group (median MIC, 2 μg/mL) in contrast to the placebo group (median MIC, 1 μg/mL). The same laboratory studied the prevalence of *P.* aeruginosa antibiotic resistance in 2008 and evaluated whether the prevalence of other CF pathogens has changed. In the decade since TIS licensure, antibiotic usage in the care of patients in CF has changed. The study recruited a contemporary cohort of CF patients meeting similar eligibility criteria as the 520 patients in the phase III trial of inhaled tobramycin (historical cohort). Sputum samples from 33 U.S. CF centers with a total of 656 *P. aeruginosa* isolates were identified from 253 culture-positive patients. Comparison between cohorts revealed an increase in the prevalence of patients with tobramycin-resistant (11.8% vs. 30.4%, $p < .001$) and amikacin-resistant (24.2% vs. 42.7%, $p < .001$) *P. aeruginosa* between 1995 and 2008. Prevalence of ciprofloxacin resistance was similar (34.4% vs. 33.6%, $p = .81$). Within the contemporary cohort, potential associations between recent antibiotic exposures and prevalence of *P. aeruginosa* resistance were examined; findings included that exposure to intravenous carbapenems was significantly associated with aztreonam resistance, meropenem resistance, and multidrug resistance ($p = .0002$, $p = .0003$, and $p = .0002$, respectively). Prevalence of *S aureus*, MRSA, *S maltophilia*, and *A xylosoxidans* was also increased in the contemporary cohort.[119]

Buoyed by the success of aerosolized tobramycin, other aerosolized agents have been developed. The effectiveness and safety of aztreonam lysine for inhalation in patients with CF on maintenance treatment for chronic *P. aeruginosa* airway infection and moderate-to-severe lung disease was evaluated in two 28-day treatment multicenter phase III, double-blind, placebo-controlled, randomized treatment trials. The 28-day treatments with aztreonam lysine significantly delayed need for inhaled or intravenous antipseudomonal antibiotics, improved respiratory symptoms and pulmonary function, and was well tolerated.[249,324] A third study was an international 18-month, open-label study that evaluated the safety and efficacy of repeated courses of aztreonam lysine for inhalation solution in patients with chronic *P. aeruginosa* infection and moderate-to-severe lung disease. With each course of aztreonam lysine, FEV_1 and scores on the Cystic Fibrosis Questionnaire–Revised Respiratory Symptom scale improved and bacterial density in sputum was reduced. In the 28 days off therapy, benefits waned, but weight gain was sustained over the 18 months. There were no sustained decreases in *P. aeruginosa* susceptibility. A dose response was observed; aztreonam lysine in patients treated thrice daily demonstrated greater improvements in lung function and respiratory symptoms. Clinical benefits in pulmonary function, health-related quality of life, and weight were observed with each course of therapy.[281]

An additional double-blind, multicenter, randomized, placebo-controlled trial of aztreonam lysine enrolled 157 patients with CF with chronic *P. aeruginosa* infection who had mild lung disease. Treatment effects favoring aztreonam lysine were larger for patients with baseline FEV_1 less than 90 percent of predicted in contrast to 90 percent

or greater of predicted. Aztreonam lysine was well-tolerated. The effects on respiratory symptoms were modest; however, FEV_1 improvements and bacterial density reductions support a possible role for aztreonam lysine in these relatively healthy patients.[433]

In 2010, aerosolized aztreonam lysine (now marketed as Cayston) was approved for long-term intermittent, 28 days on and 28 days off, suppressive therapy in patients with moderate-to-severe lung disease, with a dose of 75 mg three times per day. Aztreonam lysine is delivered via the Altera nebulizer system, a proprietary eFlow nebulizer device optimized with a short (<3 minutes) delivery time.

Many controversies surround inhaled antibiotics and include whether there is a role or benefit for (1) aerosolized antibiotics in the treatment of a pulmonary exacerbation with chronic *P. aeruginosa*, (2) continuous alternating therapy, (3) treatment of non-*Pseudomonas* chronic infection, or (4) alternative dosing regimens (e.g., 2 weeks on and 2 weeks off regimen, use of multiple inhaled antibiotics within the same month). A clinical trial comparing continuous-alternating therapy of aztreonam and TIS versus tobramycin as a 28 days on and 28 day off suppressive therapy, is currently underway. Recognizing that new treatment options for CF pulmonary infections are needed has led to the development and study of additional inhaled antimicrobials agents for suppressive therapy, which include inhaled levofloxacin,[145] dry powder ciprofloxacin, tobramycin inhalation powder, and amikacin. Aztreonam lysinate and amikacin for inhalation delivered via liposome formulations are being studied as a way to penetrate mucoid biofilms and prolong the residence time of the antibiotic in the lungs. Light, porous, dry-powder formulations are also in clinical trials to reduce delivery time. These new formulations and delivery systems promise to expand our armamentarium against microbes while reducing the time burden for patients. Guidelines and further studies are needed to guide their optimal and judicious use.

Macrolide Antibiotics. Much interest has been generated more recently in the use of macrolide agents in CF[180] as long-term suppressive therapy. The rationale for macrolide therapy in CF stems from the successful treatment of diffuse panbronchiolitis with erythromycin, azithromycin, and clarithromycin.[207,216] Diffuse panbronchiolitis is a chronic lung disease, diagnosed primarily in Asian adults, with several clinical features similar to those found in CF, including progressive lung disease caused by mucoid and nonmucoid strains of *P. aeruginosa*.[392] Long-term administration of low-dose macrolide agents to patients with diffuse panbronchiolitis has reduced morbidity and mortality rates.

In vitro studies have provided the scientific rationale for this clinical efficacy. Although macrolides are not cidal for *P. aeruginosa*, subinhibitory concentrations of macrolide agents reduce the production of several virulence factors by *P. aeruginosa*,[257,258] including the formation of biofilm.[187,209] Macrolide antibiotics also may have an anti-inflammatory effect and decrease cytokine production by neutrophils, monocytes, and bronchial epithelial cells.[186,456]

Six azithromycin trials have been conducted in patients with CF so far.*

Four trials were conducted in patients chronically infected with *P. aeruginosa*[122,195,346,449] and had similar results; all showed improvement in lung function using similar treatment regimens of azithromycin. Improvements in secondary outcomes, such as decreased hospitalization, decreased antibiotic use, and increased weight gain, also were noted. In a large study performed in 260 children and adolescents, with negative cultures for *P. aeruginosa*, pulmonary exacerbations occurred in 21 percent of the azithromycin group and 39 percent of the placebo group. Participants in the azithromycin group had a 50 percent reduction in exacerbations and an increase in body weight in contrast to placebo participants. Treatment with azithromycin for 24 weeks did not result in improved pulmonary function.[344] Cochrane review of azithromycin use in patients with CF provides evidence of improved respiratory function after 6 months of treatment. Data beyond 6 months were less clear, although reduction in pulmonary exacerbation was sustained and the studies concluded that short-term (i.e., 6 months) azithromycin seemed effective in CF, but long-term safety and efficacy remain unknown.[378,379] Patients treated with azithromycin should be screened for NTM before initiating therapy and annually thereafter because of concern about macrolide resistance in these microorganisms.

Lung Transplantation

Lung transplantation currently is considered a viable therapy for selected patients with end-stage pulmonary disease of CF.[457] Bilateral lung, heart and lung, and living donor lobar lung transplantation all have been performed in patients with CF, but most operations in recent years have used the bilateral lung transplant approach with lungs from deceased donors. By 2012, more than 3000 individuals with CF had undergone lung transplantation worldwide, with nearly 150 to 200 procedures performed annually as of 2012. The survival of lung transplant recipients with CF exceeds that of any other diagnostic group for all ages, with projected survival half-life of 6.2 years.[414] Patients with CF nonetheless present special challenges for successful lung transplantation, complicated by other comorbidities such as CF-related diabetes, gastroesophageal reflux, CF liver disease, and bone metabolism. The life-threatening manifestations of CF generally are limited to the lungs, except for patients with advanced liver disease with portal hypertension.[39] Advantages of a lung transplant recipient with CF include relative young age, giving the potential of many years of productive life ahead. In addition, patients with CF have experience with complex medical regimens.

The optimal time to refer potential candidates for evaluation for lung transplant is difficult to determine because the natural history of CF cannot be predicted precisely. In May 2005 in the United States, the United Network for Organ Sharing introduced a new system of

*References 66, 122, 195, 344, 348, 449.

lung distribution that assigns a lung allocation score to each potential candidate. Key parameters used in the lung allocation score include lung function testing, the need for oxygenation, the presence of diabetes mellitus, age, body mass index, the need for ventilatory support, hospitalization in the intensive care unit, and hemodynamic data from cardiac catheterization. Waiting time is usually not considered except in individuals younger than 12 years of age. There are no microbiologic criteria.[115]

Although transplanted lungs do not develop CF, they can become infected with the pretransplant pathogens because the trachea and paranasal sinuses continue to manifest the pathophysiology of CF. Lung transplant recipients with CF are at risk for development of infection via pretransplant microbial flora or newly acquired pathogens.[197,230,375,386] Controversy has ensued about whether microbiologic criteria, specifically pretransplant infection with fungi, NTM, or multidrug-resistant pathogens, should be considered contraindications to performing lung transplantation in patients with CF. Several centers have published their experiences with infections that developed from lung transplantation pathogens; they include invasive aspergillosis; sepsis with *S. maltophilia*, *B. cepacia* complex organisms, and *B. gladioli;* and sternal wound infection.[197] Other centers have reported that morbidity and mortality caused by *P. aeruginosa* infections after transplant are not higher in patients with CF than in patients who do not have CF.[135,278]

A more recent study indicated that lung transplant recipients with CF and with panresistant, gram-negative organisms other than *Burkholderia* spp. had significantly decreased survival rates after transplantation than a comparable group with antibiotic-sensitive organisms.[167] Patients with CF who are infected with *B. cenocepacia* have increased morbidity and mortality rates from recurrent infection, sepsis, or both after lung transplantation. Although Aris and colleagues[11] did not detect patient-to-patient transmission, increased mortality rates were noted with *B. cenocepacia* but not with multidrug-resistant *P. aeruginosa*. Patients infected with genomovar III had increased mortality rates after lung transplantation in contrast to patients infected with other genomovars before transplantation.[11]

Infection with *M. abscessus* before undergoing transplant can be associated with severe posttransplant complications and death.[62,405] Acquisition of NTM after transplant can be treated successfully with prolonged combination of antimicrobial therapy.[62,239] Patients with CF are at risk for developing invasive aspergillosis after transplant, but at lower rates than patients without CF.[278,295] Many of these patients with CF were colonized with *Aspergillus* spp. before undergoing transplantation. Treatment with antifungal therapy after surgery should be considered. The U.S. Cystic Fibrosis Foundation held a consensus conference on lung transplantation in 1996.[457] The conferees concluded that microbiologic contraindications to transplantation were limited to active hepatitis B and human immunodeficiency virus. The decision to perform transplants patients infected with *B. cepacia* complex or multidrug-resistant *P. aeruginosa* or colonized with *Aspergillus* spp., should be made on a case-by-case basis. Potentially, an understanding of the genomovar infecting a patient may be a useful predictor of mortality.

Anti-Inflammatory Therapy

Inflammation associated with chronic bacterial colonization plays a crucial role in CF lung disease. Nonsteroidal agents and glucocorticoids have been used as potential medications for anti-inflammatory therapy. A randomized trial using oral prednisone 1 to 2 mg/kg every other day showed a reduction in the rate of decline in pulmonary function of patients with CF but was associated with significant steroid-related side effects.[17] Three short-term trials using inhaled steroids have led to mixed results and no clear direction for long-term use.[18,276,421]A systematic review of inhaled steroids for CF was equivocal with respect to efficacy.[100] A multicenter randomized trial of withdrawal of inhaled steroids in CF did not find a difference in pulmonary exacerbations, infection with *P. aeruginosa*, or pulmonary function after inhaled steroids were discontinued.[19] A pilot study in of 24 patients with CF being treated with antimicrobial standard therapy for a pulmonary exacerbation were randomized to receive oral prednisone 2 mg/kg/day up to 60 mg or placebo on days 1 to 5. Study evaluations on days 1 to 6, 14, and 42 included spirometry, glucose measurements, sputum analysis, and symptom scores. Mean increase in FEV_1 percentage of predicted did not differ significantly between prednisone and placebo groups, with a trend toward improvement in pulmonary function with the prednisone therapy group; sputum inflammatory markers and symptom scores did not differ between groups. Glucosuria occurred in six prednisone subjects, two of whom developed hyperglycemia. Based on these results, a definitive study of a much larger group of subjects is required to detect a treatment effect before routine recommendation of steroid use can be made.[107] High-dose ibuprofen 20 to 30 mg/kg twice daily used over a 4-year period showed a decreased rate of decline in pulmonary function with few reported side effects.[211] Macrolide antibiotics decrease inflammation by modulation of inflammatory signaling pathways and decreased cytokine production from inflammatory cells.[118] Several anti-inflammatory agents, including antiproteases, statins, and antioxidants, have been proposed as potential therapies, but they remain under investigation and are not a part of routine clinical practice.

PREVENTION

Immunizations

The use of currently available routine childhood and adolescent vaccines is strongly advocated for patients with CF. Although patients with CF are not at increased risk for developing *S. pneumoniae* infections, they should receive the pneumococcal 13-valent conjugate vaccine. Annual influenza vaccination is recommended for patients with CF and household members.[126,132] The RSV subunit vaccine PFP-2 (purified fusion protein vaccine is a fusion glycoprotein that induces serum neutralizing antibodies)

TABLE 25-5 **Risk Factors Associated with Acquisition of *Burkholderia cepacia* Complex**

Risk Factors	Comment
Attendance at CF summer camp[299]	Risk for acquisition (6% incidence) increased with time spent at camp and prevalence of *B. cepacia* at the camp
Slept in same cabin	
Shared a personal item	
Danced with *B. cepacia*–infected camper	
Attendance at summer educational program[229]	3 (20%)/15 patients acquired same ribotype
Participation in adult CF group	Disband meetings thought to represent extensive social contact
Social contact[159]	
Kissing	
Intimate contact	
Prolonged car rides	
Sibling with *B. cepacia* complex	
Handshaking	2/68 cultures positive, 1 from patient and 1 from investigator[300]
	3 patients' hands became contaminated after coughing[159]
Inpatient exposures	Risk for acquisition increased if hospitalized within 3 mo and if hospitalized longer
Recent hospitalization[300]	Interviews with health care workers indicated poor adherence to contact precautions
Use of specific shower	
Sharing hospital room with another patient infected with *B. cepacia*	
Cared for by a medical student	
Respiratory therapy equipment	Reservoirs of large-volume nebulizers grew *B. cepacia*
Sharing equipment	
Hospital nebulizers	
Spirometer[188]	
Mouthpiece filters[159]	

CF, Cystic fibrosis.

has been studied in children with CF for two RSV seasons.[306] Initial studies showed that the RSV subunit vaccine was safe and immunogenic and that it reduced the incidence of lower respiratory tract infections.[305] A large multicenter trial in patients with mild disease failed to show a reduction in the incidence of lower respiratory tract infection, however.[304] A placebo-controlled trial of RSV monoclonal antibody (palivizumab) was performed in 186 children.[380] Although palivizumab was well tolerated, two children were hospitalized (one in each group); the study was not adequately powered for efficacy. Consideration should be made for palivizumab use in infants with CF.

The results of a phase III multicenter study of a *P. aeruginosa* vaccine showed that a bivalent, antiflagella vaccine was immunogenic and was found to reduce the incidence of *P. aeruginosa* infection and serum antibody titers. It did not prevent chronic *P. aeruginosa* infection and did not affect lung function.[105] Current studies are ongoing evaluating the safety and effectiveness of a PEGylated, recombinant Anti–*P. aeruginosa* PcrV Fab' antibody targeted for use in patients with chronic *P. aeruginosa* infection.

Infection Control Precautions

Although the sources of most pathogens in patients with CF are unknown, it is increasingly recognized that patient-to-patient spread and acquisition from the contaminated health care environment can occur. To date, reports of transmission of bacterial pathogens occurring between individuals with and without CF are rare.[152,178,246] Direct contact, indirect contact, and spread of droplets with infectious secretions all have been implicated as modes of transmission of CF pathogens. Risk factors for transmission were described first for *B. cepacia* complex, as shown in Table 25-5.*

Perhaps of most concern have been several reports of clonal spread of *P. aeruginosa* among patients with CF.[245,359,360,418] These examples have involved obvious phenotypes that triggered an investigation of possible patient-to-patient spread, including an increase in ceftazidime-resistant *P. aeruginosa*[60] or initial colonization of young children with mucoid strains of *P. aeruginosa*.[14,277] These reports have led CF centers in Europe to favor cohorting and segregating patients with *P. aeruginosa*, particularly patients with antibiotic-resistant strains, in hospital and clinic settings.[136,208,245] In the United States, infection control strategies have focused on containment of respiratory tract secretions as described subsequently.[350] Finally, occasional cases of patient-to-patient spread of other potential pathogens, including *S. maltophilia* and *A. xylosoxidans*, have been reported in patients with CF.[329,417]

The U.S. Cystic Fibrosis Foundation convened an expert panel to develop revised recommendations for infection control that addressed inpatient, outpatient (i.e., CF clinic and pulmonary function test laboratories),

*References 58, 159, 229, 299, 300, 408.

TABLE 25-6 **Infection Control Strategies for Cystic Fibrosis**

Guideline	Setting		
	Inpatient	Outpatient	Non–Health Care
Quarterly cultures of respiratory tract	X		
Appropriate processing of CF respiratory tract cultures	X	X	
Educate patients and families about proper hand hygiene	X	X	X
Implement contact precautions for MDROs, including MRSA	X	X	
Hospitalize patients with MDROs in single-patient room	X	X	
Segregate patients with *B. cepacia* complex from other CF patients	X	X	X
Clean and disinfect respiratory therapy equipment	X	X	X
Avoid socialization among CF patients	X	X	X
Maintain at least 3 ft between CF patients to prevent droplet transmission	X	X	X

CF, Cystic fibrosis; *MDROs,* multidrug-resistant organisms; *MRSA,* methicillin-resistant *Staphylococcus aureus.*

and non–health care settings, because so much CF care is delivered in the home. These recommendations have implications for treatment, transplantation, and the psychosocial well-being of patients, families, and staff. Several strategies are emphasized (Table 25-6). The guidelines emphasize that all patients with CF could harbor potentially transmissible respiratory tract pathogens, and containing respiratory tract secretions is of paramount importance.

The consensus is that patients harboring *B. cepacia* complex should be cared for apart from other patients with CF and not grouped together because of concern that some strains of *B. cepacia* complex may be more virulent and replace other strains.[236] Patients are hospitalized in single rooms and seen in outpatient settings geographically or temporally apart from other patients with CF. Similarly, patients with CF and MRSA in the respiratory tract should be placed in private rooms without access to common areas.

CONCLUSION

The microbiology of patients with CF is complex and changing. Although the pathogenesis of lung infections is still under active investigation, the current hypothesis suggests multiple etiologies. Appropriate microbiologic processing of respiratory tract specimens is crucial to ensure an accurate understanding of the epidemiology of CF lung disease and provide appropriate treatment and infection control. Current treatment strategies are directed largely at management of deteriorations of pulmonary function, but increasingly strategies are directed at prevention and preservation of lung function.

NEW REFERENCES SINCE THE SIXTH EDITION

8. Andersen HU, Lanng S, Pressler T, et al. Cystic fibrosis-related diabetes: the presence of microvascular diabetes complications. *Diabetes Care* 2006;**29**:2660–3.
19. Balfour-Lynn IM, Lees B, Hall P, et al. Multicenter randomized controlled trial of withdrawal of inhaled corticosteroids in cystic fibrosis. *Am J Respir Crit Care Med* 2006;**173**:1356–62.
24. Bhattacharyya S, Balakathiresan NS, Dalgard C, et al. Elevated miR-155 promotes inflammation in cystic fibrosis by driving hyperexpression of interleukin-8. *J Biol Chem* 2011;**286**:11604–15.
25. Bittar F, Richet H, Dubus JC, et al. Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients. *PLoS One* 2008;**3**:e2908.
27. Blackman SM, Hsu S, Ritter SE, et al. A susceptibility gene for type 2 diabetes confers substantial risk for diabetes complicating cystic fibrosis. *Diabetologia* 2009;**52**:1858–65.
29. Blyth CC, Harun A, Middleton PG, et al. Detection of occult *Scedosporium* species in respiratory tract specimens from patients with cystic fibrosis by use of selective media. *J Clin Microbiol* 2010;**48**:314–16.
36. Boucher RC. New concepts of the pathogenesis of cystic fibrosis lung disease. *Eur Respir J* 2004;**23**:146–58.
39. Braun AT, Merlo CA. Cystic fibrosis lung transplantation. *Curr Opin Pulm Med* 2011;**17**:467–72.
51. Campodonico VL, Gadjeva M, Paradis-Bleau C, et al. Airway epithelial control of *Pseudomonas aeruginosa* infection in cystic fibrosis. *Trends Mol Med* 2008;**14**:120–33.
55. Cardines R, Giufre M, Pompilio A, et al. *Haemophilus influenzae* in children with cystic fibrosis: antimicrobial susceptibility, molecular epidemiology, distribution of adhesins and biofilm formation. *Int J Med Microbiol* 2012;**302**:45–52.
56. Carlyle BE, Borowitz DS, Glick PL. A review of pathophysiology and management of fetuses and neonates with meconium ileus for the pediatric surgeon. *J Pediatr Surg* 2012;**47**:772–81.
72. Collaco JM, Blackman SM, McGready J, et al. Quantification of the relative contribution of environmental and genetic factors to variation in cystic fibrosis lung function. *J Pediatr* 2010;**157**:802–7, e801–3.
71. Collaco JM, Cutting GR. Update on gene modifiers in cystic fibrosis. *Curr Opin Pulm Med* 2008;**14**:559–66.
74. Colombo C, Ellemunter H, Houwen R, et al. Guidelines for the diagnosis and management of distal intestinal obstruction syndrome in cystic fibrosis patients. *J Cyst Fibros* 2011;**10**(suppl 2): S24–28.
76. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev* 2009;**61**:75–85.
80. Cox DW, Kelly C, Rush R, et al. The impact of MRSA infection in the airways of children with cystic fibrosis: a case-control study. *Ir Med J* 2011;**104**:305–8.
81. Cox MJ, Allgaier M, Taylor B, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. *PLoS One* 2010;**5**:e11044.
84. Cutting GR. Modifier genes in Mendelian disorders: the example of cystic fibrosis. *Ann N Y Acad Sci* 2010;**1214**:57–69.
88. Cystic Fibrosis Foundation. *Patient Registry 2010*. Bethesda, MD: Cystic Fibrosis Foundation; 2010.
92. Dasenbrook EC. Update on methicillin-resistant *Staphylococcus aureus* in cystic fibrosis. *Curr Opin Pulm Med* 2011;**17**:437–41.
93. Davies JC, Griesenbach U, Alton E. Modifier genes in cystic fibrosis. *Pediatr Pulmonol* 2005;**39**:383–91.

96. Delhaes L, Monchy S, Frealle E, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community—implications for therapeutic management. *PLoS One* 2012;**7**: e36313.
109. Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med* 2005; **353**:1443–53.
112. Edenborough FP, Mackenzie WE, Stableforth DE. The outcome of 72 pregnancies in 55 women with cystic fibrosis in the United Kingdom 1977–1996. *BJOG* 2000;**107**:254–61.
113. Edenborough FP, Stone HR, Kelly SJ, et al. Genotyping of *Pseudomonas aeruginosa* in cystic fibrosis suggests need for segregation. *J Cyst Fibros* 2004;**3**:37–44.
119. Emerson J, McNamara S, Buccat AM, et al. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. *Pediatr Pulmonol* 2010;**45**:363–70.
125. Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med* 2010;**363**:2233–47.
126. Farrell PM, Kosorok MR, Laxova A, et al. Nutritional benefits of neonatal screening for cystic fibrosis. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *N Engl J Med* 1997;**337**: 963–9.
128. Farrell PM, Li Z, Kosorok MR, et al. Longitudinal evaluation of bronchopulmonary disease in children with cystic fibrosis. *Pediatr Pulmonol* 2003;**36**:230–40.
129. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008;**153**:S4–S14.
141. Galli F, Battistoni A, Gambari R, et al. Oxidative stress and antioxidant therapy in cystic fibrosis. *Biochim Biophys Acta* 2012; **1822**:690–713.
145. Geller DE, Flume PA, Staab D, et al. Levofloxacin inhalation solution (MP-376) in patients with cystic fibrosis with *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2011;**183**:1510–16.
150. Gilljam M, Antoniou M, Shin J, et al. Pregnancy in cystic fibrosis: fetal and maternal outcome. *Chest* 2000;**118**:85–91.
156. Goss CH, Muhlebach MS. Review: *Staphylococcus aureus* and MRSA in cystic fibrosis. *J Cyst Fibros* 2011;**10**:298–306.
158. Goss CH, Rubenfeld GD, Otto K, et al. The effect of pregnancy on survival in women with cystic fibrosis. *Chest* 2003;**124**: 1460–8.
160. Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 1996;**60**:539–74.
173. Hillian AD, Londono D, Dunn JM, et al. Modulation of cystic fibrosis lung disease by variants in interleukin-8. *Genes Immun* 2008;**9**:501–8.
182. Hubert D, Le Roux E, Lavrut T, et al. Continuous versus intermittent infusions of ceftazidime for treating exacerbation of cystic fibrosis. *Antimicrob Agents Chemother* 2009;**53**:3650–6.
195. Kabra SK, Pawaiya R, Lodha R, et al. Long-term daily high and low doses of azithromycin in children with cystic fibrosis: a randomized controlled trial. *J Cyst Fibros* 2010;**9**:17–23.
199. Keel RA, Schaeftlein A, Kloft C, et al. Pharmacokinetics of intravenous and oral linezolid in adults with cystic fibrosis. *Antimicrob Agents Chemother* 2011;**55**:3393–8.
205. Knowles MR. Gene modifiers of lung disease. *Curr Opin Pulm Med* 2006;**12**:416–21.
219. Lambiase A, Catania MR, Del Pezzo M, et al. *Achromobacter xylosoxidans* respiratory tract infection in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 2011;**30**:973–80.
222. Latzin P, Fehling M, Bauernfeind A, et al. Efficacy and safety of intravenous meropenem and tobramycin versus ceftazidime and tobramycin in cystic fibrosis. *J Cyst Fibros* 2008;**7**:142–6.
228. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;**23**:299–323.
248. McColley SA, Ren CL, Schechter MS, et al. Risk factors for onset of persistent respiratory symptoms in children with cystic fibrosis. *Pediatr Pulmonol* 2012;**47**:966–72.
249. McCoy KS, Quittner AL, Oermann CM, et al. Inhaled aztreonam lysine for chronic airway *Pseudomonas aeruginosa* in cystic fibrosis. *Am J Respir Crit Care Med* 2008;**178**:921–8.
250. McGrath LT, Mallon P, Dowey L, et al. Oxidative stress during acute respiratory exacerbations in cystic fibrosis. *Thorax* 1999;**54**: 518–23.
259. Moran A, Brunzell C, Cohen RC, et al. Clinical care guidelines for cystic fibrosis-related diabetes: a position statement of the American Diabetes Association and a clinical practice guideline of the Cystic Fibrosis Foundation, endorsed by the Pediatric Endocrine Society. *Diabetes Care* 2010;**33**:2697–708.
262. Morris MR, Doull IJ, Dewitt S, et al. Reduced iC3b-mediated phagocytotic capacity of pulmonary neutrophils in cystic fibrosis. *Clin Exp Immunol* 2005;**142**:68–75.
263. Moskowitz SM, Chmiel JF, Sternen DL, et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet Med* 2008;**10**:851–68.
264. Moskowitz SM, Emerson JC, McNamara S, et al. Randomized trial of biofilm testing to select antibiotics for cystic fibrosis airway infection. *Pediatr Pulmonol* 2011;**46**:184–92.
268. Mueller C, Braag SA, Keeler A, et al. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol* 2011;**44**:922–9.
281. Oermann CM, Retsch-Bogart GZ, Quittner AL, et al. An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr Pulmonol* 2010;**45**:1121–34.
307. Pier GB, Grout M, Zaidi TS. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. *Proc Natl Acad Sci U S A* 1997;**94**:12088–93.
308. Pier GB, Grout M, Zaidi TS, et al. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science* 1996;**271**:64–7.
318. Ratjen F, Munck A, Kho P, et al. Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 2010;**65**:286–91.
319. Ratner D, Mueller C. Immune responses in cystic fibrosis: are they intrinsically defective? *Am J Respir Cell Mol Biol* 2012;**46**:715–22.
320. Razvi S, Quittell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. *Chest* 2009;**136**:1554–60.
324. Retsch-Bogart GZ, Quittner AL, Gibson RL, et al. Efficacy and safety of inhaled aztreonam lysine for airway pseudomonas in cystic fibrosis. *Chest* 2009;**135**:1223–32.
325. Ribeiro CM, Boucher RC. Role of endoplasmic reticulum stress in cystic fibrosis-related airway inflammatory responses. *Proc Am Thorac Soc* 2010;**7**:387–94.
333. Rosenfeld M, Emerson J, McNamara S, et al. Risk factors for age at initial *Pseudomonas* acquisition in the cystic fibrosis epic observational cohort. *J Cyst Fibros* 2012;**11**:446–53.
344. Saiman L, Anstead M, Mayer-Hamblett N, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa:* a randomized controlled trial. *JAMA* 2010;**303**:1707–15.
351. Salvatore D, Buzzetti R, Baldo E, et al. An overview of international literature from cystic fibrosis registries 2. Neonatal screening and nutrition/growth. *J Cyst Fibros* 2010;**9**:75–83.
358. Schwarzer C, Illek B, Suh JH, et al. Organelle redox of CF and CFTR-corrected airway epithelia. *Free Radic Biol Med* 2007;**43**: 300–16.
362. Seidler MJ, Salvenmoser S, Muller FM. *Aspergillus fumigatus* forms biofilms with reduced antifungal drug susceptibility on bronchial epithelial cells. *Antimicrob Agents Chemother* 2008;**52**: 4130–6.
364. Shilo N, Quach C. Pulmonary infections and community associated methicillin resistant *Staphylococcus aureus:* a dangerous mix? *Paediatr Respir Rev* 2011;**12**:182–9.
366. Sibley CD, Grinwis ME, Field TR, et al. Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PLoS One* 2011;**6**:e22702.
379. Southern KW, Barker PM, Solis-Moya A, et al. Macrolide antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2011;CD002203.
385. Stanke F, Becker T, Kumar V, et al. Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. *J Med Genet* 2011;**48**: 24–31.
388. Strom CM, Huang D, Chen C, et al. Extensive sequencing of the cystic fibrosis transmembrane regulator gene: assay validation and unexpected benefits of developing a comprehensive test. *Genet Med* 2003;**5**:9–14.

409. Thomson E, Brennan S, Senthilmohan R, et al. Identifying peroxidases and their oxidants in the early pathology of cystic fibrosis. *Free Radic Biol Med* 2010;**49**:1354–60.
412. Tramper-Stranders GA, Wolfs TF, van Haren Noman S, et al. Controlled trial of cycled antibiotic prophylaxis to prevent initial *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Thorax* 2010;**65**:915–20.
413. Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, et al. Comparative efficacy and safety of 4 randomized regimens to treat early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Arch Pediatr Adolesc Med* 2011;**165**:847–56.
424. Vanscoy LL, Blackman SM, Collaco JM, et al. Heritability of lung disease severity in cystic fibrosis. *Am J Respir Crit Care Med* 2007;**175**:1036–43.
426. Velsor LW, van Heeckeren A, Day BJ. Antioxidant imbalance in the lungs of cystic fibrosis transmembrane conductance regulator protein mutant mice. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**:L31–38.
428. Verhaeghe C, Remouchamps C, Hennuy B, et al. Role of IKK and ERK pathways in intrinsic inflammation of cystic fibrosis airways. *Biochem Pharmacol* 2007;**73**:1982–94.
431. Verregghen M, Heijerman HG, Reijers M, et al. Risk factors for *Mycobacterium abscessus* infection in cystic fibrosis patients: a case-control study. *J Cyst Fibros* 2012;**11**:340–3.
432. Wagener JS, Zemanick ET, Sontag MK. Newborn screening for cystic fibrosis. *Curr Opin Pediatr* 2012;**24**:329–35.
433. Wainwright CE, Quittner AL, Geller DE, et al. Aztreonam for inhalation solution (AZLI) in patients with cystic fibrosis, mild lung impairment, and *P. aeruginosa*. *J Cyst Fibros* 2011;**10**: 234–42.
434. Wainwright CE, Vidmar S, Armstrong DS, et al. Effect of bronchoalveolar lavage-directed therapy on *Pseudomonas aeruginosa* infection and structural lung injury in children with cystic fibrosis: a randomized trial. *JAMA* 2011;**306**:163–71.
445. Wilschanski M, Dupuis A, Ellis L, et al. Mutations in the cystic fibrosis transmembrane regulator gene and in vivo transepithelial potentials. *Am J Respir Crit Care Med* 2006;**174**:787–94.
460. Zemanick ET, Sagel SD, Harris JK. The airway microbiome in cystic fibrosis and implications for treatment. *Curr Opin Pediatr* 2011;**23**:319–24.
461. Zemanick ET, Wagner BD, Sagel SD, et al. Reliability of quantitative real-time PCR for bacterial detection in cystic fibrosis airway specimens. *PLoS One* 2010;**5**:e15101.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aaron SD, Ferris W, Henry DA, et al. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. *Am J Respir Crit Care Med* 2000;**161**:1206–12.
2. Abman SH, Ogle JW, Butler-Simon N, et al. Role of respiratory syncytial virus in early hospitalizations for respiratory distress of young infants with cystic fibrosis. *J Pediatr* 1988;**113**:826–30.
3. Abman SH, Ogle JW, Harbeck RJ, et al. Early bacteriologic, immunologic, and clinical courses of young infants with cystic fibrosis identified by neonatal screening. *J Pediatr* 1991;**119**: 211–17.
4. Accurso FJ, Sontag MK, Wagener JS. Complications associated with symptomatic diagnosis in infants with cystic fibrosis. *J Pediatr* 2005;**147**:S37–41.
5. Al-Aloul M, Crawley J, Winstanley C, et al. Increased morbidity associated with chronic infection by an epidemic *Pseudomonas aeruginosa* strain in CF patients. *Thorax* 2004;**59**:334–6.
6. Amaral MD. Processing of CFTR: traversing the cellular maze: how much CFTR needs to go through to avoid cystic fibrosis? *Pediatr Pulmonol* 2005;**39**:479–91.
7. American Thoracic Society. Diagnosis and treatment of disease caused by nontuberculous mycobacteria. This official statement of the American Thoracic Society was approved by the Board of Directors, March 1997. Medical Section of the American Lung Association. *Am J Respir Crit Care Med* 1997;**156**:S1–25.
8. Andersen HU, Lanng S, Pressler T, et al. Cystic fibrosis-related diabetes: the presence of microvascular diabetes complications. *Diabetes Care* 2006;**29**:2660–3.
9. Anderson DH. Therapy and prognosis of fibrocystic disease of the pancreas. *Pediatrics* 1949;406–17.
10. Anguiana A, Oates RD, Amos JA, et al. Congenital bilateral absence of the vas deferens: a primarily genital form of cystic fibrosis. *JAMA* 1992;**267**:1704–97.
11. Aris RM, Gilligan PH, Neuringer IP, et al. The effects of panresistant bacteria in cystic fibrosis patients on lung transplant outcome. *Am J Respir Crit Care Med* 1997;**155**:1699–704.
12. Armstrong D, Grimwood K, Carlin JB, et al. Severe viral respiratory infections in infants with cystic fibrosis. *Pediatr Pulmonol* 1998;**26**:371–9.
13. Armstrong DS. *Pseudomonas aeruginosa:* clinical research. *Prog Respir Res* 2006;**34**:131–7.
14. Armstrong DS, Grimwood K, Carlin JB, et al. Lower airway inflammation in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med* 1997;**156**:1197–204.
15. Armstrong DS, Grimwood K, Carzino R, et al. Lower respiratory infection and inflammation in infants with newly diagnosed cystic fibrosis. *BMJ* 1995;**310**:1571–2.
16. Armstrong DS, Nixon GM, Carzino R, et al. Detection of a widespread clone of *Pseudomonas aeruginosa* in a pediatric cystic fibrosis clinic. *Am J Respir Crit Care Med* 2002;**166**: 983–7.
17. Auerbach HS, Williams M, Kirkpatrick JA, et al. Alternate-day prednisone reduces morbidity and improves pulmonary function in cystic fibrosis. *Lancet* 1985;**2**:686–8.
18. Balfour-Lynn IM, Klein NJ, Dinwiddie R. Randomized controlled trial of inhaled corticosteroids fluticasone propionate in cystic fibrosis. *Arch Dis Child* 1997;**77**:124–30.
19. Balfour-Lynn IM, Lees B, Hall P, et al. Multicenter randomized controlled trial of withdrawal of inhaled corticosteroids in cystic fibrosis. *Am J Respir Crit Care Med* 2006;**173**:1356–62.
20. Baltimore RS, Christie CD, Smith GJ. Immunohistopathologic localization of *Pseudomonas aeruginosa* in lungs from patients with cystic fibrosis: implications for the pathogenesis of progressive lung deterioration. *Am Rev Respir Dis* 1989;**140**:1650–61.
21. Bange FC, Bottger EC. Improved decontamination method for recovering mycobacteria from patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2002;**21**:546–8.
22. Bargon J, Dauletbaev N, Kohler B, et al. Prophylactic antibiotic therapy is associated with an increased prevalence of *Aspergillus* colonization in adult cystic fibrosis patients. *Respir Med* 1999;**93**: 835–8.
23. Beardsmore CS, Thompson JR, Williams A, et al. Pulmonary function in infants with cystic fibrosis: the effect of antibiotic treatment. *Arch Dis Child* 1994;**71**:133–7.
24. Bhattacharyya S, Balakathiresan NS, Dalgard C, et al. Elevated miR-155 promotes inflammation in cystic fibrosis by driving hyperexpression of interleukin-8. *J Biol Chem* 2011;**286**:11604–15.
25. Bittar F, Richet H, Dubus JC, et al. Molecular detection of multiple emerging pathogens in sputa from cystic fibrosis patients. *PLoS One* 2008;**3**:e2908.
26. Blackman SM, Deering-Brose R, McWilliams R, et al. Relative contribution of genetic and nongenetic modifiers to intestinal obstruction in cystic fibrosis. *Gastroenterology* 2006;**131**:1030–9.
27. Blackman SM, Hsu S, Ritter SE, et al. A susceptibility gene for type 2 diabetes confers substantial risk for diabetes complicating cystic fibrosis. *Diabetologia* 2009;**52**:1858–65.
28. Blumer JL, Saiman L, Konstan MW, et al. The efficacy and safety of meropenem and tobramycin vs ceftazidime and tobramycin in the treatment of acute pulmonary exacerbations in patients with cystic fibrosis. *Chest* 2005;**128**:2336–46.
29. Blyth CC, Harun A, Middleton PG, et al. Detection of occult *Scedosporium* species in respiratory tract specimens from patients with cystic fibrosis by use of selective media. *J Clin Microbiol* 2010;**48**:314–16.
30. Bonfield TL, Konstan MW, Burfeind P, et al. Normal bronchial epithelial cells constitutively produce the anti-inflammatory cytokine interleukin-10, which is downregulated in cystic fibrosis. *Am J Respir Cell Mol Biol* 1995;**13**:257–61.
31. Borgo G, Mastella G, Gasparini P, et al. Pancreatic function and gene deletion F508 in cystic fibrosis. *J Med Genet* 1990;**27**: 665–9.
32. Bosso JA, Black PG. Controlled trial of aztreonam vs. tobramycin and azlocillin for acute pulmonary exacerbations of cystic fibrosis. *Pediatr Infect Dis J* 1988;**7**:171–6.
33. Bosso JA, Flume PA, Gray SL. Linezolid pharmacokinetics in adult patients with cystic fibrosis. *Antimicrob Agents Chemother* 2004;**48**:281–4.
34. Bosworth DG, Nielson DW. Effectiveness of home versus hospital care in the routine treatment of cystic fibrosis. *Pediatr Pulmonol* 1997;**24**:42–7.
35. Boucher RC. An overview of the pathogenesis of cystic fibrosis lung disease. *Adv Drug Deliv Rev* 2002;**54**:1359–71.
36. Boucher RC. New concepts of the pathogenesis of cystic fibrosis lung disease. *Eur Respir J* 2004;**23**:146–58.
37. Boucher RC. Evidence for airway surface dehydration as the initiating event in CF airway disease. *J Intern Med* 2007;**261**:5–16.
38. Branger C, Gardye C, Lambert-Zechovsky N. Persistence of *Staphylococcus aureus* strains among cystic fibrosis patients over extended periods of time. *J Med Microbiol* 1996;**45**:294–301.
39. Braun AT, Merlo CA. Cystic fibrosis lung transplantation. *Curr Opin Pulm Med* 2011;**17**:467–72.
40. Brown-Elliott BA, Crist CJ, Mann LB, et al. In vitro activity of linezolid against slowly growing nontuberculous mycobacteria. *Antimicrob Agents Chemother* 2003;**47**:1736–8.
41. Burns JL, Saiman L. Burkholderia cepacia infections in cystic fibrosis. *Pediatr Infect Dis J* 1999;**18**:155–6.
42. Burns JL, Emerson J, Stapp JR, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin Infect Dis* 1998;**27**:158–63.
43. Burns JL, Gibson RL, McNamara S, et al. Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *J Infect Dis* 2001;**183**:444–52.
44. Burns JL, Saiman L, Whittier S, et al. Comparison of agar diffusion methodologies for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *J Clin Microbiol* 2000;**38**:1818–22.
45. Burns JL, Saiman L, Whittier S, et al. Comparison of two commercial systems (Vitek and MicroScan-WalkAway) for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *Diagn Microbiol Infect Dis* 2001;**39**: 257–60.
46. Burns JL, Van Dalfsen JM, Shawar RM, et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory microbial flora in patients with cystic fibrosis. *J Infect Dis* 1999; **179**:1190–6.
47. Cacalano G, Kays M, Saiman L, et al. Production of the *Pseudomonas aeruginosa* neuraminidase is increased under hyperosmolar conditions and is regulated by genes involved in alginate expression. *J Clin Invest* 1992;**89**:1866–74.

48. Campbell PW 3rd, Saiman L. Use of aerosolized antibiotics in patients with cystic fibrosis. *Chest* 1999;**116**:775–88.
49. Campbell PW 3rd, White TB. Newborn screening for cystic fibrosis: an opportunity to improve care and outcomes. *J Pediatr* 2005;**147**:S2–5.
50. Campbell PW, Phillips JA, Krishnamani MRS, et al. Cystic fibrosis: relationship between clinical status and F508 deletion. *J Pediatr* 1991;**118**:239.
51. Campodonico VL, Gadjeva M, Paradis-Bleau C, et al. Airway epithelial control of *Pseudomonas aeruginosa* infection in cystic fibrosis. *Trends Mol Med* 2008;**14**:120–33.
52. Cantin AM, Bilodeau G, Ouellet C, et al. Oxidant stress suppresses CFTR expression. *Am J Physiol Cell Physiol* 2006;**290**: C262–270.
53. Cantin AM, North SL, Hubbard RC, et al. Normal alveolar epithelial lining fluid contains high levels of glutathione. *J Appl Physiol* 1987;**63**:152–7.
54. Cantin AM, White TB, Cross CE, et al. Antioxidants in cystic fibrosis: conclusions from the CF antioxidant workshop, Bethesda, Maryland, November 11-12, 2003. *Free Radic Biol Med* 2007;**42**: 15–31.
55. Cardines R, Giufre M, Pompilio A, et al. *Haemophilus influenzae* in children with cystic fibrosis: antimicrobial susceptibility, molecular epidemiology, distribution of adhesins and biofilm formation. *Int J Med Microbiol* 2012;**302**:45–52.
56. Carlyle BE, Borowitz DS, Glick PL. A review of pathophysiology and management of fetuses and neonates with meconium ileus for the pediatric surgeon. *J Pediatr Surg* 2012;**47**:772–81.
57. Cash HA, Woods DE, McCullough B, et al. A rat model of chronic respiratory infection with *Pseudomonas aeruginosa*. *Am Rev Respir Dis* 1979;**119**:453–9.
58. Centers for Disease Control and Prevention. *Pseudomonas cepacia* at summer camps for persons with cystic fibrosis. *MMWR Morb Mortal Wkly Rep* 1993;**42**:456–9.
59. Chalermskulrat W, Sood N, Neuringer IP, et al. Nontuberculous mycobacteria in end stage cystic fibrosis: implications for lung transplantation. *Thorax* 2006;**61**:507–13.
60. Cheng K, Smyth RL, Govan JR, et al. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 1996;**348**:639–42.
61. Cheng SH, Gregory RJ, Marshall J, et al. Defective intracellular transport and processing of CFTR is the molecular basis of most cystic fibrosis. *Cell* 1990;**63**:827–34.
62. Chernenko SM, Humar A, Hutcheon M, et al. *Mycobacterium abscessus* infections in lung transplant recipients: the international experience. *J Heart Lung Transplant* 2006;**25**:1447–55.
63. Chernick VC, Kendig EL. *Disorders of the respiratory tract in children*. 5th ed. Philadelphia: W. B. Saunders; 1990. p. 692–730.
64. Church DA, Kanga JF, Kuhn RJ, et al. Sequential ciprofloxacin therapy in pediatric cystic fibrosis: comparative study vs. ceftazidime/tobramycin in the treatment of acute pulmonary exacerbations. The Cystic Fibrosis Study Group. *Pediatr Infect Dis J* 1997;**16**:97–105; discussion 123–106.
65. Cimon B, Carrere J, Vinatier JF, et al. Clinical significance of *Scedosporium apiospermum* in patients with cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2000;**19**:53–6.
66. Clement A, Tamalet A, Leroux E, et al. Long term effects of azithromycin in patients with cystic fibrosis: a double blind, placebo controlled trial. *Thorax* 2006;**61**:895–902.
67. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; 17th informational supplement M100-S17*. Wayne, Pa: Clinical and Laboratory Standards Institute; 2007.
68. Coenye T, Spilker T, Reik R, et al. Use of PCR analyses to define the distribution of *Ralstonia* species recovered from patients with cystic fibrosis. *J Clin Microbiol* 2005;**43**:3463–6.
69. Coenye T, Vandamme P, Govan JR, et al. Taxonomy and identification of the *Burkholderia cepacia* complex. *J Clin Microbiol* 2001;**39**:3427–36.
70. Cohn JA, Friedman KL, Noone PG, et al. Relation beween mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 1998;**339**:653–8.
71. Collaco JM, Cutting GR. Update on gene modifiers in cystic fibrosis. *Curr Opin Pulm Med* 2008;**14**:559–66.
72. Collaco JM, Blackman SM, McGready J, et al. Quantification of the relative contribution of environmental and genetic factors to variation in cystic fibrosis lung function. *J Pediatr* 2010;**157**:802–7, e801–3.
73. Collinson J, Nicholson KG, Cancio E, et al. Effects of upper respiratory tract infections in patients with cystic fibrosis. *Thorax* 1996;**51**:1115–22.
74. Colombo C, Ellemunter H, Houwen R, et al. Guidelines for the diagnosis and management of distal intestinal obstruction syndrome in cystic fibrosis patients. *J Cyst Fibros* 2011;**10**(Suppl 2): S24–28.
75. Colombo C, Russo MC, Zazzeron L, et al. Liver disease in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 2006;**43**(Suppl 1):S49–55.
76. Cone RA. Barrier properties of mucus. *Adv Drug Deliv Rev* 2009;**61**:75–85.
77. Corey M, Farewell V. Determinants of mortality from cystic fibrosis in Canada, 1970-1989. *Am J Epidemiol* 1996;**143**:1007–17.
78. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;**284**: 1318–22.
79. Cowley EA, Linsdell P. Oxidant stress stimulates anion secretion from the human airway epithelial cell line Calu-3: implications for cystic fibrosis lung disease. *J Physiol* 2002;**543**:201–9.
80. Cox DW, Kelly C, Rush R, et al. The impact of MRSA infection in the airways of children with cystic fibrosis; a case-control study. *Ir Med J* 2011;**104**:305–8.
81. Cox MJ, Allgaier M, Taylor B, et al. Airway microbiota and pathogen abundance in age-stratified cystic fibrosis patients. *PLoS One* 2010;**5**:e11044.
82. Cross CE, Halliwell B, Allen A. Antioxidant protection: a function of tracheobronchial and gastrointestinal mucus. *Lancet* 1984;**1**: 1328–30.
83. Cutting GR. Genotype defect: Its effect on cellular function and phenotypic expression. *Semin Respir Crit Care Med* 1994;**15**:356.
84. Cutting GR. Modifier genes in Mendelian disorders: the example of cystic fibrosis. *Ann N Y Acad Sci* 2010;**1214**:57–69.
85. Cystic Fibrosis Foundation. *Clinical practice guidelines for cystic fibrosis*. Bethesda, Md; 1997.
86. Cystic Fibrosis Foundation. *Patient Registry 1996*. Bethesda, Md: Cystic Fibrosis Foundation; 1997.
87. Cystic Fibrosis Foundation. *Patient Registry 2005*. Bethesda, Md: Cystic Fibrosis Foundation; 2006.
88. Cystic Fibrosis Foundation. *Patient Registry 2010*. Bethesda, Md: Cystic Fibrosis Foundation; 2010.
89. Cystic Fibrosis Genetic Analysis Consortium. n.d. Cystic Fibrosis Mutation Database.
90. Cystic Fibrosis Genotype-Phenotype Consortium. Correlation between genotype and phenotype in patients with cystic fibrosis. *N Engl J Med* 1993;**329**:1308–13.
91. Dakin CJ, Numa AH, Wang H, et al. Inflammation, infection, and pulmonary function in infants and young children with cystic fibrosis. *Am J Respir Crit Care Med* 2002;**165**:904–10.
92. Dasenbrook EC. Update on methicillin-resistant *Staphylococcus aureus* in cystic fibrosis. *Curr Opin Pulm Med* 2011;**17**:437–41.
93. Davies JC, Griesenbach U, Alton E. Modifier genes in cystic fibrosis. *Pediatr Pulmonol* 2005;**39**:383–91.
94. Davis SD, Fordham LA, Brody AS, et al. Computed tomography reflects lower airway inflammation and tracks changes in early cystic fibrosis. *Am J Respir Crit Care Med* 2007;**175**:943–50.
95. de Groot R, Smith AL. Antibiotic pharmacokinetics in cystic fibrosis. Differences and clinical significance. *Clin Pharmacokinet* 1987;**13**:228–53.
96. Delhaes L, Monchy S, Frealle E, et al. The airway microbiota in cystic fibrosis: a complex fungal and bacterial community—implications for therapeutic management. *PLoS One* 2012;**7**: e36313.
97. Demko CA, Stern RC, Doershuk CF. *Stenotrophomonas maltophilia* in cystic fibrosis: incidence and prevalence. *Pediatr Pulmonol* 1998;**25**:304–8.
98. Denton M, Kerr K, Mooney L, et al. Transmission of colistin-resistant *Pseudomonas aeruginosa* between patients attending a pediatric cystic fibrosis center. *Pediatr Pulmonol* 2002;**34**:257–61.
99. Deretic V, Schurr MJ, Yu H. *Pseudomonas aeruginosa*, mucoidy and the chronic infection phenotype in cystic fibrosis. *Trends Microbiol* 1995;**3**:351–6.
100. Dezateux C, Walters S, Balfour-Lynn I. Inhaled corticosteroids for cystic fibrosis. *Cochrane Database Syst Rev* 2000;(6):CD001915.

101. Di Sant'Agnese PA. Fibrocystic disease of the pancreas with normal or partial pancreatic function: current views on pathogenesis and diagnosis. *Pediatrics* 1955;**15**:683–97.
102. DiMango E, Ratner AJ, Bryan R, et al. Activation of NF-kappaB by adherent *Pseudomonas aeruginosa* in normal and cystic fibrosis respiratory epithelial cells. *J Clin Invest* 1998;**101**:2598–605.
103. Dodge JA. Male fertility in cystic fibrosis. *Lancet* 1995;**346**: 587–8.
104. Doring G. Mechanisms of airway inflammation in cystic fibrosis. *Pediatr Allergy Immunol* 1996;**7**:63–6.
105. Doring G, Meisner C, Stern M, et al. A double-blind randomized placebo-controlled phase III study of a *Pseudomonas aeruginosa* flagella vaccine in cystic fibrosis patients. *Proc Natl Acad Sci U S A* 2007;**104**:11020–5.
106. Doull IJ, Ryley HC, Weller P, et al. Cystic fibrosis-related deaths in infancy and the effect of newborn screening. *Pediatr Pulmonol* 2001;**31**:363–6.
107. Dovey M, Aitken ML, Emerson J, et al. Oral corticosteroid therapy in cystic fibrosis patients hospitalized for pulmonary exacerbation: a pilot study. *Chest* 2007;**132**:1212–18.
108. Drumm ML. Modifier genes and variation in cystic fibrosis. *Respir Res* 2001;**2**:125–8.
109. Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med* 2005;**353**: 1443–53.
110. Dunne WM Jr, Maisch S. Epidemiological investigation of infections due to *Alcaligenes* species in children and patients with cystic fibrosis: use of repetitive-element-sequence polymerase chain reaction. *Clin Infect Dis* 1995;**20**:836–41.
111. Eaton TE, Weiner Miller P, Garrett JE, et al. Cystic fibrosis transmembrane conductance regulator gene mutations: do they play a role in the aetiology of allergic bronchopulmonary aspergillosis? *Clin Exp Allergy* 2002;**32**:756–61.
112. Edenborough FP, Mackenzie WE, Stableforth DE. The outcome of 72 pregnancies in 55 women with cystic fibrosis in the United Kingdom 1977–1996. *BJOG* 2000;**107**:254–61.
113. Edenborough FP, Stone HR, Kelly SJ, et al. Genotyping of *Pseudomonas aeruginosa* in cystic fibrosis suggests need for segregation. *J Cyst Fibros* 2004;**3**:37–44.
114. Efthimiou J, Hodson ME, Taylor P, et al. Importance of viruses and *Legionella pneumophila* in respiratory exacerbations of young adults with cystic fibrosis. *Thorax* 1984;**39**:150–4.
115. Egan TM, Murray S, Bustami RT, et al. Development of the new lung allocation system in the United States. *Am J Transplant* 2006;**6**:1212–27.
116. Elizur A, Orscheln RC, Ferkol TW, et al. Panton-Valentine Leukocidin-positive methicillin-resistant *Staphylococcus aureus* lung infection in patients with cystic fibrosis. *Chest* 2007;**131**:1718–25.
117. Elliott MW, Newman Taylor AJ. Allergic bronchopulmonary aspergillosis. *Clin Exp Allergy* 1997;**27**(suppl 1):55–9.
118. Elston C, Geddes D. Inflammation in cystic fibrosis: when and why? Friend or foe? *Semin Respir Crit Care Med* 2007;**28**:286–94.
119. Emerson J, McNamara S, Buccat AM, et al. Changes in cystic fibrosis sputum microbiology in the United States between 1995 and 2008. *Pediatr Pulmonol* 2010;**45**:363–70.
120. Emerson J, Rosenfeld M, McNamara S, et al. *Pseudomonas aeruginosa* and other predictors of mortality and morbidity in young children with cystic fibrosis. *Pediatr Pulmonol* 2002;**34**: 91–100.
121. Engelhardt JF, Yankaskas JR, Ernst SA, et al. Submucosal glands are the predominant site of CFTR expression in the human bronchus. *Nat Genet* 1992;**2**:240–8.
122. Equi A, Balfour-Lynn IM, Bush A, et al. Long term azithromycin in children with cystic fibrosis: a randomised, placebo-controlled crossover trial. *Lancet* 2002;**360**:978–84.
123. Ernst RK, Adams KN, Moskowitz SM, et al. The *Pseudomonas aeruginosa* lipid A deacylase: selection for expression and loss within the cystic fibrosis airway. *J Bacteriol* 2006;**188**:191–201.
124. Fabbri A, Tacchella A, Manno G, et al. Emerging microorganisms in cystic fibrosis. *Chemioterapia* 1987;**6**:32–7.
125. Fahy JV, Dickey BF. Airway mucus function and dysfunction. *N Engl J Med* 2010;**363**:2233–47.
126. Farrell PM, Kosorok MR, Laxova A, et al. Nutritional benefits of neonatal screening for cystic fibrosis. Wisconsin Cystic Fibrosis Neonatal Screening Study Group. *N Engl J Med* 1997;**337**: 963–9.
127. Farrell PM, Li Z, Kosorok MR, et al. Bronchopulmonary disease in children with cystic fibrosis after early or delayed diagnosis. *Am J Respir Crit Care Med* 2003;**168**:1100–8.
128. Farrell PM, Li Z, Kosorok MR, et al. Longitudinal evaluation of bronchopulmonary disease in children with cystic fibrosis. *Pediatr Pulmonol* 2003;**36**:230–40.
129. Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. *J Pediatr* 2008;**153**:S4–14.
130. Feranchak AP, Sokol RJ. Cholangiocyte biology and cystic fibrosis liver disease. *Semin Liver Dis* 2001;**21**:471–88.
131. Fick RB Jr, Sonoda F, Hornick DB. Emergence and persistence of *Pseudomonas aeruginosa* in the cystic fibrosis airway. *Semin Respir Infect* 1992;**7**:168–78.
132. Fiore AE, Shay DK, Haber P, et al. Prevention and control of influenza. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2007. *MMWR Recomm Rep* 2007; **56**:1–54.
133. Firoved AM, Ornatowski W, Deretic V. Microarray analysis reveals induction of lipoprotein genes in mucoid *Pseudomonas aeruginosa:* implications for inflammation in cystic fibrosis. *Infect Immun* 2004;**72**:5012–18.
134. FitzSimmons SC. The changing epidemiology of cystic fibrosis. *J Pediatr* 1993;**122**:1–9.
135. Flume PA, Egan TM, Paradowski LJ, et al. Infectious complications of lung transplantation. Impact of cystic fibrosis. *Am J Respir Crit Care Med* 1994;**149**:1601–7.
136. Frederiksen B, Koch C, Hoiby N. Antibiotic treatment of initial colonization with *Pseudomonas aeruginosa* postpones chronic infection and prevents deterioration of pulmonary function in cystic fibrosis. *Pediatr Pulmonol* 1997;**23**:330–5.
137. Frederiksen B, Koch C, Hoiby N. Changing epidemiology of *Pseudomonas aeruginosa* infection in Danish cystic fibrosis patients (1974-1995). *Pediatr Pulmonol* 1999;**28**:159–66.
138. Frederiksen B, Pressler T, Hansen A, et al. Effect of aerosolized rhDNase (Pulmozyme) on pulmonary colonization in patients with cystic fibrosis. *Acta Paediatr* 2006;**95**:1070–4.
139. Gabriel SE, Clarke LL, Boucher RC, et al. CFTR and outward rectifying chloride channels are distinct proteins with a regulatory relationship. *Nature* 1993;**363**:263–8.
140. Gales AC, Sader HS, Andrade SS, et al. Emergence of linezolid-resistant *Staphylococcus aureus* during treatment of pulmonary infection in a patient with cystic fibrosis. *Int J Antimicrob Agents* 2006;**27**:300–2.
141. Galli F, Battistoni A, Gambari R, et al. Oxidative stress and antioxidant therapy in cystic fibrosis. *Biochim Biophys Acta* 2012; **1822**:690–713.
142. Garcia DF, Hiatt PW, Jewell A, et al. Human metapneumovirus and respiratory syncytial virus infections in older children with cystic fibrosis. *Pediatr Pulmonol* 2007;**42**:66–74.
143. Garland JS, Chan YM, Kelly KJ, et al. Outcome of infants with cystic fibrosis requiring mechanical ventilation for respiratory failure. *Chest* 1989;**96**:136–8.
144. Garske LA, Kidd TJ, Gan R, et al. Rifampicin and sodium fusidate reduces the frequency of methicillin-resistant *Staphylococcus aureus* (MRSA) isolation in adults with cystic fibrosis and chronic MRSA infection. *J Hosp Infect* 2004;**56**:208–14.
145. Geller DE, Flume PA, Staab D, et al. Levofloxacin inhalation solution (MP-376) in patients with cystic fibrosis with *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2011;**183**:1510–16.
146. Geller DE, Kaplowitz H, Light MJ, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis: reported prevalence, regional distribution, and patient characteristics. Scientific Advisory Group, Investigators, and Coordinators of the Epidemiologic Study of Cystic Fibrosis. *Chest* 1999;**116**:639–46.
147. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med* 2003;**168**:918–51.
148. Gibson RL, Emerson J, McNamara S, et al. Significant microbiological effect of inhaled tobramycin in young children with cystic fibrosis. *Am J Respir Crit Care Med* 2003;**167**:841–9.
149. Gilligan PH. Microbiology of airway disease in patients with cystic fibrosis. *Clin Microbiol Rev* 1991;**4**:35–51.

150. Gilljam M, Antoniou M, Shin J, et al. Pregnancy in cystic fibrosis. Fetal and maternal outcome. *Chest* 2000;**118**:85–91.
151. Gilljam M, Berning SE, Peloquin CA, et al. Therapeutic drug monitoring in patients with cystic fibrosis and mycobacterial disease. *Eur Respir J* 1999;**14**:347–51.
152. Givney R, Vickery A, Holliday A, et al. Methicillin-resistant *Staphylococcus aureus* in a cystic fibrosis unit. *J Hosp Infect* 1997; **35**:27–36.
153. Gold R, Carpenter S, Heurter H, et al. Randomized trial of ceftazidime versus placebo in the management of acute respiratory exacerbations in patients with cystic fibrosis. *J Pediatr* 1987;**111**: 907–13.
154. Gold R, Overmeyer A, Knie B, et al. Controlled trial of ceftazidime vs. ticarcillin and tobramycin in the treatment of acute respiratory exacerbations in patients with cystic fibrosis. *Pediatr Infect Dis* 1985;**4**:172–7.
155. Goldman MJ, Anderson GM, Stolzenberg ED, et al. Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* 1997;**88**:553–60.
156. Goss CH, Muhlebach MS. *Staphylococcus aureus* and MRSA in cystic fibrosis [Review]. *J Cyst Fibros* 2011;**10**:298–306.
157. Goss CH, Aitken ML, Otto K, Rubenfeld GD. Acquiring *Stenotrophomonas maltophilia* does not reduce survival in patients with cystic fibrosis. *Pediatr Pulmonol* 2000;**S20**:101–2.
158. Goss CH, Rubenfeld GD, Otto K, et al. The effect of pregnancy on survival in women with cystic fibrosis. *Chest* 2003;**124**: 1460–8.
159. Govan JR, Brown PH, Maddison J, et al. Evidence for transmission of *Pseudomonas cepacia* by social contact in cystic fibrosis. *Lancet* 1993;**342**:15–19.
160. Govan JR, Deretic V. Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev* 1996;**60**:539–74.
161. Govan JR, Nelson JW. Microbiology of lung infection in cystic fibrosis. *Br Med Bull* 1992;**48**:912–30.
162. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
163. Grosse SD, Rosenfeld M, Devine OJ, et al. Potential impact of newborn screening for cystic fibrosis on child survival: a systematic review and analysis. *J Pediatr* 2006;**149**:362–6.
164. Guggino WB. Cystic fibrosis and the salt controversy. *Cell* 1999;**96**:607–10.
165. Gutierrez JP, Grimwood K, Armstrong DS, et al. Interlobar differences in bronchoalveolar lavage fluid from children with cystic fibrosis. *Eur Respir J* 2001;**17**:281–6.
166. Haardt M, Benharouga M, Lechardeur D, et al. C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. *J Biol Chem* 1999;**274**:21873–7.
167. Hadjiliadis D, Steele MP, Chaparro C, et al. Survival of lung transplant patients with cystic fibrosis harboring panresistant bacteria other than *Burkholderia cepacia*, compared with patients harboring sensitive bacteria. *J Heart Lung Transplant* 2007;**26**: 834–8.
168. Hanna SL, Sherman NE, Kinter MT, et al. Comparison of proteins expressed by *Pseudomonas aeruginosa* strains representing initial and chronic isolates from a cystic fibrosis patient: an analysis by 2-D gel electrophoresis and capillary column liquid chromatography-tandem mass spectrometry. *Microbiology* 2000;**146**(Pt 10):2495–508.
169. Hauber HP, Manoukian JJ, Nguyen LH, et al. Increased expression of interleukin-9, interleukin-9 receptor, and the calcium-activated chloride channel hCLCA1 in the upper airways of patients with cystic fibrosis. *Laryngoscope* 2003;**113**:1037–42.
170. Henry D, Campbell M, McGimpsey C, et al. Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. *J Clin Microbiol* 1999;**37**:1004–7.
171. Hiatt PW, Grace SC, Kozinetz CA, et al. Effects of viral lower respiratory tract infection on lung function in infants with cystic fibrosis. *Pediatrics* 1999;**103**:619–26.
172. Hill D, Rose B, Pajkos A, et al. Antibiotic susceptibilities of *Pseudomonas aeruginosa* isolates derived from patients with cystic fibrosis under aerobic, anaerobic, and biofilm conditions. *J Clin Microbiol* 2005;**43**:5085–90.
173. Hillian AD, Londono D, Dunn JM, et al. Modulation of cystic fibrosis lung disease by variants in interleukin-8. *Genes Immun* 2008;**9**:501–8.
174. Hilliard T, Edwards S, Buchdahl R, et al. Voriconazole therapy in children with cystic fibrosis. *J Cyst Fibros* 2005;**4**:215–20.
175. Ho SA, Ball R, Morrison LJ, et al. Clinical value of obtaining sputum and cough swab samples following inhaled hypertonic saline in children with cystic fibrosis. *Pediatr Pulmonol* 2004;**38**: 82–7.
176. Hodson ME, Roberts CM, Butland RJ, et al. Oral ciprofloxacin compared with conventional intravenous treatment for *Pseudomonas aeruginosa* infection in adults with cystic fibrosis. *Lancet* 1987; **1**:235–7.
177. Hohn DC, MacKay RD, Halliday B, et al. Effect of O2 tension on microbicidal function of leukocytes in wounds and in vitro. *Surg Forum* 1976;**27**:18–20.
178. Holmes A, Nolan R, Taylor R, et al. An epidemic of *Burkholderia cepacia* transmitted between patients with and without cystic fibrosis. *J Infect Dis* 1999;**179**:1197–205.
179. Hordvik NL, Konig P, Hamory B, et al. Effects of acute viral respiratory tract infections in patients with cystic fibrosis. *Pediatr Pulmonol* 1989;**7**:217–22.
180. Howe RA, Spencer RC. Macrolides for the treatment of *Pseudomonas aeruginosa* infections? *J Antimicrob Chemother* 1997;**40**: 153–5.
181. Hubeau C, Le Naour R, Abely M, et al. Dysregulation of IL-2 and IL-8 production in circulating T lymphocytes from young cystic fibrosis patients. *Clin Exp Immunol* 2004;**135**:528–34.
182. Hubert D, Le Roux E, Lavrut T, et al. Continuous versus intermittent infusions of ceftazidime for treating exacerbation of cystic fibrosis. *Antimicrob Agents Chemother* 2009;**53**:3650–6.
183. Hudson VL, Wielinski CL, Regelmann WE. Prognostic implications of initial oropharyngeal bacterial flora in patients with cystic fibrosis diagnosed before the age of two years. *J Pediatr* 1993; **122**:854–60.
184. Hyatt AC, Chipps BE, Kumor KM, et al. A double-blind controlled trial of anti-*Pseudomonas* chemotherapy of acute respiratory exacerbations in patients with cystic fibrosis. *J Pediatr* 1981;**99**: 307–14.
185. Hyde SC, Emsley P, Hartshorn MJ, et al. Structural model of ATP-binding proteins associated with cystic fibrosis, multidrug resistance and bacterial transport. *Nature* 1990;**346**:362–5.
186. Ichikawa Y, Ninomiya H, Koga H, et al. Erythromycin reduces neutrophils and neutrophil-derived elastolytic-like activity in the lower respiratory tract of bronchiolitis patients. *Am Rev Respir Dis* 1992;**146**:196–203.
187. Ichimiya T, Takeoka K, Hiramatsu K, et al. The influence of azithromycin on the biofilm formation of *Pseudomonas aeruginosa* in vitro. *Chemotherapy* 1996;**42**:186–91.
188. Isles A, Maclusky I, Corey M, et al. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* 1984;**104**: 206–10.
189. Jackson MA, Kusmiesz H, Shelton S, et al. Comparison of piperacillin vs. ticarcillin plus tobramycin in the treatment of acute pulmonary exacerbations of cystic fibrosis. *Pediatr Infect Dis* 1986;**5**:440–3.
190. Jayaraman S, Joo NS, Reitz B, et al. Submucosal gland secretions in airways from cystic fibrosis patients have normal [Na(+)] and pH but elevated viscosity. *Proc Natl Acad Sci U S A* 2001;**98**: 8119–23.
191. Jensen T, Pedersen SS, Hoiby N, et al. Use of antibiotics in cystic fibrosis. The Danish approach. *Antibiot Chemother* 1989;**42**: 237–46.
192. Jewett CV, Ledbetter J, Lyrene RK, et al. Comparison of cefoperazone sodium vs methicillin, ticarcillin, and tobramycin in treatment of pulmonary exacerbations in patients with cystic fibrosis. *J Pediatr* 1985;**106**:669–72.
193. Johansen HK, Hoiby N. Seasonal onset of initial colonisation and chronic infection with *Pseudomonas aeruginosa* in patients with cystic fibrosis in Denmark. *Thorax* 1992;**47**:109–11.
194. Jung JS, Lee JY, Oh SO, et al. Effect of t-butylhydroperoxide on chloride secretion in rat tracheal epithelia. *Pharmacol Toxicol* 1998;**82**:236–42.

195. Kabra SK, Pawaiya R, Lodha R, et al. Long-term daily high and low doses of azithromycin in children with cystic fibrosis: a randomized controlled trial. *J Cyst Fibros* 2010;**9**:17–23.
196. Kalish LA, Waltz DA, Dovey M, et al. Impact of *Burkholderia dolosa* on lung function and survival in cystic fibrosis. *Am J Respir Crit Care Med* 2006;**173**:421–5.
197. Kanj SS, Tapson V, Davis RD, et al. Infections in patients with cystic fibrosis following lung transplantation. *Chest* 1997;**112**:924–30.
198. Kazachkov MY, Muhlebach MS, Livasy CA, et al. Lipid-laden macrophage index and inflammation in bronchoalveolar lavage fluids in children. *Eur Respir J* 2001;**18**:790–5.
199. Keel RA, Schaeftlein A, Kloft C, et al. Pharmacokinetics of intravenous and oral linezolid in adults with cystic fibrosis. *Antimicrob Agents Chemother* 2011;**55**:3393–8.
200. Kerem E, Corey M, Gold R, et al. Pulmonary function and clinical course in patients with cystic fibrosis after pulmonary colonization with *Pseudomonas aeruginosa*. *J Pediatr* 1990;**116**:714–19.
201. Kerem E, Corey M, Kerem B, et al. Clinical and genetic comparisons of patients with cystic fibrosis, with or without meconium ileus. *J Pediatr* 1989;**114**:767–73.
202. Kerem E, Corey M, Kerem BS, et al. The relation between genotype and phenotype in cystic fibrosis: analysis of the most common mutation (delta F508). *N Engl J Med* 1990;**323**:1517–22.
203. Khair OA, Davies RJ, Devalia JL. Bacterial-induced release of inflammatory mediators by bronchial epithelial cells. *Eur Respir J* 1996;**9**:1913–22.
204. Knowles M, Gatzy J, Boucher R. Relative ion permeability of normal and cystic fibrosis nasal epithelium. *J Clin Invest* 1983; **71**:1410–17.
205. Knowles MR. Gene modifiers of lung disease. *Curr Opin Pulm Med* 2006;**12**:416–21.
206. Knowles MR, Boucher RC. Mucus clearance as a primary innate defense mechanism for mammalian airways. *J Clin Invest* 2002; **109**:571–7.
207. Kobayashi H, Takeda H, Sakayori S, et al. [Study on azithromycin in treatment of diffuse panbronchiolitis]. *Kansenshogaku Zasshi* 1995;**69**:711–22.
208. Koch C, Frederiksen B, Hoiby N. Patient cohorting and infection control. *Semin Respir Crit Care Med* 2003;**24**:703–16.
209. Kondoh K, Hashiba M, Baba S. Inhibitory activity of clarithromycin on biofilm synthesis with *Pseudomonas aeruginosa*. *Acta Otolaryngol Suppl* 1996;**525**:56–60.
210. Konstan MW, Butler SM, Wohl ME, et al. Growth and nutritional indexes in early life predict pulmonary function in cystic fibrosis. *J Pediatr* 2003;**142**:624–30.
211. Konstan MW, Byard PJ, Hoppel CL, et al. Effect of high-dose ibuprofen in patients with cystic fibrosis. *N Engl J Med* 1995; **332**:848–54.
212. Koscik RL, Farrell PM, Kosorok MR, et al. Cognitive function of children with cystic fibrosis: deleterious effect of early malnutrition. *Pediatrics* 2004;**113**:1549–58.
213. Koscik RL, Lai HJ, Laxova A, et al. Preventing early, prolonged vitamin E deficiency: an opportunity for better cognitive outcomes via early diagnosis through neonatal screening. *J Pediatr* 2005;**147**:S51–6.
214. Kosorok MR, Jalaluddin M, Farrell PM, et al. Comprehensive analysis of risk factors for acquisition of *Pseudomonas aeruginosa* in young children with cystic fibrosis. *Pediatr Pulmonol* 1998; **26**:81–8.
215. Krilov LR, Blumer JL, Stern RC, et al. Imipenem/cilastatin in acute pulmonary exacerbations of cystic fibrosis. *Rev Infect Dis* 1985;**7**(Suppl 3):S482–9.
216. Kudoh S, Azuma A, Yamamoto M, et al. Improvement of survival in patients with diffuse panbronchiolitis treated with low-dose erythromycin. *Am J Respir Crit Care Med* 1998;**157**:1829–32.
217. Kunzelmann K, Schreiber R, Nitschke R, et al. Control of epithelial Na+ conductance by the cystic fibrosis transmembrane conductance regulator. *Pflugers Arch* 2000;**440**:193–201.
218. Lai HC, Kosorok MR, Sondel SA, et al. Growth status in children with cystic fibrosis based on the National Cystic Fibrosis Patient Registry data: evaluation of various criteria used to identify malnutrition. *J Pediatr* 1998;**132**:478–85.
219. Lambiase A, Catania MR, Del Pezzo M, et al. Achromobacter xylosoxidans respiratory tract infection in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 2011;**30**:973–80.
220. Lambiase A, Raia V, Del Pezzo M, et al. Microbiology of airway disease in a cohort of patients with cystic fibrosis. *BMC Infect Dis* 2006;**6**:4.
221. Lang BJ, Aaron SD, Ferris W, et al. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multiresistant strains of *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2000;**162**:2241–5.
222. Latzin P, Fehling M, Bauernfeind A, et al. Efficacy and safety of intravenous meropenem and tobramycin versus ceftazidime and tobramycin in cystic fibrosis. *J Cyst Fibros* 2008;**7**:142–6.
223. Lechtzin N, John M, Irizarry R, et al. Outcomes of adults with cystic fibrosis infected with antibiotic-resistant *Pseudomonas aeruginosa*. *Respiration* 2006;**73**:27–33.
224. Linsdell P, Hanrahan JW. Glutathione permeability of CFTR. *Am J Physiol* 1998;**275**:C323–6.
225. LiPuma JJ. Burkholderia cepacia epidemiology and pathogenesis: implications for infection control. *Curr Opin Pulm Med* 1998;**4**:337–41.
226. LiPuma JJ. *Burkholderia cepacia* complex: a contraindication to lung transplantation in cystic fibrosis? *Transpl Infect Dis* 2001;**3**: 149–60.
227. LiPuma JJ. Burkholderia and emerging pathogens in cystic fibrosis. *Semin Respir Crit Care Med* 2003;**24**:681–92.
228. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;**23**:299–323.
229. LiPuma JJ, Dasen SE, Nielson DW, et al. Person-to-person transmission of *Pseudomonas cepacia* between patients with cystic fibrosis. *Lancet* 1990;**336**:1094–6.
230. LiPuma JJ, Spilker T, Gill LH, et al. Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. *Am J Respir Crit Care Med* 2001;**164**: 92–6.
231. Lloyd-Still JD, Khaw KT, Shwachman H. Severe respiratory disease in infants with cystic fibrosis. *Pediatrics* 1974;**53**:678–82.
232. Long FR, Williams RS, Castile RG. Structural airway abnormalities in infants and young children with cystic fibrosis. *J Pediatr* 2004;**144**:154–61.
233. Luna RA, Fasciano LR, Jones SC, et al. DNA pyrosequencing-based bacterial pathogen identification in a pediatric hospital setting. *J Clin Microbiol* 2007;**45**:2985–92.
234. Macfarlane M, Leavy A, McCaughan J, et al. Successful decolonization of methicillin-resistant *Staphylococcus aureus* in paediatric patients with cystic fibrosis (CF) using a three-step protocol. *J Hosp Infect* 2007;**65**:231–6.
235. Mahenthiralingam E, Bischof J, Byrne SK, et al. DNA-Based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. *J Clin Microbiol* 2000;**38**:3165–73.
236. Mahenthiralingam E, Vandamme P, Campbell ME, et al. Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. *Clin Infect Dis* 2001;**33**: 1469–75.
237. Maiz L, Canton R, Mir N, et al. Aerosolized vancomycin for the treatment of methicillin-resistant *Staphylococcus aureus* infection in cystic fibrosis. *Pediatr Pulmonol* 1998;**26**:287–9.
238. Mall M, Grubb BR, Harkema JR, et al. Increased airway epithelial Na+ absorption produces cystic fibrosis-like lung disease in mice. *Nat Med* 2004;**10**:487–93.
239. Malouf MA, Glanville AR. The spectrum of mycobacterial infection after lung transplantation. *Am J Respir Crit Care Med* 1999; **160**:1611–16.
240. Mandell G, Bennett JE, Dolin R. *Principles and practice of infectious diseases*. 6th ed. Philadelphia: W. B. Saunders; 2005.
241. Marchac V, Equi A, Le Bihan-Benjamin C, et al. Case-control study of *Stenotrophomonas maltophilia* acquisition in cystic fibrosis patients. *Eur Respir J* 2004;**23**:98–102.
242. Martinez TM, Llapur CJ, Williams TH, et al. High-resolution computed tomography imaging of airway disease in infants with cystic fibrosis. *Am J Respir Crit Care Med* 2005;**172**:1133–8.
243. Mastella G, Rainisio M, Harms HK, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis. A European epidemiological study. Epidemiologic Registry of Cystic Fibrosis. *Eur Respir J* 2000;**16**:464–71.

244. Matsui H, Grubb BR, Tarran R, et al. Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease. *Cell* 1998;**95**: 1005–15.
245. McCallum SJ, Corkill J, Gallagher M, et al. Superinfection with a transmissible strain of *Pseudomonas aeruginosa* in adults with cystic fibrosis chronically colonised by *P aeruginosa*. *Lancet* 2001; **358**:558–60.
246. McCallum SJ, Gallagher MJ, Corkill JE, et al. Spread of an epidemic *Pseudomonas aeruginosa* strain from a patient with cystic fibrosis (CF) to non-CF relatives. *Thorax* 2002;**57**:559–60.
247. McCallum TJ, Milunsky JM, Cunningham DL, et al. Fertility in men with cystic fibrosis: an update on current surgical practices and outcomes. *Chest* 2000;**118**:1059–62.
248. McColley SA, Ren CL, Schechter MS, et al. Risk factors for onset of persistent respiratory symptoms in children with cystic fibrosis. *Pediatr Pulmonol* 2012;**47**:966–72.
249. McCoy KS, Quittner AL, Oermann CM, et al. Inhaled aztreonam lysine for chronic airway *Pseudomonas aeruginosa* in cystic fibrosis. *Am J Respir Crit Care Med* 2008;**178**:921–8.
250. McGrath LT, Mallon P, Dowey L, et al. Oxidative stress during acute respiratory exacerbations in cystic fibrosis. *Thorax* 1999; **54**:518–23.
251. McKone EF, Shao J, Frangolias DD, et al. Variants in the glutamate-cysteine-ligase gene are associated with cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2006;**174**:415–19.
252. McLaughlin FJ, Matthews WJ Jr, Strieder DJ, et al. Clinical and bacteriological responses to three antibiotic regimens for acute exacerbations of cystic fibrosis: ticarcillin-tobramycin, azlocillin-tobramycin, and azlocillin-placebo. *J Infect Dis* 1983;**147**:559–67.
253. Mearns MB. Treatment and prevention of pulmonary complications of cystic fibrosis in infancy and early childhood. *Arch Dis Child* 1972;**47**:5–11.
254. Meyer KC, Sharma A. Regional variability of lung inflammation in cystic fibrosis. *Am J Respir Crit Care Med* 1997;**156**:1536–40.
255. Miall LS, McGinley NT, Brownlee KG, et al. Methicillin resistant *Staphylococcus aureus* (MRSA) infection in cystic fibrosis. *Arch Dis Child* 2001;**84**:160–2.
256. Milla CE, Billings J, Moran A. Diabetes is associated with dramatically decreased survival in female but not male subjects with cystic fibrosis. *Diabetes Care* 2005;**28**:2141–4.
257. Mizukane R, Hirakata Y, Kaku M, et al. Comparative in vitro exoenzyme-suppressing activities of azithromycin and other macrolide antibiotics against Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 1994;**38**:528–33.
258. Molinari G, Guzman CA, Pesce A, et al. Inhibition of *Pseudomonas aeruginosa* virulence factors by subinhibitory concentrations of azithromycin and other macrolide antibiotics. *J Antimicrob Chemother* 1993;**31**:681–8.
259. Moran A, Brunzell C, Cohen RC, et al. Clinical care guidelines for cystic fibrosis-related diabetes: a position statement of the American Diabetes Association and a clinical practice guideline of the Cystic Fibrosis Foundation, endorsed by the Pediatric Endocrine Society. *Diabetes Care* 2010;**33**:2697–708.
260. Moran A, Pyzdrowski KL, Weinreb J, et al. Insulin sensitivity in cystic fibrosis. *Diabetes* 1994;**43**:1020–6.
261. Morgan WJ, Butler SM, Johnson CA, et al. Epidemiologic study of cystic fibrosis: design and implementation of a prospective, multicenter, observational study of patients with cystic fibrosis in the U.S. and Canada. *Pediatr Pulmonol* 1999;**28**:231–41.
262. Morris MR, Doull IJ, Dewitt S, et al. Reduced iC3b-mediated phagocytotic capacity of pulmonary neutrophils in cystic fibrosis. *Clin Exp Immunol* 2005;**142**:68–75.
263. Moskowitz SM, Chmiel JF, Sternen DL, et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet Med* 2008;**10**:851–68.
264. Moskowitz SM, Emerson JC, McNamara S, et al. Randomized trial of biofilm testing to select antibiotics for cystic fibrosis airway infection. *Pediatr Pulmonol* 2011;**46**:184–92.
265. Moskowitz SM, Foster JM, Emerson J, et al. Clinically feasible biofilm susceptibility assay for isolates of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *J Clin Microbiol* 2004;**42**: 1915–22.
266. Moss RB. Cystic fibrosis: pathogenesis, pulmonary infection, and treatment. *Clin Infect Dis* 1995;**21**:839–49; quiz 850-831.
267. Moss RB, King VV. Management of sinusitis in cystic fibrosis by endoscopic surgery and serial antimicrobial lavage: reduction in recurrence requiring surgery. *Arch Otolaryngol Head Neck Surg* 1995;**121**:566–72.
268. Mueller C, Braag SA, Keeler A, et al. Lack of cystic fibrosis transmembrane conductance regulator in CD3+ lymphocytes leads to aberrant cytokine secretion and hyperinflammatory adaptive immune responses. *Am J Respir Cell Mol Biol* 2011;**44**:922–9.
269. Muhlebach MS, Stewart PW, Leigh MW, et al. Quantitation of inflammatory responses to bacteria in young cystic fibrosis and control patients. *Am J Respir Crit Care Med* 1999;**160**:186–91.
270. Murphy TM, Rosenstein BJ. *Cystic fibrosis lung disease: approaching the 21st century*. University of Chicago, Pritzker School of Medicine; 1995.
271. Mussaffi H, Rivlin J, Shalit I, et al. Nontuberculous mycobacteria in cystic fibrosis associated with allergic bronchopulmonary aspergillosis and steroid therapy. *Eur Respir J* 2005;**25**:324–8.
272. National Committee Clinical Laboratory Standards. *Performance standards for antimicrobial susceptibility testing*. Wayne, Pa; 2001.
273. Nazer D, Abdulhamid I, Thomas R, et al. Home versus hospital intravenous antibiotic therapy for acute pulmonary exacerbations in children with cystic fibrosis. *Pediatr Pulmonol* 2006;**41**:744–9.
274. Nepomuceno IB, Esrig S, Moss RB. Allergic bronchopulmonary aspergillosis in cystic fibrosis: role of atopy and response to itraconazole. *Chest* 1999;**115**:364–70.
275. Nguyen TD, Canada AT. Modulation of human colonic T84 cell secretion by hydrogen peroxide. *Biochem Pharmacol* 1994;**47**: 403–10.
276. Nikolaizik WH, Schoni MH. Pilot study to assess the effect of inhaled corticosteroids on lung function in patients with cystic fibrosis. *J Pediatr* 1996;**128**:271–4.
277. Nixon GM, Armstrong DS, Carzino R, et al. Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001;**138**:699–704.
278. Nunley DR, Grgurich W, Iacono AT, et al. Allograft colonization and infections with pseudomonas in cystic fibrosis lung transplant recipients. *Chest* 1998;**113**:1235–43.
279. O'Carroll MR, Syrmis MW, Wainwright CE, et al. Clonal strains of *Pseudomonas aeruginosa* in paediatric and adult cystic fibrosis units. *Eur Respir J* 2004;**24**:101–6.
280. O'Connor GT, Quinton HB, Kneeland T, et al. Median household income and mortality rate in cystic fibrosis. *Pediatrics* 2003;**111**:e333–9.
281. Oermann CM, Retsch-Bogart GZ, Quittner AL, et al. An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr Pulmonol* 2010;**45**:1121–34.
282. Ogle JW, Reller LB, Vasil ML. Development of resistance in *Pseudomonas aeruginosa* to imipenem, norfloxacin, and ciprofloxacin during therapy: proof provided by typing with a DNA probe. *J Infect Dis* 1988;**157**:743–8.
283. Ogle JW, Janda JM, Woods DE, et al. Characterization and use of a DNA probe as an epidemiological marker for *Pseudomonas aeruginosa*. *J Infect Dis* 1987;**155**:119–26.
284. Olesen HV, Nielsen LP, Schiotz PO. Viral and atypical bacterial infections in the outpatient pediatric cystic fibrosis clinic. *Pediatr Pulmonol* 2006;**41**:1197–204.
285. Oliver A, Canton R, Campo P, et al. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 2000;**288**:1251–4.
286. Olivier KN, Yankaskas JR, Knowles MR. Nontuberculous mycobacterial pulmonary disease in cystic fibrosis. *Semin Respir Infect* 1996;**11**:272–84.
287. Olivier KN, Weber DJ, Lee JH, et al. Nontuberculous mycobacteria. II: nested-cohort study of impact on cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2003;**167**:835–40.
288. Olivier KN, Weber DJ, Wallace RJ Jr, et al. Nontuberculous mycobacteria. I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med* 2003;**167**:828–34.
289. Ong EL, Ellis ME, Webb AK, et al. Infective respiratory exacerbations in young adults with cystic fibrosis: role of viruses and atypical microorganisms. *Thorax* 1989;**44**:739–42.
290. Ordonez CL, Kartashov AI, Wohl ME. Variability of markers of inflammation and infection in induced sputum in children with cystic fibrosis. *J Pediatr* 2004;**145**:689–92.

291. Orenstein DM, Pattishall EN, Nixon PA, et al. Quality of well-being before and after antibiotic treatment of pulmonary exacerbation in patients with cystic fibrosis. *Chest* 1990;**98**:1081–4.
292. Padoan R, Cambisano W, Costantini D, et al. Ceftazidime monotherapy vs. combined therapy in *Pseudomonas* pulmonary infections in cystic fibrosis. *Pediatr Infect Dis J* 1987;**6**:648–53.
293. Pamukcu A, Bush A, Buchdahl R. Effects of *Pseudomonas aeruginosa* colonization on lung function and anthropometric variables in children with cystic fibrosis. *Pediatr Pulmonol* 1995;**19**:10–15.
294. Parad RB, Gerard CJ, Zurakowski D, et al. Pulmonary outcome in cystic fibrosis is influenced primarily by mucoid *Pseudomonas aeruginosa* infection and immune status and only modestly by genotype. *Infect Immun* 1999;**67**:4744–50.
295. Paradowski LJ. Saprophytic fungal infections and lung transplantation: revisited. *J Heart Lung Transplant* 1997;**16**:524–31.
296. Park MK, Myers RA, Marzella L. Oxygen tensions and infections: modulation of microbial growth, activity of antimicrobial agents, and immunologic responses. *Clin Infect Dis* 1992;**14**:720–40.
297. Park RW, Grand RJ. Gastrointestinal manifestations of cystic fibrosis: a review. *Gastroenterology* 1981;**81**:1143–61.
298. Parry MF, Neu HC. Tobramycin and ticarcillin therapy for exacerbations of pulmonary disease in patients with cystic fibrosis. *J Infect Dis* 1976;**134**(Suppl):S194–7.
299. Pegues DA, Carson LA, Tablan OC, et al. Acquisition of *Pseudomonas cepacia* at summer camps for patients with cystic fibrosis. Summer Camp Study Group. *J Pediatr* 1994;**124**:694–702.
300. Pegues DA, Schidlow DV, Tablan OC, et al. Possible nosocomial transmission of *Pseudomonas cepacia* in patients with cystic fibrosis. *Arch Pediatr Adolesc Med* 1994;**148**:805–12.
301. Peloquin CA. Using therapeutic drug monitoring to dose the antimycobacterial drugs. *Clin Chest Med* 1997;**18**:79–87.
302. Petersen NT, Hoiby N, Mordhorst CH, et al. Respiratory infections in cystic fibrosis patients caused by virus, chlamydia and mycoplasma: possible synergism with *Pseudomonas aeruginosa*. *Acta Paediatr Scand* 1981;**70**:623–8.
303. Philippon S, Streckert HJ, Morgenroth K. In vitro study of the bronchial mucosa during *Pseudomonas aeruginosa* infection. *Virchows Arch A Pathol Anat Histopathol* 1993;**423**:39–43.
304. Piedra PA, Cron SG, Jewell A, et al. Immunogenicity of a new purified fusion protein vaccine to respiratory syncytial virus: a multi-center trial in children with cystic fibrosis. *Vaccine* 2003;**21**:2448–60.
305. Piedra PA, Grace S, Jewell A, et al. Purified fusion protein vaccine protects against lower respiratory tract illness during respiratory syncytial virus season in children with cystic fibrosis. *Pediatr Infect Dis J* 1996;**15**:23–31.
306. Piedra PA, Grace S, Jewell A, et al. Sequential annual administration of purified fusion protein vaccine against respiratory syncytial virus in children with cystic fibrosis. *Pediatr Infect Dis J* 1998;**17**:217–24.
307. Pier GB, Grout M, Zaidi TS. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung. *Proc Natl Acad Sci U S A* 1997;**94**:12088–93.
308. Pier GB, Grout M, Zaidi TS, et al. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science* 1996;**271**:64–7.
309. Plotkowski MC, Chevillard M, Pierrot D, et al. Differential adhesion of *Pseudomonas aeruginosa* to human respiratory epithelial cells in primary culture. *J Clin Invest* 1991;**87**:2018–28.
310. Przyklenk B, Bauernfeind A, Bertele RM, et al. Viral infections of the respiratory tract in patients with cystic fibrosis. *Serodian Immunther Infect Dis* 1988;**2**:217.
311. Raman V, Clary R, Siegrist KL, et al. Increased prevalence of mutations in the cystic fibrosis transmembrane conductance regulator in children with chronic rhinosinusitis. *Pediatrics* 2002;**109**:E13.
312. Ramphal R, Houdret N, Koo L, et al. Differences in adhesion of *Pseudomonas aeruginosa* to mucin glycopeptides from sputa of patients with cystic fibrosis and chronic bronchitis. *Infect Immun* 1989;**57**:3066–71.
313. Ramsey BW. Management of pulmonary disease in patients with cystic fibrosis. *N Engl J Med* 1996;**335**:179–88.
314. Ramsey BW, Gore EJ, Smith AL, et al. The effect of respiratory viral infections on patients with cystic fibrosis. *Am J Dis Child* 1989;**143**:662–8.
315. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med* 1999;**340**:23–30.
316. Ramsey DM, Wozniak DJ. Understanding the control of *Pseudomonas aeruginosa* alginate synthesis and the prospects for management of chronic infections in cystic fibrosis. *Mol Microbiol* 2005;**56**:309–22.
317. Ratjen F, Doring G, Nikolaizik WH. Effect of inhaled tobramycin on early *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. *Lancet* 2001;**358**:983–4.
318. Ratjen F, Munck A, Kho P, et al. Treatment of early *Pseudomonas aeruginosa* infection in patients with cystic fibrosis: the ELITE trial. *Thorax* 2010;**65**:286–91.
319. Ratner D, Mueller C. Immune responses in cystic fibrosis: are they intrinsically defective? *Am J Respir Cell Mol Biol* 2012;**46**:715–22.
320. Razvi S, Quittell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. *Chest* 2009;**136**:1554–60.
321. Regelmann WE, Elliott GR, Warwick WJ, et al. Reduction of sputum *Pseudomonas aeruginosa* density by antibiotics improves lung function in cystic fibrosis more than do bronchodilators and chest physiotherapy alone. *Am Rev Respir Dis* 1990;**141**:914–21.
322. Regelmann WE, Lunde NM, Porter PT, et al. Increased monocyte chemiluminescence in cystic fibrosis patients and in their parents. *Pediatr Res* 1986;**20**:619–22.
323. Ren CL, Morgan WJ, Konstan MW, et al. Presence of methicillin resistant *Staphylococcus aureus* in respiratory cultures from cystic fibrosis patients is associated with lower lung function. *Pediatr Pulmonol* 2007;**42**:513–18.
324. Retsch-Bogart GZ, Quittner AL, Gibson RL, et al. Efficacy and safety of inhaled aztreonam lysine for airway pseudomonas in cystic fibrosis. *Chest* 2009;**135**:1223–32.
325. Ribeiro CM, Boucher RC. Role of endoplasmic reticulum stress in cystic fibrosis-related airway inflammatory responses. *Proc Am Thorac Soc* 2010;**7**:387–94.
326. Riordan JR. Assembly of functional CFTR chloride channels. *Annu Rev Physiol* 2005;**67**:701–18.
327. Riordan JR, Rommens JM, Kerem B, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 1989;**245**:1066–73.
328. Robinson M, Bye PT. Mucociliary clearance in cystic fibrosis. *Pediatr Pulmonol* 2002;**33**:293–306.
329. Ronne Hansen C, Pressler T, Hoiby N, et al. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients; a retrospective case control study. *J Cyst Fibros* 2006;**5**:245–51.
330. Rosenfeld M. Overview of published evidence on outcomes with early diagnosis from large US observational studies. *J Pediatr* 2005;**147**:S11–14.
331. Rosenfeld M, Davis R, FitzSimmons S, et al. Gender gap in cystic fibrosis mortality. *Am J Epidemiol* 1997;**145**:794–803.
332. Rosenfeld M, Emerson J, Accurso F, et al. Diagnostic accuracy of oropharyngeal cultures in infants and young children with cystic fibrosis. *Pediatr Pulmonol* 1999;**28**:321–8.
333. Rosenfeld M, Emerson J, McNamara S, et al. Risk factors for age at initial *Pseudomonas* acquisition in the cystic fibrosis epic observational cohort. *J Cyst Fibros* 2012;**11**:446–53.
334. Rosenfeld M, Gibson RL, McNamara S, et al. Early pulmonary infection, inflammation, and clinical outcomes in infants with cystic fibrosis. *Pediatr Pulmonol* 2001;**32**:356–66.
335. Rosenfeld M, Ramsey BW, Gibson RL. Pseudomonas acquisition in young patients with cystic fibrosis: pathophysiology, diagnosis, and management. *Curr Opin Pulm Med* 2003;**9**:492–7.
336. Rosenstein BJ. Cystic fibrosis diagnosis: new dilemmas for an old disorder. *Pediatr Pulmonol* 2002;**33**:83–4.
337. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. Cystic Fibrosis Foundation Consensus Panel. *J Pediatr* 1998;**132**:589–95.
338. Rowe SM, Miller S, Sorscher EJ. Cystic fibrosis. *N Engl J Med* 2005;**352**:1992–2001.
339. Rozen R, Schwartz RH, Hilman BC, et al. Cystic fibrosis mutations in North American populations of French ancestry: analysis of Quebec French-Canadian and Louisiana Acadian families. *Am J Hum Genet* 1990;**47**:606–10.
340. Rubin BK. Exposure of children with cystic fibrosis to environmental tobacco smoke. *N Engl J Med* 1990;**323**:782–8.

341. Rubin BK. Mucus structure and properties in cystic fibrosis. *Paediatr Respir Rev* 2007;**8**:4–7.
342. Saiman L, Prince A. Pseudomonas aeruginosa pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. *J Clin Invest* 1993;**92**:1875–80.
343. Saiman L, Schidlow D, Smith A. *The diagnosis of cystic fibrosis: consensus statement*. Cystic Fibrosis Foundation; 1996.
344. Saiman L, Anstead M, Mayer-Hamblett N, et al. Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa:* a randomized controlled trial. *JAMA* 2010;**303**:1707–15.
345. Saiman L, Burns JL, Whittier S, et al. Evaluation of reference dilution test methods for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* strains isolated from patients with cystic fibrosis. *J Clin Microbiol* 1999;**37**:2987–91.
346. Saiman L, Cacalano G, Gruenert D, et al. Comparison of adherence of *Pseudomonas aeruginosa* to respiratory epithelial cells from cystic fibrosis patients and healthy subjects. *Infect Immun* 1992;**60**:2808–14.
347. Saiman L, Chen Y, Tabibi S, et al. Identification and antimicrobial susceptibility of *Alcaligenes xylosoxidans* isolated from patients with cystic fibrosis. *J Clin Microbiol* 2001;**39**:3942–5.
348. Saiman L, Marshall BC, Mayer-Hamblett N, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa:* a randomized controlled trial. *JAMA* 2003;**290**:1749–56.
349. Saiman L, Mehar F, Niu WW, et al. Antibiotic susceptibility of multiply resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, including candidates for transplantation. *Clin Infect Dis* 1996;**23**:532–7.
350. Saiman L, Siegel J, Cystic Fibrosis F. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. *Infect Control Hosp Epidemiol* 2003;**24**:S6–52.
351. Salvatore D, Buzzetti R, Baldo E, et al. An overview of international literature from cystic fibrosis registries 2. Neonatal screening and nutrition/growth. *J Cyst Fibros* 2010;**9**:75–83.
352. San Gabriel P, Zhou J, Tabibi S, et al. Antimicrobial susceptibility and synergy studies of *Stenotrophomonas maltophilia* isolates from patients with cystic fibrosis. *Antimicrob Agents Chemother* 2004;**48**:168–71.
353. Saralaya D, Peckham DG, Hulme B, et al. Serum and sputum concentrations following the oral administration of linezolid in adult patients with cystic fibrosis. *J Antimicrob Chemother* 2004;**53**:325–8.
354. Schaad UB, Wedgwood-Krucko J, Guenin K, et al. Antipseudomonal therapy in cystic fibrosis: aztreonam and amikacin versus ceftazidime and amikacin administered intravenously followed by oral ciprofloxacin. *Eur J Clin Microbiol Infect Dis* 1989;**8**:858–65.
355. Schechter MS, Shelton BJ, Margolis PA, et al. The association of socioeconomic status with outcomes in cystic fibrosis patients in the United States. *Am J Respir Crit Care Med* 2001;**163**:1331–7.
356. Schlichting C, Branger C, Fournier JM, et al. Typing of *Staphylococcus aureus* by pulsed-field gel electrophoresis, zymotyping, capsular typing, and phage typing: resolution of clonal relationships. *J Clin Microbiol* 1993;**31**:227–32.
357. Schottelius AJ, Mayo MW, Sartor RB, et al. Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J Biol Chem* 1999;**274**:31868–74.
358. Schwarzer C, Illek B, Suh JH, et al. Organelle redox of CF and CFTR-corrected airway epithelia. *Free Radic Biol Med* 2007;**43**:300–16.
359. Scott FW, Pitt TL. Identification and characterization of transmissible *Pseudomonas aeruginosa* strains in cystic fibrosis patients in England and Wales. *J Med Microbiol* 2004;**53**:609–15.
360. Scott FW, Pitt TL. Pseudomonas aeruginosa: Basic research. *Prog Respir Res* 2006;**34**:138–44.
361. Scully BE, Ores CN, Prince AS, et al. Treatment of lower respiratory tract infections due to *Pseudomonas aeruginosa* in patients with cystic fibrosis. *Rev Infect Dis* 1985;**7**(suppl 4):S669–74.
362. Seidler MJ, Salvenmoser S, Muller FM. *Aspergillus fumigatus* forms biofilms with reduced antifungal drug susceptibility on bronchial epithelial cells. *Antimicrob Agents Chemother* 2008;**52**:4130–6.
363. Shelhamer JH, Levine SJ, Wu T, et al. NIH conference. Airway inflammation. *Ann Intern Med* 1995;**123**:288–304.
364. Shilo N, Quach C. Pulmonary infections and community associated methicillin resistant *Staphylococcus aureus:* a dangerous mix? *Paediatr Respir Rev* 2011;**12**:182–9.
365. Shreve MR, Butler S, Kaplowitz HJ, et al. Impact of microbiology practice on cumulative prevalence of respiratory tract bacteria in patients with cystic fibrosis. *J Clin Microbiol* 1999;**37**:753–7.
366. Sibley CD, Grinwis ME, Field TR, et al. Culture enriched molecular profiling of the cystic fibrosis airway microbiome. *PLoS One* 2011;**6**:e22702.
367. Simpson DA, Ramphal R, Lory S. Genetic analysis of *Pseudomonas aeruginosa* adherence: distinct genetic loci control attachment to epithelial cells and mucins. *Infect Immun* 1992;**60**:3771–9.
368. Singh PK, Schaefer AL, Parsek MR, et al. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 2000;**407**:762–4.
369. Smith AL, Doershuk C, Goldmann D, et al. Comparison of a beta-lactam alone versus beta-lactam and an aminoglycoside for pulmonary exacerbation in cystic fibrosis. *J Pediatr* 1999;**134**:413–21.
370. Smith JJ, Travis SM, Greenberg EP, et al. Cystic fibrosis airway epithelia fail to kill bacteria because of abnormal airway surface fluid. *Cell* 1996;**85**:229–36.
371. Smyth A. Prophylactic antibiotics in cystic fibrosis: a conviction without evidence? *Pediatr Pulmonol* 2005;**40**:471–6.
372. Smyth A, Walters S. Prophylactic antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2003;(12)CD001912.
373. Smyth A, Tan KH, Hyman-Taylor P, et al. Once versus three-times daily regimens of tobramycin treatment for pulmonary exacerbations of cystic fibrosis: the TOPIC study: a randomised controlled trial. *Lancet* 2005;**365**:573–8.
374. Smyth AR, Smyth RL, Tong CY, et al. Effect of respiratory virus infections including rhinovirus on clinical status in cystic fibrosis. *Arch Dis Child* 1995;**73**:117–20.
375. Snell GI, de Hoyos A, Krajden M, et al. *Pseudomonas cepacia* in lung transplantation recipients with cystic fibrosis. *Chest* 1993;**104**:466–71.
376. Solis A, Brown D, Hughes J, et al. Methicillin-resistant *Staphylococcus aureus* in children with cystic fibrosis: An eradication protocol. *Pediatr Pulmonol* 2003;**36**:189–95.
377. Sorensen RU, Waller RL, Klinger JD. Cystic fibrosis. Infection and immunity to *Pseudomonas*. *Clin Rev Allergy* 1991;**9**:47–74.
378. Southern KW, Barker PM, Solis A. Macrolide antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2004;(3):CD002203.
379. Southern KW, Barker PM, Solis-Moya A, et al. Macrolide antibiotics for cystic fibrosis. *Cochrane Database Syst Rev* 2011;(12):CD002203.
380. Speer ME, Fernandes CJ, Boron M, Groothuis J. *Use of palivizumab in infants with cystic fibrosis: Results from the 2000–2004 Outcomes Registry*. Orlando, Fla: American Thoracic Society International Conference; 2004.
381. Speert DP, Campbell ME, Farmer SW, et al. Use of a pilin gene probe to study molecular epidemiology of *Pseudomonas aeruginosa*. *J Clin Microbiol* 1989;**27**:2589–93.
382. Speert DP, Campbell ME, Henry DA, et al. Epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis in British Columbia, Canada. *Am J Respir Crit Care Med* 2002;**166**:988–93.
383. Speert DP, Henry D, Vandamme P, et al. Epidemiology of *Burkholderia cepacia* complex in patients with cystic fibrosis, Canada. *Emerg Infect Dis* 2002;**8**:181–7.
384. Spencer DH, Kas A, Smith EE, et al. Whole-genome sequence variation among multiple isolates of *Pseudomonas aeruginosa*. *J Bacteriol* 2003;**185**:1316–25.
385. Stanke F, Becker T, Kumar V, et al. Genes that determine immunology and inflammation modify the basic defect of impaired ion conductance in cystic fibrosis epithelia. *J Med Genet* 2011;**48**:24–31.
386. Steinbach S, Sun L, Jiang RZ, et al. Transmissibility of *Pseudomonas cepacia* infection in clinic patients and lung-transplant recipients with cystic fibrosis. *N Engl J Med* 1994;**331**:981–7.
387. Stevens DA, Moss RB, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis: state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003;**37**(suppl 3):S225–64.

388. Strom CM, Huang D, Chen C, et al. Extensive sequencing of the cystic fibrosis transmembrane regulator gene: assay validation and unexpected benefits of developing a comprehensive test. *Genet Med* 2003;**5**:9–14.
389. Stroobant J. Viral infection in cystic fibrosis. *J R Soc Med* 1986;**79**(suppl 12):19–22.
390. Stutman HR, Lieberman JM, Nussbaum E, et al. Antibiotic prophylaxis in infants and young children with cystic fibrosis: a randomized controlled trial. *J Pediatr* 2002;**140**:299–305.
391. Sueblinvong V, Whittaker LA. Fertility and pregnancy: common concerns of the aging cystic fibrosis population. *Clin Chest Med* 2007;**28**:433–43.
392. Sugiyama Y. Diffuse panbronchiolitis. *Clin Chest Med* 1993;**14**: 765–72.
393. Sun L, Jiang RZ, Steinbach S, et al. The emergence of a highly transmissible lineage of cbl+ *Pseudomonas* (*Burkholderia*) *cepacia* causing CF centre epidemics in North America and Britain. *Nat Med* 1995;**1**:661–6.
394. Suter S. The role of bacterial proteases in the pathogenesis of cystic fibrosis. *Am J Respir Crit Care Med* 1994;**150**:S118–22.
395. Szaff M, Hoiby N, Flensborg EW. Frequent antibiotic therapy improves survival of cystic fibrosis patients with chronic *Pseudomonas aeruginosa* infection. *Acta Paediatr Scand* 1983;**72**:651–7.
396. Tablan OC, Carson LA, Cusick LB, et al. Laboratory proficiency test results on use of selective media for isolating *Pseudomonas cepacia* from simulated sputum specimens of patients with cystic fibrosis. *J Clin Microbiol* 1987;**25**:485–7.
397. Tablan OC, Chorba TL, Schidlow DV, et al. *Pseudomonas cepacia* colonization in patients with cystic fibrosis: risk factors and clinical outcome. *J Pediatr* 1985;**107**:382–7.
398. Tablan OC, Martone WJ, Doershuk CF, et al. Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis. Risk factors and outcomes. *Chest* 1987;**91**:527–32.
399. Takeyama K, Dabbagh K, Jeong Shim J, et al. Oxidative stress causes mucin synthesis via transactivation of epidermal growth factor receptor: role of neutrophils. *J Immunol* 2000;**164**:1546–52.
400. Talmaciu I, Varlotta L, Mortensen J, et al. Risk factors for emergence of *Stenotrophomonas maltophilia* in cystic fibrosis. *Pediatr Pulmonol* 2000;**30**:10–15.
401. Tamai H, Kachur JF, Baron DA, et al. Monochloramine, a neutrophil-derived oxidant, stimulates rat colonic secretion. *J Pharmacol Exp Ther* 1991;**257**:887–94.
402. Tang H, Kays M, Prince A. Role of *Pseudomonas aeruginosa* pili in acute pulmonary infection. *Infect Immun* 1995;**63**:1278–85.
403. Tarran R, Donaldson S, Boucher RC. Rationale for hypertonic saline therapy for cystic fibrosis lung disease. *Semin Respir Crit Care Med* 2007;**28**:295–302.
404. Tarran R, Button B, Picher M, et al. Normal and cystic fibrosis airway surface liquid homeostasis: the effects of phasic shear stress and viral infections. *J Biol Chem* 2005;**280**:35751–9.
405. Taylor JL, Palmer SM. *Mycobacterium abscessus* chest wall and pulmonary infection in a cystic fibrosis lung transplant recipient. *J Heart Lung Transplant* 2006;**25**:985–8.
406. Taylor RF, Gaya H, Hodson ME. Temocillin and cystic fibrosis: outcome of intravenous administration in patients infected with *Pseudomonas cepacia*. *J Antimicrob Chemother* 1992;**29**:341–4.
407. Thomas SR, Gyi KM, Gaya H, et al. Methicillin-resistant *Staphylococcus aureus:* impact at a national cystic fibrosis centre. *J Hosp Infect* 1998;**40**:203–9.
408. Thomassen MJ, Demko CA, Doershuk CF, et al. *Pseudomonas cepacia:* decrease in colonization in patients with cystic fibrosis. *Am Rev Respir Dis* 1986;**134**:669–71.
409. Thomson E, Brennan S, Senthilmohan R, et al. Identifying peroxidases and their oxidants in the early pathology of cystic fibrosis. *Free Radic Biol Med* 2010;**49**:1354–60.
410. Tomashefski JF Jr, Konstan MW, Bruce MC, et al. The pathologic characteristics of interstitial pneumonia cystic fibrosis: a retrospective autopsy study. *Am J Clin Pathol* 1989;**91**:522–30.
411. Tosi MF, Zakem H, Berger M. Neutrophil elastase cleaves C3bi on opsonized pseudomonas as well as CR1 on neutrophils to create a functionally important opsonin receptor mismatch. *J Clin Invest* 1990;**86**:300–8.
412. Tramper-Stranders GA, Wolfs TF, van Haren Noman S, et al. Controlled trial of cycled antibiotic prophylaxis to prevent initial *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Thorax* 2010;**65**:915–20.
413. Treggiari MM, Retsch-Bogart G, Mayer-Hamblett N, et al. Comparative efficacy and safety of 4 randomized regimens to treat early *Pseudomonas aeruginosa* infection in children with cystic fibrosis. *Arch Pediatr Adolesc Med* 2011;**165**:847–56.
414. Truluck EP, Christie JD, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: Twenty-fourth Official Adult Lung and Health-Lung Transplantation Report 2007. *J Heart Lung Transplant* 2007;**26**:782–95.
415. Ulrich M, Herbert S, Berger J, et al. Localization of *Staphylococcus aureus* in infected airways of patients with cystic fibrosis and in a cell culture model of S. aureus adherence. *Am J Respir Cell Mol Biol* 1998;**19**:83–91.
416. Valerius NH, Koch C, Hoiby N. Prevention of chronic *Pseudomonas aeruginosa* colonisation in cystic fibrosis by early treatment. *Lancet* 1991;**338**:725–6.
417. Van Daele S, Verhelst R, Claeys G, et al. Shared genotypes of *Achromobacter xylosoxidans* strains isolated from patients at a cystic fibrosis rehabilitation center. *J Clin Microbiol* 2005;**43**: 2998–3002.
418. Van Daele SG, Franckx H, Verhelst R, et al. Epidemiology of *Pseudomonas aeruginosa* in a cystic fibrosis rehabilitation centre. *Eur Respir J* 2005;**25**:474–81.
419. Van Ewijk BE, van der Zalm MM, Wolfs TF, et al. Viral respiratory infections in cystic fibrosis. *J Cyst Fibros* 2005;**4**(Suppl 2):31–6.
420. Van Ewijk BE, Wolfs TF, Aerts PC, et al. RSV mediates *Pseudomonas aeruginosa* binding to cystic fibrosis and normal epithelial cells. *Pediatr Res* 2007;**61**:398–403.
421. van Haren EH, Lammers JW, Festen J, et al. The effects of the inhaled corticosteroid budesonide on lung function and bronchial hyperresponsiveness in adult patients with cystic fibrosis. *Respir Med* 1995;**89**:209–14.
422. Vandamme P, Henry D, Coenye T, et al. Burkholderia anthina sp. nov. and *Burkholderia pyrrocinia*, two additional *Burkholderia cepacia* complex bacteria, may confound results of new molecular diagnostic tools. *FEMS Immunol Med Microbiol* 2002;**33**: 143–9.
423. Vandamme P, Holmes B, Coenye T, et al. Burkholderia cenocepacia sp. nov.: a new twist to an old story. *Res Microbiol* 2003;**154**: 91–6.
424. Vanscoy LL, Blackman SM, Collaco JM, et al. Heritability of lung disease severity in cystic fibrosis. *Am J Respir Crit Care Med* 2007;**175**:1036–43.
425. Vazquez C, Municio M, Corera M, et al. Early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Acta Paediatr* 1993;**82**:308–9.
426. Velsor LW, van Heeckeren A, Day BJ. Antioxidant imbalance in the lungs of cystic fibrosis transmembrane conductance regulator protein mutant mice. *Am J Physiol Lung Cell Mol Physiol* 2001; **281**:L31–8.
427. Venkatakrishnan A, Stecenko AA, King G, et al. Exaggerated activation of nuclear factor-kappaB and altered IkappaB-beta processing in cystic fibrosis bronchial epithelial cells. *Am J Respir Cell Mol Biol* 2000;**23**:396–403.
428. Verhaeghe C, Remouchamps C, Hennuy B, et al. Role of IKK and ERK pathways in intrinsic inflammation of cystic fibrosis airways. *Biochem Pharmacol* 2007;**73**:1982–94.
429. Verkman AS, Song Y, Thiagarajah JR. Role of airway surface liquid and submucosal glands in cystic fibrosis lung disease. *Am J Physiol Cell Physiol* 2003;**284**:C2–15.
430. Vermis K, Coenye T, LiPuma JJ, et al. Proposal to accommodate *Burkholderia cepacia* genomovar VI as *Burkholderia dolosa* sp. nov. *Int J Syst Evol Microbiol* 2004;**54**:689–91.
431. Verregghen M, Heijerman HG, Reijers M, et al. Risk factors for *Mycobacterium abscessus* infection in cystic fibrosis patients: a case-control study. *J Cyst Fibros* 2012;**11**:340–3.
432. Wagener JS, Zemanick ET, Sontag MK. Newborn screening for cystic fibrosis. *Curr Opin Pediatr* 2012;**24**:329–35.
433. Wainwright CE, Quittner AL, Geller DE, et al. Aztreonam for inhalation solution (AZLI) in patients with cystic fibrosis, mild lung impairment, and *P. aeruginosa*. *J Cyst Fibros* 2011;**10**: 234–42.
434. Wainwright CE, Vidmar S, Armstrong DS, et al. Effect of bronchoalveolar lavage-directed therapy on *Pseudomonas aeruginosa*

infection and structural lung injury in children with cystic fibrosis: a randomized trial. *JAMA* 2011;**306**:163–71.
435. Wang EE, Prober CG, Manson B, et al. Association of respiratory viral infections with pulmonary deterioration in patients with cystic fibrosis. *N Engl J Med* 1984;**311**:1653–8.
436. Weaver LT, Green MR, Nicholson K, et al. Prognosis in cystic fibrosis treated with continuous flucloxacillin from the neonatal period. *Arch Dis Child* 1994;**70**:84–9.
437. Weber AJ, Soong G, Bryan R, et al. Activation of NF-kappaB in airway epithelial cells is dependent on CFTR trafficking and Cl- channel function. *Am J Physiol Lung Cell Mol Physiol* 2001;**281**:L71–78.
438. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. *Cell* 1993;**73**:1251–4.
439. Welsh MJ, Ramsey BW, Accurso F, Cutting G. Cystic fibrosis. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editor. *The metabolic and molecular basis of inherited diseases*. 8th ed. New York: McGraw-Hill; 2001. p. 5121–88.
440. Whittier S, Hopfer RL, Knowles MR, et al. Improved recovery of mycobacteria from respiratory secretions of patients with cystic fibrosis. *J Clin Microbiol* 1993;**31**:861–4.
441. Whittier S, Olivier K, Gilligan P, et al. Proficiency testing of clinical microbiology laboratories using modified decontamination procedures for detection of nontuberculous mycobacteria in sputum samples from cystic fibrosis patients. The Nontuberculous Mycobacteria in Cystic Fibrosis Study Group. *J Clin Microbiol* 1997;**35**:2706–8.
442. Wientzen R, Prestidge CB, Kramer RI, et al. Acute pulmonary exacerbations in cystic fibrosis: a double-blind trial of tobramycin and placebo therapy. *Am J Dis Child* 1980;**134**:1134–8.
443. Wiesemann HG, Steinkamp G, Ratjen F, et al. Placebo-controlled, double-blind, randomized study of aerosolized tobramycin for early treatment of *Pseudomonas aeruginosa* colonization in cystic fibrosis. *Pediatr Pulmonol* 1998;**25**:88–92.
444. Wilmott RW, Kassab JT, Kilian PL, et al. Increased levels of interleukin-1 in bronchoalveolar washings from children with bacterial pulmonary infections. *Am Rev Respir Dis* 1990;**142**:365–8.
445. Wilschanski M, Dupuis A, Ellis L, et al. Mutations in the cystic fibrosis transmembrane regulator gene and in vivo transepithelial potentials. *Am J Respir Crit Care Med* 2006;**174**:787–94.
446. Wilschanski M, Zielenski J, Markiewicz D, et al. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. *J Pediatr* 1995;**127**:705–10.
447. Winnie GB, Cowan RG. Association of Epstein-Barr virus infection and pulmonary exacerbations in patients with cystic fibrosis. *Pediatr Infect Dis J* 1992;**11**:722–6.
448. Witko-Sarsat V, Allen RC, Paulais M, et al. Disturbed myeloperoxidase-dependent activity of neutrophils in cystic fibrosis homozygotes and heterozygotes, and its correction by amiloride. *J Immunol* 1996;**157**:2728–35.
449. Wolter J, Seeney S, Bell S, et al. Effect of long term treatment with azithromycin on disease parameters in cystic fibrosis: a randomised trial. *Thorax* 2002;**57**:212–16.
450. Wong K, Roberts MC, Owens L, et al. Selective media for the quantitation of bacteria in cystic fibrosis sputum. *J Med Microbiol* 1984;**17**:113–19.
451. Wood RE, Board TF, Doershuk CF. State of the art: Cystic fibrosis. *Am Rev Respir Dis* 1976;**113**:833.
452. Wood RE, Boat TF, Doershuk CF. Cystic fibrosis. *Am Rev Respir Dis* 1976;**113**:833–78.
453. World Health Organization. *World Health Organization classification of cystic fibrosis and related disorders*. Stockholm: WHO; 2000. WHO/CF/HGN/00.2.
454. Worlitzsch D, Tarran R, Ulrich M, et al. Effects of reduced mucus oxygen concentration in airway Pseudomonas infections of cystic fibrosis patients. *J Clin Invest* 2002;**109**:317–25.
455. Wright PF, Khaw KT, Oxman MN, et al. Evaluation of the safety of amantadine-HC1 and the role of respiratory viral infections in children with cystic fibrosis. *J Infect Dis* 1976;**134**:144–9.
456. Yanagihara K, Tomono K, Sawai T, et al. Effect of clarithromycin on lymphocytes in chronic respiratory *Pseudomonas aeruginosa* infection. *Am J Respir Crit Care Med* 1997;**155**:337–42.
457. Yankaskas JR, Mallory GB Jr. Lung transplantation in cystic fibrosis: consensus conference statement. *Chest* 1998;**113**:217–26.
458. Zabner J, Smith JJ, Karp PH, et al. Loss of CFTR chloride channels alters salt absorption by cystic fibrosis airway epithelia in vitro. *Mol Cell* 1998;**2**:397–403.
459. Zaidy AG, Davis PB. Infection versus inflammation. *Prog Respir Res* 2006;**34**:122–30.
460. Zemanick ET, Sagel SD, Harris JK. The airway microbiome in cystic fibrosis and implications for treatment. *Curr Opin Pediatr* 2011;**23**:319–24.
461. Zemanick ET, Wagner BD, Sagel SD, et al. Reliability of quantitative real-time PCR for bacterial detection in cystic fibrosis airway specimens. *PLoS One* 2010;**5**:e15101.
462. Zemel BS, Jawad AF, FitzSimmons S, et al. Longitudinal relationship among growth, nutritional status, and pulmonary function in children with cystic fibrosis: analysis of the Cystic Fibrosis Foundation National CF Patient Registry. *J Pediatr* 2000;**137**:374–80.
463. Zheng S, De BP, Choudhary S, et al. Impaired innate host defense causes susceptibility to respiratory virus infections in cystic fibrosis. *Immunity* 2003;**18**:619–30.
464. Zheng S, Xu W, Bose S, et al. Impaired nitric oxide synthase-2 signaling pathway in cystic fibrosis airway epithelium. *Am J Physiol Lung Cell Mol Physiol* 2004;**287**:L374–81.
465. Zhou J, Chen Y, Tabibi S, et al. Antimicrobial susceptibility and synergy studies of *Burkholderia cepacia* complex isolated from patients with cystic fibrosis. *Antimicrob Agents Chemother* 2007;**51**:1085–8.
466. Zhou J, Garber E, Desai M, et al. Compliance of clinical microbiology laboratories in the United States with current recommendations for processing respiratory tract specimens from patients with cystic fibrosis. *J Clin Microbiol* 2006;**44**:1547–9.
467. Zielenski J, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. *Annu Rev Genet* 1995;**29**:777–807.

SECTION **III**

INFECTIONS OF THE HEART

CHAPTER 26

Infective Endocarditis

Jeffrey R. Starke

Infective endocarditis results when microorganisms adhere to the endocardial surface of the heart. This process usually occurs on heart valves, although septal defects and mural surfaces can be affected. Most episodes of endocarditis begin on endocardium that has been altered by congenital defects, previous disease, surgery, or trauma. The clinical manifestations depend on the degree of compromise of cardiac function and the occurrence of embolic phenomena. Although bacteria are responsible for most cases, instances of infective endocarditis caused by fungi, chlamydiae, rickettsiae, and perhaps viruses have been described. Advances in the practice of general pediatrics and cardiology during the past 3 decades have contributed to changes in the predisposing conditions and etiologic agents of infective endocarditis. Before the 1950s, rheumatic fever was the major underlying condition, but its incidence has declined greatly since then.[331] Improvements in the medical and surgical management of children with congenital heart disease have increased survival rates. Eighty to 90 percent of children with infective endocarditis have congenital heart disease. Many cases occur after cardiac surgery, especially for replacement of valves and creation of shunts with prosthetic materials.[367] The reported incidence of infective endocarditis in neonates is increasing, probably owing to the use of sophisticated and highly invasive techniques in neonatal intensive care nurseries.[98,172,354,435]

Infective endocarditis has been classified as acute or subacute, based on the progression of untreated disease.[474] The acute form has a fulminant course, with high fever, systemic toxicity, and death from sepsis in several days to 6 weeks. The most common etiologic agents are *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae*. Children with the acute form often have no underlying cardiac lesion. Subacute disease usually occurs in patients with previous valvular disease or those who have undergone cardiac surgical intervention.[62] It is characterized by a more indolent course (6 weeks to several months) and with low-grade fever, vague systemic complaints, and various embolic phenomena. Viridans streptococci are the most common etiologic agents. This classification ignores the frequent overlap in clinical manifestations caused by various organisms, especially the staphylococci and fungi, which are causes of an increasing number of subacute cases in the postcardiac surgical setting. Classification based on specific etiologic agents is preferable because it has implications for the usual clinical course, predisposing factors, and appropriate medical and surgical management.[22]

EPIDEMIOLOGY

The incidence of infective endocarditis in adults has been difficult to determine because the methods of study and criteria for diagnosis vary among series.[33,131,381] Accurate figures on the incidence of infective endocarditis in children are difficult to obtain. The most common method of reporting the incidence in pediatric series expresses the number of cases of infective endocarditis as the numerator and the total number of hospital admissions during the analyzed period as the denominator. Zakrzewski and Keith[589] reported an incidence of endocarditis of 1 in 4500 pediatric admissions at the Hospital for Sick Children in Toronto from 1952 to 1962, whereas Van Hare and colleagues[553] at Case Western Reserve in Cleveland, Ohio, found an incidence of 1 in 1280 in the period from 1972 to 1982. In a large series from Boston Children's Hospital spanning the period between 1933 and 1972, the incidence before 1963 was 1 in 4500 pediatric admissions, whereas that for 1963 to 1972 was 1 in 1800 admissions.[248] A study from a children's hospital in Australia reported an incidence of 1 in 4500 hospital admissions between 1971 and 1983.[490] More recently, one Japanese center reported an annual incidence of 0.9 cases per 1000 children seen at the cardiology clinic.[169] Although differences in referral patterns at these centers may have introduced bias into these figures, the incidence of infective endocarditis in children appears to be rising. This rise may be explained by the increased survival rate of children with all forms of cardiovascular disease and an increase in the percentage of cases that occur after cardiac surgery[29] (especially implantation of foreign material[564]) and are related to intravascular catheters.[153,256,461] Early surgical correction of many types of congenital heart disease along with effective perioperative antibiotic prophylaxis regimens ultimately may lower the incidence of postoperative infective endocarditis. However, the increasing use of invasive therapeutic modalities, especially intravenous catheters and pacemakers, may lead to an increased incidence of health care–associated endocarditis.[153,159,338,436,565] In general, children with predisposing cardiac conditions who develop infective endocarditis while hospitalized have longer hospitalizations and higher mortality than patients with community-associated endocarditis.[335]

The average age of children with infective endocarditis is increasing, a phenomenon that may reflect the longer life expectancy created by improved therapy for

TABLE 26-1 **Underlying Heart Disease in 266 Children with Infective Endocarditis**

Underlying Heart Disease	Percentage Affected (%)
Congenital heart disease	78
Tetralogy of Fallot	24
Ventricular septal defect	16
Congenital aortic stenosis	8
Patent ductus arteriosus	7
Transposition of great vessel	4
Others	19
Rheumatic heart disease	14
No heart disease	8

From Kaplan EL. Infective endocarditis in the pediatric age group: an overview. In: Kaplan EL, Taranta AV, editors. *Infective endocarditis: an American Heart Association symposium.* Dallas: American Heart Association; 1977, p. 51–4.

children at risk.[521] From 1930 to 1950, the mean age for children with infective endocarditis was close to 5 years.[248] Between 1960 and the present, it increased to 8.5 and then to 13 years.[169,180,284,337,461] The number of reports of infective endocarditis in children younger than 2 years of age had been small but has increased significantly since the late 1980s.[43,180,354,573] The clinical course of infective endocarditis in these young children often is atypical, and some cases are diagnosed at autopsy.[248,409] Before the 1950s, this disease was a rare event in neonates, with only eight autopsy cases reported.[321] Several reports suggest a rapidly increasing rate associated with the development of intensive supportive care in neonates.* Symchych and colleagues[519] found a 3 percent incidence of bacterial endocarditis among all neonatal autopsies. Endocarditis in neonates frequently occurs on the tricuspid valve when associated with an indwelling central venous catheter.[539] Congenital heart defects also predispose neonates to the development of infectious endocarditis.[98]

Any form of structural cardiac disease may predispose to infective endocarditis, especially disorders associated with turbulence of blood flow.[176,498] In autopsy and clinical series, children with ventricular septal defect, tetralogy of Fallot, left-sided valvular disease, and systemic-pulmonary arterial communication were at highest risk, whereas those with pulmonary stenosis, coarctation of the aorta, and secundum atrial septal defect were at low risk.[451,461] Hypertrophic obstructive cardiomyopathy rarely is associated with infective endocarditis.[81] Isolated pulmonic or tricuspid valve endocarditis can occur in "otherwise normal" children and adolescents with sepsis or focal bacterial infection,[382] but usually it is associated with congenital heart disease, intravenous catheters, or intravenous drug abuse.[72,379] A bicuspid aortic valve is recognized as an important risk factor for the development of infective endocarditis, especially in elderly men.[345] The underlying heart diseases in 266 pediatric cases of infective endocarditis are listed in Table 26-1.[256]

*References 50, 344, 354, 361, 395, 396.

A cooperative study on the natural history of aortic stenosis, pulmonary stenosis, and ventricular septal defect reported data from a controlled pediatric population collected over a period of 4 to 15 years.[178] In patients not undergoing surgical correction, the risk of acquiring endocarditis by 30 years of age in those with ventricular septal defects was 9.7 percent versus 1.4 percent for aortic stenosis and 0.9 percent for pulmonic stenosis. Aortic valvotomy in children with aortic stenosis actually increases the relative risk, whereas successful repair of ventricular septal defect significantly decreases long-term susceptibility to infective endocarditis.[179] Similarly, endocarditis is an extremely rare occurrence after ligation of patent ductus arteriosus has been performed. At present, palliative systemic-to-pulmonary shunting is the surgical procedure most often complicated by infective endocarditis.[461] In a review of 115 patients with tetralogy of Fallot, Kaplan and colleagues[258] reported an 8 percent incidence of infective endocarditis after placement of a Pott shunt.

The increasing use of prosthetic valves and valved conduit repairs in children with complex heart disease may lead to a larger number of cases of infective endocarditis in the future.[257,265,296,529,535] Most medical centers report an incidence of prosthetic valve endocarditis of 2 to 4 percent after surgery,[61,181,456,498] with the aortic and mitral valves affected most frequently.[237,323]

Older studies arbitrarily divided prosthetic valve endocarditis into two categories—early and late—based on whether the infection occurred within 60 days of valve placement or later.[36] The rationale for categorizing by time was based on apparent differences in bacteriologic, pathogenetic, and prognostic associations. So-called early cases most often were caused by coagulase-negative staphylococci (CONS), gram-negative bacilli, and fungi, whereas oral and enterococcal streptococci, along with staphylococci, predominated in late cases.[262] These older reports suggested that early cases were acquired by contamination of an intraoperative valve or were secondary to postoperative extracardiac infections, whereas late cases were acquired by the same mechanisms as native-valve endocarditis. Nosocomial bacteremia that develops at any time after the patient has undergone valve placement is a significant risk factor for development of endocarditis.[141,335,548] Finally, the mortality rate was thought to be higher in early versus late infection.

However, more recent studies have blurred this arbitrary time distinction between early and late prosthetic valve endocarditis.[61,237] The risk probably is highest in the first 6 to 12 months and decreases to its lowest point beyond 1 year after valve replacement. CONS are the dominant organisms both before and after the 60th postoperative day.[61,261] Clinical and epidemiologic data also suggest that prosthetic valve infection caused by staphylococci within the first year after placement probably is acquired at the time of surgery.[36] Identified risk factors for the development of prosthetic valve endocarditis in adults include native valve endocarditis, black race, male sex, a mechanical (vs. biologic) prosthesis, and prolonged cardiopulmonary bypass time[237]; no comparable information is available for children.

Mitral valve endocarditis occurs frequently on an anatomically normal valve in patients with other predisposing factors.[154] An association between mitral valve prolapse and infective endocarditis has been recognized in adults and children. This heart lesion is detected with increasing frequency in adolescent girls and may be only one component of a developmental syndrome.[481] In adults, 40 to 50 percent of cases of infective endocarditis associated with isolated insufficient mitral valves occur in patients with mitral prolapse.[93] In some series of native valve endocarditis, mitral valve prolapse has been the most common underlying lesion.[345] The reported incidence of infective endocarditis in patients with mitral valve prolapse has varied markedly among studies, from low rates of 14 per 100,000 per year to 5 of 58 patients monitored prospectively for 9 to 22 years.[213] A retrospective epidemiologic analysis involving matched cases and controls yielded an odds ratio of 8.2, indicative of a substantially higher risk for development of endocarditis in patients with mitral valve prolapse than in normal controls.[90]

That the risk of developing infective endocarditis is not uniform for all patients with mitral valve prolapse has become apparent. The risk is increased in patients with a preexisting systolic murmur (but not for those with an isolated click and no murmur), echocardiographically demonstrated regurgitation, and valvular redundancy.[100,322,333] The signs and symptoms of endocarditis associated with mitral valve prolapse may be more subtle than those of other types of left-sided endocarditis.[154,393] However, significant complications are relatively common occurrences and sometimes require valve replacement during the acute illness or during convalescence.[20,505]

Fungal endocarditis is a rare disorder in children but should be suspected in certain clinical and epidemiologic settings. It is more likely to occur after cardiac surgery and rarely occurs on native heart valves. It occurs more commonly in neonates treated in intensive care settings than in older children.[98] Other predisposing factors include (1) the presence of an indwelling vascular catheter, (2) prolonged use of antibiotics, (3) intrinsic (immunodeficiency diseases, malignancy, malnutrition) or extrinsic (corticosteroids, cytotoxic drugs) immunosuppression, (4) bowel surgery resulting in transient fungemia, (5) intravenous drug abuse, and (6) preexisting or concomitant bacterial endocarditis.

Many conditions other than structural heart disease predispose children to the development of infective endocarditis. The most important is the presence of an indwelling central venous catheter, especially in patients who are seriously ill or immunocompromised.[172,180,306,542,572] The catheter acts as a foreign body and presumably causes microscopic damage by abrading endocardial and valve surfaces; such damage results in nonbacterial thrombotic vegetation.[27] Infection of intracardiac pacemaker wires also can lead to endocarditis.[14] Infection acquired during the placement procedure and infection of the pacemaker pouch are most common. Infective endocarditis, usually of the tricuspid valve, has developed in children with ventriculoatrial shunts placed for the treatment of hydrocephalus.[256] In patients with arteriovenous fistulas created for hemodialysis, bacterial vegetations may develop in the fistula and on heart valves.[298,439] Rarely, penetrating wounds or foreign bodies can initiate endocarditis.[218,332] Piercings of various body parts also have been associated with endocarditis.[2,432] One important group of patients with an increased risk for development of infective endocarditis is intravenous drug users.[340,575] In this group of patients, two thirds have no evidence of underlying heart disease. A predilection for involvement of the tricuspid valve, followed by the mitral and aortic valves, has been noted.[168] Radiographic evidence of septic pulmonary emboli and signs of tricuspid insufficiency dominate the clinical findings.[474] Within this group of patients, increased rates of infective endocarditis and mortality are associated with infection by human immunodeficiency virus (HIV), particularly as CD4 cell counts fall to less than 200/mm^3.[425]

Several recent studies have found an apparent shift in the epidemiology of pediatric infective endocarditis toward a higher proportion of children without preexisting heart disease, which accounted for 35 to 58 percent of all the infective endocarditis cases.[112,294,309] In these patients, *S. aureus* was the most common causative organism and delay in diagnosis was common.

Although the incidence of infective endocarditis in children may be rising, the prognosis has improved dramatically during the past several decades. Current mortality rates usually are close to 10 percent.[370,461,480] Most survivors remain hemodynamically stable at long-term follow-up.[157,480] However, patients who experience infective endocarditis appear to be at higher risk for developing recurrent endocarditis than are those with similar cardiac abnormalities who have not had previous endocarditis.[498] The patient's functional class before treatment appears to be most predictive of long-term functional status. In one study, 22 percent of children who survived infective endocarditis required surgery related to the infection, including vegetectomy, evacuation of a hematoma, atrioventricular valve replacement, and placement or replacement of a graft or intracardiac shunt.[461]

PATHOPHYSIOLOGY

Clinical observations, autopsy studies, and work with experimental animal models have demonstrated that the occurrence of several independent events is required for the development of subacute infectious endocarditis. The endocardial surface usually is disrupted by stress or injury commonly caused by the turbulence of blood. This surface damage results in the deposition of fibrin and platelets, which form nonbacterial thrombotic vegetations. If bacteria adhere to these deposits, infective endocarditis will result. The surface of the infected vegetation becomes protected by a cover of fibrin and platelets. A tremendous proliferation of organisms (as many as 10^9 colony-forming units per gram) may ensue.[127] The protective sheath isolates the organisms from the action of host neutrophils and antibiotics. The clinical manifestations and complications of infective endocarditis are related to both the hemodynamic changes caused by local infection and the occurrence of embolization and metastatic infection.

In experimental animals, the valvular surface must be damaged, usually by an intravenous catheter, to produce infective endocarditis.[21] The first step in the pathogenesis of subacute infective endocarditis in humans is the development of hemodynamic factors that favor endocardial damage. In an autopsy study of 1024 patients with infective endocarditis, Lepeschkin[299] showed that the location of the endocardial lesions correlated with the impact of pressure; this finding makes a strong argument for the role of mechanical stress as a critical factor in the evolution of the lesions. When associated with valvular insufficiency, infective endocarditis usually occurs on the atrial surface of the mitral valve and the ventricular surface of the aortic valve. Injection of a bacterial aerosol into the air stream passing through a Venturi tube demonstrates how high pressure drives an infected fluid into a low-pressure sink.[446] This process establishes maximal deposition of bacteria in the low-pressure sink immediately beyond the orifice. Mitral insufficiency creates a Venturi effect when blood is driven from the high-pressure left ventricle into a low-pressure atrium; maximal deposition occurs around the mitral annulus on the atrial side. Similarly, with aortic valve insufficiency, the high-pressure source is the aorta and the low-pressure sink is the left ventricle, which leads to deposition on the ventricular surface of the valve.

Lesions also are created more directly by a jet stream causing endocardial damage. For example, in a small, restrictive ventricular septal defect with a left-to-right shunt, a Venturi effect leads to the development of lesions on the right ventricular septal side of the defect, whereas secondary lesions created by the jet effect are located on the right ventricular wall opposite the defect.[567] Heart defects with a surface area sufficiently large to prevent a significant pressure gradient and those in which smaller volumes minimize the gradient do not create the jet and Venturi effects. This difference helps to explain the rarity of endocarditis in patients with atrial septal defects and the increased risk of infection complicating small, but not large, ventricular septal defects.

Once endocardial damage has occurred, collagen is exposed and platelet and fibrin deposition ensues in a manner analogous to formation of the primary plug of normal hemostasis after vascular injury.[246,568] The sterile platelet-fibrin thrombus that is formed subsequently is referred to as a nonbacterial thrombotic vegetation.[315,401] In experimental animals, many exogenous stresses, including exposure to cold, high altitude, high cardiac output states, hormonal manipulations, and passage of a sterile catheter across a heart valve, lead to formation of this lesion. Formation of the vegetation reflects two pathogenic mechanisms: hypercoagulability and endothelial damage.[474] To establish experimental infective endocarditis without initial formation of the vegetation is nearly impossible. Microscopic examination demonstrates that this lesion is the one to which microorganisms attach during the early stages of experimental endocarditis. Nonbacterial thrombotic vegetations have been found in both adults and children with malignancy, chronic wasting diseases, uremia, connective tissue diseases, and congenital heart disease and after the placement of intracardiac catheters,[341,439] and they have been associated with embolism and infarction in distant organs.[46]

Once a nonbacterial thrombotic vegetation has been established, transient bacteremia or fungemia may result in colonization of the lesion. Transient bacteremias are common occurrences, especially with traumatization of a mucosal surface. The incidence of bacteremia in adults and children after various procedures is listed in Table 26-2.[139,444] The bacteremia usually is of low grade and is proportional to the amount of trauma produced by the procedure and the number of organisms inhabiting the surface. In addition, "silent" bacteremia probably occurs frequently. Many persons have circulating antibodies to their own oral flora, as well as an increase in peripheral T cells sensitized to the flora of their dental plaque.[474]

TABLE 26-2 **Bacteremia After Various Procedures in Adults and Children**

Initiating Event	Percentage of Positive Blood Cultures (%)	Predominant Organisms
Dental extraction (children)	30-65	*Streptococcus*, diphtheroids
Chewing gum, candy, paraffin	0-51	*Streptococcus, Staphylococcus epidermidis*
Tooth brushing	0-26	*Streptococcus*
Tonsillectomy	28-38	*Streptococcus, Haemophilus*, diphtheroids
Bronchoscopy (rigid scope)	15	*Streptococcus, S. epidermidis*
Bronchoscopy (fiberoptic)	0	
Orotracheal intubation	0	
Nasotracheal intubation/suctioning	16	*Streptococcus*, aerobic gram-negative rods
Sigmoidoscopy/colonoscopy	0-9.5	*Enterococcus*, aerobic gram-negative rods
Upper gastrointestinal endoscopy	8-12	*Streptococcus, Neisseria, S. epidermidis,* diphtheroids, other
Percutaneous liver biopsy	3-14	Pneumococcus, aerobic gram-negative rods, *Staphylococcus aureus*, other
Urethral catheterization	8	Not stated
Manipulation of *S. aureus* suppurative foci	54	

From Everett ED, Hirschmann JU. Transient bacteremia and endocarditis prophylaxis: a review. *Medicine (Baltimore)* 1977;**56**:61–77.
© 1977, The Williams & Wilkins Company, Baltimore.

Some children with congenital heart disease may be at increased risk for having gingival colonization and subsequent development of bacteremia with organisms associated with infectious endocarditis, such as the HACEK *(Haemophilus* spp., *Actinobacillus actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella kingae)* microbes.[501]

The ability of microorganisms to adhere to the platelet-fibrin thrombus is a critical factor in the development of infective endocarditis.[105,202,246] In a canine model, *S. aureus* and the viridans streptococci, which frequently cause infective endocarditis, adhere more readily to normal aortic leaflets than do organisms uncommon in endocarditis.[191] Within isolates of *S. aureus*, strains devoid of microencapsulation are less capable of inducing endocarditis in an experimental model than are encapsulated strains.[22] Specific products released by these organisms, including dextran, mannan, teichoic acid, and slime, may enhance their ability to colonize the vegetation.[246,274] The amount of dextran produced by various viridans streptococci in broth correlates with both their adherence and their ability to produce endocarditis in the rabbit model.[362,477] *Candida albicans* is readily adherent and produces infective endocarditis in rabbits more easily than does *C. krusei*, a nonadherent yeast rarely implicated in human infective endocarditis.[475] In addition, endocarditis-producing strains of streptococci and staphylococci are more potent stimulators of platelet aggregation than are other bacteria that do not produce infective endocarditis.[89,211,362] This action may accelerate the formation of an infected vegetation or may increase the removal of organisms from the circulation. The importance of adherence by organisms has been studied by preincubating organisms with many classes of antibiotics. After incubation at subinhibitory concentrations, adhesion of streptococcal species to fibrin-platelet matrices and damaged canine valves is decreased.[478] Antibiotics may prevent development of infective endocarditis by both bacterial killing and inhibition of adherence to the vegetation.[185]

Host tissue factors undoubtedly play an important role in adherence of bacteria to the developing thrombus. Activation of the coagulation system ensues once bacteria become adherent to a nonbacterial thrombus. Some organisms that produce endocarditis may be able to initiate procoagulant activity through microbial enzymes. Activation of the intrinsic coagulation pathway is triggered by exposed connective tissue components and platelet aggregation.[274] However, activation of the extrinsic coagulation pathway probably is the major stimulus for growth of vegetations. Elements of the extracellular matrix, including fibronectin, laminin, and collagen, have been shown to facilitate the adherence of bacteria on fibrin-platelet matrices.[516,541] Fibronectin may be the host receptor for organisms within the nonbacterial thrombotic vegetation.[285,317] Laminin-binding proteins have been found on the cell walls of organisms recovered from patients with endocarditis.[495]

The platelet-organism interaction is complex and not understood completely. *Streptococcus sanguinis* produces two cell surface antigens that promote platelet aggregation: a class I antigen promotes adhesion of *S. sanguinis* to platelets, whereas coexpression of a class II antigen promotes platelet adhesion or aggregation.[212] The induced platelet aggregation appears to be an important determinant of further development of vegetation and progression of disease in experimental endocarditis. In addition, production of streptococcal exopolysaccharide inversely correlates with platelet adhesion while inhibiting aggregation, thus indicating that surface molecules may enhance endocarditis at only certain pathogenic steps.[513] Platelets also may be involved in host defense within the vegetation. After exposure to thrombin, platelets may release microbicidal proteins with bactericidal activity against some gram-positive cocci; resistance to these proteins may be a virulence factor for *S. aureus* in the development of endocarditis.[389,585]

As bacterial colonization of a nonbacterial thrombotic vegetation progresses, it enlarges by further bacterial proliferation and platelet-fibrin deposition (Fig. 26-1).[365] Kissane[279] described three histologic zones: (1) necrotic endocardium; (2) a broad zone of bacterial colonies, pyknotic nuclear debris, and fibrin; and (3) a thin coating on the surface of fibrin and leukocytes. The location of bacterial colonies below the surface and the minimal infiltration by phagocytic cells create an environment of impaired host resistance that results in extreme bacterial proliferation. The structure of the vegetation diminishes the penetration of antibiotics into the bacterial layer. In addition, the metabolic activity of bacteria within this lesion is slowed, thus rendering antibiotics less effective. The formation of vegetations and erosion of heart valves may cause valvular incompetence and thereby may result in cardiac failure.

Immunopathologic factors may have important roles in both the development and sequelae of infective endocarditis.[37] The susceptibility of a gram-negative bacillus to complement-mediated bactericidal activity is critical to its potential to create endocarditis.[126] Gram-positive cocci are a more frequent cause of infective endocarditis than are gram-negative bacilli. Gram-positive organisms are resistant to this bactericidal activity; phagocytosis is required for killing.

The frequent presence of hypergammaglobulinemia, splenomegaly, and monocytes in the blood of patients with infective endocarditis indicates stimulation of the humoral and cellular immune systems. Macroglobulins, cryoglobulins, and agglutinating, opsonic, and complement-fixing antibodies have been associated with infective endocarditis.[221,293] Studies in animals preimmunized with heat-killed streptococci before aortic valve trauma and infection are induced suggest that circulating antibody has a protective role.[476,549] However, antibody to *S. aureus* or *Staphylococcus epidermidis* does not prevent the development of endocarditis in immunized animals, perhaps because this antibody does not enhance opsonophagocytosis.[474] The continuous antigenic challenge created by intravascular organisms leads to increased production of specific antibody (including opsonic, agglutinating, and complement-fixing antibodies), cryoglobulins, macroglobulins, and antibodies to bacterial heat shock protein,[428] as well as to the subsequent formation of circulating immune complexes. These complexes are found with increased frequency in patients with a long duration of illness, hypocomplementemia, extravalvular

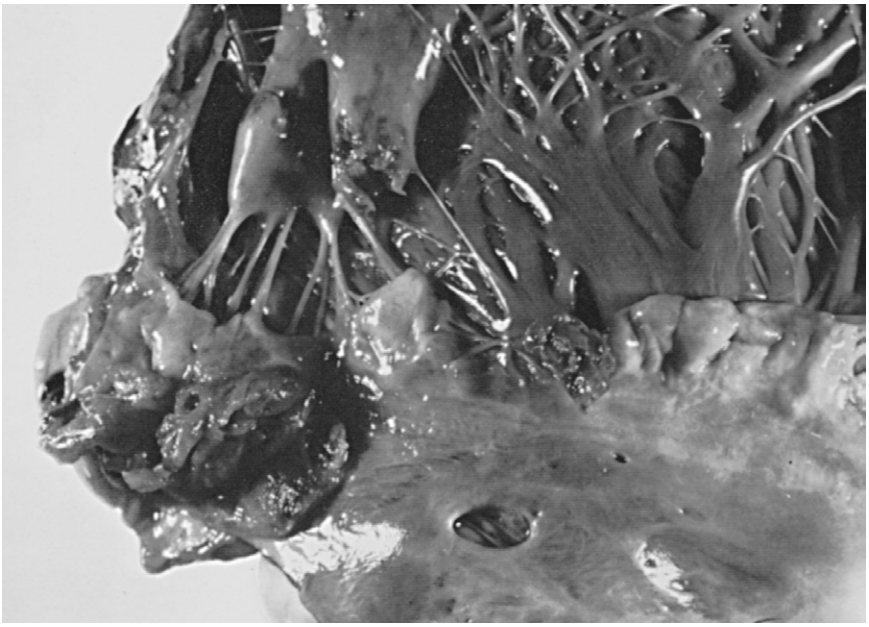

FIGURE 26-1 ■ Subacute endocarditis of the mitral valve with vegetation and rupture of the papillary muscle caused by *Staphylococcus aureus.* (Courtesy of Dr. Edith P. Hawkins, Texas Children's Hospital, Houston.)

manifestations, and right-sided disease.[39] Quantitative levels of circulating immune complexes may be helpful in distinguishing endocarditic from nonendocarditic sepsis and in monitoring anti-infective therapy. Effective treatment usually leads to a prompt decrease in these levels,[38] whereas relapses may be characterized by rising titers.[266] The diffuse glomerulonephritis occasionally noted with infective endocarditis is caused by subepithelial deposition of immune complexes and complement.[197] Immune complexes can be demonstrated in some diffuse purpuric lesions seen with endocarditis.[316] Bacterial antigens have been found within these complexes.[234]

Further evidence of stimulation of the immune system in infective endocarditis is the development of rheumatoid factor in approximately 50 percent of adults with disease lasting longer than 6 weeks.[574] Titers of rheumatoid factor correlate with hypergammaglobulinemia and, as with immune complex levels, decrease with therapy and increase during relapse. The role of rheumatoid factor in the disease process is unknown, but it may be involved by blocking immunoglobulin G opsonic activity, stimulating phagocytosis, or accelerating microvascular damage.[474] Antinuclear, antiendocardial, antisarcolemmal, and antimyolemmal antibodies also have been identified in patients with infective endocarditis; their role in pathogenesis is unclear.[325]

The pathologic changes that occur in the heart in association with infective endocarditis are secondary to local extension of the infection. The vegetations vary from a millimeter to several centimeters; frequently they are singular, but they may be multiple. Valvular stenosis may result from large lesions. Vegetations secondary to certain organisms, especially *Candida*, *Haemophilus*, and *S. aureus* in acute cases, often are large and friable, with a propensity for embolization.[586] Ulcerative lesions may occur and may lead to perforation of the valve and subsequent congestive heart failure. Other local complications include rupture of the chordae tendineae or papillary muscle (see Fig. 26-1), valve ring abscess with subsequent fistula formation and pericardial empyema,[51,67] aneurysms of the sinus of Valsalva or ventricle,[80,173,471] myocarditis, and myocardial infarction.[145] Persistent fever occurring during appropriate medical therapy for infective endocarditis may reflect a persistent vegetation, especially with right-sided disease, or extension of infection into a valve ring and adjacent structures.[120] In such cases, surgery frequently is required.

The pathologic changes in distant organs usually are secondary to embolization with subsequent infarction or metastatic infection. In many cases of infective endocarditis, the causative organism is of low pathogenicity; infections caused by septic emboli often are low-grade because of the reduced propensity of these organisms to invade tissue. However, the emboli in acute *S. aureus* endocarditis frequently cause severe metastatic infections and overwhelming sepsis. Emboli from right-sided heart lesions lodge in the lungs and cause pulmonary infarcts and abscesses, which usually are small and multiple. Left-sided lesions may embolize to any organ but most commonly affect the brain, kidney, spleen, and skin.[280,383] Cerebral emboli have been detected in 30 percent of cases in adults and children and have caused infarction, abscess, mycotic aneurysm, subarachnoid hemorrhage, meningitis, and acute hemiplegia of childhood.* Kidney abscess is a rare occurrence, but infarcts are noted in most patients at autopsy.[357] Amyloidosis involving primarily the kidneys is a rare complication of chronic infective endocarditis.[210] Splenic abscess also is a rare event but can be a fatal complication if undetected.[250] The most common manifestation of embolization to the skin is petechiae. Janeway lesions are septic emboli consisting of bacteria, neutrophils, necrosis, and subcutaneous hemorrhage. Osler nodes are areas of thrombosis and necrosis. They may be related to both immune complex deposition and septic emboli.[5]

*References 64, 193, 205, 252, 339, 463, 472, 555.

CLINICAL MANIFESTATIONS

The signs and symptoms of infective endocarditis are determined by the extent of local cardiac disease, the continuous bacteremia, and the degree of involvement of distant organs as a result of embolization, metastatic infection, and circulating immune complexes.[25] Consequently, the clinical findings are highly variable and mimic those of many other diseases.[78,459] Unexplained embolic phenomena in any organ should suggest the diagnosis of endocarditis, especially in children with known heart disease. Patients with acute bacterial endocarditis initially may be seen with florid sepsis; the endocarditis is diagnosed at autopsy. The indolent manifestations of subacute endocarditis may evolve for weeks or months before medical care is sought. Endocarditis frequently occurs in children with preexisting heart disease, so subtle changes in cardiac function may be difficult to detect early in the course. The frequency of the major clinical manifestations of bacterial endocarditis in infants and children is listed in Table 26-3.

Fever is the most common symptom of infective endocarditis, but it is absent in 10 percent of cases. It usually is of low grade and has no specific pattern. Chills may accompany the fever, but they rarely are seen in children. Persistent fever during antimicrobial therapy is an uncommon occurrence. Prolonged (≥2 weeks) fever is associated with certain etiologic agents (*S. aureus*, gram-negative bacilli, fungi), with culture-negative endocarditis, and with complications such as embolization of major vessels, intracardiac or peripheral abscess, tissue infarction, a need for cardiac surgery, and a higher mortality rate.[52,295] Nonspecific symptoms such as malaise, anorexia, weight loss, and fatigue are common findings. Arthralgia occurs in 24 percent of patients. The arthralgia frequently is multiple and most commonly affects the large joints. Although adults initially may have synovitis,[87] this finding is rare in children. Osteoarticular infection in association with infective endocarditis in adults occurs almost exclusively in intravenous drug users.[470] It is seen very rarely in children except those with disseminated *S. aureus* infection. Gastrointestinal complaints are noted in 16 percent of cases and include nausea, vomiting, and abdominal pain. Chest pain occurs in approximately 10 percent of older children and generally is mild and nonspecific. Although chest pain usually is related to diffuse myalgias, it may be secondary to pulmonary complications or cardiac lesions, especially if the tricuspid valve is involved.

Heart murmurs occur in more than 90 percent of children with infective endocarditis, but most patients have underlying heart disease with existing murmurs. The appearance of a new murmur or appreciation of a significant change in a previous one occurs in only 25 percent of cases. Significant blood flow turbulence caused by compromised valvular function must have occurred for a murmur to be detected or to change. The frequent absence of changes in the cardiac examination early in the disease contributes to the long average delay in establishing the diagnosis, especially in children with preexisting heart disease. Congestive heart failure occurs in 30 percent of children with infective endocarditis and is especially common in those in whom a new murmur of valvular insufficiency develops. Endocarditis should be suspected in any child who has rheumatic or congenital heart disease and unexplained deterioration in cardiac function. Although valvular regurgitation is the most common hemodynamic complication of endocarditis, significant obstruction of a valve or shunt requiring rapid surgery rarely occurs.[79]

Neurologic signs and symptoms are reported in approximately 20 percent of children with endocarditis. These signs and symptoms may dominate the clinical findings, especially in patients with endocarditis caused by *S. aureus*.[205,463] The sudden development of cerebral lesions in an infant or child should suggest this diagnosis. The manifestations are those that commonly accompany a cerebral infarct or abscess, namely, acute hemiplegia of childhood, seizures, ataxia, aphasia, sensory loss, focal neurologic deficits, and alterations in mental status.[64] They may be the initial features of endocarditis or may occur years after the infection has been eradicated.[590] Mycotic aneurysms of the cerebral vessels occur rarely in cases of pediatric endocarditis.[68] They usually are single, small, and peripheral but may lead to subarachnoid hemorrhage. Whereas computed tomographic scanning of the brain is useful for delineating central nervous system involvement in patients with infective endocarditis, magnetic resonance imaging may be more sensitive for detecting small infarctions and changes secondary to cerebral edema.[44] Other neurologic manifestations associated with endocarditis include cranial nerve palsies,

TABLE 26-3 **Clinical Manifestations of Bacterial Endocarditis in Children**

Symptom	Average (%)	Range (%)	Physical Finding	Average (%)	Range (%)
Fever	90	56-100	Splenomegaly	55	36-67
Malaise	55	40-79	Petechiae	33	10-50
Anorexia/weight loss	31	8-83	Embolic phenomena	28	14-50
Heart failure	30	9-47	New or change in heart murmur	24	9-44
Arthralgia	24	16-38	Clubbing	14	2-42
Neurologic findings	18	12-21	Osler nodes	7	7-8
Gastrointestinal findings	16	9-36	Roth spots	5	0-6
Chest pain	9	5-20	Janeway lesion	5	0-10
			Splinter hemorrhages	5	0-10

Data from references 53, 94, 96, 169, 248, 284, 337, 391, 459, 490, 497, 521, 530, and 553.

neuropathy, visual changes, choreoathetosis, seizures, and toxic encephalopathy.

Splenomegaly, a common manifestation of endocarditis in children, occurs in 55 percent of cases. It is found frequently in patients with long-standing disease and other evidence of immune system activation. The spleen generally is nontender and may be associated with mild hepatomegaly. Splenic infarction and abscess are rare events but should be suspected in patients with left upper quadrant abdominal pain that radiates to the left shoulder, a pleural friction rub, or left pleural effusion.

Skin manifestations occur less commonly in children than in adults.[316] Clubbing is found in 10 to 20 percent of children with endocarditis but frequently is related to underlying heart disease. Petechiae are noted in approximately one third of patients, especially those with long-standing disease. These lesions are found most commonly on the extremities, oral mucosa, and conjunctivae. Splinter hemorrhages are linear red or brown streaks seen in the nail beds. They are present in only 5 percent of children with endocarditis and are associated with other conditions.[276] Three other types of lesions are more specific for infective endocarditis but occur in only 5 to 7 percent of patients: Osler nodes, which are small (2 to 10 mm), painful nodular lesions found in the pads of the fingers or toes[5]; Janeway lesions, which usually are painless hemorrhagic macular plaques that frequently occur on the palms and soles[143]; and Roth spots, which are small, pale retinal lesions associated with areas of hemorrhage located near the optic disk.

Other than fever and, perhaps, splenomegaly, no single sign or symptom occurs in more than 50 percent of children with endocarditis. That no classic clinical manifestation exists for this disease is obvious because the chance that even three or more signs will be present is extremely low. The appearance of any one of these clinical features in a child with predisposing heart disease should raise suspicion of infective endocarditis and should lead to an appropriate diagnostic evaluation.[80]

The clinical findings of infective endocarditis in infants and neonates are less specific than are those in older children. The onset more often is acute and related to overwhelming infection.[249,348] In the preantibiotic era, these children often had other foci of infection, such as osteomyelitis, meningitis, and pneumonia, that dominated the clinical picture. The widespread use of antibiotics since 1941 has caused a decrease in the number of cases of infective endocarditis secondary to other suppurative infections.[348] Although early studies concluded that congenital heart disease was not an important predisposing factor to endocarditis in infants, more recent reports suggest that it is. At present, infants with heart defects undergo corrective and palliative surgery at a younger age than in the past. Infants in whom postoperative endocarditis does develop probably will have clinical findings more similar to those in older children.

Infective endocarditis is an uncommon occurrence in neonates and frequently is associated with indwelling vascular catheters.[172,334] It may affect the tricuspid valve and have a fairly "silent" clinical manifestation. Persistent bacteremia or fungemia should lead to a search for a cardiac focus of infection. Deterioration in pulmonary function, coagulopathies, thrombocytopenia, and low-grade murmurs often develop in neonates. Skin abscesses and hepatomegaly also are common findings. The prognosis of infective endocarditis associated with neonatal intensive care usually is favorable, perhaps because the diagnosis often is established relatively early and antibiotics are given rapidly.

Reported series of infective endocarditis in children with prosthetic valves are scarce. In early stages of disease, fever may be the only finding because the other signs of endocarditis are masked by the medical and surgical complications occurring in the immediate postoperative period. Late infections generally produce clinical findings similar to those in native valve endocarditis. Clinical evidence of systemic embolization occurs in as many as 40 percent of patients.[82] Neurologic complications carry a particularly poor prognosis for survival.[272] A new or changing murmur often indicates valvular insufficiency caused by a paravalvular leak. Florid cardiac failure is the major manifestation if local infection or an abscess creates valve instability and acute, severe regurgitation.

The signs and symptoms of infective endocarditis in intravenous drug users may be similar, but these patients have several more distinctive features of their illness. Two thirds of these patients have no predisposing heart disease. The valve most commonly affected is the tricuspid, which leads to a predominance of pulmonary signs and symptoms resulting from pleural effusion, pulmonary infarction, and lung abscesses. Signs of tricuspid insufficiency (gallop rhythm, pulsatile liver, regurgitant murmur) are found in one third of cases.[474] Many patients have extracardiac sites of infection that are helpful in establishing the diagnosis.[528]

LABORATORY FINDINGS

The most important diagnostic procedure is the blood culture. Because many bacteria that usually are not pathogenic cause infective endocarditis, scrupulous aseptic technique must be used to distinguish causative agents from contaminants.[404] The yield of organisms is not increased by obtaining blood from arterial puncture or cardiac catheterization.[40] The bacteremia usually is of low grade and continuous. The first two cultures yield the organism 90 percent of the time; in two thirds of cases, all blood cultures are positive.[571] Therefore, isolated positive cultures generally are not significant. Previous outpatient antibiotic therapy may change the yield significantly.[270] In one study, culture positivity in cases of proven endocarditis was 64 percent in patients who received antibiotics before blood was drawn for culture versus 100 percent in patients without exposure to antibiotics.[405]

When *Candida* endocarditis is suspected, several additional points should be considered. Isolation of *Candida* spp. may require incubation for 1 week or longer. All blood cultures from a patient with *Candida* endocarditis may not be positive, in contrast to the usual situation with bacterial endocarditis; several positive cultures may be interspersed among negative cultures. In patients with fungal endocarditis, *Candida* is isolated commonly from

other infected sites, such as urine, sputum, synovial fluid, cerebrospinal fluid, lymph nodes, and bone marrow.[465]

Three to five samples of blood for culture should be obtained from different sites within the first 24 hours in children with suspected endocarditis. Although difficult to obtain in smaller children, 3 to 5 mL of blood per culture is desirable for optimal yield. The samples should be injected into thioglycolate and trypticase soy (or brain-heart infusion) broth and held for at least 3 weeks to detect slow-growing organisms. If gram-positive cocci grow in the broth but fail to grow on subculture, nutritionally variant streptococci should be suspected and subculture should be performed on media with either L-cysteine or pyridoxal phosphate.[65,503]

Negative blood cultures are noted in 10 to 15 percent of patients with clinically diagnosed endocarditis.[544] However, when patients have not received antibiotic therapy previously and blood for culture is obtained properly, these cases account for less than 5 percent of the total. Potential reasons for negative cultures include the following: (1) right-sided endocarditis; (2) previous administration of antibiotics; (3) fungal (especially *Aspergillus*) endocarditis; (4) endocarditis caused by *Bartonella* spp., rickettsiae, chlamydiae, or viruses; (5) mural endocarditis; (6) slow growth of organisms (*Candida*, *Haemophilus*, *Brucella*, nutritionally variant streptococci); (7) anaerobic infection; and (8) nonbacterial thrombotic endocarditis or an incorrect diagnosis.[200,204,414,544] In some instances, intraleukocytic organisms may be seen in layered peripheral blood, even when cultures are negative.[422] If surgical resection of vegetations or valve replacement is performed, a cause may be demonstrated by appropriate histologic examination and stains for bacteria and fungi.[365] Organisms also may be isolated from extracardiac sites (bone marrow, urine).

Many nonspecific laboratory findings are abnormal in patients with infective endocarditis (Table 26-4). The total white blood cell count rarely is helpful, but peripheral eosinophilia may be seen with Loeffler endocarditis.[220] The erythrocyte sedimentation rate is elevated in 80 to 90 percent of cases. However, frequently it is normal or low when congestive heart failure or renal failure is present. Serum C-reactive protein levels usually are elevated initially and return to normal during the course of successful therapy.[342] An increase during therapy may result from treatment failure, but it can also be caused by drug allergy or intercurrent infection. Rheumatoid factor rarely has been measured in a series of pediatric patients, but when measurements have been made, they have been positive in 25 to 50 percent of children with endocarditis. A positive test may be a diagnostic aid in cases of culture-negative endocarditis when other causes are excluded. Serial measurements may provide evidence of efficacy of therapy, although a fall in the titer of rheumatoid factor may lag behind the clinical and bacteriologic response.[561] Hypocomplementemia is seen in association with glomerulonephritis. Anemia is present in approximately 40 percent of patients, especially those with long-standing disease. Although hemolysis may occur in the areas of turbulence in the heart, more often it is anemia of chronic disease. Because many patients with cyanotic heart disease normally have a compensatory polycythemia, a serial drop in hematocrit is of more significance than is a single measurement. Leukocytosis occurs in a few patients, but leukopenia is a rare finding in the absence of acute endocarditis with overwhelming sepsis. Hematuria and proteinuria, present in 25 to 50 percent of cases, usually are secondary to microemboli in the kidneys and may be accompanied by "pyuria," casts, and bacteriuria.

Circulating immune complexes are present in most adults with subacute endocarditis, as measured by Raji cell radioimmunoassay[531] or the ^{125}I-Clq binding assay.[591] These immune complexes frequently are absent in acute endocarditis. Low levels of immune complexes have been found in 32 percent of adults with septicemia but not endocarditis, in 10 percent of normal controls, and in 40 percent of noninfected intravenous drug users.[39] However, levels higher than 100 μg/mL are correlated highly with the presence of endocarditis. Serial measurement of immune complex levels may aid in monitoring therapeutic efficacy.[38] Systematic investigation of immune complexes has been reported infrequently in children with endocarditis. When immune complexes have been sought, most patients, including two of three children with culture-negative endocarditis, have had significant levels.

When infective endocarditis is suspected but blood cultures remain negative, serologic testing for specific organisms may prove helpful. Several techniques measure antibody to teichoic acids, which are major components of the cell wall of *S. aureus*. These antibodies are present in more than 85 percent of adults with staphylococcal endocarditis, but the false-positive rate is as high as 10 percent.[380] False-negative results correlate with a short (<2 weeks) duration of illness. Specific information about the accuracy of this test in children is lacking, and the tests are not readily available. Serologic testing is available or under investigation for many other organisms that cause infective endocarditis, including *Bartonella*, *Brucella*, *Candida*, *Aspergillus*, *Histoplasma*, *Cryptococcus*, *Chlamydia*, and *Coxiella*.[160,162,253,277] In general, the usefulness of these tests in children with endocarditis is unproven. Some patients with nonspirochetal bacterial endocarditis who reside in locales endemic for Lyme disease have significantly elevated levels of antibodies reactive to *Borrelia burgdorferi*.[255] Diagnostic confusion may occur because the signs and symptoms of infective endocarditis and Lyme disease can be quite similar. Other techniques, such as broad-range polymerase chain reaction, have

TABLE 26-4 **Selected Laboratory Findings of Bacterial Endocarditis in Children**

Laboratory Finding	Average (%)	Range (%)
Positive blood culture	87	68-98
Elevated erythrocyte sedimentation rate	80	71-96
Low hemoglobin (anemia)	44	19-79
Positive rheumatoid factor	38	25-55
Hematuria	35	28-47

Data from references 52, 94, 169, 248, 284, 337, 490, 497, 530, and 553.

been used to identify *Bartonella* and other causative agents of endocarditis.[55,160,559]

Radiographic techniques have not been a great aid in establishing the diagnosis of infective endocarditis. The findings on plain chest radiographs are nonspecific, but evidence of complications, such as septic pulmonary emboli or congestive heart failure, may be helpful. Computed tomography may help in establishing the diagnosis of an infected shunt.[545] Immunoscintigraphy using technetium-labeled antigranulocyte antibodies may yield useful information when the echocardiographic findings are equivocal.[363] Cineangiography is the definitive method to determine the anatomic alterations in the heart resulting from infective endocarditis, but it rarely needs to be performed in children.

The electrocardiogram also is useful in the evaluation of patients with endocarditis because it detects arrhythmias and conduction disturbances that complicate the disease. Ventricular ectopy may be related to myocardial ischemia, myocarditis, or myocardial abscess. New conduction defects imply extension of infection beyond the valve ring into the myocardium. Any degree of atrioventricular block, a new left bundle branch block, or a new right bundle branch block with a left anterior hemiblock may represent extension of infection from the aortic valve into the ventricular septum. Junctional tachycardia, Wenckebach atrioventricular block, or complete heart block may be produced by extension of the infection from the mitral valve anulus into the atrioventricular node or proximal His bundle. In general, an unstable conduction block is more likely to develop in patients with aortic valve endocarditis than in those with mitral infection.[118]

Echocardiography has become a valuable adjunct to the diagnosis and treatment of endocarditis in children.* Color Doppler imaging is a sensitive modality for detection of valvular insufficiency, and the results may influence surgical and medical treatment decisions.[155] Echocardiography can be performed by the traditional transthoracic approach or the transesophageal approach.[419] The sensitivity and specificity of transthoracic echocardiography continue to be defined, with positive results obtained in 36 to 100 percent of children in various series of pediatric patients.†

In general, two-dimensional echocardiography is more sensitive than is the M-mode technique, especially in cases of right-sided endocarditis,[399] and it is superior in diagnosing complications of the destructive process.[25,355] The smallest vegetation detectable is approximately 2 mm, but the acoustic impedance of the mass relative to the surrounding structures is a more important factor than is size in identifying the vegetation. Echocardiography has identified vegetations in culture-negative cases.[267] Its accuracy in prosthetic-valve endocarditis is diminished by the difficulty in resolution around the prosthetic device.[374,507] Serial evaluation of valvular vegetations generally does not assist in assessing the efficacy of antibiotic therapy because diminution or disappearance of vegetations may take place long after successful medical treatment has been completed.[278,449,558]

The use of transthoracic echocardiography to predict the clinical course and need for operative intervention in patients with endocarditis is controversial.[8,190]A synopsis of many reports that have assessed the role of transthoracic echocardiography in the diagnosis and management of infective endocarditis suggests the following: (1) because of variable sensitivity among studies for detection of vegetations, a negative study does not rule out endocarditis, especially when foreign material is present within the heart; (2) false-positive studies are quite rare (the specificity is high); (3) the reliability of transthoracic echocardiography depends on the experience of the examiner and the technical adequacy of the study; (4) transthoracic echocardiography is valuable in assessing local complications of endocarditis on native valves; and (5) in most but not all studies, patients with a vegetation identified by transthoracic echocardiography have an increased risk for the development of systemic emboli and congestive heart failure.*

Although some investigators contend that the presence of a vegetation should hasten early surgery, most suggest that a positive echocardiogram is adjunctive evidence that should be considered along with other clinical parameters when considering surgical intervention. One study suggested that the relative risk for having embolic events associated with echocardiographically visualized lesions is microorganism dependent, with a significant attributable risk seen, for instance, in patients with viridans streptococcal infection.[499] The absence of a vegetation on transthoracic echocardiography may define a subset of patients at low risk for the development of embolic complications.

Transesophageal echocardiography has been studied extensively in adults with infective endocarditis.[104] It uses a 5-MHz phased-array transducer with Doppler and color flow encoding capabilities mounted on the tip of a flexible endoscope.[474] Biplane transesophageal echocardiography is considered the standard technique and is superior to transthoracic echocardiography because of improved spatial resolution, lack of acoustic interference from the lungs and chest wall, and closer proximity to posterior structures, such as the mitral valve and left atrium.[103] Multiplane transesophageal echocardiography facilitates and abbreviates the examination procedure and may be more accurate in providing the dimensions of a vegetation associated with infective endocarditis.[103,244]

Transesophageal echocardiography generally is well tolerated by children, even with the use of an adult probe (when the child's weight is more than 7 kg),[482] and rarely is associated with bacteremia.[102,289,418] Transesophageal echocardiography usually is more sensitive than is transthoracic echocardiography in the detection of intracardiac vegetations and is positive in 70 to 95 percent of adults with strongly suspected endocarditis.[371,489] One recent study showed that transesophageal echocardiography significantly increased the detection of vegetations in bigger (>60 kg) children but did not improve on the results of transthoracic echocardiography in smaller

*References 116, 186, 286, 441, 457, 458, 482.
†References 56, 94, 116, 267, 300, 307, 461.

*References 190, 241, 319, 346, 443, 468, 506.

children.[411] It is significantly more sensitive in the detection of vegetations and complications in infected prosthetic valves.[273,406,448,507,520] Transesophageal echocardiography is particularly useful for detecting an aortic root abscess or involvement of the sinus of Valsalva in adults, and it should be considered in children with aortic valve endocarditis and changing aortic root dimensions on a standard transthoracic echocardiogram.[155,346] It appears to be less helpful for detection of vegetations in right-sided endocarditis.[469] Although a negative transesophageal echocardiographic study does not exclude endocarditis,[494] the procedure should be considered for patients with suspected endocarditis and a negative transthoracic echocardiogram, when the transthoracic echocardiographic windows are suboptimal, and when perivalvular extension of infection is suspected.[15,230]

To aid in establishing the diagnosis of infective endocarditis, various sets of clinical criteria have been suggested. The von Reyn criteria were described in 1982, but echocardiographic findings were not included in the case definitions.[557] In addition, isolation of a typical infective endocarditis pathogen from blood cultures was not considered. New case definitions and diagnostic criteria were proposed by investigators from Duke,[129] and they have been modified subsequently (Tables 26-5 and 26-6).[288,305] The inclusion of echocardiographic and blood culture findings in these new criteria has resulted in more flexibility, a higher proportion of "definite" cases, and a more accurate reflection of current clinical practice.[351] These criteria have been validated in large series of infective endocarditis in adults and children.[114,129,215,442,467] In two pediatric series of clinically defined endocarditis, no cases were rejected by the Duke criteria, whereas 25 and 19 percent were rejected by the von Reyn criteria.[114,508] In one study, three of six pathologically confirmed cases were rated as only probable or rejected by the von Reyn criteria whereas all were definite by the Duke criteria.[114] However, a more recent study found that 12 percent of pediatric endocarditis cases were not classified as "definite" by the modified Duke criteria.[533] In addition, the presence of an indwelling venous catheter causing prolonged bacteremia may cause an overestimation of the rate of infective endocarditis using the Duke criteria.[42]

TABLE 26-5 **Definition of Terms Used in the Modified Duke Criteria for Infective Endocarditis**

Major Criteria

1. Positive blood culture
   a. Typical microorganisms for IE from ≥2 blood cultures
      (1) Viridans streptococci, *Streptococcus bovis*, HACEK group, *Staphylococcus aureus or*
      (2) Enterococci, in the absence of another primary focus, *or*
   b. Persistently positive blood cultures, with recovery of a microorganism consistent with IE from
      (1) Blood cultures drawn ≥12 hours apart *or*
      (2) All of three or a majority of four or more separate blood cultures, with first and last drawn ≥1 hour apart
2. Evidence of endocardial involvement
   a. Positive echocardiogram for IE
      (1) Oscillating intracardiac mass on valve or supporting structures, in the path of regurgitant jets, or on implanted material, in the absence of an alternative anatomic explanation, *or*
      (2) Abscess *or*
      (3) New partial dehiscence of a prosthetic valve *or*
      (4) New valvular regurgitation (increase or change in preexisting murmur is not sufficient)

Minor Criteria

1. Predisposing heart condition or intravenous drug use
2. Fever ≥38°C (100.4°F)
3. Vascular phenomena: major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial hemorrhage, conjunctival hemorrhages, Janeway lesions
4. Immunologic phenomena: glomerulonephritis, Osler nodes, Roth spots, rheumatoid factor
5. Microbiologic evidence: positive blood culture but not meeting major criteria as noted previously* or serologic evidence of active infection with organism consistent with IE

*Excluding single positive cultures for coagulase-negative staphylococci and organisms that do not cause IE.
HACEK, Haemophilus species, *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae; IE,* infective endocarditis.
From Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 2000;**30**:633–8.

MICROBIOLOGY

Many different microorganisms are capable of causing infective endocarditis in humans.[235] A list of the organisms isolated from patients in major pediatric series is presented in Table 26-7. Gram-positive cocci are the etiologic agents in 90 percent of cases in which an organism is isolated. Streptococci remain the bacteria isolated most frequently, although the percentage of cases caused by staphylococci and fungi has been increasing during the past 2 decades.[164,461,553,586] Polymicrobial infective endocarditis, especially in nosocomial settings, also appears to be increasing in incidence.[23] The characteristics of selected organisms and the type of disease that they produce are considered next.

Streptococci

Several terminologies have been used to classify streptococci. The Lancefield system defines groups (A, B, C, D, E, F, G, H) by serologic reactions. The viridans streptococci are α-hemolytic or nonhemolytic, may be Lancefield nontypeable (*Streptococcus anginosus* [formerly *S. milleri*], *S. mitior*, *S. salivarius*, most *S. mutans*, and *S. sanguinis*) or typeable (*S. bovis* group D, some *S. sanguinis* group H, some *S. anginosus* group F), and display similar characteristics in vivo. They are the most frequent etiologic agents in subacute infective endocarditis and cause 40 percent of cases in children. They may cause rapidly progressive invasive disease.[222,515]

Viridans streptococci are common pathogens in patients with underlying heart disease but are less common in postoperative patients. They are part of the

TABLE 26-6 Modified Duke Criteria for the Diagnosis of Infective Endocarditis

Definite

1. Pathologic criteria
   a. Microorganisms: demonstrated by culture or histology in a vegetation, in a vegetation that has embolized, or in an intracardiac abscess *or*
   b. Pathologic lesions: vegetation or intracardiac abscess present and confirmed by histology showing endocarditis
2. Clinical criteria (see Table 26-5)
   a. Two major criteria *or*
   b. One major and three minor criteria *or*
   c. Five minor criteria

Possible

1. One major criteria and one minor criterion, or
2. Three minor criteria

Rejected

1. Firm alternative diagnosis explaining evidence of IE *or*
2. Resolution of IE syndrome with antimicrobial therapy for ≤4 days *or*
3. No pathologic evidence of IE at surgery or autopsy with antibiotic therapy for ≤4 days

IE, infective endocarditis.

From Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 2000;**30**:633–8.

TABLE 26-7 Etiologic Agents of Bacterial Endocarditis in Children

Organism	Average (%)	Range (%)
Streptococci		
Viridans	40.3	17-72
Enterococci	4.0	0-12
Pneumococci	3.3	0-21
β-Hemolytic	2.7	0-8
Other	1.1	0-16
Staphylococci		
Staphylococcus aureus	23.8	5-40
Coagulase-negative	4.7	0-15
Gram-negative aerobic bacilli	4.0	0-15
Fungi	1.1	0-12
Miscellaneous bacteria	2.4	0-10
Culture-negative	12.6	2-32

Data from references 53, 63, 96, 247-249, 284, 348, 461, 497, 530, and 553.

indigenous flora of the human mouth and gastrointestinal tract, and procedures that disrupt mucosal integrity in these areas predispose patients to development of viridans streptococcal bacteremia. In the pediatric population, most blood and cerebrospinal fluid isolates of viridans and nonhemolytic streptococci are not from patients with infective endocarditis.[203] Most strains are exquisitely susceptible to penicillin, although resistance has been increasing related to previous administration of antibiotics.[282,301] Nutritionally variant viridans streptococci, reclassified as *Abiotrophia defectiva* or *Granulicatella* spp., are recognized as one cause of culture-negative endocarditis in children.* These organisms grow in broth but will not grow on subculture agar-based plates. Bacteriologic failure has occurred in 40 percent of reported cases of endocarditis caused by these organisms despite susceptibility to the antibiotics used.[25,503] Most viridans streptococci have low pathogenicity; however, the *S. anginosus* group has a predilection for suppurative complications.[376] The prognosis of endocarditis caused by nonenterococcal streptococci is excellent with good medical and surgical management; the cure rate is more than 90 percent, although complications (emboli, congestive heart failure) occur in as many as 30 percent of cases.

Enterococcal endocarditis occurs much less frequently in children than in adults[349,527,529] and accounts for only 4 percent of pediatric cases. The organism normally inhabits the gastrointestinal and genitourinary tracts; instrumentation of these areas may cause enterococcal bacteremia. More than 40 percent of adult patients have no underlying heart disease.[440] Endocarditis should be considered in all infants and children with unexplained enterococcal bacteremia. Although the incidence of enterococcal bacteremia appears to be increasing in some neonatal intensive care units, the incidence of associated endocarditis seems to be very low. Factors that may suggest endocarditis in patients with enterococcal bacteremia include (1) preexisting heart disease, (2) community acquisition, (3) a cryptogenic source, and (4) the absence of polymicrobial bacteremia.[326] Differentiation of enterococci from other group D streptococci *(S. bovis)* is important because their respective therapeutic approaches are different.

Endocarditis caused by β-hemolytic streptococci occurred more commonly in the preantibiotic era than today. Most cases are caused by Lancefield group B or G organisms,[1,17,63,171,566] whereas group C and A streptococci rarely cause endocarditis.[49,121,187,314,433] Group A, B, or C streptococcal infection may lead to large, bulky vegetations, easily seen by echocardiography, and to embolic complications.[18,360,464] Although group B streptococcal bacteremia is a common finding in neonates, endocarditis caused by this organism occurs rarely in this age group. Similarly, *S. pneumoniae* accounted for 10 to 15 percent of endocarditis cases in the preantibiotic era but currently causes less than 1 percent.[175,240,397] Pneumococcal endocarditis may involve either the aortic or the mitral valve.[135,184,537] In older studies, fewer than 50 percent of affected children had underlying heart disease, but in more recent series, most children have had existing heart disease.[236] The clinical course often is fulminant.[132,297,420] Concurrent meningitis or pneumonia (or both) occurs frequently. Valvular dysfunction and cardiac decompensation are common findings.[52,60,83] Early surgical intervention may be required because the mortality rate is 75 percent when medical management alone is used.[240]

Staphylococci

Staphylococci cause 20 to 30 percent of cases of infective endocarditis in children, but the relative incidence

*References 77, 146, 308, 384, 430, 445.

appears to be increasing, and in some series this organism has been more common than streptococci.[6,164] *S. aureus* is the etiologic agent in most cases of acute endocarditis and frequently infects normal heart valves.[165,369] The course often is fulminant when the mitral or aortic valve is involved, with frequent suppurative complications occurring both in the heart (myocardial abscess, pericarditis, valve ring abscess) and in other organs.[138,254,356] *S. aureus* is responsible for more than 50 percent of cases of endocarditis in intravenous drug users, but the disease tends to be less severe in these patients.[75,76] The origin of the infecting organism is the addict's own nose or skin, not the injection paraphernalia.[543] Endocarditis associated with indwelling vascular catheters or prosthetic valves frequently is caused by *S. aureus.*[245] Endocarditis must be suspected in any patient with *S. aureus* bacteremia, even when a peripheral focus of infection is present.[452] However, most patients with *S. aureus* bacteremia do not have endocarditis. The rise of methicillin-resistant *S. aureus* (MRSA) has rendered treatment more difficult but has had little impact on the rate of local complications.[228]

The incidence of endocarditis resulting from CONS is rising rapidly.[13] CONS is a common etiologic agent of endocarditis occurring after cardiac surgery,[271,310] and it is occurring more frequently on native valves.[84,85] This organism is the leading agent in prosthetic valve endocarditis, for which it causes 25 to 67 percent of early cases and 25 to 33 percent of late cases.[181,237,261] CONS endocarditis also has been associated with mitral valve prolapse and the use of intravascular catheters in premature neonates.[24,392] Although metastatic infection rarely occurs, CONS can be locally invasive; the mortality rate of prosthetic valve endocarditis caused by CONS approaches 75 percent when valve replacement is not performed.

Gram-Negative Organisms

Although gram-negative bacteria cause 4 to 5 percent of cases of infective endocarditis in children, the percentage of children with gram-negative enteric bacteremia in whom endocarditis develops is extremely low. Endocarditis should be suspected in patients with gram-negative infection when bacteremia persists despite administration of usually appropriate antibiotic therapy.[70] Burn patients,[232] immunosuppressed hosts, narcotic addicts, and patients with implanted endovascular devices[364] are at an increased risk for development of gram-negative endocarditis. However, in the early postoperative period after cardiac surgery, sustained gram-negative bacillary bacteremia commonly is caused by other foci of infection and does not imply the presence of endocarditis. Many species of gram-negative enteric organisms have caused infective endocarditis in children, but no clear pattern has emerged. Among the gram-negative organisms more commonly reported are *Brucella*, *Escherichia coli*, *Serratia*, *Klebsiella-Enterobacter*, *Salmonella*, and *Pseudomonas.** Endocarditis caused by *Salmonella* has been reported in patients with HIV infection.[152] It most often affects previously abnormal heart valves. Endocarditis is a rare complication of tularemia.[523] Cure of left-sided endocarditis caused by the Enterobacteriaceae seldom is achieved with medical therapy alone.[474] Most information about gram-negative enteric endocarditis is limited to case reports and general medicine reviews; discussion of individual organisms is beyond the scope of this review.

Other gram-negative organisms associated with infective endocarditis are the so-called HACEK coccobacilli.[108,147,229] These organisms caused 57 percent of cases of gram-negative endocarditis seen at the Mayo Clinic in Rochester, Minnesota, from 1958 to 1979.[177] Endocarditis caused by *Haemophilus influenzae* has been reported in only several children.[101,330] Cases caused by *H. parainfluenzae* and *H. aphrophilus* occur slightly more commonly.* They generally are seen in the setting of preexisting valvular disease and run a subacute course. However, central nervous system complications and emboli to major peripheral arteries are frequent occurrences.[86] Infective endocarditis caused by other organisms of the HACEK group is an extremely rare event in children.† Infection caused by *K. kingae* is being recognized more frequently due to recognition and enhanced culture techniques.[123,124,219] All the bacteria in this group are fastidious, may require 2 to 3 weeks for primary isolation, and need subculturing onto chocolate agar in an atmosphere of 5 to 10 percent carbon dioxide for optimal growth. These procedures should be performed in all cases of culture-negative endocarditis.

Neisseria gonorrhoeae was responsible for 10 percent of cases in the preantibiotic era, but fewer episodes have been reported since 1942.[151,238] This pathogen frequently attacks previously normal heart valves and is manifested as an acute illness.[524] Valvular destruction with a need for valve replacement occurs commonly. At present, nonpathogenic *Neisseria* spp. are isolated more frequently in endocarditis than are gonococci, but they usually attack abnormal or prosthetic valves.‡ Although 1 percent of cases of infective endocarditis in adults are caused by anaerobic bacteria,[149] reports of anaerobic endocarditis in children are exceedingly rare.[91,385,492,511]

Gram-Positive Bacilli

Infective endocarditis caused by *Corynebacterium* spp. is an unusual finding but may occur on normal or previously abnormal valves.[41,350] Both toxigenic[111] and nontoxigenic[195,479,493,532] strains of *C. diphtheriae* cause endocarditis in children, a finding demonstrating that the toxigenic and invasive properties of the organism are independent. Infection occurs most often on native valves and may be quite aggressive and lead to major vascular complications. *Listeria monocytogenes* endocarditis rarely occurs, has a high mortality rate, and, unlike other forms of listeriosis, usually is not associated with immunocompromised hosts.[35,71] It has not been associated with listeriosis in neonates. Fewer than 40 cases of *Lactobacillus* endocarditis have been reported.[194,231,514] Endocarditis caused by *Erysipelothrix rhusiopathiae* is found predominantly in

*References 115, 283, 318, 364, 517, 525.

*References 45, 86, 107, 229, 243, 320, 412.
†References 12, 148, 324, 368, 402, 429, 570.
‡References 57, 208, 223, 233, 417, 453, 483.

adults who are farmers or are exposed to farm animals or products.[189,206] Most cases of *Bacillus* endocarditis involve the tricuspid valve in intravenous drug users, but other patients have been affected, including those with prosthetic valves.[502] *Gemella morbillorum* (formerly *Streptococcus morbillorum*) is a gram-positive coccus that normally resides in the gastrointestinal tract and is a rare cause of endocarditis.[142,290,426]

Other Organisms

Many different bacteria, including *Acinetobacter*,[192] *Stenotrophomonas*,[447] *Nocardia*,[563] *Actinomyces*,[287] *Streptobacillus*,[455] and *Rothia*,[512] have been associated rarely with endocarditis.[59] Mycobacterial endocarditis is an exceedingly infrequent event.[170]

Infective endocarditis caused by *Coxiella burnetii*, the causative agent of Q fever, is well documented in northern Africa, Europe, and Australia.[4,58,291,304,359] Most cases are chronic (occurring over a 6- to 12-month period) and involve the aortic valve.[328] Clues to establishing the diagnosis include exposure to parturient cats or rabbits, massive splenomegaly, hypergammaglobulinemia, and thrombocytopenia.[415] The diagnosis usually is confirmed by measurement of antibodies against phase I and phase II antigens, but the organism has been isolated from leukocytes in a shell vial assay and has been demonstrated by immunohistologic techniques.[58,183] At least 20 well-documented cases of infective endocarditis caused by *Chlamydia psittaci* and *Chlamydophila pneumoniae* (formerly *Chlamydia pneumoniae*) have been reported.[216,253,336,488] Most patients have had preexisting heart disease and a subacute course.[329] *Mycoplasma* endocarditis is exceedingly rare.[119] *Legionella* has been implicated in several cases of prosthetic valve endocarditis.[538] *Bartonella quintana* and *B. henselae* have been identified as the cause of endocarditis in "culture-negative" cases.[106,122,217,242,496] Most described cases have been in immunocompetent individuals.[416] The diagnosis was established by serology, polymerase chain reaction, or special culture techniques.[28,150,160,434,559]

Although culture of bacteria remains the primary method for establishing the microbial cause of infective endocarditis, the number of organisms causing endocarditis that cannot be cultivated by standard culture methods is growing.[161,225] More recently, universal and species-specific primers have been designed to amplify bacterial DNA directly from resected valves. Among the organisms causing endocarditis identified by these methods are *Bartonella*, *Tropheryma whippelii*, *Coxiella*, *Mycoplasma*, *Haemophilus*, *Abiotrophia*, *Gemella*, *Cardiobacterium*, and *Streptococcus*.[174,188,225,312,427]

Fungi

Most cases of fungal endocarditis in children have been described as occurring after cardiovascular surgery and prolonged intravenous and antibiotic therapy.[134,140,353,534] More recently, cases have been reported in neonates[341] and after prosthetic valve placement.[388] The most common causative organism is *C. albicans*, although disease has been attributed to other *Candida* spp., including *C. krusei*, *C. parapsilosis*, *C. stellatoidea*, *C. tropicalis*, and *C. guilliermondii*.[424,465,484] Among intravenous drug users, *Candida* spp. other than *C. albicans* are more common causes of endocarditis.[454] The clinical manifestation usually is indolent and not specific, with symptoms occurring weeks to months before the diagnosis is established. Signs and symptoms caused by emboli to large vessels, especially those supplying the brain, kidney, spleen, and extremities, should alert the physician to the possible presence of fungal endocarditis. Large, friable vegetations occur frequently and can be detected by echocardiography.[509] Cutaneous and ocular manifestations of systemic *Candida* infection may be present.[54] The prognosis of *Candida* endocarditis is poor and is related to the propensity for septic emboli, the tendency for invasion into the myocardium, and the poor penetration of antifungal agents into the bulky vegetation. The diagnosis frequently is delayed by the tendency for negative or intermittently positive blood cultures to occur in this disease.[251] Surgical intervention usually is required.

Aspergillus spp., including *A. flavus*, *A. fumigatus*, *A. terreus*, and *A. niger*, are the second most frequent causes of fungal endocarditis, and such infections have been reported in 16 children.[31,32,390] Two thirds of these patients had underlying heart disease. *Aspergillus* endocarditis has been found in immunocompromised hosts with no previous cardiac problems.[583] The most common initial manifestations are fever and embolic phenomena, especially to the central nervous system.[560] Fewer than 25 cases have been diagnosed ante mortem, several by culture of peripheral emboli. Most cases occur after open heart surgery; the most likely source of the organism is airborne inoculation of the heart during the operation.[31,343] Surgical removal of all infected material is recommended, although only several children have been treated successfully. Other fungi that rarely cause endocarditis include *Histoplasma capsulatum*, *Coccidioides immitis*,[292] *Cryptococcus neoformans*, *Torulopsis glabrata*, *Trichosporon beigelii*,[269] and *Fusarium* spp.[198,226]

TREATMENT

In the preantibiotic era, infective endocarditis was a uniformly fatal disease. With the current improved methods of diagnosis and therapy, 80 to 90 percent of children with this disease can be expected to survive. Mortality rates are higher for acute staphylococcal infection, fungal endocarditis, and prosthetic valve endocarditis, although the tendency toward earlier surgical intervention for these entities may improve survival rates. The cornerstone of successful therapy is selection of antibiotics with specific activity against the causative organism. Better analysis of pharmacodynamic variables, such as bactericidal activity and the postantibiotic effects of various drugs, may assist in the selection of optimal therapeutic regimens.[95,275,578] Although persistent infection occasionally complicates treated endocarditis,[438] deterioration in cardiac function is the major cause of morbidity and mortality.

Several general principles provide the basis for the current recommendations for treatment of endocarditis.

Parenteral administration of antibiotics is preferred because erratic absorption of oral antibiotics, especially in infants, can lead to therapeutic failure. Although patient selection criteria for the use of outpatient parenteral antibiotic therapy for endocarditis in adults have been suggested, no data have been published about this practice for children.[10] Prolonged treatment, usually 4 to 6 weeks or longer, is necessary to sterilize the vegetations and to prevent relapse. Bacteriostatic antibiotics are not effective and lead to frequent relapses or failure to eradicate the infection, or both. Antibiotic combinations may produce a rapid bactericidal effect through synergistic mechanisms of action. When synergy exists, smaller doses of each drug may be used, thereby reducing toxic side effects. However, certain drug combinations can be antagonistic, and their use should be avoided.

Blood should be drawn for culture for several days to evaluate the effect of the antibiotics. Negative follow-up cultures do not guarantee the success of therapy, but persistent positive cultures usually require that a change or addition to the antibiotic regimen be made. Observation of the patient's clinical course is extremely important. When fever is present initially, the temperature often returns to normal within a few days after therapy is started. However, fever can persist for weeks in patients whose eventual outcome is good. Such patients must be monitored closely for cardiac arrhythmias and congestive heart failure, which may require intensive care observation and electrocardiographic monitoring. Evidence of major embolic phenomena must be sought diligently by physical examination.

Several laboratory tests may aid in monitoring therapy. In all cases of bacterial endocarditis, the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) ideally are determined for the antibiotics being used because disk susceptibility testing may not be as reliable and is not quantitative. The role of monitoring the inhibitory and bactericidal activity of the patient's serum is highly controversial. The Schlichter test determines the maximal dilutions of a patient's serum that in vitro inhibit and kill an inoculum of the organism causing the endocarditis.[437] Standardization of this test is poor, with laboratories using variations in inoculum size, in composition of the broth, in timing of samples (at expected peak or trough antibiotic concentrations in serum), in methods of dilution, and in determining the bactericidal end point. In the rabbit endocarditis model, peak serum bactericidal titers greater than 1:8 correlate with therapeutic success.[69] A retrospective review of 17 reports of serum bactericidal activity in patients with endocarditis failed to show any correlation between titers greater than 1:8 and therapeutic success.[92] A prospective study suggested adjusting antibiotic doses to achieve peak titers of 1:64 or greater and trough titers of 1:32 or greater.[569] At present, no generally accepted recommendation can be made. In general, to attempt to achieve a peak serum bactericidal titer of at least 1:8 or greater seems reasonable if serious drug toxicity is not encountered. However, this level may not be attainable with certain organisms such as enterococci and gram-negative bacilli. Serum bactericidal testing may be particularly useful when synergistic combinations or less well-established antibiotic regimens are used or when response to therapy is suboptimal.[582]

TABLE 26-8 Suggested Intravenous Antibiotic Doses and Schedules for Infective Endocarditis in Children

Antibiotic	Daily Dose/kg	Divided Doses Every:
Aqueous crystalline penicillin G sodium	300,000 U	4-6 hr
Ampicillin sodium	300 mg	4-6 hr
Ampicillin-sulbactam	300 mg	4-6 hr
Cefazolin	100 mg	6-8 hr
Ceftriaxone	100 mg	12 hr
Ciprofloxacin	20-30 mg	12 hr
Doxycycline	2-4 mg	12 hr
Gentamicin sulfate	3 mg	8 hr or 24 hr
Imipenem/cilastatin	60-100 mg	6 hr
Linezolid	30 mg	8 hr
Meropenem	60 mg	8 hr
Nafcillin sodium	200 mg (max., 12 g)	4-6 hr
Oxacillin sodium	200 mg (max., 12 g)	4-6 hr
Rifampin	20 mg	8-12 hr
Vancomycin hydrochloride	40-60 mg	6-12 hr

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

Little information is available concerning optimal antibiotic therapy for infective endocarditis in children; most treatment regimens are adapted from studies of adults with endocarditis.[25,579] In general, these regimens have been equally successful (and generally less toxic) in children. Recommended doses of the antibiotics commonly used are listed in Table 26-8.

After performing the initial evaluation of a patient with suspected infective endocarditis, the physician must make a clinical judgment about when to initiate therapy. If the findings are strongly indicative of the diagnosis or the child is very ill, treatment should be started as soon as blood has been drawn for culture. Initial empirical therapy depends on the clinical setting in which the tentative diagnosis is made. If the infection is subacute, a combination of penicillin G and an aminoglycoside usually is recommended for its activity against viridans streptococci, enterococci, and most gram-negative organisms. If *S. aureus* endocarditis is a strong consideration (acute manifestation, narcotic addicts), vancomycin and a penicillinase-resistant penicillin should be added to this regimen. Patients who recently have undergone cardiac surgery, especially prosthetic valve placement, are treated best with an aminoglycoside and vancomycin to "cover" for health care–associated infection caused by MRSA or CONS; some physicians add penicillin G to this regimen to improve activity against streptococci. When culture and susceptibility data are known, antibiotic therapy can be changed as needed.

Most strains of viridans streptococci, *S. pyogenes*, and nonenterococcal group D streptococci are exquisitely susceptible to penicillin, with an MIC of less than 0.2 μg/mL. However, 15 to 20 percent of viridans streptococci have an MIC of 0.2 μg/mL or greater and are defined arbitrarily as relatively resistant.[227] In addition, some strains (particularly *S. mutans* and *S. mitior*) demonstrate tolerance, that is, an MIC to penicillin of less than 0.1 μg/mL but an MBC that is more than 10-fold higher (1.25 to 50 μg/mL). Most strains of nutritionally dependent streptococci are tolerant to penicillin.[24] Clinical failure may occur in endocarditis caused by these tolerant organisms when penicillin alone is used for treatment.[9,227] However, except for nutritionally dependent streptococci, therapy for tolerant viridans streptococci generally should be the same as for susceptible strains.

Although most experts recommend that patients with endocarditis caused by relatively resistant streptococci be treated with high doses of penicillin combined with 2 to 4 weeks of an aminoglycoside, some authorities consider that penicillin alone usually is adequate therapy.[48,117,327] Synergy in vitro between penicillin or vancomycin and streptomycin or gentamicin can be demonstrated against virtually all penicillin-susceptible streptococci.[562] This observation correlates with a faster rate of eradication of bacteria from cardiac vegetations in the rabbit endocarditis model when synergistic combinations of antibiotics are used.[127,130] However, streptomycin is not synergistic for strains with high-level streptomycin resistance; gentamicin is the preferred second drug for these rare isolates.[136] In pediatric patients, gentamicin usually is substituted for streptomycin because of its lower toxicity.

Several regimens have been examined in adults with penicillin-susceptible viridans streptococcal native valve endocarditis (Table 26-9). A 2-week course of penicillin alone leads to an unacceptable relapse rate. However, a 2-week course of intramuscular procaine penicillin and streptomycin cured 99 percent of adults with penicillin-susceptible streptococcal endocarditis in one report.[580] These results are similar to those obtained with β-lactams alone for 4 weeks[263] or with penicillin for 4 weeks combined with streptomycin for the first 2 weeks. Gentamicin may be substituted for streptomycin. The 2-week penicillin-gentamicin regimen is the least expensive and is the preferred therapy in uncomplicated cases of penicillin-susceptible streptococcal endocarditis in young adults.[474] In general, the regimen of 4 weeks of penicillin alone is preferred for patients in renal failure or at high risk for developing aminoglycoside-induced ototoxicity. Vancomycin or ceftriaxone administered for 4 weeks can be used in patients with penicillin-susceptible viridans streptococcal endocarditis who have a penicillin allergy.[166,167,485,510] The regimen of 4 weeks of penicillin plus an initial 2 weeks of gentamicin is recommended in adults with a complicated course (symptoms for >3 months) or with infection caused by relatively penicillin-resistant organisms (Table 26-10). Most nutritionally

TABLE 26-9 Suggested Regimens for Treatment of Native Valve Endocarditis Caused by Highly Penicillin-Susceptible Viridans Streptococci and *Streptococcus bovis*

Antibiotic(s)	Duration (wk)	Comments
Aqueous crystalline penicillin G sodium	4	Preferred for patients with impairment of the eighth cranial nerve or renal function
Ceftriaxone sodium	4	
Aqueous crystalline penicillin G sodium *or* ceftriaxone *plus*	2	Gentamicin peak serum concentration of approximately 3 μg/mL is desirable
Gentamicin sulfate	2	
Vancomycin hydrochloride	4	Recommended for patients allergic to β-lactam antibiotics

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

TABLE 26-10 Suggested Therapy for Native-Valve Endocarditis Caused by Strains of Viridans Streptococci and *Streptococcus bovis* Relatively Resistant to Penicillin G (Minimum Inhibitory Concentration, 0.12 and 0.5 μg/mL)

Antibiotic(s)	Duration (wk)	Comments
Aqueous crystalline penicillin G sodium	4	Cefazolin or other first-generation cephalosporins may be substituted for penicillin in patients whose penicillin hypersensitivity is not of the immediate type
or		
Ceftriaxone sodium	4	
plus		
Gentamicin sulfate	2	
Vancomycin hydrochloride	4	Recommended for patients allergic to β-lactam antibiotics

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

TABLE 26-11 **Suggested Therapy for Endocarditis Caused by Enterococcal Strains Susceptible to Penicillin, Gentamicin, and Vancomycin and Other Selected Streptococci***

Antibiotic(s)	Duration (wk)	Comments
Aqueous crystalline penicillin G sodium	4-6	4-wk therapy recommended for patients with symptoms <3 mo in duration; 6-wk therapy recommended for patients with symptoms >3 mo in duration
plus		
Gentamicin sulfate	4-6	
Ampicillin sodium	4-6	
plus		
Gentamicin sulfate	4-6	
Vancomycin hydrochloride	6	Recommended for patients allergic to β-lactam antibiotics
plus		
Gentamicin sulfate	6	

*Viridans streptococci with a minimal inhibitory concentration >0.5 μg/mL, *Abiotrophia* species, *Granulicatella* species, or *Gemella* species.

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

TABLE 26-12 **Suggested Therapy for Endocarditis of Prosthetic Valves or Other Prosthetic Material Caused by Viridans Group Streptococci and *Streptococcus bovis***

Antibiotic(s)	Duration (wk)
Penicillin-Susceptible Strain	
Aqueous crystalline penicillin G sodium	6
or	
Ceftriaxone	6
with or without	
Gentamicin sulfate	2
Vancomycin hydrochloride	6
Penicillin Relatively or Fully Resistant	
Aqueous crystalline penicillin G sodium	6
or	
Ceftriaxone	6
plus	
Gentamicin sulfate	6
Vancomycin hydrochloride	6

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

deficient streptococci are tolerant to penicillin. For patients with endocarditis caused by these organisms, a course of 4 to 6 weeks of penicillin with the addition of an aminoglycoside is recommended (Table 26-11).[25,579] In patients with streptococcal infection of prosthetic valves or other prosthetic materials, a 6-week regimen of penicillin usually supplemented with an aminoglycoside is recommended (Table 26-12). None of the regimens discussed has been evaluated specifically in children with endocarditis.

Most strains of enterococci have an MIC to penicillin of 0.4 μg/mL or greater and an MBC of 6.25 μg/mL or greater.[375] All β-lactam antibiotics are bacteriostatic against enterococci and cannot be used alone. However, plasmid-mediated β-lactamase production has been found in rare strains of *Enterococcus faecalis*. Ampicillin-sulbactam overcomes the enzyme production and may be effective as therapy.[529] Although therapy with penicillin alone is ineffective, the combination of penicillin and an aminoglycoside is synergistic and produces a bactericidal effect on most enterococcal strains. Unfortunately, 20 to 50 percent of enterococcal strains demonstrate very high resistance (MIC >2000 μg/mL) to streptomycin, and synergy between penicillin and streptomycin does not occur.[209,473] High-level resistance to gentamicin has been found in some isolates, and the incidence is increasing in certain locales.[133,311,403] When these isolates are encountered, all aminoglycosides should be tested because the organism may be susceptible to one while resistant to others.[567] Fortunately, these strains rarely cause endocarditis.[375] Although vancomycin-resistant enterococci have emerged as important nosocomial pathogens, they rarely cause endocarditis in children. Optimal therapy for these strains has not been established, but a combination of high-dose penicillin plus vancomycin and gentamicin may be effective in some cases.[66] Vancomycin-resistant enterococcal endocarditis has been treated successfully with oral linezolid.[11,16] The usual regimens for enterococcal endocarditis are listed in Tables 26-11 and 26-13.

Most isolates of *S. aureus* are resistant to penicillin, but endocarditis caused by penicillin-susceptible (MIC <0.1 μg/mL) isolates can be treated with this agent. In general, a semisynthetic penicillinase-resistant penicillin given for 6 weeks is the drug of choice (Table 26-14).[313,408,500] The addition of gentamicin to nafcillin produces an enhanced bactericidal effect in vitro and in experimental staphylococcal endocarditis in rabbits.[466] However, the value of this combination in patients has not been proved, and generally it is reserved for children with overwhelming infection. In penicillin-allergic patients or in those

TABLE 26-13 Suggested Therapy for Native or Prosthetic Valve Enterococcal Endocarditis Caused by Strains Resistant to Penicillin, Aminoglycoside, and Vancomycin

Antibiotic(s)	Duration (wk)
Enterococcus faecium	
Linezolid	≥8
Enterococcus faecalis	
Imipenen/cilastatin	≥8
plus	
Ampicillin sodium	≥8
or	
Ceftriaxone	≥8
plus	
Ampicillin sodium	≥8

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

TABLE 26-14 Suggested Therapy for Endocarditis Caused by Staphylococci in the Absence of Prosthetic Material

Antibiotic(s)	Duration	Comments
Methicillin-Susceptible Staphylococci*		
Nafcillin sodium or oxacillin sodium	6 wk	Benefit of additional aminoglycoside has not been established.
with optional addition of		
Gentamicin sulfate	3-5 days	
Cefazolin	6 wk	For patients with non–immediate-type hypersensitivity to penicillin
with optional addition of		
Gentamicin sulfate	3-5 days	
Methicillin-Resistant Staphylococci		
Vancomycin hydrochloride	6 wk	

*If Staphylococcus is penicillin-susceptible (minimal inhibitory concentration ≤0.1 μg/mL), aqueous crystalline penicillin G sodium can be used for 6 weeks instead of nafcillin or oxacillin.

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

with MRSA, vancomycin alone has been recommended, although treatment failures in children with endocarditis have been reported.[144,239,302] The addition of rifampin or the use of β-lactam drugs after desensitization is necessary in some cases.[34,369] Ciprofloxacin has been used, but

TABLE 26-15 Suggested Therapy for Endocarditis Caused by HACEK Organisms in Adults

Antibiotic(s)	Duration (wk)	Comments
Ceftriaxone sodium	4	Cefotaxime sodium or other third-generation cephalosporins may be substituted
Ampicillin sulbactam	4	
or		
Ciprofloxacin	4	

HACEK, Haemophilus species, *Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae.*

From Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.

treatment failures have occurred because of the emergence of resistance.[373,526] Vancomycin for at least 6 weeks, plus the addition of gentamicin for the first 2 weeks, is often recommended for the treatment of endocarditis caused by MRSA, although clinical trial data are not available. Daptomycin and linezolid also have been used successfully to treat endocarditis caused by MRSA.[163,358] Nosocomial infections with *S. epidermidis* usually are treated with vancomycin because of the high incidence of methicillin resistance among these isolates. The addition of rifampin and gentamicin to either nafcillin or vancomycin may increase bactericidal activity and is recommended in cases of prosthetic valve endocarditis secondary to any staphylococci (see Table 26-13).[25]

Therapy for endocarditis caused by gram-negative organisms must be individualized in accordance with in vitro susceptibility studies. A regimen of 6 to 8 weeks of combination therapy with two or more drugs may be required, especially with endocarditis caused by *Klebsiella* or *Pseudomonas*.[156,407] Surgical intervention frequently is necessary, especially for infection of the mitral or aortic valves. Endocarditis caused by *Haemophilus* and other fastidious gram-negative organisms usually is responsive to ampicillin, ceftriaxone, or ciprofloxacin alone,[25] but the addition of an aminoglycoside may improve the outcome (Table 26-15).[25,86] Anaerobic bacilli generally are susceptible to penicillin, but infection caused by resistant *Bacteroides fragilis* is treated best by combinations including metronidazole, ticarcillin-clavulanate, piperacillin-tazobactam, imipenem, or meropenem.

Survival rates of only 10 to 20 percent in patients with fungal endocarditis are related to the poor ability of currently available antifungal agents to sterilize the vegetations. Only rare cures with medical therapy alone have been reported.[465] Most investigators contend that early surgical intervention is mandatory in every patient who has conclusive evidence of intracardiac fungal infection.[504,546,547] Eradication of fungal prosthetic valve endocarditis has been reported rarely, even when surgery was performed.[182]

Although a prolonged course of antifungal therapy before surgery does not improve the outcome, chemotherapy should be given in conjunction with operative treatment. The drug of choice is usually amphotericin B or liposomal amphotericin. This antibiotic may be either fungistatic or fungicidal, depending on the infecting organism. Even though the toxicity of amphotericin B appears to be less severe in children than in adults, side effects that may necessitate alterations in the usual regimen may occur and include fever, chills, phlebitis, anemia, hypocalcemia, renal tubular acidosis, nephrotoxicity, and thrombocytopenia. The optimal dosage of amphotericin B is unknown; total doses of 20 to 50 mg/kg commonly are used. 5-Fluorocytosine[347] may act synergistically with amphotericin B against many strains of fungi, but their roles in fungal endocarditis are unproven. Fluconazole is less effective than is amphotericin B for the prophylaxis or treatment of experimental *Candida* endocarditis,[581] but it has been used successfully in a few patients.[97,554] Some newer antifungal agents, such as caspofungin, have been used successfully to treat fungal endocarditis, but the reported experience in children is limited.[522]

Treatment of culture-negative endocarditis is problematic.[30] In general, the same criteria used to choose empirical therapy for infective endocarditis can be followed. Antibiotics usually are continued for 6 weeks, and ongoing surveillance for an etiologic agent must be performed. In 52 adults with culture-negative endocarditis, survival correlated with the initial clinical response to antibiotics; most deaths were caused by systemic emboli or congestive heart failure.[414]

Surgery has become a valuable adjunct to medical therapy in the management of infective endocarditis.[33,398,413,421,462] The general trend has been for surgical intervention to be undertaken earlier and more frequently to prevent complications of endocarditis and lower mortality.[224,453,587] Several echocardiographic findings suggest a possible need for surgical intervention (Table 26-16).[33,155] Among the generally accepted indications for surgical intervention during active endocarditis are (1) refractory congestive heart failure,[352,536,577] (2) uncontrolled infection,[329] (3) more than one serious embolic episode, (4) fungal endocarditis, (5) most cases of prosthetic valve endocarditis,* and (6) local suppurative complications including perivalvular or myocardial abscess with conduction system abnormalities.† The usual indication for surgical intervention is congestive heart failure in left-sided lesions and persistent infection in right-sided disease.[112,556] Among children with endocarditis after cardiac surgery, repair or takedown of infected graft material commonly is the reason for surgery.[88,394] Recent studies have demonstrated that preservation of the child's native valve is frequently possible despite advanced clinical disease.[113,213,214,259] In general, operative mortality is low even if surgery is performed during the active infection.[372,387] The hemodynamic status of the patient, rather than the activity of the infection, is the critical factor in determining the timing of cardiac surgery or valve replacement.[3,260] The aortic valve is the site most often requiring surgical intervention.[366,423,540] Treatment with recombinant tissue plasminogen activator has been used successfully in some cases of endocarditis when surgery could not be performed safely.[196,303]

*References 7, 110, 137, 377, 460, 588.
†References 47, 264, 378, 450, 486, 518, 584.

TABLE 26-16 **Echocardiographic Features Suggesting a Possible Need for Surgical Intervention in Endocarditis**

Vegetation
Persistent vegetation after systemic embolization
Anterior mitral valve leaflet vegetation, particularly >10 mm
Embolic event during first 2 weeks of therapy
Increase in vegetation size after 4 weeks of therapy
Valvular Dysfunction
Acute aortic or mitral insufficiency with signs of ventricular failure
Heart failure unresponsive to medical therapy
Valve perforation or rupture
Perivalvular Extension
Valvular dehiscence, rupture, or fistula
New heart block
Large abscess or extension of abscess

Data from Bayer A., Bolger A, Taubert K, et al. Diagnosis and management of infective endocarditis and its complications. *Circulation* 1998;**98**:2936–48; and Ferrieri P, Gewitz MH, Gerber MA, et al. Unique features of infective endocarditis in children. *Pediatrics* 2002;**109**:931–43.

PREVENTION

Accepted medical practice has been to give prophylactic antibiotics to susceptible patients in an attempt to prevent infective endocarditis.[125,268,491] The rationale for such treatment is based on studies indicating that antibiotics can reduce the incidence of bacteremia after various procedures in humans[139] and can prevent experimental endocarditis in animals.[410] However, no controlled trials have documented the efficacy of endocarditis prophylaxis in humans.[551] Prevention of bacterial infection is most likely to be successful and cost effective when a single antibiotic is directed against a single pathogen and when the disease occurs with high frequency in the absence of prophylaxis. Prevention of endocarditis has not met these ideals because various drugs have been used against numerous organisms, and the disease rarely occurs even if prophylaxis is not given.[123] Fewer than 10 percent of all endocarditis cases can be attributed to bacteremia caused by previous medical, surgical, or dental procedures.[550] Many cases of prophylaxis failure have been reported,[128] but only 12 percent of such patients received antibiotic regimens recommended by the American Heart Association. For reasons that are not clear, mitral valve prolapse was the condition associated most frequently with failure of prophylaxis.

The most common errors in attempted prevention of endocarditis included inadequate medical histories taken

by dentists and other health care professionals to identify high-risk patients, initiation of prophylactic antibiotics too early, continuation of preventive therapy too long, the use of low-dose antibiotics, lack of prophylaxis for minor dental procedures, and confusion between prevention of rheumatic fever and prevention of infective endocarditis.[158,201,207] Several studies have shown that adult patients at risk for development of infective endocarditis often have inadequate knowledge of their cardiac lesion, endocarditis, and recommended prophylaxis.[74,281,552] Several studies demonstrated that the parents of children with heart defects have a low level of knowledge about the importance of good oral health in preventing endocarditis.[26,109,199,386,431] Another study cast doubt on the cost-effectiveness of endocarditis prophylaxis for urinary catheterization in children.[73]

The American Heart Association published new and radically different guidelines for the prevention of infective endocarditis in 2007.[576] These new guidelines eliminated many of the procedures for which prophylaxis previously was recommended.[99] It is now recommended that prophylaxis be undertaken for all dental procedures that involve manipulation of gingival tissue or the periapical region of teeth or perforation of the oral mucosa. Prophylaxis also can be considered for procedures on the respiratory tract or infected skin, skin structures, or musculoskeletal tissue. Prophylaxis no longer is recommended for gastrointestinal or genitourinary tract procedures. In addition, the number of heart lesions for which prophylaxis is recommended has been reduced to only those with the highest risk for endocarditis (Table 26-17).[576] Finally, the specific regimens for prophylaxis have been changed and simplified to encourage more judicious use (Table 26-18). Although some experts have expressed doubt about the validity of these newer recommendations,[487] there is no evidence that the release of these guidelines has been associated with a significant change in admissions for infective endocarditis.[400]

Immunization against bacteria that commonly cause endocarditis (e.g., viridans streptococci) has been proposed, but this approach remains a theoretical possibility.[19]

TABLE 26-17 Heart Conditions with the Highest Risk for Adverse Outcome from Endocarditis for Which Prophylaxis with Dental Procedures Is Recommended

- Prosthetic heart valve
- Previous infective endocarditis
- Congenital heart disease
  - Unrepaired cyanotic congenital heart disease, including palliative shunts and conduits
  - Completely repaired congenital heart defect with prosthetic material or device, whether placed by surgery or catheter, during the first 6 months after the procedure
  - Repaired congenital heart disease with residual defects at or adjacent to the site of a prosthetic patch or device
- Cardiac transplantation recipients who develop cardiac valvulopathy

From Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis: guidelines from the American Heart Association. *Circulation* 2007;**116**:1736–54.

TABLE 26-18 Endocarditis Prophylaxis Regimens for a Dental Procedure

		Regimen: Single Dose 30 to 60 Minutes Before Procedure	
Situation	**Agent**	ADULTS	CHILDREN
Oral	Amoxicillin	2 g	50 mg/kg
Unable to take oral	Ampicillin	2 g IM or IV	50 mg/kg IM or IV
	or		
	Cefazolin *or* Ceftriaxone	1 g IM or IV	50 mg/kg IM or IV
Allergic to penicillins oral	Cephalexin*	2 g	50 mg/kg
	or		
	Clindamycin	600 mg	20 mg/kg
	or		
	Azithromycin or clarithromycin	500 mg	15 mg/kg
Allergic to penicillins and unable to take oral	Cefazolin		
	or		
	Ceftriaxone*	1 g IM or IV	50 mg/kg IM or IV
	or		
	Clindamycin	600 mg IM or IV	20 mg/kg IM or IV

*Cephalosporins not to be used in an individual with a history of anaphylaxis, angioedema, or urticaria with penicillins.
IM, intramuscularly; *IV,* intravenously.
From Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis: guidelines from the American Heart Association. *Circulation* 2007;**116**:1736–54.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Alshammary A, Hervas-Malo M, Robinson JL. Pediatric infective endocarditis: has *Staphylococcus aureus* overtaken viridans group streptococci as the predominant etiologic agent? *Can J Infect Dis Med Microbiol* 2008;**19**:63–8.
26. Balmer R, Booras G, Parsons J. The oral health of children considered very high risk for infective endocarditis. *Int J Paediatr Dent* 2010;**20**:173–8.
29. Barker GM, O'Brien SM, Welke KF, et al. Major infection after pediatric cardiac surgery: a risk estimation model. *Ann Thorac Surg* 2010;**89**:843–50.
42. Bendig EA, Singh J, Butler TJ, et al. The impact of central venous catheter on the diagnosis of infectious endocarditis using Duke criteria in children with *Staphylococcus aureus* bacteremia. *Pediatr Infect Dis J* 2008;**27**:636–9.
63. Camarillo D, Banerjee R, Greenhow TL, et al. Group B streptococcal endocarditis after elective abortion in an adolescent. *Pediatr Infect Dis J* 2009;**28**:67–9.
106. Das BB, Wasser E, Bryant KA, et al. Culture negative endocarditis caused by *Bartonella henselae* in a child with congenital heart disease. *Pediatr Infect Dis J* 2009;**28**:922–5.
112. Day MD, Gauvreau K, Shulman S, et al. Characteristics of children hospitalized with infective endocarditis. *Circulation* 2009;**119**:865–70.
123. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
124. Dubnov-Raz G, Scheuerman O, Chodick G, et al. Invasive *Kingella kingae* infections in children: clinical and laboratory characteristics. *Pediatrics* 2008;**122**:1305–9.
140. Falcone M, Barzaghi N, Carosi G, et al. *Candida* infective endocarditis: report of 15 cases from a prospective multicenter study. *Medicine* 2009;**88**:160–8.
158. Fitzgerald K, Fleming P, Franklin O, et al. Dental health and management for children with congenital heart disease. *Prim Dent Care* 2010;**17**:21–5.
159. Fitzsimmons K, Bamber AI, Smalley HB. Infective endocarditits: changing aetiology of disease. *Br J Biomed Sci* 2010;**67**:35–41.
162. Fournier PE, Thuny F, Richet H, et al. Comprehensive diagnostic strategy for blood culture–negative endocarditis: a prospective study of 819 new cases. *Clin Infect Dis* 2010;**51**:131–40.
199. Haag F, Casonato S, Varela F, et al. Parents' knowledge of infective endocarditis in children with congenital heart disease. *Rev Bras Cir Cardiovasc* 2011;**26**:413–18.
214. Hickey EJ, Jung G, Manlhiot C, et al. Infective endocarditis in children: native valve preservation is frequently possible despite advanced clinical disease. *Eur J Cardiothorac Surg* 2009;**35**:130–5.
219. Holmes AA, Hung T, Human DG, et al. *Kingella kingae* endocarditis: a rare case of mitral valve perforation. *Ann Pediatr Cardiol* 2011;**4**:210–12.
224. Hosono M, Sasaki Y, Hirai H, et al. Considerations in timing of surgical intervention for infective endocarditis with cerebrovascular complications. *J Heart Valve Dis* 2010;**19**:321–5.
259. Karaci AR, Aydemir NA, Harmandar B, et al. Surgical treatment of infective valve endocarditis in children with congenital heart disease. *J Card Surg* 2012;**27**:93–8.
264. Kareem BA, Kamarulzaman H, Koh GT. Surgical management of patent ductus arteriosus with endocarditis. *Ann Thorac Surg* 2010;**90**:1703–5.
294. Le Guillou S, Casalta JP, Fraisse A, et al. Infective endocarditis in children without underlying heart disease: a retrospective study analyzing 11 cases. *Arch Pediatr* 2010;**17**:1047–55.
296. Lee JH, Burner KD, Fealey ME, et al. Prosthetic valve endocarditis: clinicopathological correlates in 122 surgical specimens from 116 patients (1985-2004). *Cardiovasc Pathol* 2011;**20**:26–35.
309. Lin YT, Hsieh KS, Chen YS, et al. Infective endocarditis in children without underlying heart disease. *J Microbiol Immunol Infect* 2012. [Epub ahead of print].
313. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:285–92.
324. Maekawa Y, Sakamoto T, Umezu K, et al. Infective endocarditis in a child caused by *Cardiobacterium hominis* after right ventricular outflow tract reconstruction using an expanded tetrafluoroethylene conduit. *Gen Thorac Cardiovasc Surg* 2011;**59**:429–32.
335. Marom D, Levy I, Gutwein O, et al. Healthcare-associated versus community-associated infective endocarditis in children. *Pediatr Infect Dis J* 2011;**30**:585–8.
343. McCormick J, Pollard J. *Aspergillus* endocarditis 2003-2009. *Med Mycol* 2011;**49**(Suppl 1):S30–4.
358. Miyamoto T, Tomoyasu T, Miyaji K. Successful treatment of pediatric endocarditis and pericarditis due to MRSA with linezolid. *Jpn J Antibiot* 2011;**64**:109–12.
359. Mogollón MV, Anguita MP, Aguado JM, et al. Q fever endocarditis in Spain: clinical characteristics and outcome. *Enferm Infecc Microbiol Clin* 2011;**29**:109–16.
377. Musci M, Hubler M, Amiri A, et al. Surgical treatment for active infective prosthetic valve endocarditis: 22-year single-centre experience. *Eur J Cardiothorac Surg* 2010;**38**:528–38.
378. Musci M, Weng Y, Hubler M, et al. Predictors of early mortality in patients with active infective native or prosthetic aortic root endocarditis undergoing homograft aortic root replacement. *Clin Res Cardiol* 2009;**98**:443–50.
386. Nath P, Kiran V, Maheshwari S. Awareness of infective endocarditis prophylaxis in parents of children with congenital heart disease: a prospective study. *Ann Pediatr Cardiol* 2008;**1**:54–5.
390. Nikolousis E, Velangi M. Two cases of *Aspergillus* endocarditis in non-neutropenic children on chemotherapy for acute lymphoblastic leukemia. *Hematol Rep* 2011;**3**:e7.
400. Pasquali SK, He X, Mohamad Z, et al. Trends in endocarditis hospitalizations at U.S. children's hospitals: impact of the 2007 American Heart Association Prophylaxis Guidelines. *Am Heart J* 2012;**163**:894–9.
411. Penk JS, Webb CL, Shulman ST, et al. Echocardiography in pediatric infective endocarditis. *Pediatr Infect Dis J* 2011;**30**:1109–11.
412. Pereira RM, Bucaretchi F, Tresoldi AT. Infective endocarditis due to *Haemophilus aphrophilus:* a case report. *J Pediatr* 2008;**84**:178–80.
426. Purcell LK, Finley JP, Chen R, et al. *Gemella* species endocarditis in a child. *Can J Infect Dis* 2001;**12**:317–20.
431. Rai K, Supriya S, Hegde AM. Oral health status of children with congenital heart disease and the awareness, attitude and knowledge of their parents. *J Clin Pediatr Dent* 2009;**33**:315–18.
442. Richet H, Casalta JP, Thuny F, et al. Development and assessment of a new early scoring system using non-specific clinical signs and biological results to identify children and adult patients with a high probability of infective endocarditis on admission. *J Antimicrob Chemother* 2008;**62**:1434–40.
452. Ross AC, Toltzis P, O'Riordan MA, et al. Frequency and risk factors for deep focus of infection in children with *Staphylococcus aureus* bacteremia. *Pediatr Infect Dis J* 2008;**27**:396–8.
453. Rossi M, Gallo A, De Silva RJ, et al. What is the optimal timing for surgery in infective endocarditis with cerebrovascular complications? *Interact Cardiovasc Thorac Surg* 2012;**14**:72–80.
472. Saxena A, Aggarwal N, Gupta P, et al. Predictors of embolic events in pediatric infective endocarditis. *Indian Heart J* 2011;**63**:237–40.
479. Schnell D, Beyler C, Lanternier F, et al. Nontoxigenic *Corynebacterium diphtheriae* as a rare cause of native endocarditis in childhood. *Pediatr Infect Dis J* 2010;**29**:886–8.
487. Shanson D. New British and American guidelines for the antibiotic prophylaxis of infective endocarditis: do the changes make sense? A critical review. *Curr Opin Infect Dis* 2008;**21**:191–9.
522. Talarmin JP, Boutoille D, Tattevin P, et al. *Candida* endocarditis: role of the new antifungal agents. *Mycoses* 2009;**52**:60–6.
525. Tasdemir K, Kaya MG, Mavili E, et al. Surgical approach to the management of *Brucella* endocarditis. *Eur J Cardiothorac Surg* 2010;**37**:1021–4.
555. Venkatesan C, Wainwright MS. Pediatric endocarditis and stroke: a single-center retrospective review of seven cases. *Pediatr Neurol* 2008;**38**:243–7.
564. Weber R, Berger C, Balmer C, et al. Interventions using foreign material to treat congenital heart disease in children increase the risk for infective endocarditis. *Pediatr Infect Dis J* 2008;**27**:544–50.
565. Wei HH, Wu KG, Sy LB, et al. Infectious endocarditis in pediatric patients: analysis of 19 cases presenting at a medical center. *J Microbiol Immunol Infect* 2010;**43**:430–7.
587. Yoshinaga M, Niwa K, Niwa A, et al. Risk factors for in-hospital mortality during infective endocarditis in patients with congenital heart disease. *Am J Cardiol* 2008;**101**:114–18.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agarwala BN. Group B streptococcal endocarditis in a neonate. *Pediatr Cardiol* 1988;**9**:51–3.
2. Akhondi H, Rahimi AR. *Haemophilus aphrophilus* endocarditis after tongue piercing. *Emerg Infect Dis* 2002;**8**:850–1.
3. Aksoy O, Sexton DJ, Wang A, et al. Early surgery in patients with infective endocarditis: a propensity score analysis. *Clin Infect Dis* 2007;**44**:364–72.
4. Al-Hajjar S, Qadri SMH, Al-Sabban E, Jager C. *Coxiella burnetii* endocarditis in a child. *Pediatr Infect Dis J* 1997;**16**:911–12.
5. Alpert JS, Krous HF, Dalen JE, et al. Pathogenesis of Osler's nodes. *Ann Intern Med* 1976;**85**:471–6.
6. Alshammary A, Hervas-Malo M, Robinson JL. Pediatric infective endocarditis: has *Staphylococcus aureus* overtaken viridans group streptococci as the predominant etiologic agent? *Can J Infect Dis Med Microbiol* 2008;**19**:63–8.
7. Alsip SG, Blackstone EH, Kirklin JW, et al. Indications for cardiac surgery in patients with infective endocarditis. *Am J Med* 1985;**78**:138–42.
8. Aly AM, Simpson PM, Humes RA. The role of transthoracic echocardiography in the diagnosis of infective endocarditis in children. *Arch Pediatr Adolesc Med* 1999;**153**:950–4.
9. Anderson AW, Cruickshank JG. Endocarditis due to viridans-type streptococci tolerant to beta-lactam antibiotics: therapeutic problems. *BMJ* 1982;**285**:85.
10. Andrews MM, von Reyn CF. Patient selection criteria and management guidelines for outpatient parenteral antibiotic therapy for native valve infective endocarditis. *Clin Infect Dis* 2001;**33**:203–9.
11. Ang JY, Lua JL, Turner DR, Asmar BI. Vancomycin-resistant *Enterococcus faecium* endocarditis in a premature infant successfully treated with linezolid. *Pediatr Infect Dis J* 2003;**22**:1101–3.
12. Anolik R, Berkowitz RJ, Campos JM, et al. *Actinobacillus* endocarditis associated with periodontal disease. *Clin Pediatr (Phila)* 1981;**20**:633–55.
13. Arber N, Militano A, Ben-Yehuda A, et al. Native valve *Staphylococcus epidermidis* endocarditis: report of seven cases and review of the literature. *Am J Med* 1991;**90**:758.
14. Arber N, Pras E, Copperman Y, et al. Pacemaker endocarditis: report of 44 cases and review of the literature. *Medicine (Baltimore)* 1994;**73**:299–305.
15. Ayres NA, Miller-Hance W, Fyfe DA, et al. Indications and guidelines for performance of transesophageal echocardiography in the patient with pediatric acquired or congenital heart disease. *J Am Soc Echocardiogr* 2005;**18**:91–8.
16. Babcock HM, Ritchie DJ, Christiansen E, et al. Successful treatment of vancomycin-resistant *Enterococcus* endocarditis with oral linezolid. *Clin Infect Dis* 2001;**32**:1373–5.
17. Backes RJ, Wilson WR, Geraci JE. Group B streptococcal infective endocarditis. *Arch Intern Med* 1985;**145**:693–6.
18. Baddour LM. Infective endocarditis caused by β-hemolytic streptococci: the Infectious Diseases Society of America's Emerging Infections Network. *Clin Infect Dis* 1998;**26**:66–71.
19. Baddour LM. Immunization for prevention of infective endocarditis. *Curr Infect Dis Rep* 1999;**1**:126–8.
20. Baddour LM, Bisno AL. Infective endocarditis complicating mitral valve prolapse: epidemiologic, clinical and microbiological aspects. *Rev Infect Dis* 1986;**8**:117–37.
21. Baddour LM, Christensen GD, Lowrance JH, et al. Pathogenesis of experimental endocarditis. *Rev Infect Dis* 1989;**11**:452–63.
22. Baddour LM, Lowrance C, Albus A, et al. *Staphylococcus aureus* microcapsule expression attenuates bacterial virulence in a rat model of experimental endocarditis. *J Infect Dis* 1992;**165**: 749–53.
23. Baddour LM, Meyer J, Henry B. Polymicrobial infective endocarditis in the 1980's. *Rev Infect Dis* 1991;**13**:963–70.
24. Baddour LM, Phillips TN, Bisno AL. Coagulase-negative staphylococcal endocarditis: occurrence in patients with mitral valve prolapse. *Arch Intern Med* 1986;**146**:119–21.
25. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications. *Circulation* 2005;**111**:e394–434.
26. Balmer R, Booras G, Parsons J. The oral health of children considered very high risk for infective endocarditis. *Int J Paediatr Dent* 2010;**20**:173–8.
27. Baltimore RS. Infective endocarditis in children. *Pediatr Infect Dis J* 1992;**11**:907–12.
28. Baorto E, Payne M, Slater L, et al. Culture-negative endocarditis caused by *Bartonella henselae*. *J Pediatr* 1998;**132**:1051–4.
29. Barker GM, O'Brien SM, Welke KF, et al. Major infection after pediatric cardiac surgery: a risk estimation model. *Ann Thorac Surg* 2010;**89**:843–50.
30. Barnes PD, Crook DWM. Culture negative endocarditis. *J Infect* 1997;**35**:209–13.
31. Barst RJ, Prince AS, Neu HC. *Aspergillus* endocarditis: case report and review of the literature. *Pediatrics* 1981;**68**:73–8.
32. Barst RJ, Prince AS, Neu HC. Echocardiography in *Aspergillus* endocarditis. *Pediatrics* 1982;**69**:252–3.
33. Bayer A, Bolger A, Taubert K, et al. Diagnosis and management of infective endocarditis and its complications. *Circulation* 1998; **98**:2936–48.
34. Bayer AS. Infective endocarditis. *Clin Infect Dis* 1993;**17**:313–32.
35. Bayer AS, Chow AW, Guze LB. *Listeria monocytogenes* endocarditis: report of a case and review of the literature. *Am J Med Sci* 1977;**273**:319–23.
36. Bayer AS, Nelson RJ, Slama TG. Current concepts in prevention of prosthetic valve endocarditis. *Chest* 1990;**97**:1203–7.
37. Bayer AS, Theofilopoulos AN. Immunopathogenetic aspects of infective endocarditis. *Chest* 1990;**97**:204–12.
38. Bayer AS, Theofilopoulos AN, Dixon FJ, et al. Circulating immune complexes in experimental streptococcal endocarditis: a monitor of therapeutic efficacy. *J Infect Dis* 1979;**139**:1–8.
39. Bayer AS, Theofilopoulos AN, Eisenberg R, et al. Circulating immune complexes in infective endocarditis. *N Engl J Med* 1976;**295**:1500–5.
40. Beeson PB, Brannon ES, Warren JV. Observations on the sites of removal of bacteria from the blood in patients with bacterial endocarditis. *J Exp Med* 1945;**81**:9–23.
41. Belmares J, Detterline S, Pak JB, Parada JP. *Corynebacterium* endocarditis species-specific risk factors and outcomes. *B C M Infect Dis* 2007;**7**:4.
42. Bendig EA, Singh J, Butler TJ, et al. The impact of central venous catheter on the diagnosis of infectious endocarditis using Duke criteria in children with *Staphylococcus aureus* bacteremia. *Pediatr Infect Dis J* 2008;**27**:636–9.
43. Berkowitz FE, Dansky R. Infective endocarditis in black South African children: report of 10 cases with some unusual features. *Pediatr Infect Dis J* 1989;**8**:787–91.
44. Bertorini TE, Laster RE, Thompson BF, et al. Magnetic resonance imaging of the brain in bacterial endocarditis. *Arch Intern Med* 1989;**149**:815–17.
45. Bieger RC, Brewer NS, Washington JA. *Haemophilus aphrophilus*: a microbiological and clinical review and report of 42 cases. *Medicine (Baltimore)* 1978;**57**:345–55.
46. Biller J, Challa VR, Toole JF, et al. Nonbacterial thrombotic endocarditis: a neurologic perspective of clinicopathologic correlations of 99 patients. *Arch Neurol* 1982;**39**:95–8.
47. Bishara J, Leibovici L, Gartman-Israel D, et al. Long-term outcome of infective endocarditis: the impact of early surgical intervention. *Clin Infect Dis* 2001;**33**:1636–43.
48. Bisno AL, Dismukes WE, Durack DT, et al. Antimicrobial treatment of infective endocarditis due to viridans streptococci, enterococci and staphylococci. *JAMA* 1989;**261**:1471–7.
49. Blair DC, Martin DB. Beta hemolytic streptococcal endocarditis: predominance of non–group A organisms. *Am J Med Sci* 1978; **276**:269–77.
50. Blieden LC, Morehead RR, Burke B, et al. Bacterial endocarditis in the neonate. *Am J Dis Child* 1972;**124**:747–9.
51. Blumberg EA, Karlis DA, Chandrasekaran K, et al. Endocarditis-associated paravalvular abscesses. *Chest* 1995;**107**:898–903.
52. Blumberg EA, Robbins N, Adimora A, et al. Persistent fever in association with infective endocarditis. *Clin Infect Dis* 1992;**15**: 983–90.
53. Blumenthal S, Griffiths SP, Morgan BC. Bacterial endocarditis in children with heart disease: a review based on the literature and experience with 58 cases. *Pediatrics* 1960;**26**:993–1017.
54. Bodey GP, Luna M. Skin lesions associated with disseminated candidiasis. *JAMA* 1974;**229**:1466–8.
55. Bosshard PP, Kronenberg A, Zbinden R, et al. Etiologic diagnosis of infective endocarditis by broad-range polymerase chain reaction: a 3-year experience. *Clin Infect Dis* 2003;**37**:167–72.

56. Bricker J, Latson L, Huhta J, et al. Echocardiographic evaluation of infective endocarditis in children. *Clin Pediatr (Phila)* 1985; **24**:312–9.
57. Brodie E, Adler JL, Daly AK. Bacterial endocarditis due to an unusual species of encapsulated *Neisseria*. *Am J Dis Child* 1972;**122**:433–7.
58. Brouqui P, Dumler JS, Raoult D. Immunohistologic demonstration of *Coxiella burnetii* in the valves of patients with Q fever endocarditis. *Am J Med* 1994;**97**:451–8.
59. Brouqui P, Raoult D. Endocarditis due to rare and fastidious bacteria. *Clin Microbiol Rev* 2001;**14**:177–207.
60. Bruyn GAW, Thompson J, Van Der Meer JWM. Pneumococcal endocarditis in adult patients: a report of five cases and review of the literature. *Q J Med* 1990;**74**:33–40.
61. Calderwood S, Swinski L, Waternaux C, et al. Risk factors for the development of prosthetic valve endocarditis. *Circulation* 1985;**72**:31–7.
62. Caldwell RL, Hurwitz RA, Girod DA. Subacute bacterial endocarditis in children. *Am J Dis Child* 1971;**122**:312–15.
63. Camarillo D, Banerjee R, Greenhow TL, et al. Group B streptococcal endocarditis after elective abortion in an adolescent. *Pediatr Infect Dis J* 2009;**28**:67–9.
64. Canter MC, Hart RG. Neurologic complications of infective endocarditis. *Neurology* 1991;**41**:1015.
65. Carey RB, Gross KC, Roberts RB. Vitamin B_6–dependent *Streptococcus mitior* (*mitis*) isolated from patients with systemic infections. *J Infect Dis* 1975;**131**:722–5.
66. Caron F, Carbon C, Gutmann L. Triple-combination penicillin-vancomycin-gentamicin for experimental endocarditis caused by a moderately penicillin- and highly glycopeptide-resistant isolate of *Enterococcus faecium*. *J Infect Dis* 1991;**164**:888–93.
67. Carpenter JL. Perivalvular extension of infection in patients with infectious endocarditis. *Rev Infect Dis* 1991;**13**:127–38.
68. Carr P, Wright M, Handler LC. Endocarditis-related cerebral aneurysms: radiologic changes with treatment. *AJNR Am J Neuroradiol* 1995;**16**:745.
69. Carrizosa J, Kaye D. Antibiotic concentrations in serum, serum bactericidal activity, and results of therapy of streptococcal endocarditis in rabbits. *Antimicrob Agents Chemother* 1977;**12**: 479–83.
70. Carruthers M. Endocarditis due to enteric bacilli other than salmonellae: case reports and literature review. *Am J Med Sci* 1977;**273**:203.
71. Carvajal A, Frederiksen W. Fatal endocarditis due to *Listeria monocytogenes*. *Rev Infect Dis* 1988;**10**:616–23.
72. Cassling RS, Rogler WC, McManus BM. Isolated pulmonic valve infective endocarditis: a diagnostically elusive entity. *Am Heart J* 1985;**109**:558–67.
73. Caviness AC, Cantor SB, Allen CH, Ward MA. A cost-effectiveness analysis of bacterial endocarditis prophylaxis for febrile children who have cardiac lesions and undergo urinary catheterization in the emergency department. *Pediatrics* 2004;**113**:1291–6.
74. Cetta F, Warnes CA. Adults with congenital heart disease: patient knowledge of endocarditis prophylaxis. *Mayo Clin Proc* 1995;**70**:50–4.
75. Chambers HF, Korzeniowski OM, Sande MA, et al. *Staphylococcus aureus* endocarditis: clinical manifestation in addicts and nonaddicts. *Medicine (Baltimore)* 1983;**62**:170–4.
76. Chambers HF, Miller RT, Newman MD. Right-sided *Staphylococcus aureus* endocarditis in intravenous drug abusers: two-week combination therapy. *Ann Intern Med* 1988;**109**:619–24.
77. Chang HH, Lu CY, Hsueh PR, et al. Endocarditis caused by *Abiotrophia defectiva* in children. *Pediatr Infect Dis J* 2002;**21**: 697–700.
78. Channer KS, Joffe HS, Jordan SC. Presentation of infective endocarditis in childhood and adolescence. *J R Coll Physicians Lond* 1989;**23**:152–5.
79. Charney R, Keltz TN, Attai L, et al. Acute valvular obstruction from streptococcal endocarditis. *Am Heart J* 1993;**125**:544.
80. Chatzis AC, Saroglou G, Giannopoulos NM, Sarris GE. Subtle infective endocarditis and congenital heart disease. *Cardiol Young* 2005;**15**:617–20.
81. Chen MR. Infective endocarditis in hypertrophic obstructive cardiomyopathy. *J Clin Ultrasound* 1992;**20**:612–14.
82. Chirouze C, Cabell CH, Fowler Jr VG, et al. Prognostic factors in 61 cases of *Staphylococcus aureus* prosthetic valve infective endocarditis from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* 2004;**38**:1323–7.
83. Choi M, Mailman TL. Pneumococcal endocarditis in infants and children. *Pediatr Infect Dis J* 2004;**23**:166–71.
84. Chu VH, Cabell CH, Abrutyn E, et al. Native valve endocarditis due to coagulase-negative staphylococci: report of 99 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* 2004;**39**:1527–30.
85. Chu VH, Woods CW, Miro JM, et al. Emergence of coagulase-negative staphylococci as a cause of native valve endocarditis. *Clin Infect Dis* 2008;**46**:232–42.
86. Chunn CJ, Jones SR, McCutchan JA, et al. *Haemophilus parainfluenzae* infective endocarditis. *Medicine (Baltimore)* 1977;**56**: 99–113.
87. Churchill MA, Geraci JE, Hunder GG. Musculoskeletal manifestations of bacterial endocarditis. *Ann Intern Med* 1977;**87**:754–9.
88. Citak M, Rees A, Mavroudis C. Surgical management of infective endocarditis in children. *Ann Thorac Surg* 1992;**54**:755–8.
89. Clawson CC, Rao Gunda HR, White JG. Platelet interaction with bacteria, part IV: stimulation of the release reaction. *Am J Pathol* 1975;**81**:411–17.
90. Clemens JO, Horwitz RI, Jaffee CC, et al. A controlled evaluation of the risk of bacterial endocarditis in persons with mitral-valve prolapse. *N Engl J Med* 1982;**307**:776–81.
91. Cofsky RD, Seligman SJ. *Peptococcus magnus* endocarditis. *South Med J* 1985;**78**:361–2.
92. Coleman DL, Horwitz RI, Andriole VT. Association between serum inhibitory and bactericidal concentrations and therapeutic outcome in bacterial endocarditis. *Am J Med* 1982;**73**:260–7.
93. Corrigan D, Bolen J, Hancock EW, et al. Mitral valve prolapse and endocarditis. *Am J Med* 1977;**63**:315–18.
94. Coward K, Tucker N, Darville T. Infective endocarditis in Arkansan children from 1990 through 2002. *Pediatr Infect Dis J* 2003;**22**:1048–52.
95. Cremieux AC, Carbon C. Pharmacokinetics and pharmacodynamic requirements for antibiotic therapy of experimental endocarditis. *Antimicrob Agents Chemother* 1992;**36**:2069–74.
96. Cutler JG, Ongley PA, Schwachman H, et al. Bacterial endocarditis in children with heart disease. *Pediatrics* 1958;**22**:706–14.
97. Czwerwiec FS, Bilsker MS, Kamerman ML, et al. Long-term survival after fluconazole therapy of candidal prosthetic valve endocarditis. *Am J Med* 1993;**94**:545–6.
98. Daher AH, Berkowitz FE. Infective endocarditis in neonates. *Clin Pediatr (Phila)* 1995;**20**:198–206.
99. Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis: recommendations by the American Heart Association. *Clin Infect Dis* 1997;**75**:1448–58.
100. Danchin N, Voiriot P, Briancon S, et al. Mitral valve prolapse as a risk factor for infective endocarditis. *Lancet* 1989;**1**:743–5.
101. Danford DA, Kugler JD, Cheatham JP, et al. *Haemophilus influenzae* endocarditis: successful treatment with ampicillin and early valve replacement. *Neb Med J* 1984;**38**:88–91.
102. Daniel WG, Erbel R, Kasper W, et al. Safety of transesophageal echocardiography: a multicenter survey of 10,419 examinations. *Circulation* 1991;**83**:817–21.
103. Daniel WG, Mugge A, Grote J, et al. Evaluation of endocarditis and its complications by biplane and multiplane transesophageal echocardiography. *Am J Card Imaging* 1995;**9**:100–5.
104. Daniel WG, Mugge A, Martin RP, et al. Improvement in the diagnosis of abscesses associated with endocarditis by transesophageal echocardiography. *N Engl J Med* 1991;**324**:795–800.
105. Dankert J, Krijgsveld J, van der Werff J, et al. Platelet microbicidal activity is an important defense factor against viridans streptococcal endocarditis. *J Infect Dis* 2001;**184**:597–605.
106. Das BB, Wasser E, Bryant KA, et al. Culture negative endocarditis caused by *Bartonella henselae* in a child with congenital heart disease. *Pediatr Infect Dis J* 2009;**28**:922–5.
107. Das I, DeGiovanni JV, Gray J. Endocarditis caused by *Haemophilus parainfluenzae* identified by 16 S ribosomal RNA sequencing. *J Clin Pathol* 1997;**50**:72–4.
108. Das M, Badley AD, Cockerill FR, et al. Infective endocarditis caused by HACEK microorganisms. *Annu Rev Med* 1997;**48**: 25–33.
109. da Silva DB, Sovza IP, Cunha MC. Knowledge, attitudes and status of oral health in children at risk for infective endocarditis. *Int J Pediatr Dent* 2002;**12**:124–31.

110. David TE. The surgical treatment of patients with prosthetic valve endocarditis. *Semin Thorac Cardiovasc Surg* 1995;**7**:47–53.
111. Davidson S, Rotem Y, Bogkowski B, et al. *Corynebacterium diphtheriae* endocarditis. *Am J Med Sci* 1976;**271**:351–3.
112. Day MD, Gauvreau K, Shulman S, et al. Characteristics of children hospitalized with infective endocarditis. *Circulation* 2009;**119**:865–70.
113. Delmo Walter EM, Musci M, Nagdyman N, et al. Mitral valve repair for infective endocarditis in children. *Ann Thorac Surg* 2007;**84**:2059–65.
114. Del Pont JM, DeCicco LT, Vartalitis C, et al. Infective endocarditis in children: clinical analyses and evaluation of two diagnostic criteria. *Pediatr Infect Dis J* 1995;**14**:1079–86.
115. Delvecchio G, Fracasetti O, Lorenzi N. *Brucella* endocarditis. *Int J Cardiol* 1991;**33**:328–9.
116. Dillon T, Meyer RA, Korfhagen JC, et al. Management of infective endocarditis using echocardiography. *J Pediatr* 1980; **96**:552–8.
117. DiNubile MJ. Treatment of endocarditis caused by relatively resistant nonenterococcal streptococci: is penicillin enough? *Rev Infect Dis* 1990;**12**:112–17.
118. DiNubile MJ, Calderwood SB, Steinhaus DM, et al. Cardiac conduction abnormalities complicating native valve active infective endocarditis. *Am J Cardiol* 1986;**58**:1213–17.
119. Dominguez SR, Littlehorn C, Nyquist AC. *Mycoplasma hominis* endocarditis in a child with complex congenital heart disease. *Pediatr Infect Dis J* 2006;**25**:851–2.
120. Douglas A, Moore-Gillon J, Eykyn S. Fever during treatment of infective endocarditis. *Lancet* 1986;**1**:1341–3.
121. Downing GJ, Spirazza C. Group C beta-hemolytic streptococcal endocarditis. *Pediatr Infect Dis J* 1986;**5**:703–4.
122. Drancourt M, Mainardi JL, Brouqui P, et al. *Bartonella (Rochalimaea) quintana* endocarditis in three homeless men. *N Engl J Med* 1995;**332**:419–23.
123. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
124. Dubnov-Raz G, Scheuerman O, Chodick G, et al. Invasive *Kingella kingae* infections in children: clinical and laboratory characteristics. *Pediatrics* 2008;**122**:1305–9.
125. Durack DT. Prevention of infective endocarditis. *N Engl J Med* 1995;**332**:38–44.
126. Durack DT, Beeson PB. Protective role of complement in experimental *Escherichia coli* endocarditis. *Infect Immun* 1977;**16**:213–14.
127. Durack DT, Beeson PB, Petersdorf RG. Experimental bacterial endocarditis, part III: production and progress of the disease in rabbits. *Br J Exp Pathol* 1973;**54**:142–51.
128. Durack DT, Kaplan EL, Bisno AL. Apparent failure of endocarditis prophylaxis: analysis of 52 cases submitted to a national registry. *JAMA* 1983;**250**:2318–22.
129. Durack DT, Lukes AS, Bright DK, et al. New criteria for diagnosis of infective endocarditis: utilization of specific echocardiographic findings. *Am J Med* 1994;**96**:200–9.
130. Durack DT, Pelletier LL, Petersdorf RG. Chemotherapy of experimental streptococcal endocarditis, part II: synergism between penicillin and streptomycin against penicillin-sensitive streptococci. *J Clin Invest* 1974;**53**:829–36.
131. Durack DT, Petersdorf RG. Changes in the epidemiology of endocarditis. In: Kaplan EL, Taranta AV, editors. *Infective endocarditis: an American Heart Association symposium*. Dallas: American Heart Association; 1977. p. 3.
132. Edwards K, Hruby N, Christy C. Pneumococcal endocarditis in infants and children: report of a case and review of the literature. *Pediatr Infect Dis J* 1990;**9**:652–7.
133. Eliopoulos GM, Thauvin-Eliopoulos C, Moellering Jr RC. Contribution of animal models in the search for effective therapy for endocarditis due to enterococci with high-level resistance to gentamicin. *Clin Infect Dis* 1992;**15**:58–62.
134. Ellis ME, Al-Abdely H, Sandridge A, et al. Fungal endocarditis: evidence in the world literature, 1965-1995. *Clin Infect Dis* 2001;**32**:50–62.
135. Elward K, Hruby N, Christy C. Pneumococcal endocarditis in infants and children: report of a case and review of the literature. *Pediatr Infect Dis J* 1990;**9**:652–7.
136. Enzler MJ, Rouse MS, Henry NK, et al. In vitro and in vivo studies of streptomycin-resistant, penicillin-susceptible streptococci from patients with infective endocarditis. *J Infect Dis* 1987;**155**:954–8.
137. Ergin MA. Surgical techniques in prosthetic valve endocarditis. *Semin Thorac Cardiovasc Surg* 1995;**7**:54–6.
138. Esperson F, Frimodt-Moller N. *Staphylococcus aureus* endocarditis: a review of 119 cases. *Arch Intern Med* 1986;**146**:1118–21.
139. Everett ED, Hirschmann JU. Transient bacteremia and endocarditis prophylaxis: a review. *Medicine (Baltimore)* 1977;**56**: 61–77.
140. Falcone M, Barzaghi N, Carosi G, et al. *Candida* infective endocarditis: report of 15 cases from a prospective multicenter study. *Medicine* 2009;**88**:160–8.
141. Fang G, Keys TF, Gentry LO, et al. Prosthetic valve endocarditis resulting from nosocomial bacteremia. *Ann Intern Med* 1993; **119**:560.
142. Farmaki E, Roilides E, Darilis E, et al. *Gemella morbillorum* endocarditis in a child. *Pediatr Infect Dis J* 2000;**19**:751–3.
143. Farrior JB, Silverman ME. A consideration of the differences between a Janeway lesion and an Osler's node in infectious endocarditis. *Chest* 1976;**70**:239–43.
144. Faville RJ, Zaska DE, Kaplan EL, et al. *Staphylococcus aureus* endocarditis: combined therapy with vancomycin and rifampin. *JAMA* 1978;**240**:1963–5.
145. Feder Jr HM, Chameides L, Diana DJ. Bacterial endocarditis complicated by myocardial infarction in a pediatric patient. *JAMA* 1982;**247**:1315–6.
146. Feder Jr HM, Olsen N, McLaughlin JC, et al. Bacterial endocarditis caused by vitamin B_6–dependent viridans group *Streptococcus*. *Pediatrics* 1980;**66**:309–12.
147. Feder Jr HM, Roberts JC, Salazar JC, et al. HACEK endocarditis in infants and children: two cases and a literature review. *Pediatr Infect Dis J* 2003;**22**:557–62.
148. Felius A, Fleer A, Mouloert A. *Actinobacillus actinomycetemcomitans* endocarditis in a child with a prosthetic heart valve. *Infection* 1984;**12**:260–1.
149. Felmer JM, Dowell VR. Anaerobic bacterial endocarditis. *N Engl J Med* 1970;**283**:1188–92.
150. Fenollar F, Lepidi H, Raoult D. Whipple's endocarditis: review of the literature and comparisons with Q fever, *Bartonella* infection and blood culture-positive endocarditis. *Clin Infect Dis* 2001;**33**: 1309–16.
151. Fernandez GC, Chapman AJ, Bolli R, et al. Gonococcal endocarditis: a case series demonstrating modern presentation of an old disease. *Am Heart J* 1984;**108**:1326–34.
152. Fernandez-Guerrero ML, Torres-Perea R, Gomez-Rodrigo J, et al. Infectious endocarditis due to non–*typhi Salmonella* in patients infected with human immunodeficiency virus: report of two cases and review. *Clin Infect Dis* 1996;**22**:853–5.
153. Fernandez-Guerrero ML, Verdejo C, Azofra J, et al. Hospital-acquired infectious endocarditis not associated with cardiac surgery: an emerging problem. *Clin Infect Dis* 1995;**20**:16–23.
154. Fernicola DJ, Roberts WC. Clinicopathologic features of active infective endocarditis isolated to the native mitral valve. *Am J Cardiol* 1993;**71**:1186–97.
155. Ferrieri P, Gewitz MH, Gerber MA, et al. Unique features of infective endocarditis in children. *Pediatrics* 2002;**109**:931–43.
156. Fichtenbaum CH, Smith MJ. Treatment of endocarditis due to *Pseudomonas aeruginosa* with imipenem. *Clin Infect Dis* 1992;**14**: 353–4.
157. Fisher RG, Moodie DS, Rice R. Pediatric bacterial endocarditis: long-term follow-up. *Cleve Clin Q* 1985;**52**:41–5.
158. Fitzgerald K, Fleming P, Franklin O, et al. Dental health and management for children with congenital heart disease. *Prim Dent Care* 2010;**17**:21–5.
159. Fitzsimmons K, Bamber AI, Smalley HB. Infective endocarditits: changing aetiology of disease. *Br J Biomed Sci* 2010;**67**: 35–41.
160. Fournier PE, Lelievre H, Eykyn SJ, et al. Epidemiologic and clinical characteristics of *Bartonella henselae* endocarditis: a study of 48 patients. *Medicine (Baltimore)* 2001;**80**:245–51.
161. Fournier PE, Raoult D. Non-culture laboratory methods for the diagnosis of infectious endocarditis. *Curr Infect Dis Rep* 1999;**1**:136–41.
162. Fournier PE, Thuny F, Richet H, et al. Comprehensive diagnostic strategy for blood culture-negative endocarditis: a prospective study of 819 new cases. *Clin Infect Dis* 2010;**51**:131–40.

163. Fowler Jr VG, Boucher HW, Corey GR, et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *N Engl J Med* 2006;**355**:653–65.
164. Fowler Jr VG, Miro JM, Hoen B, et al. *Staphylococcus aureus* endocarditis: a consequence of medical progress. *JAMA* 2005; **293**:3012–21.
165. Fowler Jr VG, Sanders LC, Kong LK, et al. Infective endocarditis due to *Staphylococcus aureus*: 59 prospectively identified cases with follow-up. *Clin Infect Dis* 1999;**28**:106–14.
166. Francioli P, Etienne J, Hoigue R, et al. Treatment of streptococcal endocarditis with a single daily dose of ceftriaxone sodium for 4 weeks: efficacy and outpatient treatment feasibility. *JAMA* 1992;**267**:264–7.
167. Francioli P, Ruch W, Stamboulian D, et al. Treatment of streptococcal endocarditis with a single daily dose of ceftriaxone and netilmicin for 14 days: a prospective multicenter study. *Clin Infect Dis* 1995;**21**:1406–10.
168. Frontera JA, Graclon JD. Right-side endocarditis in injection drug users: review of proposed mechanisms of pathogenesis. *Clin Infect Dis* 2000;**30**:374–9.
169. Fukushige J, Igarashi H, Veda K. Spectrum of infective endocarditis during infancy and childhood: 20-year review. *Pediatr Cardiol* 1994;**15**:127–31.
170. Galil K, Thurer R, Glatter K, et al. Disseminated *Mycobacterium chelonae* infection resulting in endocarditis. *Clin Infect Dis* 1996;**23**:1322–3.
171. Gallagher PG, Watanakunakorn C. Group B streptococcal endocarditis: report of seven cases and review of the literature. *Rev Infect Dis* 1986;**8**:175–88.
172. Garcia-Teresa MA, Casado-Flores J, Dominguez MAD, et al. Infectious complications of percutaneous central venous catheterization in pediatric patients. *Intensive Care Med* 2007; **33**:466–76.
173. Garty B, Berant M, Weinhouse E, et al. False aneurysm of the right ventricle due to endocarditis in a child. *Pediatr Cardiol* 1987;**8**:275–7.
174. Gauduchon V, Benito Y, Celard M, et al. Molecular diagnosis of recurrent *Streptococcus mutans* endocarditis by PCR amplification and sequencing. *Clin Microbiol Infect* 2001;**7**:36–7.
175. Gelfand MS, Threlkeld MG. Subacute bacterial endocarditis secondary to *Streptococcus pneumoniae*. *Am J Med* 1992;**93**:91.
176. Gelfman R, Levine SA. The incidence of acute and subacute bacterial endocarditis in congenital heart disease. *Am J Med Sci* 1942;**204**:324–33.
177. Geraci JE, Wilson WR. Endocarditis due to gram-negative bacteria. *Mayo Clin Proc* 1982;**57**:145–8.
178. Gersony WM, Hayes CJ. Bacterial endocarditis in patients with pulmonary stenosis, aortic stenosis or ventricular septal defect. *Circulation* 1977;**56**:84–9.
179. Gersony WM, Hayes CJ, Driscoll DJ, et al. Bacterial endocarditis in patients with aortic stenosis, pulmonary stenosis, or ventricular septal defect. *Circulation* 1993;**87**(Suppl 1):121–6.
180. Geva T, Frand M. Infective endocarditis in children with congenital heart disease: the changing spectrum, 1965-85. *Eur Heart J* 1988;**9**:1244–9.
181. Ghann JW, Dismukes WE. Prosthetic valve endocarditis: an overview. *Kardiovaskulare Erkrankungan* 1983;**8**:320–31.
182. Gilbert HM, Peters ED, Lang SJ, et al. Successful treatment of fungal prosthetic valve endocarditis: case report and review. *Clin Infect Dis* 1996;**22**:348–54.
183. Gil-Grande R, Aguado JM, Pastor C, et al. Conventional viral cultures and shell vial assay for diagnosis of apparently culture-negative *Coxiella burnetii* endocarditis. *Eur J Clin Microbiol Infect Dis* 1995;**14**:64–7.
184. Givner LB, Mason Jr EO, Tan TQ, et al. Pneumococcal endocarditis in children. *Clin Infect Dis* 2004;**38**:1273–8.
185. Glauser MP, Bernard JP, Moreillon P, et al. Successful single-dose amoxicillin prophylaxis against experimental streptococcal endocarditis: evidence for two mechanisms of protection. *J Infect Dis* 1983;**147**:568–75.
186. Goessler MC, Riggs TW, DeLeon S, Paul MH. Echocardiographic diagnosis of tricuspid valve endocarditis in a child with a normal heart. *Pediatr Cardiol* 1982;**2**:141–3.
187. Goldberg P, Shulman ST, Yogev R. Group C streptococcal endocarditis. *Pediatrics* 1985;**75**:114–16.
188. Goldenberg D, Kunzli A, Vogt P, et al. Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. *J Clin Microbiol* 1997;**35**:2733–9.
189. Gorby GL, Peacock JE. *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic and clinical features of an occupational disease. *Rev Infect Dis* 1988;**10**:317–25.
190. Gotsman I, Meirovitz A, Meizlish N, et al. Clinical and echocardiographic predictors of morbidity and mortality in infective endocarditis: the significance of vegetation size. *Isr Med Assoc J* 2007;**9**:365–9.
191. Gould K, Ramirez-Ronda CH, Holmes RK, et al. Adherence of bacteria to heart valves in vitro. *J Clin Invest* 1975;**56**:1364–70.
192. Gradon JD, Chapnick EK, Lutwick LI. Infective endocarditis of a native valve due to *Acinetobacter*: case report and review. *Clin Infect Dis* 1992;**14**:1145–8.
193. Gransden WR, Eykyn SJ, Leach RM. Neurologic presentations of native valve endocarditis. *Q J Med* 1989;**73**:1135–42.
194. Griffiths JK, Daly JS, Dodge RA. Two cases of endocarditis due to *Lactobacillus* species: antimicrobial susceptibility, review and discussion of therapy. *Clin Infect Dis* 1992;**15**:250–5.
195. Guard RW. Non-toxigenic *Corynebacterium diphtheriae* causing subacute bacterial endocarditis: case report. *Pathology* 1979;**11**: 533–5.
196. Gunes AM, Bostan OM, Bayton B, Semizel E. Treatment of infective endocarditis with recombinant tissue plasminogen activator. *Pediatr Blood Cancer* 2008;**50**:132–4.
197. Gutman RA, Striker GE, Gilliland BC, et al. The immune complex glomerulonephritis of bacterial endocarditis. *Medicine (Baltimore)* 1972;**51**:1–5.
198. Guzman-Cottrill JA, Zheng X, Chadwick EG. *Fuserium solani* endocarditis successfully treated with liposomal amphotericin B and voriconazole. *Pediatr Infect Dis J* 2004;**23**:1059–61.
199. Haag F, Casonato S, Varela F, et al. Parents' knowledge of infective endocarditis in children with congenital heart disease. *Rev Bras Cir Cardiovasc* 2011;**26**:413–18.
200. Hall B, Dowling HF. Negative blood cultures in bacterial endocarditis: a decade's experience. *Med Clin North Am* 1966;**50**: 159–70.
201. Hall G, Heimdahl A, Nord CE. Bacteremia after oral surgery and antibiotic prophylaxis for endocarditis. *Clin Infect Dis* 1999; **29**:1–10.
202. Hall LH, Herndon BL. Association of cell adherent glycocalyx and endocarditis production by viridans group streptococci. *J Clin Microbiol* 1990;**28**:1698–700.
203. Hamoudi AC, Hriban MM, Marcon MJ, et al. Clinical relevance of viridans and nonhemolytic streptococci isolated from blood and cerebrospinal fluid in a pediatric population. *Am J Clin Pathol* 1990;**93**:270.
204. Hampton JR, Harrison MJ. Sterile blood cultures in bacterial endocarditis. *Q J Med* 1967;**36**:167–74.
205. Hart RG, Foster JW, Luther MF, et al. Stroke in infective endocarditis. *Stroke* 1990;**21**:695–700.
206. Hayek LJ. *Erysipelothrix* endocarditis affecting a porcine xenograft heart valve. *J Infect* 1993;**27**:203.
207. Hayes PA, Fasules J. Dental screening of pediatric cardiac surgical patients. *ASDC J Dent Child* 2001;**68**:255–8.
208. Heiddal S, Sverrisson JT, Ynguason EE, et al. Native-valve endocarditis due to *Neisseria sicca*: case report and review. *Clin Infect Dis* 1993;**16**:667–70.
209. Hellinger WC, Rouse MS, Robadan PM, et al. Continuous intravenous versus intermittent ampicillin therapy of experimental endocarditis caused by aminoglycoside-resistant enterococci. *Antimicrob Agents Chemother* 1992;**36**:1272–5.
210. Herbert MA, Milford DV, Silove ED, et al. Secondary amyloidosis from long-standing bacterial endocarditis. *Pediatr Nephrol* 1995; **9**:33–5.
211. Herzberg MC, Brintzenhote KC, Clawson CC. Aggregation of human platelets and adhesion of *Streptococcus sanguis*. *Infect Immun* 1983;**39**:1457–69.
212. Herzberg MC, MacFarlane GD, Gong K, et al. The platelet interactivity phenotype of *Streptococcus sanguis* influences the course of experimental endocarditis. *Infect Immun* 1992;**60**:4809–18.
213. Hickey AJ, MacMahon SW, Wilcken DEL. Mitral valve prolapse and bacterial endocarditis: when is antibiotic prophylaxis necessary? *Am Heart J* 1985;**109**:431–5.

214. Hickey EJ, Jung G, Manlhiot C, et al. Infective endocarditis in children: native valve preservation is frequently possible despite advanced clinical disease. *Eur J Cardiothorac Surg* 2009;**35**:130–5.
215. Hoen B, Selton-Suty C, Danchin N, et al. Evaluation of the Duke criteria versus the Beth Israel criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 1995;**21**:905.
216. Hoen B, Selton-Suty C, Lacassin F, et al. Infective endocarditis in patients with negative blood cultures: analysis of 88 cases from a one-year nationwide survey in France. *Clin Infect Dis* 1995;**20**:501–6.
217. Hoffman RM, AboulHosn J, Child JS, et al. *Bartonella* endocarditis in complex congenital heart disease. *Congenit Heart Dis* 2007;**2**:79–84.
218. Holland F II, Fernandez L, Jacobs J, Bolooki H. Clostridial endocarditis following penetrating cardiac trauma. *Clin Infect Dis* 1997;**24**:87–8.
219. Holmes AA, Hung T, Human DG, et al. *Kingella kingae* endocarditis: a rare case of mitral valve perforation. *Ann Pediatr Cardiol* 2011;**4**:210–12.
220. Horenstein MS, Humes R, Epstein ML, Draper D. Loeffler's endocarditis presenting in 2 children as fever with eosinophilia. *Pediatrics* 2002;**110**:1014–18.
221. Horwitz D, Quismorio FP, Friou GJ. Cryoglobulinemia in patients with infective endocarditis. *Clin Exp Immunol* 1975;**19**: 131–7.
222. Hosea SW. Virulent *Streptococcus viridans* bacterial endocarditis. *Am Heart J* 1981;**101**:174–6.
223. Hoshimo T, Ohkusu K, Sudo F, et al. *Neisseria elongata* subsp. *nitroreducens* endocarditis in a seven year-old boy. *Pediatr Infect Dis J* 2005;**24**:391–2.
224. Hosono M, Sasaki Y, Hirai H, et al. Considerations in timing of surgical intervention for infective endocarditis with cerebrovascular complications. *J Heart Valve Dis* 2010;**19**:321–5.
225. Houpikian P, Raoult D. Diagnostic methods: current best practices and guidelines for identification of difficult-to-culture pathogens in infective endocarditis. *Infect Dis Clin North Am* 2002;**16**:377–92.
226. Hsu CM, Lee PI, Chen JM, et al. Fatal *Fusarium* endocarditis complicated by hemolytic anemia and thrombocytopenia in an infant. *Pediatr Infect Dis J* 1994;**13**:1146–8.
227. Hsu RB, Lin FY. Effect of penicillin resistance on presentation and outcome of nonenterococcal streptococcal infective endocarditis. *Cardiology* 2006;**105**:234–9.
228. Hsu RB, Lin FY. Methicillin resistance and risk factors for embolism in *Staphylococcus aureus* infective endocarditis. *Infect Control Hosp Epidemiol* 2007;**28**:860–6.
229. Huang ST, Lee HC, Lee NY, et al. Clinical characteristics of invasive *Haemophilus aphrophilus* infections. *J Microbiol Immunol Infect* 2005;**38**:271–6.
230. Humpl T, McCrindle BW, Smallhorn JF. The relative roles of transthoracic compared with transesophageal endocardiography in children with suspected infective endocarditis. *J Am Coll Cardiol* 2003;**41**:2068–71.
231. Husni RN, Gordon SM, Washington JA, Longworth DL. *Lactobacillus* bacteremia and endocarditis: review of 45 cases. *Clin Infect Dis* 1997;**25**:1048–55.
232. Hyams KC, Mader JT, Pollard RB, et al. *Serratia* endocarditis in a pediatric burn patient. *JAMA* 1981;**246**:983–4.
233. Ingram RJH, Cornere B, Ellis-Pegler RB. Endocarditis due to *Neisseria mucosa*: two case reports and review. *Clin Infect Dis* 1992;**15**:321–4.
234. Inman RD, Redecha PB, Knechtle SJ, et al. Identification of bacterial antigens in circulating immune complexes of infective endocarditis. *J Clin Invest* 1982;**70**:271–80.
235. Ishiwada N, Niwa K, Tateno S, et al. Causative organism influences clinical profile and outcome of infective endocarditis in pediatric patients and adults with congenital heart disease. *Circ J* 2005;**69**:1266–70.
236. Ishiwada N, Niwa K, Tateno S, et al. Pneumococcal endocarditis in children: a nationwide survey in Japan. *Int J Cardiol* 2008; **123**:298–301.
237. Ivert TS, Dismukes WE, Cobbs CG, et al. Prosthetic valve endocarditis. *Circulation* 1984;**69**:223–32.
238. Jackman Jr JD, Glamann DB. Gonococcal endocarditis: twenty-five year experience. *Am J Med Sci* 1991;**301**:221.
239. Jackson MA, Hicks RA. Vancomycin failure in staphylococcal endocarditis. *Pediatr Infect Dis J* 1987;**6**:750–2.
240. Jackson MJ, Rutledge J. Pneumococcal endocarditis in children. *Pediatr Infect Dis J* 1982;**1**:120–2.
241. Jaffe WM, Morgan DE, Pearlman AS, et al. Infective endocarditis, 1983-1988: echocardiographic findings and factors influencing morbidity and mortality. *J Am Coll Cardiol* 1990;**15**:1227–33.
242. Jalava J, Kotilainen P, Nikkari S, et al. Use of the polymerase chain reaction and DNA sequencing for detection of *Bartonella quintana* in the aortic valve of a patient with culture-negative infective endocarditis. *Clin Infect Dis* 1995;**21**:891–6.
243. Jemsek JG, Greenberg SB, Gentry LO, et al. *Haemophilus parainfluenzae* endocarditis: two cases and review of the literature in the past decade. *Am J Med* 1979;**66**:51–7.
244. Job FP, Franke S, Lethen H, et al. Incremental valve of biplane and multiplane transesophageal echocardiography for the assessment of active infective endocarditis. *Am J Cardiol* 1995;**75**: 1033–7.
245. John MD, Hibberd PL, Karchmer AW, et al. *Staphylococcus aureus* prosthetic valve endocarditis: optimal management and risk factors for death. *Clin Infect Dis* 1998;**26**:1307–9.
246. Johnson CM. Adherence events in the pathogenesis of infective endocarditis. *Infect Dis Clin North Am* 1993;**7**:21–36.
247. Johnson CM, Rhodes KH. Pediatric endocarditis. *Mayo Clin Proc* 1982;**57**:86–94.
248. Johnson DH, Rosenthal A, Nadas A. A forty-year review of bacterial endocarditis in infancy and childhood. *Circulation* 1975;**51**:581–8.
249. Johnson DH, Rosenthal A, Nadas A. Bacterial endocarditis in children under 2 years of age. *Am J Dis Child* 1975;**129**:183–6.
250. Johnson JD, Raff MJ, Barnwell PA, et al. Splenic abscess complicating infectious endocarditis. *Arch Intern Med* 1983;**143**: 906–12.
251. Johnson PG, Lee J, Domanski M, et al. Late recurrent *Candida* endocarditis. *Chest* 1991;**99**:1531–3.
252. Jones HK, Siekert RG. Neurologic manifestations of infective endocarditis. *Brain* 1989;**112**:1295–315.
253. Jones RB, Priest JB, Kuo C. Subacute chlamydial endocarditis. *JAMA* 1982;**247**:655–8.
254. Julander I. Unfavourable prognostic factors in *Staphylococcus aureus* septicemia and endocarditis. *Scand J Infect Dis* 1985;**17**:179–87.
255. Kaell AT, Volkman DJ, Gorevic PD, et al. Positive Lyme serology in subacute bacterial endocarditis: a study of four patients. *JAMA* 1990;**264**:2916–18.
256. Kaplan EL. Infective endocarditis in the pediatric age group: an overview. In: Kaplan EL, Taranta AV, editors. *Infective endocarditis: an American Heart Association symposium*. Dallas: American Heart Association; 1977. p. 51–4.
257. Kaplan EL, Rich H, Gersony W, et al. A collaborative study of infective endocarditis in the 1970's: emphasis on infections in patients who have undergone cardiovascular surgery. *Circulation* 1979;**59**:327–35.
258. Kaplan S, Helmworth JA, Ahern EN, et al. Results of palliative procedures for tetralogy of Fallot in infants and young children. *Ann Thorac Surg* 1968;**5**:489–95.
259. Karaci AR, Aydemir NA, Harmandar B, et al. Surgical treatment of infective valve endocarditis in children with congenital heart disease. *J Card Surg* 2012;**27**:93–8.
260. Karalis DG, Blumberg AE, Vilaro JF, et al. Prognostic significance of valvular regurgitation in patients with infective endocarditis. *Am J Med* 1991;**90**:193–7.
261. Karchmer AW, Archer GL, Dismukes WE. *Staphylococcus epidermidis* causing prosthetic valve endocarditis: microbiologic and clinical observations as guides to therapy. *Ann Intern Med* 1983;**98**:447–55.
262. Karchmer AW, Dismukes WE, Buckley MJ, et al. Late prosthetic valve endocarditis: clinical features influencing therapy. *Am J Med* 1978;**64**:199–206.
263. Karchmer AW, Moellering RC, Maki DG, et al. Single-antibiotic therapy for streptococcal endocarditis. *JAMA* 1979;**241**:1801–6.
264. Kareem BA, Kamarulzaman H, Koh GT. Surgical management of patent ductus arteriosus with endocarditis. *Ann Thorac Surg* 2010;**90**:1703–5.
265. Karl T, Wensley D, Starke J, et al. Infective endocarditis in children with congenital heart disease: comparison of selected features

in patients with surgical correction or palliation and those without. *Br Heart J* 1987;**58**:57–65.
266. Kauffman RH, Thompson J, Valentijn RM, et al. The clinical implications and the pathogenetic significance of circulating immune complexes in infective endocarditis. *Am J Med* 1981; **71**:17–25.
267. Kavey RW, Frank DM, Byrum CJ, et al. Two-dimensional echocardiographic assessment of infective endocarditis in children. *Am J Dis Child* 1983;**137**:851–6.
268. Kaye D. Prevention of bacterial endocarditis: 1991. *Ann Intern Med* 1991;**114**:803–4.
269. Keay S, Denning DW, Stevens DA. Endocarditis due to *Trichosporon beigelii*: in vitro susceptibility of isolates and review. *Rev Infect Dis* 1991;**13**:383–6.
270. Kennedy MJ, Jackson MA, Kearns GL. Delayed diagnosis of penicillin-resistant *Streptococcus mitis* endocarditis following single-dose amoxicillin prophylaxis in a child. *Clin Pediatr* 2004;**43**:773–6.
271. Keys TF, Hewitt WL. Endocarditis due to micrococci and *Staphylococcus epidermidis*. *Arch Intern Med* 1973;**132**:216–20.
272. Keyser DL, Biller J, Coffman TT, et al. Neurologic complications of late prosthetic valve endocarditis. *Stroke* 1990;**21**:472.
273. Khandheria BK. Transesophageal echocardiography in the evaluation of prosthetic valves. *Am J Card Imaging* 1995;**9**: 106–14.
274. Kielhofner MA, Hamill RJ. Role of adherence in infective endocarditis. *Tex Heart J* 1989;**16**:239–49.
275. Kihuchi K, Enari T, Minami S, et al. Postantibiotic effects and postantibiotic sub-MIC effects of benzyl penicillin on viridans streptococci isolated from patients with infective endocarditis. *J Antimicrob Chemother* 1994;**34**:687–96.
276. Kilpatrick ZM, Greenberg PA, Sanford JP. Splinter hemorrhages: their clinical significance. *Arch Intern Med* 1965;**115**:730–5.
277. Kimbrough RC, Ormsbee RA, Peacock M, et al. Q fever endocarditis in the United States. *Ann Intern Med* 1979;**91**:400–2.
278. King ME, Weyman AE. Echocardiographic findings in infective endocarditis. *Cardiovasc Clin* 1983;**13**:147–65.
279. Kissane JM. *Pathology of infancy and childhood*. 2nd ed. St. Louis: C. V. Mosby; 1975. p. 417–18.
280. Knirsch W, Haas NA, Uhlemann F, et al. Clinical course and complications of infective endocarditis in patients growing up with congenital heart disease. *Int J Cardiol* 2005;**101**:285–91.
281. Knirsch W, Hassberg D, Beyer A, et al. Knowledge, compliance and practice of antibiotic endocarditis prophylaxis of patients with congenital heart disease. *Pediatr Cardiol* 2003;**24**:344–9.
282. Knoll B, Tleyjeh IM, Steckelberg JM, et al. Infective endocarditis due to penicillin-resistant viridans group streptococci. *Clin Infect Dis* 2007;**44**:1585–92.
283. Komshian SV, Tablan OC, Palutke W, et al. Characteristics of left-sided endocarditis due to *Pseudomonas aeruginosa* in the Detroit Medical Center. *Rev Infect Dis* 1990;**12**:693–702.
284. Kramer H, Bourgeois M, Liersch R, et al. Current clinical aspects of bacterial endocarditis in infancy, childhood and adolescence. *Eur J Pediatr* 1983;**140**:253–9.
285. Kuypers JM, Proctor RA. Reduced adherence to traumatized rat heart valves by a low-fibronectin-binding mutant of *Staphylococcus aureus*. *Infect Immun* 1989;**57**:2306–12.
286. Laird WP, Nelson JD, Weinberg AG, et al. Fatal *Haemophilus influenzae* endocarditis diagnosed by echocardiography in an infant. *Pediatrics* 1979;**64**:292–5.
287. Lam S, Samraj J, Rahman S, et al. Primary actinomycotic endocarditis: case report and review. *Clin Infect Dis* 1993;**16**: 481–5.
288. Lamas CC, Eykyn SJ. Suggested modifications to the Duke criteria for the clinical diagnosis of native valve and prosthetic valve endocarditis: analysis of 118 pathologically proven cases. *Clin Infect Dis* 1997;**25**:713–19.
289. Lamich R, Alonso C, Guma JR, et al. Prospective study of bacteremia during transesophageal echocardiography. *Am Heart J* 1993;**125**:1454.
290. La Scola B, Raoult D. Molecular identification of *Gemella* species from three patients with endocarditis. *J Clin Microbiol* 1998; **36**:866–71.
291. Laufer D, Lew PD, Obertiansli I, et al. Chronic Q fever endocarditis with massive splenomegaly in childhood. *J Pediatr* 1986; **108**:535–9.
292. La Via WV, Koulouri S, Ross LA, et al. Right atrial mass in a child with disseminated coccidioidomycosis. *Pediatr Infect Dis J* 2005;**24**:470–1.
293. Laxdal T, Messner RP, Williams RS. Opsonic, agglutinating and complement-fixing antibodies in patients with subacute bacterial endocarditis. *J Lab Clin Med* 1968;**71**:638–75.
294. Le Guillou S, Casalta JP, Fraisse A, et al. Infective endocarditis in children without underlying heart disease: a retrospective study analyzing 11 cases. *Arch Pediatr* 2010;**17**:1047–55.
295. Lederman MM, Sprague L, Wallis RS, et al. Duration of fever during treatment of infective endocarditis. *Medicine (Baltimore)* 1992;**71**:52.
296. Lee JH, Burner KD, Fealey ME, et al. Prosthetic valve endocarditis: clinicopathological correlates in 122 surgical specimens from 116 patients (1985-2004). *Cardiovasc Pathol* 2011;**20**:26–35.
297. Lefort A, Mainordi JL, Selton-Suty C, et al. *Streptococcus pneumoniae* endocarditis in adults: a multi center study in France in the era of penicillin resistance (1991-1998). *The Pneumococcal Endocarditis Study Group Medicine (Baltimore)* 2000;**79**:327–37.
298. Leonard A, Raij L, Shapiro FC. Bacterial endocarditis in regularly dialyzed patients. *Kidney Int* 1973;**4**:407–22.
299. Lepeschkin E. On the relation between the side of valvular involvement in endocarditis and the blood pressure resting on the valve. *Am J Med Sci* 1952;**224**:318–19.
300. Lertsapcharoen P, Khongphatthanayothin A, Chotirivittayatarakorn P, et al. Infective endocarditis in pediatric patients: an eighteen-year experience from King Chilalongkorn Hospital. *J Med Assoc Thai* 2005;**88**:512–16.
301. Levin RM, Pulliam L, Mondry C, et al. Penicillin-resistant *Streptococcus constellatus* as a cause of endocarditis. *Am J Dis Child* 1982;**136**:42–5.
302. Levine DP, Fromm BS, Reddy BR. Slow response to vancomycin or vancomycin plus rifampin in methicillin-resistant *Staphylococcus aureus* endocarditis. *Ann Intern Med* 1991;**115**:674–80.
303. Levitas A, Zucker N, Zalzstein E, et al. Successful treatment of infective endocarditis with recombinant tissue plasminogen activator. *J Pediatr* 2003;**143**:649–52.
304. Levy PY, Drancourt M, Etienne J, et al. Comparison of different antibiotic regimens for therapy of 32 cases of Q fever endocarditis. *Antimicrob Agents Chemother* 1991;**35**:533–7.
305. Li JS, Sexton DJ, Mick N, et al. Proposed modifications to the Duke criteria for the diagnosis of infective endocarditis. *Clin Infect Dis* 2000;**30**:633–8.
306. Liepman MK, Jones PG, Kauffman CA. Endocarditis as a complication of indwelling right atrial catheters in leukemic patients. *Cancer* 1984;**54**:804–7.
307. Liew WK, Tan TH, Wong KY. Infective endocarditis in childhood: a seven-year experience. *Singapore Med* 2004;**45**: 525–9.
308. Lin CH, Hsu RB. Infective endocarditis caused by nutritionally variant streptococci. *Am J Med Sci* 2007;**334**:235–9.
309. Lin YT, Hsieh KS, Chen YS, et al. Infective endocarditis in children without underlying heart disease. *J Microbiol Immunol Infect* 2012. [Epub ahead of print].
310. Lina B, Celard M, Vandenesch F, et al. Infective endocarditis due to *Staphylococcus capitis*. *Clin Infect Dis* 1992;**15**:173–4.
311. Lipman ML, Silva J. Endocarditis due to *Streptococcus faecalis* with high-level resistance to gentamicin. *Rev Infect Dis* 1989;**11**: 325–8.
312. Lisby G, Gutschik E, Durack DT. Molecular methods for diagnosis of infective endocarditis. *Infect Dis Clin North Am* 2002;**16**:393–412.
313. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:285–92.
314. Liu VC, Stevenson JG, Smith AL. Group A *Streptococcus* mural endocarditis. *Pediatr Infect Dis J* 1992;**11**:1060–2.
315. Liwnicz BH, Lepow H. Nonbacterial thrombotic endocarditis in a premature child: clinical significance and possible relationships to subvalvular hematoma. *N Y State J Med* 1976;**76**:912–16.
316. Lowenstein MB, Urman JD, Abeles M, et al. Skin immunofluorescence in infective endocarditis. *JAMA* 1977;**238**:1163–5.
317. Lowrance JH, Baddour LM, Simpson WA. The role of fibronectin binding in a rat model of experimental endocarditis caused by *Streptococcus sanguis*. *J Clin Invest* 1990;**86**:7.

318. Lubani M, Sharda D, Helin I. Cardiac manifestations in brucellosis. *Arch Dis Child* 1986;**61**:569–72.
319. Lutas EM, Roberts RB, Devereux RB, et al. Relation between the presence of echocardiographic vegetations and the complication rate in infective endocarditis. *Am Heart J* 1986;**112**:107–13.
320. Lynn DC, Kane JG, Parker RH. *Haemophilus parainfluenzae* endocarditis: a review of forty cases. *Medicine (Baltimore)* 1977;**56**:115–28.
321. Macauley D. Acute endocarditis in infancy and early childhood. *Am J Dis Child* 1954;**88**:715–21.
322. MacMahon SW, Hickey AJ, Wilcken DEL, et al. Risk of infective endocarditis in mitral valve prolapse with and without precordial systolic murmurs. *Am J Cardiol* 1986;**58**:105–8.
323. Madison J, Wang K, Gobel FL, et al. Prosthetic aortic valve endocarditis. *Circulation* 1975;**51**:940–9.
324. Maekawa Y, Sakamoto T, Umezu K, et al. Infective endocarditis in a child caused by *Cardiobacterium hominis* after right ventricular outflow tract reconstruction using an expanded tetrafluoroethylene conduit. *Gen Thorac Cardiovasc Surg* 2011;**59**:429–32.
325. Maisch B, Eichstadt H, Kochsiek K. Immune reactions in infective endocarditis, part I: clinical data and diagnostic relevance of antimyocardial antibodies. *Am Heart J* 1983;**106**:329–44.
326. Maki DG, Agger WA. Enterococcal bacteremia: clinical features, the risk of endocarditis and management. *Medicine (Baltimore)* 1988;**67**:248–69.
327. Malacoff RF, Frank E, Andriole VT. Streptococcal endocarditis (non-enterococcal, non-group A): single vs. combination therapy. *JAMA* 1979;**241**:1807–10.
328. Maltezou HC, Raoult D. Q fever in children. *Lancet Infect Dis* 2002;**2**:686–91.
329. Mansur AJ, Grinberg M, da Luz PL, Bellotti G. The complications of infective endocarditis. *Arch Intern Med* 1992;**152**: 2428–32.
330. Marinell PV, Diana DJ, Todd WA. Survival of a child after *Haemophilus influenzae* b endocarditis. *Pediatr Infect Dis J* 1983;**2**:46–7.
331. Markowitz M. The decline of rheumatic fever: role of medical intervention. Lewis W. Wannamaker Memorial Lecture. *J Pediatr* 1985;**106**:545–50.
332. Markowitz SM, Szentpetery S, Lower RR, et al. Endocarditis due to accidental penetrating foreign bodies. *Am J Med* 1976;**60**:571–6.
333. Marks AR, Choong CY, Sanfilippo AJ, et al. Identification of high-risk and low-risk subgroups of patients with mitral-valve prolapse. *N Engl J Med* 1989;**370**:1031–6.
334. Marks KA, Zucker N, Kopelushnik J, et al. Infective endocarditis successfully treated in extremely low birth weight infants with recombinant tissue plasminogen activator. *Pediatrics* 2002;**109**: 153–8.
335. Marom D, Levy I, Gutwein O, et al. Healthcare-associated versus community-associated infective endocarditis in children. *Pediatr Infect Dis J* 2011;**30**:585–8.
336. Marrie TJ, Harczy M, Mann OE, et al. Culture-negative endocarditis probably due to *Chlamydia pneumoniae*. *J Infect Dis* 1990;**161**:127–9.
337. Martin JM, Neches WH, Wald ER. Infective endocarditis: 35 years of experience at a children's hospital. *Clin Infect Dis* 1997;**24**:669–75.
338. Martino P, Micozzi A, Venditti M, et al. Catheter-related right-sided endocarditis in bone marrow transplant recipients. *Rev Infect Dis* 1990;**12**:250–7.
339. Masuda J, Yutani C, Waki R, et al. Histopathologic analysis of the mechanisms of intracranial hemorrhage complicating infective endocarditis. *Stroke* 1992;**23**:843.
340. Mathew J, Addai T, Anand A, et al. Clinical features, site of involvement, bacteriologic findings and outcome of infective endocarditis in intravenous drug users. *Arch Intern Med* 1995; **155**:1641–8.
341. Mayayo E, Moralejo J, Camps J, et al. Fungal endocarditis in premature infants: case report and review. *Clin Infect Dis* 1996;**22**:366–8.
342. McCartney AC, Orange GU, Pringle SD, et al. Serum C reactive protein in infective endocarditis. *J Clin Pathol* 1988;**41**:44–8.
343. McCormick J, Pollard J. *Aspergillus* endocarditis 2003-2009. *Med Mycol* 2011;**49**(Suppl 1):S30–4.
344. McGuinness GA, Schieken RM, Maguire GF. Endocarditis in the newborn. *Am J Dis Child* 1980;**134**:577–80.
345. McKinsey DS, Ratts TE, Bisno AL. Underlying cardiac lesions in adults with infective endocarditis: the changing spectrum. *Am J Med* 1987;**82**:681–8.
346. McMahon CJ, Ayers N, Pignatelli RH, et al. Echocardiographic presentations of endocarditis and risk factors for rupture of a sinus of Valsalva in childhood. *Cardiol Young* 2003;**13**:168–72.
347. Medoff G, Comfort M, Kabayashi G. Synergistic action of amphotericin B and 5-fluorocytosine against yeast-like organisms. *Proc Soc Exp Biol Med* 1971;**138**:571–4.
348. Mendelsohn G, Hutchins GM. Infective endocarditis during the first decade of life. *Am J Dis Child* 1979;**133**:619–22.
349. Megran DW. Enterococcal endocarditis. *Clin Infect Dis* 1992;**15**: 63–71.
350. Merzbach D, Freundlich E, Metzker A, et al. Endocarditis due to *Corynebacterium*. *J Pediatr* 1965;**67**:792–6.
351. Michelfelder EC, Ochsner JE, Khoury P, Kimball TR. Does assessment of pretest probability of disease improve the utility of echocardiography in suspected endocarditis in children? *J Pediatr* 2003;**142**:263–7.
352. Middlemost S, Wisenbaugh T, Meyerowitz C, et al. A case for early surgery in native left-sided endocarditis complicated by heart failure: results in 203 patients. *J Am Coll Cardiol* 1991;**18**: 663–7.
353. Millar BC, Jugo J, Moore JE. Fungal endocarditis in neonates and children. *Pediatr Cardiol* 2005;**26**:517–36.
354. Millard DD, Shulman ST. The changing spectrum of neonatal endocarditis. *Clin Perinatol* 1988;**15**:587–608.
355. Mintz GS, Kotler MN, Segal BL, et al. Comparison of two-dimensional and M-mode echocardiography in the evaluation of patients with infective endocarditis. *Am J Cardiol* 1979;**43**: 738–45.
356. Miro JM, Anguera I, Cabell CH, et al. *Staphylococcus aureus* native valve infective endocarditis: report of 566 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* 2005;**41**:507–14.
357. Mittal BV. Renal lesions in infective endocarditis. *J Postgrad Med* 1987;**33**:193–7.
358. Miyamoto T, Tomoyasu T, Miyaji K. Successful treatment of pediatric endocarditis and pericarditis due to MRSA with linezolid. *Jpn J Antibiot* 2011;**64**:109–12.
359. Mogollón MV, Anguita MP, Aguado JM, et al. Q fever endocarditis in Spain: clinical characteristics and outcome. *Enferm Infecc Microbiol Clin* 2011;**29**:109–16.
360. Mohan UR, Walters S, Kroll JS. Endocarditis due to group A β-hemolytic *Streptococcus* in children with potentially lethal sequelae: 2 cases and review. *Clin Infect Dis* 2000;**30**:624–5.
361. Moodie DS, Gallen WJ. Pneumococcal endocarditis in a 7 week old infant. *Am J Dis Child* 1975;**129**:980–3.
362. Moreillon P, Que YA, Bayer AS. Pathogenesis of streptococcal and staphylococcal endocarditis. *Infect Dis Clin North Am* 2002;**16**:297–318.
363. Morguet AJ, Munz DL, Ivancevic V, et al. Immunoscintigraphy using technetium-99m–labeled anti-NCA-95 antigranulocyte antibodies as an adjunct to echocardiography in subacute infective endocarditis. *J Am Coll Cardiol* 1994;**23**:1171–88.
364. Morpeth S, Murdoch D, Cabell CH, et al. Non-HACEK gram-negative bacillus endocarditis. *Ann Intern Med* 2007;**147**:829–35.
365. Morris AJ, Drinkovic D, Pottumarthy S, et al. Gram stain, culture and histopathologic examination findings from heart valves removed because of infective endocarditis. *Clin Infect Dis* 2003;**36**:697–704.
366. Morris AJ, Drinkovic D, Pottumarthy S, et al. Bacteriological outcome after valve surgery for active infective endocarditis: implications for duration of treatment after surgery. *Clin Infect Dis* 2005;**41**:187–94.
367. Morris CD, Reller MD, Menosch VD. Thirty year incidence of infective endocarditis after surgery for congenital heart defect. *JAMA* 1998;**279**:599–603.
368. Morrison VA, Wagner KF. Clinical manifestations of *Kingella kingae* infections: case report and review. *Rev Infect Dis* 1989;**11**:776–82.
369. Mortara LA, Bayer AS. *Staphylococcus* bacteremia and endocarditis: new diagnostic and therapeutic concepts. *Infect Dis Clin North Am* 1993;**7**:53–67.
370. Moy RJD, George RH, DeGiovanni JV, et al. Improving survival in bacterial endocarditis. *Arch Dis Child* 1986;**61**:394–9.

371. Mugge A, Daniel WG, Frank G, et al. Echocardiography in infective endocarditis: reassessment of prognostic implications of vegetation size determined by the transthoracic and transesophageal approach. *J Am Coll Cardiol* 1989;**14**:631–8.
372. Mullany CJ, Chau YL, Schaff HV, et al. Early and late survival after surgical treatment of culture-positive active endocarditis. *Mayo Clin Proc* 1995;**70**:517–25.
373. Munoz P, Berenguer J, Rodriguez-Greixems M, et al. Ciprofloxacin and infective endocarditis. *Infect Dis Clin Pract* 1993;**2**:119.
374. Murphy JG, Foster-Smith K. Management of complications of infective endocarditis with emphasis on echocardiographic findings. *Infect Dis Clin North Am* 1993;**7**:153–65.
375. Murray BE. The life and times of the *Enterococcus*. *Clin Microbiol Rev* 1990;**3**:46–65.
376. Murray HW, Gross KC, Masur H, et al. Serious infections caused by *Streptococcus milleri*. *Am J Med* 1978;**64**:759–65.
377. Musci M, Hubler M, Amiri A, et al. Surgical treatment for active infective prosthetic valve endocarditis: 22-year single-centre experience. *Eur J Cardiothorac Surg* 2010;**38**:528–38.
378. Musci M, Weng Y, Hubler M, et al. Predictors of early mortality in patients with active infective native or prosthetic aortic root endocarditis undergoing homograft aortic root replacement. *Clin Res Cardiol* 2009;**98**:443–50.
379. Musewe NN, Hecht BM, Hesslein PS, et al. Tricuspid valve endocarditis in two children with normal hearts: diagnosis and therapy of an unusual clinical entity. *J Pediatr* 1987;**110**:735–8.
380. Nagel JG, Tuazon CV, Cardella TA, et al. Teichoic acid serologic diagnosis of staphylococcal endocarditis. *Ann Intern Med* 1975; **82**:13–8.
381. Nagunuma M. Infective endocarditis in children. *Jpn Circ J* 1985;**49**:545–52.
382. Naidoo DP. Right-sided endocarditis in the non–drug addict. *Postgrad Med J* 1993;**69**:615–20.
383. Nakayama DK, O'Neill JA, Wagner H, et al. Management of vascular complications of bacterial endocarditis. *J Pediatr Surg* 1986;**21**:636–9.
384. Narasimhan SL, Weinstein AJ. Infective endocarditis due to a nutritionally deficient *Streptococcus*. *J Pediatr* 1980;**96**:61–2.
385. Nastro LJ, Finegold SM. Endocarditis due to anaerobic gram-negative bacilli. *Am J Med* 1973;**54**:482–96.
386. Nath P, Kiran V, Maheshwari S. Awareness of infective endocarditis prophylaxis in parents of children with congenital heart disease: a prospective study. *Ann Pediatr Cardiol* 2008;**1**: 54–5.
387. Nelson RJ, Harley DP, French WJ, et al. Favorable ten-year experience with valve procedures for active infective endocarditis. *J Thorac Cardiovasc Surg* 1984;**87**:493–502.
388. Nguyen MH, Nguyen ML, Yu VL, et al. *Candida* prosthetic valve endocarditis: prospective study of six cases and review of the literature. *Clin Infect Dis* 1996;**22**:262–7.
389. Nicolau DP, Freeman CD, Nightingale CH, et al. Reduction of bacterial titers by low-dose aspirin in experimental aortic valve endocarditis. *Infect Immun* 1993;**61**:1593–5.
390. Nikolousis E, Velangi M. Two cases of *Aspergillus* endocarditis in non-neutropenic children on chemotherapy for acute lymphoblastic leukemia. *Hematol Rep* 2011;**3**:e7.
391. Niwa K, Nakazawa M, Tateno S, et al. Infective endocarditis in congenital heart disease: Japanese national collaboration data. *Heart* 2005;**91**:795–800.
392. Noel GJ, O'Loughlin JE, Edelson PJ. Neonatal *Staphylococcus epidermidis* right-sided endocarditis: Description of five catheterized infants. *Pediatrics* 1988;**82**:234–9.
393. Nolan CM, Kane JJ, Grunow WA. Infective endocarditis and mitral prolapse: a comparison with other types of endocarditis. *Arch Intern Med* 1981;**141**:447–50.
394. Nomura F, Penny DJ, Menahem S, et al. Surgical intervention for infective endocarditis in infancy and childhood. *Ann Thorac Surg* 1995;**60**:90–5.
395. O'Callaghan C, McDougall P. Infective endocarditis in neonates. *Arch Dis Child* 1988;**63**:53–7.
396. Oelberg DG, Fisher DJ, Gross DM, et al. Endocarditis in high-risk neonates. *Pediatrics* 1983;**71**:392–7.
397. Okumura A, Ito K, Kondo M, et al. Infective endocarditis caused by highly penicillin-resistant *Streptococcus pneumoniae:* successful treatment with cefuzonam, ampicillin and imipenem. *Pediatr Infect Dis J* 1995;**14**:327–9.
398. Olaison L, Pettersson G. Current best practices and guidelines: indications for surgical intervention in infective endocarditis. *Infect Dis Clin North Am* 2002;**16**:453–76.
399. Panidis IP, Kotler MN, Mintz GS, et al. Right heart endocarditis: clinical and echocardiographic features. *Am Heart J* 1984;**107**: 759–64.
400. Pasquali SK, He X, Mohamad Z, et al. Trends in endocarditis hospitalizations at U.S. children's hospitals: impact of the 2007 American Heart Association Prophylaxis Guidelines. *Am Heart J* 2012;**163**:894–9.
401. Patchell RA, White CL, Clark AW, et al. Nonbacterial thrombotic endocarditis in bone marrow transplant patients. *Cancer* 1985; **55**:631–5.
402. Patrick WD, Brown WD, Bowmer MI, et al. Infective endocarditis due to *Eikenella corrodens:* case report and review of the literature. *Can J Infect Dis* 1990;**1**:139.
403. Patterson JE, Zervos MJ. High-level gentamicin resistance in *Enterococcus:* microbiology, genetic basis and epidemiology. *Rev Infect Dis* 1990;**12**:644–52.
404. Pavlovsky M, Press J, Peled N, Yagupsky P. Blood culture contamination in pediatric patients: young children and young doctors. *Pediatr Infect Dis J* 2006;**25**;611–14.
405. Pazin GJ, Saul S, Thompson ME. Blood culture positivity: suppression by outpatient antibiotic therapy in patients with bacterial endocarditis. *Arch Intern Med* 1982;**142**:263–9.
406. Pedersen WR, Walker M, Olson JD, et al. Value of transesophageal echocardiography as an adjunct to transthoracic echocardiography in evaluation of native and prosthetic valve endocarditis. *Chest* 1991;**100**:351–6.
407. Pefanis A, Giamarellou H, Karayiannakos P, et al. Efficacy of ceftazidime and aztreonam alone or in combination with amikacin in experimental left-sided *Pseudomonas aeruginosa* endocarditis. *Antimicrob Agents Chemother* 1993;**37**:308–13.
408. Pefanis A, Thauvin-Eliopoulos C, Eliopoulos GM, et al. Activity of ampicillin-sulbactam and oxacillin in experimental endocarditis caused by beta-lactamase hyperproducing *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1993;**37**:507–11.
409. Peled N, Pitlik S, Livni G, et al. Impact of age on clinical features and outcome of infective endocarditis. *Eur J Clin Microbial Infect Dis* 2006;**25**:473–5.
410. Pelletier LL, Durack DT, Petersdorf RG. Chemotherapy of experimental streptococcal endocarditis, part IV: further observations on prophylaxis. *J Clin Invest* 1975;**56**:319–30.
411. Penk JS, Webb CL, Shulman ST, et al. Echocardiography in pediatric infective endocarditis. *Pediatr Infect Dis J* 2011;**30**: 1109–11.
412. Pereira RM, Bucaretchi F, Tresoldi AT. Infective endocarditis due to *Haemophilus aphrophilus:* a case report. *J Pediatr* 2008;**84**: 178–80.
413. Perry KS, Tresch DD, Brooks HL, et al. Operative approach to endocarditis. *Am Heart J* 1984;**108**:561–6.
414. Pesanti EL, Smith IM. Infective endocarditis with negative blood cultures: an analysis of 52 cases. *Am J Med* 1979;**66**:43–50.
415. Peter O, Flepp M, Bestetti G, et al. Q fever endocarditis: diagnostic approaches and monitoring of therapeutic effects. *Clin Invest* 1992;**70**:932.
416. Pitchford CW, Creech CB, Peters TR, Vnencak-Jones CL. *Bartonella henselae* endocarditis in a child. *Pediatr Cardiol* 2006;**27**:769–71.
417. Pollack S, Mogtader A, Lange M. *Neisseria subflava* endocarditis: case report and review of the literature. *Am J Med* 1984; **76**:752–8.
418. Pongratz G, Henneke KH, von der Grun M, et al. Risk of endocarditis in transesophageal echocardiography. *Am Heart J* 1993;**125**:190–3.
419. Popp RL. Echocardiography. *N Engl J Med* 1990;**323**:165.
420. Powderly WG, Stanley SL, Medoff G. Pneumococcal endocarditis: report of a series and review of the literature. *Rev Infect Dis* 1986;**8**:786–91.
421. Powell DC, Bivens BA, Bell RM, et al. Endocarditis: increasingly a surgical disease. *Am Surg* 1982;**48**:5–10.
422. Powers DL, Mandell GL. Intraleukocytic bacteria in endocarditis patients. *JAMA* 1974;**227**:313–15.
423. Prager RL, Maples MD, Hammon JW, et al. Early operative intervention in aortic bacterial endocarditis. *Ann Thorac Surg* 1981;**32**:347–50.

424. Prinsloo JG, Pretorius PJ. *Candida albicans* endocarditis. *Am J Dis Child* 1966;**111**:446–7.
425. Pulvirenti JJ, Kerns E, Benson C, et al. Infective endocarditis in injection drug users: importance of human immunodeficiency virus serostatus and degree of immunosuppression. *Clin Infect Dis* 1996;**22**:40–5.
426. Purcell LK, Finley JP, Chen R, et al. *Gemella* species endocarditis in a child. *Can J Infect Dis* 2001;**12**:317–20.
427. Qin X, Urdahl KB. PCR and sequencing of independent genetic targets for the diagnosis of culture negative bacterial endocarditis. *Diagn Microbiol Infect Dis* 2001;**40**:145–9.
428. Qoronfleh MW, Weraarchakul W, Wilkinson BS. Antibodies to a range of *Staphylococcus aureus* and *Escherichia coli* heat shock proteins in sera from patients with *S. aureus* endocarditis. *Infect Immun* 1993;**61**:1567–70.
429. Rabin RL, Wong P, Noonan JA, et al. *Kingella kingae* endocarditis in a child with a prosthetic aortic valve and bifurcation graft. *Am J Dis Child* 1983;**137**:403–4.
430. Raff GW, Gray BM, Torres Jr A, Hasselman TE. Aortitis in a child with *Abiotrophia defectiva* endocarditis. *Pediatr Infect Dis J* 2004;**23**:574–6.
431. Rai K, Supriya S, Hegde AM. Oral health status of children with congenital heart disease and the awareness, attitude and knowledge of their parents. *J Clin Pediatr Dent* 2009;**33**:315–18.
432. Ramage IJ, Wilson N, Thomson RB. Fashion victim: infective endocarditis after nasal piercing. *Arch Dis Child* 1997;**77**:187.
433. Ramirez CA, Naragi S, McCulley DJ. Group A beta-hemolytic streptococcus endocarditis. *Am Heart J* 1984;**108**:1383–6.
434. Raoult D, Fournier PE, Dramcourt M, et al. Diagnosis of 22 new cases of *Bartonella* endocarditis. *Ann Intern Med* 1996;**125**: 646–52.
435. Rastogi A, Luken JA, Pildes RS, et al. Endocarditis in the neonatal intensive care unit. *Pediatr Cardiol* 1993;**14**:183–6.
436. Rech A, Loss JF, Machado A, Brunetto AL. Infective endocarditis in children receiving treatment for cancer. *Pediatr Blood Cancer* 2004;**43**:159–63.
437. Reller LB. The serum bactericidal test. *Rev Infect Dis* 1986;**8**:803–7.
438. Reymann MT, Holley HP, Cobbs CG. Persistent bacteremia in staphylococcal endocarditis. *Am J Med* 1978;**65**:729–39.
439. Ribot S, Rothfeld D, Frankel HJ. Infectious endocarditis in maintenance hemodialysis patients. *Am J Med Sci* 1972;**264**:183–8.
440. Rice LB, Calderwood SB, Eliopoulos GM, et al. Enterococcal endocarditis: a comparison of prosthetic and native valve disease. *Rev Infect Dis* 1991;**13**:1–7.
441. Rice MJ, McDonald RW, Reller MD, et al. Pediatric echocardiography: current role and a review of technical advances. *J Pediatr* 1996;**128**:1–14.
442. Richet H, Casalta JP, Thuny F, et al. Development and assessment of a new early scoring system using non-specific clinical signs and biological results to identify children and adult patients with a high probability of infective endocarditis on admission. *J Antimicrob Chemother* 2008;**62**:1434–40.
443. Robbins MJ, Frater RWM, Soeiro R, et al. Influence of vegetation size on clinical outcome of right-sided infective endocarditis. *Am J Med* 1986;**80**:165–71.
444. Roberts GJ, Gardner P, Simmons NA. Optimum sampling time for detection of dental bacteremia in children. *Int J Cardiol* 1992;**35**:311–15.
445. Roberts KB, Sidlak MJ. Satellite streptococci: a major cause of "negative" blood cultures in bacterial endocarditis? *JAMA* 1979;**241**:2293–4.
446. Rodbard S. Blood velocity and endocarditis. *Circulation* 1963;**27**:18–28.
447. Rodero FG, del Mar Masia M, Cortes J, et al. Endocarditis caused by *Stenotrophomonas maltophilia:* case report and review. *Clin Infect Dis* 1996;**23**:1261–5.
448. Rogers J, Walker M, Olson JD, et al. Value of transesophageal echocardiography as an adjunct to transthoracic echocardiography in evaluation of native and prosthetic valve endocarditis. *Chest* 1991;**100**:351–5.
449. Rohmann S, Erbel R, Darius H, et al. Prediction of rapid versus prolonged healing of infective endocarditis by monitoring vegetation size. *J Am Soc Echocardiogr* 1991;**4**:465–74.
450. Rohmann S, Seifert T, Erbel R, et al. Identification of abscess formation in native-valve infective endocarditis using transesophageal echocardiography: implications for surgical treatment. *Thorac Cardiovasc Surg* 1991;**39**:273–80.
451. Rose AG. Infective endocarditis complicating congenital heart disease. *S Afr Med J* 1978;**53**:739–43.
452. Ross AC, Toltzis P, O'Riordan MA, et al. Frequency and risk factors for deep focus of infection in children with *Staphylococcus aureus* bacteremia. *Pediatr Infect Dis J* 2008;**27**:396–8.
453. Rossi M, Gallo A, De Silva RJ, et al. What is the optimal timing for surgery in infective endocarditis with cerebrovascular complications? *Interact Cardiovasc Thorac Surg* 2012;**14**:72–80.
454. Rubinstein E, Noreiga ER, Simberkoff MS, et al. Fungal endocarditis: analysis of 24 cases and review of the literature. *Medicine (Baltimore)* 1975;**54**:331–44.
455. Rupp ME. *Streptobacillus moniliformis* endocarditis: case report and review. *Clin Infect Dis* 1992;**14**:769–72.
456. Rutledge R, Kim BJ, Applebaum RE. Actuarial analysis of the risk of prosthetic valve endocarditis in 1,598 patients with mechanical and bioprosthetic valves. *Arch Surg* 1985;**120**:469–72.
457. Sable CA, Rome JJ, Martin GR, et al. Indications for echocardiography in the diagnosis of infective endocarditis in children. *Am J Cardiol* 1995;**75**:801–4.
458. Sachdev M, Peterson GE, Jollis JG. Imaging techniques for diagnosis of infective endocarditis. *Infect Dis Clin North Am* 2002;**16**:319–38.
459. Sadiq M, Nazir M, Sheikh SA. Infective endocarditis in children: incidence, pattern, diagnosis and management in a developing country. *Int J Cardiol* 2001;**78**:175–82.
460. Saffle JR, Gardner P, Schoenbaum SC, et al. Prosthetic valve endocarditis: the case for prompt valve replacement. *J Thorac Cardiovasc Surg* 1977;**73**:416–20.
461. Saiman L, Prince A, Gersony WM. Pediatric infective endocarditis in the modern era. *J Pediatr* 1993;**122**:847–53.
462. Saleh A, Dawkins K, Monro J. Surgical treatment of infective endocarditis. *Acta Cardiol* 2004;**59**:658–62.
463. Salgado AV, Furlan AJ, Keys TF, et al. Neurologic complications of endocarditis: a 12-year experience. *Neurology* 1989;**39**:173–8.
464. Sambola A, Miro JM, Tornos MP, et al. *Streptococcus agalactiae* infective endocarditis: analysis of 30 cases and review of the literature, 1962-1998. *Clin Infect Dis* 2002;**34**:1576–84.
465. Sanchez PJ, Siegel JD, Fishbein J. *Candida* endocarditis: successful medical management in three preterm infants and review of the literature. *Pediatr Infect Dis* 1991;**10**:239–43.
466. Sande MA, Courtney KB. Nafcillin-gentamicin synergism in experimental *Staphylococcus* endocarditis. *J Lab Clin Med* 1976;**88**:118–24.
467. Sandre RM, Shatran SD. Infective endocarditis: review of 135 cases over 9 years. *Clin Infect Dis* 1996;**22**:276–86.
468. Sanfilippo AJ, Picard MH, Newell JB, et al. Echocardiographic assessment of patients with infectious endocarditis: prediction of risk for complications. *J Am Coll Cardiol* 1991;**18**:1191–9.
469. San Roman JA, Vilacosta I, Zamorano JL, et al. Transesophageal echocardiography in right-sided endocarditis. *J Am Coll Cardiol* 1993;**21**:1226–30.
470. Sapico FL, Liquete JA, Sarma RJ. Bone and joint infections in patients with infective endocarditis: review of a 4-year experience. *Clin Infect Dis* 1996;**22**:783–7.
471. Sapsford RN, Fitchett DH, Tarin D, et al. Aneurysm of left ventricle secondary to bacterial endocarditis. *J Thorac Cardiovasc Surg* 1979;**78**:79–86.
472. Saxena A, Aggarwal N, Gupta P, et al. Predictors of embolic events in pediatric infective endocarditis. *Indian Heart J* 2011;**63**:237–40.
473. Scheld WM, Mandell GL. Enigmatic enterococcal endocarditis. *Ann Intern Med* 1984;**100**:904–5.
474. Scheld WM, Sande MA. Endocarditis and intravascular infections. In: Mandell G, Bennett JE, Dolin R, editors. *Principles and practices of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 740–82.
475. Scheld WM, Calderone RA, Alliegro GM, et al. Yeast adherence in the pathogenesis of *Candida* endocarditis. *Proc Soc Exp Biol Med* 1981;**168**:208–17.
476. Scheld WM, Thomas JH, Sande MA. Influence of preformed antibody on experimental *Streptococcus sanguis* endocarditis. *Infect Immun* 1979;**25**:781–5.
477. Scheld WM, Valone JA, Sande MA. Bacterial adherence in the pathogenesis of endocarditis: interaction of bacterial dextran, platelets and fibrin. *J Clin Invest* 1978;**61**:1394–8.

478. Scheld WM, Zak O, Vosbeck K, et al. Bacterial adhesion in the pathogenesis of endocarditis: effect of subinhibitory antibiotic concentrations on streptococcal adhesion in vitro and the development of endocarditis in rabbits. *J Clin Invest* 1981;**68**:1381.
479. Schnell D, Beyler C, Lanternier F, et al. Nontoxigenic *Corynebacterium diphtheriae* as a rare cause of native endocarditis in childhood. *Pediatr Infect Dis J* 2010;**29**:886–8.
480. Schollin J, Bjarke B, Wesstrom G. Follow-up study on children with infective endocarditis. *Acta Paediatr* 1989;**78**:615–19.
481. Schulte JE, Gaffney FA, Bland L, et al. Distinctive anthropometric characteristics of women with mitral valve prolapse. *Am J Med* 1981;**71**:553–8.
482. Scott PJ, Blackburn ME, Wharton GA, et al. Transesophageal echocardiography in neonates, infants and children: applicability and diagnostic value in everyday practice of a cardiothoracic unit. *Br Heart J* 1992;**68**:488–92.
483. Scott RM. Bacterial endocarditis due to *Neisseria flava*. *J Pediatr* 1971;**78**:673–5.
484. Seeling MS, Speth CP, Kozinn PJ, et al. Patterns of *Candida* endocarditis following cardiac surgery: importance of early diagnosis and therapy (an analysis of 91 cases). *Prog Cardiovasc Dis* 1974;**17**:125–60.
485. Sexton DJ, Tenenbaum MJ, Wilson WR, et al. Ceftriaxone once daily for four weeks compared with ceftriaxone plus gentamicin once daily for two weeks for treatment of endocarditis due to penicillin-susceptible streptococci. *Clin Infect Dis* 1998;**27**:1470–4.
486. Shah FS, Fennelly G, Weingarten-Arams J, et al. Endocardial abscesses in children: case report and review of the literature. *Clin Infect Dis* 1999;**29**:1478–82.
487. Shanson D. New British and American guidelines for the antibiotic prophylaxis of infective endocarditis: do the changes make sense? A critical review. *Curr Opin Infect Dis* 2008;**21**:191–9.
488. Shapiro DS, Kenney SC, Johnson M, et al. *Chlamydia psittaci* endocarditis diagnosed by blood culture. *N Engl J Med* 1992;**326**:1192–5.
489. Shively BK, Gurule FT, Roldan CA, et al. Diagnostic value of transesophageal compared with transthoracic echocardiography in infective endocarditis. *J Am Coll Cardiol* 1991;**18**:391–7.
490. Sholler GF, Hawker RE, Celermajer JM. Infective endocarditis in childhood. *Pediatr Cardiol* 1986;**6**:183–6.
491. Simmons NA. Recommendations for endocarditis prophylaxis. *J Antimicrob Chemother* 1993;**31**:437–8.
492. Singhi SC, Singh S, Bidwai PS. *Peptococcus* endocarditis. *Indian J Pediatr* 1988;**25**:876–8.
493. Sirisanthana V, Sirisanthana T. *Corynebacterium diphtheriae* endocarditis. *Pediatr Infect Dis J* 1983;**2**:470–1.
494. Sochowski RA, Chan KL. Implication of negative results on a monoplane transesophageal echocardiographic study in patients with suspected infective endocarditis. *J Am Coll Cardiol* 1993;**21**:216.
495. Sommer P, Gleyzal C, Guerret S, et al. Induction of a putative laminin-binding protein of *Streptococcus gordonii* in human infective endocarditis. *Infect Immun* 1992;**60**:360–5.
496. Spach DH, Kanter AS, Daniels NA, et al. *Bartonella (Rochalimaea)* species as a cause of apparent "culture-negative" endocarditis. *Clin Infect Dis* 1995;**20**:1044–7.
497. Stanton BF, Baltimore RS, Clemens JD. Changing spectrum of infective endocarditis in children. *Am J Dis Child* 1984;**138**:720–5.
498. Steckelberg JM, Wilson WR. Risk factors for infective endocarditis. *Infect Dis Clin North Am* 1993;**7**:9–19.
499. Steckelberg JM, Murphy JG, Ballard D, et al. Emboli in infective endocarditis: the prognostic value of echocardiography. *Ann Intern Med* 1991;**114**:635–40.
500. Steckelberg JM, Rouse MS, Tallan BM, et al. Relative efficacies of broad-spectrum cephalosporins for treatment of methicillin-susceptible *Staphylococcus aureus* experimental infective endocarditis. *Antimicrob Agents Chemother* 1993;**37**:554–8.
501. Steelman R, Einzig S, Balian A, et al. Increased susceptibility to gingival colonization by specific HACEK microbes in children with congenital heart disease. *J Clin Pediatr Dent* 2000;**25**:91–4.
502. Steen MK, Bruno-Murtha LA, Chaux G, et al. *Bacillus cereus* endocarditis: report of a case and review. *Clin Infect Dis* 1992;**14**:945–6.
503. Stein DS, Nelson KE. Endocarditis due to nutritionally deficient streptococci: therapeutic dilemma. *Rev Infect Dis* 1987;**9**:908–16.
504. Steinbach WJ, Perfect JR, Cabell CH, et al. A meta-analysis of medical versus surgical therapy for *Candida* endocarditis. *J Infect* 2005;**51**:230–47.
505. Sternik L, Zehr KJ, Orszulak TA, et al. The advantage of repair of mitral valve in acute endocarditis. *J Heart Valve Dis* 2002;**11**:91–7.
506. Stewart JA, Silamperi D, Harris P, et al. Echocardiographic documentation of vegetative lesions in infective endocarditis: clinical implications. *Circulation* 1980;**61**:374–80.
507. Stewart WJ, Shan K. The diagnosis of prosthetic valve endocarditis by echocardiography. *Semin Thorac Cardiovasc Surg* 1995;**7**:7–12.
508. Stockheim JA, Chadwick EG, Kessler S, et al. Are the Duke criteria superior to Beth Israel criteria for diagnosis of infective endocarditis in children? *Clin Infect Dis* 1998;**27**:1451–6.
509. Stopfuchen H, Benzing F, Jungst B, et al. Echocardiographic diagnosis of *Candida* endocarditis of the tricuspid valve and of the right atrium in a young infant. *Pediatr Cardiol* 1983;**4**:49–51.
510. Stramboulian D, Bonvehi P, Arevalo C, et al. Antibiotic management of outpatients with endocarditis due to penicillin-susceptible streptococci. *Rev Infect Dis* 1991;**13**(Suppl 2):160–3.
511. Stuart G, Wren C. Endocarditis with acute mitral regurgitation caused by *Fusobacterium necrophorum*. *Pediatr Cardiol* 1992;**13**:230–2.
512. Sudduth EJ, Rozich JD, Farrar WE. *Rothia dentocariosa* endocarditis complicated by perivalvular abscess. *Clin Infect Dis* 1993;**17**:772–5.
513. Sullam PM, Costerton JW, Yamasaki R, et al. Inhibition of platelet binding and aggregation by streptococcal exopolysaccharide. *J Infect Dis* 1993;**167**:1123–30.
514. Sussman JI, Baron EJ, Goldberg SM, et al. Clinical manifestations and therapy of *Lactobacillus* endocarditis: report of a case and review of the literature. *Rev Infect Dis* 1986;**8**:771–6.
515. Sussman JI, Baron EJ, Tenenbaum MJ, et al. Viridans streptococcal endocarditis: clinical, microbiological and echocardiographic correlations. *J Infect Dis* 1986;**154**:597–603.
516. Switalski LM, Murchison H, Timpl R, et al. Binding of laminin to oral and endocarditis strains of viridans streptococci. *J Bacteriol* 1987;**169**:1095–101.
517. Sykes RM. *Salmonella* endocarditis in a Nigerian child. *East Afr Med J* 1984;**61**:326–7.
518. Symbas PN, Vlasis SE, Zacharupoulos L, et al. Immediate and long-term outlook for valve replacement in acute bacterial endocarditis. *Ann Surg* 1982;**195**:721–4.
519. Symchych PS, Krauss AW, Winchester P. Endocarditis following intracardiac placement of umbilical venous catheters in neonates. *J Pediatr* 1977;**90**:287–9.
520. Taams MA, Gussenhoven EJ, Bos E, et al. Enhanced morphological diagnosis in infective endocarditis by transesophageal echocardiography. *Br Heart J* 1990;**63**:109–13.
521. Takeda S, Nakanishi T, Nakazawa M, et al. A 28-year trend of infective endocarditis associated with congenital heart disease: a single institute experience. *Pediatr Int* 2005;**47**:392–6.
522. Talarmin JP, Boutoille D, Tattevin P, et al. *Candida* endocarditis: role of the new antifungal agents. *Mycoses* 2009;**52**:60–6.
523. Tancik CA, Dillaha JA. *Francisella tularensis* endocarditis. *Clin Infect Dis* 2000;**30**:399–400.
524. Tanowitz HB, Alder JJ, Chirito E. Gonococcal endocarditis. *N Y State J Med* 1972;**42**:2782–3.
525. Tasdemir K, Kaya MG, Mavili E, et al. Surgical approach to the management of *Brucella* endocarditis. *Eur J Cardiothorac Surg* 2010;**37**:1021–4.
526. Tebas P, Martinez R, Roman F, et al. Early resistance to rifampin and ciprofloxacin in the treatment of right-sided *Staphylococcus aureus* endocarditis. *J Infect Dis* 1991;**163**:204–5.
527. Teixeira OH, Carpenter B, Vlad P. Enterococcal endocarditis in early infancy. *Can Med Assoc J* 1982;**127**:612–3.
528. Thadelpall H, Francis CK. Diagnostic clues in metastatic lesions of endocarditis in addicts. *West J Med* 1978;**128**:1–7.
529. Thal LA, Vazquez J, Perri MB, et al. Activity of ampicillin plus sulbactam against β-lactamase producing enterococci in experimental endocarditis. *J Antimicrob Chemother* 1993;**31**:182.
530. Thapar MK, Rao PS, Feldman D, et al. Infective endocarditis: a review. *Paediatrician* 1978;**7**:65–84.

531. Theofilopoulos AN, Wilson CB, Dixon FJ. The Raji cell radioimmune assay for detecting immune complexes in human sera. *J Clin Invest* 1976;**57**:169–82.
532. Tiley SM, Kociuba KR, Heron LG, et al. Infective endocarditis due to nontoxigenic *Corynebacterium diphtheriae:* report of seven cases and review. *Clin Infect Dis* 1993;**16**:271–5.
533. Tissieres P, Gervaix A, Beghetti M, Jaeggi ET. Value and limitation of the von Reyn, Duke and modified Duke criteria for the diagnosis of infective endocarditis in children. *Pediatrics* 2003; **112**:e467–71.
534. Tissieres P, Jaeggi ET, Beghetti M, Gervaix A. Increase in fungal endocarditis in children. *Infection* 2005;**33**:267–72.
535. Tleyjeh IM, Steckelberg JM, Murad HS, et al. Temporal trends in infective endocarditis: a population-based study in Olmstead County, Minnesota. *JAMA* 2005;**293**:3022–8.
536. Tolan Jr RW, Kleiman MB, Frank M, et al. Operative intervention in active endocarditis in children: report of a series of cases and review. *Clin Infect Dis* 1992;**14**:852–62.
537. Tolaymat A, Rhatigan RM, Levin S. Pneumococcal endocarditis in infants. *South Med J* 1979;**72**:448–51.
538. Tompkins LS, Roessler BJ, Redd SC, et al. *Legionella* prosthetic-valve endocarditis. *N Engl J Med* 1988;**318**:530–5.
539. Tornos MP, Castro A, Toran N, et al. Tricuspid valve endocarditis in children with normal valves. *Am Heart J* 1989;**118**: 624–5.
540. Tornos MP, Permanyer-Miralda G, Olona M, et al. Long-term complications of native valve infective endocarditis in non-addicts: a 15-year follow-up study. *Ann Intern Med* 1992;**117**:567–72.
541. Toy PT, Lai W, Drake TA, Sande MA. Effect of fibronectin on adherence of *Staphylococcus aureus* to fibrin thrombi in vitro. *Infect Immun* 1985;**48**:83–6.
542. Tsao MM, Katz D. Central venous catheter-induced endocarditis: human correlate of the animal experimental model of endocarditis. *Rev Infect Dis* 1984;**6**:783–90.
543. Tuazon CV, Sheagren JW. Staphylococcal endocarditis in parenteral drug abusers: source of the organism. *Ann Intern Med* 1975;**82**:788–90.
544. Tunkel AR, Kaye D. Endocarditis with negative blood cultures. *N Engl J Med* 1992;**326**:1215–17.
545. Turner SW, Wyllie JP, Hamilton JR, Bain HH. Diagnosis of infected modified Blalock-Taussig shunt by computed tomography. *Ann Thorac Surg* 1995;**59**:1216–17.
546. Turnier E, Kay JH, Bernstein S, et al. Surgical treatment of *Candida* endocarditis. *Chest* 1975;**67**:262–8.
547. Utley JR, Mills J, Roe BB. The role of valve replacement in the treatment of fungal endocarditis. *J Thorac Cardiovasc Surg* 1975;**69**:255–8.
548. Valente AM, Jain R, Scheurer M, et al. Frequency of infective endocarditis among infants and children with *Staphylococcus aureus* bacteremia. *Pediatrics* 2005;**115**:e15–19.
549. van de Rijn I. Analysis of cross-protection between serotypes and passively transferred immune globulin in experimental nutritionally variant streptococcal endocarditis. *Infect Immun* 1988;**56**:117–21.
550. van der Meer JTM, Thompson J, Valkenburg HA, et al. Epidemiology of bacterial endocarditis in the Netherlands, part II: antecedent procedures and use of prophylaxis. *Arch Intern Med* 1992;**152**:1869–73.
551. van der Meer JTM, van Wijk W, Thompson J, et al. Efficacy of antibiotic prophylaxis for prevention of native valve endocarditis. *Lancet* 1992;**339**:135.
552. van der Meer JTM, van Wijk W, Thompson J, et al. Awareness of need and actual use of prophylaxis: lack of patient compliance in the prevention of bacterial endocarditis. *J Antimicrob Chemother* 1992;**29**:187–94.
553. Van Hare GF, Ben-Shacher G, Liebman J, et al. Infective endocarditis in infants and children during the past 10 years: a decade of change. *Am Heart J* 1984;**107**:1235–40.
554. Venditti M, De Bernardis F, Micozzi A, et al. Fluconazole treatment of catheter-related right-sided endocarditis caused by *Candida albicans* and associated with endophthalmitis and folliculitis. *Clin Infect Dis* 1992;**14**:422–6.
555. Venkatesan C, Wainwright MS. Pediatric endocarditis and stroke: a single-center retrospective review of seven cases. *Pediatr Neurol* 2008;**38**:243–7.
556. Vikram HR, Buenconsejo J, Hasbun R, Quagliarello VJ. Impact of valve surgery on 6-month mortality in adults with complicated, left-sided native valve endocarditis. *JAMA* 2003; **290**:3207–14.
557. Von Reyn CF, Levy BS, Arbert RD, et al. Infective endocarditis: an analysis based on strict case definitions. *Ann Intern Med* 1982;**94**:505–17.
558. Vuille C, Nidor FM, Weyman A, Picard MH. Natural history of vegetations during successful medical treatment of endocarditis. *Am Heart J* 1994;**128**:1200–9.
559. Walls T, Michael K, Trounce J, et al. Broad-range polymerase chain reaction for the diagnosis of *Bartonella henselae* endocarditis. *J Paediatr Child Health* 2006;**42**:469–71.
560. Walsh TJ, Hutchins GM. *Aspergillus* mural endocarditis. *Am J Clin Pathol* 1979;**71**:640–4.
561. Walterspiel JN, Kaplan SL. Incidence and clinical characteristics of "culture negative" infective endocarditis in a pediatric population. *Pediatr Infect Dis J* 1986;**5**:328–32.
562. Watanakunakorn C, Glotzbecker C. Synergism with aminoglycosides of penicillin, ampicillin and vancomycin against nonenterococcal group D streptococci and viridans streptococci. *J Med Microbiol* 1977;**10**:133–7.
563. Watson A, French P, Wilson M. *Nocardia asteroides* native valve endocarditis. *Clin Infect Dis* 2001;**32**:660–1.
564. Weber R, Berger C, Balmer C, et al. Interventions using foreign material to treat congenital heart disease in children increase the risk for infective endocarditis. *Pediatr Infect Dis J* 2008; **27**:544–50.
565. Wei HH, Wu KG, Sy LB, et al. Infectious endocarditis in pediatric patients: analysis of 19 cases presenting at a medical center. *J Microbiol Immunol Infect* 2010;**43**:430–7.
566. Weinberg AG. Group B streptococcal endocarditis detected by echocardiography. *J Pediatr* 1978;**92**:335–6.
567. Weinstein AJ, Moellering RC. Penicillin and gentamicin therapy for enterococcal infections. *JAMA* 1973;**223**:1030–2.
568. Weinstein L, Schlesinger JJ. Pathoanatomic, pathophysiologic and clinical correlations in endocarditis. *N Engl J Med* 1974;**291**:832–7, 1122–6.
569. Weinstein MP, Stratton CW, Ackley A, et al. Multicenter collaborative evaluation of a standardized bactericidal test as a prognostic indicator in infective endocarditis. *Am J Med* 1985;**78**:262–9.
570. Wells L, Ritter N, Donald F. *Kingella kingae* endocarditis in a sixteen-month-old child. *Pediatr Infect Dis J* 2001;**20**:454–5.
571. Werner AS, Cobbs CG, Kaye D, et al. Studies on the bacteremia of bacterial endocarditis. *JAMA* 1967;**202**:199–203.
572. Wheeler JG, Weesner KM. *Staphylococcus aureus* endocarditis and pericarditis in an infant with a central venous catheter. *Clin Pediatr (Phila)* 1984;**23**:46–7.
573. White PD. The incidence of endocarditis in earliest childhood. *Am J Dis Child* 1926;**32**:536–49.
574. Williams RC, Kunkel HG. Rheumatoid factor, complement and conglutinin aberrations in patients with subacute bacterial endocarditis. *J Clin Invest* 1962;**41**:666–75.
575. Wilson LE, Thomas DL, Astemborski J, et al. Prospective study of infective endocarditis among injection drug users. *J Infect Dis* 2002;**185**:1761–6.
576. Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis: guidelines from the American Heart Association. *Circulation* 2007;**116**:1736–54.
577. Wilson WR, Davidson GK, Guiliani E, et al. Cardiac valve replacement in congestive heart failure due to infective endocarditis. *Mayo Clin Proc* 1979;**54**:223–6.
578. Wilson WR, Gilbert DN, Bisno AL, et al. Evaluation of new anti-infective drugs for the treatment of infective endocarditis. *Clin Infect Dis* 1992;**15**(Suppl 1):89–95.
579. Wilson WR, Karchmer AW, Dajani AS, et al. Antibiotic treatment of adults with infective endocarditis due to streptococci, enterococci, staphylococci, and HACEK microorganisms. *JAMA* 1995; **274**:1706–13.
580. Wilson WR, Thompson RL, Wilkowske CJ, et al. Short-term therapy for streptococcal infective endocarditis. *JAMA* 1981; **245**:360–3.
581. Witt MD, Bayer AS. Comparison of fluconazole and amphotericin B for prevention and treatment of experimental *Candida* endocarditis. *Antimicrob Agents Chemother* 1991;**35**:2481–5.

582. Wolfson JS, Swartz MN. Serum bactericidal activity as a monitor of antibiotic therapy. *N Engl J Med* 1985;**312**:968–75.
583. Woods GL, Wood RP, Shaw BW. *Aspergillus* endocarditis in patients without prior cardiovascular surgery: report of a case in a liver transplant recipient and review. *Rev Infect Dis* 1989;**11**:263–72.
584. Yankah AC, Klose H, Petzina R, et al. Surgical management of acute aortic root endocarditis with viable homograft: 13 years experience. *Eur J Cardiothorac Surg* 2002;**21**:260–7.
585. Yeaman MR, Norman DC, Bayer AS. *Staphylococcus aureus* susceptibility to thrombin-induced platelet microbicidal protein is independent of platelet adherence and aggregation in vitro. *Infect Immun* 1992;**60**:2368–74.
586. Yokochi K, Sakamato H, Mikajima T, et al. Infective endocarditis in children: a current diagnostic trend and the embolic complications. *Jpn Circ J* 1986;**50**:1294–7.
587. Yoshinaga M, Niwa K, Niwa A, et al. Risk factors for in-hospital mortality during infective endocarditis in patients with congenital heart disease. *Am J Cardiol* 2008;**101**:114–18.
588. Yu VL, Fang GD, Keys TF, et al. Prosthetic valve endocarditis: superiority of surgical valve replacement versus medical therapy alone. *Ann Thorac Surg* 1994;**58**:1073–7.
589. Zakrzewski T, Keith JD. Bacterial endocarditis in infants and children. *J Pediatr* 1965;**67**:1179–93.
590. Ziment I. Nervous system complications in bacterial endocarditis. *Am J Med* 1969;**47**:593–607.
591. Zubler RH, Lange G, Lambert PH, et al. Detection of immune complexes in unheated sera by a modified ^{125}I-Clq binding test. *J Immunol* 1976;**116**:232–9.

CHAPTER 27

Infectious Pericarditis

Sheldon L. Kaplan

Purulent pericarditis generally refers to bacterial infection of the pericardium. Inflammation of the pericardium may result from numerous nonbacterial microorganisms, however, or may occur with a variety of noninfectious illnesses (Table 27-1). Regardless of the cause of pericarditis, the responses of the pericardium are limited to acute inflammation, effusion with or without tamponade, and fibrosis with or without constriction.[20] Because untreated purulent pericarditis is rapidly fatal, suspecting the disease early and approaching the diagnosis aggressively are important.

ANATOMY AND FUNCTION

The pericardium is composed of two loosely approximated layers: visceral and parietal. The visceral pericardium is composed of mesothelial tissue, which closely follows the contour of the heart and extends for a short distance beyond the atria and ventricles to the great vessels. The outer parietal pericardium is a more fibrous structure, composed of layers of collagen interlaced with elastic fibers. The pericardial sac is attached to the diaphragm below; to the sternum in front; and to the thoracic vertebrae, esophagus, and aorta posteriorly. It is surrounded by the lungs on either side and is related closely to the main bronchi and the mediastinal lymph nodes. The phrenic and vagus nerves supply a network of pain fibers to the parietal pericardium.

The dynamics of the pericardial fluid are poorly understood. The pericardial membrane is active in the transfer of water, electrolytes, and small molecules. Molecules of large molecular weight are absorbed poorly from the pericardial space because lymphatic channels are sparse, and drainage must occur primarily through the epicardial capillaries.[73]

Ainger[1] summarized the function of the pericardium as follows: prevention of overdistention of the heart, protection of the heart from infection and adhesions, maintenance of the heart within a fixed geometric position within the chest, and regulation of the interaction between the stroke volumes of the two ventricles.

BACTERIAL PERICARDITIS

Population and Incidence

Although purulent pericarditis is not a common infection in pediatric patients, it is an important one to recognize because of its life-threatening nature. In an extensive early review of the literature on purulent pericarditis, half of 425 cases occurred in children younger than 13 years of age.[6] In a review of 162 reported children with pericarditis from 1950 to 1977, 67 percent of the children were 48 months old or younger.[31] From 1962 to 1974, 67 cases were recognized at St. Louis Children's Hospital (Table 27-2).[93] During this 12-year period, pericardial disease of all causes occurred in approximately 1 of every 850 hospital admissions.[93] Twelve (18%) of these children had purulent pericarditis.

Most cases in younger children are infectious. Acute pericarditis was found in 20 children between 1987 and 1997 in a hospital in Iran.[82] The causes of pericarditis were bacterial in eight (40%), collagen vascular disease in six (30%), viral in four (20%), and secondary to mediastinal mass invasion in two (10%). In another series from Turkey, 18 children with purulent pericarditis were encountered from 1990 to 2000.[10] At the Boston Children's Hospital, fewer than 10 patients seen among more than 1700 patients in consultation by the pediatric cardiologists had pericarditis during the period July 1, 2001, to June 30, 2002.[36] Over a 21-year period, 31 children with an inflammatory large pericardial effusion requiring drainage were admitted to one tertiary care children's hospital; 12 of the effusions were caused by bacterial infections.[70] Although rare, purulent pericarditis also can occur in neonates.[57] In most series, a marked male predominance has been noted.

Etiology

Primary purulent pericarditis is a rare disease; it accounted for only 7 of 50 cases of pericarditis reported by Gersony and McCracken.[37] The disease is associated most often with infection from another site, with hematogenous or direct spread to the pericardium. Feldman[31] reviewed all cases of bacterial pericarditis reported in the English language literature from 1950 to 1977. Bacteria were isolated in 146 (90%) of 162 cases. No other infection was found in 10 patients. The most common concomitant site involved was the lung, especially for *Staphylococcus aureus*, *Haemophilus influenzae*, and *Streptococcus pneumoniae*. When septic arthritis, osteomyelitis, or skin infections were found, *S. aureus* usually was the cause of pericarditis. *Neisseria meningitidis* and *H. influenzae* most often were responsible for concomitant meningitis and pericarditis.

Before the introduction of antibiotics, pneumococcal and streptococcal organisms were the most frequent causes of purulent pericarditis in children. Most cases

TABLE 27-1 Causes of Pericarditis

Idiopathic
Benign
Recurrent

Infectious
Purulent
Bacterial—*Staphylococcus aureus, Haemophilus influenzae,* streptococci, *Neisseria meningitidis, Streptococcus pneumoniae,* anaerobes, *Francisella tularensis, Salmonella,* enteric bacilli, *Pseudomonas, Listeria, Neisseria gonorrhoeae, Actinomyces, Nocardia*
Tuberculosis
Fungal—*Histoplasma, Coccidioides, Aspergillus, Candida, Blastomyces, Cryptococcus*

Viral
Coxsackieviruses B
Other—influenza A and B, mumps, echoviruses, adenoviruses, Epstein-Barr virus, hepatitis, measles, human immunodeficiency virus, cytomegalovirus

Other
Rickettsial—typhus, Q fever
Mycoplasmal—*Mycoplasma pneumoniae*
Parasitic—*Entamoeba histolytica, Echinococcus*
Spirochetal—syphilis, leptospirosis
Chlamydial—psittacosis
Protozoal—toxoplasmosis

Noninfectious
Postpericardiotomy syndrome
Kawasaki disease
Rheumatic fever
Connective tissue disorders—juvenile rheumatoid arthritis, systemic lupus erythematosus, dermatomyositis, periarteritis nodosa
Trauma—blunt or penetrating
Metabolic—uremia, myxedema
Hypersensitivity—serum sickness, pulmonary infiltrates with eosinophilia, Stevens-Johnson syndrome, drugs (hydralazine, procainamide, chemotherapy)
Neoplasm—leukemia, metastatic
Post irradiation

TABLE 27-2 Pericarditis in Children, 1962 to 1974 (St. Louis Children's Hospital)*

Etiology	No. Patients
Unknown	28
Purulent	12
Juvenile rheumatoid arthritis	9
Acute rheumatic fever	8
Uremia	5
Viral	2
Blunt chest trauma	2
Dermatomyositis	1

*Patients with postpericardiotomy pericarditis and patients with small exclusions at autopsy were excluded from consideration.
From Strauss AW, Santa-Maria M, Goldring D. Constrictive pericarditis in children. *Am J Dis Child* 1975;**129**:822–6.

were associated with pulmonary infections. Nearly half of patients with streptococcal pericarditis had associated postinfluenzal pneumonia. Hemolytic streptococci were isolated most often; 10 percent were nonhemolytic streptococci, and 5 percent were viridans streptococci. Kauffman and colleagues[59] reviewed 113 cases of pneumococcal pericarditis reported since 1900. Preceding pneumonia was present in 93 percent, and empyema was present in 66 percent. Pericarditis was thought to be a late event resulting from delay in administering appropriate therapy for pneumonia.

S. aureus is the organism most commonly responsible for purulent pericarditis in children.[5,31,37,49,82] Most cases are the result of hematogenous seeding of the pericardium from staphylococcal pneumonia with empyema, acute osteomyelitis, or soft tissue abscesses. Among 117 children with *S. aureus* pneumonia at Texas Children's Hospital, 13 children had an echocardiogram; 1 had a large pericardial effusion.[14] Occasionally, the pericardium is infected during the course of staphylococcal endocarditis. *S. aureus* is the most frequently recovered organism when purulent pericarditis develops within 3 months after the patient has undergone open heart surgery. The clinical course of acute staphylococcal pericarditis is dominated by severe toxemia. In addition to the necrotizing infection produced by *S. aureus*, the organism may release exotoxins, which produce shock and contribute to the high mortality. Community-associated methicillin-resistant *S. aureus* isolates have been recovered from some patients with acute pericarditis.[52]

S. aureus was isolated from 73 percent of infants who died of purulent pericarditis in the series reported by Gersony and McCracken.[37] It was responsible for 50 percent of cases in children 1 to 4 years old in the review by Feldman.[31] In 7 patients younger than 1 month of age, *S. aureus* was isolated from 4. This finding is corroborated in literature from other countries.[21,54,82] Thebaud and colleagues[97] reported 19 patients with purulent pericarditis in a children's hospital in Paris between 1979 and 1994. The mean age of the children was 3 years (range, 3 months to 10 years). The organisms isolated were *S. aureus* (three cases), *H. influenzae* (four cases), group A streptococci (three cases), *S. pneumoniae* (three cases), and *N. meningitidis* (one case). Concomitant infections included pneumonia (six cases), osteomyelitis (three cases), cellulitis (one case), and sinusitis (one case). In the series from Turkey, *S. aureus* was isolated from 5 patients and *S. pneumoniae* was isolated from 1 patient.[10] *S. aureus* pericarditis as a complication of varicella has been reported in several children.[8] *S. aureus* has also caused pericarditis associated with disseminated infection in a child with IRAK-4 deficiency.[19]

In the prevaccine era, the second most frequently encountered organism was *H. influenzae* type b.[7] It was responsible for 22 percent (35 of 163) of the cases in Feldman's review.[31] A single site of coexisting infection, the lung, was identified in 16 of the 35 cases. Meningitis as a single other site of infection was found in 5 of 35 patients, and multiple involvement was found in 7 of 35.[31] Echeverria and colleagues[26] summarized 33 cases from the literature. Pulmonary infiltrates and empyema were seen in 64 percent of patients. Because *H. influenzae* type

b conjugate vaccine has been given routinely to young infants in developed countries, this organism has been eliminated as a cause of invasive infections, including pericarditis.

Pneumococcal, streptococcal, and meningococcal pericarditis have diminished in frequency since the introduction of penicillin.[6] Go and coworkers[38] summarized the 15 reported cases of pneumococcal pericarditis from 1980 to 1998. One was a child. Only 4 cases did not have an underlying risk factor. In a surveillance study of invasive pneumococcal infections in eight pediatric hospitals, only 3 cases of pericarditis have been observed in more than 2500 cases of systemic pneumococcal infection during the 6-year period of 1993 through 1999.[58] Nevertheless, *S. pneumoniae* remains an important, although rare, cause of acute bacterial pericarditis.[30] The routine administration of the pneumococcal conjugate vaccine to young children has likely resulted in *S. pneumoniae* being an even less common cause of acute pericarditis.

Pericardial involvement occurs in approximately 5 percent of young adults with meningococcemia.[23,44] The clinical course generally is milder than that observed with other types of purulent pericarditis. Pericardial involvement rarely is detected at the time of hospital admission. Pericarditis became apparent by the third day in 13 of 17 patients reported by Dixon and Sanford.[23] In some patients, it did not occur until late in the course of therapy. Whether this late-onset pericardial effusion is a part of the meningococcal infection or is related to immune complexes is unclear.[23,76,87] Primary meningococcal pericarditis that occurs without clinical evidence of meningococcemia, meningitis, or any other focal infection has been reported in 16 patients, including 6 children 18 years old or younger (range, 2 to 18 years).[3] Meningococcal serotype C was identified in 11 (79%) of 14 cases for which the serotype was known. Cardiac tamponade developed in 88 percent of the patients. Pericarditis also has been reported in 2 children with W135 meningococcal infection.[27]

Occasionally, other microorganisms cause acute purulent pericarditis. Feldman[31] reported that 11 (8%) of 146 cases of pericarditis in children were caused by *Pseudomonas aeruginosa*. *P. aeruginosa* caused pericarditis in an immunocompetent adult with cystic fibrosis.[2] Pericarditis can occur with pneumonic tularemia, salmonellosis, sepsis from enteric bacilli, listeriosis, and disseminated gonococcal disease.[6] Anaerobic bacteria should be suspected when pericarditis develops in association with lung abscess, intra-abdominal infection including ruptured appendicitis,[95] or a penetrating wound. Callanan and colleagues[12] reported the rapid development of constrictive pericarditis after purulent pericarditis caused by anaerobic streptococcal infection. The child had a history of blunt trauma to the chest with no evidence of a penetrating wound 3 weeks before cardiac tamponade developed. The incidence of anaerobic infection may be underestimated because of improper handling of specimens for culture.[31] Prolonged symptoms related to pericarditis can be associated with *Mycoplasma pneumoniae* infection.[28]

Mycobacterium tuberculosis, previously a common cause of acute pericarditis in the United States,[5] now is responsible more often for chronic pericardial disease. This infection is a complication of miliary tuberculosis and rarely a primary infection. In the series of 2500 children with tuberculosis reported by Lincoln and Savell,[66] pericarditis was diagnosed in 0.4 percent and found at necropsy in 5 percent of patients. A review of 100 cases of tuberculous pericarditis in South African blacks by Desai[22] revealed a marked male predominance (72%). The duration of symptoms, consisting of cough and peripheral edema, in most patients was 0 to 120 days. Most patients were febrile and had congestive heart failure. Generalized lymphadenopathy occurred in nearly 30 percent of patients, pulsus paradoxus occurred in 50 percent, and a friction rub was audible in 25 percent. Of the 52 patients who had pericardiocentesis, 40 percent yielded fluid, but none was positive for acid-fast bacilli. Pericardial effusion was shown in 82 patients, 16 of whom died of tamponade and another 16 of whom developed constricting pericarditis.

The four stages of tuberculous pericarditis have been described as dry, effusive, absorptive, and constrictive.[78] Granulomas usually are found in the dry stage and heal with no sequelae. The effusive stage occurs commonly with tuberculous lymphadenitis, and 15 to 200 mL of fluid usually accumulates in the pericardial space. The absorptive stage is characterized by thickening of the pericardium with fibrin deposition. Further fibrin deposition and calcification occur during the constrictive phase. The disease may progress through all stages or remain in one stage.

Latent infection in the mediastinal lymph nodes with spread directly into the pericardium is thought to be the mode of involvement with *M. tuberculosis*.[78] The lymph nodes at the tracheal bifurcation often are the source.

Histoplasma pericarditis generally occurs with pulmonary, rather than disseminated, disease.[80] Coccidioidomycosis[16] and, rarely, blastomycosis[45] also may cause pericardial disease. Other pathogenic fungi include *Aspergillus* and *Candida*. These fungi are more serious considerations in patients who are immunosuppressed, have serious burns, or are receiving long-term, broad-spectrum antibiotics after undergoing cardiac surgery.[84]

Finally, several parasites such as *Trypanosoma cruzi* and *Toxoplasma gondii* can attack the pericardium.[46]

Pathology and Pathogenesis

Pericarditis begins with fine deposits of fibrin adjacent to the great vessels; it causes the pericardial membrane to lose its smoothness and translucency. Numerous granulocytes may extend into the myocardium.[40]

Bacterial pericarditis most commonly results from direct extension of infection from involved lung and pleura. Pulmonary infections may spread to the pericardium through the bronchial circulation.[43] Pericarditis also can develop through hematogenous dissemination from infection elsewhere, and it also may be the result of an immunologically induced response to a primary infection.

As pericardial fluid accumulates, intrapericardial pressure increases. The rate of increase is a function of the speed of accumulation and the compliance of the

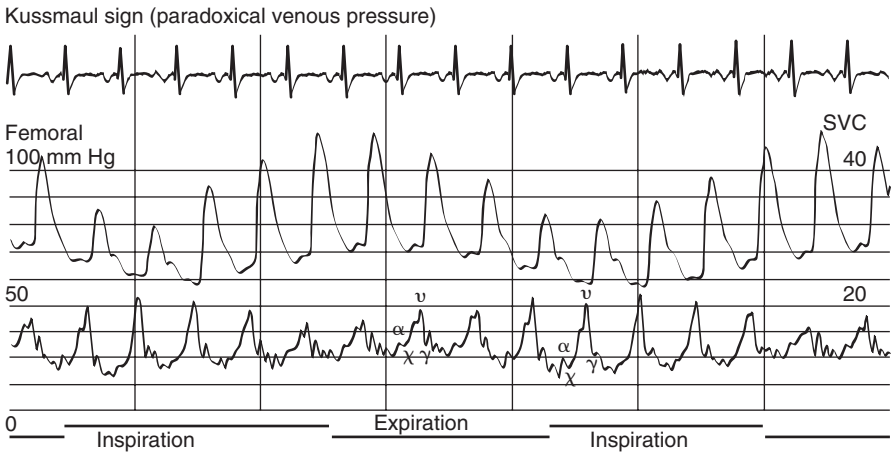

FIGURE 27-1 ■ Simultaneous recording of right atrial and femoral artery pressures. Notice the increased V wave and exaggerated decrease in the femoral artery pulse with inspiration.

pericardium. With slow accumulation of fluid, large volumes can be accommodated because of the gradual expansion of the parietal pericardium. As the compliance of the pericardium reaches its maximum, however, further accumulation of even small volumes of fluid results in an abrupt increase in intrapericardial pressure. If pericardial fluid accumulates at a rapid rate, marked elevation in intrapericardial pressure may occur with much smaller volumes of fluid. In a small child, 100 mL can cause severe tamponade, whereas 3 L may accumulate slowly in an older child and not result in tamponade.[1]

The most significant hemodynamic effect of pericardial effusion is restriction of ventricular filling. Ventricular end-diastolic, atrial, and venous pressures increase on the right and left sides of the heart equally. When restriction of ventricular filling becomes more pronounced, the ventricular stroke volume and cardiac output decrease. In an attempt to maintain cardiac output, tachycardia and peripheral vasoconstriction occur. Systemic arterial blood pressure and pulse pressure are reduced markedly. Tamponade occurs when these compensatory mechanisms fail to maintain adequate cardiac output.

Clinical Manifestations

A diagnosis of purulent pericarditis should be suspected in any patient with septicemia who develops cardiomegaly. The classic signs and symptoms of pericarditis are precordial pain, pericardial friction rub, evidence of cardiac fluid, and muffled heart sounds.[15] Chest pain is not a common symptom, especially in small children; the reported rates vary from 15 to 80 percent.* However, in one study focusing on 22 children (ages 6 to 17 years old) who presented to an emergency center and ultimately were found to have acute pericarditis, 95 percent had chest pain.[81] Acute abdominal symptoms may be the presenting complaints of some children.[24]

The most common symptoms and signs of pericarditis are fever, tachypnea, and tachycardia, which also are presenting features of associated systemic infection. If the cardiac shadow is radiographically enlarged, with or without a friction rub, and the tachypnea and tachycardia are out of proportion to the fever, myocardial dysfunction or pericarditis should be suspected.

An evanescent or ubiquitous rub may be detected. The typical sound of a rub is that of a high-frequency murmur,[79] which may have a to-and-fro or triphasic pattern but may not have any correlation with the cardiac cycle.[32] Frequently, the rub is heard better with the patient leaning forward or kneeling.[32] A rub may be differentiated from a murmur by pressing the diaphragm of the stethoscope firmly against the chest wall; this pressure amplifies the rub, and the typical scratchy quality becomes more apparent as the examiner opposes the visceral and parietal pericardium by compression of the chest. Rubs have been known to increase with inspiration.[92] Although a rub is less likely to be heard in the presence of a large effusion, it still may exist.[32] The heart sounds usually are muffled, and the palpable ventricular impulse generally is diminished. Both findings may be present in congestive heart failure, but they may be absent with tamponade.

Cardiac tamponade may be an early complication of pericarditis associated with a systemic infection. Cardiac tamponade means that there is compression of the heart by a tense pericardial sac, usually full of fluid, resulting in a decrease in venous return to the cardiac chambers and a decrease in cardiac output. During inspiration, the intrathoracic pressure decreases and venous return to the venae cavae increases. The tense pericardial sac limits the amount of blood that can enter the right atrium because of diastolic compression; a paradoxical increase in jugular venous pressure occurs during inspiration (i.e., Kussmaul sign) (Fig. 27-1).[61]

During inspiration, a small decrease in systolic blood pressure and cardiac output normally occurs and is caused by an increase in pulmonary venous capacitance. It is exaggerated with pericardial tamponade (>10 mm Hg decrease in blood pressure) because of the restricted inflow into the cardiac chambers. This clinical sign has been called *paradoxical pulse*, but it actually is an exaggeration of the normal respiratory cycle (Fig. 27-2).[39]

*References 4, 6, 37, 49, 73, 77, 98.

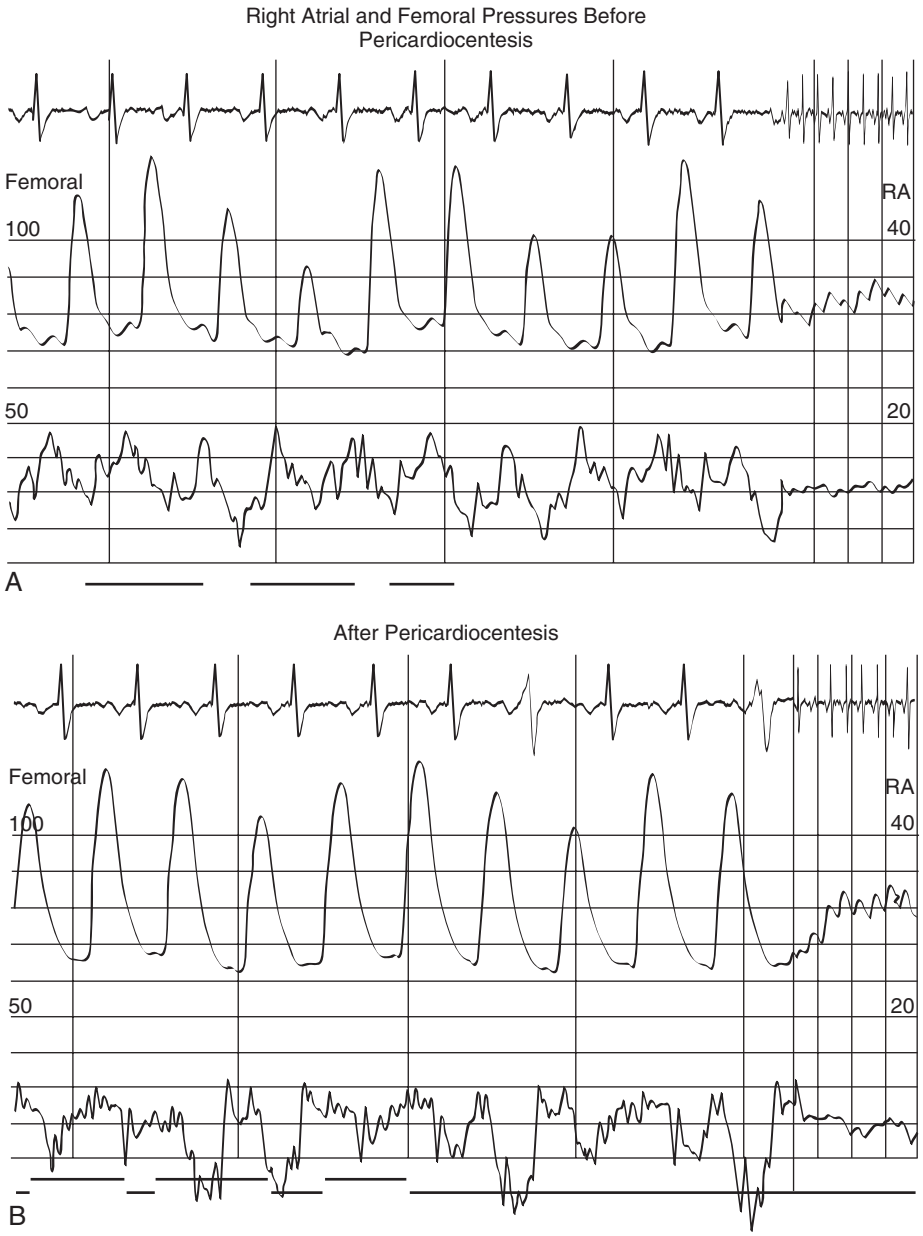

FIGURE 27-2 ■ Recordings of femoral artery and right atrial pressures before **(A)** and after **(B)** pericardiocentesis. **A,** There is an exaggerated decrease in the fall of femoral artery pressure with inspiration and a sustained increase in right atrial pressure. **B,** The recording shows a more normal variation of femoral pressure and a lower right atrial pressure.

Diagnosis

The radiographic appearance of a rapidly increasing cardiothoracic ratio without increasing pulmonary vascular markings is more suggestive of pericardial effusion than of congestive heart failure caused by myocardial dysfunction (Fig. 27-3). Fluoroscopy alone generally is of little value; myocardial dysfunction and pericarditis can impair cardiac contractility.

The size of the pericardial shadow does not indicate the severity of hemodynamic effects. It is a function of the rapidity of accumulation and the volume of pericardial fluid. When acute infection results in sudden cardiac tamponade, the heart size may be normal. A large, globular heart shadow with no evidence of increased pulmonary vasculature, particularly in a patient who has signs of right-sided heart failure, is strong evidence for pericardial disease. The lack of pulmonary overcirculation helps to distinguish this condition from myocarditis; however, determining whether pulmonary infiltrates also exist may be difficult.

A plain lateral chest radiograph may show findings consistent with a pericardial effusion.[63] Separation of more than 2 mm between the anterior mediastinal and subepithelial "fat stripes" suggests an effusion. Obliteration of the retrosternal space without evidence of thymic or right ventricular enlargement also indicates pericarditis.

The extent of electrocardiographic abnormalities may be explained by the amount of pericardial effusion and

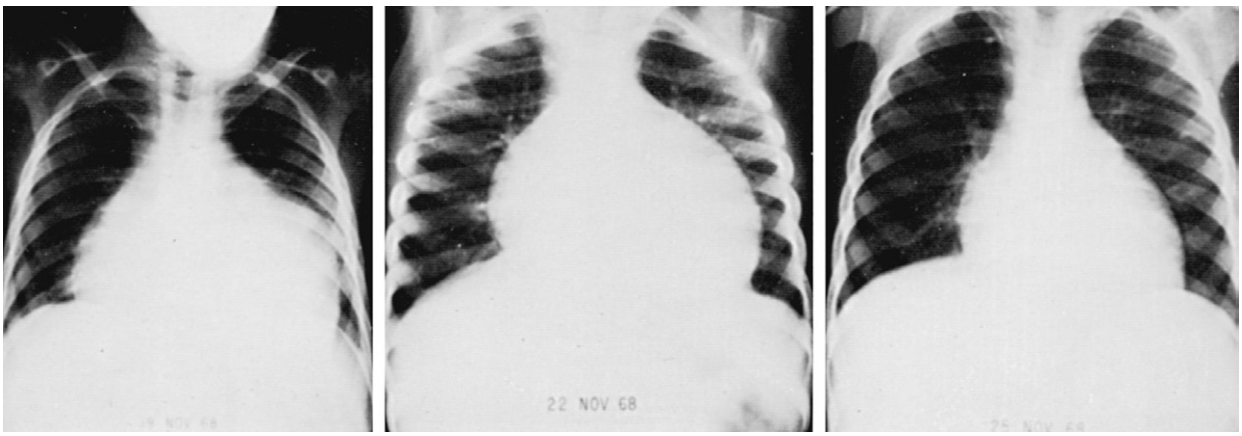

FIGURE 27-3 ■ In a patient with pericarditis, the first two radiographs show an enlarged cardiac shadow without an increase in pulmonary vascular markings. The third radiograph shows a marked decrease in apparent heart size after pericardiocentesis.

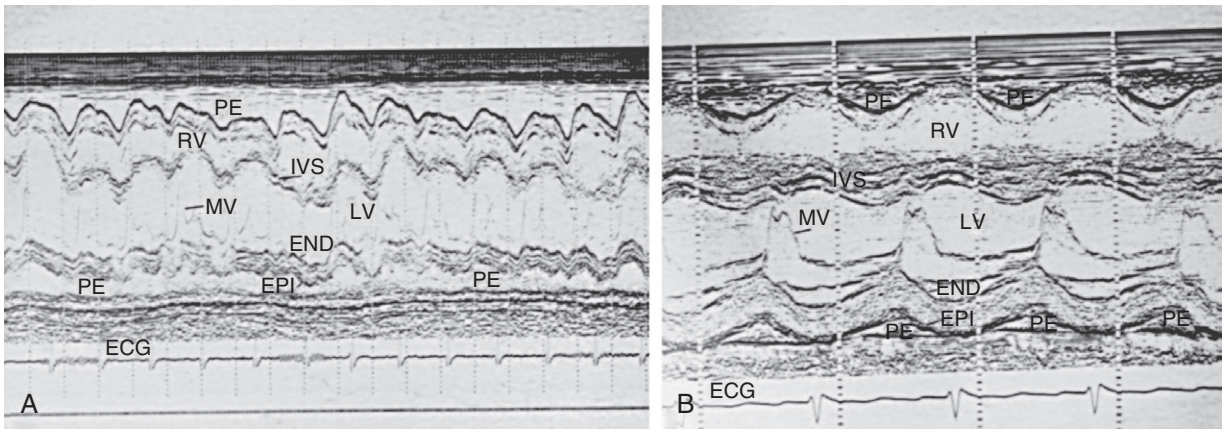

FIGURE 27-4 ■ Serial echocardiograms of a child before pericardiocentesis **(A)** and after pericardiocentesis **(B)**. **A,** Note the large effusion anteriorly and posteriorly with the "swinging" movement of the septum and anterior and posterior walls. **B,** The heart movement is normal, and there remains only a small effusion anteriorly and posteriorly. *ECG,* electrocardiogram; *END,* endocardium; *EPI,* epicardium; *IVS,* interventricular septum; *LV,* left ventricle; *MV,* mitral valve; *PE,* pericardial effusion; *RV,* right ventricle.

the presence of superficial myocardial injury or myocarditis. Pericardial effusion gives rise to low-voltage QRS complexes as a result of the damping effect of pericardial fluid between the chest wall and the myocardium. Accumulation of fluid and fibrin under pressure also may produce an injury pattern manifested by ST-segment deviation. More than 90 percent of patients have elevation of the ST segment, which occurs most frequently in leads I, II, V_5, and V_6. Widespread T-wave inversion indicative of epicarditis may be seen in the same leads in which ST-segment elevation occurs.

Spodick[90] described four stages of electrocardiographic changes in acute pericarditis. In stage I, ST-segment elevation is pronounced and the PR segment may be depressed. In stage II, the ST segment begins to return to the isoelectric line, the amplitude of the T wave diminishes, and the PR segment is depressed. By stage III, the ST segment has returned to the isoelectric line, and the T-wave inversion occurs. An incompletely inverted T wave (i.e., a diphasic wave or an upright T wave with a notched summit) sometimes is observed. In stage IV, these changes may resolve completely. T-wave abnormalities may persist for life, however, and do not indicate active disease.

Electrical alternans is seen in a large pericardial effusion. Electrical alternans refers to the alternation in electric amplitude of the T wave and the QRS complex with each cardiac cycle. It is thought to result from the rotational and pendular motion of the heart suspended in pericardial fluid.

Deviations from classic patterns occasionally occur, and single electrocardiographic changes are common findings. All 12 children reported by Okoroma and colleagues[77] had ST-segment elevation, whereas only 3 had concomitant low voltage.

Although many textbooks cite the frequent occurrence of dysrhythmias with pericarditis, these occurrences are unusual in the absence of coexisting heart disease. In one study, 20 of 49 patients with acute pericarditis had no underlying heart disease.[91] Seven patients had no dysrhythmias documented on 24-hour Holter monitoring, and 10 of 20 had infrequent single ectopic beats. Only 3 patients had supraventricular tachycardia.

M-mode echocardiography is the most sensitive method for diagnosing significant pericardial effusion (Fig. 27-4).[42,50] With a small to moderate effusion, only a "fluid space" is seen posteriorly (see Fig. 27-4B). With a larger effusion, fluid is seen anteriorly and posteriorly

and the septal motion becomes grossly abnormal. The heart may give the appearance of freely swinging (see Fig. 27-4A). Newer echocardiographic techniques, such as two-dimensional sector scanning, are not more useful than the conventional M-mode.

Friedland and colleagues[34] prospectively performed transthoracic two-dimensional echocardiography in 36 children with staphylococcal bacteremia; 89 percent had at least one suspected focus of infection, and 19 had community-acquired infections. Vegetations were detected in 4 children without clinical manifestations of endocarditis. Two other children, including one without suggestive clinical features, had significant pericarditis detected. The researchers concluded that echocardiography should be considered for children with bacteremia caused by *S. aureus*, even when an obvious noncardiac source of infection exists.

Computed tomography and magnetic resonance imaging of the chest are other modalities used to examine the pericardium.[7] They may help to differentiate a bacterial pericarditis from other conditions involving the pericardium. Occult or unsuspected pericarditis has been discerned with radionuclide techniques in immunocompromised patients and in trauma patients.[41,86]

A pericardial effusion may be diagnosed by noticing a discrepancy between the position of a catheter placed adjacent to the lateral wall of the right atrium and the right cardiac border. An injection of radiopaque contrast material into the right atrium may delineate these findings further. Pressure measurements at the time of cardiac catheterization reveal the elevated right atrial pressure and emphasize further the exaggeration of venous, systemic, and left ventricular pressures imposed by inspiration (see Fig. 27-2). Injection of carbon dioxide or air into the pericardium percutaneously may delineate further the pericardial effusion fluoroscopically and differentiate freely moving fluid from loculated areas (Fig. 27-5).

The diagnosis of purulent pericarditis is established definitively only by direct examination of pericardial fluid. Purulent fluid is characterized by a predominance of polymorphonuclear leukocytes; however, it also may occur early in the course of viral and tuberculous pericarditis. Proper handling of pericardial fluid is crucial to recovery and identification of the etiologic agent, as follows:

1. Fluid should be placed directly into broth capable of supporting aerobic and anaerobic microorganisms. The fluid should be plated directly onto agar media, such as blood agar, chocolate agar, or MacConkey agar.
2. Cultures also should be submitted for identification of *M. tuberculosis*, fungi, and viruses.
3. Several slides should be prepared for immediate examination by Gram stain and stain for acid-fast bacilli. Unstained slides should be stored in case of controversy or the need for special histochemical stains.
4. Antigen detection for *S. pneumoniae* or polymerase chain reaction for other microorganisms may be useful in selected cases, particularly when the patient has received prior antimicrobial therapy.[11,65]

The causative microorganism is isolated from blood cultures in many patients. When indicated, cerebrospinal fluid also should be cultured. Because purulent pericarditis often occurs after infections of the lung or pleural space, thoracentesis can reveal the etiologic agent in many cases. Documentation of empyema together with evidence of pericardial disease correlates highly with purulent pericarditis.

Acid-fast bacilli are seen on stained smears of pericardial fluid from 15 to 42 percent of patients with tuberculous pericarditis.[5] Examination of pericardial biopsy specimens by routine methods and with special stains such as the auramine O can increase the frequency of identification of *M. tuberculosis*.[75] A negative purified protein derivative skin test does not exclude the diagnosis of tuberculous pericarditis.

Grossly bloody pericardial fluid is observed frequently in patients with *Histoplasma* pericarditis, and an aspirate of the effusion reveals a predominance of mononuclear leukocytes. Growth of *H. capsulatum* from pericardial fluid rarely is successful. Showing the typical intracellular yeast forms on special stain of pericardial tissue also is helpful. Elevation of the yeast phase of the

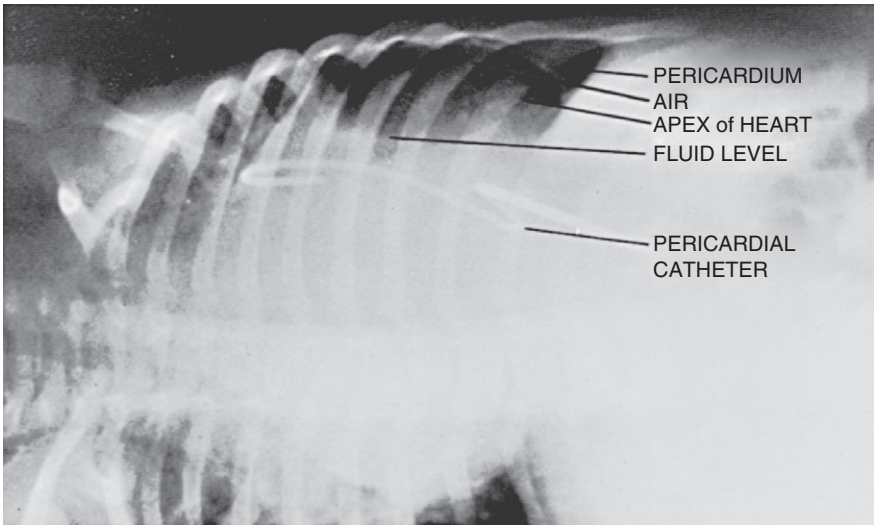

FIGURE 27-5 ■ Chest radiograph of a patient lying on the right side with a catheter in the pericardium. Air has been injected through the catheter, outlining the pericardium and fluid within the sac.

complement-fixation titer in pericardial fluid allows one to make a more rapid diagnosis.[80] Serum precipitin antibodies to *H. capsulatum* also indicate acute histoplasmosis and are helpful when present. Detecting the polysaccharide of *H. capsulatum* in urine or other body fluids is a rapid and sensitive means by which to establish the diagnosis of histoplasmosis.

Differential Diagnosis

Any patient with a rapidly increasing heart size in the absence of increasing pulmonary vascular markings should be suspected to have a pericardial effusion. Purulent pericarditis must be differentiated from pericardial effusion caused by collagen diseases, other infectious agents (e.g., viral, tuberculous, rickettsial, protozoan), neoplastic disorders, metabolic disorders, and congestive heart failure.[15] Glycogen storage disease, congenital heart disease, primary myocardial disease, cardiac tumors, and coronary artery aberrations (i.e., anomalous origin from the pulmonary artery, medial wall necrosis, and Kawasaki disease) may be confused with pericardial effusion.[15] Appropriate analysis of pericardial fluid usually permits differentiation of purulent pericarditis from pericarditis caused by other disorders. A high eosinophil count in the pericardial fluid suggests a parasite or a hypereosinophilic syndrome.[89]

Treatment

Purulent pericarditis is a potentially life-threatening illness that requires pericardial decompression and open drainage, appropriate antimicrobial therapy, and intense supportive therapy. Ainger[1] stated that more than half of children with purulent pericarditis require early or emergency drainage of the pericardium for relief of critical tamponade. Although bedside needle pericardiocentesis may be lifesaving or necessary for establishing a rapid diagnosis, Fowler and Manitsas[32] reported three deaths related to pericardiocentesis performed by inexperienced physicians. Complications include arrhythmias resulting from myocardial injury, laceration of the coronary arteries leading to hemopericardium and tamponade, and pneumothorax. Ledbetter[64] described a 10-year-old girl with staphylococcal pericarditis who developed an aortic aneurysm after undergoing multiple pericardiocentesis procedures for recurrent tamponade. The subxiphoid approach is recommended.[48]

Decompression and drainage of the pericardium are safest in a controlled environment, such as in an operating room or under fluoroscopy in the catheterization laboratory. If the patient is awake and agitated, premedication may be required.

If pericardiocentesis does not relieve symptoms successfully, and evidence of tamponade continues, immediate surgical drainage is necessary. Multiple attempts may prove unsuccessful and can lead to serious complications. The pus surrounding the heart may be too thick to be aspirated, as has been seen especially with *H. influenzae* infection.[71] Surgical creation of a pericardial window with a drain occasionally is necessary for complete removal of fluid, which accumulates rapidly. In preparation for evacuation of the pericardial fluid during tamponade, adequate cardiac output can be maintained by stimulating the heart with pharmacologic agents that cause a chronotropic and an inotropic effect.

TABLE 27-3 **Influence of Pericardial Drainage on Survival in Purulent Pericarditis in Children**

Treatment	Survived	Died
Antibiotics alone	5	28
Antibiotics and pericardial drainage	45	10

Data from references 4, 33, 59, and 74.

Medications that tend to decrease heart rate and intravascular volume are contraindicated because they compromise the patient further.[99] Wyler and colleagues warn against the use of halothane anesthesia because of its known depressant effect on myocardial function.[99]

Controversy exists regarding the approach and extent of surgery.* A left anterolateral thoracotomy through the fifth intercostal space or a subxiphoid approach with removal of the xiphoid process seems best. Some surgeons favor the creation of a pericardial "window"; however, others favor more extensive removal of pericardial tissue. This decision may be influenced by the severity of pericardial inflammation or the presence of bloody pericardial fluid because these conditions have greater potential for producing acute or chronic constriction. Care must be taken during the procedure not to injure the phrenic nerves.

Morgan and colleagues[71] reviewed 15 children with purulent pericarditis between 1971 and 1981. *H. influenzae* occurred in 7 of these children. Most patients had pericardiocentesis followed by an anterior interphrenic pericardiectomy and recovered completely. In a series of 9 children with *H. influenzae* pericarditis, all received a limited left thoracotomy with subxiphoid approach for pericardiostomy, and no deaths occurred.[17] Video-assisted thoracoscopic approaches to managing pericarditis have been described, but further experience is necessary for pediatric patients.[68] For selected patients for whom surgery cannot be performed in a timely manner, the instillation of intrapericardial streptokinase or urokinase or other thrombolytic agents has been successful in draining purulent pericarditis and preventing the need for a more extensive surgical procedure.[56]

Antimicrobial therapy alone is insufficient for the successful treatment of purulent pericarditis. The survival of patients with purulent pericarditis is improved significantly when early pericardial drainage is performed (Table 27-3). In the preantibiotic era, draining the pericardium decreased the mortality rate from nearly 100 percent to 45 percent.[6] Occasionally, patients with meningococcal pericarditis have been managed successfully without pericardial drainage.[20] Fyfe and colleagues[35] described 73 of 79 patients with *H. influenzae* pericarditis seen between 1928 and 1984. The mortality rate before 1960 was 64 percent (7 of 11 patients), although five of

*References 29, 32, 33, 62, 72, 85.

seven deaths were reported before the antibiotic era. From 1960 to 1969, the mortality rate was 36 percent, and from 1970 to 1979, it decreased to 11.5 percent. From 1980 to 1984, 25 cases with no mortality were reported.

When the etiologic agent cannot be detected rapidly, the initial antibiotic regimen should consist of two or more drugs. Because *S. aureus* is a major pathogen and community-associated methicillin-resistant *S. aureus* isolates are common in almost all areas, a penicillinase-resistant penicillin, such as nafcillin or oxacillin, in a dose of 200 mg/kg/24 hr (maximum 12 g) plus vancomycin in a dose of 60 mg/kg/day in four divided doses is usually recommended for initial empirical therapy. Vancomycin is also recommended when strains of *S. pneumoniae* resistant to the extended-spectrum cephalosporins are present or when the infection is nosocomially acquired. Cefotaxime 200 to 300 mg/kg/day in three or four divided doses or ceftriaxone 100 mg/kg/day in one or two doses should be administered to provide protection against *S. pneumoniae* (including penicillin-resistant strains), *N. meningitidis*, and *H. influenzae* type b (for children who may be inadequately immunized).

An aminoglycoside antibiotic should be added to the just-mentioned combined drug therapy when purulent pericarditis occurs after cardiac surgery, in association with genitourinary infections, or in the immunocompromised host. The antibiotics selected for empirical therapy for acute pericarditis complicating recent cardiac surgery are influenced by the hospital's antibiogram. For patients who are allergic to penicillin, vancomycin, clindamycin, or cefazolin is substituted for the treatment of susceptible *S. aureus*.

The duration of therapy is empirical and is determined partly by the nature of concomitant infection. Generally, after a pathogen is isolated and the antimicrobial susceptibilities are known, the most specific antimicrobial agent is continued intravenously for 3 to 4 weeks. Monitoring the C-reactive protein levels may help in determining the length of treatment.[53]

Using antimicrobial agents to treat tuberculous pericarditis has had a major impact on mortality. Before their use, the mortality rate in the acute phase was 80 to 90 percent. The other 10 to 20 percent of patients died of constrictive pericarditis or miliary tuberculosis.[78] The use of three or four drugs, including isoniazid, pyrazinamide, rifampin, and possibly streptomycin, for 9 to 12 months is recommended. The role of corticosteroids in preventing progression to constriction or decreasing mortality is unclear.[25,69,78] In selected cases, pericardiectomy may be indicated to prevent constrictive pericarditis. The reader is referred to Chapter 203 on histoplasmosis for details regarding treatment of pericarditis due to this organism.

As with bacterial pericarditis, open pericardiectomy is crucial for the successful treatment of *Candida* pericarditis.[74,84]

General supportive therapy in the acute stage of infection may include the administration of oxygen, volume expansion to increase ventricular filling pressure, and cardiovascular agents to facilitate systolic emptying. The input of pediatric cardiologists is critical for the optimal management of these patients. Serial electrocardiograms may indicate the presence of occult arrhythmias and alert the physician to the degree of myocardial involvement. The patient must be monitored carefully for signs of reaccumulation of pericardial fluid and for the development of acute constrictive pericarditis. Strauss and colleagues[93] reported this complication in 2 of 12 children with purulent pericarditis. Acute constriction may develop within weeks of the initial pericardial infection[4,9] and has been reported at 8 days.[83] Constrictive pericarditis may be suspected by increasing jugular and central venous pressure, weight gain, enlarging liver, worsening dyspnea, and decreased urinary output. The persistence of heart failure when the cardiac silhouette is becoming smaller also suggests the development of constrictive pericarditis. Complete pericardiectomy should be performed promptly when constriction is suspected.

Prognosis

Accurate mortality rates are difficult to compute from the literature because of the relative rarity of this infection as well as the nature and severity of any underlying disease. Factors that contribute to mortality are delay in recognition, absence of early surgical drainage, presence of cardiac tamponade, degree of myocardial involvement, etiologic agent (particularly *S. aureus*), and age of the patient. Long-term follow-up of children with purulent pericarditis is recommended. They should be observed carefully for the presence of a constrictive component as a sequela to the acute infection. Most children recover fully, however, and return to normal activity.

VIRAL PERICARDITIS

In 1951, Christian[18] suggested that viral infections were responsible for cases of idiopathic or benign pericarditis. A viral cause has not been substantiated in many patients, however.

Etiology

The principal viruses implicated in pericarditis are the coxsackieviruses.[74] Adenoviruses have been recovered less frequently.[55] Associations with varicella,[96] cytomegalovirus,[13] smallpox vaccinations, influenza,[47] (including H1N1),[60] influenza vaccinations,[94] and infectious mononucleosis[51,88] have been reported.

Clinical Manifestations

In 40 to 75 percent of cases, the patient has a history of upper respiratory tract infection for 10 days to 2 weeks preceding the onset of symptoms. Fever and chest and abdominal pain are the most common symptoms.[18] A friction rub may be heard in 50 to 80 percent of cases.[98] Children with viral pericarditis generally are less toxic and experience smaller elevations in body temperature than do children with purulent pericarditis. Some appear acutely ill, however. Large amounts of pericardial fluid accumulation and tamponade are rare findings.

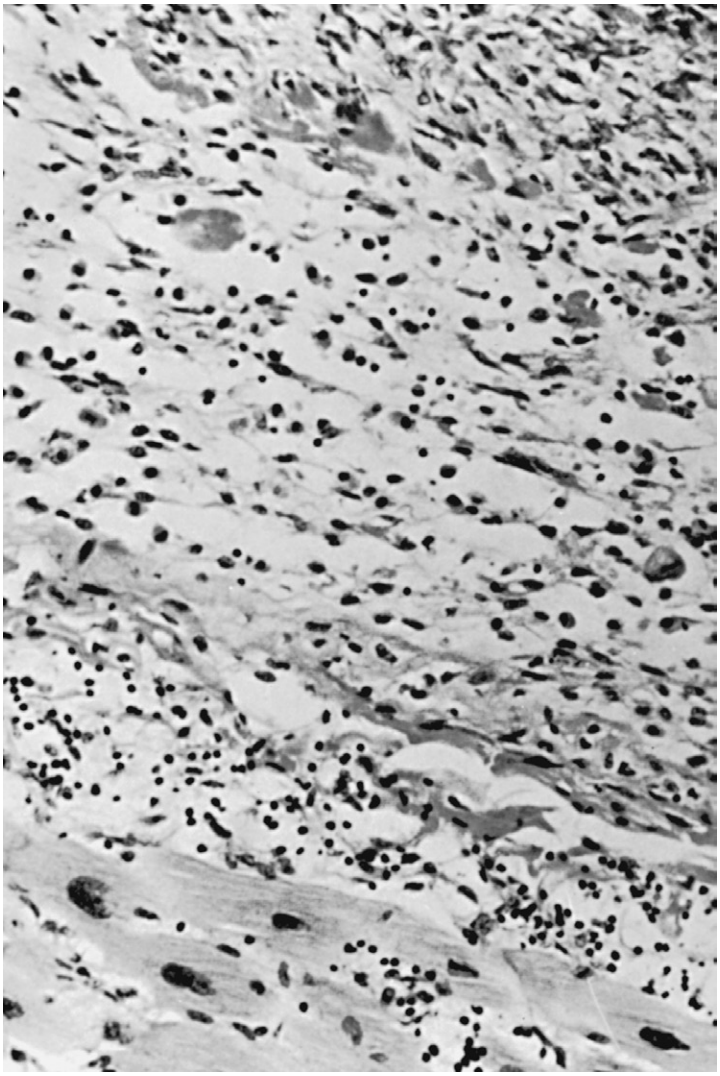

FIGURE 27-6 ■ In this case of viral pericarditis there is a layer of fibrin and fibroblasts along the pericardial surface. The mononuclear cell infiltrate in the epicardium extends into the outer myocardium (H&E, ×400). (Courtesy Edith P. Hawkins, MD, Texas Children's Hospital, Houston.)

Investigative Techniques

The electrocardiographic, radiographic, echocardiographic, and nuclear scanning findings described for patients with purulent pericarditis also are observed in patients with viral pericarditis. The peripheral leukocyte count may reveal fewer polymorphonuclear leukocytes than in patients with bacterial pericarditis. Mononuclear cell infiltrates in the pericardium with extension into the myocardium may be seen (Fig. 27-6).

If obtained, pericardial fluid should be sent for cell count and viral culture. Nasopharyngeal and rectal samples also should be obtained and cultured for viruses. Molecular methods may detect evidence of a specific virus. Acute and convalescent sera should be obtained so that appropriate titers can be measured if a virus is isolated.

Course and Prognosis

Viral pericarditis generally resolves spontaneously over the course of 3 to 4 weeks.[74] Large pericardial effusions and tamponade are rare occurrences.[15] Generally, bed rest for approximately 1 week and analgesics for pain are the only therapy that is required. Constrictive pericarditis is a rare occurrence, but pericarditis may recur.[74] In adults with recurrent pericarditis, colchicine appears to be of more benefit than corticosteroids in terms of treatment effect and decreased recurrences.[67]

NEW REFERENCES SINCE THE SIXTH EDITION

11. Calabrese F, Carturan E, Thiene G. Cardiac infections: focus on molecular diagnosis. *Cardiovasc Pathol* 2010;**19**:171–82.
14. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
19. Comeau JL, Lin TJ, Macken MB, et al. Staphylococcal pericarditis and liver and paratracheal abscesses as presentations in two new cases of interleukin-1 receptor associated kinase 4 deficiency. *Pediatr Infect Dis J* 2008;**27**:170–4.
30. Feinstein Y, Falup-Pecurariu O, Mitrica M, et al. Acute pericarditis caused by *Streptococcus pneumoniae* in young infants and children: three case reports and a literature review. *Int J Infect Dis* 2010; **14**:e175–8.

46. Hidron A, Vogenthaler N, Santos-Preciado JI, et al. Cardiac involvement with parasitic infections. *Clin Microbiol Rev* 2010; **23**:324–49.
52. Hussam MA, Ragai MF, Iman MF, Zakaria A. Community-acquired methicillin-resistant *Staphylococcus aureus* pericarditis presenting as cardiac tamponade. *South Med J* 2010;**103**:834–6.
53. Imazio M, Brucato A, Mayosi BM, et al. Medical therapy of pericardial diseases, part II: Noninfectious pericarditis, pericardial effusion and constrictive pericarditis. *J Cardiovasc Med (Hagerstown)* 2010;**11**:785–94.
60. Koranyi K, Yontz D, Rohrer Z, et al. Pericardial effusion complicating novel influenza A (H1N1) infection in an infant. *Pediatr Infect Dis J* 2010;**29**:782–3.
81. Ratnapalan S, Brown K, Benson L. Children presenting with acute pericarditis to the emergency department. *Pediatr Emerg Care* 2011;**27**:581–5.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 28

Myocarditis

Jesus G. Vallejo

Myocarditis is defined clinically and pathologically as inflammation of the myocardium. The clinical presentation and cause may be quite varied. This entity may go unrecognized in numerous patients whose illness may resolve spontaneously, or it may lead to significant morbidity and mortality.

In the early part of the 20th century, most cases of myocarditis were classified as idiopathic, and a diffuse or focal interstitial inflammation was identified on histologic examination. Rheumatic fever, diphtheria, and other bacterial infections were the only diseases recognized as associated with myocarditis, although some experts suspected that viruses might play a significant etiologic role in many cases.[151] After the discovery in 1947 of the coxsackievirus group and the subsequent isolation and identification of other viruses, the number of cases of myocarditis classified as idiopathic diminished rapidly.[34]

EPIDEMIOLOGY

The diverse clinical manifestations have made the true incidence of myocarditis difficult to determine. The clinical course of acute myocarditis can be insidious, with limited inflammation and cardiac dysfunction, or it can be overwhelming, leading to severe cardiac injury and cardiac failure. As a clinical entity, myocarditis is an uncommon occurrence in children. At Texas Children's Hospital, Houston, Texas, between 1954 and 1977, myocarditis represented 0.3 percent of the 14,322 patients seen by the Cardiology Service.

Because not all cases of myocarditis are recognized clinically, a much higher incidence is recorded in autopsy series. An autopsy incidence of 1.15 percent was found from 4343 studies performed between 1954 and 1977 at Texas Children's Hospital. This rate is considerably lower than the incidence of 6.83 percent reported by Saphir and Simon[150] in 1944 for 1420 autopsies performed on children. In Saphir's series, 32 of 97 cases had or probably had rheumatic carditis,[151] whereas only 2 cases occurred in the Texas Children's Hospital series. The discrepancy is even more pronounced when these observations are compared with those of Burch and colleagues,[25] who demonstrated evidence of interstitial myocarditis in the hearts of 29 of 50 infants and who showed evidence of interstitial myocarditis in the hearts of 29 of 50 infants and young children undergoing routine postmortem studies.

Some of the discrepancies between the clinical and autopsy series may be explained by the fact that the manifestations of myocarditis are subclinical in many cases and may be recognized only by changes on electrocardiogram (ECG) or perhaps not at all. In many instances, myocarditis is only one component of a generalized illness, and the cardiac dysfunction, if mild, may be overlooked.

ETIOLOGIES

Myocarditis may occur with many common infectious illnesses that affect infants and children (Table 28-1). In most cases of myocarditis, the etiologic agent is never identified, however. In the United States and Western

REFERENCES

1. Ainger LE. Diseases of the pericardium. In: Kelley VC, editor. Practice of pediatrics, vol. III. Hagerstown, Md: Harper Medical, Looseleaf Reference Services; 1969.
2. Altemeier WA, Tonelli MR, Aitken ML. Pseudomonal pericarditis complicating cystic fibrosis. *Pediatr Pulmonol* 1999;**27**:62–4.
3. Baevsky RH. Primary meningococcal pericarditis. *Clin Infect Dis* 1999;**29**:213–15.
4. Benzing GI, Kaplan S. Purulent pericarditis. *Am J Dis Child* 1963;**106**:287–94.
5. Boyd GL. Tuberculous pericarditis in children. *AMA Am J Dis Child* 1953;**86**:293–300.
6. Boyle JD, Pearce ML, Guze LB. Purulent pericarditis: review of literature and report of eleven cases. *Medicine (Baltimore)* 1961;**40**:119–44.
7. Breen JF. Imaging of the pericardium. *J Thorac Imaging* 2001;**16**:47–54.
8. Brumund MR, Truemper EJ, Lutin WA, Pearson-Shaver AL. Disseminated varicella and staphylococcal pericarditis after topical steroids. *J Pediatr* 1997;**131**:162–3.
9. Caird R, Conway N, McMillan IK. Purulent pericarditis followed by early constriction in young children. *Br Heart J* 1973;**35**: 201–3.
10. Cakir O, Gurkan F, Balci AE, et al. Purulent pericarditis in childhood: ten years of experience. *J Pediatr Surg* 2002;**37**:1404–8.
11. Calabrese F, Carturan E, Thiene G. Cardiac infections: focus on molecular diagnosis. *Cardiovasc Pathol* 2010;**19**:171–82.
12. Callanan DL, Morriss MJ, Kaplan SL, Park I. Constrictive pericarditis due to *Streptococcus sanguis*. *South Med J* 1981;**74**:377–8.
13. Campbell PT, Li JS, Wall TC, et al. Cytomegalovirus pericarditis: a case series and review of the literature. *Am J Med Sci* 1995;**309**: 229–34.
14. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
15. Cayler GG, Riley HD. Non-rheumatic inflammatory cardiovascular diseases. In: Moss AJ, Adams FH, editors. *Heart disease in infants, children and adolescents*. Baltimore: Williams & Wilkins; 1968. p. 851.
16. Chapman MG, Kaplan L. Cardiac involvement in coccidioidomycosis. *Am J Med* 1957;**23**:87–98.
17. Cheatham Jr JE, Grantham RN, Peyton MD, et al. *Hemophilus influenzae* purulent pericarditis in children: diagnostic and therapeutic considerations. *J Thorac Cardiovasc Surg* 1980;**79**:933–6.
18. Christian HA. Nearly ten decades of interest in idiopathic pericarditis. *Am Heart J* 1951;**42**:654.
19. Comeau JL, Lin TJ, Macken MB, et al. Staphylococcal pericarditis and liver and paratracheal abscesses as presentations in two new cases of interleukin-1 receptor associated kinase 4 deficiency. *Pediatr Infect Dis J* 2008;**27**:170–4.
20. Connolly DC, Burchell HB. Pericarditis: a ten year survey. *Am J Cardiol* 1961;**7**:7–13.
21. Corachan M, Poore P, Hadley GP, Tanner A. Purulent pericarditis in Papua New Guinea: report of 12 cases and review of the literature in a tropical environment. *Trans R Soc Trop Med Hyg* 1983;**77**:341–3.
22. Desai HN. Tuberculous pericarditis: a review of 100 cases. *S Afr Med J* 1979;**55**:877–80.
23. Dixon LM, Sanford HS. Meningococcal pericarditis in the antibiotic era. *Mil Med* 1971;**136**:433–8.
24. Donnelly LF, Kimball TR, Barr LL. Purulent pericarditis presenting as acute abdomen in children: abdominal imaging findings. *Clin Radiol* 1999;**54**:691–3.
25. Dooley DP, Carpenter JL, Rademacher S. Adjunctive corticosteroid therapy for tuberculosis: a critical reappraisal of the literature. *Clin Infect Dis* 1997;**25**:872–87.
26. Echeverria P, Smith EW, Ingram D, et al. *Hemophilus influenzae* b pericarditis in children. *Pediatrics* 1975;**56**:808–18.
27. El Bashir H, Klaber R, El Baki A, Booy R. W135 meningococcal pericarditis: report of two cases and review of the literature. *Pediatr Infect Dis J* 2004;**23**:969–70.
28. Farraj RS, McCully RB, Oh JK, Smith RF. *Mycoplasma*-associated pericarditis. *Mayo Clin Proc* 1997;**72**:33–6.
29. Farrow Jr CD, Brom AG, Nauta J. The surgical treatment of pericarditis: a follow-up study. *Dis Chest* 1965;**48**:478–83.
30. Feinstein Y, Falup-Pecurariu O, Mitrica M, et al. Acute pericarditis caused by *Streptococcus pneumoniae* in young infants and children: three case reports and a literature review. *Int J Infect Dis* 2010; **14**:e175–8.
31. Feldman WE. Bacterial etiology and mortality of purulent pericarditis in pediatric patients: review of 162 cases. *Am J Dis Child* 1979;**133**:641–4.
32. Fowler NO, Manitsas GT. Infectious pericarditis. *Prog Cardiovasc Dis* 1973;**16**:323–36.
33. Fredriksen RT, Cohen LS, Mullins CB. Pericardial windows or pericardiocentesis for pericardial effusions. *Am Heart J* 1971; **82**:158–62.
34. Friedland IR, du Plessis J, Cilliers A. Cardiac complications in children with *Staphylococcus aureus* bacteremia. *J Pediatr* 1995; **127**:746–8.
35. Fyfe DA, Hagler DJ, Puga FJ, Driscoll DJ. Clinical and therapeutic aspects of *Haemophilus influenzae* pericarditis in pediatric patients. *Mayo Clin Proc* 1984;**59**:415–22.
36. Geggel RL. Conditions leading to pediatric cardiology consultation in a tertiary academic hospital. *Pediatrics* 2004;**114**:e409–17.
37. Gersony WM, McCracken Jr GH. Purulent pericarditis in infancy. *Pediatrics* 1967;**40**:224–32.
38. Go C, Asnis DS, Saltzman H. Pneumococcal pericarditis since 1980. *Clin Infect Dis* 1998;**27**:1338–40.
39. Golinko RJ, Kaplan N, Rudolph AM. The mechanism of pulsus paradoxus during acute pericardial tamponade. *J Clin Invest* 1963;**42**:229.
40. Gore I, Kline IK. Pericarditis and myocarditis: A. Pericarditis. In: Gould SE, editor. *Pathology of the heart and great vessels*. 3rd ed. Springfield, Ill: Charles C Thomas; 1968. p. 724.
41. Greenberg ML, Niebulski HI, Uretsky BF, et al. Occult purulent pericarditis detected by indium-111 leukocyte imaging. *Chest* 1984;**85**:701–3.
42. Gutgesell HP, Paquet M. *Atlas of pediatric echocardiography*. Hagerstown, Md: Harper & Row; 1978. p. 161.
43. Hahn RS, Holman E, Frerichs JB. The role of the bronchial artery circulation in the etiology of pulmonary and pericardial suppuration: an experimental study. *J Thorac Surg* 1954;**27**:121–9.
44. Herrick WW. Meningococcal pericarditis. *Med Clin North Am* 1918;**2**:411.
45. Herrmann GR, Marchand EJ, Greer GH, Hejtmancik MR. Pericarditis: clinical and laboratory data of 130 cases. *Am Heart J* 1952;**43**:641–52.
46. Hidron A, Vogenthaler N, Santos-Preciado JI, et al. Cardiac involvement with parasitic infections. *Clin Microbiol Rev* 2010; **23**:324–49.
47. Hildebrandt HM, Maassab HF, Willis 3rd PW. Influenza virus pericarditis: report of a case with isolation of Asian influenza virus from the pericardial fluid. *Am J Dis Child* 1962;**104**:579–82.
48. Hoffman JIE, Stanger P. Diseases of the pericardium. In: Rudolph A, editor. *Pediatrics*. New York: Appleton-Century-Crofts; 1977. p. 1474–7.
49. Horan JM. Acute staphylococcal pericarditis. *Pediatrics* 1957; **19**:36–43.
50. Horowitz MS, Schultz CS, Stinson EB, et al. Sensitivity and specificity of echocardiographic diagnosis of pericardial effusion. *Circulation* 1974;**50**:239–47.
51. Hudgins JM. Infectious mononucleosis complicated by myocarditis and pericarditis. *JAMA* 1976;**235**:262.
52. Hussam MA, Ragai MF, Iman MF, Zakaria A. Community-acquired methicillin-resistant *Staphylococcus aureus* pericarditis presenting as cardiac tamponade. *South Med J* 2010;**103**:834–6.
53. Imazio M, Brucato A, Mayosi BM, et al. Medical therapy of pericardial diseases: part II: Noninfectious pericarditis, pericardial effusion and constrictive pericarditis. *J Cardiovasc Med (Hagerstown)* 2010;**11**:785–94.
54. Jaiyesimi F, Abioye AA, Antia AU. Infective pericarditis in Nigerian children. *Arch Dis Child* 1979;**54**:384–90.
55. Johnson RT, Portnoy B, Rogers NG, Buescher EL. Acute benign pericarditis: virologic study of 34 patients. *Arch Intern Med* 1961;**108**:823–32.
56. Juneja R, Kothari SS, Saxena A, et al. Intrapericardial streptokinase in purulent pericarditis. *Arch Dis Child* 1999;**80**:275–7.

57. Kanarek KS, de Brigard T, Coleman J, Silbiger ML. Purulent pericarditis in a neonate. *Pediatr Infect Dis J* 1991;**10**:549–50.
58. Kaplan SL, Mason Jr EO, Wald E, et al. Six year multicenter surveillance of invasive pneumococcal infections in children. *Pediatr Infect Dis J* 2002;**21**:141–7.
59. Kauffman CA, Watanakunakorn C, Phair JP. Purulent pneumococcal pericarditis: a continuing problem in the antibiotic era. *Am J Med* 1973;**54**:743–50.
60. Koranyi K, Yontz D, Rohrer Z, et al. Pericardial effusion complicating novel influenza A (H1N1) infection in an infant. *Pediatr Infect Dis J* 2010;**29**:782–3.
61. Kussmaul A. Ueber schwieglige mediastino-perikarditis und der paradoxen puls. *Klin Wochenschr* 1873;**10**:443.
62. Lajos TZ, Black HE, Cooper RG, Wanka J. Pericardial decompression. *Ann Thorac Surg* 1975;**19**:47–53.
63. Lane, Jr EJ Carsky EW. Epicardial fat: lateral plain film analysis in normals and in pericardial effusion. *Radiology* 1968;**91**:1–5.
64. Ledbetter MK. Aortic aneurysm complicating staphylococcal pericarditis. *J Okla State Med Assoc* 1981;**74**:222–5.
65. Levy PY, Fournier PE, Charrel R, et al. Molecular analysis of pericardial fluid: a 7-year experience. *Eur Heart J* 2006;**27**:1942–6.
66. Lincoln EM, Savell EM. *Tuberculosis in children*. New York: McGraw-Hill; 1963.
67. Lotrionte M, Biondi-Zoccai G, Imazio M, et al. International collaborative systematic review of controlled clinical trials on pharmacologic treatments for acute pericarditis and its recurrences. *Am Heart J* 2010;**160**:662–70.
68. Mack M, Acuff T, Hazelrigg S, Landreneau R. Thoracoscopic approach for the pericardium. *Endosc Surg Allied Technol* 1993;**1**: 271–4.
69. Mayosi BM, Burgess LJ, Doubell AF. Tuberculous pericarditis. *Circulation* 2005;**112**:3608–16.
70. Mok GC, Menahem S. Large pericardial effusions of inflammatory origin in childhood. *Cardiol Young* 2003;**13**:131–6.
71. Morgan RJ, Stephenson LW, Woolf PK, et al. Surgical treatment of purulent pericarditis in children. *J Thorac Cardiovasc Surg* 1983;**85**:527–31.
72. Mullen DC, Dillon ML, Young Jr WG, Sealy WC. Pericardiectomy in nontuberculous pericarditis. *J Thorac Cardiovasc Surg* 1969;**58**:517–29.
73. Nadas AS, Levy JM. Pericarditis in children. *Am J Cardiol* 1961;**7**:109–17.
74. Neill CA, Harouturuan LM. Diseases of the pericardium. In: Watson H, editor. *Paediatric cardiology*. London: Lloyd-Luke; 1968. p. 703.
75. Nelson CT, Taber LH. Diagnosis of tuberculous pericarditis with a fluorochrome stain. *Pediatr Infect Dis J* 1995;**14**:1004–7.
76. O'Connell B. Pericarditis following meningococcic meningitis. *Am J Dis Child* 1973;**126**:265–7.
77. Okoroma EO, Perry LW, Scott 3rd LP. Acute bacterial pericarditis in children: report of 25 cases. *Am Heart J* 1975;**90**:709–13.
78. Ortbals DW, Avioli LV. Tuberculous pericarditis. *Arch Intern Med* 1979;**139**:231–4.
79. Phillips Jr JH, Burch GE. Selected clues in cardiac auscultation. *Am Heart J* 1962;**63**:1–8.
80. Picardi JL, Kauffman CA, Schwarz J, et al. Pericarditis caused by *Histoplasma capsulatum*. *Am J Cardiol* 1976;**37**:82–8.
81. Ratnapalan S, Brown K, Benson L. Children presenting with acute pericarditis to the emergency department. *Pediatr Emerg Care* 2011;**27**:581–5.
82. Roodpeyma S, Sadeghian N. Acute pericarditis in childhood: a 10-year experience. *Pediatr Cardiol* 2000;**21**:363–7.
83. Rubenstein JJ, Goldblatt A, Daggett WM. Acute constriction complicating purulent pericarditis in infancy. *Am J Dis Child* 1972;**124**:591–4.
84. Schrank Jr JH, Dooley DP. Purulent pericarditis caused by *Candida* species: case report and review. *Clin Infect Dis* 1995;**21**:182–7.
85. Sethi GK, Nelson RM, Jenson CB. Surgical management of acute septic pericarditis. *Chest* 1973;**63**:732–5.
86. Shreiner DP, Krishnaswami V, Murphy JH. Unsuspected purulent pericarditis detected by gallium-67 scanning: a case report. *Clin Nucl Med* 1981;**6**:411–2.
87. Simon HB, Tarr PI, Hutter Jr AM, Erdmann 3rd AJ. Primary meningococcal pericarditis: diagnosis by counter-current immunoelectrophoresis. *JAMA* 1976;**235**:278–80.
88. Smith Jr JN. Complications of infectious mononucleosis. *Ann Intern Med* 1956;**44**:861–73.
89. Spiegel R, Miron D, Fink D, et al. Eosinophilic pericarditis: a rare complication of idiopathic hypereosinophilic syndrome in a child. *Pediatr Cardiol* 2004;**25**:690–2.
90. Spodick DH. *Acute pericarditis*. New York: Grune & Stratton; 1959. p. 17.
91. Spodick DH. Frequency of arrhythmias in acute pericarditis determined by Holter monitoring. *Am J Cardiol* 1984;**53**:842–5.
92. Spodick DH. Pericardial rub: prospective, multiple observer investigation of pericardial friction in 100 patients. *Am J Cardiol* 1975;**35**:357–62.
93. Strauss AW, Santa-Maria M, Goldring D. Constrictive pericarditis in children. *Am J Dis Child* 1975;**129**:822–6.
94. Streifler JJ, Dux S, Garty M, Rosenfeld JB. Recurrent pericarditis: a rare complication of influenza vaccination. *BMJ (Clin Res Ed)* 1981;**283**:526–7.
95. Tan EC, Rieu PN, Nijveld A, et al. Pericarditis as complication of appendicitis. *Ann Thorac Surg* 2004;**78**:1086–8.
96. Tatter D, Gerard PW, Silverman AH, et al. Fatal varicella pancarditis in a child. *Am J Dis Child* 1964;**108**:88–93.
97. Thebaud B, Sidi D, Kachaner J. [Purulent pericarditis in children: a 15 year-experience]. *Arch Pediatr* 1996;**3**:1084–90.
98. Weir EK, Joffe HS. Purulent pericarditis in children: an analysis of 28 cases. *Thorax* 1977;**32**:438–43.
99. Wyler F, Knusli D, Rutishauser M, et al. Pericarditis purulenta in children. *Helv Paediatr Acta* 1977;**32**:135–40.

TABLE 28-1 Causes of Myocarditis

Viruses	Coxsackieviruses A and B Echoviruses Polioviruses Rubella Measles Adenoviruses Vaccinia Mumps Herpes simplex Epstein-Barr Cytomegalovirus Rhinoviruses Hepatitis viruses Arboviruses Influenza viruses Varicella
Rickettsia	*Rickettsia rickettsii* *Rickettsia tsutsugamushi*
Bacteria	*Meningococcus* *Klebsiella* *Leptospira* *Staphylococcus* *Treponema pallidum* *Haemophilus influenzae* Hemolytic streptococci *Mycobacterium tuberculosis* *Salmonella enterica* subsp. *enterica* serovar Typhi (typhoid)
Mycoplasma	*Mycoplasma pneumoniae* *Chlamydia psittaci*
Protozoa	*Trypanosoma cruzi* African trypanosomiasis *Toxoplasma* Amebiasis
Other parasites	*Toxocara canis* *Trichinella spiralis*
Fungi and yeasts	*Actinomyces* *Coccidioides* *Histoplasma* *Candida*
Toxin	Diphtheria Scorpion
Drugs	Sulfonamides Phenylbutazone Cyclophosphamide NeoMercazole
Hypersensitivity/ autoimmunity	Rheumatoid arthritis Rheumatic fever Ulcerative colitis Systemic lupus erythematosus
Other	Sarcoidosis Scleroderma Idiopathic Cornstarch

Europe, viruses are the most common causes of acute myocarditis. Myocarditis generally is a sporadic disease, but epidemics have been reported. Most epidemics have been caused by coxsackievirus group B and have affected infants in the newborn period.[42,47] Gear and Measroch[58] were the first to identify coxsackievirus B in association with myocarditis after an epidemic occurred in a nursery in a maternity home in southern Rhodesia.

The association between virus infection and the development of myocardial disease also was made by Grist and Bell,[66] who presented comprehensive serologic data correlating enterovirus infection with acute viral myocarditis. In the World Health Organization record during the 10-year period from 1975 through 1985, the coxsackieviruses B represented the most frequent inflammatory agents in cardiovascular disease (34.6/1000), followed by influenza B virus (17.4/1000), influenza A virus (11.7/1000), coxsackievirus A (9.1/1000), and cytomegalovirus (CMV) (8/1000).[26] Karjalainen and colleagues[85] prospectively examined 104 conscripts during the 1978 influenza A virus (H1N1) epidemic in Sweden. The incidence of myocarditis was 9 percent of the 67 verified cases of influenza virus infection. Randolph and colleagues[139] reported that of 838 children with pandemic H1N1 admitted to a pediatric intensive care unit, 1.4 percent were diagnosed with myocarditis. Although H1N1-related acute myocarditis was uncommon, it was found to be an independent risk factor for death.

The development of molecular techniques such as polymerase chain reaction (PCR) has improved the testing of endomyocardial biopsy specimens for potential viral pathogens. A study using PCR identified viral genome in 38 percent of endomyocardial biopsy specimens from patients diagnosed with acute myocarditis.[23] Of the positive PCR samples, 23 percent were positive for adenovirus, 14 percent for enterovirus, and 3 percent for CMV. Parvovirus B19, influenza A virus, Epstein-Barr virus, herpes simplex virus (HSV), and respiratory syncytial virus were detected in less than 1 percent of cases.

Investigators also have speculated for decades on the possibility that acute myocarditis is a common forerunner of idiopathic dilated cardiomyopathy. Evidence supporting this hypothesis was presented first by Orinius and Pernow,[133] who found cardiac disease in humans years after an apparent uncomplicated coxsackievirus infection. Subsequently, Bowles and colleagues[22] using a slot-blot hybridization technique provided conclusive evidence for the presence of enterovirus in endomyocardial biopsy samples from patients with dilated cardiomyopathy. In another study, Bowles and associates[23] detected viral genomes in 20 percent of 149 patients with the diagnosis of dilated cardiomyopathy. In these patients, adenovirus was identified in 12 percent and enterovirus in 8 percent of cases. In all age groups, adenovirus and enterovirus were the viruses most commonly detected in acute myocarditis and dilated cardiomyopathy.

In December 2002, the U.S. Department of Defense began mandatory smallpox vaccination for select service members and employees without contraindications to vaccination, and in January 2003, the U.S. Department of Health and Human Services implemented a voluntary civilian smallpox vaccination program. As of June 15, 2003, the Department of Defense identified more than 50 cases of suspected, probable, or confirmed myopericarditis occurring within 30 days of vaccination in these individuals, based on clinical evaluation of symptoms, ECG, cardiac enzyme assays, echocardiography, and the exclusion of ischemic coronary artery disease. Myocarditis occurred in 7.8/100,000 primary vaccinees in the U.S. army, an incidence that was 3.6-fold more than that in unvaccinated individuals.[68]

PATHOLOGY

Isolated or idiopathic myocarditis is a rare pathologic entity. The pathologic cardiac findings usually are nonspecific; similar gross and microscopic changes occur regardless of the causative agent.[62,129,137,145] Grossly, all four chambers of the heart are enlarged, and the cardiac weight is increased. The heart usually is flabby and pale. In some instances, especially with coxsackievirus B infections, petechial hemorrhages may be seen on the epicardial surfaces; pericardial fluid may be tinged with blood. On cut section, the ventricular muscle walls may be thinned. Occasionally, the ventricles are hypertrophied or increased in thickness because of edema. The valves are spared. The endocardial surface usually is unaffected but occasionally may be thickened and appear glistening white. This important observation suggested to some investigators that endocardial fibroelastosis, which manifests as congestive cardiomyopathy, represented a progression from acute viral myocarditis.[71,80] In a study of 64 hearts of children who had myocarditis or endocardial fibroelastosis, Hutchins and Vie[80] found 18 with endocardial fibroelastosis only, 5 with myocarditis only, and 41 with features of both diseases. When time from onset of illness to death was 2 weeks or less, only myocarditis was evident. When the time interval was 2 weeks to 4 months, a combined picture was seen, whereas only endocardial fibroelastosis with trivial myocarditis was evident when the time from onset of disease to death was more than 4 months.

These findings were supported further by Hastreiter and Miller,[71] who found microscopic evidence of myocarditis after transthoracic needle biopsy of the myocardium in a child who had the classic clinical picture of endocardial fibroelastosis, including left ventricular hypertrophy on ECG. Fruhling and associates[53] extended these observations by showing coxsackievirus B3 in the myocardium of 13 of 28 infants with endocardial fibroelastosis. Ni and associates[128] analyzed 29 myocardial samples from patients with autopsy-proven endocardial fibroelastosis using specific PCR for enterovirus, adenovirus, mumps, CMV, parvovirus, influenza, and HSV. In 90 percent of samples, viral genome was amplified; more than 70 percent of the samples were positive for mumps viral RNA, and 28 percent amplified adenovirus. These data suggest that endocardial fibroelastosis also is a sequela of mumps virus infection.

The microscopic picture of acute myocarditis typically shows a focal or diffuse interstitial collection composed predominantly of mononuclear cells, lymphocytes, plasma cells, and eosinophils. Polymorphonuclear leukocytes rarely are seen, unless the cause of the carditis is bacterial. Virus particles and inclusion bodies rarely are recognized.[140,145] In severe infections caused by any agent, but especially coxsackieviruses and diphtheria, a loss of cross-striation in the muscle fibers, edema, and, sometimes, extensive necrosis of the myocardium occur. The diphtheria exotoxin has a particular affinity for conductive tissue; dysrhythmias, including complete heart block, are common findings in this form of myocarditis. The exotoxin interferes with protein synthesis by inhibiting a translocating enzyme in the delivery of amino acids.

Myocarditis seen with bacterial infections usually differs from that of presumed viral origin (Figs. 28-1 to 28-4). Microabscesses and patchy focal suppurative changes may be observed. Frequently, perimyocarditis may be seen with concomitant bacterial infection of the pericardium and myocardium.

Giant cells with or without granulomata are markers for the diagnosis of giant cell myocarditis.[79] Granulomata have been observed in the myocardium of patients with tuberculosis, syphilis, rheumatoid arthritis, rheumatic heart disease, sarcoidosis, and certain fungal and parasitic infections. Occasionally, giant cells have been seen in interstitial myocarditis (idiopathic or Fiedler). In many cases, giant cell myocarditis occurs, but no cause is found.

Two types of giant cells are recognized, one of which seems to be myogenic in origin and is thought to represent transitional forms of myocardial fibers. This type of cell has been found without granulomata. The second,

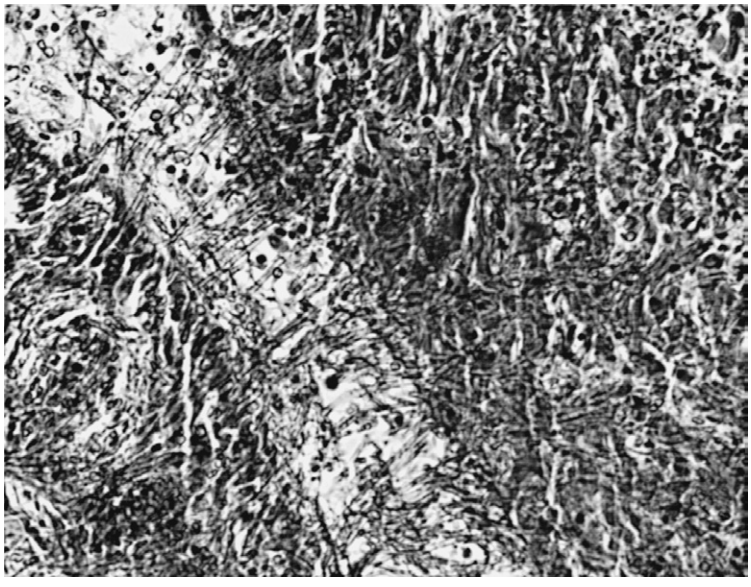

FIGURE 28-1 ■ *Candida albicans* myocarditis. Note the focal necrosis of the myocardium with central masses of hyphae and necrotic debris surrounded by mononuclear cell infiltrate. (Hematoxylin-eosin stain; ×160.) (Courtesy Edith Hawkins, MD. Houston, Tex.)

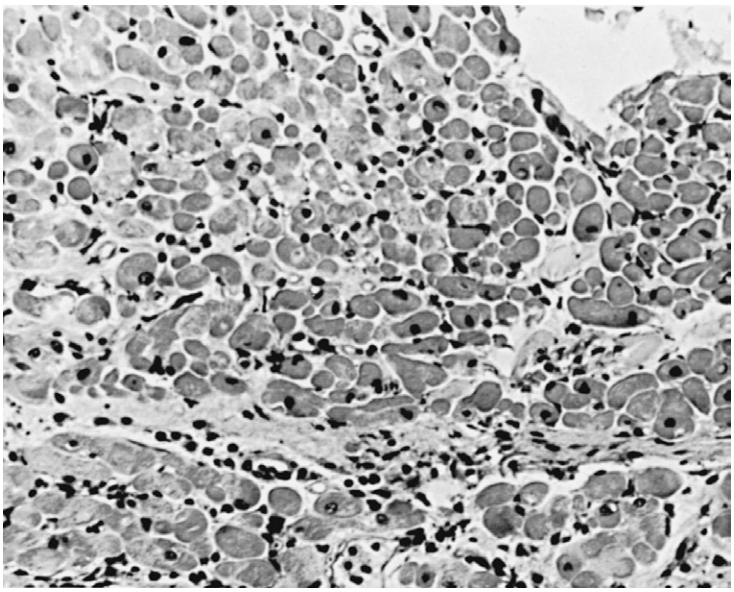

FIGURE 28-2 ■ Right ventricular biopsy specimen. The presumed viral myocarditis is characterized by focal mononuclear cell infiltrates. (Hematoxylin-eosin stain; ×160.) (Courtesy Edith Hawkins, MD. Houston, Tex.)

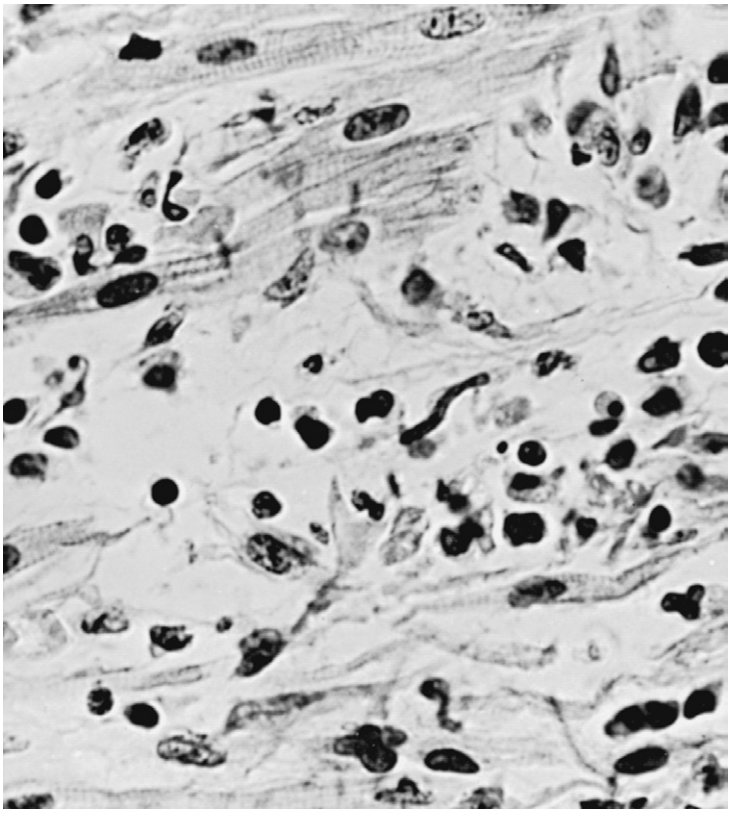

FIGURE 28-3 ■ Picornavirus myocarditis characterized by interstitial edema, mononuclear cell infiltrates, and focal myofiber disruption. (Hematoxylin-eosin stain; ×400). (Courtesy Edith Hawkins, MD. Houston, Tex.)

more characteristic giant cell probably is derived from interstitial histiocytes. The latter type typically is seen in patients with myocarditis of nonviral cause, whereas the former is a response to viral infection.

PATHOGENESIS

The pathogenesis of myocarditis is poorly understood, insofar as the disease progresses through different phases with distinctly different mechanisms and clinical manifestations. The pathophysiology of myocarditis in humans was derived largely from experimental models of coxsackievirus infection. More recently, Liu and Mason[104] suggested that myocarditis should be viewed as a continuum that comprises three separate phases: acute viral infection (phase I), autoimmunity (phase II), and dilated cardiomyopathy (phase III).

Phase I of the disease is triggered by entry and proliferation in the myocardium of the causative virus. Impairment of left ventricular function in mice with histopathologically graded moderate cellular infiltration

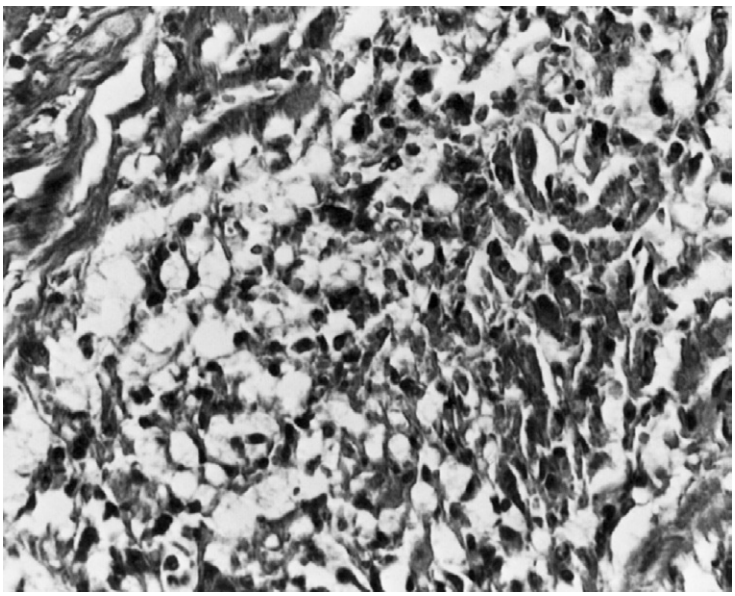

FIGURE 28-4 ■ Section of myocardium. Vaccinia (smallpox vaccine) myocarditis. Note mononuclear cell infiltrates and fatty degenerative changes. (Hematoxylin-eosin stain; ×400). (Courtesy Edith Hawkins, MD. Houston, Tex.)

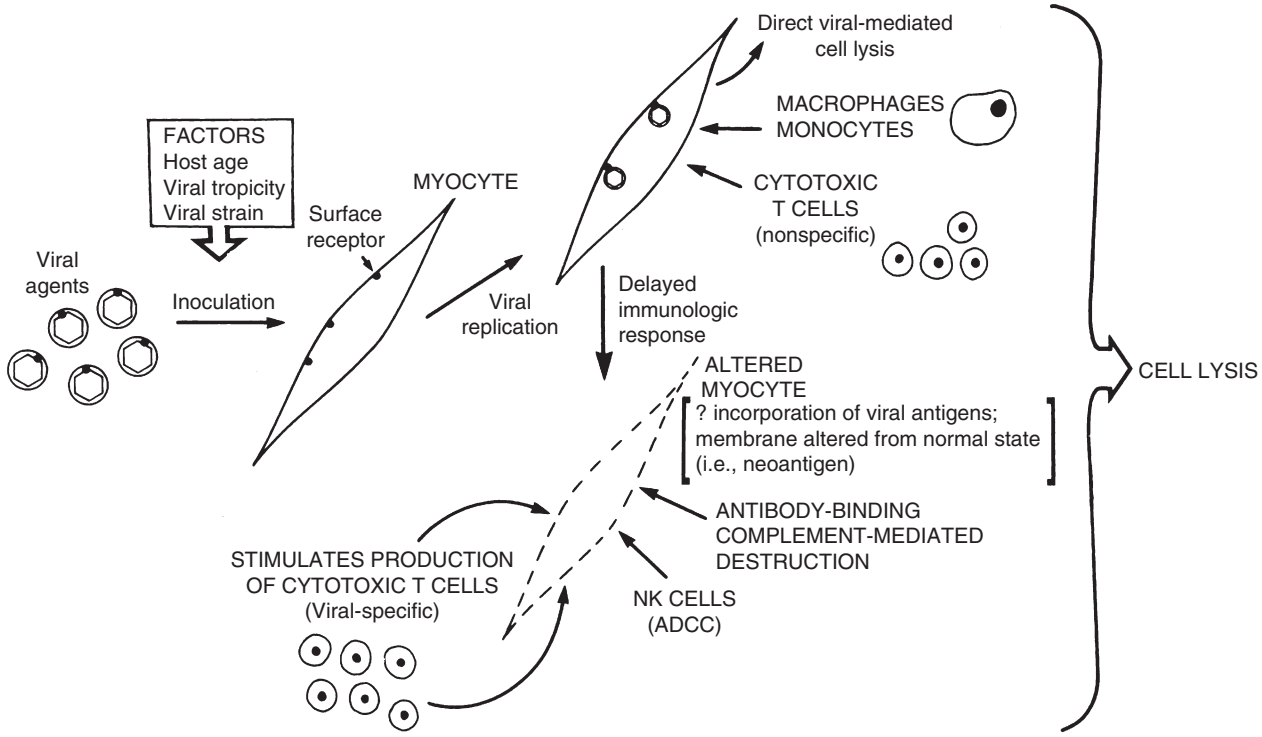

FIGURE 28-5 ■ Schema for pathogenesis of myocarditis. Viral agents attach to cells by means of surface receptors. After a cell is infected, the cell cycle is changed. Direct virus-mediated cytolysis occurs. Cellular effectors of injury (i.e., macrophages, monocytes, and nonspecific cytotoxic T cells) are involved in the primary reaction. Myocytes that survive are altered in their structure. Cytotoxic T cells specifically targeted against the altered myocyte, natural killer (NK) cells, and complement-activated, antibody-mediated cardiocytolysis or antibody-dependent cellular cytotoxicity (ADCC) participate in the secondary reaction. (Modified from Maisch B, Trostel-Soeder R, Stechemesser E, et al. Diagnostic relevance of humoral and cell-mediated immune reactions in patients with acute viral myocarditis. *Clin Exp Immunol* 1982;**48**:533.)

after coxsackievirus B3 infection supports the important pathophysiologic role of direct viral damage of the myocardium.[167] Phase I concludes with activation of the cellular immune response, which attenuates viral proliferation but also may enhance cardiac injury. Ideally, the immune response should downregulate to a resting state when viral proliferation is controlled. If immune activation continues unabated despite elimination of the virus, autoimmune disease may result, initiating phase II of the disease. The continuous activation of T cells long after viral clearance occurs is detrimental to the host because cytokine-mediated and direct T-cell–mediated myocyte injury leads to impairment of contractile function (Fig. 28-5). Long-term remodeling and progression

to dilated cardiomyopathy characterize phase III of the disease.[76]

The basic function of innate immunity is restriction of the early proliferation of infectious agents. Numerous effector cells and molecules work in concert to restrict this initial spread of an infectious focus. The responding cells include natural killer cells, natural killer/T cells, and γδ T cells. Several lines of evidence suggest that mediators of the innate immune system, such as tumor necrosis factor (TNF) and nitric oxide, play an important role in the pathogenesis of viral myocarditis.[114,189] Elevated levels of TNF have been reported in patients with viral myocarditis, and TNF mRNA and protein are consistently upregulated in the hearts of these patients.[116,152] In mice, the exogenous administration of TNF aggravates myocarditis and the neutralization of TNF by antibodies or soluble receptors attenuates the disease.[94,186] These data suggest that TNF production is detrimental in the setting of myocarditis.

More recent studies also have shown that TNF and nitric oxide are beneficial to the host by virtue of their antiviral effects. Mice with defective TNF or nitric oxide expression have increased myocardial injury, a significant increase in viral titers in the heart, and significantly higher mortality rates after infection with encephalomyocarditis virus (EMCV) or coxsackievirus B3.[175,189] These observations highlight the complexity of the innate immune response and suggest that innate immune mechanisms play a dual role in the setting of viral myocarditis. Although the prevailing notion has been that production of cytokine in the heart during viral infection is detrimental, the host–pathogen relationship is changed fundamentally when the host is unable to produce molecules such as TNF or nitric oxide.[175,189]

An important component of the innate immune system uses pattern recognition receptors, such as the Toll-like receptors (TLRs), to recognize pathogen-associated molecular patterns present in microbes.[4] TLRs induce the production of cytokines and interferons (IFNs) and modulate the adaptive immune response. Understanding of the role of TLRs in the pathogenesis of viral myocarditis is still evolving. However, recent studies suggest that cardiac inflammation during viral infection depends on TLRs. The viral genome replicates using the positive-strand RNA as its template, resulting in the formation of double-strand RNA (dsRNA) intermediates. Accordingly, both single-strand RNA (ssRNA) and dsRNA are present in virally infected cells. TLR3 and TLR7/8 signaling are activated by dsRNA and ssRNA, respectively, whereas TLR9 recognizes bacterial and viral CpG DNA motifs. Thus, viral infection can activate innate immune signaling in the heart through myeloid differentiation factor (MyD)88 dependent (TLR7/8/9) and MyD88 independent pathways (TLR3). Fairweather and colleagues[48] reported that mice with defective TLR4 signaling had decreased coxsackievirus B3 replication and less severe myocarditis 12 days after infection compared with wild-type mice. The presence of TLR4 also was associated with increased production of interleukin (IL)-1β and IL-18 and increased viral replication in the heart.

In a similar study, Fuse and associates[55] reported that mice deficient in MyD88, an adapter protein involved in TLR signaling (except TLR3), also had less myocarditis and attenuated viral replication in the heart after infection with coxsackievirus B3. Coxsackievirus B3-infected, MyD88-deficient mice had significantly higher levels of IFN-β but reduced expression of the coxsackievirus-adenovirus receptor in the heart. The enhanced IFN expression and lower expression of the coxsackie-adenovirus receptor in the heart could explain the attenuation of disease in the MyD88-deficient mice. Infection of TLR3-deficient mice with EMCV, a positive ssRNA virus, resulted in earlier mortality in TLR3-deficient mice that was associated with increased viral replication and myocardial injury in contrast to wild-type mice.[69] Similar observations have been reported for coxsackievirus group B serotype 3 in TLR3-deficient mice.[127] The final answer on the role of TLRs in the pathogenesis of viral myocarditis awaits further investigation, however.

Although the host genetic factors responsible for the changes observed in the three phases of myocarditis have yet to be defined completely, Woodruff and Woodruff,[183] using a murine model, were the first to show a role for T lymphocytes in the pathogenesis of viral myocarditis. In this study, depletion of T lymphocytes using antithymocyte serum or thymectomy and irradiation led to a decrease in mortality rates and in the inflammatory infiltrate after CVB3 infection. Huber and associates,[78] using BALB/c mice infected with coxsackievirus B3, showed that cytolytic T cells were the agents responsible for the major part of myocardial cell injury. In addition, proinflammatory mediators, such as TNF, released by infiltration cells also adversely affect cardiac function.

Opavsky and colleagues[132] defined the specific contributions of T-cell subsets (CD4 and CD8) and the T-cell receptor β chain to the pathogenesis of viral myocarditis. When $CD4^{-/-}$ or $CD8^{-/-}$ mice were exposed to CVB3, loss of $CD8^{+/+}$ immune cells did not affect survival significantly, but viral proliferation was attenuated. In contrast, $CD4^{-/-}$ mice showed a trend toward an improvement in survival and a small but significant decrease in the inflammatory infiltrate at 14 days after infection. Mice deficient in CD4/CD8 immune cells and T-cell receptor β had the best outcome in terms of decreased mortality. A marked decrease in inflammatory infiltrate was noted in CD4/CD8 double knockout mice. Although no significant change occurred in viral titers, a marked decrease in myocardial TNF mRNA 4 days after infection was seen in CD4/CD8 double knockout mice. These same investigators have shown that the T-cell receptor–associated tyrosine kinase $p56^{lck}$ is crucial for coxsackievirus B3 proliferation in the heart and activation of T cells to target the heart.[103] Mice deficient in $p56^{lck}$ were protected against the development of myocarditis, providing further support for the hypothesis that T-cell activation during viral myocarditis contributes to increased inflammation and myocyte destruction in the host.

T-regulatory (T-reg) cells are important in modulating the inflammatory response and preventing development of autoimmunity through production of cytokines such as IL-10 and tumor growth factor (TGF)-β.[181] T-regs express CD4, but they also express the α-subunit of the IL-2 receptor, CD25. They are high expressors of

the transcription factor FoxP3. Li and associates[100] have shown that the allograft of M2 (anti-inflammatory) macrophages led to improvement of virus-induced myocarditis, which was associated with enhanced levels of T-regs. Similarly, Huber and colleagues[77] reported decreased viral titers and inflammation after adoptive transfer of a $CD4^+$ $CD25^+$ regulatory-like T-cell population into a mouse model of coxsackievirus B3 infection. Thus, modulating the immune response may be critical for the prevention of chronic virus replication.

The ongoing injury that persists may be considered an autoimmune process.[78,182] At least in murine models, it is clear that both cellular and humoral autoimmunity are involved in the progression to chronic heart disease. The recently identified T helper 17 (Th17) subset has been implicated in the onset of chronic myocarditis.[70] These cells secrete high levels of IL-17 and have been implicated in the production of autoantibodies.[70,187] In a mouse model of coxsackievirus B3 myocarditis, the Th17 subset contributes to chronic myocarditis through persistent inflammatory signaling involving the secretion of IL-17. Consistent with this, considerable Th17 expansion occurs approximately 2 weeks after coxsackievirus B3 infection in mice.[188] IL-17 and its various isotypes can induce expression of TNF and Th2 responses, which leads to a prolonged inflammatory milieu that might foster the production of autoantibodies.

Autoantibodies that have been associated with myocarditis in patients and mice include those directed at cardiac myosin, adrenoreceptor adenine nucleotide translocator, and sarcolemmal and myolemmal proteins. The role played by these autoantibodies in the pathogenesis of myocarditis is unclear; antisarcolemmal antibodies that cross-react with enteroviral epitopes have been shown to damage cardiac myocytes.

More recent studies also have shed light on the mechanisms by which coxsackievirus B3 may contribute directly to the development of myocarditis and dilated cardiomyopathy. Badorff and colleagues[9-11] reported that the 2A protease encoded by coxsackievirus B3 cleaves dystrophin in cultured myocytes and in infected mouse hearts, leading to disruption of dystrophin and the dystrophin-associated glycoprotein α-sarcoglycan and β-sarcoglycan complex. Dystrophin provides a structural link between the muscle cytoskeleton and extracellular matrix to maintain muscle integrity. Xiong and associates[185] compared the effects of coxsackievirus B3 infection in dystrophin-deficient *(mdx)* and wild-type mice. Coxsackievirus B3 infection significantly enhanced sarcolemmal disruption in the *mdx* mice in contrast to wild-type mice; the disruption was detectable 2 days after infection and continued to increase after initial infection. Viral titers were higher in the hearts of *mdx* mice than in the hearts of wild-type mice, indicating greater viral replication in the absence of dystrophin. The observed differences seemed to be a result of more efficient release of coxsackievirus B3 from dystrophin-deficient myocytes. The expression of wild-type dystrophin in cultured cells decreased the cytopathic effect induced by coxsackievirus B3 and the release of virus from the cell. The expression of a cleavage-resistant mutant of the dystrophin protein inhibited coxsackievirus B3–mediated cytopathic effect and viral release further.

PATHOPHYSIOLOGY OF VENTRICULAR DYSFUNCTION IN MYOCARDITIS

With extensive interstitial inflammation, muscle cell injury, or both, myocardial contractility is reduced. Consequently, the heart enlarges and the end-diastolic volume of the ventricle increases. In the normal heart, an increase in filling volume leads, by the Starling mechanism, to an increased force of contraction, ejection fraction, and cardiac output. In patients with myocarditis, the myocardium is unable to respond in this manner and cardiac output is reduced. Systemic blood flow may be maintained, however, by use of the cardiac reserve, mediated by the sympathetic nervous system and leading to vasoconstriction of the skin vessels and an increase in heart rate. With progressive disease or any stress (e.g., infection, anemia, fever), the heart may be unable to meet the oxygen demands of the tissues, and the clinical picture of congestive cardiac failure may become evident. Increased end-diastolic volume leads to progressive increase in ventricular end-diastolic pressure, which leads to increased filling pressure, and left atrial and pulmonary venous hydrostatic pressure may be elevated above the colloid osmotic pressure, which normally prevents transudation of fluid across the capillary membranes. Pulmonary congestion and edema and systemic venous engorgement (manifested in infants primarily as hepatic enlargement) are common findings in the more acute forms of myocarditis. In some infants and young children, the presentation is predominantly that of right-sided heart failure.[144]

An appreciation of the disturbance of myocardial function may be gained from the angiographic frames shown in Figure 28-6. The left ventricle is dilated considerably, and the outline is irregular in diastole and systole. The ejection fraction is reduced significantly at 35 percent instead of the normal 60 to 75 percent.

Another means of evaluating left ventricular function is the noninvasive technique of cardiac ultrasound. Gutgesell and colleagues[67] established normal standards for children. An example is shown in Figure 28-7A.

The normal shortening fraction (i.e., percentage change in ventricular dimensions between end-diastole and end-systole) is 35 ± 4 percent, regardless of age (range 28% to 44%). Figure 28-7B illustrates the case of a 4-year-old child with idiopathic myocarditis and shows ventricular dilation with markedly reduced motion of the left ventricular posterior wall and septum, leading to a shortening fraction of only 12 percent. Further assessment of ventricular function can be achieved by measuring systolic time intervals obtained from simultaneous recording of the ECG and the semilunar valve opening and closing points on the echocardiogram.[67]

The clinical presentation of myocarditis varies with the age of the patient and the virulence of the organism. At one end of the spectrum is a fulminant, rapidly fatal illness, and at the other, no apparent clinical disturbance at all. A newborn especially is susceptible to the severe form of myocarditis usually caused by the coxsackieviruses B,[36,87] but it also is recognized with rubella[3] and HSV[184] infections and with toxoplasmosis.[79]

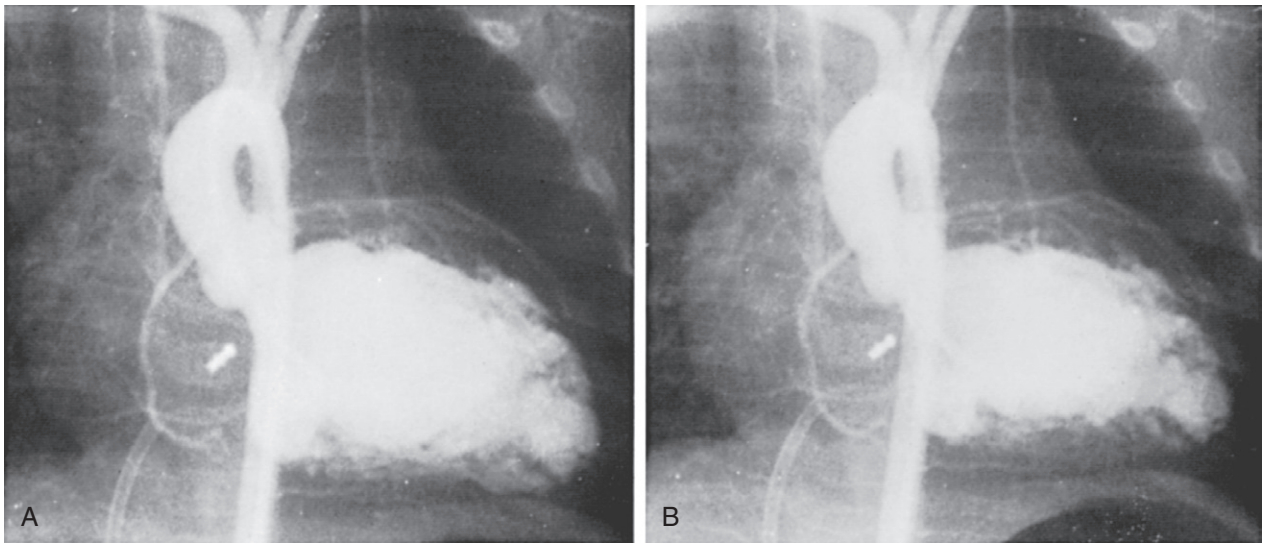

FIGURE 28-6 ■ The end-diastolic **(A)** and end-systolic **(B)** frames from a left ventriculogram of a patient with idiopathic myocarditis show irregularity of the wall and poor contractility.

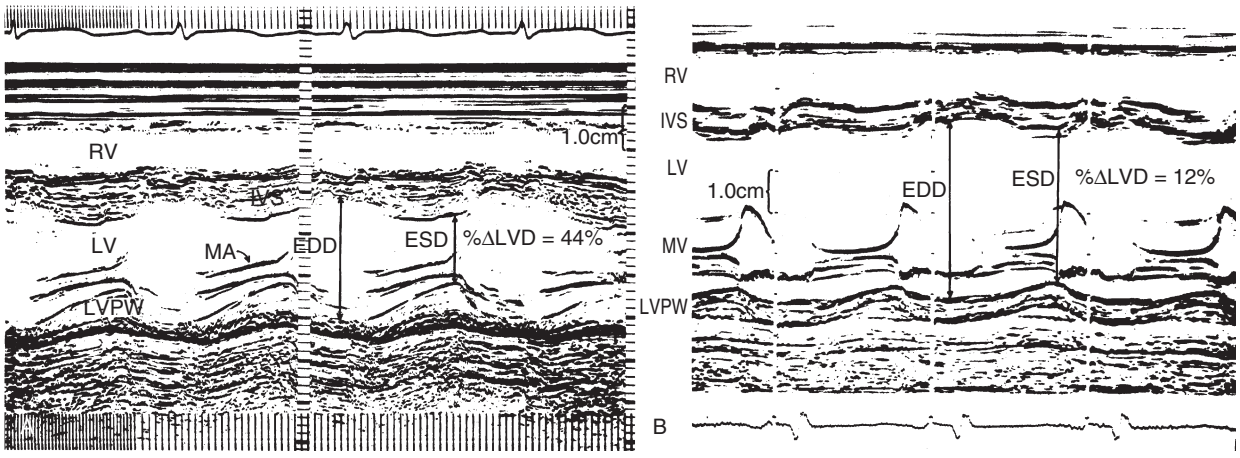

FIGURE 28-7 ■ **A,** Normal echocardiogram of a 4-year-old child. **B,** Echocardiogram of a 4-year-old child with idiopathic myocarditis shows left ventricular dilation and severely reduced shortening fraction. *EDD,* End-diastolic dimension; *ESD,* end-systolic dimension; *IVS,* interventricular septum; *LV,* left ventricle; *LVPW,* left ventricular posterior wall; *MA,* mitral apparatus; *MV,* mitral valve, %ΔLVD, percent change in left ventricular dimension (shortening fraction); *RV,* right ventricle.

In many of these infections, myocarditis is only one component of a generalized illness, often with severe hepatitis and encephalitis.[17,87] In some instances, however, infections with these organisms may produce only a mild clinical disturbance.[24,81] In the report by Brightman and colleagues,[24] a nursery epidemic of coxsackievirus B5 infection in preterm infants was recognized only by chance because a virologic survey was in progress at the time in the institution. No cases of myocarditis were documented, and all of the infants recovered. Findings included lethargy, failure to gain weight, and, in some infants, evidence of aseptic meningitis.

As described in the review by Kibrick and Benirschke[87] of 25 infants with coxsackievirus B myocarditis, vague symptoms such as lethargy and anorexia may herald the onset of the severe disease, emphasizing that close attention should be paid to all symptoms, especially in a newborn, no matter how nonspecific. Four infants had episodes of vomiting, and fever was documented for more than half of the cases; occasionally, the temperature was subnormal. Cyanosis, respiratory distress, and tachycardia, cardiomegaly, or ECG changes occurred in 19 of 23 infants. Tachypnea (a respiratory rate >60/min in a newborn) is an early sign of heart failure in a young infant and should alert the clinician to this diagnosis.

In older infants and children, the manifestations of myocarditis generally are less fulminant than manifestations in newborns.[144,149,176,180] An acute and fatal illness has been associated, however, with idiopathic myocarditis[102] and the myocarditis associated with enteroviruses,[87] adenoviruses,[72] mumps,[96] chickenpox,[134] diphtheria,[15] CMV,[170] and many of the other causative agents listed in Table 28-1. Some older children have been reported with acute, substernal chest pain consistent with angina and

have ECG changes of acute myocardial infarction.[75,118] The usual clinical picture is that of an acute or a subacute illness, which often begins with a mild upper respiratory tract infection and a low-grade fever.[8] Some infants have only vague, nonspecific suggestions of disease (e.g., irritability, periodic episodes of pallor) before the onset of cardiorespiratory symptoms, which begin a few days to 2 weeks after the onset of the initial symptoms. Abdominal pain may be a prominent complaint in some children.[176]

On examination, these infants and children often are anxious and apprehensive, but some appear apathetic and listless. Pallor may be striking, and mild cyanosis may be present. The skin may be cold and mottled. Respirations are rapid and labored, and grunting may be prominent. The pulse is thready, and blood pressure usually is normal or slightly reduced, unless the infant is in profound shock. The precordium is quiet, without a prominent cardiac impulse. Resting tachycardia invariably is present in children who are critically ill with myocarditis. The heart sounds are muffled, and a prominent gallop rhythm usually is heard. Fine and colleagues[50] found the most sensitive clinical sign of myocarditis to be a soft S_1 at the apex. A prolonged PR interval, which may be a nonspecific finding in many febrile illnesses, also can cause a soft S_1, however, without any other evidence of myocarditis.[156] A high-pitched systolic murmur of mitral insufficiency is heard in some cases. The breath sounds are harsh. Scattered rhonchi and, occasionally, fine crepitations in the lung bases may be detected.

Almost uniformly, the liver is enlarged; edema is a rare finding. Some infants are less distressed and have signs of only mild congestive cardiac failure, without the signs of peripheral circulatory failure. Other infants have no signs of cardiac compromise, and myocarditis is recognized only as part of a generalized illness by a disturbance in the ECG pattern.

DIAGNOSIS

Clinical Characteristics and Radiographic Evidence

Myocarditis often is difficult to diagnose, but it should be suspected in any infant or child who presents with congestive heart failure and who has or recently had a febrile illness. The history should include information regarding travel, exposure to tuberculosis, recent drug ingestion, and illnesses in other family members or schoolmates. A quiet precordium in the presence of a gallop rhythm and decreased intensity or muffling of the heart sounds are findings that strongly suggest the diagnosis. A tachycardia out of proportion to the level of fever also should be viewed with suspicion. A physiologic S_3 is a common finding in normal healthy children and in children with anemia and fever. Sometimes, as with fever and associated tachycardia, the cardiac rhythm may have a gallop cadence. In association with it, however, the precordium is hyperactive and the heart sounds are crisp and have increased intensity. An unusually prominent S_3 suggests a disturbance of ventricular compliance without other evidence of compromised cardiac function and should be investigated further with an echocardiogram, a chest radiograph, and an ECG.

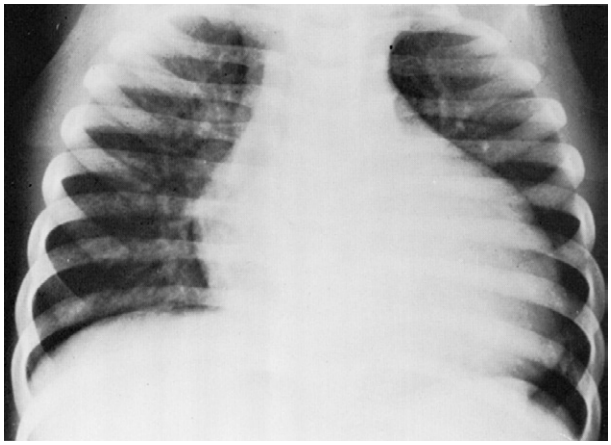

FIGURE 28-8 ■ Marked cardiomegaly with a mild increase in the pulmonary venous pattern in the upper lobes.

Chest radiographs of infants and children who have signs of congestive cardiac failure invariably show cardiomegaly, usually of a severe degree (Fig. 28-8). All four chambers may be enlarged, and evidence of pulmonary venous congestion often is found.

Sometimes, especially in newborns, the first sign of illness is acute circulatory collapse, and, in this circumstance, the cardiac size may be normal. The same is true of children who have an arrhythmia rather than congestive heart failure. Other patients may present with Stokes-Adams attacks caused by complete heart block.[101]

The occurrence of an arrhythmia, especially after a febrile illness, should alert the clinician to look for other signs of myocarditis.[32,159] Lind and Hulquist[102] detected significant dysrhythmias in five infants with isolated myocarditis. Four of the five infants died, and three of these infants had paroxysmal atrial tachycardia. Paroxysmal atrial tachycardia has been reported in patients with viral myocarditis[32,159] and has been described in patients with diphtheritic myocarditis.[15] Atrial ectopic tachycardia may mimic sinus tachycardia and, if not carefully evaluated, may be the primary cause for significant myocardial dysfunction. Complete heart block has been described in children in association with acute idiopathic myocarditis[83,101] and with rubella,[105] coxsackievirus,[153] and respiratory syncytial virus[12,59] infections. In some instances, complete heart block is permanent, and in others, it is temporary.[12,60,83] The ECG is an essential diagnostic tool for all patients with suspected myocarditis.

The classic ECG pattern in myocarditis is one of diffuse low-voltage QRS complexes (<5 mm total amplitude) with low-amplitude or slightly inverted T waves and a small or absent Q wave in leads V_5 and V_6 (Fig. 28-9). The low-voltage signal may be present in the standard leads and the precordial leads. Figure 28-10 shows the ECG of an infant with acute myocarditis and shows a pattern of acute myocardial ischemia. Figure 28-11A shows multifocal extrasystoles and severe intraventricular conduction delay in a patient with diphtheritic

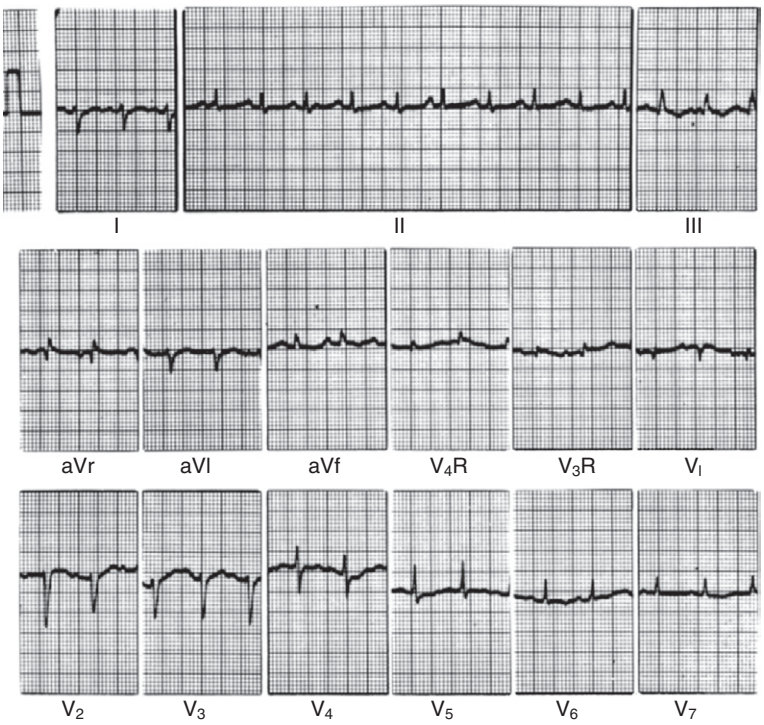

FIGURE 28-9 ■ Diffuse low-voltage or QRS complexes with T-wave flattening and 1-mm Q waves in the lateral precordial leads represents the classic pattern in myocarditis.

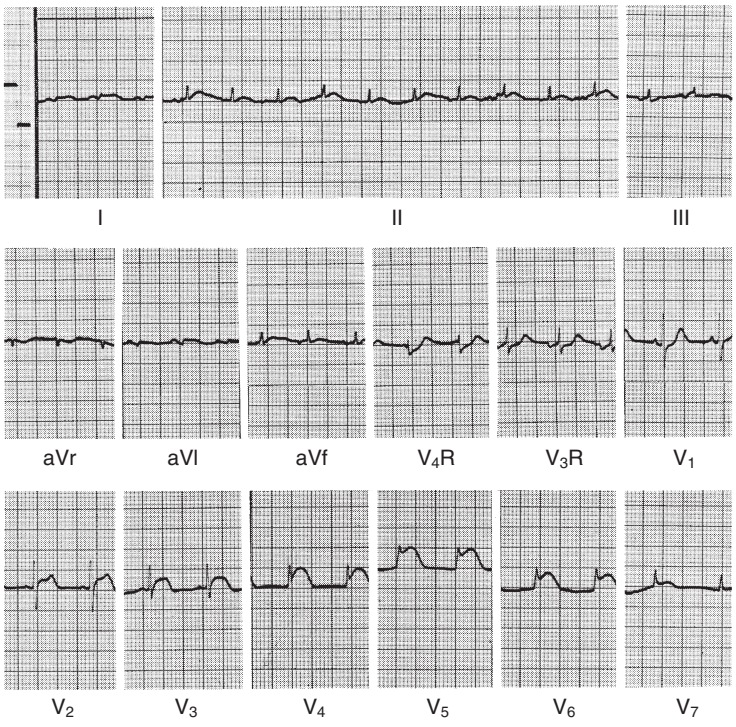

FIGURE 28-10 ■ In addition to low voltage, there is evidence of acute myocardial ischemia with 4- to 5-mm ST-segment elevation dominantly in the middle and lateral precordial leads.

myocarditis; the ECG of this child returned to normal over the course of 3 months (see Fig. 28-11B). The ECG from a 5-month-old infant who had mild fever, diarrhea, and vomiting for 3 to 4 days before admission is shown in Figure 28-12. The 2 : 1 atrioventricular block was associated with normal QRS complexes. This abnormality persisted in the absence of clinical symptoms for 1 year. Figure 28-13 shows a left bundle branch block that was identified in a 10-month-old infant with acute idiopathic myocarditis. Anomalous origin of the left coronary artery from the pulmonary artery was suspected but was excluded by catheterization. This ECG pattern persisted for at least 6 months.

Karjalainen and colleagues[85] studied the ECGs of 87 conscripts between 18 and 30 years old, 28 of whom had myocarditis. The most frequent findings were T-wave changes of reduced amplitude or inversion in the left chest leads. Sinus tachycardia followed by premature

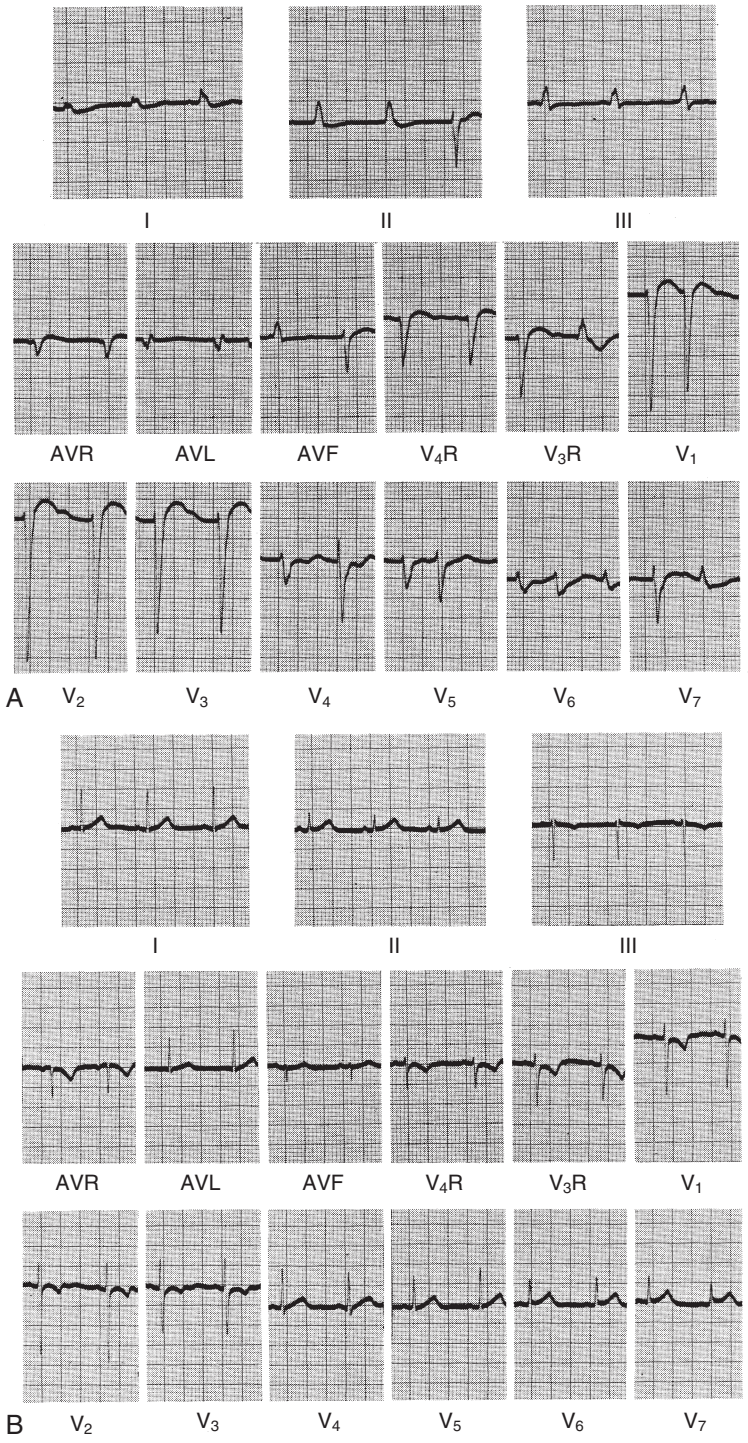

FIGURE 28-11 ■ **A,** Multifocal premature beats are caused by atrioventricular dissociation and left bundle branch block resulting from diphtheritic myocarditis. **B,** A normal electrocardiogram is shown for the same patient 3 months after an episode of myocarditis.

ventricular depolarizations was the most common dysrhythmia. Take and colleagues[165] examined serial ECGs of 16 patients with confirmed viral myocarditis. They found four patterns: (1) complete normalization in the presence of severe myocardial damage in the acute stage; (2) "pseudoinfarction" patterns with Q waves and poor R-wave progression; (3) permanent conduction disturbances that might require pacemaker support; and (4) chronic dysrhythmias, predominantly ventricular tachycardia and supraventricular tachycardia.

Although T-wave and ST-segment changes are the most sensitive indices of myocardial ischemia, they also seem to be nonspecific. Prolongation of the PR interval is another nonspecific ECG finding frequently noted in patients with febrile illnesses. Scott and colleagues[156] showed a 1.49 percent prevalence of these findings in a group of 737 infants and children with respiratory tract infections, but they also found a similar incidence among 108 control children without respiratory infection or other febrile illness. Abt and Vinnecour[1] recorded PR

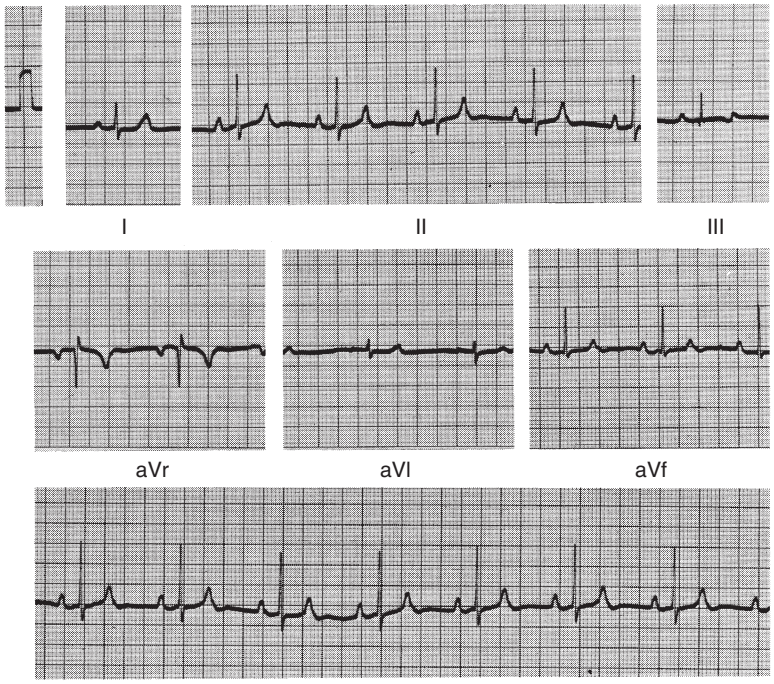

FIGURE 28-12 ■ Second-degree atrioventricular block with an effective ventricular rate of 60 beats/min. The blocked P wave is placed on top of the T wave in each cycle.

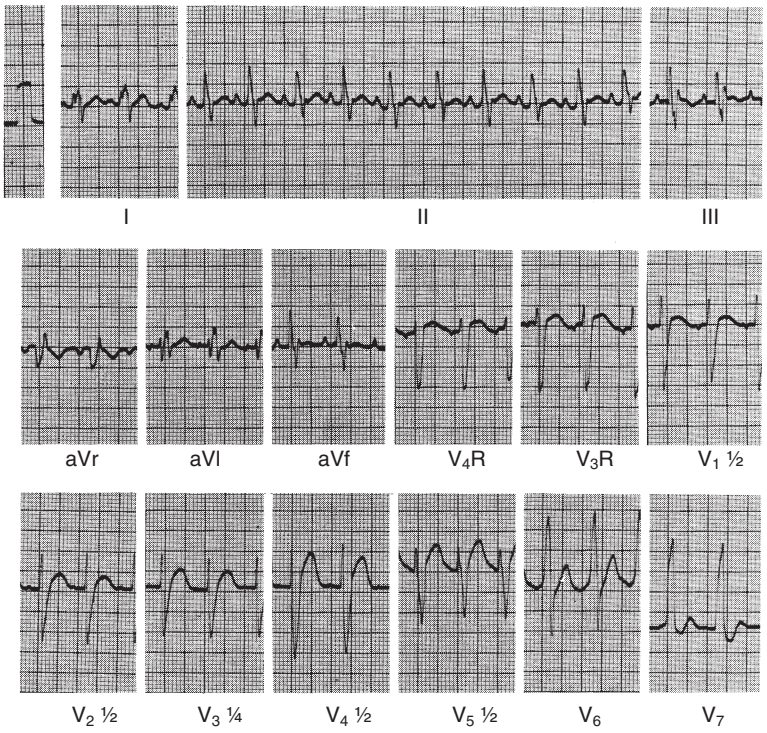

FIGURE 28-13 ■ Sinus tachycardia (rate 150 beats/min) and left bundle branch block.

prolongation and T-wave changes in infants and children who were suffering from pneumonia without other signs of myocarditis. The QT interval has been prolonged in cases of acute myocarditis, but it also appears to be a rather nonspecific finding associated with other infectious diseases.[84,146] A diagnosis of myocarditis cannot be established with certainty on the basis of these nonspecific changes.

Noninvasive diagnostic myocardial imaging techniques that are useful in the detection of myocarditis include echocardiography and magnetic resonance imaging (MRI). Echocardiography is currently recommended in the initial diagnostic evaluation of all patients with suspected myocarditis.[148] The echocardiogram is useful in assessing ventricular function and helps exclude pericardial effusion as the cause of the cardiomegaly.

Cardiovascular MRI is a safe, noninvasive technique that can be used to assess myocardial function and tissue damage in suspected myocarditis. In particular, cardiac MRI can be used to differentiate between acute and chronic myocarditis and can be used for long-term follow-up without the use of radioactive tracers or radiation. Gagliardi et al.[56] reported the first case series on the use of cardiac MRI for the diagnosis of acute myocarditis in 11 infants and children. The authors reported 100 percent specificity and 100 percent sensitivity for T-2 weighted spin echo cardiac MRI sequences in contrast to endocardial biopsy. Cardiac MRI may be a good tool for selecting patients for endomyocardial biopsy.

Endomyocardial biopsy used for establishing the diagnosis of myocarditis and possibly classifying the phase (i.e., active, healing, or healed) of viral infection may have a direct impact on the type of therapy employed. Classification of myocarditis based on histologic evidence found on biopsy specimens has proved to be a difficult and sometimes controversial task. No widespread agreement exists on the criteria for establishing the diagnosis of myocarditis from biopsy samples. Sampling error resulting from the small amounts of tissue obtained, the focal nature of sampling, and the disease process may lead to misdiagnosis. Samples usually are obtained from the right ventricular septum or apex and should contain at least three and optimally five pieces of tissue. Some investigators have found sampling from other areas of the heart (e.g., left ventricle) to be more sensitive,[173] but these techniques have not been applied widely.

Cases of "borderline myocarditis" (i.e., specimens containing increased numbers of inflammatory cells, but without evidence of myocyte necrosis) may require a repeat biopsy to confirm the diagnosis. Dec and colleagues[37] confirmed the diagnosis of myocarditis in four of six patients with an initial diagnosis of borderline myocarditis. These investigators did not show any significant advantage to sampling the left ventricle during the repeat study. Overinterpretation or misinterpretation has been cited as a major problem in the reading of biopsy specimens. Edwards and associates[46] looked at 170 endomyocardial biopsy samples and found that more than five lymphocytes per high-power field were consistent with a diagnosis of active lymphocytic myocarditis.

Using endomyocardial biopsy, Fenoglio and coworkers[49] diagnosed myocarditis in 34 patients presenting with congestive heart failure of unknown origin. They classified these patients on the basis of clinical and histologic findings in an attempt to establish subgroups of patients who might benefit from immunosuppressive therapy. Three groups were established—acute, rapidly progressive, and chronic. Immunosuppressive therapy was thought to be significantly beneficial for the last group only in terms of clinical improvement.

Dec and colleagues[38] studied 27 patients referred for endomyocardial biopsy because of congestive heart failure of unknown origin. Two thirds of the patients had biopsy samples read as positive for myocarditis, but in contrast to the study of Fenoglio and coworkers,[49] in which the histologic grouping was slightly different, no correlation was found between histologic classification and outcome. Outcome did not differ between the group receiving immunosuppressives and the group not receiving immunosuppressives. Biopsy results were negative for 30 percent of the patients who already met all the clinical criteria of myocarditis and were positive for two of five patients without any clinical evidence of myocarditis (i.e., viral-like illness, pericarditis, or laboratory evidence of viral infection).

Olsen[131] reviewed 1200 biopsy specimens from patients with a clinical diagnosis of idiopathic dilated cardiomyopathy and found that slightly more than 25 percent had a diagnosis of myocarditis established on the basis of critical evaluation of their tissue specimens. In clinical practice, endomyocardial biopsy should be used in those scenarios in which the incremental prognostic and therapeutic information gained from biopsy outweighs the risks. No large study of pediatric patients has been conducted to confirm this finding in the young.

Most physicians would agree that many cases of idiopathic dilated cardiomyopathy probably are the sequelae of unrecognized acute viral myocarditis. The role of endomyocardial biopsy in attempting to salvage patients by selecting them for specific therapy has some validity. The hope is that early intervention in some patients guided by this technique would prevent them from progressing to needing transplantation or to death from intractable heart failure.

Molecular Diagnostic Studies

Polymerase Chain Reaction

Jin and associates[82] first described the usefulness of PCR in identifying the viral genome in myocardial samples obtained from patients with suspected myocarditis. Using reverse-transcription PCR, which employs RNA to amplify the corresponding complementary DNA before final DNA amplification, the researchers were able to identify an enteroviral genome from cardiac tissue samples. Patients with dilated cardiomyopathy were shown to harbor an enteroviral genome within myocardial specimens. Confirmation of the utility of PCR in the etiologic diagnosis of a viral genome in patients with clinical myocarditis and idiopathic dilated cardiomyopathy quickly followed.* Controversy existed, however, because reports of high levels of false-positive results,[45] contamination,[31] and low sensitivity[179] were published. The strength of this rapid (<5 hours) and powerful method of amplification of a specific viral genome is also its weakness. The method depends on the quality and quantity of nucleic acid extraction, but contamination may be commonplace in some laboratories. If any requirements are altered, amplification may not occur, leading to false-negative or false-positive (i.e., contamination) results.

Numerous publications have shown that PCR is a rapid and sensitive method for establishing the diagnosis of enteroviral myocarditis and that it is probably the test of choice in the diagnosis of all virus-induced cardiac disease. Towbin and colleagues[172] used PCR to diagnose adenoviral myocarditis in a fetus with nonimmune

*References 31, 45, 63, 73, 125, 141, 177.

hydrops fetalis. In this case, the adenoviral genome was amplified from fetal blood and maternal blood at 29 weeks gestation and again at delivery at 34 weeks gestation using blood from the infant and mother and placental specimens. Using viral primers designed to amplify enterovirus, adenovirus, CMV, and HSV nucleic acid, Martin and associates[110] reported 34 patients with suspected acute myocarditis, for whom 68 percent of samples analyzed were PCR-positive. In this report, samples from 17 control patients were PCR-negative. Adenovirus was the most common viral genome identified (58%), and enteroviruses were the second most common (29%). A few reported cases were PCR-positive for HSV and CMV.

Lozinski and coworkers[106] confirmed the importance of adenovirus in their study of cases of myocarditis for which no cause had been found previously; in this case, 66 percent of the previously unidentified cases were identified as adenovirus. In a more recent study by Bowles and colleagues,[23] viral genomes were detected in 20 percent of 149 patients with the diagnosis of dilated cardiomyopathy. In these patients, adenovirus was identified in 12 percent and enterovirus in 8 percent of dilated cardiomyopathy cases. In all age groups, adenovirus and enterovirus were the viruses most commonly detected in acute myocarditis and dilated cardiomyopathy.

Schowengerdt and colleagues[155] showed that a variety of viruses might be the inciting cause of rejection in patients after undergoing heart transplantation. Using PCR of endomyocardial biopsy specimens, the investigators showed a direct correlation between histologic rejection and PCR-positive viral study results. Studying patients undergoing serial endomyocardial biopsies, they found that the viral genome could be amplified in transplant-rejecting patients who previously had negative PCR analyses. In these cases, as the rejection grade improved, the PCR results again became negative. The most common viruses correlated with rejection were adenoviruses, CMV, and parvovirus. The researchers postulated that this form of rejection probably is another form of myocarditis, and this hypothesis has been supported by other clinical studies.[178]

Virologic and Bacteriologic Studies

For each infant or child with a diagnosis of acute myocarditis, an attempt should be made to identify the offending organism. If the patient is seen early in the illness, isolation of the virus from throat washings, stool, blood, or the myocardium may be possible.

Lerner and colleagues[98] suggested criteria that would help define an etiologic association between a coxsackievirus infection and myocarditis. High-order associations included isolation of the virus from the myocardium, the endocardium, or pericardial fluid and localization of type-specific virus in myocardium, endocardium, or pericardium at sites of pathologic change.

Moderate-order associations are determined when virus is isolated from pharynx or feces, and a fourfold increase in type-specific, neutralizing, hemagglutination-inhibiting, or complement-fixing antibodies is shown, or when virus is isolated from pharynx or feces with a concurrent serum titer of 1 : 32 or greater of type-specific, IgM-neutralizing or hemagglutination-inhibiting antibodies. In chronic illness, attempts at virologic identification are less fruitful.[65] Blood for aerobic and anaerobic cultures should be obtained from any infant with fever and signs of compromised cardiovascular function.

Serum Biomarkers

In the setting of acute myocarditis, increased levels of creatine kinase and troponin indicate inflammatory myocardial injury. However, limited myocardial necrosis during myocarditis may contribute to the limited prevalence of elevated troponin T values in biopsy-proven myocarditis cases. Although most available studies involve adult patients, it is acknowledged that cardiospecific troponins provide evidence of myocyte injury in patients with myocarditis more sensitively than do conventional cardiac enzymes. The sensitivity of cardiac biomarkers of myocardial injury varies depending on the time from symptom onset to testing and the cutoff values used. In pediatric patients with acute myocarditis, the sensitivity and specificity of troponin T were 75 and 75 percent when the cutoff was set at 0.026 ng/mL and 63 and 89 percent with a cutoff value of 0.071 ng/mL.[157] In contrast to creatine kinase activities, troponin T also provides improved sensitivity for detection of micronecrosis because of a proportionally higher and longer-lasting increase in the blood.[157] The erythrocyte sedimentation rate, C-reactive protein, and leukocyte count may be elevated, but these are considered nonspecific.

DIFFERENTIAL DIAGNOSIS

Any cause of circulatory failure, especially when acute in onset, may mimic myocarditis. In newborns, heart failure associated with hypoxia, hypoglycemia, and hypocalcemia is well recognized, whereas circulatory collapse may occur with any infection and without direct involvement of the myocardium. A careful history may help elucidate possible precipitating factors. Biochemical investigations to exclude hypoglycemia and hypocalcemia always should be conducted in any newborn with signs of heart failure. Blood cultures should be obtained when infection is suspected.

Many infants with structural cardiac defects (e.g., hypoplastic left heart syndrome, aortic valve stenosis) may not have audible murmurs when severely ill. Murmurs usually appear, however, with treatment and improvement in cardiac function. The precordium usually is hyperactive, rather than quiet, and the heart sounds are clear and increased in intensity, rather than muffled. The ECG usually shows severe right ventricular hypertrophy in the former condition and right ventricular or left ventricular hypertrophy in the latter; the ECG is useful in the differential diagnosis. The findings on an echocardiogram often are diagnostic.

Beyond the immediate neonatal period, the major disease entities that require differentiation from myocarditis are endocardial fibroelastosis, anomalous left coronary artery arising from the pulmonary artery, Cori type

II glycogen storage disease (i.e., Pompe disease), medial necrosis of the coronary arteries, left atrial myxoma,[126] and other congestive cardiomyopathies of undetermined cause.

Common to all of these disorders is moderate to severe cardiomegaly, usually associated with congestive cardiac failure, gallop rhythm, and the infrequent occurrence or absence of murmurs. The murmurs are associated primarily with an anomalous left coronary artery and endocardial fibroelastosis. They are not more than grade 3/6 in intensity, are high-pitched, are apically located, and represent some degree of mitral insufficiency. Idiopathic myocarditis occurs primarily in patients older than 6 months of age,[144,145] whereas most of the conditions described earlier manifest before the infant is 6 months old.

Endocardial fibroelastosis, a common cause of congestive cardiac failure in infants, is impossible to differentiate from acute myocarditis on the basis of clinical examination alone. An anomalous origin of the left coronary artery should be identified. The ECG usually shows left axis deviation of the QRS complex in the frontal plane, left ventricular hypertrophy, and a pattern of anterolateral myocardial infarction. It is recognized as a QR pattern with inverted T waves in standard leads I and aV_L, a broad Q wave with inverted T waves in precordial leads V_5 and V_6, and loss of anterior forces in the mid-precordial leads. For definitive diagnosis, cardiac catheterization is essential.

Pericarditis, frequently caused by viruses, usually occurs in children rather than in infants. The clinical history may be identical to that of patients with myocarditis; however, considering the degree of cardiomegaly, cardiovascular function is compromised less than in patients with myocarditis, although cardiac tamponade may occur in some cases. Differentiation from myocarditis may be made clinically if the patient has a friction rub, no gallop rhythm, and a typical pattern of chest pain. Further studies may be required, however, to make a conclusive diagnosis. The echocardiogram is invaluable in establishing this diagnosis. It is the most sensitive and least traumatic technique available and easily identifies an effusion. Myocarditis and pericarditis may occur together. This combination is seen most frequently in the pancarditis of rheumatic fever, but it also may occur in coxsackievirus B infections and in many collagen vascular and autoimmune diseases. Myocarditis has been associated with rheumatoid arthritis,[119] systemic lupus erythematosus,[40] and ulcerative colitis.[51,124]

TREATMENT

Standard Approaches

Intensive medical care is required during the acute stage of the illness. Heart rate, respiratory rate, and blood pressure should be monitored frequently, and a careful assessment of urine output and fluid intake is mandatory. All patients require bed rest. Experimental studies in mice have shown that exercise increases replication of virus in the myocardium and increases the mortality rate from myocarditis by 100 percent.[57] Although extrapolating directly from experimental studies in animals to the human situation always is dangerous, suggesting strict bed rest during the early stages of acute myocarditis seems to be a prudent measure. For infants or children with signs of congestive cardiac failure or shock, oxygen should be administered to maintain a normal arterial blood oxygen tension.

No specific therapeutic modality is known that can reverse the myocardial injury directly, but much can be done to maintain adequate tissue perfusion, prevent metabolic disturbances, and support myocardial function. When congestive cardiac failure is identified, digitalis should be administered. This agent should be used with caution, given the increased expression of proinflammatory cytokine and increased mortality rates observed in murine myocarditis treated with high-dose digitalis.[115,116] Diuretics also are used frequently to treat cardiac failure. Diuretics have no direct beneficial effect on the myocardium; they should be used cautiously because rapid reduction in extracellular fluid volume may lead to shock, and the loss of potassium associated with vigorous diuresis may precipitate digitalis toxicity. The frequency of administration depends on the clinical state of the patient.

In some instances, especially in newborns, the primary presentation may be shock. The blood pressure usually is maintained close to normal levels until late in the course of disease; it cannot be a reliable index of the severity of the patient's condition. Cold extremities, increasing heart rate, and low urine output are much more sensitive indicators of a reduction in effective circulating blood volume. Although the hearts of these infants and children respond poorly to volume loading, a colloid transfusion may help in selected patients. Albumin as a 5 percent solution in lactated Ringer solution or whole blood may be administered.

If the patient remains in shock despite administration of these high filling pressures, a positive inotropic agent is required. Dopamine exerts an inotropic effect on the heart and concomitantly dilates the renal vessels, improving urine output. The usual dose is 2 to 10 μg/kg/min. As the dose increases to 20 μg/kg/min, dopamine has a more dominant α-adrenergic effect and may increase systemic peripheral resistance; experts avoid doses greater than 15 μg/kg/min. Dobutamine, which is a sympathomimetic amine that stimulates β_1-adrenergic, β_2-adrenergic, and α-adrenergic receptors, may be useful in combination with dopamine. It has significant inotropic activity while decreasing left ventricular filling pressure. Dobutamine does not induce the positive chronotropic effect and increased ventricular irritability that are seen with dopamine. Used in combination with low doses of dopamine (<10 μg/kg/min), dobutamine (in doses ≤10 μg/kg/min) may result in significant positive inotropism, while preventing a sinus tachycardia that may compromise cardiac output further.

Isoproterenol, a commonly used inotropic agent, should be avoided because it causes a significant increase in heart rate and may affect cardiac function adversely. When these drugs are used, ensuring normal acid-base balance is important because action of the agents is decreased significantly by acidosis.

Sodium nitroprusside, phentolamine, and the nitrates have been used in adults. These agents have been used less extensively in children, primarily in the early postoperative period after open heart surgery, to improve cardiac function. They improve cardiac output by indirectly reducing systemic arterial resistance, venous filling pressure, or both. One study showed a marked improvement in the reduction of inflammation, necrosis, and dystrophic calcification in mice infected with coxsackievirus B3 when they were treated with captopril, an angiotensin-converting enzyme inhibitor, early after development of infection.[143] They improve cardiac output by indirectly reducing systemic arterial resistance or venous filling pressure, or both. One study showed a marked improvement in the reduction of inflammation, necrosis, and dystrophic calcification in mice infected with coxsackievirus B3 when they were treated with captopril, an angiotensin-converting enzyme inhibitor, early after development of infection.

A temporary pacing catheter should be inserted when complete heart block occurs. In many instances, the temporary pacing catheter allows time for spontaneous recovery of atrioventricular conduction. A permanent pacemaker may be required; if so, it should be inserted as an elective, rather than emergency, procedure. Only a demand pacemaker should be used because in most instances a return to normal rhythm is expected; use of this type of pacemaker avoids the risk for competition between the patient's inherent rhythm and the pacemaker, as would occur with a fixed-rate device. If infants or children experience Stokes-Adams attacks, a transthoracic pacing wire may have to be inserted as an emergency procedure.

Antibiotics should not be given routinely, unless a bacterial infection is suspected. Appropriate cultures need to be obtained before use of antibiotics.

Immune Modulating Agents

The use of immunosuppressive agents in the treatment of viral or suspected viral myocarditis is controversial. Immunosuppressant therapy in animal models of viral myocarditis has not been shown to be beneficial. One reason for the lack of efficacy of immunosuppressant therapies may relate to the duality of the effects of the immune system. Corticosteroids enhanced viral titers in the early phase of viral murine myocarditis, whereas cyclosporine caused greater mortality and cardiac insufficiency in EMCV myocarditis.[120,171] The use of immunosuppressive agents in the treatment of viral or suspected viral myocarditis is controversial. Immunosuppressant therapy in animal models of viral myocarditis has not been shown to be beneficial. One reason for the lack of efficacy of immunosuppressant therapies may relate to the duality of the effects of the immune system. Corticosteroids enhanced viral titers in the early phase of viral murine myocarditis, whereas cyclosporine caused greater mortality and cardiac insufficiency in EMCV myocarditis.[88,89] A report of 13 children with biopsy-confirmed myocarditis treated with prednisone approximately 3 weeks after symptoms occurred showed a marked reduction in inflammation on the follow-up biopsy specimen.[30] This study was uncontrolled, however, and may not truly represent an accurate account of the role of immunosuppressive therapy.

Mason and colleagues[112] used endomyocardial biopsy as a means of diagnosing and following the effects of immunosuppressive therapy in 10 patients. Eight patients received a combination of prednisone and azathioprine, and two patients received prednisone alone. Four patients improved clinically and histologically while on therapy. Two patients who had medications discontinued had relapses, which were reversed with reinstitution of therapy. Only one patient worsened while on therapy, and that patient died. Although the study by Mason and colleagues[112] was uncontrolled, the reversal of congestive heart failure seen in the two patients who were restarted on therapy suggests the beneficial effect of these agents.

Kereiakes and Parmley[86] tabulated most of the studies showing the effects of immunosuppressive therapy in patients with myocarditis. Sixty percent of 82 biopsy-confirmed cases of myocarditis showed improvement with steroids alone or in combination with azathioprine. Patients with lower grade inflammatory changes seem to do better than patients with higher grade changes. Complications of immunosuppressive therapy, including opportunistic infections and a cushingoid state, have been reported and may limit the amount and type of therapy.[112,163]

Hobbs and associates[74] used combined prednisone and azathioprine to treat 34 adults with biopsy-confirmed myocarditis. Survival was no better for patients with histologic improvement than for patients with persistent infiltrates. Most patients experienced side effects, some of them lethal, from the corticosteroids. The potential benefits of this type of therapy must be weighed against the risk for immunosuppression in each patient with myocarditis. This therapy apparently does not prevent the observed ECG changes seen in untreated patients or the associated neuritis.[169]

Although numerous anecdotal and small case series suggested that patients with viral myocarditis might benefit from early steroid or immunosuppressive therapy, the results of the Myocarditis Treatment Trial suggested that treating patients who received a histopathologic diagnosis of myocarditis with immunosuppressive therapy for 24 weeks (prednisone plus cyclosporine or prednisone plus azathioprine) did not lead to an improvement in ejection fraction in contrast to conventional therapy.[113] The major limitation of this study was the unexpectedly low rate of positive biopsy specimens (<10%) and the extension of enrollment to 2 years after the initial clinical presentation. For many patients, the disease possibly already had progressed from ongoing immune-mediated cardiac injury to dilated cardiomyopathy, and any form of therapy may have been ineffective in this setting. Frustaci and colleagues[54] reported a favorable treatment response in adult patients with chronic active myocarditis. Of 43 patients (88%) on immunosuppressive therapy (prednisone 1 mg/kg/day for 4 weeks followed by 0.33 mg/kg/day for 5 months and azathioprine 2 mg/kg/day for 6 months), 38 showed an improvement in cardiac function and dimensions, defined as an increase of more

than 10 percentage points in the absolute ejection fraction and a reduction of left ventricular end-diastolic volume. None of the untreated patients showed improvement in cardiac function. Immunosuppressive therapy in adult patients with biopsy-proven, virus-negative inflammatory cardiomyopathy may be an effective and safe option in addition to supportive treatment for cardiac failure.

Data regarding the efficacy of immunosuppressive therapy for myocarditis in children are limited. Accordingly, routine immunosuppressive therapy is no longer recommended for pediatric myocarditis patients with a stable clinical course. However, many clinicians still recommend aggressive immunosuppressive therapy for patients with fulminant myocarditis or a deteriorating clinical course.

Various immunomodulatory therapies have been proposed for the autoimmune phase of viral myocarditis. Kishimoto and colleagues[93] found that immunoglobulin therapy suppressed coxsackievirus B3–induced murine myocarditis and increased survival in C3H/He mice after infection with EMCV. Intravenous immunoglobulin (IVIG) therapy reduced various proinflammatory markers, including TNF, IFN-γ, macrophage inflammatory protein–2, interleukin-6, plasma catecholamines, and soluble intercellular adhesion molecule–1.[93] Exogenous immunoglobulins modulate diverse immune response mechanisms; however, the exact mechanism by which IVIG modifies myocarditis remains to be determined. Drucker and associates,[43] who investigated the use of IVIG in 21 of 46 children with myocarditis, showed that patients who received this drug had better left ventricular function at follow-up. Survival tended to be higher at 1 year, although the data did not reach statistical significance because of the small number of patients in the study. More recently, in a prospective placebo-controlled trial with IVIG in adult patients with recent-onset dilated cardiomyopathy or myocarditis, treatment with IVIG did not result in an improvement in left ventricular function compared with placebo-treated patients.[117] Kim and colleagues[90] retrospectively assessed the effects of IVIG therapy in pediatric patients with presumed myocarditis on survival and recovery of ventricular function. These patients were divided into two groups. Group 1 consisted of 23 patients (69.6%) who received IVIG alone or IVIG in combination with steroids, and group 2 consisted of 10 patients (30.3%) who received neither IVIG nor other immunosuppressive agents. One year after the initial presentation, no difference was seen between the IVIG-treated patients and the control patients in the degree of recovery of left ventricular function and survival. Although no randomized controlled trials of IVIG for the treatment of myocarditis in children have been reported, some clinical evidence suggests it may be beneficial.

IFN-β serves as a natural defense against many viral infections. Its innate production is associated with clinical recovery from viral infection and subsequent sequelae, whereas exogenous administration may be protective. Studies have suggested that IFN-β may have a role in the treatment of chronic viral cardiomyopathy. Kuhl and colleagues[95] reported that patients with persistent enterovirus and adenovirus infections of the myocardium responded well to a 6-month course of IFN-β1a. Follow-up biopsies taken 3 months after completion of treatment showed complete elimination of enteroviral and adenoviral genomes. Virus clearance was associated with improvement of mean left ventricular function, a decrease in ventricular size, improvement of heart failure symptoms, and a decrease of infiltrating inflammatory cells. Patients with severely affected left ventricular dysfunction experienced the greatest improvement. Additional studies have shown that parvovirus B19 responds less well to IFN-β treatment with respect to virus clearance and hemodynamic changes.[154] Treated patients, however, do show some clinical improvement despite incomplete virus clearance. Data regarding the use of IFN-β therapy for myocarditis in children are not available.

PROGNOSIS

The prognosis of acute myocarditis caused by coxsackievirus B infection in a newborn is poor. Kibrick and Benirschke[87] reported a 75 percent mortality rate among 25 infants with coxsackievirus B myocarditis. The greatest number of deaths occurred in the first week of the illness. No apparent sequelae occurred in the six infants who survived, although no long-term follow-up data were available. The outlook in other infants and children with clinically recognized myocarditis is better, but mortality rates remain significant (10% to 25%). Hastreiter and Miller[71] observed complete recovery in 50 percent of patients. Another 25 percent became asymptomatic, but abnormal ECGs or chest radiographs persisted. An abnormality may not be evident on the ECG unless the patients are exercised.[16] Despite lack of symptoms, many adult patients have a reduced working capacity associated with exercise stress testing.[18]

The outcome of myocarditis is related partly to the cause. Patients with diphtheritic myocarditis who have arrhythmias or conduction abnormalities have a very poor prognosis. Tahernia[164] found in his study that all patients with disturbances of conduction died. Begg[15] also reported a 100 percent mortality rate for patients with diphtheritic myocarditis who developed supraventricular tachycardia.

Chronic arrhythmias may persist long after the acute disease has passed. Friedman and colleagues[52] performed a retrospective analysis of 12 patients with biopsy-confirmed myocarditis and complex ventricular arrhythmias at the time of presentation (11 with ventricular tachycardia). Of the 12 patients, 5 were still receiving antiarrhythmic therapy at a median follow-up of 50 months. Complex ventricular arrhythmias were still present in these patients (3 with ventricular tachycardia and 2 with couplets or multiforms), requiring ongoing therapy. The investigators concluded that although the arrhythmias were controlled more easily than at presentation of the patients, ongoing surveillance was essential in ensuring the suppression of these potentially life-threatening arrhythmias. Children who recover from myocarditis, regardless of cause, should be followed indefinitely.

MYOCARDITIS IN CASES OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION

Infection with human immunodeficiency virus (HIV) may affect the heart adversely. Cardiac dysfunction, including congestive heart failure, may occur; many patients who have died and undergone autopsy are shown to have myocarditis. Anderson and associates[5] retrospectively analyzed 71 consecutive necropsy patients who died of acquired immunodeficiency syndrome (AIDS) and found that 52 percent had evidence of myocarditis. Opportunistic agents could account for only a few of the cases, and most were considered idiopathic.

Another study examined autopsy specimens of 26 consecutive cases. Lymphocytic myocarditis was seen in nine patients (35%), and another seven patients had lymphocytic infiltrates without myocytolysis.[14] Acierno[2] correctly pointed out that a distinction must be made between AIDS-associated myocarditis and secondary myocarditis caused by known pathogens or idiopathic myocarditis.

Reilly and colleagues[142] found a 45 percent incidence of myocarditis in 58 consecutive autopsy cases. Congestive heart failure, ventricular tachycardia, and other ECG abnormalities were seen in nearly 60 percent of the patients. Two patients died suddenly, both with myocarditis. In a prospective study of asymptomatic HIV-infected patients, the reported mean annual incidence of progression to dilated cardiomyopathy was 15.9 cases per 1000 patients. Endomyocardial biopsy specimens revealed myocardial inflammation in 63 of 76 (83%) of these high-risk patients. HIV-infected cardiac myocytes were detected by in situ hybridization in 58 of 76 (76%) patients.

In a prospective multicenter study of 205 children infected vertically with HIV enrolled at a median age of 1.9 years and 600 HIV-exposed children enrolled prenatally or as neonates, the 5-year cumulative incidence of cardiac dysfunction ranged from 18 to 39 percent in HIV-infected children.[162] Myocardial inflammation, with or without cell destruction, is a frequent finding in patients infected with HIV. Whether this response is due to the virus itself or to opportunistic agents or other toxic reactions is unclear.

Another important cause of cardiomyopathy in HIV and AIDS is drug cardiotoxicity. Zidovudine is associated with diffuse destruction of cardiac mitochondrial ultrastructure and inhibition of mitochondrial DNA replication that may contribute to myocardial cell dysfunction.[99]

The use of highly active antiretroviral therapy (HAART) regimens, by reducing the incidence of myocarditis, has reduced the prevalence of HIV-associated cardiomyopathy by about 30 percent in developed countries.[13]

PARASITIC MYOCARDITIS

Chagas Disease

Parasitic myocarditis is an uncommon form of heart disease in the United States. Chagas disease is caused by infection with *Trypanosoma cruzi*, however, and it is a long-lived infection that affects approximately 17 million individuals in Latin America.[147] This disease is endemic in Mexico and Central and South America, where vector-borne transmission of *T. cruzi* typically occurs in persons living in rural areas. Emigration from Chagas-endemic areas has changed the epidemiology of this disease in the United States and other nonendemic regions. Bern and Montgomery[20] estimated that 300,000 persons living in the United States are chronically infected with *T. cruzi*.

After inoculation, the protozoa multiply and migrate widely throughout the body. Host control of this parasite has been shown to depend on humoral and cell-mediated adaptive responses and elements of the innate immune system.[61]

Immune-mediated cardiac injury, caused primarily by infiltrating mononuclear cells, probably is the main mechanism responsible for the development of chronic Chagas heart disease. This hypothesis is supported by several observations. Animal models of *T. cruzi* infection have shown lysis of nonparasitized cardiac myocytes by immune effector cells[7]; depletion of $CD4^+$ T-lymphocyte subpopulations abrogates myocardial injury in a murine model of chronic Chagas disease; and myocardial damage can be induced in healthy animals by passive transfer of $CD4^+$ T cells from *T. cruzi*–infected mice.[41] Although $CD4^+$ T cells seem to be crucial in myocardial injury, $CD8^+$ T cells may have a protective role. Mice depleted of $CD8^+$ T cells have a robust parasitemia, but almost no inflammatory infiltrates are seen in the parasite-infected tissues.[166]

More recently, a role for TLR signaling in resistance to *T. cruzi* is suggested by the observations that mice deficient in MyD88, an adapter molecule required for signaling events by most TLRs, show enhanced susceptibility to infection with this protozoan parasite.[27] Acute Chagas disease usually is an illness of children, but it can occur at any age.[121] Histologic examination of the heart during the acute phase reveals intracellular parasites with a marked cellular infiltrate, particularly around myocytes that have ruptured and released the parasites.[135] A well-established fact is that intracellular parasites are found in cardiac myocytes only during the acute phase of illness.

Histologic examination reveals focal but widespread areas of cellular infiltrates composed of plasma cells, eosinophils, mast cells, and macrophages.[122] Extensive fibrosis occurs, replacing previously damaged myocardial tissue. In contrast to the situation observed in acute disease, the presence of parasites (a rare finding) in tissue has little correlation with myocardial pathology. Inflammatory changes in the right bundle branch and the anterior fascicle of the left bundle branch explain the frequent occurrence of right bundle branch and left anterior fascicular block.[123]

Vector-borne acute Chagas disease is usually mild, and most acute infections are subclinical. After an incubation period of 1 to 2 weeks, a newly infected individual may develop nonspecific signs and symptoms such as fever, chills, myalgias, tachycardia, rash, and meningeal irritation. A raised inflammatory lesion at the site of parasite entry (a chagoma), unilateral periorbital edema (Romaña sign), conjunctivitis, lymphadenopathy, and

hepatosplenomegaly may also be observed in acute infection.[108] The clinical manifestations of chronic Chagas disease range from isolated rhythm disturbances to advanced disease characterized by cardiomegaly, chronic congestive heart failure, and arrhythmias. Syncope also is a frequent problem of the disease. In one series of 53 patients with chronic Chagas disease, the most frequent causes of recurrent syncope were ventricular tachycardia (43%) with a poor prognosis and paroxysmal atrioventricular block (21%) with a favorable prognosis.[111] Sudden death caused by ventricular fibrillation is a constant threat and may develop before cardiomegaly or heart failure is diagnosed.[29]

Severe myocarditis develops in only a few acute cases, and most deaths are caused by the resultant congestive heart failure and pericardial effusion. Nonspecific ECG changes are seen, but the life-threatening arrhythmias that are frequent occurrences in chronic Chagas disease generally do not occur. In most patients with more acute disease (90% of cases), symptoms resolve gradually over weeks to months.

Chronic progressive Chagas disease develops in 10 to 20 percent of previously asymptomatically infected individuals.[44] It is manifested by a chronic, diffuse, progressive fibrosing myocarditis that involves the myocytes and the atrioventricular conduction system.[122,123] On gross examination, the heart usually is enlarged and flaccid. Thrombus formation frequently occurs, and thrombus may fill much of the apex of the left ventricle in some cases.

The diagnosis of acute *T. cruzi* infection in a patient is usually made by the detection of parasites in wet mounts of blood and in Giemsa-stained slides. Testing for anti–*T. cruzi* immunoglobulin M antibodies is not useful because tests for this antibody isotype are not well standardized. The diagnosis of chronic Chagas disease cannot be made solely on the basis of histologic examination of the heart. PCR testing is thought to be the most sensitive method for detecting acute *T. cruzi* infection. This test is available at the Centers for Disease Control and Prevention (CDC) in the United States.

Serologic testing is the method of choice for establishing the diagnosis of chronic Chagas disease. Several highly sensitive serologic tests for the detection of anti–*T. cruzi* antibodies, such as indirect hemagglutination, complement fixation, indirect immunofluorescence, and enzyme-linked immunosorbent assay, are available.[91,158] A radioimmunoprecipitation assay based on iodinated *T. cruzi* proteins is sensitive and specific and is currently being used for confirmatory testing of many U.S. blood donor samples that are positive in 1 of the 2 screening tests currently approved by the Food and Drug Administration (FDA) for donor screening.[108]

The treatment of Chagas disease involves both parasite-specific therapy and adjunctive therapy for the management of the clinical manifestations. Benznidazole and nifurtimox are the only available drugs with activity against *T. cruzi*. Neither drug is FDA approved, but both are available under investigational use protocols.* Although these two drugs have been thought to be ineffective or too toxic for treating chronic infections, in a recent report a 60-day course of benznidazole therapy eliminated infection in more than 60 percent of chronically infected children.[6] These drugs are most effective for the treatment of acute and congenital infection. Parasitologic cure is believed to occur in 60 to 85 percent of persons with acute infection who complete a full course of either drug, although no large-scale studies support these data. Patients with acute infections, including congenitally acquired infections in neonates, and chronically infected children should be treated. Bern and colleagues[19,21] have described the management of persons newly diagnosed with Chagas disease as a result of screening prompted by country of origin or blood donation. The antifungal agent posaconazole[136] has been used to treat a patient with chronic Chagas disease, but its overall usefulness has not been determined. A large clinical trial designed to address the efficacy of benznidazole treatment in patients with early manifestations of cardiac Chagas disease is underway.* Other ongoing clinical trials include the use of oral posaconazole in the treatment of asymptomatic chronic Chagas disease† (ClinicalTrials.gov).

Other Parasitic Causes of Myocarditis

Myocarditis is one of the most serious complications of trichinosis. The disease develops when undercooked meat contaminated with infective larvae of *Trichinella* is eaten. Myocardial invasion by *Trichinella spiralis* has been well described, but encystment within the myocardium has been reported only rarely.[92] At autopsy, the heart may be dilated and a pericardial effusion may be identified. Histologically, a prominent focal infiltrate composed of lymphocytes and eosinophils with interstitial edema and scattered hemorrhages commonly is found.[160]

Myocarditis usually is mild, with few clinical signs and symptoms. This myocarditis may range from chest pain to fatal congestive heart failure, however, and may mimic acute myocardial infarction.[33,64] The frequencies of ECG abnormalities among 154 cases of trichinellosis were determined to be 56 percent.[138] The abnormalities on ECG most frequently observed were a nonspecific ventricular repolarization disturbance (with ST-T wave changes), followed by bundle branch conduction disturbances and sinus tachycardia. Despite ECG evidence of myocardial involvement, less than 0.1 percent of patients with trichinosis die of this complication.[64]

The definitive diagnosis of trichinosis is based on the presence of the larval forms in tissue biopsy samples, usually from a large tender area such as the gastrocnemius muscle. Serologic testing is available through state laboratories and the CDC. Typically, serum antibody titers become positive during or after the third week of illness. The efficacy of mebendazole or albendazole in the treatment of myocarditis caused by *Trichinella* infection has not been evaluated adequately.

*CDC Drug Service: http://www.cdc.gov/laboratory/drugservice/index.html; 404-639-3670.

*The BENEFIT Trial: http://ClinicalTrials.gov.
†http://ClinicalTrials.gov.

Toxocara canis, the principal cause of visceral larva migrans, is a rare cause of myocarditis. Most reported cases have occurred in children younger than 3 years of age.[35,174] Children especially are susceptible to infection with *Toxocara* because of their habit of crawling on the ground and putting objects into their mouths. The myocardial lesions noted on histologic examination have included granulomata and extensive eosinophilic infiltrates with foci of muscle necrosis. The clinical presentation may be acute respiratory distress caused by congestive heart failure, requiring administration of oxygen and diuretic therapy. Asymptomatic infection involving the heart also has been reported.[35]

The definitive diagnosis of *T. canis* infection requires microscopic identification of the larvae in biopsy specimens of the liver or heart, but this finding is infrequent. An enzyme immunoassay for *Toxocara* serum antibodies, which is performed at the CDC, can provide presumptive evidence of toxocariasis.

Toxoplasma gondii may cause myocarditis as part of disseminated infection or, less frequently, as an isolated cardiac infection. In infants with congenital toxoplasmosis, the clinical manifestations usually are those of meningoencephalitis, but at autopsy, extensive myocardial involvement has been documented.[190] Outside the newborn period, infection with this intracellular parasite most commonly occurs in immunosuppressed patients with malignant diseases, patients with AIDS,[28] and patients who have undergone cardiac or bone marrow transplantation.[107] Histologic examination of the heart reveals focal interstitial infiltrates consisting of histiocytes, lymphocytes, plasma cells, eosinophils, and very few polymorphonuclear cells.[168] Toxoplasma is seen as basophilic masses within a pseudocyst in normal or damaged myocardial fibers.

Clinical manifestations may include arrhythmias (atrial and ventricular), atrioventricular block, atypical chest pain, pericarditis, and heart failure. The diagnosis of toxoplasmic myocarditis requires the exclusion of other specific forms of heart disease and the establishment of evidence of toxoplasmosis with serologic testing. The diagnosis may be aided by endomyocardial biopsy.[107] Treatment with pyrimethamine and sulfonamides (especially sulfadiazine) has been reported in patients with isolated toxoplasmic myocarditis, but the response to therapy has varied. In one series of toxoplasmic myocarditis, relapses occurred in 17 percent of cases after therapy.[97]

Myocardial disease also has been reported after infection with *Echinococcus granulosus* and *Plasmodium falciparum*.[92] Cardiac involvement is estimated to occur in fewer than 2 percent of cases of echinococcosis.[39] When it does occur, the cysts usually are located in the intramyocardial region and protrude into the adjacent cardiac chambers. The clinical manifestations depend primarily on the location and size of the cyst. Rupture of the cyst is the most dreaded complication because it may lead to pericarditis, anaphylactic shock, or pulmonary emboli. Two-dimensional echocardiography is the preferred imaging study to detect and localize cysts.[130] Myocardial changes also have been documented in fatal malaria, particularly when caused by *P. falciparum*. Histologically, blocking of the coronary arteries and capillaries with parasites, local hemorrhage, and deposit of pigment occurs. Clinical findings suggestive of cardiac involvement are rare, however. In a series of 49 patients with *Falciparum* malaria, no ECG evidence of cardiac involvement was found.[161]

Myocarditis also has occurred in association with primary amebic meningoencephalitis caused by *Naegleria*. In a retrospective study, focal or diffuse myocarditis was documented in more than 40 percent of cases.[109] Myocardial involvement is not a clinically significant manifestation of this uniformly fatal central nervous system infection.

NEW REFERENCES SINCE THE SIXTH EDITION

13. Barbaro G. Heart and HAART: Two sides of the coin for HIV-associated cardiology issues. *World J Cardiol* 2010;**2**:53–7.
19. Bern C. Antitrypanosomal therapy for chronic Chagas' disease. *N Engl J Med* 2011;**364**:2527–34.
54. Frustaci A, Russo MA, Chimenti C. Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study. *Eur Heart J* 2009;**30**:1995–2002.
70. Harrington LE, Hatton RD, Mangan P, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005; **6**:1123–32.
90. Kim HJ, Yoo GH, Kil HR. Clinical outcome of acute myocarditis in children according to treatment modalities. *Korean J Pediatr* 2010;**53**:745–52.
100. Li K, Xu W, Guo Q, et al. Differential macrophage polarization in male and female BALB/c mice infected with coxsackievirus B3 defines susceptibility to viral myocarditis. *Circ Res* 2009;**105**: 353–64.
108. Machado FS, Jelicks LA, Kirchhoff LV, et al. Chagas heart disease: report on recent developments. *Cardiol Rev* 2012;**20**:53–65.
125. Muir P. Enteroviruses and heart disease. *Br J Biomed Sci* 1993;**50**:258–71.
127. Negishi H, Osawa T, Ogami K, et al. A critical link between Toll-like receptor 3 and type II interferon signaling pathways in antiviral innate immunity. *Proc Natl Acad Sci USA* 2008;**105**: 20446–51.
136. Pinazo MJ, Espinosa G, Gallego M. Successful treatment with posaconazole of a patient with chronic Chagas disease and systemic lupus erythematosus. *Am J Trop Med Hyg* 2010; **82**:583–7.
139. Randolph A, Vaughn GF, Sullivan R, et al. Critically ill children during the 2009-2010 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
154. Schmidt-Lucke C, Spillmann F, Bock T, et al. Interferon beta modulates endothelial damage in patients with cardiac persistence of human parvovirus B19 infection. *J Infect Dis* 2010;**201**: 936–45.
181. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat Immunol* 2010;**11**:7–13.
187. Yamashita T, Iwakura T, Matsui K, et al. IL-6-mediated Th17 differentiation through RORγt is essential for the initiation of experimental autoimmune myocarditis. *Cardiovasc Res* 2011;**91**: 640–8.
188. Yuan J, Yu M, Lin QW, et al. Th17 cells contribute to viral replication in coxsackievirus B3-induced acute viral myocarditis. *J Immunol* 2010;**185**:4004–10.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abt AF, Vinnecour MI. Electrographic studies during pneumonia in infants and children. *Am J Dis Child* 1934;**47**:737.
2. Acierno LJ. Cardiac complications in acquired immunodeficiency syndrome (AIDS): a review. *J Am Coll Cardiol* 1989;**13**:1144–54.
3. Ainger LE, Lawyer NG, Fitch CW. Neonatal rubella myocarditis. *Br Heart J* 1966;**28**:691–7.
4. Akira S. Pathogen recognition and signaling pathways of toll-like receptors. *J Invest Dermatol* 2003;**121**:1234.
5. Anderson D, Virmani WR, Reilly JM, et al. Prevalent myocarditis at necropsy in the acquired immunodeficiency syndrome. *J Am Coll Cardiol* 1988;**11**:792–9.
6. Andrade AL, Martelli CM, Oliveira RM, et al. Short report: benznidazole efficacy among *Trypanosoma cruzi*-infected adolescents after a six-year follow-up. *Am J Trop Med Hyg* 2004;**71**:594–7.
7. Andrade ZA, Andrade SG, Correa R, et al. Myocardial changes in acute *Trypanosoma cruzi* infection; ultrastructural evidence of immune damage and the role of microangiopathy. *Am J Pathol* 1994;**144**:1403–11.
8. Archard LC, Richardson PJ, Olsen EG, et al. The role of coxsackie B viruses in the pathogenesis of myocarditis, dilated cardiomyopathy and inflammatory muscle disease. *Biochem Soc Symp* 1987;**53**:51–62.
9. Badorff C, Knowlton KU. Dystrophin disruption in enterovirus-induced myocarditis and dilated cardiomyopathy: from bench to bedside. *Med Microbiol Immunol (Berl)* 2004;**193**:121–6.
10. Badorff C, Berkely N, Mehrotra S, et al. Enteroviral protease 2A directly cleaves dystrophin and is inhibited by a dystrophin-based substrate analogue. *J Biol Chem* 2000;**275**:11191–7.
11. Badorff C, Fichtlscherer B, Rhoads RE, et al. Nitric oxide inhibits dystrophin proteolysis by coxsackieviral protease 2A through S-nitrosylation: A protective mechanism against enteroviral cardiomyopathy. *Circulation* 2000;**102**:2276–81.
12. Bairan AC, Cherry JD, Fagan LF, Codd Jr JE. Complete heart block and respiratory syncytial virus infection. *Am J Dis Child* 1974;**127**:264–5.
13. Barbaro G. Heart and HAART: two sides of the coin for HIV-associated cardiology issues. *World J Cardiol* 2010;**2**:53–7.
14. Baroldi G, Corallo S, Moroni M, et al. Focal lymphocytic myocarditis in acquired immunodeficiency syndrome (AIDS): a correlative morphologic and clinical study in 26 consecutive fatal cases. *J Am Coll Cardiol* 1988;**12**:463–9.
15. Begg MD. Diphtheric myocarditis: EKG study. *Lancet* 1937;**1**:857.
16. Bengtsson E, Lamberger B. Five-year follow-up study of cases suggestive of acute myocarditis. *Am Heart J* 1966;**72**:751–63.
17. Benirschke K, Kibrick S. Acute aseptic myocarditis and meningoencephalitis in the newborn child infected with coxsackievirus group B, type 3. *N Engl J Med* 1956;**255**:883–9.
18. Bergstrom K, Erikson U, Nordbring F, et al. Acute non-rheumatic myopericarditis: a follow-up study. *Scand J Infect Dis* 1970;**2**:7–16.
19. Bern C. Antitrypanosomal therapy for chronic Chagas' disease. *N Engl J Med* 2011;**364**:2527–34.
20. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis* 2009;**49**:e52–4.
21. Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of Chagas disease in the United States: a systematic review. *JAMA* 2007;**298**:2171–81.
22. Bowles NE, Richardson PJ, Olsen EG, Archard LC. Detection of coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. *Lancet* 1986;**1**:1120–3.
23. Bowles NE, Ni J, Kearney DL, et al. Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol* 2003;**42**:466–72.
24. Brightman VJ, Scott TF, Westphal M, Boggs TR. An outbreak of coxsackie B-5 virus infection in a newborn nursery. *J Pediatr* 1966;**69**:179–92.
25. Burch GE, Sun SC, Chu KC, et al. Interstitial and coxsackievirus B myocarditis in infants and children: a comparative histologic and immunofluorescent study of 50 autopsied hearts. *JAMA* 1968;**203**:1–8.
26. Calabrese F, Thiene G. Myocarditis and inflammatory cardiomyopathy: microbiological and molecular biological aspects. *Cardiovasc Res* 2003;**60**:11–25.
27. Campos MA, Closel M, Valente EP, et al. Impaired production of proinflammatory cytokines and host resistance to acute infection with *Trypanosoma cruzi* in mice lacking functional myeloid differentiation factor 88. *J Immunol* 2004;**172**:1711–8.
28. Cappell MS, Mikhail N, Ortega A. Toxoplasma myocarditis in AIDS. *Am Heart J* 1992;**123**:1728–9.
29. Carrasco H, Parada AH, Guerrero L, et al. Prognostic implications of clinical, electrocardiographic and hemodynamic findings in chronic Chagas' disease. *Int J Cardiol* 1994;**43**:27–38.
30. Chan KY, Iwahara M, Benson LN, et al. Immunosuppressive therapy in the management of acute myocarditis in children: a clinical trial. *J Am Coll Cardiol* 1991;**17**:458–60.
31. Chapman NM, Tracy S, Gauntt CJ, Fortmueller U. Molecular detection and identification of enteroviruses using enzymatic amplification and nucleic acid hybridization. *J Clin Microbiol* 1990;**28**:843–50.
32. Cherry JD, Jahn CL, Meyer TC. Paroxysmal atrial tachycardia associated with ECHO 9 virus infection. *Am Heart J* 1967;**73**:681–6.
33. Compton SJ, Celum CL, Lee C, et al. Trichinosis with ventilatory failure and persistent myocarditis. *Clin Infect Dis* 1993;**16**:500–4.
34. Dalldorf G, Gifford R. Clinical and epidemiologic observations of coxsackie-virus infection. *N Engl J Med* 1951;**244**:868–73.
35. Dao AH, Virmani R. Visceral larva migrans involving the myocardium: report of two cases and review of literature. *Pediatr Pathol* 1986;**6**:449–56.
36. De Jager H, Van Creveld S. Myocarditis in newborns, caused by coxsackie virus; clinical and pathological data. *Ann Paediatr* 1956;**187**:100–18.
37. Dec GW, Fallon JT, Southern JF, Palacios I. "Borderline" myocarditis: an indication for repeat endomyocardial biopsy. *J Am Coll Cardiol* 1990;**15**:283–9.
38. Dec Jr GW, Palacios IF, Fallon JT, et al. Active myocarditis in the spectrum of acute dilated cardiomyopathies: clinical features, histologic correlates, and clinical outcome. *N Engl J Med* 1985;**312**:885–90.
39. Dighiero J, Canabal EJ, Aguirre CV, et al. Echinococcus disease of the heart. *Circulation* 1958;**17**:127–32.
40. Doria A, Iaccarino L, Sarzi-Puttini P, et al. Cardiac involvement in systemic lupus erythematosus. *Lupus* 2005;**14**:683–6.
41. dos Santos RR, Rossi MA, Laus JL, et al. Anti-CD4 abrogates rejection and reestablishes long-term tolerance to syngeneic newborn hearts grafted in mice chronically infected with *Trypanosoma cruzi*. *J Exp Med* 1992;**175**:29–39.
42. Drew JH. Echo 11 virus outbreak in a nursery associated with myocarditis. *Aust Paediatr J* 1973;**9**:90–5.
43. Drucker NA, Colan SD, Lewis AB, et al. Gamma-globulin treatment of acute myocarditis in the pediatric population. *Circulation* 1994;**89**:252–7.
44. Dutra WO, Rocha MO, Teixeira MM. The clinical immunology of human Chagas disease. *Trends Parasitol* 2005;**21**:581–7.
45. Easton AJ, Eglin RP. The detection of coxsackievirus RNA in cardiac tissue by in situ hybridization. *J Gen Virol* 1988;**69**(Pt 2):285–91.
46. Edwards WD, Holmes Jr DR, Reeder GS. Diagnosis of active lymphocytic myocarditis by endomyocardial biopsy: quantitative criteria for light microscopy. *Mayo Clin Proc* 1982;**57**:419–25.
47. Eichenwald HF, Shinefield HR. Viral infections of the fetus and of the premature and newborn infant. *Adv Pediatr* 1962;**12**:249–305.
48. Fairweather D, Yusung S, Frisancho S, et al. IL-12 receptor beta 1 and Toll-like receptor 4 increase IL-1 beta- and IL-18-associated myocarditis and coxsackievirus replication. *J Immunol* 2003;**170**:4731–7.
49. Fenoglio Jr JJ, Ursell PC, Kellogg CF, et al. Diagnosis and classification of myocarditis by endomyocardial biopsy. *N Engl J Med* 1983;**308**:12–8.
50. Fine I, Brainerd H, Sokolow M. Myocarditis in acute infectious diseases; a clinical and electrocardiographic study. *Circulation* 1950;**2**:859–71.

51. Frid C, Bjarke B, Eriksson M. Myocarditis in children with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1986;**5**: 964–5.
52. Friedman RA, Kearney DL, Moak JP, et al. Persistence of ventricular arrhythmia after resolution of occult myocarditis in children and young adults. *J Am Coll Cardiol* 1994;**24**:780–3.
53. Fruhling L, Korn R, Lavillaureix J, et al. Chronic fibroelastic myoendocarditis of the newborn and the infant (fibroelastosis): new morphological, etiological and pathogenic data—relation to certain cardiac abnormalities. *Ann Anat Pathol (Paris)* 1962;**7**: 227–303.
54. Frustaci A, Russo MA, Chimenti C. Randomized study on the efficacy of immunosuppressive therapy in patients with virus-negative inflammatory cardiomyopathy: the TIMIC study. *Eur Heart J* 2009;**30**:1995–2002.
55. Fuse K, Chan G, Liu Y, et al. Myeloid differentiation factor-88 plays a crucial role in the pathogenesis of coxsackievirus B3-induced myocarditis and influences type I interferon production. *Circulation* 2005;**112**:2276–85.
56. Gagliardi MG, Bevilacqua M, Di Renzi P, et al. Usefulness of magnetic resonance imaging for diagnosis of acute myocarditis in infants and children, and comparison with endomyocardial biopsy. *Am J Cardiol* 1991;**68**:1089–91.
57. Gatmaitan BG, Chason JL, Lerner AM. Augmentation of the virulence of murine coxsackie-virus B-3 myocardiopathy by exercise. *J Exp Med* 1970;**131**:1121–36.
58. Gear JH, Measroch V. *South African Institute for Medical Research Annual Report for 1952. Johannesburg*. South Africa: South African Institute for Medical Research; 1953. p. 38–9.
59. Giles TD, Gohd RS. Respiratory syncytial virus and heart disease: a report of two cases. *JAMA* 1976;**236**:1128–30.
60. Goldfinger D, Schreiber W, Wosika PH. Permanent heart block following German measles. *Am Heart J* 1947;**2**:320.
61. Golgher D, Gazzinelli RT. Innate and acquired immunity in the pathogenesis of Chagas disease. *Autoimmunity* 2004;**37**: 399–409.
62. Gore I, Saphir O. Myocarditis: a classification of 1402 cases. *Am Heart J* 1947;**34**:827.
63. Grasso M, Arbustini E, Silini E, et al. Search for coxsackievirus B3 RNA in idiopathic dilated cardiomyopathy using gene amplification by polymerase chain reaction. *Am J Cardiol* 1992;**69**: 658–64.
64. Gray DF, Morse BS, Phillips WF. Trichinosis with neurologic and cardiac involvement: review of the literature and report of three cases. *Ann Intern Med* 1962;**57**:230–44.
65. Grist NR, Bell EJ. Coxsackie viruses and the heart. *Am Heart J* 1969;**77**:295–300.
66. Grist NR, Bell EJ. A six-year study of coxsackievirus B infections in heart disease. *J Hyg (Lond)* 1974;**73**:165–72.
67. Gutgesell HP, Paquet M, Duff DF, McNamara DG. Evaluation of left ventricular size and function by echocardiography: results in normal children. *Circulation* 1977;**56**:457–62.
68. Halsell JS, Riddle JR, Atwood JE, et al. Myopericarditis following smallpox vaccination among vaccinia-naive US military personnel. *JAMA* 2003;**289**:3283–9.
69. Hardarson HS, Baker JS, Yang Z, et al. Toll-like receptor 3 is an essential component of the innate stress response in virus-induced cardiac injury. *Am J Physiol Heart Circ Physiol* 2007;**292**: H251–8.
70. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;**6**: 1123–32.
71. Hastreiter AR, Miller RA. Management of primary endomyocardial disease: the myocarditis-endocardial fibroelastosis syndrome. *Pediatr Clin North Am* 1964;**11**:401–30.
72. Henson D, Mufson MA. Myocarditis and pneumonitis with type 21 adenovirus infection: association with fatal myocarditis and pneumonitis. *Am J Dis Child* 1971;**121**:334–6.
73. Hilton DA, Variend S, Pringle JH. Demonstration of coxsackie virus RNA in formalin-fixed tissue sections from childhood myocarditis cases by in situ hybridization and the polymerase chain reaction. *J Pathol* 1993;**170**:45–51.
74. Hobbs RE, Pelegrin D, Ratliff NB, et al. Lymphocytic myocarditis and dilated cardiomyopathy: treatment with immunosuppressive agents. *Cleve Clin J Med* 1989;**56**:628–35.
75. Hoyer MH, Fischer DR. Acute myocarditis simulating myocardial infarction in a child. *Pediatrics* 1991;**87**:250–3.
76. Huber SA. Autoimmunity in myocarditis: relevance of animal models. *Clin Immunol Immunopathol* 1997;**83**:93–102.
77. Huber SA, Feldman AM, Sartini D. Coxsackievirus B3 induces T regulatory cells, which inhibit cardiomyopathy in tumor necrosis factor-alpha transgenic mice. *Circ Res* 2006;**99**:1109–16.
78. Huber SA, Job LP, Woodruff JF. Lysis of infected myofibers by coxsackievirus B-3-immune T lymphocytes. *Am J Pathol* 1980;**98**: 681–94.
79. Hudson RE. The cardiomyopathies: order from chaos. *Am J Cardiol* 1970;**25**:70–7.
80. Hutchins GM, Vie SA. The progression of interstitial myocarditis to idiopathic endocardial fibroelastosis. *Am J Pathol* 1972;**66**: 483–96.
81. Jahn CL, Cherry JD. Mild neonatal illness associated with heavy enterovirus infection. *N Engl J Med* 1966;**274**:394–5.
82. Jin O, Sole MJ, Butany JW, et al. Detection of enterovirus RNA in myocardial biopsies from patients with myocarditis and cardiomyopathy using gene amplification by polymerase chain reaction. *Circulation* 1990;**82**:8–16.
83. Johnson JL, Lee LP. Complete atrioventricular heart block secondary to acute myocarditis requiring intracardiac pacing. *J Pediatr* 1971;**78**:312–6.
84. Joos HA, Yu PN. Electrocardiographic observations in poliomyelitis: changes of the Q-T interval in 23 cases. *Am J Dis Child* 1950;**80**:22–33.
85. Karjalainen J, Nieminen MS, Heikkila J. Influenza A1 myocarditis in conscripts. *Acta Med Scand* 1980;**207**:27–30.
86. Kereiakes DJ, Parmley WW. Myocarditis and cardiomyopathy. *Am Heart J* 1984;**108**:1318–26.
87. Kibrick S, Benirschke K. Severe generalized disease (encephalohepatomyocarditis) occurring in the newborn period and due to infection with coxsackie virus, group B: evidence of intrauterine infection with this agent. *Pediatrics* 1958;**22**:857–75.
88. Kilbourne ED, Smart KM, Pokorny BA. Inhibition by cortisone of the synthesis and action of interferon. *Nature* 1961;**190**: 650–1.
89. Kilbourne ED, Wilson CB, Perrier D. The induction of gross myocardial lesions by a coxsackie (pleurodynia) virus and cortisone. *J Clin Invest* 1956;**35**:362–70.
90. Kim HJ, Yoo GH, Kil HR. Clinical outcome of acute myocarditis in children according to treatment modalities. *Korean J Pediatr* 2010;**53**:745–52.
91. Kirchhoff LV, Gam AA, Gusmao RA, et al. Increased specificity of serodiagnosis of Chagas' disease by detection of antibody to the 72- and 90-kilodalton glycoproteins of *Trypanosoma cruzi*. *J Infect Dis* 1987;**155**:561–4.
92. Kirchhoff LV, Weiss LM, Wittner M, Tanowitz HB. Parasitic diseases of the heart. *Front Biosci* 2004;**9**:706–23.
93. Kishimoto C, Takada H, Kawamata H, et al. Immunoglobulin treatment prevents congestive heart failure in murine encephalomyocarditis viral myocarditis associated with reduction of inflammatory cytokines. *J Pharmacol Exp Ther* 2001;**299**:645–51.
94. Kubota T, Bounoutas GS, Miyagishima M, et al. Soluble tumor necrosis factor receptor abrogates myocardial inflammation but not hypertrophy in cytokine-induced cardiomyopathy. *Circulation* 2000;**101**:2518–25.
95. Kuhl U, Pauschinger M, Schwimmbeck PL, et al. Interferon-beta treatment eliminates cardiotropic viruses and improves left ventricular function in patients with myocardial persistence of viral genomes and left ventricular dysfunction. *Circulation* 2003; **107**:2793–8.
96. Kussy JC. Fatal mumps myocarditis. *Minn Med* 1974;**57**:285–6.
97. Leak D, Meghji M. Toxoplasmic infection in cardiac disease. *Am J Cardiol* 1979;**43**:841–9.
98. Lerner A, Wilson FM, Reyes MP. Enteroviruses and the heart (with special emphasis on the probable role of coxsackieviruses, group B, types 1-5). I. Epidemiological and experimental studies. *Mod Concepts Cardiovasc Dis* 1975;**44**:7–10.
99. Lewis W, Grupp IL, Grupp G, et al. Cardiac dysfunction occurs in the HIV-1 transgenic mouse treated with zidovudine. *Lab Invest* 2000;**80**:187–97.
100. Li K, Xu W, Guo Q, et al. Differential macrophage polarization in male and female BALB/c mice infected with coxsackievirus B3

defines susceptibility to viral myocarditis. *Circ Res* 2009;**105**:353–64.
101. Lim CH, Toh CC, Chia BL. Stokes-Adams attacks due to acute nonspecific myocarditis. *Am Heart J* 1949;**38**:123.
102. Lind J, Hulquist GT. Isolated myocarditis in newborn and young infants. *Am Heart J* 1949;**38**:123.
103. Liu P, Aitken K, Kong YY, et al. The tyrosine kinase p56(lck) is essential in coxsackievirus B3-mediated heart disease. *Nat Med* 2000;**6**:429–34.
104. Liu PP, Mason JW. Advances in the understanding of myocarditis. *Circulation* 2001;**104**:1076–82.
105. Logue BL, Hanson JL. Complete heart block in German measles. *Am Heart J* 1945;**30**:205.
106. Lozinski GM, Davis GG, Krous HF, et al. Adenovirus myocarditis: retrospective diagnosis by gene amplification from formalin-fixed, paraffin-embedded tissues. *Hum Pathol* 1994;**25**:831–4.
107. Luft BJ, Billingham M, Remington JS. Endomyocardial biopsy in the diagnosis of toxoplasmic myocarditis. *Transplant Proc* 1986;**18**:1871–3.
108. Machado FS, Jelicks LA, Kirchhoff LV, et al. Chagas heart disease: report on recent developments. *Cardiol Rev* 2012;**20**:53–65.
109. Markowitz SM, Martinez AJ, Duma RJ, Shiel FO. Myocarditis associated with primary amebic (*Naegleria*) meningoencephalitis. *Am J Clin Pathol* 1974;**62**:619–28.
110. Martin A, Webber BS, Fricker FJ, et al. Acute myocarditis: rapid diagnosis by PCR in children. *Circulation* 1994;**90**:330–9.
111. Martinelli FM, Sosa E, Nishioka S, et al. Clinical and electrophysiologic features of syncope in chronic chagasic heart disease. *J Cardiovasc Electrophysiol* 1994;**5**:563–70.
112. Mason JW, Billingham ME, Ricci DR. Treatment of acute inflammatory myocarditis assisted by endomyocardial biopsy. *Am J Cardiol* 1980;**45**:1037–44.
113. Mason JW, O'Connell JB, Herskowitz A, et al. A clinical trial of immunosuppressive therapy for myocarditis. The Myocarditis Treatment Trial Investigators. *N Engl J Med* 1995;**333**:269–75.
114. Matsumori A. Molecular and immune mechanisms in the pathogenesis of cardiomyopathy: role of viruses, cytokines, and nitric oxide. *Jpn Circ J* 1997;**61**:275–91.
115. Matsumori A, Igata H, Ono K, et al. High doses of digitalis increase the myocardial production of proinflammatory cytokines and worsen myocardial injury in viral myocarditis: a possible mechanism of digitalis toxicity. *Jpn Circ J* 1999;**63**:934–40.
116. Matsumori A, Yamada T, Suzuki H, et al. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. *Br Heart J* 1994;**72**:561–6.
117. McNamara D, Holubkov MR, Starling RC, et al. Controlled trial of intravenous immune globulin in recent-onset dilated cardiomyopathy. *Circulation* 2001;**103**:2254–9.
118. Miklozek CL, Crumpacker CS, Royal HD, et al. Myocarditis presenting as acute myocardial infarction. *Am Heart J* 1988;**115**:768–76.
119. Miller III JJ, French JW. Myocarditis in juvenile rheumatoid arthritis. *Am J Dis Child* 1977;**131**:205–9.
120. Monrad ES, Matsumori A, Murphy JC, et al. Therapy with cyclosporine in experimental murine myocarditis with encephalomyocarditis virus. *Circulation* 1986;**73**:1058–64.
121. Montoya R, Dias JC, Coura JR. Chagas disease in a community in southeast Brazil. I. A serologic follow-up study on a vector controlled area. *Rev Inst Med Trop Sao Paulo* 2003;**45**:269–74.
122. Morris SA, Tanowitz HB, Wittner M, Bilezikian JP. Pathophysiological insights into the cardiomyopathy of Chagas' disease. *Circulation* 1990;**82**:1900–9.
123. Mott KE, Hagstrom JW. The pathologic lesions of the cardiac autonomic nervous system in chronic Chagas' myocarditis. *Circulation* 1965;**31**:273–86.
124. Mowat NA, Bennett PN, Finlayson JK, et al. Myopericarditis complicating ulcerative colitis. *Br Heart J* 1974;**36**:724–7.
125. Muir P. Enteroviruses and heart disease. *Br J Biomed Sci* 1993;**50**:258–71.
126. Neches WH, Park SC, Lenox CC, et al. Left atrial myxoma: clinical presentation suggesting acute myocarditis. *JAMA* 1974;**229**:1906–7.
127. Negishi H, Osawa T, Ogami K, et al. A critical link between Toll-like receptor 3 and type II interferon signaling pathways in antiviral innate immunity. *Proc Natl Acad Sci USA* 2008;**105**:20446–51.
128. Ni J, Bowles NE, Kim YH, et al. Viral infection of the myocardium in endocardial fibroelastosis: molecular evidence for the role of mumps virus as an etiologic agent. *Circulation* 1997;**95**:133–9.
129. Okuni M, Takamiya Y. Primary myocardial disease in children, with special references to idiopathic myocarditis. *Jpn Circ J* 1971;**35**:771–6.
130. Oliver JM, Sotillo JF, Dominguez FJ, et al. Two-dimensional echocardiographic features of echinococcosis of the heart and great blood vessels: clinical and surgical implications. *Circulation* 1988;**78**:327–37.
131. Olsen EG. The role of biopsy in the diagnosis of myocarditis. *Herz* 1985;**10**:21–6.
132. Opavsky MA, Penninger J, Aitken K, et al. Susceptibility to myocarditis is dependent on the response of alphabeta T lymphocytes to coxsackieviral infection. *Circ Res* 1999;**85**:551–8.
133. Orinius E, Pernow B. Primary cardiomyopathy: a prospective clinical and physiological study. *Acta Med Scand* 1972;**192**:55–66.
134. Osama SM, Krishnamurti S, Gupta DN. Incidence of myocarditis in varicella. *Indian Heart J* 1979;**31**:315–20.
135. Palacios-Pru E, Carrasco H, Scorza C, Espinoza R. Ultrastructural characteristics of different stages of human chagasic myocarditis. *Am J Trop Med Hyg* 1989;**41**:29–40.
136. Pinazo MJ, Espinosa G, Gallego M, et al. Successful treatment with posaconazole of a patient with chronic Chagas disease and systemic lupus erythematosus. *Am J Trop Med Hyg* 2010;**82**:583–7.
137. Pomerance A. Classification of the secondary cardiomyopathies: the pathologist's view. *Postgrad Med J* 1972;**48**:714–21.
138. Puljiz I, Beus A, Kuzman I, Seiwerth S. Electrocardiographic changes and myocarditis in trichinellosis: a retrospective study of 154 patients. *Ann Trop Med Parasitol* 2005;**99**:403–11.
139. Randolph AG, Vaughn F, Sullivan R, et al. Critically ill children during the 2009-2010 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
140. Rantakallio P, Lapinleimu K, Mantyjarvi R. Coxsackie B 5 outbreak in a newborn nursery with 17 cases of serous meningitis. *Scand J Infect Dis* 1970;**2**:17–23.
141. Redline RW, Genest DR, Tycko B. Detection of enteroviral infection in paraffin-embedded tissue by the RNA polymerase chain reaction technique. *Am J Clin Pathol* 1991;**96**:568–71.
142. Reilly JM, Cunnion RE, Anderson DW, et al. Frequency of myocarditis, left ventricular dysfunction and ventricular tachycardia in the acquired immune deficiency syndrome. *Am J Cardiol* 1988;**62**:789–93.
143. Rezkalla S, Kloner RA, Khatib G, Khatib R. Beneficial effects of captopril in acute coxsackievirus B3 murine myocarditis. *Circulation* 1990;**81**:1039–46.
144. Rosenbaum HD, Nadas AS, Neuhauser EB. Primary myocardial disease in infancy and childhood. *Am J Dis Child* 1953;**86**:28–44.
145. Rosenberg HS, McNamara DG. Acute myocarditis in infancy and childhood. *Prog Cardiovasc Dis* 1964;**7**:179–97.
146. Ross LJ. Electrocardiographic findings in measles. *Am J Dis Child* 1952;**83**:282–91.
147. Rossi MA, Ramos SG, Bestetti RB. Chagas' heart disease: clinical-pathological correlation. *Front Biosci* 2003;**8**:e94–109.
148. Sahn DJ, Vaucher Y, Williams DE, et al. Echocardiographic detection of large left to right shunts and cardiomyopathies in infants and children. *Am J Cardiol* 1976;**38**:73–9.
149. Sainani GS, Dekate MP, Rao CP. Heart disease caused by coxsackie virus B infection. *Br Heart J* 1975;**37**:819–23.
150. Saphir O, Simon WA. Myocarditis in children. *Am J Dis Child* 1944;**67**:294–312.
151. Saphir O, Field M. Complications of myocarditis in children. *J Pediatr* 1954;**45**:457–63.
152. Satoh M, Tamura G, Segawa I, et al. Expression of cytokine genes and presence of enteroviral genomic RNA in endomyocardial biopsy tissues of myocarditis and dilated cardiomyopathy. *Virchows Archiv* 1996;**427**:503–9.
153. Schieken RM, Myers MG. Complete heart block in viral myocarditis. *J Pediatr* 1975;**87**:831–2.
154. Schmidt-Lucke C, Spillmann F, Bock T, et al. Interferon beta modulates endothelial damage in patients with cardiac persistence of human parvovirus b19 infection. *J Infect Dis* 2010;**201**:936–45.

155. Schowengerdt KO, Ni J, Denfield SW, et al. Diagnosis, surveillance, and epidemiologic evaluation of viral infections in pediatric cardiac transplant recipients with the use of the polymerase chain reaction. *J Heart Lung Transplant* 1996;**15**:111–23.
156. Scott III LP, Gutelius MF, Parrott RH. Children with acute respiratory tract infections: an electrocardiographic survey. *Am J Dis Child* 1970;**119**:111–3.
157. Soongswang J, Durongpisitkul K, Nana A, et al. Cardiac troponin T: a marker in the diagnosis of acute myocarditis in children. *Pediatr Cardiol* 2005;**26**:45–9.
158. Spencer HC, Allain DS, Sulzer AJ, Collins WE. Evaluation of the micro enzyme-linked immunosorbent assay for antibodies to *Trypanosoma cruzi*. *Am J Trop Med Hyg* 1980;**29**:179–82.
159. Spencer MJ, Cherry JD, Adams FH, Byatt PH. Supraventricular tachycardia in an infant associated with a rhinoviral infection [Letter]. *J Pediatr* 1975;**86**:811–2.
160. Spink WW. Cardiovascular complications of trichinosis. *Arch Intern Med* 1935;**56**:238–45.
161. Sprague WW. The effects of malaria on the heart. *Am Heart J* 1946;**31**:426–30.
162. Starc TJ, Lipshultz SE, Easley KA, et al. Incidence of cardiac abnormalities in children with human immunodeficiency virus infection: the prospective P2C2 HIV study. *J Pediatr* 2002;**141**: 327–34.
163. Strain JE, Grose RM, Factor SM, Fisher JD. Results of endomyocardial biopsy in patients with spontaneous ventricular tachycardia but without apparent structural heart disease. *Circulation* 1983;**68**: 1171–81.
164. Tahernia AC. Electrocardiographic abnormalities and serum transaminase evels in diphtheritic myocarditis. *J Pediatr* 1969;**75**: 1008–14.
165. Take M, Sekiguchi M, Hiroe M, Hirosawa K. Long-term follow-up of electrocardiographic findings in patients with acute myocarditis proven by endomyocardial biopsy. *Jpn Circ J* 1982;**46**:1227–34.
166. Tarleton R, Zhang LL, Downs MO. "Autoimmune rejection" of neonatal heart transplants in experimental Chagas disease is a parasite-specific response to infected host tissue. *Proc Natl Acad Sci USA* 1997;**94**:3932–7.
167. Taylor LA, Carthy CM, Yang DC, et al. Host gene regulation during coxsackievirus B3 infection in mice: assessment by microarrays. *Circ Res* 2000;**87**:328–34.
168. Theologides A, Kennedy BJ. Toxoplasmic myocarditis and pericarditis. *Am J Med* 1969;**47**:169–74.
169. Thisyakorn U, Wongvanich J, Kumpeng V. Failure of corticosteroid therapy to prevent diphtheritic myocarditis or neuritis. *Pediatr Infect Dis* 1984;**3**:126–8.
170. Tiula E, Leinikki P. Fatal cytomegalovirus infection in a previously healthy boy with myocarditis and consumption coagulopathy as presenting signs. *Scand J Infect Dis* 1972;**4**:57–60.
171. Tomioka N, Kishimoto C, Matsumori A, Kawai C. Effects of prednisolone on acute viral myocarditis in mice. *J Am Coll Cardiol* 1986;**7**:868–72.
172. Towbin JA, Griffin LD, Martin AB, et al. Intrauterine adenoviral myocarditis presenting as nonimmune hydrops fetalis: diagnosis by polymerase chain reaction. *Pediatr Infect Dis J* 1994;**13**: 144–50.
173. Unverferth DV, Fetters KK, Uretsky B. Right versus left heart biopsies: different information. *Circulation* 1984;**70**(Suppl II):402.
174. Vargo TA, Singer DB, Gillatte PC, Fernbach DJ. Myocarditis due to visceral larva migrans. *J Pediatr* 1977;**90**:322–3.
175. Wada H, Saito K, Kanda T, et al. Tumor necrosis factor-alpha (TNF-alpha) plays a protective role in acute viralmyocarditis in mice: a study using mice lacking TNF-alpha. *Circulation* 2001; **103**:743–9.
176. Weber MW, Baldwin JS, Hall JW. Acute isolated myocarditis: review of the literature and a report of a case in a 10-year-old child. *Pediatrics* 1949;**3**:829–36.
177. Weiss LM, Liu XF, Chang KL, Billingham ME. Detection of enteroviral RNA in idiopathic dilated cardiomyopathy and other human cardiac tissues. *J Clin Invest* 1992;**90**:156–9.
178. Weiss LM, Movahed LA, Berry GJ, Billingham ME. In situ hybridization studies for viral nucleic acids in heart and lung allograft biopsies. *Am J Clin Pathol* 1990;**93**:675–9.
179. Weiss LM, Movahed LA, Billingham ME, Cleary ML. Detection of coxsackievirus B3 RNA in myocardial tissues by the polymerase chain reaction. *Am J Pathol* 1991;**138**:497–503.
180. Williams H, O'Reilly RN, Williams A. Fourteen cases of idiopathic myocarditis in infants and children. *Arch Dis Child* 1953; **28**:271–83.
181. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat Immunol* 2010;**11**:7–13.
182. Woodruff JF. Viral myocarditis: a review. *Am J Pathol* 1980; **101**:425–84.
183. Woodruff JF, Woodruff JJ. Involvement of T lymphocytes in the pathogenesis of coxsackie virus B3 heart disease. *J Immunol* 1974;**113**:1726–34.
184. Wright Jr HT, Miller A. Fatal infection in a newborn infant due to herpes simplex virus: report of a case diagnosed before death. *J Pediatr* 1965;**67**:130–2.
185. Xiong D, Lee GH, Badorff C, et al. Dystrophin deficiency markedly increases enterovirus-induced cardiomyopathy: a genetic predisposition to viral heart disease. *Nat Med* 2002;**8**:872–7.
186. Yamada T, Matsumori A, Sasayama S. Therapeutic effect of antitumor necrosis factor-alpha antibody on the murine model of viral myocarditis induced by encephalomyocarditis virus. *Circulation* 1994;**89**:846–51.
187. Yamashita T, Iwakura T, Matsui K, et al. IL-6-mediated Th17 differentiation through RORγt is essential for the initiation of experimental autoimmune myocarditis. *Cardiovasc Res* 2011;**91**: 640–8.
188. Yuan J, Yu M, Lin QW, et al. Th17 cells contribute to viral replication in coxsackievirus B3-induced acute viral myocarditis. *J Immunol* 2010;**185**:4004–10.
189. Zaragoza C, Ocampo C, Saura M, et al. The role of inducible nitric oxide synthase in the host response to coxsackievirus myocarditis. *Proc Natl Acad Sci USA* 1998;**95**:2469–74.
190. Zwelzer WM. Infantile toxoplasmosis. *Arch Pathol* 1944;**38**: 1–19.

CHAPTER 29

Acute Rheumatic Fever

Diana R. Lennon

Acute rheumatic fever is an inflammatory disease of the heart, joints, central nervous system, and subcutaneous tissues that develops after a nasopharyngeal infection by one of the group A β-hemolytic streptococci. The pathogenesis of this disease, a clinical syndrome without a specific diagnostic test, remains an enigma, and specific treatment is unavailable. Prevention of initial and recurrent attacks is possible, however, with penicillin treatment and prophylaxis. Rheumatic fever is especially important because of the heart disease that often ensues, and such disease may lead to chronic progressive damage and premature death. As succinctly stated by Lasègue many years ago, "Rheumatic fever licks the joints and bites the heart"[221]—a statement that holds true today.

The unexpected upsurge in this disease in the United States in the late 1980s and 1990s, reports of invasive group A streptococcal infections, the honing of echocardiography in the diagnosis of rheumatic heart disease, and increased effort in the search for a vaccine have renewed interest in group A streptococci.

Globally, recognition that rheumatic fever is the leading cause of acquired heart disease in children and young adults worldwide led to action at the close of the century.[178] Action has intensified recently after a landmark paper suggesting the global rheumatic heart disease burden may be considerably more than previously thought.[149]

EPIDEMIOLOGY

The overall incidence and severity of acute rheumatic fever have decreased in recent years in developed Western countries and in prosperous countries of Asia.[2] Reliable morbidity data on the occurrence of acute rheumatic fever in total populations are lacking because studies often consider only a segment of a population. The trend seems clear, however. Some of the best long-term data come from Denmark, where rheumatic fever has been a reportable disease for many years.[243] A steady decline has been occurring since 1900, except for a peak during World War II (Fig. 29-1). In the United States, rheumatic fever is not a reportable disease, but mortality rates (Fig. 29-2) and hospital discharge rates (Fig. 29-3 and Table 29-1) have shown a steady decline. Although this decline was already under way, it seems to have been accelerated by the introduction of penicillin.[153]

The dramatic decline in the incidence of rheumatic fever began in the United States in the late 1940s (see Fig. 29-2). During the late 1950s, the 1960s, and the early 1970s, studies in the United States showed annual rates of 13.5 to 62.5 first attacks per 100,000 children 5 to 14 years of age; however, these studies are not strictly comparable in design.* Some of them were conducted in low-income urban areas, locations in which the incidence of rheumatic fever was thought to be higher. Secondary prevention of rheumatic fever with penicillin prophylaxis to protect against recurrent attacks probably became widespread in the 1960s. Denny and colleagues[75] showed the possibility of preventing initial attacks with injectable penicillin in 1950 in military camps after the original observation by Massell and colleagues.[159] Similar conclusive controlled studies were not repeated in the general or pediatric populations or with oral penicillin.

The most compelling evidence that appropriate medical intervention helps reduce the number of initial attacks of rheumatic fever comes from a study by Gordis[97] of health care availability in an at-risk inner-city Baltimore population. The rate of acute rheumatic fever was reduced by 60 percent over the course of a decade in only the census tracts receiving a comprehensive care program, with that reduction occurring only in patients with an identifiable preceding clinical respiratory tract infection. A similar trend with small numbers of patients was seen for the Navajo and Papago American Indian populations in school-based intervention programs[17,61] and in an Alaskan program.[37] Other school-based interventions[184] have reduced streptococcal prevalence rates but have not gone the necessary further step to show a reduction in rheumatic fever morbidity in a controlled, carefully demarcated population. A recent systematic review and meta-analysis of available evidence supports the premise that a community- or school-based initiative can reduce rheumatic fever by an estimated approximately 60 percent (relative risk 0.41, 95% confidence interval [CI] 0.23, 0.70) acknowledging the eligible studies are of variable quality but are unlikely to be replicated.[135]

An unexplained high incidence of acute rheumatic fever persists in Hawaii, especially in Polynesian and part-Polynesian children.[55,129] Similarly, rates are higher in larger populations of Polynesian (including indigenous Maori) children in New Zealand,[140,168] although increasing evidence from that country points toward low socioeconomic status, including housing and income issues and associated factors such as a lesser degree of education playing a role.[62,115] In some areas of the continental United States, the rate of endemic rheumatic fever continues to exceed that of the general population in some children traditionally considered to be at risk (urban

*References 40, 61, 97, 180, 200, 209.

FIGURE 29-1 ■ Reported annual incidence of acute rheumatic fever in Denmark, 1862 to 1962. (Modified from Public Health Board of Denmark: reported rheumatic fever incidence in Denmark, 1862-1962. In: Vendsborg P, Hansen LF, Olesen KH. Decreasing incidence of a history of acute rheumatic fever in chronic rheumatic heart disease. *Cardiologia* 1968;**53**:332–40. Used with permission of S. Karger A. G., Basel.)

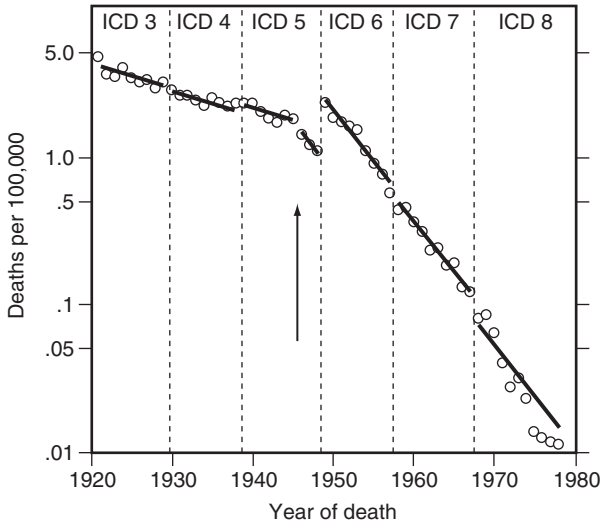

FIGURE 29-2 ■ U.S. national mortality rates from rheumatic fever in individuals 5 to 19 years old, 1921 through 1978. Values are age-adjusted to the 1950 U.S. population. Trend lines are fitted for each era (1921 through 1945 and 1946 through 1978) separately and take International Classification of Diseases (ICD) revisions into account. The *arrow* separates the two eras. (From Massell BF, Chute CG, Walker AM, Kurland GS. Penicillin and the marked decrease in morbidity and mortality from rheumatic fever in the United States. *N Engl J Med* 1988;**318**:280–6.)

blacks), although not in others (recent Hispanic immigrants).[84] In contrast, in a hospital-based study in another urban area, Hispanic children, reflecting the pediatric population being served, were the most affected.[104]

From the beginning of 1985, the number of patients with acute rheumatic fever in several centers on the U.S. mainland increased. Although these numbers were not large, they represented a definite increase in the outbreak areas (Table 29-2).[232] The resurgence in Utah since 1985 led to more than 600 cases.[157] Nationally, however, the number of diagnoses of acute rheumatic fever, in the absence of robust surveillance data, appeared to continue to decline gradually from 1984 through 1990.[232] The populations (aside from clusters in military populations)[9,247] generally did not seem to be those considered to have been at risk in the past; most patients were white and middle class and lived in suburban or rural communities with ready access to medical care. In the Utah and Tennessee outbreaks,[242,255] the families were larger than the state average. The military outbreaks[9,249] were the first in 2 decades in U.S. military personnel. Factors other than the widespread use of antibiotics and improved availability of health care are likely to be important because group A streptococci are rated moderately infectious agents in close contacts.[72,201] Overcrowded living circumstances long have been considered a risk factor.[183] This hypothesis was not substantiated as an important risk factor by conditional logistic regression in a modern (Yugoslavian) study[245] but was supported in a recent ecologic study, also exploring multivariant relationships where acute rheumatic fever rates are high.[114] The disquieting feature in these more recent U.S. outbreaks was the severity of the illness, especially in the Salt Lake City outbreak: 72 percent of patients had clinical carditis (Table 29-3), 19 percent had severe carditis with or without congestive heart failure, and 3 patients required mitral valve replacement. However, this report was from a cardiology center, which is likely to reflect a referral bias. Earlier U.S. reports documented clinical carditis in initial attacks of rheumatic fever in 40 to 51 percent of patients (1951 through 1965).[152] Longitudinal observations at the same institution suggest a decreasing frequency of clinical carditis (73% from 1921 to 1930, 51% from 1951 to 1960).[36,158]

With the use of Doppler echocardiography, carditis rates in the Utah patients increased to 91 percent (see Table 29-3). The duration of secondary penicillin prophylaxis and endocarditis prophylaxis are important considerations for patients with nonclinical carditis. The place of echocardiography in the diagnosis and management of rheumatic fever continues to evolve (see section on laboratory findings). Rheumatic fever presenting with arthritis may be misdiagnosed or not be admitted to

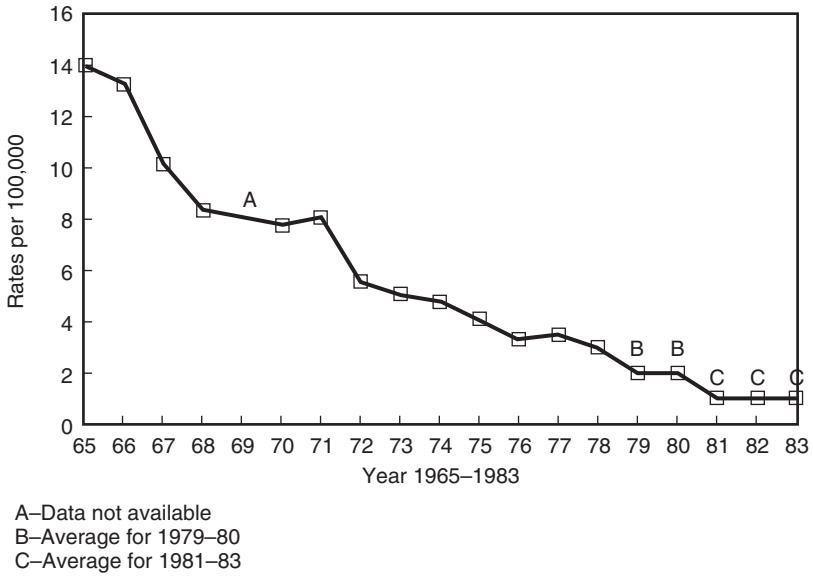

FIGURE 29-3 ■ Annual rates (per 100,000) of discharge of patients with rheumatic fever from short-stay nonfederal hospitals in the United States, 1965 to 1983. (From National Hospital Discharge Survey, NCHS. Modified from Gordis L. The virtual disappearance of rheumatic fever in the United States: lessons in the rise and fall of disease. *Circulation* 1985;**72**:1155–62.)

TABLE 29-1 **Reported Incidence of Acute Rheumatic Fever in Studies in the United States: 1970 to 1981**

Location	Years of Study	Rate/100,000	Age Range (Yr)
Fairfax, Va.	1970-1980	1.14	0-18
Rhode Island	1976-1980	0.23	5-17
Memphis, Tenn.	1977-1981	1.88	5-17
Baltimore, Md.	1977-1981	0.5	5-19
San Fernando, Calif.	1971-1980	0.63	5-17

Modified from Veasy LG, Wiedmeier SE, Orsmond GS, et al. Resurgence of acute rheumatic fever in the intermountain area of the United States. *N Engl J Med* 1987;**316**:421–7; and Markowitz M, Kaplan EL. Reappearance of rheumatic fever. *Adv Pediatr* 1989;**36**:44.

hospital, which is likely to affect estimates of rates of carditis in studies that are not population-based.[105]

A more recent assessment of hospitalized acute rheumatic fever disease in the United States in children younger than 21 years of age found a low rate of 14.8/100,000 hospitalized children (503 cases).[169] The prevalence of rheumatic heart disease, which represents the result of many years of exposure to the risks for acquiring acute rheumatic fever, seems to have declined in the United States over the course of many years.[174]

Many different racial and ethnic groups have been deemed unusually susceptible to rheumatic fever. They usually have been minority groups within a given area who are of lower socioeconomic status than the general population (e.g., Malays in Singapore, Arabs in Israel, Bantus in South Africa, indigenous Maori and Pacific Polynesians in New Zealand, blacks in the United States, aborigines in Australia).[2,62,107,151] In the United States, when differences in socioeconomic status or degree of crowding were taken into account,[100] the differences in the incidence of rheumatic fever,[99] prevalence of rheumatic heart disease,[174] and mortality from acute rheumatic fever and rheumatic heart disease generally declined or disappeared,[192] at least in the population studied. Rates in blacks were slow to decline, however. Rates became very low in the 1970s, at least in some areas, but cases persisted in other areas.[84,133]

Gordis and associates[98] caution that some other socioeconomically determined factor closely paralleling crowding could be the actual determinant of rheumatic fever. Most authorities agree that the reduction in the incidence and severity of rheumatic fever that has been noted in the United States and Western Europe might be due partly to a higher standard of living and less crowding. In a case-control study in the former Yugoslavia, home dampness, change of place of residence during the previous 5 years, low maternal education, body weight below normal, frequent sore throats, and a positive family history of rheumatic fever were found to be significant risk factors.[245]

A relationship between group A streptococcal throat infection and rheumatic fever was recognized in observations of the latter occurring after outbreaks of scarlet fever.[180] The attack rate noted after group A streptococcal throat infection varied widely (from 3% at Warren Air Force Base[194] to 0.39% in Chicago children).[211] Further observations in the latter study revealed that exudative pharyngitis, a positive throat culture with persistence of group A streptococci beyond 21 days, and the development of significant antibody (antistreptolysin O [ASO]) responses were associated with a higher attack rate that approached 3 percent in the children studied.

Rheumatic fever, similar to streptococcal pharyngeal infection, occurs most commonly in children 5 to 15 years of age. First attacks of rheumatic fever rarely occur in children younger than 3 years of age or in adults older than 40 years because of the relative infrequency of streptococcal infection at these ages and perhaps other factors.

The incidence of acute rheumatic fever is highest in the spring and winter months in temperate zones and coincides with the seasonal variation in streptococcal

TABLE 29-2 Reported Outbreaks of Acute Rheumatic Fever in the United States: Selected Epidemiologic Features

	Salt Lake City, UT[130,131]	Columbus, OH[59]	Akron, OH[33]	Pittsburgh, PA[132,144]	San Diego, CA[126]	Tennessee[137]*
Time	1985-1992	6/84-9/86	1986	1987-6/90	12/86-7/87	1/87-7/88
No. cases	274	40	23	60	50	26
White (%)	93	80	96	97	50	80
Family income	80% middle	73% middle	$20,000-$40,000	3 of 17 on assistance[132]	NA	$18,000
Suburban-rural residents	Most[130]	85%	Many	75%[137]	NA	20%
History of sore throat (*n*†)	77 (46)	22 (NA)	18 (NA)	4/17 (3/17)[132]; 28/43 (11/43)[144‡]	6 (3)	15 (7)
Family history of rheumatic fever (%)	NA	5	16	64[132]	NA	NA
Recurrences	27	1	0	2	1	0
M types of group A streptococci isolated from:						
Patients	1 × M-1; 1 × M-5	NA	M-1, M-5, M-18	NA	§	Mucoid M-18/T-1; mucoid nontypeable
Families	3 × M-3; 1 × M-1; 1 × M-18; 1 × M-78	NA	M-6	NA	NA	NA
Community	9 × M-18 (8/9 mucoid); 1 × M-4, M-5, M-6, M-9, M-11, M-12; 6 nontypeable	Mucoid M-18	NA	NA	NA	Mucoid M-18

Modified from Veasy LG, Wiedmeier SE, Orsmond GS, et al. Resurgence of acute rheumatic fever in the intermountain area of the United States. *N Engl J Med* 1987;**316**:421–7; and Markowitz M, Kaplan EL. Reappearance of rheumatic fever. *Adv Pediatr* 1989; **36**:39–68.
*Family size greater than the state average.
†Number who sought treatment.
‡Respiratory illness.
§San Diego: Five of seven available sera positive for antibodies to M-18; three of seven, positive for M-1 antibodies; two of seven, positive for M-5 and M-6 antibodies; and one of seven, positive for M-24 antibodies.
NA, Not available.

TABLE 29-3 Clinical Manifestations in Six Outbreaks of Acute Rheumatic Fever

Manifestation	Salt Lake City, Utah, 1985-1992 (274 Patients) (%)	Columbus, Ohio, 1984-1986 (40 Patients) (%)	Northeastern Ohio, 1986 (23 Patients) (%)	Pittsburgh, Penn, 1985-6/1990 (60 Patients) (%)	San Diego, Calif, Naval Base, 1986-1987 (10 Patients) (%)	Tennessee, 1987-1988 (26 Patients) (%)
Arthritis	36	62	78	43	100	58
Carditis	68*	50	30	52	30	73
Chorea	37	17	9	37	0	31
Erythema marginatum	4	12	1	0	0	4
Subcutaneous nodules	3	0	0	0	10	0

Modified from Markowitz M, Kaplan EL. Reappearance of rheumatic fever. *Adv Pediatr* 1989;**36**:39–68.
*85 percent with Doppler ultrasound examination.

pharyngitis. This incidence may be related to the greater tendency for spread of streptococcal infection by closer contact during the colder and damper months,[140,224,245] at least in some climates. In other climates, a seasonal peak for acute rheumatic fever is less pronounced.[34]

PATHOGENESIS

Evidence points toward acute rheumatic fever being preceded by a group A streptococcal upper respiratory tract infection. The events that occur after such infection and that culminate in rheumatic fever are poorly defined, suggesting a complex interaction of numerous factors. With the resurgence of interest in this disease after the outbreaks in the 1980s, laboratory data collected with the use of modern technologies have led to some new insights.[213,214] Pathogenesis involves the host, the environment (see section on epidemiology), and group A streptococci, individually and collectively.

So-called rheumatogenic strains of group A streptococci have been the subject of much discussion, more recently in relation to the latest focal upsurges in rheumatic fever.[30,31,119] Because no factor has been described or isolated, however, such strains remain a hypothesis. To date, rheumatic fever has been shown to occur only after nasopharyngeal infection,[251] although debate and research continue.[65,117] Why the site of infection seems to predispose individuals to the development of rheumatic fever remains an enigma, perhaps related to skin lipids.[118] Acute glomerulonephritis develops after skin or throat infections with a nephritogenic type of group A streptococci (e.g., M-49 and M-12).[251]

Certain streptococcal M protein serotypes are implicated strongly and repetitively in epidemics of acute rheumatic fever. Serotypes M-3, M-5, M-14, M-18, and M-24 have been reported more than once in outbreaks, and M-1, M-6, M-19, M-27, and M-29 have been reported once only.[29] Distinct nucleotide sequences that determine different gene subfamilies encoding the M or M-like protein antigenic domain (*emm* gene) may be the cause of these variations in streptococcal rheumatogenic potential, depending on the site of infection.[28] Other equally prevalent M types rarely, if ever, have been associated with epidemics of the disease[29] or have failed to cause recurrences in susceptible patients.[32] The current resurgence lends limited support for this concept: No predominance of a single serotype within a specific geographic zone was identified in any of the published outbreaks (see Table 29-2). Specific M types and their production of mucoidal colonies, considered to be related to the amount of M protein and virulence,[252] may be more relevant to epidemic than to endemic rheumatic fever.

In Auckland, New Zealand, where rheumatic fever is endemic, in a large urbanized minority population an average of 50 new cases of acute rheumatic fever currently occur annually in children aged 5 to 14 years of age (annual age-specific rate of 25/100,000/yr; Maori and Pacific 60/100,000).[114] Nine years (1984 to 1992) of surveillance of group A streptococcal isolates from hospitalized pediatric patients (one centralized children's facility for 8 of the 9 years in question) yielded 2410 isolates. Only 3 of 38 throat isolates (32 from well-documented cases of rheumatic fever, 6 from siblings) were strains described as possibly rheumatogenic (one each of M-1, M-3, and M-6).[156] None was described as mucoidal.[156] In that series, M types 6, 53, 55, and 66 (and NZ 1437, now known as M-89, when sibling isolates were included as cases) were statistically more likely to be associated with a case of acute rheumatic fever. In the more recent era, isolates associated with acute rheumatic fever and pharyngitis in Hawaii (Table 29-4) are rarely seen in the continental United States.[78] Of importance, the assumed link between rheumatic fever, the disease, and the disease-causing *emm* types is only temporal. It may itself not be sufficient to build a hypothesis for a possible association of initial infection site and acute rheumatic fever.

Strain selectivity is a further consideration. No documented evidence has shown that all members of an M type may be equally able to elicit acute rheumatic fever. Some streptococci from a particular serotype may be associated with acute rheumatic fever and acute poststreptococcal glomerulonephritis, although the two sequelae rarely occur simultaneously.[146,153,156] M types have been shown to be composed of genetically diverse streptococci, not all of which may be established within a community.[155] Genetic analysis of serotype M18 from acute rheumatic fever cases in Utah separated by 12 years showed strains nearly genetically identical.[213] The M type denotes possibly nothing more than a shared type-specific marker, with the property of rheumatogenicity as yet remaining elusive. Streptococcal strains that are opacity

TABLE 29-4 **Distribution of *emm* Types Among Patients with Acute Rheumatic Fever (ARF) in Hawaii Compared with Pharyngitis Isolates from Hawaii and Sterile Site Isolates from the Continental United States**

emm Types Isolated from Patients with ARF in Hawaii	No. of Pharyngitis Isolates from Hawaii	No. of Isolates from Continental United States
65/69*	12	4
71	14	1
92	16	50
93	2	0
98	6	1
103	4	1
122	1	0

From Erdem G, Mizumoto C, Esaki D, et al. Group A streptococcal isolates temporally associated with acute rheumatic fever in Hawaii: differences from the continental United States. *Clin Infect Dis* 2007;**45**:e20–4.

Note: There were 1258 total pharyngitis isolates from Hawaii and 3424 isolates (from the period 1995-2001) included in the Centers for Disease Control and Prevention surveillance data from the United States.

*Isolated from 2 patients.

factor–negative (a lipoprotein lipase) are unlikely to be rheumatogenic, according to some investigators.[119,255] This finding does not hold up in all geographic areas.[156] Surveillance of group A streptococci in multiple geographic zones is ongoing to guide vaccine development.

Although current evidence strongly implicates an immunologic mechanism in the pathophysiology of rheumatic fever, the details of how the disease develops are unclear.[31,64,157,222] Evidence to date strongly suggests an abnormal cell-mediated and humoral immune response to cell membrane streptococcal antigens, which, because of molecular mimicry of human tissues, may result in continued damage to the cardiovascular and nervous systems.[42,105,222] The findings of circulating immune complexes in most patients[63] and the deposition of C3 and immunoglobulin in the myocardium of patients dying of acute rheumatic fever support an abnormal immune response in rheumatic fever.[122]

M proteins from some rheumatogenic group A streptococcal types share antigenic determinants with myosin, with the sarcolemma of cardiac muscle,[68,69] and with antigens of articular cartilage and synovium.[21] The immune response to streptococci may mistake the host antigens as foreign and result in tissue damage. Other streptococcal antigens, such as the group A carbohydrate component, are candidates for mistaken cross-reaction with a glycoprotein in human heart valves.[96] Group A streptococci have components that can amplify or downregulate the immune response.[42] The mechanisms for molecular mimicry leading to central nervous system dysfunction are less defined.[157]

The site of the initial streptococcal infection may be important—lymphatic channels have been shown between the tonsils and the heart.[42] Unusual compartmentalization of rheumatic antigen–positive non-T cells has been shown in patients with acute rheumatic fever, with no positive cells detected in rheumatic tonsils but increased numbers in peripheral blood.[102]

Cell-mediated immunity to streptococcal antigens also is enhanced in patients with rheumatic fever.[222] The lymphocytic infiltrate of heart valves was found to be composed predominantly of $CD4^+$ helper cells.[193] Increased expression of human leukocyte antigen (HLA)-DR on fibroblasts, which can present antigens to $CD4^+$ lymphocytes (cytotoxic/suppressor T cells), has been observed on the heart valves of patients with acute carditis.[76] The cytotoxicity induced in normal human helper and suppressor cells in vitro by purified protein from a type M-5 group A streptococcal organism has been shown to destroy several human cell types, including cultured myocardial cells.[70] T-cell subset study results are conflicting,[172,222] but production of interleukins is reported to be enhanced.[172,173,268] The role of M protein and streptococcal pyrogenic exotoxins as superantigens is being explored and perhaps might explain the exaggeration of the streptococcal immune response.[154,235]

The genetic background of the human host seems to influence susceptibility to rheumatic fever.[41] Aggregation of rheumatic fever cases in families has been recognized for some time.[186] Low concordance for inheritance has been reported in monozygotic twins,[229] although affected siblings have significant concordance for arthritis, residual rheumatic heart disease, and chorea.[215] A recent systematic review and meta-analysis of twin studies estimated heritability across all studies of 60 percent, suggesting whole-genome scanning might provide a clinically useful genetic risk prediction tool for rheumatic fever and rheumatic heart disease.[77] Patarroyo and associates[179] found that the B lymphocytes of patients with rheumatic fever have a specific marker (883 alloantigen) associated with host rheumatic susceptibility.[265,266] It seems to transcend ethnicity,[110,179] although studies in India and Africa were less supportive, and may be similar to an immune response gene.[222,248] This work has been extended with the use of monoclonal antibodies to family members of patients with rheumatic fever.[83,197] Class I HLA molecules have not been associated with acute rheumatic fever. Many studies in different populations have shown an association with HLA-DR, but without a single HLA marker for susceptibility.[222] Genetic factors alone seem highly unlikely to be responsible for susceptibility to rheumatic fever.[153] No predictive marker for susceptibility to rheumatic fever has been defined to date. Studies in different populations have yielded conflicting results exploring the possibility that gene polymorphisms influence severity of heart disease in rheumatic fever patients.[20]

VACCINE DEVELOPMENT

Immunity to group A streptococci and to rheumatic fever is thought to depend largely on antibodies to M protein, a major virulence determinant; such antibodies can opsonize the bacteria in the presence of neutrophils.[132] Immunity is thought to be strain-specific and to depend on antibodies to the variable serotype-specific regions of the protein, and earliest vaccine development followed this pathway.[24] Two early landmark studies showed that vaccines containing purified M protein evoked protective immune response in humans.[25,189] Antibodies against the variable amino-terminal end of the M protein opsonize streptococci in a type-specific manner, but the results of experiments in animals suggest that the conserved carboxyl-terminal end also may be an immune target. Some human evidence suggests that this conserved epitope acts as a subunit vaccine.[190] Complexities in this area include the risk for inducing cross-reacting antibodies that could injure rather than protect.[19,160] Separation of the peptide fragments of M proteins (epitopes) that evoke type-specific and not cross-reacting antibodies was an important step.[39,63] Most of the cross-reactive epitopes identified have been located in the middle of the M proteins (Fig. 29-4) and can be separated from the N-terminal, hypervariable protective epitopes that evoke antibodies with the greatest bactericidal activity.[67]

More than 120 different defined serotypes (M types) of group A streptococci have been identified. In the 1990s, classic M-protein typing was largely replaced by sequence typing of the 5′ end of the M protein *(emm)* gene.[80] Vaccine development[31,35] falls into two broad approaches: vaccines based on common protective antigens of group A streptococci and multivalent vaccines based on type-specific N-terminal regions of the M protein.[67,106,219] A 26-valent vaccine with components

FIGURE 29-4 ■ Erythema marginatum in an 8-year-old girl with acute rheumatic fever.

guided by North American surveillance of acute rheumatic fever, invasive infections, and pharyngitis has been shown to be safe and immunogenic in adults.[166] This vaccine includes 80 to 90 percent of important serotypes in North America. More recent epidemiologic studies with additional data from North America and Europe, as well as some from developing countries, led to a 30-valent vaccine with broader coverage and promising animal model data against vaccine serotypes.[71] In addition, the researchers reported significant levels of bactericidal antibodies against a further 24 of 40 nonvaccine serotypes of group A streptococci.

However, despite 98 percent coverage of pharyngitis isolates in North America, preliminary data from prospective studies of pharyngitis infections in Mali and South Africa indicate only 40 percent and 59 percent coverage.[71] Epidemiologic studies of group A streptococci from low-income countries are sparse.[220] In general, a wider diversity of *emm* types are seen, indicating that vaccine coverage with current tools may be incomplete in other areas, such as in Hawaii,[78] Asia,[191] or New Zealand,[156] where rheumatic fever is endemic in some populations. More comprehensive information for serotype prevalence in such areas of high endemicity is urgently needed.

RHEUMATIC FEVER IN DEVELOPING COUNTRIES

Rheumatic heart disease is considered one of the few preventable chronic diseases.[12] Despite impressive declines in incidence in developed countries (see Figs. 29-1 to 29-3), globally, rheumatic heart disease remains the most common form of acquired heart disease.[128,266] Four fifths of the world's population live in developing countries, where the prevalence of rheumatic heart disease suggests that the incidence of acute rheumatic fever remains at high levels in areas characterized by crowded living quarters and lower socioeconomic conditions. In Soweto, South Africa, the prevalence of rheumatic heart disease in the 1970s was estimated by cardiac auscultation at 7.1/1000 schoolchildren.[165] A recent estimate using echocardiography in two developing country settings

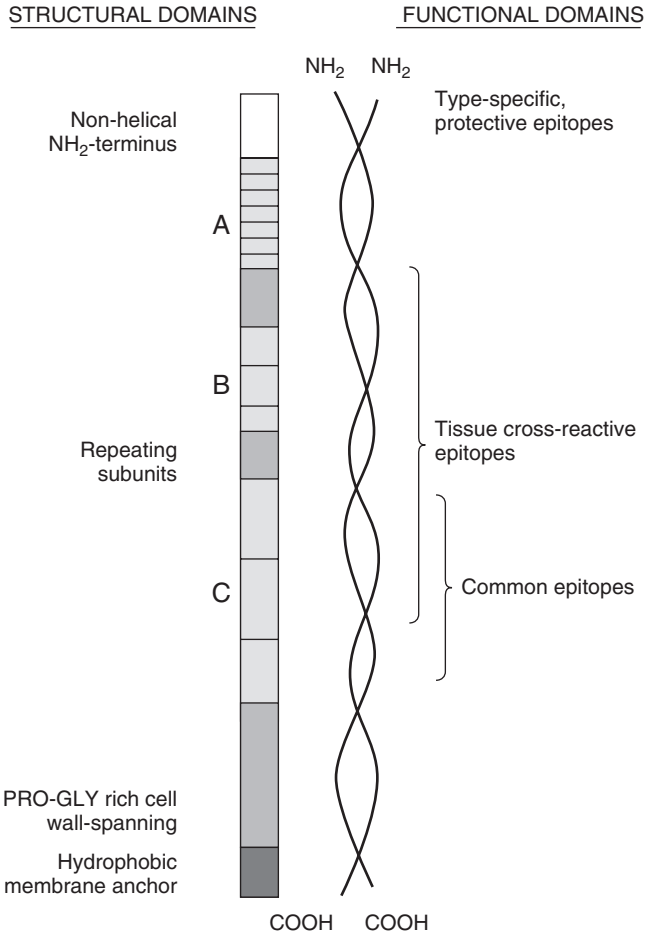

FIGURE 29-5 ■ Schematic representation of the structural and functional domains of streptococcal M protein (From Dale J. Current status of group A streptococcal vaccine development. In: Finn A, Pollard A, editors. *Hot topics in infection and immunity in children IV: Advances in experimental medicine and biology*, vol. 609, New York: Springer; 2008. p. 53–63.)

increased this estimate by nearly 10-fold.[149] Studies have been repeated in other settings (Fig. 29-5) with differing echocardiographic criteria but similar estimates of burden.[148] The true meaning of these findings in asymptomatic subjects in high-prevalence populations requires

a better understanding of the natural history of subclinical carditis, standardization of the echocardiographic criteria, and cost-benefit analyses in various settings. A recent consensus standardization guideline has been published.[199] Reliable, quality-controlled global supplies of benzathine penicillin for prevention of further attacks may need to be considered.[38]

An important recent prospective study in patients 14 years of age or older from Soweto highlights a significant disease burden of new-onset rheumatic heart disease presenting in the third and fourth decades of life,[212] suggesting a presumed unrecognized or subclinical earlier acute rheumatic fever episode most likely in childhood. The contribution of rheumatic heart disease to the burden of hospitalized disease resulting from strokes, atrial fibrillation, and other sequelae requires more elucidation.

Given the difference in medical care delivery, evaluation of the incidence of acute rheumatic fever must be viewed with caution. Estimates suggest, however, an annual incidence of 200 to 400 cases/100,000 population in Soweto.[127] The incidence of rheumatic fever (as judged by hospital admissions for rheumatic heart disease between 1966 and 1980) has remained stable in India during this period of rapid decline in the United States and the West.[2] Some recent publications have attempted to better define the global burden of acute rheumatic fever and rheumatic heart disease (and other group A streptococcal diseases) with variable rigor.[48,206,234] More detail is available from the World Health Organization. Community-based secondary penicillin prophylaxis programs in developing countries are considered cost-effective and more achievable than is primary prevention.[127]

PATHOLOGY

The unique pathologic lesion of rheumatic fever is the Aschoff body, which generally is considered to be a granuloma that results from injury to collagen fibers. Classically, Aschoff bodies are found in the heart, usually in the left atrial appendage, but similar foci can be found in the synovia of the joints and in and around joint capsules, tendons, and fascia.[202]

The early pathologic response to rheumatic fever may be an exudative reaction with Aschoff-like bodies as an inflammatory focus. They are cardiac or extracardiac, with a central area of fibrinoid necrosis surrounded mostly by polymorphonuclear leukocytes. Clinically, this condition may be manifested as arthritis and spontaneously subside in 2 to 4 weeks. No residual joint damage results. The proliferative phase of classic Aschoff nodules, which consists of central necrosis surrounded by a rosette of large mononuclear cells, giant multinuclear cells, and other cell types, is confined to the heart and usually causes pancarditis with simultaneous involvement of all three layers (i.e., the pericardium, the myocardium, and the endocardium). This event may result in permanent valvular damage in the following order of frequency: the mitral valve, the aortic valve, the tricuspid valve, and, rarely, the pulmonary valve.

The heart disease encountered clinically usually is mitral regurgitation, aortic regurgitation, or both. The scarring that leads to valvular stenosis (mitral or aortic) typically takes decades to develop but may occur much faster in hyperendemic areas. This process is not the full story, however, because although rheumatic mitral valve stenosis occurs more commonly in India[205] and occasionally in other less advantaged populations, it was never a common occurrence in the United States or the United Kingdom at the height of rheumatic fever incidence.[231]

The presence of Aschoff bodies is not evidence of rheumatic activity because these lesions are found in biopsy specimens of the left atrial appendage many years after an acute attack of rheumatic fever. Little is known about the pathology of Sydenham chorea, and the pathologic changes cannot be related to the clinical manifestations. Patients rarely die of this form of rheumatic fever.

CLINICAL COURSE

The stage is set for the development of rheumatic fever in a susceptible host after a pharyngeal infection by one of the types of group A β-hemolytic streptococci.[152,216] Not all serotypes (M or *emm* types) appear to be equally rheumatogenic (see earlier section on pathogenesis). Rheumatic fever develops in approximately 1 to 3 percent of children with known epidemic untreated exudative pharyngitis and a culture positive for group A streptococci. The frequency decreases to less than 1 percent, as shown in the one controlled study involving children,[211] when patients with less severe or less precisely diagnosed streptococcal infections are included. This finding has been replicated more recently in a large community- and school-based randomized, control trial with high consent and retention rates in New Zealand in an area with endemic rheumatic fever. The attack rate observed was approximately 0.2 percent.[140]

The preceding pharyngitis is not recognized as an illness by the patient or parents in approximately 10 to 33 percent of cases of acute rheumatic fever, although 50 to 60 percent of patients remember having a sore throat.[97] In the New Zealand trial, episodes of sore throat (with appropriately increased streptococcal serology) in this carefully monitored series preceded development of acute rheumatic fever in 14 of 19 (74%) of the cases enrolled in the program at the time of presentation.[141] In some case series, this figure is lower but the methodology is less vigorous (see Table 29-2). The infection is followed by a latent period that averages 19 days in duration,[194] during which time the patient seems well. The range seems to be between 1 and 5 weeks but has been difficult to establish.[50] In the New Zealand trial, the average latent period was 27 days (range, 2 to 49 days) for seven rheumatic cases with proven group A streptococcal pharyngitis and raised streptococcal titers.[141] The average latent period is the same for recurrent attacks as for initial episodes.[50]

Acute rheumatic fever then begins. Table 29-3 suggests a clinical profile in the United States, although

recurrent cases with their increased risk for carditis are included. In a prospective study in India, 67 percent of initial episodes were associated with migrating arthritis involving one or more of the large joints[204] accompanied by a fever of 38° to 39° C (100.4° to 102.2°F), malaise, and anorexia. Just as the redness, swelling, and pain in a knee subside, the whole process may start again in the ankle. The elbows and wrists are likely to be involved. Arthritis of the hip also occurs.[162] The joint involved may be exquisitely tender, with the patient exhibiting extreme reluctance to move the joint or mobilize. Typically, multiple joints are involved in tandem, with overlap over the course of time, when symptoms are not suppressed by anti-inflammatory therapy. The whole polyarthritic episode usually subsides over 4 weeks, with no residua remaining. The joint inflammation may be of low grade in some individuals, without limitation of motion or outward manifestations of redness and swelling (arthralgia). The literature supporting monoarthritis that does not develop into migrating polyarthritis in acute rheumatic fever is unconvincing.* The clinician should act cautiously, however, in an area endemic for rheumatic fever in the early phase of presentation. The development of polyarthritis in a patient with a culture-negative monoarthritis (e.g., a hip joint in a patient without prior antibiotic exposure) may be aborted by nonsteroidal anti-inflammatory drugs (NSAIDs).[258] An early echocardiogram may assist in establishing the diagnosis (see section on laboratory findings).[3]

Carditis generally appears early in the illness (first 2 to 3 weeks) if it is going to occur.[1] It may be silent in the absence of elevated fever or symptoms of pericarditis or cardiac failure. The clinical signs are the development of a new heart murmur, cardiac enlargement, congestive cardiac failure, a pericardial friction rub, or signs of cardiac effusion.

However, the mode of onset of acute rheumatic fever can be quite variable, and access to medical care, the health knowledge of the patient or family, or the awareness of health care personnel may be factors determining when in the illness the patient initially seeks medical care. Indolent carditis, as an insidious or even subclinical presentation now recognized by the Jones criteria (Table 29-5), may present without other manifestations to fulfill the Jones criteria as the initial episode is sometime in the past. This was a recognized presentation in the New Zealand school trial.[141]

In a classic presentation on examination, the striking findings are the patient's pallor and discomfort, especially on movement of the affected joints. Carditis usually appears within 3 weeks of the start of the illness, and in the absence of high fever or symptoms of acute pericarditis or congestive heart failure, it may be symptomatic.[1] Examination of the heart may reveal in at least half of patients a grade 2/4 apical pansystolic murmur that is transmitted to the axilla (mitral insufficiency) with or without an apical mid-diastolic flow murmur (Carey-Coombs murmur); half of these patients also may experience an early diastolic grade 2/4 murmur at the left sternal edge (aortic insufficiency). The latter can also occur alone. The tricuspid valve is not usually involved and the pulmonary valve very rarely. In addition, less commonly, the child can have congestive heart failure or cardiac enlargement, indicative of active carditis. Carditis is more likely to occur in younger children. Pericarditis may be suspected with muffled heart sounds, a frictional rub, or chest pain. It becomes less common as acute rheumatic fever in a population becomes less severe. Ruptured chordae tendineae in a patient with fulminant carditis may present as unilateral pulmonary edema.[8] Death is a rare but well-described sequela of the acute phase of the disease. Murmurs of mitral and aortic stenosis are associated with chronic but not with acute rheumatic valve disease.

Echocardiography in acute rheumatic fever cases with congestive cardiac failure has shown preserved left ventricular systolic function and/or severe mitral or aortic regurgitation.[79,91,239] Serum levels of cardiac troponin I are not elevated in acute rheumatic fever patients with heart failure.[257]

The distinctive rash, erythema marginatum, was observed in up to 12 percent of patients in these case series (Fig. 29-6; see Table 29-3). It is neither pruritic nor

*References 46, 109, 127, 141, 187, 258.

TABLE 29-5 Guidelines for the Diagnosis of an Initial Attack of Rheumatic Fever (Jones Criteria: 1992 Update)*

Major Manifestations†
Carditis
Polyarthritis
Chorea
Erythema marginatum
Subcutaneous nodules
Minor Manifestations†
Clinical findings
Arthralgia
Fever
Laboratory findings
Elevated acute-phase reactants
Elevated erythrocyte sedimentation rate
Elevated C-reactive protein
Prolonged PR interval
Supporting Evidence of Antecedent Group A Streptococcal Infection
Positive throat culture or rapid streptococcal antigen test
Elevated or rising streptococcal antibody titer

From Special Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease in the Young, American Heart Association. Guidelines for the diagnosis of rheumatic fever: Jones criteria, 1992 update. *JAMA* 1992;**268**:2069–73. Copyright 1992, American Medical Association.

*If supported by evidence of a preceding group A streptococcal infection, the presence of two major manifestations or of one major and two minor manifestations indicates a high probability of acute rheumatic fever.

†See text for details.

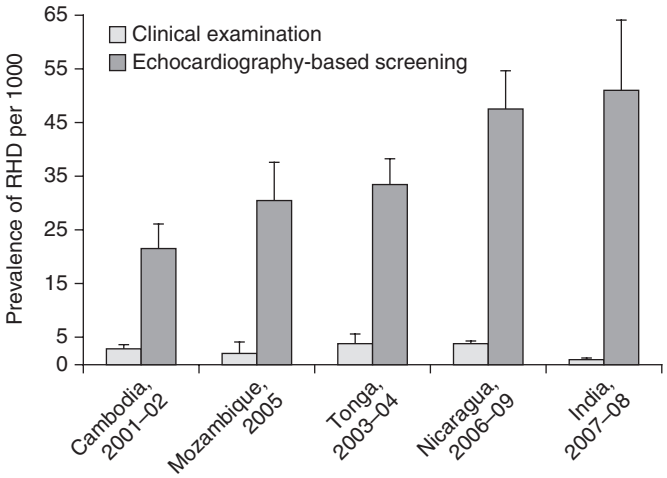

FIGURE 29-6 ■ Rheumatic heart disease (RHD) prevalence rates in children: echocardiography-based screening versus clinical examination. (From Marijon E, Mirabel M, Celermajer DS, Jouven X. Rheumatic heart disease. *Lancet* 2012;**379**:953–64.)

painful. The pink, slightly raised macules, usually seen initially on the trunk and proximal ends of the extremities and never on the face, fuse centrally and coalesce to form a serpiginous pattern. The lesions may disappear after a few hours or may reappear intermittently over the course of weeks, especially after a warm shower or bath.

Subcutaneous nodules, usually associated with severe carditis, also occur uncommonly (<10% of patients). They are firm and painless and are found over bony surfaces or prominences and over tendons. Acute rheumatic fever is not likely to be diagnosed on the basis of the latter two major criteria without another major criterion.

Sydenham chorea, or St. Vitus dance, may be the only manifestation of rheumatic fever, or it may be associated with other disease manifestations. All laboratory parameters, including streptococcal serology, may be normal. It becomes less common as acute rheumatic fever becomes less severe in a population, presumably as a result of improved awareness and earlier presentation of other manifestations of acute rheumatic fever. Chorea is characterized by purposeless (most often bilateral, uncoordinated, involuntary) movements, mostly of the hands, feet, and face, that develop over the course of weeks and are accentuated by excitement and emotional stress. They disappear during sleep. Sensation remains intact. The speech can be explosive and indistinct, and the handwriting can be clumsy. Handwriting is a useful objective means of monitoring the course of the disease. The child has difficulty counting rapidly and holding the protruded tongue still. The fingers and wrists are hyperextended when the fingers are outstretched, and the palms usually are turned outward when the arms are held above the head. Handgrip generally is weak and may consist of spasmodic contractions followed by rapid relaxation (so-called milkmaid grip). The patient may be easily irritated and quarrelsome. Chorea typically is a delayed manifestation of rheumatic fever and may develop after other signs of the disease have subsided, commonly appearing 2 to 6 months after the streptococcal infection. Historically, observers think residual heart disease occurs less commonly when chorea is the only manifestation of rheumatic fever, but in the echocardiographic era this hypothesis may prove not to be the case.[3] The importance of prophylaxis in those with chorea to prevent recurrent attacks and possible subsequent carditis was reaffirmed in Kuwait.[145] Permanent serious residual neurologic deficits have not been observed. A 25-year review found the duration of chorea to be 1 to 22 weeks, with a median of 12 weeks.[176] Rare cases may last 2 to 3 years.[4,15,44] Recurrent attacks are common and may occur despite faithful adherence to prophylaxis with intramuscular benzathine penicillin.[27,233] The neuropsychiatric sequelae of chorea were reviewed recently.[170]

Some cases of chorea are mild or atypical and may be confused with motor tics or the involuntary jerks of Tourette syndrome. Confusion may ensue between Sydenham chorea and these conditions. The term *pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection (PANDAS)* refers to a subgroup of children with tic or obsessive-compulsive disorder whose symptoms may develop or worsen after an episode of group A streptococcal infection. The following five criteria have been used to define the PANDAS subgroup[130,227]:

1. The presence of a tic disorder, obsessive-compulsive disorder, or both
2. Prepubertal age of onset (usually between 3 and 12 years of age)
3. Abrupt onset of symptoms, episodic course of symptom severity, or both
4. Temporal association between exacerbations of symptoms and streptococcal infection (approximately 7 to 14 days)
5. Presence of neurologic abnormalities during periods of exacerbation of symptoms (typically adventitious movements or motoric hyperactivity)

The evidence supporting PANDAS as a distinct disease entity has been questioned.[130] In any population with a high prevalence of acute rheumatic fever, clinicians should rarely (if ever) make a diagnosis of PANDAS and rather should err on the side of overdiagnosis of acute rheumatic fever and secondary prophylaxis. If acute rheumatic fever is excluded, secondary prophylaxis is not needed, but these patients should be followed carefully to ensure they do not develop carditis in the long term (see Chapter 82).

The average duration of an attack of acute rheumatic fever is approximately 3 months when unaltered by anti-inflammatory therapy.[152] Fewer than 5 percent of cases persist longer than 6 months with active symptoms, so-called chronic rheumatic fever.[229]

LABORATORY FINDINGS

The degree of inflammation in patients with acute rheumatic fever is measured by nonspecific indicators, such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).[152,216] Unless the patient has taken corticosteroids or salicylates, these test results almost always are

positive in patients with polyarthritis or acute carditis but are normal in patients with chorea. The magnitude of the ESR is proportional to the intensity of the inflammatory reaction but is not site-specific (i.e., it can be high with polyarthritis or carditis). The ESR is typically more than 80 mm/hr in acute rheumatic fever.[142] The ESR may be decreased in congestive heart failure, whereas the CRP may be elevated in congestive heart failure attributable to any cause. The ESR may remain elevated for 6 weeks to 3 months in an untreated attack of acute rheumatic fever. Anti-inflammatory agents may reduce the ESR, but it rebounds if the drugs are stopped before the rheumatic process has run its course. Although chronic elevation of the ESR (>6 months) is not understood, it is not sufficient reason on its own to limit a patient's activities.[230] The CRP may reflect the patient's rheumatic activity more precisely than may the ESR.[152]

Chest radiographs are useful for detecting cardiomegaly, which may be caused by dilation, preexisting heart disease, or pericardial effusion. The degree of enlargement is helpful in judging severity. The electrocardiogram may show a prolonged atrioventricular conduction time, usually evidenced by a prolonged PR interval or even greater degrees of heart block.[196] Generally, an increase in the PR interval in tracings with comparable rates is considered significant.[152] Upper limits of normal by age are available.[142] Atrioventricular conduction abnormalities per se bear no relation to the ultimate prognosis of patients. Changes of myocarditis and pericarditis also are seen.

Every patient with suspected rheumatic fever should be considered for an echocardiogram. The roles of two-dimensional and Doppler echocardiography in establishing the diagnosis and determining the prognosis of acute rheumatic fever are becoming clearer.* In a prospective blinded study using febrile controls and strict color and pulsed Doppler criteria, pathologic left-sided heart regurgitation could be differentiated from physiologic regurgitation.[1] Several centers using similar strict echocardiographic guidelines have observed subclinical carditis in acute rheumatic fever.[87,88,241,242] The status of echocardiographic evidence as a major or minor Jones criterion remains to be settled by the American Heart Association.[85] It has important implications for patients with polyarthritis or chorea as a sole major criterion[3,216] or patients without major criteria and only echocardiographic evidence of mitral or aortic regurgitation. In an area of high endemicity (New Zealand), strict criteria have evolved and are incorporated into common usage.[16,142]

However, in a prospective randomized trial of prevention of acute rheumatic fever (see earlier discussion), only three additional cases (3 of 59 [5%]) met the case definition for acute rheumatic fever using the New Zealand Rheumatic Fever diagnostic criteria, which allows echocardiographic carditis to be a major or minor criterion in the presence of other major or minor criteria and evidence of preceding streptococcal infection.[141] Every case was scrutinized by an independent group of clinicians, including a cardiologist.[141] Australia also has incorporated echocardiographic criteria into guidelines.[47] Publications included in a systematic review found a prevalence of 17 percent of subclinical carditis in acute rheumatic fever.[237] However, no comment was made in this paper on the use of echocardiographic-only carditis as a stand-alone criterion, beyond the current Jones criteria.[216] Careful studies on the long-term prognostic significance of subclinical carditis need to be performed.[43]

In 2012, an international consortium published guidelines on minimal criteria for a diagnosis of rheumatic heart disease with echocardiography.[199] These criteria do not address the differentiation between acute carditis and chronic rheumatic heart disease. The criteria for pathologic regurgitation are unlikely to be different and have been incorporated into other guidelines.[16,47,142] Morphologic changes may be different in acute rheumatic fever and require clarification. Assessment of left ventricular size and function are an important part of the evaluation of a patient with rheumatic fever, especially if surgery is being considered.[91]

A positive throat culture for group A β-hemolytic streptococci as evidence of a recent streptococcal infection seldom is found in an acute rheumatic fever case, although 50 percent of such patients could be carriers of the organism.[123] In the New Zealand randomized trial, of patients with rheumatic fever who reported sore throat episodes (n = 14), 57 percent (8 of 14) had a positive throat culture and appropriately increased serology.[141] A positive culture may be helpful if it can be related to the time of the acute infection.

Corroboration of a previous streptococcal infection is better determined serologically. Many streptococcal antibody tests are available.[207,256] Antibody titers may be elevated in the absence of clinical or bacteriologic evidence of streptococcal pharyngitis (5 of 19 acute rheumatic fever cases in the New Zealand program).[141] The ASO titer is the most popular antibody test. It measures the inhibition of rabbit red blood cells by specific antibody to streptolysin O, an extracellular product of β-hemolytic streptococci that in its reduced form hemolyzes red blood cells. The "normal" level for an ASO titer usually is defined as the highest titer exceeded by only 20 percent of a population, but it is influenced significantly by age, geography, season, and other factors.[120,207] ASO titers of 500 Todd units or greater are rare findings in normal schoolchildren and are good evidence of a recent streptococcal infection. ASO titers less than 250 Todd units could be considered normal; titers of 250 to 320 Todd units should be considered borderline elevated. Approximately 50 percent of patients with acute rheumatic fever have ASO titers in this range, and approximately 60 percent have titers of 500 Todd units or greater.[216] Conversely, ASO titers can be normal in 20 percent of patients with acute rheumatic fever.[223]

A recent streptococcal infection is more likely to be shown if more than one antibody titer is measured (e.g., antistreptokinase and antihyaluronidase).[223] Anti–deoxyribonuclease B is currently the most favored test because of its better reproducibility.

The Jones criteria (see Table 29-5) call for an elevated or increasing titer of an antistreptococcal antibody.[216] The onset of clinical acute rheumatic fever usually

*References 1, 85, 86, 142, 216, 240, 241, 242, 244, 260.

coincides with the peak of the streptococcal antibody response. It may stay elevated for many weeks. The absence of an elevated antistreptococcal titer, if three different antibodies are measured, means, however, that the clinician can be 95 percent certain that the patient has not had a streptococcal infection within the recent past. In patients with pure chorea or indolent carditis, antibody levels may have declined to normal because of the length of the latent period between the development of streptococcal infection and the manifestation of this symptom. A slide agglutination test is available, but cannot be recommended at this time because of inconsistencies in results caused by variations in different lots of test material.[263]

The synovial fluid in joints affected by acute rheumatic fever contains 10,000 to 100,000 white blood cells/mm^3, which are mostly neutrophils. The protein concentration is approximately 4 g/dL, glucose levels are normal, and a good mucin clot is present.[112] A more recent report corroborates the cellular findings and highlights similarities with septic arthritis.[109]

DIAGNOSIS

An accurate diagnosis of acute rheumatic fever is vital because a misdiagnosis may lead to future serious cardiac disease but overdiagnosis will commit the patient to a long-term unpleasant therapy—benzathine penicillin every 4 weeks.

The signs and symptoms of rheumatic fever vary greatly, depending on the stage of the disease, the epidemiology of the rheumatic fever in that place at that time, the severity of the disease, and the sites of involvement. In the absence of a diagnostic test or pathognomonic sign, Jones suggested a series of criteria (major and minor) (see Table 29-5) that have stood the test of time, with ongoing modifications.[85,150] These are an estimate of probability of the likelihood of acute rheumatic fever. The keystone on which the Jones criteria[216] rest is the demonstration of a recent streptococcal infection. Because few patients with acute rheumatic fever have positive throat cultures, demonstration of a previous streptococcal infection by a rising titer of one or more of the extracellular streptococcal antibodies is crucial confirmatory evidence to establish a recent streptococcal infection. However, in the absence of the appropriate constellation of signs and symptoms for acute rheumatic fever, the mere presence of an elevated titer to one or more of the streptococcal antibodies (see the section on laboratory findings) means only that the subject has had a recent group A β-hemolytic streptococcal infection, site unspecified.

Clinical manifestations in outbreaks in the 1980s are summarized in Table 29-3. Before this time, during the period of declining incidence in the United States, carditis was found in fewer than half of rheumatic patients and generally was less severe.[158] Joint involvement alone was the most common manifestation, so arriving at diagnostic certainty was difficult. A common avoidable error is the premature administration of NSAIDs or corticosteroids before the signs and symptoms become distinct; such therapy leaves in doubt the necessity of administering secondary prophylaxis without a firm diagnosis.[258]

In contrast to the revised Jones criteria (1965), more updated criteria (1992)[216] are designed to establish a diagnosis of the initial attack of acute rheumatic fever; a previous attack of rheumatic fever or rheumatic heart disease no longer is a minor manifestation. Echocardiography currently is not a stand-alone criterion for the diagnosis of acute rheumatic fever in the United States using these criteria (see the section on laboratory findings); however, chorea and indolent carditis are considered stand-alone criteria for establishing the diagnosis of rheumatic fever. Recurrences of rheumatic fever in patients with a reliable history of rheumatic fever or clear-cut rheumatic heart disease can be diagnosed by the demonstration of a single major or several minor criteria if supporting evidence of a recent group A streptococcal infection is present.

So-called poststreptococcal arthritis has been discussed as a possible entity when the initial symptoms and signs are atypical for acute rheumatic fever, fail to respond to salicylate therapy, or both. In some cases, rheumatic heart disease has ensued.[74] In all such patients who fulfill the Jones criteria, a diagnosis of rheumatic fever should be considered, particularly for the purpose of administering secondary penicillin prophylaxis.[19]

Criteria have been proposed for establishing the diagnosis of poststreptococcal reactive arthritis that have yet to stand the test of time.[18] The variable response to salicylates in published series of this proposed disease entity has not often been well documented with serum salicylate levels.[95] A shorter latent period is proposed, but the latent period between a group A streptococcal throat infection in original investigations of rheumatic fever varied (mean, 18.6 days), with 10 percent developing disease within 8 days (see the section on clinical course).[138,194] If this diagnosis is considered and rheumatic fever per se is not supported, vigilant follow-up for cardiac sequelae should ensue and penicillin prophylaxis should be reconsidered.[93] This diagnosis should be considered with caution in areas endemic for rheumatic fever.

A prospective epidemiologic study with rigorous application of an agreed case definition is needed to clarify this proposed disease entity.

An atypical presentation of chorea (e.g., hemichorea) warrants consideration of neuroimaging.[269]

DIFFERENTIAL DIAGNOSIS

Rheumatic fever can present in diverse ways (acute polyarthritis, congestive heart failure, chorea, or a combination of these), and no specific diagnostic test for the disease exists; thus, the differential diagnoses in an individual patient may be extensive. Many other diseases might be confused with acute rheumatic fever.[134] In polyarthritis without clinical or echocardiographic evidence of carditis and fulfilling the Jones criteria, rheumatic fever is a diagnosis of exclusion. A search for alternative diagnoses is recommended,[142] including rheumatoid arthritis, suppurative bacterial arthritis (including gonococcal arthritis in adolescents), reactive arthritis (e.g.,

after *Yersinia*[131] or *Mycoplasma*[171] infection), infective endocarditis, sickle-cell anemia, leukemia, Lyme disease,[188] rubella, and poststreptococcal reactive arthritis (see earlier discussion). Before presentation to medical care, the first swollen joint may be attributed to injury. A follow-up echocardiogram after 2 to 3 weeks may reveal late-presenting carditis.[1,246]

With the help of the Jones criteria and time, alternative diagnoses usually can be excluded. Heart involvement with rheumatoid arthritis is a rare occurrence. In suppurative arthritis, showing the infecting bacteria by smear and recovery by culture provide the answer. However in an area endemic for rheumatic fever, aseptic monoarthritis without previous antibiotic exposure should be considered rheumatic fever until proved otherwise.[162] Treatment for possible septic arthritis may need to ensue while the diagnosis evolves. In the absence of an audible cardiac murmur, echocardiography revealing subclinical mitral or aortic valve regurgitation may be very helpful. With sickle-cell disease, the bone is affected and not the joint, and a sickle-cell preparation helps establish the diagnosis. A blood smear usually establishes the diagnosis of leukemia.

The diagnosis of isolated Sydenham chorea based on presenting symptoms may be reinforced by an abnormal echocardiogram in the absence of clinical carditis. Other, much rarer considerations, especially in an area not endemic for rheumatic fever, include systemic lupus erythematosus, Wilson disease, juvenile Huntington chorea, and various medications.[49,134]

Common errors include diagnosing acute rheumatic fever and prescribing NSAIDs when a single joint is involved (see the section on clinical course)[258]; when an innocent murmur is present; when a nonspecific rash, especially an urticarial or erythema multiforme rash, erroneously is identified as erythema marginatum; and when other symptoms similar to chorea (e.g., tics, phenothiazine-induced extrapyramidal syndrome) are misinterpreted.[116] Committing a child to many years of penicillin prophylaxis requires careful decision making at the time of establishing the diagnosis.

TREATMENT

Therapy for acute rheumatic fever is symptomatic to control the inflammation, decrease the fever, and keep cardiac failure in check.[50,66,134,165,228] Neither salicylates nor corticosteroids are thought to affect severity or outcome.[12] Cardiac drugs (e.g., diuretics, angiotensin-converting enzyme inhibitors, β blockers) may improve impaired function. The long-term benefits have not been studied systematically. If the physician thinks a patient has acute rheumatic fever, a trial of salicylates may be useful as symptomatic therapy. If the diagnosis is uncertain, however, pain relief such as acetaminophen should be used as an interim measure to allow migrating polyarthritis to occur. This approach has not been evaluated critically.

The most experience and published evidence for management of the arthritis of acute rheumatic fever is with salicylates. Characteristically, the joint inflammation and fever subside in 24 to 48 hours with salicylate treatment if the serum level is 10 to 20 mg/dL, which usually is achieved by a dose of 60 to 100 mg/kg/24 hr (not exceeding 6 g/day in divided doses). This dose may be increased, but the clinician is advised to measure serum salicylate levels and adjust the dose regimen accordingly. A higher dose may result in the undesirable development of salicylism (tinnitus and hyperpnea). Except for occasional patients with rheumatoid arthritis, no other forms of arthritis respond in this dramatic way to aspirin. Salicylate therapy is recommended for 1 to 2 weeks and then can be reduced gradually, keeping in mind that inflammatory markers suggesting ongoing disease activity may persist 3 months or longer. Joint symptoms may recur, obviating a more gradual withdrawal of aspirin.

Most acute rheumatic fever episodes subside within 6 weeks, and 90 percent resolve within 12 weeks. Approximately 5 percent of cases require 6 months or more of salicylate therapy.[224] If lengthy therapy is required, influenza and varicella vaccines are important considerations to reduce the potential for developing Reye syndrome.[59] Newer NSAIDs for the treatment of acute rheumatic fever have been studied in limited fashion.[111,238] Naproxen 10 to 20 mg/kg/day given in two divided doses (maximum dose 1250 mg) had a dramatic effect similar to that of aspirin and is well-tolerated. Advantages are twice-daily administration, the availability of elixir, less hepatotoxicity, and no need to determine serum levels. No data have been published on the use of ibuprofen in rheumatic fever. Breakthrough symptoms of acute arthritis in patients on ibuprofen have been observed and subsequently controlled by naproxen (personal observation).

No evidence has substantiated that steroid therapy is superior or that treatment with steroid or aspirin decreases the severity or prevents the development of residual heart disease.[6,12,56] Both treatments are palliative and not curative. They are, however, effective anti-inflammatory agents for controlling the acute exudative manifestations of rheumatic fever. Steroids are more likely to reduce acute symptoms promptly and may be indicated for severely ill patients in whom inflammatory edema of the myocardium may be life-threatening during the acute stage of the illness.[13,142] The effect of more modern NSAIDs has not been evaluated in this way.

A randomized controlled trial of intravenous immunoglobulin, a proven immunomodulator, in acute rheumatic fever (excluding chorea) failed to alter the natural history. No detectable difference was noted in the clinical, laboratory, or echocardiographic parameters found during the subsequent 12 months.[246]

Bed rest has not been studied critically.[152] Restriction of physical activity until the rheumatic process has become quiescent is a time-honored method of treatment. It has been based on the assumption that the workload of the inflamed heart is related to the degree of residual scarring. Suggested guidelines include 6 weeks of bed rest, depending on whether carditis is present, followed by gradual ambulation indoors over the course of a further 6 weeks before outside activity, in modified fashion, occurs.[16] Patients with severe carditis who have congestive heart failure are managed more conservatively.

All patients at diagnosis should receive penicillin, even if the throat culture does not reveal group A β-hemolytic streptococci. Patients then can be placed on the secondary preventive treatment regimen, which may be either oral penicillin V 250 mg twice per day, or injections of benzathine penicillin 1.2 million U every 4 weeks. The parenteral route has been shown to be more effective in the New Zealand setting[89,177] (Figure 29-7) and by others (Table 29-6).[262] In high-risk situations (e.g., after a recurrence in a compliant patient), administration of benzathine penicillin every 3 weeks has been advised (see the section on prevention for more detail).[60]

Rarely, a patient has congestive heart failure.[142] A diuretic, fluid restrictions, or both are recommended for mild to moderate failure. Angiotensin-converting enzyme inhibitors should be considered for more severe failure, particularly if aortic regurgitation is present. Experience with β blockers in acute rheumatic carditis is very limited, and their use is not recommended.

Chorea is benign and self-limited. Mild or moderate chorea does not require any specific treatment aside from rest and a calm environment, perhaps in the hospital. Overstimulation or stress can exacerbate the symptoms. The potential toxicity of recommended medication for severe distressing or limiting chorea should be taken into consideration. Aspirin does not have a significant treatment effect.[152] A randomized trial of prednisone in Sydenham chorea showed efficacy.[181] More studies are awaited to confirm this finding.

Small studies of intravenous immunoglobulin have suggested more rapid recovery from chorea,[226] but they have not shown reduced incidence of long-term valve disease in acute rheumatic fever without chorea.[246] Until more evidence is available, intravenous immunoglobulin is not recommended except for severe chorea refractory to other treatments.

Carbamazepine and valproic acid now are preferred to haloperidol, which previously was considered the first-line medical treatment for chorea.[73,90] A small prospective comparison of these three agents concluded that valproic acid was the most effective.[182]

Other antichorea medications should be avoided because of potential toxicity. Because of the small potential for liver toxicity with valproic acid, the recommendation is that carbamazepine be used initially for severe chorea requiring treatment and that valproic acid be considered for refractory cases. A response may not be seen for 1 to 2 weeks, and successful medication may reduce, but not eliminate, the symptoms. Medication should be continued for 2 to 4 weeks after chorea has subsided and then withdrawn gradually. Recurrences of chorea usually are mild and can be managed conservatively, but in severe recurrences, the medication can be restarted if necessary.

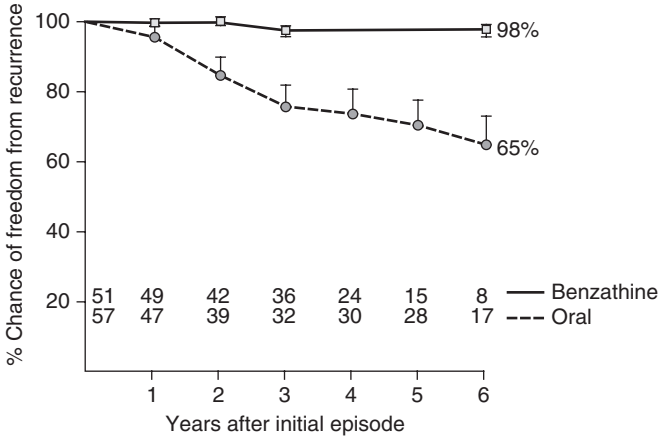

FIGURE 29-7 ■ Actuarial curves showing chance of freedom from recurrence of rheumatic fever after a first attack depending on type of penicillin prophylaxis initially instituted 1968-1982. The bars on the curves refer to the standard error. The figures under the curves refer to the number of patients at risk at each time interval. (From Frankish JD. Rheumatic fever prophylaxis: Gisborne experience. *N Z Med J* 1984;**97**:674–5.)

CARDIAC SURGERY

Aggressive surgical therapy, with increasing acceptance that mitral repair rather than replacement is the treatment of choice for mitral regurgitation,[14,250] may be indicated in patients with severe active rheumatic heart disease. A meta-analysis provides strong support for repair relative to replacement surgery in adult patients.[208] Repair was associated with higher repeat operation in the presence of a risk for disease progression. Data specific to children with rheumatic heart disease (mean age 12.7 years) for long-term outcomes of mitral valve repair have recently become available, demonstrating superiority to replacement.[198] Repair offers a survival advantage and a greater freedom from valve-related morbidity (e.g., a thrombotic, embolic, or hemorrhagic event), without compromised long-term durability. In the

TABLE 29-6 **Prophylaxis and Attack Rates of Streptococcal Infection and Rheumatic Fever Recurrence**

	Parenteral Benzathine Penicillin	Oral Penicillin	Oral Sulfadiazine
No. years	560	545	576
No./rate of all streptococcal infections, exclusive of carrier state (per 100,000)	24/4.3	101/18.5	102/17.7
No./rate of rheumatic recurrences (per 100,000)	2/0.4	30/5.5	16/2.8

Modified from Wood HF, Feinstein AR, Taranta A, et al. Rheumatic fever in children and adolescents: a long-term epidemiologic study of subsequent prophylaxis, streptococcal infections, and clinical sequelae. III. Comparative effectiveness of three prophylaxis regimens in preventing streptococcal infections and rheumatic recurrences. *Ann Intern Med* 1964;**60**(suppl 5):31–45.

short term, repair was associated with a higher early repeat operation rate but freedom from repeat operation over the 11-year mean follow-up period was equal to replacement.

Extensive published experience in South Africa[22,53] and France[53] has challenged the concept that congestive heart failure and death during active carditis are caused exclusively by myocarditis, rather than incompetence of the valve. Studies on left ventricular mechanics support this with new evidence.[91] In addition, primary myocardial involvement as evidenced by histologic abnormalities of the myocardium has been well documented during acute rheumatic fever.[175,228] Careful postoperative management, including at least 4 months of physical rest, diuretics, and vasodilation with angiotensin-converting enzyme inhibitors, is thought to improve the long-term outcome by avoiding increased blood pressure and myocardial contractility before the repair has consolidated.

Surgery is ideally deferred until active inflammation as best judged by CRP measurements has subsided. Rarely, valve leaflet or chordae tendineae rupture leads to severe regurgitation; this event requires emergency surgery, which can be performed safely by experienced surgeons, although the risk seems to be slightly higher than when surgery is performed after active inflammation has resolved.[5,8]

PROGNOSIS

Carditis is the most serious manifestation of acute rheumatic fever and is the only aspect that has well-defined permanent sequelae.[250] Up to approximately 80 percent of patients have cardiac involvement during their acute episode, with 20 percent with moderate or severe rheumatic heart disease in modern series using echocardiography.[141,242] The rate of progression of valvular regurgitation resulting from rheumatic carditis is subject to wide individual variation; however, many cases tend to progress over the next 5 to 10 years, particularly if acute rheumatic fever recurs.[167] Moderate to severe valve lesions during the initial attack of acute rheumatic fever is a strong predictor of rheumatic heart disease in the long term.[46,58,167] Valve lesions of a mild nature are likely to resolve over time in the absence of recurrences of acute rheumatic fever.[246] According to a study conducted in Brazil by Meira and colleagues,[167] 69.5 percent of patients with moderate or severe carditis (in the initial attack of acute rheumatic fever) had clinical evidence of rheumatic heart disease, and all of the patients with severe carditis had persistent rheumatic heart disease, at a mean follow-up of 5.4 years.

Before the availability of penicillin, the prognosis for patients with acute rheumatic fever was even more stark. In a 20-year follow-up study by Bland and Duckett Jones,[36] a patient with marked cardiomegaly, congestive heart failure, or pericarditis had about a 70 to 80 percent chance of dying in the 10 years before the advent of secondary prevention programs, open heart surgery, and use of prosthetic valves. The prognosis today is not as poor, although the recurrence rates (with the attendant increased risk for carditis in individual patients) reported after some outbreaks[241,267] suggest a careful look at secondary prevention and its delivery. The risk for rheumatic heart disease at 1-year[12] and 10-year[58] follow-up is 70 percent for patients with cardiomegaly or heart failure and who survive. Most of these patients have mitral insufficiency, and approximately 50 percent also have aortic insufficiency.

Of patients with no or questionable carditis[12] during their attack of rheumatic fever, only 6 percent were found to have heart murmurs when re-examined 10 years later. Heart disease was present at follow-up in 30 percent of the patients initially found to have only apical systolic murmurs and in 40 percent of patients with basal diastolic murmurs during the acute phase. Patients with chorea may have a slightly lower incidence of residual heart disease.[144] Robust data on prognosis from the echocardiographic era are required.

PREVENTION

Most (approximately two thirds) but not all episodes of rheumatic fever have a streptococcal throat infection that comes to medical attention. Denny and associates[75] and Wannamaker and colleagues[254] made one of the most important research contributions in the last 50 years when they showed that rheumatic fever can be prevented in most susceptible subjects if the preceding pharyngeal infection by one of the group A β-hemolytic streptococci is treated adequately. These studies used intramuscular depot procaine penicillin G in oil, now replaced by benzathine penicillin. The effectiveness of other antimicrobial agents (benzathine penicillin, chlortetracycline, sulfadiazine, oxytetracycline) in the prevention of rheumatic fever also was studied. Eradication of the streptococcus was shown to be essential in the prevention of rheumatic fever.[50] A 10-day course of oral penicillin treatment was found essential for this aim.[253] From these studies, penicillin, a bactericidal agent with activity against streptococci, became the drug of choice.[93] Group A streptococci resistant to penicillin have never to date been documented.

Efficacy studies against rheumatic fever per se were performed mainly in military populations with injectable penicillin. Only one inconclusive study was done in children.[211] These studies more recently have been subjected to meta-analysis,[203] with a relative risk of 0.20 (95% CI 0.11, 0.36; $p < .00001$) favoring the intervention and demonstrating that rheumatic fever can be prevented approximately 80 percent of the time. The ability of oral penicillin to eradicate streptococci in throats is not equal to the ability of injectable penicillin to do so.[23]

In the absence of clear direction from the published literature for the control of rheumatic fever in an endemic area with inadequate health care access, a cluster-randomized, school-based, controlled trial of sore throat clinics in schools using oral penicillin in New Zealand (>80,000 person-years of observation) was performed. Trained and highly supervised lay workers working with public health nurses were the main workforce. Over the 4-year study period, consent, participation, antibiotic

adherence, and retention rates remained high. The outcome suggested a clinically useful result, but with uncertainty about the effect size. Possible reasons for the failure to demonstrate a statistically significant reduction in rheumatic fever were persistent house crowding,[115] an inability to follow symptomatic siblings (who may have been at a control school) because of the school-based design, use of oral rather than injectable penicillin, or other reasons. Exclusion from school of streptococcal pharyngitis cases was not possible. A meta-analysis of published literature on community-based or school-based controlled studies to prevent first attacks of rheumatic fever revealed a relative risk of 0.62 (95% CI 0.45, 0.85; $p = .003$), which favors these interventions, suggesting approximately 40 percent efficacy.[135] The New Zealand study used supervised oral penicillin treatment[141]; the treatment regimen was unclear in the other studies.[54,61,185] Peer-reviewed guidelines for rheumatic fever control have led to population-based initiatives in New Zealand* that are ongoing at this time as a result of these endeavors.[16,125,126,136,139]

A comprehensive explanation for the decline in rheumatic fever in most areas of the developed world remains unclear. Improved housing after World War II, especially in Western Europe, improved access to health care, public health messages highlighting the desirability to manage sore throats appropriately, the availability of penicillin, and other factors have been cited (see section on epidemiology above).

Streptococcal pharyngeal infection should be identified in most situations before treatment is started. Guidelines using clinical parameters have evolved[52,125,137] and have been tested empirically to guide rational management.[163,164] Streptococcal pharyngeal infection can be diagnosed by throat culture or by using a rapid diagnostic antigen-detection kit.[33,92,93] Most tests have high specificity, so a patient with acute pharyngitis and a positive test result should be treated. Many of the tests have suboptimal sensitivity and should be confirmed by a throat culture.[93] One study found that in one third of individuals with false-negative rapid antigen-detection test results, streptococcal antibody titers increased subsequently, suggesting infection.[94]

One study supports the notion that primary preventive treatment with penicillin is effective even if started 9 days after the infection develops,[51] so physicians can wait 24 to 48 hours for verification of infection by recovery of group A β-hemolytic streptococci.[93] Delays should be minimized. In the New Zealand study discussed earlier, this finding has been challenged with 3 students developing acute rheumatic fever 5, 6, and 7 days after presenting with culture proven group A streptococcal pharyngitis.[141]

A dose of 1.2 million U of benzathine penicillin intramuscularly (0.6 million U if weight is ≤27 kg) usually is adequate treatment. Because of the discomfort and a possible, but small, risk associated with intramuscular penicillin,[113] oral penicillin V 250 mg two or three times per day for children ≤27 kg or 500 mg two or three times per day for adolescents and adults may be preferred. Amoxicillin[57,81,93,143,210] 50/mg/kg once per day (maximum 1 g/day) for 10 days is the other antibiotic of choice. Although of somewhat broader spectrum, it has the advantage of once-daily dosing, is relatively inexpensive, and the suspension is considered more palatable than penicillin V suspension.[93] Erythromycin estolate 20 to 40 mg/kg/day in two to four divided doses (maximum 1 g/day) may be used in patients who are allergic to penicillin. Erythromycin ethyl succinate 40 mg/kg/day two to four times per day (maximum 1 g/day) is an alternative therapy.[66] The latter macrolides have been superseded in many countries by azithromycin 12 mg/kg/day (maximum 500 mg for 5 days) or clarithromycin 15 mg/kg/day in two divided doses (maximum 250 mg two times per day for 10 days). Clindamycin 20 mg/kg/day divided in three doses (maximum 1.8 g/day for 10 days) is also an alternative for the penicillin allergic or a narrow-spectrum cephalosporin.[93]

All oral treatments, except azithromycin, should be given for 10 days.[93] Although certain new wider spectrum agents such as azithromycin have been administered in shorter courses with the desired streptococcal eradication, on the basis of penicillin's narrow spectrum of antimicrobial activity, the infrequency with which it produces adverse reactions, and its modest cost, it is the drug of choice for nonallergic patients.[93]

Reappearance of acute rheumatic fever in a specific geographic region should draw attention to therapeutic, preventive, and epidemiologic measures for control of the disease. A targeted approach to particularly high-risk population groups in schools may be cost-effective and efficacious.* Because treatment of pharyngitis seems likely to have contributed to the declining incidence of rheumatic fever, obtaining throat cultures (or a rapid antigen-detection test) and administering penicillin treatment, if positive, for group A streptococci, still are recommended in low-risk populations, although this recommendation is being challenged by some physicians.[163,164] In addition, a negative culture avoids unnecessarily prescribing antibiotics in the 70 to 80 percent of children with a sore throat attributable to viral pharyngitis, although clinical assessment may obviate having to obtain a throat culture.[164,209]

Prompt administration of antibiotic therapy may shorten the duration of symptoms in patients with group A β-hemolytic streptococcal pharyngitis.[195] Cultures should be used selectively in age groups in which rheumatic fever rarely occurs (e.g., <4 years of age or >20 years). Signs and symptoms usually not associated with streptococcal infection, such as simple coryza, hoarseness, cough, conjunctivitis, anterior stomatitis, and diarrhea,[93,164] may help target the approach in a low-risk population.

A follow-up throat culture taken after a course of treatment for streptococcal pharyngitis is not recommended routinely, unless the patient remains symptomatic or is from a family with a rheumatic member.[33] Such follow-up cultures probably identify long-term carriers

*Heart Foundation: http://www.heartfoundation.org.nz.

*References 11, 37, 61, 184, 200, 236.

for whom repeated courses of antibiotics generally are not indicated.[33] Streptococcal carriers seem to pose little threat to themselves regarding the development of sequelae from streptococcal infection or dissemination of the organism to people around them. When a symptomatic viral upper respiratory tract infection develops in such a carrier, distinguishing whether the group A streptococci isolated indicate a current streptococcal infection or identify that individual as a chronic carrier is usually impossible. In one study, only 43 percent of children with paired sera from whom group A streptococci were recovered showed a significant antibody response to one of two different streptococcal antibodies.[123]

Often, a reasonable approach is to administer a single course of therapy. Indications for obtaining cultures from household contacts vary according to circumstances.[66] Family contacts of high-risk patients should have a culture performed and receive treatment if the culture is positive.

In the era before penicillin, 75 percent of individuals developed a recurrence of acute rheumatic fever.[36] Continuous penicillin prophylaxis is well established in its ability to prevent recurrent attacks (Fig. 29-7), with intramuscular long-acting preparations being superior to oral twice-daily delivery.[89,148] Persistent worsening rheumatic heart disease is prevented.[152] Secondary prophylaxis is considered the most cost-effective management of acute rheumatic fever and its sequelae.[225]

The parenteral route has been shown to be more effective (1.2 million U of benzathine penicillin intramuscularly at 28-day intervals).[262] In a comprehensive study, children experienced a recurrence rate of only 0.4/100 patient-years of observation (Table 29-6). A Cochrane systematic review found an 87 to 90 percent reduction with parenteral penicillin in four studies.[147] An international study of allergic reactions to long-term benzathine penicillin prophylaxis found that the benefits of preventing recurrence far outweighed the risk for development of a serious allergic reaction.[113] In areas of particularly high risk, administration of benzathine penicillin every 21 days may be more efficacious, although this study was not performed systematically.[144] Serum levels of penicillin toward the end of the time can be unreliable,[121] although this decision should be offset against practicability, cost, and probable compliance.

The need for benzathine penicillin every 21 days was challenged in a carefully monitored, community-based program evaluation with high compliance rates.[103,217] On four weekly injections of penicillin, the penicillin failure rate per se was very low at 0.07/100 patient-years.[217] The program failure rate was 1.4/100 patient-years, with failure primarily resulting from nonadherence. This is in an environment endemic for rheumatic fever, where children had a 1:3 annual chance of having group A streptococci detected in their throats.[141] A study in Egypt[124] showed that two weekly injections of penicillin resulted in an almost 50 percent reduction in recurrences. This result occurred, however, in an environment in which the rate of recurrences was approximately 45 percent of cases in the four-weekly penicillin comparison arm, suggesting imperfect adherence to the treatment regimen. Data for the latter are not presented. A publication supporting the use of 1 percent lidocaine hydrochloride as a diluent for benzathine penicillin G to increase tolerability may be useful for increased program adherence.[7] Increased frequency of benzathine penicillin injections beyond those necessary will add complexity and cost to secondary prevention programs. Re-evaluation in the modern era is pressing.[225] Patients allergic to penicillin may be given erythromycin. Penicillin and sulfadiazine are oral regimens that have been studied for efficacy (see Table 29-6). Sulfadiazine is not readily available in the United States.[10] The lesser efficacy of oral regimens is related at least partly to compliance difficulties.

The risk for rheumatic fever occurring after a group A streptococcal infection increases from an attack rate of 1 to 3 percent with the first attack of streptococcal pharyngitis to 25 to 75 percent with subsequent attacks.[75] Patients who have had carditis are at increased risk for development of further carditis. Patients who have not had clinical carditis have considerably less risk for having cardiac involvement after a recurrence.[82,145] In the echocardiographic era, the dogma that a patient who escapes carditis in the first attack is highly likely to remain free of rheumatic heart disease, even if prophylaxis fails in subsequent attacks, has been challenged.[82,217]

The risk for recurrence depends on several other factors, such as the length of time since the most recent attack, multiple previous attacks, and the risk for acquiring streptococcal throat infections according to occupation or living circumstances.[36,228,259] If possible, the length of prophylaxis should be individualized. The suggested length ranges from a minimum of 5 years of prophylaxis to a maximum of lifelong prophylaxis.[59,93] This approach has been validated in a study from Chile.[26]

The New Zealand approach* to recurrence prevention has been in place in Auckland for more than 2 decades.[16,217] All patients, regardless of cardiac status, receive at least 10 years of prophylaxis or until they reach 21 years of age (see earlier details for recurrence prevention). Evaluation of criteria for discharge from prophylaxis revealed only two recurrences in this series, which occurred in teenagers inadvertently discharged after at least 5 years of prophylaxis. It was concluded that it is safe to discharge individuals with mild or no clinically evident carditis at the time of discharge, per the recommendations in this highly rheumatic fever endemic environment.[217] Recommended length of prophylaxis varies by authority.[218] Rigorous evaluation is lacking.

The American Heart Association has recently published updated recommendations for the use of prophylactic antibiotics for prevention of infective endocarditis, most notably no longer suggesting prophylaxis for patients with rheumatic heart disease.[261] Some exceptions are necessary, such as those with prosthetic valves or prosthetic material used in valve repair. Some other international scientific bodies have made similar recommendations.[101] However, this approach has not been followed in New Zealand, where rheumatic valvular disease

*Heart Foundation: http://www.heartfoundation.org.nz.

remains common in disadvantaged individuals[168] who in addition have important dental and periodontal disease.*[62]

CONCLUSION

Although having effective prophylaxis against a disease[161] for which the pathophysiology is understood incompletely and for which no pharmacologic cure exists is gratifying, rheumatic fever and its sequelae still occur in numerous young people, especially in the developing world and in disadvantaged populations in the developed world.[264] This rate is largely a reflection of complacency about rheumatic fever and rheumatic heart disease by physicians and health care funders, although it also is likely to reflect living conditions and health care access. Renewed educational efforts regarding prevention of rheumatic fever are needed by physicians and the public. The available preventive methods should be applied vigorously. Applying modern research tools may be beneficial.

NEW REFERENCES SINCE THE SIXTH EDITION

8. Anderson Y, Wilson N, Nicholson R, et al. Fulminant mitral regurgitation due to ruptured chordae tendinae in acute rheumatic fever. *J Paediatr Child Health* 2008;**44**:134–7.
16. Atatoa-Carr P, Lennon D, Wilson N, et al. Rheumatic fever diagnosis, management, and secondary prevention: a New Zealand guideline. *N Z Med J* 2008;**121**:59–69.
20. Azevedo PM, Bauer R, Caparbo Vde F, et al. Interleukin-1 receptor antagonist gene (IL1RN) polymorphism possibly associated to severity of rheumatic carditis in a Brazilian cohort. *Cytokine* 2010;**49**:109–13.
38. Broderick MP, Hansen CJ, Faix DJ. Factors associated with loss of penicillin G concentrations in serum after intramuscular benzathine penicillin G injection: a meta-analysis. *Pediatr Infect Dis J* 2012;**31**:722–5.
41. Bryant PA, Robins-Browne R, Carapetis JR, et al. Some of the people, some of the time: susceptibility to acute rheumatic fever. *Circulation* 2009;**119**:742–53.
43. Caldas AM, Terreri MT, Moises VA, et al. What is the true frequency of carditis in acute rheumatic fever? A prospective clinical and Doppler blind study of 56 children with up to 60 months of follow-up evaluation. *Pediatr Cardiol* 2008;**29**:1048–53.
71. Dale JB, Penfound TA, Chiang EY, et al. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. *Vaccine* 2011; **29**:8175–8.
77. Engel ME, Stander R, Vogel J, et al. Genetic susceptibility to acute rheumatic fever: a systematic review and meta-analysis of twin studies. *PLoS One* 2011;**6**:e25326.
93. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute Streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. *Circulation* 2009;**119**:1541–51.
107. Hanna JN, Clark MF. Acute rheumatic fever in indigenous people in North Queensland: some good news at last? *Med J Aust* 2010;**192**:581–4.
114. Jackson C, Lennon D. *25 Years of lack of progress with acute rheumatic fever control: Auckland Plan for 2011 (abstr)*. Auckland: Annual Scientific Meeting of the Paediatric Society of New Zealand; 2011.
115. Jaine R, Baker M, Venugopal K. Acute rheumatic fever associated with household crowding in a developed country. *Pediatr Infect Dis J* 2011;**30**:315–9.
125. Kerdemelidis M, Lennon D, Arroll B, et al. Guidelines for sore throat management in New Zealand. *N Z Med J* 2009; **122**:10–8.
126. Kerdemelidis M, Lennon DR, Arroll B, et al. The primary prevention of rheumatic fever. *J Paediatr Child Health* 2010; **46**:534–48.
136. Lennon D, Kerdemelidis M, Arroll B. Prevention of rheumatic fever. *Pediatr Infect Dis J* 2010;**29**:92 10.1097/INF.1090b1013e3181bf5419.
139. Lennon D, Kerdemelidis M, Arroll B, et al. Once-daily amoxicillin for group A streptococcal (GAS) sore throat as the other first-line option: a clarification of the NZ sore throat guidelines. *N Z Med J* 2011;**124**:116–9.
141. Lennon D, Stewart J, Farrell E, et al. School-Based Prevention of Acute Rheumatic Fever: A Group Randomized Trial in New Zealand. *Pediatr Infect Dis J* 2009;**28**:787–94.
143. Lennon DR, Farrell E, Martin DR, et al. Once-daily amoxicillin versus twice-daily penicillin V in group A beta-haemolytic streptococcal pharyngitis. *Arch Dis Child* 2008;**93**:474–8.
148. Marijon E, Mirabel M, Celermajer DS, Jouven X. Rheumatic heart disease. *Lancet* 2012;**379**:953–64.
162. Mataika R, Carapetis JR, Kado J, et al. Acute rheumatic fever: an important differential diagnosis of septic arthritis. *J Trop Paediatr* 2008;**54**:205–7.
168. Milne RJ, Lennon DR, Stewart JM, et al. Incidence of acute rheumatic fever in New Zealand children and youth. *J Paediatr Child Health* 2012;**48**:685–91.
198. Remenyi B, Webb R, Russell P, et al. Long term outcomes of isolated mitral valve surgery for rheumatic heart disease in the young: New Zealand 1990-2006. *Heart Lung and Circulation*. Annual Scientific Meeting New Zealand of the Cardiac Society of Australia and New Zealand, Adelaide, SA Australia, August 5-8, 2010;**19**:S251.
199. Remenyi B, Wilson N, Steer A, et al. World Heart Federation criteria for echocardiographic diagnosis of rheumatic heart disease—an evidence-based guideline. *Nat Rev Cardiol* 2012;**28**: 297–309.
206. Seckeler MD, Hoke TR. The worldwide epidemiology of acute rheumatic fever and rheumatic heart disease. *Clin Epidemiol* 2011;**3**:17.
212. Sliwa K, Carrington M, Mayosi BM, et al. Incidence and characteristics of newly diagnosed rheumatic heart disease in urban African adults: insights from the Heart of Soweto Study. *Eur Heart J* 2010;**31**:719–27.
217. Spinetto H, Lennon D, Horsburgh M. Rheumatic fever recurrence prevention: a nurse-led programme of 28-day penicillin in an area of high endemnicity. *J Paediatr Child Health* 2011;**47**: 228–34.
218. Steer AC, Batzloff MR, Mulholland K, et al. Group A streptococcal vaccines: facts versus fantasy. *Curr Opin Infect Dis* 2009; **22**(6):544–52.
219. Steer AC, Carapetis JR. Prevention and treatment of rheumatic heart disease in the developing world. *Nat Rev Cardiol* 2009;**6**: 689–98.
220. Steer AC, Law I, Matatolu L, et al. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. *Lancet Infect Dis* 2009;**9**:611–6.
234. Tibazarwa KB, Volmink JA, Mayosi BM. Incidence of acute rheumatic fever in the world: a systematic review of population-based studies. *Heart* 2008;**94**:1534–40.
250. Walsh W, Brown A, Carapetis J. The diagnosis and management of chronic rheumatic heart disease: an Australian guideline. *Heart Lung Circ* 2008;**17**:271–89.
257. Williams RV, Minich LL, Shaddy RE, et al. Evidence for lack of myocardial injury in children with acute rheumatic carditis. *Cardiol Young* 2002;**12**:519–23.

The full reference list for this chapter is available at expertconsult.com.

*For details see http://www.heartfoundation.org.nz.

REFERENCES

1. Abernethy M, Bass N, Sharpe N, et al. Doppler echocardiography and the early diagnosis of carditis in acute rheumatic fever. *Aust N Z J Med* 1994;**24**:530–5.
2. Agarwal BL. *Rheumatic fever and rheumatic heart disease in developing countries*. India: Arnold Publishers; 1988.
3. Agarwal PK, Misra M, Sarkari NB, et al. Usefulness of echocardiography in detection of subclinical carditis in acute rheumatic polyarthritis and rheumatic chorea. *J Assoc Phys India* 1998;**46**: 937–8.
4. al-Eissa A. Sydenham's chorea: a new look at an old disease. *Br J Clin Pract* 1993;**47**:14–6.
5. al Kasab S, al Fagih MR, Shahid M, et al. Valve surgery in acute rheumatic heart disease: one- to four-year follow-up. *Chest* 1988; **94**:830–3.
6. Albert DA, Harel L, Karrison T. The treatment of rheumatic carditis: a review and meta-analysis. *Medicine* 1995;**74**:1–12.
7. Amir J, Ginat S, Cohen YH, et al. Lidocaine as a diluent for administration of benzathine penicillin G. *Pediatr Infect Dis J* 1998;**17**:890–3.
8. Anderson Y, Wilson N, Nicholson R, et al. Fulminant mitral regurgitation due to ruptured chordae tendinae in acute rheumatic fever. *J Paediatr Child Health* 2008;**44**:134–7.
9. Anonymous. Acute rheumatic fever among army trainees: Fort Leonard Wood, Missouri, 1987-1988. *MMWR Morb Mortal Wkly Rep* 1988;**37**:519–22.
10. Anonymous. Availability of sulfadiazine: United States. *MMWR Morb Mortal Wkly Rep* 1992;**41**:950–1.
11. Anonymous. Prevention of rheumatic heart disease. *Lancet* 1982; **1**:143–4.
12. Anonymous. The natural history of rheumatic fever and rheumatic heart disease. *Circulation* 1965;**32**:457–76.
13. Anonymous. Treatment of rheumatic fever [editorial]. *N Engl J Med* 1965;**272**:101–2.
14. Antunes MJ. Mitral valvuloplasty, a better alternative. *Eur J Cardiothorac Surg* 1990;**4**:257–62; discussion 263–4.
15. Aron AM, Freeman JM, Carter S. The natural history of Sydenham's chorea. *Am J Med* 1965;**38**:83–95.
16. Atatoa-Carr P, Lennon D, Wilson N, et al. Rheumatic fever diagnosis, management, and secondary prevention: a New Zealand guideline. *N Z Med J* 2008;**121**:59–69.
17. Atha M, Enos E, Frank C, et al. How an American Indian tribe controlled the Streptococcus. *World Health Forum* 1982;**3**:423–8.
18. Ayoub EM, Ahmed S. Update on complications of group A streptococcal infections. *Curr Prob Pediatr* 1997;**27**:90–101.
19. Ayoub EM, Majeed HA. Poststreptococcal reactive arthritis. *Curr Opin Rheumatol* 2000;**12**:306–10.
20. Azevedo PM, Bauer R, Caparbo Vde F, et al. Interleukin-1 receptor antagonist gene (IL1RN) polymorphism possibly associated to severity of rheumatic carditis in a Brazilian cohort. *Cytokine* 2010;**49**:109–13.
21. Baird RW, Bronze MS, Kraus W, et al. Epitopes of group A streptococcal M protein shared with antigens of articular cartilage and synovium. *J Immunol* 1991;**146**:3132–7.
22. Barlow JB. Aspects of active rheumatic carditis. *Aust N Z J Med* 1992;**22**:592–600.
23. Bass JW, Crast FW, Knowles CR, et al. Streptococcal pharyngitis in children: a comparison of four treatment schedules with intramuscular penicillin G benzathine. *JAMA* 1976;**235**:1112–6.
24. Beachey EH, Bronze M, Dale JB, et al. Protective and autoimmune epitopes of streptococcal M proteins. *Vaccine* 1988;**6**:192–6.
25. Beachey EH, Stollerman GH, Johnson RH, et al. Human immune response to immunization with a structurally defined polypeptide fragment of streptococcal M protein. *J Exp Med* 1979;**150**: 862–77.
26. Berrios X, del Campo E, Guzman B, et al. Discontinuing rheumatic fever prophylaxis in selected adolescents and young adults: a prospective study. *Ann Intern Med* 1993;**118**:401–6.
27. Berrios X, Quesney F, Morales A, et al. Are all recurrences of "pure" Sydenham chorea true recurrences of acute rheumatic fever? *J Pediatr* 1985;**107**:867–72.
28. Bessen DE, Sotir CM, Readdy TL, et al. Genetic correlates of throat and skin isolates of group A streptococci. *J Infect Dis* 1996;**173**:896–900.
29. Bisno A. The concept of rheumatogenic and nephritogenic group A streptococci. In: Read SE, Zabriskie JB, editors. *Streptococcal diseases and the immune response*. New York: Academic; 1980. p. 789–804.
30. Bisno AL. Group A streptococcal infections and acute rheumatic fever. *N Engl J Med* 1991;**325**:783–93.
31. Bisno AL, Brito MO, Collins CM. Molecular basis of group A streptococcal virulence. *Lancet Infect Dis* 2003;**3**:191–200.
32. Bisno AL, Pearce IA, Stollerman GH. Streptococcal infections that fail to cause recurrences of rheumatic fever. *J Infect Dis* 1977;**136**:278–85.
33. Bisno AL, Gerber MA, Gwaltney Jr JM, et al. Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. Infectious Diseases Society of America. *Clin Infect Dis* 2002;**35**:113–25.
34. Bisno AL, Pearce IA, Wall HP, et al. Contrasting epidemiology of acute rheumatic fever and acute glomerulonephritis. *N Engl J Med* 1970;**283**:561–5.
35. Bisno AL, Rubin FA, Cleary PP, et al. Prospects for a group a streptococcal vaccine: rationale, feasibility, and obstacles: report of a National Institute of Allergy and Infectious Diseases workshop. *Clin Infect Dis* 2005;**41**:1150–6.
36. Bland EF, Duckett Jones T. Rheumatic fever and rheumatic heart disease: a twenty year report on 1000 patients followed since childhood. *Circulation* 1951;**4**:836–43.
37. Brant LJ, Bender TR, Bross DS. Evaluation of an Alaskan streptococcal control program: importance of the program's intensity and duration. *Prev Med* 1986;**15**:632–42.
38. Broderick MP, Hansen CJ, Faix DJ. Factors associated with loss of penicillin G concentrations in serum after intramuscular benzathine penicillin G injection: a meta-analysis. *Pediatric Infect Dis J* 2012;**31**:722–5.
39. Bronze MS, Beachey EH, Dale JB. Protective and heart-crossreactive epitopes located within the NH2 terminus of type 19 streptococcal M protein. *J Exp Med* 1988;**167**:1849–59.
40. Brownell KD, Bailen-Rose F. Acute rheumatic fever in children: incidence in a borough of New York City. *JAMA* 1973;**224**: 1593–7.
41. Bryant PA, Robins-Browne R, Carapetis JR, et al. Some of the people, some of the time: susceptibility to acute rheumatic fever. *Circulation* 2009;**119**:742–53.
42. Cairns LM. Immunological studies in rheumatic fever: the immunology of rheumatic fever. *N Engl J Med* 1988;**101**: 388–91.
43. Caldas AM, Terreri MT, Moises VA, et al. What is the true frequency of carditis in acute rheumatic fever? A prospective clinical and Doppler blind study of 56 children with up to 60 months of follow-up evaluation. *Pediatr Cardiol* 2008;**29**:1048–53.
44. Carapetis JR, Currie BJ. Rheumatic chorea in northern Australia: a clinical and epidemiological study. *Arch Dis Child* 1999;**80**: 353–8.
45. Reference deleted in proofs.
46. Carapetis JR, Currie BJ, Mathews JD. Cumulative incidence of rheumatic fever in an endemic region: a guide to the susceptibility of the population? *Epidemiol Infect* 2000;**124**:239–44.
47. Carapetis JR, Brown A, Wilson NJ, et al. An Australian guideline for rheumatic fever and rheumatic heart disease: an abridged outline. *Med J Aust* 2007;**186**:581–6.
48. Carapetis JR, Steer AC, Mulholland EK, et al. The global burden of group A streptococcal diseases. *Lancet Infect Dis* 2005;**5**: 685–94.
49. Cardoso F. Chorea: non-genetic causes. *Curr Opin Neurol* 2004; **17**:433–6.
50. Catanzaro FJ, Rammelkamp Jr CH, Chamovitz R. Prevention of rheumatic fever by treatment of streptococcal infections. *N Engl J Med* 1958;**259**:53–7.
51. Catanzaro FJ, Jetson CA, Morris AJ, et al. The role of the streptococcus in the pathogenesis of rheumatic fever. *Am J Med* 1954;**17**:749–56.
52. Centor RM, Witherspoon JM, Dalton HP, et al. The diagnosis of strep throat in adults in the emergency room. *Med Decis Making* 1981;**1**:239–46.
53. Chauvaud S, Perier P, Touati G, et al. Long-term results of valve repair in children with acquired mitral valve incompetence. *Circulation* 1986;**74**:I104–9.

54. Chun LT, Reddy V, Rhoads GG. Occurrence and prevention of rheumatic fever among ethnic groups of Hawaii. *Am J Dis Child* 1984;**138**:476–8.
55. Chun LT, Reddy DV, Yim GK, et al. Acute rheumatic fever in Hawaii: 1966 to 1988. *Hawaii Med Jl* 1992;**51**:206–11.
56. Cilliers AM, Manyemba J, Saloojee H. Anti-inflammatory treatment for carditis in acute rheumatic fever (review). *Cochrane Database Syst Rev* 2003;(2)CD003176.
57. Clegg HW, Ryan AG, Dallas SD, et al. Treatment of streptococcal pharyngitis with once-daily compared with twice-daily amoxicillin: a noninferiority trial. *Pediatr Infect Dis J* 2006;**25**:761–7.
58. Combined Rheumatic Fever Study Group. A comparison of short-term, intensive prednisone and acetylsalicylic acid therapy in the treatment of acute rheumatic fever. *N Engl J Med* 1965;**272**:63–70.
59. Committee on Infectious Diseases, American Academy of Pediatrics. *Red book: 2006 report of the Committee on Infectious Diseases*. Elk Grove, Ill: American Academy of Pediatrics; 2006.
60. Committee on Infectious Diseases, American Academy of Pediatrics. Group A Streptococcal Infections. In: *Red book: 2006 report of the Committee on Infectious Diseases*. Elk Grove, Ill: American Academy of Pediatrics; 2009. p. 616–28.
61. Coulehan J, Grant S, Reisinger K, et al. Acute rheumatic fever and rheumatic heart disease on the Navajo reservation, 1962-77. *Public Health Rep* 1980;**95**:62–8.
62. Craig E, Jackson C, Han DY, et al. *Monitoring the health of New Zealand children and young people: indicator handbook*. Auckland: Paediatric Society of New Zealand, New Zealand Child and Youth Epidemiology Service; 2007.
63. Cunningham M, Russell M. Study of heart-reactive antibody in antisera and hybridoma culture fluids against group A streptococci. *Infect Immun* 1983;**42**:531–8.
64. Cunningham MW. Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev* 2000;**13**:470–511.
65. Currie BJ. Group A streptococcal infections of the skin: molecular advances but limited therapeutic progress. *Curr Opin Infect Dis* 2006;**19**:132–8.
66. Dajani AS, Taubert K, Ferrieri P, et al. Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. *Pediatrics* 1995;**96**:758–64.
67. Dale JB. Current status of group A streptococcal vaccine development. In: Finn A, Pollard AJ, editors. *Hot topics in infection and immunity in children IV: Advances in experimental medicine and biology*, vol. 609. New York: Springer; 2008. p. 53–63.
68. Dale JB, Beachey EH. Protective antigenic determinant of streptococcal M protein shared with sarcolemmal membrane protein of human heart. *J Exp Med* 1982;**156**:1165–76.
69. Dale JB, Beachey EH. Epitopes of streptococcal M proteins shared with cardiac myosin. *J Exp Med* 1985;**162**:583–91.
70. Dale JB, Beachey EH. Human cytotoxic T lymphocytes evoked by group A streptococcal M proteins. *J Exp Med* 1987;**166**: 1825–35.
71. Dale JB, Penfound TA, Chiang EY, et al. New 30-valent M protein-based vaccine evokes cross-opsonic antibodies against non-vaccine serotypes of group A streptococci. *Vaccine* 2011;**29**: 8175–8.
72. Danchin MH, Rogers S, Kelpie L, et al. Burden of acute sore throat and group A streptococcal pharyngitis in school-aged children and their families in Australia. *Pediatrics* 2007;**120**:950–7.
73. Daoud AS, Zaki M, Shakir R, et al. Effectiveness of sodium valproate in the treatment of Sydenham's chorea. *Neurology* 1990; **40**:1140–1.
74. De Cunto CL, Giannini EH, Fink CW, et al. Prognosis of children with poststreptococcal reactive arthritis. *Pediatr Infect Dis J* 1988;**7**:683–6.
75. Denny FW, Wannamaker LW, Brink WR, et al. Prevention of rheumatic fever; treatment of the preceding streptococcic infection. *J Am Med Assoc* 1950;**143**:151–3.
76. Eckerd JM, McJunkin JE. Recent increase in incidence of acute rheumatic fever in southern West Virginia. *W V Med J* 1989; **85**:323–5.
77. Engel ME, Stander R, Vogel J, et al. Genetic susceptibility to acute rheumatic fever: a systematic review and meta-analysis of twin studies. *PLoS One* 2011;**6**:e25326.
78. Erdem G, Mizumoto C, Esaki D, et al. Group A streptococcal isolates temporally associated with acute rheumatic fever in Hawaii: differences from the continental United States. *Clin Infect Dis* 2007;**45**:e20–24.
79. Essop MR, Wisenbaugh T, Sareli P. Evidence against a myocardial factor as the cause of left ventricular dilation in active rheumatic carditis. [see comment]. *J Am Coll Cardiol* 1993;**22**:826–9.
80. Facklam RR, Beall B, Efstratiou A, et al. *emm* typing and validation of provisional M types for group A streptococci. *Emerg Infect Dis* 1999;**5**:247–53.
81. Feder Jr HM, Gerber MA, Randolph MF, et al. Once-daily therapy for streptococcal pharyngitis with amoxicillin. *Pediatrics* 1999;**103**:47–51.
82. Feinstein AR, Spagnuolo M. Mimetic features of rheumatic fever recurrences. *N Engl J Med* 1960;**262**:533–40.
83. Feldman BM, Zabriskie JB, Silverman ED, et al. Diagnostic use of B-cell alloantigen D8/17 in rheumatic chorea. *J Pediatr* 1993; **123**:84–6.
84. Ferguson GW, Shultz JM, Bisno AL. Epidemiology of acute rheumatic fever in a multiethnic, multiracial urban community: the Miami-Dade County experience. *J Infect Dis* 1991;**164**:720–5.
85. Ferrieri P, Jones Criteria Working Group. Proceedings of the Jones Criteria workshop. *Circulation* 2002;**106**:2521–3.
86. Figueroa FE, Fernandez MS, Valdes P, et al. Prospective comparison of clinical and echocardiographic diagnosis of rheumatic carditis: long term follow up of patients with subclinical disease. *Heart* 2001;**85**:407–10.
87. Folger GM, Hajar R, Robida A, et al. Doppler echocardiographic findings of mitral and aortic valve regurgitation in children manifesting only rheumatic arthritis. *Am J Cardiol* 1989;**63**: 1278–80.
88. Folger GM, Hajar R, Robida A, et al. Occurrence of valvular heart disease in acute rheumatic fever without evident carditis: Colour flow Doppler identification. *Br Heart J* 1992;**67**:434–8.
89. Frankish JD. Rheumatic fever prophylaxis: Gisborne experience. *N Z Med J* 1984;**97**:674–5.
90. Genel F, Arslanoglu S, Uran N, et al. Sydenham's chorea: clinical findings and comparison of the efficacies of sodium valproate and carbamazepine regimens. *Brain Dev* 2002;**24**:73–6.
91. Gentles TL, Colan SD, Wilson NJ, et al. Left ventricular mechanics during and after acute rheumatic fever: contractile dysfunction is closely related to valve regurgitation. *J Am Coll Cardiol* 2001; **37**:201–7.
92. Gerber MA, Shulman ST. Rapid diagnosis of pharyngitis caused by group A streptococci. *Clin Microbiol Rev* 2004;**17**:571–80.
93. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute Streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research: endorsed by the American Academy of Pediatrics. *Circulation* 2009;**119**:1541–51.
94. Gerber MA, Randolph MF, Chanatry J, et al. Antigen detection test for streptococcal pharyngitis: evaluation of sensitivity with respect to true infections. *J Pediatr* 1986;**108**:654–8.
95. Gibofsky A, Zabrigkie J. Rheumatic fever: new insights into an old disease. *Bull Rheum Dis* 1994;**42**:5–7.
96. Goldstein I, Halpern B, Robert L. Immunological relationship between streptococcus a polysaccharide and the structural glycoproteins of heart value. *Nature* 1967;**213**:44–7.
97. Gordis L. Effectiveness of comprehensive-care programs in preventing rheumatic fever. *N Engl J Med* 1973;**289**:331–5.
98. Gordis L, Lilienfeld A, Rodriguez R. A community-wide study of acute rheumatic fever in adults. epidemiologic and preventive factors. *JAMA* 1969;**210**:862–5.
99. Gordis L, Lilienfeld A, Rodriguez R. Studies in the epidemiology and preventability of rheumatic fever. I. Demographic factors and the incidence of acute attacks. *J Chronic Dis* 1969;**21**:645–54.
100. Gordis L, Lilienfeld A, Rodriguez R. Studies in the epidemiology and preventability of rheumatic fever. II. Socio-economic factors and the incidence of acute attacks. *J Chronic Dis* 1969;**21**: 655–66.
101. Gould FK, Elliott TS, Foweraker J, et al. Guidelines for the prevention of endocarditis: report of the Working Party of the British Society for Antimicrobial Chemotherapy. *J Antimicrob Chemother* 2006;**57**:1035–42.

102. Gray ED, Regelmann WE, Abdin Z, et al. Compartmentalisation of cells bearing "rheumatic" cell surface antigens in peripheral blood and tonsils in rheumatic heart disease. *J Infect Dis* 1987;**155**:247–52.
103. Grayson S, Horsburgh M, Lennon D. An Auckland regional audit of the nurse-led rheumatic fever secondary prophylaxis programme. *N Z Med J* 2006;**119**:U2255.
104. Griffiths SP, Gersony WM. Acute rheumatic fever in New York City (1969-1988): a comparative study of two decades. *J Pediatr* 1990;**116**:882–7.
105. Guilherme L, Ramasawmy R, Kalil J. Rheumatic fever and rheumatic heart disease: Genetics and pathogenesis. *Scand J Immunol* 2007;**66**(2-3):199–207.
106. Guilherme L, Fae KC, Higa F, et al. Towards a vaccine against rheumatic fever. *Clin Dev Immunol* 2006;**13**(2-4):125–32.
107. Hanna JN, Clark MF. Acute rheumatic fever in indigenous people in North Queensland: some good news at last? *Med J Aust* 2010;**192**(10):581–4.
108. Hanna JN, Heazlewood RJ. The epidemiology of acute rheumatic fever in indigenous people in north Queensland. *Aust N Z J Public Health* 2005;**29**:313–7.
109. Harlan GA, Tani LY, Byington CL. Rheumatic fever presenting as monoarticular arthritis. *Pediatr Infect Dis J* 2006;**25**:743–6.
110. Harrington Z, Visvanathan K, Skinner NA, et al. B-cell antigen D8/17 is a marker of rheumatic fever susceptibility in Aboriginal Australians and can be tested in remote settings. [comment]. *Med J Aust* 2006;**184**:507–10.
111. Hashkes PJ, Tauber T, Somekh E, et al. Naproxen as an alternative to aspirin for the treatment of arthritis of rheumatic fever: a randomized trial. *J Pediatr* 2003;**143**:399–401.
112. Homer C, Shulman S. Clinical aspects of acute rheumatic fever. *J Rheumatol* 1991;**18**:2–13.
113. International Rheumatic Fever Study Group. Allergic Reactions to Long-Term Benzathine Penicillin Prophylaxis for Rheumatic Fever. *Lancet* 1991;**337**:1308–10.
114. Jackson C, Lennon D. *25 Years of lack of progress with acute rheumatic fever control: Auckland Plan for 2011 (abstr)*. Auckland: Annual Scientific Meeting of the Paediatric Society of New Zealand; 2011.
115. Jaine R, Baker M, Venugopal K. Acute rheumatic fever associated with household crowding in a developed country. *Pediatr Infect Dis J* 2011;**30**:315–9.
116. Kaplan E. Acute rheumatic fever. *Pediatr Clin North Am* 1978;**25**:817–29.
117. Kaplan EL, Bisno AL. Antecedent Streptococcal infection in acute rheumatic fever. *Clin Infect Dis* 2006;**43**:690–2.
118. Kaplan E, Wannamaker L. Streptolysin O: suppresion of its antigenicity by lipids extracted from skin. *Proc Soc Exp Biol Med* 1974;**146**:205–8.
119. Kaplan E, Johnson D, Cleary P. Group A streptococcal serotypes isolated from patients and sibling contacts during the resurgence of rheumatic fever in the United States in the mid-1980s. *J Infect Dis* 1989;**159**:101–3.
120. Kaplan EL, Rothermel CD, Johnson DR. Antistreptolysin O and anti-deoxyribonuclease b titers: normal values for children ages 2 to 12 in the United States. *Pediatrics* 1998;**101**:86–8.
121. Kaplan E, Berrios X, Speth J, et al. Pharmacokinetics of benzathine penicillin G: serum levels during the 28 days after intramuscular injection of 1,200,000 units. *J Pediatr* 1989;**115**: 146–51.
122. Kaplan M, Bolande R, Rakita L, et al. Presence of bound immunoglobulins and complement in the myocardium in acute rheumatic fever: association with cardiac failure. *N Engl J Med* 1964;**271**:637–45.
123. Kaplan E, Top F, Dudding B, et al. Diagnosis of streptococcal pharyngitis: differentiation of active infection from the carrier state in the symptomatic child. *J Infect Dis* 1971;**123**:490–501.
124. Kassem AS, Zaher SR, Abou Shleib H, et al. Rheumatic fever prophylaxis using benzathine penicillin G (BPG): two-week versus four-week regimens: comparison of two brands of BPG. *Pediatrics* 1996;**97**:992–5.
125. Kerdemelidis M, Lennon D, Arroll B, et al. Guidelines for sore throat management in New Zealand. *N Z Med J* 2009;**122**:10–8.
126. Kerdemelidis M, Lennon DR, Arroll B, et al. The primary prevention of rheumatic fever. *J Paediatr Child Health* 2010;**46**: 534–48.
127. Khriesat I, Najada AH. Acute rheumatic fever without early carditis: an atypical clinical presentation. *Eur J Pediatr* 2003;**162**: 868–71.
128. Kumar R. Controlling rheumatic heart disease in developing countries. *World Health Forum* 1995;**16**:47–51.
129. Kurahara D, Tokuda A, Grandinetti A, et al. Ethnic differences in risk for pediatric rheumatic illness in a culturally diverse population. *J Rheumatol* 2002;**29**:379–83.
130. Kurlan R, Kaplan EL. The pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS) etiology for tics and obsessive-compulsive symptoms: hypothesis or entity? Practical considerations for the clinician. [comment]. *Pediatrics* 2004;**113**:883–6.
131. Laitinen O, Leirisalo M, Allander E. Rheumatic fever and Yersinia arthritis criteria and diagnostic problems in a changing disease pattern. *Scand J Rheumatol* 1975;**4**:145–57.
132. Lancefield RC. Persistence of type-specific antibodies in man following infection with group A streptococci. *J Exp Med* 1959; **110**:271–92.
133. Land M, Bisno A. Acute rheumatic fever: a vanishing disease in suburbia. *J Am Med Assoc* 1983;**249**:895–8.
134. Lennon D. Acute rheumatic fever in children: recognition and treatment. *Pediatr Drugs* 2004;**6**:363–73.
135. Lennon D, Kerdemelidis M, Arroll B. Meta-analysis of trials of streptococcal throat treatment programs to prevent rheumatic fever. *Pediatr Infect Dis J* 2009;**28**:e259–64.
136. Lennon D, Kerdemelidis M, Arroll B. Prevention of rheumatic fever. *Pediatr Infect Dis J* 2010;**29**:92 10.1097/INF.1090b1013e31 81bf5419.
137. Lennon D, Peat B, Arroll B. New Zealand guidelines for rheumatic fever. II. Sore throat management. 2008. Available at: http://www.heartfoundation.org.nz.
138. Lennon D, Stewart J, Farrell E. A randomised controlled trial of once a day amoxicillin for streptococcal pharyngitis. *Arch Dis Child* 2008;**93**:474–78.
139. Lennon D, Kerdemelidis M, Arroll B, et al. Once-daily amoxicillin for group A streptococcal (GAS) sore throat as the other first-line option: a clarification of the NZ sore throat guidelines. *N Z Med J* 2011;**124**:116–9.
140. Lennon D, Martin D, Wong E, et al. Longitudinal study of post-streptococcal disease in Auckland; rheumatic fever, glomerulonephritis, epidemiology and M typing 1981-86. *N Z Med J* 1988; **101**:396–8.
141. Lennon D, Stewart J, Farrell E, et al. School-based prevention of acute rheumatic fever: a group randomized trial in New Zealand. *Pediatr Infect Dis J* 2009;**28**:787–94.
142. Lennon D, Wilson N, Peat B, et al. New Zealand guidelines for rheumatic fever: 1. diagnosis, management & secondary prophylaxis. 2006. Available at: http://www.nhf.org.nz/files/Rheumatic%20fever%20guideline%201.pdf.
143. Lennon DR, Farrell E, Martin DR, et al. Once-daily amoxicillin versus twice-daily penicillin V in group A beta-haemolytic streptococcal pharyngitis. *Arch Dis Child* 2008;**93**:474–8.
144. Lue HC, Wu MH, Wang JK, et al. Three- versus four-week administration of benzathine penicillin G: effects on incidence of streptococcal infections and recurrences of rheumatic fever. *Pediatrics* 1996;**97**:984–8.
145. Majeed H, Yousof A, Khuffash F, et al. The natural history of acute rheumatic fever in Kuwait: a prospective 6-year follow-up report. *J Chronic Dis* 1986;**39**:361–9.
146. Majeed H, Yousof A, Rotta J, et al. Group A streptococcal strains in Kuwait: a nine-year prospective study of prevalence and associations. *Pediatr Infect Dis J* 1992;**11**:295–300.
147. Manyemba J, Mayosi BM. Penicillin for secondary prevention of rheumatic fever. *Cochrane Database Syst Rev* 2002;(3):CD002227.
148. Marijon E, Mirabel M, Celermajer DS, Jouven X. Rheumatic heart disease. *Lancet* 2012;**379**:953–64.
149. Marijon E, Ou P, Celermajer DS, et al. Prevalence of rheumatic heart disease detected by echocardiographic screening. *N Engl J Med* 2007;**357**:470–6.
150. Markowitz M. Evolution and critique of changes in the Jones criteria for diagnosis of rheumatic fever. *N Z Med J* 1988;**101**:392–4.
151. Markowitz M. Streptococcal disease in developing countries. *Pediatr Infect Dis J* 1991;**10**:S11–38.
152. Markowitz M, Gordis L. *Rheumatic fever*. Philadelphia: W.B. Saunders; 1972.

153. Markowitz M, Kaplan EL. Reappearance of rheumatic fever. *Adv Pediatr* 1989;**36**:39–65.
154. Marrack P, Kappler J. The staphylococcal enterotoxins and their relatives. *Science* 1990;**248**:705–11.
155. Martin D, Single L. Molecular epidemiology of group A *Streptococcus* M type 1 infections. *J Infect Dis* 1993;**167**:1112–7.
156. Martin D, Voss L, Walker S, et al. Acute rheumatic fever in Auckland, New Zealand: spectrum of associated group A streptococci different from expected. *Pediatr Infect Dis J* 1994;**13**:264–9.
157. Martins TB, Veasy LG, Hill HR. Antibody responses to group A streptococcal infections in acute rheumatic fever. *Pediatr Infect Dis J* 2006;**25**:832–7.
158. Massell B, Amezcuac F, Pelargonio S. Evolving picture of rheumatic fever: data from 40 years at the House of the Good Samaritan. *J Am Med Assoc* 1964;**188**:287–94.
159. Massell BF, Dow JW, Jones TD. Orally administered penicillin in patients with rheumatic fever. *J Am Med Assoc* 1948;**138**:1030–6.
160. Massell BF, Honikman LH, Amezcua J. Rheumatic fever following streptococcal vaccination: report of three cases. *JAMA* 1969; **207**:1115–9.
161. Massell BF, Chute CG, Walker AM, Kurland GS. Penicillin and the marked decrease in morbidity and mortality from rheumatic fever in the United States. *N Engl J Med* 1988;**318**:280–6.
162. Mataika R, Carapetis JR, Kado J, et al. Acute rheumatic fever: an important differential diagnosis of septic arthritis. *J Trop Paediatr* 2008;**54**:205–7.
163. McIsaac WJ, Goel V, Slaughter PM, et al. Reconsidering sore throats. II. Alternative approach and practical office tool. [comment]. *Can Fam Phys* 1997;**43**:495–500.
164. McIsaac WJ, Kellner JD, Aufricht P, et al. Empirical validation of guidelines for the management of pharyngitis in children and adults. *JAMA* 2004;**291**:1587–95.
165. McLaren MJ, Hawkins DM, Koornhof HJ, et al. Epidemiology of rheumatic heart disease in black schoolchildren of Soweto, Johannesburg. *Br Med J* 1975;**3**:474–8.
166. McNeil SA, Halperin SA, Langley JM, et al. Safety and immunogenicity of 26-valent group A streptococcus vaccine in healthy adult volunteers. *Clin Infect Dis* 2005;**41**:1114–22.
167. Meira ZM, Goulart EM, Colosimo EA, et al. Long term follow up of rheumatic fever and predictors of severe rheumatic valvar disease in Brazilian children and adolescents. *Heart* 2005;**91**: 1019–22.
168. Milne RJ, Lennon DR, Stewart JM, et al. Incidence of acute rheumatic fever in New Zealand children and youth. *J Paediatr Child Health* 2012;**48**:685–91.
169. Miyake CY, Gauvreau K, Tani LY, et al. Characteristics of children discharged from hospitals in the United States in 2000 with the diagnosis of acute rheumatic fever. *Pediatrics* 2007;**120**:503–8.
170. Moore D. Neuropsychiatric aspects of Sydenham's chorea: a comprehensive review. *J Clin Psychiatry* 1996;**57**:407–14.
171. Moore P, Mortland T. *Mycoplasma pneumoniae* infection mimicking acute rheumatic fever. *Pediatr Infect Dis J* 1994;**13**:81–2.
172. Morris K, Mohan C, Wahi P. Increased inactivated T cells and reduction in suppressor/cytotoxic T cells in acute rheumatic fever and active heart disease: a longitudinal study. *J Infect Dis* 1993;**167**:3979–83.
173. Morris K, Mohan C, Wahi P, et al. Enhancement of IL-1, IL-2 production and IL-2 receptor generation in patients with acute rheumatic fever and active rheumatic heart disease; a prospective study. *Clin Exp Immunol* 1993;**91**:429–36.
174. Morton W, Huhn L, Litchy J. Rheumatic heart disease epidemiology: observations on 17,366 Denver schoolchildren. *JAMA* 1967; **199**:879–84.
175. Narula J, Chopra P, Talwar K, et al. Does endomyocardial biopsy aid in the diagnosis of active rheumatic carditis? *Circulation* 1993;**88**:2198–205.
176. Nausieda P, Grossman B, Koller W, et al. Sydenham chorea: an update. *Neurology* 1980;**30**:331–4.
177. Newman JE, Lennon DR, Wong-Toi W. Patients with rheumatic fever recurrences. *N Z Med J* 1984;**97**:678–80.
178. Nordet P, Lopez, R, Duenas A, et al. Prevention and control of rheumatic fever and rheumatic heart disease: the Cuban experience (1986-2002). *Cardiovasc J of Africa* 2008;**19**:135–40.
179. Patarroyo ME, Winchester RJ, Vejerano A, et al. Association of a B-cell alloantigen with susceptibility to rheumatic fever. *Nature* 1979;**278**:173–4.
180. Paul JR. *Epidemiology of rheumatic fever*. 3rd ed. New York: American Heart Association; 1957.
181. Paz JA, Silva CAA, Marques-Dias MJ. Randomized double-blind study with prednisone in Sydenham's chorea. *Pediatr Neurol* 2006;**34**:264–9.
182. Pena J, Mora E, Cardozo J, et al. Comparison of the efficacy of carbamazepine, haloperidol and valproic acid in the treatment of children with Sydenham's chorea: clinical follow-up of 18 patients. *Arq Neuropsiquiatr* 2002;**60**:374–7.
183. Perry CB, Roberts JAF. A study on the variability in the incidence of rheumatic heart disease within the city of Bristol. *Br Med J* 1937;**2**:154–8.
184. Phibbs B, Lundin S, Watson W, et al. Experience of a Wyoming County streptococcal control project. *West J Med* 1988;**148**: 546–50.
185. Phibbs BP. Streptococcal control in school children. Is it worthwhile? *Minn Med* 1975;**58**:607–10.
186. Pickles W. A rheumatic family. *Lancet* 1943;**2**:241.
187. Pileggi GCS, Ferriani PL. Atypical arthritis in children with rheumatic fever. *J Pediatr* 2000;**76**:49–54.
188. Pinals R. Polyarthritis and fever. *N Engl J Med* 1994;**330**: 769–74.
189. Polly SM, Waldman RH, High P, et al. Protective studies with a group A streptococcal M protein vaccine. II. Challenge of volunteers after local immunization in the upper respiratory tract. *J Infect Dis* 1975;**131**:217–24.
190. Pruksakorn S, Currie B, Brandt E, et al. Towards a vaccine for rheumatic fever: identification of a conserved target epitope on M protein of group A streptococci. *Lancet* 1994;**344**:639–42.
191. Pruksakorn S, Sittisombut N, Phornphutkul C, et al. Epidemiological analysis of non-M-typeable group A streptococcus isolates from a Thai population in Northern Thailand. *J Clin Microbiol* 2000;**38**:1250–4.
192. Quinn R, Federspiel C. The incidence of rheumatic fever in metropolitan Nashville, 1963-1969. *Am J Epidemiol* 1974;**99**:273–80.
193. Raizada V, Williams R, Chopra P. Tissue distribution of lymphocytes in rheumatic heart valves as defined by monoclonal anti-T cell antibodies. *Am J Med* 1983;**74**:90–6.
194. Rammelkamp C, Wannamaker W, Denny F. Studies on the epidemiology of rheumatic fever in the armed services. In: Thomas L, editor. *Rheumatic fever*. Minneapolis: University of Minnesota Press; 1972.
195. Randolph MF, Gerber MA, DeMeo KK, Wright L. Effect of antibiotic therapy on the clinical course of streptococcal pharyngitis. *J Pediatr* 1985;**106**:870–5.
196. Reddy D, Chun L, Yamamoto L. Acute rheumatic fever with advanced degree AV block. *Clin Pediatr* 1989;**28**:326–8.
197. Regelmann WE, Talbot R, Cairns L, et al. Distribution of cells bearing "rheumatic" antigens in peripheral blood of patients with rheumatic fever/rheumatic heart disease. *J Rheumatol* 1989; **16**:931–5.
198. Remenyi B, Webb R, Russell P, et al. Long term outcomes of isolated mitral valve surgery for rheumatic heart disease in the young: New Zealand 1990-2006. *Heart Lung and Circulation* Annual Scientific Meeting New Zealand of the Cardiac Society of Australia and New Zealand, Adelaide, SA Australia, August 5-8, 2010;**19**:S251.
199. Remenyi B, Wilson N, Steer A, et al. World Heart Federation criteria for echocardiographic diagnosis of rheumatic heart disease—an evidence-based guideline. *Nat Rev Cardiol* 2012; **28**:297–309.
200. Rhodes P, Jackson H. Rheumatic fever in Colorado: a conquered disease? *JAMA* 1975;**234**:157–8.
201. Richardson M, Elliman D, Maguire H, et al. Evidence base of incubation periods, periods of infectiousness and exclusion policies for the control of communicable diseases in schools and preschool. [see comment] [erratum appears in Pediatr Infect Dis J 2001 Jul;20(7):653]. *Pediatr Infect Dis J* 2001;**20**:380–91.
202. Robbins SL. *The heart: rheumatic fever and rheumatic heart disease*. 4th ed. Philadelphia: W.B. Saunders; 1994.
203. Robertson KA, Volmink JA, Mayosi BM. Antibiotics for the primary prevention of acute rheumatic fever: a meta-analysis. *BMC Cardiovasc Disord* 2005;**5**:1–9.
204. Sanyal SK, Berry AM, Duggal S, et al. Sequelae of the initial attack of acute rheumatic fever in children from north India. A prospective 5-year follow-up study. *Circulation* 1982;**65**:375–9.

205. Sanyal SK, Thapar MK, Ahmed SH, et al. The initial attack of acute rheumatic fever during childhood in North India: a prospective study of the clinical profile. *Circulation* 1974;**49**:7–12.
206. Seckeler MD, Hoke TR. The worldwide epidemiology of acute rheumatic fever and rheumatic heart disease. *Clin Epidemiol* 2011;**3**:17.
207. Shet A, Kaplan EL. Clinical use and interpretation of group A streptococcal antibody tests: a practical approach for the pediatrician or primary care physician. *Pediatr Infect Dis J* 2002;**21**:420–6; quiz 427–30.
208. Shuhaiber J, Anderson RJ. Meta-analysis of clinical outcomes following surgical mitral valve repair or replacement. *Eur J Cardiothorac Surg* 2007;**31**:267–75.
209. Shulman ST. The decline of rheumatic fever: what impact on our management of pharyngitis? *Am J Dis Child* 1984;**138**:426–7.
210. Shvartzman P, Tabenkin H, Rosentzwaig A, et al. Treatment of streptococcal pharyngitis with amoxycillin once a day. *Br Med J* 1993;**306**:1170–2.
211. Siegel AC, Johnson EE, Stollerman GH. Controlled studies of streptococcal pharyngitis in a pediatric population. *N Engl J Med* 1961;**265**:559–66.
212. Sliwa K, Carrington M, Mayosi BM, et al. Incidence and characteristics of newly diagnosed rheumatic heart disease in urban African adults: insights from the Heart of Soweto Study. *Eur Heart J* 2010;**31**:719–27.
213. Smoot JC, Barbian KD, Van Gompel JJ, et al. Genome sequence and comparative microarray analysis of serotype M18 group A streptococcus strains associated with acute rheumatic fever outbreaks. *Proc Natl Acad Sci USA* 2002;**99**:4668–73.
214. Smoot JC, Korgenski EK, Daly JA, et al. Molecular analysis of group A streptococcus type *emm18* isolates temporally associated with acute rheumatic fever outbreaks in Salt Lake City, Utah. *J Clin Microbiol* 2002;**40**:1805–10.
215. Spagnuolo M, Taranta A. Rheumatic fever in siblings: similarity of its clinical manifestations. *N Engl J Med* 1968;**278**:183–8.
216. Special Writing Group. Guidelines for the diagnosis of rheumatic fever: Jones criteria, 1992 update. *JAMA* 1992;**268**:2069–73.
217. Spinetto H, Lennon D, Horsburgh M. Rheumatic fever recurrence prevention: a nurse-led programme of 28-day penicillin in an area of high endemnicity. *J Paediatr Child Health* 2011;**47**: 228–34.
218. Steer AC, Batzloff MR, Mulholland K, et al. Group A streptococcal vaccines: facts versus fantasy. *Curr Opin Infect Dis* 2009;**22**: 544–52.
219. Steer AC, Carapetis JR. Prevention and treatment of rheumatic heart disease in the developing world. *Nat Rev Cardiol* 2009;**6**: 689–98.
220. Steer AC, Law I, Matatolu L, et al. Global emm type distribution of group A streptococci: systematic review and implications for vaccine development. *Lancet Infect Dis* 2009;**9**:611–6.
221. Stollerman G. The relative rheumatogenicity of strains of group A streptococci. *Mod Concepts Cardiovasc Dis* 1975;**64**:35–40.
222. Stollerman G. Rheumatogenic streptococci and autoimmunity. *Clin Immunol Immunopathol* 1991;**61**:131–42.
223. Stollerman GH, Lewis AJ, Schultz I, Taranta A. Relationship of immune response to group A streptococci to the course of acute chronic and recurrent rheumatic fever. *Am J Med* 1956;**20**: 163–9.
224. Stollerman GH. *Rheumatic fever and streptococcal infection*. New York: Grune & Stratton; 1975.
225. Strasser T. Cost-effective control of rheumatic fever in the community. *Health Policy* 1985;**5**:159–64.
226. Swedo S. Sydenham's chorea: a model for childhood autoimmune neuropsychiatric disorders. *JAMA* 1994;**272**:1788–91.
227. Swedo S, Leonard H, Mittleman B, et al. Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. *Am J Psychiatry* 1997;**154**:110–2.
228. Taranta A, Markowitz M. *Rheumatic fever*. 2nd ed. Boston: Kluwer Academic Publications; 1989.
229. Taranta A, Spagnuolo M, Feinstein AR. Rheumatic fever in monozygotic and dizygotic twins. *Circulation* 1959;**20**:778.
230. Taranta A, Spagnuolo M, Feinstein A. "Chronic" rheumatic fever. *Ann Intern Med* 1962;**56**:367–88.
231. Taranta A, Kleinberg E, Feinstein A, et al. Rheumatic fever in children and adolescents: a long-term epidemiologic study of subsequent prophylaxis, streptococcal infections and clinical sequelae. *Ann Intern Med* 1964;**60**:58–67.
232. Taubert KA, Rowley AH, Shulman ST. Seven-year national survey of Kawasaki disease and acute rheumatic fever. *Pediatr Infect Dis J* 1994;**13**:704–8.
233. Terreri MT, Roja SC, Len CA, et al. Sydenham's chorea: clinical and evolutive characteristics. *São Paulo Med J* 2002;**120**:16–9.
234. Tibazarwa KB, Volmink JA, Mayosi BM. Incidence of acute rheumatic fever in the world: a systematic review of population-based studies. *Heart* 2008;**94**:1534–40.
235. Tomai M, Kotb M, Majumdar G, et al. Superantigenicity of streptococcal M protein. *J Exp Med* 1990;**172**:359–62.
236. Tompkins R, Burnes D, Cable W. An analysis of the cost-effectiveness of pharyngitis management and acute rheumatic fever prevention. *Ann Intern Med* 1977;**86**:481–92.
237. Tubridy-Clark M, Carapetis JR. Subclinical carditis in rheumatic fever: a systematic review. *Int J Cardiol* 2007;**119**:54–8.
238. Uziel Y, Hashkes P, Kassem E, et al. The use of naproxen in the treatment of children with rheumatic fever. *J Pediatr* 2000;**137**: 269–71.
239. Vasan R, Shrivastava S, Vijayakumar M, et al. Echocardiographic evaluation of patients with acute rheumatic carditis. *Circulation* 1996;**94**:73–82.
240. Veasy LG. Time to take soundings in acute rheumatic fever [commentary]. *Lancet* 2001;**357**:1994–5.
241. Veasy L, Tani L, Hill H. Persistence of acute rheumatic fever in the intermountain area of the United States. *J Pediatr* 1994; **124**:9–16.
242. Veasy LG, Wiedmeier SE, Orsmond GS, et al. Resurgence of acute rheumatic fever in the intermountain area of the United States. *N Engl J Med* 1987;**316**:421–7.
243. Vendsborg P, Hansen LF, Olesen KH. Decreasing incidence of a history of acute rheumatic fever in chronic rheumatic heart disease. *Cardiologia* 1968;**53**:332–40.
244. Vijayalakshmi IB, Vishnuprabhu RO, Chitra N, et al. The efficacy of echocardiographic criterions for the diagnosis of carditis in acute rheumatic fever. *Cardiology in the Young* 2008;**18**:586–92.
245. Vlajinac H, Adanja B, Marinkovic J, et al. Influence of socioeconomic and other factors on rheumatic fever occurrence. *Eur J Epidemiol* 1991;**7**:702–4.
246. Voss LM, Wilson NJ, Neutze JM, et al. Intravenous immunoglobulin in acute rheumatic fever: a randomized controlled trial. *Circulation* 2001;**103**:401–6.
247. Wald E, Dashefsky B, Feidt C, et al. Acute rheumatic fever in Western Pennsylvania and the tristate area. *Pediatrics* 1987;**80**: 371–4.
248. Walker KG, Cooper M, McCabe K, et al. Markers of susceptibility to acute rheumatic fever: the B-cell antigen D8/17 is not robust as a marker in South Africa. *Cardiol Young* 2011;**21**:328–33.
249. Wallace M, Garst P, Papadimos T, et al. The return of acute rheumatic fever in young adults. *JAMA* 1989;**262**:2557–61.
250. Walsh W, Brown A, Carapetis J. The diagnosis and management of chronic rheumatic heart disease: an Australian guideline. *Heart Lung Circ* 2008;**17**:271–89.
251. Wannamaker L. Differences between streptococcal infections of the skin and of the throat. *N Engl J Med* 1970;**282**:23–30, 78–85.
252. Wannamaker L. Virulence factors in streptococci. *Scand J Infect Dis* 1982;**31**(suppl):22–7.
253. Wannamaker L, Denny F, Perry W, et al. Effect of penicillin prophylaxis on streptococcal disease rates and the carrier state. *N Engl J Med* 1953;**249**:1–7.
254. Wannamaker L, Rammelkamp Jr CH, Denny FW, et al. Prophylaxis of acute rheumatic fever: by treatment of the preceding streptococcal infection with various amounts of depot penicillin. *Am J Med* 1951;**10**:673–94.
255. Westlake R, Graham T, Edwards K. An outbreak of acute rheumatic fever in Tennessee. *Pediatr Infect Dis J* 1990;**9**:97–100.
256. Widdowson JP, Maxted WR, Notley CM, et al. The antibody respones in man to infection with different serotypes of group-A streptococci. *J Med Microbiol* 1974;**7**:483–96.
257. Williams RV, Minich LL, Shaddy RE, et al. Evidence for lack of myocardial injury in children with acute rheumatic carditis. *Cardiol Young* 2002;**12**:519–23.
258. Wilson E, Wilson N, Voss L, et al. Monoarthritis in rheumatic fever? [comment]. *Pediatr Infect Dis J* 2007;**26**:369–70.

259. Wilson MG, Luhschez R. Recurrence rates in rheumatic fever: the evaluation of etiologic concepts and consequent preventive therapy. *JAMA* 1944;**126**:477–80.
260. Wilson NJ, Neutze JM. Echocardiographic diagnosis of subclinical carditis in acute rheumatic fever. *Int J Cardiol* 1995;**50**:1–6.
261. Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation* 2007;**116**:1736–54.
262. Wood HF, Feinstein AR, Taranta A, et al. Rheumatic fever in children and adolescents: a long-term epidemiologic study of subsequent prophylaxis, streptococcal infections, and clinical sequelae. III. Comparative effectiveness of three prophylaxis regimens in preventing streptococcal infections and rheumatic recurrences. *Ann Intern Med* 1964;**60**(suppl 5):31–45.
263. World Health Organisation. WHO evaluation of the streptozyme test for streptococcal antibodies. *Bull WHO* 1986;**64**:504.
264. World Health Organization. *The current evidence for the burden of Group A streptococcal diseases*. WHO/FCH/CAH/05.07 WHO/IVB/05.12:60, 2005.
265. Zabriskie JB. Rheumatic fever: a model for the pathological consequences of microbial-host mimicry. *Clinical & Experimental Rheumatology* 1986;**4**:65–73.
266. Zabriskie J, Lavenchy D, Williams R, et al. Rheumatic fever-associated b cell alloantigens as identified by monoclonal antibodies. *Arthritis Rheum* 1985;**28**:1047–51.
267. Zangwill K, Wald E, Londino A. Acute rheumatic fever in Western Pennsylvania: a persistent problem into the 1990s. *J Pediatr* 1991;**118**:561–3.
268. Zedan M, El-Shennawy F, Abou-Bakr H, et al. Interleukin-2 in relation to T cell subpopulations in rheumatic heart disease. *Arch Dis Child* 1992;**67**:1373–5.
269. Zomorrodi A, Wald ER. Sydenham's chorea in western Pennsylvania. *Pediatrics* 2006;**117**:e675–9.

CHAPTER 30

Mediastinitis

Morven S. Edwards

The mediastinum is the extrapleural portion of the thoracic cavity situated between the two pleural sacs. The superior and inferior portions are separated arbitrarily by a line extending from the lower manubrium to the fourth thoracic vertebra. The superior mediastinum contains the thymus gland, trachea, esophagus, and aortic arch. The inferior mediastinum is divided into the anterior compartment, containing lymphatic tissue and fat; the middle compartment, containing the heart, pericardium, aorta, bifurcation of the trachea, main bronchi, and numerous lymph nodes; and the posterior compartment, containing the esophagus, thoracic duct, descending aorta, and vagus nerve. *Mediastinitis* refers to inflammation of the tissues located in the mediastinum. Infections of the mediastinum are uncommon, but often they pose a serious threat to vital structures.

Acute mediastinitis is a fulminant, septic process, and chronic mediastinitis is an indolent infection that produces late symptoms caused by compression of adjacent structures. Acute mediastinitis is subclassified as (1) traumatic, occurring as a consequence of perforation of the esophagus; (2) infection extending to the mediastinum from adjacent structures; and (3) postoperative, usually occurring after thoracic surgery (Table 30-1).

ACUTE MEDIASTINITIS

Mediastinitis due to Esophageal Perforation

The esophagus is a thin-walled organ, and esophageal perforation is the most common cause of acute mediastinitis.[42] Perforation usually occurs at one of the three sites of anatomic narrowing of the esophagus: (1) the proximal end, located at the level of the cricopharyngeal muscle; (2) the midthoracic segment, where the aortic arch and left main stem bronchus indent the esophagus; or (3) the transdiaphragmatic segment. A correlation exists between the location of the perforation and the injury. Proximal perforations usually are caused by instrumentation or ingestion of a foreign body. The proximal segment is the narrowest, and perforations generally are located in the posterior wall, adjacent to the prevertebral and retrovisceral spaces. Perforations at the aortic arch usually are caused by ingested foreign bodies. Transdiaphragmatic perforations usually are spontaneous. In most such cases, a longitudinal tear occurs on the left posterolateral wall just above the cardia, where the esophagus has little connective tissue support and its intrinsic musculature is weak.

Retching or vomiting can generate sufficient force to cause esophageal perforation, also known as Boerhaave syndrome. Traumatic perforation can occur after blunt trauma, from ingestion of a foreign body, or as a complication of endoscopic or open surgical procedures. Ingestion of coins, teeth, and food particles such as corn chips as well as objects such as a toy soldier or the spring from a clothespin have caused mediastinitis.[17] Children with mediastinitis from erosion of the esophagus caused by a foreign body tend to have small and well-contained perforations. Sharp objects, such as pins and bone fragments, can cause immediate transmural penetration. More commonly, and especially when a blunt object is ingested, the foreign body becomes impacted in the esophagus.[39] Eventually, suppurative necrosis of the wall occurs, with symptoms occurring days, weeks, or months after the ingestion.[23]

Perforations from instrumentation can produce precipitous clinical deterioration during the procedure from transmural laceration.[42] Alternatively, a superficial tear can occur from which infection subsequently extends, and several hours or days can elapse between instrumentation and the onset of symptoms. Repair of esophageal atresia is a common condition associated with a tear of the esophagus in childhood. In one review, 7 of 41 infants had a clinically significant esophageal disruption requiring reoperation 1 to 18 days after repair of esophageal atresia.[6] Postoperative perforations of the esophagus usually represent infectious complications of anastomotic leaks occurring after esophageal resection or of esophageal-pleural fistulas that develop after thoracic surgery. Most of these infections do not become apparent until weeks or months after surgery.

Presenting findings of acute mediastinitis after perforation of the esophagus include neck and chest pain, respiratory distress, and dysphagia. Chills, a temperature of 37.8° C to 39° C (100° F to 102° F), and leukocytosis also are common. Infants can present with tachypnea, tachycardia, stridor, or a supplemental oxygen requirement. Some patients have a staccato breathing pattern characterized by an inspiratory halt with resumption of inspiration after a brief rest.[12] The onset of symptoms usually is abrupt, and the course is fulminant. Physical examination often reveals cervical tenderness and subcutaneous emphysema in patients with proximal perforations, whereas patients with perforations of the lower esophagus are more likely to have signs suggesting an acute abdominal catastrophe. Examination of the lung fields often shows nonspecific abnormalities. The Hamman sign (a "crunching" sound heard in synchrony with the heartbeat along the left sternal border or cardiac

TABLE 30-1 **Classification of Mediastinitis**

I. Acute mediastinitis
  A. Due to traumatic perforation of the esophagus
    1. Spontaneous or postemetic
    2. Foreign body associated
    3. Instrumentation or surgery
  B. Due to extension of infection from adjacent structures
    1. Infection of the head and neck
    2. Infections of lungs, pleura, lymph nodes, or pericardium
    3. Subphrenic infection
    4. Vertebral osteomyelitis
    5. Hematogenous dissemination
  C. Postoperative
II. Chronic mediastinitis

apex) is observed in approximately 50 percent of cases of mediastinal emphysema, but it also occurs with left pneumothorax, dilated esophagus, gastric dilation, bullous emphysema, and pneumoperitoneum. Children with chronically retained foreign bodies can present with respiratory signs suggestive of asthma or reflux.[9]

The principal findings on chest radiographs are a widened mediastinum, subcutaneous and mediastinal emphysema, and pleural effusions. Pleural effusions occur more commonly with perforations of the lower than the upper esophagus and usually involve the left side. Basilar or retrocardiac infiltrates ascribed to chemical pneumonitis can occur in the pulmonary segment adjacent to the site of perforation. Additional changes often include basilar atelectasis, pneumothorax, or a hydropneumothorax. Radiopaque foreign bodies can be detected with plain radiographs, but often they are seen better with mediastinal computed tomography (CT) or magnetic resonance imaging (MRI).

Gas in the soft tissues seen on radiography is highly suggestive of perforation of the esophagus if interpreted in the context of a compatible clinical presentation. Gas in the prevertebral tissue or superior mediastinum occurs most commonly with perforation of the upper esophagus. Other conditions, such as chest wall trauma or perforation of the trachea, also can cause mediastinal emphysema.

The diagnosis of acute mediastinitis caused by perforation of the esophagus often can be based on clinical coupled with radiographic findings. Contrast-enhanced CT or MRI can provide additional anatomic detail and confirmation of the diagnosis.[47] Findings include esophageal thickening, fluid collections in the mediastinum adjacent to the perforation, and extraluminal air.[1] Other routine laboratory tests are not helpful. Analysis of pleural fluid usually shows a sterile exudate early in the disease course. Pleural fluid amylase levels often are normal within the first 24 hours after perforation. After 24 hours, the pleural fluid amylase level is elevated disproportionately compared with serum levels. Esophagoscopy is unnecessary and is contraindicated except for removing a foreign body.

Surgical drainage and repair and antimicrobial therapy are indicated for large perforations, when there is communication with the pleural space or abdomen, when vascular erosion is a concern, and in the setting of underlying esophageal pathology. Nonsurgical management can be considered for children in whom the perforation is a small, well-contained lesion in the upper esophagus in the absence of an underlying esophageal pathologic process.[10,23] Supportive measures include intravenous fluid support, maintenance of an adequate airway, esophageal rest, and careful monitoring of vital functions. Blood and pleural fluid cultures should be obtained, but they usually are sterile except late in the course. Antimicrobial agents provided empirically should be directed against streptococci, staphylococci, and oral anaerobic bacteria.[5,7] Mortality rates can be high when recognition of the infection is delayed.[42]

Mediastinitis due to Extension of Infection from Adjacent Structures

The mediastinum is anatomically well situated for involvement when infection extends downward from the oropharynx. Fascial planes from the supraclavicular region and abdomen traverse the mediastinum, and the lymphatic duct is located in the mediastinum. The lung, situated laterally to the mediastinum, is a frequent locus of potentially serious infection. Despite its position as an anatomic crossroad, however, extension of infection to the mediastinum from adjacent structures occurs infrequently.

In the preantibiotic era, retropharyngeal or peritonsillar abscess, Ludwig angina, dental abscess, and other infections of the head and neck were common causes of acute mediastinitis.[14,46,53] Since the advent of penicillin, infections of the head and neck usually are contained at the site of origin. The principal spaces that serve as conduits to the mediastinum when these infections extend are the visceral division of the deep cervical fascia that envelops the esophagus, trachea, larynx, and thyroid gland and the carotid sheath, which extends from the base of the skull, passes through the posterior pharyngomaxillary space along the prevertebral fascia, and enters the chest. Mediastinitis can occur as a complication of retropharyngeal abscess, peritonsillar and dental abscesses, as well as mastoiditis, laryngectomy, mediastinotomy, tracheostomy, and surgery or trauma of the oropharynx. Mediastinitis can complicate placement of airway stents for management of tracheal or bronchial stenoses in children.[21]

Children have developed mediastinitis after incurring intraoral injuries caused by falling with an object such as a toothbrush in their mouths.[25] Infection spreads to the mediastinum through the retropharyngeal space. As noted earlier, sharp objects, such as fish bones, can perforate the esophagus, with resultant infection.[36] A penetrating wound to the oropharynx can be caused by falling on a sharp or pointed object such as a pencil. Mediastinitis rarely results in a complication of suppurative pleuropulmonary infection. Extension of infection from vertebrae, ribs, or sternum also is unusual. The main radiographic feature by plain chest radiograph is widening of the mediastinum. Imaging by contrast-enhanced cervicothoracic CT or MRI is crucial for establishing the

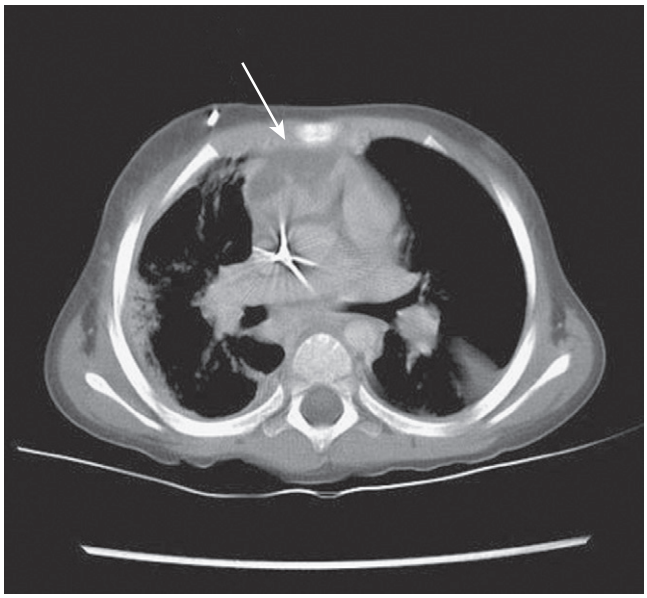

FIGURE 30-1 ■ Contrast-enhanced CT scan shows a 3 × 3-cm heterogeneous enhancing abscess *(arrow)* in the anterior mediastinum of a child acutely ill with *Streptococcus pneumoniae* bacteremia and pneumonia with empyema. Cultures from the mediastinal abscess at the time of surgical drainage were sterile.

diagnosis and can reveal heterogeneous infiltration, gas in tissues, abscesses, and fluid collections (Fig. 30-1).

The major bacteria responsible for suppurative infections that originate in the oral cavity and extend to the mediastinum are streptococci and anaerobic oral flora.[7,35] Methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MRSA) should be considered potential pathogens, especially in mediastinitis complicating retropharyngeal abscess.[43,51] Mixed infection with aerobes and anaerobes should be anticipated when empirical therapy is initiated. Group A streptococcus is a common pathogen when the oropharynx is the original portal of entry. Other streptococci, including *Streptococcus anginosus* (formerly *S. milleri*) and group F streptococcus, are common isolates. Anaerobic bacteria, principally *Prevotella* spp., *Bacteroides* spp., *Fusobacterium* spp., and peptostreptococci, are the major anaerobic pathogens. Gram-negative aerobic bacteria, including *Pseudomonas aeruginosa*, *Serratia* spp., and *Neisseria* spp., are isolated less often.[28] Clindamycin often is regarded as the agent of choice for streptococci and anaerobes, although some authorities prefer other regimens, including penicillin plus metronidazole, cefoxitin, cefotetan, meropenem, or a β-lactam/β-lactamase inhibitor.[7,13] Consideration should be given to including vancomycin in empirical regimens for MRSA coverage until culture results are available.

Surgical drainage combined with appropriate antibiotic therapy is the cornerstone of treatment. Transcervical incisions usually are employed when spread to the superior mediastinum has occurred. Extension of the infection below the level of the fourth thoracic vertebra requires a parasternal or paravertebral approach, depending on whether the anterior or posterior mediastinum is involved.

TABLE 30-2 **Risk Factors for Pediatric Poststernotomy Mediastinitis**

Known or possible genetic syndrome
Young age
High American Society of Anesthesiologists score
Asplenia
Prolonged duration of surgery
Cardiac transplantation
Intracardiac pacing wires longer than 3 days
Delayed sternal closure

Data from references 2, 22, 31, and 48.

Postoperative Mediastinitis

Sternal wound infections are an uncommon complication of median sternotomy for cardiac surgery in children.* The Centers for Disease Control and Prevention classifies surgical site infections in cardiac patients as superficial or deep wound infection, sternal osteomyelitis, or mediastinitis. Mediastinitis is a serious complication, with potential involvement of contiguous structures, including prosthetic valves, grafts, pericardium, lung, and chest wall. Contemporary incidence data for mediastinitis after pediatric cardiac surgery is 0.2 to 1.4 percent of procedures.[2,27,31,48] The incidence is higher after cardiac transplantation.[27] Postoperative wound infection is more often a complication of double-outlet right ventricle repair, truncus repair, atrial switch, or valvulatory or conduit procedures than of repair of atrial septal defect, ventricular septal defect, or tetralogy of Fallot.[38] Mediastinal infection has developed from an infection of a retained epicardial pacemaker lead.[19]

Some of the well-established risk factors for development of poststernotomy mediastinitis in adults are not applicable to children. Factors that are linked to risk for development of mediastinitis after median sternotomy in children are shown in Table 30-2.[2,22,31,48] Obesity and diabetes mellitus, which are risk associations in adults, have not been assessed as potential risk factors in more recent pediatric series. Factors not associated with risk for infection include emergency surgery or prior infection.[31] Intraoperative introduction of organisms is considered the source of most infections. Epidemics have been traced to operating room personnel, who can serve as the source of *S. aureus* or other bacteria.[15]

In three pediatric series, median age at diagnosis of mediastinitis after pediatric cardiac surgery ranged from 6 weeks to 3 years.[2,27,48] Median time to onset of infection after surgery was 10 to 14 days, with a range from 4 to 50 days. The time to infection after initial sternotomy was longer for infections caused by gram-positive organisms than for infections caused by gram-negative bacteria or fungi in one report.[48] The presenting features include erythema, purulent drainage or tenderness of the sternal incision, wound dehiscence, sternal instability, persistent or recurrent postoperative fever,

*References 2, 4, 27, 31, 38, 48.

and leukocytosis. A common clinical sequence is fever and systemic toxicity followed by signs of a sternal wound infection with cellulitis or purulent drainage. Imaging abnormalities include mediastinal soft tissue swelling, pleural effusion, and sternal dehiscence or erosion, but CT does not always reveal abnormalities.[33,52] Consideration should be given to use of MRI, when infection is suspected. Lack of compelling radiographic evidence of infection should not delay surgical drainage when clinical signs are evident.

Empirical antibiotic treatment should be initiated based on the expected pathogens and modified according to the results of blood and wound cultures. Approximately two thirds of infections are caused by gram-positive bacteria, one fourth by gram-negative bacteria, and the remainder by yeasts and fungi. The most frequent pathogen is *S. aureus*, which accounts for more than half of infections; coagulase-negative staphylococci and enterococci (including vancomycin-resistant isolates) constitute the remainder of the gram-positive isolates.[2,27,48] In adults, methicillin-resistant *S. aureus* mediastinitis is associated with higher rates of overall mortality, mediastinitis-related mortality, and treatment failure than infection caused by methicillin-sensitive *S. aureus*.[32]

Approximately half of children have associated bacteremia, and infection with *S. aureus* is an independent risk factor for development of bacteremic postoperative mediastinitis.[44] Among gram-negative organisms, *P. aeruginosa*, *Serratia* spp., and *Citrobacter* are common isolates. Infection is polymicrobial in one fourth of patients.[2,27,48] *Candida* spp. should be considered in any patient with infection of the mediastinum, particularly when broad-spectrum antimicrobial agents have been used.[8] Almost any microorganism gaining entry to the mediastinum theoretically can serve as the nidus for infection, and *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Nocardia*, and *Aspergillus* all have been reported, albeit rarely.[16,26,30,50] Heart and lung transplant patients are prone to acquisition of infection with less common and more resistant pathogens, such as *Aspergillus fumigatus* and *Burkholderia cepacia*.[27] *M. fortuitum* can cause apparently culture-negative mediastinitis and has been reported in a child who had undergone a Fontan operation.[45]

Adequate surgical débridement is crucial to successful treatment of postoperative mediastinitis. Surgical procedures to reexplore the sternum should be undertaken and infected and devitalized tissue débrided. After wound débridement, continuous irrigation and vacuum-assisted closure and use of simple primary closed drainage has been an effective management option.[3,49] Open wound packing and delayed rectus abdominis flap, pectoralis muscle flap, or omental reconstruction is required in some children.[37,48] Pectoralis muscle flap has been an effective treatment even in neonates.[11,48] Early consultation with the plastic surgery and reconstructive team is advisable when sternal reconstruction is anticipated. Infection superficial to the sternum can be treated simply with incision, packing, and a short course of antibiotics, usually for 10 to 14 days. Systemic antibiotics must be given for at least 3 to 6 weeks for children with postoperative mediastinitis.

CHRONIC MEDIASTINITIS

Chronic mediastinitis histologically can be fibrosing or granulomatous. Both are rare conditions in which a definite cause often remains elusive. The clinical presentations are identical, and considerable overlap can occur in histologic findings. Fibrosing mediastinitis can be focal or diffuse; calcification within the lesion is observed more often when the process is focal. Some authors propose that mediastinal fibrosis represents the end stage of mediastinal granuloma, whereas others suggest that the two are distinct entities.[41] Fibrosing mediastinitis can be associated with a fibrotic process at another anatomic site, such as the retroperitoneum.

Chronic mediastinitis is rare in very young children but has been described in toddlers.[40] Many patients are asymptomatic, and the lesion initially is detected by routine chest radiographs showing a widened superior mediastinum near the tracheal bifurcation or the hilum with a lobulated configuration. Concomitant changes in the pulmonary parenchyma that are seen on chest radiograph vary. Common clinical presentations include recurrent pneumonia and hemoptysis.[20] Symptoms, when manifested, can reflect compression of adjacent structures, such as the superior vena cava, pulmonary vessels, esophagus, and tracheobronchial tree. Patients can present with low-grade fever, cough, pleuritic chest pain and dyspnea, with anemia and weight loss.[41]

When an inciting cause for chronic mediastinitis can be identified, it usually is linked to infection with *Histoplasma capsulatum*. Two types of mediastinal fibrosis caused by histoplasmosis are recognized. Mediastinal granuloma is caused by coalescence of a cluster of mediastinal lymph nodes to form an encapsulated mass that may be large and compress adjacent structures, especially the superior vena cava or esophagus. Surgical resection often is feasible but is advocated only when the obstruction is significant. Fibrosing mediastinitis is thought to result from an exuberant inflammatory response to *H. capsulatum* and to represent a resolved, rather than an active, infectious process. Less common causes of fibrosing mediastinitis include tuberculosis, blastomycosis, cryptococcosis, aspergillosis, zygomycosis, autoimmune disease, nocardiosis, actinomycosis, and lymphoma.*

The diagnostic evaluation of patients with possible chronic mediastinitis should include a chest radiograph and contrast-enhanced CT or MRI. A vascular imaging study may be required if there is evidence of venous or vena caval obstruction or if arterial involvement is present. Testing for tuberculosis and histoplasmosis serology should be obtained. Cultures of sputum for *Mycobacterium tuberculosis* and pathogenic fungi are rarely positive. Antigen assay for *H. capsulatum* using blood and urine usually is negative, and skin tests are not helpful. Complement-fixation titers usually exceed 1 : 8 in patients with chronic histoplasmosis.

Surgical resection can be associated with high morbidity rates but can be curative. Biopsy of the lesion by mediastinoscopy can exclude a malignant process and

*References 18, 24, 29, 34, 40, 41.

provide tissue for histopathologic evaluation. Antifungal therapy is not likely to affect resolution of the process when surgically excised tissue shows fungi consistent with *H. capsulatum* with sterile cultures. Dense fibrosis of mediastinal structures without a granulomatous component does not respond to antifungal treatment.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Barker GM, O'Brien SM, Welke KF, et al. Major infection after pediatric cardiac surgery: a risk estimation model. *Ann Thorac Surg* 2009;**89**:843–50.
9. Cole S, Kearns D, Magit A. Chronic esophageal foreign bodies and secondary mediastinitis in children. *Ann Otol Rhinol Laryngol* 2011;**120**:542–5.
20. Hammoud ZT, Rose AS, Hage CA, et al. Surgical management of pulmonary and mediastinal sequelae of histoplasmosis: a challenging spectrum. *Ann Thorac Surg* 2009;**88**:399–404.
43. Shah RK, Chun R, Choi SS. Mediastinitis in infants from deep neck space infections. *Otolaryngol Head Neck Surg* 2009;**140**:936–8.
49. Ugaki S, Kasahara S, Arai S, et al. Combination of continuous irrigation and vacuum-assisted closure is effective for mediastinitis after cardiac surgery in small children. *Interactive Cardiovasc Thorac Surg* 2010;**11**:247–51.
51. Wright CT, Stocks RMS, Armstrong DL, et al. Pediatric mediastinitis as a complication of methicillin-resistant *Staphylococcus aureus* retropharyngeal abscess. *Arch Otolaryngol Head Neck Surg* 2008; **134**:408–13.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Akman C, Kantarci F, Cetinkaya S. Imaging in mediastinitis: a systemic review based on aetiology. *Clin Radiol* 2004;**59**:573–85.
2. Al-Sehly AA, Robinson JL, Lee BE, et al. Pediatric poststernotomy mediastinitis. *Ann Thorac Surg* 2005;**80**:2314–20.
3. Anslot C, Hulin S, Durandy Y. Postoperative mediastinitis in children: improvement of simple primary closed drainage. *Ann Thorac Surg* 2007;**84**:423–8.
4. Barker GM, O'Brien SM, Welke KF, et al. Major infection after pediatric cardiac surgery: a risk estimation model. *Ann Thorac Surg* 2009;**89**:843–50.
5. Brook I. The role of anaerobic bacteria in mediastinitis. *Drugs* 2006;**66**:315–20.
6. Chavin K, Field G, Chandler J, et al. Save the child's esophagus: management of major disruption after repair of esophageal atresia. *J Pediatr Surg* 1996;**31**:48–52.
7. Civen R Vaisanen ML, Finegold SM. Peritonsillar abscess, retropharyngeal abscess, mediastinitis and nonclostridial anaerobic myonecrosis: a case report. *Clin Infect Dis* 1992;**16**(Suppl 4):S299–303.
8. Clancy CJ, Nguyen MH, Morris AJ. Candidal mediastinitis: an emerging clinical entity. *Clin Infect Dis* 1997;**25**:608–13.
9. Cole S, Kearns D, Magit A. Chronic esophageal foreign bodies and secondary mediastinitis in children. *Ann Otol Rhinol Laryngol* 2011;**120**:542–5.
10. Demirbag S, Tiryaki T, Atabek C, et al. Conservative approach to the mediastinitis in childhood secondary to esophageal perforation. *Clin Pediatr* 2005;**44**:131–4.
11. Erez E, Katz M, Sharoni E, et al. Pectoralis major muscle flap for deep sternal wound infection in neonates. *Ann Thorac Surg* 2000;**69**:572–7.
12. Feldman R, Gromischm DS. Acute suppurative mediastinitis. *Am J Dis Child* 1971;**121**:79–81.
13. Finegold SM, Wexler HM. Therapeutic implications of bacteriologic findings in mixed aerobic-anaerobic infections. *Antimicrob Agents Chemother* 1988;**32**:611–6.
14. Garatea-Crelgo J, Gay-Escoda C. Mediastinitis from odontogenic infection: report of three cases and review of the literature. *Int J Oral Maxillofac Surg* 1991;**20**:65–8.
15. Gaynes R, Marosok R, Mowry-Hanley J, et al. Mediastinitis following coronary artery bypass surgery: a 3-year review. *J Infect Dis* 1991;**163**:117–21.
16. Geers TA, Taege AJ, Longworth DL, et al. *Ureaplasma urealyticum*: unusual cause of culture-negative mediastinitis. *Clin Infect Dis* 1999;**29**:949–50.
17. Gilchrist BF, Valerie EP, Nguyen M, et al. Pearls and perils in the management of prolonged, peculiar, penetrating esophageal foreign bodies in children. *J Pediatr Surg* 1997;**32**:1429–31.
18. Goodwin RA, Nickell JA, Des Prez RM. Mediastinal fibrosis complicating healed primary histoplasmosis and tuberculosis. *Medicine (Baltimore)* 1972;**51**:227–46.
19. Hachiro Y, Kikuchi S, Ito M, et al. Infection of a retained permanent epicardial pacemaker lead. *Ann Thorac Surg* 2001;**71**:2038–9.
20. Hammoud ZT, Rose AS, Hage CA, et al. Surgical management of pulmonary and mediastinal sequelae of histoplasmosis: a challenging spectrum. *Ann Thorac Surg* 2009;**88**:399–404.
21. Jacobs JP, Quintessenza JA, Botero LM, et al. The role of airway stents in the management of pediatric tracheal, carinal, and bronchial disease. *Eur J Cardiothorac Surg* 2000;**18**:505–12.
22. Kagen J, Lautenbach E, Bilker WB, et al. Risk factors for mediastinitis following median sternotomy in children. *Pediatr Infect Dis J* 2007;**26**:613–8.
23. Kerschner JE, Beste DJ, Conley SF, et al. Mediastinitis associated with foreign body erosion of the esophagus in children. *Int J Pediatr Otorhinolaryngol* 2001;**59**:89–97.
24. Langerstrom CF, Mitchell HG, Graham BS, et al. Chronic fibrosing mediastinitis and superior vena caval obstruction from blastomycosis. *Ann Thorac Surg* 1992;**54**:764–5.
25. Law RC, Fouque CA, Waddell A, et al. Penetrating intra-oral trauma in children. *BMJ* 1997;**314**:50–1.
26. Lequier L, Robinson J, Vaudry W. Sternotomy infection with *Mycoplasma hominis* in a neonate. *Pediatr Infect Dis J* 1995;**14**:1010–1.
27. Long CB, Shah SS, Lautenbach E, et al. Postoperative mediastinitis in children: epidemiology, microbiology and risk factors for Gram-negative pathogens. *Pediatr Infect Dis J* 2005;**24**:315–9.
28. Makeieff M, Gresillon N, Berthet JP, et al. Management of descending necrotizing mediastinitis. *Laryngoscope* 2004;**114**:772–5.
29. Mathisen DJ, Grillo HC. Clinical manifestations of mediastinal fibrosis and histoplasmosis. *Ann Thorac Surg* 1992;**54**:1053–7.
30. Mattila PS, Carlson P, Sivonen A, et al. Life-threatening *Mycoplasma hominis* mediastinitis. *Clin Infect Dis* 1999;**29**:1529–37.
31. Mehta PA, Cunningham CK, Colella CB, et al. Risk factors for sternal wound and other infections in pediatric cardiac surgery patients. *Pediatr Infect Dis J* 2000;**19**:1000–4.
32. Mekontso-Dessap A, Kirsch M, Brun-Buisson C, et al. Poststernotomy mediastinitis due to *Staphylococcus aureus:* comparison of methicillin-resistant and methicillin-susceptible cases. *Clin Infect Dis* 2001;**32**:877–83.
33. Misawa Y, Fuse K, Hasegawa T. Infectious mediastinitis after cardiac operations: computed tomographic findings. *Ann Thorac Surg* 1998;**65**:622–4.
34. Mole TM, Glover J, Sheppard MN. Sclerosing mediastinitis: a report on 18 cases. *Thorax* 1995;**50**:280–3.
35. Murray PM, Finegold SM. Anaerobic mediastinitis. *Rev Infect Dis* 1984;**6**:5123–7.
36. Nozoe T, Kitamura M, Adachi Y, et al. Successful conservative treatment for esophageal perforation by a fish bone associated with mediastinitis. *Hepatogastroenterology* 1998;**45**:2190–2.
37. Ohye RG, Maniker RB, Graves HL, et al. Primary closure for postoperative mediastinitis in children. *J Thorac Cardiovasc Surg* 2004;**128**:480–6.
38. Pollock EMM, Ford-Jones EL, Rebeyka I, et al. Early nosocomial infections in pediatric cardiovascular surgery patients. *Crit Care Med* 1990;**18**:378–84.
39. Reino AJ, Jahn AF, Parsons J, et al. Traumatic pneumomediastinum in a child secondary to corn chip perforation of the esophagus. *Pediatr Emerg Care* 1993;**9**:211–5.
40. Robertson BD, Bautista MA, Russell TS, et al. Fibrosing mediastinitis secondary to zygomycosis in a twenty-two-month-old child. *Pediatr Infect Dis J* 2002;**21**:441–2.
41. Rossi SE, McAdams HP, Rosado-de-Christenson ML, et al. Fibrosing mediastinitis. *RadioGraphics* 2001;**21**:737–57.
42. Salo JA, Isolauri JO, Heikkila LJ, et al. Management of delayed esophageal perforation with mediastinal sepsis. *J Thorac Cardiovasc Surg* 1993;**106**:1088–91.
43. Shah RK, Chun R, Choi SS. Mediastinitis in infants from deep neck space infections. *Otolaryngol Head Neck Surg* 2009;**140**:936–8.
44. Shah SS, Lautenbach E, Long CB, et al. *Staphylococcus aureus* as a risk factor for bloodstream infection in children with postoperative mediastinitis. *Pediatr Infect Dis J* 2005;**24**:834–7.
45. Syed AU, Hussain R, Bhat AN, et al. Mediastinitis due to *Mycobacterium fortuitum* infection following Fontan operation in a child. *Scand Cardiovasc J* 1997;**31**:311–3.
46. Sztajnbok J, Grassi MS, Katayama DM, et al. Descending suppurative mediastinitis: nonsurgical approach to this unusual complication of retropharyngeal abscesses in childhood. *Pediatr Emerg Care* 1999;**15**:341–3.
47. Tecce PM, Fishman EK, Kuhlman JE. CT evaluation of the anterior mediastinum: Spectrum of disease. *RadioGraphics* 1994; **14**:973–90.
48. Tortoriello TA, Friedman JD, McKenzie ED, et al. Mediastinitis after pediatric cardiac surgery: a 15-year experience at a single institution. *Ann Thorac Surg* 2003;**76**:1655–60.
49. Ugaki S, Kasahara S, Arai S, et al. Combination of continuous irrigation and vacuum-assisted closure is effective for mediastinitis after cardiac surgery in small children. *Interactive Cardiovasc Thorac Surg* 2010;**11**:247–51.
50. Wenger PN, Brown JM, McNeil MM, et al. *Nocardia farcinica* sternotomy site infections in patients following open heart surgery. *J Infect Dis* 1998;**178**:1539–43.
51. Wright CT, Stocks RMS, Armstrong DL, et al. Pediatric mediastinitis as a complication of methicillin-resistant *Staphylococcus aureus* retropharyngeal abscess. *Arch Otolaryngol Head Neck Surg* 2008; **134**:408–13.
52. Yamaguchi H, Yamauchi H, Yamada T, et al. Diagnostic validity of computed tomography for mediastinitis after cardiac surgery. *Ann Thorac Cardiovasc Surg* 2001;**7**:94–8.
53. Zeitoun IM, Dhanarajani PJ. Cervical cellulitis and mediastinitis caused by odontogenic infections: report of two cases and review of the literature. *J Oral Maxillofac Surg* 1995;**53**:203–8.

SECTION IV

CENTRAL NERVOUS SYSTEM INFECTIONS

CHAPTER 31

Bacterial Meningitis Beyond the Neonatal Period

Kwang Sik Kim

Bacterial meningitis is an inflammation of the meninges affecting the pia, the arachnoid, and the subarachnoid space that occurs in response to bacteria and bacterial products. A discussion of infections of neonates, including bacterial meningitis, is presented here, and infections of the central nervous system (CNS) caused by mycobacteria are discussed in other chapters.

INCIDENCE AND EPIDEMIOLOGY

Before the discovery and use of antibiotics, bacterial meningitis generally was fatal. Antibiotic therapy has dramatically improved the prognosis in patients with bacterial meningitis, although it continues to be a significant cause of morbidity and mortality in infants and children. The number of deaths attributed to many other infectious diseases in the United States decreased by 10-fold to 200-fold between 1935 and 1968, whereas the number of reported deaths caused by bacterial meningitis decreased by only half during the same period.[366,367]

The epidemiology of bacterial meningitis has changed by several recent preventive measures introduced since the early 1990s, such as introduction of the *Haemophilus influenzae* type b conjugate vaccine and the pneumococcal conjugate vaccine for infants and screening of pregnant women for group B streptococcus and intrapartum antibiotic prophylaxis since 1996.*

Before the widespread use of the *H. influenzae* type b conjugate vaccine, *H. influenzae* type b was the most common cause of bacterial meningitis in children in the United States, Canada, and Scandinavia, but this pattern was not universal. Davey and associates[89] reported that between 1968 and 1977, *Neisseria meningitidis* was the most common cause and *H. influenzae* type b was the second most common cause of bacterial meningitis for children and young adults in Great Britain. Data from 2001 showed that meningococcal meningitis accounted for 48 percent of meningitis in children in England.[90]

Studies in the 1970s and 1980s showed that three pathogens (*H. influenzae*, *Streptococcus pneumoniae*, and *N. meningitidis*) are predominant in infants, children, and young adults, whereas group B streptococcus, *Escherichia coli*, and *Listeria monocytogenes* are the most common bacteria causing meningitis in neonates. Between 1986 and 1995, the incidence of bacterial meningitis declined markedly, largely owing to the introduction of *H. influenzae* type b conjugate vaccines in infants. After the introduction of the heptavalent pneumococcal conjugate vaccine (PCV7), including pneumococcal serotypes 4, 6B, 9V, 14, 8C, 19F, and 23F, to infants in 2000, invasive pneumococcal disease in the United States declined by 75 percent among children younger than 5 years of age as well as by 31 percent among adults 65 years or older and in unvaccinated populations through herd immunity.[181,200]

In 1972, the Centers for Disease Control and Prevention (CDC) estimated that in the United States, 29,000 cases of meningitis were caused by *H. influenzae* type b, 4,800 cases were caused by *S. pneumoniae*, and 4,600 cases were caused by *N. meningitidis*. However, based on cases of bacterial meningitis from 2003 to 2007, an estimate of an average of 4,100 cases of bacterial meningitis, including 500 that were fatal, occurred annually in the United States during 2003 to 2007.[358]

Population-based studies in South Carolina, Minnesota, Vermont, and New Mexico in the 1970s suggested that the actual incidence of bacterial meningitis ranged from 5.4 to 7.3 cases per 100,000 population.[62,131-134] Studies reported from the CDC in 2011 indicated that the incidence of bacterial meningitis ranged from 6.91 cases per 100,000 population for children aged 2 to 23 months to 0.43 case per 100,000 population for adolescents aged 11 to 17 years (Table 31-1).[358]

A recent report of bacterial meningitis in the United States from 1998 to 2007 showed that the overall incidence of meningitis declined by 31 percent from 2.00 cases per 100,000 population in 1998 to 1999 to 1.38 cases per 100,000 population in 2006 to 2007, but the incidence remained unchanged in patients younger than 2 months of age (see Table 31-1).[358] The incidence of bacterial meningitis was highest for patients younger than 2 months of age and for black patients of any age. The median age of patients increased from 30.3 years during 1998 to 1999 to 41.9 years during 2006 to 2007, and this shift was significant, suggesting that the burden of bacterial meningitis is borne more by older adults. However, the case-fatality rate did not change significantly from 15.7 percent in 1997 to 1998 to 14.3 percent in 2006 to 2007.[358]

In 2003 to 2007 in the United States, the most frequent cause of bacterial meningitis in children younger than 2 months of age was group B streptococcus (86.1% of cases), and *N. meningitidis* caused 45.9 percent of cases

*References 81, 200, 238, 276, 322, 358.

TABLE 31-1 **Age-Based Incidence of Bacterial Meningitis in the United States, 1998-2007, per 100,000 Population**

Age	1998-1999	2006-2007	Percent Change 2006-2007 vs. 1998-1999
<2 mo	74.46	80.69	10
2-23 mo	14.20	6.91	–51
2-10 yr	1.55	0.56	–64
11-17 yr	1.03	0.43	–58
18-34 yr	0.99	0.66	–33
35-49 yr	1.23	0.95	–23
50-64 yr	2.15	1.73	–19
≥65 yr	2.64	1.92	–27
All ages	2.00	1.38	–31

From Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States 1998-2007. *N Engl J Med* 2011;**364**:2016–25.

TABLE 31-2 **Pathogen-Based Incidence of Bacterial Meningitis in the United States, 1998-2007, per 100,000 Population**

Pathogen	1998-1999	2006-2007	Percent Change 2006-2007 vs. 1998-1999
S. pneumoniae	1.09	0.81	–26
N. meningitidis	0.44	0.19	–58
Group B streptococcus	0.24	0.25	4
H. influenzae	0.12	0.08	–35
L. monocytogenes	0.10	0.05	–46

From Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States 1998-2007. *N Engl J Med* 2011;**364**:2016–25.

among those 11 to 17 years of age. Among the other pediatric age groups, *S. pneumoniae* was the most common cause (Table 31-2).[358] The case-fatality rate was 6.9 percent among pediatric patients, and nearly 10 percent had underlying immunocompromising or chronic medical conditions. *E. coli* and *L. monocytogenes* are other common causes of meningitis in neonates 2 to 6 weeks of age.[358]

Epidemiology of Meningitis Caused by *Streptococcus pneumoniae*

S. pneumoniae is the most common pathogen causing bacterial meningitis in the United States. The risk for development of sepsis or meningitis caused by *S. pneumoniae* depends to some extent on the serotype with which the child is colonized and against which there is no opsonic antibody. Although 90 pneumococcal serotypes have been identified, invasive disease, including sepsis and meningitis, in children younger than 6 years old was associated most commonly with serotypes 14, 6B, 19F, 18C, 23F, 4, and 9V in the United States.[57] The 7-valent pneumococcal conjugate vaccine (PCV7), added to the national vaccination schedule in 2000, contains each of the just-mentioned most common strains.[67]

Nasopharyngeal colonization in children mainly depends on age (young age), crowding, attendance at daycare centers, and ethnicity (the Native American population). The serotype distribution of colonization isolates is by and large a reliable indicator for invasive disease, antibiotic resistance profiles, and potential vaccine coverage.[44] Daycare attendance, underlying disease, and lack of breast-feeding were risk factors for acquiring invasive pneumococcal infections in a large case-control study that included children from the United States and Canada.[228] Although cigarette smoking is a major risk factor for the development of invasive pneumococcal disease in adults, whether exposure to cigarette smoke increases the risk for developing systemic pneumococcal infection in children remains unclear.[271]

TABLE 31-3 **Age-Based Incidence of Pneumococcal Meningitis in the United States, 1998-2007, per 100,000 Population**

Age	1998-1999	2006-2007	Percent Change 2006-2007 vs. 1998-1999
2-23 mo	9.69	3.67	–62
2-10 yr	0.54	0.36	–34
11-17 yr	0.20	0.21	7
18-34 yr	0.40	0.27	–33
35-49 yr	0.83	0.76	–8
50-64 yr	1.47	1.34	–9
≥65 yr	1.88	1.43	–24
All ages	1.09	0.81	–26

From Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States 1998-2007. *N Engl J Med* 2011;**364**:2016–25.

In the prevaccine era (before 2000 in the United States), the age-specific incidence for pneumococcal meningitis was 21, 12, 6, 2, and 0.5 cases per 100,000 for the age groups 0 to 6 months, 7 to 12 months, 13 to 24 months, 25 to 60 months, and older than 60 months, respectively.[394] The overall incidence of *S. pneumoniae* meningitis, however, decreased by 26 percent from 1.09 cases per 100,000 population in 1998 to 1999 to 0.81 case per 100,000 based on the recent report of bacterial meningitis in the United States.[358] This decline included a decrease of 62 percent among children 2 to 23 months of age (Table 31-3). The incidence of meningitis from *S. pneumoniae* PCV7 serotypes declined by 92 percent from 0.61 case per 100,000 population in 1998 to 1999 to 0.05 case per 100,000 population in 2006 to 2007. However, the incidence of meningitis from non-PCV7 serotypes increased by 62 percent from 0.48 case per 100,000 population in 1998 to 1999 to 0.77 case per 100,000 population in 2006 to 2007.[358] Meningitis with *S. pneumoniae* had a case-fatality rate of 17.9 percent in 1998 to 1999 and 14.7 percent in 2006 to 2007.[358]

Among children ages 2 months to 10 years, *S. pneumoniae* was the most common cause of bacterial meningitis. PCV7 serotypes accounted for 15.5 percent of the pediatric cases overall and 13.0 percent of cases in the 2- to 23-month age groups. The case-fatality rates were similar among children infected with PCV7 isolates and those infected with non-PCV7 isolates (10.7% and 7.6%, respectively). Use of PCV7 also reduced invasive pneumococcal disease including meningitis among unvaccinated populations through herd immunity (see Table 31-3).[200] PCV13 serotypes (PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F, and 19A) accounted for 60.0 percent of cases in children between 2 and 23 months of age and 57.2 percent in children of any age. PCV7 and PCV13 serotypes accounted for 16.0 and 41.6 percent of the adult meningitis cases, respectively.[358] The overall fatality rate was 16.4 percent, and the rate increased linearly with increasing age (8.9% among patients 18 to 34 years of age to 22.7% among those 65 years and older, $p<.001$).[358] Vaccine serotype coverages by PCV7 and PCV13 are less in European and other countries than those in the United States.[108] Surveys of nasopharyngeal carriage of *S. pneumoniae* in Australia revealed that the percentages of serotypes included in PCV13 were 40 percent in 2- to 15-year-old children and 29 percent in adults.[236]

Pneumococcal infections generally occur sporadically. Household contacts of a patient with pneumococcal disease are not considered to be at increased risk for acquiring secondary infection. The occurrence of concurrent pneumococcal disease (meningitis and bacteremia) in the household setting has been reported, however.[19,332] Although uncommon in the United States, *S. pneumoniae* has been associated with epidemics in locations such as Northern Ghana.[226]

The incidence of systemic infection with penicillin-resistant *S. pneumoniae* has been increasing steadily worldwide since it first was reported in Australia in the 1960s.[173] Systemic infection with penicillin-resistant pneumococci has become an increasing problem in the United States since the mid 1980s.[345,352] In 1995, a CDC multistate surveillance study showed that 35 percent of cerebrospinal fluid (CSF) isolates of *S. pneumoniae* were resistant to penicillin.[319]

The first report of meningitis caused by resistant pneumococci was published in 1974,[264] and numerous case reports appeared subsequently. Patients with systemic infections caused by penicillin-resistant pneumococci were more likely to have received a course of antibiotics within 1 month before acquiring their infection than were matched control subjects who had infections caused by pneumococci but whose isolates were susceptible to penicillin.[352]

By 1998, CDC surveillance of multiple areas around the United States found that 24 percent of pneumococcal isolates associated with invasive infections were nonsusceptible to penicillin; 14 percent of isolates were nonsusceptible to cefotaxime.[386] As with many studies, rates of resistance were higher among children younger than 5 years old. In contrast, penicillin- and cefotaxime-nonsusceptible pneumococcal isolates were 10.5 and 8.7 percent, respectively, in 2010.[358] In a prospective study involving eight children's hospitals nationwide during a 6-year period starting September 1993, a significant increase in the proportion of isolates nonsusceptible to penicillin or ceftriaxone was found. In the sixth year of the study ending August 1998, 37 percent and 11 percent of invasive isolates were nonsusceptible to penicillin and ceftriaxone, respectively.[192] Isolates of penicillin-resistant and third-generation cephalosporin-resistant *S. pneumoniae* have been recovered in other regions of the world as well.* Treatment failures in patients with *S. pneumoniae* meningitis resistant to penicillin and third-generation cephalosporins have led to changes in the empirical therapy of suspected bacterial meningitis, as discussed in the section on treatment.[140,205,335,362]

Data from the Active Bacterial Core Surveillance of the Emerging Infections Program Network after introduction of the 7-valent pneumococcal conjugate vaccine showed an 87 percent decrease in rates of antibiotic-resistant disease by vaccine serotypes.[216] These results show that rates of invasive pneumococcal disease with penicillin-nonsusceptible strains and strains nonsusceptible to multiple antibiotics "peaked in 1999 and decreased by 2004."[216] In children younger than 2 years old, invasive pneumococcal disease with penicillin-nonsusceptible strains decreased from 70.3 to 13.1 cases per 100,000.[216] The proportion of disease caused by penicillin-nonsusceptible strains varied by location within the United States from 9.4 percent in California to 29.5 percent in Tennessee.[216] The incidence of invasive *S. pneumoniae* disease caused by cefotaxime-resistant *S. pneumoniae* strains increased in children younger than 2 years of age, however, in the postvaccination period, from approximately 10 percent in 1999 to almost 15 percent in 2003.[216] The decreases in disease caused by vaccine-type *S. pneumoniae* strains were coupled with an increase in nonvaccine serotype *S. pneumoniae* strains, especially with serotype 19A (increased from 2.0 to 8.3 cases per 100,000).[216,361]

Recent data from 1998 to 2009 from continuous surveillance of invasive pneumococcal disease in eight geographic areas of the United States indicate that reductions in rates ranged by site from 19.0 to 29.9 cases per 100,000 population during 1998 to 1999 to 11.2 to 18.0 cases per 100,000 population during 2009 (rate reduction of 5.1 to 15.3 cases per 100,000 population).[302] Reductions among children aged younger than 5 years ranged from 35.7 to 117.2 cases per 100,000 population across the sites. Reductions in rates due to PCV7 were seen in all age groups and at all sites, ranging from 12.0 to 21.4 cases per 100,000 population during 1998 to 1999 to less than 2.0 cases per 100,000 population during 2009 (92% to 98% reductions). Serotype 19A rates ranged from 0.4 to 1.5 cases per 100,000 population during 1998 to 1999 to 1.3 to 3.4 cases per 100,000 population during 2009 (rate difference of 0.9 to 2.8 cases per 100,000 population). Rates due to all other serotypes ranged from 6.3 to 10.3 cases per 100,000 population during 1998 to 1999 to 8.3 to 13.6 cases per 100,000 population during 2009 (rate difference of –0.04 to 5.67 cases per 100,000 population). Across the sites, the greatest increases were seen in the 50- to 64-year and older than 65-year age groups.[302]

*References 94, 137, 251, 298, 377.

Epidemiology of Meningococcal Meningitis

In the United States, the incidence of *N. meningitidis* meningitis decreased by 58 percent from 0.44 case per 100,000 population in 1998 to 1999 to 0.19 case per 100,000 population in 2006 to 2007 (see Table 31-2).[358] The rates of meningococcal meningitis caused by serogroups B, C, and Y declined by 55 percent. Meningitis with *N. meningitidis* had a case-fatality rate of 10.1 percent. *N. meningitidis* remained a major cause of bacterial meningitis among older children and young adults between 11 to 17 years of age (45.9% of cases), despite the fact that rates declined significantly between 1997 and 2007. Because the declines were similar among all major serogroups and because quadrivalent meningococcal vaccines (A, C, Y, and W135) do not include serogroup B, these declines are not likely from a vaccine effect.[358] Serogroups B, C, and Y were most commonly identified (59.1%, 21.0%, and 11.4% of cases, respectively) in meningococcal isolates. Serogroup B caused 70.5 percent of the cases among children younger than 11 years of age. Serogroups included in quadrivalent meningococcal vaccines accounted for 66.7 percent of infections among children 11 to 17 years of age. Serogroups B and C were the predominant causes of meningococcal meningitis among adults 18 to 34 years of age, accounting for 34.4 and 45.9 percent of cases, respectively, whereas in adults 35 years of age or older, serogroups B and Y were the most common, each serogroup accounting for 30.4 percent of cases.[358] As the proportion of children receiving quadrivalent meningococcal vaccines continues to increase, additional reductions in the incidence of meningococcal disease are likely to occur (Fig. 31-1).

From 1990 to 2002 in the United States, mortality rates for meningococcal disease were 0.1 deaths per 100,000 population per year, with 58 percent of deaths occurring among individuals younger than 25 years old. Infants had a higher mortality rate than that of individuals in different age groups, but they experienced a decline from 1.3 deaths per 100,000 in 1990 to 0.42 deaths per 100,000 population in 2002.[327] Meningococcal disease mortality rates increased each year in individuals between the ages of 10 and 19 years, which was followed by a decline in mortality rates in the years following and throughout most of adulthood.[327]

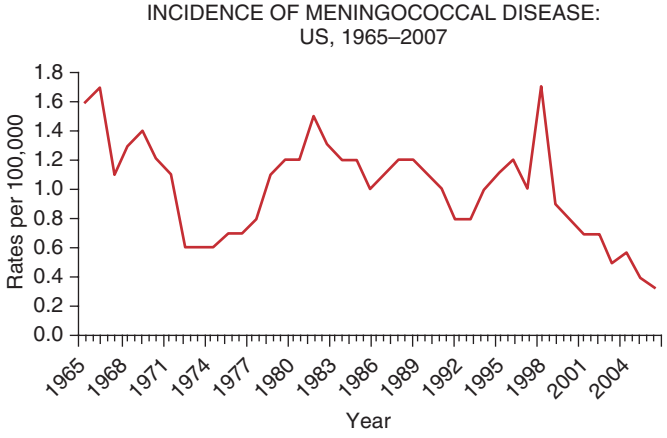

FIGURE 31-1 ■ Trends in annual incidence of invasive *N. meningitidis* disease in the United States, 1965-2007. (See companion website on Expert Consult for a color version of this figure.) (Data based on Active Bacterial Core Surveillance Report, CDC.)

In the United States, in the 1990s, serogroup Y became more common among older patients and caused more pneumonia than did the other serogroups. The overall incidence of serogroup Y infection increased from 2 percent during 1989 to 1991 to 37 percent during 1997 to 2002.[69] A study of the epidemiology of meningococcal disease in New York City from 1989 to 2000 revealed a threefold decrease in rates of serogroup B disease and almost no cases in children younger than 5 years old. The authors noted the coincident increase in the use of *H. influenzae* type b conjugate vaccine and postulated the existence of a possible correlation.[254] CDC data reveal that 75 percent of meningococcal infections in individuals older than 11 years of age are caused by serogroups C, Y, and W-135, all of which are included in the newly available conjugate vaccine. An increased risk for contracting meningococcal infection also exists in college students, especially freshmen who reside on campus in dormitories.[176]

The meningococcus is pathogenic only in humans. The carriage rate for *N. meningitidis* in the civilian population has been estimated at various times to range from 1 to 15 percent. Carriage rates in military personnel during epidemic periods have been considerably greater. Meningococcal carriers generally are adults (>21 years old) who harbor the organism for months, and meningococcus colonizes the nasopharynx asymptomatically in up to 40 percent of the adult population.[351]

No correlation has been noted between meningococcal meningitis and crowding within households, but disease seems to be more prevalent in urban than in rural areas. In a civilian population, meningococcal meningitis generally is a disease of children and young adults who have been exposed to an adult carrier, usually in the same family, or to individuals with disease or who are carrying the organism in a daycare setting. The estimated likelihood of severe meningococcal disease in family contacts occurring simultaneously with the first case is 1 percent.[223] The rate is 1000-fold greater than the risk in the community. The risk of meningitis being acquired in daycare center contacts of children with meningococcal disease is 1 per 1000.

Another change in the epidemiology of meningococcal disease in the United States relates to the increasing number of outbreaks (>10 cases per 100,000 population during 3 months), generally caused by serogroup C. Although these outbreaks account for only 2 to 3 percent of the total number of cases, they cause tremendous public concern and anxiety, which frequently result in misunderstanding of the nature of an outbreak by the media and the public.[184]

An otherwise unprecedented outbreak of meningococcal disease occurred in a sixth-grade elementary school classroom.[113] Five children from a class of 24 developed meningococcal meningitis. In addition, two siblings of one of the index patients also developed meningococcal infection. Detailed epidemiologic investigation suggested that close contact in the classroom (nose-to-nose

distances of ≤34 inches) correlated with an increased rate of carriage of *N. meningitidis* and with an increased risk for development of invasive meningococcal disease.

Major outbreaks of meningococcal meningitis have occurred worldwide. In the late 1980s and early 1990s, major epidemics of meningococcal meningitis caused by a specific clone (III-1) of serogroup A *N. meningitidis* occurred throughout sub-Saharan Africa.[2,166,170,286,310] The origins of a pandemic spread of clone III-1 were traced to epidemics in Asia in the early 1980s, with spread through the Near East.[1] An outbreak occurred during the annual pilgrimage to Mecca in 1987,[252,253] with pilgrims carrying clones back to their countries of origin, including the United States and the United Kingdom. Epidemics of closely related strains of clone III-1 serogroup A meningococcal meningitis occurred in the Sudan,[310] Ethiopia,[170] and Chad in 1988[253] and in Kenya[286] in 1989. A report in 1992 described an epidemic of clone III-1 in the Central African Republic in an area traditionally outside the "meningitis belt."[166] These epidemics generally begin during the dry season and decline at onset of the rainy season. According to the World Health Organization, "in major African epidemics, attack rates range from 100 to 800 per 100,000 population, but individual communities have reported rates as high as 1000 per 100,000."[245] Major outbreaks have occurred in Brazil,[96] in Finland,[277] and at multiple sites in Africa.[387] A clonal outbreak of serogroup W135 (ET-37ST-11 clonal complex) occurred in 2000 among Hajj pilgrims returning to Europe from Saudi Arabia. In 2001, outbreaks with serogroup W135 also occurred in Niger and Burkina Faso.[350]

Shifts within a community or within a country as a whole from one serogroup to another also are associated with an increased incidence of disease for several years after the new serogroup is introduced into the community.[303] The mechanisms underlying the changing patterns of meningococcal serogroups that cause diseases are unknown.

Protection from meningococcal infection depends on a functional complement system as well as the humoral antibody response. The serum bactericidal activity assay (SBA), which measures killing of *N. meningitidis* in the presence of specific antibodies and addition of complement is the standard immunologic correlate of protection. SBA titers of 4 or higher with the use of human complement (or 8 or higher with the use of rabbit complement) are considered protection against subsequent diseases.[22] Meningococcal infections occur more frequently in patients with a deficiency of the terminal components (C5-C9) of the complement system, and the risk for developing meningococcal infection in persons with deficiency of complement factor C5-C9 is approximately 600-fold higher compared with the general population.[54,107,126,231,311] More recently, an increased risk for contracting meningococcal meningitis has been reported in individuals with an inherited deficiency of C9[263] and with properdin deficiency.[340] Individuals with a complement-depleting underlying illness also are at particular risk for acquiring invasive diseases.[282] Screening for complement deficiency in pediatric patients with meningococcal disease is recommended.[224]

Epidemiology of *Haemophilus influenzae* Meningitis

The most dramatic change in the epidemiology of bacterial meningitis since the advent of antibiotics has occurred in the past 2 decades as a result of licensure of conjugate vaccines against *H. influenzae* type b.[68,238,276,319,358] The first vaccine available was *H. influenzae* type b capsular polysaccharide (polyribosylribitol) phosphate [PRP], which was licensed in April 1985 for use in children 18 to 59 months of age. Subsequently, vaccines with improved immunogenicity for children of younger ages were developed by covalently linking the capsular polysaccharide with protein antigens. In October 1990, the first conjugate, PRP diphtheria CRM_{197} protein conjugate (HbOC), was approved for infant use. In 1991, the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Pediatrics (AAP) recommended universal infant immunization at 2, 4, and 6 months of age with either HbOC or PRP-meningococcal protein conjugate (PRP-OMP) vaccines.[8,63] Currently, four different conjugate vaccine preparations exist: PRP-CRM (HbOC), PRP-OMP, PRP-T (tetanus toxoid), and PRP-D (diphtheria toxoid), which is not licensed in the United States.[382]

Before universal vaccination was initiated, the incidence of *H. influenzae* meningitis varied worldwide. The incidence of *H. influenzae* meningitis in Scandinavian children younger than 5 years old averaged 16 to 28 cases per 100,000 children from 1975 to 1984.[278] The incidence of *H. influenzae* type b meningitis in three Pacific Island countries was 70 to 84 cases per 100,000 children younger than 5 years old in 2003[306] and 67 to 158 cases per 100,000 in Indonesian children younger than 5 years old in 1998 to 2002.[153] The incidence of *H. influenzae* meningitis in the Netherlands was 22 cases per 100,000 children younger than 5 years old.[367] A universal vaccination program in the Netherlands led to a marked decrease in the incidence, with six reported cases of meningitis caused by *H. influenzae* in a 1-year period after implementation of universal vaccination of infants compared with 34 cases per 100,000 children in a 1-year period before vaccination.[369]

The incidence of *H. influenzae* meningitis is markedly higher in nonindustrialized populations. Before the vaccination era, Alaskan Eskimos had an annual incidence of 282 cases per 100,000 children younger than 5 years of age.[380] The Navajo and White Mountain Apache Native Americans had a much higher incidence compared with that of Native Americans in other regions of the United States,[84,234] and the incidence among Australian Aboriginals and among certain African populations, such as those in Gambia and Senegal, was 3 to 10 times higher than that of populations in the United States and Europe.[40,156,174] The incidence of *H. influenzae* meningitis was decreased in Gambia from more than 60 cases per 100,000 children to none per 100,000 in children younger than 5 years old after implementation of *Haemophilus influenzae* type b vaccination in May 1997.[6]

Good epidemiologic data concerning the incidence of *H. influenzae* infection are, however, lacking in many other areas of the world. Measuring the extent of *H.*

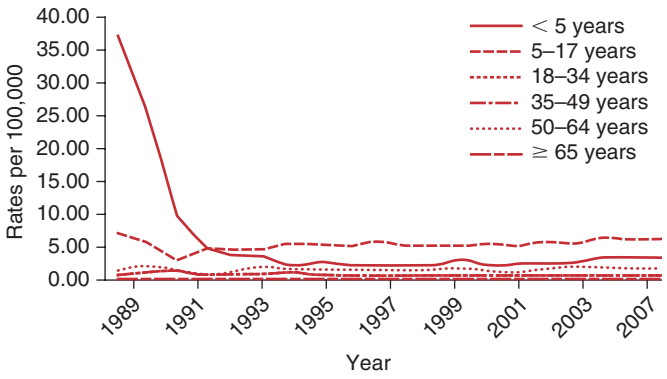

FIGURE 31-2 ■ Trends in incidence of invasive *H. influenzae* disease, by age group in the United States, 1989-2008. (See companion website on Expert Consult for a color version of this figure.) (From MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* diseases—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.)

influenzae disease often is difficult, time consuming, and expensive. In an attempt to decrease complexity, cost, and time required to collect data concerning the incidence of *H. influenzae* infection, the *Haemophilus influenzae* type b Rapid Assessment Tool was developed. More and more countries are using such tools to estimate the local *H. influenzae* disease burden and to help determine the need for implementation of a local vaccination program.[382]

In one study, the incidence of *H. influenzae* meningitis was 3.5-fold higher in blacks than in whites, but this distribution of cases seemed to be related more closely to poverty than to race.[131] In whites, no increase in incidence occurred in overcrowded households, but the incidence was higher in rural than in urban areas, which allowed the postulation that the increased incidence in rural whites and in blacks was related to lack of access to early medical care.[131]

The *Haemophilus Influenzae* Study Group[5] noted that the number of cases of *H. influenzae* meningitis in children younger than 5 years old reported through the National Bacterial Meningitis Reporting System began declining rapidly in 1988. In another CDC surveillance project conducted from 1989 to 1997, the race-adjusted incidence of invasive *H. influenzae* type b disease among children younger than age 5 years declined from 34.0 to 0.4 per 100,000, a 99 percent reduction (Fig. 31-2).[66,238] A report from Active Bacterial Core Surveillance sites during 1998 to 2007 indicated that small increases in the incidence of serotypes a, e, and f were observed, and the largest of these increases was in serotype f and was primarily among adults aged 18 years or older.[358]

The overall incidence of *H. influenzae* meningitis decreased by 35 percent between 1998 and 1999 and 2006 and 2007, from 0.12 cases per 100,000 population to 0.08 cases per 100,000 population (see Table 31-2); 9.4 percent of the cases were caused by serotype b.[358] The epidemiology of invasive *H. influenzae* disease in the post–*H. influenzae* type b vaccine era seems to be shifting from *H. influenzae* type b in children toward non–type b strains in older individuals.[358] For example, among adult cases of *H. influenzae* meningitis, a majority (73.8%) of the *H. influenzae* isolates were not typable whereas serotypes e and f were the most common serotypes identified (with each found in 11.5% of cases). The case-fatality rates were significantly higher among adults with typable *H. influenzae* than among cases from nontypable *H. influenzae* (18.8% vs. 2.2%, $p = .02$).[358]

Schoendorf and colleagues[321] evaluated national trends in mortality from *H. influenzae* meningitis from 1980 to 1991. From 1980 through 1987, mortality rates from *H. influenzae* meningitis decreased an average of 8.5 percent a year, from 1.72 per 100,000 children in 1980 to 0.94 per 100,000 children in 1987. From 1988 to 1991, mortality rates decreased an average of 48 percent a year, with a death rate of 0.11 per 100,000 children in 1991. The estimated case-fatality rate for *H. influenzae* meningitis was 3.3 percent from 1980 to 1987 and 2.3 percent from 1988 to 1991. In comparison, mortality rates from *S. pneumoniae* decreased 10 percent annually from 1980 to 1987 and 3 percent annually from 1988 to 1991. Similarly, mortality rates from meningococcal meningitis decreased by 13 percent annually from 1980 to 1987 and 12 percent annually from 1988 to 1991.

The dramatic decrease in the incidence of *H. influenzae* meningitis probably has been affected by several factors. The precipitous drop that occurred shortly after universal immunization of infants strongly suggests that this practice has affected the epidemiology of this disease. Conjugate vaccination protects against nasopharyngeal colonization,[259] decreasing the carriage rate of *H. influenzae* type b and diminishing the reservoir for transmission and providing immunity from infection. These factors would also lessen the likelihood of development of infection in unvaccinated populations through herd immunity. Other changes in medical practice, such as the widespread use of outpatient antibiotics and improvements in supportive care, may have some effect, as shown by the decrease in case-fatality rates for *H. influenzae* meningitis, the steady decrease in mortality rates before vaccination, and the decrease in instances of meningococcal and pneumococcal disease.

The risk for spread of severe *H. influenzae* illness among household contacts of patients with *H. influenzae* meningitis was studied prospectively in 19 states.[378] The risk in children younger than 1 year of age was 6 percent; in children younger than 4 years, 2.1 percent; and in children younger than 6 years, 0.5 percent. The risk for *H. influenzae* disease occurring in household contacts younger than 6 years old is similar to the risk for secondary meningococcal disease occurring in all household contacts, indicating a need for effective antimicrobial prophylaxis. Spread of *H. influenzae* disease in daycare centers also is well documented.[30,158,244,379] The precise risk for acquiring secondary *H. influenzae* type b infection in daycare centers is unclear.

Recurrent invasive *H. influenzae* type b disease has been reported. Detailed studies suggest that age and high incidence of disease alone are not the only factors contributing to the recurrence of the disease.[53] Patients who have recurrent disease caused by *H. influenzae* type b may represent a subset of a population with unusual disease susceptibility. In addition, children who develop invasive infection caused by *H. influenzae* type b after receiving appropriate conjugate vaccine frequently have subnormal immunoglobulin concentrations and should undergo immune evaluation.[180]

PATHOPHYSIOLOGY

Organisms Encountered

Almost all microorganisms that are pathogenic to humans have the potential to cause meningitis, but a relatively small number of pathogens (i.e., group B streptococcus, *E. coli*, *L. monocytogenes*, *H. influenzae*, *S. pneumoniae*, and *N. meningitidis*) account for most cases of acute bacterial meningitis in neonates and children.[199,200] The reasons for this association remain incompletely understood. In immunocompromised hosts, infection with other organisms may occur. The specific organism sometimes may be predicted on the basis of the type of deficit that is present in the host.

Routes of Infection

Bacterial infection of the normally sterile leptomeningeal spaces can occur from hematogenous spread, direct extension from paranasal and dental infections as well as skull base fracture, direct implantation (e.g., after surgery, cochlear implant), or rarely secondary to infections in the epidural or subdural spaces.[198-200] Bacterial meningitis in children with otitis media generally follows bacteremia, although direct invasion of the meninges may occur as a complication of otitis media.[198]

The meninges are seeded with microorganisms during a bacteremic period. Bacterial meningitis has been induced in infant rats[255] and monkeys[336] after intranasal inoculation of *H. influenzae* type b. Bacteremia developed hours before meningitis could be detected histologically, a finding that supports the concept that meningitis follows hematogenous dissemination from nasopharyngeal colonization or infection. Marginating bacteria could be detected by fluorescent staining initially in the lateral and dorsal longitudinal (sagittal) sinuses and subsequently spread to the leptomeninges. Several lines of evidence from human cases of group B streptococcus and *E. coli* meningitis and animal models of experimental hematogenous meningitis, however, reveal that cerebral capillaries are the portal of entry into the brain for circulating group B streptococcus and *E. coli*.[198-200]

Meningitis can also develop after bacterial invasion from a contiguous focus of infection, as in infection of the mastoid or paranasal sinuses or as a complication of otitis media. For example, *S. pneumoniae* has been shown to enter the CNS through a nonhematogenous route in experimental animals after intranasal infection and otitis media.[198] Fracture through the paranasal sinuses as a result of head trauma may precede development of meningitis caused by *S. pneumoniae* and *H. influenzae*, which may be recurrent. Direct invasion also may occur in individuals with dermoid sinus tracts or meningomyeloceles, when a direct communication between the skin and the meninges is present. In this setting, infection usually is produced by organisms found on the skin. Recurrent meningitis has been reported in patients with basiethmoidal encephaloceles[348] or a congenital defect in the stapedial footplate.[179] Surgical obliteration of the fistula with temporal muscle and fascia prevented the recurrence of meningitis. Meningitis also may develop subsequent to osteomyelitis of the skull or vertebral column. Rarely, meningitis may develop in the normal host with commensal microorganisms after having a tooth extraction or getting dental fillings.[79,328]

Neurosurgical procedures, particularly procedures designed for diversion of CSF in children who have hydrocephalus, may lead to development of meningitis. A chemical meningitis also may occur after neurosurgical procedures, especially procedures involving the posterior fossa.[130] In these patients, evidence of inflammation develops rapidly with elevation of temperature occurring on the first postoperative day.

Infection of the CNS may result from environmental contamination or manipulation. Meningeal infection may be acquired in utero transplacentally or during delivery through contact with the cervix or vaginal canal, which may be colonized with a variety of organisms, particularly group B streptococci, *E. coli*, and *L. monocytogenes*.[7,23,198-200] A neonate, a patient with cystic fibrosis, or a burned child may develop septicemia and meningitis as a result of persistent heavy colonization with *Staphylococcus aureus*. A humidified atmosphere promotes the colonization and growth of such organisms as *Serratia marcescens* and *Pseudomonas aeruginosa*. Placing a patient in this setting leads to an increased frequency of infection with these organisms. Indwelling catheters can predispose a patient to infection by bacterial (and fungal) organisms that generally are of low virulence in the normal host.

PATHOGENESIS

Most cases of bacterial meningitis develop through four steps: (1) colonization of the mucosal membranes such as upper respiratory or gastrointestinal tract, (2) invasion of the blood from the mucosal colonization, (3) traversal of the blood-brain barrier, and (4) inflammation of the meninges and brain.[198,199] Less commonly, infection of the leptomeninges can occur by contiguous spread or hematogenous dissemination from another remote site.

Mucosal Colonization

Most meningitis-causing bacteria colonize the mucosal membranes of the nasopharynx (*S. pneumoniae*, *N. meningitidis*, and *H. influenzae*) and gastrointestinal tract (*E. coli* and *L. monocytogenes*).[44,54,199,351] This attachment is mediated by specific microbial cell surface components and affected by co-colonization with other microorganisms and host immune response.

Several pneumococcal factors have been shown to enhance nasopharyngeal colonization and binding to nasopharyngeal epithelium: pneumococcal phosphorylcholine, IgA1-protease, capsular switching, pneumolysin, pneumococcal surface protein (Psp) C (also known as choline binding protein [CbpA]), pneumococcal surface adhesin A (PsaA), pneumococcal adherence and virulence factor A (PavA), neuraminidase A (NanA), and pili. Additionally, prior exposure to influenza virus has been associated with increased colonization and secondary invasive pneumococcal disease.[44,199,385] Pneumococcal phosphorylcholine binds to the platelet-activating factor (PAF)

receptor on activated host epithelial cells,[44] whereas the PspC binds to epithelial polymeric immunoglobulin receptor (pIgR).[395] The pIgRs are expressed in a decreasing gradient from the upper to lower respiratory tract, whereas the opposite pattern of expression is observed for the PAF receptor, suggesting that pIgR serves mainly as a pneumococcal receptor for the nasopharynx but the PAF receptor facilitates attachment and invasion of the pulmonary epithelium.[44]

N. meningitidis strains use IgA1 protease, pili, and outer membrane proteins Opa, Opc, and porin A and B for binding to cell surface receptors on nasopharyngeal mucosal cells[54,97,199] and apparently are transported across specialized cells within phagocytic vacuoles.[349] Human embryonic antigen cell adhesion molecules (CEACAMs or CD66) are cell-surface molecules in nasopharyngeal epithelial cells that interact with meningococcal Opa proteins.[54]

Bacteremia

When in the bloodstream, the common meningitis-causing organisms (*S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *E. coli* K1, and group B streptococcus) are capable of evading host defense mechanisms,[198,199,292] which involves capsular polysaccharides and other bacterial factors, resulting in a high level of bacteremia (Table 31-4).

Several studies of humans and experimental animals suggest a relationship between the magnitude of bacteremia and development of meningitis due to *E. coli*, group B streptococcus, *H. influenzae* type b, and *S. pneumoniae*.[198,199] For example, a significantly higher incidence of *E. coli* meningitis was noted in neonates who had bacterial counts in blood greater than 10^3 colony-forming units (CFU)/mL (6 of 11, 55%) compared with those with bacterial counts less than 10^3 CFU/mL (1 of 19, 5%). Similarly, meningitis due to *S. pneumoniae* was observed more frequently in patients whose bacterial counts in blood were greater than 1×10^2 to 5×10^2 CFU/mL (6 of 7, 86%) than in those with less bacterial counts in blood (2 of 50, 4%). For *H. influenzae* type b, the level of bacteremia is around 10^2 CFU/mL of blood. Consistent with these clinical findings, a high degree of bacteremia was a primary determinant for meningeal invasion of experimental hematogenous meningitis caused by *E. coli*, group B streptococcus, *H. influenzae* type b, and *S. pneumoniae*.[198,199] All patients with *N. meningitidis* detected in their CSF had *N. meningitidis* detected in their blood, thus also suggesting a relationship between bacteremia and development of meningitis caused by *N. meningitidis*.[87]

TABLE 31-4 **Microbial Factors Contributing to a High Level of Bacteremia**

Pathogens	Bacterial Factors
E. coli	Capsular polysaccharides, O-LPS, type 1 fimbriae, NlpI
Group B streptococcus	Capsular polysaccharides, C5a peptidase? SodA
S. pneumoniae	Capsular polysaccharides, pneumolysin
N. meningitidis	Capsular polysaccharides, LOS, factor-H binding protein, PorA

LOS, lipo-oligosaccharides; LPS, lipopolysaccharides; NlpI, new lipoprotein I; SodA, superoxide dismutase.

Bacterial Traversal of the Blood-Brain Barrier

The bacteria in the bloodstream subsequently traverse the blood-brain barrier, most likely at the cerebral capillaries (*S. pneumoniae*, *N. meningitidis*, *E. coli* K1, and group B streptococcus) and choroid plexus (*H. influenzae*).[198,199,201] Meningitis-causing bacteria cross the blood-brain barrier transcellularly, paracellularly, and/or by means of infected phagocytes (the so-called Trojan horse mechanism).[198,199] Transcellular traversal of the blood-brain barrier has been demonstrated for most meningitis-causing bacteria in infants and children, including group B streptococcus, *E. coli*, and *S. pneumoniae*, whereas *N. meningitidis* has been shown to utilize both transcellular and paracellular mechanisms for traversal of the blood-brain barrier.[29,83,198,199]

Recent studies, however, have shown that a high degree of bacteremia is necessary, but not sufficient, for the development of meningitis and that bacterial binding to and invasion of the blood-brain barrier is a prerequisite for penetration into the brain.[198,199] This concept was well demonstrated with *E. coli* K1, when mutants deleted of the *E. coli* factors contributing to binding to and invasion of the blood-brain barrier were significantly less able to traverse the blood-brain barrier than the parent strain despite causing similar levels of bacteremia.[198,199]

Bacterial traversal of the blood-brain barrier requires specific bacterial factors and their interactions with host receptors (Table 31-5),[198,199] although the complete information on microbial-host factors contributing to penetration of the blood-brain barrier is currently lacking for most meningitis-causing bacteria.

Recent studies have also shown that meningitis-causing bacteria exploit host cell signaling molecules for host cell actin cytoskeleton rearrangements and bacterial invasion of the blood-brain barrier (see Table 31-5).[198,199] *E. coli* invasion of the blood-brain barrier involves focal adhesion kinase (FAK), paxillin, phosphatidylinositol 3-kinase (PI3-K), Src kinase, Rho guanosine triphosphates, signal transducers and activators of transcription (STAT)3, cytosolic phospholipase $A_2\alpha$, ($cPLA_2\alpha$), 5-lipoxygenase, cyteinyl leukotrienes, and protein kinase C, whereas group B streptococcus invasion of the blood-brain barrier is independent of Src activation and *L. monocytogenes* invasion of the blood-brain barrier is independent of FAK and $cPLA_2\alpha$.[198] *N. meningitidis* has been shown to invade the blood-brain barrier involving type IV pili, PilC, NadA, Opa, and Opc proteins.[54,198] The detailed information on how meningitis-causing bacteria exploit host signaling molecules for invasion of the blood-brain barrier, however, remains incompletely understood. For example, the invasion of the blood-brain barrier by unencapsulated meningococcus is mediated by Opc

TABLE 31-5 **Microbial-Host Factors Contributing to Penetration of the Blood-Brain Barrier by Common Meningitis-Causing Bacteria**

Pathogens	Bacterial Factors	Host Receptors	Host Signaling Molecules
E. coli	Type 1 fimbriae, OmpA, flagella, NlpI, IbeA, IbeB, IbeC, CNF1, AslA	CD48, gp96, 37LRP, VEGFR1	FAK, paxillin, PI3-K, Src kinase, Rho GTPases, $cPLA_2\alpha$, 5-lipoxygenase, cyteinyl leukotrienes, STAT3, PKCα
Group B streptococcus	Lmb, FbsA, IagA, HvgA, PilA, LTA, β-hemolysin, ScpB, αC protein	Integrin, GAGs	FAK, Rho GTPases, $cPLA_2\alpha$, 5-lipoxygenase, and cyteinyl leukotrienes
L. monocytogenes	InlB	gC1qR, Met, gp96	PI3-K
S. pneumoniae	Phosphorylcholine, PspC (CbpA), pneumolysin, NanA	PAF receptor, 37LRP	β-arrestin, MAPK
N. meningitidis	Type IV pili, PilC, Opa, Opc, NadA	CD46, CD66, integrin, 37LRP, β_2-adrenoreceptor	JNK1 and 2, β-arrestin

37LRP, 37 laminin receptor precursor; *AslA,* arylsulfatase-like gene; *CNF1,* cytotoxic necrotizing factor 1; *$cPLA_2\alpha$,* cytosolic phospholipase $A_2\alpha$; *FAK,* focal adhesion kinase; *FbsA,* fibrinogen-binding protein A; *GAGs,* glycol aminoglycans; *gC1qR,* globular head of complement component C1q; *HvgA,* hypervirulent group B streptococcus adhesin; *IagA,* invasion-associated gene A; *Ibe,* invasion of brain endothelial cell; *InlB,* internalin B; *JNK,* c-Jun kinase; *Lmb,* laminin-binding protein; *LTA,* lipoteichoic acid; *MAPK,* mitogen-activated protein kinase; *Met,* Met tyrosine kinase; *NadA,* neisserial adhesin A; *NanA,* neuraminidase A; *NlpI,* new lipoprotein I; *OmpA,* outer membrane protein A; *PI-3K,* phosphatidylinositol-3 kinase; *PilA,* pilus protein A; *PKCα,* protein kinase Cα; *PspC,* pneumococcal surface protein C; *ScpB,* C5a peptidase; *STAT3,* signal transducers and activators of transcription; *VEGFR1,* vascular endothelial growth factor receptor 1.

binding to fibronectin, which anchors the bacteria to the integrin receptor on the blood-brain barrier. However, in the bloodstream, *N. meningitidis* is encapsulated and the in vivo relevance of Opc-fibronectin–mediated binding to integrin requires clarification[199,341,365]; and type IV pili have been shown to contribute to paracellular penetration of *N. meningitidis* across the blood-brain barrier.[83] *S. pneumoniae* has been shown to invade the blood-brain barrier via complex interaction of CbpA protein and PAF on activated endothelial cells.[54,199,210]

The mechanisms involved in entry of meningitis-causing bacteria into the blood-brain barrier differ from those involved in the inflammation occurring in response to those bacteria. This concept has been demonstrated for *E. coli*, group B streptococcus, and *N. meningitidis*.[29,143,199,341,365] *N. meningitidis* invasion of the blood-brain barrier involves c-Jun kinases 1 and 2 (JNK1 and JNK2), but the release of interleukin (IL)-6 and IL-8 from the blood-brain barrier involves the p38-mitogen–activated protein kinase (MAPK) pathways.[341]

Blood-Brain Barrier Dysfunction and Intracranial Inflammation

Once bacteria enter the CSF, because of insufficient opsonic and phagocytic activity in the CSF, organisms multiply rapidly, liberating bacterial cell wall or membrane components (lipopolysaccharide, lipoteichoic acid, peptidoglycan, lipoproteins, bacterial toxins), and induce release of inflammatory mediators and toxic compounds, which leads to CSF pleocytosis and increased blood-brain barrier permeability, the hallmarks of bacterial meningitis. However, pleocytosis and increased blood-brain barrier permeability can develop independently of each other in some patients with bacterial meningitis[198,199] (see later).

Complement and opsonic proteins either are found at very low concentrations or are absent entirely within normal CSF,[331] and the CSF is devoid of the factors required for enhancing bacterial killing. When bacteria first invade the meninges, the lack of complement and opsonic proteins within the sanctuary of the CNS may permit the bacteria to multiply unrestrained for some time.[38,111,154,396]

The specific pathophysiologic changes in bacterial meningitis are the result of the bacterial products and the inflammatory response of the host to those products (Fig. 31-3). Initial bactericidal antibiotic therapy results in a rapid release of bacterial products, such as lipopolysaccharides (endotoxins), teichoic acid, and peptidoglycans. For gram-positive bacterial meningitis, lipoteichoic acid and peptidoglycans are the bacterial surface elements that induce inflammation.[198,363] The threshold concentration that triggers inflammation is approximately 10^5 bacterial cell equivalents of cell wall pieces.[355] For gram-negative meningitis, lipopolysaccharides (endotoxins) are the major inflammatory components, with peptidoglycan serving as an important cofactor.[56] The inflammatory threshold is approximately 2 pg of endotoxin, or approximately 10^5 bacterial cell equivalents.[389]

Immune activation in the CNS is initiated by the recognition of different bacterial pathogen-associated molecular patterns (PAMPs). Major pattern recognition receptors (PRRs) involved in initial sensing of bacteria in the CNS include Toll-like receptors (TLRs) 2, 4, 5, and 9 and Nod-like receptors (NLRs), which are involved in triggering an intracellular signaling pathway that leads to activation of transcription factors such as nuclear factor-kB (NF-κB) and the subsequent gene transcription of proinflammatory cytokines and chemokines such as tumor necrosis factor-α (TNF-α), IL-1, and IL-8.[54]

Cytokines and chemokines seem to be the primary drivers of the inflammatory response. The following

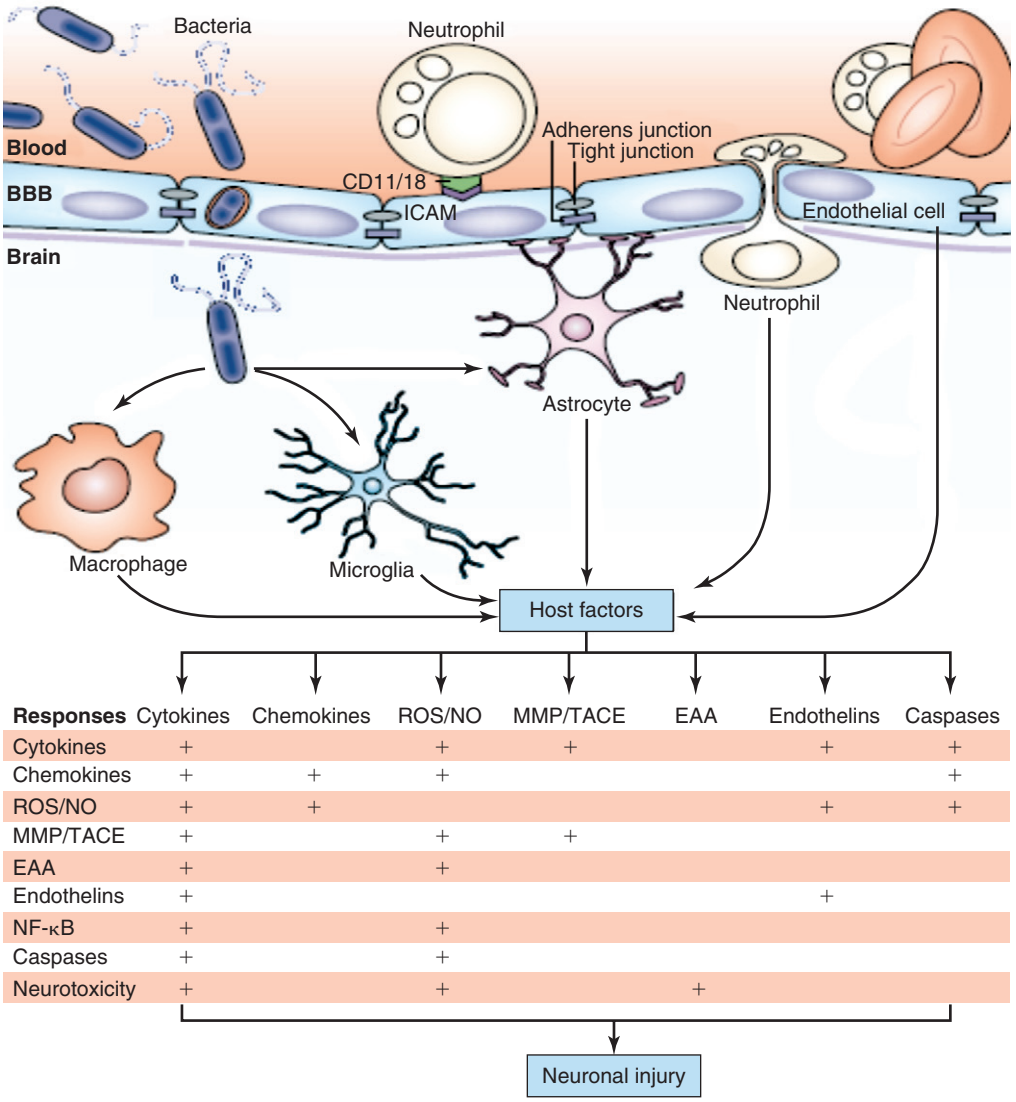

FIGURE 31-3 ■ Host response during bacterial meningitis contributing to neuronal injury. Bacterial entry into the central nervous system elicits the release of many factors from host cells (macrophage, microglia, astrocyte, ependymal cell, endothelial cell, infiltrating inflammatory cell), which along with cytoxicity of bacterial compounds exacerbate host cellular responses, resulting in neuronal injury. + denotes upregulation of specific host factors. BBB, blood-brain barrier; EAA, excitatory amino acids; ICAM, intercellular adhesion molecule; MMP, matrix metalloproteases; NO, nitric oxide; ROS, reactive oxygen species; TACE, tumor necrosis factor-α (TNF-α)–converting enzyme. (See companion website on Expert Consult for a color version of this figure.) (Modified from Kim KS. Pathogenesis of bacterial meningitis: from bacteremia to neuronal injury. *Nat Rev Neurosc* 2003;**4**:376–85.)

cytokines and chemokines are involved in the inflammatory response noted in bacterial meningitis: IL-1, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, interferon-γ, macrophage inflammatory protein, transforming growth factor-β, and TNF-α.[98,135,198,370,398]

TNF-α and IL-1β seem to be key mediators in initiation of meningeal inflammation. Both proteins stimulate vascular endothelial cells to induce adhesion and passage of leukocytes into the CNS and trigger inflammatory processes. Astrocytes and microglia are capable of producing TNF-α.[222,359] TNF-α concentrations are elevated (1) in CSF but not in serum; (2) in animal models of bacterial meningitis; and (3) in patients with bacterial meningitis caused by *H. influenzae*, *N. meningitidis*, *S. pneumoniae*, and *Streptococcus agalactiae*,[198,260] but not in patients with culture-proven viral meningitis.[294] TNF-α levels can be correlated with meningeal inflammation and severity of disease, but lack of TNF-α in the animal model is associated with increased mortality and stronger deficits in spatial memory.[148]

IL-1β can be detected in infants and children with bacterial meningitis, and its presence is correlated significantly with CSF inflammatory abnormalities, TNF-α concentrations, and adverse outcome.[260] The role of IL-1β in bacterial meningitis, however, is inconsistent. Levels of IL-1β were not associated with the degree of the blood-brain barrier disruption in patients with bacterial meningitis.[198]

The chemokines are a superfamily of small chemoattractant cytokines that play an important role in the initiation and modulation of inflammation in bacterial meningitis.[344] IL-8 is one of the well-characterized

chemokines in bacterial meningitis and was found to be chemotactic for neutrophils in the CSF of patients with bacterial meningitis.[143,363]

Complement factors are upregulated in bacterial meningitis. The activated complement cascade in CSF, acting on upregulated complement receptors on brain cells, potentially mediates direct brain damage.[51] Complement factors also are chemoattractants that enhance CSF leukocytosis and indirectly produce brain damage in patients with bacterial meningitis.[110]

Intracisternal administration of antibodies to TNF-α and IL-1β decreased pleocytosis and blood-brain barrier permeability in response to intracisternal injection of *H. influenzae* and *S. pneumoniae*, and blockade of pleocytosis was beneficial in reducing blood-brain barrier permeability in experimental meningitis induced by intracisternal inoculation of *S. pneumoniae*, *H. influenzae*, and *N. meningitidis*.[198] In contrast, intracisternal inoculation of *H. influenzae* and *S. pneumoniae* resulted in increased blood-brain barrier permeability in both normal and leukopenic animals, indicating that increased blood-brain barrier permeability in bacterial meningitis can occur in the absence of pleocytosis.[201] However, no change in blood-brain barrier permeability and absence of pleocytosis after bacterial entry into the CNS usually reflects an early stage of bacterial meningitis.

Neuronal Injury

Neuronal damage is caused by vasculitis; focal ischemia; increased intracranial pressure; cytotoxic, vasogenic, and interstitial edema; transmigrating leukocytes; stimulation of glial cells and astrocytes; cortical necrosis and hippocampal neuronal loss stemming from an inflammatory response to bacteria or bacterial products (bacterial cell wall, teichoid and lipoteichoid acids, lipopolysaccharides, lipoproteins, bacterial DNA); and direct effects of bacterial toxins in the CNS. The various host factors contributing to neuronal injury are shown in Figure 31-3.

Reactive oxygen species (superoxide and hydrogen peroxide) are secreted by TNF-α–stimulated macrophages, including brain microglia, and leukocytes.[198,210] Hydrogen peroxide induces extensive neuronal damage.[198,210] Nitric oxide also can be induced in brain cells in response to bacterial products.[203] Nitric oxide and superoxide radicals react to form peroxynitrite anion, which decomposes and forms nitrogen dioxide, hydroxyl radicals, and strong oxidant compounds.[33] Peroxynitrite seems to be an important neuronal toxin.[209] Neuronal damage caused by reactive oxygen species and reactive nitrate species occurs via at least two separate pathways—via lipid peroxidation and subsequent cell membrane instability and via DNA fragmentation and subsequent activation of poly(adenosine diphosphate ribose) polymerase (PARP) and cellular energy depletion.[198,210] Reactive oxygen species also activate NF-κB, a transcription factor affecting genes such as cytokines and chemokines, and peroxynitrite initiates activation of caspases.[210]

In addition, macrophages secrete excitatory amino acids, such as glutamate, which potentially kill *N*-methyl-D-aspartate receptor–positive cells[201] and glutamate concentrations in the CSF are shown to be increased in patients with bacterial meningitis.[198]

Meningitis causes damage in the cortex and the hippocampal region via different mechanisms.[198] Cortical damage is mainly via cellular necrosis surrounded by an area of caspase-3–dependent apoptosis. Damage in the hippocampus is mediated not only via the classic caspase-3–dependent pathway but also by caspase-independent apoptosis mediated by apoptosis-initiating factor.[37,39,248]

The increasing concentrations of chemotactic factors in the subarachnoid space lead to accumulation of large numbers of neutrophils in the CSF, but the growth of bacteria is not slowed significantly by this response.[110] Impaired phagocytosis by neutrophils in the meningeal spaces may be related to weak activity in fluid medium, lack of complement activity and opsonization, and poor penetration of IgM and IgG through the blood-brain barrier, even during acute *H. influenzae* and *S. pneumoniae* meningitis.[155] The contribution of leukocyte entry into the CSF to bacterial meningitis remains inconsistent. Blockage of leukocyte entry into the CSF was associated with poorer prognosis (likely secondary to an increase in bacterial counts in the blood) but did not affect the risk for brain damage,[50] whereas inhibition of leukocyte migration or of products released by leukocytes is shown to interfere with the pathogenesis of bacterial meningitis.[198]

Bacterial cell wall fragments, endotoxin, or both also contribute to vascular permeability. In experimental *E. coli* meningitis, the CSF endotoxin concentration increased markedly after treatment with β-lactam antibiotics. This increase was associated with an increase in brain water content. This effect could be blocked by polymyxin or a monoclonal antibody, both of which inactivate endotoxin.[198]

The inflammatory and vascular events described earlier act synergistically to produce the clinical symptoms and long-term sequelae that are noted in patients with bacterial meningitis. Altered vascular permeability leads to vasogenic edema, inflammatory and electrolyte changes lead to cytotoxic edema, and alterations in production and absorption of CSF lead to interstitial edema. The cytokines also trigger increased cerebral blood flow and further formation of edema, resulting in increased intracranial pressure.[18,198] The increased intracranial pressure and vasculitis lead to a subsequent decrease in cerebral blood flow, and maintenance of an appropriate cerebral perfusion pressure is crucial to maintaining cerebral blood flow.[18,198] Activation of the coagulation cascade predisposes the patient to venous, microvascular, and, rarely, arterial thrombosis. Direct neurotoxic damage by inflammatory cells also may contribute to the neuropathologic changes seen in bacterial meningitis.[198,210]

Studies in experimental animals and human autopsy cases of bacterial meningitis reveal that neuronal injury occurs mostly as apoptosis in the dentate gyrus of the hippocampus formation and as necrosis in the neocortex and the CA1-4 region of the hippocampus.[149,198] In other regions of the brain, neurons die with necrotic morphology.[149] Not only cellular destruction but also proliferation of neuronal progenitor cells has been observed in patients after bacterial meningitis.[151] Increased neural progenitor

cell proliferation and differentiation were also observed after experimental bacterial meningitis.[147] These findings suggest an endogenous potential of cell renewal in the hippocampal formation in bacterial meningitis. It remains to be determined whether stimulation of endogenous neurogenesis can be used as a potential option in survivors of meningitis.

In the adult brain, neural progenitor cells maintain the ability to proliferate and differentiate into new neurons. The progenitor cells are located predominantly in the subgranular zone of the dentate gyrus and the subventricular zone of the lateral ventricle. In the subgranular zone, the stem cells proliferate and differentiate into progenitor cells. The newly formed cells subsequently migrate into the granular layer of the dentate gyrus, differentiate into mature, functional neurons, and become integrated into the hippocampal network. The hippocampus is therefore potentially equipped for repair. However, because neurofunctional sequelae of bacterial meningitis related to hippocampus function persist throughout childhood and adulthood, the capacity of self-repair seems insufficient to compensate for the brain damage. It remains to be determined whether the regenerative capacity of the hippocampus is compromised by bacterial meningitis.

Factors Predisposing the Host to Bacterial Meningitis

Factors that predispose the host to the development of bacterial infection in other sites also predispose the host to the development of bacterial infection of the CNS. A strong interrelationship exists among factors relating to the host, the organism, and the environment with regard to the pathogenesis and outcome of meningitis. Although presented separately, they must be considered a complex interplay of factors that leads to infection.

An increased incidence of bacterial meningitis is observed in the very young; boys are affected more frequently than girls, and the severity of disease also is increased in these groups. A neonate is predisposed to development of septicemia and meningitis by factors that reflect physiologic deficiencies or immaturity of host defense mechanisms, including (1) decreased phagocytic and bactericidal activity of polymorphonuclear leukocytes, (2) defects in the response of neonatal leukocytes to chemotactic factors, (3) a deficiency in the capacity of leukocytes to support opsonization, and (4) defects in microtubular length and number that decrease the motility of the neonatal leukocytes compared with leukocytes from older children. Deficiencies in serum complement components (C1q, C3, and C5), low levels of serum properdin, and low concentrations of serum IgM and IgA have been documented. Despite transplacental acquisition of IgG, antibodies against specific infective agents may be lacking.[200] The precise age at which each of these factors reaches the concentration and functional activity noted in older children and adults is unclear and undoubtedly varies from individual to individual. In part, meningitis in children aged 1 month to 1 year may reflect qualitative or quantitative differences of the inflammatory and immunologic responses compared with older children.

The increased risk for development of meningitis in the normal host with less than completely mature immunologic and inflammatory responses to infection may be attributable to age alone. This factor is exemplified in the report,[77] where the risk for recurrent bacteremia was studied in young children. Within 18 months of having bacteremic illness, none of 42 children older than 24 months of age had a documented additional episode of bacteremia or systemic infection. Fifteen of 135 children (11%) younger than age 24 months at the time of the initial bacteremic disease had at least one additional documented bacteremic illness, however. Of these 15 children, 14 contracted both infections before reaching 2 years of age. Seven of these 15 children had meningitis. Only 2 patients had documented congenital or hereditary disorders of immunoglobulin or complement concentration or function.

There is increasing evidence that genetic polymorphisms in the inflammatory response, complement system, coagulation, and fibrinolysis pathways influence the genetic predisposition and outcome in bacterial meningitis.[54] A genetic determination for the predilection of some normal children for the development of bacteremia and meningitis has been suggested.[54,357] The ability of the host to produce, within the CSF, IL-12 and TNF-α–induced interferon-γ is important in the natural immunity to various microorganisms that may cause meningitis.[54,214]

Deficiencies and polymorphisms in the PRRs downstream signaling cascade have been associated with invasive pneumococcal meningitis.[285] IL-1 receptor–associated kinase 4 (IRAK-4) is an intracellular kinase that is involved in signal transduction in the TLR pathways, and MyD88 is a general adaptor molecule for TLR signaling. Individuals with IRAK-4 and MyD88 deficiency are at a high risk for developing pneumococcal meningitis, with increasing mortality.[283,284,374] IRAK-4 and MyD88, however, appear to be important at a young age, because no fatal disease after 8 years of age and no invasive bacterial infection after 14 years of age have been reported.[374]

Congenital or acquired abnormalities of the immune system may predispose the host to the acquisition of bacterial infections. Congenital deficiency of the three major immunoglobulin classes may predispose the host to the acquisition of severe bacterial infection. Congenital defects of thymic-dependent, small lymphocyte function or combined T- and B-cell defects are detrimental to host defense. A deficiency of $CD4^+$ helper-inducer T cells in patients with bacterial meningitis has been reported and may contribute to the impaired antibody synthesis to bacterial capsular polysaccharides in this disease.[296] Multiple studies have shown that deficiencies of various components of the complement system and increased consumption or loss of complement have been associated with increased risk for development of bacterial meningitis caused by encapsulated organisms.[54]

An increased incidence of overwhelming infection, including meningitis, occurs after splenectomy, but the likelihood of development of such infection depends on

the age of the child at the time of splenectomy, the time since splenectomy, and the original indication for splenectomy.[109] Congenital asplenia or polysplenia also has been associated with an increased incidence of septicemia and meningitis caused by *S. pneumoniae*,[109] *H. influenzae* type b, and gram-negative enteric microorganisms. Children with sickle-cell disease and other hemoglobinopathies have meningitis caused by *S. pneumoniae*, *H. influenzae*, and *Salmonella* spp. more frequently than do normal children.

Children with malignant neoplasms with or without neutropenia seem to be susceptible to development of meningitis caused by organisms of low virulence that pose a minimal threat to healthy children, presumably because of abnormalities in immunologic function.[342] Decreased production of normal immunoglobulins, delayed and defective antibody responses to antigenic stimuli, production of abnormal immunoglobulins, depression in the clearance mechanisms of the reticuloendothelial system, and depression of cellular immunity have been documented in children with malignant neoplasms involving the reticuloendothelial system. In addition, the use of irradiation or immunosuppressive agents and antimetabolites predisposes the host to the development of infection in the CNS. Attributing the occurrence of bacterial meningitis in this population directly to these agents rather than to the disease for which this therapy has been provided may be difficult. Meningitis occurring after neurosurgical manipulation for tumors of the CNS in non-neutropenic children usually develops within 1 month of the neurosurgery.[342]

Malnutrition also predisposes children and adults to infectious disease. Impaired cellular immune responses, low levels of serum complement, impaired phagocytic activity of neutrophils, and decreased serum concentrations of transferrin have been documented in malnourished children.[117]

Patients with systemic diseases, such as diabetes mellitus, renal insufficiency, adrenal insufficiency, cystic fibrosis, hypoparathyroidism, and exudative enteropathy, have an increased frequency and severity of CNS infections.[45] Children with diabetes mellitus, coma caused by drug overdose, and Cushing syndrome have been shown to be at increased risk for development of bacteremia or meningitis caused by *H. influenzae* type b.[227] Some type of underlying condition was noted for 21 percent (37 of 181) of the children with pneumococcal meningitis in a multicenter study.[16] The most common of these conditions was some disorder of the CNS, which occurred in 16 children (9%). Defective chemotaxis, phagocytosis, and bactericidal function accompany these disorders and may explain in part the increased susceptibility of these individuals to infection.[117,118]

In the normal host, bacterial infections at sites other than the leptomeninges are associated with an increased incidence of CNS infection. Infection may spread hematogenously to the meninges in children with endocarditis, pneumonia, or thrombophlebitis or by direct extension from sinusitis, mastoiditis, or osteomyelitis of the skull.[198]

An increased risk for development of pneumococcal meningitis also occurs in children after placement of cochlear implants. The incidence of meningitis caused by *S. pneumoniae* in patients after receiving cochlear implants was 138.2 cases per 100,000 person-years, which represents a more than 30-fold increase over the age-controlled general population.[297] From 1999 to 2002, some children were implanted with cochlear devices, including a positioner, which were subsequently removed from the market as a result of an associated marked increase in incidence of pneumococcal meningitis. After removing the influence of cochlear devices containing a positioner, the incidence of pneumococcal meningitis among children with cochlear implants was still 16 times higher than that of an age-matched control population.

PATHOLOGY

The most detailed account in English of the pathologic changes occurring with meningitis was written in 1948 by Adams and colleagues.[4] They described the meningeal, cerebral, and vascular changes found post mortem in 14 patients who died of *H. influenzae* infection 14 hours to 76 days after the onset of disease. Although most patients in their series received inadequate treatment (effective antibiotic therapy was unavailable), the pathologic findings they describe differ little from the findings of subsequent reports for patients who died despite administration of antibiotics.[102,301,337]

Meningeal exudates of variable thickness may be found (Fig. 31-4). Purulent material is distributed widely but may accumulate around the veins and venous sinuses, over the convexity of the brain, in the depths of the sulci, in the sylvian fissures, within the basal cisterns, and around the cerebellum. The spinal cord may be encased in pus. Ventriculitis (purulent material within the ventricles) has been noted in children who died of their diseases. Subsequent experience suggests that ventriculitis may be a common finding in children with bacterial meningitis who survive, particularly neonates. Invasion of the ventricular wall with perivascular collections of purulent material has been noted. Loss of ependymal

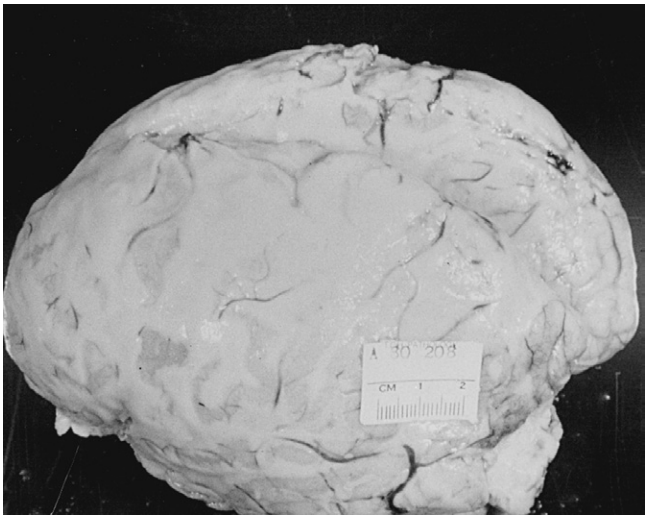

FIGURE 31-4 ■ Note extensive purulent exudates over entire cerebral cortex in a patient who died as a result of bacterial meningitis.

lining and subependymal gliosis may be seen. In some studies, purulent exudates tended to be thicker over the convexity of the brain in patients with pneumococcal meningitis than in patients with other forms of meningitis.[102,301]

Vascular and parenchymatous changes have been shown at necropsy. Polymorphonuclear infiltrates extending to the subintimal region of small arteries and veins have been associated with the exudative meningeal process. Thrombosis of small cortical veins associated with necrosis of the cerebral cortex may be noted. Occlusion of one of the major venous sinuses, subarachnoid hemorrhage secondary to necrotizing arteritis, and necrosis of the cerebral cortex in the absence of identifiable thrombosis of small vessels rarely may be observed. Reactive microglia and astrocytes may be identified in the cerebral cortex, particularly subadjacent to regions of heavy subarachnoid exudate. Because no bacteria are found in the cerebral cortex, these pathologic changes should be viewed as a noninfectious encephalopathy. "Toxic or circulatory factors" were suggested as possible causes by Adams and associates.[5] Dodge and Swartz[102] suggested systemic hypoxia and fever as additional possible causes. They also noted that an increase in intracranial pressure might interfere with cerebral circulation.

Impaired consciousness, deficits in motor and sensory functions, seizures, and retardation may be observed. Damage to the cerebral cortex, reflecting the effects of vascular occlusion, hypoxia, toxic encephalopathy, bacterial factors, inflammatory mediators, small molecule effectors, or some combination of these factors, provides an adequate explanation for these observations.

Hydrocephalus that develops in patients beyond the newborn period is an uncommon complication of meningitis. Most often, hydrocephalus is communicating and is the result of adhesive thickening of the arachnoid around the cisterns at the base of the brain. Less frequently, the aqueduct of Sylvius or the foramina of Magendie and Luschka are obstructed by fibrosis and reactive gliosis. The ensuing ventricular dilation may be coupled with coexistent necrosis of nervous tissue because of the meningitis itself or because of occlusion of cerebral veins and, rarely, arteries.

Subdural effusions occur frequently during the course of meningitis. The exact pathogenesis is unknown. The high incidence of effusion and the fact that subdural fluid collections may be found early in the course of bacterial meningitis in children suggest, however, that subdural effusions should be considered a concomitant occurrence with meningeal inflammation rather than a complication of the disease. Numerous veins traverse the subdural space, and inflammation of these veins and of the dural capillaries could produce an increase in vascular permeability and loss of albumin-rich fluid into the subdural space. The ratio of albumin to gamma globulin is higher in the subdural fluid of children with meningitis than in serum.[160] When the inflammatory process subsides, formation of fluid generally ceases, but its presence may persist because of a continued transudation through newly formed vessels in the subdural membrane. Subdural empyema, as opposed to subdural effusion, occurs rarely.[5,102,337]

Many factors contribute to the increase in intracranial pressure in patients with meningitis. Endotoxin and fragments of the cell wall of gram-positive organisms are capable of inducing the release of IL-1β and TNF-α from macrophages and other sources.[198,354] These substances, in addition to other interleukins and metabolites, affect endothelial cells, affecting the function of the vasculature and its interaction with neutrophils and other inflammatory cells. They also play an important role in the pathogenesis of increased intracranial pressure and cerebral edema in patients with meningitis by altering cerebral blood flow, intracranial blood volume, and permeability of the cerebral vasculature.[268] Experimentally, the main components of the blood-brain barrier—endothelial cells, astrocyte-end feet, and pericytes—have been found to be sensitive to the effects of such inflammatory mediators.[27] Intercellular junctions, which normally are tight, are open in experimental meningitis, which is associated with an increased permeability to circulating albumin.[291] Pinocytotic vesicles also are noted within the cytoplasm of endothelial cells. Swelling of cellular elements (cytotoxic edema) also has been noted.

Alterations in CSF resorption exacerbate cerebral edema and increase intracranial pressure further. In experimental meningitis, resorption of CSF is diminished because an accumulation of proteins, leukocytes, and other materials interferes with the function of the arachnoid villus.[318]

During the course of meningitis, excess secretion of antidiuretic hormone (ADH) occurs, which induces water retention and exacerbates electrolyte abnormalities already created secondary to the inflammatory process occurring in the CNS. Cellular electrolyte disturbances may depolarize neuronal membranes, predisposing the host to seizure activity. Increased oxidation of glucose, increased lactate production, and depletion of high-energy compounds such as adenosine 5′-triphosphate and phosphocreatine are observed. Hypoglycorrhachia results primarily from decreased transport of glucose across the inflamed blood-brain barrier and from increased use of glucose by host tissues. Use of glucose by bacteria and polymorphonuclear leukocytes is of less relative importance.[102,373]

CLINICAL MANIFESTATIONS AND PATHOPHYSIOLOGIC RELATIONSHIPS

The clinical features of bacterial meningitis in infants and children can be subtle, variable, and nonspecific, or even absent.[200] In infants, they might include fever, hypothermia, lethargy, irritability, poor feeding, vomiting, diarrhea, respiratory distress, seizures, or bulging fontanelles. In older children, clinical features might include fever, headaches, photophobia, nausea, vomiting, confusion, lethargy, or irritability. Signs of meningeal irritation are present in 75 percent of children with bacterial meningitis at the time of presentation.[229] In contrast, a retrospective review of 326 children presenting to a pediatric emergency department in the Netherlands between 1988 and 1998 with meningeal irritation revealed that 30 percent had bacterial meningitis.[275] Absence of meningeal

TABLE 31-6 **Frequency (%) of Selected Findings in Infants, Children, and Adults with Bacterial Meningitis on Presentation**

Selected Findings	Infants	Children	Adults
Underlying disorders	27-32	10-21	53-92
Headache	NA	15-92	31-87
Fever	34-98	88-97	42-97
Neck stiffness	9-54	40-80	50-88
Bulging fontanelle	14-42	NA	NA
Altered mental status	34-79	53-83	32-91
Coma	1-40	7-11	11-19
Seizures	16-62	19-41	5-21
Focal neurologic deficits	4-16	8-34	10-42
Cranial nerve palsies	10	10	10-21
Hearing loss	NA	23-34	9

NA, not available.

irritation in children with bacterial meningitis was substantially more common in those younger than age 12 months (Table 31-6).[200]

Inflammation of the meninges generally is associated with nausea, vomiting, irritability, anorexia, headache, confusion, back pain, and nuchal rigidity. In many cases, Kernig and Brudzinski signs are noted. The Kernig sign is present when the leg is flexed 90 degrees at the hips and cannot be extended more than 135 degrees. The Brudzinski sign is present if the thighs and legs are flexed involuntarily when the neck is flexed. All of these findings suggest irritation of inflamed sensory nerves, which produces a reflex contraction of certain muscles in an attempt to minimize pain. These findings also can be the result of increased intracranial pressure and an associated distortion of nerve roots. These signs can be accompanied by hyperesthesia and photophobia. Currently, no satisfactory pathophysiologic explanation for photophobia exists.

Signs of meningeal inflammation may be minimal in the infant, but irritability, restlessness, and poor feeding may be noted. Nuchal rigidity and Kernig and Brudzinski signs may occur late in a young child. Nuchal rigidity may not be elicited in comatose patients or when signs of focal or diffuse neurologic impairment are present. At the time of initial evaluation, 60 to 80 percent of children have a stiff neck.[199] A review of 1064 cases of bacterial meningitis in children beyond the neonatal period revealed that 16 (1.5%) had no meningeal signs during their entire period of hospitalization, despite the presence of CSF pleocytosis.[145] Fever, a hallmark of infection, generally is present; its absence in a patient with signs of meningeal inflammation, although infrequent, is not unusual.

The relative frequency with which selected findings in infants, children, and adults with bacterial meningitis are summarized in Table 31-6.*

Increased intracranial pressure is the rule; it may be reflected by complaints of headache in older children and by a bulging fontanelle and diastasis of sutures in infants. Papilledema is an uncommon finding in acute meningitis, presumably because of the brief duration of increased pressure at the time of diagnosis. When papilledema is observed, venous sinus occlusion, subdural empyema, or brain abscess should be sought.

A major contributing factor to increased intracranial pressure in bacterial meningitis is the development of cerebral edema. Vasogenic edema occurs as a consequence of increased permeability of the blood-brain barrier. Interstitial edema may occur secondary to decreased clearance of CSF at the arachnoid villi and subsequent obstructive hydrocephalus. Cytotoxic cerebral edema mediated by the release of toxic factors from neutrophils and bacteria leads to increased concentration of intracellular water and sodium and loss of intracellular potassium. In many cases,[114] meningitis is associated with the release of ADH, causing water retention and a relative dumping of sodium by the kidney. If the patient is given excessive free water during therapy, a further increase in intracranial pressure may be noted.

Transient or permanent paralysis of cranial nerves may be noted. Deafness or disturbances in vestibular function are common findings; optic nerve involvement with blindness rarely occurs. Involvement of the eighth cranial nerve may reflect disease at the level of the cochlear and vestibular end organs, which may be related to concomitant infection of the inner ear. Paralysis of extraocular and facial nerves may be noted. Torticollis has been reported in two children with partially treated meningitis.[242] Obtundation, stupor, coma, and focal neurologic signs may be seen in children with bacterial meningitis.

Table 31-7 shows data for 235 children with bacterial meningitis in a prospective study analyzed according to the type of organism responsible for meningitis. Overall, 14.9 percent of children were semicomatose or comatose at the time of admission; rates for children with pneumococcal or meningococcal meningitis were higher than rates for children with *H. influenzae* disease. Focal neurologic signs were present at the time of admission in 16.5 percent of the total group (34.3% of children with pneumococcal meningitis). The presence of focal neurologic signs at the time of admission indicated a poor prognosis and could be correlated with persistent abnormal neurologic examinations at 1, 3, and 6 months ($p < .01$) and at 1 year after discharge ($p < .03$). The presence of focal signs at the time of admission also correlated with the presence of retardation ($p < .001$), as determined by detailed psychometric testing after discharge.

Generally, when focal signs are noted in the absence of seizures, cortical necrosis, occlusive vasculitis, or thrombosis of cortical veins has occurred. Thrombosis of meningeal vessels or cortical necrosis may be associated with hemiparesis or quadriparesis and with focal seizures. These signs may appear during the first 3 or 4 days of illness or, less commonly, may be noted after the first or second week of infection. A highly significant association ($p < .001$) between neurologic signs indicative of cerebral injury and late (1 to 15 years after the acute infection) afebrile seizures has been noted.[287] Ataxia has been a presenting sign of meningitis in numerous children and adults.[324] Adolescents with meningitis may present with

*References 20, 73, 74, 85, 129, 146, 368, 384.

TABLE 31-7 **Frequency of Selected Findings in Children with Bacterial Meningitis**

	Total Group	*H. influenzae*	*S. pneumoniae*	*N. meningitidis*
No. patients	235	151	35	26
Level of consciousness (%)				
Irritable or lethargic	184 (78.3)	117 (77.5)	24 (68.6)	21 (80.8)
Somnolent	16 (6.8)	13 (8.6)	1 (2.8)	1 (3.8)
Obtunded-semicomatose	27 (11.5)	15 (9.9)	8 (22.9)	4 (15.4)
Comatose	8 (3.4)	6 (4)	2 (5.7)	0 (0)
Focal neurologic signs on admission (%)	37 (16.5)	22 (14.6)	12 (34.3)	2 (7.7)
Seizures before admission (%)	48 (20.4)	35 (23.2)	8 (23)	3 (11.5)
Seizures in hospital (%)	61 (26)	43 (28)	12 (34)	5 (19)

behavioral abnormalities that may be confused with drug abuse or psychiatric disorders.[26]

Approximately 20 percent of children with bacterial meningitis experience seizures before admissions, and approximately 26 percent have them during the first or second day in the hospital. Green and colleagues[163] retrospectively examined the frequency of seizures before or at the time of presentation in children with meningitis. They found that 111 of 410 (27%) children with bacterial meningitis had seizures at or before the time of diagnosis; 88 of these children had complex seizures (focal, prolonged, or more than one in a 24-hour period). They found that all children with bacterial meningitis who presented with seizures had other signs or symptoms of meningitis, such as altered level of consciousness, nuchal rigidity, or complex seizures and petechial rash. The frequency of seizure activity is similar for children with *H. influenzae* type b or pneumococcal meningitis; seizures occur in children with meningitis caused by these organisms approximately twice as frequently as in children with meningococcal meningitis. In a multicenter study of 181 children with pneumococcal meningitis, 41 (23%) developed seizures before admission; 74 percent of preadmission seizures were generalized.[16]

Overall, seizures are noted in approximately 30 percent of children with bacterial meningitis. Seizures noted before or during the first several days of hospitalization are of no particular prognostic significance. Specifically, their occurrence does not herald the development of a permanent seizure disorder. Seizures that are difficult to control or that persist beyond the fourth hospital day and seizures that occur for the first time late in the hospital course may be of greater significance and have been associated with permanent sequelae of meningitis. Children with focal seizures have a greater likelihood for development of sequelae of meningitis than do children with generalized seizure activity. Focal or prolonged seizures probably indicate serious cerebral vascular disturbances or cerebral infarction. Seizures that occur before admission have correlated positively with abnormal audiometric studies and permanent hearing handicaps. Seven percent of patients with bacterial meningitis have focal or generalized seizures 3 months to 15 years after recovery from bacterial meningitis.[287]

Collections of fluid in the subdural space can be shown in 50 percent of infants and children during acute illness.[102] In a prospective study of infants 1 to 18 months old with bacterial meningitis, subdural effusions were noted in 43 percent of infants with *H. influenzae* meningitis, 30 percent of infants with pneumococcal meningitis, and 22 percent of infants with meningococcal meningitis. Subdural effusions were found in 24 percent (25 of 103) of children undergoing neuroimaging in the multicenter pediatric surveillance study of pneumococcal meningitis.[16,338] No greater incidence of neurologic sequelae or developmental delay was found on long-term follow-up in patients with effusion compared with patients with bacterial meningitis who did not have effusion.[338] Subdural effusions may cause enlargement in head circumference or may be responsible for abnormal transillumination of the skull, and vomiting, seizures, a full fontanelle, focal neurologic signs, or persistent fever sometimes may be noted.

Blindness and optic atrophy may be related to optic arachnoiditis or infarction of the occipital lobe. Spastic paraparesis with sensory loss in the lower extremities may be secondary to meningomyelitis, spinal cord infarction, or both.

Shock may be associated with any form of overwhelming bacteremia, but it occurs most often in patients with fulminant meningococcemia. In a prospective study, 3.8 percent of children with meningococcal meningitis developed profound hypotension. In the same study, shock occurred in 5.5 percent of children with *H. influenzae* meningitis. Endotoxin has been detected by limulus lysate assay in the blood and CSF of children with meningococcal and *H. influenzae* meningitis.[304] Sixteen percent of the children in the multicenter pneumococcal meningitis study were in shock on admission.[16] Signs of disseminated intravascular coagulation may accompany hypotension in these patients.

Facial cellulitis (including buccal and periorbital cellulitis), pneumonia, epiglottitis, endophthalmitis, and other suppurative manifestations can manifest at the time of admission in any patient with bacterial meningitis. In one study of children with buccal cellulitis, less than 10 percent (7 of 73 children) had concomitant bacterial meningitis documented by lumbar puncture as part of their initial evaluation.[24] Five of the 7 children had no clinical evidence of meningeal irritation. *H. influenzae* and *S. pneumoniae* were cultured from 2 patients with periorbital cellulitis, and no clinical evidence of

meningeal irritation or abnormal CSF cell counts or results of chemistries was found.[312] In the largest review of facial cellulitis caused by *S. pneumoniae*,[161] 15 of 52 children had a lumbar puncture performed; 2 (13.3%) had a pleocytosis (18 white blood cells [WBCs]—52% polymorphonuclear leukocytes; and 9 WBCs—91% polymorphonuclear leukocytes). The Gram stain and cultures of the CSF specimens were negative. Lumbar puncture should be considered for children with facial cellulitis who are younger than age 18 months and possibly bacteremic with *S. pneumoniae*.

Arthralgia and myalgia are noted in many patients with bacterial meningitis, reflecting the systemic nature of the disease. Arthritis also may occur and does so most commonly during the course of meningococcal disease; generally, it is transient. Early findings of arthritis may be related to direct invasion of the joint by the meningococcus. Arthritis that develops late in the course of meningococcal or *H. influenzae* meningitis may be an immune complex–mediated event. Petechial or purpuric lesions may be seen in 50 percent of patients with meningococcal meningitis[102] but also may accompany any infectious or noninfectious disease process in which vasculitis occurs. Purpura, shock, and hypothermia indicate a poor prognosis.

Pericardial effusions may be present; they generally resolve during the course of antibiotic therapy. In some cases, pericardial effusions are the cause of persistent fever, and pericardiocentesis or an open drainage procedure may be required.

DIFFERENTIAL DIAGNOSIS

The signs and symptoms described earlier suggest meningeal or intracranial pathologic processes but are not pathognomonic of acute bacterial infection. Tuberculous meningitis, fungal meningitis, aseptic meningitis, brain abscess, intracranial or spinal epidural abscesses, cranial osteomyelitis, subdural empyema, bacterial endocarditis with embolism, ruptured dermoid cysts, ruptured spinal ependymomas, and brain tumors may show similar signs and symptoms. Differentiation of these disorders depends on careful examination of CSF obtained by lumbar puncture and additional immunologic, radiographic, and imaging studies, as delineated later.

Recurrent bacterial meningitis may be the result of a bacterial infection in the setting of an immunocompromised condition or a communication between the nasal passage or ear and the meninges from congenital anatomic defect or the result of trauma. If rhinorrhea or otorrhea is present, a leak may be suspected, but documenting that CSF is present and locating the site of leakage are difficult when the sample is small or contaminated. Sectional (2 mm) coronal cranial CT has been reported to be a noninvasive method for delineating anatomic abnormalities in children with recurrent meningitis.[348]

Meurman and associates[246] showed that an extra band of transferrin is located in the β_2-fraction after protein electrophoresis of CSF. This extra β_2-transferrin band could not be shown in serum, nasal secretions, saliva, tears, or perilymph and endolymph, suggesting that this immunochemical method is useful for documenting that fluid found draining from the nose or ear was CSF. The amount of sample required is small (<50 μL). Differential suction may permit demonstration of the site of the anatomic communication among the nose, the ear, and the meninges. Moderate contamination with other body fluids does not invalidate the method. The method is noninvasive and safe for the patient.

Noninfectious causes of meningitis include medications such as nonsteroidal anti-inflammatory drugs (NSAIDs), trimethoprim-sulfamethoxazole, isoniazid, metronidazole, intravenous immune globulin treatment, Behçet syndrome, systemic lupus erythematosus, mixed connective tissue disease, sarcoidosis, familial Mediterranean fever, Vogt-Koyanagi syndrome, procedures involving the CNS (neurosurgery, spinal anesthesia, intrathecal injection), subarachnoid hemorrhage, vein of Galen aneurysm, Mollaret meningitis, intracranial/intraspinal tumors, and cysts.

DIAGNOSIS

Early diagnosis and treatment of bacterial meningitis are imperative in reducing mortality rates and morbidity. Physicians must perform a lumbar puncture on any child in whom they suspect the diagnosis after a careful history and physical examination have been performed, unless specific contraindications to this procedure (e.g., clinical signs of increased intracranial pressure in a patient with a closed fontanelle and closed sutures, evidence of mass lesions, cardiopulmonary compromise) are present.

Measurement of opening pressure, often neglected in infants and young children, is an important component of each CSF examination. When the pressure is very high, only enough fluid to permit a careful examination should be removed. Compression of the jugular vein should be avoided unless compression of the spinal cord is suspected. The CSF appearance can be cloudy, stemming from high concentrations of bacteria, protein, WBCs and/or red blood cells (RBCs). Xanthochromic CSF derives its color primarily from bilirubin pigment. Hemorrhage, bilirubin staining in icteric patients who have meningitis (i.e., neonates, patients with leptospirosis), or an elevated protein concentration of CSF may be associated with xanthochromia.

CSF should be examined immediately. The total number of WBCs should be counted in a counting chamber; and after cytocentrifugation, a differential cell count should be done on a Wright-stained smear of the sediment. The normal CSF of children age 3 months or older contains less than 6 WBCs/mm^3. Ninety-five percent of children older than age 3 months have no polymorphonuclear leukocytes in the CSF; the presence of a polymorphonuclear leukocyte in the CSF may be regarded as abnormal. When a lumbar puncture has been performed in a febrile child and a single polymorphonuclear leukocyte has been noted, careful clinical observation is imperative, and treatment should be considered until the results of the culture of the CSF are known.

If the lumbar puncture has been traumatic, a total cell count can be done in a counting chamber. The RBCs can be lysed by acetic acid, and a cell count can be repeated. If the total number of WBCs compared with the number of RBCs is greater than that in whole blood, one can assume the presence of CSF pleocytosis. One simple way to estimate the WBC count in the presence of RBCs is to allow 1 WBC per 1000 RBCs/mm^3. However, this adjustment cannot be reliably performed in the presence of clot. CSF protein should be measured (usually elevated in bacterial meningitis), and the CSF glucose concentration should be compared with the blood glucose concentration that has been obtained concomitantly. In patients with bacterial meningitis, depression of CSF glucose and of the ratio of CSF to blood glucose (normally 40% to 60%) is the rule (Table 31-8).

Separate smears should be made, and one smear should be Gram stained for bacteria. A Kinyoun stain for mycobacteria is performed if tuberculous meningitis is suspected. The probability of visualizing bacteria on a Gram stain of CSF depends on the number of organisms present.[41,122] The percentage of positive smears is 25 percent with less than 10^3 CFU/mL, 60 percent in the range of 10^3 to 10^5 CFU/mL, and 97 percent with greater than 10^5 CFU/mL.[217] Gram stain is positive in about 90 percent of children with pneumococcal meningitis, about 80 percent of children with meningococcal meningitis, half of patients with gram-negative bacillary meningitis, and a third of patients with *Listeria* meningitis.[200] Cytospin centrifugation increases the chances of detecting organisms in Gram-stained CSF.[200] A low CSF WBC count with positive Gram stain is a risk factor for an unfavorable outcome.[16,200] Quellung and agglutination reactions can provide immediate identification of various organisms if the appropriate type of specific antisera is available.

Treating a child with bacterial meningitis with an antibiotic before performing initial lumbar puncture usually does not alter markedly the cell counts or chemical results obtained (Table 31-9). Even when children received appropriate antibiotics for meningitis intravenously for 44 to 68 hours, the bacterial character of the chemical and cellular findings could be discerned in most cases.[43] CSF culture, however, can be negative in children who receive antibiotic treatment before CSF examination. Complete sterilization of *N. meningitidis* occurred within 2 hours of giving a parenteral third-generation cephalosporin and the beginning of sterilization of *S. pneumoniae* from CSF by 4 hours into treatment.[188] In such children, pleocytosis and an increased CSF protein concentration are usually sufficient to establish the diagnosis of bacterial meningitis. Blood cultures or nonculture diagnostic tests can help in identifying the infecting organism.

The CSF should be cultured on a blood agar plate and a chocolate agar plate. The CSF specimens always should be cultured, even when the fluid appears to be crystal clear and acellular.

In the 1970s, countercurrent immunoelectrophoresis was shown to be a useful technique for rapid diagnosis (within 1 hour) and management of bacterial meningitis caused by *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis* (groups A, C, W135, and D), and group B streptococcus. It was also used to detect antigens from K1 strains of *E. coli* or *L. monocytogenes*.[120,200,241] The methodology was sensitive and could detect nonviable bacteria, permitting the detection of bacterial antigen, even in patients who have been pretreated with appropriate antibiotics. If this technique is employed, it is imperative to use antisera that have the greatest possible sensitivity and specificity, although it is rarely available currently.[182,326] A negative result of countercurrent immunoelectrophoresis does not exclude the diagnosis of bacterial meningitis.

Latex particle agglutination commercial kits are available for detecting the polysaccharide antigens of *H. influenzae* type b, *S. pneumoniae*, *N. meningitidis*, and group B streptococcus. Latex particle agglutination is superior to countercurrent immunoelectrophoresis in detecting PRP antigen of *H. influenzae* type b in CSF and serum. Nonspecific agglutination of latex particles in serum, urine, and other body fluids may result in an indeterminate test result.[88,183,317,381] A commercial latex particle agglutination kit containing antibody-coated latex particles for *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis* serogroups is available. In the multicenter pneumococcal meningitis surveillance study, latex agglutination was positive in 49 (66%) of 74 CSF specimens that grew *S. pneumoniae* and in 4 of 14 CSF specimens that were culture negative.[16] The U.S. Food and Drug Administration (FDA) does not recommend latex agglutination testing of urine of infants for group B streptococcus as a method for inferring invasive disease with this organism. Latex agglutination of urine for group B streptococcus or *N. meningitidis* should be avoided because of the high frequency of false-positive results.

The clinical utility of rapid antigen detection in CSF has been questioned in recent years since the decline in frequency of invasive infections caused by *H. influenzae* type b. A positive test response rarely alters the approach to therapy. Bacterial antigen tests should be considered for patients with suspected bacterial meningitis whose initial CSF Gram stain was negative and whose culture was negative after 48 hours of incubation.[240,281] Finlay and colleagues[127] suggested that latex agglutination testing of CSF be used only when the Gram stain is consistent with meningococci. In the latter case, clarifying the meningococcal serotype quickly may have implications in large outbreaks for recommending meningococcal vaccine for type A, C, Y, or W135 disease.

The rapidity with which results of bacterial antigen tests are obtained renders them tempting as a means to establish an early diagnosis. The degree to which they affect clinical decisions is unclear. Performing these tests is unnecessary for every patient suspected to have bacterial meningitis, but they could play a role in certain circumstances, such as when a patient's clinical presentation suggests bacterial meningitis and pretreatment with antibiotics or with a traumatic lumbar puncture. Antigen detection is useful in developing countries, where CSF culture yields are lower.[93,200]

Standard or sequential-multiplex polymerase chain reaction (PCR) has been shown to be useful in identification of infecting pathogens in patients who have previously received antibiotics or in resource-poor

TABLE 31-8 **Cerebrospinal Fluid Findings in Infectious and Inflammatory Diseases of the Central Nervous System and Meninges**

Condition	Pressure (mm H_2O)	Leukocytes/μL	Protein (mg/dL)	Sugar (mg/dL)	Specific Findings
Acute bacterial meningitis	Usually elevated; average 300	Several hundred to >10,000; usually a few thousand; occasionally <100 (especially meningococcal or early in disease); PMLs predominate	Usually 100-500, occasionally >1000	<40 in more than half the cases	Organism usually seen on smear or recovered on culture in >90% of cases
Subdural empyema	Usually elevated; average 300	<100 to a few thousand; PMLs predominate	Usually 100-500	Normal	No organisms on smear or by culture unless concurrent meningitis
Brain abscess	Usually elevated	Usually 10-200; fluid rarely is acellular; lymphocytes predominate	Usually 75-400	Normal	No organisms on smear or by culture
Ventricular empyema (rupture of brain abscess)	Elevated	Several thousand to 100,000; usually >90% PMLs	Usually several hundred	Usually <40	Organisms may be seen on smear or cultured
Cerebral epidural abscess	Slight to modest elevation	Few to several hundred or more cells; lymphocytes predominate	Usually 50-200	Normal	No organisms on smear or by culture
Spinal epidural abscess	Usually reduced with spinal block	Usually 10-100; lymphocytes predominate	Usually several hundred	Normal	No organisms on smear or by culture
Thrombophlebitis (often associated with subdural empyema)	Often elevated	Few to several hundred; PMLs and lymphocytes	Slightly to moderately elevated	Normal	No organisms on smear or by culture
Bacterial endocarditis (with embolism)	Normal or slightly elevated	Few to <100; lymphocytes and PMLs	Slightly elevated	Normal	No organisms on smear or by culture
Acute hemorrhagic encephalitis	Usually elevated	Few to >1000; PMLs predominate	Moderately elevated	Normal	No organisms on smear or by culture
Tuberculous infection	Usually elevated	Usually 25-100, rarely >500; lymphocytes predominate except in early stages when PMLs may account for 80% of cells	Nearly always elevated; usually 100-200; may be much higher if block in CSF flow	Usually reduced; <50 in 75% of cases	Acid-fast organisms may be seen on smear of protein coagulum (pellicle) or recovered from inoculated guinea pig or by culture
Cryptococcal infection	Usually elevated	Average 50 (0-800); lymphocytes predominate	Average 100; usually 20-500	Reduced in more than half of cases; often higher in patients with concomitant diabetes mellitus	Organisms may be seen in India ink preparation and on culture (Sabouraud medium); usually grow on blood agar; may produce alcohol in CSF from fermentation of glucose
Syphilis (acute)	Usually elevated	Average 500; usually lymphocytes; rarely PMLs	Average, 100; gamma-globulin often high, with abnormal colloidal gold curve	Normal (rarely reduced)	Positive reagin test result for syphilis; spirochete not demonstrable by usual techniques of smear or by culture
Sarcoidosis	Normal to considerably elevated	0 to <100 mononuclear cells	Slight to moderate elevation	Normal	No specific findings

PMLs, polymorphonuclear leukocytes.

TABLE 31-9 **Comparison of Cerebrospinal Fluid Findings in Patients with Untreated and Pretreated Meningitis**

	Untreated	Pretreated
No. patients	143	91
Total white blood cell count? 10^3		
Mean ± 1 SD	4.9 ± 6.5	4.1 ± 5
Range	0-55	0.006-25.5
Percentage polys		
Mean ± 1 SD	84 ± 21	81 ± 25
Range	0-100	0-100
Glucose (mg/dL)		
Mean ± 1 SD	35 ± 28	32 ± 25
Range	0-109	0-100
CSF/blood glucose (%)		
Mean ± 1 SD	29 ± 21	29 ± 21
Range	0-78	0-94
Protein (mg/dL)		
Mean ± 1 SD	226 ± 228	174 ± 193
Range	13-2290	10-1640
Culture positive	135	71
Gram stain positive	114	62

settings.[76,82,200,215,235] PCR analysis of CSF has been used to detect microbial DNA in patients with bacterial meningitis. Primers are available for the detection of *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b simultaneously. Species-specific amplicons have been detected in 89 percent of patients with proven streptococcal, meningococcal, or *H. influenzae* type b meningitis.[293] No false-positive results were noted.

Multiplex real-time PCR or broad-range PCR aimed at the 16S ribosomal RNA gene of eubacteria is promising for the detection of pathogens from CSF. The detection rate was substantially higher with PCR than with cultures in patients who had previously received antibiotics.[82] However, the limit of detection differs between assays. Real-time PCR has been shown to detect as few as two copies of *E. coli*, *N. meningitidis*, and *S. pneumoniae*, 16 copies of *L. monocytogenes*, and 28 copies of group B streptococcus,[82] whereas the sensitivity for broad-range 16S ribosomal DNA PCR was 10 to 200 organisms per milliliter of CSF.[84,85] The time needed for the whole process from DNA extraction to the end of real-time PCR was 1.5 hours,[82] an attractive time frame for its application in clinical practice.

A Gram stain–specific, probe-based, real-time PCR using 16S ribosomal RNA has been shown to allow simultaneous detection and discrimination of clinically relevant gram-positive and gram-negative bacteria directly from blood samples,[200] which might provide more rapid and accurate diagnosis of bacterial infection in infants and children. In addition, sequential PCR-based serotyping of *S. pneumoniae* using serotype-specific primers could improve ascertainment of pneumococcal serotype distribution in settings in which prior use of antibiotics is high.[200] Dicuonzo and associates[99] also documented the great sensitivity of oligoprobes on amplified DNA for the diagnosis of *H. influenzae*, streptococcal, and *Mycobacterium tuberculosis* meningitis. No false-negative results occurred in culture-positive CSF specimens. The possible addition of gene microarray technology offers the potential to probe for multiple different bacterial species simultaneously.[175]

PCR also is useful in rapidly documenting viral antigens within CSF, thus reducing the use of antibiotics in selected patients who are treated for presumptive bacterial disease but who may have viral meningitis.[305,320,371]

Measurement of C-reactive protein (CRP) has been proposed as a test that may be valuable in distinguishing bacterial from viral meningitis. In some studies, overlap in CRP determinations between these groups of patients has been observed and one cannot rely on the CRP result to distinguish bacterial from viral meningitis with sufficient certainty.[152] Nonetheless, serum CRP was superior to CSF parameters in distinguishing gram-negative bacterial from viral meningitis.[343] Among 92 patients with viral meningitis, 93 percent had serum CRP levels within the normal range (<20 mg/L). Only one child with gram-negative bacterial meningitis had a serum CRP value within the normal range.

Elevated serum concentrations of the polypeptide procalcitonin were shown to be useful in differentiating between bacterial and viral meningitis. At a threshold of 0.5 ng/mL, procalcitonin had a sensitivity of 99 percent and a specificity of 83 percent by one report and has been incorporated into the Meningitest (see later).[104]

Numerous metabolic changes have been reported to occur in the CSF and blood of patients with meningitis. CSF lactate has been noted to be elevated significantly in patients with bacterial meningitis. The increase in CSF lactate apparently is related to decreased cerebral blood flow, cerebral hypoxia, and a change to anaerobic metabolism by the brain. Concentration of CSF lactate tends to parallel the CSF cellular response.[307] Although the concentration of CSF lactate in patients with bacterial meningitis generally is greater than that in patients with aseptic meningitis, such is not always the case. In some patients with aseptic meningitis, CSF lactate has been in the range generally observed in patients with bacterial infections. Conversely, in patients who proved to have bacterial meningitis but who had equivocal clinical and CSF findings, measurement of CSF lactate failed to differentiate bacterial from nonbacterial infection.[307] The determination of the concentration of CSF lactate cannot be used reliably to differentiate viral from bacterial meningitis in an individual patient.[211]

Depression of the pH of CSF also has been described in patients with bacterial meningitis. The depression of pH in CSF is more transient than is the elevation of CSF lactic acid, and its measurement is of even less value in the differential diagnosis.

Lactate dehydrogenase, creatine phosphokinase, and aspartate transaminase may be elevated in patients with bacterial meningitis. In some cases, total lactate dehydrogenase activity within CSF may be similar in patients with bacterial and aseptic meningitis but lactate dehydrogenase isoenzymic analysis may permit differentiation of bacterial from nonbacterial infection. This procedure is time consuming and cumbersome and does not permit establishing a specific etiologic diagnosis in any patient.[266]

Despite the application of impeccable clinical judgment, examination of CSF, and use of one or more of the

rapid diagnostic techniques, situations arise in which differentiation of bacterial from aseptic meningitis remains problematic. In these cases, a predominance of polymorphonuclear leukocytes generally is found in the CSF, the CSF cell count is less than 100 cells/mm^3, the CSF glucose concentration is normal or nearly so, and the Gram stain result is negative. Most children with aseptic meningitis have a predominance of polymorphonuclear leukocytes on their initial CSF examination.[267] In addition, although patients exhibit signs and symptoms suggestive of meningitis, they do not appear acutely ill. Some investigators have advocated withholding antibiotic therapy in these patients and repeating the lumbar puncture after 6 to 12 hours of close observation.[3,116] Usually, the repeated examination of CSF either substantiates the impression of aseptic meningitis (a shift to a lymphocytic differential is noted) or points more conclusively to a bacterial process. This course of action is not recommended if the patient has been pretreated with antibiotics or is younger than 1 year old. Occasionally, children have a mild CSF pleocytosis, which may have a predominance of polymorphonuclear leukocytes, after they experience seizures.[391,393] Generally, CSF abnormalities should not be attributed to seizures, unless other causes of CNS inflammation have been excluded.

Other approaches to differentiate bacterial from viral meningitis have been suggested. Evaluation of CSF ferritin concentration has been proposed as a test with considerable predictive value. CSF ferritin concentration is significantly elevated in pediatric patients with bacterial meningitis compared with those with viral meningitis.[202] Using a proposed cutoff of 15.6 ng/mL of CSF ferritin, the test had a sensitivity of 96.2 percent and a specificity of 96.6 percent in differentiating bacterial from viral meningitis.

The ability to distinguish between bacterial and nonbacterial aseptic meningitis in infants and children in the emergency department could contribute to limiting hospital admissions or unnecessary use of antibiotics. The bacterial meningitis score was developed for assessing infants and children with meningitis, and outpatient management might be considered for children who had pleocytosis (7 cells/μL or more) and none of the following five criteria on presentation (history of a seizure with the illness, blood neutrophil count of at least 10×10^3 cells/μL, positive CSF Gram stain, CSF protein of at least 80 mg/dL, or CSF neutrophil count of at least 1×10^3 cells/μL), but this proposed diagnostic tool achieved 95 percent sensitivity and failed to identify 5 patients with bacterial meningitis.[103,269] This tool, called the Meningitest, was refined with incorporation of a serum procalcitonin level of greater than or equal to 0.5 ng/mL and was claimed to have the sensitivity of 100 percent and specificity of 37 percent.[104] The usefulness of both the bacterial meningitis score and the Meningitest for differentiating between bacterial and aseptic meningitis remains to be determined.

Additional laboratory data are helpful and should be obtained. Blood cultures should be obtained in every patient suspected to have bacterial meningitis. In one prospective study in which blood was obtained for culture from every patient, the cultures were positive in 80 percent of children with *H. influenzae* meningitis, in 52 percent of children with pneumococcal meningitis, and in 33 percent of children with meningococcal meningitis.[114] Forty-four percent of the entire group had received some form of antibiotic therapy before admission to the hospital and before these blood cultures were performed. If these individuals were excluded, positive blood cultures were obtained from 90 percent, 80 percent, and 91 percent of children with meningitis caused by *H. influenzae*, *S. pneumoniae*, and *N. meningitidis*, respectively.

A thorough search for foci of infection adjacent to or remote from the meninges should be conducted. Repetitive neurologic evaluation also should be performed, and appropriate laboratory studies should be undertaken to define the extent of neurologic dysfunction.

When the concentration of bacteria within the blood is high, a Gram-stained smear of a buffy coat obtained from the blood may reveal the presence of microorganisms. If petechial lesions are present, a smear of the lesions after puncture with a small lancet may reveal microorganisms on Gram stain. A chest radiograph may be helpful in disclosing a focus of infection.

Radioisotope scanning may be helpful in selected patients, such as patients with a leak of CSF. The pattern of distribution of radioactivity recorded by gamma camera coincides with the accumulation of purulent material. Increased concentration of isotope may relate to the inflammatory response within the meninges or in the periventricular region or to alteration in the blood-brain barrier.[157] Localized concentrations of radionuclide may be seen in children with meningitis, most likely as a result of cerebral vasculitis or infarction.[102] Confirmation of impaired cerebral circulation, including occlusion and narrowing of arteries, sluggish circulation, and retrograde flow, has been described.[141] In these studies, resolution of the arterial lesions was shown in subsequent angiograms in two patients, despite the persistence of neurologic deficits; these findings prompted the authors to suspect vascular spasm at the earlier stage of disease. Hydrocephalus contributed to sluggish circulation through intracerebral vessels in two patients. At least transient disturbance in the circulation of CSF was noted in 45 percent of patients with meningitis, but persistent hydrocephalus is a rare complication of purulent meningitis.[364]

Computed tomography (CT) and magnetic resonance imaging (MRI) are noninvasive techniques that permit the prospective and repetitive assessment of children with meningitis. These techniques permit detection of ventricular dilation, subdural effusion, decrease in brain mass, and presence of vascular lesions or of brain infarcts (Fig. 31-5). With these procedures, ventricular dilation may be noted acutely in many children who never develop hydrocephalus after recovery from their disease.

CT is widely available and useful for rapid assessment of hydrocephalus, mass lesions, hemorrhage or acute brain edema prior to lumbar puncture. In contrast, MRI is required to detect more subtle findings. MRI is more sensitive for assessing CSF involvement, leptomeningitis, empyema, ventriculitis, and infarctions but is not as widely available and can be logistically challenging to obtain in the acutely ill patient. Noncontrast CT and MRI can be normal in early cases of bacterial meningitis. Administration of a contrast agent may be helpful to

FIGURE 31-5 ■ CT scan of a 2-year-old child with bacterial meningitis. Moderately severe ventricular dilation and the presence of bilateral extracerebral fluid collections overlying the convexities of the brain (subdural effusions) are noted. Note the several prominent vessels that run through the subdural space.

detect meningeal enhancement, with MRI being more sensitive than CT. Meningeal enhancement, however, is not specific to the diagnosis of bacterial meningitis and can be seen with other diagnoses, such as leptomeningeal carcinomatosis. Fluid-attenuated inversion recovery (FLAIR)-weighted MRI can demonstrate high signal in the subarachnoid space, reflecting high protein content in the CSF, but high signal in the subarachnoid space can also be seen with leptomeningeal carcinomatosis and subarachnoid hemorrhage. The findings of rhombencephalitis suggest *L. monocytogenes* as the likely causative organism.

The most important role of neuroimaging is to identify potential complications of bacterial meningitis, such as infarction, hydrocephalus, ventriculitis, brain empyema, abscess, and venous sinus thrombosis. Neuroimaging (MRI with gadolinium contrast) may be indicated in the following situations: (1) focal neurologic signs, (2) persistently positive CSF cultures despite administration of appropriate antibiotic therapy, (3) persistent elevation of CSF polymorphonuclear leukocytes (>30% to 40%) after more than 10 days of therapy, and (4) recurrent meningitis.

TREATMENT

Antimicrobial Therapy

Prompt treatment of bacterial meningitis with an appropriate antibiotic is essential. Several retrospective and prospective studies showed that delay in antibiotic treatment was associated with adverse outcomes.[21,200,247] In patients with suspected bacterial meningitis for whom immediate lumbar puncture is delayed due to pending brain imaging study or the presence of disseminated intravascular coagulation, blood cultures must be obtained and antimicrobial treatment should be initiated immediately.

TABLE 31-10 **Likely Pathogens for Meningitis Based on Age, Immunization Status, and Risk Factors**

Age, Immunization Status, Risk Factors	Likely Pathogens
<1 mo	Group B streptococci, *E. coli*, *L. monocytogenes* (neonatal pathogens)
1-3 mo	
No immunization or one dose of primary immunization	Neonatal pathogens, *S. pneumoniae*, *N. meningitidis*, *H. influenzae*
3-6 mo	
No immunization	*S. pneumoniae*, *N. meningitidis*, *H. influenzae*
At least two doses of Hib-OMP vaccine	*S. pneumoniae*, *N. meningitidis*
>7 mo to 5 yr	
No immunization	*S. pneumoniae*, *N. meningitidis*, *H. influenzae*
Primary immunization completed	*S. pneumoniae* (non-PCV serotypes), *N. meningitidis*
6-21 yr	*S. pneumoniae*, *N. meningitidis*
CSF leak, cochlear implant, nephrotic syndrome	*S. pneumoniae*
Complement deficiencies	*S. pneumoniae*, *N. meningitidis*
Asplenia, sickle cell disease	*S. pneumoniae*, *N. meningitidis*, *H. influenzae*

Antibiotic selection should include consideration of such factors as the likely pathogens, the CSF penetration of the antibiotic, the activity of the drug in purulent CSF, the mode of administration of the drug, and the intrinsic pharmacodynamic relationships of CSF drug concentrations to bactericidal activity.[334] The initial selection always should be made before definitive cultures are available, and empirical antimicrobial regimens should cover likely pathogens based on age of the patient and specific risk factors (Table 31-10) with modification if the CSF Gram stain is positive, as well as based on incidence and susceptibility patterns in the local community.[200]

For many years, ampicillin and chloramphenicol were preferred as the initial empirical therapy for children older than 3 months of age and thought to have bacterial meningitis. Ampicillin and chloramphenicol continue to be effective as initial treatment of bacterial meningitis when organisms are susceptible to these agents, but they are used infrequently. Ampicillin may be given intravenously in a dose of 300 mg/kg/24 hours in six divided doses. An initial bolus of 100 mg/kg is given. Chloramphenicol is administered intravenously in a dose of 100 mg/kg/24 hours in four divided doses. No loading dose of chloramphenicol is required.

The development of third-generation cephalosporins and other antibiotics that have excellent bactericidal activity against *H. influenzae* type b, *N. meningitidis*, and *S. pneumoniae* within the CSF as well as emergence of penicillin-resistant pneumococcal isolates led to the current approaches to initial therapy of childhood meningitis. Cefotaxime and ceftriaxone are included in the empirical treatment regimen of choice in most centers.[204]

Cefotaxime is a third-generation cephalosporin that has a broad spectrum of activity against gram-positive and gram-negative organisms. It possesses a high level of resistance to hydrolysis by β-lactamase. Cefotaxime penetrates the blood-brain barrier and provides bactericidal activity in the CSF equivalent to or greater than that of antibiotics that have been used conventionally for treatment of bacterial meningitis in children.[256] It is an excellent choice for inclusion in empirical therapy in children 1 month old or older but must be used with ampicillin for initial therapy in children younger than 1 month old because *L. monocytogenes* and enterococci are sensitive to ampicillin and cannot be treated with cefotaxime. Cefotaxime is given as a daily dose of 225 to 300 mg/kg/day in three or four divided doses intravenously. The high dosage is preferred by some experts because the higher CSF concentrations achieved by high-dose therapy may be beneficial for patients whose disease may be caused by *S. pneumoniae* when the organisms are of intermediate susceptibility to third-generation cephalosporins.[80]

Ceftriaxone is another third-generation cephalosporin that possesses broad antimicrobial activity against the organisms that cause bacterial meningitis. Ceftriaxone readily penetrates the CSF of patients with inflamed meninges. In patients who receive adjunctive therapy with dexamethasone, meningeal inflammation may be reduced, possibly decreasing the penetration of antibiotics into the CSF. Concentration of ceftriaxone in the CSF of children with bacterial meningitis treated with dexamethasone, however, was similar to that found in children not treated with corticosteroids.[142] The half-life of ceftriaxone in serum is approximately 4 hours; a twice-daily dose regimen provides serum and CSF concentrations far in excess of the minimal bactericidal concentrations of most organisms that cause bacterial meningitis. Several prospective randomized studies demonstrated that ceftriaxone is comparable to ampicillin plus chloramphenicol for the treatment of bacterial meningitis in children.[17,31,55,81,95]

Ceftriaxone therapy has been associated with an increased incidence of diarrhea, which is mild and self-limited. An increased incidence of "gallbladder sludge," or precipitation of ceftriaxone salts in the gallbladder, diagnosed by ultrasound, also occurs and generally is asymptomatic but occasionally is associated with clinical symptoms of cholecystitis.[17,316] Ceftriaxone also has a high protein-binding capacity and can displace bilirubin from albumin in vitro[167] and needs to be used cautiously in neonates.

When ceftriaxone is used for the treatment of bacterial meningitis, it can be administered in a dose of 100 mg/kg/24 hr in two divided doses or one daily dose intravenously. Although a once-daily dose has proved to be effective,[47,136,165] is convenient, and lends itself particularly to home therapy for selected patients (after an initial period of hospitalization), single daily dosing is not advocated; dosing errors, delayed doses, or missed doses undoubtedly occur, and inadequate treatment could result. Although ceftriaxone can be given intramuscularly, a single dose by this route may be impractical.[47] The solution used for intramuscular administration should contain no more than 250 mg/mL. A 15-kg child receiving a daily dose of 80 mg/kg would require 4.8 mL of fluid, a volume too large for injection in a single site in an infant. In addition, for penicillin-nonsusceptible but ceftriaxone-susceptible organisms, administration twice a day may be preferred.[80]

An increasing number of strains of *S. pneumoniae* that are relatively or completely resistant to penicillin and third-generation cephalosporins have been identified.[52] Tan and associates[353] reported a retrospective analysis of five children who had pneumococcal meningitis caused by strains that were penicillin resistant and that had minimal inhibitory concentrations (MICs) to cefotaxime or ceftriaxone of 0.5 to 2 μg/mL compared with strains that were penicillin-resistant but susceptible to cefotaxime or ceftriaxone (MIC ≤0.25 μg/mL); there was no difference in clinical outcome at the time of discharge. The Clinical and Laboratory Standards Institute established the current guidelines for interpreting the MIC for cefotaxime or ceftriaxone for pneumococci isolated from patients with bacterial meningitis. The guidelines indicate that strains with an MIC of 2 μg/mL or greater are considered resistant, strains with an MIC of 1 μg/mL are intermediate, and strains with an MIC of 0.5 μg/mL or less are considered fully susceptible.[265]

Vancomycin in a dose of 60 mg/kg/day (15 mg/kg every 6 hr for children older than 1 month old not to exceed 4 g per day; some experts recommend up to 20 mg/kg every 6 hr for these patients.) is recommended in addition to cefotaxime for empirical therapy of children with meningitis because of the frequency with which penicillin-resistant and cephalosporin-resistant pneumococci have been isolated in recent years worldwide. Vancomycin has been used successfully to treat penicillin-resistant pneumococcal meningitis[48] and experimental models of cephalosporin-resistant pneumococcal meningitis.[139] In eight reports of treatment failures with third-generation cephalosporins, a variety of treatment regimens were used, all successfully. Vancomycin, alone or in combination with rifampin, chloramphenicol, or both, was used most frequently. The recommendation for empirical therapy for bacterial meningitis advocated by the AAP Committee on Infectious Diseases is to include vancomycin in addition to a third-generation cephalosporin in patients aged 1 month or older.[10] If nonsusceptibility to penicillin (MIC ≥0.1 μg/mL) and cephalosporins (MIC >0.5μg/mL) is documented, treatment is continued with vancomycin plus cefotaxime or ceftriaxone (a synergistic effect is achieved when they are used together) with or without rifampin to complete an appropriate course. For pneumococcal meningitis, a serum trough concentration greater than 10 to 15 μg/mL is generally recommended; monitoring of vancomycin peak concentrations is not recommended.

Antibiotic tolerance is the ability of an organism to be susceptible to inhibition of growth (as MIC values show susceptible), but resistant to the killing activity of antibiotics, which differs from conventional resistance when resistance is shown by bacterial growth in the presence of antibiotics, resulting in high MIC.[200] Clinical isolates of *S. pneumoniae* that are tolerant to vancomycin have been reported in the United States.[270] Meningitis caused by a vancomycin-tolerant organism in a patient was treated successfully with cefotaxime (300 mg/kg/day in four divided doses) plus vancomycin (80 mg/kg/day in four divided doses) and rifampin (20 mg/kg/day in two divided doses). Chloramphenicol also may be a suitable alternative for the treatment of these organisms if the pneumococcus proves to be susceptible to this antibiotic. Chloramphenicol therapy, however, was shown to have a significantly greater unsatisfactory outcome for children with meningitis caused by penicillin-resistant pneumococci compared with those with penicillin-susceptible pneumococci, although pneumococcal isolates were susceptible to chloramphenicol with MICs of less than or equal to 4 μg/mL.[138]

Two carbapenem agents, imipenem and meropenem, have been studied in patients with bacterial meningitis. These carbapenems are active against the bacteria that cause meningitis, but the use of imipenem-cilastatin has been associated with drug-induced seizures.[393]

The safety and efficacy of meropenem were compared with those of cefotaxime in a prospective randomized trial of 190 children with bacterial meningitis.[207] Seizures occurred within 24 hours before administration of antibiotic therapy in 16 percent of patients randomly assigned to receive meropenem and in 7 percent of patients randomly assigned to receive cefotaxime. Seizures occurred in patients after administration of therapy in 6 percent of children receiving meropenem and in 1 percent of children receiving cefotaxime. None of these seizures could be attributed to drug therapy. All patients responded to therapy with clinical improvement, and bacterial eradication was proved by repeated lumbar puncture in 100 percent of patients in both groups. No significant difference in short-term outcomes occurred between the two groups. Similarly, the efficacy and safety of meropenem with cefotaxime were compared for the treatment of bacterial meningitis in 258 children who were randomly assigned to the meropenem or cefotaxime group.[273] Clinical cure with or without sequelae was achieved in 97 percent and 96 percent of the meropenem-treated and cefotaxime-treated patients, respectively. At 7 weeks after treatment was concluded, 54 percent of patients treated with meropenem and 58 percent of patients treated with cefotaxime had no sequelae. Seizures were noted in 12 percent of the patients treated with meropenem and in 17 percent of patients treated with cefotaxime; none of the seizures was considered to be drug related. These data suggest that meropenem is effective in the treatment of bacterial meningitis in children. Few children with pneumococcal meningitis in either study had isolates that were nonsusceptible to cefotaxime. No conclusions can be drawn regarding the usefulness of meropenem in treating pneumococcal meningitis caused by isolates with a cefotaxime or ceftriaxone MIC equal to or greater than 2 μg/mL. However, of 20 cefotaxime-resistant *S. pneumoniae* isolates, 4 were intermediate and 13 were resistant to meropenem, suggesting that meropenem may not be a reliable alternative agent for pneumococcal isolates that are highly resistant to penicillin and cephalosporins. Meropenem may be indicated for patients who have meningitis that is caused by organisms resistant to extended-spectrum cephalosporins (e.g., nosocomial meningitis due to extended-spectrum β-lactamases producing gram-negative bacteria), but its efficacy has not been demonstrated.

Cefepime is a fourth-generation cephalosporin that has been studied for the treatment of bacterial meningitis. In vitro, cefepime offers no advantage over cefotaxime or ceftriaxone for penicillin-resistant *S. pneumoniae*.[196] In addition, no in vitro data exist for the efficacy of cefepime against cefotaxime-resistant pneumococci. In two clinical trials, the efficacy of cefepime was found to be equivalent to the efficacy of either cefotaxime or ceftriaxone.[308,309] No penicillin-resistant or ceftriaxone-resistant pneumococci were encountered in either study, however. Cefepime has been used for the treatment of bacterial meningitis as an alternative agent.

Ceftazidime has been efficacious in the treatment of meningitis caused by *P. aeruginosa*.[177,249,299] Cefpirome concentrations in CSF of patients with bacterial meningitis were found to be significantly higher than the minimal bactericidal concentrations for *N. meningitidis*, *H. influenzae*, and *S. pneumoniae*,[391] but studies documenting its effectiveness in large numbers of children have not been done.

Cefoperazone and cefoxitin within CSF may fail to reach concentrations required to kill all susceptible strains of *H. influenzae* and *S. pneumoniae* and cannot be recommended for the treatment of bacterial meningitis in children.[58,124]

Cefuroxime is a second-generation cephalosporin that has been shown to be effective in vitro against *H. influenzae* type b, *S. pneumoniae*, and *N. meningitidis*. Studies showed delayed sterilization of the CSF, and also relapse during or after treatment was higher; and more frequent sensorineural hearing loss occurred than with use of ampicillin, chloramphenicol, cefotaxime, and ceftriaxone.[15,219,221,315] Cefuroxime should not be used to treat bacterial meningitis in children.

Aztreonam is an antimicrobial agent that belongs to the monobactam family of antibiotics. It is effective against most gram-negative organisms, including *P. aeruginosa*. Limited data suggest its efficacy in the treatment of *Pseudomonas* and *H. influenzae* meningitis, suggesting a potential role for this agent in the treatment of patients who are allergic to penicillin and who are infected with these or other gram-negative organisms.[197,220,360]

If a history of *significant* allergy to penicillin or cephalosporin (anaphylaxis, urticaria, exfoliative dermatitis) is documented, vancomycin plus rifampin or aztreonam may be used. A cross-reactivity of 10 to 15 percent has been noted for cephalosporins in penicillin-allergic patients.

When meningitis is caused by *Streptococcus pyogenes*, penicillin or ampicillin provides effective therapy. If meningitis is caused by a penicillin-resistant strain of *S. aureus*, oxacillin or nafcillin 200 mg/kg/24 hr intravenously in

four or six divided doses should be used. Vancomycin is effective against *S. aureus* strains resistant to penicillin and to semisynthetic penicillin derivatives[168] or *S. aureus* meningitis in patients who are penicillin allergic. Vancomycin also may be useful in treatment of meningitis caused by *Flavobacterium meningosepticum*.[100] Metronidazole is effective in treatment of anaerobic infection of the CNS when response to conventional therapy has been suboptimal. A dose of 340 mg/kg/24 hr in four divided doses results in CSF concentrations of greater than 10 μg/mL.[36]

In animal models, the addition of a nonbacteriolytic antibiotic such as rifampin[150] has been associated with a neuroprotective effect, suggesting the possibility of its use as an adjunct therapy. Bottcher and associates[46] showed decreased bacterial cell wall release, a lessened proinflammatory response (including decreased leukocyte recruitment), less free radical formation, and decreased apoptosis and cellular damage after the addition of clindamycin to standard antibiotic therapy.

Table 31-11 provides current recommendations for antibiotic treatment of various microorganisms. An appropriate antibiotic should be continued until the patient is afebrile for 5 days, but for at least 7 to 10 days in every patient. Although some data support a shorter course of therapy, at least 10 days of treatment is recommended for pneumococcal and *H. influenzae* type b meningitis and 7 days for *N. meningitidis* meningitis.[185,232] If clinical improvement is noted within 24 hours, a repeated lumbar puncture is unnecessary, in most cases, during the course of treatment or after treatment has been completed. If infection is caused by *S. pneumoniae* that is resistant to penicillin and to third-generation cephalosporins, a repeated lumbar puncture is recommended at 48 to 72 hours to document bacterial sterilization of the CSF. If clinical improvement is slower than anticipated or is not noted, a repeated examination of the CSF is indicated at any time.

In the 1970s, lumbar puncture frequently was performed at the conclusion of therapy. Data from studies performed at that time (Table 31-12) reveal that WBC counts and protein concentrations within the CSF generally had not returned completely to normal and that the CSF-to-blood glucose ratios may have remained depressed. In every case, Gram stain of the CSF should reveal no organisms and cultures should be sterile. If a lumbar puncture is performed at the conclusion of therapy, we consider re-treatment to be mandatory if organisms are seen or grown. It also may be considered if more than 30 percent of the cells are polymorphonuclear leukocytes, or if the CSF glucose concentration is less than 20 m/dL, and the CSF-to-blood ratio is less than 20 percent (see Table 31-12).

Bacteriologic relapse after treatment of meningitis (particularly that caused by *H. influenzae* and treated with ampicillin) was highlighted in numerous reports.[25,75,78,171,172] Precise assessment of the frequency of relapse in children who have received an appropriate antibiotic to which the organism is susceptible or an appropriate dose intravenously and for an extended period has been difficult. The relapse rate is currently less than 1 percent.

Some physicians have discharged children with meningitis from the hospital before the conclusion of a course of therapy and prescribed home management. Benefits of home therapy include a decreased risk for acquiring nosocomial infection, a return of the child to his or her normal environment sooner, and a decrease in the total cost of therapy. Financial savings of outpatient, once-daily ceftriaxone for pediatric meningitis have been estimated to be $200 per day.[289]

Fifty-four children with bacterial meningitis were treated as outpatients for 1 to 8 days (mean 4.6 days) with intramuscular ceftriaxone given once daily.[47] Each dose was given in conjunction with a physician's examination. Each child had to be afebrile for 24 to 48 hours before initiation of home therapy, free of neurologic dysfunction except for auditory or vestibular dysfunction, and without evidence of inappropriate secretion of ADH before being considered for outpatient therapy. No child required readmission or developed neurologic sequelae or relapse. A retrospective review of 26 patients with meningitis or other serious bacterial infections who received some portion of their therapy as an outpatient with ceftriaxone revealed that none of the patients experienced relapse or recurrence.[289]

Waler and Rathore[378] suggest 10 criteria for considering outpatient therapy for children with bacterial meningitis: (1) the child has received inpatient therapy for at least 6 days, (2) the child is afebrile for at least 24 to 48 hours before initiation of outpatient therapy, (3) the child has no significant neurologic dysfunction or focal findings, (4) the child has no seizure activity, (5) the child is clinically stable, (6) the child is taking all fluids by mouth, (7) the first dose of outpatient antibiotic is received in the hospital, (8) the antibiotic is administered in the office or emergency department setting or by qualified home health nursing, (9) daily examination is performed by a physician, and (10) parents are reliable and have transportation and a telephone. The dose of ceftriaxone of 100 mg/kg/24 hr intramuscularly may need to be aliquoted to account for the volume of diluents needed to achieve a concentration of no greater than 250 mg/mL.

H. influenzae type b organisms have been recovered from the throats of patients after completion of a course of treatment for *H. influenzae* type b meningitis. When members of a household to which the patient will return include children 4 years of age or younger, the patient should be given rifampin 20 mg/kg once daily for 4 days, to prevent the occurrence of secondary cases. Children with meningococcal meningitis also should receive chemoprophylaxis to eradicate nasopharyngeal carriage before discharge, unless they were treated with cefotaxime or ceftriaxone (see Prevention).

Adjunctive Therapy

As described earlier, the pathogenesis and subsequent sequelae of bacterial meningitis are as much a consequence of the host response to infection as of the bacterial organisms themselves. Anti-inflammatory and anti-osmotic agents used as adjuncts to antimicrobial therapy may decrease the degree of tissue injury during the course of the disease.

TABLE 31-11 **Recommendations for Antibiotic Therapy for Bacterial Meningitis**

Organism	Antibiotic(s)	Recommended Dosages (IV)
Bacteroides fragilis	Metronidazole	30 mg/kg/day in 4 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
Bacteroides other than *B. fragilis*	Penicillin G	300,000 U/kg/day in 6 dd
Clostridium	Penicillin G	300,000 U/kg/day in 6 dd
Corynebacterium	Penicillin G	300,000 U/kg/day in 6 dd
	Erythromycin	50 mg/kg/day in 4 dd
Enterobacter, Klebsiella, Escherichia coli[*]	Cefotaxime	200 mg/kg/day in 4 dd
	Ceftriaxone	100 mg/kg/day in 2 dd
	Meropenem	120 mg/kg/day in 3 dd
	Ampicillin	300 mg/kg/day in 6 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Amikacin	15 mg/kg/day in 3 dd
Haemophilus influenzae	Cefotaxime	200 mg/kg/day in 4 dd
	Ceftriaxone	100 mg/kg/day in 1 or 2 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
	Ampicillin	300 mg/kg/day in 6 dd
Listeria monocytogenes	Ampicillin	300 mg/kg/day in 6 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	TMP-SMX	20 mg/kg/day in 4 dd (TMP component)
Neisseria meningitidis	Penicillin G	300,000 U/kg/day in 6 dd
	Cefotaxime	200 mg/kg/day in 4 dd
	Ceftriaxone	100 mg/kg/day in 1 or 2 dd
Proteus mirabilis (indole-negative)	Ampicillin	300 mg/kg/day in 6 dd
P. mirabilis (indole-positive)	Cefotaxime	200 mg/kg/day in 4 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Amikacin	22.5 mg/kg/day in 3 dd
	Ticarcillin	300 mg/kg/day in 4-6 dd
Pseudomonas	Ceftazidime	150-200 mg/kg/day in 3 dd
	Meropenem	120 mg/kg/day in 3 dd
	Ticarcillin	450 mg/kg/day in 4 or 6 dd
	Piperacillin	300 mg/kg/day in 4 or 6 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Amikacin	15-20 mg/kg/day in 3 dd
Salmonella	Cefotaxime	200 mg/kg/day in 4 dd
	Ampicillin	300 mg/kg/day in 6 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
Staphylococcus aureus (penicillinase-negative)[†]	Penicillin G	300,000 U/kg/day in 6 dd
S. aureus (penicillinase-positive)[†]	Oxacillin or nafcillin	200 mg/kg/day in 4 or 6 dd
S. aureus (resistant to semisynthetic penicillins)	Vancomycin *plus*	80 mg/kg/day in 4 dd
	Rifampin	20 mg/kg/day in 2 dd
Staphylococcus (coagulase-negative)	Vancomycin	80 mg/kg/day in 4 dd
	Rifampin	20 mg/kg/day in 2 dd
Streptococcus pneumoniae[†]	Cefotaxime/ceftriaxone	225-300 mg/kg/day in 3 or 4 dd/100 mg/kg/day in 1 or 2 dd
	Vancomycin	60-80 mg/kg/day in 4 dd
	Rifampin[‡]	20 mg/kg/day in 2 dd
	Penicillin G	300,000 U/kg/day in 6 dd
	Chloramphenicol	100 mg/kg/day in 4 dd
Unknown (<1 mo old)	Ampicillin *plus*	300 mg/kg/day in 6 dd
	Cefotaxime *or*	200 mg/kg/day in 4 dd
	Gentamicin	7.5 mg/kg/day in 3 dd
Unknown (>1 mo old)	Cefotaxime *or*	225-300 mg/kg/day in 4 dd
	Ceftriaxone *plus*	100 mg/kg/day in 1 or 2 dd
	Vancomycin	60-80 mg/kg/day in 4 dd
	Nafcillin or oxacillin (if question of staphylococcal infection)	200 mg/kg/day in 6 dd

*Trimethoprim-sulfamethoxazole (TMP-SMX) in a dose of 20 mg/kg/day (TMP) and 100 mg/kg/day (SMX) IV in 4 dd has been used successfully in selected patients with gram-negative enteric meningitis.

†Vancomycin may be provided in a dose of 80 mg/kg/day in 4 dd IV (20 mg/kg every 8 hr, or 60 mg/kg/day in 3 dd in older adolescents) if patients are allergic to penicillin or penicillin derivatives or, in the case of *S. pneumoniae*, for multidrug-resistant pneumococci or pneumococci that are highly resistant to penicillin. In these cases, addition of rifampin may be considered.

‡Should never be used alone.

dd, divided doses.

Note: In the 1970s, lumbar puncture frequently was performed at the conclusion of therapy. Data from studies performed at that time (see Table 31-12) reveal that white blood cell counts and protein concentrations within the CSF generally had not returned completely to normal and that the CSF-to-blood glucose ratios may have remained depressed. In every case, Gram stain of the CSF should reveal no organisms and cultures should be sterile. If a lumbar puncture is performed at the conclusion of therapy, we consider re-treatment to be mandatory if organisms are seen or grown. It also may be considered if more than 30 percent of the cells are polymorphonuclear leukocytes or if the CSF glucose concentration is less than 20 m/dL and the CSF-to-blood ratio is less than 20 percent.

TABLE 31-12 **Cerebrospinal Fluid Findings at Conclusion of Antibiotic Treatment**

	Total White Blood Cell Count		Polymorphonuclear Leukocytes (%)		CSF-to-Blood Protein (mg/dL)		Glucose (mg/dL)		CSF-to-Blood Glucose Ratio (%)	
	MEAN ±SD	RANGE	MEAN ±SD	RANGE	MEAN ±SD	RANGE	MEAN ±SD	RANGE	MEAN ±SD	RANGE
Total group	41 ± 80	0-850	5.5 ± 12	0-90	46 ± 72	7-970	47 ± 12.7	21-91	55.7 ± 17	23-156
H. influenzae	53 ± 98	0-850	5.5 ± 11	0-90	43 ± 37	10-334	47 ± 13	31-100	55 ± 17	31-100
H. influenzae (ampicillin)	56 ± 107	0-850	5.9 ± 12	0-90	44 ± 43	13-334	46 ± 14	21-91	53 ± 17	33-89
H. influenzae (chloramphenicol)	49 ± 83	0-325	5.1 ± 10.7	0-50	41 ± 26	7-127	48 ± 11	27-90	57 ± 13	30-100
S. pneumoniae	29 ± 30	0-110	5.5 ± 11.5	0-45	42 ± 39	7-211	48 ± 9	22-68	57 ± 13	22-91
N. meningitidis	16 ± 27	0-132	3.4 ± 7.9	0-27	39 ± 46	7-188	47 ± 11	29-77	47 ± 19	23-100
Others	11 ± 18	0-77	7.3 ± 19	0-75	70 ± 197	10-970	48 ± 18	37-73	70 ± 28	44-156

CSF, cerebrospinal fluid; SD, standard deviation.

Anti-inflammatory Therapy

Research studies seek additional therapies that could be used to help reduce further mortality, morbidity, or both. Five main areas serve as the targets for therapeutic development: (1) modulation of bacterial killing and the release of bacterial products by using nonbacteriolytic antibiotics such as rifampin, (2) host recognition of bacteria or its products and the initiation of the inflammatory response, (3) modulation of the inflammatory response with adjuvant dexamethasone, (4) inhibition or interruption of the host inflammatory/neurotoxic mediators, and (5) modulation of the apoptotic pathways.[148,162,198,210] Inhibitors of inflammatory mediators and mediator effector molecules such as TNF-α, matrix metalloproteinases, and nitric oxide and antioxidants, neuroprotective factors (melatonin and brain-derived neurotrophic factor), and other anti-inflammatory therapies (triptans) are being studied in experimental animal models of bacterial meningitis.[149,198,210] Although their use is attractive theoretically because of the damage produced by the inflammatory cascade (Fig. 31-3), none has emerged as a realistic potential candidate for general clinical use at present.

Corticosteroids

Corticosteroids have been suggested as an adjunct to therapy for bacterial meningitis because they may (1) decrease intracranial pressure by decreasing meningeal inflammation and brain water content; (2) modulate the production of cytokines, which lessens the meningeal inflammatory response; and (3) decrease the incidence of sensorineural hearing loss or other neurologic complications of meningitis.[218,272]

Corticosteroids may play a role in acute management of increased intracranial pressure and cerebral herniation, although no data specifically indicate that corticosteroids decrease cerebral edema caused by bacterial meningitis. Dexamethasone therapy in children with bacterial meningitis was shown to decrease opening lumbar CSF pressure 12 hours after administration of the first dose, but this effect was lost by 24 hours of treatment.[270] The significance of these findings is unclear because the corticosteroids were not given as specific therapy for increased intracranial pressure and most of the subjects were not showing signs of impending herniation.

In experimental *H. influenzae* meningitis, administration of dexamethasone 1 hour before, but not 1 hour after, administration of ceftriaxone was associated with significantly reduced TNF-α concentration and indices of inflammation in the CSF.[261]

Administration of dexamethasone has been associated with decreased concentration in CSF of prostaglandin E_2 and decreased leakage of some proteins from serum into CSF in rabbits with experimental pneumococcal meningitis.[186] Among patients with bacterial meningitis, steroid-treated patients had significantly lower concentrations of IL-1β, TNF-α, PAF, and prostaglandin E_2 in their CSF than those of patients who received antibiotics alone.[262,272] Patients tend to become afebrile sooner when they receive dexamethasone, but they have an increased incidence of secondary fevers.[16,376]

Although the understanding of the pathophysiologic events associated with initiation of the acute inflammatory response has been enhanced by more recent data, the ability of dexamethasone to reduce long-term complications of bacterial meningitis in pediatric patients remains controversial. In a prospective, randomized, double-blinded, placebo-controlled study evaluating adults in Europe with bacterial meningitis, de Gans and colleagues[92] showed a decrease in mortality rates in all patients and in unfavorable outcomes in patients with pneumococcal meningitis. By contrast, no pediatric studies of the use of dexamethasone for treatment of bacterial meningitis have shown an overall change in mortality rates.

In randomized, placebo-controlled trials of dexamethasone as adjunctive therapy in bacterial meningitis[218,272,314,376] and in retrospective studies[159,195] published since 1988, 68 percent (227 of 333) of corticosteroid recipients in the six randomized trials had meningitis caused by *H. influenzae* type b; the remaining 32 percent had meningitis caused by *S. pneumoniae* (50 of 333) or *N.*

meningitidis (56 of 333).[290] A meta-analysis of nine controlled trials published before 1991 failed to document a reduced risk for neurologic abnormality at hospital discharge or follow-up examination.[178] A more recent meta-analysis of randomized clinical trials of dexamethasone as adjunctive therapy for bacterial meningitis suggests a beneficial effect of this agent for *H. influenzae* meningitis and a possible beneficial effect in preventing severe hearing loss in *S. pneumoniae* meningitis, but only if it is given early.[243]

Most patients with *S. pneumoniae* meningitis in the studies that were reviewed were not treated with vancomycin. This issue is important because the penetrance of vancomycin across the blood-brain barrier is not optimal, and selected studies show that it is reduced further when dexamethasone is used concomitantly.[16,49,250] Despite the theoretical clinical concerns, CSF concentrations of vancomycin and ceftriaxone are adequate for treatment of penicillin-susceptible and penicillin-nonsusceptible *S. pneumoniae* when dexamethasone is given at recommended dosages.[112,208]

Administration of dexamethasone immediately before the initiation of cefotaxime therapy was associated with a reduced incidence of neurologic sequelae (14% compared with 38% in patients receiving cefotaxime alone).[272] These investigators found no significant difference in auditory sequelae compared with control subjects. The frequency of neurologic sequelae in placebo-treated patients, however, was significantly higher than that noted in other studies.[16,314,376]

In contrast, other studies failed to show a significant reduction in the incidence of neurologic sequelae in comparisons of corticosteroid-treated and placebo-treated patients.[218,314] A prospective, multicenter, placebo-controlled study evaluated 143 children with bacterial meningitis caused by *H. influenzae* type b (58%), *S. pneumoniae* (23%), and *N. meningitidis* (17%).[376] Patients were treated with ceftriaxone and placebo or ceftriaxone and dexamethasone administered within 4 hours of the first dose of antibiotics. No significant difference in neurologic or developmental outcome was found between patients who received corticosteroids or placebo.

Sensorineural hearing loss is a significant sequel of bacterial meningitis. In the two randomized studies,[218] patients treated with dexamethasone were significantly less likely to have moderate or severe bilateral sensorineural hearing loss; however, in one study, patients were treated with cefuroxime, which has been shown to result in delayed sterilization of CSF and a higher rate of hearing loss compared with ceftriaxone.[315] An additional 100 infants and children with bacterial meningitis were treated with ceftriaxone for 10 days and either dexamethasone or placebo for 4 days. A significant reduction in moderate to severe hearing loss in children with *H. influenzae* type b meningitis (p <.001) was reported. No significant differences in other neurologic sequelae were found between the two groups. In addition, two patients receiving dexamethasone developed gastrointestinal bleeding severe enough to require transfusion and two others developed heme-positive stools.[218] Odio and associates found no significant difference in the incidence of moderate or severe hearing impairment between placebo and corticosteroid groups (16% and 6%), respectively.[272]

The Swiss Meningitis Group[314] found that treatment of children with dexamethasone 10 minutes before administration of ceftriaxone and then for 2 days subsequently resulted in persistent hearing loss in 5 percent (three) of children who had received dexamethasone and in 15 percent (eight) of children who received placebo, a difference that was not significant. One child treated with corticosteroid and five children treated with placebo had unilateral hearing loss only. The group also documented a transient mild to moderate hearing impairment in five children treated with dexamethasone and four children treated with placebo; in six of the nine children, the impairment was caused by a conductive disturbance. Dexamethasone did not alter the incidence or natural history of the transient hearing impairment.

In a multicenter study,[376] audiologic measurements were made early in the course of the disease (within 24 hours of admission) and 6 weeks to 12 months after recovery from disease. The authors found no significant difference in the incidence of persistent moderate or severe hearing loss between children who received dexamethasone within 4 hours of antibiotics and children who received placebo, with the exception of bilateral deafness in children with *H. influenzae* type b meningitis (5 of 72 in the placebo-treated group vs. 0 of 67 in the dexamethasone-treated group; p = .02). The overall incidence of moderate to severe hearing loss was 14.7 percent (10.3% unilateral and 4.4% bilateral) in the dexamethasone-treated group and 22.9 percent (13.5% unilateral and 9.4% bilateral; p = .33 for bilateral loss) in the placebo-treated group. These authors found that 22 children (8 in the dexamethasone-treated group and 14 in the placebo-treated group) had bilateral moderate or severe hearing loss at the initial evaluation. Only one child with *H. influenzae* meningitis and unilateral deafness at initial examination progressed to bilateral deafness. At follow-up, the resolution of hearing loss was nearly identical for each group, with 8 of the 22 children having normal hearing at follow-up, 5 having unilateral deafness, and 9 having bilateral deafness.

These results suggest that hearing loss occurs early in the course of meningitis and that early auditory brain stem response results need to be interpreted cautiously with regard to long-term audiologic sequelae. A strong relationship was noted between hearing loss early in the disease and a low concentration of CSF glucose at manifestation of meningitis, a finding that has been reported previously.[101]

Data from animal studies highlight a potential negative effect of dexamethasone therapy on neuronal injury, including hippocampal apoptosis in experimental animals with pneumococcal and *E. coli* meningitis who received dexamethasone.[225,346]

The AAP Committee on Infectious Diseases states that dexamethasone therapy should be considered for pneumococcal meningitis in infants and children 6 weeks old and older.[10] Dexamethasone also is recommended for treatment of infants and children with *H. influenzae* type b meningitis.[10] The reluctance on the part of some experts to recommend dexamethasone for pneumococcal

meningitis is predicated on data that fail to show any diminution in morbidity or mortality rates from meningitis when meningitis is caused by *S. pneumoniae* and dexamethasone is used. Dexamethasone should be used cautiously when vancomycin is used to treat meningitis caused by *S. pneumoniae* that may be resistant to penicillin, third-generation cephalosporins, or both.[49] The CSF concentrations of vancomycin, ceftriaxone, cefotaxime, and rifampin when they are given in dosages recommended for meningitis in children treated with dexamethasone generally are shown to be adequate to treat meningitis caused by most nonsusceptible strains of *S. pneumoniae*. Dexamethasone plus vancomycin may decrease the transport of vancomycin into the CSF of experimental animals with pneumococcal meningitis, but this finding was not observed in nine children who were treated with vancomycin and dexamethasone (0.6 mg/kg/day).[208] Dexamethasone can lead to decreased fever and a misleading impression of clinical improvement, even though sterilization of the CSF has not been achieved.

Dexamethasone should not be used if aseptic or nonbacterial meningitis is suspected; if it is started before the diagnosis of nonbacterial meningitis is made, it should be discontinued immediately. It should not be used in "partially treated" meningitis. No data exist on which to base a recommendation for use of dexamethasone in the treatment of bacterial meningitis in infants younger than 6 weeks of age or in infants with congenital or acquired abnormalities of the CNS, with or without a prosthetic device. One prospective study in infants concluded that adjunctive dexamethasone therapy does not improve the outcome of neonatal bacterial meningitis.[86] If dexamethasone is used, it should be used in all patients, regardless of disease severity, and it should be administered as early as possible in the course of treatment in a dose of 0.15 mg/kg/dose intravenously every 6 hours for no more than 4 days. One study[314] found no difference in children treated for 2 days instead of 4 days with 0.4 mg/kg/dose every 12 hours.

Except for hearing loss after *H. influenzae* type b meningitis, no clear evidence establishes that dexamethasone dramatically alters the long-term sequelae of meningitis, and its use is not without risk for causing adverse events. The markedly decreased frequency of meningitis caused by *H. influenzae* type b and the increased frequency of meningitis caused by *S. pneumoniae* nonsusceptible to penicillin (for which therapy with vancomycin may be necessary) suggest that initiation of dexamethasone should be considered carefully and that the clinician caring for the patient should evaluate the risk-to-benefit ratio of such therapy.

Glycerol

A recent multicenter double-blind randomized study in six Latin American countries showed that adjunctive treatment with oral glycerol 1.5 g/kg every 6 hours for 48 hours prevents severe neurologic sequelae in childhood meningitis (odds ratio 0.31; 95% CI 0.31 to 0.76) compared with placebo.[279] Glycerol is a hyperosmolar agent, and because of its safety, wide availability, low cost, and oral administration, its use as adjunctive treatment in children with bacterial meningitis, particularly in resource-limited settings, is attractive, but subsequent studies in experimental animals and children with bacterial meningitis did not reveal any benefits.[280]

Supportive Care

In addition to antibiotic therapy, management of bacterial meningitis includes measures that apply generally to critically ill children. Careful monitoring and attention to detail are essential. Pulse rate, blood pressure, and respiratory rate should be measured carefully every 15 minutes until stable and then every hour while the patient is in the intensive care unit. Temperature should be measured every 4 hours. A thorough neurologic examination should be done at the time of admission and at least daily thereafter. A rapid assessment of neurologic function should be done several times a day for the first several days of treatment. Body weight should be measured daily for at least the first 3 or 4 days. Head circumference should be measured in children younger than 18 months at the time of admission and repeated daily if concerns about increased intracranial pressure persist.

The following laboratory data are suggested if results of lumbar puncture indicate bacterial meningitis: (1) total peripheral WBC count and differential, (2) hemoglobin concentration, (3) hematocrit, (4) platelet count, and (5) serum electrolytes (serum and urine osmolalities may be useful in selected patients). Urine volume and specific gravity should be monitored. A low WBC count may suggest a poor prognosis. Anemia associated with *H. influenzae* type b septicemia has been reported[329,330] and has been attributed to immune hemolysis of RBCs that are coated with soluble bacterial antigens.

Every child with meningitis should be evaluated carefully to identify inappropriate secretion of ADH, recognize seizure activity, and detect the development of subdural effusions. Determinations of body weight, serum electrolytes (serum and urine osmolalities in selected patients), urine volume, and specific gravity should be made at the time of admission and observed closely (every 6 to 12 hours) for the first 24 to 36 hours that the child is in the hospital and daily for several days thereafter. Initially, the child should receive nothing by mouth because of the risk for vomiting and aspiration. In addition, delivery of all fluid intravenously ensures greater accuracy in measurement of intake and output during the critical early days of therapy. Inappropriate secretion of ADH has been documented in 88 percent of children enrolled in a prospective study of bacterial meningitis.[114] Elevated serum concentrations of ADH in the presence of hyponatremia have been documented by direct measurement of ADH concentration in serum obtained from the same children.[115]

An electrolyte solution containing approximately 40 mEq/L of sodium and chloride, 35 mEq/L of potassium, and 20 mEq/L of acetate or lactate should be administered at a rate of 1000 to 1200 mL/m^2/24 hr in a patient without evidence of dehydration or shock. Fluid restriction is continued until it can be documented (frequently within 2 hours), on the basis of objective measures, that ADH secretion is not a factor or has resolved.

The best indicators of retention of fluid in excess of solute are body weight and serum sodium concentration. As serum sodium concentration approaches normal (135 to 140 mEq/L), fluid administration may be liberalized progressively to normal maintenance levels of 1500 to 1700 mL/m^2/24 hr.

The syndrome of inappropriate secretion of ADH should not be diagnosed in the presence of dehydration. Decreased intravascular volume is a physiologic stimulus for the release of ADH, and its release is not inappropriate. Fluid restriction is not advocated for patients who are dehydrated; rehydration should be performed with careful and frequent assessment of fluid and electrolyte status.

Singhi and colleagues[333] examined the effect of fluid restriction on body water and outcome of 50 consecutive children who had been hospitalized with acute meningitis. These children were divided into two groups: patients with hyponatremia and patients without hyponatremia. Patients in both groups were randomly assigned to receive either normal maintenance or restricted fluids (65% to 70% of the volume of that received by the maintenance subgroup). Eleven to 15 patients were randomly assigned to any of the four subgroups in the study. No significant difference in overall outcome or intact survival was found when comparisons were made between fluid-restricted and non–fluid-restricted groups or within each group between the subgroups that received restricted fluids or maintenance fluids. After combination of the subgroups that received restricted fluids with the subgroups that received maintenance fluids, however, a trend toward higher intact survival and lower mortality rates was noted in the non–fluid-restricted groups. Nonetheless, children who had an extracellular water reduction of 10 mL/kg or greater in 48 hours had a significantly lower intact survival rate (10 of 28, 36%) than that of children with less than 10 mL/kg or no reduction of extracellular water (15 of 22, 64%). The mortality rate also was higher in the former group (7 of 28, 25%) than in the latter group (2 of 22, 9%). The authors concluded that fluid restriction did not improve the outcome of acute meningitis and that a decrease in extracellular water volume at 48 hours may increase the likelihood of having an adverse outcome.

In contrast, in studies of large numbers of children with bacterial meningitis, evidence of inappropriate secretion of ADH correlated significantly (p <.01) with abnormal neurologic findings, even 3 months after discharge, and with low IQ scores.[119] As a result of these findings, coupled with documentation of inappropriate secretion of ADH, a recommendation was made to restrict fluids in patients who are hyponatremic at admission. Fluids are restricted only until evidence of inappropriate secretion of ADH can be excluded (usually within 2 hours). The average patient in subsequent studies[190] was fluid restricted for only 18 hours.

Because cerebral edema and increased intracranial pressure have been noted as major disturbances in seriously ill patients with meningitis and because many of the deaths and some of the sequelae have been related to the effects of cerebral edema and intracranial hypertension, it appears prudent to recommend fluid restriction in patients with hyponatremia who are not dehydrated and liberalization of fluids as soon as the effects of excess ADH secretion have been dissipated (usually <1 day).

Meningitis complicated by shock creates a complex fluid management problem. Shock associated with meningitis is secondary to septicemia and generally is treated with intravenous infusion of large quantities of fluid to maintain blood pressure and adequate tissue perfusion. Patients with meningitis without shock or dehydration benefit from initial fluid restriction to avoid worsening of cerebral edema and severe hyponatremia with subsequent seizures. Children with meningitis and shock should receive sufficient quantities of isotonic fluid to maintain a systolic blood pressure of 80 to 90 mm Hg, a urine output equal to or greater than 500 mL/m^2/24 hr, and adequate cerebral perfusion as indicated by mental status. Central venous pressure monitoring is useful to guide fluid resuscitation and to prevent fluid overload. The addition of albumin (1 g/kg) to intravenously administered fluids may decrease the total volume of fluid needed to maintain adequate perfusion. Vasopressors, such as dopamine, dobutamine, and isoproterenol, also may provide support of blood pressure and perfusion and reduce requirements for intravenously administered fluids.

When increased intracranial pressure is suggested by such signs as progressive lethargy, increased muscle tone, or bulging anterior fontanelle, elevating the head approximately 30 degrees may be helpful. Increased intracranial pressure associated with deterioration in mental status or signs of cerebral herniation may be treated more vigorously with mannitol administered intravenously at 0.5 g/kg infused during 30 minutes and repeated as necessary. If corticosteroids are used for this purpose, the recommended agent is dexamethasone in a dose of 10 to 12 mg/m^2/day in four divided doses for no more than 4 or 5 days.[190] Other supportive measures to ensure appropriate delivery of oxygen and nutrients to the brain include a quiet environment, elective intubation, and the use of sedatives.[32]

Head circumference measurement and transillumination permit assessment of the development of subdural effusions or may suggest other causes for an enlarging head. CT may be helpful in detecting large subdural effusions or hydrocephalus. Because effusions can be considered part of the pathophysiologic changes that occur with bacterial meningitis, obtaining CT scans to evaluate effusions does not need to be part of the routine evaluation of a child with meningitis. Neuroimaging (CT or MRI with contrast) should be performed in children with focal neurologic signs. In children with hemiparesis or quadriparesis, CT or MRI may document cerebrovascular abnormalities. CT also should be performed in children with papilledema on an emergency basis before proceeding with the initial lumbar puncture. *Administration of antibiotics should not be delayed for diagnostic imaging in patients in whom bacterial meningitis is suspected.*

Subdural effusions should be treated with subdural paracentesis only when one suspects that the effusions are responsible for seizures or for prolonged fever as a result of subdural empyema. Paracentesis also may be useful if the effusion is responsible for symptoms of increased

intracranial pressure or is the cause of focal neurologic signs.[114,119] In most cases, subdural taps are not required.

Seizures should be carefully monitored particularly in patients with high bacterial counts in CSF (many organisms on Gram stain) but insufficient inflammation (few polymorphonuclear leukocytes in CSF) and, when noted, are treated expeditiously. A patent airway must be maintained, and appropriate anticonvulsants must be administered. Sodium phenobarbital (7 mg/kg loading dose) may be administered parenterally, followed by a maintenance dose of 5 mg/kg/day in two divided doses. If necessary, diazepam (≤0.2 mg/kg) or lorazepam (0.05 mg/kg/dose, 4 mg maximum) infused intravenously for 1 to 2 minutes may be used. If prolonged seizure control is needed, phenytoin (5 mg/kg/day) in two divided doses may be used. Phenytoin generally does not depress the respiratory center to the same extent as phenobarbital does, and it may benefit the patient by inhibiting the secretion of ADH. If the seizure activity no longer is apparent after the second hospital day and the patient has no focal neurologic signs at the time of discharge from the hospital, anticonvulsants may be discontinued. Phenytoin and phenobarbital can induce hepatic microsomal enzymes; their use may increase the metabolism rate of chloramphenicol and possibly cause a significant decrease in the serum concentration of this antibiotic if it has been used for treatment of meningitis.[288]

An electroencephalogram is indicated in patients with meningitis and seizures when focal seizures are noted, seizures persist more than 72 hours after presentation, seizures occur after the third day of hospitalization, a subdural effusion is noted, or prolonged alteration in sensorium is present. An electroencephalogram may be valuable in distinguishing abnormal intermittent posturing from movements associated with seizure activity.

Persistent fever (>8 or 9 days' duration) has been noted.[25] In the multicenter pneumococcal study, the mean duration of fever was 4.4 ± 3.9 days.[16] Suppurative complications, including subdural or pleural empyema, septic arthritis, and pericarditis, should be sought carefully. The rare occurrence of brain abscess in association with bacterial meningitis also may lead to persistent fever. Nosocomial intercurrent infection, usually viral, may cause prolonged fever in a child with meningitis. Complications of therapy, such as suppurative thrombophlebitis or a urinary tract infection after prolonged catheterization, are additional considerations. Persistent fever may be related to the severity of the infection. Poor therapeutic response (especially in multidrug-resistant organisms) occurs, and repeated lumbar puncture must be considered on an individual basis. Drug fever often is cited but rarely is the cause of persistent fever and remains a diagnosis of exclusion.

PROGNOSIS AND SEQUELAE

The prognosis in patients with bacterial meningitis depends on many factors: (1) the age of the patient, (2) the time course or progression of illness before antibiotic therapy is effective, (3) the specific microorganism causing the disease, (4) the number of organisms or the quantity of capsular polysaccharide material present in the meninges and CSF at the time of diagnosis, (5) the rapidity with which CSF is sterilized after initiation of antibiotic therapy, (6) the presence of disorders that may compromise host response to infection, and geographic location of the patient (low-income countries).[105,200,221,355]

The younger the patient and the greater the antigenic load at the time of admission, the worse the prognosis. Bacterial colony counts seem to be a more reliable indication of sequelae than is antigen concentration. Seizures, subdural effusions, bacteremia, and a more prolonged period of fever occur more frequently in children who have more than 10^7 CFU/mL of a particular organism in the CSF at the time of admission.[123] Children with colony counts equal to or greater than 10^7 CFU/mL also are significantly more likely to experience hearing loss and speech disturbance than children with meningitis but lower concentrations of bacteria within CSF specimens.[123] The concentration of TNF in serum has been associated with a fatal outcome in patients with meningococcal meningitis.[375] Elevated concentrations of IL-1β and TNF within the CSF of patients with bacterial meningitis also have been correlated significantly ($p < .002$) with a higher incidence of neurologic sequelae of disease.[260]

The mortality rate for bacterial meningitis in children who are beyond the neonatal period has been reduced to 1 to 5 percent. Although antibiotic therapy has reduced the mortality rate, up to 50 percent of the survivors of meningitis have some sequelae of disease.[102,105,119,144,347] Most studies from which estimates of sequelae have been derived have been retrospective, and the patients were enrolled for many years (1951-1968). Although antibiotic treatment of these individuals may have been relatively standardized during this period, ancillary methods employed in their care were not controlled.

Neurologic sequelae are common in survivors of meningitis and include hearing loss, cognitive impairment, and developmental delay.[105] For example, in 1991 the Metropolitan Atlanta Developmental Disabilities Surveillance Program identified bacterial meningitis as the leading postnatal cause of developmental disabilities, including cerebral palsy and mental retardation.[64] Hearing loss happens in 22 to 30 percent of survivors of pneumococcal meningitis compared with 1 to 8 percent after meningococcal meningitis.*

The frequency of complications of meningitis can be assessed most appropriately by prospective evaluation. In 1975, Sell[325] began a prospective study of 50 infants and children who recovered from *H. influenzae* meningitis. Fifty percent of this group were entirely normal, 9 percent were normal except for behavioral problems, and 28 percent had significant handicaps. The major handicaps noted included hearing loss (10% to 11%), language disorders or delayed language development (15%), impaired vision (2% to 4%), mental retardation (10% to 11%), motor abnormalities (3% to 7%), and seizures (2% to 8%).

Twenty-one postmeningitic children were paired with a sibling and tested by the Wechsler Intelligence Scale for Children. The mean IQ of the postmeningitic

*References 16, 101, 200, 362, 367, 376.

children was 86, and the mean IQ of control children was 97 (p <.05). Comparison of results for individual pairs revealed that 29 percent of postmeningitic children scored 1 full standard deviation below their siblings; no survivor had a score 1 standard deviation higher than a sibling.

Feldman and Michaels[121] reported that children who recovered from meningitis caused by *H. influenzae* and who were evaluated 10 to 12 years later maintained grades and scores comparable with those of their siblings as they progressed to middle school. Their academic success may require more school and family support to compensate for the minor differences in IQs that had been noted.

In 1995, Grimwood and colleagues[164] reported the results of a prospective cohort study of 158 meningitis survivors, 3 months to 14 years old, who were treated in a single center between 1983 and 1986. Between 1991 and 1993, 130 children (82% of the original cohort) were evaluated at a mean age of 8.4 years and a mean of 6.7 years after their meningitis. Results of blinded, audiologic, behavioral, neurologic, neuropsychologic, and sociodemographic assessments were compared with those of sex-matched and grade-matched control children. A systematic increase in the risk for abnormality or for poorer functioning was noted across all categories tested in children with meningitis versus control children. The differences reached statistical significance for tests of fine motor function, intelligence, neuropsychological function, school behavior, and auditory figure-ground differentiation.

Eleven children who had experienced meningitis (8.5% of the cohort studied) had major deficits (hydrocephalus, persistent seizures, spasticity, blindness, IQ <70, or profound hearing loss). Twenty-four (18.5%) of the survivors of meningitis and 14 (10.8%) of the control children had minor deficits (IQ of 70 to 80, inability to read, abnormalities in speech discrimination possibly referable to mild to moderate hearing loss, and school behavior problems). Overall, one in four of the children in this study had either a serious disabling sequela or a functionally important behavior disorder or neuropsychologic or auditory dysfunction that adversely affected academic performance.

This prospectively followed cohort was reevaluated 12 years post meningitis; impairment, although not severe, still persisted in the postmeningitic patients compared with controls.[14] After having meningitis, the children were "more than twice as likely as controls to require special educational assistance" and "took longer to complete tasks, made more errors, were less organized, and struggled within problem-solving situations." Decreased performance in language tasks and executive skills also were seen in children whose age was younger than 12 months at onset of meningitis.

A prospective study of bacterial meningitis in children revealed that 32.8 percent of children had abnormalities detectable on neurologic examination at the time of discharge, but by 5 years after discharge specific deficits were noted in only 11.1 percent of the total group. As a result of the onset of late seizures in some of these patients, the frequency of neurologic sequelae 15 years after discharge was 14 percent.[287] Specific complications or sequelae of meningitis in these patients are shown in Table 31-13. Shortly after discharge, hemiparesis or quadriparesis was noted in 30 patients (12.4% of the total group), but at 1 year after discharge, paralysis was noted in only 5 patients. These data reflect the tendency for even major neurologic defects to clear unpredictably with time. This important observation suggests the need to maintain cautious optimism in discussing long-term complications of meningitis with parents.

The mean IQ (±1 standard deviation) of the entire group of patients (235) after recovery was 94 (±23 standard deviations), with a range of 33 to 150. Twenty-nine children (17.3%) had IQs less than 80, and 22 (11.6%) had IQs less than 70. A comparison of these patients with their siblings and other control children revealed no significant difference in mean IQ. A significantly greater

TABLE 31-13 **Complications or Sequelae of Meningitis**

	Total	*H. influenzae*	*H. influenzae* (Ampicillin)	*H. influenzae* (Chloramphenicol)	*S. pneumoniae*	*N. meningitidis*
No.	235	151	90	61	35	26
Deaths <12 hr in hospital	4	4	3	1	0	0
Deaths >12 hr in hospital	1	0	0	0	0	1
Shock	8	6	5	1	1	1
Paralysis						
Early	30	18	7	11	7	3
Persistent	5	4	1	3	4	0
Persistent Tone	5	4	1	3	1	2
Ataxia						
Early	7	5	2	3	2	0
Persistent	1	1	0	1	0	0
Visual Problems	7	4	2	2	3	0
Clinically Significant Hearing Deficit	25	17	6	11	5	2
Hydrocephalus	1	1	1	0	0	0

proportion (p <.01) of children who recovered from meningitis had IQs less than 80 compared with children from control groups. These results differ from those of Sell.[325] Tejani and associates[356] also prospectively evaluated children who had recovered from bacterial meningitis using siblings as controls. They reported no significant differences in verbal performances or full-scale IQs between patients with meningitis and the sibling control population.

The prospective nature of these studies has permitted an assessment of factors that herald a poor prognosis and that may be discernible at or near the time of admission. Evidence of inappropriate secretion of ADH was correlated significantly (p <.01) with abnormal neurologic examinations at 3 months after discharge and with low IQs. The age of the child correlated inversely with the development of subdural effusion (p <.01), the occurrence of hearing deficits (p <.02), and low IQ (p <.05). The presence of focal neurologic findings in patients who were not postictal at the time of admission correlated significantly (p <.001) with abnormal neurologic examination, which was noted previously. Focal deficits indicating cerebral injury noted at admission or during the course of hospitalization were associated significantly (p <.001) with the development of late (1 to 15 years after discharge) afebrile seizures.[287] Focal neurologic findings at the time of admission proved to be a reliable predictor of permanent sequelae of bacterial meningitis. Focal deficits at admission also correlated significantly with low IQs (p <.001), even at 2 and 3 years after discharge from the hospital. The quantity of antigen in the initial CSF specimen and the number of organisms present also correlated significantly (p <.01) with sequelae of meningitis.[119] A prospective multicenter study of 180 children with pneumococcal meningitis between 1993 and 1996 revealed that 14 (7.7%) of 180 children died. No deaths were related to treatment failure by an antibiotic-resistant strain. Of the 166 surviving children, 41 (25%) developed motor defects and 48 (32%) of 151 children had moderate to severe unilateral or bilateral hearing loss. By CT or MRI of 103 patients, brain infarcts were noted in 39 (38%); subdural effusions in 25 (24%); hydrocephalus in 22 (21%); cerebritis in 12 (12%); and brain edema in 6 (6%).[16]

The outcome of patients with pneumococcal meningitis caused by penicillin-nonsusceptible or cefotaxime-nonsusceptible isolates has not differed from that caused by susceptible strains.[16,128,194] This finding is explained in part because vancomycin has been administered empirically to most children with suspected bacterial meningitis since the mid 1990s in the United States and in other parts of the world where treatment failures have been reported as a result of antibiotic-resistant *S. pneumoniae*.[274]

Meta-analysis of 90 published reports from 1980 to 2008 revealed that the most common types of major sequelae after bacterial meningitis were hearing loss (33.6%), followed by seizures (12.6%), motor deficit (11.6%), cognitive impairment (9.1%), hydrocephalus (7.1%), and visual disturbance (6.3%) (Table 31-14). The risk for at least one major sequela was almost three times greater in the World Health Organization African region (pooled risk estimate 25.1%) and southeast Asian region (21.6%) compared with the European region (9.4%).[105]

Other specific sequelae or complications of bacterial meningitis that have been observed include cranial nerve involvement, hemiparesis or quadriparesis, muscle hypertonia, ataxia, permanent seizure disorders, and development of obstructive hydrocephalus. Subdural effusions (as noted earlier) are so frequent in young children that they can be considered a part of the general disease process, rather than a persistent or troublesome complication of the meningeal infection. Development of brain abscess after bacterial meningitis is exceedingly rare[125]; when it is found, the possibility that it preceded the development of meningeal infection must be considered, and a careful search for other sites of infections such as endocarditis should be initiated.

Significant hearing loss after bacterial meningitis has been reported frequently. The mechanisms responsible for hearing deficits include spread of infection along the auditory canal and cochlear aqueduct, serous or purulent labyrinthitis, and, with time, replacement of the membranous labyrinth with fibrous tissue and new bone.[35,187,230,233,300] Damage to inner ear structures and subsequent hearing loss also is affected by the immune response of the host. TNF-α has been shown to produce cytolytic effects by the production of oxygen-free

TABLE 31-14 **Median Risk (%) of Sequelae After Bacterial Meningitis**

Sequelae	All Causes	*S. pneumoniae*	*H. influenzae*	*N. meningitidis*
At least one major sequelae	12.	24.7	9.5	7.2
Cognitive	1.1	3.1	1.0	0.4
Seizure	1.6	2.5	1.5	0.5
Hearing	4.3	6.7	3.2	2.1
Motor	1.5	3.3	1.2	0.8
Visual	0.8	1.1	0.1	2.1
At least one minor sequelae	8.6	18.6	5.7	2.3
Cognitive	2.2	3.6	2.4	0.9
Hearing	2.1	4.6	0.6	0.5
Motor	1.4	2.7	1.3	0.4
Visual	0.5	1.8	0.1	0.2
Behavioral	1.5	4.0	0.8	0.1

From a review of 90 published papers from 1980 to 2008 (Edmond K, Clark A, Korczak VS, et al. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:317–28.)

radicals. Animal studies have shown a decreased incidence of hearing loss when animals are treated with antibody blockade of TNF-α[13] and scavenging of oxygen-free radicals by antioxidant therapy.[206]

Deafness generally is noted early in the course of bacterial meningitis and is independent of the therapy provided.[101,189,258,325,372] Ataxia has been reported as a presenting sign of bacterial meningitis in children with hearing loss noted at a later date.[191,324] Presumably, the insult to the vestibular and auditory systems occurred concomitantly in these children. The early loss of hearing noted by several investigators suggests that hearing loss is *not* associated specifically with the use of a particular antimicrobial agent. Early diagnosis and treatment apparently do not prevent the development of deafness in many children who develop hearing loss as a consequence of bacterial meningitis.

Estimates of the frequency of hearing loss in retrospective studies vary from 2.4 to 29 percent.[101,105] Overall, 48 of 151 (32%) of the children with pneumococcal meningitis in the multicenter study had unilateral or bilateral moderate to severe hearing loss.[16] Occasionally, hearing loss noted early may improve over weeks to months.[300]

No correlation has been found between hearing loss and either the age of the patient at the onset of meningitis or the duration of illness before admission.[101] A significant correlation was noted between hearing loss and the presence of seizures before admission, the duration of fever in the hospital after therapy has been initiated (which presumably reflects more severe disease), treatment with antibiotics administered orally before a definitive diagnosis of bacterial meningitis was established, and a depressed CSF-to-blood glucose ratio at the time of admission.[101,193] Koomen and associates[212] developed a nomogram to predict the probability of development of hearing loss using five factors: (1) duration of symptoms before admission, (2) absence of petechiae, (3) CSF glucose level, (4) infection by *S. pneumoniae*, and (5) ataxia; they were thus able to identify effectively patients at risk for developing hearing loss with excellent sensitivity (100%) using a cutoff of 0 on the point scale.

Because hearing deficits occur so commonly in patients with bacterial meningitis, hearing evaluation by evoked response audiometry in young, uncooperative children is recommended routinely at the time of or shortly after discharge from the hospital. Repeated audiometric evaluation is recommended after discharge if the results of the initial examination are abnormal. Pure tone audiometry can be used for older, cooperative children. Differentiation of hearing deficits resulting from conductive disturbances from deficits related to damage to the eighth cranial nerve is important. Some children who have repetitive episodes of otitis media may experience conductive loss that is unrelated to the meningitis.

Systematic review of neurocognitive impairment in children after CNS infection revealed deficits in cognition and motor function to be very common.[59,105] Koomen and associates[213] reported one third of Dutch children with non–*H. influenzae* type b meningitis were found on follow-up to have academic or behavior limitations or both. Childhood meningitis survivors also were found to have a higher incidence of nonspecific symptoms, such as headaches, and significantly more symptoms of inattention, hyperactivity, and impulsiveness compared with nearest-age siblings.[34]

Only one study assessed the value of the Pediatric Risk of Mortality (PRISM) score in predicting outcomes of bacterial meningitis.[239] This study was done in a subgroup of children requiring mechanical ventilation. The best predictor of death and functional states on follow-up evaluation was the PRISM score. When the score was less than 20 within the first 24 hours of admission to the pediatric intensive care unit, a favorable outcome was noted in 82 percent. When the score was 20 or higher, a favorable outcome was noted in only 30 percent ($p < .009$).

CT has revealed evidence of cerebral infarction in children who had a diagnosis of bacterial meningitis established within 1 or 2 days of the onset of symptoms of a febrile illness.[339] In most of these cases, abnormalities in cerebrovascular dynamics (arteritis, thrombosis, thrombophlebitis), ventricular dilation, or both have been observed.

PREVENTION

Pneumococcal Infection

Chemoprophylaxis

Antibiotic chemoprophylaxis of children exposed to pneumococcal meningitis is not recommended regardless of immunization status.[10] In asplenic patients (functional and anatomic), daily chemoprophylaxis against pneumococcal infection is recommended with oral penicillin therapy.[10] The reader is referred to the CDC and AAP statements for the details of these recommendations.

Immunoprophylaxis

In a large randomized trial,[42] more than 37,000 infants received either the 7-valent pneumococcal conjugate vaccine (PCV7) containing polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to CRM_{197} or meningococcal serogroup C oligosaccharide conjugated to the same protein. Vaccines were administered at 2, 4, 6, and 12 to 15 months of age. The PCV7 was found to be 97.4 percent efficacious in preventing acquisition of invasive pneumococcal infections caused by vaccine serotype isolates. As a result of this study, in 2000, the PCV7 was recommended by the ACIP and the AAP Committee on Infectious Diseases for all children younger than 24 months of age to prevent invasive pneumococcal infections.[9,67]

In February 2010, a 13-valent pneumococcal conjugate vaccine (PCV13, Prevnar 13) was licensed by the FDA for prevention of invasive pneumococcal diseases caused by the 13 pneumococcal serotypes covered by the vaccine (PCV7 serotypes plus 1, 3, 5, 6A, 7F, and 19A) and for prevention of otitis media caused by serotypes in PCV7.[70,72] PCV13 is approved for use among children aged 6 weeks to 71 months and has replaced PCV7. Use of PCV13 is recommended by the ACIP for routine vaccination for all children aged 2 to 59 months, vaccination of children aged 60 to 71 months with underlying

medical conditions that increase their risk for pneumococcal disease or complications (e.g., chronic heart disease, chronic lung disease, diabetes mellitus, CSF leaks, cochlear implant, functional or anatomic asplenia, human immunodeficiency virus infection, chronic renal failure and nephrotic syndrome, diseases associated with treatment with immunosuppressive drugs or radiation therapy, congenital immunodeficiency), and children who previously received one or more doses of PCV7.[70,72]

Meningococcal Infection

Chemoprophylaxis

Chemoprophylaxis is recommended to all household members of a patient with meningococcal meningitis and in daycare and nursery school contacts, preferably within 24 hours of the diagnosis of the primary care.[12] Prophylaxis may be provided for individuals who had contact with the patient's oral secretions through kissing or sharing toothbrushes or eating utensils during the 7 days before onset of disease in the index child. Prophylaxis is not recommended routinely for health care personnel unless they have had close exposure through mouth-to-mouth resuscitation, intubation, or suctioning before antibiotic therapy was initiated. Schoolroom classmates and hospital contacts of patients usually are not given prophylactic treatment.

Minocycline and rifampin have proved to be 80 to 90 percent effective in eradicating carriage of meningococci.[169] Both drugs are secreted in the saliva in concentrations greater than the MICs for meningococci. The use of minocycline has been accompanied by frequent and significant vestibular reactions, even after a single dose of 100 mg, and generally should not be used.[60-62,388]

Rifampin is the drug of choice in most instances and can be used in a dose of 600 mg twice daily for four doses in adults and in doses of 10 mg/kg/dose twice daily for four doses in children 1 to 12 years of age. A dose of 5 mg/kg every 12 hours for four doses can be used in children 3 months to 1 year of age.[257] The emergence of rifampin-resistant strains in treated meningococcal carriers has been reported to occur with a frequency of 0 to 27 percent.[91,106,383]

A single intramuscular dose of ceftriaxone has proved to be an effective alternative to rifampin for prophylaxis in meningococcal contacts.[323] This approach to prophylaxis may be particularly useful in circumstances in which compliance with the use of oral rifampin is considered questionable. The efficacy of ceftriaxone has been confirmed for only group A strains, but its effect is likely to be similar for other serogroups. Ceftriaxone has the advantage of easier dosage and administration and safety in pregnancy.[12] Ceftriaxone may be given intramuscularly in a dose of 125 mg for individuals younger than 12 years old and in a dose of 250 mg for individuals 12 years and older.

Ciprofloxacin given to adults in a single oral dose of 500 mg has been effective in eradicating meningococcal carriage.[12] Ciprofloxacin presently is not recommended for individuals younger than 18 years old or for pregnant women.

Immunoprophylaxis

Because secondary cases may occur several weeks or more after onset of disease in the index case, meningococcal vaccine may be used as an adjunct to chemoprophylaxis when an outbreak is caused by a serogroup contained in the vaccine. The CDC has outlined recommendations for the administration of the meningococcal serogroup C vaccine to control outbreaks.[65]

A serogroup-specific quadrivalent polysaccharide meningococcal vaccine against group A, C, Y, and W135 *N. meningitidis* is approved for use in the United States in children 2 years old and older. The vaccine consists of 50 μg each of the respective purified bacterial polysaccharides. It may be given subcutaneously as a single 0.5-mL dose and can be given concurrently with other vaccines.

The FDA approved a quadrivalent meningococcal conjugate vaccine (MenACWY-D, Menactra) in January 2005 and a quadrivalent meningococcal conjugate vaccine (MenACWY-CRM, Menveo) in February 2010.[69,71] Because of the conjugate vaccine's ability to induce a T-cell–dependent immune response and subsequently longer immunologic memory compared with polysaccharide vaccines, it is advantageous. Both vaccines include serotypes A, C, Y, and W-135 and were initially licensed for use for persons aged 11 to 55 years. The ACIP recommended that persons aged 2 through 55 years at increased risk for meningococcal diseases and all adolescents aged 11 through 18 years be immunized with meningococcal conjugate vaccine.

As of January 2011, the FDA lowered the approval age range for use of both vaccines to include persons aged 2 through 55 years. MenACWY-D is also licensed as a two-dose series for children aged 9 through 23 months (see later). The ACIP recommended vaccination with meningococcal conjugate vaccine for children aged 2 through 10 years at increased risk for meningococcal disease. A two-dose primary series is recommended for children with terminal complement deficiencies (e.g., C5-C9, properdin, factor H, or factor D deficiencies) or anatomic or functional asplenia. A single primary dose is recommended for children with increased risk for disease if they are travelers to or residents of countries in which meningococcal disease is hyperendemic or epidemic (e.g., the meningococcal belt of sub-Saharan Africa). No meningococcal vaccine is recommended in children aged 2 through 10 years with no increased risk for meningococcal disease. The ACIP also recommended a single booster dose of meningococcal conjugate vaccine for adolescents who received a previous dose before age 16 years. For persons aged 2 through 55 years at increased risk for meningococcal disease (i.e., persons with asplenia or terminal complement deficiencies or laboratory workers who work with *N. meningitidis*), a booster dose is recommended if they remain at increased risk.

In April 2011, the FDA approved the use of MenACWY-D as a two-dose primary series for children aged 9 months through 23 months at high risk for invasive meningococcal disease (Table 31-15).[71]

Similar to other conjugate vaccines, meningococcal C conjugate vaccines are more immunogenic than the pure

TABLE 31-15 **Summary of MenACWY-D Recommendation for Children Aged 9 Months Through 23 Months at High Risk for Invasive Meningococcal Disease**

Risk Group	Primary Series	Booster Dose
Children aged 9 through 23 months at high risk for invasive meningococcal disease (except children with functional or anatomic asplenia)*	Two doses, 3 months apart Catch-up dose if the second is not received on schedule: at the earliest opportunity	Initial booster 3 years after completing the primary series if person remains at increased risk Continued boosters at 5-year intervals after the initial booster if person remains at increased risk
Children at high risk for invasive meningococcal disease with functional or anatomic asplenia	Two doses, 2 months apart, beginning at age 2 years and ≥4 weeks after completion of PCV13 vaccine series	Initial booster 3 years after completing the primary series if person remains at increased risk Continued boosters at 5-year intervals after the initial booster if person remains at increased risk

*Children who have persistent complement component deficiencies (e.g., C5-C9, properdin, factor H, or factor D), children who are traveling to or residents of countries where meningococcal disease is hyperendemic or epidemic, and children who are in a defined risk group during a community or institutional outbreak.

polysaccharide vaccine in infants younger than 2 years of age.[237] Because of a high incidence of meningococcal disease in the United Kingdom, a meningococcal serogroup C-CRM_{197} was administered in a phased program beginning in November 1999 to all children younger than 18 years of age.[295] Surveillance studies to assess short-term efficacy have shown a 97 percent efficacy for teenagers and a 92 percent efficacy for toddlers in the prevention of meningococcal serogroup C infection.

An effective serogroup B meningococcal vaccine has not been produced to date. The polysaccharide capsule cross reacts with fetal neural tissues, causing autoantibodies. Different strategies have been tried to develop a safe vaccine against serogroup B meningococcus, including use of outer membrane proteins (OMP) and lipopolysaccharide components. Reverse vaccinology was used to develop a serogroup B meningococcal vaccine using the genome from the strain MC58,[22] which resulted in a vaccine formulation of the five genome-derived neisserial antigens (GNAs) with factor H binding protein (GNA1870), neisserial adhesin A (NadA or GNA1994), neisserial heparin-binding antigen (GNA2132), GNA2091, and GNA1030. This vaccine (Bexsero) has been shown to induce immune responses for all age groups including infants and offers broader coverage of serogroup B meningococcal isolates; results from additional phase III trials are pending.[22]

Haemophilus influenzae Meningitis

Chemoprophylaxis

The most comprehensive study concerning the spread of *H. influenzae* type b infection among household contacts was coordinated by the CDC.[378] Data collected from 19 states were analyzed prospectively. *H. influenzae* meningitis was reported in 1403 patients. Eighty-two percent of exposed families were investigated for the occurrence of *H. influenzae* disease within 30 days of its onset in the index patient. Systemic disease caused by *H. influenzae* type b developed in 9 of 1687 contacts (0.5%) who were younger than age 6 years. The risk for infection in patients younger than age 4 years was 2.1 percent; the risk in children younger than age 1 year was 6 percent. The risk for secondary infection of household contacts in the 30 days after onset of meningitis in the index case was 585 times greater than the age-adjusted risk in the general population and was similar to the risk for secondary meningococcal disease in household contacts. This nationwide study provided an important impetus for finding a chemoprophylactic regimen that could prevent secondary infection in household contacts.

A nationwide, collaborative, placebo-controlled trial was conducted subsequently among household (children <6 years old) and daycare center contacts of individuals with invasive *H. influenzae* type b disease.[28] Four of 765 placebo-treated contacts experienced secondary disease versus none of 1112 rifampin-treated contacts ($p = .027$).

The AAP Committee on Infectious Diseases recommends that rifampin be provided orally once a day for 4 days in a dose of 20 mg/kg (maximum dose 600 mg/day) to all household contacts (children and adults) regardless of age in households with at least one unvaccinated contact younger than 4 years.[11] The dose for infants younger than 1 month is not established, but it may be reduced to 10 mg/kg. Rifampin prophylaxis is not required, however, when all of the household contacts younger than 4 years have been fully immunized. All members of households with a fully immunized but immunocompromised child, regardless of age, should receive rifampin because of concern that the immunization may not have been effective.

When two or more cases of invasive disease have occurred within 60 days and unimmunized or incompletely immunized children attend a daycare facility, rifampin should be administered to all personnel and attendees. When a single case has been reported, the use of rifampin is controversial, and many experts recommend *no* prophylaxis.[11] Unimmunized or incompletely immunized children should receive a dose of vaccine and should be scheduled for completion of the recommended age-specific immunization schedule.

No data document the safety of rifampin administered during pregnancy. Prophylaxis with rifampin is not

recommended for pregnant women who are contacts of infected infants.

Patients receiving rifampin should be advised routinely that their urine, sweat, and tears will be stained orange. Individuals should be advised to refrain from using contact lenses while receiving rifampin therapy because the lenses may be stained permanently.

Immunoprophylaxis

The incidence of *H. influenzae* type b invasive disease and meningitis decreased dramatically after the introduction of vaccines that initially were found to be effective at 15 months of age[8] and then later found to be effective at 2 months of age.[321] Currently, three conjugate vaccines are approved for infants beginning at 2 months of age: HbOC, PRP-OMP, and PRP conjugated to tetanus toxoid (PRP-T). The ACIP and the AAP recommend universal infant immunization with HbOC or PRP-T at 2.4 and 6 months of age or PRP-OMP at 2 and 4 months of age.[19,63]

Passive immunization of infants also has been studied with use of bacterial polysaccharide immunoglobulin.[313] This preparation given in a single intramuscular dose of 0.5 mL/kg provides significant protection for infants from *H. influenzae* type b disease for 3 or 4 months.

Acknowledgment

The author acknowledges the contribution of Ralph D. Feigin in the previous editions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

22. Bai X, Findlow J, Borrow R. Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles. *Expert Opin Biol Ther* 2011;**11**:969–85.
29. Banerjee A, Kim BJ, Carmona EM, et al. Bacterial pili exploit integrin machinery to promote immune activation and efficient blood-brain barrier penetration. *Nat Commun* 2011;**2**:139–51.
54. Brouwer MC, de Gans J, Heckenberg SGB, et al. Host genetic susceptibility to pneumococcal and meningococcal disease: a systematic review and a meta-analysis. *Lancet Infect Dis* 2009;**9**: 31–44.
70. Centers for Disease Control and Prevention. Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendation for use among children—Advisory Committee on Immunization Practice (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2010;**50**:258–61.
71. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391–2.
72. Centers for Disease Control and Prevention. Invasive pneumococcal disease and 13-valent pneumococcal conjugate vaccine (PCV13) coverage among childrens aged ≤ 59 months—selected U.S. regions, 2010-2011. *MMWR Morb Mortal Wkly Rep* 2011; **60**:1477–81.
76. Chiba N, Murayama SY, Morozumi M, et al. Rapid detection of eight causative pathogens for the diagnosis of bacterial meningitis by real-time PCR. *J Infect Chemother* 2009;**15**:92–8.
83. Coureuil M, Mikaty G, Miller F, et al. Meningococcal type IV pili recruit the polarity complex to cross the brain endothelium. *Science* 2009;**325**:83–7.
85. Curtis S, Stobart K, Vandermeer B, et al. Clinical features suggestive of meningitis in children: a systematic review of prospective data. *Pediatrics* 2010;**126**:952–60.
87. Darton TC, Guiver M, Naylor S, et al. Bacterial genomic detection within cerebrospinal fluid of patients with meningococcal disease is influenced by microbial and host characteristics. *Clin Infect Dis* 2011;**53**:463–7.
94. Dejsirilert S, Tienkrim S, Ubonyaem N, et al. National antimicrobial resistance surveillance among clinical isolates of *Streptococcus pneumoniae* in Thailand. *J Med Assoc Thai Suppl* 2009;**4**:S19–33.
103. Dubos F, De La Rocque F, Levy C, et al. Sensitivity of the bacterial meningitis score in 889 children with bacterial meningitis. *J Pediatr* 2008;**152**:378–82.
104. Dubos F, Martinet A, Gendrel D, et al. Clinical decision rules for evaluating meningitis in children. *Curr Opin Neurol* 2009; **22**:288–93.
105. Edmond K, Clark A, Korczak VS, et al. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:317–28.
108. Elston JW, Santaniello-Newton A, Meigh JA, et al. Increasing incidence of invasive pneumococcal disease and pneumonia despite improved vaccination uptake: surveillance in Hull and East Yorkshire, UK, 2002-2009. *Epidemiol Infect* 2012;**140**:1252–66.
129. Fitch MT, Abrahamian FM, Moran GJ, Talan DA. Emergency department management of meningitis and encephalitis. *Infect Dis Clin North Am* 2008;**22**:33–52.
149. Gerber J, Nau R. Mechanisms of injury in bacterial meningitis. *Curr Opin Neurol* 2010;**23**:312–8.
151. Gerber J, Tauber SC, Armbrecht I, et al. Increased neuronal proliferation in human bacterial meningitis. *Neurology* 2009;**73**: 1026–32.
181. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009;**360**:244–56.
199. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. *Nat Rev Microbiol* 2008;**6**:625–34.
200. Kim KS. Acute bacterial meningitis in infants and children. *Lancet Infect Dis* 2010;**10**:32–42.
210. Koedel U, Klein M, Pfister H. New understandings on the pathophysiology of bacterial meningitis. *Curr Opin Infect Dis* 2010;**23**:217–23.
236. Mackenzie GA, Leach AJ, Carapetis JR, et al. Epidemiology of naropharyngeal carriage of respiratory pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disdease. *BMC Infect Dis* 2010;**10**:304.
238. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* diseases—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.
280. Peltola H, Roine I, Fernandez J, et al. Hearing impairment in childhood bacterial meningitis is little relieved by dexamethasone or glycerol. *Pediatrics* 2010;**125**:e1–e8.
283. Picard C, Casanova JL, Puel A. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. *Clin Microbiol Rev* 2011;**24**:490–7.
302. Rosen JB, Thomas AR, Lexau CA, et al. Geographical variation in invasive pneumococcal disease following pneumococcal conjugate vaccine introduction in the United States. *Clin Infect Dis* 2011;**53**:137–43.
351. Tan LKK, Carlon GM, Borrow R. Advances in the development of vaccines against *Neisseria meningitidis*. *N Engl J Med* 2010; **362**:1511–20.
358. Thigpen MC, Whitney CG, Messonnier NE. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011;**364**: 2016–25.
361. Tsai CJ, Griffin MR, Pekka Nuorti J, Grijalva CG. Changing epidemiology of pneumococcal meningitis after the introduction of pneumococcal conjugate vaccine in the United States. *Clin Infect Dis* 2008;**46**:1664–72.
373. Viola GM. Extreme hypoglycorrhachia: not always bacterial meningitis. *Nat Rev Neurol* 2010;**6**:637–41.
374. Von Bernuth H, Picard C, Jin Z, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 2008;**321**:691–6.
377. Waiserbourd-Zinman O, Bilavsky E, Tirosh N, et al. Penicillin and ceftriaxone susceptibility of *Streptococcus pneumoniae* isolated from cerebrospinal fluid of children with meningitis hospitalized in a tertiary hospital in Israel. *Isr Med Assoc J* 2010;**12**:225–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Achtman M. Molecular epidemiology of epidemic bacterial meningitis. *Rev Med Microbiol* 1990;**1**:29–38.
2. Achtman M. Clonal properties of meningococci from epidemic meningitis. *Trans R Soc Trop Med Hyg* 1991;**85** (Suppl.):24–31.
3. Adair CV, Gould RL, Smadel JE. Aseptic meningitis, a disease of diverse etiology: clinical and etiologic studies on 854 cases. *Ann Intern Med* 1953;**39**:675–704.
4. Adams RD, Kubik CS, Bonner FJ. The clinical and pathological aspects of influenzal meningitis. *Arch Pediatr* 1948;**65**:354–76.
5. Adams WG, Deaver KA, Cochi SL, et al. Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. *JAMA* 1993;**269**:221–6.
6. Adegnola R, Secka O, Lahai G, et al. Elimination of *Haemophilus influenzae* type b (Hib) disease from The Gambia after introduction of routine immunization with Hib conjugate vaccine: a prospective study. *Lancet* 2005;**366**:144–50.
7. Allbritton WL, Wiggins GL, Feeley JC. Neonatal listerioses: distribution of serotypes in relation to age at onset of disease. *J Pediatr* 1976;**88**:481–3.
8. American Academy of Pediatrics. *Haemophilus influenzae* type b conjugate vaccines: Recommendations for immunization of infants and children 2 months of age and older: Update. *Pediatrics* 1991; **88**:169–72.
9. American Academy of Pediatrics Committee on Infectious Diseases. Policy Statement: Recommendations for the prevention of pneumococcal infections, including the use of pneumococcal conjugate vaccine (Prevnar), pneumococcal polysaccharide vaccine, and antibiotic prophylaxis. *Pediatrics* 2000;**106**:362–6.
10. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, editor. *2006 Red Book: Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 525–37.
11. American Academy of Pediatrics. *Haemophilus influenzae* infections. In: Pickering LK, editor. *2006 Red Book: Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 310–18.
12. American Academy of Pediatrics. Meningococcal infections. In: Pickering LK, editor. *2006 Red Book: Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 452–60.
13. Aminpour S, Tinling S, Brodie H. Role of tumor necrosis factor-α in sensorineural hearing loss after bacterial meningitis. *Otol Neurotol* 2005;**26**:602–9.
14. Anderson V, Anderson A, Grimwood K, et al. Cognitive and executive function 12 years after childhood bacterial meningitis: effect of acute neurologic complications and age of onset. *J Pediatr Psychol* 2004;**29**:67–81.
15. Arditi M, Herold BC, Yogev R. Cefuroxime treatment failure and *Haemophilus influenzae* meningitis: case report and review of the literature. *Pediatrics* 1989;**84**:132–5.
16. Arditi M, Mason Jr E, Bradley J, et al. Three-year multicenter surveillance of pneumococcal meningitis in children: clinical characteristics, and outcome related to penicillin susceptibility and dexamethasone use. *Pediatrics* 1998;**102**:1087–97.
17. Aronoff SC, Reed MO, O'Brien CA, et al. Comparison of the efficacy and safety of ceftriaxone to ampicillin/chloramphenicol in the treatment of childhood meningitis. *Antimicrob Agents Chemother* 1984;**13**:143–51.
18. Ashwal S, Tomasi L, Schneider S, et al. Bacterial meningitis in children: pathophysiology and treatment. *Neurology* 1992;**42**: 739–48.
19. Asmar BI, Dajani AS. Concurrent pneumococcal disease in two siblings. *Am J Dis Child* 1982;**136**:946–7.
20. Attia J, Hatala R, Cook DJ, Wong JG. The rational clinical examination. Does this adult patient have acute meningitis? *JAMA* 1999;**282**:175–81.
21. Auburtin M, Wolff M, Charpentier J, et al. Detrimental role of delayed antibiotic administration and penicillin-nonsusceptible strains in adult intensive care unit patients with pneumococcal meningitis: the PNEUMOREA prospective multicenter study. *Crit Care Med* 2006;**34**:2758–65.
22. Bai X, Findlow J, Borrow R. Recombinant protein meningococcal serogroup B vaccine combined with outer membrane vesicles. *Expert Opin Biol Ther* 2011;**11**:969–85.
23. Baker CJ, Barrett FF. Transmission of group B streptococci among parturient women and their neonates. *J Pediatr* 1972; **83**:919–25.
24. Baker RC, Bausher JD. Meningitis complicating acute bacteremic facial cellulitis. *Pediatr Infect Dis* 1986;**5**:421–3.
25. Balagtas RC, Levin S, Nelson KE, et al. Secondary and prolonged fevers in bacterial meningitis. *J Pediatr* 1970;**77**:957–64.
26. Baldwin LN, Henderson A, Thomas P, et al. Acute bacterial meningitis in young adults mistaken for substance abuse. *BMJ* 1993;**306**:775–6.
27. Ballabh P, Braun A, Nedergaard M. The blood-brain barrier: an overview: structure, regulation, and clinical implications. *Neurobiol Dis* 2004;**16**:1–13.
28. Band J, Fraser DW, Ajello G. Prevention of *Hemophilus influenzae* type b disease. *JAMA* 1984;**25**:2381–6.
29. Banerjee A, Kim BJ, Carmona EM, et al. Bacterial pili exploit integrin machinery to promote immune activation and efficient blood-brain barrier penetration. *Nat Commun* 2011;**2**:139–51.
30. Barenkamp SJ, Granoff DM, Munson Jr RS. Outer-membrane protein subtypes of *Haemophilus influenzae* type b and spread of disease in day-care centers. *J Infect Dis* 1981;**144**:210–7.
31. Barson WJ, Miller MA, Brady MT, et al. Prospective comparative trial of ceftriaxone versus conventional therapy for treatment of bacterial meningitis in children. *Pediatr Infect Dis* 1985;**4**: 362–8.
32. Bashir H, Laundy M, Booy R. Diagnosis and treatment of bacterial meningitis. *Arch Dis Child* 2003;**88**:614–20.
33. Beckman JS, Beckman TW, Chen J, et al. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proc Natl Acad Sci USA* 1990;**87:**1620–4.
34. Berg S, Trollfors B, Hugosson S, et al. Long-term follow-up of children with bacterial meningitis with emphasis on behavioral characteristics. *Eur J Pediatr* 2002;**161**:330–6.
35. Berlow SJ, Cakdarelli DD, Matz GJ, et al. Bacterial meningitis: a prospective investigation. *Laryngoscope* 1980;**90**:1445–52.
36. Berman BW, King Jr FH, Rubenstein DS, et al. *Bacteroides fragilis* meningitis in a neonate successfully treated with metronidazole. *J Pediatr* 1078;**93**:793–5.
37. Bermpohl D, Halle A, Freyer D, et al. Bacterial programmed cell death of cerebral endothelial cells involves dual death pathways. *J Clin Invest* 2005;**115**:1607–15.
38. Bernhardt LL, Semberkoff MS, Rahal Jr JJ. Deficient cerebrospinal fluid opsonization in experimental *Escherichia coli* meningitis. *Infect Immun* 1981;**32**:411–13.
39. Bifrare Y, Gianinazzi C, Imboden H, et al. Bacterial meningitis causes two distinct forms of cellular damage in the hippocampal dentate gyrus in infant rats. *Hippocampus* 2003;**13**:481–8.
40. Bijilmer HA. World-wide epidemiology of *Haemophilus influenzae* meningitis: industrialized versus non-industrialized countries. *Vaccine* 1991;**9**(Suppl.):S5–9.
41. Bingen E, Lambert-Zechovsky N, Mariani-Kurkdjian P, et al. Bacterial counts in cerebrospinal fluid of children with meningitis. *Eur J Clin Microbiol Infect Dis* 1990;**9**:278–81.
42. Black S, Shinfield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal vaccine in children. *Pediatr Infect Dis J* 2000;**19**:187–95.
43. Blazer S, Berant M, Alon U. Bacterial meningitis: effect of antibiotic treatment on cerebrospinal fluid. *Am J Clin Pathol* 1983;**80**:386–7.
44. Bogaert D, de Groot R, Hermans PWM. *Streptococcus pneumoniae* colonization: the key to pneumococcal disease. *Lancet Infect Dis* 2004;**4**:144–54.
45. Bohr V, Hansen B, Jessen O, et al. Eight hundred and seventy-five cases of bacterial meningitis, part I: Clinical data, prognosis, and the role of specialized hospital departments. *J Infect* 1983;**7**: 21–30.
46. Bottcher T, Ren H, Goiny M, et al. Clindamycin is neuroprotective in experimental *Streptococcus pneumoniae* meningitis compared with ceftriaxone. *J Neurochem* 2004;**91**:1450–60.
47. Bradley JS, Ching DK, Phillips SE. Outpatient therapy of serious pediatric infections with ceftriaxone. *Pediatr Infect Dis J* 1988;**7**: 160–4.
48. Bradley JS, Conner JD. Ceftriaxone failure in meningitis caused by *Streptococcus pneumoniae* with reduced susceptibility to beta-lactam antibiotics. *Pediatr Infect Dis J* 1991;**10**:871–3.

49. Brady MT, Kaplan SL, Taber LH. Association between persistence of pneumococcal meningitis and dexamethasone administration. *J Pediatr* 1981;**99**:924–6.
50. Brandt C, Lundgren J, Frimolt-Moller N, et al. Blocking of leukocyte accumulation in the cerebrospinal fluid augments bacteremia and increased lethality in experimental pneumococcal meningitis. *J Neuroimmunol* 2005;**166**:126–31.
51. Braun JS, Tuomanen EI. Molecular mechanisms of brain damage in bacterial meningitis. *Adv Pediatr Infect Dis* 1999;**14**:49–71.
52. Breiman RF, Butler JC, Tenover FC, et al. Emergence of drug-resistant pneumococcal infections in the United States. *JAMA* 1994;**271**:1831–5.
53. Brenneman G, Silimperi D, Ward J. Recurrent invasive *Haemophilus influenzae* type b disease in Alaskan natives. *Pediatr Infect Dis J* 1987;**6**:388–92.
54. Brouwer MC, de Gans J, Heckenberg SGB, et al. Host genetic susceptibility to pneumococcal and meningococcal disease: a systematic review and a meta-analysis. *Lancet Infect Dis* 2009;**9**: 31–44.
55. Bryan JP, Rocha H, da Silva HR, et al. Comparison of ceftriaxone and ampicillin plus chloramphenicol for the therapy of acute bacterial meningitis. *Antimicrob Agents Chemother* 1985;**28**:361–8.
56. Burroughs M, Cabellos C, Prasad S, et al. Bacterial components and the pathophysiology of injury to the blood-brain barrier: does cell wall add to the effects of endotoxin in gram-negative meningitis? *J Infect Dis* 1992;**165**:S82–5.
57. Butler JC, Breiman RF, Lipman HB. Serotype distribution of *Streptococcus pneumoniae* infections among preschool children in the United States. 1978-1994: implications for development of conjugate vaccine. *J Infect Dis* 1995;**171**:885–9.
58. Cable D, Overturf G, Edralin G. Concentrations of cefoperazone in cerebrospinal fluid during bacterial meningitis. *Antimicrob Agents Chemother* 1983;**23**:688–91.
59. Carter J, Neville B, Newton C. Neuro-cognitive impairment following acquired central nervous system infections in childhood: a systematic review. *Brain Res Rev* 2003;**43**:57–69.
60. Centers for Disease Control. Vestibular reactions to minocycline after meningococcal prophylaxis—New Jersey. *MMWR Morb Mortal Wkly Rep* 1975;**24**:9–11.
61. Centers for Disease Control. Vestibular reactions to minocycline followup—Georgia, New York, Vermont. *MMWR Morb Mortal Wkly Rep* 1975;**24**:55–6.
62. Centers for Disease Control. Bacterial meningitis and meningococcemia: United States—1978. *MMWR Morb Mortal Wkly Rep* 1979;**28**:277–8.
63. Centers for Disease Control. *Haemophilus* b conjugate vaccines for prevention of *Haemophilus influenzae* type b disease among infants and children two months of age and older: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Morb Mortal Wkly Rep* 1991;**40**:1–7.
64. Centers for Disease Control and Prevention. Postnatal causes of developmental disabilities in children aged 3-10 years, Atlanta, Georgia, 1991. *MMWR Morb Mortal Wkly Rep* 1996;**45**:130–34.
65. Centers for Disease Control and Prevention. Control and prevention of meningococcal disease and control and prevention of serogroup C meningococcal disease: evaluation and management of suspected outbreaks. *MMWR Morb Mortal Wkly Rep* 1997;**46**(RR-5):1–21.
66. Centers for Disease Control and Prevention. Progress toward eliminating *Haemophilus influenzae* type b disease among infants and children—United States, 1987-1997. *MMWR Morb Mortal Wkly Rep* 1998;**47**:993–8.
67. Centers for Disease Control and Prevention. Recommendations of the Advisory Committee on Immunization Practices: preventing pneumococcal disease among infants and young children. *MMWR Morb Mortal Wkly Rep* 2000;**49**(RR-9):1–35.
68. Centers for Disease Control and Prevention. Progress toward elimination of *Haemophilus influenzae* type b invasive disease among infants and children—United States 1998-2000. *MMWR Morb Mortal Wkly Rep* 2002;**51**:234–7.
69. Centers for Disease Control and Prevention. Prevention and control of meningococcal disease recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2005;**54**(RR-7):1–17.
70. Centers for Disease Control and Prevention. Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendation for use among children—Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2010;**50**:258–61.
71. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391–2.
72. Centers for Disease Control and Prevention. Invasive pneumococcal disease and 13-valent pneumococcal conjugate vaccine (PCV13) coverage among children aged ≤ 59 months—selected U.S. regions, 2010-2011. *MMWR Morb Mortal Wkly Rep* 2011; **60**:1477–81.
73. Chang CJ, Chang WN, Huang LT, et al. Cerebral infarction in perinatal and childhood bacterial meningitis. *Q J Med* 2003; **96**:755–62.
74. Chang CJ, Chang WN, Huang LT, et al. Bacterial meningitis in infants: the epidemiology, clinical features, and prognostic factors. *Brain Dev* 2004;**26**:168–75.
75. Cherry JD, Sheenan CP. Bacteriologic relapse in *Haemophilus influenzae* meningitis: inadequate ampicillin therapy. *N Engl J Med* 1968;**278**:1001–3.
76. Chiba N, Murayama SY, Morozumi M, et al. Rapid detection of eight causative pathogens for the diagnosis of bacterial meningitis by real-time PCR. *J Infect Chemother* 2009;**15**:92–8.
77. Cole FS, Saryan JA, Smith AL. The risk of additional systemic bacterial illness in infants with systemic *Streptococcus pneumoniae* disease. *J Pediatr* 1981;**99**:91–4.
78. Coleman SJ, Auld EB, Connor JD, et al. Relapse of *Hemophilus influenzae* type b meningitis during intravenous therapy with ampicillin. *J Pediatr* 1969;**74**:781–4.
79. Colville A, Davies W, Heneghan M, et al. A rare complication of dental treatment: *Streptococcus oralis* meningitis. *Br Dent J* 1993;**175**:133–4.
80. Committee on Infectious Diseases of the American Academy of Pediatrics. Therapy for children with invasive pneumococcal infections. *Pediatrics* 1997;**99**:289–99.
81. Congeni BL. Comparison of ceftriaxone and traditional therapy of bacterial meningitis. *Antimicrob Agents Chemother* 1984;**25**: 40–4.
82. Corless CE, Guiver M, Borrow R, et al. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J Clin Microbiol* 2001;**39**:1553–58.
83. Coureuil M, Mikaty G, Miller F, et al. Meningococal type IV pili recruit the polarity complex to cross the brain endothelium. *Science* 2009;**325**:83–7.
84. Coulehan JL, Michaels RH, Hallowell C, et al. Epidemiology of *Haemophilus influenzae* type b disease among Navajo Indians. *Public Health Rep* 1984;**99**:404–9.
85. Curtis S, Stobart K, Vandermeer B, et al. Clinical features suggestive of meningitis in children: a systematic review of prospective data. *Pediatrics* 2010;**126**:952–60.
86. Daoud AS, Batieha A, Al-Sheyyab M, et al. Lack of effectiveness of dexamethasone in neonatal bacterial meningitis. *Eur J Pediatr* 1999;**158**:230–3.
87. Darton TC, Guiver M, Naylor S, et al. Bacterial genomic detection within cerebrospinal fluid of patients with meningococcal disease is influenced by microbial and host characteristics. *Clin Infect Dis* 2011;**53**:463–7.
88. Daum RS, Silber GR, Kamon JS, et al. Evaluation of a commercial latex particle agglutination test for rapid diagnosis of *Haemophilus influenzae* type b infection. *Pediatrics* 1982;**69**:466–71.
89. Davey PG, Cruikshank JK, McManus IC, et al. Bacterial meningitis—ten years' experience. *J Hyg* 1982;**88**:383–401.
90. Davison K, Ramsay M. The epidemiology of acute meningitis in England and Wales. *Arch Dis Child* 2003;**88**:662–4.
91. Deal WB, Sanders E. Efficacy of rifampin in treatment of meningococcal carriers. *N Engl J Med* 1969;**281**:641–5.
92. De Gans J, van de Beek D. European Dexamethasone in Adulthood Bacterial Meningitis Study Investigators. Dexamethasone in adults with bacterial meningitis. *N Engl J Med* 2002;**347**:1549–56.
93. Deivanayagam N, Ashaok TP, Nedunchelian K, et al. Evaluation of CSF variables as a diagnostic test for bacterial meningitis. *J Trop Pediatr* 1993;**39**:284–7.

94. Dejsirilert S, Tienkrim S, Ubonyaem N, et al. National antimicrobial resistance surveillance among clinical isolates of *Streptococcus pneumoniae* in Thailand. *J Med Assoc Thai Suppl* 2009;**4**:S19–33.
95. Del Rio M, Chrane D, Shelton S, et al. Ceftriaxone versus ampicillin and chloramphenicol for treatment of bacterial meningitis in children. *Lancet* 1983;**1**:1241–4.
96. De Niraus JS, Munford RS, Rise JN, et al. Epidemic disease due to serogroup L *Neisseria meningitidis* in San Paulo, Brazil. *J Infect Dis* 1974;**129**:568–71.
97. Devoe IW, Gilchrist JE. Pili on meningococci from primary cultures of nasopharyngeal carriers and cerebrospinal fluid of infants with acute disease. *J Exp Med* 1975;**141**:297–305.
98. Diab A, Zhu J, Lindquist L, et al. *Haemophilus influenzae* and *Streptococcus pneumoniae* induce different intracerebral mRNA cytokine patterns during the course of experimental bacterial meningitis. *Clin Exp Immunol* 1997;**109**:233–41.
99. Dicuonzo G, Lorino G, Lilli D, et al. Use of oligoprobes on amplified DNA in the diagnosis of bacterial meningitis. *Eur J Clin Microbiol Infect Dis* 1999;**18**:352–7.
100. Di Pentima MC, Mason Jr EO, Kaplan SL. In vitro antibiotic synergy against *Flavobacterium meningosepticum:* implications for therapeutic options. *Clin Infect Dis* 1998;**26**:1169–76.
101. Dodge PR, Davis H, Feigin RD, et al. Prospective evaluation of hearing impairment as a sequel of acute bacterial meningitis. *N Engl J Med* 1994;**311**:869–74.
102. Dodge PR, Swartz MN. Bacterial meningitis: a review of selected aspects, II: Special neurologic problems, postmeningitic complications and clinicopathological correlations. *N Engl J Med* 1965;**272**:1003–10.
103. Dubos F, De La Rocque F, Levy C, et al. Sensitivity of the bacterial meningitis score in 889 children with bacterial meningitis. *J Pediatr* 2008;**152**:378–82.
104. Dubos F, Martinet A, Gendrel D, et al. Clinical decision rules for evaluating meningitis in children. *Curr Opin Neurol* 2009;**22**: 288–93.
105. Edmond K, Clark A, Korczak VS, et al. Global and regional risk of disabling sequelae from bacterial meningitis: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:317–28.
106. Eickhoff TC. In-vitro and in-vivo studies of resistance to rifampin in meningococci. *J Infect Dis* 1971;**123**:414–20.
107. Ellison III RT, Kohler PF, Curd JG, et al. Prevalence of congenital or acquired complement deficiency in patients with sporadic meningococcal disease. *N Engl J Med* 1983;**308**:913–6.
108. Elston JW, Santaniello-Newton A, Meigh JA, et al. Increasing incidence of invasive pneumococcal disease and pneumonia despite improved vaccination uptake: surveillance in Hull and East Yorkshire, UK, 2002-2009. *Epidemiol Infect* 2012;**140**:1252–66.
109. Eraklis AJ, Kevy SV, Diamond LK, et al. Hazard of overwhelming infection after splenectomy in childhood. *N Engl J Med* 1967; **276**:1225–9.
110. Ernst JD, Decazes JM, Sande MA. Experimental pneumococcal meningitis: the role of leukocytes in pathogenesis. *Infect Immun* 1983;**41**:275–9.
111. Ernst JD, Hartiala KT, Goldstein IM, et al. Complement (C5)-derived chemotactic activity accounts for accumulation of polymorphonuclear leukocytes in cerebrospinal fluid of rabbits with pneumococcal meningitis. *Infect Immun* 1984;**46**:81–6.
112. Feigin R. Use of corticosteroids in bacterial meningitis. *Pediatr Infect Dis J* 2004;**23**:355–7.
113. Feigin RD, Baker CJ, Herwaldt LA, et al. Epidemic meningococcal disease in an elementary-school classroom. *N Engl J Med* 1982;**307**:1255–7.
114. Feigin RD, Dodge PR. Bacterial meningitis: newer concepts of pathophysiology and neurologic sequelae. *Pediatr Clin North Am* 1976;**23**:541–56.
115. Feigin RD, Kaplan S. Inappropriate secretion of antidiuretic hormone (ADH) in children with bacterial meningitis. *Am J Clin Nutr* 1977;**30**:1482–4.
116. Feigin RD, Shackelford PG. Sequential lumbar puncture as a diagnostic aid in aseptic meningitis. *N Engl J Med* 1973;**289**: 571–4.
117. Feigin RD, Shearer WT. Opportunistic infection in children, part I: In the compromised host. *J Pediatr* 1975;**87**:507–14.
118. Feigin RD, Shearer WT. Opportunistic infection in children, part II: In the compromised host. *J Pediatr* 1975;**87**:677–94.
119. Feigin RD, Stechenberg BW, Chang MJ, et al. Prospective evaluation of treatment of *Hemophilus influenzae* meningitis. *J Pediatr* 1976;**88**:773–5.
120. Feigin RD, Wong M, Shackelford PG, et al. Countercurrent immunoelectrophoresis of urine as well as CSF and blood for the diagnosis of bacterial meningitis. *J Pediatr* 1976;**89**:773–4.
121. Feldman HM, Michaels RH. Academic achievement in children 10 to 12 years after *Haemophilus influenzae* meningitis. *Pediatrics* 1988;**81**:339–44.
122. Feldman WE. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. *J Pediatr* 1976;**88**:549–52.
123. Fekdnab WE, Ginsburg CB, McCracken GH, et al. Relation of concentrations of *Haemophilus influenzae* type b in cerebrospinal fluid to late sequelae of patients with meningitis. *J Pediatr* 1982;**100**:209–18.
124. Feldman WE, Moffitt S, Manning NS. Penetration of cefoxitin into cerebrospinal fluid of infants and children with bacterial meningitis. *Antimicrob Agents Chemother* 1982;**21**:468–71.
125. Feldman WE, Schwartz J. *Haemophilus influenzae* type b brain abscess complicating meningitis: case report. *Pediatrics* 1983;**72**: 473–5.
126. Fijen CA, Kuijper EJ, te Bulte MT, et al. Assessment of complement deficiency in patients with meningococcal diseases in The Netherlands. *Clin Infect Dis* 1999;**28**:98–105.
127. Finlay FO, Witherow H, Rudd PT. Latex agglutination testing in bacterial meningitis. *Arch Dis Child* 1995;**73**:160–1.
128. Flore AE, Moroney JF, Farley MM, et al. Clinical outcomes of meningitis caused by *Streptococcus pneumoniae* in the era of antibiotic resistance. *Clin Infect Dis* 2000;**30**:71–7.
129. Fitch MT, Abrahamian FM, Moran GJ, Talan DA. Emergency department management of meningitis and encephalitis. *Infect Dis Clin North Am* 2008;**22**:33–52.
130. Forgacs P, Geyer CA, Freidberg SR. Characterization of chemical meningitis after neurological surgery. *Clin Infect Dis* 2001;**32**:179–85.
131. Fraser DW, Darby CP, Koehler RE, et al. Risk factors in bacterial meningitis: Charleston County, South Carolina. *J Infect Dis* 1973;**127**:271–7.
132. Fraser DW, Geil CC, Feldman RA. Bacterial meningitis in Bernalillo County, New Mexico: a comparison with three other American populations. *Am J Epidemiol* 1974;**100**:29–34.
133. Fraser DW, Henke CE, Feldman RA. Changing patterns of bacterial meningitis in Olmstead County, Minnesota, 1935-1970. *J Infect Dis* 1973;**128**:300–7.
134. Fraser DW, Mitchell JE, Silverman LP, et al. Undiagnosed bacterial meningitis in Vermont children. *Ann J Epidemiol* 1975; **102**:394–9.
135. Frei K, Piani D, Pfister HW, et al. Immune-mediated injury in bacterial meningitis. *Int Rev Exp Pathol* 1993;**34**:183–92.
136. Frenkel LD, The Multicenter Ceftriaxone Pediatric Study Group. Once-daily administration of ceftriaxone for the treatment of selected serious bacterial infections in children. *Pediatrics* 1988;**82**:486–91.
137. Friedland IR, Klugman KP. Antibiotic-resistant pneumococcal disease in South African children. *Am J Dis Child* 1992;**146**: 920–3.
138. Friedland IR, Klugman KP. Failure of chloramphenicol therapy in penicillin-resistant pneumococcal meningitis. *Lancet* 1992; **339**:405–8.
139. Friedland IR, Paris M, Ehrett S, et al. Evaluation of antimicrobial regimens for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother* 1993;**37**:1630–6.
140. Friedland IR, Shelton S, Paris M, et al. Dilemmas in diagnosis and management of cephalosporin-resistant *Streptococcus pneumoniae* meningitis. *Pediatr Infect Dis J* 1993;**12**:196–200.
141. Gado M, Axley J, Appleton DB, et al. Angiography in the acute and post-treatment phases of *Haemophilus influenzae* meningitis. *Radiology* 1974;**110**:439–44.
142. Gaillard JL, Abadie G, Cheron J, et al. Concentrations of ceftriaxone in cerebrospinal fluid of children with meningitis receiving dexamethasone therapy. *Antimicrob Agents Chemother* 1994;**38**:1209–10.
143. Galanakis E, Di Cello F, Paul-Satyaseela M, Kim KS. *Escherichia coli* K1 induces IL-8 expression in human brain microvascular endothelial cells. *Eur Cytokine Netw* 2006;**17**:260–65.

144. Gary N, Powers N, Todd JK. Clinical identification and comparative prognosis of high-risk patients with *Haemophilus influenzae* meningitis. *Am J Dis Child* 1989;**143**:307–11.
145. Geiseler PJ, Nelson KE. Bacterial meningitis without clinical signs of meningeal irritation. *South Med J* 1982;**75**:448–50.
146. Geiseler PJ, Nelson KE, Levin S. Community-acquired purulent meningitis: a review of 1,316 cases during the antibiotic era, 1954-1976. *Rev Infect Dis* 1980;**2**:725–44.
147. Gerber J, Bottcher T, Bering J, et al. Increased neurogenesis after experimental *Streptococcus pneumoniae* meningitis. *J Neurosci Res* 2003;**73**:441–6.
148. Gerber J, Bottcher T, Hahn M, et al. Increased mortality and spatial memory deficits in TNF-alpha-deficient mice in ceftriaxone-treated experimental pneumococcal meningitis. *Neurobiol Dis* 2004;**16**:133–8.
149. Gerber J, Nau R. Mechanisms of injury in bacterial meningitis. *Curr Opin Neurol* 2010;**23**:312–8.
150. Gerber J, Pohl K, Sander V, et al. Rifampin followed by ceftriaxone for experimental meningitis decreases lipoteichoic acid concentrations in cerebrospinal fluid and reduces neuronal damage in comparison to ceftriaxone alone. *Antimicrob Agents Chemother* 2003;**47**:1313–7.
151. Gerber J, Tauber SC, Armbrecht I, et al. Increased neuronal proliferation in human bacterial meningitis. *Neurology* 2009;**73**: 1026–32.
152. Gerdes LU, Jorgensen PE, Nexo E, et al. C-reactive protein and bacterial meningitis: a meta-analysis. *Scand J Clin Lab Invest* 1998;**58**:383–93.
153. Gessner B, Sutanto A, Linehan M, et al. Incidences of vaccine-preventable *Haemophilus influenzae* type b pneumonia and meningitis in Indonesian children: hamlet-randomised vaccine-probe trial. *Lancet* 2005;**365**:43–52.
154. Giampaolo C, Scheld WM, Boyd J, et al. Leukocyte and bacterial interrelationships in experimental meningitis. *Ann Neurol* 1982;**9**: 328–33.
155. Gigliotti F, Lee D, Insel RA, et al. IgG penetration into the cerebrospinal fluid in a rabbit model of meningitis. *J Infect Dis* 1987;**156**:394–8.
156. Gilbert G. Epidemiology of *Haemophilus influenzae* type b disease in Australia and New Zealand. *Vaccine* 1991;**9**(Suppl.):S10–3.
157. Gilday DL. Various radionuclide patterns of cerebral inflammation in infants and children. *AJR Am J Roentgenol* 1974;**120**: 247–53.
158. Ginsburg CM, McCracken Jr GH, Parke JJ. *Haemophilus influenzae* type b disease in a day-care center. *Pediatr Res* 1977;**11**:435.
159. Girgis NI, Farid Z, Mikhail IA, et al. Dexamethasone treatment for bacterial meningitis in children and adults. *Pediatr Infect Dis J* 1989;**5**:210–5.
160. Gitlin D. Pathogenesis of subdural collections of fluid. *Pediatrics* 1955;**16**:345–51.
161. Givner LB, Mason Jr EO, Barson WJ, et al. Pneumococcal facial cellulitis in children. *Pediatrics* 2000;**106**:e61.
162. Gradgirard D, Leib S. Strategies to prevent neuronal damage in pediatric bacterial meningitis. *Curr Opin Pediatr* 2006;**18**:112–8.
163. Green SM, Rothrock SG, Clem KJ, et al. Can seizures be the sole manifestation of meningitis in febrile children? *Pediatrics* 1993;**92**:527–34.
164. Grimwood K, Anderson VA, Bond L, et al. Adverse outcomes of bacterial meningitis in school-age survivors. *Pediatrics* 1995;**95**: 646–56.
165. Grubbauer HM, Dornbusch HJ, Dittrich P, et al. Ceftriaxone monotherapy for bacterial meningitis in children. *Chemotherapy* 1990;**36**:441–7.
166. Guibourdenche M, Caugant DA, Herv V, et al. Characteristics of serogroup A *Neisseria meningitidis* strains isolated in the Central African Republic in February 1992. *Eur J Clin Microbiol Infect Dis* 1994;**13**:174–7.
167. Gulian J-M, Gonard V, Daimasso C, et al. Bilirubin displacement by ceftriaxone in neonates: evaluation by determination of "free" bilirubin and erythrocyte-bound bilirubin. *J Antimicrob Chemother* 1987;**19**:823–9.
168. Gump DW. Vancomycin for treatment of bacterial meningitis. *Rev Infect Dis* 1981;**3**:S289–92.
169. Guttler RB, Counts GW, Avent CK, et al. Effect of rifampin and minocycline on meningococcal carrier rates. *J Infect Dis* 1971;**124**:199–205.
170. Haimanot RT, Caugant DA, Fekadu D, et al. Characteristics of serogroup A *Neisseria meningitidis* responsible for an epidemic in Ethiopia, 1988-89. *Scand J Infect Dis* 1990;**22**:171–4.
171. Hall BD. Failure of ampicillin in meningitis. *Lancet* 1968;**1**:1033.
172. Haltalin KC, Smith JB. Reevaluation of ampicillin therapy for *Haemophilus influenzae* meningitis: an appraisal based on a review of cases of persistent or recurrent infection. *Am J Dis Child* 1971;**122**:328–36.
173. Hansman D, Andrews GA. A resistant pneumococcus. *Lancet* 1967;**2**:264–5.
174. Hansman D, Hanna J, Morey F. High prevalence of invasive *Haemophilus influenzae* disease in central Australia. *Lancet* 1986;**2**: 927.
175. Hanson EH, Niemeyer DM, Folio L, et al. Potential use of microarray technology for rapid identification of central nervous system pathogens. *Milit Med* 2004;**169**:594–9.
176. Harrison LH, Dwyer DM, Maples CT, et al. Risk of meningococcal infection in college students. *JAMA* 1999;**281**:1906–10.
177. Hatch D, Overturf GD, Kovacs A, et al. Treatment of bacterial meningitis with ceftazidime. *Pediatr Infect Dis* 1986;**5**:416–20.
178. Haven PL, Wendelgerger KJ, Hoffman GM, et al. Corticosteroids as adjunctive therapy in bacterial meningitis. *Am J Dis Child* 1989;**143**:1051–5.
179. Hirakawa K, Kurokawa M, Yajin K, et al. Recurrent meningitis due to a congenital fistula in the stapedial footplate. *Arch Otolaryngol* 1983;**109**:697–700.
180. Holmes SJ, Lucas AH, Osterholm MT, et al. Immunoglobulin deficiency and idiotype expression in children developing *Haemophilus influenzae* type b disease after vaccination with conjugate vaccine. The Collaborative Study Group. *JAMA* 1991;**266**: 1960–5.
181. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009;**360**:244–56.
182. Ingram DL, Anderson P, Smith DH. Counter-current immunoelectrophoresis in the diagnosis of systemic disease caused by *Hemophilus influenzae*, type b. *J Pediatr* 1972;**81**:1156–9.
183. Ingram DL, Pearson AW, Occhiuti AR. Detection of bacterial antigens in body fluids with the Wellcogen *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* (ACYW135) latex agglutination tests. *J Clin Microbiol* 1983;**18**:1119–21.
184. Jackson LA, Schuchat A, Reeves MW, et al. Serogroup C meningococcal outbreaks in the United States: an emerging threat. *JAMA* 1995;**273**:383–9.
185. Jadavji T, Biggar WD, Gold R, et al. Sequelae of acute bacterial meningitis in children treated for seven days. *Pediatrics* 1985; **78**:21–5.
186. Kadurugamuwa JL, Hengstler B, Zak O. Effects of anti-inflammatory drugs on arachidonic-acid metabolites and cerebrospinal fluid (CSF) proteins during infectious pneumococcal meningitis in rabbits. *Pediatr Infect Dis* 1987;**6**(Suppl.):1153–4.
187. Kaene WM, Postic WP, Rowe LD, et al. Meningitis and hearing loss in children. *Otolaryngology* 1979;**105**:39–44.
188. Kanegaye JT, Soliemanzadeh P, Bradley JS. Lumbar puncture in pediatric bacterial meningitis: defining the time interval for recovery of cerebrospinal fluid pathogens after parenteral antibiotic pretreatment. *Pediatrics* 2001;**108**:1169–74.
189. Kaplan SL, Catlin FI, Weaver T, et al. Onset of hearing loss in children with bacterial meningitis. *Pediatrics* 1984;**73**:575–8.
190. Kaplan SL, Fishman MA. Supportive therapy for bacterial meningitis. *Pediatr Infect Dis J* 1987;**6**:670–7.
191. Kaplan SL, Goddard J, VanKleeck M, et al. Ataxia and deafness in children due to bacterial meningitis. *Pediatrics* 1981;**68**:8–13.
192. Kaplan SL, Mason Jr EO, Wald ER, et al. Six-year multicenter surveillance of invasive pneumococcal infections in children. *Pediatr Infect Dis J* 2002;**21**:141–7.
193. Kaplan SL, Smith EO, Wills C, et al. Association between preadmission oral antibiotic therapy and cerebrospinal fluid findings and sequelae caused by *Haemophilus influenzae* type b meningitis. *Pediatr Infect Dis* 1986;**5**:626–32.
194. Kellner J, Scheifele D, Halperin S, et al. Outcome of penicillin-nonsusceptible *Streptococcus pneumoniae* meningitis: a nested case-control study. *Pediatr Infect Dis J* 2002;**21**:903–9.
195. Kennedy WA, Hoyt MJ, McCracken Jr GH. The role of corticosteroids in children with pneumococcal meningitis. *Am J Dis Child* 1991;**145**:1374–8.

196. Kessler RE. Cefepime microbiologic profile and update. *Pediatr Infect Dis J* 2001;**20**:331–6.
197. Kilpatrick M, Girgis N, Farid Z, et al. Aztreonam for treating meningitis caused by gram-negative rods. *Scand J Infect Dis* 1991;**23**:1125–6.
198. Kim KS. Pathogenesis of bacterial meningitis; from bacteremia to neuronal injury. *Nat Rev Neurosci* 2003;**4**:376–84.
199. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. *Nat Rev Microbiol* 2008;**6**:625–34.
200. Kim KS. Acute bacterial meningitis in infants and children. *Lancet Infect Dis* 2010;**10**:32–42.
201. Kim KS, Itabashi H, Gemski P, et al. The K1 capsule is the critical determinant in the development of *Escherichia coli* meningitis in the rat. *J Clin Invest* 1992;**90**:897–905.
202. Kim YO, Kang JS, Youm MH, et al. Diagnostic capability of CSF ferritin in children with meningitis. *Pediatr Neurol* 2003;**28**:271–6.
203. Kim YS, Tauber MG. Neurotoxicity of glia activated by gram-positive bacterial products depends on nitric oxide production. *Infect Immun* 1996;**64**:3148–53.
204. Klass PE, Klein JO. Therapy of bacterial sepsis, meningitis and otitis media in infants and children: 1992 poll of directors of programs in pediatric infectious diseases. *Pediatr Infect Dis J* 1992;**11**:702–5.
205. Kleiman MD, Weinberg GA, Reynolds JK, et al. Meningitis with beta-lactam–resistant *Streptococcus pneumoniae:* the need for early repeat lumbar puncture. *Pediatr Infect Dis J* 1993;**12**: 782–4.
206. Klein M, Koedel U, Pfister H, Kastenbauer S. Meningitis-associated hearing loss: protection by adjunctive antioxidant therapy. *Ann Neurol* 2003;**54**:451–8.
207. Klugman KP, Dagan R. The Meropenem Meningitis Study Group. Randomized comparison of meropenem with cefotaxime for treatment of bacterial meningitis. *Antimicrob Agents Chemother* 1995;**39**:1140–6.
208. Klugman KP, Friedland IR, Bradley JS. Bacterial activity against cephalosporin-resistant *Streptococcus pneumoniae* in cerebrospinal fluid of children with acute bacterial meningitis. *Antimicrob Agents Chemother* 1995;**39**:1988–92.
209. Koedel U, Bernatowicz A, Paul R, et al. Experimental pneumococcal meningitis: cerebrovascular alterations, brain edema, and meningeal inflammation are linked to the production of nitric oxide. *Ann Neurol* 1995;**37**:313–23.
210. Koedel U, Klein M, Pfister H. New understandings on the pathophysiology of bacterial meningitis. *Curr Opin Infect Dis* 2010; **23**:217–23.
211. Komorowski RN, Farmer SG, Hause LL. Cerebrospinal fluid lactic acid in diagnosis of meningitis. *J Clin Microbiol* 1978;**8**: 89–92.
212. Koomen I, Grobbee D, Roord J, et al. Hearing loss at school age in survivors of bacterial meningitis: assessment, incidence and prediction. *Pediatrics* 2003;**112**:1049–53.
213. Koomen I, van Furth A, Kraak M, et al. Neuropsychology of academic and behavioral limitations in school-age survivors of bacterial meningitis. *Dev Med Child Neurol* 2004;**46**:724–32.
214. Kornelisse RF, Hack CE, Savelkoul HF, et al. Intrathecal production of interleukin-12 and gamma interferon in patients with bacterial meningitis. *Infect Immun* 1997;**65**:877–81.
215. Kotilainen P, Jalava J, Meurman O, et al. Diagnosis of meningococcal meningitis by broad-range bacterial PCR with cerebrospinal fluid. *J Clin Microbiol* 1998;**36**:2205–9.
216. Kyaw M, Lynfield R, Schaffner W, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006;**254**:1455–63.
217. LaScolea Jr LJ, Dryja D. Quantitation of bacteria in cerebrospinal fluid and blood of children with meningitis and its diagnostic significance. *J Clin Microbiol* 1984;**19**:187–90.
218. Lebel MH, Freij BJ, Syrogiannopoulos GA, et al. Dexamethasone therapy for bacterial meningitis: results of two double-blind, placebo-controlled trials. *N Engl J Med* 1988;**319**:964–71.
219. Lebel MH, Hoyt MJ, McCracken GH. Comparative efficacy of ceftriaxone and cefuroxime for treatment of bacterial meningitis. *J Pediatr* 1989;**114**:1049–54.
220. Lebel MH, McCracken Jr GH. Aztreonam: review of the clinical experience and potential uses in pediatrics. *Pediatr Infect Dis J* 1988;**7**:331–9.
221. Lebel MH, McCracken Jr GH. Delayed cerebrospinal fluid sterilization and adverse outcome of bacterial meningitis in infants and children. *Pediatrics* 1989;**83**:161–7.
222. Lee J, Shin S, Teng CH, et al. FimH adhesin of *Escherichia coli* K1 type 1 fimbriae activates BV-2 microglia. *Biochem Biophys Res Commun* 2005;**334**:917–23.
223. Leedom JM, Ivler D, Mathies AW, et al. The problem of sulfadiazine-resistant meningococci. *Antimicrob Agents Chemother* 1966;**6**:281–92.
224. Leggiadro RJ, Winkelstein JA. Prevalence of complement deficiencies in children with systemic meningococcal disease. *Pediatr Infect Dis J* 1987;**6**:75–6.
225. Leib S, Heimgartner C, Bifrare Y, et al. Dexamethasone aggravates hippocampal apoptosis and learning deficiency in pneumococcal meningitis in infant rats. *Pediatr Res* 2003;**54**: 353–7.
226. Leimkugel J, Adams Forgor A, Gagneux S, et al. An outbreak of serotype 1 *Streptococcus pneumoniae* meningitis in northern Ghana with features that are characteristic of *Neisseria meningitidis* meningitis epidemics. *J Infect Dis* 2005;**192**:192–9.
227. Lerman SJ. Systemic *Hemophilus influenzae* infection: a study of risk factors. *Clin Pediatr* 1982;**21**:360–4, 1982.
228. Levine OS, Farley M, Harrison LE, et al. Risk factors for invasive pneumococcal disease in children: a population-based case-control study in North America. *Pediatrics* 1999;**103**:e28.
229. Levy M, Wong E, Fried D. Diseases that mimic meningitis: analysis of 650 lumbar punctures. *Clin Pediatr* 1990;**29**:254–55, 258–61.
230. Liebman EP, Ronis ML, Loyrinic JH, et al. Hearing improvement following meningitis deafness. *Arch Otolaryngol* 1969;**90**:470–3.
231. Lim D, Gerurz A, Lint TF, et al. Absence of the sixth component of complement in a patient with repeated episodes of meningococcal meningitis. *J Pediatr* 1976;**89**:42–7.
232. Lin TY, Chrane DF, Nelson JD, et al. Seven days of ceftriaxone therapy is as effective as ten days' treatment for bacterial meningitis. *JAMA* 1985;**253**:3559–63.
233. Lindsay J. Profound childhood deafness: inner ear pathology. *Ann Otol Rhinol Laryngol* 1973;**82** (Suppl. 5):88–102.
234. Losonsky GA, Santosham M, Sehgal VM, et al. *Haemophilus influenzae* disease in the White Mountain Apaches: molecular epidemiology of a high risk population. *Pediatr Infect Dis* 1984;**3**:539–47.
235. Lu JJ, Perng CL, Lee SY, Wan CC. Use of PCR with universal primers and restriction endonuclease digestions for detection and identification of common bacterial pathogens in cerebrospinal fluid. *J Clin Microbiol* 2000;**38**:2076–80.
236. Mackenzie GA, Leach AJ, Carapetis JR, et al. Epidemiology of naropharyngeal carriage of respiratory pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. *BMC Infect Dis* 2010;**10**:304.
237. MacLennan JM, Shackley F, Heath PT, et al. Safety, immunogenicity, and induction of immunologic memory by a serogroup C meningococcal conjugate vaccine in infants: a randomized controlled trial. *JAMA* 2000;**283**:2795–801.
238. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* diseases—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.
239. Madagame ET, Havens PL, Bresnahan JM, et al. Survival and functional outcome of children requiring mechanical ventilation during therapy for acute bacterial meningitis. *Crit Care Med* 1995;**23**:1279–83.
240. Maxson S, Lewno MJ, Schutze GE. Clinical usefulness of cerebrospinal fluid bacterial antigen studies. *J Pediatr* 1994;**125**: 235–8.
241. McCracken GH, Sarff LD, Glode MP, et al. Relation between *Escherichia coli* K1 capsular polysaccharide antigen and clinical outcome in neonatal meningitis. *Lancet* 1974;**2**:246–50.
242. McIntosh D, Brown J, Hanson R, et al. Torticollis and bacterial meningitis. *Pediatr Infect Dis J* 1993;**12**:160–1.
243. Mcintyre PB, Berkey CS, King SM, et al. Dexamethasone as adjunctive therapy in bacterial meningitis. *JAMA* 1997;**278**: 925–31.
244. Melish ME, Nelson AJ, Martin TE, et al. Epidemic spread of *Hemophilus influenzae* type b disease in a day care center. *Pediatr Res* 1976;**10**:348.
245. Meningococcal meningitis. *Wkly Epidemiol Rec* 2003;**78**:292–6.

246. Meurman OH, Irjala K, Suonpaa J, et al. A new method for the identification of cerebrospinal fluid leakage. *Acta Otolaryngol* 1979;**87**:366–9.
247. Miner JR, Heegaard W, Mapes A, Biros M. Presentation, time to antibiotics, and mortality of patients with bacterial meningitis at an urban county medical center. *J Emerg Med* 2001;**21**:387–92.
248. Mitchell L, Smith S, Braun J, et al. Dual phases of apoptosis in pneumococcal meningitis. *J Infect Dis* 2004;**190**:2039–46.
249. Modal J, Vittecoq D, Decazes JM, et al. Penetration of ceftazidime into cerebrospinal fluid of patients with bacterial meningitis. *Antimicrob Agents Chemother* 1983;**24**:126–8.
250. Moellering RC. Pharmacokinetics of vancomycin. *J Antimicrob Chemother* 1984;**14**:43–52.
251. Moon SY, Chung DR, Kim SW, et al. Changing etiology of community-acquired bacterial meningitis in adults: a nationwide multicenter study in Korea. *Eur J Clin Microbiol Infect Dis* 2010;**29**:793–800.
252. Moore PS, Harrison LH, Telzak EE, et al. Group A meningococcal carriage in travelers returning from Saudi Arabia. *JAMA* 1988;**260**:2686–9.
253. Moore PS, Reeves MW, Schwartz B, et al. Intercontinental spread of an epidemic group A *Neisseria meningitidis* strain. *Lancet* 1989;**2**:260–3.
254. Moura A, Pablos-Mendez A, Layton M, Weiss D. Epidemiology of meningococcal disease, New York City, 1989-2000. *Emerg Infect Dis* 2003;**9**:355–61.
255. Moxon ER, Smith AL, Averill DR, et al. *Hemophilus influenzae* meningitis in infant rats after intranasal inoculation. *J Infect Dis* 1974;**129**:154–62.
256. Mullaney DT, John JF. Cefotaxime therapy: evaluation of its effect on bacterial meningitis, CSF drug levels, and bactericidal activity. *Arch Intern Med* 1983;**143**:1705–8.
257. Mumford RJ, deVasconelas ZJS, Philips CJ, et al. Eradication of carriage of *Neisseria meningitidis* in families: a study in Brazil. *J Infect Dis* 1974;**129**:644–9.
258. Munoz O, Benitez-Diaz L, Martinez MC, et al. Hearing loss after *Hemophilus influenzae* meningitis: follow-up study with auditory brainstem potentials. *Ann Otol Rhinol Laryngol* 1983;**92**:272–5.
259. Murphy TV, Pastor P, Medley F, et al. Decreased *Haemophilus* colonization in children vaccinated with *Haemophilus influenzae* type b conjugate vaccine. *J Pediatr* 1993;**122**:517–23.
260. Mustafa MM, Lebel MH, Ramilo O, et al. Correlation of interleukin 1β and cachectin concentrations in cerebrospinal fluid and outcome from bacterial meningitis. *J Pediatr* 1989;**115**:208–13.
261. Mustafa MM, Ramilo O, Mertsola J, et al. Modulation of inflammation and cachectin activity in relation to treatment of experimental *Haemophilus influenzae* type b meningitis. *J Infect Dis* 1989;**160**:818–25.
262. Mustafa MM, Ramilo O, Saez-Llorens X, et al. Cerebrospinal fluid prostaglandins, interleukin 1β, and tumor necrosis factor in bacterial meningitis: clinical and laboratory correlations in placebo and dexamethasone-treated patients. *Am J Dis Child* 1990;**144**:883–7.
263. Nagata M, Hara T, Aoki T, et al. Inherited deficiency of ninth component of complement: an increased risk of meningococcal meningitis. *J Pediatr* 1989;**114**:260–4.
264. Naraqui S, Kirkpatrick GP, Kabins S. Relapsing pneumococcal meningitis: isolation of an organism with decreased susceptibility to penicillin G. *J Pediatr* 1974;**85**:671–3.
265. National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Testing. Tenth information supplement (aerobic dilution). M100-S10(M7) Table 2G. MIC Interpretive Standards (mg/mL) for Streptococcus pneumoniae*. Wayne, Penn.: NCCLS; 2000.
266. Neches W, Platt M. Cerebrospinal fluid LDH in 287 children including 53 cases of meningitis of bacterial and non-bacterial etiology. *Pediatrics* 1968;**41**:1097–103.
267. Negrini B, Kelleher KJ, Wald ER. Cerebrospinal fluid findings in aseptic versus bacterial meningitis. *Pediatrics* 2000;**105**:316–9.
268. Niemoller UM, Tauber MG. Brain edema and increased intracranial pressure in the pathophysiology of bacterial meningitis. *Eur J Clin Microbiol Infect Dis* 1989;**8**:109–17.
269. Nigrovic LE, Kuppermann N, Macias CG, et al. Clinical prediction rule for identifying children with cerebrospinal fluid pleocytosis at very low risk of bacterial meningitis. *JAMA* 2007; **297**:52–60.
270. Novak R, Henriques B, Charpentier E, et al. Emergence of vancomycin tolerance in *Streptococcus pneumoniae*. *Nature* 1999; **399**:590–3.
271. Nuorti JP, Butler JC, Farley MM, et al. Cigarette smoking and invasive pneumococcal disease. *N Engl J Med* 2000;**342**:681–9.
272. Odio CM, Faingezicht I, Paris M. The beneficial effects of early dexamethasone administration in infants and children with bacterial meningitis. *N Engl J Med* 1991;**324**:1515–31.
273. Odio CM, Puig JR, Feris JM, et al. Prospective, randomized investigator-blinded study of the efficacy and safety of meropenem vs. cefotaxime therapy in bacterial meningitis in children. *Pediatr Infect Dis J* 1999;**18**:581–90.
274. Olivier C, Cohen R, Begué P, et al. Bacteriologic outcome of children with cefotaxime- or ceftriaxone-susceptible and -nonsusceptible Streptococcus pneumoniae meningitis. *Pediatr Infect Dis J* 2000;**19**:1015–7.
275. Oostenbrink R, Moons KG, Theunissen CC, et al. Signs of meningeal irritation at the emergency department: how often bacterial meningitis? *Pediatr Emerg Care* 2001;**17**:161–4.
276. Peltola H. Worldwide *Haemophilus influenzae* type b diseases at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. *Clin Microbiol Rev* 2000;**13**:302–17.
277. Peltola H, Makela PH, Kayhty H, et al. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. *N Engl J Med* 1977;**297**:686–91.
278. Peltola H, Rod TO, Jonsdottir K, et al. Life-threatening *Haemophilus influenzae* infections in Scandinavia: a five-country analysis of the incidence and the main clinical and bacteriologic characteristics. *Rev Infect Dis* 1990;**12**:708–15.
279. Peltola H, Roine I, Fernandez J, et al. Adjuvant glycerol and/or dexamethasone to improve the outcomes of childhood bacterial meningitis: a prospective, randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2007;**45**:1277–86.
280. Peltola H, Roine I, Fernandez J, et al. Hearing impairment in childhood bacterial meningitis is little relieved by dexamethasone or glycerol. *Pediatrics* 2010;**125**:e1–e8.
281. Perkins MD, Mirrett S, Reller RB. Rapid bacterial antigen detection is not clinically useful. *J Clin Microbiol* 1995;**33**:1486–91.
282. Peter G, Weigart MB, Bissel AR, et al. Meningococcal meningitis in familial deficiency of the fifth component of complement. *Pediatrics* 1981;**67**:882–6.
283. Picard C, Casanova JL, Puel A. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. *Clin Microbiol Rev* 2011;**24**:490–7.
284. Picard C, Puel A, Bonnet M, et al. Pyogenic bacterial infections in humans with IRAK-4 deficiency. *Science* 2003;**299**:2076–9.
285. Picard C, Puel A, Bustamante J, et al. Primary immunodeficiencies associated with pneumococcal disease. *Curr Opin Allergy Clin Immunol* 2003;**3**:451–9.
286. Pinner RW, Onyango F, Perkins BA, et al. Epidemic meningococcal disease in Nairobi, Kenya, 1989. *J Infect Dis* 1992;**166**:359–64.
287. Pomeroy SL, Holmes SJ, Dodge PR, et al. A prospective evaluation of the neurologic sequelae of bacterial meningitis in children with special emphasis on late seizures. *N Engl J Med* 1990;**323**: 1651–7.
288. Powell DA, Nahata MC, Durrell DC, et al. Interactions among chloramphenicol, phenytoin and phenobarbital in a pediatric patient. *J Pediatr* 1981;**98**:1001–3.
289. Powell KR, Mawhorter S. Outpatient treatment of serious infections in infants and children with ceftriaxone. *J Pediatr* 1987;**110**:889–901.
290. Prober CG. The role of steroids in the management of children with bacterial meningitis. *Pediatrics* 1995;**95**:29–31.
291. Quagliarello VJ, Long WJ, Scheld WM. Morphologic alterations of the blood brain barrier with experimental meningitis in the rat: temporal sequence and role of encapsulation. *J Clin Invest* 1986;**77**:1084–95.
292. Quin LR, Moore QC, McDaniel LS. Pneumolysin, PspA and PspC contribute to pneumococcal evasion of early innate immune responses during bacteremia in mice. *Infect Immun* 2007;**75**: 2067–70.
293. Radstrom P, Backman A, Qian N, et al. Detection of bacterial DNA in cerebrospinal fluid by an assay for simultaneous detection

of *Neisseria meningitidis*, *Haemophilus influenzae*, and streptococci using a semi-nested PCR strategy. *J Clin Microbiol* 1994;**32**: 1738–44.
294. Ramilo O, Mustafa MM, Porter J, et al. Detection of interleukin-1β but not tumor necrosis factor-alpha in cerebrospinal fluid of children with aseptic meningitis. *Am J Dis Child* 1990;**144**: 349–52.
295. Ramsay ME, Andrews N, Kaczmarski EB, et al. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. *Lancet* 2001;**357**:195–6.
296. Raziuddin S, El-Awad ME, Mir NA. Bacterial meningitis: T cell activation and immunoregulatory CD4-T cell subset alteration. *J Allergy Clin Immunol* 1991;**87**:1115–20.
297. Reelhuis J, Honein M, Whitney C, et al. Risk of bacterial meningitis in children with cochlear implants. *N Engl J Med* 2003;**349**:435–45.
298. Ridgway EJ, Allen KD, Neal TJ, et al. Penicillin-resistant pneumococcal meningitis. *Lancet* 1992;**339**:931.
299. Rodriguez WJ, Puig JR, Khan WN, et al. Ceftazidime vs. standard therapy for pediatric meningitis: therapeutic, pharmacologic and epidemiologic observations. *Pediatr Infect Dis* 1986;**5**:408–15.
300. Roeses RJ, Campbell JC. Recovery of auditory function following meningitis deafness. *J Speech Hear Dis* 1975;**40**:405–11.
301. Rorke LB, Pitts FW. Purulent meningitis: the pathological basis of clinical manifestations. *Clin Pediatr* 1963;**2**:64–71.
302. Rosen JB, Thomas AR, Lexau CA, et al. Geographical variation in invasive pneumococcal disease following pneumococcal conjugate vaccine introduction in the United States. *Clin Infect Dis* 2011;**53**:137–43.
303. Rosenstein NE, Perkins BA, Stephens DS, et al. The changing epidemiology of meningococcal disease in the United States, 1992-1996. *J Infect Dis* 1999;**180**:1894–901.
304. Ross S, Rodriguez W, Controni G, et al. Limulus lysate test for gram-negative bacterial meningitis: bedside application. *JAMA* 1975;**233**:1366–9.
305. Rotbart HA. Diagnosis of enteroviral meningitis with the polymerase chain reaction. *J Pediatr* 1990;**117**:85–9.
306. Russell F, Carapetis J, Mansoor O, et al. High incidence of *Haemophilus influenzae* type b infection in children in Pacific Island Countries. *Clin Infect Dis* 2003;**37**:1593–9.
307. Rutledge J, Benjamin D, Hood L, et al. Is the CSF lactate measurement useful in the management of children with suspected bacterial meningitis? *J Pediatr* 1981;**98**:20–4.
308. Sáez-Llorens X, Castaño E, García R, et al. Prospective randomized comparison of cefepime and cefotaxime for treatment of bacterial meningitis in infants and children. *Antimicrob Agents Chemother* 1995;**39**:937–40.
309. Sáez-Llorens X, O'Ryan M. Cefepime in the empiric treatment of meningitis in children. *Pediatr Infect Dis* 2001;**20**:356–61.
310. Salih MA, Ahmed HS, Karrar ZA, et al. Features of a large epidemic of group A meningococcal meningitis in Khartoum, Sudan, in 1988. *Scand J Infect Dis* 1990;**22**:161–70.
311. Sanal O, Loos M, Ersoy F, et al. Complement component deficiencies and infection: C5, C8, C3. *Eur J Pediatr* 1992;**151**: 676–9.
312. Sankrithi UM, Lipuma JJ. Clinically inapparent meningitis complicating periorbital cellulitis. *Pediatr Emerg Care* 1991;**7**:28–9.
313. Santosham M, Reid R, Ambrosino DM. Prevention of *Haemophilus influenzae* type b infections in high-risk infants treated with bacterial polysaccharide immune globulin. *N Engl J Med* 1987;**317**:923–9.
314. Schaad UB, Lips U, Gnehm HE, et al. Dexamethasone therapy for bacterial meningitis in children. *Lancet* 1993;**342**:457–61.
315. Schaad UB, Suter S, Gianella-Borradori A, et al. A comparison of ceftriaxone and cefuroxime for the treatment of bacterial meningitis in children. *N Engl J Med* 1990;**322**:141–7.
316. Schaad UB, Wedgwood-Krucko J, Tschaeppeler H. Reversible ceftriaxone-associated biliary pseudolithiasis in children. *Lancet* 1988;**2**:1411–3.
317. Scheifele DW, Ward JI, Siber GR. Advantage of latex agglutination over countercurrent immunoelectrophoresis in the detection of *Haemophilus influenzae* type b antigen in serum. *Pediatrics* 1981;**68**:888–91.
318. Scheld WM. Pathogenesis and pathophysiology of pneumococcal meningitis. In: Sande MA, Smith AL, Root RK, editors. *Bacterial meningitis*. New York: Churchill Livingstone; 1985. p. 37–69.
319. Schlech WF 3rd, Ward JI, Band JD, et al. Bacterial meningitis in the United States, 1978-1981: The National Bacterial Meningitis Surveillance Study. *JAMA* 1985;**253**:1749–54.
320. Schlesinger Y, Sawyer MH, Storch GA. Enteroviral meningitis in infancy: potential role for polymerase chain reaction in patient management. *Pediatrics* 1994;**94**:157–62.
321. Schoendorf KC, Adams WG, Kiely JL, et al. National trends in *Haemophilus influenzae* meningitis mortality and hospitalization among children, 1980 through 1991. *Pediatrics* 1994;**93**:663–8.
322. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. *N Engl J Med* 1997;**337**:970–6.
323. Schwartz B, Al-Tobaiqi A, Al-Ruwais A, et al. Comparative efficacy of ceftriaxone and rifampin in eradicating pharyngeal carriage of group A *Neisseria meningitidis*. *Lancet* 1988;**1**:1239–42.
324. Schwartz JF. Ataxia in bacterial meningitis. *Neurology* 1972;**22**: 1071–4.
325. Sell SH. Long-term sequelae of bacterial meningitis in children. *Pediatr Infect Dis* 1983;**2**:90–3.
326. Shackelford PG, Campbell J, Feigin RD. Countercurrent immunoelectrophoresis in the evaluation of childhood infection. *J Pediatr* 1974;**85**:478–81.
327. Sharp A, Sorvillo F, Redelings M, et al. Population-based analysis of meningococcal disease mortality in the United States: 1990-2002. *Pediatr Infect Dis J* 2006;**25**:191–4.
328. Shetty N, de Keyser P, Ridgway GL. Acute bacterial meningitis after dental fillings. *J Infect* 1998;**37**:89–90.
329. Shurin SB, Anderson P. Anemia associated with *H. influenzae* b (Hib) septicemia is due to immune hemolysis of RBC coated with soluble bacterial antigens. *Pediatr Res* 1983;**17**:932.
330. Sills RH, Caserta MT, Landaw SA. Decreased erythrocyte deformability in the anemia of bacterial meningitis. *J Pediatr* 1982;**101**:395–8.
331. Simberkoff MD, Moldovec NH, Rahal Jr JJ. Absence of detectable bactericidal and opsonic activities in normal and infected human cerebrospinal fluids: a regional host defense deficiency. *J Lab Clin Med* 1980;**95**:362–72.
332. Singer JI, Berger OG. Simultaneous occult pneumococcal bacteremia in identical twins. *J Pediatr* 1981;**98**:250–1.
333. Singhi SC, Singhi PD, Srinivas B, et al. Fluid restriction does not improve the outcome of acute meningitis. *Pediatr Infect Dis J* 1995;**14**:495–503.
334. Sinner S, Tunkel A. Antimicrobial agents in the treatment of bacterial meningitis. *Infect Dis Clin North Am* 2004;**18**: 581–602.
335. Sloas MM, Barrett FF, Chesney PJ, et al. Cephalosporin treatment failure in penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* meningitis. *Pediatr Infect Dis J* 1992;**11**:662–6.
336. Smith AL, Roberts MC, Haas JE, et al. Mechanisms of *Haemophilus influenzae* type b meningitis. In: Sande MA, Smith AL, Root RK, editors. *Bacterial meningitis*. New York: Churchill Livingstone; 1985. p. 11–21.
337. Smith JF, Landing BH. Mechanisms of brain damage in *H. influenzae* meningitis. *J Neuropathol Exp Neurol* 1960;**19**:248–65, 1960.
338. Snedeker JD, Kaplan SL, Dodge PR, et al. Subdural effusion and its relationship with neurologic sequelae of bacterial meningitis in infancy: a prospective study. *Pediatrics* 1990;**86**:163–70.
339. Snyder RD, Stovring J, Cushing AH, et al. Cerebral infarction in childhood bacterial meningitis. *J Neurol Neurosurg Psychiatry* 1981;**44**:581–5.
340. Soderstrom C, Sjoholm AG, Svensson R, et al. Another Swedish family with complete properdin deficiency: association with fulminant meningococcal disease in one male family member. *Scand J Infect Dis* 1989;**21**:259–65.
341. Sokolova O, Heppel N, Jagerhuber R, et al. Interaction of *Neisseria meningitidis* with human brain microvascular endothelial cells: role of MAP- and tyrosine kinases in invasion and inflammatory cytokine release. *Cell Microbiol* 2004;**6**:1153–66.
342. Sommers LM, Hawkins DS. Meningitis in pediatric cancer patients: a review of forty cases from a single institution. *Pediatr Infect Dis J* 1999;**18**:902–7.
343. Sormunen P, Kallio MJT, et al. C-reactive protein is useful in distinguishing Gram stain-negative bacterial meningitis from viral meningitis. *J Pediatr* 1999;**134**:725–9.
344. Spanaus KS, Nadal D, Pfister HW, et al. C-C-C and C-C chemokines are expressed in the cerebrospinal fluid in bacterial meningitis and mediate chemotactic activity on peripheral

blood-derived polymorphonuclear and mononuclear cells in vitro. *J Immunol* 1997;**158**:1956–64.
345. Spika JS, Facklam RR, Plikaytis BD (The Pneumococcal Surveillance Working Group), et al. Antimicrobial resistance of *Streptococcus pneumoniae* in the United States, 1979-1987. *J Infect Dis* 1991;**163**:1273–8.
346. Spreer A, Gerber J, Hanssen M, et al. Dexamethasone increases hippocampal neuronal apoptosis in a rabbit model of *Escherichia coli* meningitis. *Pediatr Res* 2006;**60**:210–15.
347. Sproles III ET, Azerrad J, Williamson C, et al. Meningitis due to *Hemophilus influenzae:* long-term sequelae. *J Pediatr* 1969;**75**: 782–8.
348. Steele RW, McConnell JR, Jacobs RF, et al. Recurrent bacterial meningitis: coronal thin-section cranial computed tomography to delineate anatomic defects. *Pediatrics* 1985;**76**:950–3.
349. Stephen DS, McGee ZA. Attachment of *Neisseria meningitidis* to human mucosal surfaces: influence of pili and type of receptor cell. *J Infect Dis* 1981;**143**:525–32.
350. Taha M, Giorgini D, Ducos-Galand M, Alonso J. Continuing diversification of *Neisseria meningitidis* W135 as a primary cause of meningococcal disease after emergence of the serogroup in 2000. *J Clin Microbiol* 2004;**42**:4158–63.
351. Tan LKK, Carlon GM, Borrow R. Advances in the development of vaccines against *Neisseria meningitidis*. *N Engl J Med* 2010; **362**:1511–20.
352. Tan TQ, Mason Jr EO, Kaplan SL. Penicillin-resistant systemic pneumococcal infections in children: a retrospective case-control study. *Pediatrics* 1993;**92**:761–7.
353. Tan TQ, Schutze GE, Mason Jr EO, et al. Antibiotic therapy and acute outcome of meningitis due to *Streptococcus pneumoniae* considered intermediately susceptible to broad-spectrum cephalosporins. *Antimicrob Agents Chemother* 1994;**38**:918–23.
354. Tauber MG, Shibi AM, Hackbarth CJ, et al. Antibiotic therapy endotoxin concentration in cerebrospinal fluid and brain edema in experimental *Escherichia coli* meningitis in rabbits. *J Infect Dis* 1987;**156**:456–62.
355. Taylor HG, Mills EL, Ciampi A, et al. The sequelae of *Haemophilus influenzae* meningitis in school-age children. *N Engl J Med* 1990;**323**:1657–63.
356. Tejani A, Dobias B, Sambursky J. Long-term prognosis after *H. influenzae* meningitis: prospective evaluation. *Dev Med Child Neurol* 1982;**24**:338–43.
357. Tejani A, Mahadevan R, Dobias B. Occurrence of HLA types in H. influenzae type b disease. *Tissue Antigens* 1981;**17**:205–11.
358. Thigpen MC, Whitney CG, Messonnier NE. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011;**364**: 2016–25.
359. Tracey KJ, Viassara J, Cerami A. Cachectin/tumor necrosis factor. *Lancet* 1989;**1**:1122–5.
360. Trujillo H, Harry N, Arango A, et al. Aztreonam in the treatment of aerobic, gram-negative bacillary infections in pediatric patients. *Chemotherapy* 1989;**35**(Suppl.):25–30.
361. Tsai CJ, Griffin MR, Pekka Nuorti J, Grijalva CG. Changing epidemiology of pneumococcal meningitis after the introduction of pneumococcal conjugate vaccine in the United States. *Clin Infect Dis* 2008;**46**:1664–72.
362. Tunkel AR, Hartman BJ, Kaplan SL, et al. Practice guidelines for the management of bacterial meningitis. *Clin Infect Dis* 2004;**39**:1267–84.
363. Tuomanen E, Liu H, Hengstler B, et al. The induction of meningeal inflammation by components of the pneumococcal cell wall. *J Infect Dis* 1985;**151**:859–68.
364. Tyson JE, Gilmartin Jr RC, Friedman BI, et al. ^{131}I-HAS cisternography in children with meningitis. In: Harbert JC, editor. *Cisternography and hydrocephalus*. Springfield, Ill: Charles C Thomas; 1972. p. 413–32.
365. Unkmeir A, Latsch K, Dietrich G, et al. Fibronectin mediates Opc dependent internalization of *Neisseria meningitidis* by human brain microvascular endothelial cells. *Mol Microbiol* 2002;**46**: 933–46.
366. U.S. Department of Commerce, Bureau of the Census. *Mortality Statistics*, *1935*. Washington, D.C.: Government Printing Office; 1937.
367. U.S. Department of Health, Education and Welfare, Public Health Service. *Vital Statistics of the United States*, *1968*, vol. II. Mortality, Washington, D.C.: Government Printing Office; 1970.
368. Valmari P, Peltola H, Ruuskanen O, Korvenranta H. Childhood bacterial meningitis: initial symptoms and signs related to age, and reasons for consulting a physician. *Eur J Pediatr* 1987; **146**:515–18.
369. Van Alphen L, Spanjaard L, van der Ende A, et al. Predicted disappearance of *Haemophilus influenzae* type b meningitis in Netherlands. *Lancet* 1994;**344**:195.
370. Van Furth AM, Roord JJ, van Furth R. Roles of proinflammatory and anti-inflammatory cytokines in pathophysiology of bacterial meningitis and effect of adjunctive therapy. *Infect Immun* 1996; **64**:4883–90.
371. Van Vliet KE, Glimaker M, Lebon P, et al. Multicenter evaluation of the Amplicor Enterovirus PCR test with cerebrospinal fluid from patients with aseptic meningitis. *J Clin Microbiol* 1998;**36**: 2652–7.
372. Vienny H, Despland PA, Lutschg J, et al. Early diagnosis and evolution of deafness in childhood bacterial meningitis: a study using brainstem auditory evoked potentials. *Pediatrics* 1994;**73**: 579–86.
373. Viola GM. Extreme hypoglycorrhachia: not always bacterial meningitis. *Nat Rev Neurol* 2010;**6**:637–41.
374. Von Bernuth H, Picard C, Jin Z, et al. Pyogenic bacterial infections in humans with MyD88 deficiency. *Science* 2008;**321**: 691–6.
375. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. *Lancet* 1987;**1**:355–7.
376. Wald ER, Kaplan SL, Mason Jr EO, et al. Dexamethasone therapy for children with bacterial meningitis. *Pediatrics* 1995;**95**:21–8.
377. Waiserbourd-Zinman O, Bilavsky E, Tirosh N, et al. Penicillin and ceftriaxone susceptibility of *Streptococcus pneumoniae* isolated from cerebrospinal fluid of children with meningitis hospitalized in a tertiary hospital in Israel. *Isr Med Assoc J* 2010;**12**:225–8.
378. Waler JA, Rathore MH. Outpatient management of pediatric bacterial meningitis. *Pediatr Infect Dis J* 1995;**14**:89–92.
379. Ward JI, Fraser DW, Baraff LJ, et al. *Hemophilus influenzae* meningitis: a national study of secondary spread in household contacts. *N Engl J Med* 1979;**19**:122–6.
380. Ward JI, Gorman C, Philips C, et al. *Haemophilus influenzae* type b disease in a day care center. *J Pediatr* 1978;**92**:713–7.
381. Ward JI, Lum MKW, Hall DB, et al. Invasive *Haemophilus influenzae* type b disease in Alaska: background epidemiology for a vaccine efficacy trial. *J Infect Dis* 1986;**153**:17–26.
382. Ward JI, Siber GI, Scheifele DW, et al. Rapid diagnosis of *Hemophilus influenzae* type b infections by latex particle agglutination and counterimmunoelectrophoresis. *J Pediatr* 1978;**93**: 37–42.
383. Watt J, Levine O, Santosham M. Global reduction of Hib disease: what are the next steps? Proceedings of the meeting in Scottsdale, Arizona, September 22-25, 2002. *J Pediatr* 2003;**143**: (6 Suppl):S163–87.
384. Weidmer CE, Dunkel TB, Pettyjohn FS, et al. Effectiveness of rifampin in eradicating the meningococcal carrier state in a relatively closed population: emergence of resistant strains. *J Infect Dis* 1971;**24**:172–8.
385. Weisfelt M, de Gans J, van der Poll T, van de Beek D. Pneumonoccal meningitis in adults: new approaches to management and prevention. *Lancet Neurol* 2006;**5**:332–42.
386. Weiser JN, Ausrain R, Sreenivasan PK, Masure HR. Phase variation in pneumococcal opacity: relationship between colonical morphology and nasopharyngeal colonization. *Infect Immun* 1994;**62**:2582–9.
387. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multi-drug resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000;**343**:1917–24.
388. Whittle HC, Greenwood BM. Meningococcal meningitis in the northern savanna of Africa. *Trop Doct* 1976;**6**:99–104.
389. Williams DN, Laughlin LW, Lee YH. Minocycline: possible vestibular side-effects. *Lancet* 1974;**2**:744–6.
390. Wispelwey B, Lesse AJ, Hansen EJ, et al. *Haemophilus* lipopolysaccharide-induced blood brain barrier permeability during experimental meningitis in the rat. *J Clin Invest* 1988; **82**:1339–46.
391. Wolff M, Chavanet P, Kazmierczak A, et al. Diffusion of cefpirome into the cerebrospinal fluid of patients with purulent meningitis. *J Antimicrob Chemother* 1992;**29A**:59–62.

392. Wong M, Schlaggar BL, Landt M. Postictal cerebrospinal fluid abnormalities in children. *J Pediatr* 2001;**138**:373–7.
393. Wong VK, Wright Jr HT, Ross LA, et al. Imipenem/cilastatin treatment of bacterial meningitis in children. *Pediatr Infect Dis J* 1991;**10**:122–5.
394. Woody RC, Yamauchi T. Cerebrospinal fluid cell counts in childhood idiopathic status epilepticus. *Pediatr Infect Dis J* 1988;**7**:298–9.
395. Zangwell KM, Vadheim CM, Vannier AM, et al. Epidemiology of invasive pneumococcal disease in Southern California: Implications for the design and conduct of a pneumococcal conjugate vaccine efficacy trial. *J Infect Dis* 1996;**174**:752–9.
396. Zhang JR, Mostov KE, Lamm ME, et al. The polymeric immunoglobuilin receptor translocates pneumococci across human nasopharyngeal epithelial cells. *Cell* 2000;**102**:827–37.
397. Zwahien A, Nydegger UE, Vaudaux P, et al. Complement-mediated opsonic activity in normal and infected human cerebrospinal fluid: early response during bacterial meningitis. *J Infect Dis* 1982;**145**:635–46.
398. Zwienburg P, van der Poll T, Florquin S, et al. Interleukin-18 gene–deficient mice show enhanced defense and reduced inflammation during pneumococcal meningitis. *J Neuroimmun* 2003; **138**:31–7.

CHAPTER 32

Parameningeal Infections

Xavier Sáez-Llorens • Javier Nieto Guevara

BRAIN ABSCESS

Although it is uncommon, pediatric brain abscess remains a serious, life-threatening neurologic problem. Those with congenital heart disease, an ongoing infection, or an immunocompromised state are particularly at risk. The symptoms on presentation may include those associated with a space-occupying lesion in the brain, and neuroimaging has made the diagnosis of brain abscess more reliable. Abscess formation may occur in the parenchyma of the central nervous system, in the subdural space, or in the epidural space. It can originate from contiguous site infections (e.g., skull osteomyelitis, chronic otitis),[46] from underlying vascular anomalies (e.g., congenital cyanotic heart disease), after head trauma or neurosurgical procedures, or from cryptogenic sources. These conditions can alter neurologic function by direct destruction of nervous tissue, by infarction after inflammatory occlusion of veins and arteries, or by compression caused by mass effect.

Advances in diagnostic and therapeutic modalities since the 1990s have improved the prognosis of this serious disease. The mortality rate associated with brain abscesses seems to be decreasing. This reduction probably is the result of the advent of head imaging and its use to guide precise location and management, improvement in surgical techniques, and advances in antibiotic development. Goodkin and coworkers[18] identified 54 patients aged 5 days to 34 years of age who were diagnosed with a brain abscess at the Boston Children's Hospital from 1945 to 1980 and 1981 to 2000. They found a reduction in the number of abscesses that occurred in the setting of sinus or middle ear infection (26% vs. 11%, respectively) and an increase in the number of children who were treated with antibiotics alone (1% vs. 22%). These investigators did not observe, however, any additional decline in the mortality rates in their institution between 1981 and 2000. Fischer and associates[16] found a reduction in mortality rate from 36 percent before 1970 to 14 percent after 1970. Tekkok and Erbengi[50] described a similar decline, from 30 percent in the era before the use of computed tomography (CT) to 3 to 6 percent in the last 5 years of their study and zero in the last 3 years of their study. Other case series also have reported a mortality rate of zero.[27]

Although the mortality rate seems to be decreasing, a significant percentage of children continue to have residual neurologic deficits, including epilepsy, permanent motor or sensory dysfunction, visual field defects, and personality changes.[16,20,23] Some children also require placement of a ventriculoperitoneal shunt.[11]

Pathogenesis and Pathology

Brain abscess is a focal, intracerebral infection that begins as a localized area of cerebritis and eventually ends in a collection of pus surrounded by a well-vascularized capsule.[1] For practical purposes, brain abscesses usually are classified according to the likely entry point of the infection (Table 32-1). Ear and mastoid infections are associated with formation of an abscess at the temporal or cerebellar locations; sinus and dental infections give rise to purulent collections in the frontal lobe; and metastatic spread from distant foci in children with congenital cardiac or pulmonary right-to-left shunts commonly results in involvement of any parenchymal area, including parietal or occipital regions.[26,46]

The most common origin of microbial infection in children remains direct or indirect cranial infection arising from the middle ear, paranasal sinuses, or teeth. Seeding of the brain presumably occurs via transit of infecting microbes through the valves and emissary veins that serve these regions. A direct erosion of skull and dura by osteomyelitis-induced sinus or middle ear infection can be another mechanism of bacterial spread.[8] Resulting abscesses tend to be solitary and superficial. Metastatic inoculation of the brain from distant extracranial sources (pulmonary infection, endocarditis) tends to provoke multiple cerebral abscesses, with a distribution that reflects the regional cerebral blood flow of the area affected, usually the middle cerebral artery network.[8]

In children with cyanotic congenital heart disease, bacteria are not filtered out by the pulmonary vascular bed, which allows for systemic spread.[11] This situation rarely occurs in patients younger than age 2 years, and the abscess or abscesses usually are in areas of brain perfused by the middle cerebral arteries. Evidence of associated endocarditis is rare in these cases, although acute bacterial endocarditis may be complicated by septic infarction of the brain and abscess formation.[41]

Formation of an abscess by direct bacterial implantation may complicate compound skull fractures, scalp wounds, anterior cranial fossa or temporal bone fractures, and chronic cerebrospinal fluid (CSF) fistula. Abscesses also rarely may develop during the course of bacterial meningitis.[45] Despite identification of all these potential routes, 20 to 30 percent of cases are classified as cryptic brain abscess for which no obvious predisposing factor can be identified.

The brain is remarkably resistant to microbial infection. Despite the common occurrence of occult bacteremia in infants and children, cerebral abscess is a quite rare

TABLE 32-1 **Primary Source, Usual Location of Lesion, and Associated Neurologic Findings in Children with Brain Abscess**

Primary Source	Location of Abscess	Associated Neurologic Findings
Sinusitis	Frontal lobe	Headache, behavioral changes, motor/speech disorders, depressed consciousness, forced grasping and sucking, hemiparesis
Chronic otitis/mastoiditis	Temporal lobe	Dyspraxia and aphasia (dominant hemisphere), ipsilateral third cranial nerve palsy, ipsilateral headache, upper homonymous hemianopsia, motor dysfunction of face and arm
	Cerebellum	Dizziness, vomiting, ipsilateral ataxia and tremor, sixth cranial nerve palsy, nystagmus (toward lesion)
Dental infection	Frontal lobe	
Head trauma	Related to injured site	Variable by region involved
Postoperative	At operative site	Variable by region involved
Metastatic spread	Multiple lesions	Variable by region involved
	If parietal lobe involved	Visual field defects in inferior quadrant, homonymous hemianopsia, dysphasia (dominant hemisphere), dyspraxia, and contralateral spatial neglect (no dominant hemisphere)

disease. This resistance is attributable in part to the abundant blood supply of the brain and the relative impermeable blood-brain barrier.[1] Although certain underlying brain morbidities, such as previous stroke, intracerebral hematoma, or underlying neoplasm, may serve as a nidus of abscess formation in adults, affected children have no apparent predisposing brain lesion. In animal models of infection, induction of abscess usually requires direct inoculation of numerous organisms into the animal's cerebrum.[35]

Experimental animal studies and use of CT have provided evidence of the clinical evolution of a brain abscess.[2] In the early stage of cerebritis (days 1 to 3), a focal area of acute inflammation, vascular dilation, microthrombosis, rupture of small vessels, and edema is present. The center of the lesion then undergoes liquefaction. Expansion of the cerebritis and formation of a necrotic central focus are seen in the late cerebritis stage (days 4 to 9). Establishment of a ring-enhancing dense collagenous capsule of well-vascularized tissue with peripheral gliosis or fibrosis or both occurs at the early capsule stage (days 10 to 14). Finally, during the late capsule stage (>14 days), host defenses act to wall off the abscess, and a well-developed capsule results.

Death can occur if the volume of pus and surrounding edema induce a significant increase of intracranial pressure leading to brain herniation. In addition, cerebral abscesses can rupture into the ventricular system or through the cortex into the subarachnoid space, resulting in acute deterioration and a life-threatening event.[11] Figure 32-1 shows the gross appearance of a well-defined abscess of hematogenous origin. Various microscopic features are illustrated in Figures 32-2 through 32-4.

The spectrum of microorganisms cultured from brain abscesses has changed with time. This change reflects improved microbiologic isolation techniques, early and aggressive treatment of primary infections, and better neurosurgical procedures. In more recent series, the predominance of *Staphylococcus aureus* has decreased and identification of anaerobes has increased.[7,14,54]

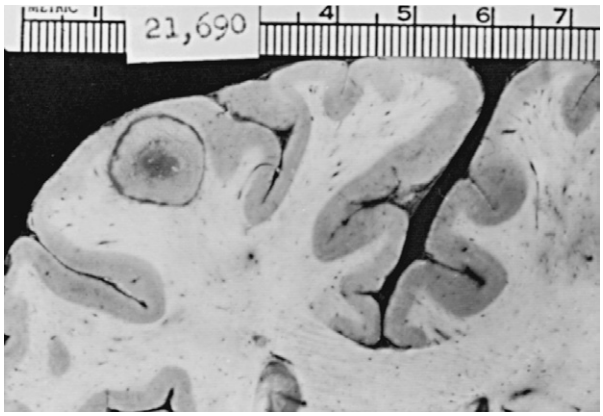

FIGURE 32-1 ■ Circumscribed cerebral abscess of hematogenous origin.

Anaerobic bacteria isolated from brain abscesses include species of *Bacteroides*, *Peptostreptococcus*, *Fusobacterium*, *Veillonella*, *Propionibacterium*, *Prevotella*, and *Actinomyces*. Aerobic and microaerophilic streptococci, staphylococci, *Haemophilus* spp., gram-negative enteric bacilli, and *Pseudomonas aeruginosa* also are implicated frequently. In children with impaired host defenses, fungal etiology (*Candida*, *Aspergillus*, *Cryptococcus*, *Histoplasma*, *Coccidioides*, and *Mucor* spp.) or uncommon pathogens, such as *Toxoplasma*, *Nocardia*, *Mycobacterium*, and *Listeria* spp., can be identified.[16] Parasites such as amebae, *Cysticercus*, *Schistosoma*, or *Paragonimus* are very rare causative pathogens.[35] *Citrobacter koseri (diversus)* and *Enterobacter sakazakii* are particularly able to cause brain abscesses in neonates.

In order of etiologic importance, the predominant organisms causing brain abscess in children are aerobic and anaerobic streptococci (60% to 70% of cases), gram-negative anaerobic bacilli (20% to 40%), Enterobacteriaceae (20% to 30%), *S. aureus* (10% to 15%), and fungi (1% to 5%).* Multiple aerobic and anaerobic organisms

*References 3, 4, 6, 7, 14, 16, 26, 35, 38, 46, 54.

are isolated in approximately one third of patients, especially in patients with chronic otitis. No growth is reported from 30 percent of properly handled purulent specimens. A reasonable speculation of the likely causative microbes can be made according to the predisposing source of infection (Table 32-2).

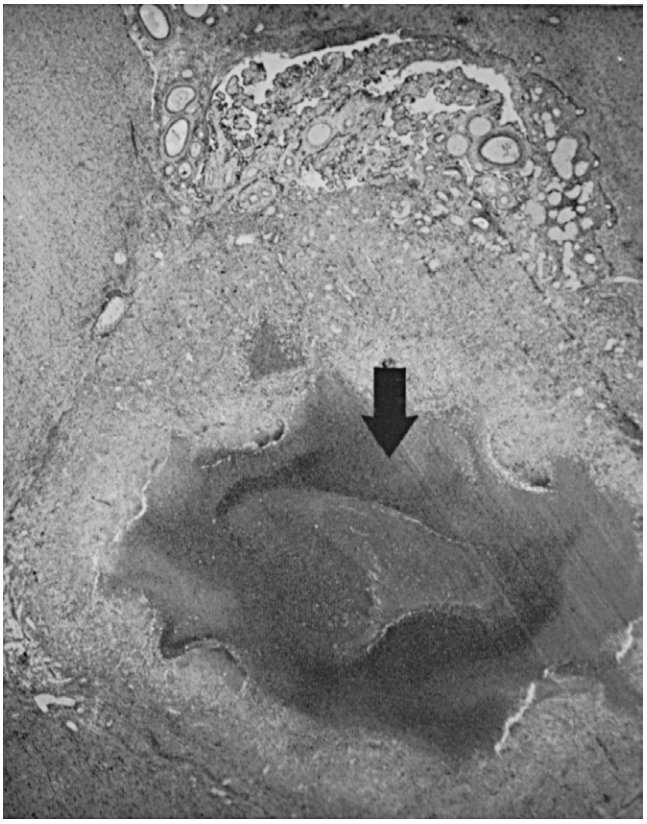

FIGURE 32-2 ■ Area of necrosis and liquefaction in a cerebral abscess *(arrow)*.

Clinical Manifestations

The clinical presentation of a brain abscess depends on the size of the collection, its location, the multiplicity of lesions, the host's immune status, and the age of the patient. Generally, symptoms and signs can be related to the effect of a space-occupying mass, to the focal neuronal dysfunction of the parenchymal region involved (see Table 32-1), or to accompanying clinical findings of the underlying predisposing infection. Because on early evaluation most children with cerebral abscess present with vague or nonspecific signs and symptoms, the physician must have a high index of suspicion to recognize the condition as early as possible (Table 32-3). When a patient develops significant alterations in mental status, the prognosis is most ominous.[11,46]

In older children and adolescents, headache is the most common initial symptom. Irritability occurs more commonly in infants and small children. The nature of the headache has no particular distinguishing features and usually is poorly localized. Sudden worsening of a preexisting headache can indicate rupture of the brain abscess into the ventricular space, which has a poor prognosis.[9]

Fever is usually a nonspecific symptom at some phase in the illness in about 50% of patients; and, overall, it may be found in up to 80% of children.[36] Drowsiness, confusion, and vomiting occur frequently during the acute phase of disease. Lethargy, stupor, and coma usually are later events and potentially associated with adverse outcome. Papilledema is present in less than one fourth of the cases, but its presence requires immediate neurosurgical assessment and CT.

Focal neurologic disturbances reflect the location of the abscess and can be detected in 30 to 50 percent of cases on presentation (see Tables 32-1 and 32-3).[55] Frontal location is characterized by development of motor speech disorders, memory deficits, personality changes, and

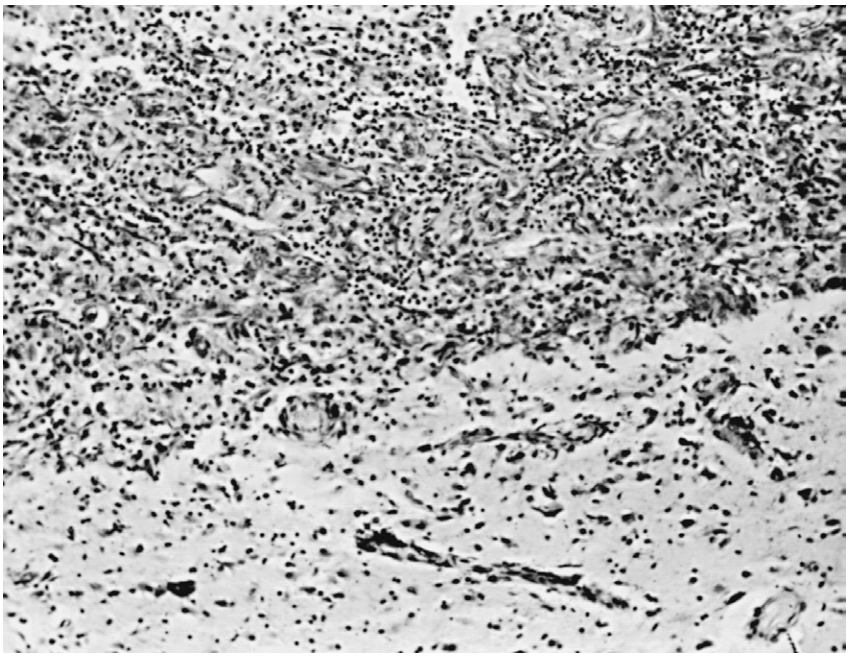

FIGURE 32-3 ■ Astroglia and fibroblasts forming the capsule of a cerebral abscess.

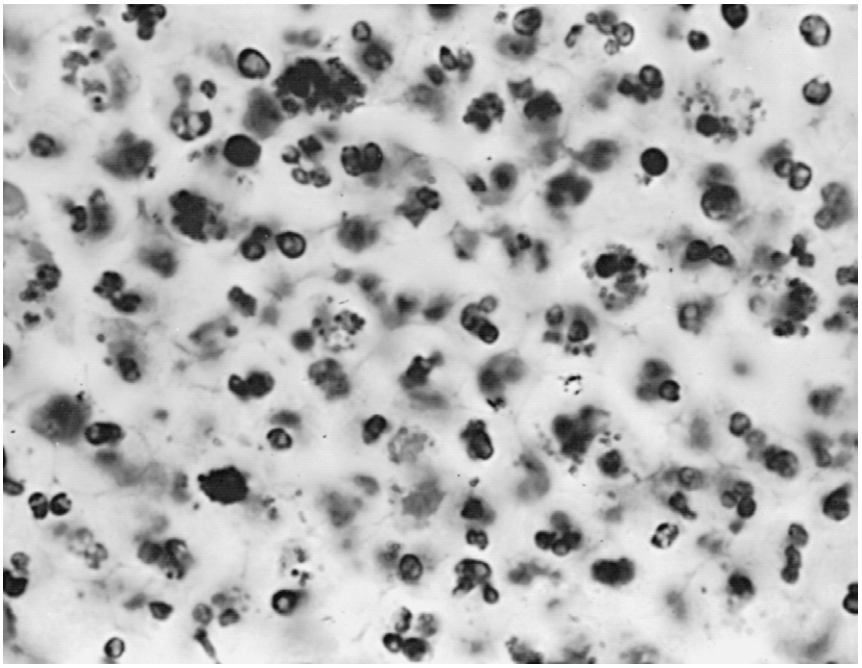

FIGURE 32-4 ■ Mononuclear and polymorphonuclear leukocytes are in the center of a cerebral abscess.

TABLE 32-2 **Primary Source, Usual Etiologic Pathogens, and Recommended Empirical Antibiotic Therapy in Children with Brain Abscess**

Primary Source	Usual Etiologic Microorganisms	Recommended Empirical Antibiotic Combination
Upper respiratory tract infection		
Sinusitis/dental infection	Viridans and anaerobic streptococci, *Haemophilus* spp., *Fusobacterium* spp., *Bacteroides* spp. (non-*fragilis*)	Penicillin or cefotaxime/ceftriaxone + metronidazole or ampicillin + chloramphenicol or sulbactam
Chronic otitis/mastoiditis	Aerobic and anaerobic streptococci, gram-negative enteric bacilli, *Bacteroides* spp. (including *B. fragilis*), *Pseudomonas aeruginosa*	Penicillin + metronidazole + ceftazidime
Head trauma	*Staphylococcus aureus,* aerobic streptococci, gram-negative enteric bacilli	Oxacillin/nafcillin (vancomycin) + cefotaxime/ceftriaxone
Postoperative	*Staphylococcus epidermidis, S. aureus,* gram-negative rods, *P. aeruginosa*	Vancomycin + ceftazidime
Metastatic spread		
Endocarditis	*S. aureus,* viridans streptococci	Oxacillin/nafcillin (vancomycin) + cefotaxime/ceftriaxone + metronidazole
Pulmonary infection	Aerobic streptococci, *Actinomyces, Fusobacterium*	Oxacillin/nafcillin (vancomycin) + cefotaxime/ceftriaxone + metronidazole
Congenital heart disease	Viridans streptococci, *Haemophilus* spp., *Haemophilus aphrophilus*	Cefotaxime/ceftriaxone + metronidazole or ampicillin + chloramphenicol or sulbactam
Bacterial meningitis	*Streptococcus pneumoniae, Haemophilus influenzae* type b, *Salmonella* spp., *Citrobacter* (neonates)	Cefotaxime/ceftriaxone ± vancomycin (resistant pneumococcal strains)
Cryptogenic source and immunosuppression*	Any type of microorganisms *Nocardia,* fungi, *Mycobacterium tuberculosis*	Oxacillin/nafcillin (vancomycin) + ceftazidime + metronidazole Oxacillin/nafcillin (vancomycin) + ceftazidime + metronidazole

*In areas with significant prevalence of methicillin-resistant staphylococcal strains or penicillin allergy, immunosuppressed children should be treated with broad coverage and with consideration of amphotericin B therapy. Antituberculous therapy should be considered for children with exposure to tuberculosis. Antibiotic regimens are likely to vary by geographic location based on resistant organisms.

TABLE 32-3 **Frequency of Presenting Signs and Symptoms in Children with Brain Abscess**

Symptoms	%	Signs	%
Headache	60-70	Focal neurologic deficits	35-50
Fever	50-80	Papilledema	30-40
Vomiting	35-55	Meningeal signs	25-35
Seizures	30-45	Hemiparesis	20-30
Mental changes	30-40	Nerve palsy	10-20
Coma	15-20	Ataxia	5-15

depressed consciousness. Hemiparesis occurs with lesions in the postfrontal region or as a consequence of uncal herniation. Abscess in the temporal lobe is characterized by a contralateral homonymous upper quadranopia. If the dominant hemisphere is affected, nominal dysphasia and aphasia are characteristic symptoms. Patients with cerebellar abscesses classically exhibit dizziness, nystagmus, defective conjugate eye movements, ataxia, tremor, and hypotonia. Seizures occur in at least 30 to 45 percent of patients, may be focal or generalized, and may occur at any time during the course of the disease. If the parietal region is involved, visual field defects, homonymous hemianopsia, dysphasia, and dyspraxia can be present.

The rapidity with which symptoms develop can vary substantially. Most patients are symptomatic within 1 week of the onset of formation of an abscess. Immunocompromised children can have a more insidious progression of clinical findings. The presentation of brain abscess in infancy can be suspected by bulging fontanelle, vomiting, irritability, and an enlarging head circumference. Seizures occur commonly, particularly in small infants, and at any time during the course of the disease.[14] School-aged children with cyanotic congenital heart disease, notably tetralogy of Fallot or transposition of the great vessels, also can exhibit symptoms and signs related to their chronic cardiac disease.

Rupture of Brain Abscess into the Ventricular System

Rupture of an abscess into the ventricle with consequent ventricular empyema is a dreaded complication because the mortality rate is greater than 50 percent, and residual neurologic deficits, including hydrocephalus, are the rule in patients who survive. Frequently, rupture occurs before the diagnosis of abscess has been established and surgical removal can occur. A sudden worsening in the patient's clinical state heralds this event. High fever, shock, meningismus, and altered consciousness are prominent clinical signs.

Rupture of the abscess into the ventricle is seen more frequently in patients with deep-seated abscesses or in immunocompromised patients.[49] Although a modest pleocytosis and elevated protein concentration in CSF may have been identified earlier, 50,000 to 100,000 polymorphonuclear leukocytes per microliter and markedly reduced glucose concentration in the CSF are usual findings. Organisms may be seen on smear of the CSF and cultured from the fluid. In other words, the patient has developed purulent meningitis, and treatment must include high doses of antibiotics and surgery (see Treatment).

The concurrence of abscess and meningitis in the past has led to the assumption that brain abscess can be a complication of meningitis; this rarely, if ever, is the case, although meningitis may develop during the incipient stages of abscess formation after intracranial invasion of organisms from a contiguous extracranial source. In such circumstances, the abscess may seem to be a consequence of the leptomeningitis. Given a potential source of infection in the ear or paranasal sinuses, the clinician must be wary and appreciate the possibility of this sequence of events. Abscess has been reported to complicate *Citrobacter* meningitis in infants, but careful pathologic study has shown vasculitis and liquefaction necrosis of the white matter without capsule formation.[54]

Laboratory Diagnosis

Laboratory tests frequently are not helpful in supporting the clinical diagnosis of brain abscess.[11,34,46] Children usually have unremarkable leukocyte counts, and the erythrocyte sedimentation rate can be normal. Blood cultures rarely are positive. Performance of a lumbar puncture is potentially dangerous because it can be associated with brain stem herniation.[10] In addition, CSF analysis uncommonly provides useful clinical information. Usual CSF findings include an elevated protein, mild mononuclear pleocytosis, and hypoglycorrhachia. CSF cultures usually are negative, unless the abscess has drained into the ventricular system or has been a complication of meningitis.

Culturing abscess material obtained at the time of surgical drainage provides the best opportunity to make a microbiologic diagnosis. Proper handling and processing, with attention given to optimal anaerobic and aerobic isolation techniques, can identify the causative organism in most cases, especially when antimicrobial therapy has not been instituted previously.[35] The high incidence of sterile cultures reported in the literature is probably because of the inadequacy of bacteriological procedures. Under such conditions, sterile cultures may reach even unjustified rates higher than 32.2 or 40 percent.[39]

Diagnosis

Magnetic resonance imaging (MRI) is used at an increasing rate in evaluation of cerebral abscess both at the time of diagnosis and in follow-up. Similar MRI findings can be observed in infarction, demyelinating disorders, and neoplastic processes. Diffusion-weighted MRI and magnetic resonance spectroscopy have been shown to be effective methods in differentiating cerebral abscesses from tumors.[6,35] Figure 32-5 illustrates the appearance on MRI of a right frontal cerebral abscess in a 10-year-old girl. CT remains an excellent alternative, however, if MRI is unavailable (Fig. 32-6). Intravenous administration of contrast material is advised because the abscess may be missed otherwise.[13,24] Serial CT or MRI

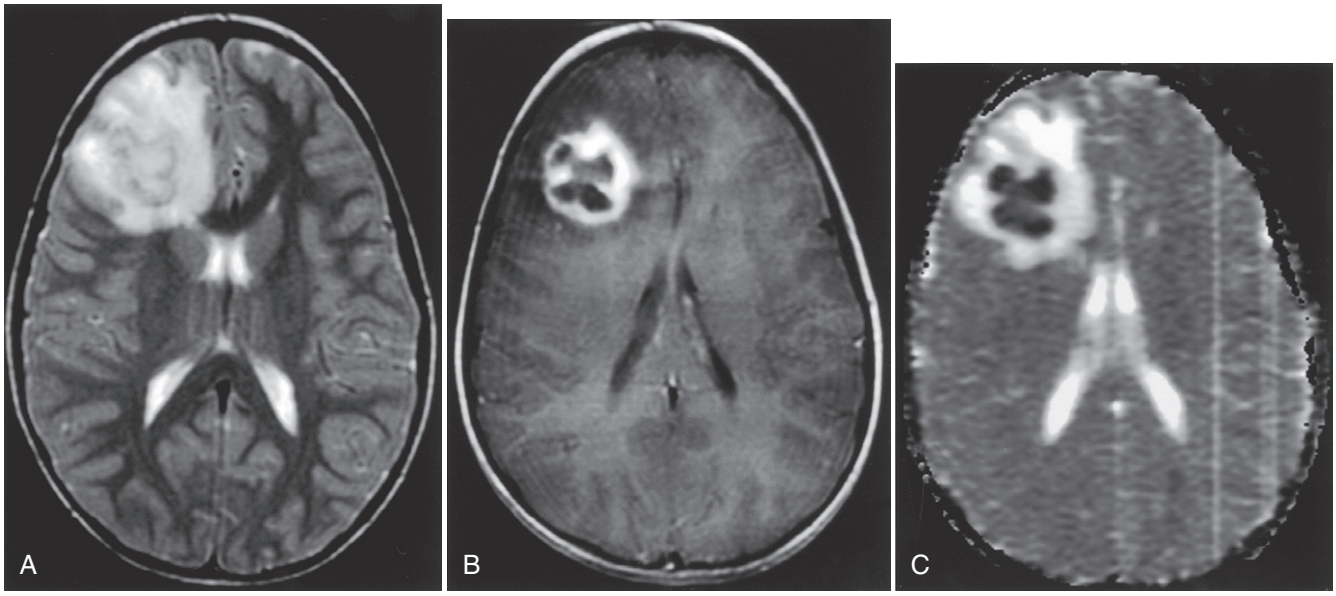

FIGURE 32-5 ■ Bacterial abscess in a 10-year-old girl. **A,** Axial T2-weighted MR image shows an irregular mass in the right frontal lobe with a hypointense wall and surrounding vasogenic edema. **B,** Gadolinium-enhanced T1-weighted MR image shows intense, smooth enhancement of the capsule. **C,** Apparent diffusion coefficient map from a line-scan, diffusion-weighted study shows decreased signal within the cavity, compatible with restricted diffusion and suggesting an abscess cavity rather than tumor necrosis. (Courtesy Kim F, MD, Northwestern University School of Medicine, Chicago.)

performed weekly or biweekly provides evaluation of the response to therapy and of the need for repeating the surgical procedure. MRI is more accurate than is CT for establishing the diagnosis of cerebritis, cerebellar abscess, edema formation, and brain stem purulent collections.[19,22] On T1-weighted sequences, brain abscesses appear hypointense and show ring enhancement after administration of intravenous gadolinium. In contrast, on T2-weighted images, the typical mature abscess has a hyperintense central area of pus surrounded by a well-defined hypointense capsule and surrounding edema.

Because specific images allow precise lesion staging, this information provides important clues for the surgeon to suspect the likelihood of encountering purulent material of a well-developed abscess at the moment of drainage. The presence of gas within the abscess cavity on head imaging suggests communication of the abscess with air outside the skull, although gas-forming bacteria in the abscess cavity rarely may be responsible.[33]

Plain skull films generally are normal in children with brain abscess. In small infants, separation of cranial sutures indicates intracranial hypertension. Radionuclide brain scan, arteriography, and ventriculography have been replaced largely by CT and MRI for the diagnosis of brain abscess. Indium-labeled leukocyte scans and thallium-brain single photon emission computed tomography (SPECT) to differentiate brain abscess from tumor in questionable cases have not been studied adequately in the pediatric population, but these methods seem promising in affected adults.[44]

Abnormalities on electroencephalography may be localized and can help in excluding a more generalized, bilateral intracranial disease, such as encephalitis. Unilateral slow waves (delta, 1 to 3/sec) characterize the usual

FIGURE 32-6 ■ Contrast-enhanced CT scan shows a ring-enhancing left frontal cerebral abscess *(arrows)* in a 3-week-old boy with concurrent *Enterobacter* meningitis. (Courtesy Robson CD, MD, Harvard Medical School, Boston, Mass., and Barnes PD, MD, Stanford University School of Medicine, Stanford, Calif.)

electroencephalographic findings in cases of cerebral abscess.

After the diagnosis of brain abscess has been established, a careful search should be made for a source of infection serving as a site of origin for hematogenous spread or direct inoculation of organisms into the central nervous system. In addition to obtaining data from the history and physical examination, the physician should extend the MRI or CT evaluation to include the mastoids and paranasal sinuses. An echocardiogram should be obtained to assess for concurrent endocarditis. Other testing should be guided by the history and physical examination findings.

Treatment

No prospective clinical trials have compared various surgical and medical treatment strategies available to guide the management of cerebral abscesses in children. Most surgical and medical treatment guidelines are based on populations consisting primarily of adult patients. Appropriate management of brain abscesses apparently requires a combined surgical and medical approach,[30] with isolated medical management limited to patients who are neurologically intact and in whom the abscess is in the cerebritis stage[21] or the abscess or abscesses are small,[43] or to patients who are too unstable to undergo a surgical procedure.

The initial treatment of solitary and multiple brain abscesses relies on the aspiration of the cavity contents followed by the initiation of empirical antibiotic coverage while the cultures are processing. CT- or MRI-guided stereotactic aspiration is accurate, minimally invasive, and associated with few complications.[17] Mamelak and colleagues[32] suggested performing head imaging biweekly or on any sign of clinical deterioration, with further aspiration if the cavity enlarges or fails to diminish after 3 to 4 weeks. Because excisions may be associated with increased risk for developing neurologic sequelae, surgery should be reserved for patients who do not respond to the strategy of repeated aspiration and medical management and when an abscess results in a significant mass effect. Madhugiri and colleagues[31] evaluated 231 children who underwent treatment for focal intradural abscess/empyema at the National Institute of Mental Health and Neurosciences, Bangalore in India. These included 57 children with cerebral abscess, 65 with supratentorial empyema, 82 with cerebellar abscess, and 27 with infratentorial empyema. All patients underwent emergency surgery (which was either bur hole and aspiration of the lesion or craniotomy/craniectomy and excision/evacuation), along with antibiotic therapy, typically 2 weeks of intravenous and 4 weeks of oral therapy. The antibiotic regimen was empirical to begin with and was altered based on the antimicrobial susceptibilities of the causative organism(s) when isolated. Hydrocephalus was managed with external ventricular drainage initially and with ventriculoperitoneal shunt if warranted. Mortality rates were 4.8 percent for cerebral abscess, 9.6 percent for cerebellar abscess, 10.8 percent for supratentorial subdural empyema, and 3.7 percent for posterior fossa subdural empyema. The choice of surgery was found to have a strong bearing on the recurrence rates and outcome in most groups, with aggressive surgery with craniotomy leading to excellent outcomes with a low incidence of residual/recurrent lesions.

Although results of cultures are pending, the selection of antibiotics should be guided by the primary source of infection, pertinent patient history, and results of microscopic examination of the pus (see Table 32-3). Meropenem monotherapy can be considered as treatment in cerebral abscess.[33] The appropriate duration of antimicrobial therapy for brain abscess is unclear. A 6- to 8-week course of parenteral antibiotics has been recommended traditionally, provided that the etiologic organisms are susceptible and adequate surgical drainage is achieved.[34] In selected cases with uncomplicated infection and complete surgical removal of a well-delineated abscess, shorter courses of treatment (3 to 4 weeks) are likely to be sufficient.[47] Nonetheless, many authorities recommend 2 to 3 months of additional oral antimicrobial therapy to prevent relapses.[11] Some guidelines recommend switching to oral antibiotics after 2 weeks if the culture was positive and continued the same for 4 weeks. The reasons for the switch to oral antibiotics after 2 weeks are mainly logistical and economic because patients in developing countries find it difficult to stay in hospital (far away from their homes) for 6 weeks.[31] In fungal, nocardial, and helminthic infection, surgical intervention frequently is required because of failure of medical treatment. Surgical procedures can be used to reduce increased intracranial pressure, obtain pus for microbiologic diagnosis, and enhance the antibiotic efficacy. In carefully selected patients (illness duration ≤2 weeks, neurologically intact, no signs of increased intracranial pressure, and abscess ≤3 cm in diameter), medical therapy alone can be successful.

With the advent of head imaging and the current methods of treatment employing head imaging, an overall reduction in the mortality rate has occurred; however, a high proportion of children with cerebral abscesses develop neurologic deficits. Eradication of potential sources of infection before the abscess has developed is logical preventive medicine. Early diagnosis and treatment are imperative and can be facilitated by the liberal use of MRI or CT when this diagnosis is even a remote consideration.

Antibiotic therapy, emergency surgery, and management of associated complications are the mainstays of treatment of these lesions.

ADJUNCTIVE AGENTS

The use of corticosteroids for treatment of brain abscess is controversial. These agents usually are indicated to control life-threatening intracranial hypertension that is associated with risk for herniation or when significant symptoms and signs are presumed to be the result of cerebral edema. Severe brain edema may necessitate the additional administration of intravenous mannitol. Corticosteroids interfere with the inflammatory response,

retard the encapsulation process, increase development of necrosis, potentially reduce antibiotic concentrations inside the purulent collection, and alter CT images.[42]

Anticonvulsants are recommended in children who have developed seizures potentially to prevent further episodes. Duration of anticonvulsant therapy should be individualized and guided by an electroencephalographic study in the follow-up phase of disease. Most authors recommend providing at least 3 months of prophylaxis if no more seizures have occurred.[11]

SUBDURAL EMPYEMA

Subdural empyema is defined as a collection of pus in the preformed space between the cranial dura mater and arachnoid mater, while epidural abscess is produced by suppurative infection in the potential epidural space.[51]

Pyogenic infection in the subdural space is designated as subdural empyema or sometimes, but less correctly, as subdural abscess. The sources of infection and microorganisms responsible are the same as those encountered in brain abscess. It is a rare disease; only one case was seen at St. Louis Children's Hospital during a period when 122 patients with bacterial meningitis and 3 patients with cerebral abscess were treated. The primary source of subdural empyema in this single case was not found, which increasingly is true for the pediatric experience. Farmer and Wise[15] and Jacobson and Farmer[25] found associated meningitis in six of eight infants with subdural empyema (seen over a 16-year period), suggesting that the latter was a complication of the former. We also have encountered this situation, albeit rarely, and the subdural fluid usually is turbid, rather than frankly purulent.

In older children, the infection apparently does not follow leptomeningitis. Although leptomeningitis may complicate subdural empyema, infections of the paranasal sinuses and mastoid region, usually chronic, spread to the subdural space directly because of osteomyelitis or by way of infected veins that penetrate the skull. Extension to cortical veins and to major venous sinuses frequently is associated with subdural empyema, as discussed elsewhere in this section. Why in some cases the infection is restricted to one or another anatomic site or sites (e.g., epidural space, subdural space, parenchyma of the brain, or blood vessels) is unknown. Subdural empyema may be hematogenous in origin. It is an infrequent but recognized complication of intracranial surgery. The anatomy of the subdural space is such that the infection often extends widely over one or both cerebral hemispheres, and accumulation of pus in the parafalcine region occurs commonly.

Clinical Manifestations

The symptoms and signs of the primary source of infection may be prominent, subtle, or absent. Increasingly severe headache, high fever, signs of meningeal irritation, and progressive neurologic deficits referable to the site of the lesion are reported in the typical untreated case. Focal or generalized seizures are prominent, especially in cases of cortical injury from associated vasculitis. Signs of increased intracranial pressure become prominent as the mass of pus enlarges. In infants, a fullness of fontanelle, vomiting, and depressed responsiveness are seen. Transillumination of the skull can be positive. Older children also may develop papilledema. As the intracranial pressure increases, symptoms and signs progress, ultimately leading to temporal lobe or cerebellar herniation and the characteristic syndromes of these complications.

Diagnosis

The laboratory findings reflect the active infectious process. Peripheral leukocytosis and a predominance of polymorphonuclear leukocytes with immature cells frequently are seen. The CSF in infants reflects the common association with leptomeningitis, and the findings depend on when the fluid is examined. Before the meningitis has been treated adequately, organisms may be cultured; the glucose concentration may be low, and the protein concentration high in the CSF. Later, the CSF findings are the same as those for treated meningitis, but viable organisms can be recovered from the subdural collections when the CSF is sterile. Specific antigens may be shown in subdural collections by tests such as latex agglutination in the absence of viable organisms. In older children, the characteristic CSF findings include elevated pressure with a few to a few hundred or more leukocytes per microliter, with polymorphonuclear leukocytes predominating. The protein concentration frequently is elevated, the glucose concentration is normal, and the fluid is sterile.

In the past 2 or more decades, subdural empyema in children has become more difficult to diagnose clinically because of antimicrobial therapy. When initiated early, antimicrobial therapy may attenuate the dramatic nature of the disease, especially the symptoms and signs of acute infection. Because the diagnosis of subdural empyema often is confused with that of brain abscess, radiographic studies are necessary to establish the correct diagnosis. CT may be the most cost-effective imaging modality in epidural abscess and subdural empyema because of its accessibility and sensitivity.[5] CT scans can appear normal in up to 50% of the patients; therefore, a high index of suspicion is required, coupled with subtle CT scan clues, including minimal midline shift, cortical enhancement, and edema in the adjacent cerebral hemisphere.[40] The use of thin-slice coronal CT scans may help to reduce uncertainty in diagnosis.[37] In our series, the initial CT scan revealed negative findings in 10 cases, which led to delayed diagnosis (Fig. 32-7).

MRI, if available in the acute setting, may be the imaging modality of choice because it provides a better anatomic delineation of any collections that may be present than does CT, and it can adequately display areas of localized meningeal infection.[10] The use of MRI, however, must be weighed against the need for urgent diagnosis and intervention for patients in extremis (Fig. 32-8).

FIGURE 32-7 ■ CT scan shows a subdural empyema with marked displacement of the ventricular system.

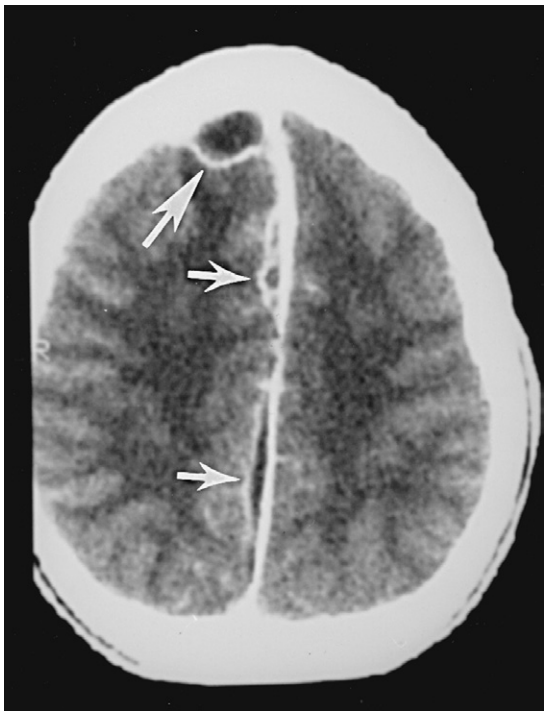

FIGURE 32-8 ■ Contrast-enhanced CT scan shows a right frontal epidural abscess *(large arrow)* concurrent with an interhemispheric subdural abscess *(small arrows)* in a 13-year-old boy who developed headache, fever, and vomiting after fracture of the right frontal sinus. (Courtesy Robson CD, MD, Harvard Medical School, Boston, Mass., and Barnes PD, MD, Stanford University School of Medicine, Stanford, Calif.)

Treatment

Until a causative organism is identified, the patient should receive broad-spectrum antibiotics intravenously in dosages appropriate for bacterial meningitis. After the offending organism is identified, the sensitivities of that organism to specific medications dictate selection of more precise antibiotic therapy. In infants, antibiotic therapy may be sufficient when the fluid is cloudy only, but if thick, purulent material is obtained by subdural paracentesis or, in older children, if the clinical and radiographic evidence indicates a subdural empyema, surgery is requisite. Depending on individual circumstances, craniotomy or multiple bur holes with irrigation of the subdural space should be accomplished.

Mortality and morbidity rates are high (20% to 40%) in most series but lower in some.[48,56] Early diagnosis and treatment are imperative. The early use of CT seems to be responsible for improved therapeutic results.[56]

EPIDURAL ABSCESS

Because the dura mater is adherent to the inner aspect of the cranium, epidural abscesses rarely attain a large size, and they consequently do not exert significant pressure on the brain. They are important because they can serve as a focus for spread of infection into the subdural space, leptomeninges, or brain. The infection may involve local penetrating vessels and lead to their occlusion, with extension to venous sinuses and other vessels. Infection of the middle ear, mastoid bone, or ancillary air sinuses may lead to epidural abscess, as in the case of subdural empyema and brain abscess. Often, epidural abscess coexists with the other lesions and presumably develops first. Osteomyelitis may be evident, which usually is the case with mastoiditis and often is evident after head injury with comminuted fracture of the skull or after intracranial surgery. A case of an adolescent boy with osteomyelitis and epidural abscess complicating a wrestling injury has been reported.[52]

Local pain, tenderness, and fever may be the only signs. Treatment usually includes antibiotics and surgery in the presence of osteomyelitis. Exclusion of associated intracranial infections is mandatory; the epidural infection serves to raise these diagnostic possibilities.

SPINAL EPIDURAL INFECTIONS

Spinal epidural infections may be acute or chronic, and they may be restricted in their extent or extend longitudinally over many segments of the spinal cord because the epidural space offers no resisting structures. Most often, the process affects the posterior region of the spine, with maximal pus or granulomatous tissue found over the dorsal aspect of the cord or spinal roots. In Danner and Hartman's[12] series from the New York Hospital, however, in approximately half of the patients, the pus primarily was anterior to the cord. In spinal osteomyelitis, the pus may be more viscous ventrally. Occasionally, the purulent material may encircle the neural

elements. In infants and children, this condition is rare; no case was encountered at the St. Louis Children's Hospital during the 39 months in which 122 cases of bacterial meningitis were seen. In an extensive report by Baker and colleagues[4] covering a 27-year period at the Massachusetts General Hospital, only 6 of 39 patients were younger than 20 years of age, and the youngest was 11 years old. The incidence ranged from 0.2 to 1.2 hospital admissions each year. In approximately half of the patients in this series, acute purulent material was discovered at operation or autopsy, with *S. aureus* incriminated in more than half of the cases. In the remainder, a granulomatous process was found, associated with a wide variety of bacteria. No instance of tuberculous infection occurred, although in many regions of the world this organism remains an important consideration. Nonbacterial infective agents may include fungi or parasites.

The neural dysfunction probably results from direct compression of the spinal cord, roots, and nerves, but impaired circulation from associated inflammation and occlusion of vessels is at least a contributory factor in some cases. Determining with certainty the extent to which each of the previous mechanisms contributes to neural dysfunction often is difficult. Extensive necrosis of the cord may result in advanced cases in which a prompt diagnosis has not been made.

Sources of Infection

In tuberculous and other chronic infections, osteomyelitis and intervertebral disk infection are common occurrences, but adults primarily are affected. Acute spinal epidural infections usually occur after hematogenous spread from furuncles, pharyngitis, dental abscesses, decubitus ulcers, and urinary tract and wound infections.[28] They may complicate spinal surgery, and lumbar puncture rarely has been implicated as a source.[6] Osteomyelitis seldom occurs. The patient may have a history of minor trauma to the back. Presumably, trauma results in local tissue injury or hemorrhage, forming a nidus for the developing infection.

Clinical Manifestations

Fever is the rule, and the temperature is higher in patients with acute infections. Patients appear septic, and toxic delirium occurs frequently. Heusner,[21] in his classic article dealing primarily with acute epidural abscesses, divided the clinical phases as follows: (1) spinal ache, (2) root pain, (3) weakness, and (4) paralysis. Although certain therapeutic implications render this separation a useful way to consider the disorder, phases 3 and 4 are combined in the following discussion. That the various stages often overlap is axiomatic.

Phase 1: Spinal Ache

Spinal ache was a universal finding in Heusner's experience.[21] In a report from Baker and associates,[4] all 39 patients had backache of various degrees of severity. Local tenderness was absent in only 2 of their patients and should be searched for by carefully tapping over the spine.

Phase 2: Root Pain

Root pain is characteristic and is an early symptom that may assist in localization of the pathologic process. Root pain especially is prominent with lumbosacral disease in which roots are implicated without involvement of the spinal cord. The diagnosis is suspected infrequently until functional motor and sensory losses occur, which is unfortunate because therapy is most effective during the early stages. Progression to symptoms and signs of spinal cord involvement usually occurs within a few days except when the process is granulomatous, in which case the course tends to be prolonged, extending over several or more weeks.

Phases 3 and 4: Weakness and Paralysis

After weakness and impaired sensation referable to disease of the spinal cord appear, the progression to paralysis can be rapid, and immediate surgical treatment is imperative to maximize the likelihood of reasonable functional recovery. Even appropriate therapy at this stage often is ineffective, however, in restoring normal neurologic functions. Death occurs in at least 20 percent of cases; this rate has not changed significantly since 1948, despite the availability of a wide range of antibiotic agents.

Diagnosis

The value of MRI in the diagnosis and differential diagnosis of pediatric spinal infection is well established.[29] Diagnostic MRI findings of spinal infection include bone marrow edema, destruction of the affected vertebrae, abnormal signal intensity of the contiguous disc, and paraspinal or epidural extension of infection (Fig. 32-9). Epidural and paravertebral soft tissue changes on short-term follow-up MRI correlated better with patients' clinical symptoms compared with bone and disc destruction. Bone and disc changes can appear to progress more so than soft tissue infection despite adequate clinical response to therapy. Although MRI is essential for initial diagnosis, serial routine follow-up MR images were not necessary for the vast majority of cases. Wang and colleagues[53] examined 35 whole-spine and 16 localized spinal scans from 17 patients (age 2 months to 16 years, 9F:8M) who had 51 follow-up imaging studies done 2 weeks to 4.75 years after baseline. Seven children (41%) younger than 3 years of age underwent 33 follow-ups (65%); most required general anesthesia or conscious sedation. Short-term follow-up scans demonstrated epidural and/or paraspinal soft tissue changes correlating with clinical status and laboratory findings in all cases. However, MRI showed that bone and/or disk abnormalities continued and progressed in some cases despite clinical improvement.[53]

Follow-up MRI is not needed for patients with good clinical response; these patients should be observed

FIGURE 32-9 ■ Gadolinium-enhanced, sagittal, T1-weighted MR image shows a rim-enhancing lumbar spinal epidural abscess *(arrows)* in a 15-year-old boy with a 3-week history of lower back pain, followed by rapidly progressing left leg numbness and decreased bowel and bladder function. *Staphylococcus aureus* was cultured from purulent fluid removed after L4-L5 laminectomy was performed. (Courtesy Robson CD, MD, Harvard Medical School, Boston, Mass., and Barnes PD, MD, Stanford University School of Medicine, Stanford, Calif.)

clinically and/or have laboratory markers of inflammation tested. The segment of the pediatric population that might benefit from follow-up MRI includes those whose condition deteriorates clinically and those too young for a clinical response to be accurately assessed. If follow-up MRI is indicated, a localized spine scan rather than a whole-spine scan is recommended unless disease extension beyond the original focus is suggested. After epidural abscess is discovered, immediate neurosurgical intervention is necessary to prevent long-term neurologic sequelae. At the time of surgery, stains and cultures for aerobic and anaerobic bacteria, mycobacteria, and fungi should be obtained. If lumbar puncture is attempted when epidural abscess is suspected, the spinal needle (with stylet) is advanced slowly into the lumbar region, with periodic removal of the stylet and with suction applied gently *before* the thecal sac is entered.

If purulent material is obtained, the diagnosis is established, and the pus must be examined by a Gram-stained smear and cultured on various media under aerobic and anaerobic conditions. The leptomeninges should not be penetrated if purulent material is encountered; otherwise, CSF should be obtained. Characteristically, the CSF is clear or slightly opalescent and yellow if there is a block. Pleocytosis with a few too many hundred cells (with lymphocytes predominating) reflects a contiguous infectious process, but in the absence of meningitis no organisms can be identified and the CSF glucose concentration should be normal. The protein concentration always is elevated, and the level may be very high (several hundred to 2000 mg/dL) in the case of a partial or total manometric block.

The differential diagnosis includes myelitis caused by bacterial meningitis, syphilis, viruses, and a parainfectious process and by the syndrome of acute transverse myelopathy of unknown cause. Spinal ache is most prominent in acute transverse myelopathy, but, as a general rule, the entire illness is compressed in time, with paresis or paralysis evolving over the course of hours or a few days from the onset of disease. Impaired circulation of CSF does not occur in this or the aforementioned disorders. Rarely, a lymphoma may mimic a spinal epidural abscess. Spinal cord tumors, vascular malformations, and arachnoiditis are considerations when the course of disease is prolonged and evidence of sepsis is minimal or absent, as occurs with chronic epidural infections.

Treatment

Owing to variable etiology, identification of the responsible microorganism through neurosurgical drainage followed by long-term intravenous antibiotics remains the mainstay in treating extra-axial CNS infections. Optimal outcome is achieved with early diagnosis and therapy. Initial empirical antibiotic coverage is similar to that for brain abscess, although antifungal therapy need not be included, unless cultures or stains are positive for fungi. Treatment typically should be continued for 3 to 4 weeks, but it should be prolonged for twice this period if the patient has osteomyelitis. Despite advances, the morbidity and mortality rates for spinal epidural abscess remain high. One third of children with the disease die, and another third are left with permanent neurologic sequelae, including weakness, incontinence, and sensory abnormalities. Rapid diagnosis and treatment are essential to ensure a successful outcome.

NEW REFERENCES SINCE THE SIXTH EDITION

17. Ghannane H, Laghmari M, Aniba K, et al. Diagnostic and management of pediatric brain stem abscess, a case-based update. *Childs Nerv Syst* 2011;**27**:1053–62.
31. Madhugiri VS, Sastri BV, Srikantha U, et al. Focal intradural brain infections in children: an analysis of management and outcome. *Pediatr Neurosurg* 2011;**47**:113–24.
40. Osman Farah J, Kandasamy J, May P, et al. Subdural empyema secondary to sinus infection in children. *Childs Nerv Syst* 2009; **25**:199–205.
53. Wang Q, Babyn P, Branson H, et al. Utility of MRI in the follow-up of pyogenic spinal infection in children. *Pediatr Radiol* 2010;**40**: 118–30.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Amano K, Kamano S. Cerebellar abscess due to penetrating orbital wound. *J Comput Assist Tomogr* 1982;**6**:1163–6.
2. Armstrong D. Central nervous system infections in the immunocompromised host. *Infection* 1984;**12**(Suppl 1):S58–64.
3. Arseni C, Horvath L, Dumitrescu L. Cerebral abscesses in children. *Acta Neurochir (Wien)* 1966;**14**:197–224.
4. Baker AS, Ojemann RG, Swartz MN, Richardson Jr EP. Spinal epidural abscess. *N Engl J Med* 1975;**293**:463–8.
5. Banerjee AD, Pandey P, Devi BI, et al. Pediatric supratentorial subdural empyemas: a retrospective analysis of 65 cases. *Pediatr Neurosurg* 2009;**45**:11–18.
6. Barlas O, Sencer A, Erkan K, et al. Stereotactic surgery in the management of brain abscess. *Surg Neurol* 1999;**52**:404–10; discussion 411.
7. Bergman I, Wald ER, Meyer JD, Painter MJ. Epidural abscess and vertebral osteomyelitis following serial lumbar punctures. *Pediatrics* 1983;**72**:476–80.
8. Brook I. Brain abscess in children: microbiology and management. *J Child Neurol* 1995;**10**:283–8.
9. Choudhari KA. Prodromal signs and clinical factors influencing outcome in patients with intraventricular rupture of purulent brain abscess. *Neurosurgery* 2001;**49**:481–3.
10. Chun CH, Johnson JD, Hofstetter M, Raff MJ. Brain abscess: a study of 45 consecutive cases. *Medicine (Baltimore)* 1986;**65**: 415–31.
11. Cochrane DD. Consultation with the specialist. Brain abscess. *Pediatr Rev* 1999;**20**:209–15.
12. Danner RL, Hartman BJ. Update on spinal epidural abscess: 35 cases and review of the literature. *Rev Infect Dis* 1987;**9**:265–74.
13. de Louvois J, Gortvai P, Hurley R. Antibiotic treatment of abscesses of the central nervous system. *BMJ* 1977;**2**:985–7.
14. Domingo Z, Peter JC. Brain abscess in childhood: a 25-year experience. *S Afr Med J* 1994;**84**:13–15.
15. Farmer TW, Wise GR. Subdural empyema in infants, children and adults. *Neurology* 1983;**23**:254–61.
16. Fischer EG, McLennan JE, Suzuki Y. Cerebral abscess in children. *Am J Dis Child* 1981;**135**:746–9.
17. Ghannane H, Laghmari M, Aniba K, et al. Diagnostic and management of pediatric brain stem abscess, a case-based update. *Childs Nerv Syst* 2011;**27**:1053–62.
18. Goodkin HP, Harper MB, Pomeroy SL. Intracerebral abscess in children: historical trends at Children's Hospital Boston. *Pediatrics* 2004;**113**:1765–70.
19. Haimes AB, Zimmerman RD, Morgello S, et al. MR imaging of brain abscesses. *AJR Am J Roentgenol* 1989;**152**:1073–85.
20. Hegde AS, Venkataramana NK, Das BS. Brain abscess in children. *Childs Nerv Syst* 1986;**2**:90–2.
21. Heusner AP. Nontuberculous spinal epidural infections. *N Engl J Med* 1948;**239**:845–54.
22. Hunter JV, Morriss MC. Neuroimaging of central nervous system infections. *Semin Pediatr Infect Dis* 2003;**14**:140–64.
23. Idriss ZH, Gutman LT, Kronfol NM. Brain abscesses in infants and children: current status of clinical findings, management and prognosis. *Clin Pediatr (Phila)* 1978;**17**:738–40, 45–6.
24. Itakura T, Yokote H, Ozaki F, et al. Stereotactic operation for brain abscess. *Surg Neurol* 1987;**28**:196–200.
25. Jacobson PL, Farmer TW. Subdural empyema complicating meningitis in infants: improved prognosis. *Neurology* 1981;**31**:190–3.
26. Jadavji T, Humphreys RP, Prober CG. Brain abscesses in infants and children. *Pediatr Infect Dis* 1985;**4**:394–8.
27. Jamjoom A. Childhood brain abscess in Saudi Arabia. *Ann Trop Paediatr* 1997;**17**:95–9.
28. Kaufman DM, Kaplan JG, Litman N. Infectious agents in spinal epidural abscesses. *Neurology* 1980;**30**:844–50.
29. Khoury NJ, Hourani MH, Arabi MM, et al. Imaging of back pain in children and adolescents. *Curr Probl Diagn Radiol* 2006; **35**:224–44.
30. Leys D, Christiaens JL, Derambure P, et al. Management of focal intracranial infections: is medical treatment better than surgery? *J Neurol Neurosurg Psychiatry* 1990;**53**:472–5.
31. Madhugiri VS, Sastri BV, Srikantha U, et al. Focal intradural brain infections in children: an analysis of management and outcome. *Pediatr Neurosurg* 2011;**47**:113–24.
32. Mamelak AN, Mampalam TJ, Obana WG, Rosenblum ML. Improved management of multiple brain abscesses: a combined surgical and medical approach. *Neurosurgery* 1995;**36**:76–85; discussion 86.
33. Martin-Canal G, Saavedra A, Asensi JM, et al. Meropenem monotherapy is as effective as and safer than imipenem to treat brain abscesses. *Int J Antimicrob Agents* 2010;**35**:301–4.
34. Mathisen GE, Johnson JP. Brain abscess. *Clin Infect Dis* 1997;**25**:763–79; quiz 80–1.
35. Molinari GF, Smith L, Goldstein MN, Satran R. Brain abscess from septic cerebral embolism: an experimental model. *Neurology* 1983;**23**:1205–10.
36. Mutluer S. Bacterial infections of the central nervous system. In: Choux M, Di Rocco C, Hockley A, Walker M, editors. *Pediatric neurosurgery*. London: Churchill-Livingstone; 1999. p. 617–36.
37. Nathoo N, Nadvi SS, van Dellen JR, Gouws E. Intracranial subdural empyemas in the era of computed tomography: a review of 699 cases. *Neurosurgery* 1999;**44**:529–35; discussion 35–6.
38. Nielsen H. Cerebral abscess in children. *Neuropediatrics* 1983;**14**: 76–80.
39. Osenbach RK, Loftus CM. Diagnosis and management of brain abscess. *Neurosurg Clin North Am* 1992;**3**:403–20.
40. Osman Farah J, Kandasamy J, May P, et al. Subdural empyema secondary to sinus infection in children. *Childs Nerv Syst* 2009; **25**:199–205.
41. Pruitt AA, Rubin RH, Karchmer AW, Duncan GW. Neurologic complications of bacterial endocarditis. *Medicine (Baltimore)* 1978; **57**:329–43.
42. Quartey GR, Johnston JA, Rozdilsky B. Decadron in the treatment of cerebral abscess: an experimental study. *J Neurosurg* 1976;**45**: 301–10.
43. Rosenblum ML, Hoff JT, Norman D, et al. Nonoperative treatment of brain abscesses in selected high-risk patients. *J Neurosurg* 1980;**52**:217–25.
44. Ruiz A, Ganz WI, Post MJ, et al. Use of thallium-201 brain SPECT to differentiate cerebral lymphoma from *Toxoplasma* encephalitis in AIDS patients. *AJNR Am J Neuroradiol* 1994;**15**:1885–94.
45. Sáez-Llorens X, McCracken Jr GH. Bacterial meningitis in neonates and children. *Infect Dis Clin North Am* 1990;**4**:623–44.
46. Sáez-Llorens XJ, Umana MA, Odio CM, et al. Brain abscess in infants and children. *Pediatr Infect Dis J* 1989;**8**:449–58.
47. Skoutelis AT, Gogos CA, Maraziotis TE, Bassaris HP. Management of brain abscesses with sequential intravenous/oral antibiotic therapy. *Eur J Clin Microbiol Infect Dis* 2000;**19**:332–5.
48. Smith HP, Hendrick EB. Subdural empyema and epidural abscess in children. *J Neurosurg* 1983;**58**:392–7.
49. Takeshita M, Kagawa M, Izawa M, Takakura K. Current treatment strategies and factors influencing outcome in patients with bacterial brain abscess. *Acta Neurochir (Wien)* 1998;**140**:1263–70.
50. Tekkok IH, Erbengi A. Management of brain abscess in children: review of 130 cases over a period of 21 years. *Childs Nerv Syst* 1992;**8**:411–16.
51. Tsai YD, Chang WN, Shen CC, et al. Intracranial suppuration: a clinical comparison of subdural empyemas and epidural abscesses. *Surg Neurol* 2003;**59**:191–6; discussion 6.
52. Tudor RB, Carson JP, Pulliam MW, Hill A. Pott's puffy tumor, frontal sinusitis, frontal bone osteomyelitis, and epidural abscess secondary to a wrestling injury. *Am J Sports Med* 1981;**9**:390–1.
53. Wang Q, Babyn P, Branson H, et al. Utility of MRI in the follow-up of pyogenic spinal infection in children. *Pediatr Radiol* 2010;**40**: 118–30.
54. Woods Jr CR. Brain abscess and other intracranial suppurative complications. *Adv Pediatr Infect Dis* 1995;**10**:41–79.
55. Yogev R. Focal suppurative infections of the central nervous system. In: Long SS, Pickering LK, Prober CG, editors. Principles and practice of pediatric infectious diseases. 2nd ed, vol. 1. New York: Churchill Livingstone; 2003. p. 302–312.
56. Zimmerman RD, Leeds NE, Danziger A. Subdural empyema: CT findings. *Radiology* 1984;**150**:417–22.

CHAPTER 33

Fungal Meningitis

José R. Romero • Richard F. Jacobs

Fungi are rare causes of meningitis in children. *Candida albicans*, *Cryptococcus neoformans*, and *Coccidioides immitis* are the isolates found most commonly in pediatric patients. Making a diagnosis can be difficult. Fungal meningitis frequently is chronic, and patients may have few symptoms. The lack of obvious meningeal signs and symptoms and the uncommon incidence of a fungal cause often delay establishing the diagnosis. Inherent problems with the diagnosis of fungal infections of the central nervous system (CNS) begin with the basic microbiology of the organisms. Their fastidious growth, the prolonged time needed for culture, and the requirement of special media can render making a diagnosis of meningitis difficult.

Because cultivating many fungi from the cerebrospinal fluid (CSF) frequently is difficult, the use of serologic tests for antibodies and antigens helps define the infection more quickly and with more sensitivity. These tests can be done on CSF, serum, and, in some instances, urine.[168] For most infections, lipid formulations of amphotericin B have become the drug of choice, although its supremacy is being tested by some of the newer triazoles. CNS disease caused by fungi generally has high morbidity and mortality rates.

EPIDEMIOLOGY

The epidemiology of fungal meningitis depends on many factors. The geographic location of the patient or travel to an endemic area can be an important clue to defining the cause of the infection. The geographic distribution of fungal meningitis varies in the United States and worldwide. Histoplasmosis generally occurs in endemic areas of the Mississippi River valley.[167] Coccidioidomycosis occurs in the San Joaquin Valley and the desert Southwest of the United States and in Mexico.[3,112] Cryptococcosis distribution is worldwide, rather than in discrete endemic areas, but it seems to be associated with pigeon droppings and nesting areas of other birds.[93] Blastomycosis has a sporadic pattern of infectivity but generally occurs in states bordering the Mississippi and Ohio River basins, with occasional outbreaks occurring in the Great Lakes region and Canada.[44,79,138] *Candida* spp., *Aspergillus* spp., *Sporothrix schenckii*, and other fungal pathogens generally are not defined by geographic boundaries but depend more on environmental exposures and the immunocompetence of the individual. Uncommon species of yeasts and fungi, including *Rhodotorula rubra*, *Aureobasidium mansoni*, *Clavispora lusitaniae*, and *Bipolaris spicifera*, have been reported in nosocomial cases of meningitis.[64,85]

Many fungal infections (particularly infections caused by *Histoplasma* and *Candida* spp.) do not usually cause meningitis, unless the host is immunocompromised. The number of cases of cryptococcal meningitis has increased dramatically in association with the acquired immunodeficiency syndrome (AIDS) epidemic. A review from the mid-1980s by the Centers for Disease Control and Prevention showed an incidence rate of 6.8 percent for cryptococcal meningitis in patients with AIDS; however, the rate in pediatric patients with AIDS was 10 times lower (0.6%).[136] In the pre-AIDS era, patients known to be at risk for acquiring disseminated cryptococcosis included patients with lymphoma, patients on high-dose corticosteroid therapy, and patients with underlying cellular immune dysfunction.[19] Risk factors for developing *Candida* meningitis are similar to those for candidemia and include prolonged antimicrobial therapy, indwelling venous or ventricular catheters, hyperalimentation, corticosteroid use, recent neurosurgery or intra-abdominal surgery, and intravenous drug abuse.[109,132,150] Pediatric cases occur most commonly in neonates, particularly in newborns with very low birth-weight.[8,52,66,69,143] Of neonates with candidemia, 5 to 9 percent have associated meningitis.[26]

Inhalation is the most common means by which many fungi infect the host. Other fungi can be inoculated directly into the skin; this inoculation can occur in the outdoor environment or in the hospital setting (e.g., intravenous catheters), especially in infants with very low birth-weight.[8]

CLINICAL MANIFESTATIONS

Infections caused by *Candida* spp. in newborns with very low birth-weight can be particularly difficult to diagnose because of the broad range of symptoms. Most infants with disseminated candidiasis with meningitis present with respiratory distress and a supplemental oxygen requirement, and most progress to require mechanical ventilation.[8] *Candida* usually is identified in endotracheal washings, urine, and blood in patients with *Candida* meningitis, but infants have symptoms on average 11 days before the diagnosis is made. Ophthalmologic examinations may be very important in identifying disseminated *Candida* infections.[23] A careful funduscopic examination also can help determine whether increased intracranial pressure is present with papilledema. Marked

abdominal distention also occurs commonly in disseminated *Candida* infections in infants with very low birthweight and frequently is associated with guaiac-positive stools. Most patients also have temperature instability, elevated white blood cell counts, and feeding intolerance. Hepatomegaly may indicate the presence of systemic infection.

Physical manifestations diagnostic for other causes of fungal meningitis (e.g., *Cryptococcus*, *Blastomyces*, and *Histoplasma*) rarely are found because the infections usually are chronic. Looking carefully for other manifestations of fungal infections, especially the presence of skin lesions, is very important. All superficial lesions, nodules, and draining abscesses should be investigated because they may give a clue to the cause of subacute and chronic infections (e.g., *Coccidioides*, *Blastomyces*, and *Cryptococcus*).[58] Fungal stains, including India ink, should be performed on all biopsy specimens and drainage material, and all specimens should be processed for culture. Bone involvement is a common occurrence with certain fungal infections (e.g., *Cryptococcus* and *Blastomyces*).

The significance of isolating a fungus from CSF cannot be overemphasized. The finding of fungal organisms should be considered a true infection, and appropriate antifungal therapy should be initiated. A single CSF culture for *Candida*, in an otherwise immunocompetent host, or an unlikely meningeal pathogen *(Paecilomyces)* with an otherwise normal CSF should lead the physician to consider the possibility of contamination.[5] Repeat CSF cultures should be sought in such patients.

Infection with Specific Organisms

Candidal Meningitis

Candidal meningitis is rare in children. Arisoy and associates[5] reported that 2 percent of all positive CSF cultures were fungal organisms but *Candida* spp. accounted for 94.5 percent of the fungal isolates, including 9 of 23 newborn patients, 8 of whom were infants with very low birth-weight. Risk factors for positive CSF fungal cultures from neonates included antimicrobial therapy, umbilical catheterization, total parenteral nutrition, intubation, and prematurity.[37,38] In many cases of candiasis in neonates, concurrent meningitis exists but is not discovered until the time of autopsy.[26] Risk factors in children beyond the neonatal period included concurrent bacterial infection, chronic systemic or CNS disease, and the presence of central venous catheters. Histopathology models in animals have revealed hyphal invasion, vasculitis, abscesses, and acute and chronic inflammatory infiltration of meninges and brain parenchyma.[68]

Children with human immunodeficiency virus (HIV) infection are at risk for acquiring disseminated *Candida* infections, including meningitis.[90] In one study, 27 percent of HIV-infected patients with disseminated *Candida* infections had CNS involvement.[90] Nearly all HIV-infected patients who develop *Candida* infection do so as a result of nosocomial infection. Predisposing factors include oral candidiasis, central venous catheters, prolonged antibiotic therapy, and total parenteral nutrition. In HIV-infected patients, neutropenia is not a major risk factor. Simultaneous pulmonary disease, particularly viral, bacterial, or *Pneumocystis jiroveci* pneumonias, exists in most HIV-infected patients with disseminated *Candida* infection. Most patients are febrile for more than 14 days, with peak temperatures greater than 39° C (102.2° F) before the diagnosis is established. Recently, a homozygous mutation in *CARD9* has been associated with chronic mucocutaneous candidiasis and meningitis.[50] *CARD9* is an intracellular adapter molecule that receives signals from several antifungal pattern-recognition receptors.

The incidence of infection with *Candida* spp. other than *C. albicans* has increased dramatically in immunocompromised children, particularly children with malignancies. In a case series of children with leukemia, *Candida tropicalis* meningitis was uniformly fatal.[41] In addition to patients with malignancies and HIV infections, other children at risk for developing infection have been reported, including a child with myeloperoxidase deficiency.[95]

Development of resistance in *Candida* spp. is a concern. Intensive efforts to develop standardized, reproducible, and clinically relevant susceptibility testing methods for the fungi have resulted in the development of the standardized methodology for antifungal susceptibility testing of yeasts for the azole agents.[128] Isolates of *Candida glabrata* and *Candida krusei* with increasing resistance to amphotericin B have been described.[165] *C. lusitaniae* has shown intrinsic resistance to amphotericin B, and meningitis in an adult patient with a resistant strain has been reported.[133] *C. krusei* has been noted to have near uniform azole resistance, particularly in immunocompromised patients receiving suppressive azole therapy. Patients with meningitis caused by *C. glabrata* or any *Candida* spp. who are slow to clear infection, or in whom unexpected relapse occurs, should have antifungal susceptibility testing performed on their isolate.[114]

Candida meningitis usually responds to therapy with intravenous amphotericin B alone or in combination with oral flucytosine. The use of flucytosine is controversial; however, most reports support its use in meningitis. Flucytosine has been difficult to use in infants with low birth-weight because of the immaturity of their gastrointestinal tracts and the risk for developing necrotizing enterocolitis, as well as the lack of an intravenous formulation in the United States. Amphotericin B alone for systemic candidiasis, including meningitis, in neonates has been used successfully in patients who could not tolerate oral medication.[18] The most important factor in successful treatment is the initiation of therapy *quickly*, as soon as systemic *Candida* infection is suspected and frequently before the organism is isolated. Prolonged delays in initiating therapy result in high mortality rates.

Amphotericin B for children and neonates should begin at the desired 1 mg/kg daily dose and not be preceded by smaller test doses, because of the importance of early and effective therapy in improving outcome. In nonneonatal patients, a lipid formulation of amphotericin B can be used. Flucytosine can be used in nonneonatal patients at a dose of 150 mg/kg/day, divided every 6 hours. The use of flucytosine often requires careful monitoring and adjustment of the dosage based on serum

determinations. Flucytosine is not recommended for neonates.

Large, controlled trials using the azoles and newer agents for *Candida* meningitis are lacking. Huttova and coworkers[63] reported eight neonates with candidal meningitis treated with intravenous fluconazole with and without amphotericin B. Numbers from the study were too small to make conclusive recommendations. However, because of rapid clearance and differences in the volume of distribution of fluconazole in neonates, infants, and children, daily fluconazole doses in pediatric patients need to be doubled (12 mg/kg daily),[114] and more recent studies suggest a fluconazole loading dose in neonates of 25 mg/kg.[122] Lipid formulations of amphotericin B increasingly are preferred in adults, and liposomal amphotericin B was used successfully in five of six cases of *Candida* meningitis in newborns.[134] Optimal dosing is unknown, but many physicians prescribe liposomal amphotericin B or amphotericin B lipid complex at a dose of 5 mg/kg daily. Marr and associates[98] reported on the use of fluconazole plus flucytosine for the treatment of *Candida* meningitis in an infant with a very low birthweight. Voriconazole has excellent penetration of the CNS and is active against most *Candida* isolates causing CNS infections; however, the clinical experience with voriconazole is too limited to recommend its use at this time.[70] Caspofungin and the other echinocandins do not achieve adequate CSF concentrations for treatment of *Candida* meningitis,[30,124] but they do achieve appropriate brain parenchyma concentrations and have been successfully used for therapy.[62]

Cryptococcosis

Cryptococcal meningitis was rare in the United States in the pre-HIV era. With the growth of the AIDS epidemic, however, this infection has become the most common cause of fungal meningitis in immunocompromised patients and infects 2 to 9 percent of adults with AIDS.[24,33,81,175] In the United States and worldwide it remains an uncommon finding in pediatric patients.[91,107,129,148] Cryptococcosis is a systemic fungal infection, and meningitis is its most serious manifestation. Patients with initial pulmonary involvement may have few symptoms but can present with fever, cough, weight loss, and dyspnea on exertion.[99] In adult studies, progressively severe headaches without the presence of fever were common manifestations.[152] Patients with cryptococcal meningitis frequently have few symptoms but can present with nausea, dizziness, and irritability. Nuchal rigidity is usually absent. Careful examination for cranial nerve palsies, which are found in approximately one fifth of adult patients, should be performed. Diplopia, in particular, is one of the most common manifestations of cryptococcal meningitis. Papilledema is seen in nearly one third of patients with cryptococcal meningitis.[52] Patients with coexistent AIDS frequently have very few symptoms.[33]

Pediatric patients with cryptococcal meningitis usually have signs and symptoms not referable to the CNS.[142] Leggiadro and associates[89] reported 13 children with AIDS, compiled from 11 institutions, who were diagnosed with extrapulmonary cryptococcosis. Meningitis was diagnosed in 62 percent of these patients and was the most common clinical manifestation of extrapulmonary disease. Patients who initially responded to antifungal therapy did not die of the fungal infection but died of other illnesses related to their immunodeficiency. Death can occur, however, even in an immunocompetent child receiving effective therapy.[131]

Leggiadro and associates[88] also reported on eight children with acute lymphoblastic leukemia who developed extrapulmonary cryptococcosis. Of these immunocompromised children, 63 percent had meningitis. Fever was the most common symptom, occurring in 60 percent of the children with meningitis. Headache was present in only 40 percent. A significant number of the children (40%) were completely asymptomatic and had lumbar punctures performed as routine management of acute lymphoblastic leukemia, with the subsequent unexpected growth of *C. neoformans* on culture. One child had cutaneous lesions, and another had ptosis and an unsteady gait. Treatment in this series of patients included amphotericin B (intravenous, intrathecal, or both), alone or combined with oral flucytosine. In these patients with acute lymphoblastic leukemia, relapse was a major complication, occurring in 60 percent of patients thought to have been treated successfully. The relapses occurred within 2 to 6 months of completing therapy. Treatment of the relapses generally included combination therapy of amphotericin B and flucytosine, with the occasional use of intrathecal amphotericin B.

Other illnesses that may lead to cryptococcal meningitis include systemic lupus erythematosus being treated with corticosteroids alone or in combination with azathioprine,[2,88,92,126] chronic mucocutaneous candidiasis,[65,160] and hyper-IgE syndrome.[146] Zoonotic transmission from a pet cockatoo was described in an immunocompromised adult.[108]

Direct examination of the CSF using the India ink test can provide an immediate presumptive diagnosis of cryptococcal meningitis. The sensitivity of this test varies, but in studies of adult patients with AIDS, positive results with the stain approached 75 percent.[24] Other useful stains include silver, periodic acid–Schiff, and mucicarmine. Gram stain of CSF is insensitive and unreliable.

Diagnosis of cryptococcal meningitis is aided by the use of serologic tests. The most common test is the cryptococcal capsular polysaccharide antigen test, which can be performed on serum, CSF, or other sterile body fluids. The sensitivity of this test is nearly 100 percent for the serum of patients who are HIV-positive.[25,81,175] The CSF antigen test in some studies seems to be less sensitive (91%).[24] False-positive results caused by cross-reactions of antigens in disseminated infections with *Trichosporon beigelii* have been reported.[101] Culture is still the gold standard for establishing the diagnosis and monitoring the success of therapy.[121]

Treatment of cryptococcal meningitis is prolonged and, in immunocompromised patients, frequently requires lifelong maintenance therapy. Practice guidelines have been published for children with cryptococcal meningitis.[121] For HIV-negative, non–transplant recipient children, amphotericin B deoxycholate 1 mg/kg/day

plus flucytosine 100 mg/kg/day in four divided doses should be given for at least 2 weeks, as induction therapy, if no neurologic complications are present and CSF culture performed at 2 weeks of therapy is negative. Liposomal amphotericin B 5 mg/kg/day or amphotericin B lipid complex 5 mg/kg/day may be substituted for amphotericin B deoxycholate in those intolerant to the latter. In patients with neurologic complications, consideration for extension of the induction phase to 4 to 6 weeks using a lipid formulation of amphotericin B for the final 4 weeks of therapy should be made. Consolidation using oral fluconazole 10 to 12 mg/kg/day for 8 weeks follows. On completion of the consolidation phase, maintenance therapy with fluconazole 6 mg/kg/day should be started and continued for 6 to 12 months.

For HIV-infected children, the practice guideline study group recommends induction therapy consisting of amphotericin B 1 mg/kg/day plus flucytosine 100 mg/kg/day in four divided doses for 2 weeks, followed by consolidation with fluconazole 10 to 12 mg/kg/day for 8 weeks. In patients who cannot tolerate amphotericin B, the use of lipid formulations of amphotericin B should be considered, as described earlier.[87,121] A trial from Thailand evaluated sterilization of the CSF in 64 patients and revealed that the combination of amphotericin B plus flucytosine was the most rapidly fungicidal regimen.[17] Fluconazole 6 mg/kg/day is used for maintenance therapy for 1 year or longer. For adults receiving highly active antiretroviral therapy (HAART) with a CD4 cell count ≥100 cells/μL, and low or undetectable viral RNA level for ≥3 months, maintenance therapy may be discontinued after a minimum of 1 year, but should be restarted if the CD4 cell count falls below 100 cells/μL. In children receiving HAART, discontinuation of maintenance therapy is poorly studied and requires individualization. HIV and AIDS guidelines change frequently, and the physician should consult the most recent guidelines for changes, particularly with regard to need for prolonged maintenance therapy.

In patients with renal compromise associated with amphotericin B, flucytosine levels must be monitored carefully. The recommendation is that the dose of flucytosine be reduced based on glomerular filtration rate and continuing doses be adjusted to maintain serum flucytosine levels of 25 to 60 μg/mL.[6,42] Serum cryptococcal antigens are not useful in monitoring response to therapy, and the use of CSF cryptococcal antigens to monitor response to therapy also is controversial. Therapy is best judged to be successful by the demonstration of sterility of CSF fungal cultures.

Histoplasmosis

Infection with *Histoplasma capsulatum*, usually a benign and self-limited disease, is endemic in many parts of the United States. Disseminated disease, including meningitis, is a rare occurrence in children.[76,118] Case reports of adults generally describe immunocompromised individuals.[48,157] Clinical presentations reported in the literature show a wide variability in the manifestation of meningitis. In these cases, 39 percent of patients presented with meningitis associated with acute dissemination, 25 percent presented with single histoplasmoma that manifested as symptomatic mass lesions alone or with dissemination, 25 percent presented with chronic meningitis without evidence of dissemination, and the remaining patients presented with meningitis as a manifestation of recurrent disease. Rarely, embolization to the brain caused by *Histoplasma* endocarditis has been associated with meningitis.[167]

Meningitis occurring in patients with AIDS has become common in endemic areas. In one report, disseminated histoplasmosis caused 8 percent of the AIDS-defining illnesses in children.[136] The duration of symptoms varies. In patients who do not have AIDS, the symptoms generally last longer than 6 months and can last 7 years.[35,48,116,161] In patients with AIDS, symptoms usually manifest more acutely and in a much shorter time frame.[167] In one series, neurologic findings occurred in all but 6 percent of the patients.[167] The most common signs and symptoms include depressed consciousness (29%), headaches (24%), confusion (22%), cranial nerve deficits (19%), other focal deficits (16%), seizures (14%), personality changes (12%), and ataxia (11%). Findings more commonly observed with acute bacterial meningitis, such as meningismus, a Babinski sign, or papilledema, were seen in fewer than 8 percent of the cases. In adult patients who do not have AIDS, the death rate is approximately 12 percent, with a relapse rate of 44 percent. In adult patients with AIDS, the death rate is 100 percent in some series.[4,167]

Diagnosis can be aided by serologic testing. High levels of anti–*H. capsulatum* antibodies were detected in the serum of 70 percent of patients tested. CSF serology was helpful in 75 percent of patients who were tested. Culture of the CSF was positive for fewer than half of the cases in one review.[76] In a second series in patients with AIDS, blood cultures were positive for 49 percent, bone marrow cultures were positive for 53 percent, respiratory secretions cultures were positive for 58 percent, and brain or meninges cultures were positive for 75 percent of those tested. Serologic testing can be negative because 10 to 25 percent of patients with disseminated disease lack a positive antibody response. The serology also can be false-positive in patients with other fungal diseases or tuberculosis. The antibody response to acute *Histoplasma* infection may remain elevated for years, without evidence of dissemination or meningeal disease. The use of *Histoplasma* antigen has become widely held as a useful test in immunocompromised patients with disseminated *Histoplasma*. Data from Wheat and associates[167] showed that antigen was found in the urine of six of seven patients with AIDS and meningitis.

Treatment with liposomal amphotericin B 5 mg/kg/day given over 4 to 6 weeks, followed by itraconazole 200 mg or 5 mg/kg/dose 2 or 3 times daily for a minimum of 1 year and until CSF abnormalities have resolved, including *Histoplasma* antigen levels, is currently recommended.[166,169] The liposomal form provides higher CNS levels than the deoxycholate form. However, it should be noted that none of the amphotericin B formulations achieve detectable concentrations in the CSF.[169] The use of intrathecal amphotericin B is not recommended for *H. capsulatum* meningitis. Determination of itraconazole

serum trough blood levels should be performed to ensure appropriate drug levels.[170] Johnson and colleagues[71] reported on a trial comparing amphotericin B with liposomal amphotericin B (AmBisome, LAmB) as induction therapy and showed the agents had similar efficacy for treating disseminated histoplasmosis.

Patients with AIDS for whom induction therapy was successful must remain on an anti-*Histoplasma* agent indefinitely. A few cases of children with disseminated histoplasmosis and AIDS have been reported, but none with meningitis, and in these children, antifungal therapy must be continued indefinitely.[20,135] The case report by Schutze and associates[135] showed that ketoconazole was ineffective for preventing recurrence of nonmeningeal disseminated disease, and because ketoconazole has poor CNS penetration, it is likely to be ineffective for prophylaxis of meningitis. In patients with AIDS, some success has been achieved with the use of itraconazole for suppressive therapy in disseminated disease. However, few data exist on the use of itraconazole for suppressive therapy for meningitis or other CNS lesions caused by *Histoplasma*. For maintenance therapy, fluconazole (high-dose), itraconazole, or intravenous amphotericin B in a weekly dose of 1 mg/kg are available. In one case report, fluconazole was effective in an adult with *Histoplasma* meningitis refractory to amphotericin B therapy.[151]

Coccidioidomycosis

C. immitis (*Coccidioides posadasii* in Texas and Central and South America) meningitis is a more common cause of chronic meningitis than *Histoplasma*.[72] In endemic regions of the United States (southwestern United States, Central Valley of California), 200 to 300 cases of coccidioidal meningitis occur annually.[171] Approximately 1 percent of children with symptomatic pulmonary disease develop disseminated disease.[73] Of patients with disseminated coccidioidomycosis, 15 to 20 percent develop meningitis.[73,78] History of exposure is a crucial factor in diagnosing this disease and relies on careful questioning of the patient about travel to or residence in an endemic area.[147,149] Exposure of wounds to colonized soil has been implicated in at least one pediatric case.[106] An association between facial cutaneous coccidioidomycosis and meningitis has been described.[7]

CSF shows a mononuclear pleocytosis with an elevated protein and decreased glucose concentration. Elevated CSF pressures may herald the development of hydrocephalus.[171] Diagnosing coccidioidal meningitis is made easier by the availability of reliable serologic tests. Smith and associates[144] showed that complement-fixing antibodies appeared in the CSF only in patients with meningitis and provided a sensitivity of 76 percent. Sensitivity of 96 percent was seen when the complement-fixation test was incubated at 4° C.[113] McGinnis,[100] in his review of the literature based on pooling five studies, showed that *Coccidioides* could be cultured from the CSF in 76 percent of patients with meningitis and was seen on direct examination of the CSF in only 8 percent of the cases. He concluded that these values were too high and were likely skewed because of reporting bias. Other experts in the field also consider that the rate of positive CSF cultures is much lower (approximately 33%) and that finding the organism in the CSF by direct examination rarely occurs.[145]

Coccidioidomycosis in infancy was described more than 40 years ago and continues to be a problem today.[153] Infants usually have severe disease with high mortality rates and morbidity. In the 1980s, two studies described children with *Coccidioides* spp. meningitis who were treated with oral, intravenous, or intrathecal imidazoles.[60,139] The authors reported promising results with the use of ketoconazole and imidazole therapy compared with standard intravenous or intrathecal amphotericin B.

If untreated, *Coccidioides* meningitis is uniformly fatal. Therapy with intravenous and intrathecal amphotericin B reduced the mortality rate to 30 percent.[113] The use of fluconazole has been found in adult studies to have a success rate of 79 percent.[46] It seems to be safe in clinical use, despite a case report of a pregnant woman with *Coccidioides* meningitis treated with fluconazole who delivered an infant with congenital malformations. The infant was thought to have an autosomal recessive disorder (Antley-Bixler syndrome), however, and not teratogenic malformations caused by fluconazole.[86] Fluconazole may be the superior agent for sustaining remission of meningitis caused by *Coccidioides* because of its oral bioavailability, the toxicity associated with amphotericin B, and the elimination of the need for intrathecal administration.[120]

Galgiani and coworkers[45] have written practice guidelines for the treatment of coccidioidomycosis in adults. Therapy with oral fluconazole is preferred, with most authorities recommending 400 mg/day orally, although some physicians begin with higher doses of 800 to 1000 mg/day. Fluconazole 400 to 600 mg/day for 9 to 12 months has been shown effective in adults.[46,155] A corresponding dose of 6 mg/kg/day in pediatric patients also has been used, but no controlled studies using this agent in pediatric patients have been published. If treatment is begun with azole therapy, it should be continued for life.[31]

Itraconazole has proven efficacy in nonmeningeal coccidioidomycosis, with 63 percent of adult patients treated showing a complete response.[156] Itraconazole 400 to 600 mg/day has been reported effective in adults. Because itraconazole has variable oral bioavailability, many authorities recommend monitoring for adequate serum drug levels.[159] The use of itraconazole for meningeal involvement led to great hope for the use of an oral agent in this disease.[154] Adult patients with *Coccidioides* meningitis had high relapse rates (40% to 50%), which rendered them dependent on lifelong therapy with itraconazole.[54] The alternative of continued intrathecal amphotericin B renders this treatment much more appealing.

Well-developed, controlled clinical trials with large numbers of children do not exist for the azoles; some authorities consider intravenous and intrathecal amphotericin B to be the standard therapy for coccidioidal meningitis. It also is recommended for patients who do not respond to fluconazole or itraconazole treatment. Some experts initially use a combination of oral azole with intrathecal amphotericin B with the thought that

responses are more prompt with this approach. Amphotericin B can be administered intrathecally into the lumbar area or cisterna magna or by using an Ommaya reservoir.[29,47,83,174] In younger children, intraventricular or intracisternal therapy using the Ommaya reservoir allows ease of administration but has the disadvantage of increasing susceptibility to secondary bacterial infection.

The initial dose of amphotericin B administered into the CSF is 0.025 mg. The dose is increased by doubling until a maintenance dose of 0.1 to 0.5 mg is attained. After this dose has been achieved, therapy can be given every other day, alternating with intravenous administration of amphotericin B. Therapy is continued until the child's condition has stabilized, at which point intrathecal therapy gradually can be stretched out to every 3 weeks. This program is continued until the CSF indices are normal and culture results have been negative for at least 1 year.

Use of voriconazole has been successful in two case reports, and other similar anecdotal references have been reported at referral centers; some centers use voriconazole 4 mg/kg every 12 hours orally for salvage therapy.[27,45,125]

Blastomycosis

Blastomyces dermatitidis is an uncommon cause of chronic meningitis and is difficult to diagnose premortem unless the patient has other signs of systemic blastomycosis. When systemic blastomycosis occurs, it can involve the CNS in 5 to 10 percent of cases.[15] Importantly, a review of CNS cases of blastomycosis revealed that approximately 23 percent were not associated with disease outside the CNS.[9] Common sites for this organism include bone, genitourinary tract, and skin.[10,16] Previously, blastomycosis most often affected patients who were immunocompetent. A total of 55 percent of cases of CNS blastomycosis involve individuals with immunosuppressive conditions.[9] Patients with AIDS are at high risk for developing chronic infection.[59] Because of the difficulty in diagnosing meningitis caused by *B. dermatitidis* and its similarities to tuberculous meningitis, patients usually are treated for presumptive tuberculous meningitis.[51,105] Although meningitis is the most common form of CNS blastomycosis, solitary mass lesions also can occur.[130] A review of 22 cases treated from 1990 to 2008 demonstrated a mortality rate of 18 percent.[9]

Examination of CSF obtained by lumbar puncture usually is negative.[9] However, the yield from ventricular fluid (four of four patients tested) seems to have a higher yield.[82] Diagnosis relies on the characteristic histopathologic appearance in tissues and occasionally on culture of CSF obtained from the ventricles. Isolation of *Blastomyces* from the CSF by culture is possible in approximately half of cases.[9] Testing for the presence of *B. dermatitidis* antigen may yield better results. In one report, all three CSF samples tested using a commercially available assay were found to be positive. Blastomycosis meningitis is associated with a pleocytosis that may be lymphocytic or neutrophilic in nature.[9] The CSF protein is elevated in the majority of cases.[9] Meningitis caused by *B. dermatitidis* frequently has a neutrophilic predominance.[9,59] If the difficulty in performing these procedures is prohibitive, looking for other sources of blastomycosis, including sputum and urine, is indicated. A study in patients with AIDS showed that examination of sputum was useful for establishing the diagnosis of disseminated blastomycosis.[115]

Treatment of CNS blastomycosis relies on the administration of lipid formulation of amphotericin B in a dose of 5 mg/kg/day for 4 to 6 weeks, followed by oral azole therapy (i.e., fluconazole 800 mg/day, itraconazole 200 mg 2 or 3 times per day, voriconazole 200 to 400 mg/day) for a minimum of 12 months or until CSF abnormalities have resolved.[22] For children, no specific recommendations exist for the treatment of CNS disease, although for children with severe blastomycosis amphotericin B deoxycholate 0.7 to 1 mg/kg/day or lipid formulation of amphotericin 3 to 5 mg/kg/day is recommended for initial therapy. Itraconazole 10 mg/kg/day (up to 400 mg/day) divided twice daily is recommended as follow-up therapy for 12 months.[22] A recently published review favors the use of voriconazole as the azole of choice after initial therapy with lipid formulation of amphotericin.[9] Azoles should not be considered for primary treatment of CNS blastomycosis. Schutze and colleagues[136] reported that children respond less satisfactorily to oral azole therapy than do adults. Although only a few published reports existed for the use of lipid formulations of amphotericin B to treat CNS blastomycosis, these drugs are preferred because of the need of prolonged duration of therapy in these patients. Experimental animal data exist demonstrating superior penetration of liposomal amphotericin B compared with lipid complex or the deoxycholate formulations of amphotericin.[55]

Aspergillosis

CNS infections with *Aspergillus fumigatus* and other *Aspergillus* spp. in immunocompromised patients usually are fatal. Conditions that place the patient at risk include organ transplantation, malignancies, and neutropenia. In most reported cases, patients were receiving high-dose corticosteroid therapy in addition to broad-spectrum antibiotics.[11,84] Many patients had fever, and pulmonary findings preceded neurologic manifestations. *Aspergillus* infection of the CNS usually is acquired by hematogenous spread from the lungs. Other modes of acquisition include extension from a contiguous focus (sinuses) and intravenous drug abuse.[104]

Brain abscesses are the most typical manifestation of *Aspergillus* spp. infection in the CNS, but meningoencephalitis, isolated spinal cord lesions, aqueductal stenosis, and mycotic aneurysms also have been described.[21,39,158,162,173] Magnetic resonance imaging has been suggested to be superior to computed tomography for the delineation of CNS lesions in patients with bone marrow transplantation.[102] The lesions are consistent with acute infarcts.

Some early reports of CNS aspergillosis were in infants who appeared to be normal.[1,96] The diagnosis of meningitis relies on CSF cultures, but the results of bronchoscopy or biopsy frequently are more helpful and provide a more expedient diagnosis. Use of *Aspergillus*

antigen in the CSF has been helpful, and it may be used for serial observations during the course of therapy.[163] Because the disease is uniformly fatal, the importance of treating pulmonary aspergillosis before the development of meningeal involvement cannot be overemphasized.

No specific recommendations for therapy of CNS *Aspergillus* infections are published for children. Published practice guidelines for the therapy of adults supports the use of voriconazole as the primary recommendation for antifungal therapy of CNS aspergillosis.[164] For those intolerant to or who fail to respond to voriconazole therapy with itraconazole, posaconazole or lipid formulations of amphotericin B should be attempted. Itraconazole has been used successfully in one case report, as has voriconazole.[103,163] Combination therapy using voriconazole and an echinocandin antifungal shows promise, but no definitive studies exist to date.

Sporotrichosis

Although primarily a lymphocutaneous disease, *S. schenckii* has been reported to cause meningitis.* Many of the cases described more recently have occurred in adults with AIDS as an underlying risk factor.[34,56] Meningeal seeding may occur through hematogenous spread from the lungs, as is seen in most other fungal infections.[36] Meningeal involvement with sporotrichosis produces CSF indices and abnormalities similar to those seen with the other fungal meningitides.[35,56] CSF fungal culture is insensitive for the diagnosis of meningitis caused by sporotrichosis. The use of *S. schenckii* antibody in the CSF has been effective in diagnosing meningitis in patients without other overt signs of this infection.[137]

Treating this infection is very difficult. Practice guidelines for adults recommend amphotericin B given as a lipid formulation at a dosage of 5 mg/kg/day for 4 to 6 weeks as initial therapy.[77] The expert panel did not prefer amphotericin B deoxycholate over a lipid formulation. Step-down therapy using itraconazole 200 mg twice daily is recommended once the patient responds to initial therapy using amphotericin B. Itraconazole should be continued for at least 12 months. Monitoring of serum levels is recommended to ensure adequate drug exposure.[53] For patients with immunosuppressive diseases or conditions, itraconazole suppressive therapy, 200 mg daily, is recommended.

Mucormycosis

Meningitis caused by *Mucor* spp. or other Zygomycetes usually occurs as a result of direct extension from paranasal sinus disease. Infection with these organisms most commonly is seen in the immunocompromised host and particularly in patients with diabetes mellitus or patients receiving high doses of corticosteroids. Patients (particularly dialysis patients) undergoing chelation therapy with deferoxamine are at risk for developing infection.[13,14,75,172] The association with deferoxamine therapy is seen with *Cunninghamella*.[127] *Mucor* spp. have been reported as a cause of CNS disease in children but very rarely.[57,67] Treatment employs amphotericin B in doses of 1 to 1.5 mg/kg/day or lipid formulations. Improved survival has been seen among cancer patients with mucormycosis of the head and neck with treatment using liposomal amphotericin B.[28] Surgical excision of rhinocerebral infection is recommended along with antifungal therapy. In two compassionate use case series of posaconazole in patients with refractory mucormycosis or intolerant to standard therapy, an oral azole produced encouraging results.[28] Importantly, voriconazole has no antifungal activity against mucormycosis.

Other Fungal Infections

Acremonium spp. are common soil fungi that may cause chronic meningitis in humans.[111] *Xylohypha bantiana*, an uncommon dematiaceous fungus, caused a fungal brain abscess in an adolescent girl; this report increases the number of cases reported in the literature to nearly 40.[110] Cerebral chromoblastomycosis also has been reported.[140] Other fungal organisms causing CNS infection include *Paracoccidioides brasiliensis* (i.e., South American blastomycosis),[119] *Prototheca wickerhamii*,[74] *Blastoschizomyces capitatus*,[49] *Rhodotorula* spp.,[97,123] *Pseudallescheria boydii*,[12] *A. mansoni*,[64] *C. lusitaniae*,[64] and *B. spicifera*.[85]

DIAGNOSIS

Specific information about the diagnosis of individual organisms is provided in the previous sections. Table 33-1 provides specific data for some fungal meningitides. Overall, the problem with diagnosing many of these infections is that most patients have nonspecific signs and symptoms without reference to the CNS. Standard culture media may not be useful for these organisms, and it can take weeks before an organism is identified on culture. The use of antigen and antibody testing has proved quite useful for the diagnosis of *Cryptococcus*, *Coccidioides*, and *Histoplasma* infections. The hope is that polymerase chain reaction technology may be helpful in the future, as it has been for bacterial and mycobacterial diagnoses. Neonates may have only a mild CSF pleocytosis. In one series of 16 neonates with definite *Candida* meningitis, the median CSF white blood cell count was 53 cells/mm^3 (range, 0 to 1120 cells/mm^3).[40]

CSF examination may be helpful in some instances. As much CSF as can be removed safely should be obtained, especially at the time of ventriculography or pneumoencephalography.[35] A minimum of 5 mL of spinal fluid has been suggested, based on experimental work.[94] Repeated cultures of large volumes of CSF may be helpful.[35] The fluid obtained should be centrifuged and the sediment saved for culture and India ink preparation. The supernatant is sent for serologic tests. The India ink test should be interpreted with caution and must be followed with cultures because artifacts frequently can cause misinterpretation.[35] The cumulative efficacy of repeated lumbar punctures for cryptococcal meningitis improved the sensitivity of the India ink smear from 26 percent in one lumbar puncture to 52.6 percent with the second.[100] If large volumes are available, membrane filtration may be

*References 36, 43, 56, 80, 117, 141.

TABLE 33-1 **Fungal Cerebrospinal Fluid Characteristics**

Organism	WBCs	Protein	Glucose	Smears	Serology	Cultures
Blastomyces	Variable up to 15,000 cells/mm^3 with PMNs or lymphocytes	Elevated up to 300 mg/dL	Normal or low	Rare on smear	No good serology	CSF cultures rarely positive; increased yield with ventricular taps
Candida	Mean 600 cells/mm^3 up to 1900 cells/mm^3 with lymphocytes or PMNs	Elevated	Low or normal	40% positive on smears	Serology not helpful	CSF cultures useful
Coccidioides	100-750 WBCs, mostly lymphocytes	150-2000 mg/dL	21%-62% serum	Rare on smear	CSF CF antibody positive in 75%-95%	CSF cultures positive in 33%-60%
Cryptococcus	40-400 WBCs, mostly lymphocytes	High	Low	India ink positive in 25%-50%	CSF and serum cryptococcal antigen positive in 85%-90%	CSF cultures positive in 75%
Histoplasma	0-300 WBCs, lymphocytes, or PMNs; most 11-101/mm^3	Usually elevated, but can be normal	Usually low (<40 mg/dL) to normal	Rare on smear	Polysaccharide antigen in urine, blood, CSF positive in 61%	CSF cultures positive in 27%-65%

Data from references 51, 61, 100, 165.
CF, Complement fixation; *CSF,* cerebrospinal fluid; *PMNs,* polymorphonuclear leukocytes; *WBCs,* white blood cells.

used to concentrate the fungal elements. The membrane containing the fungi is placed aseptically on isolation media and incubated at 30° C for 4 weeks. The CSF that passes through the membrane can be used for serology or chemistry determinations.[100] The remaining CSF can be inoculated onto Sabouraud glucose agar, blood agar, and brain-heart infusion agar or into broth media or into both types of media. CSF cultures generally are unhelpful for *Histoplasma*, *Blastomyces*, and other dimorphic fungi.

Serologic techniques are extremely useful for *Cryptococcus* and *Coccidioides*. Approximately 100 percent sensitivity is seen with serum cryptococcal antigen tests in HIV-positive patients.[25,81,175] The CSF antigen test in HIV-positive patients seems to be less sensitive (91%) in some studies.[24] In patients not infected with HIV, the sensitivity of the serologic test in the CSF approaches 90 percent.[61] For coccidioidal meningitis, the sensitivity rate is greater than 76 percent.[113,144]

Candida can be cultured but may require a prolonged incubation period, necessitating institution of empirical therapy while awaiting results of cultures. For other organisms, such as *Histoplasma*, *Blastomyces*, and *Coccidioides*, culturing other body fluids, such as blood, urine, sputum, or draining wounds, can be helpful.

CONCLUSION

With the large increase in the number of immunocompromised children caused by the epidemic of HIV, the advent of new antineoplastic agents, the growth of organ transplantation, and the increased use of corticosteroids, the relative rarity of fungal meningitides has been replaced by a burgeoning upswing in the incidence and prevalence of these infections. New modalities for diagnosis are needed because many of these fungal infections still require weeks for identification. Future trials in progress will help determine the role of azoles, amphotericin B, lipid formulations of amphotericin B, or combinations of these drugs as the optimal treatment of many fungal meningitides.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Aronoff GR, Bennett WM, Berns JS, et al. *Drug Prescribing in Renal Failure: Dosing Guidelines for Adults and Children*. 5th ed. Philadelphia, Pa: American College of Physicians; 2007. p. 73.
9. Bariola JR, Perry P, Pappas PG, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin Infect Dis* 2010;**50**:797–804.
26. Cohen-Wolkowiez M, Smith PB, Mangum B, et al. Neonatal Candida meningitis: significance of cerebrospinal fluid parameters and blood cultures. *J Perinatol* 2007;**27**:97–100.
28. Dan M. Mucormycosis of the head and neck. *Curr Infect Dis Rep* 2011;**13**:123–31.
50. Glocker EO, Hennigs A, Nabavi M, et al. A homozygous CARD9 mutation in a family with susceptibility to fungal infections. *N Engl J Med* 2009;**361**:1727–35.
56. Gutierrez-Galhardo MC, do Valle AC, Fraga BL, et al. Disseminated sporotrichosis as a manifestation of immune reconstitution inflammatory syndrome. *Mycoses* 2010;**53**:78–80.
62. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous Candida meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008;**197**:163–71.
77. Kauffman CA, Bustamante B, Chapman SW, et al. Clinical practice guidelines for the management of sporotrichosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:1255–65.
91. Leimann BC, Koifman RJ. Cryptococcal meningitis in Rio de Janeiro, Brazil, 1994-2004. *Cad Saude Publica* 2008;**24**:2582–92.
109. O'Brien D, Stevens NT, Lim CH, et al. Candida infection of the central nervous system following neurosurgery: a 12-year review. *Acta Neurochir (Wien)* 2011;**153**:1347–50.
114. Pappas PG, Kauffman CA, Ades D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**:503–35.

118. Pereira GH, Pádua SS, Park MV, et al. Chronic meningitis by histoplasmosis: report of a child with acute myeloid leukemia. *Braz J Infect Dis* 2008;**12**:555–77.
121. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
122. Piper L, Smith PB, Hornik CP, et al. Fluconazole loading dose pharmacokinetics and safety in infants. *Pediatr Infect Dis J* 2011;**30**:375–8.
148. Tay ST, Rohani MY, Hoo TS, et al. Epidemiology of cryptococcosis in Malaysia. *Mycoses* 2010;**53**:509–14.
164. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
168. Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:807–25.
171. Williams PL. Coccidioidal meningitis. *Ann N Y Acad Sci* 2007; **1111**:377–84.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 34

Eosinophilic Meningitis

Barbara W. Stechenberg

Eosinophilic meningitis may include any meningitis, infectious or noninfectious, in which cerebrospinal fluid (CSF) pleocytosis with a significant percentage of eosinophils is found. The diagnosis of eosinophilic meningitis can be made by the presence of 10 or more eosinophils in the CSF or if at least 10 percent of the CSF leukocytes are eosinophils.[20] Such a finding strongly suggests invasion of the central nervous system (CNS) by a helminthic parasite. In the last 40 years, the term *eosinophilic meningitis* has been applied more specifically to a typical form of meningitis caused by *Angiostrongylus cantonensis*, a rat lungworm found primarily in the Pacific Islands and Southeast Asia. *Gnathostoma spinigerum* and *Baylisascaris procyonis* also may be considered in particular circumstances.

The first documented case of eosinophilic meningitis was reported from Taiwan in 1945.[1] The patient was a 15-year-old boy who developed severe headache and vomiting. Examination of the CSF revealed 528 leukocytes, of which 50 percent were eosinophils. Ten actively moving nematodes also were recovered from the specimen. Since the early 1960s, many new cases have been reported, with particularly large numbers coming from Thailand,[29,30] Tahiti,[34] Taiwan,[49] and Hawaii.[14,17]

ETIOLOGIC AGENTS

The organism that presumably is involved in most cases is the rodent lungworm. *A. cantonensis*, which as an adult is 17 to 25 mm long and 0.25 to 0.36 mm at its maximum width, has a smooth cuticle and three minute lips at the cephalic end. The male has a copulatory bursa supported by bursal rays.

In its life cycle, rodents such as *Rattus rattus* are the principal hosts. These rodents ingest mollusks containing third-stage larvae, which travel from the liver and lung into the general circulation. The larvae selectively leave the circulation to enter the CNS within the first 48 hours. There they develop into young adults in approximately 2 weeks.[46] From the brain, they travel to the pulmonary arteries, where their eggs are laid. The eggs hatch in the pulmonary capillaries; the first-stage forms then travel from the alveolar spaces up the rat trachea to the gastrointestinal tract, from which they are eliminated in the feces. The larvae can survive for approximately 2 weeks under humid conditions. The third-stage larvae develop in the intermediate hosts in approximately 2 weeks.

Another form of eosinophilic meningitis has been attributed to the nematode *G. spinigerum*. The adult *G. spinigerum* is stout and reddish and has a globose cephalic bulb that is separated from the body by a slight constriction. The head and anterior part of the body have spines. Males may be 11 to 25 mm long, and females may be 25 to 54 mm long. The adults lie coiled in lesions along the alimentary canal, from which they release eggs into the feces, where they become embryonated. The eggs hatch on reaching water, releasing a larva that is ingested by a copepod *(Cyclops)* and continues to develop. When the infected copepod is eaten by a fish, frog, snake, or bird, a third-stage larva develops and becomes encapsulated in the intermediate host. When it is ingested by the definitive host (i.e., cats, dogs, hogs, mink, and humans), the parasite localizes in the stomach wall.[37]

The adult worms of *B. procyonis*, the raccoon ascarid, reside in the intestines. They are large, tan-colored roundworms; the female is 20 to 22 cm long, and the male is 9 to 11 cm long. Infection in raccoons usually is

REFERENCES

1. Allan GW, Anderson DH. Generalized aspergillosis in an infant 18 days of age. *Pediatrics* 1960;**26**:432–40.
2. al-Rasheed SA, al-Fawaz IM. Cryptococcal meningitis in a child with systemic lupus erythematosus. *Ann Trop Paediatr* 1990;**10**:323–6.
3. Ampel NM, Wieden MA, Galgiani JN. Coccidioidomycosis: clinical update. *Rev Infect Dis* 1989;**11**:897.
4. Anaissie E, Fainstein V, Samo T, et al. Central nervous system histoplasmosis: an unappreciated complication of the acquired immunodeficiency syndrome. *Am J Med* 1988;**84**:215–7.
5. Arisoy ES, Arisoy AE, Dunne Jr WM. Clinical significance of fungi isolated from cerebrospinal fluid in children. *Pediatr Infect Dis J* 1994;**13**:128–33.
6. Aronoff GR, Bennett WM, Berns JS, et al. *Drug Prescribing in Renal Failure: Dosing Guidelines for Adults and Children*, 5th ed. Philadelphia, Pa: American College of Physicians; 2007. p. 73.
7. Arsura EL, Kilgore WB, Caldwell JW, et al. Association between facial cutaneous coccidioidomycosis and meningitis. *West J Med* 1998;**169**:13–6.
8. Baley JE, Kliegman RM, Fanaroff AA. Disseminated fungal infections in very low-birth-weight infants: clinical manifestations and epidemiology. *Pediatrics* 1984;**73**:144–52.
9. Bariola JR, Perry P, Pappas PG, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin Infect Dis* 2010;**50**:797–804.
10. Baumgardner DJ, Buggy BP, Mattson BJ, et al. Epidemiology of blastomycosis in a region of high endemicity in north central Wisconsin. *Clin Infect Dis* 1992;**15**:629–35.
11. Beal MF, O'Carroll CP, Kleinman GM, et al. Aspergillosis of the central nervous system. *Neurology* 1982;**32**:473–9.
12. Berenguer J, Diaz-Mediavilla J, Urra D, et al. Central nervous system infection caused by *Pseudallescheria boydii*: case report and review. *Rev Infect Dis* 1989;**11**:890–6.
13. Boelaert JR, Vergauwe PL, Vandepitte JM. Mucormycosis infection in dialysis patients. *Ann Intern Med* 1987;**107**:782–3.
14. Boelaert JR, de Locht M, Van Cutsem J, et al. Mucormycosis during deferoxamine therapy is a siderophore-mediated infection: in vitro and in vivo animal studies. *J Clin Invest* 1993;**91**:1979–86.
15. Bradsher RW. Blastomycosis. *Clin Infect Dis* 1992;**14**(Suppl. 1): S582–90.
16. Bradsher RW, Rice DC, Abernathy RS. Ketoconazole therapy for endemic blastomycosis. *Ann Intern Med* 1985;**103**:872–9.
17. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomized trial. *Lancet* 2004;**363**:1764–7.
18. Butler KM, Rench MA, Baker CJ. Amphotericin B as a single agent in the treatment of systemic candidiasis in neonates. *Pediatr Infect Dis J* 1990;**9**:51–6.
19. Butler WT, Alling DW, Spickard A, et al. Diagnostic and prognostic value of clinical and laboratory findings in cryptococcal meningitis: a follow-up study of forty patients. *N Engl J Med* 1964;**270**:59–66.
20. Byers M, Feldman S, Edwards J. Disseminated histoplasmosis as the acquired immunodeficiency syndrome-defining illness in an infant. *Pediatr Infect Dis J* 1992;**11**:127–8.
21. Casey AT, Wilkins P, Uttley D. Aspergillosis infection in neurosurgical practice. *Br J Neurosurg* 1994;**8**:31–9.
22. Chapman SW, Dismukes WE, Proia LA, et al. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:1801–12.
23. Chen JY. Neonatal candidiasis associated with meningitis and endophthalmitis. *Acta Pediatr Jpn* 1994;**36**:261–5.
24. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. *N Engl J Med* 1989;**321**:794–9.
25. Clark RA, Greer D, Atkinson W, et al. Spectrum of *Cryptococcus neoformans* infection in 68 patients infected with human immunodeficiency virus. *Rev Infect Dis* 1990;**12**:768–77.
26. Cohen-Wolkowiez M, Smith PB, Mangum B, et al. Neonatal Candida meningitis: significance of cerebrospinal fluid parameters and blood cultures. *J Perinatol* 2007;**27**:97–100.
27. Cortez KJ, Walsh TJ, Bennett JE. Successful treatment of coccidioidal meningitis with voriconazole. *Clin Infect Dis* 2003;**36**: 1619–22.
28. Dan M. Mucormycosis of the head and neck. *Curr Infect Dis Rep* 2011;**13**:123–31.
29. Dennis M, Rasch JR. The Ommaya reservoir in fungal meningitis: a case report. In: Ajello L, editor. *Coccidioidomycosis*. Tucson, Ariz: University of Arizona Press; 1967. p. 119–22.
30. Deresinski SC, Stevens DA. Caspofungin. *Clin Infect Dis* 2003;**36**:1445–57.
31. Dewsnup DH, Galgiani JN, Graybill JR, et al. Is it ever safe to stop azole therapy for *Coccidioides immitis* meningitis? *Ann Intern Med* 1996;**124**:305–10.
32. Diamond RD. Cryptococcus neoformans. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 2331–40.
33. Dismukes WE. Cryptococcal meningitis in patients with AIDS. *J Infect Dis* 1988;**157**:624–8.
34. Donabedian H, O'Donnell E, Olszewski C, et al. Disseminated cutaneous and meningeal sporotrichosis in an AIDS patient. *Diagn Microbiol Infect Dis* 1994;**18**:111–5.
35. Ellner JJ, Bennett JE. Chronic meningitis. *Medicine (Baltimore)* 1976;**55**:341–96.
36. Ewing GE, Bosl GJ, Peterson PK. *Sporothrix schenckii* meningitis in a farmer with Hodgkin's disease. *Am J Med* 1980;**68**:455–7.
37. Faix RG. Systemic *Candida* infections in infants in intensive care nurseries: high incidence of central nervous system involvement. *J Pediatr* 1984;**105**:616–22.
38. Faix RG, Kovarik SM, Shaw TR, et al. Mucocutaneous and invasive candidiasis among very low birth weight (<1500 grams) infants in intensive care nurseries: a prospective study. *Pediatrics* 1989;**83**:101–7.
39. Feely M, Steinberg M. *Aspergillus* infection complicating transsphenoidal yttrium-90 pituitary implant. *J Neurosurg* 1977;**46**: 530–2.
40. Fernandez M, Moylett EH, Noyala DE, et al. *Candida* meningitis in neonates. *Clin Infect Dis* 2000;**31**:458–63.
41. Flynn PM, Marina NM, Rivera GK, et al. *Candida tropicalis* infections in children with leukemia. *Leuk Lymphoma* 1993;**10**: 369–76.
42. Francis P, Walsh TJ. Evolving role of flucytosine in immunocompromised patients: new insights into safety, pharmacokinetics, and antifungal therapy. *Clin Infect Dis* 1992;**15**:1003–8.
43. Freeman JW, Ziegler DK. Chronic meningitis caused by *Sporotrichum schenckii*. *Neurology* 1977;**27**:989–92.
44. Furcolow ML, Chick EW, Busey JF, et al. Prevalence and incidence studies of human and canine blastomycosis. I. Cases in the United States, 1885-1968. *Am Rev Respir Dis* 1970;**102**: 60–7.
45. Galgiani JN, Ampel NM, Blair JE, et al. Practice guidelines for treatment of coccidioidomycosis. *Clin Infect Dis* 2005;**41**:1217.
46. Galgiani JN, Catanzaro A, Cloud GA, et al. Fluconazole therapy for coccidioidal meningitis: the NIAID-Mycoses Study Group. *Ann Intern Med* 1993;**119**:28–35.
47. Gallis HA, Drew RH, Pickard WW. Amphotericin B: 30 years of clinical experience. *Rev Infect Dis* 1990;**12**:308–29.
48. Gelfand JA, Bennett JE. Active *Histoplasma* meningitis of 22 years duration. *JAMA* 1975;**233**:1294–5.
49. Girmenia C, Micozzi A, Venditti M, et al. Fluconazole treatment of *Blastoschizomyces capitatus* meningitis in an allogeneic bone marrow recipient. *Eur J Clin Microbiol Infect Dis* 1991;**10**: 752–6.
50. Glocker EO, Hennigs A, Nabavi M, et al. A homozygous *CARD9* mutation in a family with susceptibility to fungal infections. *N Engl J Med* 2009;**361**:1727–35.
51. Gonyea EF. The spectrum of primary blastomycotic meningitis: a review of central nervous system blastomycosis. *Ann Neurol* 1978;**3**:26–39.
52. Gordon MA, Holzman RS, Senter H, et al. *Aspergillus oryzae* meningitis. *JAMA* 1976;**235**:2122–3.
53. Grant SM, Clissold SP. Itraconazole: a review of its pharmacodynamic and pharmacokinetic properties and therapeutic use in superficial and systemic mycoses. *Drugs* 1989;**37**:310–44.
54. Graybill JR. Future directions of antifungal chemotherapy. *Clin Infect Dis* 1992;**14** (suppl 1):S170–81.
55. Groll AH, Giri N, Petraitis V, et al. Comparative efficacy and distribution of lipid formulations of amphotericin B in experimental *Candida albicans* infection of the central nervous system. *J Infect Dis* 2000;**182**:274–82.

56. Gutierrez-Galhardo MC, do Valle AC, Fraga BL, et al. Disseminated sporotrichosis as a manifestation of immune reconstitution inflammatory syndrome. *Mycoses* 2010;**53**:78–80.
57. Hale LM. Orbital-cerebral phycomycosis: report of a case and a review of the disease in infants. *Arch Ophthalmol* 1971;**86**: 39–43.
58. Hamner RW, Baum EW, Pritchett PS. Coccidioidal meningitis diagnosed by skin biopsy. *Cutis* 1982;**29**:603–10.
59. Harley WB, Lomis M, Haas DW. Marked polymorphonuclear pleocytosis due to blastomycotic meningitis: case report and review. *Clin Infect Dis* 1994;**18**:816–8.
60. Harrison HR, Galgiani JN, Reynolds Jr AF, et al. Amphotericin B and imidazole therapy for coccidioidal meningitis in children. *Pediatr Infect Dis* 1983;**2**:216–21.
61. Hay RJ, Mackenzie DWR, Campbell CK, et al. Cryptococcosis in the United Kingdom and the Irish Republic: an analysis of 69 cases. *J Infect* 1980;**2**:13–22.
62. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous Candida meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008;**197**:163–71.
63. Huttova M, Hartmanova I, Kralinsky K, et al. *Candida* fungemia in neonates treated with fluconazole: report of forty cases, including eight with meningitis. *Pediatr Infect Dis J* 1998;**17**:1012–5.
64. Huttova M, Kralinsky K, Horn J, et al. Prospective study of nosocomial fungal meningitis in children: report of 10 cases. *Scand J Infect Dis* 1998;**30**:485–7.
65. Imperato PJ, Buckley CE, Callaway JL. *Candida* granuloma. *Arch Dermatol* 1968;**97**:139–46.
66. Isaacs D, Barfield CP, Grimwood K, et al. Systemic bacterial and fungal infections in infants in Australian neonatal units. Australian Study Group for Neonatal Infections. *Med J Aust* 1995;**162**: 198–201.
67. Isaacson C, Levin SE. Gastrointestinal mucormycosis in infancy. *S Afr Med J* 1961;**35**:581–4.
68. Jafari HS, Saez-Llorens X, Grimprel E, et al. Characteristics of experimental *Candida albicans* infection of the central nervous system in rabbits. *J Infect Dis* 1991;**164**:389–95.
69. Johnson DE, Thompson TR, Green TP, et al. Systemic candidiasis in very low-birth-weight infants (<1500 grams). *Pediatrics* 1984;**73**:138–43.
70. Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. *Clin Infect Dis* 2003;**36**:630–7.
71. Johnson P, Wheat LJ, Cloud G, et al. A multicenter randomized trial comparing amphotericin B (AmB) and liposomal amphotericin B (AmBisome, LAmB) as induction therapy of disseminated histoplasmosis (DH) in AIDS patients. Abstract L40e. In: *Proceedings of the 7th Conference on Retroviruses and Opportunistic Infections, San Francisco, January 30-February 2, 2000*. San Francisco: Foundation for Retrovirology and Human Health; 2000.
72. Johnson RH, Einstein HE. Coccidioidal meningitis. *Clin Infect Dis* 2006;**42**:103–7.
73. Kafka JA, Catanzaro A. Disseminated coccidioidomycosis in children. *J Pediatr* 1981;**98**:355–61.
74. Kaminski ZC, Kapila R, Sharer LR, et al. Meningitis due to *Prototheca wickerhamii* in a patient with AIDS. *Clin Infect Dis* 1992;**15**:704–6.
75. Kaneko T, Abe F, Ito M, et al. Intestinal mucormycosis in a hemodialysis patient treated with desferrioxamine. *Acta Pathol Jpn* 1991;**41**:561–6.
76. Karalakulasingam R, Arora KK, Adams G. Meningoencephalitis caused by *Histoplasma capsulatum*: occurrence in a renal transplant and review of the literature. *Arch Intern Med* 1976;**136**: 217–20.
77. Kauffman CA, Bustamante B, Chapman SW, et al. Clinical practice guidelines for the management of sporotrichosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:1255–65.
78. Kelly PC. Coccidioidal meningitis. In: Stevens DA, editor. *Coccidioidomycosis: a text*. New York: Plenum Medical Book; 1980. p. 163–94.
79. Klein BS, Vergeront JM, Weeks RJ, et al. Isolation of *Blastomyces dermatitidis* in soil associated with a large outbreak of blastomycosis in Wisconsin. *N Engl J Med* 1986;**314**:529–34.
80. Klein RC, Ivens MS, Seabury JH, et al. Meningitis due to *Sporotrichum schenckii*. *Arch Intern Med* 1966;**118**:145–9.
81. Kovacs JA, Kovacs AA, Polis M, et al. Cryptococcosis in the acquired immunodeficiency syndrome. *Ann Intern Med* 1985; **103**:533–8.
82. Kravitz GR, Davies SF, Eckman MR, et al. Chronic blastomycotic meningitis. *Am J Med* 1981;**71**:501–5.
83. Kucers A, Bennett N McK. Amphotericin B. In: Kucers A, Bennett N, editors. *The use of antibiotics*. 4th ed. Philadelphia: J. B. Lippincott; 1987. p. 1441–77.
84. Lammens M, Robberecht W, Waer M, et al. Purulent meningitis due to aspergillosis in a patient with systemic lupus erythematosus. *Clin Neurol Neurosurg* 1992;**94**:39–43.
85. Latham RH. *Bipolaris spicifera* meningitis complicating a neurosurgical procedure. *Scand J Infect Dis* 2000;**32**:102–3.
86. Lee BE, Feinberg M, Abraham JJ, et al. Congenital malformations in an infant born to a woman treated with fluconazole. *Pediatr Infect Dis J* 1992;**11**:1062–4.
87. Leenders AC, Riss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. *AIDS* 1997;**11**:1463–71.
88. Leggiadro RJ, Barrett FF, Hughes WT. Extrapulmonary cryptococcosis in immunocompromised infants and children. *Pediatr Infect Dis J* 1992;**11**:43–7.
89. Leggiadro RJ, Kline MW, Hughes WT. Extrapulmonary cryptococcosis in children with acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 1991;**10**:658–62.
90. Leibovitz E, Rigaud M, Chandwani S, et al. Disseminated fungal infections in children infected with human immunodeficiency virus. *Pediatr Infect Dis J* 1991;**10**:888–94.
91. Leimann BC, Koifman RJ. Cryptococcal meningitis in Rio de Janeiro, Brazil, 1994-2004. *Cad Saude Publica* 2008;**24**:2582–92.
92. Lesser RL, Simon RM, Leon H, et al. Cryptococcal meningitis and internal ophthalmoplegia. *Am J Ophthalmol* 1979;**87**:682–7.
93. Littman ML, Walter JE. Cryptococcosis: current status. *Am J Med* 1968;**45**:922–32.
94. Louria DB, Feder N, Mitchell W, et al. Influence of fungus strain and lapse on time in experimental histoplasmosis and of volume of inoculum in cryptococcosis upon recovery of the fungi. *J Lab Clin Med* 1959;**53**:311–7.
95. Ludviksson BR, Thorarensen O, Gudnason T, et al. *Candida albicans* meningitis in a child with myeloperoxidase deficiency. *Pediatr Infect Dis J* 1993;**12**:162–4.
96. Luke JL, Bolande RP, Gross S. Generalized aspergillosis and *Aspergillus endocarditis* in infancy. *Pediatrics* 1963;**31**:115–22.
97. Marinova I, Szabadosova V, Brandeburova O, et al. *Rhodotorula* spp. fungemia in an immunocompromised boy after neurosurgery successfully treated with miconazole and 5-flucytosine: case report and review of the literature. *Chemotherapy* 1994;**40**:287–9.
98. Marr B, Gross S, Cunningham C, Weiner L. Candidal sepsis and meningitis in a very-low-birth-weight infant successfully treated with fluconazole and flucytosine. *Clin Infect Dis* 1994;**19**:795–6.
99. McDonald R, Greenberg EN, Kramer R. Cryptococcal meningitis. *Arch Dis Child* 1970;**45**:417–20.
100. McGinnis MR. Detection of fungi in cerebrospinal fluid. *Am J Med* 1983;**75**:129–38.
101. McManus EJ, Jones JM. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcus neoformans* capsular polysaccharide in serum from a patient with disseminated *Trichosporon* infection. *J Clin Microbiol* 1985;**21**:681–5.
102. Miaux Y, Ribaud P, Williams M, et al. MR of cerebral aspergillosis in patients who have had bone marrow transplantation. *AJNR Am J Neuroradiol* 1995;**16**:555–62.
103. Mikolich DJ, Kinsella LJ, Skowron G, et al. *Aspergillus* meningitis in an immunocompetent adult successfully treated with itraconazole. *Clin Infect Dis* 1996;**23**:1318–9.
104. Morrow R, Wong B, Finkelstein WE, et al. Aspergillosis of the cerebral ventricles in a heroin abuser: case report and review of the literature. *Arch Intern Med* 1983;**143**:161–4.
105. Morse HG, Nichol WP, Cook DM, et al. Central nervous system and genitourinary blastomycosis: confusion with tuberculosis. *West J Med* 1983;**139**:99–103.
106. Morwood DT, Nichter LS, Wong V. An unusual complication of an open-head injury: coccidioidal meningitis. *Ann Plast Surg* 1989;**23**:437–41.
107. Nicholas SW, Dondheimer DL, Willoughby AD, et al. Human immunodeficiency virus infection in childhood, adolescence, and

pregnancy: a status report and national research agenda. *Pediatrics* 1989;**83**:293–308.
108. Nosanchuk JD, Shoham S, Fries BC, et al. Evidence of zoonotic transmission of *Cryptococcus neoformans* from a pet cockatoo to an immunocompromised adult. *Ann Intern Med* 2000;**132**:205–8.
109. O'Brien D, Stevens NT, Lim CH, et al. Candida infection of the central nervous system following neurosurgery: a 12-year review. *Acta Neurochir (Wien)* 2011;**153**:1347–50.
110. Palaoglu S, Sav A, Basak T, et al. Cerebral phaeohyphomycosis. *Neurosurgery* 1993;**33**:894–7.
111. Papadatos C, Pavatou M, Alexiou D. *Cephalosporium* meningitis. *Pediatrics* 1969;**44**:749–51.
112. Pappagianis D. Epidemiology of coccidioidomycosis. In: Stevens DA, editor. *Coccidioidomycosis: A text*. New York: Plenum Medical; 1980. p. 63.
113. Pappagianis D, Crane R. Survival in coccidioidal meningitis since introduction of amphotericin B. In: Ajello L, editor. *Coccidioidomycosis: Current clinical and diagnostic status*. Miami, Fla: Symposia Specialists Medical Books; 1977. p. 223–237.
114. Pappas PG, Kauffman CA, Ades D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
115. Pappas PG, Pottage JC, Powderly WG, et al. Blastomycosis in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1992;**116**:847–53.
116. Parsons RJ, Zarafonetis CJD. Histoplasmosis in man: Report of seven cases and a review of seventy-one cases. *Arch Intern Med* 1945;**75**:1–23.
117. Penn CC, Goldstein E, Bartholomew MR. *Sporothrix schenckii* meningitis in a patient with AIDS. *Clin Infect Dis* 1992;**15**:741–3.
118. Pereira GH, Pádua SS, Park MV, et al. Chronic meningitis by histoplasmosis: report of a child with acute myeloid leukemia. *Braz J Infect Dis* 2008;**12**:555–77.
119. Pereira WC, Tenuto RA, Raphael A, et al. Localizacaco encefalica da blastomicose Sul-Americana. *Arq Neuro-Psiquiat (São Paulo)* 1965;**23**:113–26.
120. Perez Jr JA, Johnson RA, Caldwell JW, et al. Fluconazole therapy in coccidioidal meningitis maintained with intrathecal amphotericin B. *Arch Intern Med* 1995;**155**:1665–8.
121. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
122. Piper L, Smith PB, Hornik CP, et al. Fluconazole loading dose pharmacokinetics and safety in infants. *Pediatr Infect Dis J* 2011;**30**:375–8.
123. Pore RS, Chen J. Meningitis caused by *Rhodotorula*. *Sabouraudia* 1976;**14**:331–5.
124. Prabhu RM, Orenstein R. Failure of caspofungin to treat brain abscesses secondary to *Candida albicans* prosthetic valve endocarditis. *Clin Infect Dis* 2004;**39**:1253–4.
125. Proia LA, Tenorio AR. Successful use of voriconazole for treatment of *Coccidioides* meningitis. *Antimicrob Agents Chemother* 2004;**48**:2341.
126. Rapaport SI, Ames SB, Duvall BJ. A plasma coagulation defect in systemic lupus erythematosus arising from hypoprothrombinemia combined with antiprothrombinase activity. *Blood* 1960;**15**: 212–27.
127. Rex JH, Ginsberg AM, Fries LF, et al. *Cunninghamella bertholetiae* infection associated with deferoxamine therapy. *Rev Infect Dis* 1988;**10**:1187–94.
128. Rex JH, Pfaller MA, Galgiani JN, et al. Development of interpretative breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro-in vivo correlation data for fluconazole, itraconazole, and *Candida* infections. *Clin Infect Dis* 1997;**24**:235–47.
129. Rogers MF, Thomas PA, Starcher ET, et al. Acquired immunodeficiency syndrome in children: report of the Centers for Disease Control national surveillance, 1982-1985. *Pediatrics* 1987;**79**: 1008–14.
130. Ross KL, Bryan JP, Maggio WW, et al. Intracranial blastomycoma. *Medicine (Baltimore)* 1987;**66**:224–35.
131. Ruggierei M, Polizzi A, Vitaliti MC, et al. Fatal biphasic brainstem and spinal leptomeningitis with *Cryptococcus neoformans* in a non-immunocompromised child. *Acta Paediatr* 1999;**88**:671–4.
132. Salaki JS, Louria DB, Chmel H. Fungal and yeast infections of the central nervous system: a clinical review. *Medicine (Baltimore)* 1984;**63**:108–32.
133. Sarma PS, Durairaj P, Padhye AA. *Candida lusitaniae* causing fatal meningitis. *Postgrad Med J* 1993;**69**:878–80.
134. Scarcella A, Pasquariello MB, Guigliano B, et al. Liposomal amphotericin B treatment for neonatal fungal infections. *Pediatr Infect Dis J* 1998;**17**:146–8.
135. Schutze GE, Tucker NC, Jacobs RF. Histoplasmosis and perinatal human immunodeficiency virus. *Pediatr Infect Dis J* 1992;**11**: 501–2.
136. Schutze GE, Hickerson SL, Fortin EM, et al. Blastomycosis in children. *Clin Infect Dis* 1996;**22**:496–502.
137. Scott EN, Kaufman L, Brown AC, et al. Serologic studies in the diagnosis and management of meningitis due to *Sporothrix schenckii*. *N Engl J Med* 1987;**317**:935–40.
138. Sekshon AS, Borgorus MS, Sims HV. Blastomycosis: report of three cases from Alberta with a review of Canadian cases. *Mycopathologia* 1979;**1**:53–63.
139. Shehab ZM, Britton H, Dunn JH. Imidazole therapy of coccidioidal meningitis in children. *Pediatr Infect Dis J* 1988;**7**:40–4.
140. Shimosaka S, Waga S. Cerebral chromoblastomycosis complicated by meningitis and multiple fungal aneurysms after resection of a granuloma: case report. *J Neurosurg* 1983;**59**:158–61.
141. Shoemaker EH, Bennett HD, Fields WS, et al. Leptomeningitis due to *Sporotrichum schenckii*. *Arch Pathol* 1957;**64**:222.
142. Siewers CMF, Cramblett HG. Cryptococcosis (torulosis) in children: a report of four cases. *Pediatrics* 1964;**34**:393–400.
143. Smego RA, Perfect JR, Durack DT. Combined therapy with amphotericin B and 5-fluorocytosine for *Candida* meningitis. *Rev Infect Dis* 1984;**6**:791–801.
144. Smith CE, Saito MT, Simons SA. Pattern of 39,500 serologic tests in coccidioidomycosis. *JAMA* 1956;**160**:546–52.
145. Stevens D. Coccidioides immitis. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 2365–75.
146. Stone BD, Wheeler JG. Disseminated cryptococcal infection in a patient with hyperimmunoglobulinemia E syndrome. *J Pediatr* 1990;**117**:92–5.
147. Takeda K, Oritsu M, Sakuta M. A case of coccidioidomycosis with central nervous system involvement. *Rinsho Shinkeigaku* 1993;**33**:1184–7.
148. Tay ST, Rohani MY, Hoo TS, et al. Epidemiology of cryptococcosis in Malaysia. *Mycoses* 2010;**53**:509–14.
149. Taylor GD, Boettger DW, Miedzinski LJ, et al. Coccidioidal meningitis acquired during holidays in Arizona. *Can Med Assoc J* 1990;**142**:1388–90.
150. Taylor GD, Buchanan-Chell M, Kirkland T, et al. Trends and sources of nosocomial fungaemia. *Mycoses* 1994;**37**:187–90.
151. Tiraboschi I, Parera IC, Pikielny R, et al. Chronic *Histoplasma capsulatum* infection of the central nervous system successfully treated with fluconazole. *Eur Neurol* 1992;**32**:70–3.
152. Tjia TL, Yeow YK, Tan CB. Cryptococcal meningitis. *J Neurol Neurosurg Psychiatry* 1985;**48**:853–8.
153. Townsend TE, McKay RW. Coccidioidomycosis in infants. *Am J Dis Child* 1953;**86**:51–3.
154. Tucker RM, Denning DW, Dupont B, et al. Itraconazole therapy for chronic coccidioidal meningitis. *Ann Intern Med* 1990;**112**: 108–12.
155. Tucker RM, Galgiani JN, Denning DW, et al. Treatment of coccidioidal meningitis with fluconazole. *Rev Infect Dis* 1990;**12**(suppl. 3):S390–9.
156. Tucker RM, Williams PL, Arathoon EG, et al. Treatment of mycoses with itraconazole. *Ann N Y Acad Sci* 1988;**544**: 451–70.
157. Tynes BS, Crutcher JC, Utz JP. Histoplasma meningitis. *Ann Intern Med* 1963;**59**:615–21.
158. van Landeghem FK, Stiller B, Lehmann TN, et al. Aqueductal stenosis and hydrocephalus in an infant due to aspergillus infection. *Clin Neuropathol* 2000;**19**:26–9.
159. Van Peer A, Woestenborghs R, Heykants J, et al. The effects of food and dose on the oral systemic availability of itraconazole in healthy subjects. *Eur J Clin Pharmacol* 1989;**36**:423–6.
160. Van't Wout JW, DeGraeff-Meeder ER, Paul LC, et al. Treatment of two cases of cryptococcal meningitis with fluconazole. *Scand J Infect Dis* 1988;**20**:193–8.

161. Venger BH, Landon G, Rose JE. Solitary histoplasmoma of the thalamus: case report and literature review. *Neurosurgery* 1987;**20**:784–7.
162. Venugopal PV, Venugopal TV, Thiruneelakantan D, et al. Cerebral aspergillosis: report of two cases. *Sabouraudia* 1977; **15**:225–30.
163. Verweij PE, Brinkman K, Kremer HPH, et al. *Aspergillus* meningitis: diagnosis by nonculture-based microbiological methods and management. *J Clin Microbiol* 1999;**37**:1186–9.
164. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
165. Wanger A, Mills K, Nelson PW, Rex JH. Comparison of Etest and National Committee for Clinical Laboratory Standards broth microdilution method for antifungal susceptibility testing: enhanced ability to detect amphotericin B-resistant isolates. *Antimicrob Agents Chemother* 1995;**39**:2520–2.
166. Wheat LJ, Musial CE, Jenny-Avital E. Diagnosis and management of central nervous system histoplasmosis. *Clin Infect Dis* 2005;**40**:844–52.
167. Wheat LJ, Batteiger BE, Sathapatayavongs B. *Histoplasma capsulatum* infections of the central nervous system: a clinical review. *Medicine (Baltimore)* 1990;**69**:244–60.
168. Wheat LJ, Connolly-Stringfield PA, Baker RL, et al. Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment and review of the literature. *Medicine (Baltimore)* 1990;**69**:361–74.
169. Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:807–25.
170. Wheat J, Sarosi G, McKinsey D, et al. Practice guidelines for the management of patients with histoplasmosis. *Clin Infect Dis* 2000;**30**:688–95.
171. Williams PL. Coccidioidal meningitis. *Ann N Y Acad Sci* 2007;**1111**:377–84.
172. Windusi DW, Stokes JJ, Julian BA, et al. Fatal *Rhizopus* infections in hemodialysis patients receiving deferoxamine. *Ann Intern Med* 1987;**107**:678–80.
173. Young RC, Bennett JE, Vogel CL, et al. Aspergillosis: the spectrum of the disease in 98 patients. *Medicine (Baltimore)* 1970;**49**:147–73.
174. Zealear DS, Winn WA. The neurosurgical approach in the treatment of coccidioidal meningitis: report of ten cases. In: Ajello L, editor. *Coccidioidomycosis*. Tucson, Ariz: University of Arizona Press; 1967. p. 43–53.
175. Zuger A, Louie E, Holzman RS, et al. Cryptococcal disease in patients with the acquired immunodeficiency syndrome: diagnostic features and outcome of treatment. *Ann Intern Med* 1986; **104**:234–40.

subclinical, with the adult female worm producing 115,000 to 179,000 eggs/worm/day, most of which are shed in feces.[12] Young raccoons have a higher prevalence of infection and higher parasitic burden; in their first few months of life, they ingest eggs that are in their environment or sticking to their mothers' fur.

EPIDEMIOLOGY

The geographic distribution of eosinophilic meningitis from angiostrongyliasis depends on the distribution of *A. cantonensis*. Many cases have been reported from Taiwan and Thailand. Other areas include Vietnam, the Society Islands (especially Tahiti), Hawaii, the Marshall Islands, and Ponape. Rodents infected with the worm have been documented in other areas of the Pacific Islands and Asia. Disease has been reported from Cuba, Egypt, and many other countries. As infected rodents are carried to other countries on cargo ships, spread of these organisms will continue to increase. Several cases have been diagnosed in the continental United States in travelers from endemic regions.[13,16] An outbreak was described in 12 of 23 young adults who had traveled together to Jamaica.[19,41]

The seasonal incidence of the disease varies in different areas. The months of highest prevalence usually correspond with the more humid periods in each country.

Patients with eosinophilic meningitis have eaten terrestrial snails, slugs, fish, or fresh-water shrimp, all of which can serve as intermediate hosts for the parasites.[44,45] In Thailand, the most common source is the *Pila* snails, which are eaten raw or pickled.[28] They may be served as an appetizer with alcoholic beverages, which may partly explain the increased incidence of the disease in men.[29] The giant African snail *Achatina fulica* is found commonly in Taiwan and may carry thousands of roundworm larvae, explaining the higher incidence of recovery of organisms from the patients in Taiwan.[45] The larvae also may remain infective in water for approximately 60 hours. Cases have been associated with the ingestion of leafy vegetables, presumed to be contaminated by slugs or snails.[17]

The distribution of disease among age groups varies. In Thailand, most of the cases occur in the third to fourth decades of life, but in Taiwan and Hawaii, most cases occur in children younger than 15 years old.[29,49] Although eosinophilic meningitis rarely occurs in very young children, one report documented disease in five children younger than 2 years.[40]

The first confirmed cases of *B. procyonis* neural larva migrans were described in the 1980s in 10-month-old and 18-month-old boys from Pennsylvania and Illinois, respectively.[11,15] Both boys had rapidly progressive, fatal eosinophilic meningoencephalitis. Subsequent reports of fatal and nonfatal disease have been reported from areas with large raccoon populations. They have occurred in rural, urban, and suburban areas. Two risk factors for acquisition of severe infection are contact with raccoons, particularly baby or young animals, or their feces or a contaminated environment, and pica or geophagia. Outdoor contamination of children's play areas by raccoons and proximity of raccoon communal latrines to human habitation may contribute to risk.[35]

PATHOGENESIS

For angiostrongyliasis, the pathogenesis of this disease in humans has not been studied rigorously but is presumed to parallel the pathogenesis in the rodent host. The larvae are ingested, make their way into the general circulation, and selectively enter the CNS. The number of nematodes found on autopsy specimens has varied. In a 5-year-old Taiwanese girl, 150 nematodes were found on the surface of the cerebrum and cerebellum and in the subarachnoid spaces, and more than 500 were recovered from the normal saline in which the spinal cord and meninges were placed. *A. cantonensis* also were found in the pulmonary arteries.[49]

Pathologic specimens have shown a leptomeningitis in which plasma cells and eosinophils predominate. Tortuous tracks of variable size in the brain and spinal cord parenchyma surrounded by variable reaction and degenerating neurons may be present. Granulomata may form around dead *A. cantonensis*.[26]

Hemorrhagic, necrotic tracts caused by the organisms have been associated more commonly with *G. spinigerum* infection.[4] *Angiostrongylus* is capable of causing a vascular reaction, however, including thrombosis and rupture of vessels and arteritis, leading to formation of an aneurysm.[26] The tracts caused by *G. spinigerum* may be larger and more necrotic.

In baylisascariasis, information about the pathology is based on a few fatal cases with massive larval invasion of the CNS.[12] Swelling of the brain, leptomeningeal congestion, and evidence of herniation have been seen. Necrosis is most marked in the periventricular white matter. Large deposits of eosinophils are present along necrotic migration tracts.

CLINICAL MANIFESTATIONS

On the basis of information obtained from patients with a history of ingestion of the intermediate hosts, the incubation period is 7 to 30 days. Most patients with typical eosinophilic meningitis have an abrupt onset of disease; a more insidious onset may be noted in 20 percent of cases. Headache is the most common and distressing symptom. It usually is intermittent but is frequent and severe. Other common symptoms are nausea, vomiting (often projectile), intermittent somnolence, malaise, anorexia, constipation, and fever with temperatures usually reaching a maximum of 38° to 39°C (100.4° to 102.2°F) in the early phase of the disease, although many patients have no documented fever. Nuchal rigidity is seen more commonly in older patients, often in association with severe headache. Paresthesias occur in a large variety of locations and are expressed as pain, numbness, itching, or a sense of worms crawling on the skin. Some patients also note diplopia with or without strabismus. Convulsions are unusual occurrences.

The findings on physical examination are normal in half the patients. Physical findings may include mild hepatomegaly, mild changes in deep tendon reflexes (usually decreased), nuchal rigidity, absent abdominal wall reflexes, and, less commonly, ophthalmoplegia or

facial paralysis. Twelve percent of Thai patients had abnormal ophthalmoscopic examinations.[16]

Examination of the CSF reveals grossly turbid or opalescent fluid in most patients, with leukocyte counts usually 100/mm^3 to 5000/mm^3. All counts usually reach their maximum in the first 3 weeks of disease, decreasing sharply thereafter. Generally, the percentage of eosinophils in the CSF is high, often greater than 50 percent. Fluids with higher cell counts tend to have a higher percentage of eosinophils.[30,49] The proportion of eosinophils may decrease after the first 4 weeks. CSF protein concentration is moderately high (often 50 to 200 mg/dL), but the glucose concentration usually is normal.

Peripheral white blood cell count varies, but the differential cell count often shows striking eosinophilia. Examination of the feces may reveal concurrent infestation with other parasites, such as *Ascaris* or *Trichuris*.

The clinical manifestations of the eosinophilic radiculomyeloencephalitis associated with *G. spinigerum* overlap to some degree with the manifestations of typical eosinophilic meningitis. Headache is less prominent, however. Many patients report sharp, shooting pains of the trunk or limbs, flaccid paralyses, and impairment of superficial sensation.[31] Impairment of the sensorium may be sudden in association with cerebral hemorrhage. Grossly bloody spinal fluid is a common finding when myeloencephalitis is present, but it is extremely rare in typical eosinophilic meningitis.

Patients with baylisascariasis develop an acute fulminant eosinophilic meningoencephalitis; early features include low-grade fever, increasing lethargy, ataxia, somnolence, and irritability. Progression to posturing, spasticity, and ocular or cranial nerve involvement occurs. Seizures are common occurrences. Continued deterioration to coma and death may occur, or, with survival, to a persistent vegetative state or severe deficits.

DIAGNOSIS

The diagnosis of cerebral angiostrongyliasis usually is based on the clinical presentation with associated CSF eosinophilia and an epidemiologic history consistent with possible exposure to the *A. cantonensis* larvae. Rarely, larvae may be recovered from the CSF. Two thirds of patients also have peripheral eosinophilia. Enzyme-linked immunosorbent assay helps confirm the diagnosis.[10,42] Dot-blot ELISA kits for rapid diagnosis in the field have been developed that are simple and effective.[10] Newer immunoblotting analyses show excellent specificity for the diagnosis of angiostrongyliasis and gnathostomiasis in endemic areas.[38] The absence of focal findings on computed tomography and abnormal enhancement in the globus pallidus may be valuable in establishing a diagnosis.

Gnathostomiasis often is associated with xanthochromic or bloody CSF with an eosinophilic pleocytosis. Peripheral eosinophilia often is pronounced. Computed tomography and magnetic resonance imaging show areas of hemorrhage.[35] Immunoblotting serologies are available,[10] but definitive diagnosis is by recovery of the pathogen, which may be found in subcutaneous tissue.

Baylisascariasis should be suspected in a child with CSF and peripheral eosinophilia, with severe encephalopathy with diffuse white matter disease on neuroimaging,[36] and with or without eye disease in North America or Europe. A history of exposure to raccoons or their feces would strongly suggest the diagnosis. In the absence of brain biopsy, the diagnosis is made serologically.[15]

DIFFERENTIAL DIAGNOSIS

Other helminths may invade the CNS of humans and may be associated with an eosinophilic pleocytosis,[13,47] including *Taenia solium*, the pork tapeworm causing cerebrospinal cysticercosis; *Schistosoma* spp.; *Paragonimus westermani*; and *Echinococcus*. The diseases produced generally have a chronic and intermittent course. Signs of a space-occupying lesion and convulsions are found frequently. Visceral myiasis with invasion of the CNS by botfly larvae may cause CSF eosinophilia.[3] The larvae of *Trichinella spiralis* can invade the CNS, but pleocytosis seems to be an unusual occurrence.[3,13] Neurologic involvement in visceral larva migrans caused by *Toxocara canis* can occur; it may be associated with CSF pleocytosis and eosinophilia.[23,43] Rarely, eosinophilic pleocytosis can be seen in coenosis and filariasis.[13]

Coccidioidomycosis is the one fungal infection that may be associated with CSF eosinophilia when infection involves the meninges.[33,39] In one series, 30 percent of cases with coccidioidal meningitis had significant CSF eosinophilia.[33] Neurosyphilis and tuberculous meningitis rarely have been associated with eosinophils in the CSF, as have several malignant neoplasms, particularly lymphomas, involving the CNS.[18,24] Other known causes of significant eosinophilic pleocytosis include intrathecal injection of various foreign proteins, rabies vaccination, the insertion of rubber tubing into the CNS during neurosurgery, lymphocytic choriomeningitis,[18] and Rocky Mountain spotted fever.[9] Eosinophilic pleocytosis has been documented in infants with congenital toxoplasmosis and late-onset group B streptococcal meningitis.[22,48]

Eosinophilia in the CSF indicating hypersensitivity has been difficult to document, although one case report described the association of eosinophilic meningitis and ibuprofen therapy.[32] Eosinophilic meningitis developing after implantation of a ventriculostomy catheter impregnated with rifampin and minocycline has been reported.[2] A study documented more than 5 percent eosinophils in the ventricular fluid of patients with shunt-associated pathology, particularly malfunction.[21]

TREATMENT

In a study in Thailand in which the disappearance of headache was used as the criterion for improvement in patients with *A. cantonensis* meningitis, no significant differences were noted among groups when 284 patients were treated with analgesics alone, 96 patients were treated with analgesics and steroids (prednisone 30 to

60 mg/day for 5 days), and 56 patients were treated with analgesics and antibiotics (penicillin or tetracycline).[25] A study that compared adult patients given a 2-week course of prednisolone (60 mg/day) with patients treated with a placebo showed a significant decrease in the number of patients with headache and in the duration of headache in the prednisolone-treated group.[6] The combination of albendazole or mebendazole with prednisolone has shown promise.[7,8] However, a recent randomized, controlled trial comparing prednisolone and albendazole with prednisolone alone showed no added benefit from the albendazole.[5] Treatment of gnathostomiasis is supportive, although steroids have been used to decrease meningeal inflammation. Baylisascariasis has no known effective anthelminthic therapy, although treatment with albendazole and corticosteroids has been used.

COURSE AND PROGNOSIS

In most patients, eosinophilic meningitis is a self-limited disease characterized by repeated attacks of severe headache, vomiting, intermittent fever, and somnolence. Many patients experience a dramatic improvement soon after undergoing a lumbar puncture, so repeated lumbar punctures may be performed at weekly intervals to relieve the headache. In most patients, most symptoms disappear within 4 weeks of onset, often within a few days after the first lumbar puncture is performed, leaving no sequelae. The mortality rate in large series from Thailand and Taiwan has been less than 5 percent; the incidence of permanent sequelae also has been less than 5 percent for *Angiostrongylus* infection.[13,30] The mortality in gnathostomiasis is higher, ranging from 7.7 to 25 percent.[37] No evidence suggests that immunity develops after recovery from infection; many recurrences have been reported.[30,34,49] In baylisascariasis, the mortality rate is high, and survivors often have severe sequelae. A recent case in a 4 year old documenting full recovery is encouraging.[27]

PREVENTION

Disease can be prevented only by control of rodents and proper cooking of mollusks, shrimp, fish, and other intermediate hosts. Careful washing of fruits and vegetables that may be contaminated by rodent feces also is important. The larvae may remain infective in water for 60 hours, so protection of the water supply should be attempted. Prevention of baylisascariasis depends on eliminating exposure to raccoon feces.

NEW REFERENCES SINCE THE SIXTH EDITION

5. Chotmongkol V, Kittimongkolma S, Niwattayakul K, et al. Comparison of prednisolone plus albendazole with prednisolone alone for treatment of patients with eosinophilic meningitis. *Am J Trop Med Hyg* 2009;**81**:443–5.
10. Eamsobhana P, Yong HS. Immunological diagnosis of human angiostrongyliasis due to *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae). *Int J Infect Dis* 2009;**13**:425–31.
13. Graeff-Teixeira C, Arámburu da Silva AC, Yoshimura K. Update on eosinophilic meningoencephalitis and its clinical relevance. *Clin Microbiol Rev* 2009;**22**:322–48.
27. Pai PJ, Blackburn BG, Kazacos KR, et al. Full recovery from *Baylisascaris procyonis* eosinophilic meningitis. *Emerg Infect Dis* 2007;**13**:928–30.
38. Sawanyawisuth K, Sawanyawisuth K, Intapan PM, et al. Specificity of immunoblotting analyses in eosinophilic meningitis. *Mem Inst Oswaldo Cruz* 2011;**106**:570–2.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Beaver PC, Rosen L. Memorandum on the first report of *Angiostrongylus* in man by Nomura and Lin, 1945. *Am J Trop Med Hyg* 1964;**13**:589–90.
2. Bell RS, Vo AH, Cooper PB, et al. Eosinophilic meningitis after implantation of a rifampin and minocycline-impregnated ventriculostomy catheter in a child: case report. *J Neurosurg* 2006;**104**(suppl):50–4.
3. Char DFB, Rosen L. Eosinophilic meningitis among children in Hawaii. *J Pediatr* 1967;**70**:28–35.
4. Chitanondh H, Rosen L. Fatal eosinophilic encephalomyelitis caused by the nematode *Gnathostoma spinigerum*. *Am J Trop Med Hyg* 1967;**16**:638–45.
5. Chotmongkol V, Kittimongkolma S, Niwattayakul K, et al. Comparison of prednisolone plus albendazole with prednisolone alone for treatment of patients with eosinophilic meningitis. *Am J Trop Med Hyg* 2009;**81**:443–5.
6. Chotmongkol V, Sawadpanitch K, Sawanyawisuth K, et al. Treatment of eosinophilic meningitis with a combination of prednisolone and mebendazole. *Am J Trop Med Hyg* 2006;**74**: 1122–4.
7. Chotmongkol V, Sawanyawisuth K, Thavornpirak Y. Corticosteroid treatment of eosinophilic meningitis. *Clin Infect Dis* 2000;**31**: 660–2.
8. Chotmongkol V, Wongjitrat C, Sawadpanit K, et al. Treatment of eosinophilic meningitis with a combination of albendazole and corticosteroid. *Southeast Asian J Trop Med Public Health* 2004;**35**:172–4.
9. Crennan JM, VanScoy RE. Eosinophilic meningitis caused by Rocky Mountain spotted fever. *Am J Med* 1986;**80**:288–9.
10. Eamsobhana P, Yong HS. Immunological diagnosis of human angiostrongyliasis due to *Angiostrongylus cantonensis* (Nematoda: Angiostrongylidae). *Int J Infect Dis* 2009;**13**:425–31.
11. Fox AS, Kazacos KR, Gould NS, et al. Fatal eosinophilic meningoencephalitis and visceral larva migrans caused by the raccoon ascarid *Baylisascaris procyonis*. *N Engl J Med* 1985;**312**:1619–23.
12. Gavin PJ, Kazacos KR, Shulman ST. Baylisascariasis. *Clin Microbiol Rev* 2005;**18**:703–18.
13. Graeff-Teixeira C, Arámburu da Silva AC, Yoshimura K. Update on eosinophilic meningoencephalitis and its clinical relevance. *Clin Microbiol Rev* 2009;**22**:322–48.
14. Hochberg NS, Park SY, Blackborn BG, et al. Distribution of eosinophilic meningitis cases attributable to *Angiostrongylus cantonensis*, Hawaii. *Emerg Infect Dis* 2007;**13**:1675–80.
15. Huff DS, Neatie RC, Binder MJ, et al. Case 4. The first fatal *Baylisascaris* infection in humans: an infant with eosinophilic meningoencephalitis. *Pediatr Pathol* 1984;**2**:345–52.
16. Kanchanaranya C, Punyagupta S. Case of ocular angiostrongyliasis associated with eosinophilic meningitis. *Am J Ophthalmol* 1971;**71**: 931–4.
17. Koo J, Pien F, Kliks MM. *Angiostrongylus* (parastrongylus) eosinophilic meningitis. *Rev Infect Dis* 1988;**10**:1155–62.
18. Kuberski T. Eosinophils in the cerebrospinal fluid. *Ann Intern Med* 1979;**91**:70–5.
19. Lo Re V, Glickman SJ. Eosinophilic meningitis due to *Angiostrongylus cantonensis* in a returned traveler: case report and review of the literature. *Clin Infect Dis* 2001;**33**:e112–5.
20. Lo Re V, Glickman SJ. Eosinophilic meningitis. *Am J Med* 2003;**114**:217–23.
21. McClinton D, Caraccio C, Englander R. Predictors of ventriculoperitoneal shunt pathology. *Pediatr Infect Dis J* 2001;**20**:593–7.
22. Miron D, Snelling LK, Josephson SL, et al. Eosinophilic meningitis in a newborn with group B streptococcal infection. *Pediatr Infect Dis J* 1993;**12**:966–7.
23. Moreira-Silva SF, Rodrigues MG, Pimenta JL, et al. Toxocariasis of the central nervous system: with report of two cases. *Rev Soc Brasil Med Trop* 2004;**37**:169–74.
24. Mulligan MJ, Vasu R, Grossi CE, et al. Case report. Neoplastic meningitis with eosinophilic pleocytosis in Hodgkin's disease: a case with cerebellar dysfunction and a review of the literature. *Am J Med Sci* 1988;**296**:322–6.
25. Noskin GA, McMenamin MB, Grohmann SM. Eosinophilic meningitis due to *Angiostrongylus cantonensis*. *Neurology* 1992;**42**:1423–4.
26. Nye SW, Tangchai P, Sundarakiti S. Lesion of the brain in eosinophilic meningitis. *Arch Pathol* 1970;**89**:9–19.
27. Pai PJ, Blackburn BG, Kazacos KR, et al. Full recovery from *Baylisascaris procyonis* eosinophilic meningitis. *Emerg Infect Dis* 2007; **13**:928–30.
28. Punyagupta S. Eosinophilic meningoencephalitis in Thailand: summary of nine cases and observations on *Angiostrongylus cantonensis* as a causative agent and *Pila ampullacea* as a new intermediate host. *Am J Trop Med Hyg* 1965;**14**:370–4.
29. Punyagupta S, Bunnag T, Juttijudata P, et al. Eosinophilic meningitis in Thailand: epidemiologic studies of 484 typical cases and the etiologic role of *Angiostrongylus cantonensis*. *Am J Trop Med Hyg* 1970;**19**:950–8.
30. Punyagupta S, Juttijudata P, Bunnag T. Eosinophilic meningitis in Thailand: clinical studies of 484 typical cases probably caused by *Angiostrongylus cantonensis*. *Am J Trop Med Hyg* 1975;**24**:921–31.
31. Punyagupta S, Limtrakul C, Vichipanthu P, et al. Radiculomyeloencephalitis associated with eosinophilic pleocytosis: report of nine cases. *Am J Trop Med Hyg* 1968;**17**:551–60.
32. Quinn JP, Weinstein RA, Caplan LR. Eosinophilic meningitis and ibuprofen therapy. *Neurology* 1984;**34**:108–9.
33. Ragland AS, Arsura E, Ismail Y, et al. Eosinophilic pleocytosis in coccidioidal meningitis: frequency and significance. *Am J Med* 1993;**95**:254–6.
34. Rosen L, Loison G, Laigret J, et al. Studies on eosinophilic meningitis. III. Epidemiologic and clinical observations on Pacific Islands and the possible etiologic role of *Angiostrongylus cantonensis*. *Am J Epidemiol* 1967;**85**:17–44.
35. Roussere GP, Murray WJ, Raudenbush CB, et al. Raccoon roundworm eggs near homes and risk for larva migrans disease, California communities. *Emerg Infect Dis* 2003;**9**:1516–22.
36. Rowley HA, Uht RM, Kazacos KR, et al. Radiologic-pathologic findings in raccoon roundworm *(Baylisascaris procyonis)* encephalitis. *AJNR Am J Neuroradiol* 2000;**21**:415–20.
37. Rusnak JM, Lucey DR. Clinical gnathostomiasis: case report and review of the English language literature. *Clin Infect Dis* 1993;**16**:33–50.
38. Sawanyawisuth K, Sawanyawisuth K, Intapan PM, et al. Specificity of immunoblotting analyses in eosinophilic meningitis. *Mem Inst Oswaldo Cruz* 2011;**106**:570–2.
39. Schermoly MJ, Hinthorn DR. Eosinophilia in coccidioidomycosis. *Arch Intern Med* 1988;**148**:895–6.
40. Shih S-L, Hsu C-H, Huanig F-Y, et al. *Angiostrongylus cantonensis* infection in infants and young children. *Pediatr Infect Dis J* 1992; **11**:1064–5.
41. Slom TJ, Cortese MM, Gerber SI, et al. An outbreak of eosinophilic meningitis caused by *Angiostrongylus cantonensis* in travelers returning from the Caribbean. *N Engl J Med* 2002;**346**:668–75.
42. Tsai HC, Liu YC, Kunin CM, et al. Eosinophilic meningitis caused by *Angiostrongylus cantonensis*: report of 17 cases. *Am J Med* 2001;**111**:109–14.
43. Vidal JE, Sztanjbok J, Seguro AC. Eosinophilic meningoencephalitis due to *Toxacara canis*: case report and review of the literature. *Am J Trop Med Hyg* 2003;**69**:341–3.
44. Wallace GD, Rosen L. Studies on eosinophilic meningitis. IV. Experimental infection of freshwater and marine fish with *Angiostrongylus cantonensis*. *Am J Epidemiol* 1967;**85**:395–402.
45. Wallace GD, Rosen L. Studies on eosinophilic meningitis. V. Molluscan hosts of *Angiostrongylus cantonensis* on Pacific Islands. *Am J Trop Med Hyg* 1969;**18**:206–16.
46. Wallace GD, Rosen L. Studies on eosinophilic meningitis. VI. Experimental infection of rats and other homoiothermic vertebrates with *Angiostrongylus cantonensis*. *Am J Epidemiol* 1969;**89**: 331–44.
47. Weller PF. Eosinophilic meningitis. *Am J Med* 1993;**95**:250–3.
48. Woods CR, Englund J. Congenital toxoplasmosis presenting with eosinophilic meningitis. *Pediatr Infect Dis J* 1993;**12**:347–8.
49. Yii C. Clinical observations on eosinophilic meningitis and meningoencephalitis caused by *Angiostrongylus cantonensis* on Taiwan. *Am J Trop Med Hyg* 1976;**25**:233–49.

meningitis. *Serous meningitis*, *lymphocytic meningitis*, and *nonparalytic poliomyelitis* are terms that were used in the past to denote aseptic meningitis.

HISTORY

Aseptic meningitis is a syndrome that first was described by Wallgren in 1925.[346] Wallgren's criteria for this diagnosis included (1) an acute onset with obvious signs and symptoms of meningeal involvement; (2) alteration of CSF typical of meningitis, which may show a small or large number of cells; (3) absence of bacteria in the CSF, as shown by appropriate culture; (4) a relatively short, benign course of illness; (5) absence of local parameningeal infection (e.g., otitis, sinusitis, or trauma) or a general disease that might have meningitis as a secondary manifestation; and (6) absence from the community of epidemic disease, of which meningitis is a feature. In 1951, Wallgren[345] redefined aseptic meningitis as a syndrome likely to be encountered in many different infectious diseases.

The clinical occurrence of aseptic meningitis first was recognized in epidemic poliomyelitis and in mumps at the beginning of the 20th century.[106,353] Rivers and Scott[274] reported the recovery of lymphocytic choriomeningitis virus from the CSF of several patients with aseptic meningitis in 1935, and in 1934, Johnson and Goodpasture[173] proved that mumps was caused by a virus. The discovery of coxsackieviruses in 1948 by Dalldorf and Sickles[86] and the introduction of tissue culture in 1949 by Enders and colleagues,[107] which resulted in the discovery of echoviruses, paved the way for the widespread investigation into the etiology of aseptic meningitis.

Rasmussen[268] reported on 374 cases evaluated at the Walter Reed Army Institute of Research laboratory between 1941 and 1946 and found the probable or definite etiology in 26 percent of "viral" disease of the central nervous system (CNS). Mumps and lymphocytic choriomeningitis viruses were the two etiologic agents identified in his study.

In 1953, Adair and associates[5] reviewed 480 additional cases of aseptic meningitis occurring in military personnel and their dependents from 1947 through 1952 and were able to confirm the etiology in 25 percent of those patients. Herpes simplex virus (HSV) and *Leptospira* spp. were added to the previously identified mumps and lymphocytic choriomeningitis viruses as causes of aseptic meningitis. Meyer and associates[226] extended these studies to include 713 more children and adults with acute CNS syndromes of "viral" etiology admitted to military and Veterans Administration hospitals between 1953 and 1958. Of these 713 patients, 430 had the clinical syndrome of aseptic meningitis. Approximately 80 percent of these patients were hospitalized in the United States. An etiologic diagnosis was determined in 71 percent of patients with aseptic meningitis. In addition to the agents identified earlier, poliovirus, coxsackieviruses of groups A and B, echoviruses, and arthropod-borne viruses were identified as causes of aseptic meningitis.

Lepow and colleagues[201,202] reported the probable viral etiology in 54 percent of the 407 patients they studied in Cleveland between 1955 and 1958. In 1958, Lennette and associates[200] determined a viral etiology in 65 percent of 511 children and adults with presumed viral CNS system disease in Los Angeles; 368 of these patients were diagnosed as having aseptic meningitis. Sköldenberg[302] analyzed 3117 patients admitted to the Hospital for Infectious Diseases in Stockholm between 1955 and 1964 with the diagnosis of aseptic meningitis, with or without encephalitis or myelitis, and a virologic or clinical diagnosis (or both) of an associated viral infection was established in 72.6 percent. Berlin and associates[27] performed a surveillance study of aseptic meningitis in pediatric ambulatory clinics and emergency departments of three Baltimore hospitals between July 1986 and December 1990. They identified a single viral agent in 169 (62%) of the 274 cases with laboratory study; 168 enteroviruses and 1 adenovirus were identified. Today, with the use of polymerase chain reaction (PCR) and culture and appropriate serologic study, the etiology of most cases of aseptic meningitis can be determined.

ETIOLOGY

Table 35-1 lists infectious agents and other causes of aseptic meningitis. At present, the diagnostic workup of aseptic meningitis usually is not undertaken vigorously, and the etiologic agent is identified in only approximately 10 percent of all cases. Epidemiologic study and intensive investigations at some centers indicate, however, that most cases result from viral infections. Enteroviruses account for approximately 85 percent of all cases of aseptic meningitis.[48,90-92,233] The following enteroviruses have been associated with aseptic meningitis: polioviruses 1 to 3; coxsackieviruses A 1 to 14, 16 to 18, 21, 22, and 24; coxsackieviruses B 1 to 6; echoviruses 1 to 9, 11 to 21, 24 to 27, and 29 to 33; enterovirus 71. Parechoviruses, a newly described family of viruses, closely related to enterovirus, have also been associated with aseptic meningitis. Thus far, 16 genotypes of parechoviruses have been characterized[69]; some have been associated with aseptic meningitis, but because these viruses are so newly described, it is unknown which will play important roles.[138,150]

In recent years, multiple outbreaks of aseptic meningitis caused by enteroviruses have been described, including outbreaks caused by echovirus 30 in several countries throughout Eastern and Western Europe, China, Japan, Korea, Australia, the Arabian Gulf, the United States, and Brazil.* Echovirus 13 was responsible for reported outbreaks of aseptic meningitis in the United States, England, Wales, Germany, Belgium, Spain, France, Lithuania, Israel, Japan, Korea, and Australia.† Enterovirus 71 caused a major epidemic in Taiwan from 1998 to 1999, with multiple cases of hand, foot, and mouth syndrome

*References 10, 20, 28, 37, 44, 55, 58, 64, 71, 101, 112, 134, 151, 177, 186, 204, 242, 244, 253, 257, 272, 294, 328, 338, 339, 348, 367.
†References 57, 68, 72, 98, 176, 183, 187, 236, 238, 307, 328, 330.

TABLE 35-1 Etiologic Agents, Factors, and Diseases Associated with Aseptic Meningitis

Viruses

Adenoviruses (1, 2, 3, 5, 6, 7, 12, 14, 32)
Arboviruses (in the United States: West Nile, St. Louis, California, Colorado tick fever, Eastern equine, Western equine, Venezuelan equine, and Powassan)*
Coronaviruses
Cytomegalovirus
Encephalomyocarditis
Enteroviruses (echoviruses, coxsackieviruses A and B, polioviruses, enteroviruses)
Epstein-Barr
Hendra and Nipah
Herpes simplex type 1
Herpes simplex type 2
Human herpesvirus–6
Human herpesvirus–7
Human immunodeficiency virus (HIV-1)
Human T-cell lymphotrophic virus (HTLV-1)
Influenza A and B
Lymphocytic choriomeningitis
Measles
Mumps
Parechoviruses
Parainfluenza
Parvovirus B19
Rhinoviruses
Rotaviruses
Rubella
Varicella-zoster
Variola

Bacteria

Atypical Mycobacteria
Bartonella henselae
Borrelia spp. (relapsing fever)
Borrelia burgdorferi (Lyme disease)
Brucella spp.
Leptospira spp. (leptospirosis)
Mycobacterium tuberculosis
Nocardia spp. (nocardiosis)
Pyogenic: Partially treated
Treponema pallidum (syphilis)

Rickettsia

Anaplasma phagocytophila
Coxiella burnetii
Ehrlichia chaffeensis
Rickettsia rickettsii (Rocky Mountain spotted fever)
Rickettsia prowazekii (typhus)

Mycoplasma

Mycoplasma hominis
Mycoplasma pneumoniae

Chlamydia

Chlamydia pneumoniae
Chlamydia psittaci

Ureaplasma

Ureaplasma urealyticum

Fungi

Blastomyces dermatitidis
Candida spp.
Coccidioides immitis
Cryptococcus neoformans
Histoplasma capsulatum
Other: *Acremonium* spp., *Alternaria* spp., *Aspergillus* spp., *Blastoschizomyces capitus, Cephalosporium* spp., *Cladosporium trichoides, Drechslera hawaiiensis, Fusarium* spp., *Paecilomyces* spp., *Paracoccidioides brasiliensis, Penicillium marneffei,* Phaeohyphomycosis, *Pseudallescheria boydii, Sporothrix schenckii, Trichosporon beigelii, Ustilago* spp., *Zygomycetes* spp.

Parasites (Eosinophilic Meningitis)

Flukes: *Paragonimus westermani*, schistosomiasis, fascioliasis
Roundworms: *Angiostrongylus cantonensis, Gnathostoma spinigerum, Baylisascaris procyonis, Strongyloides stercoralis, Trichinella spiralis, Toxocara canis*
Tapeworms: Cysticercosis
Protozoa and free-living amoeba (noneosinophilic meningitis)
Acanthamoeba
Naegleria fowleri
Toxoplasma gondii (toxoplasmosis)

Vaccine-Associated

Measles
Mumps
Polio
Rabies
Vaccinia

Parameningeal Infection

Malignancy
Central nervous system tumor
Leukemia

Immune Diseases
Behçet syndrome
Lupus erythematosus
Sarcoidosis

Miscellaneous
Antimicrobial agents
Epidermoid, dermoid, other cysts
Foreign bodies (shunt, reservoir)
Heavy metal poisoning
Intrathecal injections (e.g., contrast media, antibiotics)
Kawasaki disease
Nonsteroidal anti-inflammatory drugs
Other drugs

*In other areas of the world, many other arboviruses are important.

associated with aseptic meningitis and other neurologic manifestations.* Similar outbreaks of aseptic meningitis caused by enterovirus 71 were reported in Malaysia, Japan, Hong Kong, and Australia.† Other enteroviruses involved in more recent outbreaks include echovirus 4 in Italy, Greece, Israel and Palestine, and Australia[147,216,220,261]; echovirus 6 in China[218]; echovirus 9 in Japan and regions of the United States[8,60]; echovirus 11 among institutionalized children in Israel[308]; echovirus 16 in Cuba[290]; echovirus 18 in Taiwan and Missouri[331,335,349]; echovirus 33 in New Zealand[166]; and coxsackievirus A9 in Latvia and China.[83,253] In the United States, the most common serotypes are coxsackie B1; echovirus 6, 9, and 18; and coxsackievirus A9, with echoviruses 9 and 30 being the most frequently identified etiologies of aseptic meningitis since 2003.[58,59,61,62,275,323]

Sharing seasonality with the enteroviruses, several arboviruses cause CNS disease in North America. Although encephalitis is the most recognizable manifestation of many of these infections, some arboviruses commonly are associated with aseptic meningitis as well.[63,132,275,279] Since the mid-1990s, outbreaks of West Nile virus (WNV) meningitis and encephalitis have occurred in Romania, Russia, and Israel.[73,258,332] First detected in the Western Hemisphere in 1999 in New York City, WNV subsequently spread across North America from the Atlantic to the Pacific coasts and into Canada and Mexico.[39,109,167,239,255] Between 1999 and 2008, almost 29,000 cases were reported in the United States, with more than 1100 deaths.[153,213] An estimated 1/150 infections results in severe neurologic illness, with meningitis as the primary manifestation in 16 to 40 percent of hospitalized patients.[175] Although the incidence of neuroinvasive disease increases with age, WNV is more likely to manifest as meningitis in children than in older adults and occurred in at least one quarter of the 150 pediatric cases diagnosed in the United States in 2002.[75,117,152,169,212] Even in regions with increased incidence of WNV, episodes of meningitis caused by enterovirus greatly outnumber those caused by WNV.[175]

Before the introduction of WNV, arboviruses accounted for approximately 5 percent of cases of aseptic meningitis in North America, with St. Louis encephalitis virus being the most common.[40,45,50,52] Infection with La Crosse encephalitis virus (a California encephalitis virus subtype) often resembles herpes encephalitis, but it may manifest as aseptic meningitis in children.[224] Unlike WNV, the majority of severe La Crosse encephalitis virus cases occur in children 15 years of age and younger, and in a study of 282 patients with La Crosse encephalitis virus infections in the Eastern United States from 2003 to 2007, 17 percent had aseptic meningitis.[63,144] Other California serogroup viruses, such as Jamestown Canyon virus and snowshoe hare virus, and other arboviruses, such as Colorado tick fever, result in aseptic meningitis more frequently than encephalitis.[132,275,313] Tick-borne encephalitis can manifest as aseptic meningitis in endemic areas. Tick-borne encephalitis virus cases were reported more recently in studies conducted in Slovenia and Sweden, and in mild cases, the clinical presentation was that of aseptic meningitis.[141,203,215]

Aseptic meningitis is an occasional manifestation of acute and recurrent genital infections with herpes simplex virus–2 (HSV-2).* In contrast to HSV-1 CNS infections, which without treatment usually are fatal, HSV-2 aseptic meningitis in otherwise immunocompetent patients is a benign, self-limited illness. Herpes family viruses other than HSV-1 and HSV-2 also are potential causes of aseptic meningitis. Although neurologic involvement in primary varicella-zoster virus (VZV) infections usually is encephalitis rather than benign meningitis, herpes zoster infection occasionally does present with concurrent meningitis.† VZV has been identified by PCR in the CSF of patients who had acute aseptic meningitis without cutaneous lesions, and meningitis associated with the VZV vaccine strain has been described.‡ One recent report detected VZV vaccine strain in a young patient with viral meningitis 11 years after vaccination.[245] A variety of neurologic disorders, including aseptic meningitis, are rare complications of Epstein-Barr virus infection.[110,119,137,317,334] Most noncongenital infections with cytomegalovirus in nonimmunocompromised patients are unrecognized; however, occasional instances of aseptic meningitis have been noted.[90-92,264,265]

The role of human herpesvirus–6 (HHV-6) in causing meningitis is unclear; although HHV-6 has been found in CSF samples from infants with meningitis, the virus also is detectable in the CSF of asymptomatic individuals.[12,42,361,365] Similarly, PCR identified HHV-7 in the CSF of six children with neurologic diseases, including aseptic meningitis, meningoencephalitis, facial palsy, vestibular neuritis, and febrile seizures.[259] The role of HHV-7 as a causative agent in aseptic meningitis remains to be determined.

Occasionally, meningitis or meningoencephalitis occurs as a manifestation of acute illness with HIV-1 infection.[18,145] Neurologic manifestations develop 3 to 6 weeks after primary infection at the same time as an infectious mononucleosis–like illness.

Lymphocytic choriomeningitis virus was an important historical cause of aseptic meningitis. In 1974, eight cases of aseptic meningitis caused by lymphocytic choriomeningitis virus were found in New York State.[93] Today, it is rarely recognized as a cause of meningitis, which is likely a result of both decreasing incidence of disease and decreased detection.§ Seroprevalence studies conducted over 2 decades ago found seroprevalence of 4.7 percent, whereas more recent studies show a much lower seroprevalence of 0.4 percent.[70,190] Nevertheless, physicians should be alert to the possibility in all situations of rodent (pet or wild) exposure and test appropriately. Encephalomyocarditis virus is another rodent virus that rarely is recognized in humans.[350] It is associated with a variety of neurologic manifestations, including aseptic meningitis.[121]

*References 165, 207, 209, 214, 298, 347, 348, 360, 362.
†References 65, 120, 159, 217, 225, 297-299.

*References 17, 26, 81, 100, 303, 327.
†References 90, 146, 168, 250, 262, 271, 352.
‡References 1, 67, 105, 122, 124, 126, 168, 172, 189, 197, 205, 206, 245, 311.
§References 9, 16, 19, 23, 30, 93, 226, 309.

Adenoviral types 1, 2, 3, 5, 6, 7, 12, 14, and 32 have been associated with meningitis and meningoencephalitis.* Although they occur infrequently, adenoviral CNS infections tend to be more severe than enteroviral infections. Rarely, aseptic meningitis has been noted during illnesses caused by influenza A viruses, including 2009 pandemic influenza (H1N1), influenza B, rhinoviruses, parainfluenza viruses, parvovirus B19 virus, rotaviruses, and coronaviruses.† Most infections with measles, rubella, and variola viruses that involve the CNS are encephalitic.[46,47,49,230]

In the prevaccine era, mumps virus was the agent responsible for the greatest number of cases of aseptic meningitis; today in the United States, use of vaccine has rendered mumps rare, although mumps outbreaks with associated cases of aseptic meningitis occur occasionally.[51,60] Aseptic meningitis and encephalitis resulting from administration of mumps vaccine have been noted in Canada, Brazil, Japan, and Europe.‡ The Leningrad 3, Urabe Am 9, and three Japanese strains of vaccine viruses have been implicated. In the United States, where the Jeryl Lynn vaccine strain has been used exclusively, the rate of encephalitis in vaccinees has been no higher than that of the observed background incidence of similar illness in the population.[45] A preliminary analysis of the Vaccine Safety Datalink project showed a possible increased risk for developing aseptic meningitis 8 to 14 days after receiving immunization with Jeryl Lynn mumps vaccine strain. A follow-up case-control evaluation of hospitalized cases failed to show an increased risk, however.[33]

Neurologic illness is a rare complication of measles, smallpox, polio, and rabies viral vaccines. In most instances, the illnesses are complex and severe, but occasionally, aseptic meningitis is the only manifestation.§ A case of aseptic meningitis caused by vaccine-derived poliovirus was reported in the Philippines in 2001.[56] It was in association with two pediatric cases of acute flaccid paralysis that occurred during the same time period. Viral isolates from all three patients revealed type 1 poliovirus derived from the Sabin vaccine strain.

Certain bacteria are important to recognize as etiologic agents in aseptic meningitis because the illnesses are treatable and early initiation of therapy is crucial. Of greatest importance is tuberculous meningitis. Early treatment of this illness nearly always results in complete cure, whereas diagnostic delay or inadequate treatment frequently results in permanent neurologic sequelae. Lyme disease, relapsing fever, brucellosis, leptospirosis, and rickettsial infections are illnesses acquired either directly or indirectly from animals, in which aseptic meningitis may be a part of the disease process.¶ *Mycoplasma pneumoniae* has been implicated as a causative agent of neurologic illness.[44,105,145,265,339] Pönkä[260] noted that 8/560 hospitalized patients with *M. pneumoniae* infections had aseptic meningitis and 18 had encephalitis or meningoencephalitis. Despite numerous case reports and case series, the role of *M. pneumoniae* is thought by some experts to be unknown.[32] *Mycoplasma hominis* and *Ureaplasma urealyticum* are rare causes of neonatal meningitis.[123,219,342,343] Meningitis and meningoencephalitis have been associated with *Chlamydia pneumoniae* infections.[13,139,304,318] Partially treated common bacterial meningitides are a common cause of meningitis in which cultures of CSF fail to grow organisms. Antigen detection systems, such as latex agglutination, can be useful in identifying the causative agents in some of these cases.

Numerous fungi and yeasts cause meningitis.[287] Although many fungal meningitides occur almost exclusively in immunocompromised patients, children and adults with normal immune status experience meningitis most commonly caused by *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Cladosporium* spp., *Histoplasma capsulatum*, and *Paracoccidioides brasiliensis*.[22,116,222] In infants who are premature or younger than 1 month of age, *Candida albicans* is an important cause of meningitis and is associated with significant morbidity and mortality.[24,136,234]

Parasites occasionally cause aseptic meningitis. Eosinophilic meningitis is caused by *Angiostrongylus cantonensis*, a rat lungworm.* Aseptic meningitis caused by *A. cantonensis* has been observed on several islands in the Pacific, and the infection may be acquired by the consumption of freshwater shrimp.

A sterile CSF pleocytosis occurs in 12 to 13 percent of young infants with bacterial urinary tract infections[6,113,320] and in approximately one third of patients with Kawasaki disease who undergo lumbar puncture.[94,131] Numerous drugs and biologics have been implicated in aseptic meningitis.† Of most importance in pediatrics are trimethoprim-sulfamethoxazole and intravenous immunoglobulin. Other causes of aseptic meningitis are listed in Table 35-1.‡

EPIDEMIOLOGY

No unified epidemiologic pattern exists because so many different types of organisms cause aseptic meningitis. The epidemiology of the specific individual infectious agents or diseases is presented in detail in the various chapters of this book, and only a brief overview is presented here.

Because approximately 85 percent of all cases of aseptic meningitis are caused by enteroviral infections, the basic epidemiologic pattern of aseptic meningitis reflects these agents. In temperate climates, most cases occur in the summer and fall; infection with enteroviruses is spread directly from person to person, and the incubation period usually is 4 to 6 days. Epidemiologic considerations in aseptic meningitis caused by agents other than

*References 27, 79, 90-92, 111, 184, 252, 253, 300, 306.
†References 14, 43, 82, 90-92, 97, 135, 162, 182, 241, 243, 246, 251, 273, 340, 357, 364.
‡References 11, 15, 76, 77, 84, 85, 102, 223, 228, 237.
§References 27, 46, 49, 56, 229, 295, 312.
¶References 29, 31, 34, 36, 108, 130, 163, 170, 171, 174, 179, 191, 198, 247, 254, 310, 324, 326, 341, 344, 356, 359, 366.

*References 66, 145, 160, 161, 208, 266, 277, 351.
†References 19, 41, 95, 114, 133, 143, 221, 225, 227, 235, 267, 358.
‡References 53, 99, 118, 129, 155, 158, 188, 191, 193, 199, 221, 296, 319.

enteroviruses depend markedly on season, geography, climatic conditions, animal exposures, and many other factors related to the specific pathogens.

CLINICAL MANIFESTATIONS

Aseptic meningitis has many causes (see Table 35-1), and clinical manifestations vary with the different diseases. In some instances, the signs and symptoms resulting from meningeal inflammation dominate the clinical illness, whereas in other instances the main signs and symptoms reflect other organ system involvement. Clinical manifestations in aseptic meningitis, regardless of etiology, also vary markedly by patient age.

Enteroviruses

Enteroviruses are the most common cause of aseptic meningitis, and they can be considered the prototype for a description of general clinical manifestations of aseptic meningitis.* Even among the enteroviruses, however, significant differences in clinical manifestations exist among the different viral types. Some general aspects of epidemic enteroviral aseptic meningitis are presented by viral type in Chapter 165.

The onset of illness generally is acute, although it may be insidious over the course of a week or so, or may be preceded by a nonspecific acute febrile illness of a few days' duration. Almost all children have fever, and most older children have headache, which most often is retroorbital or frontal in location. Photophobia is common. Temperature elevation varies, ranging from 38° to 40.5° C (100.4° to 105° F), and usually lasts approximately 5 days. Occasionally, fever is biphasic, with the initial elevation occurring before the onset of neurologic signs and symptoms. Anorexia, nausea, and vomiting are common, and abdominal pain and diarrhea also are reported frequently.

Meningeal signs (i.e., stiff neck and back, tightness of the hamstring muscles, and Brudzinski and Kernig signs) usually are present, but deep tendon reflexes usually are normal or hyperactive. Seizures occur occasionally, usually when concomitant high fever is present. Muscle weakness rarely is reported, but myalgia occasionally is noted. In young children, fever, irritability, and lethargy are the most common findings. Infants may be irritable and show resentment to handling, and the fontanelle may be tense.

Other manifestations of enteroviral infections also occur in children with aseptic meningitis. The most common is pharyngitis, which may occur during infection with all of the neurotropic enteroviral types. Rash occurs commonly, but varies by viral type. With echovirus 9 meningitis, 30 to 50 percent of children have rashes, whereas with echovirus 6, exanthem is rare. Cases of meningitis caused by enterovirus 71 and coxsackie A 16 frequently are accompanied by hand, foot, and mouth syndrome. Enanthem, pleurodynia, pericarditis, myocarditis, and conjunctivitis are other findings noted in children with enteroviral aseptic meningitis. Illness often is biphasic with fever, an interlude, then return of fever and neurologic manifestations.

CSF leukocyte counts vary from a few cells to a few thousand cells; the median is in the range of 100 to 500 cells/mm^3. The percentage of neutrophils also varies greatly. Initially, a predominance of neutrophils commonly occurs; later, CSF examinations show a decline in the percentage of neutrophils. The CSF protein usually is elevated mildly, and the glucose concentration usually is normal; rarely, hypoglycorrhachia is noted.

The duration of illness varies. Usually, disability because of neurologic involvement lasts 1 to 2 weeks.

Aseptic Meningitis Caused by Other Agents

Of 1478 pediatric WNV cases reported from 1999 through 2007, 30 percent were classified as West Nile neuroinvasive disease (WNND).[212] Unlike in older adults who often have encephalitis, most WNND in pediatric patients manifests as meningitis. Seizures occur more commonly in arboviral meningitides than in enteroviral illnesses of otherwise comparable severity.[149] The CSF findings generally are similar to those in enteroviral disease, although some reports suggest that neutrophils are more commonly seen with WNV than with other viral entities.[88,269] Examination of the CSF in mild cases of mumps often reveals pleocytosis, and mumps is one of the few viral infections that can cause hypoglycorrhachia. When neurologic disease caused by mumps is recognized, usually evidence of brain involvement is present.

Tuberculous meningitis usually has a gradual onset over the course of 2 to 3 weeks.[185,231,276] Initially, personality changes, irritability, anorexia, listlessness, and low-grade fever may be present, followed by signs of increased intracranial pressure, such as drowsiness, stiff neck, cranial nerve palsies, inequality of the pupils, vomiting, and seizures. Finally, coma, irregular pulse and respirations, and high fever occur. In fungal diseases, the course of meningitis is similar to the course of tuberculosis. In tuberculosis and several fungal meningitides, such as those caused by *C. immitis*, *H. capsulatum*, and *C. neoformans*, historical and radiographic evidence of pulmonary disease may be present.

Aseptic meningitis associated with *M. pneumoniae* is unique in that it frequently occurs a few days to 3 weeks after a respiratory illness (i.e., pharyngitis, bronchitis, or pneumonia).[140,210,305,333] Generally, the likelihood of a predominance of neutrophils is less in other aseptic meningitides, and low glucose levels are likely in parameningeal bacterial infections, partially treated bacterial meningitides, brain tumors, leukemic infiltration, *M. pneumoniae* infections, fungal infections, and tuberculosis.

Recurrent Aseptic Meningitis (Mollaret Meningitis)

In 1944, Mollaret[232] described three patients with recurrent aseptic meningitis whom he had observed over the

*References 3, 6, 10, 35, 54-57, 64, 87, 98, 115, 125, 138, 142, 147, 148, 154, 156, 165, 177, 180, 181, 195, 209, 270, 278, 283, 284, 286, 288, 301, 316, 321, 354.

course of 15 years. Subsequently, many other cases have been reported, and some cases have been noted in children.* The illness is characterized by recurrent attacks of fever with meningeal signs and symptoms. The attacks last several days and are separated by symptom-free periods lasting weeks or months. In addition to a lymphocyte-predominant pleocytosis, CSF samples obtained from certain patients contain large mononuclear cells (Mollaret cells). The disease remits spontaneously. HSV-2 has been identified by PCR or DNA probes in the CSF of most patients with recurrent meningitis.† Other viruses, such as HSV-1 and Epstein-Barr virus, and noninfectious causes, such as systemic lupus erythematosus, intracranial cysts, antibiotics such as amoxicillin, and environmental exposures, also have been identified as less frequent etiologies of recurrent meningitis.‡

DIFFERENTIAL DIAGNOSIS

Careful analysis of the history and epidemiologic circumstances may point toward one of the specific causes listed in Table 35-1. During the summer and autumn, the presence of pleurodynia, herpangina, or unexplained febrile eruptions in the community suggests the possibility of enteroviral infections. Acute paralytic disorders in other patients suggests poliomyelitis, enterovirus 71, or WNV. Exposure to mosquitoes and encephalitis in horses implicates certain arboviruses, and exposure to ticks may be suggestive of Lyme disease, relapsing fever, or rickettsial disease, depending on the geographic location and other symptoms of the illness. A history of swimming in waters contaminated by urine from infected animals and exposure to rats in urban slums suggest leptospiral infection. Knowledge of clear-cut exposure to or concurrent evidence of mumps or of one of the common exanthems is helpful in delineating the differential diagnosis. The association of pneumonia or other respiratory illness preceding aseptic meningitis strongly suggests the possibility of *M. pneumoniae* as the etiologic agent.

Most difficult from the diagnostic, therapeutic, and prognostic points of view are instances of incipient or partially treated bacterial (especially when caused by *Haemophilus influenzae*) or mycobacterial meningitis. The clinical findings; the dosage of antibiotic previously used; the spinal fluid smear, latex agglutination, or other rapid antigen identification test; the culture; and the glucose level may be helpful in diagnosing bacterial meningitis. The quantitative determination of C-reactive protein in the CSF also may be useful in differentiating bacterial from viral meningitis.[2,80,89,248,249] Lindquist and associates[211] found that the determination of CSF concentrations of lactate was the most useful test in differentiating bacterial from nonbacterial causes of meningitis. Studies suggest that the presence of tumor necrosis factor-α in the CSF is rare in viral infections, but common in bacterial disease.[7,103,128] When tuberculous meningitis is suspected, a careful evaluation of contacts, a careful examination of an appropriately stained smear from the pellicle of the CSF that was allowed to settle, and a positive tuberculin reaction or positive interferon-γ release assay may confirm the diagnosis. Because combined bacterial and viral infection has occurred, examinations of CSF should be repeated if any doubt exists. The possibility that the observed meningeal reaction is of neither viral nor bacterial origin must be considered. Finally, CNS tumor must be considered in the differential diagnosis, particularly if hypoglycorrhachia and prominent signs of increased intracranial pressure are present.[193]

SPECIFIC DIAGNOSIS

Obtaining a meticulous history is essential. The clinician must evaluate exposure of the patient in the past 2 to 3 weeks to illness in contacts; exposure to mosquitoes, ticks, and animals during recent vacations, picnics, and so on; awareness of illness in animals, especially horses and other Equidae, in the patient's environment; recent travel from the home area; recent injections or medications of any kind; and the possibility of accidental exposure to heavy metals.

The CSF must be examined carefully to exclude disorders that respond to specific therapy. Smears for bacteria, appropriate rapid antigen identification tests, and cultures of the CSF are mandatory; the history and clinical findings may indicate the need for performing acid-fast stain and culture of the sediment for mycobacteria. Other circumstances may indicate the need for excluding fungal or protozoal infection; atypical cells may require cytopathologic study to exclude neural neoplasms, which may manifest acutely.

The introduction of PCR has facilitated the etiologic diagnosis of CNS viral infections, particularly infections caused by enteroviruses and herpesviruses.[127,164,178,292,329,363] PCR detects enterovirus in the CSF more rapidly than does cell culture and has been shown to shorten the duration of hospitalization for children with meningitis, reducing costs.[240,293,315] Enterovirus PCR tests do not detect parechoviruses; specific PCR testing for parechoviruses should be ordered in the appropriate population (especially children younger than 3 years).

PCR is the test of choice for detecting CNS infections caused by HSV, and molecular techniques also have been used to identify in CSF such causes of meningitis and meningoencephalitis as VZV, HHV-6, parvovirus B19, and rotavirus.[21,172,192,337]

For many arboviruses, serologic examination is more sensitive than molecular methods, especially for arboviruses that have a short period of viremia and presence in the CSF such as WNV. Molecular methods may have a complementary role, as in one study in which WNV PCR analysis had a 57 percent sensitivity and 100 percent specificity.[74,196]

In any patient suspected to have viral meningitis, spinal fluid, serum, feces, and throat swabs should be collected and either held in hospital laboratory or sent to a public health laboratory with viral diagnostic services. An additional serum specimen should be collected 10 to 21 days later so that paired sera can be examined for

*References 38, 77, 78, 157, 256, 314, 325.
†References 20, 77, 104, 194, 256, 291, 322, 325, 336.
‡References 137, 188, 263, 285, 289, 314.

antibody titer increases. This pairing is particularly useful in arboviral, lymphocytic choriomeningitis, encephalomyocarditis, leptospiral, borrelial, rickettsial, mycoplasmal, and toxoplasmal infections. Although these studies may not provide an immediate diagnosis, they may give early warning of a specific epidemic, and they are useful for prognostication, particularly in very young infants.

TREATMENT

Hospitalization usually is necessary because of the possibility of treatable bacterial disease, the frequent need for fluid therapy for dehydration, and sometimes for analgesics. Headache and hyperesthesia are treated with rest; analgesics; and a reduction in room light, noise, and visitors. Antipyretics are recommended for fever. Using acetaminophen rather than aspirin is prudent because of the risk for developing Reye syndrome associated with the latter antipyretic. Codeine, morphine, and the phenothiazine derivatives often are used for pain and vomiting but are rarely necessary in children and should be avoided because they may induce misleading signs and symptoms. The investigational antiviral drug pleconaril has been shown effective in the treatment of enteroviral meningitis; however, this drug presently is unavailable.[96,280,281,282] Treatment for illnesses such as tuberculous meningitis, fungal meningitides, and other illnesses for which specific therapies are available is covered in specific chapters of this book.

Several weeks after the patient has apparently recovered, a careful neuromuscular assessment should be conducted to ensure that muscular weakness is not a sequela. Bilateral audiometry is recommended, especially when mumps virus was involved.

PROGNOSIS

The prognosis in aseptic meningitis depends on the etiology. Some illnesses have an ominous prognosis (i.e., tuberculous meningitis, parameningeal infections, rickettsial infections), but patients usually do well if appropriate specific therapy is instituted early in the course of the illness. In *C. immitis* meningitis, the prognosis for cure is guarded even with early optimal therapy.

In enteroviral and other viral meningitides, children usually recover completely. Some patients complain of fatigue, irritability, decreased ability to concentrate, muscle pain, muscle weakness and spasm, and incoordination for several weeks after an acute illness. Although the outcome of enteroviral meningitis most often is without residual, some infants who have enteroviral meningitis in the first few months of life have an increased risk for altered language development.[25,355] Formally evaluating such children at age 3 to 6 years is important.

PREVENTION

The universal use of polio and mumps vaccines in children clearly is effective in controlling these two diseases. Control of insect vectors by suitable spraying methods and eradication of insect breeding sites is important in the control of many arboviruses. The control of animal vectors such as mice and rats alters the incidence of infections with lymphocytic choriomeningitis and encephalomyocarditis viruses.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Al-Zein N, Boyce TG, Correa AG, et al. Meningitis caused by lymphocytic choriomeningitis virus in a patient with leukemia. *J Pediatr Hematol Oncol* 2008;**30**:781–4.
22. Bariola JR, Perry P, Pappas PG, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin Infect Dis* 2010;**50**:797–804.
32. Bitnun A, Richardson SE. *Mycoplasma pneumoniae:* innocent bystander or a true cause of central nervous system disease? *Curr Infect Dis Rep* 2010;**12**:282–90.
44. Castro CM, Oliveira DS, Macedo O, et al. Echovirus 30 associated with cases of aseptic meningitis in state of Para, Northern Brazil. *Mem Inst Oswaldo Cruz* 2009;**104**:444–50.
62. Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance: United States, 2006-2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1577–80.
63. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1009–13.
69. Chieochansin T, Vichiwattana P, Korkong S, et al. Molecular epidemiology, genome characterization, and recombination event of human parechovirus. *Virology* 2011;**421**:159–66.
71. Choi YJ, Park KS, Baek KA, et al. Molecular characterization of echovirus 30-associated outbreak of aseptic meningitis in Korea in 2008. *J Microbiol Biotechnol* 2010;**20**:643–9.
83. Cui A, Yu D, Zhu Z, et al. An outbreak of aseptic meningitis caused by coxsackievirus A9 in Gansu, the People's Republic of China. *Virol J* 2010;**7**:72.
97. Dickey M, Jamison L, Michaud L, et al. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. *Pediatr Infect Dis J* 2009;**28**:318–21.
122. Galea SA, Sweet A, Beninger P, et al. The safety profile of varicella vaccine: a 10-year review. *J Infect Dis* 2008;**197**(Suppl 2):S165–169.
124. Garnier A, Frascari F, Decramer S. Varicella-zoster virus meningoencephalitis without skin lesions in a paediatric kidney recipient. *Nephrol Dial Transplant* 2011;**26**:378–9; author reply 379.
126. Gilden D, Cohrs RJ, Mahalingam R, et al. Neurological disease produced by varicella zoster virus reactivation without rash. *Curr Top Microbiol Immunol* 2010;**342**:243–53.
144. Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. *PLoS One* 2009;**4**:e6145.
146. Han JY, Hanson DC, Way SS. Herpes zoster and meningitis due to reactivation of varicella vaccine virus in an immunocompetent child. *Pediatr Infect Dis J* 2011;**30**:266–8.
150. Harvala H, Wolthers KC, Simmonds P. Parechoviruses in children: understanding a new infection. *Curr Opin Infect Dis* 2010;**23**:224–30.
160. Hochberg NS, Blackburn BG, Park SY, et al. Eosinophilic meningitis attributable to *Angiostrongylus cantonensis* infection in Hawaii: clinical characteristics and potential exposures. *Am J Trop Med Hyg* 2011;**85**:685–90.
168. Iyer S, Mittal MK, Hodinka RL. Herpes zoster and meningitis resulting from reactivation of varicella vaccine virus in an immunocompetent child. *Ann Emerg Med* 2009;**53**:792–5.
170. Jha S, Ansari MK. Leptospirosis presenting as acute meningoencephalitis. *J Infect Dev Ctries* 2010;**4**:179–82.
189. Klein NC, McDermott B, Cunha BA. Varicella-zoster virus meningoencephalitis in an immunocompetent patient without a rash. *Scand J Infect Dis* 2010;**42**:631–3.
190. Knust B, Macneil A, Wong SJ, et al. Exposure to lymphocytic choriomeningitis virus, New York, USA. *Emerg Infect Dis* 2011;**17**:1324–5.
197. Leahy TR, Webb DW, Hoey H, et al. Varicella zoster virus associated acute aseptic meningitis without exanthem in an immunocompetent 14-year-old boy. *Pediatr Infect Dis J* 2008;**27**:362–3.

204. Leveque N, Jacques J, Renois F, et al. Phylogenetic analysis of Echovirus 30 isolated during the 2005 outbreak in France reveals existence of multiple lineages and suggests frequent recombination events. *J Clin Virol* 2010;**48**:137–41.
206. Levin MJ, DeBiasi RL, Bostik V, et al. Herpes zoster with skin lesions and meningitis caused by 2 different genotypes of the Oka varicella-zoster virus vaccine. *J Infect Dis* 2008;**198**:1444–7.
208. Li H, Xu F, Gu JB, et al. A severe eosinophilic meningoencephalitis caused by infection of Angiostrongylus cantonensis. *Am J Trop Med Hyg* 2008;**79**:568–70.
212. Lindsey NP, Hayes EB, Staples JE, et al. West Nile virus disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084–9.
216. Logotheti M, Pogka V, Horefti E, et al. Laboratory investigation and phylogenetic analysis of enteroviruses involved in an aseptic meningitis outbreak in Greece during the summer of 2007. *J Clin Virol* 2009;**46**:270–4.
217. Ma E, Chan KC, Cheng P, et al. The enterovirus 71 epidemic in 2008: public health implications for Hong Kong. *Int J Infect Dis* 2010;**14**:e775–80.
218. Mao N, Zhao L, Zhu Z, et al. An aseptic meningitis outbreak caused by echovirus 6 in Anhui province, China. *J Med Virol* 2010;**82**:441–5.
220. Markey PG, Davis JS, Harnett GB, et al. Meningitis and a febrile vomiting illness caused by echovirus type 4, Northern Territory, Australia. *Emerg Infect Dis* 2010;**16**:63–8.
222. Mathisen G, Shelub A, Truong J, et al. Coccidioidal meningitis: clinical presentation and management in the fluconazole era. *Medicine (Baltimore)* 2010;**89**:251–84.
242. Ortner B, Huang CW, Schmid D, et al. Epidemiology of enterovirus types causing neurological disease in Austria 1999-2007: detection of clusters of echovirus 30 and enterovirus 71 and analysis of prevalent genotypes. *J Med Virol* 2009;**81**:317–24.
243. Ozdemir H, Karbuz A, Ciftci E, et al. Aseptic meningitis in a child due to 2009 pandemic influenza A (H1N1) infection. *Turk J Pediatr* 2011;**53**:91–3.
245. Pahud BA, Glaser CA, Dekker CL, et al. Varicella zoster disease of the central nervous system: epidemiological, clinical, and laboratory features 10 years after the introduction of the varicella vaccine. *J Infect Dis* 2011;**203**:316–23.
250. Pena JA, Pirics ML, DiCaprio HS, et al. Varicella reactivation presenting as shingles and aseptic meningitis in an immunocompetent 11-year-old boy. *Clin Pediatr (Phila)* 2009;**48**:435–7.
253. Perevoscikovs J, Brila A, Firstova L, et al. Ongoing outbreak of aseptic meningitis in South-Eastern Latvia, June-August 2010. *Euro Surveill* 2010;15.
257. Pinto Junior VL, Rebelo MC, Costa EV, et al. Description of a widespread outbreak of aseptic meningitis due to echovirus 30 in Rio de Janeiro state, Brazil. *Braz J Infect Dis* 2009;**13**:367–70.
263. Prieto-Gonzalez S, Escoda R, Coloma E, et al. Amoxicillin-induced acute aseptic meningitis. *J Clin Neurosci* 2011;**18**:443–4.
265. Rafailidis PI, Mourtzoukou EG, Varbobitis IC, et al. Severe cytomegalovirus infection in apparently immunocompetent patients: a systematic review. *Virol J* 2008;**5**:47.
266. Ramirez-Avila L, Slome S, Schuster FL, et al. Eosinophilic meningitis due to *Angiostrongylus* and *Gnathostoma* species. *Clin Infect Dis* 2009;**48**:322–7.
309. Sosa LE, Gupta S, Juthani-Mehta M, et al. Meningitis in a college student in Connecticut, 2007. *J Am Coll Health* 2009;**58**:12–4.
311. Spiegel R, Miron D, Lumelsky D, et al. Severe meningoencephalitis due to late reactivation of varicella-zoster virus in an immunocompetent child. *J Child Neurol* 2010;**25**:87–90.
323. Tavakoli NP, Wang H, Nattanmai S, et al. Detection and typing of enteroviruses from CSF specimens from patients diagnosed with meningitis/encephalitis. *J Clin Virol* 2008;**43**:207–11.
324. Tayal S, Chadwick D, Chawla G. Considering syphilis in aseptic meningitis. *Clin Med* 2009;**9**:626–7.
331. Tsai HP, Huang SW, Wu FL, et al. An echovirus 18-associated outbreak of aseptic meningitis in Taiwan: epidemiology and diagnostic and genetic aspects. *J Med Microbiol* 2011;**60**:1360–5.
335. Turabelidze G, Lin M, Butler C, et al. Outbreak of echovirus 18 meningitis in a rural Missouri community. *Mo Med* 2009;**106**:420–4.
341. Waespe N, Steffen I, Heininger U. Etiology of aseptic meningitis, peripheral facial nerve palsy, and a combination of both in children. *Pediatr Infect Dis J* 2010;**29**:453–6.
349. Wang SM, Ho TS, Shen CF, et al. Echovirus 18 meningitis in southern Taiwan. *Pediatr Infect Dis J* 2011;**30**:259–60.
364. Yildizdas D, Kendirli T, Arslankoylu AE, et al. Neurological complications of pandemic influenza (H1N1) in children. *Eur J Pediatr* 2011;**170**:779–88.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aberle SW, Aberle JH, Steininger C, et al. Quantitative real time PCR detection of varicella-zoster virus DNA in cerebrospinal fluid in patients with neurological disease. *Med Microbiol Immunol* 2005;**194**:7–12.
2. Abramson JS, Hampton KD, Babu S, et al. The use of C-reactive protein from cerebrospinal fluid for differentiating meningitis from other central nervous system diseases. *J Infect Dis* 1985; **151**:854–8.
3. Abzug MJ, Levin MJ, Rotbart HA. Profile of enterovirus disease in the first two weeks of life. *Pediatr Infect Dis J* 1993;**12**:820–4.
4. Abzug MJ, Loeffelholz M, Rotbart HA. Diagnosis of neonatal enterovirus infection by polymerase chain reaction. *J Pediatr* 1995;**126**:447–50.
5. Adair CV, Gauld RL, Smadel JE. Aseptic meningitis, a disease of diverse etiology: clinical and etiologic studies on 854 cases. *Ann Intern Med* 1953;**39**:675–704.
6. Adler-Shohet FC, Cheung MM, Hill M, et al. Aseptic meningitis in infants younger than six months of age hospitalized with urinary tract infections. *Pediatr Infect Dis J* 2003;**22**:1039–42.
7. Akalin H, Akdis AC, Mistik R, et al. Cerebrospinal fluid interleukin-1 beta/interleukin-1 receptor antagonist balance and tumor necrosis factor-alpha concentrations in tuberculous, viral and acute bacterial meningitis. *Scand J Infect Dis* 1994;**26**: 667–74.
8. Akasu Y. Outbreak of aseptic meningitis due to ECHO-9 in northern Kyushu island in the summer of 1997. *Kurume Med J* 1999;**46**:97–104.
9. Al-Zein N, Boyce TG, Correa AG, et al. Meningitis caused by lymphocytic choriomeningitis virus in a patient with leukemia. *J Pediatr Hematol Oncol* 2008;**30**:781–4.
10. Amvrosieva TV, Titov LP, Mulders M, et al. Viral water contamination as the cause of aseptic meningitis outbreak in Belarus. *Cent Eur J Public Health* 2001;**9**:154–7.
11. Anonymous. Mumps meningitis and MMR vaccination. *Lancet* 1989;**2**:1015–6.
12. Ansari A, Li S, Abzug MJ, et al. Human herpesviruses 6 and 7 and central nervous system infection in children. *Emerg Infect Dis* 2004;**10**:1450–4.
13. Anton E, Otegui A, Alonso A. Meningoencephalitis and *Chlamydia pneumoniae* infection. *Eur J Neurol* 2000;**7**:586.
14. Arisoy ES, Demmler GJ, Thakar S, et al. Meningitis due to parainfluenza virus type 3: report of two cases and review. *Clin Infect Dis* 1993;**17**:995–7.
15. Arruda WO, Kondageski C. Aseptic meningitis in a large MMR vaccine campaign (590,609 people) in Curitiba, Parana, Brazil, 1998. *Rev Inst Med Trop Sao Paulo* 2001;**43**:301–2.
16. Asnis DS, Muana O, Kim do G, et al. Lymphocytic choriomeningitis virus meningitis, New York, NY, USA, 2009. *Emerg Infect Dis* 2010;**16**:328–30.
17. Atia WA, Ratnatunga CS, Greenfield C, et al. Aseptic meningitis and herpes simplex proctitis: a case report. *Br J Vener Dis* 1982;**58**:53–8.
18. Atwood WJ, Berger JR, Kaderman R, et al. Human immunodeficiency virus type 1 infection of the brain. *Clin Microbiol Rev* 1993;**6**:339–66.
19. Auxier GG. Aseptic meningitis associated with administration of trimethoprim and sulfamethoxazole. *Am J Dis Child* 1990;**144**: 144–5.
20. Bailly JL, Brosson D, Archimbaud C, et al. Genetic diversity of echovirus 30 during a meningitis outbreak, demonstrated by direct molecular typing from cerebrospinal fluid. *J Med Virol* 2002;**68**:558–67.
21. Barah F, Vallely PJ, Chiswick ML, et al. Association of human parvovirus B19 infection with acute meningoencephalitis. *Lancet* 2001;**358**:729–30.
22. Bariola JR, Perry P, Pappas PG, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin Infect Dis* 2010;**50**:797–804.
23. Barton LL, Hyndman NJ. Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. *Pediatrics* 2000; **105**:835.
24. Benjamin Jr DK, Stoll BJ, Fanaroff AA, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. *Pediatrics* 2006;**117**:84–92.
25. Bergman I, Painter MJ, Wald ER, et al. Outcome in children with enteroviral meningitis during the first year of life. *J Pediatr* 1987;**110**:705–9.
26. Bergstrom T, Vahlne A, Alestig K, et al. Primary and recurrent herpes simplex virus type 2-induced meningitis. *J Infect Dis* 1990;**162**:322–30.
27. Berlin LE, Rorabaugh ML, Heldrich F, et al. Aseptic meningitis in infants < 2 years of age: diagnosis and etiology. *J Infect Dis* 1993;**168**:888–92.
28. Bernit E, de Lamballerie X, Zandotti C, et al. Prospective investigation of a large outbreak of meningitis due to echovirus 30 during summer 2000 in Marseilles, France. *Medicine (Baltimore)* 2004;**83**:245–53.
29. Bharti AR, Nally JE, Ricaldi JN, et al. Leptospirosis: a zoonotic disease of global importance. *Lancet Infect Dis* 2003;**3**:757–71.
30. Biggar RJ, Woodall JP, Walter PD, et al. Lymphocytic choriomeningitis outbreak associated with pet hamsters: fifty-seven cases from New York State. *JAMA* 1975;**232**:494–500.
31. Bingham PM, Galetta SL, Athreya B, et al. Neurologic manifestations in children with Lyme disease. *Pediatrics* 1995;**96**:1053–6.
32. Bitnun A, Richardson SE. *Mycoplasma pneumoniae:* innocent bystander or a true cause of central nervous system disease? *Curr Infect Dis Rep* 2010;**12**:282–90.
33. Black S, Shinefield H, Ray P, et al. Risk of hospitalization because of aseptic meningitis after measles-mumps-rubella vaccination in one- to two-year-old children: an analysis of the Vaccine Safety Datalink (VSD) Project. *Pediatr Infect Dis J* 1997;**16**:500–3.
34. Bowen AP. A cluster of children with lyme meningitis presenting to one emergency department in a Boston suburb. *J Emerg Nurs* 2002;**28**:355–7.
35. Bowen GS, Fisher MC, DeForest A, et al. Epidemic of meningitis and febrile illness in neonates caused by ECHO type 11 virus in Philadelphia. *Pediatr Infect Dis* 1983;**2**:359–63.
36. Bruhn FW. Lyme disease. *Am J Dis Child* 1984;**138**:467–70.
37. Brunel D, Leveque N, Jacques J, et al. Clinical and virological features of an aseptic meningitis outbreak in North-Eastern France, 2005. *J Clin Virol* 2008;**42**:225–8.
38. Bruyn GW, Straathof LJ, Raymakers GM. Mollaret's meningitis: differential diagnosis and diagnostic pitfalls. *Neurology* 1962;**12**: 745–53.
39. Buck PA, Sockett P, Barker IK, et al. West Nile virus: surveillance activities in Canada. *Ann Epidemiol* 2003;**13**:582.
40. Calisher CH. Medically important arboviruses of the United States and Canada. *Clin Microbiol Rev* 1994;**7**:89–116.
41. Carlson J, Wiholm BE. Trimethoprim associated aseptic meningitis. *Scand J Infect Dis* 1987;**19**:687–91.
42. Caserta MT, Hall CB, Schnabel K, et al. Neuroinvasion and persistence of human herpesvirus 6 in children. *J Infect Dis* 1994;**170**:1586–9.
43. Cassinotti P, Schultze D, Schlageter P, et al. Persistent human parvovirus B19 infection following an acute infection with meningitis in an immunocompetent patient. *Eur J Clin Microbiol Infect Dis* 1993;**12**:701–4.
44. Castro CM, Oliveira DS, Macedo O, et al. Echovirus 30 associated with cases of aseptic meningitis in state of Para, Northern Brazil. *Mem Inst Oswaldo Cruz* 2009;**104**:444–50.
45. Centers for Disease Control: Neurotropic viral diseases surveillance: aseptic meningitis, annual summary 1975, July 1977.
46. Centers for Disease Control: Neurotropic diseases surveillance: summary 1974-1976, October 1977.
47. Centers for Disease Control: Encephalitis surveillance, annual summary 1978, May 1981.
48. Centers for Disease Control. Enterovirus surveillance, summary 1970-9. November 1981.
49. Centers for Disease Control: Measles surveillance, 1977-1981, September 1982.
50. Centers for Disease Control. Arboviral infections of the central nervous system: United States, 1985. *MMWR Morb Mortal Wkly Rep* 1986;**35**:341–50.
51. Centers for Disease Control. ACIP: Mumps prevention. *MMWR Morb Mortal Wkly Rep* 1989;**38**:388–400.
52. Centers for Disease Control. Arboviral surveillance: United States, 1990. *MMWR Morb Mortal Wkly Rep* 1990;**39**:593–8.

53. Centers for Disease Control. Outbreak of Hendra-like virus: Malaysia and Singapore—1998-1999. *MMWR Morb Mortal Wkly Rep* 1999;**48**:265–9.
54. Centers for Disease Control. Enterovirus surveillance: United States, 1997-1999. *MMWR Morb Mortal Wkly Rep* 2000;**49**: 913–6.
55. Centers for Disease Control. Outbreak of aseptic meningitis associated with multiple enterovirus serotypes: Romania, 1999. *MMWR Morb Mortal Wkly Rep* 2000;**49**:669–71.
56. Centers for Disease Control. Acute flaccid paralysis associated with circulating vaccine-derived poliovirus: Philippines, 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:874–5.
57. Centers for Disease Control. Echovirus type 13: United States, 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:777–80.
58. Centers for Disease Control and Prevention. Outbreaks of aseptic meningitis associated with echoviruses 9 and 30 and preliminary surveillance reports on enterovirus activity: United States, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**:761–4.
59. Centers for Disease Control and Prevention. Enterovirus surveillance: United States, 2002-2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:153–6.
60. Centers for Disease Control and Prevention. Update: multistate outbreak of mumps—United States, January 1-May 2, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:559–63.
61. Centers for Disease Control and Prevention. Increased detections and severe neonatal disease associated with coxsackievirus B1 infection: United States, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:553–6.
62. Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance: United States, 2006-2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1577–80.
63. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1009–13.
64. Cernescu C, Tardei G, Ruta S, et al. An outbreak of aseptic meningitis due to ECHO 30 virus in Romania during the 1999 summer. *Rom J Virol* 1999;**50**:99–106.
65. Chan LG, Parashar UD, Lye MS, et al. Deaths of children during an outbreak of hand, foot, and mouth disease in Sarawak, Malaysia: clinical and pathological characteristics of the disease. Outbreak Study Group. *Clin Infect Dis* 2000;**31**:678–83.
66. Char DF, Rosen L. Eosinophilic meningitis among children in Hawaii. *J Pediatr* 1967;**70**:28–35.
67. Chaves SS, Haber P, Walton K, et al. Safety of varicella vaccine after licensure in the United States: experience from reports to the vaccine adverse event reporting system, 1995-2005. *J Infect Dis* 2008;**197**(Suppl 2):S170–177.
68. Cheon DS, Lee J, Lee K, et al. Isolation and molecular identification of echovirus 13 isolated from patients of aseptic meningitis in Korea, 2002. *J Med Virol* 2004;**73**:439–42.
69. Chieochansin T, Vichiwattana P, Korkong S, et al. Molecular epidemiology, genome characterization, and recombination event of human parechovirus. *Virology* 2011;**421**:159–66.
70. Childs JE, Glass GE, Ksiazek TG, et al. Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. *Am J Trop Med Hyg* 1991; **44**:117–21.
71. Choi YJ, Park KS, Baek KA, et al. Molecular characterization of echovirus 30-associated outbreak of aseptic meningitis in Korea in 2008. *J Microbiol Biotechnol* 2010;**20**:643–9.
72. Chomel JJ, Antona D, Thouvenot D, et al. Three ECHOvirus serotypes responsible for outbreak of aseptic meningitis in Rhone-Alpes region, France. *Eur J Clin Microbiol Infect Dis* 2003;**22**:191–3.
73. Chowers MY, Lang R, Nassar F, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. *Emerg Infect Dis* 2001;**7**:675–8.
74. Cinque P, Bossolasco S, Lundkvist A. Molecular analysis of cerebrospinal fluid in viral diseases of the central nervous system. *J Clin Virol* 2003;**26**:1–28.
75. Civen R, Villacorte F, Robles DT, et al. West Nile virus infection in the pediatric population. *Pediatr Infect Dis J* 2006;**25**: 75–8.
76. Cizman M, Mozetic M, Radescek-Rakar R, et al. Aseptic meningitis after vaccination against measles and mumps. *Pediatr Infect Dis J* 1989;**8**:302–8.
77. Cohen BA, Rowley AH, Long CM. Herpes simplex type 2 in a patient with Mollaret's meningitis: demonstration by polymerase chain reaction. *Ann Neurol* 1994;**35**:112–6.
78. Coleman WS, Lischner HW, Grover WD. Recurrent aseptic meningitis without sequelae. *J Pediatr* 1975;**87**:89–91.
79. Connor JD, Buchta RM, DeGenaro F, Jr, et al. Specialty conference: potpourri of adenoviral infections. *West J Med* 1974;**120**: 55–61.
80. Corrall CJ, Pepple JM, Moxon ER, et al. C-reactive protein in spinal fluid of children with meningitis. *J Pediatr* 1981;**99**:365–9.
81. Craig CP, Nahmias AJ. Different patterns of neurologic involvement with herpes simplex virus types 1 and 2: isolation of herpes simplex virus type 2 from the buffy coat of two adults with meningitis. *J Infect Dis* 1973;**127**:365–72.
82. Craver RD, Gohd RS, Sundin DR, et al. Isolation of parainfluenza virus type 3 from cerebrospinal fluid associated with aseptic meningitis. *Am J Clin Pathol* 1993;**99**:705–7.
83. Cui A, Yu D, Zhu Z, et al. An outbreak of aseptic meningitis caused by coxsackievirus A9 in Gansu, the People's Republic of China. *Virol J* 2010;**7**:72.
84. da Cunha SS, Rodrigues LC, Barreto ML, et al. Outbreak of aseptic meningitis and mumps after mass vaccination with MMR vaccine using the Leningrad-Zagreb mumps strain. *Vaccine* 2002;**20**:1106–12.
85. da Silveira CM, Kmetzsch CI, Mohrdieck R, et al. The risk of aseptic meningitis associated with the Leningrad-Zagreb mumps vaccine strain following mass vaccination with measles-mumps-rubella vaccine, Rio Grande do Sul, Brazil, 1997. *Int J Epidemiol* 2002;**31**:978–82.
86. Dalldorf G, Sickles GM. An unidentified, filtrable agent isolated from the feces of children with paralysis. *Science* 1948;**108**:61–2.
87. Davies JW, McDermott A, Severs D. Epidemic virus meningitis due to ECHO 9 virus in Newfoundland. *Can Med Assoc J* 1958;**79**: 162–7.
88. Davis LE, DeBiasi R, Goade DE, et al. West Nile virus neuroinvasive disease. *Ann Neurol* 2006;**60**:286–300.
89. de Beer FC, Kirsten GF, Gie RP, et al. Value of C reactive protein measurement in tuberculous, bacterial, and viral meningitis. *Arch Dis Child* 1984;**59**:653–6.
90. Deibel R, Flanagan TD. Central nervous system infections: etiologic and epidemiologic observations in New York State, 1976-1977. *N Y State J Med* 1979;**79**:689–95.
91. Deibel R, Flanagan TD, Smith V. Central nervous system infections in New York State: etiologic and epidemiologic observations, 1974. *N Y State J Med* 1975;**75**:2337–42.
92. Deibel R, Flanagan TD, Smith V. Central nervous system infections: etiologic and epidemiologic observations in New York State, 1975. *N Y State J Med* 1977;**77**:1398–404.
93. Deibel R, Woodall JP, Decher WJ, et al. Lymphocytic choriomeningitis virus in man: serologic evidence of association with pet hamsters. *JAMA* 1975;**232**:501–4.
94. Dengler LD, Capparelli EV, Bastian JF, et al. Cerebrospinal fluid profile in patients with acute Kawasaki disease. *Pediatr Infect Dis J* 1998;**17**:478–81.
95. Derbes SJ. Trimethoprim-induced aseptic meningitis. *JAMA* 1984;**252**:2865–6.
96. Desmond RA, Accortt NA, Talley L, et al. Enteroviral meningitis: natural history and outcome of pleconaril therapy. *Antimicrob Agents Chemother* 2006;**50**:2409–14.
97. Dickey M, Jamison L, Michaud L, et al. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. *Pediatr Infect Dis J* 2009;**28**:318–21.
98. Diedrich S, Schreier E. Aseptic meningitis in Germany associated with echovirus type 13. *BMC Infect Dis* 2001;**1**:14.
99. Dimmitt DC, Fishbein DB, Dawson JE. Human ehrlichiosis associated with cerebrospinal fluid pleocytosis: a case report. *Am J Med* 1989;**87**:677–8.
100. Do AN, Green PA, Demmler GJ. Herpes simplex virus type 2 meningitis and associated genital lesions in a three-year-old child. *Pediatr Infect Dis J* 1994;**13**:1014–6.
101. Dos Santos GP, Skraba I, Oliveira D, et al. Enterovirus meningitis in Brazil, 1998-2003. *J Med Virol* 2006;**78**:98–104.
102. Dourado I, Cunha S, Teixeira MG, et al. Outbreak of aseptic meningitis associated with mass vaccination with a urabe-containing measles-mumps-rubella vaccine: implications for immunization programs. *Am J Epidemiol* 2000;**151**:524–30.

103. Dulkerian SJ, Kilpatrick L, Costarino Jr AT, et al. Cytokine elevations in infants with bacterial and aseptic meningitis. *J Pediatr* 1995;**126**:872–6.
104. Dylewski JS, Bekhor S. Mollaret's meningitis caused by herpes simplex virus type 2: case report and literature review. *Eur J Clin Microbiol Infect Dis* 2004;**23**:560–2.
105. Echevarria JM, Casas I, Tenorio A, et al. Detection of varicella-zoster virus-specific DNA sequences in cerebrospinal fluid from patients with acute aseptic meningitis and no cutaneous lesions. *J Med Virol* 1994;**43**:331–5.
106. Enders JF. Mumps. In: Rivers TM, Horsfall FL, editors. *Viral and Rickettsial Infections of Man*. Philadelphia: J. B. Lippincott; 1959. pp. 780–9.
107. Enders JF, Weller TH, Robbins FC. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* 1949;**109**:85–7.
108. Eppes SC, Nelson DK, Lewis LL, et al. Characterization of Lyme meningitis and comparison with viral meningitis in children. *Pediatrics* 1999;**103**:957–60.
109. Estrada-Franco JG, Navarro-Lopez R, Beasley DW, et al. West Nile virus in Mexico: evidence of widespread circulation since July 2002. *Emerg Infect Dis* 2003;**9**:1604–7.
110. Evans AS, Niederman JC. Epstein-Barr virus. In: Evans AS, editors. *Viral Infections of Humans: Epidemiology and Control*. 2nd ed. New York: Plenum Medical; 1982. p. 253–81.
111. Faulkner R, Van Rooyen CE. Adenoviruses types 3 and 5 isolated from the cerebrospinal fluid of children. *Can Med Assoc J* 1962;**87**:1123–5.
112. Faustini A, Fano V, Muscillo M, et al. An outbreak of aseptic meningitis due to echovirus 30 associated with attending school and swimming in pools. *Int J Infect Dis* 2006;**10**:291–7.
113. Finkelstein Y, Mosseri R, Garty BZ. Concomitant aseptic meningitis and bacterial urinary tract infection in young febrile infants. *Pediatr Infect Dis J* 2001;**20**:630–2.
114. Fobelo MJ, Corzo Delgado JE, Romero Alonso A, et al. Aseptic meningitis related to valacyclovir. *Ann Pharmacother* 2001;**35**:128–9.
115. Forbes JA. Meningitis in Melbourne due to E.C.H.O. virus. I. Clinical aspects. *Med J Aust* 1958;**45**:246–8.
116. Francesconi F, Francesconi do Valle AC, Silva MT, et al. International issues: meningoencephalitis due to *Paracoccidioides brasiliensis*. *Neurology* 2008;**71**:e65–7.
117. Francisco AM, Glaser C, Frykman E, et al. 2004 California pediatric West Nile virus case series. *Pediatr Infect Dis J* 2006;**25**:81–4.
118. Fryden A, Kihlstrom E, Maller R, et al. A clinical and epidemiological study of "rnithosis" caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). *Scand J Infect Dis* 1989;**21**:681–91.
119. Fujimoto H, Asaoka K, Imaizumi T, et al. Epstein-Barr virus infections of the central nervous system. *Intern Med* 2003;**42**:33–40.
120. Fujimoto T, Chikahira M, Yoshida S, et al. Outbreak of central nervous system disease associated with hand, foot, and mouth disease in Japan during the summer of 2000: detection and molecular epidemiology of enterovirus 71. *Microbiol Immunol* 2002;**46**:621–7.
121. Gajdusek DC. Encephalomyocarditis virus infection in childhood. *Pediatrics* 1955;**16**:902–6.
122. Galea SA, Sweet A, Beninger P, et al. The safety profile of varicella vaccine: a 10-year review. *J Infect Dis* 2008;**197** (Suppl 2):S165–169.
123. Garland SM, Murton LJ. Neonatal meningitis caused by *Ureaplasma urealyticum*. *Pediatr Infect Dis J* 1987;**6**:868–70.
124. Garnier A, Frascari F, Decramer S. Varicella-zoster virus meningoencephalitis without skin lesions in a paediatric kidney recipient. *Nephrol Dial Transplant* 2011;**26**:378–9; author reply 379.
125. Gilbert GL, Dickson KE, Waters MJ, et al. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. *Pediatr Infect Dis J* 1988;**7**:484–8.
126. Gilden D, Cohrs RJ, Mahalingam R, et al. Neurological disease produced by varicella zoster virus reactivation without rash. *Curr Top Microbiol Immunol* 2010;**342**:243–53.
127. Glimaker M, Johansson B, Olcen P, et al. Detection of enteroviral RNA by polymerase chain reaction in cerebrospinal fluid from patients with aseptic meningitis. *Scand J Infect Dis* 1993;**25**:547–57.
128. Glimaker M, Kragsbjerg P, Forsgren M, et al. Tumor necrosis factor-alpha (TNF alpha) in cerebrospinal fluid from patients with meningitis of different etiologies: high levels of TNF alpha indicate bacterial meningitis. *J Infect Dis* 1993;**167**:882–9.
129. Golden SE. Aseptic meningitis associated with Ehrlichia canis infection. *Pediatr Infect Dis J* 1989;**8**:335–7.
130. Gonzalez Garcia H, Ernandez Alonso JF, de Paz Garcia M, et al. Meningitis as the first and only manifestation of brucellosis. *An Esp Pediatr* 2000;**53**:280–2.
131. Gonzalez Pascual E, Villanueva Lamas J, Ros Viladoms J, et al. [Kawasaki disease: a report of 50 cases]. *An Esp Pediatr* 1999;**50**:39–43.
132. Goodpasture HC, Poland JD, Francy DB, et al. Colorado tick fever: clinical, epidemiologic, and laboratory aspects of 228 cases in Colorado in 1973-1974. *Ann Intern Med* 1978;**88**:303–10.
133. Gordon MF, Allon M, Coyle PK. Drug-induced meningitis. *Neurology* 1990;**40**:163–4.
134. Gosbell I, Robinson D, Chant K, et al. Outbreak of echovirus 30 meningitis in Wingecarribee Shire, New South Wales. *Commun Dis Intell* 2000;**24**:121–4.
135. Goto T, Kimura H, Numazaki K, et al. A case of meningoencephalitis associated with G1P[8] rotavirus infection in a Japanese child. *Scand J Infect Dis* 2007;**39**:1067–70.
136. Gottfredsson M, Perfect JR. Fungal meningitis. *Semin Neurol* 2000;**20**:307–22.
137. Graman PS. Mollaret's meningitis associated with acute Epstein-Barr virus mononucleosis. *Arch Neurol* 1987;**44**:1204–5.
138. Grist NR, Bell EJ, Assaad F. Enteroviruses in human disease. *Prog Med Virol* 1978;**24**:114–57.
139. Guglielminotti J, Lellouche N, Maury E, et al. Severe meningoencephalitis: an unusual manifestation of *Chlamydia pneumoniae* infection. *Clin Infect Dis* 2000;**30**:209–10.
140. Guleria R, Nisar N, Chawla TC, et al. *Mycoplasma pneumoniae* and central nervous system complications: a review. *J Lab Clin Med* 2005;**146**:55–63.
141. Gunther G, Haglund M, Lindquist L, et al. Tick-borne encephalitis in Sweden in relation to aseptic meningo-encephalitis of other etiology: a prospective study of clinical course and outcome. *J Neurol* 1997;**244**:230–8.
142. Guthrie N. Coxsackie B5 meningitis: report of an outbreak in a high school football squad. *J Tn State Med Assoc* 1962;**55**:355–6.
143. Haase KK, Lapointe M, Haines SJ. Aseptic meningitis after intraventricular administration of gentamicin. *Pharmacotherapy* 2001;**21**:103–7.
144. Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. *PLoS One* 2009;**4**:e6145.
145. Hammer SM, Connolly KJ. Viral aseptic meningitis in the United States: clinical features, viral etiologies, and differential diagnosis. *Curr Clin Top Infect Dis* 1992;**12**:1–25.
146. Han JY, Hanson DC, Way SS. Herpes zoster and meningitis due to reactivation of varicella vaccine virus in an immunocompetent child. *Pediatr Infect Dis J* 2011;**30**:266–8.
147. Handsher R, Shulman LM, Abramovitz B, et al. A new variant of echovirus 4 associated with a large outbreak of aseptic meningitis. *J Clin Virol* 1999;**13**:29–36.
148. Hanninen P, Pohjonen R. Echovirus type 6 meningitis: clinical and virological observations during an epidemic in Turku in 1968. *Scand J Infect Dis* 1971;**3**:121–5.
149. Hardin SG, Erwin PC, Patterson L, et al. Clinical comparisons of La Crosse encephalitis and enteroviral central nervous system infections in a pediatric population: 2001 surveillance in East Tennessee. *Am J Infect Control* 2003;**31**:508–10.
150. Harvala H, Wolthers KC, Simmonds P. Parechoviruses in children: understanding a new infection. *Curr Opin Infect Dis* 2010;**23**:224–30.
151. Hauri AM, Schimmelpfennig M, Walter-Domes M, et al. An outbreak of viral meningitis associated with a public swimming pond. *Epidemiol Infect* 2005;**133**:291–8.
152. Hayes EB, O'Leary DR. West Nile virus infection: a pediatric perspective. *Pediatrics* 2004;**113**:1375–81.
153. Hayes EB, Sejvar JJ, Zaki SR, et al. Virology, pathology, and clinical manifestations of West Nile virus disease. *Emerg Infect Dis* 2005;**11**:1174–9.

154. Haynes RE, Cramblett HG, Kronfol HJ. Echovirus 9 meningoencephalitis in infants and children. *JAMA* 1969;**208**:1657–60.
155. Haynes RE, Sanders DY, Cramblett HG. Rocky Mountain spotted fever in children. *J Pediatr* 1970;**76**:685–93.
156. Helin I, Widell A, Borulf S, et al. Outbreak of coxsackievirus A-14 meningitis among newborns in a maternity hospital ward. *Acta Paediatr Scand* 1987;**76**:234–8.
157. Hermans PE, Goldstein NP, Wellman WE. Mollaret's meningitis and differential diagnosis of recurrent meningitis: report of case, with review of the literature. *Am J Med* 1972;**52**:128–40.
158. Heusner AP. Nontuberculous spinal epidural infections. *N Engl J Med* 1948;**239**:845–54.
159. Ho M. Enterovirus 71: the virus, its infections and outbreaks. *J Microbiol Immunol Infect* 2000;**33**:205–16.
160. Hochberg NS, Blackburn BG, Park SY, et al. Eosinophilic meningitis attributable to *Angiostrongylus cantonensis* infection in Hawaii: clinical characteristics and potential exposures. *Am J Trop Med Hyg* 2011;**85**:685–90.
161. Hochberg NS, Park SY, Blackburn BG, et al. Distribution of eosinophilic meningitis cases attributable to *Angiostrongylus cantonensis:* Hawaii. *Emerg Infect Dis* 2007;**13**:1675–80.
162. Holzel A, Smith PA, Tobin JO. A new type of meningo-encephalitis associated with a rhinovirus. *Acta Paediatr Scand* 1965;**54**:168–74.
163. Hoppa E, Bachur R. Lyme disease update. *Curr Opin Pediatr* 2007;**19**:275–80.
164. Huang C, Morse D, Slater B, et al. Multiple-year experience in the diagnosis of viral central nervous system infections with a panel of polymerase chain reaction assays for detection of 11 viruses. *Clin Infect Dis* 2004;**39**:630–5.
165. Huang CC, Liu CC, Chang YC, et al. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999; **341**:936–42.
166. Huang QS, Carr JM, Nix WA, et al. An echovirus type 33 winter outbreak in New Zealand. *Clin Infect Dis* 2003;**37**:650–7.
167. Huhn GD, Sejvar JJ, Montgomery SP, et al. West Nile virus in the United States: an update on an emerging infectious disease. *Am Fam Physician* 2003;**68**:653–60.
168. Iyer S, Mittal MK, Hodinka RL. Herpes zoster and meningitis resulting from reactivation of varicella vaccine virus in an immunocompetent child. *Ann Emerg Med* 2009;**53**:792–5.
169. Jean CM, Honarmand S, Louie JK, et al. Risk factors for West Nile virus neuroinvasive disease, California, 2005. *Emerg Infect Dis* 2007;**13**:1918–20.
170. Jha S, Ansari MK. Leptospirosis presenting as acute meningoencephalitis. *J Infect Dev Countries* 2010;**4**:179–82.
171. Jhaveri R, Cherry JD, Phillips S, et al. Erythema migrans after ceftriaxone treatment of aseptic meningitis caused by *Borrelia burgdorferi*. *Pediatr Infect Dis J* 2001;**20**:1010–2.
172. Jhaveri R, Sankar R, Yazdani S, et al. Varicella-zoster virus: an overlooked cause of aseptic meningitis. *Pediatr Infect Dis J* 2003;**22**:96–7.
173. Johnson CD, Goodpasture EW. An investigation of the etiology of mumps. *J Exp Med* 1934;**59**:1–20.
174. Jorbeck HJ, Gustafsson PM, Lind HC, et al. Tick-borne *Borrelia*-meningitis in children; an outbreak in the Kalmar area during the summer of 1984. *Acta Paediatr Scand* 1987;**76**:228–33.
175. Julian KG, Mullins JA, Olin A, et al. Aseptic meningitis epidemic during a West Nile virus avian epizootic. *Emerg Infect Dis* 2003;**9**:1082–8.
176. Kaida A, Kubo H, Iritani N, et al. Isolation of Echovirus type 13 in Osaka City during 2001-2002. *Jpn J Infect Dis* 2004; **57**:127–8.
177. Kajiwara I, Kusaba T, Hayashida I, et al. [Clinical study of an outbreak of aseptic meningitis due to echovirus type 30 in Munakata City in 1997-1998]. *Kansenshogaku Zasshi* 2000;**74**: 231–6.
178. Kammerer U, Kunkel B, Korn K. Nested PCR for specific detection and rapid identification of human picornaviruses. *J Clin Microbiol* 1994;**32**:285–91.
179. Karande S, Patil S, Kulkarni M, et al. Acute aseptic meningitis as the only presenting feature of leptospirosis. *Pediatr Infect Dis J* 2005;**24**:390–1.
180. Karzon DT, Barron AL. An epidemic of aseptic meningitis syndrome due to echo virus type 6. I. Correlation of enterovirus isolation with illness. II. Clinical study. III. Sequelae. *Pediatrics* 1962;**29**:409–17, 418–31, 432–7.
181. Karzon DT, Eckert GL, Barron AL, et al. Aseptic meningitis epidemic due to ECHO 4 virus. *Am J Dis Child* 1961;**101**: 610–22.
182. Kehle J, Metzger-Boddien C, Tewald F, et al. First case of confirmed rotavirus meningoencephalitis in Germany. *Pediatr Infect Dis J* 2003;**22**:468–70.
183. Keino M, Kanno M, Hirasawa K, et al. Isolation of echovirus type 13 from patients of aseptic meningitis. *Jpn J Infect Dis* 2001; **54**:249–50.
184. Kelsey DS. Adenovirus meningoencephalitis. *Pediatrics* 1978;**61**: 291–3.
185. Kennedy DH, Fallon RJ. Tuberculous meningitis. *JAMA* 1979; **241**:264–8.
186. Khalfan S, Aymard M, Lina B, et al. Epidemics of aseptic meningitis due to enteroviruses following national immunization days in Bahrain. *Ann Trop Paediatr* 1998;**18**:101–9.
187. Kirschke DL, Jones TF, Buckingham SC, et al. Outbreak of aseptic meningitis associated with echovirus 13. *Pediatr Infect Dis J* 2002;**21**:1034–8.
188. Kitai I, Navas L, Rohlicek C, et al. Recurrent aseptic meningitis secondary to an intracranial cyst: a case report and review of clinical features and imaging modalities. *Pediatr Infect Dis J* 1992;**11**:671–5.
189. Klein NC, McDermott B, Cunha BA. Varicella-zoster virus meningoencephalitis in an immunocompetent patient without a rash. *Scand J Infect Dis* 2010;**42**:631–3.
190. Knust B, Macneil A, Wong SJ, et al. Exposure to lymphocytic choriomeningitis virus, New York, USA. *Emerg Infect Dis* 2011; **17**:1324–5.
191. Kochar DK, Agarwal N, Jain N, et al. Clinical profile of neurobrucellosis: a report on 12 cases from Bikaner (north-west India). *J Assoc Physicians India* 2000;**48**:376–80.
192. Kondo K, Nagafuji H, Hata A, et al. Association of human herpesvirus 6 infection of the central nervous system with recurrence of febrile convulsions. *J Infect Dis* 1993;**167**:1197–200.
193. Kriss TC, Kriss VM, Warf BC. Recurrent meningitis: the search for the dermoid or epidermoid tumor. *Pediatr Infect Dis J* 1995; **14**:697–700.
194. Kupila L, Vainionpaa R, Vuorinen T, et al. Recurrent lymphocytic meningitis: the role of herpesviruses. *Arch Neurol* 2004;**61**: 1553–7.
195. Laforest RA, McNaughton GA, Beale AJ, et al. Outbreak of aseptic meningitis (meningoencephalitis) with rubelliform rash: Toronto, 1956. *Can Med Assoc J* 1957;**77**:1–4.
196. Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. *J Clin Microbiol* 2000;**38**:4066–71.
197. Leahy TR, Webb DW, Hoey H, et al. Varicella zoster virus associated acute aseptic meningitis without exanthem in an immunocompetent 14-year-old boy. *Pediatr Infect Dis J* 2008;**27**: 362–3.
198. Lecour H, Miranda M, Magro C, et al. Human leptospirosis: a review of 50 cases. *Infection* 1989;**17**:8–12.
199. Lee KE, Umapathi T, Tan CB, et al. The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. *Ann Neurol* 1999;**46**:428–32.
200. Lennette EH, Magoffin RL, Knouf EG. Viral central nervous system disease: An etiologic study conducted at the Los Angeles County General Hospital. *JAMA* 1962;**179**:687–95.
201. Lepow ML, Carver DH, Wright Jr HT, et al. A clinical, epidemiologic and laboratory investigation of aseptic meningitis during the four-year period, 1955-1958. I. Observations concerning etiology and epidemiology. *N Engl J Med* 1962;**266**:1181–7.
202. Lepow ML, Coyne N, Thompson LB, et al. A clinical, epidemiologic and laboratory investigation of aseptic meningitis during the four-year period, 1955-1958. II. The clinical disease and its sequelae. *N Engl J Med* 1962;**266**:1188–93.
203. Lesnicar G, Poljak M, Seme K, et al. Pediatric tick-borne encephalitis in 371 cases from an endemic region in Slovenia, 1959 to 2000. *Pediatr Infect Dis J* 2003;**22**:612–7.
204. Leveque N, Jacques J, Renois F, et al. Phylogenetic analysis of Echovirus 30 isolated during the 2005 outbreak in France reveals

existence of multiple lineages and suggests frequent recombination events. *J Clin Virol* 2010;**48**:137–41.
205. Levin MJ, Dahl KM, Weinberg A, et al. Development of resistance to acyclovir during chronic infection with the Oka vaccine strain of varicella-zoster virus, in an immunosuppressed child. *J Infect Dis* 2003;**188**:954–9.
206. Levin MJ, DeBiasi RL, Bostik V, et al. Herpes zoster with skin lesions and meningitis caused by 2 different genotypes of the Oka varicella-zoster virus vaccine. *J Infect Dis* 2008;**198**: 1444–7.
207. Li CC, Yang MY, Chen RF, et al. Clinical manifestations and laboratory assessment in an enterovirus 71 outbreak in southern Taiwan. *Scand J Infect Dis* 2002;**34**:104–9.
208. Li H, Xu F, Gu JB, et al. A severe eosinophilic meningoencephalitis caused by infection of *Angiostrongylus cantonensis*. *Am J Trop Med Hyg* 2008;**79**:568–70.
209. Liao HT, Hung KL. Neurologic involvement in an outbreak of enterovirus 71 infection: a hospital-based study. *Acta Paediatr Taiwan* 2001;**42**:27–32.
210. Lin WC, Lee PI, Lu CY, et al. *Mycoplasma pneumoniae* encephalitis in childhood. *J Microbiol Immunol Infect* 2002;**35**:173–8.
211. Lindquist L, Linne T, Hansson LO, et al. Value of cerebrospinal fluid analysis in the differential diagnosis of meningitis: a study in 710 patients with suspected central nervous system infection. *Eur J Clin Microbiol Infect Dis* 1988;**7**:374–80.
212. Lindsey NP, Hayes EB, Staples JE, et al. West Nile virus disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**: e1084–9.
213. Lindsey NP, Staples JE, Lehman JA, et al. Surveillance for human West Nile virus disease: United States, 1999-2008. *MMWR Surveill Summ* 2010;**59**:1–17.
214. Liu CC, Tseng HW, Wang SM, et al. An outbreak of enterovirus 71 infection in Taiwan, 1998: epidemiologic and clinical manifestations. *J Clin Virol* 2000;**17**:23–30.
215. Logar M, Arnez M, Kolbl J, et al. Comparison of the epidemiological and clinical features of tick-borne encephalitis in children and adults. *Infection* 2000;**28**:74–7.
216. Logotheti M, Pogka V, Horefti E, et al. Laboratory investigation and phylogenetic analysis of enteroviruses involved in an aseptic meningitis outbreak in Greece during the summer of 2007. *J Clin Virol* 2009;**46**:270–4.
217. Ma E, Chan KC, Cheng P, et al. The enterovirus 71 epidemic in 2008: public health implications for Hong Kong. *Int J Infect Dis* 2010;**14**:e775–80.
218. Mao N, Zhao L, Zhu Z, et al. An aseptic meningitis outbreak caused by echovirus 6 in Anhui province, China. *J Med Virol* 2010;**82**:441–5.
219. Mardh PA. *Mycoplasma hominis* infection of the central nervous system in newborn infants. *Sex Transm Dis* 1983;**10**:331–4.
220. Markey PG, Davis JS, Harnett GB, et al. Meningitis and a febrile vomiting illness caused by echovirus type 4, Northern Territory, Australia. *Emerg Infect Dis* 2010;**16**:63–8.
221. Martin MA, Massanari RM, Nghiem DD, et al. Nosocomial aseptic meningitis associated with administration of OKT3. *JAMA* 1988;**259**:2002–5.
222. Mathisen G, Shelub A, Truong J, et al. Coccidioidal meningitis: clinical presentation and management in the fluconazole era. *Medicine (Baltimore)* 2010;**89**:251–84.
223. McDonald JC, Moore DL, Quennec P. Clinical and epidemiologic features of mumps meningoencephalitis and possible vaccine-related disease. *Pediatr Infect Dis J* 1989;**8**:751–5.
224. McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med* 2001;**345**:148–9.
225. McMinn P, Stratov I, Nagarajan L, et al. Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. *Clin Infect Dis* 2001;**32**:236–42.
226. Meyer Jr HM, Johnson RT, Crawford IP, et al. Central nervous system syndromes of "vital" etiology: a study of 713 cases. *Am J Med* 1960;**29**:334–47.
227. Mifsud AJ. Drug-related recurrent meningitis. *J Infect* 1988; **17**:151–3.
228. Miller E, Goldacre M, Pugh S, et al. Risk of aseptic meningitis after measles, mumps, and rubella vaccine in UK children. *Lancet* 1993;**341**:979–82.
229. Miller HG, Stanton JB. Neurological sequelae of prophylactic inoculation. *Q J Med* 1954;**23**:1–27.
230. Miller HG, Gibbons JL, Stanton JB. Para-infectious encephalomyelitis and related syndromes; a critical review of the neurological complications of certain specific fevers. *Q J Med* 1956;**25**: 427–505.
231. Molavi A, LeFrock JL. Tuberculous meningitis. *Med Clin North Am* 1985;**69**:315–31.
232. Mollaret P. La meningite endothelio-leukocytaire multirecurrente benigne: syndrome nouveau ou maladie nouvelle? *Rev Neurol* 1944;**72**:57–76.
233. Moore M. Centers for Disease Control. Enteroviral disease in the United States, 1970-1979. *J Infect Dis* 1982;**146**:103–8.
234. Moylett EH. Neonatal *Candida* meningitis. *Semin Pediatr Infect Dis* 2003;**14**:115–22.
235. Muller MP, Richardson DC, Walmsley SL. Trimethoprim-sulfamethoxazole induced aseptic meningitis in a renal transplant patient. *Clin Nephrol* 2001;**55**:80–4.
236. Mullins JA, Khetsuriani N, Nix WA, et al. Emergence of echovirus type 13 as a prominent enterovirus. *Clin Infect Dis* 2004;**38**:70–7.
237. Nagai T, Okafuji T, Miyazaki C, et al. A comparative study of the incidence of aseptic meningitis in symptomatic natural mumps patients and monovalent mumps vaccine recipients in Japan. *Vaccine* 2007;**25**:2742–7.
238. Narkeviciute I, Vaiciuniene D. Outbreak of echovirus 13 infection among Lithuanian children. *Clin Microbiol Infect* 2004;**10**:1023–5.
239. Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 2001;**344**:1807–14.
240. Nigrovic LE, Chiang VW. Cost analysis of enteroviral polymerase chain reaction in infants with fever and cerebrospinal fluid pleocytosis. *Arch Pediatr Adolesc Med* 2000;**154**:817–21.
241. Okumura A, Ichikawa T. Aseptic meningitis caused by human parvovirus B19. *Arch Dis Child* 1993;**68**:784–5.
242. Ortner B, Huang CW, Schmid D, et al. Epidemiology of enterovirus types causing neurological disease in Austria 1999-2007: detection of clusters of echovirus 30 and enterovirus 71 and analysis of prevalent genotypes. *J Med Virol* 2009;**81**:317–24.
243. Ozdemir H, Karbuz A, Ciftci E, et al. Aseptic meningitis in a child due to 2009 pandemic influenza A (H1N1) infection. *Turk J Pediatr* 2011;**53**:91–3.
244. Ozkaya E, Hizel K, Uysal G, et al. An outbreak of aseptic meningitis due to echovirus type 30 in two cities of Turkey. *Eur J Epidemiol* 2003;**18**:823–6.
245. Pahud BA, Glaser CA, Dekker CL, et al. Varicella zoster disease of the central nervous system: epidemiological, clinical, and laboratory features 10 years after the introduction of the varicella vaccine. *J Infect Dis* 2011;**203**:316–23.
246. Paisley JW, Bruhn FW, Lauer BA, et al. Type A2 influenza viral infections in children. *Am J Dis Child* 1978;**132**:34–6.
247. Panicker JN, Mammachan R, Jayakumar RV. Primary neuroleptospirosis. *Postgrad Med J* 2001;**77**:589–90.
248. Peltola H, Valmari P. Serum C-reactive protein as detector of pretreated childhood bacterial meningitis. *Neurology* 1985;**35**: 251–3.
249. Peltola HO. C-reactive protein for rapid monitoring of infections of the central nervous system. *Lancet* 1982;**1**:980–2.
250. Pena JA, Pirics ML, DiCaprio HS, et al. Varicella reactivation presenting as shingles and aseptic meningitis in an immunocompetent 11-year-old boy. *Clin Pediatr (Phila)* 2009;**48**:435–7.
251. Pereira AC, Barros RA, do Nascimento JP, et al. Two family members with a syndrome of headache and rash caused by human parvovirus B19. *Braz J Infect Dis* 2001;**5**:37–9.
252. Pereira MS, Maccallum FO. Infection with adenovirus type 12. *Lancet* 1964;**1**:198–9.
253. Perevoscikovs J, Brila A, Firstova L, et al. Ongoing outbreak of aseptic meningitis in South-Eastern Latvia, June-August 2010. *Euro Surveill* 2010;15:pii:19639.
254. Peter G. Leptospirosis: a zoonosis of protean manifestations. *Pediatr Infect Dis* 1982;**1**:282–8.
255. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med* 2002;**137**:173–9.
256. Picard FJ, Dekaban GA, Silva J, et al. Mollaret's meningitis associated with herpes simplex type 2 infection. *Neurology* 1993;**43**: 1722–7.

257. Pinto Junior VL, Rebelo MC, Costa EV, et al. Description of a widespread outbreak of aseptic meningitis due to echovirus 30 in Rio de Janeiro state, Brazil. *Braz J Infect Dis* 2009;**13**: 367–70.
258. Platonov AE, Shipulin GA, Shipulina OY, et al. Outbreak of West Nile virus infection, Volgograd region, Russia, 1999. *Emerg Infect Dis* 1999;**7**:128–32.
259. Pohl-Koppe A, Blay M, Jager G, et al. Human herpes virus type 7 DNA in the cerebrospinal fluid of children with central nervous system diseases. *Eur J Pediatr* 2001;**160**:351–8.
260. Pönkä A. Central nervous system manifestations associated with serologically verified *Mycoplasma pneumoniae* infection. *Scand J Infect Dis* 1980;**12**:175–84.
261. Portolani M, Pecorari M, Pietrosemoli P, et al. Outbreak of aseptic meningitis by echo 4: prevalence of clinical cases among adults. *New Microbiol* 2001;**24**:11–5.
262. Preblud SR. Age-specific risks of varicella complications. *Pediatrics* 1981;**68**:14–7.
263. Prieto-Gonzalez S, Escoda R, Coloma E, et al. Amoxicillin-induced acute aseptic meningitis. *J Clin Neurosci* 2011;**18**:443–4.
264. Rafailidis PI, Kapaskelis A, Falagas ME. Cytomegalovirus meningitis in an immunocompetent patient. *Med Sci Monit* 2007;**13**: CS107–9.
265. Rafailidis PI, Mourtzoukou EG, Varbobitis IC, et al. Severe cytomegalovirus infection in apparently immunocompetent patients: a systematic review. *Virol J* 2008;**5**:47.
266. Ramirez-Avila L, Slome S, Schuster FL, et al. Eosinophilic meningitis due to *Angiostrongylus* and *Gnathostoma* species. *Clin Infect Dis* 2009;**48**:322–7.
267. Rao SP, Teitlebaum J, Miller ST. Intravenous immune globulin and aseptic meningitis. *Am J Dis Child* 1992;**146**:539–40.
268. Rasmussen AF. *The laboratory diagnosis of lymphocytic choriomeningitis and mumps: Rocky Mountain Conference on Infantile Paralysis*. Denver: University of Colorado School of Medicine; 1946. p. 45.
269. Rawal A, Gavin PJ, Sturgis CD. Cerebrospinal fluid cytology in seasonal epidemic West Nile virus meningo-encephalitis. *Diagn Cytopathol* 2006;**34**:127–9.
270. Reeves WC, Quiroz E, Brenes MM, et al. Aseptic meningitis due to echovirus 4 in Panama City, Republic of Panama. *Am J Epidemiol* 1987;**125**:562–75.
271. Reimer LG, Beller LB. CSF in herpes zoster meningoencephalitis. *Arch Neurol* 1981;**38**:668.
272. Reintjes R, Pohle M, Vieth U, et al. Community-wide outbreak of enteroviral illness caused by echovirus 30: a cross-sectional survey and a case-control study. *Pediatr Infect Dis J* 1999;**18**: 104–8.
273. Riski H, Hovi T. Coronavirus infections of man associated with diseases other than the common cold. *J Med Virol* 1980;**6**: 259–65.
274. Rivers TM, McNair Scott TF. Meningitis in man caused by a filterable virus. *Science* 1935;**81**:439–40.
275. Romero JR, Newland JG. Viral meningitis and encephalitis: traditional and emerging viral agents. *Semin Pediatr Infect Dis* 2003; **14**:72–82.
276. Roos KL. *Mycobacterium tuberculosis* meningitis and other etiologies of the aseptic meningitis syndrome. *Semin Neurol* 2000;**20**: 329–35.
277. Rosen L, Loison G, Laigret J, et al. Studies on eosinophilic meningitis. III. Epidemiologic and clinical observations on Pacific islands and the possible etiologic role of *Angiostrongylus cantonensis*. *Am J Epidemiol* 1967;**85**:17–44.
278. Rotbart HA. Enteroviral infections of the central nervous system. *Clin Infect Dis* 1995;**20**:971–81.
279. Rotbart HA. Viral meningitis. *Semin Neurol* 2000;**20**:277–92.
280. Rotbart HA, Webster AD. Treatment of potentially life-threatening enterovirus infections with pleconaril. *Clin Infect Dis* 2001;**32**:228–35.
281. Rotbart HA, O'Connell JF, McKinlay MA. Treatment of human enterovirus infections. *Antiviral Res* 1998;**38**:1–14.
282. Rotbart HA, Sawyer MH, Fast S, et al. Diagnosis of enteroviral meningitis by using PCR with a colorimetric microwell detection assay. *J Clin Microbiol* 1994;**32**:2590–2.
283. Rotem CE. Meningitis of virus origin. *Lancet* 1957;**272**:502–4.
284. Rothenberg R, Murphy W, O'Brien CL, et al. Aseptic meningitis associated with ECHO virus, type 9: an outbreak in Norfolk, Virginia. *South Med J* 1970;**63**:280–5.
285. Rottach K, Scheglmann K, Pfadenhauer K. Mollaret's meningitis: a new aetiologic feature. *Eur Neurol* 1996;**36**:172–3.
286. Sabin AB, Krumbiegel ER, Wigand R. ECHO type 9 virus disease. *AMA J Dis Child* 1958;**96**:197–219.
287. Salaki JS, Louria DB, Chmel H. Fungal and yeast infections of the central nervous system: a clinical review. *Medicine (Baltimore)* 1984;**63**:108–32.
288. Samuda GM, Chang WK, Yeung CY, et al. Monoplegia caused by enterovirus 71: an outbreak in Hong Kong. *Pediatr Infect Dis J* 1987;**6**:206–8.
289. Sands ML, Ryczak M, Brown RB. Recurrent aseptic meningitis followed by transverse myelitis as a presentation of systemic lupus erythematosus. *J Rheumatol* 1988;**15**:862–4.
290. Sarmiento L, Mas P, Goyenechea A, et al. First epidemic of echovirus 16 meningitis in Cuba. *Emerg Infect Dis* 2001;**7**:887–9.
291. Sato R, Ayabe M, Shoji H, et al. Herpes simplex virus type 2 recurrent meningitis (Mollaret's meningitis): a consideration for the recurrent pathogenesis. *J Infect* 2005;**51**:e217–20.
292. Sawyer MH, Holland D, Aintablian N, et al. Diagnosis of enteroviral central nervous system infection by polymerase chain reaction during a large community outbreak. *Pediatr Infect Dis J* 1994;**13**:177–82.
293. Schlesinger Y, Sawyer MH, Storch GA. Enteroviral meningitis in infancy: potential role for polymerase chain reaction in patient management. *Pediatrics* 1994;**94**:157–62.
294. Schumacher JD, Chuard C, Renevey F, et al. Outbreak of echovirus 30 meningitis in Switzerland. *Scand J Infect Dis* 1999;**31**: 539–42.
295. Sejvar JJ, Labutta RJ, Chapman LE, et al. Neurologic adverse events associated with smallpox vaccination in the United States, 2002-2004. *JAMA* 2003;**294**:2744–50.
296. Shaked Y, Samra Y. Q fever meningoencephalitis associated with bilateral abducens nerve paralysis, bilateral optic neuritis and abnormal cerebrospinal fluid findings. *Infection* 1989;**17**:394–5.
297. Shekhar K, Lye MS, Norlijah O, et al. Deaths in children during an outbreak of hand, foot and mouth disease in Peninsular Malaysia: clinical and pathological characteristics. *Med J Malaysia* 2005;**60**:297–304.
298. Shimizu H, Utama A, Yoshii K, et al. Enterovirus 71 from fatal and nonfatal cases of hand, foot and mouth disease epidemics in Malaysia, Japan and Taiwan in 1997-1998. *Jpn J Infect Dis* 1999; **52**:12–5.
299. Shinohara M, Uchida K, Shimada S, et al. [Characterization of enterovirus type 71 isolated in Saitama Prefecture in 2000]. *Kansenshogaku Zasshi* 2001;**75**:490–4.
300. Simila S, Jouppila R, Salmi A, et al. Encephaloningitis in children associated with an adenovirus type 7 epidemic. *Acta Paediatr Scand* 1970;**59**:310–6.
301. Singer JI, Maur PR, Riley JP, et al. Management of central nervous system infections during an epidemic of enteroviral aseptic meningitis. *J Pediatr* 1980;**96**:559–63.
302. Sköldenberg B. On the role of viruses in acute infectious diseases of the central nervous system: clinical and laboratory studies on hospitalized patients. *Scand J Infect Dis* 1975;**3**:5–95.
303. Sköldenberg B, Jeansson S, Wolontis S. Herpes simplex virus type 2 and acute aseptic meningitis: clinical features of cases with isolation of herpes simplex virus from cerebrospinal fluids. *Scand J Infect Dis* 1975;**7**:227–32.
304. Socan M, Beovic B, Kese D. *Chlamydia* pneumonia and meningoencephalitis. *N Engl J Med* 1994;**331**:406.
305. Socan M, Ravnik I, Bencina D, et al. Neurological symptoms in patients whose cerebrospinal fluid is culture- and/or polymerase chain reaction-positive for *Mycoplasma pneumoniae*. *Clin Infect Dis* 2001;**32**:E31–5.
306. Sohier R, Chardonnet Y, Prunieras M. Adenoviruses. Status of current knowledge. *Prog Med Virol* 1965;**7**:253–325.
307. Somekh E, Cesar K, Handsher R, et al. An outbreak of echovirus 13 meningitis in central Israel. *Epidemiol Infect* 2003;**130**:257–62.
308. Somekh E, Shohat T, Handsher R, et al. An outbreak of echovirus 11 in a children's home. *Epidemiol Infect* 2001;**126**:441–4.
309. Sosa LE, Gupta S, Juthani-Mehta M, et al. Meningitis in a college student in Connecticut, 2007. *J Am Coll Health* 2009;**58**: 12–4.
310. Southern Jr PM. Relapsing fever. In: Tice F, editor. *Practice of Medicine*, vol. 3, Scranton, Pa: Hoeber Medical Div., Harper & Row; 1969. p. 1–19.

311. Spiegel R, Miron D, Lumelsky D, et al. Severe meningoencephalitis due to late reactivation of varicella-zoster virus in an immunocompetent child. *J Child Neurol* 2010;**25**:87–90.
312. Spillane JD, Wells CE. The neurology of Jennerian vaccination. a clinical account of the neurological complications which occurred during the smallpox epidemic in South Wales in 1962. *Brain* 1964;**87**:1–44.
313. Srihongse S, Grayson MA, Deibel R. California serogroup viruses in New York State: the role of subtypes in human infections. *Am J Trop Med Hyg* 1984;**33**:1218–27.
314. Steel JG, Dix RD, Baringer JR. Isolation of herpes simplex virus type I in recurrent (Mollaret) meningitis. *Ann Neurol* 1982;**11**: 17–21.
315. Stellrecht KA, Harding I, Woron AM, et al. The impact of an enteroviral RT-PCR assay on the diagnosis of aseptic meningitis and patient management. *J Clin Virol* 2002;**25**(Suppl 1): S19–26.
316. Sumaya CV, Corman LI. Enteroviral meningitis in early infancy: significance in community outbreaks. *Pediatr Infect Dis* 1982;**1**: 151–4.
317. Sumaya CV, Ench Y. Epstein-Barr virus infectious mononucleosis in children. I. Clinical and general laboratory findings. *Pediatrics* 1985;**75**:1003–10.
318. Sundelof B, Gnarpe H, Gnarpe J. An unusual manifestation of *Chlamydia pneumoniae* infection: meningitis, hepatitis, iritis and atypical erythema nodosum. *Scand J Infect Dis* 1993;**25**: 259–61.
319. Suzuki N, Terada S, Inoue M. Neonatal meningitis with human parvovirus B19 infection. *Arch Dis Child Fetal Neonatal Ed* 1995;**73**:F196–197.
320. Syrogiannopoulos GA, Grivea IN, Anastassiou ED, et al. Sterile cerebrospinal fluid pleocytosis in young infants with urinary tract infection. *Pediatr Infect Dis J* 2001;**20**:927–30.
321. Syverton JT, McLean DM, Martins Da Silva M, et al. Outbreak of aseptic meningitis caused by coxsackie B5 virus; laboratory, clinical, and epidemiologic study. *JAMA* 1957;**164**:2015–9.
322. Tang YW, Cleavinger PJ, Li H, et al. Analysis of candidate-host immunogenetic determinants in herpes simplex virus-associated Mollaret's meningitis. *Clin Infect Dis* 2000;**30**:176–8.
323. Tavakoli NP, Wang H, Nattanmai S, et al. Detection and typing of enteroviruses from CSF specimens from patients diagnosed with meningitis/encephalitis. *J Clin Virol* 2008;**43**:207–11.
324. Tayal S, Chadwick D, Chawla G. Considering syphilis in aseptic meningitis. *Clin Med* 2009;**9**:626–7.
325. Tedder DG, Ashley R, Tyler KL, et al. Herpes simplex virus infection as a cause of benign recurrent lymphocytic meningitis. *Ann Intern Med* 1994;**121**:334–8.
326. Tena D, Gonzalez-Praetorius A, Lopez-Alonso A, et al. Acute meningitis due to *Brucella* spp. *Eur J Pediatr* 2006;**165**:726–7.
327. Terni M, Caccialanza P, Cassai E, et al. Aseptic meningitis in association with herpes progenitalis. *N Engl J Med* 1971;**285**: 503–4.
328. Thoelen I, Lemey P, Van Der Donck I, et al. Molecular typing and epidemiology of enteroviruses identified from an outbreak of aseptic meningitis in Belgium during the summer of 2000. *J Med Virol* 2003;**70**:420–9.
329. Thoren A, Widell A. PCR for the diagnosis of enteroviral meningitis. *Scand J Infect Dis* 1994;**26**:249–54.
330. Trallero G, Casas I, Avellon A, et al. First epidemic of aseptic meningitis due to echovirus type 13 among Spanish children. *Epidemiol Infect* 2003;**130**:251–6.
331. Tsai HP, Huang SW, Wu FL, et al. An echovirus 18-associated outbreak of aseptic meningitis in Taiwan: epidemiology and diagnostic and genetic aspects. *J Med Microbiol* 2011;**60**:1360–5.
332. Tsai TF, Popovici F, Cernescu C, et al. West Nile encephalitis epidemic in southeastern Romania. *Lancet* 1998;**352**:767–71.
333. Tsiodras S, Kelesidis I, Kelesidis T, et al. Central nervous system manifestations of *Mycoplasma pneumoniae* infections. *J Infect* 2005;**51**:343–54.
334. Tsutsumi H, Kamazaki H, Nakata S, et al. Sequential development of acute meningoencephalitis and transverse myelitis caused by Epstein-Barr virus during infectious mononucleosis. *Pediatr Infect Dis J* 1994;**13**:665–7.
335. Turabelidze G, Lin M, Butler C, et al. Outbreak of echovirus 18 meningitis in a rural Missouri community. *Mo Med* 2009;**106**: 420–4.
336. Tyler KL. Herpes simplex virus infections of the central nervous system: encephalitis and meningitis, including Mollaret's. *Herpes* 2004;**11**(Suppl 2):57A–64A.
337. Ushijima H, Xin KQ, Nishimura S, et al. Detection and sequencing of rotavirus VP7 gene from human materials (stools, sera, cerebrospinal fluids, and throat swabs) by reverse transcription and PCR. *J Clin Microbiol* 1994;**32**:2893–7.
338. Uysal G, Ozkaya E, Guven A. Echovirus 30 outbreak of aseptic meningitis in Turkey. *Pediatr Infect Dis J* 2000;**19**:490.
339. Vieth UC, Kunzelmann M, Diedrich S, et al. An echovirus 30 outbreak with a high meningitis attack rate among children and household members at four day-care centers. *Eur J Epidemiol* 1999;**15**:655–8.
340. Vreede RW, Schellekens H, Zuijderwijk M. Isolation of parainfluenza virus type 3 from cerebrospinal fluid. *J Infect Dis* 1992; **165**:1166.
341. Waespe N, Steffen I, Heininger U. Etiology of aseptic meningitis, peripheral facial nerve palsy, and a combination of both in children. *Pediatr Infect Dis J* 2010;**29**:453–6.
342. Waites KB, Duffy LB, Crouse DT, et al. Mycoplasmal infections of cerebrospinal fluid in newborn infants from a community hospital population. *Pediatr Infect Dis J* 1990;**9**:241–5.
343. Waites KB, Rudd PT, Crouse DT, et al. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of central nervous system in preterm infants. *Lancet* 1988;**1**:17–21.
344. Wallach JC, Baldi PC, Fossati CA. Clinical and diagnostic aspects of relapsing meningoencephalitis due to *Brucella suis*. *Eur J Clin Microbiol Infect Dis* 2002;**21**:760–2.
345. Wallgren A. [Etiology of meningoencephalitis in children, especially the syndrome of acute aseptic meningitis]. *Acta Paediatr* 1951;**40**:541–65.
346. Wallgren A. Une nouvelle maladie infectieuse du système nerveux central? *Acta Paediatr Scand* 1925;**4**:158–82.
347. Wang JR, Tsai HP, Chen PF, et al. An outbreak of enterovirus 71 infection in Taiwan, 1998. II. Laboratory diagnosis and genetic analysis. *J Clin Virol* 2000;**17**:91–9.
348. Wang JR, Tsai HP, Huang SW, et al. Laboratory diagnosis and genetic analysis of an echovirus 30-associated outbreak of aseptic meningitis in Taiwan in 2001. *J Clin Microbiol* 2002;**40**: 4439–44.
349. Wang SM, Ho TS, Shen CF, et al. Echovirus 18 meningitis in southern Taiwan. *Pediatr Infect Dis J* 2011;**30**:259–60.
350. Warren J. Encephalomyocarditis viruses. In: Horsfall FL, Tamm I, editors. *Viral and Rickettsial Infections of Man*. Philadelphia: J. B. Lippincott; 1965. p. 562–8.
351. Weller PF. Eosinophilic meningitis. *Am J Med* 1993;**95**:250–3.
352. Weller TH. Varicella-Herpes zoster virus. In: Evans AS, editor. *Viral Infections of Humans: Epidemiology and Control*. 2nd ed. New York: Plenum Medical; 1982. p. 569–95.
353. Wickman I. *Studien über poliomyelitis acuta: Zugleich ein beitrag zur kenntnis der myelitis acuta: Engl. Trans. Nev. and Ment. Dis. Monog. Ser. No. 16*. Berlin: S. Karger; 1905. p. 1913.
354. Wilfert CM, Lehrman SN, Katz SL. Enteroviruses and meningitis. *Pediatr Infect Dis* 1983;**2**:333–41.
355. Wilfert CM, Thompson Jr RJ, Sunder TR, et al. Longitudinal assessment of children with enteroviral meningitis during the first three months of life. *Pediatrics* 1981;**67**:811–5.
356. Williams CL, Strobino B, Lee A, et al. Lyme disease in childhood: clinical and epidemiologic features of ninety cases. *Pediatr Infect Dis J* 1990;**9**:10–4.
357. Wong CJ, Price Z, Bruckner DA. Aseptic meningitis in an infant with rotavirus gastroenteritis. *Pediatr Infect Dis* 1984;**3**:244–6.
358. Wong JG, Hathaway SC, Paat JJ, et al. Drug-induced meningitis: a case involving trimethoprim-sulfamethoxazole. *Postgrad Med* 1994;**96**:117–8, 121,124.
359. Wong ML, Kaplan S, Dunkle LM, et al. Leptospirosis: a childhood disease. *J Pediatr* 1977;**90**:532–7.
360. Yan JJ, Wang JR, Liu CC, et al. An outbreak of enterovirus 71 infection in Taiwan 1998: a comprehensive pathological, virological, and molecular study on a case of fulminant encephalitis. *J Clin Virol* 2000;**17**:13–22.
361. Yanagihara K, Tanaka-Taya K, Itagaki Y, et al. Human herpesvirus 6 meningoencephalitis with sequelae. *Pediatr Infect Dis J* 1995; **14**:240–2.
362. Yang TT, Huang LM, Lu CY, et al. Clinical features and factors of unfavorable outcomes for non-polio enterovirus infection of the

central nervous system in northern Taiwan, 1994-2003. *J Microbiol Immunol Infect* 2005;**38**:417–24.
363. Yerly S, Gervaix A, Simonet V, et al. Rapid and sensitive detection of enteroviruses in specimens from patients with aseptic meningitis. *J Clin Microbiol* 1996;**34**:199–201.
364. Yildizdas D, Kendirli T, Arslankoylu AE, et al. Neurological complications of pandemic influenza (H1N1) in children. *Eur J Pediatr* 2011;**170**:779–88.
365. Yoshikawa T, Ihira M, Suzuki K, et al. Invasion by human herpesvirus 6 and human herpesvirus 7 of the central nervous system in patients with neurological signs and symptoms. *Arch Dis Child* 2000;**83**:170–1.
366. Young EJ. Human brucellosis. *Rev Infect Dis* 1983;**5**:821–42.
367. Zhao YN, Jiang QW, Jiang RJ, et al. Echovirus 30, Jiangsu Province, China. *Emerg Infect Dis* 2005;**11**:562–7.

associated meningeal inflammation, thus representing an overlap with meningoencephalitis. For the purposes of this chapter, these terms are used interchangeably. The diagnosis of encephalitis can be established only by the microscopic examination of brain tissue or the recovery of a neurotropic agent. In clinical practice, however, the diagnosis is presumptive and frequently based on clinical features.

Encephalitis is often classified as primary or as postinfectious or parainfectious. Primary encephalitis is an illness in which encephalitis is the major manifestation. Symptoms are caused by direct invasion and replication of an infectious agent in the central nervous system (CNS), resulting in objective clinical evidence of cerebral or cerebellar dysfunction. Postinfectious or parainfectious encephalitis occurs after or in combination with other illnesses that are not CNS illnesses or after a vaccine or other product has been administered. Manifestations may be mediated immunologically. Clinical symptoms can result from noninfectious causes and are often indistinguishable from those of infectious encephalitis.

Frequently, when encephalitis occurs, other regions of the nervous system, such as the spinal cord (myelitis), nerve roots (radiculitis), and nerves (neuritis), also are involved. When neurologic clinical findings suggest encephalitis but inflammation of the brain has not occurred (e.g., influenza encephalopathy), the condition is identified by the less-specific term *encephalopathy.*

HISTORY

Rabies encephalitis was recognized in ancient times in Europe and Asia.[224] In AD 100, Celsus noted the relationship of animal rabies to human disease. "Sleeping sickness" associated with epidemic influenza was noted early in the 18th century.[445] For the past 100 years, epizootics of encephalitis in equine animals have been observed in the United States; and in 1933, St. Louis encephalitis virus was isolated from the brains of humans dying of epidemic encephalitis.[303,329] Meningoencephalitis was recognized at the beginning of the 20th century as a complication of mumps.[136] Nonpolio enteroviruses have been known for the past 60 years to be a cause of encephalitis; during the same period, more than 400 zoonotic arthropod-borne viruses have been discovered; and of these, 100 or more cause encephalitis in humans.[33] In countries where vaccines are widely available there has been a decline in vaccine-preventable neurologic complications from measles, mumps, and rubella. However, there has been a continually expanding list of emerging or reemerging pathogens, including *Balamuthia mandrillaris*, *Baylisascaris procyonis*, parechoviruses, Nipah virus, and Chandipura virus. Moreover, there has been expansion of certain agents into geographic regions.[177] West Nile virus, for instance, has expanded its geographic range from Africa to North and South America, Europe, the Middle East, western Asia, and Australia.[184] Japanese encephalitis virus has spread into India, Nepal, and northern Australia.[402] Chikungunya is the most recent example of a striking geographic virus expansion with outbreaks in the Indian Ocean and subsequent spread to India and Europe. Simultaneously, the neurologic complications from Chikungunya appear to be increasing as well.[19]

ETIOLOGY

The many causes of encephalitis include both infectious and noninfectious entities. The infectious causes include viruses, bacteria, fungi, and parasites. Etiologic agents of acute encephalitis, meningoencephalitis, or illnesses that clinically resemble encephalopathy are listed in Table 36-1. Many of the infectious agents or diseases are discussed more fully and are referenced more completely elsewhere in this book (see Index).

Despite extensive testing and evaluation, the causes of many cases of encephalitis remained unexplained in recent prospective studies.[163,247,353] During the first seven years of the California Encephalitis Project, 1570 patients were identified who met the case definition of having encephalitis (immunocompetent patients older than 6 months of age hospitalized with at least 24 hours of encephalopathy and one or more of the following characteristics: fever, seizure, focal neurologic findings, or electroencephalographic [EEG] or neuroimaging findings consistent with encephalitis). A confirmed or probable agent was identified in only 251 (16%) cases; of these agents, 69 percent were viral, 20 percent were bacterial, 3 percent were prion, 3 percent were parasitic, and 1 percent were fungal. An additional 13 percent had a possible cause identified, and a noninfectious cause was identified in 8 percent of cases.[164] Similar percentages of "unknown" causes are seen in studies across the globe—in Australia from 1990 to 2007, 69.8 percent unknown; in England from 1989 to 1998, 60 percent unknown; and in another study in the United States, 60 percent unknown.[112,215,242]

Viruses

Some types of viral encephalitides are an uncommon complication of a relatively common infection, such as enteroviruses and herpesviruses, whereas others are clinical manifestations of newly emerging or rare pathogens such as Hendra virus, lymphocytic choriomeningitis virus, Chikungunya, or rabies.

Enteroviruses and Parechoviruses

Enteroviruses and parechoviruses are small, nonenveloped single-stranded RNA viruses within the Picornaviridae. Encephalitis is a rare complication of enteroviruses, but because enteroviruses are so common and estimated to cause 10 to 15 million illnesses per year in the United States they are a leading cause of encephalitis, particularly in children.[410] Of encephalitis cases with a cause identified, enteroviruses comprise 10 to 15 percent of cases.[308] Enteroviruses are now the leading viral cause of neurologic disease in children in the United States, and

Text continued on p. 499

TABLE 36-1 **Encephalitis (with a Focus on Immunocompetent Patients and Pathogens in the United States)**

Etiology	Association with Encephalitis	Epidemiology	Clinical and Laboratory Hallmarks	Recommended Tests and "Pitfalls"
Viruses				
Adenovirus	Mostly anecdotal data; unclear neurotropic potential	Sporadic; children and immunocompromised persons at greatest risk	Respiratory symptoms common	Viral culture or PCR from respiratory site, CSF, or brain tissue
Eastern equine encephalitis virus	Proven neurotropic potential, but uncommon	Atlantic and Gulf U.S. states	Subclinical to fulminant; 50%-70% mortality	Serology
Enteroviruses (include coxsackieviruses and enterovirus 71)	Most common cause of encephalitis in pediatric population	Highest incidence in late summer and early fall but can occur year round; large outbreaks of enterovirus 71 infection in Asia have occurred.	Aseptic meningitis most common but also encephalitis; hand, foot and mouth rash may be present; enterovirus 71 can cause rhomboencephalitis.	CSF PCR single best test but not always sensitive; to increase sensitivity of detection add serum/plasma, throat PCR or culture.
Epstein-Barr virus	Relatively common	During acute infection	Infectious mononucleosis during acute infection, cerebellar ataxia, sensory distortion ("Alice-in-Wonderland" syndrome)	Serology and CSF PCR. Beware of PCR false-positive results (detection of low levels may represent latent infection) and false-negative results (not all cases are CSF positive).
Hendra virus	Less common	Endemic in Australia; associated with equine exposure	Nonspecific	Contact local health department or Special Pathogens Branch at CDC.
Hepatitis C	Mostly anecdotal data; unclear neurotropic potential; neurologic symptoms may be related to vasculitis.	Hepatitis C–seropositive patients		CSF PCR
Herpes B virus	Proven neurotropic potential; rare	Transmitted by bite of Old World macaque	Vesicular eruption at site of bite followed by neurologic symptoms, including transverse myelitis	Culture and PCR of vesicles, CSF; contact CDC or Dr. Julia Hilliard.
Herpes simplex virus (HSV) types 1 and 2	Relatively common	HSV type 1 accounts for 5%-10% of encephalitis; typically a reactivation disease, HSV type 2 occurs in neonates.	Temporal lobe seizures (apraxia, lip smacking), olfactory hallucinations, behavioral abnormalities, but children often have extratemporal lesions as well	CSF PCR single best test, but false-negative results can occur; if HSE strongly suspected, repeat lumbar puncture in a few days and recheck HSV PCR and intrathecal antibodies.
Human herpes virus–6	Unknown, especially owing to difficult interpretation of CSF PCR false-positive results	Young children (≤2 yr) or immunocompromised patients, particularly bone marrow transplant recipients	May be associated with "roseola rash"	CSF PCR. Beware of false-positive findings due to chromosomal integration and latent infections.
Human metapneumovirus	Anecdotal evidence only	Newly described; almost exclusively in children	Often with associated respiratory symptoms	Respiratory tract PCR (most CSF PCR negative)
Influenza virus	Unclear neurotropic potential; good data to support flu-associated encephalopathy but unclear if encephalitis and unclear mechanism	Neurologic complications occur; sporadically reported during influenza seasons; higher numbers reported in Japan and Southeast Asia	Upper respiratory tract symptoms CSF often acellular in 10% with bilateral thalamic necrosis	Respiratory tract culture, PCR, or rapid antigen. CSF and brain PCR infrequently positive

Japanese encephalitis virus	Relatively common (but only in endemic areas)	Mosquito-borne; most common worldwide cause of encephalitis; endemic throughout Asia; vaccine preventable	Seizures, parkinsonian features, acute flaccid paralysis variably seen. MRI classically shows thalamic and basal ganglia involvement	CSF and serum antibodies
La Crosse virus	Relatively common	Mosquito-borne; endemic in U.S. East and Midwest; highest incidence in school-aged children	Varies from subclinical illness to seizures and coma	CSF and serum antibodies
Lymphocytic choriomeningitis virus	Rare	Highest incidence in fall and winter; rodent exposure	One of few viral causes of hypoglycorrhachia	Serology
Measles virus	Less common in countries where vaccine is routinely used	Vaccine preventable; measles inclusion body encephalitis onset 1-6 mo after infection; SSPE can manifest >5 years after infection.	Measles encephalitis nonspecific. SSPE has a subacute onset with progressive dementia, myoclonus, seizures, and, ultimately, death. EEG changes are often diagnostic.	Acute form: measles IgM SSPE: measles IgG in CSF and serum
Mumps virus	Less common	Vaccine preventable; used to be leading cause of encephalitis-meningitis, now rarely seen	Parotitis, orchitis, hearing loss; one of few viral causes of hypoglycorrhachia	Serology, throat swab PCR, CSF culture or PCR
Murray Valley encephalitis virus	Less common	Highest incidence in Aboriginal children in Australia and New Guinea	Nonspecific presentation; case fatality 15%-30%	Serology (may cross react with other flaviviruses)
Nipah virus	Less common	Epidemics in Southeast Asia; contact with pigs	Myoclonus, dystonia, pneumonitis	Serology (Special Pathogens Branch, CDC)
Parainfluenza 1-4	Unknown neurotropic potential; anecdotal evidence	Worldwide	Associated with respiratory symptoms	Respiratory DFA or PCR; CSF PCR rarely positive
Parechoviruses	Proven neurotropic potential but frequency unknown	Children <3 yr	In young infants periventricular white matter changes resemble hypoxic ischemic encephalopathy.	Parechovirus PCR (enterovirus PCR will not detect)
Parvovirus B19	Anecdotal evidence only	Sporadic cases	Variably associated with rash	IgM antibody, CSF PCR
Powassan virus	Less common	Tick-borne; endemic to New England, Canada	Nonspecific	Serology
Rabies virus	Uncommon in developed countries; relatively common in Africa, Asia, South America	Vaccine preventable; most common vector is bat (bites often unrecognized); dogs important source in developing countries; worldwide distribution	Paresthesia at site of bite. Furious form: hydrophobia, agitation, delirium, autonomic instability, coma Paralytic form: ascending paralysis in 30% Two forms sometimes overlap.	Multiple tests and assays needed for antemortem testing: antibodies (serum, CSF), PCR of saliva or CSF, IFA of nuchal biopsy or CNS tissue. Coordinate testing with health department.
Rotavirus	Correlation with seizures in young child but unclear association with encephalitis	Typically children; winter; vaccine preventable	Usually with diarrhea	Stool antigen, CSF PCR (CDC)

Continued on following page

TABLE 36-1 **Encephalitis (with a Focus on Immunocompetent Patients and Pathogens in the United States)** (Continued)

Etiology	Association with Encephalitis	Epidemiology	Clinical and Laboratory Hallmarks	Recommended Tests and "Pitfalls"
Rubella virus	Less common in countries where vaccine is routinely used	Vaccine preventable	Neurologic findings typically occur at same time as rash and fever	Serology, CSF antibodies
St. Louis encephalitis virus	Relatively common	Mosquito-borne; endemic to western U.S.; occasional outbreaks in central/eastern U.S.; highest incidence in adults >50 yr	Tremors, seizures, paresis, urinary symptoms, SIADH variably present	Serology (cross reacts with other flaviviruses)
Tick-borne encephalitis virus	Relatively common in affected geographic areas	Vaccine-preventable; transmitted via tick or ingestion of unpasteurized milk; endemic to Asia, Europe and areas of former Soviet Union	Weakness ranging from mild paresis to acute flaccid paralysis	Serology
Vaccinia	Less common	Primarily associated with vaccination	Vaccinia rash (localized or disseminated)	CSF antibodies, serum IgM (natural infection)
Venezuelan equine encephalitis virus	Less common	Central and South America; sometimes in U.S. border states (Texas, Arizona)	Myalgias, pharyngitis, upper respiratory tract infection variably present	Serology, viral cultures (blood, oropharynx), CSF antibody
Varicella zoster virus	Relatively common	Acute infection (chickenpox) or reactivation (shingles)	Vesicular rash (disseminated or dermatome), cerebellar ataxia, large vessel vasculitis	DFA or PCR of skin lesions, CSF PCR, serum IgM (acute infection)
Western equine encephalitis virus	Less common	Onset in summer and early fall; western U.S. and Canada, Central and South America	Nonspecific	Serology
West Nile virus	Relatively common	Mosquito-borne; emerging cause of epidemic encephalitis in U.S., Europe; endemic in Middle East; highest incidence in adults >50 yr; documented transmission through organ and blood	Weakness and acute flaccid paralysis, tremors, myoclonus, parkinsonian features; MRI shows basal ganglia and thalamic lesions.	CSF IgM, serum IgM/IgG, paired serology (cross reactivity with West Nile virus and SLE)
Bacteria				
Bartonella henselae (and other *Bartonella* spp.)	Relatively common	Often occurs after scratch or bite from kitten	Encephalopathy with seizures (often status epilepticus); peripheral lymphadenopathy. CSF is usually paucicellular.	Serology (acute usually diagnostic), PCR of lymph node; CSF PCR rarely positive
Borrelia burgdorferi	Less common	Tick-borne infection; in U.S. mostly in New England and eastern Mid-Atlantic states	Facial nerve palsy (often bilateral), meningitis, radiculitis; may be associated with or follow erythema migrans rash.	Serology (serial EIA and Western blot), CSF antibody index, CSF PCR
Chlamydia spp.	Anecdotal evidence only	Associated with *C. psittaci* and *Chlamydophila pneumoniae*	Often with associated respiratory symptoms	NP swab, respiratory, or CSF PCR
Coxiella burnetti	Less common	Animal exposures, particularly placenta and amniotic fluid	Flulike symptoms	Serology

Ehrlichia /Anaplasma	Relatively common	Tick-borne bacteria causing human monocytic and human granulocytic ehrlichiosis (HME, HGE) respectively; HME endemic to southern and central U.S.; HGE endemic to northeastern U.S. and Midwest	Acute onset of fever and HA. Rash seen in <30% of cases. Leukopenia, thrombocytopenia, and elevated LFTs frequent manifestations	Morulae in white blood cells, PCR of whole blood, serology (seroconversion may occur several weeks after symptoms)
Mycoplasma pneumoniae	One of most frequently identified agents in case series but mostly anecdotal evidence	Worldwide distribution	Respiratory symptoms variably present, but pneumonia rare. Often with white matter involvement consistent with ADEM	PCR of NP swab or respiratory culture, serum IgM; CSF PCR rarely positive
Mycobacterium tuberculosis	Relatively common	Most common in developing countries; disease of very young and very old or immunocompromised	Subacute basilar meningitis, lacunar infarcts, hydrocephalus. CSF often with low glucose, high protein levels. Pulmonary findings often associated	CSF AFB smear, culture, PCR, respiratory cultures highly suggestive
Rickettsia rickettsii	Relatively common in affected geographic areas	Tick-borne infection in North America; highest incidence in southeast and south central U.S.	Acute onset of fever and headache. Petechial rash in 85% of cases beginning 3 days after onset of symptoms	Serology (seroconversion may occur several weeks after symptoms), PCR or IHC on skin biopsy of rash
Treponema pallidum	Rare (especially in pediatrics)	Sexually transmitted disease; meningoencephalitis in early disseminated disease; progressive dementia in late disease	Protean manifestations including temporal lobe focality (mimics HSV), general paresis, psychosis, dementia	CSF VDRL (sensitive but not specific), serum RPR with confirmatory FTA-ABS
Tropheryma whippelii	Rare (especially in pediatrics)		Progressive subacute encephalopathy, oculomasticatory myorhythmia pathognomonic. Variable enteropathy, uveitis	CSF PCR, PAS-positive cells in CSF, small bowel biopsy
Protozoa				
Acanthamoeba spp.	Less common; more common in immunocompromised	Worldwide, inhalation of wind-blown soil	Subacute progressive	Contact CDC/Parasitology.
Balamuthia mandrillaris	Less common	Worldwide (but most case reports in United States and South America), inhalation of wind-blown soil	Subacute progressive disease characterized by space-enhancing lesions, often with cranial nerve palsies and hydrocephalus (similar to tuberculosis)	Serology (research laboratories), brain histopathology, CDC laboratories Contact CDC/Parasitology for testing.
Naegleria fowleri	Less common	Summer; swimming or diving in brackish water or poorly chlorinated pools	Anosmia, progressive obtundation; CSF resembles bacterial meningitis, but sterile.	Mobile trophozoites on wet mount of warm CSF, brain histopathology

Continued on following page

TABLE 36-1 **Encephalitis (with a Focus on Immunocompetent Patients and Pathogens in the United States)** (Continued)

Etiology	Association with Encephalitis	Epidemiology	Clinical and Laboratory Hallmarks	Recommended Tests and "Pitfalls"
Toxoplasma gondii	Rare in normal hosts	Worldwide; cats definitive hosts but humans often infected via consumption of undercooked meats, unwashed produce		
Helminths				
Angiostrongylus cantonensis	Most common cause of eosinophilic meningitis worldwide; rare in United States	In the U.S. in Louisiana and Hawaii; South Pacific, Asia, Australia and Caribbean	Meningitis or encephalitis; eosinophils in CSF; also associated with eosinophilic pneumonitis	Identification of worm in tissues
Baylisascaris procyonis	Less common	North America, Europe, and Asia; pica, particularly near raccoon latrines	Obtundation, coma; significant CSF and peripheral eosinophilia	CSF and serum antibodies; contact CDC/Parasitology for testing.
Gnasthostoma spinigerum	Relatively common in affected geographic areas	Southeast Asia, some areas of South/Central America; undercooked freshwater fish, chicken, or pork; also reported with reports of ingestion of frogs/snakes	Eosinophilic myeloencephalitis; can cause intermittent symptoms for 10-15 years because larvae are long lived	Identification of worm in tissues
Fungi				
Coccidioides spp.	Relatively common	Southwest U.S., northern Mexico, areas of Central and South America	Neurologic manifestations are result of disseminated disease; more often meningitis than encephalitis. CSF eosinophils sometimes seen	CSF fungal culture (but need to alert lab); CSF and serum antigen and antibody. EDTA-heat–treated antigen increases sensitivity of CSF and serum.
Histoplasma capsulatum	Relatively common	Eastern and central U.S., especially Mississippi, Ohio and Missouri River valleys. Grows on mold, bird, and bat droppings; especially found in caves, barns, or excavation areas	Neurologic manifestations are result of disseminated disease.	CSF fungal culture; CSF and serum antigen and antibody. EDTA-heat–treated antigen increases sensitivity of CSF and serum. Urine antigen
Blastomyces dermatitidis	Relatively common	Southeast, central, and midwestern U.S.; also in Canada, Africa, and India	Neurologic manifestations are result of disseminated disease.	CSF fungal culture; CSF and serum antigen and antibody. EDTA-heat–treated antigen increases sensitivity of CSF and serum.

ADEM, acute demyelinating encephalomyelitis; *AFB,* acid-fast bacilli; *CDC,* Centers for Disease Control and Prevention; *CNS,* central nervous system; *CSF,* cerebrospinal fluid; *DFA,* direct fluorescent antibody test; *EDTA,* ethylenediaminetetraacetic acid; *EEG,* electroencephalogram; *EIA,* enzyme immunoassay; *FTA-ABS,* fluorescent treponemal antibody absorption test; *HA,* hemagglutination assay; *IFA,* indirect fluorescent antibody test; *IHC,* immunohistochemistry; *LFTs,* liver function tests; *MRI,* magnetic resonance imaging; *NP,* nasopharyngeal; *PAS,* periodic acid–Schiff; *PCR,* polymerase chain reaction; *RPR,* rapid plasma reagin; *SIADH,* syndrome of inappropriate secretion of antidiuretic hormone; *SLE,* systemic lupus erythematosus; *SSPE,* subacute sclerosing panencephalitis; *VDRL,* Venereal Disease Research Laboratory.

they are a major cause of encephalitis.* Of the 1750 patients with encephalitis enrolled in the California encephalitis project, enteroviruses were the leading causative agent.[175] Several enterovirus types have been associated with encephalitis: coxsackieviruses A2, A4 to A7, A9, A10, A16, A21 and B1 to B5; echoviruses 1 to 9, 11 to 25, 27, 30, and 33; and enteroviruses 71, 75, 76, and 89. In 1998, an extensive epidemic of enterovirus 71 disease occurred in Taiwan.† Outside the United States, outbreaks of enterovirus 71 have been observed, often with neurologic manifestations and relatively high morbidity and mortality.[277] Manifestations of illness in this epidemic varied, and many children had severe neurologic events, including meningitis, encephalitis, cerebellitis, and polio-like syndrome. In particular, numerous children had brain stem encephalitis, with many fatalities occurring.

The first two parechoviruses characterized, formerly echovirus 22 and echovirus 23, were originally described as enteroviruses. To date, 16 genotypes of parechovirus have been characterized.[92] As a group, parechoviruses can cause similar clinical diseases as enteroviruses, including respiratory, gastrointestinal, and sepsis-like syndromes and CNS infections. Neonates and young children have more severe clinical disease than older children. Parechoviruses 1 and 2 are most often associated with respiratory and gastrointestinal infections, but acute flaccid paralysis and encephalitis have occasionally been associated with parechovirus 1 (when formerly classified as echovirus 22).[140] Parechovirus 3 has been associated with sepsis, meningitis, and encephalitis and during the neonatal period has been associated with white matter changes, often suggestive of hypoxic-ischemia injuries.[43,188,195,437] The relative frequency of parechovirus infections is unknown, especially in neurologic illnesses, because these viruses are often difficult to grow and molecular testing for enteroviruses would not have detected parechoviruses.

Herpesviruses

Neurologic manifestations of herpesviruses are uncommon complications, but, like enterovirus infections, herpesvirus infections are common. Indeed, as a group the herpesviruses are among the most frequently identified agents responsible for viral encephalitis.[172,215,287,432] Herpes simplex encephalitis is the most common cause of sporadic fatal encephalitis in the United States, with approximately one third of cases occurring in patients younger than age 20 years but older than 6 months and with approximately half occurring in patients older than age 50 years.[303,366,431,451] Molecular analyses of paired oral or labial and brain sites have indicated that herpes simplex encephalitis can be the result of a primary infection, a reactivation of latent herpes simplex virus, or a reinfection by a second herpes simplex virus.[453] Although herpes simplex virus type 2 is a leading cause of severe and frequently fatal encephalitis in neonates,[452] type 1 is the usual cause in older children.* Meningitis is the most frequent neurologic sequela of herpes simplex virus type 2 genital infection outside the neonatal period but rarely can cause encephalitis.

Other herpesviruses, including varicella-zoster virus, Epstein-Barr virus, cytomegalovirus, and human herpesvirus–6, can also cause encephalitis.[370] Encephalitis can occur in association with primary infection with varicella-zoster virus (chickenpox) or with endogenous recurrent disease (herpes zoster).† In a study in Finland of more than 3000 patients with acute CNS infections of suspected viral origin, varicella-zoster virus constituted 29 percent of all confirmed or probable etiologic agents.[251] In chickenpox, the rate of encephalitis is approximately 0.3 per 1000 cases[61] and the case-fatality rate is approximately 17 percent.[346] Of patients with herpes zoster, 0.5 to 5 percent have encephalitis.[223] This complication occurs more commonly in immunocompromised patients.

Epstein-Barr virus encephalitis occurs in less than 1 percent of cases, and most patients with Epstein-Barr virus encephalitis are adolescents and young adults. Patients typically present 1 to 3 weeks after the onset of mononucleosis syndrome, but encephalitis may be the presenting complaint in Epstein-Barr virus infection.‡ Caruso and associates[57] reported five children with subacute and chronic neurologic deficits associated with apparent primary Epstein-Barr virus infections. Severe chronic involvement of the brain is a common finding in congenital cytomegalovirus infection.[191] Encephalitis caused by acquired cytomegalovirus infection is uncommon and usually occurs in immunocompromised children.[180,203,393]

Human herpesvirus–6 is an important cause of acute febrile illness and roseola infantum in young children and is commonly associated with febrile convulsions.[133] Encephalitis is a rare complication of this infection in children with and without roseola.§ Human herpesvirus–6 increasingly has become recognized as an important cause of encephalitis in immunocompromised, posttransplant patients, occasionally manifesting as severe amnesia after they have undergone bone marrow transplantation.¶ Human herpesvirus–7 has also been implicated as a causative agent in encephalitis.[331,344,424,444]

Arboviruses

More than 400 different arboviruses exist, and detailed information about illnesses caused by specific types in various areas of the world is provided in Chapters 171, 174, 175, 189, and 190.[33,203,234] Arboviruses that are endemic to the United States and have been associated with encephalitis include eastern equine, western equine, Venezuelan equine, St. Louis, Powassan, West Nile, California serogroup viruses including La Crosse and Jamestown canyon, and Colorado tick fever. Historically,

*References 30, 63, 71, 72, 94, 118-120, 129, 181, 203, 233, 238, 251, 264, 303, 308, 311, 322, 339.
†References 85, 204, 209, 276, 281, 442, 458, 459.

*References 94, 217, 246, 293, 306, 344, 355, 434.
†References 1, 54, 98, 110, 135, 198, 221, 252, 353, 394.
‡References 102, 108, 127, 203, 223, 275, 431.
§References 10, 59, 105, 190, 217-219, 294, 325, 464.
¶References 45, 93, 100, 153, 286, 288, 375, 379, 397, 438.

arboviruses have been cited as one of the most common causes of encephalitis; however, recent studies found arboviruses to be relatively uncommon in the pediatric age group in the United States.[164,242]

First detected in the Western Hemisphere in 1999 in New York City, West Nile virus subsequently spread across North America from the Atlantic to the Pacific coasts and into Canada and Mexico.* Between 1999 and 2008, almost 29,000 cases of West Nile virus were reported in the United States, with more than 1,100 deaths.[200,279] An estimated 1 in 150 infections results in severe neurologic illness, and, although the incidence of neuroinvasive disease increases with age, West Nile virus has been responsible for encephalitis in young children and adolescents, occurring in 37 percent of the 443 pediatric cases diagnosed in the United States between 1999 and 2007.[97,149,199,278] Although West Nile virus was the most common cause of neuroinvasive arboviral disease in the United States in 2010, the most common cause among children was La Crosse virus.[81,189,333,360,380] Of all North American causes of arboviral encephalitis, eastern equine encephalitis virus results in the most severe disease and has the highest case-fatality rate, particularly among infants and children.[365]

Outside the United States, dengue is the most common mosquito-borne viral disease in the world, responsible for 50 to 100 million infections and 25,000 deaths each year.[162] In 2009 and 2010, 89 cases of dengue virus occurred in Key West, Florida.[78,171] With the exception of a few cases that occurred along the Texas-Mexico border, the cases in Florida represented the first local transmission of dengue virus in the continental United States since 1946.[67,68] Although encephalopathy is a well-reported neurologic complication of dengue fever, there has been increasing evidence that encephalitis with direct neuronal infiltration by dengue virus occurs.[46,436] Japanese encephalitis virus, also a mosquito-borne flavivirus, although not quite as common as dengue, is the most important cause of epidemic encephalitis worldwide, with an estimated 35,000 to 50,000 cases and 10,000 deaths annually.[400,402,428] Most cases occur in Southeast Asia, China, and the Indian subcontinent.[401]

Chikungunya, a mosquito-borne arbovirus of the Togaviridae, was first isolated in 1953 and has caused outbreaks in sub-Saharan Africa, India, and Southeast Asia.[342,433] In 2005 to 2006, an outbreak occurred on the island of Réunion, a French territory in the Indian Ocean involving 266,000 cases, which represented more than one third of the island's population. The epidemic subsequently spread to the Indian subcontinent, where 1.4 million cases were reported in 2006. Although neurologic features due to Chikungunya are rare, encephalitis has been diagnosed in the United States among travelers returning from endemic areas.[74,261,363,396] Tick-borne encephalitis, as the name implies, is transmitted by ticks and is caused by a group of tick-borne enteroviruses in the Flaviviridae. Within this group there are subtypes, including European or Western tick-borne encephalitis virus, Siberian tick-borne encephalitis virus, and Russian Spring-Summer encephalitis virus (a.k.a. Far Eastern tick-borne encephalitis virus). These viruses are found in Asia, parts of Europe, and areas of the former Soviet Union.

Vaccine-Preventable Viruses

Prior to the widespread availability of vaccines in the United States, measles, mumps, and rubella were a common cause of encephalitis. Although still occurring elsewhere in the world, including Europe,* measles is now a relatively rare occurrence in the United States.[76] Approximately 30 million cases of measles occur each year worldwide. Encephalitis occurs uncommonly, at a rate of 0.74 per 1,000 cases of measles; the case-fatality rate is 14 percent. Subacute sclerosing panencephalitis, an indolent and progressive form of encephalitis, generally occurs many years after the initial infection. Before the use of mumps vaccine became widespread, this virus was the leading cause of meningoencephalitis in the United States. Today, mumps is rare, although extensive mumps outbreaks with associated cases of encephalitis occurred in 2006.[73] The incidence of encephalitis among individuals with mumps is approximately 3 per 1,000 cases; the case-fatality rate is 1.4 percent.[65] Neurologic involvement is a common manifestation of congenital rubella virus infection,[192] and encephalitis is a rare complication of noncongenital disease.[84,186,267,306] Some data suggest a rate of encephalitis in rubella between 1 per 5,000 and 1 per 10,000 cases.[63] In the prevaccine era, the encephalitis rate in one epidemic in 1964 was 1 per 5,000 cases, and in another epidemic in 1942, it was 1 per 6,000 cases.[290,388] Smallpox (variola virus infection), before its worldwide eradication, was a rare cause of encephalitis. Human rabies is uncommon in the United States, but approximately 55,000 cases occur worldwide primarily in Asia, Africa, and Latin America.[243] Since the early 1970s, approximately two cases of rabies have occurred per year in the United States and approximately 50 percent of the cases have occurred in children and teenagers.[8,64,66]

Rare and/or Newly Emerging Viruses

Lymphocytic choriomeningitis virus is an arenavirus acquired from infected rodents. It was previously a relatively common cause of encephalitis in the United States but now is rarely recognized, except for occasional outbreaks.[35] Clusters of cases of lymphocytic choriomeningitis have been identified among recipients of solid organ transplants in 2003 and 2005.[144] Encephalitis caused by herpesvirus B is rare; it occurs predominantly in monkey handlers and usually after monkey bites.[214] During the 2003 monkeypox outbreak in the midwestern United States, one child developed severe acute encephalitis and seizures; no additional encephalitis cases were identified.[381]

Nipah virus, a new paramyxovirus, is the first widescale epizoonotic encephalitis with direct animal-to-human, rather than vectorial, transmission.[87,283] The initial outbreak of Nipah virus encephalitis occurred among pig farmers in Malaysia and Singapore in 1999,

*References 50, 69, 137, 213, 289, 316, 341.

*References 15, 80, 141, 142, 314, 350, 460.

and subsequently outbreaks have been identified in Bangladesh and India.* A fatal case of encephalitis caused by Hendra virus (equine morbillivirus), another novel paramyxovirus, was reported in an adult[324]; previously, this virus had been noted in association with fatal respiratory tract infections in horses and humans. In 2003, another emerging pathogen, Chandipura virus, was responsible for a large outbreak of acute encephalitis in 329 children in southern India, with a case-fatality rate of 56 percent.[1,354] This rhabdovirus, transmitted to humans by sandflies, was responsible for a second outbreak in western India in 2004, with an even higher case-fatality rate of 78 percent.[83]

Bacteria

Patients with acute bacterial meningitis can sometimes have signs and symptoms that are indistinguishable from those of encephalitis. For example, drowsiness, coma, convulsions, and mental confusion commonly occur in *Haemophilus influenzae*, *Neisseria meningitidis*, and *Streptococcus pneumoniae* bacterial meningitides. Spirochetal infections are more common causes of nervous system disease, specifically encephalitis, than generally is appreciated. Encephalitis is a recognized complication of leptospirosis, Lyme disease, and relapsing fever.† *Brucella* spp. are infrequent causes of meningoencephalitis.[305] *Bartonella henselae* encephalopathy is an uncommon complication of cat-scratch disease.‡ Infection with *Listeria monocytogenes* has been shown to mimic herpetic and West Nile virus encephalitis.[106,107,347]

CNS involvement has been described in most forms of rickettsial infections. Rickettsial diseases include infections caused by organisms of the genera *Rickettsia* (spotted fever rickettsioses, typhus fever group), *Orientia* (scrub typhus), *Coxiella* (Q fever), *Ehrlichia*, and *Anaplasma*. In particular, neurologic involvement commonly occurs in Rocky Mountain spotted fever.[201,236] In one study, two thirds of the ill children had evidence of encephalitis. Neurologic sequelae are common.[170] Neurologic involvement also occurs in non–spotted fever rickettsial infections but is generally not as severe.§ *Coxiella burnetii*, *Ehrlichia canis*, *Rickettsia typhi*, *Rickettsia canada*, and *Rickettsia conorii* all have been implicated.

Parasites and Free-Living Amoebae

In the United States, *Baylisascaris procyonis* has been associated with severe and often fatal eosinophilic encephalitis in children.[70,96,156,157,456] *B. procyonis* is a common roundworm in raccoons, and children typically acquire the infection by ingestion of raccoon feces. Outside the United States, parasitic causes of eosinophilic meningitis/encephalitis include *Toxocara* spp., *Gnasthostoma spinigerum*, *Angiostrongylus cantonensis*, and neurocysticercosis.

Protozoan parasites associated with encephalitis include *Plasmodium falciparium* and *Toxoplasma gondii*, and the reader is referred to Section XXII, Parasitic Diseases, for a complete review. Cerebral malaria is a common complication of *P. falciparum* infection. Meningoencephalitis and enlarging cerebral mass lesions rarely occur in acute acquired toxoplasmosis.[426]

The free-living amoebae that cause human disease are divided into two major categories: primary amoebic meningoencephalitis and granulomatous amoebic encephalitis. *Naegleria fowleri* affects immunocompetent hosts; *Acanthamoeba* predominantly affects immunocompromised hosts, and *Balamuthia* is found in both host groups. All three pathogens are found in soil and water. Primary amoebic meningoencephalitis is caused by the free-living amoeba *N. fowleri* and is a rare, but usually fatal, cause of encephalitis.* Most cases occur in children and young adults and are caused by swimming or playing in contaminated water. Granulomatous amoebic encephalitis, caused by *Acanthamoeba* or *B. mandrillaris*, can be subacute or chronic. Although formerly thought to be innocuous, neurologic infections with *Acanthamoeba* and *Balamuthia* are often fatal.

Fungi

Fungal CNS infections often present as meningitis or brain abscesses, but in some instances patients have encephalitis-like symptoms. These illnesses occur most often in immunocompromised patients, but some infections occur in apparently normal individuals. Meningitis and brain abscess are the most common pathologic events, but encephalitis is associated commonly with meningitis. The following fungal agents are the most common causes of meningoencephalitis in children and adults with normal immunologic status: *Blastomyces dermatitidis*, *Coccidioides immitis*, *Cryptococcus neoformans*, *Cladosporium* spp., *Histoplasma capsulatum*, and *Paracoccidioides brasiliensis*.[373]

Other Putative Agents of Encephalitis

Many other infections have been associated with encephalitis, but their relevance is unclear. This is particularly true for agents with an unclear neurotropic potential, especially when the infection is identified only outside the CNS.

Although encephalitis associated with *Mycoplasma* is identified with increasing frequency,† the true role of *Mycoplasma* in encephalitis is unknown. In most case reports and case series in which *Mycoplasma pneumoniae* is identified as a cause, there is evidence of acute infection by serology and/or respiratory tract specimens.[38,95] The significance of these findings is unclear given the high background incidence of infection. Furthermore, serologic assays for *M. pneumoniae* are notoriously problematic.[29] Similar issues arise with other agents that have been associated with encephalitis cases, including parvovirus B19, parainfluenza virus, respiratory syncytial virus,

*References 31, 83, 167, 194, 206-208, 260, 270, 310, 416, 417.
†References 36, 49, 126, 335, 340, 403.
‡References 55, 148, 159, 301, 302, 321, 406, 465.
§References 131, 168, 254, 280, 336, 355, 357, 386.

*References 41, 58, 75, 124, 160, 176, 202, 231, 237, 241, 258, 291, 338, 378, 392, 395, 439.
†References 37-39, 60, 95, 101, 113, 114, 128, 187, 248, 277, 304, 345, 389, 399, 423, 427, 429, 462.

Chlamydia, adenovirus, rotavirus, human metapneumovirus, and hepatitis B and C viruses.*

Although the association of influenza and neurologic manifestations is probably better documented than the aforementioned agents, the mechanism by which influenza causes neurologic illness is not well understood. Neurologic complications associated with influenza include Guillain-Barré syndrome, Reye syndrome, myelitis, and encephalopathy/encephalitis.†

Evidence of neurologic invasion by influenza is almost never seen, and these cases are most accurately described as an "encephalopathy" rather than an encephalitis. Influenza-associated encephalopathy is more commonly described in children than in adults and is typically characterized by a rapidly progressive encephalopathy. Although particularly notable during 1997 to 2001 influenza A epidemics in Japan, cases of encephalitis and encephalopathy associated with influenza A and influenza B also have been described in North America and Europe.[313,317,412,421,443,463] Similarly, the 2009 influenza A H1N1 pandemic was associated with encephalitis as well as acute necrotizing encephalopathy.‡

Neurologic disease is a common reported complication of pertussis. A 10-year study in the United States indicated a rate of neurologic disease in infants of approximately 9 per 1000 cases.[138] An extensive review by Miller and associates[161] suggested that the neurologic disease occurring with pertussis rarely is inflammatory and it is better classified as an encephalopathy.

Postimmunization Encephalitis

Neurologic disease, including encephalitis and meningoencephalitis, has been reported after various immunizations, but establishing causality is difficult. Depending on the type of immunizing agent, the encephalitis can be the result of an immunologic reaction, a CNS infection with the vaccine virus (e.g., with live vaccines), or a combination of infection and immunologic reaction. Historically, many of the observed neurologic reactions occurred after the administration of antisera prepared in animals in the treatment of specific diseases. Antisera to the following diseases or infectious agents have been noted in association with neurologic illness: tetanus, diphtheria, scarlet fever, tuberculosis, gas gangrene, pneumococcus, gonococcus, meningococcus, and streptococci.[4,212,239,306,364] Of 100 neurologic syndromes complicating administration of serum reviewed by Miller and Stanton,[306] only 10 percent were of a cerebral or meningeal type.

Neurologic disease was a common complication of rabies vaccine derived from animal nervous tissue.[306] The incidence of complication was between 3 per 1000 and 1 per 6000 cases.[40,306] Approximately 10 percent of the neurologic disease attributed to this rabies vaccine was meningoencephalitic or encephalomyelitic. Five cases of CNS disease (Guillain-Barré syndrome, demyelination, meningoradiculitis) have been reported in temporal association with the administration of human diploid cell rabies vaccine.[343] This occurrence is so rare that a causal relationship with vaccine is uncertain.

Encephalitis was an important complication of smallpox vaccination.* The rate of encephalitis varied markedly from one study to another, from 1 in 4000 primary vaccinations in the Netherlands[315] to approximately 1 in 80,000 primary vaccinations in the United States.[266] After reinstatement of the vaccination among military personnel and selected civilian groups in the United States in 2002, cases of suspected encephalitis or myelitis have been identified.[300,383,435]

Neurologic disease, including encephalitis, occurs rarely after the administration of typhoid-paratyphoid vaccine.[306] Neurologic disease also rarely has been attributed to administration of tetanus toxoid and diphtheria toxoid, but the manifestations seldom are central.

Encephalitis and encephalopathy have been observed after the administration of influenza immunization.[158,175,367,457] In the extensive surveillance that occurred in the United States during the period October 1, 1976, to December 16, 1976, when 45,651,113 people received the A/New Jersey/76 influenza vaccine, no epidemiologic evidence of an association between vaccine and encephalitis was noted.[185] Two cases in children who developed acute disseminated encephalomyelitis after receiving Japanese B encephalitis vaccination have been reported.[326]

Neurologic disease developing after administration of a whole-cell pertussis vaccine has also been reported but is controversial.[89-91] Pathologic evidence in fatal cases suggests encephalopathy rather than encephalitis.[104] Because neurologic illness similar to that which occurs after the administration of pertussis vaccination is a frequent development in infants who have not been vaccinated, establishing a true rate of pertussis vaccine encephalopathy, or that such an entity exists at all, has been difficult. The analysis of studies suggests that encephalopathy caused by pertussis vaccine does not occur.[89-91,179,365]

Neurologic disease, including encephalitis, is a rare complication after measles immunization.[48,61,147,263] The rate in vaccinees in the United States is less than 1 per 1 million. In contrast, the finding in the National Childhood Encephalopathy Study in England, Scotland, and Wales indicated a rate of 1 per 87,000 immunizations.[2] This high rate of encephalopathy may be an artifact due to the misclassification of complicated febrile convulsions as encephalopathy. Meningoencephalitis has been shown to be a rare complication of mumps immunization with some vaccine virus strains,[193,295] although it has not been a problem in the United States. More recent studies in the United States and Finland have failed to show an association between measles-mumps-rubella vaccination and encephalitis or encephalopathy.[288,358] A fatal encephalitis occurred in a 3-year-old child after receiving 17D yellow fever vaccine.[349]

*References 18, 22, 23, 44, 56, 62, 63, 118, 120, 125, 129, 130, 150, 152, 169, 244, 250, 255, 284, 312, 318, 319, 322, 323, 330, 339, 391, 407, 408, 413, 415, 440.

†References 7, 30, 63, 118, 120, 145, 165, 166, 322, 328, 333, 353, 374, 409, 411, 432, 468.

‡References 3, 52, 77, 88, 111, 138, 151, 216, 256, 285, 291, 352, 361, 441, 461.

*References 9, 48, 146, 174, 233, 265, 315, 404.

Postinfectious Encephalitis

Postinfectious or parainfectious encephalitis occurs after a demonstrated or presumed viral infection and is thought to be immune mediated rather than due to a direct effect of the virus in nerve cells.[178,227-229,371] This theory has been studied extensively by Johnson[229] and Griffin[178] in encephalitis associated with measles. These researchers have described a periventricular demyelinating disease and have not been able to isolate measles virus or identify measles antigens in nervous tissue. Other investigators have recovered measles virus from the cerebrospinal fluid (CSF) and brain of affected patients.[147,299,349,385,420] We suggest that immune mechanisms play a role in the pathogenesis of measles and perhaps other postinfectious neurologic illnesses but that the process is stimulated by the direct presence of the antigen in the nervous system. The mechanism of disease is important regarding possible treatment: corticosteroids might be useful in immune-mediated disease but could be detrimental in an acute viral infection.

In contrast to measles, other apparent postinfectious encephalitides that usually have a subacute onset are immune mediated and have multifocal white matter lesions.* Specifically, acute demyelinating encephalomyelitis (ADEM) usually is subacute in onset and is usually a monophasic polysymptomatic disorder that can affect any region of the brain and/or spinal cord. Thus, clinical manifestations may include optic neuritis, myelitis, ataxia, hemiparesis, cranial nerve palsies, and multifocal white matter lesions that are easily confused with multiple sclerosis. Subtle features help distinguish between the two disorders, however.[398] If solitary or unilateral lesions are present, it most likely is ADEM, whereas lesions in the corpus callosum are much more common in multiple sclerosis. Lesions of greater than 4 cm diameter suggest ADEM, and ADEM lesions generally have indistinct borders compared with multiple sclerosis lesions, which generally have sharp borders. Patients with ADEM may respond dramatically to treatment with corticosteroids and perhaps intravenous immunoglobulin or plasmapheresis.

Chronic Encephalitic or Encephalopathic Illnesses

"Slow infections" that cause encephalitic and encephalopathic illness in humans have been recognized for many years. Many of these illnesses now are recognized as viral infections or caused by prions. Viral illnesses include progressive multifocal leukoencephalopathy in primarily immunocompromised hosts (JC, SV40, and BK viruses), subacute sclerosing panencephalitis (measles virus), and acquired immunodeficiency syndrome (AIDS) (human immunodeficiency virus [HIV] type 1 [HIV-1] and HIV-2). Prion diseases, termed *transmissible spongiform encephalopathies*, include kuru, Jakob-Creutzfeldt disease, and Gerstmann-Sträussler-Scheinker disease.[348] Prion diseases are related to scrapie of sheep and bovine spongiform encephalopathy (mad cow disease), which are prion diseases of animals. These chronic illnesses are discussed in Chapter 192.

*References 5, 13, 21, 47, 109, 183, 240, 268, 297, 301, 315, 334, 418.

EPIDEMIOLOGY

Because of the diversity of the infectious disease agents causing encephalitis, the incidence, geographic distribution, age distribution, and seasonality vary tremendously. The specific epidemiology of each infectious agent or disease is presented in detail in the respective chapters of this book; only a brief overview is presented here.

Accurate information on the overall incidence of encephalitis is lacking owing to the vast number of agents and the imprecise case definition. Nevertheless, incidence rates for encephalitis range from 1.5 to 8.8 per 100,000 population.[98,112,249,353] In the United States, a study of hospital discharge data from 1988 through 1997 determined a rate of 7.3 encephalitis hospitalizations per 100,000.[242] The incidence of ADEM (a form of postinfectious encephalitis) is estimated to account for 10 to 15 percent of encephalitis overall, with specific ADEM incidence numbers estimated to be 0.4 to 0.8 per 100,000.[24,269,419]

Although encephalitis per se does not appear to follow a seasonal pattern, specific pathogens, particularly arthropod-borne viruses, do have well-defined temporal patterns. Encephalitis caused by arboviruses can sometimes occur in localized outbreaks and epidemics or sporadic cases may be seen. In temperate areas, most cases occur in summer and fall. The occurrence of human infection and disease is influenced by the abundance of mosquito vectors and natural reservoir in animals. Although enteroviral disease, including aseptic meningitis, occurs in epidemics, severe encephalitis caused by these agents usually is a sporadic event. Influenza-associated encephalopathy has been described sporadically but when it occurs it follows a seasonal pattern with illnesses occurring primarily during the winter months in temperate climates. Sporadic cases of encephalitis occur in any season. Epidemiologic considerations that must be reviewed in a search for the causative agent include geographic area; climatic conditions; animal, water, food, soil, and personal exposures; and host factors.

For most viral agents, the incubation periods range from a few days to a few weeks. Rabies is an important exception to this. Although the incubation for rabies is typically 20 to 60 days, it can be up to 7 years.[225] Additionally, measles, which can cause an acute form of encephalitis, also can cause an indolent, slowly progressive form of encephalitis, termed subacute sclerosing panencephalitis, that can manifest years after the original infection.[32]

PATHOGENESIS

Because encephalitis has multiple causes, the lack of a unified pathogenesis is not surprising.* Clinical

*References 9, 34, 60, 101, 132, 143, 147, 161, 170, 178, 182, 192, 203, 228, 303, 304, 306, 309, 449.

manifestations of encephalitis can result from a direct or an indirect effect of an infectious agent on the brain. Rabies, arboviruses, herpes simplex, and enteroviral encephalitides are examples in which the viral infections directly involve tissue cells within the brain. In contrast, agents such as measles can trigger immunologic events responsible for the pathogenesis of postinfectious or parainfectious encephalitides. Encephalitic symptoms in bacterial meningitides and in rickettsial infections may be caused by the vasculitis and liberated toxins of the surrounding infection. In addition, there is yet another group of organisms whose role and mechanism is unknown. This includes agents such as *M. pneumoniae* where there is anecdotal evidence of infection but minimal neurotropism and limited laboratory data to support direct CNS invasion.[95]

In many viral encephalitides, such as those caused by arboviruses, mumps, and enteroviruses, the CNS infection occurs after a primary viral infection elsewhere in the body. Generally, the infectious agents, whether from ingestion, as in enteroviral infections, or from the bite of a mosquito, as in an arboviral infection, enter the lymphatic system. In the lymphatics, viral multiplication occurs, which results in seeding of the bloodstream and infection of other organs in the body. Viral multiplication occurs at these secondary infection sites; extensive secondary viremia occurs, and then the CNS becomes infected. The reason certain viruses are more "neurotropic" than others is not well known. One hypothesis is that the small size of arboviruses allows them to escape clearance of the reticuloendothelial system. Arboviruses may enter the CNS via the cerebral capillaries with vascular endothelial cell infection.[230] Indeed, recent studies have found that hypertension and diabetes are a risk factor for the development of West Nile virus neuroinvasive disease.[222] Actual involvement of nervous tissue may result from growth across or passive diffusion through brain capillaries or centripetal axonal transport of virus from the olfactory neuroepithelium to the olfactory bulb.[309]

Infection of the brain also may occur through the peripheral nerves. This retrograde spread of virus is important in encephalitis caused by herpes simplex virus, polio virus, and rabies virus. In the case of rabies, after the virus is introduced through the skin, usually via a bite, the rabies virus replicates in skeletal muscle and travels to the CNS via the peripheral nerves.[220] Rabies is unique among viral diseases with respect to incubation period. In some cases of prolonged incubation periods the virus likely remains close to the viral entry site.[220] Once the virus has spread to the brain via peripheral nerves, rabies virus disseminates throughout the CNS. Another mechanism organisms use to gain entry to the CNS is exemplified by free-living amoebae such as *N. fowleri;* these organisms enter transnasally, pass through the cribriform plate, and invade the frontal lobes of the brain.[439]

Postinfectious or parainfectious encephalitis is an acute demyelinating disease of the brain in which the findings suggest an autoimmune process. Usually, little evidence of an active infectious process is present when symptoms occur. Viral or other agents probably invaded the CNS initially and then were cleared but were a trigger for the subsequent development of disease. An immune (T cell) response to myelin basic protein occurs.

PATHOLOGY

The classic findings in viral encephalitis include perivascular mononuclear cell inflammation, neuronal destruction, neuronophagia, and microglial nodules,[226] with most of the abnormalities seen in the gray matter of the brain. Tissue sections of the brain generally reveal meningeal congestion and mononuclear infiltration, perivascular cuffs of lymphocytes and plasma cells, some perivascular tissue necrosis with myelin breakdown, neuronal disruption in various stages (including ultimately neuronophagia), and endothelial proliferation or necrosis. The severity and the extent of observed lesions vary with the infectious agent and with the degree of reaction of the host. The cerebral cortex, especially the temporal lobe, often is affected severely by herpes simplex virus, and arboviruses tend to affect the entire brain.[220] Intranuclear inclusions are suggestive of a member of the Herpesviridae. Rabies has a predilection for the basal structures; involvement of the spinal cord, nerve roots, and peripheral nerves varies. Negri bodies, if identified, are pathognomonic for rabies. Although rabies has one of the highest fatality rates of any infectious disease, brain tissue shows relatively benign neuropathologic changes without evidence of neuronal death. In contrast, *Naegleria* causes marked hemorrhagic necrosis, especially in the olfactory bulbs and cerebral cortex.[253] The pathologic process seen in patients with herpes simplex encephalitis is also characterized by a hemorrhagic necrosis but in different locations (i.e., the temporal and frontal lobes).[271]

In contrast to acute viral encephalitis where gray matter is predominantly affected, the white matter is most affected in postinfectious encephalitis. A marked degree of demyelination with preservation of neurons and their axons is considered to be predominantly postinfectious or parainfectious (autoimmune) encephalitis.

Finally, some infectious agents such as *Mycobacterium tuberculosis* and fungi can cause encephalitis-like symptoms without parenchymal involvement. For example, tuberculosis can lead to hydrocephalus and cranial nerve palsies. Other agents, such as varicella-zoster virus and coccidioidomycosis, can cause vasculitis resulting in infarctions in the brain with resultant focal neurologic deficits mimicking encephalitis.[454]

CLINICAL MANIFESTATIONS

The clinical findings in encephalitis are determined by the severity of involvement and anatomic localization of the affected portions of the nervous system, the inherent pathogenicity of the offending agent, and the immune and other reactive mechanisms of the patient ("host factors"). Evidence of brain parenchymal involvement is the hallmark of encephalitis. Children with encephalitis may show evidence of diffuse disease, such as behavioral or personality changes; decreased consciousness; and generalized seizures or localized changes, such as focal

seizures, hemiparesis, movement disorders, cranial nerve defects, and ataxia. Some children may seem to be mildly affected initially only to lapse into coma and sudden death. In others, the illness is ushered in by high fever, violent convulsions interspersed with bizarre movements, and hallucinations alternating with brief periods of clarity and the children emerge with relatively few sequelae.

Specific forms of encephalitis or complicating manifestations of encephalitis include Guillain-Barré syndrome and related syndromes, acute transverse myelitis, acute hemiplegia, brain stem encephalitis, and acute cerebellar ataxia. Acute cerebellar ataxia is characterized by an abrupt onset of truncal ataxia resulting in varying degrees of gait disturbance and balance abnormalities. Children with this illness have tremulousness of the head and trunk when in the upright position and of the extremities when attempting to move them against gravity. The duration of illness varies from 3 to 4 days to several weeks. Acute cerebellar ataxia often follows chickenpox or other viral illnesses. In one study, 3 percent were related to immunization.[103] Approximately 90 percent of patients recovered completely from the ataxia, but 20 percent had transient behavioral or intellectual disturbances. Five percent had persistent learning problems.

Most commonly, the initial manifestations resemble an undifferentiated acute systemic illness with fever, headache, or, in infants, screaming spells, abdominal distress, nausea, and vomiting. Signs of an associated mild nasopharyngitis may suggest a respiratory tract infection. As the temperature increases, new findings direct attention to the nervous system: mental dullness eventuating in stupor; bizarre movements; convulsions; nuchal rigidity, often not as pronounced as in purely meningitic illness; and focal neurologic signs, which may be stationary, progress, or fluctuate. Loss of bowel and bladder control and unprovoked emotional outbursts may occur.

A wide range of severity of clinical manifestations exists even with the same etiologic agent. Nevertheless, some organisms show tropism for a specific area of the brain and consequently specific clinical characteristics are appreciated. Arboviruses often can cause diffuse brain involvement with global impairment, whereas herpes simplex virus type 1 almost universally involves the temporal lobe. The classic features of herpes simplex encephalitis include fever, altered level of consciousness, dysphagia, focal motor seizures, and hemiparesis; almost all adults with this disease exhibit these features. Children, however, may have atypical features and can have extratemporal lobe involvement.[134] One study identified up to one fourth of children with atypical features, such as ataxia, decreased visual acuity, or tonic-clonic seizures.[134] Primary varicella infection is often associated with cerebellar inflammation, and patients present with ataxia and nystagmus and may or may not have cognitive impairment. Individuals with Epstein-Barr virus encephalitis can sometimes have micropsia, macropsia, and/or size distortion, called "Alice in Wonderland" syndrome. The occurrence of seizures is variable depending on the pathogen. In *Bartonella* encephalopathy, generalized seizures are common,[54] whereas they are unusual in West Nile virus infection.[382] Individuals with rabies often have rapidly progressive encephalitis. Paresthesia at or near the bite site is unique to rabies. Most (~80%) patients with rabies have the "furious" form, characterized by agitation, hydrophobia, delirium, and seizures. Patients with the "paralytic" form (~20%) have ascending paralysis, followed by confusion and coma. Although these two forms are described, it is not unusual for patients to have features of both.

Brain stem encephalitis is a rare disorder, but it is important because clinical signs appear similar to those of a brain stem glioma. The differentiation is important because treatment is radically different. The differentiation is made by the time of onset of symptoms and by the course. Brain stem glioma usually has slowly progressive symptoms developing over the course of several weeks or months. Brain stem encephalitis evolves over 1 to 7 days. Both disorders may be associated with radiographic evidence of brain stem enlargement. Brain stem encephalitis resolves after 1 to 4 weeks, whereas a tumor continues to progress until radiation therapy is given.

Most cases of brain stem encephalitis seem to be postinfectious and are similar to postinfectious cerebellar ataxia, Miller-Fisher syndrome, or Guillain-Barré syndrome. The conditions often overlap.[14] In postinfectious cases, the onset of brain stem encephalitis begins 1 to 3 weeks after a nonspecific viral infection. Brain stem encephalitis has been reported to occur, however, as a result of specific, identifiable, and some treatable infectious agents, including herpes simplex virus,[16,282,337,362,377] varicella-zoster virus,[368,414] cytomegalovirus,[154,232] enterovirus 71,[209,211] West Nile virus,[116] *M. pneumoniae*,[262,405] *L. monocytogenes*,[6,11,12,17,26] *Propionibacterium acnes*,[53] and *Campylobacter jejuni*.[466]

Some patients with a typical clinical picture of brain stem encephalitis have had anti-GQ1b antibodies in the serum,[86] which may represent a subgroup of postinfectious brain stem encephalitis cases. Brain stem encephalitis may arise in HIV-infected patients and may be due to a treatable cause, such as herpes simplex encephalitis.[154,191,367] In an enterovirus outbreak in Taiwan, the most common neurologic complication was rhombencephalitis, and a 14 percent mortality rate was reported.[209,211]

DIFFERENTIAL DIAGNOSIS

The evaluation of a patient with an acute CNS illness (encephalopathy) must be considered carefully, and the sequence of tests should be dictated by the specific circumstances of the individual patient. Several disease processes may have presentations similar to infectious causes.

The differential diagnosis of acute encephalopathy includes the following:

- Autoimmune (anti–*N*-methyl-D-aspartate receptor [NMDAR] encephalitis has recently been found to be one of the leading causes of noninfectious encephalitis in children)
- Acute demyelinating disorders, including ADEM, acute multiple sclerosis, and acute hemorrhagic leukoencephalitis

- Other postinfectious diseases, including Guillain-Barré syndrome (including Miller-Fisher variant), brain stem encephalitis, and acute cerebellar ataxia
- Status epilepticus, especially nonconvulsive status epilepticus, such as complex-partial status or absence status
- Metabolic diseases, such as hypoglycemia, uremic encephalopathy, hepatic encephalopathy, and rare genetic inborn errors of metabolism, including disorders of glucose or ammonia metabolism
- Toxic disorders, such as drug ingestion or Reye syndrome
- Mass lesions, such as tumor or abscess
- Subarachnoid hemorrhage from arteriovenous malformation or aneurysm
- Embolic lesions caused by bacterial endocarditis
- Acute confusional migraine

EVALUATION OF A PATIENT WITH ENCEPHALOPATHY OR POSSIBLE ENCEPHALITIS

Although many cases of encephalitis remain without a cause, and many of the causes are not treatable per se, a thorough diagnostic evaluation is important. For example, the identification of a specific cause may be helpful for prognosis, lead to the discontinuation of unnecessary antimicrobial therapy, and be useful for potential prophylaxis of contacts and initiation of public health interventions. Obtaining a careful history and performing a physical and neurologic examination are essential in all patients who have a history consistent with encephalitis. The differential diagnosis previously presented indicates that encephalitis is only one of many disorders that can manifest as an acute or subacute picture of encephalopathy. Although the diagnosis of encephalitis may be determined best with a lumbar puncture and evaluation of the CSF, lumbar puncture may be contraindicated in some disorders and, if performed inappropriately, may lead to serious complications and even death. A child who has a cerebellar tumor with acute obstruction of the fourth ventricle may have a decreasing level of consciousness caused by the rapidly increasing intracranial pressure. Nuchal rigidity may be present. The family may not have recognized the more subtle changes in cerebellar functions for the months before the acute obstruction developed and may give a history of acute encephalopathy. In that case, a lumbar puncture could result in herniation through the foramen magnum. It is essential that the patient be assessed for the possibility of increased intracranial pressure and the potential for herniation.

The patient's history should be reviewed carefully, questioning specifically for symptoms of neurologic problems that manifested in the days or weeks before the acute disorder occurred. The physical examination must be performed with special attention given to focal neurologic abnormalities, cerebellar signs, and evidence of increased intracranial pressure. Conducting a careful funduscopic examination is important but may be impossible in an agitated patient or young child. The presence of papilledema indicates that neuroimaging should be performed before doing the lumbar puncture. If spontaneous venous pulsations are noted on funduscopic examination, intracranial pressure is not increased and the lumbar puncture can be done without imaging.

In addition to a lumbar puncture, neuroimaging and obtaining an EEG can help in determining the cause of the encephalopathy and the most appropriate course of therapy. Results of the history and physical examination provide a guide to the most appropriate first test to perform, but generally neuroimaging is the most likely to be helpful. The exception would be a child in nonconvulsive status epilepticus. The history may suggest encephalitis as the most likely diagnosis, but, in some patients, nonconvulsive status epilepticus may be clinically indistinguishable from encephalitis.

Neuroimaging

Most patients with encephalopathy should undergo neuroimaging to aid diagnosis of treatable conditions, such as herpes simplex encephalitis. Computed tomography (CT) is helpful in the acute setting to identify abnormalities, such as tumor or abscess, and to decide whether performing a lumbar puncture is safe. However, CT is not as helpful as magnetic resonance imaging (MRI) in detecting the subtle changes associated with encephalitis.[51,273,387] In many cases of viral encephalitis, CT and MRI yield normal results or only nonspecific changes, such as swelling[448] or edema.[273] An important exception is herpes simplex encephalitis.

As previously noted, MRI is more sensitive than is CT. In one study, CT initially was positive in only 42 percent of cases whereas MRI was positive in all cases.[245] MRI in HSV encephalitis characteristically shows abnormalities in the medial temporal lobes, inferior frontal cortex, and insula.[272,450] The likelihood of finding the abnormalities may be increased by using T2-weighted imaging and fluid-attenuated inversion recovery (FLAIR) sequences[20,235] or diffusion-weighted imaging.[384,422] Diffusion-weighted MRI seems to be more sensitive than is FLAIR or T2-weighted sequences in the detection of herpes simplex virus or other encephalitides.[430] The localization of abnormalities may differ, however, from the classic pattern in young children. In neonatal herpes encephalitis, widespread changes occur in the periventricular white matter, often sparing the medial temporal and inferior frontal lobes. Another pattern has been described in children aged 4 to 13 months in whom the cortex and adjacent white matter of the hemispheres were abnormal.[272]

In addition to herpes, other encephalitides may yield abnormal neuroimaging. CT and MRI results often are abnormal in disorders caused by arbovirus or enterovirus infections. When imaging is abnormal, it usually is nonspecific, showing areas of decreased density (with CT) or increased signal intensity (with MRI) in the gray or white matter. A variety of MRI abnormalities with certain viral encephalitides have been reported. The basal ganglia, brain stem, and thalami have been reported to be

abnormal on MR images of patients with eastern equine encephalitis,[122] Japanese encephalitis,[210,257] and enterovirus 71.[209,211] These differences help to distinguish herpes simplex virus from other, nontreatable causes of viral encephalitis.

Postinfectious disorders most often are associated with selective oligodendrocyte involvement.[28] Imaging shows increased signal in white matter with T2-weighted MRI or low-density white matter with CT.[25,45,197,240,327] Patients with acute hemorrhagic leukoencephalitis, a rare disease that is rapidly progressive and often fatal, may have a clinical picture similar to that of herpes simplex encephalitis. In contrast to herpes simplex virus infection, the CT results often are abnormal within the first 1 or 2 days.[369,446] If a patient with suspected herpes simplex encephalitis has abnormal CT results early in the course, acute hemorrhagic leukoencephalitis should be considered.

Another imaging technique that has been reported to be helpful in establishing the diagnosis of encephalitis is single-photon emission computed tomography (SPECT). Initial reports suggest that SPECT is more sensitive than is CT. Ackerman and colleagues[2] found that SPECT showed greater sensitivity and more precise localization than did conventional radionuclide scanning and CT. Launes and associates[268] studied 14 patients with encephalitis and found that SPECT detected temporal lobe abnormalities in all 6 of the patients with herpes simplex encephalitis and yielded normal results in the remaining 8 whose disease had other causes. A few cases of normal MR images but abnormal SPECT scans in patients with herpes simplex encephalitis have been reported[196,292]; this is less likely to occur with newer MRI sequences such as diffusion-weighted imaging. If SPECT is performed, technetium-99m hexamethylpropyleneamine oxime seems to be superior to technetium-99m ethyl cysteinate dimer.[115,139] Generally, SPECT should be reserved for cases in which the MRI results are normal and the EEG is nondiagnostic but in which herpes simplex encephalitis is still strongly suspected. Intracranial ultrasonography in neonates has been shown to be helpful in establishing the diagnosis and in follow-up of infants with herpes simplex virus or cytomegalovirus infections.[293]

Electroencephalography

Generally, an EEG should be obtained in most patients with encephalitis. The EEG results of patients with encephalitis are often nonspecifically abnormal, showing diffuse slowing. Crucial exceptions to the general rule exist, however.

In acute encephalopathy, comatose patients may be in nonconvulsive status epilepticus,[424] which requires immediate and appropriate intervention. The presence of periodic lateralized epileptiform discharges (PLEDs) on an EEG strongly suggests the possibility of herpes simplex encephalitis but also may be an indication of seizures.[27] Early in the course of herpes encephalitis, generalized slowing of the background frequencies and focal slowing over the affected temporal lobe may occur. Within a few days, the characteristic PLEDs pattern develops in most cases. Later in the course, the background activity between the bursts of PLEDs gradually may flatten. Occasionally, other areas of the brain seem to be involved, primarily with herpes simplex virus. PLEDs, although strongly suggestive of herpes simplex encephalitis, are not diagnostic. PLEDs have been reported with stroke and infectious mononucleosis encephalitis,[173] and periodic complexes are characteristic of the slow virus and prion disorders, including Jakob-Creutzfeldt disease and subacute sclerosing panencephalitis.

The EEG abnormalities in neonatal herpes encephalitis are similar to those in older patients. The characteristic EEG results yield periodic or pseudoperiodic complexes, usually triangular or sharp waves, occurring in a multifocal pattern.[307] In one study of 34 infants with herpes simplex encephalitis, EEGs were obtained in 21, the results in 19 being abnormal. The results of 3 showed only focal slowing, but the other 16 showed the characteristic periodic or pseudoperiodic complexes.[372] The authors reviewed 500 other neonatal EEG records and found 20 with similar complexes; 11 patients had meningoencephalitis of unknown etiology, 3 had hemorrhage, and 2 had asphyxia. Four were placed in a miscellaneous category. Periodic or pseudoperiodic complexes on a neonatal EEG strongly suggest herpes simplex encephalitis, but they are not diagnostic.

DIAGNOSIS

A meticulous history is essential and must evaluate exposure in the previous 2 to 3 weeks to illness in contacts; exposure to mosquitoes, ticks, and animals during recent vacations or picnics or other outdoor activities; awareness of illness in animals in the patient's environment; recent travel from the home area; recent injections of any kind; and the possibility of accidental exposure to heavy metals, pesticides, or other questionable substances. The CSF must be examined carefully to exclude other disorders that respond to specific therapy. Smears for bacteria, appropriate rapid antigen-identification tests, and cultures of the CSF are mandatory; the history and clinical findings may indicate the need for acid-fast stain and culture of the sediment for mycobacteria. Although often not done because of inconvenience, opening CSF pressures should be measured. Molecular testing has advanced the diagnostics for encephalitis overall, but there are a number of important limitations of molecular diagnostics (both false-positive and false-negative findings). In particular, it is important to understand that a positive polymerase chain reaction (PCR) test in the CSF does not necessarily equate with disease as well as a negative PCR test does not rule it out. Additionally, there is still a very important role for culture and serologic assays for the diagnosis of encephalitis. Recommended test types and "pitfalls" in testing are outlined in Table 36-1. A diagnostic algorithm is presented in Table 36-2.

The availability of PCR has revolutionalized the diagnosis of herpes simplex encephalitis. It has allowed the definitive and rapid diagnosis of HSV encephalitis to be established, eliminating the need for brain biopsy.[123,259,376,434,452] If, however, initial testing is negative for herpes simplex virus and herpes simplex encephalitis is strongly suspected (e.g., from results of temporal lobe

Table 36-2 **Diagnostic Algorithm**

All Cases

CSF

White blood cells, protein, glucose

Bacterial culture

Herpes simplex virus-1 PCR

Enterovirus PCR

Blood/Serum

Routine blood culture

Epstein-Barr virus (EBV) antibodies (if positive for acute infection, check CSF EBV PCR)

Hold acute serum and collect convalescent serum 10-14 days later for paired antibody testing.

Respiratory, Stool

Enterovirus PCR (respiratory, stool)

Enterovirus (stool)

Conditional

Host Factors

Neonate: herpes simplex virus-2 PCR (CSF), swabs of skin vesicles, mouth, nasopharynx, conjunctivae, and rectum (viral culture)

≤3 yr: parechovirus PCR (CSF and respiratory)

Immunocompromised: cytomegalovirus, human herpesvirus–6/7, JC virus, human immunodeficiency virus PCR (CSF)

Season and Exposure

Summer/fall: West Nile virus (WNV) IgM (CSF, serum), WNV IgG (paired serum) and other appropriate arboviruses as geographically relevant

Cat (particularly if with seizures and paucicellular CSF): *Bartonella* antibody (serum)

Animal bite exposure: rabies test*

Rodent exposure: LCM antibody (serum)

Tick and/or camping exposure: *Rickettsia* spp., antibody (serum), *Anaplasma* phagocytophila antibody (serum)

Swimming or diving in brackish water: *Naegleria fowleri* (wet mount)*

If history of sexual activity: herpes simplex virus-2 (CSF PCR)

Signs and Symptoms

Psychotic component or movement disorder: anti-NMDAR antibody (CSF and serum), and abdominal ultrasound evaluation for teratoma

Vesicular rash: varicella-zoster virus PCR (CSF)

Rapid decompensation (especially with bite history or foreign travel): rabies test*

Respiratory (during influenza season): influenza PCR (respiratory)

Diarrhea and seizure (especially young child): rotavirus (check stool for antigen), if positive then rotavirus PCR (CSF)

Laboratory Features

CSF protein >100 mg/dL or CSF glucose less than two thirds peripheral glucose and/or lymphocytic pleocytosis:

Mycobacterial tuberculosis: culture (CSF, respiratory), place PPD, and check IGRA, chest radiograph, fungal culture (CSF).

Fungal (specific types depend on geographic residence and/or travel to endemic areas): culture CSF *and* check antibody and antigen.

Balamuthia mandrillaris: contact health department/CDC for assistance with testing.

CSF eosinophilia: *Baylisascaris procyonis* antibody

Travel

Consider consultation with public health department concerning specific diseases such as arboviruses, rabies, and other diseases.

*Contact health department for assistance with testing.

CDC, Centers for Disease Control and Prevention; *CSF,* cerebrospinal fluid; *IGRA,* interferon gamma release assay; *LCM,* lymphocytic choriomeningitis; *NMDAR, N*-methyl-D-aspartate receptor; *PCR,* polymerase chain reaction; *PPD,* purified protein derivative.

neuroimaging), a second CSF specimen should be obtained and retested for herpes simplex virus by PCR because false-negative results can occur.[134,447] The etiology of encephalitis caused by other herpesviruses also has been determined by PCR assay of CSF.[110,294,431] Physicians should be aware, however, of some of the limitations in interpretation of PCR results in patients with encephalitis. For example, because Epstein-Barr virus infections result in latency in the lymphoreticular system (including B cells), when PCR on CSF is examined for this virus, low levels can be detected when it is not necessarily the cause of encephalitis. Serology as well as Epstein-Barr virus PCR should be performed. Similarly, testing the CSF by PCR for human herpesvirus–6 should be performed in some cases but can lead to false-positive results owing to latency. Additionally, for human herpesvirus–6 infections, the phenomenon of chromosomal integration of the virus can further confound the diagnosis.[444]

In addition to its use in herpesvirus infections, PCR can be useful for detected enteroviruses and parechoviruses, and in the future it is likely to be useful to identify other encephalitides.[38,247,274,334,359] For possible enteroviral encephalitis, because the CSF PCR is not always positive, it is therefore important to look for enteroviruses in multiple sites outside the CNS to maximize detection.[339]

In viral encephalitis, the CSF frequently is clear; the leukocyte count ranges from none to several thousand, often with a significant percentage of polymorphonuclear cells initially, moderate or no elevation of protein, and an initially normal level of glucose relative to the simultaneously determined blood glucose level. A nonviral entity should be considered in cases in which the CSF white blood cell count is high (particularly for values >1000 cells/mm^3), protein is greater than 100 mg/dL, and/or CSF glucose is less than two thirds of the serum value. For example, a lymphocytic pleocytosis with high protein

and low glucose values is often found in patients with fungal or *M. tuberculosis* meningitis-encephalitis. If eosinophils are identified in the CSF, a helminthic-parasitic infection should be considered such as *B. procyonis*, *A. cantonensis*, or *G. spinigerum*. Eosinophils in the CSF can also be seen in patients with coccidioidomycosis and *M. tuberculosis* infection.[351]

In any patient suspected to have viral meningoencephalitis, CSF, blood, feces, and throat swabs should be collected and sent to a laboratory offering viral diagnostic services. An additional serum specimen should be collected 10 to 21 days later. Although these studies may not provide an immediate diagnosis, they may give early warning of a specific epidemic and the use of specific antiviral chemotherapy may be indicated by the preliminary culture results.

Inquiry regarding recent illness, recent injections, and, especially, recent exposures away from the home environment sometimes is helpful. The incubation periods of some arboviruses are such that mosquito bites acquired at least 1 week earlier or insect bites now healed may give a clue. Occasionally, patients who have traveled to Africa or Asia in preceding weeks have encephalitis caused by viruses, trypanosomiasis, or falciparum malaria with bizarre systemic and CNS signs and symptoms.

Rabies should be considered in any patient with a rapid progression of illness. Hydrophobia, autonomic instability, extreme agitation, and seizures are often present. Paresthesia at the site of inoculation, if present, is an important diagnostic clue unique to rabies.

Anti-NMDAR, although not an infectious agent, deserves special mention given its relatively high frequency in the young population.[155] In a recent study, anti-NMDAR encephalitis occurred more than four times as frequently as herpes simplex virus type 1, West Nile virus, and varicella-zoster encephalitides and was the leading entity identified in California Encephalitis Project patients younger than 30 years of age. Seizures, language dysfunction, psychosis, and EEG abnormalities were significantly more frequent in patients with anti-NMDAR. Testing for this entity could lessen the need for additional testing and allow for a clear diagnosis.[155]

TREATMENT

Acyclovir should be used to treat herpes simplex and varicella-zoster virus encephalitis and perhaps encephalitis caused by Epstein-Barr virus. Cytomegalovirus encephalitis should be treated with ganciclovir, and oseltamivir should be considered for treatment of encephalitis caused by influenza viruses A or B. Specific antimicrobial treatment should be used for infections caused by spirochetes, *Chlamydia*, *Mycoplasma*, fungi, and parasites.

General treatment is nonspecific and empirical, aimed at maintaining life and supporting each involved organ system. The effectiveness of various recommended regimens in most instances has not been evaluated objectively. Until a bacterial etiology and, in particular, a brain abscess are excluded, parenteral antibiotic therapy should be administered.

Anticipating and being prepared for convulsions, cerebral edema, hyperpyrexia, inadequate respiratory exchange, disturbed fluid and electrolyte balance, aspiration and asphyxia, abrupt cardiac and respiratory arrest of central origin, cardiac decompensation, and gastrointestinal bleeding is crucial. The syndrome of disseminated intravascular coagulation may be an additional complication.

For these reasons, all patients with severe encephalitis should receive care in intensive care units. Cardiac monitoring should be maintained. Repeat CT and MRI are helpful in following the status of comatose patients and often show signs of brain swelling before the patient has the typical clinical indicators of intracranial pressure, such as Cushing triad (systolic hypertension, bradycardia, and slowing of respirations), dilated pupils, and decorticate or decerebrate posturing. The Cushing triad is an unreliable indicator of increased intracranial pressure, and, when the other signs of increased intracranial pressure occur, they often do so late in the course, when the patient's cerebral perfusion already is at risk.[296] As mentioned previously, the opening pressure should be determined when CSF is obtained. If a patient has a high opening pressure, close monitoring is advised with consideration of measures to decrease intracranial pressure. If brain swelling becomes a problem, placement of an intracranial pressure monitor may be necessary. The intracranial pressure should be maintained at less than 15 mm Hg if possible using the standard techniques for reduction of intracranial pressure, including hyperventilation, osmotic diuretics, and removal of CSF. As a last resort, inducing a barbiturate coma may be necessary. A related consequence of intracranial pressure is the syndrome of inappropriate antidiuretic hormone secretion. Careful monitoring of the fluid and electrolyte balance is essential in all seriously ill patients with encephalitis.

All fluids, electrolytes, and medications initially are given parenterally. In patients with prolonged coma, parenteral hyperalimentation is indicated. Normal blood levels of glucose, magnesium, and calcium must be maintained to minimize the threat of convulsions.

Status epilepticus caused by encephalitis should be treated vigorously using a structured protocol to ensure optimal control.[390,427] The current standard initial therapy is intravenous lorazepam 0.1 to 0.2 mg/kg, up to 4 mg maximum. Seizures associated with encephalitis may be refractory to the usual therapy, and other anticonvulsants may be required to achieve and maintain control of seizures. In patients who fail initial therapy and are in medically refractory status epilepticus, continuous EEG monitoring usually is recommended to monitor the efficacy of the therapy, especially when the patient is in nonconvulsive status epilepticus.[121,425]

If, after a second attempt, lorazepam fails to control the seizures, intravenous phenytoin (preferably phosphenytoin in children) is the next drug of choice. The dose is 18 to 20 mg/kg, maximum 1000 mg, given over 20 minutes. Phosphenytoin is preferred because it can be administered faster and does not cause sclerosis of the veins as does phenytoin and is not as likely to cause cardiac arrhythmias. Virtually all patients who require

therapy beyond lorazepam need to be intubated to prevent respiratory embarrassment. If phosphenytoin is unsuccessful, or as an alternative to phosphenytoin, intravenous midazolam has gained favor in recent years.[99,336] The initial dose is 0.1 to 0.2 mg/kg over 5 minutes, with a maintenance infusion starting at 0.05 mg/kg/hr up to a maximum of 0.4 mg/kg/hr. Another alternative therapy is propofol, which generally is administered by an anesthesiologist.

Many methods have been proposed to minimize cerebral edema and to diminish the consequences of cerebral anoxia. The following measures are difficult to evaluate and generally are reserved for patients with severe illness whose condition apparently is desperate.

1. Dexamethasone 0.1 to 0.2 mg/kg intravenously in an initial dose followed by 0.05 to 0.1 mg/kg intravenously every 4 to 6 hours is given. This large dose should be reduced gradually after a few days if recovery or improvement is evident. Dexamethasone probably should not be used in acute viral diseases because corticosteroids may potentiate the viral infection.
2. Other substances employed in an effort to reduce elevated intracranial pressure include mannitol, given intravenously as a 20 percent solution in a dose of 0.25 to 1 g/kg over a 30- to 60-minute period (this may be repeated every 8 to 12 hours), and glycerol, given by nasogastric tube using 0.5 to 1 mL/kg diluted with twice that volume of orange juice. This regimen is nontoxic and may be repeated every 6 hours for an extended period.

For more than 40 years, corticosteroids and adrenocorticotropic hormone frequently have been used as empirical therapy for encephalitis. No controlled studies have shown any efficacy, however. In two comparative studies of measles encephalitis, corticosteroids were found to offer no benefit; and in both studies the corticosteroid recipients seemed to have had worse outcomes.[42,467] More recently, in a carefully controlled study, no benefit of high-dose dexamethasone was found in the treatment of acute encephalitis caused by Japanese encephalitis virus.[205]

In contrast to these investigations are more recent clinical experiences in the treatment of acute disseminated encephalomyelitis, in which MRI studies have indicated multifocal white matter lesions.[47,183,240,297] Patients treated with corticosteroids often have responded dramatically, with clinical improvement and resolution of the lesions as shown by MRI. Corticosteroids, along with specific antibiotic therapy, also may be beneficial in the treatment of encephalitis caused by *M. pneumoniae* infection.[248,389]

Plasmapheresis and intravenous immunoglobulin have been used empirically to treat brain stem encephalitis and other encephalitides, but no studies have been done that indicate such therapies are helpful.[320]

Equipment and personnel for handling emergencies such as cardiac and respiratory arrest always must be available. Early consultation with an anesthesiologist or intensive care specialist is useful in anticipating the need for artificially assisted respiration.

Supportive and rehabilitative efforts are important after the patient recovers. Motor incoordination, convulsive disorders, squint, total or partial deafness, or behavioral disturbances may appear only after some time. Visual disturbances caused by chorioretinopathy and perceptual amblyopia also may make a delayed appearance. Special facilities and sometimes institutional placement may become necessary.

PROGNOSIS

The prognosis in all patients with encephalitides is guarded with respect to immediate outcome and sequelae. Sequelae involving the CNS may be intellectual, motor, psychiatric, epileptic, visual, or auditory. The short-term and long-term prognoses depend to some extent on etiology and age. Unfortunately, quality data for most agents are not available, and even less is known about the "unknown" causes.

Rautonen and associates[356] examined prognostic factors in childhood acute encephalitis at the Children's Hospital, University of Helsinki, during a 20-year period from 1968 to 1987. This study comprised 462 cases with the following causes: mumps virus, measles virus, rubella virus, varicella-zoster virus, herpes simplex virus, enteroviruses, respiratory viruses, *M. pneumoniae*, other agents, and cause undetermined. The investigators found that mortality was fivefold greater in infants compared with older children. Children who were disorientated or unconscious before admission had 4-fold and 25-fold greater risks for death and severe damage than did children whose level of consciousness had been normal. Patients with herpes simplex virus or (putative) *M. pneumoniae* infection had the greatest risks for death or serious residual damage compared with children with encephalitis of other causes. Generally, herpes simplex virus carries a worse prognosis for survival and residual disability than do the enteroviruses.

The data on prognosis for arboviruses are more complete than for most other entities owing to the Centers for Disease Control and Prevention's excellent surveillance system. Prognosis of children affected with arboviruses is highly variable. West Nile virus tends to be less severe in children than adults; children accounted for only 4 percent of West Nile neuroinvasive disease in cases reported between 1999 and 2007.[278] There were only 3 fatalities (1%) in the pediatric age group compared with 45 (15%) in adults.[279] Conversely, La Crosse virus (in the California serogroup of viruses) and eastern equine encephalitis are more severe in children than adults.[298] Eastern equine encephalitis has a high mortality rate. Infants and children younger than 5 years of age who survive usually have severe sequelae consisting of mental retardation, convulsions, and paralysis. These consequences are in contrast to adults older than 40 years of age who survive, who recover completely, or have only slight damage.

St. Louis encephalitis has a low mortality rate. Although neurologic sequelae are reported, their incidence is low in the pediatric age group. The prognosis in

encephalitis caused by western equine virus is guarded; 56 percent of infants younger than 1 month of age have had recurring seizures with marked motor and behavioral changes. After they reach 1 year of age, the sequelae appear to diminish; only 5 percent of adults have neurologic sequelae. Fifty-seven percent of infants who survived western equine virus infection and who were younger than 1 year of age at the time of infection had major neurologic sequelae requiring either a special school or institutionalization late in life. Severe retardation, paralysis, spasticity, recurrent convulsions, hearing deficits, and speech difficulties all were reported as complications.[133,143]

Nevertheless, progress has been made recently for some causative agents that were thought to always have a poor outcome, and the paradigm seems to be changing. For example, rabies previously was considered to be universally fatal, but in 2004 a 15-year-old girl in Wisconsin developed rabies and survived, representing the first person to survive documented clinical rabies without previous vaccination.[455] Since then, two additional human rabies cases have occurred in the United States and both patients survived without significant neurologic sequelae.[79,82]

Correspondingly, *B. mandrillaris* encephalitis was also considered to have a grave prognosis but recent case reports described successful outcomes.[58,117] Similarly, all previously reported human cases of *Baylisascaris* meningoencephalitis had resulted in either severe neurologic sequelae or death; in 2007, a case report was published concerning a 4-year-old child with *Baylisascaris* meningoencephalitis who survived without any recognizable neurologic deficits.[332]

PREVENTION

The widespread use of effective attenuated viral vaccines for measles, mumps, and rubella almost has eliminated CNS complications from these diseases in the United States. The control of encephalitis caused by arboviruses is primarily through control of insect vectors. Specific vaccines for the arbovirus diseases that occur in North America are unavailable. Until we learn more about the "unknowns," it will be difficult to know how to prevent many of the causes.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Al-Baghli F, Al-Ateeqi W. Encephalitis-associated pandemic A (H1N1) 2009 in a Kuwaiti girl. *Med Princ Pract* 2011;**20**:191–5.
7. Amin R, Ford-Jones E, Richardson SE, et al. Acute childhood encephalitis and encephalopathy associated with influenza: a prospective 11-year review. *Pediatr Infect Dis J* 2008;**27**:390–5.
16. Arita JH, Lin J, Peruchi MM, et al. Herpes simplex type 1 encephalitis restricted to the brainstem in a pediatric patient. *Case Report Med* 2010;**2010**:606584.
18. Arnold JC, Singh KK, Milder E, et al. Human metapneumovirus associated with central nervous system infection in children. *Pediatr Infect Dis J* 2009;**28**:1057–60.
19. Arpino C, Curatolo P, Rezza G. Chikungunya and the nervous system: what we do and do not know. *Rev Med Virol* 2009;**19**:121–9.
24. Banwell B, Kennedy J, Sadovnick D, et al. Incidence of acquired demyelination of the CNS in Canadian children. *Neurology* 2009;**72**:232–9.
39. Bitnun A, Richardson SE. *Mycoplasma pneumoniae:* innocent bystander or a true cause of central nervous system disease? *Curr Infect Dis Rep* 2010;**12**:282–90.
41. Bodi I, Dutt N, Hampton T, et al. Fatal granulomatous amoebic meningoencephalitis due to *Balamuthia mandrillaris*. *Pathol Res Pract* 2008;**204**:925–8.
46. Borawake K, Prayag P, Wagh A, et al. Dengue encephalitis. *Indian J Crit Care Med* 2011;**15**:190–3.
52. Calitri C, Gabiano C, Garazzino S, et al. Clinical features of hospitalised children with 2009 H1N1 influenza virus infection. *Eur J Pediatr* 2010;**169**:1511–5.
58. Cary LC, Maul E, Potter C, et al. *Balamuthia mandrillaris* meningoencephalitis: survival of a pediatric patient. *Pediatrics* 2010; **125**:e699–703.
76. Centers for Disease Control and Prevention. Outbreak of measles—San Diego, California, January-February 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:203–6.
77. Centers for Disease Control and Prevention. Neurologic complications associated with novel influenza A (H1N1) virus infection in children—Dallas, Texas, May 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:773–8.
78. Centers for Disease Control and Prevention. Locally acquired Dengue—Key West, Florida, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:577–81.
79. Centers for Disease Control and Prevention. Presumptive abortive human rabies—Texas, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:185–90.
80. Centers for Disease Control and Prevention. Increased transmission and outbreaks of measles—European Region, 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1605–10.
81. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1009–13.
82. Centers for Disease Control and Prevention. Recovery of a patient from clinical rabies—California, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:61–5.
88. Chen YC, Lo CP, Chang TP. Novel influenza A (H1N1)-associated encephalopathy/encephalitis with severe neurological sequelae and unique image features—a case report. *J Neurol Sci* 2010;**298**:110–3.
92. Chieochansin T, Vichiwattana P, Korkong S, et al. Molecular epidemiology, genome characterization, and recombination event of human parechovirus. *Virology* 2011;**421**:159–66.
96. Chun CS, Kazacos KR, Glaser C, et al. Global neurologic deficits with *Baylisascaris* encephalitis in a previously healthy teenager. *Pediatr Infect Dis J* 2009;**28**:925–7.
111. Davis LE. Neurologic and muscular complications of the 2009 influenza A (H1N1) pandemic. *Curr Neurol Neurosci Rep* 2010; **10**:476–83.
124. Diaz JH. The public health threat from *Balamuthia mandrillaris* in the southern United States. *J La State Med Soc* 2011;**163**: 197–204.
125. Dickey M, Jamison L, Michaud L, et al. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. *Pediatr Infect Dis J* 2009;**28**:318–21.
128. Domenech C, Leveque N, Lina B, et al. Role of *Mycoplasma pneumoniae* in pediatric encephalitis. *Eur J Clin Microbiol Infect Dis* 2009;**28**:91–4.
130. Douvoyiannis M, Litman N, Goldman DL. Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis* 2009;**48**:1713–23.
138. Farooq O, Faden HS, Cohen ME, et al. Neurologic complications of 2009 influenza-A H1N1 infection in children. *J Child Neurol* 2012;**27**:431–8.
142. Filia A, Tavilla A, Bella A, et al. Measles in Italy, July 2009 to September 2010. *Eur Surveill* 2011;**16**(29):19925.
150. Frange P, Peffault de Latour R, Arnaud C, et al. Adenoviral infection presenting as an isolated central nervous system disease without detectable viremia in two children after stem cell transplantation. *J Clin Microbiol* 2011;**49**:2361–4.
151. Frobert E, Sarret C, Billaud G, et al. Pediatric neurological complications associated with the A(H1N1)pdm09 influenza infection. *J Clin Virol* 2011;**52**:307–13.
155. Gable MS, Sheriff H, Dalmau J, et al. The frequency of autoimmune *N*-methyl-D-aspartate receptor encephalitis surpasses

that of individual viral etiologies in young individuals enrolled in the California Encephalitis Project. *Clin Infect Dis* 2012;**54**: 899–904.
160. Ghosh PS, Ghosh D, Loddenkemper T, et al. Necrotizing granulomatous meningoencephalitis due to *Balamuthia* in an immunocompetent child. *Neurology* 2011;**77**:801–2.
171. Graham AS, Pruszynski CA, Hribar LJ, et al. Mosquito-associated dengue virus, Key West, Florida, USA, 2010. *Emerg Infect Dis* 2011;**17**:2074–5.
172. Granerod J, Ambrose HE, Davies NW, et al. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis* 2010;**10**:835–44.
177. Griffin DE. Emergence and re-emergence of viral diseases of the central nervous system. *Prog Neurobiol* 2010;**91**:95–101.
186. Guler E, Davutoglu M, Guler S, et al. Encephalitis in a child during atypical course of rubella. *Infection* 2009;**37**:65–6.
188. Gupta S, Fernandez D, Siddiqui A, et al. Extensive white matter abnormalities associated with neonatal parechovirus (HPeV) infection. *Eur J Paediatr Neurol* 2010;**14**:531–4.
189. Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. *PLoS One* 2009;**4**:e6145.
193. Hara M, Mizuochi T, Kawano G, et al. A case of clinically mild encephalitis with a reversible splenial lesion (MERS) after mumps vaccination. *Brain Dev* 2011;**33**:842–4.
195. Harvala H, Wolthers KC, Simmonds P. Parechoviruses in children: understanding a new infection. *Curr Opin Infect Dis* 2010;**23**:224–30.
202. Hill CP, Damodaran O, Walsh P, et al. *Balamuthia* amebic meningoencephalitis and mycotic aneurysms in an infant. *Pediatr Neurol* 2011;**45**:45–8.
206. Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect* 2010;**138**:1630–6.
207. Homaira N, Rahman M, Hossain MJ, et al. Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One* 2010;**5**:e13570.
215. Huppatz C, Durrheim DN, Levi C, et al. Etiology of encephalitis in Australia, 1990-2007. *Emerg Infect Dis* 2009;**15**:1359–65.
216. Incecik F, Ozlem Herguner M, Altunbasak S, et al. Fatal encephalitis associated with novel influenza A (H1N1) virus infection in a child. *Neurol Sci* 2012;**33**:677–9.
225. Johnson N, Fooks A, McColl K. Human rabies case with long incubation, Australia. *Emerg Infect Dis* 2008;**14**:1950–1.
231. Kansagra AP, Menon JP, Yarbrough CK, et al. *Balamuthia mandrillaris* meningoencephalitis in an immunocompromised patient: case report. *J Neurosurg* 2009;**111**:301–5.
237. Kaushal V, Chhina DK, Kumar R, et al. *Acanthamoeba* encephalitis. *Indian J Med Microbiol* 2008;**26**:182–4.
244. Kobayashi S, Negishi Y, Ando N, et al. Two patients with acute rotavirus encephalitis associated with cerebellar signs and symptoms. *Eur J Pediatr* 2010;**169**:1287–91.
256. Kulkarni R, Kinikar A. Encephalitis in a child with H1N1 infection: first case report from India. *J Pediatr Neurosci* 2010;**5**:157–9.
274. Levorson RE, Jantausch BA, Wiedermann BL, et al. Human parechovirus-3 infection: emerging pathogen in neonatal sepsis. *Pediatr Infect Dis J* 2009;**28**:545–7.
278. Lindsey NP, Hayes EB, Staples JE, et al. West Nile virus disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**: e1084–9.
279. Lindsey NP, Staples JE, Lehman JA, et al. Surveillance for human West Nile virus disease—United States, 1999-2008. *MMWR Surveill Summ* 2010;**59**:1–17.
283. Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. *Clin Infect Dis* 2009;**49**:1743–8.
285. Lyon JB, Remigio C, Milligan T, et al. Acute necrotizing encephalopathy in a child with H1N1 influenza infection. *Pediatr Radiol* 2010;**40**:200–5.
287. Mailles A, Stahl JP. Infectious encephalitis in France in 2007: a national prospective study. *Clin Infect Dis* 2009;**49**:1838–47.
291. Martin A, Reade EP. Acute necrotizing encephalopathy progressing to brain death in a pediatric patient with novel influenza A (H1N1) infection. *Clin Infect Dis* 2010;**50**:e50–2.
300. Melekhin VV, Karem KL, Damon IK, et al. Encephalitis after secondary smallpox vaccination. *Clin Infect Dis* 2009;**48**:e1–2.
304. Meyer Sauteur PM, Streuli JC, Iff T, et al. *Mycoplasma pneumoniae*-associated encephalitis in childhood—nervous system disorder during or after a respiratory tract infection. *Klin Padiatr* 2011; **223**:209–13.
310. Montgomery JM, Hossain MJ, Gurley E, et al. Risk factors for Nipah virus encephalitis in Bangladesh. *Emerg Infect Dis* 2008;**14**:1526–32.
312. Mori T, Morii M, Kuroiwa Y, et al. Rotavirus encephalitis and cerebellitis with reversible magnetic resonance signal changes. *Pediatr Int* 2011;**53**:252–5.
314. Muscat M, Bang H, Wohlfahrt J, et al. Measles in Europe: an epidemiological assessment. *Lancet* 2009;**373**:383–9.
331. Ouattara LA, Barin F, Barthez MA, et al. Novel human reovirus isolated from children with acute necrotizing encephalopathy. *Emerg Infect Dis* 2011;**17**:1436–44.
352. Randolph AG, Vaughn F, Sullivan R, et al. Critically ill children during the 2009-10 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
357. Ravid S, Shahar E, Genizi J, et al. Acute Q fever in children presenting with encephalitis. *Pediatr Neurol* 2008;**38**:44–6.
360. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg* 2008;**79**:974–9.
361. Rellosa N, Bloch KC, Shane AL, et al. Neurologic manifestations of pediatric novel H1N1 influenza infection. *Pediatr Infect Dis J* 2011;**30**:165–7.
363. Robin S, Ramful D, Le Seach F, et al. Neurologic manifestations of pediatric chikungunya infection. *J Child Neurol* 2008;**23**: 1028–35.
378. Schuster FL, Yagi S, Gavali S, et al. Under the radar: *Balamuthia* amebic encephalitis. *Clin Infect Dis* 2009;**48**:879–87.
392. Siddiqui R, Khan NA. *Balamuthia* amoebic encephalitis: an emerging disease with fatal consequences. *Microb Pathog* 2008;**44**: 89–97.
395. Silva RA, Araujo Sde A, Avellar IF, et al. Granulomatous amoebic meningoencephalitis in an immunocompetent patient. *Arch Neurol* 2010;**67**:1516–20.
409. Streng A, Grote V, Liese JG. Severe influenza cases in paediatric intensive care units in Germany during the pre-pandemic seasons 2005 to 2008. *BMC Infect Dis* 2011;**11**:233.
415. Takanashi J, Miyamoto T, Ando N, et al. Clinical and radiological features of rotavirus cerebellitis. *AJNR Am J Neuroradiol* 2010;**31**:1591–5.
423. Tong CY, Menson E, Lin JP, et al. Prevalence of *Mycoplasma* encephalitis. *Lancet Infect Dis* 2011;**11**:425–6.
433. Tyler KL. Emerging viral infections of the central nervous system, part 2. *Arch Neurol* 2009;**66**:1065–74.
435. Van Dam CN, Syed S, Eron JJ, et al. Severe postvaccinia encephalitis with acute disseminated encephalomyelitis: recovery with early intravenous immunoglobulin, high-dose steroids, and vaccinia immunoglobulin. *Clin Infect Dis* 2009;**48**:e47–9.
436. Varatharaj A. Encephalitis in the clinical spectrum of dengue infection. *Neurol India* 2010;**58**:585–91.
437. Verboon-Maciolek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white matter injury in neonates. *Ann Neurol* 2008;**64**:266–73.
441. Wang GF, Li W, Li K. Acute encephalopathy and encephalitis caused by influenza virus infection. *Curr Opin Neurol* 2010;**23**: 305–11.
460. Yasunaga H, Shi Y, Takeuchi M, et al. Measles-related hospitalizations and complications in Japan, 2007-2008. *Intern Med* 2010; **49**:1965–70.
461. Yildizdas D, Kendirli T, Arslankoylu AE, et al. Neurological complications of pandemic influenza (H1N1) in children. *Eur J Pediatr* 2011;**170**:779–88.
462. Yis U, Kurul SH, Cakmakci H, et al. *Mycoplasma pneumoniae:* nervous system complications in childhood and review of the literature. *Eur J Pediatr* 2008;**167**:973–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aberle SW, Aberle JH, Steininger C, et al. Quantitative real time PCR detection of varicella-zoster virus DNA in cerebrospinal fluid in patients with neurological disease. *Med Microbiol Immunol* 2005;**194**:7–12.
2. Ackerman ES, Tumeh SS, Charron M, et al. Viral encephalitis: imaging with SPECT. *Clin Nucl Med* 1988;**13**:640–3.
3. Al-Baghli F, Al-Ateeqi W. Encephalitis-associated pandemic A (H1N1) 2009 in a Kuwaiti girl. *Med Princ Pract* 2011;**20**:191–5.
4. Allen IM. The neurological complications of serum treatment: with report of a case. *Lancet* 1931;**2**:1128–31.
5. Alper G, Schor NF. Toward the definition of acute disseminated encephalitis of childhood. *Curr Opin Pediatr* 2004;**16**:637–40.
6. Alstadhaug KB, Antal EA, Nielsen EW, et al. *Listeria* rhombencephalitis—a case report. *Eur J Neurol* 2006;**13**:93.
7. Amin R, Ford-Jones E, Richardson SE, et al. Acute childhood encephalitis and encephalopathy associated with influenza: a prospective 11-year review. *Pediatr Infect Dis J* 2008;**27**:390–5.
8. Anderson LJ, Nicholson KG, Tauxe RV, et al. Human rabies in the United States, 1960 to 1979: epidemiology, diagnosis, and prevention. *Ann Intern Med* 1984;**100**:728–35.
9. Angulo JJ, De Campos EP, De Gomes LF. Postvaccinal meningoencephalitis; isolation of the virus from the brain. *JAMA* 1964; **187**:151–3.
10. Ansari A, Li S, Abzug MJ, et al. Human herpesviruses 6 and 7 and central nervous system infection in children. *Emerg Infect Dis* 2004;**10**:1450–4.
11. Antal EA, Dietrichs E, Loberg EM, et al. Brain stem encephalitis in listeriosis. *Scand J Infect Dis* 2005;**37**:190–4.
12. Antal EA, Loberg EM, Dietrichs E, et al. Neuropathological findings in 9 cases of *Listeria monocytogenes* brain stem encephalitis. *Brain Pathol* 2005;**15**:187–91.
13. Apak RA, Kose G, Anlar B, et al. Acute disseminated encephalomyelitis in childhood: report of 10 cases. *J Child Neurol* 1999; **14**:198–201.
14. Arai M, Odaka M, Yuki N, et al. A patient with overlapping Bickerstaff's brainstem encephalitis, Miller Fisher syndrome and Guillain-Barré syndrome during the clinical course. *Eur J Neurol* 2002;**9**:115–6.
15. Arenz S, Fischer R, Wildner M. Measles outbreak in Germany: clinical presentation and outcome of children hospitalized for measles in 2006. *Pediatr Infect Dis J* 2009;**28**:1030–2.
16. Arita JH, Lin J, Peruchi MM, et al. Herpes simplex type 1 encephalitis restricted to the brainstem in a pediatric patient. *Case Report Med* 2010;**2010**:606584.
17. Armstrong RW, Fung PC. Brainstem encephalitis (rhombencephalitis) due to *Listeria monocytogenes:* case report and review. *Clin Infect Dis* 1993;**16**:1089–93.
18. Arnold JC, Singh KK, Milder E, et al. Human metapneumovirus associated with central nervous system infection in children. *Pediatr Infect Dis J* 2009;**28**:1057–60.
19. Arpino C, Curatolo P, Rezza G. Chikungunya and the nervous system: what we do and do not know. *Rev Med Virol* 2009;**19**: 121–9.
20. Ashikaga R, Araki Y, Ishida O. MR FLAIR imaging of herpes simplex encephalitis. *Radiat Med* 1996;**14**:349–52.
21. Assa A, Watemberg N, Bujanover Y, et al. Demyelinative brainstem encephalitis responsive to intravenous immunoglobulin therapy. *Pediatrics* 1999;**104**:301–3.
22. Assaad F, Borecka I. Nine-year study of WHO virus reports on fatal viral infections. *Bull World Health Organ* 1977;**55**:445–53.
23. Balfour Jr HH, Schiff GM, Bloom JE. Encephalitis associated with erythema infectiosum. *J Pediatr* 1970;**77**:133–6.
24. Banwell B, Kennedy J, Sadovnick D, et al. Incidence of acquired demyelination of the CNS in Canadian children. *Neurology* 2009;**72**:232–9.
25. Barnes PD, Poussaint TY, Burrows PE. Imaging of pediatric central nervous system infections. *Neuroimaging Clin North Am* 1994;**4**:367–91.
26. Barontini F, Leoncini F. Brainstem encephalitis due to *Listeria monocytogenes:* favourable outcome after early antibiotic therapy. *Ital J Neurol Sci* 1989;**10**:85–7.
27. Baykan B, Kinay D, Gokyigit A, et al. Periodic lateralized epileptiform discharges: association with seizures. *Seizure* 2000;**9**:402–6.
28. Becker LE. Infections of the developing brain. *AJNR Am J Neuroradiol* 1992;**13**:537–49.
29. Beersma MF, Dirven K, van Dam AP, et al. Evaluation of 12 commercial tests and the complement fixation test for *Mycoplasma pneumoniae*-specific immunoglobulin G (IgG) and IgM antibodies, with PCR used as the "gold standard." *J Clin Microbiol* 2005; **43**:2277–85.
30. Beghi E, Nicolosi A, Kurland LT, et al. Encephalitis and aseptic meningitis, Olmsted County, Minnesota, 1950-1981, part I: epidemiology. *Ann Neurol* 1984;**16**:283–94.
31. Bellini WJ, Harcourt BH, Bowden N, et al. Nipah virus: an emergent paramyxovirus causing severe encephalitis in humans. *J Neurovirol* 2005;**11**:481–7.
32. Bellini WJ, Rota JS, Lowe LE, et al. Subacute sclerosing panencephalitis: more cases of this fatal disease are prevented by measles immunization than was previously recognized. *J Infect Dis* 2005; **192**:1686–93.
33. Berge TO, editor. *International catalogue of arboviruses including certain other viruses of vertebrates*. 2nd ed. Atlanta: U.S. Department of Health, Education and Welfare, Public Health Service; 1975.
34. Bergey GK, Coyle PK, Krumholz A, et al. Herpes simplex encephalitis with occipital localization. *Arch Neurol* 1982;**39**: 312–3.
35. Biggar RJ, Woodall JP, Walter PD, et al. Lymphocytic choriomeningitis outbreak associated with pet hamsters: fifty-seven cases from New York State. *JAMA* 1975;**232**:494–500.
36. Bingham PM, Galetta SL, Athreya B, et al. Neurologic manifestations in children with Lyme disease. *Pediatrics* 1995;**96**: 1053–6.
37. Bitnun A, Ford-Jones E, Blaser S, et al. *Mycoplasma pneumoniae* encephalitis. *Semin Pediatr Infect Dis* 2003;**14**:96–107.
38. Bitnun A, Ford-Jones EL, Petric M, et al. Acute childhood encephalitis and *Mycoplasma pneumoniae*. *Clin Infect Dis* 2001;**32**: 1674–84.
39. Bitnun A, Richardson SE. *Mycoplasma pneumoniae:* Innocent bystander or a true cause of central nervous system disease? *Curr Infect Dis Rep* 2010;**12**:282–90.
40. Blatt NH, Lepper MH. Reactions following antirabies prophylaxis; report on sixteen patients. *AMA Am J Dis Child* 1953; **86**:395–402.
41. Bodi I, Dutt N, Hampton T, et al. Fatal granulomatous amoebic meningoencephalitis due to *Balamuthia mandrillaris*. *Pathol Res Pract* 2008;**204**:925–8.
42. Boe J, Solberg CO, Saeter T. Corticosteroid treatment for acute meningoencephalitis: a retrospective study of 346 cases. *BMJ* 1965;**1**:1094–5.
43. Boivin G, Abed Y, Boucher FD. Human parechovirus 3 and neonatal infections. *Emerg Infect Dis* 2005;**11**:103–5.
44. Bolay H, Soylemezoglu F, Nurlu G, et al. PCR detected hepatitis C virus genome in the brain of a case with progressive encephalomyelitis with rigidity. *Clin Neurol Neurosurg* 1996;**98**: 305–8.
45. Bollen AE, Wartan AN, Krikke AP, et al. Amnestic syndrome after lung transplantation by human herpes virus–6 encephalitis. *J Neurol* 2001;**248**:619–20.
46. Borawake K, Prayag P, Wagh A, et al. Dengue encephalitis. *Indian J Crit Care Med* 2011;**15**:190–3.
47. Boulloche J, Parain D, Mallet E, et al. Postinfectious encephalitis with multifocal white matter lesions. *Neuropediatrics* 1989;**20**: 173–5.
48. Brown EH. Complications of smallpox vaccination. *Postgrad Med J* 1965;**41**:634–5.
49. Bruhn FW. Lyme disease. *Am J Dis Child* 1984;**138**:467–70.
50. Buck PA, Sockett P, Barker IK, et al. West Nile Virus: Surveillance activities in Canada. *Ann Epidemiol* 2003;**13**:582.
51. Cakmakci H, Kovanlikaya A, Obuz F, et al. Herpes encephalitis in children. MRI assessment. *Turk J Pediatr* 1998;**40**:559–66.
52. Calitri C, Gabiano C, Garazzino S, et al. Clinical features of hospitalised children with 2009 H1N1 influenza virus infection. *Eur J Pediatr* 2010;**169**:1511–5.
53. Camarata PJ, McGeachie RE, Haines SJ. Dorsal midbrain encephalitis caused by *Propionibacterium acnes:* report of two cases. *J Neurosurg* 1990;**72**:654–9.
54. Carithers HA, Margileth AM. Cat-scratch disease: acute encephalopathy and other neurologic manifestations. *Am J Dis Child* 1991;**145**:98–101.
55. Carmack MA, Twiss J, Enzmann DR, et al. Multifocal leukoencephalitis caused by varicella-zoster virus in a child with

leukemia: successful treatment with acyclovir. *Pediatr Infect Dis J* 1993;**12**:402–6.
56. Carr-Locke DL, Mair HJ. Neurological presentation of psittacosis during a small outbreak in Leicestershire. *BMJ* 1976;**2**:853–4.
57. Caruso JM, Tung GA, Gascon GG, et al. Persistent preceding focal neurologic deficits in children with chronic Epstein-Barr virus encephalitis. *J Child Neurol* 2000;**15**:791–6.
58. Cary LC, Maul E, Potter C, et al. *Balamuthia mandrillaris* meningoencephalitis: survival of a pediatric patient. *Pediatrics* 2010;**125**: e699–703.
59. Caserta MT, Hall CB, Schnabel K, et al. Neuroinvasion and persistence of human herpesvirus 6 in children. *J Infect Dis* 1994;**170**:1586–9.
60. Cassell GH, Cole BC. Mycoplasmas as agents of human disease. *N Engl J Med* 1981;**304**:80–9.
61. Centers for Disease Control and Prevention. Measles surveillance: 1973-6; July 1977.
62. Centers for Disease Control and Prevention. Encephalitis surveillance: annual summary 1977. Atlanta, Ga.: U.S. Dept. of Health, Education, and Welfare, Public Health Service; December 1979.
63. Centers for Disease Control and Prevention. Encephalitis surveillance: annual summary 1978. Atlanta, Ga.: U.S. Dept. of Health, Education, and Welfare, Public Health Service; May 1981.
64. Centers for Disease Control and Prevention. Rabies surveillance: annual summary 1980-2; August 1983.
65. Centers for Disease Control and Prevention. Mumps surveillance, January 1977–December 1982; September 1984.
66. Centers for Disease Control and Prevention. Rabies prevention: United States, 1984. *MMWR Morb Mortal Wkly Rep* 1984;**33**: 393–408.
67. Centers for Disease Control and Prevention. Dengue fever at the U.S.-Mexico border, 1995-1996. *MMWR Morb Mortal Wkly Rep* 1996;**45**:841–4.
68. Centers for Disease Control and Prevention. Underdiagnosis of dengue—Laredo, Texas, 1999. *MMWR Morb Mortal Wkly Rep* 2001;**50**:57–9.
69. Centers for Disease Control and Prevention. West Nile virus activity—eastern United States, 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:617–9.
70. Centers for Disease Control and Prevention. Raccoon roundworm encephalitis—Chicago, Illinois, and Los Angeles, California, 2000. *MMWR Morb Mortal Wkly Rep* 2002;**50**:1153–5.
71. Centers for Disease Control and Prevention. Enterovirus surveillance—United States, 1970-2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1–20.
72. Centers for Disease Control and Prevention. Enterovirus surveillance—United States, 2002-2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:153–6.
73. Centers for Disease Control and Prevention. Update: multistate outbreak of mumps—United States, January 1–May 2, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:559–63.
74. Centers for Disease Control and Prevention. Update: chikungunya fever diagnosed among international travelers—United States, 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:276–7.
75. Centers for Disease Control and Prevention. *Balamuthia* amebic encephalitis—California, 1999-2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:768–71.
76. Centers for Disease Control and Prevention. Outbreak of measles—San Diego, California, January-February 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:203–6.
77. Centers for Disease Control and Prevention. Neurologic complications associated with novel influenza A (H1N1) virus infection in children—Dallas, Texas, May 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:773–8.
78. Centers for Disease Control and Prevention. Locally acquired dengue—Key West, Florida, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:577–81.
79. Centers for Disease Control and Prevention. Presumptive abortive human rabies—Texas, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:185–90.
80. Centers for Disease Control and Prevention. Increased transmission and outbreaks of measles—European Region, 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1605–10.
81. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1009–13.
82. Centers for Disease Control and Prevention. Recovery of a patient from clinical rabies—California, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:61–5.
83. Chadha MS, Arankalle VA, Jadi RS, et al. An outbreak of Chandipura virus encephalitis in the eastern districts of Gujarat state, India. *Am J Trop Med Hyg* 2005;**73**:566–70.
84. Chang DI, Park JH, Chung KC. Encephalitis and polyradiculoneuritis following rubella virus infection—a case report. *J Korean Med Sci* 1997;**12**:168–70.
85. Chang LY, Lin TY, Huang YC, et al. Comparison of enterovirus 71 and coxsackie-virus A16 clinical illnesses during the Taiwan enterovirus epidemic, 1998. *Pediatr Infect Dis J* 1999;**18**:1092–6.
86. Chataway SJ, Larner AJ, Kapoor R. Anti-GQ1b antibody status, magnetic resonance imaging, and the nosology of Bickerstaff's brainstem encephalitis. *Eur J Neurol* 2001;**8**:355–7.
87. Chaudhuri A, Kennedy PG. Diagnosis and treatment of viral encephalitis. *Postgrad Med J* 2002;**78**:575–83.
88. Chen YC, Lo CP, Chang TP. Novel influenza A (H1N1)-associated encephalopathy/encephalitis with severe neurological sequelae and unique image features—a case report. *J Neurol Sci* 2010;**298**:110–3.
89. Cherry JD. The epidemiology of pertussis and pertussis immunization in the United Kingdom and the United States: a comparative study. *Curr Probl Pediatr* 1984;**14**:1–78.
90. Cherry JD. "Pertussis vaccine encephalopathy": it is time to recognize it as the myth that it is. *JAMA* 1990;**263**:1679–80.
91. Cherry JD, Brunell PA, Golden GS, et al. Report of the Task Force on Pertussis and Pertussis Immunization—1988. *Pediatrics* 1988;**81**:939–84.
92. Chieochansin T, Vichiwattana P, Korkong S, et al. Molecular epidemiology, genome characterization, and recombination event of human parechovirus. *Virology* 2011;**421**:159–66.
93. Chik KW, Chan PK, Li CK, et al. Human herpesvirus-6 encephalitis after unrelated umbilical cord blood transplant in children. *Bone Marrow Transplant* 2002;**29**:991–4.
94. Chonmaitree T, Menegus MA, Schervish-Swierkosz EM, et al. Enterovirus 71 infection: report of an outbreak with two cases of paralysis and a review of the literature. *Pediatrics* 1981;**67**: 489–93.
95. Christie LJ, Honarmand S, Talkington DF, et al. Pediatric encephalitis: what is the role of *Mycoplasma pneumoniae? Pediatrics* 2007;**120**:305–13.
96. Chun CS, Kazacos KR, Glaser C, et al. Global neurologic deficits with *Baylisascaris* encephalitis in a previously healthy teenager. *Pediatr Infect Dis J* 2009;**28**:925–7.
97. Civen R, Villacorte F, Robles DT, et al. West Nile virus infection in the pediatric population. *Pediatr Infect Dis J* 2006;**25**:75–8.
98. Cizman M, Jazbec J. Etiology of acute encephalitis in childhood in Slovenia. *Pediatr Infect Dis J* 1993;**12**:903–8.
99. Claassen J, Hirsch LJ, Emerson RG, et al. Continuous EEG monitoring and midazolam infusion for refractory nonconvulsive status epilepticus. *Neurology* 2001;**57**:1036–42.
100. Clark DA. Human herpesvirus 6 and human herpesvirus 7: emerging pathogens in transplant patients. *Int J Hematol* 2002;**76**(Suppl 2):246–52.
101. Clyde WA, Jr. Neurological syndromes and mycoplasmal infections. *Arch Neurol* 1980;**37**:65–6.
102. Connelly KP, DeWitt LD. Neurologic complications of infectious mononucleosis. *Pediatr Neurol* 1994;**10**:181–4.
103. Connolly AM, Dodson WE, Prensky AL, et al. Course and outcome of acute cerebellar ataxia. *Ann Neurol* 1994;**35**:673–9.
104. Corsellis JA, Janota I, Marshall AK. Immunization against whooping cough: a neuropathological review. *Neuropathol Appl Neurobiol* 1983;**9**:261–70.
105. Crawford JR, Kadom N, Santi MR, et al. Human herpesvirus 6 rhombencephalitis in immunocompetent children. *J Child Neurol* 2007;**22**:1260–8.
106. Cunha BA, Fatehpuria R, Eisenstein LE. *Listeria monocytogenes* encephalitis mimicking herpes simplex virus encephalitis: the differential diagnostic importance of cerebrospinal fluid lactic acid levels. *Heart Lung* 2007;**36**:226–31.
107. Cunha BA, Filozov A, Reme P. *Listeria monocytogenes* encephalitis mimicking West Nile encephalitis. *Heart Lung* 2004;**33**:61–4.
108. Dagan R, Shahak E. Prolonged meningoencephalitis due to Epstein-Barr virus with favorable outcome in a young infant. *Infection* 1993;**21**:400–2.

109. Dale RC. Acute disseminated encephalomyelitis. *Semin Pediatr Infect Dis* 2003;**14**:90–5.
110. Dangond F, Engle E, Yessayan L, et al. Pre-eruptive varicella cerebellitis confirmed by PCR. *Pediatr Neurol* 1993;**9**:491–3.
111. Davis LE. Neurologic and muscular complications of the 2009 influenza A (H1N1) pandemic. *Curr Neurol Neurosci Rep* 2010; **10**:476–83.
112. Davison KL, Crowcroft NS, Ramsay ME, et al. Viral encephalitis in England, 1989-1998: what did we miss? *Emerg Infect Dis* 2003;**9**:234–40.
113. Daxboeck F. *Mycoplasma pneumoniae* central nervous system infections. *Curr Opin Neurol* 2006;**19**:374–8.
114. Daxboeck F, Blacky A, Seidl R, et al. Diagnosis, treatment, and prognosis of *Mycoplasma pneumoniae* childhood encephalitis: systematic review of 58 cases. *J Child Neurol* 2004;**19**:865–71.
115. De Deyn PP, Van den Broucke PW, Pickut BA, et al. Perfusion and thallium single photon emission computed tomography in herpes simplex encephalitis. *J Neurol Sci* 1998;**157**:96–9.
116. Debiasi RL, Tyler KL. West Nile virus meningoencephalitis. *Nat Clin Pract Neurol* 2006;**2**:264–75.
117. Deetz TR, Sawyer MH, Billman G, et al. Successful treatment of *Balamuthia* amoebic encephalitis: presentation of 2 cases. *Clin Infect Dis* 2003;**37**:1304–12.
118. Deibel R, Flanagan TD. Central nervous system infections: etiologic and epidemiologic observations in New York State, 1976-1977. *N Y State J Med* 1979;**79**:689–95.
119. Deibel R, Flanagan TD, Smith V. Central nervous system infections in New York State: etiologic and epidemiologic observations, 1974. *N Y State J Med* 1975;**75**:2337–42.
120. Deibel R, Flanagan TD, Smith V. Central nervous system infections: etiologic and epidemiologic observations in New York State, 1975. *N Y State J Med* 1977;**77**:1398–404.
121. DeLorenzo RJ, Waterhouse EJ, Towne AR, et al. Persistent nonconvulsive status epilepticus after the control of convulsive status epilepticus. *Epilepsia* 1998;**39**:833–40.
122. Deresiewicz RL, Thaler SJ, Hsu L, et al. Clinical and neuroradiographic manifestations of eastern equine encephalitis. *N Engl J Med* 1997;**336**:1867–74.
123. DeVincenzo JP, Thorne G. Mild herpes simplex encephalitis diagnosed by polymerase chain reaction: a case report and review. *Pediatr Infect Dis J* 1994;**13**:662–4.
124. Diaz JH. The public health threat from *Balamuthia mandrillaris* in the southern United States. *J La State Med Soc* 2011;**163**: 197–204.
125. Dickey M, Jamison L, Michaud L, et al. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. *Pediatr Infect Dis J* 2009;**28**:318–21.
126. Dimopoulou I, Politis P, Panagyiotakopoulos G, et al. Leptospirosis presenting with encephalitis-induced coma. *Intensive Care Med* 2002;**28**:1682.
127. Doja A, Bitnun A, Ford Jones EL, et al. Pediatric Epstein-Barr virus–associated encephalitis: 10-year review. *J Child Neurol* 2006; **21**:384–91.
128. Domenech C, Leveque N, Lina B, et al. Role of *Mycoplasma pneumoniae* in pediatric encephalitis. *Eur J Clin Microbiol Infect Dis* 2009;**28**:91–4.
129. Donat JF, Rhodes KH, Groover RV, et al. Etiology and outcome in 42 children with acute nonbacterial meningoencephalitis. *Mayo Clin Proc* 1980;**55**:156–60.
130. Douvoyiannis M, Litman N, Goldman DL. Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis* 2009;**48**:1713–23.
131. Drancourt M, Raoult D, Xeridat B, et al. Q fever meningoencephalitis in five patients. *Eur J Epidemiol* 1991;**7**:134–8.
132. Dutt MK, Johnston ID. Computed tomography and EEG in herpes simplex encephalitis: their value in diagnosis and prognosis. *Arch Neurol* 1982;**39**:99–102.
133. Earnest MP, Goolishian HA, Calverley JR, et al. Neurologic, intellectual, and psychologic sequelae following western encephalitis: a follow-up study of 35 cases. *Neurology* 1971;**21**: 969–74.
134. Elbers JM, Bitnun A, Richardson SE, et al. A 12-year prospective study of childhood herpes simplex encephalitis: is there a broader spectrum of disease? *Pediatrics* 2007;**119**:e399–407.
135. Elliott KJ. Other neurological complications of herpes zoster and their management. *Ann Neurol* 1994;**35**(Suppl):S57–61.
136. Enders JF. Mumps. In: Rivers TM, Horsfall Jr FL, editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1959. p. 780–9.
137. Estrada-Franco JG, Navarro-Lopez R, Beasley DW, et al. West Nile virus in Mexico: evidence of widespread circulation since July 2002. *Emerg Infect Dis* 2003;**9**:1604–7.
138. Farooq O, Faden HS, Cohen ME, et al. Neurologic complications of 2009 influenza-A H1N1 infection in children. *J Child Neurol* 2012;**27**:431–8.
139. Fazekas F, Roob G, Payer F, et al. Technetium-99m-ECD SPECT fails to show focal hyperemia of acute herpes encephalitis. *J Nucl Med* 1998;**39**:790–2.
140. Figueroa JP, Ashley D, King D, et al. An outbreak of acute flaccid paralysis in Jamaica associated with echovirus type 22. *J Med Virol* 1989;**29**:315–9.
141. Filia A, Brenna A, Pana A, et al. Health burden and economic impact of measles-related hospitalizations in Italy in 2002-3. *BMC Public Health* 2007;**7**:169.
142. Filia A, Tavilla A, Bella A, et al. Measles in Italy, July 2009 to September 2010. *Eur Surveill* 2011;**16**(29):19925.
143. Finley KH, Fitzgerald LH, Richter RW, et al. Western encephalitis and cerebral ontogenesis. *Arch Neurol* 1967;**16**:140–64.
144. Fischer SA, Graham MB, Kuehnert MJ, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N Engl J Med* 2006;**354**:2235–49.
145. Flewett TH, Hoult JG. Influenzal encephalopathy and postinfluenzal encephalitis. *Lancet* 1958;**2**:11–5.
146. Flexner S. Postvaccinal encephalitis and allied conditions. *JAMA* 1930;**94**:305–11.
147. Forman ML, Cherry JD. *Isolation of measles virus from the cerebrospinal fluid of a child with encephalitis following measles vaccination*. Evanston, Ill.: The Program for the American Pediatric Society; 1967.
148. Fouch B, Coventry S. A case of fatal disseminated *Bartonella henselae* infection (cat-scratch disease) with encephalitis. *Arch Pathol Lab Med* 2007;**131**:1591–4.
149. Francisco AM, Glaser C, Frykman E, et al. 2004 California pediatric West Nile virus case series. *Pediatr Infect Dis J* 2006; **25**:81–4.
150. Frange P, Peffault de Latour R, Arnaud C, et al. Adenoviral infection presenting as an isolated central nervous system disease without detectable viremia in two children after stem cell transplantation. *J Clin Microbiol* 2011;**49**:2361–4.
151. Frobert E, Sarret C, Billaud G, et al. Pediatric neurological complications associated with the A(H1N1)pdm09 influenza infection. *J Clin Virol* 2011;**52**:307–13.
152. Fryden A, Kihlstrom E, Maller R, et al. A clinical and epidemiological study of "ornithosis" caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). *Scand J Infect Dis* 1989; **21**:681–91.
153. Fujimaki K, Mori T, Kida A, et al. Human herpesvirus 6 meningoencephalitis in allogeneic hematopoietic stem cell transplant recipients. *Int J Hematol* 2006;**84**:432–7.
154. Fuller GN, Guiloff RJ, Scaravilli F, et al. Combined HIV-CMV encephalitis presenting with brainstem signs. *J Neurol Neurosurg Psychiatry* 1989;**52**:975–9.
155. Gable MS, Sheriff H, Dalmau J, et al. The frequency of autoimmune *N*-methyl-D-aspartate receptor encephalitis surpasses that of individual viral etiologies in young individuals enrolled in the California Encephalitis Project. *Clin Infect Dis* 2012;**54**:899–904.
156. Gavin PJ, Kazacos KR, Shulman ST. Baylisascariasis. *Clin Microbiol Rev* 2005;**18**:703–18.
157. Gavin PJ, Shulman ST. Raccoon roundworm *(Baylisascaris procyonis)*. *Pediatr Infect Dis J* 2003;**22**:651–2.
158. Gens RD, Beecham 3rd HJ. Meningoencephalitis after influenza inoculation. *N Engl J Med* 1978;**299**:721–2.
159. Gerber JE, Johnson JE, Scott MA, et al. Fatal meningitis and encephalitis due to *Bartonella henselae* bacteria. *J Forensic Sci* 2002;**47**:640–4.
160. Ghosh PS, Ghosh D, Loddenkemper T, et al. Necrotizing granulomatous meningoencephalitis due to *Balamuthia* in an immunocompetent child. *Neurology* 2011;**77**:801–2.
161. Gibbons JL, Miller HG, Stanton JB. Para-infectious encephalomyelitis and related syndromes; a critical review of the neurological complications of certain specific fevers. *Q J Med* 1956;**25**: 427–505.

162. Gibbons RV, Vaughn DW. Dengue: an escalating problem. *BMJ* 2002;**324**:1563–6.
163. Glaser CA, Gilliam S, Schnurr D, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000. *Clin Infect Dis* 2003;**36**:731–42.
164. Glaser CA, Honarmand S, Anderson LJ, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. *Clin Infect Dis* 2006;**43**:1565–77.
165. Glezen WP. Considerations of the risk of influenza in children and indications for prophylaxis. *Rev Infect Dis* 1980;**2**: 408–20.
166. Glezen WP, Paredes A, Taber LH. Influenza in children: relationship to other respiratory agents. *JAMA* 1980;**243**:1345–9.
167. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000;**342**:1229–35.
168. Golden SE. Aseptic meningitis associated with *Ehrlichia canis* infection. *Pediatr Infect Dis J* 1989;**8**:335–7.
169. Goldwater PN, Rowland K, Thesinger M, et al. Rotavirus encephalopathy: pathogenesis reviewed. *J Paediatr Child Health* 2001;**37**:206–9.
170. Gorman RJ, Saxon S, Snead 3rd OC. Neurologic sequelae of Rocky Mountain spotted fever. *Pediatrics* 1981;**67**:354–7.
171. Graham AS, Pruszynski CA, Hribar LJ, et al. Mosquito-associated dengue virus, Key West, Florida, USA, 2010. *Emerg Infect Dis* 2011;**17**:2074–5.
172. Granerod J, Ambrose HE, Davies NW, et al. Causes of encephalitis and differences in their clinical presentations in England: a multicentre, population-based prospective study. *Lancet Infect Dis* 2010;**10**:835–44.
173. Greenberg DA, Weinkle DJ, Aminoff MJ. Periodic EEG complexes in infectious mononucleosis encephalitis. *J Neurol Neurosurg Psychiatry* 1982;**45**:648–51.
174. Greenberg M. Complications of vaccination against smallpox. *Am J Dis Child* 1948;**76**:492–502.
175. Greenberg SB, Taber L, Septimus E, et al. Computerized tomography in brain biopsy–proven herpes simplex encephalitis: early normal results. *Arch Neurol* 1981;**38**:58–9.
176. Griesemer DA, Barton LL, Reese CM, et al. Amebic meningoencephalitis caused by *Balamuthia mandrillaris*. *Pediatr Neurol* 1994; **10**:249–54.
177. Griffin DE. Emergence and re-emergence of viral diseases of the central nervous system. *Prog Neurobiol* 2010;**91**:95–101.
178. Griffin DE. Post-infectious and post-vaccinal disorders of the central nervous system. *Immunol Allergy Clin North Am* 1988;**8**: 239–49.
179. Griffith AH. Permanent brain damage and pertussis vaccination: is the end of the saga in sight? *Vaccine* 1989;**7**:199–210.
180. Griffiths P. Cytomegalovirus infection of the central nervous system. *Herpes* 2004;**11**(Suppl 2):95A–104A.
181. Grist NR, Bell EJ, Assaad F. Enteroviruses in human disease. *Prog Med Virol* 1978;**24**:114–57.
182. Gross WL, Ravens KG, Hansen HW. Meningoencephalitis syndrome following influenza vaccination. *J Neurol* 1978;**217**: 219–22.
183. Grossman M, Azimi PH. An encephalitis syndrome in a seven-year-old. *Pediatr Infect Dis J* 1995;**14**:550–5.
184. Gubler DJ. The continuing spread of West Nile virus in the western hemisphere. *Clin Infect Dis* 2007;**45**:1039–46.
185. Guerrero IC, Retailliau HF, Brandling-Bennett AD, et al. No increased meningoencephalitis after influenza vaccine. *N Engl J Med* 1979;**300**:565.
186. Guler E, Davutoglu M, Guler S, et al. Encephalitis in a child during atypical course of rubella. *Infection* 2009;**37**:65–6.
187. Guleria R, Nisar N, Chawla TC, et al. *Mycoplasma pneumoniae* and central nervous system complications: a review. *J Lab Clin Med* 2005;**146**:55–63.
188. Gupta S, Fernandez D, Siddiqui A, et al. Extensive white matter abnormalities associated with neonatal parechovirus (HPeV) infection. *Eur J Paediatr Neurol* 2010;**14**:531–4.
189. Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. *PLoS One* 2009;**4**:e6145.
190. Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus–6 infection in children: a prospective study of complications and reactivation. *N Engl J Med* 1994;**331**:432–8.
191. Hamilton RL, Achim C, Grafe MR, et al. Herpes simplex virus brainstem encephalitis in an AIDS patient. *Clin Neuropathol* 1995;**14**:45–50.
192. Hanshaw JB, Dudgeon JA, Marshall WC. Congenital cytomegalovirus. In: Hanshaw JB, Dudgeon JA, Marshall WC, editors. *Viral diseases of the fetus and newborn*. 2nd ed. Philadelphia: W. B. Saunders; 1985. p. 92–131.
193. Hara M, Mizuochi T, Kawano G, et al. A case of clinically mild encephalitis with a reversible splenial lesion (MERS) after mumps vaccination. *Brain Dev* 2011;**33**:842–4.
194. Harit AK, Ichhpujani RL, Gupta S, et al. Nipah/Hendra virus outbreak in Siliguri, West Bengal, India in 2001. *Indian J Med Res* 2006;**123**:553–60.
195. Harvala H, Wolthers KC, Simmonds P. Parechoviruses in children: understanding a new infection. *Curr Opin Infect Dis* 2010; **23**:224–30.
196. Hasegawa Y, Morishita M, Ikeda T, et al. [Early diagnosis of herpes simplex virus encephalitis by single photon emission computed tomography (SPECT) in patients with normal MRI]. *Rinsho Shinkeigaku* 1996;**36**:475–80.
197. Hattori H, Kawamori J, Takao T, et al. Computed tomography in postinfluenzal encephalitis. *Brain Dev* 1983;**5**:564–7.
198. Hausler M, Schaade L, Kemeny S, et al. Encephalitis related to primary varicella-zoster virus infection in immunocompetent children. *J Neurol Sci* 2002;**195**:111–6.
199. Hayes EB, O'Leary DR. West Nile virus infection: a pediatric perspective. *Pediatrics* 2004;**113**:1375–81.
200. Hayes EB, Sejvar JJ, Zaki SR, et al. Virology, pathology, and clinical manifestations of West Nile virus disease. *Emerg Infect Dis* 2005;**11**:1174–9.
201. Haynes RE, Sanders DY, Cramblett HG. Rocky Mountain spotted fever in children. *J Pediatr* 1970;**76**:685–93.
202. Hill CP, Damodaran O, Walsh P, et al. *Balamuthia* amebic meningoencephalitis and mycotic aneurysms in an infant. *Pediatr Neurol* 2011;**45**:45–8.
203. Ho DD, Hirsch MS. Acute viral encephalitis. *Med Clin North Am* 1985;**69**:415–29.
204. Ho M. Enterovirus 71: the virus, its infections and outbreaks. *J Microbiol Immunol Infect* 2000;**33**:205–16.
205. Hoke Jr CH, Vaughn DW, Nisalak A, et al. Effect of high-dose dexamethasone on the outcome of acute encephalitis due to Japanese encephalitis virus. *J Infect Dis* 1992;**165**:631–7.
206. Homaira N, Rahman M, Hossain MJ, et al. Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007. *Epidemiol Infect* 2010;**138**:1630–6.
207. Homaira N, Rahman M, Hossain MJ, et al. Cluster of Nipah virus infection, Kushtia District, Bangladesh, 2007. *PLoS One* 2010;**5**:e13570.
208. Hsu VP, Hossain MJ, Parashar UD, et al. Nipah virus encephalitis reemergence, Bangladesh. *Emerg Infect Dis* 2004;**10**:2082–7.
209. Huang CC, Liu CC, Chang YC, et al. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999; **341**:936–42.
210. Huang CR, Chang WN, Lui CC, et al. Neuroimages of Japanese encephalitis: report of three patients. *Zhonghua Yi Xue Za Zhi (Taipei)* 1997;**60**:105–8.
211. Huang MC, Wang SM, Hsu YW, et al. Long-term cognitive and motor deficits after enterovirus 71 brainstem encephalitis in children. *Pediatrics* 2006;**118**:e1785–8.
212. Hughes RR. Neurological complications of serum and vaccine therapy. *Lancet* 1944;**2**:464–7.
213. Huhn GD, Sejvar JJ, Montgomery SP, et al. West Nile virus in the United States: an update on an emerging infectious disease. *Am Fam Physician* 2003;**68**:653–60.
214. Hummeler K, Davidson WL, Henle W, et al. Encephalomyelitis due to infection with herpesvirus simiae (herpes B virus); a report of two fatal, laboratory-acquired cases. *N Engl J Med* 1959; **261**:64–8.
215. Huppatz C, Durrheim DN, Levi C, et al. Etiology of encephalitis in Australia, 1990-2007. *Emerg Infect Dis* 2009;**15**:1359–65.
216. Incecik F, Ozlem Herguner M, Altunbasak S, et al. Fatal encephalitis associated with novel influenza A (H1N1) virus infection in a child. *Neurol Sci* 2012;**33**:677–9.
217. Irving WL, Chang J, Raymond DR, et al. Roseola infantum and other syndromes associated with acute HHV-6 infection. *Arch Dis Child* 1990;**65**:297–300.

218. Isaacson E, Glaser CA, Forghani B, et al. Evidence of human herpesvirus 6 infection in 4 immunocompetent patients with encephalitis. *Clin Infect Dis* 2005;**40**:890–3.
219. Ishiguro N, Yamada S, Takahashi T, et al. Meningo-encephalitis associated with HHV-6 related exanthem subitum. *Acta Paediatr Scand* 1990;**79**:987–9.
220. Jackson AC. Rabies pathogenesis. *J Neurovirol* 2002;**8**:267–9.
221. Jackson MA, Burry VF, Olson LC. Complications of varicella requiring hospitalization in previously healthy children. *Pediatr Infect Dis J* 1992;**11**:441–5.
222. Jean CM, Honarmand S, Louie JK, et al. Risk factors for West Nile virus neuroinvasive disease, California, 2005. *Emerg Infect Dis* 2007;**13**:1918–20.
223. Jemsek J, Greenberg SB, Taber L, et al. Herpes zoster–associated encephalitis: clinicopathologic report of 12 cases and review of the literature. *Medicine (Baltimore)* 1983;**62**:81–97.
224. Johnson HN. Rabies. In: Rivers TM, Horsfall Jr FJ, editors. *Viral and rickettsial infections of man*. 3rd ed. Philadelphia: J. B. Lippincott; 1959. p. 405–31.
225. Johnson N, Fooks A, McColl K. Human rabies case with long incubation, Australia. *Emerg Infect Dis* 2008;**14**:1950–1.
226. Johnson RT. Acute encephalitis. *Clin Infect Dis* 1996;**23**:219–24; quiz 225–216.
227. Johnson RT. The pathogenesis of acute viral encephalitis and postinfectious encephalomyelitis. *J Infect Dis* 1987;**155**:359–64.
228. Johnson RT. *Viral infections of the nervous system*. New York: Raven Press; 1982.
229. Johnson RT. The virology of demyelinating diseases. *Ann Neurol* 1994;**36**:S54–60.
230. Johnson RT. Virus invasion of the central nervous system: a study of sindbis virus infection in the mouse using fluorescent antibody. *Am J Pathol* 1965;**46**:929–43.
231. Kansagra AP, Menon JP, Yarbrough CK, et al. *Balamuthia mandrillaris* meningoencephalitis in an immunocompromised patient: case report. *J Neurosurg* 2009;**111**:301–5.
232. Kanzaki A, Yubuki S, Yuki N. Bickerstaff's brainstem encephalitis associated with cytomegalovirus infection. *J Neurol Neurosurg Psychiatry* 1993;**58**:260–1.
233. Kaplan MH, Klein SW, McPhee J, et al. Group B coxsackievirus infections in infants younger than three months of age: a serious childhood illness. *Rev Infect Dis* 1983;**5**:1019–32.
234. Kappus KD, Sather GE, Kaplan JE, et al. Human arboviral infections in the United States in 1980. *J Infect Dis* 1982;**145**:283–6.
235. Kato T, Ishii C, Furusho J, et al. Early diagnosis of herpes encephalopathy using fluid-attenuated inversion recovery pulse sequence. *Pediatr Neurol* 1998;**19**:58–61.
236. Katz DA, Dworzack DL, Horowitz EA, et al. Encephalitis associated with Rocky Mountain spotted fever. *Arch Pathol Lab Med* 1985;**109**:771–3.
237. Kaushal V, Chhina DK, Kumar R, et al. *Acanthamoeba* encephalitis. *Indian J Med Microbiol* 2008;**26**:182–4.
238. Kennedy C. Acute viral encephalitis in childhood. *BMJ* 1995; **310**:139–40.
239. Kennedy F. Certain nervous complications following the use of therapeutic and prophylactic sera. *Am J Med Sci* 1929;**177**: 555–9.
240. Kesselring J, Miller DH, Robb SA, et al. Acute disseminated encephalomyelitis. MRI findings and the distinction from multiple sclerosis. *Brain* 1990;**113**(Pt 2):291–302.
241. Khan NA. *Acanthamoeba* invasion of the central nervous system. *Int J Parasitol* 2007;**37**:131–8.
242. Khetsuriani N, Holman RC, Anderson LJ. Burden of encephalitis-associated hospitalizations in the United States, 1988-1997. *Clin Infect Dis* 2002;**35**:175–82.
243. Knobel DL, Cleaveland S, Coleman PG, et al. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 2005;**83**:360–8.
244. Kobayashi S, Negishi Y, Ando N, et al. Two patients with acute rotavirus encephalitis associated with cerebellar signs and symptoms. *Eur J Pediatr* 2010;**169**:1287–91.
245. Koelfen W, Freund M, Guckel F, et al. MRI of encephalitis in children: comparison of CT and MRI in the acute stage with long-term follow-up. *Neuroradiology* 1996;**38**:73–9.
246. Kohl S, James AR. Herpes simplex virus encephalitis during childhood: importance of brain biopsy diagnosis. *J Pediatr* 1985; **107**:212–5.
247. Kolski H, Ford-Jones EL, Richardson S, et al. Etiology of acute childhood encephalitis at The Hospital for Sick Children, Toronto, 1994-1995. *Clin Infect Dis* 1998;**26**:398–409.
248. Koskiniemi M. CNS manifestations associated with *Mycoplasma pneumoniae* infections: summary of cases at the University of Helsinki and review. *Clin Infect Dis* 1993;**17**(Suppl 1):S52–57.
249. Koskiniemi M, Korppi M, Mustonen K, et al. Epidemiology of encephalitis in children: a prospective multicentre study. *Eur J Pediatr* 1997;**156**:541–5.
250. Koskiniemi M, Manninen V, Vaheri A, et al. Acute encephalitis: a survey of epidemiological, clinical and microbiological features covering a twelve-year period. *Acta Med Scand* 1981;**209**:115–20.
251. Koskiniemi M, Rantalaiho T, Piiparinen H, et al. Infections of the central nervous system of suspected viral origin: a collaborative study from Finland. *J Neurovirol* 2001;**7**:400–8.
252. Kovacs SO, Kuban K, Strand R. Lateral medullary syndrome following varicella infection. *Am J Dis Child* 1993;**147**:823–5.
253. Krogstad DJ. In: Linette EH, editor. *Manual of clinical microbiology*. 4th ed. Washington, D. C.: American Society for Microbiology; 1985.
254. Kubota H, Tanabe Y, Komiya T, et al. Q fever encephalitis with cytokine profiles in serum and cerebrospinal fluid. *Pediatr Infect Dis J* 2001;**20**:318–9.
255. Kubota T, Suzuki T, Kitase Y, et al. Chronological diffusion-weighted imaging changes and mutism in the course of rotavirus-associated acute cerebellitis/cerebellopathy concurrent with encephalitis/encephalopathy. *Brain Dev* 2011;**33**:21–7.
256. Kulkarni R, Kinikar A. Encephalitis in a child with H1N1 infection: first case report from India. *J Pediatr Neurosci* 2010;**5**: 157–9.
257. Kumar S, Misra UK, Kalita J, et al. MRI in Japanese encephalitis. *Neuroradiology* 1997;**39**:180–4.
258. Lackner P, Beer R, Broessner G, et al. Acute granulomatous *Acanthamoeba* encephalitis in an immunocompetent patient. *Neurocrit Care* 2010;**12**:91–4.
259. Lakeman FD, Whitley RJ. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J Infect Dis* 1995;**171**:857–63.
260. Lam SK, Chua KB. Nipah virus encephalitis outbreak in Malaysia. *Clin Infect Dis* 2002;**34**(Suppl 2):S48–51.
261. Lanciotti RS, Kosoy OL, Laven JJ, et al. Chikungunya virus in US travelers returning from India, 2006. *Emerg Infect Dis* 2007; **13**:764–7.
262. Lanczik O, Lecei O, Schwarz S, et al. *Mycoplasma pneumoniae* infection as a treatable cause of brainstem encephalitis. *Arch Neurol* 2003;**60**:1813; author reply 1813–4.
263. Landrigan PJ, Witte JJ. Neurologic disorders following live measles-virus vaccination. *JAMA* 1973;**223**:1459–62.
264. Landry ML, Fonseca SN, Cohen S, et al. Fatal enterovirus type 71 infection: rapid detection and diagnostic pitfalls. *Pediatr Infect Dis J* 1995;**14**:1095–100.
265. Lane JM, Ruben FL, Neff JM, et al. Complications of smallpox vaccination, 1968. *N Engl J Med* 1969;**281**:1201–8.
266. Lane JM, Ruben FL, Neff JM, et al. Complications of smallpox vaccination, 1968: results of ten statewide surveys. *J Infect Dis* 1970;**122**:303–9.
267. Lau KK, Lai ST, Lai JY, et al. Acute encephalitis complicating rubella. *Hong Kong Med J* 1998;**4**:325–8.
268. Launes J, Nikkinen P, Lindroth L, et al. Diagnosis of acute herpes simplex encephalitis by brain perfusion single photon emission computed tomography. *Lancet* 1988;**1**:1188–91.
269. Leake JA, Albani S, Kao AS, et al. Acute disseminated encephalomyelitis in childhood: epidemiologic, clinical and laboratory features. *Pediatr Infect Dis J* 2004;**23**:756–64.
270. Lee KE, Umapathi T, Tan CB, et al. The neurological manifestations of Nipah virus encephalitis, a novel paramyxovirus. *Ann Neurol* 1999;**46**:428–32.
271. Leetsmo JE. Viral infection of the nervous system. In: Davis RL, Robertson DM, editors. *Textbook of neuropathology*. Baltimore: Williams & Wilkins; 1985. p. 704–87.
272. Leonard JR, Moran CJ, Cross 3rd DT, et al. MR imaging of herpes simplex type 1 encephalitis in infants and young children: a separate pattern of findings. *AJR Am J Roentgenol* 2000;**174**: 1651–5.

273. Lester Jr JW, Carter MP, Reynolds TL. Herpes encephalitis: MR monitoring of response to acyclovir therapy. *J Comput Assist Tomogr* 1988;**12**:941–3.
274. Levorson RE, Jantausch BA, Wiedermann BL, et al. Human parechovirus-3 infection: emerging pathogen in neonatal sepsis. *Pediatr Infect Dis J* 2009;**28**:545–7.
275. Lewis P, Glaser CA. Encephalitis. *Pediatr Rev* 2005;**26**:353–63.
276. Li CC, Yang MY, Chen RF, et al. Clinical manifestations and laboratory assessment in an enterovirus 71 outbreak in southern Taiwan. *Scand J Infect Dis* 2002;**34**:104–9.
277. Lin WC, Lee PI, Lu CY, et al. *Mycoplasma pneumoniae* encephalitis in childhood. *J Microbiol Immunol Infect* 2002;**35**:173–8.
278. Lindsey NP, Hayes EB, Staples JE, et al. West Nile virus disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084–9.
279. Lindsey NP, Staples JE, Lehman JA, et al. Surveillance for human West Nile virus disease—United States, 1999-2008. *MMWR Surveill Summ* 2010;**59**:1–17.
280. Linnemann Jr CC, Pretzman CI, Peterson ED. Acute febrile cerebrovasculitis: a non-spotted fever group rickettsial disease. *Arch Intern Med* 1989;**149**:1682–4.
281. Liu CC, Tseng HW, Wang SM, et al. An outbreak of enterovirus 71 infection in Taiwan, 1998: epidemiologic and clinical manifestations. *J Clin Virol* 2000;**17**:23–30.
282. Livorsi D, Anderson E, Qureshi S, et al. Brainstem encephalitis: an unusual presentation of herpes simplex virus infection. *J Neurol* 2010;**257**:1432–7.
283. Luby SP, Gurley ES, Hossain MJ. Transmission of human infection with Nipah virus. *Clin Infect Dis* 2009;**49**:1743–8.
284. Lynch M, Lee B, Azimi P, et al. Rotavirus and central nervous system symptoms: cause or contaminant? Case reports and review. *Clin Infect Dis* 2001;**33**:932–8.
285. Lyon JB, Remigio C, Milligan T, et al. Acute necrotizing encephalopathy in a child with H1N1 influenza infection. *Pediatr Radiol* 2010;**40**:200–5.
286. MacLean HJ, Douen AG. Severe amnesia associated with human herpesvirus 6 encephalitis after bone marrow transplantation. *Transplantation* 2002;**73**:1086–9.
287. Mailles A, Stahl JP. Infectious encephalitis in France in 2007: a national prospective study. *Clin Infect Dis* 2009;**49**:1838–47.
288. Makela A, Nuorti JP, Peltola H. Neurologic disorders after measles-mumps-rubella vaccination. *Pediatrics* 2002;**110**:957–63.
289. Marfin AA, Gubler DJ. West Nile encephalitis: an emerging disease in the United States. *Clin Infect Dis* 2001;**33**:1713–9.
290. Margolis FJ, Wilson JL, Top FH. Postrubella encephalomyelitis: report of cases in Detroit and review of literature. *J Pediatr* 1943;**23**:158–65.
291. Martin A, Reade EP. Acute necrotizing encephalopathy progressing to brain death in a pediatric patient with novel influenza A (H1N1) infection. *Clin Infect Dis* 2010;**50**:e50–2.
292. Masdeu JC, Van Heertum RL, Abdel-Dayem H. Viral infections of the brain. *J Neuroimaging* 1995;**5**(Suppl 1):S40–44.
293. Matsumoto N, Yano S, Miyao M, et al. Two-dimensional ultrasonography of the brain: its diagnostic usefullness in herpes simplex encephalitis and cytomegalic inclusion disease. *Brain Dev* 1983;**5**: 327–33.
294. McCullers JA, Lakeman FD, Whitley RJ. Human herpesvirus 6 is associated with focal encephalitis. *Clin Infect Dis* 1995;**21**: 571–6.
295. McDonald JC, Moore DL, Quennec P. Clinical and epidemiologic features of mumps meningoencephalitis and possible vaccine-related disease. *Pediatr Infect Dis J* 1989;**8**:751–5.
296. McDowall DG. Monitoring the brain. *Anesthesiology* 1976;**45**: 117–34.
297. McHugh K, McMenamin JB. Acute disseminated encephalomyelitis in childhood. *Ir Med J* 1987;**80**:412–4.
298. McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med* 2001;**344**:801–7.
299. McLean DM, Best JM, Smith PA, et al. Viral infections of Toronto children during 1965, part II: measles encephalitis and other complications. *Can Med Assoc J* 1966;**94**:905–10.
300. Melekhin VV, Karem KL, Damon IK, et al. Encephalitis after secondary smallpox vaccination. *Clin Infect Dis* 2009;**48**:e1–2.
301. Melis K, Bochner A, Vandenberghe P, et al. Cat-scratch disease with reversible encephalopathy. *Eur J Pediatr* 1989;**149**:2–25.
302. Menge T, Hemmer B, Nessler S, et al. Acute disseminated encephalomyelitis: an update. *Arch Neurol* 2005;**62**:1673–80.
303. Meyer Jr HM, Johnson RT, Crawford IP, et al. Central nervous system syndromes of "vital" etiology: a study of 713 cases. *Am J Med* 1960;**29**:334–47.
304. Meyer Sauteur PM, Streuli JC, Iff T, et al. *Mycoplasma pneumoniae*-associated encephalitis in childhood—nervous system disorder during or after a respiratory tract infection. *Klin Padiatr* 2011; **223**:209–13.
305. Miguel PS, Fernandez G, Vasallo FJ, et al. Neurobrucellosis mimicking cerebral tumor: case report and literature review. *Clin Neurol Neurosurg* 2006;**108**:404–6.
306. Miller HG, Stanton JB. Neurological sequelae of prophylactic inoculation. *Q J Med* 1954;**23**:1–27.
307. Mizrahi EM, Tharp BR. A characteristic EEG pattern in neonatal herpes simplex encephalitis. *Neurology* 1982;**32**:1215–20.
308. Modlin JF, Dagan R, Berlin LE, et al. Focal encephalitis with enterovirus infections. *Pediatrics* 1991;**88**:841–5.
309. Monath TP, Cropp CB, Harrison AK. Mode of entry of a neurotropic arbovirus into the central nervous system: reinvestigation of an old controversy. *Lab Invest* 1983;**48**:399–410.
310. Montgomery JM, Hossain MJ, Gurley E, et al. Risk factors for Nipah virus encephalitis in Bangladesh. *Emerg Infect Dis* 2008; **14**:1526–32.
311. Moore M. Centers for Disease Control. Enteroviral disease in the United States, 1970-1979. *J Infect Dis* 1982;**146**:103–8.
312. Mori T, Morii M, Kuroiwa Y, et al. Rotavirus encephalitis and cerebellitis with reversible magnetic resonance signal changes. *Pediatr Int* 2011;**53**:252–5.
313. Morishima T, Togashi T, Yokota S, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002;**35**:512–7.
314. Muscat M, Bang H, Wohlfahrt J, et al. Measles in Europe: an epidemiological assessment. *Lancet* 2009;**373**:383–9.
315. Nanning W. Prophylactic effect of antivaccinia gamma-globulin against post-vaccinal encephalitis. *Bull World Health Organ* 1962; **27**:317–24.
316. Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 2001;**344**:1807–14.
317. Newland JG, Romero JR, Varman M, et al. Encephalitis associated with influenza B virus infection in 2 children and a review of the literature. *Clin Infect Dis* 2003;**36**:e87–95.
318. Ng YT, Cox C, Atkins J, et al. Encephalopathy associated with respiratory syncytial virus bronchiolitis. *J Child Neurol* 2001;**16**: 105–8.
319. Nigrovic LE, Lumeng C, Landrigan C, et al. Rotavirus cerebellitis? *Clin Infect Dis* 2002;**34**:130.
320. Nishikawa M, Ichiyama T, Hayashi T, et al. Intravenous immunoglobulin therapy in acute disseminated encephalomyelitis. *Pediatr Neurol* 1999;**21**:583–6.
321. Noah DL, Bresee JS, Gorensek MJ, et al. Cluster of five children with acute encephalopathy associated with cat-scratch disease in south Florida. *Pediatr Infect Dis J* 1995;**14**:866–9.
322. Noah ND, Urquhart AM. Virus meningitis and encephalitis in 1979. *J Infect* 1980;**2**:379–83.
323. Nolan RC, Chidlow G, French MA. Parvovirus B19 encephalitis presenting as immune restoration disease after highly active antiretroviral therapy for human immunodeficiency virus infection. *Clin Infect Dis* 2003;**36**:1191–4.
324. O'Sullivan JD, Allworth AM, Paterson DL, et al. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* 1997;**349**:93–5.
325. Ohsaka M, Houkin K, Takigami M, et al. Acute necrotizing encephalopathy associated with human herpesvirus-6 infection. *Pediatr Neurol* 2006;**34**:160–3.
326. Ohtaki E, Murakami Y, Komori H, et al. Acute disseminated encephalomyelitis after Japanese B encephalitis vaccination. *Pediatr Neurol* 1992;**8**:137–9.
327. Okuno T, Takao T, Ito M, et al. Contrast enhanced hypodense areas in a case of acute disseminated encephalitis following influenza A virus. *Comput Radiol* 1982;**6**:215–7.
328. Olgar S, Ertugrul T, Nisli K, et al. Influenza A–associated acute necrotizing encephalopathy. *Neuropediatrics* 2006;**37**:166–8.
329. Olitsky PK, Casals J. Arthropod-borne group A virus infections of man. In: Rivers RM, Horsfall Jr FL, editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1959. p. 285–304.

330. Osamura T, Mizuta R, Yoshioka H, et al. Isolation of adenovirus type 11 from the brain of a neonate with pneumonia and encephalitis. *Eur J Pediatr* 1993;**152**:496–9.
331. Ouattara LA, Barin F, Barthez MA, et al. Novel human reovirus isolated from children with acute necrotizing encephalopathy. *Emerg Infect Dis* 2011;**17**:1436–44.
332. Pai PJ, Blackburn BG, Kazacos KR, et al. Full recovery from *Baylisascaris procyonis* eosinophilic meningitis. *Emerg Infect Dis* 2007;**13**:928–30.
333. Paisley JW, Bruhn FW, Lauer BA, et al. Type A2 influenza viral infections in children. *Am J Dis Child* 1978;**132**:34–6.
334. Palacios G, Quan PL, Jabado OJ, et al. Panmicrobial oligonucleotide array for diagnosis of infectious diseases. *Emerg Infect Dis* 2007;**13**:73–81.
335. Panicker JN, Mammachan R, Jayakumar RV. Primary neuroleptospirosis. *Postgrad Med J* 2001;**77**:589–90.
336. Parent JM, Lowenstein DH. Treatment of refractory generalized status epilepticus with continuous infusion of midazolam. *Neurology* 1994;**44**:1837–40.
337. Pelligra G, Lynch N, Miller SP, et al. Brainstem involvement in neonatal herpes simplex virus type 2 encephalitis. *Pediatrics* 2007;**120**:e442–6.
338. Perez MT, Bush LM. *Balamuthia mandrillaris* amebic encephalitis. *Curr Infect Dis Rep* 2007;**9**:323–8.
339. Perez-Velez CM, Anderson MS, Robinson CC, et al. Outbreak of neurologic enterovirus type 71 disease: a diagnostic challenge. *Clin Infect Dis* 2007;**45**:950–7.
340. Peter G. Leptospirosis: a zoonosis of protean manifestations. *Pediatr Infect Dis* 1982;**1**:282–8.
341. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med* 2002;**137**:E173–9.
342. Pialoux G, Gauzere BA, Jaureguiberry S, et al. Chikungunya, an epidemic arbovirosis. *Lancet Infect Dis* 2007;**7**:319–27.
343. Plotkin SA, Rupprecht CE, Koprowski H. Rabies vaccine. In: *Vaccines*. 4th ed. Philadelphia: W. B. Saunders; 2004. p. 1017–38.
344. Pohl-Koppe A, Blay M, Jager G, et al. Human herpes virus type 7 DNA in the cerebrospinal fluid of children with central nervous system diseases. *Eur J Pediatr* 2001;**160**:351–8.
345. Ponka A. Central nervous system manifestations associated with serologically verified *Mycoplasma pneumoniae* infection. *Scand J Infect Dis* 1980;**12**:175–84.
346. Preblud SR. Age-specific risks of varicella complications. *Pediatrics* 1981;**68**:14–7.
347. Protopsaltis J, Kokkoris S, Brestas PS, et al. Neurolisteriosis mimicking herpes simplex encephalitis in an immunocompromized patient. *Scand J Infect Dis* 2006;**38**:825–8.
348. Prusiner SB, Hsiao KK. Human prion diseases. *Ann Neurol* 1994;**35**:385–95.
349. Public Health Service, U.S. Department of Health, Education and Welfare, joint statement. Fatal viral encephalitis following 17D yellow fever vaccine inoculation: report of a case in a 3-year-old child. *JAMA* 1966;**198**:203–4.
350. Purdham DR, Batty PF. A case of acute measles meningoencephalitis with virus isolation. *J Clin Pathol* 1974;**27**:994–6.
351. Ragland AS, Arsura E, Ismail Y, et al. Eosinophilic pleocytosis in coccidioidal meningitis: frequency and significance. *Am J Med* 1993;**95**:254–7.
352. Randolph AG, Vaughn F, Sullivan R, et al. Critically ill children during the 2009-10 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
353. Rantala H, Uhari M. Occurrence of childhood encephalitis: a population-based study. *Pediatr Infect Dis J* 1989;**8**:426–30.
354. Rao BL, Basu A, Wairagkar NS, et al. A large outbreak of acute encephalitis with high fatality rate in children in Andhra Pradesh, India, in 2003, associated with Chandipura virus. *Lancet* 2004;**364**:869–74.
355. Raoult D, Marrie T. Q fever. *Clin Infect Dis* 1995;**20**:489–95; quiz 496.
356. Rautonen J, Koskiniemi M, Vaheri A. Prognostic factors in childhood acute encephalitis. *Pediatr Infect Dis J* 1991;**10**:441–6.
357. Ravid S, Shahar E, Genizi J, et al. Acute Q fever in children presenting with encephalitis. *Pediatr Neurol* 2008;**38**:44–6.
358. Ray P, Hayward J, Michelson D, et al. Encephalopathy after whole-cell pertussis or measles vaccination: lack of evidence for a causal association in a retrospective case-control study. *Pediatr Infect Dis J* 2006;**25**:768–73.
359. Read SJ, Jeffery KJ, Bangham CR. Aseptic meningitis and encephalitis: the role of PCR in the diagnostic laboratory. *J Clin Microbiol* 1997;**35**:691–6.
360. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg* 2008;**79**:974–9.
361. Rellosa N, Bloch KC, Shane AL, et al. Neurologic manifestations of pediatric novel H1N1 influenza infection. *Pediatr Infect Dis J* 2011;**30**:165–7.
362. Robb L, Butt W. Brain stem encephalitis due to herpes simplex virus. *Aust Paediatr J* 1989;**25**:246–7.
363. Robin S, Ramful D, Le Seach F, et al. Neurologic manifestations of pediatric chikungunya infection. *J Child Neurol* 2008;**23**:1028–35.
364. Robinson LJ. Neurologic complications following the administration of vaccines and serums: report of a case of peripheral paralysis following the injection of typhoid vaccine. *N Engl J Med* 1937;**216**:831–7.
365. Romero JR, Newland JG. Viral meningitis and encephalitis: traditional and emerging viral agents. *Semin Pediatr Infect Dis* 2003;**14**:72–82.
366. Roos KL. Encephalitis. *Neurol Clin* 1999;**17**:813–33.
367. Rosenberg GA. Meningoencephalitis following an influenza vaccination. *N Engl J Med* 1970;**283**:1209.
368. Rosenblum MK. Bulbar encephalitis complicating trigeminal zoster in the acquired immune deficiency syndrome. *Hum Pathol* 1989;**20**:292–5.
369. Rothstein TL, Shaw CM. Computerized tomography as a diagnostic aid in acute hemorrhagic leukoencephalitis. *Ann Neurol* 1983;**13**:331–3.
370. Rowley AH, Whitley RJ, Lakeman FD, et al. Rapid detection of herpes-simplex-virus DNA in cerebrospinal fluid of patients with herpes simplex encephalitis. *Lancet* 1990;**335**:440–1.
371. Rubeiz H, Roos RP. Viral meningitis and encephalitis. *Semin Neurol* 1992;**12**:165–77.
372. Sainio K, Granstrom ML, Pettay O, et al. EEG in neonatal herpes simplex encephalitis. *Electroencephalogr Clin Neurophysiol* 1983;**56**:556–61.
373. Salaki JS, Louria DB, Chmel H. Fungal and yeast infections of the central nervous system: a clinical review. *Medicine (Baltimore)* 1984;**63**:108–32.
374. Sato S, Kumada S, Koji T, et al. Reversible frontal lobe syndrome associated with influenza virus infection in children. *Pediatr Neurol* 2000;**22**:318–21.
375. Savolainen H, Lautenschlager I, Piiparinen H, et al. Human herpesvirus–6 and –7 in pediatric stem cell transplantation. *Pediatr Blood Cancer* 2005;**45**:820–5.
376. Schlesinger Y, Buller RS, Brunstrom JE, et al. Expanded spectrum of herpes simplex encephalitis in childhood. *J Pediatr* 1995;**126**:234–41.
377. Schmidbauer M, Budka H, Ambros P. Herpes simplex virus (HSV) DNA in microglial nodular brainstem encephalitis. *J Neuropathol Exp Neurol* 1989;**48**:645–52.
378. Schuster FL, Yagi S, Gavali S, et al. Under the radar: *Balamuthia* amebic encephalitis. *Clin Infect Dis* 2009;**48**:879–87.
379. Seeley WW, Marty FM, Holmes TM, et al. Post-transplant acute limbic encephalitis: clinical features and relationship to HHV6. *Neurology* 2007;**69**:156–65.
380. Sejvar JJ. The evolving epidemiology of viral encephalitis. *Curr Opin Neurol* 2006;**19**:350–7.
381. Sejvar JJ, Chowdary Y, Schomogyi M, et al. Human monkeypox infection: a family cluster in the midwestern United States. *J Infect Dis* 2004;**190**:1833–40.
382. Sejvar JJ, Haddad MB, Tierney BC, et al. Neurologic manifestations and outcome of West Nile virus infection. *JAMA* 2003;**290**:511–5.
383. Sejvar JJ, Labutta RJ, Chapman LE, et al. Neurologic adverse events associated with smallpox vaccination in the United States, 2002-2004. *JAMA* 2005;**294**:2744–50.
384. Sener RN. Herpes simplex encephalitis: diffusion MR imaging findings. *Comput Med Imaging Graph* 2001;**25**:391–7.
385. Shaffer MF, Rake G, Hodes HL. Isolation of virus from a patient with fatal encephalitis complicating measles. *Am J Dis Child* 1942;**64**:815–9.
386. Shaked Y, Samra Y. Q fever meningoencephalitis associated with bilateral abducens nerve paralysis, bilateral optic neuritis

and abnormal cerebrospinal fluid findings. *Infection* 1989;**17**: 394–5.
387. Shaw DW, Cohen WA. Viral infections of the CNS in children: imaging features. *AJR Am J Roentgenol* 1993;**160**:125–33.
388. Sherman FE, Michaels RH, Kenny FM. Acute encephalopathy (encephalitis) complicating rubella. Report of cases with virologic studies, cortisol-production determinations, and observations at autopsy. *JAMA* 1965;**192**:675–81.
389. Sheth RD, Goulden KJ, Pryse-Phillips WE. The focal encephalopathies associated with *Mycoplasma pneumoniae*. *Can J Neurol Sci* 1993;**20**:319–23.
390. Shields WD. Status epilepticus. *Pediatr Clin North Am* 1989; **36**:383–93.
391. Shiihara T, Watanabe M, Honma A, et al. Rotavirus associated acute encephalitis/encephalopathy and concurrent cerebellitis: report of two cases. *Brain Dev* 2007;**29**:670–3.
392. Siddiqui R, Khan NA. *Balamuthia* amoebic encephalitis: an emerging disease with fatal consequences. *Microb Pathog* 2008;**44**: 89–97.
393. Siegman-Igra Y, Michaeli D, Doron A, et al. Cytomegalovirus encephalitis in a noncompromised host. *Isr J Med Sci* 1984;**20**: 163–6.
394. Silliman CC, Tedder D, Ogle JW, et al. Unsuspected varicella-zoster virus encephalitis in a child with acquired immunodeficiency syndrome. *J Pediatr* 1993;**123**:418–22.
395. Silva RA, Araujo Sde A, Avellar IF, et al. Granulomatous amoebic meningoencephalitis in an immunocompetent patient. *Arch Neurol* 2010;**67**:1516–20.
396. Simon F, Parola P, Grandadam M, et al. Chikungunya infection: an emerging rheumatism among travelers returned from Indian Ocean islands: report of 47 cases. *Medicine (Baltimore)* 2007;**86**: 123–37.
397. Singh N, Paterson DL. Encephalitis caused by human herpesvirus-6 in transplant recipients: relevance of a novel neurotropic virus. *Transplantation* 2000;**69**:2474–9.
398. Singh S, Prabhakar S, Korah IP, et al. Acute disseminated encephalomyelitis and multiple sclerosis: magnetic resonance imaging differentiation. *Australas Radiol* 2000;**44**:404–11.
399. Socan M, Ravnik I, Bencina D, et al. Neurological symptoms in patients whose cerebrospinal fluid is culture- and/or polymerase chain reaction-positive for *Mycoplasma pneumoniae*. *Clin Infect Dis* 2001;**32**:E31–5.
400. Solomon T. Control of Japanese encephalitis—within our grasp? *N Engl J Med* 2006;**355**:869–71.
401. Solomon T, Dung NM, Kneen R, et al. Japanese encephalitis. *J Neurol Neurosurg Psychiatry* 2000;**68**:405–15.
402. Solomon T, Ni H, Beasley DW, et al. Origin and evolution of Japanese encephalitis virus in southeast Asia. *J Virol* 2003;**77**: 3091–8.
403. Southern Jr PM. Relapsing fever. In: Tice F, editor. *Practice of medicine*. Hagerstown, Md.: Harper & Row, Hoeber Medical Division; 1969. vol. 3. p. 1–19.
404. Spillane JD, Wells CE. The neurology of Jennerian vaccination: a clinical account of the neurological complications which occurred during the smallpox epidemic in South Wales in 1962. *Brain* 1964;**87**:1–44.
405. Steer AC, Starr M, Kornberg AJ. Bickerstaff brainstem encephalitis associated with *Mycoplasma pneumoniae* infection. *J Child Neurol* 2006;**21**:533–4.
406. Steiner MM, Vuckovitch D, Hadawi SA. Cat-scratch disease with encephalopathy: case report and review of the literature. *J Pediatr* 1963;**62**:514–20.
407. Steinfort DP, Dixon B. Parvovirus encephalitis and pneumonia in an immunocompetent adult. *Intern Med J* 2006;**36**:209–10.
408. Straussberg R, Harel L, Levy Y, et al. A syndrome of transient encephalopathy associated with adenovirus infection. *Pediatrics* 2001;**107**:E69.
409. Streng A, Grote V, Liese JG. Severe influenza cases in paediatric intensive care units in Germany during the pre-pandemic seasons 2005 to 2008. *BMC Infect Dis* 2011;**11**:233.
410. Strikas RA, Anderson LJ, Parker RA. Temporal and geographic patterns of isolates of nonpolio enterovirus in the United States, 1970-1983. *J Infect Dis* 1986;**153**:346–51.
411. Studahl M. Influenza virus and CNS manifestations. *J Clin Virol* 2003;**28**:225–32.
412. Surtees R, DeSousa C. Influenza virus associated encephalopathy. *Arch Dis Child* 2006;**91**:455–6.
413. Sweetman LL, Ng YT, Butler IJ, et al. Neurologic complications associated with respiratory syncytial virus. *Pediatr Neurol* 2005; **32**:307–10.
414. Tagawa Y, Yuki N. Bickerstaff's brainstem encephalitis associated with shingles. *J Neurol* 2000;**247**:218–9.
415. Takanashi J, Miyamoto T, Ando N, et al. Clinical and radiological features of rotavirus cerebellitis. *AJNR Am J Neuroradiol* 2010;**31**:1591–5.
416. Tambyah PA, Tan JH, Ong BK, et al. First case of Nipah virus encephalitis in Singapore. *Intern Med J* 2001;**31**:132–3.
417. Tan CT, Goh KJ, Wong KT, et al. Relapsed and late-onset Nipah encephalitis. *Ann Neurol* 2002;**51**:703–8.
418. Tenembaum S, Chamoles N, Fejerman N. Acute disseminated encephalomyelitis: a long-term follow-up study of 84 pediatric patients. *Neurology* 2002;**59**:1224–31.
419. Tenembaum S, Chitnis T, Ness J, et al. Acute disseminated encephalomyelitis. *Neurology* 2007;**68**:S23–36.
420. ter Meulen V, Muller D, Kackell Y, et al. Isolation of infectious measles virus in measles encephalitis. *Lancet* 1972;**2**:1172–5.
421. Togashi T, Matsuzono Y, Narita M, et al. Influenza-associated acute encephalopathy in Japanese children in 1994-2002. *Virus Res* 2004;**103**:75–8.
422. Tokunaga Y, Kira R, Takemoto M, et al. Diagnostic usefulness of diffusion-weighted magnetic resonance imaging in influenza-associated acute encephalopathy or encephalitis. *Brain Dev* 2000;**22**:451–3.
423. Tong CY, Menson E, Lin JP, et al. Prevalence of *Mycoplasma* encephalitis. *Lancet Infect Dis* 2011;**11**:425–6.
424. Torigoe S, Koide W, Yamada M, et al. Human herpesvirus–7 infection associated with central nervous system manifestations. *J Pediatr* 1996;**129**:301–5.
425. Towne AR, Waterhouse EJ, Boggs JG, et al. Prevalence of nonconvulsive status epilepticus in comatose patients. *Neurology* 2000;**54**:340–5.
426. Townsend JJ, Wolinsky JS, Baringer JR, et al. Acquired toxoplasmosis: a neglected cause of treatable nervous system disease. *Arch Neurol* 1975;**32**:335–43.
427. Treatment of convulsive status epilepticus. Recommendation of the Epilepsy Foundation of America's Working Group on Status Epilepticus. *JAMA* 1993;**270**:854–9.
428. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13-15 October 1998. *Vaccine* 2000;**18**(Suppl 2):1–25.
429. Tsiodras S, Kelesidis I, Kelesidis T, et al. Central nervous system manifestations of *Mycoplasma pneumoniae* infections. *J Infect* 2005;**51**:343–54.
430. Tsuchiya K, Katase S, Yoshino A, et al. Diffusion-weighted MR imaging of encephalitis. *AJR Am J Roentgenol* 1999;**173**:1097–9.
431. Tsutsumi H, Kamazaki H, Nakata S, et al. Sequential development of acute meningoencephalitis and transverse myelitis caused by Epstein-Barr virus during infectious mononucleosis. *Pediatr Infect Dis J* 1994;**13**:665–7.
432. Tyler KL. Herpes simplex virus infections of the central nervous system: encephalitis and meningitis, including Mollaret's. *Herpes* 2004;**11**(Suppl 2):57A–64A.
433. Tyler KL. Emerging viral infections of the central nervous system, part 2. *Arch Neurol* 2009;**66**:1065–74.
434. Uren EC, Johnson PD, Montanaro J, et al. Herpes simplex virus encephalitis in pediatrics: diagnosis by detection of antibodies and DNA in cerebrospinal fluid. *Pediatr Infect Dis J* 1993;**12**: 1001–6.
435. Van Dam CN, Syed S, Eron JJ, et al. Severe postvaccinia encephalitis with acute disseminated encephalomyelitis: recovery with early intravenous immunoglobulin, high-dose steroids, and vaccinia immunoglobulin. *Clin Infect Dis* 2009;**48**:e47–9.
436. Varatharaj A. Encephalitis in the clinical spectrum of dengue infection. *Neurol India* 2010;**58**:585–91.
437. Verboon-Maciolek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white matter injury in neonates. *Ann Neurol* 2008;**64**:266–73.
438. Visser AM, van Doornum GJ, Cornelissen JJ, et al. Severe amnesia due to HHV-6 encephalitis after allogenic stem cell transplantation. *Eur Neurol* 2005;**54**:233–4.

439. Visvesvara GS, Moura H, Schuster FL. Pathogenic and opportunistic free-living amoebae: *Acanthamoeba* spp., *Balamuthia mandrillaris*, *Naegleria fowleri*, and *Sappinia diploidea*. *FEMS Immunol Med Microbiol* 2007;**50**:1–26.
440. Wallace SJ, Zealley H. Neurological, electroencephalographic, and virological findings in febrile children. *Arch Dis Child* 1970; **45**:611–23.
441. Wang GF, Li W, Li K. Acute encephalopathy and encephalitis caused by influenza virus infection. *Curr Opin Neurol* 2010;**23**: 305–11.
442. Wang SM, Liu CC, Tseng HW, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. *Clin Infect Dis* 1999;**29**:184–90.
443. Wang YH, Huang YC, Chang LY, et al. Clinical characteristics of children with influenza A virus infection requiring hospitalization. *J Microbiol Immunol Infect* 2003;**36**:111–6.
444. Ward KN, Kalima P, MacLeod KM, et al. Neuroinvasion during delayed primary HHV-7 infection in an immunocompetent adult with encephalitis and flaccid paralysis. *J Med Virol* 2002;**67**:538–41.
445. Warren J. Encephalitis lethargica. In: Rivers TM, Horsfall Jr FL, editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1959. p. 914–5.
446. Watson RT, Ballinger Jr WE, Quisling RG. Acute hemorrhagic leukoencephalitis: diagnosis by computed tomography. *Ann Neurol* 1984;**15**:611–2.
447. Weil AA, Glaser CA, Amad Z, et al. Patients with suspected herpes simplex encephalitis: rethinking an initial negative polymerase chain reaction result. *Clin Infect Dis* 2002;**34**:1154–7.
448. Weisberg LA. The role of CT in the evaluation of patients with intracranial CNS infectious-inflammatory disorders. *Comput Radiol* 1984;**8**:29–36.
449. Whitley RJ. Viral encephalitis. *N Engl J Med* 1990;**323**:242–50.
450. Whitley RJ, Cobbs CG, Alford Jr CA, et al. Diseases that mimic herpes simplex encephalitis: diagnosis, presentation, and outcome. NIAD Collaborative Antiviral Study Group. *JAMA* 1989;**262**: 234–9.
451. Whitley RJ, Kimberlin DW. Herpes simplex encephalitis: children and adolescents. *Semin Pediatr Infect Dis* 2005;**16**:17–23.
452. Whitley RJ, Lakeman F. Herpes simplex virus infections of the central nervous system: therapeutic and diagnostic considerations. *Clin Infect Dis* 1995;**20**:414–20.
453. Whitley RJ, Lakeman AD, Nahmias A, et al. DNA restriction-enzyme analysis of herpes simplex virus isolates obtained from patients with encephalitis. *N Engl J Med* 1982;**307**:1060–2.
454. Williams PL, Johnson R, Pappagianis D, et al. Vasculitic and encephalitic complications associated with *Coccidioides immitis* infection of the central nervous system in humans: report of 10 cases and review. *Clin Infect Dis* 1992;**14**:673–82.
455. Willoughby Jr RE, Tieves KS, Hoffman GM, et al. Survival after treatment of rabies with induction of coma. *N Engl J Med* 2005;**352**:2508–14.
456. Wise ME, Sorvillo FJ, Shafir SC, et al. Severe and fatal central nervous system disease in humans caused by *Baylisascaris procyonis*, the common roundworm of raccoons: a review of current literature. *Microbes Infect* 2005;**7**:317–23.
457. Woods CA, Ellison GW. Encephalopathy following influenza immunization. *J Pediatr* 1964;**65**:745–8.
458. Yan JJ, Wang JR, Liu CC, et al. An outbreak of enterovirus 71 infection in Taiwan 1998: a comprehensive pathological, virological, and molecular study on a case of fulminant encephalitis. *J Clin Virol* 2000;**17**:13–22.
459. Yang TT, Huang LM, Lu CY, et al. Clinical features and factors of unfavorable outcomes for non-polio enterovirus infection of the central nervous system in northern Taiwan, 1994-2003. *J Microbiol Immunol Infect* 2005;**38**:417–24.
460. Yasunaga H, Shi Y, Takeuchi M, et al. Measles-related hospitalizations and complications in Japan, 2007-2008. *Intern Med* 2010; **49**:1965–70.
461. Yildizdas D, Kendirli T, Arslankoylu AE, et al. Neurological complications of pandemic influenza (H1N1) in children. *Eur J Pediatr* 2011;**170**:779–88.
462. Yis U, Kurul SH, Cakmakci H, et al. *Mycoplasma pneumoniae:* nervous system complications in childhood and review of the literature. *Eur J Pediatr* 2008;**167**:973–8.
463. Yoshikawa H, Yamazaki S, Watanabe T, et al. Study of influenza-associated encephalitis/encephalopathy in children during the 1997 to 2001 influenza seasons. *J Child Neurol* 2001;**16**: 885–90.
464. Yoshikawa T, Nakashima T, Suga S, et al. Human herpesvirus–6 DNA in cerebrospinal fluid of a child with exanthem subitum and meningoencephalitis. *Pediatrics* 1992;**89**:888–90.
465. Young EJ. Human brucellosis. *Rev Infect Dis* 1983;**5**:821–42.
466. Yuki N, Odaka M, Hirata K. Bickerstaff's brainstem encephalitis subsequent to *Campylobacter jejuni* enteritis. *J Neurol Neurosurg Psychiatry* 2000;**68**:680–1.
467. Ziegra SR. Corticosteroid treatment for measles encephalitis. *J Pediatr* 1961;**59**:322–3.
468. Zinserling AV, Aksenov OA, Melnikova VF, et al. Extrapulmonary lesions in influenza. *Tohoku J Exp Med* 1983;**140**:259–72.

CHAPTER 37

Parainfectious and Postinfectious Disorders of the Nervous System

37A ■ PARAINFECTIOUS AND POSTINFECTIOUS DEMYELINATING DISORDERS OF THE CENTRAL NERVOUS SYSTEM

J. Lloyd Holder, Jr. • Timothy E. Lotze

ACUTE DISSEMINATED ENCEPHALOMYELITIS

Acute disseminated encephalomyelitis (ADEM) is a monophasic demyelinating disease of the central nervous system (CNS) that results in acute, polysymptomatic neurologic disability. It also has been termed *postinfectious encephalomyelitis*. It is related to other central inflammatory demyelinating conditions of childhood, including optic neuritis, transverse myelitis, neuromyelitis optica (Devic disease), and multiple sclerosis. Although no single diagnostic test can be used to distinguish among these conditions at the initial presentation, certain clinical features, laboratory results, and imaging findings can be used to ensure a correct diagnosis. Most of these conditions are thought to be caused by autoimmune dysregulation triggered by an infectious agent in a genetically susceptible host.

EPIDEMIOLOGY

The estimated incidence of ADEM is 0.8/100,000 population per year.[37] ADEM occurs just as frequently in children as it does in adults.[11] It has no specific gender predilection or ethnic distribution.[14,15] In contrast to multiple sclerosis, which generally has a higher incidence at more northern latitudes, ADEM has no appreciable geographic distribution.

DIAGNOSTIC CRITERIA

The term *acute disseminated encephalomyelitis* has been used variably in the literature in describing clinical characteristics of this disease. Discrepancies exist among descriptive studies regarding (1) the occurrence of encephalopathy, (2) the association with preceding infection, (3) symptoms that are monofocal or multifocal, and (4) the possibility for recurrence. Lack of standardized diagnostic criteria for ADEM has impeded the ability to understand this distinct disease better as part of the spectrum of demyelinating conditions occurring in childhood.

The International Pediatric Multiple Sclerosis Study Group developed diagnostic criteria for ADEM.[35] The group created working definitions for monophasic ADEM and for recurrent forms of the disease. Table 37A-1 lists the diagnostic criteria. An absolute criterion for a diagnosis of ADEM is the presence of encephalopathy. This criterion is defined to include either behavioral changes, such as lethargy or irritability, or more severe alterations in level of consciousness, such as coma. The onset of the encephalopathy must correspond with the occurrence of the disease state. Premorbid cognitive difficulties, such as learning difficulties or mental retardation, would not be included in this definition. Magnetic resonance imaging (MRI) shows multiple lesions in both hemispheres distributed throughout the white matter (Fig. 37A-1). A distinguishing characteristic of ADEM is prominent involvement of the cortical gray matter and deep gray nuclei (basal ganglia and thalamus). Such involvement is atypical for multiple sclerosis and other demyelinating conditions. The lesions of ADEM are asymmetric, showing variable size, shape, and distribution between the hemispheres. MRI showing symmetric and confluent lesions should prompt the clinician to consider other diagnoses, such as leukodystrophies and inborn errors of metabolism. If MRI shows evidence of previous demyelination, the clinician should query the history further for previous attacks, which would suggest either a recurrent form of the disease or a chronic demyelinating condition. Cerebrospinal fluid (CSF) analysis may show a pleocytosis of greater than 50 cells, in contrast to pediatric multiple sclerosis, in which only a slight pleocytosis (<50 cells) is observed.

TABLE 37A-1 Diagnostic Criteria of Acute Disseminated Encephalomyelitis

Clinical Features
First clinical attack of demyelinating disease in CNS
Acute or subacute onset
Polysymptomatic presentation
Must include encephalopathy Acute behavioral change (e.g., irritability, lethargy) Alteration in consciousness (e.g., somnolence, coma)
Attack should be followed by improvement
Lesion Characteristics on MRI FLAIR and T2-Weighted Images
Multifocal, hyperintense, bilateral, asymmetric lesions in the white matter
At least one or more lesions >1-2 cm
Gray matter, especially basal ganglia and thalamus, may be involved
Spinal cord MRI may show confluent intramedullary lesions
No radiologic evidence of previous destructive white matter changes
Cerebrospinal Fluid
Pleocytosis ≥50 WBCs can be observed
Other
No other etiologies can explain the event
New or fluctuating symptoms and signs occurring within 3 mo of the inciting ADEM event are part of the same acute event

Note: Symptoms that vary during periods of steroid taper within 3 mo of the inciting event or occur <30 days after discontinuation of all steroids are considered part of the initial inciting event.

ADEM, Acute disseminated encephalomyelitis; *CNS,* central nervous system; *FLAIR,* fluid-attenuated inversion recovery; *MRI,* magnetic resonance imaging; *WBCs,* white blood cells.

The International Pediatric Multiple Sclerosis Study Group has defined criteria to distinguish between an evolving pattern for the initial event and recurrent forms of ADEM. Any new and fluctuating symptoms occurring within 3 months of the initial event are considered part of the same inciting event. In addition, symptoms occurring during steroid taper or within 1 month of the patient completing a steroid taper are considered part of the same inciting event. Relapsing ADEM and multiphasic ADEM are the two recurrent forms of the disease. Both forms are defined to occur more than 3 months after the initial event and more than 1 month after completion of steroids. By definition for ADEM, both forms must include a clinical presentation with encephalopathy. Relapsing ADEM describes the recurrence of the same symptoms that occurred at the time of the initial presentation. The MRI findings also are similar to the initial event, although they may show enlargement of the previous lesions. Multiphasic ADEM describes children with recurrent disease characterized by symptoms that differ from those of the initial event. MRI likewise shows development of new lesions different from those seen with the first attack.

CLINICAL MANIFESTATIONS

A febrile illness occurs in 50 to 75 percent of children in the 4 weeks before the onset of typical neurologic symptoms.[41] Preceding vaccinations have been associated with the occurrence of ADEM, but this is less common.[15,51] Fever, headache, vomiting, and meningismus often are present at the time of initial presentation and may persist during the hospitalization.[9,24] Neurologic symptoms typically appear 4 to 13 days after the infection develops or vaccination is administered.[15,24,43,51] New clinical symptoms may continue during hospitalization and may alter treatment. Per current diagnostic criteria, all children with ADEM have an encephalopathy at the time of presentation.[35] The degree of altered mental status varies, ranging from irritability to somnolence to coma. Encephalopathy may be the initial symptom that brings the child to medical attention. Although alteration in mental state often raises concern for the possibility of seizures, they occur in only one third of patients.[16,51] In addition to having encephalopathy, patients exhibit various other neurologic features. The most common of these are long tract signs, acute hemiparesis, cerebellar ataxia, and cranial neuropathy.[9,16,25,43,51] Aphasia, movement disorders, and sensory deficits occur less commonly.

Demyelination of the optic nerves (optic neuritis) or spinal cord (transverse myelitis) may occur. Symptoms of optic neuritis include vision loss, pain with eye movement, and an afferent papillary defect. Inflammation of the optic disk may be seen on direct funduscopic examination if there is extensive involvement of the optic nerve. Patients with retrobulbar optic neuritis typically have a normal funduscopic examination. Optic neuritis may occur in one or both eyes, with differing degrees of involvement. Symptoms of transverse myelitis include flaccid paralysis of the legs, with a sensory level on examination. The arms can be involved as well if the demyelinating lesion is in the cervical cord. Respiratory failure may occur with high cervical lesions that extend into the brain stem. Bowel and bladder involvement secondary to spinal cord disease results in constipation and urinary retention.

The extent of demyelination in the CNS may not be recognized fully at the time of initial presentation, particularly if the patient has severe encephalopathy. Imaging of the entire CNS to include the brain, orbits, and spinal cord should be done in all patients meeting diagnostic criteria for ADEM because co-occurrence of transverse myelitis or optic neuritis can have a significant impact on rehabilitation needs and long-term outcome.

CLINICAL VARIANTS

Relapsing ADEM and multiphasic ADEM are the two recurrent forms of the disease. Both forms are defined by reoccurrence of neurologic symptoms more than 3 months after the initial event and more than 1 month after completion of steroids. Most often, recurrence of disease is singular. Repeated events are unusual and should prompt assessment for a different underlying

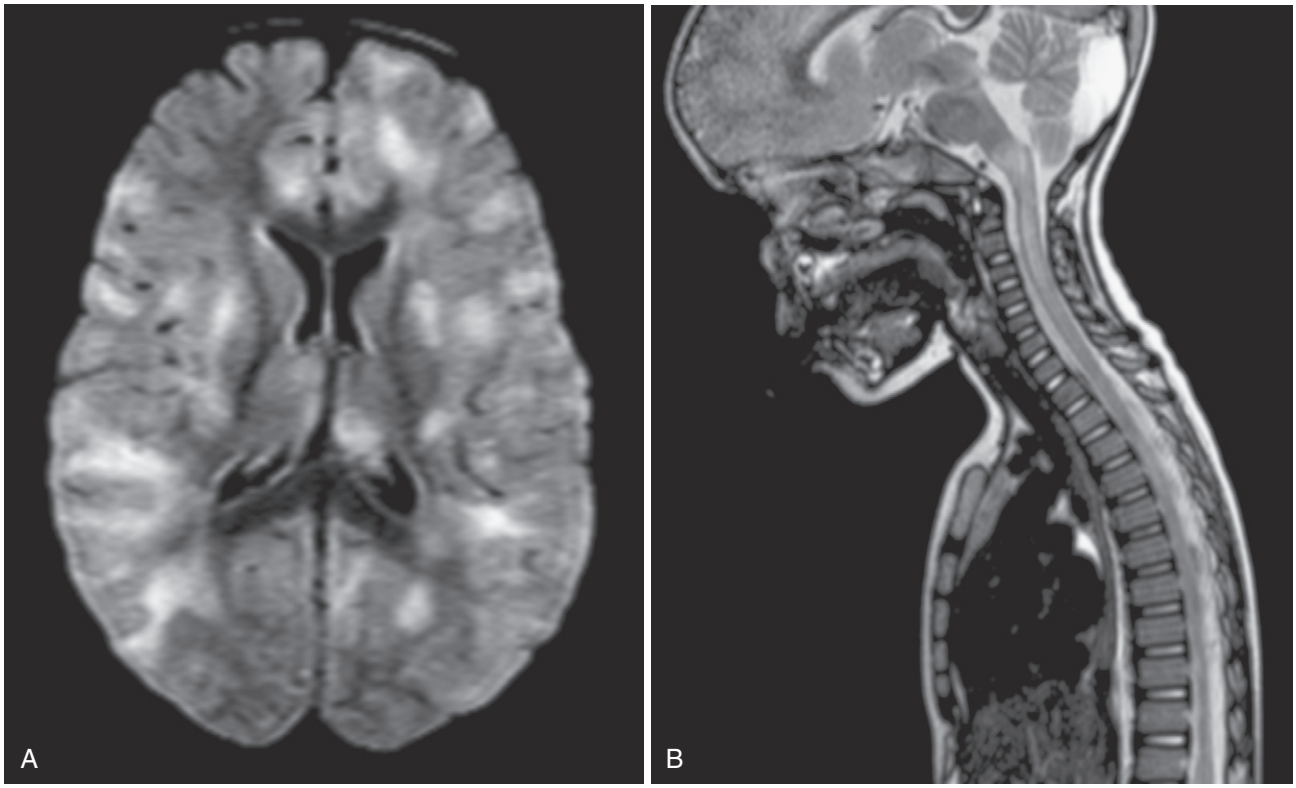

FIGURE 37A-1 ■ **A,** Axial fluid-attenuated inversion recovery (FLAIR) image showing multifocal areas of hyperintensity in both cerebral hemispheres involving cortical gray matter, centrum semiovale, and deep gray nuclei. **B,** Sagittal T2-weighted spine image with increased intrinsic signal consistent with longitudinally extensive transverse myelitis in the same patient. (Courtesy Tim Lotze, MD.)

disease process, including metabolic disorders or primary inflammatory diseases such as multiple sclerosis.

Acute hemorrhagic leukoencephalitis is considered a severe variant of ADEM. It accounts for 2 percent of patients with ADEM.[51] Clinical presentation is similar to that of ADEM, including an acute onset of neurologic deficits 1 to 3 weeks after an upper respiratory tract infection or vaccination. Seizures and coma ensue within hours. The mortality rate is extremely high with fulminant disease. Survivors often have severe residual neurologic deficits. The clinical presentation and imaging features mimic those typically seen in herpes simplex virus encephalitis. Evidence of inflammatory changes and hemorrhage on MRI often is not present for the first few days in herpes simplex virus encephalitis, which may help distinguish the two conditions.[20] Early recognition with prompt institution of steroids or other immunosuppressive agents can be lifesaving.

Historically, site-restricted forms of demyelination, such as optic neuritis or transverse myelitis, have been included as part of the spectrum of ADEM. Although these conditions share similar underlying pathology, clinical presentations and prognosis are different, and they should be considered to be separate entities. They are considered clinically isolated syndromes by the current consensus definitions for demyelinating disease.[35] Clinically isolated syndromes may carry a greater risk for development of multiple sclerosis or other recurrent forms of demyelinating disease.[53] *Neuromyelitis optica*, also known as *Devic disease*, describes coincident or sequential optic neuritis and longitudinally extensive myelitis. It is not always peri-infectious in onset and is associated with antibodies directed against aquaporin-4 water channels in the CNS.[54]

Multiple sclerosis is the second most common cause of acquired neurologic disability in adults. It is an uncommon finding in pediatric patients. Five percent of adults with multiple sclerosis had onset of disease before reaching 18 years of age.[5] Clinical phenotypes consistent with ADEM, relapsing ADEM, and multiphasic ADEM should not be confused with multiple sclerosis. Although they may share some of the same initiating pathologic mechanisms, they are different diseases.[11] The diagnosis of multiple sclerosis requires clinical or radiographic evidence for demyelinating events separated in space and time in the CNS. Patients typically follow a relapsing course of neurologic attacks, with resolution or remission of disability between attacks. Evolution into a course of progressive disability occurs in 50 percent of patients who have had the disease for more than 15 years.[8] Treatment consists of medications that modulate the immune response by decreasing migration into the CNS, altering cytokine profiles, and interrupting antigen presentation.

PATHOLOGY AND PATHOGENESIS

Role of Infection

ADEM is preceded by a viral or bacterial infection in 75 percent of the cases. This infection usually is in the form of a nonspecific upper respiratory tract infection. Many

TABLE 37A-2 **Infectious Pathogens Associated with Acute Disseminated Encephalomyelitis**

Viral
Coxsackie
Cytomegalovirus
Epstein-Barr
Hepatitis A or B
Herpes simplex
Human herpesvirus–6
Human T-lymphotropic virus–1
Human immunodeficiency virus
Influenza A or B
Measles
Mumps
Rocky Mountain spotted fever
Rubella
Vaccinia
Varicella
Bacterial
Borrelia burgdorferi
Campylobacter
Chlamydia
Legionella
Leptospira
Mycoplasma pneumoniae
Rickettsia rickettsii
Streptococcus

TABLE 37A-3 **Vaccines Associated with Acute Disseminated Encephalomyelitis**

Diphtheria-tetanus-polio
Hepatitis B
Hog vaccine
Japanese B encephalitis
Measles
Mumps
Polio
Rabies
Smallpox
Tetanus
Tick-borne encephalitis

different pathogens have been identified in association with the illness (Table 37A-2). No well-defined latency period has been identified in which an infection can be related to ADEM. Generally, patients present within 1 month of their illness. Correlation between ADEM and an infection occurring more than 30 days before is more difficult because children are diagnosed with a viral infection four to six times per year, resulting in a positive infectious history in 33 to 50 percent of patients.[41]

The phenotypic presentation for ADEM varies depending on the infectious agent. Some organisms have been associated with typical clinical features. Measles virus infection is associated with ADEM in 1/1000 cases. The clinical course often is fulminating, with severe neurologic sequelae or death in most patients. Introduction of vaccination has reduced markedly the occurrence of measles in developed countries, although vaccine-associated ADEM is described in 2 cases per 1 million.[7]

Postvaricella ADEM is characterized by ataxia in more than 90 percent of patients, occasionally with an explosive onset.[26] Headache and meningismus are typical constitutional signs. Pyramidal symptoms include bilateral symmetric upper motor neuron weakness. Patients also may have mood disturbances with a depressed affect or irritability.

ADEM that develops after a rubella infection is similar to measles, with an explosive onset in most children.[26] Profound lethargy, coma, and generalized seizures often occur. Most patients with explosive onset of disease have pyramidal signs and myelitis, whereas a milder phenotype is more likely to have ataxia. Both forms also have been associated with brain stem signs.

Group A β-hemolytic streptococcal infection has been associated with a dystonic extrapyramidal syndrome. Abnormal movements include dystonia, tremor, and parkinsonism. Tics and chorea have not been associated with this condition. Similar to Sydenham chorea and PANDAS, behavioral disturbances, such as emotional lability, obsessive-compulsive tendencies, and inappropriate speech, can occur. Changes in mental status can vary from irritability to coma. MRI shows a predilection for demyelination within the basal ganglia, which includes the caudate, putamen, and globus pallidus. Other deep gray structures, including the thalamus, subthalamus, and substantia nigra, may be affected as well. This finding is distinct from Sydenham chorea and PANDAS, in which imaging is unremarkable. It has been associated with anti–basal ganglia antibodies that cross-react with certain strains of streptococcus.[14]

Role of Immunization

Vaccine-associated ADEM has been described with nearly every immunization (Table 37A-3). The Collaborating Center for Reference and Research on Viral Hepatitis of the World Health Organization has suggested a maximal period of 3 months to diagnose vaccination-associated ADEM. Postvaccination ADEM accounts for fewer than 5 percent of ADEM cases.

Historically, Pasteur rabies vaccination was associated with prototypic ADEM in approximately 1/1000 individuals.[7] The inoculum was derived from rabbit spinal cord injected with fixed rabies virus. The disease was thought to result principally from the neural tissue contaminating the vaccine, rather than the virus itself. This suggestion is supported further by the continued higher incidence of vaccine-associated encephalomyelitis in patients receiving the Semple or duck embryo vaccinations, both of which contain neural tissue. These forms of the vaccination usually are found in developing countries. Experimental allergic encephalomyelitis, the animal model of demyelinating disease, is induced by inoculating myelin or myelin antigens into a suitable experimental animal, further supporting a role of CNS tissue as the causal agent.

Currently, measles, mumps, and rubella vaccination is associated most often with postvaccination encephalomyelitis. A significant difference exists between the incidence of live-measles vaccination associated ADEM (1 to 2/1 million) and the incidence of ADEM previously associated with the measles virus infection (1/1000).

Smallpox was eradicated in 1980 after a successful vaccination program that was discontinued in the United States in 1971.[12] The World Health Organization has allowed retention of the variola virus at a designated laboratory in the United States and Russia for the purpose of creating a safer vaccination than the presently available live vaccinia virus. More recent concerns for bioterrorism have resulted in the reinstitution of smallpox vaccination to certain identified groups within the United States that might come into contact with the virus as first responders to a possible smallpox outbreak. The previously reported incidence of ADEM associated with smallpox vaccination was 10 to 300 cases for every 1 million vaccinations. It is estimated, based on these figures, that vaccination of the entire British population would result in approximately 600 cases of vaccine-associated ADEM. Administration of antivaccinia gamma globulin at the time of vaccination can help prevent this complication.[48]

Immunologic Factors

A review of the normal process of immune system regulation and surveillance of the CNS is useful when discussing immune dysfunction in the pathogenesis of ADEM. Similar to other disorders of immune dysfunction, activated $CD4^+$ T cells play a principal role in the disease process. T cells reactive against self-antigens, including myelin components, are present in the normal immune system. Several regulatory mechanisms are thought to prevent activation of these lymphocytes, and failure of such processes results in autoimmune conditions. The thymus plays a crucial role in normal T-cell development by deleting many autoreactive lymphocytes from the immune system. Other peripheral mechanisms also are needed because this thymic depletion often is incomplete. Clonal anergy describes the unresponsive state of T cells that have encountered their antigen without costimulatory factors.[44] A risk for failure of this mechanism exists through release of nonspecific, costimulatory factors from damaged tissue. Another peripheral process is immunologic ignorance, in which no productive reaction exists between the T cell and its corresponding peptide/major histocompatibility complex on an antigen-presenting cell. Changes in antigen availability, such as through increased antigen presentation by an invading microorganism, can disrupt this safeguard.[28]

Active regulation is a third process by which regulatory T cells prevent expansion of self-reactive lymphocytes.[34] Regulatory T cells exert their suppressive effects through direct cell-to-cell contact with membrane-bound molecules (CTLA-4) or indirectly through soluble suppressive cytokines, such as interleukin (IL)-10 and transforming growth factor-β (TGF-β).[27] Natural killer cells expressing a T-cell receptor also can regulate autoimmune diseases.[40] The presence of such cells within demyelinating regions suggests that some of the inflammation associated with ADEM may have a protective effect.

Although failure of these mechanisms may result in the activation of T cells reactive against myelin antigens, migration of lymphocytes into the CNS is required to produce disease. The CNS has been considered a site of limited immunologic surveillance because of the blood-brain barrier and lack of classic lymph vessels. The blood-brain barrier is principally a mechanical diffusion barrier, however, for hydrophilic molecules formed by specialized endothelial cells at the level of the capillaries. Leukocytes readily cross through endothelial cells in postcapillary venules to occupy the perivenular spaces or move on to the neuropil.[23] In addition, antigens and antigen-presenting cells drain from the brain into cervical lymph nodes via the cribriform plate and perineural sheath of the cranial nerves.[13,30] As discussed later, these factors allow for the presentation of antigen in the systemic immune compartment and passage of leukocytes into the CNS for production of disease.

Children with demyelinating disease of the CNS are thought to have a genetic predisposition for such conditions. The strongest data relate to susceptibility genes on chromosome 6p21 in the area of the histocompatibility leukocyte antigen (HLA). Much of the research into these genetic determinants has been related to adult-onset multiple sclerosis.[50] Limited research is available for pediatric demyelinating diseases. Some of the findings show similarities, however, among age groups and various demyelinating diseases. Linkage studies in Russian children found an association between ADEM and *HLA-DRB1*01* and *HLA-DRB1*017(03)* alleles.[26] Korean children with ADEM were found to have higher frequencies of *HLA-DRB4*0101* and *HLA-DRB1*1501* compared with controls. In adults with multiple sclerosis, the specific genes that confer the highest risk include *HLA-DRB1*1501* and *HLA-DRB5*0101* among others. In multiple sclerosis, these genes have been linked to earlier disease onset, female gender, a relapsing remitting course, and optic neuritis or spinal involvement as the initial symptom. In addition, genes associated with this haplotype include TGF-β family members, CTLA-4, the tumor necrosis factor cluster, IL-1 receptor antagonist, IL-1, and estrogen receptor. Polymorphisms in these genes also have been associated with increased risk for development of demyelinating disease.[18]

The precise mechanism by which these HLA class II genes confer risk for development of demyelinating disease is unclear. Possibilities include (1) preferential binding of self-antigens by these peptides, (2) preferential linkage of autoreactive T cells to the antigen-presenting cell expressing these peptides, (3) abnormal antigen presentation by certain DR molecules, and (4) engagement of HLA class II molecules leading to intracellular signaling events.[50]

Humoral factors also play a role in the production of demyelinating disease. This finding is based on disease-associated laboratory findings and treatment responses. Analysis of CSF in one third of patients with ADEM shows production of oligoclonal bands, which are not found in the serum.[15] In addition, some patients with

post–rabies inoculation ADEM have been found to have positive serum antibodies against myelin basic protein and galactocerebroside.[36]

Pathogenesis

ADEM is considered to occur in genetically susceptible individuals prone to immune system dysregulation after an encounter with an appropriate environmental stimulus. Mechanisms of disease production are based principally on two animal models that closely resemble the disease. The first is experimental autoimmune encephalomyelitis.[47] In this model, animals develop monophasic neurologic disease similar to ADEM after receiving immunization with CNS homogenate or encephalitogenic myelin peptides emulsified in Freund complete adjuvant. The second model is Theiler murine encephalomyelitis, in which susceptible mouse strains develop disease after receiving direct injection of Theiler murine encephalomyelitis virus into the cerebrum.[24] In both of these models, increased exposure of the immune system to myelin proteins produces disease.

By comparing these models with ADEM in humans, two pathogenetic concepts have been developed. The inflammatory cascade concept implies a direct CNS infection with a neurotropic pathogen, which results in CNS tissue damage and systemic leakage of CNS-confined autoantigens through a damaged blood-brain barrier into the systemic circulation. Presentation of these antigens within systemic lymphatic organs leads to tolerance breakdown and a self-reactive encephalitogenic T-cell response. The molecular mimicry concept suggests structural amino acid homology between the invading pathogen and myelin basic protein in the host.[19] Although similar to myelin peptides, the amino acid sequence contains subtle differences that fail to prevent immune tolerance and result in activation of myelin-reactive T cells against similar "self" myelin antigens. The Epstein-Barr virus provides an example of this. The virus contains a pentapeptide sequence in its nuclear antigen (EBNA) that shares sequence homology with an epitope of myelin basic protein, a major protein of the myelin sheath.[10] Epstein-Barr virus has been implicated in the pathogenesis of multiple sclerosis partly based on this concept of molecular mimicry.[2] Recently, antibodies to myelin oligodendrocyte glycoprotein have been shown to be present during the period of active demyelination in ADEM but quickly fall in most patients.[46] In those with persistent or rebounding of myelin oligodendrocyte glycoprotein antibody levels, a propensity was seen to develop childhood multiple sclerosis, suggesting this might be prognostic.

The immunopathologic events leading to ADEM can be divided into two major phases: (1) initial T-cell priming and activation and (2) subsequent recruitment and effector phase.[6] The priming phase occurs in systemic secondary lymphoid organs, in which the antigen-presenting cell presents myelin protein antigen and peptides to neuroantigen-reactive T cells. The activated T cells expand and then migrate to the CNS via the postcapillary venules into the perivascular space. In the Virchow-Robin space, the T cells re-encounter their cognate antigen, in the context of HLA class II molecules expressed by dendritic cells.[21] This reactivation allows the T cells to migrate through the glial limitans and enter the brain parenchyma.

Further recruitment occurs through the production of cytokines and chemokines by antigen-presenting cells and activated T cells, promoting migration into the CNS of additional T cells and other leukocytes, such as polymorphonuclear and monomorphonuclear phagocytes.[50] Breakdown of the blood-brain barrier occurs by release of proteases from recruited mast cells, T cells, and monocytes. In addition, production of reactive oxygen radicals occurs, causing further endothelial injury, which leads to the effector phase in which T cells have more of a secondary role to other inflammatory processes that cause demyelination and axonal injury. These inflammatory processes include oxygen and nitrogen radicals, tumor necrosis factor-α, direct and indirect complement activation, antibody-dependent cellular toxicity, myelin phagocytosis, direct axonal injury by $CD8^+$ cytotoxic T lymphocytes, protease secretion, and oligodendrocyte apoptosis.[50] Glutamate-mediated excitotoxic injury of the oligodendrocytes also occurs.[45] The inflammatory process continues for a few days to 2 weeks, resulting in stretches of demyelinated axons, some of which may be transected.

The repair process begins with activation and proliferation of astrocytes. Clearing of debris by macrophages and increased production of anti-inflammatory cytokines and various growth factors by resident cells and T cells occur. Oligodendrocyte precursors become activated and, along with surviving oligodendrocytes, begin the process of remyelination. The clinical and imaging outcome of ADEM most often shows complete recovery. Subtle differences in repaired myelin, including altered thickness and redistribution of sodium channels, may occur, however.[52] In addition, the relative composition of myelin peptides is altered to forms that may have increased vulnerability to further damage[48] and may explain recurrent forms of ADEM.

CLINICAL EVALUATION

ADEM should be considered in a child seen several days after a febrile illness with subacute onset of encephalopathy and polysymptomatic neurologic deficits. The clinical features require ruling out other possible diagnoses, however, through additional diagnostic tests.

Diagnostic imaging is the most useful tool in establishing the diagnosis. Computed tomography (CT) scans usually are done on an emergent basis for encephalopathic patients. Areas of demyelination may appear as darker areas of hypodensity, with any hemorrhagic component being hyperdense. CT does not adequately show the full burden of disease, however, and may be completely normal. MRI is the most sensitive means for showing the widespread demyelination typical of the disease (see Fig. 37A-1). T2-weighted sequences provide the best assessment of the disease, with demyelinating areas being hyperintense and accompanied by surrounding edema. Administration of gadolinium contrast

material may show breakdown of the blood-brain barrier with areas of enhancement. This enhancement may appear homogeneous throughout the lesion or show a "broken ring" appearance, with the open edge pointing toward the cortex. Lesions may be large, measuring more than 1 cm in diameter. They typically are rounded with poorly defined margins.

Of most common concern in children presenting with a febrile encephalopathy is the possibility of an underlying CNS infection. A lumbar puncture often is done to investigate this possibility. A lymphocytic pleocytosis may occur (commonly >50 cells). Elevated albumin levels also may be present. Findings of neutrophils and elevated red blood cells alternatively would raise concern for the possibility of herpes encephalitis.

Intrathecal oligoclonal bands may be positive, and IgG synthesis may be increased in 30 percent of patients with ADEM. These abnormalities usually are associated with multiple sclerosis. In ADEM, convalescent testing for both should normalize, whereas tests remain positive in multiple sclerosis. Their relationship to the underlying disease pathophysiology is unclear but is thought to represent a response of B cells within the CNS to the inflammatory process.[3] Serologic studies of the CSF may be useful to uncover the causal agent, although results should be interpreted with caution because many neurotropic viruses have a high prevalence in the general population.

Rarely, patients may present with very large demyelinating lesions, characteristically described as *tumefactive*. In such circumstances, a biopsy may be needed to determine the underlying etiology. Findings typical of demyelination help rule out a CNS malignancy. Before a biopsy is done, the clinician should investigate for other evidence of demyelinating disease, including imaging of the spinal cord. A comorbid transverse myelitis would provide more evidence of a demyelinating condition and reduce the need for a biopsy. In addition, normal CSF cytology helps discount an underlying malignancy.

Primary and secondary CNS vasculitis associated with collagen vascular diseases, such as systemic lupus erythematosus or antiphospholipid antibody syndrome, may manifest with a similar clinical and radiologic picture. These diseases are typified by recurrent ischemic strokes. Distinguishing features for antiphospholipid antibody syndrome include a family history of strokes or other thromboses at a young age and fetal loss. Testing for antiphospholipid antibody and lupus anticoagulant is positive. Primary CNS vasculitis with strokes is best investigated through conventional angiography showing an irregular vessel lumen. Leptomeningeal biopsy is considered the gold standard to confirm the diagnosis.

A severe course that principally affects the optic nerves and spinal cord should raise concern for neuromyelitis optica. Serum or CSF testing for the aquaporin-4 water channel antibody that is associated with this disease is clinically available. The test is greater than 90 percent specific for neuromyelitis optica.[34]

Neurophysiologic studies can be useful to evaluate comorbidities and burden of disease. An electroencephalogram should be obtained in patients with seizures and severe encephalopathy to characterize the seizure focus and investigate for subclinical seizures. Findings often are consistent with a diffuse disturbance in brain function noting slowing of the normal electric rhythms. Visual evoked potentials can evaluate for demyelination of the optic nerves, which may not be appreciated on imaging. The examination measures the time for an electric impulse to travel from the retina to the occipital lobe, with delays suggesting a demyelinating lesion. The patient needs to be attentive to a flashing light stimulus, rendering this test difficult to perform in patients with severe encephalopathy.

TREATMENT

To date, no controlled clinical trials have been conducted regarding the optimal treatment for ADEM. Based on empirical evidence, intravenous high-dose corticosteroids are accepted as first-line treatment.[49] Methylprednisolone is given at 30 mg/kg per dose (maximum 1000 mg) for a total of five doses. Alternatively, dexamethasone, 1 mg/kg per dose for five doses, may be used. A prednisone taper typically is instituted on completion of this regimen, with an initial dosing of 1 mg/kg/day and tapering by 5 mg every 5 days. Alternative anti-inflammatory and immunosuppressive therapies may be used depending on the response to the initial steroid treatment. Intravenous immunoglobulin (IVIG) has been found to be beneficial in some patients.[49] IVIG is given 2 g/kg divided over 2 to 5 days.

Plasmapheresis also may be used.[31,33] The patient receives a total of seven treatments consisting of 1.1 to 1.4 plasma volume exchanges over a 14-day course. IVIG and plasmapheresis typically are not considered as first-line treatment for ADEM, partly related to availability of IVIG or pheresis units, cost of treatment in contrast to steroids, prolonged hospitalization, and need for adequate venous access. Nonetheless, they should be considered secondary treatment options, especially in the occasional patient who continues to deteriorate during steroid treatment or who fails to show adequate recovery 7 days after completing intravenous steroids. The choice between the two agents is arbitrary. Particular consideration for plasmapheresis might be given in the setting of a comorbid myelopathy because this has been noted to be beneficial in patients presenting with transverse myelitis.[29] Severe cases failing to respond to any of these measures may require immunosuppressive agents, such as cyclophosphamide.[39] A few cases of severe ADEM with massive edema have required hemicraniectomy to prevent severe neurologic sequelae or death.[1,17]

Additional treatment that may be needed depends on the patient's neurologic deficits. Anticonvulsants should be given for management of seizures. Attention given to bowel and bladder care is important to avoid secondary complications of retention. Impaired swallowing requires adequate nutrition to be provided by gavage feedings. Transverse myelopathy can be associated with autonomic dysfunction, including orthostatic hypotension, necessitating the use of an abdominal binder. Rehabilitation should begin at the time of admission, with the assistance of physical medicine and rehabilitation specialists. During

the long-term recovery period, modifications to the home and school environment may be needed, depending on residual deficits.

OUTCOME AND PROGNOSIS

Historically, ADEM was associated with high morbidity and mortality rates principally related to disease associated with the measles virus. Measles-associated ADEM was associated with death in 25 percent of affected patients, with an additional 30 percent of patients having severe neurologic sequelae.[38] This dismal outcome has improved dramatically with institution of measles vaccination. The introduction of high-dose steroid treatment in modifying the disease course also may have improved the current outcome findings. A 5 percent mortality rate remains associated with this disease.

Currently, 80 to 90 percent of patients show excellent recovery of functional and cognitive deficits.[4,15,26,51] Children typically have a less severe course and fewer long-term neurologic sequelae than adults.[32] Mild neurologic disabilities, with weakness and fine motor difficulties being the most common deficits, may be present in some patients. Epilepsy may occur in 6 percent of patients after an episode of ADEM.[15] Neurocognitive testing of children recovering from ADEM has shown mild impairments in attention and executive function and reduced visuospatial and visuomotor skills.[22] The greatest risk for development of neurologic sequelae may be in older patients with sudden onset of severe neurologic symptoms.

Recurrent forms of the disease often raise concern for pediatric multiple sclerosis. The exact relationship between ADEM and multiple sclerosis remains to be better defined. The conditions are distinct, however, based on the clinical presentations, imaging, and clinical courses. Studies investigating risk for disease recurrence or evolution into multiple sclerosis have been limited and do not adequately define ADEM in the context of the current definition, and they do not distinguish between recurrent forms of the disease and multiple sclerosis. Disease recurrence occurs in 30 percent of patients.[41] Most patients have their second attack within the first year after their initial event.[4,42] It often occurs soon after discontinuation of steroid taper, or after an infection or vaccination encountered shortly after the initial event. Treatment and outcome for recurrences are not different from those described for the initial event.

NEW REFERENCES SINCE THE SIXTH EDITION

17. Dombrowski KE, Meta AI, Turner DA, et al. Life-saving hemicraniectomy for fulminant acute disseminated encephalomyelitis. *Br J Neurosurg* 2011;**25**:249–52.
32. Ketelslegers IA, Visser IE, Neuteboom RF, et al. Disease course and outcome of acute disseminated encephalomyelitis is more severe in adults than in children. *Mult Scler* 2010;**17**:441–8.
46. Pröbstel AK, Dornmair K, Bittner R, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. *Neurology* 2011;**77**:580–8.

The full reference list for this chapter is available at expertconsult.com.

37B INFECTION-ASSOCIATED MYELITIS AND MYELOPATHIES OF THE SPINAL CORD

J. Lloyd Holder, Jr. • Timothy E. Lotze

ACUTE TRANSVERSE MYELITIS

The sudden and progressive onset of paresis, sensory deficits and bowel and bladder dysfunction that characterizes acute transverse myelitis (ATM) terrifies affected children and their families. Successful management requires prompt differentiation from other myelopathic disorders to determine proper treatment. Because of the association of ATM with numerous infections, specialists in infectious diseases frequently participate in the care of children with ATM. The clinical presentation, radiologic features, differential diagnosis, pathophysiology, treatment, and prognosis of pediatric ATM are reviewed here.

DIAGNOSTIC CRITERIA

ATM is an acquired demyelinating disorder consisting of progressive signs and symptoms reflecting bilateral sensory, motor, or autonomic dysfunction attributable to the spinal cord. This constellation of signs and symptoms can be caused by a heterogeneous group of disorders, including ATM. Several disorders, including direct infections of the spinal cord, can mimic ATM.

Diagnostic criteria were established by the Transverse Myelitis Working Group Consortium in 2002 and consist of both inclusionary and exclusionary criteria. The criteria were established to rapidly differentiate ATM from other myelopathies that require different treatment regimens. Differentiation from extramedullary compression such as that caused by extrinsic tumor, abscess, hematoma, and other intramedullary lesions such as infarction and intrinsic tumors are necessary to guide treatment and facilitate discussion of prognosis. Table 37B-1 lists both the inclusionary and exclusionary criteria for ATM.

REFERENCES

1. Ahmed AI, Eynon CA, Kinton L, et al. Decompressive craniectomy for acute disseminated encephalomyelitis. *Neurocrit Care* 2010;**13**: 393–5.
2. Alotaibi S, Kennedy J, Tellieret R, et al. Epstein-Barr virus in pediatric multiple sclerosis. *JAMA* 2004;**291**:1875–9.
3. Antel J, Bar-Or A. Roles of immunoglobulins and B cells in multiple sclerosis: from pathogenesis to treatment. *J Neuroimmunol* 2006; **180**:3–8.
4. Anlar B, Basaran C, Kose G, et al. Acute disseminated encephalomyelitis in children: outcome and prognosis. *Neuropediatrics* 2003; **34**:194–9.
5. Banwell BL. Pediatric multiple sclerosis. *Curr Neurol Neurosci Rep* 2004;**4**:245–52.
6. Becher B, Bechmann I, Greter M. Antigen presentation in autoimmunity and CNS inflammation: how T lymphocytes recognize the brain. *J Mol Med* 2006;**84**:532–43.
7. Bennetto L, Scolding N. Inflammatory/post-infectious encephalomyelitis. *J Neurol Neurosurg Psychiatry* 2004;**75**(suppl. 1):i22–8.
8. Boiko A, Vorobeychik G, Paty D, et al. Early onset multiple sclerosis: a longitudinal study. *Neurology* 2002;**59**:1006–10.
9. Brass SD, Caramanos Z, Santos C, et al. Multiple sclerosis vs acute disseminated encephalomyelitis in childhood. *Pediatr Neurol* 2003;**29**:227–31.
10. Bray PF, Luka J, Bray PF, et al. Antibodies against Epstein-Barr nuclear antigen (EBNA) in multiple sclerosis CSF, and two pentapeptide sequence identities between EBNA and myelin basic protein. *Neurology* 1992;**42**:1798–804.
11. Brinar VV, Poser CM. The spectrum of disseminated encephalomyelitis. *Clin Neurol Neurosurg* 2006;**108**:295–310.
12. Casey CG, Iskander JK, Roper MH, et al. Adverse events associated with smallpox vaccination in the United States, January-October 2003. *JAMA* 2005;**294**:2734–43.
13. Cserr HF, Knopf PM. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. *Immunol Today* 1992;**13**:507–12.
14. Dale RC, Church AJ, Cardoso F, et al. Poststreptococcal acute disseminated encephalomyelitis with basal ganglia involvement and auto-reactive antibasal ganglia antibodies. *Ann Neurol* 2001; **50**:588–95.
15. Dale RC, de Sousa C, Chong WK, et al. Acute disseminated encephalomyelitis, multiphasic disseminated encephalomyelitis and multiple sclerosis in children. *Brain* 2000;**123**(Pt. 12):2407–22.
16. Davis LE, Booss J. Acute disseminated encephalomyelitis in children: a changing picture. *Pediatr Infect Dis J* 2003;**22**:829–31.
17. Dombrowski KE, Mehta AI, Turner DA, et al. Life-saving hemicraniectomy for fulminant acute disseminated encephalomyelitis. *Br J Neurosurg* 2011;**25***(2)*:249–52.
18. Dyment DA, Ebers GC, Sadovnick AD. Genetics of multiple sclerosis. *Lancet Neurol* 2004;**3**:104–10.
19. Fujinami RS, Oldstone MB. Amino acid homology between the encephalitogenic site of myelin basic protein and virus: mechanism for autoimmunity. *Science* 1985;**230**:1043–5.
20. Gibbs WN, Kriedi MA, Kim RC, et al. Acute hemorrhagic leukoencephalitis: neuroimaging features and neuropathologic diagnosis. *J Comput Assist Tomogr* 2005;**29**:689–93.
21. Greter M, Heppner FL, Lemoa MP, et al. Dendritic cells permit immune invasion of the CNS in an animal model of multiple sclerosis. *Nat Med* 2005;**11**:328–34.
22. Hahn CD, Miles BS, MacGregor DL, et al. Neurocognitive outcome after acute disseminated encephalomyelitis. *Pediatr Neurol* 2003;**29**:117–23.
23. Hickey WF. Basic principles of immunological surveillance of the normal central nervous system. *Glia* 2001;**36**:118–24.
24. Humphries JM. Encephalitis complicating measles. *J Med Assoc State Ala* 1955;**25**:113–6.
25. Hynson JL, Kornberg AJ, Coleman LT, et al. Clinical and neuroradiologic features of acute disseminated encephalomyelitis in children. *Neurology* 2001;**56**:1308–12.
26. Idrissova ZR, Boldyreva MN, Dekonenko EP, et al. Acute disseminated encephalomyelitis in children: clinical features and HLA-DR linkage. *Eur J Neurol* 2003;**10**:537–46.
27. Jonuleit H, Schmitt E. The regulatory T cell family: distinct subsets and their interrelations. *J Immunol* 2003;**171**:6323–7.
28. Kamradt T, Mitchison NA. Tolerance and autoimmunity. *N Engl J Med* 2001;**344**:655–64.
29. Kaplin AI, Krishnan C, Deshpande DM, et al. Diagnosis and management of acute myelopathies. *Neurologist* 2005;**11**:2–18.
30. Karman J, Ling C, Sandor M, et al. Initiation of immune responses in brain is promoted by local dendritic cells. *J Immunol* 2004; **173**:2353–61.
31. Keegan M, Pineda AA, McClelland RL, et al. Plasma exchange for severe attacks of CNS demyelination: predictors of response. *Neurology* 2002;**58**:143–6.
32. Ketelslegers IA, Visser IE, Neuteboom RF, et al. Disease course and outcome of acute disseminated encephalomyelitis is more severe in adults than in children. *Mult Scler* 2010;**17***(4)*:441–8.
33. Khurana DS, Melvin JJ, Kothare SV, et al. Acute disseminated encephalomyelitis in children: discordant neurologic and neuroimaging abnormalities and response to plasmapheresis. *Pediatrics* 2005;**116**:431–6.
34. King C, Llic A, Koelsch K, Sarvetnik N, et al. Homeostatic expansion of T cells during immune insufficiency generates autoimmunity. *Cell* 2004;**117**:265–77.
35. Krupp LB, Banwell B, Tenembaum S. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. *Neurology* 2008;**68**:S7–12.
36. Laouini D, Kennou MF, Khoufi S, et al. Antibodies to human myelin proteins and gangliosides in patients with acute neuroparalytic accidents induced by brain-derived rabies vaccine. *J Neuroimmunol* 1998;**91**:63–72.
37. Leake JA, Albani S, Kao AS, et al. Acute disseminated encephalomyelitis in childhood: epidemiologic, clinical and laboratory features. *Pediatr Infect Dis J* 2004;**23**:756–64.
38. Lipton LH. Theiler's virus infection in mice: an unusual biphasic disease process leading to demyelination. *Infect Immun* 1975;**11**: 1147–55.
39. Markus R, Brew BJ, Turner J, et al. Successful outcome with aggressive treatment of acute haemorrhagic leukoencephalitis. *J Neurol Neurosurg Psychiatry* 1997;**63**:551.
40. McHugh RS, Shevach EM. Cutting edge: depletion of CD4+CD25+ regulatory T cells is necessary, but not sufficient, for induction of organ-specific autoimmune disease. *J Immunol* 2002; **168**:5979–83.
41. Menge T, Hemmer B, Nessler S, et al. Acute disseminated encephalomyelitis: an update. *Arch Neurol* 2005;**62**:1673–80.
42. Mikaeloff Y, Suissa S, Vallée L, et al. First episode of acute CNS inflammatory demyelination in childhood: Prognostic factors for multiple sclerosis and disability. *J Pediatr* 2004;**144**:246–52.
43. Murthy SN, Faden HS, Cohen ME, et al. Acute disseminated encephalomyelitis in children. *Pediatrics* 2002;**110**(2 Pt. 1):e21.
44. Nossal GJV. A purgative mastery. *Nature* 2001;**412**:685–6.
45. Pitt D, Werner P, Raine CS. Glutamate excitotoxicity in a model of multiple sclerosis. *Nat Med* 2000;**6**:67–70.
46. Pröbstel AK, Dornmair K, Bittner R, et al. Antibodies to MOG are transient in childhood acute disseminated encephalomyelitis. *Neurology* 2011;**77**:580–8.
47. Rivers TM, McNair-Scott TF. Meningitis in man caused by a filterable virus. *Science* 1935;**31**:439–40.
48. Sejvar JJ, Labutta RJ, Chapman LE, et al. Neurologic adverse events associated with smallpox vaccination in the United States, 2002-2004. *J A M A* 2005;**294**:2744–50.
49. Shahar E, Andraus J, Savitizki D, et al. Outcome of severe encephalomyelitis in children: effect of high-dose methylprednisolone and immunoglobulins. *J Child Neurol* 2002;**17**:810–4.
50. Sospedra M, Martin R. Immunology of multiple sclerosis. *Annu Rev Immunol* 2005;**23**:683–747.
51. Tenembaum S, Chamoles N, Fejerman N. Acute disseminated encephalomyelitis: a long-term follow-up study of 84 pediatric patients. *Neurology* 2002;**59**:1224–31.
52. Whitaker JN, Kirk KA, Herman PK, et al. An immunochemical comparison of human myelin basic protein and its modified, citrullinated form, C8. *J Neuroimmunol* 1992;**36**:135–46.
53. Wilejto M, Shroff M, Buncic JR, et al. The clinical features, MRI findings, and outcome of optic neuritis in children. *Neurology* 2006;**67**:258–62.
54. Wingerchuk DM, Lennon VA, Pittock SJ, et al. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006;**66**:1485–9.

TABLE 37B-1 **Transverse Myelitis Consortium Working Group Diagnostic Criteria**

Inclusion Criteria	Exclusion Criteria
Bilateral signs/symptoms (not necessarily symmetric)	Clear arterial distribution clinical deficit consistent with thrombosis of anterior spinal artery
Clearly defined sensory level	Abnormal flow voids on surface of the spinal cord consistent with AVM
Exclusion of extra-axial compressive etiology by neuroimaging (MRI or myelography; CT of spine inadequate)	Serologic or clinical evidence of connective tissue disease (sarcoidosis, Behçet disease, Sjögren syndrome, SLE, mixed connective tissue disorder)*
Inflammation within spinal cord shown by CSF pleocytosis or elevated IgG index or gadolinium enhancement. If no inflammatory criteria are met at symptom onset, repeat MRI and lumbar puncture evaluation 2-7 days after symptom onset meet criteria	CNS manifestations of syphilis, Lyme disease, HIV, HTLV-1, *Mycoplasma,* other viral infection (e.g., HSV-1, HSV-2, VZV, EBV, CMV, HHV-6, enteroviruses)* Brain MRI abnormalities suggestive of MS* History of clinically apparent optic neuritis*
Progression to nadir 4 hr to 21 days after onset of symptoms (if patient awakens with symptoms, symptoms must become more pronounced from point of awakening)	

Data from Transverse Myelitis Working Group. Proposed diagnostic criteria and nosology of acute myelitis. *Neurology* 2002;**59**:499–505.

*Do not exclude disease-associated acute transverse myelitis.

AVM, Arteriovenous malformation; *CMV,* cytomegalovirus; *CNS,* central nervous system; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *EBV,* Epstein-Barr virus; *HIV,* human immunodeficiency virus; *HHV-6,* human herpesvirus-6; *HSV,* herpes simplex virus; *HTLV-1,* human T-cell lymphotrophic virus-1; *MRI,* magnetic resonance imaging; *MS,* multiple sclerosis; *SLE,* systemic lupus erythematosus; *VZV,* varicella-zoster virus.

EPIDEMIOLOGY

Two prospective studies have evaluated the incidence of acquired ATM in children.[7,16] Based on these studies, the incidence in pediatric populations is approximately 1.72 to 2 persons per 1 million. Although early case series had suggested a slight female predominance,[14,15,20,37,56,58] the more recent prospective studies have found a male predominance in the pediatric population. When stratifying for children younger than 10 years of age at onset and older than 10 years of age, one study found a striking 1:2 female to male ratio in children younger than 10 years old. In children with age of onset older than 10 years, the female to male ratio was 1.2:1, similar to those of other inflammatory demyelinating disorders.[7] The mean age of onset is between 9 and 10.5 years; however, onset has been reported as early as 6 months. Of interest, one study found a bimodal distribution with peak ages of onset at 4 and 15 years.[16]

CLINICAL PRESENTATION

Three phases occur in patients with ATM: initial, plateau, and recovery. The initial phase begins with the presenting symptom and concludes with reaching the neurologic nadir. The plateau phase begins with reaching the neurologic nadir and is a period during which the symptoms are more or less stable. The last phase is recovery, which is the period of slow improvement in neurologic function. The length of time in each phase has been useful for prognostication of recovery and will be discussed later. Patients typically present acutely to subacutely with a combination of sensory and motor deficits. The most common motor symptom is bilateral leg weakness (82%), with a paraplegia developing in 36 percent of cases, although upper extremity weakness is a rare initial feature (4%).[16] Involvement of the arms occurs in 37 percent of patients at some point. Approximately 70 to 90 percent of patients develop bowel and bladder dysfunction, ranging from urinary retention and constipation to incontinence.[14,16] A similar percentage of patients report sensory symptoms, including paresthesias and numbness. Most patients reach their neurologic nadir within 1 week, and patients rarely have worsening of symptoms more than 4 weeks after onset of symptoms.[14,16] Children with extremely rapid evolution of their symptoms (1 to 4 hours) should cause the clinician to take pause and consider a possible ischemic etiology.

The general examination, although usually unremarkable, may reveal signs suggestive of an underlying systemic infection or autoimmune disorder. Fever is not uncommon, with one study reporting its presence in 20 percent of children with ATM.[7] Abdominal examination may reveal a distended bladder. A diffuse or dermatomal vesicular rash or its sequelae suggest concurrent or preceding chickenpox or shingles.

The presence of any mental status changes suggests that the myelitis is a component of a more diffuse process (i.e., acute disseminated encephalomyelitis). In the acute stages, muscle tone is low in affected limbs, but with time may become spastic. Detailed muscle strength testing with objective grading serves as a crucial baseline to compare with serial examinations to determine whether the patient is improving. All sensory modalities should be assessed carefully. In one series, 65 percent of children with ATM had sensory symptoms, with over 80 percent of those children having a clear sensory level.[16] An early case series had suggested that severe pain is a common symptom (88%); however, this is potentially skewed by selection bias of this retrospective study.[14] A spinal cord sensory level usually is located in the thoracic region (58%) and less commonly in the cervical (7%) or lumbar (31%) area.[16] In the acute phase, deep tendon reflexes are depressed in approximately 70 percent of patients and later become hyperactive. Similarly, the Babinski sign

may be negative early in the acute phase but soon becomes positive, indicating upper motor neuron dysfunction.

RADIOLOGIC FEATURES

Every patient with suspected ATM should undergo emergent gadolinium-enhanced magnetic resonance imaging (MRI) of the entire spine to confirm the diagnosis and rule out alternative diagnoses, particularly compressive lesions, such as epidural abscess, epidural hematoma, and extramedullary tumors, which are all potentially neurosurgical emergencies. T1-weighted and T2-weighted sagittal imaging of the entire spine can serve as an initial screen, followed by axial imaging in areas of suspected pathology. In addition, every patient with any neurologic symptoms not referable to the spinal cord should undergo MRI of the brain with gadolinium-enhancement. Optimally, this should include dedicated fine sections through the orbits to evaluate for optic neuritis associated with neuromyelitis optica (NMO). The distinction between idiopathic ATM and NMO is important for both treatment and prognosis. At least one study identified over 40 percent of children diagnosed with ATM with lesions on brain MRI, and the majority of these (65%) did not have symptoms or signs of cerebral involvement, leading the authors to recommend MRI brain scanning in all children with ATM.

Spinal MRI in ATM typically reveals T1-isointense (Fig. 37B-1A) and T2-hyperintense (see Fig. 37B-1B) signals over several contiguous spinal cord segments[14] and may involve the entire spine.[1] The most common finding is a T2-hyperintense lesion involving more than three segments, with one study finding the average length of lesion being more than six segments. The hyperintense signal can extend into the lower brain stem.[1] Spinal cord swelling with effacement of the surrounding cerebrospinal fluid (CSF) spaces may be present in severe cases (see Fig. 37B-1A). Axial imaging typically reveals lesions involving both gray and white matter (Fig. 37B-1D). Contrast enhancement is variably present and may appear diffuse, patchy, or nodular (see Fig. 37B-1C). In some patients with very suggestive clinical features, initial spine MRI may be normal and should be repeated several days later.[14,37,56] In rare cases, the MRI remains normal but does not rule out the diagnosis. A new modality still used exclusively in research that has been applied to ATM is diffusion tensor imaging (DTI). DTI is an MRI sequence that evaluates for the presence and integrity of white matter tracts. One study of a small number of adults with ATM revealed that those individuals with more severe disruption of white matter tracts within the lesion and distal to the lesion had a worse prognosis.[42] In the future, DTI might be useful for stratifying patients for likely prognosis and determining who might benefit from more aggressive treatment.

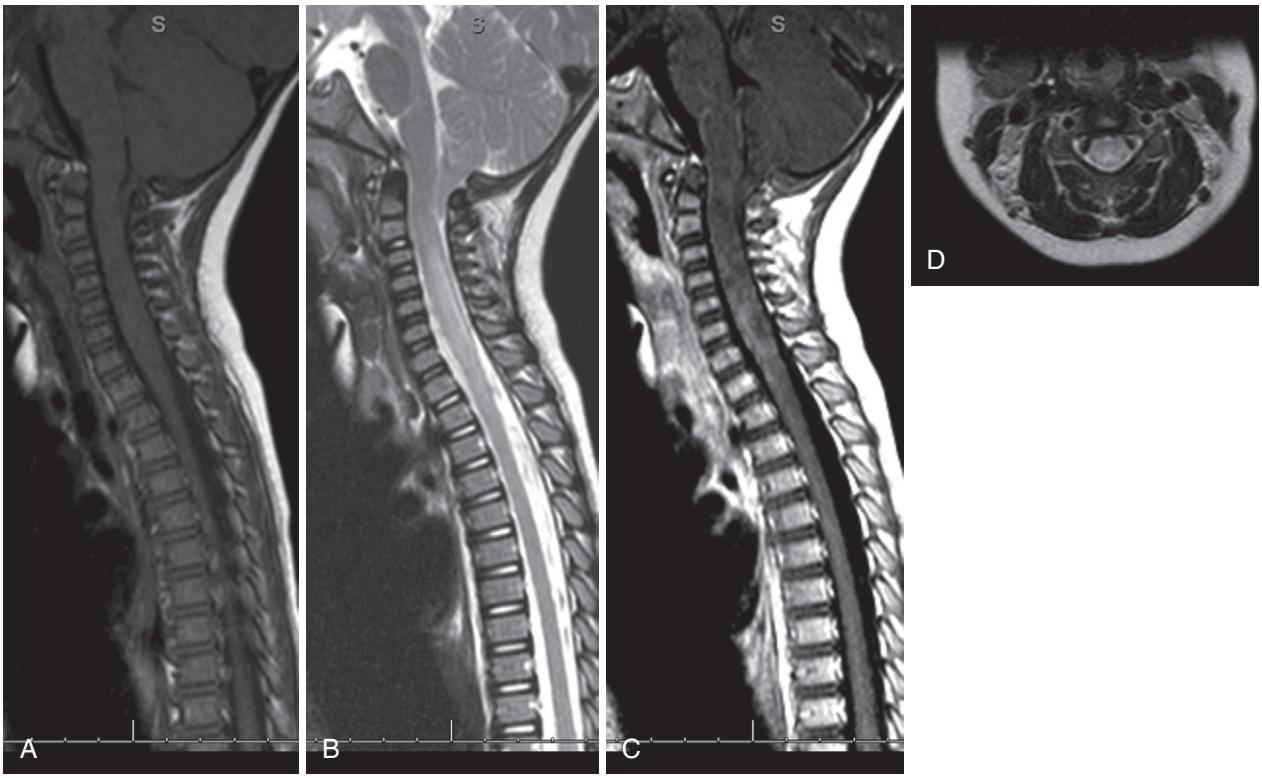

FIGURE 37B-1 ■ Acute transverse myelitis of the cervical cord. **A,** Sagittal T1-weighted magnetic resonance imaging (MRI) sequence through the cervical spinal cord demonstrating swelling of the cord. **B,** T2-weighted MRI sequence of same patient revealing longitudinally extensive hyperintensity through the cervical cord. **C,** T1-weighted MRI sequence with gadolinium revealing patchy enhancement of the cervical cord. **D,** T2-weighted axial MRI sequence through the cervical cord revealing hyperintensity of both gray and white matter.

LUMBAR PUNCTURE

Approximately 50 percent of pediatric patients with ATM have CSF pleocytosis, typically with a lymphocytic predominance.[14,16] Elevated CSF protein levels, either in isolation or in conjunction with pleocytosis, also are detected in approximately 50 percent of patients. Glucose typically is normal. A normal CSF profile does not rule out ATM because this pattern is seen in approximately 25 percent of patients. CSF cytology should be considered for patients with concern for neoplasm. Additional CSF testing is discussed further subsequently.

DIFFERENTIAL DIAGNOSIS

Conditions That Mimic Acute Transverse Myelitis

ATM can be associated with more widespread central nervous system (CNS) demyelinating disorders or systemic autoimmune disorders; in such cases, the term *disease-associated transverse myelitis* has been used. Isolated ATM can be triggered by identifiable preceding infections. Alternatively, ATM can be associated with a nonspecific preceding infection or have no apparent cause; for both of these subgroups, the term *idiopathic ATM* has been used. This last group constitutes the most common category in pediatric ATM. Although the precise pathophysiology of idiopathic ATM is uncertain, the frequent association with preceding infections and vaccinations and accumulating immunologic data suggest an inflammatory cause for the disorder.[11,14,33,55]

Numerous disorders can affect the spinal cord and produce identical symptoms and signs that mimic idiopathic ATM (Table 37B-2). Such conditions must be ruled out through a combination of history, physical examination, neuroimaging, and laboratory evaluation.

Extramedullary Lesions

Extramedullary compressive lesions are neurosurgical emergencies that must be diagnosed rapidly for effective treatment. Spinal epidural abscesses are rare in children. In one series of eight children compiled over a 15-year period at a major children's hospital, seven had fever and all eight had back pain.[5] *Staphylococcus aureus*, some of which were methicillin-resistant, were the most common isolates in this and other series. Other species that have been isolated from spinal epidural abscesses in children include *Salmonella enteritides* (primarily in sickle-cell patients),[5] *Streptococcus pneumoniae*,[8] *Bartonella henselae*,[31] and *Mycobacterium tuberculosis*.[36]

Spinal epidural hematomas also are rare occurrences in children. They can occur spontaneously or may be associated with anticoagulant use, clotting disorders, trauma, or lumbar puncture.[61] Spinal epidural hematomas after lumbar puncture typically self-resolve with no residual sequelae.[38] The first episode of significant bleeding in individuals with hemophilia A has been reported to be a spinal epidural hematoma either forming spontaneously or after lumbar puncture.[29,77] Although they typically cause slowly progressive symptoms (Foix-Alajouainine syndrome), paraspinal arteriovenous malformations can manifest acutely as a result of hemorrhage.[35] Unruptured AVMs usually can be detected as abnormal, dilated vasculature on MRI of the spine; subsequent spinal angiography can confirm the diagnosis.

Spinal cord compression resulting from metastatic disease occurred in about 6 percent of children with solid tumors at some point during their course in one series.[64] The most common responsible tumor types were primitive neuroectodermal tumor, soft tissue sarcoma, and neuroblastoma. When spinal cord compression is the initial manifestation of cancer, as was the case for 67 percent of the patients in this series, it can mimic ATM, although symptoms would in general be expected to more slowly progress than in ATM. Spinal MRI readily distinguishes spinal cord compression from ATM.

TABLE 37B-2 **Conditions That Mimic Idiopathic Acute Transverse Myelitis**

Direct infectious myelopathies
Extramedullary compressive lesions
Epidural abscess
Epidural hematoma
Extramedullary tumors
Guillain-Barré syndrome
Intramedullary spinal cord tumors
Astrocytomas
Ependymomas
Ischemia and infarction
Syringomyelia
Radiation injury
Traumatic spinal cord injury
Vascular malformations

Intramedullary Lesions

Spinal cord infarction is exceedingly rare in children but must be considered in the setting of acute onset of neurologic symptoms referable to the spinal cord. Clinical features and neuroimaging can be used to distinguish ATM from spinal cord infarction, although this can be difficult at initial presentation. Spinal cord infarct typically manifests in a hyperacute fashion (<4 hours from onset to severe neurologic symptoms) with dissociated sensory loss (absent temperature and pain sensation with preserved vibration and proprioception) from thrombosis of the anterior spinal artery.[57] One should inquire about recent trauma, even minor, including to the neck, because this can result in vertebral artery dissection and resultant embolic thrombi to the anterior spinal artery (Fig. 37B-2). Coagulopathies also have been associated with spinal cord ischemia and should be considered.[57] Accompanying MRI signal abnormality in the adjacent vertebral body is highly suggestive of the diagnosis but not universally present at onset of symptoms.[60] Diffusion-weighted MRI also may reveal the presence of ischemia and aid in making the diagnosis; however, diffusion restriction can be difficult to appreciate in the spinal cord.

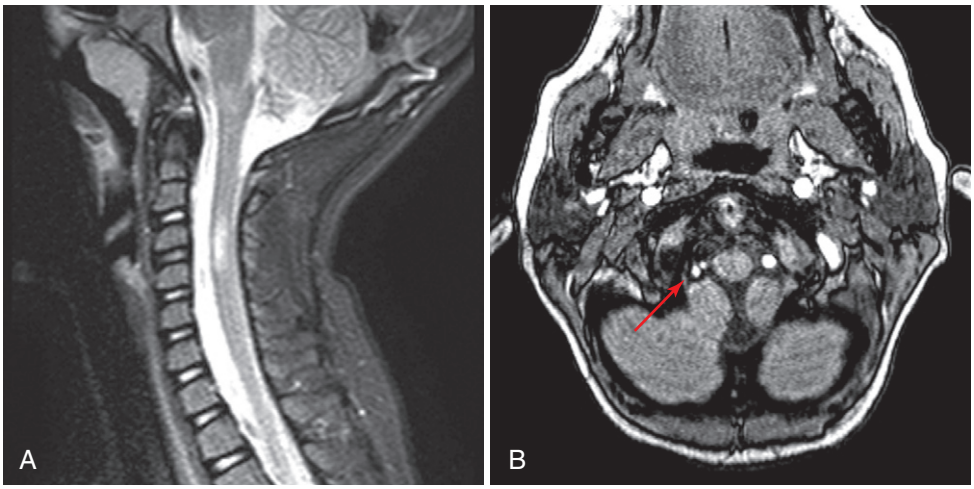

FIGURE 37B-2 ■ Ischemic infarction of the cervical cord. **A,** Sagittal T2-weighted magnetic resonance imaging sequence through the cervical spinal cord revealing hyperintensity of the cord. **B,** Axial magnetic resonance angiogram of the neck revealing a dissection of the vertebral artery on the right of the same patient *(arrow)*. (See companion Expert Consult website for color version.)

The most common intramedullary spinal cord tumors in children are astrocytomas (60%), followed by ependymomas (30%), and finally gangliogliomas (15%).[69] Although biopsy of the tumors is necessary to establish the exact histologic diagnosis, biopsy of the spinal cord rarely is needed to distinguish tumor from ATM. Despite similarities in presenting symptoms, clinical features can help distinguish intramedullary tumors from ATM. Intramedullary spinal cord tumors manifest more subacutely or chronically than does ATM, reflecting their low cellular growth rate.[69] In one study, the mean duration of symptoms before presentation of children with intramedullary spinal cord tumors was 254 days[18] in contrast to ATM, which manifests full symptoms in about 1 week. On examination, the presence of scoliosis or torticollis suggests the presence of longer-standing tumors rather than ATM.[69]

ATM and spinal cord tumors typically are isointense on T1-weighted MRI and hyperintense on T2-weighted images. Although intramedullary tumors display swelling and contrast enhancement more frequently than does ATM, these features also can be seen in the latter. Other radiographic features may be used to distinguish the two entities. The eccentric location of astrocytomas within the spinal cord can be used to differentiate them from ATM, which usually produces symmetric findings.[69] Although ependymomas can produce symmetric spinal cord expansion because of their origin from the central canal, these tumors usually span three to four vertebral segments,[4] whereas pediatric ATM typically involves longer areas of the spinal cord. In addition, an area of low signal intensity reflecting hemosiderin deposits from chronic hemorrhage at the edge of ependymomas also can serve as a radiographic clue.[4] Because patients with intramedullary spinal cord tumors may be diagnosed on the basis of CSF cytologic examination, this test should be considered in all patients with suspected ATM.

The clinical presentation of spinal cord dysfunction in the setting of prior irradiation suggests radiation-induced transverse myelitis as the most likely diagnosis. This entity typically manifests 9 to 18 months after radiotherapy, but it can occur earlier in children.[75]

Syringomyelia typically begins with pain and temperature sensory loss in a capelike distribution over the shoulders. However, if these early symptoms are missed and the syrinx grows, motor disturbances can develop that might be mistaken for ATM. The time course of developing sensory and motor dysfunction in syringomyelia is typically longer than that in ATM. The most common causes of syrinxes in children are tumors and Chiari I malformations.[70] Syringomyelia is readily distinguishable from ATM on MRI.

Peripheral Lesions

The initial clinical presentation of ATM can be quite similar to that of Guillain-Barré syndrome. Both disorders can manifest with back pain, paraparesis, and sensory abnormalities. Although frequently absent in the acute phase, the presence of deep tendon reflexes would strongly indicate ATM. When deep tendon reflexes are absent, the presence of a spinal cord sensory level and bowel and bladder involvement suggests ATM. Some patients with ATM have albuminocytologic dissociation, which is indistinguishable from Guillain-Barré syndrome. In some cases, ATM and Guillain-Barré syndrome can be distinguished only with MRI. Spinal cord, nerve root, and peripheral nerve pathology can coexist in myeloradiculoneuritis, suggesting that ATM and Guillain-Barré syndrome may share a common pathophysiologic mechanism in some cases.[46]

Disease-Associated Acute Transverse Myelitis

Acute transverse myelitis with a presumed inflammatory basis can be associated with recurrent CNS demyelinating disorders and systemic autoimmune disorders. In these cases, the term *disease-associated acute transverse myelitis* can be applied (Table 37B-3).[17]

The presence of encephalopathy and multifocal MRI abnormalities of cerebral white matter points toward acute disseminated encephalomyelitis as a possible diagnosis (Table 37B-4). Spinal cord demyelination in multiple sclerosis tends to be limited to fewer than two vertebral segments with partial cord involvement seen in the transverse plane, resulting in mild, asymmetric symptoms compared with the typically severe, symmetric symptoms in ATM.[67] In addition, the recurrence of lesions over the course of time and in different parts of the CNS is required for establishing the diagnosis of multiple sclerosis.[40] Previous subtle visual or sensory symptoms suggestive of a recurrent demyelinating disorder may be elicited only with detailed questioning. Oligoclonal bands are present more commonly in multiple sclerosis–related myelitis[40] but they also have been reported in idiopathic ATM (Table 37B-5).[57]

ATM is a necessary feature of NMO, a severe, relapsing demyelinating disorder. Specific diagnostic criteria for NMO have been developed and require both ATM and optic neuritis (Table 37B-6).[80] However, ATM may precede optic neuritis by months,[47] and rarely does not occur at all. A novel biomarker (NMO-IgG) targeting the predominant CNS water channel protein aquaporin-4, which is concentrated in astrocytic foot processes in the blood-brain barrier, has been detected in adult patients with NMO.[43,44] Its utility in pediatrics has been confirmed, with one series demonstrating 7/8 children diagnosed with NMO with positive serum NMO-IgG antibodies.[47] In contrast to early criteria that excluded anyone with cerebral MRI findings from a diagnosis of NMO, recent reports have clearly demonstrated that NMO is frequently associated with abnormal brain MRIs and resulting neurologic sequelae to include seizures, intractable hiccups, and aphasia.[47] Because of the potential lag in time from ATM to occurrence of optic neuritis in some children, and the difference in recurrence risk,[78] severity, prognosis, and need for maintenance therapy, every child with ATM should have serum NMO-IgG testing and CSF testing if possible.

ATM also can occur secondary to a variety of systemic autoimmune disorders. Involvement of other organ systems, particularly the skin, lungs, kidneys, and joints, may point to a particular diagnosis, including systemic lupus erythematosus, sarcoidosis, and Sjögren syndrome. A personal or family history of recurrent miscarriages or hypercoagulability is suggestive of antiphospholipid antibody syndrome.

The long list of demyelinating and rheumatologic disorders potentially associated with ATM precludes diagnostic testing for every possible disorder. Table 37B-6 presents a schema to guide which tests should be obtained in patients with ATM.

TABLE 37B-3 Central Nervous System and Systemic Autoimmune Disorders Associated with Acute Transverse Myelitis

Central Nervous System Disorders
Acute disseminated encephalomyelitis
Multiple sclerosis
Neuromyelitis optica
Systemic Autoimmune Disorders
Antiphospholipid antibody syndrome
Behçet disease
Mixed connective tissue disorder
Neurosarcoidosis
Sjögren syndrome
Systemic lupus erythematosus

TABLE 37B-4 Distinguishing Acute Transverse Myelitis from Other Central Nervous System Demyelinating Disorders

Finding	ATM	ADEM	MS	NMO
Myelitis	+	+/–	+/– (partial)	+
Acute mental status changes	–	+	–	–
Optic neuritis	–	+/–	+/–	+
Abnormal brain MRI	–	+	+	+/–
CSF oligoclonal bands	–	+/–	+	–
Serum NMO-IgG	–	–	–	+/–
Recurrences	+/–	+/–	+	+

+, Always present; +/–, variably present; –, usually absent.
ADEM, Acute disseminated encephalomyelitis; *ATM,* acute transverse myelitis; *CSF,* cerebrospinal fluid; *MRI,* magnetic resonance imaging; *MS,* multiple sclerosis; *NMO,* neuromyelitis optica.

TABLE 37B-5 Suggested Diagnostic Workup for Recurrent Central Nervous System Demyelinating Disorders and Systemic Autoimmune Disorders Associated with Acute Transverse Myelitis

All Patients	Suggestive of Neuromyelitis Optica	Also Consider
Brain MRI with gadolinium	Ophthalmology consultation	Angiotensin-converting enzyme (serum, CSF)
CSF oligoclonal bands	Visual evoked potentials	Other autoantibodies
Antinuclear antibodies	Formal visual field testing	Anti-dsDNA
Antiphospholipid antibodies		Anti-La
Serum NMO-IgG		Anti-Ro
		Anti-Smith

CSF, Cerebrospinal fluid; *MRI,* magnetic resonance imaging; *NMO,* neuromyelitis optica.

TABLE 37B-6 Diagnostic Criteria for Neuromyelitis Optica

Required	Must Have One of Two
Transverse myelitis	Longitudinally extensive (≥3 segments) transverse myelitis
Optic neuritis	Serum NMO-IgG

TABLE 37B-7 Partial Listing of Microbes That Cause Infectious Myelopathies

- Cytomegalovirus[49]
- Epstein-Barr virus[46,48]
- *Echinococcus granulosus*[11]
- Enteroviruses[11]
  - Coxsackieviruses
  - Echoviruses[76]
  - Enterovirus 71[52]
  - Polioviruses (vaccine associated and wild-type)
- Herpes simplex virus–1[23,53]
- Herpes simplex virus-2[25]
- Human herpesvirus-6[16,30]
- Human immunodeficiency virus[10,62]
- Human T-cell lymphotrophic virus-1 and -2[9]
- *Mycobacterium tuberculosis*[12]
- *Mycoplasma pneumoniae*[3]
- *Schistosoma haematobium* and *Schistosoma mansoni*[2]
- *Taenia solium* (neurocysticercosis)[11]
- *Treponema pallidum*[12,24]
- Varicella-zoster virus[24]
- West Nile virus[65]

TABLE 37B-8 Partial Listing of Microbes and Vaccines Associated with Postinfectious or Postvaccinial Acute Transverse Myelitis

Bacteria/ Spirochetes	Viruses	Vaccinations
Bartonella henselae[66]	Adenovirus[46]	Hepatitis B[72]
Borrelia burgdorferi[45]	Cytomegalovirus[22]	Influenza[21]
Brucellosis[39]	Coxsackieviruses[55]	Measles, mumps, and rubella[81]
Chlamydia psittaci[79]	Enterovirus 71[27,52]	Poliovirus[34]
Mycoplasma pneumoniae[73]	Epstein-Barr virus[27]	Rabies[21]
	Hepatitis A[13,74]	Smallpox[21]
	Hepatitis B[50]	
	Hepatitis C[26]	
	Herpes simplex virus–1[28]	
	Herpes simplex virus–2[31]	
	Human herpesvirus–6[34]	
	Mumps[58]	
	Rubella[24]	
	Varicella-zoster virus[51]	

ROLE OF INFECTIONS IN TRANSVERSE MYELITIS

Infectious Myelopathies

Infectious agents can cause spinal cord dysfunction by directly infecting the spinal cord parenchyma (infectious myelopathy). The etiology of an infectious myelopathy usually is established by positive CSF culture or polymerase chain reaction (PCR) results. Biopsy or autopsy results also have provided evidence of direct CNS infection in some patients. Some authors consider elevated CSF titers without positive culture or PCR results to be sufficient evidence of direct CNS infection, but this issue has not been resolved. In one prospective study, only 3 of 41 cases could be attributed directly to a specific infectious agent (influenza virus A, influenza virus B, and human herpesvirus–6).[16] Viruses constitute the most frequently reported category of infectious myelopathies, with bacteria and parasitic causes being less frequent (Table 37B-7).

Although cases of cytomegalovirus (CMV) myelitis have been reported in immunocompetent adults,[22,74] no cases have been reported in immunocompetent children. Immunocompromised children have been reported to develop CMV myelitis.[28,49] Treatment with ganciclovir or foscarnet should be considered.[9] The combined treatment of CMV-associated myelitis with ganciclovir and methylprednisolone was associated with marked recovery in an immunocompetent adult.[22] As illustrated by CMV, myelitis associated with viral infections may be produced by direct infection, accompanying secondary processes, such as vasculitis, or postinfectious immune-mediated mechanisms. Whether optimal treatment requires antiviral agents or immunomodulation or both is unclear.

Postinfectious Acute Transverse Myelitis

In postinfectious cases, a specific etiology can be suggested by elevated acute or rising convalescent serum titers, isolation of the agent from systemic sources in the setting of a suggestive clinical picture, or both. In such cases, the presumptive pathophysiology entails an aberrant autoimmune response to the spinal cord via mechanisms such as molecular mimicry, rather than direct CNS invasion by the microbe. Over 50 percent of cases of pediatric ATM have been attributed to postinfectious or parainfectious causes.[16] A variety of bacterial and viral pathogens and vaccinations have been associated with postinfectious ATM (Table 37B-8). Within the postinfectious category of ATM, the preceding infection usually is a nonspecific upper respiratory tract infection without an identifiable, specific microbe.[11,14,59] Approximately 50 percent of patients report having had such an infection with an intervening symptom-free interval of 5

TABLE 37B-9 Suggested Diagnostic Workup for Infections Associated with Acute Transverse Myelitis

Blood	Cerebrospinal Fluid	Other
Blood cultures	Bacterial culture	Viral culture of stool and respiratory secretions
Acute and convalescent titers to *Borrelia burgdorferi*	Viral culture	Consider stool ova and parasite testing and serum titers if parasitic infection is suspected
EBV	PCR testing for CMV	
Mycoplasma pneumoniae	EBV	
	Enterovirus	
	HSV	
	M. pneumoniae	
	VZV	

CMV, cytomegalovirus; *EBV,* Epstein-Barr virus; *HSV,* herpes simplex virus; *PCR,* polymerase chain reaction; *VZV,* varicella-zoster virus.

TABLE 37B-10 Additional Diagnostic Workup for Infections Associated with Acute Transverse Myelitis

Blood	Cerebrospinal Fluid	Other
Bartonella titers	Cryptococcal antigen	PPD placement
HIV antibody	HTLV antibody	Stool ova and parasite testing
HTLV-1 antibody	Fungal culture	
Parasitic infection titers	VDRL	
RPR		

HIV, Human immunodeficiency virus; *HTLV,* human T-cell lymphotrophic virus; *PPD,* purified protein derivative; *RPR,* rapid plasma reagin; *VDRL,* Venereal Disease Research Laboratory.

to 10 days.[11,14,59] In instances in which the specific microbe is not identified and in cases in which no associated infection, systemic disorder, or recurrent demyelinating disorder is identified, the term *idiopathic acute transverse myelitis* has been applied.

The long list of potential pathogens precludes performing diagnostic testing for every possible agent. The workup instead should focus on the pathogens that are common, treatable, or suggested by particular clues in the history or examination. Table 37B-9 lists the infectious disease tests that should be performed in every patient with ATM. Additional infectious disease tests that should be considered, depending on the clinical scenario and immune status, are listed in Table 37B-10.

ROLE OF THE IMMUNE SYSTEM IN IDIOPATHIC ACUTE TRANSVERSE MYELITIS

Several lines of evidence have implicated derangements in the immune system as the primary mechanism of damage in idiopathic ATM. First, neuropathologic studies have identified inflammatory cell invasion within the spinal cord parenchyma.[6] Both antibody-mediated damage and cell-mediated damage have been implicated, similar to findings in multiple sclerosis. Second, the production of interleukin (IL)-6 by astrocytes seems to lead to nitric oxide–induced injury to spinal cord oligodendrocytes and axons in patients with idiopathic ATM. IL-6 levels are markedly elevated in the CSF of patients with ATM and correlate with long-term disability.[33] Third, immunosuppressive therapies, including intravenous methylprednisolone, plasma exchange, and cyclophosphamide, have been used successfully as treatment for idiopathic ATM.

TREATMENT

All patients with ATM should be hospitalized for further monitoring and treatment. Most patients can receive care on the regular ward, although the approximately 5 percent of patients with respiratory involvement from cervical myelitis require intensive care monitoring and may need endotracheal tube placement and mechanical ventilation.

No randomized, controlled treatment trials in ATM in children have been done. Based on case reports and series that have suggested a beneficial effect,[15,41,68] high-dose corticosteroids have become the standard of care in ATM. In one series of 12 children with severe ATM compared with a historical control group of 17 patients, the use of high-dose intravenous methylprednisolone significantly increased the proportion of children walking independently at 1 month (66% vs. 18%) and with full recovery at 1 year (55% vs. 12%).[15] Although a variety of agents and courses have been used, we use intravenous methylprednisolone 30 mg/kg per dose for 5 days, with a maximum of 1 g per day. The need for a prednisone taper is controversial; however, we use a slow taper over 3 to 4 weeks beginning with 1 mg/kg/day. Most patients improve, often dramatically, with intravenous steroid treatment. Although some patients may improve spontaneously,[59] the collective data suggest that high-dose intravenous methylprednisolone should be used in all patients with ATM. The use of adjunctive immunomodulating therapies has been demonstrated to be beneficial in a subset of adults with ATM—those with complete motor and sensory loss below the level of the spinal cord lesion. The adjunctive therapies include plasma exchange and cyclophosphamide. Furthermore, a recent evidence-based guideline from the American Academy of Neurology cites class II evidence that plasmapheresis should be used in addition to intravenous methylprednisolone for fulminant demyelination. In our experience, any child with complete motor and sensory loss or without improvement with 1 or 2 doses of intravenous methylprednisolone should begin every other day plasma exchange with complete plasma volume exchange with each treatment for six sessions. Children who continue to have severe impairment with minimal improvement after both high-dose intravenous methylprednisolone and plasma

exchange warrant consideration of immune depletion with cyclophosphamide.

The use of antimicrobial agents in patients with ATM is controversial. Because most cases are caused by secondary, immune-mediated mechanisms, such treatment may not have significant benefit. Antimicrobial therapy is indicated, however, in cases with highly suspected or proven direct or associated infections, such as doxycycline for *Mycoplasma*,[73] ganciclovir for CMV,[22] and acyclovir for herpes simplex virus and varicella-zoster virus.[24] When antimicrobials are used, agents with good CSF penetration are preferred because direct invasion of the CNS may be present in some cases.

Additional treatment includes neuropathic pain management with gabapentin or pregabalin, urinary bladder catheterization, bowel regimens, and peptic ulcer and deep venous thrombosis prophylaxis. The early institution of clean intermittent catheterization may improve long-term neurourologic outcomes in children with ATM.[71] Physical therapy should be instituted early to maximize the chance for recovery and continued in inpatient rehabilitation or an outpatient setting after the patient has been discharged from the acute care hospital.

OUTCOME AND PROGNOSIS

Recurrences

In contrast to adult patients with idiopathic ATM, who have an approximately 25 percent likelihood of experiencing relapses,[17] most pediatric patients with idiopathic ATM do not have any recurrences. Three series have evaluated the recurrence risk for further episodes of demyelination after an episode of ATM.[54,76] A total of 82 pediatric patients were followed, with 4 developing a second demyelinating event. Of these, 2 were ultimately diagnosed with multiple sclerosis and another 2 eventually met criteria for NMO. This suggests that in contrast to adults with ATM, the risk for a more widespread demyelinating disorder is low after an initial event of ATM. However in our experience, rarely ATM can recur in children.

Disability

During recovery, motor function typically returns first, with an average time to independent ambulation of 56 days in one study[14] and 25 days in a group of patients treated with high-dose intravenous methylprednisolone.[15] Bowel and bladder control recover more slowly, with an average time to recovery of normal urinary function of 7 months.[14] Another study confirmed worse prognosis for sphincter control with more than half of children with ATM having modified or complete sphincter dependence at, on average, 8 years of follow-up.[63] Mobility and locomotion were also at least partially impaired in 36 and 33 percent, respectively, at follow-up, although full recovery is possible.[19]

Multiple studies have evaluated prognostic factors in children with ATM. Unfavorable outcomes have been linked with early age of onset, complete paraplegia, rapid evolution to neurologic nadir, absence or diminished deep tendon reflexes, and high rostral border of sensory level. Favorable outcomes were associated with a plateau phase of 8 days or less, normal white blood cell count in CSF, diagnosis within seven days of symptom onset, few segments of spinal cord involved, and treatment with high-dose methylprednisolone.[14,56,63] Levels of the intracellular neuronal 14-3-3 protein in the CSF of patients with ATM may reflect the extent of neuronal injury and correlate with outcome.[32,33]

NEW REFERENCES SINCE THE SIXTH EDITION

6. Awad A, Stuve O. Idiopathic transverse myelitis and neuromyelitis optica: clinical profiles, pathophysiology and therapeutic choices. *Curr Neuropharm* 2011;**9**:417–28.
7. Banwell B, Kennedy J, Sadovnick D, et al. Incidence of acquired demyelination of the CNS in Canadian children. *Neurology* 2009; **72**:232–9.
8. Bhattacharya M, Joshi N. Spinal epidural abscess with myelitis and meningitis caused by *Streptococcus pneumoniae* in a young child. *J Spinal Cord Med* 2011;**34**:340–3.
16. DeGoede CG, Holmes EM, Pike MG. Acquired transverse myelopathy in children in the United Kingdom: a two year prospective study. *Eur J Pediatr Neurol* 2010;**14**:479–87.
29. Heer JS, Enriquez EG, Carter AJ. Spinal epidural hematoma as a first presentation of hemophilia A. *J Emerg Med* 2008;**34**:159–62.
31. Hussain S, Rathore MH. Cat scratch disease with epidural extension while on antimicrobial treatment. *Pediatr Neurosurg* 2007;**43**: 164–6.
35. Kitagawa RS, Mawad ME, Whitehead WE, et al. Paraspinal arteriovenous malformations in children. *J Neurosurg Pediatr* 2009;**3**: 425–8.
38. Koch BL, Moosbrugger EA, Egelhoff JC. Symptomatic spinal epidural collections after lumbar puncture in children. *AJNR* 2007;**28**:1811–6.
40. Krupp LB, Banwell B, Tenembaum S. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. *Neurology* 2007;**68**:S7–S12.
47. Lotze TE, Northrop JL, Hutton GJ, et al. Spectrum of pediatric neuromyelitis optic. *Pediatrics* 2008;**122**:e1039–47.
57. Nance JR, Golomb MR. Ischemic spinal cord infarction in children without vertebral fracture. *Pediatr Neurol* 2007;**36**:209–16.
63. Pidcock FS, Kishnan C, Crawford TO, et al. Acute transverse myelitis in childhood: center-based analysis of 47 cases. *Neurology* 2007;**68**:1474–80.
69. Smith AB, Soderlund KA, Rushing EJ, et al. Radiologic-pathologic correlation of pediatric and adolescent spinal neoplasms. I. Intramedullary spinal neoplasms. *AJR Am J Roentgenol* 2012;**198**: 34–43.
70. Strahle J, Muraszo KM, Kapurch J, et al. Chiari malformation type I and syrinx in children undergoing magnetic resonance imaging. *J Neurosurg Pediatr* 2011;**8**:205–13.
77. van Veen JJ, Nokes TJ, Makris M. The risk of spinal haematoma following neuraxial anaesthesia or lumbar puncture in thrombocytopenic individuals. *Br J Haematol* 2010;**148**:15–25.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Andronikou S, Albuquerque-Jonathan G, Wilmshurst J, et al. MRI findings in acute idiopathic transverse myelopathy in children. *Pediatr Radiol* 2003;**33**:624–9.
2. Anonymous. Acute schistosomiasis with transverse myelitis in American students returning from Kenya. *MMWR Morb Mortal Wkly Rep* 1984;**33**:445–7.
3. Anonymous. Proposed diagnostic criteria and nosology of acute transverse myelitis. *Neurology* 2002;**59**:499–505.
4. Auguste KI, Gupta N. Pediatric intramedullary spinal cord tumors. *Neurosurg Clin N Am* 2006;**17**:51–61.
5. Auletta JJ, John CC. Spinal epidural abscesses in children: a 15-year experience and review of the literature. *Clin Infect Dis* 2001;**32**: 9–16.
6. Awad A, Stuve O. Idiopathic transverse myelitis and neuromyelitis optica: clinical profiles, pathophysiology and therapeutic choices. *Curr Neuropharm* 2011;**9**:417–28.
7. Banwell B, Kennedy J, Sadovnick D, et al. Incidence of acquired demyelination of the CNS in Canadian children. *Neurology* 2009; **72**:232–9.
8. Bhattacharya M, Joshi N. Spinal epidural abscess with myelitis and meningitis caused by *Streptococcus pneumoniae* in a young child. *J Spinal Cord Med* 2011;**34**:340–3.
9. Berger JR, Sabet A. Infectious myelopathies. *Semin Neurol* 2002;**22**:133–42.
10. Berger JR, Raffanti S, Svenningsson A, et al. The role of HTLV in HIV-1 neurologic disease. *Neurology* 1991;**41**:197–202.
11. Berman M, Feldman S, Alter M, et al. Acute transverse myelitis: incidence and etiologic considerations. *Neurology* 1981;**31**:966–71.
12. Bhigjee AI, Madurai S, Bill PL, et al. Spectrum of myelopathies in HIV seropositive South African patients. *Neurology* 2001;**57**: 348–51.
13. Breningstall GN, Belani KK. Acute transverse myelitis and brainstem encephalitis associated with hepatitis A infection. *Pediatr Neurol* 1995;**12**:169–71.
14. Defresne P, Hollenberg H, Husson B, et al. Acute transverse myelitis in children: clinical course and prognostic factors. *J Child Neurol* 2003;**18**:401–6.
15. Defresne P, Meyer L, Tardieu M, et al. Efficacy of high dose steroid therapy in children with severe acute transverse myelitis. *J Neurol Neurosurg Psychiatry* 2001;**71**:272–4.
16. DeGoede CG, Holmes EM, Pike MG. Acquired transverse myelopathy in children in the United Kingdom: a two year prospective study. *Eur J Pediatr Neurol* 2010;**14**:479–87.
17. de Seze J, Lanctin C, Lebrun C, et al. Idiopathic acute transverse myelitis: application of the recent diagnostic criteria. *Neurology* 2005;**65**:1950–3.
18. DeSousa AL, Kalsbeck JE, Mealey Jr J, et al. Intraspinal tumors in children: a review of 81 cases. *J Neurosurg* 1979;**51**:437–45.
19. Dubnov G, Constantini NW. Rapid recovery from acute transverse myelitis in an elite female swimmer. *Br J Sports Med* 2006;**40**:e8; discussion e8.
20. Dunne K, Hopkins IJ, Shield LK. Acute transverse myelopathy in childhood. *Dev Med Child Neurol* 1986;**28**:198–204.
21. Fenichel GM. Neurological complications of immunization. *Ann Neurol* 1982;**12**:119–28.
22. Fux CA, Pfister S, Nohl F, et al. Cytomegalovirus-associated acute transverse myelitis in immunocompetent adults. *Clin Microbiol Infect* 2003;**9**:1187–90.
23. Galanakis E, Bikouvarakis S, Mamoulakis D, et al. Transverse myelitis associated with herpes simplex virus infection. *J Child Neurol* 2001;**16**:866–7.
24. Gilden DH, Beinlich BR, Rubinstien EM, et al. Varicella-zoster virus myelitis: an expanding spectrum. *Neurology* 1994;**44**:1818–23.
25. Gobbi C, Tosi C, Stadler C, et al. Recurrent myelitis associated with herpes simplex virus type 2. *Eur Neurol* 2001;**46**:215–8.
26. Grewal AK, Lopes MB, Berg CL, et al. Recurrent demyelinating myelitis associated with hepatitis C viral infection. *J Neurol Sci* 2004;**224**:101–6.
27. Grose C, Feorino PM. Epstein-Barr virus and transverse myelitis. *Lancet* 1973;**1**:892.
28. Gungor T, Funk M, Linde R, et al. Cytomegalovirus myelitis in perinatally acquired HIV. *Arch Dis Child* 1993;**68**:399–401.
29. Heer JS, Enriquez EG, Carter AJ. Spinal epidural hematoma as a first presentation of hemophilia A. *J Emerg Med* 2008;**34**:159–62.
30. Hill AE, Hicks EM, Coyle PV. Human herpes virus 6 and central nervous system complications. *Dev Med Child Neurol* 1994;**36**: 651–2.
31. Hussain S, Rathore MH. Cat scratch disease with epidural extension while on antimicrobial treatment. *Pediatr Neurosurg* 2007;**43**: 164–6.
32. Irani DN, Kerr DA. 14-3-3 protein in the cerebrospinal fluid of patients with acute transverse myelitis. *Lancet* 2000;**355**:901.
33. Kaplin AI, Deshpande DM, Scott E, et al. IL-6 induces regionally selective spinal cord injury in patients with the neuroinflammatory disorder transverse myelitis. *J Clin Invest* 2005;**115**:2731–41.
34. Kelly H. Evidence for a causal association between oral polio vaccine and transverse myelitis: a case history and review of the literature. *J Paediatr Child Health* 2006;**42**:155–9.
35. Kitagawa RS, Mawad ME, Whitehead WE, et al. Paraspinal arteriovenous malformations in children. *J Neurosurg Pediatr* 2009;**3**: 425–8.
36. Kiymaz N, Yilmaz N, Demir O. Spinal cord compression from spinal tuberculosis in a child. *Pediatr Neurosurg* 2006;**42**:180–2.
37. Knebusch M, Strassburg HM, Reiners K. Acute transverse myelitis in childhood: nine cases and review of the literature. *Dev Med Child Neurol* 1998;**40**:631–9.
38. Koch BL, Moosbrugger EA, Egelhoff JC. Symptomatic spinal epidural collections after lumbar puncture in children. *AJNR* 2007; **28**:1811–6.
39. Krishnan C, Kaplin AI, Graber JS, et al. Recurrent transverse myelitis following neurobrucellosis: immunologic features and beneficial response to immunosuppression. *J Neurovirol* 2005;**11**: 225–31.
40. Krupp LB, Banwell B, Tenembaum S. Consensus definitions proposed for pediatric multiple sclerosis and related disorders. *Neurology* 2007;**68**:S7–S12.
41. Lahat E, Pillar G, Ravid S, et al. Rapid recovery from transverse myelopathy in children treated with methylprednisolone. *Pediatr Neurol* 1998;**19**:279–82.
42. Lee JW, Park KS, Kim JH, et al. Diffusion tensor imaging in idiopathic acute transverse myelitis. *AJR Am J Roentgenol* 2008;**191**: W52–7.
43. Lennon VA, Kryzer TJ, Pittock SJ, et al. IgG marker of optic-spinal multiple sclerosis binds to the aquaporin-4 water channel. *J Exp Med* 2005;**202**:473–7.
44. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004;**364**:2106–12.
45. Lesca G, Deschamps R, Lubetzki C, et al. Acute myelitis in early *Borrelia burgdorferi* infection. *J Neurol* 2002;**249**:1472–4.
46. Linssen WH, Gabreels FJ, Wevers RA. Infective acute transverse myelopathy: report of two cases. *Neuropediatrics* 1991;**22**:107–9.
47. Lotze TE, Northrop JL, Hutton GJ, et al. Spectrum of pediatric neuromyelitis optic. *Pediatrics* 2008;**122**:e1039–47.
48. Majid A, Galetta SL, Sweeney CJ, et al. Epstein-Barr virus myeloradiculitis and encephalomyeloradiculitis. *Brain* 2002;**125**: 159–65.
49. Marriage SC, Booy R, Hermione Lyall EG, et al. Cytomegalovirus myelitis in a child infected with human immunodeficiency virus type 1. *Pediatr Infect Dis J* 1996;**15**:549–51.
50. Matsui M, Kakigi R, Watanabe S, et al. Recurrent demyelinating transverse myelitis in a high titer HBs-antigen carrier. *J Neurol Sci* 1996;**139**:235–7.
51. McCarthy JT, Amer J. Postvaricella acute transverse myelitis: a case presentation and review of the literature. *Pediatrics* 1978;**62**: 202–4.
52. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS Microbiol Rev* 2002;**26**:91–107.
53. Mewasingh LD, Christiaens FJ, Dachy B, et al. Cervical myelitis from herpes simplex virus type 1. *Pediatr Neurol* 2004;**30**:54–6.
54. Mikaeloff Y, Suissa S, Vallee L, et al. First episode of acute CNS inflammatory demyelination in childhood: prognostic factors for multiple sclerosis and disability. *J Pediatr* 2004;**144**:246–52.
55. Minami K, Tsuda Y, Maeda H, et al. Acute transverse myelitis caused by coxsackie virus B5 infection. *J Paediatr Child Health* 2004;**40**:66–8.

56. Miyazawa R, Ikeuchi Y, Tomomasa T, et al. Determinants of prognosis of acute transverse myelitis in children. *Pediatr Int* 2003;**45**:512–6.
57. Nance JR, Golomb MR. Ischemic spinal cord infarction in children without vertebral fracture. *Pediatr Neurol* 2007;**36**:209–16.
58. Nussinovitch M, Brand N, Frydman M, et al. Transverse myelitis following mumps in children. *Acta Paediatr* 1992;**81**:183–4.
59. Paine RS, Byers RK. Transverse myelopathy in childhood. *Am J Dis Child* 1953;**85**:151–63.
60. Parazzini C, Rossi L, Righini A, et al. Spinal cord and vertebral stroke: a paediatric case. *Neuropediatrics* 2006;**37**:107–9.
61. Patel H, Boaz JC, Phillips JP, et al. Spontaneous spinal epidural hematoma in children. *Pediatr Neurol* 1998;**19**:302–7.
62. Petito CK, Navia BA, Cho ES, et al. Vacuolar myelopathy pathologically resembling subacute combined degeneration in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1985;**312**:874–9.
63. Pidcock FS, Kishnan C, Crawford TO, et al. Acute transverse myelitis in childhood: center-based analysis of 47 cases. *Neurology* 2007;**68**:1474–80.
64. Pollono D, Tomarchia S, Drut R, et al. Spinal cord compression: a review of 70 pediatric patients. *Pediatr Hematol Oncol* 2003;**20**: 457–66.
65. Roos KL. West Nile encephalitis and myelitis. *Curr Opin Neurol* 2004;**17**:343–6.
66. Salgado CD, Weisse ME. Transverse myelitis associated with probable cat-scratch disease in a previously healthy pediatric patient. *Clin Infect Dis* 2000;**31**:609–11.
67. Scott TF, Bhagavatula K, Snyder PJ, et al. Transverse myelitis: comparison with spinal cord presentations of multiple sclerosis. *Neurology* 1998;**50**:429–33.
68. Sebire G, Hollenberg H, Meyer L, et al. High dose methylprednisolone in severe acute transverse myelopathy. *Arch Dis Child* 1997;**76**:167–8.
69. Smith AB, Soderlund KA, Rushing EJ, et al. Radiologic-pathologic correlation of pediatric and adolescent spinal neoplasms. I. intramedullary spinal neoplasms. *AJR Am J Roentgenol* 2012;**198**: 34–43.
70. Strahle J, Muraszo KM, Kapurch J, et al. Chiari malformation type I and syrinx in children undergoing magnetic resonance imaging. *J Neurosurg Pediatr* 2011;**8**:205–13.
71. Tanaka ST, Stone AR, Kurzrock EA. Transverse myelitis in children: long-term urological outcomes. *J Urol* 2006;**175**:1865–8; discussion 1868.
72. Tartaglino LM, Heiman-Patterson T, Friedman DP, et al. MR imaging in a case of postvaccination myelitis. *AJNR* 1995;**16**: 581–2.
73. Tsiodras S, Kelesidis T, Kelesidis I, et al. *Mycoplasma pneumoniae*-associated myelitis: a comprehensive review. *Eur J Neurol* 2006; **13**:112–24.
74. Tyler KL, Gross RA, Cascino GD. Unusual viral causes of transverse myelitis: hepatitis A virus and cytomegalovirus. *Neurology* 1986;**36**:855–8.
75. Ullrich NJ, Marcus K, Pomeroy SL, et al. Transverse myelitis after therapy for primitive neuroectodermal tumors. *Pediatr Neurol* 2006;**35**:122–5.
76. Unay B, Kendirli T, Meral C, et al. Transverse myelitis due to echovirus type 30. *Acta Paediatr* 2005;**94**:1863–4.
77. van Veen JJ, Nokes TJ, Makris M. The risk of spinal haematoma following neuraxial anaesthesia or lumbar puncture in thrombocytopenic individuals. *Br J Haematol* 2010;**148**:15–25.
78. Weinshenker BG, Wingerchuk DM, Vukusic S, et al. Neuromyelitis optica IgG predicts relapse after longitudinally extensive transverse myelitis. *Ann Neurol* 2006;**59**:566–9.
79. Williams W, Sunderland R. As sick as a pigeon: psittacosis myelitis. *Arch Dis Child* 1989;**64**:1626–8.
80. Wingerchuk DM, Lennon VA, Pittock SJ, et al. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006;**66**:1485–9.
81. Zanoni G, Nguyen TM, Destefani E, et al. Transverse myelitis after vaccination. *Eur J Neurol* 2002;**9**:696–7.

SECTION V

GENITOURINARY TRACT INFECTIONS

CHAPTER 38

URETHRITIS

Ellen R. Wald

Urethritis is an inflammation of the urethra and periurethral tissues in males and females. It may be associated with a variety of infectious and noninfectious disorders.

EPIDEMIOLOGY

The cause of urethritis varies with the age of the patient, sexual practices, and hygienic standards.[9,39] *Chlamydia* infections and gonorrhea are common occurrences in adolescents; fecal contamination or irritation caused by physical or chemical substances is a more usual occurrence in preschool-aged children. Transmission during sexual activity is the usual means of spread of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in teenagers and in sexually abused patients; nonvenereal transmission has been described in prepubertal children.[16] Manifestations after nonvenereal spread has occurred may include urethritis, vaginitis, balanitis, conjunctivitis, and complications of disseminated infection (arthritis, meningitis).[11,16]

The home and social environments of prepubertal children must be examined to identify fully the pattern of spread and infection in the patient and contacts because complex psychosocial diagnoses and therapies often are involved.[33] Children with gonorrhea and chlamydial infections are concentrated in large urban centers, usually in poor socioeconomic environments.

Although nonvenereal transmission of *N. gonorrhoeae* is well-established, gonococcal infections in children 2 to 10 years of age should be considered evidence of probable sexual abuse.[1,17,19-21] Household contacts have been found to have positive cultures in 27 to 63 percent of such cases. Prepubertal girls infected with *N. gonorrhoeae* as a result of sexual abuse outnumber boys by a ratio of at least 3 : 1 and in one report 8 : 1.[13]

PATHOPHYSIOLOGY

Infection caused by *N. gonorrhoeae* usually is localized to the urethra in boys and to the vagina in girls; however, rectal and pharyngeal carriage sometimes occurs in the absence of urethral colonization. Gonococcal virulence factors include pili, the ability to attach to urethral epithelial cells, and production of extracellular proteases that cleave IgA. Initial attachment of gonococci to the surface of columnar epithelial cells is mediated by pili, which are filamentous outer membrane appendages composed of multiple subunits, the most important of which is pilin.[38] Local invasion involves multiple adhesins interacting with host receptors at the mucosal cell level. After attachment occurs, gonococci become engulfed in a process known as parasite-directed endocytosis.[14] The organisms are able to undergo intracellular replication within phagocytic vacuoles and columnar epithelial cells, which is a successful adaptive response promoting survival.

Chlamydia infections are the most frequent cause of sexually transmitted infection in the United States.[5,7,44] Chlamydiae are structurally complex organisms that are obligate intracellular parasites and contain DNA and RNA. Attachment, which is not understood completely, is the first step in the infectious process of the susceptible host cell. It is followed by phagocytosis and then the failure of cellular lysosomes to fuse with the phagosome containing the elementary body, which may be mediated partly by macromolecules in the chlamydial cell envelope. After these two crucial events occur, the elementary bodies undergo biologic changes, and after approximately 72 hours, they are released from the host cell as new infective elementary bodies (Fig. 38-1).

Urethritis in younger children also may be caused by the introduction of fecal bacteria or pinworms into the urethra during the early years of toilet training, particularly in girls. Inflammation may be related to bubble baths and other chemical and physical irritants. The entity of idiopathic urethritis is also observed in young boys and girls as a cause of dysuria and hematuria.[24] Edema of the mucosa and the presence of inflammation and red blood cells are common histopathologic features of urethritis that lead to dysuria, hematuria, and microscopic pyuria.

CLINICAL PRESENTATION

Gonococcal urethritis is characterized by a 2- to 7-day incubation period after sexual intercourse. The onset often is sudden, with dysuria and copious urethral discharge in boys and leukorrhea in girls. The urethral discharge often is thick, profuse, and yellow. The patient usually has no fever. In prepubertal girls, leukorrhea is more prominent as a sign of gonococcal infection (reflecting a vulvovaginitis) and urethritis occurs less commonly. This difference may be related to the method of infection and the different sensitivity of the vaginal epithelial surface to infection in a prepubertal child. Leukorrhea may be minimal in adolescent girls, and dysuria may be absent.[1]

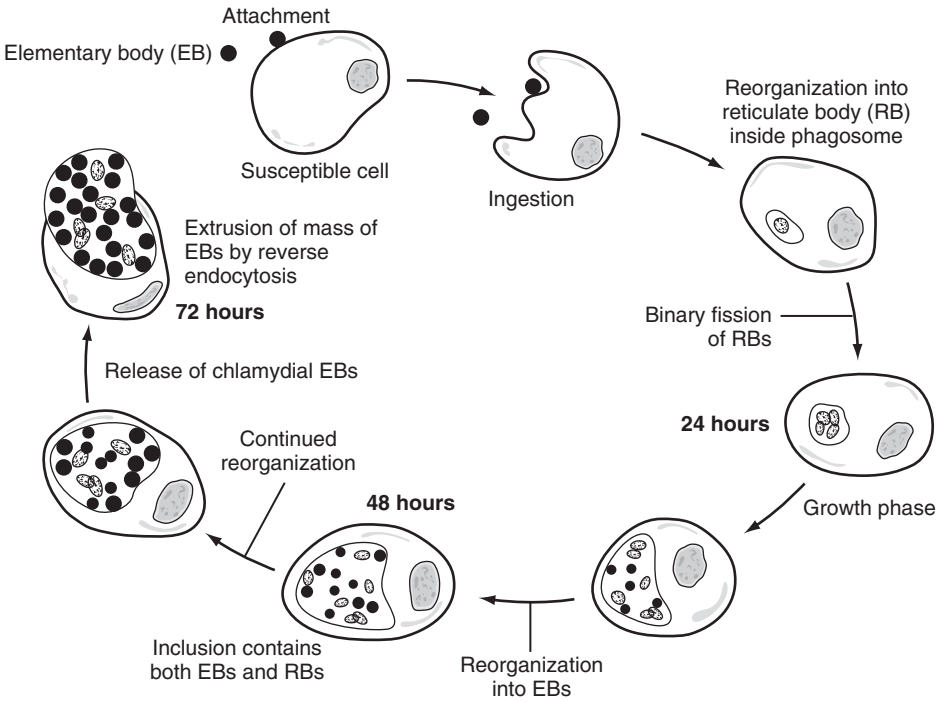

FIGURE 38-1 ■ Schematic description of the growth cycle of *Chlamydia trachomatis*. (From Batteiger BE, Jones RB. Chlamydial infections. *Infect Dis Clin North Am* 1987;1:55–81.)

Diagnosis often is made earlier in adolescent boys than in girls, perhaps because of the prominence of urethral discharge in boys and misinterpretation of the significance of leukorrhea in girls. Gonococcal urethritis also may cause asymptomatic pyuria in boys. Occasionally, prepubertal patients have conjunctivitis or balanitis without significant urethritis. Clinical presentations include systemic illness with fever, arthritis, and skin lesions secondary to bacteremia in 3 percent of untreated individuals with mucosal gonorrhea.[1] These lesions often begin on the extremities as small erythematous macules that progress to circular papules with an area of central necrosis.

The clinical presentation of nongonococcal urethritis may be similar to that described for gonorrhea, but it more commonly has a longer incubation period (often 8 to 14 days after sexual intercourse) and a scanty exudate, which may be clear and intermittent. This condition also is referred to as *nonspecific urethritis* and may be present in association with or subsequent to gonococcal urethritis. In the latter case, the scant urethral discharge may persist after the patient has been treated for gonorrhea. Asymptomatic urethral colonization with *C. trachomatis* also is reported in males.[15,42]

An equivalent syndrome, acute urethral syndrome, has been described in sexually active females. The patient experiences an acute onset of dysuria and increased frequency, and pyuria (≥10 white blood cells/mm^3 of midstream urine) is a common finding. Bacterial cultures of the urine often are sterile or show less than 10^5 bacteria/mL; coliform bacteria, *Staphylococcus saprophyticus*, and *C. trachomatis* are the most common causes. Clinical expression of infection with *C. trachomatis* in adolescent girls is characterized by a yellowish, mucopurulent secretion at the cervical os.[6] However, infection with *C. trachomatis* may be asymptomatic in both sexes, an important consideration in designing effective strategies for diagnosis and management of sexual contacts.

A patient with urethritis caused by trauma may have hematuria and dysuria without fever. The trauma may be obvious or related to masturbation or introduction of foreign bodies into the urethra. Patients with urethritis secondary to bubble bath or detergent usually have transient dysuria and no systemic signs. Fecal contamination of the urethra may be accompanied by hematuria, dysuria, and pyuria.

DIFFERENTIAL DIAGNOSIS

Table 38-1 lists the differential diagnoses for urethritis.

Noninfectious

Trauma, bubble bath, detergents found in shampoos, masturbation, radiation, dysfunctional elimination syndrome, and caustic substances may lead to the development of urethritis.[24] Idiopathic urethritis of childhood is characterized by blood spotting of underwear between voids or microhematuria with or without dysuria and urethral discharge.[24] Urethritis also may be a component of several systemic syndromes, including erythema multiforme (Stevens-Johnson syndrome), Kawasaki disease, and occasionally other forms of allergy. Reiter syndrome denotes the association of nongonococcal urethritis with conjunctivitis and arthritis.

Infectious

The most common forms of urethritis in sexually active adolescents and young adults are gonococcal and so-called nongonococcal urethritis. They may occur together or sequentially. Nongonococcal urethritis has been related causally to infections with *C. trachomatis* in approximately 15 to 40 percent of cases. *Mycoplasma genitalium* is associated with signs and symptoms of urethritis in 15 to 25 percent of nongonococcal urethritis in the United States.[29,37] The role, if any, of *Ureaplasma urealyticum* is currently unclear. The remaining cases of infectious urethritis in postpubescent, sexually active patients may be caused by a variety of pathogenic microorganisms, including *Gardnerella vaginalis*, *Mycoplasma hominis*, *Trichomonas vaginalis*, *Candida albicans*, herpes simplex virus type 2, *Treponema pallidum* (syphilis), and other bacteria, such as staphylococci, Enterobacteriaceae, and occasionally streptococci, including group B.[15] Studies also have implicated a causative role in *Chlamydia*-negative nongonococcal urethritis for anaerobic organisms of the *Bacteroides* spp., in particular *Bacteroides urealyticus*.

In younger children, urethritis usually has noninfectious causes as outlined earlier. Gonorrhea, *Chlamydia*, and fecal bacteria may be important as well.

TABLE 38-1 **Etiology of Urethritis**

Infectious	Noninfectious
Sexually Transmitted Infections	**Vasculitides**
Neisseria gonorrhoeae	Reiter syndrome
Chlamydia trachomatis	Erythema multiforme
Trichomonas vaginalis	Kawasaki disease
Herpes simplex virus type 2	
Mycoplasma spp.	**Mechanical**
	Masturbation
Non-Sexually Transmitted Infections	Foreign body
	Trauma
Staphylococcus saprophyticus	
Enterobacteriaceae	**Dysfunctional Elimination Chemical**
Gardnerella vaginalis	
Streptococcus spp.	Soaps
Enterobius vermicularis	Detergents
	Drugs

SPECIFIC DIAGNOSIS

A standard method for diagnosing gonorrhea in a sexually active male is to obtain urethral discharge by manually stripping the urethra or, if that is unproductive, by gently inserting a swab 2 to 3 cm into the distal urethra. The best culture technique for isolating *N. gonorrhoeae*, a fastidious organism, is immediate inoculation of this material onto a selective growth medium, such as regular or modified Thayer-Martin agar. Any delay in inoculation of the plates necessitates the use of a transport method with growth media in a carbon dioxide environment that support the gonococcus at ambient temperatures. These media protect the organism from its marked susceptibility to the effects of drying, cold, and overgrowth by other bacteria. Urethral exudate from a male patient should be gram-stained at the same time; typical kidney bean–shaped, gram-negative, intracellular diplococci are presumptively diagnostic, with a sensitivity and specificity approaching 100 percent (Fig. 38-2).

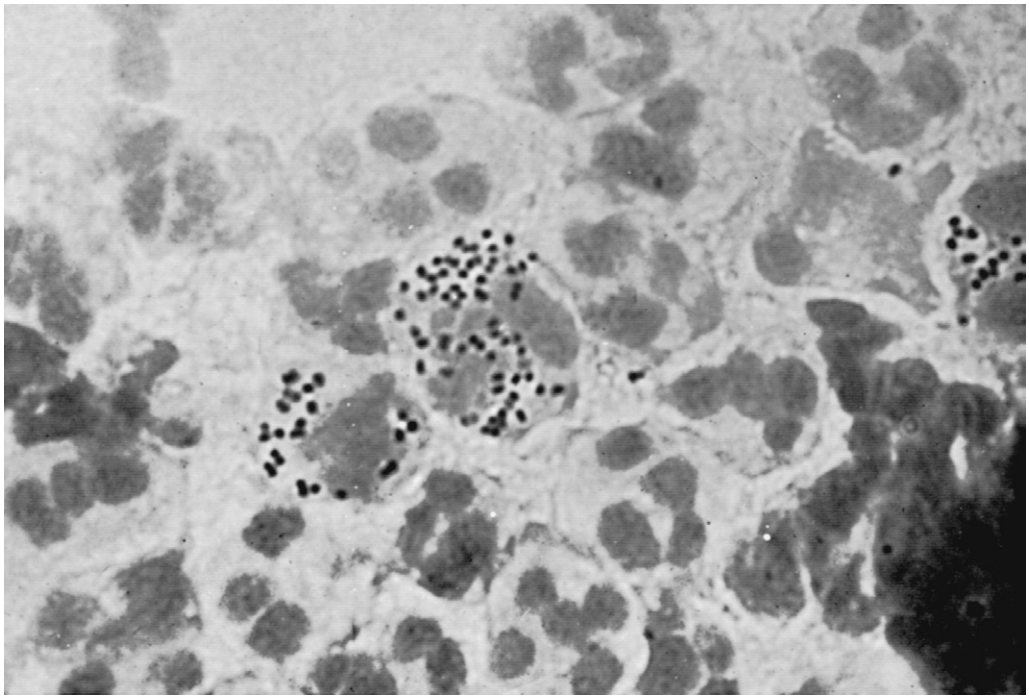

FIGURE 38-2 ■ Gram-stained smear of urethral discharge from a teenage boy with gonorrhea.

Putative gonococcal colonies should be confirmed by oxidase reaction, Gram staining, sugar use tests, rapid enzyme tests, nucleic acid probes, or agglutination reactions with antibodies specific for *N. gonorrhoeae*. The last four tests are especially important in evaluating sites of infection (e.g., the pharynx) or populations of patients with a low prevalence of gonorrhea.

Sexually active females with urethritis also should undergo urethral culture. Although the Gram stain of cervical secretions is only 66 percent sensitive in detecting *N. gonorrhoeae* in adolescent girls, the finding of kidney bean–shaped, intracellular gram-negative diplococci is highly specific and helpful.[45] Vaginal, cervical, and rectal swabs are recommended. Asymptomatic colonization with gonococci seems to occur most commonly in female patients, although it has been described in adolescent boys.[1,23] Pharyngitis, conjunctivitis, balanitis, and other, less common, manifestations of gonorrhea may coexist with urethritis. Samples obtained from these sites should be handled as described earlier. Blood agar and other specialized media may be indicated to identify nongonococcal causes of urethritis.

Nucleic acid amplification tests are highly sensitive and specific when used on urethral swab (males), endocervical swab, patient-obtained vaginal swabs, and urine specimens.[7] These tests include polymerase chain reaction (PCR), transcription-mediated amplification, and strand displacement assays. Only the transcription-mediated assay is approved by the U.S. Food and Drug Administration (FDA) for testing vaginal swabs from postmenarcheal females.[1] Use of urine specimens increases the feasibility of initial testing and follow-up of hard-to-access populations such as adolescents.[1] These techniques also permit dual testing of urine for *C. trachomatis* and *N. gonorrhoeae*. Nucleic acid amplification tests are not recommended for rectal and pharyngeal swabs.[1] However, some noncommercial laboratories have initiated nucleic amplification testing of rectal and pharyngeal swab specimens after establishing the performance of the test to meet requirements of the Clinical Laboratory Improvement Amendments (CLIA). Although none of these tests is superior to culture, their ease of use (i.e., for urine or self-obtained specimens) renders them extremely attractive.

Gonococcal urethritis in prepubescent boys is diagnosed as described earlier for adolescent boys. Vaginal swabs are most useful in female patients, although vaginal discharge may not be prominent. Endocervical cultures are not recommended for the diagnosis of gonorrhea in prepubescent girls. The yield of vaginal swabs seems to be adequate for most diagnostic purposes; rectal swabs also may be useful in female patients. Showing kidney bean–shaped, gram-negative intracellular diplococci in a prepubescent boy and girl is useful for establishing a presumptive diagnosis and instituting therapy. Confirmation by culture as described for a sexually active male is necessary.

Other infectious causes of urethritis may be diagnosed by specific techniques, including wet mount or nucleic acid amplification for *Trichomonas*, Gram stain, and culture on Sabouraud dextrose agar for *C. albicans*, and culture or PCR for herpes simplex virus, used in the patients and their contacts. New culture techniques for *Chlamydia*, such as the use of microtiter cell monolayers, have increased the recovery rates, decreased the cost, and shortened the turnaround time for the isolation of *C. trachomatis*.[2] The rigorous transport conditions and the small number of laboratories with cell culture techniques have limited the availability of *Chlamydia* cultures; however, nonculture methods, including direct immunofluorescence staining of smears with use of monoclonal antibodies,[2,18,25,28,40] enzyme-linked immunosorbent assay (ELISA) techniques, and nucleic acid amplification testing, are available.

The use of immunofluorescence and ELISA techniques has been surpassed in sensitivity and ease of use by nucleic acid amplification testing. Nucleic acid amplification tests such as PCR, transcription-mediated amplification, and strand displacement assays are available and are more sensitive than cell culture, DNA probe, direct fluorescent antibody tests, or ELISA, although specificity varies in contrast to culture.[1] PCR, strand displacement assay, and transcription-mediated amplification are useful for evaluating urine specimens from either sex. The FDA has approved transcription-mediated amplification for testing vaginal swabs from postmenarcheal adolescents. Nucleic acid amplification tests are not yet recommended for specimens obtained from rectal or pharyngeal swabs or for urethral swabs from females.

When documentation of infection is being sought in cases of suspected child abuse, performing cultures is strongly preferred and may be the only acceptable test in certain jurisdictions.[1] When culture is unavailable, some experts support using nucleic acid amplification testing if a positive result can be verified by another nucleic acid amplification test. ELISA and fluorescent antibody tests should not be used for testing rectal, vaginal, or urethral specimens from infants and children because of low sensitivity and specificity.[1] A recent multicenter investigation showed nucleic acid amplification tests for detection of *C. trachomatis* and *N. gonorrhoeae* in children being evaluated for child abuse were superior to culture.[4]

Ureaplasma and other genital *Mycoplasma* spp. currently can be identified only by culture. This test should be reserved for the evaluation of recurrent cases of urethritis with poor response to treatment.[25] Specimens of urethral and vaginal discharge secondary to fecal contamination and foreign bodies can be examined by conventional diagnostic bacterial techniques.

TREATMENT

Table 38-2 presents antibiotic regimens for treatment of urethritis. The treatment of urethritis should include treatment of the sexual partners of the index case to avoid reinfection and further spread of infection. Patients with urethritis frequently have mixed infections with *N. gonorrhoeae* and the pathogens linked with nongonococcal urethritis, such as *C. trachomatis*. Concurrent infection with *N. gonorrhoeae* and *C. trachomatis* is documented frequently. In a survey of adolescents admitted to juvenile detention centers throughout the United States, screening for sexually transmitted infections was performed on

TABLE 38-2 **Antibiotic Regimen for Urethritis**

Nongonococcal Urethritis	
Recommended regimen	Azithromycin 1 g orally in a single dose *or* Doxycycline 100 mg orally twice daily for 7 days
Alternative regimen	Erythromycin base 500 mg four times daily for 7 days *or* Erythromycin ethylsuccinate 800 mg four times daily for 7 days *or* Ofloxacin 300 mg twice daily for 7 days *or* Levofloxacin 500 mg once daily for 7 days
Chlamydial Infection	
Recommended regimen (adults and adolescents)	Azithromycin 1 g orally in a single dose *or* Doxycycline 100 mg orally twice daily for 7 days
Alternative regimens	Erythromycin base 500 mg four times daily for 7 days *or* Erythromycin ethylsuccinate 800 mg four times daily for 7 days Ofloxacin 300 mg twice daily for 7 days *or* Levofloxacin 500 mg once daily for 7 days
Children	
<45 kg	Erythromycin 50 mg/kg/day in 4 divided doses for 14 days
≥45 kg and <8 years old	Azithromycin 1 g orally in a single dose
>8 years old	Use same regimen as for adults
Gonococcal Infection	
Recommended regimen (adults and adolescents)	Ceftriaxone 125 mg IM in a single dose *or* Cefixime 400 mg orally in a single dose *plus* Regimen effective for *Chlamydia*
Children	
<45 kg	Ceftriaxone 125 mg IM in a single dose Cefixime 8 mg/kg (maximum 400 mg) orally in a single dose
If bacteremia, arthritis, or meningitis	Ceftriaxone 50-100 mg/kg/day (maximum 2 g/day) IV for 7-14 days
≥45 kg	Use adult regimen

IM, Intramuscularly; *IV,* intravenously.

adolescent girls with gonorrhea. Fifty-four percent were coinfected with chlamydia; of adolescent boys infected with gonorrhea, 51 percent were coinfected with *Chlamydia*.[26] A study determining chlamydia and gonorrhea co-occurrence in a high school population showed similar results: of 117 students with gonorrhea, 50 (42.7%) had chlamydia; of 451 with chlamydia, 50 (11.1%) had gonorrhea.[34] Patients with gonococcal urethritis also are at risk for development of early-incubating syphilis. One must consider these factors and the possibility of systemic infection when choosing a treatment regimen for urethritis.[31]

Sexual abuse is the most common cause of gonococcal infection among children 2 to 10 years of age. Anorectal and pharyngeal infections with *N. gonorrhoeae* are common and frequently asymptomatic occurrences among these patients.[32]

In 2010, the Centers for Disease Control and Prevention (CDC) published treatment guidelines for sexually transmitted diseases, including urethritis (see Table 38-2). Every attempt should be made to ascertain the specific diagnosis. If doing so is impossible, the treatment regimen chosen always should be appropriate for nongonococcal urethritis and gonococcal infection. The emergence of penicillin-resistant and tetracycline-resistant strains of *N. gonorrhoeae* has led to the abandonment of penicillin and tetracycline for the treatment of gonorrhea.[12]

Single-dose azithromycin for nongonococcal urethritis and either intramuscular or oral single-dose regimens for uncomplicated gonococcal infections are likely to increase compliance.* Unfortunately, rare reports of isolates of *N. gonorrhoeae* resistant to cefixime and ceftriaxone have been noted in Japan and France.[43] Test of cure is not indicated for uncomplicated gonococcal or chlamydial infection when single-dose treatment regimens are used.[7] Emergence of resistance to quinolones initially restricted the use of these drugs in cases of gonorrhea that originated from California, Hawaii, and Asia,[1,3,7] However, in 2007, the CDC recommended that fluoroquinolones no longer be used for treatment of gonorrhea.[8] If these regimens fail, infections with other pathogens, such as herpes simplex virus, *T. vaginalis*, or bacterial urethritis should be considered. Appropriate testing and specific treatment should be provided when indicated.

PROGNOSIS

Gonococcal urethritis may subside and lead to asymptomatic carriage in female patients. Carriage may last for weeks to months in adults; this period is undefined in children. Untreated gonococcal urethritis also may lead to prostatitis and epididymitis in male patients and urethral stricture. Asymptomatic genital infections in women can progress to pelvic inflammatory disease with tubal scarring and infertility. Systemic complications of asymptomatic gonorrheal infections include arthritis, endocarditis, and necrotic skin lesions.

Chlamydial infections frequently have been associated with pelvic inflammatory disease in women, sometimes

*References 21, 22, 27, 30, 35, 36, 41, 46.

resulting in infertility or ectopic pregnancy. *Chlamydia* can be transmitted to a newborn in the birth canal, which can result in conjunctivitis, pneumonia, or both. Prenatal screening for *C. trachomatis* has limited this problem in the United States.

PREVENTION

Despite substantial efforts, a specific gonococcal vaccine has not been developed. The mainstays of prevention continue to be education and screening. Prepubescent gonorrhea can be prevented from recurring only by careful family counseling and psychosocial therapy; legal intervention may be necessary.

The availability of diagnostic tests performed on urine (a noninvasive alternative to specimens obtained from the urethra or endocervix) renders extensive screening possible in nontraditional settings such as schools and recreation centers.[20] Large-scale efforts that have implemented screening for all sexually active teens attending school-based clinics have been successful in reducing the prevalence of *Chlamydia* infection in boys.[10]

Prevention of noninfectious causes of urethritis usually depends on education and specific counseling of the family. Physical agents or allergens identified as a cause of urethritis must be removed.

NEW REFERENCES SINCE THE SIXTH EDITION

1. American Academy of Pediatrics. Gonococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *2012 Red book: Report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 336.
4. Block CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for child abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
7. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010; **59**:1–110.
15. Geisler WM. Diagnosis and management of uncomplicated *Chlamydia trachomatis* infections in adolescents and adults: summary of evidence reviewed for the 2010 Centers for Disease Control and Prevention sexually transmitted diseases treatment guidelines. *Clin Infect Dis* 2011;**53**(suppl 3):S92–8.
43. Unemo M, Golparian D, Nicholas R, et al. High-level cefixime- and ceftriaxone-resistant *N. gonorrhoeae* in Europe (France): novel pnA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012;**56**:1273–80.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 39

Cystitis and Pyelonephritis

Ellen R. Wald

Urinary tract infections (UTIs) are the most common serious bacterial infections in children.[124] In several series of children evaluated for fever, UTIs accounted for 5 to 6 percent of infections. They are more common than occult bacteremia, bacterial pneumonia, and bacterial meningitis. UTIs are especially common as causes of infection in white female infants, and they may explain febrile episodes in nearly 20 percent of such infants.

EPIDEMIOLOGY

UTIs occur in all age groups and may be symptomatic or asymptomatic. Factors that affect the incidence of UTIs are associated with gender, age, race, circumcision status, and general health.[96] The site of infection may be the bladder (cystitis), ureters (ureteritis), pelvis (pyelitis), and renal parenchyma (pyelonephritis). Infections in neonates and infants are common occurrences. In the first 3 months of life, infections in uncircumcised male infants are most common.[126,137] Beyond 6 months, infections in female infants are substantially more common than are infections in male infants; the female predominance of UTIs is maintained throughout the remainder of childhood and adolescence.[30]

Risk for Urinary Tract Infection

The risk for developing a UTI during childhood seems to have increased since early studies by Winberg and colleagues[172] in 1960. These investigations showed that the risk for developing a UTI during the first 10 years of

REFERENCES

1. American Academy of Pediatrics. Gonococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *2012 Red book: Report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 336.
2. Batteiger BE, Jones RB. Chlamydial infections. *Infect Dis Clin North Am* 1987;**1**:55–81.
3. Bauer HM, Mark KE, Samuel M, et al. Prevalence of and associated risk factors for fluoroquinolone-resistant *Neisseria gonorrhoeae* in California, 2000-2003. *Clin Infect Dis* 2005;**41**:795–803.
4. Block CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for child abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
5. Braverman PK. Sexually transmitted diseases in adolescents. *Med Clin North Am* 2000;**84**:869–90.
6. Brunham RC, Paavonen J, Stevens CE, et al. Mucopurulent cervicitis: the ignored counterpart in women of urethritis in men. *N Engl J Med* 1984;**311**:1–6.
7. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010; **59**:1–110.
8. Centers for Disease Control and Prevention. Updated recommended treatment regimens for gonococcal infections and associated conditions—United States. *MMWR Morb Mortal Wkly Rep* 2007;**56**:332–6.
9. Chacko MR, Lovchik JC. *Chlamydia trachomatis* infection in sexually active adolescents: prevalence and risk factors. *Pediatrics* 1984;**73**:836–40.
10. Cohen DA, Nsaumi M, Martin DH, Farley TA. Repeated school-based training for sexually transmitted disease: a feasible strategy for reaching adolescents. *Pediatrics* 1999;**104**:1281–5.
11. Dayan L. Transmission of *Neisseria gonorrhoeae* from a toilet seat. www.st.journal.com *Sex Transm Infect* 2004;**80**:327.
12. De Maria Jr A. Challenges of sexually transmitted disease prevention and control: no magic bullets, but some bullets would be appreciated. *Clin Infect Dis* 2005;**41**:804–7.
13. Folland DS, Burke RE, Hinman AR, et al. Gonorrhea in preadolescent children: an inquiry into source of infection and mode of transmission. *Pediatrics* 1977;**60**:153–6.
14. Fox KK, Leone PA. Epidemiology and pathogenesis of *Neisseria gonorrhoeae* infection. *Up To Date* 2008.
15. Geisler WM. Diagnosis and management of uncomplicated *Chlamydia trachomatis* infections in adolescents and adults: summary of evidence reviewed for the 2010 Centers for Disease Control and Prevention sexually transmitted diseases treatment guidelines. *Clin Infect Dis* 2011;**53**(suppl 3):S92–8.
16. Goodyear-Smith F. What is the evidence for non-sexual transmission of gonorrhoea in children after the neonatal period? A systematic review. *J Forensic Legal Med* 2007;**148**:489–502.
17. Hadlich S, Kohl PK. Sexually transmitted diseases in children: a practical approach. *Dermatol Clin* 1998;**16**:859–61.
18. Hagay ZJ, Sarov B, Sachs J. Detecting *Chlamydia trachomatis* in men with urethritis: serology v isolation in cell culture. *Genitourin Med* 1988;**65**:166–70.
19. Hammershlag MR. The transmissibility of sexually transmitted diseases in sexually abused children. *Child Abuse Neglect* 1998;**22**: 623–35.
20. Hammershlag MR. Appropriate use of non-culture tests for the detection of sexually transmitted diseases in children and adolescents. *Semin Pediatr Infect Dis* 2003;**14**:54–9.
21. Hammerschlag MR, Golden NH, Oh MK, et al. Single dose of azithromycin for the treatment of genital chlamydial infections in adolescents. *J Pediatr* 1993;**122**:961–5.
22. Handsfield HH, McCormack WM, Hook EW, et al. A comparison of single-dose cefixime with ceftriaxone as treatment for uncomplicated gonorrhea. *N Engl J Med* 1991;**325**:1337–40.
23. Hein K, Marks A, Cohen M. Asymptomatic gonorrhea: prevalence in a population of urban adolescents. *J Pediatr* 1977;**90**:634–5.
24. Herz D, Weiser A, Collette T, et al. Dysfunctional elimination syndrome as an etiology of idiopathic urethritis in childhood. *J Urol* 2005;**173**:2132–7.
25. Hooton TM, Barnes RC. Urethritis in men. *Infect Dis Clin North Am* 1987;**1**:165–78.
26. Kahn RH, Mosure DJ, Blank S, et al. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* prevalence and coinfection in adolescents entering selected U.S. juvenile detention centers, 1997-2002. *Sex Transm Dis* 2005;**32**:255–9.
27. Lauharanta J, Saarinen K, Mustonen MT, et al. Single-dose oral azithromycin versus seven-day doxycycline in treatment of non-gonococcal urethritis and cervicitis. *J Antimicrob Chemother* 1993; **31**(suppl.):177–83.
28. Mahony J, Castriciano S, Sellors J, et al. Diagnosis of *Chlamydia trachomatis* genital infections by cell culture and two enzyme immunoassays detecting different chlamydial antigens. *J Clin Microbiol* 1989;**27**:1934–8.
29. Manhart LE, Holmes KK, Hughes JP, et al. *Mycoplasma genitalium* among young adults in the United States: an emerging sexually transmitted infection. *Am J Public Health* 2007;**97**:1118–25.
30. Martin DH, Mroczkowski TF, Dalu ZA, et al. A controlled trial of single dose of azithromycin for the treatment of chlamydial urethritis and cervicitis. *N Engl J Med* 1992;**327**:921–5.
31. Moran JS, Levine WC. Drugs of choice for the treatment of uncomplicated gonococcal infections. *Clin Infect Dis* 1995;**20** (suppl.):S47–65.
32. Nair P, Glazer-Semmel E, Gould C, Ruff E. *Neisseria gonorrhoeae* in asymptomatic prepubertal household contacts of children with gonococcal infection. *Clin Pediatr* 1986;**25**:160–3.
33. Neinstein LS, Goldenring J, Carpenter S. Non-sexual transmission of sexually transmitted diseases: an infrequent occurrence. *Pediatrics* 1984;**74**:67–76.
34. Nsaumi M, Cammarata CL, Brooks BN, et al. Chlamydia and gonorrhea co-occurrence in a high school population. *Sex Transm Dis* 2004;**31**:424–7.
35. Odugbemi T, Oyewold F, Isichei CS, et al. Single oral dose of azithromycin for therapy of susceptible sexually transmitted diseases: a multicenter open evaluation. *W Afr J Med* 1993;**12**: 136–40.
36. Plourde PJ, Tyndall M, Agoki E, et al. Single-dose cefixime versus single-dose ceftriaxone in the treatment of antimicrobial-resistant *Neisseria gonorrhoeae* infection. *J Infect Dis* 1992;**166**:919–22.
37. Ross JD, Jensen JS. *Mycoplasma genitalium* as a sexually transmitted infection: Implications for screening, testing and treatment. *Sex Trans Infect* 2006;**82**:269–71.
38. Scheuerpflug I, Rudel T, Ryll R, et al. Roles of PilC and PilE proteins in pilus-mediated adherence of *Neisseria gonorrhoeae* and *Neisseria meningitidis* to human erythrocytes and endothelial and epithelial cells. *Infect Immun* 1999;**67**:834–43.
39. Shafer MA, Beck A, Blain B, et al. *Chlamydia trachomatis*: important relationships to race, contraception, lower genital tract infection, and Papanicolaou smear. *J Pediatr* 1984;**104**:141–6.
40. Stamm WE. Diagnosis of *Chlamydia trachomatis* genitourinary infections. *Ann Intern Med* 1988;**108**:710–7.
41. Stamm WE. Azithromycin in the treatment of uncomplicated genital chlamydial urethritis and cervicitis. *Am J Med* 1991;**9**: 195–225.
42. Stamm WE, Koutsky LA, Benedetti JK, et al. *Chlamydia trachomatis* urethral infections in men: prevalence, risk factors, and clinical manifestations. *Ann Intern Med* 1984;**100**:47–51.
43. Unemo M, Golparian D, Nicholas R, et al. High-level cefixime- and ceftriaxone-resistant *N. gonorrhoeae* in Europe (France): novel pnA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012;**56**:1273–80.
44. U.S. Preventive Services Task Force. USPSTF guidelines: screening for gonorrhea—recommendation statement. *Ann Fam Med* 2005;**3**:263–7.
45. Wald ER. Gonorrhea: diagnosis by Gram stain in the female adolescent. *Am J Dis Child* 1977;**131**:1094–6.
46. Weber JT, Johnson RE. New treatments for *Chlamydia trachomatis* genital infection. *Clin Infect Dis* 1995;**20**(suppl.):S66–71.

life was 3 percent in girls and 1.1 percent in boys. In a more recent retrospective study of a cohort of 3556 school entrants, 7.8 percent of girls and 1.6 percent of boys were found to have had symptomatic UTIs as confirmed by significant bacteriuria.[48] In approximately half of these cases, the clinical presentation was consistent with acute pyelonephritis (APN).[43] Another population-based study was performed in Göteborg, Sweden, to describe the incidence of first-time symptomatic UTI in children younger than 6 years old. The prevalence during the first 6 years of life was 6.6 percent for girls and 1.8 percent for boys.[97] The apparent increase in risk most likely relates to an increased awareness of the diagnosis of UTI as an explanation for fever in children and the more frequent practice of culturing the urine of children who are ill.

Several studies have investigated systematically the prevalence of UTI as the explanation for fever in febrile young children presenting to the emergency department. Although the definition of significant bacteriuria has varied among studies, the overall prevalence of UTI is 3.3 to 5.3 percent.[51,144] White female infants had significantly more UTIs than did black male infants. Higher prevalences occurred in uncircumcised male infants or male infants with abdominal or suprapubic tenderness on examination.[144] White female infants with a temperature of 39°C (102.2°F) or greater had a prevalence of UTIs of 17 percent.[58] In a large prospective study of febrile (temperature ≥38°C [100.4°F]) infants 3 months or younger evaluated in pediatric office settings, 54 percent of infants had urine tested and 10 percent had UTIs.[106]

A recent meta-analysis searched all publications on UTI in children from 1966 to 2005.[141] The overall prevalence of UTI was found to be 7 percent, but it varied greatly depending on age, gender, and circumcision status.[125] The prevalence was higher in white infants (8%) than black infants (4.7%) and was highest in uncircumcised male infants younger than 3 months of age and in female infants younger than 12 months of age.

Risk Factors for Urinary Tract Infection

Uncircumcised Boys

The common problem of UTIs in uncircumcised boys, although suspected in the 1970s, was first documented in the 1980s by Ginsburg and McCracken.[34] The strongest evidence of a causal link between an intact foreskin and a UTI comes from several studies conducted by Wiswell and colleagues.[175] In their series, an overall 10-fold increased incidence of UTI was found in uncircumcised compared with circumcised male infants (1.12% vs. 0.11%; $p < .001$).[175] Wiswell and other investigators have continued to document this problem.[137,145,174] In the recent meta-analysis by Shaikh and associates, among febrile male infants younger than 3 months of age, 2.4 percent (confidence interval [CI], 1.4 to 3.5) of circumcised males and 20.1 percent (CI, 16.8 to 23.4) of uncircumcised males had a UTI.[141] Where and when rates of circumcision have decreased, the frequency of UTIs in boys has increased. The presence of preputial folds in uncircumcised boys encourages a high density of bacterial growth and contamination of the urethral opening.[15] Circumcision reduces meatal contamination, decreasing the ascent of bacteria into the bladder.[174] The high risk for acquiring UTIs in uncircumcised boys diminishes with age (as the foreskin becomes more retractable) but is still present in the toddler age group.[22]

Dysfunctional Voiding

Dysfunctional voiding is a risk factor for the development of a UTI and an occasional consequence of UTI. Dysfunctional voiding refers to a lack of coordination between the two functions that are essential for normal voiding to occur—relaxation of the urethral sphincter and contraction of the detrusor muscle of the bladder. Ordinarily, the sphincter must relax as the detrusor contracts.[5] The failure of the sphincter to relax causes an obstruction to the outflow of urine. Consequently, voiding pressures and intravesicular pressure are high, the bladder becomes overdistended, dribbling instead of a good flow occurs, and residual urine remains in the bladder after the void. This dyscoordination is termed *dyssynergia*.[5]

Clinical manifestations typically appear after toilet training and include incontinence, enuresis, urinary urgency, and UTI.[28] Constipation is a common occurrence because of the inability to relax the musculature of the pelvic floor. The presence of dysfunctional voiding also may promote the persistence of vesicoureteral reflux (VUR) and lead to recurrence or contralateral reflux after attempts are made at surgical correction of reflux.[75]

Constipation

The distended rectum in constipated children has been suggested to press on the bladder wall and produce an obstruction to bladder outflow that may cause dysfunctional voiding. Urodynamic studies have shown instability of the detrusor muscle in patients with functional constipation and associated enuresis or UTI.[176] Loening-Baucke[90] studied a group of children referred with encopresis and constipation. The history indicated that many were incontinent of urine, and 11 percent had histories of UTIs. When a vigorous regimen to alleviate the constipation was prescribed, a dramatic improvement occurred in the enuresis and the frequency of recurrent UTI.

Kasirga and coworkers studied 38 children with chronic functional constipation and 31 children as the control group. A detailed past and present history of UTIs or symptoms pointing to this diagnosis, enuresis, encopresis, urgency, and urge incontinence was obtained from both groups. Frequency of UTI and urgency was significantly higher in the group with constipation.[70]

Sexual Activity

The well-recognized association in women of acute cystitis with sexual intercourse is reflected in the popular, now perhaps outdated, term *honeymoon cystitis*.[108] This phenomenon is often related to the new onset of sexual activity or a recent change in sexual partners. A novel study of 15 patients with a history of recurrent UTIs

involved daily monitoring for the presence of UTI with dipslides and calendars that recorded episodes of intercourse, menses, and the occurrence of symptoms.[108] Eleven patients experienced 16 infections; 12 infections occurred within 24 hours of engaging in intercourse. In 12 control subjects, three infections occurred, all within 24 hours of having intercourse. The authors concluded that in sexually active women, most UTIs are related to intercourse.

These results were reinforced by a large prospective study from Seattle, Washington, which confirmed that the incidence of symptomatic UTI is high in sexually active young women and that a strong and independent association exists between UTI and recent sexual intercourse, recent use of a diaphragm with spermicide, and a history of recurrent UTIs.[58] Most of these same risk factors, including frequency of sexual intercourse and use of spermicide, were documented as risk factors associated with development of APN in healthy women.[138]

Catheters

In the hospital, urinary catheters are major risk factors for acquisition of nosocomial infection. In adults, the risk for developing an infection is approximately 5 percent per day of catheterization.[107] The usual infecting strains include *Escherichia coli*, *Proteus*, *Pseudomonas*, *Klebsiella*, and *Serratia*. Many strains of bacteria that cause infection display antibiotic susceptibilities that are more resistant than usual. The route of infection may be either intraluminal or periurethral. Bacteremia is an unusual complication of nosocomial UTI. In a study of nosocomial UTIs in a pediatric intensive care unit, catheter-associated UTI occurred at a rate of 0.95 per 100 admissions. Nosocomial UTIs were associated with previous cardiovascular surgery and with urinary tract catheterization of at least 3 days.[99]

PATHOGENESIS

Bacteriology

Most uncomplicated UTIs are caused by members of a large family of gram-negative bacteria known as Enterobacteriaceae. In most instances, the urinary tract becomes infected by the ascending route. Bacteria derived from the fecal flora colonize the periurethral area and gain access to the urethra. The most common bacterial species in primary and recurrent infections is *E. coli*. Other gram-negative species that commonly cause UTI are *Klebsiella*, *Proteus*, *Enterobacter*, and *Citrobacter*, although virtually any enteric organism can be the cause of these infections. Gram-positive bacterial species account for approximately 5 percent of UTIs and primarily include *Staphylococcus saprophyticus* and enterococcal species. After *E. coli*, *S. saprophyticus* is the most common cause of uncomplicated UTIs in teenagers and young adults of both sexes.

Rarely, the urinary tract may become infected hematogenously in the course of a bacteremic infection. This mechanism is thought to account for at least some cases of neonatal UTIs. Increasingly, evidence indicates that even in neonates, most infections occur by the ascending route.

Virulence Factors

The key virulence factor for isolates of *E. coli* is the mechanism by which they attach or adhere to the uroepithelial cell.[62] Bacterial adherence is an essential initiating step in all infections. So-called uropathogenic bacteria, derived from the numerous species found in the fecal flora, can attach to specific receptor sites on the uroepithelium and can bind in a nonspecific manner by electrostatic and hydrophobic bonds.[131] The primary adherence factors encoded by uropathogenic *E. coli*, and many other microbes, are supramolecular, filamentous adhesive organelles known as pili or fimbriae.[167] Some of these pili are referred to as *P fimbriae* because they can recognize and agglutinate erythrocytes of the P1 blood group; this P blood group antigen also is present on human uroepithelial cells. Other common adhesive organelles elaborated by *E. coli* are types 1, 5, and F1C pili encoded by the *fim*, *sfa*, and *foc* operons, respectively.[167]

Evidence to support the notion of the increased pathogenicity or virulence of the P fimbriae comes from studies of *E. coli* recovered from children with infection at different levels of the urinary tract. When *E. coli* strains recovered from patients with pyelonephritis are examined, 76 to 94 percent are P fimbriated; in contrast, strains of *E. coli* recovered from patients with cystitis or asymptomatic bacteriuria are 19 to 23 percent and 14 to 18 percent P fimbriated, respectively.[69,158] Although P fimbriated strains of *E. coli* are common findings in patients with pyelonephritis whose urinary tracts are completely normal, their frequency decreases considerably when strains of *E. coli* are examined from patients with pyelonephritis associated with VUR. Apparently, this virulence characteristic (and others described later) is unnecessary when reflux is present.[92]

The principal adhesin on the tip of the P fimbriae that fosters adherence to the uroepithelial cell is known as the *PapG adhesin*. More recently, 153 *E. coli* organisms recovered from the urinary tracts of infants and children with pyelonephritis were analyzed by polymerase chain reaction for class I, II, and III alleles of the pyelonephritis-associated adhesin gene *papG*. Strains with any class II *papG* alleles were found significantly more often in infants with normal anatomy and function or in infants with clinically insignificant abnormalities than in infants with significant abnormalities (90 of 119 vs. 14 of 34 infants; $p < .001$).[64] This virulence factor is more important when the urinary tract is structurally normal than when anatomic features predispose the individual to infection.

Other virulence factors related to the bacterial species causing UTIs are the K antigen, lipopolysaccharides, hemolysins, colicins, resistance to the bactericidal action of serum, and increased iron-binding capacity. In addition to the immunogenic activity of lipopolysaccharides (activating an intense host response), they have been shown to have direct toxic effects on renal cells via their biologically active component lipid A.[156] The K antigen is a capsular polysaccharide that constitutes an outer

surface of the *E. coli* organism. The capsule has the capacity to impede phagocytosis and to shield the bacteria from lysis induced by complement.[59] Hemolysins are cytotoxic proteins that can damage renal tubular cells in vitro.

Approximately 50 percent of the types of *E. coli* isolated from the urine of patients with APN produce a pore-forming toxin, α-hemolysin. α-Hemolysin damages the cell membrane and may activate apoptosis in renal tubular cells.[18] Colicins, elaborated by "uropathogenic" strains of *E. coli*, kill other bacteria that are in their vicinity. In the presence of human serum, many bacteria are killed after activation of complement. Virulent *E. coli* organisms have the capacity to resist this bactericidal effect of serum. Another virulence factor found in bacteria is their ability to acquire and bind iron. Most bacteria require iron for optimal growth and metabolism and have developed mechanisms to acquire iron when the supply is limited. One strategy to acquire iron is the use of siderophores to scavenge iron from the environment and subsequently concentrate it in the bacterial cytosol.[167] Siderophores such as enterobactin or aerobactin are secreted low-molecular-weight molecules that have a high affinity for ferric (Fe^{3+}) iron. Bacteria retrieve iron bound to siderophores through receptors that facilitate the transport of iron through the bacterial membrane.

After reaching the bladder, P fimbriated *E. coli* organisms can colonize the ureter even in the absence of VUR.[126] Bacterial colonization of the ureter affects ureteral peristalsis, leading to dilation and a physiologic obstruction. This dilation of the ureter and calyces favors a change in the shape of the renal papillae, which facilitates intrarenal reflux of colonizing bacteria at low pressure. APN develops because the receptors for the P fimbriated *E. coli* are present in the collecting duct and proximal tubules.[109,126]

Experimental studies conducted by Roberts[126] have led to a theory of the chain of events involved in the process that ultimately leads to renal scarring. The initial event is the inoculation of the renal parenchyma with bacteria, which leads to an intense inflammatory response. Liberation of proinflammatory cytokines (interleukin-1, interleukin-6, and interleukin-8) is followed by recruitment of inflammatory cells and a second cytokine burst.[67] This inflammation results in the release of toxic enzymes within the granulocytes and tubular lumen. Superoxide is released simultaneously, generating oxygen radicals that are toxic to the bacteria and to the tubular cells.[127] The resultant death of the tubules intensifies and extends the inflammatory process into the interstitium. At the same time, focal ischemia results from the intravascular aggregation of granulocytes and edema.[66] The tissue damage that results from the toxic enzymes, oxygen radicals, inflammatory response, and ischemia culminates in the creation of renal scars.[62,126]

CLINICAL PRESENTATION

Cystitis

Most children older than 5 years of age with cystitis have urgency, frequency, or dysuria.[6] Children who have the urge to urinate may have a history of difficulty in initiating the urinary stream. Occasionally, children may complain of abdominal or suprapubic pain. If fever is present, it is low grade. Suprapubic tenderness may be present on palpation. The urine may be foul smelling and cloudy.

Pyelonephritis

Many children who have APN have impressive chills, spiking fevers, and complaints of back pain.[142] They may have associated gastrointestinal complaints of vomiting and diarrhea, especially vomiting. Lower urinary tract symptoms, such as frequency, urgency, dysuria, and suprapubic discomfort, may or may not be present.

Other findings, such as irritability, poor feeding, vomiting, decreased urinary output, and clinical evidence of dehydration, vary. The youngest children with APN usually have high fever without other localizing features.[53,96,178]

Physical Examination

Features of the physical examination that should be emphasized include (1) an accurate measurement of blood pressure (hypertension may be present in patients who have chronic renal disease), (2) general growth and development (failure to thrive may be a sign of more chronic or recurrent UTI), and (3) a careful abdominal examination (which might reveal tenderness or a mass caused by either an enlarged bladder or an obstructed urinary tract).[154] An effort should be made to elicit the finding of costovertebral angle tenderness in children of all ages. The perineum should be inspected carefully to search for signs of irritation, scars, tears, signs of trauma, labial adhesions, or evidence of vulvovaginitis. In uncircumcised infants, the foreskin may not be retractable, leading to phimosis. A rectal examination should be considered to detect masses or poor sphincter tone, which might be associated with a neurogenic bladder.[154] The lower back should be observed for any lipoma, sinus, pigmentation, or tufts of hair that may be signs of an occult myelodysplasia.

Neurologic examination of the lower extremities and evaluation of the bulbocavernosal reflex often reflect the neurologic integrity of the lower motor neuron reflex arcs. The bulbocavernosus reflex is elicited by squeezing the glans penis or clitoris and observing or feeling a reflex contraction at the external anal sphincter. Absence of this reflex suggests a possible sacral lesion.[5]

Asymptomatic Bacteriuria

A large body of work has been produced dealing with the issue of asymptomatic bacteriuria. Data were accumulated during a long-term study by Kunin[76] of the natural history of recurrent bacteriuria among school-aged girls in a well-defined community in central Virginia. Girls were identified in the first grade and were observed prospectively for 10 years. Each year, approximately 0.5 percent of school-aged girls developed asymptomatic bacteriuria. The cumulative prevalence was 5 percent for the years between entrance to grade school and

graduation from high school. Although it was billed as asymptomatic bacteriuria, approximately one third of the girls did have symptoms, and some were known to have had infection or, rarely, abnormalities of the urinary tract before the first screening.

Just a few years after Kunin[76] began his investigations, a similar study was conducted in Göteborg, Sweden. Beginning in 1970, 19,000 girls a year were screened routinely for bacteriuria in Göteborg schools at ages 7, 11, 14, and 16 years.[88] A significant minority had a history of previous infection or symptoms that were referable to the urinary tract.

Savage and colleagues[134] also studied covert bacteriuria in school-aged girls. In the three studies, the point prevalence of bacteriuria ranged from 0.7 percent[88] to 1.1 percent[76] to 1.6 percent.[134] The risks associated with asymptomatic bacteriuria are difficult to assess from these studies because patients who were truly asymptomatic were difficult to separate from patients with symptoms.

Several prospective studies of infants and school-aged girls from Scandinavia have provided important information regarding the natural history of asymptomatic bacteriuria.[43,165] Most children identified as having asymptomatic bacteriuria among a large cohort of infants (N = 3581) spontaneously cleared the bacteriuria within months. Only 2 of 45 went on to develop symptomatic bacteriuria. In contrast, none of 42 infants who developed symptomatic UTIs had been identified previously as having asymptomatic bacteriuria, suggesting that asymptomatic bacteriuria rarely is a precursor to symptomatic UTI. In addition, prophylactic antibiotics used to treat children with asymptomatic bacteriuria seemed to predispose them to the development of pyelonephritis, usually with microorganisms that had not been present at the outset.[43]

Currently, physicians have little enthusiasm for screening children of any age to discover the presence of asymptomatic bacteriuria. The absence of pyuria in these specimens of urine provides additional evidence that the host is not perturbed by the presence of asymptomatic bacteriuria. The presence of bacteria of low virulence in the urine in asymptomatic patients seems to be protective. These strains apparently prevent invasion by other bacteria and provide a kind of biologic prophylaxis.[43,71]

Rather than screening asymptomatic populations of children, an appropriate approach is vigorous evaluation for the presence of UTIs in febrile children without an obvious focus of infection. In addition, health maintenance examinations should be used as an opportunity to screen for historical information that might suggest the need to collect a urine specimen for culture (Table 39-1). Important items include frequent episodes of unexplained fever, dribbling when urinating, enuresis, encopresis, constipation, urgency, frequency, and dysuria. In addition, it is valuable to know when toilet training was accomplished; frequency of voiding; frequency of stooling; and any apparent difficulties associated with voiding, such as in initiating the urinary stream. The practitioner also should inquire about so-called avoidance maneuvers (e.g., repetitive habitual squirming, crossing of legs, or sitting on heels)[140] and family history of UTI.

TABLE 39-1 **Renal-Focused History and Physical Examination**

History
Age at toilet training
Characteristics and frequency of voiding (urgency, dysuria, dribbling)
Frequency and characteristics of stooling
Family history of renal disease
Habitual squirming
Color and odor of urine
Unexplained episodes of fever
Physical Examination
Temperature
Blood pressure
Abdominal tenderness
Costovertebral angle tenderness
Suprapubic tenderness
Genital examination (irritation, scars, tears)
Rectal examination (sphincter tone, bulbocavernosus reflex)
Lower back (sinus, pigmentation, lipoma, tufts of hair)

DIFFERENTIAL DIAGNOSIS

Infectious

E. coli is the most common cause of infection in the urinary tract for primary infections (in which *E. coli* causes 85% to 90% of infections) and recurrent infections (in which *E. coli* causes approximately 75% of infections). Virtually any other gram-negative enteric bacteria may cause infection. Common etiologic agents include *Klebsiella*, *Proteus*, *Enterobacter*, *Serratia*, and *Pseudomonas*. *Proteus mirabilis* is a common cause of UTIs in some series of boys and in nosocomial UTIs associated with catheterization.[74,112] *Enterococcus* and *Pseudomonas* were the most common cause of nosocomial UTI after *E. coli* in a large series of cases reported from Austria.[122] In young women, *S. saprophyticus* is second only to *E. coli* as a cause of cystitis. Rarely, *Staphylococcus epidermidis* has been reported as a cause of pyelonephritis in young boys with anatomic abnormalities of the urinary tract.[41]

Acute lobar nephronia is a localized nonliquefactive inflammatory infection of the kidney that has also been called acute focal bacterial nephritis.[19,128] It represents the midpoint in the spectrum of acute uncomplicated pyelonephritis and renal abscess. From a clinical perspective, it may be signaled by protracted fever before and after diagnosis and may warrant a longer duration of antimicrobial treatment than uncomplicated APN. The diagnosis depends on the performance of an enhanced computed tomographic (CT) scan. The bacterial etiology is the same as for other cases of APN.

Xanthogranulomatous pyelonephritis is a rare, chronic, suppurative renal infection. Although it can occur at any age, it typically involves middle-aged women. Cases in children have been reported across all age groups, including infants.[1,40,179] The patient usually presents with what appears to be an acute UTI, caused most often by *E. coli*

or *Proteus* spp. Evaluation of the patient usually reveals a unilateral enlargement of the kidney, often accompanied by urolithiasis and sometimes a staghorn calculus. The differential diagnosis of the mass lesion includes neuroblastoma, Wilms tumor, tuberculosis, and renal carcinoma. The lesion is characterized histologically by granulomas, abscesses, and lipid-laden foam cells. Nephrectomy is the usual means of management.

In the context of bacteremia or septicemia, occasionally the blood culture and the urine culture are positive for the same bacterial species. In these instances, the kidney has been seeded as part of a hematogenous dissemination. Any organism that is responsible for sepsis, such as *Haemophilus influenzae* type b, *Neisseria meningitidis*, *Neisseria gonorrhoeae*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, or *Streptococcus pyogenes*, may be found in the urine.

Anaerobic infections of the urinary tract are rare occurrences in children despite the high density of gram-positive and gram-negative anaerobes in the fecal flora; this fact relates to the probable lack of adherence of these bacterial species to the uroepithelium. Anaerobic infections of the urinary tract should be suspected when organisms are seen on Gram stain but do not grow in conventional culture or when the urine of symptomatic children shows no bacterial growth.[13] Another unusual infecting agent that should be suspected in instances in which the Gram stain of the urine shows gram-negative rods but the urine culture is negative is *H. influenzae*.[103]

Fungal infections of the urinary tract usually are caused by *Candida* spp., but they also may be caused by *Cryptococcus neoformans*, *Aspergillus* spp., and the endemic mycoses.[152] Candiduria is an increasingly common form of nosocomial infection that may involve any level of the urinary tract.[50,118] It often occurs in immunosuppressed patients, especially patients who are receiving broad-spectrum antibiotics for treatment of documented or undocumented systemic infections. In many immunosuppressed patients, the infection is complicated by the presence of an indwelling urinary catheter.

Viruses also may cause infection of the urinary tract. For the most part, these infections involve the bladder rather than the kidney, although infection of any part of the urinary tract may occur. The principal etiologic agents are adenoviruses, enteroviruses, coxsackieviruses, and echoviruses. Mumps virus and hepatitis viruses occasionally have been implicated. Type 11 adenovirus has been the most common cause of acute hemorrhagic cystitis in school-age boys; type 21 also has been documented to be a cause of infection in this age group. In immunosuppressed patients, especially children who have undergone bone marrow transplantation or are recipients of kidney transplants, BK polyomavirus and adenovirus may cause hemorrhagic cystitis.[20,85,113]

Granulomatous cystitis is the histopathologic description of cystitis caused by *Mycobacterium tuberculosis* and by schistosomiasis and other parasitic infections. Granulomas formed in response to certain parasites, such as *Toxocara* and microfilariae, also may contain numerous eosinophils. *Enterobius vermicularis* infection occasionally leads to signs and symptoms of cystitis and inflammatory changes of the bladder wall.

Infectious urethritis caused by *N. gonorrhoeae* or *Chlamydia trachomatis* is a common cause of symptoms suggestive of UTI. In addition, any etiologic agents of vulvovaginitis may cause inflammation of the distal urethra, with urgency, frequency, or dysuria; they include *Candida* spp., *Gardnerella vaginalis*, *Trichomonas vaginalis*, *S. pyogenes*, *S. pneumoniae*, *H. influenzae*, *C. trachomatis*, *Shigella* spp., and *Yersinia enterocolitica*.

Noninfectious

Urethral symptoms, such as urgency, frequency, and dysuria, may be caused by any factor or process that gives rise to inflammation in the lower urinary tract. Examples include mechanical irritation (which might result from insertion of foreign bodies, migration of pinworms, or masturbation) and chemical irritation (which might arise from bubble baths or shampoos). Chemical cystitis has been reported from the inadvertent insertion of a vaginal contraceptive suppository (nonoxinol 9) into the bladder. Pharmacologic causes of urethral symptoms include cyclophosphamide and methenamine mandelate, both of which can lead to inflammatory changes in the urinary bladder. Several other agents used in the topical treatment of bladder cancer have been noted to cause cystitis.

DIAGNOSIS

Collection of a Urine Specimen

Proper collection of a urine specimen is crucial to facilitate interpretation of the culture. In toilet-trained children, a midstream clean-catch specimen is appropriate for evaluation. When this specimen is used, the definition of significant bacteriuria is 10^5 colony-forming units (CFU)/mL or more. The child is asked to void into the toilet. Straddling the commode in a reverse position creates a natural separation between the urethra and the vulva. Cleansing of the perineum does not result in less contamination of the specimen and is no longer encouraged.[136] The child is asked to begin voiding. A second or two after the void has been initiated, a sterile cup is passed into the stream. The hope is that the initial void succeeds in washing out the distal urethra, the site from which the urine specimen is most likely to be contaminated.

If the child is not toilet trained, a specimen may be collected by urethral catheterization or suprapubic aspiration. When the urine is collected by urethral catheterization, the perineum is cleaned with 1 percent iodine. A properly sized catheter (10 or 12 French) or a size 5 feeding tube may be used. The catheter is lubricated and inserted into the urethra and threaded a short distance. The first few drops of urine should be collected outside the sterile container. This part of the specimen is the most likely to be contaminated with fecal flora from the distal urethra; these bacteria are not eliminated by the process of perineal cleansing. The remaining urine is collected in a sterile container and sent to the laboratory. When a urine specimen is collected by urethral

catheterization, significant bacteriuria is defined as 50,000 CFU/mL or more.[56] This method is preferred when a small volume of urine in the bladder is anticipated and collecting a specimen of urine is necessary so that antibiotic therapy can be initiated. Unfortunately, there is a high likelihood that even a urine sample obtained by catheter will be contaminated (14%), especially in infants younger than 6 months of age, when the catheterization is difficult, and in uncircumcised boys.[173] Use of bladder ultrasonography increases the success of the procedure.[17]

An alternative to urethral catheterization is suprapubic aspiration. Some physicians contend that catheterization is less traumatic than suprapubic aspiration.[154] There is modest evidence that when suprapubic aspiration is performed in very young infants it may be associated with greater discomfort than catheterization of the urine. The procedure can be done in children of any age; it has been used to obtain specimens of urine in pregnancy.[100] Urine culture specimens obtained by suprapubic aspiration are easy to interpret because the usual source of contamination, the distal urethra, has been bypassed. The presence of any bacteria in a specimen collected by suprapubic aspiration is significant, although most samples contain 10^5 CFU/mL or more. Use of bedside ultrasonography increases the likelihood of a successful procedure.[104]

To perform a suprapubic aspiration, the patient is placed in a supine position with the lower extremities flexed (Fig. 39-1 and Table 39-2). Iodine and alcohol are used to clean the suprapubic area. The symphysis pubis is located with the index finger. A 3-mL syringe is attached to a 1½-inch, 22-gauge needle. A spinal needle (2½- or 3-inch, 21-gauge) can be used in older patients. The needle is passed in the midline about 1.5 cm above the symphysis pubis. It is angled 10 to 20 degrees from the vertical, pointing in a slightly cephalad direction (Fig. 39-2). Negative pressure is applied while the needle is inserted. The procedure is most likely to be successful when the infant can be encouraged to drink and the diaper has been dry for at least 60 minutes before the procedure is done. The success rate of suprapubic aspiration can be improved with the use of a portable ultrasound device.[35] If the suprapubic aspiration is unsuccessful, a catheterized specimen should be obtained. Complications of suprapubic aspiration are rare and include formation of a hematoma, perforation of the bowel, and formation of a suprapubic abscess.[120] Suprapubic aspiration is contraindicated if the patient has a bleeding diathesis.

The last method of urine collection is the bag technique. The perineum is washed with soap and water and allowed to dry. A sterile plastic bag is attached. The bag is removed as soon as the patient voids. If the patient has not voided in 20 minutes, the bag is removed, the

TABLE 39-2 Suprapubic Aspiration Technique

Step 1	Child should not have voided within 1 hour of the procedure.
Step 2	Restrain the infant in a supine, frog-leg position.
Step 3	Clean the suprapubic area with povidone-iodine and alcohol.
Step 4	Identify the site of puncture at 1 to 2 cm above the symphysis pubis in the midline.
Step 5	Use a 22-gauge, 1½-inch needle (with 3-mL syringe attached to it) and puncture at 10- to 20-degree angle of the true vertical aiming cephalad; a second attempt can be made at a similar angle aiming caudad.
Step 6	Exert suction gently as the needle is advanced until urine enters syringe; aspirate urine with gentle suction. If urine is not obtained, further trials are unlikely to be successful.

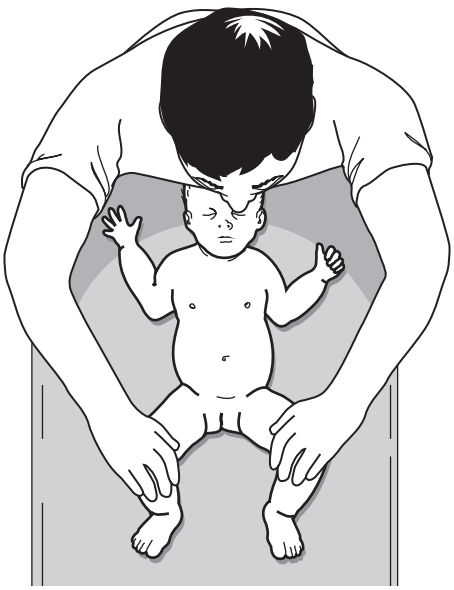

FIGURE 39-1 ■ Suprapubic aspiration technique, position of patient.

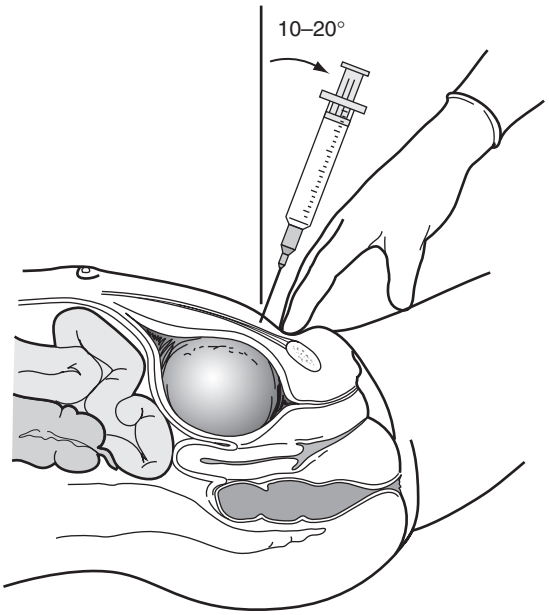

FIGURE 39-2 ■ Suprapubic aspiration technique, position of needle.

perineum is cleaned again, and a new bag is attached. If this procedure is followed meticulously, a reasonable specimen can be collected. This specimen is susceptible to contamination from periurethral flora,[135] however, and the technique is not recommended if the patient appears ill and antibiotic therapy needs to be started immediately after the specimen of urine is collected. The results of the culture of a bagged specimen are useful only if they are negative. If the culture is positive, a second specimen must be collected by a more reliable method.[3]

Diagnosis of Urinary Tract Infection

The diagnosis of UTI hinges on the results of culture of a properly collected urine specimen. Disagreement within the literature is substantial regarding the definition of significant bacteriuria.[37] As indicated in the previous section, the definition of UTI varies according to the method by which the urine is collected. This variability in definition acknowledges that although the bladder urine is regarded as a sterile body fluid, contamination of the urine specimen may occur as it passes through the urethra. The distal urethra frequently is colonized with coliforms derived from the gastrointestinal tract. The only urine specimen that bypasses the urethra and is free of contamination is obtained by suprapubic aspiration. When a specimen is collected by this technique, any colony count of coliforms is significant.[178]

In urine that is collected by a midstream clean-catch method, significant bacteriuria is defined, most stringently, by the recovery of 100,000 CFU/mL or more (Table 39-3). For specimens of urine obtained by catheter, significant bacteriuria is defined as 50,000 CFU/mL or more. In each of these instances, physicians recognize that although urine specimens containing lower colony counts rarely may represent true infection, for the most part lower colony counts usually are the result of contamination of the specimen.[56]

Specimens of urine usually are inoculated onto two different kinds of solid media—one that supports the growth of only gram-negative enteric bacteria (e.g., MacConkey agar) and another that supports gram-positive and gram-negative bacteria (e.g., 5% sheep blood agar), with use of a 0.001 calibrated loop. Colonies are counted the next day (18 hours later), and the total is multiplied by 1000 to determine the colony count. The results of the urine culture are unavailable during the practitioner's first encounter with the ill child. Consequently, there is great interest in the development of a method that would predict the results of the urine culture so that appropriate antimicrobial therapy could be initiated presumptively at the time of the initial encounter. Microscopic methods (to evaluate pyuria and bacteriuria) and biochemical tests (which can be evaluated with a dipstick) have been evaluated and will be discussed. However, the performance of urine culture is mandatory because no rapid urine test is sufficiently sensitive to identify all children with UTI.[170]

TABLE 39-3 **Urinary Tract Infection—Definitions**

Method of Collection	Colony Count (CFU/mL)
Clean catch	$\geq 10^5$
Catheter	$\geq 5 \times 10^4$
Suprapubic	Any

CFU, colony-forming unit.

Microscopy

Two surrogate markers for UTI on microscopic assessment are pyuria and bacteriuria. A problem in assessing pyuria and bacteriuria has been the issue of the definition of significant microscopic pyuria and bacteriuria. How many white blood cells (WBCs) in the urine are too many? Should the specimen of urine that is examined be centrifuged or uncentrifuged? How should the WBCs in a specimen of urine be enumerated? Should the number of WBCs on a centrifuged specimen be enumerated as the number per high-power field, or should they be enumerated on a counting chamber as the number of cells per cubic millimeter, as they would be in a sample of cerebrospinal fluid? If the urine is centrifuged, additional variables are introduced: the initial volume of urine, the duration of the spin, and the volume of urine used to resuspend the sediment. All of these variables influence substantially the enumeration of WBCs per high-power field, especially the volume used to resuspend the sediment.

Methods to assess bacteriuria also have raised issues of definition. Should bacteriuria be assessed on a centrifuged specimen or an uncentrifuged specimen, and should the bacteria be evaluated on a wet mount or a Gram-stained specimen? Should bacteria be enumerated as the number per high-power field?

The standard definition of pyuria in the pediatric literature has been 5 WBCs per high-power field on a centrifuged specimen. Traditionally, microscopic bacteriuria has been expressed as the number of bacteria per high-power field on a Gram stain of a centrifuged specimen of urine. Several investigations undertaken by Hoberman and colleagues[55] have shown, however, that a so-called enhanced urinalysis has greater sensitivity, specificity, and positive predictive value than the standard urinalysis. An enhanced urinalysis is performed on an uncentrifuged sample of urine that has been obtained by catheter. The urine is placed on a counting chamber, and the cells are enumerated as the number per cubic millimeter. A Gram stain is performed in a manner that standardizes the number of drops of urine that are assessed and the number of oil immersion fields that are reviewed.

An enhanced urinalysis is considered to be positive when 10 WBCs/mm^3 or more are present and at least one gram-negative rod in 10 oil immersion fields is present. This definition of significant pyuria is much more sensitive than were previous definitions, and it has performed well for numerous investigators and in neonates and in older infants.[49,86-88,178] A systematic review of the existing literature to assess the performance of rapid diagnostic tests for UTI concluded that use of the traditional definition of pyuria (>5 WBCs per high-power

field on a centrifuged specimen) is sufficiently poor that it cannot be recommended for making a presumptive diagnosis of UTI.[37] Huicho and coworkers[61] also performed a meta-analysis of urine screening tests to determine the risk for UTI in children. This study concluded that pyuria of at least 10 WBCs/mm^3 and bacteriuria are best suited for assessing the risk for UTIs in children. The most recent meta-analysis concluded that detection of bacteriuria by microscopy with Gram stain is the single best test and, if available locally and reported rapidly, is the optimum guide to empirical treatment of children with antibiotics.[170]

Automated methods to perform urinalysis are being used in many hospitals and laboratories. The most updated automated image-based urinalysis system, the iQ200, received clearance from the U.S. Food and Drug Administration more recently. The system uses flow imaging analysis technology and so-called Auto Particle Recognition (APR) software to classify particles found in an uncentrifuged urine specimen based on multiple parameters. Images are stored and can be viewed later on the workstation screen, eliminating the need for manual microscopy in most cases.[160] The iQ200 provides for a rapid turnaround time, and results correlate well with manual methods, especially for red blood cells, WBCs, and squamous epithelial cells.[77,89]

Pyuria is not specific for UTI. In numerous other conditions, including fever, streptococcal infections, and Kawasaki disease, and after exercise, WBCs are found in the urine. Finding pyuria does not ensure that an infection of the urinary tract is present. Despite reports to the contrary, finding true UTI without pyuria is unusual.[162] Generally, inflammation is expected to accompany infection. The absence of pyuria in children with UTIs is rare; it may occur when a child is being evaluated so early in the clinical course of the infection that the inflammatory response has not yet developed. It also may occur when a child is experiencing an episode of asymptomatic bacteriuria.

The most likely explanation for significant bacteriuria by culture in the absence of pyuria is a contaminated specimen. In most cases when UTI has been reported to occur in the absence of pyuria, the definition of pyuria has been at fault. The requirement for 5 WBCs per high-power field on a centrifuged specimen corresponds to approximately 25 WBCs/mm^3; this is too stringent a requirement, with a low sensitivity for the detection of UTIs in infants and children.

Urine Dipsticks

Urine dipsticks (or reagent strips) have been used to indicate the presence of leukocyte esterase (as a surrogate marker for pyuria) and urinary nitrite (which is converted from dietary nitrates by the presence of gram-negative bacteria in the urine). The conversion of dietary nitrates to nitrites by bacteria takes approximately 4 hours. The test result is most likely to be positive when the urine tested is the first morning void (representing a urine that has incubated in the bladder overnight) or a urine that has been in the bladder for at least 4 hours (e.g., obtained from an older child who may hold urine in the bladder for several hours at a time). Most gram-positive cocci will not produce nitrites.

The performance characteristics of leukocyte esterase and nitrites vary according to the definition used for a positive urine culture, the age and symptoms of the population being studied, and the method of urine collection. A nitrite test, although not a sensitive marker in children, is helpful when the result is positive because it is highly specific. A negative nitrite test result has little value in ruling out UTI, however.[25] The leukocyte esterase test has an average sensitivity of 83 percent.[26] It can have a sensitivity of 94 percent in settings in which UTIs are suspected clinically and a sensitivity of 52.9 percent when it is performed on febrile children, most of whom do not have a UTI.[56] The specificity of leukocyte esterase (average, 72%; range, 64% to 92%) generally is not as good as the sensitivity, reflecting the nonspecificity of pyuria in general. A positive leukocyte esterase test result should be interpreted with caution, depending largely on the population being evaluated.[25]

If both leukocyte esterase and nitrites are positive, the sensitivity and specificity are very high. The combination of a positive leukocyte esterase and nitrate predicts a positive urine culture very accurately; and when both are negative, the likelihood of UTI is low.[124]

Determining the Site of Infection

Urinalysis is useful for detecting infection but not for determining the location of the infection within the urinary tract (i.e., upper tract vs. lower tract). To determine the site of infection (i.e., kidney vs. bladder), many investigations of the discriminatory ability of C-reactive protein, erythrocyte sedimentation rate, and total peripheral WBCs have been performed. Several studies have evaluated the accuracy of procalcitonin levels compared with C-reactive protein levels to predict renal involvement among children with febrile UTIs.[8,33,115,121,151] These studies showed that serum procalcitonin concentrations, measured with either an immunoluminetric quantitative test or a rapid semiquantitative test, diagnosed APN with a sensitivity of 70.3 to 94.1 percent and a specificity of 82.6 to 93.6 percent. The specificity of procalcitonin was always higher than that of C-reactive protein, and a highly significant correlation was noted between elevated procalcitonin levels and severity of renal involvement as measured by dimercaptosuccinic acid (DMSA) scintigraphic scores.[118]

In a recent meta-analysis, 12 studies representing 526 patients with UTI (10% with VUR of grade III or higher) were included.[82] The sensitivity of procalcitonin greater than or equal to 0.5 mg/mL as an indicator of VUR of grade III or higher was 83 percent (95% CI, 71 to 91) with a 43 percent specificity rate (95% CI, 38 to 47). In the subgroup of children with positive results on DMSA scan, procalcitonin greater than or equal to 0.5 mg/mL was also associated with high-grade VUR (adjusted odds ratio, 4.8; 95% CI, 1.3 to 17.6). More research is necessary before measurement of procalcitonin becomes routine in the evaluation of children with UTI.[81]

TABLE 39-4 **Imaging Procedures in Children with Urinary Tract Infection**

1. Renal ultrasonography
2. Renal scintigraphy
3. Magnetic resonance imaging
4. Voiding cystourethrography
5. Computed tomography

Imaging

The recommendations for performing imaging procedures on children with a diagnosis of UTI have undergone change and remain somewhat controversial. The imaging studies that usually are considered are renal ultrasonography, contrast voiding cystourethrography (VCUG) to detect VUR, renal cortical scintigraphy, and magnetic resonance imaging (MRI) (Table 39-4).

Renal Ultrasonography

The renal ultrasound examination has replaced intravenous pyelography as a means to assess the gross anatomy of the urinary tract. Generally, ultrasonography has been performed promptly after diagnosis of the UTI has been made. It is a noninvasive test that can describe the size and shape of the urinary tract, the presence of duplication and dilation of the ureters, the presence of ureteroceles, and the existence of gross anatomic abnormalities such as a horseshoe kidney.[3] It is not sensitive enough, however, to signal consistently the presence of hydronephrosis, hydroureter, VUR, or renal scarring.[29] When ultrasonography was compared with intravenous pyelography for the detection of renal scars, wide interobserver variations were noted, with sensitivity ranging from 40 to 90 percent.[36]

Widespread application of prenatal ultrasonography has reduced the prevalence of previously unsuspected obstructive uropathy in infants, but the consequences of prenatal screening with respect to the risk for renal abnormalities in infants with UTIs have not yet been well defined.[3] There is considerable variability in the timing and quality of prenatal ultrasonograms, and the report of "normal" ultrasonographic results cannot necessarily be relied on to dismiss completely the possibility of a structural abnormality unless the study was a detailed anatomic survey (with measurements), was performed during the third trimester, and was performed and interpreted by qualified individuals. Unfortunately, a negative prenatal ultrasound evaluation does not significantly alter the probability of finding an abnormality on a postnatal ultrasound evaluation after a UTI.[72]

Some investigators have questioned whether routine performance of renal ultrasonography is essential.[52] Several studies of a large number (N = 561) of young children with their first febrile UTI disclosed that abnormalities found on ultrasound evaluation were infrequent and rarely influenced management.[54,177] In the most recent investigation of this question, the renal ultrasound study was abnormal in 23 of 155 (14.8%) children.[63] Management of 4 patients was changed by the results. Because of the seriousness of the uncommon but potentially correctable abnormalities in 1 to 2 percent of children with their first UTI and because ultrasonography is noninvasive and poses minimal risk, the American Academy of Pediatrics has recommended performance of renal and bladder ultrasonography in all febrile infants with a UTI.[3]

The timing of renal and bladder ultrasonography depends on the clinical situation. The imaging study is recommended during the first 2 days of treatment to identify serious complications such as renal or perirenal abscesses or pyonephrosis associated with obstructive uropathy when the clinical illness is unusually severe or substantial clinical improvement is not occurring. For febrile infants with UTIs who demonstrate substantial clinical improvement, however, imaging does not need to occur early during the acute infection and the results can even be misleading. Changes in the size and shape of the kidneys and the echogenicity or renal parenchyma attributable to edema also are common during acute infection. The presence of these abnormalities makes it inappropriate to consider renal and bladder ultrasonography performed early during acute infection to be a true baseline study for later comparisons in the assessment of renal growth. An optimum time to perform the ultrasound is one week after diagnosis.

Renal Scintigraphy

In patients with presumed APN, renal scintigraphy with technetium-99m DMSA or technetium-99m glucoheptonate has been shown to be the most practical and reliable method for detecting APN.[140] DMSA and glucoheptonate are amino acids that are cleared by the renal tubules. When these amino acids are labeled with technetium and injected intravenously, they can be used to create an image of the kidney, which reflects vascular flow and tubular function (Fig. 39-3). In experimentally induced APN in piglets, the DMSA scan had a sensitivity of 87 percent and a specificity of 100 percent in showing lesions consistent with APN compared with histology as the gold standard.[132]

In most patients with APN, renal scintigraphy performed during the acute phase of the illness shows a decreased uptake of DMSA (Fig. 39-4). High-resolution pinhole images of the kidney reveal focal, multifocal, or diffuse areas of decreased uptake of isotope in the kidney without loss of volume and with maintenance of the contour of the kidney.[140] DMSA renal scintigraphy can be used to localize the level at which the urinary tract is infected, specifically distinguishing between acute cystitis and APN with a sensitivity of slightly less than 90 percent. Rarely, a patient may have classic signs and symptoms of APN but renal scintigraphy does not show the usual findings.[31] Nonetheless, renal scintigraphy is the most sensitive study to indicate the presence of APN.

The DMSA and glucoheptonate scans also can be used to indicate the presence of renal scars that result from an episode of APN. The scar is indicated by an area in which uptake of the radioisotope is decreased (Fig. 39-5). In this case, the contour of the kidney is not preserved. Scarring

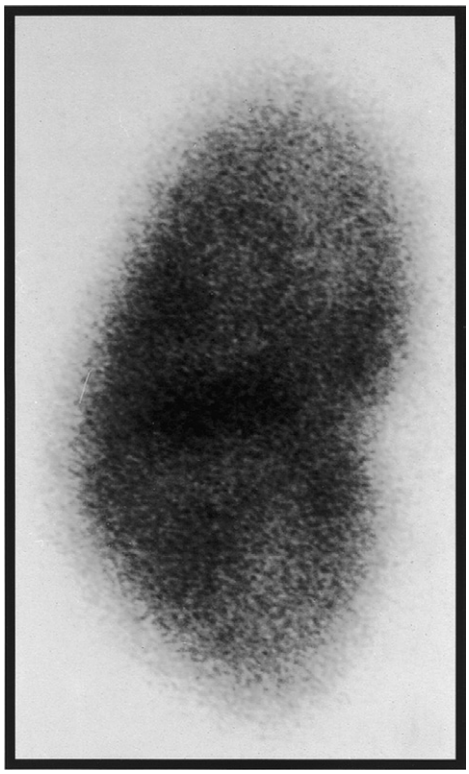

FIGURE 39-3 ■ Normal renal scintigram.

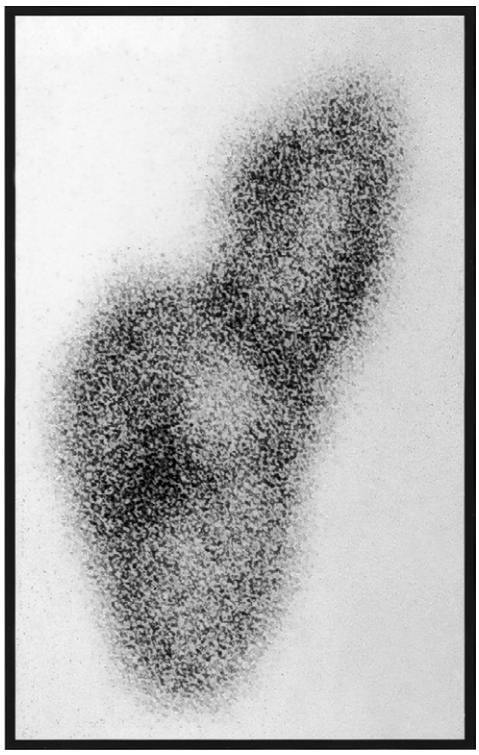

FIGURE 39-5 ■ Scintigram showing a renal scar with loss of the normal contour.

FIGURE 39-4 ■ Scintigram showing acute pyelonephritis, manifesting as a photon-deficient area in the upper pole.

also may be indicated by an overall reduction of the size of the kidney. Hoberman and colleagues[52] prospectively evaluated 309 infants who were 1 to 24 months old with their first febrile UTI. An initial DMSA scan was performed on all infants and showed that 61 percent (190 of 309) had findings compatible with APN and only 1 infant had evidence of previous scars. Repeat scintigraphic scanning was done 6 months after entry in 89 percent to detect renal scarring. A small percentage of renal parenchymal involvement (mean 8.2%) was noted in 9.6 percent of the infants. All infants whose initial scans were normal had normal scans at follow-up.

DMSA scintigraphy has been suggested as the preferred imaging study to evaluate a child with a first febrile UTI rather than ultrasonography or VCUG.[42,47] A strategy to perform VCUG only in patients with an abnormal DMSA scan has been proposed. The rationale is based on the high negative predictive value of a normal DMSA for high degrees of reflux (III to V) and rarity of renal scarring when the initial DMSA is normal.[72] Reflux definitely may be present, however, when DMSA scans are normal (30 of 99 in Hoberman's data).[52] Absence of scarring may be attributable to the effect of antibiotics.

Magnetic Resonance Imaging

MRI can be used to detect the presence of APN. Two studies by Lonergan and colleagues[94,116] showed a sensitivity and a specificity of MRI that are equal to and perhaps even greater than those of renal scintigraphy

with DMSA. In both instances, the best pulse sequence to show areas of APN with MRI was gadolinium-enhanced, fast spin-echo inversion recovery. These results have been confirmed by others.[80] Several investigators have urged, however, that MRI not become a screening test for children with UTIs until the detection of minor changes in the renal parenchyma (by either DMSA or MRI) has been proved to be of clinical importance.[80,93] The time and sedation requirements for MRI render it an unattractive choice for routine management.

Voiding Cystourethrography

Two types of cystography are available for the diagnosis of VUR: fluoroscopic contrast VCUG and radionuclide cystography. Contrast VCUG has excellent anatomic resolution and provides detailed images of the bladder and the urethra. It can be used specifically to label the degree of reflux (according to the international classification)[78]; to look for trabeculations, diverticula, or ureteroceles of the bladder; and to outline the urethra (which is essential to identify posterior urethral valves causing obstruction). Radionuclide cystography provides less anatomic detail and is most useful when urethral disease is not suspected and when the patient has no history of voiding dysfunction. It is used to observe patients who are known to have reflux (to assess whether reflux is still present) and can be used for screening purposes in siblings of children with reflux and in children of adults known to have had reflux. Compared with conventional contrast VCUG, the amount of radiation exposure is reduced substantially when the radioisotope is used.

The international classification of reflux is a universally used system involving five degrees of reflux.[78] Grade I is reflux into the distal ureter; grade II is reflux into the proximal ureter; and grades III, IV, and V are reflux with mild, moderate, and severe dilation of the pelvis and calyces (Fig. 39-6).

VCUG is performed on children who have experienced a symptomatic UTI to determine the presence of VUR. Children who have VUR are at risk for developing reflux nephropathy—permanent renal damage secondary to the reflux of infected urine into the kidney. Because reflux often resolves spontaneously over the course of several years, the purpose of identifying a child with VUR in the past was to recommend antibiotic prophylaxis until the reflux either had resolved spontaneously or, in cases of high degrees of reflux, had been corrected surgically.

Although imaging of children with UTI has been routine, little evidence exists that diagnostic imaging of children after their first UTI results in prevention of renal scarring, hypertension, or renal failure.[21] The value of identifying abnormalities on the renal ultrasound or VCUG is predicated on the benefits of treatment for these findings, which is a subject of recent controversy.[72] If studies can show with certainty that antibiotic prophylaxis to prevent UTIs in children with VUR is superior to placebo in the prevention of renal scarring, the necessity and importance of performing VCUG would be established. Such a study was funded by the National Institutes of Health to begin in 2007[38] (see later section on VUR under Treatment). In the past 5 years, several studies have been published casting doubt on the effectiveness of antibiotic prophylaxis.[12,32,102,117,130]

Computed Tomography

In general, CT of the kidneys is not recommended. However, when the clinical response to treatment is sluggish, this study is occasionally performed. Both xanthogranulomatous pyelonephritis and lobar nephronia are best imaged with this technique.[19,40]

TREATMENT

Antibiotics for Treatment of Acute Infection

The treatment of UTI is influenced by the age of the patient, the probable site of infection (cystitis or APN), the degree of toxicity, and the likelihood of adherence to the treatment regimen. Oral antimicrobial therapy is appropriate for children with cystitis and for older children with suspected APN who are neither toxic nor

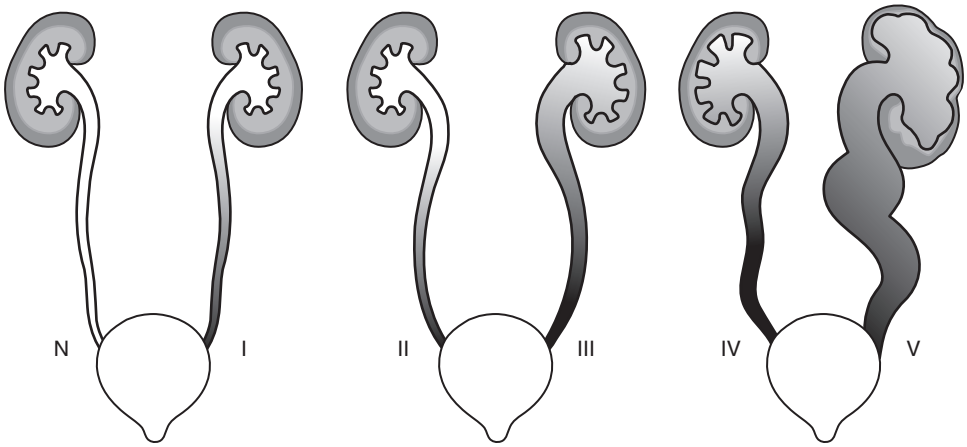

FIGURE 39-6 ■ International classification of vesicoureteral reflux.

TABLE 39-5 **Antibiotic Treatment**

Drug Name	Dosage
Oral	
Amoxicillin + potassium clavulanate	45 mg/kg/day in two divided doses
Cefuroxime	30 mg/kg/day in two divided doses
Cefprozil	30 mg/kg/day in two divided doses
Cefixime	10 mg/kg/day in one dose
Cefpodoxime	9 mg/kg/day in one dose
Ceftibuten	10 mg/kg/day in one dose
Cefdinir	14 mg/kg/day in one dose
Trimethoprim-sulfamethoxazole	10 mg/kg/day (trimethoprim) in two divided doses
Parenteral	
Ampicillin + sulbactam	200 mg/kg/day in four divided doses
Cefuroxime	150 mg/kg/day in three divided doses
Cefotaxime	200 mg/kg/day in four divided doses
Ceftriaxone	80 mg/kg/day in one dose
Ceftazidime	150 mg/kg/day in three divided doses
Cefepime	100 mg/kg/day in two divided doses
Gentamicin	7.5 mg/kg/day in three divided doses

vomiting. The more complex issue is the management of a young infant with high fever in whom the likely diagnosis is APN. These children traditionally have been admitted to the hospital for parenteral administration of antibiotics.

Numerous choices of oral antimicrobial agents are available for patients who have presumed cystitis (Table 39-5). Alternatives include amoxicillin + potassium clavulanate; second-generation cephalosporins, such as cefuroxime and cefprozil; third-generation cephalosporins, such as cefixime, cefpodoxime, ceftibuten, and cefdinir; and the combination agent trimethoprim-sulfamethoxazole. Generally, neither amoxicillin nor first-generation cephalosporins are recommended for first-line therapy because 30 to 50 percent of *E. coli* now are inherently resistant to these agents. Because of some geographic variability in the prevalence of resistance, the practitioner should check with local infectious disease specialists to verify these susceptibility patterns.

In an older patient who appears ill but is not toxic, a repeated visit or telephone follow-up is indicated to ensure that recovery is progressing as predicted. If fever is present, the patient generally becomes afebrile within 1 or 2 days, although occasionally it may take longer. If other indices of recovery, such as general well-being, appetite, and playfulness, are improving appropriately, the persistence of fever is not alarming. If susceptibility test results are available and the organism causing infection is susceptible to the antimicrobial agent being used, repeating the urine culture after 24 hours is unnecessary.[54] The rapid sterilization of urine is a testimony to the fact that virtually all the antibiotics that are used to treat UTIs are concentrated in the kidney and excreted in the urine.

Duration of treatment for patients who are presumed to have cystitis has been controversial. Conventional recommendations are for 7 to 10 days of antimicrobial therapy. Short courses of therapy, varying from single-dose regimens to 3- or 4-day courses, have been evaluated with mixed results. The potential advantages of short-course therapy include the likelihood of improved adherence to the drug regimen; lower cost of drug; and fewer undesirable side effects, including less alteration of normal flora.[143] A meta-analysis (and the most comprehensive review of the data) was performed by Tran and coworkers[155] after review of 22 published trials and a total of 1279 patients. Amoxicillin in a single dose and trimethoprim-sulfamethoxazole as either a single dose or a 3-day regimen were the short-course regimens most commonly evaluated. The authors found that short-course antimicrobial therapy is less effective than therapy of conventional duration, largely because of the ineffectiveness of single-dose amoxicillin. A 3-day course of trimethoprim-sulfamethoxazole was an effective alternative to the standard course of treatment.

A more recent meta-analysis evaluated 10 trials in 652 children with lower tract UTIs.[57] No significant difference was found in the frequency of positive urine cultures between short-duration (2 to 4 days) and standard-duration (7 to 14 days) oral antibiotic therapy for UTIs in children 1 to 15 months after treatment. In children with clear evidence of cystitis without APN, short-course therapy may be acceptable.[161]

Management of a young infant with presumed APN has been controversial. A study conducted by Hoberman and colleagues[54] compared oral therapy with cefixime (for 14 days) with a combination of intravenous therapy with cefotaxime (for 3 or 4 days) plus oral cefixime for 9 to 10 days. Children were eligible for this study if they were 1 to 24 months of age and presented to the physician with temperature greater than 38.3°C (100.9°F) and were found to have a positive enhanced urinalysis (≥10 WBCs/mm^3 and 1 organism/10 oil immersion fields). After blood and urine cultures, complete blood cell count, C-reactive protein determination, and erythrocyte sedimentation rate were performed, the children were randomly assigned to treatment groups. Outcome was evaluated with regard to the short-term measures of sterilization of the urine and time to defervescence and the long-term measures of reinfection and scarring 6 months after the initial infection. No statistically significant differences in any outcome were found.

Hodson and colleagues[101] performed a systematic review of the treatment of APN in children. They assessed 23 trials of 3295 children aged up to 18 years with proven UTIs and APN. Their results suggested that children with APN could be treated effectively with oral antibiotics (cefixime, ceftibuten, and amoxicillin-clavulanate) or with short courses (2 to 4 days) of intravenous therapy followed by oral therapy to complete a 10-day course. If intravenous therapy is chosen, single-dose treatment

daily with an aminoglycoside is safe and effective.[101] If the diagnosis of lobar nephronia is made secondary to a sluggish response to antimicrobial therapy, 2 or 3 weeks of antibiotic therapy (dependent on the pattern of abnormality seen on the CT scan) is recommended rather than a 10-day course.[19]

Corticosteroids

A 2011 publication examining the potential role of corticosteroid therapy, as an adjunct to antibiotics, in the acute phase of APN suggests a beneficial effect.[60] Nineteen patients treated with methylprednisolone were compared with 65 in the placebo group. Adjunctive oral corticosteroids reduced the occurrence and severity of renal scarring in children with APN. Larger studies must be performed before this strategy is adopted.

Dysfunctional Voiding

If a history of dysfunctional voiding is obtained, a behavioral modification approach may be helpful. The key features include frequent volitional voiding every 2 to 3 hours until the voiding pattern has been reestablished, increased water intake (1 L/day) in addition to all other fluids consumed, correction of constipation, and adequate perineal hygiene.[27] Referral to a pediatric urologist may be necessary. Children who exhibit persistent inability to relax their sphincters may require training that involves relaxation techniques reinforced with biofeedback. Pharmacologic treatment with anticholinergics may be necessary if uninhibited bladder contractions are shown with urodynamic studies.[27] Special attention must be paid to the management of constipation if anticholinergic agents are initiated because they will exaggerate the problem.

Antibiotic Prophylaxis

Although antibiotic prophylaxis commonly is recommended for children with recurrent UTIs,[83] only limited data support this position. In a review of the literature, Le Saux and colleagues[83] found sparse data of low quality to support the use of antibiotic prophylaxis in children with a normal urinary tract. A meta-analysis by Williams and colleagues[168] revealed five randomized controlled trials assessing the use of prophylactic therapy. Of these, only two trials with 71 children evaluated the effectiveness of long-term, low-dose antibiotics to prevent UTIs.[91,148] The authors concluded that well-designed, randomized, placebo-controlled trials still are required to evaluate this commonly used intervention.

Vesicoureteral Reflux

The clinical significance of VUR as a predisposing factor for UTIs in general or APN in particular and its contribution to the formation of renal scarring have been questioned more recently. VUR is thought to increase the risk for UTI by increasing postvoid residual and allowing less virulent strains of bacteria to ascend into the upper tract and infect the kidney.[72] As a corollary to the latter, the question also has been framed as to whether long-term antibiotic prophylaxis is effective in reducing reinfection, thereby preventing renal damage in patients with VUR.[31] The authors concluded that the role of VUR in UTIs needs to be redefined through well-designed, multicenter, prospective, randomized, controlled studies using state-of-the-art renal imaging techniques.

Epidemiology

The incidence of VUR in the general population is thought to be approximately 1 percent.[24] Children usually are tested for the possibility of VUR in one of two clinical situations—during the assessment of prenatally diagnosed hydronephrosis and in the evaluation of a UTI. Reflux is diagnosed in 10 to 20 percent of cases of prenatally identified hydronephrosis; it often is high grade and occurs more frequently in boys than in girls in this situation.[42,135] The incidence of reflux in girls evaluated after a first UTI is 25 to 40 percent.[26,159]

An increased incidence of VUR within families suggests a genetic mode of transmission.[68] Rates for prevalence of VUR in identical and fraternal twins were 80 percent and 35 percent, providing evidence that this trait is transmitted in an autosomal dominant fashion. Siblings of children identified to have VUR as part of an evaluation for UTI have a much greater chance of having reflux than the normal population.[4,114] The presence of VUR in these siblings is accompanied by silent renal damage.[16] In addition, a girl who has reflux has a 65 percent risk for having affected offspring.[24] Reflux is much more common among white than black children.

Natural History

The natural history of reflux is for spontaneous resolution to occur over time, with a longer period being necessary in more severe types of reflux. Other factors that may affect the rate of resolution include age at diagnosis, gender, unilaterality versus bilaterality, and whether there is dysfunctional voiding. Average figures for spontaneous resolution are 80 to 86 percent for grade II reflux and 40 to 46 percent for grade III reflux during a 5-year period.

In a report from Skoog and associates,[146] spontaneous resolution occurred within 1.65 years of diagnosis in patients with grade II reflux, but 1.97 years was required for resolution in patients with grade III reflux ($p < .04$). Ninety percent of patients with reflux grades between I and III who ultimately had resolution of reflux experienced this result within 5 years. The rate of resolution was 30 to 35 percent per year, although the duration of reflux was shorter for patients in whom the diagnosis was made before they reached 1 year of age compared with patients who were older at the time of diagnosis.[7]

In another study of the natural history of reflux, Schwab and coworkers[139] determined the resolution rate by patient for 179 girls and 35 boys with UTIs and diagnoses of primary VUR between 1981 and 1984. Reflux spontaneously resolved in 68 percent of patients during the study. Grades I to III reflux resolved at a rate of 13 percent per year for the first 5 years of follow-up and then at a rate of 3.5 percent per year during subsequent follow-up. Grades IV and V reflux resolved at a rate of 5

TABLE 39-6 **Recurrences of Febrile UTI/Pyelonephritis in Infants 2 to 24 Months of Age with and Without Antimicrobial Prophylaxis, According to Grade of VUR**

	Prophylaxis		*No Prophylaxis*		
Reflux Grade	**No. of Recurrences**	**Total *N***	**No. of Recurrences**	**Total *N***	***P***
None	7	210	11	163	.15
I	2	37	2	35	1.00
II	11	133	10	124	.95
III	31	140	40	145	.29
IV	16	55	21	49	.14

percent per year. Bilateral reflux resolved more slowly than unilateral reflux and more rapidly in boys than in girls. There is no specific age in adolescence at which one can assume reflux will not resolve. Leneghan and colleagues[79] reported cessation of reflux after 14 years of age in 27 percent of patients observed without surgical intervention.

Management

The optimal management of children with high grades of reflux (grades III to V) has been the subject of numerous retrospective and prospective studies.[65,163,164] Since 1970, two large prospective randomized trials, the Birmingham Reflux study[9-11] and the International Reflux Study in Children,[125] have been performed to compare medical therapy (antibiotic prophylaxis) with surgical therapy (reimplantation of ureters). The International Reflux Study in Children reported results from Europe and the United States. Because entry criteria were different, the results have been reported separately.

In Europe and the United States, the surgical management of VUR is neither superior nor inferior to medical treatment. In both groups, new scars were acquired during the 5-year follow-up period, some in patients who did not show scarring at the time of entry. In other children, scars present at entry worsened.[119] No detectable differences were found between study groups in either renal function or renal growth. The number of episodes of infection also was similar in both groups. The only exception was a greater frequency of episodes of febrile UTIs (presumed pyelonephritis) in children receiving medical therapy.

Wheeler and colleagues[166] conducted a systematic review of randomized trials of the effects of various interventions in patients with VUR on the development of subsequent UTI and renal parenchymal injury. The aim was to evaluate whether any intervention for reflux (surgical) is better than nonsurgical treatment. Eight trials involving 859 evaluable children were reviewed. Seven trials compared antimicrobial agents alone with surgery plus antimicrobial prophylaxis. The risk for a UTI developing by 1 or 2 and 5 years was not significantly different between surgical and medical groups, and the risk for renal scarring or progressive renal damage occurring was similar. The choice of treatment remains a value judgment governed by such local factors as preference of the parents, availability of skilled surgeons, availability of closely supervised medical treatment, and willingness to comply with prolonged periods of prophylaxis.

Does VUR predispose to pyelonephritis? Garin and associates[31] reviewed 10 studies of children with acute UTIs who underwent DMSA scanning and VCUG. Selection of patients in at least one of the studies was biased toward the association between reflux and APN because the VCUG often was performed after the DMSA scan was reported to be abnormal. Six of 10 studies showed statistically significant results indicating that the presence of reflux definitely was associated with the occurrence of APN; all 4 of the remaining studies showed a trend in the same direction. Kerem, in his review of imaging, endorsed the observation that children with high-grade VUR (grades IV and V) are four to six times more likely to develop scarring than those with low-grade reflux and 8 to 10 times as likely as those with no reflux.[72]

Antimicrobial Prophylaxis

Although the use of antimicrobial prophylaxis had become routine in the management of children with VUR, substantial controversy currently surrounds this approach, and an increasing amount of literature challenges its benefit. In 2003, a Cochrane Review of the effectiveness of long-term antibiotics for preventing recurrent UTIs in children indicated that most studies published before this date were poorly designed without proper blinding.[169] Six recent randomized clinical trials have been performed to evaluate the effectiveness of antimicrobial prophylaxis in patients with VUR.* To provide comparable data from these six trials, authors of the 2011 American Academy of Pediatrics Clinical Practice Guideline for UTIs contacted the respective researchers and requested raw data from their studies. This allowed creation of a data set for 1091 infants, 2 to 24 months of age, according to grade of VUR. Table 39-6 and Figure 39-7 show that there are no significant differences in the rate of recurrences of febrile UTI/APN in young infants, with or without antimicrobial prophylaxis, according to the grade of VUR.[3]

Accepting for the moment that prophylaxis may be effective medical therapy that can be offered to children with severe degrees of reflux, the duration of prophylaxis also is an issue. VUR spontaneously remits in most cases during the first 3 to 5 years of life. Conventional wisdom

*References 12, 23, 40, 102, 117, 130.

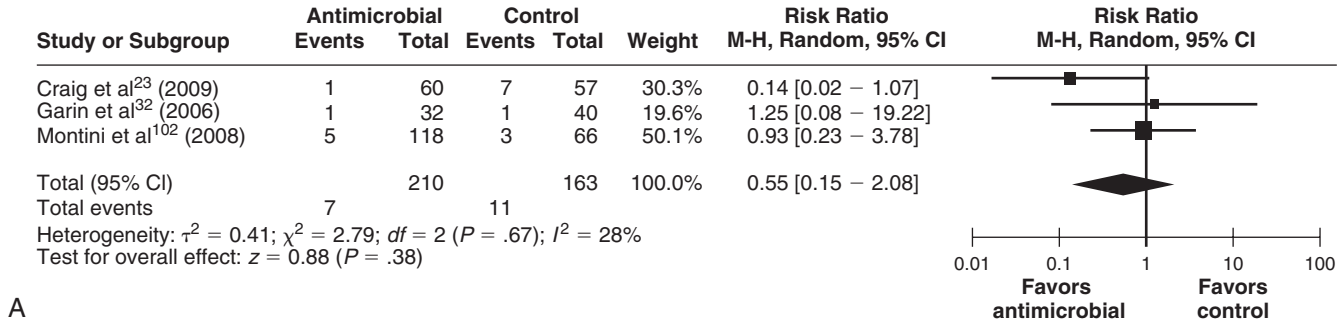
Antimicrobial
Control
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Craig et al[23] (2009) 1 60 7 57 30.3% 0.14 [0.02 − 1.07]
Garin et al[32] (2006) 1 32 1 40 19.6% 1.25 [0.08 − 19.22]
Montini et al[102] (2008) 5 118 3 66 50.1% 0.93 [0.23 − 3.78]
Total (95% CI) 210 163 100.0% 0.55 [0.15 − 2.08]
Total events 7 11
Heterogeneity: τ² = 0.41; χ² = 2.79; df = 2 (P = .67); I² = 28%
Test for overall effect: z = 0.88 (P = .38)
0.01 0.1 1 10 100
Favors antimicrobial
Favors control

A

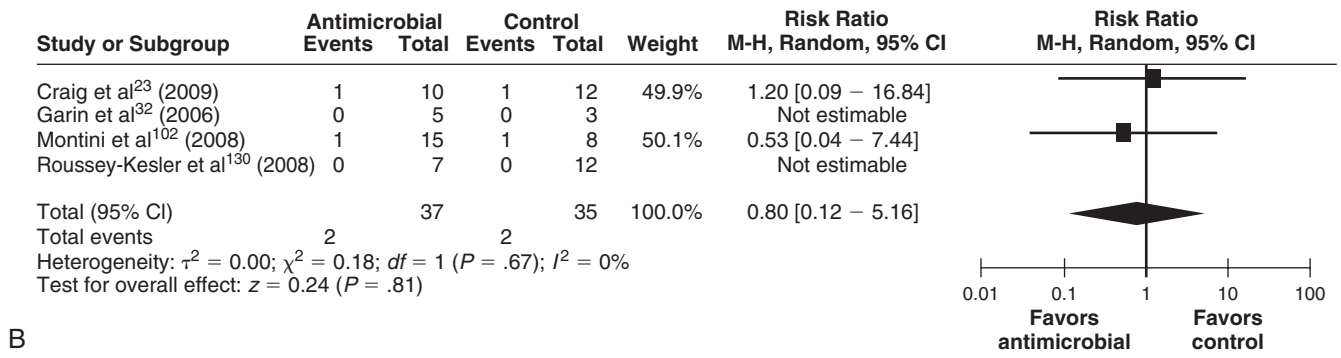
Antimicrobial
Control
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Craig et al[23] (2009) 1 10 1 12 49.9% 1.20 [0.09 − 16.84]
Garin et al[32] (2006) 0 5 0 3 Not estimable
Montini et al[102] (2008) 1 15 1 8 50.1% 0.53 [0.04 − 7.44]
Roussey-Kesler et al[130] (2008) 0 7 0 12 Not estimable
Total (95% CI) 37 35 100.0% 0.80 [0.12 − 5.16]
Total events 2 2
Heterogeneity: τ² = 0.00; χ² = 0.18; df = 1 (P = .67); I² = 0%
Test for overall effect: z = 0.24 (P = .81)
0.01 0.1 1 10 100
Favors antimicrobial
Favors control

B

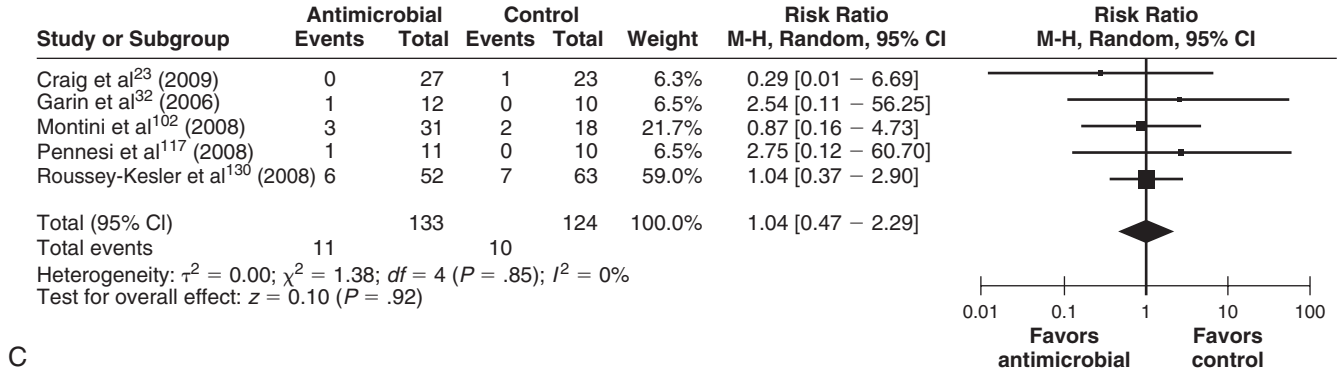
Antimicrobial
Control
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Craig et al[23] (2009) 0 27 1 23 6.3% 0.29 [0.01 − 6.69]
Garin et al[32] (2006) 1 12 0 10 6.5% 2.54 [0.11 − 56.25]
Montini et al[102] (2008) 3 31 2 18 21.7% 0.87 [0.16 − 4.73]
Pennesi et al[117] (2008) 1 11 0 10 6.5% 2.75 [0.12 − 60.70]
Roussey-Kesler et al[130] (2008) 6 52 7 63 59.0% 1.04 [0.37 − 2.90]
Total (95% CI) 133 124 100.0% 1.04 [0.47 − 2.29]
Total events 11 10
Heterogeneity: τ² = 0.00; χ² = 1.38; df = 4 (P = .85); I² = 0%
Test for overall effect: z = 0.10 (P = .92)
0.01 0.1 1 10 100
Favors antimicrobial
Favors control

C

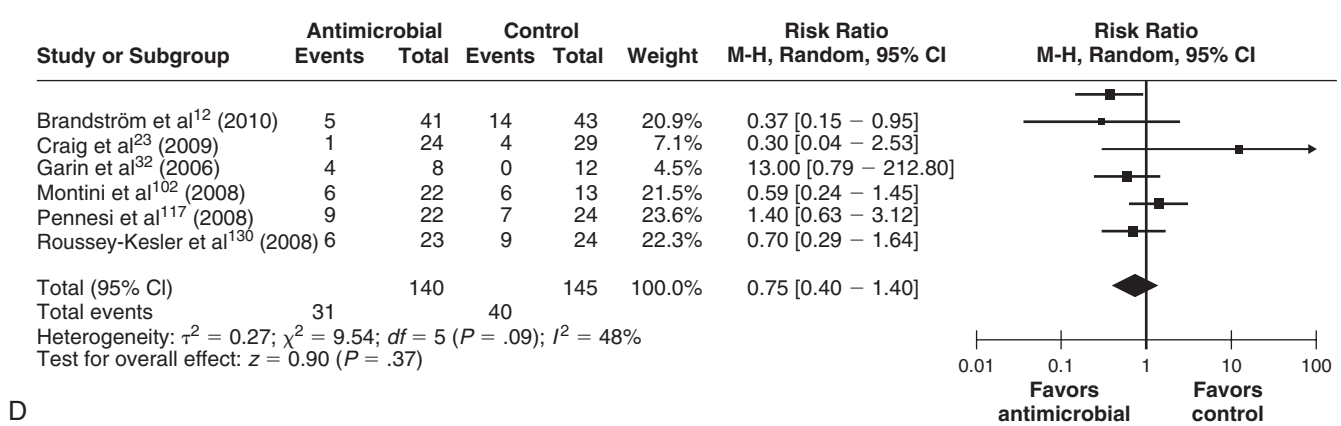
Antimicrobial
Control
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Brandström et al[12] (2010) 5 41 14 43 20.9% 0.37 [0.15 − 0.95]
Craig et al[23] (2009) 1 24 4 29 7.1% 0.30 [0.04 − 2.53]
Garin et al[32] (2006) 4 8 0 12 4.5% 13.00 [0.79 − 212.80]
Montini et al[102] (2008) 6 22 6 13 21.5% 0.59 [0.24 − 1.45]
Pennesi et al[117] (2008) 9 22 7 24 23.6% 1.40 [0.63 − 3.12]
Roussey-Kesler et al[130] (2008) 6 23 9 24 22.3% 0.70 [0.29 − 1.64]
Total (95% CI) 140 145 100.0% 0.75 [0.40 − 1.40]
Total events 31 40
Heterogeneity: τ² = 0.27; χ² = 9.54; df = 5 (P = .09); I² = 48%
Test for overall effect: z = 0.90 (P = .37)
0.01 0.1 1 10 100
Favors antimicrobial
Favors control

D

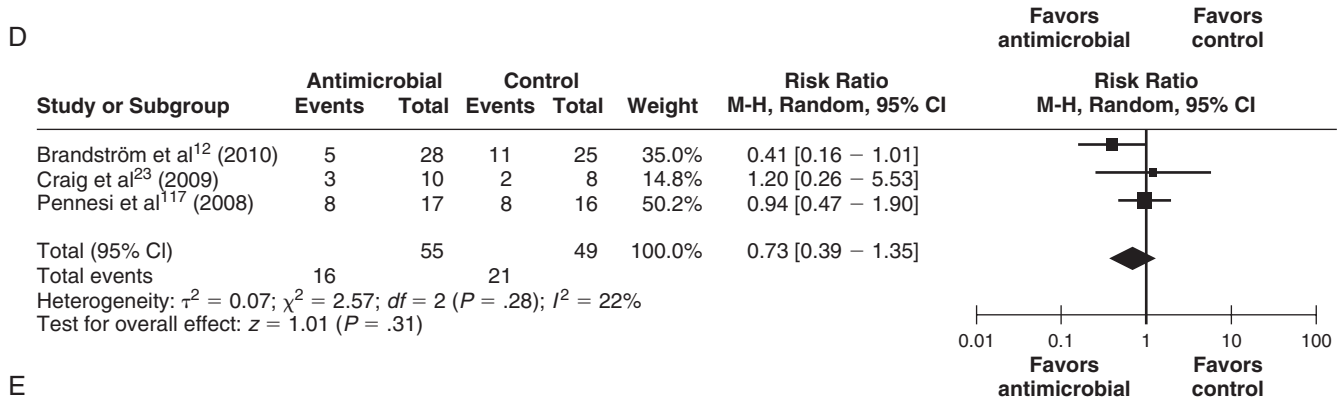
Antimicrobial
Control
Risk Ratio
Risk Ratio
Study or Subgroup Events Total Events Total Weight M-H, Random, 95% CI
M-H, Random, 95% CI
Brandström et al[12] (2010) 5 28 11 25 35.0% 0.41 [0.16 − 1.01]
Craig et al[23] (2009) 3 10 2 8 14.8% 1.20 [0.26 − 5.53]
Pennesi et al[117] (2008) 8 17 8 16 50.2% 0.94 [0.47 − 1.90]
Total (95% CI) 55 49 100.0% 0.73 [0.39 − 1.35]
Total events 16 21
Heterogeneity: τ² = 0.07; χ² = 2.57; df = 2 (P = .28); I² = 22%
Test for overall effect: z = 1.01 (P = .31)
0.01 0.1 1 10 100
Favors antimicrobial
Favors control

E

FIGURE 39-7 ■ **A,** Recurrences of febrile urinary tract infection (UTI)/pyelonephritis in 373 infants, ages 2 to 24 months, without vesicoureteral reflux (VUR), with and without antimicrobial prophylaxis (based on three studies; data provided by Drs. Craig, Garin, and Montini). **B,** Recurrences of febrile UTI/pyelonephritis in 72 infants, ages 2 to 24 months, with grade I VUR, with and without antimicrobial prophylaxis (based on four studies; data provided by Drs. Craig, Garin, Montini, and Roussey-Kesler). **C,** Recurrences of febrile UTI/pyelonephritis in 257 infants, ages 2 to 24 months, with grade II VUR, with and without antimicrobial prophylaxis (based on five studies; data provided by Drs. Craig, Garin, Montini, Pennesi, and Roussey-Kesler). **D,** Recurrences of febrile UTI/pyelonephritis in 285 infants, ages 2 to 24 months, with grade III VUR, with and without antimicrobial prophylaxis (based on six studies; data provided by Drs. Brandström, Craig, Garin, Montini, Pennesi, and Roussey-Kesler). **E,** Recurrences of febrile UTI/pyelonephritis in 104 infants, ages 2 to 24 months, with grade IV VUR, with and without antimicrobial prophylaxis (based on three studies; data provided by Drs. Brandström, Craig, and Pennesi). *CI,* confidence interval; *M-H,* Mantel-Haenszel test.

is that prophylaxis should be maintained until reflux ceases, either spontaneously or with surgical intervention. Greenfield and colleagues[39] suggested that two normal VCUGs performed 12 months apart are necessary before prophylactic antibiotics are discontinued. This recommendation is based on the observation that 27 percent of children exhibit VUR again after a single normal study.

Greenfield and colleagues[33] maintained that administration of prophylactic antibiotics should continue until reflux resolves, no matter what the age. They supported this position by noting that older children with reflux may have new scars evolve when they become infected. In contrast, other authors suggest that prophylaxis should be discontinued in older children, even if reflux persists.[2,7,21,153,171] The only two antimicrobial agents that are recommended for prophylaxis of the urinary tract are nitrofurantoin and trimethoprim-sulfamethoxazole. Each agent is used in half the usual therapeutic dose and given before bedtime. These two agents can often be used for months to years without the emergence of antibiotic resistance. However, some bacteria, such as *Pseudomonas*, are inherently resistant to both agents. In contrast, most other agents that are used for treatment of UTIs are not recommended for prophylaxis. Invariably, if agents such as amoxicillin, cephalexin, and second-generation and third-generation cephalosporins are used for prophylaxis, infection with resistant strains emerges within weeks.

Surgery

When antireflux surgery is undertaken, a variety of approaches are available, no one of which seems to be superior. In most cases, the surgical approach is intravesicular. Interest has been gaining in evaluation of an extravesicular approach to reimplantation of the ureters.[98] The advantage of extravesicular approaches seems to be a diminution of the intensity and frequency of bladder spasms and less requirement for postoperative analgesia. This technique is indicated primarily in unilateral VUR.[45] When experienced senior surgeons embark on reimplantation of ureters, the outcome usually is successful.[24] Generally, all surgical techniques have a high success rate of 92 to 98 percent.[45] Repeated operation for persistence or recurrence of reflux seldom is required. Rarely, when children younger than 2 years old undergo surgery, ureteral obstruction may result; its persistence mandates a second operation.[123]

A newer technique for the management of VUR is endoscopic injection therapy using a variety of materials. Developed as a minimally invasive approach to the treatment of VUR in 1984, dextranomer/hyaluronic acid copolymer (Deflux) has emerged as the favored bulking agent since its approval by the U.S. Food and Drug Administration.[44,95] A single injection of Deflux has been shown to correct VUR (decrease grade to 0 or I/V) in approximately 70 percent of patients, with long-term success rates of 80 to 95 percent for grades I and II VUR but significantly lower (43% to 73%) for grades III to IV VUR.[14,73] Furthermore, long-term outcome of the procedure has been assessed primarily for reduction in grade of VUR rather than prevention of infection or renal scarring. The bulking agent is injected beneath the ureteral orifice and effectively closes the distal ureter. The procedure is done in the outpatient setting and is associated with less pain and a quicker recovery compared with traditional reimplantation procedures.[95] The success rates are lower, however, than those of traditional therapy, and the results have questionable durability. Long-term prospective studies are necessary to evaluate endoscopic treatments critically.

PROGNOSIS

Generally, the short-term prognosis for previously normal children who experience an episode of UTI is excellent. In a large cohort of 306 children experiencing their first febrile episode of UTI, 40 percent were found to have reflux.[54] Only five (1.6%) of the children had grade IV reflux; none had grade V reflux. Most of the children with grades I, II, and III reflux can be expected to have spontaneous resolution of the process during the subsequent several years. Although many reports indicate that recurrent UTIs are common occurrences in children who have recovered from UTIs, recurrence during the first 6 months after recovery from the index episode of UTI was an infrequent event in this cohort of children. Recurrences in general, after a first UTI, vary according to the degree of VUR, as shown in Table 39-6.

Recurrence is seen most commonly in the early months after a symptomatic or asymptomatic UTI has occurred. Symptomatic reinfections (fever, pyuria, and positive urine culture) occurred in 7 children treated orally and in 11 children treated intravenously (for a total of 5.9% of 306 children) during the 6-month follow-up period.[54] Asymptomatic bacteriuria (positive urine culture in the absence of fever and pyuria) occurred in 1 child treated orally and in 2 children treated intravenously (1%). Renal

scarring occurred in 9.5 percent of all children; the average percentage of renal parenchymal involvement was 8.2 percent. In children who had an abnormal renal scintigram at the time of diagnosis, the frequency of scarring was 15.3 percent. None of the children with a normal renal scintigram at the time of diagnosis developed scars. This experience, reflecting an aggressive approach to the early diagnosis of UTI in infancy, is encouraging with regard to outcome.

Two recent studies have suggested that childhood UTIs are a rare cause of chronic kidney disease.[129,133] Using data from the literature, renal registries and medical records from a tertiary care hospital, it was concluded that in the absence of structural kidney abnormalities evident on imaging studies performed after the first UTI, the fraction of recurrent UTI as a main cause of chronic renal disease was small, ranging from 1/154 to 3/1000 or less.

When children with a diagnosis of UTI are found to have anomalies of the urinary tract, congenital dysplasias, or massive degrees of reflux, the prognosis is less optimistic. The most profound degrees of scarring are associated with advanced degrees of reflux. Reflux nephropathy is a major cause of severe hypertension in children and young adults. It occasionally progresses to chronic renal failure,[149] which accounts for approximately 25 percent of the children in the United Kingdom with end-stage renal failure requiring regular dialysis or transplantation.[84] Although surgical correction may relieve the reflux, the overall outcome for children with reflux who have undergone surgical correction has not been shown to be materially better than that for children who have been managed medically.[110,111,150]

Smellie and colleagues[147] undertook a randomized trial of medical management versus surgical correction in 53 children with bilateral severe VUR and bilateral nephropathy. The glomerular filtration rate of the children at enrollment was 20 mL/min/1.73 m^2 body surface area. Children with this degree of severity of renal impairment are rare; recruitment to this study took 5 years. No significant differences were observed in glomerular filtration rate, renal growth, or scarring during a 10-year follow-up period. The failure to find differences may be due to the small sample size and broad heterogeneity of the study group. Nonetheless, no convincing evidence that outcome for renal function is improved by surgical correction of VUR in children with bilateral disease exists.

PREVENTION

Primary prevention of UTI can be accomplished in infant boys by promoting the practice of circumcision. Whether this procedure can be justified simply to prevent UTIs in the context of social rituals is uncertain. Circumcision should be recommended, however, for selected groups of patients, including newborns with prenatal hydronephrosis who are found to have VUR in the neonatal period, boys with high grades of VUR, and boys in whom VUR is associated with unilateral renal agenesis or multicystic kidney.[15]

Other commonly recommended strategies to prevent UTI, such as the avoidance of bathing in tubs or swimming and the instruction in correct wiping techniques, are not accompanied by convincing evidence.[46] The relationship between constipation and UTI is well known. Effective treatment of constipation results in normalization of bladder function and cessation of UTIs.[90]

The role of prophylactic antibiotics for patients with VUR was discussed previously in the section on treatment. Prophylaxis has not been shown in large, prospective, placebo-controlled, randomized trials to reduce effectively the frequency of UTI and subsequent renal scarring either in patients with reflux or in patients who have experienced an episode of APN.* Although definite biologic plausibility to this likelihood exists, strong evidence is lacking.

An additional preventive strategy for UTI is immunization. A small pilot study was reported from Turkey.[105] Ten otherwise healthy girls aged 5 to 11 years old with recurrent UTIs were immunized with inactivated uropathogenic bacteria intramuscularly once a week for 3 consecutive weeks. A booster injection was given after 6 months. The frequency of infection was compared with that in a group of 10 age-matched girls with UTIs who were not immunized. Immunization therapy caused a significant reduction in the frequency of infection and an increase in the concentration of the secretory component of IgA in the urine. Other mucosa, such as vaginal and oral surfaces, are alternative targets for vaccine delivery.

A vaginal mucosal vaccine composed of a mixture of heat-killed bacteria from 10 human uropathic strains (6 *E. coli* and 4 other gram-negative enteric bacteria) was developed for women with recurrent UTIs.[157] Fifty-four women completed a double-blind, placebo-controlled, phase II trial. Time until reinfection was longest for women who received primary and booster immunization compared with women receiving a placebo. This strategy has potential for further development.

NEW REFERENCES SINCE THE SIXTH EDITION

3. American Academy of Pediatrics, Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnoses and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.
12. Brandström P, Esbjörner E, Herthelius M, et al. The Swedish reflux trial in children, part III: urinary tract infection pattern. *J Urol* 2010;**184**:286–91.
19. Cheng CH, Tsau Y, Chen S, Lin T. Clinical courses of children with acute lobar nephronia correlated with computed tomographic patterns. *Pediatr Infect Dis J* 2009;**28**:300–3.
23. Craig JC, Simpson JM, Williams GJ. Antibiotic prophylaxis and recurrent urinary tract infection in children. *N Engl J Med* 2009;**361**:1748–59.
40. Gupta S, Araya CE, Dharmidharka VR. Xanthogranulomatous pyelonephritis in pediatric patients: case report and review of the literature. *J Pediatr Urol* 2010;**6**:355–8.
60. Huang YY, Chen MJ, Chin NT, et al. Adjunctive oral methylprednisolone in pediatric acute pyelonephritis alleviates renal scarring. *Pediatrics* 2011;**128**:e496.

*References 12, 23, 32, 102, 117, 130.

81. Leroy S, Gervaix A. Procalcitonin: a key marker in children with urinary tract infection. *Adv Urol* 2011;**2011**:397618. [Epub 2011 Jan 17.]
82. Leroy S, Romanello C, Galetto-Lacour A, et al. Procalcitonin is a predictor for high grade vesicoureteral reflux in children: meta-analysis of individual patient data. *J Pediatr* 2011;**159**:644–51.
102. Montini G, Rigon L, Zuchetta P, et al. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, non-inferiority trial. *Pediatrics* 2008;**122**:1064–71.
117. Pennesi M, Travan L, Peratoner L, et al. Is antibiotic prophylaxis in children with vesicoureteral reflux effective in preventing pyelonephritis and renal scars? A randomized controlled trial. Available at www.pediatrics.org/cgi/content/full/121/6/e1489.
124. Quigley R. Diagnosis of urinary tract infections in children. *Curr Opin Pediatr* 2009;**21**:194–8.
129. Round J, Fitzgerald AC, Hulme C, et al. Urinary tract infections in children and the risk of ESRF. *Acta Pediatr* 2012;**101**:278–82.
130. Roussey-Kesler G, Gadjos V, Idres N, et al. Antibiotic prophylaxes for the prevention of recurrent urinary tract infection in children with low grade vesicoureteral reflux: results from a prospective randomized study. *J Urol* 2008;**179**:674–9.
133. Salo J, Ikäheimo R, Tapiainen T, Uhari M. Childhood urinary tract infections as a cause of chronic kidney disease. *Pediatrics* 2011;**128**:840–7.
141. Shaikh N, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood. *Pediatr Infect Dis* 2008;**27**:302–8.
159. Venhola M, Huttunen NP, Renko M, et al. Practice guidelines for imaging studies in children after the first urinary tract infection. *J Urol* 2010;**184**:325–8.
167. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Exp Mol Pathol* 2008;**85**:11–9.
170. Williams GJ, Macaskill P, Chan SF, et al. Absolute and relative accuracy of rapid urine tests for urinary tract infection in children: a meta-analysis. *Lancet Infect Dis* 2010;**10**:240–50.
173. Wingerter S, Bachur R. Risk factors for contamination of catheterized urine specimens in febrile children. *Pediatr Emerg Care* 2011;**27**:1–4.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 40

Renal Abscess

Edmond T. Gonzales, Jr. • Sheldon L. Kaplan

Although acute pyelonephritis is a common infection in children, the primary development of a renal abscess or progression of pyelonephritis to a renal or perinephric abscess is an uncommon occurrence. In one study conducted over a 10-year period, 8 children with a renal abscess were found among 43,224 discharge diagnoses—approximately 1 case/5400 pediatric admissions.[5] Six of the eight children were 11 years or older. In a study from a children's hospital in Taiwan, 45 children were found to have a renal abscess over a 10-year period.[9] Although rare, renal abscesses have been reported in neonates.[10] Renal abscess may be a primary problem, that is, one that develops in a kidney without an antecedent infection or underlying anatomic abnormality, or it may occur secondarily in a patient with previously recognized acute pyelonephritis or in a child with congenital urologic abnormalities known to predispose to the development of pyelonephritis.

A primary renal abscess is thought to develop most often after an episode of bacteremia and frequently occurs in younger children. Hematogenous spread of bacteria to the kidney usually results in a cortical abscess.[5] The most common organisms involved in these abscesses are gram-positive cocci, primarily *Staphylococcus aureus* and less often *Streptococcus* spp. In some cases, a cutaneous infection might have been present before development of the renal abscess, and this infection is thought to be the primary source for the bacteremia.[16] Most children in whom a renal abscess develops hematogenously are normal hosts.[5,12,22,24] *Bartonella henselae* has been reported to cause microabscesses in the kidney.[17]

In older children and teenagers, tuberculous abscesses and caseous necrosis of the renal parenchyma also should be included in this primary classification. These infections tend to be indolent, although renal tuberculosis may be complicated by bacterial infection because of associated ureteral strictures and severe tuberculous cystitis. Pediatricians should become familiar with this "adult" malady because of the resurgence of tuberculosis and the generally older age group for which many pediatricians now provide care.

When a renal abscess occurs in association with a recognized urologic disorder, the organism responsible most often is a gram-negative bacillus or an enterococcus, bacteria usually seen in simple urinary tract infections and pyelonephritis.[19,21] Examples of urologic disorders that one might encounter with these infections include congenital and acquired obstructions (e.g., ureteropelvic

REFERENCES

1. Aia-ul-Miraj M, Cheema MA. Xanthogranulomatous pyelonephritis presenting as a pseudotumor in a 2 month old boy. *J Pediatr Surg* 2000;**35**:1256–8.
2. Al-Sayyad AJ, Pike JG, Leonard MP. Can prophylactic antibiotics safely be discontinued in children with vesicoureteral reflux? *J Urol* 2005;**174**:1587–8.
3. American Academy of Pediatrics, Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnoses and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**: 595–610.
4. Ataei N, Madani A, Esfahani ST, et al. Screening for vesicoureteral reflux and renal scars in siblings of children with known reflux. *Pediatr Nephrol* 2004;**19**:1127–31.
5. Austin PF, Ritchey ML. Dysfunctional voiding. *Pediatr Rev* 2000; **21**:336–40.
6. Azzarone G, Liewehr S, O'Connor T. Cystitis. *Pediatr Rev* 2007; **28**:474–6.
7. Belman AB. Vesicoureteral reflux. *Pediatr Clin North Am* 1997; **44**:1171–90.
8. Benador N, Siegrist CA, Gendrel D, et al. Procalcitonin is a marker of severity of renal lesions in pyelonephritis. *Pediatrics* 1998;**102**:1422–5.
9. Birmingham Reflux Study Group. Prospective trial of operative versus nonoperative treatment of severe vesicoureteric reflux: two years' observation in 96 children. *BMJ* 1983;**287**:171–4.
10. Birmingham Reflux Study Group. A prospective trial of operative versus nonoperative treatment of severe vesicoureteric reflux: 2 years' observation in 96 children. *Contrib Nephrol* 1984;**39**: 169–85.
11. Birmingham Reflux Study Group. Prospective trial of operative versus nonoperative treatment of severe vesicoureteric reflux in children: five years' observation. *BMJ* 1987;**295**:237–41.
12. Brandström P, Esbjörner E, Herthelius M, et al. The Swedish reflux trial in children, part III: urinary tract infection pattern. *J Urol* 2010;**184**:286–91.
13. Brook I. Urinary tract infection caused by anaerobic bacteria in children. *Urology* 1980;**16**:596–8.
14. Capozza N, Carone P. Dextranomer/hyaluronic acid copolymer implantation for vesico-ureteral reflux: a randomized comparison with antibiotic prophylaxis. *J Pediatr* 2002;**140**:230–4.
15. Cascio S, Colhoun E, Puri P. Bacterial colonization of the prepuce in boys with vesicoureteral reflux who receive antibiotic prophylaxis. *J Pediatr* 2001;**139**:160–2.
16. Cascio S, Yoneda A, Chertin B, et al. Renal parenchymal damage in sibling vesicoureteric reflux. *Acta Pediatr* 2003;**92**:17–20.
17. Chen L, Hsaio AL, Moore CL, et al. Utility of bedside bladder ultrasound before urethral catheterization in young children. *Pediatrics* 2005;**115**:108–11.
18. Chen M, Bao W, Aizman R, et al. ERK activation mediates apoptosis induced by uropathogenic *Escherichia coli* toxins via nitric oxide synthesis: protective role of heme-oxygenase-1. *J Infect Dis* 2004;**190**:127–35.
19. Cheng CH, Tsau Y, Chen S, Lin T. Clinical courses of children with acute lobar nephronia correlated with computed tomographic patterns. *Pediatr Infect Dis J* 2009;**28**:300–3.
20. Childs R, Sanchez C, Engler H, et al. High incidence of adeno- and polyomavirus-induced hemorrhagic cystitis in bone marrow allotransplantation for hematological malignancy following T cell depletion and cyclosporine. *Bone Marrow Transplant* 1998;**22**: 889–93.
21. Cooper CS, Chung BI, Kirsch AJ, et al. The outcome of stopping prophylactic antibiotics in older children with vesicoureteral reflux. *J Urol* 2000;**163**:269–72.
22. Craig JC, Knight JF, Sureshkuman P, et al. Effect of circumcision on incidence of urinary tract infection in preschool boys. *J Pediatr* 1996;**128**:23–7.
23. Craig JC, Simpson JM, Williams GJ. Antibiotic prophylaxis and recurrent urinary tract infection in children. *N Engl J Med* 2009;**361**:1748–59.
24. Decter RM. Vesicoureteral reflux. *Pediatr Rev* 2001;**22**:205–10.
25. Downs SM. Diagnostic testing strategies in childhood urinary tract infections. *Pediatr Ann* 1999;**28**:670–6.
26. Downs SM. Technical report: urinary tract infections in febrile infants and young children. The Urinary Tract Subcommittee of the American Academy of Pediatrics Committee on Quality Improvement. *Pediatrics* 1999;**103**:e54.
27. Farhat W, McLorie G. Urethral syndromes in children. *Pediatr Rev* 2001;**22**:17–20.
28. Feldman AS, Bauer SB. Diagnosis and management of dysfunctional voiding. *Curr Opin Pediatr* 2006;**18**:139–47.
29. Foresman WH, Hulbert Jr WC, Rabinowitz R. Does urinary tract ultrasonography at hospitalization for acute pyelonephritis predict vesicoureteral reflux? *J Urol* 2001;**165**:2232–4.
30. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity and economic costs. *Am J Med* 2002;**113**:5S–13S.
31. Garin EH, Campos A, Homsy Y. Primary vesicoureteral reflux: review of current concepts. *Pediatr Nephrol* 1998;**12**:249–56.
32. Garin EH, Olavarria F, Garcia-Nieto V, et al. Clinical significance of primary vesicoureteral reflux and urinary antibiotic prophylaxis after acute pyelonephritis: a multicenter, randomized, controlled study. *Pediatrics* 2006;**117**:626–32.
33. Gervaix A, Galletto-Lacour A, Gueron T, et al. Usefulness of procalcitonin and C-reactive protein rapid tests for the management of children with urinary tract infection. *Pediatr Infect Dis J* 2001;**20**:507–11.
34. Ginsburg CM, McCracken Jr GH. Urinary tract infections in young infants. *Pediatrics* 1982;**69**:409–12.
35. Gochman RF, Karasic RB, Heller MB. Use of portable ultrasound to assist urine collection by suprapubic aspiration. *Ann Emerg Med* 1991;**20**:631–5.
36. Gordon AC, Thomas DFM, Arthur RJ, et al. Prenatally diagnosed reflux: a follow-up study. *Br J Urol* 1990;**65**:407–12.
37. Gorelick MH, Shaw KN. Screening tests for urinary tract infection in children: a meta-analysis. *Pediatrics* 1999;**104**:e54.
38. Greenfield SP, Cherry RW, Carpenter M, et al. Vesicoureterial reflux: the RIVUR study and the way forward. *J Urol* 2008;**179**: 405–7.
39. Greenfield SP, Ng M, Wan J. Experience with vesicoureteral reflux in children: clinical characteristics. *J Urol* 1997;**158**:574–7.
40. Gupta S, Araya CE, Dharmidharka VR. Xanthogranulomatous pyelonephritis in pediatric patients: case report and review of the literature. *J Pediatr Urol* 2010;**6**:355–8.
41. Hall DE, Snitzer III JA. *Staphylococcus epidermidis* as a cause of urinary tract infections in children. *J Pediatr* 1994;**124**:437–8.
42. Hansson S, Dhamey M, Sigstrom O, et al. Dimercapto-succinic acid scintigraphy instead of voiding cystourethrography for infants with urinary tract infection. *J Urol* 2004;**172**:1071–4.
43. Hansson S, Martinell J, Stokland E, et al. The natural history of bacteriuria in childhood. *Infect Dis Clin North Am* 1997;**11**: 499–512.
44. Harrell WB, Snow BW. Endoscopic treatment of vesicoureteral reflux. *Curr Opin Pediatr* 2005;**17**:409–11.
45. Heidenreich A, Ozgur E, Becker T, Haupt G. Surgical management of vesicoureteral reflux in pediatric patients. *World J Urol* 2004;**22**:96–106.
46. Hellerstein S. Urinary tract infections in children: why they occur and how to prevent them. *Am Fam Physician* 1998;**57**:2440–6.
47. Hellerstein S. Acute urinary tract infection—evaluation and treatment. *Curr Opin Pediatr* 2006;**18**:134–8.
48. Hellstrom A, Hanson E, Hansson S, et al. Association between urinary symptoms at 7 years old and previous urinary tract infection. *Arch Dis Child* 1991;**66**:232–4.
49. Herr SM, Wald ER, Pitetti RD, Choi SS. Enhanced urinalysis improves identification of febrile infants ages 60 days and younger at low risk for serious bacterial illness. *Pediatrics* 2001;**108**: 866–71.
50. Hitchcock RJ, Pallett A, Hall MA, et al. Urinary tract candidiasis in neonates and infants. *Br J Urol* 1995;**76**:252–6.
51. Hoberman A, Chao HP, Keller DM, et al. Prevalence of urinary tract infection in febrile infants. *J Pediatr* 1993;**123**:17–23.
52. Hoberman A, Charron M, Hickey RW, et al. Imaging studies after a first febrile urinary tract infection in young children. *N Engl J Med* 2003;**248**:195–202.
53. Hoberman A, Wald ER. Urinary tract infections in young febrile children. *Pediatr Infect Dis J* 1997;**16**:11–7.
54. Hoberman A, Wald ER, Hickey RW, et al. Oral versus initial intravenous therapy for urinary tract infections in young febrile children. *Pediatrics* 1999;**104**:79–86.

55. Hoberman A, Wald ER, Penchansky L, et al. Enhanced urinalysis as a screening test for urinary tract infection. *Pediatrics* 1993; **91**:1196–9.
56. Hoberman A, Wald ER, Reynolds EA, et al. Pyuria and bacteriuria in urine specimens obtained by catheter from young children with fever. *J Pediatr* 1994;**124**:513–9.
57. Hodson EM, Willis NS, Craig JC. Antibiotics for acute pyelonephritis in children. *Cochrane Database Syst Rev* 2007;(4):CD003772.
58. Hooten TM, Scholes D, Hughes JP, et al. A prospective study of risk factors for symptomatic urinary tract infection in young women. *N Engl J Med* 1996;**335**:468–74.
59. Horwitz MA, Silverstein SC. Influence of *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. *J Clin Invest* 1980;**65**:82–94.
60. Huang YY, Chen MJ, Chin NT, et al. Adjunctive oral methylprednisolone in pediatric acute pyelonephritis alleviates renal scarring. *Pediatrics* 2011;**128**:e496.
61. Huicho L, Campos-Sanchez M, Alamo C. Meta-analysis of urine screening tests for determining the risk of urinary tract infection in children. *Pediatr Infect Dis J* 2002;**21**:1–11.
62. Jahnukainen T, Chen M, Celsi G. Mechanism of renal damage owing to infection. *Pediatr Nephrol* 2005;**20**:1043–53.
63. Jahnukainen T, Honkinen O, Ruuskanen O, Mertsola J. Ultrasonography after the first febrile urinary tract infection in children. *Eur J Pediatr* 2006;**165**:556–9.
64. Jantunen ME, Siitonen A, Koskimies O, et al. Predominance of class II *papG* allele of *Escherichia coli* in pyelonephritis in infants with normal urinary tract anatomy. *J Infect Dis* 2000;**181**: 1822–4.
65. Jodal U, Hansson S, Hjalmas K. Medical or surgical management for children with vesicoureteric reflux? *Acta Paediatr* 1999;**431** (Suppl):53–61.
66. Kaack MB, Dowling KJ, Patterson GM, et al. Immunology of pyelonephritis, part VIII: *E. coli* causes granulocytic aggregation and renal ischemia. *J Urol* 1986;**136**:1117–22.
67. Kabore AF, Simord M, Bergeron MG. Local production of inflammatory mediators in an experimental model of acute obstructive pyelonephritis. *J Infect Dis* 1999;**179**:1162–72.
68. Kaefer M, Curran M, Treves ST, et al. Sibling vesicoureteral reflux in multiple gestation births. *Pediatrics* 2000;**105**:800–4.
69. Kallenius G, Mollby R, Svensson SB, et al. The pK antigen as receptor for the hemagglutination of pyelonephritogenic *Escherichia coli*. *FEMS Microbiol Lett* 1980;**7**:297.
70. Kasirga E, Akel L, Yilmaz O, et al. Evaluation of voiding dysfunction in children with chronic functional constipation. *Turk J Pediatr* 2006;**48**:340–3.
71. Kemper KJ, Avner ED. The case against screening urinalyses for asymptomatic bacteriuria in children. *Am J Dis Child* 1992;**146**: 343–6.
72. Kerem R. Imaging and treatment strategies for children after first urinary tract infection. *Curr Opin Pediatr* 2007;**19**:705–10.
73. Kersch AJ, Perez-Brayfull MR, Scherz HC. Minimally invasive treatment of vesicoureteral reflux with endoscopic injection of dextranomer/hyaluronic acid copolymer: the Children's Hospital of Atlanta experience. *J Urol* 2003;**170**:211–5.
74. Khan AJ, Ubriani RS, Bombach E, et al. Initial urinary tract infection caused by *Proteus mirabilis* in infancy and childhood. *J Pediatr* 1978;**93**:791–3.
75. Koff SA, Wagner TT, Jayanthi VR. The relationship among dysfunctional elimination syndromes, primary vesicoureteral reflux and urinary tract infections in children. *J Urol* 1998;**160**: 1019–22.
76. Kunin CM. A ten-year study of bacteriuria in schoolgirls: final report of bacteriologic, urologic and epidemiologic findings. *J Infect Dis* 1970;**122**:382–93.
77. Lamchiogdhase P, Preechaborisutkul K, Lomsomboon P, et al. Urine sediment examination: a comparison between manual method and the iQ200 automated urine microscopy analyzer. *Clin Chim Acta* 2005;**359**:167–74.
78. Lebowitz RL, Olbing H, Parkkulainen KV, et al. International system of radiographic grading of vesicoureteral reflux. International Reflux Study in Children. *Pediatr Radiol* 1985;**15**:105–9.
79. Leneghan D, Whitaker JG, Jensen F, Stephens FD. The natural history of reflux and long term effects of reflux on the kidney. *J Urol* 1976;**115**:728–30.
80. Leonidas JC, Berdon WE. MR imaging of urinary tract infections in children. *Radiology* 1999;**210**:582–3.
81. Leroy S, Gervaix A. Procalcitonin: a key marker in children with urinary tract infection. *Adv Urol* 2011;**2011**:397618. [Epub 2011 Jan 17.]
82. Leroy S, Romanello C, Galetto-Lacour A, et al. Procalcitonin is a predictor for high grade vesicoureteral reflux in children: meta-analysis of individual patient data. *J Pediatr* 2011;**159**:644–51.
83. Le Saux N, Pham BA, Moher D. Evaluating the benefits of antimicrobial prophylaxis to prevent urinary tract infections in children: a systematic review. *Can Med Assoc J* 2000;**163**:523–9.
84. Lewis M. Report of the Paediatric Renal Registry 1999. In: Ansell D, Feest T, editors. *The second annual report of the UK Renal Registry*. Bristol, U.K.: Renal Association; 1999. p. 175–87.
85. Liles WC, Cushing H, Holt S, et al. Severe adenoviral nephritis following bone marrow transplantation: successful treatment with intravenous ribavirin. *Bone Marrow Transplant* 1993;**12**:409–12.
86. Lin DS, Huang FY, Chiu NC, et al. Comparison of hemocytometer leukocyte counts and standard urinalyses for predicting urinary tract infections in febrile infants. *Pediatr Infect Dis J* 2000;**19**:223–7.
87. Lin DS, Huang S, Lin C, et al. Urinary tract infection in febrile infants younger than eight weeks of age. *Pediatrics* 2000;**105**:e20.
88. Lindberg U, Claesson I, Hanson LA, et al. Asymptomatic bacteriuria in schoolgirls: clinical and laboratory findings. *Acta Paediatr Scand* 1975;**64**:425–31.
89. Linko S, Kauri TK, Toivonen E, et al. Analytic performance of the Iris iQ200 automated urine microscopy analyzer. *Clin Chim Acta* 2006;**372**:54–6.
90. Loening-Baucke V. Urinary incontinence and urinary tract infection and their resolution with treatment of chronic constipation of childhood. *Pediatrics* 1997;**100**:228–32.
91. Lohr JA, Nunley DH, Howards SS, et al. Prevention of recurrent urinary tract infections in girls. *Pediatrics* 1977;**59**:562–5.
92. Lomberg L, Hellstrom M, Jodal U, et al. Virulence-associated traits in *Escherichia coli* causing first and recurrent episodes of urinary tract infection in children with or without vesicoureteral reflux. *J Infect Dis* 1984;**150**:561–9.
93. Lonergan DJ, Pennington DJ. MR imaging of urinary tract infections in children. *Reply Radiology* 1999;**210**:583–4.
94. Lonergan GJ, Pennington DJ, Morrison JC, et al. Childhood pyelonephritis: comparison of gadolinium-enhanced MR imaging and renal cortical scintigraphy. *Radiology* 1998;**207**:377–84.
95. Lorenzo AJ, Khoury AE. Endoscopic treatment of reflux: management of pros and cons. *Curr Opin Urol* 2006;**16**:299–304.
96. Ma JF, Shortliffe LMD. Urinary tract infection in children: Etiology and epidemiology. *Urol Clin North Am* 2004;**31**:517–26.
97. Marild S, Jodal U. Incidence rate of first-time symptomatic urinary tract infection in children under 6 years of age. *Acta Paediatr* 1998;**87**:549–52.
98. Marotte JB, Smith DP. Extravesical ureteral reimplantations for the correction of primary reflux can be done as outpatient procedures. *J Urol* 2001;**165**:2228–31.
99. Matlow AG, Wray RD, Cox PN. Nosocomial urinary tract infections in children in a pediatric intensive care unit: a follow-up after 10 years. *Pediatr Crit Care Med* 2003;**4**:74–7.
100. McFadyen IR, Eykyn SJ. Suprapubic aspiration of urine in pregnancy. *Lancet* 1968;**1**:1112–4.
101. Michael M, Hodson EM, Craig EM, Martin S, et al. Short versus standard duration oral antibiotic therapy for acute urinary tract infection in children. *Cochrane Database Syst Rev* 2003;(1):CD003966.
102. Montini G, Rigon L, Zuchetta P, et al. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, non-inferiority trial. *Pediatrics* 2008;**122**: 1064–71.
103. Morgan MG, Hamilton-Miller JM. *Haemophilus influenzae* and *H. parainfluenzae* as urinary pathogens. *J Infect* 1990;**20**:143–5.
104. Munir V, Barnett P, South M. Does the use of volumetric bladder ultrasound inprove the success rate of suprapubic aspiration of urine? *Pediatr Emerg Care* 2002;**18**:346–9.
105. Nayir A, Emre S, Sirin A, et al. The effects of vaccination with inactivated uropathogenic bacteria in recurrent urinary tract infections of children. *Vaccine* 1995;**13**:987–90.
106. Newman TB, Bernzweign JA, Takayama JI, et al. Urine testing and urinary tract infections in febrile infants seen in office settings:

The Pediatric Research in Office Settings Febrile Infant Study. *Arch Pediatr Adolesc Med* 2002;**156**:44–54.
107. Nicolle LE. Catheter-related urinary tract infection. *Drugs Aging* 2005;**22**:627–39.
108. Nicolle LE, Harding GKM, Preiksaitis J, et al. The association of urinary tract infection with sexual intercourse. *J Infect Dis* 1982;**146**:579–83.
109. Nowicki B, Holthofer H, Saraneva T, et al. Location of adhesion sites for P-fimbriated and for O75X-positive *Escherichia coli* in the human kidney. *Microb Pathog* 1986;**1**:169–80.
110. Olbing H, Claesson I, Ebel KD, et al. Renal scars and parenchymal thinning in children with vesicoureteral reflux: a 5-year report of the International Reflux Study in Children (European branch). *J Urol* 1992;**148**:1653–6.
111. Olbing H, Hirche H, Koskimies O, et al. Renal growth over 10 years in a prospective study of medical or surgical treatment in children with severe vesicoureteral reflux: 10-year prospective study of medical and surgical treatment. *Radiology* 2000;**216**:731–7.
112. Orrett FA, Brooks PJ, Richardson EG, et al. Paediatric nosocomial urinary tract infection at a regional hospital. *Int Urol Nephrol* 1999;**31**:173–9.
113. Paduch DA. Viral lower urinary tract infection. *Curr Urol Rep* 2007;**8**:324–35.
114. Parekh DJ, Pope JC, Adams MC, Brock JW. Outcome of sibling vesicoureteral reflux. *J Urol* 2002;**167**:283–4.
115. Pecile P, Miorin E, Romanello C, et al. Procalcitonin: a marker of severity of acute pyelonephritis among children. *Pediatrics* 2004;**114**:e249–54.
116. Pennington DJ, Lonergan GJ, Flack CE, et al. Experimental pyelonephritis in piglets: diagnosis with MR imaging. *Radiology* 1996;**201**:199–205.
117. Pennesi M, Travan L, Peratoner L, et al. Is antibiotic prophylaxis in children with vesicoureteral reflux effective in preventing pyelonephritis and renal scars? A randomized controlled trial. Available at www.pediatrics.org/cgi/content/full/121/6/e1489.
118. Phillips JR, Karlowicz MG. Prevalence of *Candida* species in hospital-acquired urinary tract infections in a neonatal intensive care unit. *Pediatr Infect Dis J* 1997;**16**:190–4.
119. Piepsz A, Tamminen-Mobius T, Reiners C, et al. Five-year study of medical or surgical treatment in children with severe vesicoureteral reflux dimercaptosuccinic acid findings. *Eur J Pediatr* 1998;**157**:753–8.
120. Polnay L, Fraser AM, Lewis JM. Complication of suprapubic bladder aspiration. *Arch Dis Child* 1975;**50**:80–1.
121. Prat C, Dominguez J, Rodrigo C, et al. Elevated serum procalcitonin values correlate with renal scarring in children with urinary tract infection. *Pediatr Infect Dis J* 2003;**22**:438–42.
122. Prelog M, Schiefecker D, Fille M, et al. Acute nosocomial urinary tract infection in children. *Infect Control Hosp Epidemiol* 2007;**28**:1019–23.
123. Pryles CV, Atkins MD, Morse TS, et al. Comparative bacteriologic study of urine obtained from children by percutaneous suprapubic aspiration of the bladder and by catheter. *Pediatrics* 1959;**24**:983–91.
124. Quigley R. Diagnosis of urinary tract infections in children. *Curr Opin Pediatr* 2009;**21**:194–8.
125. Report of the International Reflux Study Committee. Medical versus surgical treatment of primary vesicoureteral reflux: a prospective international reflux study in children. *Pediatrics* 1981;**67**:392–400.
126. Roberts JA. Etiology and pathophysiology of pyelonephritis. *Am J Kidney Dis* 1991;**17**:1–9.
127. Roberts JA, Angel JR, Roth Jr JK. The hydrodynamics of pyelorenal reflux, part II: the effect of chronic obstructive changes on papillary shape. *Invest Urol* 1981;**18**:296–301.
128. Rathore MH, Barton LL, Luisiri A. Acute lobar nephronia: a review. *Pediatrics* 1991;**87**:728–34.
129. Round J, Fitzgerald AC, Hulme C, et al. Urinary tract infections in children and the risk of ESRF. *Acta Pediatr* 2012;**101**:278–82.
130. Roussey-Kesler G, Gadjos V, Idres N, et al. Antibiotic prophylaxes for the prevention of recurrent urinary tract infection in children with low grade vesicoureteral reflux: results from a prospective randomized study. *J Urol* 2008;**179**:674–9.
131. Rushton HG. Urinary tract infections in children: epidemiology, evaluation, and management. *Pediatr Clin North Am* 1997;**44**:1133–69.
132. Rushton HG, Majd M, Chandra R, et al. Evaluation of 99m technetium-dimercaptosuccinic acid in renal scans in experimental acute pyelonephritis in piglets. *J Urol* 1988;**140**:1169–74.
133. Salo J, Ikäheimo R, Tapiainen T, Uhari M. Childhood urinary tract infections as a cause of chronic kidney disease. *Pediatrics* 2011;**128**:840–7.
134. Savage DCL, Wilson MI, McHardy M, et al. Covert bacteriuria of childhood: a clinical and epidemiological study. *Arch Dis Child* 1973;**48**:8–20.
135. Schlager TA, Hendley JO, Dudley SM, et al. Explanation for false-positive urine cultures obtained by bag technique. *Arch Pediatr Adolesc Med* 1995;**149**:170–3.
136. Schlager TA, Smith DE, Donowitz LG. Perineal cleansing does not reduce contamination of urine samples from pregnant adolescents. *Pediatr Infect Dis J* 1995;**14**:909–11.
137. Schoen EJ, Colby CJ, Ray GT. Newborn circumcision decreases incidence and cost of urinary tract infections during the first year of life. *Pediatrics* 2000;**105**:789–93.
138. Scholes D, Hooton TM, Roberts PL, et al. Risk factors associated with acute pyelonephritis in healthy women. *Ann Intern Med* 2005;**142**:20–7.
139. Schwab CW, Wu H, Selman H, et al. Spontaneous resolution of vesicoureteral reflux: a 15-year perspective. *J Urol* 2002;**168**:2594–9.
140. Shalaby-Rana E, Lowe LH, Blask AN, et al. Imaging in pediatric urology. *Pediatr Clin North Am* 1997;**44**:1065–89.
141. Shaikh N, Morone NE, Bost JE, Farrell MH. Prevalence of urinary tract infection in childhood. *Pediatr Infect Dis* 2008;**27**:302–8.
142. Shaikh N, Morone NE, Lopez J. Does this child have a urinary tract infection? *JAMA* 2007;**298**:2895–904.
143. Shapiro ED. Short course antimicrobial treatment of urinary tract infections in children: a critical analysis. *Pediatr Infect Dis J* 1982;**1**:294–7.
144. Shaw KN, Gorelick M, McGowan KL, et al. Prevalence of urinary tract infection in febrile young children in the emergency department. *Pediatrics* 1998;**102**:e16.
145. Singh-Grewal D, Macdessi J, Craig J. Circumcision for the prevention of urinary tract infection in boys: a systematic review of randomized trials and observational studies. *Arch Dis Child* 2005;**90**:853–8.
146. Skoog SJ, Belman AB, Majd M. A nonsurgical approach to the management of primary vesicoureteral reflux. *J Urol* 1987;**138**:941.
147. Smellie JM, Barratt TM, Chantler C, et al. Medical versus surgical treatment in children with severe bilateral vesicoureteric reflux and bilateral nephropathy: a randomised trial. *Lancet* 2001;**357**:1329–33.
148. Smellie JM, Katz G, Gruneberg RN. Controlled trial of prophylactic treatment in childhood urinary tract infection. *Lancet* 1978;**2**:175–8.
149. Smellie JM, Prescod NP, Shaw PJ, et al. Childhood reflux and urinary infection: a follow-up of 10-41 years in 226 adults. *Pediatr Nephrol* 1998;**12**:727–36.
150. Smellie JM, Tamminen-Mobius T, Olbing H, et al. Five year study of medical or surgical treatment in children with severe reflux: radiological renal findings. *Pediatr Nephrol* 1992;**6**:223–30.
151. Smolken V, Koren A, Raz R, et al. Procalcitonin as a marker of acute pyelonephritis in infants and children. *Pediatr Nephrol* 2002;**17**:409–12.
152. Sobel JD, Vasquez JA. Fungal infections of the urinary tract. *World J Urol* 1999;**17**:410–4.
153. Thompson RH, Chen JJ, Pugach J, et al. Cessation of prophylactic antibiotics for managing persistent vesicoureteral reflux. *J Urol* 2001;**161**:1465–9.
154. Todd JK. Management of urinary tract infections: children are different. *Pediatr Rev* 1995;**16**:190–6.
155. Tran D, Muchant DG, Aronoff SC. Short-course versus conventional length antimicrobial therapy for uncomplicated lower urinary tract infections in children: a meta-analysis of 1279 patients. *J Pediatr* 2001;**139**:93–9.
156. Traylor LA, Mayeux PR. Nitric oxide generation mediates lipid-A–induced oxidant injury in renal proximal cells. *Arch Biochem Biophys* 1997;**338**:129–35.

157. Uehling DT, Hopkins WJ, Elkahwaji JE, et al. Phase 2 clinical trial of a vaginal mucosal vaccine for urinary tract infections. *J Urol* 2003;**170**:867–9.
158. Vaisanen-Rhen V, Elo J, Vaisanen E, et al. P-fimbriated clones among uropathogenic *Escherichia coli* strains. *Infect Immun* 1984;**43**:149–55.
159. Venhola M, Huttunen NP, Renko M, et al. Practice guidelines for imaging studies in children after the first urinary tract infection. *J Urol* 2010;**184**:325–8.
160. Wah DT, Wises PK, Butch AW. Analytic performance of the iQ200 automated urine microscopy analyzer and comparison with manual counts using Fuchs-Rosenthal cell chambers. *Am J Clin Pathol* 2005;**123**:290–6.
161. Wald E. Urinary tract infections in infants and children: a comprehensive review. *Curr Opin Pediatr* 2004;**16**:85–8.
162. Wald E. Vesicoureteral reflux: the role of antibiotics. *Pediatrics* 2006;**117**:919–22.
163. Weiss R, Duckett J, Spitzer A. Results of a randomized clinical trial of medical versus surgical management of infants and children with grades III and IV primary vesicoureteral reflux (United States). *J Urol* 1992;**148**:1667–73.
164. Weiss R, Tamminen-Mobius T, Koskimies O, et al. Characteristics at entry of children with severe primary vesicoureteral reflux recruited for a multicenter, international therapeutic trial comparing medical and surgical management. *J Urol* 1992;**148**:1644–9.
165. Wettergren B, Jodal U. Spontaneous clearance of asymptomatic bacteriuria in infants. *Acta Paediatr Scand* 1990;**79**:300–4.
166. Wheeler D, Vimalachendra D, Hodson EM, et al. Antibiotics and surgery for vesicoureteric reflux: a meta-analysis of randomized controlled trials. *Arch Dis Child* 2003;**88**:688–94.
167. Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Exp Mol Pathol* 2008;**85**: 11–9.
168. Williams GJ, Lee A, Craig JC. Antibiotics for the prevention of urinary tract infection in children: a systematic review of randomized controlled trials. *J Pediatr* 2001;**138**:868–74.
169. Williams GJ, Lee A, Craig JC. Long-term antibiotics for preventing recurrent urinary tract infection in children. *Cochrane Database Syst Rev* 2001;(4):CD001534; updated 2006;(3)CD001534.
170. Williams GJ, Macaskill P, Chan SF, et al. Absolute and relative accuracy of rapid urine tests for urinary tract infection in children: a meta-analysis. *Lancet Infect Dis* 2010;**10**:240–50.
171. Winberg J. Management of primary vesicoureteric reflux in children—operation ineffective in preventing progressive renal damage. *Infection* 1994;**22**(Suppl 1):S4–7.
172. Winberg J, Andersen HJ, Bergstrom T, et al. Epidemiology of symptomatic urinary tract infection in childhood. *Acta Paediatr Scand* 1974;**252**(Suppl):1–20.
173. Wingerter S, Bachur R. Risk factors for contamination of catheterized urine specimens in febrile children. *Pediatr Emerg Care* 2011;**27**:1–4.
174. Wiswell TE, Roscelli JD. Corroborative evidence for the decreased incidence of urinary tract infections in circumcised male infants. *Pediatrics* 1986;**78**:96–9.
175. Wiswell TE, Smith FR, Bass JW. Decreased incidence of urinary tract infections in circumcised male infants. *Pediatrics* 1985;**75**: 901–3.
176. Yazbeck S, Schick E, O'Regan S. Relevance of constipation to enuresis, urinary tract infection and reflux: a review. *Eur Urol* 1987;**13**:318–21.
177. Zamir G, Sakran W, Horowitz Y, et al. Urinary tract infection: is there a need for routine renal ultrasonography? *Arch Dis Child* 2004;**89**:466–8.
178. Zorc JJ, Kiddoo DA, Shaw KN. Diagnosis and management of pediatric urinary tract infections. *Clin Microbiol Rev* 2005;**18**: 417–22.
179. Zugor V, Schott GE, Labanaris AP. Xanthogranulomatous pyelonephritis in childhood: a critical analysis of 10 cases and of the literature. *Urology* 2007;**70**:157–60.

and ureterovesical obstruction, retrocaval ureter, ureteral stricture after surgical intervention), calculous disease (obstructing and nonobstructing), infundibular stenosis, and renal dysplasia with cystic changes. Abscesses that occur as a result of infection of the urinary tract generally are found in a corticomedullary location.[5]

Anaerobic organisms also have been implicated as a cause of renal abscess. They often are present simultaneously with the more usual aerobic bacteria, but they can cause infections and abscesses alone. These anaerobic renal infections develop most commonly in association with infections complicating bowel injury or surgery, renal transplantation, malignancy, and orodental infections.[4] The genus of the anaerobic organism may provide a clue to its source. *Bacteroides fragilis* is likely to arise from an intra-abdominal source, whereas an oral site is more common for *Prevotella oralis*.[4]

Children with human immunodeficiency virus infection seem to have an increased risk for development of renal abscesses from the more common traditional organisms[3] and from unusual opportunistic fungal organisms, especially *Aspergillus*.[14] As expected, these children also tend to have a more fulminant course that often requires extensive surgical intervention and drainage.

The presence of a renal abscess implies the destruction and liquefaction of tissue in a confined space. Two other infectious disorders of the kidney, xanthogranulomatous pyelonephritis and acute lobar nephropathy (acute focal bacterial nephritis), frequently are included in this general category, although technically, true abscesses do not always develop in these disorders. Acute lobar nephropathy may progress to renal abscess, however, if not treated appropriately.

Xanthogranulomatous pyelonephritis describes a more chronic form of severe renal parenchymal destruction that often is associated with chronic stone disease. The process may involve the whole kidney or may be focal. In children, the focal form occurs more commonly. The pathognomonic histologic finding is an accumulation of lipid-laden macrophages that coalesce into discrete yellow nodules. Small abscess cavities often are studded throughout the kidney. The organism most frequently recovered from the kidney is *Proteus*, and urinary calculi are common findings. Although these patients often have acute symptoms, the symptoms frequently are superimposed on more chronic manifestations, such as weight loss, failure to thrive, and anemia. Treatment is complete or partial nephrectomy because the renal destruction generally is severe.[23]

Acute lobar nephropathy describes a focal area of intense edema at the site of infection in acute pyelonephritis.[2] It usually is recognized as a mass effect on an initial screening renal ultrasonogram. Severe nephromegaly (renal length >3 standard deviations above the mean for age) is another finding on renal ultrasound suggestive of acute lobar nephropathy.[8] Computed tomography (CT) of the kidney shows poor uptake in the involved segment, but no well-defined liquefaction (Fig. 40-1). Whether this edema is just an exaggerated response to infection or represents a change before an abscess is unknown. Klar and colleagues[13] described 13 children, 4 months to 8 years of age, with acute lobar nephropathy in a prospective study during a 4-year period. Bacteremia was documented in only 1 child. Evolution to abscess formation occurred in 4 (31%). In another prospective study, Cheng and associates[6] found bacteremia occurred in 4 of 80 children (5%) with acute lobar nephropathy. In further studies these investigators noted that patients with acute lobar nephronia who had CT findings with heterogeneously decreased nephrographic density after contrast enhancement were more likely to develop a renal abscess than those with a homogenous decrease in density.[7] These lesions generally heal satisfactorily with administration of antibiotics alone.

CLINICAL FINDINGS

Children with acute renal abscess are febrile and have localized pain in the costovertebral location. Prolonged fever is a common finding, especially in older children. Most patients are febrile for longer than 7 days before the diagnosis is established. The initial findings generally do not differentiate, however, between acute pyelonephritis with and without an abscess. If the abscess has spread into the perinephric region, it may be possible to recognize psoas muscle irritation in the patient—that is, the patient is more comfortable with the ipsilateral leg in a position of flexion and experiences pain with full extension. A large abscess may be palpable as a flank mass.

Findings on urinalysis can be confusing. With a primary (hematogenous) abscess, the urine may be deceptively benign, and culture generally is negative.[16] Blood cultures may be positive, depending on the duration of the illness when blood is obtained for culture. Abscesses associated with underlying urologic disorders can be expected to contain organisms and pyuria. Generally, severe leukocytosis is present. The erythrocyte sedimentation rate typically is elevated; the same probably is true for C-reactive protein.[24] All of these findings are nonspecific, however, and often further studies are needed to establish the diagnosis. If a more indolent process is encountered, consider performing appropriate staining and cultures for tuberculosis should be considered.

DIAGNOSTIC EVALUATION

All children admitted to the hospital with a febrile urinary tract infection should undergo renal ultrasonography as soon as is reasonable after admission.[18] The findings on this initial study can have a significant effect on the choice of therapeutic options. If both kidneys are normal and unobstructed, organism-specific therapy is satisfactory in most patients. If significant obstruction or stones are present, antibiotic therapy may not be as effective, and interventional drainage may become necessary if the response to antibiotics is inadequate. At the time of this initial screening study, findings may suggest the presence of a renal abscess. Such findings include a mass effect within the margins of the kidney, along with a thickened wall and material of varying sonographic density within the mass (Fig. 40-2). Similar findings can be seen in

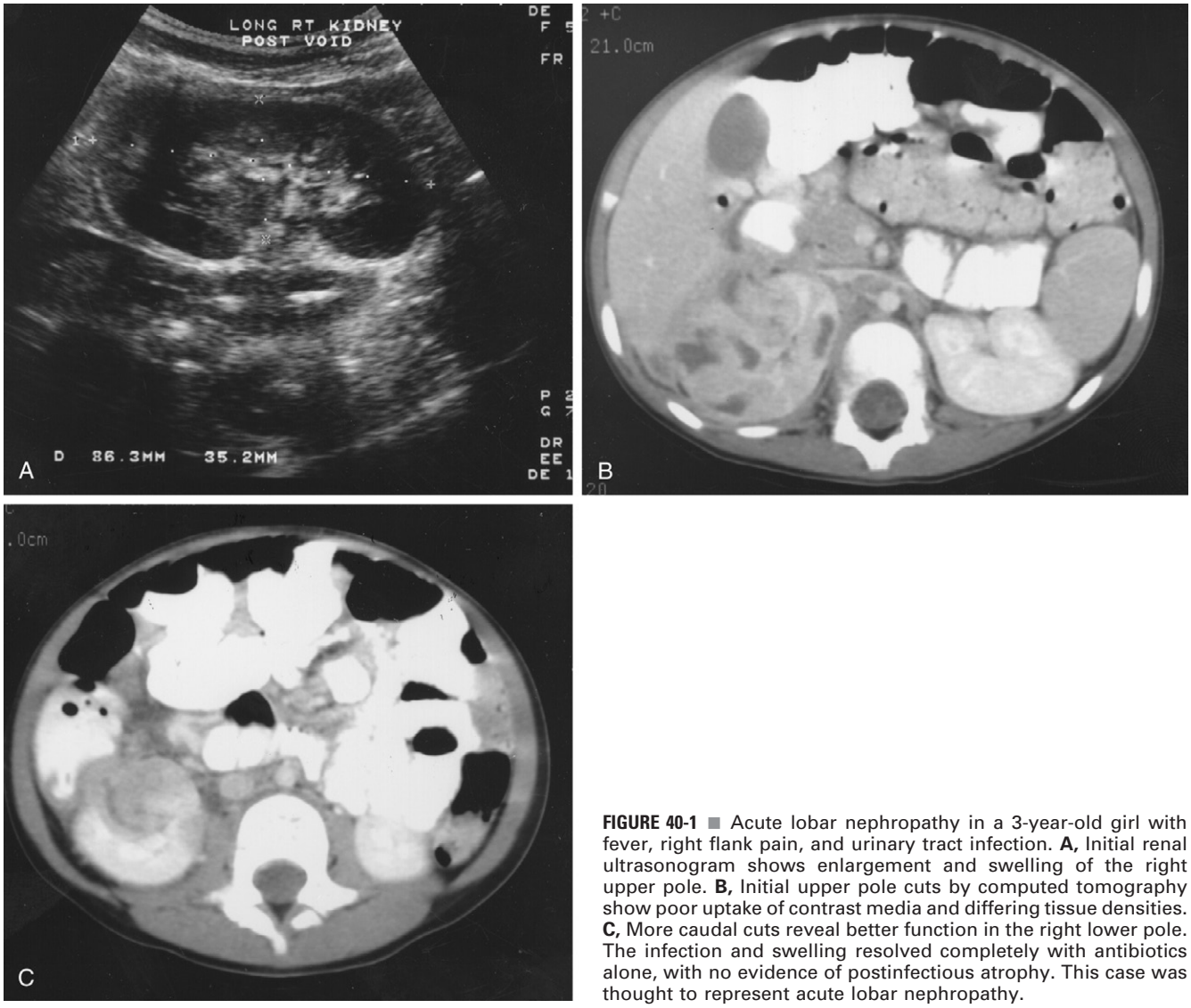

FIGURE 40-1 ■ Acute lobar nephropathy in a 3-year-old girl with fever, right flank pain, and urinary tract infection. **A,** Initial renal ultrasonogram shows enlargement and swelling of the right upper pole. **B,** Initial upper pole cuts by computed tomography show poor uptake of contrast media and differing tissue densities. **C,** More caudal cuts reveal better function in the right lower pole. The infection and swelling resolved completely with antibiotics alone, with no evidence of postinfectious atrophy. This case was thought to represent acute lobar nephropathy.

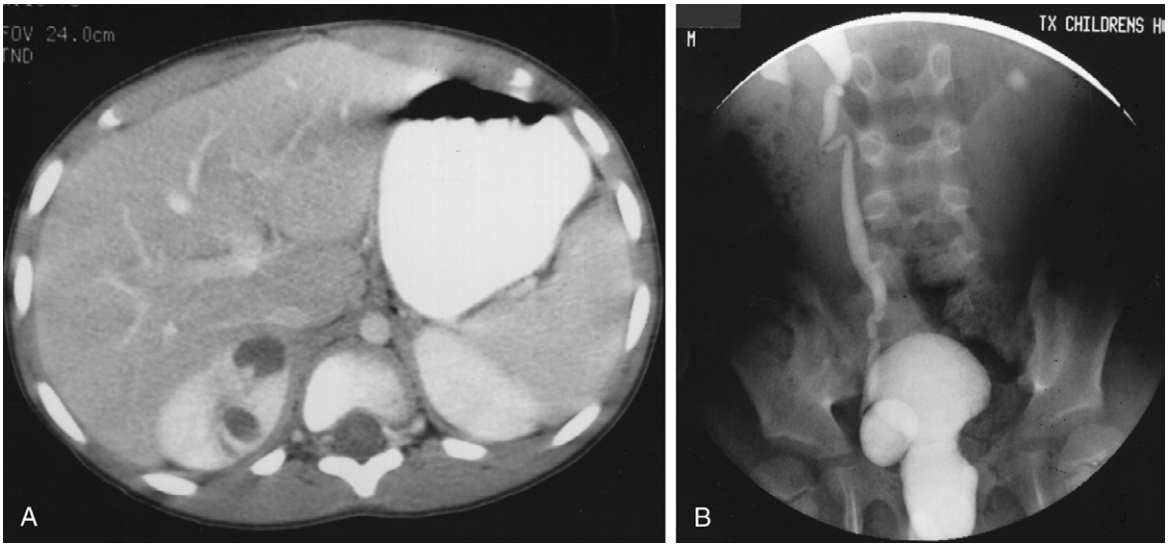

FIGURE 40-2 ■ Renal abscess (secondary) in a 6-month-old boy with urinary tract infection. A screening renal ultrasonogram showed a small mass in the right kidney. **A,** Computed tomography shows two clearly defined cystic areas consistent with small parenchymal abscesses. Treatment consisted of intravenous antibiotics only. **B,** Voiding cystogram revealed the presence of vesicoureteral reflux.

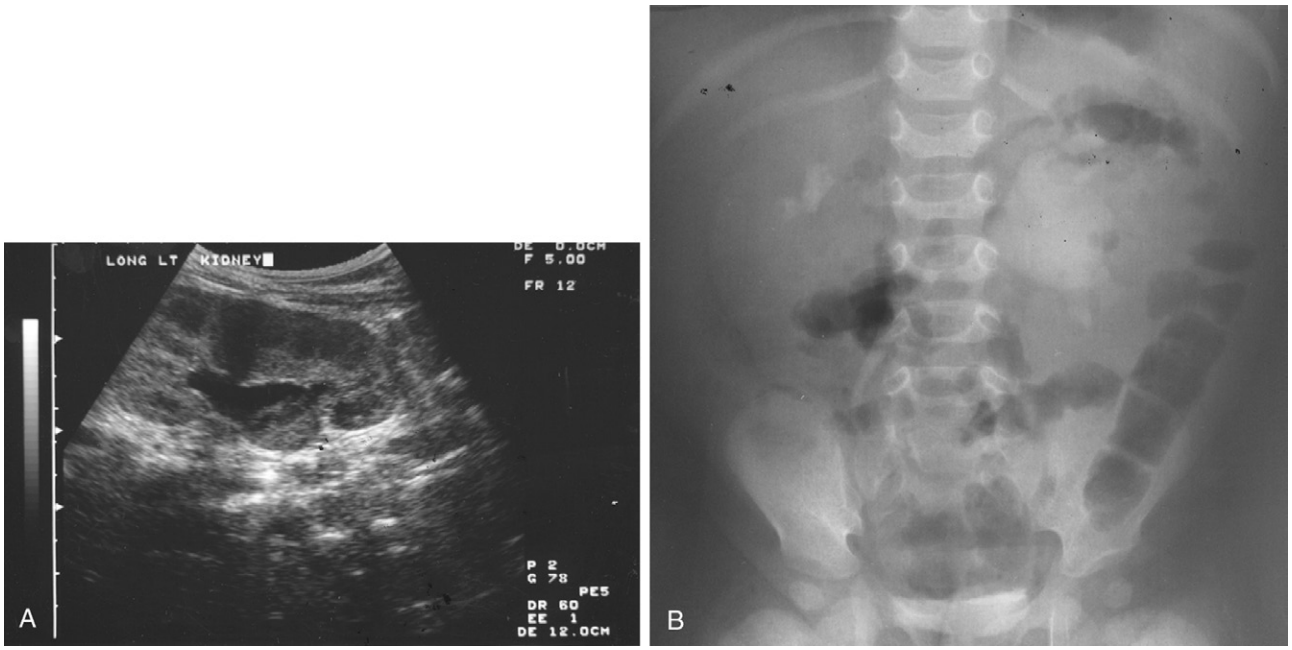

FIGURE 40-3 ■ Pyonephrosis associated with ureteropelvic junction obstruction in a 3-month-old boy with fever, left abdominal and flank tenderness, and urinary tract infection. **A,** Renal ultrasonogram shows a medially placed cystic mass with layered fluids of different density consistent with purulent material. The position of the mass is most consistent with the renal pelvis. **B,** Intravenous pyelogram confirms ureteropelvic junction obstruction. This infant was treated initially with percutaneous nephrostomy drainage in addition to appropriate antibiotics.

infections accompanied by severe ureteropelvic junction obstruction or infundibular stenosis with isolated calyceal dilation, in which case purulent material within the dilated collecting system layers out and can mimic a renal abscess (Figs. 40-3 and 40-4). Nephromegaly on ultrasonography also has been found to be a clue to recognizing a renal abscess with subsequent CT imaging studies.[9]

When the diagnosis of an abscess is suspected on ultrasonography, performing CT of the involved kidney is in order.[11] CT more clearly defines the margins of the abscess, assesses whether loss of function is significant, and can screen the remainder of the kidney for small satellite abscesses.[20] If loss of function in the affected kidney seems to be significant, a dimercaptosuccinic acid renal scan should be done because it is an even more sensitive test to quantitate overall renal function. If CT suggests the diagnosis, percutaneous aspiration can confirm clearly whether an abscess is present, without having to perform additional studies, and, at the same time, provide material for culture.

If a child with a urinary tract infection and a previously normal result on renal ultrasonogram is taking culture-specific antibiotics and a new fever subsequently develops, ultrasonography should be repeated. A previously small, unrecognized abscess may have been treated inadequately and now may be more apparent.

Occasionally, imaging studies cannot distinguish between a renal abscess and severe acute lobar nephropathy. The latter condition also may show tissues of differing density by ultrasonography or CT. In these situations, ultrasonography-guided or CT-guided percutaneous aspiration of the lesion may be necessary to determine whether purulent material can be obtained.

THERAPEUTIC CONSIDERATIONS

When the diagnosis of an abscess is confirmed, the choice of therapeutic options is dictated by several factors, including the overall status of the patient, the size and number of abscesses, whether the abscess appears to be unilocular or septate, associated uropathies, and the extent of renal function in the involved kidney. Included in these considerations is some insight into the responsible organism. If it is a solitary abscess in a child with unimpressive urinalysis findings and an otherwise normal kidney, one should suspect a staphylococcal abscess, which can be confirmed by percutaneous aspiration, Gram stain, and culture. If bacteremia caused by *S. aureus* possibly was nosocomial in origin, or if methicillin-resistant *Staphylococcus aureus* isolates are found commonly in the community, antibiotics such as vancomycin or clindamycin should be included in the initial empirical regimen.

Subsequent therapy is based on the antibiotic susceptibility pattern of the organism isolated. If urinalysis reveals an obvious infection, one can assume that the urine culture reflects the organism responsible for the abscess. In this situation, empirical therapy is directed against gram-negative enteric organisms. Cefotaxime, ceftriaxone, or ceftazidime plus an aminoglycoside would be suggested. The only caution is if the abscess is complicated by significant stone disease. In this situation, more than one organism may be in the urinary tract, and urine culture alone may be misleading. Percutaneous aspiration can identify accurately the organism responsible for the abscess. The optimal duration of antibiotic therapy for treatment of a renal abscess is not established,

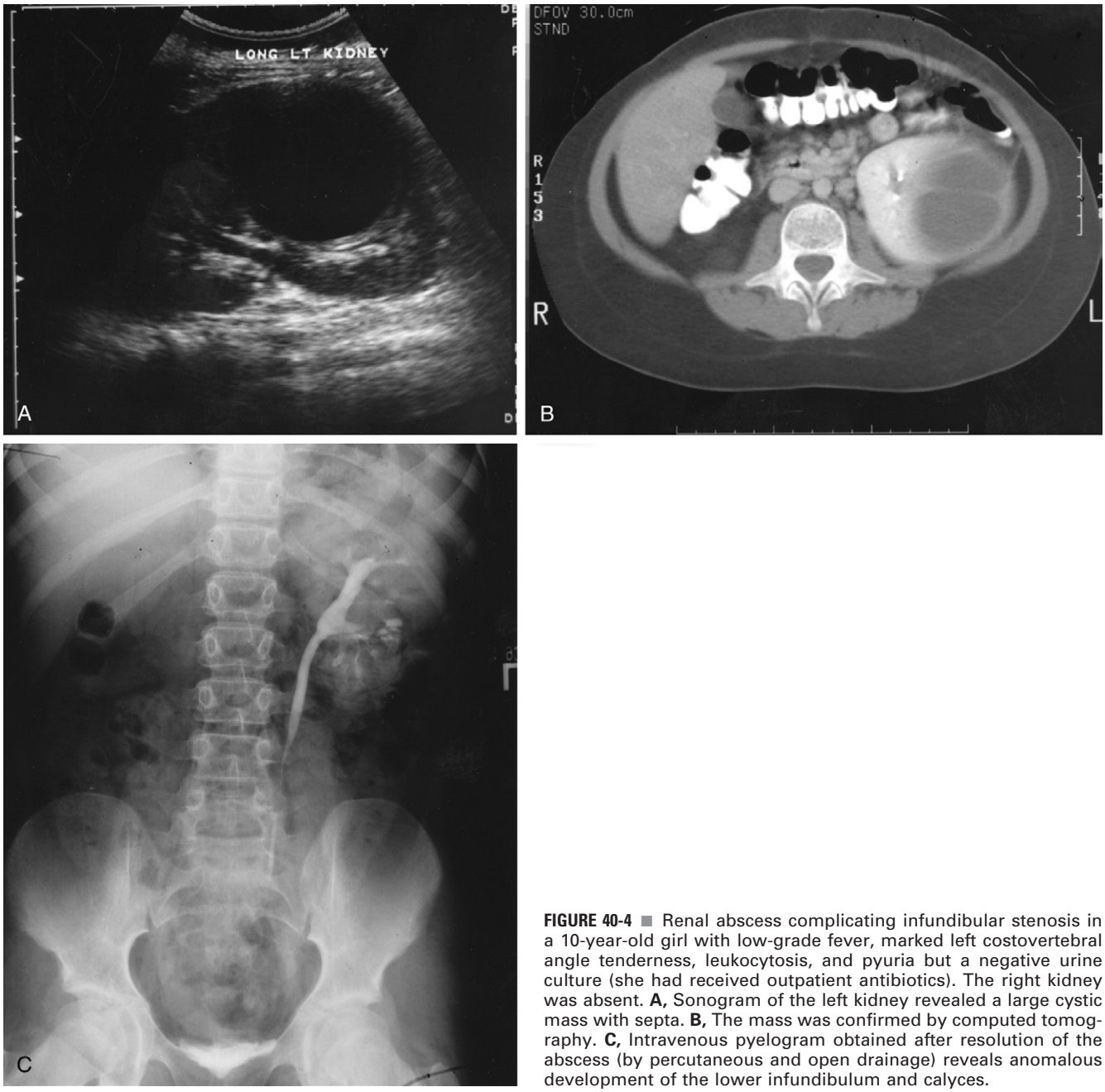

FIGURE 40-4 ■ Renal abscess complicating infundibular stenosis in a 10-year-old girl with low-grade fever, marked left costovertebral angle tenderness, leukocytosis, and pyuria but a negative urine culture (she had received outpatient antibiotics). The right kidney was absent. **A,** Sonogram of the left kidney revealed a large cystic mass with septa. **B,** The mass was confirmed by computed tomography. **C,** Intravenous pyelogram obtained after resolution of the abscess (by percutaneous and open drainage) reveals anomalous development of the lower infundibulum and calyces.

but 3 weeks of treatment is superior to 2 weeks for acute lobar nephropathy.[6]

Renal abscesses traditionally have been managed by open surgical drainage. With the introduction of safe percutaneous access, particularly when used with ultrasonography or CT guidance, single, unilocular lesions now can be managed effectively with percutaneous placement of an indwelling catheter that allows not only for primary drainage but also for irrigation of the cavity with appropriate antibiotic solutions.[1,13] Small renal abscesses have been treated successfully with antibiotics alone.[15,22] If the child does not improve with medical therapy alone, some type of drainage procedure is indicated. When function is insufficient to justify renal salvage and ultimate nephrectomy is planned, a short course of specific antibiotics and percutaneous drainage is initiated, if the abscess is large, to decrease inflammation in the perinephric tissues and to reduce the possibility of causing bacteremia at the time of surgery.

When required, surgical repair of associated congenital anomalies of the urinary tract generally is performed at a separate session after the abscess has resolved. Reconstructive surgery in the presence of active infection significantly increases the risk for development of surgical complications and the possibility that additional surgical procedures might be required.

CONCLUSION

Treatment of renal abscess today is multifaceted, and each case must be individualized. In addition to culture-specific antibiotics, treatment may include observation only, percutaneous drainage, or open surgical drainage. Diagnostic evaluation, including renal ultrasonography, CT, and percutaneous aspiration, generally can confirm that an abscess is present and assist in the decision regarding therapeutic options.

NEW REFERENCES SINCE THE SIXTH EDITION

7. Cheng CH, Tsau YK, Lin TY. Is acute lobar nephronia the midpoint in the spectrum of upper urinary tract infections between acute pyelonephritis and renal abscess? *J Pediatr* 2010;**156**:82–6.
9. Cheng CH, Tsai MH, Su LH, et al. Renal abscess in children: a ten-year clinical and radiographic experience in a tertiary medical center. *Pediatr Infect Dis J* 2008;**27**:1025–7.
17. Salehi N, Custodio H, Rathore MH. Renal microabscesses due to Bartonella infection. *Pediatr Infect Dis J* 2010;**29**:472–3.
18. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 41

Prostatitis

Sheldon L. Kaplan

Prostatitis, a major source of chronic infection and symptoms in men, occurs rarely in prepubertal children. Although prostatitis may develop in postpubertal teenagers, even in this age group the diagnosis is recognized infrequently. The disorder commonly termed *prostatitis* generally is divided into four separate clinical problems based on the National Institutes of Health Classification of prostatitis: acute bacterial prostatitis, chronic bacterial prostatitis, chronic prostatitis and chronic pelvic pain syndrome, and asymptomatic inflammatory prostatitis.[9] This chapter will focus on acute or chronic bacterial prostatitis.

Acute prostatitis is a severe infection generally associated with significant toxicity (high fever, systemic symptoms such as malaise, chills, nausea, and vomiting).[19] Marked urinary symptoms, such as frequency, suprapubic pain, dysuria, hematuria, and urinary retention, may be present. On rectal examination, the prostate is enlarged, boggy (edematous), and exquisitely tender. The urine usually is infected. Care must be taken when performing the rectal examination or when inserting a transurethral catheter because bacteremia can result. The white blood cell count and inflammatory markers are usually elevated. Urinalysis is abnormal and urine culture is positive in the majority of cases. Bacteremia has been reported in up to 20 percent of patients and blood cultures may be positive when urine cultures are negative.[5]

The organism responsible usually is one of the gram-negative pathogens that commonly causes urinary tract infection (UTI). *Escherichia coli* is most commonly isolated, but other pathogens include *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella* and *Enterococcus* spp., among others.[3] *Neisseria gonorrhea* is a consideration in sexually active males. Community-acquired methicillin-resistant *Staphylococcus aureus* has been isolated, rarely, from patients with a prostatic abscess.[18] The virulence factors of *E. coli* strains recovered from men with acute prostatitis and women with UTIs, such as adhesins and cytotoxins, were similar in one study.[15] Johnson and colleagues[7] found, however, that prostatitis *E. coli* isolates differed significantly from *E. coli* associated with cystitis or pyelonephritis by having a higher prevalence of the genes encoding P fimbriae structural subunits, P fimbriae assembly, fimbriae tip pilins, hemolysin, and cytotoxic necrotizing factor and a lower prevalence of the gene encoding invasion of brain endothelium virulence factor. In a study of *E. coli*, isolates from 18 previously healthy young men with acute bacterial prostatitis, Krieger and colleagues[10] reported that most isolates fell within the family of considered extraintestinal pathogenic *E. coli* (ExPEC) and carried a median of 12.5 virulence genes, characteristic of ExPEC gene clusters. Rarely, *Trichomonas vaginalis* can cause prostatitis.

If the patient is not systemically ill, in adults a quinolone, such as ciprofloxacin or levofloxacin, or

REFERENCES

1. Barker AP, Ahmed S. Renal abscess in childhood. *Aust N Z J Surg* 1991;**61**:217–21.
2. Boam WD, Miser WF. Acute focal bacterial pyelonephritis. *Am Fam Physician* 1995;**52**:919–24.
3. Brandels JM, Baskin LS, Kogan BA, et al. Recurrent *Staphylococcus aureus* renal abscess in a child positive for the human immunodeficiency virus. *Urology* 1995;**46**:246–8.
4. Brook I. The role of anaerobic bacteria in perinephric and renal abscesses in children. *Pediatrics* 1994;**92**:261–4.
5. Casullo VA, Bottone E, Herold BC. *Peptostreptococcus asaccharolyticus* renal abscess: a rare cause of fever of unknown origin. *Pediatrics* 2001;**107**:E11.
6. Cheng CH, Tsau YK, Lin TY. Effective duration of antimicrobial therapy for the treatment of acute lobar nephronia. *Pediatrics* 2006;**117**:e84–9.
7. Cheng CH, Tsau YK, Lin TY. Is acute lobar nephronia the midpoint in the spectrum of upper urinary tract infections between acute pyelonephritis and renal abscess? *J Pediatr* 2010; **156**:82–6.
8. Cheng CH, Tsau YK, Hsu SY, Lee TL. Effective ultrasonographic predictor for the diagnosis of acute lobar nephronia. *Pediatr Infect Dis J* 2004;**23**:11–4.
9. Cheng CH, Tsai MH, Su LH, et al. Renal abscess in children: a ten-year clinical and radiographic experience in a tertiary medical center. *Pediatr Infect Dis J* 2008;**27**:1025–7.
10. Dougherty FE, Gottlieb RP, Gross GW, Denison MR. Neonatal renal abscess caused by *Staphylococcus aureus*. *Pediatr Infect Dis J* 1991;**10**:463.
11. Gerzof SG, Gale ME. Computed tomography and ultrasonography for diagnosis and treatment of renal and retroperitoneal abscesses. *Urol Clin North Am* 1982;**9**:185–93.
12. Higham M, Santos JI, Grodin M, Klein JO. Renal abscess without preexisting structural abnormality. *Pediatr Infect Dis J* 1984;**3**: 138–41.
13. Klar A, Hurvitz H, Berkun Y, et al. Focal bacterial nephritis (lobar nephronia) in children. *J Pediatr* 1996;**128**:850–3.
14. Martinez-Jabalonas J, Osca JM, Ruiz JL, et al. Renal aspergillosis and AIDS. *Eur Urol* 1995;**27**:167–9.
15. Pedersen JF, Hancke S, Kristensen JV. Renal carbuncle: antibiotic therapy governed by ultrasonically guided aspiration. *J Urol* 1973; **109**:777–8.
16. Rote AR, Bauer SB, Retik AB. Renal abscess in children. *J Urol* 1978;**119**:254–8.
17. Salehi N, Custodio H, Rathore MH. Renal microabscesses due to Bartonella infection. *Pediatr Infect Dis J* 2010;**29**:472–3.
18. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.
19. Schiff M, Glickman M, Weiss RM, et al. Antibiotic treatment of renal carbuncle. *Ann Intern Med* 1977;**8**:305.
20. Thornbury JR. Acute renal infections. *Urol Radiol* 1991;**12**:209–13.
21. Timmons JW, Perlmutter AD. Renal abscess: a changing concept. *J Urol* 1976;**115**:299–301.
22. Vachvanichsanong P, Dissaneewate P, Patrapinyokul S, et al. Renal abscess in healthy children: report of three cases. *Pediatr Nephrol* 1992;**6**:273–5.
23. Watson AR, Marsden HB, Cendon M, et al. Renal pseudotumors caused by xanthogranulomatous pyelonephritis. *Arch Dis Child* 1982;**57**:635.
24. Wippermann CF, Schofer O, Beetz R, et al. Renal abscess in childhood: diagnostic and therapeutic progress. *Pediatr Infect Dis J* 1991;**10**:446–50.

trimethoprim-sulfamethoxazole is generally recommended because these agents have been more carefully studied for this indication than oral cephalosporins.[12] These same agents also are reasonable for male adolescents. Modification of antibiotic therapy is based on the organism isolated and its antibiotic susceptibilities. The duration of treatment is generally 14 to 28 days. If the patient is more seriously ill, parenteral therapy is recommended. Empirical treatment generally begins with administration of broad-spectrum β-lactam agents, such as cefotaxime or piperacillin-tazobactam, possibly combined with an aminoglycoside, that are effective against gram-negative enteric organisms, pending the availability of culture-proven sensitivity results and a satisfactory clinical response. Once the patient has clearly improved and is stable, therapy can be completed with an oral agent such as a fluoroquinolone or trimethoprim-sulfamethoxazole based on susceptibility results.

Chronic bacterial prostatitis is a more indolent infection associated with intermittent UTI, bladder irritative symptoms, and perineal discomfort. Recurrent urethritis or epididymitis may be present. Ejaculation may be painful. Expressed prostatic secretions usually show an increase in the white blood cell count. Common organisms associated with chronic bacterial prostatitis are similar to those causing acute bacterial prostatitis, including *Klebsiella* spp., *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *Enterococcus faecalis*.[19] *E. coli* isolates associated with chronic prostatitis possess urovirulence profiles similar to those of strains from women with acute uncomplicated pyelonephritis.[1]

The diagnosis of chronic bacterial prostatitis is related partly to detecting inflammation in expressed prostatic secretions. More precise methods of determining inflammatory parameters, such as counting the white blood cells per cubic millimeter with a hemocytometer, have been proposed.[16] In a large cohort study, the severity of symptoms in men with chronic prostatitis did not correlate with leukocyte or bacterial counts, however.[20]

Chronic bacterial prostatitis accounts for only a small fraction of men with chronic prostatitis syndromes. The cause of nonbacterial chronic prostatitis and chronic pelvic pain syndrome remains enigmatic. Prostatic secretions do not show a common organism consistently, and UTI does not recur. Culture of prostatic secretions is notoriously unreliable, because of the means by which this material is collected; these secretions are contaminated easily as they pass through the urethra.[14] A specific causal relationship in nonbacterial prostatitis has not been found for either *Ureaplasma urealyticum* or *Chlamydia trachomatis*, two organisms commonly implicated in urethritis.[21] Molecular studies using polymerase chain reaction have shown DNA evidence of the presence of bacteria despite negative cultures for the typical bacteria associated with prostatitis. Using these new techniques may lead to greater understanding of the role of infection in the pathogenesis of prostatitis.

Treatment of chronic bacterial prostatitis is frustrating because few patients truly are cured, and relapse occurs commonly after discontinuation of antibiotic therapy. Several studies have confirmed satisfactory concentrations of most common antibiotics in prostatic tissues,[6,8,11,13] but no study has provided a convincing explanation for the high treatment failure rate. Levofloxacin and other fluoroquinolones penetrate well into seminal and prostatic fluids and reach concentrations equivalent to corresponding plasma levels.[4] Researchers have postulated that antibiotic concentrations may be inadequate within the acini of the prostatic glands and their secretions, but this theory has not been proved.[17] Typically, fluoroquinolones for 4 to 6 weeks of administration are the agents of choice for treating chronic bacterial prostatitis.[12]

In the older age group still seen in a pediatric practice, symptoms similar to those of the chronic form of prostatitis also might occur with urethritis. As noted earlier, *Chlamydia* and *Ureaplasma* spp. commonly are found in the urethral discharge in these patients[2] and are thought to be responsible for these specific symptoms. These organisms also have been implicated as a cause of epididymitis in young men. The organisms are transmitted sexually and are treated effectively with a tetracycline (minocycline or doxycycline) or a macrolide (erythromycin or azithromycin).[22] To prescribe these drugs empirically for sexually active young adults with lower tract irritative symptoms and clinical findings consistent with urethritis while awaiting culture results seems reasonable.

Nonspecific urethritis is the term commonly used for the infection in men with irritative voiding symptoms and a clear mucoid discharge and in whom culture of the discharge reveals a mixture of common perineal and gram-negative organisms but without a predominant organism thought to be responsible for the infection. Some of these patients likely have undiagnosed *Chlamydia* or *Ureaplasma* infection. Although specific treatment is empirical, a tetracycline or macrolide seems to be a prudent first choice in most cases. Finally, one must not overlook the possibility of gonococcal urethritis.

In males, ectopic ureters can drain into the prostatic urethra or the seminal vesicle. With this anomaly, the ipsilateral renal parenchyma generally is dysplastic, the ureter is highly dilated, and the seminal vesicle may be distended markedly and form a large cystlike structure behind the prostate, the bladder neck, and the trigone. If this anomaly becomes infected, pain in the perineum and on rectal examination generally is significant. Occasionally, frankly purulent material is passed at the urethral meatus or expressed from the urethra after rectal examination.

In most cases, the kidney drained by the ectopic ureter is highly dysplastic and shows little or no function. Ultimate management usually consists of nephroureterectomy (ureteroneocystostomy if the kidney works) and, if the seminal vesicle is unusually large, partial excision and decompression of the seminal vesicle. Although these abnormalities are congenital and easily recognized by fetal ultrasonography, they can occur at almost any age if not identified before birth. Unless a palpable flank or lower abdominal mass is felt, infection usually is the initial symptom. Despite the severe degree of abnormality and ureteral and seminal vesicle dilation, the development of infection can be delayed well into the adult years. Infrequently, distortion at the bladder neck can result in obstruction to urinary flow.

SUMMARY

Prostatitis as it is seen and described in adults is a rare event in pediatric patients. When it does occur, it affects pubertal adolescents. Even in these patients, distinguishing true prostatitis from simple urethritis may be difficult.

Appropriate evaluation of an adolescent boy with a lower tract urinary infection generally begins with renal ultrasonography. Special attention should be directed to the region of the bladder and prostate. With this imaging modality, the presence and normalcy of both kidneys, the degree of thickening of the bladder wall, the ability of the bladder to empty, and whether any cystic masses are located behind the bladder or prostate can be assessed. Any obvious abnormality justifies obtaining a voiding cystourethrogram. A thickened detrusor or recurrence of infection suggests the possibility of urethral obstruction. Although properly performed voiding cystourethrography does image the entire urethra, other diagnostic tests might include retrograde urethrography or determination of the urinary flow rate, especially if a urethral stricture is suspected. Performing cystoscopy usually is unnecessary if all imaging study results are normal, although cystoscopy may be used to confirm, and sometimes manage, obvious urethral obstruction.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Brede CM, Shoskes DA. The etiology and management of acute prostatitis. *Nat Rev Urol* 2011;**8**:207–12.
5. Etienne M, Pestel-Caron M, Chapuzet C, et al. Should blood cultures be performed for patients with acute prostatitis? *J Clin Microbiol* 2010;**48**:1935–8.
9. Krieger JN, Nyberg L, Nickel JC. NIH consensus definition and classification of prostatitis. *JAMA* 1999;**282**:236–7.
10. Krieger JN, Dobrindt U, Riley DE, Oswald E. Acute *Escherichia coli* prostatitis in previously healthy young men: bacterial virulence factors, antimicrobial resistance, and clinical outcomes. *Urology* 2011;**77**:1420–5.
12. Lipsky BA, Byren I, Hoey CT. Treatment of bacterial prostatitis. *Clin Infect Dis* 2010;**50**:1641–52.
18. Park SC, Lee JW, Rim JS. Prostatic abscess caused by community-acquired methicillin-resistant *Staphylococcus aureus*. *Int J Urol* 2011;**18**:536–9.
19. Rothman JR, Jaffe WI. Prostatitis: updates on diagnostic evaluation. *Curr Urol Report* 2007;**8**:301–6.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 42

Genital Infections

Mariam R. Chacko • Mary Allen Staat • Charles R. Woods, Jr

This chapter reviews female genital tract infections in the pediatric and adolescent age groups. An initial review of normal vaginal microenvironment in the prepubertal, pubertal, and postpubertal period is followed by a section on lower genital tract infection in the prepubertal, pubertal and postpubertal period. This section addresses infections of the external genital area, including vulvovaginitis; infections of the clitoris, urethra, and Bartholin ducts and glands; vulvovaginal lesions; ulcerative and granulomatous disorders; and infections of the cervix. Upper genital tract infections are rare in the prepubertal age group; thus, discussion of pelvic inflammatory disease (PID), tubo-ovarian abscess, and oophoritis, which follows, is predominantly addressed for the postpubertal period. Additional information on the microbes and the specific detailed treatment recommendations can be found in the respective chapters on each pathogen. Genital infections associated with pregnancy are not addressed in this chapter.

Supplemental information concerning the principles of gynecologic infections in children and adolescents can be found in referenced sources.[8,59,102,104]

GENERAL APPROACH TO EVALUATION OF PREPUBERTAL CHILD

When genital tract symptoms are present in young girls, the history should include information about the manner and circumstances surrounding the onset of the complaint, the characteristics of any discharge, the duration of symptoms, whether the problem is recurrent, and the nature of any recent treatments or medications. Information should be elicited regarding the use of bubble baths,

REFERENCES

1. Andreu A, Stapleton AE, Fennell C, et al. Urovirulence determinants in *Escherichia coli* strains causing prostatitis. *J Infect Dis* 1997;**176**:464–9.
2. Bowie WR, Wang SP, Alexander ER, et al. Etiology of nongonococcal urethritis: evidence for *C. trachomatis* and *U. urealyticum*. *J Clin Invest* 1977;**59**:735–42.
3. Brede CM, Shoskes DA. The etiology and management of acute prostatitis. *Nat Rev Urol* 2011;**8**:207–12.
4. Bundrick W, Heron SP, Ray P, et al. Levofloxacin versus ciprofloxacin in the treatment of chronic bacterial prostatitis: a randomized double-blind multicenter study. *Urology* 2003;**62**:537–41.
5. Etienne M, Pestel-Caron M, Chapuzet C, et al. Should blood cultures be performed for patients with acute prostatitis? *J Clin Microbiol* 2010;**48**:1935–8.
6. Hensle TW, Prout Jr GR, Griffin P. Minocycline diffusion into benign prostatic hyperplasia. *J Urol* 1977;**118**:609–11.
7. Johnson JR, Kuskowski MA, Gajewski A, et al. Extended virulence genotypes and phylogenetic background of *Escherichia coli* isolates from patients with cystitis, pyelonephritis, or prostatitis. *J Infect Dis* 2005;**191**:46–50.
8. Krieger JN, Riley DE. Prostatitis: what is the role of infection? *Int J Antimicrob Agents* 2002;**19**:475–9.
9. Krieger JN, Nyberg L, Nickel JC. NIH consensus definition and classification of prostatitis. *JAMA* 1999;**282**:236–7.
10. Krieger JN, Dobrindt U, Riley DE, Oswald E. Acute *Escherichia coli* prostatitis in previously healthy young men: bacterial virulence factors, antimicrobial resistance, and clinical outcomes. *Urology* 2011;**77**:1420–5.
11. Larsen EH, Grasser TC, Dorflinger T, et al. The concentration of various quinolone derivatives in the human prostate. In: Weidner W, Brunner H, Krause W, et al, editors. *Therapy of Prostatitis*. Munich: W. Zucksweidt Verlag; 1986. p. 40–4.
12. Lipsky BA, Byren I, Hoey CT. Treatment of bacterial prostatitis. *Clin Infect Dis* 2010;**50**:1641–52.
13. Meares Jr EM. Prostatitis: review of pharmacokinetics and therapy. *Rev Infect Dis* 1982;**4**:475–83.
14. Meares Jr EM, Stamey TA. Bacteriologic localization patterns in bacterial prostatitis and urethritis. *Invest Urol* 1968;**5**:492–518.
15. Mitsumori K, Terai A, Yamamoto S, et al. Virulence characteristics of *Escherichia coli* in acute bacterial prostatitis. *J Infect Dis* 1999; **180**:1378–81.
16. Muller CH, Berger RE, Mohr LE, Krieger JN. Comparison of microscopic methods for detecting inflammation in expressed prostatic secretions. *J Urol* 2001;**166**:2518–24.
17. Nielsen OS, Frimodt-Moeller N, Maiqaard S, et al. Penicillanic acid derivatives in the canine prostate. *Prostate* 1980;**1**:79–85.
18. Park SC, Lee JW, Rim JS. Prostatic abscess caused by community-acquired methicillin-resistant *Staphylococcus aureus*. *Int J Urol* 2011;**18**:536–9.
19. Rothman JR, Jaffe WI. Prostatitis: updates on diagnostic evaluation. *Curr Urol Rep* 2007;**8**:301–6.
20. Schaeffer AJ, Knauss JS, Landis JR, et al. Leukocyte and bacterial counts do not correlate with severity of symptoms in men with chronic prostatitis. The National Institutes of Health Chronic Prostatitis Cohort Study. *J Urol* 2002;**168**:1048–53.
21. Shortliffe LM, Wehner N. The characterization of bacterial and non-bacterial prostatitis by prostatic immunoglobulins. *Medicine (Baltimore)* 1986;**65**:399–414.
22. Skerk V, Markekovic I, Markovinovic L, et al. Comparative randomized study of azithromycin and doxycycline efficacy and tolerability in the treatment of prostate infection caused by *Ureaplasma urealyticum*. *Chemotherapy* 2006;**52**:9–11.

enuresis, history of atopic dermatitis or allergies, anal pruritus (associated with pinworm infection), recent respiratory or skin infections in family members, and hygienic practices. The possibility that the child may have had or has a systemic disorder affecting the genitalia should be explored.

Sexual abuse also must be considered when a child has a genital infection, regardless of the nature of the infection or the socioeconomic status of the family. This concern is particularly relevant when the child has a sexually transmissible disease. Sexual abuse does not require penile penetration of the vagina. The history of vaginal or penile discharge in other family members should be ascertained. Behavioral changes, nightmares, fears, abdominal pain, headaches, and enuresis can be indicators of abuse or other psychosocial stressors. The possibility of nonsexual transmission of these diseases often is raised, particularly when a preliminary investigation cannot elicit a history of sexual abuse. The rate of eliciting evidence of sexual abuse in prepubertal children can be 67 percent, however, in children with sexually transmitted infections (STIs).[12] Based on a review of the literature, common STIs in premenarcheal children and their potential mode of transmission are also summarized.

A general physical examination should precede assessment of the genital tract. The gynecologic examination begins with abdominal inspection and palpation followed by external examination of the perineum and genitalia, including the vulva, urethral meatus, clitoris, hymen, and anus. The examination should be done with the child in the supine, frog-leg position. The labia can be retracted gently to visualize the anterior of the vagina. Speculum and bimanual examinations generally are inappropriate in prepubertal children.[57,160] Note should be made of structural abnormalities, inflammation, sores and ulcerations, and excoriations. If complaints consist only of vulvitis, and findings on external examination are limited to a scanty mucoid discharge and an erythematous introitus, further examination generally is unnecessary.[58]

If the vaginal discharge is purulent, persistent, or recurrent, a thorough gynecologic assessment is warranted. Visualization of the vagina and cervix without instrumentation is possible with the child in the prone knee-chest position.[57] This method is useful in children older than 2 years. In this position, with labial traction, the vaginal muscles relax and stretch the hymenal membrane open. An otoscope head or a magnifying lens with a good wall light is used to visualize the cervix. Because the vagina of a prepubertal child is short, a foreign body or a lesion may be seen.[57]

A rectal examination may be important when persistent discharge, bleeding, or pelvic or abdominal pain is present. The rectal examination may help express discharge from the vagina that previously was not recognized and can permit palpation of hard foreign bodies or abnormal masses.

Visualization with instrumentation (vaginoscopy) is required in some situations. General anesthesia may be required for girls who are small or unable to cooperate or who may experience significant anxiety or discomfort during the procedure. Conscious sedation for the procedure may be an option in some situations. When a purulent, persistent, or recurrent vaginal discharge is present, samples should be obtained for culture and Gram stain. Wet mount preparations may be useful if fungal infection (potassium hydroxide preparations) or pinworms are suspected. Saline wet mount may be indicated for trichomoniasis if sexual abuse is suspected. Urinalysis with microscopic examination should be performed. A complete blood cell count may be useful when pyogenic infection is suspected or bleeding has occurred.

When cultures are indicated, separate vulvar and vaginal specimens may be necessary. The type and duration of discharge and considerations of potential sexual abuse influence this decision. Vulvar specimens alone may be appropriate when etiologies such as group A streptococci are suspected. Exudates emanating from the vagina in prepubertal girls also are adequate specimens for evaluation for gonorrhea, such that vaginal swabs or aspirates are not required. Further information on types of specimens required for specific sexually transmitted infections such as gonorrhea or *Chlamydia trachomatis* is provided in Chapters 89 (gonorrhea) and 193 (*C. trachomatis*).

Options for obtaining vaginal specimens in young girls, when needed, include use of (1) a nasopharyngeal Dacron swab moistened in nonbactericidal saline, inserted carefully through the hymenal opening; (2) a catheter-within-a-catheter technique (Fig. 42-1)[160]; or (3) a sterile newborn suction catheter, with insertion 2 to 3 cm into the vagina.[202] For the catheter-within-a-catheter technique, the distal 4 inches of a soft, size 12 bladder catheter and the proximal 4 inches of butterfly needle intravenous tubing are excised from their parent devices by sterile technique. The catheter-within-a-catheter is inserted into the vagina, and fluid is flushed in and out of the upper part of the vagina several times before final aspiration into the syringe and removal of the device.

NORMAL VAGINAL FLORA

The lower female genital tract is colonized by nonpathogenic bacteria from birth. Throughout life, this colonization is dynamic and complex. A wide range of aerobic and

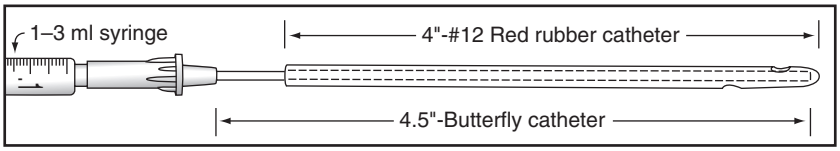

FIGURE 42-1 ■ Assembled catheter-within-a-catheter for obtaining specimens from a prepubertal child. (From Pokorny SF, Stormer J. A traumatic removal of secretions from the prepubertal vagina. *Am J Obstet Gynecol* 1987;**156**:581.)

TABLE 42-1 **Vaginal Organisms Isolated from Asymptomatic Girls 2 Months to 16 Years of Age**

Organism	%*
Coagulase-negative staphylococci	35-73
Diphtheroids	14-78
Streptococcus viridans	13-39
Enterococci	29-62
Group B streptococcus	5-11
Group D streptococcus	
Staphylococcus epidermidis	
Staphylococcus aureus	
Streptococcus pneumoniae	
Micrococcus spp.	
Gaffkya (Aerococcus) spp.	
Lactobacillus spp.	10-39†
Escherichia coli	12-67
Klebsiella spp.	15-52
Enterobacter spp.	
Proteus spp.	3-5
Pseudomonas aeruginosa	5-6.5
Citrobacter spp.	
Haemophilus influenzae	
Neisseria spp. other than gonococci	
Moraxella (Branhamella) catarrhalis	
Flavobacterium spp.	
Alcaligenes spp.	
Acinetobacter spp.	
Mycoplasma hominis	
Ureaplasma urealyticum	
Gardnerella vaginalis‡	
Peptostreptococcus spp.	29-56
Peptococcus spp.	39-76
Veillonella spp.	
Eubacterium spp.	
Propionibacterium acnes	
Bacteroides fragilis	
Bacteroides melaninogenicus	
Other *Bacteroides* spp.	
Prevotella spp.	
Bifidobacterium spp.	
Clostridium perfringens	
Other *Clostridium* spp.	
Fusobacterium spp.	
Candida spp.	3-18
Other yeasts	
Actinomyces spp.	

Data from references 37, 58, 73, 87, 94, 99, 126, 127.
*Percentage range when the organism was isolated from patients in at least two studies. If no range is present, the organism was isolated from 3% to 33% of patients in a single study.
†In one series, 88% of girls were older than 11 years.
‡Isolated in numerous cases without discharge.

anaerobic species have been cultured from asymptomatic girls. The results of several modern series are summarized in Table 42-1. The vagina and its microbial flora form an ecosystem that changes over time from infancy to childhood to adolescence and adulthood.[99] The major forces that influence these changes are fluctuations in estrogen levels and the advent of sexual activity. Hygienic practices and medications, including oral contraceptives and antimicrobial agents, also affect the complex interactions among the various flora present in the vagina in terms of persistence, predominance, and overgrowth.

Most girls harbor several organisms in the vagina at any given time. Younger adolescents have a greater prevalence of anaerobic bacteria than do women. From puberty, aerobic colonization increases with age, onset of sexual activity, and parity.[127] Vaginal specimens obtained for culture during anesthesia for elective surgery from 19 healthy girls aged 3 months to 5.7 years old yielded a mean of 12 bacterial species.[98] Anaerobes predominated, with a mean of 8.7 species versus 3.4 aerobic species. In a series of 25 asymptomatic girls aged 2 months to 15 years, a mean of 8.7 different species (approximately four aerobes and five anaerobes) per vaginal specimen were detected.[87] Another series of adolescents and young adults aged 13 to 21 years in which only aerobic flora were evaluated noted a mean of approximately three organisms in non–sexually active subjects versus six in sexually active patients.[186] This heterogeneity in vaginal microflora during childhood and adolescence is similar to that found in women.[126]

Gram-negative enteric bacteria and enterococci commonly are encountered in infants and toddlers before completion of toilet training, but less frequently thereafter.[87,98] In children, lactobacilli are present more often in girls younger than 2 years than in older prepubertal girls. Lactobacilli are the predominant flora in most girls by the end of adolescence and may play a protective role in limiting the overgrowth of other flora.[99] The vaginal flora of most healthy women is dominated by one or more *Lactobacillus* spp., but a substantial minority may harbor other predominant flora, including *Bifidobacterium*, *Gardnerella*, *Prevotella*, *Pseudomonas*, or *Streptococcus*, in the absence of lactobacilli.[105]

The increasing presence of yeast and *Gardnerella vaginalis* from puberty to adulthood, although much less common in all ages, parallels that of lactobacilli.[87] *Mycoplasma hominis* and *Ureaplasma urealyticum* are present more commonly in sexually active adolescents and in girls of any age who have been sexually abused than in non–sexually active girls. Genital mycoplasmas were found in 17 percent of one series of young girls who were not known to have undergone any sexual abuse.[90,186]

Microbes that usually behave as commensals sometimes are associated with vulvovaginitis. The difference between colonization and disease is at least partially a function of the magnitude of the replication and the quantity of a given bacterial species. For example, in women with bacterial vaginosis in which *G. vaginalis* is a predominant microbe, colony counts generally are greater than 10^7 colony-forming units/g of vaginal fluid. Asymptomatic colonization with *G. vaginalis* usually is associated with colony counts of less than 10^5 colony-forming units/g of vaginal fluid.[127] Alteration in the vaginal microenvironment by factors such as poor hygiene, foreign bodies, or hormonal fluctuations results in loss of environmental constraints on bacterial replication and facilitates the overgrowth of one or more commensals.

LOWER GENITAL TRACT INFECTIONS

This section includes the following topics according to anatomic areas: vulvovaginitis; infections of the clitoris, urethra, and vulvar glands; genital lesions, ulcerations, and granulomatous infections and cervicitis. Each topic addresses the prepubertal and postpubertal aspects of infections where relevant.

Vulvovaginitis

Prepubertal

Infections and inflammation of the vulva and vagina account for 85 to 90 percent of all genital problems in prepubertal girls. These conditions are encountered most commonly in children 2 to 7 years old.[159,171] Infections of the vulva and vagina usually occur together and generally are considered one entity—vulvovaginitis. The various types of vulvovaginitis are differentiated by determining the presence or absence of specific microbial or other agents associated with the inflammation in a particular case.

Genital discharge does not always indicate infection or inflammation. Most female infants have a grayish white, mucoid discharge from the vagina during the newborn period. This discharge consists of desquamated vaginal mucosa and cervical epithelium that has undergone hypertrophy because of prenatal stimulation by placental and maternal hormones. Microscopic examination of the material reveals masses of large, superficial vaginal epithelial cells (Fig. 42-2). The condition may last for several weeks, is not pathogenic, and does not require treatment.

Similarly, a pubertal girl nearing menarche may develop a copious viscous, transparent secretion that exudes from the vagina. This normal state sometimes raises concern about possible vaginal infection. The vulvar and vaginal tissues appear thick and moist, a sign of increased estrogen stimulation, without erythema. Microscopic examination of the vaginal fluid reveals masses of estrogenized superficial vaginal epithelial cells and few leukocytes (Fig. 42-3).

Genital discharge and perineal or vulvar discomfort are the most common symptoms of vulvovaginitis.[59,159,192] The discomfort may range from minor pain or soreness to intense perineal burning or pruritus. Genital erythema is the most common sign in girls, and vulvovaginitis can be found in more than 80 percent of cases. Visible discharge is present in one third of cases.[159] Infections of the vulva and vagina are more likely to be accompanied by moderate or severe inflammation and prominent discharge than is nonspecific vulvovaginitis.[112] Infection can be present at times, however, with neither discomfort nor discharge.

The characteristics of the discharge frequently do not help identify a specific cause. At the onset of an infection, the discharge is often thick, purulent, and profuse. It may become scanty and seropurulent in later stages of infection.[58] Discharges that are odorless and bloody or serosanguineous may result from noninfectious conditions, such as vulvar irritation, trauma, precocious puberty, urethral prolapse, and tumor. Vulvovaginitis caused by *Shigella* or group A streptococci also can cause bleeding, as can an eroding foreign body. Foul-smelling discharge suggests a foreign body but also may result from a necrotic tumor. Urinary leakage from an ectopic ureter opening into the genital tract may mimic a vaginal discharge. Specific diagnoses are made more often when symptoms have been present for less than 1 month.[214]

In a series of 80 prepubertal and pubertal girls aged 2 to 12 years old with vulvovaginitis and no suspicion of sexual abuse, probable bacterial pathogens were found in cultures of 36 percent. Group A streptococci, *Staphylococcus aureus*, and *Haemophilus influenzae* (non–type b) were the most common isolates.[202] In another series of 74 girls, group A streptococci and *H. influenzae* were isolated in 47 percent and 12 percent. Most of these girls had

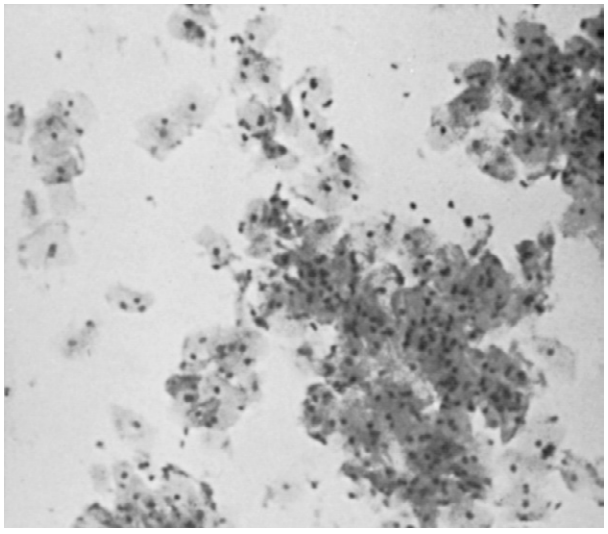

FIGURE 42-2 ■ Cytosmear from the vaginal discharge of a newborn.

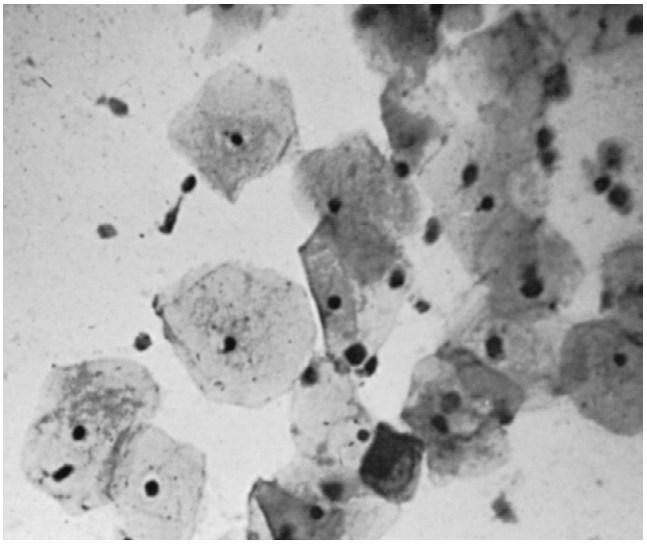

FIGURE 42-3 ■ Cytosmear from the vaginal secretion of a pubertal girl. Masses of epithelial cells and few bacteria or leukocytes attest.

symptoms for 7 days or less at the time treatment was sought.[44] These bacteria, especially group A streptococci, causing vulvovaginitis may lead to more severe symptoms that result in care being sought soon after onset, whereas pathogenic bacteria generally are less likely to be found when girls initially are examined after several weeks of symptoms.

Postpubertal

Infections of the pubertal and postpubertal vulva and vagina produce a variety of overlapping symptoms, including vulvar pruritus, dysuria, and increased or altered vaginal discharge and spotting.[226] As a result, distinguishing among various lower genital tract infections based solely on symptoms is difficult. The history, physical examination, and laboratory tests play an important role in assisting the clinician in differentiating urethritis, vaginitis, and cervicitis.

Nonspecific Vulvovaginitis

Nonspecific vulvovaginitis is a frequently encountered condition in prepubertal girls and is addressed in this section. Nonspecific vaginitis in postpubertal females is now called bacterial vaginosis and is addressed in its respective section in this chapter.

Prepubertal. Nonspecific vulvovaginitis is responsible for 25 to 75 percent of cases of vulvovaginitis diagnosed in the prepubertal age group in referral centers.[214] In most cases, as noted in Table 42-2, identifiable secondary factors contribute to nonspecific vulvovaginitis. The presence of specific genital tract bacterial or viral pathogens excludes this diagnosis.

Poor hygiene with subsequent overgrowth of a mixed aerobic and anaerobic bacterial flora that are typically nonpathogenic on mucosal surfaces is the most common cause of premenarcheal vulvovaginitis.[156] Contact irritation and allergic reactions induced by soaps, detergents, and medications are frequent causes of vulvovaginitis. Some systemic diseases and focal skin disorders may mimic vulvovaginitis or allow it to develop as a secondary process when these conditions involve the vulvar or perineal tissues.[63] Chronic vulvitis in young girls is often associated with atopic dermatitis or psoriasis.[64] Anatomic abnormalities also may be associated with vulvovaginitis. An increased incidence of vulvovaginitis and urinary tract infections has been reported in girls with high posterior commissures.[222] Drainage from an ectopic ureter into the vagina can cause chronic vulvovaginitis and lead to the formation of a vaginal calculus. Labial adhesions were present in 7 percent of girls with vulvovaginitis in one series.[159] Table 42-2 outlines the causes of vulvovaginitis in premenarcheal girls.

Several factors other than those listed in Table 42-2 contribute to the occurrence of vulvovaginal infections in young children. The developing immature labia minora and majora flare outward as a young girl squats or sits. As a result, they do not protect the vestibular and vulvar mucosae from contamination, as occurs later in life. The nonestrogenized, prepubertal vulvar and vaginal epithelium, which consists of only a few layers of cells, is traumatized easily and infected readily; however, no evidence suggests that estrogen deficiency is a causative factor in premenarcheal vulvovaginitis. The alkaline vaginal reaction during childhood also is not as resistant to infection as is the acidic vaginal secretion of postmenarcheal girls and women. Because children frequently do not cleanse themselves properly after defecation, the perineum and vulva often are contaminated by fecal material.

Vulvovaginitis Secondary to Poor Perineal Hygiene. A vulvovaginal infection is considered secondary to poor perineal hygiene when bacteria native to the lower gastrointestinal tract are found in properly obtained cultures from the vagina. In a large series of cases of vulvovaginitis subjected to culture, *Escherichia coli* or other coliform organisms were found in 70 percent of patients in the series.[94] Other studies also have found a higher prevalence of *E. coli* and other enteric organisms in vaginal cultures from girls with vulvovaginitis than in asymptomatic controls.[58] Reappearance and disappearance of premenarcheal vulvovaginitis secondary to poor perineal hygiene are related directly to the appearance and disappearance of coliform organisms in vulvovaginal cultures. The primary role of vulvar and vaginal contamination with fecal material as a result of inadequate cleansing after defecation is supported by the observation that symptoms resolve in most cases when proper perineal hygiene is the only treatment recommended. In children, 15 to 20 percent have recurrences, usually 1 month or more after resolution of the initial episode. In most instances, recurrences can be attributed to poor perineal hygiene.[94]

Children with nonspecific vulvovaginitis secondary to poor perineal hygiene do not have any uniform historical findings. On examination, the vulvar mucosa and outer third of the vagina usually are hyperemic with associated scant, light gray mucoid discharge. Many children wipe themselves from back to front after defecation. In girls, this practice easily results in fecal contamination of the vulvar area. Fecal soiling around the anus or on the perineum or in undergarments can be a clue to the etiology.

Proper cleansing of the perineum and anus after defecation and sitz baths lead to the resolution of symptoms and infection in most cases. Sitz baths (warm water with or without colloidal oatmeal or baking soda) 10 to 15 minutes in duration should be taken 2 to 6 times per day, depending on the severity of the vulvovaginal inflammation.[58] For intense inflammation, wet compresses with Burow solution (1:40) or plain water applied every 3 to 4 hours may be used instead of sitz baths.[7] In mild cases, the vulva may be washed twice per day with water or a mild, unscented, nonmedicated soap. Witch hazel pads may be used for cleansing after defecating and to provide mild analgesia.[190]

Loose-fitting clothing should be worn for several days to a few weeks after the symptoms resolve. Continued wearing of such clothing also may be helpful in preventing recurrences of vulvovaginitis, especially in warmer climates. Instructing parents regarding perineal and

TABLE 42-2 **Etiologic Factors in Premenarcheal Vulvovaginitis**

Bacterial Infections

Nonspecific mixed infections secondary to
- Poor perineal hygiene
- Foreign body in vagina
- Respiratory tract infections
- Skin infections (impetigo)
- Urinary tract infection

Specific nonvenereal infection
- Hemolytic streptococci (groups A, B, F)
- *Escherichia coli*
- *Shigella flexneri, Shigella sonnei*
- *Neisseria meningitidis, Neisseria sicca*
- *Haemophilus influenzae* type b, nontypeable strains
- *Streptococcus pneumoniae*
- *Corynebacterium diphtheriae*
- *Yersinia enterocolitica*
- *Mycobacterium tuberculosis*
- *Moraxella (Branhamella) catarrhalis*
- *Staphylococcus aureus*

Specific venereal infections
- *Neisseria gonorrhoeae*
- *Treponema pallidum*
- *Chlamydia trachomatis*
- Chancroid *(Haemophilus ducreyi)*
- Granuloma inguinale

Bacterial vaginosis
- *Gardnerella vaginalis*
- *Mobiluncus* species

Fungal Infections

Candida albicans

Other yeasts

Dermatophytes

Protozoan and Parasitic Infections

Trichomoniasis

Amebiasis

Enterobius vermicularis

Hirudiniasis

Schistosomiasis

Other parasitic infections (ascariasis, trichuriasis)

Viral Infections

Venereal
- Herpes simplex
- Condyloma acuminatum (papillomavirus)
- Molluscum contagiosum

Involvement as part of systemic infection
- Measles
- Varicella
- Mononucleosis (Epstein-Barr virus)
- Coxsackievirus
- Smallpox

Infestations

Pediculosis

Scabies

Contact Irritation or Allergic Reactions

Bubble bath preparations

Hair shampoos

Vulvar deodorant sprays

Soaps, laundry detergents

Other medications

Vulvar or Perineal Skin Diseases

Local
- Seborrhea
- Lichen sclerosus et atrophicus
- Lichen planus
- Lichen simplex chronicus
- Premalignant leukoplakia
- Erythrasma *(Corynebacterium minutissimum)*
- Bartholinitis
- Skenitis

Involvement as part of a systemic disorder
- Psoriasis
- Bullous pemphigoid
- Atopic dermatitis
- Drug eruption
- Generalized pruritus with excoriation
- Chronic liver disease
- Chronic renal disease
- Metabolic errors
- Psychosomatic
- Crohn disease
- Sjögren syndrome
- Henoch-Schönlein purpura
- Histiocytosis
- Kawasaki disease
- Stevens-Johnson syndrome
- Typhoid
- Zinc deficiency

Physical Factors

Sand (sandbox)

Chemical or thermal trauma

Physical trauma (accidents, abuse, masturbation)

Nylon, rayon underclothing

Tight garments (maceration in warm climates)

Anatomic abnormalities
- Neoplasms (sarcoma botryoides)
- Polyps
- Labial agglutination, adhesion
- Prolapsed urethra
- Ectopic ureter
- Rectal fistula
- Draining pelvic abscess via fistula

Data from references 9, 10, 20, 40, 41, 58, 63, 65, 104, 125, 141, 166, 192.

vulvar cleansing when girls are bathed decreases the likelihood that nonspecific vulvovaginitis will develop. Young girls should be taught proper hygiene and that they should wash their hands before and after urinating and defecating. Shampooing the hair while sitting in a bathtub and using harsh soaps, bubble baths, or other preparations that might lead to chemical irritation of the vulvar skin and vaginal mucosa should be avoided throughout the course of vulvovaginitis.[16,26] The application of powders should be avoided, at least until the acute symptoms have resolved.

As the inflammation and exudate subside over 1 to 2 days, sitz baths may be reduced in frequency and alternated two to four times per day with the application of either calamine lotion or protective ointments, such as zinc oxide. If pruritus is a significant symptom, an oral agent such as hydroxyzine or diphenhydramine may be administered. Topical application of 1 percent

hydrocortisone cream or triamcinolone acetonide cream may be used as the inflammation resolves but should be avoided in the acute phase.[190]

Patients who do not improve after 2 to 3 days of hygienic measures should be reevaluated. Specimens taken from the vagina should be sent for aerobic and anaerobic bacterial cultures, if these were not done initially. Intractable nonspecific vulvovaginal infections that are not caused by foreign bodies, intestinal parasites, or poor perineal hygiene are encountered occasionally. Reducing vaginal pH from an alkaline or neutral to an acid reaction often helps in these difficult cases and may be achieved by the local use of estrogen. Topical estrogens are not recommended for the treatment of routine cases of premenarcheal vulvovaginitis because the results do not seem to be superior to nonhormonal therapies in these cases[94] and prolonged administration may cause isosexual pseudoprecocity. After 7 to 10 days, the vulvar and vaginal tissues usually thicken and a mucoid vaginal secretion may appear.

Oral or parenteral antibiotics may be indicated if symptoms persist for 2 to 3 weeks, or if a specific pathogen that requires antibiotic treatment is isolated. When possible, selection of antibiotic agents and the route of administration should be based on susceptibility testing of organisms isolated from vaginal cultures. Nonspecific vulvovaginal infections generally are benign, superficial, localized mucosal inflammations that usually respond to less potent chemotherapeutic agents when these are needed.

Vulvovaginitis Secondary to Intestinal Parasites. Pinworms *(Enterobius vermicularis)* are the causative factor in many cases of recurrent or intractable nonspecific vulvovaginitis in children.[58] Infection occurs when the worms in the lower bowel crawl out of the anus onto the perineum and migrate into the vagina, where they deposit ova. The pinworms may carry *E. coli* and other coliform bacteria into the vagina, which may lead to vulvovaginitis.

Infestation with pinworms occurs commonly and does not indicate poor hygiene. Pinworm ova may be deposited in playground soil, on toys or books, and on the hands of infected individuals. Infection frequently is asymptomatic. A child with a pinworm infection and vulvovaginitis usually has a history of a chronic vaginal discharge that has recurred despite repeated attempts to eradicate it. Parents may note worms on the perineum of the child and that she awakens at night because of perineal itching. Other family members or the girl herself may have had pinworms previously.

Examination reveals a low-grade inflammation of the vulva and vagina. Excoriations from scratching may be seen on the perineum. Vaginoscopy (if indicated) shows an inflammatory reaction extending to, but not including, the cervix. Vaginal cultures produce a mixed growth of nonpathogenic bacteria, with *E. coli* and other coliform bacteria generally predominating.

The diagnosis depends on finding pinworm ova on smears from the perineum or in the vaginal discharge or a report from parents that worms are visible on the child's perianal skin. A perineal smear is most likely to show the presence of pinworms, but pinworm ova may be detected in a wet smear of vaginal secretions (pinworms Fig. 42-4).

FIGURE 42-4 ■ Pinworm ova discovered in a vaginal smear from a child with intractable vulvovaginitis.

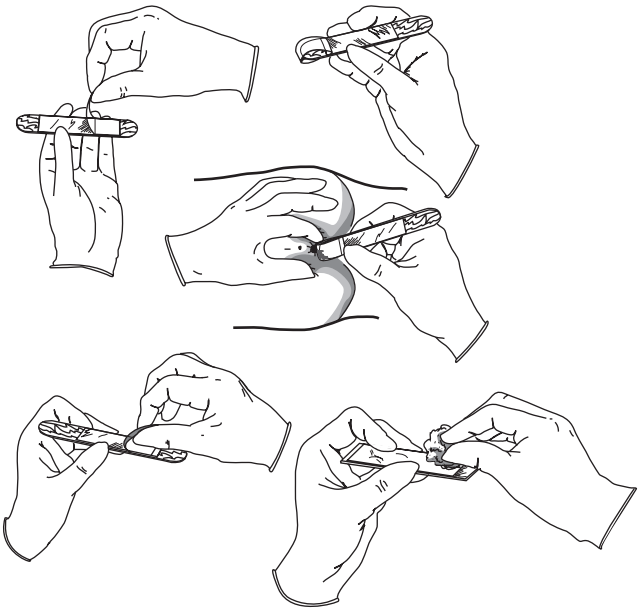

FIGURE 42-5 ■ Technique for obtaining a perianal smear for detection of pinworm ova, using Scotch tape and a tongue depressor.

When a perineal smear for the ova of *E. vermicularis* is to be obtained, the parent is given a wooden tongue blade, a piece of Scotch tape, and a glass microscopic slide. The tape is attached to the tongue blade, adhesive side out (Fig. 42-5), and applied firmly to several areas around the anus. It is removed and applied, adhesive side down, to the glass slide, which is sent to the laboratory for examination.

The type of discharge, the appearance of the vaginal mucosa, or the presence or absence of pruritus does not aid in establishing a diagnosis. A history of a previous pinworm infection in the patient, a member of the family, or a playmate is significant. Pinworms should be suspected when a child has recurrent episodes of intractable nonspecific vulvovaginitis.

Treatment consists of eradicating the pinworms. All members of the family are presumed to be infected and should be treated. Recommended treatment regimens are mebendazole 100 mg orally; albendazole 400 mg orally; or pyrantel pamoate 11 mg/kg base, not to exceed 1 g. Each is given in a single dose and repeated in 2 weeks. The vulvovaginitis caused by coliform organisms carried on pinworms is treated the same as in other cases of nonspecific vulvovaginitis caused by poor perineal hygiene (see earlier discussion).

Vulvovaginitis Secondary to Vaginal Foreign Bodies. Foreign bodies account for approximately 4 percent of cases of vaginal discharge in premenarcheal girls.[7] When a foreign body remains in the vagina for some time, it inevitably causes nonspecific vulvovaginitis. Many types of vaginal foreign bodies, including safety pins, glass beads, coins, beans, bits of crayon, and parts of toys, have been reported. The most common objects are bits of toilet paper or shreds of cloth from nightclothes or bedding (Fig. 42-6).[96,104]

The history does not contribute to the diagnosis unless the child is known to have previously put objects in her vagina. The child usually is brought to a physician because of a profuse, foul-smelling, sometimes blood-tinged discharge. The presence of such a discharge is almost pathognomonic for the presence of a foreign body. Even without a profuse discharge or bleeding, a foul odor from the vagina suggests a foreign body.

Examination reveals inflammation of the vulvar and vaginal mucosa. Although foreign material may be seen when the labia are separated, vaginoscopy often must be performed to explore the full length of the vagina. Rarely, a metallic object that has been in the vagina for some time erodes the mucosa and becomes hidden in granulation tissue. When such a condition is suspected, a radiograph should be obtained. Most foreign bodies, such as glass, plastics, paper, or cloth, are not radiopaque, however, and radiographic examination fails to detect the foreign material. Soft foreign bodies, such as toilet paper, can be flushed out of the vaginal canal.

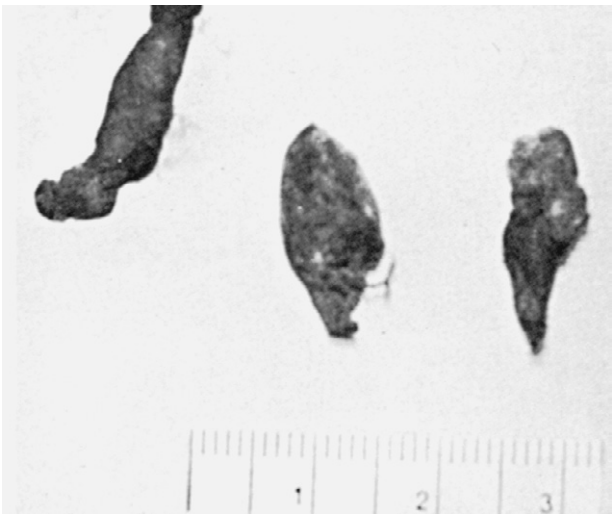

FIGURE 42-6 ■ Bits of paper or cloth are the vaginal foreign bodies most frequently found in children.

The nonspecific vulvovaginitis caused by a foreign body disappears gradually after removal. Recovery can be hastened by using the hygienic treatments previously described. Repeat episodes are common.

Specific Non–Sexually Transmitted Vulvovaginitis

Specific acquired non–sexually transmitted infections are addressed in this section. These include nongonorrheal neisserial vulvovaginitis, group A streptococcal vulvovaginitis, vulvovaginitis secondary to bacteria that colonize the nasopharynx, vulvovaginitis secondary to skin infections, *Shigella* vulvovaginitis, other specific bacterial causes of vulvovaginitis and mycotic (fungal) vulvovaginitis. The majority of infections addressed in this section occur during the prepubertal period. The prepubertal and postpubertal period are addressed for mycotic (fungal) vulvovaginitis.

Nongonorrheal Neisserial Vulvovaginitis. Occasionally, other species of *Neisseria* are the causative agents in cases of premenarcheal vulvovaginitis. These gram-negative, intracellular and extracellular diplococci resemble *Neisseria gonorrhoeae* on stained smears. *Moraxella catarrhalis* also is identical on Gram stain. Gram-negative diplococci should be speciated completely by the microbiology laboratory to avoid misidentifying of nongonococcal diplococci as gonococci and vice versa. Especially for pediatric patients, misidentification can result in serious medicolegal consequences for the family when unwarranted intervention is initiated, or it can result in failure to protect the child's welfare when appropriate measures are deemed unnecessary.[6]

Neisseria sicca, generally considered nonpathogenic, has been isolated from children with vulvovaginal infections that clinically resembled gonorrhea.[218] *Neisseria meningitidis* also has been reported as a cause of vulvovaginitis.[61,80] Nongonococcal *Neisseria* spp. may be considered the cause of vulvovaginal infections when isolated as the predominant flora in the setting of vaginal inflammation and discharge.

Treatment of vulvovaginitis caused by nongonococcal *Neisseria* spp. or *M. catarrhalis* is the same as for *N. gonorrhoeae*. Most nongonococcal *Neisseria* spp. are not resistant to penicillin, but the need for therapy generally is based on the Gram stain finding of gram-negative diplococci, which for therapeutic purposes should be considered gonococci pending culture results. Although data are unavailable, a single dose of ceftriaxone probably should be as effective for these noninvasive *Neisseria* infections as it is for gonorrhea. Chemoprophylaxis, generally with rifampin, should be considered for family members and other contacts in cases of meningococcal vulvovaginitis.

Group A Streptococcal Vulvovaginitis. Group A streptococci are a frequent cause of vulvovaginitis in premenarcheal girls and accounted for 9 to 20 percent of cases in several series.* Most cases occur in girls aged 2 to 7 years, but cases in infants and teenagers have been reported.[21,52,75,201] A marked seasonal variation in incidence in some geographic regions, with peak rates occurring in late fall and winter, may explain the low number of cases of vulvovaginitis caused by group A streptococci in some series.[62,140] The nasopharynx seems to be the primary reservoir for group A streptococci in these girls. Infection may occur from self-inoculation by hand to nose to the vulvovaginal area.[182] The skin also may serve as the source of group A streptococcal vulvovaginitis.[75,201] Preceding or concurrent symptoms of upper respiratory tract infection are uncommon findings, but many girls with group A streptococcal vulvovaginitis have throat cultures positive for *Streptococcus pyogenes*.[75,201] Perineal symptoms have preceded pharyngeal symptoms in some patients.[62] Group A streptococcal vulvovaginitis may occur during the course of scarlet fever.[21,93,94]

The signs and symptoms of group A streptococcal vulvovaginitis often overlap those caused by other bacterial infections, but symptoms usually are abrupt in onset. Most patients seek medical care within 1 week of onset. Vaginal discharge and dysuria are the most common complaints. These girls usually are afebrile. Localized tenderness and an intense, fiery red erythema of the vulvar tissues are frequent findings, but some cases have only mild erythema. Pruritus and excoriation may be present. The discharge usually is seropurulent but may be serosanguineous. The color may be white or green. Petechiae may be present on the vaginal mucosa, and regional papular or scarlatiniform rashes can occur.[62,75,182,201] Acute poststreptococcal glomerulonephritis has been reported in association with group A streptococcal vulvovaginitis.[150] Labial abscesses caused by *S. pyogenes* have been noted rarely in prepubertal girls.[214]

Concomitant streptococcal proctitis and perianal skin infections have been reported.[62,120,197] Perianal pruritus, erythema, and tenderness are common findings.

The diagnosis of *S. pyogenes* infection may be missed if vaginal secretions are not plated onto sheep blood agar or other media that readily support the growth of streptococci.[201] The vulvovaginitis caused by group A streptococci usually shows initial response to oral antimicrobial therapy within 24 hours. A 10-day course of oral penicillin, amoxicillin, or erythromycin generally is sufficient. A second course sometimes is necessary when perianal disease is present.[197] Adjunctive use of the hygienic measures discussed for nonspecific vulvovaginitis hastens clinical improvement. Group B and group F streptococci also have been isolated from girls with acute vulvovaginitis.[187]

Vulvovaginitis Secondary to Bacteria That Colonize the Nasopharynx. Often, a history of an upper respiratory tract infection precedes the onset of vulvovaginitis by a few days. Suspicion that the two conditions are related is strengthened when vaginal cultures yield organisms that commonly colonize the nasopharynx. Vulvovaginitis is assumed to result from autoinoculation of microbes from the nasopharynx to the genitalia. The onset of vulvovaginal symptoms in these infections tends to be acute, the inflammation and discomfort marked, and the discharge less profuse and less purulent than in nonspecific vulvovaginitis.[58]

H. influenzae and *S. aureus* are the species isolated most frequently from cultures.* *Streptococcus pneumoniae* is seen occasionally. *H. influenzae* was the organism isolated most frequently in a series of 200 girls with vulvovaginitis.[159] Acute and chronic cases of *H. influenzae* vulvovaginitis do occur, and the discharge usually is mucoid or mucopurulent, yellow, and odorless. Vulvovaginitis can be caused by serotypes a, b, and c and by nontypeable strains.[94,139] Concurrent otitis media or urinary tract infection may be present. All three of these species occasionally are found in vaginal cultures from asymptomatic children. Their isolation in pure culture from symptomatic girls is what leads to the clinical conclusion of cause and effect.

Vulvovaginitis caused by these organisms often responds to the treatment described earlier for nonspecific vulvovaginitis secondary to poor perineal hygiene. Systemic antibiotics may be required for persistent cases or may be helpful early in severely symptomatic cases. The choice of agent depends on the anticipated or known antimicrobial susceptibilities of the specific organism. The vagina is a well-recognized source of *S. aureus* colonization in cases of toxic shock syndrome associated with this organism. Such cases generally have occurred in adolescent girls and women in association with the use of tampons. Vulvovaginitis caused by *S. aureus* in prepubertal girls has not been reported in connection with toxic shock syndrome. Labial abscesses caused by *S. aureus* have been described in prepubertal girls.[58]

Vulvovaginitis Secondary to Skin Infections. Similar to a child with an upper respiratory tract infection, a child with impetigo or an infected superficial wound may transmit bacteria from the wound to the genitalia by hand contamination. Cultures in such cases usually yield hemolytic streptococci or *S. aureus*. Treatment is the same as that described for nonspecific vulvovaginitis secondary to respiratory tract infections.

Shigella Vulvovaginitis. Vulvovaginitis may be caused by infection with pathogens from the intestinal tract, especially when the organisms are endemic in a community. *Shigella* spp., mainly *Shigella flexneri* and *Shigella sonnei*, seem to account for most of these cases.[20,24,25,49,147] Vaginal discharge without pain, pruritus, or dysuria is the most frequent manifestation of *Shigella* vulvovaginitis. The course can be acute, but discharge that persists for 4 weeks to several months before the diagnosis is made is common. Bloody discharge has been observed in approximately half the cases reported from developed countries, but it was not seen in any of 27 girls with *Shigella* vulvovaginitis in Rwanda between 1988 and 1991.[20,24] The discharge may be purulent and heavy;

*References 40, 41, 52, 54, 94, 166, 192, 201, 214.

*References 40, 41, 137, 159, 166, 192.

occasionally, it is absent. The vulvar tissues usually appear inflamed.

In most instances, *Shigella* vulvovaginitis is not associated with current or recent diarrhea.[20,147] The clinical presentation can be very similar to that of a vaginal foreign body. Local application of triple-sulfa cream (Sultrin) may clear the infection in some cases. Refractory cases have been described, however.[49,147] Systemic treatment with an antibiotic to which the *Shigella* isolate is susceptible is recommended. Trimethoprim-sulfamethoxazole and cefixime are reasonable choices for susceptible isolates; however, most *Shigella* isolates are resistant to ampicillin and trimethoprim-sulfamethoxazole. Ciprofloxacin and ceftriaxone are also effective treatment options.[8,15] As with other causes of vulvovaginitis, adjunctive use of hygienic measures may help resolve the process and prevent recurrence.

Other Specific Bacterial Causes of Vulvovaginitis. Diphtheritic, amebic, and other types of specific vulvovaginitis in children have been reported in the literature.[67,125,158] Most such cases have had associated primary disease elsewhere.

Diphtheritic vulvovaginitis can be the primary site of infection, but most reported cases have been secondary to nasopharyngeal infection. Although diphtheria seldom occurs today, sporadic cases occasionally appear in areas where immunization coverage is inadequate. The vulva is the most common genital site involved in diphtheria,[158] but diphtheritic lesions may occur in the vagina without vulvar involvement. The diagnosis is suspected when a child has the severe systemic symptoms produced by upper respiratory tract diphtheria and a local ulceration covered by a gray adherent membrane; it is confirmed by finding *Corynebacterium diphtheriae* in discharge from the lesion.[56] Treatment of diphtheria is the same regardless of the site of the infection and is discussed in Chapter 90.

One case of vulvovaginitis with *Yersinia enterocolitica* isolated as the predominant organism was reported in a 4-year-old girl who also had a positive stool culture and associated fever and abdominal pain but no diarrhea.[216] In other members of the community in which she lived, diarrhea developed with cultures positive for *Y. enterocolitica.* The outbreak was linked to contaminated food. Infections with this organism may be missed because special culture techniques are required for isolation.

Rarely, ulcerative vulvovaginitis caused by *Entamoeba histolytica* or related to typhoid fever has been reported. Generally, specific genital infections with pathogenic organisms usually found in the gastrointestinal tract are the result of fecal contamination of the vulva and vagina. Treatment of infections caused by *E. histolytica* is described in Chapter 209.

Mycotic (Fungal) Vulvovaginitis. *Candida albicans* and *Torulopsis glabrata* are the yeastlike fungi found most often in the vagina. *C. albicans* is responsible for 80 to 95 percent and *T. glabrata* for 3 to 16 percent of fungal infections. Other *Candida* spp. are detected less often in the vagina but can be pathogenic. *Candida tropicalis*, similar to *C. albicans*, produces systemic infection in immunosuppressed hosts.

Prepubertal. *C. albicans* and other fungi can cause vulvovaginitis in infants and children. Although these infections can occur in any child, children with a history of recent antibiotic use, uncontrolled diabetes mellitus, or immunosuppression are at an increased risk for development of fungal vulvovaginitis. In children with recurrent or persistent infection, especially with no history of antibiotic use, immunosuppressive conditions, such as diabetes or human immunodeficiency virus (HIV) infection, should be considered. Mycotic infections are not considered sexually transmitted infections, but they are discussed in this section because they can cause genital infections.

C. albicans and other fungi have been isolated from the vagina in asymptomatic and symptomatic girls. In a study examining the microbiology of the vagina, yeast was cultured from 48 percent of girls aged 2 months to 2 years, from 12.5 percent of girls aged 3 to 10 years, and from 35 percent of girls aged 11 to 15 years.[96] *C. albicans* (37%) and *C. tropicalis* (26%) were the species most frequently isolated, but *Candida parapsilosis*, *T. glabrata*, non-*Candida* yeast species, and other *Candida* spp. were isolated as well.

In studies evaluating prepubertal girls with vulvovaginitis, fungi have been recovered at varying rates. In one study, *C. albicans* was isolated from 25 percent of 31 symptomatic prepubertal girls with an abnormal vaginal discharge or vulvovaginitis, whereas only 3 percent of asymptomatic girls had *C. albicans* isolated.[73] Another study found different results, with none of the 50 asymptomatic girls and only 2 percent of the 50 girls with vulvovaginitis having *C. albicans.*[112] Similarly, in a study evaluating girls younger than 12 years with vaginal complaints, only 3 percent of the 74 girls had *C. albicans* isolated.[187] Neither of these groups of girls had a discharge on examination, and both had been treated recently with antibiotics. In a study that examined recovery of microorganisms from girls with vaginitis, 8 percent of the vaginal cultures of the 35 girls evaluated grew yeast.[79] In these latter two studies, assessing the role of yeast in the disease is difficult because there was no control population for comparison.

Mycotic vulvovaginitis causes severe vulvar and vaginal itching; usually, the patient does not have an excessive discharge. The discharge is white, thick, and curdled and has a yeasty sour odor. The patient may have pain during and after voiding or external dysuria as a result of urine coming in contact with excoriated areas on the urethra and vulva. Young children with fungal vulvovaginitis have similar symptoms. In diapered children, an erythematous rash in the child's diaper area may be noticed. If the condition has been long-standing, the edema, secondary infection, and repeated scratching may produce thickened and fissured lesions closely resembling chronic eczema or lichen sclerosus et atrophicus (Fig. 42-7).

Postpubertal. Several factors play roles in causing the increased incidence of vaginal candidiasis after menarche. These are menstruation, fecal contamination of the vulva, wearing tight-fitting underclothes, widespread use of antibiotics, and the use of oral contraceptives. Other predisposing factors include pregnancy, obesity,

FIGURE 42-7 ■ Mycotic vulvovaginitis. The child has diabetes.

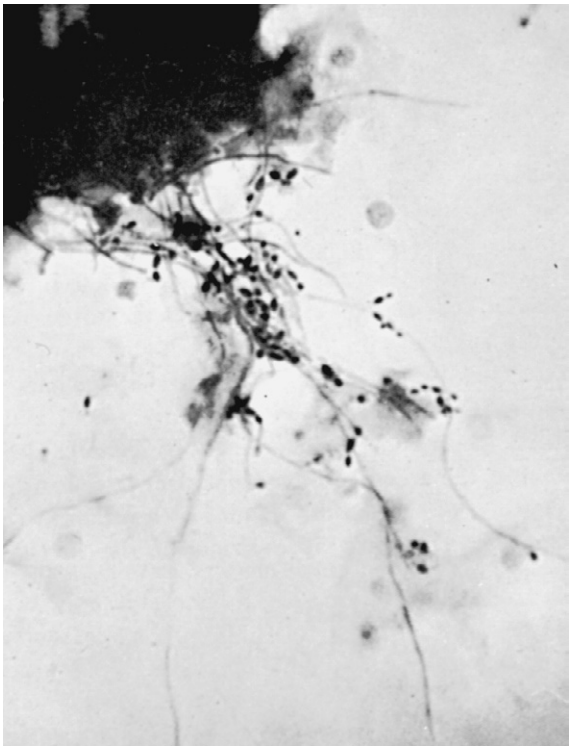

FIGURE 42-8 ■ Hyphae of *Candida albicans* discovered on a wet smear of vaginal discharge.

uncontrolled diabetes mellitus, immunosuppressive therapy, and heroin or other drug addiction.[226]

Mycotic vulvovaginitis causes severe vulvar and vaginal itching. The discharge is white, thick, and curdled and has a sour odor. The patient may have external dysuria. Examination in the acute stage reveals intense inflammation of the vulva and vagina with shiny and beefy red mucosa with linear excoriations and edema. Long-standing infection can cause lichenification and hyperpigmentation of the perineal skin. *C. albicans* typically forms white plaques scattered over the inflamed surfaces. *C. tropicalis* produces a cottage cheese–like discharge similar to that found with *C. albicans*. Vaginal pH usually is normal (<4.5).[226]

Diagnosis. The diagnosis in all age groups is frequently based on clinical findings and can be established by a vaginal saline wet preparation under microscopy and by finding hyphae and buds of the fungus in vaginal fluid (Fig. 42-8) in the curdled discharge. If debris and cellular material render identifying the fungus difficult, a smear using 10 percent potassium hydroxide solution is helpful. The potassium hydroxide solution dissolves other extraneous material without affecting the fungus. Even with an experienced microscopist, this method yields a sensitivity range of 40 to 86 percent in symptomatic girls; however, the yield may be only 40 percent. Further confirmation can be obtained by identifying fungus by culture of material from the vagina or vulva on Sabouraud or Nickerson media, but this is generally unnecessary.

Treatment. Several modes of therapy can be used for the management of fungal vulvovaginitis in premenarcheal females.[58] Numerous antifungal creams, including nystatin, miconazole, clotrimazole, or terconazole, are available. The creams should be applied as prescribed to the affected area after cleansing. In diapered children, the cream should be applied with each diaper change.

Curing fungal infections often is difficult in children who are taking antibiotics or who receive repeated courses of antibiotic therapy. In such cases, a 1-mL dose of nystatin suspension (Mycostatin), 100,000 U/mL, can be injected into the vagina three times per day for 10 days in infants and small children. In addition, the suspension should be administered at the same dose orally four times daily. Fluconazole (Diflucan) also can be administered as a one-time oral dose. A second dose may need to be administered for severe infections.[58]

In adolescents a variety of imidazoles are available as over-the-counter vaginal creams, tablets, and coated tampons. Intravaginal treatment with one 100-mg vaginal tablet of clotrimazole every night for 7 days produces a cure rate of 90 percent. Comparable cure rates are observed with 100 to 200 mg of clotrimazole or miconazole (2 tablets) for 3 days. The shorter regimen may result in better compliance. Creams and tablets are equally effective.[35] Oral fluconazole 150 mg as a single dose is an effective preparation for the treatment of uncomplicated vulvovaginal candidiasis.[35] Low-dose prophylactic treatment also may be appropriate in these cases. If recurrent unexplainable or resistant infection occurs, diabetes mellitus should be suspected.

SPECIFIC SEXUALLY TRANSMITTED VULVOVAGINITIS

Specific sexually transmitted vulvovaginitis in this section includes discussion of gonorrhea and chlamydia in prepubertal girls and trichomoniasis and bacterial vaginosis in prepubertal and postpubertal females. Gonorrhea and *Chlamydia* infections in postpubertal period females cause

cervicitis instead of vulvovaginitis and are addressed under the discussion of cervicitis.

Gonorrhea

Prepubertal

Gonococcal infections of the prepubertal genital tract are manifested as vulvovaginitis.* The alkaline environment of the unestrogenized vaginal tissues of young girls apparently limits spread of infection to the upper genital tract. Gonorrhea is found less commonly in children now than in the past but must be considered whenever a girl has vulvovaginitis.

In infants, perinatal nonsexual transmission is considered the most likely cause of gonococcal infection. Branch and Paxton[23] found that in 1- to 11-month-old infants, all the mothers were found to have gonococcal infection and no history of sexual contact could be elicited. The authors concluded that transmission of the infection was perinatal, from freshly contaminated hands, or through fomites.

Prepubertal children with gonococcal infection frequently are found to have a history of sexual contact.[107] The rate of eliciting a history of sexual contact in prepubertal children with gonorrhea ranges from 36 to 93 percent.[23,66,109,110] Branch and Paxton[23] reported that 93 percent of children aged 1 to 9 years with gonorrhea had sexual contact with relatives in the household. Ingram and colleagues[109,110] found that 35 percent of 1- to 4-year-old children and 100 percent of children older than 4 years with gonorrhea reported having sexual contact with an older male family member. Folland and coworkers[66] elicited a history of sexual contact from 34 percent of children with gonococcal urethritis and vaginitis.

Other infected adults and children often are found in an infected child's environment.[163] Testing of household members can detect infected adults at a rate of 18 to 29 percent.[5,149] In a retrospective study of 14 Native Alaskan children with gonococcal infection, 3 reported having sexual contact. Seven children slept with their parents, one or both of whom had gonorrhea; the authors assumed that these children acquired the infection by nonsexual means.[189] Nonsexual transmission conceivably occurs in children who sleep and bathe with their parents.

N. gonorrhoeae has been shown to survive for 20 to 24 hours in infected secretions on towels and handkerchiefs.[199] Although *N. gonorrhoeae* has survived on toilet seats for 2 hours, no gonococci were recovered from toilet seats in public restrooms or in a clinic for STI.[74,169] Nonsexual transmission to adults is rare.

Sexual transmission is the more common and most likely mode of transmission, however.[86] Thus, the presence of gonorrhea should be considered diagnostic of sexual abuse. An investigation for sexual abuse must be pursued in a child with gonorrhea by reporting the case to the appropriate legal authority (Table 42-3).[35]

N. gonorrhoeae was the most common cause of vulvovaginal discharge among prepubertal girls in Rwanda in the late 1980s. Sexual contact was considered likely in all cases because of a cultural belief that a man with a purulent urethral discharge could be cured by rubbing his penis on the external genitalia of a prepubertal girl.[20] In a prospective study of girls 12 months to 12 years of age in the mid-1990s in Cincinnati, Ohio, who had vaginal discharge and no suspicion of sexual abuse, 4 of 43 had positive cultures for *N. gonorrhoeae*.[187] Such cases should lead to investigation for probable sexual abuse.

The acute stage of gonorrheal vulvovaginitis is characterized by inflammation and a purulent discharge. The child may complain of vulvar discomfort, dysuria, frequent urination, and pain on walking. The child usually is well otherwise. Asymptomatic vaginal infection is rare. On examination, the vulvar tissues are edematous, hyperemic, and covered by a profuse, thick, yellowish discharge that exudes from the vagina. The entire vaginal mucosa is inflamed.

The urethra, paraurethral glands, and major vestibular (Bartholin) glands rarely are involved in a premenarcheal gonorrheal infection. Vulvovaginal infections, including gonorrhea, in prepubertal children rarely, if ever, affect the upper genitalia (uterus, uterine tubes, ovaries, or pelvic peritoneum). Symptoms suggestive of pelvic peritonitis have been reported in premenarcheal children who had gonorrheal vulvovaginitis; all the patients recovered promptly after the administration of penicillin.[33,68]

The acute phase of infection lasts for a few weeks, after which most of the symptoms, except the discharge, disappear. The vulvar and vaginal tissues may remain hyperemic and macerated in some cases. The discharge becomes scanty and seropurulent, which may persist chronically.

Diagnosis

The diagnosis of gonorrheal vulvovaginitis and its differentiation from other types of vulvovaginitis are established by vaginal smears and cultures. Specimens from the pharynx and rectum to test for *N. gonorrhoeae* also should be obtained. Because of the potential medicolegal use of the test results for *N. gonorrhoeae* among children, standard culture systems should be used for the diagnosis of *N. gonorrhoeae* in children. More recently nucleic amplification testing for gonorrhea using urine and vaginal swabs was conducted in 485 premenarcheal girls with alleged sexual abuse from four cities in the United States. The sensitivity of urine nucleic amplification testing for gonorrhea relative to vaginal swabs was 100 percent.[19] As a result, the Centers for Disease Control and Prevention (CDC) has approved use of nucleic amplification testing of vaginal or urine specimens in girls suspected of having experienced sexual abuse, as long as specimens are retained for additional testing if necessary.[35]

Treatment

The presence of gram-negative intracellular diplococci in vaginal smears from a child with a history of exposure or with typical clinical findings is sufficient reason to start treatment, but it does not establish a definitive

*References 33, 59, 95, 184, 213, 227.

TABLE 42-3 **Sexually Transmitted Diseases in Prepubertal Children: Modes of Transmission and Implications for Child Sexual Abuse**

Infectious Agent	Persistence After Perinatal Acquisition	STI Rate in Alleged Sexual Abuse Cases (%)	Alleged Sexual Abuse Rate in STI Cases	Infections in Household Members of Infected Children	Survival on Fomites	AAP CCAN 2005 Guideline Recommendations for Abuse Report	Other Comments
Neisseria gonorrhoeae	Up to 1 yr old[23]	5-7[49]	35%, 1-4 yr old[109] 100%, >4 yr old[109] 93%, 1-9 yr old[23,66]	18%-29% of adults in household[87,149,192] (abuse was thought unlikely in these series)	24 hr on wet fomites; 2 hr on dry fomites. None from public toilet seats[74,169]	Diagnostic for abuse; report to Child Protective Services*	Although fomite transmission can occur, sexual transmission must be considered the likely mode, unless strong evidence of an alternative mode is found. Fomite or maternal nonsexual transmission plausible <1 yr old[23]
Chlamydia trachomatis	Up to 6 yr old[178,211] Rectal and vaginal sites up to 54 wk	6-7[69,108,110] 6-8 cases vs. 0 controls[107,110]	75%		No data	Diagnostic for abuse; report to Child Protective Services*	Perinatal acquisition likely <1 yr old; if >1 yr old, genital infection strongly suggests sexual transmission.[107,108,110]
Trichomonas vaginalis	Up to 1 yr old [43,138]	0 cases vs. 0 controls[71,191]	Case report[114]	Case report with no contacts[33] 43% fathers, 72% mothers, with no history of sexual abuse elicited (Poland)[123]	Up to 6 hr on wood surface and in discharge.[118] Isolated from bathing implements[152], bathing waters, mud baths[123]	Highly suspicious for abuse; report to Child Protective Services*	If >1 yr old, genital infection strongly suggests sexual transmission. Bathing in tanks or rivers may increase infection risk in girls (India).[37] Has been isolated from mud baths, warm mineral waters, water from toilets.[49,123]
Bacterial vaginosis (*Gardnerella vaginalis*)	No systematic studies; colonization at birth potentially persists for years	7-13 by Amsel criteria[87] *G. vaginalis* 15 cases vs. 1-4 controls[15,70]	*G. vaginalis* 6%-15%[15,70]	None	None	Inconclusive; conduct medical follow-up	*G. vaginalis* can be found as part of normal flora in nonabused virginal children

TABLE 42-3 **Sexually Transmitted Diseases in Prepubertal Children: Modes of Transmission and Implications for Child Sexual Abuse** (Continued)

Infectious Agent	Persistence After Perinatal Acquisition	STI Rate in Alleged Sexual Abuse Cases (%)	Alleged Sexual Abuse Rate in STI Cases	Infections in Household Members of Infected Children	Survival on Fomites	AAP CCAN 2005 Guideline Recommendations for Abuse Report	Other Comments
Herpes simplex virus (genital sites)	Symptomatic congenital infection usually develops within first 6 wk of life		Case reports only	Case report only (mother with an infected finger)[116]	2 hr on latex gloves, toilet seats; 24 hr on speculum; 72 hr on gauze[129]	Suspicious for abuse; report to Child Protective Services*†	Autoinoculation from oral lesions is possible,[148] but fomite transmission is unlikely because it requires direct contact of viable virus with broken skin or mucous membranes
Human papillomavirus‡ (anogenital warts)	Up to at least 3 yr old and probably years longer[48]	27-90[38,97]		Digital infection[183]	None	Suspicious for abuse; report to Child Protective Services*§	The possibility of sexual abuse should be considered when oral warts or laryngeal papillomas are found in older children
Syphilis	Months to years if untreated	0-5[49,76,107]	Case reports		Unlikely, based on difficulty in culturing the microbes, but no data	Diagnostic for abuse; report to Child Protective Services*	Infection not known to be congenital should be considered sexually transmitted. Clinical manifestations (e.g., late congenital syphilis) may be helpful in discerning mode in some cases[3]

Modified from Chacko M, Staat MA, Woods CR. Genital infections in childhood and adolescence. In: Feigin RD, Cherry JD, Demmler GJ, Kaplan SL, eds. *Textbook of Pediatric Infectious Diseases*. 5th ed. Philadelphia: W. B. Saunders; 2009:575; and Kellogg N, American Academy of Pediatrics Committee on Child Abuse and Neglect. The Evaluation of sexual abuse in children. *Pediatrics* 2005;116:506-512.

*Or other local agency mandated to receive reports of suspected child sexual abuse.

†Report unless there is a clear history of autoinoculation. Type of herpes simplex virus (1 vs. 2) is generally not helpful in determining whether acquisition was by abuse or autoinoculation, although presence of type 1 may increase the likelihood of nonsexual transmission.

‡Laryngeal papillomas in young children are likely perinatally acquired.

§Some experts do not believe reporting is warranted in all cases. In children younger than 3-4 years old for whom a thorough evaluation by an experienced child sexual abuse investigator reveals no concerns of abuse, reporting to Child Protective Services may be unnecessary. Close medical follow-up may be appropriate. Physicians must be clear when reporting that anogenital human papillomavirus is not always acquired by sexual abuse even in older children.

STI, Sexually transmitted infection.

diagnosis. Further details on treatment are addressed in Chapter 89.

Chlamydia

Prepubertal

Sexual abuse as a potential mode of transmission of *C. trachomatis* infection should be considered in children older than 1 year.[69,89] An investigation for sexual abuse must be pursued in a child with *Chlamydia* infection by reporting the case to the appropriate legal authority (see Table 42-3).[35]

In a retrospective study, Ingram and associates[108] found that 6 percent of girls who allegedly were sexually abused and no girls who denied sexual abuse had *C. trachomatis* infection. Except for one child, all the children in the control group were determined later to have been sexually abused. In a prospective study, Ingram and associates[107,110] found *C. trachomatis* in 8 percent of girls with a history of sexual abuse compared with none in girls with no history of sexual abuse. In the former group, three girls also were found to have rectal infection and one to have pharyngeal infection.

Perinatally acquired genital *Chlamydia* infection is a strong possibility in children younger than 1 year. Perinatal transmission is possible in children aged 3 years. After 1 year of age, however, sexual transmission should be considered diagnostic of sexual abuse in children with genital *Chlamydial* infection.[34] An investigation for sexual abuse must be pursued in a child in whom genital chlamydial infection is diagnosed.[34,89] Studies have evaluated the presence of *C. trachomatis* on fomites and its coexistence in family members of infected children.

Although *C. trachomatis* appears to be an uncommon cause of vaginitis in prepubertal girls, vaginal infection with *C. trachomatis* can occur in children with or without a known history of sexual activity and should be considered in the evaluation of a child with vaginal symptoms. Only a few studies have evaluated young children for *C. trachomatis.* These studies have involved mostly children being evaluated for sexual abuse or vulvovaginal symptoms, or both, and some have included a control group in an attempt to control for either a history of sexual activity or vaginal symptoms.

In studies evaluating premenarcheal children with vulvovaginitis, recovery of *C. trachomatis* has been low in children with symptoms and healthy control groups. In one study, none of the 35 children with symptoms had *C. trachomatis*, whereas 1 of 35 without symptoms had *C. trachomatis* isolated.[156] In another study conducted in a pediatric gynecology clinic, 4 of 29 (14%) premenarcheal girls were found to have *C. trachomatis.*[30] All four had a homogeneous white discharge, and one had a bloody discharge. Sexual abuse occurred in two of the children and was considered possible in another. In a Cincinnati study evaluating vaginitis in girls younger than 12 years in whom sexual abuse was not suspected, none of the 87 children had a positive culture for *C. trachomatis.*[187] Similarly, in a smaller study of 11 girls with vaginitis, none was found to have *C. trachomatis.*[170]

In studies evaluating children suspected of being sexually abused, rates of recovery tend to be higher than those seen in children with vaginitis. In one study, a history of sexual abuse was discovered in two control children only after *C. trachomatis* was identified.[84] Initially, 2 percent of children with a history of sexual abuse versus 4 percent of healthy controls had positive vaginal cultures for *Chlamydia*. On further questioning, however, the two children in the control group with *C. trachomatis* were siblings who were sexually abused 3 years earlier, changing the rate of *C. trachomatis* vaginal infection in the sexual abuse group to 6 percent and decreasing the rate in the control group to zero. This misclassification, if not discovered, would have changed the conclusion of the study, which suggested that similar rates of *C. trachomatis* infection occur in children with and without a history of sexual contact. This incidence illustrates the importance of thoroughly investigating the possibility of sexual abuse whenever a sexually transmitted pathogen is isolated in a young child. In a Cincinnati study evaluating children for sexual abuse, rates were low in prepubertal girls compared with pubertal girls (0.8% vs. 7%),[191] whereas in a study in Raleigh, North Carolina, 6 percent of sexually abused girls younger than 12 years versus none of control children had *C. trachomatis* isolated from their vagina.[110] Four of the 10 children in the latter study had a vaginal discharge.

The highest rates of *C. trachomatis* infection were seen in a Los Angeles study, in which 47 prepubertal girls were examined for alleged sexual abuse and 17 percent had *C. trachomatis* isolated from vaginal specimens.[69] The variation in rates among studies may reflect differences in the prevalence of *C. trachomatis* in different communities. Although coinfection with *C. trachomatis* has not been studied well in prepubertal children, experience from adult populations has shown that it should be suspected in children with *N. gonorrhoeae* infection. In one study in prepubertal girls, 30 percent with *N. gonorrhoeae* also tested positive for *C. trachomatis.*[170]

One possibility is that a positive chlamydial culture in a young child, especially a child younger than 3 years, may be due to persistence of perinatal infection. *C. trachomatis* has been isolated from the conjunctiva, nasopharynx, vagina, and rectum of infants born to infected mothers.[179] Subclinical infections have been reported in 14 percent of infants of mothers with active *C. trachomatis* infection.[178] Many infants infected with *C. trachomatis* at birth remain infected for 372 days in the vagina; 383 days in the rectum; and 866 days in the conjunctiva, nasopharynx, and oropharynx.[17] Persistent chlamydial infection of the pharynx in infants can persist for 2 years.[17] Persistence of perinatal infection for 18 months and for 6 years has been reported in other studies as well.[134,177,211] Although persistence of perinatal infection can occur, when *C. trachomatis* is identified in vaginal specimens, sexual abuse still must be suspected strongly.

In contrast to prepubertal gonorrhea infections, chlamydial vaginitis in children often seems to be asymptomatic. Because of study design issues in evaluating children with *C. trachomatis* infection, in which only children with a history of sexual abuse or vaginal symptoms, or both, are included, fully understanding the spectrum of

symptoms seen in young children is difficult. In one study evaluating symptomatic children with *C. trachomatis* vaginal infection, 25 percent had a vaginal discharge and 12 percent had vaginal bleeding.[69]

Diagnosis

Numerous diagnostic methods for *Chlamydia* are available.[18] Direct tissue culture isolation of *C. trachomatis* remains the gold standard and is the only test that should be used in prepubertal children or in cases in which sexual abuse is under consideration.[35] Culturing for *Chlamydia* requires isolation of the organism in tissue culture and confirmation of the characteristic intracytoplasmic inclusions by fluorescent monoclonal antibody staining.[35] Although the specificity of tissue culture approaches 100 percent, tissue culture is only 70 to 85 percent sensitive in contrast to DNA amplification techniques.[18] The low recovery rate in studies of prepubertal children may be due to the low sensitivity of tissue culture or the use of vaginal cultures in young children. Isolation rates from the vagina usually are lower than rates of recovery from the endocervix.[136]

Nonculture tests for *Chlamydia*, including enzyme immunoassays, direct fluorescent antibody tests, DNA hybridization, and DNA amplification tests, have not been field-tested for large numbers of vaginal specimens or rectal or pharyngeal specimens from premenarcheal girls. False-positive results have been reported in vaginal specimens for some of these tests in children, and these tests should not be used in premenarcheal children.[86,161] False-positive results probably are caused by cross-reactivity with common anogenital organisms, such as group A and B streptococci, *N. gonorrhoeae*, *G. vaginalis*, *E. coli*, and *Proteus*, *Acinetobacter*, and *Staphylococcus* spp.[121,172,174,175,210]

However, more recently, nucleic amplification *Chlamydia* testing using urine and vaginal swabs was conducted in 485 premenarcheal girls with alleged sexual abuse from four cities in the United States. The sensitivity of urine nucleic acid amplification (NAAT) for chlamydia relative to vaginal swabs was 100 percent.[19] As a result the Center for Disease Control and Prevention has approved use of NAAT testing of vaginal or urine specimens in girls suspected with sexual abuse, as long as specimens are retained for additional testing if necessary.[34]

Treatment

Treatment for *Chlamydia* infection is addressed in Chapter 193.

Trichomoniasis

Trichomonas vaginalis is a triflagellated protozoan (Fig. 42-9). The organism, which is larger than a polymorphonuclear leukocyte, has a distinctive vibrating or whiplike movement when seen microscopically in fresh wet smears taken from the vagina. It quickly succumbs to reduction of the pH, drying, cooling, and changing the osmotic pressure of the fluid surrounding it. Most infections are encountered in sexually active adolescents and young adult women. Chapter 214 addresses trichomoniasis, including epidemiology, in more detail.

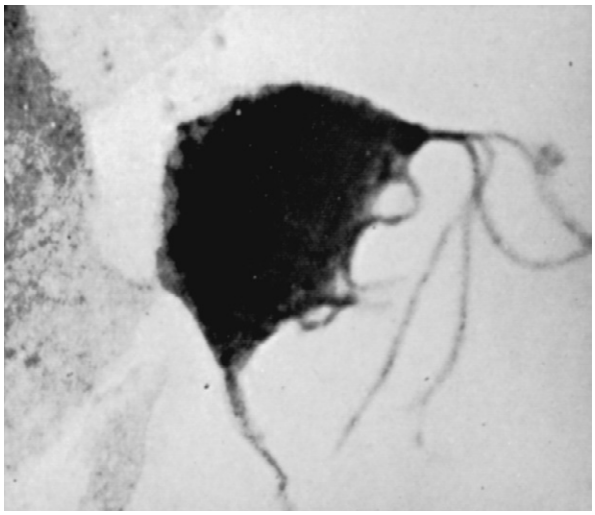

FIGURE 42-9 ■ *Trichomonas vaginalis* is a triflagellated protozoan that, when motile, is identified easily in wet smears of vaginal discharge.

Prepubertal

Vaginal trichomoniasis is reported infrequently in prepubertal children. This low prevalence may be accurate or could be due to lack of appropriate testing or limitations of the diagnostic techniques used in studies in prepubertal children.

Trichomoniasis in newborns through the acquisition of *T. vaginalis* from the mother's vagina during delivery, with recovery of *T. vaginalis* from urine, vaginal, and respiratory tract specimens, has been described in several case reports.* The prevalence of *T. vaginalis* in healthy, vaginally delivered infants of mothers with *T. vaginalis* is unknown. In one study of 868 infants, *T. vaginalis* could not be identified in any of the 14 female infants of mothers in whom *T. vaginalis* was diagnosed. The overall prevalence of *T. vaginalis* was 4 percent, but the denominator of mothers of female infants was not described.[22] In another study of 984 female infants, direct smear or culture (or both) of infant vaginal specimens identified 3 infants with *T. vaginalis*, a prevalence of 0.3 percent.[6] Of the 3 infants, 2 had a vaginal discharge. The overall prevalence of infection in the mothers of the 984 infants was not reported. Of the three infants with *T. vaginalis*, one mother had a history of vaginal discharge and a negative direct smear for *T. vaginalis*, and the other two mothers had no history of *T. vaginalis*. In conclusion, transmission of *T. vaginalis* in infants aged 1 year probably is perinatal.

The mode of transmission in children older than 1 year is controversial. Two cases of *T. vaginalis* infection in premenarcheal girls who were sexually abused have been reported.[113] Numerous reports confirm the occurrence of *T. vaginalis* outside the newborn period in prepubertal girls evaluated for vaginitis. The prevalence of

*References 4, 22, 43, 46, 131, 138, 162, 196.

T. vaginalis in prepubertal girls with vaginitis but no history of sexual abuse ranged from 0 to 4 percent.[94,112,156,187] In studies with a healthy control population,[79,94,112] *T. vaginalis* was not identified in any of the children in the asymptomatic control group.

T. vaginalis has been described in varying frequency in sexually abused prepubertal girls. A case report described *T. vaginitis* in two sexually abused children with vaginal discharge.[113] In studies systematically evaluating children for sexual abuse regardless of symptoms, *T. vaginalis* has not been recovered. In an Australian study evaluating 160 children younger than 10 years and 95 healthy age-matched controls, none of the children had *T. vaginalis* isolated from vaginal cultures.[71] Similarly, none of the 119 prepubertal girls evaluated for sexual abuse in a Cincinnati, Ohio, study had *T. vaginalis* identified by urinalysis or wet mount of vaginal secretions.[191] Without additional studies using culture techniques and conducted in diverse populations of prepubertal girls, determining the true prevalence of *T. vaginalis* in prepubertal girls who have vaginitis, with or without a history of sexual abuse, is difficult.

One case report suggests that *T. vaginalis* could be transmitted within a family without sexual abuse or contact.[2] In this report, *T. vaginalis* was diagnosed in the mother, and her three prepubertal daughters were symptomatic with a vaginal discharge. Two of the three girls had *T. vaginalis* identified on wet-mount evaluation of their vaginal specimens. They had no history or evidence of sexual abuse, the father was asymptomatic, and microscopy of an early morning urine specimen was negative for *T. vaginalis*.

A study from Poland found one case of *T. vaginalis* infection in children aged 2 to 7 years and a significantly higher number of cases in 8- to 10-year-old girls. The numbers increased even further for children older than 10 years, suggesting a strong association between *T. vaginalis* and the presence of an estrogenic environment, which promotes glycogen production and decreases vaginal pH.[123]

The Polish investigation tested families of women infected with *T. vaginalis* and found that almost a third of their sexual partners and 8 percent of the children (mostly girls) had *T. vaginalis*. When families of men infected with *T. vaginalis* were tested, 91 percent of their sexual partners and 13 percent of the children had *T. vaginalis*. When families of children (mostly girls) infected with *T. vaginalis* were tested, 72 percent of the mothers and 43 percent of the fathers had the infection. The investigators considered that the infection in the latter group originated from mothers and that the primary mode of transmission was nonsexual (beds, sponges, towels, overcrowding). Information regarding sharing of potentially infected fomites, sexual abuse, or physically intimate behavior between parents and children was not gathered in these cases.[123]

Although *T. vaginalis* has been known to survive on fomites in controlled experiments, its ability to spread by these means is unknown. No cases of adults being infected by fomites have been documented. *T. vaginalis* has been found to survive for 6 hours on droplets of discharge and enameled surfaces of wood blocks.[118] It has been isolated from droplets of water splashed from toilets containing the urine of an infected individual.[32] *T. vaginalis* also has been found to survive in mud baths, bathing waters, and warm mineral waters and on moist bathing implements.[123,152] In rural India, a survey found that young girls who bathed in tanks or rivers had a significantly higher risk for acquiring *T. vaginalis* than did girls who used pipe or well water.[37]

In summary, *T. vaginalis* conceivably is transmitted to children nonsexually. The likelihood of perinatal transmission is high in infants younger than 1 year. A child or an infant younger than 1 year with *T. vaginalis* also may have been sexually abused. In a child older than 1 year, the probability of sexual abuse must be considered highly suspicious, and the case must be investigated and reported. Perinatal transmission and transmission of infection through fomites should not be assumed without an investigation for sexual abuse (see Table 42-3).[35]

Although these cases may have resulted from transmission within a family without sexual abuse, identification of *T. vaginalis* in a prepubertal child outside the newborn period should prompt further medical and social evaluation for sexual abuse. Additionally, if *T. vaginalis* is recovered from a child, the child should be evaluated for other STIs, including syphilis, *N. gonorrhoeae*, *C. trachomatis*, hepatitis B, and HIV infection.[58]

Postpubertal

Trichomoniasis is common and frequently is asymptomatic in sexually active adolescents (see Chapter 214). When it is symptomatic, patients complain of a profuse, irritating discharge. The discharge and the pruritus tend to be more severe just before and immediately after a menstrual period. Patients occasionally report dysuria and abdominal pain. Recurrent exacerbations of the infection occur commonly.

Examination reveals diffuse vulvitis with erythema and excoriations and copious leukorrhea that covers the vulvar tissues. The discharge typically is frothy or bubbly, grayish yellow, and watery or mucopurulent. It has a pH of 5.0 to 7.0 and an acrid or musty odor. A "strawberry" or punctate vaginal eruption with hemorrhagic spots has been described as being typical of trichomoniasis. Such eruptions frequently are not present, however, even in severe cases. More often, diffuse inflammation causes the vaginal mucosa to be brilliant red.

Diagnosis

The diagnosis of trichomoniasis usually is confirmed clinically by finding trichomonads in a wet saline smear of vaginal fluid. It is important that the slide be viewed under the microscope (dry high power) promptly because the organisms do not remain viable for long outside the vagina and are difficult to detect when they cease to be motile. The observer sees numerous ovoid-shaped, motile organisms (see Fig. 42-9). The sensitivity of this test with immediate evaluation is 60 to 70 percent. A Papanicolaou (Pap) smear to detect trichomoniasis is not recommended because of the high rate of error in identifying trichomonads in stained smears. Other methods used to diagnose

trichomoniasis include isolation by special culture medium, direct fluorescent immunoassay, and nucleic acid amplification test. Culture is the most sensitive, but it is not routinely available. The nucleic acid amplification test is available in some clinical settings (point-of-care test); however, a false-positive result may occur in low-prevalence populations.[35]

Treatment

Treatment for trichomoniasis is addressed in Chapter 214.

Bacterial Vaginosis

Bacterial vaginosis (previously termed *nonspecific vaginitis* in adult women) is a cause of vaginal discharge in adolescents and occasionally may be seen in premenarcheal girls. Bacterial vaginosis is caused by a change in the relative proportions of bacteria in the vaginal flora: an overgrowth of anaerobes, especially *Bacteroides* and *Mobiluncus* spp., *G. vaginalis*, and *M. hominis;* and a decrease in hydrogen peroxide–producing lactobacilli.[10] Identification of *G. vaginalis* by culture of the vaginal discharge is not diagnostic because *G. vaginalis* may be present in girls with or without bacterial vaginosis.[35]

The clinical criteria used for establishing the diagnosis of bacterial vaginosis are the presence of three of four findings: a grayish homogeneous discharge, the presence of clue cells on a wet-mount evaluation (Fig. 42-10), a pH greater than 4.5, and a positive amine test result (amine or fishy odor when vaginal secretions are mixed with 10% potassium hydroxide).[35]

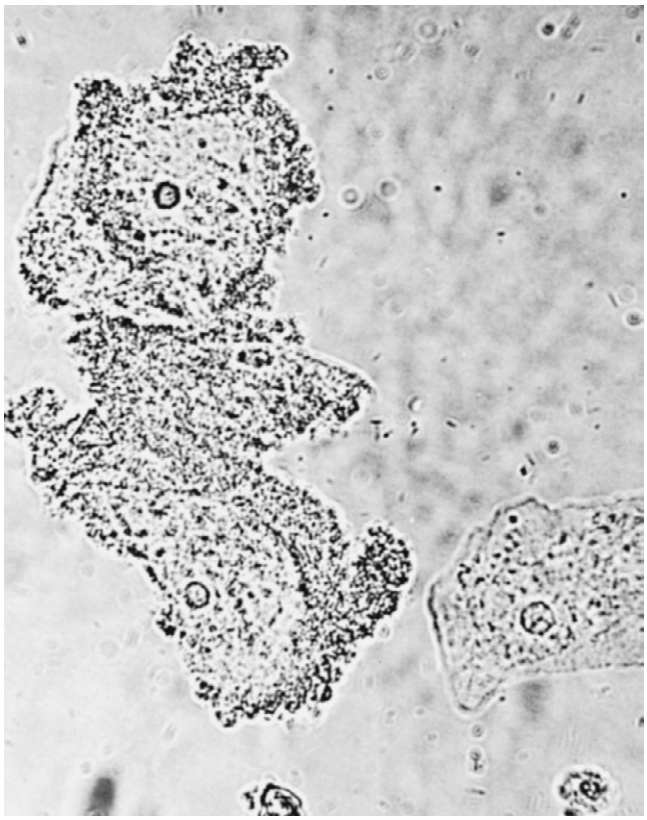

FIGURE 42-10 ■ The finding of bacteria clinging to the sides of a vaginal epithelial cell (clue cell) is significant in bacterial vaginosis. (Courtesy Herman L. Gardner, MD.)

Prepubertal

Bacterial vaginosis is defined poorly in premenarcheal girls. No published studies using the recommended diagnostic criteria exist. A few studies have used some of the criteria, however, in an attempt to examine bacterial vaginosis in premenarcheal girls. In a study examining STIs in girls aged 1 to 12 years who were evaluated for possible sexual abuse, 99 of the 245 girls with a vaginal discharge had an amine test and were examined for clue cells. Of the 99 girls, 7 (7%) had clue cells, a positive amine test, or both.[107] In a similar study, 22 of 51 girls with a history of sexual contact and a vaginal discharge had an amine test performed and were evaluated for clue cells; all of them were negative for both tests.[110] In the same study, in a second group of girls defined as being at risk for having had undetected previous sexual contact, 30 girls had a vaginal discharge and 10 had wet-mount preparations performed. One had a positive amine test and clue cells, and one had a positive amine test only. In both of these studies, the full criteria were not used, and only subgroups of girls with vaginal discharge were evaluated. In another study, 31 abused and 23 nonabused girls aged 2.5 to 13 years had vaginal washes performed along with amine tests and testing for clue cells.[88] The abused girls had an initial visit and a follow-up visit more than 7 days after the abuse occurred. The nonabused girls had only an initial evaluation. Of the 23 nonabused girls, 1 had a positive amine test with normal examination results and was asymptomatic. Similarly, 1 of the 31 abused girls had a positive amine test on the initial visit. On follow-up evaluation, clue cells and a positive amine test developed in 4 of the 31 abused girls and either clue cells or a positive amine test was noted in another 4 of the 31 girls. Either a new vaginal discharge or dysuria developed in 5 of these 8 girls. Of the 8 girls, 3 were postmenarcheal. Whether the testing itself could have been responsible for the positive results is unclear because the control group did not undergo the follow-up evaluation.[107]

In a study of premenarcheal girls older than 2 years with vulvovaginitis, none of the 50 girls or their age-matched controls had *G. vaginalis* isolated from vaginal specimens.[112] In a survey of vaginal flora in children without a vaginal discharge, *G. vaginalis* was isolated from 14 percent.[86] Prepubertal girls with a history of sexual abuse were more likely to have *G. vaginalis* isolated from vaginal specimens (15%) than were girls with no history of sexual abuse and either genitourinary complaints (4%) or no genitourinary complaints (4%).[15] In a study examining the vaginal flora of sexually abused and nonabused 3- to 10-year-old girls, 6 percent of abused versus 1 percent of nonabused girls had *G. vaginalis* in their vaginal secretions.[71] Although the prevalence of *G. vaginalis* and bacterial vaginosis is higher in sexually abused children than in nonabused children, their significance as a marker for sexual abuse is unclear. No data are available regarding the survival of *G. vaginalis* on fomites.

Until studies using the proper and complete criteria are done in young girls with and without a vaginal discharge, the prevalence of bacterial vaginosis will be unknown and the significance of bacterial vaginosis as a cause of vaginal discharge in premenarcheal girls will remain unclear. At this time, the presence of manifestations of bacterial vaginosis should prompt the health care provider to consider treatment of bacterial vaginosis. In addition, the possibility of sexual abuse should be contemplated because a diagnosis of bacterial vaginosis does not provide evidence of sexual abuse. Because bacterial cultures may be performed in the evaluation of a vaginal discharge in a premenarcheal girl and *G. vaginalis* may be identified, identification of *G. vaginalis* should not be considered evidence of sexual abuse or diagnostic of bacterial vaginosis (see Table 42-3).

Postpubertal

Bacterial vaginosis in adolescents is considered a sexually transmitted condition based on the occurrence of bacterial vaginosis with other STIs. Bacterial vaginosis also has been described in adolescent girls who are not sexually active.[31] Other risk factors are foreign bodies such as a forgotten tampon, vaginal douching,[103,224] no condom use, a new sexual partner, anal intercourse, unclean insertive sex toys, and an intrauterine device. In addition, the association among bacterial vaginosis and postpartum endometritis, premature rupture of membranes, and PID has implicated it as the cause for these gynecologic and obstetric complications.[35,226]

The primary complaint is an offensive fishy odor with moderately profuse, gray leukorrhea. A mild pruritus may be reported. Examination shows little or no vulvar or vaginal erythema. The vagina contains a thick, homogeneous, grayish-white discharge. The pH of vaginal secretions in bacterial vaginosis is 5.0 to 6.0.[35]

Diagnosis

Two methods are used to establish the diagnosis of bacterial vaginosis—Gram stain of vaginal discharge and clinical criteria,[35,58,226] with clinical criteria being used more commonly. A Gram stain of vaginal secretions is considered the most reliable diagnostic test for bacterial vaginosis. A predominance of gram-variable cocci and curved rods (anaerobes) and occasional long gram-positive rods (lactobacilli) is seen.[35] A saline wet mount of vaginal secretions is a rapid and helpful test for clue cells in a busy clinical setting (see Fig. 42-10). The Gram stain method is used to determine the relative concentrations of bacterial morphotypes and evaluate for the overgrowth of anaerobes. This method requires an examiner with expertise in evaluating specimens for bacterial vaginosis and is less practical than is the use of clinical criteria; thus, clinical criteria are used more widely.

The clinical criteria used for establishing the diagnosis of bacterial vaginosis in adolescents are the presence of any three of the four criteria[35,226]: (1) a homogeneous, gray-white malodorous discharge that smoothly coats the vaginal walls, (2) a pH of nonbloody vaginal secretions greater than 4.5, (3) a fishy odor caused by the release of amines when 10 percent potassium hydroxide is added to a nonbloody vaginal specimen (whiff test), and (4) the presence of clue cells in a nonbloody specimen. Microscopic examination of some of the discharge mixed with normal saline solution in a wet-mount preparation shows masses of desquamated vaginal epithelial cells and cellular debris. Clusters of bacteria adhere to the surface of many of the vaginal cells; these clue cells (see Fig. 42-10) are characteristic of the condition. Absence of erythrocytes and leukocytes in the discharge is another characteristic finding. Vaginal cultures for *G. vaginalis* and anaerobes are available; however, they are not useful clinically in children or adolescents and are not recommended.

Treatment

Numerous regimens for the treatment of bacterial vaginosis exist, but no specific recommendations are available for premenarcheal girls. Probably because bacterial vaginosis is diagnosed infrequently, clinical trials evaluating treatment of bacterial vaginosis in premenarcheal girls are lacking. The drugs recommended for use in young girls are those recommended for postmenarcheal women, with dosages based on the child's body weight. In small children (<45 kg), metronidazole can be given at 15 mg/kg/day divided two times per day for 7 days (maximum dose 1 g/day).[35,58]

The goal of therapy for symptomatic bacterial vaginosis in adolescents is to relieve vaginal symptoms and prevent an ecologic environment that predisposes to the development of PID. Treatment of asymptomatic bacterial vaginosis may be considered before performing a surgical abortion procedure to prevent postpartum PID. In most cases, bacterial vaginosis responds to metronidazole 500 mg orally twice per day for 7 days[35] or 2 percent clindamycin cream intravaginally every night for 7 days or 0.75 percent metronidazole gel intravaginally once per day for 5 days. Alternative regimens include clindamycin 300 mg orally twice per day for 7 days, or clindamycin ovules, 100 mg intravaginally once per day for 3 days. These regimens provide effective coverage of anaerobes and *G. vaginalis*. Bacterial vaginosis that occurs during pregnancy generally is treated during the first trimester with clindamycin cream. During the second and third trimesters of pregnancy, it can be treated with clindamycin cream or metronidazole gel. Treatment of the sexual partner has not proved to be beneficial.[35]

Infections of the Clitoris

Cellulitis with induration, edema, and erythema occasionally develops in the clitoral hood in the prepubertal and pubertal period.[58] Clitoral hood piercings can cause cellulitis in older adolescents. Staphylococci and streptococci are the most common etiologies. Oral antibiotics with efficacy against these organisms usually are effective. Warm soaks or sitz baths also may provide symptomatic relief.[58]

Clitoromegaly with erythema can occur with vulvovaginitis of any etiology but usually is associated with herpes simplex virus (HSV) infections.[54] Edematous enlargement of the clitoris and the labia without erythema has been reported in patients with Crohn disease.[146]

Urethritis

Urethritis is manifested clinically by dysuria, urinary urgency, or both and occurs particularly in sexually active postmenarcheal girls. Urethritis with internal dysuria can occur as a result of sexually transmitted infections, such as *T. vaginalis*, *N. gonorrhoeae*, and *C. trachomatis*, and has been implicated as an important cause of dysuria in sexually active girls.[200] Urethral infection by *C. trachomatis* may occur with or without cervical infection; this infection was associated with sterile pyuria in 50 percent of girls with acute-onset dysuria and frequency, and *C. trachomatis* was isolated in 31 percent of these cases.[200]

When a sexually active adolescent girl has internal dysuria, in addition to being tested for conventional uropathogens, she should be screened for common STIs, such as gonorrhea, chlamydia, and trichomonas infection.[50] Urinalysis and microscopy for the presence of leukocytes and bacteria should be done in these patients as well. For treatment of urethritis caused by sexually transmitted organisms, the sections in this chapter on trichomoniasis, gonorrhea, and chlamydial infections should be reviewed.

Bartholinitis and Bartholin Abscess

Infections of the Bartholin ducts occasionally occur in adolescent girls. The Bartholin glands are small, bean-shaped glands that lie on each side of the vaginal opening, behind the hymen. Each gland opens by means of a long single duct immediately external to the hymen.

Bartholinitis, or inflammation of the Bartholin ducts, causes pain, tenderness, and a linear rope-shaped swelling, best palpated by holding the vulvar mucosa and labia majora between the fingers. Purulent or mucoid exudate can be expressed occasionally from the Bartholin duct.

N. gonorrhoeae and *C. trachomatis* have been isolated almost a third of the time from ductal exudates in women with bartholinitis.[47,168] A Bartholin abscess does occur in sexually active adolescent girls. Risk factors for development of Bartholin abscess are similar to those for sexually transmitted infections.[3] Infection of a Bartholin cyst results in a markedly tender abscess (Fig. 42-11). The abscess can rupture spontaneously and drain foul-smelling, purulent material externally through the skin. Multiple organisms are isolated from Bartholin abscesses. An early study using percutaneous aspirates from abscesses showed predominantly anaerobes and facultative organisms. *N. gonorrhoeae* was isolated in 8 percent of cases, and gram-negative bacilli were isolated in 16 percent. Although genital Mycoplasmataceae organisms were isolated from the duct secretions, they were not isolated directly from abscesses.[130] *Porphyromonas asaccharolytica* (a black-pigmented, gram-negative anaerobe), *Salmonella panama* (after an attack of *Salmonella* enteritis), and *Mycobacterium tuberculosis* have been isolated from a Bartholin abscess.[45,51,55,104]

FIGURE 42–11 ■ Acute Bartholin duct abscess.

A Bartholin abscess should be incised and drained and not aspirated because it is likely to cause recurrent abscess formation. Incision plus drainage and primary suture of the abscess cavity along with administration of an antibiotic (clindamycin) has been found to lead to more rapid healing and decrease significantly the incidence of recurrent abscesses.[11] The cavity should be packed with sterile gauze. Alternatively, marsupialization or fistulization can be used to treat recurrent Bartholin abscesses.[219] A Word catheter inflated with water can be inserted into the cavity. Antibiotic coverage for anaerobes, *N. gonorrhoeae*, and *C. trachomatis* should be provided for at least 2 weeks, and a nonsteroidal medication is recommended for inflammation and pain. Frequent sitz baths help further with drainage and healing. Close follow-up during the first week is advised. The packing or the catheter may be removed after 4 days of antibiotics. Healing should be anticipated in 2 weeks.

VULVOVAGINAL LESIONS, ULCERATIONS, AND GRANULOMATOUS INFECTIONS

Vulvovaginal lesions, ulcerations, and granulomatous infections include genital warts, molluscum contagiosum, herpes genitalis, syphilis, chancroid, granuloma

inguinale, and tuberculosis. Human papillomavirus (HPV) and genital warts, molluscum contagiosum, and lymphogranuloma venereum will be addressed in detail in this chapter. All other conditions are described in detail in Chapters 130, 134, 143, and 157. The modes of transmission of all the previously mentioned infections and implications for child sexual abuse will also be discussed in this chapter (see Table 42-3).

Human Papillomavirus and Genital Warts

HPV is a small, slow-growing virus of the papovavirus group. HPV types 6 and 11 are responsible for 90 percent of genital warts (condyloma acuminata); HPV types 16, 18, and 31 are associated with premalignant and malignant cervical carcinoma in women, with HPV types 16 and 18 causing 70 percent of premalignant and malignant lesions.[115]

Prepubertal

Perinatal transmission of HPV from an infected mother to her infant is well documented. The incubation period after exposure to the virus may range from 1 to 20 months.[48] Data on the presence of HPV DNA in children beyond the neonatal period are inconsistent—1.2 to 27 percent in three different studies.[128,164,217] In addition, the relationship between the presence of HPV DNA and the ultimate development of disease in children is unclear. Because the exact incubation period for the development of genital lesions is unknown, perinatal transmission has been found to be the most likely cause of genital warts in almost 96 percent of patients younger than 3 years.[48] In children 3 years or older, a history of sexual abuse has been elicited in 27 to 90 percent with venereal warts.[38,97]

Genital warts are acquired by children as a result of close physical contact with an infected individual, by digital infection of the child's genitalia by an infected individual, or by sexual contact with an infected individual.[183] A history of genital warts in other members of the family, particularly the mother or older sisters who care for the child, sometimes may be elicited. Perinatal exposure, poor hygiene, and shared bathing have been suggested sources of infection.[203,204] HPV types 6, 11, 16, and 18 are the most common genital types detected in children. This finding has raised questions about warts in children resulting from sexual abuse. In many cases, however, the mode of transmission is unknown.[48] Based on failure to identify sexual abuse, a report from a dermatology clinic concluded that transmission of HPV possibly occurs by fomites.[38] No reports in the literature address survival of the virus on fomites.

Sexual abuse is the most common means of acquiring anogenital HPV infection and should be considered suspicious for sexual abuse and investigated and reported in all prepubertal children older than 3 years who are infected. In children younger than 3 years, although perinatal transmission is likely, sexual abuse should be suspected and investigated (see Table 42-3).[35]

As recommended by the CDC,[34] the possibility of sexual abuse should be considered strongly if no conclusive explanation for nonsexual transmission of an STI has been identified. When the only evidence of sexual abuse is the isolation of an organism or the detection of antibodies to a sexually transmitted agent, findings should be confirmed and implications should be considered carefully.

FIGURE 42-12 ■ Condylomata acuminata usually are single or scattered, small warts in premenarcheal children.

Genital warts (condyloma acuminata) in premenarcheal children may cover the entire vulva. Most often, however, warts in this population are single or scattered cauliflower-like lesions (Fig. 42-12). They have a predilection for the smooth, moist mucosa covering the vestibular mucosa, inner surfaces of the labia minora, and mucosa around the urethral meatus. Warts seldom become ulcerative, but those arising within the vagina may become necrotic and produce a bloody vaginal discharge. Although not tumors, genital warts should be considered in the differential diagnosis of vaginal neoplasms. HIV infection should be considered in infants and children with severe, extensive warts.[38,48,115]

Postpubertal

The prevalence of HPV in adolescents as detected by DNA isolation techniques is far higher than the prevalence of active disease. The cell-mediated or T-cell immune system seems to play an important role in whether the presence of virus results in clinically manifested disease. Cofactors of HPV infection in adolescents are herpes, cervicitis, and tobacco use. Immunosuppression from a variety of conditions, including renal transplantation, Hodgkin disease, and HIV infection, is associated with a greater likelihood of development of HPV disease.[115] With regard to the natural history of HPV infection, Moscicki and colleagues[142,143] found that high-grade squamous intraepithelial lesions (HSILs) were more likely to develop in individuals with persistent

FIGURE 42-13 ■ Condylomata acuminata may form large masses covering the vulva in postmenarcheal patients.

positive tests showing oncogenic HPV types. During the course of a 2-year period, 70 percent of cases showed regression. Daily cigarette smoking was strongly linked to the development of low-grade squamous intraepithelial lesions (LSILs).[142] Regression was more likely in young women with LSIL than in those with HSIL.

In postmenarcheal girls, genital warts usually form discrete, sessile, vegetative, wartlike growths covered with folded grayish-pink epithelium. They are found most frequently on the smooth mucosa of the vulvar vestibule. Vaginal and cervical lesions commonly accompany the lesions on the vulva. Usually, they are accompanied by more or less vaginal discharge. Most often, associated pruritus and secondary infection are present. Contact lesions frequently appear on contiguous surfaces. Huge condylomatous masses (Fig. 42-13) that completely hide the introitus can develop in adolescents; if not treated, these huge growths may invade the rectum.

Diagnosis

Diagnosis is usually made clinically. HPV testing and biopsy of warts are not recommended in children and adolescents. A biopsy may be considered if genital warts appear necrotic or the morphologic appearance is not classic for warts.

Treatment

The goal of treatment is removal of warts and amelioration of symptoms, not eradication of HPV. No therapy has been shown to eradicate HPV. When only a few lesions are present, they can be treated in the outpatient setting with 85 percent trichloracetic acid. In infants and children with extensive lesions, electrocautery, electrocoagulation, or laser treatment, general anesthesia may be needed and may result in deep scarring and distortion of the vulva. Older children and young adolescents usually tolerate cryotherapy without general anesthesia if they know that some tingling or burning sensation is associated with it. Liquid nitrogen may be used in the same manner as solid carbon dioxide. Reports in the international literature demonstrate successful treatment of extensive warts in children using imiquimod 5 percent cream.[115,133]

The therapeutic methods available are 22 to 94 percent effective in clearing exophytic genital warts, but recurrence rates are high, at least 25 percent within 3 months. Treatment seems to be more successful for genital warts that are small and present for less than 1 year.[35] When a few lesions are present on the vulvar and labial areas, they can be treated in the office setting with 10 to 25 percent podophyllin resin or a solution of 85 percent trichloroacetic acid. Urethral, anal, vaginal, and cervical lesions should not be treated with podophyllin. The medication is applied to the wart only (not to the surrounding skin), and treatment is repeated once weekly until the lesions have disappeared (usually 3 to 4 weeks). Four hours after podophyllin has been applied, the treated area should be washed off with soap and water. Self-application of 0.5 percent podofilox gel may be considered in older nonpregnant adolescents who are comfortable using this approach and can understand the treatment regimen so as to avoid dermatologic side effects.

Imiquimod 5 percent cream also is recommended for the treatment of warts in patients older than 18 years.[35] This medication is self-applied to the lesions, three times per week for 16 weeks. Laser treatment or cryocautery is recommended if the lesions do not respond to chemical cautery or if they progressively increase in size. Large perianal warts may need to be removed surgically if cryotherapy does not work. Alternative treatments for adolescents are topical 5-fluorouracil in 5 percent creams and intralesional interferon. 5-Fluorouracil is preferable for vaginal and intraurethral warts and seems to be more effective than is the laser in treating exophytic warts in the vagina.[35,48]

The first endocervical Pap smear to screen for HPV-related changes is recommended at age 21 years.[9,176] The quadrivalent (types 6, 11, 16, and 18) and bivalent (types 16, 18) HPV vaccines are approved by the U.S. Food and Drug Administration, the former to prevent genital warts and cervical cancer and the latter to prevent cervical cancer.[135]

Molluscum Contagiosum

Molluscum contagiosum is a viral infection of the skin caused by a DNA pox virus characterized by small, discrete, translucent, grayish-pink, umbilicated, wartlike papules that sometimes are surrounded by a narrow ring of erythema. The lesions, which are asymptomatic, usually are less than 5 mm in diameter and may be missed by the patient and the examiner. The disease is transmitted by close physical contact, including sexual activity,

and is encountered most often in postmenarcheal patients on the inner surfaces of the thighs, the perineum, and the lower part of the abdomen. The average incubation period is 2 to 7 weeks and can be as long as 6 months. Pediatricians frequently encounter molluscum contagiosum on the nongenital skin of children. It can be contracted from contaminated towels, bedding, and garments. Lesions of molluscum contagiosum on the lower part of the abdomen, pubis, thighs, or perineum of a child are tacit evidence of sexual contact.[39,132]

The appearance of the lesions usually is sufficient to establish the diagnosis. It can be confirmed by finding large, intracytoplasmic inclusion bodies in a smear or biopsy specimen from a lesion.

Lesions may go away on their own and usually last 6 to 8 weeks. They do not recur. A variety of treatment approaches are available if the lesions continue to spread. Curettage is the most efficacious treatment, with a low rate of side effects; however, the procedure requires anesthesia and is time-consuming. Cryotherapy gives good results. Topical imiquimod holds promise, but an optimal treatment schedule has not been determined.[35,39]

Ulcerations and Granulomatous Infections

Sexually transmitted genital ulcerative and granulomatous infections occur throughout the world but are found most frequently in tropical countries. Genital ulcers are a common finding on evaluation of adolescent and adult patients with genital symptoms. They are less common findings in young children. Ulcers usually are secondary lesions that result from the breakdown of vesicles, papules, or pustules. By the time that many patients with genital infections seek medical attention for their symptoms, the primary lesion has proceeded to ulceration.[193] Microbial causes vary by geographic region and socioeconomic status: HSV is the most common cause in Western Europe and North America, whereas chancroid is the most common in the tropics; in the United States, syphilis and chancroid are more common occurrences in urban minority groups, whereas HSV is found more commonly in more affluent groups. Complications of sexually transmitted genital ulcerative diseases also occur more commonly in developing countries and often are the reason for seeking medical care.[145]

HSV infection, syphilis, chancroid, lymphogranuloma venereum, granuloma inguinale, and tuberculosis all may have genital ulcers as a major clinical finding (Table 42-4). Although each of these diseases has a characteristic lesion and course, considerable overlap exists, so a diagnosis based on the history and physical appearance alone often is inaccurate. Herpetic ulcers, contrary to classic manifestations, can be painless, and syphilis chancres can be painful at times (see Chapters 143 and 157). Secondary infection of an ulcerated area may cause pain in lesions that characteristically are painless. In addition, more than one STI may be present in at least 3 to 10 percent of patients with genital ulcers.

Genital ulcers also may result from infestation, fixed drug eruption, mechanical or chemical trauma, autoimmune processes (e.g., Behçet syndrome and Crohn disease[146]), and neoplasia. The presence of ulcers in sites other than the genital regions or oropharynx suggests a noninfectious etiology. The vulvar lesions of Behçet syndrome appear as destructive, deep, relatively painless ulcerations that, on recurrence, can result in scarring and distortion with progressive loss of vulvar tissue.[119,165]

In the United States, most patients with genital ulcers have genital herpes, syphilis, chancroid, or a combination thereof. Empirical treatment often must be given before diagnostic test results are available, and laboratory confirmation of a specific diagnosis is lacking in at least a quarter of patients with genital ulcer disease. Treatment of syphilis and chancroid should be considered in such circumstances, especially in geographic regions where chancroid morbidity is notable (see later discussion).[34,154]

Genital ulcers and granulomatous infections of any etiology, but especially those caused by herpes, syphilis, chancroid, and granuloma inguinale, are associated with an increased risk for acquiring HIV infection. Serologic testing for HIV infection should be considered in the management of patients with genital ulcers.[35] Improved treatment of STIs can affect the rate of HIV seroconversion in a population.[81]

Perinatal transmission of HSV types 1 and 2 in the form of stomatitis occurs in infants. HSV infection of the genitalia rarely occurs in children.[91,116] The presence of genital herpes lesions in a child or any disease that is known to be sexually transmitted in an adult should raise a question about whether the child has been subjected to some type of sexual molestation. HSV types 1 and 2 have been isolated in the genital area in children alleging sexual abuse.[72,107] The mode of acquisition of the infection is not always known, but case reports describe the possibility of autoinoculation from oral lesions, physical contact by a mother's infected finger, and sexual abuse.[116] No studies have reported the coexistence of HSV genital infection in household members and infected children. HSV has been known to survive for 2 hours on latex gloves and toilet seats, 24 hours on a speculum, and 72 hours on gauze.[129] However, transmission of HSV from fomites requires direct contact of viable virus with either a mucous membrane or a break in the skin, rendering fomite transmission unlikely. In conclusion, a child with genital herpes infection should be considered suspicious and evaluated for sexual abuse, and the case should be reported to the authorities (see Table 42-3).[35]

Syphilis infections not found to be acquired congenitally should be considered sexually transmitted. Sexually acquired infection is a strong possibility in all prepubertal children with syphilis. The presence of syphilis should be considered diagnostic of sexual abuse, and an investigation must be pursued (see Table 42-3). In three studies, syphilis has been identified in 0, 0.3, and 5 percent of cases of alleged sexual abuse.[49,76,106] Syphilitic lesions and positive serologic results have been detected in alleged sexual abusers of children with syphilis.[1] No data are available on the survival of *Treponema pallidum* on fomites.

Lymphogranuloma venereum will be addressed in this chapter. The remaining listed infectious etiologies of genital ulcerative and granulomatous infections, including herpes, syphilis, chancroid, and granuloma inguinale,

TABLE 42-4 **Diagnostic Features of Genital Ulcerations Caused by Sexually Transmitted Diseases**

Feature	Primary Syphilis	Genital Herpes	Chancroid	Lymphogranuloma Venereum	Granuloma Inguinale
Incubation period	9-90 days; avg, 2-4 wk	2-7 days	1-35 days; avg, 3-7 days	3 days to 3 wk; avg, 10-14 days	Precise data unavailable; probably a few days to several months
No. lesions	Usually one; may be multiple	Multiple; may coalesce, more with primary episodes than with recurrences	Usually one to three, may be multiple	Usually single	Single or multiple
Description of genital ulcers	Sharply demarcated, round or oval ulcer with slightly elevated edges; may be irregular, symmetric "kissing chancre"	Small, superficial, grouped vesicles, erosions, or both; lesions may coalesce and form bullae or large areas of ulceration; lesions have irregular borders	Superficial, shallow, sharply demarcated ulcer; irregular, ragged, undermined edge; a few millimeters to 2 cm in diameter	Papule, pustule, vesicle, or ulcer discrete and transient; frequently overlooked	Sharply defined, irregular ulcerations or hypertrophic, verrucous, necrotic, or cicatricial granulomata
Base	Red, smooth, and shiny or crusty; oozing serous exudate when squeezed	Bright red and smooth	Rough, uneven, yellow to gray	Variable	Usually friable, rough, beefy granulations; can be necrotic, verrucous, or cicatricial
Induration	Firm; does not change shape with pressure	None	Soft; changes shape with pressure	None	Firm granulation tissue
Pain	Painless; may become tender if secondarily infected	Common; more prominent with initial infection than with recurrences	Common	Variable	Rare
Inguinal lymphadenopathy	Unilateral or bilateral, firm, movable, and nontender; does not suppurate	Usually bilateral, firm, and tender; more common in primary episodes than in recurrences	Unilateral; bilateral rarely occurs; overlying erythema; matted, fixed, and tender; suppuration may occur	Unilateral or bilateral; initially movable, firm, and tender; later indolent; fixed and matted; "sign of groove" may suppurate; fistulas	Pseudobuboes; subcutaneous perilymphatic granulomatous lesions that produce inguinal swellings
Constitutional symptoms	Rare	Common in primary episode; less likely in recurrences	Rare	Frequent	Rare
Course of untreated disease	Slowly (2-6 wk) resolves to latency	Recurrence is the rule	May progress to erosive lesions	Local lesions heal; systemic disease may progress; disfiguring; late complications	Worsens slowly
Diagnostic tests	*Treponema pallidum:* Darkfield examination, direct immunofluorescence, RPR, TPPA, FTA-ABS, VDRL	*Herpes simplex types 1 and 2:* Culture, PCR, direct immunofluorescence, Tzanck smear, electron microscopy, direct immunoperoxidase staining, serology	*Haemophilus ducreyi:* Culture, biopsy (rarely used), Gram-stained smears have low specificity	*Chlamydia trachomatis LGV 1, 2, 3:* Complement fixation, isolation of microorganism by culture	*Klebsiella granulomatis:* Donovan bodies in tissue smears; biopsy

Modified from Mroczkowski TF, Martin DH. Genital ulcer disease. *Dermatol Clin* 1994;12:753–764.[145]

avg, Average, *FTA-ABS,* fluorescent treponemal antibody absorption test; *RPR,* rapid plasma reagin; *TPPA,* treponema pallidum particle agglutination; *VDRL,* Venereal Disease Research Laboratory.

are described in more detail in Chapters 130, 134, 143, and 157.

Lymphogranuloma Venereum

Lymphogranuloma venereum is caused by *C. trachomatis*, subtypes L1 to L3. It is characterized by indolent inflammatory infiltration, granulomatous ulceration, abscess formation, and fibrotic cicatrization of the inguinal, perineal, and rectal lymphatics. It occurs more often in tropical than temperate climates.

Adolescents acquire lymphogranuloma venereum through sexual activity. Lymphogranuloma venereum has been reported in children[14,77,220] and always should raise suspicion for child sexual abuse. Nonsexual transmission by accidental inoculation of infected material from family members via handling of garments or towels that have been contaminated by drainage from ulcerative lesions or buboes of other household members has been postulated. Some evidence suggests that transplacental or perinatal transmission of the lymphogranuloma venereum serovars of *C. trachomatis* can occur.[77]

The primary lesion, a small papule or superficial and relatively asymptomatic ulcer (Fig. 42-14), seldom is seen in either children or older patients. A prodromal episode of fever, malaise, and joint pain accompanied by leukocytosis and anemia may precede the local signs. These symptoms often are mild and not diagnostically significant.

The most common manifestation is inguinal or femoral lymphadenopathy that is usually unilateral. When untreated, the adenitis may progress to abscess formation (buboes), followed by rupture, with the development of draining sinuses. Spontaneous regression sometimes occurs. Rectal exposure can lead to proctocolitis that may be associated with anal pain, mucoid or hemorrhagic rectal discharge, constipation, or tenesmus. Fever also can occur. Untreated proctocolitis can progress to colorectal fistulas and strictures over time. Reactive manifestations, as with other forms of *C. trachomatis* infection, can occur and include arthritis, usually of the knees, and erythema nodosum.[77]

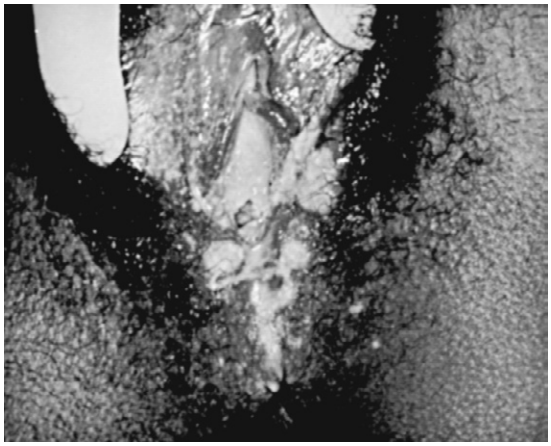

FIGURE 42-14 ■ Early lesions of lymphogranuloma venereum in the form of herpetic-like ulcers in an adolescent. The diagnosis was confirmed serologically.

Diagnosis can be made by culture, direct immunofluorescence, or nucleic acid tests of genital or lymph node specimens for *C. trachomatis*. Swabs of ulcerative lesions or aspirates of buboes may be used. Nucleic acid amplification tests are not FDA-approved for rectal specimens though some laboratories may have performed sufficient validation studies to offer these. Serum complement fixation titers greater than 1:64 can be used to support the diagnosis of lymphogranuloma venereum in appropriate clinical contexts.

Lymphogranuloma venereum may coexist with syphilis, and false-positive Venereal Disease Research Laboratory test results have been reported. Specific tests for antitreponemal antibodies should be obtained to confirm coinfection with syphilis when nontreponemal tests are positive. The disease is differentiated from other granulomatous and ulcerative disorders by specific tests for each, biopsy, and clinical appearance of the lesions (see Table 42-3).

The preferred treatment of lymphogranuloma venereum is doxycycline 100 mg orally twice per day for 21 days.[35] The alternative regimen is erythromycin base 500 mg orally four times per day for 21 days. Azithromycin 1 g orally once per week for 3 weeks is probably effective, but clinical data are lacking. A macrolide-based regimen is preferred for children younger than 8 years of age. Treatment can prevent progression of tissue damage but may not reverse scarring that has already occurred. Aspiration or incision and drainage of buboes may be required in some cases. Sexual contacts of patients with lymphogranuloma venereum within the 60 days before onset of the patient's symptoms should be evaluated and treated with a chlamydia regimen.[35]

Cervicitis

Prepubertal

The cervix is not involved when a prepubertal child has vaginitis because most vaginal infections affect only the distal half of the vagina in children; the exception is gonococcal vaginitis, which in addition usually involves the squamous epithelium over the external cervix. Cervicitis also may occur when a vaginal foreign body is present. Endocervicitis is an unusual finding in premenarcheal girls because the endocervical glands and mucosa are developed poorly before menarche, providing a poor environment for invading organisms.[59,226]

Postpubertal

The structure of the cervix during the reproductive years renders it vulnerable to infections induced by numerous factors. The long, narrow, deeply pocketed cervical canal, which is lined by open cryptic glands bathed in alkaline secretion and washed periodically by menstrual blood, is an excellent nidus for the growth of bacteria. Pathogenic bacteria in the vagina find ready access to the cervical canal. The use of oral contraceptive pills promotes cervical ectopy, increasing the vulnerability of endocervical cells to chlamydial infection.[101,102,226]

Early recognition and aggressive treatment of cervicitis in sexually active adolescents are important to prevent serious complications, including PID and tubo-ovarian abscess. Some confusion tends to occur, however, with regard to differentiating normal ectopic columnar epithelium on the exocervix (ectopy) from cervicitis. Ectopy is not an abnormal finding. When the squamocolumnar junction is exposed on the exocervix, it is called *ectopy*. It appears bright shiny red, in contrast to the dull pearly pink appearance of the exocervix. This area does not bleed easily when touched with a swab. During adolescence, the normal cervix may exhibit no ectopy, a small area of ectopy, or sometimes 50 percent ectopy. When the ectopy appears edematous, raised, and friable (often with marked bleeding when touched lightly with a swab), cervicitis should be suspected.[101,226]

Cervicitis primarily is endocervicitis most commonly associated with *C. trachomatis* and *N. gonorrhoeae*. However, the etiology in most cases is not determined and could be from *Mycoplasma genitalium*, *G. vaginalis*, group B streptococci, and *T. vaginalis*. Because of the high prevalence of coexisting infections of the cervix, associating any one organism with the clinical signs and symptoms of cervicitis often is difficult.[101,173,185]

Cervicitis often is asymptomatic, but it should be clinically suspected if an adolescent reports a vaginal discharge and bleeding, especially after having sexual intercourse. Criteria for a clinical diagnosis of mucopurulent cervicitis include the presence of mucopurulent secretion from the endocervix, erythema of the cervix, friable ectopy, and bleeding from the cervix.[28] The mucopurulent secretion in chlamydial cervicitis is tenacious mucoid material mixed with yellow exudate and is difficult to remove from the endocervix. In contrast a profuse, yellowish-green acrid discharge is present in gonococcal cervicitis. Cervical erosion ulcerations can occur in sexually active adolescents and can be caused by HSV. Rarer causes are the primary lesion of syphilis, tuberculosis, granuloma inguinale, lymphogranuloma venereum, chancroid, schistosomiasis, and actinomycosis.

Diagnosis

Tests for chlamydial and gonococcal infection should be performed in all sexually active adolescents. Nucleic acid amplification tests are considered the most sensitive and specific tests today, and testing of urine and self-obtained vaginal specimens has revolutionized screening and diagnosis. The reliability of amplification tests such as polymerase chain reaction, strand displacement amplification, and transcription-mediated amplification in detecting chlamydia and gonorrhea seems to be equivalent for vaginal, cervical, and urine specimens as long as the urine collected is a first-catch specimen; the sensitivities range from 87 to 99 percent and specificity from 97 to 100 percent.[18,178,180,190]

Other tests, including culture, direct fluorescent antibody tests, enzyme immunoassay, nucleic acid hybridization tests, enhanced optical immunoassays, and nucleic acid amplification tests, continue to be available today to diagnose *C. trachomatis* infection. The regular culture and tissue culture method was considered the gold standard for both gonorrhea and chlamydia[179] but because of the high cost of this technique, it is not used widely for adolescents. In some large-volume, community-based clinics, the DNA hybridization assay or DNA probe (Gen-Probe) and enzyme immunoassay (Abbot, Wampole, Kodak, Gen-Probe) are still used for gonorrhea and chlamydia.[122,181,190,195]

Commercial multiplex tests are available for *M. genitalium*, *U. urealyticum*, *T. vaginalis*, and *G. vaginalis*. However, the utility of testing for other organisms in adolescents is questioned, especially in clinical settings in which microscopy is readily available. However, in settings without microscopy, using commercial tests for *T. vaginalis* and *G. vaginalis* may be of value.

Although the reliability of detecting gram-negative intracellular diplococci on a Gram stain of cervical secretions is low, this test can be useful in areas where more sophisticated tests are unavailable. The presence of at least eight pairs of gram-negative intracellular diplococci in at least three polymorphonuclear cells strongly suggests gonococcal cervicitis.

Treatment

The same guidelines for the treatment of gonococcal and chlamydial cervicitis are used for the treatment of presumptive cervicitis in nonpregnant adolescents. Azithromycin 1 g orally in a single dose or doxycycline 100 mg once per day for 7 days is recommended for presumptive *C. trachomatis*, *M. genitalium*, and *U. urealyticum* cervicitis.[35] Current treatment of gonococcal infection is influenced by the emergence of antibiotic-resistant strains, including penicillinase-producing *N. gonorrhoeae*, fluoroquinolone-resistant strains, tetracycline-resistant strains, and chromosomally resistant strains.[35] The high frequency of chlamydial infection in individuals with gonorrhea (45% in certain populations) and the serious complications resulting from untreated gonorrhea and chlamydial infections also have influenced the treatment approach. Treatment options include intramuscular ceftriaxone 250 mg in a single dose plus azithromycin 1 g orally in a single dose, or doxycycline 100 mg orally twice per day for 7 days.[35]

In the case of penicillin allergy, cephalosporins are not contraindicated unless the patient has a history of a severe reaction (e.g., anaphylaxis, Stevens-Johnson syndrome, and toxic epidermal necrolysis). Spectinomycin is an alternative but is unavailable in the United States.[35] Azithromycin 2 g is effective to treat gonococcal cervicitis; however, because of emerging resistance, its use should be limited.[35] Pregnant adolescent girls or women should not be treated with doxycycline. If pharyngeal or rectal gonorrhea is suspected, the recommended treatment is intramuscular ceftriaxone 250 mg. When *T. vaginalis* vaginitis is diagnosed or HSV is suspected strongly, the appropriate treatment for these infections should be given (see discussions on trichomoniasis and genital herpes). Currently no CDC consensus exists on the treatment of asymptomatic individuals with positive tests for *M. genitalium* and *U. urealyticum*.[34]

Integral to treating the patient is notification, examination, and expedited treatment of the patient's sexual partners if gonococcal or chlamydial infection, or both, is confirmed. Direct notification by the patient should be encouraged, and, based on state laws, a prescription for partner therapy can be given. Written information should be provided with names of diseases and medication side effects to assist with this process. When tests are positive for gonorrhea or chlamydial infection, retesting for these infections is recommended—in 3 weeks if treated with erythromycin or if symptoms persist and in 3 months if treated with other regimens to detect reinfection from an untreated or new partner.[35]

Chlamydia

Chlamydia infections are the most frequently reported STIs in the United States, and the highest rates are reported in adolescents 15 to 19 years old.[34] Prevalence rates of chlamydial cervicitis in adolescents range from 8 to 25 percent in urban clinics located in hospital, community, family planning, and juvenile detention centers. It is three to four times more common than gonococcal cervicitis.[34] *C. trachomatis* causes cervicitis by infecting the columnar and transitional epithelium of the cervix. Cervical ectopy seems to be a predisposing factor for acquisition of *C. trachomatis* infection.[13] *C. trachomatis* cervicitis is predominantly an asymptomatic disease in adolescent girls.[36] A clinical diagnosis is made when friable ectopy with a mucopurulent cervical discharge (mucopus) is noted. Chlamydial cervicitis is suspected when a Gram stain of an adequate endocervical smear shows more than 5 to 10 polymorphonuclear cells per oil immersion field in the absence of gram-negative intracellular diplococci.[28,144]

Treatment of chlamydial cervicitis was discussed in the earlier section on cervicitis.

Gonorrhea

The highest rates of gonorrhea infections are reported in adolescents 15 to 19 years old.[34] The risk for acquiring infection varies with the population. Adolescents seen in private practices in the suburbs have a significantly lower rate of gonococcal cervicitis than that noted in adolescents seen in large outpatient hospital clinics or community clinics serving inner-city populations. The prevalence rate of gonorrhea in adolescent girls in urban hospital settings ranges from 3 to 12 percent in hospital, community, family planning, and juvenile detention centers.[34] Anal gonorrhea usually is secondary to discharge from gonococcal cervicitis infecting the anus. Primary anal gonorrhea as a result of anal intercourse should be suspected in a sexually active adolescent with anal discomfort. In addition, gonococcal infection should be considered in the differential diagnosis of pharyngitis in sexually active adolescents. Gonococcal endocarditis, arthritis, and dermal abscesses occur, but they are beyond the scope of this chapter.

Gonococcal cervicitis may be symptomatic or asymptomatic. Although gonococcal cervicitis is reported to be predominantly an asymptomatic condition in girls and women, many patients, after careful questioning, are found to have symptoms. The discharge is profuse and prevalent. On examination, the vulvar tissues may be inflamed and edematous. Discharge also may be seen exuding out of the urethra and paraurethral ducts in severe cases. On speculum examination, the patient has a normal-appearing cervix or the cervix appears erythematous and friable, with a foul-smelling purulent discharge draining from the cervical os.

Treatment of gonococcal cervicitis was discussed in the earlier section on cervicitis.

UPPER GENITAL TRACT INFECTIONS

Pelvic Inflammatory Disease

PID is defined as an acute clinical syndrome attributed to the ascent of microorganisms from the vagina and endocervix to the endometrium, fallopian tubes, or contiguous structures that result in pelvic and generalized peritonitis. The use of terms specifically describing the anatomic sites involved is preferable (e.g., endometritis, salpingitis, salpingo-oophoritis, tubo-ovarian abscess).

PID is an extremely rare finding in prepubertal girls and often is undiagnosed until advanced infection occurs.[27,53] With the exception of gonorrhea and the inflammation caused by a foreign body, most vulvar and vaginal infections do not involve the upper third of the vagina and do not approach the cervix of premenarcheal patients. The prepubertal cervix and endometrium apparently are barriers rather than passageways for bacteria causing all types of vulvovaginitis in children.

When intrapelvic infection has been reported in prepubertal girls, it usually has been part of either generalized primary peritonitis or peritonitis secondary to a ruptured appendix or some other intra-abdominal infection.[58] Pelvic infection that is part of an intra-abdominal infection characteristically affects the surfaces of the uterine tubes, uterus, and ovaries and produces perisalpingitis and periovaritis; periappendicitis also is present even if the infection did not begin as appendicitis. An ascending infection from the lower genital tract, such as that caused by gonorrhea, involves the tubal mucosa and produces endosalpingitis and pyosalpingitis. Gonococcal pelvic infection has been reported after sexual abuse has occurred. *S. pneumoniae* serotypes 1 and 2 and *E. coli* also have been reported in pubertal girls with salpingitis and peritonitis and tubo-ovarian abscess.[27]

PID in the postpubertal period occurs primarily in sexually active adolescents at risk for STIs.[60,190] Salpingitis has been reported in virgins, and, although the etiology is unknown, the organisms are thought to spread hematogenously, lymphatically, or transmurally from intestines or ascending infection from the vagina.[53] National epidemiologic data on PID specific to the adolescent age group are not available. However, the CDC reported a significant decline in the number of hospitalizations and initial visits to physician offices for PID among women 15 to 44 years of age between 2000 to 2009/2010.[34] This decline has been attributed to aggressive screening for chlamydia and gonorrhea infections in sexually active

adolescents in a variety of health care settings that serve urban sexually active adolescents.[34]

Reasons for the development of PID in adolescents are physiologic and social and include sexual activity and multiple sexual partners. Cervical mucus is less viscous during menses and at midcycle, rendering it more permeable to ascending infection. In vitro experiments show that *N. gonorrhoeae*, *C. trachomatis*, *U. urealyticum*, and other aerobic and anaerobic agents can adhere to spermatozoa and migrate with them.[212] Whether this event can occur in vivo is unknown. Most organisms found in nongonococcal and nonchlamydial PID also are found in bacterial vaginosis, suggesting that bacterial vaginosis may be a predisposing or a precipitating factor for the development of PID. In addition, researchers have suggested that *T. vaginalis* can act as a vector for transporting bacteria.[117] Another possible factor is upward transport of bacteria from orgasmic myometrial contractions.[155] Finally vaginal douching has been reported to be a risk factor in promoting ascending infection.[153,155,224]

Oral contraceptive pills are used commonly by adolescents and may have a protective or facilitating risk factor for PID. Laparoscopic studies also have shown that women with PID who were using oral contraceptive pills have significantly milder degrees of fallopian tube inflammation than do women who do not use the pills. This finding suggests that the oral contraceptive pill may reduce potential tubal damage. Other studies report, however, that by promoting cervical ectopy, oral contraceptives are a potential risk factor for acquiring chlamydial PID.[186,209,225] In contrast, compared with other methods of contraception, women using depot medroxyprogesterone acetate (Depo-Provera) had a significantly decreased risk for developing PID.[225] Risk for acquiring PID with the intrauterine device seems to be related to the individual's background risk for acquiring an STI and a direct complication of the device.[190]

Women who have had one episode of PID have a 20 to 25 percent chance of having subsequent ones. Reasons for this susceptibility may be reinfection from untreated sexual partners, an inadequately treated first infection, or increased susceptibility of the tubal epithelium to infection.[60,155]

Endometritis

Infections of the endometrium in postmenarcheal girls and women are as rare as previously thought. The gonococcus, on its way upward to the uterine tubes, produces a fleeting asymptomatic infection of the endometrium, or the symptoms produced are overshadowed by those of the vulvar and cervical infections. Evidence also suggests that endometritis occurs in 40 percent of patients with asymptomatic endocervical chlamydial infection.[114] It also is called *subclinical PID*, and repeated episodes have been linked to infertility.[114,221] The role of HIV infection in the development of subclinical PID is unclear. An increased prevalence of endometritis in HIV-infected women compared with uninfected women has been noted, and altered immune function might contribute to difficulty clearing endometritis in these cases.[29]

Symptoms specific to endometritis are uncommon. It is commonly an asymptomatic condition. Symptoms include recurrent intermittent or acute suprapubic pain and tenderness with vaginal bleeding or spotting. On bimanual examination, the uterus is tender, and cervical motion tenderness may or may not be present. Endometritis in adolescent girls and young women also has been associated with postabortive and puerperal endometritis. Poor compliance with prophylactic antibiotics places an adolescent at an increased risk for development of endometritis after having an abortion. Endometrial tuberculosis is a rare occurrence in the United States and is encountered most often in patients with pulmonary tuberculosis. This entity needs to be considered in HIV-infected girls and women and in geographic areas and regions where tuberculosis is endemic. In a study from a province in Iran, tuberculous endometritis was detected in 72 percent of genital tuberculosis cases in women.[151] The disease may not manifest for years after initial seeding, and the infection is thought to reach the uterus via the gastrointestinal tract through hematogenous seeding and by lymphatic spread. It is not acquired as a result of an ascending infection or through coitus with an infected man. The symptoms of endometrial tuberculosis vary from none to amenorrhea, pelvic pain, dysmenorrhea, abnormal uterine bleeding, and tuberculosis. Often, the patient has a history of healed or active pulmonary disease. Overall, 60 percent of patients with pelvic tuberculosis have tuberculous endometritis.[92]

Endometrial tuberculosis is diagnosed by endometrial biopsy and demonstration of acid-fast *M. tuberculosis* in cultures of curettage material or menstrual blood. Menstrual fluid has been reported to be culture-positive more frequently than are biopsy specimens.[92] Endometrial tuberculosis and the management of tuberculosis are described further in Chapter 96.

Salpingitis

The pathogenesis of salpingitis has been studied best with *N. gonorrhoeae* and *C. trachomatis*. In the fallopian tube, gonococci attach to nonciliated epithelial cells and induce sloughing of ciliated epithelial cells into the lumen.[155,207] Gonococci also enter the subepithelial space, where a local inflammatory response, predominantly polymorphonuclear leukocytic infiltrate and anaerobic bacteria, is produced and may reach the bloodstream. A purulent exudate is produced within the tube and may cause pelvic peritonitis. Chlamydial infection produces a similar picture except that it causes a predominantly lymphocytic infiltrate in the submucosa.[155,157,207]

Salpingitis also is caused by several other microbial agents that have been isolated directly from the fallopian tubes. The organisms most commonly recovered from the upper tract in salpingitis are mixed anaerobes (25% to 84%), followed by *N. gonorrhoeae* (25% to 40%) and *C. trachomatis* (25% to 40%). Mixed aerobic and anaerobic infections, including *Bacteroides* and *Peptostreptococcus* spp., account for 25 to 60 percent of cases. These mixed infections occur more frequently in girls and women with severe, chronic, and recurrent PID. PID develops in approximately 10 to 17 percent of girls and women with

endocervical *N. gonorrhoeae* and in 10 to 30 percent with endocervical *C. trachomatis*. At first, researchers thought that *N. gonorrhoeae* initiated the infection and that superinfection with anaerobes followed. They now realize that apparently anaerobes and aerobes can initiate PID without *C. trachomatis* or *N. gonorrhoeae*.[155,190,223]

Differentiating salpingitis caused by different microbiologic organisms is difficult. *N. gonorrhoeae* has been identified most frequently within the initial 24 hours of development of symptoms. Beyond 48 hours, the most frequent isolates are anaerobes. Gonococcal or chlamydial salpingitis seems to occur most often within 1 week of menstruation.[208] The clinical presentation varies from asymptomatic to severe disease. The severity of infection depends on duration of symptoms and the etiologic agent. Chlamydial PID tends to be less symptomatic than infection from *N. gonorrhoeae*.

Diagnosis

The classic manifestation of acute PID is lower abdominal pain, which usually is bilateral and continuous and may worsen with movement. After the onset of abdominal pain, fever, nausea, and vomiting develop. The temperature may reach 39° to 39.5° C (102° to 103° F). A chill seldom precedes the fever. Other common initial symptoms include vaginal discharge, irregular vaginal bleeding, and urinary symptoms.

On physical examination, the patient usually looks sick and uncomfortable. Abdominal examination reveals a markedly tender and often tense lower part of the abdomen. Rebound tenderness indicates generalized peritonitis. The genital examination may show a purulent vaginal discharge in the vaginal vault. Even gentle bimanual rectovaginal abdominal palpation causes great distress. Attempted mobilization of the cervix is extremely painful. The uterus is tense and tender. The adnexa may not be outlined because of discomfort produced by the examination. Palpation of the adnexa unilaterally or bilaterally may be exceptionally painful. An adnexal swelling may be palpated, but this finding is unreliable in diagnosing an adnexal mass. In a less acutely tender patient, the examination may reveal a thickened, tender tube.

Common laboratory findings in patients with PID include a peripheral white blood cell count greater than 10,000/mm^3 and an erythrocyte sedimentation rate greater than 15 mm/hr. In addition, endocervical gonorrhea and *Chlamydia* infection may be present simultaneously in 10 to 30 percent of cases. Ultrasonography of the pelvic cavity helps detect pelvic abscesses. A study involving the use of pelvic ultrasonography in adolescents with PID showed that the presence of fluid in the cul-de-sac was not helpful in differentiating patients with and without PID.[78] Almost 20 percent of patients with PID had a tubo-ovarian abscess; in the absence of a tubo-ovarian abscess, adnexal volume was significantly larger in adolescents with PID than in adolescents without it.[78] Ultrasonography (transvaginal) can be very useful in ruling out other diagnoses and defining adnexal masses.

The clinical diagnosis of PID is made by having a high index of suspicion of this entity. For practical reasons, the clinical criteria developed by Jacobsen and Westrom[111] and revised by the CDC[35] are recommended to aid in making the diagnosis and subsequently to increase the index of suspicion for PID (Table 42-5). When comparing the reliability of clinical criteria using endometrial sampling as the reference test,[35] the sensitivity of using one criterion—adnexal tenderness—was 95 percent, and the sensitivity of using three criteria—abdominal pain, cervical motion tenderness, and adnexal tenderness—was 83 percent.

TABLE 42-5 **Clinical Criteria for the Diagnosis of Acute Pelvic Inflammatory Disease**

Minimal Criteria
Lower abdominal or pelvic pain with one or more of the following: uterine or adnexal tenderness or cervical motion tenderness
Additional Criteria to Enhance Specificity
Temperature >38.3° C (>101.9° F)
Abnormal cervical or vaginal mucopurulent discharge
Presence of white blood cells on saline microscopy of vaginal secretions
Elevation of erythrocyte sedimentation rate
Elevated C-reactive protein
Laboratory documentation of cervical infection with *Neisseria gonorrhoeae* or *Chlamydia trachomatis* or both
Specific Criteria
Endometrial biopsy specimen with histopathologic evidence of endometritis
Transvaginal sonography or magnetic resonance imaging technique showing thickened fluid-filled or tubo-ovarian complex
Laparoscopic abnormalities consistent with pelvic inflammatory disease

From Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2010. *MMWR Recomm Rep* 2010;59(RR-12):1–110.

The minimum criteria recommended for empirical treatment include abdominal pain with uterine tenderness or cervical motion tenderness or adnexal tenderness.[35] The more criteria that can be met, the more likely it is that a tubal infection is present. The differential diagnosis of PID is an acute abdomen. Conditions of the urinary tract that should be considered include cystitis, pyelonephritis, and urethritis. Gastrointestinal tract conditions include appendicitis, constipation, diverticulitis, gastroenteritis, inflammatory bowel disease, and irritable bowel syndrome. Gynecologic conditions include dysmenorrhea, ectopic pregnancy, endometriosis, endometritis, mittelschmerz, torsion or rupture of an ovarian cyst, ruptured follicle, septic abortion, threatened abortion, and pyogenic sacroiliitis. As in PID, establishing an early diagnosis of acute appendicitis and ectopic pregnancy is important.

Treatment

The major goals of treatment of PID are preservation of fertility and prevention of other long-term sequelae, including ectopic pregnancy. Although some data suggest

that women younger than 25 years old have a better fertility prognosis overall after having PID and ectopic pregnancy, no difference exists among age groups regarding tubal infertility specifically after PID.[155] Earlier initiation of treatment results in lower risk for developing infertility. Girls and women initially evaluated after more than 3 days of abdominal pain are found to have a 2.8-fold increased risk for having impaired fertility (tubal infertility or ectopic pregnancy) compared with those evaluated within 3 days of the onset of abdominal pain.[100] Generally, more severe PID has a higher risk for future infertility. In addition, more episodes of PID result in a higher risk for future infertility. After one episode, the risk for infertility ranges from 8 to 13 percent; after two episodes, the risk is 20 to 35 percent; and after three or more episodes, it is 40 to 75 percent.[190,206]

To reduce the incidence of sequelae associated with this condition, early recognition of PID, the use of broad-spectrum antibiotics to treat polymicrobial disease, careful clinical reevaluation 48 hours after initiating antibiotic treatment to assess antibiotic response, and evaluation and treatment of sexual partners are important. Screening the patient for other STIs, such as trichomoniasis, bacterial vaginosis, and syphilis, also is important.

Patients with PID often are treated on an ambulatory basis. The primary reason to hospitalize an adolescent with PID is to ensure compliance with medication when she is unable to follow or tolerate an outpatient oral regimen because of the seriousness of the sequelae and problems with compliance in this age group. Other reasons for hospitalization are an uncertain diagnosis, the presence of a tubo-ovarian abscess, pregnancy, infection with HIV, temperature greater than 38.5°C (101.3°F), nausea and vomiting precluding the use of oral medications, and lack of improvement after 48 hours of oral antibiotic treatment.

The recommendations of the CDC for treating PID in ambulatory and hospitalized patients address antibiotic coverage for the polymicrobial etiology of this condition (Tables 42-6 and 42-7).[35] In addition to antibiotic treatment, bed rest is recommended. Intravenous fluids are administered for hydration when necessary. When the diagnosis of PID is certain, oral analgesic agents may be prescribed. Close follow-up is recommended, with a bimanual examination performed after 48 hours of antibiotic treatment to assess the patient's response to treatment.[35] Overall, most studies show that response to antibiotic regimens is similar between HIV-positive and HIV-negative patients. The treatment recommendations for HIV-positive patients are no different.[34] Complications associated with PID are perihepatitis, tubo-ovarian abscess, hydrosalpinx (obstruction of the tube caused by scarring [Fig. 42-15]), chronic abdominal pain from adhesions surrounding the fallopian tubes and ovaries, recurrent PID, ectopic pregnancy, and infertility.[190,215]

Perihepatitis

The classic manifestation of perihepatitis, or Fitz-Hugh–Curtis syndrome,[155] is severe right upper abdominal pain that often radiates to the shoulder. Concurrent left upper abdominal pain also may be present. Lower abdominal pain and evidence of acute or subacute PID are frequent

FIGURE 42-15 ■ Large hydrosalpinx, the result of gonorrheal salpingitis in an older teenage girl who had repeated gonorrheal infections. The other tube also was diseased.

TABLE 42-6 Ambulatory Management of Pelvic Inflammatory Disease

Ceftriaxone 250 mg IM in a single dose *or* cefoxitin 2 g IM in a single dose *plus* probenecid 1 g orally in a single dose *or* other parenteral third-generation cephalosporins (e.g., ceftizoxime or cefotaxime)
Plus
Doxycycline 100 mg PO two times a day for 14 days
With or without
Metronidazole 500 mg PO twice a day for 14 days

From Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2010. *MMWR Recomm Rep* 2010;59(RR-12):1–110.

TABLE 42-7 Inpatient Treatment of Pelvic Inflammatory Disease

Regimen A
Cefoxitin 2 g IV every 6 hr *or* cefotetan 2 g IV every 12 hr
Plus
Doxycycline 100 mg every 12 hr PO or IV
Regimen B
Clindamycin 900 mg IV every 8 hr
Plus
Gentamicin loading dose 2 mg/kg IV or IM, followed by a maintenance dose 1.5 mg/kg every 8 hr

From Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2010. *MMWR Recomm Rep* 2010;59(RR-12):1–110.

Note: The above regimens are given for at least 24 hr after the patient improves. After discharge from the hospital, the patient is continued on doxycycline 100 mg PO two times per day for a total of 14 days.

findings. The right upper quadrant pain lasts about 48 hours. Nausea, fever, and leukocytosis are common manifestations. Elevation of the erythrocyte sedimentation rate and liver enzymes may be present. The pathogenesis of perihepatitis is thought to be from direct spread of *N. gonorrhoeae* and *C. trachomatis* from the fallopian tubes into the peritoneal cavity, along the paracolic sulci. From there, they reach the subphrenic space and hepatic surface. Spread from the reproductive tract also is possible via the retroperitoneal lymphatics.[155]

The diagnosis is made by having a high index of suspicion. Perihepatitis frequently mimics cholelithiasis, hepatitis, pleuritis, subphrenic abscess, perforated peptic ulcer, nephrolithiasis, appendicitis, ectopic pregnancy, abdominal trauma, and pancreatitis. Treatment of this condition is similar to treatment of PID.

Tubo-ovarian Abscess

The formation of a tubo-ovarian abscess is a late manifestation of PID. The incidence of tubo-ovarian abscess ranges from 14 to 38 percent in hospital-based adolescents and adults with salpingitis.[78,82,124,194] Tubo-ovarian abscesses rarely are seen in adolescents and young women who are not sexually active, and alternative diagnoses involving congenital anomalies must be considered.[53,188] Organisms isolated from tubo-ovarian abscesses in adolescents who do not report sexual activity include *E. coli*, α-hemolytic streptococci and *Pasteurella multocida*. The actual cause for ascending infection in these cases is unknown. Alterations in cervical secretions, which usually serve as a barrier, have been hypothesized. The abscess typically results from a mixture of facultative and anaerobic bacteria, with facultative bacteria dominating the early phase of infection, and bacterial metabolic products producing an environment of low oxygen tension that favors the growth of anaerobic bacteria.[155]

The most common organisms recovered from tubo-ovarian abscesses in sexually active women include: *E. coli*, *Bacteroides fragilis*, other *Bacteroides* spp., *Peptostreptococcus*, *Peptococcus*, and aerobic streptococci.[167] Diagnosing the presence of a tubo-ovarian abscess clinically usually is difficult. Adolescents with a tubo-ovarian abscess tend to seek care later in their menstrual cycle than do girls without a tubo-ovarian abscess.[194] Bimanual examination frequently does not reveal a pelvic mass.[42] Four potentially useful clinical features that suggest the presence of a pelvic abscess are pain, persistent fever, adnexal tenderness (for 7 days), and an erythrocyte sedimentation rate greater than 30 mm/hr.[42] Ultrasonography of the pelvis is valuable in confirming the presence of an abscess.[78]

The prompt administration of antibiotics has reduced greatly the incidence of pelvic abscess. Most of those encountered today are in adolescents who have not had adequate care and have delayed seeking treatment.[83,198]

A conservative approach is favored for the treatment of a tubo-ovarian or ovarian abscess (i.e., bed rest, supportive care, intravenous antibiotics, and nonsteroidal analgesics).[194] This approach is appropriate when the adolescent (including an immunocompromised adolescent) is hemodynamically stable and the abscess is 9 cm or less in diameter. An abscess larger than 10 cm has a 60 percent chance, a 7- to 9-cm abscess has a 35 percent chance, and a 4- to 6-cm abscess has a 20 percent chance of requiring surgical intervention.[167] Surgical intervention may be needed during the initial period if hemodynamic instability and sepsis with imminent rupture is suspected. The choice of antibiotics should be based on effectiveness against lactamase-producing anaerobes, adequate coverage against resistant *Bacteroides* spp., penetration into the abscess, and ability to remain stable in an abscess environment. The antibiotic regimens for inpatient treatment of PID fulfill these considerations and are appropriate for the treatment of tubo-ovarian abscess (see Table 42-7). As is the case with cefoxitin, cefotetan, and clindamycin, metronidazole provides good activity against anaerobes.[35] Many hospitals prefer the use of triple antibiotics cefoxitin and gentamicin plus clindamycin or metronidazole.

A clinical response to treatment consisting of a decrease in pain, fever, and total leukocyte count should be noted in 72 hours. Pelvic ultrasonography should be repeated at this time to note any further increase in the size of the abscess. The duration of intravenous and oral antibiotic therapy for a tubo-ovarian abscess should be at least 21 days. The patient may begin oral antibiotics when she is afebrile and asymptomatic and when the size of the abscess has stabilized. The abscess either resolves without drainage or becomes encapsulated. The latter eventually "points" either in the cul-de-sac or anteriorly in the abdominal wall.

The fertility rate after treatment of tubo-ovarian abscesses may be 20 to 50 percent with conservative medical and surgical approaches.

Oophoritis

Oophoritis is a rare condition today and refers to inflammation of the substance of the ovaries, the oocytes in particular. Viral exanthems such as mumps and cytomegalovirus can be complicated by oophoritis, especially in immunosuppressed children and adolescents. Improved immunization practices have decreased the prevalence of mumps and its complications, including oophoritis. However, an outbreak of mumps in children occurred in Centerville, Ohio, in 1981.[205] The severity of mumps (including oophoritis) was higher in children who had not been previously immunized in contrast to those who were immunized against mumps.[205] The presence of an enlarged, tender, boggy, smooth, mobile ovary in a child with mumps or one of the exanthems suggests oophoritis. Treatment is palliative, with analgesics given for discomfort and fever.

NEW REFERENCES SINCE THE SIXTH EDITION

8. American Academy of Pediatrics. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
9. American College of Obstetrics and Gynecology, Committee on Practice Bulletins—Gynecology. Cervical cytology screening. Practice Bull. *Obstet Gynecol* 2009;**114**:1409–20.
22. Bramley M. Study of female babies of women entering confinement with vaginal trichomoniasis. *Br J Vener Dis* 1976;**52**:58–62.

34. Centers for Disease Control and Prevention. *Sexually Transmitted Disease Surveillance 2010*. Atlanta: U.S. Department of Health and Human Services; 2011.
35. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2010. *MMWR Recomm Rep* 2010;**59** (No.RR-12):1–110.
39. Colven R. Generalized cutaneous manifestations of STD and HIV infection: Typical presentations, differential diagnosis, and management. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 1177–98.
40. Cox RA. *Haemophilus influenzae:* an underrated cause of vulvovaginitis in young girls. *J Clin Pathol* 1997;**50**:765–8.
41. Cox RA, Slack MPE. Clinical and microbiological features of *Haemophilus influenzae* vulvovaginitis in young girls. *J Clin Pathol* 2002;**55**:961–4.
102. Holmes KK, Stamm WE. Lower genital tract infection syndromes in women. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 987–1016.
115. Kahn JA, Hillard PA. Human papillomavirus infection in children and adolescents. In: Emans SJ, Laufer MR, Goldstein DP, editors. *Pediatric & Adolescent Gynecology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 649–684.
155. Paavonen J, Westrom L, Eschenbach D. Pelvic inflammatory disease. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008.
166. Randelović G, Mladenović V, Ristić L, et al. Microbiological aspects of vulvovaginitis in prepubertal girls. *Eur J Pediatr* 2012;**171**:1203–8.
176. Sawaya GF. Cervical-cancer screening: new guidelines and the balance between benefits and harms. *N Engl J Med* 2009;**361**: 2503–5.
192. Šikanić-Dugić N, Pustišek N, Hiršl-Hećej V, et al. Microbiological findings in prepubertal girls with vulvovaginitis. *Acta Dermatovenerol Croat* 2009;**17**:267–72.
205. Sullivan KM, Halpin TJ, Kim-Farley R, et al. Mumps disease and its health impact: an outbreak-based report. *Pediatrics* 1985;**76**: 533–6.
219. Wechter ME, Wu JM, Marzano D, et al. Management of Bartholin duct cysts and abscesses. *Obstet Gynecol Surv* 2009;**64**: 395–404.
226. Woods ER, Emans SJ. Vulvovaginal complaints in the adolescent. In: Emans SJ, Laufer MR, Goldstein DR, editors. *Pediatric & Adolescent Gynecology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 525–64.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ackerman AB, Goldfaden G, Cosmides JC. Acquired syphilis in prepubertal children. *Arch Dermatol* 1972;**106**:92–3.
2. Adu-Sarkodie Y. *Trichomonas vaginalis* transmission in a family. *Genitourin Med* 1995;**71**:199–200.
3. Aghajanian A, Bernstein L, Grimes DA. Bartholin's duct abscess and cyst: a case-control study. *South Med J* 1994;**87**:26–9.
4. Al-Salihi FL, Curran JP, Wang J. Neonatal *Trichomonas vaginalis*: report of three cases and review of the literature. *Pediatrics* 1974;**53**:196–200.
5. Alexander ER. Misidentification of sexually transmitted organisms in children: medicolegal implications. *Pediatr Infect Dis* 1988;**7**:1–2.
6. Alexander WJ, Griffith H, Housch JG, et al. Infections in sexual contacts and associates of children with gonorrhea. *Sex Transm Dis* 1983;**11**:156–8.
7. Altchek A. Vulvovaginitis, vulvar skin disease, and pelvic inflammatory disease. *Pediatr Clin North Am* 1981;**28**:397–432.
8. American Academy of Pediatrics. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
9. American College of Obstetrics and Gynecology, Committee on Practice Bulletins—Gynecology. Cervical cytology screening. Practice Bull. 109. *Obstet Gynecol* 2009;**114**:1409–11.
10. Amsel R, Totten PA, Speigel CA, et al. Nonspecific vaginitis: diagnostic criteria, microbial and epidemiologic associations. *Am J Med* 1983;**74**:14–22.
11. Andersen PG, Christensen S, Detlefsen GJ, et al. Treatment of Bartholin's abscess: marsupialization versus incision, curettage and suture under antibiotic cover—a randomized study with 6 months' follow up. *Acta Obstet Gynecol Scand* 1992;**71**:59–62.
12. Argent AC, Lachman PI, Hanslo D, et al. Sexually transmitted diseases in children and evidence of sexual abuse. *Child Abuse Negl* 1995;**19**:1303–10.
13. Arya OP, Mallinson H, Goddard AD. Epidemiological and clinical correlates of chlamydial infection of the cervix. *Br J Vener Dis* 1981;**57**:118–24.
14. Banov Jr L. Rectal lesions of lymphogranuloma venereum in childhood. *Am J Dis Child* 1952;**83**:600–2.
15. Bartley DL, Morgan L, Rimsza ME. *Gardnerella vaginalis* in prepubertal girls. *Am J Dis Child* 1987;**141**:1014–7.
16. Bass HN. Bubble bath as an irritant to the urinary tract of children. *Clin Pediatr* 1968;**7**:174.
17. Bell TA, Stamm WE, Wang SP, et al. Chronic *Chlamydia trachomatis* infections in infants. *JAMA* 2001;**267**:400–2.
18. Black CM. Current methods of laboratory diagnosis of *Chlamydia trachomatis* infections. *Clin Microbiol* 1997;**10**:160–84.
19. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
20. Bogaerts J, Leparge P, De Clercq A, et al. Shigella and gonococcal vulvovaginitis in prepubertal Central African girls. *Pediatr Infect Dis* 1992;**11**:890–2.
21. Boisvert P, Walcher D. Hemolytic streptococcal vaginitis in children. *Pediatrics* 1948;**2**:24–9.
22. Bramley M. Study of female babies of women entering confinement with vaginal trichomoniasis. *Br J Vener Dis* 1976;**52**:58–62.
23. Branch G, Paxton R. A study of gonococcal infections among infants and children. *Public Health Rep* 1965;**80**:347–52.
24. Braverman P, Polaneczky M. Adolescent gynecology. I. Common disorders. *Pediatr Clin North Am* 1999;**46**:489–503.
25. Braverman P, Polaneczky M. Adolescent gynecology. II. The sexually active adolescent. *Pediatr Clin North Am* 1999;**46**:649–70.
26. Brown JL. Hair shampooing technique and pediatric vulvovaginitis. *Pediatrics* 1989;**83**:146.
27. Brown-Harrison MC, Christenson JC, Harrison AM, Matlak ME. Group A streptococcal salpingitis in a prepubertal girl. *Clin Pediatr Phila* 1995;**34**:556–8.
28. Brunham RC, Paavonen J, Stevens CE, et al. Mucopurulent cervicitis: the ignored counterpart in women of urethritis in men. *N Engl J Med* 1984;**311**:1–6.
29. Bukusi EA, Cohen CR, Stevens CE, et al. Effects of human immunodeficiency virus 1 infection on microbial origins of pelvic inflammatory disease and on efficacy of ambulatory oral therapy. *Am J Obstet Gynecol* 1999;**181**:1374–81.
30. Bump RC. Chlamydia trachomatis as a cause of prepubertal vaginitis. *Obstet Gynecol* 1985;**65**:384–8.
31. Bump RC, Buesching WJ. Bacterial vaginosis in virginal and sexually active adolescent females: evidence against exclusive sexual transmission. *Am J Obstet Gynecol* 1988;**158**:935–9.
32. Burgess JA. *Trichomonas vaginalis* infection from splashing in water closets. *Br J Vener Dis* 1963;**39**:248–50.
33. Burry V. Gonococcal vulvovaginitis and possible peritonitis in prepubertal girls. *Am J Dis Child* 1971;**121**:536–7.
34. Centers for Disease Control and Prevention. *Sexually Transmitted Disease Surveillance 2010*. Atlanta: U.S. Department of Health and Human Services; 2011.
35. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2010. *MMWR Recomm Rep* 2010;**59** (No.RR-12):1–109.
36. Chacko MR, Lovchik JC. *Chlamydia trachomatis* infection in sexually active adolescents: prevalence and risk factors. *Pediatrics* 1984;**73**:836–40.
37. Charles SX. Epidemiology of *Trichomonas vaginalis* in rural adolescent and juvenile children. *J Trop Pediatr* 1991;**37**:90.
38. Cohen BA, Honig P, Androphy E. Anogenital warts in children. *Arch Dermatol* 1990;**126**:1575–80.
39. Colven R. Generalized cutaneous manifestations of STD and HIV infection: typical presentations, differential diagnosis, and management. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 1177–88.
40. Cox RA. *Haemophilus influenzae*: an underrated cause of vulvovaginitis in young girls. *J Clin Pathol* 1997;**50**:765–8.
41. Cox RA, Slack MPE. Clinical and microbiological features of *Haemophilus influenzae* vulvovaginitis in young girls. *J Clin Pathol* 2002;**55**:961–4.
42. Cromer BA, Brandstaetter LA, Fischer RA, et al. Tubo-ovarian abscess in adolescents. *Adolesc Pediatr Gynecol* 1990;**3**:21–4.
43. Crowther I. *Trichomonas vaginitis* in infancy. *Lancet* 1962;**1**:1074.
44. Cuadros J, Mazon A, Martinez R, et al. The aetiology of paediatric inflammatory vulvovaginitis. *Eur J Pediatr* 2004;**163**:105–7.
45. Cummins AJ, Atia WA. Bartholin's abscess complicating food poisoning with *Salmonella panama*: a case report. *Genitourin Med* 1994;**70**:46–8.
46. Danesh IS, Stephen JM, Gorbach J. Neonatal *Trichomonas vaginalis* infection. *J Emerg Med* 1995;**13**:51–4.
47. Davies JA, Res E, Hobson D. Isolation of *Chlamydia trachomatis* from Bartholin's ducts. *Br J Vener Dis* 1978;**54**:409–13.
48. Davis AM, Emans SJ. Human papilloma virus in the pediatric and adolescent patients. *J Pediatr* 1989;**115**:1–9.
49. DeJong AR. Sexually transmitted diseases in sexually abused children. *Sex Transm Dis* 1986;**13**:123–6.
50. Demetriou E, Emans SJ, Masland RP. Dysuria in adolescent girls: urinary tract infection or vaginitis? *Pediatrics* 1982;**70**:299–301.
51. Dhall K, Das SS, Dey P. Tuberculosis of Bartholin's gland. *Int J Gynaecol Obstet* 1995;**48**:223–4.
52. Dhar V, Roker K, Adhami Z. et al. Streptococcal vulvovaginitis in girls. *Pediatr Dermatol* 1993;**10**:366–7.
53. Dogan E, Altuyurt S, Altindog T, et al. Tubo-ovarian abscess mimicking ovarian tumor in a sexually inactive girl. *J Pediatr Adolesc Gynecol* 2004;**17**:351–2.
54. Donald FE, Slack DB, Colman G. *Streptococcus pyogenes* vulvovaginitis in children in Nottingham. *Epidemiol Infect* 1991;**106**: 459–65.
55. Duerden BI. Black-pigmented gram-negative anaerobes in genitourinary tract and pelvic infections. *FEMS Immunol Med Microbiol* 1993;**6**:223–7.
56. Eigen L. Vaginal diphtheria. *J Med Assoc NJ* 1932;**29**:778.
57. Emans SJ. Office evaluation of the child and adolescent. In: Emans SJH, Laufer MR, Goldstein DP, editors. *Pediatric & Adolescent Gynecology*. Philadelphia: Lippincott-Raven; 2005. p. 1–50.
58. Emans SJ. Vulvovaginal problems in the prepubertal child. In: Emans SJ, Laufer MR, Goldstein DP, editors. *Pediatric & Adolescent Gynecology*. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 83–119.
59. Emans SJ, Laufer MR, Goldstein DP, editors. *Pediatric & Adolescent Gynecology*. Philadelphia: Lippincott Williams & Wilkins; 2005.
60. Eschenbach DA. Acute pelvic inflammatory disease. *Urol Clin North Am* 1984;**11**:65–81.

61. Fallon P, Robinson ET. Meningococcal vulvovaginitis. *Scand J Infect Dis* 1974;**6**:295–6.
62. Figueroa-Colon R, Grunow JE, Torres-Pinedo T, et al. Group A streptococcal proctitis and vulvovaginitis in a prepubertal girl. *Pediatr Infect Dis J* 1984;**3**:439–42.
63. Fink CW. A perineal rash in Kawasaki disease. *Pediatr Infect Dis* 1983;**2**:140–1.
64. Fischer G. Chronic vulvitis in pre-pubertal girls. *Australas J Dermatol* 2010;**51**:118–23.
65. Fischer G, Rogers M. Vulvar disease in children: a clinical audit of 130 cases. *Pediatr Dermatol* 2000;**17**:1–6.
66. Folland DS, Burke RE, Hinman AR, et al. Gonorrhea in preadolescent children: an inquiry into source of infection and mode of transmission. *Pediatrics* 1977;**60**:153–6.
67. Forssner H. Vaginaltresia mit diptherischer pathogenese. *Acta Med Scand* 1923;**59**:690.
68. Fuld G. Gonococcal peritonitis in a prepubertal child. *Am J Dis Child* 1968;**115**:621–22.
69. Fuster C, Neinstein LS. Vaginal *Chlamydia trachomatis*: prevalence in sexually abused pubertal girls. *Pediatrics* 1987;**79**:235–8.
70. Gardner H, Dukes C. A newly defined specific infection previously classified "non-specific" vaginitis. *Am J Obstet Gynecol* 1955; **69**:962–76.
71. Gardner JJ. Comparison of the vaginal flora in sexually abused and nonabused girls. *J Pediatr* 1992;**120**:872–7.
72. Gardner M, Jones JG. Genital herpes acquired by sexual abuse of children. *J Pediatr* 1984;**104**:243–4.
73. Gerstner GJ, Grunberger W, Boschitsch E, et al. Vaginal organisms in prepubertal children with and without vulvovaginitis. *Arch Gynecol* 1982;**231**:247–52.
74. Gilbaugh JH, Fuchs PC. The gonococcus and the toilet seat. *N Engl J Med* 1979;**301**:91–3.
75. Ginsberg CM. Group A streptococcal vaginitis in children. *Pediatr Infect Dis J* 1982;**1**:36–7.
76. Girardet RG, Lahoti S, Howard LA, et al. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 2009;**124**:79–86.
77. Goh BT, Forster GE. Sexually transmitted diseases in children: Chlamydia oculo-genital infection. *Genitourin Med* 1993;**69**: 213–21.
78. Golden N, Cohen H, Gennari G, et al. The use of pelvic ultrasonography in evaluation of adolescents with pelvic inflammatory disease. *Am J Dis Child* 1987;**141**:1235–8.
79. Gray LA, Kutcher E. Vulvovaginitis in childhood. *Clin Obstet Gynecol* 1960;**3**:165–74.
80. Gregory JE, Abramson E. Meningococci in vaginitis. *Am J Dis Child* 1971;**121**:423.
81. Grosskurth H, Mosha F, Todd J, et al. Impact of improved treatment of sexually transmitted diseases on HIV infection in rural Tanzania: randomised controlled trial. *Lancet* 1995;**346**:530–6.
82. Hager WD. Followup of patients with tubo-ovarian abscess(es) in association with salpingitis. *Obstet Gynecol* 1983;**61**:680–4.
83. Hager WD, Eschenbach DA, Spence MR, et al. Criteria for diagnosis and grading of salpingitis. *Obstet Gynecol* 1983;**61**:113–4.
84. Hammerschlag MR. *Chlamydia trachomatis* in children. *Pediatr Ann* 1994;**23**:349–53.
85. Hammerschlag MR. Sexually transmitted diseases in sexually abused children: medical and legal implications. *Sex Transm Infect* 1998;**74**:167–74.
86. Hammerschlag MR, Rettig PJ, Shields WE. False-positive results with the use of chlamydial antigen detection tests in the evaluation of suspected sexual abuse in children. *Pediatr Infect Dis* 1988;**7**: 11–4.
87. Hammerschlag MR, Alpert S, Rosner I, et al. Microbiology of the vagina in children: normal and potentially pathogenic organisms. *Pediatrics* 1978;**62**:57–62.
88. Hammerschlag MR, Cummings M, Doraiswamy B, et al. Nonspecific vaginitis following sexual abuse in children. *Pediatrics* 1985;**75**:1028–31.
89. Hammerschlag MR, Doraiswamy B, Alexander ER, et al. Are rectogenital chlamydial infections a marker of sexual abuse in children? *Pediatr Infect Dis* 1984;**3**:100–4.
90. Hammerschlag MR, Doraiswamy B, Cox P, et al. Colonization of sexually abused children with genital mycoplasmas. *Sex Transm Dis* 1987;**14**:23–5.
91. Hare M, Mowla A. Genital herpesvirus infection in a prepubertal girl. *Br J Dermatol Gynaecol* 1977;**84**:141–2.
92. Hassoun A, Jacquette G, Huang A, et al. Female genital tuberculosis: uncommon presentation of tuberculosis in the United States. *Am J Med* 2005;**118**:1295–6.
93. Hedlund P. Acute vulvovaginitis in patients with streptococcal infections. *Nord Med* 1953;**49**:566–8.
94. Heller RH, Joseph JM, Davis HJ. Vulvovaginitis in the premenarcheal child. *J Pediatr* 1969;**74**:370–7.
95. Hellgren L. Gonorre-tonsillit efter genitooral kontakt. *Lakartidningen* 1971;**68**:569–71.
96. Henderson P, Scott R. Foreign body vaginitis caused by toilet tissue. *Am J Dis Child* 1966;**111**:529–32.
97. Herman-Giddens ME, Gutman LT, Berson NL. Association of coexisting vaginal infections and multiple abusers in female children with genital warts. *Sex Transm Dis* 1987;**1**:63–7.
98. Hill GB, St Claire KK, Gutman LT. Anaerobes predominate among the vaginal microflora of prepubertal girls. *Clin Infect Dis* 1995;**20**(suppl 2):269–70.
99. Hill GB, Eschenbach DA, Holmes KK. Bacteriology of the vagina. *Scand J Urol Nephrol* 1984;**86**(suppl):23–39.
100. Hillis SD, Joesoef R, Marchbanks PA, et al. Delayed care of pelvic inflammatory disease as a risk factor for impaired fertility. *Am J Obstet Gynecol* 1993;**168**:1503–9.
101. Holmes KK, Stamm WE. *Lower Genital Tract Infection Syndromes in Women*. New York: McGraw-Hill; 1999.
102. Holmes KK, Stamm WE. Lower genital tract infection syndromes in women. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 987–1016.
103. Holzman C, Leventhal JM, Qiu H, et al. Factors linked to bacterial vaginosis in nonpregnant women. *Am J Pub Health* 2001;**91**: 1664–70.
104. Huffman JW, Dewhurst CJ, Capraro VJ. *The Gynecology of Childhood and Adolescence*. Philadelphia: W.B. Saunders; 1981.
105. Hyman RW, Fukushima M, Diamond L, et al. Microbes on the human vaginal epithelium. *Science* 2005;**102**:7952–7.
106. Ingram DL. *Neisseria gonorrhoeae* in children. *Pediatr Ann* 1994; **23**:341–5.
107. Ingram DL, Everett VD, Lyna PR, et al. Epidemiology of adult sexually transmitted disease agents in children being evaluated for sexual abuse. *Pediatr Infect Dis* 1992;**11**:945–50.
108. Ingram DL, Runyan DK, Collins AD, et al. Vaginal *Chlamydia trachomatis* infection in children with sexual contact. *Pediatr Infect Dis J* 1984;**3**:97–9.
109. Ingram DL, White ST, Durfee MF, et al. Sexual contact in children with gonorrhea. *Am J Dis Child* 1982;**136**:994–6.
110. Ingram DL, White ST, Occhiuti AR, et al. Childhood vaginal infections: association of *Chlamydia trachomatis* with sexual contact. *Pediatr Infect Dis J* 1986;**5**:226–9.
111. Jacobsen L, Westrom L. Objectivized diagnosis of acute pelvic inflammatory disease. *Am J Obstet Gynecol* 1969;**105**:1088–99.
112. Jaquiery A, Stylianopoulos A, Hogg G, et al. Vulvovaginitis: clinical features, aetiology, and microbiology of the genital tract. *Arch Dis Child* 1999;**81**:64–7.
113. Jones JG, Yamauchi T, Lambert B. *Trichomonas vaginalis* infestation in sexually abused girls. *Am J Dis Child* 1985;**139**:846–7.
114. Jones RB, Mammel JB, Shepard MK, et al. Recovery of *Chlamydia trachomatis* from the endometrium of women at risk for chlamydial infection. *Am J Obstet Gynecol* 1986;**155**:35–9.
115. Kahn JA, Hillard PA. Human papillomavirus infection in children and adolescents. In: Emans SJ, Laufer MR, Goldstein DR, editors. *Pediatric & Adolescent Gynecology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 649–684.
116. Kaplan KM, Fleisher GR, Paradise JE, et al. Social relevance of genital herpes simplex in children. *Am J Dis Child* 1984;**138**: 872–4.
117. Keith LG, Berger GS, Edelman DA, et al. On the causation of pelvic inflammatory disease. *Am J Obstet Gynecol* 1984;**149**: 215–24.
118. Kessel JF, Thomspon CF. Survival of *Trichomonas vaginalis* infestation in vaginal discharge. *Proc Soc Exp Biol Med* 1950;**74**:755–8.
119. Kim DK, Chang SN, Bang D, et al. Clinical analysis of 40 cases of childhood-onset Behçet's disease. *Pediatr Dermatol* 1994;**11**: 95–101.

120. Kokx NP, Comstock JA, Facklam RR. Streptococcal perianal disease in children. *Pediatrics* 1987;**80**:659–63.
121. Krech T, Gerhard-Fsadni D, Hoffman N, et al. Interference of *Staphylococcus aureus* in the detection of *Chlamydia trachomatis* by monoclonal antibodies. *Lancet* 1985;**1**:1161–2.
122. Krowchuk DP, Anglin TM, Lembo RM, et al. Use of enzyme immunoassay for the rapid diagnosis of *Chlamydia trachomatis* endocervical infection in female adolescents. *J Adolesc Health Care* 1988;**9**:296–300.
123. Kurnatowska A, Komorowska A. Urogenital trichomoniasis in children. In: Honigsberg BM, editor. *Trichomonads Parasites in Humans*. New York: Springer-Verlag; 1989. p. 246.
124. Landers DV, Sweet RL. Tubo-ovarian abscess: contemporary approach to management. *Rev Infect Dis* 1983;**5**:876–84.
125. Lang W. Pediatric vaginitis. *N Engl J Med* 1955;**253**:1153–60.
126. Larsen B, Galask RP. Vaginal microbial flora: composition and influences of host physiology. *Ann Intern Med* 1982;**96**:926–30.
127. Larsen B, Monif GRG. Understanding the bacterial flora of the female genital tract. *Clin Infect Dis* 2001;**32**: e69–77.
128. Larson J, Kaye JM, Jewers RJ, et al. Perinatal transmission and persistence of human papillomavirus types 16 and 18 in infants. *J Med Virol* 1995;**47**:209–18.
129. Larson T, Bryson YJ. Fomites and herpes simplex virus. *J Infect Dis* 1985;**151**:746–7.
130. Lee Y-H, Rankin JS, Alpert S, et al. Microbiological investigation of Bartholin's gland abscesses and cysts. *Am J Obstet Gynecol* 1977;**129**:150–3.
131. Littlewood JM, Kohler HG. Urinary tract infection by *Trichomonas vaginalis* in a newborn baby. *Arch Dis Child* 1966;**41**: 693–5.
132. Lynch P. Molluscum contagiosum venereum. *Clin Obstet Gynecol* 1972;**15**:966–75.
133. Majewski S, Pniewski T, Malejczyk M, Jablonska S. Imiquimod is highly effective for extensive, hyperproliferative condyloma in children. *Pediatr Dermatol* 2003;**20**:440–2.
134. Mardh P-A, Stenberg K. Long-term vaginal carriage of chlamydia? *Lancet* 1987;**1**:804.
135. Markowitz LE, Dunne EF, Saraiya M, et al. Centers for Disease Control and Prevention Advisory Committee on Immunization Practices (ACIP). Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007; **56**:1–24.
136. McCormack WW, Alpert SA, McComb DE, et al. Fifteen-month follow-up study of women infected with *Chlamydia trachomatis*. *N Engl J Med* 1979;**300**:123–5.
137. Macfarlane DE, Sharma DP. *Haemophilus influenzae* and genital tract infections in children. *Acta Paediatr Scand* 1987;**76**:363–4.
138. McLaren LC, Davis LE, Healy GR, James CG. Isolation of *Trichomonas vaginalis* from the respiratory tract of infants with respiratory disease. *Pediatrics* 1983;**71**:888–90.
139. Mishaw CO. Sexual abuse and sexually transmitted diseases in prepubertal children. *Semin Pediatr Infect Dis* 1993;**4**:131.
140. Morris CA. Seasonal variation of streptococcal vulvo-vaginitis in an urban community. *J Clin Pathol* 1971;**24**:805–7.
141. Morrison JC, Fish SA. Adolescent genital dermatoses. *South Med J* 1976;**69**:1136–40.
142. Moscicki AB, Shiboski S, Broering J, et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr* 1998;**132**: 277–84.
143. Moscicki AB, Hills N, Shiboski S, et al. Risks for incident human papilloma virus infection and low-grade squamous intraepithelial lesion development in young females. *JAMA* 2001;**285**: 2995–3002.
144. Moscicki B, Shafer MA, Millstein SG, et al. The use and limitations of endocervical gram stains and mucopurulent cervicitis as predictors for *Chlamydia trachomatis* in female adolescents. *Am J Obstet Gynecol* 1987;**157**:65–71.
145. Mroczkowski TF, Martin DH. Genital ulcer disease. *Dermatol Clin* 1994;**12**:753–64.
146. Muram D, Savage MO, Harries JT, et al. Crohn's disease of the vulva in a prepubertal girl. *Pediatr Adolesc Gynecol* 1983;**1**:189–95.
147. Murphy TV, Nelson JD. Shigella vaginitis: report of 38 patients and review of the literature. *Pediatrics* 1979;**63**:511–6.
148. Nahamias AJ, Dowdle WR, Naib ZM, et al. Genital infection with herpes virus hominis types 1 and 2 in children. *Pediatrics* 1968;**42**:659–66.
149. Nair P, Glazer-Semmel E, Gould C, et al. *Neisseria gonorrhoeae* in asymptomatic prepubertal household contacts of children with gonococcal infection. *Clin Pediatr* 1986;**25**:160–3.
150. Nair S, Schoeneman MJ. Acute glomerulonephritis with group A streptococcal vulvovaginitis. *Clin Pediatr* 2000;**39**:721–2.
151. Namavar JB, Parsanezhad ME, Grane-Shirazi R. Female genital tuberculosis and infertility. *Int J Gynaecol Obstet* 2001;**75**: 269–72.
152. Neinstein LS, Goldenring J. Nonsexual transmission of sexually transmitted diseases: an infrequent occurrence. *Pediatrics* 1984;**74**: 67–76.
153. Ness RB, Soper DE, Holley RL, et al. Douching and endometritis: results from the PID evaluation and clinical health (PEACH) study. *Sex Transm Dis* 2001;**28**:240–5.
154. O'Farrell N, Hoosen AA, Coetzee KD. Genital ulcer disease: accuracy of clinical diagnosis and strategies to improve control in Durban, South Africa. *Genitourin Med* 1994;**70**:7–11.
155. Paavonen J, Westrom L, Eschenbach D. Pelvic inflammatory disease. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 1017–50.
156. Paradise JE, Campos JM, Friedman HM. Vulvovaginitis in premenarchal girls: clinical features and diagnostic evaluation. *Pediatrics* 1982;**70**:193–8.
157. Patton DC. Immunopathy and histopathology of experimental chlamydial salpingitis. *Rev Infect Dis* 1985;**7**:746–53.
158. Peter R, Vesely K. *Kindergynakologie*. Leipzig, Germany: Georg Thieme; 1966.
159. Pierce AM, Hart CA. Vulvovaginitis: causes and management. *Arch Dis Child* 1992;**67**:509–12.
160. Pokorny S. Prepubertal vulvaginopathies. *Obstet Gynecol Clin North Am* 1992;**19**:39–58.
161. Porder K, Sanchez N, Roblin PM, et al. Lack of specificity of Chlamydiazyme for detection of vaginal chlamydial infection in prepubertal girls. *Pediatr Infect Dis J* 1989;**8**:385–60.
162. Postlethwaite RJ. *Trichomonas* vaginitis and *Escherichia coli* urinary infection in a newborn infant. *Clin Pediatr* 1975;**14**:866–7.
163. Potterat JJ, Markewich GS, King RD, et al. Child-to-child transmission of gonorrhea: report of asymptomatic genital infection in a boy. *Pediatrics* 1986;**70**:711–2.
164. Puranen M, Yliskoski M, Saarikoski S, et al. Vertical transmission of human papilloma virus from infected mothers to their newborn babies and persistence of the virus in childhood. *Am J Clin Oncol* 1996;**174**:694–9.
165. Rakover Y, Ada H, Tal I, et al. Behçet disease: long-term follow-up of three children and review of the literature. *Pediatrics* 1989; **83**:986–92.
166. Randelović G, Mladenović V, Ristić L, et al. Microbiological aspects of vulvovaginitis in prepubertal girls. *Eur J Pediatr* 2012;**171**:1203–8.
167. Reed SD, Landers DV, Sweet RL. Antibiotic treatment of tubo-ovarian abscess: comparison of broad-spectrum beta-lactam agents versus clindamycin-containing regimens. *Am J Obstet Gynecol* 1991;**164**:1556–61.
168. Rees E. Gonococcal bartholinitis. *Br J Vener Dis* 1967;**43**:150–6.
169. Rein MF. Survival of gonococci outside the body. *N Engl J Med* 1979;**301**:1347.
170. Rettig PJ, Nelson JD. Genital tract infection with *Chlamydia trachomatis* in prepubertal children. *J Pediatr* 1981;**99**:206–10.
171. Rey-Stocker I. Vulvitis and vaginitis in the infant. *Gynaecologia* 1969;**168**:413–5.
172. Riordon T, Ellis DA, Mathews PI, et al. False positive results with an ELISA for detection of Chlamydia antigen. *J Clin Pathol* 1986;**39**:1276–7.
173. Rosenfeld WD, Clark J. Vulvovaginitis and cervicitis. *Pediatr Clin North Am* 1989;**36**:489–511.
174. Rothburn MM, Mallinson H, Mutton KJ. False-positive EIA for *Chlamydia trachomatis* recognized by atypical morphology on fluorescent staining. *Lancet* 1986;**2**:982–3.
175. Saikku P, Puolakkainen M, Nissinen A. Cross reactivity between Chlamydiazyme and *Acinetobacter* strains. *N Engl J Med* 1986;**314**: 922–3.

176. Sawaya GF. Cervical-cancer screening: new guidelines and the balance between benefits and harms. *N Engl J Med* 2009;**361**: 2503–5.
177. Schachter J, Dattel BJ. Sexually transmitted diseases in victims of sexual assault. *N Engl J Med* 1987;**316**:1023–5.
178. Schachter J, Stephens RS. Biology of *Chlamydia trachomatis*. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 555–74.
179. Schachter J, Grossman M, Sweet RL, et al. Prospective study of perinatal transmission of *Chlamydia trachomatis*. *JAMA* 1986; **255**:3374–7.
180. Schachter J, Stamm WE, Quinn TC, et al. Ligase chain reaction to detect *C. trachomatis* infection of the cervix. *J Clin Microbiol* 1994;**32**:2540–3.
181. Schubiner HH, Lebar W, Jemal C, et al. Comparison of three new nonculture tests in the diagnosis of Chlamydia genital infections. *J Adolesc Health Care* 1990;**11**:505–9.
182. Schwartz RH, Wientzen RL, Barsanti RG. Vulvovaginitis in prepubertal girls: the importance of group A Streptococcus. *South Med J* 1982;**75**:446–7.
183. Seidel J, Zonang J, Totten E. Condylomata acuminata as a sign of sexual abuse in children. *J Pediatr* 1979;**95**:553–4.
184. Sgroi S. Pediatric gonorrhea beyond infancy. *Pediatr Ann* 1979;**8**:326–36.
185. Shafer MA, Beck A, Blain B, et al. *Chlamydia trachomatis*: important relationships to race, contraception, lower genital tract infection, and Papanicolaou smear. *J Pediatr* 1984;**104**:141–6.
186. Shafer MA, Sweet RL, Ohm-Smith MJ, et al. Microbiology of the lower genital tract in postmenarcheal adolescent girls: differences by sexual activity, contraception, and presence of nonspecific vaginitis. *J Pediatr* 1985;**107**:974–81.
187. Shapiro RA, Schubert CJ, Siegel RM. Neisseria gonorrhea infection in girls younger than 12 years of age evaluated for vaginitis. *Pediatrics* 1999;**104**: e72.
188. Shavell VI, Abdallah ME, Triest JA, et al. Ureterocele misdiagnosed as pelvic inflammatory disease in an adolescent. *Obstet Gynecol* 2009;**113**:522–5.
189. Shore WB, Winkelstein JA. Nonvenereal transmission of gonococcal infections to children. *J Pediatr* 1971;**79**:661–3.
190. Shrier LA. Bacterial sexually transmitted infections: Gonorrhea, chlamydia, pelvic inflammatory disease and syphilis. In Emans SJ, Laufer MR, Goldstein DR, editors. *Pediatric & Adolescent Gynecology*. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 565–614.
191. Siegel RM, Schubert CJ, Myers PA, Shapiro RA. The prevalence of sexually transmitted diseases in children and adolescents evaluated for sexual abuse in Cincinnati: rationale for limited STD testing in prepubertal girls. *Pediatrics* 1995;**96**:1090–4.
192. Šikanić-Dugić N, Pustišek N, Hiršl-Hećej V, et al. Microbiological findings in prepubertal girls with vulvovaginitis. *Acta Dermatovenerol Croat* 2009;**17**:267–72.
193. Silber T. Genital ulcer syndrome. *Semin Adolesc Med* 1986;**2**: 155–62.
194. Slap GB, Forke CM, Cnaan A, et al. Recognition of tubo-ovarian abscess in adolescents with pelvic inflammatory disease. *J Adolesc Health* 1996;**18**:397–403.
195. Smith KR, Ching S, Lee H, et al. Evaluation of ligase chain reaction for use with urine for identification of *N. gonorrhoeae* in females attending a sexually transmitted disease clinic. *J Clin Microbiol* 1995;**33**:455–7.
196. Sokol AB, Min DS. Trichomonas cystitis in a six-week old infant-effective treatment with metronidazole. *J Indiana State Med Assoc* 1972;**65**:1084–6.
197. Spear RM, Rothbaum RJ, Keating JP, et al. Perianal streptococcal cellulitis. *J Pediatr* 1985;**107**:557–9.
198. Spence MR, Adler J, McLellan R. Pelvic inflammatory disease. *J Adolesc Health Care* 1990;**11**:304–9.
199. Srivastava A. Survival of gonococci in urethral secretions with reference to the nonsexual transmission of gonococcal infection. *J Med Microbiol* 1980;**13**:593–6.
200. Stamm WE, Wagner KF, Amsel R, et al. Causes of acute urethral syndrome in women. *N Engl J Med* 1980;**303**:409–15.
201. Straumanis JP, Bocchini JA. Group A beta-hemolytic streptococcal vulvovaginitis in prepubertal girls: a case report and review of the past twenty years. *Pediatr Infect Dis J* 1990;**9**:845–8.
202. Stricker T, Navratil F, Sennhauser FH. Vulvovaginitis in prepubertal girls. *Arch Dis Child* 2003;**88**:324–6.
203. Stringel G, Spence J, Corsini L. Genital warts in children. *Can Med Assoc J* 1985;**132**:1397–8.
204. Stumph PG. Increasing occurrence of condyloma acuminata in premenarcheal children. *Obstet Gynecol* 1980;**56**:262–4.
205. Sullivan KM, Halpin TJ, Kim-Farley R, et al. Mumps disease and its health impact: an outbreak-based report. *Pediatrics* 1985;**76**: 533–6.
206. Sweet RL. Pelvic inflammatory disease and infertility in women. *Infect Dis Clin North Am* 1987;**1**:199–215.
207. Sweet RL, Banks J, Sung M, et al. Experimental chlamydial salpingitis in the guinea pig. *Am J Obstet Gynecol* 1980;**138**:952–6.
208. Sweet RL, Banks J, Sung M, et al. The occurrence of chlamydial and gonococcal salpingitis during the menstrual cycle. *JAMA* 1986;**255**:2062–4.
209. Swensson L, Westrom T, Mardh P-A. Contraceptives and acute salpingitis. *JAMA* 1984;**251**:2553–5.
210. Taylor-Robinson D, Thomas BJ, Osborn MF. Evaluation of enzyme immunoassay (Chlamydiazyme) for detecting *Chlamydia trachomatis* in genital tract specimens. *J Clin Pathol* 1987;**40**: 194–9.
211. Thompson C, Macdonal M, Sutherland A. A family cluster of *Chlamydia trachomatis* infection. *BMJ* 2001;**322**:1473–4.
212. Toth A, O'Leary WM, Ledger W. Evidence for microbial transfer by spermatozoa. *Obstet Gynecol* 1982;**59**:556–9.
213. Tunnessen Jr WW, Jastermski M. Prepubescent gonococcal vulvovaginitis. *Clin Pediatr (Phila)* 1974;**13**:675–6.
214. Vandeven AM, Emans J. Vulvovaginitis in the child and adolescent. *Pediatr Rev* 1993;**14**:141–7.
215. Washington AE, Sweet RL, Shafer MB. Pelvic inflammatory disease and its sequelae in adolescents. *J Adolesc Health Care* 1985;**6**:298–310.
216. Watkins S, Quan L. Vulvovaginitis caused by *Yersinia enterocolitica*. *Pediatr Infect Dis J* 1984;**3**:444–5.
217. Watts DM, Koustdky LA, Holmes KK, et al. Low risk of perinatal transmission of human papillomavirus: results from a prospective cohort study. *Am J Obstet Gynecol* 1998;**178**:365–73.
218. Weaver J. Non-gonorrheal vulvovaginitis due to gram-negative intracellular diplococci. *Am J Obstet Gynecol* 1950;**60**:257–60.
219. Wechter ME, Wu JM, Marzano D, et al. Management of Bartholin duct cysts and abscesses. *Obstet Gynecol Surv* 2009;**64**: 395–404.
220. Weinstock H, Keesol S. Lymphogranuloma venereum: report of 24 cases in children. *Urol Cutan Rev* 1946;**50**:520–2.
221. Weisenfeld HC, Hillier SL, Krohn MA, et al. Lower genital tract infection and endometritis: insight into subclinical pelvic inflammatory disease. *Obstet Gynecol* 2002;**100**:456–63.
222. Weissenbacher G, Wiltschke H. Chronic urinary tract infections and vulvitis in girls with high posterior commissure. *Padiatr Padol* 1974;**9**:60–5.
223. Westrom L, Eschenbach D. Pelvic inflammatory disease. In: Holmes KK, Sparling PF, Mardh PA, et al, editors. *Sexually Transmitted Disease*. New York: McGraw-Hill; 1999. p. 783–809.
224. Wolner-Hanssen P, Eschenbach DA, Paavonen J, et al. Association between vaginal douching and acute pelvic inflammatory disease. *JAMA* 1990;**263**:1936–41.
225. Wolner-Hanssen P, Sensoon L, Mardh P-A. Laparoscopic findings and contraceptive use in women with signs and symptoms suggestive of acute salpingitis. *Obstet Gynecol* 1985;**66**:233–8.
226. Woods ER, Emans SJ. Vulvovaginal complaints in the adolescent. In: Emans SJ, Laufer MR, Goldstein DR, editors: *Pediatric & Adolescent Gynecology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2005. p. 525–64.
227. Woods CR. Gonococcal infections in children and adolescents. *Semin Pediatr Rev* 1993;**14**:141–7.

SECTION VI

GASTROINTESTINAL TRACT INFECTIONS

CHAPTER 43

Esophagitis

Paul Krogstad • Robert S. Venick

Infectious esophagitis is distinct from other gastrointestinal infections in that it is a rare finding in previously healthy individuals, is predominantly of fungal and viral etiology, and usually is an indicator of a primary or secondary immunodeficiency state. In nonimmunocompromised children, infectious esophagitis frequently is associated with conditions that compromise the esophageal defense mechanisms.[46]

In view of the numerous predisposing conditions, the incidence of infectious esophagitis in children is difficult to quantify. It has been reported frequently after patients have undergone chemotherapy or radiation therapy for hematologic malignancies and after solid organ and hematopoietic stem cell transplantation. Infectious esophagitis has been reported in 3 percent of patients with ataxia-telangiectasia.[29] Before the availability of highly active antiretroviral therapy (HAART), 8 to 10 percent of children infected with human immunodeficiency virus (HIV) developed *Candida* esophagitis.[4,7,37] Despite the availability of HAART, *Candida* esophagitis remains one of the most common opportunistic infections in HIV-infected children and generally is associated with low $CD4^+$ T-cell count (<100/μL), prior episodes of oropharyngeal candidiasis, high plasma HIV-1 load, and neutropenia (<500/μL).[7,8,45]

PATHOPHYSIOLOGY AND CAUSATIVE ORGANISMS

The defense mechanisms of the esophagus against infection include motility (continuous flow of luminal contents, which discourages colonization by microbes), a mucosal lining of stratified squamous epithelium that is resistant to microbial invasion, and local and systemic immune responses. Disruption of any of these protective factors may lead to esophageal infections. Immunosuppression (as seen in patients with the acquired immunodeficiency syndrome [AIDS] or after organ transplantation), chronic mucocutaneous candidiasis, malignancy, chemotherapy, and prolonged corticosteroid treatment are the most common predisposing factors for fungal and viral infection of the esophagus.[6-8,19,27,37] In immunocompetent children, dysmotility of the esophagus, in addition to mucosal injury secondary to gastroesophageal reflux, can render the esophagus vulnerable to infections.

Most esophageal infections are caused by fungi and viruses.[3] *Candida* spp. (especially *Candida albicans*), herpes simplex virus (HSV), and cytomegalovirus (CMV) are the major pathogens. The esophagus can be infected by local invasion (*Candida*, HSV, bacteria), as a result of systemic infection (CMV, *Candida*, *Pneumocystis*), or by contiguous spread from the mediastinum or neck (tuberculosis, retropharyngeal abscess).[4,37,46] Infections with fungi such as *Pneumocystis*, *Aspergillus*, and *Histoplasma* spp.; viruses such as varicella-zoster virus (VZV), human papillomaviruses, Epstein-Barr virus, and HIV; and protozoa such as *Cryptosporidium* and *Leishmania donovani* are less frequent, yet well-documented causes of esophagitis.*

Bacterial infections of the esophagus probably are underreported. Ten to 16 percent of esophageal infections can have a bacterial etiology, mostly in granulocytopenic patients and as secondary infection in fungal or viral esophagitis. Gram-positive and gram-negative oropharyngeal bacterial flora and *Mycobacterium tuberculosis* are the common pathogens causing esophagitis.[13,44] *Helicobacter pylori* has been isolated from involved tissue in Barrett esophagitis. This "infection" seems, however, to be related more to gastroesophageal reflux disease rather than to direct invasion of the esophagus by *H. pylori*.[31]

CLINICAL FEATURES

Patients with infectious esophagitis can present with esophageal, abdominal, or systemic symptoms (Table 43-1). Oropharyngeal lesions suggestive of HSV or *Candida* may serve as a clue that esophageal involvement is also present. Odynophagia and dysphagia are the most common symptoms of esophagitis, but they may not be apparent in small children.[7,8,35] In adults, 59 to 79 percent of patients with documented esophageal infection had these symptoms.[3] Children also may describe a sensation of food "sticking" behind the sternum or a feeling of a food or liquid bolus passing through the chest.

Nearly all patients with AIDS and oral candidiasis and odynophagia have endoscopic evidence of esophageal candidiasis.[5,7,39] Some patients ultimately proven to have esophagitis have no signs or symptoms at presentation, however. Children who develop esophageal candidiasis despite being treated with HAART are less likely to have typical symptoms (e.g., odynophagia and retrosternal pain) or to have concomitant oropharyngeal candidiasis.[8] Similarly, approximately one fourth of adult patients with esophageal candidiasis are asymptomatic.[3]

Drooling is an unusual manifestation in esophagitis per se but may be present with pharyngeal involvement.

*References 9, 14, 15, 17, 18, 23, 30, 34, 41.

TABLE 43-1 **Signs and Symptoms of Infectious Esophagitis**

Dysphagia
Odynophagia
Oral lesions
Nausea/vomiting
Abdominal pain
Fever
Diarrhea
Cough
Rash
Hematemesis

Fever is caused mostly by systemic or secondary infection, such as disseminated CMV disease or tuberculosis, or complications that occur after esophageal perforation. Fever does not occur commonly in bacterial esophagitis. Cough is characteristic of tuberculosis, tracheobronchial fistulas, or high-grade esophageal obstruction.

Nausea and vomiting are associated more commonly with CMV esophagitis. The abdominal pain in esophagitis may be caused by referred pain from inflammation of the distal one third of the esophagus, associated gastritis (as in CMV infection), or concomitant intra-abdominal infections in immunocompromised hosts. A maculopapular truncal rash and fever may be present in patients with idiopathic esophageal ulceration during acute HIV seroconversion.[34] Diarrhea can be present in CMV infection (diffuse involvement of the gastrointestinal tract) or HIV infection (opportunistic enteral infections, HIV enteropathy). In one report of fungal esophagitis in children, hematemesis was the most common initial symptom.[46] Finally, because physicians seldom seek evidence of esophagitis in the absence of dysphagia or odynophagia, infectious esophagitis may be underdiagnosed in infants and children.

Oral lesions are seen in patients with *Candida*, HSV, or HIV esophagitis, but they rarely are present with CMV infection and tuberculosis. Oral thrush is a common occurrence, however, in infants and immunocompromised patients. The frequency of *Candida* esophagitis in infants with oral thrush is unknown. The presence of oral thrush and esophageal symptoms does not exclude concomitant esophageal infection with pathogens other than *Candida* or the esophageal ulcerations associated with acute HIV infection. Oropharyngeal lesions are less common findings in HSV esophagitis than in *Candida* esophagitis. Recurrent cold sores, vesicular lesions on the nasolabial folds, and esophageal symptoms sometimes are seen as initial features of HSV esophagitis in immunocompetent adults and children, but their absence does not exclude HSV infection.[3,35]

DIFFERENTIAL DIAGNOSIS

Esophagitis can be caused by the following mechanisms: gastroesophageal reflux, chemical irritation (including medications or caustic ingestions), immunologic factors (i.e., cow's milk protein allergy), traumatic factors (nasogastric tube), radiation therapy, cancer chemotherapy, systemic disease (e.g., Crohn disease or chronic granulomatous disease), eosinophilic esophagitis, or motility disorders.

"Pill" esophagitis may be identified by history. Pathologic gastroesophageal reflux is the most common cause of esophagitis in children. In previously healthy children, esophageal symptoms are likely to be caused by reflux esophagitis, whereas in immunocompromised patients the physician needs to rule out infectious esophagitis. Secondary bacterial or fungal infections can be present in reflux esophagitis and Chagas disease, especially with severe inflammation and obstruction. Absence of reflux symptoms (long-standing heartburn, a water brash taste in the mouth, vomiting, spitting up [in infants], pillow wetting, or coughing) tends to exclude reflux esophagitis. Achalasia, diffuse esophageal spasm, foreign body impaction, and mediastinal or retropharyngeal abscesses can cause esophageal symptoms and may result in secondary infection. Besides imaging and endoscopy (see later), other available tools include 24-hour intraesophageal pH probe, esophageal impedance, and manometry.

DIAGNOSIS

Establishing a specific diagnosis is essential for management of infectious esophagitis, particularly because fungal and bacterial superinfections occur commonly in the setting of viral esophagitis. Although the clinical profile, barium esophagogram, and endoscopic appearance can provide some clue as to the etiology of esophagitis, histopathology, immunohistochemistry, and culture of endoscopic and brush biopsy specimens are essential for confirmation of specific pathogens. Serology for HSV, CMV, or Epstein-Barr virus may help establish the diagnosis in some cases by providing evidence of acute infection. DNA detection methods also may provide evidence of viremia with these herpesviruses. Detection of fungal or viral esophagitis in an ostensibly healthy host should prompt an evaluation for the presence of primary or secondary immunodeficiency states, including HIV infection.

Barium Esophagography

The usefulness of an esophagogram in diagnosing infectious esophagitis is limited because it does not separate specific causes; normal or nonspecific findings also can be found in some cases of esophagitis. These studies are useful for assessing dysmotility, which can be a predisposing factor for esophagitis, and for excluding obstruction, perforation, and fistulas of the esophagus. Barium studies are not indicated if endoscopy is planned. Double-contrast barium esophagography with air and barium, which details the mucosal lining, is the radiologic investigation of choice, but many children may not tolerate this procedure.[25]

Candida esophagitis generally is diffuse, whereas HSV or CMV lesions are found more frequently in the mid to distal portion of the esophagus. Discrete longitudinal

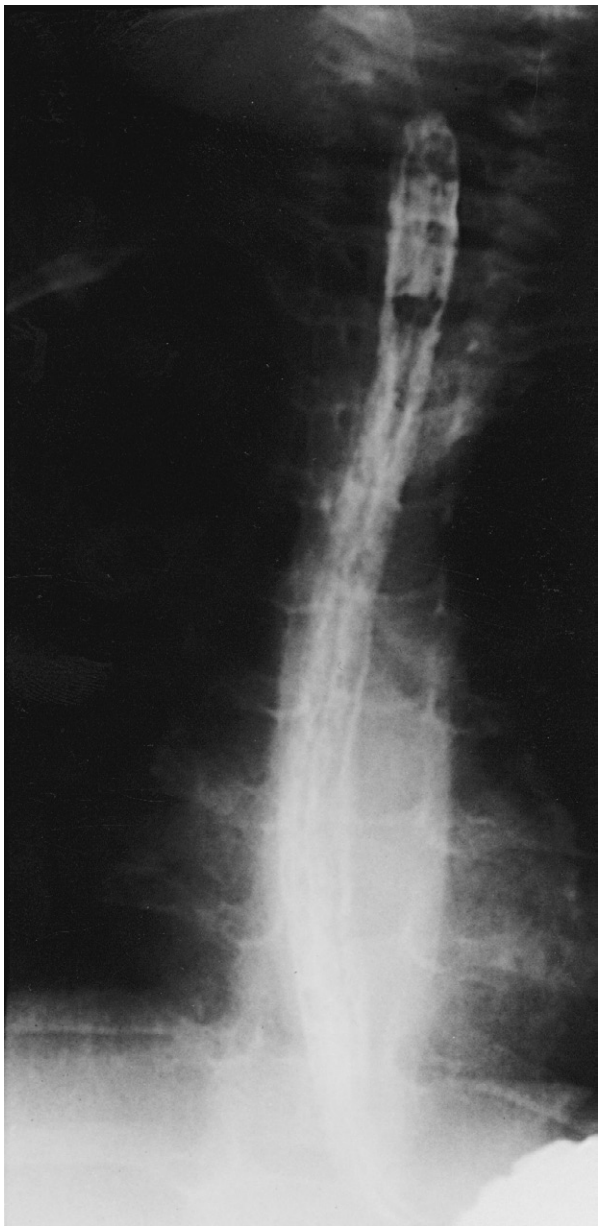

FIGURE 43-1 ■ *Candida* esophagitis. Barium esophagogram shows diffuse mucosal irregularity suggestive of inflammation and longitudinal filling defects suggestive of plaques. (Courtesy Sjirk Westra, MD, Division of Pediatric Radiology, David Geffen School of Medicine at UCLA, Los Angeles.)

plaques, a grossly irregular or "shaggy" appearance, or tiny nodular lesions with a granular radiographic appearance are characteristic of *Candida* esophagitis (Fig. 43-1). The presence of discrete superficial stellate ulcers in the mid esophagus with normal-appearing surrounding mucosa is characteristic of HSV esophagitis, whereas CMV lesions may mimic HSV lesions on barium esophagography. Oval or elongated large ulcers are found mostly in CMV infection. Idiopathic esophageal ulceration is found in HIV infection.[24,43] Esophagograms of patients with tuberculosis can show intramural pseudodiverticula, extrinsic compression, or esophageal displacement by mediastinal lymph nodes and sinus tracts.[10]

FIGURE 43-2 ■ Endoscopic image of *Candida* esophagitis. Examination of the distal one third of the esophagus reveals whitish plaques with superficial ulceration of the esophageal mucosa. (See companion website on Expert Consult for a color version of this figure.)

Esophagoscopy

The diagnosis of esophagitis can be made on upper endoscopy with biopsies, although esophageal brushings often are of greater diagnostic yield. The technique for performing endoscopy with video-endoscopes miniaturized to a diameter of 4.8 mm has made the procedure far more tolerable in immunocompromised patients, infants, and small children. Such instruments allow for procedures to be performed with greater technical care, and these instruments have biopsy capabilities.

Characteristic macroscopic lesions are associated with some infectious agents, but the features of these lesions overlap considerably, and histopathologic or immunohistochemical analysis (or both) of endoscopic and brush biopsy specimens is essential for specific diagnosis. A diffuse esophageal lesion is characteristic of *Candida* infection, whereas CMV and HSV infections mainly involve the distal part of the esophagus. White, longitudinal plaques adhering to the mucosa are characteristic of *Candida* infection (Fig. 43-2).[20] Plaques from oral thrush, common findings in infants and immunocompromised children, can be washed away and reveal a nonulcerated underlying mucosa. Similar-appearing plaques also may be seen in CMV, HSV, bacterial, and "pill" esophagitis and after sucralfate ingestion.

Small, 1- to 3-mm vesicles are characteristic of HSV esophagitis, but by the time endoscopy is done these vesicles usually slough off and reveal sharply demarcated ulcers with a raised edge, necrotic base, and normal-appearing surrounding mucosa.[35] In progressive disease, these ulcers may coalesce to resemble *Candida* esophagitis.[1] Multiple superficial ulcers in the distal portion of the esophagus often are seen in CMV esophagitis. Large elongated ulcers also are typical manifestations of CMV infection, but they may occur in idiopathic esophageal ulcerations in HIV infection as well.[24] Complete

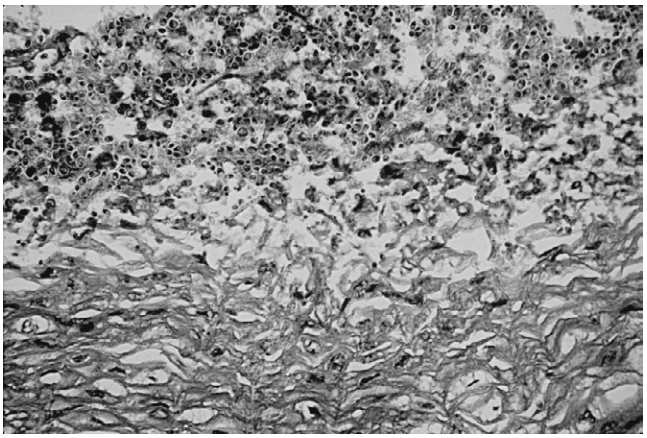

FIGURE 43-3 ■ *Candida* esophagitis. Photomicrograph shows yeastlike organisms in the esophageal mucosa. (Methenamine silver.) (Courtesy Klaus Lewin, MD, Division of Pediatric Radiology, David Geffen school of Medicine at UCLA, Los Angeles.)

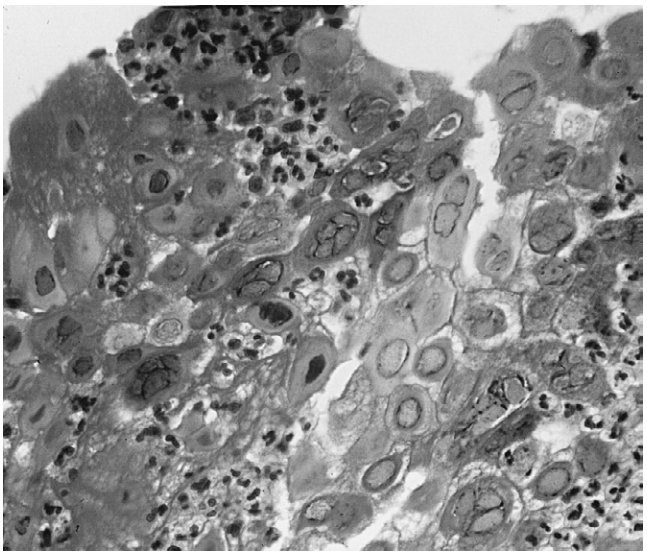

FIGURE 43-4 ■ Herpes simplex virus esophagitis. Photomicrograph shows viral inclusions in squamous epithelium. (Hematoxylin-eosin.) (Courtesy Klaus Lewin, MD, Department of Pathology, UCLA Medical Center, Los Angeles.)

denudation of the mucosa is an unusual finding with CMV infection.[40] Endoscopy done in patients with VZV esophagitis can show vesicles, discrete ulcers, or necrotizing esophagitis, depending on the stage of the disease. Tubercular ulcers of the esophagus usually are of varying size, distinct, and shallow with a necrotic base.

Endoscopic biopsy specimens should be obtained from the edge and the base of the lesions. The pathologist should be alerted to the possibility of fungal, viral, and polymicrobial infection. Appropriate fixatives should be used for routine hematoxylin and eosin stain, Gram stain, and special stains for fungi and bacteria such as *Mycobacterium*. *Candida* and *Aspergillus* can be shown by silver stain, periodic acid–Schiff stain, or Gram stain (Fig. 43-3). Diagnostic histopathologic changes, such as multinucleated giant cells, ballooning degeneration, intranuclear Cowdry type A inclusion bodies, and margination of chromatin in HSV infection (Fig. 43-4) and

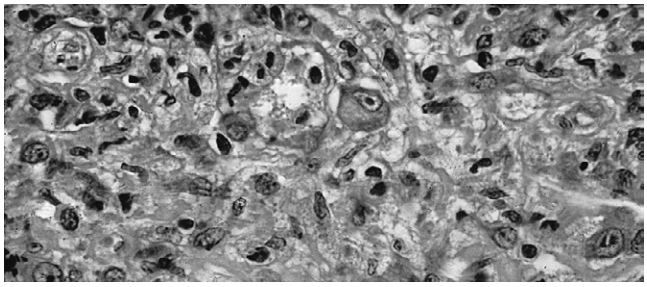

FIGURE 43-5 ■ Cytomegalovirus esophagitis. Photomicrograph shows intracytoplasmic inclusion bodies in the lamina propria. (Hematoxylin-eosin.) (Courtesy Klaus Lewin, MD, Department of Pathology, David Geffen School of Medicine at UCLA, Los Angeles.)

amphophilic intranuclear inclusions and small multiple cytoplasmic inclusion bodies in CMV infection (Fig. 43-5), can be diagnostic. Immunohistochemical studies and DNA hybridization techniques often are required to establish the diagnosis, however.

Viral culture, with or without immunohistochemical techniques, may aid in confirmation. Material obtained by endoscopy-guided brush biopsy can reveal the features of *Candida* or viral infection described earlier. If abdominal pain, fever, or other unusual symptoms are present, the possibility of disseminated or abdominal infection should be excluded by appropriate investigations.

TREATMENT

Candida Esophagitis

Treatment of esophageal candidiasis requires systemic antifungal therapy. Topical therapy may produce an initial response, but early treatment failures are common.[33] Systemic therapy with newer triazole medications (fluconazole, itraconazole solution, or voriconazole) is now the preferred approach.[32] Numerous trials have been done comparing the azole agents, most often in adults with AIDS. Patients are less likely to respond and more likely to experience drug-related toxicity when treated with ketoconazole compared with fluconazole.[11] Oral fluconazole and itraconazole suspension seem comparable in efficacy for initial therapy, and some patients whose disease fails to respond to fluconazole may see improvement with subsequent itraconazole therapy.[12,36,44] Regardless of the initial severity of esophagitis, voriconazole and fluconazole seemed comparable in efficacy in one study, although toxicity occurred more commonly with voriconazole.[2,8] However, the pharmacologic interactions between triazole antifungal medications with immunosuppressive medications and other drugs may be problematic in some patients.

Overall, experience with the triazole medications supports recommendations for the initial use of oral fluconazole therapy for *Candida* esophagitis. Therapy generally is given for 14 to 21 days. Intravenous administration of azole medications may be used for patients with severe dysphagia.[26,32]

Infection with azole-resistant variants of *Candida*, including species with intrinsic or acquired resistance to

fluconazole (e.g., *Candida krusei*), may occur.[2,8] Although pediatric data currently are limited, large-scale studies in adults have shown that intravenous therapy with echinocandin agents (caspofungin, anidulafungin, or micafungin) is an alternative to azole therapy.[26,32,42]

Intravenous amphotericin B deoxycholate remains an alternative for patients with disease that is refractory to treatment with azole medications.[21,32] Nephrotoxicity is a major toxic effect of amphotericin B, but brief, low-dose therapy (7 to 14 days, 0.3 to 0.7 mg/kg/day) generally is sufficient to treat esophagitis. Lipid formulations of amphotericin B also may be useful for treating refractory disease, but they are more expensive and not known to be superior in efficacy or lower in toxicity for the treatment of *Candida* esophagitis than low doses of deoxycholate formulation.

OTHER CAUSES OF FUNGAL ESOPHAGITIS

Cases of esophagitis caused by *Aspergillus*, *Mucor*, *Cryptococcus*, and *Histoplasma* spp. have been described. Optimal therapy has not been established, but systemic therapy, as for other manifestations of invasive infection with these organisms, has been successful. Currently, this approach would involve the use of intravenous or oral triazole agents or an amphotericin B preparation. Echinocandins (micafungin, caspofungin, or anidulafungin) may be useful for *Aspergillus* or *Histoplasma* infections, but they have no activity against cryptococci.

VIRAL ESOPHAGITIS

Although spontaneous resolution of HSV esophagitis may occur in some individuals, antiviral therapy for HSV esophagitis generally is advised. Immunocompromised patients usually are treated with intravenous acyclovir, but in older patients valacyclovir and famciclovir may be useful alternatives because of their clinical efficacy and convenient dosing schedule.[28] Ganciclovir is used for CMV infection.[3,38] Acyclovir is the treatment of choice for VZV infection, and the prophylactic use of acyclovir has reduced effectively the rate of VZV infection after transplant surgery. Foscarnet is an alternative treatment for patients with HSV, VZV, or CMV who cannot tolerate or do not respond to initial therapy with acyclovir or ganciclovir. Therapy for 14 to 21 days usually is recommended for esophagitis caused by these herpesviruses. HIV-associated idiopathic ulcers do not respond to empirical antiviral or antifungal therapy, but they may respond to treatment with thalidomide.[16]

BACTERIAL ESOPHAGITIS

Bacterial esophagitis typically occurs in immunocompromised hosts and may involve gram-positive or gram-negative bacteria, or a mixture of both. In immunocompromised hosts, esophagitis may be associated with bacteremia. Consequently, empirical therapy should include broad-spectrum antibacterial agents active against *Staphylococcus aureus*, viridans streptococci, and aerobic gram-negative organisms. Blood cultures always should be performed to exclude bacteremia.[44]

Esophageal infections with *M. tuberculosis* or atypical mycobacteria are treated with regimens appropriate for other systemic infections with these organisms. Lack of response to appropriate therapy may indicate concomitant superinfection by other organisms or resistance to the drugs used. Repeat endoscopy is indicated for documenting eradication of infection.

PROGNOSIS

Candida esophagitis carries a poor prognosis for patients with AIDS. Survival is approximately 1 year after an episode of *Candida* esophagitis in children with untreated HIV infection, and *Candida* esophagitis is an indication for administration of antiretroviral therapy.[4,34] Fungal esophagitis, if not treated successfully, can lead to esophageal strictures, obstruction, perforation, and fistulas.[22,27] Viral esophagitis usually does not have any significant long-term sequelae.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agha FP, Lee HH, Nostrant TT. Herpetic esophagitis: a diagnostic challenge in immunocompromised patients. *Am J Gastroenterol* 1986;**81**:246–53.
2. Ally R, Schurmann D, Kreisel W, et al. A randomized, double-blind, double-dummy, multicenter trial of voriconazole and fluconazole in the treatment of esophageal candidiasis in immunocompromised patients. *Clin Infect Dis* 2001;**33**:1447–54.
3. Baehr PH, McDonald GB. Esophageal infections: risk factors, presentation, diagnosis, and treatment. *Gastroenterology* 1994;**106**: 509–32.
4. Blanche S, Tardieu M, Duliege A, et al. Longitudinal study of 94 symptomatic infants with perinatally acquired human immunodeficiency virus infection: evidence for a bimodal expression of clinical and biological symptoms. *Am J Dis Child* 1990;**144**:1210–15.
5. Bonacini M, Laine L, Gal AA, et al. Prospective evaluation of blind brushing of the esophagus for *Candida* esophagitis in patients with human immunodeficiency virus infection. *Am J Gastroenterol* 1990;**85**:385–9.
6. Brouillette DE, Alexander J, Yoo YK, et al. T-cell populations in liver and renal transplant recipients with infectious esophagitis. *Dig Dis Sci* 1989;**34**:92–6.
7. Chiou CC, Groll AH, Gonzalez CE, et al. Esophageal candidiasis in pediatric acquired immunodeficiency syndrome: clinical manifestations and risk factors. *Pediatr Infect Dis J* 2000;**19**:729–34.
8. Chiou CC, Groll AH, Mavrogiorgos N, et al. Esophageal candidiasis in human immunodeficiency virus-infected pediatric patients after the introduction of highly active antiretroviral therapy. *Pediatr Infect Dis J* 2002;**21**:388–92.
9. Datry A, Similowski T, Jais P, et al. AIDS-associated leishmaniasis: an unusual gastro-duodenal presentation. *Trans R Soc Trop Med Hyg* 1990;**84**:239–40.
10. de Silva R, Stoopack PM, Raufman JP. Esophageal fistulas associated with mycobacterial infection in patients at risk for AIDS. *Radiology* 1990;**175**:449–53.
11. De Wit S, Weerts D, Goossens H, et al. Comparison of fluconazole and ketoconazole for oropharyngeal candidiasis in AIDS. *Lancet* 1989;**1**:746–8.
12. Eichel M, Just-Nubling G, Helm EB, et al. [Itraconazole suspension in the treatment of HIV-infected patients with fluconazole-resistant oropharyngeal candidiasis and esophagitis]. *Mycoses* 1996;**39**(Suppl. 1):102–6.
13. Eng J, Sabanathan S. Tuberculosis of the esophagus. *Dig Dis Sci* 1991;**36**:536–40.
14. Grimes MM, LaPook JD, Bar MH, et al. Disseminated *Pneumocystis carinii* infection in a patient with acquired immunodeficiency syndrome. *Hum Pathol* 1987;**18**:307–8.
15. Hirsch MS. Herpes group virus infections in the compromised host. In: Rubin RH, Young LS, editors. *Clinical approach to infections in the compromised host*. 2nd ed. New York: Plenum; 1988. p. 347–66.
16. Jacobson JM, Spritzler J, Fox L, et al. Thalidomide for the treatment of esophageal aphthous ulcers in patients with human immunodeficiency virus infection. National Institute of Allergy and Infectious Disease AIDS Clinical Trials Group. *J Infect Dis* 1999; **180**:61–7.
17. Javdan P, Pitman ER. Squamous papilloma of esophagus. *Dig Dis Sci* 1984;**29**:317–20.
18. Kazlow PG, Shah K, Benkov KJ, et al. Esophageal cryptosporidiosis in a child with acquired immune deficiency syndrome. *Gastroenterology* 1986;**91**:1301–3.
19. Kesten S, Hyland RH, Pruzanski WR, et al. Esophageal candidiasis associated with beclomethasone dipropionate aerosol therapy. *Drug Intell Clin Pharm* 1988;**22**:568–9.
20. Kodsi BE, Wickremesinghe C, Kozinn PJ, et al. *Candida* esophagitis: a prospective study of 27 cases. *Gastroenterology* 1976;**71**: 715–9.
21. Lake DE, Kunzweiler J, Beer M, et al. Fluconazole versus amphotericin B in the treatment of esophageal candidiasis in cancer patients. *Chemotherapy* 1996;**42**:308–14.
22. Lal DR, Foroutan HR, Su WT, et al. The management of treatment-related esophageal complications in children and adolescents with cancer. *J Pediatr Surg* 2006;**41**:495–9.
23. Lee JH, Neumann DA, Welsh JD. Disseminated histoplasmosis presenting with esophageal symptomatology. *Am J Dig Dis* 1977; **22**:831–4.
24. Levine MS, Loercher G, Katzka DA, et al. Giant, human immunodeficiency virus-related ulcers in the esophagus. *Radiology* 1991; **180**:323–6.
25. Levine MS, Macones Jr AJ, Laufer I. *Candida* esophagitis: accuracy of radiographic diagnosis. *Radiology* 1985;**154**:581–7.
26. Mofenson LM, Oleske J, Serchuck L, et al. Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. *Clin Infect Dis* 2005; **40**(Suppl. 1):S1–84.
27. Naito Y, Yoshikawa T, Oyamada H, et al. Esophageal candidiasis. *Gastroenterol Jpn* 1988;**23**:363–70.
28. Nassar NN, Gregg CR. Esophageal infections. *Curr Treat Options Gastroenterol* 1998;**1**:56–63.
29. Nowak-Wegrzyn A, Crawford TO, Winkelstein JA, et al. Immunodeficiency and infections in ataxia-telangiectasia. *J Pediatr* 2004;**144**:505–11.
30. Obrecht Jr WF, Richter JE, Olympio GA, et al. Tracheoesophageal fistula: a serious complication of infectious esophagitis. *Gastroenterology* 1984;**87**:1174–9.
31. O'Connor HJ, Cunnane K. *Helicobacter pylori* and gastro-oesophageal reflux disease—a prospective study. *Irish J Med Sci* 1994;**163**:369–73.
32. Pappas PG, Rex JH, Sobel JD, et al. Guidelines for treatment of candidiasis. *Clin Infect Dis* 2004;**38**:161–89.
33. Pons V, Greenspan D, Debruin M. Therapy for oropharyngeal candidiasis in HIV-infected patients: a randomized, prospective multicenter study of oral fluconazole versus clotrimazole troches. The Multicenter Study Group. *J Acquir Immune Defic Syndr* 1993;**6**:1311–16.
34. Rabeneck L, Popovic M, Gartner S, et al. Acute HIV infection presenting with painful swallowing and esophageal ulcers. *JAMA* 1990;**263**:2318–22.
35. Rodrigues F, Brandao N, Duque V, et al. Herpes simplex virus esophagitis in immunocompetent children. *J Pediatr Gastroenterol Nutr* 2004;**39**:560–3.
36. Saag MS, Fessel WJ, Kaufman CA, et al. Treatment of fluconazole-refractory oropharyngeal candidiasis with itraconazole oral solution in HIV-positive patients. *AIDS Res Hum Retroviruses* 1999;**15**: 1413–17.
37. Scott GB, Hutto C, Makuch RW, et al. Survival in children with perinatally acquired human immunodeficiency virus type 1 infection. *N Engl J Med* 1989;**321**:1791–6.
38. Stratta RJ, Shaefer MS, Cushing KA, et al. Successful prophylaxis of cytomegalovirus disease after primary CMV exposure in liver transplant recipients. *Transplantation* 1991;**51**:90–7.
39. Tavitian A, Raufman JP, Rosenthal LE. Oral candidiasis as a marker for esophageal candidiasis in the acquired immunodeficiency syndrome. *Ann Intern Med* 1986;**104**:54–5.
40. Theise ND, Rotterdam H, Dieterich D. Cytomegalovirus esophagitis in AIDS: Diagnosis by endoscopic biopsy. *Am J Gastroenterol* 1991;**86**:1123–6.
41. Tilbe KS, Lloyd DA. A case of viral esophagitis. *J Clin Gastroenterol* 1986;**8**:494–5.
42. Wagner C, Graninger W, Presterl E, et al. The echinocandins: comparison of their pharmacokinetics, pharmacodynamics and clinical applications. *Pharmacology* 2006;**78**:161–77.
43. Wall SD, Jones B. Gastrointestinal tract in the immunocompromised host: opportunistic infections and other complications. *Radiology* 1992;**185**:327–35.
44. Walsh TJ, Belitsos NJ, Hamilton SR. Bacterial esophagitis in immunocompromised patients. *Arch Intern Med* 1986;**146**: 1345–8.
45. Ylitalo N, Brogly S, Hughes MD, et al. Risk factors for opportunistic illnesses in children with human immunodeficiency virus in the era of highly active antiretroviral therapy. *Arch Pediatr Adolesc Med* 2006;**160**:778–87.
46. Young C, Chang MH, Chen JM. Fungal esophagitis in children. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1993;**34**: 436–42.

CHAPTER 44

Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

Theresa J. Ochoa • Mara Zambruni • Elsa Chea-Woo

Gastrointestinal infections can be caused by a wide array of pathogens, including bacteria, viruses, and parasites. Chemical food poisoning further broadens the spectrum of diseases. The cardinal symptoms of enteric infections/intoxications are diarrhea, emesis, and abdominal cramps in various combinations, but some enteropathogens cause less specific systemic syndromes and neurologic disorders that are the hallmark of a few food intoxications. A high degree of suspicion is necessary in these cases to formulate the correct diagnosis.

Irrespective of the underlying cause, the approach to a patient with suspected gastrointestinal infection or food poisoning starts with the assessment of patient dehydration status and need for immediate clinical intervention: the timely correction of fluid loss is the single most important intervention to decrease mortality. The next step consists of a thorough interview and physical examination aimed at formulating a presumptive differential diagnosis: circumstances of disease onset, constellation of symptoms and signs, characteristics of stools, geographic location and season of the year, and presence of other sick contacts normally are sufficient to orient the physician toward the correct cause of infection. A careful choice of diagnostic tests will not only confirm the diagnosis and establish a plan of treatment but also provide grounds for infection control actions and prevention of outbreaks.

In this chapter we review the essential information regarding epidemiology and epidemiologic classifications of enteric diseases, characteristics of common pathogens, general management of patients with diarrhea, laboratory diagnosis, and updated recommendations for treatment and prevention.

EPIDEMIOLOGY

Gastrointestinal infections represent a major cause of morbidity and mortality among children. Worldwide it is estimated that 1.6 billion episodes occur per year among children younger than 5 years of age, leading to 1.3 million deaths per year.[49,192,291] The World Health Organization (WHO) and United Nations International Children's Emergency Fund (UNICEF) Child Health Epidemiology Reference Group (CHERG) have published estimates of the distribution of child deaths by cause in 2010, with time trends since 2000.[291]

Worldwide rates for diarrhea-associated deaths in children younger than 5 years of age in 2010 were estimated to be 0.7 million, a figure consistently lower than the 1.8 million estimated in 2003. Significant differences, however, exist among geographic areas related to age, race, household income, access to health care, and sanitation level. Diarrheal diseases accounted for 4 percent of deaths among children younger than age 5 in the Americas, Europe, and western Pacific regions, whereas in Africa, South East Asia, and eastern Mediterranean areas they caused up to 12 percent of deaths.[291] In the United States, diarrhea-associated morbidity and mortality among children younger than 5 years of age significantly decreased over the past century owing to improved living standards, sanitation systems, access to clinical care, and clinical management. In 2005 to 2006 the estimated rates of diarrhea-associated deaths in this country were 2.3 per 100,000 children per year: case-fatality ratios were higher among young infants, males (M:F ratio 1.3), black children (relative risk 3.9 compared with white children), and children from southern counties.[147] Racial and geographic differences likely reflect disparities in socioeconomic status and access to quality health care. Since rotavirus vaccination was included in the U.S. vaccination program in 2006, a substantial reduction in gastroenteritis among young children has been observed: from 2008 to 2009 reported rates of all-cause gastroenteritis hospitalizations and rotavirus-associated hospitalizations were reduced, respectively, 31 to 33 percent and 61 to 72 percent compared with the prevaccination era.[125] Similar declines in all-cause gastroenteritis and rotavirus-enteric infections rates were observed in other high and middle income countries where vaccination programs were implemented. It is hoped that vaccine use in low-income countries, as endorsed by the WHO, will have a substantial impact on diarrheal overall burden of disease.[457]

There is also growing awareness of potential long-term disability caused by repeated episodes of diarrheal disease, especially in children living in developing countries: malnutrition, failure to thrive, and impaired cognition have all been described as consequences of protracted diarrhea.[191,278,369]

Gastrointestinal infections are fundamentally acquired in two ways: by fecal-oral transmission and by contaminated food or water ingestion. The relative importance of the two routes, the risk for a child to be infected, and

the type of microbes involved vary with age, immune status, and type of environmental exposures. Most cases of sporadic diarrhea occur in children younger than 5 years old, although all age groups can be affected. Breast-fed infants are less likely to acquire gastrointestinal infections because of the lower exposure to contaminated food and water and the protection granted by human milk components such as secretory antibodies, glycans, lactoferrin, leukocytes, cytokines, and other components produced by the mother's immune system. During and after weaning the risk of diarrhea increases.

Epidemiologic Categories of Diarrhea

Information regarding host immune status and environmental exposures normally allows for classification of disease into broad epidemiologic categories helpful to narrow the differential diagnosis: (1) diarrhea acquired in institutional settings, (2) antimicrobial-associated diarrhea, (3) diarrhea in immunosuppressed hosts, (4) traveler's diarrhea, and (5) foodborne or waterborne diarrhea (food poisoning).

Diarrhea Acquired in Institutional Centers: Childcare Centers

Gastrointestinal infections acquired in institutional settings are normally caused by highly contagious pathogens transmitted either by person-to-person contact, fomites, or ingestion of contaminated food. A typical setting for the pediatric population is daycare centers. Rotavirus, *Shigella* spp., and *Giardia* are the most common pathogens reported, but, as laboratory diagnosis improved, a number of other pathogens have been implicated, including *Cryptosporidium*, astrovirus, enteric adenovirus, calicivirus, hepatitis A, sapovirus, *Escherichia coli* O157:H7, and *Salmonella*.[114,284,471] Rigorous hygiene practices, exclusion or cohorting of sick children from daycare, and judicious use of antimicrobial agents are all effective strategies for infection control.[272] Criteria for child exclusion from daycare attendance and duration of exclusion have been published.[56,284]

Antimicrobial-Associated Diarrhea

Intestinal microbiota play a major role in gastrointestinal function and immune system homeostasis. Antimicrobial agents induce significant modification of enteric flora both in terms of diversity and bacterial biomass.[492] Such modification, which can persist long after the inciting agent has been discontinued, can result in altered gastrointestinal function and increased susceptibility to opportunistic pathogens such as *Clostridium difficile* or *Candida albicans*.[492] Diarrhea is a common side effect of antibiotics, occurring in 11 to 40 percent of the children treated with antimicrobial agents.[245] The spectrum of disease ranges from a mild self-resolving illness to pseudomembranous colitis, toxic megacolon, and possibly death.[38,51] Type and dose of antibiotic and host characteristics affect the severity of clinical manifestations. The epidemiology of *C. difficile* among children is not well understood.

C. difficile is the most common bacterial agent associated with severe antimicrobial-associated diarrhea. Enterotoxin A and cytotoxin B account for most of its virulence. Since the early 2000s, rates and severity of *C. difficile* infections have been increasing among adults and to a less extent in children, in part owing to the emergence of a hypervirulent strain called BI/NAP1/027.[304] This strain produces higher amounts of toxins A and B, and a third toxin called binary toxin is frequently resistant to fluoroquinolones and is associated with more severe disease.

Prior use of antibiotics is the single most important risk factor for *C. difficile* disease. Nearly all the antibiotics have been implicated, but penicillins, cephalosporins, clindamycin, and fluoroquinolones are the most frequently involved.[259] Additional risk factors for *C. difficile* infection in children include gastrostomy or jejunostomy tubes, use of proton pump inhibitors, altered intestinal mobility, and prolonged hospitalization.[408] Certain underlying diseases, such as immunodeficiency, malignancy, cystic fibrosis, and inflammatory bowel disease also predispose to *C. difficile* infection.[260]

Guidelines for diagnosis and treatment of *C. difficile* infection have been published by the Infectious Diseases Society of America (IDSA) and Society for Health Care Epidemiology of America (SHEA).*

Diarrhea in Immunosuppressed Host

Enteric infections are often a major problem in people with primary and secondary immunologic disorders.[24,405,506] The spectrum of disease and the type of involved pathogens can vary with the type of underlying deficit and the degree of immunosuppression. Patients with defects in cellular immunity (e.g., solid-organ or bone-marrow recipients, patients on chemotherapy for malignancies, children infected by human immunodeficiency virus [HIV] or born with congenital disorders) are mainly susceptible to pathogens with partial or predominant intracellular localization. Among common enteric pathogens, *Salmonella* and *Listeria* infections can result in disseminated, life-threatening diseases.[186,225] Viral infections by routine enteric pathogens such as rotavirus or norovirus can become chronic and debilitating.[155,265,363,502] Opportunistic agents such as cytomegalovirus can cause severe colitis in patients with advanced immune suppression, whereas Cryptosporidiosis and Microsporidiosis have been associated with persistent diarrhea.[314]

HIV-infected children represent a major group of immunosuppressed hosts, especially in some developing countries. Gastrointestinal dysfunction is common in these patients and can be related to a number of causes: concomitant infections, HIV enteropathy, malignancy, or highly active antiretroviral therapy (HAART) medicines. Besides the conventional enteric pathogens, opportunistic agents can cause significant disease in patients with advanced disease. In addition to protozoal infections like Microsporidiosis and Cryptosporidiosis, *Mycobacterium avium* complex is a common cause of persistent diarrhea and mainly affects the small intestine.[314] Cytomegalovirus

*http://www.idsociety.org/IDSA_Practice_Guidelines/

TABLE 44-1 Organisms That Cause Gastrointestinal Tract Infections in Patients with AIDS

	Organisms
Esophagus	*Candida albicans**
	Cytomegalovirus*
	Herpes simplex virus*
Hepatobiliary	Cytomegalovirus
	*Cryptosporidium**
	Hepatotropic viruses
	Mycobacterium avium complex*
Small Intestine	*Campylobacter* species
	Cytomegalovirus*
	*Cryptosporidium**
	*Giardia lamblia**
	*Isospora belli**
	Mycobacterium avium complex*
	Microsporidia* (*Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*)
	Salmonella species*
	Enteroaggregative *E. coli*
	Strongyloides stercoralis
Large Intestine	*Campylobacter* species
	Clostridium difficile
	Cytomegalovirus*
	Entamoeba histolytica
	Herpes simplex virus*
	Salmonella species*
	Enteroaggregative *E. coli*
	Shigella species

*Diseases of the gastrointestinal tract that fulfill the Centers for Disease Control and Prevention surveillance case definition of AIDS.

can cause diffuse and severe ileocolitis and dysentery-like illness. *C. difficile* can play a role, especially in children previously treated with antibiotics. *Salmonella* infection is associated with a high risk of septicemia and should be treated aggressively.[148] A description of enteropathogens causing infections in HIV-infected patients is provided in Table 44-1.

The advent of antiretroviral treatment has dramatically changed the epidemiology of opportunistic infections in HIV-infected children.[331,385] A study in adults examined the annual incidence of bacterial diarrhea before and during the HAART era in a large cohort of people with HIV infection.[331] Between 1992 and 2002, the overall rate of bacterial diarrhea in people with clinical acquired immunodeficiency syndrome (AIDS) decreased (odds ratio, 0.4; 95% confidence interval, 0.2 to 0.6). HAART can result in complete clinical, microbiologic, and histologic responses in patients with AIDS infected with *Cryptosporidium parvum* and *Enterocytozoon bieneusi*[69] and in patients with HIV enteropathy.[273] Guidelines for prevention of opportunistic infections, including those involving the gastrointestinal tract, can be found on the internet.*

*www.aidsinfo.nih.gov/

Severe defects in humoral immunity, such as X-linked agammaglobulinemia or common variable immunodeficiency, are often associated with persistent diarrhea and malabsorption syndrome; rotavirus and *Giardia lamblia* can be etiologic factors.[363] Enteric infections caused by *Campylobacter jejuni* and *Salmonella* spp. are more frequent and can be more severe and prolonged than in immunocompetent individuals.

Traveler's Diarrhea

Traveler's diarrhea is the most common illness reported by individuals traveling from high-income countries to middle and low income areas: occurrence as high as 40 to 60 percent is reported.* The most important determinant of risk is travel destination. World regions are divided into three risk categories: low-risk areas include United States, Canada, Western and Northern Europe, Australia, New Zealand, and Japan; intermediate-risk areas include Eastern Europe, South Africa, and some Caribbean Islands; and high-risk areas include most of Asia, Africa, Middle East, and Central and South America.[105,358]

Half of travelers experience diarrhea within the first few weeks after arrival. A variety of intestinal pathogens can be responsible for traveler's diarrhea.[48,216,348,358,422] Bacteria account for 80 to 90 percent of the cases: enterotoxigenic *E. coli* (ETEC) is the most common pathogen, followed by *Campylobacter jejuni*, *Shigella* spp., *Salmonella* spp., enteroaggregative *E. coli*, *Aeromonas* spp., *Plesiomonas* spp., and *Vibrio* spp. Parasites account for 10% of the cases: *Giardia lamblia* and *Cryptosporidium* are the most common pathogens; *Cyclospora*, *Entamoeba*, and *Dientamoeba* are occasionally encountered. Viruses represent 5 to 8 percent of cases: rotavirus, norovirus, and calicivirus are the most frequently reported.[105] However, based on the GeoSentinel Surveillance Network, the main pathogens isolated from international travelers with gastroenteritis are parasites (65%), followed by bacteria (31%) and viruses (3%).[449]

Clinical illness varies, reflecting the diversity of causative agents. Typically, illness presents as loose stools over a 24-hour period, accompanied by fever, nausea, vomiting, and abdominal cramping. Tenesmus and bloody diarrhea are less common. An incubation period of 6 to 48 hours normally suggests a bacterial or viral etiology, whereas parasites tend to have longer incubation periods and rarely present in the first week after travel; an exception is *Cyclospora cayetanensis*, which can present as an acute illness resembling bacterial pathogens. Traveler's diarrhea is normally a mild, self-limited illness that can resolve spontaneously in 3 to 5 weeks; protozoal diarrhea, however, can persist for months if untreated.

Children who travel are at risk of developing the same, well-known illnesses that affect adult travelers.[200] The etiology, treatment, and actual risk of these illnesses are not as well defined as in adults.[296] Treatment of traveler's diarrhea in children consists of fluid replacement and antibiotics. Antidiarrheal agents are not recommended for young children, although they can be considered for

*References 105, 134, 181, 216, 217, 358.

older children and adults, provided there is no gross blood in the stool or fever higher than 38.5 °C (101.3 °F).[17,105,143,217,296] Because access to good quality health care can be problematic in certain regions, travelers should be advised to take with them medications with expected activity against the prevalent bacteria. Antibiotic therapy has been shown to reduce duration of disease from several days to approximately 1 day. Recommended antibiotics for empirical treatment of traveler's diarrhea in adults and children are fluoroquinolones or azithromycin. Rifamixin is an alternative for afebrile nondysenteric diarrhea.[1,3,135,136,143]

Traveler's diarrhea is mainly a foodborne or waterborne disease: poor hygiene practices of food handlers, inadequate food storage and refrigeration, and high levels of environment contamination by fecal flora due to poor sanitation infrastructure are risk factors for acquiring enteric infections in developing countries. Travelers should be advised on safe practices regarding food and beverage consumption. No effective vaccinations are currently available for traveler's diarrhea. Similarly, chemoprophylaxis with antibiotics is not recommended in healthy travelers. Prophylactic treatment can be taken into consideration for hosts at increased risk of acquiring severe diarrhea, including patients with immunodeficiencies, malignancies, or chronic intestinal illnesses.[217]

Foodborne and Waterborne Diseases: Food Poisoning

Foodborne or waterborne diseases can result from ingestion of food or water contaminated with bacteria, viruses, parasites, or other types of chemical poisoning.[19,75] They can present either as sporadic cases or as outbreaks; the latter are defined as clusters of two or more individuals experiencing a similar illness after ingesting common food or water.[142,288,294] Foodborne and waterborne diseases represent major public health concerns and are subject to different degrees of surveillance across countries. In the United States, several surveillance systems coordinated by the Centers for Disease Control and Prevention (CDC) are in place to monitor foodborne and waterborne disease occurrence, track sources of contaminated food, identify emerging pathogens, and provide administrative guidance.

The Foodborne Disease Active Surveillance Network (FoodNet) was established by the CDC in 1996 to conduct population-based, active surveillance investigation of foodborne disease in the United States.[416] The surveillance includes laboratory-confirmed infections for nine common pathogens (*Campylobacter* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp., Shiga toxin–producing *E. coli* (STEC), *Vibrio* spp., *Yersinia* spp., *Cryptosporidium* spp., and *Cyclospora* spp.). Although some other important pathogens known to be transmitted by food are not actively tracked by the system, the control measures triggered by FoodNet inputs will likely have an impact on all foodborne infection. The National Notifiable Disease Surveillance System (NNDSS) is a passive surveillance database. Reportable foodborne diseases include botulism, listeriosis, salmonellosis, STEC infections, hemolytic-uremic syndrome, and vibriosis. A complete list of other CDC surveillance systems is available at the CDC website.*

The 2010 preliminary data from FoodNet surveillance showed that although foodborne infections caused by *Campylobacter* spp., *Listeria* spp., STEC O157, and *Yersinia* spp. decreased by an overall 23 percent compared with the period 1996 to 1998, rates for *Salmonella* spp. infections did not change significantly over the same period of time and incidence for *Vibrio* spp. infections actually increased. Population at extremes of age were the most affected, with highest incidence for *Salmonella*, *Campylobacter*, *Shigella*, STEC O157 and non-O157, *Yersinia*, and *Cryptosporidium* being reported among children younger than 5 years of age, whereas individuals older than 60 years of age had the highest rates for infection with *Listeria* and *Vibrio*. Case-fatality ratio ranged from 0 percent for *Cyclospora* and *Shigella* to 13 percent for *Listeria* infections. The highest number of deaths was reported among the elderly.[91,92]

In 2011 the CDC released new national estimates for foodborne illnesses acquired in the United States. Data for modeling were gathered through five different surveillance systems and collected during 2000 to 2008.[418] Based on existing data, 31 identified known agents of foodborne disease were estimated to cause 9.4 million illnesses, 55,961 hospitalizations, and 1,351 deaths per year. Viral infections were expected to represent 59 percent of the cases, bacterial infections, 39 percent, and parasites, 2 percent. Norovirus, nontyphoidal *Salmonella*, *Clostridium perfringens*, and *Campylobacter* were estimated to be the pathogens most commonly involved. Norovirus, nontyphoidal *Salmonella*, *Campylobacter*, and *Toxoplasma* were responsible for the most hospitalizations. *Salmonella*, *Toxoplasma*, and *Listeria* accounted for the majority of documented deaths.[418] When modeling included foodborne illnesses caused by unspecified pathogens—either microbes or toxins for which there are no surveillance systems in place or pathogens yet to be discovered—the number of expected foodborne diseases rose to 48 million per year, leading to 128,000 hospitalizations and 3,000 deaths annually.[417]

Clinical manifestations of foodborne disease can affect the gastrointestinal tract and the central nervous system or cause systemic symptoms with little or no intestinal tract involvement. Incubation period and type of consumed food are helpful tools for establishing a presumptive diagnosis.

1. Foodborne diseases with incubation time less than 1 hour are generally caused by chemical poisoning from mushrooms or marine biotoxins, inorganic toxins such as monosodium glutamate (Chinese restaurant syndrome), or metals.
2. Foodborne diseases manifesting as nausea, vomiting, and diarrhea within 1 to 7 hours after food ingestion are normally caused by preformed bacterial toxins, mainly from *Staphylococcus aureus* and *Bacillus cereus*. Biotoxins are less frequently a cause but must be considered in cases of acute onset of neurologic symptoms following seafood or mushroom consumption.

*http://cdc.gov/foodborneburden/surveillance-systems.html

3. Illnesses manifesting as abdominal cramps and diarrhea within a period of 8 to 14 hours are commonly caused by bacteria such as *Clostridium perfringens* or *B. cereus*, through the in vivo production of enterotoxins.
4. Diarrhea, abdominal cramps, and fever with onset in 8 to 48 hours suggest a bacterial etiology. *Campylobacter*, *E. coli*, *Salmonella*, *Shigella*, and *Vibrio parahaemolyticus* are the most common species involved. In the presence of frankly bloody diarrhea, *E. coli* O157, *Shigella*, and *Campylobacter* need to be ruled out.
5. Watery diarrhea presenting more than 15 hours after consumption of suspected food is normally due to viral infections. Noroviruses are the most common foodborne pathogens.
6. Foodborne diseases presenting as diarrhea protracted for days or weeks are normally due to parasites. *Giardia lamblia* and the microsporidia are the most common among children, whereas *Cyclospora* has been described mainly in adults.
7. Some foodborne diseases present as systemic symptoms and longer incubation periods. Examples include typhoid fever, brucellosis, Q fever, toxoplasmosis, tapeworm infection, anisakariasis, and anthrax. *Trichinella* can cause gastrointestinal symptoms in the early phase, but later systemic symptoms prevail. *Listeria* and *Vibrio* spp. can be particularly dangerous in immunosuppressed patients.

Foodborne Disease due to Bacteria, Viruses, and Parasites. Table 44-2 illustrates clinical characteristics and confirmation tests for the diagnosis of common foodborne and waterborne diseases. Detailed guidelines for clinical diagnosis and instructions for collecting stool specimens are available on the CDC website.*

A long list of foods have been associated with enteric infections:

- Unpasteurized milk and dairy products have been associated with salmonellosis and campylobacteriosis and, less frequently, infections with *Brucella* spp., *E. coli*, *L. monocytogenes*, *Mycobacterium* spp., *S. aureus*, *Streptococcus* spp., *Streptobacillus moniliformis*, and *Yersinia enterocolitica*.[295] A nationwide outbreak of *Salmonella enteritidis* gastroenteritis involved 224,000 people in the United States and was associated with ingestion of contaminated ice cream.[209] Mexican-style soft cheese made with unpasteurized milk has been described as source of infection among children.[477] Consumption of raw milk has been associated with a chronic diarrhea syndrome of unknown cause, initially identified in Minnesota and referred to as *Brainerd diarrhea*.[355] Several additional outbreaks have been reported as Brainerd diarrhea, each characterized by watery diarrheal illness of impressively long duration with acute onset and the same constellation of symptoms, including extreme fecal urgency and fecal incontinence. However, despite intensive laboratory examinations, the cause of Brainerd diarrhea remains unknown.[262,312]
- Fresh fruit and vegetables have been linked with a number of pathogens; radish sprouts, lettuce, apple juice, alfalfa sprouts, and spinach have been described as sources of enterohemorrhagic *E. coli*.[73,82] Hot peppers, tomatoes, and cantaloupes have been described as potential sources of *Salmonella*.[76,89]
- Cold salads were described as a vehicle for *Shigella* and *Listeria* infection.[32] Homemade canned food can be associated with *Clostridium botulinum* infection in adults[438]; honey is associated with *Botulinum* intoxication in infants.
- Poultry, especially raw chicken, and eggs have been identified as sources of foodborne infection due to *Campylobacter* and *Salmonella*.[108]
- Pork and chitterlings have been associated with *Yersinia* infection.[80]
- Ingestion of raw fish (sushi or sashimi) has led to infection with *Vibrio parahaemolyticus* and various parasites.[118,295] *V. parahaemolyticus* outbreaks have

*http://www.cdc.gov/outbreaknet/index.html.

TABLE 44-2 **Foodborne Disease Agents and Clinical Presentation**

Usual Incubation Periods	Causative Agent	Clinical Illness			Epidemiologic and Laboratory Diagnosis
		Fever	Diarrhea	Vomiting	
5 min-6 hr (usually <3 hr)	Chemical or toxin—see Tables 44-2 and 44-3	Rare	Occasional (see Table 44-3)	Common	Demonstration of toxin or chemical from food or epidemiologic incrimination of food
1-6 hr (usually <1 hr)	*Staphylococcus aureus* enterotoxin	Rare	Occasional	Profuse	Isolation of organisms in food (>10^5/g)/vomitus/ stool; detection of enteroxin in food
	Bacillus cereus emetic toxin	Rare	Occasional	Profuse	Isolation of organisms in food (>10^5/g)/vomitus/ stool
6-24 hr	*Clostridium perfringens* enterotoxin	Rare	Typical	Occasional	Isolation of organisms or toxin from food (>g) or stools of ill persons, epidemiologic incrimination of food; detection of enteroxin in food
	B. cereus enterotoxin	Rare	Typical	Occasional	

TABLE 44-2 **Foodborne Disease Agents and Clinical Presentation** (Continued)

Usual Incubation Periods	Causative Agent	Clinical Illness			Epidemiologic and Laboratory Diagnosis
		Fever	Diarrhea	Vomiting	
12-72 hr	*Clostridium botulinum*	Clinical syndrome compatible with botulism	Constipation more common		Isolation of organism or toxin from food (10^5/g) or stools; demonstration of toxin in serum or food
16-96 hr	*Shigella*	Common	Typical, often bloody	Occasional	Isolation of organism from clinical specimens from two or more ill persons; isolation of organism from epidemiologically implicated food
	Nontyphoidal *Salmonella*	Common	Typical	Occasional	
	Enteroinvasive *E. coli* (EIEC)	Common	Typical, may be bloody	Occasional	
	Enteropathogenic *E. coli* (EPEC)	Occasional	Typical	Occasional	
	Enterotoxigenic *E. coli* (ETEC)	Rare	Typical	Rare	
	Vibrio parahaemolyticus; V. cholerae enterotoxin	Occasional	Typical	Occasional	
1-3 days	Caliciviruses (noroviruses) Rotavirus	Occasional	Typical	Common	Antigen detection (enzyme immunoassay) in stool; immune electron microscopy of stool; detection of viral RNA in stool or vomitus by PCR
1-10 days	*Yersinia*	Uncommon	Typical, severe abdominal pain	Uncommon	Isolation of organisms from food or clinical specimens of ill persons
2-10 days	*Campylobacter jejuni*	Common	Typical, often bloody	Uncommon	Isolation of organisms from food or clinical specimens of ill persons
1-11 days	*Cryptosporidium*	Occasional	Common	Occasional	Demonstration of oocysts in stool or in small bowel biopsy of ill persons; demonstration of organism in epidemiologically implicated food
	Cyclospora	Occasional	Common	Occasional	Demonstration of parasite in stool or in small bowel biopsy of ill persons; demonstration of organism in epidemiologically implicated food
	Giardia intestinalis	Occasional	Common	Occasional	Demonstration of parasite in stool or in small bowel biopsy of ill persons; demonstration of organism in epidemiologically implicated food
2 days-weeks	*Bacillus anthracis*	Common	Typical	Frequent	Isolation of organism from blood or contaminated meat
1-7 days	*E. coli* O157:H7 and other Shiga toxin–producing *E. coli*	Uncommon	Typical	Frequent	Isolation of organism from food or stool or identification of toxin in stools of ill persons
3-60 days, usually 7-14	*Salmonella typhi*	Common	Diarrhea or constipation	Uncommon	Isolation of organisms from food or clinical specimens of ill persons
7-21 days	*Brucella* spp.	Common	Common	Rare	Isolation of organisms from blood or bone marrow culture of ill persons; fourfold increase in standard agglutination titer overall several weeks or single titer 1:160 in person with compatible clinical syndrome
1-4 wk	*Giardia lamblia*	Rare	Common	Rare	Stool for ova and parasite examination enzyme immunoassay
2 days-8 wk	*Trichinella spiralis*	Common	Common	Common	Serology, muscle biopsy

TABLE 44-3 **Fungal and Inorganic Toxins: Food Poisoning**

Type of Chemical, Toxin, or Poison	Food	Clinical Symptoms	Onset of Symptoms (hr)
Heavy metals*	Water (through metallic container) and food	Gastrointestinal	1
Monosodium glutamate	Chinese food	Burning sensation, heavy feeling in chest, pressure over face, flushing, gastrointestinal	1
Ibotenic acid, muscimol	Mushroom	CNS: confusion, delirium, visual disturbances, lethargy	2
Coprine	Mushroom	Disulfiram-like effect: nausea, vomiting, headache, hypotension, flushing, paresthesia, and tachycardia	2
Muscarine	Mushroom	Parasympathetic: sweating, salivation, lacrimation, blurred vision, diarrhea, bradycardia, hypotension	2
Psilocybin, psilocin	Mushroom	CNS: hallucinations, anxiety, mood elevation, weakness	2
Diverse, mostly unknown	Mushroom	Gastrointestinal	2
Monomethylhydrazine, gyromitrin	Mushroom	Cellular destruction, gastrointestinal, loss of coordination, convulsion, coma, death	6-12
Amatoxins, phallotoxins	Mushroom	Cellular destruction, gastrointestinal, hepatic and renal necrosis	6-24
Vomitoxin (deoxynivalenol)	Cereals contaminated with *Fusarium* species	Vomiting acutely Altered mucosal immunity with chronic exposure	<3

CNS, central nervous system.
*Includes antimony, arsenic, cadmium, copper, mercury, thallium, tin, and zinc.

been also associated with raw oysters and clams from Northeastern and Pacific Northwestern coasts of the United States.[85] Parasites acquired through ingestion of raw fish include larval nematodes of the family Anisakidae, fish tapeworm of the species *Diphyllobothrium*, the fluke *Nanophyetus salmincola* from salmon, and many other helminths.[330]

- Consumption of raw and lightly cooked shellfish (mussels, clams, oysters, lobsters, and other mollusks) is associated with infection by agents that are native to the marine environment or released by sewage effluents contaminating environmental waters. Viruses such as caliciviruses and hepatitis A virus are commonly concentrated and transmitted through shellfish.[323] The *Vibrio* genus (specifically *V. vulnificus*), acquired either by eating shellfish or by contaminating open wounds by swimming, presents a serious problem in terms of the severity of human illness and death, especially in people with liver disease.[221,333]

Food Poisoning by Chemicals. Intoxication from seafood is caused by toxins accumulated in fish, shellfish, and crustaceans. Marine toxins are tasteless and odorless and are not inactivated by cooking: once ingested they normally cause symptoms within a few hours. Symptoms can be gastrointestinal, neurologic, or systemic. Clinical characteristics of shellfish poisoning are listed in Table 44-3. Prevention of such illness is accomplished by monitoring of toxin concentration in seafood samples.[437] Scombroid fish poisoning is caused by inadequate conservation of certain types of fish. If fish are not adequately refrigerated after being caught, marine bacteria proliferate and catalyze decarboxylation of fish tissue histidine into histamine; once the fish is consumed, patients present with symptoms of histamine toxicity. A large reported outbreak in the United States was associated with escolar fish. Puffer fish syndrome is a lethal intoxication resulting from consumption of certain specific species of puffer. It is very rare in the United States but more common in Japan.[437]

Chinese restaurant syndrome appears to be caused by excessive amounts of monosodium glutamate in foods. Symptoms include paresthesias, reversal of hot-cold sensations, loss of proprioception, flushing, weakness, and burning sensations.

Mushrooms toxins produce several clinical syndromes, generally within 2 hours of ingestion (Table 44-4),[202] except for poisoning caused by amatoxins, phallotoxins, amantin, monomethylhydrazine, and gyromitrin, which may produce symptoms, including death, up to 24 hours after ingestion.[202]

Heavy metals, such as antimony, arsenic, cadmium, copper, mercury, thallium, tin, and zinc, cause irritation of the gastric mucosa, with nausea, vomiting, and abdominal cramps, which usually resolves 2 to 3 hours after the offending agent has been removed.[19,75]

Prevention of Foodborne Disease. Changes in human behavior, increased consumption of ready-to-eat food, centralization of food production, processing, and distribution, and globalization of food market are all risk factors for foodborne disease. Among outbreaks reported from 1998 through 2002, 46 percent were associated with at least one pitfall in the food chain process.[295,505] Major risk factors for food contamination were food handling by a person infected or colonized by a pathogen, bare-handed contact with food, inadequate cleaning of preparation equipment or utensils, and cross contamination from a raw ingredient of animal origin. Factors associated

TABLE 44-4 **Marine Biotoxins**

Syndrome	Symptoms	Incubation period	Toxin	Mechanism of Action	Food Source	Geographic Distribution	Seasonality
Paralytic shellfish poisoning	Facial and perioral paresthesias, headache, muscular weakness, mental status changes, nausea, vomiting, death	30 min-4 hr	Saxitoxin	Blocks sodium channels	Shellfish	NW and NW United States, Chile, Japan	May-Nov
Diarrheic shellfish poisoning	Diarrhea, nausea, vomiting, abdominal pain, chills	30 min-12 hr	Okadaic acid	Inhibits protein phosphatase	Shellfish	Japan, Europe, Africa	
Amnesic shellfish poisoning	Nausea vomiting, abdominal cramps, diarrhea, headache, visual disturbance, anterograde amnesia, confusion, coma	15 min-6 hr	Domoic acid	Stimulates glutamate receptors	Shellfish	Canada, NE and NW United States	
Neurotoxic shellfish poisoning	Paresthesia, abdominal pain, dizziness, diplopia, gait disturbance, reversed temperature perception, respiratory difficulty	5 min-4 hr	Brevetoxins	Opens sodium channels	Shellfish and other fish	Western Florida, Caribbean	Spring-fall
Ciguatera	Nausea, vomiting, abdominal cramps, facial and perioral paresthesias, headache, reverse temperature perception, extremity pain, arthralgia, myalgia, sharp pain in the legs and teeth	1-6 hr	Ciguatoxin, maitotoxine	Opens Na^+ channels; opens Ca^{2+} channels	Predatory fish reef	Tropical areas	February-September
Scombroid fish poisoning	Pruritic rash, flushing, sweating, perioral tingling, dizziness, facial and lingual swelling, vomiting, diarrhea, urticaria, bronchospasm	Minutes-hours	Histamine	Histamine receptors	Tuna, mahi-mahi, mackerei, sardines	Worldwide	Year-round
Puffer fish	Facial paresthesia, ascending paralysis, respiratory failure, circulatory collapse, death	30 min-3 hr	Tetrodotoxin, saxitoxin	Blocks Na^+ channels	Puffer fish	East Asia	

with bacterial proliferation in food include inadequate refrigeration, slow cooking, insufficient time or temperature during hot holding, or long delay between food preparation and consumption.

Detailed description regarding safe food processing can be retrieved on the website of the U.S. Department of Agriculture (USDA) and other websites.*

Waterborne Disease. The waterborne disease and outbreak surveillance system in the United States is responsible for collecting data and reporting on waterborne disease. The database collects information on outbreaks associated with recreational water, drinking water, and water not intended for drinking. During 2007 to 2008, 36 outbreaks associated with drinking water were reported: of those, 61 percent manifested as acute gastrointestinal illnesses and 3 percent as hepatitis; the remainder were either associated with respiratory symptoms or skin irritation. Bacterial agents accounted for 41 percent of acute gastrointestinal disease outbreaks: *Campylobacter* spp., *E. coli* O157:H7, *Salmonella* spp., and *Providencia* were identified as etiologic agents. Viruses (norovirus and hepatitis A) were responsible for 18 percent of outbreaks, and parasites (*Cyclospora cayetanensis* and *Giardia intestinalis*) were the cause of 13.6 percent. The remaining outbreaks were either caused by unknown agents or multiple agents. More than half of the outbreaks were associated with untreated or inadequately treated ground water.[63] During the same period (2007-2008) a total of 134 recreational-water outbreaks were reported: 86 percent of outbreaks involved treated recreational water, and the remainder involved untreated fresh or marine water. Sixty percent of outbreaks manifested as acute gastrointestinal illness, with the remainder as respiratory or dermatologic disease. *Cryptosporidium* was responsible for 74 percent of the waterborne diseases presenting as gastrointestinal symptoms, followed by norovirus (6%), *Shigella* (4.9%), *Giardia* (3.7%), *E. coli* O157:7 (3.7%), and *Plesiomonas shigelloides* (1.2%).[232]

CLINICAL CLASSIFICATION OF DIARRHEAL EPISODES

Diarrhea in children can be classified in several ways. Based on duration, diarrhea can be acute (usually self-limited and lasting <7 days), prolonged (7 to 14 days), persistent (>14 days), or chronic (usually more than 30 days or relapsing).[318] The importance of this classification is that, in addition to focusing on the importance of subsequent development of undernutrition,[319] it can help to identify the most likely causes. Acute diarrhea can be associated with many bacterial and viral pathogens, whereas prolonged and persistent diarrhea is associated with parasites, especially *Giardia duodenalis*, *Cryptosporidium*, and other coccidia, and some bacterial pathogens including enteroaggregative *E. coli* (EAEC) and enteropathogenic *E. coli* (EPEC).[319,334,345,348,467] On the other hand, chronic diarrhea, although it can start as an infectious diarrhea, is often the result of a postinfectious process (e.g., malabsorption, irritable bowel syndrome, inflammatory bowel disease).[508]

Diarrhea can also be classified as inflammatory or noninflammatory and as osmotic or secretory, based on its pathogenesis. Inflammatory diarrhea can be defined by the presence of abundant leukocytes in the stools or by measuring other markers of inflammation such as fecal lactoferrin.[231,352,475] Inflammatory diarrhea is associated with "invasive" bacterial pathogens such as *Shigella*, *Salmonella*, and *Campylobacter;* therefore, identifying this type of diarrhea can help raise suspicion of these agents. On the other hand, osmotic diarrhea is important in young children. Because rotavirus, the most common cause of diarrhea in young children worldwide, can be associated with accumulation of carbohydrates in the intestinal lumen and malabsorption, measurement of reducing substance in stool may be helpful in resource-limited situations to aid in its diagnosis.[293]

Finally, diarrhea can be classified by its characteristics as watery or dysenteric. Watery diarrhea is characterized by liquid or semi-liquid stools, usually of large volume, with or without fever, vomiting, and abdominal pain. This type of diarrhea can be associated with any type of viral, bacterial, or parasitic agents. However, dysentery, a syndrome characterized by fever, abdominal cramps, and tenesmus with small, frequent bloody stools, sometimes with mucus (colitis or colonic inflammation), is associated with a narrower list of pathogens, including *Shigella*, *Salmonella*, *Campylobacter*, Shiga toxin–producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), *Yersinia*, *Clostridium*, and some parasites—*Entamoeba histolytica*, *Strongyloides*, and *Balantidium coli*. Recognition of this syndrome affects both diagnostic evaluation and empirical therapy.[370]

ORGANISMS THAT CAUSE DIARRHEA

Many viral, bacterial, and parasitic organisms produce diarrhea in children. The major enteric pathogens associated with infectious diarrhea are shown in Table 44-5.[102]

Viruses

Acute infectious diarrhea of viral origin generally is a self-limited disease characterized by various combinations of diarrhea, nausea, vomiting, abdominal cramps, headaches, myalgias, and low-grade fever.[313,339,461] Bowel movements are watery and generally do not contain mucus or blood. Vomiting is the most common manifestation of this condition. Rotavirus, norovirus, enteric adenovirus, and astrovirus are common causes of viral gastroenteritis.[121,388] Other viruses, including coronaviruses, Breda virus, parvoviruses, pestiviruses, picobirnaviruses, and toroviruses, have been linked to gastroenteritis in humans with varying degrees of certainty.[40,269,507]

Rotaviruses

Worldwide, rotavirus is the leading cause of severe gastroenteritis in children. Rotavirus is responsible for the deaths of nearly 450,000 children younger than 5 years

*www.fisis.usda.gov/OA/pubs/consumerpubs.htm; www.ama-assn.org/foodborne; www.cdc.gov/foodsafety; and www.fightbac.org.

TABLE 44-5 **Pathogens Associated with Infectious Diarrhea in Children**[102]

Type	Enteropathogen	Clinical and Epidemiologic Characteristics
Virus	Rotavirus	Acute watery dehydrating diarrhea with vomiting and fever, particularly in young children during winter
	Norovirus	Acute watery diarrhea with vomiting; short duration; most common cause of outbreaks of nonbacterial gastroenteritis worldwide
	Astrovirus	Short duration mild watery diarrhea with fever and vomiting in young children
	Enteric adenovirus	Mild watery diarrhea with vomiting and low-grade fever in young children; serotypes 40 and 41
Bacteria	*Shigella*	Severe diarrhea, often dysenteric with fever; high risk of person-to-person transmission
	Salmonella	Acute watery diarrhea, occasionally dysenteric, and systemic infections; exposure to carriers, food, and animals (poultry, reptiles)
	Campylobacter	Acute watery diarrhea, often dysenteric with fever; food and animal exposure (poultry)
	Shiga toxin–producing *E. coli*	Watery diarrhea progressing to blood streaked or grossly bloody diarrhea usually without fever; some serotypes are associated with hemolytic-uremic syndrome; food exposure, person-to-person spread, water, and contact with animals[102]
	Other diarrheagenic *E. coli*	Watery diarrhea; some pathotypes with bloody diarrhea (EIEC); usually acute; some pathotypes with prolonged diarrhea (EAEC, EPEC); food exposure[102]
	Vibrio cholerae	Acute watery dehydrating diarrhea in endemic regions; food or water exposure
	Vibrio parahaemolyticus	Watery diarrhea, often dysenteric; seafood related
	Yersinia enterocolitica	Acute watery diarrhea, may cause fever, dysentery and pseudoappendicitis; most common in northern countries; food or animal exposure (swine)
	Aeromonas hydrophila	Acute watery diarrhea; water, food, or animal exposure
	Plesiomonas shigelloides	Acute watery diarrhea; water, fish, or animal exposure
	Clostridium difficile	Diarrhea often with fever and blood after administration of antimicrobial agents
	Clostridium perfringens	Foodborne outbreaks; short-duration acute watery diarrhea with abdominal pain and fever
	Staphylococcus aureus	Foodborne outbreaks; short-duration vomiting sometimes with diarrhea
	Bacillus cereus	Foodborne outbreaks; two distinct short-duration illnesses: emetic and diarrheal
	Listeria monocytogenes	Foodborne outbreaks; systemic infection or acute watery diarrhea, often with fever
Parasite	*Entamoeba histolytica*	Watery or bloody diarrhea (amebic dysentery) with fever and abdominal pain; hepatic amebiasis
	Giardia	Watery diarrhea; usually persistent or chronic diarrhea; food or water exposure
	Cryptosporidium	Watery diarrhea; usually persistent; severe diarrhea in patients with AIDS; food or water exposure; outbreaks
	Isospora belli	Watery diarrhea, usually persistent; severe diarrhea in patients with AIDS; food or water exposure
	Cyclospora	Watery diarrhea, usually persistent; severe in young children; food or water exposure
	Microsporidia	Watery diarrhea; usually persistent; severe diarrhea in patients with AIDS; food or water exposure
	Strongyloides	Watery diarrhea, often with mucus, blood, abdominal pain, and eosinophilia; hyperinfection syndrome

of age each year, mainly in developing countries.[357,457] Rotavirus gastroenteritis affects more than 90 percent of children by the time they are 3 years of age and may cause moderate to severe vomiting that precedes diarrhea. It accounts for 10 to 50 percent of the cases of diarrhea in children and is the most common cause of diarrhea in infants and children during winter months in colder climates. Rotavirus accounts for 35 to 50 percent of infants and young children hospitalized for acute diarrhea. Stools usually are watery or soft, and the presence of blood or leukocytes is rare. Asymptomatic rotavirus infections occur frequently,[339,374] and reinfection appears to be a common event.[473,474] The mechanism of spread is fecal-oral. Shedding of virus most frequently occurs from a few days before to 10 days after the onset of illness.[374]

Natural rotavirus infection efficiently protects against severe disease associated with reinfection. Two virus surface proteins with antigenic properties, VP4 and VP7, are found in various conformations and are the basis for a binary serologic classification scheme that defines the G/P type of the virus[21,365] (Fig. 44-1). Five genotypes (G1 to G4, and G9) accounted for 88 percent of all strains, although extensive geographic and temporal differences exist.[34]

The first rotavirus vaccine licensed in the United States was associated with an increased rate of intussusception, resulting in its withdrawal from the market within 14 months of being licensed.[325,391] Two new vaccines have recently been shown to be safe and effective in protecting young children against severe rotavirus gastroenteritis and are now marketed worldwide.[400,476] Rotavirus vaccines have reduced the health burden of rotavirus gastroenteritis in both developed and developing countries.[122,393]

Noroviruses

Noroviruses are in the family Caliciviridae and have been identified as the second most common viral cause of severe gastroenteritis in children younger than 5 years of age in developing and developed countries, preceded only by rotavirus.[175] Norovirus is considered the most

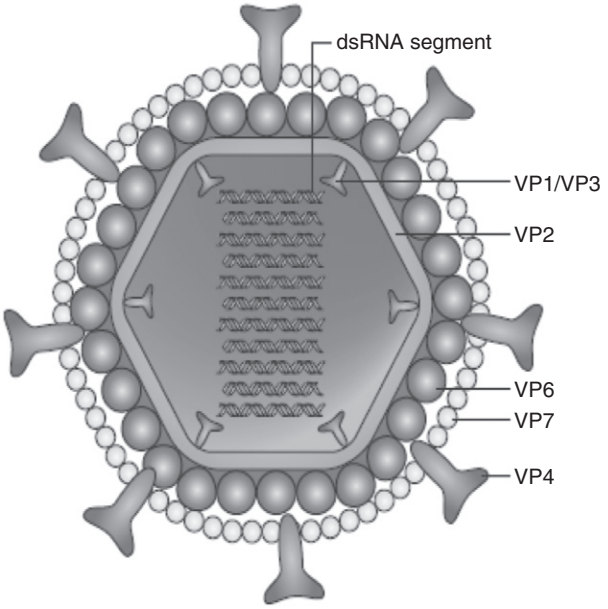

FIGURE 44-1 ■ Rotavirus: schematic representation of a virion. VP4 and VP7, two virus surface proteins, are the basis for the serologic classification of the virus. (See companion website on Expert Consult for a color version of this figure.) (From Angel J, Franco MA, Greenberg HB. Rotavirus vaccines recent developments and future considerations. *Nat Rev Microbiol* 2007;**5**:529–39.)

common cause of outbreaks of nonbacterial gastroenteritis worldwide.[50,302,472,511] It accounts for 12 percent of severe gastroenteritis cases among children younger than 5 years of age and 12 percent of mild and moderate diarrhea cases among persons of all ages.[362] It is estimated that each year norovirus causes 64,000 episodes of diarrhea requiring hospitalization, 900,000 clinic visits among children in industrialized countries, and up to 200,000 deaths of children younger than 5 years of age in developing countries.[362] The mean incubation period for norovirus is 24 to 48 hours. The primary routes of transmission are fecal-oral, including consumption of fecally contaminated food or water; direct person-to-person contact, especially in schools, childcare centers, restaurants, summer camps, hospitals, nursing homes, and cruise ships*; and through contaminated objects or environments. A low inoculum dose is required for infection.[460] Clinical manifestations include acute onset of vomiting, nonbloody diarrhea, or both lasting 12 to 60 hours.[175] The identification of human histo-blood group antigens as norovirus receptors opens a new approach for evaluation of susceptibility and therapy of norovirus infection.[150,175,454]

Noroviruses are genetically and antigenically diverse. Molecular epidemiology techniques have identified substantial diversity in strains, indicating that epidemic strains might be more virulent or more environmentally persistent than nonepidemic strains.[50] Five genogroups (G) and several subgenogroups have been assigned. Strains of three genogroups GI, GII, and GIV are found in humans; genogroups GI and GII are the more frequently found, with GII most prevalent worldwide.[302,509,510]

*References 5, 77, 241, 242, 268, 389.

Astroviruses

Astrovirus gastroenteritis occurs worldwide and has been associated with outbreaks of mild gastroenteritis in schools, daycare centers, pediatric wards, and nursing homes.[124,174,193,214,313] Illness is restricted primarily to children younger than the age of 2 years, elderly people, and immunocompromised individuals.[239,324] Astrovirus infections can be asymptomatic[308] and associated with other enteric viruses.[394] Eighty percent or more of adults have antibodies against the virus. The incubation period is 2 to 4 days.[174,239] Symptoms include fever and malaise, followed by watery diarrhea that may last approximately 3 days. Vomiting is an uncommon symptom. Short-term intolerance of monosaccharides and more prolonged intolerance of cow's milk protein have been reported after astrovirus infection.[174] Until 2008, eight different antigenic types were described in humans, but recently new species have been found in stool samples from patients suffering gastroenteritis.[152-154,255] The virus can be detected by electron microscopy or by more specific molecular tools such as reverse transcriptase–PCR (RT-PCR).[174,239,481]

Enteric Adenoviruses

Human adenoviruses of subgroups A to F have been identified as etiologic agents in a wide range of human diseases, including conjunctivitis, upper respiratory tract infections, and pneumonia. A subgroup of fastidious adenoviruses (group F) with a distinct set of antigenic determinants and specific tissue culture growth characteristics has been shown to be associated with acute gastroenteritis and is referred to as enteric adenovirus.[59,274,470] The enteric adenoviruses in group F include serotypes 40 and 41,[274] which are responsible for approximately 5 percent of cases of endemic pediatric diarrhea worldwide,[127,430-432] with higher prevalence in children younger than age 24 months.[127] The clinical manifestations include watery diarrhea accompanied by vomiting, low-grade fever, and mild dehydration.[274] Antibody prevalence to enteric adenovirus increases from 20 percent during the first 6 months of life to 50 percent or greater by the third or fourth year of life.[433] Seasonal shifts in the predominance of types 40 and 41, similar to those described for rotavirus, may occur.[59] Infection with enteric adenovirus appears to increase in the summer, although with a less marked seasonal variation than that exhibited by rotavirus, which peaks in the winter.[59] Outbreaks of enteric adenovirus diarrhea have been described in childcare centers, where asymptomatic excretion is a common occurrence.[470,483]

Bacteria

Shigella

Shigella is the principal cause of clinical dysentery and an important cause of morbidity and mortality among children in impoverished regions of the developing world.[270] In the United States, shigellosis is the third most frequent FoodNet pathogen in sentinel states but appears to be

decreasing in incidence. There are four serogroups of *Shigella*, containing 46 serotypes: *S. dysenteriae*, *S. flexneri*, *S. boydii*, and *S. sonnei*. In developing countries *S. flexneri* is the predominant species, whereas *S. sonnei* predominates in industrialized regions. Infection by *Shigella* spp. rarely occurs in the first few months of life but is a common occurrence in children between the ages of 6 months and 10 years.[88] In highly endemic areas, peak infection rates occur in the second year of life. Children infected with *Shigella* can have asymptomatic excretion, watery diarrhea, or dysentery. Hemolytic-uremic syndrome may occur after infection with Shiga toxin 1 (Stx1)–producing strains (primarily *S. dysenteriae* serotype 1). The postinfectious complications include reactive arthritis[464] and postinfectious irritable bowel syndrome.[198] Shigellosis results from the exposure to low inoculums of the bacteria; in addition to person-to-person spread, shigellae can be transmitted through contaminated foods, sexual contact, and water used for drinking or recreational purposes.[142,295] Mild symptoms are self-limiting but, in more severe cases, antibiotics are recommended for cure and preventing relapse. The progressive development of antibiotic resistance in *Shigella* isolates is a worldwide problem.[270]

Salmonella

Salmonella infections are associated with several clinical syndromes: asymptomatic carriage, acute gastroenteritis, bacteremia, enteric fever, and dissemination with localized suppuration, such as abscess, osteomyelitis, or meningitis.[96,406] Identification of *Salmonella* spp. in the laboratory is not difficult, but the various terminologies used to classify *Salmonella* spp. are confusing. Based on the current classification, the genus *Salmonella* contains only two species, *S. bongori* and *S. enterica*, but there are more than 2500 serovars of *S. enterica*. *S. enterica* serovar typhi *(S. typhi)* and nontyphoidal salmonellae (NTS) are important causes of infection and disease in children. Typhoid fever is particularly common in South and South East Asia and is estimated to cause more than half a million deaths each year. NTS are a major cause of foodborne infections including gastroenteritis worldwide.[20,182,187] In the United States, in the past decade, the *Salmonella* isolates most frequently reported have been *S. enterica* serotype *typhimurium* and *S. enterica* serotype *enteritidis*.[78,88]

Salmonella gastroenteritis occurs most commonly in the first 5 years of life, decreases in frequency during childhood, and remains relatively constant throughout the adult years.[88] Although most episodes of *Salmonella* infection are foodborne, reptiles, including turtles, snakes, lizards, and iguanas, carry certain serotypes of *Salmonella* in their intestinal tracts and have been associated with episodes of salmonellosis.[376,445] Numerous outbreaks of disease caused by *Salmonella* after ingestion of contaminated food products, including eggs, milk, ice cream, peanut butter, and fresh produce, have been reported.* Foodborne outbreaks associated with *Salmonella* have been reported from fairs or festivals, hospitals, nursing homes, and prisons.[88]

Campylobacter

Campylobacter spp. are recognized as among the most important causes of acute diarrheal disease in humans throughout the world,[12,67,243] including the United States.[12,87] The organism is a microaerophilic, curved gram-negative rod, carried in the intestine of many wild and domestic animals, particularly avian species including poultry. Healthy animals may be intestinal carriers.[435] Water can be a direct source of human infection, although food contamination from food-producing animals is a more significant problem. The main risk factors for *Campylobacter* infection are age younger than 5 years and age 20 to 29 years; male sex; handling and consumption of raw or undercooked poultry and other meats; consumption of unpasteurized milk and dairy products and untreated water; and contact with pets and farm animals.[163] Most *C. jejuni* diarrheal illness in the United States is foodborne.[295]

Currently, 20 *Campylobacter* spp. and subspecies are recognized.[435] *C. jejuni* and *C. coli* are the two predominant species, although use of selective media and failure to use stool filtration techniques may preclude isolation of other *Campylobacter* spp. Many clinical microbiology laboratories do not differentiate *C. jejuni* and *C. coli*. Emerging *Campylobacter* spp. have also been associated with a range of gastrointestinal diseases, particularly gastroenteritis, inflammatory bowel disease, and periodontitis.[298] People infected with *C. jejuni* may develop diarrhea, cramping abdominal pain, chills, and fever. Gross rectal bleeding may occur, and mucus and fecal leukocytes may be present, resembling the illness produced by *Shigella*. The clinical presentation also may mimic that of inflammatory bowel disease. *Campylobacter fetus*, recognized as a cause of fever, bacteremia, and meningitis in immunocompromised hosts and of abortion, rarely causes diarrhea. *C. jejuni* has been associated with reactive arthritis and Guillain-Barré syndrome.[379,380]

Diarrheagenic Escherichia coli

E. coli associated with diarrhea is referred to as diarrheagenic *E. coli*.[328] There are six well-described categories of diarrheagenic *E. coli*, classified based on clinical, microbiologic, and epidemiologic characteristics. Current classification is based mainly on the identification of specific virulence genes associated with each pathotype (Table 44-6).[254,328] In children, diarrheagenic *E. coli* as a group are responsible for 30 to 40 percent of all diarrhea cases worldwide.[342,343,347,354]

8. Shiga toxin–producing *E. coli* (STEC), also referred to as enterohemorrhagic *E. coli* (EHEC) or verotoxin-producing *E. coli* (VTEC), is probably the most important pathogen in this group, owing to its association with hemolytic-uremic syndrome. STEC produces a spectrum of disease, from mild watery diarrhea, to afebrile bloody diarrhea, hemorrhagic colitis, and hemolytic-uremic syndrome.[254,307] Only a small proportion (5% to 10%)

*References 55, 76, 89, 209, 295, 384.

TABLE 44-6 **Diarrheagenic *E. coli* Pathotypes**

Name	Abbreviation	Clinical and Epidemiologic Characteristics	Diagnosis
Shiga toxin-producing or enterohemorrhagic or verotoxin-producing *E. coli*	STEC or EHEC or VTEC	Watery diarrhea progressing to bloody diarrhea usually without fever; may develop hemolytic-uremic syndrome (5%-10%), especially in children <5 years, associated with specific strains including both O157 and non-O157 serotypes; food exposure, person-to-person spread, water, and contact with animals; important reservoir in cattle	Simultaneous culture for O157 STEC and non–culture assay for Shiga toxin: stool culture on sorbitol-MacConkey media to detect sorbitol-nonfermenting *E. coli* O157; detection of Shiga toxins on stool sample by enzyme immunoassays; detection of Shiga toxin genes (*stx1* and *stx2*) and intimin gene (*eae*) by PCR
Enteropathogenic *E. coli*	EPEC	Acute watery diarrhea; may cause prolonged or persistent diarrhea in young children in developing countries; associated with hospital nursery outbreaks; atypical strains (aEPEC) are more prevalent than typical strains (tEPEC) worldwide	Detection of intimin gene (*eae*) ± bundle-forming pili (*bfp*A) by PCR and absence of Shiga toxins; HEp-2 cells adherence assay and serotyping have been replaced by molecular methods; serotyping should be performed only for research or outbreak investigations.
Enterotoxigenic *E. coli*	ETEC	Acute watery dehydrating diarrhea; usually self-limited; important cause of diarrhea in young children in developing countries and most important cause of traveler's diarrhea	Detection of enterotoxins: heat-labile (LT) and heat-stable (ST) by enzyme immunoassays or PCR
Enteroaggregative *E. coli*	EAEC	Acute watery diarrhea; may cause prolonged or persistent diarrhea in young children in developing countries and in HIV-infected patients; second most important cause of traveler's diarrhea	Detection of the *agg*R regulatory gene and other virulence genes: *aap, aat*A, *ast*A by PCR. Characteristic "stacked-brick" attachment patterns on HEp-2 cells, used only in research laboratories
Enteroinvasive *E. coli*	EIEC	Acute watery diarrhea, usually dysenteric; *Shigella*-like pediatric diarrhea; an occasional cause of foodborne outbreaks in industrialized areas	Detection of invasion plasmid antigen of *Shigella* (*ipa*H) by PCR
Diffusely adherent *E. coli*	DAEC	Acute watery diarrhea, particularly in children >12 mo of age in developing countries; may be an important cause of childhood diarrhea in the United States	Detection of Dr adhesins (*daa*D) or Dr-associated genes by PCR; characteristic "diffuse" attachment patterns in HEp-2 cells, used only in research laboratories

PCR, polymerase chain reaction.

of children infected with STEC, usually younger than 5 years of age, develop hemolytic-uremic syndrome.[292] This syndrome is characterized by the triad of hemolytic anemia, thrombocytopenia, and renal insufficiency and is associated most frequently with *E. coli* O157:H7. However, strains of many other serotypes are also associated with hemolytic-uremic syndrome. For example, the large European outbreak that began in Germany in 2011 was produced by an O104:H4 strain.[161] STEC has two potent cytotoxins encoded by lambdoid bacteriophages: Shiga toxin 1 (Stx1, also called verotoxin 1) and Stx2 (or verotoxin 2). Multiple variants of Stx2 exist, as well as several uncommon variants of Stx1. Sxt1 is identical to Shiga toxin made by *S. dysenteriae* type 1.[244,307] These strains usually possess genes like those found in EPEC (see later) for colonizing the gut, although the recent O104:H4 strain had colonization genes like those of EAEC (see later).[62] Many STEC strains appear to be nonvirulent for humans. Most reported outbreaks result after ingestion of contaminated food or water, although person-to-person transmission is also important, particularly in families and daycare settings. Healthy cattle harbor the organism as part of their intestinal flora and are the main animal reservoir for STEC. Direct transmission from animals and their environments to humans in public settings where children come in contact with farm animals, such as petting zoos, represents a public health concern.[376] The diagnosis of STEC is based on the identification of Shiga toxin by immunoassays or PCR or isolation of pathogenic STEC strains.[185,211]

9. Enteropathogenic *E. coli* (EPEC) is associated with both sporadic and epidemic diarrhea in children, especially in developing countries.[131,254] EPEC most commonly causes acute diarrhea and may also cause persistent diarrhea in young children. EPEC was originally serogroup-defined as *E. coli* associated with infantile diarrhea. Subsequently, it was defined by its characteristic localized adherence pattern in tissue cultured cells. Currently, it is identified

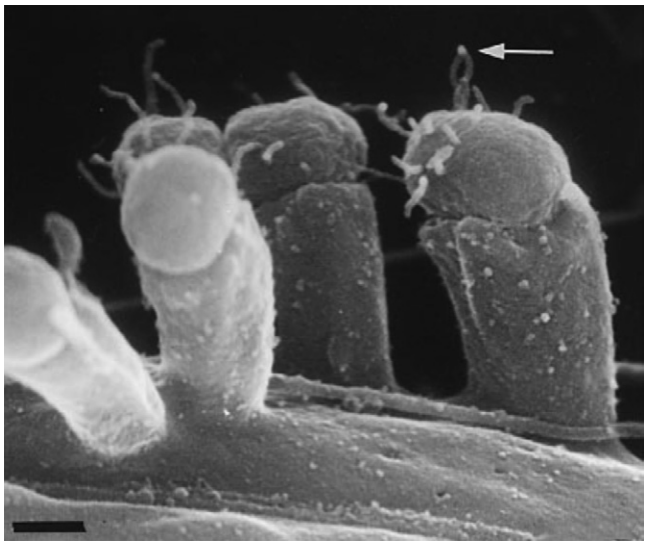

FIGURE 44-2 ■ Enteropathogenic *E. coli* (EPEC): intimate attachment of bacteria to the epithelial surface and effacement of host cell microvilli (pedestal formation lesion). (From Knutton S, Rosenshine I, Pallen MJ, et al. A novel EspA-associated surface organelle of enteropathogenic *Escherichia coli* involved in protein translocation into epithelial cells. *EMBO J* 1998;**17**:2166–76.)

mainly based on the presence of a specific virulence gene *(eae)*.[341] EPEC induces a distinctive histopathology known as the "attaching and effacing" lesion, which is characterized by the intimate attachment of bacteria to the epithelial surface and effacement of host cell microvilli (Fig. 44-2).[131] EPEC is currently divided into two groups, typical EPEC (tEPEC) and atypical EPEC (aEPEC), based on the presence of the EPEC adherence factor plasmid, which is only found in tEPEC. Recent epidemiologic studies indicate that aEPEC is more prevalent than tEPEC in both developed and developing countries.[341] However, the large variety of serotypes and genetic virulence properties of aEPEC strains makes it difficult to determine which strains are truly pathogenic.[212]

10. Enterotoxigenic *E. coli* (ETEC) is an important cause of diarrhea in infants and children living in developing countries and is the most important bacterial agent of traveler's diarrhea.[254,386,448] ETEC causes watery diarrhea, which can be a mild, self-limited disease or severe dehydrating diarrhea. ETEC colonizes the small bowel mucosa and elaborates enterotoxins, which gives rise to intestinal secretion. Colonization is mediated by one or more colonization factors (CFs). ETEC strains express heat-labile (LT) and/or heat-stable (ST) enterotoxins. LT is closely related in structure and function to cholera enterotoxin (CT) expressed by *Vibrio cholerae*.[448]
11. Enteroinvasive *E. coli* (EIEC) is related antigenically and biochemically to *Shigella* and causes either a dysentery-like illness or watery diarrhea. EIEC is distinguished from *Shigella* by a few minor biochemical tests, but these pathotypes share essential virulence factors.[254] EIEC infection is thought to represent an inflammatory colitis, although many patients seem to manifest a secretory, small bowel syndrome. Infections generally occur in adults; foodborne outbreaks have been reported.
12. Enteroaggregative *E. coli* (EAEC) causes acute and persistent diarrhea in children in developing countries, adult traveler's diarrhea, and diarrhea in HIV-infected patients.* The pathogenesis of EAEC infection comprises colonization of the intestinal mucosa in an aggregative, "stacked brick" pattern by means of one of several different aggregative adherence fimbriae (AAF). Some strains of EAEC may then elaborate cytotoxins, including the plasmid-encoded toxin and the enterotoxins EAST1 and ShET1. EAEC virulence factors are under the control of a global regulator, called AggR, in "typical EAEC strains."[204,327] EAEC infections elicit mucosal inflammation. Some infected subjects develop bloody diarrhea as well as a subset who develop chronic persistent diarrhea; persistent illness is particularly important in young children.[44,227,415] EAEC may be an important, unrecognized cause of childhood diarrhea in the United States.[104]
13. Diffusely adherent *E. coli* (DAEC) has been implicated as a cause of diarrhea in several studies, particularly in children older than 12 months of age.[328,414,442] DAEC is defined by the presence of a characteristic, diffuse pattern of adherence to HEp-2 cell monolayers. DAEC strains produce fimbrial adhesins that belong to the Dr family of adhesins.[254,282] DAEC was isolated in 13 percent of diarrhea cases in children younger than 5 years of age in the emergency department at a children's hospital in the United States, where it may be an important, underrecognized cause of childhood diarrhea.[104]

Vibrio cholerae

Cholera is an acute, severe diarrheal disease caused by *Vibrio cholerae* that affects millions of people each year. Without prompt rehydration, death can occur within hours of the onset of symptoms. Cholera affects people of all ages, but children are involved disproportionately. Strains of *Vibrio cholerae* are classified according to somatic or O groups. *V. cholerae* strains are separated further into two main serotypes (Ogawa and Inaba) and two biotypes (classic and El Tor). *V. cholerae* responsible for epidemic cholera belong to serogroups O1 and in recent decades, O139.[93,97,253] It is estimated that *V. cholerae* causes 1 to 4 million cases of diarrhea and more than 100,000 deaths annually.[10,512] During the past several decades, despite advances in water sanitation technology and antibiotic treatment, the seventh cholera pandemic has spread.[512] The cholera burden has grown strikingly during the past 4 years and has spread to countries previously spared by this disease. The current spread has proved especially violent, as illustrated by the recent deadly epidemics in

*References 2, 44, 157, 172, 228, 422.

East Africa and Haiti.[371] Cholera emerged in Haiti in 2010, with more than 400,000 cases and over 6,000 deaths, and continues today.[487] Cholera is rare in the United States. Most clinical isolates of *V. cholerae* O1 in the United States are associated with foreign travel and with ingestion of undercooked seafood,[88,322,332] and many are resistant to antimicrobial agents.[444] Crabs harvested from the U.S. Gulf Coast are a common source of cholera.[78] After Hurricane Katrina in 2005, crabs were the source of illness for certain cases of cholera.[84,88] Most *Vibrio* infections after the hurricane were *V. vulnificus*, *V. parahaemolyticus*, and nontoxigenic *V. cholerae*. *V. cholerae* O75 has caused cholera in the U.S. Gulf Coast states. Since epidemic cholera began in Haiti in 2010, a total of 23 cholera cases caused by toxigenic *Vibrio cholerae* O1 have been confirmed in the United States.[332]

V. cholerae O1 adheres to and multiplies on small intestinal mucosa. Diarrhea occurs after elaboration of several toxins, the most important of which is cholera toxin (CT) composed of one A and five B subunits.[423] The B subunits bind the toxin to the terminal galactose of G_{M1} ganglioside receptors present on intestinal mucosal cells. Inside the epithelial cell, the A subunit activates adenylate cyclase, initiating continuous cyclic adenosine monophosphate production. This results in chloride and fluid secretion into the small intestine lumen.[238,253,423] Strains of *V. cholerae* belonging to serotypes other than O1 and O139 are much less significant pathogens, although they can cause mild and occasionally profuse, watery diarrhea. Other *Vibrio* spp., including *V. fluvialis*, *V. mimicus*, *V. hollisae*, and *V. furnissii*, have been shown occasionally to cause gastrointestinal tract disease.[238]

Vibrio parahaemolyticus

V. parahaemolyticus inhabits warm estuarine waters worldwide. The organism has been found in water, shellfish, fish, and plankton[221] and has caused outbreaks of gastroenteritis after ingestion of contaminated seafood.[305,322] Although widely distributed in coastal waters, *V. parahaemolyticus* is an uncommon cause of diarrhea where consumption of raw seafood is common. Clinical manifestations of infection with *V. parahaemolyticus* are gastroenteritis in 59 percent of cases and include abdominal cramps, nausea, and, less frequently, vomiting, headache, low-grade fever, and chills; wound infections, including hemorrhagic cellulitis in 34 percent; and septicemia in 5 percent.[118] A dysentery-like syndrome has been described in India and Bangladesh.[229] Preexisting liver disease predisposes infected patients to development of septicemia and death.[221] Surveillance data from the United States indicate that the incidence of vibriosis increased from 1996 to 2010 overall and for each of the three most commonly reported species: *V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*.[333]

Yersinia enterocolitica

Yersinia enterocolitica is a gram-negative bacillus that appears to be a common cause of gastroenteritis among children in Europe and Canada but is a relatively uncommon cause of enteritis in the United States.[52] The ingestion of contaminated milk or food such as chitterlings[132,246,283] has been implicated as the mode of transmission in reported outbreaks. The clinical manifestations vary according to the age of the person involved. Illness in children younger than 5 years usually is self-limited gastroenteritis. Stools may contain blood and mucus or be watery. Associated symptoms consist of fever, vomiting, and abdominal pain. Older children may present with abdominal pain associated with mesenteric adenitis that mimics acute appendicitis. Adults develop diarrhea and abdominal pain less frequently than do children but may present with polyarthritis, arthralgia, or erythema nodosum. Patients with β-thalassemia and iron overload are at an increased risk for development of severe yersiniosis.[4]

Aeromonas hydrophila

Aeromonas spp. are gram-negative bacteria found in soil and fresh and brackish water worldwide. *Aeromonas* spp. are recognized as colonizers and pathogens of cold-blooded animals, including fish, reptiles, and amphibians. *Aeromonas* spp. have been associated with a wide spectrum of human disease, most frequently gastroenteritis, soft tissue infection, and bacteremia, especially in immunocompromised hosts.[237] There are many species of *Aeromonas* associated with human diseases; however, *A. hydrophila*, *A. veroni*, and *A. caviae* are the major species associated with gastroenteritis. Because many clinical laboratories cannot perform precise identification, most species isolated are reported as *A. hydrophila*.

The role of *A. hydrophila* in human diarrhea remains controversial. Supporting evidence for aeromonads as intestinal pathogens stems from detailed case reports, epidemiologic case-controlled investigations on *Aeromonas*-associated diarrhea, and generally very low colonization rates in asymptomatic persons.[237] However, one of the impediments to establishing *Aeromonas* as a true gastrointestinal pathogen is the failure to identify a single clonally related outbreak of diarrhea caused by this agent. In addition, volunteers who have been fed the organism have not become ill and no animal model has been established that can faithfully reproduce the diarrheal syndrome.[237] Nevertheless, *Aeromonas*-associated diarrhea occurs worldwide; however, the exact prevalence of this infection on a global scale is unknown.[425] Children with watery diarrhea associated with *Aeromonas* are significantly more likely to have organisms that possess genes for both heat-labile and heat-stable enterotoxins than control children.[6] *Aeromonas* has been linked to cholera-like and dysentery-like illness.[237]

Plesiomonas shigelloides

Plesiomonas shigelloides is a gram-negative bacillus that has been associated with opportunistic infections in immunocompromised hosts and with sporadic cases of diarrhea in immunocompetent hosts in a variety of countries.[145,224,250,257] In some case-control studies, the organism has been found to be associated with diarrhea, whereas in others it has not.[7] The organism has been isolated from surface water and the intestines of

freshwater fish and many animals, including dogs and cats.[462] *Plesiomonas* occurs commonly in tropical and subtropical areas from which most stool isolates have been reported.[224] Patients with *P. shigelloides* infection describe self-limited diarrhea, occasionally characterized by blood and mucus. The organism has failed to produce illness when fed to volunteers, and its role as an enteric pathogen remains uncertain.[213]

Clostridium difficile

Clostridium difficile is the most common bacterial agent associated with severe antimicrobial-associated diarrhea. Enterotoxin A and cytotoxin B account for most of its virulence. The epidemiology of *C. difficile* among children is not well understood. Asymptomatic colonization is very common in neonates and young children; reported rates range from 0 to 50 percent in neonates and up to 70 percent in infants. Frequency of colonization gradually decreases with age: after 4 years of age, rates are less than 5 percent, similar to those of healthy adults.[64] Despite the high frequency of colonization by *C. difficile*, infants rarely develop symptomatic disease. In older children, *C. difficile* disease was traditionally considered a hospital-acquired infection but community-acquired infection has been increasingly described.[90]

Clostridium perfringens

Clostridium perfringens types A, C, and D produce an enterotoxin that is implicated in the pathogenesis of a short-duration food poisoning syndrome.[423] Most foodborne outbreaks are caused by type A strains. Within 14 hours after ingesting contaminated food (meat or poultry products), patients experience watery diarrhea and abdominal pain with minimal nausea, vomiting, or fever; illness resolves in less than 24 hours. *C. perfringens* type C also is associated with a rare destructive intestinal disease called *enteritis necroticans* or *pigbel*, characterized by vomiting, abdominal pain, bloody diarrhea, and small bowel necrosis, with peritonitis, shock, and death.[326,484] These strains produce three toxins (α-toxin, β-toxin, and an enterotoxin) of potential pathogenetic significance.[310,422] Enteritis necroticans occurs after ingestion of undercooked pork.

Staphylococcus aureus

Staphylococcus aureus produces a wide variety of toxins including staphylococcal enterotoxins and staphylococcal-like proteins.[25] Staphylococcal enterotoxins are a major cause of food poisoning, which typically occurs after ingestion of processed meat or dairy products, which have been contaminated with *S. aureus* due to improper handling and subsequent storage at elevated temperatures. Symptoms include nausea and vomiting, with or without diarrhea. The illness is usually self-limiting, and only occasionally is it severe enough to warrant hospitalization.[420] The severity of the illness depends on the amount of food ingested, the amount of toxin in the ingested food, and the general health of the host. Staphylococcal enterotoxin type A has been responsible for more than half of the reported outbreaks of staphylococcal food poisoning in the United States.[223,287]

Bacillus cereus

Bacillus cereus is an aerobic, spore-forming, gram-positive bacillus that is widely distributed environmentally; it causes an emetic or a diarrheal food-associated illness primarily in the industrialized world.[53] *B. cereus* is associated mainly with food poisoning but can also cause potentially fatal nongastrointestinal infections.[53] Although frequently present in food, *B. cereus* should be suspected as the cause of gastrointestinal tract illness if appropriate symptoms are present and if incriminated food, particularly fried rice, contains 10^5 or more *B. cereus* organisms per gram.[72,75]

Two distinct forms of gastrointestinal tract illness can be produced by this organism: the emetic disease, a food intoxication caused by cereulide, a small ring-formed peptide; and a diarrheal syndrome caused by vegetative cells, ingested as viable cells or spores, that produce protein enterotoxins in the small intestine.[446] If the strain causing illness produces the emetic toxin, a syndrome occurs that resembles illness produced by staphylococcal enterotoxin, with nausea, vomiting, and abdominal cramps that begin within 1 to 6 hours of ingestion. If the strain produces enterotoxins, profuse watery diarrhea and abdominal pain begin within 6 to 24 hours, with minimal or no vomiting. Some strains produce both toxins, whereas others produce only one toxin. Symptoms caused by either toxin usually resolve in less than 24 hours and fever rarely occurs. Spores of *B. cereus* are resistant to heat and, therefore, may withstand a brief period of cooking or boiling.

Listeria monocytogenes

Listeria monocytogenes is the causative agent of listeriosis, a potentially fatal opportunistic foodborne infection. *Listeria* spp. are isolated from a diversity of environmental sources, including soil, water, foods, and the feces of humans and animals. The foods most frequently contaminated include soft cheeses and dairy products; pâtés and sausages; smoked fish; and industrially produced, refrigerated, ready-to-eat products that are eaten without cooking or reheating.[32,74,184] *L. monocytogenes* can cause gastroenteritis in otherwise healthy individuals and more severe invasive disease in immunocompromised patients. Common symptoms include fever, watery diarrhea, nausea, headache, cramps, and myalgia.[35,350]

Parasites

The most important parasites known to cause diarrhea are the protozoa (*Entamoeba histolytica*, *Giardia lamblia*, *Cryptosporidium parvum*, *Isospora belli*, *Cyclospora cayetanensis*), and the Microsporidia (*Encephalitozoon intestinalis* and *Enterocytozoon bieneusi*). Among helminths, *Strongyloides stercoralis* and *Trichuris trichiura* may produce diarrhea. Data associating *Ascaris* and hookworm with diarrhea are lacking, but both cause abdominal pain. *Balantidium coli* is a cause of bloody diarrhea in humans. The roles of

Blastocystis hominis[447] and *Dientamoeba fragilis* as causes of diarrhea are controversial.

Entamoeba histolytica

Entamoeba histolytica is the causative agent of amebiasis, a disease that is a major source of morbidity and mortality in the developing world.[387,443] The genus *Entamoeba* contains many species, six of which can reside in the human intestinal lumen. *E. histolytica* is the only species definitely pathogenic. *E. dispar* and *E. moshkovskii* do not appear to be pathogenic. New approaches to identifying *E. histolytica* are based on detection of *E. histolytica*–specific antigen and DNA in stool and other clinical specimens.[160] Clinical patterns that occur in patients with amebiasis consist of intestinal amebiasis, with the gradual onset of colicky abdominal pain and frequent bowel movements, tenesmus, and little or no constitutional disturbance; amebic dysentery, characterized by profuse diarrhea containing blood and mucus and the presence of constitutional signs, such as fever, dehydration, and electrolyte alterations; hepatic amebiasis, which usually presents as abscess formation without gastrointestinal tract symptoms; and asymptomatic colonization.[203] Patients may experience tender hepatomegaly, jaundice, weight loss, fever, and anorexia. The frequency of liver abscess in patients with amebiasis is between 1 and 5 percent. The complications of intestinal amebiasis include perforation, ameboma, stricture, hemorrhage secondary to erosion into a blood vessel, intussusception, ischiorectal abscess, fistulas, and rectal prolapse.[203]

Giardia intestinalis

Giardia intestinalis (synonyms: *G. lamblia* and *G. duodenalis*) is a flagellated protozoan parasite that reproduces in the small intestine, causing giardiasis. *Giardia* is one of the most common intestinal parasites in the world; it contributes to diarrhea and nutritional deficiencies in children in developing regions.[11] In the United States it is the most common intestinal parasite identified by public health laboratories.[81] *Giardia* infection is transmitted by the fecal-oral route and occurs after ingestion of *Giardia* cysts in fecally contaminated food or water or through person-to-person transmission.[142,288,295] The low infectious dose of 10 cysts places people in close contact, including children in daycare, at risk of acquiring infection.[390] Children appear to be more susceptible to *Giardia* than adults. Specific conditions that predispose to giardiasis are hypogammaglobulinemia, secretory immunoglobulin A (IgA) deficiency, peptic ulcer disease, biliary tract disease, and pancreatitis. The parasite may exist in two forms: cyst and trophozoite. The trophozoites usually are seen in duodenal aspirates and loose stools, whereas cysts can be found in formed stools and can remain viable and infectious in water for longer than 3 months. Diagnostic assays using antigen detection are available.[11]

Giardia infection can be asymptomatic or present as an acute illness with a sudden onset of explosive, watery, foul-smelling stools and flatulence, abdominal distention, nausea, and anorexia, with the absence of blood and mucus or as chronic diarrhea and malabsorption, with exacerbations and remissions of flatulence, abdominal distention, and abdominal pain often lasting for months.[372] *Giardia* infections can result in chronic gastrointestinal disorders such as postinfectious irritable bowel syndrome, and symptoms may manifest at extraintestinal sites, even though the parasite does not disseminate beyond the gastrointestinal tract.[112]

Cryptosporidium

Cryptosporidium is an intestinal coccidian protozoan parasite that causes diarrheal diseases in humans worldwide. Children and immunosuppressed individuals, especially those with HIV infection or AIDS, are disproportionately affected.[65] In developing countries there is a high prevalence of cryptosporidiosis in young children, particularly among those who are malnourished or HIV infected.[226,320,352] Fecal-oral transmission of *Cryptosporidium* oocysts occurs from person to person, through ingestion of contaminated drinking water or recreational water, through consumption of contaminated food, or by contact with infected animals (cattle and sheep).[86,88,142,222,288] Unlike bacterial pathogens, *Cryptosporidium* is resistant to chlorine disinfection and can survive for days in treated recreational water, including swimming pools and recreational water parks.[142] Children aged 4 years old and younger appear to be at a particularly high risk for acquiring this organism.[88] *Cryptosporidium* has been implicated as a cause of diarrhea in travelers and of epidemics in hospitals, daycare centers, and other institutional settings worldwide. The largest waterborne outbreak of diarrhea documented in the United States was caused by *Cryptosporidium*. The incubation period in humans has been estimated to be 2 to 14 days.

Cryptosporidiosis can be manifested with a wide spectrum of symptoms, including asymptomatic excretion, acute diarrhea, chronic diarrhea, epidemic diarrhea, severe life-threatening watery diarrhea, and biliary tract disease.[101] Watery diarrhea is the hallmark of symptomatic infections, but few, if any, features distinguish gastroenteritis caused by *Cryptosporidium* in the immunocompetent patient from other enteric infections. Stools do not contain blood or leukocytes. Vomiting, flatulence, abdominal pain, and low-grade fever routinely accompany diarrhea.[207] Symptoms usually subside in an average of 9 days. Patients may have cholera-like illness, transient diarrhea, relapsing episodes, or a protracted clinical course with unremitting, profuse diarrhea lasting for months accompanied by profound malabsorption and weight loss.

In immunocompetent individuals, infection with this parasite may be asymptomatic or cause a self-limiting diarrheal illness. However, in immunocompromised patients such as those with HIV infection or AIDS, *Cryptosporidium* spp. may cause severe, chronic, and possibly fatal diarrhea and wasting. Before HAART was instituted, enteritis caused by *Cryptosporidium* occurred in 10 to 15 percent of patients with AIDS in the United States and approximately 15 percent of patients with AIDS in the developing world.[299] Identification of the organisms and symptoms is more frequent when the CD4 count is less than 200 cells/μL. Antiretroviral therapy is

protective against disease.[69,299] The emergence of drug-resistant HIV variants and failure or discontinuation of HAART has been associated with reemergence of *Cryptosporidium* spp. infection in HIV patients.[338]

Isospora belli

Isospora was established as a cause of diarrhea in humans in the early 1900s, when sporadic cases of isosporiasis and a few clinical series were reported in the medical literature.[130] *I. belli* gained importance with the advent of AIDS and before HAART was shown to be an important cause of severe and prolonged gastroenteritis.[356] Infection can occur in adults and children and has been reported in infants with severe diarrhea.[289] This organism also has been implicated as a cause of traveler's diarrhea.[171] Transmission is fecal-oral, through ingestion of oocysts contaminating food, water, or environmental surfaces.[289] The clinical spectrum of disease caused by *Isospora* is indistinguishable from that described for *Cryptosporidium*. The spectrum includes asymptomatic infection, acute diarrhea in children in developing countries, and chronic diarrhea or severe protracted life-threatening diarrhea in patients with AIDS.[194] Fever, malaise, abdominal pain, and headache have been reported. Stools are watery and do not contain blood or leukocytes. Malabsorption, steatorrhea, severe weight loss, and chronic diarrhea lasting months to years are the most likely occurrences in immunocompromised hosts.[120]

Cyclospora

Cyclospora cayetanensis, a coccidian protozoon, is transmitted by the fecal-oral route. Direct person-to-person transmission is unlikely to occur because excreted oocysts require days to weeks under favorable environmental conditions to sporulate and to become infectious. An animal reservoir has not been described. Outbreaks of diarrhea caused by consumption of water and fresh fruits contaminated with *Cyclospora* have been described,[215,295,297] and travelers to developing countries are at increased risk for development of diarrhea caused by *Cyclospora*. Most of the reported cases have occurred during the spring and summer. The mean incubation period appears to be 7 days.

Clinical manifestations include asymptomatic excretion, acute watery diarrhea, and diarrhea that may be protracted for days to weeks with frequent, watery stools, which may remit and relapse.The clinical presentation is somewhat different in areas of endemicity, where asymptomatic infections are more frequent. Nevertheless, younger children have more severe clinical symptoms. In endemic settings, infections tend to be milder as children get older, because the duration of the infection is shorter and the severity of disease decreases. As in young children, the elderly may also present with a more severe illness.[353]

Microsporidia

Microsporidia have emerged as causes of opportunistic infections associated with diarrhea and wasting in AIDS patients. Among non–HIV-infected but immunesuppressed individuals, microsporidia have infected organ transplant recipients, diabetics, children, the elderly, and patients with malignant disease. In otherwise healthy immune-competent HIV-seronegative populations, self-limiting diarrhea occurred in travelers and as a result of a foodborne outbreak associated with contaminated cucumbers. Greater awareness and implementation of better diagnostic methods are demonstrating that microsporidia contribute to a wide range of clinical syndromes in HIV-infected and non–HIV-infected people.[129]

The nontaxonomic term *human Microsporidia* can refer to any of the order of Microsporidia known to cause disease in humans: *Enterocytozoon* spp., *Encephalitozoon* spp., *Pleistophora* spp., and *Nosema* spp. Of these, *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis* are the most important in gastrointestinal tract disease of humans.[31,486] Both species have been detected in intestinal biopsy specimens of patients with AIDS, with a clinical picture of prolonged diarrhea and weight loss.[111] The clinical spectrum appears to depend on the immune status of the host. The primary location of all intestinal spore-forming protozoal infection is the small intestine, but colonic infection has been reported with *E. bieneusi*.[180] Infection of the biliary tract with *E. bieneusi* and *E. intestinalis* in patients with AIDS can cause sclerosing cholangitis[378,429,493] and acalculous cholecystitis. *E. intestinalis* can infect lamina propria macrophages, fibroblasts, and endothelial cells and can disseminate to other organs, including liver, respiratory tract, and kidney.[180,493] Extraintestinal infection develops after infection with other species of the order of Microsporidia occurs.

Strongyloides stercoralis

Strongyloides stercoralis is a nematode that infects humans through the intestinal tract or through skin if either comes in contact with soil that contains larvae. About one third of people with strongyloidiasis are asymptomatic, and the remainder may have skin, pulmonary, or, more frequently, gastrointestinal tract involvement.[434] People at risk include residents and travelers to endemic areas; natives and residents of the Appalachian region in the United States; institutionalized patients; and people treated with corticosteroids, cimetidine, and antacids.[167] Epigastric abdominal pain occurs and is associated with diarrhea that contains mucus and blood. Some patients may complain of nausea, vomiting, and weight loss with evidence of malabsorption. Eosinophilia and an urticarial rash are prominent features of infection. Diagnosis of *Strongyloides* is often delayed, owing to patients presenting with nonspecific gastrointestinal complaints, a low parasite load, and irregular larval output. Novel diagnostic methods are expected to improve epidemiologic studies and control efforts for prevention and treatment of strongyloidiasis.[317]

The hyperinfection syndrome caused by *Strongyloides stercoralis* has a high mortality rate (15% to 87%). Risk factors include new immunosuppressive therapies, human T-lymphotropic virus (HTLV)-1 infection, cadaveric transplantation, immune reconstitution syndrome, hematologic malignancies (especially lymphoma), tuberculosis,

TABLE 44-7 **Diagnostic Evaluation of the Main Enteropathogens Associated with Pediatric Diarrhea**

Type	Enteropathogen	Laboratory Test
Virus	Rotavirus	Immunoassays: ELISA (preferred method), EIA, immune-chromatography, latex agglutination; reverse transcriptase-PCR (RT-PCR)
	Norovirus	Real time or conventional RT-PCR (preferred method); EIA (less sensitive)
Bacteria	*Shigella*	Conventional stool culture; fecal leukocytes
	Salmonella	Conventional stool culture; blood and bone marrow culture for systemic disease; serotype
	Campylobacter	Stool culture with special media, incubated at 42°C, with microaerophilia; Gram stain; EIA
	Shiga toxin–producing *E. coli*	Simultaneous culture for O157 STEC (sorbitol-MacConkey) and non-culture assay for Shiga toxin detection (ELISA and other enzyme immunoassays) (preferred method); PCR (see Table 44-6)
	Other diarrheagenic *E. coli*	Conventional stool culture for initial isolation of *E. coli* colonies, followed by a specific assay; conventional or real time PCR; HEp-2 cells adherence assays; toxin detection by EIA (see Table 44-6)
	Vibrio cholerae	Stool culture in salt-containing media, with study of isolates for O1 serotype
	Vibrio parahaemolyticus	Stool culture in salt-containing media
	Yersinia enterocolitica	Stool culture in selective media
	Aeromonas hydrophila	Conventional stool culture
	Plesiomonas shigelloides	Conventional stool culture
	Clostridium difficile	Detection of toxins A and B in stools by enzyme immunoassay; cytotoxicity tissue-culture assay; stool culture
Parasites	Intestinal parasites associated with diarrhea	Examination of stools for ova and parasites (*Giardia, E. hystolytica, Strongyloides*), and with special stains for coccidia (*Cryptosporidium, Isospora, Cyclospora*) and Microsporidia; enzyme immunoassays (*E. hystolytica, Giardia, Cryptosporidium*) and PCR for all

malnutrition secondary to chronic *Strongyloides* diarrhea, international travel, and immigration.[301,306]

DIAGNOSIS

Determination of the cause of an episode of infectious diarrhea depends on epidemiologic information, the clinical syndrome, laboratory tests, and knowledge or assessment of an organism for virulence factors. Because virulence properties determine clinical manifestations of disease, an understanding of pathophysiologic mechanisms guides the laboratory evaluation and empirical therapy. The major virulence properties of enteropathogens include adherence, epithelial cell invasion, and production of toxins. Some enteropathogens produce diarrhea by other mechanisms, and enteric pathogens may possess one or several of these virulence properties. Both host and microbiologic factors ultimately determine clinical expression in the individual patient. Not all of the recognized virulence properties of a given species are obvious clinically in a given episode of disease.

The diagnosis of gastroenteritis has traditionally been based on cultures and microscopy. However, this pattern may be changing because acute diarrhea diagnostics are moving away from culture toward rapid nonculture methods. These infections are mainly foodborne and therefore preventable, and it is of paramount importance that public health surveillance for these infections is consistent and reliable.[247] Isolation of cultured organisms is still an invaluable tool for determining sensitivity to antimicrobial agents in clinical settings and for identifying specific strains, virulence factors, or toxins during investigations of outbreaks.[366] However, for some pathogens it can be more important to identify the toxins than the organisms themselves; this is the case, for example, for Shiga toxins of STEC/EHEC or for toxins A and B for *C. difficile*. The use of molecular diagnostics is increasing; improvements include multiplex and quantitative PCR, fluorescence in situ hybridization, and metagenomic analyses.[190,247,351,377] It is likely that many isolation-based methods for serotyping, pulsed-field gel electrophoresis, and antimicrobial drug testing will need to transition to sequence-based techniques to remain epidemiologically useful.[247]

Proper identification of the causative agent of an episode of acute infectious diarrhea will help facilitate initiation of appropriate management. In this section we will discuss the basic concepts for enteropathogen identification by direct microscopic examination, stool cultures, immunologic methods, and molecular methods. Laboratory tests used to detect enteropathogens are listed in Table 44-7.

Macroscopic Stool Examination

A gross examination of the stool specimen should be routine in all patients with diarrhea, even if no laboratory studies are performed. Diarrheal stool that is watery and without mucus or blood usually is caused by an enterotoxin, virus, or protozoan organism, or it may be caused by infection outside the gastrointestinal tract. The color of stools generally conveys little information if the stool does not contain blood. Infectious causes to be considered when stools contain blood or mucus include a cytotoxin-producing bacterium, an enteroinvasive bacterium causing mucosal inflammation, and an enteric parasite associated with blood in stools, such as *E. histolytica*,

B. coli, and *T. trichiura*. When it is present, blood usually is mixed evenly into the stool, except in the case of *E. histolytica* infections, in which blood often is on the surface of the stool, and some STEC infections, in which the stool may be blood streaked. Stools that are particularly foul smelling are consistent with *Salmonella* and other bacteria as well as *Giardia*, *Cryptosporidium*, and *Strongyloides* spp. Stools with little odor suggest an enterotoxin, such as cholera toxin or ETEC, or a viral enteropathogen.

Microscopic Examination

Fecal Leukocytes

Microscopic examination of stool specimens for evidence of fecal leukocytes provides information about the cause of diarrhea and helps determine the anatomic location and presence of mucosal inflammation.[373] Fecal leukocytes are produced in response to bacteria that diffusely invade the colonic mucosa and indicate that the patient has colitis. No inflammatory bacterial enteritis exists in which results of the fecal leukocyte examination are uniformly positive.[218] Thus, results of examination are more helpful when they are positive than when negative. When results are positive, the patient probably has an invasive or cytotoxin-producing organism, such as *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., invasive *E. coli*, STEC, *C. difficile*, or *Y. enterocolitica*, although ulcerative colitis and Crohn disease also are associated with fecal leukocytes.

Diarrheagenic *E. coli* is associated with a mild inflammatory response during symptomatic infection in children.[310] Fecal leukocytes generally are not present in stools from patients with diarrhea secondary to viruses, enterotoxin-producing bacteria, or parasites. The leukocytes seen in cytotoxin-associated and invasive bacterial diarrhea syndromes are polymorphonuclear leukocytes. If the fecal leukocyte examination shows evidence of inflammatory enteritis, further laboratory evaluation is indicated. Fecal lactoferrin and fecal calprotectin are new available markers associated with intestinal inflammation. Fecal lactoferrin is a much more sensitive indicator of inflammatory diarrhea than the fecal leukocyte or occult blood test,[94,230] but it may not be useful in differentiating inflammatory from noninflammatory diarrhea in diarrhea-endemic countries.[30] Fecal calprotectin can also be used to screen for bacterial diarrhea and to differentiate between bacterial and viral pathogens in children with gastroenteritis.[95,451]

Ova and Parasites

Normally, examination of stools for ova and parasites is unnecessary unless the patient has a history of recent travel to high-risk areas, stool cultures are negative for other enteropathogens, the patient is involved in an outbreak of parasitic diarrhea, diarrhea persists for longer than 1 week, or the patient is immunosuppressed. Both trophozoites and cysts of *G. lamblia* and larvae of *Strongyloides* spp. can be identified on direct smears of stool specimens. However, the sensitivity of stool examination for most parasites can be improved by use of a concentration technique and by placement of stools in vials containing a stool preservative. Trichrome and iron hematoxylin both are useful as permanent stains for *Giardia* spp. Pooling of preserved fecal samples is an efficient and economical procedure for detecting ova and parasites.[8] Rational use of the stool ova and parasite examination relies on communication between the clinician and laboratory personnel.[57] For patients in whom giardiasis, cryptosporidiosis, isosporiasis, or strongyloidiasis is considered and in whom stool cultures are negative, aspiration or biopsy of the duodenum or upper jejunum may be indicated. Because these organisms live in the upper intestine, this procedure is more reliable than is examination of stool specimens.[396] *E. histolytica* can be diagnosed by microscopic examination of fresh stool specimens or bowel wall scrapings for cysts or trophozoites. A concentration technique may be helpful in demonstrating amebic cysts. Examination of several stool samples by an experienced technician may be necessary because excretion of cysts often is intermittent and interpretation is difficult. Confusion in differentiating amebic cysts from fecal leukocytes may occur. Microscopy can be used only as presumptive evidence of *E. histolytica* because the nonpathogen *E. dispar* is morphologically identical.[146]

Special Stains for Coccidia

Diagnosis of *Cryptosporidium*, *Isospora*, *Cyclospora*, and the Microsporidia is based on morphologic appearance and staining of stool or histologic examination of tissue sections.[180] Among the most widely used stains to visualize these coccidia are standard acid-fast or modified acid-fast stains, which are based on the use of reagents that enhance the penetration of fuchsin into the organism without the need for heating (modified Kinyoun acid-fast stain). In a modified acid-fast stain, *Cryptosporidium* oocysts, which are 4 to 6 μm with four crescentic sporozoites, stain red and can be differentiated readily from yeasts that stain green.[210] In a study of seven microscopy-based *Cryptosporidium* oocyst detection methods, false-positive results were detected by acid-fast and auramine-rhodamine stains but not by monoclonal antibody–based methods.[27] Oocysts of *I. belli* often are visualized by wet-mount preparations because of their size, which is 20 to 30 μm with four sporozoites in two sporocysts. *Cyclospora* oocysts are 8 to 10 μm in diameter and are nonrefractile spherical organisms containing two sporozoites in two sporocysts that are seen easily on wet mount preparations and are variably acid fast. Microsporidia are difficult to differentiate from bacteria and debris because of the small size of the spores, which measure 1 to 2 μm. For detection, formalin-fixed stool or duodenal fluid can be stained with a calcofluor stain, a modified trichrome stain, or a fluorescent stain.[128,485] Gram, acid-fast, periodic acid–Schiff, and Giemsa stains also have been used to stain the organism.[426] Small bowel biopsy may be more sensitive than is stool examination for establishing the diagnosis of intestinal microsporidiosis.[41] Spores are Gram positive, and parts of the internal structure are positive for acid-fast or periodic acid–Schiff stains.

Stool Cultures

Obtaining of stool cultures cannot be justified in all patients with acute diarrhea.[218] Patients with mild, self-limited illness do not need to have stool specimens cultured. When culture is indicated, the specimen should be inoculated onto culture plate media adequate to isolate *E. coli*, *Shigella*, *Salmonella*, and *Campylobacter*. Fecal specimens can be transported to the laboratory in a non-nutrient holding medium, such as Cary-Blair, when culture cannot be performed immediately. This medium prevents drying or overgrowth of specific organisms. All bloody stool specimens should be evaluated with MacConkey sorbitol medium for *E. coli* O157:H7.[54] Some bacterial enteropathogens require modified laboratory procedures for identification.[218] If these agents are suspected (e.g., *Vibrio* or *Yersinia*), the laboratory should be notified so that appropriate culture methods can be used.

Shigella and *Salmonella* organisms are isolated routinely by clinical microbiology laboratories. *Shigella* species are determined in most clinical laboratories. Speciation is important in salmonellosis because *Shigella* serotype *choleraesuis* and *Shigella* serotype *typhi* cause more severe disease than do other *Salmonella* spp. Serotyping of *Shigella* serotype *enteritidis* usually is not helpful in the individual case, although serotyping is crucial in evaluation of an outbreak. Because so many *Salmonella* serotypes exist, isolation of an unusual serotype can be of use in the investigation of a foodborne epidemic or identification of a potential reptile source of infection. As with *Shigella*, isolation of a species of *Salmonella*, even without demonstration of virulence properties, is considered adequate to make an etiologic diagnosis. Serologic studies are of no value in the individual patient.

Culture of *Campylobacter jejuni* from stools requires special methods and special media. It can be accomplished with media that contain antibiotics or using membrane filters. Inoculated plates should be incubated with microaerophilia at 42 °C and read at 72 hours.

V. cholerae strains can be isolated from stool with use of thiosulfate–citrate–bile salt–sucrose agar, which is the most convenient and frequently used selective medium. This medium is suitable for most enteropathogenic *Vibrio* spp. except *V. hollisae*. Placement of the specimen into an enrichment broth, such as alkaline peptone water with 1 percent sodium chloride (pH 8.5) for 5 hours before placement on thiosulfate–citrate–bile salt–sucrose agar enhances the isolation of vibrios. Serotyping is necessary to classify organisms into those that cause typical epidemic cholera (i.e., O1 and O139 serotypes) and those that cause less severe disease (i.e., non-O1, or nonagglutinating vibrios).[253] *V. parahaemolyticus*, like other vibrios, can be cultured on thiosulfate–citrate–bile salt–sucrose agar. Strains associated with diarrhea are Kanagawa-positive on Wagatsuma agar (i.e., show hemodigestion resembling β-hemolysis), which is a marker for pathogenicity. *V. parahaemolyticus* can be serotyped on the basis of the O and K antigens.[118]

A. hydrophila can be overlooked easily on standard stool cultures. A specialized blood agar has been suggested for isolation.[13] Oxidase testing of organisms that resemble *E. coli* can select organisms as possible *Aeromonas* spp.[13] If oxidase-positive colonies are found, they can be evaluated biochemically to determine species.

C. difficile can be isolated by anaerobic stool culture on agar containing cycloserine, cefoxitin, and fructose. For establishing a definitive diagnosis, demonstration of the presence of cytotoxin in stool specimens and neutralization with antitoxin or by use of enzyme immunoassay or PCR-based assays are necessary.[9,381,404] *C. perfringens* is isolated commonly from feces of well people. Diagnosis of *C. perfringens* food poisoning requires isolation of the organisms from epidemiologically implicated food in a significant quantity (more than 10^5 organisms/g) or demonstration of 10^6 organisms/g of stool from two or more ill people or demonstration of enterotoxin in stools of two or more ill people.[295]

S. aureus may be isolated from food and may not be the cause of illness because not all strains of *Staphylococcus* produce enterotoxin. Conversely, the absence of *S. aureus* from food that has been reheated just before being eaten does not exclude staphylococcal food poisoning because heating may destroy the organism without inactivating the toxin. Confirmation as a cause of foodborne disease requires isolation of the same phage type *S. aureus* from stools or vomitus of two or more ill persons or detection of enterotoxin in epidemiologically implicated food or isolation of 10^5 organisms/g from epidemiologically implicated food, provided the specimen is properly handled.[295] *B. cereus* can be diagnosed by demonstration of greater than 10^5 organisms/g in the incriminated food or isolation of the organism from stool of two or more ill people and not from stool of control patients.[295]

Listeria is cultured on blood agar plates rather than on the usual enteric media.[468] *Y. enterocolitica* can be isolated from routine media, but a differential selective medium, such as cefsulodin–triclosan (Irgasan)–novobiocin agar, is more effective.[52] Cold-enrichment techniques may increase the yield of the organism from contaminated specimens, such as feces. Stool cultures positive for *Y. enterocolitica* only after prolonged cold enrichment may represent environmental strains of low virulence, unrelated to human disease. Biotyping and serotyping for O3, O8, and O9 are helpful in determining the clinical relevance of such isolates.

Immunologic Methods

Detection of antigens, toxins, and/or antibodies of several important enteric pathogens can be performed using commercially available kits such as enzyme-linked immunosorbent assay (ELISA), enzyme immunoassay (EIA), immune-chromatography, latex agglutination kits, and so on. The sensitivity, specificity, and associated positive and negative predictive values of antigen tests for enteric pathogens differ among them and from those of culture. Rapid tests are available for enteric viruses, bacteria, and parasites.

Among viruses, commercially available enzyme immunoassay and latex agglutination kits are available to detect rotavirus antigen in stool specimens. Assay procedures using monoclonal antibodies have improved the sensitivity and specificity to greater than 95 percent.[123,266] Non–group A rotaviruses are not detected by the commercially

available assays. After the Norwalk virus was cloned and sequenced in 1990,[240] two major types of assays for diagnosing human noroviruses were developed. One detects the viral antigens or antibodies against the antigens by recombinant enzyme immunoassays, and the other detects the viral RNA by RT-PCR.[321] Diagnosis of enteric adenovirus can be established by immune electron microscopy of stool specimens, enzyme immunoassay of stool specimens, or propagation in a line of human embryonic kidney cells transformed by adenovirus type 5 (293 cells).[274] Restriction enzyme analysis is the definitive method for classifying individual enteric adenovirus isolates. Commercially available assays for detection of enteric adenovirus are available.[470] Astroviruses grow well in human embryo kidney cells in the presence of trypsin. Electron microscopy, immune electron microscopy, immunofluorescence on cell culture, enzyme immunoassay, and PCR can be used as detection methods.[174]

Several rapid antigen detection tests are available for enteric bacteria, such as *Campylobacter*, *E. coli* O157:H7, STEC (detection of Shiga toxin 1 and 2), ETEC (detection of ST and LT enterotoxins), and *C. difficile* (detection of cytotoxin A and B).[185,248,264,448]

Numerous serologic tests for amebiasis to detect different types and antibodies are available. Serologic test results for amebae almost always are positive in acute amebic dysentery and hepatic amebiasis. *G. lamblia* and *Cryptosporidium* antigens in feces can be detected by use of one of several rapid and sensitive diagnostic tests.[165,267] Enzyme immunoassays[165,256] and fluorescent monoclonal antibody–based assays [164,401] for detection of *Cryptosporidium* antigen in stool specimens are available. For the Microsporidia, nonspecific fluorescence methods or enzyme immunoassay may enhance speed and sensitivity. After preliminary identification by these stains has been achieved, further examination by electron microscopy is needed to classify adequately the Microsporidia into an appropriate genus. Routine histopathologic studies can provide presumptive identification in infected biopsy tissue; diagnostic confirmation requires electron microscopy. Reliable serologic tests are not available.

Molecular Methods

Although molecular diagnostics are still used primarily in research laboratories, they are highly sensitive and specific in detecting infections in small samples and can simultaneously identify multiple pathogens. Multiplex genetic assays are used to detect different toxins, pathogens, and species or genotypes of the same pathogen.[366] All enteric viruses can be detected by PCR; however, the most widely used is the RT-PCR for norovirus.[175] Several molecular techniques have been developed for detection and differentiation of *E. histolytica*, *E. dispar*, and *E. moshkovskii*[160] and for Microsporidia species *E. bieneusi* and *E. intestinalis*.[119,149] Among bacterial agents, there are several multiplex assay systems to detect for combinations of common enteric bacteria such as *Shigella*, *Salmonella*, *Campylobacter*, *Vibrio*, and other foodborne and waterborne pathogens.* However, worldwide, the most widely used are the PCR systems to detect STEC and other diarrheagenic *E. coli*.

STEC, including O157 and non-O157 serotypes, is a significant foodborne pathogen that requires sensitive and discriminatory methods for detection and characterization. In addition to available immunoassays, there are numerous PCR-based methods, conventional or real-time, for the detection of STEC virulence factors and common serotypes.[22,168,169,170,364] Recent technologic advancements have combined the high-throughput performance of the microarray with the specificity and sensitivity of real-time qPCR to make large-scale screening efforts both time and cost effective.[178]

Diarrheagenic *E. coli* were originally serogroup-defined *E. coli* associated with infantile diarrhea, defined also by their characteristic adherence pattern in tissue-cultured cells.[322] Currently, they are identified mainly based on the presence of specific virulence genes for each pathotype.[254] Conventional methods such as colony-based serotyping are not routinely performed outside reference laboratories and as part of outbreaks investigations. Many "classic" serotypes of each pathotype do not harbor the specific virulence genes of that particular pathotype, and, conversely, some genetically defined strains do not belong to the classic serogroups.[68,504] Therefore, O-serogroup identification of diarrheagenic *E. coli*, especially of EPEC, should not be used in clinical laboratories, except as part of outbreak investigations. One exception is STEC; because *E. coli* O157:H7 is the most virulent strain, it should always be sought in clinical laboratories. Other STEC serotypes (e.g., O104:H4) have also caused massive outbreaks of serious disease and should be defined when the epidemiology so dictates. Tissue-cultured cell methods for the identification of specific adherence patterns (e.g., EAEC and tEPEC) are done only in research laboratories; are laborious and time consuming; are not always specific for virulent strains; and are a challenge when the sample produces few colonies, such as a sample from a patient with early-phase gastroenteritis.[234] These traditional methods for differentiating *E. coli* strains have been replaced with more-sensitive PCR methods performed in *E. coli*–isolated colonies or stool samples. Several multiplex PCR-based platforms have been developed (Fig. 44-3) and validated in different countries.*

TREATMENT

In caring for children with diarrhea and dehydration, the major therapeutic considerations include fluid and electrolyte therapy, dietary management, nonspecific therapy with antidiarrheal compounds, and specific therapy with antimicrobial agents to shorten the duration of the illness and eradicate fecal shedding of the organism (Table 44-8). The timely replacement of fluid loss is the single most important intervention to decrease mortality.

Several guidelines for the management of acute gastroenteritis in children have been developed in the United

*References 115, 162, 177, 261, 290, 336, 490.

*References 23, 36, 37, 48, 58, 195, 455, 463, 477.

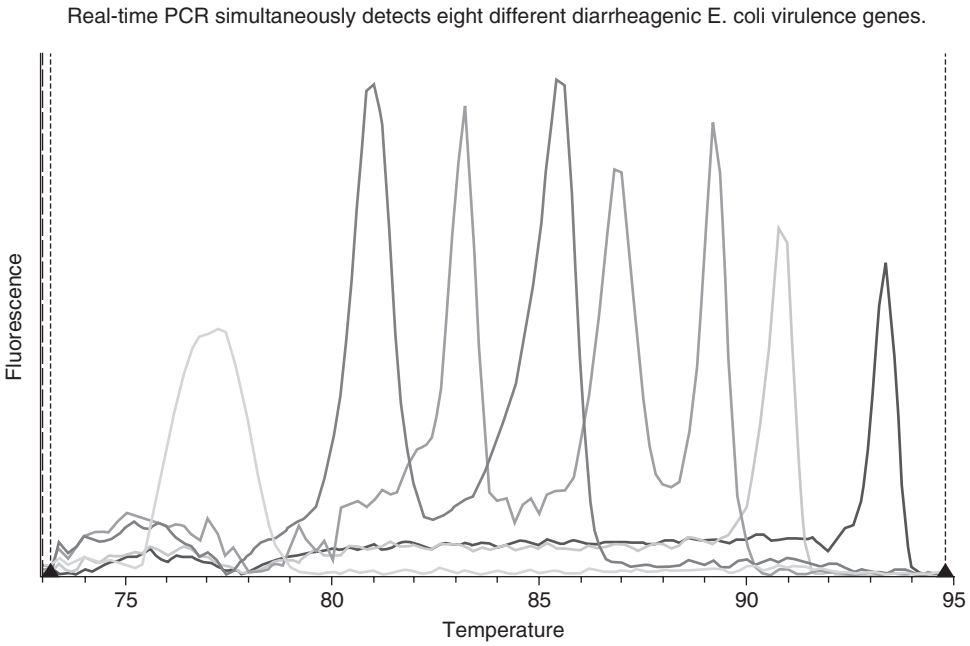

FIGURE 44-3 ■ Real time PCR simultaneously detects eight different diarrheagenic *E. coli* virulence genes. (See companion website on Expert Consult for a color version of this figure.) (From Guion CE, Ochoa TJ, Walker CM, et al. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. J Clin Microbiol 2008;**46**:1752–7.)

TABLE 44-8 **Management of Diarrhea in Children**

Key Points	Description
Rehydration	Clinical assessment of hydration status
	Prevention of dehydration: continue breast-feeding and other milk feeds, encourage intake of fluids available at home
	Oral rehydration therapy with oral rehydration salts (ORS) solutions using the current WHO formulation (reduced osmolarity solution) in mild or moderate dehydration
	Intravenous therapy is required only in hypovolemic shock, obtunded mental status, or ileus.
Nutrition	Start refeeding with the usual age-appropriate diet at the earliest opportunity (4-6 hr after onset of rehydration); no need of a special diet
Antibiotics	Most diarrhea cases in pediatrics do not require antibiotics.
	Antibiotics are recommended for dysentery *(Shigella, Salmonella, Campylobacter),* dysenteric amebiasis, cholera, bacteremia or extraintestinal spread of the infection, and in malnourished or immunocompromised children.
	Antibiotics are not recommended in patients with STEC infection.
Additional therapy	Zinc supplementation (10-20 mg/day) for 10 to 14 days in developing countries
Prevention	Rotavirus vaccination in all young infants
	Exclusive breast-feeding for the first 6 months of age
	Safe and clean weaning foods starting at 6 months of age
	Hand washing with soap, improved water quality, and adequate excreta disposal, especially in resource-limited conditions

States[17,263] and elsewhere* as well as practice guidelines for management of infectious diarrhea in adults[192] and recommendations for management of foodborne illnesses.[19,75] Here we highlight the most important aspects of the management of diarrhea in children.

Fluid and Electrolyte Therapy

Patients who develop diarrhea lose fluid and electrolytes through the gastrointestinal tract by several mechanisms: vomiting, loss of fecal fluid caused by the infecting enteropathogen, and fecal water loss in excess of sodium caused by the intraluminal osmotic effect of unabsorbed nutrients.[219] The composition and amount of lost fluid depend on the rate of stool loss and the causative agent. The higher the rate of stool loss, the greater the sodium loss, probably as a result of rapid passage of intestinal contents through the colon, where sodium-potassium exchange occurs. Stools from patients with cholera or ETEC infection contain sodium in a concentration of 80 to 120 mEq/L, whereas stools from patients with virus infection have sodium concentrations of less than 50 mEq/L.[206,263,410] In secretory diarrheal disorders, loss of fluid generally is derived from the small intestine, and colonic reabsorption is overwhelmed. In viral gastroenteritis, small bowel absorptive capacity primarily is impaired; and in dysenteric or invasive diarrhea, reabsorptive capacity of the large intestine is reduced. If

*References 45, 188, 196, 197, 205, 329, 497.

vomiting also is a manifestation, this loss is compounded. Continued loss of fluid or electrolytes may lead to dehydration, with potentially severe sequelae. Children, especially infants, are more susceptible to dehydration because they have greater basal fluid and electrolyte requirements per kilogram and because they depend on others to meet these needs.

Important factors to be considered in evaluating patients with diarrhea and possible dehydration include an estimation of deficiency, ongoing daily requirements, continued losses and their replacement, and correction of the underlying cause.[17,189,219] The clinical signs and symptoms that may help in estimating deficiencies and determining the severity of dehydration include thirst, dryness of the mucous membranes, decrease in urinary output, tachycardia, loss of skin elasticity and turgor, and mottling and coolness of the skin.[17,79] Various scores have been proposed for the assessment of dehydration and disease severity, but they have not been validated for the assessment of dehydration in individual patients.[188] These signs may be misleading in patients who are malnourished or in patients with hypertonic dehydration.[498,499] In clinical practice, a physical examination enables the clinician to classify patients into the three groups of none, mild/moderate, or severe dehydration. This general assessment can then be used to guide clinical management.

The main complication associated with gastroenteritis is dehydration; it should be prevented with appropriate fluid management. In children with gastroenteritis but without clinical dehydration, it is recommended as primary prevention of dehydration to continue breastfeeding and other milk feeds, encourage intake of fluids available at home, offer oral rehydration salt (ORS) solution as supplemental fluid to those at increased risk of dehydration, and discourage the drinking of fruit juices and carbonated drinks, especially in those at increased risk of dehydration. All these juices and drinks contain inadequate amounts of sodium and potassium and excessive carbohydrate concentrations, which may exceed the absorptive capacity of the intestine and may exacerbate diarrhea due to high osmotic activity.[262,428] The homemade sugar and salt solutions are not recommended because errors in preparing the solutions may result in dangerously high or low sodium concentrations.[424,491]

Oral rehydration should be used as first-line therapy for the management of mild-to-moderate dehydration in children with gastroenteritis. Oral therapy consists of rehydration with replacement of ongoing losses during the first 4 to 8 hours of therapy with a glucose-electrolyte solution.[98,263,410] Children with moderate dehydration should receive ORS ad libitum, approximately 75 mL/kg in 4 or 6 hours, following "Plan B" of the WHO recommendations.[497] When oral rehydration is not feasible, enteral rehydration by the nasogastric route is as effective if not better than intravenous rehydration. Enteral rehydration is associated with significantly fewer major adverse events and a shorter hospital stay compared with intravenous therapy and is successful in most children.[158,208] Once the patient is rehydrated, feeding should be restarted.

The ORS solution must contain glucose, sodium, and other electrolytes that are lost in diarrhea (Table 44-9). The glucose in the ORS solution is necessary to promote intestinal absorption of sodium and water in the small intestine. The formulation ORS solution must preserve the 1:1 molar ratio of sodium to glucose that is critical for efficient cotransport of sodium and glucose. The composition of the original WHO ORS solution was selected to allow for use of a single solution that would effectively treat dehydration secondary to diarrhea caused by various infectious agents and resulting in varying degrees of electrolyte loss, such as cholera or ETEC infection.[263] However, viral gastroenteritis is common in

TABLE 44-9 **Compositions of Rehydration Solutions and Chemical Composition of Diarrheal Stools in Children**

	Osmolarity (mOsm/L)	Glucose (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Potassium (mmol/L)	Citrate (mmol/L)	Bicarbonate (mol/L)
Oral Rehydration Solutions							
Original or standard WHO solution	311	111	90	80	20	10	—
New WHO reduced osmolarity solution	245	75	75	65	20	10	—
Hypotonic osmolarity solution*	240	90	60	60	20	10	—
ReSoMal†	300	125	45	70	40	7	—
Intravenous Fluids							
Ringer's lactate‡	272	—	130	111	4	—	28
Cholera saline (Dhaka solution)§	432	140	133	98	13	—	48
Normal saline	300	—	154	154	—	—	—
Losses in Children							
Cholera stool		—	105	30	25	—	30
Noncholeraic stool		—	52	14	25	—	14

*This is one example; there are several other commercially available ORS solutions with different sodium (50 and 45 mmol/L) and glucose concentrations.
†ReSoMal is a modified oral rehydration solution for patients with severe malnutrition.
‡Preferred IV therapy by WHO recommendations. The base is lactate, not bicarbonate.
§Some preparations do not have glucose and therefore have a lower osmolarity.[294]

pediatric patients and is associated with less severe salt losses, so there was concern that the sodium content of the original WHO ORS solution might be excessive.[133] The current WHO ORS formulation, so-called reduced osmolarity solution, adopted in 2002 (see Table 44-9) has a reduced osmolar load (245 mOsm/L) compared with the original formulation (311 mOsm/L). The so-called hypotonic osmolarity solution, containing sodium 60 mmol/L, is also accepted. Both solutions have reduced the need for unscheduled intravenous fluid infusion and have less stool output and less risk of vomiting compared with the original formulation.[98,201] The rice-based ORS that contains glucose polymers and amino acids may be more beneficial than the glucose-based oral rehydration solution in children with cholera diarrhea.[183] Unlike reduced osmolarity ORS, rice-based ORS does not reduce the need for intravenous infusion.[159]

Intravenous therapy is required if the patient is in hypovolemic shock, has an obtunded mental status, or has an ileus. Children with severe dehydration should receive intravenous fluids immediately. If the patient can drink, ORS should be given by mouth until the drip is set up. Children should receive 100 mL/kg lactated Ringer solution divided as follows: infants (<12 months) 30 mL/kg in 1 hour, followed by 70 mL/kg in 5 hours; older children (>12 months) 30 mL/kg in 30 minutes, followed by 70 mL/kg in 2½ hours, following "Plan C" of the WHO recommendations.[497] If the child is able to tolerate orally administered fluids, intravenous infusion should be stopped and further rehydration completed with oral rehydration therapy alone.[98]

Nutritional Management

Adequate feeding is important to reduce the nutritional defects caused by diarrhea.[17,263] Children with diarrhea who are not dehydrated should be fed age-appropriate diets.[480] Children who require rehydration should be started on refeeding with the usual diet at the earliest opportunity, while the oral glucose-electrolyte solution is continued to replace ongoing losses from stools. It is recommended to feed starting 4 to 6 hours after the onset of rehydration. Early feeding may reduce duration of diarrhea and improve weight gain.[61,188,220]

Breast-feeding in infants should be continued at any time during an episode of diarrhea. Continuing breast-feeding during acute gastroenteritis has been shown to exert a beneficial effect by reducing the number and volume of diarrheal stools and reducing the duration of diarrhea in rotavirus gastroenteritis.[199,259] Refeeding with undiluted or full-strength nonhuman milk (cow's or formula milk) should continue. Weaned children should be fed whatever they eat normally. Full feeding of appropriate-for-age foods is well tolerated and is better than the practice of withholding food, resulting in better weight gain, without increasing complication rates of treatment failures.[406] Controlled clinical trials suggest that complex carbohydrates (rice, wheat, potatoes, bread, and cereals), lean meats, yogurt, fruits, and vegetables are well tolerated in children with mild to moderate diarrhea and should be continued unrestricted as age-appropriate foods after the period of rehydration.[188]

Transient lactose intolerance occurs in some children with gastroenteritis. However, routine use of lactose-free formula is not necessary and only should be indicated in patients with severe and prolonged diarrhea. There is not enough evidence to support the routine use of soy-based formula or hydrolyzate formula in infants with acute gastroenteritis.[26,107] Beverages such as tea, soft drinks, and fruit juices that contain a high sugar concentration should be avoided, owing to the risk of inducing further fluid losses as a consequence of increased osmotic loads.[392,469]

Antimicrobial Therapy

Because viruses are the predominant cause of acute diarrhea in children in developed and developing countries, the routine use of antimicrobial agents for treating diarrhea wastes resources and might lead to increased antimicrobial resistance. Even when a bacterial cause is suspected in an outpatient setting, antimicrobial therapy is not usually indicated in children because the majority of cases of acute diarrhea are self-limited and not shortened by administration of antimicrobial agents. Exceptions to these rules involve special needs of individual children (e.g., immune-compromised hosts, premature infants, or children with underlying disorders) and particular organisms and conditions.[263]

Therapy with antimicrobial agents is important in most cases of diarrhea caused by invasive or inflammatory bacterial pathogens and can be useful in other noninvasive forms of bacterial diarrhea. Recommendations for antibiotic therapy in each form of bacterial diarrhea are provided in Table 44-10. Antibiotics should be used for the treatment of children with suspected or confirmed septicemia; for those with extraintestinal spread of bacterial infection; for children younger than 6 months of age with *Salmonella* gastroenteritis; for those who are malnourished or immunocompromised with *Salmonella* gastroenteritis; and for those with *C. difficile*–associated pseudomembranous enterocolitis, giardiasis, dysenteric shigellosis, dysenteric amebiasis, or cholera.[329]

When antimicrobial therapy is administered to selected patients with gastroenteritis, the aim of the therapy is to abbreviate the duration of the clinical course and to decrease excretion of the causative organisms. A stool culture specimen should be obtained when antibiotic treatment is anticipated, and antibiotic susceptibility testing of any suspected pathogen should be performed to ensure optimal therapy. Changing susceptibility patterns renders the initial selection of an antimicrobial agent difficult. Antimicrobial agents should not be used routinely for gastroenteritis of unknown cause.

Therapy for Dysentery

Dysentery is defined as acute bloody diarrhea typically with abdominal pain and fever caused by invasive microbial infection. The child with bloody diarrhea is at higher risk for complications, including sepsis and other systemic diseases; therefore, the threshold for admission of such children to the hospital for close observation is lower. Stool cultures are indicated in the setting of acute

TABLE 44-10 **Antimicrobial Therapy for Children with Bacterial Diarrhea**

Pathogen	Antibiotic*	Comments
Shigella	Ciprofloxacin	First-line therapy by WHO recommendations; increasing resistance rates in some countries
	Azithromycin, ceftriaxone, pivmecillinam	Second-line therapy by WHO recommendations
	Cefixime, nalidixic acid, furazolidone	Alternative drugs for nonsevere dysentery
	TMP-SMX; ampicillin	High frequency of resistance; use only for susceptible strains
Salmonella	None	For nontyphoidal *Salmonella* diarrhea, except in special patients
	Ciprofloxacin; azithromycin, ceftriaxone	For severe diarrhea in special patients†; or for systemic infection or typhoid fever
	TMP-SMX; chloramphenicol	High frequency of resistance; use only for susceptible strains
Campylobacter	Azithromycin; erythromycin	First-line therapy. High frequency of ciprofloxacin resistance in many countries
STEC	None	May increase the risk of hemolytic-uremic syndrome
ETEC, EAEC, EPEC	Ciprofloxacin; azithromycin; ceftriaxone	Based on recommendations from adults with traveler's diarrhea
	TMP-SMX	High frequency of resistance; use only for susceptible strains
EIEC	Treat as shigellosis	
Vibrio cholerae	Azithromycin, erythromycin	Azithromycin resistance is rare; it is the preferred therapy
	Ciprofloxacin, doxycycline, tetracycline	Alternative drugs; potential toxicity of tetracyclines, use in children >8 year
Diarrhea due to noncholeraic vibrios	None or treat as *Shigella*	
Aeromonas, Plesiomonas	Treat as shigellosis	
Clostridium difficile	Metronidazole, vancomycin	Stop offending antibiotic, if possible

TMP-SMX, trimethoprim-sulfamethoxazole.
*Ceftriaxone is administered intravenously or intramuscularly; all other listed drugs are given orally.
†Infants <6 months of age, malnourished and immunocompromised children.

bloody diarrhea and are helpful for guiding therapy.[370] The four major causes of bloody diarrhea (including both dysenteric and nondysenteric forms) in the United States, in descending order of frequency of occurrence, are *Shigella*, *Campylobacter*, nontyphoid *Salmonella*, and Shiga toxin–producing *E. coli* (STEC).[137] Other organisms may also cause dysentery, including *Aeromonas* species, noncholeraic vibrios, and *Yersinia enterocolitica*. Once laboratory diagnosis is made, pathogen-specific antimicrobial therapy should be initiated for all forms of infectious colitis (*Shigella*, *Salmonella*, and *Campylobacter*) other than STEC.[138] Treatment of dysentery in cases in which stool culture is not available should be targeted at *Shigella*.

Shigella

Mild symptoms are self-limited, but in more severe cases antibiotics are recommended for cure and for preventing complications and relapse. The progressive development of antibiotic resistance in *Shigella* isolates is not new. Ampicillin and trimethoprim-sulfamethoxazole, once affordable mainstays of therapy, have long ago lost efficacy in most *Shigella* endemic regions. Resistance to nalidixic acid, fluoroquinolones, ceftriaxone, and azithromycin and multidrug–resistant strains are also being reported in many countries.[29,270,271] Therefore, regularly updated local or regional antibiotic sensitivity patterns to different species and strains of *Shigella* are required to guide empirical therapy.[100, 270] In the absence of local data, the recommendations from the WHO should be followed. First-line therapy is ciprofloxacin (in all age groups, including pediatrics), and second-line therapy is pivmecillinam (where available), ceftriaxone, or azithromycin.[496] Azithromycin has the added advantage of also treating most isolates of *Campylobacter*, a second major cause of dysenteric diarrhea in children younger than the age of 2 years in developing countries.[271] The antibiotics recommended by the WHO are effective in reducing the clinical and bacteriologic signs and symptoms of dysentery and thus can be expected to decrease diarrhea mortality attributable to dysentery.[465]

Campylobacter

Isolation of *Campylobacter* from stool does not imply the need for antimicrobial therapy. In a meta-analysis of eight randomized, controlled trials of antimicrobial therapy versus placebo, antimicrobial therapy shortened the duration of intestinal symptoms by 1.3 days.[459] The decision concerning therapy should be individualized, but therapy should be considered in patients with high temperatures, bloody diarrhea, severe diarrhea, and in immunocompromised hosts. *Campylobacter* is increasingly resistant to antibiotics, especially fluoroquinolones and macrolides, which are the most frequently used antimicrobial agents for the treatment of campylobacteriosis.[294] Either erythromycin or azithromycin is preferred. In addition to resistance, antibiotic management has also become more complex because postinfectious irritable

bowel syndrome has been associated with a longer duration of untreated infection.[264]

Salmonella

The type of syndrome produced by *Salmonella* (acute gastroenteritis; bacteremia and enteric fever; dissemination with localized suppuration [e.g., abscess, osteomyelitis, or meningitis] influences the selection and duration of antimicrobial therapy. Antibiotics should not be used in the treatment of patients who are nontyphoid *Salmonella* carriers or in most patients with mild gastroenteritis. Antimicrobial therapy may, on occasion, convert intestinal carriage into systemic disease with bacteremia,[397] prolong excretion of *Salmonella*,[28] produce a bacteriologic or symptomatic relapse,[28] or encourage development or selection of resistant strains.

Acute diarrheal disease caused by nontyphoid salmonellosis can be complicated with bacteremia in approximately 8 percent of normal healthy persons. Patients with bacteremia often present with high fever and systemic toxic effects. Risk factors in the host that are associated with a higher than 8 percent risk of systemic *Salmonella* infection during bouts of gastroenteritis include extremes of age (<3 months and ≥65 years), corticosteroid use, inflammatory bowel disease, immunosuppression, hemoglobinopathy including most cases of sickle cell disease, and hemodialysis. People with one of these risk factors and nontyphoid salmonellosis should be treated with antibacterial drugs.[137] Antibiotic treatment of *Salmonella* infection should be given to all patients with typhoid fever, bacteremia caused by nontyphoidal strains, and dissemination with localized suppuration.

Increasing occurrence of antimicrobial resistance in both typhoidal and nontyphoidal *Salmonella* infection is a major public health problem. Recent studies have documented the occurrence of resistance, with particular reference to quinolones and extended-spectrum cephalosporins. Occurrence of multidrug resistance has declined in some areas, but the incidence of decreased ciprofloxacin susceptibility has reached high levels, particularly in the Indian subcontinent, and isolates with full resistance to this antimicrobial agent are increasingly reported. Patterns of resistance in *Salmonella* are constantly changing. Continual surveillance of resistance levels is critical for clinicians to keep abreast of treatment options, but it is often lacking in resource-poor regions of the world with the highest disease burden.[359,406]

Several outbreaks of multiresistant *Salmonella* infection in the United States have been traced to animals.* Resistant strains of *Salmonella* are common findings in retail ground meats, possibly because of overuse of antibiotics in animals used for food.[489] Human isolates of *Salmonella* should have susceptibility testing performed to guide therapy. Recommended antimicrobial agents include chloramphenicol, third-generation cephalosporins, fluoroquinolone, and azithromycin. In the presence of multidrug resistance, a fluoroquinolone is the first line of therapy and a third-generation cephalosporin is the second choice.[406]

*References 71, 166, 176, 252, 421, 450.

Shiga Toxin–Producing E. coli (STEC)

Some antibacterial drugs, including fluoroquinolones and trimethoprim-sulfamethoxazole, may increase the induction of phage-mediated production of Shiga toxin and may increase the risk of development of hemolytic-uremic syndrome.[509] However, an association between the use of these antibiotics and an increased risk of this syndrome has not been proven.[42,402,495] In one study in the United States, individuals with STEC O157 infection presenting with a more severe illness were at an increased risk of developing hemolytic-uremic syndrome; the use of bactericidal antibiotics to treat STEC O157 infection, particularly β-lactams, was associated with the subsequent development of hemolytic-uremic syndrome.[436] In a large prospective surveillance study in Argentina the main risk factor for progression to hemolytic-uremic syndrome was neutrophilic leukocytosis in the early course of the diarrheal episode in STEC-positive children with bloody diarrhea.[292] On the other hand, other drugs, including fosfomycin, azithromycin, and rifaximin, do not appear to increase production of Shiga toxin.[233,235,340] In a mouse model, azithromycin inhibited a Shiga toxin–induced inflammatory response and prevented death.[346] During the STEC O104:H4 outbreak in Germany in 2011, STEC-infected patients were monitored and observed for approximately 40 days; treatment with azithromycin was associated with a lower frequency of long-term STEC O104:H4 carriage.[337] Additional studies are needed to determine the effects of azithromycin and rifaximin on reducing diarrhea and decreasing the risk of the hemolytic-uremic syndrome among patients with STEC colitis. Pending such data, most authorities recommend supportive treatment only; antibiotic therapy is not generally recommended.[137,456] Currently, there is no cure for STEC infection; however, chimeric or humanized monoclonal antibodies have been developed that neutralize the Shiga toxins, and those therapies may be able to prevent the development of hemolytic-uremic syndrome in a STEC-infected patient.[307]

Therapy for Other Bacterial Agents

Diarrheagenic E. coli

Antibiotics are not used routinely for treatment of diarrheagenic *E. coli*, except in traveler's diarrhea. There are no clinical trials on the efficacy of antibiotics for the treatment of the different *E. coli* pathotypes in children, because simple and rapid diagnostic methods are lacking in order to conduct proper trials. Most of the current information on therapy for diarrheagenic *E. coli* comes from case series in children or data from studies in adults. Antimicrobial agents have been employed frequently in attempts to treat infantile gastroenteritis caused by EPEC and as a means of controlling the spread of EPEC strains in hospital nurseries. Although no definitive studies support the effectiveness of these drugs, they may be useful in certain situations, particularly when life-threatening infection occurs or when epidemic spread of the strains continues, despite the use of strict hand washing and appropriate isolation. The treatment in

patients with diarrhea caused by EIEC is similar to that in patients with shigellosis. Diarrhea caused by ETEC and EAEC usually is self-limited, but studies in adults have shown that antimicrobial agents such as ciprofloxacin, azithromycin, and rifaximin are effective therapies.[358] Antimicrobial resistance in diarrheagenic *E. coli* is an increasing problem worldwide[16,335,344,347] as well as the resistance of commensal *E. coli*.[39,141] Therefore, regional antibiotic sensitivity patterns to different *E. coli* pathotypes are required to guide empirical therapy when a decision is made to treat an individual patient.

Cholera

Effective antibiotic treatment reduces the volume and duration of diarrhea in patients with cholera and reduces the number of days that symptomatic patients shed viable *V. cholerae*. Tetracyclines, quinolones, and macrolides are active against *V. cholerae*; however, antibiotic resistance occurs frequently, and resistant strains can emerge rapidly. Therefore, the choice of antibiotic agent is based on results of local antibiotic resistance patterns. Stool cultures performed as part of a surveillance program are also useful for monitoring trends in antimicrobial resistance. In areas of endemicity, many strains have become resistant to tetracycline and to fluoroquinolones. In these areas, azithromycin treatment is preferred and is administered as a single dose of 1 g in adults and 20 mg/kg in children. At present, azithromycin resistance remains rare; and owing to the potential toxicity of tetracyclines and fluoroquinolones in children and pregnant women, azithromycin is also preferred for these patients.[258,487]

Therapy for Intestinal Parasites

Entamoeba histolytica

Metronidazole is the most commonly used medication for invasive amebiasis caused by infection with *Entamoeba histolytica*. The efficacy of available treatments was assessed in a meta-analysis: tinidazole was better than metronidazole at reducing clinical failure and was better tolerated; combination drug therapy was more effective in reducing parasitologic failure compared with metronidazole alone.[179]

Giardia

Metronidazole, tinidazole, secnidazole, and ornidazole are effective therapies for *Giardia*.[501] Occasionally, the initial improvement is followed by a subsequent relapse of symptoms, which requires repeat treatment. Some patients experiencing relapse needed combination therapy. A recent meta-analysis has shown that albendazole, given as a single dose of 400 mg/day for 5 days, was comparable to metronidazole, with fewer side effects.[439] There are new antigiardial drugs being developed.[458]

Cryptosporidium

Children and immunosuppressed individuals, especially with HIV infection or AIDS, are disproportionately affected. Nitazoxanide given to HIV-negative Zambian children with diarrhea eradicated infection, stopped diarrhea, and reduced mortality, whereas no benefit was seen in children who were HIV positive.[15] Recent research confirms the limited effectiveness of antiparasitic drugs to treat cryptosporidiosis in AIDS.[65] Questions remain about using partially active drugs paromomycin and nitazoxanide for treatment. Potent antiretroviral combinations modify disease epidemiology and are key components of therapy in AIDS. However, it is unclear whether this is due solely to immune reconstitution or due in part to antiparasitic effects of HIV protease inhibitors.[65]

Cyclospora

The treatment of choice for infection with *Cyclospora* is trimethoprim-sulfamethoxazole. Ciprofloxacin or nitazoxanide can be used as an alternative therapy, especially in patients who are allergic to sulfa products.[353]

Microsporidia

For microsporidial infections other than with *Enterocytozoon bieneusi*, albendazole is effective. A randomized trial showed that albendazole therapy was effective in improving the clinical manifestations and decreasing the duration of the illness of children with diarrhea caused by the Microsporidia.[466] *Encephalitozoon intestinalis* is susceptible to albendazole,[106,394] which has been reported to stop diarrhea and weight loss as well as to promote weight gain in patients infected with *E. intestinalis*,[315,466] although improvement has not been uniform. *E. bieneusi* does not respond to albendazole but does respond to fumagillin, which is more toxic.[316] For all patients with HIV/AIDS antiretroviral treatment to raise the CD4 count is a major element in management.[501]

Strongyloides

Patients infected with *S. stercoralis* should be treated with ivermectin or, alternatively, thiabendazole or albendazole. In disseminated strongyloidiasis, ivermectin or thiabendazole therapy should be continued for at least 5 days. In immunocompromised patients and patients with *Strongyloides* hyperinfection, longer duration of therapy may be necessary[286]; however, the mortality rate is high despite therapy. In hyperinfection syndrome, rapid initiation of therapy with broad-spectrum antibiotics and anthelminthic drugs is the first step in management. A thorough examination should be performed before immunosuppressive therapy is given to a patient with a history of infection with *S. stercoralis*.[301]

Additional Therapy

Zinc

Zinc is an important trace element in gastrointestinal structure and function. It is involved in epithelial barrier integrity, tissue repair, and immune function.[43,382,427] Zinc deficiency, which is common in young children in developing countries, is worse because intestinal losses of zinc

are considerably increased during acute diarrhea.[70,275] Several studies and meta-analysis have shown that zinc supplementation can reduce the incidence of respiratory and diarrheal diseases.[46,60,399] In developing countries, zinc supplementation results in clinically important reduction in the duration and severity of acute diarrhea when given as an adjunct to oral rehydration therapy.[33,368,398,412] The WHO recommends zinc supplementation to all children with diarrhea in a dosage of 10 mg/day in those younger than 6 months of age and 20 mg/day in older infants and children for 10 to 14 days.[499,500] Zinc decreases the severity and reduces the number of episodes of diarrhea occurring within 2 to 3 months after the intake of zinc. Recent meta-analyses have shown that in areas where the prevalence of zinc deficiency or the prevalence of moderate malnutrition is high, zinc supplementation in children is associated with a reduction in the morbidity and mortality of diarrhea,[479,503] especially among children older than 6 months of age.[281] However, the role of zinc supplements in developed countries needs further evaluation.[113,275]

Probiotics

Probiotics are living nonpathogenic microorganisms that have been studied for prevention and treatment of a variety of disorders, including diarrhea, irritable bowel syndrome, and inflammatory bowel disease.[14,461] The most commonly used strains are lactic acid bacteria, such as lactobacilli or bifidobacteria, and the nonpathogenic yeast *Saccharomyces boulardii.* The rationale for the use of probiotics to treat and prevent diarrheal diseases is based on the assumption that they modify the composition of the colonic microflora and act against enteric pathogens. However, the exact mechanism by which probiotics exert their activity against enteropathogens in humans remains unknown. The possible mechanisms of action include competition with pathogens for binding sites and substrates, lowering of intestinal luminal pH, production of bacteriocins, promotion of mucin production, upregulation of genes mediating immunity, and production of trophic short-chain fatty acids to promote mucosal cell growth and differentiation.[236] Several meta-analyses have shown moderate clinical benefit of selected probiotic strains in treatment of acute watery diarrhea (primarily rotaviral) mainly in infants and young children.[403,413,451,452] *Lactobacillus* GG and *Saccharomyces boulardii* were found to be beneficial in meta-analyses devoted to single probiotics. The beneficial effects of probiotics in acute diarrhea in children seem to be moderate, strain-dependent, dose-dependent (greater for doses $>10^{10}$ to 10^{11} colony-forming units), significant for watery diarrhea and viral gastroenteritis but not for invasive bacterial diarrhea, more evident when treatment with probiotics is initiated early in the course of disease, and more evident in children in developed countries.[188] A recent meta-analysis suggests a protective effect of probiotics in preventing antibiotic-associated diarrhea in pediatric patients.[245]

Antisecretory Agents

Racecadotril (also known as acetorphan) is an antisecretory drug that exerts its antidiarrheal effects by inhibiting intestinal enkephalinase, thereby preventing breakdown of endogenous enkephalins in the gastrointestinal tract and reducing secretion of water and electrolytes into the gut.[383] Racecadotril as an adjunct to oral rehydration therapy has been shown to decrease 48-hour stool output, median duration of diarrhea, and intake of oral rehydration solution in children with severe watery diarrhea. Racecadotril should be considered in the treatment of gastroenteritis in children.[188,196] A recent individual patient data meta-analysis showed that racecadotril has an effect on reducing diarrhea (duration, stool output, and stool number), irrespective of baseline conditions (dehydration, rotavirus, or age) and treatment conditions (inpatient or outpatient studies).[285]

Bismuth subsalicylate and other bismuth salts preparations are used to control diarrhea and other gastrointestinal symptoms. Although the precise mechanism of their action remains unknown, their effect is thought to be due to intestinal antisecretory[144] and antimicrobial properties.[300] Laboratory studies showed that bismuth subsalicylate inhibited intestinal secretion caused by *E. coli* and cholera enterotoxins, reduced diarrhea in adult students who became ill in Mexico,[140] and prevented diarrhea among U.S. students traveling to Mexico.[139] Studies supporting its use in children are limited.[440,441] Three randomized controlled trials that compared bismuth subsalicylate with placebo in infants with acute watery diarrhea found that bismuth subsalicylate only modestly reduced the duration and severity of diarrhea.[99,151,441] In addition to temporary side effects (i.e., darkening of the tongue and stool), bismuth subsalicylate has been reported to cause salicylate toxicity in children.[375]

Loperamide is an opioid receptor agonist that reduces intestinal lumen motility. It is used for short-term symptomatic relief of acute diarrhea in adults; however, it should not be used in the management of diarrhea in children.[188]

PREVENTION

Despite the continued reduction in overall disease burden with increased public health measures, such as improved sanitation, use of antimicrobial agents, and greater public awareness, enteric infections continue to cause significant morbidity and mortality in vulnerable populations. New methods for prevention, management, and treatment of diarrhea, including an improved oral rehydration formulation, zinc supplementation, and rotavirus vaccines, make now the time to revitalize efforts to reduce diarrhea mortality worldwide.[409] In addition to these important preventive measures and the development of new enteric vaccines, breast-feeding, safe weaning foods, water quality, hygiene, and sanitation are critical for prevention of diarrhea in children, especially in developing countries, where the disease burden is higher.

Breast-feeding is the most cost-effective intervention for protecting children against diarrhea and all causes of mortality.[246] Lack of exclusive breast-feeding among neonates from birth to 5 months of age and no breast-feeding among infants 6 to 23 months of age are associated with increased morbidity and mortality from diarrhea in developing countries. There is a large body of evidence

regarding the protective effects of breast-feeding against diarrhea incidence, prevalence, hospitalizations, diarrhea mortality, and all-cause mortality. These findings support the current WHO recommendation for exclusive breast-feeding during the first 6 months of life as a key child survival intervention and also highlight the importance of breast-feeding to protect against diarrhea-specific morbidity and mortality throughout the first 2 years of life.[279]

Contaminated foods, especially in the weaning groups, play a major role in the occurrence of diarrheal diseases. The frequency of contamination of weaning foods with enteropathogens is high in developing countries and is dependent on the food type, storage time, and ambient temperature of storage, the method used, and the temperature reached on rewarming before refeeding. Other considerations are the bacterial content of cooking and feeding utensils. Fruit and raw vegetables can become contaminated with pathogenic microorganisms by sewage-containing irrigation water and by washing produce and fruits with contaminated water. In most studies, the level of contamination is higher in weaning foods than in drinking water.[280] Food safety education is a critical and essential element in control and prevention of diarrheal diseases.[430]

The effect of water quality, hygiene, and sanitation in preventing diarrhea deaths has always been debated. In a recent systematic review, diarrhea risk reduction was estimated to be 48% with hand washing with soap, 17% with improved water quality, and 36% with excreta disposal. Most of the evidence is of poor quality, and more trials are needed; nevertheless, the evidence is strong enough to support the provision of water supply, sanitation, and hygiene for all.[66] A growing understanding of what drives hygiene behavior and creative partnerships are providing new approaches to change behavior.[116]

Vaccines

Enteric infections are a major cause of morbidity and mortality in developing countries. To date, vaccines have played a limited role in public health efforts to control enteric infections. Licensed vaccines exist for cholera and typhoid, but these vaccines are used primarily for travelers. There are two internationally licensed vaccines for rotavirus, but they are mainly used in affluent countries. The reasons that enteric vaccines are little used in developing countries are multiple and certainly include financial and political constraints.[103] In populations in industrialized countries, live attenuated vaccines (e.g., poliovirus, typhoid, and rotavirus) mimic natural infection and generate robust protective immune responses. In contrast, a major challenge is to understand and overcome the barriers responsible for the diminished immunogenicity and efficacy of the same enteric vaccines in underprivileged populations in developing countries. Success in developing vaccines against some enteric pathogens has heretofore been elusive (e.g., *Shigella*).[360] Therefore, there is an urgent need for vaccines that induce effective and long-lasting intestinal immunity against diarrheal infections, especially during infancy and early childhood. Mucosal vaccines would be appropriate for a number of pathogens. These include pathogens that are noninvasive, such as *Vibrio cholerae* and ETEC; pathogens that produce gut inflammation, such as *Shigella* and rotavirus; and pathogens that invade through the intestine and enter the systemic circulation, such as *Salmonella typhi*, poliovirus, and human immunodeficiency virus. Although the first category may need a mucosally administered vaccine for adequate protection, especially in immunologically unprimed individuals such as infants and young children, the latter, more invasive infections can probably be prevented by vaccines given either mucosally or parenterally or by a combination of such routes. Although 30 or more parenteral vaccines are licensed, only a handful of oral vaccines are licensed: oral live attenuated typhoid vaccine, oral inactivated cholera, oral live attenuated cholera CVD 103-HgR, oral live attenuated rotavirus, and oral polio virus.[117] Many oral vaccines (e.g., poliovirus, rotavirus vaccine, live attenuated cholera vaccine CVD 103-HgR, and *Shigella flexneri* 2a SC602 vaccine) have performed poorly in developing countries when compared with industrialized countries, a finding attributed mainly to chronic environmental enteropathy, also called tropical enteropathy, which is characterized by disturbances of digestive and absorptive functions. Factors that may contribute to chronic environmental enteropathy include poor sanitation, intestinal flora overgrowth, and histologic changes characterized by inflammation and blunting of small intestinal villi leading to malabsorption. Children living under extreme poverty are especially sensitive.[117]

Rotavirus Vaccine

The first rotavirus vaccine licensed in the United States was associated with an increased rate of intussusception, resulting in its withdrawal from the market within 14 months of being licensed.[325,391] Two new vaccines have recently been shown to be safe and effective in protecting young children against severe rotavirus gastroenteritis and are now marketed worldwide.[400,476] In February 2006, a bovine-based pentavalent rotavirus vaccine[476] was licensed by the U.S. Food and Drug Administration for use in infants in the United States[18,83] as a three-dose vaccine administered orally at 2, 4, and 6 months of age, with all doses administered by the time the child reaches 32 weeks of age. Later, a two-dose orally administered, attenuated monovalent G1P[8] human rotavirus vaccine[400] was recommended by the U.S. Advisory Committee on Immunization Practices[110] and now is available in many countries throughout the world. Before routine rotavirus vaccination was recommended, rotavirus diarrhea caused an estimated 400,000 visits to physician's offices, 200,000 emergency department visits, 55,000 hospitalizations, and 20 to 60 deaths annually among children younger than 5 years of age in the United States.[156,173,489] Since the introduction of rotavirus vaccine, diarrhea-associated health care utilization and medical expenditures for U.S. children have decreased substantially.[109,367,482] Rotavirus vaccines have reduced the health burden of rotavirus gastroenteritis in both developed and developing countries.[122,393] It is hoped the diffusion of the vaccine use in low-income countries, endorsed by the WHO, will have a substantial impact on diarrheal overall burden of disease.[361,457]

Vaccines for Enteric Bacteria

Although several strategies have been used to develop vaccines targeting shigellosis, none has been licensed for use outside China. Owing to the wide range of *Shigella* serotypes and subtypes, there is a need for a multivalent vaccine representing prevalent species and serotypes. Strategies for *Shigella* vaccine development include live attenuated, conjugate, broad-spectrum, and proteosome-based vaccines. The greatest barrier to the use of vaccine against shigellosis in developing areas is a poor immune response to oral vaccines in children who have minimal maternal antibodies.[277] Several nonliving *Shigella* vaccines are currently at different stages of development and show substantial promise.[251] In relation to cholera, the availability of new oral vaccines and supporting data have prompted the WHO to recommend vaccination be used with other prevention and control strategies where disease is endemic and their use should be considered in outbreak situations. New variant strains appear to cause more severe clinical disease and may be displacing earlier seventh pandemic organisms as the major cause of cholera. Licensed newer-generation oral vaccines have proven to be well tolerated, protective (including against new variant strains), and affordable and offer a new tool to control cholera.[126] Finally, no effective vaccine for ETEC is available. Several inactivated and live candidate ETEC vaccines consisting of toxin antigens, alone or together with colonization factors, have been evaluated in clinical trials. Intense efforts are in progress to try to improve the immunogenicity of different available candidate vaccines, particularly in children, as well as to develop new types of ETEC vaccines.[448]

NEW REFERENCES SINCE THE SIXTH EDITION

10. Ali, M, Lopez AL, You YA, et al. The global burden of cholera. *Bull World Health Organ* 2012;**90**:209–18A.
16. Amaya E, Reyes D, Vilchez S, et al. Antibiotic resistance patterns of intestinal *Escherichia coli* isolates from Nicaraguan children. *J Med Microbiol* 2011;**60**:216–22.
20. Andrews-Polymenis HL, Bäumler AJ, McCormick BA, Fang FC. Taming the elephant: *Salmonella* biology, pathogenesis, and prevention. *Infect Immun* 2010;**78**:2356–69.
22. Anklam KS, Kanankege KS, Gonzales TK, et al. Rapid and reliable detection of Shiga toxin-producing *Escherichia coli* by real-time multiplex PCR. *J Food Prot* 2012;**75**:643–50.
23. Antikainen J, Tarkka E, Haukka K, et al. New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. *Eur J Clin Microbiol Infect Dis* 2009;**28**: 899–908.
25. Argudín MÁ, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins (Basel)* 2010;**2**: 1751–73.
34. Bányai K, László B, Duque J, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: for understanding the impact of rotavirus vaccination programs. *Vaccine* 2012;27;**30**(Suppl 1):A122–30.
35. Barbuddhe SB, Chakraborty T. *Listeria* as an enteroinvasive gastrointestinal pathogen. *Curr Top Microbiol Immunol* 2009;**337**: 173–95.
36. Barletta F, Ochoa TJ, Ecker L, et al. Validation of five-colony pool analysis using multiplex real-time PCR for detection of diarrheagenic *Escherichia coli*. *J Clin Microbiol* 2009;**47**:1915–7.
38. Barletta F, Ochoa TJ, Mercado E, et al. Quantitative real-time polymerase chain reaction for enteropathogenic *Escherichia coli:* a tool for investigation of asymptomatic versus symptomatic infections. *Clin Infect Dis* 2011;**53**:1223–9.
39. Bartoloni A, Pallecchi L, Riccobono E, et al. Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in *Escherichia coli:* 20 years of surveillance in resource-limited settings from Latin America. *Clin Microbiol Infect* 2012. Feb 9; [Epub ahead of print.]
43. Berni Canani R, Buccigrossi V, Passariello A. Mechanisms of action of zinc in acute diarrhea. *Curr Opin Gastroenterol* 2011;**27**: 8–12.
47. Bhutta ZA, Nelson EA, Lee WS, et al. Persistent Diarrhea Working Group. Recent advances and evidence gaps in persistent diarrhea. *J Pediatr Gastroenterol Nutr* 2008;**47**:260–5.
49. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet* 2010;**375**:1969–87.
53. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* 2010;**23**:382–98.
62. Brzuszkiewicz E, Thürmer A, Schuldes J, et al. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). *Arch Microbiol* 2011;**193**:883–91.
63. Brunkard JM, Ailes E, Roberts VA, et al. Surveillance for waterborne disease outbreaks associated with drinking water—United States, 2007–2008. *MMWR Surveill Summ* 2011;**60**:38–68.
64. Bryant K, McDonald LC. *Clostridium difficile* infections in children. *Pediatr Infect Dis J* 2009;**28**:145–6.
65. Cabada MM, White Jr AC. Treatment of cryptosporidiosis: do we know what we think we know? *Curr Opin Infect Dis* 2010;**23**: 494–9.
66. Cairncross S, Hunt C, Boisson S, et al. Water, sanitation and hygiene for the prevention of diarrhoea. *Int J Epidemiol* 2010;**1**: i193–205.
71. Cavallaro E, Date K, Medus C, et al. *Salmonella typhimurium* Outbreak Investigation Team: *Salmonella typhimurium* infections associated with peanut products. *N Engl J Med* 2011;**365**:601–10.
89. Centers for Disease Control and Prevention. Outbreak of *Salmonella* serotype *saintpaul* infections associated with multiple raw produce items—United States, 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:929–34.
90. Centers for Disease Control and Prevention. Surveillance for community-associated *Clostridium difficile*—Connecticut, 2006. *MMWR Morb Mortal Wkly Rep* 2008;**57**:340–3.
91. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:418–22.
92. Centers for Disease Control and Prevention. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food—foodborne diseases active surveillance network, 10 U.S. Sites, 1996–2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 749–55.
93. Charles RC, Ryan ET. Cholera in the 21st century. *Curr Opin Infect Dis* 2011;**24**:472–7.
94. Chen CC, Chang CJ, Lin TY, et al. Usefulness of fecal lactoferrin in predicting and monitoring the clinical severity of infectious diarrhea. *World J Gastroenterol* 2011;**17**:4218–24.
95. Chen CC, Huang JL, Chang CJ, Kong MS. Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. *J Pediatr Gastroenterol Nutr* 2012 Jun 13. [Epub ahead of print].
96. Chimalizeni Y, Kawaza K, Molyneux E. The epidemiology and management of nontyphoidal *Salmonella* infections. *Adv Exp Med Biol* 2010;**659**:33–46.
97. Cho YJ, Yi H, Lee JH, et al. Genomic evolution of *Vibrio cholerae*. *Curr Opin Microbiol* 2010;**13**:646–51.
100. Christopher PR, David KV, John SM, Sankarapandian V. Antibiotic therapy for *Shigella* dysentery. *Cochrane Database Syst Rev* 2010;4:CD006784.
103. Clemens J. Evaluation of vaccines against enteric infections: a clinical and public health research agenda for developing countries. *Philos Trans R Soc Lond B Biol Sci* 2011;**366**:2799–805.
105. Connor BA. *Traveler's diarrhea: the yellow book*. Atlanta, GA: Centers for Disease Control and Prevention; 2012.
109. Cortes JE, Curns AT, Tate JE, et al. Rotavirus vaccine and health care utilization for diarrhea in US children. *N Engl J Med* 2011;**365**:1108–17.

110. Cortese MM, Parashar UD. Centers for Disease Control and Prevention (CDC). Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;**58**:1–25.
112. Cotton JA, Beatty JK, Buret AG. Host parasite interactions and pathophysiology in *Giardia* infections. *Int J Parasitol* 2011;**41**: 925–33.
113. Crane JK, Hoque KM. Zinc for infectious diarrhea in developed countries: should we be sprinkling our own lawns? *J Pediatr Gastroenterol Nutr* 2008;**46**:484–5.
114. Cunliffe NA, Booth JA, Elliot C, et al. Healthcare-associated viral gastroenteritis among children in a large pediatric hospital, United Kingdom. *Emerg Infect Dis* 2010;**16**:55–62.
115. Cunningham SA, Sloan LM, Nyre LM, et al. Three-hour molecular detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* species in feces with accuracy as high as that of culture. *J Clin Microbiol* 2010;**48**:2929–33.
116. Curtis V, Schmidt W, Luby S, et al. Hygiene: new hopes, new horizons. *Lancet Infect Dis* 2011;**11**:312–21.
117. Czerkinsky C, Holmgren J. Enteric vaccines for the developing world: a challenge for mucosal immunology. *Mucosal Immunol* 2009;**2**:284–7.
121. Dennehy PH. Viral gastroenteritis in children. *Pediatr Infect Dis J* 2011;**30**:63–4.
122. Dennehy PH. Effects of vaccine on rotavirus disease in the pediatric population. *Curr Opin Pediatr* 2012;**24**:76–84.
125. Desai R, Curns AT, Steiner CA, et al. All cause gastroenteritis and rotavirus-coded hospitalizations among US children from 2000–2009. *Clin Infect Dis* 2012;**55**:e28–34.
126. Desai SN, Clemens JD. An overview of cholera vaccines and their public health implications. *Curr Opin Pediatr* 2012;**24**:85–91.
127. Dey RS, Ghosh S, Chawla-Sarkar M, et al. Circulation of a novel pattern of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. *J Clin Microbiol* 2011;**49**:500–5.
129. Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr Opin Infect Dis* 2011;**24**:490–5.
132. Drummond N, Murphy BP, Ringwood T, et al. *Yersinia enterocolitica:* a brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. *Foodborne Pathog Dis* 2012;**9**:179–89.
137. DuPont HL. Clinical practice. Bacterial diarrhea. *N Engl J Med* 2009;**361**:1560–9.
138. DuPont HL. Approach to the patient with infectious colitis. *Curr Opin Gastroenterol* 2012;**28**:39–46.
141. Dyar OJ, Hoa NQ, Trung NV, et al. High prevalence of antibiotic resistance in commensal *Escherichia coli* among children in rural Vietnam. *BMC Infect Dis* 2012;**12**:92.
145. Escobar JC, Bhavnani D, Trueba G, et al. *Plesiomonas shigelloides* infection, Ecuador, 2004–2008. *Emerg Infect Dis* 2012;**18**: 322–4.
147. Esposito DH, Holman RC, et al. Baseline estimates of diarrhea-associated mortality among United States children before rotavirus vaccine introduction. *Pediatr Infect Dis J* 2011;**30**:942–7.
148. Feasey NA, Dougan G, Kingsley PA, et al. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet* 2012;**379**:2489–99.
152. Finkbeiner SR, Allred AF, Tarr PI, et al. Metagenomic analysis of human diarrhea: viral detection and discovery. *PLoS Pathog* 2008;**4**:e1000011.
153. Finkbeiner SR, Holtz LR, Jiang Y, et al. Human stool contains a previously unrecognized diversity of novel astroviruses. *Virol J* 2009;**6**:161.
154. Finkbeiner SR, Le BM, Holtz LR, et al. Detection of newly described astrovirus MLB1 in stool samples from children. *Emerg Infect Dis* 2009;**15**:441–4.
155. Fischer SA. Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV. *Transplantation* 2008;**86**:1327–39.
157. Flores J, Okhuysen PC. Enteroaggregative *Escherichia coli* infection. *Curr Opin Gastroenterol* 2009;**25**:8–11.
161. Frank C, Werber D, Cramer JP, et al. HUS Investigation Team: Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *N Engl J Med* 2011;**365**: 1771–80.
168. Gerner-Smidt P. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;**58**:1–14.
169. Gerritzen A, Wittke JW, von Ahsen N, Wolff D. Direct faecal PCR for diagnosis of Shiga-toxin-producing *Escherichia coli*. *Lancet Infect Dis* 2012;**12**:102.
170. Gerritzen A, Wittke JW, Wolff D. Rapid and sensitive detection of Shiga toxin-producing *Escherichia coli* directly from stool samples by real-time PCR in comparison to culture, enzyme immunoassay and Vero cell cytotoxicity assay. *Clin Lab* 2011;**57**: 993–8.
175. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med* 2009;**361**:1776–85.
177. Gómez-Duarte OG, Bai J, Newell E. Detection of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, and *Campylobacter* spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2009;**63**: 1–9.
178. Gonzales TK, Kulow M, Park DJ, et al. A high-throughput open-array qPCR gene panel to identify, virulotype, and subtype O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Mol Cell Probes* 2011;**25**:222–30.
179. Gonzalez ML, Dans LF, Martinez EG. Antiamoebic drugs for treating amoebic colitis. *Cochrane Database Syst Rev* 2009;2: CD006085.
182. Gordon MA. Invasive nontyphoidal *Salmonella* disease: epidemiology, pathogenesis and diagnosis. *Curr Opin Infect Dis* 2011;**24**: 484–9.
185. Gould LH, Bopp C, Strockbine N, et al. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;**58**:1–14.
186. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis* 2012;**54**:652–60.
188. Guarino A, Albano F, Ashkenazi S, et al. Evidence-based guidelines for the management of acute gastroenteritis in children in Europe. *J Pediatric Gastroent Nutr* 2008;**46**:S81–S184.
189. Guarino A, Dupont C, Gorelov AV, et al. The management of acute diarrhea in children in developed and developing areas: from evidence base to clinical practice. *Expert Opin Pharmacother* 2012; **13**:17–26.
190. Guarino A, Giannattasio A. New molecular approaches in the diagnosis of acute diarrhea: advantages for clinicians and researchers. *Curr Opin Gastroenterol* 2011;**27**:24–9.
191. Guerrant RL, Oria RB, Moore SR, et al. Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutr Rev* 2008;**66**:487–505.
195. Guion CE, Ochoa TJ, Walker CM, et al. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *J Clin Microbiol* 2008;**46**:1752–7.
196. Gutiérrez-Castrellón P, Polanco I, Salazar Lindo E. Manejo de la gastroenteritisaguda en menores de 5 años: un enfoque basado en la evidencia. *An Pediatr (Barc)* 2009;1–19.
197. Gutiérrez-Castrellón P, Polanco I, Salazar Lindo E. An evidence based Iberic-Latin American guideline for acute gastroenteritis management in infants and prescholars. *An Pediatr (Barc)* 2010; **72**:220.e1–220.e20.
198. Haagsma JA, Siersema PD, De Wit NJ, Havelaar AH. Disease burden of postinfectious irritable bowel syndrome in The Netherlands. *Epidemiol Infect* 2010;**138**:1650–6.
200. Hagmann S, Neugebauer R, Schwartz, E, et al. GeoSentinel Surveillance Network. Illness in children after international travel: analysis from the GeoSentinel Surveillance Network. *Pediatrics* 2010;**125**:e1072–80.
205. Harris C, Wilkinson F, Mazza D, Turner T. Health for Kids Guideline Development Group. Evidence-based guideline for the management of diarrhea with or without vomiting in children. *Aust Fam Physician* 2008;**37**:22–9.
206. Harris JB, Ivers LC, Ferraro MJ. Case records of the Massachusetts General Hospital Case 19-2011. A 4-year-old Haitian boy with vomiting and diarrhea. *N Engl J Med* 2011;**23**;364: 2452–61.
211. Hermos CR, Janineh M, Han LL, McAdam AJ. Shiga toxin–producing *Escherichia coli* in children: diagnosis and clinical manifestations of O157:H7 and non-O157:H7 infection. *J Clin Microbiol* 2011;**49**:955–9.

212. Hernandes RT, Elias WP, Vieira MA, Gomes TA. An overview of atypical enteropathogenic *Escherichia coli*. *FEMS Microbiol Lett* 2009;**297**:137–49.
216. Hill DR, Beeching NJ. Travelers' diarrhea. *Curr Opin Infect Dis* 2010;**23**:481–7.
225. Hsu RB, Lin FY. Nontyphoid *Salmonella* infection in heart transplant recipients. *Am J Med Sci* 2008;**336**:393–6.
232. Hlavsa MC, Roberts VA, Anderson AR, et al. Surveillance for waterborne disease outbreaks and other health events associated with recreational water—United States, 2007–2008. *MMWR Surveill Summ* 2011;**60**:1–32.
233. Ichinohe N, Ohara-Nemoto Y, Nemoto TK, et al. Effects of fosfomycin on Shiga toxin–producing *Escherichia coli:* quantification of copy numbers of Shiga toxin–encoding genes and their expression levels using real-time PCR. *J Med Microbiol* 2009;**58**: 971–3.
237. Janda JM, Abbott SL. The genus *Aeromonas:* taxonomy, pathogenicity, and infection. *Clin Microbiol Rev* 2010;**23**:35–73.
239. Jeong HS, Jeong A, Cheon DS. Epidemiology of astrovirus infection in children. *Korean J Pediatr* 2012;**55**:77–82.
244. Johannes L, Romer W. Shiga toxins—from cell biology to biomedical applications. *Nat Rev Microbiol* 2010;**8**:105–16.
245. Johnston BC, Goldenberg JZ, Vandvik PO, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2011;11:CD004827.
248. Jones TF, Gerner-Smidt P. Nonculture diagnostic tests for enteric diseases. *Emerg Infect Dis* 2012;**18**:513–14.
249. Kachrimanidou M, Malisiovas N. *Clostridium difficile* infection: a comprehensive review. *Crit Rev Microbiol* 2011;**37**:178–87.
251. Kaminski RW, Oaks EV. Inactivated and subunit vaccines to prevent shigellosis. *Expert Rev Vaccines* 2009;**8**:1693–704.
252. Kaneene JB, Miller R, May K, Hattey JA. An outbreak of multidrug-resistant *Salmonella enterica* serotype Oranienburg in Michigan dairy calves. *Foodborne Pathog Dis* 2010;**7**:1193–201.
255. Kapoor A, Li L, Victoria J, et al. Multiple novel astrovirus species in human stool. *J Gen Virol* 2009;**90**:2965–72.
260. Kim J, Shaklee JF, Smathers S, et al. Risk factors and outcomes associated with severe *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2012;**31**:134–8.
264. Kirkpatrick BD, Tribble DR. Update on human *Campylobacter jejuni* infections. *Curr Opin Gastroenterol* 2011;**27**:1–7.
265. Kiulia NM, Nyaundi JK, Peenze I, et al. Rotavirus infections among HIV-infected children in Nairobi, Kenya. *J Trop Pediatr* 2009;**55**:318–23.
270. Kosek M, Yori PP, Olortegui MP. Shigellosis update: advancing antibiotic resistance, investment empowered vaccine development, and green bananas. *Curr Opin Infect Dis* 2010;**23**: 475–80.
271. Kosek M, Yori PP, Pan WK, et al. Epidemiology of highly endemic multiply antibiotic-resistant shigellosis in children in the Peruvian Amazon. *Pediatrics* 2008;**122**:e541–9.
275. Kouame KS, Verga ME, Pittet A, et al. Zinc and diarrhoea in children under 5 years: WHO recommendations implemented in Switzerland. *Rev Med Suisse* 2012;**8**:1244–7.
277. Kweon MN. Shigellosis: the current status of vaccine development. *Curr Opin Infect Dis* 2008;**21**:313–18.
278. Lamberti LM, Fischer-Walker CL, Black RE, et al. Systematic review of diarrhea duration and severity in children and adults in low- and middle-income countries. *BMC Public Health* 2012;**12**:276.
279. Lamberti LM, Fischer Walker CL, Noiman A, et al. Breastfeeding and the risk for diarrhea morbidity and mortality. *BMC Public Health* 2011;**3**:S15.
281. Lazzerini M, Ronfani L. Oral zinc for treating diarrhea in children. *Cochrane Database Syst Rev* 2012;6:CD005436.
284. Lee MB, Greig JD. A review of enteric outbreaks in child care centers: effective infection control recommendations. *J Environ Health* 2008;**71**:24–32, 46.
285. Lehert P, Chéron G, Calatayud GA, et al. Racecadotril for childhood gastroenteritis: an individual patient data meta-analysis. *Dig Liver Dis* 2011;**43**:707–13.
290. Liu J, Gratz J, Maro A, et al. Simultaneous detection of six diarrhea-causing bacterial pathogens with an in-house PCR-luminex assay. *J Clin Microbiol* 2012;**50**:98–103.
291. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012;**379**:2151–61.
292. López EL, Contrini MM, Glatstein E, et al. An epidemiologic surveillance of Shiga-like toxin-producing *Escherichia coli* infection in Argentinean children: risk factors and serum Shiga-like toxin 2 values. *Pediatr Infect Dis J* 2012;**31**:20–4.
294. Luangtongkum T, Jeon B, Han J, et al. Antibiotic resistance in *Campylobacter:* emergence, transmission and persistence. *Future Microbiol* 2009;**4**:189–200.
298. Man SM. The clinical importance of emerging *Campylobacter* species. *Nat Rev Gastroenterol Hepatol* 2011;**8**:669–85.
301. Marcos LA, Terashima A, Dupont HL, Gotuzzo E. *Strongyloides* hyperinfection syndrome: an emerging global infectious disease. *Trans R Soc Trop Med Hyg* 2008;**102**:314–18.
302. Marshall JA, Bruggink LD. The dynamics of norovirus outbreak epidemics: recent insights. *Int J Environ Res Public Health* 2011;**8**: 1141–9.
306. Mejia R, Nutman TB. Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by *Strongyloides stercoralis*. *Curr Opin Infect Dis* 2012;**25**:458–63.
307. Melton-Celsa A, Mohawk K, Teel L, O'Brien A. Pathogenesis of Shiga-toxin producing *Escherichia coli*. *Curr Top Microbiol Immunol* 2012;**357**:67–103.
310. Mercado EH, Ochoa TJ, Ecker L, et al. Fecal leukocytes in children infected with diarrheagenic *Escherichia coli*. *J Clin Microbiol* 2011;**49**:1376–81.
311. Miclard J, Van Baarlen J, Wyder M, et al. *Clostridium perfringens* beta-toxin binding to vascular endothelial cells in a human case of enteritis necroticans. *J Med Microbiol* 2009;**58**:826–8.
314. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1–166.
317. Montes M, Sawhney C, Barros N. *Strongyloides stercoralis:* there but not seen. *Curr Opin Infect Dis* 2010;**23**:500–4.
318. Moore SR. Update on prolonged and persistent diarrhea in children. *Curr Opin Gastroenterol* 2011;**27**:19–23.
319. Moore SR, Lima NL, Soares AM, et al. Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children. *Gastroenterology* 2010;**139**:1156–64.
320. Mor SM, Tzipori S. Cryptosporidiosis in children in sub-Saharan Africa: a lingering challenge. *Clin Infect Dis* 2008;**47**:915–21.
329. National Collaborating Centre for Women's and Children's Health (UK). *Diarrhoea and vomiting caused by gastroenteritis: diagnosis, assessment and management in children younger than 5 years*. London: National Institute for Health and Clinical Excellence (NICE); 2009.
332. Newton A, Heiman KE, Schmitz A, et al. Cholera in United States associated with epidemic in Hispaniola. *Emerg Infect Dis* 2011;**17**: 2166–8.
333. Newton A, Kendall M, Vugia DJ, et al. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. *Clin Infect Dis* 2012;**54**(Suppl 5):S391–5.
337. Nitschke M, Sayk F, Härtel C, et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin–producing enteroaggregative *Escherichia coli* O104:H4. *JAMA* 2012;**307**:1046–52.
338. O'Connor RM, Shaffie R, Kang G, Ward HD. Cryptosporidiosis in patients with HIV/AIDS. *AIDS* 2011;**25**:549–60.
341. Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. *Curr Opin Infect Dis* 2011;**24**:478–83.
342. Ochoa TJ, Ecker L, Barletta F, et al. Age/related susceptibility to infection with diarrheagenic *Escherichia coli* among infants from Periurban areas in Lima, Peru. *Clin Infect Dis* 2009;**49**: 1694–702.
343. Ochoa TJ, Mercado EH, Durand D, et al. Frequency and pathotypes of diarrheagenic *Escherichia coli* in Peruvian children with and without diarrhea. *Rev Peru Med Exp Salud Publica* 2011;**28**:13–20.
344. Ochoa TJ, Ruiz J, Molina M, et al. High frequency of antimicrobial drug resistance of diarrheagenic *Escherichia coli* in infants in Peru. *Am J Trop Med Hyg* 2009;**81**:296–301.
347. Okeke IN. Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *J Infect Dev Ctries* 2009;**3**: 817–42.

349. Okhuysen PC, Dupont HL. Enteroaggregative *Escherichia coli* (EAEC): a cause of acute and persistent diarrhea of worldwide importance. *J Infect Dis* 2010;**202**:503–5.
351. Operario DJ, Houpt E. Defining the causes of diarrhea: novel approaches. *Curr Opin Infect Dis* 2011;**24**:464–71.
352. Opintan JA, Newman MJ, Ayeh-Kumi PF, et al. Pediatric diarrhea in southern Ghana: etiology and association with intestinal inflammation and malnutrition. *Am J Trop Med Hyg* 2010;**83**:936–43.
353. Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a foodborne and waterborne parasite. *Clin Microbiol Rev* 2010;**23**: 218–34.
358. Paredes-Paredes M, Flores-Figueroa J, Dupont HL. Advances in the treatment of travelers' diarrhea. *Curr Gastroenterol Rep* 2011;**13**:402–7.
359. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. *Curr Opin Infect Dis* 2008;**21**:531–8.
360. Pasetti MF, Simon JK, Sztein MB, Levine MM. Immunology of gut mucosal vaccines. *Immunol Rev* 2011;**239**:125–48.
361. Patel MM, Glass R, Desai R, et al. Fulfilling the promise of rotavirus vaccines: how far have we come since licensure? *Lancet Infect Dis* 2012;**12**:561–70.
362. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis* 2008;**14**:1224–31.
363. Patel NC, Hertel PM, Hanson IC, et al. Chronic rotavirus infection in an infant with severe combined immunodeficiency: successful treatment by hematopoietic stem cell transplantation. *Clin Immunol* 2012;**142**:399–401.
365. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med* 2012;**13**:85–97.
366. Pawlowski SW, Warren CA, Guerrant R. Diagnosis and treatment of acute or persistent diarrhea. *Gastroenterology* 2009;**136**:1874–86.
367. Payne DC, Staat MA, Edwards KM, et al. New Vaccine Surveillance Network (NVSN). Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US Counties, 2006–2009. *Clin Infect Dis* 2011;**53**:245–53.
369. Petri Jr WA, Miller M, Binder HJ, et al. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest* 2008;**118**:1277–90.
370. Pfeiffer ML, DuPont HL, Ochoa TJ. The patient presenting with acute dysentery—a systematic review. *J Infect* 2012;**64**:374–86.
371. Piarroux R, Faucher B. Cholera epidemics in 2010: respective roles of environment, strain changes, and human-driven dissemination. *Clin Microbiol Infect* 2012;**18**:231–8.
377. Platts-Mills JA, Operario DJ, Houpt ER. Molecular diagnosis of diarrhea: current status and future potential. *Curr Infect Dis Rep* 2011;**14**:41–6.
380. Poropatich KO, Walker CL, Black RE. Quantifying the association between *Campylobacter* infection and Guillain-Barré syndrome: a systematic review. *J Health Popul Nutr* 2010;**28**:545–52.
382. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clin Nutr Metab Care* 2009;**12**:646–52.
387. Ralston KS, Petri Jr WA. Tissue destruction and invasion by *Entamoeba histolytica*. *Trends Parasitol* 2011;**27**:254–63.
388. Ramani S, Kang G. Viruses causing childhood diarrhoea in the developing world. *Curr Opin Infect Dis* 2009;**22**:477–82.
393. Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *N Engl J Med* 2010;**362**:299–305.
403. Salari P, Nikfar S, Abdollahi M. A meta-analysis and systematic review on the effect of probiotics in acute diarrhea. *Inflamm Allergy Drug Targets* 2012;**11**:3–14.
406. Sánchez-Vargas FM, Abu-El-Haija MA, Gómez-Duarte OG. *Salmonella* infections: an update on epidemiology, management, and prevention. *Travel Med Infect Dis* 2011;**9**:263–77.
408. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2011;**30**:580–4.
409. Santosham M, Chandran A, Fitzwater S, et al. Progress and barriers for the control of diarrhoeal disease. *Lancet* 2010;**376**:63–7.
417. Scallan E, Griffin PM, Angulo FJ, et al. Foodborne illness acquired in the United States—unspecified agents. *Emerg Infect Dis* 2011;**17**: 16–22.
418. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.
420. Schelin J, Wallin-Carlquist N, Cohn MT, et al. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2011;**2**:580–92.
421. Schneider JL, White PL, Weiss J, et al. Multistate outbreak of multidrug-resistant *Salmonella newport* infections associated with ground beef, October to December 2007. *J Food Prot* 2011;**74**: 1315–19.
425. Senderovich Y, Ken-Dror S, Vainblat I, et al. A molecular study on the prevalence and virulence potential of *Aeromonas* spp. recovered from patients suffering from diarrhea in Israel. *PLoS One* 2012;**7**:e30070.
435. Silva J, Leite D, Fernandes M, et al. *Campylobacter* spp. as a foodborne pathogen: a review. *Front Microbiol* 2011;**2**:200.
436. Smith KE, Wilker PR, Reiter PL, et al. Antibiotic treatment of *Escherichia coli* O157 infection and the risk of hemolytic uremic syndrome, Minnesota. *Pediatr Infect Dis J* 2012;**31**:37–41.
439. Solaymani-Mohammadi S, Genkinger JM, Loffredo CA, Singer SM. A meta-analysis of the effectiveness of albendazole compared with metronidazole as treatments for infections with *Giardia duodenalis*. *PLoS Negl Trop Dis* 2010;**4**:e682.
442. Spano LC, Sadovsky AD, Segui PN, et al. Age-specific prevalence of diffusely adherent *Escherichia coli* in Brazilian children with acute diarrhoea. *J Med Microbiol* 2008;**57**:359–63.
446. Stenfors-Arnesen LP, Fagerlund A, Granum PE. From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol Rev* 2008;**32**:579–606.
448. Svennerholm AM, Lundgren A. Recent progress toward an enterotoxigenic *Escherichia coli* vaccine. *Expert Rev Vaccines* 2012; **11**:495–507.
449. Swaminathan A, Torresi J, Schlagenhauf P, et al. A global study of pathogens and host risk factors associated with infectious gastrointestinal disease in returned international travellers. *J Infect* 2009; **59**:19–27.
451. Sýkora J, Siala K, Huml M, et al. Evaluation of faecal calprotectin as a valuable non-invasive marker in distinguishing gut pathogens in young children with acute gastroenteritis. *Acta Paediatr* 2010;**99**: 1389–95.
454. Tan M, Jiang X. Norovirus-host interaction: multi-selections by human histo-blood group antigens. *Trends Microbiol* 2011;**19**: 382–8.
455. Taniuchi M, Walters CC, Gratz J, et al. Development of a multiplex polymerase chain reaction assay for diarrheagenic *Escherichia coli* and *Shigella* spp. and its evaluation on colonies, culture broths, and stool. *Diagn Microbiol Infect Dis* 2012;**73**:121–8.
457. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:136–41.
458. Tejman-Yarden N, Eckmann L. New approaches to the treatment of giardiasis. *Curr Opin Infect Dis* 2011;**24**:451–6.
460. Teunis PF, Moe CL, Liu P, et al. Norwalk virus: how infectious is it? *J Med Virol* 2008;**80**:1468–76.
461. Thomas DW, Greer FR. Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**:1217–31.
463. Tobias J, Vutukuru SR. Simple and rapid multiplex PCR for identification of the main human diarrheagenic *Escherichia coli*. *Microbiol Res* 2011 Dec 20 [Epub ahead of print].
464. Townes JM. Reactive arthritis after enteric infections in the United States: the problem of definition. *Clin Infect Dis* 2010;**50**: 247–54.
465. Traa BS, Walker CL, Munos M, Black RE. Antibiotics for the treatment of dysentery in children. *Int J Epidemiol* 2010;**39**: i70–4.
471. Vandenberg O, Robberecht F, et al. Management of a *Cryptosporidium hominis* outbreak in a day-care center. *Pediatr Infect Dis J* 2012;**31**:10–15.
472. Vega E, Barclay L, Gregoricus N, et al. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. *Emerg Infect Dis* 2011;**17**:1389–95.
479. Walker CL, Black RE. Zinc for the treatment of diarrhoea: effect on diarrhoea morbidity, mortality and incidence of future episodes. *Int J Epidemiol* 2010;**1**:i63–9.
482. Wang FT, Mast TC, Glass RJ, et al. Effectiveness of the pentavalent rotavirus vaccine in preventing gastroenteritis in the United States. *Pediatrics* 2010;**125**:e208–13.

487. Weil AA, Ivers LC, Harris JB. Cholera: lessons from Haiti and beyond. *Curr Infect Dis Rep* 2012;**14**:1–8.
490. Wiemer D, Loderstaedt U, von Wulffen H, et al. Real-time multiplex PCR for simultaneous detection of *Campylobacter jejuni*, *Salmonella*, *Shigella*, and *Yersinia* species in fecal samples. *Int J Med Microbiol* 2011;**301**:577–84.
492. Willing BP, Russell SL, Finlay BB, et al. Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nat Rev Microbiol* 2011;**9**:233–43.
501. Wright SG. Protozoan infections of the gastrointestinal tract. *Infect Dis Clin North Am* 2012;**26**:323–39.
502. Wunderli W, Meerbach A, Güngör, et al. Astrovirus infection in hospitalized infants with severe combined immunodeficiency after allogeneic hematopoietic stem cell transplantation. *PLoS One* 2011;**6**:e27483.
503. Yakoob MY, Theodoratou E, Jabeen A, et al. Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhea, pneumonia and malaria. *BMC Public Health* 2011;**3**:S23.
508. Zella GC, Israel EJ. Chronic diarrhea in children. *Pediatr Rev* 2012;**33**:207–17.
511. Zheng DP, Widdowson MA, Glass RI, Vinjé J. Molecular epidemiology of genogroup II-genotype 4 noroviruses in the United States between 1994 and 2006. *J Clin Microbiol* 2010;**48**:168–77.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 45

Antibiotic-Associated Colitis

Robin Schmucker • Kristina Bryant

More than 130 million courses of antibiotics are prescribed in the United States each year, and diarrhea is a common side effect of antibiotic therapy. *Clostridium difficile* is the most common infectious cause of antibiotic-associated diarrhea, accounting for 15 to 25 percent of cases.[8] According to a 2012 report from the Centers for Disease Control and Prevention, the incidence, mortality, and medical care costs of *C. difficile* infection (CDI) have reached "historic highs," in part, because of the emergence of a hypervirulent stain of *C. difficile* identified as North American pulsed-field type 1 (NAP1).[21] Although most cases of CDI have traditionally occurred in hospitalized adults, infections are reported with increasing frequency in children.[54,74,103] CDI is recognized as a common cause of health care–acquired infection, but community transmission is also identified.[21]

HISTORY

In 1935, Hall and O'Toole described the identification of a new, anaerobic, gram-positive, spore-forming bacillus in the stools of 4 of 10 healthy, breast-fed neonates. They named it *Bacillus difficilis*, in recognition of the fact that the organism was difficult to isolate and study in the laboratory. *B. difficilis* was noted to be highly pathogenic in guinea pigs and rabbits; guinea pigs inoculated subcutaneously with the bacteria commonly developed convulsions, which the investigators attributed to a soluble exotoxin isolated from broth culture filtrates.[42] A link between intestinal colonization and illness in human infants was much less clear, although the authors postulated a potential association with occult blood in the stools of neonates or convulsions of unexplained origin. Neither association was proved, and, for decades, *B. difficilis* was thought to be a harmless, intestinal commensal in infants.[6] The role of *C. difficile* as a human pathogen was finally elucidated in the 1970s when toxin-producing *Clostridia* were isolated from the stools of adult patients with clindamycin-associated pseudomembranous colitis.[7]

ETIOLOGY AND PATHOGENESIS

Over the past decade, cases of CDI have increased in both frequency and severity. From 2000 to 2009, the number of hospitalized patients with CDI doubled.[21] Deaths attributable to CDI increased from 3,000 deaths per year during 1999 to 2000 to 14,000 during 2006 to 2007; more than 90% of the deaths occurred in individuals 65 years of age or older.[41] The increased incidence and mortality of CDI in adults has been attributed in large part to the emergence of spread of a hypervirulent, fluoroquinolone-resistant strain of *C. difficile*. The NAP1 strain produces more toxin A (TcdA) and toxin B (TcdB) than historic strains. The role of a third toxin, the binary toxin, is less clear but may contribute to enhanced virulence of toxin A and toxin B.[48]

REFERENCES

1. Adachi JA, Ericsson CD, Jiang ZD, et al. Azithromycin found to be comparable to levofloxacin for the treatment of US travelers with acute diarrhea acquired in Mexico. *Clin Infect Dis* 2003;**37**: 1165–71.
2. Adachi JA, Jiang ZD, Mathewson JJ, et al. Enteroaggregative *Escherichia coli* as a major etiologic agent in traveler's diarrhea in 3 regions of the world. *Clin Infect Dis* 2001;**32**:1706–9.
3. Adachi JA, Ostrosky-Zeichner L, DuPont HL, et al. Empirical antimicrobial therapy for traveler's diarrhea. *Clin Infect Dis* 2000;**31**: 1079–83.
4. Adamkiewicz TV, Berkovitch M, Krishnan C, et al. Infection due to *Yersinia enterocolitica* in a series of patients with beta-thalassemia: incidence and predisposing factors. *Clin Infect Dis* 1998;**27**: 1362–6.
5. Akihara S, Phan TG, Nguyen TA, et al. Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. *Arch Virol* 2005;**150**:2061–75.
6. Albert MJ, Ansaruzzaman M, Talukder KA, et al. Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhea, healthy controls, and the environment. *J Clin Microbiol* 2000;**38**:3785–90.
7. Albert MJ, Faruque AS, Faruque SM, et al. Case-control study of enteropathogens associated with childhood diarrhea in Dhaka, Bangladesh. *J Clin Microbiol* 1999;**37**:3458–64.
8. Aldeen WE, Whisenant J, Hale D, et al. Comparison of pooled formalin-preserved fecal specimens with three individual samples for detection of intestinal parasites. *J Clin Microbiol* 1993;**31**: 144–5.
9. Alfa MJ, Kabani A, Lyerly D, et al. Characterization of a toxin A–negative, toxin B–positive strain of *Clostridium difficile* responsible for a nosocomial outbreak of *Clostridium difficile*–associated diarrhea. *J Clin Microbiol* 2000;**38**:2706–14.
10. Ali, M, Lopez AL, You YA, et al. The global burden of cholera. *Bull World Health Organ* 2012;**90**:209–18A.
11. Ali SA, Hill DR. Giardia intestinalis. *Curr Opin Infect Dis* 2003;**16**:453–60.
12. Allos BM. *Campylobacter jejuni* infections: update of emerging issues and trends. *Clin Infect Dis* 2001;**32**:1201–6.
13. Altwegg M, Geiss HK. *Aeromonas* as a human pathogen. *C R C Crit Rev Microbiol* 1989;**16**:253.
14. Alvarez-Olmos MI, Oberhelman RA. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. *Clin Infect Dis* 2001;**32**:1567–76.
15. Amadi B, Mwiya M, Musuku J, et al. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomized controlled trial. *Lancet* 2002;**360**:1375–80.
16. Amaya E, Reyes D, Vilchez S, et al. Antibiotic resistance patterns of intestinal *Escherichia coli* isolates from Nicaraguan children. *J Med Microbiol* 2011;**60**:216–22.
17. American Academy of Pediatrics, Subcommittee on Acute Gastroenteritis. Practice parameter: the management of acute gastroenteritis in young children. *Pediatrics* 1996;**97**:424–36.
18. American Academy of Pediatrics. Prevention of rotavirus disease: guidelines for use of rotavirus vaccine. *Pediatrics* 2007;**119**: 171–82.
19. American Medical Association; American Nurses Association-American Nurses Foundation; Centers for Disease Control and Prevention; Center for Food Safety and Applied Nutrition, Food and Drug Administration; Food Safety and Inspection Service, US Department of Agriculture. Diagnosis and management of foodborne illnesses: a primer for physicians and other health care professionals. *MMWR Recomm Rep* 2004;**53**:1–33.
20. Andrews-Polymenis HL, Bäumler AJ, McCormick BA, Fang FC. Taming the elephant: *Salmonella* biology, pathogenesis, and prevention. *Infect Immun* 2010;**78**:2356–69.
21. Angel J, Franco MA, Greenberg HB. Rotavirus vaccines recent developments and future considerations. *Nat Rev Microbiol* 2007;**5**:529–39.
22. Anklam KS, Kanankege KS, Gonzales TK, et al. Rapid and reliable detection of Shiga toxin-producing *Escherichia coli* by real-time multiplex PCR. *J Food Prot* 2012;**75**:643–50.
23. Antikainen J, Tarkka E, Haukka K, et al. New 16-plex PCR method for rapid detection of diarrheagenic *Escherichia coli* directly from stool samples. *Eur J Clin Microbiol Infect Dis* 2009;**28**: 899–908.
24. Arbo A, Santas JI. Diarrheal disease in the immunocompromised host. *Pediatr Infect Dis J* 1987;**6**:894–906.
25. Argudín MÁ, Mendoza MC, Rodicio MR. Food poisoning and *Staphylococcus aureus* enterotoxins. *Toxins (Basel)* 2010;**2**:1751–73.
26. Armitstead J, Kelly D, Walker-Smith J. Evaluation of infant feeding in acute gastroenteritis. *J Pediatr Gastroenterol Nutr* 1989;**8**:240–4.
27. Arrowood MJ, Sterling CR. Comparison of conventional staining methods and monoclonal antibody–based methods for *Cryptosporidium* oocyst detection. *J Clin Microbiol* 1989;**27**:1490.
28. Aserkoff B, Bennett JV. Effect of antibiotic therapy in acute salmonellosis on the fecal excretion of salmonellae. *N Engl J Med* 1969;**281**:636–40.
29. Ashkenazi S. *Shigella* infections in children: new insights. *Semin Pediatr Infect Dis* 2004;**15**:246–52.
30. Ashraf H, Beltinger J, Alam NH, et al. Evaluation of faecal occult blood test and lactoferrin latex agglutination test in screening hospitalized patients for diagnosing inflammatory and non-inflammatory diarrhoea in Dhaka, Bangladesh. *Digestion* 2007; **76**:256–61.
31. Asmuth DM, DeGirolami PC, Federman M, et al. Clinical features of microsporidiosis in patients with AIDS. *Clin Infect Dis* 1994;**18**:819–25.
32. Aureli P, Fiorucci GC, Caroli D, et al. An outbreak of febrile gastroenteritis associated with corn contaminated by *Listeria monocytogenes*. *N Engl J Med* 2000;**342**:1236–41.
33. Bahl R, Bhandari N, Saksena M, et al. Efficacy of zinc-fortified oral rehydration solution in 6- to 35-month-old children with acute diarrhea. *J Pediatr* 2002;**141**:677–82.
34. Bányai K, László B, Duque J, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: for understanding the impact of rotavirus vaccination programs. *Vaccine* 2012;27;**30**(Suppl 1):A122–30.
35. Barbuddhe SB, Chakraborty T. *Listeria* as an enteroinvasive gastrointestinal pathogen. *Curr Top Microbiol Immunol* 2009;**337**: 173–95.
36. Barletta F, Ochoa TJ, Ecker L, et al. Validation of five-colony pool analysis using multiplex real-time PCR for detection of diarrheagenic *Escherichia coli*. *J Clin Microbiol* 2009;**47**:1915–7.
37. Barletta F, Ochoa TJ, Mercado E, et al. Quantitative real-time polymerase chain reaction for enteropathogenic *Escherichia coli:* a tool for investigation of asymptomatic versus symptomatic infections. *Clin Infect Dis* 2011;**53**:1223–9.
38. Bartlett JG. Narrative review: the new epidemic of *Clostridium difficile*–associated enteric disease. *Ann Intern Med* 2006;**145**: 758–64.
39. Bartoloni A, Pallecchi L, Riccobono E, et al. Relentless increase of resistance to fluoroquinolones and expanded-spectrum cephalosporins in *Escherichia coli:* 20 years of surveillance in resource-limited settings from Latin America. *Clin Microbiol Infect* 2012. Feb 9;[Epub ahead of print.]
40. Beards GM, Green JG, Hall C, et al. An enveloped virus in stools of children and adults with gastroenteritis that resembles the Breda virus of calves. *Lancet* 1984;**1**:1050.
41. Beauvais B, Sarfati C, Molina JM, et al. Comparative evaluation of five diagnostic methods for demonstrating microsporidia in stool and intestinal biopsy specimens. *Ann Trop Med Parasitol* 1993;**87**:99–102.
42. Bennish ML, Khan WA, Begum M, et al. Low risk of hemolytic uremic syndrome after early effective antimicrobial therapy for *Shigella* dysenteriae type 1 infection in Bangladesh. *Clin Infect Dis* 2006;**42**:356–62.
43. Berni Canani R, Buccigrossi V, Passariello A. Mechanisms of action of zinc in acute diarrhea. *Curr Opin Gastroenterol* 2011;**27**: 8–12.
44. Bhan MK, Raj P, Levine MM, et al. Enteroaggregative *E. coli* associated with persistent diarrhea in a cohort of rural children in India. *J Infect Dis* 1989;**159**:1061.
45. Bhatnagar S, Lodha R, Choudhury P, et al. IAP Guidelines 2006 on management of acute diarrhea. *Indian Pediatr* 2007;**44**:380–9.
46. Bhutta ZA, Black RE, Brown KH, et al. Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: pooled analysis of randomized controlled trials. Zinc investigators Collaborative Group. *J Pediatr* 1999;**135**:689–97.

47. Bhutta ZA, Nelson EA, Lee WS, et al. Persistent Diarrhea Working Group. Recent advances and evidence gaps in persistent diarrhea. *J Pediatr Gastroenterol Nutr* 2008;**47**:260–5.
48. Bischoff C, Lüthy J, Altwegg M, Baggi F. Rapid detection of diarrheagenic *E. coli* by real-time PCR. *J Microbiol Methods* 2005;**61**: 335–41.
49. Black RE, Cousens S, Johnson HL, et al. Global, regional, and national causes of child mortality in 2008: a systematic analysis. *Lancet* 2010;**375**:1969–87.
50. Blanton LH, Adams SM, Beard RS, et al. Molecular and epidemiologic trends of caliciviruses associated with outbreaks of acute gastroenteritis in the United States, 2000–2004. *J Infect Dis* 2006; **193**:413–21.
51. Blossom DB, McDonald LC. The challenges posed by reemerging *Clostridium difficile* infection. *Clin Infect Dis* 2007;**45**:222–7.
52. Bottone EJ. *Yersinia enterocolitica*: The charisma continues. *Clin Microbiol Rev* 1997;**10**:257–76.
53. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* 2010;**23**:382–98.
54. Boyle TG, Pemberton AG, Wells JG, et al. Screening for *Escherichia coli* O157:H7: A nationwide survey of clinical laboratories. *J Clin Microbiol* 1995;**33**:3275–7.
55. Braden CR. *Salmonella enterica* serotype enteridis and eggs: a national epidemic in the United States. *Clin Infect Dis* 2006;**43**: 512–17.
56. Brady MT. Infectious disease in pediatric out-of-home child care. *Am J Infect Control* 2005;**33**:276–85.
57. Branda JA, Lin T-Y, Rosenberg ES, et al. A rational approach to the stool ova and parasite examination. *Clin Infect Dis* 2006;**42**: 972–8.
58. Brandal LT, Lindstedt BA, Aas L, et al. Octaplex PCR and fluorescence-based capillary electrophoresis for identification of human diarrheagenic *Escherichia coli* and *Shigella* spp. *J Microbiol Methods* 2007;**68**:331–41.
59. Brandt CD, Kim HW, Rodriguez WJ, et al. Adenoviruses and pediatric gastroenteritis. *J Infect Dis* 1985;**151**:437–43.
60. Brooks WA, Santosham M, Roy SK, et al. Efficacy of zinc in young children with acute watery diarrhea. *Am J Clin Nutr* 2005; **82**:605–10.
61. Brown KH, Gastanaduy AS, Saavedra JM, et al. Effect of continued oral feeding on clinical and nutritional outcomes of acute diarrhea in children. *J Pediatr* 1988;**112**:191–200.
62. Brzuszkiewicz E, Thürmer A, Schuldes J, et al. Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). *Arch Microbiol* 2011;**193**:883–91.
63. Brunkard JM, Ailes E, Roberts VA, et al. Surveillance for waterborne disease outbreaks associated with drinking water—United States, 2007–2008. *MMWR Surveill Summ* 2011;**60**:38–68.
64. Bryant K, McDonald LC. *Clostridium difficile* infections in children. *Pediatr Infect Dis J* 2009;**28**:145–6.
65. Cabada MM, White Jr AC. Treatment of cryptosporidiosis: do we know what we think we know? *Curr Opin Infect Dis* 2010;**23**: 494–9.
66. Cairncross S, Hunt C, Boisson S, et al. Water, sanitation and hygiene for the prevention of diarrhoea. *Int J Epidemiol* 2010;**1**: i193–205.
67. Calva JJ, Ruiz-Palacios GM, Lopez-Vidal AB, et al. Cohort study of intestinal infection with *Campylobacter* in Mexican children. *Lancet* 1988;**1**:503.
68. Campos LC, Franzolin MR, Trabulsi LR. Diarrheagenic *Escherichia coli* categories among the traditional enteropathogenic *E. coli* O serogroups—a review. *Mem Inst Oswaldo Cruz* 2004;**99**: 545–52.
69. Carr A, Marriott D, Field A, et al. Treatment of HIV-1–associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy. *Lancet* 1998;**351**:256–61.
70. Castillo-Duran C, Vial P, Uauy R. Trace mineral balance during acute diarrhea in infants. *J Pediatr* 1988;**113**:452–7.
71. Cavallaro E, Date K, Medus C, et al. *Salmonella typhimurium* Outbreak Investigation Team: *Salmonella typhimurium* infections associated with peanut products. *N Engl J Med* 2011;**365**: 601–10.
72. Centers for Disease Control and Prevention. *Bacillus cereus* food poisoning associated with fried rice at two child care centers: Virginia, 1993. *MMWR Morb Mortal Wkly Rep* 1994; **43**:177–8.
73. Centers for Disease Control and Prevention. Outbreaks of *Escherichia coli* O157:H7 infection and cryptosporidiosis associated with drinking unpasteurized apple cider—Connecticut and New York, October 1996. *JAMA* 1997;**277**:781–2.
74. Centers for Disease Control and Prevention. Multistate outbreak of *Listeriosis*—United States, 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:1129–30.
75. Centers for Disease Control and Prevention. Diagnosis and management of foodborne illnesses: A primer for physicians. *MMWR Morb Mortal Wkly Rep* 2001;**50**(RR-02):1–69.
76. Centers for Disease Control and Prevention. Multistate outbreaks of *Salmonella* serotype Poona infections associated with eating cantaloupe from Mexico—United States and Canada, 2000–2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:1044–7.
77. Centers for Disease Control and Prevention. Outbreak of gastroenteritis associated with noroviruses on cruise ships—United States, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:1112–15.
78. Centers for Disease Control and Prevention. National Antimicrobial Resistance Monitoring System (NARMS): Enteric Bacteria. Human Isolates Final Report, 2003. Available at: www.cdc.gov/NARMS.
79. Centers for Disease Control and Prevention. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Morb Mortal Wkly Rep* 2003; **52**(RR-16):1–16.
80. Centers for Disease Control and Prevention. *Yersinia enterocolitica* gastroenteritis among infants exposed to chitterlings—Chicago, Illinois, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:956–8.
81. Centers for Disease Control and Prevention. Giardiasis surveillance. United States, 1998–2002. *MMWR Morb Mortal Wkly Rep* 2005;**54**(SS-1):9–16.
82. Centers for Disease Control and Prevention. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach—United States, September 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1045–6.
83. Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2006;**55**(RR-12):1–13.
84. Centers for Disease Control and Prevention. Two cases of toxigenic *Vibrio cholera* O1 infection after Hurricanes Katrina and Rita—Louisiana, October 2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:31–2.
85. Centers for Disease Control and Prevention. *Vibrio parahaemolyticus* infections associated with consumption of raw shellfish—three states, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:854–6.
86. Centers for Disease Control and Prevention. Cryptosporidiosis outbreaks associated with recreational water use—five states, 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:729–32.
87. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:336–9. Available at: www.cdc.gov/foodnet.
88. Centers for Disease Control and Prevention. Summary of notifiable diseases, United States, 2005. *MMWR Morb Mortal Wkly Rep* 2007;**54**:1–92.
89. Centers for Disease Control and Prevention. Outbreak of *Salmonella* serotype *saintpaul* infections associated with multiple raw produce items—United States, 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:929–34.
90. Centers for Disease Control and Prevention. Surveillance for community-associated *Clostridium difficile*—Connecticut, 2006. *MMWR Morb Mortal Wkly Rep* 2008;**57**:340–3.
91. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:418–22.
92. Centers for Disease Control and Prevention. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food—foodborne diseases active surveillance network, 10 U.S. Sites, 1996–2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 749–55.
93. Charles RC, Ryan ET. Cholera in the 21st century. *Curr Opin Infect Dis* 2011;**24**:472–7.

94. Chen CC, Chang CJ, Lin TY, et al. Usefulness of fecal lactoferrin in predicting and monitoring the clinical severity of infectious diarrhea. *World J Gastroenterol* 2011;**17**:4218–24.
95. Chen CC, Huang JL, Chang CJ, Kong MS. Fecal calprotectin as a correlative marker in clinical severity of infectious diarrhea and usefulness in evaluating bacterial or viral pathogens in children. *J Pediatr Gastroenterol Nutr* 2012 Jun 13. [Epub ahead of print].
96. Chimalizeni Y, Kawaza K, Molyneux E. The epidemiology and management of nontyphoidal *Salmonella* infections. *Adv Exp Med Biol* 2010;**659**:33–46.
97. Cho YJ, Yi H, Lee JH, et al. Genomic evolution of *Vibrio cholerae*. *Curr Opin Microbiol* 2010;**13**:646–51.
98. CHOICE Study Group. Multicenter, randomized, double-blind clinical trial to evaluate the efficacy and safety of a reduced osmolarity oral rehydration salts solution in children with acute watery diarrhea. *Pediatrics* 2001;**107**:613–18.
99. Chowdhury HR, Yunus M, Zaman K, et al. The efficacy of bismuth subsalicilate in the treatment of acute diarrhoea and the prevention of persistent diarrhoea. *Acta Paediatr* 2001;**90**: 605–10.
100. Christopher PR, David KV, John SM, Sankarapandian V. Antibiotic therapy for *Shigella* dysentery. *Cochrane Database Syst Rev* 2010;4:CD006784.
101. Clark DP. New insights into human cryptosporidiosis. *Clin Microbiol Rev* 1999;**12**:554–63.
102. Cleary TG, Guerrant RL, Pickering LK. Microorganisms responsible for neonatal diarrhea. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 5th ed. Philadelphia: W. B. Saunders; 2000. p. 1249.
103. Clemens J. Evaluation of vaccines against enteric infections: a clinical and public health research agenda for developing countries. *Philos Trans R Soc Lond B Biol Sci* 2011;**366**:2799–805.
104. Cohen MB, Nataro JP, Bernstein DI, et al. Prevalence of diarrheagenic *Escherichia coli* in acute childhood enteritis: a prospective controlled study. *J Pediatr* 2005;**146**:54–61.
105. Connor BA. *Traveler's diarrhea: the yellow book*. Atlanta, GA: Centers for Disease Control and Prevention; 2012.
106. Conteas CN, Berlin OG, Ash LR, Pruthi JS. Therapy for human gastrointestinal microsporidiosis. *Am J Trop Med Hyg* 2000;**63**: 121–7.
107. Conway SP, Ireson A. Acute gastroenteritis in well nourished infants: comparison of four feeding regimens. *Arch Dis Child* 1989;**64**:87–91.
108. Cornick NA, Gorbach SL. *Campylobacter*. *Infect Dis Clin North Am* 1988;**2**:643–54.
109. Cortes JE, Curns AT, Tate JE, et al. Rotavirus vaccine and health care utilization for diarrhea in US children. *N Engl J Med* 2011;**365**:1108–17.
110. Cortese MM, Parashar UD. Centers for Disease Control and Prevention (CDC). Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;**58**:1–25.
111. Cotte L, Rabodonirina M, Chapuis F, et al. Waterborne outbreak of intestinal microsporidiosis in persons with and without human immunodeficiency virus infection. *J Infect Dis* 1999;**180**: 2003–8.
112. Cotton JA, Beatty JK, Buret AG. Host parasite interactions and pathophysiology in *Giardia* infections. *Int J Parasitol* 2011;**41**: 925–33.
113. Crane JK, Hoque KM. Zinc for infectious diarrhea in developed countries: should we be sprinkling our own lawns? *J Pediatr Gastroenterol Nutr* 2008;**46**:484–5.
114. Cunliffe NA, Booth JA, Elliot C, et al. Healthcare-associated viral gastroenteritis among children in a large pediatric hospital, United Kingdom. *Emerg Infect Dis* 2010;**16**:55–62.
115. Cunningham SA, Sloan LM, Nyre LM, et al. Three-hour molecular detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* species in feces with accuracy as high as that of culture. *J Clin Microbiol* 2010;**48**:2929–33.
116. Curtis V, Schmidt W, Luby S, et al. Hygiene: new hopes, new horizons. *Lancet Infect Dis* 2011;**11**:312–21.
117. Czerkinsky C, Holmgren J. Enteric vaccines for the developing world: a challenge for mucosal immunology. *Mucosal Immunol* 2009;**2**:284–7.
118. Daniels NA, MacKinnon L, Bishop R, et al. *Vibrio parahaemolyticus* infections in the United States, 1973–1998. *J Infect Dis* 2000;**181**: 161–6.
119. DaSilva AJ, Schwartz DA, Visvesvara GS, et al. Sensitive PCR diagnosis of infections by *Enterocytozoon bieneusi* (microsporidia) using primers based on the region coding for small-subunit rRNA. *J Clin Microbiol* 1996;**34**:986–7.
120. DeHovitz JA, Pape JW, Boncy M, et al. Clinical manifestations and therapy of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1986;**315**:87.
121. Dennehy PH. Viral gastroenteritis in children. *Pediatr Infect Dis J* 2011;**30**:63–4.
122. Dennehy PH. Effects of vaccine on rotavirus disease in the pediatric population. *Curr Opin Pediatr* 2012;**24**:76–84.
123. Dennehy PH, Gauntlett DR, Tente W. Comparison of nine commercial immunoassays for the detection of rotavirus in fecal samples. *J Clin Microbiol* 1988;**26**:1630.
124. Dennehy PH, Nelson SM, Spangenberger S, et al. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. *J Infect Dis* 2001;**184**: 10–15.
125. Desai R, Curns AT, Steiner CA, et al. All cause gastroenteritis and rotavirus-coded hospitalizations among US children from 2000–2009. *Clin Infect Dis* 2012;**55**:e28–34.
126. Desai SN, Clemens JD. An overview of cholera vaccines and their public health implications. *Curr Opin Pediatr* 2012;**24**:85–91.
127. Dey RS, Ghosh S, Chawla-Sarkar M, et al. Circulation of a novel pattern of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. *J Clin Microbiol* 2011;**49**:500–5.
128. Didier ES, Orenstein JM, Aldras A, et al. Comparison of three staining methods for detecting microsporidia in fluids. *J Clin Microbiol* 1995;**33**:3138–45.
129. Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr Opin Infect Dis* 2011;**24**:490–5.
130. Dobell C. A revision of the coccidia parasitic in man. *Parasitology* 1919;**11**:147.
131. Donnenberg MD, Kaper JB. Enteropathogenic *Escherichia coli*. *Infect Immun* 1992;**60**:3953–61.
132. Drummond N, Murphy BP, Ringwood T, et al. *Yersinia enterocolitica:* a brief review of the issues relating to the zoonotic pathogen, public health challenges, and the pork production chain. *Foodborne Pathog Dis* 2012;**9**:179–89.
133. Duggan C, Fontaine O, Pierce NF, et al. Scientific rationale for a change in the composition of oral rehydration solution. *JAMA* 2004;**291**:2628–31.
134. DuPont HL. New insights and directions in travelers' diarrhea. *Gastroenterol Clin North Am* 2006;**35**:337–53.
135. DuPont HL. Azithromycin for the self-treatment of traveler's diarrhea. *Clin Infect Dis* 2007;**44**:347–9.
136. DuPont HL. Therapy for and prevention of traveler's diarrhea. *Clin Infect Dis* 2007;**45**:S78–84.
137. DuPont HL. Clinical practice. Bacterial diarrhea. *N Engl J Med* 2009;**361**:1560–9.
138. DuPont HL. Approach to the patient with infectious colitis. *Curr Opin Gastroenterol* 2012;**28**:39–46.
139. DuPont HL, Evans DG, Sullivan P, et al. Prevention of travelers' diarrhea (emporiatric enteritis) by prophylactic administration of bismuth subsalicylate. *JAMA* 1980;**243**:237–41.
140. DuPont HL, Sullivan P, Pickering LK, et al. Symptomatic treatment of diarrhea with bismuth subsalicylate among students attending a Mexican university. *Gastroenterology* 1977;**73**:715–18.
141. Dyar OJ, Hoa NQ, Trung NV, et al. High prevalence of antibiotic resistance in commensal *Escherichia coli* among children in rural Vietnam. *BMC Infect Dis* 2012;**12**:92.
142. Dziuban EJ, Liang JL, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with recreational water—United States, 2003–2004. *M M W R Surveill Summ* 2006; **55**(SS-12):1–30.
143. Ericsson C, DuPont H, Okhuysen P, et al. Loperamide plus azithromycin more effectively treats travelers' diarrhea in Mexico than azithromycin alone. *J Travel Med* 2007;**14**:312–19.
144. Ericsson CD, Tannenbaum C, Charles TT. Antisecretory and antiinflamatory properties of bismuth subsalycilate. *Rev Infect Dis* 1990;**12**:S16–20.

145. Escobar JC, Bhavnani D, Trueba G, et al. *Plesiomonas shigelloides* infection, Ecuador, 2004–2008. *Emerg Infect Dis* 2012;**18**:322–4.
146. Espinosa-Cantellano M, Martinez-Palomo A. Pathogenesis of intestinal amebiasis: from molecules to disease. *Clin Microbiol Rev* 2000;**13**:318–31.
147. Esposito DH, Holman RC, et al. Baseline estimates of diarrhea-associated mortality among United States children before rotavirus vaccine introduction. *Pediatr Infect Dis J* 2011;**30**:942–7.
148. Feasey NA, Dougan G, Kingsley RA, et al. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet* 2012;**379**:2489–99.
149. Fedorko DP, Nelson NA, Cartwright CP. Identification of Microsporidia in stool specimens by using PCR and restriction endonucleases. *J Clin Microbiol* 1995;**33**:1739–41.
150. Feng X, Jiang X. Library screen for inhibitors targeting norovirus binding to histo-blood group antigen receptors. *Antimicrob Agents Chemother* 2007;**51**:324–31.
151. Figueroa-Quintanilla D, Salazar-Lindo E, Sack RB, et al. A controlled trial of bismuth subsalycilate in infants with acute watery diarrhea disease. *N Engl J Med* 1993;**328**:1653–8.
152. Finkbeiner SR, Allred AF, Tarr PI, et al. Metagenomic analysis of human diarrhea: viral detection and discovery. *PLoS Pathog* 2008;**4**:e1000011.
153. Finkbeiner SR, Holtz LR, Jiang Y, et al. Human stool contains a previously unrecognized diversity of novel astroviruses. *Virol J* 2009;**6**:161.
154. Finkbeiner SR, Le BM, Holtz LR, et al. Detection of newly described astrovirus MLB1 in stool samples from children. *Emerg Infect Dis* 2009;**15**:441–4.
155. Fischer SA. Emerging viruses in transplantation: there is more to infection after transplant than CMV and EBV. *Transplantation* 2008;**86**:1327–39.
156. Fischer TK, Viboud C, Parashar U, et al. Hospitalizations and deaths from diarrhea and rotavirus among children 5 years of age in the United States, 1993–2003. *J Infect Dis* 2007;**195**:1117–25.
157. Flores J, Okhuysen PC. Enteroaggregative *Escherichia coli* infection. *Curr Opin Gastroenterol* 2009;**25**:8–11.
158. Fonseca BK, Holdgate A, Craig JC. Enteral vs intravenous rehydration therapy for children with gastroenteritis: a meta-analysis of randomized controlled trials. *Arch Pediatr Adolesc Med* 2004; **158**:483–90.
159. Fontaine O, Gore SM, Pierce NF. Rice-based oral rehydration solution for treating diarrhea. *Cochrane Database Syst Rev* 2000;2:CD001264.
160. Fotedar R, Stark D, Beebe N, et al. Laboratory diagnostic techniques for *Entamoeba* species. *Clin Microbiol Rev* 2007;**20**:511–32.
161. Frank C, Werber D, Cramer JP, et al. HUS Investigation Team: Epidemic profile of Shiga-toxin-producing *Escherichia coli* O104:H4 outbreak in Germany. *N Engl J Med* 2011;**365**: 1771–80.
162. Fukushima H, Tsunomori Y, Seki R. Duplex real-time SYBR green PCR assays for detection of 17 species of food- or waterborne pathogens in stools. *J Clin Microbiol* 2003;**41**:5134–46.
163. Galanis E. *Campylobacter* and bacterial gastroenteritis. *Can Med Assoc J* 2007;**177**:570–1.
164. Garcia LS, Brewer TC, Bruckner DA. Incidence of *Cryptosporidium* in all patients submitting stool specimens for ova and parasites examination: monoclonal antibody IFA method. *Diagn Microbiol Infect Dis* 1988;**11**:25.
165. Garcia LS, Shimizu RY. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection for *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J Clin Microbiol* 1997;**35**:1526–9.
166. Gebreyes WA, Davies PR, Morrow WEM, et al. Antimicrobial resistance of *Salmonella* isolates from swine. *J Clin Microbiol* 2000;**38**:4633–6.
167. Genta RM. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Rev Infect Dis* 1989;**11**:755.
168. Gerner-Smidt P. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;**58**:1–14.
169. Gerritzen A, Wittke JW, von Ahsen N, Wolff D. Direct faecal PCR for diagnosis of Shiga-toxin-producing *Escherichia coli*. *Lancet Infect Dis* 2012;**12**:102.
170. Gerritzen A, Wittke JW, Wolff D. Rapid and sensitive detection of Shiga toxin-producing *Escherichia coli* directly from stool samples by real-time PCR in comparison to culture, enzyme immunoassay and Vero cell cytotoxicity assay. *Clin Lab* 2011;**57**: 993–8.
171. Girard DE, Keefe EB. *Isospora* and travelers' diarrhea. *Ann Intern Med* 1987;**106**:908.
172. Glandt M, Adachi JA, Mathewson JJ, et al. Enteroaggregative *Escherichia coli* as a cause of traveler's diarrhea: clinical response to ciprofloxacin. *Clin Infect Dis* 1999;**29**:335–8.
173. Glass RI, Kilgore PE, Holman RC, et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. *J Infect* 1996;**1**:S5–11.
174. Glass RI, Noel J, Mitchell D, et al. The changing epidemiology of astrovirus-associated gastroenteritis. *Arch Virol* 1996;**12**(Suppl.): 287–300.
175. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med* 2009;**361**:1776–85.
176. Glynn MK, Bopp D, Dewitt W, et al. Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. *N Engl J Med* 1998;**338**:1333–8.
177. Gómez-Duarte OG, Bai J, Newell E. Detection of *Escherichia coli*, *Salmonella* spp., *Shigella* spp., *Yersinia enterocolitica*, *Vibrio cholerae*, and *Campylobacter* spp. enteropathogens by 3-reaction multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2009;**63**: 1–9.
178. Gonzales TK, Kulow M, Park DJ, et al. A high-throughput open-array qPCR gene panel to identify, virulotype, and subtype O157 and non-O157 enterohemorrhagic *Escherichia coli*. *Mol Cell Probes* 2011;**25**:222–30.
179. Gonzalez ML, Dans LF, Martinez EG. Antiamoebic drugs for treating amoebic colitis. *Cochrane Database Syst Rev* 2009;2: CD006085.
180. Goodgame RW. Understanding intestinal spore-forming protozoa: Cryptosporidia, Microsporidia, *Isospora*, and *Cyclospora*. *Ann Intern Med* 1996;**124**:429–41.
181. Gorbach SL, Carpenter CCJ, Grayson R, et al. Travelers' diarrhea: consensus conference. *JAMA* 1985;**253**:2700–4.
182. Gordon MA. Invasive nontyphoidal *Salmonella* disease: epidemiology, pathogenesis and diagnosis. *Curr Opin Infect Dis* 2011;**24**: 484–9.
183. Gore SM, Fontaine O, Pierce N. Impact of rice based oral rehydration solution on stool output and duration on diarrhoea: meta-analysis of 13 clinical trials. *BMJ* 1992;**304**:287–91.
184. Gottlieb SL, Newbern EC, Griffin PM, et al. Multistate outbreak of listeriosis linked to turkey deli meat and subsequent changes in US regulatory policy. *Clin Infect Dis* 2006;**42**:29–36.
185. Gould LH, Bopp C, Strockbine N, et al. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;**58**:1–14.
186. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis* 2012;**54**:652–60.
187. Graham SM. Salmonellosis in children in developing and developed countries and populations. *Curr Opin Infect Dis* 2002;**15**: 507–12.
188. Guarino A, Albano F, Ashkenazi S, et al. Evidence-based guidelines for the management of acute gastroenteritis in children in Europe. *J Pediatric Gastroent Nutr* 2008;**46**:S81–184.
189. Guarino A, Dupont C, Gorelov AV, et al. The management of acute diarrhea in children in developed and developing areas: from evidence base to clinical practice. *Expert Opin Pharmacother* 2012;**13**:17–26.
190. Guarino A, Giannattasio A. New molecular approaches in the diagnosis of acute diarrhea: advantages for clinicians and researchers. *Curr Opin Gastroenterol* 2011;**27**:24–9.
191. Guerrant RL, Oria RB, Moore SR, et al. Malnutrition as an enteric infectious disease with long-term effects on child development. *Nutr Rev* 2008;**66**:487–505.
192. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis* 2001;**32**:331–50.
193. Guerrero ML, Noel JS, Mitchell DK, et al. A prospective study of astrovirus diarrhea of infancy in Mexico City. *Pediatr Infect Dis J* 1998;**17**:723–7.

194. Guiguet M, Furco A, Tattevin P, et al. French Hospital Database on HIV Clinical Epidemiology Group: HIV-associated *Isospora belli* infection: incidence and risk factors in the French Hospital Database on HIV. *HIV Med* 2007;**8**:124–30.
195. Guion CE, Ochoa TJ, Walker CM, et al. Detection of diarrheagenic *Escherichia coli* by use of melting-curve analysis and real-time multiplex PCR. *J Clin Microbiol* 2008;**46**:1752–7.
196. Gutiérrez-Castrellón P, Polanco I, Salazar Lindo E. Manejo de la gastroenteritisaguda en menores de 5 años: un enfoque basado en la evidencia. *An Pediatr (Barc)* 2009;1–19.
197. Gutiérrez-Castrellón P, Polanco I, Salazar Lindo E. An evidence based Iberic-Latin American guideline for acute gastroenteritis management in infants and prescholars. *An Pediatr (Barc)* 2010; **72**:220.e1–220.e20.
198. Haagsma JA, Siersema PD, De Wit NJ, Havelaar AH. Disease burden of postinfectious irritable bowel syndrome in The Netherlands. *Epidemiol Infect* 2010;**138**:1650–6.
199. Haffejee IE. Cow's milk-based formula, human milk, and soya feeds in acute infantile diarrhea: a therapeutic trial. *J Pediatr Gastroenterol Nutr* 1990;**10**:193–8.
200. Hagmann S, Neugebauer R, Schwartz E, et al. GeoSentinel Surveillance Network. Illness in children after international travel: analysis from the GeoSentinel Surveillance Network. *Pediatrics* 2010;**125**:e1072–80.
201. Hahn S, Kim Y, Garner P. Reduced osmolarity oral rehydration solution for treating dehydration due to diarrhea in children: systematic review. *BMJ* 2001;**323**:81–5.
202. Hanrahan JP, Gordon MA. Mushroom poisoning: case reports and a review of therapy. *JAMA* 1984;**251**:1057–61.
203. Haque R, Huston CD, Hughes M, et al. Amebiasis. *N Engl J Med* 2003;**348**:1565–73.
204. Harrington SM, Dudley EG, Nataro JP. Pathogenesis of enteroaggregative *Escherichia coli* infection. *FEMS Microbiol Lett* 2006; **254**:12–18.
205. Harris JB, Ivers LC, Ferraro MJ. Case records of the Massachusetts General Hospital Case 19-2011. A 4-year-old Haitian boy with vomiting and diarrhea. *N Engl J Med* 2011;**23**;364: 2452–61.
206. Harris C, Wilkinson F, Mazza D, Turner T. Health for Kids Guideline Development Group. Evidence-based guideline for the management of diarrhea with or without vomiting in children. *Aust Fam Physician* 2008;**37**:22–9.
207. Hart CA, Baxby D, Blundell N. Gastroenteritis due to *Cryptosporidium:* a prospective survey in a children's hospital. *J Infect* 1984;**9**:264.
208. Hartling L, Bellemare S, Wiebe N, et al. Oral versus intravenous rehydration for treating dehydration due to gastroenteritis in children. *Cochrane Database Syst Rev* 2006;3:CD004390.
209. Hennessy TW, Hedberg CW, Slutsker L, et al. A national outbreak of *Salmonella enteritidis* infections from ice cream. *N Engl J Med* 1996;**334**:1281–6.
210. Henricksen SA, Pohlenz JFL. Staining of cryptosporidia by a modified Ziehl-Neelsen technique. *Acta Vet Scand* 1981; **22**:594.
211. Hermos CR, Janineh M, Han LL, McAdam AJ. Shiga toxin–producing *Escherichia coli* in children: diagnosis and clinical manifestations of O157:H7 and non-O157:H7 infection. *J Clin Microbiol* 2011;**49**:955–9.
212. Hernandes RT, Elias WP, Vieira MA, Gomes TA. An overview of atypical enteropathogenic *Escherichia coli*. *FEMS Microbiol Lett* 2009;**297**:137–49.
213. Herrington DA, Tzipori S, Robins-Browne RM, et al. In vitro and in vivo pathogenicity of *Plesiomonas shigelloides*. *Infect Immun* 1987;**55**:979.
214. Herrmann JE, Taylor DN, Echeverria P, Blacklow NR. Astroviruses as a cause of gastroenteritis in children. *N Engl J Med* 1991;**324**:1757–60.
215. Herwaldt BL. *Cyclospora cayetanensis:* a review, focusing on the outbreaks of cyclosporiasis in the 1990s. *Clin Infect Dis* 2000;**31**: 1040–57.
216. Hill DR, Beeching NJ. Travelers' diarrhea. *Curr Opin Infect Dis* 2010;**23**:481–7.
217. Hill DR, Ericsson CD, Pearson RD, et al. The practice of travel medicine: guidelines by the Infectious Diseases Society of America. *IDSA Guidelines* 2006;**43**:1499.
218. Hines J, Nachamkin I. Effective use of the clinical microbiology laboratory for diagnosing diarrheal diseases. *Clin Infect Dis* 1996;**23**:1292–301.
219. Hirschhorn N. Treatment of acute diarrhea in children: historical and physiological perspective. *J Clin Nutr* 1980;**33**:637–63.
220. Hjelt K, Paerregaard A, Petersen W, et al. Rapid versus gradual refeeding in acute gastroenteritis in childhood: energy intake and weight gain. *J Pediatr Gastroenterol Nut* 1989;**8**:75–80.
221. Hlady WG, Klontz KC. The epidemiology of *Vibrio* infections in Florida, 1981–1993. *J Infect Dis* 1996;**173**:1176–83.
222. Hlavsa MC, Watson JC, Beach MJ. Cryptosporidiosis surveillance—United States 1999–2002. *MMWR Morb Mortal Wkly Rep* 2005;**54**(SS-1):1–8.
223. Holmberg SD, Blake PA. Staphylococcal food poisoning in the United States: new facts and old misconceptions. *JAMA* 1984; **251**:487–9.
224. Holmberg SD, Wachsmuth IK, Hickman-Brenner FW, et al. *Plesiomonas* enteric infections in the United States. *Ann Intern Med* 1986;**105**:690.
225. Hsu RB, Lin FY. Nontyphoid *Salmonella* infection in heart transplant recipients. *Am J Med Sci* 2008;**336**:393–6.
226. Huang DB, Chappell C, Okhuysen PC. Cryptosporidiosis in children. *Semin Pediatr Infect Dis* 2004;**15**:253–9.
227. Huang DB, Dupont HL. Enteroaggregative *Escherichia coli:* an emerging pathogen in children. *Semin Pediatr Infect Dis* 2004; **15**:266–71.
228. Huang DB, Nataro JP, DuPont HL, et al. Enteroaggregative *Escherichia coli* is a cause of acute diarrheal illness: a meta-analysis. *Clin Infect Dis* 2006;**43**:556–63.
229. Hughes JM, Boyce JM, Aleem AR, et al. *Vibrio parahaemolyticus* enterocolitis in Bangladesh: report of an outbreak. *Am J Trop Med Hyg* 1978;**27**:106–12.
230. Huicho L, Campos M, Rivera J, et al. Fecal screening tests in the approach to acute infectious diarrhea: a scientific overview. *Pediatr Infect Dis J* 1996;**15**:486–94.
231. Huicho L, Garaycochea V, Uchima N, et al. Fecal lactoferrin, fecal leukocytes and occult blood in the diagnostic approach to childhood invasive diarrhea. *Pediatr Infect Dis J* 1997;**16**:644–7.
232. Hlavsa MC, Roberts VA, Anderson AR, et al. Surveillance for waterborne disease outbreaks and other health events associated with recreational water—United States, 2007–2008. *MMWR Surveill Summ* 2011;**60**:1–32.
233. Ichinohe N, Ohara-Nemoto Y, Nemoto TK, et al. Effects of fosfomycin on Shiga toxin–producing *Escherichia coli:* quantification of copy numbers of Shiga toxin–encoding genes and their expression levels using real-time PCR. *J Med Microbiol* 2009;**58**:971–3.
234. Iijima Y, Tanaka S, Miki K, et al. Evaluation of colony-based examinations of diarrheagenic *Escherichia coli* in stool specimens: low probability of detection because of low concentrations, particularly during the early stage of gastroenteritis. *Diagn Microbiol Infect Dis* 2007;**58**:303–8.
235. Ikeda K, Ida O, Kimoto K, et al. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. *Clin Nephrol* 1999;**52**: 357–62.
236. Isolauri E. Probiotics for infectious diarrhoea. *Gut* 2003;**52**: 436–7.
237. Janda JM, Abbott SL. The genus *Aeromonas:* taxonomy, pathogenicity, and infection. *Clin Microbiol Rev* 2010;**23**:35–73.
238. Janda JM, Powers C, Bryant RG, et al. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin Microbiol Rev* 1988;**1**:245.
239. Jeong HS, Jeong A, Cheon DS. Epidemiology of astrovirus infection in children. *Korean J Pediatr* 2012;**55**:77–82.
240. Jiang X, Graham DY, Wang K, et al. Norwalk virus genome cloning and characterization. *Science* 1991;**250**:1580–3.
241. Jiang X, Matson DO, Velazquez FR, et al. A study of Norwalk-related viruses in Mexican children. *J Med Virol* 1995;**47**:309–16.
242. Jiang X, Turf E, Hu J, et al. Outbreaks of gastroenteritis in elderly nursing homes and retirement facilities associated with human caliciviruses. *J Med Virol* 1996;**50**:335–41.
243. Jimenez SG, Heine RG, Ward PB, et al. *Campylobacter upsaliensis* gastroenteritis in childhood. *Pediatr Infect Dis J* 1999;**18**: 988–92.

244. Johannes L, Romer W. Shiga toxins—from cell biology to biomedical applications. *Nat Rev Microbiol* 2010;**8**:105–16.
245. Johnston BC, Goldenberg JZ, Vandvik PO, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2011;11:CD004827.
246. Jones G, Steketee RW, Black RE, et al. Bellagio Child Survival Study Group. How many child deaths can we prevent this year? *Lancet* 2003;**362**:65–71.
247. Jones TF, Buckingham SC, Bobb CA, et al. From pig to pacifier: chitterling-associated yersiniosis outbreak among black infants. *Emerg Infect Dis* 2003;**9**:1007–9.
248. Jones TF, Gerner-Smidt P. Nonculture diagnostic tests for enteric diseases. *Emerg Infect Dis* 2012;**18**:513–14.
249. Kachrimanidou M, Malisiovas N. *Clostridium difficile* infection: a comprehensive review. *Crit Rev Microbiol* 2011;**37**:178–87.
250. Kain KC, Kelly MT. Clinical features, epidemiology, and treatment of *Plesiomonas shigelloides* diarrhea. *J Clin Microbiol* 1989; **27**:998.
251. Kaminski RW, Oaks EV. Inactivated and subunit vaccines to prevent shigellosis. *Expert Rev Vaccines* 2009;**8**:1693–704.
252. Kaneene JB, Miller R, May K, Hattey JA. An outbreak of multidrug-resistant *Salmonella enterica* serotype Oranienburg in Michigan dairy calves. *Foodborne Pathog Dis* 2010;**7**:1193–201.
253. Kaper JB, Morris Jr JG, Levine MM. *Cholera Clin Microbiol Rev* 1995;**8**:48–86.
254. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2004;**2**:123–40.
255. Kapoor A, Li L, Victoria J, et al. Multiple novel astrovirus species in human stool. *J Gen Virol* 2009;**90**:2965–72.
256. Katanik MT, Schneider SK, Rosenblatt JE, et al. Evaluation of ColorPAC *Giardia/Cryptosporidium* rapid assay and ProSpecT *Giardia/Cryptosporidium* microplate assay for detection of *Giardia* and *Cryptosporidium* in fecal specimens. *J Clin Microbiol* 2001;**39**: 4523–5.
257. Khan AM, Faruque AS, Hossain MS, et al. *Plesiomonas shigelloides*–associated diarrhoea in Bangladeshi children: a hospital-based surveillance study. *J Trop Pediatr* 2004;**50**:354–6.
258. Khan WA, Saha D, Rahman A. Comparison of a single-dose azithromycin and 12-dose, 3-day erythromycin for childhood cholera: a randomized, double-blind trial. *Lancet* 2002;**360**: 1722–7.
259. Khin MU, Nyunt W, Myo K, et al. Effect on clinical outcome of breast feeding during acute diarrhea. *BMJ* 1985;**290**:587–9.
260. Kim J, Shaklee JF, Smathers S, et al. Risk factors and outcomes associated with severe *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2012;**31**:134–8.
261. Kim JS, Lee GG, Park JS, et al. A novel multiplex PCR assay for rapid and simultaneous detection of five pathogenic bacteria: *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Vibrio parahaemolyticus*. *J Food Prot* 2007;**70**: 1656–62.
262. Kimura AC, Mead P, Walsh B, et al. A large outbreak of Brainerd diarrhea associated with a restaurant in the Red River Valley, Texas. *Clin Infect Dis* 2006;**43**:55–61.
263. King CK, Glass R, Bresee JS, Duggan C. Centers for Disease Control and Prevention: Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* 2003;**52**:1–16.
264. Kirkpatrick BD, Tribble DR. Update on human *Campylobacter jejuni* infections. *Curr Opin Gastroenterol* 2011;**27**:1–7.
265. Kiulia NM, Nyaundi JK, Peenze I, et al. Rotavirus infections among HIV-infected children in Nairobi, Kenya. *J Trop Pediatr* 2009;**55**:318–23.
266. Knisley CV, Bednarz-Prashad AJ, Pickering LK. Detection of rotavirus in stool specimens with monoclonal and polyclonal antibody-based assay systems. *J Clin Microbiol* 1986;**23**:897–900.
267. Knisley CV, Englekirk PG, Pickering LK, et al. Rapid detection of *Giardia* antigen in stool using enzyme immunoassays. *Am J Clin Pathol* 1989;**91**:704.
268. Koo D, Maloney K, Tauxe R. Epidemiology of diarrheal disease outbreaks on cruise ships, 1986 through 1993. *JAMA* 1996;**275**: 545–7.
269. Koopmans MPG, Goosen ESM, Lima AAM, et al. Association of torovirus with acute and persistent diarrhea in children. *Pediatr Infect Dis J* 1997;**16**:504–7.
270. Kosek M, Yori PP, Olortegui MP. Shigellosis update: advancing antibiotic resistance, investment empowered vaccine development, and green bananas. *Curr Opin Infect Dis* 2010;**23**:475–80.
271. Kosek M, Yori PP, Pan WK, et al. Epidemiology of highly endemic multiply antibiotic-resistant shigellosis in children in the Peruvian Amazon. *Pediatrics* 2008;**122**:e541–9.
272. Kotch JB, Isbell P, Weber DJ, et al. Hand-washing and diapering equipment reduces disease among children in out-of-home child care centers. *Pediatrics* 2007;**120**:e29–36.
273. Kotler DP. Characterization of intestinal disease associated with human immunodeficiency virus infection and response to antiretroviral therapy. *J Infect Dis* 1999;**179**(Suppl):454–6.
274. Kotloff KL, Losonsky GA, Morris JG, et al. Enteric adenovirus infection and childhood diarrhea: an epidemiologic study in three clinical settings. *Pediatrics* 1989;**84**:219.
275. Kouame KS, Verga ME, Pittet A, et al. Zinc and diarrhoea in children under 5 years: WHO recommendations implemented in Switzerland. *Rev Med Suisse* 2012;**8**:1244–7.
276. Kourtis AP, Bansel P, Posner SF, et al. Trends in hospitalizations of HIV-infected children and adolescents in the United States: analysis of data from the 1994–2003 Nationwide Inpatient Sample. *Pediatrics* 2007;**120**:e236–43.
277. Kweon MN. Shigellosis: the current status of vaccine development. *Curr Opin Infect Dis* 2008;**21**:313–18.
278. Lamberti LM, Fischer Walker CL, Black RE, et al. Systematic review of diarrhea duration and severity in children and adults in low- and middle-income countries. *BMC Public Health* 2012;**12**:276.
279. Lamberti LM, Fischer Walker CL, Noiman A, et al. Breastfeeding and the risk for diarrhea morbidity and mortality. *BMC Public Health* 2011;**3**:S15.
280. Lanata CF. Studies of food hygiene and diarrhoeal disease. *J Environ Health Res* 2003;**1**:S175–83.
281. Lazzerini M, Ronfani L. Oral zinc for treating diarrhea in children. *Cochrane Database Syst Rev* 2012;6:CD005436.
282. Le Bouguénec C, Servin AL. Diffusely adherent Escherichia coli strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. *FEMS Microbiol Lett* 2006;**256**:185–94.
283. Lee LA, Gerber AR, Lonsway DR, et al. *Yersinia enterocolitica* O:3 infections in infants and children, associated with the household preparation of chitterlings. *N Engl J Med* 1990;**322**:984.
284. Lee MB, Greig JD. A review of enteric outbreaks in child care centers: effective infection control recommendations. *J Environ Health* 2008;**71**:24–32, 46.
285. Lehert P, Chéron G, Calatayud GA, et al. Racecadotril for childhood gastroenteritis: an individual patient data meta-analysis. *Dig Liver Dis* 2011;**43**:707–13.
286. Lessnau KD, Can S, Talavera W. Disseminated *Strongyloides stercoralis* in human immunodeficiency virus–infected patients: treatment failure and a review of the literature. *Chest* 1993;**104**: 119–22.
287. Levine WC, Bennett RW, Choi Y, et al. Staphylococcal food poisoning caused by imported canned mushrooms. *J Infect Dis* 1996;**173**:1263–7.
288. Liang JL, Dziuban EJ, Craun GF, et al. Surveillance for waterborne disease and outbreaks associated with drinking water and water not intended for drinking—United States, 2003–2004. *M M W R Surveill Summ* 2006;**55**(SS-12):31–65.
289. Lindsay DS, Dubey JP, Blagburn BL. Biology of *Isospora* spp. from humans, non-human primates, and domestic animals. *Clin Microbiol Rev* 1997;**10**:19–34.
290. Liu J, Gratz J, Maro A, et al. Simultaneous detection of six diarrhea-causing bacterial pathogens with an in-house PCR-luminex assay. *J Clin Microbiol* 2012;**50**:98–103.
291. Liu L, Johnson HL, Cousens S, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. *Lancet* 2012;**379**:2151–61.
292. López EL, Contrini MM, Glatstein E, et al. An epidemiologic surveillance of Shiga-like toxin-producing *Escherichia coli* infection in Argentinean children: risk factors and serum Shiga-like toxin 2 values. *Pediatr Infect Dis J* 2012;**31**:20–4.
293. Lorrot M, Vasseur M. Physiopathology of rotavirus diarrhea. *Arch Pediatr* 2007;**3**:S145–51.
294. Luangtongkum T, Jeon B, Han J, et al. Antibiotic resistance in *Campylobacter:* emergence, transmission and persistence. *Future Microbiol* 2009;**4**:189–200.

295. Lynch M, Painter J, Woodruff R, et al. Surveillance for foodborne-disease outbreaks—United States, 1998–2002. *MMWR Surveill Summ* 2006;**55**(SS-10):1–42.
296. Mackell S. Traveler's diarrhea in the pediatric population: etiology and impact. *Clin Infect Dis* 2005;**8**:S547–52.
297. Madico G, McDonald J, Gilman RH, et al. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. *Clin Infect Dis* 1997;**24**:977–81.
298. Man SM. The clinical importance of emerging *Campylobacter* species. *Nat Rev Gastroenterol Hepatol* 2011;**8**:669–85.
299. Manabe YC, Clark DP, Moore RD, et al. Cryptosporidiosis in patients with AIDS: correlates of disease and survival. *Clin Infect Dis* 1998;**27**:536–42.
300. Manhart MD. In vitro antimicrobial activity of bismuth subsalycilate and other bismuth salts. *Rev Infect Dis* 1990;**12**: S11–15.
301. Marcos LA, Terashima A, Dupont HL, Gotuzzo E. *Strongyloides* hyperinfection syndrome: an emerging global infectious disease. *Trans R Soc Trop Med Hyg* 2008;**102**:314–18.
302. Marshall JA, Bruggink LD. The dynamics of norovirus outbreak epidemics: recent insights. *Int J Environ Res Public Health* 2011;**8**: 1141–9.
303. Maslanka SE, Kerr JG, Williams G, et al. Molecular subtyping of *Clostridium perfringens* by pulsed-field gel electrophoresis to facilitate food-borne-disease outbreak investigations. *J Clin Microbiol* 1999;**37**:2209–14.
304. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene–variant strain of *Clostridium difficile*. *N Engl J Med* 2005;**353**:2433–41.
305. McLaughlin JB, DePaola A, Bopp CA, et al. Outbreak of *Vibrio parahaemolyticus* gastroenteritis associated with Alaskan oysters. *N Engl J Med* 2005;**353**:1463–70.
306. Mejia R, Nutman TB. Screening, prevention, and treatment for hyperinfection syndrome and disseminated infections caused by *Strongyloides stercoralis*. *Curr Opin Infect Dis* 2012;**25**:458–63.
307. Melton-Celsa A, Mohawk K, Teel L, O'Brien A. Pathogenesis of Shiga-toxin producing *Escherichia coli*. *Curr Top Microbiol Immunol* 2012;**357**:67–103.
308. Mendelowitz PC, Hoffman RS, Weber S. Bismuth absorption and myoclonic encephalopathy during bismuth subsalicylate therapy. *Ann Intern Med* 1990;**112**:140.
309. Méndez-Toss M, Griffin DD, Calva J, et al. Prevalence and genetic diversity of human astroviruses in Mexican children with symptomatic and asymptomatic infections. *J Clin Microbiol* 2004;**42**:151–7.
310. Mercado EH, Ochoa TJ, Ecker L, et al. Fecal leukocytes in children infected with diarrheagenic *Escherichia coli*. *J Clin Microbiol* 2011;**49**:1376–81.
311. Miclard J, Van Baarlen J, Wyder M, et al. *Clostridium perfringens* beta-toxin binding to vascular endothelial cells in a human case of enteritis necroticans. *J Med Microbiol* 2009;**58**:826–8.
312. Mintz E. A riddle wrapped in a mystery inside an enigma: Brainerd diarrhoea turns 20. *Lancet* 2003;**362**:2037–8.
313. Mitchell DK, Monroe SS, Jiang X, et al. Virologic features of an astrovirus diarrhea outbreak in a day care center revealed by reverse transcriptase–polymerase chain reaction. *J Infect Dis* 1995;**172**:1437–44.
314. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1–166.
315. Molina JM, Chastang C, Goguel J, et al. Albendazole for treatment and prophylaxis of microsporidiosis due to *Encephalitozoon intestinalis* in patients with AIDS: a randomized double-blind controlled trial. *J Infect Dis* 1998;**177**:1373–7.
316. Molina JM, Tourneur M, Sarfati C, et al. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med* 2002;**346**: 1963–9.
317. Montes M, Sawhney C, Barros N. *Strongyloides stercoralis:* there but not seen. *Curr Opin Infect Dis* 2010;**23**:500–4.
318. Moore SR. Update on prolonged and persistent diarrhea in children. *Curr Opin Gastroenterol* 2011;**27**:19–23.
319. Moore SR, Lima NL, Soares AM, et al. Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children. *Gastroenterology* 2010;**139**:1156–64.
320. Mor SM, Tzipori S. Cryptosporidiosis in children in sub-Saharan Africa: a lingering challenge. *Clin Infect Dis* 2008;**47**: 915–21.
321. Moreno-Espinosa S, Farkas T, Jiang X. Human caliciviruses and pediatric gastroenteritis. *Semin Pediatr Infect Dis* 2004;**15**: 237–45.
322. Morris JG. Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell. *Clin Infect Dis* 2003; **37**:272–80.
323. Morse DL, Guzewich JJ, Hanrahan JP, et al. Widespread outbreaks of clam- and oyster-associated gastroenteritis: role of Norwalk virus. *N Engl J Med* 1986;**314**:678–81.
324. Moser LA, Schultz-Cherry S. Pathogenesis of astrovirus infection. *Viral Immunol* 2005;**18**:4–10.
325. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. *N Engl J Med* 2001;**344**:564.
326. Murrell TGC, Egerton JR, Rampling A, et al. The ecology and epidemiology of the pig-bel syndrome in man in New Guinea. *J Hyg* 1966;**64**:375–96.
327. Nataro JP. Enteroaggregative *Escherichia coli* pathogenesis. *Curr Opin Gastroenterol* 2005;**21**:4–8.
328. Nataro JP, Kaper JB. Diarrheagenic Escherichia coli. *Microbiol Rev* 1998;**11**:142–201.
329. National Collaborating Centre for Women's and Children's Health (UK). *Diarrhoea and vomiting caused by gastroenteritis: diagnosis, assessment and management in children younger than 5 years*. London: National Institute for Health and Clinical Excellence (NICE); 2009.
330. Nawa Y, Hatz C, Blum J, et al. Sushi delights and parasites: the risk of fishborne and foodborne parasitic zoonoses in Asia. *Clin Infect Dis* 2005;**419**:1297–303.
331. Nesheim SR, Kapogiannis BG, Soe MM, et al. Trends in opportunistic infections in the pre- and post–highly active antiretroviral therapy eras among HIV-infected children in the Perinatal AIDS Collaborative Transmission Study, 1986–2004. *Pediatrics* 2007;**120**: 100–9.
332. Newton A, Heiman KE, Schmitz A, et al. Cholera in United States associated with epidemic in Hispaniola. *Emerg Infect Dis* 2011; **17**:2166–8.
333. Newton A, Kendall M, Vugia DJ, et al. Increasing rates of vibriosis in the United States, 1996–2010: review of surveillance data from 2 systems. *Clin Infect Dis* 2012;**54**(Suppl 5):S391–5.
334. Nguyen RN, Taylor LS, Tauschek M, Robins-Browne RM. Atypical enteropathogenic *Escherichia coli* infection and prolonged diarrhea in children. *Emerg Infect Dis* 2006;**12**:597–603.
335. Nguyen TV, Le PV, Le CH, Weintraub A. Antibiotic resistance in diarrheagenic *Escherichia coli* and *Shigella* strains isolated from children in Hanoi, Vietnam. *Antimicrob Agents Chemother* 2005;**49**: 816–9.
336. Nhung PH, Ohkusu K, Miyasaka J, et al. Rapid and specific identification of 5 human pathogenic *Vibrio* species by multiplex polymerase chain reaction targeted to dnaJ gene. *Diagn Microbiol Infect Dis* 2007;**59**:271–5.
337. Nitschke M, Sayk F, Härtel C, et al. Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin–producing enteroaggregative *Escherichia coli* O104:H4. *JAMA* 2012;**307**:1046–52.
338. O'Connor RM, Shaffie R, Kang G, Ward HD. Cryptosporidiosis in patients with HIV/AIDS. *AIDS* 2011;**25**:549–60.
339. O'Ryan M, Matson DO, Estes MK, et al. Molecular epidemiology of rotaviruses in children attending day care centers in Houston. *J Infect Dis* 1990;**162**:810.
340. Ochoa TJ, Chen J, Walker CM, et al. Rifaximin does not induce toxin production or phage-mediated lysis of Shiga toxin–producing *Escherichia coli*. *Antimicrob Agents Chemother* 2007;**51**:2837–41.
341. Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. *Curr Opin Infect Dis* 2011;**24**:478–83.
342. Ochoa TJ, Ecker L, Barletta F, et al. Age/related susceptibility to infection with diarrheagenic *Escherichia coli* among infants from Periurban areas in Lima, Peru. *Clin Infect Dis* 2009;**49**: 1694–702.

343. Ochoa TJ, Mercado EH, Durand D, et al. Frequency and pathotypes of diarrheagenic *Escherichia coli* in Peruvian children with and without diarrhea. *Rev Peru Med Exp Salud Publica* 2011;**28**:13–20.
344. Ochoa TJ, Ruiz J, Molina M, et al. High frequency of antimicrobial drug resistance of diarrheagenic *Escherichia coli* in infants in Peru. *Am J Trop Med Hyg* 2009;**81**:296–301.
345. Ochoa TJ, Salazar-Lindo E, Cleary TG. Management of children with infection-associated persistent diarrhea. *Semin Pediatr Infect Dis* 2004;**15**:229–36.
346. Ohara T, Kojio S, Taneike I, et al. Effects of azithromycin on shiga toxin production by *Escherichia coli* and subsequent host inflammatory response. *Antimicrob Agents Chemother* 2002;**46**:3478–83.
347. Okeke IN. Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *J Infect Dev Ctries* 2009;**3**: 817–42.
348. Okhuysen PC. Traveler's diarrhea due to intestinal protozoa. *Clin Infect Dis* 2001;**33**:110–14.
349. Okhuysen PC, Dupont HL. Enteroaggregative *Escherichia coli* (EAEC): a cause of acute and persistent diarrhea of worldwide importance. *J Infect Dis* 2010;**202**:503–5.
350. Ooi ST, Lorger B. Gastroenteritis due to *Listeria monocytogenes*. *Clin Infect Dis* 2005;**40**:1327–32.
351. Operario DJ, Houpt E. Defining the causes of diarrhea: novel approaches. *Curr Opin Infect Dis* 2011;**24**:464–71.
352. Opintan JA, Newman MJ, Ayeh-Kumi PF, et al. Pediatric diarrhea in southern Ghana: etiology and association with intestinal inflammation and malnutrition. *Am J Trop Med Hyg* 2010;**83**:936–43.
353. Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a foodborne and waterborne parasite. *Clin Microbiol Rev* 2010;**23**: 218–34.
354. O'Ryan M, Prado V, Pickering LK. A millennium update on pediatric diarrheal illness in the developing world. *Semin Pediatr Infect Dis* 2005;**16**:125–36.
355. Osterholm MT, MacDonald KL, White KE, et al. An outbreak of brainerdiasis: an outbreak of a newly recognized chronic diarrhea syndrome associated with raw milk consumption. *JAMA* 1986;**256**:484–90.
356. Pape JW, Johnson Jr WD. *Isospora belli* infection. *Prog Clin Parasitol* 1991;**2**:119–27.
357. Parashar UD, Hummelman EG, Bresee JS, et al. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003;**9**:565–72.
358. Paredes-Paredes M, Flores-Figueroa J, Dupont HL. Advances in the treatment of travelers' diarrhea. *Curr Gastroenterol Rep* 2011;**13**:402–7.
359. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. *Curr Opin Infect Dis* 2008;**21**:531–8.
360. Pasetti MF, Simon JK, Sztein MB, Levine MM. Immunology of gut mucosal vaccines. *Immunol Rev* 2011;**239**:125–48.
361. Patel MM, Glass R, Desai R, et al. Fulfilling the promise of rotavirus vaccines: how far have we come since licensure? *Lancet Infect Dis* 2012;**12**:561–70.
362. Patel MM, Widdowson MA, Glass RI, et al. Systematic literature review of role of noroviruses in sporadic gastroenteritis. *Emerg Infect Dis* 2008;**14**:1224–31.
363. Patel NC, Hertel PM, Hanson IC, et al. Chronic rotavirus infection in an infant with severe combined immunodeficiency: successful treatment by hematopoietic stem cell transplantation. *Clin Immunol* 2012;**142**:399–401.
364. Paton AW, Paton JC. Multiplex PCR for direct detection of Shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin. *J Clin Microbiol* 2005;**43**:2944–7.
365. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med* 2012;**13**:85–97.
366. Pawlowski SW, Warren CA, Guerrant R. Diagnosis and treatment of acute or persistent diarrhea. *Gastroenterology* 2009;**136**: 1874–86.
367. Payne DC, Staat MA, Edwards KM, et al. New Vaccine Surveillance Network (NVSN). Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US Counties, 2006–2009. *Clin Infect Dis* 2011;**53**:245–53.
368. Penny ME, Peerson JM, Marin RM, et al. Randomized, community-based trial of the effect of zinc supplementation, with and without other micronutrients, on the duration of persistent childhood diarrhea in Lima, Peru. *J Pediatr* 1999;**135**:208–17.
369. Petri Jr WA, Miller M, Binder HJ, et al. Enteric infections, diarrhea, and their impact on function and development. *J Clin Invest* 2008;**118**:1277–90.
370. Pfeiffer ML, DuPont HL, Ochoa TJ. The patient presenting with acute dysentery—a systematic review. *J Infect* 2012;**64**:374–86.
371. Piarroux R, Faucher B. Cholera epidemics in 2010: respective roles of environment, strain changes, and human-driven dissemination. *Clin Microbiol Infect* 2012;**18**:231–8.
372. Pickering LK, Bartlett AV, Reves RR, et al. Asymptomatic excretion of rotavirus before and after rotavirus diarrhea in children in day care centers. *J Pediatr* 1988;**112**:361.
373. Pickering LK, Engelkirk PG. *Giardia lamblia*. *Pediatr Clin North Am* 1988;**35**:565.
374. Pickering LK, DuPont HL, Olarte J, et al. Fecal leukocytes in enteric infections. *Am J Clin Pathol* 1977;**68**:562–5.
375. Pickering LK, Feldman S, Ericsson CD, et al. Absorption of salicylate and bismuth from a bismuth subsalicylate containing compound (Pepto-Bismol). *J Pediatr* 1981;**99**:654–6.
376. Pickering LK, Marano N, Bocchini JA, Angulo FJ. Exposure to nontraditional pets at home and to animals in public settings: risks to children. Prepared for the Committee on Infectious Diseases, American Academy of Pediatrics. *Pediatrics* 2008;**122**:876.
377. Platts-Mills JA, Operario DJ, Houpt ER. Molecular diagnosis of diarrhea: current status and future potential. *Curr Infect Dis Rep* 2011;**14**:41–6.
378. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993;**328**:95–9.
379. Pope JE, Krizova A, Garg AX, et al. *Campylobacter* reactive arthritis: a systematic review. *Semin Arthritis Rheum* 2007;**37**:48–55.
380. Poropatich KO, Walker CL, Black RE. Quantifying the association between *Campylobacter* infection and Guillain-Barré syndrome: a systematic review. *J Health Popul Nutr* 2010;**28**:545–52.
381. Pothoulakis C. Effects of *Clostridium difficile* toxins on epithelial cell barrier. *Ann N Y Acad Sci* 2000;**915**:347–56.
382. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. *Curr Opin Clin Nutr Metab Care* 2009;**12**:646–52.
383. Primi MP, Bueno L, Baumer P, et al. Racecadotril demonstrates intestinal antisecretory activity in vivo. *Aliment Pharmacol Ther* 1999;**13**:3–7.
384. Proctor ME, Hamacher M, Tortorello ML, et al. Multistate outbreak of *Salmonella* serovar Muenchen infections associated with alfalfa sprouts grown from seeds pretreated with calcium hypochlorite. *J Clin Microbiol* 2001;**39**:3461–5.
385. Puthanakit T, Aurpibul L, Oberdorfer P. Hospitalization and mortality among HIV-infected children after receiving highly active antiretroviral therapy. *Clin Infect Dis* 2007;**44**:599–604.
386. Qadri F, Das SK, Faruque ASG, et al. Prevalence of toxin types and colonization factors in enterotoxigenic *Escherichia coli* isolated during a 1-year period from diarrheal patients in Bangladesh. *J Clin Microbiol* 2000;**38**:27–31.
387. Ralston KS, Petri Jr WA. Tissue destruction and invasion by *Entamoeba histolytica*. *Trends Parasitol* 2011;**27**:254–63.
388. Ramani S, Kang G. Viruses causing childhood diarrhoea in the developing world. *Curr Opin Infect Dis* 2009;**22**:477–82.
389. Reid JA, White DG, Caul EO, et al. Role of infected food handler in hotel outbreak of Norwalk-like viral gastroenteritis: implications for control. *Lancet* 1988;**2**:321.
390. Rendtorff RC. The experimental transmission of human intestinal protozoan parasites, part II. *Giardia lamblia* cysts given in capsules. *Am J Hyg* 1954;**59**:209–20.
391. Rennels MB. The rotavirus vaccine story: a clinical investigator's view. *Pediatrics* 2000;**106**:123–5.
392. Ribeiro Jr H, Ribeiro TC, Valois S, et al. Incomplete carbohydrate absorption from fruit juice consumption after acute diarrhea. *J Pediatr* 2001;**139**:325–7.
393. Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *N Engl J Med* 2010;**362**:299–305.
394. Ridoux O, Drancourt M. In vitro susceptibilities of the Microsporidia *Encephalitozoon cuniculi*, *Encephalitozoon hellem*, and *Encephalitozoon intestinalis* to albendazole and its sulfoxide and sulfone metabolites. *Antimicrob Agents Chemother* 1998;**42**:3301–3.
395. Román E, Wilhelmi I, Colomina J, et al. Acute viral gastroenteritis: proportion and clinical relevance of multiple infections in Spanish children. *J Med Microbiol* 2003;**52**:435–40.

396. Rosenthal P, Liebman WM. Comparative study of stool examination, duodenal aspiration and pediatric Entero-test for giardiasis in children. *J Pediatr* 1980;**96**:278–9.
397. Rosenthal SL. Exacerbation of *Salmonella enteritis* due to ampicillin. *N Engl J Med* 1969;**280**:147–8.
398. Roy SK, Tomkins AM, Akramuzzaman SM, et al. Randomised, controlled trial of zinc supplementation in malnourished Bangladeshi children with acute diarrhea. *Arch Dis Child* 1997;**77**: 196–200.
399. Ruel MT, Rivera JA, Santizo MC, et al. Impact of zinc supplementation on morbidity from diarrhea and respiratory infections among rural Guatemalan children. *Pediatrics* 1979;**99**:808–13.
400. Ruiz-Palacios GM, Pérez-Schael I, Velázquez FR, et al. Human Rotavirus Vaccine Study Group. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006;**354**:11–22.
401. Rusmak J, Hadfield TL, Rhodes M, et al. Detection of *Cryptosporidium* oocysts in human fecal specimens by an indirect immunofluorescence assay with monoclonal antibodies. *J Clin Microbiol* 1989;**27**:1135.
402. Safdar N, Said A, Gangnon RE, et al. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis. *JAMA* 2002;**288**:996–1001.
403. Salari P, Nikfar S, Abdollahi M. A meta-analysis and systematic review on the effect of probiotics in acute diarrhea. *Inflamm Allergy Drug Targets* 2012;**11**:3–14.
404. Sambol JSP, Merrigan MM, Lyerly D, et al. Toxin gene analysis of a variant strain of *Clostridium difficile* that causes human clinical disease. *Infect Immun* 2000;**68**:5480–7.
405. Sanchez TH, Brooks JT, Sullivan PS, et al. Bacterial diarrhea in persons with HIV infection, United States, 1992–2002. *Clin Infect Dis* 2005;**41**:1621–7.
406. Sánchez-Vargas FM, Abu-El-Haija MA, Gómez-Duarte OG. *Salmonella* infections: an update on epidemiology, management, and prevention. *Travel Med Infect Dis* 2011;**9**:263–77.
407. Sandhu BK, Isolauri E, Walker-Smith JA, et al. A multicentre study on behalf of the European Society of Paediatric Gastrenterology and Nutrition Working Group on Acute Diarrhoea: Recommendations for feeding on childhood gastroenteritis. *J Pediatr Gastroenterol Nut* 1997;**24**:619–20.
408. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2011;**30**:580–4.
409. Santosham M, Chandran A, Fitzwater S, et al. Progress and barriers for the control of diarrhoeal disease. *Lancet* 2010;**376**: 63–7.
410. Santosham M, Greenough III WB. Oral rehydration therapy: global prospective. *J Pediatr* 1991;**118**:44–51.
411. Santosham M, Fayad IM, Hashem M, et al. A comparison of rice-based oral rehydration solution and "early feeding" for the treatment of acute diarrhea in infants. *J Pediatr* 1990;**116**:868–76.
412. Sazawal S, Black RE, Bhan MK, et al. Zinc supplementation in young children with acute diarrhea in India. *N Engl J Med* 1994;**333**:839–44.
413. Sazawal S, Hiremath G, Dhingra U, et al. Efficacy of probiotics in prevention of acute diarrhoea: a meta-analysis of masked, randomised, placebo-controlled trials. *Lancet Infect Dis* 2006;**6**: 374–82.
414. Scaletsky IC, Fabbricotti SH, Carvalho RL, et al. Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in northeast Brazil: a case-control study. *J Clin Microbiol* 2002; **40**:645–8.
415. Scaletsky IC, Pedroso MD, Morais MB, et al. Association of patterns of *Escherichia coli* adherence to Hep-2 cells with acute and persistent diarrhea. *Arch Gastroenterol* 1999;**36**:54–60.
416. Scallan E. Activities, achievements, and lesson learned during the first 10 years of the Foodborne Diseases Active Surveillance Network: 1996–2006. *Clin Infect Dis* 2007;**44**:718–25.
417. Scallan E, Griffin PM, Angulo FJ, et al. Foodborne illness acquired in the United States—unspecified agents. *Emerg Infect Dis* 2011;**17**: 16–22.
418. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.
419. Schantz PM. The dangers of eating raw fish. *N Engl J Med* 1989;**320**:1143.
420. Schelin J, Wallin-Carlquist N, Cohn MT, et al. The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2011;**2**:580–92.
421. Schneider JL, White PL, Weiss J, et al. Multistate outbreak of multidrug-resistant *Salmonella newport* infections associated with ground beef, October to December 2007. *J Food Prot* 2011;**74**: 1315–19.
422. Schultsz C, van den Ende J, Cobelens F, et al. Diarrheagenic *Escherichia coli* and acute and persistent diarrhea in returned travelers. *J Clin Microbiol* 2000;**38**:3550–4.
423. Sears CL, Kaper JB. Enteric bacterial toxins: mechanisms of action and linkage to intestinal secretion. *Microbiol Rev* 1996;**60**: 167–215.
424. Sena LV, Maranhão H de S, Moraes MB. Evaluation of mothers' knowledge about oral rehydration therapy and sodium concentration in homemade sugar salt solutions. *J Pediatr (Rio J)* 2001;**77**: 481–6.
425. Senderovich Y, Ken-Dror S, Vainblat I, et al. A molecular study on the prevalence and virulence potential of *Aeromonas* spp. recovered from patients suffering from diarrhea in Israel. *PLoS One* 2012;**7**:e30070.
426. Shadduck JA, Greeley E. Microsporidia and human infections. *Clin Microbiol Rev* 1989;**2**:158.
427. Shankar AH, Prasad AS. Zinc and immune function: the biological basis of altered resistance to infection. *Am J Clin Nutr* 1998; **68**:S47–63.
428. Shedl HP, Clifton JA. Solute and water absorption by the human small intestine. *Nature* 1963;**199**:1264–7.
429. Sheikh RA, Prindiville TP, Yenamandra S, et al. Microsporidial AIDS cholangiopathy due to *Encephalitozoon intestinalis:* case report and review. *Am J Gastroenterol* 2000;**95**:2364–71.
430. Sheth M, Dwivedi R. Complementary foods associated diarrhea. *Indian J Pediatr* 2006;**73**:61–4.
431. Shimizu H, Phan TG, Nishimura S, et al. An outbreak of adenovirus serotype 41 infection in infants and children with acute gastroenteritis in Maizura City, Japan. *Infect Genet Evol* 2007;**7**: 279–84.
432. Shinozaki T, Araki K, Fujita Y, et al. Epidemiology of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children in the Tokyo area. *Scand J Infect* 1991;**23**: 543–7.
433. Shinozaki T, Araki K, Ushijima H, et al. Antibody response to enteric adenovirus types 40 and 41 in sera from people in various age groups. *J Clin Microbiol* 1987;**25**:1679.
434. Siddiqui AA, Berk SL. Diagnosis of *Strongyloides stercoralis* infection. *Clin Infect Dis* 2001;**33**:1040–7.
435. Silva J, Leite D, Fernandes M, et al. *Campylobacter* spp. as a foodborne pathogen: a review. *Front Microbiol* 2011;**2**:200.
436. Smith KE, Wilker PR, Reiter PL, et al. Antibiotic treatment of *Escherichia coli* O157 infection and the risk of hemolytic uremic syndrome, Minnesota. *Pediatr Infect Dis J* 2012;**31**:37–41.
437. Sobel J, Painter J. Illnesses caused by marine toxins. *Clin Infect Dis* 2005;**41**:1290–6.
438. Sobel J, Tucker N, Sulka A, et al. Foodborne botulism in the United States, 1990–2000. *Emerg Infect Dis* 2004;**10**:1606–11.
439. Solaymani-Mohammadi S, Genkinger JM, Loffredo CA, Singer SM. A meta-analysis of the effectiveness of albendazole compared with metronidazole as treatments for infections with *Giardia duodenalis*. *PLoS Negl Trop Dis* 2010;**4**:e682.
440. Soriano-Brücher HE, Avendaño P, O'Ryan M, et al. Use of bismuth subsalicylate in acute diarrhea in children. *Rev Infect Dis* 1990;**12**:S51.
441. Soriano-Brücher H, Avendaño P, O'Ryan M, et al. Bismuth subsalicylate in the treatment of acute diarrhea in children: a clinical study. *Pediatrics* 1991;**87**:18–27.
442. Spano LC, Sadovsky AD, Segui PN, et al. Age-specific prevalence of diffusely adherent *Escherichia coli* in Brazilian children with acute diarrhoea. *J Med Microbiol* 2008;**57**:359–63.
443. Stauffer W, Ravdin JI. *Entamoeba histolytica:* an update. *Curr Opin Infect Dis* 2003;**16**:479–85.
444. Steinberg EB, Greene KD, Bopp CA, et al. Cholera in the United States, 1995–2000: trends at the end of the twentieth century. *J Infect Dis* 2001;**184**:799–802.
445. Steinmuller N, Demma L, Bender JB, et al. Outbreaks of enteric disease associated with animal contact: Not just a foodborne problem anymore. *Clin Infect Dis* 2006;**43**:1596–602.

446. Stenfors-Arnesen LP, Fagerlund A, Granum PE. From soil to gut: *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol Rev* 2008;**32**:579–606.
447. Stenzel DJ, Boreham PFL. *Blastomycosis hominis* revisited. *Clin Microbiol Rev* 1996;**9**:563–84.
448. Svennerholm AM, Lundgren A. Recent progress toward an enterotoxigenic *Escherichia coli* vaccine. *Expert Rev Vaccines* 2012; **11**:495–507.
449. Swaminathan A, Torresi J, Schlagenhauf P, et al. A global study of pathogens and host risk factors associated with infectious gastrointestinal disease in returned international travellers. *J Infect* 2009;**59**:19–27.
450. Swanson SJ, Snider C, Braden CR, et al. Multidrug-resistant *Salmonella enterica* serotype Typhimurium associated with pet rodents. *N Engl J Med* 2007;**356**:21–8.
451. Sýkora J, Siala K, Huml M, et al. Evaluation of faecal calprotectin as a valuable non-invasive marker in distinguishing gut pathogens in young children with acute gastroenteritis. *Acta Paediatr* 2010; **99**:1389–95.
452. Szajewska H, Skorka A, Dylag M. Meta-analysis: *Saccharomyces boulardii* for treating acute diarrhoea in children. *Aliment Pharmacol Ther* 2007;**25**:257–64.
453. Szajewska H, Skorka A, Ruszczyski M, et al. Meta-analysis: lactobacillus GG for treating acute diarrhoea in children. *Aliment Pharmacol Ther* 2007;**25**:871–81.
454. Tan M, Jiang X. Norovirus-host interaction: multi-selections by human histo-blood group antigens. *Trends Microbiol* 2011;**19**:82–8.
455. Taniuchi M, Walters CC, Gratz J, et al. Development of a multiplex polymerase chain reaction assay for diarrheagenic *Escherichia coli* and *Shigella* spp. and its evaluation on colonies, culture broths, and stool. *Diagn Microbiol Infect Dis* 2012;**73**:121–8.
456. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin–producing *Escherichia coli* and haemolytic uremic syndrome. *Lancet* 2005;**365**: 1073–86.
457. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:136–41.
458. Tejman-Yarden N, Eckmann L. New approaches to the treatment of giardiasis. *Curr Opin Infect Dis* 2011;**24**:451–6.
459. Ternhag A, Asikainen T, Giesecke J, et al. A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with *Campylobacter* species. *Clin Infect Dis* 2007;**44**: 696–700.
460. Teunis PF, Moe CL, Liu P, et al. Norwalk virus: how infectious is it? *J Med Virol* 2008;**80**:1468–76.
461. Thomas DW, Greer FR. Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**:1217–31.
462. Tippen PS, Meyer A, Blank EC, et al. Aquarium-associated *Plesiomonas shigelloides* infection: Missouri. *MMWR Morb Mortal Wkly Rep* 1989;**38**:617.
463. Tobias J, Vutukuru SR. Simple and rapid multiplex PCR for identification of the main human diarrheagenic *Escherichia coli*. *Microbiol Res* 2011 Dec 20 [Epub ahead of print].
464. Townes JM. Reactive arthritis after enteric infections in the United States: the problem of definition. *Clin Infect Dis* 2010;**50**:247–54.
465. Traa BS, Walker CL, Munos M, Black RE. Antibiotics for the treatment of dysentery in children. *Int J Epidemiol* 2010;**39**: i70–4.
466. Tremoulet AH, Avila-Aguero ML, Paris MM, et al. Albendazole therapy for *Microsporidium* diarrhea in immunocompetent Costa Rican children. *Pediatr Infect Dis J* 2004;**23**:915–18.
467. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, et al. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. *Am J Trop Med Hyg* 2005;**73**: 921–5.
468. Valazquez-Boland JA, Kuhn M, Berche P, et al. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001;**14**:584–640.
469. Valois S, Costa-Ribeiro Jr H, Mattos A, et al. Controlled, double-blind, randomized clinical trial to evaluate the impact of fruit juice consumption on the evolution of infants with acute diarrhea. *Nutr J* 2005;**4**:23.
470. Van R, Wun CC, O'Ryan ML, et al. Outbreaks of human enteric adenovirus types 40 and 41 in Houston day care centers. *J Pediatr* 1992;**120**:516–21.
471. Vandenberg O, Robberecht F, et al. Management of a *Cryptosporidium hominis* outbreak in a day-care center. *Pediatr Infect Dis J* 2012;**31**:10–15.
472. Vega E, Barclay L, Gregoricus N, et al. Novel surveillance network for norovirus gastroenteritis outbreaks, United States. *Emerg Infect Dis* 2011;**17**:1389–95.
473. Velazquez FR, Calva JJ, Guerrero ML, et al. Cohort study of rotavirus serotype patterns in symptomatic and asymptomatic infections in Mexican children. *Pediatr Infect Dis J* 1993;**12**: 56–61.
474. Velazquez FR, Matson DO, Calva JJ, et al. Natural protection conferred by rotavirus infections: implications for vaccine strategies. *N Engl J Med* 1996;**335**:1022–8.
475. Venkataraman S, Ramakrishna BS, Kang G, et al. Faecal lactoferrin as a predictor of positive faecal culture in south Indian children with acute diarrhoea. *Ann Trop Paediatr* 2003;**23**:9–13.
476. Vesikari, T, Matson DO, Dennehy P, et al. Rotavirus Efficacy and Safety Trial (REST) Study Team: Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006;**354**:23–33.
477. Vidal M, Kruger E, Durán C, et al. Single multiplex PCR assay to identify simultaneously the six categories of diarrheagenic *Escherichia coli* associated with enteric infections. *J Clin Microbiol* 2005;**43**:5362–5.
478. Villar RG, Macek MD, Simons S, et al. Investigation of multidrug-resistant *Salmonella* serotype *typhimurium* DT104 infections linked to raw-milk cheese in Washington State. *JAMA* 1999;**281**: 1811–16.
479. Walker CL, Black RE. Zinc for the treatment of diarrhoea: effect on diarrhoea morbidity, mortality and incidence of future episodes. *Int J Epidemiol* 2010;**1**:i63–9.
480. Walker-Smith JA, Sandhu BK, Isolauri E, et al. Recommendations for feeding on childhood gastroenteritis. *J Pediatr Gastroenterol Nutr* 1997;**24**:619–20.
481. Walter JE, Briggs J, Lourdes GM, et al. Molecular characterization of a novel recombinant strain of human astrovirus associated with gastroenteritis in children. *Arch Virol* 2001;**146**:235.
482. Wang FT, Mast TC, Glass RJ, et al. Effectiveness of the pentavalent rotavirus vaccine in preventing gastroenteritis in the United States. *Pediatrics* 2010;**125**:e208–13.
483. Waters V, Ford-Jones EL, Petric M, et al. Etiology of community-acquired pediatric viral diarrhea: a prospective longitudinal study in hospitals, emergency departments, pediatric practices and child care centers during the winter rotavirus outbreak, 1997 to 1998. The Pediatric Rotavirus Epidemiology Study for Immunization Study Group. *Pediatr Infect Dis J* 2000;**19**:843–8.
484. Watson DA, Andrew JH, Banting S, et al. Pig-bel but no pig: *Enteritis necroticans* acquired in Australia. *Med J Aust* 1991;**155**: 47–50.
485. Weber R, Bryan RT, Owen RL, et al. Improved light-microscopical detection of microsporidia spores in stool and duodenal aspirates. *N Engl J Med* 1992;**326**:161–6.
486. Weber R, Bryan RT, Schwartz DA, et al. Human microsporidial infections. *Clin Microbiol Rev* 1994;**7**:426–61.
487. Weil AA, Ivers LC, Harris JB. Cholera: lessons from Haiti and beyond. *Curr Infect Dis Rep* 2012;**14**:1–8.
488. White DG, Zhoa S, Sudler R, et al. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *N Engl J Med* 2001;**345**:1147–54.
489. Widdowson MA, Meltzer MI, Zhang X, et al. Cost-effectiveness and potential impact of rotavirus vaccination in the United States. *Pediatrics* 2007;**119**:684–97.
490. Wiemer D, Loderstaedt U, von Wulffen H, et al. Real-time multiplex PCR for simultaneous detection of *Campylobacter jejuni*, *Salmonella*, *Shigella*, and *Yersinia* species in fecal samples. *Int J Med Microbiol* 2011;**301**:577–84.
491. Wilcox WD, Miller JJ. Inaccuracy of three-finger pinch method of determining salt content in homemade sugar salt solutions. *Wilderness Environ Med* 1996;**2**:122–6.
492. Willing BP, Russell SL, Finlay BB, et al. Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nat Rev Microbiol* 2011;**9**:233–43.
493. Reference deleted in proofs.

494. Wittner M, Turner JW, Jacquette G, et al. Eustrongylidiasis: a parasitic infection acquired by eating sushi. *N Engl J Med* 1989;**320**:1124.
495. Wong CS, Jelacic S, Habeeb RL, et al. The risk of the hemolytic uremic syndrome after antibody treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000;**342**:1930–6.
496. World Health Organization. *Management of severe malnutrition: a manual for physicians and other senior health workers*. Geneva: WHO; 1999.
497. World Health Organization. *Guidelines for the inpatient treatment of severely malnourished children*. Geneva: WHO; 2003.
498. World Health Organization. *Guidelines for the control of shigellosis, including epidemics due to* Shigella dysenteriae *type 1*. Geneva: WHO; 2005.
499. World Health Organization. *The treatment of diarrhoea: a manual for physicians and other senior health workers*. Geneva: WHO; 2005.
500. World Health Organization/UNICEF Joint Statement. *Clinical Management of Acute Diarrhoea*. New York, NY, and Geneva, Switzerland: The United Nations Children's Fund/WHO; WHO, Geneva; 2004.
501. Wright SG. Protozoan infections of the gastrointestinal tract. *Infect Dis Clin North Am* 2012;**26**:323–39.
502. Wunderli W, Meerbach A, Güngör T, et al. Astrovirus infection in hospitalized infants with severe combined immunodeficiency after allogeneic hematopoietic stem cell transplantation. *PLoS One* 2011;**6**:e27483.
503. Yakoob MY, Theodoratou E, Jabeen A, et al. Preventive zinc supplementation in developing countries: impact on mortality and morbidity due to diarrhea, pneumonia and malaria. *BMC Public Health* 2011;**3**:S23.
504. Yang JR, Wu FT, Tsai JL, et al. Comparison between O serotyping method and multiplex real-time PCR to identify diarrheagenic *Escherichia coli* in Taiwan. *J Clin Microbiol* 2007;**45**:3620–5.
505. Yang S, Leff MG, McTague D, et al. Multistate surveillance for food handling, preparation and consumption behaviors associated with foodborne diseases: 1995 and 1996 BRFSS food-safety questions. *M M W R CDC Surveill Sum* 1998;**47**(SS-4):33–57.
506. Yolken RH, Bishop CA, Townsend TR, et al. Infectious gastroenteritis in bone-marrow transplant recipients. *N Engl J Med* 1982; **306**:1009–12.
507. Yolken R, Leister F, Dubovi E, et al. Infantile gastroenteritis associated with excretion of pestivirus antigens. *Lancet* 1989; **1**:517.
508. Zella GC, Israel EJ. Chronic diarrhea in children. *Pediatr Rev* 2012;**33**:207–17.
509. Zhang X, McDaniel AD, Wolf LE, et al. Quinolone antibiotics induce Shiga toxin–encoding bacteriophages, toxin production, and death in mice. *J Infect Dis* 2000;**181**:664–70.
510. Zheng DP, Ando T, Fankhauser RL, et al. Vorovirus classification and proposed strain nomenclature. *Virology* 2006;**346**:312–23.
511. Zheng DP, Widdowson MA, Glass RI, Vinjé J. Molecular epidemiology of genogroup II-genotype 4 noroviruses in the United States between 1994 and 2006. *J Clin Microbiol* 2010;**48**:168–77.
512. Zuckerman JN, Rombo L, Fisch A. The true burden and risk of cholera: implications for prevention and control. *Lancet Infect Dis* 2007;**7**:521–30.

Antibiotic use remains a principal risk factor for the development of CDI. The risk of CDI increases 7- to 10-fold during a course of antibiotics and for 1 month after antibiotic discontinuation.[43] Although the earliest reports of pseudomembranous colitis were associated with administration of clindamycin, nearly every antibiotic class has been implicated in the development of CDI, particularly cephalosporins, other β-lactam antibiotics, and fluoroquinolones. Diarrhea typically begins during antibiotic use or up to 30 days after antibiotic therapy.[87] Symptoms may occur after a single dose of an intravenous antibiotic.[57] Other risk factors for the development of CDI in adults include age older than 65 years, increased severity of underlying illness, prior hospitalization, gastrointestinal surgery, and use of feeding tubes.[10] Proton-pump inhibitor use has been associated not only with an increased risk of CDI but also with increased complication rates and increased mortality.[26,71,97]

CDI has historically been less common and less severe in children. However, over the past decade, rates of CDI in children have increased in both hospitals[54,74,103] and the community.[9] Moreover, CDI has been reported in children previously thought to be at low risk for the disease, including those without prior antibiotic or hospital exposure, as well as infants.[20]

Forty to 70 percent of asymptomatic, healthy newborns may be colonized with *C. difficile* in the first 10 to 28 days of life; colonization rates decrease to 3 to 10 percent by the second year of life.[47,49] Rates of *C. difficile* colonization in children older than 2 years of age approximate those in healthy adults and may be as low as 2 to 3 percent.[5,8,50]

Colonization occurs with both toxin-producing and nontoxigenic strains.[61] Factors associated with increased colonization rates in infants include longer duration of hospitalization, hospitalization in an intensive care setting, low birth weight, and formula feeding.[47] Even with a large number of toxigenic *C. difficile* bacteria and high levels of toxin A and B, infants usually remain asymptomatic,[65] and most studies have failed to show an epidemiologic association between colonization and disease in infants younger than 1 year of age. For example, in a study of outpatient children, *C. difficile* was isolated from 7% of patients with diarrhea and 14.8% of healthy controls. Children with *C. difficile* were younger than children without the organism (mean age 8.2 to 9.8 months).[11] *C. difficile* was isolated with equal frequency in healthy Swedish children 1 week to 1 year of age (17%) and children younger than 6 years with diarrhea (18%).[88] Most controlled studies in neonatal intensive care units corroborate high rates of asymptomatic colonization in young infants. *C. difficile* toxin was recovered from the stools of 55% of patients in one neonatal intensive care unit, but signs of enteric disease, including necrotizing enterocolitis, occurred with equal frequency in both toxin-positive and toxin-negative infants.[27] Although severe CDI has occasionally been reported in young infants, especially those with underlying gastrointestinal disease, it has historically been thought to be rare.[79]

Coincident with the emergence of the epidemic NAP1 strain, the epidemiology of CDI in children may be changing. In a study that included 21 free-standing children's hospitals, CDI increased 53% in hospitalized children between 2001 to 2006, from 2.6 cases to 4.0 cases per 1000 admissions.[54] Twenty-six percent of the children treated for CDI were younger than 1 year of age, and 5 percent were newborns. An analysis of two large administrative databases demonstrated a nearly twofold increase in CDI-associated hospitalization between 1997 and 2006.[103] Children younger than 1 year of age had the second highest rate of CDI hospitalizations. Investigators acknowledged that their study methodology precluded an assessment of whether these children had true CDI or *C. difficile* colonization in the setting of diarrhea attributable to another cause. Nevertheless, significant increases in CDI in hospitalized children have been documented, even when children younger than 1 year of age are excluded.[74]

C. difficile is a common health care–acquired pathogen, and most cases of CDI are health care associated.[21] In a prospective cohort study of children younger than 18 years of age, *C. difficile* was the most common cause of health care–associated diarrhea, occurring in 32 percent of cases.[60] Outbreaks of CDI among hospitalized pediatric patients are well documented.[17,37,67] Nevertheless, several studies have documented the importance of CDI in ambulatory settings. In a prospective cohort study of children presenting for outpatient care for diarrhea, nearly 8% were found to be positive for *C. difficile* toxin; other bacterial pathogens were identified in approximately 5 percent.[24] In a similar study of children presenting to an emergency department with diarrhea, 6.7 percent were positive for *C. difficile* toxin.[56] A 5-year retrospective study performed at a tertiary care children's hospital revealed an approximate 2.5-fold increase in the rates of community-acquired CDI in children who visited the emergency department.[9] Daycare center outbreaks of *C. difficile* have also been reported.[55]

Some of these changes may be related to the emergence of the epidemic NAP1 strain, which has been isolated from children with diarrhea.[53,85,94] Nevertheless, it is not yet known if the NAP1 strain is associated with more severe disease in children, as it is in adults. Ten percent of isolates from hospitalized children with CDI identified through the Canadian Nosocomial Infections Surveillance Program between November 1, 2004, and April 30, 2005, were the B1/NAP1/027 strain.[85] Children with the NAP1 strain were more likely to suffer complications from CDI than were children with other strains (29% vs. 6%; relative risk 4.6; 95% confidence interval 1.1 to 17.2; $p = .04$), although none died. Similar rates of NAP1 isolation were identified in a prospective study of children at two U.S. hospitals with CDI. NAP1 was not associated with increased disease severity.[53]

As in adults, antibiotic exposure is a risk factor for the development of CDI in children.[53,84] Use of amoxicillin-clavulanic acid for acute otitis media[69] and long-term antibiotic use for acne[34] have both been occasionally linked to the development of CDI in pediatric patients. In one study of hospitalized children, receipt of three or more antibiotic classes in the 30 days before diagnosis of CDI was a risk factor for severe disease.[53] Nevertheless, antibiotic use is not a prerequisite for the development of CDI in children. Severe disease has been reported in

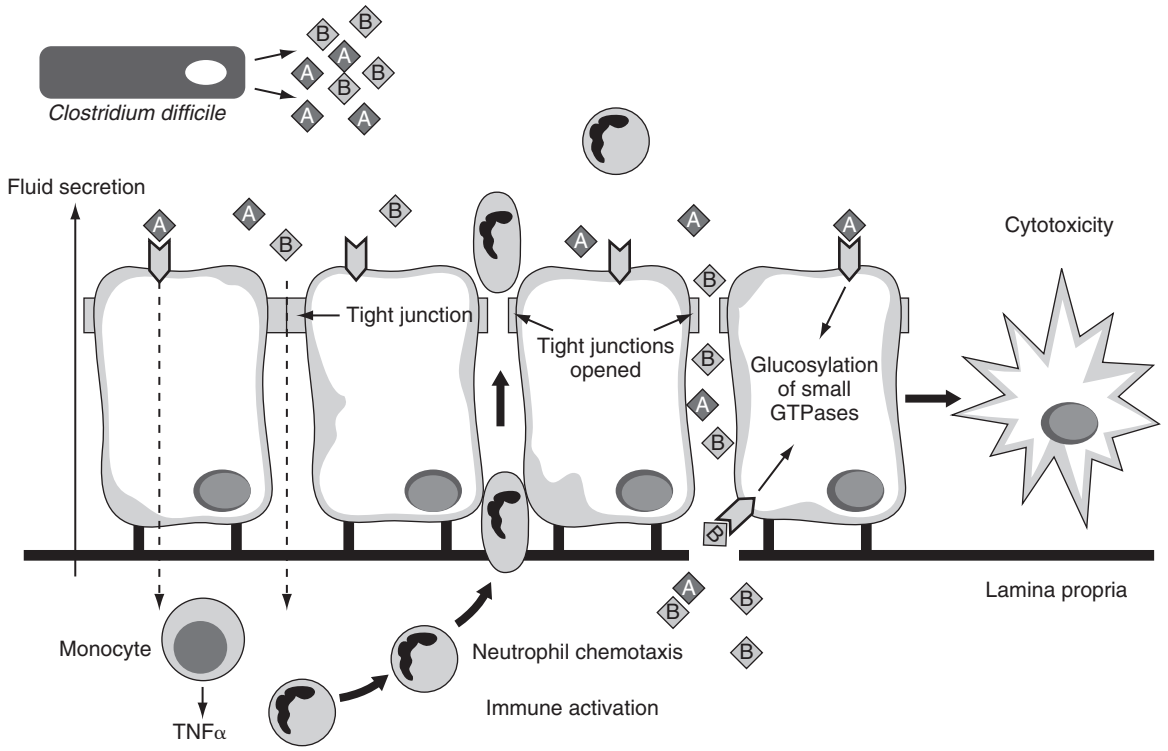

FIGURE 45-1 ■ Actions of *Clostridium difficile* toxins A and B on intestinal epithelium. (Courtesy Ian R. Poxton, Professor of Microbial Infection and Immunity, Medical Microbiology, University of Edinburgh School of Medicine.)

the absence of antibiotic exposure,[20] and in one study of community-acquired CDI, 43 percent of patients lacked a history of antibiotic use.[9]

Several studies have demonstrated that CDI is more common in boys.[74,79] Underlying gastrointestinal disease, particularly Hirschsprung disease, is a risk factor for severe, complicated CDI. Other risk factors for CDI in children include proton pump inhibitor use, solid organ transplant, inflammatory bowel disease, immune suppression, and cancer.[70,74,77,84] In a prospective study of 141 children undergoing chemotherapy for a solid tumor or lymphoma, 9 (6%) tested positive for *C. difficile* toxin A and were symptomatic.[19] Another study suggests that rates of *C. difficile* diarrhea in children after chemotherapy are as high as approximately 15 percent.[32] In an analysis of 3 years of data from the Kids' Inpatient Database (2000, 2003, 2006), children with cancer accounted for 21 percent of all CDI cases and rates of CDI were 15 times higher in children with cancer than in those without cancer.[89]

PATHOGENESIS

Ingestion of *C. difficile* spores and subsequent intestinal colonization precedes the development of CDI. In older children and adults, colonization is inhibited by normal intestinal flora, which is thought to compete for intestinal nutrients and space on the mucosal surface. Antibiotics, by altering normal flora, facilitate *C. difficile* colonization. Disease manifestations are related to toxin production; most toxigenic strains produce both toxin A and B.[80] Early animal studies suggested that toxin A was the major virulence factor, but recent studies have documented severe CDI caused by toxin A–negative, toxin B–positive strains.[1,66] Approximately 25 percent of *C. difficile* isolated by culture do not produce toxins[35] and are not clinically significant.

C. difficile toxins act at the level of the epithelial cells (Fig. 45-1) and produce effects by two main pathophysiologic pathways.[73,80] Toxin A and B cause the disorganization of actin microfilaments of the enterocyte cytoskeleton. The change in enterocyte structure leads to epithelial cell destruction and opening of tight junctions between enterocytes.[73,80] Toxin A also induces activation of neutrophils and results in local inflammation. Release of proinflammatory cytokines, including interleukin-6 and interleukin-8, occurs from enterocytes, causing further damage to the intestinal mucosa.[48,80] Both pathways lead to increased permeability of, and damage to, enterocytes, resulting in the clinical symptoms of *C. difficile*.[48]

Why young infants are commonly colonized with toxin-producing *C. difficile* strains yet rarely have symptoms is not yet completely understood. Colonization density does not appear to be important, because asymptomatic infants may have *C. difficile* colony counts as high as $10^{8.1}$ bacteria per gram of feces; these colony counts are similar to those in adults with pseudomembranous colitis.[47] Experiments in newborn rabbits suggested that protection against disease may result from lack of receptors for toxin A.[31] Interestingly, the appearance of receptors with age may be a species-specific phenomenon. Toxin receptors have been identified in the intestines of neonatal pigs, and these animals are susceptible to

disease.[51] The immaturity of the toxin receptor sites may also play a role in the absence of disease in neonates.[67]

Transmission of *C. difficile* is fecal-oral. The organism is generally spread by person-to-person contact or environment-to-person contact. Both modes of spread are important in health care environments, where *C. difficile* is shed in the environment by infected and colonized patients and occasionally from asymptomatic hospital personnel. Patients may acquire the *C. difficile* via the unwashed hands of health care workers, but direct or indirect transmission through contaminated objects and surfaces also occurs. *C. difficile* spores can survive for up to 5 months in the environment and are difficult to eradicate through routine cleaning and disinfection.[8,35,48] Objects likely to harbor organisms are those contaminated with feces and include toilet seats, sinks, and scales, but *C. difficile* can also be isolated from the hands and feces of asymptomatic hospital personnel.[35] Stethoscopes may be vectors for *C. difficile* transmission when not effectively cleaned after use on a patient with CDI.[98] Electronic rectal thermometers have also been implicated in hospital spread of *C. difficile*.[15]

Foodborne transmission has been postulated as a source of *C. difficile* spread in the community. Although *C. difficile* spores have been recovered from retail food products, there is limited evidence to link contamination of food to human illness.[39] Likewise, the role of zoonotic transmission has been explored because *C. difficile* is known to be both a pathogen and a commensal organism in domestic and food animals. To date, no study has convincingly demonstrated human *C. difficile* infection as a result of animal contact, although further research is needed.[39]

CLINICAL MANIFESTATIONS

C. difficile infection can cause a broad spectrum of disease from asymptomatic colonization or mild diarrhea to severe diarrhea, pseudomembranous colitis (PMC), toxic megacolon, and death.[6,96] Most commonly, *C. difficile* infection results in watery diarrhea in 90 to 95 percent of cases and bloody diarrhea in 5 to 10 percent.[57] Most pediatric patients develop self-resolving diarrhea associated with fever, abdominal pain and cramps, and abdominal distention, with or without bloody diarrhea, but some patients progress to PMC or chronic diarrhea, causing failure to thrive.[93] If severe disease develops, anasarca can occur because of protein-losing enteropathy.[6] Chronic diarrhea secondary to *C. difficile* can occur without evidence of colitis.[87] Uncommon complications of severe disease include gastrointestinal perforation, pneumatosis intestinalis,[53] or rectal prolapse.[45]

Mucoid stools are a hallmark of PMC.[57] PMC lesions almost always affect the colon, but involvement of the small intestine has been reported in adults.[46] Focal necrosis and inflammation are initially found in PMC lesions, but these can progress to extensive involvement of the colon covered by confluent pseudomembranes.[6] Although uncommon, fulminant colitis occurs and can lead to bowel perforation, peritonitis, and a high mortality rate.[58]

The role of *C. difficile* in the pathogenesis of necrotizing enterocolitis (NEC) among infants has been debated. Early studies reported no difference in colonization with *C. difficile* among infants with NEC and those without NEC.[101] A prospective study of 50 preterm infants also demonstrated that *C. difficile* colonization was not associated with a higher incidence of NEC.[33] In a retrospective cohort study of children with *C. difficile*–toxin positive stool specimens, 50 percent of 22 patients in a neonatal intensive care unit had suspected or confirmed NEC, although causality was not proved.[9]

In adults, *C. difficile* colitis has been reported to mimic acute peritonitis with fever, leukocytosis, and signs and symptoms of peritonitis on physical examination.[28,72,83,96] Rare extraintestinal manifestations of *C. difficile* include bacteremia, sepsis, visceral abscesses, pleural involvement, and reactive arthritis. Most cases of *C. difficile* bacteremia are usually polymicrobial with other bowel flora being isolated. The mortality rate of bacteremia is low.[46]

LABORATORY STUDIES

Stool culture is rarely used in clinical practice for the diagnosis of CDI. Although culture is highly sensitive, it requires selective media because it yields both toxigenic and nontoxigenic strains of *C. difficile*. Isolates must be tested for the presence of toxin genes or the expression of toxin gene proteins before the diagnosis of CDI can be confirmed.[58,62] Stool culture is generally reserved for epidemiologic investigations.[23]

The cell cytotoxicity neutralization assay (CCNA) has historically been considered the "gold standard" for the diagnosis of CDI and involves the addition of a diluted, buffered, and filtered stool sample to a monolayer of cultured cells. A positive CCNA test requires cytopathic changes in 50 percent or more of cells.[59] Although this test is highly specific, its sensitivity may be as low as 67 percent. Other limitations of CCNA include the need for skilled personnel to interpret the findings in tissue culture, the subjective nature of test interpretation, and an approximate 72-hour turn-around time.[18,59]

Enzyme immunoassays (EIAs) to detect toxin A and B or the glutamate dehydrogenase (GDH) antigen are commonly used for the clinical diagnosis of CDI. EIAs offer the advantages of rapid turn-around time, ease of use, and lower cost,[2,67,102] compared with CCNAs. EIAs for toxin A or toxins A and B have a reported sensitivity of 60 to 70 percent and specificities in the 90-percent range.[18] However, positive tests should be interpreted with caution in children because both lower sensitivity and specificity have been observed.[64,95] In one study of hospitalized children, the positive predictive value of a positive EIA was only 64 percent; one third of all positive EIA tests were ultimately found to be falsely positive.

In general, EIAs for GDH offer improved sensitivity but lower specificity because GDH is present in both toxigenic and nontoxigenic strains of *C. difficile* as well as other *Clostridium* species. The high negative predictive value of the GDH assay makes it useful as a screening tool.[18]

TABLE 45-1 **Laboratory Tests to Diagnose *Clostridium difficile* Infection**[59]

Testing Method	Sensitivity	Specificity	Advantage(s)	Disadvantage(s)
Culture	High	Low	Often considered gold standard	Long turn-around time; no differentiation between toxigenic and nontoxigenic strains
Cell cytoxicity neutralization assay (CCNA)	Moderate	Moderate to high	Highly specific	2 to 3 day turn-around time; requires skilled experts to interpret
Enzyme immunoassays for toxin detection	Low to moderate	High	Inexpensive; quick turn-around time; easy to perform	Overall low sensitivity
Enzyme immunoassay for glutamate dehydrogenase (GDH)	High	Low	Quick; useful for screening purposes	Low specificity; no differentiation between toxigenic and nontoxigenic strains
Nucleic acid amplification tests	High	High	High sensitivity and specificity; quick turn-around time; easy to perform	Expensive; increased detection of asymptomatic colonization

Nucleic acid amplification tests (NAATs) that detect toxin A *(tcdA)* or B *(tcdB)* genes, often by polymerase chain reaction, are increasingly available. Although expensive, these tests are rapid, specific, and more sensitive than EIAs.[25,64]

Because no single diagnostic test is presently optimal for the diagnosis of CDI (Table 45-1), some laboratories have adopted a multistep, diagnostic algorithm for stool testing. In one approach, stools are initially screened with a rapid immunoassay that can detect GDH and toxins A and B. Specimens that test both positive or negative for both GDH and toxin are immediately reported as positive or negative for toxigenic *C. difficile*. GDH-positive but toxin-negative specimens are subject to further testing by either a nucleic NAAT or CCNA.[18,59] The sensitivity and specificity of this approach in adult populations has been reported from 98 to 100 percent and 88 to 97 percent, respectively.[18] In a tertiary pediatric population, the sensitivity of the GDH-based algorithms was less but still superior to screening by toxin immunoassay (81% vs. 56%).[75]

Stool specimens submitted for *C. difficile* testing should be fresh and of liquid consistency. Specimens should be stored at less than 4° C because toxin rapidly degrades at room temperature.[23] Approximately 90 percent of patients can be diagnosed with CDI on a single stool sample and do not need repeat stool testing, because it does not increase diagnostic yield significantly and is not cost effective.[25] Because asymptomatic colonization is common in children younger than 1 year of age, testing for *C. difficile* should not be performed routinely in this age group and other causes for diarrhea should be sought.[59]

Leukocytosis, thrombocytosis, and hypoalbuminemia are commonly present in CDI. A leukocyte count greater than 15,000 cells/μL and a platelet count exceeding 400,000 cells/μL are more common in patients with CDI as compared to those without CDI.[76] Leukocytosis may be an early finding of CDI among hospitalized patients, even in the setting of mild or absent symptoms of colitis.[16,100] The presence of fecal leukocytes is an insensitive method for CDI screening.[82]

In seriously ill children in whom CDI is suspected but cannot be proved by standard laboratory testing, colonoscopy or sigmoidoscopy may be helpful. If pseudomembranes are found and clinical symptoms are consistent, the diagnosis of CDI can be made because almost all cases of PMC are caused by *C. difficile*. On examination, PMC has a characteristic appearance of yellowish white, raised plaques that are usually 2 to 10 mm in diameter.[67] Among infants with CDI, nonspecific colitis and not characteristic pseudomembranes are commonly found by endoscopy.[4]

Other radiographic modalities may provide supplemental data to aid in the diagnosis of CDI. Plain abdominal radiographs in patients with PMC may demonstrate colonic ileus, small bowel ileus, ascites, and/or nodular haustral thickening.[12] Abdominal computed tomography (CT) can be performed, but severity of colitis present on CT does not always correlate to clinical disease severity, and CT findings are less specific when compared with laboratory and clinical findings. Abnormalities on CT include nodular haustral thickening, colonic wall thickening, ascites, and pericolonic edema.[67] Abdominal ultrasonography has also been used to aid in the diagnosis of CDI because bowel wall thickening can be visualized.[81]

DIFFERENTIAL DIAGNOSIS

CDI must be distinguished from diarrhea that occurs as an adverse effect of antibiotics, other medications, or enteral nutrition, as well as diarrhea from other infectious causes. Viruses, for example, account for most cases of health care–associated diarrhea. *C. difficile* infection should be considered in hospitalized patients who develop diarrhea and who have had exposure to antibiotics.[60] Other bacteria that have occasionally been associated with antibiotic-associated diarrhea include *Staphylococcus aureus*, enterotoxin-producing strains of *Clostridium perfringens*, *Salmonella* species, and *Klebsiella oxytoca*.[6] Cytotoxin-producing *K. oxytoca* was recently reported as a cause of antibiotic-associated hemorrhagic colitis

(AAHC) in children.[44] In contrast to the diarrhea associated with CDI, the diarrhea associated with *K. oxytoca* AAHC is always bloody. Colitis is typically segmental and concentrated in the right colon; pseudomembranes are usually absent. AAHC is most commonly associated with exposure to amoxicillin-clavulanate.[44]

The differential diagnosis of community-onset diarrhea is broad compared with diarrhea with onset in the hospital setting. Bacteria, viruses, and parasites can all cause diarrhea in children, although viruses are still the most common.[24] Common causative viruses include astrovirus, adenovirus, and norovirus.[56] The incidence of rotavirus gastroenteritis has decreased with routine childhood immunization against this pathogen, but infections still occur.[91] Common bacterial causes of community-onset diarrhea, including *Escherichia coli*, *Campylobacter*, *Salmonella*, *Shigella*, and *Yersinia*, can be diagnosed by stool culture.[38,56] Like *C. difficile*, *Giardia intestinalis* can cause acute or protracted watery diarrhea. When clinical symptoms suggest CDI, testing should be considered even without a history of antibiotic use, especially in ambulatory patients. CDI in the absence of prior antibiotic exposure is more common in the community setting than the hospital setting and has been reported in 24 to 60 percent of patients with community-associated CDI.[30]

Inflammatory bowel disease, either Crohn disease or ulcerative colitis, should be considered in the setting of relapsing CDI or if colitis persists despite treatment.[13] In children previously diagnosed with inflammatory bowel disease, diarrhea and abdominal pain may be related to progression of inflammatory bowel disease and not CDI.[68]

TREATMENT

Among children with mild disease who do not require antimicrobial therapy, symptoms usually resolve within 7 to 10 days. Those with prolonged symptoms or more severe disease will require antimicrobial interventions.[14] Evidence-based guidelines have been published for the treatment of CDI in adults.[22] The American Academy of Pediatrics has made similar recommendations for the treatment of children and adolescents.[3] If possible, precipitating antibiotics should be discontinued. At least 10 days of oral or intravenous metronidazole (30 mg/kg/day in four divided doses, maximum 2 g/day) is recommended as initial treatment for most children and adolescents with CDI. Oral vancomycin (40 mg/kg/day in 4 divided doses, maximum 2 g/day) is generally reserved for patients with severe disease. Intravenous metronidazole in combination with vancomycin by mouth or nasogastric tube has been recommended for adults with severe CDI complicated by hypotension, shock, ileus, or megacolon; vancomycin may be administered rectally in cases of complete ileus.[22] These recommendations may have applicability for children with severe, complicated disease. Intravenous vancomycin is not effective for the treatment of CDI. Colectomy may be required for treatment of severe, complicated cases.

Relapse occurs in up to 20 to 30 percent of patients who initially improve with treatment with either metronidazole or vancomycin.[36] Most patients respond to a second course of the same treatment. A tapering and/or pulsed regimen of oral vancomycin has been recommended for adults who develop a second recurrence of CDI.[22]

In 2011, the U.S. Food and Drug Administration approved the use of fidaxomicin for the treatment of CDI in adults.[99] This monocyclic antibiotic acts as an RNA polymerase inhibitor and is bactericidal against *C. difficile*. In a large, multicenter randomized phase III trial, fidaxomicin was noninferior to vancomycin for the treatment of CDI (clinical cure 88.2% vs. 85.5%). However, recurrence of CDI within 4 weeks of clinical cure was significantly less in fidaxomicin-treated patients.[63] Published data in children are lacking.

Because perturbations in the normal bacterial flora of the gastrointestinal tract play a crucial role in the development of CDI, adjunctive therapies that promote the restoration of normal flora have received increasing attention. In fecal microbiota transplantation (FMT), stool from a healthy volunteer is administered to a patient with severe or relapsing CDI in an effort to restore normal intestinal flora.[52] FMT may be delivered by enema, colonoscopy, nasogastric tube or nasojejunal tube. Although randomized, controlled trials are lacking, data from case reports and case series suggest that symptoms resolve in more than 90% of patients treated with FMT.[52]

Probiotics have been studied alone or in combination with antibiotics for the treatment of CDI. One study of adults 18 years of age or older demonstrated that *Saccharomyces boulardii* administered in combination with high-dose vancomycin (2 g/day) decreased recurrences compared with vancomycin administered with placebo (16.7% vs. 50%).[86] Other clinical trials have failed to show a benefit of probiotics in treatment or prevention of CDI in adults.[78] Probiotic treatment of children with CDI has not been well studied.[92]

PREVENTION AND CONTROL

Because CDI is commonly precipitated by antibiotic therapy, judicious use of antibiotics is essential. Antimicrobial stewardship programs that reduce the use of broad-spectrum antibiotics have been linked to reductions in CDI in hospitalized patients.[90]

Contact precautions, in addition to standard precautions, are recommended for hospitalized patients for the duration of the illness.[3] Contact precautions include placement in a single-patient room when feasible, donning of gown and gloves by health care personnel before room entry, and hand hygiene after glove removal. The most appropriate method of hand hygiene when caring for patients with CDI has been the subject of some debate. Evidence-based guidelines published by the Infectious Disease Society of America (IDSA) and the Society for Healthcare Epidemiology of America (SHEA) recommend the preferential use of soap and water for hand hygiene over alcohol-based hand hygiene products only in outbreak settings,[22,29] while the AAP recommends soap and water routinely when caring for patients with CDI.[3]

The IDSA/SHEA recommendations may seem counterintuitive. Alcohol does not kill *C. difficile* spores. Additionally, in several studies, hand washing with soap and water was more effective than use of alcohol-based products in removing *C. difficile* spores from the hands of volunteers inoculated with the pathogen. Nevertheless, there are no data to support an increase in CDI with the use of alcohol-based hand hygiene products, nor are there studies that demonstrate a decrease in CDI when soap and water is used for hand hygiene. The recommendation to use soap and water for hand hygiene when caring for a patient with CDI in an outbreak setting is based on expert opinion.[22,29]

Appropriate environmental cleaning and disinfection of patient rooms and medical equipment are considered important measures in preventing health care–associated spread of *C. difficile*. Because quaternary compounds that are commonly used as hospital disinfectants are not sporicidal, chlorine-containing agents (e.g., household bleach diluted 1 : 10 in water) are recommended for cleaning in settings with increased rates of CDI.[22,29]

Measures to prevent the transmission of CDI in the community are less well defined, but include judicious antibiotic use. Children with *C. difficile* diarrhea should be excluded from daycare centers for the duration of illness.[3]

The observation that serum antibody levels against toxin A and B are associated with protection against recurrent CDI has fueled interest in the development of *C. difficile* vaccine. Although no vaccine is currently available for the prevention of CDI, in a recent phase I study, an adjuvanted *C. difficile* toxoid vaccine induced antibodies against toxin A in all healthy adult volunteers; some also developed antibodies against toxin B.[40]

NEW REFERENCES SINCE THE SIXTH EDITION

3. American Academy of Pediatrics. Clostridium difficile. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red Book: 2012 Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 285–7.
4. Barakat M, El-Kady Z, Mostafa M. Antibiotic-associated bloody diarrhea in infants: clinical, endoscopic, and histopathologic profiles. *J Pediatr Gastroenterol Nutr* 2011;**52**:60–4.
6. Bartlett JG. Historical perspectives on studies of *Clostridium difficile* and *C. difficile* infection. *Clin Infect Dis* 2008;**46**:S4–11.
8. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008;**46**:S12–18.
13. Brandt LJ, Reddy SS. Fecal microbiota transplantation for recurrent *Clostridium difficile* infection. *J Clin Gastroenterol* 2011;**45**: S159–67.
17. Bustinza A, Solona MJ, Padilla B, et al. Nosocomial outbreak of *Clostridium difficile*–associated disease in a pediatric intensive care unit in Madrid. *Infect Control Hosp Epidemiol* 2009;**30**: 199–201.
18. Carroll KC. Tests for the diagnosis of *Clostridium difficile* infection: the next generation. *Anaerobe* 2011;**17**:170–4.
19. Castagnola E, Battaglia T, Bandettini R, et al. *Clostridium difficile*–associated disease in children with solid tumors. *Support Care Cancer* 2009;**17**:321–4.
21. Centers for Disease Control and Prevention. Vital signs: preventing *Clostridium difficile* infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:157–62.
22. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of American (IDSA). *Infect Cont Hosp Epidemiol* 2010;**31**:431–55.
25. Deshpande A, Pasupuleti V, Patel P, et al. Repeat stool testing to diagnose *Clostridium difficile* infection using enzyme immunoassay does not increase diagnostic yield. *Clin Gastroenterol Hepatol* 2011;**9**:665–9.
29. Dubberke ER, Gerding DN, Classen D, et al. Strategies to prevent *Clostridium difficile* infections in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**:S81–92.
30. Dumyati G, Stevens V, Hannett GE, et al. Community-associated *Clostridium difficile* infections, Monroe County, New York, USA. *Emerg Infect Dis* 2012;**18**:392–400.
39. Gould LH, Limbago B. *Clostridium difficile* in food and domestic animals: a new foodborne pathogen? *Clin Infect Dis* 2010;**51**: 577–82.
40. Greenberg RN, Marbury TC, Foglia G, et al. Phase I dose finding studies of an adjuvanted *Clostridium difficile* toxoid vaccine. *Vaccine* 2012;**30**:2245–9.
41. Hall AC, Curns AT, McDonald LC, et al. The roles of norovirus and *Clostridium difficile* among gastroenteritis deaths in the United States, 1999-2007. Presentation at the 49th annual meeting of the Infectious Diseases Society of America; October 22, 2011; Boston, MA.
43. Hensgens MP, Goorhuis A, Dekkers OM, et al. Time interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics. *J Antimicrob Chemother* 2012;**67**:742–8.
44. Hoffman KM, Deutschmann A, Weitzer Z, et al. Antibiotic-associated hemorrhagic colitis caused by cytotoxin-producing *Klebsiella oxytoca*. *Pediatrics* 2010;**125**:e960–e963.
45. Huang S, Yang Y, Lee C. Rectal prolapse in a child: an unusual presentation of *Clostridium difficile*–associated pseudomembranous colitis. *Pediatr Neonatol* 2011;**52**:110–2.
47. Jangi S, Lamont JT. Asymptomatic colonization by *Clostridium difficile* in infants: implications for disease in later life. *JPGN* 2010;**51**:2–7.
48. Kachrimanidou M, Malisiovas N. *Clostridium difficile* infection: a comprehensive review. *Crit Rev Microbiol* 2011;**37**:178–87.
49. Kaslow DC, Shiver JW. *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus*: emerging concepts in vaccine development. *Annu Rev Med* 2011;**62**:201–15.
52. Khoruts A, Dicksved J, Jansson J, et al. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*–associated diarrhea. *J Clin Gastroenterol* 2010;**44**:354–60.
53. Kim J, Shaklee JF, Smathers S, et al. Risk factors and outcomes associated with severe *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2012;**31**:134–8.
54. Kim J, Smathers SA, Prasad P, et al. Epidemiological features of *Clostridium difficile*–associated disease among inpatients at children's hospitals in the United States, 2001-2006. *Pediatrics* 2008; **122**:1266–70.
59. Kufelnicka AM, Kirn TJ. Effective utilization of evolving methods for the laboratory diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2011;**52**:1451–7.
63. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N Engl J Med* 2011; **364**:422–31.
64. Luna RA, Boyanton BL, Mehta S, et al. Rapid stool-based diagnosis of *Clostridium difficile* infection by real-time PCR in a children's hospital. *J Clin Microbiol* 2011;**49**:851–7.
66. Lyras D, O'Connor JR, Howarth PM, et al. Toxin B is essential for virulence of *Clostridium difficile*. *Nature* 2009;**458**:1176–9.
68. Mezoff E, Mann EA, Hart KW. *Clostridium difficile* infection and treatment in the pediatric inflammatory bowel disease population. *J Pediatr Gastroenterol Nutr* 2011;**52**:437–41.
71. Morrison RH, Hall NS, Said M, et al. Risk factors associated with complications and mortality in patients with *Clostridium difficile* infection. *Clin Infect Dis* 2011;**53**:1173–8.
74. Nyland CM, Goudie A, Garza JM, et al. *Clostridium difficile* infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011;**165**:451–7.
75. Ota KV, McGowan KL. *Clostridium difficile* testing algorithms using glutamate dehydrogenase antigen and *C. difficile* toxin enzyme immunoassays with *C. difficile* nucleic acid amplification testing increase diagnostic yield in a tertiary pediatric population. *J Clin Microbiol* 2012;**50**:1185–8.

76. Pant C, Madonia P, Minocha A. Does PPI therapy predispose to *Clostridium difficile* infection? *Nat Rev Gastroenterol Hepatol* 2009;**6**:555–7.
77. Pascarella F, Martinelli M, Miele E, et al. Impact of *Clostridium difficile* infection on pediatric inflammatory bowel disease. *J Pediatr* 2009;**154**:854–8.
78. Pillai A, Nelson R. Probiotics for treatment of *Clostridium difficile*–associated colitis in adults. *Cochrane Database Syst Rev* 2008. CD004611.
79. Pokorn M, Radsel A, Cizman M, et al. Severe *Clostridium difficile*–associated disease in children. *Pediatr Infect Dis J* 2008;**27**:944–6.
84. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2011;**30**:580–4.
85. Suh KN, Gravel D, Mulvey MR, et al. *Clostridium difficile*–associated diarrhea in children admitted to acute care hospitals participating in the Canadian Nosocomial Infections Surveillance Program (CNISP), 2004-5 [abstract 306]. In: Program of the 18th Annual Scientific Meeting of the Society of Healthcare Epidemiology of America, Orlando, Fla, 2008.
89. Tai E, Richardson LC, Townsend J, et al. *Clostridium difficile* infection among children with cancer. *Pediatr Infect Dis J* 2011; **30**:610–2.
90. Talpaert MJ, Rao GG, Cooper BS, et al. Impact of guidelines and enhanced antibiotic stewardship on reducing broad-spectrum antibiotic usage and its effect on the incidence of *Clostridium difficile* infection. *J Antimicrob Chemother* 2011;**66**:2168–74.
91. Tate JE, Cortese MM, Payne DC, et al. Uptake, impact, and effectiveness of rotavirus vaccination in the United States—review of the first 3 years of postlicensure data. *Pediatr Infect Dis J* 2011;**30**:S56–60.
92. Thomas DW, Greer FR. Committee on Nutrition: Section on Gastroenterology, Hepatology and Nutrition, American Academy of Pediatrics. Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**:1217–31.
94. Toltzis P, Kim J, Dul M, et al. Presence of epidemic North American pulsed field type I *Clostridium difficile* strain in hospitalized children. *J Pediatr* 2009;**154**:607–8.
95. Toltzis P, Nerandzic MM, Saade E, et al. High proportion of false-positive *Clostridium difficile* enzyme immunoassays for toxin A and B in pediatric patients. *Infect Contr Hosp Epidemiol* 2012; **33**:175–9.
97. Turco R, Martinelli M, Miele E, et al. Proton pump inhibitors as a risk factor for paediatric *Clostridium difficile* infection. *Aliment Pharmacol Ther* 2010;**31**:754–9.
98. Vajravelu RK, Guerrero DM, Jury LA, et al. Evaluation of stethoscopes as vectors of *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 2012;**33**:96–8.
99. Venugopal AA, Johnson S. Fidaxomicin: a novel macrocyclic antibiotic approved for treatment of *Clostridium difficile* infection. *Clin Infect Dis* 2012;**54**:568–74.
103. Zilberberg MD, Tillotson GS, McDonald C. *Clostridium difficile* infections among hospitalized children, United States, 1997-2006. *Emerg Infect Dis* 2010;**16**:604–9.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 46

Whipple Disease

Zev Davidovics • Mark A. Gilger

Over the past 20 years there has been an explosion of knowledge about Whipple disease. It is now known that Whipple bacillus causes not only classic Whipple disease, a rare, systemic bacterial infection that can be fatal without therapy, but also acute infection such as gastroenteritis, pneumonia, and bacteremia. In its most common form, Whipple disease affects white, middle-aged men, causing diarrhea, weight loss, abdominal pain, arthralgias, and fever. Although it is extraordinarily rare in children, its recognition may be crucial, because simple treatment with appropriate antibiotics may be curative and lifesaving.[28]

HISTORY

Whipple disease was described in 1907 by George Hoyt Whipple,[91] at that time an instructor in pathology at The Johns Hopkins University.[7] Whipple's description probably was not the first; Allchin and Webb apparently described a patient with "Whipple disease" in 1895.[59]

In Whipple's account, a 37-year-old medical missionary was admitted to The Johns Hopkins Hospital with low-grade fever, steatorrhea, and an abdominal mass. The patient had a 5-year history of sporadic migratory polyarthritis. These attacks of arthritis were associated with a gradual loss of weight and strength. His skin was pigmented with a brownish hue. Laboratory evaluation found severe anemia and an enormous number of fatty acid crystals in the stool. Explorative laparotomy revealed large, firm mesenteric lymph nodes, and a diagnosis of either Hodgkin disease or tuberculosis was made. The patient died 1 week later, and autopsy revealed marked fatty deposition within intestinal mucosa and the mesenteric and retroperitoneal lymph nodes. Other findings included polyserositis (peritonitis, pleuritis, and

REFERENCES

1. Alfa MJ, Kabani A, Lyerly D. Characterization of a toxin A–negative, toxin B–positive strain of *Clostridium difficile* responsible for a nosocomial outbreak of *Clostridium difficile*–associated diarrhea. *J Clin Microbiol* 2000;**38**:2706–14.
2. Altaie SS, Meyer P, Dryja D. Comparison of two commercially available enzyme immunoassays for detection of *Clostridium difficile* in stool specimens. *J Clin Microbiol* 1994;**32**:51–3.
3. American Academy of Pediatrics. Clostridium difficile. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red Book: 2012 Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 285–7.
4. Barakat M, El-Kady Z, Mostafa M. Antibiotic-associated bloody diarrhea in infants: clinical, endoscopic, and histopathologic profiles. *J Pediatr Gastroenterol Nutr* 2011;**52**:60–4.
5. Bartlett JG. *Clostridium difficile*: history of its role as an enteric pathogen and the current state of knowledge about the organism. *Clin Infect Dis* 1994;**18**:S265–73.
6. Bartlett JG. Historical perspectives on studies of *Clostridium difficile* and *C. difficile* infection. *Clin Infect Dis* 2008;**46**:S4–11.
7. Bartlett JG, Chang TW, Gurwith M, et al. Antibiotic-associated pseudomembranous colitis due to toxin-producing clostridia. *N Engl J Med* 1978;**298**:531–4.
8. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008;**46**:S12–18.
9. Benson L, Song X, Campos J, et al. Changing epidemiology of *Clostridium difficile*–associated disease in children. *Infect Control Hosp Epidemiol* 2007;**28**:1233–5.
10. Bignardi GE. Risk factors for *Clostridium difficile* infection. *J Hosp Infect* 1998;**40**:1–15.
11. Boenning DA, Fleisher GR, Campos JM, et al. *Clostridium difficile* in a pediatric outpatient population. *Pediatr Infect Dis J* 1982;**1**:336–8.
12. Boland GW, Lee MJ, Cats A, et al. Pseudomembranous colitis: diagnostic sensitivity of the abdominal plain radiograph. *Clin Radiol* 1994;**49**:473–5.
13. Brandt LJ, Reddy SS. Fecal microbiota transplantation for recurrent *Clostridium difficile* infection. *J Clin Gastroenterol* 2011;**45**:S159–67.
14. Brook I. Pseudomembranous colitis in children. *J Gastroen Hepatol* 2005;**20**:182–6.
15. Brooks S, Khan A, Stoica D, et al. Reduction in vancomycin-resistant *Enterococcus* and *Clostridium difficile* infections following change to tympanic thermometers. *Infect Control Hosp Epidemiol* 1998;**19**:333–6.
16. Bulusu M, Narayan S, Shetler K, et al. Leukocytosis as a harbinger and surrogate marker of *Clostridium difficile* infection in hospitalized patients with diarrhea. *Am J Gastroenterol* 2000;**95**:3137–41.
17. Bustinza A, Solona MJ, Padilla B, et al. Nosocomial outbreak of *Clostridium difficile*–associated disease in a pediatric intensive care unit in Madrid. *Infect Control Hosp Epidemiol* 2009;**30**:199–201.
18. Carroll KC. Tests for the diagnosis of *Clostridium difficile* infection: the next generation. *Anaerobe* 2011;**17**:170–4.
19. Castagnola E, Battaglia T, Bandettini R, et al. *Clostridium difficile*–associated disease in children with solid tumors. *Support Care Cancer* 2009;**17**:321–4.
20. Centers for Disease Control and Prevention. Severe *Clostridium difficile*–associated disease in populations previously at low risk—four states, 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1201–5.
21. Centers for Disease Control and Prevention. Vital signs: preventing *Clostridium difficile* infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:157–62.
22. Cohen SH, Gerding DN, Johnson S, et al. Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the Society for Healthcare Epidemiology of America (SHEA) and the Infectious Diseases Society of American (IDSA). *Infect Cont Hosp Epidemiol* 2010;**31**:431–55.
23. Delmee M. Laboratory diagnosis of *Clostridium difficile* disease. *Clin Microbiol Infect* 2001;**7**:411–6.
24. Denno DM, Stapp JR, Boster DR, et al. Etiology of diarrhea in pediatric outpatient settings. *Pediatr Infect Dis J* 2005;**24**:142–8.
25. Deshpande A, Pasupuleti V, Patel P, et al. Repeat stool testing to diagnose *Clostridium difficile* infection using enzyme immunoassay does not increase diagnostic yield. *Clin Gastroenterol Hepatol* 2011;**9**:665–9.
26. Dial S, Delaney JA, Barkun AN. Use of gastric acid–suppressive agents and the risk of community acquired *Clostridium difficile*–associated disease. *JAMA* 2005;**294**:2989–95.
27. Donta ST, Myers MG. *Clostridium difficile* toxin in asymptomatic neonates. *J Pediatr* 1982;**100**:431–4.
28. Drapkin MS, Worthington MG, Chang TW, et al. *Clostridium difficile* colitis mimicking acute peritonitis. *Arch Surg* 1985;**120**:1321–2.
29. Dubberke ER, Gerding DN, Classen D, et al. Strategies to prevent *Clostridium difficile* infections in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**:S81–92.
30. Dumyati G, Stevens V, Hannett GE, et al. Community-associated *Clostridium difficile* infections, Monroe County, New York, USA. *Emerg Infect Dis* 2012;**18**:392–400.
31. Eglow R, Pothoulakis C, Itzkowitz S, et al. Diminished *Clostridium difficile* toxin A sensitivity in newborn rabbit ileum is associated with decreased toxin A receptor. *J Clin Invest* 1992;**90**:822–9.
32. El-Mahallawy HA, El-Din NH, Salah F, et al. Epidemiologic profile of symptomatic gastroenteritis in pediatric oncology patients receiving chemotherapy. *Pediatr Blood Cancer* 2004;**42**:338–42.
33. El-Mohandes AE, Keiser JF, Refat M, et al. Prevalence and toxigenicity of *Clostridium difficile* isolates in fecal microflora of preterm infants in the intensive care nursery. *Biol Neonate* 1993;**63**:225–9.
34. Facklam DP, Gardner JS, Neidert GL, et al. An epidemiologic postmarketing surveillance study of prescription acne medications. *Am J Public Health* 1990;**80**:50–3.
35. Fekety R, Kim KH, Brown D, et al. Epidemiology of antibiotic-associated colitis; isolation of *Clostridium difficile* from the hospital environment. *Am J Med* 1981;**70**:906–8.
36. Fekety R, McFarland LV, Surawicz CM, et al. Recurrent *Clostridium difficile* diarrhea: characteristics of and risk factors for patients enrolled in a prospective, randomized, double-blinded trial. *Clin Infect Dis* 1997;**24**:324–33.
37. Ferroni A, Merckx J, Ancelle T, et al. Nosocomial outbreak of *Clostridium difficile* diarrhea in a pediatric service. *Eur J Clin Microbiol Infect Dis* 1997;**16**:928–33.
38. Gadewar S, Fasano A. Current concepts in the evaluation, diagnosis and management of acute infectious diarrhea. *Curr Opin Pharmacol* 2005;**5**:559–65.
39. Gould LH, Limbago B. *Clostridium difficile* in food and domestic animals: a new foodborne pathogen? *Clin Infect Dis* 2010;**51**:577–82.
40. Greenberg RN, Marbury TC, Foglia G, et al. Phase I dose finding studies of an adjuvanted *Clostridium difficile* toxoid vaccine. *Vaccine* 2012;**30**:2245–9.
41. Hall AC, Curns AT, McDonald LC, et al. The roles of norovirus and *Clostridium difficile* among gastroenteritis deaths in the United States, 1999-2007. Presentation at the 49th annual meeting of the Infectious Diseases Society of America; October 22, 2011; Boston, MA.
42. Hall IC, O'Toole E. Intestinal flora in newborn infants with a description of a new pathogenic anaerobe. *Am J Dis Child* 1935;**49**:390–402.
43. Hensgens MP, Goorhuis A, Dekkers OM, et al. Time interval of increased risk for *Clostridium difficile* infection after exposure to antibiotics. *J Antimicrob Chemother* 2012;**67**:742–8.
44. Hoffman KM, Deutschmann A, Weitzer Z, et al. Antibiotic-associated hemorrhagic colitis caused by cytotoxin-producing *Klebsiella oxytoca*. *Pediatrics* 2010;**125**:e960–e963.
45. Huang S, Yang Y, Lee C. Rectal prolapse in a child: an unusual presentation of *Clostridium difficile*–associated pseudomembranous colitis. *Pediatr Neonatol* 2011;**52**:110–2.
46. Jacobs A, Barnard K, Fishel R, et al. Extracolonic manifestations of *Clostridium difficile* infections. *Medicine* 2001;**80**:88–101.
47. Jangi S, Lamont JT. Asymptomatic colonization by *Clostridium difficile* in infants: implications for disease in later life. *JPGN* 2010;**51**:2–7.
48. Kachrimanidou M, Malisiovas N. *Clostridium difficile* infection: a comprehensive review. *Crit Rev Microbiol* 2011;**37**:178–87.
49. Kaslow DC, Shiver JW. *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus*: emerging concepts in vaccine development. *Annu Rev Med* 2011;**62**:201–15.

50. Kato H, Kita H, Karasawa T, et al. Colonisation and transmission of *Clostridium difficile* in healthy individuals examined by PCR ribotyping and pulsed-field gel electrophoresis. *J Med Microbiol* 2001;**50**:720–7.
51. Keel MK, Songer JG. The distribution and density of *Clostridium difficile* toxin receptors on the intestinal mucosa of neonatal pigs. *Vet Pathol* 2007;**44**:814–22.
52. Khoruts A, Dicksved J, Jansson J, et al. Changes in the composition of the human fecal microbiome after bacteriotherapy for recurrent *Clostridium difficile*–associated diarrhea. *J Clin Gastroenterol* 2010;**44**:354–60.
53. Kim J, Shaklee JF, Smathers S, et al. Risk factors and outcomes associated with severe *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2012;**31**:134–8.
54. Kim J, Smathers SA, Prasad P, et al. Epidemiological features of *Clostridium difficile*–associated disease among inpatients at children's hospitals in the United States, 2001-2006. *Pediatrics* 2008;**122**:1266–70.
55. Kim K, DuPont HL, Pickering LK. Outbreaks of diarrhea associated with *Clostridium difficile* and its toxin in day-care centers: evidence of person-to-person spread. *J Pediatr* 1983;**102**:376–82.
56. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. *Clin Infect Dis* 2006;**43**:807–13.
57. Knoop FC, Owens M, Crocker IC. *Clostridium difficile*: clinical disease and diagnosis. *Clin Microbiol Rev* 1993;**6**:251–65.
58. Koss K, Clark MA, Sanders DS, et al. The outcome of surgery in fulminant *Clostridium difficile* colitis. *Colorectal Dis* 2006;**8**: 149–54.
59. Kufelnicka AM, Kirn TJ. Effective utilization of evolving methods for the laboratory diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2011;**52**:1451–7.
60. Langley JM, LeBlanc JC, Hanakowski M, et al. The role of *Clostridium difficile* and viruses as causes of nosocomial diarrhea in children. *Infect Control Hosp Epidemiol* 2002;**23**:660–4.
61. Larson HE, Barclay FE, Honour P, et al. Epidemiology of *Clostridium difficile* in infants. *J Infect Dis* 1982;**146**:727–33.
62. Lashner BA, Todorczuk J, Sahm DF, et al. *Clostridium difficile* culture-positive toxin-negative diarrhea. *Am J Gastroenterol* 1986; **81**:940–3.
63. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *N Engl J Med* 2011;**364**:422–31.
64. Luna RA, Boyanton BL, Mehta S, et al. Rapid stool-based diagnosis of *Clostridium difficile* infection by real-time PCR in a children's hospital. *J Clin Microbiol* 2011;**49**:851–7.
65. Lyerly DM, Krivan HC, Wilkins TD. *Clostridium difficile*: its disease and toxins. *Clin Microbiol Rev* 1988;**1**:1–18.
66. Lyras D, O'Connor JR, Howarth PM, et al. Toxin B is essential for virulence of *Clostridium difficile*. *Nature* 2009;**458**:1176–9.
67. McFarland LV, Brandmarker SA, Guandalini S. Pediatric *Clostridium difficile*: a phantom menace or clinical reality? *J Pediatr Gastroenterol Nutr* 2000;**31**:220–31.
68. Mezoff E, Mann EA, Hart KW. *Clostridium difficile* infection and treatment in the pediatric inflammatory bowel disease population. *J Pediatr Gastroenterol Nutr* 2011;**52**:437–41.
69. Mitchell DK, Van R, Mason EH, et al. Prospective study of toxigenic *Clostridium difficile* in children given amoxicillin/clavulanate for otitis media. *Pediatr Infect Dis J* 1996;**15**:514–19.
70. Morinville V, McDonald J. *Clostridium difficile*–associated diarrhea in 200 Canadian children. *Can J Gastroenterol* 2005;**19**:497–501.
71. Morrison RH, Hall NS, Said M, et al. Risk factors associated with complications and mortality in patients with *Clostridium difficile* infection. *Clin Infect Dis* 2011;**53**:1173–8.
72. Muehlhausen VL, Blount BW. *Clostridium difficile* colitis presenting as an acute abdomen: case report and review of the literature. *J Fam Pract* 1997;**44**:97–100.
73. Nusrat A, Eichel-Streiber C, Turner JR, et al. *Clostridium difficile* toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect Immun* 2001;**69**:1329–36.
74. Nyland CM, Goudie A, Garza JM, et al. *Clostridium difficile* infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011;**165**:451–7.
75. Ota KV, McGowan KL. *Clostridium difficile* testing algorithms using glutamate dehydrogenase antigen and *C. difficile* toxin enzyme immunoassays with *C. difficile* nucleic acid amplification testing increase diagnostic yield in a tertiary pediatric population. *J Clin Microbiol* 2012;**50**:1185–8.
76. Pant C, Madonia P, Minocha A. Does PPI therapy predispose to *Clostridium difficile* infection? *Nat Rev Gastroenterol Hepatol* 2009;**6**: 555–7.
77. Pascarella F, Martinelli M, Miele E, et al. Impact of *Clostridium difficile* infection on pediatric inflammatory bowel disease. *J Pediatr* 2009;**154**:854–8.
78. Pillai A, Nelson R. Probiotics for treatment of *Clostridium difficile*–associated colitis in adults. *Cochrane Database Syst Rev* 2008. CD004611.
79. Pokorn M, Radsel A, Cizman M, et al. Severe *Clostridium difficile*–associated disease in children. *Pediatr Infect Dis J* 2008;**27**:944–6.
80. Pothoulakis C. Effects of *Clostridium difficile* toxins on epithelial cell barrier. *Ann N Y Acad Sci* 2000;**915**:347–56.
81. Razzaq R, Sukumar SA. Ultrasound diagnosis of clinically undetected *Clostridium difficile* toxin colitis. *Clin Radiol* 2006;**61**: 446–52.
82. Reddymasu S, Sheth A, Banks DE. Is fecal leukocyte test a good predictor of *Clostridium difficile* associated with diarrhea? *Ann J Clin Microbiol Antimicrob* 2006;**5**:9.
83. Reihner E, Hellers G, Lindqvist L, et al. Pseudomembranous colitis presenting as acute abdomen. *Eur J Surg* 1996;**162**: 579–82.
84. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2011;**30**:580–4.
85. Suh KN, Gravel D, Mulvey MR, et al. *Clostridium difficile*–associated diarrhea in children admitted to acute care hospitals participating in the Canadian Nosocomial Infections Surveillance Program (CNISP), 2004-5 [abstract 306]. In: Program of the 18th Annual Scientific Meeting of the Society of Healthcare Epidemiology of America, Orlando, Fla, 2008.
86. Surawicz CM, McFarland LV, Greenberg RN, et al. The search for a better treatment for recurrent *Clostridium difficile* disease: use of high-dose vancomycin combined with *Saccharomyces boulardii*. *Clin Infect Dis* 2000;**31**:1012–7.
87. Sutphen JL, Grand RJ, Flores A, et al. Chronic diarrhea associated with *Clostridium difficile* in children. *Am J Dis Child* 1983;**137**: 275–8.
88. Svedhem Å, Kaijser B, MacDowall I. Intestinal occurrence of *Campylobacter fetus* subspecies jejuni and *Clostridium difficile* in children in Sweden. *Eur J Clin Microbiol* 1982;**1**:29–32.
89. Tai E, Richardson LC, Townsend J, et al. *Clostridium difficile* infection among children with cancer. *Pediatr Infect Dis J* 2011;**30**:610–2.
90. Talpaert MJ, Rao GG, Cooper BS, et al. Impact of guidelines and enhanced antibiotic stewardship on reducing broad-spectrum antibiotic usage and its effect on the incidence of *Clostridium difficile* infection. *J Antimicrob Chemother* 2011;**66**:2168–74.
91. Tate JE, Cortese MM, Payne DC, et al. Uptake, impact, and effectiveness of rotavirus vaccination in the United States—review of the first 3 years of postlicensure data. *Pediatr Infect Dis J* 2011;**30**:S56–60.
92. Thomas DW, Greer FR. Committee on Nutrition: Section on Gastroenterology, Hepatology and Nutrition, American Academy of Pediatrics. Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**:1217–31.
93. Thompson Jr CM, Gilligan PH, Fisher MC, et al. *Clostridium difficile* cytotoxin in a pediatric population. *Am J Dis Child* 1983;**137**:271–4.
94. Toltzis P, Kim J, Dul M, et al. Presence of epidemic North American pulsed field type I *Clostridium difficile* strain in hospitalized children. *J Pediatr* 2009;**154**:607–8.
95. Toltzis P, Nerandzic MM, Saade E, et al. High proportion of false-positive *Clostridium difficile* enzyme immunoassays for toxin A and B in pediatric patients. *Infect Contr Hosp Epidemiol* 2012;**33**: 175–9.
96. Triadafilopoulos G, Hallstone AE. Acute abdomen as the first presentation of pseudomembranous colitis. *Gastroenterology* 1991; **101**:685–91.

97. Turco R, Martinelli M, Miele E, et al. Proton pump inhibitors as a risk factor for paediatric *Clostridium difficile* infection. *Aliment Pharmacol Ther* 2010;**31**:754–9.
98. Vajravelu RK, Guerrero DM, Jury LA, et al. Evaluation of stethoscopes as vectors of *Clostridium difficile* and methicillin-resistant *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 2012;**33**:96–8.
99. Venugopal AA, Johnson S. Fidaxomicin: a novel macrocyclic antibiotic approved for treatment of *Clostridium difficile* infection. *Clin Infect Dis* 2012;**54**:568–74.
100. Wanahita A, Goldsmith EA, Marino BJ, et al. *Clostridium difficile* infection in patients with unexplained leukocytosis. *Am J Med* 2003;**115**:543–6.
101. Westra-Meijer CM, Degener JE, Dzoljic-Danilovic G, et al. Quantitative study of the aerobic and anaerobic fecal flora in neonatal necrotizing enterocolitis. *Arch Dis Child* 1983;**58**: 523–8.
102. Wilkins TD, Lyerly DM. *Clostridium difficile* testing: after 20 years, still challenging. *J Clin Microbiol* 2003;**41**:531–4.
103. Zilberberg MD, Tillotson GS, McDonald C. *Clostridium difficile* infections among hospitalized children, United States, 1997-2006. *Emerg Infect Dis* 2010;**16**:604–9.

pericarditis) and endocarditis. Histologic examination revealed infiltration of the lamina propria of the small intestine by large, foamy mononuclear cells that did not stain for fat. Fatty acids and triglycerides were found in dilated lymph channels. Silver stains of the mesenteric lymph nodes showed "great numbers of rod-shaped organisms" that resembled the tubercle bacillus. Whipple suggested that these bacillus-like organisms in the nodes could be the cause.

Whipple[91] reported "a hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal and mesenteric lymphatic tissues." He concluded that the patient had "an obscure disease of fat metabolism," and proposed the term *intestinal lipodystrophy*.[21] Whipple recognized the most important features of this disease except for the involvement of the central nervous system (CNS). In 1949, Black-Schaffer showed that macrophages within the intestinal mucosa of patients with Whipple disease are stained intensely by the periodic acid–Schiff (PAS) method,[45] proving that the macrophages contained glycoprotein or mucopolysaccharide, not fat, as Whipple had suggested.

In 1992, Relman and colleagues[71] identified a gram-positive bacillus in association with Whipple disease by use of polymerase chain reaction (PCR). They reported a unique 1321-base-pair, 16S ribosomal RNA sequence amplified by PCR on intestinal and lymph node tissue from five unrelated patients with Whipple disease. They suggested that the responsible bacillus is a member of the Actinomycetes. Relman and colleagues[71] concluded that the phylogenetic relationships of the Whipple disease bacillus, the features of the illness, and its distinct morphologic characteristics provided sufficient grounds to propose a new genus and species name: *Tropheryma whippelii* from the Greek *trophe*, or "nourishment"; *eryma*, or "barrier," because of the malabsorption it causes; and *whippelii* (now *whipplei*), in honor of Whipple.

EPIDEMIOLOGY

Classic Whipple disease characteristically occurs in white middle-aged men. Its true incidence and prevalence are unknown because fewer than 1000 cases have been reported worldwide. It is an extremely rare disease in children,* with fewer than 10 cases reported. The youngest patient was a neonate,[17] and the oldest was 83 years old.[53] The peak age at presentation is 40 to 49 years.[27] In a literature review of 114 patients,[53] 88 percent were men and 12 percent were women. Most of these patients were white. Most patients reported as having Whipple disease are from continental Europe or the United States.[15] In an extensive review of 741 cases, Dobbins[23] found that most academic centers in the United States had records of three or four unreported cases. He estimated that for every published report at least two or three unpublished cases exist and that 1500 to 2000 individuals probably have had Whipple disease.[18]

Although classic Whipple disease is quite rare, the incidence of *T. whipplei*–related infection may be higher than previously thought. Raoult and associates[68] found stool PCR evidence of *T. whipplei* in 36 of 241 (15 percent) children with acute gastroenteritis but in none of 47 control subjects. Thirty-three percent of children with a positive stool PCR also had another identified diarrheal pathogen. The bacterial load in the children's stool was higher than in asymptomatic controls, and more in line with numbers found in classic Whipple disease. These children showed seropositivity with IgM during the acute infection and recovered without treatment within 2 weeks. *T. whipplei* has also been identified in patients with pneumonia[10] and bacteremia.[29]

The prevalence of asymptomatic carriage of *T. whipplei* varies by country and exposure.[28,31,32] For example, in stools, prevalence estimates range from 2 to 4 percent of the general population, 12 percent in sewer workers, and 44 percent in healthy children in Senegal.[28,31,32] *T. whipplei* has been identified in 0.5 to 30 percent of healthy adults in saliva and gingival plaque.[73,77,84] Such studies confirm the existence of asymptomatic carriage.

Etiology and Pathogenesis

Although humans are the only species to display clinical signs of *T. whipplei* infection, they are likely not the only reservoir. In a study of sewage water in Austria, *T. whipplei* was found by PCR in 17 of 46 (37 percent) of samples.[79] Transmission of *T. whipplei* is most likely fecal-oral. Among asymptomatic workers in those plants, 16 of 64 (25 percent) were PCR positive for *T. whipplei* in their stool. This compared to a baseline rate of 7 percent of PCR-positive stools in controls. A study in France identified an asymptomatic control population with PCR-positive *T. whipplei* in 4 percent in their stool.[31] Similar to the Austrian study, sewage workers had a higher carrier rate of 12 percent. The same study did not identify *T. whipplei* in the stool of monkeys or apes.

Classic Whipple disease is caused by an organism known as Whipple bacillus or *T. whipplei*.[27,53,67,91] Despite Whipple's account of "great numbers of rod-shaped organisms," culture of the organism was unsuccessful until more recently.[91] In 2000, Raoult and associates[67] reported that the bacterium *T. whipplei* had been cultured successfully from an aortic valve vegetation in a patient with prolonged endocarditis. The bacteria were isolated after inoculation in a human fibroblast cell line (HEL). Analysis by PCR confirmed that the 16S ribosomal RNA gene of the cultured bacterium was identical to the *T. whipplei* sequence. Subcultures of the bacterium also were obtained, and high-titer polyclonal antibodies against *T. whipplei* were produced. Such antibodies potentially may allow serologic diagnosis to become a reality.

T. whipplei may be a member of the actinomycetales,[71] which are gram-positive bacteria with DNA rich in guanine and cytosine.[92] The genus consists of actinomycetes, streptomycetes, and the nocardioforms.[16] *T. whipplei* seems to be related most closely to the four actinobacteria *Dermatophilus congolensis*, *Arthrobacter globiformis*, *Terrabacter tumescens*, and *Micrococcus luteus*.[71] Using so-called bootstrap analysis, some researchers have argued that the Whipple bacillus is only 67 percent associated with actinobacteria, far from the level needed for

*References 1, 2, 5, 12, 43, 87.

scientific conclusion.[81] The Whipple bacillus may represent another, separate, fourth line of descent with the actinomycetes. Amplification, cloning, and sequencing of a 620-base-pair fragment of *T. whipplei* heat shock protein led to the conclusion that *T. whipplei* is a member of the actinomycetales.[60]

Scant support exists for a primary humoral immunodeficiency in Whipple disease,[36] but stronger evidence exists for a distinct defect in the cell-mediated immune function. Dobbins[22] reviewed data of 30 patients with HLA-A and HLA-B locus typing and 47 patients with HLA-B27 typing. He found an increased incidence of patients who were positive for HLA-B27 (28%), even with absence of concomitant sacroiliitis. Other reports have failed to confirm the increased association with the HLA-B27 antigen.[3] Marth and associates[55] studied 27 patients with Whipple disease. They found a significantly reduced number of cells expressing the complement receptor 3 L-chain (CD11B), a reduced proliferation to phytohemagglutinin and to sheep red blood cells, and a hypoergic skin reaction. These findings indicated a defect of cell-mediated immunity.

In patients with active disease, the number of $CD8^+$ cells is increased, which results in a reduced CD4/CD8 ratio. Such defects of cellular immunity seem to persist in patients for several years, despite complete remission of the disease. Schoeden and associates[78] were able to culture *T. whipplei* in mononuclear phagocytes deactivated with interleukin (IL)-4, IL-10, and dexamethasone. IL-4 was found to be the crucial deactivating signal that rendered monocytes permissive for intracellular multiplication of *T. whipplei*. IL-4 is an immunoregulatory cytokine. Schoeden and associates[78] suggested that host factors, such as an imbalance in the T-helper 1 and T-helper 2 immune response, may contribute to the pathogenesis of Whipple disease.

Desnues and colleagues[19,20] reported a specific gene expression pattern associated with replication of *T. whipplei* in macrophages. *T. whipplei* organisms are killed by monocytes. The addition of exogenous IL-16 enabled *T. whipplei* to replicate in monocytes and increased bacterial replication in macrophages. *T. whipplei* replication in macrophages was completely prevented after blocking IL-16 activity with the use of anti–IL-16 antibodies. Untreated patients with Whipple disease were noted to have significantly higher circulating IL-16 than that of control subjects and patients treated for Whipple disease. They concluded that response of monocytes and macrophages to IL-16 likely is crucial for replication of *T. whipplei* to occur, in patients with Whipple disease.

Oral acquisition of the Whipple bacillus seems most likely, emphasizing greater involvement of the duodenum and proximal jejunum than the more distal small intestine.[27] Only three reports of siblings with this disease exist; contagious spread of Whipple disease seems unlikely.[41] *T. whipplei* has been identified free in the small intestine next to the glycocalyx of the enterocyte's microvilli, in epithelial cells, and in the lamina propria.[27] Even in patients with extraintestinal Whipple disease, the organism usually is identified in the small bowel.[27] The bacillus seems to spread through the lymphatics and through the systemic circulation[27,46,61] and then can involve several extraintestinal organs. *T. whipplei* can be seen faintly by light microscopy. The bacilli are seen best by transmission electron microscopy, which reveals a rod-shaped organism 0.2 μm wide and 1.5 to 2.5 μm long (Fig. 46-1). The ultrastructure of the wall of *T. whipplei* is similar to that of other gram-positive bacteria, with the exception of an additional surface membrane. This membrane is different from the outer membrane of gram-negative bacteria because it is thinner, has a symmetric profile, and has no PAS-positive components.[81] After the bacillus has been ingested by the macrophage, the degenerative process that occurs leads to the accumulation of bacterial remnants that are resistant to degradation. The polysaccharide-containing portion of the bacillus wall correlates with these remnants, and its progressive accumulation leads to the typical intramacrophagic inclusions. These inclusions are PAS positive and one of the key features in the histologic diagnosis of Whipple disease.[7] Biopsy specimens from the small intestine in patients with Whipple disease usually show characteristic changes. The intestinal villi are preserved,[27] but distortion of the architecture occurs.[36] A clubbed appearance of the intestinal villi[53] usually is present caused by the accumulation of foamy macrophages in the lamina propria.[27] The enterocytes may appear normal,[24,53] or they may show flattening and vacuolization and occasionally appear cuboidal (Fig. 46-2).[24,53] Lipid accumulation, with large fat droplets within the lamina propria and smaller droplets within and between the absorptive cells, occurs commonly.[53] In some instances, prominent, dilated lacteals are present.[27] Ectors and associates[24] reported reduced and absent lactase and major histocompatibility complex class II (HLA-DR) expression that normalized within 3 to 6 months of starting antibiotic therapy.

The characteristic feature of Whipple disease is the presence of PAS-positive, diastase-resistant macrophages.[41] These findings are not pathognomonic, however, because intestinal PAS-positive macrophages can be found in other conditions,[27] such as histiocytosis, melanosis coli, and *Mycobacterium avium-intracellulare*

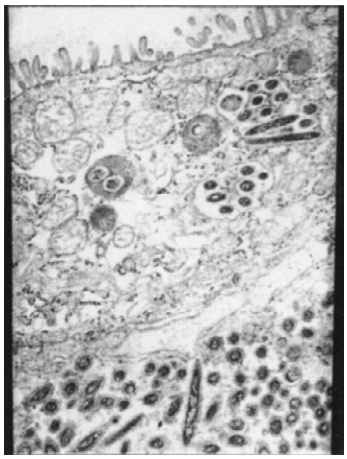

FIGURE 46-1 ■ Electron micrograph of the invasion of enterocytes by Whipple bacilli. There are numerous bacilli within the lamina propria. (×25,000.) (From Tyor MP. Whipple's disease: the Duke connection. *N C Med J* 1994;**55**:237–40.)

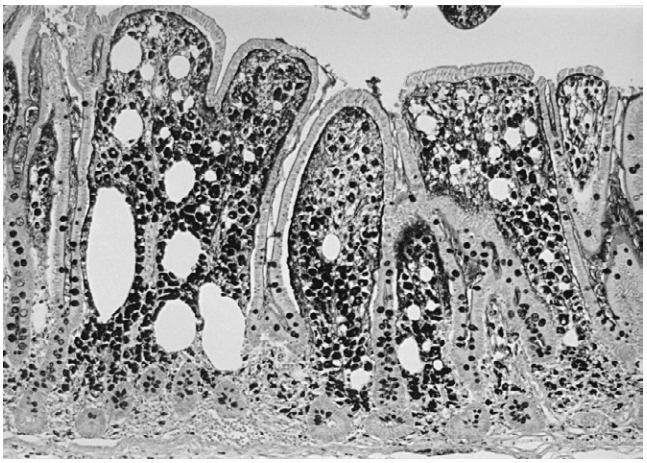

FIGURE 46-2 ■ Light microscopic photograph of intestinal mucosa from the proximal jejunum of a patient with untreated Whipple disease. The villi appear blunted and swollen with periodic acid–Schiff-positive macrophages stuffed in the lamina propria. (Courtesy Kenneth P. Batts, MD, Mayo Clinic, Rochester, MN; Jeffrey Craver, MD, DePaul Health Center, Bridgeton, MO; and Milton J. Finegold, MD, Baylor College of Medicine, Houston, TX.)

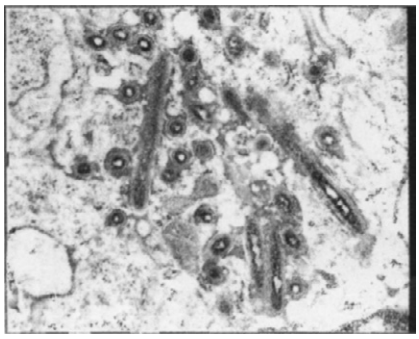

FIGURE 46-3 ■ Electron micrograph illustrating numerous rod-shaped bacilliform bodies within the cytoplasm of a macrophage. (×13,750.) (From Tyor MP. Whipple's disease: the Duke connection. *N C Med J* 1994;**55**:237–40.)

infection, and even within macrophages in healthy individuals. The macrophages in Whipple disease do not stain with Ziehl-Neelsen.[24,41] A sickle-shaped appearance of the PAS-positive granules often is present in the macrophages of patients with Whipple disease.[53]

Sieracki and Fine[80] observed systemic involvement in the autopsies of five patients with Whipple disease. The sickle-shaped, PAS-positive macrophages were thought to be specific for Whipple disease, showing involvement of the entire gastrointestinal tract and the pancreas; diffuse involvement of the retroperitoneum and lymph nodes, the adrenals, the liver with sickle-form particles in Kupffer cells and in histiocytes, the brain, the heart, and the visceral pleura of the lungs; and minimal involvement of the genitourinary tract, skeletal muscles, and bone marrow. James and associates[46] examined the vessels of the gastrointestinal system and found abundant bacilli in the arteries of the small intestinal serosa and liver. They noted focal degeneration and fibrosis in the tunica media with arteritis and intimal proliferation. Rickman and coworkers[60] reported a case that confirmed the presence of *T. whipplei* in the vitreous of the eye.

T. whipplei produces a predominantly histiocytic inflammatory reaction, with infiltration by macrophages.[26] Noncaseating, epithelioid cell, sarcoid-like granulomas are located preferentially in peripheral lymph nodes and the liver.[18,26] These granulomas occasionally can be seen in different tissues, including three reports of granulomas in the intestinal tract.[26] Mesenteric lymph nodes often are strikingly enlarged.[15]

Electron microscopy can be useful, often revealing the presence of the rod-shaped bacterium (see Fig. 46-1).[28] Electron microscopy also shows intestinal macrophages containing bacteria with signs of lysis.[81] Silva and associates[81] described steps of a degradative process of the bacillus that starts with disorganization of the surface membrane and the thick outer wall. With the loss of intracellular material, bacterial ghosts composed of the three inner layers of the envelope are present. The two electron-dense layers of the cytoplasmic membrane become disorganized and solubilized, leaving the inner dense layer of the cell wall as the final bacterial remnant (Fig. 46-3).

TABLE 46-1 Clinical Presentations of *T. whipplei* Infection

Acute infection
Gastroenteritis
Bacteremia
Pneumonia
Asymptomatic carriage
"Classic" Whipple disease

Data from references 1-3, 12, and 13.

CLINICAL MANIFESTATIONS

The manifestations of *T. whipplei* infections now include at least three forms, "classic" Whipple disease; acute infection such as gastroenteritis and bacteremia; and asymptomatic carriage (Table 46-1).[19,20]

Acute Infection

Gastroenteritis

T. whipplei is associated with a mild acute gastroenteritis in young children.[68] Diarrhea typically lasts from 4 to 5 days and is associated with weight loss and 1 to 2 days of fever. Additional side effects include vomiting (55%), abdominal pain (25%), and bloody diarrhea (8%). The infection has no seasonality.

Bacteremia

In rural West Africa, *T. whipplei* was found by PCR in the blood of adults and children with unexplained fever.[29] Patients with *T. whipplei* bacteremia also have associated cough and sleep disorders. The bacteremia has seasonality, occurring primarily during the months of December

and January. Fevers are not commonly associated with gastrointestinal symptoms, and patients rarely have PCR-positive stool or saliva.

Pneumonia

Severe cases of pneumonia in adults and children are associated with *T. whipplei*.[10,44] PCR has detected *T. whipplei* in the bronchoalveolar lavage of mechanically ventilated patients in intensive care units. Initial presentations include aspiration pneumonia, community-acquired pneumonia, and ventilator-associated pneumonia. Duration of mechanical ventilation ranges from 3 to 81 days, and the mortality rate is high (33 percent).

"Classic" Whipple Disease

Whipple disease is viewed best as a multisystem illness. It usually manifests as arthralgias and then progresses to involve the gastrointestinal tract.[6] Malabsorption is the key feature of clinical disease, but no specific signs or symptoms for Whipple disease are known. The major symptoms and signs of Whipple disease are listed in Table 46-2. In children, failure to thrive, malnutrition, and chronic diarrhea appear most frequently. Abdominal distention, abdominal pain, and generalized lymphadenopathy may be found.[2] Response to antibiotic treatment may be dramatic, with rapid weight gain and resolution of symptoms.[5]

Gastrointestinal Tract

One of the most common symptoms is weight loss, which is found in 65 to 100 percent of patients.[15,25,27,37,53] Weight loss may be the only symptom.[12] Diarrhea is reported in 60 to 85 percent of patients.[15,27,37,53] The diarrhea usually is watery or fatty.[53] Several mechanisms have been proposed to explain the malabsorption and steatorrhea in Whipple disease.[27] Direct infection and secondary enterocyte dysfunction may prevent the esterification of fatty acids to triglycerides and inhibit the uptake of carbohydrates and amino acids. Blockage of transport of triglyceride-rich chylomicrons into lacteals may result from the deposition of foamy macrophages in the lamina propria. Lymphatic obstruction may occur by involvement of the mesenteric lymph nodes. Malabsorption and diarrhea tend to resolve within a few days after initiation of antibiotic treatment, whereas the lacteal dilation and PAS-positive macrophages can remain for months to years. Occult gastrointestinal bleeding frequently is found, but melena and gross gastrointestinal bleeding are rare. Hematemesis has been reported.[13] Endoscopy revealed diffuse hemorrhagic duodenitis with bleeding on contact.

Abdominal pain is experienced by 60 percent of patients.[15,27,37,53] The pain is nonspecific, is generally epigastric, and may be worse after meals.[53] Abdominal pain and anorexia may lead to reduced calorie intake and further weight loss.[27] Abdominal distention occurs commonly and may be secondary to intra-abdominal lymphadenopathy or to thickening of loops of diseased intestine.[27,53] Ascites occasionally is seen and may be chylous, secondary to lymphatic obstruction.[12,27]

TABLE 46-2 **Major Symptoms and Signs in Whipple Disease**

	Cases (%)
Symptoms	
Weight loss	65-100
Chronic diarrhea	60-85
Arthralgia	65-80
Abdominal pain	60
Fever	10-55
Central nervous system–related complaints	10-40
Signs	
Malnutrition	90-95
Hypotension	70
Lymphadenopathy	55
Hyperpigmentation	45-55
Abdominal tenderness	50
Edema	30
Abdominal mass	20
Hepatomegaly	1-14
Splenomegaly	5-10
Ascites	8

Data from references 12, 26, 27, 45, and 48.

Joints

Arthralgia, the most frequent nongastrointestinal symptom in Whipple disease,[27,53] is present in 65 percent of adult cases.[53] Arthralgia may precede manifestation of gastrointestinal symptoms by many years or decades[27,49] and occurs less frequently in children. Generally, joint symptoms continue unchanged with the onset of gastrointestinal symptoms.[27] Acute migratory arthralgia or arthritis may last days or weeks[37,41,53] and may persist as the disease progresses. The involved joints, in decreasing order of frequency, are knees, ankles, hips, fingers, wrists, elbows, hands, and spine.[27] Examination may reveal joint pain, swelling, limited range of motion, and warmth.[76] Fever sometimes is present.[27] Spondylitis, with or without sacroiliitis, may develop.[5] Permanent joint destruction and deformity are uncommon occurrences but can be severe.[27,76] Arthrocentesis may reveal an inflammatory arthritis, with cell counts of 6000 to 75,000/mm^3, often with a polymorphonuclear leukocyte predominance.[27] Synovial biopsy may show PAS-positive macrophages.[27]

Central Nervous System

Whipple disease can be confined to the brain[74] but usually is accompanied by other manifestations.[37] CNS and neurologic manifestations, such as headache, diplopia, meningoencephalitis, depression, confusion, and personality changes, are uncommon manifestations[15,27,53] but may be significant.* The spectrum of potential CNS involvement is listed in Table 46-3.

*References 15, 27, 36, 53, 62, 64.

TABLE 46-3 **Central Nervous System Symptoms and Signs of Whipple Disease**

Ataxia
Confusion
Convulsions
Dementia
Depression
Diplopia
Dizziness
Facial pain
Headache
Hearing loss
Hemiparesis
Hyperphagia
Hyperreflexia (± Babinski sign)
Incoordination
Lethargy, coma
Loss of vibratory and position sense
Meningoencephalitis
Mental and personality changes
Motor weakness
Muscle jerks and twitches
Muscle rigidity
Numbness
Nystagmus
Ophthalmoplegia
Papilledema
Polydipsia
Ptosis
Pupillary abnormalities
Sensory loss
Sleep disorders
Slurred speech
Stiff neck
Tinnitus
Visual difficulties (diplopia, blurring)

Eye

Indirect involvement of the CNS and direct involvement of the eye can produce visual problems.[27] Ophthalmoplegia and diplopia can occur with involvement of cranial nerves III, IV, and VI.[24] Reduced visual acuity and papilledema can occur with compromise of the optic nerve.[27] Numerous ophthalmologic findings, including vasculitis, vitritis, optic atrophy, uveitis, chorioretinitis, vitreous opacities, glaucoma, keratitis, retinal hemorrhages, disk edema, and lacrimal duct obstruction, have been reported.[27,89,90] Rickman and associates[72] reported a case of ocular disease without marked CNS or gastrointestinal disease.

Skin

Hyperpigmentation of the skin in sun-exposed areas occurs in roughly half of cases.[36,53] The mechanism of hyperpigmentation is uncertain but is not related to adrenal insufficiency.[27] Subcutaneous nodules may be found and can reveal PAS-positive macrophages and bacilli on electron microscopy.[40]

Heart

The heart frequently is involved in Whipple disease. Endocarditis, myocarditis, pericarditis, pancarditis, and coronary arteritis have been found.[27,57,88] A blood culture–negative endocarditis may be caused by Whipple disease.[27] Chronic aortic regurgitation is the most common clinical finding of endocardial involvement.[27] Pericarditis with polyserositis (pleuritis, peritonitis) has been found.[35] The abnormalities on electrocardiogram are nonspecific but include first-degree atrioventricular block, left ventricular hypertrophy, sinus tachycardia, left bundle branch block, intraventricular conduction delay, old inferior wall infarct, and short PR interval.[36] Sossai and associates[83] reported a case of regression of a right bundle branch block after treatment with antibiotics, but the block spontaneously recurred 2 years later.

Skeletal Muscle

Skeletal muscle may be involved, diagnosed by electromyography or muscle biopsy.[85] Proximal muscle weakness occurs most commonly. Muscle biopsy reveals a nonspecific myopathy.

Lymph Nodes and Spleen

Whipple disease may involve any lymph node in the body.[27] Mesenteric lymph nodes frequently are involved, and splenomegaly is found in 5 to 10 percent of cases.[53]

Lungs

Cough, pleuritic chest pain, and dyspnea have been reported.[86] Pleural involvement may appear as a pleural rub. Pleural adhesions and granulomas have been reported at autopsy.[5,86] Chest radiography often reveals pleural thickening, parenchymal shadowing, and elevation of the diaphragm.[86] Lung function tests may show decreased lung volumes.[86]

Kidney

Renal involvement in Whipple disease is rare.[27] Granulomas have been found, however, as has focal glomerulonephritis.[48]

Blood

Anemia occurs commonly and usually is of a hypochromic, microcytic variety.[27,36,37] Macrocytic anemia caused by malabsorption of folate also may be seen. Leukocytosis, thrombocytosis,[63] and bone marrow involvement may be found.[69] Low serum iron concentration and an elevated erythrocyte sedimentation rate frequently are seen.[36] A single case of extraintestinal lymphoma in association with Whipple disease found at autopsy has been reported.[39]

DIAGNOSIS

Despite the successful culture of the organism in 2000,[67] no specific diagnostic tests have been developed. Anemia and low albumin level probably are most common, being found in approximately 90 percent of patients. Low serum iron and folate concentrations are seen in approximately 30 percent of cases. Hypokalemia, hypocalcemia, low cholesterol and carotene, prolonged prothrombin time, and increased transaminase levels are less common findings. Elevated fecal fat is found in more than 90 percent of cases. The D-xylose malabsorption test result frequently is abnormal.

Barium x-ray studies of the small bowel may show marked thickening of the mucosal folds and separation of bowel loops, suggesting a malabsorptive disease. These findings usually resolve completely with successful antibiotic therapy. For CNS Whipple disease, characteristic abnormalities seen on computed tomography of the brain include atrophy, focal gray matter lesions, hydrocephalus, and white matter alterations.[87]

A key feature in the histologic diagnosis of Whipple disease is the accumulation of periodic acid–Schiff-positive, diastase-resistant macrophages in the lamina propria of the small intestine.[5,36,54] These findings are not pathognomonic, and infection with *M. avium-intracellulare* must be excluded.[54] *T. whipplei* does not stain acid-fast,[54] whereas mycobacteria are identified readily. Small bowel involvement by *M. avium* complex can have endoscopic, histologic, and radiographic findings similar to those of Whipple disease.[66] Periodic acid–Schiff-positive macrophages occasionally can be seen in the mucosa of the large bowel and rectum in unrelated diseases, such as histiocytosis, and in benign conditions, such as melanosis coli and pneumatosis intestinalis.[54] Although electron microscopy can confirm the diagnosis by visualization of the characteristic bacillus,[37,81] immunohistochemistry has shown to be not only more efficient, but also more sensitive and specific than periodic acid–Schiff in the diagnosis and monitoring of *T. whipplei* infection.[4,50] Using a polyclonal rabbit antibody produced against *T. whipplei*, Baisden and associates[4] were the first to show sensitivity equal to periodic acid–Schiff without cross reactivity with other bacterial species.

Endoscopy of the small bowel may be helpful because characteristic lesions may be seen and biopsy specimens can be obtained. No clear data suggest where the intestinal specimens should be taken[54] because intestinal involvement usually is patchy. Random biopsy samples of the small bowel are suggested, beginning at the ligament of Treitz.[58] Endoscopic findings include yellow-white plaques and an erythematous, erosive, friable mucosa.[38]

PCR amplification may be used to detect and identify bacterial pathogens. To set up a PCR, the only prerequisite information is the nucleotide sequences flanking each end of the target.[70] PCR has become an important method for establishing the diagnosis of Whipple disease. It may be especially helpful if the histopathologic examination findings are normal or in patients with unusual extraintestinal involvement. Specific identification of *T. whipplei* can be achieved by amplification of the 1321-base-pair bacterial, 16S ribosomal RNA gene isolated from infected tissue.[71] Muller and associates[61] applied this PCR technique to show *T. whipplei* in peripheral blood mononuclear cells and cells derived from pleural effusion in a patient with Whipple disease. PCR techniques have been used to detect *T. whipplei* in erythrocytes,[52] cerebrospinal fluid,[7] and resected heart valves with infective endocarditis.[12,14,61]

Sloan and colleagues[82] reported another real-time PCR method using a 213-base-pair target sequence of the heat shock protein *(hsp65)* gene of *T. whipplei*. The sensitivity, specificity, and positive and negative values of this LightCycler real-time PCR were compared with the conventional 16S rRNA gene sequence PCR assay and were 98 percent (sensitivity), 99 percent (specificity), 94 percent (positive), and 100 percent (negative). The completion time of the LightCycler assay was approximately 5 hours, which was significantly less than the 2 to 3 days required for the traditional PCR assay.[82]

The diagnostic utility of PCR in Whipple disease is clear, but it cannot be taken as the sole basis for diagnosis. Ehrbar and associates[26] studied the specificity of PCR for *T. whipplei*. They performed elective gastroscopy on patients without known Whipple disease. PCR analysis was positive in 4.8 percent of duodenal biopsy specimens and 11.4 percent of gastric juice samples. Compared with the gold standard of histology and clinical signs, the specificity of PCR for *T. whipplei* was 95.2 percent for duodenal biopsy specimens and 88.6 percent for gastric juice. These findings suggest that *T. whipplei* or a closely related bacterium may be present in some people without known Whipple disease. Street and colleagues[84] detected *T. whipplei* DNA by PCR in the saliva of 35 percent of 40 healthy adults tested, which suggests that this organism can be an oral commensal.

In a study by Fenollar and associates[28], when real-time PCR detection of *T. whipplei* in saliva and stool were combined, the positive predictive value of the patient having Whipple disease was 95.2 percent. Additionally, if the bacterial load in the stool was more than 10^4 CFU/g, the positive predictive value increased to 100 percent. Conversely, classic Whipple disease was unlikely in patients with saliva or stool specimens negative for *T. whipplei* by real-time PCR, having a negative predictive value of 99.2 percent. The authors argue that following these negative results, additional specific investigations for classic Whipple disease could be stopped. Localized Whipple disease was poorly detected by PCR of the saliva and stool samples, with a sensitivity of only 58 percent.

TREATMENT

Antibiotics are the mainstay of therapy for Whipple disease.[15] In vitro antibiotic susceptibility testing using real-time PCR has identified penicillin, doxycycline, macrolides, ketolides, rifampin, aminoglycosides, teicoplanin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) with activity.[8,9,56] Current antibiotic treatment strategies are empirical, based only on the accumulated anecdotal experience. Generally, response to antibiotics is good and often dramatic. Symptoms such as diarrhea quickly resolve, and weight gain is rapid. Several

TABLE 46-4 **Treatment of Whipple Disease: Initial Antibiotic Regimen and Relapse**

Antibiotics	No. Patients	Total No. Relapses	CNS Relapses
TCN* alone	49	21	9†
PCN + STM + TCN*	15	2	0
PCN/PCN*	8	3	2
PCN + STM	5	2	0
TMP-SMX*	3	0	0
Other	8	3	2
Totals	*88*	*31*	*13*

*Oral therapy.
†Includes 2 patients treated with TCN only in whom CNS relapse was the second relapse.
Data from Keinath RD, Merrell DE, Vlietstra R, et al. Antibiotic treatment and relapse in Whipple's disease: long-term follow-up of 88 patients. *Gastroenterology* 1985;**88**:1867–73.
CNS, central nervous system; PCN, penicillin; STM, streptomycin; TCN, tetracycline; TMP-SMX, trimethoprim-sulfamethoxazole.

antibiotics that have been tried alone or in combination include chloramphenicol, penicillin, streptomycin, ampicillin, TMP-SMX, erythromycin, and doxycycline.[15,27,34,37] Keinath and associates[47] analyzed the antibiotic response rate of 88 patients with documented Whipple disease. Thirty-one patients experienced relapse, with a mean time to relapse of 4.2 years after initial diagnosis. CNS relapse occurred in 13 of 88, and all CNS and cardiac relapses were late (Table 46-4). In addition to diagnosis, PCR may prove useful in the monitoring of response to antibiotic therapy.[42,65] Disease relapse may be secondary to genetic mutations in *T. whipplei*. Fenollar and associates[30] discovered amino acid changes in the *T. whipplei folP* gene, which encodes dihydropteroate synthase, the target of sulfonamides. Two amino acid positions, N4S and S234F, significantly predicted treatment failure to sulfamethoxazole.

All patients with Whipple disease should receive antibiotics that penetrate the blood-brain barrier.[47,75] Tetracycline and penicillin do not penetrate the blood-brain barrier well, unless the meninges are inflamed.[75] The preferred treatment in adults and children is TMP-SMX given orally twice a day for 1 year.[75] If the small bowel is involved, repeated small bowel biopsy is suggested at least 6 months to 1 year after treatment to document the disappearance of the bacillus. For patients who do not tolerate TMP-SMX, penicillin or ampicillin is recommended. Levy and associates[51] described a case of acquired resistance to TMP-SMX that responded to oral penicillin. Feurle and Marth[34] noted that TMP-SMX was more efficacious than tetracycline in inducing clinical remission of Whipple disease. They observed the development of aqueductal stenosis with hydrocephalus in a patient receiving TMP-SMX treatment, however, and indicated that even TMP-SMX is no safeguard against cerebral recurrence.

Treatment with IV antibiotics before a course of TMP-SMX may improve remission rates. In a randomized control trial, Feurle and associates[33] studied the use of ceftriaxone or meropenem IV for 2 weeks before initiating a 1-year course of TMP-SMX. At a 3-year follow-up, all 38 subjects, except for 1 with asymptomatic CNS infection with *T. whipplei*, had clinical and biopsy evidence of disease remission.

CNS relapse has a poor prognosis.[11,27,34,37,47] Feurle and Marth[34] suggested an experimental therapy for CNS recurrence with a highly active bactericidal compound, such as a third-generation cephalosporin, a quinolone, or intrathecal antibiotic therapy, that readily crosses the blood-brain barrier. In adult patients, Keinath and associates[47] recommended treatment with parenteral penicillin 1.2 million units daily plus streptomycin 1 g daily for 10 to 14 days, followed by TMP-SMX one double-strength tablet twice daily for 1 year. Because of the extreme rarity of the occurrence of Whipple disease in children, prudent management dictates long-term surveillance after resolution of symptoms.

Because Whipple disease is a malabsorptive disorder, the patient's nutritional needs must be assessed carefully. Specific attention must be paid to replacement of any vitamin or mineral deficiencies. Iron, folate, vitamin D, and calcium typically are given until the steatorrhea resolves.

CONCLUSION

T. whipplei appears ubiquitous in our environment but appears to only rarely cause disease. It is involved in not only classic Whipple disease but also acute infection and asymptomatic carriage. These findings suggest a genetic predisposition to classic Whipple disease, but causative genes or immune factors have not yet been identified.

NEW REFERENCES SINCE THE SIXTH EDITION

10. Bousbia S, Papazian L, Auffray JP, et al. *Tropheryma whipplei* in patients with pneumonia. *Emerg Infect Dis* 2010;**16**:258–63.
19. Desnues B, Al Moussawi K, Fenollar F. New insights into Whipple's disease and *Tropheryma whipplei* infections. *Microbes Infect* 2010;**12**:1102–10.
28. Fenollar F, Laouira S, Lepidi H, et al. Value of *Tropheryma whipplei* quantitative polymerase chain reaction assay for the diagnosis of Whipple disease: usefulness of saliva and stool specimens for first-line screening. *Clin Infect Dis* 2008;**47**:659–67.
29. Fenollar F, Mediannikov O, Socolovschi C, et al. *Tropheryma whipplei* bacteremia during fever in rural West Africa. *Clin Infect Dis* 2010;**51**:515–21.
30. Fenollar F, Rolain JM, Alric L, et al. Resistance to trimethoprim/sulfamethoxazole and *Tropheryma whipplei*. *Int J Antimicrob Agents* 2009;**34**:255–9.
31. Fenollar F, Trani M, Davoust B, et al. Prevalence of asymptomatic *Tropheryma whipplei* carriage among humans and nonhuman primates. *J Infect Dis* 2008;**197**:880–7.
32. Fenollar F, Trape JF, Bassene H, et al. *Tropheryma whipplei* in fecal samples from children, Senegal. *Emerg Infect Dis* 2009;**15**:922–4.
33. Feurle GE, Junga NS, Marth T. Efficacy of ceftriaxone or meropenem as initial therapies in Whipple's disease. *Gastroenterology* 2010;**138**:478–86; quiz 11–12.
50. Lepidi H, Fenollar F, Gerolami R, et al. Whipple's disease: immunospecific and quantitative immunohistochemical study of intestinal biopsy specimens. *Hum Pathol* 2003;**34**:589–96.
68. Raoult D, Fenollar F, Rolain JM, et al. *Tropheryma whipplei* in children with gastroenteritis. *Emerg Infect Dis* 2010;**16**:776–82.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ament ME. Malabsorption syndromes in infancy and childhood. *J Pediatr* 1972;**81**:867–84.
2. Aust CH, Smith EB. Whipple's disease in a 3-month-old infant. *Am J Clin Pathol* 1962;**37**:66–74.
3. Bai JC, Mota AH, Maurino E, et al. Class I and class II HLA antigens in a homogeneous Argentinian population with Whipple's disease: lack of association with HLA-B27. *Am J Gastroenterol* 1991;**86**:992–4.
4. Baisden BL, Lepidi H, Raoult D, et al. Diagnosis of Whipple disease by immunohistochemical analysis: a sensitive and specific method for the detection of *Tropheryma whipplei* (the Whipple bacillus) in paraffin-embedded tissue. *Am J Clin Pathol* 2002;**118**: 742–8.
5. Barakat AY, Bitar J, Nassar VH. Whipple's disease in a seven-year-old child: report of a case. *Am J Proctol* 1973;**24**:312–15.
6. Bayless TM, Knox DL. Whipple's disease: a multisystemic infection. *N Engl J Med* 1979;**300**:920.
7. Birch CA. Whipple's disease: George Hoyt Whipple (born 1878). *Practitioner* 1974;**212**(1270 Spec. No.):581–2.
8. Boulos A, Rolain JM, Mallett MN, Raoult D. Molecular evaluation of antibiotic susceptibility of *Tropheryma whipplei* in axenic medium. *J Antimicrob Chemother* 2005;**55**:178.
9. Boulos A, Rolain JM, Raoult D. Antibiotic susceptibility of *Tropheryma whipplei* in MRC5 cells. *Antimicrob Agents Chemother* 2004;**48**:7477.
10. Bousbia S, Papazian L, Auffray JP, et al. *Tropheryma whipplei* in patients with pneumonia. *Emerg Infect Dis* 2010;**16**:258–63.
11. Brandle M, Ammann P, Spinas G, et al. Relapsing Whipple's disease presenting with hypopituitarism. *Clin Endocrinol* 1999;**50**: 399–403.
12. Bruni R, Massimo L. Descrizione un rarissimo caso di mallatia de Whipple nel eta pediatrica. *Minerva Pediatr* 1959;**11**:935–43.
13. Carney PW, Murugasu A, Duggan AE. Whipple's disease: a rare cause of haematemesis and melaena. *Eur J Gastroenterol Hepatol* 2005;**17**:779.
14. Celard M, De Gevigney G, Mosnier S, et al. Polymerase chain reaction analysis for the diagnosis of *Tropheryma whippelii* infective endocarditis in two patients with no previous evidence of Whipple's disease. *Clin Infect Dis* 1999;**29**:1348–9.
15. Comer GM, Brandt LJ, Abissi CJ. Whipple's disease: a review. *Am J Gastroenterol* 1983;**78**:107–14.
16. Couper R. Whipple's disease: the bacillus unmasked. *J Pediatr Gastroenterol Nutr* 1993;**17**:339–44.
17. DePra M, Casagrande A, Guarino M. Whipple's disease: apropos of a case demonstrating the existence of the congenital form. *Minerva Pediatr* 1974;**26**:1723–43.
18. Desmet VJ, Geboes K. Review article: liver lesions in inflammatory bowel disorders. *J Pathol* 1987;**151**:247–55.
19. Desnues B, Al Moussawi K, Fenollar F. New insights into Whipple's disease and *Tropheryma whipplei* infections. *Microbes Infect* 2010;**12**:1102–10.
20. Desnues B, Raoult D, Mege JL. IL-16 is critical for *Tropheryma whipplei* replication in Whipple's disease. *J Immunol* 2005;**175**: 4575–82.
21. Dobbins III WO. Whipple's disease: an historical perspective. *Q J Med* 1985;**56**:523–31.
22. Dobbins III WO. HLA antigens in Whipple's disease. *Arthritis Rheum* 1987;**30**:102–5.
23. Dobbins III WO. Whipple's disease. *Mayo Clin Proc* 1988;**63**: 623–4.
24. Ectors NL, Geboes KJ, DeVos RM, et al. Whipple's disease: a histological, immunocytochemical and electron microscopic study of the small intestinal epithelium. *J Pathol* 1994;**172**:73–9.
25. Ectors N, Geboes K, Wynants P, et al. Granulomatous gastritis and Whipple's disease. *Am J Gastroenterol* 1992;**87**:509–15.
26. Ehrbar H, Bauerfeind P, Dutly F, et al. PCR-positive tests for *Tropheryma whippelii* in patients without Whipple's disease. *Lancet* 1999;**353**:2214.
27. Feldman M. Southern Internal Medicine Conference: Whipple's disease. *Am J Med Sci* 1986;**291**:56–7.
28. Fenollar F, Laouira S, Lepidi H, et al. Value of *Tropheryma whipplei* quantitative polymerase chain reaction assay for the diagnosis of Whipple disease: usefulness of saliva and stool specimens for first-line screening. *Clin Infect Dis* 2008;**47**:659–67.
29. Fenollar F, Mediannikov O, Socolovschi C, et al. *Tropheryma whipplei* bacteremia during fever in rural West Africa. *Clin Infect Dis* 2010;**51**:515–21.
30. Fenollar F, Rolain JM, Alric L, et al. Resistance to trimethoprim/sulfamethoxazole and *Tropheryma whipplei*. *Int J Antimicrob Agents* 2009;**34**:255–9.
31. Fenollar F, Trani M, Davoust B, et al. Prevalence of asymptomatic *Tropheryma whipplei* carriage among humans and nonhuman primates. *J Infect Dis* 2008;**197**:880–7.
32. Fenollar F, Trape JF, Bassene H, et al. *Tropheryma whipplei* in fecal samples from children, Senegal. *Emerg Infect Dis* 2009;**15**: 922–4.
33. Feurle GE, Junga NS, Marth T. Efficacy of ceftriaxone or meropenem as initial therapies in Whipple's disease. *Gastroenterology* 2010;**138**:478–86; quiz 11–12.
34. Feurle GE, Marth T. An evaluation of antimicrobial treatment for Whipple's disease: tetracycline versus trimethoprim-sulfamethoxazole. *Dig Dis Sci* 1995;**39**:1642–8.
35. Finch W. Arthritis and the gut. *Postgrad Med* 1989;**86**:229–35.
36. Flemin JL, Wiesner RH, Shorter RG. Whipple's disease: clinical, biochemical and histopathologic features and assessment of treatment in 29 patients. *Mayo Clin Proc* 1988;**63**:539–51.
37. Gaist D, Ladefoged K. Whipple's disease. *Scand J Gastroenterol* 1994;**29**:97–101.
38. Geboes K, Ectors N, Heidbuchel H, et al. Whipple's disease: endoscopic aspects before and after therapy. *Gastrointest Endosc* 1990;**36**:247–52.
39. Gillen CD, Coddington R, Montieth PG, et al. Extraintestinal lymphoma in association with Whipple's disease. *Gut* 1993;**34**: 1627–9.
40. Good AE, Beals TF, Simmons JL, et al. A subcutaneous nodule with Whipple's disease: key to early diagnosis? *Arthritis Rheum* 1980;**23**:856–9.
41. Gran JT, Husby G. Joint manifestations in gastrointestinal diseases. *Dig Dis* 1992;**10**:295–312.
42. Gras E, Matias O, Guiu X, et al. PCR analysis in the pathological diagnosis of Whipple's disease: emphasis on the extraintestinal involvement or atypical morphological features. *J Pathol* 1999; **188**:318–21.
43. Hamilton JR. Whipple disease. In: Behrman RE, Vaughn V, Nelson, WE, editors. *Textbook of pediatrics*. Philadelphia: W.B. Saunders; 1983. p. 935.
44. Harris JK, De Groote MA, Sagel SD, et al. Molecular identification of bacteria in bronchoalveolar lavage fluid from children with cystic fibrosis. *Proc Natl Acad Sci U S A* 2007;**104**:20529–33.
45. Harvey AM. Teacher and distinguished pupil: William Henry Welch and George Hoyt Whipple. *Johns Hopkins Med J* 1974; **135**:178–90.
46. James TN, Bulkley BH, Kent SP. Case report: vascular lesions of the gastrointestinal system in Whipple's disease. *Am J Med Sci* 1984;**288**:125–9.
47. Keinath RD, Merrell DE, Vlietstra R, et al. Antibiotic treatment and relapse in Whipple's disease: long-term follow-up of 88 patients. *Gastroenterology* 1985;**88**:1867–73.
48. Kraunz RF. Whipple's disease with cardiac and renal abnormalities. *Arch Intern Med* 1969;**123**:701–6.
49. Leirisalo-Repo M. Enteropathic arthritis, Whipple's disease, juvenile spondyloarthropathy and uveitis. *Curr Opin Rheumatol* 1994; **6**:385–90.
50. Lepidi H, Fenollar F, Gerolami R, et al. Whipple's disease: immunospecific and quantitative immunohistochemical study of intestinal biopsy specimens. *Hum Pathol* 2003;**34**:589–96.
51. Levy M, Poyart C, Lamarque D, et al. Whipple's disease: acquired resistance to trimethoprim-sulfamethoxazole. *Am J Gastroenterol* 2000;**95**:2390–1.
52. Lowsky R, Archer GL, Fyles G, et al. Brief report: diagnosis of Whipple's disease by molecular analysis of peripheral blood. *N Engl J Med* 1994;**331**:1343–6.
53. Maizel H, Ruffin JM, Dobbins III WO. Whipple's disease: a review of 19 patients from one hospital and a review of the literature since 1950. *Medicine (Baltimore)* 1970;**49**:175–205.
54. Marbet VA, Stalder GA, Gyr KE. Whipple's disease: a multisystemic disease with changing presentation. *Dig Dis* 1986;**4**: 119–28.
55. Marth T, Roux M, VonHerbay A, et al. Persistent reduction of complement receptor 3 L-chain expressing mononuclear blood

cells and transient inhibitory serum factors in Whipple's disease. *Clin Immunol Immunopathol* 1994;**72**:217–26.
56. Masselot F, Boulos A, Maurin M, et al. Molecular evaluation of antibiotic susceptibility: *Tropheryma whipplei* paradigm. *Antimicrob Agents Chemother* 2003;**47**:1658.
57. McAllister HA, Fenoglio JJ. Cardiac involvement in Whipple's disease. *Circulation* 1975;**52**:152–6.
58. Moorthy S, Nolley G, Hermos JA. Whipple's disease with minimal intestinal involvement. *Gut* 1977;**18**:152–5.
59. Morgan AD. The first recorded case of Whipple's disease. *Gut* 1961;**2**:370–2.
60. Morgenegg S, Dutly F, Altwegg M. Cloning and sequencing of a part of the heat shock protein 65 gene (hsp 65) of *Tropheryma whippelii* and its use for detection in clinical specimens by PDR. *J Clin Microbiol* 2000;**38**:2248–53.
61. Muller C, Stain C, Burghuber O. *Tropheryma whippelii* in peripheral blood mononuclear cells and cells of pleural effusion. *Lancet* 1993;**341**:701.
62. Naegeli B, Bannwart F, Bertel O. An uncommon cause of recurrent strokes: *Tropheryma whippelii* endocarditis. *Stroke* 2000;**31**:2002–3.
63. Nuzum CT, Sandler RS, Paulk HT. Thrombocytosis in Whipple's disease. *Gastroenterology* 1981;**80**:1465–7.
64. Ojeda E, Redondo J, Lapaza J, et al. Enfermedad de Whipple: manifestaciones neurologicas y revision de los casos publicados en la literatura nacional. *Rev Clin Esp* 1981;**183**:1465–7.
65. Petrides P, Muller-Hocker J, Fredricks D, et al. PCR analysis of *T. whippelii* DNA in a case of Whipple's disease: effect of antibiotics and correlation with histology. *Am J Gastroenterol* 1998;**93**: 1579–82.
66. Poorman JC, Katon RM. Small bowel involvement by *Mycobacterium avium* complex in a patient with AIDS: endoscopic, histologic and radiographic similarities to Whipple's disease. *N Engl J Med* 2000;**342**:620–5.
67. Raoult D, Birg ML, Scola BL, et al. Cultivation of the bacillus of Whipple's disease. *N Engl J Med* 2000;**342**:620–5.
68. Raoult D, Fenollar F, Rolain JM, et al. *Tropheryma whipplei* in children with gastroenteritis. *Emerg Infect Dis* 2010;**16**:776–82.
69. Rausing A. Bone marrow biopsy in diagnosis of Whipple's disease. *Acta Med Scand* 1973;**193**:5–8.
70. Relman DA. Whipple's disease: a disorder associated with a visible but uncultured bacillus. *J Infect Dis* 1993;**168**:1–8.
71. Relman DA, Schmidt TM, MacDermott RP, et al. Identification of the uncultured bacillus of Whipple's disease. *N Engl J Med* 1992;**327**:293–300.
72. Rickman LS, Freeman WR, Green WR, et al. Brief report: uveitis caused by *Tropheryma whippelii* (Whipple's bacillus). *N Engl J Med* 1995;**332**:363–6.
73. Rolain JM, Fenollar F, Raoult D. False positive PCR detection of *Tropheryma whipplei* in the saliva of healthy people. *BMC Microbiol* 2007;**7**:48.
74. Romanul FCA, Radvany J, Rosales RK. Whipple's disease confined to the brain: a case studied clinically and pathologically. *J Neurol Neurosurg Psychiatry* 1977;**40**:901–9.
75. Ryser RJ, Locksley RM, Eng SC, et al. Reversal of dementia associated with Whipple's disease by trimethoprim-sulfamethoxazole: drugs that penetrate the blood-brain barrier. *Gastroenterology* 1984; **86**:745–52.
76. Scheib JS, Quinet RJ. Whipple's disease with axial and peripheral joint destruction. *South Med J* 1990;**83**:684–7.
77. Schneemann M, Schoedon G. Whipple's DNA is not Whipple's disease. *J Clin Microbiol* 2005;**43**:5414.
78. Schoeden G, Goldenberger D, Forrer R, et al. Deactivation of macrophages with interleukin-4 is the key to the isolation of *Tropheryma whippelii*. *J Infect Dis* 1997;**176**:672–7.
79. Schoniger-Hekele M, Petermann D, Weber B, Muller C. *Tropheryma whipplei* in the environment: survey of sewage plant influxes and sewage plant workers. *Appl Environ Microbiol* 2007; **73**:2033–5.
80. Sieracki JC, Fine G. Whipple's disease: observations on systemic involvement, part II: Gross and histologic observations. *Arch Pathol* 1959;**67**:81–93.
81. Silva MT, Macedo PM, Moura Nunes JF. Ultrastructure of bacilli and the bacillary origin of the macrophagic inclusions in Whipple's disease. *J Gen Microbiol* 1985;**131**:1001–13.
82. Sloan LM, Rosenblatt JE, Cockerill III FJ. Detection of *Tropheryma whipplei* DNA in clinical specimens by LightCycler Real-Time PCR. *J Clin Microbiol* 2005;**43**:3516–8.
83. Sossai P, DeBoni M, Cielo R. The heart and Whipple's disease. *Int J Cardiol* 1989;**23**:275–6.
84. Street S, Donoghue HD, Neild GH. *Tropheryma whippelii* DNA in saliva of healthy people. *Lancet* 1999;**354**:1178–9.
85. Swash M, Schwartz MS, Vandenberg MJ, et al. Myopathy in Whipple's disease. *Gut* 1977;**18**:800–4.
86. Symmons DPN, Shepard AN, Boardman PL, et al. Pulmonary manifestations of Whipple's disease. *Q J Med* 1985;**56**:497–504.
87. Tan T, Vogel H, Tharp BR, et al. Presumed central nervous system Whipple's disease in a child: case report. *Clin Infect Dis* 1995; **20**:883–9.
88. Tyor MP. Whipple's disease: the Duke connection. *N C Med J* 1994;**55**:237–40.
89. Vine AK. Retinal vasculitis. *Semin Neurol* 1994;**14**:354–60.
90. von Weizsacker F, Blum HE. Impact of molecular biology in gastroenterology. *Digestion* 1993;**54**:125–9.
91. Whipple GH. A hitherto undescribed disease characterized anatomically by deposits of fat and fatty acids in the intestinal mesenteric lymphatic tissues. *Johns Hopkins Hosp Bull* 1907;**18**: 382–91.
92. Woese CR. Bacterial evolution. *Microbiol Rev* 1987;**51**:221–71.

SECTION **VII**

LIVER DISEASES

CHAPTER 47

Hepatitis

Daniel H. Leung • Leon L. Chen

Hepatitis, or inflammation of the liver, may be caused by a variety of infectious agents. Some agents, such as hepatitis A, B, C, D, and E viruses, are primarily hepatotropic and produce disease almost exclusively in the liver, whereas other agents, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), adenovirus, and the hemorrhagic fever viruses, produce hepatitis as part of a systemic or disseminated illness. With many infectious agents, hepatitis may be subclinical or silent, with very few, if any, signs or symptoms manifested in the patient. Symptomatic or clinical hepatitis may be acute or chronic at initial evaluation, and it may be associated with mild to even fulminant or fatal disease.[78] Children of all ages, both immunocompetent and immunocompromised, may contract infectious hepatitis, and the age and immune status of the host, as well as the history of exposure, may provide clues to identification of the most likely etiologic agent. Noninfectious causes such as autoimmune, storage, and metabolic disorders, exposure to toxins, or hypersensitivity reactions to medications or drugs are also important causes of hepatitis in children. Hepatic steatosis associated with childhood obesity and type 2 diabetes is a rapidly growing public health concern for our youth. Vasculitis, cardiac disease, hypoxic injury, or trauma also may damage the liver.

A general approach to an infant or child with hepatitis or hepatic dysfunction, from an infectious perspective, is presented in this chapter. Detailed information regarding the diagnosis and management of each particular pathogen can be found in the respective chapters in the section of this textbook dedicated to infections with specific microorganisms.

HISTORY

Originally described by Hippocrates in the second century BC, hepatitis has an ancient historical perspective. The earliest descriptions of outbreaks of hepatitis in the ancient world most likely involved hepatitis A virus (HAV), and it was known as epidemic *jaundice* and *catarrhal jaundice* or *acute yellow atrophy of the liver* if the disease was severe or fulminant.[25] The earliest recorded outbreak in the United States occurred in Norfolk, Virginia, in 1812.[11] During wartimes, outbreaks of "camp jaundice" or "field jaundice" occurred and probably were caused by HAV, yellow fever, or leptospirosis. Not until 1973 was the cause of "infectious hepatitis" determined to be a 27-nm, nonenveloped viral particle, originally designated enterovirus 72, now known as HAV.[45] Infection with hepatitis B virus (HBV) has a relatively more recent history. It probably was described originally in the late 1880s in an epidemiologic study published by Luerman in 1885 in Germany, where an outbreak of hepatitis in shipyard and warehouse workers who received smallpox vaccine contaminated with human material was associated with a prolonged form of hepatitis.[88] Other outbreaks of "serum hepatitis" associated with percutaneous therapies, such as gold used for rheumatoid arthritis or contaminated vaccines that were stabilized with human serum have been described.[1,14,69] In the early 1970s, the complete HBV infectious particle identified in serum was called the *Dane particle*. A specific serologic marker, originally called the *Australian (Au) antigen* (because it was discovered first in the blood of an Australian aborigine) and later became what is now known as hepatitis B surface antigen (HBsAg), was characterized and subsequently used to diagnose HBV.[16,87,130] Not long thereafter, in the mid 1970s, parenterally transmitted "non-A, non-B hepatitis" was described and subsequently identified in 1989 as hepatitis C virus (HCV).[23,70,131,152] The 1970s also marked the discovery of hepatitis delta virus (HDV), a "defective helper virus" that replicates only in the presence of HBV and is associated with chronic or fulminant hepatitis.[135,136] In the 1990s, another enterically transmitted non-A, non-B hepatitis virus was found to be associated with outbreaks of infectious hepatitis in developing parts of the world.[12,59] It was characterized originally as a calicivirus-like particle and subsequently named hepatitis E virus (HEV).[134] No doubt, as our medical knowledge evolves, even more viruses and other infectious agents associated with hepatitis will be discovered and named. Antiviral treatment of viral hepatitis is also now routine, and with the new millennium we also have entered an antiviral era for specific genomic targeted treatment of hepatitis B and C viruses.[38,54]

CLINICAL MANIFESTATIONS AND EVALUATION

Patient History

The clinical approach to evaluating and managing a child with hepatitis includes careful attention to the age of presentation; evolution of the initial signs and symptoms; history of exposure to potential pathogens, toxins, or medications; the presence of underlying conditions; and a family history of intravenous drug use or liver or metabolic disorders. Presenting symptoms in older children with acute hepatitis are nonspecific and may include fever, vomiting, anorexia or aversion to specific foods, change in taste and smell, lethargy and malaise, weight loss, dark urine, pale stool, jaundice, icterus, or even

pruritus. Abdominal pain is a common complaint, and it usually is mild, dull, or aching; located in the right upper quadrant; and unaffected by meals, body position, or defecation. Often, an antecedent viral syndrome or "flulike" illness is noted 7 to 14 days before the onset of hepatitis. Rarely, a "serum sickness–like syndrome" may occur at the onset of the illness, before jaundice occurs, and is characterized by fever, rash, and arthritis. In contrast, neonates rarely have signs and symptoms from infection with hepatotropic viruses and are more likely to have clinical disease with agents that cause congenital or perinatal infection, such as CMV, herpes simplex virus (HSV), rubella virus, parvovirus B19, or *Treponema pallidum*, especially if extrahepatic signs such as splenomegaly, skin lesions, microcephaly, or hearing loss are present. Neonates and young infants often become seriously ill with disseminated HSV, enteroviruses, or adenoviruses and show signs of acute, massive hepatocellular necrosis. These signs can include irritability or lethargy, jaundice, ascites, abdominal distention, hepatomegaly, acholic stools, anasarca, and coagulopathy. Patients from developing countries with a personal or maternal history of blood transfusions should be screened for HBV, HCV, and HDV and CMV, whereas previous attendance at summer camp or an institutional environment might warrant HAV screening. Recent treatment with antifungal azoles, antibiotics (macrolides, cyclines, isoniazid), antiepileptics, or even hepatotoxic over-the-counter medications such as acetaminophen may suggest drug-induced hepatitis.[133] Exposure of older children to feral kittens can be suggestive of infection with *Bartonella henselae* or *Toxoplasma gondii*. Children receiving cancer chemotherapy may have drug-induced hepatitis, and those who have experienced prolonged neutropenia may have fungal disease of the liver. Children with fulminant hepatitis are critically ill and initially may have persistent fever, protracted nausea and vomiting, severe abdominal pain, worsening jaundice, fluid retention with ascites, easy bruising, bleeding, and encephalopathy with seizures or coma. Patients with chronic hepatitis, on the other hand, often are clinically asymptomatic unless complications such as cirrhosis or chronic liver failure develop.

Physical Findings

Physical examination of a child with hepatitis should focus on the abdomen, liver, and spleen but also should include a careful evaluation for extrahepatic manifestations of systemic disease. Tender hepatomegaly, with or without ascites, scleral icterus, and jaundice, often is noted on physical examination of patients with acute hepatitis. Percussion over the right lower part of the thorax may produce right upper quadrant pain. Fever may or may not be present. Extrahepatic physical findings associated with the hepatotropic viruses, especially HAV, HBV, and HCV, include systemic vasculitis with rash or urticaria, polyarthralgia, and polyarthritis, similar to serum sickness or polyarteritis nodosa. A generalized papular rash, known as *Gianotti disease*, may accompany HBV infection, especially in young children. Rarely, a patient with acute viral hepatitis may exhibit Raynaud phenomenon, bullous lesions, or erythema nodosa on the extensor surfaces. Skin excoriations may be present if jaundice-associated pruritus is severe, and older children and adolescents may have vascular spiders or exacerbation of acne. A child who also has conjunctivitis, pneumonitis, and a maculopapular rash may have disseminated adenoviral disease. Generalized lymphadenopathy accompanied by pharyngitis and splenomegaly, in contrast, suggests systemic infection with CMV or EBV. Idiosyncratic reactions involving the liver may occur at any time; however, drug-related hypersensitivity hepatitis occurs 1 to 5 weeks after exposure to the agent and usually is accompanied by rash and fever on examination and evidence of nephritis, eosinophilia, and neutropenia. Neonates with acute fulminant hepatitis from disseminated HSV, enteroviruses, or adenoviruses may have fever or hypothermia, respiratory distress, abdominal distention, coagulopathy, myocarditis, seizure-like activity, or a sepsis-like syndrome. Physical examination of a child with fulminant hepatitis and liver failure may actually reveal a normal or small liver with new-onset irritability, confusion, lethargy, or personality changes. As hepatic failure ensues, the patient may become deeply jaundiced, icteric, and encephalopathic with hyperreflexia, decerebrate posturing, involuntary movements, and asterixis or may even become comatose. A distinctive sweet, fecal smell (also called *fetor hepaticus*) likely from ammonia, mercaptans, and ketones shunted from the liver to the lungs of a patient also may be appreciated by an astute observer. Physical examination of a child with chronic hepatitis, on the other hand, may reveal a normal or enlarged liver with splenomegaly. Splenomegaly is often the hallmark of portal hypertension related to chronic liver disease. If the child is obese, fatty infiltration of the liver should be considered as a cause of liver inflammation and dysfunction.

Laboratory Diagnosis

Laboratory evaluation of a child with acute hepatitis should include general blood and urine chemistries (complete blood cell count with differential, electrolytes, blood urea nitrogen, creatinine, urinalysis), tests of hepatic function (albumin, glucose, ammonia, coagulopathy panel, factors V and VII), and tests of hepatic inflammation/obstruction (serum aspartate aminotransferase and alanine aminotransferase [AST/ALT], alkaline phosphatase, γ-glutamyltranspeptidase, bilirubin). Serologic tests (i.e., HBsAg, HCV Ab), cultures, or detection assays (i.e., HCV RNA quantitative polymerase chain reaction [PCR]) for specific pathogens of interest according to the patient's screening laboratory results, history, or physical findings should also be pursued. Neonates with elevated aminotransferase levels should be tested for enterovirus and herpes simplex virus with viral cultures of secretions and virus-specific blood and spinal fluid PCR tests to assess for viremia and meningoencephalitis.

In many forms of viral hepatitis, the white blood cell count may be low, usually between 3000 and 4000/mm^3. Atypical lymphocytes also may be seen. Eosinophilia suggests a drug hypersensitivity reaction. If significant

leukocytosis is present, sepsis or fulminant hepatitis should be considered. A rising creatinine value may indicate development of hepatorenal syndrome. Urinalysis may reveal dark urine and the presence of urobilinogen. Hematuria with casts or other signs of nephritis in an older child may signify an autoimmune disorder or a drug-induced process as the cause of the hepatitis.

The hepatic aminotransferases, including AST and ALT, will be elevated in patients with acute hepatitis, often before the onset of clinical symptoms occurs. Most forms of acute hepatitis will be accompanied by elevations of 500 IU/mL or greater. A neonate with aminotransferase levels higher than 1000 IU/mL may have a potentially life-threatening infection with bacterial sepsis, HSV, enterovirus, or adenovirus. Similarly, older children with fulminant hepatitis or hepatic necrosis may have significantly elevated aminotransferase levels. These values may begin to fall after reaching a pinnacle; however, if the γ-glutamyltranspeptidase or conjugated bilirubin level continues to rise, this may indicate disease progression with massive hepatocyte necrosis rather than improvement. Prothrombin time, international normalized ratio, albumin, and glucose levels usually are normal in cases of uncomplicated acute viral hepatitis, but if coagulation factors become prolonged and hypoalbuminemia and hypoglycemia persist, fulminant hepatitis should be considered. Direct markers of hepatic synthetic function such as factors V and VII will also be low in the setting of liver failure. Serum bilirubin, both conjugated and unconjugated, may be elevated, but it is rarely higher than 4 mg/dL unless fulminant hepatitis with hepatic failure is present. Exceptions to this rule include patients with underlying hemolytic states such as glucose-6-dehydrogenase deficiency or sickle-cell disease; such patients may exhibit marked jaundice and high indirect hyperbilirubinemia even if the viral hepatitis otherwise is mild. Some patients will have low levels of nonspecific autoantibodies, such as an elevated homogeneous pattern of antinuclear antibodies, decreased complement levels, or false-positive Venereal Disease Research Laboratory (VDRL) test reactions. The erythrocyte sedimentation rate usually is normal or slightly increased in acute viral hepatitis.

Laboratory investigation to determine the specific cause of the patient's hepatitis includes an evaluation for the hepatotropic viruses. This often includes detection of serum hepatitis B surface antigen (HBsAg) and hepatitis B core antibody (HBcAb; IgM), serum hepatitis A virus antibody (HAV Ab; IgM), serum hepatitis C antibody (HCV Ab), serum hepatitis D antibody (HDV Ab) especially if the hepatitis is fulminant, and hepatitis E antibody (HEV Ab) if the travel history suggests exposure).[3,4] Both HBV and HCV have qualitative and quantitative PCR testing. Many of the nonhepatitis viruses may be identified by serologic tests that detect virus-specific IgM antibody or by fourfold rises in viral-specific IgG antibody. However, isolation of the specific viral agent in cell culture or detection of viral nucleic acid by PCR in blood, cerebrospinal fluid, body fluids such as saliva or stool, or liver tissue provides the most convincing evidence. Bacterial pathogens may be detected by culture for agents associated with granulomatous hepatitis, such as *Brucella* and *Mycobacterium*, or by a variety of culture, serology, or appropriate skin tests. In neonates, gram-negative enteric organisms (e.g., *Escherichia coli*) identified from the blood or urine are common causes of jaundice and hepatic dysfunction. Noninfectious causes of hepatitis should also be considered. Autoimmune hepatitis may be identified by persistent hypergammaglobulinemia and the presence of autoantibodies, such as F-actin and anti–liver-kidney-microsome antibodies (anti-LKM). Laboratory tests that evaluate for metabolic diseases include serum amino acids and urine-reducing substances and organic acids, sweat chloride testing for cystic fibrosis, α_1-antitrypsin levels and pi typing, serum ceruloplasmin and 24-hour urine collection for copper to rule out Wilson disease, and an iron panel for hemochromatosis. Acute hepatotoxicity from overdose of acetaminophen may be predicted from elevated acetaminophen levels. A urine or serum toxicology screen can assess for other toxic substances. Anatomic causes of hepatic dysfunction, such as biliary atresia in infants and hepatic tumors or steatosis in older children, usually require diagnostic imaging such as ultrasonography or liver biopsy for diagnosis.

INFECTIOUS CAUSES

Viruses

Hepatitis Viruses

Five hepatotropic viruses are known to cause infectious viral hepatitis in children: hepatitis A, B, C, D, and E (Table 47-1).

Hepatitis A Virus. HAV is a member of the Picornaviridae and formerly was known as enterovirus 72.[93] A small, nonenveloped RNA virus with icosahedral symmetry (Fig. 47-1), HAV is transmitted by the fecal-oral route, and transplacental transmission has been documented on rare occasion.[31,50] It is transmitted most commonly among young and school-aged children receiving care in group settings such as daycare, summer camp, schools, and institutions.[72] The incubation period generally is 30 days but ranges from 15 to 50 days. HAV infection in young children often is asymptomatic, and usually outbreaks in children are recognized first when symptoms occur in adult caretakers. Older children are more likely to have the classic symptoms of nausea, malaise, jaundice, and tender hepatomegaly. Rarely, HAV causes fulminant hepatitis. Acute infection with HAV is diagnosed serologically by detecting the presence of HAV IgM antibody in serum. No licensed, specific antiviral treatment is available. However, prevention may be accomplished by passive immunization with immune globulin or active immunization with licensed inactivated HAV vaccines.

Hepatitis B Virus. HBV, also known as *Dane particle* and *hepadnavirus type 1*, is a member of the Hepadnaviridae and is a DNA virus that is a 42-nm, nonenveloped spherical particle (Fig. 47-2).[93] There are eight known genotypes, of which A through D are the most predominant in the United States. It is transmitted through close

TABLE 47-1 **Causes of Hepatitis in Children**

Infectious

Viral

Primary hepatotropic
- Hepatitis A virus
- Hepatitis B virus
- Hepatitis C virus
- Hepatitis D virus
- Hepatitis E virus

DNA viruses
- Adenovirus
- Cytomegalovirus
- Epstein-Barr virus
- Erythrovirus (human parvovirus B-19)
- Herpes B virus
- Herpes simplex viruses 1 and 2
- Human herpesviruses 6, 7, and 8
- Varicella-zoster virus

RNA viruses
- Enteroviruses
- Hemorrhagic fever viruses
- Human immunodeficiency virus
- Measles virus
- Rubella virus
- Syncytial giant-cell hepatitis

Bacterial

Atypical mycobacteria

Bacille Calmette-Guérin (BCG)

Bacillus cereus toxin

Bartonella henselae and *Bartonella quintana*

Brucella species

Listeria monocytogenes

Mycobacterium tuberculosis

Sepsis syndrome with cholestatic jaundice

Urinary tract infection in neonates

Spirochetes
- Leptospira species
- *Treponema pallidum*

Rickettsiae
- *Coxiella burnetii*

Parasites

Ascaris lumbricoides

Entamoeba histolytica

Plasmodium species

Toxoplasma gondii

Fungal

Aspergillus species

Candida species

Cryptococcus neoformans

Histoplasma capsulatum

Noninfectious

Anoxic liver damage

Autoimmune hepatitis

Biliary atresia

Drugs and toxins

Hemophagocytic syndrome

Histiocytosis

Kawasaki disease

Lymphoma

Metabolic and genetic disorders

Obesity with hepatic steatosis (fatty infiltration)

Reye syndrome

Sarcoidosis

Sickle-cell crisis

Toxic shock syndrome

Tumors

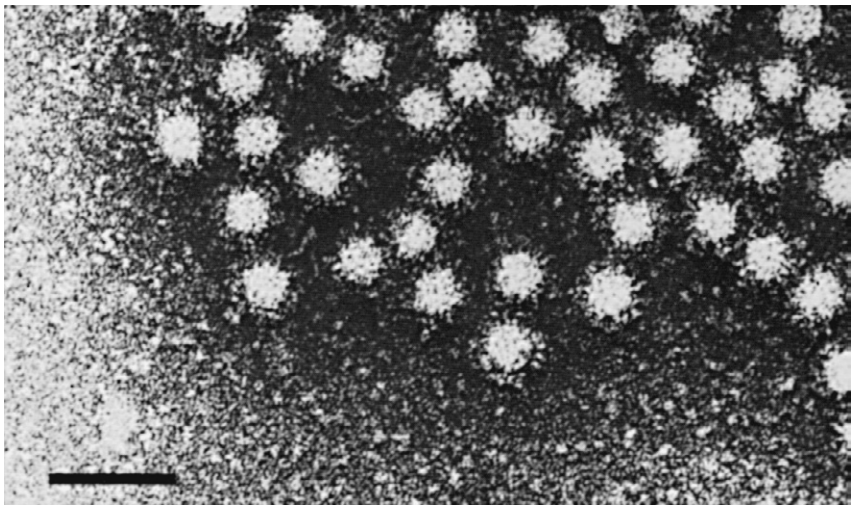

FIGURE 47-1 ■ A 27-nm hepatitis A virus (HAV) isolated from the stool filtrate of a patient with acute HAV infection. The HAV particles are aggregated by convalescent serum containing anti-HAV antibodies. The *line* represents 100 nm. (Courtesy of Dr. Jules Dienstag, taken at the Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health, Education, and Welfare, Bethesda, MD.)

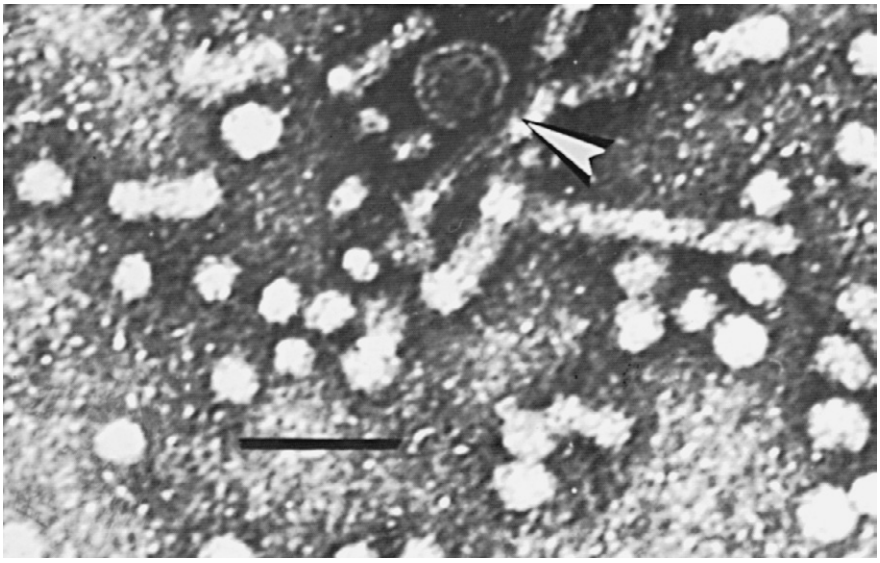

FIGURE 47-2 ■ Electron micrograph of hepatitis B virus particles. Most particles are 20 to 25 nm in diameter and consist of both spheres and tubules. A larger, 42-nm Dane particle also is present *(arrowhead)*. Hepatitis B surface antigen determinants are present on the surface of all three forms.

contact with blood or blood-contaminated secretions, objects, or products.[50] It also has been transmitted by organ transplantation, intravenous drug use, and sexual contact. The incubation period is prolonged, usually 90 days, with ranges of 45 to 160 days reported. Neonates may acquire HBV vertically from mothers who are actively infected with HBV, especially if the mothers also are positive for hepatitis B e antigen (HBeAg). Whereas neonates and toddlers rarely are symptomatic, older children and adults may have mild to moderate symptoms of acute hepatitis or progress to fulminant or fatal disease. HBV also is a common cause of chronic hepatitis in children. Chronic hepatitis will develop in approximately 10 percent of older children and adults infected with HBV; the figure rises to approximately 30 percent if the infection occurs during infancy or early childhood and reaches a striking 90 to 95 percent in infants born to infected mothers who are HBeAg positive. Acute hepatitis B is diagnosed serologically by the presence of HBsAg and HBcAb, which can identify infected patients in the window period. HBV DNA also may be detected and quantified in serum by PCR during acute hepatitis. Individuals with HBeAg in their serum are highly infectious. Diagnosis of chronic hepatitis B requires a combination of persistent clinical symptoms and laboratory abnormalities, persistence of specific serologic markers such as HBsAg over a period of at least 6 months, and histopathologic characteristics on liver biopsy. Primary hepatocellular carcinoma also is a long-term complication of infection with HBV. Successful resolution of infection with HBV is marked serologically by the presence of antibody to HBsAg, known as HBsAb in the nomenclature. For patients with chronic hepatitis B, the decision to treat is largely based on ALT level, HBV viral load, and liver histology.[68] First-line therapy in children with compensated disease remains interferon-α.[77] Three nucleos(t)ide analogs are also age approved for use. Of these, lamivudine and adefovir can be used in younger children, although due to a high rate of resistance even after one year of treatment, these are not recommended; however, entecavir has been shown to have higher potency as well as lower resistance rates, and while it has a product indication in children older than 16 years, it is not yet FDA approved.[22,77,154] Tenofovir is now a first-line therapy in adults but is currently limited to use as therapy for HIV infection in children.[163] Both entecavir and tenofovir are awaiting results of phase III trials in children and will likely soon be first-line options for children. Evidence also suggests that therapeutic response to these medications may vary by HBV genotype.[38] Liver transplantation has been attempted in patients with end-stage HBV-associated cirrhosis, with variable results. Prevention is achieved by passive immunization with hepatitis B immune globulin (HBIG) in the delivery room and active immunization with licensed recombinant vaccines.[57] Pregnant women with chronic hepatitis B should have their HBV viral load and HBeAg status determined to assess their risk for vertical transmission. All infants born to infected women should receive hepatitis B immunoglobulin (HBIG) at delivery and a vaccination within 12 hours of birth. However, if there is high risk for transmission, lamivudine or telbivudine may be administered to the mother during pregnancy.[60,118,119]

Hepatitis C Virus. HCV is a member of the Flaviviridae. It is a small RNA virus that is highly heterogeneous. Eleven genotypes with several distinct subtypes have been identified throughout the world and appear to have a variable effect on the development of clinical disease and response to antiviral therapy. It is transmitted most commonly to children and adolescents who have been exposed to blood products, clotting factor concentrates before 1987, hemodialysis, organ transplantation, intravenous drug use, cocaine snorting, or tattoos and piercing procedures performed at parlors that do not practice sterile technique. Perinatal transmission from an HCV-seropositive mother to her infant also occurs at an estimated risk of 5 percent, especially if the mother is HCV RNA positive at delivery.[109,114] Infection with HCV usually does not cause acute hepatitis; however, chronic hepatitis develops in more than 50 percent of children infected with HCV.[28] In adults, cirrhosis, end-stage liver

failure, and hepatocellular carcinoma also may develop. Infection with HCV is diagnosed serologically by detecting the presence of HCV antibody and confirming with HCV RNA quantification by PCR.[83] Use of peginterferon-α2b (PegIntron) plus ribavirin is considered first-line therapy in children. Achieving a sustained virologic response, defined as being HCV RNA negative 6 months after discontinuation of treatment, is the standard of care. The rates of achieving a sustained virologic response with treatment in children with genotypes 2 and 3 are much higher (75% to 80%) than in children with genotype 1 (35% to 40%).[144,160] Treatment response, and even spontaneous clearance without treatment, seem to be strongly influenced by a genetic polymorphism near the *IL28B* gene.[54,141] Directly acting antiviral agents, such as the protease inhibitors telaprevir and boceprevir, have significantly increased sustained virologic response rates (by nearly twofold) in adults when used in combination with peginterferon-α2b plus ribavirin and were approved in 2011.[75,128] Pediatric studies will soon be underway. Liver transplantation may be performed in patients with end-stage liver disease. Currently, no biologic products or vaccines are licensed for prevention of infection with HCV. The heterogeneity of HCV has hindered the development of such products because vaccine antigens from multiple serotypes will probably be necessary for global protection.

Hepatitis D Virus. HDV, also known as the *delta agent*, *delta virus*, *helper virus*, and *defective virus*, is a small, 37-nm RNA virus that requires the presence of HBV, especially HBsAg, to replicate.[127] HDV can coinfect a patient simultaneously or subsequent to infection with HBV. Like HBV, HDV is acquired by exposure to blood products or clotting factors, intravenous drug use, or sexual contact. It also may be transmitted by liver transplantation, and vertical transmission has been reported. Infection with HDV occurs more commonly in Europe, South America, Africa, and the Middle East and appears to be less common in the United States. The incubation period after superinfection occurs is 1 to 2 months, but it is similar to that for HBV (90 days) if coinfection occurs simultaneously. Coinfection with HDV is associated with more severe disease or progression to fulminant hepatitis in HBV-infected patients.[127] Laboratory diagnosis includes detection of HDV antibody (IgG, IgM) and HDV RNA by PCR, although this is not commercially available. Treatment with peginterferon-α2b results in sustained HDV clearance in about one fourth of patients.[71,156] Because HDV cannot be transmitted to or infect humans without HBV, prevention of HBV infection by vaccination prevents the acquisition of HDV infection. HDV coinfection of individuals already infected with HBV, however, cannot be prevented by any licensed biologic product or vaccine.

Hepatitis E Virus. HEV is a small RNA virus that is transmitted by the fecal-oral route.[12,120,129,134] HEV infection in U.S. residents is a rare occurrence, but it may be a common cause of acute, self-limited viral hepatitis in developing countries and has been linked to outbreaks associated with contaminated water supplies.[52,101,108] The incubation period is an average of 6 to 7 weeks.[2] HEV appears to cause a mild to moderate, acute hepatitis, especially in adults, and may have a high case-fatality rate in pregnant women. Chronic hepatitis is rare but has been seen in immunosuppressed patients.[2] Laboratory diagnosis is established by detection of IgG and IgM antibody to HEV in serum or detection of HEV RNA by PCR in serum or feces performed in reference laboratories or at the Centers for Disease Control and Prevention (CDC). No antiviral agent is licensed for HEV, although peginterferon-α2b has been shown to achieve a sustained virologic response. Two candidate vaccines have achieved 95 to 100 percent protective efficacy, but neither has been licensed for marketing.[2]

Herpesviruses

Herpesviruses are large DNA viruses with icosahedral symmetry and a glycoprotein envelope, and they share the biologic properties of latency and reactivation. Infections with this family of viruses, the Herpesviridae, may be primary or recurrent. All the human herpesviruses (HHVs) and one of the primate herpesviruses (herpes B virus) can cause acute hepatitis during the course of a systemic illness, but primary hepatitis with these viral agents is an unusual event.[48]

Herpes Simplex Virus. HSV-1 and HSV-2 most often cause mucocutaneous vesicles or ulcers, and dissemination usually occurs during periods of relative immune compromise, such as pregnancy, the neonatal period, malnutrition, congenital or acquired immunodeficiency syndromes, or organ transplantation. Transient, subclinical hepatitis may occur during acute mucocutaneous HSV disease, but fulminant hepatitis with hepatic necrosis rarely has been documented in a normal host.[44,51,79,98] A special exception to this observation is HSV-associated primary hepatic necrosis and disseminated HSV disease in pregnant women.[51] Most cases occur during the late second or early third trimester, and most, but not all, cases are associated with primary infection with HSV-2.[159,162] Obvious skin lesions may not be present. This disease is associated with high mortality rates in both the mother and infant. Neonates, both term and preterm, are at risk for the development of neonatal HSV hepatitis as part of a disseminated HSV disease that includes viral sepsis–like syndrome, coagulopathy, abdominal distention with hepatomegaly and ascites, pneumonitis with respiratory distress, and meningoencephalitis.[19,74] Skin lesions often are absent in this form of HSV disease. Neonatal HSV hepatitis develops most often in the child's first 2 weeks of life, and aminotransferase levels may initially be slightly elevated, then rise to be more than a thousand times higher than normal as disease progresses. Recipients of solid organ, bone marrow, and stem cell transplants may have HSV infection with dissemination within the first 3 weeks after undergoing transplantation, most often as a result of reactivation.[49] Patients with hepatitis secondary to HSV infection may be shedding HSV from a mucocutaneous source or may be viremic with virus detected in the blood by DNA PCR. Some patients may require analysis of ascites fluid or liver

biopsy for confirmation. Acyclovir therapy is recommended for HSV-associated hepatitis, and post-transplant prophylaxis is very effective in preventing post-transplant HSV disease.[159] Children with HSV fulminant hepatitis have significantly better survival rates than adults before and after liver transplantation.[36,102]

Varicella-Zoster Virus. Varicella-zoster virus also causes mucocutaneous vesicles in both immunocompetent and immunocompromised hosts. Primary infection is known as *varicella* (also chickenpox) and is associated with primary and secondary viremias that often seed the visceral organs. Approximately a fourth of healthy children experiencing varicella will have silent hepatitis with aminotransferase levels at least twice normal.[47,108,126] Fulminant hepatitis with varicella is rare and generally is seen in immunocompromised hosts.[6,105,121,147] Patients have severe abdominal pain with little nausea or vomiting, skin lesions may or may not be present, and aminotransferase levels may be more than a thousand times higher than normal.[104,140,143] Zoster in a normal host is not associated with fulminant hepatitis, but immunocompromised hosts may experience disseminated zoster with hepatitis and hepatic necrosis.[46] The diagnosis often is based on clinical findings, but viral culture or direct fluorescent antibody testing of cutaneous lesions, if present, or viral culture or viral DNA detection by PCR of blood and liver biopsy also may help establish the diagnosis. Treatment with acyclovir is recommended. Varicella may be prevented or attenuated by passive immunization with varicella-zoster immune globulin (VZIG) or intravenous immunoglobulin (IVIG) or by active immunization with a licensed live virus vaccine.[20,29]

Cytomegalovirus. CMV infection usually is asymptomatic or "silent." However, CMV may cause gastroenteritis, pneumonitis, or a mononucleosis-like syndrome that consists of fever, lymphadenopathy, and atypical lymphocytosis. Hepatitis occurs as part of these syndromes but often is silent or mild and rarely is accompanied by jaundice.[81,97] Granulomatous hepatitis also may be associated with CMV.[32] Infants born congenitally infected with CMV frequently have hepatosplenomegaly, elevated aminotransferase levels, and conjugated hyperbilirubinemia.[32,33,153] The hepatitis that neonates and infants experience is self-limited and generally resolves within the first few months of life. If hepatitis with cholestasis persists, other diseases such as extrahepatic biliary atresia should be considered. Solid organ and marrow transplant recipients and patients with acquired immunodeficiency syndrome (AIDS) and other immunodeficiency states may experience persistent fever, malaise, leukopenia, and hepatitis caused by primary or recurrent infection with CMV. Severe liver disease can occur in transplant recipients and may be associated with graft-versus-host disease or graft rejection.[41,42] CMV-associated hepatitis may be diagnosed clinically and supported by virologic evidence of positive cultures, CMV viremia (or a viremia surrogate marker such as CMV antigenemia or CMV DNAemia), or demonstration of involvement of the end-organ by liver biopsy.[40,111] Treatment with ganciclovir, valganciclovir, foscarnet, or cidofovir appears to be beneficial in immunocompromised hosts, and prophylaxis or preemptive therapy with antiviral agents or CMV hyperimmune globulin may prevent the development of severe CMV disease in transplant recipients.[40,43,52]

Epstein-Barr Virus. EBV infection usually is asymptomatic or "silent," but it may cause a mononucleosis-like syndrome with mild hepatitis. However, in rare patients with a genetic X-linked predisposition, a severe, often fatal lymphoproliferative syndrome with prominent liver involvement may develop. Transplant recipients also may be susceptible to post-transplant lymphoproliferative disease (PTLD), in which the liver may be involved.[9,26,137] In addition, patients with tumors associated with EBV, such as lymphoma, may have hepatic involvement. The diagnosis of mononucleosis usually is clinical and supported by a positive heterophile or "Monospot" test or by specific EBV serologic tests such as detection of IgM antibody to viral capsid antigen (VCA).[13] The immune response to EBV in immunocompromised hosts may be unusual, and the diagnosis of PTLD generally is suspected by an exponential increase in EBV DNA genome copies in peripheral blood, generalized adenopathy, visualization by positron emission tomography, and the presence of histopathologic features on biopsy. Treatment options for PTLD include reducing immunosuppression, rituximab (anti-CD20 monoclonal antibody), adoptive immunotherapy, interferon-α, and anti–interleukin-6 antibody.[30,58,62,116,142] Antiviral agents such as acyclovir or foscarnet should be used only as an adjunct therapy because they are less effective in the latent phase of PTLD.[62]

Human Herpesviruses 6, 7, and 8. These viruses may involve the liver, especially in immunocompromised patients.[48] Infection with HHV-6 in recipients of liver transplants is usually a result of reactivation and has been associated with acute rejection, portal lymphocyte infiltration, and graft dysfunction. Even though HHV-6 commonly can be detected in blood and tissue, high viral loads have been associated with decreased graft survival.[125] Normal hosts with primary HHV-6 infection may have a silent hepatitis with mild elevation of aminotransferase levels. Rarely, severe disseminated disease with fulminant hepatitis has been linked to HHV-6 and HHV-7.[3,10,63,94] HHV-8 (also known as *Kaposi sarcoma virus*) causes a complex neoplasm involving the skin, mucous membranes, and internal organs, most often in severely immunocompromised patients. Although rare, it has been reported in human immunodeficiency virus (HIV)-infected children with advanced AIDS. Recipients of solid organ transplants may be infected with HHV-8. The liver is a common site of visceral disease caused by HHV-8, which most often is associated with tumors rather than hepatitis.[27] Diagnosing infection with HHV-6, HHV-7, or HHV-8 is difficult because of the ubiquity of these viruses and their viral DNA in humans, but the diagnosis is supported by serologic evidence and by detection of viral DNA in blood, in secretions, or, more specifically, in tissue.[117] No specific licensed antiviral therapy is available, but these viruses may be inhibited by ganciclovir, foscarnet, and cidofovir. Response also may be noted after withdrawal or reduction of immunosuppression. Chemotherapy may be indicated in some cases of HHV-8.

Herpes B Virus. Also known as *herpesvirus simiae* or *cercopithecine herpesvirus 1*, herpes B virus is an α-herpesvirus of monkeys that causes severe disease in humans.[158] It can be transmitted from Asian monkeys, such as rhesus and cynomolgus monkeys, to humans through bites or contact with mucous membrane secretions from infected monkeys. The human disease associated with herpes B virus involves skin vesicles at the portal of entry, regional lymphadenitis, and hemorrhagic encephalitis. The virus also may disseminate to the liver and lungs and produce hemorrhagic necrosis, with a high mortality rate. The diagnosis is made by isolation of virus or detection of viral DNA by PCR in specialized reference laboratories, and the virus is inhibited by acyclovir and ganciclovir.

Adenoviruses

Adenoviruses are small DNA viruses that are members of the viral family Adenoviridae. They usually are respiratory or enteric pathogens, but they can cause disseminated disease with hepatitis and hepatic necrosis in both immunocompetent and immunocompromised hosts and neonates.[67,96,113] Patients often have fever, malaise or lethargy, conjunctivitis, pharyngitis, cough, respiratory distress, vomiting and diarrhea, and a viral sepsis–like syndrome. Hepatitis is marked by hepatomegaly and by elevated aminotransferases and bilirubin. A specific viral diagnosis is made by isolation of adenovirus or detection of adenoviral DNA by PCR from respiratory secretions, blood, stool, or tissue. Adenovirus serotype 5 generally is associated with severe hepatitis, followed in frequency by types 1 and 2.[106] Viral surveillance cultures, blood DNA PCR testing, and virus serotyping and genotyping in immunocompromised patients, such as bone marrow and stem cell transplant recipients and liver transplant recipients at high risk for acquiring severe or fatal adenovirus-associated disease, may allow early intervention before severe, disseminated disease develops.[21,57,96] No licensed antiviral therapy is available, but the virus is inhibited to some extent by antiviral agents such as ribavirin and ganciclovir, and case reports suggest a potential clinical benefit in some patients with severe adenoviral hepatitis treated with cidofovir, an antiviral agent with broad-spectrum activity against many DNA viruses.[18,86]

Erythroviruses—Human Parvovirus B19

Erythroviruses (e.g., human parvovirus B19) are small DNA viruses. They are members of the family Parvoviridae and are responsible for a variety of illnesses, including fifth disease (i.e., erythema infectiosum), arthritis, and anemia. Liver involvement, often severe, may be seen in intrauterine infection with hydrops fetalis.[5,95] Fulminant liver failure with massive hepatic necrosis also has been reported in patients with aplastic anemia.[82] Pediatric patients with fulminant liver failure from the hepatotropic viruses have more severe disease and high mortality rates when coinfected with human parvovirus B19.[35] Persistent infection is seen in immunocompromised hosts. Parvovirus infection can be diagnosed by detection of virus-specific IgM antibodies or detection of viral DNA in blood, serum/plasma, bone marrow, secretions, urine, or tissue. No specific antiviral therapy is available, but immunocompromised patients with chronic infection may benefit from receiving IVIG. Severe fetal hydrops usually requires in utero and neonatal blood transfusions for anemia.

Enteroviruses

Enteroviruses are small RNA viruses and members of the Picornaviridae, along with polioviruses and rhinoviruses. The nonpolio enteroviruses often are associated with mild respiratory or gastrointestinal illnesses, myocarditis, or aseptic meningitis, most often occurring in late summer or early fall. Significant hepatic necrosis can occur in neonates with disseminated disease, particularly with serotype echovirus 11. It often is accompanied by hepatomegaly, thrombocytopenia, viral sepsis syndrome, aseptic meningoencephalitis, myocarditis, and elevated aminotransferase and serum bilirubin levels.[99-101] Coxsackievirus B and echoviruses 9 and 30 also are associated with fatal disease. Enteroviruses may be transmitted to neonates perinatally from the mother or through contact with ill family members. Health care–associated nursery outbreaks with enteroviruses also have been reported.[100] The diagnosis is established by isolation of the virus or detection of viral RNA by PCR in throat, stool, urine, blood, cerebrospinal fluid, or tissue samples. Treatment is supportive; however, case reports suggest that IVIG and pleconaril may have some clinical benefit in severely ill neonates.[7,24,76,133]

Measles Virus

Measles virus is an RNA virus that is a member of the Paramyxoviridae, along with parainfluenza viruses, respiratory syncytial virus, metapneumovirus, and mumps virus. Of all the paramyxoviruses, measles virus is associated most often with hepatitis. Ten to 20 percent of children with measles will have subclinical hepatitis, although severe disease of the liver, lungs, and brain may occur in immunocompromised patients.[53,110,145] Rare reports of severe giant-cell hepatitis, often leading to liver failure, have implicated paramyxoviruses of undetermined type.[124] Measles and other paramyxoviruses may be identified by detection of virus-specific IgM antibody in serum and by isolation of virus or detection of RNA by PCR in secretions, blood, or tissue. No specific antiviral therapy is licensed for measles virus; however, ribavirin, a broad-spectrum antiviral agent, may have some activity against the virus. Prevention is achieved by vaccination with the live measles vaccine or by postexposure administration of immunoglobulin or IVIG.

Rubella Virus

Rubella virus is a member of the Togaviridae of RNA viruses. Clinical disease associated with infection by rubella virus generally is mild, but as many as 10 percent of children with rubella may have subclinical hepatitis with transient elevation of aminotransferase levels.[150] Congenital rubella syndrome caused by intrauterine infection with rubella virus, however, is associated with significant liver involvement, and hepatomegaly with

jaundice is noted at birth.[39,103,149] Congenital rubella syndrome also is associated with intrauterine growth retardation, cataracts, congenital heart disease, thrombocytopenia, purpura, and hearing loss.[80] Because isolating the virus is technically challenging, the diagnosis of rubella most often is made serologically by detection of virus-specific IgM antibody or by detection of viral RNA by PCR in reference laboratories. No specific antiviral therapy is available. Rubella can be prevented, however, by vaccination with the live rubella virus vaccine.

Hemorrhagic Fever Viruses

The hemorrhagic fever viruses are a diverse group of RNA viruses from a variety of different virus families. They include arenaviruses such as Lassa fever virus, bunyaviruses such as hantavirus, filoviruses such as Marburg and Ebola, and flaviviruses such as yellow fever virus and dengue. Hemorrhagic fever is characterized by fever, malaise, or lethargy, headache, retro-orbital pain, myalgia, conjunctivitis, rash, and intravascular coagulation with hemorrhage.[37] Liver involvement with these viruses is a very common event, and elevation of aminotransferase levels to 500 IU/mL occurs in almost every patient, with a thousand times the normal range seen in patients who are severely ill.[37] Jaundice is a significant component of yellow fever.[73] The diagnosis is established by serologic means or by detection of the viral agent with electron microscopy or PCR techniques, which in most cases should be attempted only in biosafety level IV reference laboratories. Treatment of most hemorrhagic fevers is supportive; however, intravenous ribavirin reduces the mortality rate associated with Lassa fever and also may be of benefit to patients with hemorrhagic fever caused by other arenaviruses.[88,92]

Bacteria

Nonviral acute hepatitis can be caused by bacterial illnesses (see Table 47-1).[112] Sepsis with gram-positive organisms, especially pneumococci, or with gram-negative organisms, particularly gram-negative enteric bacteria, can produce hepatic dysfunction, primarily from the cholestatic effects induced by bacterial endotoxins.[112,166] In this form of hepatic dysfunction, the patient will appear jaundiced with mild hepatomegaly and conjugated bilirubin levels will be elevated out of proportion to the modest elevation in aminotransferase or alkaline phosphatase levels. Neonates also may have jaundice secondary to urinary tract infection with gram-negative enteric organisms. The diagnosis is made by isolating the offending bacteria from blood, urine, or other usually sterile site. Treatment involves specific antimicrobial therapy.

Other bacterial diseases may cause chronic or granulomatous hepatitis, including actinomycosis, brucellosis, listeriosis, nocardiosis, bartonellosis (cat-scratch disease), and tuberculosis.[8,115] Diseases caused by atypical mycobacteria, especially *Mycobacterium avium-intracellulare* complex (MAC complex) or *Mycobacterium mucogenicum* may be seen, particularly in patients with congenital or acquired immunodeficiency states.[55] Rarely, disseminated disease with hepatitis can occur as a complication of vaccination with bacille Calmette-Guérin (BCG) in children or, in adults, as a complication of bladder irrigation for bladder carcinoma.[8,56,85] Bacterial toxins, such as the emetic toxin of *Bacillus cereus*, also have been linked to fulminant hepatic failure in some patients.[89] A diagnosis of hepatitis caused by these unusual or indolent bacterial pathogens usually is accomplished by isolating the organism from blood or the affected organ. The diagnosis may be supported by positive skin test results in the case of tuberculosis or atypical mycobacterial disease, serologically by elevated titers to *Bartonella quintana* or *Bartonella henselae*, and by positive imaging studies that show hepatic microabscesses, as in the case of hepatic involvement with cat-scratch disease. Antimicrobial therapy is guided by the susceptibility of the offending pathogen.

Spirochetes

Acute infection with *T. pallidum*, the agent of primary or early secondary syphilis in adolescents or adults, may cause acute or granulomatous hepatitis with serum aminotransferase levels up to 5 to 10 times normal.[84] Jaundice rarely develops, but a chancre of primary disease or a rash of secondary disease often is present. Congenital syphilis also is associated commonly with hepatosplenomegaly in the neonate, along with elevated aminotransferase and bilirubin levels. Other clinical manifestations of congenital syphilis include petechiae or purpura, osteitis, and meningitis. Laboratory diagnosis is confirmed by reactive VDRL and positive fluorescent treponemal antibody tests.[66] Treatment with penicillin is recommended.

Leptospirosis, or infection with the pathogenic bacterium *Leptospira interrogans*, can cause acute hepatitis.[64] Leptospirosis usually is an abrupt, anicteric, flulike illness, but approximately 10 percent of patients will have an icteric or septicemic syndrome with a biphasic clinical course. Patients with the icteric or severe form will exhibit jaundice, hepatomegaly, and characteristic conjunctival injection. Usually, levels of serum bilirubin are elevated out of proportion to the more modest elevations in serum aminotransferases, suggesting a defect in excretion of bilirubin rather than direct hepatic necrosis as the pathogenesis of the jaundice. Meningitis, renal failure, and even liver failure may occur in some patients. The diagnosis should be considered in older children and adolescents with a history of exposure to wild and domestic mammals, especially dogs, rats, and livestock, which may excrete *Leptospira* organisms in their urine, or with a history of exposure to contaminated water in ditches, lakes, or streams. The diagnosis is established by isolating the organisms on special media during the acute phase of illness. Serologic and PCR tests also are available in reference laboratories. Treatment with penicillin or doxycycline is recommended.

Rickettsiae

The rickettsial organism *Coxiella burnetii* causes Q fever, both the acute and chronic forms, in which hepatitis is a prominent feature along with persistent fever, malaise,

rash, weight loss, and pneumonitis.[15] Clinical jaundice is a rare finding, and most often the hepatitis is subclinical. A history of exposure to mammals or birds suggests the diagnosis, which can be confirmed serologically by reference clinical laboratories. Treatment with antibiotics, usually doxycycline, is recommended.

Parasites and Fungi

A variety of parasites may invade the liver and occasionally cause hepatic dysfunction or disease.[112,164] Such parasites include *Plasmodium* spp. (malaria), *Entamoeba histolytica* (liver abscess), *T. gondii* (toxoplasmosis), and *Toxocara canis* (visceral larval migrans). *Ascaris lumbricoides* (roundworms) may invade the common bile duct and cause acute obstructive jaundice.

Fungi also may invade the liver, usually with only minimal elevation of aminotransferase levels and rarely causing jaundice or elevated levels of bilirubin. Patients with a compromised immune system may have hepatic abscesses with *Candida* spp. or abscesses or necrotic lesions with *Aspergillus* spp., as well as other unusual fungal species. Both immunocompromised and normal hosts may have liver involvement with *Histoplasma capsulatum* or *Cryptococcus neoformans*.[112]

NONINFECTIOUS CAUSES

An important noninfectious cause of acute hepatitis is drug-related hepatitis (see Table 47-1).[107,161,165] It can range in severity from mild, with subclinical elevation of aminotransferase levels, to fulminant hepatic failure.[78] A careful history of ingestion of prescription or over-the-counter medications, as well as exposure to toxins or herbal remedies, should be elicited. Although almost any drug can cause acute hepatitis, the most common agents associated with hepatitis in children include acetylsalicylic acid (i.e., aspirin), acetaminophen, isoniazid, rifampin, phenytoin, valproic acid, phenobarbital, β-lactam antibiotics (e.g., oxacillin, nafcillin, and third-generation cephalosporins), sulfa drugs, and antifungal agents such as ketoconazole and fluconazole. Many of the above medications, including allopurinol and carbamazepine, have been implicated in the potentially life-threatening DRESS syndrome (Drug Reaction with Eosinophilia and Systemic Symptoms). The majority of patients with DRESS syndrome have hepatic involvement, and the treatment is withdrawal of the offending agent and corticosteroid therapy.[17] The anesthetic drug halothane also can cause acute hepatitis with jaundice.[132] Gold and other metals used to treat arthritis, carbon tetrachloride, and ingestion of *Amanita* mushroom, can all lead to liver injury. In addition, chronic hepatitis can develop in children, especially premature infants, who receive prolonged total parenteral nutrition (for 2 weeks or more).

Anoxic liver injury also can resemble acute viral hepatitis. It occurs in critically ill children after a period of hypotension, heart failure, or cardiopulmonary arrest. A history of such an inciting event supports the diagnosis. Anoxic liver injury is characterized by an abrupt onset of markedly elevated levels of aminotransferases, often hundreds of times higher than normal, without jaundice, and rapid recovery of enzyme levels to normal or nearly normal after the event has resolved.[65]

Other diseases such as Kawasaki syndrome and toxic shock syndrome, which have been linked to or associated with infectious pathogens, may have hepatitis as part of their manifestation. Similarly, Reye syndrome, characterized by hepatomegaly with fatty infiltration of the liver and often fatal encephalopathy, may occur after a viral syndrome such as varicella or influenza.

Metabolic disorders may manifest as hepatitis, especially in infants. Such disorders include cystic fibrosis, α_1-antitrypsin deficiency, galactosemia, hereditary fructose intolerance, glycogen storage disease, urea cycle deficiencies, organic acidemias, tyrosinemia, and lipid storage diseases such as Gaucher and Niemann-Pick disease.[122,151] Of note, cystic fibrosis in neonates may be associated with cholestatic jaundice in the absence of pulmonary disease, similar to biliary atresia.[146] Disorders of metal metabolism, such as Wilson disease or hereditary hemochromatosis, may cause acute, chronic, or even fulminant hepatitis in older children.[123,138] A distinct disorder, neonatal hemochromatosis, is hypothesized to be an alloimmune-mediated (maternal) hepatitis that often manifests as severe liver injury within hours of birth and notable coagulopathy.[157] In addition, patients with congenital disorders of bilirubin metabolism or transport may become jaundiced without significant elevation of aminotransferase levels, especially during an intercurrent viral illness. Of these, Rotor and Dubin-Johnson syndromes predominantly cause a conjugated hyperbilirubinemia and do not lead to hepatic injury.[148] Gilbert syndrome and Crigler-Najjar type 1 and 2 are on the same spectrum of disease causing unconjugated hyperbilirubinemia, with the complete loss of bilirubin glucuronidation being fatal.[148] Sickle-cell crisis also may manifest as hepatitis.[139]

Tumors of the liver such as hepatoblastoma and hemangioendothelioma and infiltrative diseases such as lymphoma, leukemia, and hemophagocytic lymphohistiocytosis syndrome may have hepatitis or hepatic failure as part of the initial manifestation.[61,90] Another multisystem disorder that may produce chronic granulomatous hepatitis is sarcoidosis.[34]

Anatomic causes of hepatic dysfunction in infants include extrahepatic biliary atresia, which usually develops in the first 2 months of life.[91,153]

Autoimmune hepatitis or other autoimmune disorders with liver involvement also may cause acute or chronic hepatitis, especially in older children and adolescents.[155] These disorders often are associated with rash, arthritis, inflammatory bowel disease, thyroiditis, malaise, and persistent fever. Autoimmune hepatitis may respond to corticosteroids or other immunosuppressive agents.

Hepatic steatosis (fatty infiltration) is becoming more and more common in obese children, especially if insulin resistant. These children may have enlarged or tender livers on examination, elevated aminotransferase levels in serum, abnormal imaging studies showing fatty infiltrates of the liver, and liver biopsies that show inflammation, fibrosis, and even cirrhosis in some cases. Treatment

currently includes diet and lifestyle changes with weight loss and exercise.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Aggarwal R, Jameel S. Hepatitis E. *Hepatology* 2011;**54**(6):2218–26.
17. Cacoub P, Mussette P, Descamps V, et al. The DRESS syndrome: a literature review. *Am J Med* 2011;**124**(7):588–97.
19. Caviness AC, Demmler GJ, Selwyn BJ. Clinical and laboratory features of neonatal herpes simplex virus infection: a case control study. *Pediatr Infect Dis J* 2008;**27**(5):425–30.
22. Chang TT, Lai CC, Kew Yoo S, et al. Entecavir treatment in patients for up to 5 years in patients with e antigen positive chronic hepatitis B. *Hepatology* 2010;**51**(2):422–30.
35. Dwivedi M, Manocha H, Tiwari S, et al. Coinfection of parvovirus B19 with other hepatitis viruses leading to fulminant hepatitis of unfavorable outcome in children. *Pediatr Infect Dis J* 2009;**28**(7):649–50.
38. Erhardt A, Blondin D, Hauck K, et al. Response to interferon alpha is hepatitis B genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* 2009;**54**(7):1009–13.
54. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;**461**(7262):399–401.
60. Han L, Zhang HW, Xie JX, et al. A meta-analysis of lamivudine for interruption of mother-to-child transmission of hepatitis B virus. *World J Gastroenterol* 2011;**17**(38):4321–33.
62. Hartmann C, Schuchmann M, Zimmermann T. Posttransplant lymphoproliferative disease in liver transplant patients. *Curr Infect Dis Rep* 2011;**13**(1):53–9.
65. Henrion J. Hypoxic hepatitis. *Liver Int* 2012;**32**(7):1039–52.
66. Herremans T, Kortbeek L, Notermans DW. A review of diagnostic tests for congenital syphilis in newborns. *Eur J Clin Microbiol Infect Dis* 2010;**29**(5):495–501.
71. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet* 2011;**378**(9785):73–85.
75. Jacobson IM, McHutchison JG, Dusheiko G. et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011;**364**(25):2405–16.
77. Jonas MM, Block JM, Haber BA, et al. Treatment of children with chronic hepatitis B infection in the United States: patient selection and therapeutic options. *Hepatology* 2010;**52**(6):2192–205.
90. Makin E, Davenport M. Fetal and neonatal liver tumours. *Early Hum Dev* 2010;**86**(10):637–42.
102. Moldovan B, Mentha G, Maino P, et al. Demographics and outcomes of severe herpes simplex virus hepatitis: a registry-based study. *J Hepatol* 2011;**55**(6):1222–6.
107. Murray KF, Hadzic N, Wirth S, et al. Drug-related hepatotoxicity and acute liver failure. *J Pediatr Gastroenterol Nutr* 2008;**47**(4):395–405.
118. Pan CQ, Han GR, Jiang HX, et al. Telbivudine prevents vertical transmission from HBeAg-positive women with chronic hepatitis B. *Clin Gastroenterol Hepatol* 2012;**10**(5):520–6.
119. Pan CQ, Duan JP, Bhamidimarri KR, et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. *Clin Gastroenterol Hepatol* 2012;**10**(5):452–9.
125. Pischke S, Gosling J, Engelmann I, et al. High intrahepatic HHV-6 virus loads but neither CMV nor EBV are associated with decreased graft survival after diagnosis of graft hepatitis. *J Hepatol* 2012;**56**(5):1063–9.
128. Poordad F, McCone Jr J, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;**364**(13):1195–206.
141. Ruiz-Extremera A, Munoz-Gamez JA, Salmeron-Ruiz MA, et al. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011;**53**(6):1830–8.
144. Schwarz KB, Gonzalez-Peralta RP, Murray KF, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. *Gastroenterology* 2011;**140**(2):450–8.
148. Strassburg CP. Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, and Rotor syndrome). *Best Pract Res Clin Gastroenterol* 2010;**24**(5):555–71.
154. Tenney DJ, Rose RE, Baldwick CJ, et al. Long term monitoring shows hepatitis B resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009;**49**(5):1503–14.
156. Wedemeyer H, Yurdaydin C, Dalekos GN, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;**364**(4):322–31.
160. Wirth S. Current treatment options and response rates in children with chronic hepatitis C. *World J Gastroenterol* 2012;**18**(2):99–104.
163. Yuen MF, Lai CL. Treatment of chronic hepatitis B: evolution over two decades. *J Gastroenterol Hepatol* 2011;**26**(Suppl 1):138–43.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aach RD, Lander JJ, Sherman LA, et al. Transfusion-transmitted viruses: interim analysis of hepatitis among transfused and non-transfused patients. In: Vyas GN, Cohen SN, Schmid R, editors. *Viral hepatitis*. Philadelphia: Franklin Institute Press; 1978. p. 383.
2. Aggarwal R, Jameel S. Hepatitis E. *Hepatology* 2011;**54**(6): 2218–26.
3. Akashi K, Eizuru Y, Sumiyoshi Y, et al. Brief report: severe infectious mononucleosis–like syndrome and primary human herpesvirus 6 infection in an adult. *N Engl J Med* 1993;**329**:168–71.
4. Amado LA, Villar LM, dePaula VS, Gaspar AM. Comparison between serum and saliva for the detection of hepatitis A virus RNA. *J Virol Methods* 2008;**148**:74–80.
5. Anand A, Gray E, Brown T, et al. Human parvovirus infection in pregnancy and hydrops fetalis. *N Engl J Med* 1987;**316**:183–6.
6. Anderson DR, Schwartz J, Hunter NJ, et al. Varicella hepatitis: a fatal case in a previously healthy, immunocompetent adult. Report of a case, autopsy, and review of the literature. *Arch Intern Med* 1994;**154**:2101–6.
7. Aradottir E, Alonoso EM, Shulman ST. Severe neonatal enteroviral hepatitis treated with pleconaril. *Pediatr Infect Dis J* 2001; **20**:457–9.
8. Arisoy ES, Correa AG, Wagner ML, et al. Hepatosplenic catscratch disease in children: selected clinical features and treatment. *Clin Infect Dis* 1999;**28**:778–84.
9. Armes JE, Angus P, Southey MD, et al. Lymphoproliferative disease of donor origin arising in patients after orthotopic liver transplantation. *Cancer* 1994;**74**:2436–41.
10. Asano Y, Yoshikawa T, Suga S, et al. Fatal fulminant hepatitis in an infant with human herpesvirus-6 infection. *Lancet* 1990;**335**: 862–3.
11. Bachmann L. Infectious hepatitis in Europe. In: Rodenwalt E, editor. *World atlas of epidemic diseases, part I*. Hamburg, Germany: Falk-Verlag; 1952. p. 67.
12. Balayan MS, Andjaparidze AG, Savinskaya SS, et al. Evidence for a virus in non-A/non-B hepatitis transmitted via the fecal oral route. *Intervirology* 1983;**20**:23–6.
13. Basson V, Sharp AA. Monospot: a differential slide test for infectious mononucleosis. *J Clin Pathol* 1969;**225**:324–5.
14. Beeson PB, Chesney G, McFarlan AM, et al. Hepatitis following injection of mumps convalescent plasma. *Lancet* 1944;**1**:814–16.
15. Bernstein M, Edmondson HA, Barhour BH. The liver lesion in Q fever: clinical and pathologic features. *Arch Intern Med* 1965;**116**:491–500.
16. Blumberg BS, Stunick AI, London WT. Hepatitis and leukemia: their relation to Australia antigen. *Bull N Y Acad Med* 1968;**44**: 1566–86.
17. Cacoub P, Mussette P, Descamps V, et al. The DRESS syndrome: a literature review. *Am J Med* 2011;**124**(7):588–97.
18. Carter BA, Karpen SJ, Quiros-Tejeira RE, et al. Intravenous cidofovir therapy for disseminated adenovirus in a pediatric liver transplant recipient. *Transplantation* 2002;**74**:1050–2.
19. Caviness AC, Demmler GJ, Selwyn BJ. Clinical and laboratory features of neonatal herpes simplex virus infection: a case control study. *Pediatr Infect Dis J* 2008;**27**(5):425–30.
20. Centers for Disease Control and Prevention. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1996;**45** (RR-11):1–36.
21. Chakrabarti S, Mauther V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood* 2002;**100**:1619–27.
22. Chang TT, Lai CC, Kew Yoo S, et al. Entecavir treatment in patients for up to 5 years in patients with e antigen positive chronic hepatitis B. *Hepatology* 2010;**51**(2):422–30.
23. Choo Q-L, Kuo G, Weiner AJ, et al. Isolation of cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;**244**:359–62.
24. Chuang E, Maller E, Hoffman M, et al. Successful treatment of fulminant echovirus 11 infection in a neonate by orthotopic liver transplantation. *J Pediatr Gastroenterol Nutr* 1993;**17**:211–4.
25. Cockayne EA. Catarrhal jaundice sporadic and epidemic, and its relation to acute yellow atrophy of the liver. *Q W J Med* 1912;**6**:1–5.
26. Cohen J. Epstein-Barr virus lymphoproliferative disease with acquired immunodeficiency. *Medicine (Baltimore)* 1991;**70**:137–59.
27. Colina F, Lopez-Rios F, Lumbreras C, et al. Kaposi's sarcoma developing in a liver graft. *Transplantation* 1996;**61**:1779–81.
28. Committee on Infectious Diseases, American Academy of Pediatrics. Hepatitis C virus infection. *Pediatrics* 1998;**101**:481–5.
29. Committee on Infectious Diseases, American Academy of Pediatrics. Varicella-zoster infections. In: Pickering LK, editor. *2006 Red book: report of the committee on infectious diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 711.
30. Cook RC, Connors JM, Gascoyne RD, et al. Treatment of post-transplant lymphoproliferative disease with rituximab monoclonal antibody after lung transplantation. *Lancet* 1999;**354**:1698–9.
31. Cuthbert JA. Hepatitis A: old and new. *Clin Microbiol Rev* 2001;**14**:38–58.
32. de la Serna-Higuera C, Gonzalez-Garcia M, Milicua JM, Munoz V. Cytomegalovirus granulomatous hepatitis in an immunocompetent patient. *Gastroenterol Hepatol* 1999;**22**:230–1.
33. Demmler GJ. Summary of a workshop on surveillance for congenital cytomegalovirus disease. Rev Infect Dis 1991;**13**: 315–329.
34. Devaney K, Goodman ZD, Epstein MS, et al. Hepatic sarcoidosis: clinicopathologic features in 100 patients. *Am J Surg Pathol* 1993;**17**:1273–80.
35. Dwivedi M, Manocha H, Tiwari S, et al. Coinfection of parvovirus B19 with other hepatitis viruses leading to fulminant hepatitis of unfavorable outcome in children. *Pediatr Infect Dis J* 2009; **28**(7):649–50.
36. Egawa H, Inomata Y, Nakayama S, et al. Fulminant hepatic failure secondary to herpes simplex virus infection in a neonate: a case report of successful treatment with liver transplantation and perioperative acyclovir. *Liver Transpl Surg* 1998;**4**:513–15.
37. Elisaf M, Stafanaki S, Reparti M, et al. Liver involvement in hemorrhagic fever with renal syndrome. *J Clin Gastroenterol* 1993;**17**:33–7.
38. Erhardt A, Blondin D, Hauck K, et al. Response to interferon alpha is hepatitis B genotype dependent: genotype A is more sensitive to interferon than genotype D. *Gut* 2009;**54**(7):1009–13.
39. Esterly J, Slusser R, Ruebner B. Hepatic lesions in the congenital rubella syndrome. *J Pediatr* 1967;**71**:676–85.
40. Evans PC, Soin A, Wreghitt TG, et al. Qualitative and semiquantitative polymerase chain reaction testing for cytomegalovirus DNA in serum allows prediction of CMV related disease in liver transplant recipients. *J Clin Pathol* 1998;**51**:914–21.
41. Falagas ME, Paya C, Ruthazer R, et al. Significance of cytomegalovirus for long-term survival after orthotopic liver transplantation: a prospective derivation and validation cohort analysis. *Transplantation* 1998;**66**:1020–8.
42. Falagas ME, Snydman DR, Griffith J, et al. Effect of cytomegalovirus infection status on first-year mortality rates among orthotopic liver transplant recipients. The Boston Center for Liver Transplantation CMVIG Study Group. *Ann Intern Med* 1997; **126**:275–9.
43. Falagas ME, Snydman DR, Ruthazer R, et al. Cytomegalovirus immune globulin (CMVIG) prophylaxis is associated with increased survival after orthotopic liver transplantation. The Boston Center for Liver Transplantation CMVIG Study Group. *Clin Transplant* 1997;**11**:432–7.
44. Farr RW, Short S, Weissman D. Fulminant hepatitis during herpes simplex virus infection in apparently immunocompetent adults: report of two cases and review of the literature. *Clin Infect Dis* 1997;**24**:1191–4.
45. Feinstone SM, Kapikian AZ, Purcell RH. Hepatitis A: detection by immune electron microscopy of a virus-like antigen associated with the acute illness. *Science* 1973;**182**:1026–8.
46. Feldman S, Chaudary S, Ossi M, et al. A viremic phase for herpes zoster in children with cancer. *J Pediatr* 1977;**91**:597–600.
47. Feldman S, Crout JD, Andrew ME. Incidence and natural history of chemically defined varicella-zoster virus hepatitis in children and adolescents. *Scand J Infect Dis* 1997;**29**:33–6.
48. Fingeroth JD. Herpesvirus infections of the liver. *Infect Dis Clin North Am* 2000;**14**:1–34.
49. Fishman JA, Rubin RH. Infections in organ-transplant recipients. *N Engl J Med* 1998;**338**:1741–51.
50. Fishman LN, Jonas MM, Lavine JE. Update on viral hepatitis in children. *Pediatr Clin North Am* 1996;**43**:57–74.

51. Flewett TH, Parker RG, Philip WM. Acute hepatitis due to herpes simplex virus in an adult. *J Clin Pathol* 1969;**22**:60–6.
52. Gane E, Saliba F, Valdecasa GJ, et al. Randomized trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver-transplant recipients. The Oral Ganciclovir International Transplantation Study Group. *Lancet* 1997;**350**:1729–33.
53. Gavish D, Kleinman Y, Morag A, et al. Hepatitis and jaundice associated with measles in young adults. *Arch Intern Med* 1983; **143**:674–7.
54. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;**461**(7262):399–401.
55. Goldblatt MR, Ribes JA. Mycobacterium mucogenicum isolated from a patient with granulomatous hepatitis. *Arch Pathol Lab Med* 2002;**126**:73–5.
56. Gottke MU, Wong P, Muhn C, et al. Hepatitis in disseminated bacillus Calmette-Guérin infection. *Can J Gastroenterol* 2000;**14**: 333–6.
57. Gray GC, McCarthy T, Lebeck MG, et al. Genotype prevalence and risk factors for severe clinical adenovirus infections, United States, 2004–2006. *Clin Infect Dis* 2007;**45**:1120–31.
58. Grillo-Lopez AJ, White CA Varns C, et al. Overview of the clinical development of rituximab: first monoclonal antibody approved for the treatment of lymphoma. *Semin Oncol* 1999; **26**:66–73.
59. Gust ED, Purcell RH. Waterborne non-A, non-B hepatitis. *J Infect Dis* 1987;**156**:630–6.
60. Han L, Zhang HW, Xie JX, et al. A meta-analysis of lamivudine for interruption of mother-to-child transmission of hepatitis B virus. *World J Gastroenterol* 2011;**17**(38):4321–33.
61. Harrison HB, Middleton HM, Crosby JH, et al. Fulminant hepatic failure: an unusual presentation of metastatic liver disease. *Gastroenterology* 1981;**80**:820–5.
62. Hartmann C, Schuchmann M, Zimmermann T. Posttransplant lymphoproliferative disease in liver transplant patients. *Curr Infect Dis Rep* 2011C;**13**(1):53–9.
63. Hashida T, Komura E, Yoshida M, et al. Hepatitis in association with human herpes virus 7 infection. *Pediatrics* 1995;**96**:783–5.
64. Heath Jr CW, Alexander AD, Galton MM. Leptospirosis in the United States: analysis of 483 cases in man 1949–1961. *N Engl J Med* 1965;**273**:857–67.
65. Henrion J. Hypoxic hepatitis. *Liver Int* 2012;**32**:1039–52.
66. Herremans T, Kortbeek L, Notermans DW. A review of diagnostic tests for congenital syphilis in newborns. *Eur J Clin Microbiol Infect Dis* 2010;**29**(5):495–501.
67. Hierholzer JC. Adenoviruses in the immunocompromised host. *Clin Microbiol Rev* 1992;**5**:262–74.
68. Hilleman MR. Overview of the pathogenesis, prophylaxis, and therapeutics of viral hepatitis B, with focus on reduction to practical applications. *Vaccine* 2001;**19**:1837–48.
69. Hillis WD. An outbreak of infectious hepatitis among chimpanzee handlers at a United States Air Force base. *Am J Hyg* 1961; **73**:316–20.
70. Hollinger FB, Gitnick GL, Aach RD, et al. Non-A, non-B hepatitis transmission in chimpanzees: a project of the transfusion-transmitted viruses study group. *Intervirology* 1978;**10**:60–8.
71. Hughes SA, Wedemeyer H, Harrison PM. Hepatitis delta virus. *Lancet* 2011;**378**(9785):73–85.
72. Hurwitz ES, Deseda CC, Shapiro CN, et al. Hepatitis infections in the day-care setting. *Pediatrics* 1994;**94**(Suppl.):1023–4.
73. Ishak K, Walker D, Coetzer J, et al. Viral hemorrhagic fevers with hepatic involvement: pathologic aspects with clinical correlations. *Prog Liver Dis* 1982;**7**:495–515.
74. Jacobs RF. Neonatal herpes simplex virus infections. *Semin Perinatol* 1998;**22**:64–71.
75. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011;**364**(25):2405–16.
76. Johnston J, Overall J. Intravenous immune globulin in disseminated neonatal echovirus 11 infection. *Pediatr Infect Dis J* 1989;**8**: 254–6.
77. Jonas MM, Block JM, Haber BA, et al. Treatment of children with chronic hepatitis B infection in the United States: patient selection and therapeutic options. *Hepatology* 2010;**52**(6):2192–205.
78. Katelaris PH, Jones DB. Fulminant hepatic failure. *Med Clin North Am* 1989;**73**:955–70.
79. Kaufman B, Gandhi SA, Louie E, et al. Herpes simplex virus hepatitis: case report and review. *Clin Infect Dis* 1997;**24**: 334–8.
80. Korones S, Ainger L, Monif G, et al. Congenital rubella syndrome: study of 22 infants. *Am J Dis Child* 1965;**110**: 434–40.
81. Lamb SG, Stern H. Cytomegalovirus mononucleosis with jaundice as a presenting sign. *Lancet* 1966;**2**:1003–6.
82. Langnas A, Markin R, Cattral J, et al. Parvovirus B19 as a possible causative agent of fulminant liver failure and associated aplastic anemia. *Hepatology* 1995;**22**:1661–5.
83. Lauer GM, Walker BD. Hepatitis C infection. *N Engl J Med* 2001;**345**:41–52.
84. Lee RV, Thornton GF, Conn HO. Liver disease associated with secondary syphilis. *N Engl J Med* 1971;**284**:1423–30.
85. Leebeek FWG, Ouwendijk RJT, Kolk AHU, et al. Granulomatous hepatitis caused by bacillus Calmette-Guérin (BCG) infection after bladder instillation. *Gut* 1996;**38**:616–8.
86. Legrand F, Berrebi D, Houhou N, et al. Early diagnosis of adenovirus infection and treatment with cidofovir after bone marrow transplantation in children. *Bone Marrow Transplant* 2001;**27**:621–6.
87. London WT, Sutnick AI, Blumberg BS, et al. Australian antigen and acute viral hepatitis. *Ann Intern Med* 1969;**70**:55–9.
88. Luerman A. Eine Icterusepidemie. *Berl Klin Wochenschr* 1885;**22**:20–5.
89. Mahler H, Pasi A, Kramer JM, et al. Fulminant liver failure in association with the emetic toxin of Bacillus cereus. *N Engl J Med* 1997;**33**:1142–8.
90. Makin E, Davenport M. Fetal and neonatal liver tumours. *Early Hum Dev* 2010;**86**(10):637–42.
91. Manolaki B, Larcher VF, Mowar AP, et al. The prelaparotomy diagnosis of extra-hepatic biliary atresia. *Arch Dis Child* 1983; **58**:591–4.
92. McCormick JB, King IT, Webb PA, et al. Lassa fever: Effective therapy with ribavirin. *N Engl J Med* 1986;**314**:20–6.
93. Melnick JL. Classification of hepatitis A as enterovirus 72 and of hepatitis B virus as hepadnavirus, type 1. *Intervirology* 1982; **18**:105–10.
94. Mendel I, de Matteis M, Bertin C, et al. Fulminant hepatitis in neonates with human herpesvirus 6 infection. *Pediatr Infect Dis J* 1995;**14**:993–7.
95. Metzman R, Anand A, DeGiulio P, et al. Hepatic disease associated with intrauterine parvovirus B19 infection in a newborn premature infant. *J Pediatr Gastroenterol Nutr* 1989;**9**:112–4.
96. Michaels MG, Green M, Wald ER, et al. Adenovirus infection in pediatric liver transplant recipients. *J Infect Dis* 1992;**165**: 170–4.
97. Miguelez M, Gonzalez A, Perez F. Severe cytomegalovirus hepatitis in a pregnant woman treated with ganciclovir. *Scand J Infect Dis* 1998;**30**:304–5.
98. Minuk GY, Nicolle LE. Genital herpes and hepatitis in healthy young adults. *J Med Virol* 1986;**19**:269–72.
99. Modlin J. Fatal echovirus 11 disease in premature neonates. *Pediatrics* 1980;**66**:775–9.
100. Modlin J. Perinatal echovirus infection: insights from a literature review of 61 cases of serious infection and 16 outbreaks in nurseries. *Rev Infect Dis* 1986;**8**:918–26.
101. Modlin J. Perinatal echovirus and group B Coxsackie virus infections. *Clin Perinatol* 1988;**15**:233–45.
102. Moldovan B, Mentha G, Maino P, et al. Demographics and outcomes of severe herpes simplex virus hepatitis: a registry-based study. *J Hepatol* 2011;**55**:1222–6.
103. Monif G, Asofsky R, Sever J. Hepatic dysfunction in the congenital rubella syndrome. *BMJ* 1966;**1**:1086–8.
104. Morgan ER, Smalley LA. Varicella in immunocompromised children: incidence of abdominal pain and organ involvement. *Am J Dis Child* 1983;**137**:883–5.
105. Morishita K, Kodo H, Asano S, et al. Fulminant varicella hepatitis following bone marrow transplantation. *JAMA* 1985;**253**:511–15.
106. Munoz F, Piedra P, Demmler GJ. Disseminated adenovirus disease in immunocompromised and immunocompetent children. *Clin Infect Dis* 1998;**27**:1194–200.

107. Murray KF, Hadzic N, Wirth S, et al. Drug-related hepatotoxicity and acute liver failure. *J Pediatr Gastroenterol Nutr* 2008;**47**(4): 395–405.
108. Myers MG. Hepatic cellular injury during varicella. *Arch Dis Child* 1982;**57**:317–19.
109. Narkewicz MR, Cabrera R, Gonzalez-Peralta RP. The "C" of viral hepatitis in children. *Semin Liver Dis* 2007;**27**:295–311.
110. Nickell M, Cannady P, Schwitzer G. Subclinical hepatitis in rubeola infections in young adults. *Ann Intern Med* 1979;**90**: 354–5.
111. Niubo J, Perez JL, Martinez-Lacasa JT, et al. Associations of quantitative cytomegalovirus antigenemia with symptomatic infection in solid organ transplant recipients. *Diagn Microbiol Infect Dis* 1996;**24**:19–24.
112. Novak DA, Doloson DJ. Bacterial, parasitic, and fungal infections of the liver. In: Suchy FJ, editor. *Liver disease in children*. St. Louis: C. V. Mosby; 1994. p. 550–68.
113. Odio C, McCracken Jr GH, Nelson Jr JD, Nelson JD. Disseminated adenovirus infection: a case report and review of the literature. *Pediatr Infect Dis* 1984;**3**:46–9.
114. Ohto H, Terazawa S, Saski N, et al. Transmission of hepatitis C virus from mothers to infants. *N Engl J Med* 1994;**330**:744–50.
115. Oliva A, Duarte B, Jonasson O, Nadimpalli V. The nodular form of local hepatic tuberculosis: a review. *J Clin Gastroenterol* 1990;**12**:166–73.
116. O'Reilly RJ, Small TN, Papadopoulos E, et al. Biology and adoptive cell therapy of Epstein-Barr virus–associated lymphoproliferative disorders in recipients of marrow allografts. *Immunol Rev* 1997;**157**:195–216.
117. Ozaki Y, Tajiri H, Tanaka-Taya K, et al. Frequent detection of the human herpesvirus 6–specific genomes in the livers of children with various liver diseases. *J Clin Microbiol* 2001;**39**: 2173–7.
118. Pan CQ, Han GR, Jiang HX, et al. Telbivudine prevents vertical transmission from HBeAg-positive women with chronic hepatitis B. *Clin Gastroenterol Hepatol* 2012;**10**(5):520–6.
119. Pan CQ, Duan JP, Bhamidimarri KR, et al. An algorithm for risk assessment and intervention of mother to child transmission of hepatitis B virus. *Clin Gastroenterol Hepatol* 2012;**10**(5):452–9.
120. Panda SK, Thakral D, Rehman S. Hepatitis E virus. *Rev Med Virol* 2007;**17**:151–80.
121. Patti ME, Selvaggi KJ, Kroboth FJ. Varicella hepatitis in the immunocompromised adult: a case report and review of the literature. *Am J Med* 1990;**88**:77–80.
122. Perlmutter DH. Alpha-1-antitrypsin deficiency. *Semin Liver Dis* 1998;**18**:217–25.
123. Perman JA, Werlin SL, Grand RJ, et al. Laboratory measures of copper metabolism in the differentiation of chronic active hepatitis and Wilson disease in children. *J Pediatr* 1979;**94**:564–8.
124. Phillips MJ, Blendis LM, Poucell S, et al. Syncytial giant-cell hepatitis: sporadic hepatitis with distinctive pathologic features, a severe clinical course, and paramyxoviral features. *N Engl J Med* 1991;**324**:455–60.
125. Pischke S, Gosling J, Engelmann I, et al. High intrahepatic HHV-6 virus loads but neither CMV nor EBV are associated with decreased graft survival after diagnosis of graft hepatitis. *J Hepatol* 2012;**56**(5):1063–9.
126. Pitel PA, McCormick KL, Fitzgerald T, et al. Subclinical hepatic changes in varicella infection. *Pediatrics* 1980;**65**:631–3.
127. Polish LB, Gallagher M, Fields HA, et al. Delta hepatitis: molecular biology and clinical and epidemiologic features. *Clin Microbiol Rev* 1993;**6**:211–29.
128. Poordad F, McCone Jr J, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;**364**(13):1195–206.
129. Purcell RH, Emerson SU. Hepatitis E: An emerging awareness of an old disease. *J Hepatol* 2008;**48**:494–503.
130. Prince AM. An antigen detected in blood during the incubation period of serum hepatitis. *Proc Natl Acad Sci U S A* 1968; **60**:814–821.
131. Prince AM, Brotman B, Grady GF, et al. Long-incubation, post-transfusion hepatitis without serological evidence of exposure to hepatitis B virus. *Lancet* 1974;**2**:241–6.
132. Ray DC, Drummond GB. Halothane hepatitis. *Br J Anaesth* 1991;**67**:84–99.
133. Rentz AC, Libbey JE, Fujinami RS, et al. Investigation of treatment failure in neonatal echovirus 7 infection. *Pediatr Infect Dis J* 2006;**25**:259–62.
134. Reyes GR, Purdy MA, Kim JP, et al. Isolation of cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 1990;**247**:1335–9.
135. Rizzetto M. The delta agent. *Hepatology* 1983;**3**:729–35.
136. Rizzetto M, Canese MG, Avico S, et al. Immunofluorescence detection of a new antigen/antibody system (delta/anti-delta) associated with hepatitis B virus in liver and serum of HBsAg carriers. *Gut* 1977;**18**:977–1003.
137. Robinson JE, Brown N, Andiman W, et al. Diffuse polyclonal B-cell lymphoma during primary infection with Epstein-Barr virus. *N Engl J Med* 1980;**302**:1293–7.
138. Roche-Sicot J, Benhamou JP. Acute intravascular hemolysis and acute liver failure associated with a first manifestation of Wilson's disease. *Ann Intern Med* 1977;**86**:301–10.
139. Rosenblate HJ, Eisenstein R, Holmes AW. The liver in sickle cell anemia. A clinical-pathologic study. *Arch Pathol* 1970;**90**:235–45.
140. Rowland P, Wald ER, Mirro Jr JR, et al. Progressive varicella presenting with pain and minimal skin involvement in children with acute lymphoblastic leukemia. *J Clin Oncol* 1995;**13**: 1697–703.
141. Ruiz-Extremera A, Munoz-Gamez JA, Salmeron-Ruiz MA, et al. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011;**53**(6):1830–8.
142. Savoldo B, Goss JA, Hammer MM, et al. Treatment of solid organ transplant recipients with autologous Epstein Barr virus–specific cytotoxic T lymphocytes (CTLs). *Blood* 2006;**108**:2942–9.
143. Schiller GJ, Nimer SD, Gajewski JL, et al. Abdominal presentation of varicella-zoster infection in recipients of allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1991;**7**:489–91.
144. Schwarz KB, Gonzalez-Peralta RP, Murray KF, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. *Gastroenterology* 2011;**140**(2):450–8.
145. Shalev-Zimels H, Weizman Z, Lotan C, et al. Extent of measles hepatitis in various ages. *Hepatology* 1988;**8**:1138–9.
146. Sokol RJ, Durie PR. Recommendations for management of liver and biliary tract disease in cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1999;**28**:511–13.
147. Soriano V, Bru F, Gonzalez-Laoz J. Fatal varicella hepatitis in a patient with AIDS. *J Infect* 1992;**25**:107–9.
148. Strassburg CP. Hyperbilirubinemia syndromes (Gilbert-Meulengracht, Crigler-Najjar, Dubin-Johnson, and Rotor syndrome). *Best Pract Res Clin Gastroenterol* 2010;**24**(5):555–71.
149. Strauss L, Bernstein J. Neonatal hepatitis in congenital rubella. *Arch Pathol* 1968;**86**:317–27.
150. Sugaya N, Nirasawa M, Mitamura K, et al. Hepatitis in acquired rubella infection in children. *Am J Dis Child* 1988;**142**:817–8.
151. Svegar T. The natural history of liver disease in alpha-1-antitrypsin deficient children. *Acta Pediatr* 1995;**77**:847–51.
152. Tabor E, Gerety RJ, Drucker JA, et al. Transmission of non-A, non-B hepatitis from man to chimpanzees. *Lancet* 1978;**1**:463–6.
153. Tarr PI, Haas JE, Christie DL. Biliary atresia, cytomegalovirus, and age at referral. *Pediatrics* 1996;**97**:828–31.
154. Tenney DJ, Rose RE, Baldwick CJ, et al. Long term monitoring shows hepatitis B resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009;**49**(5): 1503–14.
155. Van den Berg AP. Autoimmune hepatitis: pathogenesis, diagnosis, and treatment. *Scand J Gastroenterol Suppl* 1998;**225**:66–9.
156. Wedemeyer H, Yurdaydin C, Dalekos GN, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;**364**(4):322–31.
157. Whitington PF. Neonatal hemochromatosis: a congenital alloimmune hepatitis. *Semin Liver Dis* 2007;**27**(3):243–50.
158. Whitley RJ. Cercopithecine herpes virus 1 (B virus). In: Fields BN, Knipe DM, Howley PM, editors. *Fields virology*. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 2623–30.
159. Whitley RJ, Kimberlin DW. Treatment of viral infections during pregnancy and the neonatal period. *Clin Perinatol* 1997; **24**:267–83.

160. Wirth S. Current treatment options and response rates in children with chronic hepatitis C. *World J Gastroenterol* 2012;**18**(2): 99–104.
161. Wu SS, Chao CS, Vargas JH, et al. Isoniazid-related hepatic failure in children: a survey of liver transplantation centers. *Transplantation* 2007;**27**:173–9.
162. Young EJ, Chafizadek E, Oliveira VL, et al. Disseminated herpesvirus infection during pregnancy. *Clin Infect Dis* 1996; **22**:51–8.
163. Yuen MF, Lai CL. Treatment of chronic hepatitis B: evolution over two decades. *J Gastroenterol Hepatol* 2011;**26**(Suppl 1): 138–43.
164. Zimmerman HJ. The differential diagnosis of jaundice. *Med Clin North Am* 1968;**52**:1417–50.
165. Zimmerman HJ. *Hepatotoxicity: the adverse effects of drugs and other chemicals on the liver*. New York: Appleton-Century-Crofts; 1978.
166. Zimmerman HJ, Fang M, Utili R, et al. Clinical conference: jaundice due to bacterial infection. *Gastroenterology* 1979;**77**:362–73.

One specific pediatric population—children with biliary atresia and a hepatic portoenterostomy (HPE)—is particularly susceptible to cholangitis and its sequelae. This population and other patient groups at high risk for developing infectious cholangitis, such as patients with immunodeficiency states, congenital or acquired bile duct abnormalities, or liver transplants, are highlighted in this chapter. Biliary tract obstruction secondary to gallstone disease is discussed in the section on cholecystitis.

ETIOLOGY AND PATHOGENESIS

The central mechanism leading to biliary tract infection is obstruction of normal bile flow. Bile typically is sterile, owing to mechanical flushing with normal bile flow, the anatomic gate of the choledochal sphincter, bacteriostatic properties of bile, and biliary IgA.[34,144] When obstruction and stagnation occur, bacteria gain access either by ascending from the gut or via hematogenous spread. The exact route of bacterial invasion and spreading remains controversial. Ascent may occur from the duodenum, most commonly in the presence of abnormal function of the sphincter of Oddi. In addition, bacterial invasion may occur hematogenously via increased gut translocation across the intestine into the portal circulation,[250] followed by flow across the gallbladder wall.[59] Although the exact mechanism has not been firmly established, the consensus is that transient episodes of bactibilia combined with biliary obstruction may lead to higher concentrations of bacteria in the biliary tract. As biliary pressure increases, bacteria likely migrate from the bile ducts into lymphatic and blood vessels, resulting in bacteremia and clinical signs and symptoms of cholangitis.[34,111] Iatrogenic introduction of bacteria into the biliary tract can occur during endoscopic retrograde cholangiopancreatography (ERCP) or with indwelling stents.[144]

In adults, cholangitis typically occurs in the presence of biliary tract obstruction secondary to impaction of gallstones in the common bile duct, leading to bile stasis and secondary infection. In approximately 85 percent of cases of cholangitis in adults, evidence of a common bile duct stone is present.[18] In adults with documented gallstones in the common bile duct (choledocholithiasis), the incidence of positive bile cultures is 30 to 99 percent.[146,153,231] Other potential causes of biliary obstruction in adults, possibly leading to a more chronic or recurrent presentation, include neoplasm of the biliary tree or head of the pancreas, other intrinsic or extrinsic strictures, parasitic disease, inflammatory conditions (e.g., primary biliary cirrhosis, primary sclerosing cholangitis [PSC]), and congenital bile duct anomalies. More recently, with improved imaging modalities and a better understanding of the pathogenesis of biliary lithiasis, it has become accepted that biliary sludge or microcalculi may be at the root of nonobstructive cholangitis and recurrent biliary infections.

In children, acute cholangitis occurs most frequently in patients with biliary atresia who have undergone HPE surgery, also known as a *Kasai operation*. HPE provides a permissive setup for cholangitis: poor bile flow combined with damaged intrahepatic bile ducts and obligate bacterial colonization with enteric flora in the intestinal conduit.[105,135,150,217] In this setting, cholangitis is thought to arise from reflux of jejunal flora through the HPE (Roux-en-Y loop), directly contiguous with the hepatic porta. Incidence is between 40 to 93 percent of patients and tends to occur more frequently within the first year after surgery.[48,66,217] Decreased biliary and duodenal motility generally is accepted as being associated with a higher incidence of cholangitis,[63,252] but most predictors and exacerbants for the development of cholangitis remain unknown.

Microbiologic evidence of biliary tract infection ideally should involve at least 10^5 organisms per milliliter of bile.[59] The frequencies of specific organisms found in adult and pediatric studies are summarized in Table 48-1.[19,28,127,149,206] *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* spp. and *Enterococcus* spp. are most commonly isolated from infected bile. Many cases of cholangitis are polymicrobial. Anaerobes, such as *Bacteroides fragilis* and *Clostridium perfringens*, also may play a significant role and have been identified in 40 percent of biliary tract infections.[29] Specifically, the latter organism has been implicated in acute emphysematous cholecystitis. Despite these findings in bile, many patients with cholangitis have

TABLE 48-1 **Bacterial Pathogens Isolated from Bile Cultures in Cholangitis***

Organism	Keighley 1975[127] (n = 231)	Boey 1980[19] (n = 99)	Lewis 1987[149] (n = 23)	Brook 1989[28] (n = 123)	Rerknimitr 2002[206] (n = 69)
Escherichia coli	65	46	15	71	16
Klebsiella/Enterobacter spp.	28	28	17	65	16
Pseudomonas spp.	1	17	0	2	6
Proteus spp.	13	8	0	15	0
Enterococcus spp.	22	14	0	42	15
Staphylococcus spp.	5	0	1	7	10
Streptococcus spp.	3	0	6	16	14
Bacteroides spp.	2	0	2	29	0
Clostridium spp.	11	0	9	27	0
Other anaerobes	7	0	0	14	4
Other spp.	7	23	10	8	3

*Pediatric and adult data.

TABLE 48-2 **Diagnostic Criteria for Acute Cholangitis**

Definitive Diagnosis	Charcot Triad, 1877	Tokyo Guidelines, 2006
Clinical signs of infection and finding of purulent bile by:	Fever	Two of three Charcot triad criteria plus:
Endoscopic retrograde cholangiopancreatography	Right upper quadrant pain	Evidence of inflammatory response
Surgery	Jaundice	Abnormal white blood cell count
Percutaneous puncture	**Reynold Pentad, 1959**	Elevated C-reactive protein
	Fever	Abnormal liver tests
	Right upper quadrant pain	Aspartate aminotransferase
	Jaundice	Alanine aminotransferase
	Septic shock	Alkaline phosphatase
	Altered mental status	γ-Glutamyl transpeptidase
		Imaging evidence of etiology (e.g., stone, stricture, stent)

negative blood cultures, requiring clinicians to have a high index of suspicion for cholangitis in selected circumstances and patient populations.

In addition to bacteria, viral, fungal, and parasitic pathogens have been reported in cases of pediatric cholangitis, particularly in immunodeficient patients (primarily patients infected with human immunodeficiency virus [HIV]) and patients with environmental exposure to parasites in endemic settings. Cholangitis due to viral infection is most commonly associated with hepatocellular disease with hepatotropic viruses, primarily cytomegalovirus (CMV), hepatitis C, and hepatitis B.[32,87,145] Depending on the clinical setting and age of the patient, CMV can have a markedly varied presentation, ranging from bile duct paucity seen in congenital CMV infection to hepatomegaly and jaundice in infants and children with primary CMV infection. The role of CMV hepatitis and cholangitis is significant in immunocompromised patients and solid-organ transplant recipients.

Fungal cholangitis, specifically with *Cryptococcus neoformans* or *Candida albicans*, has been reported in immunocompromised and immunocompetent patients.[44,140,151] Hepatobiliary dysfunction may be the initial manifestation of disseminated infection.

The biliary tree also is susceptible to parasitic infections in normal hosts. Nematodes—*Ascaris* and, rarely, *Strongyloides*—commonly cause biliary disease in endemic regions. Migrating *Ascaris* larvae may cause a direct inflammatory response if they pass through the biliary system, or, more commonly, a high worm burden can cause an obstruction leading to secondary pyogenic cholangitis.[131,235] Liver flukes, such as *Opisthorchis* (formerly *Clonorchis*) *sinensis*, *O. viverrini*, and *O. felineus*, also may pass through the biliary system through their life cycle.[162] Of these trematodes, *Clonorchis* is found frequently in cases of recurrent pyogenic cholangitis in Asian children.[282] Similar to the worms, the migration of the flukes *(Fasciola hepatica)* through the biliary tree may induce primary inflammation and, later, a secondary bacterial infection. Echinococcal cholangitis has been described in the setting of obstruction secondary to cyst formation from infection with either *Echinococcus granulosus* or *E. multilocularis*.[34,90] Although *Cryptosporidium* spp. typically are known to cause cholangitis in the immunocompromised host, one case of a child without a documented immunodeficiency has been reported.[84] A thorough travel history should be obtained in all children presenting with new-onset cholangitis, in particular in patients in whom the most common causes are ruled out rapidly.

Finally, several cases of *Mycobacterium tuberculosis* cholangitis mimicking cholangiocarcinoma in immunocompromised and immunocompetent hosts have been reported in the adult literature.*

CLINICAL PRESENTATION

The classic presentation of cholangitis first described by Charcot in 1877 and known commonly as the *Charcot triad* is reported in 50 to 70 percent of patients with cholangitis in most studies (Table 48-2).[19,186,251,272] It combines the clinical findings of fever, right upper quadrant pain, and jaundice.[41] The Reynold pentad added septic shock and altered mental status to the Charcot triad.[208] More recently, the Tokyo guidelines updated diagnostic criteria for cholangitis to include laboratory evidence of an inflammatory response, abnormal liver test results, and imaging findings of biliary dilation or evidence of etiology of underlying disease.[272] Studies in children show that fever is the most common presenting symptom, occurring in 100 percent of 105 patients with cholangitis after undergoing HPE.[63] In addition, either acholic stools or an increase in serum bilirubin concentration occurs in 68 percent of patients. Older children and teenagers with cholangitis typically report abdominal pain that may or may not be associated with meals or localize to the right upper quadrant. Patients may report new-onset pruritus as a consequence of retained biliary constituents. Laboratory findings include elevated white blood cell count and C-reactive protein, bilirubin, and alkaline phosphatase values.[285]

The findings of cholangitis may be markedly attenuated in infants and immunocompromised patients, suggesting that a low threshold for suspicion be employed in the evaluation of these children at risk. The presentation of cholangitis in an infant with biliary atresia, status post Kasai portoenterostomy, may be as varied as lethargy, increasing jaundice, abdominal tenderness, or fever alone. Finally, patients with ongoing hemolysis (e.g., patients with hemoglobin SS disease) are at high risk for

*References 14, 70, 136, 193, 202, 267.

developing cholecystitis and cholangitis from bilirubin stones, yet the symptoms may readily overlap with other causes of abdominal pain.

DIAGNOSTIC EVALUATION

Physical examination may reveal a varied range of toxic appearances, with vital signs suggesting serious systemic infection in more advanced cases (fever, tachycardia, hypotension). Physical examination typically reveals icteric sclera and a distended abdomen, with tenderness localized to the mid or right upper quadrant. Palpation of the liver edge may reveal tenderness. Laboratory studies may reveal an elevated erythrocyte sedimentation rate (81%), leukocytosis or leukopenia (56%), and increased serum levels of conjugated bilirubin.[63] Laboratory evaluations in children should include a standard complete blood cell count with differential and liver panel (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyltransferase, and fractionated bilirubin [unconjugated and conjugated]). The differential diagnosis of fever, abdominal pain, and jaundice also should include sepsis and hepatitis. No single laboratory test or combination of tests can establish a diagnosis of cholangitis.[251,285]

Attempts at identifying a microbiologic agent should be made. Blood and bile cultures should be performed at all available opportunities and specimens drawn before antimicrobial therapy is initiated.[255] Most cases are polymicrobial, with a predominance of gut-derived organisms (see Table 48-1). Some studies report positive blood cultures in 21 to 71 percent of patients with acute cholangitis.[96,251,277] Other investigators have reported identification of organisms in blood in 10 to 25 percent of patients, which is similar to our experience.[277] In patients with a history of hepatobiliary surgery, aerobic and anaerobic cultures should be considered; and in virtually all cases of young children with abdominal pain and jaundice, a urine culture should be considered.

The role of liver biopsy to evaluate the parenchyma for evidence of cholangitis is controversial, but occasionally this testing may have a place in the setting of negative blood cultures. When used along with blood cultures, hepatic cultures have been shown to increase the diagnostic yield of identifying a microbiologic organism to 75 percent of patients, whereas histologic confirmation of cholangitis may be the only firm evidence of biliary tract infection in some cases.[63] In practice, liver biopsy is performed infrequently and patients are treated empirically. In cases of gallstone-associated biliary tract obstruction with cholangitis that requires endoscopic or surgical decompression, bile should be obtained and cultured at the time of the procedure. In an adult study, 22 of 23 patients with gallstone-associated cholangitis were found to have positive bile cultures.[149]

The initial and principal radiologic evaluation for suspected cholangitis is transabdominal ultrasonography, with the primary goal being to search for evidence of biliary tract obstruction, usually associated with dilation of the common bile duct or intrahepatic ducts. Ultrasound examination may reveal anatomic biliary tract abnormalities (choledochal cyst), intrahepatic cysts or fluid collections, and hepatic or other intra-abdominal masses. Other noninvasive imaging modalities include computed tomography (CT) and magnetic resonance cholangiopancreatography (MRCP), which provide increasingly detailed images of the intrahepatic and extrahepatic biliary anatomy.*

Taken together, these noninvasive imaging techniques should be able to detect the presence of biliary tract obstruction and some congenital anatomic anomalies, although even experienced ultrasonographers still may miss small stones and sludge, especially in the common bile duct. It is important to recognize the technical limitations of the methodology and the possible presence of undetected stones or sludge in the gallbladder or common bile duct (choledocholithiasis) as a cause of biliary tract dilation. The sensitivity of ultrasound for choledocholithiasis is poor, ranging from 30 to 50 percent.[46,47,234] Sensitivity improves to approximately 90 percent with CT, and from 95 to 99 percent with ERCP.[203]

Newer modalities, including endoscopic ultrasound, laparoscopic ultrasound, and helical CT cholangiography, may improve detection in certain patient populations. The 2002 National Institutes of Health State-of-the-Science Consensus conference regarded endoscopic ultrasound, MRCP, and ERCP to have comparable sensitivity and specificity in detecting common bile duct stones.[182] In the hands of an experienced echoendoscopist, endoscopic ultrasound has been shown in adults to be the procedure of choice to identify small stones and sludge in the common bile duct. This method may prove useful in children as well and may prevent the unnecessary use of ERCP and the risk of its associated complications.[214,234,270]

After HPE has been performed, imaging may be able to identify lakes of retained bile (bilomas), which may be a source of infection, in a patient with biliary atresia. Nuclear medicine (hepatobiliary iminodiacetic acid [HIDA]) scans have a limited role in the evaluation of cholangitis. HIDA scans may be most helpful in determining if a patient has a complete obstruction of the common bile duct or if an isolated obstruction of the cystic duct (e.g., cholecystitis) is present.

Because of the inherent risks associated with invasive procedures in these infants, percutaneous drainage of bilomas for culture or other purposes rarely is performed. Similarly, percutaneous drainage is preferred to ERCP for anatomic reasons. Invasive imaging has a role, especially when it may be coupled with therapeutic decompression of an obstructed, and potentially infected, biliary tree. ERCP has proved to be extremely useful in the evaluation and management of biliary tract obstruction and should be considered in the management of a child with evidence of an obstructed common bile duct.† The role of ERCP in infants and small children is limited by the number of trained pediatric gastroenterologists and should be considered in tertiary or quaternary care centers that have the requisite expertise. ERCP allows obtaining bile for microbial and chemical analysis and

*References 72, 174, 213, 223, 232, 262.
†References 5, 74, 157, 236, 237, 241.

provides detailed imaging of the biliary tree. Significant technical advances have occurred in biliary endoscopy, such that direct visualization of the bile ducts (choledochoscopy) with the ability to obtain direct sampling is possible in children.[98,238] Acute cholangitis alone is an indication for ERCP when choledocholithiasis is suggested.[161]

Practically, ERCP may detect (and remove) small stones and sludge that are missed by ultrasound, CT, or MRCP. ERCP is helpful in establishing the differential diagnosis of biliary tract diseases, such as PSC, which may manifest in a fashion similar to that of infectious cholangitis but have a characteristic radiographic appearance. In the future, as the use of endoscopic ultrasound in pediatrics increases, ERCP may be reserved for those patients in whom stones or sludge have been found by endoscopic ultrasound or in patients with a high pretest probability of having choledocholithiasis (e.g., patients with sickle-cell conditions).[75,161,234] Any child with a first episode of cholangitis warrants a detailed investigation of a possible underlying biliary tract anatomic abnormality using at least one of the available diagnostic modalities.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis for an acutely ill child with fever and clinical evidence of hyperbilirubinemia is broad; a thorough workup must include consideration of infectious and noninfectious causes (Table 48-3). A thorough investigation for any hepatic and biliary tract pathologic process with blood and radiologic studies must be initiated to evaluate for stones or other obstructive processes that may cause symptoms characteristic of the Charcot triad.

Acute viral hepatitis may manifest in numerous ways but often begins with nonspecific signs: fever, headache, anorexia, and jaundice.[71] Laboratory studies typically reveal a greater elevation of serum aminotransferase levels (alanine aminotransferase and aspartate aminotransferase) than the biliary tract enzymes alkaline phosphatase and γ-glutamyltransferase. In a child with no other evidence of biliary tract obstruction, screening assays for hepatitis infection are warranted.

TABLE 48-3 **Differential Diagnosis of Fever, Abdominal Pain, and Jaundice**

Cholangitis
Cholecystitis
Cholelithiasis
Sepsis
Hepatitis
Choledochal cyst
Pancreatitis
Urinary tract infection
Leptospirosis and other systemic infections with hepatic involvement
Spontaneous perforation of common bile duct
Biliary cyst
Appendicitis

Pyogenic liver abscesses and amebic abscesses tend to manifest as fever, abdominal pain, hepatomegaly, and focal right upper quadrant tenderness.[171] Laboratory findings vary. CT is considered to be the most sensitive technique for evaluation of these diagnoses.[174] Numerous bacterial, parasitic, and spirochetal infections also should be considered in the differential diagnosis, including, but not limited to, typhoid fever, brucellosis, leptospirosis, borreliosis, amebiasis, and malaria. It is beyond the scope of this chapter to expand on the systemic infections associated with hepatic involvement.[192] Recurrent pyogenic cholangitis is another consideration, although it is rare in the Western Hemisphere and even more so in the pediatric population.[222]

Jaundice may occur with sepsis of any cause, more commonly in a critically ill infant or child. Clinical evaluation may show a predominantly direct (conjugated) hyperbilirubinemia usually accompanied by a modest elevation of γ-glutamyltransferase, although serum transaminase levels may not be elevated. These findings are caused by a hepatocellular cholestasis, owing to humoral mediators of sepsis (i.e., endotoxin and proinflammatory cytokines), but they can be exacerbated by biliary sludging that accompanies septicemia. Although sepsis-associated cholestasis historically was linked primarily to gram-negative sepsis, it can be seen in all forms of infection. In some cases, radiologic workup or biopsy is warranted to rule out ductal obstruction or a pathologic process of the biliary tract. Jaundice may occur as the sole presentation of sepsis in infants and children[61]; however, research into sepsis-induced cholestasis points to a complex, multifactorial etiopathogenesis.[40,80,175,264]

Drug-induced cholestasis tends to occur acutely with the onset of jaundice, pruritus, and other symptoms that may mimic cholangitis. It rarely is associated with fever, however. Among the main classes of drugs commonly used in pediatric practice that may lead to significant hepatotoxicity are antimicrobial agents (mainly trimethoprim-sulfamethoxazole [TMP-SMX], amoxicillin/clavulanate, clindamycin, minocycline, nitrofurantoin, erythromycin, cephalosporins, isoniazid, rifampicin, fluconazole), anticonvulsants (phenytoin, carbamazepine, valproate, felbamate), nonsteroidal anti-inflammatory drugs (aspirin, ibuprofen), antihypertensive agents (propranolol, diltiazem),[30,45] and herbal remedies such as kava kava. Although rarely used in pediatric patients, statins may also lead to significant hepatotoxicity.[45] If the offending agent is identified in time, removing it typically leads to rapid improvement. Ceftriaxone warrants special mention because it has been linked directly to cholangitis and cholecystitis, possibly as a result of its biliary concentration and precipitation, leading to sludge and stones.[134,183]

Other systemic illnesses may occur with fever and evidence of a pathologic process of the biliary tract, and their presentations may overlap with presentations of infectious cholangitis. PSC is defined as a chronic inflammation of the intrahepatic or extrahepatic ducts leading to a range of biliary tract pathologic processes, from

dilation to obliteration and periductular fibrosis. PSC is seen most commonly in patients with inflammatory bowel disease and affects adolescent boys with inflammatory bowel disease more commonly than girls. Symptoms include systemic findings, such as fatigue, malaise, and weight loss, and evidence of cholangitis, including fever. PSC must be suspected in a jaundiced patient with inflammatory bowel disease or in an adolescent presenting for the first time with jaundice.[147] Diagnosis is established best by ERCP or magnetic resonance cholangiography showing irregular narrowing and stricture of the hepatic and common bile ducts and the intrahepatic ducts.[159,210] Patients with PSC are at risk for developing intrahepatic and common bile duct strictures, with subsequent obstruction, sludge or stone formation, cholangitis, and, ultimately, cholangiocarcinoma.[113,147]

TREATMENT

Therapeutic goals in the treatment of cholangitis should include general support of the patient, early initiation of appropriate antibiotic therapy, and, in cases of obstruction by stones or stricture, decompression of biliary obstruction via ERCP or surgery. Consulting pediatric surgeons early in the course of evaluating a patient with suspected cholangitis generally is helpful. The importance of decompression and drainage of the bile tract in the presence of systemic infection secondary to cholangitis cannot be overemphasized. Other potential causes of obstruction, such as choledochal cysts, ultimately require surgical consultation and repair.

Initial and conservative management should include appropriate inpatient monitoring, cessation of oral intake with intravenous fluid support, parenteral antibiotic therapy, and supportive management as warranted by the child's clinical status. Antimicrobial agents are selected to cover suspected or documented organisms based on local or documented susceptibility patterns and the ability to achieve adequate serum and tissue concentrations. Currently, no single antibiotic or combination is recognized universally as being the definitive therapy for cholangitis in children. Parenteral antibiotics are indicated in almost all cases of children with suspected biliary tract sepsis. Theoretically, the ability to achieve high biliary concentrations (Table 48-4) should be considered. However, clinical and experimental data are lacking to strongly support the recommendation for sole use of antimicrobial agents with excellent biliary penetration. Adequate serum levels may be sufficient.[2,59]

Empirical antibiotics should cover the most commonly isolated enteric pathogens, including *E. coli*, *Klebsiella* spp., and *Enterococcus* spp. Previously, a combination of ampicillin or penicillin with an aminoglycoside was considered a standard regimen for cholangitis, with a clinical cure rate of 40 to 95 percent in various adult studies.[39,81,177,255,260] Multiple recent studies show equivalent effectiveness of newer agents, including penicillin plus β-lactamase inhibitor combinations, third- and fourth-generation cephalosporins, and carbapenems.[81,116,259,260]

TABLE 48-4 **Distribution of Antibiotics Used to Treat Cholangitis**

Antibiotic	Blood	Bile	Tissues
Aminoglycosides	+++	+	+
Carbapenems	+++	++	+++
Cephalosporins*	+++	+++	++
Penicillins†	++	++	++
Quinolones	+++	+++	+++
Trimethoprim-sulfamethoxazole	+++	+++	++

*Anionic cephalosporins with molecular weight >500 such as cefpiramide, cefoperazone, ceftriaxone, cefotetan, and cefixime.
†Ureidopenicillins such as pipercillin.
Data from references 16, 27, 106, 128, and 277.

The general recommendation for treatment of a pediatric patient with suspected cholangitis is a semisynthetic penicillin plus β-lactamase combination or a third-generation cephalosporin with or without an aminoglycoside.[66,139,277] The ureidopenicillins (e.g., pipercillin plus tazobactam) exhibit broad-spectrum activity against gram-positive streptococci, gram-negative bacilli, and many anaerobes. If a cephalosporin is used in a child with clinical sepsis, it is reasonable to consider the addition of metronidazole for anaerobic species, particularly *Bacteroides* spp.[29] Rothenberg and colleagues reported a 63 percent success rate in the treatment of pediatric patients with cholangitis after HPE using imipenem-cilastatin or third-generation cephalosporins with or without aminoglycosides and a 58 percent success rate using semisynthetic penicillins with aminoglycosides.[217] As mentioned previously, ceftriaxone probably should be avoided because it has been associated with biliary sludging and cholecystitis.[117] Although carbapenems provide very broad-spectrum coverage of enteric organisms associated with bacterial cholangitis,[217,242,269] these agents should be reserved for treatment of extended-spectrum β-lactamase producers or other multidrug-resistant pathogens. Antibiotic resistance is encountered more often in patients with repeated episodes of cholangitis.[63] Empirical broad-spectrum treatment should be narrowed to target specific pathogens as soon as culture results are available to avoid emergence of antimicrobial resistance or overgrowth of flora leading to secondary infection (e.g., *Clostridium difficile*, yeast).[144,255]

Antibiotic efficacy is determined by clinical and laboratory parameters, such as defervescence and improvement in biliary excretion. Duration of antibiotic treatment in adult guidelines is as short as 2 to 3 days; however, a short course may result in recurrence in pediatric patients.[217] The recommended course of therapy is 14 to 21 days, with longer duration recommended in special cases, such as recurrent or refractory cholangitis, and in the case of intrahepatic abscesses or hepatic surgery. Long-term administration of antibiotics without rationale should be avoided.[255] Although oral antibiotic therapy generally has no place in the treatment of cholangitis in children, oral ciprofloxacin has been used in some adult populations.[277]

Ultimately, cholangitis, or the risk thereof, will not resolve in the presence of ongoing biliary obstruction. Although antibiotic therapy may treat septicemia, timely establishment of biliary drainage is imperative to relieve the obstruction and to increase biliary penetration of antibiotics.[65,144] In 20 to 25 percent of adults with cholangitis, medical therapy is insufficient and decompression via ERCP (or laparotomy) is indicated.[9,65,127,221] Furthermore, almost all patients who improve with medical therapy alone will ultimately require bile duct clearance to prevent recurrence of cholangitis.[144] ERCP and percutaneous transhepatic cholangiography generally are recommended as first-line approaches because they pose a lower risk than does open surgical intervention.[195] Although ERCP can be diagnostic in the case of biliary obstruction, it also provides therapeutic options with sphincterotomy, stone removal or destruction, or stent placement.

Treatment of parasitic cholangitis should include appropriate treatment of biliary parasites based on regional sensitivities in addition to antibiotic coverage of secondary bacterial infections. Endoscopic or surgical intervention may be required to remove worms or cysts from the biliary tree.[85,201]

PREVENTION

Treatment of biliary obstruction is the primary means to prevent acute bacterial cholangitis. Long-term prophylactic antibiotics are not recommended. Although a few case reports and small case series have documented success with TMP-SMX, ciprofloxacin, and neomycin as prophylactic antibiotics,[31,43,109,173] there are no data showing significant benefit compared with patients without prophylaxis in controlled studies. Moreover, development of highly resistant bacteria selected by continuous use of antibiotics has been documented.[35,63,226]

Antibiotics immediately prior to biliary tract surgery do play a role in prevention of wound infection rates.[169] The role for antibiotic prophylaxis before ERCP is less clear.[277] Antibiotics are recommended if biliary stone clearance is not anticipated during a single ERCP session; otherwise, prophylaxis is unlikely to be useful.[11]

COMPLICATIONS

Regardless of the patient's age at the time of presentation, cholangitis can be life threatening. Other morbidities associated with cholangitis include biloma formation and pancreatitis. Because pediatric patients who present with cholangitis typically have an underlying biliary or liver pathologic process, cholangitis may precipitate a rapid exacerbation of the underlying disease and potentiate cirrhosis, portal hypertension, severe gastroesophageal bleeding, or ongoing sepsis. In diseases such as biliary atresia, cholangitis may hasten the patient's course toward requiring organ replacement. Cholangitis is an important determinant of long-term survival after HPE, but it is unclear if it is a determinant of transplant survival.[50-52,278]

SPECIFIC POPULATIONS AND CHOLANGITIS

Cholangitis and Biliary Atresia

In pediatrics, cholangitis is encountered most frequently in the setting of a patient with biliary atresia who has undergone a Kasai procedure. In 1959, Kasai and Suzuki first reported relief of biliary obstruction in children with biliary atresia by HPE.[124] The procedure, a Roux-en-Y hepatic portoenterostomy, is considered to be standard first-line surgical therapy for infants with biliary atresia, and it is associated with the highest success rates and long-term survival when performed early, usually by 60 days of life. Without this procedure, 90 percent of patients with biliary atresia die before reaching age 3 years, at an average age of 19 months.[99,123,125]

Cholangitis remains the most frequent complication of the Kasai procedure, occurring in 40 to 60 percent of patients.[189] A subgroup of patients with the pathologic finding of cystic dilation of the intrahepatic bile ducts seems to have the highest risk for developing postoperative cholangitis.[107] The development of cholangitis has been associated with a worsening in long-term prognosis of children with biliary atresia.[50,52,110] Complications of cholangitis are severe, ranging from inflammation and scarring, to alterations in biliary flow, and, eventually, to biliary cirrhosis. Repeated bouts of cholangitis are associated with worsening liver function, impaired growth, and need for early transplantation. For this reason, early detection, appropriate management, and prompt intervention and treatment of cholangitis are important in this population.

Most cases of post–Kasai procedure cholangitis occur within 1 year postoperatively. In a 1976 review of 49 cases of children who underwent the procedure, 31 achieved good bile flow restoration. Of these, 20 subsequently developed cholangitis, with 15 cases occurring within the first postoperative month and 3 within the second month.[187] Likewise, in a study of 105 cases of cholangitis developing in 101 children after they underwent hepatic portoenterostomies, Ecoffey and colleagues[63] showed that 63 percent of the cases occurred within 3 months after surgery and 93 percent occurred within 1 year. Rarely, late cholangitis may occur and has been reported to occur several years after HPE.[86]

The generally accepted mechanism for development of cholangitis after a Kasai procedure is ascending bacterial colonization. This theory is reinforced by reports of a higher incidence of cholangitis developing in patients with good or partial bile flow after the Kasai procedure (78%) than in patients with no obvious bile flow (13%).[63] A 1978 study of 19 patients concluded that all bilioenteric conduits after the procedure were colonized within 1 month, correlating with the high incidence of symptomatic infection at this early stage.[105,106] The retrograde bacterial colonization may be enhanced by overall changes in intestinal motility after the Roux-en-Y loop.[252] Over time, the intestinal conduit from hepatic porta to jejunum seems to "mature," and the number of episodes of cholangitis diminishes. How this adaptation happens is unknown. Late episodes of cholangitis, occurring 1 to 2

years after a Kasai procedure, likely are related to mechanical obstruction, such as adhesions, and warrant investigation.[150]

Gram-negative enteric organisms constitute most organisms causing ascending cholangitis after HPE. *E. coli* has been found in 50 percent of the first and second cholangitis episodes and in decreasing frequency in subsequent episodes.[63] Anaerobes also are common findings and should be considered when selecting antibiotic treatment.[29] Refractory or recurrent cases occurring after surgery may warrant consideration of fungal disease, principally *Candida* spp.[44] Attempts to isolate an organism generally are made, but blood cultures typically have a low yield.[217] Bile cultures tend to reflect the multiple enteric organisms that colonize the conduit but may not specify the true pathogens. Percutaneous liver biopsy has been used successfully to obtain cultures, but as in other forms of cholangitis it rarely is used for this purpose.[188]

In the intraoperative and postoperative management of children with biliary atresia, attempts have been made to prevent the incidence of cholangitis. Various modifications in the surgical configuration of the intestinal conduit have been suggested to reduce enteric reflux. Initial studies have documented a reduced incidence of ascending cholangitis in the presence of a surgically placed antireflux valve.[24] Despite the reduction of intestinal reflux in these cases, cholangitis continues to occur.[24] Cases of refractory cholangitis may require surgical intervention, especially if obstruction of the conduit or porta hepatis is suspected. Absolute indications for reoperation after HPE are unclear; however, a recent series demonstrated positive outcomes with the primary indication of recurrent jaundice.[22]

The role of corticosteroids in improving biliary drainage is controversial and has not been the subject of rigorous controlled trials. Perioperative corticosteroid pulses, typically a 3- to 5-day course of intravenous methylprednisolone, have been shown, however, to be clinically beneficial by decreasing temperature, increasing bile flow, and improving liver biochemistries.[143,178,217] A multicenter, randomized, double-blind, placebo-controlled study sponsored by National Institutes of Health is in progress in the United States to assess the safety and efficacy of using corticosteroids after the Kasai procedure.* The forthcoming results of this study should yield definitive information regarding the postoperative management of these children. The incidence of cholangitis in the treated and untreated arms is one of the outcomes.

In addition, some centers advocate the use of prophylactic antibiotics, typically TMP-SMX. The benefits of this practice are not well established, however (see Prevention).[43] In adults with recurrent cholangitis secondary to fixed obstruction, as in the case of malignancy, TMP-SMX and ciprofloxacin have been shown to be helpful in preventing episodes.[268] When used, TMP-SMX is used as a first-line agent. In selected patients with recurrent episodes of cholangitis on TMP-SMX and a functional HPE, we have used oral ciprofloxacin successfully.

*http://clinicaltrials.gov/ct2/show/NCT00294684?term=basitrank=3

In patients with post–Kasai procedure biliary atresia, each episode of cholangitis is associated with a 1 percent mortality risk. In a recent longitudinal series, 30 percent of patients who died with biliary atresia without transplant had sepsis, several with suspected or proven cholangitis.[50] Some studies suggest that no significant clinical difference exists in overall survival rates between patients who have undergone the Kasai procedure and have or have not had cholangitis.[189] Previous studies suggest, however, an 88 percent mortality rate among patients who develop cholangitis within 1 month after undergoing the Kasai procedure and 16 percent in patients who develop it more than 1 month later. More recent studies suggest that the occurrence of cholangitis is related to early postoperative mortality, and the number of repeated episodes is inversely related to survival.[283] Thus, patients with recurrent episodes of cholangitis are considered for liver transplantation.

Cholangitis After Liver Transplantation

Infection remains the most common reason for morbidity and mortality after pediatric liver transplantation, accounting for 20 to 30 percent of postoperative deaths.[219,247] Bacterial infection that develops after transplantation tends to occur within the first 2 months postoperatively and generally is of either respiratory tract or intra-abdominal origin. Among patients with severe bacterial infections of intra-abdominal origin, cholangitis and biliary tract infections commonly develop. Gram-negative aerobic bacteria are encountered most commonly in this setting.[224] One adult study showed that 18 percent of 284 patients receiving liver transplantation had confirmed episodes of cholangitis.[273] Pediatric data suggest that the rate of cholangitis after liver transplantation for biliary atresia is 5 to 11 percent.

Biliary tract disease frequently occurs after the post–liver transplantation complication of hepatic artery thrombosis.[158,165] This complication was more prevalent in the early days of pediatric liver transplantation but decreased owing to the use of microsurgical techniques. Hepatic artery thrombosis eventually leads to damage of the bile duct because the biliary blood supply is exclusively arterial, whereas the hepatic parenchyma is vascularized by the portal vein and the hepatic artery. Biliary injury leads to altered biliary anatomy and drainage, increasing the risk for development of infection in these patients. Although the majority of pediatric liver transplant recipients have a Roux-en-Y biliary-enteric anastomosis from the donor bile duct to the recipient bowel, ascending cholangitis is rare in this population. In patients with a duct-to-duct anastomoses, cholangitis secondary to stricture formation or intraductal lithiasis can be treated by ERCP.

Cholangitis that develops after liver transplantation may manifest as fever, jaundice, elevated aminotransferase and bilirubin levels, and bacteremia. The variable nature of an immunocompromised patient's response to infection renders the reliability of each of these signs and symptoms unique to each occurrence. Distinguishing these symptoms from the presentation of posttransplant rejection or infection (e.g., with CMV or Epstein-Barr

virus) is important. For this reason, liver biopsy often is a necessary part of the evaluation of a posttransplant patient with fever and elevated liver tests.

Cholangitis in Immunocompromised Patients

Although biliary disease related to acquired immunodeficiency syndrome (AIDS) may be considered the most common cholangiopathy of immunocompromised hosts, it is not the most frequent one in pediatric practice. Cholangitis also may occur in children with non-AIDS immunodeficiency. Sclerosing cholangitis has been reported in cases of primary immunodeficiency, including familial T-cell deficiency, IgA/IgG deficiency, and X-linked hyper-IgM syndrome.[89,148] Patients with sclerosing cholangitis often present with superimposed bacterial cholangitis because of poor biliary drainage. Opportunistic hepatobiliary infection also has been reported in children with leukemia.[79,137,240]

AIDS-related cholangitis is a well-known complication of AIDS. It tends to occur late in the course of the illness and is more common in adults than in children. With the decline in the number of new AIDS cases in the United States, especially in pediatric patients, this diagnosis has become rare. In 1989, Cello described four distinct patterns of disease in AIDS-related cholangitis as seen on cholangiography—papillary stenosis, sclerosing cholangitis, combined papillary stenosis and sclerosing cholangitis, and long extrahepatic bile duct strictures.[38] The pathogenesis of these changes is unknown and may be related to biliary inflammation secondary to immunodeficiency, infiltration of the mucosa by HIV itself, or opportunistic infection by known gut culprits in AIDS infection.

Opportunistic agents most commonly responsible are CMV, *Cryptosporidium*, Microsporidia, and, uncommonly, *Mycobacterium avium-intracellulare* and *Isospora*.[181,289] One report of unexplained cholangitis in a small cohort of HIV-positive men identified *Enterocytozoon bieneusi* in the bile of all of the patients.[198] Although AIDS cholangiopathy is extremely rare in children, these pathogens can be found in pediatric patients with primary immunodeficiencies and associated PSC and with secondary immunosuppression after undergoing solid-organ transplantation.[1,57,167,211,279]

Clinical presentation is similar to that in non-HIV patients, with the exception of jaundice, which tends to be less common. In a series of 45 adults with AIDS-related cholangitis, abdominal pain was reported as the most common presenting symptom, occurring in 64 percent of patients, followed by diarrhea (22%), fever (20%), and jaundice (7%).[60] Twenty percent of patients were asymptomatic and identified by routine blood work alone.[60] A 1997 review reported that 90 percent of adults present with right upper quadrant or epigastric pain.[181] Cholangitis has been reported as the initial presentation of HIV infection in a few patients.[23,176]

Diagnostic steps include noninvasive imaging with ultrasonography and CT. ERCP can also be useful. Abdominal ultrasonography is abnormal in 75 percent of patients and typically shows dilation or wall thickening of the common bile duct. These findings, along with liver function studies, may be suggestive of the disease even in children.[37,218] ERCP can show further characteristic changes in the biliary tract and has the added advantage of obtaining specimens for biopsy and culture and the possibility of therapeutic intervention.[284] Although AIDS-related cholangitis typically is not directly associated with mortality, most patients with AIDS-related cholangitis die within 1 year of being diagnosed because cholangitis usually occurs in patients with end-stage disease.[156] Therapy generally is symptomatic and should include coverage of bacterial cholangitis, which often is associated because of impaired biliary drainage.

Cholangitis in Association with Congenital Anatomic Abnormalities: Choledochal Cysts and Caroli Disease

Choledochal cysts occur in 1 in 15,000 births in Western nations and 1 in 1000 live births in Japan.[64,154,243,246] Typically, presenting signs suggest cholestasis and may include jaundice, dark urine, and acholic stools, or patients may present with abdominal masses with or without jaundice. If diagnosed late or untreated, choledochal cysts may result in severe complications secondary to biliary tract obstruction, including cholangitis and pancreatitis. One series of 36 Indian patients reported 13 patients, more frequently children than infants, who presented with cholangitis.[197] Cholangitis is more likely to be the presenting sign for choledochal cyst in adult patients.[42]

Treatment of cholangitis associated with choledochal cysts involves supportive treatment of the patient, including appropriate antibiotic treatment. In the past, cystenteric drainage procedures were used as temporizing treatments, and this type of repair was associated with high rates of complications, including recurrent bouts of cholangitis, stones, and cholangiocarcinoma in the remnant duct.[209] The current surgical goal is complete surgical excision; compared with internal drainage procedures, this approach is associated with lower rates of postoperative cholangitis and mortality.[181] Five types of choledochal cysts have been identified, with solitary extrahepatic cysts (type I) being encountered most frequently. After this type of cyst has been excised, reconstruction of the biliary tract may involve a Roux-en-Y choledochojejunostomy or hepatojejunostomy.

Patients who undergo this surgery also are at risk for developing postoperative ascending cholangitis, with an incidence of 8 to 19 percent.[73,227] Although recurrent cholangitis may lead to chronic liver disease in the long-term, antibiotic prophylaxis is not recommended routinely.[119]

Caroli disease describes congenital dilation of the intrahepatic and extrahepatic biliary tree characterized by pure ductal ectasia.[54,55] More commonly, dilation of the intrahepatic ducts is attributed to ductal plate malformation in association with congenital hepatic fibrosis. The association of ductal ectasia with congenital hepatic fibrosis is more common than without and is termed

Caroli syndrome. Both of these diseases may manifest as clinical signs of liver disease or renal disease secondary to the associated condition of autosomal recessive polycystic kidney disease, or both.[120,152,190] Dilation of the intrahepatic bile ducts results in biliary obstruction and places the patient at increased risk for development of intrahepatic stones and cholangitis, which significantly increases morbidity and mortality rates in Caroli disease and congenital hepatic fibrosis. Diagnosis is suspected in the case of recurrent cholangitis or portal hypertension of unknown etiology and can be confirmed by ultrasound or cholangiography (MRCP, ERCP, or percutaneous transhepatic cholangiography).[69]

Portosystemic shunting is considered the treatment of choice because this condition typically does not progress to liver failure. Suspicion of cholangitis, whether owing to signs of infection or sepsis or to laboratory results suggesting inflammation, should be confirmed via a diagnostic liver biopsy for culture. Treatment of cholangitis in the setting of Caroli disease may be difficult. Recurrent episodes may occur even after administration of intensive intravenous antibiotic therapy.[274] In some cases, drainage procedures may be used for refractory or recurrent infections. Orthotopic liver transplantation is the treatment of choice in recurrent, life-threatening episodes of cholangitis.[97,274]

Typically, patients with Caroli disease or Caroli syndrome present with cholangitis because the biliary malformation communicates with the extrahepatic biliary tree. Rarely, patients are seen with hepatic cysts associated with autosomal dominant polycystic kidney disease. These lesions are noncommunicating lesions and as such are not prone to infection. In the absence of a history of cholangitis, it is advisable that these rare patients not undergo invasive procedures of the biliary tree because of the risk for microbial seeding and subsequent suppurative cholangitis.[6,130,287]

Cholangitis After Endoscopic and Other Biliary Procedures

The overall complication rate for pediatric ERCP is reported from 0 to 11 percent and in adults cholangitis is a known complication of ERCP. In a series of 50 pediatric patients, low-grade fever, abdominal pain, nausea, and vomiting were reported as the most common complaints after undergoing ERCP; 1 patient was treated for mild cholangitis.[257] Another group reported three cases of sepsis in adults caused by multiresistant *Pseudomonas aeruginosa* after ERCP, ascribed to nosocomial transmission from the endoscope despite negative surveillance cultures.[58] In adults, and rarely in children, biliary sphincterotomy with or without stent placement can be used to alleviate a common bile duct obstruction, most frequently malignant.

The major complication of biliary stents is obstruction and subsequent upstream infection. The role of antibiotics or ursodeoxycholic acid to maintain stent patency has been examined and no conclusive evidence was demonstrated in favor of either one in the prevention of stent occlusion.[77,256] In certain children and adolescents with a history of recalcitrant cholangitis, percutaneous transhepatic biliary drainage is used by experienced centers to decompress the biliary tree upstream of the stricture or to dilate the stricture. This procedure is used primarily in patients with PSC and in transplant patients with biliary stenoses secondary to ischemia or rejection.* These patients may benefit from oral antibiotic prophylaxis, especially with ciprofloxacin because of its excellent biliary penetration.

CHOLECYSTITIS

Cholecystitis and its related complications are most commonly associated with cholelithiasis (gallstones); however, cholecystitis can occur without stone disease. An estimated 20 million individuals have gallbladder disease in the United States based on the National Health and Nutrition Examination Survey (NHANES) III report.[67] Although gallbladder-related diseases occur less often in children, there is a trend toward increasing rates of disease.[20,168] In one report from 2009, gallstones accounted for 4 to 6 per 100,000 hospital discharges in children younger than 15 years of age.[68] Recent data suggest that the obesity epidemic and related metabolic syndrome account for a greater proportion of gallstones in children.[122,168] Despite the predominance of obesity-related disease, children with hemolytic disorders remain at high risk for gallstones and related comorbidities. Similarly, several studies have reported much higher rates of complicated gallbladder disease in children than for adults, with common bile duct obstruction and gallstone pancreatitis occurring in up to 30 percent of children.[20,100,168]

Cholecystitis refers to inflammation of the gallbladder, which can occur in a variety of clinical scenarios. In adults, acute cholecystitis almost always is associated with gallstones—90 percent of adult cases occur secondary to gallstones. In contrast, 30 to 50 percent of pediatric cases are acalculous.[266] Acalculous cholecystitis is discussed separately at the end of this section. The most likely cause of calculous cholecystitis is gallstone obstruction of the cystic duct, which leads to increased intraluminal pressure with gallbladder distention, mucosal damage, and release of inflammatory mediators. The end result is acute inflammation of the gallbladder. Any bacterial infection is likely a secondary occurrence to biliary obstruction.[134]

ETIOLOGY AND PATHOGENESIS

Gallstones are divided into cholesterol or pigmented stones (black or brown), although they often are mixed

*References 12, 49, 77, 82, 95, 256.

in constituents. Stone formation occurs secondary to precipitation of insoluble bile content (cholesterol, bile pigments, and calcium salts). Cholesterol stones are formed when the balance of cholesterol, lecithin, and bile salts is such that cholesterol is no longer soluble and becomes supersaturated. Black pigmented stones can form when there is supersaturated bile or a decrease in the bile salt pool. This process includes an increase in bilirubin anions (as seen in unconjugated hyperbilirubinemia), an increase in unbound calcium, or a decrease in other factors that solubilize calcium and bilirubin.[126,191] Brown pigmented stones differ from black stones in that brown stones typically are more often related to infection and form within the common bile duct rather than the gallbladder in both children and adults. *O. sinensis* and *Ascaris lumbricoides* are major sources of brown pigmented stones in rural Asian countries.[103] Pigmented stones have been described with *E. coli*, *Pseudomonas* spp., *Staphylococcus* spp., *Enterobacter* spp., *Citrobacter* spp., and *Salmonella enterica* serovar Virchow.[15,53,265] There is also a strong relationship to urinary tract infections.[101] Ceftriaxone as well as other third-generation cephalosporins have been associated with cholelithiasis and sludge in this population, being reported in up to 38 percent in one series of children with biliary tract disease in Israel.[132]

The majority of data on children with pigmented stones comes from patients with hemolytic disease (e.g., sickle-cell anemia and thalassemia). There is a significant population of patients with biliary disease secondary to hereditary spherocytosis and other red cell membrane defects, pyruvate kinase deficiency, glucose-6-phosphate dehydrogenase deficiency, and autoimmune hemolytic anemia.[20,207,233] The prevalence of pigment gallstones in hemolytic disease increases with age. In children younger than 10 years old, the frequency is 12 to 14 percent; the frequency increases to 36 to 42 percent in individuals 10 to 20 years old.[21,230]

Until recently, pigmented stones were the predominant cause of gallstone disease and related morbidity in children in which up to 72 percent were involved.[76] However, in a recent 3-year report of over 400 pediatric patients with gallbladder disease, there was more than a threefold difference in obese patients with cholesterol stones compared with those with hemolytic disease.[168] This suggests a trend that children and adolescents are now more similar to adults with gallbladder disease than in years past.

Cholesterol stones are found most frequently in adults and have an increased prevalence in women from puberty to menopause and in obese patients.[225] Although boys and girls exhibit an equal incidence in gallstones at young ages, the incidence in girls increases significantly after puberty, and female predominance of gallstone disease continues through menopause.[68,185] Related risk factors for the presence of cholesterol stones in children and adolescents include ethnic background (Hispanic), obesity, pregnancy, and the use of oral contraceptives.[133,155,168,184] In addition, conditions that are associated with decreased ileal bile salt resorption predispose to stone formation. This association includes patients who have undergone ileal resection or bypass and patients with Crohn disease.[215]

TABLE 48-5 Conditions Associated with Pediatric Gallstone Disease

Biliary tract anomaly (e.g., choledochal cyst)
Cephalosporin use
Cystic fibrosis
Crohn disease
Genetic predisposition (e.g., *ABCB4, UGT1A1* mutations)
Hispanic or Latino ancestry
Hemolytic disorders (e.g., sickle cell anemia, hereditary spherocytosis)
Malabsorption
Metabolic syndrome
Obesity
Parasitic disease *(Ascaris lumbricoides)*
Pregnancy
Rapid weight loss
Solid-organ and hematologic transplant (e.g., liver, kidney, heart, bone marrow)
Total parenteral nutrition

Data from references 20, 76, 78, 141, and 168.

Total parenteral nutrition remains a significant risk factor for children to develop biliary tract disease and, in particular, gallstone formation.[204] This condition occurs more commonly in premature neonates, especially neonates with enteral diseases, in which prolonged fasting, sepsis, immaturity of the enterohepatic circulation of bile acids, small bowel bacterial overgrowth, and prolonged duration of total parenteral nutrition contribute to biliary stasis and increased prevalence of gallstones and sludge.* The occurrence of gallstones secondary to total parenteral nutrition does not lead to increased prevalence of complications, because many of these gallstones are clinically silent.

Gallstones in cystic fibrosis are common, with an estimated prevalence of gallstones in 10 to 30 percent. Symptomatic gallstones have been reported in 3.6 percent of affected pediatric patients.[244,271] Gallstones in cystic fibrosis are predominantly composed of a mixed pigment and smaller amounts of cholesterol. Stone formation is multifactorial but is related to altered lipid composition and altered motility.[102] Pathophysiology of pigmented stones in cystic fibrosis is also linked to ΔF508 mutations with fecal bile loss and altered enterohepatic circulation as well as *UGT1A1* mutations. Alterations in enterohepatic circulation have also been suggested as the mechanism for pigmented gallstone formation in Crohn disease.[26] Finally, children with chronic liver disease and, in particular, cholestasis are at increased risk for gallstone formation, owing to a deficit of biliary excretion.[280] The characteristics of the pediatric patient populations at risk for development of gallstones are summarized in Table 48-5.

In addition to the observations made in *UGT1A1* mutations, knowledge regarding the heredity of cholelithiasis continues to develop. A combination of genetic

*References 7, 129, 138, 196, 229, 253, 276.

predisposition and environmental risk factors is likely. In adults, a distinction is made between the "common" polygenic predisposition to gallstones and the rarer oligogenic predisposition. The first, polygenic group refers to patients with evidence of increased risk for developing biliary stone formation, including lithogenic *(LITH)* genes. The oligogenic group refers to a small group of patients with known mutations in genes involved in bile synthesis and export.[92,280] The clearest examples to date are defects in the *ABCB4* transporter in humans and the *Mdr2* homozygous null mutant in mice.[141,142,180,216] Both conditions lead to an increased propensity toward cholelithiasis and choledocholithiasis; however, whether these subjects have a profile of biliary tract infections different from that of the polygenic group is unknown.

CLINICAL PRESENTATION

Acute cholecystitis is characterized by abdominal pain with vomiting or nausea. The pain is referred to as "biliary colic" but is nonparoxysmal with a constant, sharp pain lasting 4 to 6 hours. Pain relents only if the obstructing stone in the cystic or common bile duct mobilizes but may persist or return with gallbladder wall inflammation. In children, differentiating between other forms of acute abdomen can be difficult because pain can be in the right upper quadrant but also exist in the mid epigastrium. Radiation to the left upper quadrant or the right shoulder can occur and, with peritoneal inflammation, poorly localize in the abdomen, corresponding to the gallbladder's somatic innervation.[239]

Jaundice is reported more frequently in younger children with acute cholecystitis and infrequently with acholic stools in infants. Although jaundice is typically associated in younger children, in one series of 50 children, no child younger than 6 years of age had jaundice.[204] If jaundice is present, choledocholithiasis or cholangitis should be strongly considered. Fever is not universally present in cholecystitis and has been reported to be as low as 11 percent in pediatric patients. Similarly, in a retrospective study of 198 adults who presented to an emergency department with acute cholecystitis, 71 percent were afebrile; of those with gangrenous acute cholecystitis, 59 percent were afebrile.[91]

Physical examination may reveal a tender right upper quadrant. On light palpation a small mass may be appreciated, corresponding to the swollen gallbladder. Contrasting this, the Murphy sign is positive when abdominal pain is elicited on deep palpation of the right upper quadrant corresponding to the liver edge or gallbladder and most apparent with deep inspiration. This can be appreciated during ultrasound examination and may be reported by the ultrasonographer. With a tense abdomen, rigidity suggests cholecystitis with local peritonitis and the patient may exhibit voluntary guarding or other peritoneal signs.

EVALUATION

Laboratory evaluation may show leukocytosis, although this finding is not universally present in adults or children with cholecystitis.[91,204] Even in gangrenous cholecystitis, up to 25% of patients may fail to have leukocytosis. Bilirubin levels may be elevated, especially in the setting of hemolysis. Conjugated hyperbilirubinemia may suggest cholangitis, choledocholithiasis, or sepsis-associated cholestasis. Friesen and Roberts reported 38 percent of patients with hyperbilirubinemia in a pediatric series, and levels of bilirubin to 4 mg/dL are reported in adults.[76,239] Serum aminotransferase, alkaline phosphatase, and γ-glutamyltransferase levels are also useful. Amylase and lipase values should be obtained because of the overlap of pancreatitis with both gallstone-related disease as well as viral hepatitides. Pediatric surgical consultation should be obtained in patients with possible acute cholecystitis or other forms of acute abdomen. However, if common bile duct obstruction or gallstone pancreatitis is suspected, pediatric gastroenterology consultation should be obtained for possible endoscopic management with ERCP.

Abdominal ultrasonography is the test of choice in establishing the diagnosis of cholecystitis. Findings include a thickened or irregular, hyperreflexive gallbladder wall with or without the presence of gallstones. Experienced sonographers may be able to elicit tenderness over the gallbladder, the "sonographic Murphy sign." Ultrasound is useful for detecting gallstones within the gallbladder in up to 90 percent of cases. However, the sensitivity of common bile duct stone detection is poor, ranging from 30 to 50 percent.[46,47,75] The finding of gallstones alone by sonogram does not indicate cholecystitis. Other false-positive studies may occur with the finding of thickened gallbladder walls in patients with hypoalbuminemia, renal failure, or heart failure.[194] The inability to visualize the gallbladder by ultrasonography may indicate the finding of a diseased, chronically obstructed gallbladder.[88]

Hepatobiliary scintigraphy (HIDA scan) can be useful in confirming the diagnosis of acute cholecystitis. The study employs a technetium-labeled iminodiacetic acid agent that is excreted into the bile ducts. Subsequent images are taken, which in a normal study should fill the gallbladder, extrahepatic ducts, and duodenum. In the case of cystic duct obstruction, a positive study fails to show the gallbladder filling. The sensitivity and specificity of this study are high, with results approaching 90 to 100 percent and 90 to 95 percent.[172,179,207] The test may be augmented by attempts to stimulate gallbladder contraction using a cholecystokinin analog.[94] Plain abdominal radiographs and oral cholecystography are rarely used in the modern era of biliary disease.

MANAGEMENT

The treatment plan for patients with gallstones depends on several factors, including patient symptoms, underlying conditions, and the presence of associated/concomitant choledocholithiasis. Asymptomatic patients with gallstones identified incidentally can often be managed conservatively, although guidelines in children are lacking. Symptomatic patients with large stones (>2 cm) may be considered for conservative nonoperative management or elective cholecystectomy.

Recommendations for evaluation of patients with sickle-cell disease include abdominal ultrasonography at 10 years of age.[249] When stones are detected, patients should be considered for early cholecystectomy, although this plan is controversial.[4,93,118,168,275] In patients with sickle-cell anemia, the benefit of timely surgery may outweigh the morbidity of associated abdominal pain crises and the overall morbidity and mortality of surgery in these patients, which increases with age. In patients with hereditary spherocytosis, ultrasound evaluation is recommended annually beginning at age 4, but severity of disease or coexistence of Gilbert syndrome may alter this.[254]

In acute calculous cholecystitis, laparoscopic cholecystectomy is the most common surgical treatment for children and is performed in over 95% of cases.[168] The preoperative management of patients includes hospital admission for monitoring, intravenous hydration, and pain control. Antibiotic therapy often is initiated, even without clinical evidence of sepsis or perforation. The choice of antibiotics is similar to that for cholangitis and typically covers gut luminal flora (e.g., a semisynthetic penicillin/β-lactamase combination or third- or fourth-generation cephalosporin with or without an aminoglycoside).[96,277,288] In high-risk patients in whom laparoscopic or open cholecystectomy is not feasible, cholecystotomy can be performed by interventional radiology or surgery. In adults, data suggest that early cholecystectomy should be performed in those with acute cholecystitis secondary to gallstone disease[205] and in biliary pancreatitis.[205]

The management of acalculous cholecystitis may vary based on whether it is acute or chronic, but overall limited data are available. In addition to treatment of underlying causes, options for therapy in acute acalculous cholecystitis include nonoperative therapy with nothing by mouth and intravenous hydration and nutrition, cholecystostomy, or cholecystectomy.[114,266] The majority of patients with chronic acalculous cholecystitis undergo cholecystectomy. With findings of microlithiasis by endoscopic sampling or by endoscopic ultrasonography, consideration for ERCP or cholecystectomy may be appropriate to prevent cholangitis or recurrent pancreatitis.[121] The role of cholecystectomy for biliary dyskinesia, a motility disorder related to acalculous cholecystitis, is debated in children.[172]

COMPLICATIONS

Even with prompt recognition and therapy, acute cholecystitis may lead to complications. Perforation of the gallbladder is rare but can also occur in the cystic duct or bile duct. Mirizzi syndrome is a spectrum in which a cystic duct stone obstructs the common bile duct and in advanced forms can lead to fistulas and perforation. An inflamed, obstructed gallbladder may wall off to form an intraluminal abscess or empyema, although gangrenous inflammation of the gallbladder is rare in children. A localized perforation may develop into a pericholecystic abscess that is palpable as a tender right upper quadrant mass on examination. Free perforation with peritonitis is associated with a 30 percent mortality rate, but it is found in only a few patients, estimated at 1 to 2 percent of adult patients.[144] In both gangrene and perforation, intravenous fluids, antibiotic therapy, and surgical evaluation are critical.

However, the most common complication of cholecystitis is common bile duct obstruction or choledocholithiasis. Recent reports in children describe rates of obstructive jaundice and pancreatitis in up to 30 percent in those with symptomatic gallbladder disease.[20,100,168,204] Choledocholithiasis can lead to ascending cholangitis but is a rare cause of cholangitis in pediatric patients. Choledocholithiasis should be suspected in all patients with gallstones, along with right or left upper quadrant pain, jaundice, fever, or pancreatitis. Common bile duct stones originating from the gallbladder can be found in patients with acute cholecystitis by transabdominal or endoscopic ultrasonography, MRCP, ERCP, or intraoperative cholangiography. Although no formal recommendations are available in children, adult guidelines recommend the consideration of ERCP with stone clearance if suspicion is high for common bile duct stones. Patients with a total bilirubin greater than 4 mg/dL or a visualized stone by abdominal ultrasonography are at high risk for stones, as well as patients who have *both* a common bile duct diameter greater than 6 mm and a total bilirubin value greater than 1.8 mg/dL.[161] Modifications are likely necessary in children, owing to a smaller bile duct diameter. In patients at low risk for common bile duct stone or recently passed stones, laparoscopic cholecystectomy with or without intraoperative cholangiography is appropriate.[205]

Acalculous Cholecystitis

Acalculous cholecystitis is a rare but important cause of cholecystitis in pediatric patients. Generally, acalculous gallbladder disease in children occurs in various clinical settings, ranging from congenital gallbladder abnormalities, to idiopathic gallbladder distention without inflammation (acute hydrops of the gallbladder), to acute or chronic cholecystitis (Table 48-6). The exact pathogenesis of acute acalculous cholecystitis is unclear;

TABLE 48-6 **Conditions Associated with Acalculous Gallbladder Disease**

Biliary tract anomaly (e.g., choledochal cyst)
Bone marrow transplant
Burns
Chemotherapy in oncology patients
Critical illness in patients in intensive care units
Crohn disease, Henoch-Schönlein purpura, Kawasaki disease, systemic lupus erythematosus
Infectious agents (atypical microbes)
Microlithiasis
Postoperative state (e.g., cardiac surgery)
Sepsis
Sludge
Systemic inflammatory states
Total parenteral nutrition
Traumatic spinal cord injury

Data from references 112, 115, 212, 245, 261, and 263.

however, stagnation of the bile may be an important factor, as suggested by several predisposing clinical conditions that lead to bile stasis. In addition, sphincter of Oddi spasm or dysfunction, as occurs after administration of opiates, could lead to retrograde reflux of inflammatory enzymes or infectious agents. Alternatively, changes in the gallbladder vascular supply may weaken the gallbladder mucosa and allow biliary components to damage the gallbladder wall.[83] Acalculous cholecystitis has been associated with many infectious and noninfectious clinical scenarios. Bacterial, viral, and parasitic agents have been implicated in the setting of acalculous cholecystitis.

Frequently, gallstone or biliary microlithiasis missed by standard diagnostic evaluations may lead the clinician to the presumptive diagnosis of acalculous cholecystitis. One study in adults revealed that 33 of 35 patients with abdominal pain and a negative transabdominal sonogram had gallbladder sludge and stones, and 21 of those patients also had common bile duct involvement.[170]

Reports of true acalculous disease have been described with systemic infection from *Salmonella* spp., *Mycoplasma pneumoniae*, *Leptospira* spp., *Brucella* spp., Rocky Mountain spotted fever, group A streptococci, group B streptococci, and *Staphylococcus aureus*.* Examination and culture of cholecystectomy specimens from patients with acalculous cholecystitis have revealed positive cultures for *Helicobacter* spp. and *Campylobacter jejuni*. Associations with viral disease include CMV, mumps virus, Epstein-Barr virus, and hepatitis A virus.† Acalculous cholecystitis has been associated with *Plasmodium falciparum* infections (malaria).[62,160,220,228,286] In immunosuppressed hosts, acalculous cholecystitis also has been described in fungal infections with *Candida* spp. or *Aspergillus* spp. or in parasitic infections with *Giardia lamblia* and *Cryptosporidium parvum*.[181]

Noninfectious systemic diseases associated with acalculous cholecystitis or hydrops include neoplastic disease (i.e., leukemia), Henoch-Schönlein purpura, lupus erythematosus, and Kawasaki disease.[33,108,164,248] Table 48-5 includes several conditions with which acalculous cholecystitis has been reported.‡

Clinical presentation is similar to that of patients with calculous disease, with right upper quadrant pain, nausea, vomiting, anorexia, and fever being the most common features. Leukocytosis, jaundice, or a palpable right upper quadrant mass also may be present.[266] The most common setting for acalculous disease is in critically ill or chronically ill patients with concurrent acute or chronic symptoms. A high degree of clinical suspicion is crucial in making the diagnosis in these populations because often the findings suggestive of cholecystitis are obscured by the patient's systemic disease. The most common sonographic finding is a thickened gallbladder wall and a possible sonographic Murphy sign. Gallbladder distention, sludge, or pericholecystic fluid also may be found on transabdominal ultrasonography.[114] In addition to ultrasound, the HIDA scan is an important tool in the evaluation of acalculous cholecystitis. Failure to visualize the gallbladder on scintigraphy and a low ejection fraction in response to a cholecystokinin analog are suggestive of acalculous disease.[163,179,200,290]

*References 8, 10, 56, 105, 166, 248, 258, 281.
†References 3, 13, 17, 25, 36, 80, 199.
‡References 112, 115, 212, 245, 261, 263.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Attasaranya S, Fogel EL, Lehman GA. Choledocholithiasis, ascending cholangitis, and gallstone pancreatitis. *Med Clin North Am* 2008;**92**:925–60.
11. Banerjee S, Shen B, Baron TH, et al. Antibiotic prophylaxis for GI endoscopy. *Gastrointest Endosc* 2008;**67**:791–8.
22. Bondoc AJ, Taylor JA, Alonso MH, et al. The beneficial impact of revision of Kasai portoenterostomy for biliary atresia: an institutional study. *Ann Surg* 2012;**255**:570–6.
37. Catalano OA, Sahani DV, Forcione DG, et al. Biliary infections: spectrum of imaging findings and management. *RadioGraphics* 2009;**29**:2059–80.
50. de Vries W, de Langen ZJ, Aronson DC, et al. Mortality of biliary atresia in children not undergoing liver transplantation in the Netherlands. *Pediatr Transplant* 2011;**15**:176–83.
51. de Vries W, de Langen ZJ, Groen H, et al. Biliary atresia in the Netherlands: outcome of patients diagnosed between 1987 and 2008. *J Pediatr* 2012;**160**:638–44.e2.
52. de Vries W, Homan-Van der Veen J, Hulscher JB, et al. Twenty-year transplant-free survival rate among patients with biliary atresia. *Clin Gastroenterol Hepatol* 2011;**9**:1086–91.
64. Edil BH, Cameron JL, Reddy S, et al. Choledochal cyst disease in children and adults: a 30-year single-institution experience. *J Am Coll Surg* 2008;**206**:1000–5; discussion 1005–8.
68. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States, part III: Liver, biliary tract, and pancreas. *Gastroenterology* 2009;**136**:1134–44.
71. Fawaz R, Jonas MM. Acute and chronic hepatitis. In: Wyllie R, Hyams JS, editors. *Pediatric Gastrointestinal and Liver*, 4th ed. Philadelphia: Saunders; 2011. p. 811–28.
98. Harpavat S, Raijman I, Hernandez JA, Fishman DS. Single-center experience of choledochoscopy in pediatric patients. *Gastrointest Endosc* 2012;**76**:685–8.
100. Herzog D, Bouchard G. High rate of complicated idiopathic gallstone disease in pediatric patients of a North American tertiary care center. *World J Gastroenterol* 2008;**14**:1544–8.
113. Ibrahim SH, Lindor KD. Current management of primary sclerosing cholangitis in pediatric patients. *Paediatr Drugs* 2011;**13**:87–95.
133. Ko CW, Beresford SA, Schulte SJ, et al. Insulin resistance and incident gallbladder disease in pregnancy. *Clin Gastroenterol Hepatol* 2008;**6**:76–81.
143. Lao OB, Larison C, Garrison M, et al. Steroid use after the Kasai procedure for biliary atresia. *Am J Surg* 2010;**199**:680–4.
144. Lee JG. Diagnosis and management of acute cholangitis. *Nat Rev Gastroenterol Hepatol* 2009;**6**:533–41.
155. Liu B, Beral V, Balkwill A. Childbearing, breastfeeding, other reproductive factors and the subsequent risk of hospitalization for gallbladder disease. *Int J Epidemiol* 2009;**38**:312–8.
161. Maple JT, Ben-Menachem T, Anderson MA, et al. The role of endoscopy in the evaluation of suspected choledocholithiasis. *Gastrointest Endosc* 2010;**71**:1–9.
162. Marcos LA, Terashima A, Gotuzzo E. Update on hepatobiliary flukes: fascioliasis, opisthorchiasis and clonorchiasis. *Curr Opin Infect Dis* 2008;**21**:523–30.
168. Mehta S, Lopez ME, Chumpitazi BP, et al. Clinical characteristics and risk factors for symptomatic pediatric gallbladder disease. *Pediatrics* 2012;**129**:e82–8.
171. Mishra K, Basu S, Roychoudhury S, et al. Liver abscess in children: an overview. *World J Pediatr* 2010;**6**:210–6.
172. Misra S. Is biliary scintigraphy a reliable diagnostic tool for biliary dyskinesia in children? *J Clin Gastroenterol* 2011;**45**:814–7.
180. Nakken KE, Labori KJ, Rodningen OK, et al. *ABCB4* sequence variations in young adults with cholesterol gallstone disease. *Liver Int* 2009;**29**:743–7.
192. Pashankar DS, Schreiber RA. The liver: bacterial, parasitic, and other infections. In: Kleinman RE, Goulet OJ, Mieli-Vergani G, et al. editors. *Walker's Pediatric Gastrointestinal Disease*, vol 2. 5th ed. Hamilton, Ontario: B. C. Decker; 2008. p. 865–74.

205. Reinders JS, Goud A, Timmer R, et al. Early laparoscopic cholecystectomy improves outcomes after endoscopic sphincterotomy for choledochocystolithiasis. *Gastroenterology* 2010;**138**:2315–20.
232. Seale MK, Catalano OA, Saini S, et al. Hepatobiliary-specific MR contrast agents: role in imaging the liver and biliary tree. *RadioGraphics* 2009;**29**:1725–48.
239. Silen W. Cholecystitis and other causes of acute pain in the right upper quadrant of the abdomen. In: Silen W, editor. *Cope's Early Diagnosis of the Acute Abdomen*. 22nd ed. New York: Oxford University Press; 2010. p. 131.
262. Tipnis NA, Dua KS, Werlin SL. A retrospective assessment of magnetic resonance cholangiopancreatography in children. *J Pediatr Gastroenterol Nutr* 2008;**46**:59–64.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 49

Pyogenic Liver Abscess

Sheldon L. Kaplan

Pyogenic liver abscesses are encountered infrequently in healthy children and generally have been reported more commonly in the compromised pediatric host. The rarity of liver abscesses may be explained partly by the rich blood supply, unique architecture, and extensive reticuloendothelial system of the liver, all of which present an effective barrier against bacterial invasion.

The precise incidence of pyogenic liver abscesses in children is unknown. Adult patients with hepatic abscesses constitute approximately 8 to 20/100,000 admissions; a 0.29 to 0.57 percent incidence of liver abscesses has been found in autopsies of adult patients.[11,50] In an early large series of liver abscesses in children, Dehner and Kissane[10] reported a 0.38 percent incidence at autopsy in patients younger than 15 years old; 11 of 27 (41%) patients were younger than 2 years, and 18 of 27 (67%) patients were younger than 6 years. In a review of admissions to Milwaukee Children's Hospital, Chusid[9] found 5 children (4 of whom were <6 months old) with at least one hepatic abscess, and estimated an incidence of 3 cases/100,000 admissions. Pineiro-Carrero and Andres[45] estimated an incidence of approximately 25 cases/100,000 admissions in their pediatric population (11 patients >14 years old). Pyogenic liver abscess in children is encountered more frequently in developing countries than in developed countries.[52] In Taiwan the overall incidence of pyogenic liver abscesses increased from 11.2/100,000 population in 1996 to 17.6/100,000 in 2004.[57]

PATHOGENESIS

Bacteria can establish an inflammatory focus in the liver by four major routes. Direct extension from contiguous structures is the most common mode in adults, accounting for up to 60 percent of hepatic abscesses in adults.[7,25,46,50] Biliary tract infection (cholangitis, cholecystitis), pancreatitis, and penetrating gastric or duodenal ulcer are examples of diseases associated with liver abscesses caused by extension from a contiguous focus of infection. In a review of this problem at St. Louis Children's Hospital, 3 of 27 children (11%) were considered to have a liver abscess secondary to inflammation of contiguous organs.[10] Although biliary tract disease occurs infrequently in children, ascending cholangitis is a particularly frequent complication of the hepatic portoenterostomy procedure for congenital biliary atresia and may lead to infections of the liver in such patients.[14,49] Liver abscesses also may develop as a complication of liver transplantation, especially if technical problems related to vascular supply or biliary drainage develop.[31]

The portal system is the second most common route by which bacteria may reach the liver in adults; 6 to 27 percent of liver abscesses in adults derive from this source.[25,33,50] In newborns, solitary liver abscesses, especially abscesses caused by gram-negative organisms, have complicated the use of umbilical vein catheterization or have been secondary to omphalitis.[6] Prematurity and necrotizing enterocolitis also are important predisposing conditions.[13]

Portal vein inflammation and bacteremia can be associated with infections within the abdominal cavity. Appendicitis, diverticulitis, perirectal abscesses, regional enteritis, ulcerative colitis, and omphalitis are possible sources of portal vein sepsis.[10] A pyogenic liver abscess may be an unusual complication of an ingested foreign body, with subsequent development of portal venous bacteremia.[39] Since antibiotics have been available, portal

REFERENCES

1. Abdo A, Klassen J, Urbanski S, et al. Reversible sclerosing cholangitis secondary to cryptosporidiosis in a renal transplant patient. *J Hepatol* 2003;**38**:688–91.
2. Acocella G, Mattiussi R, Nicolis FB, et al. Biliary excretion of antibiotics in man. *Gut* 1968;**9**:536–45.
3. Adolph MD, Bass SN, Lee SK, et al. Cytomegaloviral acalculous cholecystitis in acquired immunodeficiency syndrome patients. *Am Surg* 1993;**59**:679–84.
4. Al-Mulhim AS, Al-Mulhim FM, Al-Suwaiygh AA. The role of laparoscopic cholecystectomy in the management of acute cholecystitis in patients with sickle cell disease. *Am J Surg* 2002;**183**:668–72.
5. Allendorph M, Werlin SL, Geenen JE, et al. Endoscopic retrograde cholangiopancreatography in children. *J Pediatr* 1987; **110**:206–11.
6. Ananthakrishnan AN, Saeian K. Caroli's disease: identification and treatment strategy. *Curr Gastroenterol Rep* 2007;**9**:151–5.
7. Angelico M, Della Guardia P. Review article: hepatobiliary complications associated with total parenteral nutrition. *Aliment Pharmacol Ther* 2000;**14**(Suppl2):54–7.
8. Ashley D, Vade A, Challapalli M. Brucellosis with acute acalculous cholecystitis. *Pediatr Infect Dis J* 2000;**19**:1112–3.
9. Attasaranya S, Fogel EL, Lehman GA. Choledocholithiasis, ascending cholangitis, and gallstone pancreatitis. *Med Clin North Am* 2008;**92**:925–60.
10. Ayite A, Etey K, Tchatagba K, et al. [Acute acalculous cholecystitis of typhoid origin: a report of 5 cases]. *Tunis Med* 1996;**74**:257–60.
11. Banerjee S, Shen B, Baron TH, et al. Antibiotic prophylaxis for GI endoscopy. *Gastrointest Endosc* 2008;**67**:791–8.
12. Barrioz T, Ingrand P, Besson I, et al. Randomised trial of prevention of biliary stent occlusion by ursodeoxycholic acid plus norfloxacin. *Lancet* 1994;**344**:581–2.
13. Başar O, Kisacik B, Bozdogan E, et al. An unusual cause of acalculous cholecystitis during pregnancy: hepatitis A virus. *Dig Dis Sci* 2005;**50**:1532.
14. Behera A, Kochhar R, Dhavan S, et al. Isolated common bile duct tuberculosis mimicking malignant obstruction. *Am J Gastroenterol* 1997;**92**:2122–3.
15. Beiler HA, Kuntz C, Eckstein TM, et al. Cholecystolithiasis and infection of the biliary tract with *Salmonella virchow*—a very rare case in early childhood. *Eur J Pediatr Surg* 1995;**5**: 369–71.
16. Bergeron MG, Mendelson J, Harding GK, et al. Cefoperazone compared with ampicillin plus tobramycin for severe biliary tract infections. *Antimicrob Agents Chemother* 1988;**32**:1231–6.
17. Bigio EH, Haque AK. Disseminated cytomegalovirus infection presenting with acalculous cholecystitis and acute pancreatitis. *Arch Pathol Lab Med* 1989;**113**:1287–9.
18. Bilhartz L, Horton J. Gallstone disease and its complications. In: Feldman M, et al, editors. *Sleisenger & Fordtran's Gastrointestinal and Liver Disease*, 6th ed. Philadelphia: W. B. Saunders; 1998.
19. Boey JH, Way LW. Acute cholangitis. *Ann Surg* 1980;**191**: 264–70.
20. Bogue CO, Murphy AJ, Gerstle JT, et al. Risk factors, complications, and outcomes of gallstones in children: a single-center review. *J Pediatr Gastroenterol Nutr* 2010;**50**:303–8.
21. Bond LR, Hatty SR, Horn ME, et al. Gall stones in sickle cell disease in the United Kingdom. *BMJ (Clin Res Ed)* 1987; **295**:234–6.
22. Bondoc AJ, Taylor JA, Alonso MH, et al. The beneficial impact of revision of Kasai portoenterostomy for biliary atresia: an institutional study. *Ann Surg* 2012;**255**:570–6.
23. Bouche H, Housset C, Dumont JL, et al. AIDS-related cholangitis: diagnostic features and course in 15 patients. *J Hepatol* 1993; **17**:34–9.
24. Bowles BJ, Abdul-Ghani A, Zhang J, et al. Fifteen years' experience with an antirefluxing biliary drainage valve. *J Pediatr Surg* 1999;**34**:1711–4.
25. Brent AJ, Hull R, Jeffery KJ, et al. Acute cholecystitis complicating mumps. *Clin Infect Dis* 2006;**42**:302–3.
26. Brink MA, Slors JF, Keulemans YC, et al. Enterohepatic cycling of bilirubin: a putative mechanism for pigment gallstone formation in ileal Crohn's disease. *Gastroenterology* 1999;**116**: 1420–7.
27. Brogard JM, Caro-Sampara F, Westphal JF, et al. Biliary elimination and hepatic disposition of an association of piperacillin and tazobactam: experimental evaluation. *Drugs Exp Clin Res* 1994; **20**:247–55.
28. Brook I. Aerobic and anaerobic microbiology of biliary tract disease. *J Clin Microbiol* 1989;**27**:2373–5.
29. Brook I, Altman RP. The significance of anaerobic bacteria in biliary tract infection after hepatic portoenterostomy for biliary atresia. *Surgery* 1984;**95**:281–3.
30. Brown SJ, Desmond PV. Hepatotoxicity of antimicrobial agents. *Semin Liver Dis* 2002;**22**:157–67.
31. Bu LN, Chen HL, Chang CJ, et al. Prophylactic oral antibiotics in prevention of recurrent cholangitis after the Kasai portoenterostomy. *J Pediatr Surg* 2003;**38**:590–3.
32. Burgart LJ. Cholangitis in viral disease. *Mayo Clin Proc* 1998; **73**:479–82.
33. Büyükaşik Y, Koşar A, Demiroğlu H, et al. Acalculous acute cholecystitis in leukemia. *J Clin Gastroenterol* 1998;**27**:146–8.
34. Carpenter HA. Bacterial and parasitic cholangitis. *Mayo Clin Proc* 1998;**73**:473–8.
35. Carratala J, Fernandez-Sevilla A, Tubau F, et al. Emergence of fluoroquinolone-resistant *Escherichia coli* in fecal flora of cancer patients receiving norfloxacin prophylaxis. *Antimicrob Agents Chemother* 1996;**40**:503–5.
36. Casha P, Rifflet H, Renou C, et al. [Acalculous acute cholecystitis and viral hepatitis A]. *Gastroenterol Clin Biol* 2000;**24**:591–2.
37. Catalano OA, Sahani DV, Forcione DG, et al. Biliary infections: spectrum of imaging findings and management. *RadioGraphics* 2009;**29**:2059–80.
38. Cello JP. Acquired immunodeficiency syndrome cholangiopathy: spectrum of disease. *Am J Med* 1989;**86**:539–46.
39. Chacon JP, Criscuolo PD, Kobata CM, et al. Prospective randomized comparison of pefloxacin and ampicillin plus gentamicin in the treatment of bacteriologically proven biliary tract infections. *J Antimicrob Chemother* 1990;**26**(Suppl B):167–72.
40. Chand N, Sanyal AJ. Sepsis-induced cholestasis. *Hepatology* 2007;**45**:230–41.
41. Charcot M. *Leçons sur les maladies du fore des voices biliares et des veins. Paris, Faculte de Medicine de Paris*. Recueillies et Publiees Par Bourneville et Sevestre; 1877. p. 176–85.
42. Chaudhary A, Dhar P, Sachdev A, et al. Choledochal cysts—differences in children and adults. *Br J Surg* 1996;**83**:186–8.
43. Chaudhary S, Turner RB. Trimethoprim-sulfamethoxazole for cholangitis following hepatic portoenterostomy for biliary atresia. *J Pediatr* 1981;**99**:656–8.
44. Chen CC, Chang PY, Chen CL. Refractory cholangitis after Kasai's operation caused by candidiasis: a case report. *J Pediatr Surg* 1986;**21**:736–7.
45. Chitturi S, George J. Hepatotoxicity of commonly used drugs: nonsteroidal anti-inflammatory drugs, antihypertensives, antidiabetic agents, anticonvulsants, lipid-lowering agents, psychotropic drugs. *Semin Liver Dis* 2002;**22**:169–83.
46. Cronan JJ. US diagnosis of choledocholithiasis: a reappraisal. *Radiology* 1986;**161**:133–4.
47. Cronan JJ, Mueller PR, Simeone JF, et al. Prospective diagnosis of choledocholithiasis. *Radiology* 1983;**146**:467–9.
48. Davenport M, Kerkar N, Mieli-Vergani G, et al. Biliary atresia: the King's College Hospital experience (1974–1995). *J Pediatr Surg* 1997;**32**:479–85.
49. De Lédinghen V, Person B, Legoux JL, et al. Prevention of biliary stent occlusion by ursodeoxycholic acid plus norfloxacin: a multicenter randomized trial. *Dig Dis Sci* 2000;**45**:145–50.
50. de Vries W, de Langen ZJ, Aronson DC, et al. Mortality of biliary atresia in children not undergoing liver transplantation in the Netherlands. *Pediatr Transplant* 2011;**15**:176–83.
51. de Vries W, de Langen ZJ, Groen H, et al. Biliary atresia in the Netherlands: outcome of patients diagnosed between 1987 and 2008. *J Pediatr* 2012;**160**:638–44.e2.
52. de Vries W, Homan-Van der Veen J, Hulscher JB, et al. Twenty-year transplant-free survival rate among patients with biliary atresia. *Clin Gastroenterol Hepatol* 2011;**9**:1086–91.
53. Descos B, Bernard O, Brunelle F, et al. Pigment gallstones of the common bile duct in infancy. *Hepatology* 1984;**4**:678–83.
54. Desmet VJ. Congenital diseases of intrahepatic bile ducts: variations on the theme "ductal plate malformation." *Hepatology* 1992;**16**:1069–83.

55. Desmet VJ. Ludwig symposium on biliary disorders, part I: pathogenesis of ductal plate abnormalities. *Mayo Clin Proc* 1998;**73**:80–9.
56. Dickinson SJ, Corley G, Santulli TV. Acute cholecystitis as a sequel of scarlet fever. *Am J Dis Child* 1971;**121**:331–3.
57. Dimicoli S, Bensoussan D, Latger-Cannard V, et al. Complete recovery from *Cryptosporidium parvum* infection with gastroenteritis and sclerosing cholangitis after successful bone marrow transplantation in two brothers with X-linked hyper-IgM syndrome. *Bone Marrow Transplant* 2003;**32**:733–7.
58. Doherty DE, Falko JM, Lefkovitz N, et al. *Pseudomonas aeruginosa* sepsis following retrograde cholangiopancreatography (ERCP). *Dig Dis Sci* 1982;**27**:169–70.
59. Dooley JS, Hamilton-Miller JM, Brumfitt W, et al. Antibiotics in the treatment of biliary infection. *Gut* 1984;**25**:988–98.
60. Ducreux M, Buffet C, Lamy P, et al. Diagnosis and prognosis of AIDS-related cholangitis. *AIDS* 1995;**9**:875–80.
61. Dunham EC. Septicemia in the newborn. *Am J Dis Child* 1933;**45**:229.
62. Dylewski JS, Al-Azragi T. Acalculous cholecystitis associated with *Plasmodium falciparum* infection. *Clin Infect Dis* 1999;**29**:947–8.
63. Ecoffey C, Rothman E, Bernard O, et al. Bacterial cholangitis after surgery for biliary atresia. *J Pediatr* 1987;**111**:824–9.
64. Edil BH, Cameron JL, Reddy S, et al. Choledochal cyst disease in children and adults: a 30-year single-institution experience. *J Am Coll Surg* 2008;**206**:1000–5; discussion 1005–8.
65. Elsakr R, Johnson DA, Younes Z, et al. Antimicrobial treatment of intra-abdominal infections. *Dig Dis* 1998;**16**:47–60.
66. Ernest van Heurn LW, Saing H, Tam PK. Cholangitis after hepatic portoenterostomy for biliary atresia: a multivariate analysis of risk factors. *J Pediatr* 2003;**142**:566–71.
67. Everhart JE, Khare M, Hill M, et al. Prevalence and ethnic differences in gallbladder disease in the United States. *Gastroenterology* 1999;**117**:632–9.
68. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States, part III: liver, biliary tract, and pancreas. *Gastroenterology* 2009;**136**:1134–44.
69. Fagundes-Neto U, Schettini ST, Wehba J, et al. Caroli's disease in childhood: report of two new cases. *J Pediatr Gastroenterol Nutr* 1983;**2**:708–11.
70. Fan ST, Ng IO, Choi TK, et al. Tuberculosis of the bile duct: a rare cause of biliary stricture. *Am J Gastroenterol* 1989;**84**:413–4.
71. Fawaz R, Jonas MM. Acute and chronic hepatitis. In: Wyllie R, Hyams JS, editors. *Pediatric Gastrointestinal and Liver Disease*, 4th ed. Philadelphia, PA: Saunders; 2011. p. 811–28.
72. Fitoz S, Erden A, Boruban S. Magnetic resonance cholangiopancreatography of biliary system abnormalities in children. *Clin Imaging* 2007;**31**:93–101.
73. Fonkalsrud EW. Choledochal cysts. *Surg Clin North Am* 1973;**53**:1275–81.
74. Fox CJ, Harry RA, Cairns SR. A prospective series of out-patient endoscopic retrograde cholangiopancreatography. *Eur J Gastroenterol Hepatol* 2000;**12**:523–7.
75. Freitas ML, Bell RL, Duffy AJ. Choledocholithiasis: evolving standards for diagnosis and management. *World J Gastroenterol* 2006;**12**:3162–7.
76. Friesen CA, Roberts CC. Cholelithiasis: clinical characteristics in children. Case analysis and literature review. *Clin Pediatr (Phila)* 1989;**28**:294–8.
77. Galandi D, Schwarzer G, Bassler D, et al. Ursodeoxycholic acid and/or antibiotics for prevention of biliary stent occlusion. *Cochrane Database Syst Rev* 2002;(3):CD003043.
78. Ganschow R. Cholelithiasis in pediatric organ transplantation: detection and management. *Pediatr Transplant* 2002;**6**:91–6.
79. García-Ruiz JC, Hernández I, Muñoz F, et al. Cholangitis due to *Aspergillus fumigatus* in a patient with acute leukemia. *Clin Infect Dis* 1998;**26**:228–9.
80. Geier A, Fickert P, Trauner M. Mechanisms of disease: mechanisms and clinical implications of cholestasis in sepsis. *Nat Clin Pract Gastroenterol Hepatol* 2006;**3**:574–85.
81. Gerecht WB, Henry NK, Hoffman WW, et al. Prospective randomized comparison of mezlocillin therapy alone with combined ampicillin and gentamicin therapy for patients with cholangitis. *Arch Intern Med* 1989;**149**:1279–84.
82. Ghosh S, Palmer KR. Prevention of biliary stent occlusion using cyclical antibiotics and ursodeoxycholic acid. *Gut* 1994;**35**:1757–9.
83. Glenn F, Becker CG. Acute acalculous cholecystitis: an increasing entity. *Ann Surg* 1982;**195**:131–6.
84. Goddard EA, Mouton SC, Westwood AT, et al. Cryptosporidiosis of the gastrointestinal tract associated with sclerosing cholangitis in the absence of documented immunodeficiency: *Cryptosporidium parvum* and sclerosing cholangitis in an immunocompetent child. *J Pediatr Gastroenterol Nutr* 2000;**31**:317–20.
85. Goessling W, Chung RT. Amebic liver abscess. *Curr Treat Options Gastroenterol* 2002;**5**:443–9.
86. Gottrand F, Bernard O, Hadchouel M, et al. Late cholangitis after successful surgical repair of biliary atresia. *Am J Dis Child* 1991;**145**:213–5.
87. Grant A, Sargent C, Weller IV, et al. Disseminated cytomegalovirus infection. *Genitourin Med* 1992;**68**:75–9.
88. Greenberg M, Kangarloo H, Cochran ST, et al. The ultrasonographic diagnosis of cholecystitis and cholelithiasis in children. *Radiology* 1980;**137**:745–9.
89. Gremse DA, Bucuvalas JC, Bongiovanni GL. Papillary stenosis and sclerosing cholangitis in an immunodeficient child. *Gastroenterology* 1989;**96**:1600–3.
90. Greulich T, Kohler B. [Obstructive jaundice caused by spontaneous rupture of an echinococcus granulosus cyst into the bile duct system]. *Z Gastroenterol* 2000;**38**:301–6.
91. Gruber PJ, Silverman RA, Gottesfeld S, et al. Presence of fever and leukocytosis in acute cholecystitis. *Ann Emerg Med* 1996;**28**:273–7.
92. Grünhage F, Lammert F. Gallstone disease: pathogenesis of gallstones: a genetic perspective. *Best Pract Res Clin Gastroenterol* 2006;**20**:997–1015.
93. Haberkern CM, Neumayr LD, Orringer EP, et al. Cholecystectomy in sickle cell anemia patients: perioperative outcome of 364 cases from the national preoperative transfusion study. Preoperative Transfusion in Sickle Cell Disease Study Group. *Blood* 1997;**89**:1533–42.
94. Hadigan C, Fishman SJ, Connolly LP, et al. Stimulation with fatty meal (Lipomul) to assess gallbladder emptying in children with chronic acalculous cholecystitis. *J Pediatr Gastroenterol Nutr* 2003;**37**:178–82.
95. Halm U, Schiefke I, Fleig WE, et al. Ofloxacin and ursodeoxycholic acid versus ursodeoxycholic acid alone to prevent occlusion of biliary stents: a prospective, randomized trial. *Endoscopy* 2001;**33**:491–4.
96. Hanau LH, Steigbigel NH. Acute (ascending) cholangitis. *Infect Dis Clin North Am* 2000;**14**:521–46.
97. Harjai MM, Bal RK. Caroli syndrome. *Pediatr Surg Int* 2000;**16**:431–2.
98. Harpavat S, Raijman I, Hernandez JA, Fishman DS. Single-center experience of choledochoscopy in pediatric patients. *Gastrointest Endosc* 2012;**76**:685–8.
99. Hays DM, Snyder WH. Life-span in untreated biliary atresia. *Surgery* 1963;**54**:373–5.
100. Herzog D, Bouchard G. High rate of complicated idiopathic gallstone disease in pediatric patients of a North American tertiary care center. *World J Gastroenterol* 2008;**14**:1544–8.
101. Hes FJ, de Jong TP, Bax NM, et al. Urinary tract infections and cholelithiasis in early childhood. *J Pediatr Gastroenterol Nutr* 1995;**21**:319–21.
102. Heubi J. Diseases of the gallbladder in infancy, childhood and adolescence. In: Suchy FJ, Sokol RJ, Balistreri WF, editors. *Liver Disease in Children*, 3rd ed. Cambridge: Cambridge University Press; 2007. p. 358.
103. Hikasa Y, Takabayashi A, Sato T, et al. Mechanism of the formation of bilirubin calcium stones and black stones. *Nihon Geka Hokan* 1986;**55**:3–24.
104. Hirata K, Torigoe T, Fukuda M, et al. [A case of acute acalculous cholecystitis due to *Salmonella*]. *Nihon Shokakibyo Gakkai Zasshi* 1996;**93**:137–40.
105. Hitch DC, Lilly JR. Identification, quantification, and significance of bacterial growth within the biliary tract after Kasai's operation. *J Pediatr Surg* 1978;**13**:563–9.
106. Hitch DC, Lilly JR, Reller LB, et al. Biliary flora and antimicrobial concentrations after Kasai's operation. *J Pediatr Surg* 1979;**14**:648–52.
107. Honna T, Tsuchida Y, Kawarasaki H, et al. Further experience with the antireflux valve to prevent ascending cholangitis in biliary atresia. *J Pediatr Surg* 1997;**32**:1450–2.

108. Hou JW, Chang MH, Wu MH, et al. Kawasaki disease complicated by gallbladder hydrops mimicking acute abdomen: a report of three cases. *Zhonghua Min Guo Xiao Er Ke Yi Xue Hui Za Zhi* 1989;**30**:52–60.
109. Houwen RH, Bijleveld CM, de Vries-Hospers HG. Ciprofloxacin for cholangitis after hepatic portoenterostomy. *Lancet* 1987; **1**:1367.
110. Houwen RH, Zwierstra RP, Severijnen RS, et al. Prognosis of extrahepatic biliary atresia. *Arch Dis Child* 1989;**64**:214–8.
111. Huang T, Bass JA, Williams RD. The significance of biliary pressure in cholangitis. *Arch Surg* 1969;**98**:629–32.
112. Hyams JS, Baker E, Schwartz AN, et al. Acalculous cholecystitis in Crohn's disease. *J Adolesc Health Care* 1989;**10**:151–4.
113. Ibrahim SH, Lindor KD. Current management of primary sclerosing cholangitis in pediatric patients. *Paediatr Drugs* 2011; **13**:87–95.
114. Imamoğlu M, Sarihan H, Sari A, et al. Acute acalculous cholecystitis in children: diagnosis and treatment. *J Pediatr Surg* 2002; **37**:36–9.
115. Imhof M, Raunest J, Ohmann C, et al. Acute acalculous cholecystitis complicating trauma: a prospective sonographic study. *World J Surg* 1992;**16**:1160–6.
116. Investigators of the Pipercillin/Tazobactam Intra-Abdominal Infection Study Group. Results of the North American trial of pipercillin/tazobactam compared with clindamycin and gentamicin in the treatment of severe intra-abdominal infections. *Eur J Surg* 1994;**573**:61–6.
117. Jacobs RF. Ceftriaxone-associated cholecystitis. *Pediatr Infect Dis J* 1988;**7**:434–6.
118. Johna S, Shaul D, Taylor EW, et al. Laparoscopic management of gallbladder disease in children and adolescents. *JSLS* 1997;**1**: 241–5.
119. Joseph VT. Surgical techniques and long-term results in the treatment of choledochal cyst. *J Pediatr Surg* 1990;**25**:782–7.
120. Jung G, Benz-Bohm G, Kugel H, et al. MR cholangiography in children with autosomal recessive polycystic kidney disease. *Pediatr Radiol* 1999;**29**:463–6.
121. Jungst C, Kullak-Ublick GA, Jungst D. Gallstone disease: microlithiasis and sludge. *Best Pract Res Clin Gastroenterol* 2006;**20**:1053–62.
122. Kaechele V, Wabitsch M, Thiere D, et al. Prevalence of gallbladder stone disease in obese children and adolescents: influence of the degree of obesity, sex, and pubertal development. *J Pediatr Gastroenterol Nutr* 2006;**42**:66–70.
123. Karrer FM, Lilly JR, Stewart BA, et al. Biliary atresia registry, 1976 to 1989. *J Pediatr Surg* 1990;**25**:1076–80; discussion 1081.
124. Kasai M, Suzuki S. A new operation for "noncorrectable" biliary atresia: hepatic portoenterostomy. *Shujyutsu* 1959;**13**:733–9.
125. Kasai M, Watanabe I, Ohi R. Follow-up studies of long term survivors after hepatic portoenterostomy for "noncorrectible" biliary atresia. *J Pediatr Surg* 1975;**10**:173–82.
126. Keida Y, Nakano T, Tabata M, et al. Significance of different conjugate forms of bilirubin in the formation of pigment gallstones. *J Gastroenterol Hepatol* 1991;**6**:595–8.
127. Keighley MR, Drysdale RB, Quoraishi AH, et al. Antibiotic treatment of biliary sepsis. *Surg Clin North Am* 1975;**55**: 1379–90.
128. Keighley MR, Drysdale RB, Quoraishi AH, et al. Antibiotics in biliary disease: the relative importance of antibiotic concentrations in the bile and serum. *Gut* 1976;**17**:495–500.
129. Kelly DA. Liver complications of pediatric parenteral nutrition—epidemiology. *Nutrition* 1998;**14**:153–7.
130. Kerkar N, Norton K, Suchy FJ. The hepatic fibrocystic diseases. *Clin Liver Dis* 2006;**10**:55–71, v–vi.
131. Khuroo MS. Ascariasis. *Gastroenterol Clin North Am* 1996;**25**: 553–77.
132. Klar A, Branski D, Akerman Y, et al. Sludge ball, pseudolithiasis, cholelithiasis and choledocholithiasis from intrauterine life to 2 years: a 13-year follow-up. *J Pediatr Gastroenterol Nutr* 2005;**40**: 477–80.
133. Ko CW, Beresford SA, Schulte SJ, et al. Insulin resistance and incident gallbladder disease in pregnancy. *Clin Gastroenterol Hepatol* 2008;**6**:76–81.
134. Ko CW, Sekijima JH, Lee SP. Biliary sludge. *Ann Intern Med* 1999;**130**:301–11.
135. Kobayashi A, Utsunomiya T, Obe Y, et al. Ascending cholangitis after successful surgical repair of biliary atresia. *Arch Dis Child* 1973;**48**:697–703.
136. Kok KY, Yapp SK. Tuberculosis of the bile duct: a rare cause of obstructive jaundice. *J Clin Gastroenterol* 1999;**29**:161–4.
137. Kosloske AM. Acute abdomen due to acute cholangitis in a leukemic child. *Pediatrics* 1975;**56**:469–71.
138. Kubota A, Yonekura T, Hoki M, et al. Total parenteral nutrition–associated intrahepatic cholestasis in infants: 25 years' experience. *J Pediatr Surg* 2000;**35**:1049–51.
139. Kuhls TL, Jackson MA. Diagnosis and treatment of the febrile child following hepatic portoenterostomy. *Pediatr Infect Dis* 1985;**4**:487–90.
140. Kulaksiz H, Rudolph G, Kloeters-Plachky P, et al. Biliary *Candida* infections in primary sclerosing cholangitis. *J Hepatol* 2006;**45**:711–6.
141. Lammert F, Sauerbruch T. Mechanisms of disease: the genetic epidemiology of gallbladder stones. *Nat Clin Pract Gastroenterol Hepatol* 2005;**2**:423–33.
142. Lammert F, Wang DQ, Hillebrandt S, et al. Spontaneous cholecysto- and hepatolithiasis in mdr2–/– mice: a model for low phospholipid-associated cholelithiasis. *Hepatology* 2004;**39**: 117–28.
143. Lao OB, Larison C, Garrison M, et al. Steroid use after the Kasai procedure for biliary atresia. *Am J Surg* 2010;**199**: 680–4.
144. Lee JG. Diagnosis and management of acute cholangitis. *Nat Rev Gastroenterol Hepatol* 2009;**6**:533–41.
145. Lefkowitch JH. Pathology of AIDS-related liver disease. *Dig Dis* 1994;**12**:321–30.
146. Leung JW, Liu YL, Lau GC, et al. Bacteriologic analyses of bile and brown pigment stones in patients with acute cholangitis. *Gastrointest Endosc* 2001;**54**:340–5.
147. Levy C, Lindor KD. Primary sclerosing cholangitis: epidemiology, natural history, and prognosis. *Semin Liver Dis* 2006;**26**: 22–30.
148. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr* 1997;**131**:47–54.
149. Lewis RT, Goodall RG, Marien B, et al. Biliary bacteria, antibiotic use, and wound infection in surgery of the gallbladder and common bile duct. *Arch Surg* 1987;**122**:44–7.
150. Lilly JR, Hitch DC. Postoperative ascending cholangitis following portoenterostomy for biliary atresia: measures for control. *World J Surg* 1978;**2**:581–5.
151. Lin JI, Kabir MA, Tseng HC, et al. Hepatobiliary dysfunction as the initial manifestation of disseminated cryptococcosis. *J Clin Gastroenterol* 1999;**28**:273–5.
152. Lipschitz B, Berdon WE, Defelice AR, et al. Association of congenital hepatic fibrosis with autosomal dominant polycystic kidney disease: report of a family with review of literature. *Pediatr Radiol* 1993;**23**:131–3.
153. Lipsett PA, Pitt HA. Acute cholangitis. *Front Biosci* 2003;**8**: s1229–1239.
154. Lipsett PA, Pitt HA, Colombani PM, et al. Choledochal cyst disease: a changing pattern of presentation. *Ann Surg* 1994; **220**:644–52.
155. Liu B, Beral V, Balkwill A. Childbearing, breastfeeding, other reproductive factors and the subsequent risk of hospitalization for gallbladder disease. *Int J Epidemiol* 2009;**38**:312–8.
156. Liu KJ, Atten MJ, Donahue PE. Cholestasis in patients with acquired immunodeficiency syndrome: a surgeon's perspective. *Am Surg* 1997;**63**:519–24.
157. Livingston EH. Endoscopic biliary drainage for acute cholangitis. *N Engl J Med* 1992;**327**:1176.
158. López-Santamaria M, Martinez L, Hierro L, et al. Late biliary complications in pediatric liver transplantation. *J Pediatr Surg* 1999;**34**:316–20.
159. MacCarty RL, LaRusso NF, Wiesner RH, et al. Primary sclerosing cholangitis: findings on cholangiography and pancreatography. *Radiology* 1983;**149**:39–44.
160. Maggi P, Coppola SL, Lamargese V, et al. Acute acalculous cholecystitis associated with co-infection by *Plasmodium falciparum* and *Plasmodium vivax*. *J Infect* 2002;**44**:136–7.
161. Maple JT, Ben-Menachem T, Anderson MA, et al. The role of endoscopy in the evaluation of suspected choledocholithiasis. *Gastrointest Endosc* 2010;**71**:1–9.

162. Marcos LA, Terashima A, Gotuzzo E. Update on hepatobiliary flukes: fascioliasis, opisthorchiasis and clonorchiasis. *Curr Opin Infect Dis* 2008;**21**:523–30.
163. Mariat G, Mahul P, Prévt N, et al. Contribution of ultrasonography and cholescintigraphy to the diagnosis of acute acalculous cholecystitis in intensive care unit patients. *Intensive Care Med* 2000;**26**:1658–63.
164. McCrindle BW, Wood RA, Nussbaum AR. Henoch-Schönlein syndrome: unusual manifestations with hydrops of the gallbladder. *Clin Pediatr (Phila)* 1988;**27**:254–6.
165. McDiarmid SV. Management of the pediatric liver transplant patient. *Liver Transpl* 2001;**7**:S77–86.
166. McKiernan J, O'Brien DJ, Dundon S. Leptospirosis and acalculous cholecystitis. *Ir Med J* 1976;**69**:71–2.
167. McLauchlin J, Amar CF, Pedraza-Díaz S, et al. Polymerase chain reaction–based diagnosis of infection with *Cryptosporidium* in children with primary immunodeficiencies. *Pediatr Infect Dis J* 2003;**22**:329–35.
168. Mehta S, Lopez ME, Chumpitazi BP, et al. Clinical characteristics and risk factors for symptomatic pediatric gallbladder disease. *Pediatrics* 2012;**129**:e82–8.
169. Meijer WS, Schmitz PI, Jeekel J. Meta-analysis of randomized, controlled clinical trials of antibiotic prophylaxis in biliary tract surgery. *Br J Surg* 1990;**77**:283–90.
170. Mirbagheri SA, Mohamadnejad M, Nasiri J, et al. Prospective evaluation of endoscopic ultrasonography in the diagnosis of biliary microlithiasis in patients with normal transabdominal ultrasonography. *J Gastrointest Surg* 2005;**9**:961–4.
171. Mishra K, Basu S, Roychoudhury S, et al. Liver abscess in children: an overview. *World J Pediatr* 2010;**6**:210–6.
172. Misra S. Is biliary scintigraphy a reliable diagnostic tool for biliary dyskinesia in children? *J Clin Gastroenterol* 2011;**45**:814–7.
173. Mones RL, DeFelice AR, Preud'Homme D. Use of neomycin as the prophylaxis against recurrent cholangitis after Kasai portoenterostomy. *J Pediatr Surg* 1994;**29**:422–4.
174. Mortele KJ, McTavish J, Ros PR. Current techniques of computed tomography: helical CT, multidetector CT, and 3D reconstruction. *Clin Liver Dis* 2002;**6**:29–52.
175. Moseley RH. Sepsis and cholestasis. *Clin Liver Dis* 1999;**3**: 465–75.
176. Mukhopadhyay S, Monga A, Rana SS, et al. AIDS cholangiopathy as initial presentation of HIV infection. *Trop Gastroenterol* 2001; **22**:29–30.
177. Muller EL, Pitt HA, Thompson JE, et al. Antibiotics in infections of the biliary tract. *Surg Gynecol Obstet* 1987;**165**:285–92.
178. Muraji T, Higashimoto Y. The improved outlook for biliary atresia with corticosteroid therapy. *J Pediatr Surg* 1997;**32**:1103–6; discussion 1106–7.
179. Nadel HR. Hepatobiliary scintigraphy in children. *Semin Nucl Med* 1996;**26**:25–42.
180. Nakken KE, Labori KJ, Rodningen OK, et al. *ABCB4* sequence variations in young adults with cholesterol gallstone disease. *Liver Int* 2009;**29**:743–7.
181. Nash JA, Cohen SA. Gallbladder and biliary tract disease in AIDS. *Gastroenterol Clin North Am* 1997;**26**:323–35.
182. National Institutes of Health. NIH state-of-the-science statement on endoscopic retrograde cholangiopancreatography (ERCP) for diagnosis and therapy. *NIH Consens State Sci Statements* 2002; **19**:1–26.
183. Navarro VJ, Senior JR. Drug-related hepatotoxicity. *N Engl J Med* 2006;**354**:731–9.
184. Nervi F, Miquel JF, Alvarez M, et al. Gallbladder disease is associated with insulin resistance in a high risk Hispanic population. *J Hepatol* 2006;**45**:299–305.
185. Nilsson S. Gallbladder disease and sex hormones: a statistical study. *Acta Chir Scand* 1966;**132**:275–9.
186. O'Connor MJ, Schwartz ML, McQuarrie DG, et al. Acute bacterial cholangitis: an analysis of clinical manifestation. *Arch Surg* 1982;**117**:437–41.
187. Odiévre M, Valayer J, Razemon-Pinta M, et al. Hepatic portoenterostomy or cholecystostomy in the treatment of extrahepatic biliary atresia: a study of 49 cases. *J Pediatr* 1976;**88**:774–9.
188. Odom FC, Oliver BB, Kline M, et al. Gallbladder disease in patients 20 years of age and under. *South Med J* 1976;**69**: 1299–300.
189. Ohi R. Surgery for biliary atresia. *Liver* 2001;**21**:175–82.
190. Onuchic LF, Furu L, Nagasawa Y, et al. *Pkhd1*, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats. *Am J Hum Genet* 2002;**70**:1305–17.
191. Ostrow JD. Bilirubin solubility and the etiology of pigment gallstones. *Prog Clin Biol Res* 1984;**152**:53–69.
192. Pashankar DS, Schreiber RA. The liver: bacterial, parasitic, and other infections. In: Kleinman RE, Goulet OJ, Mieli-Vergani G, et al, editors. *Walker's Pediatric Gastrointestinal Disease*, vol 2. 5th ed. Hamilton, Ontario: B. C. Decker; 2008. p. 865–74.
193. Patino C, Fontes B, Poggetti RS, et al. Bile duct-duodenal fistula caused by AIDS/HIV-associated tuberculosis. *Rev Hosp Clin Fac Med Sao Paulo* 2003;**58**:223–6.
194. Patriquin HB, DiPietro M, Barber FE, et al. Sonography of thickened gallbladder wall: causes in children. *AJR Am J Roentgenol* 1983;**141**:57–60.
195. Pessa ME, Hawkins IF, Vogel SB. The treatment of acute cholangitis: percutaneous transhepatic biliary drainage before definitive therapy. *Ann Surg* 1987;**205**:389–92.
196. Pitt HA, King 3rd W, Mann LL, et al. Increased risk of cholelithiasis with prolonged total parenteral nutrition. *Am J Surg* 1983;**145**:106–12.
197. Poddar U, Thapa BR, Chhabra M, et al. Choledochal cysts in infants and children. *Indian Pediatr* 1998;**35**:613–8.
198. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993;**328**:95–9.
199. Prassouli A, Panagiotou J, Vakaki M, et al. Acute acalculous cholecystitis as the initial presentation of primary Epstein-Barr virus infection. *J Pediatr Surg* 2007;**42**:E11–13.
200. Prévôt N, Mariat G, Mahul P, et al. Contribution of cholescintigraphy to the early diagnosis of acute acalculous cholecystitis in intensive-care-unit patients. *Eur J Nucl Med* 1999;**26**:1317–25.
201. Rana SS, Bhasin DK, Nanda M, et al. Parasitic infestations of the biliary tract. *Curr Gastroenterol Rep* 2007;**9**:156–64.
202. Ratanarapee S, Pausawasdi A. Tuberculosis of the common bile duct. *HPB Surg* 1991;**3**:205–8.
203. Reddy SI, Grace ND. Liver imaging: a hepatologist's perspective. *Clin Liver Dis* 2002;**6**:297–310.
204. Reif S, Sloven DG, Lebenthal E. Gallstones in children: characterization by age, etiology, and outcome. *Am J Dis Child* 1991; **145**:105–8.
205. Reinders JS, Goud A, Timmer R, et al. Early laparoscopic cholecystectomy improves outcomes after endoscopic sphincterotomy for choledochocystolithiasis. *Gastroenterology* 2010;**138**:2315–20.
206. Rerknimitr R, Fogel EL, Kalayci C, et al. Microbiology of bile in patients with cholangitis or cholestasis with and without plastic biliary endoprosthesis. *Gastrointest Endosc* 2002;**56**:885–9.
207. Rescorla FJ. Cholelithiasis, cholecystitis, and common bile duct stones. *Curr Opin Pediatr* 1997;**9**:276–82.
208. Reynolds BM, Dargan EL. Acute obstructive cholangitis; a distinct clinical syndrome. *Ann Surg* 1959;**150**:299–303.
209. Rha SY, Stovroff MC, Glick PL, et al. Choledochal cysts: a ten year experience. *Am Surg* 1996;**62**:30–4.
210. Roberts EA. Primary sclerosing cholangitis in children. *J Gastroenterol Hepatol* 1999;**14**:588–93.
211. Rodrigues F, Davies EG, Harrison P, et al. Liver disease in children with primary immunodeficiencies. *J Pediatr* 2004;**145**:333–9.
212. Romero Ganuza FJ, La Banda G, Montalvo R, et al. Acute acalculous cholecystitis in patients with acute traumatic spinal cord injury. *Spinal Cord* 1997;**35**:124–8.
213. Ros PR, Mortele KJ. Hepatic imaging: an overview. *Clin Liver Dis* 2002;**6**:1–16.
214. Roseau G, Palazzo L, Dumontier I, et al. Endoscopic ultrasonography in the evaluation of pediatric digestive diseases: preliminary results. *Endoscopy* 1998;**30**:477–81.
215. Roslyn JJ, Pitt HA, Mann LL, et al. Gallbladder disease in patients on long-term parenteral nutrition. *Gastroenterology* 1983;**84**: 148–54.
216. Rosmorduc O, Hermelin B, Boelle PY, et al. *ABCB4* gene mutation–associated cholelithiasis in adults. *Gastroenterology* 2003;**125**:452–9.
217. Rothenberg SS, Schroter GP, Karrer FM, et al. Cholangitis after the Kasai operation for biliary atresia. *J Pediatr Surg* 1989;**24**:729–32.

218. Rusin JA, Sivit CJ, Rakusan TA, et al. AIDS-related cholangitis in children: sonographic findings. *AJR Am J Roentgenol* 1992;**159**: 626–7.
219. Ryckman FC, Alonso MH, Bucuvalas JC, et al. Long-term survival after liver transplantation. *J Pediatr Surg* 1999;**34**:845–849; discussion 849–50.
220. Saha A, Batra P, Vilhekar KY, et al. Acute acalculous cholecystitis in a child with *Plasmodium falciparum* malaria. *Ann Trop Paediatr* 2005;**25**:141–2.
221. Saharia PC, Cameron JL. Clinical management of acute cholangitis. *Surg Gynecol Obstet* 1976;**142**:369–72.
222. Saing H, Tam PK, Choi TK, et al. Childhood recurrent pyogenic cholangitis. *J Pediatr Surg* 1988;**23**:424–9.
223. Saini S. Imaging of the hepatobiliary tract. *N Engl J Med* 1997;**336**:1889–94.
224. Saint-Vil D, Luks FI, Lebel P, et al. Infectious complications of pediatric liver transplantation. *J Pediatr Surg* 1991;**26**:908–13.
225. Sama C, Labate AM, Taroni F, et al. Epidemiology and natural history of gallstone disease. *Semin Liver Dis* 1990;**10**: 149–58.
226. Samonis G, Gikas A, Toloudis P, et al. Prospective study of the impact of broad-spectrum antibiotics on the yeast flora of the human gut. *Eur J Clin Microbiol Infect Dis* 1994;**13**:665–7.
227. Samuel M, Spitz L. Choledochal cyst: varied clinical presentations and long-term results of surgery. *Eur J Pediatr Surg* 1996;**6**: 78–81.
228. Sánchez R, Portilla J, Boix V, et al. Acalculous cholecystitis associated with *Plasmodium falciparum* malaria. *Clin Infect Dis* 2000;**31**:622–3.
229. Sandhu IS, Jarvis C, Everson GT. Total parenteral nutrition and cholestasis. *Clin Liver Dis* 1999;**3**:489–508.
230. Sarnaik S, Slovis TL, Corbett DP, et al. Incidence of cholelithiasis in sickle cell anemia using the ultrasonic gray-scale technique. *J Pediatr* 1980;**96**:1005–8.
231. Scott AJ, Khan GA. Origin of bacteria in bile duct bile. *Lancet* 1967;**2**:790–2.
232. Seale MK, Catalano OA, Saini S, et al. Hepatobiliary-specific MR contrast agents: role in imaging the liver and biliary tree. *RadioGraphics* 2009;**29**:1725–48.
233. Senaati S, Gumruk FU, Delbakhsh P, et al. Gallbladder pathology in pediatric beta-thalassemic patients: a prospective ultrasonographic study. *Pediatr Radiol* 1993;**23**:357–9.
234. Sgouros SN, Bergele C. Endoscopic ultrasonography versus other diagnostic modalities in the diagnosis of choledocholithiasis. *Dig Dis Sci* 2006;**51**:2280–6.
235. Shah OJ, Zargar SA, Robbani I. Biliary ascariasis: a review. *World J Surg* 2006;**30**:1500–6.
236. Shah SK, Mutignani M, Costamagna G. Therapeutic biliary endoscopy. *Endoscopy* 2002;**34**:43–53.
237. Sharma AK, Wakhlu A, Sharma SS. The role of endoscopic retrograde cholangiopancreatography in the management of choledochal cysts in children. *J Pediatr Surg* 1995;**30**:65–7.
238. Siddique I, Galati J, Ankoma-Sey V, et al. The role of choledochoscopy in the diagnosis and management of biliary tract diseases. *Gastrointest Endosc* 1999;**50**:67–73.
239. Silen W. Cholecystitis and other causes of acute pain in the right upper quadrant of the abdomen. In: Silen W, editors. *Cope's Early Diagnosis of the Acute Abdomen*. 22nd ed. New York: Oxford University Press; 2010. p. 131.
240. Sindermann J, Foerster E, Kienast J. Severe hepatobiliary complication in a patient with acute promyelocytic leukemia treated with all-*trans* retinoic acid. *Acta Oncol* 1996;**35**:499–501.
241. Soetikno RM, Montes H, Carr-Locke DL. Endoscopic management of choledocholithiasis. *J Clin Gastroenterol* 1998;**27**: 296–305.
242. Sokol RJ, Mack C, Narkewicz MR, et al. Pathogenesis and outcome of biliary atresia: current concepts. *J Pediatr Gastroenterol Nutr* 2003;**37**:4–21.
243. Stain SC, Guthrie CR, Yellin AE, et al. Choledochal cyst in the adult. *Ann Surg* 1995;**222**:128–33.
244. Stern RC, Rothstein FC, Doershuk CF. Treatment and prognosis of symptomatic gallbladder disease in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr* 1986;**5**:35–40.
245. Still J, Scheirer R, Law E. Acute cholecystectomy performed through cultured epithelial autografts in a patient with burn injuries: a case report. *J Burn Care Rehabil* 1996;**17**:429–31.
246. Stringer MD, Dhawan A, Davenport M, et al. Choledochal cysts: lessons from a 20-year experience. *Arch Dis Child* 1995; **73**:528–31.
247. Sudan DL, Shaw BW, Langnas AN. Causes of late mortality in pediatric liver transplant recipients. *Ann Surg* 1998;**227**: 289–95.
248. Suddleson EA, Reid B, Woolley MM, et al. Hydrops of the gallbladder associated with Kawasaki syndrome. *J Pediatr Surg* 1987;**22**:956–9.
249. Suell MN, Horton TM, Dishop MK, et al. Outcomes for children with gallbladder abnormalities and sickle cell disease. *J Pediatr* 2004;**145**:617–21.
250. Sung JY, Shaffer EA, Olson ME, et al. Bacterial invasion of the biliary system by way of the portal-venous system. *Hepatology* 1991;**14**:313–7.
251. Takada T, Kawarada Y, Nimura Y, et al. Background: Tokyo guidelines for the management of acute cholangitis and cholecystitis. *J Hepatobiliary Pancreat Surg* 2007;**14**:1–10.
252. Takano K, Iwafuchi M, Uchiyama M, et al. Studies on intestinal motility and mechanism of cholangitis after biliary reconstruction. *J Pediatr Surg* 1989;**24**:1225–31.
253. Takiff H, Fonkalsrud EW. Gallbladder disease in childhood. *Am J Dis Child* 1984;**138**:565–8.
254. Tamary H, Aviner S, Freud E, et al. High incidence of early cholelithiasis detected by ultrasonography in children and young adults with hereditary spherocytosis. *J Pediatr Hematol Oncol* 2003;**25**:952–4.
255. Tanaka A, Takada T, Kawarada Y, et al. Antimicrobial therapy for acute cholangitis: Tokyo guidelines. *J Hepatobiliary Pancreat Surg* 2007;**14**:59–67.
256. Tarnasky PR, Cotton PB. Randomized trial of prevention of biliary stent occlusion by ursodeoxycholic acid plus norfloxacin. *Gastrointest Endosc* 1995;**42**:103–4.
257. Teng R, Yokohata K, Utsunomiya N, et al. Endoscopic retrograde cholangiopancreatography in infants and children. *J Gastroenterol* 2000;**35**:39–42.
258. Thambidorai CR, Shyamala J, Sarala R, et al. Acute acalculous cholecystitis associated with enteric fever in children. *Pediatr Infect Dis J* 1995;**14**:812–3.
259. Thompson Jr JE, Bennion RS, Roettger R, et al. Cefepime for infections of the biliary tract. *Surg Gynecol Obstet* 1993;**177**(Suppl):30–34; discussion 35–40.
260. Thompson Jr JE, Pitt HA, Doty JE, et al. Broad spectrum penicillin as an adequate therapy for acute cholangitis. *Surg Gynecol Obstet* 1990;**171**:275–82.
261. Thurston WA, Kelly EN, Silver MM. Acute acalculous cholecystitis in a premature infant treated with parenteral nutrition. *Can Med Assoc J* 1986;**135**:332–4.
262. Tipnis NA, Dua KS, Werlin SL. A retrospective assessment of magnetic resonance cholangiopancreatography in children. *J Pediatr Gastroenterol Nutr* 2008;**46**:59–64.
263. Toursarkissian B, Kearney PA, Holley DT, et al. Biliary sludging in critically ill trauma patients. *South Med J* 1995;**88**: 420–4.
264. Trauner M, Fickert P, Stauber RE. Inflammation-induced cholestasis. *J Gastroenterol Hepatol* 1999;**14**:946–59.
265. Treem WR, Malet PF, Gourley GR, et al. Bile and stone analysis in two infants with brown pigment gallstones and infected bile. *Gastroenterology* 1989;**96**:519–23.
266. Tsakayannis DE, Kozakewich HP, Lillehei CW. Acalculous cholecystitis in children. *J Pediatr Surg* 1996;**31**:127–30; discussion 130–1.
267. Valeja R, Pal S, Mann MS, et al. Isolated common bile duct tuberculosis. *Indian J Gastroenterol* 1999;**18**:125–6.
268. van den Hazel SJ, Speelman P, Tytgat GN, et al. Successful treatment of recurrent cholangitis with antibiotic maintenance therapy. *Eur J Clin Microbiol Infect Dis* 1994;**13**:662–5.
269. van Erpecum KJ. Gallstone disease: complications of bile-duct stones: acute cholangitis and pancreatitis. *Best Pract Res Clin Gastroenterol* 2006;**20**:1139–52.
270. Varadarajulu S, Wilcox CM, Eloubeidi MA. Impact of EUS in the evaluation of pancreaticobiliary disorders in children. *Gastrointest Endosc* 2005;**62**:239–44.
271. Vitek L, Carey MC. Enterohepatic cycling of bilirubin as a cause of "black" pigment gallstones in adult life. *Eur J Clin Invest* 2003;**33**:799–810.

272. Wada K, Takada T, Kawarada Y, et al. Diagnostic criteria and severity assessment of acute cholangitis: Tokyo guidelines. *J Hepatobiliary Pancreat Surg* 2007;**14**:52–8.
273. Wade JJ, Rolando N, Hayllar K, et al. Bacterial and fungal infections after liver transplantation: an analysis of 284 patients. *Hepatology* 1995;**21**:1328–36.
274. Waechter FL, Sampaio JA, Pinto RD, et al. The role of liver transplantation in patients with Caroli's disease. *Hepatogastroenterology* 2001;**48**:672–4.
275. Walker TM, Hambleton IR, Serjeant GR. Gallstones in sickle cell disease: observations from the Jamaican cohort study. *J Pediatr* 2000;**136**:80–5.
276. Wesdorp I, Bosman D, de Graaff A, et al. Clinical presentations and predisposing factors of cholelithiasis and sludge in children. *J Pediatr Gastroenterol Nutr* 2000;**31**:411–7.
277. Westphal JF, Brogard JM. Biliary tract infections: A guide to drug treatment. *Drugs* 1999;**57**:81–91.
278. Wildhaber BE, Coran AG, Drongowski RA, et al. The Kasai portoenterostomy for biliary atresia: a review of a 27-year experience with 81 patients. *J Pediatr Surg* 2003;**38**:1480–5.
279. Winkelstein JA, Marino MC, Ochs H, et al. The X-linked hyper-IgM syndrome: clinical and immunologic features of 79 patients. *Medicine (Baltimore)* 2003;**82**:373–84.
280. Wittenburg H, Lammert F. Genetic predisposition to gallbladder stones. *Semin Liver Dis* 2007;**27**:109–21.
281. Wong ML, Kaplan S, Dunkle LM, et al. Leptospirosis: a childhood disease. *J Pediatr* 1977;**90**:532–7.
282. Wong WT, Teoh-Chan CH, Huang CT, et al. The bacteriology of recurrent pyogenic cholangitis and associated diseases. *J Hyg (Lond)* 1981;**87**:407–12.
283. Wu ET, Chen HL, Ni YH, et al. Bacterial cholangitis in patients with biliary atresia: impact on short-term outcome. *Pediatr Surg Int* 2001;**17**:390–5.
284. Yabut B, Werlin SL, Havens P, et al. Endoscopic retrograde cholangiopancreatography in children with HIV infection. *J Pediatr Gastroenterol Nutr* 1996;**23**:624–7.
285. Yasuda H, Takada T, Kawarada Y, et al. Unusual cases of acute cholecystitis and cholangitis: Tokyo guidelines. *J Hepatobiliary Pancreat Surg* 2007;**14**:98–113.
286. Yombi JC, Meuris CM, Van Gompel AM, et al. Acalculous cholecystitis in a patient with *Plasmodium falciparum* infection: A case report and literature review. *J Travel Med* 2006;**13**: 178–80.
287. Yonem O, Bayraktar Y. Clinical characteristics of Caroli's syndrome. *World J Gastroenterol* 2007;**13**:1934–7.
288. Yoshida M, Takada T, Kawarada Y, et al. Antimicrobial therapy for acute cholecystitis: Tokyo guidelines. *J Hepatobiliary Pancreat Surg* 2007;**14**:83–90.
289. Yusuf TE, Baron TH. AIDS cholangiopathy. *Curr Treat Options Gastroenterol* 2004;**7**:111–7.
290. Ziessman HA. Cholecystokinin cholescintigraphy: clinical indications and proper methodology. *Radiol Clin North Am* 2001; **39**:997–1006.

vein inflammation and pyelophlebitis have become less common sources of hepatic infection in children.

Systemic bacteremia with hematogenous spread of bacteria to the liver through the hepatic artery seems to be the most common source of liver abscess in children, but it is implicated in less than 20 percent of adult patients. In the St. Louis series, the systemic hematogenous route was responsible for 21 of 27 (78%) cases of liver abscesses.[10] Of 13 patients encountered after 1940, 7 had bacteremia associated with leukemia. Anaerobic bacteremia associated with retropharyngeal or peritonsillar abscesses presumably has preceded development of anaerobic liver abscesses in several children.[8] Likewise, liver abscesses in neonates may be preceded by a systemic bacteremia without evidence of portal or biliary tract involvement.[36]

Liver abscesses occur more frequently in compromised pediatric hosts, especially those with chronic granulomatous disease, than in healthy children.[26] In a registry of 368 patients with chronic granulomatous disease from the United States, a liver abscess occurred in 27 percent of patients.[60] Over the course of 10 years, 15 children were diagnosed with pyogenic liver abscess in a large referral center for pediatric liver disease in the United Kingdom.[37] Three children (20%) had chronic granulomatous disease. In addition to functional disorders of phagocytes, chronic neutropenia predisposes to the development of liver abscesses.[44] Wintch and colleagues[61] noted that 5 of 10 children with hepatic abscesses in their institution had an underlying defect in host defense. Primary hemochromatosis predisposes to multiple liver abscesses caused by *Yersinia enterocolitica* in particular.[58] Pyogenic liver abscesses also are associated with Papillon-Lefèvre syndrome, a rare autosomal recessive disease characterized by palmoplantar keratoderma and periodontitis.[1]

Penetrating and nonpenetrating trauma to the liver may lead to liver abscesses, presumably caused by bacterial proliferation within small collections of blood and bile that result from the trauma. Hepatic abscess may be a rare complication of ventriculoperitoneal shunts after penetration of a peritoneal catheter into the liver.[23,40] This mode of infection has been reported in several children.[22] Liver abscess also is a complication of percutaneous liver biopsy.[16]

Unexplained or cryptogenic hepatic abscesses are encountered in most series and accounted for 40 to 50 percent of cases in many series.[25] Lee and Block[34] have proposed that these cryptogenic liver abscesses "originate from anaerobic bacterial invasion of hepatic infarcts." This theory is supported by reports that describe pyogenic liver abscesses as a complication of hepatic infarction in patients with sickle-cell anemia.[53] Normal gastrointestinal bacterial flora were isolated from 9 of 11 patients with liver abscesses at the Mayo Clinic. This finding suggested to Lazarchick and associates[33] that unrecognized intra-abdominal collections of pus were responsible. Although the reasons are unclear, diabetes mellitus also predisposes to the development of liver abscesses.[22,46,55] Nematode infection with larvae migrating through the liver is thought to be another predisposing factor for the development of pyogenic abscesses in children. The larvae induce liver granulomata that trap bacteria, leading to formation of an abscess.[43] In one study from Brazil, positive serology for *Toxocara canis* was significantly more frequent for patients with pyogenic liver abscess (10/16) than for the 32 age-matched controls (4/32).[48]

Biliary tract disease generally predisposes to the development of multiple liver abscesses. In contrast, blunt trauma to the liver or portal system inflammation most commonly predispose to a single abscess. In neonates, liver abscesses may be solitary or multiple because of systemic bacteria.[36,38] Solitary abscesses are the most common findings in the right lobe of the liver.[33]

Hepatic and splenic abscesses caused by *Candida* spp. are well described in patients with cancer.[54] Multiple abscesses are typical findings. These organs presumably are infected hematogenously, usually when the host is neutropenic.

MICROBIOLOGY

Gram-negative organisms have been the predominant isolates from liver abscesses in adults. *Escherichia coli* and *Klebsiella*, *Aerobacter*, *Pseudomonas*, and *Proteus* spp. have been implicated most frequently. *Klebsiella* spp. were the most common organism isolated from children with pyogenic liver abscess in Taiwan.[56] *Klebsiella* spp. also predominated in Taiwan overall and in adult Asian patients in a report from New York.[47,57] Anaerobic organisms also are important; anaerobic organisms were recovered from 45 percent of patients with liver abscesses in the University of California, Los Angeles series.[51]

In contrast to the adult experience, Dehner and Kissane[10] reported that 33 percent of liver abscesses in children were caused by *Staphylococcus aureus*, whereas gram-negative organisms were found in only 32 percent. Two or more organisms were recovered from liver abscesses in 52 percent of children. In a review of 96 children (no neonates) with pyogenic liver abscesses, *S. aureus*, gram-negative enteric organizations, and anaerobes were the organisms isolated most commonly, in that order.[29] *S. aureus* is the most common isolate that causes pyogenic liver abscess in patients with chronic granulomatous disease. In neonates, gram-negative enteric organisms are isolated most commonly. Anaerobes, particularly *Fusobacterium necrophorum*, have been isolated from liver abscesses in children without underlying disease.[15] Fungi, particularly *Candida albicans*, have been associated with liver abscesses in children with leukemia and neutropenia who have received parenteral hyperalimentation.[3] Liver or splenic abscesses also may be an unusual complication of brucellosis.[59] Human rotavirus–like particles were identified in the material aspirated from a liver abscess, but they were considered a secondary phenomenon and not the primary etiology of the liver abscess.[19]

CLINICAL MANIFESTATIONS

The clinical manifestations of pyogenic liver abscesses are nonspecific. A high index of suspicion and an

awareness of this illness are necessary to establish the diagnosis. A history of preceding abdominal surgery or trauma is helpful when present, as is the knowledge that the host's response to infection is compromised.

Fever, nausea, vomiting, anorexia, weakness, and malaise are prominent symptoms that may last several weeks. Abdominal or pleuritic pain, weight loss, and diarrhea are less common manifestations. A history of abdominal pain and fever of unknown origin in an otherwise healthy child suggests the diagnosis of pyogenic liver abscess.[28] In contrast, fever often is not observed in neonates.[13] Patients with a macroscopic or single abscess frequently experience a subacute to chronic course. In contrast, patients with multiple abscesses generally experience a more acute febrile illness.

Hepatomegaly occurs in 40 to 80 percent of patients; abdominal tenderness occurs less frequently. Right upper quadrant tenderness or even a mass may be subtle and not appreciated, unless the physician specifically and carefully examines this region. Other physical findings include jaundice (generally associated with biliary tract disease and not liver abscesses), abdominal distention, and evidence of pleuropulmonary involvement (i.e., elevated or fixed hemidiaphragm, rales, and pleural effusion).

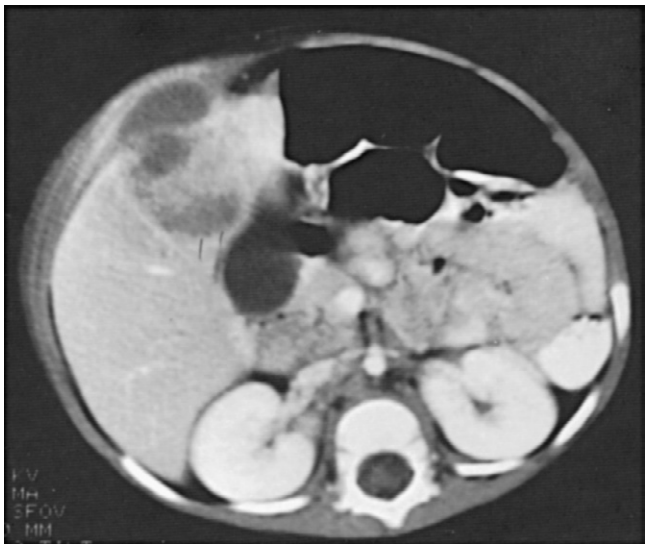

FIGURE 49-1 ■ Abdominal computed tomography scan showing a 4- × 5-cm encapsulated, septate, circular mass within the liver. A low-density soft tissue mass is noted in the abdominal wall from apparent extension from the intrahepatic mass.

DIAGNOSIS

Routine laboratory studies are of little help in establishing a diagnosis. Anemia, leukocytosis, and an elevation in C-reactive protein are common findings. Liver function tests generally reflect underlying disease of the liver itself and usually are not caused by the abscess. When abscesses occur secondary to biliary tract obstruction, alkaline phosphatase and bilirubin concentrations generally are elevated. Transaminase concentrations usually are normal to mildly elevated in most cases. A rapidly enlarging, tender liver in a patient with normal transaminase concentrations should alert the clinician to the possibility of liver abscess. Lazarchick and colleagues[33] found that the serum albumin concentration was the most important test with regard to prognosis in adults; 14 of 16 patients with a serum albumin level of less than 2 g/dL died.

Blood cultures are positive more commonly in patients with multiple abscesses than in patients with solitary abscesses. Overall, however, blood cultures usually are sterile in children with pyogenic liver abscess.

More than 50 percent of adult patients have abnormalities on chest radiography. Atelectasis, pulmonary infiltrates, pleural effusion, and elevated or fixed right hemidiaphragm are the most common findings.

Computed tomography (CT) currently provides the most accurate information concerning the size, location, and number of abscesses within the liver parenchyma (Fig. 49-1).[27,32,45] Lesions measuring 1 cm in diameter can be detected by CT. Multiple small abscesses may appear in clusters in a pattern suggesting early coalescence of the abscesses.[24] Liver abscesses appear as areas of low attenuation. The "target" lesions of hepatic candidiasis are not visualized by CT when the patient is neutropenic, and scans may need to be repeated before these characteristic lesions are observed.[54] Structures contiguous with the liver also are shown by CT; this information is important when a surgical approach to drainage is being planned.

Magnetic resonance imaging does not have any major advantages over CT for detecting or characterizing liver abscess but may show characteristic features to distinguish an abscess from other focal liver lesions in selected patients.[2,35] Ultrasonography also is a sensitive technique for detecting liver abscesses, and because it is noninvasive and does not require exposure to radiation, it is recommended for initial evaluation.[30,32] Hepatic angiography defines the vascular anatomy in the area of the liver abscess further and may provide information necessary for surgical management in selected cases. Nuclear medicine techniques rarely are indicated as a diagnostic method if liver abscess is suspected.

TREATMENT

Numerous reports have documented that patients with undiagnosed and untreated liver abscesses generally die and that surgical drainage of the solitary pyogenic liver abscess is the key to successful treatment. The choice of extraserous or transperitoneal open drainage or percutaneous closed aspiration depends on the location and size of the abscess and the experience and preference of the surgeon.[18,25,42,50]

Numerous groups have described percutaneous catheter drainage of liver abscesses in adults.[4,5,17,30] Percutaneous drainage of liver abscesses in children should be considered as the initial approach to drainage of such abscesses, especially in the right lobe of the liver.[12,45]

A catheter is placed into the cavity under CT or ultrasound guidance; material is aspirated, and, when an

abscess is documented, a draining catheter is placed. The cavity can be irrigated with saline initially. Criteria for the selection of patients for percutaneous drainage have been established.[17] The optimal route of percutaneous aspiration is directly into the abscess cavity and does not involve any uninfected organs or space. Drainage may proceed for 2 weeks or more or until drainage from the cavity is decreased, the patient is afebrile and improving, and radiography shows that the cavity is becoming smaller.[21] Surgical backup is mandatory when this drainage technique is used because spillage of abscess material into the peritoneal cavity, hemorrhage, and other complications may occur. Generally, percutaneous drainage is not indicated for patients with multiple large abscesses or multiloculated abscesses.[25] Heneghan and associates[20] have proposed that indications for surgical drainage of pyogenic liver abscesses are as follows:

1. No clinical response after 4 to 7 days of drainage via a percutaneous catheter placed into the abscess cavity
2. Multiple large or loculated abscesses
3. Thick-walled abscess with viscous pus
4. Concurrent intra-abdominal surgical pathology

Appropriate antibiotic therapy initially is based on knowledge of the organisms most commonly involved, Gram stain of the purulent material, and culture and susceptibility to antibiotics of the organisms that are recovered. If a hematogenous source of infection is suspected or the host has an immunodeficiency disease, *S. aureus* and streptococci are more likely. Biliary tract disease and blunt trauma are associated more frequently with gram-negative aerobic and anaerobic organisms. A logical antibiotic combination for the initial therapy of children with liver abscesses includes a penicillinase-resistant penicillin, such as nafcillin, plus an aminoglycoside. Vancomycin or clindamycin is selected if strains of *S. aureus* resistant to methicillin are present in the community.

The optimal duration and route of administration of antibiotics for a child with a solitary pyogenic liver abscess that has been drained have not been determined. Generally, 2 to 4 weeks of antibiotic therapy administered parenterally, followed by an appropriate oral antibiotic to complete a minimum 4-week total course, should be adequate. Penicillin, ticarcillin-clavulanate, piperacillin-tazobactam, clindamycin, cefoxitin, or metronidazole is administered for anaerobic isolates, depending on susceptibility. Meropenem is useful for polymicrobial infections, including infections caused by gram-negative aerobic and anaerobic rods or organisms that are multidrug resistant.

Multiple liver abscesses are more difficult to treat because achieving complete surgical drainage usually is impossible. Prolonged antibiotic therapy plus treatment of any underlying illnesses is the keystone to effective management. Duration of treatment can be modified, depending on the evidence for resolution of the abscesses as determined by repeated ultrasound examination or CT.

Fungal liver abscesses are difficult to document by culture of the abscess material and prolonged treatment is required. The reader is referred to Chapter 199 on candidiasis and the Infectious Diseases Society of America Clinical Practice Guidelines for the Management of Candidiasis for details of management.[41]

COMPLICATIONS AND PROGNOSIS

Complications of hepatic abscesses vary and are relatively common. Twenty-eight percent of the patients described by Rubin and associates[50] and 44 percent of the patients studied by Pitt and Zuidema[46] had one or more complications. Possible complications include pleural and pulmonary inflammation, peritonitis, subphrenic or subhepatic abscesses, and hemobilia.[10,48]

Polymicrobial bacteremia, hypoalbuminemia, multiple liver abscesses, or the presence of any complication is associated with increased mortality rates in patients with liver abscesses. Overall mortality rates depend largely on underlying pathologic processes and are difficult to interpret. Mortality figures from more recent reports range from 2.5 to 11 percent in adults.[24,45] In children, the prognosis for pyogenic liver abscess is excellent, generally with a low chance of mortality.[36] An increased awareness and suspicion of liver abscesses, in conjunction with ultrasound or CT of the liver, substantially reduces the mortality rate of this disease.

NEW REFERENCES SINCE THE SIXTH EDITION

20. Heneghan HM, Healy NA, Martin ST, et al. Modern management of pyogenic hepatic abscess: a case series and review of the literature. *BMC Res Notes* 2011;**4**:80.
41. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guideline for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
52. Srivastava A, Yachha SK, Arora V, et al. Identification of high-risk group and therapeutic options in children with liver abscess. *Eur J Pediatr* 2012;**171**:33–41.
55. Thompson RW, Jepsen P, Sorensen HT. Diabetes mellitus and pyogenic liver abscess: risk and prognosis. *Clin Infect Dis* 2007;**44**:1194–201.
57. Tsai F-C, Huang Y-T, Chang L-Y, Wang J-T. Pyogenic liver abscess as endemic disease, Taiwan. *Emerg Infect Dis* 2008;**14**: 1592–600.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Almuneef M, Khenaizan SA, Ajaji SA, Al-Anazi A. Pyogenic liver abscess and Papillon-Lefèvre syndrome: not a rare association. *Pediatrics* 2003;**111**:e85–8.
2. Balci NC, Semelka RC, Noone TC, et al. Pyogenic hepatic abscesses: MRI findings on T1- and T2-weighted and serial gadolinium-enhanced gradient-echo images. *J Magn Reson Imaging* 1999;**9**:285–90.
3. Bartley DL, Hughes WT, Parvey LS, et al. Computed tomography of hepatic and splenic fungal abscesses in leukemic children. *Pediatr Infect Dis* 1982;**1**:317–21.
4. Berger LA, Osborne DR. Treatment of pyogenic liver abscesses by percutaneous needle aspiration. *Lancet* 1982;**1**:132–4.
5. Bernardino ME, Berkman WA, Plemmons M, et al. Percutaneous drainage of multiseptated hepatic abscess. *J Comput Assist Tomogr* 1984;**8**:38–41.
6. Brans YM, Ceballos R, Cassady G. Umbilical catheters and hepatic abscesses. *Pediatrics* 1974;**53**:264–5.
7. Branum GD, Tyson GS, Branum MA, et al. Hepatic abscess: changes in etiology, diagnosis, and management. *Ann Surg* 1990;**212**:655–62.
8. Brook I, Fraizer EH. Role of anaerobic bacteria in liver abscesses in children. *Pediatr Infect Dis J* 1993;**12**:743–7.
9. Chusid MJ. Pyogenic hepatic abscess in infancy and childhood. *Pediatrics* 1978;**62**:554–9.
10. Dehner LP, Kissane JM. Pyogenic hepatic abscesses in infancy and childhood. *J Pediatr* 1969;**74**:763–73.
11. de la Maza LM, Faramary N, Berman LD. The changing etiology of liver abscess. *JAMA* 1974;**227**:161–3.
12. Diament MJ, Stanley P, Kangarloo H, et al. Percutaneous aspiration and catheter drainage of abscesses. *J Pediatr* 1986; **108**:204–8.
13. Doerr CA, Demmler GJ, Garcia-Pratts JA, et al. Solitary pyogenic liver abscess in neonates: report of three cases and review of the literature. *Pediatr Infect Dis J* 1994;**13**:64–9.
14. Ecoffey C, Rothman E, Bernard O, et al. Bacterial cholangitis after surgery for biliary atresia. *J Pediatr* 1987;**111**:824–9.
15. Embree JE, Williams T, Law BJ. Hepatic abscesses in a child caused by *Fusobacterium necrophorum*. *Pediatr Infect Dis* 1988;**7**:359–60.
16. Garcia-Arias MB, Rodriguez-Galindo C, Hoffer FA, Wang WC. Pyogenic hepatic abscess after percutaneous liver biopsy in a patient with sickle cell disease. *J Pediatr Hematol Oncol* 2005;**27**: 103–5.
17. Gerzof SG, Robbins AH, Johnson WC, et al. Percutaneous catheter drainage of abdominal abscesses: a five-year experience. *N Engl J Med* 1981;**305**:353–7.
18. Goldsmith HS, Chen WF. Management of a pyogenic abscess of the liver. *Surg Clin North Am* 1973;**53**:711–15.
19. Grunow JE, Dunton SF, Waner JL. Human rotavirus-like particles in a hepatic abscess. *J Pediatr* 1985;**106**:73–6.
20. Heneghan HM, Healy NA, Martin ST, et al. Modern management of pyogenic hepatic abscess: a case series and review of the literature. *BMC Res Notes* 2011;**4**:80.
21. Hoffer FA, Fellows KE, Wyly JB, et al. Therapeutic catheter procedures in pediatrics. *Pediatr Clin North Am* 1985;**32**:1461–76.
22. Holt JM, Spry CJF. Solitary pyogenic liver abscess in patients with diabetes mellitus. *Lancet* 1966;**2**:198–200.
23. Huang L-T, Chen C-C, Shih T-Y, et al. Pyogenic liver abscess complicating a ventriculoperitoneal shunt. *Pediatr Surg Int* 1998; **13**:6–7.
24. Jeffrey Jr RB, Tolentino CS, Chang FC, et al. CT of small pyogenic hepatic abscesses: the cluster sign. *AJR Am J Roentgenol* 1988; **151**:487–9.
25. Johannsen EC, Sifri CD, Madoff LC. Pyogenic liver abscesses. *Infect Dis Clin N Am* 2000;**14**:547–63.
26. Johnston RB, Baehner RL. Chronic granulomatous disease: correlation between pathogenesis and clinical findings. *Pediatrics* 1971;**48**:730–9.
27. Kandel G, Marcon NE. Pyogenic liver abscess: new concepts of an old disease. *Am J Gastroenterol* 1984;**79**:65–71.
28. Kaplan SL, Feigin RD. Pyogenic liver abscess in normal children with fever of unknown origin. *Pediatrics* 1976;**58**:614–16.
29. Kays DW. Pediatric liver cysts and abscesses. *Semin Pediatr Surg* 1992;**1**:107–14.
30. Kuligowska E, Connors SK, Shapiro JH. Liver abscess: sonography in diagnosis and treatment. *AJR Am J Roentgenol* 1982;**138**: 253–7.
31. Kusne S, Dummer JS, Singh N, et al. Infections after liver transplantation: an analysis of 101 consecutive cases. *Medicine (Baltimore)* 1988;**67**:132–43.
32. Laurin S, Kaude JV. Diagnosis of liver-spleen abscesses in children: with emphasis on ultrasound for the initial and follow-up examinations. *Pediatr Radiol* 1984;**14**:198–204.
33. Lazarchick J, DeSouza E, Silvia NA, et al. Pyogenic liver abscess. *Mayo Clin Proc* 1973;**48**:349–55.
34. Lee JF, Block GE. The changing clinical pattern of hepatic abscesses. *Arch Surg* 1972;**104**:456–70.
35. Mendez RJ, Schiebler ML, Outwater EK, et al. Hepatic abscesses: MR imaging findings. *Radiology* 1994;**190**:431–6.
36. Moss TJ, Pysher TJ. Hepatic abscess in neonates. *Am J Dis Child* 1981;**135**:726–8.
37. Muorah M, Hinds R, Verma A, et al. Liver abscesses in children: A single center experience in the developed world. *J Pediatr Gastroenterol Nutr* 2006;**42**:201–6.
38. Murphy FM, Baker CJ. Solitary hepatic abscess: a delayed complication of neonatal bacteremia. *Pediatr Infect Dis* 1988;**7**: 414–16.
39. Noel GJ, Karasic RB. Liver abscess following ingestion of a foreign body. *Pediatr Infect Dis* 1984;**3**:342–4.
40. Paone RF, Mercer LC. Hepatic abscess caused by a ventriculoperitoneal shunt. *Pediatr Infect Dis J* 1991;**10**:338–9.
41. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guideline for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
42. Patterson HC. Open aspiration in solitary liver abscess. *Am J Surg* 1970;**119**:326–9.
43. Pereira FE, Musso C, Castelo JS. Pathology of pyogenic liver abscess in children. *Pediatr Dev Pathol* 1999;**2**:537–43.
44. Pincus SH, Boxer LA, Stossel TP. Chronic neutropenia in childhood: analysis of 16 cases and a review of the literature. *Am J Med* 1976;**61**:849–61.
45. Pineiro-Carrero VM, Andres JM. Morbidity and mortality in children with pyogenic liver abscess. *Am J Dis Child* 1989;**143**: 1424–7.
46. Pitt HA, Zuidema GD. Factors influencing mortality in the treatment of pyogenic hepatic abscess. *Surg Gynecol Obstet* 1975;**140**: 228–34.
47. Rahimian J, Wilson T, Oram V, Holzman RS. Pyogenic liver abscess: recent trends in etiology and mortality. *Clin Infect Dis* 2004;**39**:1654–9.
48. Rayes AA, Teixeira D, Serufo JC, et al. Human toxocariasis and pyogenic liver abscess: a possible association. *Am J Gastroenterol* 2001;**96**:563–6.
49. Rogers CA, Isenberg JN, Leonard AS, et al. Ascending cholangitis diagnosed by percutaneous hepatic aspiration. *J Pediatr* 1976;**88**: 83–6.
50. Rubin RH, Swartz MN, Malt R. Hepatic abscess: changes in clinical, bacteriologic and therapeutic aspects. *Am J Med* 1974; **57**:601–10.
51. Sabbaj J. Anaerobes in liver abscess. *Rev Infect Dis* 1984;**6**: S152–6.
52. Srivastava A, Yachha SK, Arora V, et al. Identification of high-risk group and therapeutic options in children with liver abscess. *Eur J Pediatr* 2012;**171**:33–41.
53. Shulman ST, Beem MO. A unique presentation of sickle cell disease: pyogenic hepatic abscess. *Pediatrics* 1971;**47**:1019–22.
54. Thaler M, Pastakia B, Shawker TH, et al. Hepatic candidiasis in cancer patients: the evolving picture of the syndrome. *Ann Intern Med* 1988;**108**:88–100.
55. Thompson RW, Jepsen P, Sorensen HT. Diabetes mellitus and pyogenic liver abscess: risk and prognosis. *Clin Infect Dis* 2007; **44**:1194–201.
56. Tsai CC, Chung JH, Ko SF, et al. Liver abscess in children: a single institutional experience in southern Taiwan. *Acta Paediatr Taiwan* 2003;**44**:282–6.
57. Tsai F-C, Huang Y-T, Chang L-Y, Wang J-T. Pyogenic liver abscess as endemic disease, *Taiwan Emerg Infect Dis* 2008;**14**: 1592–600.

58. Vadillo M, Corbella X, Pac V. Multiple liver abscesses due to *Yersinia enterocolitica* discloses primary hemochromatosis: three case reports and review. *Clin Infect Dis* 1994;**18**:938–41.
59. Vallejo JG, Stevens AM, Dutton RV, et al. Hepatosplenic abscesses due to *Brucella melitensis:* report of a case involving a child and review of the literature. *Clin Infect Dis* 1996;**22**:485–9.
60. Winkestein JA, Marino MC, Johnston RB, et al. Chronic granulomatous disease: report on a national registry of 368 patients. *Medicine (Baltimore)* 2000;**79**:155–69.
61. Wintch RW, Reines HD, Rambo WM. Liver abscess: a changing entity. *Am J Surg* 1982;**48**:11–15.

CHAPTER 50

Reye Syndrome

Eugene S. Hurwitz

A syndrome involving the acute onset of encephalopathy associated with fatty metamorphosis of the liver and occurring primarily in children was described first by Reye and colleagues[10,12] in Australia and by Johnson and associates[9] in the United States. The similarities of these two descriptions in separate countries led to the common designation of this clinicopathologic entity as *Reye-Johnson* or *Reye syndrome*. Reye syndrome occurs most frequently after a viral illness and is characterized by the onset of severe vomiting followed by the development of encephalopathy and hepatic dysfunction. The recognition in the early 1980s that the syndrome is associated with the ingestion of aspirin during the antecedent viral illness led to public awareness of this association, a decline in aspirin use for such illnesses in children, and a dramatic decline in the occurrence of this disease in the United States.[1]

EPIDEMIOLOGY

In the United States, national surveillance for Reye syndrome was conducted first during the 1973 to 1974 nationwide outbreak of influenza B and influenza A (H1N1). Such surveillance led to the recognition of outbreaks of Reye syndrome regionally and nationally that were associated with outbreaks of influenza in these and subsequent years.[3] During the first 5 years of surveillance, 250 to 550 cases were reported nationally—an underestimate because it was based on voluntary reporting.[11] Population-based studies conducted in several geographic locations showed that the average annual incidence of the syndrome was one or two cases per 100,000 children younger than 18 years old. Adults rarely were affected. Case-fatality rates reported through national surveillance, initially 40 percent, declined to 20 to 30 percent in later years when the syndrome was prevalent, although this rate undoubtedly was an overestimate because of the tendency to report more severe and fatal cases through this system.

Between 1980 and 1982, four case-control studies reported an association between Reye syndrome and the ingestion of aspirin during an antecedent respiratory or chickenpox illness.[4,13,14] The results of these studies subsequently were confirmed in the Public Health Service Pilot and Main Studies of Reye Syndrome and Medications.[5-7] In these studies, more than 90 percent of patients with Reye syndrome compared with 40 to 70 percent of controls had received aspirin for the antecedent respiratory or chickenpox illness; reported odds ratios were 11.5 to 40. After these studies were reported, publicity and recommendations from various expert panels, including recommendations issued by the U.S. Food and Drug Administration in 1985, led to a decline in the use of aspirin and a decline in the incidence of Reye syndrome, particularly in the age group that had been affected most—children 5 to 15 years old.[1]

CLINICAL MANIFESTATIONS AND LABORATORY FINDINGS

Reye syndrome is described classically as an illness characterized by the abrupt onset of severe vomiting and progressive encephalopathy in a child who is just recovering from a viral illness, the most common of which are influenza and chickenpox. The onset of these symptoms typically occurs within several days after the onset of the viral illness and commonly during a period when the child seems to be recovering from this illness. In association with severe—often projectile—vomiting, which occurs for a transient period, are progressive encephalopathic changes that may follow stages from delirium through confusion, agitation, and lethargy to coma if untreated.

The definition used by the Centers for Disease Control and Prevention (CDC) and widely adopted for clinical purposes includes (1) evidence of acute encephalopathy manifested by alterations in consciousness and documented, when available, by cerebrospinal fluid with less than 9×10^6/L leukocytes or by biopsy or autopsy evidence of cerebral edema without perivascular or meningeal inflammation in histologic sections of the brain; (2) evidence of liver involvement, including either biopsy or autopsy findings of fatty metamorphosis of the liver if available or, in the absence of such specimens, elevations in liver enzymes (alanine aminotransaminase, aspartate aminotransaminase, or serum ammonia) that typically are more than three times normal levels; and (3) no other more reasonable explanation for the cerebral or hepatic abnormalities. The last requirement emphasizes that Reye syndrome is a diagnosis of exclusion and that every effort should be undertaken to identify other possible causes for the clinical and laboratory abnormalities.

Liver biopsy or autopsy findings are considered characteristic and include panlobular microvesicular fat and mitochondrial abnormalities on electron microscopic examination showing peroxisome swelling and enlarged pleomorphic mitochondria with loss of dense granules. Additional findings include normal bilirubin levels and absence of jaundice. Most patients also have hypoglycemia and a prolonged prothrombin time. The typically

elevated cerebrospinal fluid pressure in patients leads to progressive stages of coma.

Staging criteria for Reye syndrome have been used to define the level of encephalopathy. Patients have been reported with liver involvement, but without evidence of encephalopathy. These patients have been described as having stage 0 encephalopathy and, although they do not meet the CDC criteria for Reye syndrome, are considered to have mild disease. Patients with stage I encephalopathy are difficult to arouse and lethargic, whereas patients with stage II are delirious and combative with some movement. Patients with higher stages of encephalopathy (III to V) cannot be aroused and have progressively deeper stages of coma. These patients have a poor prognosis, with a mortality rate approaching 50 percent for patients admitted with stage III or greater and 90 percent for patients admitted with stage V.

Exclusion of other diseases that may resemble Reye syndrome, such as salicylate toxicity, is essential in patients with symptoms resembling this entity. Intensive laboratory investigations should be undertaken to exclude such disorders. In young children, particularly children younger than 3 years of age, inherited metabolic disorders frequently may mimic Reye syndrome and must be excluded. Such metabolic disorders include disorders of fatty acid oxidation, urea cycle disorders, carnitine transport defects, and organic acidemias. Laboratory studies must be performed for the younger age group to exclude these disorders before a diagnosis of Reye syndrome is made, particularly because some of these disorders can be treated effectively. With the declining incidence of Reye syndrome in the typical age group (5 to 15 years) after virtual elimination of the use of aspirin in children, an increasing number of patients with features of Reye syndrome are in this younger age group and ultimately are found, after careful evaluation, to have one of the many metabolic disorders that mimic this syndrome.

An acute encephalopathy mimicking Reye syndrome was recently noted.[8] This 11-year-old boy's illness was due to *Bacillus cereus* emetic toxin cereulide.

TREATMENT AND PREVENTION

The mainstay of treatment of Reye syndrome is early recognition of disease and supportive care focusing on various measures to control intracranial pressure and electrolyte and other abnormalities. Patients should have glucose levels monitored, and early infusion of glucose is considered by many physicians to improve outcome. Comatose patients should be transferred to tertiary care centers that have experience in caring for such patients and can monitor and treat elevated intracranial pressure. When such measures were undertaken before the decline in incidence of this disease, they were associated with improved outcome and decreased mortality. Other therapeutic measures that have been used include efforts to reduce ammonia levels, such as exchange transfusions, peritoneal dialysis, and total-body washout via cardiopulmonary bypass. With advances in supportive care, the mortality rate declined to 10 to 20 percent in later years before Reye syndrome became extremely rare.

Since the association between Reye syndrome and aspirin has been recognized, aspirin no longer is recommended or used for the treatment of febrile illnesses in children. Alternative antipyretics, including nonsteroidal anti-inflammatory drugs and acetaminophen (Tylenol), have replaced aspirin as the primary therapy for such illnesses. These medications have not been associated with an increased risk for development of Reye syndrome. Children with some disorders, including juvenile rheumatoid arthritis and Kawasaki disease, continue to be given aspirin to treat these disorders. Efforts to reduce the risk for development of Reye syndrome in these children have included influenza vaccination annually and vaccination against chickenpox. Careful monitoring of these children also is necessary to ensure early recognition and treatment of Reye syndrome should it occur.

A large number of over-the-counter aspirin-containing products are available in the United States as well as in other countries.[2] It is often not recognized by parents that these alternative medicine products contain aspirin so that children with respiratory illness may on occasion receive aspirin. Therefore, Reye syndrome may still occasionally occur.

NEW REFERENCE SINCE THE SIXTH EDITION

8. Ichikawa K, Gakumazawa M, Inaba A, et al. Acute encephalopathy of *Bacillus cereus* mimicking Reye syndrome. *Brain Dev* 2010; **32**:688–90.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Belay ED, Bresee JS, Holman RC, et al. Reye's syndrome in the United States from 1981 through 1997. *N Engl J Med* 1999;**340**:1377–82.
2. Chow EL, Cherry JD, Harrison R, et al. Reassessing Reye syndrome. *Pediatr Forum* 2003;**157**:1241–2.
3. Corey L, Rubin RJ, Hattwick MA, et al. Nationwide outbreak of Reye's syndrome: its epidemiologic relationship to influenza B. *Am J Med* 1976;**61**:615–25.
4. Halpin TJ, Holtzhauer FJ, Campbell RJ, et al. Reye's syndrome and medication use. *JAMA* 1982;**248**:687–91.
5. Hurwitz ES, Barrett MJ, Bregman D, et al. Public Health Service study on Reye's syndrome and medications: report of the pilot phase. *N Engl J Med* 1985;**313**:849–57.
6. Hurwitz ES, Barrett MJ, Bregman D, et al. Public Health Service study of Reye's syndrome and medications: report of the main study. [Erratum]. *JAMA* 1987;**257**:1905–11.
7. Hurwitz ES, Nelson DB, Davis C, et al. National surveillance for Reye syndrome: a five-year review. *Pediatrics* 1982;**70**:895–900.
8. Ichikawa K, Gakumazawa M, Inaba A, et al. Acute encephalopathy of *Bacillus cereus* mimicking Reye syndrome. *Brain Dev* 2010;**32**:688–90.
9. Johnson GM, Scurletis TD, Carroll NB. A study of sixteen fatal cases of encephalitis-like disease in North Carolina children. *N C Med J* 1963;**24**:464–73.
10. Reye RDK, Morgan G, Baral J. Encephalopathy and fatty degeneration of the viscera: a disease entity in childhood. *Lancet* 1963;**2**:749–52.
11. Reye syndrome surveillance—United States, 1987 and 1988. *MMWR Morb Mortal Wkly Rep* 1989;**38**:325–7.
12. Rowe PC, Valle D, Brusilow SW. Inborn errors of metabolism in children referred with Reye's syndrome: a changing pattern. *JAMA* 1988;**260**:3167–70.
13. Starko KM, Ray CG, Dominguez LB, et al. Reye's syndrome and salicylate use. *Pediatrics* 1980;**66**:859–64.
14. Waldman RJ, Hall WN, McGee H, Van Amburg G. Aspirin as a risk factor in Reye's syndrome. *JAMA* 1982;**247**:3089–94.

SECTION **VIII**

OTHER INTRA-ABDOMINAL INFECTIONS

CHAPTER 51

Appendicitis and Pelvic Abscess

Thomas L. Kuhls

The ability to diagnose appendicitis accurately in a child continues to be one of the most fundamental skills that a pediatric surgeon has to master, although making the diagnosis often is difficult in young patients. The surgeon ultimately is responsible for deciding whether a child is taken to the operating room for appendectomy; however, a primary care physician often is the first person to evaluate the patient who complains of abdominal pain. Pediatricians with expertise in infectious diseases frequently are involved in the care of children who present with subtle or atypical manifestations of appendicitis, have unusual microorganisms recovered from their appendices, or have complications as a result of appendiceal rupture, such as the development of wound infections, sepsis, peritonitis, intra-abdominal abscesses, and pelvic abscesses.

HISTORY

As early as the 16th century, physicians began describing patients with clinical manifestations suggestive of perforated appendicitis. Until the late 1800s, the inflammatory process was called *typhlitis* or *perityphlitis* because the illness was thought to originate from the cecum. In 1886, Reginald Fitz recognized that the source of the inflammation was the appendix and suggested that a laparotomy be performed early in the course of the illness.[63] Shortly afterward, Charles McBurney reported that in patients with appendicitis, tenderness is greatest 2 inches from the anterior iliac spine on a line drawn to the umbilicus.[129] Despite intensive research and refinement of our understanding of appendicitis during the past century, the rates of removing nondiseased appendices or finding already perforated appendices at laparotomy both remained at about 20 percent until more recently when ultrasound and computed tomography (CT) examinations have been commonly used in children with suspected appendicitis.

Despite the continued difficulties in diagnosing appendicitis, the mortality rate from appendicitis has greatly decreased since Fitz reported a 40 percent operative mortality rate. In 1936, Bancroft and Skoluda reported a mortality rate of 8 percent and a complication rate of 11 percent for appendicitis, most likely because of the availabilities of general anesthesia and better aseptic surgical techniques.[23] The second major improvement in outcome occurred in the 1940s, when sulfonamides and banked blood became widely available.[127] In the 1970s, anaerobes such as *Bacteroides fragilis* were found to cause postoperative infections frequently in patients with appendicitis, and treating these microorganisms was found to reduce further the rate of postoperative complications.[116] Currently, death from appendicitis is a rare occurrence in the United States.

EPIDEMIOLOGY

Reported incidences of acute appendicitis vary widely, depending on where the studies were performed and what methodologies were used. The consensus is that the number of cases of acute appendicitis has been decreasing during the past few decades.[96,130] Investigators have estimated that more than 70,000 children are diagnosed with appendicitis each year in the United States.[80] In 1997, appendicitis-related hospitalizations accounted for 0.6 percent of all hospitalizations in the United States, resulting in approximately 1 million hospital days and $3 billion in hospital charges.[47] Although appendicitis occurs in all age groups, the highest incidence occurs during the second decade of life.[3,123] Appendicitis is an uncommon event in children younger than 5 years of age and occurs extremely rarely in infants younger than 6 months old. Patients with acquired immunodeficiency syndrome (AIDS) may have a higher incidence of appendicitis than does the normal population.[108]

Numerous studies have demonstrated that the peak rates of appendicitis occur during the summer months, whereas the lowest rates occur during the winter months.[3,123] An Italian study found a higher rate of pediatric appendicitis in the winter, whereas appendicitis in adults was observed more frequently during the summer.[68] The reasons for these seasonal patterns are unknown, but changes in diet and exposure to allergens have been suggested as explanations.[123] Also, enteric infections occur most frequently during the summer and may play a role in increasing the incidence of appendicitis during this particular season.

Most studies show a modest increase in incidence of appendicitis in males compared with females.[3,123] An estimated lifetime risk for developing appendicitis is 8.6 percent for males and 6.7 percent for females.[3] In a study of acute appendicitis in California, the rate of appendicitis in whites was twice that of blacks and Asians.[123] More recently, Hispanics were shown to have the highest rate of appendicitis-related hospitalizations in the United States.[47] Whether the reported racial differences are due to errors of measurement, sociodemographic factors, environmental factors, factors related to body constitution, or genetic factors remains unknown. Children with appendicitis more frequently have a history of having family members who previously have had appendicitis,

suggesting that genetic background plays a role in the susceptibility to appendicitis.[62] From twin studies, less genetic susceptibility was found in males compared with females.[19] Previous breast-feeding, decreased dietary fiber, ingestion of refined carbohydrates, and atopy have all been suggested to increase the risk for developing appendicitis.[77,155]

Rates of perforation when children are diagnosed with appendicitis are significantly higher in Asian, Hispanic, and black children compared with non-Hispanic white children.[143,156,187] Similarly, children without health insurance or Medicaid have rates of perforation higher than those of children with private health insurance.[156,186] Researchers have suggested that the pediatric rupture rate is a good candidate for inclusion in the National Healthcare Disparities Report.[88] Already, studies have found no differences in perforation rates based on socioeconomic status, showing improved access to early medical care in communities and health systems.[113] Higher perforation rates have been observed in obese individuals and tobacco smokers.[20]

PATHOPHYSIOLOGY

The initial event in the development of most cases of appendicitis is thought to be obstruction of the appendiceal lumen. Microorganisms rarely invade the appendiceal mucosa and initiate the inflammatory process. Appendiceal obstruction can be caused by inspissated feces (fecalith), hypertrophied lymphoid tissue that develops during a systemic viral infection or bacterial enterocolitis, parasitic infestation, appendiceal wall hemorrhage associated with anaphylactic purpura, inspissated barium, or ingested seeds. Continued production of mucus by the appendiceal mucosa distal to the obstruction causes the appendix to distend. Vascular congestion and ischemia occur as the increased intraluminal pressure of the appendix becomes greater than the venous pressure, and edema develops as lymphatic flow becomes obstructed.

Stasis of intestinal flow and intestinal ischemia allow the microorganisms in the appendix to invade the tissues, enhancing the already developing inflammatory response. Bacteria then may translocate across the appendiceal wall and reach the peritoneal cavity.[24] If the process is severe and arteriolar blood flow to the appendix is obstructed, transmural infarction occurs and the appendix ruptures. Microorganisms are liberated into the peritoneal cavity, causing generalized peritonitis and formation of an abscess. Animal studies have suggested that synergism occurring between enteric aerobes, such as *Escherichia coli*, and anaerobes, such as *B. fragilis*, is important in the development of intra-abdominal and pelvic abscesses after perforation.[71] An association between the development of an abscess after appendiceal perforation and the presence of *Streptococcus anginosus* (formerly *S. milleri*) also apparently exists.[76]

As the appendix distends in the early stages of appendicitis, the visceral afferent autonomic nerves that enter the spinal cord at T8 to T10 are stimulated, referring the pain to the epigastric and periumbilical areas of the abdomen. When the inflammatory response reaches the serosal surface of the appendix, the parietal peritoneum is stimulated and the pain intensifies in the right lower quadrant. If perforation occurs, the peritoneal inflammatory response causes more generalized abdominal tenderness.

Although appendiceal obstruction may play an important role in the early stages of most cases of appendicitis, not all obstructed appendices become inflamed. Ten percent of normal appendices removed during abdominal surgical procedures contain inspissated fecal material. Also, children may develop recurrent, crampy abdominal pain, possibly from intermittent appendiceal obstruction.

The classic description of the pathophysiology of appendicitis does not easily explain many epidemiologic features of this disease, including its higher incidence in males and certain races. The amount and reactivity of the lymphoid tissue in the wall of the appendix have been suggested to be key determinants to the development of appendicitis. The amount of lymphoid tissue in the appendix is greatest during adolescence when the disease process is most prevalent and the amount most likely is controlled genetically.

In a case-control study from Italy, prolonged breast-feeding during infancy was associated with a decreased risk for developing acute appendicitis later in life.[155] The investigators hypothesized that breast-feeding may have decreased the amount of stimulation to intestinal lymphocytes by microbial and food antigens early in life, so that appendiceal lymphoid tissues were less reactive to antigenic challenge during adolescence and adulthood. Appendicitis may be observed less frequently during infancy and the neonatal period because the appendix is more funnel shaped, the diet is primarily liquid, recumbent posture is maintained for prolonged periods, and gastrointestinal and respiratory tract infections develop less frequently during this time.

CLINICAL MANIFESTATIONS

In school-aged children and adolescents with appendicitis, the median duration of symptoms before the time of hospital admission is 24 to 28 hours.[162] Pain in the right iliac fossa is the most common sign of appendicitis, occurring in 88 to 99 percent of patients.[162,167] Pain shifts from the periumbilical area to the right lower quadrant of the abdomen in approximately two thirds of children with appendicitis. In three fourths of children, the pain worsens during movement. The characteristics of the abdominal pain do not always predict accurately which children have appendicitis. Of children found to have mesenteric adenitis at laparotomy, 25 percent report a shift in their abdominal pain to the right iliac fossa and 33 percent experience worsening of their pain during movement.[162]

Nausea and vomiting are found in 86 to 96 percent of children with appendicitis.[162,167] Vomiting usually occurs after the onset of abdominal pain but may precede pain in nearly 20 percent of cases. If only nausea or vomiting is present, it is less likely the child has appendicitis.[162] Anorexia occurs less commonly in children than in adults, occurring in 47 to 91 percent of cases of appendicitis. Because 50 percent of children found to have normal appendices at surgery complain of anorexia,

differentiating appendicitis from other causes of right iliac fossa pain is not always helpful. Similarly, complaints of diarrhea (9% to 16%), constipation (5% to 28%), and dysuria (7%) occasionally can be elicited from children with appendicitis.[162,170]

Fever is a useful diagnostic sign of appendicitis in children with right lower quadrant abdominal pain.[38,58] A temperature greater than 37.5 °C (99.5 °F) is found in 68 to 96 percent of cases. Very high temperatures (>39 °C [102.2 °F]) suggest that perforation already has occurred or that another intra-abdominal process is present. Rarely, children present with erythema and tenderness of the scrotum as the only manifestation of acute appendicitis.[227]

During the physical examination, the child frequently lies quietly on the examination table with the right hip flexed. Tenderness in the right iliac fossa is the most sensitive sign of appendicitis, occurring in 93 to 100 percent of cases of appendicitis.[162,167] The psoas muscle may become irritated from the inflamed appendix, causing the child to feel increased pain when the right hip is flexed actively. Likewise, if the obturator internus muscle is involved, pain is elicited when the flexed thigh is rotated internally. Guarding is found in 80 to 91 percent of cases of appendicitis, compared with 50 percent of cases of mesenteric adenitis and 8 percent of cases of nonspecific abdominal pain.[162] Similarly, rebound tenderness is found in 56 to 83 percent of cases of appendicitis, 33 percent of cases of acute mesenteric adenitis, and 1 percent of cases of nonspecific abdominal pain; its presence triples the odds of the child having appendicitis.[38,58,162]

The development of diffuse abdominal tenderness and the absence of bowel sounds usually indicate perforation. Extremely hyperactive bowel sounds suggest that the patient may not have appendicitis. Occasionally, a mass can be palpated in the right lower quadrant of the abdomen in children with appendicitis who are relaxed or well sedated. Rectal tenderness is present more commonly in children with appendicitis (44% to 68%) than in children with other causes of abdominal pain (12%); however, findings during the rectal examination seldom alter the clinical decision of the surgeon.[162]

In preschool-aged children, the diagnosis of appendicitis is more difficult to establish because of the inability of young children to express their symptoms and because they often do not cooperate during the physical examination.[221,222] Young children with appendicitis are often seen early in the course of their symptoms and are prescribed antibiotics, antihistamines, or antipyretics. By the time one realizes that the child has appendicitis, the appendix usually is perforated (50% to 90%).[221,222] In contrast to older children, vomiting is the initial symptom of appendicitis most frequently observed and abdominal pain may be absent or may never localize in the right iliac fossa.[162] Sleep disturbances, irritability, restlessness, and crying are common manifestations of appendicitis in this age group. The preschool-aged child is more likely to have a palpable inflammatory mass at presentation.

During the newborn period, appendicitis is an extremely rare occurrence, although most cases are found when prematurity exists.[92] Symptoms of neonatal appendicitis include abdominal distention; vomiting; irritability; diarrhea; erythema, edema, or cellulitis of the abdominal wall; gastrointestinal hemorrhage; abdominal rigidity; lethargy; and jaundice. Usually, the symptoms of neonatal appendicitis are indistinguishable from those of necrotizing enterocolitis. Underlying conditions such as total colonic Hirschsprung disease, meconium plugs, esophageal atresia, or hernias may predispose the neonate to develop this condition.

In children who are undergoing chemotherapy for leukemia, acute appendicitis may present as only vague abdominal pain, abdominal distention, lack of abdominal guarding, fever, dehydration, diarrhea, or unusual symptoms such as gastrointestinal bleeding.[12] Symptoms of appendicitis in immunocompromised patients may be identical to those of typhlitis.

The differential diagnosis of acute abdominal pain in children is extensive. Table 51-1 includes conditions that can manifest as symptoms suggestive of acute appendicitis.

DIAGNOSIS

The diagnosis of acute appendicitis should be established without laboratory studies when a child complains of abdominal tenderness in the right lower quadrant that initially started in the periumbilical area, has fever, develops nausea and vomiting, and has rebound tenderness in the right lower quadrant with guarding during an abdominal examination. The child should be taken to the operating room for an appendectomy as soon as possible before perforation occurs.[101] Most children do not have all of the classic clinical manifestations of appendicitis; thus, a great emphasis has been placed on using various laboratory tests to help clinicians diagnose the disease accurately.[106] Similarly, patients who have received prior oral antibiotics may have milder symptoms and signs of classic appendicitis, necessitating further diagnostic studies.[61]

For decades, physicians have valued peripheral blood leukocyte counts, neutrophil counts, C-reactive protein concentrations, and erythrocyte sedimentation rates to help them distinguish appendicitis from other causes of abdominal pain. When properly evaluated, these tests have been found to be too insensitive to use as reliable tools for diagnosing appendicitis.[26,55,169] Normal results do not rule out the possibility that the child has appendicitis, although these tests help to confirm a physician's suspicions when results are positive. When the nonspecific tests are elevated, often perforation or abscess formation has occurred.[8,150] Similarly, hyperbilirubinemia found in children with symptoms of appendicitis is a sign of probable perforation.[174] Certain groups of patients commonly have normal leukocyte counts despite having acute appendicitis. Black patients with acute appendicitis frequently do not develop leukocytosis.[87] Patients with AIDS who develop appendicitis also frequently do not have elevated white blood cell counts.[34]

Routine radiographic studies for the diagnosis of appendicitis in children no longer are suggested. Radiographs of the abdomen are not sensitive nor specific enough for diagnosing childhood appendicitis. Abnormalities described in association with acute appendicitis include an abnormal bowel gas pattern, a mass, a fecalith,

TABLE 51-1 **Differential Diagnosis of Acute Appendicitis in Children**

Cecal and Colonic Diseases
Constipation
Crohn disease
Infectious colitis (bacterial, parasitic)
Intestinal obstruction
Necrotizing enterocolitis (newborns)
Typhlitis (leukemia patients)
Small Intestinal Diseases
Duodenal ulcers (acute and perforated)
Gastroenteritis (including mesenteric adenitis)
Intestinal duplication
Intestinal obstruction
Intussusception
Meckel diverticulitis
Volvulus
Hepatobiliary and Pancreatic Diseases
Cholecystitis
Hepatitis
Hydrops of the gallbladder
Pancreatitis
Other Diseases
Omental torsion
Pneumonia
Psoas abscess
Spontaneous peritonitis
Reproductive Tract Diseases
Intrauterine and ectopic pregnancy
Ovarian cysts
Ovarian torsion
Pelvic inflammatory disease
Testicular torsion
Urinary Tract Diseases
Hydronephrosis
Pyelonephritis
Urachal abscess
Urolithiasis
Wilms tumor
Systemic Illnesses
Anaphylactoid purpura
Cytomegalovirus infection (in patients with AIDS)
Diabetic ketoacidosis
Kawasaki disease
Lymphoma (Burkitt)
Porphyria
Rocky Mountain spotted fever
Sickle-cell disease
Tuberculosis

and obliteration of normal fat planes in the right lower quadrant. Gas in the appendiceal lumen is thought to be diagnostic of acute appendicitis but may occur rarely in its absence. Most children do not have radiographic findings of appendicitis or findings are nonspecific.

Graded compression ultrasonography has become the diagnostic procedure of choice in some institutions when evaluating a patient with possible appendicitis.[152] A transducer is used to apply gradual pressure to the abdomen. The technician must ensure that all gas and fluid contents from the loops of bowel are expressed for the examination to be adequate. A noncompressible, enlarged (>6 mm in diameter in adolescents) appendix or a fecalith is the major criterion used for diagnosing appendicitis by ultrasonography. Interruption in the continuity of the echogenic submucosa suggests necrosis of the appendiceal wall and impending perforation. An echogenic periappendiceal mass indicates inflammation of the mesenteric or omental fat. Loculated or generalized fluid collections suggest that perforation already has occurred.

Because graded compression ultrasonography is highly operator dependent, a meta-analysis has demonstrated that the procedure is 88 percent sensitive and 94 percent specific in diagnosing acute appendicitis in children.[52] False-positive ultrasound results occur in obese patients who have noncompressible appendices because of overlying fat and in children who have inflamed appendices caused by Crohn disease, ulcerative colitis, or adjacent salpingitis. False-negative results occur if retrocecally located appendices are not visualized properly; if the cecum is filled with gas or feces and is not compressed adequately; or if perforation has occurred, allowing the appendix to be compressible. In one study, a noncompressible appendix was identified in only 38 percent of pediatric patients with perforated appendicitis, thus rendering the other ultrasound findings of appendicitis important in diagnosing the disease.[158] The examination should be directed to diagnose other causes of abdominal pain that can mimic appendicitis when a normal appendix is found during the evaluation.

The advantages of using ultrasonography over CT are that it is relatively inexpensive, is safe, does not require sedation, lacks radiation exposure, and is widely available. It is especially useful in adolescent girls with abdominal pain because gynecologic causes of the pain can be evaluated easily at the time of appendiceal examination.

Most North American pediatric surgeons prefer CT over ultrasonography as the diagnostic procedure of choice for appendicitis.[152] High-resolution CT has higher sensitivity (94%) and similar specificity (95%) than ultrasonography in diagnosing appendicitis, and it is less operator dependent.[52] Intravenous contrast agents and high-resolution, thin-section scanning techniques must be used to visualize the appendix adequately. An enlarged appendix with a circumferentially and symmetrically thickened bowel wall is the most common CT finding in appendicitis. Periappendiceal inflammatory reaction or fluid collections may be identified. If the appendix is not well visualized, the presence of a fecalith, along with pericecal inflammatory changes, strongly suggests appendicitis. Fecaliths can be visualized in normal appendices by CT, however, and are of no clinical significance unless other inflammatory changes are present.

Helical or multidetector CT techniques using rectal or no contrast have been shown to be very accurate in diagnosing appendicitis.[54,122,197] By using a focused right lower quadrant approach, helical CT may be completed more rapidly. Because of the high accuracy rates in diagnosing appendicitis, some physicians have suggested that

all children with suspected disease should have CT.[102] In most community hospitals and medical centers, waiting for a CT scan may delay a surgical consultation and increase the rate of perforation before surgery.[114] Also, CT is expensive and uses significant amounts of ionizing radiation in children who have greater radiosensitivity of organs and tissues compared with adults. Some medical centers have implemented protocols that initially use ultrasonography as the first diagnostic procedure for possible appendicitis and only use CT when the ultrasound results are equivocal to reduce cost and radiation exposure.[152]

Whether the increasing use of ultrasonography and CT to diagnose appendicitis in children has decreased the misdiagnosis of the disease and subsequent negative appendectomy rate in hospitals is unclear.[65,93,126,229] In a single study, the misdiagnosis rate was lower in hospitals that perform frequent pediatric appendectomies.[186] Most children with appendicitis, however, have surgery in hospitals other than children's hospitals.[45]

Radiolabeled autologous leukocyte scans also have been used to diagnose appendicitis in children; however, this modality should be reserved for atypical presentations of disease when localizing signs are not present.[81] Magnetic resonance imaging may be considered in patients with nondiagnostic ultrasound tests and concerns about radiation exposure.[105]

MICROBIOLOGY

Numerous microorganisms have been implicated as a cause of acute appendicitis; however, considerable debate has ensued as to whether simply isolating an organism from the appendiceal lumen is sufficient proof to define causation (Table 51-2).[2]

TABLE 51-2 **Microorganisms Associated with Acute Appendicitis in Children**

Anaerobes
Bacteroides spp.
Bilophila wadsworthia
Catabacter hongkongensis
Clostridium spp., including *C. difficile*
Fusobacterium spp.
Peptostreptococcus spp.
Pigmented bile-resistant, gram-negative rods
Turicibacter sanguinis

Enteric Aerobes and Facultative Anaerobes
Aeromonas spp.
Campylobacter spp.
Citrobacter spp.
Enterobacter spp.
Enterococcus spp.
Escherichia coli
Klebsiella spp.
Morganella morganii
Proteus spp.
Providencia rettgeri
Salmonella spp.
Shigella spp.
Streptococcus anginosus (formerly *milleri*) group
Yersinia species

Other Bacteria
Actinomyces spp.
Atypical mycobacteria (in patients with AIDS)
Chromobacterium violaceum
Corynebacterium appendicis
Eikenella corrodens
Haemophilus spp.
Ehrlichia chaffeensis
Kluyvera ascorbata
Pasteurella multocida
Pseudomonas spp.
Staphylococcus spp.
Streptococcus pneumoniae
Streptococcus pyogenes

Parasites
Angiostrongylus costaricensis
Anisakis spp.
Ascaris lumbricoides
Balantidium coli
Cryptosporidium parvum
Entamoeba histolytica
Enterobius vermicularis
Schistosoma spp.
Strongyloides stercoralis
Taenia spp.
Trichuris trichiura

Viruses
Adenoviruses
Coxsackievirus B
Cytomegalovirus
Epstein-Barr virus
Measles virus

Fungi
Candida albicans
Coccidioides immitis
Mucor spp.
Histoplasma capsulatum

Bacteria

In most cases of appendicitis, bacteria do not appear to be involved directly in the initial stages of the inflammatory process. Microorganisms that normally inhabit the appendix are liberated into the peritoneal cavity when appendiceal perforation occurs or when translocation through the inflamed tissues is present, and polymicrobial infections develop as a complication of the disease process.[27,204] In a study of 30 adolescents and adults with nonperforated and perforated appendicitis, 223 different anaerobes and 82 aerobes were recovered from cultures of their appendiceal tissues, peritoneal fluid, and contents of abscesses.[27] An average of 10 different organisms were isolated per specimen collected.

In most microbiologic studies of appendiceal tissues and peritoneal fluid specimens from patients with appendicitis, *B. fragilis* is the strict anaerobe isolated most frequently, occurring in more than 70 percent of patients.[27,164] Other anaerobes that are isolated frequently include *Bacteroides* spp., *Bilophila wadsworthia*, *Peptostreptococcus* spp., *Fusobacterium* spp., and *Clostridium* spp.[210,225] A gram-negative anaerobic rod that develops a pigment in culture and is bile resistant also has been identified frequently.[163,164] Anaerobes such as *Turicibacter sanguinis* and *Catabacter hongkongensis* continue to be newly described from patients with appendicitis.[33,109] Rarely, acute appendicitis has been seen in the setting of *Clostridium difficile* colitis, although the role of the anaerobe's toxins in causing the appendiceal inflammation is unknown.[36]

E. coli is the aerobic or facultative anaerobic bacteria isolated most frequently from children with appendicitis. *E. coli* is found in more than 75 percent of patients.[27,164] Researchers have suggested that certain *E. coli* strains with type 1C fimbriae may contribute to the development of appendiceal inflammation.[178] Enterohemorrhagic *E. coli* O157:H7 and O111:H have been isolated infrequently from the stools and peritoneal fluid of children with appendicitis.[208,215]

Viridans streptococci of the *S. anginosus group* can be found in more than 60 percent of cultures from children with appendicitis.[89,164] Group D streptococci are isolated in 20 to 30 percent of patients with appendicitis, whereas *Pseudomonas* spp. are isolated slightly less frequently.[164] Other aerobes or facultative anaerobes that can be isolated from appendiceal tissues, abscesses, or blood include *Citrobacter* spp.; *Klebsiella* spp.; *Enterobacter* spp.; *Proteus* spp.; *Morganella morganii; Providencia rettgeri; Eikenella corrodens;* groups C, F, and G β-hemolytic streptococci; and staphylococci.*

Rarely, encapsulated organisms, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Haemophilus segnis*, and *Aggregatibacter aphrophilus* have been isolated from appendiceal tissues or peritoneal fluid of young children with appendicitis, and often these organisms have been isolated in pure culture.[17,18,134,141] Very rarely, organisms such as *Pasteurella multocida*, *Streptococcus pyogenes*, and *Actinomyces* spp. have been cultured from patients with appendicitis.[56,120,161] *Shigella*, *Salmonella*, *Campylobacter*, *Yersinia*, and *Aeromonas* spp. also have been isolated occasionally from appendiceal tissues or peritoneal fluid of patients with nonperforated and perforated appendicitis; but, again, whether they played a role in the pathogenesis of disease is unknown.* Much more commonly, these organisms cause enterocolitis or mesenteric adenitis, with symptoms mimicking appendicitis.[212]

Appendicitis has occurred during systemic infections caused by *Brucella melitensis* and *Ehrlichia chaffeensis*.[11,182] Rarely, *Kluyvera ascorbata*, *Arcobacter butzleri*, and *Chromobacterium violaceum* have been isolated from individuals with appendicitis, as well as newly described aerobes and facultative anaerobes, such as *Corynebacterium appendicis*.[40,42,111,226]

Rarely, isolated primary tuberculosis can occur in children.[29,159] The progression of disease is usually rapid; thus one should be suspicious when caseating granulomas are observed in histopathologic sections of the appendix.

Adults and children with appendicitis usually are not bacteremic at the time they are diagnosed, especially if the appendix is not perforated. Occasionally, *Klebsiella pneumoniae*, *E. coli*, *B. fragilis*, and *B. wadsworthia* are isolated from the blood of patients with nonperforated appendicitis.[30,171,173] In a review of 1000 children and adults with appendicitis, 10 percent of patients with perforation had positive blood cultures, whereas none of the patients without perforation had bacteremia.[117] A higher rate of bacteremia may occur when laparoscopic surgery is performed because of the air that is forced into the peritoneum, although the clinical significance of the induced bacteremia is unknown.[147]

In immunocompromised patients who develop appendicitis, the microorganisms that are isolated from appendiceal tissues or peritoneal cultures usually are identical to those found in immunocompetent patients. Patients with AIDS who have gastrointestinal *Mycobacterium avium* complex or *Mycobacterium tuberculosis* infections may develop symptoms that mimic appendicitis.[49,214] Atypical mycobacteria have been isolated from an appendiceal abscess from a child with AIDS.[60]

Parasites

Roundworms, such as *Ascaris lumbricoides*, may obstruct the appendiceal lumen occasionally and initiate the cascade of inflammatory events leading to perforated appendicitis.[124,148] Parasites such as *Enterobius vermicularis* can be identified in the lumen of 1 to 12 percent of surgically removed appendices obtained from patients living in highly endemic areas.[14,78] Pinworms have been found more frequently, however, in appendices with no evidence of appendiceal inflammation in some studies, suggesting that pinworms probably are a part of the normal appendiceal flora and do not play a role in the pathogenesis of appendicitis.[172,228] Whether some parasites may cause abdominal pain that mimics the symptoms of appendicitis necessitating surgical intervention remains unclear.

Scattered reports from mostly developing nations describe other worms, including *Taenia* spp., *Anisakis* spp., *Trichuris trichiura*, *Strongyloides stercoralis*, *Schistosoma* spp., and *Angiostrongylus costaricensis*, that have been identified in the lumen of appendices from patients with

*References 53, 79, 91, 160, 164, 204.

*References 25, 28, 39, 98, 110, 119, 135.

appendicitis.[4,15,73,100,142] Similarly, protozoa such as *Balantidium coli*, *Entamoeba histolytica*, and *Cryptosporidium parvum* have been found in inflamed appendices of immunocompromised and immunocompetent patients, but whether they play a role in the pathogenesis of disease is unknown.[10,37,51]

Viruses

The role that viruses play in causing appendicitis also has been debated. One suggestion is that a systemic viral infection may induce hypertrophied lymphoid aggregates that obstruct the appendiceal lumen. In the 1960s, elevated levels of antibodies against group B coxsackieviruses and adenoviruses were found in the sera of some children with appendicitis.[205] A later study, however, could not confirm this finding.[137] Six adolescents with infectious mononucleosis have developed appendicitis, and cytomegalovirus has occasionally been observed in immunodeficient and otherwise healthy patients' inflamed appendices.[95,121,144,203] Other children have had histologic evidence of measles virus or adenovirus infection.[72,190] Because of the rarity of documented simultaneous viral infections and appendicitis, whether these viruses play a major role in the pathogenesis of acute appendicitis is doubtful.

Fungi

Rarely, *Candida albicans* is isolated from inflamed appendices or abscess cultures, but its role in pathogenesis of disease is unknown. Perforation of the appendix from intestinal mucormycosis has occurred in granulocytopenic patients and premature newborns.[145,202] Also, appendicitis has been described in individuals with histoplasmosis and coccidioidomycosis.[107,188]

TREATMENT

Nonperforated Appendicitis

In previously healthy children with signs of acute appendicitis and no clinical evidence of perforation, nasogastric suctioning should be established and imbalances in fluid and electrolyte concentrations should be corrected quickly. The child should be taken to the operating room as soon as possible for appendectomy. Many teaching hospitals now perform appendectomies only during day and evening hours, because of limitations on resident work hours and decreased services available at night. No differences have been noted in perforation rates, lengths of stay, or complication rates in children who are diagnosed with appendicitis at night and given analgesics until a scheduled morning surgery if the delay time is less than 12 hours.[1,7] Morphine can be used to reduce the severity of abdominal pain in children with appendicitis until the child is taken to the operating room and does not impede establishing the diagnosis in children under observation for suggestive symptoms and signs of appendiceal disease. There is no evidence, however, that morphine reduces appendiceal pain compared with placebo.[5,22]

Prophylactic antibiotics given perioperatively decrease the rate of postoperative wound infection, even in noncomplicated cases of childhood appendicitis.[6,183] No consensus exists concerning the appropriate antimicrobial agent or agents that should be used to reduce the complication rate. Although some surgeons continue the antibiotics after surgery, prospective, randomized studies demonstrate that a single perioperative dose of proper antibiotic(s) with antimicrobial activity against *E. coli* and enteric anaerobes is as effective as continuing the antibiotic(s) for 1 to 5 days after surgery.[139,207]

Few data support the routine intraoperative collection of peritoneal fluid or appendiceal cultures in children with nonperforated appendicitis, although 5 to 20 percent of cultures grow enteric aerobes, anaerobes, or both.[69] Immunocompromised patients should undergo intraoperative cultures, including cultures for mycobacteria and cytomegalovirus.

Most pediatric surgeons are now performing laparoscopic appendectomies in children with nonperforated and perforated appendicitis. Advantages of the procedure are a reduction in wound infection, reduction in scarring, less postoperative pain at 24 hours, shorter hospital stay, and earlier return to normal activity, although the procedure must be performed by a surgeon experienced in laparoscopic technique.[118,177,219] The mean total cost of a laparoscopic appendectomy is similar to that of open appendectomy. Meta-analyses have determined that there is increased operative time and a small increase in the risk for developing a postoperative abscess using the laparoscopic technique.[118,177,219] It is unclear whether the newer single incision laparoscopic technique improves outcome compared with conventional multiple-incision laparoscopy.[165,192]

Outside North America, children with nonperforated appendicitis are often treated initially with intravenous fluids and antibiotics. Two thirds of patients may be treated successfully without surgery.[64,213,216] When the child does not clinically improve or a walled-off abscess develops, drainage of the area and appendectomy are performed. If the child responds to conservative management, it is controversial as to whether an interval appendectomy has to be performed 6 to 8 weeks after resolution of the symptoms. Proponents of initial conservative management consider that the complication rate after interval appendectomy is significantly lower than when a procedure is done during the acute stage of disease.

Perforated Appendicitis

Most surgeons advocate early intervention when perforation has occurred to prevent severe complications such as fistula formation, abscess rupture, and death, despite the high chance of developing postoperative complications.[112] Debate ensues as to whether the wound(s) should be closed primarily and whether transperitoneal drains should be placed at the time of surgery.[57] Surgeons are increasingly performing primary closures without drains in children.[59,131] A randomized prospective trial of appendiceal drains in children with perforated appendicitis showed no benefit in using drains compared with primary wound closure.[198] Appendectomy wounds can be closed

with continuous, absorbable sutures even in complicated cases.[153] If incisions are not primarily closed, simple daily wound probing may decrease the incidence of wound infection.[206]

During the surgical procedure, many surgeons irrigate the peritoneal cavity with copious amounts of saline or antibiotics to reduce the quantity of bacteria in the abdomen. To obtain sterile cultures of the lavage fluid, 6 L of lavage fluid per square meter body surface area of the child is required.[149] It is unclear whether the lavage fluid or the addition of antibiotics to the lavage fluid decreases the rate of postoperative complications in children with perforated appendicitis who are receiving systemic antibiotics.[128] Ranitidine or diphenhydramine given to children with perforated appendicitis may increase the risk for developing a postoperative abscess.[194]

Antimicrobial agents should be administered routinely to children when perforation or appendiceal abscess is suggested or discovered during surgery. Antibiotics active against aerobes and anaerobes that normally inhabit the intestinal tract have been effective in treating children with perforated appendicitis. Treatment failures occur most commonly when *B. fragilis* or *Pseudomonas* spp. are isolated from intraoperative cultures and antimicrobial agents without activity against these organisms are used.[83] Controversy continues regarding the value of obtaining routine intraoperative peritoneal cultures in cases of perforated appendicitis, although most studies demonstrate that culture results seldom change the clinical management of patients.[48,69]

The antimicrobial combination of ampicillin, gentamicin, and clindamycin has been the gold standard of therapy since the 1970s. The importance of including ampicillin in the regimen for adequate enterococcal coverage continues to be controversial. Animal studies and clinical trials using antibiotics with poor enterococcal activity have shown that ampicillin probably is not required in the treatment of perforated appendicitis.[71] Because of the increasing problem of ampicillin resistance in enterococci, ampicillin probably should be reserved for the rare child with enterococcal bacteremia or with persistent intra-abdominal infection in which enterococci have been isolated. Some medical centers use metronidazole instead of clindamycin because of its broader activity against enteric anaerobes, whereas other institutions substitute cefotaxime or ceftriaxone for gentamicin.[180,193] In a small study, once-daily dosing of ceftriaxone and metronidazole was comparable in efficacy to standard three-drug therapy in children with perforated appendicitis.[195]

Single-agent antibiotic therapy for perforated appendicitis may offer improvement in terms of pharmacy and hospital costs.[70] Agents that have been shown to be effective in treating children with perforated appendicitis include cefoxitin, imipenem-cilastatin, ticarcillin-clavulanate, piperacillin-tazobactam, ampicillin-sulbactam, meropenem, and ertapenem.* In a few medical centers, nearly 50 percent of *B. fragilis* isolates are resistant to cefoxitin, raising the question as to whether cefoxitin should be used routinely as a single agent in these institutions.[67] Generally, the convenience of monotherapy does not outweigh the potential development of resistance to these broad-spectrum agents. They may be useful in the treatment of appendicitis in children with renal disease or hearing loss when avoiding the use of gentamicin is prudent.

Most patients with perforated appendicitis are treated with intravenous antibiotics for 5 to 10 days after surgery; however, limiting the duration of antibiotic use to 3 days does not lead to higher rates of wound infections or intra-abdominal abscesses in children who are afebrile and eating.[82,189] Efforts have been made to shorten the hospital stay of children with perforated appendicitis. Some institutions have set criteria for hospital discharge and discontinuation of antibiotics, such as absence of fever for 24 hours, ability to eat well, and less than 3 percent band forms on the white blood cell differential.[84] Many surgeons will switch to oral antibiotics at home after 3 to 5 days of intravenous antibiotics in the hospital, although the benefit of adding prolonged oral antibiotics to a 3-day course of intravenous antibiotics has not been shown.[66,200] Providing home single intravenous antimicrobial therapy also can reduce costs and hasten hospital discharge in selected children with perforated appendicitis.[191]

Similar to nonperforated appendicitis, some controversy exists regarding whether immediate appendectomy should be performed on children in whom a palpable mass is associated with their appendicitis or who show evidence of appendiceal rupture with or without abscess formation at the time of presentation.[32,132] Similarly, there is a lack of prospective studies to determine if interval appendectomy is required after successful nonoperative treatment of an appendiceal mass in children.[75] Families do experience more stress when nonoperative management of perforated appendicitis is implemented.[181]

PROGNOSIS AND EARLY COMPLICATIONS

Currently in the United States, the risk of dying of appendicitis is very low. It has been stated that the risk of death from appendicitis should be the risk of death from general anesthesia.[127] The mortality rate is higher in the rare newborn or premature infant who develops appendicitis. Also, factors contributing to the death of children rarely may include delay in establishing diagnosis, inadequate fluid replacement, immunodeficiency, and postoperative vascular or infectious complications.

The most predictive factor of postoperative morbidity occurring from appendicitis is perforation.[166] Age, obesity, duration of the surgical procedure, and nutritional status also are risk factors for the development of complications. Wound infection rates in children who receive perioperative antibiotics should be less than 7 percent; infections generally are caused by the same organisms that are isolated in cultures obtained during the appendectomy.[183,207] Occasionally, children develop peritonitis, intra-abdominal abscesses, psoas abscesses, fistulas, pylophlebitis of the portal vein, scrotal abscesses, empyema, or pneumoperitoneum during the course of treatment of

*References 125, 140, 184, 185, 201, 209, 223.

appendicitis.* Postoperative morbidity rates appear similar in children with nonperforated appendicitis when surgery is performed in teaching institutions compared with community hospitals; however, the rates are higher in children with perforated appendicitis in community hospitals.[115]

CT can be successfully used to detect postoperative abscesses even in the first week after surgery.[13] If complications occur, another surgical procedure often is performed and antibiotic treatment is prolonged. Abscesses may be treated successfully with antibiotics alone and without surgical drainage in stable patients after appendectomy.[50]

PELVIC ABSCESS

The pelvic area is a common site for development of abscesses because it is the most dependent portion of the peritoneal cavity. Pelvic abscesses most commonly occur in children who have had intestinal perforations after appendicitis, have suffered penetrating abdominal or retroperitoneal injury, or have undergone an abdominal surgical procedure. Occasionally, adolescents with pelvic inflammatory disease or Crohn disease develop a pelvic abscess.

In children with perforated appendicitis, a coexisting pelvic abscess often is diagnosed at the time of laparotomy. In patients who recently have had penetrating trauma to the abdomen, have had pelvic inflammatory disease, or have undergone gastrointestinal surgery, a pelvic abscess should be suspected when they have continued fever or complain of abdominal pain despite receiving adequate treatment of the initial disease process. Symptoms may not develop until days to months after therapy is ended. No characteristic physical findings are associated with a pelvic abscess, although abdominal palpation or rectal examination may elicit tenderness or signs of intestinal obstruction may be present.

If a pelvic abscess is suspected, contrast CT evaluation of the pelvis should be completed. The bladder should be filled before the procedure so that it can displace bowel loops from the pelvis, act as an anatomic marker, and act as a standard of fluidity against which an abscess cavity can be compared. Walled-off fluid collections in the pelvis can be identified and sometimes the rectum, sigmoid colon, or bladder is compressed because of mass effect from the abscess cavity. Because most pelvic abscesses develop as complications of intestinal or pelvic infections, enteric aerobes and anaerobes are the organisms most commonly isolated from the abscess cavity. Yeasts rarely cause pelvic abscesses.[211,220] *Actinomyces*-related pelvic abscesses uncommonly are observed.[154] Tuberculous abscesses can develop as a complication of genital tuberculosis.[218]

When a pelvic abscess is identified, antibiotics covering intestinal aerobes and anaerobes, such as clindamycin and gentamicin, should be started, and the abscess contents should be drained. In most situations, reaching the abscess cavity by an anterior approach is difficult. Considerable interest has developed in using CT or ultrasonography to guide percutaneous drainage of pelvic abscesses by transgluteal, transrectal, transparacoccygeal, or transvaginal approaches.[90,146,157,175] Although placement of a transgluteal catheter is easiest, the sciatic nerve and gluteal vessels must be avoided. Also, an increased risk for development of a wound infection may occur because microorganisms may track along the outside of the catheter to the skin. Many surgeons prefer the transrectal approach because it often is the most direct route to the abscess. Transvaginal drainage has been used with good results in young women. Most often, drainage catheters can be removed after 7 to 10 days of treatment.

Abscesses also may develop within the muscles of the pelvic girdle, including the psoas and internal obturator muscles.[179,196] Similar to true pelvic abscesses, they usually cause fever and occasionally abdominal complaints in children. Most children begin to limp, refuse to walk, or complain of pain in the buttocks, thigh, or groin. Often, a suppurative hip infection is suspected initially. A pelvic muscle abscess is usually diagnosed by CT or magnetic resonance imaging. Labeled leukocyte scans sometimes are useful in localizing the infection to within the pelvis, especially when the child has no symptoms other than fever or refusing to walk.

Pelvic muscle abscesses can develop as a complication of Crohn disease or appendicitis; however, they often develop after an episode of bacteremia.[31,176] *Staphylococcus aureus* is the most common cause of a primary pelvic muscle abscess with methicillin-resistant strains being increasingly identified.[151,179] *S. pneumoniae*, *H. influenzae* type b, *E. coli*, *Enterococcus faecalis*, *S. anginosus*, *Yersinia enterocolitica*, *Salmonella* spp., *Proteus mirabilis*, *Capnocytophaga sputigena*, and *Actinomyces* spp. also have been reported to cause hematogenously acquired abscesses.* Bacteremia secondary to intravenous drug abuse or the presence of central lines occasionally predisposes patients to developing this type of infection.[103,217] Rarely, tuberculous psoas abscesses have been reported, often as a complication of vertebral osteomyelitis.[199]

Pelvic muscle abscesses usually are drained by a percutaneous or surgical approach, and antibiotic therapy is based on Gram stain and culture results. Successful therapy with antibiotics alone has been reported.[133] Duration of treatment is individualized and depends on the child's response and the drainage techniques that were used.

LATE COMPLICATIONS

Most children who have undergone appendectomy or drainage of a pelvic abscess do not have late complications. Occasionally, patients develop signs of bowel obstruction from peritoneal adhesions. Some studies have suggested that the future risk for infertility is greater in women who have perforated appendices or pelvic abscesses, presumably because of adhesion formation that impairs the migration of ova in the reproductive tract.[138]

*References 21, 74, 85, 168, 224, 227.

*References 35, 41, 43, 46, 86, 94, 99, 104.

Although some retrospective studies have suggested that patients with appendectomies have a higher rate of developing malignancies later in life, prospective and controlled studies have failed to demonstrate this association.[44,136] Patients who have had appendicitis have been reported to be three times more likely to develop right inguinal hernias than individuals who have not had the appendix removed.[16] It is unclear whether there is a higher long-term risk for developing Crohn disease after appendicitis early in life, because recent studies have not confirmed earlier observations.[9,97]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Ajmera RK, Simon GL. Appendicitis associated with Taenia species: cause or coincidental? *Vector Borne Zoonotic Dis* 2010; **10**:321–2.
7. Andersson RE. Is appendicitis an emergency? *World J Surg* 2011;**35**:1634–5.
18. Aye AM, Law CW, Sabet NS, et al. Isolation of *Aggregatibacter aphrophilus* from a patient with acute appendicitis. *Eur Rev Med Pharmacol Sci* 2011;**15**:845–7.
19. Azodi OS, Andren-Sandberg A, Larsson H. Genetic and environmental influences on the risk of acute appendicitis in twins. *Br J Surg* 2009;**96**:1336–40.
20. Azodi OS, Lindstrom D, Adami J, et al. Impact of body mass index and tobacco smoking on outcome after open appendicectomy. *Br J Surg* 2008;**95**:751–7.
25. Bartoli F, Guerra A, Dolina M, et al. *Salmonella enterica* serovar Israel causing perforating appendicitis. *Int J Infect Dis* 2010;**14**: e538.
29. Bercu TE, Rubin ZA, Agopian VG. Tuberculous appendicitis. *Am J Med* 2011;**124**:e9–10.
31. Bertrand SL, Lincoln ED, Prohaska MG. Primary pyomyositis of the pelvis in children: a retrospective review of 8 cases. *Orthopedics* 2011;**34**:e832–40.
32. Blakely ML, Williams R, Dassinger MS, et al. Early vs interval appendectomy for children with perforated appendicitis. *Arch Surg* 2011;**146**:660–5.
41. Chan JF, Wong SS, Leung SS, et al. *Capnocytophaga sputigena* primary iliopsoas abscess. *J Med Microbiol* 2010;**59**:1368–70.
48. Davies HOB, Alkhamesi NA, Dawson PM. Peritoneal fluid culture in appendicitis: review in changing times. *Int J Surg* 2010;**8**:426–9.
54. Doumit G, Abouhassan W, Reimer MW, et al. The role of multidetector computed tomography for diagnosing acute appendicitis. *Ann Intern Med* 2011;**154**:136.
56. Dumas F, Kierzek G, Coignard S, et al. Acute appendicitis, an unusual presentation of *Streptococcus pyogenes* infection. *Am J Emerg Med* 2009;**27**:254.e1–2.
57. Duttaroy DD, Jitendra J, Duttaroy B, et al. Management strategy for dirty abdominal incisions: primary or delayed primary closure? a randomized trial. *Surg Infect* 2009;**10**:129–36.
58. Ebell MK. Diagnosis of appendicitis, part 1: history and physical examination. *Am Fam Physician* 2008;**77**:828–30.
64. Fitzmaurice GJ, McWilliams B, Hurreiz H, et al. Antibiotics versus appendectomy in the management of acute appendicitis: a review of the current evidence. *Can J Surg* 2011;**54**:307–14.
66. Fraser JD, Aguayo P, Leys CM, et al. A complete course of intravenous antibiotics vs a combination of intravenous and oral antibiotics for perforated appendicitis in children: a prospective, randomized trial. *J Pediatr Surg* 2010;**45**:1198–202.
72. Grynspan D, Rabah R. Adenoviral appendicitis presenting clinically as acute appendicitis. *Pediatr Develop Pathol* 2008;**11**: 138–41.
75. Hall NJ, Jones CE, Eaton S, et al. Is interval appendicectomy justified after successful nonoperative treatment of an appendix mass in children? a systematic review. *J Pediatr Surg* 2011;**46**: 767–71.
77. Harlak A, Gulee M, Mentes O, et al. Atopy is a risk factor for acute appendicitis? a prospective clinical study. *J Gastrointest Surg* 2008;**12**:1251–6.
78. Harris A, Muttalib M. *Enterobius* infestation in suspected appendicitis. *Postgrad Med J* 2008;**84**:614.
92. Jancelewicz T, Kim G, Miniati D. Neonatal appendicitis: a new look at an old zebra. *J Pediatr Surg* 2008;**43**:e1–5.
102. Krajewski S, Brown J, Phang PT, et al. Impact of computed tomography of the abdomen on clinical outcomes in patients with acute right lower quadrant pain: a meta-analysis. *Can J Surg* 2011;**54**:43–53.
104. Lachenal F, Meeus P, Thiesse P, et al. *Streptococcus pneumoniae* retroperitoneal and pelvic abscess. *Lancet Infect Dis* 2011;**11**: 720.
105. Lam M, Singh A, Kaewlai R, et al. Magnetic resonance of acute appendicitis: pearls and pitfalls. *Curr Probl Diagn Radiol* 2008;**37**: 57–66.
106. Lameris W, van Randen A, Go MNYH, et al. Single and combined diagnostic value of clinical features and laboratory tests in acute appendicitis. *Acad Emerg Med* 2009;**16**:835–42.
112. Lee SL, Islam S, Cassidy LD, et al. Antibiotics and appendicitis in the pediatric population: an American Pediatric Surgical Association outcomes and clinical trials committee systematic review. *J Pediatr Surg* 2010;**45**:2181–5.
113. Lee SL, Shekherdimian S, Chiu VY. Effect of race and socioeconomic status in the treatment of appendicitis in patients with equal health care access. *Arch Surg* 2011;**146**:156–61.
115. Lee SL, Yaghoubian A, de Virgilio C. A multi-institutional comparison of pediatric appendicitis outcomes between teaching and nonteaching hospitals. *J Surg Educ* 2011;**68**:6–9.
118. Li X, Zhang J, Sang L, et al. Laparoscopic versus conventional appendectomy—a meta-analysis of randomized controlled trials. *BMC Gastroenterol* 2010;**10**:129.
119. Lim PL. Appendicitis associated with travelers' diarrhea caused by *Aeromonas sobria*. *J Travel Med* 2009;**16**:132–3.
120. Liu V, Val S, Kang K, et al. Case report: actinomycosis of the appendix—an unusual cause of acute appendicitis in children. *J Pediatr Surg* 2010;**45**:2050–2.
132. Meshikhes A-WN. Management of appendiceal mass: controversial issues revisited. *J Gastrointest Surg* 2008;**12**:767–75.
133. Miller NJ, Duncan RD, Huntley JS. The conservative management of primary pyomyositis abscess in children: a case series and review of the literature. *Scott Med J* 2011;**56**:181.
142. Nandipati K, Parithivel V, Niazi M. Schistosomiasis: a rare cause of acute appendicitis in the African American population in the United States. *Amer Surg* 2008;**74**:221–3.
152. Parks NA, Schroeppel TJ. Update on imaging for acute appendicitis. *Surg Clin N Am* 2011;**91**:141–54.
153. Pauniaho S-L, Lahdes-Vasama T, Helminen MT, et al. Non-absorbable interrupted versus absorbable continuous skin closure in pediatric appendectomies. *Scand J Surg* 2010;**99**: 142–6.
157. Puri R, Eloubeidi MA, Sud R, et al. Endoscopic ultrasound-guided drainage of pelvic abscess without fluoroscopy guidance. *J Gastroenterol Hepatol* 2010;**25**:1416–19.
165. Rehman R, Rao AM, Ahmed I. Single incision versus conventional multi-incision appendicectomy for suspected appendicitis. *Cochrane Database Syst Rev* 2011;CD009022.
173. Salemis NS. Acute appendicitis presenting with *Klebsiella pneumoniae* septicemia due to bacterial translocation. *Am J Emerg Med* 2009;**27**:1023.e3–e4.
174. Sand M, Bechara FG, Holland-Letz T, et al. Diagnostic value of hyperbilirubinemia as a predictive factor for appendiceal perforation in acute appendicitis. *Am J Surg* 2009;**198**:193–8.
175. Saokar A, Arellano RS, Gervais DA, et al. Transvaginal drainage of pelvic fluid collections: results, expectations, and experience. *Am J Roentgenol* 2008;**191**:1352–8.
177. Sauerland S, Jaschinski T, Neugebauer EAM. Laparoscopic versus open surgery for suspected appendicitis. *Cochrane Database Syst Rev* 2010;CD001546.
181. Schurman JV, Cushing CC, Garey CL, et al. Quality of life assessment between laparoscopic appendectomy at presentation and interval appendectomy for perforated appendicitis with abscess: analysis of a prospective randomized trial. *J Pediatr Surg* 2011; **46**:1121–5.
188. Smith G, Hoover S, Sobonya R, et al. Abdominal and pelvic coccidioidomycosis. *Am J Med Sci* 2011;**341**:308–11.
190. Stadlmann S, Lenggenhager DM, Alves VA, et al. Histopathologic characteristics of the transitional stage of measles-associated

appendicitis: case report and review of the literature. *Human Pathol* 2011;**42**:285–90.
192. St. Peter SD, Adibe OO, Juang D, et al. Single incision versus standard 3-port laparoscopic appendectomy. *Ann Surg* 2011;**254**:586–90.
194. St. Peter SD, Sharp SW, Ostlie DJ. Influence of histamine receptor antagonists on the outcome of perforated appendicitis. *Arch Surg* 2010;**145**:143–6.
195. St. Peter SD, Tsao K, Spilde TL, et al. Single daily dosing ceftriaxone and metronidazole vs standard triple antibiotic regimen for perforated appendicitis in children: a prospective randomized trial. *J Pediatr Surg* 2008;**43**:981–5.
197. Suh SW, Choi YS, Park JM, et al. Clinical factors for distinguishing perforated from nonperforated appendicitis: a comparison using multidetector computed tomography in 528 laparoscopic appendectomies. *Surg Laparosc Endosc Percutan Tech* 2011;**21**:72–5.
206. Towfigh S, Clarke T, Yacoub W, et al. Significant reduction of wound infections with daily probing of contaminated wounds. *Arch Surg* 2011;**146**:448–52.
213. Varadhan KK, Humes DJ, Neal KR, et al. Antibiotic therapy versus appendectomy for acute appendicitis: a meta-analysis. *World J Surg* 2010;**34**:199–209.
216. Vons C, Barry C, Maitre S, et al. Amoxicillin plus clavulanic acid versus appendicectomy for treatment of acute uncomplicated appendicitis: an open-label, non-inferiority, randomized controlled trial. *Lancet* 2011;**377**:1573–9.
219. Wei B, Qi C-L, Chen T-F, et al. Laparoscopic versus open appendectomy for acute appendicitis: a metaanalysis. *Surg Endosc* 2011;**25**:1199–208.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 52

Pancreatitis

Thomas L. Kuhls

Pancreatitis was thought to be an uncommon cause of abdominal pain in children and a disease primarily of adults. Because of better recognition of symptoms in children and the more frequent use of medications that cause pancreatic inflammation, acute pancreatitis currently is being diagnosed more frequently in institutions specializing in pediatric care.[109,208]

Compared with causes of acute pancreatitis in adults—primarily alcoholism, cholelithiasis, and trauma—causes of childhood pancreatitis are more diverse. Microorganisms account for a significant proportion of cases of pancreatitis in children. In addition, antimicrobial agents have been associated with severe and occasionally fatal episodes of pancreatitis and bacterial infections may complicate the natural history of acute and chronic pancreatitis. Pediatricians who care for children with pancreatitis must have expertise in the diagnosis and treatment of infectious diseases.

CLINICAL MANIFESTATIONS

More than 80 percent of children with acute pancreatitis complain of abdominal pain.[121,208] Only 30 percent of pediatric patients have epigastric pain as usually described by adults, however.[205] In children, other sites of focal tenderness or diffuse pain include the right upper quadrant of the abdomen, the periumbilical area, the entire abdomen, and, less commonly, the right lower quadrant of the abdomen. The onset of pain usually is rapid and increases to a maximal intensity in a few hours, but occasionally the onset may be slow and gradual. Most often the pain is sharp and excruciating. Only one third of children complain of pain that radiates to other areas, including the back, lower part of the abdomen, upper abdominal quadrants, and anterior chest wall. In school-aged children the pain often intensifies after meals.

Two thirds of children with acute pancreatitis have vomiting.[21] Children younger than 5 years of age occasionally experience vomiting without abdominal tenderness.[215] Fever is present in only 30 percent of children with pancreatitis, but temperatures greater than 38.5 °C [101.3 °F] are observed occasionally.[205] Children may present with only the symptom of cough, and pleural effusions are found on radiography.[118]

On physical examination, children are classically found lying quietly on their sides with their knees flexed. They usually have epigastric tenderness to palpation and decreased or absent bowel sounds. Abdominal distention is found in 30 percent of children with pancreatitis and occurs more commonly in preschool-aged children.[205,215] Rebound tenderness, guarding of the epigastrium, jaundice, an abdominal mass, or ascites occasionally is detected. Rarely, ecchymoses of the flanks (Grey Turner

REFERENCES

1. Abou-Nukta F, Bakhos C, Arroyo K, et al. Effects of delaying appendectomy for acute appendicitis for 12 to 24 hours. *Arch Surg* 2006;**141**:504–7.
2. Addiss DG, Juranek DD. Lack of evidence for a causal association between parasitic infections and acute appendicitis. *J Infect Dis* 1991;**164**:1036–7.
3. Addiss DG, Shaffer N, Fowler BS, et al. The epidemiology of appendicitis and appendectomy in the United States. *Am J Epidemiol* 1990;**132**:910–25.
4. Ajmera RK, Simon GL. Appendicitis associated with *Taenia* species: cause or coincidental? *Vector Borne Zoonotic Dis* 2010; **10**:321–2.
5. Amoli HA, Golozar A, Keshavarzi H, et al. Morphine analgesia in patients with acute appendicitis: a randomized double-blind clinical trial. *Emerg Med J* 2007;**25**:586–9.
6. Andersen BR, Kallehave FL, Andersen HK. Antibiotics versus placebo for prevention of postoperative infection after appendicectomy. *Cochrane Database Syst Rev* 2005;(3):CD001439.
7. Andersson RE. Is appendicitis an emergency? *World J Surg* 2011;**35**:1634–5.
8. Andersson RE. Meta-analysis of the clinical and laboratory diagnosis of appendicitis. *Br J Surg* 2004;**91**:28–37.
9. Andersson RE, Olaison G, Tysk T, et al. Appendectomy is followed by increased risk of Crohn's disease. *Gastroenterol* 2003; **124**:40–6.
10. Andrade JE, Mederos R, Rivero H, et al. Amebiasis presenting as acute appendicitis. *South Med J* 2007;**100**:1140–2.
11. Andriopoulos P, Tsironi M, Simakopoulos G. Acute abdomen due to *Brucella melitensis*. *Scand J Infect Dis* 2003;**35**:204–5.
12. Angel CA, Rao BN, Wrenn E, et al. Acute appendicitis in children with leukemia and other malignancies: still a diagnostic dilemma. *J Pediatr Surg* 1992;**27**:476–9.
13. Antevil JL, Egan JC, Woodbury RO, et al. Abdominal computed tomography for postoperative abscess: is it useful during the first week? *J Gastrointest Surg* 2006;**10**:901–5.
14. Arca MJ, Gates RL, Groner JI, et al. Clinical manifestations of appendiceal pinworms in children: an institutional experience and a review of the literature. *Pediatr Surg Int* 2004;**20**:372–5.
15. Arenal Vera JJ, Marcos Rodriquez JL, Borrego Pintado MH, et al. Anisakiasis as a cause of acute appendicitis and rheumatologic picture: the first case in medical literature. *Rev Esp Enferm Digest* 1991;**79**:355–8.
16. Arnbjornsson E. Development of right inguinal hernia after appendectomy. *Am J Surg* 1982;**143**:174–5.
17. Astagneau P, Goldstein FW, Francoual S, et al. Appendicitis due to both *Streptococcus pneumoniae* and *Haemophilus influenzae*. *Eur J Clin Microbiol Infect Dis* 1992;**11**:559–60.
18. Aye AM, Law CW, Sabet NS, et al. Isolation of *Aggregatibacter aphrophilus* from a patient with acute appendicitis. *Eur Rev Med Pharmacol Sci* 2011;**15**:845–7.
19. Azodi OS, Andren-Sandberg A, Larsson H. Genetic and environmental influences on the risk of acute appendicitis in twins. *Br J Surg* 2009;**96**:1336–40.
20. Azodi OS, Lindstrom D, Adami J, et al. Impact of body mass index and tobacco smoking on outcome after open appendicectomy. *Br J Surg* 2008;**95**:751–7.
21. Babcock DS. Ultrasound diagnosis of portal vein thrombosis as a complication of appendicitis. *A J R Am J Roentgenol* 1979;**133**: 317–19.
22. Bailey B, Bergeron S, Gravel J, et al. Efficacy and impact of intravenous morphine before surgical consultation in children with right lower quadrant pain suggestive of appendicitis: a randomized controlled trial. *Ann Emerg Med* 2007;**50**:371–8.
23. Bancroft FW, Skoluda ER. Appendicitis: a study of 596 cases. *N Y State J Med* 1936;**36**:507–9.
24. Baron EJ, Bennion R, Thompson J, et al. Microbiological comparison between acute and complicated appendicitis. *Clin Infect Dis* 1992;**14**:227–31.
25. Bartoli F, Guerra A, Dolina M, et al. *Salmonella enterica* serovar Israel causing perforating appendicitis. *Int J Infect Dis* 2010;**14**:e538.
26. Beltran MA, Almonacid J, Vicencio A, et al. Predictive value of white blood cell count and C-reactive protein in children with appendicitis. *J Pediatr Surg* 2007;**42**:1208–14.
27. Bennion RS, Baron EJ, Thompson JE, et al. The bacteriology of gangrenous and perforated appendicitis revisited. *Ann Surg* 1990;**211**:165–71.
28. Bennion RS, Thompson JE, Gil J, et al. The role of *Yersinia enterocolitica* in appendicitis in the southwestern United States. *Am Surg* 1991;**57**:766–8.
29. Bercu TE, Rubin ZA, Agopian VG. Tuberculous appendicitis. *Am J Med* 2011;**124**:e9–10.
30. Bernard D, Verschraegen G, Claeys G, et al. *Bilophila wadsworthii* bacteremia in a patient with gangrenous appendicitis. *Clin Infect Dis* 1994;**18**:1023–4.
31. Bertrand SL, Lincoln ED, Prohaska MG. Primary pyomyositis of the pelvis in children: a retrospective review of 8 cases. *Orthopedics* 2011;**34**:e832–40.
32. Blakely ML, Williams R, Dassinger MS, et al. Early vs interval appendectomy for children with perforated appendicitis. *Arch Surg* 2011;**146**:660–5.
33. Bosshard PP, Zbinden R, Altwegg M. *Turicibacter sanguinis* gen. nov., sp. nov., a novel anaerobic, Gram-positive bacterium. *Int J Syst Evol Microbiol* 2002;**52**:1263–6.
34. Bova R, Meagher A. Appendicitis in HIV-positive patients. *Aust N Z J Surg* 1998;**68**:337–9.
35. Brooks DJ, Cant AJ, Lambert HP, et al. Recurrent *Salmonella* septicaemia with aortitis, osteomyelitis and psoas abscess. *J Infect* 1983;**7**:156–8.
36. Brown TA, Rajappannair L, Dalton AB, et al. Acute appendicitis in the setting of *Clostridium difficile* colitis: case report and review of the literature. *Clin Gastroenterol Hepatol* 2007;**5**:969–71.
37. Buch K, Nguyen S, Divino CM, et al. Cryptosporidiosis presenting as acute appendicitis: a case report. *Am Surgeon* 2005;**71**:537–8.
38. Bundy DG, Byerley JS, Liles EAI, et al. Does this child have appendicitis? *JAMA* 2007;**298**:438–51.
39. Campbell LK, Havens JM, Scott MA, et al. Molecular detection of *Campylobacter jejuni* in archival cases of acute appendicitis. *Mod Pathol* 2006;**19**:1042–6.
40. Carter JE, Evans TN. Clinically significant *Kluyvera* infections—a report of seven cases. *Am J Clin Pathol* 2005;**123**:334–8.
41. Chan JF, Wong SS, Leung SS, et al. *Capnocytophaga sputigena* primary iliopsoas abscess. *J Med Microbiol* 2010;**59**:1368–70.
42. Chen C-H, Lin L-C, Liu C-E, et al. *Chromobacterium violaceum* bacteremia: a case report. *J Microbiol Immunol Infect* 2003;**36**: 141–4.
43. Coakham HB, Ashby EC. Actinomycosis in recurrent psoas abscess. *Proc R Soc Med* 1972;**65**:880.
44. Cope JU, Askling J, Gridley G, et al. Appendectomy during childhood and adolescence and the subsequent risk of cancer in Sweden. *Pediatrics* 2003;**111**:1343–50.
45. Cosper GH, Hamann MS, Stiles A, et al. Hospital characteristics affect outcomes for common pediatric surgical conditions. *Am Surgeon* 2006;**72**:739–45.
46. Davies D, King SM, Parekh RS, et al. Psoas abscess caused by *Haemophilus influenzae*, type b. *Pediatr Infect Dis J* 1991;**10**: 411–2.
47. Davies GM, Dasbach EJ, Teutsch S. The burden of appendicitis-related hospitalizations in the United States in 1997. *Surg Infect* 2004;**5**:160–5.
48. Davies HOB, Alkhamesi NA, Dawson PM. Peritoneal fluid culture in appendicitis: review in changing times. *Int J Surg* 2010;**8**:426–9.
49. Dezfuli MG, Oo MM, Jones BE, et al. Tuberculosis mimicking acute appendicitis in patients with human immunodeficiency virus infection. *Clin Infect Dis* 1994;**18**:650–51.
50. Dobremez E, Lavrand F, Lefevre Y, et al. Treatment of post-appendectomy intra-abdominal deep abscesses. *Eur J Pediatr Surg* 2003;**13**:393–7.
51. Dodd LG. *Balantidium coli* infestation as a cause of acute appendicitis. *J Infect Dis* 1991;**163**:1392.
52. Doria AS, Moineddin R, Kellenberger CJ, et al. US or CT for diagnosis of appendicitis in children and adults? A meta-analysis. *Radiology* 2006;**241**:83–94.
53. Dougherty SH, Saltzstein EC, Peacock JB, et al. Perforated or gangrenous appendicitis treated with aminoglycosides. *Arch Surg* 1989;**124**:1280–83.
54. Doumit G, Abouhassan W, Reimer MW, et al. The role of multidetector computed tomography for diagnosing acute appendicitis. *Ann Intern Med* 2011;**154**:136.

55. Dueholm S, Bagi P, Bud M. Laboratory aid in the diagnosis of acute appendicitis: a blinded, prospective trial concerning diagnostic value of leukocyte count, neutrophil differential count, and C-reactive protein. *Dis Colon Rectum* 1989;**32**:855–9.
56. Dumas F, Kierzek G, Coignard S, et al. Acute appendicitis, an unusual presentation of *Streptococcus pyogenes* infection. *Am J Emerg Med* 2009;**27**:254.e1–2.
57. Duttaroy DD, Jitendra J, Duttaroy B, et al. Management strategy for dirty abdominal incisions: primary or delayed primary closure? a randomized trial. *Surg Infect* 2009;**10**:129–36.
58. Ebell MK. Diagnosis of appendicitis, part 1: history and physical examination. *Am Fam Physician* 2008;**77**:828–30.
59. Emil S, Laberge J-M, Mikhail P, et al. Appendicitis in children: a ten-year update of therapeutic recommendations. *J Pediatr Surg* 2003;**38**:236–42.
60. Enami MA, Frayha HH, Halim MA. An appendiceal abscess due to *Mycobacterium kansasii* in a child with AIDS. *Clin Infect Dis* 1998;**27**:891–92.
61. England RJ, Crabbe DCG. Delayed diagnosis of appendicitis in children treated with antibiotics. *Pediatr Surg Int* 2006;**22**: 541–5.
62. Ergul E. Heredity and familial tendency of acute appendicitis. *Scand J Surg* 2007;**96**:290–2.
63. Fitz RH. Perforating inflammation of the vermiform appendix, with special reference to its early diagnosis and treatment. *Am J Med Sci* 1886;**92**:321–46.
64. Fitzmaurice GJ, McWilliams B, Hurreiz H, et al. Antibiotics versus appendectomy in the management of acute appendicitis: a review of the current evidence. *Can J Surg* 2011;**54**:307–14.
65. Flum DR, Morris A, Koepsell T, et al. Has misdiagnosis of appendicitis decreased over time? A population-based analysis. *JAMA* 2001;**286**:1748–53.
66. Fraser JD, Aguayo P, Leys CM, et al. A complete course of intravenous antibiotics vs a combination of intravenous and oral antibiotics for perforated appendicitis in children: a prospective, randomized trial. *J Pediatr Surg* 2010;**45**:1198–202.
67. Fraulin FOG, Thurston OG. Value of cultures of tissue samples taken at operation for lower intestinal perforation. *Can J Surg* 1993;**36**:261–5.
68. Gallerani M, Boari B, Anania G, et al. Seasonal variation in onset of acute appendicitis. *Clin Ter* 2006;**157**:123–7.
69. Gladman MA, Knowles CH, Gladman LJ, et al. Intra-operative culture in appendicitis: traditional practice challenged. *Ann R Coll Surg Engl* 2004;**86**:196–201.
70. Goldin AB, Sawin RS, Garrison MM, et al. Aminoglycoside-based triple-antibiotic therapy versus monotherapy for children with ruptured appendicitis. *Pediatrics* 2007;**119**:905–11.
71. Gorbach SL. Intra-abdominal infections. *Clin Infect Dis* 1993; **17**:961–7.
72. Grynspan D, Rabah R. Adenoviral appendicitis presenting clinically as acute appendicitis. *Pediatr Develop Pathol* 2008;**11**: 138–41.
73. Gupta SC, Gupta AK, Keswani NK, et al. Pathology of tropical appendicitis. *J Clin Pathol* 1989;**42**:1169–72.
74. Haas GP, Shumaker BP, Haas PA. Appendicovesical fistula. *Urology* 1984;**24**:604–9.
75. Hall NJ, Jones CE, Eaton S, et al. Is interval appendicectomy justified after successful nonoperative treatment of an appendix mass in children? A systematic review. *J Pediatr Surg* 2011;**46**: 767–71.
76. Hardwick RH, Taylor A, Thompson MH, et al. Association between *Streptococcus milleri* and abscess formation after appendicitis. *Ann R Coll Surg Engl* 2000;**82**:24–6.
77. Harlak A, Gulee M, Mentes O, et al. Atopy is a risk factor for acute appendicitis? A prospective clinical study. *J Gastrointest Surg* 2008;**12**:1251–6.
78. Harris A, Muttalib M. *Enterobius* infestation in suspected appendicitis. *Postgrad Med J* 2008;**84**:614.
79. Hasan RA, Abuhammour W. Beta-hemolytic group F streptococcal bacteremia in children. *Pediatr Infect Dis J* 2004;**23**:468–70.
80. HCUPnet, Healthcare Cost and Utilization Project. Rockville, MD: Agency for Healthcare Research and Quality. 2002. Available at http://www.ahrq.gov/data/hcup/hcupnet.htm.
81. Henneman PL, Marcus CS, Inkelis SH, et al. Evaluation of children with possible appendicitis using technetium 99m leukocyte scan. *Pediatrics* 1990;**85**:838–43.
82. Henry MCW, Walker A, Silverman BL, et al. Risk factors for the development of abdominal abscess following operation for perforated appendicitis in children. *Arch Surg* 2007;**142**:236–41.
83. Heseltine PNR, Yellin AE, Appleman MD, et al. Perforated and gangrenous appendicitis: an analysis of antibiotic failures. *J Infect Dis* 1983;**148**:322–9.
84. Hoelzer DJ, Zabel DD, Zern JT. Determining duration of antibiotic use in children with complicated appendicitis. *Pediatr Infect Dis J* 1999;**18**:979–82.
85. Hoffer FA, Ablow RC, Gryboski JD, et al. Primary appendicitis with an appendico-tuboovarian fistula. *A J R Am J Roentgenol* 1982;**138**:742–3.
86. Humphreys H, Keane CT, Marron P, et al. Infective sacroiliac arthritis and psoas abscess caused by *Streptococcus milleri*. *J Infect* 1989;**19**:77–8.
87. Hyman P, Westring DW. Leukocytosis in acute appendicitis: observed racial difference. *J A M A* 1974;**229**:1630–2.
88. Jablonski KA, Guagliardo MF. Pediatric appendicitis rupture rate: a national indicator of disparities in healthcare access. *Popul Health Met* 2005;**3**:4.
89. Jackson DS, Welch DF, Pickett DA, et al. Suppurative infections in children caused by non-beta-hemolytic members of the *Streptococcus milleri* group. *Pediatr Infect Dis J* 1995;**14**:80–2.
90. Jaffe TA, Nelson RC, DeLong DM, et al. Practice patterns in percutaneous image-guided intraabdominal abscess drainage: survey of academic and private practice centers. *Radiology* 2004; **233**:750–6.
91. Jakobsen J, Andersen JC, Klausen IC. Beta-haemolytic streptococci in acute appendicitis. *Acta Chir Scand* 1988;**154**:301–3.
92. Jancelewicz T, Kim G, Miniati D. Neonatal appendicitis: a new look at an old zebra. *J Pediatr Surg* 2008;**43**:e1–5.
93. Jones K, Pena AA, Dunn EL, et al. Are negative appendectomies still acceptable? *Am J Surg* 2004;**188**:748–54.
94. Kahn FW, Glasser JE, Agger WA. Psoas muscle abscess due to *Yersinia enterocolitica*. *Am J Med* 1984;**76**:947–9.
95. Kanafani ZA, Sharara AI, Shabb NS, et al. Cytomegalovirus appendicitis following acute Epstein-Barr virus infection in an immunocompetent patient. *Scand J Dis* 2004;**36**:505–7.
96. Kang JY, Hoare J, Majeed A, et al. Decline in admission rates for acute appendicitis in England. *Br J Surg* 2003;**90**:1586–92.
97. Kaplan GG, Pedersen BV, Andersson RE, et al. The risk of developing Crohn's disease after an appendectomy: a population-based cohort study in Sweden and Denmark. *Gut* 2007;**56**:1387–92.
98. Kazlow PG, Freed J, Rosh JR, et al. *Salmonella typhimurium* appendicitis. *J Pediatr Gastroenterol Nutr* 1991;**13**:101–3.
99. Knobel B, Sommer I, Schwartz G. Primary psoas abscess three years after ipsilateral nephrectomy. *Infection* 1985;**13**:27–8.
100. Komenaka IK, Wu GCH, Lazar EL, et al. *Strongyloides* appendicitis: unusual etiology in two siblings with chronic abdominal pain. *J Pediatr Surg* 2003;**38**:e37.
101. Kosloske AM, Love CL, Rohrer JE, et al. The diagnosis of appendicitis in children: outcomes of a strategy based on pediatric surgical evaluation. *Pediatrics* 2004;**113**:29–34.
102. Krajewski S, Brown J, Phang PT, et al. Impact of computed tomography of the abdomen on clinical outcomes in patients with acute right lower quadrant pain: a meta-analysis. *Can J Surg* 2011;**54**:43–53.
103. Kwok T, Coles J. Psoas abscess as a complication of subclavian venous catheterization. *Postgrad Med J* 1990;**66**:771–2.
104. Lachenal F, Meeus P, Thiesse P, et al. *Streptococcus pneumoniae* retroperitoneal and pelvic abscess. *Lancet Infect Dis* 2011;**11**:720.
105. Lam M, Singh A, Kaewlai R, et al. Magnetic resonance of acute appendicitis: pearls and pitfalls. *Curr Probl Diagn Radiol* 2008;**37**: 57–66.
106. Lameris W, van Randen A, Go MNYH, et al. Single and combined diagnostic value of clinical features and laboratory tests in acute appendicitis. *Acad Emerg Med* 2009;**16**:835–42.
107. Lamps LW, Molina CP, Haggitt RC, et al. The pathologic spectrum of gastrointestinal and hepatic histoplasmosis. *Am J Clin Pathol* 2000;**113**:64–72.
108. LaRaja RD, Rothenberg RE, Odom JW, et al. The incidence of intra-abdominal surgery in acquired immunodeficiency syndrome: a statistical review of 904 patients. *Surgery* 1989;**105**:175–9.
109. Lau SKP, McNabb A, Woo GKS, et al. *Catabacter hongkongensis* gen. nov., isolated from blood cultures of patients from Hong Kong and Canada. *J Clin Microbiol* 2007;**45**:395–401.

110. Lau SKP, Woo PCY, Chan CYF, et al. Typhoid fever associated with acute appendicitis caused by an H1-j strain of *Salmonella enterica* serotype *Typhi*. *J Clin Microbiol* 2005;**43**:1470–2.
111. Lau SKP, Woo PCY, Teng JLL, et al. Identification by 16S ribosomal RNA gene sequencing of *Arcobacter butzleri* bacteraemia in a patient with acute gangrenous appendicitis. *Mol Pathol* 2002; **55**:182–5.
112. Lee SL, Islam S, Cassidy LD, et al. Antibiotics and appendicitis in the pediatric population: an American Pediatric Surgical Association outcomes and clinical trials committee systematic review. *J Pediatr Surg* 2010;**45**:2181–5.
113. Lee SL, Shekherdimian S, Chiu VY. Effect of race and socioeconomic status in the treatment of appendicitis in patients with equal health care access. *Arch Surg* 2011;**146**:156–61.
114. Lee SL, Walsh AJ, Ho HS. Computed tomography and ultrasonography do not improve and may delay the diagnosis and treatment of acute appendicitis. *Arch Surg* 2001;**136**:556–62.
115. Lee SL, Yaghoubian A, de Virgilio C. A multi-institutional comparison of pediatric appendicitis outcomes between teaching and nonteaching hospitals. *J Surg Educ* 2011;**68**:6–9.
116. Leigh DA, Simmons K, Normal E. Bacterial flora of the appendix fossa in appendicitis and postoperative wound infection. *J Clin Pathol* 1974;**27**:997–1000.
117. Lewis F, Holcroft J, Boey J, et al. Appendicitis in critical review of diagnosis and treatment in 1000 cases. *Arch Surg* 1975;**110**: 677–84.
118. Li X, Zhang J, Sang L, et al. Laparoscopic versus conventional appendectomy—a meta-analysis of randomized controlled trials. *BMC Gastroenterol* 2010;**10**:129.
119. Lim PL. Appendicitis associated with travelers' diarrhea caused by *Aeromonas sobria*. *J Travel Med* 2009;**16**:132–3.
120. Liu V, Val S, Kang K, et al. Case report: actinomycosis of the appendix—an unusual cause of acute appendicitis in children. *J Pediatr Surg* 2010;**45**:2050–2.
121. Lopez-Navidad A, Domingo P, Cada Falch G. Acute appendicitis complicating infectious mononucleosis: case report and review. *Rev Infect Dis* 1990;**12**:297–302.
122. Lowe LH, Penney MW, Stein SM, et al. Unenhanced limited CT of the abdomen in the diagnosis of appendicitis in children: comparison with sonography. *A J R Am J Roentgenol* 2001; **176**:31–5.
123. Luckmann R, Davis P. The epidemiology of acute appendicitis in California: racial, gender, and seasonal variation. *Epidemiology* 1991;**2**:323–30.
124. Malde HM, Chadha D. Roundworm obstruction: sonographic diagnosis. *Abdom Imag* 1993;**18**:274–6.
125. Maltezou HC, Nikolaidis P, Lebesii E, et al. Piperacillin/tazobactam versus cefotaxime plus metronidazole for treatment of children with intra-abdominal infections requiring surgery. *Eur J Clin Microbiol Infect Dis* 2001;**20**:643–6.
126. Martin AE, Vollman D, Adler B, et al. CT scans may not reduce the negative appendectomy rate in children. *J Pediatr Surg* 2004;**39**:886–90.
127. Maxwell JM, Ragland JJ. Appendicitis: improvements in diagnosis and treatment. *Am Surg* 1991;**57**:282–5.
128. McAllister TA, Fyfe AH, Young DG, et al. Cefotaxime lavage in children undergoing appendicectomy. *Drugs* 1988;**35**(Suppl 2): 127–32.
129. McBurney C. Experience with early operative interference in cases of disease of the vermiform appendix. *NY State Med J* 1889;**50**:676.
130. McCahy P. Continuing fall in the incidence of acute appendicitis. *Ann R Coll Surg Engl* 1994;**76**:282–3.
131. Meier DE, Guzzetta PC, Barber RG, et al. Perforated appendicitis in children: is there a best treatment? *J Pediatr Surg* 2003;**38**: 1520–4.
132. Meshikhes A-WN. Management of appendiceal mass: controversial issues revisited. *J Gastrointest Surg* 2008;**12**:767–75.
133. Miller NJ, Duncan RD, Huntley JS. The conservative management of primary pyomyositis abscess in children: a case series and review of the literature. *Scott Med J* 2011;**56**:181.
134. Miron D, Dashkovsky I, Zuker M, et al. Primary *Streptococcus pneumoniae* appendicitis in a child: case report and review. *Pediatr Infect Dis J* 2003;**22**:282–4.
135. Miron D, Sochotnick I, Yardeni D, et al. Surgical complications of shigellosis in children. *Pediatr Infect Dis J* 2000;**19**:898–900.
136. Moertel CG, Nobrega FT, Elveback LR, et al. A prospective study of appendectomy and predisposition to cancer. *Surg Gynecol Obstet* 1974;**138**:549–53.
137. Morrison JD. *Yersinia* and viruses in acute non-specific abdominal pain and appendicitis. *Br J Surg* 1981;**68**:284–6.
138. Mueller BA, Daling JR, Moore DE, et al. Appendectomy and the risk of tubal infertility. *N Engl J Med* 1986;**315**:1506–8.
139. Mui LM, Ng CSH, Wong SKH, et al. Optimum duration of prophylactic antibiotics in acute non-perforated appendicitis. *ANZ J Surg* 2005;**75**:425–8.
140. Nadler EP, Reblock KK, Ford HR, et al. Monotherapy versus multi-drug therapy for the treatment of perforated appendicitis in children. *Surg Infect* 2003;**4**:327–33.
141. Namnyak SS, Martin DH, Ferguson JDM, et al. *Haemophilus segnis* appendicitis. *J Infect* 1991;**23**:339–41.
142. Nandipati K, Parithivel V, Niazi M. Schistosomiasis: a rare cause of acute appendicitis in the African American population in the United States. *Am Surg* 2008;**74**:221–3.
143. Natesha R, Barnwell S, Weaver W, et al. Is there evidence for a racial difference in the misdiagnosis in patients explored for appendicitis? *J Natl Med Assoc* 1989;**81**:269–71.
144. Neumayer LA, Makar R, Ampel NM, et al. Cytomegalovirus appendicitis in a patient with human immunodeficiency virus infection. *Arch Surg* 1993;**128**:467–8.
145. Nichol PF, Corliss RF, Rajpal S, et al. Perforation of the appendix from intestinal mucormycosis in a neonate. *J Pediatr Surg* 2004; **39**:1133–5.
146. Nielsen MB, Torp-Pedersen S. Sonographically guided transrectal or transvaginal one-step catheter placement in deep pelvic and perirectal abscesses. *AJR* 2004;**183**:1035–6.
147. Nordentoft T, Bringstrup FA, Bremmelgaard A, et al. Effect of laparoscopy on bacteremia in acute appendicitis: a randomized controlled study. *Surg Laparosc Endosc Percutan Tech* 2000;**10**: 302–4.
148. Ochoa B. Surgical complications of ascariasis. *World J Surg* 1991;**15**:222–7.
149. Ohno Y, Furui J, Kanematsu T. Treatment strategy when using intraoperative peritoneal lavage for perforated appendicitis in children: a preliminary report. *Pediatr Surg Int* 2004;**20**:534–7.
150. Okamoto T, Sano K, Ogasahara K. Receiver-operating characteristic analysis of leukocyte counts and serum C-reactive protein levels in children with advanced appendicitis. *Surg Today* 2006;**36**: 515–8.
151. Pannaraj PS, Hulten KG, Gonzalez BE, et al. Infective pyomyositis in children in the era of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* 2006;**43**: 953–60.
152. Parks NA, Schroeppel TJ. Update on imaging for acute appendicitis. *Surg Clin North Am* 2011;**91**:141–54.
153. Pauniaho S-L, Lahdes-Vasama T, Helminen MT, et al. Non-absorbable interrupted versus absorbable continuous skin closure in pediatric appendectomies. *Scand J Surg* 2010;**99**:142–6.
154. Pearlman M, Frantz AC, Floyd WS, et al. Abdominal wall *Actinomyces* abscess associated with an intrauterine device. *J Reprod Med* 1991;**36**:398–402.
155. Pisacane A, de Luca U, Impagliazzo N, et al. Breast feeding and acute appendicitis. *BMJ* 1995;**310**:836–7.
156. Ponsky TA, Huang ZJ, Kittle K, et al. Hospital- and patient-level characteristics and the risk of appendiceal rupture and negative appendectomy in children. *JAMA* 2004;**292**:1977–82.
157. Puri R, Eloubeidi MA, Sud R, et al. Endoscopic ultrasound-guided drainage of pelvic abscess without fluoroscopy guidance. *J Gastroenterol Hepatol* 2010;**25**:1416–9.
158. Quillin SP, Siegel MJ, Coffin CM. Acute appendicitis in children: value of sonography in detecting perforation. *A J R Am J Roentgenol* 1992;**159**:1265–8.
159. Rabenandrasana HA, Ahmad A, Samison LH, et al. Child primary tubercular appendicitis. *Pediatr Int* 2004;**46**:374–6.
160. Raffensperger J. *Eikenella corrodens* infections in children. *J Pediatr Surg* 1986;**21**:644–6.
161. Raffi F, David A, Mouzard A, et al. *Pasteurella multocida* appendiceal peritonitis: report of three cases and review of the literature. *Pediatr Infect Dis* 1986;**5**:695–8.
162. Rasmussen O, Hoffmann J. Assessment of the reliability of the symptoms and signs of acute appendicitis. *J R Coll Surg Edinb* 1991;**36**:372–7.

163. Rautio M, Lonnroth M, Saxen H, et al. Characteristics of an unusual anaerobic pigmented gram-negative rod isolated from normal and inflamed appendices. *Clin Infect Dis* 1997;**25**(Suppl 2):S107–10.
164. Rautio M, Saxen H, Siitonen A, et al. Bacteriology of histopathologically defined appendicitis in children. *Pediatr Infect Dis J* 2000;**19**:1078–83.
165. Rehman R, Rao AM, Ahmed I. Single incision versus conventional multi-incision appendicectomy for suspected appendicitis. *Cochrane Database Syst Rev* 2011;CD009022.
166. Reid RI, Dobbs BR, Frizelle FA. Risk factors for post-appendicectomy intra-abdominal abscess. *ANZ J Surg* 1999; **69**:373–4.
167. Reynolds SL, Jaffe DM. Diagnosing abdominal pain in a pediatric emergency department. *Pediatr Emerg Care* 1992;**8**:126–8.
168. Robertson FM, Olsen SB, Jackson MR, et al. Inguinal-scrotal suppuration following treatment of perforated appendicitis. *J Pediatr Surg* 1993;**28**:267–8.
169. Rodriguez-Sanjuan JC, Martin-Parra JI, Seco I, et al. C-reactive protein and leukocyte count in the diagnosis of acute appendicitis in children. *Dis Colon Rectum* 1999;**42**:1325–9.
170. Rothrock SG, Skeoch G, Rush JJ, et al. Clinical features of misdiagnosed appendicitis in children. *Ann Emerg Med* 1991; **20**:45–50.
171. Ruff ME, Friedland IR, Hickey SM. *Escherichia coli* septicemia in nonperforated appendicitis. *Arch Pediatr Adolesc Med* 1994;**148**: 853–5.
172. Sah SP, Bhadani PP. *Enterobius vermicularis* causing symptoms of appendicitis in Nepal. *Trop Doctor* 2006;**36**:160–2.
173. Salemis NS. Acute appendicitis presenting with *Klebsiella pneumoniae* septicemia due to bacterial translocation. *Am J Emerg Med* 2009;**27**:1023.e3–4.
174. Sand M, Bechara FG, Holland-Letz T, et al. Diagnostic value of hyperbilirubinemia as a predictive factor for appendiceal perforation in acute appendicitis. *Am J Surg* 2009;**198**:193–8.
175. Saokar A, Arellano RS, Gervais DA, et al. Transvaginal drainage of pelvic fluid collections: results, expectations, and experience. *Am J Roentgenol* 2008;**191**:1352–8.
176. Sauer C, Gutgesell M. Ballet dancer with hip and groin pain: Crohn disease and psoas abscess. *Clin Pediatr* 2005;**44**:731–3.
177. Sauerland S, Jaschinski T, Neugebauer EAM. Laparoscopic versus open surgery for suspected appendicitis. *Cochrane Database Syst Rev* 2010;CD001546.
178. Saxen H, Tarkka E, Hannikainen P, et al. *Escherichia coli* and appendicitis: phenotypic characteristics of *E. coli* isolates from inflamed and noninflamed appendices. *Clin Infect Dis* 1996;**23**: 1038–42.
179. Scharschmidt TJ, Weiner SD, Myers JP. Bacterial pyomyositis. *Curr Infect Dis Rep* 2004;**6**:393–6.
180. Schropp KP, Kaplan S, Golladay ES, et al. A randomized clinical trial of ampicillin, gentamicin and clindamycin versus cefotaxime and clindamycin in children with ruptured appendicitis. *Surgery* 1991;**172**:351–6.
181. Schurman JV, Cushing CC, Garey CL, et al. Quality of life assessment between laparoscopic appendectomy at presentation and interval appendectomy for perforated appendicitis with abscess: analysis of a prospective randomized trial. *J Pediatr Surg* 2011; **46**:1121–5.
182. Sehdev AES, Sehdev PS, Jacobs R, et al. Human monocytic ehrlichiosis presenting as acute appendicitis during pregnancy. *Clin Infect Dis* 2002;**35**:e99–102.
183. Simpson J, Speake W. Appendicitis. *Clin Evid* 2005;**14**:529–35.
184. Sirinek KR, Levine BA. Antimicrobial management of surgically treated gangrenous or perforated appendicitis: comparison of cefoxitin and clindamycin-gentamicin. *Clin Ther* 1987;**9**:420–8.
185. Sirinek KR, Levine BA. A randomized trial of ticarcillin and clavulanate versus gentamicin and clindamycin in patients with complicated appendicitis. *Surg Gynecol Obstet* 1991;**172**:30–5.
186. Smink DS, Finkelstein JA, Kleinman K, et al. The effect of hospital volume of pediatric appendectomies on the misdiagnosis of appendicitis in children. *Pediatrics* 2004;**113**:18–23.
187. Smink DS, Fishman SJ, Kleinman K, et al. Effects of race, insurance status, and hospital volume on perforated appendicitis in children. *Pediatrics* 2005;**115**:920–5.
188. Smith G, Hoover S, Sobonya R, et al. Abdominal and pelvic coccidioidomycosis. *Am J Med Sci* 2011;**341**:308–11.
189. Snelling CMH, Poenaru D, Drover JW. Minimum postoperative antibiotic duration in advanced appendicitis in children: a review. *Pediatr Surg Int* 2004;**20**:838–45.
190. Stadlmann S, Lenggenhager DM, Alves VA, et al. Histopathologic characteristics of the transitional stage of measles-associated appendicitis: case report and review of the literature. *Hum Pathol* 2011;**42**:285–90.
191. Stovroff MC, Totten M, Glick PL. PIC lines save money and hasten discharge in the care of children with ruptured appendicitis. *J Pediatr Surg* 1994;**29**:245–7.
192. St. Peter SD, Adibe OO, Juang D, et al. Single incision versus standard 3-port laparoscopic appendectomy. *Ann Surg* 2011;**254**: 586–90.
193. St. Peter SD, Little DC, Calkins CM, et al. A simple and more cost-effective antibiotic regimen for perforated appendicitis. *J Pediatr Surg* 2006;**41**:1020–4.
194. St. Peter SD, Sharp SW, Ostlie DJ. Influence of histamine receptor antagonists on the outcome of perforated appendicitis. *Arch Surg* 2010;**145**:143–6.
195. St. Peter SD, Tsao K, Spilde TL, et al. Single daily dosing ceftriaxone and metronidazole vs standard triple antibiotic regimen for perforated appendicitis in children: a prospective randomized trial. *J Pediatr Surg* 2008;**43**:981–5.
196. Street J, Lenehan B, Mulcahy D, et al. Pelvic girdle sepsis in childhood: an illustrative case of the difficulty in diagnosis. *Acta Orthop Belg* 2005;**71**:361–5.
197. Suh SW, Choi YS, Park JM, et al. Clinical factors for distinguishing perforated from nonperforated appendicitis: a comparison using multidetector computed tomography in 528 laparoscopic appendectomies. *Surg Laparosc Endosc Percutan Tech* 2011;**21**: 72–5.
198. Tander B, Pektas O, Bulut M. The utility of peritoneal drains in children with uncomplicated perforated appendicitis. *Pediatr Surg Int* 2003;**19**:548–50.
199. Tanomkiat W, Buranapanitkit B. Percutaneous drainage of large tuberculous iliopsoas abscess via a subinguinal approach: a report of two cases. *J Orthop Sci* 2004;**9**:157–61.
200. Taylor E, Berjis A, Bosch T, et al. The efficacy of postoperative oral antibiotics in appendicitis: a randomized prospective double-blinded study. *Am Surgeon* 2004;**70**:858–62.
201. Teppler H, Meibohm AR, Woods GL. Management of complicated appendicitis and comparison of outcome with other primary sites of intra-abdominal infection: results of a trial comparing ertapenem and piperacillin-tazobactam. *J Chemother* 2004;**16**: 62–9.
202. ter Borg P, Kuijper EJ, van der Lelie H. Fatal mucormycosis presenting as an appendiceal mass with metastatic spread to the liver during chemotherapy-induced granulocytopenia. *Scand J Infect Dis* 1990;**22**:499–501.
203. Terry NE, Fowler CL. Cytomegalovirus enterocolitis complicated by perforated appendicitis in a premature infant. *J Pediatr Surg* 2006;**41**:1476–8.
204. Thadepalli H, Mandal AK, Chuah SK, et al. Bacteriology of the appendix and the ileum in health and in appendicitis. *Am Surg* 1991;**57**:317–22.
205. Tobe I. Inapparent virus infection as a trigger of appendicitis. *Lancet* 1965;**1**:1343–6.
206. Towfigh S, Clarke T, Yacoub W, et al. Significant reduction of wound infections with daily probing of contaminated wounds. *Arch Surg* 2011;**146**:448–52.
207. Tsang TM, Tam PKH, Saing H. Antibiotic prophylaxis in acute non-perforated appendicitis in children: single dose of metronidazole and gentamicin. *J R Coll Surg Edinb* 1992;**37**:110–2.
208. Uchimura M, Tsuruoka Y, Hukuda T, et al. Isolation of vero toxin-producing *Escherichia coli* (enterohemorrhagic *E. coli*) 0111:H- from 2 cases diagnosed as appendicitis. *Kansenshogaku Zasshi* 1991;**65**:905–8.
209. Uhari M, Seppanen J, Heikkinen E. Imipenem-cilastatin vs. tobramycin and metronidazole for appendicitis-related infections. *Pediatr Infect Dis J* 1992;**11**:445–50.
210. Urban E, Hortobagyi A, Szentpali K, et al. Two intriguing *Bilophila wadsworthia* cases from Hungary. *J Med Microbiol* 2004;**53**: 1167–9.
211. Urizar RE, Lepow M, Neumann M, et al. Fungal peritonitis with splenic-pelvic abscess in a patient on continuous ambulatory peritoneal dialysis. *Peritoneal Dialysis Int* 1993;**13**:162–3.

212. Van Noyen R, Selderslaghs R, Bekaert J, et al. Causative role of *Yersinia* and other enteric pathogens in the appendicular syndrome. *Eur J Clin Microbiol Infect Dis* 1991;**10**:735–41.
213. Varadhan KK, Humes DJ, Neal KR, et al. Antibiotic therapy versus appendectomy for acute appendicitis: a meta-analysis. *World J Surg* 2010;**34**:199–209.
214. Visvanathan K, Jones PD, Truskett P. Abdominal mycobacterial infection mimicking acute appendicitis in an AIDS patient. *Aust N Z J Surg* 1993;**63**:558–60.
215. Volinsky JB, Karrer FM, Todd JK. Hemolytic-uremic syndrome caused by *Escherichia coli* 0157:H7 after perforated appendix. *Pediatr Infect Dis J* 1998;**17**:846–7.
216. Vons C, Barry C, Maitre S, et al. Amoxicillin plus clavulanic acid versus appendicectomy for treatment of acute uncomplicated appendicitis: an open-label, non-inferiority, randomized controlled trial. *Lancet* 2011;**377**:1573–9.
217. Walsh TR, Reilly JR, Hanley E, et al. Changing etiology of iliopsoas abscess. *Am J Surg* 1992;**163**:413–6.
218. Wehner JH, De Bruyne K, Kagawa FT, et al. Pulmonary tuberculosis, amenorrhea, and a pelvic mass. *West J Med* 1994;**161**:515–18.
219. Wei B, Qi C-L, Chen T-F, et al. Laparoscopic versus open appendectomy for acute appendicitis: a metaanalysis. *Surg Endosc* 2011;**25**:1199–208.
220. Wiesenfeld HC, Berg SR, Sweet RL. *Torulopsis glabrata* pelvic abscess and fungemia. *Obstet Gynecol* 1994;**83**:887–9.
221. Williams N, Kapila L. Acute appendicitis in the preschool child. *Arch Dis Child* 1991;**66**:1270–2.
222. Williams N, Kapila L. Acute appendicitis in the under 5-year-old. *J R Coll Surg Edinb* 1994;**39**:168–70.
223. Wilson SE. Results of a randomized, multicenter trial of meropenem versus clindamycin/tobramycin for the treatment of intra-abdominal infections. *Clin Infect Dis* 1997;**24**(Suppl. 2):S197–206.
224. Wong K, Kumar R. Fever following appendicectomy: think about thoracic empyema. *N Z Med J* 2007;**120**:U2561.
225. Woo PCY, Lau SKP, Woo GKS, et al. Bacteremia due to *Clostridium hathewayi* in a patient with acute appendicitis. *J Clin Microbiol* 2004;**42**:5947–9.
226. Yassin AF, Steiner U, Ludwig W. *Corynebacterium appendicis* sp. nov. *Int J Syst Evol Microbiol* 2002;**52**:1165–9.
227. Yasumoto R, Kawano M, Kawanishi H, et al. Left acute scrotum associated with appendicitis. *Int J Urol* 1998;**5**:108–10.
228. Yildirim S, Nursal TZ, Tarim A, et al. A rare cause of acute appendicitis: parasitic infection. *Scand J Infect Dis* 2005;**37**:757–9.
229. York D, Smith A, Phillips JD, et al. The influence of advanced radiographic imaging on the treatment of pediatric appendicitis. *J Pediatr Surg* 2005;**40**:1908–11.

sign) or the umbilical area (Cullen sign) can be identified but usually only when life-threatening hemorrhagic pancreatitis is present. In severe pancreatitis, children may present with evidence of shock and multiple-organ failure.

Chronic pancreatitis occurs when irreversible damage in the pancreatic architecture causes abnormalities in the function of the pancreas.[110] Children with chronic pancreatitis often have lengthy or recurrent bouts of abdominal pain and vomiting.

LABORATORY DIAGNOSIS

The single most common useful laboratory test for the clinical diagnosis of pancreatitis in children is measurement of the serum amylase concentration, but the level correlates poorly with the severity of the disease. In most studies of childhood pancreatitis, the diagnosis is confirmed when the serum amylase level is greater than three times the normal level for the particular laboratory completing the test. The serum concentration rises quickly within hours after symptoms develop. High serum amylase concentrations can be observed, however, in numerous other illnesses, including acute cholecystitis, intestinal obstruction, perforations of abdominal organs, appendicitis, salpingitis, ruptured ectopic pregnancy, and salivary gland disease. The serum amylase concentration can return to normal in 24 to 72 hours after the onset of symptoms; therefore, the diagnosis of pancreatitis can be missed. In this situation, the urine amylase concentration can remain elevated for at least 1 week.

Serum amylase concentrations occasionally are not elevated during the course of pancreatitis in children.[155] In a series of children with pancreatitis, 83 percent of patients had elevated serum amylase levels.[208] In addition, marked hyperlipidemia may interfere with the laboratory measurement of amylase.[23] Serum lipase is useful in these situations; however, high serum concentrations often are not detected until 24 hours after the beginning of the illness. Because lipase is produced only in the pancreas and intestinal cells, measurement of its serum concentration helps distinguish children with high serum amylase concentrations of pancreatic as opposed to salivary origin. Some children with pancreatitis have high amylase levels without an elevation of serum lipase.[111]

Laboratory findings in children with severe acute pancreatitis may include leukocytosis with increased immature polymorphonuclear leukocytes, an elevated erythrocyte sedimentation rate, and elevated C-reactive protein level. In children with fulminate hemorrhagic pancreatitis, anemia develops quickly. Other associated findings include hyperglycemia, hypertriglyceridemia, hypoalbuminemia, and hypocalcemia. Although its validity has been questioned, a scoring system for children with pancreatitis has been devised in which severe pancreatitis is more likely when a child presents who is younger than 7 years of age, weighs less than 23 kg, has a total white blood cell count of more than 18,500 cells/mm^3, has an admission lactate dehydrogenase value greater than 2000, has a 48-hour calcium level less than 8.3 mg/dL, has a 48-hour albumin level less than 2.6 g/dL, has a 48-hour fluid sequestration less than 75 mL/kg/48 hr, and has a 48-hour rise in blood urea nitrogen greater than 5 mg/dL.[44,176] Procalcitonin levels may be useful in predicting severe pancreatitis and later complications in adults.[123] Elevated aminotransferase and alkaline phosphatase levels generally are observed only when the episode of pancreatitis is caused by biliary obstruction, such as in gallstone-related disease.

The radiographic features of childhood pancreatitis also are nonspecific. Radiographs of the abdomen may show localized ileus of the jejunum in the midepigastric or left upper quadrant region adjacent to the pancreas (sentinel loop), a distended transverse colon without visualization of the descending colon because of adjacent pancreatic inflammation (colon cutoff sign), duodenal distention with air–fluid levels, or loss of the left psoas shadow.[84] Occasionally, chest radiography reveals an elevated left hemidiaphragm or pleural effusion.

In recent years, the ability to diagnose pancreatitis in children has been improved greatly by ultrasonography.[208] The echodensity of the pancreas is normally equal to or greater than that of the left lobe of the liver. During acute pancreatitis, edema causes the gland to enlarge and become less dense than the liver. These two findings can aid in establishing the diagnosis of pancreatitis, and complications such as abscesses and pseudocysts can be identified. Also, ultrasonography may delineate dilations of the pancreatic ducts due to obstruction or ductal stones. Visualization of the pancreas by ultrasonography may be obscured because of overlying bowel gas. In such cases, computed tomography (CT) is useful in detecting pancreatic size and density.

CT of the pancreas should be performed in complicated cases of pancreatitis after a few days of treatment to determine the severity of disease and extent of pancreatic necrosis.[171] It is especially useful when surgery is being considered for drainage of abscesses and pseudocysts.

Endoscopic retrograde cholangiopancreatography (ERCP) is used in children with pancreatitis to treat gallstones, strictures, or *Ascaris* infection.[146,151] Because of ongoing improvement in image quality, magnetic resonance cholangiopancreatography (MRCP) is being increasingly used as a noninvasive technique for evaluating children with chronic or recurrent pancreatitis, with results similar to those from ERCP.[38,43,184]

NONINFECTIOUS CAUSES

A cause for childhood pancreatitis can be determined in more than 90 percent of cases if diagnostic evaluation is thorough, especially in children younger than 6 years of age.[109,215] The frequency of each specific cause depends on the patient population of the particular medical center. At Children's Hospital of Michigan, 33 percent of children with pancreatitis have biliary tract–related disease because of that institution's large patient population with sickle-cell anemia.[215] At Yale–New Haven Hospital, drug-related pancreatitis accounts for 30 percent of the total cases of childhood pancreatitis because of the frequent use of immunosuppressive and cancer chemotherapeutic

agents in that hospital.[84] The most common noninfectious causes of pancreatitis in children are listed in Table 52-1.

Trauma

In many older series of patients, trauma is the leading cause of acute pancreatitis in children. Because the highly vascular pancreas is immobilized by the stomach, duodenum, and vertebrae, it is susceptible to blunt and penetrating trauma. The less-developed abdominal wall musculature in pediatric patients may enhance their susceptibility to pancreatic injury after episodes of blunt trauma. Child abuse has been recognized increasingly as a cause of trauma-related pancreatitis.[140] Postoperative pancreatitis occurs most commonly after abdominal or cardiac surgery.[56] Pancreatitis also may be associated with traumatic brain injury in children.[195]

Medications

Medications used in pediatric practice increasingly are causing episodes of pancreatitis in children. In most pediatric studies, the anticonvulsant valproic acid is the most common medication associated with the development of pancreatitis, and its occurrence may be underreported.[67,208] The risk for developing pancreatitis does not depend on the serum level of valproic acid, and it is not related to the length of therapy.[169,207] Pancreatitis has also been observed in children taking the seizure medications zonisamide and lamotrigine.[152,165] Chemotherapeutic and immunosuppressive agents such as azathioprine, steroids, 6-mercaptopurine, methotrexate, L-asparaginase, and ifosfamide have been associated with cases of childhood pancreatitis, and other immunosuppressive agents most likely play a role in post transplant pancreatitis.* Acute pancreatitis has been associated rarely with the use of diuretics, including hydrochlorothiazide and furosemide[115]; growth hormone[26]; sulfasalazine[65]; atypical antipsychotics[77,104]; statins[96]; propofol[31]; nonsteroidal inflammatory agents, including ibuprofen[112,113]; metformin[59]; proton pump inhibitors[212]; angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists[14]; estrogens; and codeine. Alcohol consumption is an uncommon cause of pancreatitis in younger children; however, it can cause illness occasionally in the adolescent population.

Physicians with expertise in the management of infectious diseases are becoming more aware of drug-induced pancreatitis because many antimicrobial agents can cause pancreatic inflammation. Pentamidine isethionate has been used in the treatment of *Pneumocystis jejuni* pneumonia, African trypanosomiasis, and leishmaniasis. It may cause hypoglycemia because of toxicity to pancreatic islet cells and is associated with severe and occasionally fatal episodes of pancreatitis.[122,216] In children and adults, aerosolized pentamidine prophylaxis for *P. jejuni* pneumonia also has been associated with severe cases of pancreatitis in patients with acquired immunodeficiency syndrome (AIDS).[80,122] Similarly, pentavalent antimonial

*References 51, 52, 66, 103, 158, 190, 204.

TABLE 52-1 **Noninfectious Causes of Childhood Pancreatitis**

Trauma
Blunt
Brain injury
Penetrating
Postoperative

Drugs
Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor antagonists
Antiepileptic agents (valproic acid, zonisamide, lamotrigine)
Antimicrobial agents (pentamidine, antimonials, sulfonamides, tetracycline, macrolides, metronidazole, dapsone, nitrofurantoin, tigecycline, isoniazid, ceftriaxone, gatifloxacin, nucleoside analogue reverse transcriptase inhibitors, interferon-α, liposomal amphotericin B, micafungin, itraconazole)
Atypical antipsychotic agents
Chemotherapeutic/immunosuppressive agents (azathioprine, corticosteroids, asparaginase, 6-mercaptopurine, tacrolimus, methotrexate, ifosfamide)
Diuretics (thiazides, furosemide)
Estrogens
Ethyl alcohol
Growth hormone
Metformin
Pain medications (nonsteroidal inflammatory agents, codeine)
Propofol
Proton pump inhibitors
Statins
Sulfasalazine

Obstructive Diseases
Anatomic abnormalities
Cholelithiasis

Genetic and Metabolic Diseases
Aminoacidurias
Cystic fibrosis
Diabetes mellitus
Glycogen storage disease type 1
Hyperlipoproteinemia types I, IV, and V
Hyperparathyroidism
Hypertriglyceridemia
Recurrent hereditary pancreatitis

Vasculitic and Autoimmune Diseases
Crohn disease
Henoch-Schönlein purpura
IgG4 autoimmune pancreatitis
Kawasaki syndrome
Systemic lupus erythematosus

Miscellaneous
Anticholinesterase insecticide intoxication
Chronic fibrosing pancreatitis
Lymphoma
Reye syndrome
Scorpion stings
Tropical chronic pancreatitis
Orthotopic liver transplantation

agents such as sodium stibogluconate and meglumine antimonite, used for the treatment of visceral leishmaniasis, can induce pancreatic inflammation.[12,161]

Sulfonamides, including trimethoprim-sulfamethoxazole, have been implicated occasionally as a cause of acute pancreatitis in adults.[8,199] Symptoms have recurred when patients have been reexposed to the medication. The abdominal pain often is accompanied by a hypersensitivity-type rash. Tetracycline-induced pancreatitis has been described in children with and without overt liver disease.[55,187] In addition, clarithromycin,[69,159] erythromycin,[179] rifampin,[145] roxithromycin,[149] dapsone,[42] nitrofurantoin,[130] isoniazid,[94] tigecycline,[148] and metronidazole[136] have been added to the list of agents that can cause pancreatitis in previously healthy individuals when given in routine doses or when high amounts are consumed. Although uncommonly used in children, quinolone antibiotics, such as gatifloxacin, have been associated with hepatotoxicity and acute pancreatitis.[40] An adolescent who was receiving ceftriaxone also has developed pancreatitis secondary to obstruction of the biliary tract from gallstones.[117]

Pancreatitis has been a major dose-limiting toxic effect of the human immunodeficiency virus (HIV)-inhibiting nucleoside analogue reverse transcriptase inhibitor (NRTI) class of medications because of mitochondrial toxicity, especially dideoxyinosine, in adult and pediatric patients.[32,61] Most episodes of pancreatitis occur when the dose is 360 mg/m^2/day or more, and usually the pancreatic inflammation resolves when the medication is discontinued. Concomitant administration of pentamidine or another NRTI, such as ribavirin, used in the treatment of hepatitis C infection with dideoxyinosine, may increase the risk for developing pancreatitis.[125] In pediatric patients with AIDS, serum amylase concentrations often are elevated in patients without pancreatic symptoms, whereas children with pancreatitis can have normal serum amylase concentrations. The serum lipase concentration is useful in evaluating HIV-infected children for possible pancreatic inflammation.[32,122] Increased liver aminotransferase or lipase concentrations before the administration of dideoxyinosine may be helpful in predicting the children in whom pancreatitis would develop.[32] In all children with symptoms consistent with pancreatitis, dideoxyinosine should be withheld pending the results of a lipase assay, and it should be discontinued if the concentration is elevated. Similarly, dideoxyinosine should be discontinued for 1 week after treatment with pentamidine for *P. jejuni* pneumonia.[60] Because of increased awareness of NRTI toxicity, the incidence rate of pancreatitis in HIV-infected children in the highly active antiretroviral therapy (HAART) era appears to be decreasing.[126]

Interferon-α, which is used in the treatment of chronic hepatitis and malignancies, also has been associated with the development of pancreatitis.[37] The antifungal agents liposomal amphotericin B, micafungin, and itraconazole rarely cause pancreatic toxicity.[144,156,175]

Obstructive Diseases

Obstruction of the common bile duct, pancreatic duct, or sphincter of Oddi may cause pancreatitis. Gallstones or congenital anatomic malformations, including an annular pancreas, pancreas divisum, choledochal cysts, and enteric duplication cysts, can cause pancreatitis by obstructing normal pancreatic flow.[7,70,87,89] Pancreatitis observed in children with sickle-cell anemia may be caused by obstruction from stones, biliary sludge, pancreatic microvascular occlusion, and ischemia during crises.[4]

Genetic and Metabolic Diseases

Metabolic diseases often are associated with chronic recurrent episodes of pancreatitis. Such diseases include hyperlipoproteinemias, hypertriglyceridemias, cystic fibrosis, diabetes mellitus, hyperparathyroidism, aminoacidurias, and glycogen storage disease type I.* Recurrent hereditary pancreatitis usually occurs in an autosomal dominant pattern, with onset taking place between infancy and adolescence. Mutations in the genes encoding cationic trypsinogen *(PRSS1)*, the serine protease inhibitor Kazak type 1 *(SPINK1)*, and chymotrypsinogen C *(CTRC)* have been associated with hereditary pancreatitis.[153] Other children and adults with chronic pancreatitis have been shown to have mutations in the cystic fibrosis transmembrane regulator *(CFTR)* gene similar to individuals with cystic fibrosis.[46,192]

Vasculitic and Autoimmune Diseases

Pancreatitis also can occur in syndromes in which vasculitis is a major component of the disease process. It has been associated with common pediatric disorders, including Kawasaki syndrome, Henoch-Schönlein purpura, Crohn disease, and systemic lupus erythematosus.[106,134,174,201]

Rarely, children develop pancreatitis from an autoimmune process. Children usually present with a mass in their pancreas and are diagnosed when IgG4 levels are elevated in the serum and there is histologic infiltration of IgG4-bearing plasma cells surrounding the medium and large interlobular ducts of the pancreas.[24,214]

Miscellaneous Causes

Although no longer common, Reye syndrome has been associated with acute pancreatitis.[68] The venom of the scorpions *Tityus trinitatus*, *Tityus asthenes*, and *Leiurus quinquestriatus* can cause pancreatitis in patients who have been stung; however, these species do not live naturally in the United States.[19,141,170] The gastrointestinal symptoms observed in children with anticholinesterase insecticide poisoning may be caused by pancreatitis.[206] Also, tropical chronic pancreatitis seen in developing countries is most likely related to malnutrition and dietary intake of toxins, such as those from the tuber cassava.[18] Rarely, primary pancreatic lymphoma may manifest in children as acute pancreatitis.[54] Chronic fibrosing pancreatitis in which the pathogenesis of the disease process remains unsolved only occurs rarely in the United States.[78]

Although a much less common occurrence than in adults, acute pancreatitis after orthotopic liver transplantation is severe in children and often results in death.[185,186]

*References 22, 30, 74, 75, 119, 197.

The cause of pancreatitis in liver transplant recipients most likely is multifactorial but probably involves traumatic injury, biliary obstruction, and immunosuppressive therapy.

INFECTIOUS CAUSES

Infections caused by various microorganisms have been shown by culture, histologic examination, or antibody titer rise during the course of acute pancreatitis in humans (Table 52-2). A true causal relationship usually is not shown. Although not all of the following infectious agents have been shown to be associated with childhood pancreatitis, they must be considered as possible etiologic agents because adult patients with infectious pancreatitis have been described. Compared with previous decades, infectious agents are being encountered less as a cause of acute pancreatitis, most likely because of mumps vaccination.

TABLE 52-2 **Microorganisms Associated with Episodes of Acute Pancreatitis**

Viruses
Adenoviruses
Cytomegalovirus
Epstein-Barr virus
Group B coxsackieviruses
Hepatitis A virus
Hepatitis B virus
Hepatitis E virus
Herpes simplex viruses
Human immunodeficiency virus
H1N1 influenza A virus
Measles virus
Mumps virus
Parainfluenza viruses
Rotavirus
Rubella virus
Varicella-zoster virus
West Nile virus

Parasites
Ascaris lumbricoides
Clonorchis sinensis
Cryptosporidium parvum
Echinococcus granulosus
Fasciola hepatica
Plasmodium falciparum
Taenia saginata
Toxoplasma gondii
Wuchereria bancrofti

Mycoplasmas and Bacteria
Brucella melitensis
Campylobacter jejuni
Escherichia coli
Legionella spp.
Leptospira spp.
Moraxella catarrhalis
Mycobacterium tuberculosis
Mycoplasma pneumoniae
Salmonella spp.
Streptococcus pyogenes
Yersinia spp.

Fungi
Aspergillus spp.
Candida spp.
Cryptococcus neoformans

Viral Infections

Group B coxsackieviruses and mumps virus are the best documented causes of pancreatitis in children. Group B coxsackieviruses usually cause pancreatitis along with other clinical manifestations, including aseptic meningitis, mild diarrhea, rash, and myocarditis.[34,90] They rarely have caused death in young infants with myocarditis and pancreatitis.[47] How commonly these enteroviruses cause pancreatic inflammation is unknown. Thirty-one percent of patients with aseptic meningitis during an epidemic of group B coxsackievirus infection had increased serum amylase concentrations.[129] Numerous studies have shown coxsackievirus B–induced damage to pancreatic acinar cells in mouse models of infection, and it is believed that the pancreas is the primary replication site for these viruses.[86,189] Coxsackievirus B strains have caused worsening bouts of pancreatic disease in children with chronic pancreatitis and the primary episode of pancreatitis in children later found to have the hereditary form of disease.[71,167]

Usually, mumps pancreatitis occurs in the presence of parotitis; however, abdominal pain and vomiting may occur for days before the development of salivary swelling.[203] Rarely, mumps virus can cause pancreatitis without other common clinical manifestations.[127,196] Because more than 80 percent of children with mumps parotitis have elevated serum amylase concentrations, ultrasonography and serum lipase concentrations should be obtained to aid in establishing the diagnosis.[76] An estimated 15 percent of children with mumps virus infection have abdominal tenderness and vomiting suggestive of the diagnosis of pancreatitis.[84] In only a single report has the pancreatitis been hemorrhagic and severe.[57] Occasionally, chronic or recurring pancreatitis develops after mumps infection.[211]

Researchers previously thought that acute pancreatitis occurred in cases of viral hepatitis only when fulminate liver disease developed. Increasingly, children with mild hepatitis A infection and pancreatitis are being described.[3] In addition, a 16-year-old with acute hepatitis A infection died of severe pancreatitis with multiple-organ failure.[100] Individuals with acute hepatitis and pancreatitis have also been found to have hepatitis E viral infection.[181] Hepatitis B viral antigens have been detected in the pancreatic glandular cells of patients with severe acute hemorrhagic pancreatitis.[164] The role of hepatitis B virus in the pathogenesis of pancreatic inflammation in these patients is unknown; however, a young adult has developed three episodes of acute pancreatitis during acute exacerbations of chronic hepatitis B infection that resolved after lamivudine therapy.[39] It has been suggested that edema of the ampulla of Vater caused by biliary

sludge formed during hepatitis viral infection leads to outflow obstruction of pancreatic fluid and the development of pancreatitis.[92]

Human herpesviruses are uncommon causes of childhood pancreatitis in immunocompetent patients. Occasionally, pancreatitis develops in children and adolescents with infectious mononucleosis.[101] Acute pancreatitis and, occasionally, pseudocyst formation also have been reported in previously healthy individuals with varicella infection.[62,188] In addition, previously healthy adults have developed pancreatitis during primary cytomegalovirus and herpes simplex virus infections.[99,105,139]

Interstitial pancreatitis occurs relatively commonly in children with congenital rubella syndrome.[124] In addition, severe pancreatitis has been identified in immunocompetent patients with mild and fatal measles virus infection.[64,198] An adolescent has been described with measles encephalitis and pancreatitis that responded to corticosteroids.[177] Rarely, other viruses including adenovirus, West Nile virus, rotavirus, and H1N1 influenza A virus have been associated with the development of pancreatitis in previously healthy adults and children.[25,28,102,143]

Viral pancreatitis also occurs in immunocompromised patients. Cytomegalovirus has been identified in pancreatic specimens obtained during autopsies of patients who had AIDS, transplant recipients, individuals taking corticosteroids for autoimmune diseases, and cancer chemotherapy patients.[91,95,180] The symptoms of pancreatitis have resolved in a few patients with AIDS treated with ganciclovir or foscarnet.[41] Adenovirus has caused hemorrhagic pancreatitis and death in children with bone marrow transplants, whereas an infant with disseminated adenoviral infection and pancreatitis survived with cidofovir therapy.[20,36,135] Researchers have suggested that stool cultures for adenoviruses should be obtained when post-transplant patients develop pancreatitis. Varicella-zoster and herpes simplex viruses have also caused pancreatitis and death in patients with various immunodeficient conditions.[58,166] A disseminated parainfluenza virus infection in an infant with severe combined immunodeficiency was associated temporally with the development of pancreatitis; however, no attempt was made to culture the virus from postmortem pancreatic tissue.[63]

Whether HIV directly causes pancreatitis is unclear. Laboratory-diagnosed episodes of pancreatitis in adults and children with AIDS do occur, but whether the pancreatic inflammation is caused by HIV or an unrecognized opportunistic pathogen is unknown.[213] HIV-infected children frequently have elevated amylase and lipase levels with no correlation to antiviral therapy.[35] Increasing numbers of adults with primary manifestations of HIV infection have presented with acute pancreatitis, suggesting a role of HIV in the pathogenesis of the disease.[193]

Parasite Infestations and Infections

Ascaris lumbricoides can migrate in the intestines to the ampulla of Vater and subsequently to the pancreatic duct or common bile duct. Obstruction of the biliary or pancreatic duct can cause acute pancreatitis.[2,13,15,178] Ascariasis is diagnosed when adult roundworms are identified in the duodenum by radiographs of the upper gastrointestinal tract (Fig. 52-1) or more commonly by ultrasonography or ERCP. Often, a history of seeing worms in the feces can be elicited. The flukes *Clonorchis sinensis* and *Fasciola hepatica* and the cestode *Taenia saginata* also can migrate to the pancreatic and biliary drainage systems and cause pancreatitis.[53,108,168] Rarely, hepatic hydatid cysts caused by *Echinococcus* can obstruct biliary drainage and cause pancreatic inflammation.[97] *Wuchereria bancrofti* occasionally has been found to cause chronic pancreatitis.[93] Parasitic infestations should be considered as a cause of pancreatitis, particularly in immigrant children and patients who have traveled to developing nations.

FIGURE 52-1 ■ An ascaris close to the ampulla of Vater, with the body and tail lying in the second and third parts of the duodenum. The patient is a 9-year-old girl with acute pancreatitis.

The protozoan *Cryptosporidium parvum* has been identified in the bile of an AIDS patient with elevated serum amylase levels and right upper quadrant abdominal pain.[72] ERCP demonstrated biliary and pancreatic ductal disease, but no other opportunistic pathogens were isolated. Cryptosporidia also have been observed in the interlobular pancreatic ducts of experimentally infected immunocompromised mice.[194] Whether cryptosporidial infection causes pancreatitis in immunocompetent patients is unknown; however, a previously healthy adolescent developed pancreatitis after having cryptosporidial diarrhea.[81] *Toxoplasma gondii* cysts have been found in the postmortem pancreatic tissue of patients with AIDS.[5,85]

Rarely, pancreatitis occurs during acute episodes of *Plasmodium falciparum* malaria.[182] Other systemic manifestations of malaria that often are present include high fever, hepatitis, intestinal malabsorption, encephalitis, and pulmonary insufficiency.

Mycoplasmal and Bacterial Infections

In older children and adults, moderately severe symptoms of pancreatitis have occurred just before or during the course of atypical pneumonia.[6,83] Most patients have had cold agglutinins in their sera, and all have had significant changes in *Mycoplasma pneumoniae* antibody titer. Some controversy has ensued over whether *M. pneumoniae* can cause acute pancreatitis without evidence of pneumonia. Although complement-fixing IgM antibodies against *M. pneumoniae* often increase significantly during the course of acute pancreatitis, researchers have argued that pancreatic cellular antigenic components similar to *Mycoplasma* lipid antigens are exposed during the disease process and that the antibodies elicited cross react in *Mycoplasma* serologic assays.[107] Rarely, *Mycoplasma* has caused severe necrotizing pancreatitis.[128]

Along with *M. pneumoniae* infection, legionnaires' disease must be considered when acute pancreatitis develops along with pneumonia.[121,209] Miliary tuberculosis also can cause symptoms of pancreatitis.[154] Pancreatitis may occasionally be the only manifestation of tuberculosis and is usually diagnosed by fine-needle aspiration of the pancreas.[79,131]

Common pyogenic bacteria usually do not cause acute pancreatitis. Secondary invasion of inflamed pancreatic tissue does occur. Some evidence exists that circulating endotoxin from *Escherichia coli* can cause extrahepatic cholestasis and pancreatitis.[48] Acute pancreatitis also has been seen in children with hemolytic-uremic syndrome.[150,157] Pancreatitis can occur during acute episodes of enteritis. *Salmonella typhimurium*, *Salmonella typhosa*, *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Yersinia pseudotuberculosis* all have been reported to cause clinically evident and laboratory-confirmed cases of pancreatitis.[11,45,120,160] There have been single reports of *Moraxella catarrhalis* and *Streptococcus pyogenes* causing severe pancreatitis in young children.[1,138]

Pancreatitis has been reported in children with leptospirosis.[137,172] *Brucella melitensis* also has been added to the list of uncommon causes of acute pancreatitis.[142] *Helicobacter pylori* has been suggested to influence the clinical course of pancreatitis in humans, but data are still lacking to imply a role in pancreatic pathology.[116]

Fungal Infections

Fungal infections have not been reported to cause acute pancreatitis in immunocompetent patients. *Aspergillus* has caused fatal hemorrhagic pancreatitis, however, in an adult patient with cancer who was undergoing chemotherapy.[73] *Candida* spp. and *Cryptococcus neoformans* have been isolated from the pancreatic tissue of patients with AIDS, but whether they cause clinical symptoms of pancreatitis is unknown.[213]

PATHOGENESIS

Enzymes for polysaccharides, fats, and proteins are produced and stored in pancreatic acinar cells. They exist intracellularly in an inactive precursor form. After cholecystokinin-pancreozymin stimulation, the proenzymes are released into the pancreatic ducts and flow into the duodenum. The enzymes normally do not become enzymatically active until they reach the intestinal lumen.

When trypsinogen is activated prematurely to trypsin within the pancreatic acinar cells, autodigestion occurs within the pancreas. The mechanisms that activate trypsinogen and other proteolytic enzymes during specific pathologic processes have not been well elucidated. Drug-induced and infectious causes of pancreatitis are thought to be due to direct toxic effects on acinar cells. Gallstones, *Ascaris* infection, and congenital abnormalities are thought to damage acinar cells by obstructing pancreatic flow. Traumatic pancreatitis probably occurs as a result of direct injury to the glandular cells, whereas vasculitis may cause changes in pancreatic blood flow, causing ischemia.

Autodigestion of the pancreas causes edema of pancreatic tissue, and microcirculation may be compromised, leading to ischemia, hemorrhage, or necrosis. An inflammatory response develops, which may be mild, as occurs in episodes of infectious pancreatitis, or may be more severe with hemorrhagic necrosis. Major mediators of the intense immune response include chemoattractant chemokines and their upregulated receptors; cytokines including tumor necrosis factor, interleukin (IL)-1, IL-6, and IL-8; and platelet-activating factor.[114,162] If an imbalance of the proinflammatory response occurs within the pancreas, a systemic inflammatory response including shock may occur, leading to high morbidity and mortality. Also, sepsis may occur because of extensive necrotic tissue within the pancreas and translocation of microorganisms from the intestines.

TREATMENT

Despite increasing recognition of cases of childhood pancreatitis, no major pharmacologic advances have been made in the treatment of the disease since the mid 1970s. Animal data have shown that medications such as glucagon, aprotinin, gabexate mesilate, 5-fluorouracil, octreotide, somatostatin, probiotics, and vitamin-based antioxidants may be useful in the treatment of pancreatitis; however, trials in adults have not substantiated their efficacy.[16,49,163] The continuing main objectives of treatment are to relieve abdominal pain and treat aggressively systemic manifestations, such as shock, electrolyte abnormalities, and anemia. Meperidine continues to be the medication most commonly used for controlling pain.

In the past, children were kept in the fasted state and nasogastric suction was applied to decrease duodenal acid–stimulated secretin release. Feeding with carbohydrate solutions was not restarted until all symptoms had

resolved. Meta-analyses in adults, however, have shown that feeding with a low-fat elemental diet decreases the complication rate of patients with acute pancreatitis and now is considered the treatment of choice over total parenteral nutrition.[9,33] Intravenous fluids and colloids are used during the acute episode to maintain intravascular volume. During the entire course of acute pancreatitis, the hematologic and biochemical parameters of the child must be monitored closely.

If the episode of pancreatitis is drug induced, use of the medication should be curtailed immediately. Often, the symptoms recur if the medication is restarted. Pancreatitis caused by *M. pneumoniae* or bacteria should be treated with proper antimicrobial agents. Obstructions to pancreatic flow (e.g., gallstones, roundworms, congenital abnormalities) may have to be excised or altered either by surgery or endoscopy.[27,98,146]

COMPLICATIONS

During the acute episode of pancreatitis, the systemic inflammatory response syndrome may develop, leading to renal, hematologic, central nervous system, pulmonary, and cardiovascular complications. In 12 percent of children with pancreatitis, an inflammatory mass develops in the first weeks after the onset of illness; however, these masses more commonly occur after trauma.[202] Continued or increasing abdominal pain, nausea, or vomiting often accompanies the development of a phlegmon, abscess, or pseudocyst. An inflammatory phlegmon usually develops into a thin-walled pseudocyst of the lesser sac but may become secondarily infected and induce the formation of an abscess. Patients in whom an inflammatory mass develops must be monitored closely with frequent physical examinations and serial CT studies. In children with pseudocysts, acute abdominal pain accompanied by hypotension often signifies bleeding into the pseudocyst or rupture of the pseudocyst into the peritoneum. Slowly leaking pseudocysts may cause pancreatic ascites. Pseudocysts should be treated conservatively but have to be resected surgically, drained externally, or drained by endoscopy when complications occur.[10,173] Approximately 33 percent of pseudocysts resolve spontaneously within 6 weeks.[147]

The development of fever and leukocytosis during the course of pancreatitis should suggest an infected pseudocyst, pancreatic abscess, or sepsis. In adults, infectious complications account for 80 percent of deaths associated with acute pancreatitis.[29] Isolates from pancreatic abscesses and necrotic pancreatic tissue have yielded intestinal flora, including anaerobes, in more than 90 percent of cases, but *Candida* spp. are being isolated more frequently in many medical centers.[50,191] Rarely, *Streptococcus pneumoniae* can be isolated from infected pancreatic tissues of adults with chronic pancreatitis.[183] Carbapenems, such as imipenem and meropenem, are used commonly to treat adult patients with suppurative complications of pancreatitis because these antibiotics penetrate well into pancreatic tissues and have activity against intestinal flora.

Even with early diagnosis, proper antibiotics, and surgical intervention using interventional drainage procedures, necrosectomy, or open surgery, the death rate from pancreatic abscesses reaches 27 to 57 percent in adults.[88] Performing percutaneous catheter drainage under CT guidance may reduce the mortality rate associated with treating pancreatic abscesses.[17] Rarely, fistulas from pseudocysts or abscesses to other abdominal organs may develop.[82]

The role of prophylactic antibiotics in preventing the suppurative complications of acute pancreatitis remains controversial despite 3 decades of debate. Most recent meta-analyses on the subject conclude that prophylactic antibiotics do not prevent pancreatic necrosis from being infected and do not prevent death, although a poorly powered 2010 Cochrane review suggests that imipenem may reduce the number of pancreatic infections.[133,200,210] Infections, when they do occur after the administration of prophylactic antimicrobial agents, often are caused by multiresistant bacteria or by fungi.

Osteolytic lesions resembling osteomyelitis may develop weeks to months after an acute episode of pancreatitis.[132] Elevated systemic levels of lipase activity possibly may cause intramedullary fat necrosis in the bone. Usually, the lesions are asymptomatic and resolve spontaneously without therapy.

NEW REFERENCES SINCE THE SIXTH EDITION

7. Alasmi MM, Assiri AM, Iqbal MA, et al. Acute pancreatitis as a presenting symptom of Caroli's disease in a child. *Trop Gastroenterol* 2011;**32**:59–62.
9. Al-Omran M, Albalawi ZH, Tashkandi MF, Al-Ansary LA. Enteral versus parenteral nutrition for acute pancreatitis. *Cochrane Database Syst Rev* 2010;(1):CD002837.
12. Aronson NE, Wortmann GW, Byrne WR, et al. A randomized controlled trial of local heat therapy versus intravenous sodium stibogluconate for the treatment of cutaneous *Leishmania major* infection. *PLoS Negl Trop Dis* 2010;**4**:e628.
13. Baba AA, Shera AH, Bhat MA, et al. Management of biliary ascariasis in children living in an endemic area. *Eur J Pediatr Surg* 2010;**20**:187–90.
14. Balani AR, Grendell JH. Drug-induced pancreatitis. *Drug Safety* 2008;**31**:823–37.
16. Bansal D, Bhalla A, Bhasin DK, et al. Safety and efficacy of vitamin-based antioxidant therapy in patients with severe acute pancreatitis: a randomized controlled trial. *Saudi J Gastroenterol* 2011;**17**:174–9.
22. Bitton S, Weinstein TA, Levine JJ, et al. A 9-year-old with stabbing stomach pain, low-grade fever. *Pediatr Ann* 2011;**40**:401–3.
25. Blum A, Podvitzky O, Shalabi R, et al. Acute pancreatitis may be caused by H1N1 influenza A virus infection. *Isr Med Assoc J* 2010;**12**:640–1.
26. Bolar K, Hoffman AR, Maneatis T, et al. Long-term safety of recombinant human growth hormone in Turner syndrome. *J Clin Endocrinol Metab* 2008;**93**:344–51.
28. Buber J, Fink N, Bin H, et al. West Nile virus–induced pancreatitis. *Travel Med Infect Dis* 2008;**6**:373–5.
33. Cao Y, Xu Y, Lu T, et al. Meta-analysis of enteral nutrition versus total parenteral nutrition in patients with severe acute pancreatitis. *Ann Nutr Metab* 2008;**53**:268–75.
38. Chavhan GB, Babyn PS, Manson D, et al. Pediatric MR cholangiopancreatography: principles, technique, and clinical applications. *Radio Graphics* 2008;**28**:1951–62.
43. Darge K, Anupindi S. Pancreatitis and the role of US, MRCP and ERCP. *Pediatr Radiol* 2009;**39**:S153–7.
46. de Cid R, Ramos MD, Aparisi L, et al. Independent contribution of common CFTR variants to chronic pancreatitis. *Pancreas* 2010;**39**:209–15.

52. Earl M. Incidence and management of asparaginase-associated adverse events in patients with acute lymphoblastic leukemia. *Clin Adv Hematol Oncol* 2009;**7**:600–6.
61. Foster C, Lyall H. HIV and mitochondrial toxicity in children. *J Antimicrobial Chemother* 2008;**61**:8–12.
62. Franco J, Fernandes R, Oliveira M, et al. Acute pancreatitis associated with varicella infection in an immunocompetent child. *J Paediatr Child Health* 2009;**45**:547–8.
64. Fusilli G, De Mitri B. Acute pancreatitis associated with the measles virus case report and review of literature data. *Pancreas* 2009;**38**:478–80.
66. Garg R, Agarwala S, Bhatnagar V. Acute pancreatitis induced by ifosfamide therapy. *J Pediatr Surg* 2010;**45**:2071–3.
70. Gowda G, Familua O, Sathyanaravana N. Acute gallstone pancreatitis in a child, a rare entity. *Am Surgeon* 2010;**76**:e35–6.
71. Groeneweg M, Poley JW, Dansen M, et al. Chronic hereditary pancreatitis in a girl with a serine protease inhibitor Kazal type 1 *(SPINK-1)* gene mutation and a coxsackie type B5 infection. *Pediatr Infect Dis J* 2009;**28**:169–70.
74. Gupta N, Moore D, Hooper AJ, et al. Pancreatitis in a child with lipemia due to novel lipoprotein lipase mutations. *J Pediatr Gastroenterol Nutr* 2010;**50**:457–9.
87. Hunter CJ, Connelly ME, Ghaffari N, et al. Enteric duplication cysts of the pancreas: a report of two cases and review of the literature. *Pediatr Surg Int* 2008;**24**:227–33.
89. Hwang SS, Paik CN, Lee K-M. Recurrent acute pancreatitis caused by an annular pancreas in a child. *Gastrointest Endosc* 2010;**72**:848–9.
97. Karakas E, Tuna Y, Basar O, et al. Primary pancreatic hydatid disease associated with acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 2010;**9**:441–2.
101. Khawcharoenporn T, Lau WKK, Chokrungvaranon N. Epstein-Barr virus infection with acute pancreatitis. *Int J Infect Dis* 2008;**12**:227–9.
102. Kir S, Aydin Y, Kocaman O, et al. Acute pancreatitis after severe ophthalmic adenoviral infection. *Acta Gastroenterol Belg* 2011; **74**:361–2.
105. Konstantinou GN, Liatsos CN, Patelaros EG, et al. Acute pancreatitis associated with herpes simplex virus infection: report of a case and review of the literature. *Eur J Gastroenterol Hepatol* 2009;**21**:114–6.
111. Lowe ME, Greer JB. Pancreatitis in children and adolescents. *Curr Gastroenterol Rep* 2008;**10**:128–35.
123. Mofidi R, Suttie SA, Patil PV, et al. The value of procalcitonin at predicting the severity of acute pancreatitis and development of infected pancreatic necrosis: systematic review. *Surgery* 2009;**146**:72–81.
126. Nachman SA, Chernoff M, Gona P, et al. Incidence of noninfectious conditions in perinatally HIV-infected children and adolescents in the HAART era. *Arch Pediatr Adolesc Med* 2009;**163**: 164–71.
128. Nakagawa M, Ogino H, Shimohira M, et al. Continuous regional arterial infusion therapy for acute necrotizing pancreatitis due to *Mycoplasma pneumoniae* infection in a child. *Cardiovasc Intervent Radiol* 2009;**32**:581–4.
133. Nicholson LJ. Acute pancreatitis: should we use antibiotics? *Curr Gastroenterol Rep* 2011;**13**:336–43.
134. Nie Y, Li W, Tong Z, et al. Acute pancreatitis associated with Henoch-Schönlein purpura. *Pancreas* 2011;**40**:315–6.
140. Oliveira PH, Silva B, Oliveira AH, et al. Acute pancreatitis and child sexual abuse. *Acta Paediatr* 2011;**100**:138–40.
143. Parri N, Innocenti L, Collini S, et al. Acute pancreatitis due to rotavirus gastroenteritis in a child. *Pediatr Emer Care* 2010;**26**: 592–3.
144. Passier JL, van Puijenbroek EP, Jonkers GJ, van Grootheest AC. Pancreatitis associated with the use of itraconazole. *Neth J Med* 2010;**68**:285–9.
148. Prot-Labarthe S, Youdaren R, Benkerrou M, et al. Pediatric acute pancreatitis related to tigecycline. *Pediatr Infect Dis J* 2010;**29**:890–1.
152. Roquin G, Peres M, Lerolle N, et al. First report of lamotrigine-induced drug rash with eosinophilia and systemic symptoms syndrome with pancreatitis. *Ann Pharmacother* 2010;**44**:1998–2000.
156. Sato K, Hayashi M, Ishizuka T, et al. Acute pancreatitis in a patient treated with micafungin. *Clin Ther* 2007;**29**:1468–73.
161. Shahian M, Alborzi A. Effect of meglumine antimonials on the pancreas during treatment of visceral leishmaniasis in children. *Med Sci Monit* 2009;**15**:CR290–3.
162. Shanmugam MK, Bhatia M. The role of pro-inflammatory molecules and pharmacological agents in acute pancreatitis and sepsis. *Inflamm Allergy Drug Targets* 2010;**9**:20–31.
163. Sharma B, Srivastava S, Singh N, et al. Role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis. *J Clin Gastroenterol* 2011;**45**:442–8.
165. Shinnar S, Pellock JM, Conry JA. Open-label, long-term safety study of zonisamide administered to children and adolescents with epilepsy. *Eur J Paediatr Neuro* 2009;**13**:3–9.
171. Spanier BWM, Nio Y, van der Hulst RWM, et al. Practice and yield of early CT scan in acute pancreatitis; a Dutch observational multicenter study. *Pancreatology* 2010;**10**:222–8.
173. Spraker HL, Spyridis GP, Pui C-H, et al. Conservative management of pancreatic pseudocysts in children with acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2009;**31**:957–9.
176. Suzuki M, Fujii T, Takahiro K, et al. Scoring system for the severity of acute pancreatitis in children. *Pancreas* 2008;**37**: 222–3.
178. Talukdar R, Chaudhuri D, Vora P, et al. A child with upper abdominal pain and pancreatitis. *Gut* 2008;**57**:103–4.
180. Terada T. Cytomegalovirus-associated severe fatal necrotizing pancreatitis in a patient with interstitial pneumonitis treated with steroids. *J Pancreas* 2011;**12**:158–61.
181. Thapa R, Biswas B, Mallick D, et al. Acute pancreatitis–complicating hepatitis E virus infection in a 7-year-old boy with glucose-6-phosphate dehydrogenase deficiency. *Clin Pediatr* 2009;**48**:199–201.
182. Thapa R, Mallick D, Biswas B. Childhood Plasmodium falciparum malaria complicated by acute pancreatitis. *Trop Doc* 2010;**40**: 184–5.
189. Tracy S, Gauntt C. Group B coxsackievirus virulence. *Curr Top Microbiol Immunol* 2008;**323**:49–63.
190. Treepongkaruna S, Thongpak N, Pakakasama S, et al. Acute pancreatitis in children with acute lymphoblastic leukemia after chemotherapy. *J Pediatr Hematol Oncol* 2009;**31**:812–5.
191. Trikudanathan G, Navaneethan U, Vege SS. Intra-abdominal fungal infections complicating acute pancreatitis: a review. *Am J Gastroenterol* 2011;**106**:1188–92.
196. Vanlioglu B, Chua TC. Presentation of mumps infection as acute pancreatitis without parotitis. *Pancreas* 2011;**40**:167–8.
200. Villatoro E, Mulla M, Larvin M. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis. *Cochrane Database Syst Rev May* 2010;(5): CD002941.
201. Wang CH, Yao TC, Huang YL, et al. Acute pancreatitis in pediatric and adult-onset systemic lupus erythematosus: a comparison and review of the literature. *Lupus* 2011;**20**:443–52.
204. Weiss B, Lerner A, Shapiro R, et al. Methotrexate treatment in pediatric Crohn disease patients intolerant or resistant to purine analogues. *J Pediatr Gastroenterol Nutr* 2009;**48**:526–30.
210. Wittau M, Mayer B, Scheele J, et al. Systematic review and meta-analysis of antibiotic prophylaxis in severe acute pancreatitis. *Scand J Gastroenterol* 2011;**46**:261–70.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adams D, Fenton SJ, Nichol PF. Streptococcal pancreatitis and toxic shock syndrome in a 2-month-old infant. *J Pediatr Surg* 2007;**42**:261–3.
2. Agarwal A, Chowdhury V, Srivastava N, et al. Pancreatic duct ascariasis: sonographic diagnosis—a case report. *Trop Gastroenterol* 2005;**26**:197–8.
3. Agarwal KS, Puliyel JM, Mathew A, et al. Acute pancreatitis with cholestatic hepatitis: an unusual manifestation of hepatitis A. *Ann Trop Paediatr* 1999;**19**:391–4.
4. Ahmed S, Siddiqui AK, Siddiqui RK, et al. Acute pancreatitis during sickle cell vaso-occlusive painful crisis. *Am J Hematol* 2003;**73**:190–3.
5. Ahuja SK, Ahuja SS, Thelmo W, et al. Necrotizing pancreatitis and multisystem organ failure associated with toxoplasmosis in a patient with AIDS. *Clin Infect Dis* 1993;**16**:432–4.
6. Al-Abassi A. Acute pancreatitis associated with *Mycoplasma pneumoniae*: a case report of missed diagnosis. *Med Prin Pract* 2002; **11**:112–5.
7. Alasmi MM, Assiri AM, Iqbal MA, et al. Acute pancreatitis as a presenting symptom of Caroli's disease in a child. *Trop Gastroenterol* 2011;**32**:59–62.
8. Alberti-Flor JJ, Hernandez ME, Ferrer JP, et al. Fulminant liver failure and pancreatitis associated with the use of sulfamethoxazole-trimethoprim. *Am J Gastroenterol* 1989;**84**:1577–9.
9. Al-Omran M, Albalawi ZH, Tashkandi MF, Al-Ansary LA. Enteral versus parenteral nutrition for acute pancreatitis. *Cochrane Database Syst Rev* 2010;(1):CD002837.
10. Al-Shanafey S, Shun A, Williams S. Endoscopic drainage of pancreatic pseudocysts in children. *J Pediatr Surg* 2004;**39**:1062–5.
11. Andrén-Sandberg A, Höjer H. Necrotizing acute pancreatitis induced by *Salmonella* infection. *Int J Pancreatol* 1994;**15**:229–30.
12. Aronson NE, Wortmann GW, Byrne WR, et al. A randomized controlled trial of local heat therapy versus intravenous sodium stibogluconate for the treatment of cutaneous *Leishmania major* infection. *PLoS Negl Trop Dis* 2010;**4**:e628.
13. Baba AA, Shera AH, Bhat MA, et al. Management of biliary ascariasis in children living in an endemic area. *Eur J Pediatr Surg* 2010;**20**:187–90.
14. Balani AR, Grendell JH. Drug-induced pancreatitis. *Drug Safety* 2008;**31**:823–37.
15. Baldwin M, Eisenman RE, Prelipp AM, et al. *Ascaris lumbricoides* resulting in acute cholecystitis and pancreatitis in the Midwest. *Am J Gastroenterol* 1993;**88**:2119–21.
16. Bansal D, Bhalla A, Bhasin DK, et al. Safety and efficacy of vitamin-based antioxidant therapy in patients with severe acute pancreatitis: a randomized controlled trial. *Saudi J Gastroenterol* 2011;**17**:174–9.
17. Baril NB, Ralls PW, Wren SM, et al. Does an infected peripancreatic fluid collection or abscess mandate operation? *Ann Surg* 2000;**23**:361–67.
18. Barman KK, Premalatha G, Mohan V. Tropical chronic pancreatitis. *Postgrad Med J* 2003;**79**:606–15.
19. Bartholomew C. Acute scorpion pancreatitis in Trinidad. *BMJ* 1970;**1**:666–8.
20. Bateman CM, Kesson AM, Shaw PJ. Pancreatitis and adenoviral infection in children after blood and marrow transplantation. *Bone Marrow Transplant* 2006;**38**:807–11.
21. Benifla M, Weizman Z. Acute pancreatitis in childhood. *J Clin Gastroenterol* 2003;**37**:169–72.
22. Bitton S, Weinstein TA, Levine JJ, et al. A 9-year-old with stabbing stomach pain, low-grade fever. *Pediatr Ann* 2011;**40**:401–3.
23. Blake RL. Acute pancreatitis. *Primary Care* 1988;**15**:187–9.
24. Blejter J, Weller S, Pace R, et al. Autoimmune pancreatitis: an adolescent case and review of literature. *J Pediatr Surg* 2008;**43**:1368–72.
25. Blum A, Podvitzky O, Shalabi R, et al. Acute pancreatitis may be caused by H1N1 influenza A virus infection. *Isr Med Assoc J* 2010;**12**:640–1.
26. Bolar K, Hoffman AR, Maneatis T, et al. Long-term safety of recombinant human growth hormone in Turner syndrome. *J Clin Endocrinol Metab* 2008;**93**:344–51.
27. Bonnard A, Seguier-Lipszyc E, Liguory C, et al. Laparoscopic approach as primary treatment of common bile duct stones in children. *J Pediatr Surg* 2005;**40**:1459–63.
28. Buber J, Fink N, Bin H, et al. West Nile virus–induced pancreatitis. *Travel Med Infect Dis* 2008;**6**:373–5.
29. Buggy BP, Nostrant TT. Lethal pancreatitis. *Am J Gastroenterol* 1983;**78**:810–4.
30. Bultron G, Seashore MR, Pashankar DS, et al. Recurrent acute pancreatitis associated with propionic acidemia. *J Pediatr Gastroenterol Nutr* 2008;**47**:370–1.
31. Bustamante SE, Appachi E. Acute pancreatitis after anesthesia with propofol in a child with glycogen storage disease type 1A. *Pediatr Anesthesia* 2006;**16**:680–3.
32. Butler KM, Venzon D, Henry N, et al. Pancreatitis in human immunodeficiency virus–infected children receiving dideoxyinosine. *Pediatrics* 1993;**91**:747–51.
33. Cao Y, Xu Y, Lu T, et al. Meta-analysis of enteral nutrition versus total parenteral nutrition in patients with severe acute pancreatitis. *Ann Nutr Metab* 2008;**53**:268–75.
34. Capner P, Lendrum R, Jeffries DJ, et al. Viral antibody studies in pancreatic disease. *Gut* 1975;**16**:866–70.
35. Carroccio A, Fontana M, Spagnuolo MI, et al. Serum pancreatic enzymes in human immunodeficiency virus–infected children. *Scand J Gastroenterol* 1998;**33**:998–1001.
36. Carter BA, Karpen SJ, Quiros-Tejeira RE, et al. Intravenous cidofovir therapy for disseminated adenovirus in a pediatric liver transplant recipient. *Pediatr Infect Dis J* 2002;**74**:1050–2.
37. Cecchi E, Forte P, Cini E, et al. Pancreatitis induced by pegylated interferon alfa-2b in a patient affected by chronic hepatitis C. *Emerg Med Australas* 2004;**16**:473–5.
38. Chavhan GB, Babyn PS, Manson D, et al. Pediatric MR cholangiopancreatography: principles, technique, and clinical applications. *Radio Graphics* 2008;**28**:1951–62.
39. Chen C, Changchien C, Lu S, et al. Lamivudine treatment for recurrent pancreatitis associated with reactivation of chronic B hepatitis. *Dig Dis Sci* 2002;**47**:564–7.
40. Cheung O, Chopra K, Yu T, et al. Gatifloxacin-induced hepatotoxicity and acute pancreatitis. *Ann Intern Med* 2004; **140**:73–4.
41. Colebunders R, Van den Abbeele K, Fleerackers Y, et al. Two AIDS patients with life-threatening pancreatitis successfully treated, one with ganciclovir, the other with foscarnet. *Acta Clin Belg* 1994;**49**:229–32.
42. Corp CC, Ghishan FK. The sulfone syndrome complicated by pancreatitis and pleural effusion in an adolescent receiving dapsone for treatment of acne vulgaris. *J Pediatr Gastroenterol Nutr* 1998;**26**:103–5.
43. Darge K, Anupindi S. Pancreatitis and the role of US, MRCP and ERCP. *Pediatr Radiol* 2009;**39**:S153–7.
44. DeBanto JR, Goday PS, Pedroso MRA, et al. Acute pancreatitis in children. *Am J Gastroenterol* 2002;**97**:1726–31.
45. de Bois MH, Schoemaker MC, van der Werf SD, et al. Pancreatitis associated with *Campylobacter jejuni* infections: diagnosis by ultrasonography. *BMJ* 1989;**298**:1004.
46. de Cid R, Ramos MD, Aparisi L, et al. Independent contribution of common *CFTR* variants to chronic pancreatitis. *Pancreas* 2010;**39**:209–15.
47. Dettmeyer RB, Padosch SA, Madea B. Lethal enterovirus-induced myocarditis and pancreatitis in a 4-month-old boy. *Forensic Sci Int* 2006;**156**:51–4.
48. Dev G, Sikka M, Sehgal S, et al. *Escherichia coli* infection producing pancreatitis and extrahepatic cholestasis. *Indian Pediatr* 1987;**24**: 249–53.
49. De Waele JJ, Hoste E. Current pharmacotherapeutic recommendations for acute pancreatitis. *Expert Opin Pharmacother* 2006;**7**: 1017–25.
50. De Waele JJ, Vogelaers D, Colardyn F. Fungal infections in patients with severe acute pancreatitis and the use of prophylactic therapy. *Clin Infect Dis* 2003;**37**:208–13.
51. Dinndorf PA, Gootenberg J, Cohen MH, et al. FDA drug approval summary: pegaspargase (Oncaspar) for the first-line treatment of children with acute lymphoblastic leukemia (ALL). *Oncologist* 2007;**12**:991–8.
52. Earl M. Incidence and management of asparaginase-associated adverse events in patients with acute lymphoblastic leukemia. *Clin Adv Hematol Oncol* 2009;**7**:600–6.
53. Echenique-Elizondo M, Amondarain J, Liron de Robles C. Fascioliasis: an exceptional cause of acute pancreatitis. *J Pancreas* 2005;**6**:36–9.

54. Eisenhuber E, Schoefl R, Wiesbauer P, et al. Primary pancreatic lymphoma presenting as acute pancreatitis in a child. *Med Pediatr Oncol* 2001;**37**:53–4.
55. Elmore MF, Rogge JD. Tetracycline-induced pancreatitis. *Gastroenterology* 1981;**81**:1134–6.
56. Feiner H. Pancreatitis after cardiac surgery: a morphologic study. *Am J Surg* 1976;**131**:684–8.
57. Feldstein JD, Johnson FR, Kallick CA, et al. Acute hemorrhagic pancreatitis and pseudocyst due to mumps. *Ann Surg* 1974; **180**:85–8.
58. Fernández RA, Varona TL, Jaquotot JMK, et al. Pancreatitis aguda asociada a infección por virus de la varicela-zoster en un paciente con sindrome de immunodeficiencia adquirida. *Med Clin (Barc)* 1992;**98**:339–41.
59. Fimognari FL, Corsonello A, Pastorell R, et al. Metformin-induced pancreatitis. *Diabetes Care* 2006;**29**:1183.
60. Foisy MM, Slayter KL, Hewitt RG, et al. Pancreatitis during intravenous pentamidine therapy in an AIDS patient with prior exposure to didanosine. *Ann Pharmacother* 1994;**28**: 1025–8.
61. Foster C, Lyall H. HIV and mitochondrial toxicity in children. *J Antimicrobial Chemother* 2008;**61**:8–12.
62. Franco J, Fernandes R, Oliveira M, et al. Acute pancreatitis associated with varicella infection in an immunocompetent child. *J Paediatr Child Health* 2009;**45**:547–8.
63. Frank JA, Warren RW, Tucker JA, et al. Disseminated parainfluenza infection in a child with severe combined immunodeficiency. *Am J Dis Child* 1983;**137**:1172–4.
64. Fusilli G, De Mitri B. Acute pancreatitis associated with the measles virus case report and review of literature data. *Pancreas* 2009;**38**:478–80.
65. Garau P, Orenstein SR, Neigut DA, et al. Pancreatitis associated with olsalazine and sulfasalazine in children with ulcerative colitis. *J Pediatr Gastroenterol Nutr* 1994;**18**:481–5.
66. Garg R, Agarwala S, Bhatnagar V. Acute pancreatitis induced by ifosfamide therapy. *J Pediatr Surg* 2010;**45**:2071–3.
67. Gerstner T, Busing D, Bell N, et al. Valproic acid–induced pancreatitis: 16 new cases and a review of the literature. *J Gastroenterol* 2007;**42**:39–48.
68. Glassman M, Tahan S, Hillemeier C, et al. Pancreatitis in patients with Reye's syndrome. *J Clin Gastroenterol* 1981;**3**:165–9.
69. Gonzalez Carro P, Perez Roldan F, Legaz Huidobro ML, et al. Acute pancreatitis and modified-release clarithromycin. *Ann Pharmacother* 2004;**38**:508–9.
70. Gowda G, Familua O, Sathyanaravana N. Acute gallstone pancreatitis in a child, a rare entity. *Am Surgeon* 2010;**76**:e35–6.
71. Groeneweg M, Poley JW, Dansen M, et al. Chronic hereditary pancreatitis in a girl with a serine protease inhibitor Kazal type 1 *(SPINK-1)* gene mutation and a coxsackie type B5 infection. *Pediatr Infect Dis J* 2009;**28**:169–70.
72. Gross TL, Wheat J, Bartlett M, et al. AIDS and multiple system involvement with *Cryptosporidium*. *Am J Gastroenterol* 1986;**81**:456–8.
73. Guice KS, Lynch M, Weatherbee L. Invasive aspergillosis: an unusual cause of hemorrhagic pancreatitis. *Am J Gastroenterol* 1987;**82**:563–5.
74. Gupta N, Moore D, Hooper AJ, et al. Pancreatitis in a child with lipemia due to novel lipoprotein lipase mutations. *J Pediatr Gastroenterol Nutr* 2010;**50**:457–9.
75. Haddad NG, Croffie JM, Eugster EA. Pancreatic enzyme elevations in children with diabetic ketoacidosis. *J Pediatr* 2004;**145**:122–4.
76. Haddock G, Coupar G, Youngson GG, et al. Acute pancreatitis in children: a 15-year review. *J Pediatr Surg* 1994;**29**:719–22.
77. Hanft A, Bourgeois J. Risperidone and pancreatitis. *J Am Acad Child Adolesc Psychiatry* 2004;**43**:1458–9.
78. Harb R, Naon H. Idiopathic fibrosing pancreatitis in a 3-year-old girl: a case report and review of the literature. *J Pediatr Surg* 2005;**40**:1335–40.
79. Hari S, Seith A, Srivastava DN, et al. Isolated tuberculosis of the pancreas diagnosed with needle aspiration: a case report and review of the literature. *Trop Gastroenterol* 2005;**26**:141–3.
80. Hart CC. Aerosolized pentamidine and pancreatitis. *Ann Intern Med* 1989;**111**:691.
81. Hawkins SP, Thomas RP, Teasdale C. Acute pancreatitis: a new finding in *Cryptosporidium* enteritis. *BMJ* 1987;**294**:483–4.
82. Henderson JM, MacDonald JAE. Fistula formation complicating pancreatic abscess. *Br J Surg* 1976;**63**:233–4.
83. Herbaut C, Tielemans C, Burette A, et al. *Mycoplasma pneumoniae* infection and acute pancreatitis. *Acta Clin Belg* 1983;**38**:186–8.
84. Hillemeier C, Gryboski JD. Acute pancreatitis in infants and children. *Yale J Biol Med* 1984;**57**:149–59.
85. Hofman P, Michiels J-F, Mondain V, et al. Pancréatite aiguë toxoplasmique. *Gastroenterol Clin Biol* 1994;**18**:895–7.
86. Huber S, Ramsingh AI. Coxsackievirus-induced pancreatitis. *Viral Immunol* 2004;**17**:358–69.
87. Hunter CJ, Connelly ME, Ghaffari N, et al. Enteric duplication cysts of the pancreas: a report of two cases and review of the literature. *Pediatr Surg Int* 2008;**24**:227–33.
88. Hurley JE, Vargish T. Early diagnosis and outcome of pancreatic abscesses in pancreatitis. *Am Surg* 1987;**53**:29–33.
89. Hwang SS, Paik CN, Lee K-M. Recurrent acute pancreatitis caused by an annular pancreas in a child. *Gastrointest Endosc* 2010;**72**:848–9.
90. Imrie CW, Ferguson JC, Sommerville RG. Coxsackie and mumps virus infection in a prospective study of acute pancreatitis. *Gut* 1977;**18**:53–6.
91. Iwasaki T, Tashiro A, Satodate R, et al. Acute pancreatitis with cytomegalovirus infection. *Acta Pathol Jpn* 1987;**37**:1661–8.
92. Jain P, Nijhawan S. Acute viral hepatitis with pancreatitis: is it due to the viruses or sludge? *Pancreatology* 2007;**7**:544–5.
93. Jesudason SRB, Mathai V, Muthusami JC, et al. *Wuchereria bancrofti* induced pancreatitis. *Trop Gastroenterol* 1992;**13**:115–8.
94. Jin C-F, Sable R. Isoniazid-induced acute hepatitis and acute pancreatitis in a patient during chemoprophylaxis. *J Clin Gastroenterol* 2002;**35**:100–1.
95. Joe L, Ansher AF, Gordin FM. Severe pancreatitis in an AIDS patient in association with cytomegalovirus infection. *South Med J* 1989;**82**:1444–5.
96. Johnson JL, Loomis IB. A case of simvastatin-associated pancreatitis and review of statin-associated pancreatitis. *Pharmacotherapy* 2006;**26**:414–22.
97. Karakas E, Tuna Y, Basar O, et al. Primary pancreatic hydatid disease associated with acute pancreatitis. *Hepatobiliary Pancreat Dis Int* 2010;**9**:441–2.
98. Kawahara H, Takahashi T, Okada A. Characteristics of duodenal duplications causing pancreatitis in children and adolescents: a case report and review of the literature. *J Pediatr Gastroenterol Nutr* 2002;**35**:372–6.
99. Keidar S, Porath EB, Naftali V, et al. Acute pancreatitis associated with rising cytomegalovirus titer. *Isr J Med Sci* 1987;**23**:296–7.
100. Khanna S, Vij JC. Severe acute pancreatitis due to hepatitis A virus infection in a patient of acute viral hepatitis. *Trop Gastroenterol* 2003;**24**:25–6.
101. Khawcharoenporn T, Lau WKK, Chokrungvaranon N. Epstein-Barr virus infection with acute pancreatitis. *Int J Infect Dis* 2008; **12**:227–9.
102. Kir S, Aydin Y, Kocaman O, et al. Acute pancreatitis after severe ophthalmic adenoviral infection. *Acta Gastroenterol Belg* 2011; **74**:361–2.
103. Kirschner BS. Safety of azathioprine and 6-mercaptopurine in pediatric patients with inflammatory bowel disease. *Gastroenterology* 1998;**115**:813–21.
104. Koller EA, Cross JT, Doraiswamy PM, et al. Pancreatitis associated with atypical antipsychotics: from the Food and Drug Administration's MedWatch surveillance system and published reports. *Pharmacotherapy* 2003;**23**:1123–30.
105. Konstantinou GN, Liatsos CN, Patelaros EG, et al. Acute pancreatitis associated with herpes simplex virus infection: report of a case and review of the literature. *Eur J Gastroenterol Hepatol* 2009;**21**:114–6.
106. Kugathasan S, Halabi I, Telega G, et al. Pancreatitis as a presenting manifestation of pediatric Crohn's disease: a report of three cases. *J Pediatr Gastroenterol Nutr* 2002;**35**:96–8.
107. Leinikki PO, Panzar P, Tykka H. Immunoglobulin M antibody response against *Mycoplasma pneumoniae* lipid antigen in patients with acute pancreatitis. *J Clin Microbiol* 1978;**8**:113–8.
108. Liu Y-M, Bair M-J, Chang W-H, et al. Acute pancreatitis caused by tapeworm in the biliary tract. *Am J Trop Med Hyg* 2005;**73**: 377–80.
109. Lopez MJ. The changing incidence of acute pancreatitis in children: a single-institution perspective. *J Pediatr* 2002;**140**:622–4.

110. Lowe ME. Pancreatitis in children. *Curr Gastroenterol Rep* 2004;**6**:240–6.
111. Lowe ME, Greer JB. Pancreatitis in children and adolescents. *Curr Gastroenterol Rep* 2008;**10**:128–35.
112. Magill P, Ridgway PF, Conlon KC, et al. A case of probable ibuprofen-induced acute pancreatitis. *J Pancreas* 2006;**7**:311–4.
113. Mahjoub W, Jarboui S, Moussa MB, et al. Indomethacin-induced pancreatitis. A second case report. *J Pancreas* 2006;**7**:321–3.
114. Makhija R, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2002;**9**:401–10.
115. Mallory A, Kern F. Drug-induced pancreatitis: a critical review. *Gastroenterology* 1980;**78**:813–20.
116. Manes G, Balzano A, Vaira D. *Helicobacter pylori* and pancreatic disease. *J Pancreas* 2003;**4**:111–6.
117. Maranan MC, Gerber SI, Miller GG. Gallstone pancreatitis caused by ceftriaxone. *Pediatr Infect Dis J* 1998;**17**:662–3.
118. Marchi A, Caimmi S, Caimmi D, et al. Recurrent pleural effusion as an unusual presentation of acute pancreatitis in children. *Pancreas* 2011;**40**:321–3.
119. Marquard J, el Scheich T, Klee D, et al. Chronic pancreatic in branched-chain organic acidurias—a case of methylmalonic aciduria and an overview of the literature. *Eur J Pediatr* 2011; **170**:241–5.
120. Martinez-Roig A, Bonet-Alcaina M, Casellas-Montagut M, et al. Pancreatitis in typhoid fever relapse. *Pediatr Infect Dis J* 2008; **28**:74.
121. Michel O, Naeije N, Csoma M, et al. Acute pancreatitis in legionnaires' disease. *Eur J Respir Dis* 1985;**66**:62–4.
122. Miller TL, Winter HS, Luginbuhl LM, et al. Pancreatitis in pediatric human immunodeficiency virus infection. *J Pediatr* 1992;**120**:223–7.
123. Mofidi R, Suttie SA, Patil PV, et al. The value of procalcitonin at predicting the severity of acute pancreatitis and development of infected pancreatic necrosis: systematic review. *Surgery* 2009; **146**:72–81.
124. Monif GRG. Rubella virus and the pancreas. *Med Chir Dig* 1974;**3**:195–7.
125. Moreno A, Quereda C, Moreno L, et al. High rate of didanosine-related mitochondrial toxicity in HIV/HCV-coinfected patients receiving ribavirin. *Antiviral Ther* 2004;**9**:133–8.
126. Nachman SA, Chernoff M, Gona P, et al. Incidence of noninfectious conditions in perinatally HIV-infected children and adolescents in the HAART era. *Arch Pediatr Adolesc Med* 2009;**163**: 164–71.
127. Naficy K, Nategh R, Ghadimi H. Mumps pancreatitis without parotitis. *BMJ* 1973;**1**:529–33.
128. Nakagawa M, Ogino H, Shimohira M, et al. Continuous regional arterial infusion therapy for acute necrotizing pancreatitis due to *Mycoplasma pneumoniae* infection in a child. *Cardiovasc Intervent Radiol* 2009;**32**:581–4.
129. Nakao T, Nitta T, Miura R, et al. Clinical and epidemiological studies on an outbreak of aseptic meningitis caused by Coxsackie B5 and A9 viruses in Aomori in 1961. *Tohoku J Exp Med* 1964;**83**:94–102.
130. Nelis GF. Nitrofurantoin-induced pancreatitis: report of a case. *Gastroenterology* 1983;**84**:1032–4.
131. Netherland NA, Chen VK, Eloubeidi MA. Intra-abdominal tuberculosis presenting with acute pancreatitis. *Digest Dis Sci* 2006;**51**:247–51.
132. Neuer FS, Roberts FF, McCarthy V. Osteolytic lesions following traumatic pancreatitis. *Am J Dis Child* 1977;**131**:738–40.
133. Nicholson LJ. Acute pancreatitis: should we use antibiotics? *Curr Gastroenterol Rep* 2011;**13**:336–43.
134. Nie Y, Li W, Tong Z, et al. Acute pancreatitis associated with Henoch-Schönlein purpura. *Pancreas* 2011;**40**:315–6.
135. Niemann TH, Trigg ME, Winick N, et al. Disseminated adenoviral infection presenting as acute pancreatitis. *Hum Pathol* 1993;**24**:1145–48.
136. Nigwekar SU, Casey KJ. Metronidazole-induced pancreatitis: a case report and review of literature. *J Pancreas* 2004;**5**:516–9.
137. O'Brien MM, Vincent JM, Person DA, et al. Leptospirosis and pancreatitis: a report of ten cases. *Pediatr Infect Dis J* 1998; **17**:436–8.
138. Ohkusu K, Nakamura A, Horie H, et al. Fatal sepsis associated with acute pancreatitis caused by *Moraxella catarrhalis* in a child. *Pediatr Infect Dis J* 2001;**20**:914–5.
139. Oku T, Maeda M, Waga E, et al. Cytomegalovirus cholangitis and pancreatitis in an immunocompetent patient. *J Gastroenterol* 2005;**40**:987–92.
140. Oliveira PH, Silva B, Oliveira AH, et al. Acute pancreatitis and child sexual abuse. *Acta Paediatr* 2011;**100**:138–40.
141. Otero R, Navio E, Cespedes FA, et al. Scorpion envenoming in two regions of Colombia: clinical, epidemiological and therapeutic aspects. *Trans R Soc Trop Med Hyg* 2004;**98**:742–50.
142. Papaioannides D, Korantzopoulos P, Sinapidis D, et al. Acute pancreatitis associated with brucellosis. *J Pancreas* 2006;**7**: 62–5.
143. Parri N, Innocenti L, Collini S, et al. Acute pancreatitis due to rotavirus gastroenteritis in a child. *Pediatr Emer Care* 2010; **26**:592–3.
144. Passier JL, van Puijenbroek EP, Jonkers GJ, van Grootheest AC. Pancreatitis associated with the use of itraconazole. *Neth J Med* 2010;**68**:285–9.
145. Perry W, Jenkins MV, Stamp TCB. Lysosomal enzymes and pancreatitis during rifampicin therapy. *Lancet* 1979;**1**:492.
146. Poddar U, Thapa BR, Bhasin DK, et al. Endoscopic retrograde cholangiopancreatography in the management of pancreaticobiliary disorders in children. *J Gastroenterol Hepatol* 2001;**16**: 927–31.
147. Pollak EW, Michas CA, Wolfman EF. Pancreatic pseudocyst: management in 54 patients. *Am J Surg* 1978;**135**:199–201.
148. Prot-Labarthe S, Youdaren R, Benkerrou M, et al. Pediatric acute pancreatitis related to tigecycline. *Pediatr Infect Dis J* 2010;**29**:890–1.
149. Renkes P, Petitpain N, Cosserat F, et al. Can roxithromycin and betamethasone induce acute pancreatitis? A case report. *J Pancreas* 2003;**4**:184–6.
150. Robitaille P, Gonthier M, Grignon A, et al. Pancreatic injury in the hemolytic-uremic syndrome. *Pediatr Nephrol* 1997;**11**: 631–2.
151. Rocca R, Castellino F, Daperno M, et al. Therapeutic ERCP in paediatric patients. *Dig Liver Dis* 2005;**37**:357–62.
152. Roquin G, Peres M, Lerolle N, et al. First report of lamotrigine-induced drug rash with eosinophilia and systemic symptoms syndrome with pancreatitis. *Ann Pharmacother* 2010;**44**:1998–2000.
153. Rosendahl J, Bodeker H, Mossner J, et al. Hereditary chronic pancreatitis. *Orphanet J Rare Dis* 2007;**2**:1.
154. Rushing JL, Hanna CJ, Selecky PA. Pancreatitis as the presenting manifestation of miliary tuberculosis. *West J Med* 1978;**129**: 432–6.
155. Ruzena S. Normal serum amylase in acute pancreatitis. *Dig Dis Sci* 1989;**34**:960–1.
156. Sato K, Hayashi M, Ishizuka T, et al. Acute pancreatitis in a patient treated with micafungin. *Clin Ther* 2007;**29**:1468–73.
157. Sass DA, Chopra KB, Regueiro MD. Pancreatitis and *E. coli* 0157:H7 colitis without hemolytic-uremic syndrome. *Dig Dis Sci* 2003;**48**:415–6.
158. Sastry J, Young S, Shaw PJ. Acute pancreatitis due to tacrolimus in a case of allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2004;**33**:867–8.
159. Schouwenberg BJ, Deinum J. Acute pancreatitis after a course of clarithromycin. *Neth J Med* 2003;**61**:266–7.
160. Schulz TB. Association of pancreas infection and yersiniosis. *Acta Med Scand* 1979;**205**:255–6.
161. Shahian M, Alborzi A. Effect of meglumine antimonials on the pancreas during treatment of visceral leishmaniasis in children. *Med Sci Monit* 2009;**15**:CR290–3.
162. Shanmugam MK, Bhatia M. The role of pro-inflammatory molecules and pharmacological agents in acute pancreatitis and sepsis. *Inflamm Allergy Drug Targets* 2010;**9**:20–31.
163. Sharma B, Srivastava S, Singh N, et al. Role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis. *J Clin Gastroenterol* 2011;**45**:442–8.
164. Shimoda T, Shikata T, Karasawa T, et al. Light microscopic localization of hepatitis B virus antigens in the human pancreas: possibility of multiplication of hepatitis B virus in the human pancreas. *Gastroenterology* 1981;**81**:998–1005.
165. Shinnar S, Pellock JM, Conry JA. Open-label, long-term safety study of zonisamide administered to children and adolescents with epilepsy. *Eur J Paediatr Neurol* 2009;**13**:3–9.
166. Shintaku M, Umehara Y, Iwaisako K, et al. Herpes simplex pancreatitis. *Arch Pathol Lab Med* 2003;**127**:231–4.

167. Shirobokov VP, Zhurba TB, Zemlyansky VV. Properties of the Coxsackie viruses isolated from pancreatic tissue of patients with chronic pancreatitis. *Mikrobiol Z* 1988;**50**:78–81.
168. Shugar RA, Ryan JJ. *Clonorchis sinensis* and pancreatitis. *Am J Gastroenterol* 1975;**65**:400–3.
169. Sinclair DB, Berg M, Breault R. Valproic acid–induced pancreatitis in childhood epilepsy: case series and review. *J Child Neurol* 2004;**19**:498–502.
170. Sofer S, Shalev H, Weizman Z, et al. Acute pancreatitis in children following envenomation by the yellow scorpion *Leiurus quinquestriatus*. *Toxicon* 1991;**29**:125–8.
171. Spanier BW, Nio Y, van der Hulst RW, et al. Practice and yield of early CT scan in acute pancreatitis: a Dutch observational multicenter study. *Pancreatology* 2010;**10**:222–8.
172. Spichler A, Spichler E, Moock M, et al. Acute pancreatitis in fatal anicteric leptospirosis. *Am J Med Hyg* 2007;**76**:886–7.
173. Spraker HL, Spyridis GP, Pui C-H, et al. Conservative management of pancreatic pseudocysts in children with acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2009;**31**:957–9.
174. Stoler J, Biller JA, Grand RJ. Pancreatitis in Kawasaki disease. *Am J Dis Child* 1987;**141**:306–8.
175. Stuecklin-Utsch A, Hasan C, Bode U, et al. Pancreatic toxicity after liposomal amphotericin B. *Mycoses* 2002;**45**:170–3.
176. Suzuki M, Fujii T, Takahiro K, et al. Scoring system for the severity of acute pancreatitis in children. *Pancreas* 2008;**37**:222–3.
177. Takebayashi K, Aso Y, Wakabayashi S, et al. Measles encephalitis and acute pancreatitis in a young adult. *Am J Med Sci* 2004;**327**: 299–303.
178. Talukdar R, Chaudhuri D, Vora P, et al. A child with upper abdominal pain and pancreatitis. *Gut* 2008;**57**:103–4.
179. Tenenbein MS, Tenenbein M. Acute pancreatitis due to erythromycin overdose. *Pediatr Emerg Care* 2005;**21**:675–6.
180. Terada T. Cytomegalovirus-associated severe fatal necrotizing pancreatitis in a patient with interstitial pneumonitis treated with steroids. *J Pancreas* 2011;**12**:158–61.
181. Thapa R, Biswas B, Mallick D, et al. Acute pancreatitis–complicating hepatitis E virus infection in a 7-year-old boy with glucose-6-phosphate dehydrogenase deficiency. *Clin Pediatr* 2009; **48**:199–201.
182. Thapa R, Mallick D, Biswas B. Childhood *Plasmodium falciparum* malaria complicated by acute pancreatitis. *Trop Doc* 2010;**40**: 184–5.
183. Thege MK, Pulay I, Balla E, et al. *Streptococcus pneumoniae* as an etiologic agent in infectious complications of pancreatic disease. *Microbial Drug Resistance* 2002;**8**:73–6.
184. Tipnis NA, Werlin SL. The use of magnetic resonance cholangiopancreatography in children. *Curr Gastroenterol Rep* 2007;**9**: 225–9.
185. Tissieres P, Durand P, Chardot C, et al. Acute pancreatitis after orthotopic liver transplantation in children. *Liver Transplant Surg* 1997;**3**:430–2.
186. Tissieres P, Simon L, Debray D, et al. Acute pancreatitis after orthotopic liver transplantation in children: incidence, contributing factors, and outcome. *J Pediatr Gastroenterol Nutr* 1998;**26**: 315–20.
187. Torosis J, Vender R. Tetracycline-induced pancreatitis. *J Clin Gastroenterol* 1987;**9**:580–1.
188. Torre JAC, Martin JJD, Garcia CB, et al. Varicella infection as a cause of acute pancreatitis in an immunocompetent child. *Pediatr Infect Dis J* 2000;**19**:1218–9.
189. Tracy S, Gauntt C. Group B coxsackievirus virulence. *Curr Top Microbiol Immunol* 2008;**323**:49–63.
190. Treepongkaruna S, Thongpak N, Pakakasama S, et al. Acute pancreatitis in children with acute lymphoblastic leukemia after chemotherapy. *J Pediatr Hematol Oncol* 2009;**31**:812–5.
191. Trikudanathan G, Navaneethan U, Vege SS. Intra-abdominal fungal infections complicating acute pancreatitis: a review. *Am J Gastroenterol* 2011;**106**:1188–92.
192. Truninger K, Malik N, Ammann RW, et al. Mutations of the cystic fibrosis gene in patients with chronic pancreatitis. *Am J Gastroenterol* 2001;**96**:2657–61.
193. Tyner R, Turett G. Primary human immunodeficiency virus infection presenting as acute pancreatitis. *South Med J* 2004;**97**:393–4.
194. Ungar BLP, Burris JA, Quinn CA, et al. New mouse models for chronic *Cryptosporidium* infection in immunodeficient hosts. *Infect Immun* 1990;**58**:961–9.
195. Urban M, Splaingard M, Werlin SL. Pancreatitis associated with remote traumatic brain injury in children. *Child Nerv Syst* 1994;**10**:388–91.
196. Vanlioglu B, Chua TC. Presentation of mumps infection as acute pancreatitis without parotitis. *Pancreas* 2011;**40**:167–8.
197. van Walraven LA, de Klerk JB, Postema RR. Severe acute necrotizing pancreatitis associated with lipoprotein lipase deficiency in childhood. *J Pediatr Surg* 2003;**38**:1407–8.
198. Vargas PA, Bernardi FDC, Alves VAF, et al. Uncommon histopathological findings in fatal measles infection: pancreatitis, sialoadenitis and thyroiditis. *Histopathology* 2000;**37**:141–6.
199. Versleijen MWJ, Naber AHJ, Riksen NP, et al. Recurrent pancreatitis after trimethoprim-sulfamethoxazole rechallenge. *Neth J Med* 2005;**63**:275–7.
200. Villatoro E, Mulla M, Larvin M. Antibiotic therapy for prophylaxis against infection of pancreatic necrosis in acute pancreatitis (review). *Cochrane Database Syst Rev* 2010;(5):CD002941.
201. Wang CH, Yao TC, Huang YL, et al. Acute pancreatitis in pediatric and adult-onset systemic lupus erythematosus: a comparison and review of the literature. *Lupus* 2011;**20**:443–52.
202. Warner RL, Othersen HB, Smith CD. Traumatic pancreatitis and pseudocyst in children: current management. *J Trauma* 1989;**29**:597–601.
203. Warren WR. Serum amylase and lipase in mumps. *Am J Med Sci* 1955;**230**:161–8.
204. Weiss B, Lerner A, Shapiro R, et al. Methotrexate treatment in pediatric Crohn disease patients intolerant or resistant to purine analogues. *J Pediatr Gastroenterol Nutr* 2009;**48**:526–30.
205. Weizman Z, Durie PR. Acute pancreatitis in childhood. *J Pediatr* 1988;**113**:24–9.
206. Weizman Z, Sofer S. Acute pancreatitis in children with anticholinesterase insecticide intoxication. *Pediatrics* 1992;**90**:204–6.
207. Werlin SL, Fish DL. The spectrum of valproic acid–associated pancreatitis. *Pediatrics* 2006;**118**:1660–3.
208. Werlin SL, Kugathasan S, Frautschy BC. Pancreatitis in children. *J Pediatr Gastroenterol Nutr* 2003;**37**:591–5.
209. Westblom TU, Hamory BH. Acute pancreatitis caused by *Legionella pneumophila*. *South Med J* 1988;**81**:1200–1.
210. Wittau M, Mayer B, Scheele J, et al. Systematic review and meta-analysis of antibiotic prophylaxis in severe acute pancreatitis. *Scand J Gastroenterol* 2011;**46**:261–70.
211. Wood CB, Bradbrook RA, Blumgart LH. Chronic pancreatitis in childhood associated with mumps virus infection. *Br J Clin Pract* 1974;**28**:67–9.
212. Youssef SS, Iskandar SB, Scruggs J, et al. Acute pancreatitis associated with omeprazole. *Intl J Clin Pharmacol Ther* 2005; **43**:558–61.
213. Zazzo JF, Pichon F, Regnier B. HIV and the pancreas. *Lancet* 1987;**2**:1212–13.
214. Zhang L, Notohara K, Levy MJ, et al. IgG4-positive plasma cell infiltration in the diagnosis of autoimmune pancreatitis. *Mod Pathol* 2007;**20**:23–8.
215. Ziegler DW, Long JA, Philippart AI, et al. Pancreatitis in childhood: experience with 49 patients. *Ann Surg* 1988;**207**: 257–61.
216. Zuger A, Wolf BZ, El-Sadr W, et al. Pentamidine-associated fatal acute pancreatitis. *JAMA* 1986;**256**:2383–5.

CHAPTER 53

Peritonitis and Intra-Abdominal Abscess

Judith R. Campbell

Intra-abdominal infection can be a life-threatening condition that occurs spontaneously or as a result of intra-abdominal disease, injury, or surgery. Given the compartmental anatomy and physiology of the abdominal cavity, intra-abdominal infection frequently is categorized as peritonitis, intraperitoneal abscess, retroperitoneal abscess, and visceral abscess.[3] In this chapter peritonitis and intra-abdominal abscess are reviewed; liver abscess, appendicitis and pelvic abscess, and retroperitoneal abscess, are reviewed in Chapters 49, 51, and 54, respectively.

PERITONITIS

ANATOMY

Knowledge of the anatomic relationships within the abdomen is important for understanding the source and routes of spread of infection. The peritoneal cavity extends from the undersurface of the diaphragm to the pelvis. In males it is a closed space, whereas in females the ends of the fallopian tubes penetrate into the peritoneal cavity. The transverse mesocolon and greater omentum separate the upper and lower peritoneal cavity. Peritoneal reflections divide the intraperitoneal space further into several compartments: the lesser sac, the paracolic gutters, and the subhepatic and subphrenic spaces (Fig. 53-1). The most dependent area of the peritoneal cavity is the pelvis. Exudate can extend to any of the recesses within the peritoneal cavity distant from the original source, however, and cause diffuse inflammation.[3] When inflamed, the anterior parietal peritoneum, which is supplied by somatic afferent nerves, gives the sensation of localized pain. Stimulation of the visceral peritoneum causes dull, poorly localized pain.

PATHOGENESIS

Peritonitis is defined as inflammation of the serosal lining of the abdominal cavity or the peritoneum and may be caused by any chemical or infectious agent that irritates the peritoneal surfaces. Noninfectious peritonitis is caused by extravasation of irritants, such as gastric juice, bile, urine, blood, pancreatic secretions, or the contents of a ruptured cyst, into the peritoneal cavity. Although chemical peritonitis generally is aseptic, it may be an important antecedent event to the development of infectious peritonitis.

After peritoneal contamination by bacteria has occurred, the first mechanism of host defense is lymphatic clearance. In experimental peritonitis, this clearance is so efficient that peritonitis and abscess formation occur only if adjuvant substances, such as hemoglobin or necrotic tissue, are present.[24,25,48] In the first hours after bacterial contamination occurs, local resident macrophages are the predominant phagocytic cells. The macrophages then are cleared by the lymphatic system. After bacterial proliferation occurs, polymorphonuclear leukocytes become more numerous in the peritoneal cavity, and inflammation ensues. These peritoneal defense mechanisms also have adverse effects. Fibrin is deposited, which potentially entraps bacteria into a sequestered environment. An increase in splanchnic blood flow causes exudation of fluid into the peritoneal space, further impairing host defenses by diluting important peritoneal opsonins.[24,25] These host responses serve as a means of containing infection, but they also may contribute to the formation of abscesses.

Infectious peritonitis is subdivided into primary and secondary peritonitis based on the pathophysiology of the infection. Peritonitis that is associated with peritoneal dialysis or the presence of a ventriculoperitoneal shunt is a unique form of peritonitis that also is reviewed in this chapter. The microbial causes of peritonitis vary with the underlying cause and are summarized in Table 53-1.

PRIMARY PERITONITIS

Primary, or spontaneous, bacterial peritonitis is a rare infection defined as bacterial peritonitis in the absence of intra-abdominal findings, such as intestinal perforation. The incidence of spontaneous peritonitis in children is unknown; however, in the early 20th century, 8 to 10 percent of abdominal emergencies requiring surgical intervention were due to spontaneous peritonitis.[17,73] Freij and colleagues[30] conducted a 22-year review of

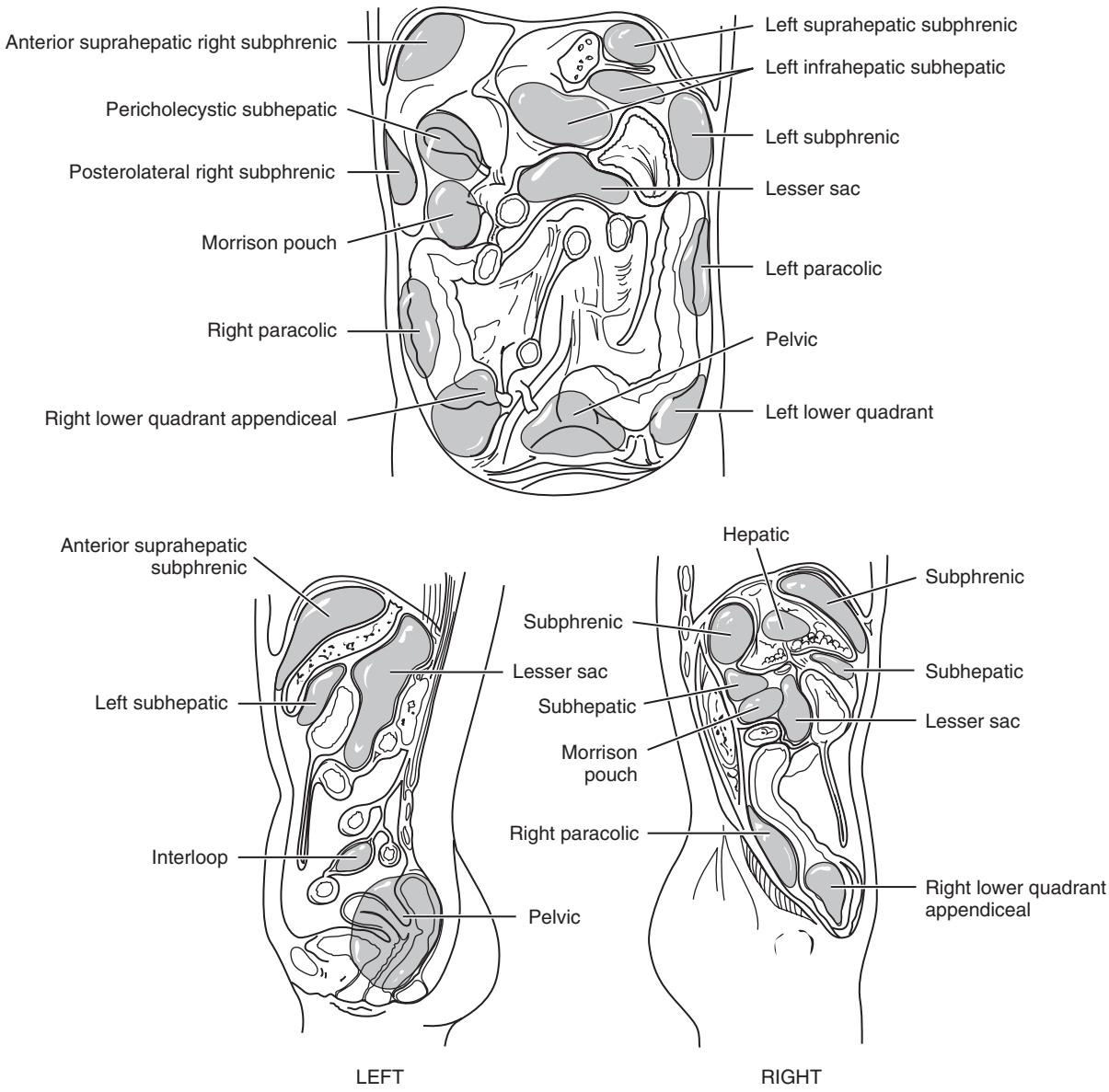

FIGURE 53-1 ■ Anterior and sagittal views of the peritoneal cavity. (From Altemeier WA, Culbertson WR, Fullen WD. Intra-abdominal sepsis. *Adv Surg* 1971;**5**:281–3.)

children with primary peritonitis in Dallas, Texas. Primary peritonitis was diagnosed in seven previously healthy children compared with 1840 cases of appendicitis during the same period. Currently, 1 to 2 percent of abdominal emergencies requiring surgical intervention are due to primary peritonitis.[39,42]

Now that this condition frequently is recognized clinically with the assistance of computed tomography (CT), the diagnosis often is made without exploratory laparotomy. The peak incidence of spontaneous peritonitis in children occurs when they are 5 to 9 years of age. In children, the most common predisposing factor is nephrotic syndrome, but this form of peritonitis also occurs in children with postnecrotic cirrhosis.* Spontaneous peritonitis rarely develops in previously healthy individuals without underlying conditions.[34,49]

The exact pathophysiologic mechanism for primary peritonitis is unknown; however, hematogenous inoculation is thought to be the most likely mechanism because the same organism frequently is recovered from cultures of blood and peritoneal fluid.[17,33,39] Alternative mechanisms include peritoneal seeding via the lymphatics, transmural migration through edematous bowel, and ascending infection from the female genitourinary tract.[33,39] In certain cases, impaired host defenses allow proliferation of bacteria that invade the peritoneal cavity, but a few children with primary peritonitis have no apparent impaired defense. Ascitic fluid from patients with nephrotic syndrome or cirrhosis contains lower levels of complement and immunoglobulin than does peritoneal fluid from a healthy host.[17,73,81] Deficiency of these important opsonins diminishes the natural clearance of organisms from the peritoneal cavity. Proliferation of organisms triggers the influx of phagocytes,

*References 3, 17, 33, 39, 43, 47, 73, 74.

TABLE 53-1 **Most Commonly Identified Etiologic Agents**

Primary Peritonitis	Secondary Peritonitis
Escherichia coli (25%-40%)	Aerobes
Haemophilus influenzae type b	*Enterobacter*
Klebsiella	*Enterococcus*
Mycobacterium bovis	*E. coli*
Mycobacterium tuberculosis	*Klebsiella*
Neisseria meningitidis	*Pseudomonas aeruginosa*
Other enteric gram-negative bacilli	*Proteus mirabilis*
Other streptococci (α-hemolytic and β-hemolytic)	*Serratia*
Staphylococcus aureus (2%-4%)	Anaerobes
Streptococcus pneumoniae (30%-50%)	*Bacteroides fragilis* group
	Peptostreptococcus
	Clostridium species
	Prevotella species
	Fusobacterium species
	Eubacterium species
CAPD-Associated Peritonitis	**VP Shunt–Associated Peritonitis**
Candida	Coagulase-negative staphylococci
Coagulase-negative staphylococci	*Enterobacter*
Enteric gram-negative bacilli	*E. coli*
Mycobacterium	*Klebsiella*
Other fungi	*Pseudomonas*
Pseudomonas	*S. aureus*
S. aureus	
Stenotrophomonas	

CAPD, continuous ambulatory peritoneal dialysis; *VP,* ventriculoperitoneal.

release of inflammatory mediators, and localized or diffuse peritoneal irritation that gives rise to symptoms of abdominal pain and fever.

Since the preantibiotic era, researchers have recognized that primary peritonitis frequently is caused by *Streptococcus pneumoniae*,[33] *Streptococcus pyogenes*,[34] and *Staphylococcus aureus.*[17] Rarely, primary peritonitis in prepubescent girls is caused by extension of upper genital tract *S. pneumoniae* infection.[39,68] Since the 1960s, the bacteriology of primary peritonitis has shifted to include an increased proportion of infections caused by gram-negative enteric organisms, such as *Escherichia coli* and *Klebsiella* spp.[17,39,43,74,81] In some instances, primary *E. coli* peritonitis may occur concurrently with bacteremic urinary tract infection.

Tuberculous peritonitis may be caused by *Mycobacterium tuberculosis* or *Mycobacterium bovis.* It may occur as a complication of primary mycobacteremia or be caused by reactivation of latent intra-abdominal infection within lymphoid tissue, but only rarely does it seem to occur as a function of the ingestion of swallowed organisms from a pulmonary primary focus.[36,37,67,78] Peritoneal infection with *M. bovis*, which is clinically similar to *M. tuberculosis* peritonitis, is acquired from unpasteurized dairy products and has been reported in children living along the border between the United States and Mexico. These organisms may cause peritonitis from either mycobacteremia or erosion of organisms through the mesenteric lymph nodes or bowel wall into the peritoneal cavity.[20] *Salmonella* spp. rarely cause primary peritonitis and have been reported primarily in patients with underlying conditions.[49]

SECONDARY PERITONITIS

Secondary peritonitis, the most common form of peritonitis, arises as a complication of intra-abdominal injury or disease when microorganisms, secretions, and the particulate material of an intra-abdominal organ enter the peritoneal cavity. Congenital or acquired conditions that result in ischemia, inflammation, or perforation of abdominal viscera may be complicated by secondary peritonitis.[23,48,70] In premature infants, necrotizing enterocolitis is the most common cause of secondary peritonitis.[53] In infants and children, appendicitis is the most common cause; however, it also may occur with volvulus, intussusception, incarcerated hernia, or rupture of a Meckel diverticulum.[53] Although less common in children than in adults, peritonitis also occurs as a complication of mucosal diseases, such as peptic ulcer, ulcerative colitis, Crohn disease, and pseudomembranous colitis.[53]

Rupture of or injury to an intra-abdominal viscus results in spillage of the luminal contents and contamination of the peritoneal cavity with bacteria, gastrointestinal secretions, and debris. Chemical and infectious sources of inflammation are introduced. The stomach and upper gastrointestinal tract contents contain only 10^3 to 10^4 or fewer organisms per gram because of the low pH of gastric secretions. Gram-negative aerobic organisms colonize the upper gastrointestinal tract. In contrast, the colonic contents have predominantly anaerobes, with 10^{11} anaerobes and 10^8 aerobes per gram.[11,23,39,66,70]

Secondary peritonitis usually is a polymicrobial infection, with 5 to 10 different bacterial species of anaerobes and facultative gram-negative bacilli. Synergy among the various bacterial species enhances bacterial proliferation.[11,70] Members of the *Bacteroides fragilis* group and *Peptostreptococcus* spp. are the anaerobic organisms reported most commonly in secondary peritonitis. Of the aerobic organisms, *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Enterococcus* spp. are isolated most often. More recently, *P. aeruginosa* has been noted to be present in 20 to 30 percent of children with complicated ruptured appendicitis.[8,23,40,70] When secondary peritonitis occurs in patients with a history of prolonged hospitalization, underlying chronic conditions, or recent antibiotic therapy, the etiology may include nosocomial pathogens that have colonized the gastrointestinal tract, such as *P. aeruginosa*, *Enterobacter* spp., *Acinetobacter* spp., or other antibiotic-resistant organisms.

Focal suppurative infection may be present within an intra-abdominal or retroperitoneal solid organ or within intra-abdominal lymphoid tissue. Organisms spread from this purulent focus through the capsule of the organ or lymphoid tissue and enter the peritoneal cavity, with the subsequent development of peritonitis. The intra-abdominal organ or lymphoid tissue may be inoculated

either via bacteremia (e.g., *S. aureus* and renal infection) or as a complication of the normal function of the organ (e.g., *E. coli* and renal infection or *Yersinia* and mesenteric adenitis).[13,38,44]

PERITONITIS AND IMPLANTED DEVICES

Peritonitis is the most significant infectious complication of long-term peritoneal dialysis. Contamination of the dialysis tubing, migration of skin flora from the exit site, or contamination of the dialysate may lead to peritonitis in patients undergoing continuous ambulatory peritoneal dialysis (CAPD). In each instance, a single pathogen usually is isolated.

Gram-positive organisms, coagulase-negative staphylococci, and *S. aureus* account for 30 to 45 percent of peritonitis episodes in children undergoing CAPD. Of CAPD-associated peritonitis episodes, 20 to 30 percent are caused by Enterobacteriaceae.[27] In these instances, contamination of the catheter site with fecal material most often occurs in young children who wear diapers and children with incontinence, an open urogenital sinus, or nephrostomy tubes. The waterborne pathogens *Pseudomonas* and *Acinetobacter* account for 6 percent and 4 percent, respectively, of peritonitis episodes in children receiving CAPD. *Pseudomonas* peritonitis is especially difficult to treat with the dialysis catheter in situ and may recur despite administration of appropriate antimicrobial therapy.[27]

Fungal peritonitis is another complication of CAPD that is difficult to treat successfully without removal of the catheter. Although fungal pathogens have accounted for only 2 percent of peritonitis episodes in children undergoing CAPD, this problem is occurring more commonly.* Most patients with fungal peritonitis have had previous episodes of bacterial peritonitis and antibiotic therapy. The most common fungal pathogens are *Candida* spp.[26,27]; however, rare fungi, such as *Curvularia* spp.,[15] *Fusarium* spp.,[27] *Trichosporon asahii*,[50] and *Aspergillus* spp.,[27] have been reported.[58,79] Other rare causes of CAPD-associated peritonitis include *Mycobacterium fortuitum* and *Mycobacterium chelonae*.[80]

Intra-abdominal infectious complications develop on average in less than 5 percent of infants and children who undergo ventriculoperitoneal shunt placement or revision for hydrocephalus.[57,65,71] Peritonitis, peritoneal pseudocyst, or perforation of the bowel by the abdominal catheter rarely occurs in children with such shunts.† Cerebrospinal fluid (CSF) in the peritoneal cavity may be seeded during transient bacteremia or a febrile illness or after abdominal trauma. In addition, peritonitis may develop as a complication of infection within the ventricles being drained[71] as organisms descend into the peritoneal cavity via the distal tubing.

A peritoneal pseudocyst containing CSF is the most common manifestation of peritoneal inflammation in patients with ventriculoperitoneal shunts. These patients often have a history of symptoms compatible with a shunt infection before the formation of a pseudocyst and may have signs of peritoneal inflammation and a palpable abdominal mass. The microbial etiology of ventriculoperitoneal shunt–associated peritonitis varies and reflects the pathogenesis of infection. Infections occurring within months of surgery often are caused by skin flora, *Staphylococcus epidermidis*, other coagulase-negative staphylococci, and *S. aureus*.[31,62] The microbiology of late shunt-associated peritonitis is similar to that of spontaneous bacterial peritonitis and may include gram-negative enteric organisms and gram-positive cocci.[62] Peritonitis caused by colonic flora also rarely has been associated with bowel perforation by the distal end of the ventriculoperitoneal shunt.[35,62,69]

CLINICAL MANIFESTATIONS

The initial signs and symptoms of primary bacterial peritonitis include nausea, vomiting, diarrhea, and diffuse abdominal pain.[39,42,43] These signs and symptoms are similar to those of secondary peritonitis caused by a ruptured appendix.

Rupture of the appendix is the most common cause of secondary peritonitis in children; the initial symptoms of anorexia, vomiting, and localized abdominal pain frequently precede the signs and symptoms of diffuse peritoneal inflammation. In primary and secondary peritonitis, patients typically lie very still because any movement exacerbates the abdominal pain. Physical findings include fever, tachycardia, abdominal distention, hypoactive bowel sounds, abdominal tenderness, rebound tenderness, abdominal wall rigidity, and tenderness on rectal or vaginal examination. Peritoneal inflammation is associated with an increase in splanchnic blood flow, capillary permeability, and a shift of fluid into the peritoneal space, which may lead to intravascular hypovolemia and shock, in addition to systemic absorption of endotoxin and bacteria.[48]

Fever and abdominal pain in any child undergoing peritoneal dialysis should be evaluated carefully. Turbid dialysate fluid raises the suspicion of CAPD-associated peritonitis. Similarly, symptomatic children with ventriculoperitoneal shunts should be evaluated for shunt-associated peritonitis.[63,65,71,83] In a retrospective report of 19 children with ventriculoperitoneal shunts and peritonitis, Reynolds and associates[63] noted that fever and abdominal pain were the most common symptoms in 14 of their patients. Stamos and colleagues[71] found that fever, lethargy, nausea, and vomiting were the most frequently reported symptoms in a review of 23 children with gram-negative infection of ventriculoperitoneal shunts.

Primary tuberculous peritonitis usually is gradual in onset and associated with weight loss, malaise, and night sweats.[36,37,39,67] The degree of tenderness is less than that present with acute pyogenic peritonitis and may be nonexistent. Palpation of the abdomen may reveal an extensive, irregular collection of masses, often described as "doughy," caused by widespread granulomatous inflammation.[36,39]

*References 15, 26, 27, 50, 58, 79.
†References 5, 31, 35, 62, 63, 65, 69, 75.

DIAGNOSIS

Laboratory findings in a child with peritonitis often are nonspecific. The peripheral white blood cell count usually is elevated (16,000 to ≥25,000 cells/mm^3), with a predominance of polymorphonuclear leukocytes and an increase in immature forms.[39] The hematocrit may be elevated because of dehydration and hemoconcentration. Mild pyuria is noted occasionally because of irritation of the urinary bladder or ureters.

Diagnostic imaging studies can be useful in evaluating intra-abdominal infections. Upright and lateral decubitus radiographs of the abdomen may show distended adynamic loops of bowel suggestive of ileus and obliteration of the peritoneal fat lines and psoas shadows. Free intraperitoneal air below the diaphragm indicates a ruptured viscus. The presence of a fecalith or right lower quadrant mass may be consistent with appendicitis. Abdominal ultrasonography and CT may reveal an underlying cause of the peritonitis.[49,53]

Analysis of peritoneal fluid aspirate or lavage material may be helpful in differentiating primary from secondary peritonitis. Free air, blood, or bile indicates peritonitis secondary to intestinal perforation. In peritonitis, the leukocyte count in peritoneal fluid usually is greater than 250 to 300 white blood cells/mm^3 and sometimes 3000 to 5000 white blood cells/mm^3, with granulocytes predominating in 80 percent of cases.[39,47,70,73] A total protein content greater than 1 g/dL, a glucose level less than 50 mg/dL, or an elevated lactate dehydrogenase concentration (>25 mg/dL) is consistent with secondary peritonitis.[17,33,39,73]

If a Gram stain of peritoneal fluid shows only gram-positive cocci, primary peritonitis is most likely. The presence of gram-negative bacilli is consistent with primary or secondary peritonitis, but the presence of many different organisms on Gram stain is diagnostic of secondary peritonitis. Bacteremia occurs in 75 percent of patients with primary peritonitis. Specimens of peritoneal fluid and blood should be sent for culture.[39] Similarly, secondary peritonitis also can be associated with bacteremia, suggesting the need for obtaining cultures of blood in addition to peritoneal fluid. Specimens of peritoneal fluid should be processed to optimize the recovery of aerobic and anaerobic organisms, and the use of specific transport tubes or an airless, capped syringe is required.[11,39,70] The wide variety of pathogens isolated from intra-abdominal infections along with the variable antibiotic susceptibility of these pathogens supports taking an aggressive approach to obtaining samples for microbiologic evaluation.

A child undergoing CAPD who is suspected of having peritonitis should have dialysate sent for cell count, Gram stain, and culture for bacterial, mycobacterial, and fungal pathogens. If a child with a ventriculoperitoneal shunt is suspected of having peritonitis, CSF from the proximal portion of the shunt should be sent for culture, cell count, and determination of glucose and protein levels in addition to Gram stain and culture of peritoneal fluid.[31] Abdominal imaging by ultrasonography or CT is useful in identifying a peritoneal pseudocyst and the location of distal tubing.

DIFFERENTIAL DIAGNOSIS

Other infectious diseases that may mimic primary or secondary bacterial peritonitis include mesenteric adenitis, gastroenteritis, hepatitis, streptococcal pharyngitis, lower lobe pneumonia, pyelonephritis, and pelvic inflammatory disease. Noninfectious diseases to be considered in the differential diagnosis are pancreatitis, diabetic ketoacidosis, Henoch-Schönlein purpura, ovarian torsion, sickle-cell pain crisis, and lead poisoning.[53]

TREATMENT

Optimal management of peritonitis involves prompt and aggressive physiologic support, surgical consultation, and antimicrobial therapy. Correction of fluid and electrolyte imbalances and hemodynamic stabilization should be initiated as soon as the diagnosis of peritonitis is suspected. Spontaneous bacterial peritonitis usually is managed medically, unless the diagnosis is uncertain, in which case exploratory laparotomy or laparoscopy is performed. Before resistant strains of *S. pneumoniae* emerged, primary peritonitis in children was treated with aqueous penicillin G.[17,33] Given the increased prevalence of *S. pneumoniae* with reduced susceptibility to penicillin, third-generation cephalosporins such as cefotaxime or ceftriaxone are recommended until susceptibility results are available.[47,74] If primary peritonitis is caused by gram-negative organisms, appropriate empirical therapy includes cefotaxime or ceftriaxone, with or without an aminoglycoside, a carbapenem, ticarcillin-clavulanate, or piperacillin-tazobactam, pending completion of culture and susceptibility testing.

Patients with secondary peritonitis may require either immediate surgery to control the source of contamination and to remove necrotic tissue, blood, and intestinal contents from the peritoneal cavity or a drainage procedure if a limited number of large abscesses can be shown.[45,49,54,70,82] In cases of phlegmon, or extensive inflammatory edema, surgery usually is not performed acutely because of the child's unstable metabolic state and friable intra-abdominal tissues. Surgery is delayed for several hours or weeks to allow the inflammation to resolve. Surgery also may be postponed indefinitely.[6,14,59,77]

Empirical antimicrobial therapy for secondary peritonitis should have activity against anaerobes, especially the *B. fragilis* group, and enteric gram-negative aerobes.[7] Although controversial, some regimens also include an antibiotic effective against enterococci. The gold standard for antimicrobial therapy historically has been clindamycin or metronidazole, gentamicin, and ampicillin.* Alternative efficacious regimens, as single or combination therapy, include aztreonam, cefotaxime, cefoxitin, imipenem-cilastatin, meropenem, piperacillin-tazobactam, and ticarcillin-clavulanate.† Rates of resistance to cefoxitin and clindamycin among the *B. fragilis* group have increased and are reported to be 49 percent;

*References 7, 11, 28, 45, 48, 54, 64, 70.
†References 7, 9, 32, 45, 48, 51, 54, 76, 82.

therefore, metronidazole is now recommended and in some institutions alternative regimens are used routinely.[2,48,70] Therapy for secondary peritonitis that is a health care–associated infection should be selected based on local antimicrobial susceptibility patterns at that institution.[70]

Other studies have examined the use of a single broad-spectrum antibiotic, which allows a portion of the therapy to be delivered less expensively on an outpatient basis. Fishman and coworkers[28] prospectively evaluated the clinical outcomes of 150 children with perforated appendicitis treated postoperatively with a 10-day course of piperacillin-tazobactam. They compared the outcome with that of historical controls treated with a 10-day course of ampicillin, gentamicin, and clindamycin. Rates of postoperative infectious complications were similar in both groups. Bradley and colleagues[8] prospectively identified 87 children with complicated appendicitis in five pediatric centers, also comparing costs and outcomes with historical controls. Although inpatient treatment courses were reduced by an average of 42 percent in meropenem-treated children, outcome measures were equivalent to those of historical controls.

Table 53-2 summarizes randomized trials of monotherapy versus combination therapy for ruptured appendicitis in children. Although no differences in outcome were observed, the potential of emerging resistance to broad-spectrum agents versus the convenience of monotherapy must be considered and balanced against the possible decreased risk for developing nosocomial infection among children who can receive a substantial component of parenteral therapy in the home.[40] Goldin and colleagues[32] retrospectively compared the use of triple antibiotic therapy versus monotherapy in a cohort of children with perforated appendicitis and found that single-agent antibiotic therapy in the treatment of perforated appendicitis was at least equal in efficacy to the traditional aminoglycoside-based combination therapy. The potential benefits of a single-agent regimen are reduced length of stay and pharmacy and hospital charges.

Empirical antibiotic treatment of CAPD-associated peritonitis should be effective against gram-positive and gram-negative organisms until culture results are available. Intraperitoneal antibiotics, with or without concomitant intravenous antibiotics, achieve adequate serum and dialysate concentrations. Vancomycin is used for empirical therapy for gram-positive infections, but if staphylococcal organisms are susceptible to β-lactam agents, treatment with cefazolin is effective.[27] Aminoglycosides (gentamicin or tobramycin) or cephalosporins are used for gram-negative infections; however, because most intraperitoneal antibiotics are absorbed into the systemic circulation, serum aminoglycoside or vancomycin concentrations should be monitored for possible toxicity. Therapy for fungal peritonitis usually is intravenous amphotericin B, although successful use of fluconazole or intraperitoneal amphotericin B has been reported.[27,58] Indications for removal of a dialysis catheter include persistent infection with *S. aureus* or *Pseudomonas*, tunnel infection, or fungal peritonitis.[27]

Treatment of peritonitis associated with ventriculoperitoneal shunts usually requires externalization of the distal end of the catheter in addition to institution of antibiotic therapy.[31] Empirical antibiotic therapy should include an antistaphylococcal agent active against coagulase-positive and coagulase-negative staphylococci. Coagulase-negative staphylococci are a common cause of ventriculoperitoneal shunt infection; vancomycin should be administered pending culture and susceptibility results. If Gram stain of ventricular CSF or peritoneal fluid reveals gram-negative organisms, cefotaxime, ceftriaxone, ceftazidime, or meropenem should be added.[5,63]

The duration of antibiotic therapy for peritonitis should be dictated by the clinical course of the patient because no single regimen or treatment course is accepted universally.[39,70] Indicators of sufficient therapy include resolution of fever and abdominal pain and return of the leukocyte and differential counts to normal.[39,54,70,72] Primary peritonitis caused by streptococci is treated successfully with a 10- to 14-day course of antibiotics.[17,39] Primary peritonitis with gram-negative organisms may require 10 days to 3 weeks of antibiotic treatment.[17] The duration of therapy for secondary peritonitis after adequate surgery usually is 5 to 10 days, but it depends on the clinical response to therapy.[39,54,70,72] Short-course

TABLE 53-2 **Monotherapy versus Combination Therapy for Ruptured Appendicitis in Children**

			No. Patients		*Complications**	
Study	**Monotherapy (A)**	**Combination Therapy (B)**	**A**	**B**	**A (%)**	**B (%)**
Meller et al.[51]	Cefoxitin	Clindamycin/gentamicin	29	27	1 (3)	4 (15)
Dougherty et al.[22]	Ticarcillin-clavulanate	Clindamycin/gentamicin ± ampicillin	79	45	14 (18)	5 (11)
Uhari et al.[76]	Imipenem-cilastatin	Metronidazole/tobramycin	9	10	2 (22)	1 (10)
Collins et al.[19]	Ampicillin-sulbactam ± aminoglycoside	Ampicillin/clindamycin ± aminoglycoside	75	39	2 (1)	1 (3)
Fishman et al.,[28] Lund and Murphy[45]	Pipercillin-tazobactam	Ampicillin/gentamicin/clindamycin	150	373	14 (9)	24 (6)
Bradley et al.[9]	Meropenem	Cefotaxime ± amikacin or tobramycin, clindamycin or metronidazole	22	13	2 (9)	1 (8)

*Wound infections, intra-abdominal abscess, or rehospitalization.
Modified from Kaplan SL. Antibiotic usage in appendicitis in children. *Pediatr Infect Dis J* 1998;**17**:1047–8.

therapy for 5 days has been shown to be efficacious in some patients,[39,54] but longer courses are required if fever persists or abdominal signs and symptoms are present. Standard therapy for tuberculous peritonitis consists of a minimum of two antituberculous drugs. As with other forms of extrapulmonary tuberculosis in children, empirical therapy with isoniazid, rifampin, and pyrazinamide is advised pending culture and susceptibility results. Although *M. bovis* is resistant to pyrazinamide, most strains are susceptible to isoniazid, rifampin, and ethambutol.

COMPLICATIONS

Acute complications associated with peritonitis include septic shock, adult respiratory distress syndrome, septic thrombophlebitis of the portal vein, acute renal failure, and multiple-organ system failure.[48] Postoperative complications include wound infection, adhesions, bowel obstruction, formation of a fistula, and formation of an intra-abdominal or retroperitoneal abscess.

Recurrent peritonitis (tertiary peritonitis) is an entity described as occurring late in the course of therapy for secondary peritonitis.[39,46,70,82] Patients with this condition continue to have symptoms despite receiving appropriate antimicrobial therapy, and peritoneal fluid reveals persistent inflammation. Bacterial cultures often are negative or may yield an organism of low virulence. Multiple-organ system failure and a poor outcome frequently are associated with tertiary peritonitis. The mechanism of ongoing peritoneal inflammation is unknown; however, some investigators have proposed that immunoregulatory dysfunction and poor nutrition are contributing factors.

INTRA-ABDOMINAL ABSCESS

Intra-abdominal abscesses often are categorized as intraperitoneal, visceral, or retroperitoneal (see Chapter 54).[4,10] In children, intraperitoneal abscesses are most common. The most common underlying conditions associated with an intra-abdominal abscess in children are appendicitis and trauma.[10,53] Reviews of gangrenous or perforated appendicitis in children indicate that 2 to 20 percent of cases are complicated by the formation of an abscess.[54,64]

Two basic mechanisms exist for the development of an intraperitoneal abscess. In the first mechanism, diffuse peritonitis may cause loculations of purulent material to form in the areas anatomically most dependent—typically the pelvic, subphrenic, and paracolic regions (see Fig. 53-1). The second mode of formation of an abscess involves a localized focus related to contiguous disease or injury in which host defenses and the inflammatory response prevent diffuse spread and peritonitis.[4] The microbiology of intraperitoneal abscesses is polymicrobial and reflects that of the intestinal flora. In a review of intra-abdominal abscess in 36 children, Brook[10,11] noted that the predominant organisms were the *B. fragilis* group, *Peptostreptococcus*, *E. coli*, and other Enterobacteriaceae.

The most common sites of visceral abscess in children are the liver (see Chapter 49), pancreas, and spleen. Underlying conditions that may lead to the development of a pancreatic abscess include pancreatic injury, pancreatitis, and biliary obstruction. Pancreatitis or surgical or accidental injury to the pancreas causes the release of pancreatic enzymes and focal necrosis.[29] Reflux of contaminated bile into the pancreatic duct is hypothesized to be the mechanism by which enteric organisms gain access to the injured pancreas and proliferate. A pancreatic abscess usually is a polymicrobial infection caused by aerobic (*E. coli*, *Klebsiella pneumoniae*, group D streptococci) or anaerobic (peptostreptococci, *B. fragilis* group) organisms that inhabit the gastrointestinal tract. Rare instances of *S. aureus* pancreatic abscess occur as a result of bacteremia.[12,13]

Splenic abscesses are unusual findings in infants and children. Before the 1970s, most reports involved solitary pyogenic abscesses. Since then, the number of reports of multiple splenic abscesses has increased.[16,41,60] Splenic abscess usually is associated with one of five underlying conditions: endocarditis, injury, hemoglobinopathy, immunodeficiency, or adjacent infection. Given the filtering function of the spleen, an abscess can form as a result of any metastatic hematogenous infection, such as endocarditis. Although rare, splenic abscess can be a delayed complication of the nonoperative management of splenic injuries. Splenic infarcts associated with hemoglobinopathies such as sickle-cell disease may become secondarily infected and form an abscess.[16] Immunodeficiency, such as malignancy or acquired immunodeficiency syndrome, is another significant risk factor for the development of multiple splenic abscesses.[41,55,60]

Rarely, infection or disease in a contiguous focus may extend to the spleen. In a review of 56 children with splenic abscesses, 7 (12.5%) were cryptogenic with no apparent cause.[41] In most instances, a single pathogen is isolated, with *S. aureus*, streptococci, *E. coli*, and *Salmonella* spp. being the most common. Fungi, most often *Candida* spp., have been isolated from splenic abscesses primarily in immunocompromised hosts.[41,55,60]

CLINICAL MANIFESTATIONS

The typical clinical features of an intra-abdominal abscess include fever, abdominal pain, and tenderness over the involved area. Subphrenic abscesses also may be

manifested as referred pain or pulmonary or pleuritic symptoms. Pancreatic abscess may be associated with a palpable epigastric mass and elevated serum lipase and amylase.[12] Splenomegaly or a splenic mass may be noted in approximately half of patients with splenic abscesses.[41] In postoperative patients, persistence of abdominal symptoms or fever warrants evaluation for an intraperitoneal abscess.[3] Leukocytosis (20,000 to 50,000 cells/mm^3) frequently is present in children with an intra-abdominal abscess.[3,39]

DIAGNOSIS

Imaging studies are helpful in diagnosing an intra-abdominal abscess. Plain radiographs are useful as an initial procedure and may show an extraintestinal air–fluid level, right lower quadrant mass, or localized ileus.[1] Chest radiographs should be obtained because subphrenic abscesses often are associated with a pleural effusion. In a series of 27 children with splenic abscesses, chest radiographs were abnormal in 20 cases, with the most common findings being left pleural effusion and an elevated left hemidiaphragm.[41] Ultrasonography is a useful noninvasive technique that can detect abdominal and pelvic abscesses. The quality of the images depends on the examiner, however. In addition, conditions such as ileus, postoperative drains, or dressings may hinder ultrasound detection of an abscess.[1,49,52] CT is the most sensitive tool for detecting an intra-abdominal abscess, and it provides good anatomic resolution. Disadvantages of CT are the radiation and, if used, exposure to intravenous, oral, or rectal contrast material.[1,49,52]

Although more recent experience with the use of magnetic resonance imaging for detecting an intraperitoneal abscess has been described in the literature,[56] this modality should be considered in children only when an abscess may not be detected more easily by other methods or in patients who should not have exposure to radiation.[56] Gallium scanning is a sensitive technique for diagnosing an abscess, but it is nonspecific, particularly in the abdomen.[1]

TREATMENT

Management of an intra-abdominal abscess includes physiologic and nutritional support, antimicrobial therapy, and drainage. After blood cultures have been obtained, empirical antibiotic therapy should be instituted with agents effective against anaerobes, Enterobacteriaceae, and other enteric flora as discussed earlier for peritonitis. Antibiotic therapy usually is begun before surgery is done to minimize any complications of bacteremia during the procedure. Abscess material should be obtained for culture of aerobic, anaerobic, fungal, and mycobacterial pathogens. Effective surgical management depends on accurate localization of the abscess, discrimination between single and multiple abscesses, and early and adequate drainage.[3,4,48] Traditional therapy for intraperitoneal abscesses has relied on open surgical drainage, although drainage of intraperitoneal abscesses percutaneously under ultrasound or CT guidance now is used often.*

In instances of multiple intraperitoneal abscesses or if the source of peritoneal contamination has not been controlled, laparotomy is indicated.[48] Pancreatic abscesses require intensive surgical and medical therapy. Antimicrobial therapy for mixed aerobic and anaerobic infection is suggested,[12] but splenectomy remains the definitive treatment of bacterial splenic abscesses. In selected patients, percutaneous drainage or splenotomy has the advantage of preserving splenic function, however.[41,55,60] Multiple small splenic abscesses and fungal lesions generally are treated medically.[41] Antibiotic therapy for a pyogenic splenic abscess should be guided by the pathogens associated with the child's underlying condition. Therapy should include antibiotics effective against *S. aureus*, streptococci, and gram-negative enteric bacilli. Specific therapy should be revised after culture and susceptibility results are available.

COMPLICATIONS

Intraperitoneal and visceral abscesses, if not adequately drained, may be associated with significant complications, including ongoing spread of the infectious process and, for splenic or pancreatic abscesses, a high mortality rate. Fistula formation, adhesions, and bowel obstruction may be late complications of intra-abdominal infection.

NEW REFERENCES SINCE THE SIXTH EDITION

23. Dumont R, Cinotti R, Lejus C, et al. The microbiology of community-acquired peritonitis in children. *Pediatr Infect Dis J* 2011;**30**:131–5.
70. Solomkin JS, Muzuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Surg Infect* 2010;**11**:79–109.

The full reference list for this chapter is available at expertconsult.com.

*References 1, 18, 21, 48, 52, 61.

REFERENCES

1. Afshani E. Computed tomography of abdominal abscesses in children. *Radiol Clin North Am* 1981;**19**:515–26.
2. Aldridge KE, Ashcraft D, Cambre K, et al. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Peptostreptococcus* species. *Antimicrob Agents Chemother* 2001;**45**:1238–43.
3. Altemeier WA, Culbertson WR, Fuller WD. Intra-abdominal sepsis. *Adv Surg* 1971;**5**:281–333.
4. Altemeier WA, Culbertson WR, Fuller WD, et al. Intra-abdominal abscesses. *Am J Surg* 1973;**125**:70–9.
5. Baird C, O'Connor D, Pittman T. Late shunt infections. *Pediatr Neurosurg* 1999;**31**:269–73.
6. Blakely ML, Spurbeck WW, Lobe TE. Current status of laparoscopic appendectomy in children. *Semin Pediatr Surg* 1998;**7**: 225–7.
7. Bohnen JM, Solomkin JS, Dellinger EP, et al. Guidelines for clinical care: anti-infective agents for intra-abdominal infection. A Surgical Infection Society policy statement. *Arch Surg* 1992; **127**:83–9.
8. Bradley JS, Behrendt CE, Arrieta AC, et al. Convalescent phase outpatient parenteral antiinfective therapy for children with complicated appendicitis. *Pediatr Infect Dis J* 2001;**20**:19–24.
9. Bradley JS, Faulkner KL, Klugman KP. Efficacy, safety and tolerability of meropenem as empiric antibiotic therapy in hospitalized pediatric patients. *Pediatr Infect Dis J* 1996;**15**:749–57.
10. Brook I. Intra-abdominal abscess in children: a 13 year experience. *Hosp Pract* 1990;**25**:20–3.
11. Brook I. Intra-abdominal infections in children: pathogenesis, diagnosis and management. *Drugs* 1993;**46**:53–62.
12. Brook I, Frazier EH. Microbiological analysis of pancreatic abscess. *Clin Infect Dis* 1996;**22**:384–5.
13. Brook I, Frazier EH. Aerobic and anaerobic microbiology of retroperitoneal abscesses. *Clin Infect Dis* 1998;**26**:938–41.
14. Bufo AJ, Shah RS, Li MH, et al. Interval appendectomy for perforated appendicitis in children. *J Laparoendosc Adv Surg Tech A* 1998;**8**:209–14.
15. Canon HL, Buckingham SC, Wyatt RJ, et al. Fungal peritonitis caused by *Curvularia* species in a child undergoing peritoneal dialysis. *Pediatr Nephrol* 2001;**16**:35–7.
16. Chun CH, Raff MJ, Contreras L, et al. Splenic abscess. *Medicine* 1980;**59**:50–65.
17. Clark JH, Fitzgerald JF, Kleiman MB. Spontaneous bacterial peritonitis. *J Pediatr* 1984;**104**:495–500.
18. Clark RA, Towbin R. Abscess drainage with CT and ultrasound guidance. *Radiol Clin North Am* 1983;**21**:445–59.
19. Collins MD, Dajani AS, Kim KS, et al. Comparison of ampicillin/sulbactam plus aminoglycoside vs. ampicillin plus clindamycin plus aminoglycoside in the treatment of intraabdominal infections in children. *Pediatr Infect Dis J* 1998;**17**(Suppl.):15–8.
20. Dankner WM, Davis CE. *Mycobacterium bovis* as a significant cause of tuberculosis in children residing along the United States–Mexico border in the Baja California region. *Pediatrics* 2000;**105**:E79.
21. Diament MJ, Stanley P, Kangarloo H, Donaldson JS. Percutaneous aspiration and catheter drainage of abscesses. *J Pediatr* 1986;**108**: 204–8.
22. Dougherty SH, Sirinek KR, Schauer PR, et al. Ticarcillin/clavulanate compared with clindamycin/gentamicin (with or without ampicillin) for the treatment of intra-abdominal infections in pediatric and adult patients. *Am Surg* 1995;**61**:297–303.
23. Dumont R, Cinotti R, Lejus C, et al. The microbiology of community-acquired peritonitis in children. *Pediatr Infect Dis J* 2011;**30**:131–5.
24. Dunn DL, Barke RA, Ahrenholz DH, et al. The adjuvant effect of peritoneal fluid in experimental peritonitis: mechanism and clinical implications. *Ann Surg* 1984;**199**:37–43.
25. Dunn DL, Barke RA, Knight NB, et al. Role of resident macrophages, peripheral neutrophils, and translymphatic absorption in bacterial clearance from the peritoneal cavity. *Infect Immun* 1985;**49**:257–64.
26. Enriquez JL, Kalia A, Travis LB. Fungal peritonitis in children on peritoneal dialysis. *J Pediatr* 1990;**117**:830–2.
27. Feinstein EI, Chesney RW, Zelikovic I. Peritonitis in childhood renal disease. *Am J Nephrol* 1988;**8**:147–65.
28. Fishman SJ, Pelosi L, Klavon SL, et al. Perforated appendicitis: prospective outcome analysis for 150 children. *J Pediatr Surg* 2000;**35**:923–6.
29. Ford EG, Hardin WD, Mahour GH, et al. Pseudocysts of the pancreas in children. *Am Surg* 1990;**56**:384–7.
30. Freij BJ, Votteler TP, McCracken GH. Primary peritonitis in previously healthy children. *Am J Dis Child* 1984;**138**: 1058–61.
31. Gaskill SJ, Marlin AE. Spontaneous bacterial peritonitis in patients with ventriculoperitoneal shunts. *Pediatr Neurosurg* 1997;**26**: 115–9.
32. Goldin AB, Sawin RS, Garrison MM, et al. Aminoglycoside-based triple-antibiotic therapy versus monotherapy for children with ruptured appendicitis. *Pediatrics* 2007;**119**:905–11.
33. Gorensek MJ, Lebel MH, Nelson JD. Peritonitis in children with nephrotic syndrome. *Pediatrics* 1988;**81**:849–56.
34. Graham JC, Moss PJ, McKendrick MW. Primary group A streptococcal peritonitis. *Scand J Infect Dis* 1995;**27**:171–2.
35. Grosfeld JL, Cooney DR, Smith J, et al. Intra-abdominal complications following ventriculoperitoneal shunt procedures. *Pediatrics* 1974;**54**:791–6.
36. Gurkan F, Ozates M, Bosnak M, et al. Tuberculous peritonitis in 11 children: clinical features and diagnostic approach. *Pediatr Int* 1999;**41**:510–3.
37. Jakubowski A, Elwood RK, Enarson DA. Clinical features of abdominal tuberculosis. *J Infect Dis* 1988;**158**:687–92.
38. Jelloul L, Fremond B, Dyon JF, et al. Mesenteric adenitis caused by *Yersinia pseudotuberculosis* presenting as an abdominal mass. *Eur J Pediatr Surg* 1997;**7**:180–3.
39. Johnson CC, Baldessarre J, Levison ME. Peritonitis: update on pathophysiology, clinical manifestations, and management. *Clin Infect Dis* 1997;**24**:1035–47.
40. Kaplan SL. Antibiotic usage in appendicitis in children. *Pediatr Infect Dis J* 1998;**17**:1047–8.
41. Keidl CM, Chusid MJ. Splenic abscesses in childhood. *Pediatr Infect Dis J* 1989;**8**:368–73.
42. Kimber CP, Hutson JM. Primary peritonitis in children. *Aust N Z J Surg* 1996;**66**:169–70.
43. Krensky AM, Ingelfinger JR, Grupe WE. Peritonitis in childhood nephrotic syndrome. *Am J Dis Child* 1982;**136**:732–6.
44. Lamps LW, Madhusukhan KT, Greenson JK, et al. The role of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in granulomatous appendicitis: a histologic and molecular study. *Am J Surg Pathol* 2001;**25**:508–15.
45. Lund DP, Murphy EU. Management of perforated appendicitis in children: a decade of aggressive treatment. *J Pediatr Surg* 1994; **29**:1130–4.
46. Malangoni MA. Evaluation and management of tertiary peritonitis. *Am Surg* 2000;**66**:157–61.
47. Markenson DS, Levine D, Schacht R. Primary peritonitis as a presenting feature of nephrotic syndrome: a case report and review of the literature. *Pediatr Emerg Care* 1999;**15**:407–9.
48. McClean KL, Sheehan GJ, Harding GKM. Intraabdominal infection: a review. *Clin Infect Dis* 1994;**19**:100–16.
49. McConkey SJ, McCarthy ND, Keane CT. Primary peritonitis due to nonenteric salmonellae. *Clin Infect Dis* 1999;**29**:211–2.
50. Melez KA, Cherry J, Sanchez C, et al. Successful outpatient treatment of *Trichosporon beigelii* peritonitis with oral fluconazole. *Pediatr Infect Dis J* 1995;**14**:1110–3.
51. Meller JL, Reyes HM, Loeff DS, et al. One drug versus two-drug antibiotic therapy in pediatric perforated appendicitis: a prospective randomized study. *Surgery* 1991;**110**:764–8.
52. Montgomery RS, Wilson SE. Intraabdominal abscesses: image-guided diagnosis and therapy. *Clin Infect Dis* 1996;**23**:28–36.
53. Neblett WW, Pietsch JB, Holcomb Jr GW. Acute abdominal conditions in children and adolescents. *Surg Clin North Am* 1988;**68**:415–30.
54. Neilson IR, Laberge JM, Nguyen LT, et al. Appendicitis in children: current therapeutic recommendations. *J Pediatr Surg* 1990;**25**:1113–6.
55. Nelken N, Ignatius J, Skinner M, et al. Changing clinical spectrum of splenic abscess: a multicenter study and review of the literature. *Am J Surg* 1987;**154**:27–34.
56. Noone TC, Semelka RC, Worawattanakul S, et al. Intraperitoneal abscesses: diagnostic accuracy of and appearances at MR imaging. *Radiology* 1998;**208**:525–8.

57. Odio C, McCracken GH, Nelson JD. CSF shunt infections in pediatrics: a seven-year experience. *Am J Dis Child* 1984;**138**: 1103–8.
58. Oh SH, Conley SB, Rose GM, et al. Fungal peritonitis in children undergoing peritoneal dialysis. *Pediatr Infect Dis J* 1985;**4**:62–6.
59. Olika D, Yamini D, Udani VM, et al. Nonoperative management of perforated appendicitis without periappendiceal mass. *Am J Surg* 2000;**179**:177–81.
60. Phillips GS, Radosevich MD, Lipsett PA. Splenic abscess: another look at an old disease. *Arch Surg* 1997;**132**:1331–6.
61. Ramakrishnan MR, Sarathy TKP. Percutaneous drainage of splenic abscess: case report and review of literature. *Pediatrics* 1987;**79**:1029–31.
62. Rekate HL, Yonas H, White RJ, et al. The acute abdomen in patients with ventriculoperitoneal shunts. *Surg Neurol* 1979;**11**: 442–5.
63. Reynolds M, Sherman JO, Mclone DG. Ventriculoperitoneal shunt infection masquerading as an acute surgical abdomen. *J Pediatr Surg* 1983;**18**:951–5.
64. Schwartz MZ, Tapper D, Solenberger RI. Management of perforated appendicitis in children: the controversy continues. *Ann Surg* 1983;**197**:407–11.
65. Sells CJ, Loeser JD. Peritonitis following perforation of the bowel: a rare complication of a ventriculoperitoneal shunt. *J Pediatr* 1973;**83**:823–4.
66. Simon GL, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 1984;**86**:174–93.
67. Sioson PB, Stechenberg BW, Courtney R, et al. Tuberculous peritonitis in a three-year-old boy: case report and review of the literature. *Pediatr Infect Dis J* 1992;**11**:409–11.
68. Sirotnak AP, Eppes SC, Klein JD. Tuboovarian abscess and peritonitis caused by *Streptococcus pneumoniae* serotype 1 in young girls. *Clin Infect Dis* 1996;**22**:993–6.
69. Snow RB, Lavyne MH, Fraser RAR. Colonic perforation by ventriculoperitoneal shunts. *Surg Neurol* 1986;**25**:173–7.
70. Solomkin JS, Muzuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Surg Infect* 2010;**11**: 79–109.
71. Stamos JK, Kaufman BA, Yogev R. Ventriculoperitoneal shunt infections with gram-negative bacteria. *Neurosurgery* 1993;**33**: 858–62.
72. Stone HH, Bourneuf AA, Stinson LD. Reliability of criteria for predicting persistent or recurrent sepsis. *Arch Surg* 1985;**120**: 17–20.
73. Such J, Runyon BA. Spontaneous bacterial peritonitis. *Clin Infect Dis* 1998;**27**:669–76.
74. Tain YL, Lin GJ, Cher TW. Microbiological spectrum of septicemia and peritonitis in nephrotic children. *Pediatr Nephrol* 1999;**13**:835–7.
75. Tchirkow G, Verhagen AD. Bacterial peritonitis in patients with ventriculoperitoneal shunt. *J Pediatr Surg* 1979;**14**:182–4.
76. Uhari M, Seppanen J, Heikkinen E. Imipenem-cilastatin vs. tobramycin and metronidazole for appendicitis-related infections. *Pediatr Infect Dis J* 1992;**11**:445–50.
77. Vargas HI, Averbook A, Stamos MJ. Appendiceal mass: conservative therapy followed by interval laparoscopic appendectomy. *Am Surg* 1994;**60**:753–8.
78. Veeragandham RS, Lynch FP, Canty TG, et al. Abdominal tuberculosis in children: review of 26 cases. *J Pediatr Surg* 1996;**31**: 170–6.
79. Warady BA, Bashir M, Donaldson LA. Fungal peritonitis in children receiving peritoneal dialysis: a report of the NAPRTCS. *Kidney Int* 2000;**58**:384–9.
80. White R, Abreo K, Flanagan R, et al. Nontuberculous mycobacterial infections in continuous ambulatory peritoneal dialysis patients. *Am J Kidney Dis* 1993;**22**:581–7.
81. Wilfert CM, Katz SL. Etiology of bacterial sepsis in nephrotic children 1963-1967. *Pediatrics* 1968;**42**:840–3.
82. Wittmann DH, Schein M, Condon RE. Management of secondary peritonitis. *Ann Surg* 1996;**224**:10–8.
83. Younger JJ, Simmons JCH, Barrett FF. Occult distal ventriculoperitoneal shunt infections. *Pediatr Infect Dis J* 1985;**4**:557–8.

CHAPTER 54

Retroperitoneal Infections

Alice Pong • John S. Bradley

Retroperitoneal infections consist primarily of suppurative bacterial infections that originate within the retroperitoneal structures or as an extension from another primary site with systemic spread. In children, these infections are much less common than are intra-abdominal infections; however, they can lead to significant morbidity if missed. Making a diagnosis can be difficult because symptoms often are indolent and poorly localized.

Several retroperitoneal spaces exist; each may harbor a localized infection.

The retroperitoneal structures are separated from the intra-abdominal organs by the posterior peritoneal fascia (Fig. 54-1). Structures posterior to this fascia layer, in the anterior retroperitoneal space, include the duodenum, pancreas, and parts of the colon. The kidneys and ureters are encased further by the renal fascia. The iliopsoas and psoas muscles lie at the posterior aspect of the retroperitoneal space and are separated from the other retroperitoneal structures by the transversalis fascia. Pelvic structures, including the bladder, uterus, and rectum, that lie inferior to the pelvic peritoneum constitute the pelvic portion of the retroperitoneal space. The fascial layers limit the spread of retroperitoneal infections. However, the deep location can be difficult to assess by physical examination.

ETIOLOGY AND PATHOGENESIS

Retroperitoneal infections in children arise in numerous anatomic structures. Brook[7] reviewed cases of retroperitoneal infections from five U.S. hospitals from 1974 to 1994. Forty-one children were identified. Twenty-one had infections in the anterior retroperitoneal space related to the pancreas (4) and intestines (13), 6 had perinephric abscesses, 7 had iliopsoas abscesses, and 7 had pelvic retroperitoneal abscesses.

Primary infection of the retroperitoneal space within the renal fascia can be hematogenous in origin or complicate an ascending urinary tract infection. Within the anterior retroperitoneal space, secondary infections occur as a direct extension from gastrointestinal perforations such as ruptured appendices or those related to Crohn disease.[7,24,26] Greenstein and associates reported retroperitoneal abscesses in 12 of 231 patients with Crohn disease.[24] Retroperitoneal infections, particularly of the pelvic space, can also develop secondary to primary infections of the vertebral spine, pelvic bones, and sacroiliac joint.[27,49,52] Suppurative iliac or retroperitoneal lymph nodes are another source of retroperitoneal infections. Prior surgery has been reported as a predisposing factor in perinephric abscesses[8,20] and in vascular grafts in adults.[10] Pancreatic abscesses are seen more commonly in adult patients and are associated with underlying biliary tract disease, alcoholism, surgery, and trauma.[9] Acute pancreatitis occurs less frequently in children than in adults, but when it occurs infections are often implicated.[29,55]

Infections of the perinephric retroperitoneal space include those involving the kidney and adrenal glands. Perinephric abscesses can result from bacteremic inoculation of renal tissue or as a consequence of an ascending urinary tract infection. Nephronia (i.e., focal renal cellulitis, focal bacterial nephritis) is thought to be an intermediate stage of renal infection between pyelonephritis and renal abscess, resulting from an ascending infection of the urinary tract.[13] Adrenal abscesses are reported more frequently in neonates than in older children and are suspected to be related to adrenal hemorrhages that become secondarily infected.[41]

Iliopsoas abscesses may be a consequence of hematogenous seeding of the muscle, with trauma as a predisposing factor (Fig. 54-2).[26,48] Although primary infection occurs most commonly,[6] the iliopsoas muscle extends from the ribs and lumbar vertebrae to its insertion on the femur and is exposed to the risk of extension of infection from numerous adjacent structures. Psoas abscesses have developed as a consequence of vertebral infections, intestinal perforations, and genitourinary sources.[26,30] Neonatal iliopsoas abscesses have also been reported and patients present with symptoms similar to that of a septic hip.[18,21,44]

Complications of retroperitoneal abscesses include both rupture into the intraperitoneal space and extension of the infection along fascial planes to adjacent muscles that extend from origins in the pelvis and trunk to insertion sites on the femur. Rupture into the thoracic cavity also has been reported.[1] Other reported complications include pneumonia, recurrent abscess, renal failure, and venous and arterial thrombosis.[15]

MICROBIOLOGY

The microbiology of retroperitoneal infections is determined by the source of the infection and the retroperitoneal compartment involved. Most primary infections thought to result from bacteremia are caused by *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA).[45] Secondary infection related to the

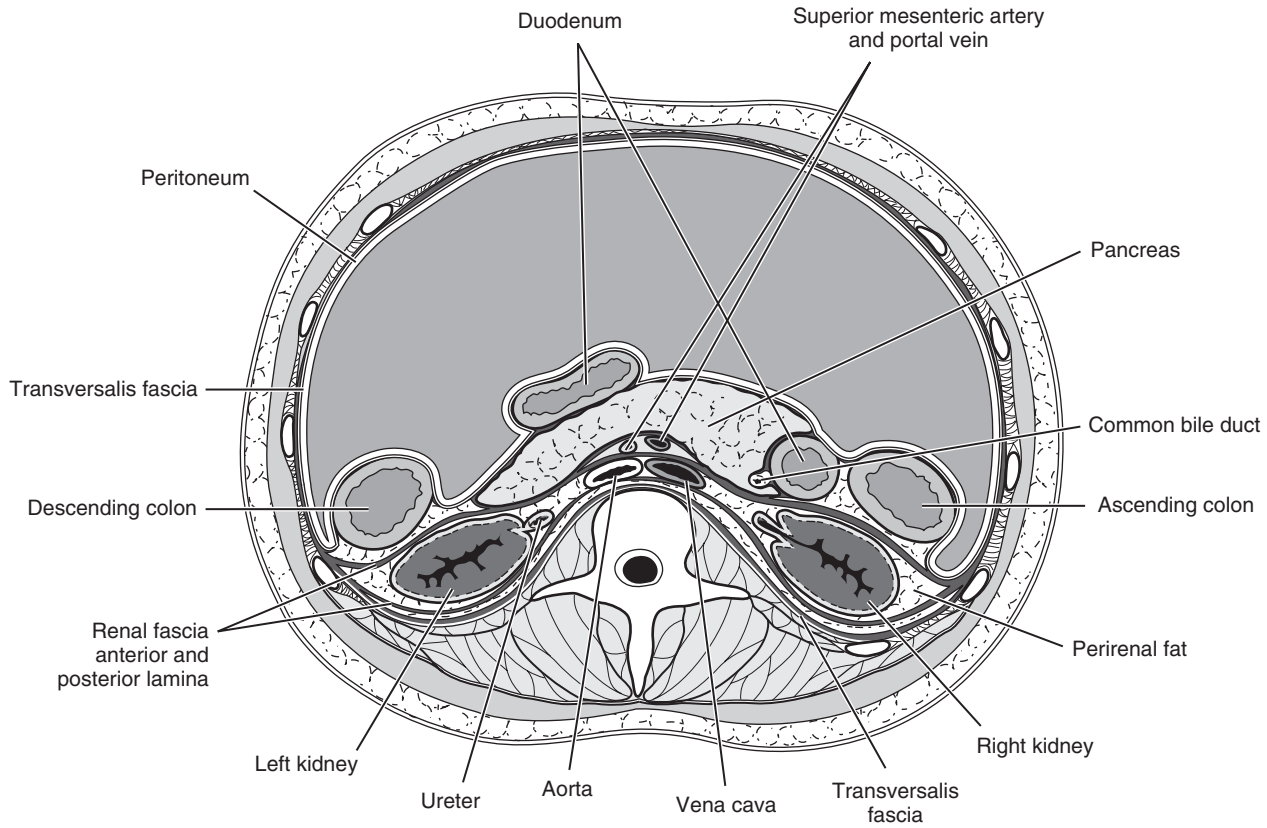

FIGURE 54-1 ■ Cross section of abdomen at L2 shows the structures within the retroperitoneal space. (From Altemeier WA, Alexander JW. Retroperitoneal abscess. *Arch Surg* 1961;**83**:515, with permission.)

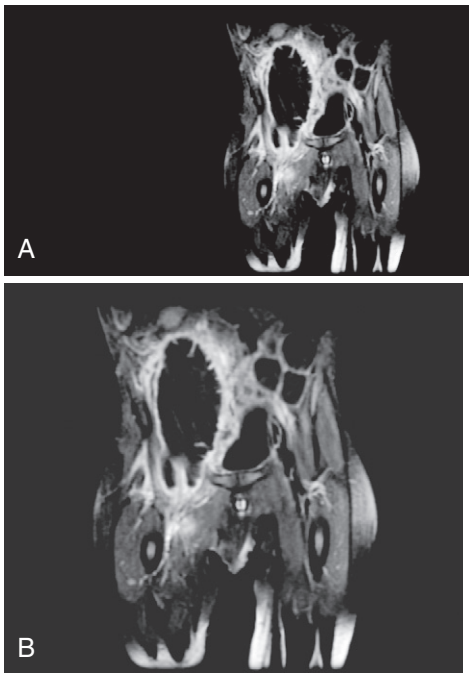

FIGURE 54-2 ■ Coronal postcontrast **(A)** and axial postcontrast **(B)** MR images of a large iliopsoas abscess.

gastrointestinal tract is caused by mixed bowel flora including *Escherichia coli*, other gram-negative enteric bacteria, *Pseudomonas* spp., and gastrointestinal anaerobes, particularly *Bacteroides fragilis* and *Peptostreptococcus*.[7] Most infections in the anterior retroperitoneal space are associated with a gastrointestinal source and may be polymicrobial. *Actinomyces* infections, although more commonly seen in the cervicofacial area, can also present as retroperitoneal infections.[4,35]

Ascending infections from the urinary tract usually are caused by *E. coli;* however, perinephric abscesses also are reported as a complication of renal infection caused by *S. aureus*, group B *Streptococcus*, and *Salmonella*.[20,56,57]

S. aureus is the leading pathogen isolated in iliopsoas abscesses unless the infection is secondary to erosion of a primary gastrointestinal focus. In that situation, gram-negative enteric bacteria and anaerobes are more likely to be the causative agents.[6,48] Retroperitoneal necrotizing fasciitis from group A *Streptococcus* has also been reported.[17]

Tuberculosis caused by *Mycobacterium tuberculosis* or *Mycobacterium bovis* may involve the retroperitoneal space as an extension of vertebral tuberculous osteomyelitis.[1,19,30] Abdominal tuberculosis usually manifests as an intraperitoneal infection but can produce retroperitoneal adenopathy.

The most common infectious agents associated with acute pancreatitis are viral pathogens including adenovirus, group B coxsackieviruses, mumps, and hepatitis

viruses (A, B, E).* Varicella, herpes simplex virus, cytomegalovirus, Epstein-Barr virus, and rotavirus have also been reported as associated infections in patients with acute pancreatitis.[16,22,33,46,47] Bacterial pathogens including *Salmonella* and *Mycoplasma*[39,42,46] are less frequently associated with pancreatitis. *Ascaris lumbricoides* can cause acute pancreatitis via obstruction of the common bile duct or other intrahepatic and pancreatic ducts.[31,46]

CLINICAL MANIFESTATIONS

Children with retroperitoneal infections present clinically in a variety of ways, ranging from nonspecific fever to overwhelming sepsis. The most common clinical symptoms associated with retroperitoneal infections include fever and pain in the hip, back, and abdomen.[15,37] Psoas abscesses often manifest as the child limping or refusing to walk.[6] Neonates with a retroperitoneal abscess may present with an abdominal mass.[50] In children, acute pancreatitis presents most commonly as abdominal pain and vomiting, rather than back pain.[25,29,55] Symptoms associated with retroperitoneal infections can be vague, and pain is not well localized. Patients often have been evaluated previously for fevers and have been treated with antibiotics before a diagnosis has been made.[6,32,49] A delay in the diagnosis is not uncommon.

DIFFERENTIAL DIAGNOSIS

Retroperitoneal infections can be confused with a variety of other infections. Limp and fever caused by pyogenic arthritis of the hip and infection of the sacroiliac joint and pelvic bones are more common than retroperitoneal infections. Abdominal pain and fever are more frequently seen in patients with intra-abdominal infections, including appendicitis and intra-abdominal abscesses. Trauma and malignant disease are more frequent causes of retroperitoneal masses compared with infectious causes and should be considered.

SPECIFIC DIAGNOSIS

Laboratory tests often are nonspecific and of minimal benefit. Sedimentation rates and leukocyte counts often are elevated.[6,7,48,49] Pancreatic enzymes including amylase and lipase are usually elevated with acute pancreatitis. Pyuria often is absent in children with perinephric and renal abscesses, and the urine culture result may be negative.[8,20,54,56] However, in patients with nephronia, pyuria and positive urine cultures are more likely.[13,32] Blood cultures may be helpful in identifying a bacterial pathogen. Brook[7] reported that 40 percent of blood cultures were positive for the same organism isolated from abscesses in children with retroperitoneal infections who had blood cultures collected. For children with psoas abscesses, Santaella and colleagues[48] reported that 71 percent of blood cultures were positive.

Imaging studies are the most useful diagnostic tools. Ultrasonography can be used to diagnose perinephric infections[8,54] and has been used to diagnose abscesses of the iliopsoas muscle.[26] Computed tomography (CT) with contrast enhancement can be the most helpful[12,15,48] because of superior delineation of organ involvement and the extent of infection. CT also can provide clues about the primary focus of the infection, thereby helping to guide empirical antibiotic therapy. Abscess fluid is seen on CT as areas of low attenuation, often with an enhancing rim.[12,34,56] Percutaneous drainage and biopsy of the lesions may also be accomplished with CT. Magnetic resonance imaging (MRI) offers the advantage over CT of superior visualization of inflamed bone and muscle tissue, although calcifications may not be as well identified.[43] Although alternative diagnoses also can be evaluated with CT,[51] hematomas and certain tumors may not be easily distinguished radiographically from infection. These noninfectious entities may be better identified by MRI. Decreased radiation exposure is an additional advantage of MRI.

TREATMENT

Percutaneous or open surgical drainage should be considered for all retroperitoneal infections for both diagnostic and treatment purposes. Culture of the aspirated fluid for aerobic and anaerobic bacteria, mycobacteria, and fungi is vital to selecting appropriate antimicrobial therapy. Reports of percutaneous drainage of deep tissue abscesses are increasing.[6,30] These procedures usually are performed with ultrasound or CT guidance. Treatment of patients with antibiotics alone without surgical drainage may not be effective, particularly in cases involving larger abscesses.[1,12]

Initial antimicrobial therapy for retroperitoneal infections should be directed by the presumed source of the infection, with definitive therapy guided by microbiologic culture results. Infections related to gastrointestinal perforation should include coverage directed primarily against enteric gram-negative bacteria and gastrointestinal anaerobes such as β-lactamase–producing *B. fragilis*. Coverage for *Pseudomonas* and *Enterococcus* spp. also should be considered. Historically, antibiotic combinations such as ampicillin for enterococcus, metronidazole or clindamycin for anaerobes, and a third-generation cephalosporin or aminoglycoside for gram-negative bacteria have been used. However, carbapenems such as meropenem, imipenem, or ertapenem as single agents may be more cost effective, particularly if any outpatient antibiotic therapy is being considered. The β-lactam and β-lactamase inhibitor combinations (e.g., ticarcillin-clavulanate, piperacillin-tazobactam), with or without an aminoglycoside, are also likely to be effective.

Infections of renal origin, usually caused by *E. coli* or other gram-negative enteric organisms, can be treated with extended-spectrum cephalosporins such as ceftriaxone or, if abscesses have been drained successfully, with aminoglycosides such as gentamicin or tobramycin. The activity of aminoglycosides may be compromised by the acidic environment of abscess cavities and may lead to

*References 3, 14, 25, 28, 40, 46, 53.

clinical failures despite in vitro susceptibility of the organism.[11] Increasingly, resistance to ampicillin is seen in *E. coli*,[2,5] rendering it unreliable for empirical use in severe urinary tract infections. *Pseudomonas* is not an uncommon pathogen in children with recurrent infections caused by anatomic genitourinary abnormalities.[2] *Pseudomonas* spp. usually are resistant to ceftriaxone; extended-spectrum cephalosporins such as ceftazidime or cefepime, or a carbapenem, may be needed. Urine or abscess culture and susceptibility results help to focus the antibiotic choice to the agent with the most narrow spectrum of effectiveness.

Psoas abscesses and primary perinephric abscesses caused by *S. aureus* should be treated with an antistaphylococcal agent such as nafcillin (or oxacillin or methicillin) or a first-generation cephalosporin such as cefazolin. The prevalence of community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has increased significantly as a soft tissue pathogen, particularly in children.[23,38] Vancomycin or clindamycin should be considered as empirical therapy for serious infections in areas with high rates of CA-MRSA (more than 5 percent of all invasive *S. aureus* infections) until culture results are available. These agents also may be effective in treating the patient who is unable to tolerate penicillin or cephalosporin antibiotics.

Infections originating in the vertebrae are most often caused by *S. aureus* or may result from tuberculosis. Empirical antistaphylococcal therapy can be started, but culture and histologic examination of tissue are needed to direct appropriate therapy. For the child with risk factors for tuberculosis, a positive tuberculin skin test or interferon-γ release assay, and a negative Gram stain result, empirical therapy with three or four antituberculous antibiotics should be considered. A chest radiograph should be obtained to look for evidence of pulmonary tuberculosis.

After adequate drainage is achieved, the duration of antimicrobial therapy depends on several factors, including the organism, the site and extent of infection, and clinical improvement. Most drained retroperitoneal bacterial abscesses of renal or muscle origin are treated for 2 to 3 weeks with initial parenteral and follow-up oral antibiotics. Infections involving bone may require 6 to 8 weeks or longer, depending on how quickly the infection responds to treatment. Radiographic studies, erythrocyte sedimentation rate, and C-reactive protein measurement can be helpful to monitor recovery. Tuberculous infections are treated for 6 to 12 months, depending on the presence of bone involvement. *Actinomyces* infection can be difficult to treat because of lack of susceptibility to many antibiotics and the need for prolonged courses of antibiotic therapy, preferentially with penicillin.

Treatment of pancreatitis is primarily supportive with elimination of enteral feedings and pain control. There have been some reports of adenovirus treatment with cidofovir, particularly in the bone marrow transplant population.[3,36,58]

PROGNOSIS

Historically, retroperitoneal infections are reported to have high morbidity and mortality rates. However, with modern imaging techniques enabling more timely diagnoses, the overall prognosis is good and most children with no underlying disease recover without sequelae.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Bateman CM, Kesson AM, Shaw PJ. Pancreatitis and adenoviral infection in children after blood and marrow transplantation. *Bone Marrow Transplant* 2006;**38**:807–11.
13. Cheng CH, Tsau YK, Lin TY. Is acute lobar nephronia the midpoint in the spectrum of upper urinary tract infections between acute pyelonephritis and renal abscess? *J Pediatr* 2010;**156**(1): 82–6.
14. Chrysos G, Kokkoris S, Protopsaltis J, et al. Coxsackie infection associated with acute pancreatitis. *J Pancreas* 2004;**5**:384–7.
16. De la Rubia L, Herrera MI, Cebrero M, de Jong JC. Acute pancreatitis associated with rotavirus infection. *Pancreas* 1996; **12**:98–9.
25. Haddock G, Coupar G, Youngson GG, et al. Acute pancreatitis in children: a 15 year review. *J Pediatr Surg* 1994;**29**:719–22.
28. Jain P, Nijhawan S, Rai RR, et al. Acute pancreatitis in acute viral hepatitis. *World J Gastroenterol* 2007;**13**:5741–4.
29. Kandula L, Lowe ME. Etiology and outcome of acute pancreatitis in infants and toddlers. *J Pediatr* 2008;**152**:106–10.
31. Khuroo MS, Zargar SA, Mahajan R. Hepatobiliary and pancreatic ascariasis in India. *Lancet* 1990;**335**:1503–6.
33. Konstantinou GN, Liatsos CN, Patelaros EG, et al. Acute pancreatitis associated with herpes simplex virus infection: report of a case and review of the literature. *Eur J Gastroenterol Hepatol* 2009;**21**:114–6.
36. Legrand F, Berrebi D, Houhou N, et al. Early diagnosis of adenovirus infection and treatment with cidofovir after bone marrow transplantation in children. *Bone Marrow Transplant* 2001; **27**:621–6.
39. Martinez-Roig A, Bonet-Alcaina M, Casellas-Montagut M, Ymbert-Pellajà L. Pancreatitis in typhoid fever relapse. *Pediatr Infect Dis J* 2009;**28**:74.
40. Mishra A, Saigal S, Gupta R, Sarin SK. Acute pancreatitis associated with viral hepatitis: a report of six cases with review of literature. *Am J Gastroenterol* 1999;**94**:2292–5.
42. Nakagawa M, Ogino H, Shimohira M, et al. Continuous regional arterial infusion therapy for acute necrotizing pancreatitis due to *Mycoplasma pneumoniae* infection in a child. *Cardiovasc Intervent Radiol* 2009;**32**:581–4.
45. Pannaraj P, Hulten JG, Gonzalez BE, et al. Infective pyomyositis and myositis in children in the era of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* 2006;**43**:953–60.
46. Parenti DM, Steinberg W, Kang P. Infectious causes of acute pancreatitis. *Pancreas* 1996;**13**:356–71.
47. Parri N, Innocenti L, Collini S, et al. Acute pancreatitis due to rotavirus gastroenteritis. *Pediatr Emer Care* 2010;**26**:592–3.
53. Thapa R, Biswas B, Mallick D, Ghosh A. Acute pancreatitis–complicating hepatitis E virus infection in a 7-year-old boy with glucose-6-phosphate dehydrogenase deficiency. *Clin Pediatr* 2009; **48**:199–201.
55. Werlin SL, Kugathasan S, Frautschy BC. Pancreatitis in children. *J Pediatr Gastroenterol Nutr* 2003;**37**:591–5.
58. Yusuf U, Hale GA, Carr J, et al. Cidofovir for the treatment of adenoviral infection in pediatric hematopoietic stem cell transplant patients. *Transplantation* 2006;**81**:1398–404.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Altemeier WA, Alexander JW. Retroperitoneal abscess. *Arch Surg* 1961;**83**:512–24.
2. Ashkenazi S, Even-Tov S, Samra Z, et al. Uropathogens of various childhood populations and their antibiotic susceptibility. *Pediatr Infect Dis J* 1991;**10**:742–6.
3. Bateman CM, Kesson AM, Shaw PJ. Pancreatitis and adenoviral infection in children after blood and marrow transplantation. *Bone Marrow Transplant* 2006;**38**:807–11.
4. Benammar S, Helardot PF, Sapin E, et al. Childhood actinomycosis: report of two cases. *Eur J Pediatr Surg* 1995;**5**:180–3.
5. Bonadio WA, Smith DS, Madagame E, et al. *Escherichia coli* bacteremia in children. *Am J Dis Child* 1991;**145**:671–4.
6. Bresee JS, Edwards MS. Psoas abscess in children. *Pediatr Infect Dis J* 1990;**9**:201–6.
7. Brook I. Microbiology of retroperitoneal abscesses in children. *J Med Microbiol* 1999;**48**:697–700.
8. Brook I. The role of anaerobic bacteria in perinephric and renal abscesses in children. *Pediatrics* 1994;**93**:261–4.
9. Brook I, Frazier EH. Microbiological analysis of pancreatic abscess. *Clin Infect Dis* 1996;**22**:384–5.
10. Brook I, Frazier EH. Aerobic and anaerobic microbiology of retroperitoneal abscesses. *Clin Infect Dis* 1998;**26**:938–41.
11. Bryant RE, Fox K, Oh G, Morthland VH. β-Lactam enhancement of aminoglycoside activity under conditions of reduced pH and oxygen tension that may exist in infected tissues. *J Infect Dis* 1992;**165**:676–82.
12. Chen WC, Huang JK, Chen KK, et al. Retroperitoneal abscesses. *Chin Med J* 1990;**46**:208–12.
13. Cheng CH, Tsau YK, Lin TY. Is acute lobar nephronia the midpoint in the spectrum of upper urinary tract infections between acute pyelonephritis and renal abscess? *J Pediatr* 2010;**156**(1):82–6.
14. Chrysos G, Kokkoris S, Protopsaltis J, et al. Coxsackie infection associated with acute pancreatitis. *J Pancreas* 2004;**5**:384–7.
15. Crepps JT, Welch JP, Orlando III R, Management and outcome of retroperitoneal abscesses. *Ann Surg* 1987;**205**:276–81.
16. De la Rubia L, Herrera MI, Cebrero M, de Jong JC. Acute pancreatitis associated with rotavirus infection. *Pancreas* 1996;**12**:98–9.
17. Devin B, McCarthy A, Mehran R, Auger C. Necrotizing fasciitis of the retroperitoneum: an unusual presentation of group A *Streptococcus* infection. *Can J Surg* 1998;**41**:156–60.
18. Dib M, Bedu A, Garel C, et al. Iliopsoas abscess in neonates: treatment by ultrasound-guided percutaneous drainage. *Pediatr Radiol* 2000;**30**:677–80.
19. Dinc H, Onder C, Turhan AU, et al. Percutaneous catheter drainage of tuberculous and nontuberculous psoas abscesses. *Eur J Radiol* 1996;**23**:130–4.
20. Edelstein H, McCabe RE. Perinephric abscess in pediatric patients: report of six cases and review of the literature. *Pediatr Infect Dis J* 1989;**8**:167–70.
21. Feo CF, Dessanti A, Franco B, et al. Retroperitoneal abscess and omphalitis in young infants. *Acta Paediatr* 2003;**92**:122–5.
22. Franco J, Fernandes R, Oliveira M, et al. Acute pancreatitis associated with varicella infection in an immunocompetent child. *J Paediatr Child Health* 2009;**45**:547–8.
23. Frank AL, Marcinak JF, Mangat D, et al. Community-acquired and clindamycin susceptible methicillin-resistant *Staphylococcus aureus* in children. *Pediatr Infect Dis J* 1999;**18**:993–1000.
24. Greenstein AJ, Dreiling DA, Aufses Jr AH. Crohn's disease of the colon. *Am J Gastroenterol* 1975;**64**:306–18.
25. Haddock G, Coupar G, Youngson GG, et al. Acute pancreatitis in children: a 15-year review. *J Pediatr Surg* 1994;**29**:719–22.
26. Hoffer FA, Shamberger RC, Teele RL. Iliopsoas abscess: diagnosis and management. *Pediatr Radiol* 1987;**17**:23–7.
27. Holliday III PO, Davis Jr CH, Shaffner LS. Intervertebral disc space infection in a child presenting as a psoas abscess: case report. *Neurosurgery* 1980;**7**:395–7.
28. Jain P, Nijhawan S, Rai RR, et al. Acute pancreatitis in acute viral hepatitis. *World J Gastroenterol* 2007;**13**:5741–4.
29. Kandula L, Lowe ME. Etiology and outcome of acute pancreatitis in infants and toddlers. *J Pediatr* 2008;**152**:106–10.
30. Kang M, Gupta S, Gulati M, Suri S. Iliopsoas abscess in the paediatric population: treatment by US-guided percutaneous drainage. *Pediatr Radiol* 1998;**28**:478–81.
31. Khuroo MS, Zargar SA, Mahajan R. Hepatobiliary and pancreatic ascariasis in India. *Lancet* 1990;**335**:1503–6.
32. Kline MW, Kaplan SL, Baker CJ. Acute focal bacterial nephritis: diverse clinical presentations in pediatric patients. *Pediatr Infect Dis J* 1988;**7**:346–9.
33. Konstantinou GN, Liatsos CN, Patelaros EG, et al. Acute pancreatitis associated with herpes simplex virus infection: report of a case and review of the literature. *Eur J Gastroenterol Hepatol* 2009;**21**:114–6.
34. Kuhns LR. Computed tomography of the retroperitoneum in children. *Radiol Clin North Am* 1981;**19**:495–501.
35. Latawiec-Mazurkiewicz I, Juszkiewicz P, Pacanowski J, et al. Tumour-like inflammatory abdominal conditions in children. *Eur J Pediatr Surg* 2005;**15**:38–43.
36. Legrand F, Berrebi D, Houhou N, et al. Early diagnosis of adenovirus infection and treatment with cidofovir after bone marrow transplantation in children. *Bone Marrow Transplant* 2001;**27**:621–6.
37. March AW, Riley LH, Robinson RA. Retroperitoneal abscess and septic arthritis of the hip in children. *J Bone Joint Surg Am* 1972;**54**:67–74.
38. Martinez-Aguilar G, Avalos-Mishaan A, Hulten K, et al. Community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* musculoskeletal infections in children. *Pediatr Infect Dis J* 2004;**23**:701–6.
39. Martinez-Roig A, Bonet-Alcaina M, Casellas-Montagut M, Ymbert-Pellajà L. Pancreatitis in typhoid fever relapse. *Pediatr Infect Dis J* 2009;**28**:74.
40. Mishra A, Saigal S, Gupta R, Sarin SK. Acute pancreatitis associated with viral hepatitis: a report of six cases with review of literature. *Am J Gastroenterol* 1999;**94**:2292–5.
41. Mondor C, Gauthier M, Garel L, et al. Nonsurgical management of neonatal adrenal abscess. *J Pediatr Surg* 1988;**23**:1048–50.
42. Nakagawa M, Ogino H, Shimohira M, et al. Continuous regional arterial infusion therapy for acute necrotizing pancreatitis due to *Mycoplasma pneumoniae* infection in a child. *Cardiovasc Intervent Radiol* 2009;**32**:581–4.
43. Negus S, Sidhu PS. MRI of retroperitoneal collections: a comparison with CT. *Br J Radiol* 2000;**73**:907–12.
44. Okada Y, Yamataka A, Ogasawara Y, et al. Iliopsoas abscess caused by methicillin-resistant *Staphylococcus aureus* (MRSA): a rare but potentially dangerous condition in neonates. *Pediatr Surg Int* 2004;**20**:73–4.
45. Pannaraj P, Hulten JG, Gonzalez BE, et al. Infective pyomyositis and myositis in children in the era of community-acquired methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* 2006;**43**:953–60.
46. Parenti DM, Steinberg W, Kang P. Infectious causes of acute pancreatitis. *Pancreas* 1996;**13**:356–71.
47. Parri N, Innocenti L, Collini S, et al. Acute pancreatitis due to rotavirus gastroenteritis. *Pediatr Emer Care* 2010;**26**:592–3.
48. Santaella RO, Fishman EK, Lipsett PA. Primary vs secondary iliopsoas abscess. *Arch Surg* 1995;**130**:1309–13.
49. Schwaitzberg SD, Pokorny WJ, Thurston RS, et al. Psoas abscess in children. *J Pediatr Surg* 1985;**20**:339–42.
50. Sedaghatian MR, Barkhordar J, Gerami S. Retroperitoneal abscess presenting as an abdominal mass in neonate. *J Pediatr Surg* 1978;**13**:544–45.
51. Siegel MJ, Balfe DM, McClennan BL, Levitt RG. Clinical utility of CT in pediatric retroperitoneal disease: 5 years' experience. *AJR Am J Roentgenol* 1982;**138**:1011–17.
52. Simons GW, Sty JR, Starshak RJ. Retroperitoneal and retrofascial abscesses. *J Bone Joint Surg Am* 1983;**65**:1041–58.
53. Thapa R, Biswas B, Mallick D, Ghosh A. Acute pancreatitis–complicating hepatitis E virus infection in a 7-year-old boy with glucose-6-phosphate dehydrogenase deficiency. *Clin Pediatr* 2009;**48**:199–201.
54. Vachvanichsanong P, Dissaneewate P, Patrapinyokul S, et al. Renal abscess in healthy children: report of three cases. *Pediatr Nephrol* 1992;**6**:273–5.
55. Werlin SL, Kugathasan S, Frautschy BC. Pancreatitis in children. *J Pediatr Gastroenterol Nutr* 2003;**37**:591–5.
56. Wippermann CF, Schofer O, Beetz R, et al. Renal abscess in childhood: diagnostic and therapeutic progress. *Pediatr Infect Dis J* 1991;**10**:446–50.
57. Woods CR, Edwards MS. Renal abscess caused by group B Streptococcus. *Clin Infect Dis* 1994;**18**:662–3.
58. Yusuf U, Hale GA, Carr J, et al. Cidofovir for the treatment of adenoviral infection in pediatric hematopoietic stem cell transplant patients. *Transplantation* 2006;**81**:1398–404.

SECTION **IX**

MUSCULOSKELETAL INFECTIONS

CHAPTER 55

Osteomyelitis

Paul Krogstad

The term *osteomyelitis* denotes inflammation of bone and marrow but generally implies the presence of infection. Osteomyelitis is considered acute if diagnosed within 2 weeks of the onset of symptoms and is subacute or chronic if symptoms have been present for more than 2 weeks at the time of presentation. Although bacteria are the most common cause, fungi, parasites, and other microorganisms also may cause osteomyelitis. These microorganisms can be introduced into bone in three ways: (1) by direct inoculation, usually traumatic but also during surgery or due to the presence of orthopedic fixation devices; (2) by local invasion from a contiguous focus of infection; and (3) by hematogenous delivery. In children, osteomyelitis is generally of hematogenous origin and occurs in long, tubular bones. Regardless of the route of infection, the goal of treatment is to arrest the infection and limit the extent of the injury to bone.

The incidence of osteomyelitis in normal children has been examined in several populations. Estimates have varied from 1 in 20,000 adolescent girls in New Zealand to 1 in 1,000 Australian Aboriginals.[28,51,90,150] Boys contract the disease 1.2 to 3.7 times more often than do girls.[90,171,202] Osteomyelitis occurs most often in the first 2 decades of life. Approximately 25 percent of children with osteomyelitis are younger than 2 years old, and 50 percent are younger than 5 years old.[91,130,165,226] The incidence is increased in patients with sickle-cell disease and in some other immunocompromised patients (see the section on special populations).

Although cultures fail to identify bacterial pathogens in approximately one fourth to half of all cases of osteomyelitis in children, most microbiologically confirmed infections are caused by single organisms.[8,24,165,175] When polymicrobial infections are encountered, they may reflect the spread of infection from contiguous infectious foci and most often occur in the skull, face, hands, or feet. Distal extremities compromised by vascular insufficiency or immobilized because of peripheral neuropathy also are sites of polymicrobial osteomyelitis.

Gram-positive bacteria are most often identified, especially *Staphylococcus aureus*[91,184,219,224] and *Streptococcus pyogenes;* together they account for 80 to 90 percent or more of cases in most series (Table 55-1). However, *Kingella kingae*, a fastidious gram-negative organism, is increasingly being identified in young children as a cause of osteoarticular infections, including osteomyelitis, diskitis, and septic arthritis. *K. kingae* osteomyelitis has been identified at a frequency equal to *S. pyogenes* in some series,[143,150] and its incidence may have been historically underestimated because of its fastidious growth. Polymerase chain reaction–based studies suggest that it accounts for many cases of culture-negative osteoarticular disease.[239] Reports demonstrating its importance as a cause of osteoarticular infections in young children have come from countries as diverse as Israel, the United States, Australia, Switzerland, and Iceland,* and in two reports *Kingella* infections exceeded those of gram-positive pathogens.[39,42]

Streptococcus pneumoniae has been a frequent cause of osteomyelitis in the past, but protein conjugate vaccines have markedly reduced the frequency of invasive pneumococcal infections, including pneumococcal osteomyelitis. Similarly, *Haemophilus influenzae* used to cause 5 to 8 percent of cases and was found primarily in infants and younger children†; however, cases of *H. influenzae* osteomyelitis are noticeably absent from most recent case series.[8,91,122,170,244]

Even in affluent countries *Salmonella* spp. are occasionally a cause of osteomyelitis in immunocompetent patients, and *Salmonella* spp. are the most common organism found in cases of osteomyelitis in patients with sickle-cell disease (see later).[91,165] Osteomyelitis caused by *Escherichia coli* and other aerobic enteric gram-negative organisms is also seen, generally in neonates and young infants.[238] Osteomyelitis caused by *Pseudomonas aeruginosa* has been associated with injection drug use.[110,137,235]

Although rare in comparison to osteomyelitis caused by aerobic bacteria, four distinct clinical entities of osteomyelitis caused by anaerobic bacteria are recognized: (1) bacteremic seeding of previously normal bones in children and young adults; (2) superinfection of a fracture site already infected with *S. aureus;* (3) indolent (months to years after surgery) infection of a prosthetic device; and (4) contiguous chronic infection,[213] which most often occurs in the skull and the extremities. *Bacteroides* spp. are found most commonly and are associated with paranasal, sinus, or mastoid infection. In most cases, a foul odor is noted when the bone is incised or the focus is opened; trauma often has been an inciting influence.[138]

Osteomyelitis caused by fungi and atypical bacterial pathogens occasionally occurs in children and is discussed in greater detail later.

HEMATOGENOUS OSTEOMYELITIS

Pathogenesis

In long tubular bones, hematogenous osteomyelitis generally begins in the metaphysis, the broad cancellous end of the bone shaft adjacent to the epiphyseal growth plate.

*References 52, 62, 96, 143, 150, 227, 241.
†References 69, 79, 97, 130, 165, 184, 211, 224.

TABLE 55-1 **Etiology of Acute Hematogenous Osteomyelitis in Children: Number of Bacteriologically Confirmed Cases**

Organisms	Nelson[165] (*n* = 296) (%)	LaMont et al.[130] (*n* = 90) (%)	Peltola et al.[170] (*n* = 252) (%)	Masson et al.[150] (*n* = 95) (%)	Goergens et al.[91] (*n* = 45) (%)
Gram-Positive Bacteria					
Staphylococcus aureus	67	70	75	65	85
Coagulase-negative staphylococci	3	1		4	
Streptococcus pneumoniae	2	5	4	2	4
Other streptococci	12	16	10	10	8
Gram-Negative Bacteria					
Haemophilus influenzae	4	8	10	1	0
Pseudomonas aeruginosa	3				
Salmonella spp.	2				
Escherichia coli	<1				
Kingella kingae	<1			7	
Mixed or unusual organisms	4		1	10	

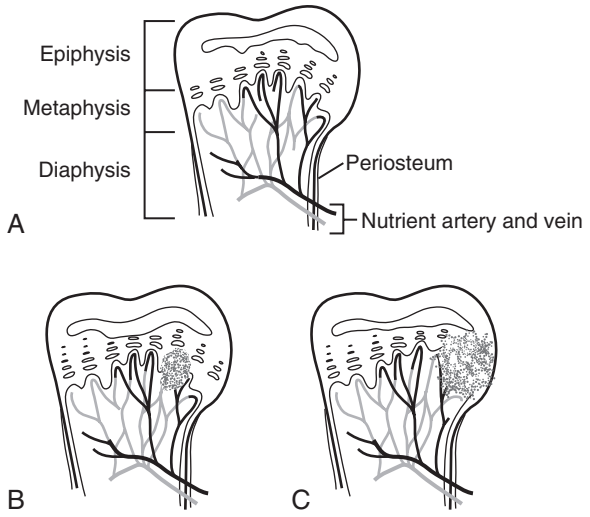

FIGURE 55-1 ■ **A,** The sluggish blood flow in the sinusoidal venous connections located at the metaphyseal-epiphyseal junction predisposes to the development of traumatic thrombosis and infarction. **B,** Bacteremic seeding of the avascular area initiates the infection, which spreads through the Volkmann canals and the haversian systems and causes septic thrombosis. **C,** Infection tends to spread laterally through the cortex and elevates or ruptures through the periosteum.

The cartilaginous epiphyseal growth plate (the physis) is nourished by diffusion of nutrients from a narrow plexus of capillaries fed by the metaphyseal branches of a nutrient artery; these capillaries drain into a large sinusoidal plexus that ultimately joins the large sinusoidal veins in the bone marrow (Fig. 55-1).[108] Trauma or emboli lead to occlusion of the slow-flowing sinusoidal vessels, establishing a nidus for infection. Blood-borne bacteria can seed the poorly perfused area and proliferate.[46,119,221]

The high frequency of *S. aureus* in osteomyelitis may reflect specific pathogenic properties of the organisms, including the ability to adhere to type I collagen of bone fibrils.[35] When *S. aureus* binds to collagen, bacterial replication gives rise to microcolonies surrounded by a glycocalyx.[102] Continued injury, elicited by *S. aureus* exoproducts and the host cellular inflammatory response to the injury, causes the accumulation of exudate under pressure. The pressure compresses blood vessels of bone and produces focal bone necrosis. The low ratio of surface area to mass, combined with the blood vessel anatomy described earlier, interferes with reabsorption of necrotic cortical bone[46,119] and the effectiveness of host defense mechanisms. The very early stages of osteomyelitis may be aborted by administration of appropriate chemotherapy. In the absence of therapy, necrosis of cortical bone and marrow continues. The exudate under pressure is forced through the haversian systems and Volkmann canals and into the cortex (see Fig. 55-1).

Beginning in the late 1990s, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) isolates became a common cause of musculoskeletal infections in the United States and other countries. Most of these CA-MRSA isolates from cases of osteomyelitis carry genes encoding Panton-Valentine leukocidin.[23] Panton-Valentine leukocidin–positive CA-MRSA has been associated frequently with sepsis, venous thrombosis, adjacent myositis, and polyostotic disease, suggesting that this or other factors contribute to the development and severity of hematogenous osteomyelitis.[29,148] However, it is important to note that similar patterns of disease are seen in Panton-Valentine leukocidin–negative organisms[120] and the precise role of this substance in *S. aureus*–associated disease remains unclear.

Clinical Manifestations

The bacteremic phase of hematogenous osteomyelitis may be entirely subclinical and associated only with malaise and low-grade fever, or it may be characterized by severe constitutional symptoms with high fevers (39° to 40°C [102.2° to 104°F]). The subsequent clinical manifestations of osteomyelitis are not related to the severity of initial constitutional signs of infection but are influenced by the age of the child and the etiologic agent (Fig. 55-2).

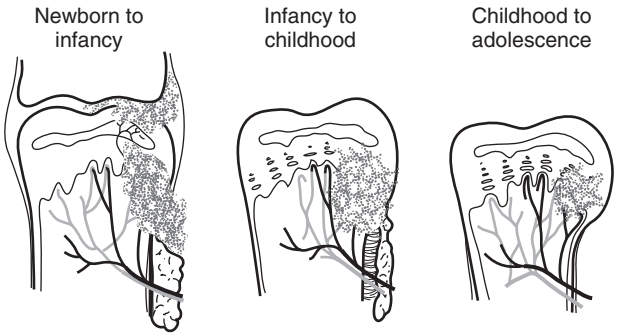

FIGURE 55-2 ■ In young infants and neonates, particularly in the hip where the epiphyseal growth plate is traversed by nutrient vessels terminating in the distal ossification center, septic thrombophlebitis of the nutrient vessels can lead to growth discrepancies. With the capsule of the joint extending to the metaphysis, rupture of the infection through the cortex leads to the development of septic arthritis. Because of the thin cortex and loose periosteum, the osteomyelitis may come to medical attention as a deep soft tissue abscess. In older infants and young children, the thicker cortex and denser periosteum are a greater barrier to infection. Local tenderness from subperiosteal edema or abscess is the rule. In late childhood and adolescence, the lesion is extremely well localized and rarely penetrates the bony cortex. In this age group, invasive procedures, such as windowing or drilling, are necessary to obtain infected material.

In neonates, the thin cortex and loosely attached periosteum are poor barriers to the spread of infection. Consequently, the purulence rapidly ruptures through both of these structures into the contiguous muscle bed. With progression of the infection, the purulent material often dissects the muscle bundles, with the swollen, discolored limb taking the appearance of a sausage. In addition, nutrient metaphyseal capillaries perforate the epiphyseal growth plate in neonates and the capsule of the diarthrodial joints frequently extends to or is slightly distal to the epiphyseal plate. These anatomic characteristics permit an infection arising in the metaphysis to involve the epiphysis and to extend into the adjacent joint cavity.

In older infants, the cortex (see Fig. 55-2) is thicker and the periosteum is slightly more dense. Consequently, the infection spreads less often to the soft tissues of the extremity. Subperiosteal abscess and contiguous edema readily develop, however. The nutrient metaphyseal capillaries in older infants are atrophic, which, although more recently disputed,[172] is thought to decrease the risk of spread of infection into the adjacent joint space. The subperiosteal purulence almost always is at the metaphysis, the area in which the cortex is the thinnest.[99]

In children and adolescents (4 to 16 years old), the metaphyseal cortex is considerably thicker, with a dense, fibrous periosteum. The pathogenesis of the infection is the same in this age group, but the infection rarely ruptures and spreads to the outer cortical lamellae. As a result, the signs and symptoms of osteomyelitis in these older children and adolescents usually are more focal.

A neonate with osteomyelitis usually is irritable and displays evidence of pain when the affected extremity is touched or moved. Pseudoparalysis may occur; and if the disease remains untreated, massive swelling of the extremity may be seen. Obtaining a plain radiograph is particularly valuable in this age group; most of these patients have changes consistent with osteomyelitis on the initial radiograph.[126,158,232] In infants and young children, pain usually is present, as is a limp because osteomyelitis occurs more commonly in the lower extremities. The child usually refuses to use the affected extremity and displays variable constitutional symptoms.

In older children and adolescents, less restriction of function of the extremity is found compared with infants and young children. The point tenderness is circumscribed more sharply and may be found only as a small area of discomfort at rest. This disease most often affects the lower extremities and produces a mild limp. Most commonly, tubular bones are involved, but osteomyelitis occurs throughout the skeleton (Table 55-2). The hallmark of the disease is the focal nature of symptoms; point tenderness and well-localized pain suggest the diagnosis. Percussion of the long bone away from the area of point tenderness often elicits pain at the site of osteomyelitis in older children and adolescents.

TABLE 55-2 **Site of Involvement in Acute Hematogenous Osteomyelitis**

Site	%
Tubular Bone	
Femur	25
Tibia	24
Humerus	13
Phalanges	5
Fibula	4
Radius	4
Ulna	2
Metatarsal	2
Clavicle	0.5
Metacarpal	0.5
Cuboidal Bone	
Calcaneus	5
Talus	0.8
Carpals	0.5
Cuneiform	0.5
Cuboid	0.3
Irregular Bone	
Ischium	4
Ilium	2
Vertebra	2
Pubis	0.8
Sacrum	0.8
Flat Bone	
Skull	1
Rib	0.5
Sternum	0.5
Scapula	0.5
Maxilla	0.3
Mandible	0.3

Data from reference 165; similar distribution of affected bones has been reported in other reports.[24,91,169]

The clinical features of osteomyelitis are also influenced by the organisms involved. Osteomyelitis caused by CA-MRSA seems to be more complex and severe than are infections caused by methicillin-susceptible *S. aureus* (MSSA). Life-threatening infections caused by CA-MRSA have been reported in adolescents, and polyostotic disease is more common (15% in one more recent series compared with 2% in a large series that preceded the emergence of CA-MRSA).[23,165] Myositis, pyomyositis, intraosseous and subperiosteal abscesses, and septic thrombophlebitis also seem to occur more frequently with CA-MRSA than with MSSA.[8,94,95,148] Osteoarticular infections caused by *Kingella* spp. arise in children younger than 36 months of age and have an indolent course, with limb pain often present for longer than a week before initial medical evaluation is made; complications are largely unknown.[62,143,239,242] Osteomyelitis caused by *H. influenzae* seems to occur primarily in the upper extremities.[68,69,97,211]

Culture-negative osteomyelitis is generally milder than diseases with microbiologically confirmed causes. In one series comparing 45 culture-positive patients with 40 patients with culture-negative osteomyelitis, symptoms were of longer duration and overlying skin changes were seen less frequently in patients with negative disease. Treatment with β-lactams generally was successful and associated with skeletal sequelae in only one case.[77] Whether these features of culture-negative osteomyelitis suggest a more effective host defense, or infection by a less virulent and more fastidious pathogen such as *K. kingae*,[227] remains unclear.

Differential Diagnosis

Infectious osteomyelitis can be confused with many other conditions associated with pain and tenderness in an extremity, including fractures, rheumatic fever, septicemia, septic arthritis, cellulitis, Ewing sarcoma and malignant bone tumors, osteoid osteoma, leukemia, complex regional pain syndrome (reflex neurovascular dystrophy), thrombophlebitis, bone infarction secondary to sickle-cell or Gaucher disease, and toxic synovitis.

Chronic recurrent multifocal osteomyelitis (CRMO) (also known as chronic nonbacterial osteomyelitis) must also be considered in some cases and merits special attention in the differential diagnosis. As originally described by Giedion and coworkers,[87] CRMO is a chronic illness generally characterized by multiple chronic focal, inflammatory lesions in bone with periodic exacerbation and remission, and moderate bone pain.[225] It occurs more commonly in girls younger than 10 years of age. Many of the cases are in children of northern European origin.[19,125] At presentation, about half of patients have fever and virtually all have an increased erythrocyte sedimentation rate (ESR) (or increased C-reactive protein [CRP] value). The lesions occur primarily in the distal femoral, distal tibial, and proximal tibial regions, although vertebral lesions are seen in approximately one fourth of cases.[192] Patients may have 1 to 18 lesions at a time; biopsy specimens show a nonspecific chronic inflammatory process, and no organisms are identified histologically or by culture. Approximately 20 percent of patients have a pustular eruption of the palms and soles *(pustulosis palmaris et plantaris)* at the same time that they come to medical attention with bone lesions.

CRMO is a sporadic illness in most cases, but it may also be found as part of syndromic illnesses. Majeed syndrome, for example, is an autosomal recessive, lifelong disorder composed of CRMO, congenital dyserythropoietic anemia, and transient inflammatory dermatosis.[71] It may also occur in the rare patients with deficiency of interleukin-1 receptor antagonist.[3]

The long-term outlook for children with sporadic CRMO disease generally is good, although numerous relapses may occur.[19] Glucocorticoids and nonsteroidal anti-inflammatory drugs are used therapeutically, although recurrences are common. Treatment with interferon-γ and tumor necrosis factor–α blocking agents, bisphosphonates, and other agents has been reported to be helpful.[3,58,83]

Diagnosis

The diagnosis of osteomyelitis generally is suspected based on the presence of fever, focal skeletal pain, warmth, swelling, and a limp or refusal to use an extremity. The diagnosis is confirmed by identification of organisms by culture or Gram stain in an aspirate of bone or by histopathologic evidence of inflammation in surgical specimens of bone. In an otherwise healthy individual, the diagnosis is probable when a patient has fever, leukocytosis, elevated acute-phase reactants (elevated CRP or ESR), or a positive blood culture plus one or more of the following: abnormal results of imaging studies (plain radiograph, magnetic resonance imaging [MRI], or computed tomography [CT]), scintigraphy, or physical findings consistent with osteomyelitis.

Microbiology

The cornerstone of the diagnosis of osteomyelitis is isolation of bacteria or other microbes from bone or from anatomic structures contiguous to bone. Overall, such cultures (aspirates or surgical specimens from bone débridement, subperiosteal exudate, or joint fluid) provide a bacteriologic diagnosis in 66 to 76 percent of cases. Blood cultures yield an organism in about half of cases (36% to 74% of patients in several series).[165,219,223,227] Recovery of *Kingella* is enhanced by placing aspirates of fluid into blood culture bottles.[239]

In neonates, needle aspiration of soft tissue or incision and drainage of bone may yield the offending organism. In infants and young children, subperiosteal needle aspiration can be done if the point tenderness is localized. In older children and adolescents, noninvasive culturing of the bone is less rewarding. In this age group, windowing or drilling to drain pus from the bone yields valuable material for culture but is controversial; some orthopedic surgeons consider the risk of causing epiphyseal damage and subsequent length discrepancy secondary to the procedure too great. Consequently, blood cultures and imaging methods often represent the extent of the diagnostic evaluation. Nonetheless, it is important to attempt to obtain bacterial cultures early in the evaluation of

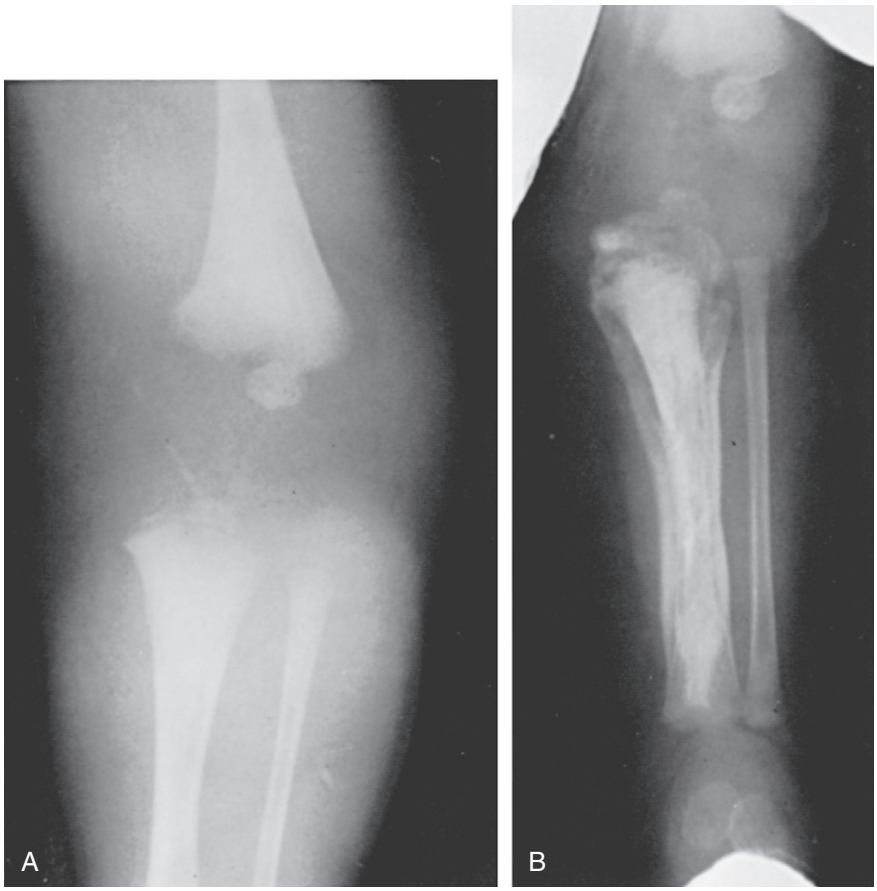

FIGURE 55-3 ■ **A,** The left knee of an infant shows diffuse soft tissue swelling around the proximal ends of the tibia and fibula. **B,** Six weeks later, the subcutaneous fat lines between the muscles and the skin can be seen, as can an involucrum involving almost all of the tibia.

suspected osteomyelitis because decisions about the type and duration of antimicrobial therapy are greatly facilitated by knowing the susceptibility profile of the etiologic agent.

Radiology

Plain Radiographs. Conventional radiographs are crucial in establishing the diagnosis of pediatric osteomyelitis and always should be obtained.[29,44,104] Because bone density must decrease 50 percent to be detected by radiographs,[11] changes in the less ossified bones of neonates are detected more readily than are changes in older children. In contrast, Waldvogel and Papageorgiou[228] found in adults that plain radiographs were of no diagnostic value in 23 percent and were misleading in an additional 16 percent.

Radiographic changes occur in three stages.[37] The first stage, which occurs approximately 3 days after the onset of symptoms, is the formation of a small area of localized, deep soft tissue swelling, usually in the region of the metaphysis (Fig. 55-3). Consequently, when diagnosis of osteomyelitis is sought soon after onset of symptoms, examination of the radiograph should be directed to the soft tissue rather than the bone. During the second stage, which occurs 3 to 7 days after the onset of symptoms, swelling of the muscles with obliteration of the interposed translucent fat planes can be noted. It is caused by continued spread of edema fluid and can progress, particularly in neonates and young infants, to superficial soft tissue edema; the skin may acquire an orange-peel texture.

Radiographic evidence of bone destruction usually is not detected until 10 to 21 days after the onset of symptoms. The first changes detected include subperiosteal bone resorption, areas of bone destruction, and periosteal new bone formation. The variability depends on the specific bone involved; generally, long tubular bones tend to show bony changes 2 to 3 weeks earlier than membranous or irregular bones.

Magnetic Resonance Imaging. MRI has become the imaging modality of choice when additional imaging is needed beyond plain radiographs and has sensitivity in excess of 90%.* The major advantages of MRI are that it accurately delineates subperiosteal or soft tissue collections of pus that might require surgical drainage without using ionizing radiation, and it can identify sinus tracts for removal (Fig. 55-4).[29,76,177,209] MRI often provides more specific anatomic information than CT or plain radiography.

In acute osteomyelitis, bone marrow edema caused by the accumulation of purulent material leads to decreased signal on T1-weighted images. On T2-weighted images of the same area, increased signal is seen. With commonly used sequences, the sensitivity of MRI for the

*References 24, 29, 44, 73, 147, 151.

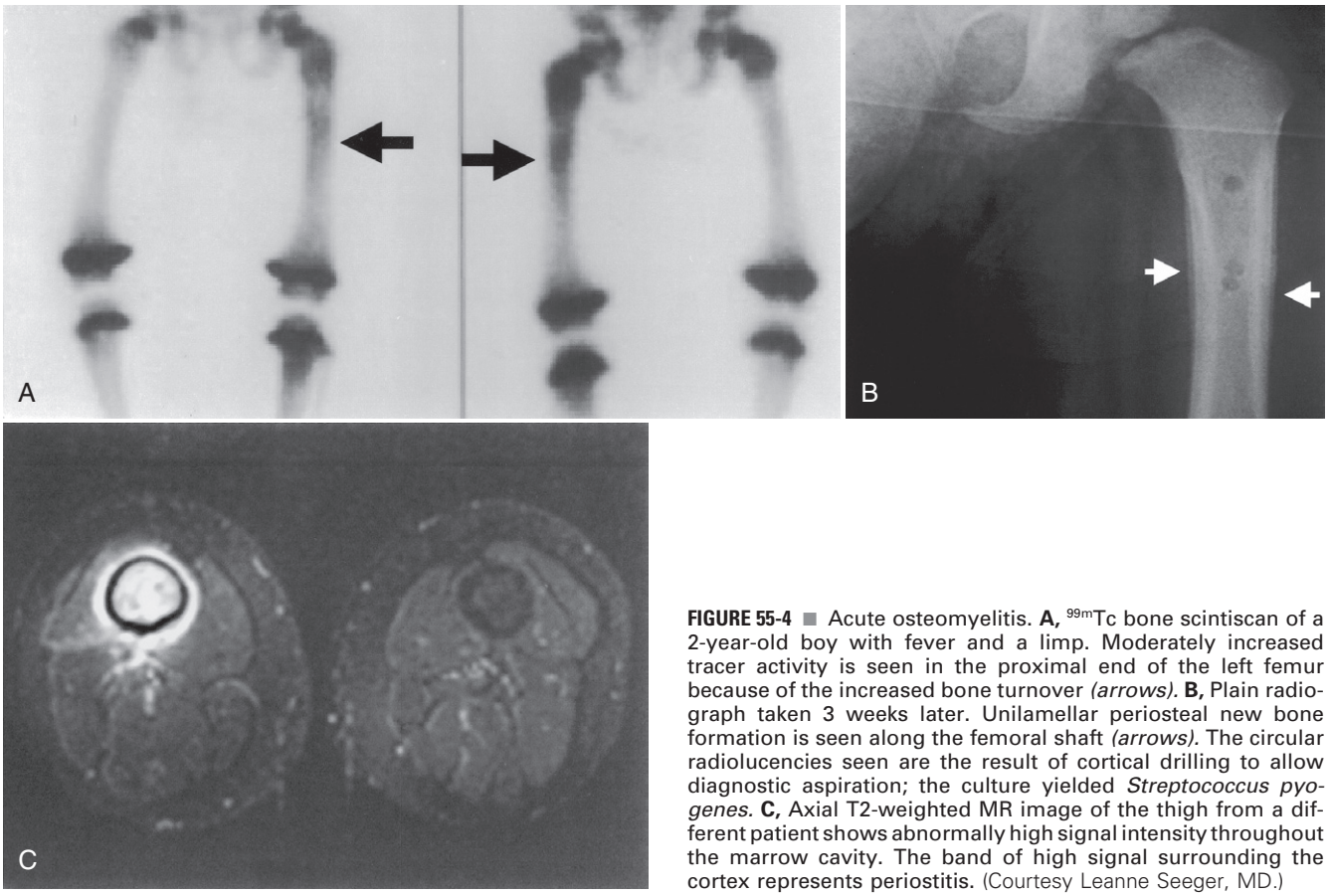

FIGURE 55-4 ■ Acute osteomyelitis. **A,** ^{99m}Tc bone scintiscan of a 2-year-old boy with fever and a limp. Moderately increased tracer activity is seen in the proximal end of the left femur because of the increased bone turnover *(arrows).* **B,** Plain radiograph taken 3 weeks later. Unilamellar periosteal new bone formation is seen along the femoral shaft *(arrows).* The circular radiolucencies seen are the result of cortical drilling to allow diagnostic aspiration; the culture yielded *Streptococcus pyogenes.* **C,** Axial T2-weighted MR image of the thigh from a different patient shows abnormally high signal intensity throughout the marrow cavity. The band of high signal surrounding the cortex represents periostitis. (Courtesy Leanne Seeger, MD.)

detection of acute osteomyelitis may approach 100 percent.[151] Fat-suppression sequences, including short-tau inversion recovery, decrease the signal from fat. Inversion recovery sequences allow more sensitive detection of bone marrow edema. MRI may have a particular advantage in establishing the diagnosis of spinal osteomyelitis because a clear distinction can be made between the vertebral body and the adjacent disk. Loss of this border is one of the first abnormalities detected by MRI in spinal osteomyelitis.

The need for sedation in most infants and children and the cost of MRI are the major disadvantages that continue to limit its use. In addition, infarction and other processes can alter the appearance of bone marrow and lower the specificity of MRI and must be considered in the interpretation of the imaging study.

Radionuclide Imaging. Radionuclide scanning has been used for decades in the evaluation of suspected osteomyelitis. Despite the fact that bone scintigraphy involves exposure to ionizing radiation, it continues to be used widely because of its ready availability and utility in detecting multifocal disease.[64] Bone imaging employing technetium-99m (^{99m}Tc) diphosphonate scintigraphy is used most frequently. After being injected, the phosphate adduct is adsorbed to the surface of the hydroxyapatite crystal in bone, and the isotope becomes concentrated in the cement line located at the junction of osteoid and mineralized bone.[216]

In most institutions a three-phase bone scintiscan is performed for evaluation of infection. Shortly after injection (2 to 5 seconds), a nuclear angiogram (flow phase) of the area of suspected osteomyelitis is obtained. The second (blood pool) phase consists of a single image obtained 5 to 10 minutes after injection. The third image is obtained 2 to 4 hours after injection. In this last phase, specificity of the diphosphonate compounds for the bone is revealed. Anything increasing local blood flow to the area, particularly if accompanied by inflammation, results in increased general uptake in the first two phases, but osteomyelitis results in focal uptake in the third phase, with the intensity of the signal detected reflecting the level of osteoblastic activity.*

Acute osteomyelitis in children is often diagnosed by a ^{99m}Tc scan and treated successfully before bone changes are detected by plain radiographs (see Fig. 55-4).[218] With sensitivity previously reported to be as high as 95 percent, bone scintiscanning has until recently been a reliable tool in establishing the diagnosis of osteomyelitis.[37] Unfortunately, in one recent report, bone scintigraphy detected only osteomyelitis caused by CA-MRSA cases in 53 percent (26/49) of children.[29] This apparent lack of sensitivity for CA-MRSA may reflect the acuity of disease caused by this pathogen, because changes in radionuclide scans are more characteristic when patients have had an illness of longer duration.[195]

*References 40, 55, 57, 64, 88, 128, 193.

In addition, bone scintiscans that use ^{99m}Tc may be nondiagnostic in neonates.[9,79] The false-negative result of bone scintiscans probably is due to the paucity of mineralization in neonates' bones. Ischemia of bone, probably caused by infarction, also has been noted on the initial bone scintiscan.[158] The sensitivity of bone scintigraphy in neonates varies in different studies of neonates and ranges from as little as 31 to as high as 90 percent.*

Older infants with osteomyelitis and a nondiagnostic ^{99m}Tc bone scintiscan also have been described.[26,103] In such instances, a gallium-67 (^{67}Ga) scan may be valuable. ^{67}Ga is a transition metal that, similar to iron, is bound to plasma proteins; the unbound portion (10% to 25%) is excreted in urine. It localizes in inflammatory foci because of increased capillary permeability (leaking plasma proteins), in vivo leukocyte labeling, binding to lactoferrin in the lesion, and perhaps direct bacterial uptake. Because of the slower elimination of gallium from blood, its uptake in an inflammatory focus depends less on blood flow. Delayed elimination often results in poor contrast of bone to soft tissue, however, and delays making a reliable interpretation for 24 to 72 hours after injection.[109] In one study, 15 of 16 cases of osteomyelitis with a nondiagnostic ^{99m}Tc scan had changes on ^{67}Ga scan typical of osteomyelitis.[201] Combined evaluation with ^{67}Ga imaging and ^{99m}Tc bone scintigraphy may lead to greater diagnostic certainty when the studies are not conclusively diagnostic.[198,238] Despite these successes, ^{67}Ga scans are seldom employed because repeating MRI or plain radiographs and clinical improvement during therapy usually are sufficient to resolve initial diagnostic uncertainties.

Numerous studies have been conducted to examine the utility of indium 111–labeled leukocyte scans for the diagnosis of osteomyelitis. This method involves removing leukocytes and injecting them back into the patient after in vitro labeling. It has a reported sensitivity of 86 percent,[190] which seems to be best for the detection of lesions in long bones. False-positive scans can result from a variety of processes, including fracture and infarction. Enthusiasm for this modality also is limited by the higher organ absorption of the radiation dose.[81] Numerous other scintigraphic methods for the detection of osteomyelitis, including ^{99m}Tc-hexamethylpropyleneamine oxime–labeled leukocytes and monoclonal antibodies, have been examined. At present, these alternative methods do not offer any advantage over MRI and older radionuclide imaging approaches.

Computed Tomography. CT is used occasionally in the diagnosis and management of osteomyelitis because it provides excellent definition of cortical bone and high spatial resolution. CT abnormalities commonly found in osteomyelitis include increased density of bone marrow caused by the accumulation of purulent material and periosteal new bone formation and purulence. CT is particularly useful in detecting sequestra and delineating subperiosteal abscesses. It previously has been used to define infections of the spine, but MRI has largely replaced CT for this indication.[73,118]

*References 9, 56, 66, 79, 126, 158, 232.

Treatment of Acute Hematogenous Osteomyelitis

The need for surgical therapy must be considered immediately when osteomyelitis is diagnosed. Reports of soft tissue, subperiosteal, and intramedullary abscesses have become common in the early 21st century, mirroring the increased prevalence of skin, soft tissue, and musculoskeletal infections caused by CA-MRSA.[8,120,148] MRI, ultrasonography, and CT may prove useful in assessing these purulent foci.[29] Drainage of these sites by surgical or interventional radiology techniques also provides the opportunity to obtain cultures to confirm a microbiologic diagnosis. Sequestra should be removed if present. If contiguous infectious foci are present, they should be débrided adequately and treated with effective antimicrobial therapy. Immobilization of the affected extremity or splinting may afford relief from pain and sometimes is used to prevent the development of pathologic fractures when extensive bone involvement is detected by plain radiography.

The initial therapy should have potent activity against *S. aureus* and group A streptococci because these pathogens represent the primary causes of osteomyelitis. Acute bacterial hematogenous osteomyelitis should be treated initially with parenteral anti-infective agents, in view of the high mortality rate in *S. aureus* osteomyelitis seen in the preantibiotic era and more recent reports of fulminant disease with CA-MRSA.[94,95,99,111,148]

The choice of initial therapy has become more complex with the marked increase in prevalence of CA-MRSA in the United States and other countries. In areas where most (≥90%) *S. aureus* isolates remain methicillin susceptible, initial therapy may consist of a penicillinase-resistant, semisynthetic penicillin, such as nafcillin or oxacillin, administered parenterally in a dosage of 150 to 200 mg/kg/day in four divided doses.[98,114,184] Cefazolin, a first-generation cephalosporin, and cefuroxime, a second-generation cephalosporin, have also been used for empirical therapy, with good results, and may be preferred in infants and children younger than 36 months of age if *Kingella kingae* is strongly suspected based on clinical features.[165,240] In areas where CA-MRSA is common, either vancomycin or clindamycin (if >90% of CA-MRSA isolates are susceptible) should be included in the initial empirical therapy for CA-MRSA.[120,149] Currently, most isolates of CA-MRSA remain susceptible to clindamycin, but clinical laboratories should screen for inducible macrolide-lincosamide-streptogramin resistance using the D-test or similar methodology.[8,120,136]

Although protein conjugate vaccines have all but eliminated *H. influenzae* type B infections in the United States, antimicrobial coverage for this possibility should be considered for younger children who have not yet completed their immunization series. In these circumstances, addition of a third-generation cephalosporin (ceftriaxone or cefotaxime) to the empirical antistaphylococcal agent (vancomycin, clindamycin, oxacillin, or nafcillin) might be warranted. Other agents may be administered when epidemiologic factors suggest the possible presence of other pathogens: third- or fourth-generation cephalosporins for *P. aeruginosa*, *Salmonella*

species, or other enteric gram-negative organisms and clindamycin for suspected anaerobic infections.[70,183]

When an organism is isolated or identified by other means, antimicrobial therapy can be chosen with greater specificity. Staphylococci should be treated with penicillin G if the organisms are susceptible to this antibiotic. In most cases, staphylococci must be treated with a penicillinase-resistant penicillin (oxacillin or nafcillin), clindamycin, or vancomycin. Ceftriaxone also has proved successful in the treatment of most cases of MSSA. Oxazolidinone (linezolid) and streptogramin (quinupristin-dalfopristin) drugs have been used successfully for the treatment of osteomyelitis with MRSA and MSSA[61] and for the treatment of vancomycin-resistant enterococci, but this experience is limited.[41]

Trimethoprim-sulfamethoxazole (cotrimoxazole) and the lipophilic tetracyclines (minocycline and doxycycline) are readily absorbed by the oral route and have been used successfully in the treatment of osteomyelitis in both children and adults. One study reported consistent success in 20 children treated with cotrimoxazole, including several children with CA-MRSA infections.[154] However, experience remains limited with using cotrimoxazole or tetracyclines, especially for treatment of CA-MRSA infections in which pneumonia and bacteremia are common, and the potential for dental staining with tetracyclines must be kept in mind. Daptomycin, a bactericidal lipopeptide antimicrobial agent, has excellent activity against CA-MRSA, but pediatric pharmacokinetic data and clinical experience also are limited. Moreover, daptomycin has poor activity in lung tissue, adding to concerns about the utility of daptomycin for the treatment of CA-MRSA osteomyelitis.[41,94,95,120]

Osteomyelitis caused by *S. pneumoniae* strains with decreased susceptibility to penicillin have been managed successfully with a variety of agents, including ceftriaxone, vancomycin, and clindamycin.[26] β-Lactam antibiotics, including oxacillin, nafcillin, and cephalosporins, have been used successfully in the treatment of *K. kingae* infection, although cefazolin may be preferable to antistaphylococcal penicillins, because the latter have relatively lower activity.[40,96,239-241]

Decades of experience and clinical investigation have led to wide acceptance of sequential use of the intravenous and oral routes of administration of antibiotics to treat pediatric osteomyelitis, which previously was controversial. Completing treatment with oral therapy avoids the cost, pain, inconvenience, and well-known complications of long-term intravenous administration of antibiotics. The relative safety of central venous catheters has not obviated these concerns. To the contrary, local skin infections, bacteremia, and malfunctions of the catheters are common occurrences when used for osteomyelitis treatment, especially in young children.[188,244]

Oral therapy is most likely to succeed, and oral therapy is an acceptable option when the following criteria are met: an organism has been identified, the patient has the ability to swallow and retain an appropriate medication, the laboratory is able to monitor the degree of antibiotic absorption, and the patient has a clear clinical response to intravenously administered antibiotics.[165,167] In most series in which oral therapy has been evaluated,[32,59,165,215,229] treatment was continued with intravenous antibiotics until the patient was afebrile, until local signs and symptoms of infection were reduced considerably, and until the patient was maintaining caloric and fluid balances by the oral route. In severe or complicated cases, an advisable approach is to delay switching to the oral route until the peripheral leukocyte count has normalized, and the ESR has decreased by 20 percent or more, or until a marked decrease in the concentration of CRP has occurred.[184,223] A review of case series employing sequential therapy affirmed these recommendations and found no evidence that a fixed period of intravenous therapy is beneficial or essential.[132] A transition to oral therapy within 7 days of diagnosis seemed to be equal in outcome to therapy with a fixed initial period of parenteral therapy.

When oral therapy is begun, most antibiotics administered orally for osteomyelitis must be given in doses higher than the doses used for the treatment of other infections.[11,166,215] Specific antibiotics and the recommended starting doses are listed in Table 55-3. Dosages of β-lactams often can be increased to 200 mg/kg/day without having serious adverse side effects. Diarrhea, an infrequent complication of high-dose, oral β-lactam therapy, can be mitigated by a reduction in dose and the administration of probenecid 40 mg/kg/day every 6 hours, with a maximal dose of 2 g/day.[32,165,215] Clindamycin readily achieves high bone levels and does not require higher dose therapy when given orally.[121,149]

The assumption implicit in successful oral therapy is that the antibiotic reaches an effective concentration at the focus of infection. Compliance with the prescribed dose and frequency and absorption into the bloodstream are necessary to fulfill this assumption. Patient (or parent) education and a continuing time commitment by a physician or nurse are essential to maintaining the compliance required for successful treatment. Although eschewed by some physicians,[146,171] therapeutic drug monitoring (TDM) is recommended by some specialists to show that adequate absorption of orally administered antibiotics is occurring.[166] This recommendation stems from the observation that rare patients have inadequate serum levels despite receiving high oral dosages. TDM also can be used to document adherence to the therapeutic regimen.[215]

TDM was initially performed by measuring serum bactericidal activity, targeting a peak serum bactericidal

TABLE 55-3 **Initial Antibiotic Doses for Oral Treatment of Osteomyelitis**

Drug	Dose (mg/kg/day)	Interval between Doses (hr)
Amoxicillin	100	6
Cephalexin	150	6
Chloramphenicol	75	8
Clindamycin	40	8
Cloxacillin	125	6
Dicloxacillin	100	6
Penicillin V	125	4

Data from references 32, 84, 165, and 215.

titer of 1:8 or greater.[178,215] However, this assay is confounded by other antibiotics present in the serum sample; for example, nafcillin administered intravenously would interfere with evaluating the oral bioavailability of oral dicloxacillin. Chemical assays of a specific agent circumvent this problem. Dicloxacillin can be administered orally and the dose adjusted based on dicloxacillin measurements before the intravenous administration of another β-lactam has been discontinued. This procedure avoids several days of inadequate therapy should the dosage of the oral agent need to be adjusted.

In most cases, clinical improvement is evident within 3 to 7 days of the initiation of appropriate therapy. To help show that effective therapy is underway, it is helpful to monitor acute-phase reactants. Although CRP and ESR usually increase during the first 3 days of therapy, CRP then begins to decrease rapidly and typically returns to normal in 7 to 10 days. CRP returns to normal levels in blood more rapidly in children with an uneventful clinical course than in children who ultimately require repeated surgical drainage.[184] A slow decline in serum CRP to normal levels has been associated with more extensive radiographic changes or persistent symptoms 1 to 2 months after discharge from the hospital. Similar to CRP, ESR generally increases during the first several days but declines in the weeks that follow.[223] Failure of ESR to decrease during the second week of treatment may indicate a need for surgical drainage or the development of chronic osteomyelitis.[208]

Prolonged antibiotic therapy is essential for successful treatment of acute osteomyelitis. In one early report of 45 cases of osteomyelitis, four treatment failures occurred; all of the patients had been treated for 10 days or less.[105] Likewise, Dich and colleagues[59] noted a 19 percent failure rate in 37 patients treated for 3 weeks or less; the rate was 2 percent in 48 patients treated for 21 to 50 days. Blockey and Watson[22] provided similar data. Thus, 3 weeks or more appears to be the minimal duration of therapy for hematogenous osteomyelitis to minimize the risk of recurrence. A conservative but individualized approach is to administer antibiotics until the ESR and CRP are within the normal range, which usually requires 4 to 6 weeks of treatment.[120,165–167,208]

Special Manifestations of Hematogenous Osteomyelitis

Brodie Abscess

Osteomyelitis occasionally is indolent in presentation. Perhaps the best defined example is subacute osteomyelitis with the development of an intraosseous abscess (Brodie abscess).[204] These patients most often are adolescents with complaints of long bone pain and tenderness. The ESR usually is normal at the time of presentation. A bony defect with sclerotic margins is detected by plain radiography in most patients. A distinctive "target" lesion has been described after MRI of Brodie abscesses.[147] Concentric layers are seen and reflect a central abscess cavity surrounded by an inner ring of granulation tissue, an outer ring of fibrotic reaction, and a peripheral rim of endosteal reaction that is hypointense on T1-weighted images.

Treatment consists of surgical drainage and curettage followed by antimicrobial therapy, as for other forms of hematogenous osteomyelitis. A variety of organisms, including *H. influenzae*, have been isolated from these lesions,[129] but *S. aureus* and other gram-positive cocci are the pathogens usually involved. The prognosis generally is good, although deformities occur in some cases.

Osteomyelitis in Patients After Closed Fractures

Acute hematogenous osteomyelitis sometimes develops after closed fractures of tubular bones occur.[36,230] A clue to diagnosis is the resumption of pain after the initial postfracture pain has subsided, usually 1 to 6 weeks after the injury occurs. The pain differs from that associated with the fracture by being progressive, and it is not relieved by immobilization. Patients are febrile and may be thought to have another focus of infection before osteomyelitis is discovered. When the cast is removed, local erythema, fluctuance, and warmth are apparent and out of proportion to the normal healing of fracture. Although usually caused by *S. aureus*, anaerobic superinfection of staphylococcal osteomyelitis at the fracture site has been described.[213] Successful therapy requires adequate débridement, administration of appropriate antibiotics, and external fixation in some cases.

Epiphyseal Osteomyelitis

Rarely, hematogenous osteomyelitis may arise in the epiphyses of the tubular bones of young children.[1,86,142,220] Although the pathogenesis is unclear, it may involve delivery of microorganisms to the epiphyses by transphyseal vessels. After the child reaches 15 to 18 months of age, these vessels are lost, and the physis acts as a physical barrier to the spread of infection from the metaphysis. The vascular anatomy of the epiphysis is very similar to that of the metaphysis, however, and in some cases infection may occur when bacteria are delivered to venous sinusoids by terminal branches of the epiphyseal arteries.

Hematogenous epiphyseal osteomyelitis may be acute or subacute. In the acute manifestation, septic arthritis initially may be diagnosed when joint swelling occurs and abnormal fluids are removed by diagnostic aspiration. In cases with a more indolent course, pain, limp, or other symptoms prompt an evaluation for the possibility of an osteoarticular infection. The correct diagnosis typically is established when a radionuclide bone scintiscan or plain radiographs taken weeks later show evidence of increased bone turnover or the lytic changes characteristic of osteomyelitis. Administration of appropriate therapy for epiphyseal osteomyelitis has been followed by complete recovery without apparent sequelae 2 to 6 years after diagnosis.

Involvement of Nontubular Bones

Less than 20 percent of all cases of osteomyelitis involve nontubular bones. Infection of the calcaneus seems to be

the most common (see Table 55-2).[165] In patients with hematogenous infection, destruction occurs just under the epiphyseal line in the metaphysis posteriorly and medially, where the blood supply is greatest. It is present in all patients, in addition to destruction of the adjacent epiphysis, particularly in its middle to superior portion. Periosteal new bone formation occurs very late, with 3 to 4 months required for reossification. Osteomyelitis in the other cuboidal bones rarely occurs.

Almost equal in frequency to infection of the calcaneus is infection of the bones of the pelvis.[165] Of the bones of the pelvis, the ischium is involved most commonly. The next most frequently involved bone is the ilium, followed by the sacroiliac joint. The pubis is involved in only 20 percent of cases of pelvic osteomyelitis.[107,163] Pelvic osteomyelitis causes an increase in the ESR in nearly all patients, and two thirds have a peripheral leukocyte count greater than 10,000 cells/mm^3.[163] The most common organism causing pelvic osteomyelitis is *S. aureus*, which is isolated from either blood or an aspirate from the bone lesions in approximately 80 percent of cases.

Establishing the diagnosis of pelvic osteomyelitis often is difficult. Most patients are judged to have disease in the hip at the time that medical attention is sought. Most often, patients with pelvic osteomyelitis have hip pain and a gait abnormality but allow their hips to be put through a passive range of motion. Point tenderness at the site of the lesion can be elicited in approximately 50 percent of these patients. Sacroiliitis frequently is difficult to identify by clinical examination. Pressing down on the pelvis, which stresses the sacroiliac joint, produces local pain. Tenderness in the buttocks or the sciatic notch, if present, is an invaluable diagnostic finding.[2,43,63,159,233] Pelvic osteomyelitis can mimic appendicitis and urinary tract infection[233]; it occurs more frequently in individuals with inflammatory bowel disease. In most patients, plain films of the pelvis are not rewarding, whereas ^{99m}Tc bone scintiscans indicate the diagnosis in approximately 90 percent of cases.[144,163] CT may reveal infection not evident by bone scintigraphy. MRI also is likely to identify pelvic osteomyelitis.[222] Antibiotic therapy alone is adequate in most cases of pelvic osteomyelitis. Surgery is indicated only when a lack of response to antimicrobial therapy occurs. Osteomyelitis in the pelvic bones has a good prognosis; chronic infection and sequelae are rare events.

Hematogenous osteomyelitis of flat bones occurs rarely and has been described in the skull, ribs, sternum, and scapula.[91,160,165,224]

Spinal Osteomyelitis

Spinal osteomyelitis can involve either the intervertebral disk or the vertebral bodies per se. Conceptualizing these infections as different entities is worthwhile because of the different pathophysiology and prognosis.[73]

Diskitis. The intervertebral disk consists of three components: the paired cartilaginous articulation, the fibrous ring (anulus fibrosus), and the nucleus pulposus.[25,73] The axial vessels that parallel the fetal notochord are atrophied by birth, with the avascular, mucilaginous nucleus pulposus remaining. The disk has two arterial supplies: periosteal vessels and vessels descending from the central portion of the vertebral body.[48,72] The vessel from the central portion of the adjacent vertebra begins to atrophy in the first year and is obliterated completely by the time the child is 10 years old. This condition leaves only the capillary network in the anulus fibrosus, which is derived from the terminal radial ramifications of the periosteal vessels. If loss of this vascular supply is precipitous, idiopathic disk necrosis ensues, usually manifested as asymptomatic calcification.[191,206,212] If bacteremia occurs during loss of the blood supply, however, infection of an intervertebral disk may occur.

Infection of an intervertebral disk has no sex preponderance, and most children are younger than 5 years old.[73,197] It occurs almost exclusively in the lumbar region (the L4 and L5 disks are involved most frequently, followed by L3 and L4). The disease comes to medical attention with the patient's refusal to walk. Backache and a progressive limp may be present. Nonambulatory infants often become irritable and refuse to sit. On examination, the most striking feature is percussion tenderness over the contiguous spine; hip pain and stiffness with loss of lordosis of the lower part of the back are observed. Occasionally, compression of the spine produces pain at the infected disk. The mean duration of symptoms before diagnosis is established ranges from 1 day to 18 months. Most patients have had symptoms for several weeks.

Lesions in higher locations (T8 to L1) can mimic gastrointestinal disease with abdominal pain, ileus, and vomiting, but the most important entities in the differential diagnosis are vertebral osteomyelitis and spinal or paraspinal tumors. Most patients have a history of a recent upper respiratory tract infection.[124,131,182,187,200] Fever generally is absent or of low grade. Peripheral leukocytosis is present in one third of patients, and virtually all have an increased ESR. A few patients undergoing biopsy have cultures that grow microorganisms. Most commonly, *S. aureus* is recovered, along with rare isolates of pneumococci and gram-negative organisms, including *K. kingae*. Evaluation for suspected diskitis usually involves careful elicitation of the history and physical examination, a complete blood cell count, determination of the ESR, a blood culture, and plain lateral radiographs of the lumbosacral spine.[73]

Typical plain radiographic findings are shown in Figure 55-5. The first finding is narrowing of the disk space, which usually is not detectable until 2 to 4 weeks after the onset of symptoms. Frequently, this narrowing is overlooked if loss of the normal progressive (from cephalad to caudad) increase in disk width is not appreciated. It is followed by destruction of the adjacent cartilaginous vertebral end plates and, subsequently, by herniation of the disk into the vertebral body. Rarely, compression or wedging of the vertebral body is noted. In older children, anterior spontaneous fusion is a common finding. In all individuals, reactive bone proliferation is a rare finding, as are paravertebral soft tissue masses.

Because of overlap of this syndrome with noninfectious disk necrosis, investigators in the earlier literature advocated treating this disease solely by bed rest. In view of the low yield and generally favorable prognosis of

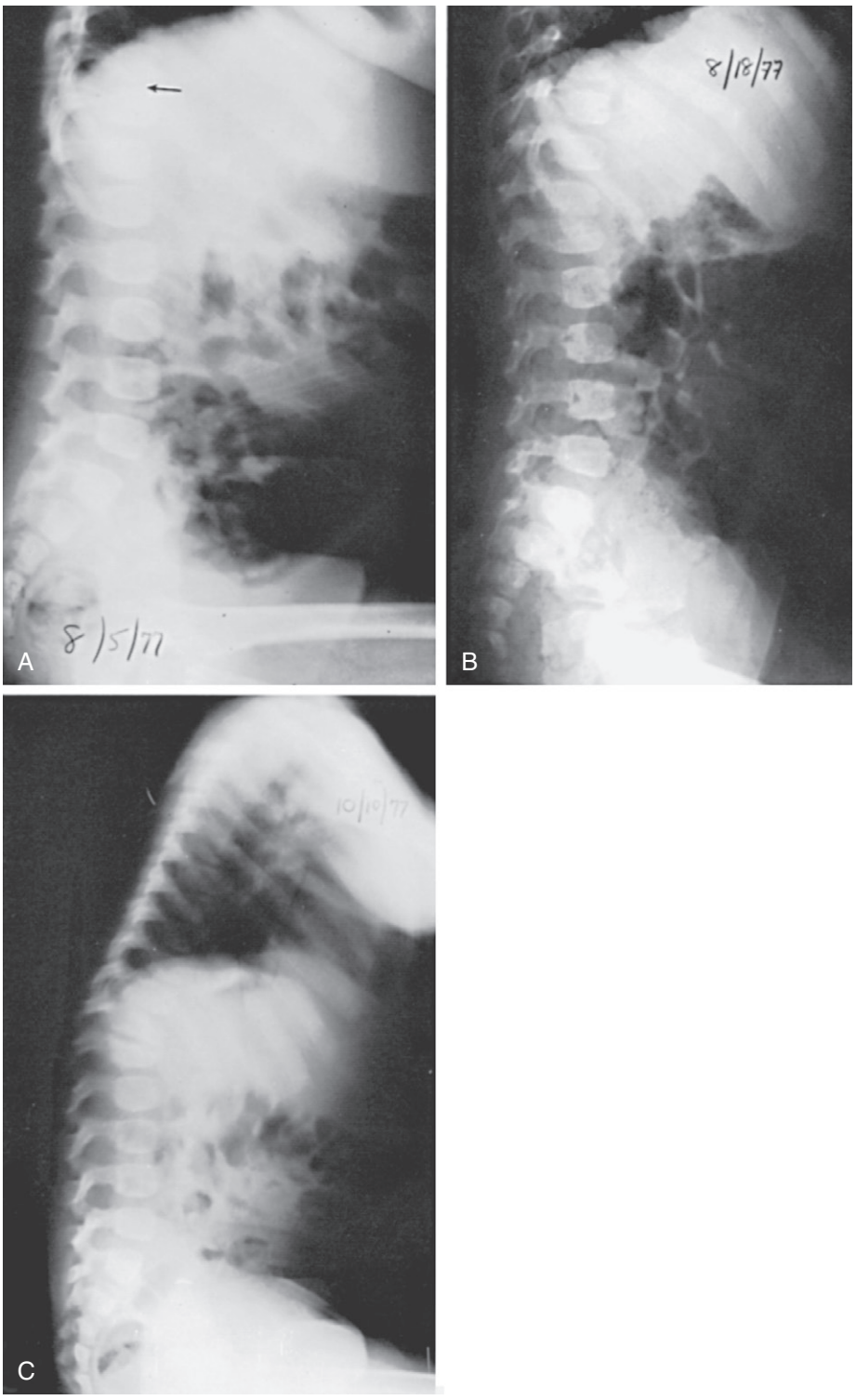

FIGURE 55-5 ■ Diskitis. **A,** Lateral lumbosacral spine of a 13-month-old child who had low-grade fever and refused to walk. The *arrow* indicates the narrow disk space. **B,** Follow-up examination 2 weeks later shows almost complete collapse of the intervertebral body. **C,** Two months later, destruction of the contiguous vertebra with marked kyphosis was apparent. (Courtesy Joel Blumhagen, MD.)

diskitis, biopsy or aspiration for culture generally is unnecessary. Although controversial, oral antistaphylococcal therapy often is administered for a prolonged period (5 to 6 months).[73] Other physicians have suggested giving 5 to 7 days of intravenous antistaphylococcal therapy, followed by 7 to 14 days of a similar oral agent.[50] Very young children generally do well, and the disk space is preserved; spontaneous spinal fusion occurs commonly in older individuals.

Vertebral Osteomyelitis. The intervertebral disk loses its vascular supply with age, a process usually completed

by 30 years of age. As a result, bacteremic pyogenic infections of the spine occur most often in the vertebral bodies. The venous drainage of the vertebral bodies is composed of three different freely communicating, but valveless, systems. Intraosseous vertebral veins drain the center of each body and form a large channel that exits through the nutrient foramen. They anastomose with the anterior and posterior internal plexus (between the dura mater and the vertebral body). The internal venous plexus has anastomotic connections with the external venous plexus through the vertebral ligaments. The external venous plexus communicates freely with the segmental veins on the ventral surface of the body wall (Batson plexus).

Reversal of blood flow or thrombosis in the sluggishly flowing vertebral veins is thought to be the first event in the development of vertebral osteomyelitis. Because of the vascular communication, osteomyelitis usually involves two adjacent vertebral bodies, with the interposed disk initially skipped. The same process of septic thrombophlebitis involving the internal venous plexus can produce an epidural abscess (with cord compression) and infarction of the vertebral body. Spread along the external venous plexus can lead to a paraspinous mass. In the thoracic area, it may produce mediastinitis[54]; and in the cervical area, a retropharyngeal abscess can occur. The lumbar area is involved much more frequently than is the thoracic area, which is involved more frequently than is the cervical region.[5,118,205]

Children with vertebral osteomyelitis usually are older than 8 years of age and seek medical attention because of constant back pain. They may appear toxic and have a low-grade fever (>39° C [102.2° F]) after an indolent course (2 weeks to several months).[73] Percussion of the spinal dorsal process frequently elicits exquisite tenderness. Usually, the paraspinous muscles around the involved vertebrae are in spasm, with rigidity of the area. Radiographic examination of a patient with vertebral osteomyelitis shows the earliest change to be localized rarefaction of one vertebral plateau, followed by involvement of adjacent vertebrae. Marked destruction of the bone, usually anteriorly, is followed by abundant anterior osteophytic reactions with bridging and bone sclerosis (Fig. 55-6).

MRI is useful in establishing the diagnosis of vertebral osteomyelitis[157] because the vertebral disk and the vertebral body are clearly discernible. Most cases of vertebral osteomyelitis begin at the margin between the disk and the anterior part of the vertebral body. In some cases, MRI has detected evidence of osteomyelitis when radionuclide imaging studies were normal.[217] Distinguishing pyogenic osteomyelitis from tuberculous lesions radiologically is impossible, but the latter usually are characterized by less bone destruction, less bone proliferation, and less sclerosis. The osteophytic bridging between adjacent vertebrae is extremely rare in spinal tuberculosis.

^{99m}Tc and ^{67}Ga are taken up by the lesion in vertebral osteomyelitis. A possible advantage of scanning with both isotopes is identification of paraspinous abscess with ^{67}Ga,[189] but MRI probably would accomplish the same goal.

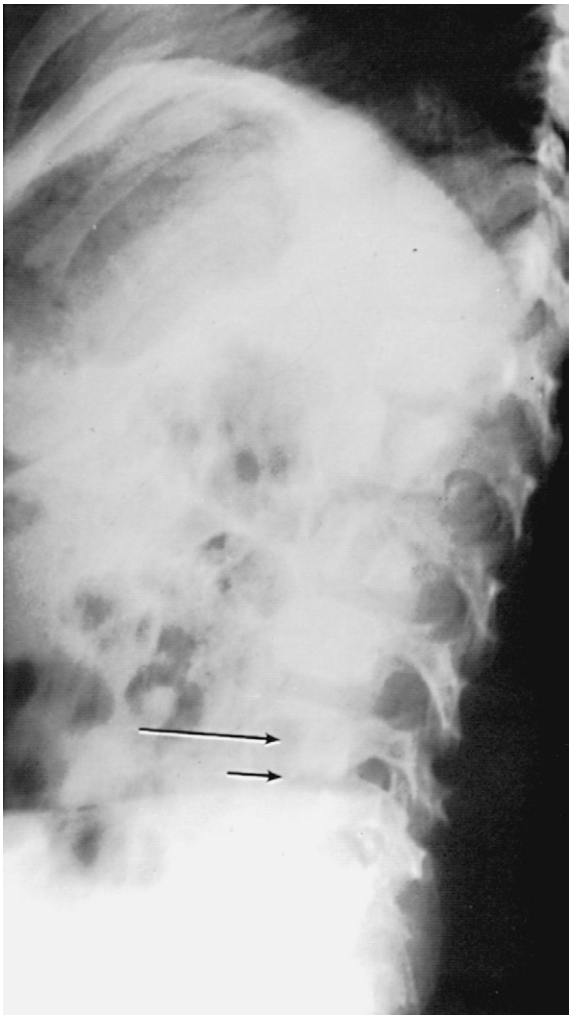

FIGURE 55-6 ■ Vertebral osteomyelitis. The *large arrow* indicates the lytic lesion with some anterior sclerosis in the lumbar vertebra. The *small arrow* indicates involvement of the adjacent lower vertebra and narrowing of the disk space. (Courtesy Joel Blumhagen, MD.)

Most cases of vertebral osteomyelitis are caused by *S. aureus*. Organisms causing urinary tract infection can cause osteomyelitis, presumably by local spread through the Batson plexus.[30,106,134] Urinary tract infection rarely precedes the development of vertebral osteomyelitis, however, and only approximately 2 percent of all cases can be shown to be related to infection of the urinary tract.[85] The best method of establishing the diagnosis is through examination of bone biopsy specimens and cultures.[164] *P. aeruginosa* is sometimes found to cause vertebral osteomyelitis in injectable drug users, and the organisms presumably were inoculated along with the illicit drug. Young heroin addicts have been found to have *P. aeruginosa* infection in their intervertebral disks.[31,194] In areas of the world where brucellosis is endemic, spinal osteomyelitis caused by *Brucella* spp. needs to be considered.[139] *Bartonella henselae* also has been found to cause vertebral osteomyelitis.[73] Fungal pathogens causing vertebral osteomyelitis include *Coccidioides* species in endemic areas and *Candida* spp. (often in immunocompromised patients).[155]

Therapy for vertebral osteomyelitis generally includes immobilization. Whether immobilization should be accomplished by simple bed rest or with a body cast is unclear, but bed rest is often used to provide pain relief. Administration of an antibiotic is always indicated. The average duration of treatment of bacterial vertebral osteomyelitis is 2 months; however, no data are available that can be used for determining the most appropriate duration. The need for surgical drainage in some cases should not be overlooked because spinal cord compression caused by an epidural or subdural abscess can lead to permanent paraplegia. A paraspinal focus of infection may rupture into the abdominal cavity or erode the aorta; both events are catastrophic complications.[80] The optimal therapy for fungal infection of the vertebra is unclear.

Hematogenous Osteomyelitis in Special Populations

Osteomyelitis in Neonates

Infection of the bones of neonates has distinct physiologic and clinical features that warrant emphasis.[10,56,238] Although it is rare, neonatal osteomyelitis may occur in neonates with certain risk factors, such as prematurity, skin infections, and complicated delivery.[80,126] As indicated earlier, the epiphyseal-metaphyseal junction frequently is within the joint capsule, and blood vessels that penetrate the epiphysis are common findings, particularly in the hip, shoulder, and knee of the neonate.

In neonates, osteomyelitis in the long tubular bones frequently (50% to 70% of cases) is accompanied by contiguous septic arthritis. Fever is present in one third to one half of these young patients, and, of these, half have a septic or toxic appearance. Fifty percent have multiple bone involvement. Antecedent infections are present in half of the cases and usually are nosocomial; infections of heel puncture sites, arterial cannulas, lungs, and cut-down sites and cephalhematomas have been described.[10] *S. aureus* is the etiologic agent in more than 90 percent of cases; gram-negative bacilli cause a few cases.[238] Osteomyelitis often is identified when a search is instituted for the source or focus of bacteremia.[238] As noted earlier, plain radiographs often reveal evidence of osteomyelitis at the time of diagnosis, whereas bone scintigraphy may be negative. Bone scintigraphy may be useful, however, in excluding the diagnosis of osteomyelitis; in one study, the negative predictive value was estimated at 82 percent.[238]

Although staphylococci seem to be the most common cause of osteomyelitis in infants, group B streptococci have been found consistently in case series.[66] Infants with group B streptococcal osteomyelitis generally are older (2 to 4 weeks old), have no recognized preceding infection, and have only a single bone involved (often the right tibia or humerus).[10]

Osteomyelitis in the skull, an uncommon disease, can occur in neonates. Frequently, it is associated with a cephalhematoma, with or without loss of skin integrity,[133] but it can be caused by fetal monitoring without a cephalhematoma.[152] Numerous bacteria have been isolated from such lesions; as one might expect, most of these bacteria have been present as vaginal flora. Radiographic changes (i.e., bony erosion) are a late finding, but CT of the skull seems to assist in establishing the diagnosis. Infection of a cephalhematoma should be considered if it is enlarging, if it is inflamed, or if laboratory evidence of infection is present, such as an increased CRP concentration or leukocyte count. In most cases, the lesion is drained and treated with an antibiotic appropriate for the infecting organism.

In older descriptions of neonatal osteomyelitis, sequelae were common findings. In more recent series,[126,236] approximately three fourths of all cases have had good outcomes, even when the hip has been involved. When seen, sequelae include avascular necrosis of the femoral head, bony deformities, and shortening of the involved limb.[174]

Osteomyelitis in Long-Term Hemodialysis Patients

Patients undergoing long-term hemodialysis, with multiple invasions of the vascular compartment, seem to be at greater risk for development of hematogenous osteomyelitis.[135] Their indwelling intravenous cannulas can be colonized with coagulase-negative staphylococci or *S. aureus*, and osteomyelitis may develop. The thoracic spine and ribs are the bones most commonly involved; other tubular and cuboidal bones that have been traumatized also can become infected.

Osteomyelitis in Children with Hemoglobinopathies

After pneumonia, osteomyelitis is the most common serious infection in children with sickle-cell disease.[14] Children at risk are children with hemoglobin SS, hemoglobin S-Thal, or hemoglobin SO-Arab and certain children with hemoglobin SC disease.[101,199] The clinical manifestations of osteomyelitis are similar to those of other children with osteomyelitis, but there is a propensity for simultaneous involvement of multiple sites, a tendency toward recurrence, and a greater frequency in children 18 to 48 months of age.

As noted earlier, the microbiology of osteomyelitis in children with sickle-cell disease is complex and dominated by *Salmonella* spp.[14,34] Although *Salmonella* osteomyelitis occurs in less than 1 percent of normal patients with *Salmonella* bacteremia,[234] the frequency of *Salmonella* osteomyelitis in sickle-cell disease is several hundred times that occurring in the general population, with an incidence estimated at 0.36 percent per annum.[14,60,176] Seventy percent of all lesions or blood cultures in children with hemoglobinopathy and presumptive osteomyelitis yield *Salmonella* microorganisms; 10 percent contain *S. aureus;* and aerobic gram-negative rods, including *Shigella sonnei*,[186] *E. coli*,[100] *Serratia* spp.,[78] and *Arizona hinshawii*,[112] are isolated in 7 percent.

Many factors probably contribute to the greater incidence of osteomyelitis in patients with sickle-cell hemoglobinopathy. Injuries to the intestinal mucosa from local thrombosis during a thrombotic crisis may facilitate the entrance of *Salmonella* and other enteric organisms

into the bloodstream.[34,234] When infection of the bloodstream occurs, the splenic dysfunction in these patients may allow a prolonged period or greater magnitude of bacteremia. Evidence of impaired production of opsonic antibodies also exists. Whether bacteria lodge in infarcted bone or whether a different pathogenesis exists is unclear. Infarction occurs in the capital femoral epiphysis, hands, feet, and vertebrae, whereas osteomyelitis involves the metaphyseal-diaphyseal junction of long tubular bones.[67,176]

Infants with hand and foot syndrome are not distinguished easily from infants with osteomyelitis of the phalanges of the hands or tarsal bones of the feet.[28,47,231] The changes caused by osteomyelitis that are seen on plain radiographs are more severe than the changes expected in uncomplicated sickle-cell disease. The most common radiographic findings are a longitudinal intracortical diaphyseal fissure and overabundant periosteal new bone formation. The cortical fissures are thought to represent a layer of purulent exudate in and between the periosteal new bone and dead bone.[60]

Acute infarction and osteomyelitis cannot always be differentiated by MRI, perhaps because of preexisting abnormalities in bone marrow. MRI may be useful, however, for presurgical evaluation. Radionuclide scans also are used frequently to help differentiate osteomyelitis from bone infarction. In theory, bone infarction should have decreased uptake of ^{99m}Tc in the early "blood pool" phase of the scan; increased uptake would be found only as the lesion healed.[123] In one series with 34 sites of infarction, increased ^{99m}Tc uptake occurred in 10, normal uptake occurred in 9, and decreased uptake surrounded by zones of increased concentration occurred in the remaining 15 sites.[89] Ultrasound imaging also has been used to distinguish osteomyelitis and sickle-cell crisis.[25]

Generally, patients with bony infarcts have had dactylitis as an infant and multiple episodes; their temperature usually is less than 39° C (102.2° F). Children with hemoglobinopathy and osteomyelitis may lack this history and have a modest leukocytosis of immature granulocytes.[114] If fever, leukocytosis, and local symptoms persist despite provision of hydration and other supportive measures, needle aspiration of the area must be considered. Identification of an infectious pathogen is particularly important because of the large number of possible organisms involved.

Osteomyelitis in Patients with Human Immunodeficiency Virus Infection

Although recurrent invasive bacterial infections often complicate human immunodeficiency virus (HIV) infection in children and adults, osteomyelitis is not a particularly common complication.[113,153,203] Many of the existing reports involve patients with recent intravenous drug use, which probably acted as a predisposing factor. *S. aureus* was recovered most commonly, although *E. coli*, *Salmonella enteritidis*, *Cryptococcus neoformans*, *Mycobacterium kansasii*, *Histoplasma capsulatum*, and other organisms have also been reported. One recent report, however, suggests that *S. pneumoniae* osteomyelitis may be more common than *S. aureus* infections among HIV-infected children[180] who have not received protein-conjugate pneumococcal vaccines.

Osteomyelitis in Patients with Chronic Granulomatous Disease

The phagocytes of patients with chronic granulomatous disease fail to kill intracellular organisms, and infections with catalase-positive bacteria or fungi are frequent complications. Although staphylococci are a common cause of cutaneous infection in chronic granulomatous disease, osteomyelitis is caused most often by *Serratia* and *Aspergillus* spp. Osteomyelitis is attributed less often to staphylococci, *Pseudomonas*, *Burkholderia*, *Nocardia*, and other bacterial and fungal species.[237]

NONHEMATOGENOUS OSTEOMYELITIS

Puncture Wound Osteomyelitis

Inoculation osteomyelitis most often involves either the patella or the bones of the foot. Soft tissue infections occur after puncture wounds of the foot approximately 15 percent of the time, and osteomyelitis occurs in 1.5 percent of these injuries.[75] Osteomyelitis of the foot that develops after children sustain puncture wounds should be termed *osteochondritis* because it commonly includes infection of the articular cartilage in the metatarsals. Most patients are aged 9 to 18 years. After the initial pain of the puncture wound subsides (in 24 to 48 hours), the signs of osteochondritis appear after another 48 to 72 hours. Typically, joint tenderness and localized swelling, erythema, and pain over the entrance of the puncture wound are present. Fever is an infrequent finding, and the patient seldom has any other constitutional symptoms. No peripheral leukocytosis is noted, and the ESR is increased minimally.

Although the offending organism most commonly is *P. aeruginosa* (90% of the time), staphylococci, streptococci, *Stenotrophomonas maltophilia*,[12] and *Serratia marcescens*[162] also have been isolated.[27,75,115-117] In one series,[116] 20 percent of the cases involved infection with *P. aeruginosa* and *S. aureus*. The predominance of *Pseudomonas* can be explained partially on the basis of the mechanism of injury. *Pseudomonas* is not found commonly in surveys of the microbial flora of the skin of the feet,[93,156,210] but often it is found by culture of the sponge liner from children's sneakers.[74] In addition, many patients in whom *P. aeruginosa* osteochondritis develops have received prophylactic treatment with oral antibiotics that have activity against gram-positive bacteria. A semisynthetic penicillinase-resistant penicillin and an aminoglycoside or ceftazidime often are used as initial therapy.[115,116]

Surgical débridement of necrotic cartilage is a key element of treatment because foreign material frequently is found embedded in the soft tissue; débridement also provides the opportunity to identify organisms resistant to the initial antibiotic agents. The local signs and symptoms of infection usually resolve 4 to 5 days after adequate débridement is performed, and the patient is able to bear weight on the foot. In contrast, antibiotic therapy

alone after diagnostic aspiration may need to be continued 6 to 8 weeks before weight bearing is possible. Long-term follow-up of *P. aeruginosa* osteochondritis indicates that many patients have asymptomatic radiographic abnormalities.[16] Radiographic abnormalities are found more commonly in patients in whom an adjacent joint was involved initially.[16]

Osteomyelitis also has been described repeatedly after puncture wounds occur from stepping on wooden toothpicks. In these cases, *Eikenella corrodens*, a member of the human oral flora, has been found with other organisms. Surgical débridement to remove toothpick fragments and drain local abscesses is essential.[181]

Osteomyelitis of the patella is a disease of children aged 5 to 15 years old, the time in life during which the patella is vascularized. By adulthood, the vessels are almost completely atrophied. In almost all cases, the diagnosis shows the cause to be by inoculation, such as kneeling on a needle. The most common etiologic agent is *S. aureus;* signs of osteomyelitis appear 1 week after the puncture occurs. No constitutional symptoms occur, but extension of the leg produces pain over the anterior aspect of the patella. The diagnosis is made by isolating the organism from the patella. Radiographic confirmation of the diagnosis often requires 2 to 3 weeks. Because the patella lacks periosteum, no periosteal elevation is present, but rarefaction and sclerosis may be seen on the profile view or on tomograms. Treatment of this disease is similar to that for other forms of osteomyelitis.

Osteomyelitis Caused by Spread of Infection from a Contiguous Focus

In children, osteomyelitis related to an infected contiguous focus is a rare finding. Almost all cases of osteomyelitis from a contiguous source in childhood are nosocomial or are associated with infected burn wounds. The probability of postoperative osteomyelitis developing is a function of the surgeon's experience, the technique, the length of time that the wound was open, and whether prophylactic antibiotics were administered. The interval between the precipitating event and pain and the appearance of persistent sinus drainage or ulceration is 2 to 4 weeks. The peripheral white blood cell count often is normal, as is ESR. More than half of cases are caused by multiple organisms. When small draining sinuses are present, correlation between sinus culture and bone biopsy findings is good. With large open areas, organisms obtained by culture of the wound may not be important etiologically, and obtaining a bone biopsy specimen is necessary for making a definitive bacteriologic diagnosis. Staphylococci and streptococci predominate, although gram-negative organisms from the hospital environment often are seen.

Orthopedic Fixator Devices

Infection involving the wires or pins used for orthopedic stabilization represents a diagnostic and therapeutic challenge. Osteomyelitis is suspected when evidence of inflammation or infection is noted near these materials, but it seldom can be proved.[21] Plain radiographs are essential because they may reveal bone destruction at the site of entry of fixation pins. No controlled studies of treatment are available. Soft tissue débridement and removal of necrotic bone should be done as soon as possible, but fixation devices generally are left in place.[13,173] Prolonged therapy usually is given, guided by the susceptibility pattern of any organisms recovered from local cultures.[173]

UNUSUAL MICROBIAL CAUSES OF OSTEOMYELITIS

Actinomyces

More than half of all actinomycotic infections involve the facial or cervical area. The most common site of actinomycotic bone infection is the jaw, with the mandible being involved more frequently than the maxilla. Local signs and symptoms of fever and discharge from a sinus indicate the presence of the disease. Radiographically, periosteal elevation is followed by lytic changes. Often, several "egg shell" areas of new bone are present.

Forty percent of all actinomycotic infections occur in the vertebral bodies. In this illness, the infection almost always is associated with a focus elsewhere; the condition comes to medical attention because of mild pain, tenderness, and some stiffness. It can be distinguished from tuberculosis radiographically by the diffuse honeycombing of the vertebral bodies and the periosteal reaction; large lytic lesions usually are absent. Although no controlled trials of therapy have been done, long-term (>3 months) penicillin G therapy is indicated at doses of 150,000 U/kg body weight per day (i.e., approximately 100 mg/kg/day). Extensive débridement may be needed and has been linked to successful short-term treatment of mandibular actinomycosis.[15]

Brucella

Although an uncommon cause of osteomyelitis in developed countries, *Brucella* spp. are a well-known cause of skeletal infections, most often following the consumption of unpasteurized milk products.[4] *Brucella* spp. can produce abscesses in the vertebral bodies or long bones, although they are not striking features of the disease. The disease often is subacute in presentation, and anorexia, weight loss, sweating, adenopathy, and hepatosplenomegaly are common initial findings. Multidrug treatment is generally successful, with the addition of streptomycin or gentamicin during the first 2 to 3 weeks of therapy.

Fungi

Osteomyelitis may be caused by numerous endemic and opportunistic fungal agents, including various species of *Coccidioides*, *Cryptococcus*, *Candida*, *Blastomyces*, and *Aspergillus*.[127] *Sporothrix* infections occur but are rare.[243] Coccidioidomycosis often follows an earlier acute illness with cough, chest pain, night sweats, and anorexia, and it often is associated with erythema nodosum or erythema multiforme. This disease commonly is found in the

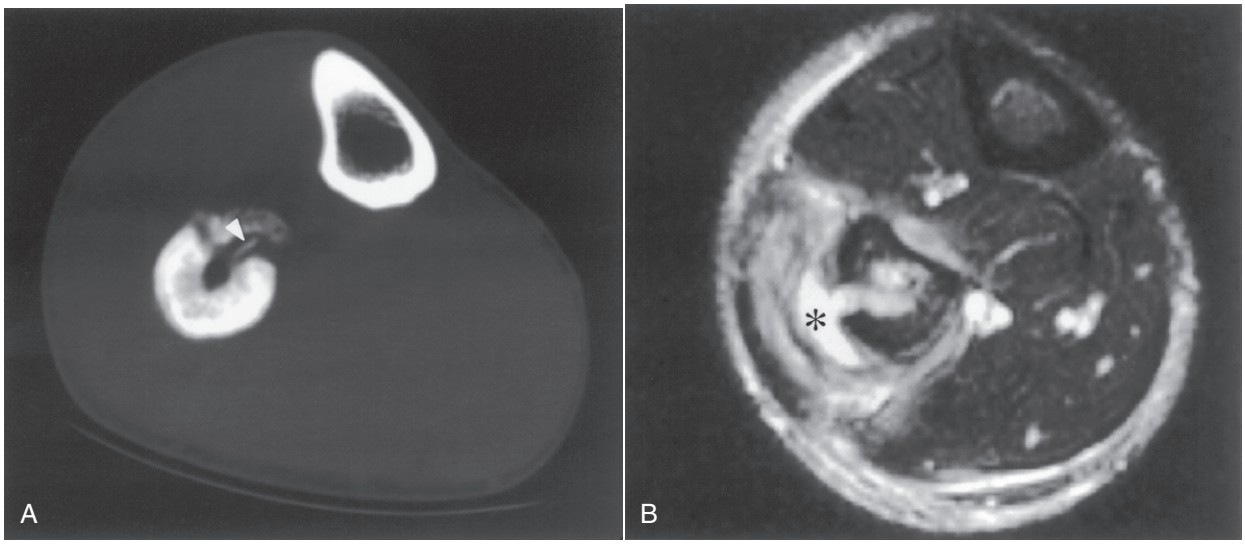

FIGURE 55-7 ■ Chronic osteomyelitis. **A,** CT reveals thickening of the fibular cortex and a sinus tract that contains a sequestrum *(arrowhead)*. **B,** T2-weighted axial MR image from a different level shows a lateral sinus tract through the cortex communicating with a soft tissue abscess *(asterisk)*. Edema is surrounding the entire fibula. (Courtesy Leanne Seeger, MD.)

southwestern United States. Extrapulmonary involvement is suggested by persistent high temperature and toxicity. *Coccidioides* osteomyelitis occurs primarily in cancellous bone (e.g., vertebral bodies, distal tubular bones, and the skull).[179] These lesions are not radiographically distinct from the lesions seen in osteomyelitis from other causes.[196] Débridement of bone lesions often is needed initially, and years of therapy are required. Oral triazole agents generally are recommended.[82]

Blastomycosis may mimic coccidioidomycosis, but the pulmonary involvement is much more varied, and fusion of the vertebral bodies rarely occurs. A propensity for the development of verrucous, reddened, weeping skin lesions has been noted, and prostate involvement may be seen. Bone involvement occurs frequently, with the skull and vertebral bodies being infected most often. Distinguishing it from other forms of osteomyelitis is impossible by radiographic examination.

Aspergillus osteomyelitis is generally a disease of immunosuppressed patients,[38] with *Aspergillus* pneumonia seen initially followed by disseminated disease. Bone disease occurring by hematogenous spread has developed, however, in normal individuals and after injectable drug use.[49,185] *Aspergillus* osteomyelitis developing after trauma also has been reported.

Other fungi are infrequent causes of osteomyelitis. The medical literature contains many reports of osteomyelitis caused by *C. neoformans*, generally in immunocompromised patients and in the setting of disseminated or pulmonary infection. The few reports in pediatric patients generally have involved immunocompromised adolescents. In adolescents and adults, usually only one bone is involved. The lesions generally are slowly destructive, very discrete lesions occurring primarily in the long tubular bones without marginal sclerosis. This radiologic reaction is confused most commonly with tumor and, occasionally, with tuberculosis. Infection of the ribs and skull also has been reported. Although experience with this disease is limited, débridement of the lesions and medical therapy seem to be highly effective.* Cases of *Rhizopus* osteomyelitis have been reported intermittently and apparently are hematogenous.[65]

CHRONIC OSTEOMYELITIS

Chronic osteomyelitis may develop after a surgical procedure, major trauma,[221] or unsuccessful treatment of acute osteomyelitis.[208] The diagnosis of chronic osteomyelitis usually is straightforward; patients generally have a painful, nonfunctional extremity and may have chronically draining sinuses. Radiographs often reveal clear evidence of devitalized bone, including the formation of sequestra or involucra. Cultures of the purulent exudate or necrotic bone usually reveal *S. aureus*, although gram-negative bacteria, including *H. influenzae*,[122] may be found in some cases. Plain radiographs, CT, and MRI all play roles in medical and surgical management by revealing details of the bony and soft tissue involvement, including the formation of abscesses and sequestra (Fig. 55-7).[45,177]

Treatment of chronic osteomyelitis involves the removal of devitalized bone, débridement and drainage of soft tissue disease, achieving wound closure and enhanced perfusion, stabilization of structural deficiencies, and long-term administration of appropriate antibiotics.[45]

Antimicrobial regimens for chronic staphylococcal osteomyelitis that have been studied include oral cloxacillin plus probenecid for 6 to 12 months; in this study 9 of 19 patients apparently were treated successfully.[18] In another study, the outcome of a 6-week course of nafcillin was compared with that of nafcillin and oral rifampin. No statistically significant differences were observed with the addition of rifampin, but most (10 of 17) of the patients showed no evidence of disease activity 2 years after the

*References 17, 33, 40, 92, 140, 141.

cessation of treatment with antibiotics.[168] The high failure rate in these and other studies has led to investigation of a variety of adjunctive measures to improve the outcome of chronic osteomyelitis. Local irrigation with antibiotic solutions, with[145] and without[6] added detergents, has been examined. Surgically implanted polymethyl methacrylate beads impregnated with an antibiotic (usually gentamicin, tobramycin, and vancomycin) are sometimes used for short periods to produce high local concentrations of drug[53] and then removed, although the added benefit from this approach is unclear.[20] Negative pressure wound therapy may also be used to achieve wound closure. Hyperbaric oxygen also has been suggested as an aid to therapy, but no comparative studies have been done.[161]

Because perpetuation of chronic infection seems to be caused by the presence of avascular bone and tissue, advances such as laser Doppler flowmetry ultimately may improve the outcome of this disease.[207] Surgical approaches to close open wounds after débridement and improve blood flow with mobilized tissue flaps also seem to bring about prolonged remission in some patients.[7] Meticulous surgical technique is essential to avoid thermal injury and to retain the vascular supply to compromised areas.[214] Complications of chronic osteomyelitis include secondary amyloidosis and local carcinomatous changes at the site of infection. The high likelihood of a poor outcome in chronic osteomyelitis and the complexities in its management must be kept in mind during treatment of acute hematogenous osteomyelitis.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Aksentijevich I, Masters SL, Ferguson PJ, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* 2009;**360**:2426–37.
39. Ceroni D, Cherkaoui A, Ferey S, et al. *Kingella kingae* osteoarticular infections in young children: clinical features and contribution of a new specific real-time PCR assay to the diagnosis. *J Pediatr Orthop* 2010;**30**:301–4.
53. Decoster TA, Bozorgnia S. Antibiotic beads. *J Am Acad Orthop Surg* 2008;**16**:674–8.
62. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
71. Ferguson PJ, Sandu M. Current understanding of the pathogenesis and management of chronic recurrent multifocal osteomyelitis. *Curr Rheumatol Rep* 2012;**14**:130–41.
150. Masson AT, Gudnason T, Jonmundsson GK, et al. [Bacterial osteomyelitis and arthritis in Icelandic children 1996–2005]. *Laeknabladid* 2011;**97**:91–6.
169. Pääkkönen M, Kallio PE, Kallio MJ, et al. Management of osteoarticular infections caused by *Staphylococcus aureus* is similar to that of other etiologies: analysis of 199 staphylococcal bone and joint infections. *Pediatr Infect Dis J* 2012;**31**:436–8.
244. Zaoutis T, Localio AR, Leckerman K, et al. Prolonged intravenous therapy versus early transition to oral antimicrobial therapy for acute osteomyelitis in children. *Pediatrics* 2009;**123**:636–42.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 56

Septic Arthritis

Paul Krogstad

Acute infection of the joints can be caused by bacteria, fungi, and viruses. Bacterial infections occur most frequently. The terms *septic arthritis*, *acute suppurative pyarthrosis*, and *infectious arthritis* are used interchangeably with regard to bacterial infections and refer to the presence of organisms in the joint space.

EPIDEMIOLOGY

Septic arthritis occurs most frequently in childhood. The overall incidence of septic arthritis in children has been estimated as 5.5 to 12 cases per 100,000 individuals.[38] Describing the age distribution of septic arthritis is difficult because of the varying age intervals selected by authors of different studies. In a review from the preantibiotic era, half of the patients were younger than 20 years old.[52] In a later study, performed after antibiotics became available for systemic use, 31 of 66 patients (47%) were younger than 20 years old.[6] Septic arthritis occurs most often in boys and young children. Males are affected more often than are females by a ratio of 1.1 to 2.1 and most cases occur in children younger than 10 years old; a median age of 2 years has been reported in several series.*

*References 10, 40, 64, 67, 69, 88, 104.

REFERENCES

1. Abdelgawad AA, Rybak LD, Sheth M, et al. Treatment of acute *Salmonella* epiphyseal osteomyelitis using computed tomography–guided drainage in a child without sickle cell disease. *J Pediatr Orthop B* 2007;**16**:415–18.
2. Ailsby RL, Staheli LT. Pyogenic infections of the sacroiliac joint in children: radioisotope bone scanning as a diagnostic tool. *Clin Orthop Relat Res* 1974;(100)96–100.
3. Aksentijevich I, Masters SL, Ferguson PJ, et al. An autoinflammatory disease with deficiency of the interleukin-1-receptor antagonist. *N Engl J Med* 2009;**360**:2426–37.
4. al-Eissa YA, Kambal AM, Alrabeeah AA, et al. Osteoarticular brucellosis in children. *Ann Rheum Dis* 1990;**49**:896–900.
5. Ambrose GB, Alpert M, Neer CS. Vertebral osteomyelitis: a diagnostic problem. *JAMA* 1966;**197**:619–22.
6. Anderson LD, Horn LG. Irrigation-suction technic in the treatment of acute hematogenous osteomyelitis, chronic osteomyelitis, and acute and chronic joint infections. *South Med J* 1970;**63**: 745–54.
7. Anthony JP, Mathes SJ. Update on chronic osteomyelitis. *Clin Plast Surg* 1991;**18**:515–23.
8. Arnold SR, Elias D, Buckingham SC, et al. Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant *Staphylococcus aureus*. *J Pediatr Orthop* 2006;**26**:703–8.
9. Ash JM, Gilday DL. The futility of bone scanning in neonatal osteomyelitis: concise communication. *J Nucl Med* 1980;**21**:417–20.
10. Asmar BI. Osteomyelitis in the neonate. *Infect Dis Clin North Am* 1992;**6**:117–32.
11. Babaiantz L. Les osteopathies atrophiques. *J Radiol Radiother Nucl Med* 1948;**19**:333.
12. Baltimore RS, Jenson HB. Puncture wound osteochondritis of the foot caused by *Pseudomonas maltophilia*. *Pediatr Infect Dis J* 1990;**9**:143–4.
13. Barret JP, Desai MH, Herndon DN. Osteomyelitis in burn patients requiring skeletal fixation. *Burns* 2000;**26**:487–9.
14. Barrett-Connor E. Bacterial infection and sickle cell anemia: an analysis of 250 infections in 166 patients and a review of the literature. *Medicine (Baltimore)* 1971;**50**:97–112.
15. Bartkowski SB, Zapala J, Heczko P, et al. Actinomycotic osteomyelitis of the mandible: review of 15 cases. *J Craniomaxillofac Surg* 1998;**26**:63–7.
16. Barton LL, Hoddy DM, Rathore MH, et al. Long term radiologic outcome of *Pseudomonas* osteomyelitis of the foot. *Pediatr Infect Dis J* 1990;**9**:476–8.
17. Behrman RE, Masci JR, Nicholas P. Cryptococcal skeletal infections: case report and review. *Rev Infect Dis* 1990;**12**:181–90.
18. Bell SM. Further observations on the value of oral penicillins in chronic staphylococcal osteomyelitis. *Med J Aust* 1976;**2**:591–3.
19. Bjorksten B, Gustavson KH, Eriksson B, et al. Chronic recurrent multifocal osteomyelitis and pustulosis palmoplantaris. *J Pediatr* 1978;**93**:227–31.
20. Blaha JD, Calhoun JH, Nelson CL, et al. Comparison of the clinical efficacy and tolerance of gentamicin PMMA beads on surgical wire versus combined and systemic therapy for osteomyelitis. *Clin Orthop Relat Res* 1993;(295)8–12.
21. Blasier RD, Aronson J, Tursky EA. External fixation of pediatric femur fractures. *J Pediatr Orthop* 1997;**17**:342–6.
22. Blockey NJ, Watson JT. Acute osteomyelitis in children. *J Bone Joint Surg Br* 1970;**52**:77–87.
23. Bocchini CE, Hulten KG, Mason Jr EO, et al. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. *Pediatrics* 2006;**117**:433–40.
24. Bonhoeffer J, Haeberle B, Schaad UB, et al. Diagnosis of acute haematogenous osteomyelitis and septic arthritis: 20 years experience at the University Children's Hospital Basel. *Swiss Med Wkly* 2001;**131**:575–81.
25. Booz MM, Hariharan V, Aradi AJ, et al. The value of ultrasound and aspiration in differentiating vaso-occlusive crisis and osteomyelitis in sickle cell disease patients. *Clin Radiol* 1999;**54**:636–9.
26. Bradley JS, Kaplan SL, Tan TQ, et al. Pediatric pneumococcal bone and joint infections. The Pediatric Multicenter Pneumococcal Surveillance Study Group (PMPSSG). *Pediatrics* 1998;**102**: 1376–82.
27. Brand RA, Black H. *Pseudomonas* osteomyelitis following puncture wounds in children. *J Bone Joint Surg Am* 1974;**56**:1637–42.
28. Bremner A, Neligan G. Pyogenic osteitis. In: Gairdners D, editor. *Recent advances in pediatrics*. London: T A Churchill; 1958. p. 354.
29. Browne LP, Mason EO, Kaplan SL, et al. Optimal imaging strategy for community-acquired *Staphylococcus aureus* musculoskeletal infections in children. *Pediatr Radiol* 2008;**38**:841–7.
30. Bruno MS, Neligan G. Embolic osteomyelitis of the spine as a complication of infection of the urinary tract. *Am J Med* 1958;**19**:865–78.
31. Bryan V, Franks L, Torres H. *Pseudomonas aeruginosa* cervical diskitis with chondro-osteomyelitis in an intravenous drug abuser. *Surg Neurol* 1973;**1**:142–4.
32. Bryson YJ, Connor JD, LeClerc M, et al. High-dose oral dicloxacillin treatment of acute staphylococcal osteomyelitis in children. *J Pediatr* 1979;**94**:673–5.
33. Burch KH, Fine G, Quinn EL, et al. *Cryptococcus neoformans* as a cause of lytic bone lesions. *JAMA* 1975;**231**:1057–9.
34. Burnett MW, Bass JW, Cook BA. Etiology of osteomyelitis complicating sickle cell disease. *Pediatrics* 1998;**101**:296–7.
35. Buxton TB, Rissing JP, Horner JA, et al. Binding of a *Staphylococcus aureus* bone pathogen to type I collagen. *Microb Pathog* 1990; **8**:441–8.
36. Canale ST, Puhl J, Watson FM, et al. Acute osteomyelitis following closed fractures: report of three cases. *J Bone Joint Surg Am* 1975;**57**:415–8.
37. Capitanio MA, Kirkpatrick JA. Early roentgen observations in acute osteomyelitis. *Am J Roentgenol Radium Ther Nucl Med* 1970;**108**:488–96.
38. Casscells SW. *Aspergillus* osteomyelitis of the tibia: a case report. *J Bone Joint Surg Am* 1978;**60**:994–5.
39. Ceroni D, Cherkaoui A, Ferey S, et al. *Kingella kingae* osteoarticular infections in young children: clinical features and contribution of a new specific real-time PCR assay to the diagnosis. *J Pediatr Orthop* 2010;**30**:301–4.
40. Charkes ND. Skeletal blood flow: implications for bone-scan interpretation. *J Nucl Med* 1980;**21**:91–8.
41. Chen CJ, Chiu CH, Lin TY, et al. Experience with linezolid therapy in children with osteoarticular infections. *Pediatr Infect Dis J* 2007;**26**:985–8.
42. Chometon S, Benito Y, Chaker M, et al. Specific real-time polymerase chain reaction places *Kingella kingae* as the most common cause of osteoarticular infections in young children. *Pediatr Infect Dis J* 2007;**26**:377–81.
43. Chung SM, Borns P. Acute osteomyelitis adjacent to the sacroiliac joint in children: report of two cases. *J Bone Joint Surg Am* 1973;**55**:630–4.
44. Chung T. Magnetic resonance imaging in acute osteomyelitis in children. *Pediatr Infect Dis J* 2002;**21**:869–70.
45. Cierny 3rd G, DiPasquale D. Treatment of chronic infection. *J Am Acad Orthop Surg* 2006;**14**:S105–110.
46. Collin DH. *Pathology of bone*. London: Butterworths; 1966.
47. Constant E, Green RL, Wagner DK. *Salmonella* osteomyelitis of both hands and the hand-foot syndrome. *Arch Surg* 1971;**102**: 148–51.
48. Conventry MB, Ghormley RK, Kernohan J. Intervertebral disc: its microscopic anatomy and pathology. *J Bone Joint Surg Am* 1945;**27**:105–12.
49. Corrall CJ, Merz WG, Rekedal K, et al. *Aspergillus* osteomyelitis in an immunocompetent adolescent: a case report and review of the literature. *Pediatrics* 1982;**70**:455–61.
50. Cushing AH. Diskitis in children. *Clin Infect Dis* 1993;**17**:1–6.
51. Dahl LB, Hoyland AL, Dramsdahl H, et al. Acute osteomyelitis in children: a population-based retrospective study 1965 to 1994. *Scand J Infect Dis* 1998;**30**:573–7.
52. de Groot R, Glover D, Clausen C, et al. Bone and joint infections caused by *Kingella kingae:* six cases and review of the literature. *Rev Infect Dis* 1988;**10**:998–1004.
53. Decoster TA, Bozorgnia S. Antibiotic beads. *J Am Acad Orthop Surg* 2008;**16**:674–8.
54. Delorimier AA, Haskin D, Massie F. Mediastinal mass caused by vertebral osteomyelitis. *Am J Dis Child* 1966;**111**:639–43.
55. Demopulos GA, Bleck EE, McDougall IR. Role of radionuclide imaging in the diagnosis of acute osteomyelitis. *J Pediatr Orthop* 1988;**8**:558–65.

56. Deshpande PG, Wagle SU, Mehta SD, et al. Neonatal osteomyelitis and septic arthritis. *Indian Pediatr* 1990;**27**:453–7.
57. Deutsch SD, Gandsman EJ, Spraragen SC. Quantitative regional blood-flow analysis and its clinical application during routine bone-scanning. *J Bone Joint Surg Am* 1981;**63**:295–305.
58. Deutschmann A, Mache CJ, Bodo K, et al. Successful treatment of chronic recurrent multifocal osteomyelitis with tumor necrosis factor-alpha blockage. *Pediatrics* 2005;**116**:1231–3.
59. Dich VQ, Nelson JD, Haltalin KC. Osteomyelitis in infants and children: a review of 163 cases. *Am J Dis Child* 1975; **129**:1273–8.
60. Diggs LW. Bone and joint lesions in sickle-cell disease. *Clin Orthop Relat Res* 1967;(52)119–43.
61. Drew RH, Perfect JR, Srinath L, et al. Treatment of methicillin-resistant *Staphylococcus aureus* infections with quinupristin-dalfopristin in patients intolerant of or failing prior therapy. For the Synercid Emergency-Use Study Group. *J Antimicrob Chemother* 2000;**46**:775–84.
62. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
63. Dunn EJ, Bryan DM, Nugent JT, Robinson RA. Pyogenic infections of the sacro-iliac joint. *Clin Orthop Relat Res* 1976;(118)113–17.
64. Duszynski DO, Kuhn JP, Afshani E, et al. Early radionuclide diagnosis of acute osteomyelitis. *Radiology* 1975;**117**:337–40.
65. Echols RM, Selinger DS, Hallowell C, et al. *Rhizopus* osteomyelitis: a case report and review. *Am J Med* 1979;**66**:141–5.
66. Edwards MS, Baker CJ, Wagner ML, et al. An etiologic shift in infantile osteomyelitis: the emergence of the group B streptococcus. *J Pediatr* 1978;**93**:578–83.
67. Epps Jr CH, Bryant 3rd DD, Coles MJ, et al. Osteomyelitis in patients who have sickle-cell disease: diagnosis and management. *J Bone Joint Surg Am* 1991;**73**:1281–94.
68. Farr H. Acute hematogenous osteomyelitis due to type B *Haemophilus* osteomyelitis and arthritis. *Lancet* 1966;**1**:517–18.
69. Farrand RJ, Johnstone JM, McCabe AF. *Haemophilus* osteomyelitis and arthritis. *BMJ* 1968;**2**:334–6.
70. Feigin RD, Pickering LK, Anderson D, et al. Clindamycin treatment of osteomyelitis and septic arthritis in children. *Pediatrics* 1975;**55**:213–23.
71. Ferguson PJ, Sandu M. Current understanding of the pathogenesis and management of chronic recurrent multifocal osteomyelitis. *Curr Rheumatol Rep* 2012;**14**:130–41.
72. Ferguson WR. Some observations on the circulation in foetal and infant spines. *J Bone Joint Surg Am* 1950;**32-A**:640–8.
73. Fernandez M, Carrol CL, Baker CJ. Discitis and vertebral osteomyelitis in children: an 18-year review. *Pediatrics* 2000;**105**: 1299–304.
74. Fisher MC, Goldsmith JF, Gilligan PH. Sneakers as a source of *Pseudomonas aeruginosa* in children with osteomyelitis following puncture wounds. *J Pediatr* 1985;**106**:607–9.
75. Fitzgerald Jr RH, Cowan JD. Puncture wounds of the foot. *Orthop Clin North Am* 1975;**6**:965–72.
76. Fletcher BD, Scoles PV, Nelson AD. Osteomyelitis in children: detection by magnetic resonance. Work in progress. *Radiology* 1984;**150**:57–60.
77. Floyed RL, Steele RW. Culture-negative osteomyelitis. *Pediatr Infect Dis J* 2003;**22**:731–6.
78. Fonk J, Coonrod JD. *Serratia* osteomyelitis in sickle cell disease. *JAMA* 1971;**217**:80–1.
79. Fox L, Sprunt K. Neonatal osteomyelitis. *Pediatrics* 1978;**62**: 535–42.
80. Freehafer AA, Furey JG, Pierce DS. Pyogenic osteomyelitis of the spine resulting in spinal paralysis. *J Bone Joint Surg Am* 1962;**44**:710–6.
81. Gainey MA, Siegel JA, Smergel EM, et al. Indium-111-labeled white blood cells: dosimetry in children. *J Nucl Med* 1988;**29**: 689–94.
82. Galgiani JN, Ampel NM, Catanzaro A, et al. Practice guideline for the treatment of coccidioidomycosis. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:658–61.
83. Gallagher KT, Roberts RL, MacFarlane JA, et al. Treatment of chronic recurrent multifocal osteomyelitis with interferon gamma. *J Pediatr* 1997;**131**:470–2.
84. Geddes AM, Finch RS, Goodall AC, et al. *The treatment of pediatric infections with clindamycin*. Baltimore: University Park Press; 1972.
85. Genster HG, Andersen MJ. Spinal osteomyelitis complicating urinary tract infection. *J Urol* 1972;**107**:109–11.
86. Gibson WK, Bartosh R, Timperlake R. Acute hematogenous epiphyseal osteomyelitis. *Orthopedics* 1991;**14**:705–7.
87. Giedion A, Holthusen W, Masel LF, et al. [Subacute and chronic "symmetrical" osteomyelitis]. *Ann Radiol (Paris)* 1972;**15**:329–42.
88. Gilday DL, Paul DJ, Paterson J. Diagnosis of osteomyelitis in children by combined blood pool and bone imaging. *Radiology* 1975;**117**:331–5.
89. Gilfand MJ, Harche HT. Skeletal imaging in sickle cell disease. *J Nucl Med* 1978;**19**:698–709.
90. Gillespie WJ. Epidemiology in bone and joint infection. *Infect Dis Clin North Am* 1990;**4**:361–76.
91. Goergens ED, McEvoy A, Watson M, Barrett IR. Acute osteomyelitis and septic arthritis in children. *J Paediatr Child Health* 2005; **41**:59–62.
92. Goldshteyn N, Zanchi A, Cooke K, et al. Cryptococcal osteomyelitis of the humeral head initially diagnosed as avascular necrosis. *South Med J* 2006;**99**:1140–1.
93. Goldstein EJ, Ahonkhai VI, Cristofaro RL, et al. Source of *Pseudomonas* in osteomyelitis of heels. *J Clin Microbiol* 1980;**12**: 711–3.
94. Gonzalez BE, Hulten KG, Dishop MK, et al. Pulmonary manifestations in children with invasive community-acquired *Staphylococcus aureus* infection. *Clin Infect Dis* 2005;**41**:583–90.
95. Gonzalez BE, Teruya J, Mahoney Jr DH, et al. Venous thrombosis associated with staphylococcal osteomyelitis in children. *Pediatrics* 2006;**117**:1673–9.
96. Goutzmanis JJ, Gonis G, Gilbert GL. *Kingella kingae* infection in children: ten cases and a review of the literature. *Pediatr Infect Dis J* 1991;**10**:677–83.
97. Granoff DM, Sargent E, Jolivette D. *Haemophilus influenzae* type b osteomyelitis. *Am J Dis Child* 1978;**132**:488–90.
98. Green JH. Cloxacillin in treatment of acute osteomyelitis. *BMJ* 1967;**2**:414–16.
99. Green WT. Osteomyelitis of infants: a disease different from osteomyelitis of older children. *Arch Surg* 1936;**32**:462–93.
100. Greenburg LW, Haynes RE. *Escherichia coli* osteomyelitis in an infant with sickle cell disease. *Clin Pediatr (Philadelphia)*. 1990;**9**:436–8.
101. Griesemer DA, Winkelstein JA, Luddy R. Pneumococcal meningitis in patients with a major sickle hemoglobinopathy. *J Pediatr* 1978;**92**:82–4.
102. Gristina AG, Oga M, Webb LX, et al. Adherent bacterial colonization in the pathogenesis of osteomyelitis. *Science* 1985;**228**: 990–3.
103. Handmaker H, Giammona ST. Improved early diagnosis of acute inflammatory skeletal-articular diseases in children: a two-radiopharmaceutical approach. *Pediatrics* 1984;**73**:661–9.
104. Harcke HT. Role of imaging in musculoskeletal infections in children. *J Pediatr Orthop* 1995;**15**:141–3.
105. Harris NH. Some problems in the diagnosis and treatment of acute osteomyelitis. *J Bone Joint Surg Br* 1960;**42**:535–41.
106. Henson NH. Some problems in the diagnosis and treatment of acute osteomyelitis. *Surg Gynecol Obstet* 1956;**102**:207–14.
107. Highland TR, LaMont RL. Osteomyelitis of the pelvis in children. *Pediatrics* 1984;**73**:661–9.
108. Hobo T. Zur Pathogenese der Vitalfarbungalehre. *Acta Sch Med Univ Kioto* 1921;**4**:1–29.
109. Hoffer P. Gallium and infection. *J Nucl Med* 1980;**21**:484–8.
110. Holzman RS, Bishko F. Osteomyelitis in heroin addicts. *Ann Intern Med* 1971;**75**:693–6.
111. Homans J. Osteomyelitis of the long bones: a study of ninety-four cases from the Children's Hospital, Boston. *Ann Surg* 1912;**55**: 375–87.
112. Hruby MA, Honig GR, Lolekha S, et al. Arizoni hinshawii osteomyelitis in sickle cell anemia. *Am J Dis Child* 1973;**125**:867–8.
113. Hughes RA, Rowe IF, Shanson D, et al. Septic bone, joint and muscle lesions associated with human immunodeficiency virus infection. *Br J Rheumatol* 1992;**31**:381–8.
114. Jackson MA, Nelson JD. Etiology and medical management of acute suppurative bone and joint infections in pediatric patients. *J Pediatr Orthop* 1982;**2**:313–23.
115. Jacobs RF, Adelman L, Sack CM, et al. Management of *Pseudomonas* osteochondritis complicating puncture wounds of the foot. *Pediatrics* 1982;**69**:432–5.

116. Jacobs RF, McCarthy RE, Elser JM. *Pseudomonas* osteochondritis complicating puncture wounds of the foot in children: a 10-year evaluation. *J Infect Dis* 1989;**160**:657–61.
117. Johanson PH. *Pseudomonas* infections of the foot following puncture wounds. *JAMA* 1968;**204**:262–4.
118. Jordan MC, Kirby WM. Pyogenic vertebral osteomyelitis: treatment with antimicrobial agents and bed rest. *Arch Intern Med* 1971;**128**:405–10.
119. Kahn DS, Pritzker KP. The pathophysiology of bone infection. *Clin Orthop Relat Res* 1973;(96):12–9.
120. Kaplan SL. Osteomyelitis in children. *Infect Dis Clin North Am* 2005;**19**:787–97, vii.
121. Kaplan SL, Mason Jr EO, Feigin RD. Clindamycin versus nafcillin or methicillin in the treatment of *Staphylococcus aureus* osteomyelitis in children. *South Med J* 1982;**75**:138–42.
122. Karwowska A, Davies HD, Jadavji T. Epidemiology and outcome of osteomyelitis in the era of sequential intravenous-oral therapy. *Pediatr Infect Dis J* 1998;**17**:1021–6.
123. Keeley K, Buchanan GR. Acute infarction of long bones in children with sickle cell anemia. *J Pediatr* 1982;**101**:170–5.
124. Kemp HB, Jackson JW, Jeremiah JD, et al. Pyogenic infections occurring primarily in intervertebral discs. *J Bone Joint Surg Br* 1973;**55**:698–714.
125. King SM, Laxer RM, Manson D, et al. Chronic recurrent multifocal osteomyelitis: a noninfectious inflammatory process. *Pediatr Infect Dis J* 1987;**6**:907–11.
126. Knudsen CJ, Hoffman EB. Neonatal osteomyelitis. *J Bone Joint Surg Br* 1990;**72**:846–51.
127. Kohli R, Hadley S. Fungal arthritis and osteomyelitis. *Infect Dis Clin North Am* 2005;**19**:831–51.
128. Kozin F, et al. Computed tomography in the diagnosis of sacroiliitis. *Am J Dis Child* 1980;**134**:828–30.
129. Kurlandsky LE, Quinn PH, Sills EM. *Haemophilus influenzae* as a cause of Brodie's abscess in an infant. *Johns Hopkins Med J* 1979;**144**:15–7.
130. LaMont RL, Anderson PA, Dajani AS, et al. Acute hematogenous osteomyelitis in children. *J Pediatr Orthop* 1987;**7**:579–83.
131. Lascari AD, Graham MH, MacQueen JC. Intervertebral disk infection in children. *J Pediatr* 1967;**70**:751–7.
132. Le Saux N, Howard A, Barrowman NJ, et al. Shorter courses of parenteral antibiotic therapy do not appear to influence response rates for children with acute hematogenous osteomyelitis: a systematic review. *BMC Infect Dis* 2002;**2**:16.
133. Lee PY. Infected cephalhaematoma and neonatal osteomyelitis. *J Infect* 1990;**21**:191–3.
134. Leigh TF, Kelly RP, Weens HS. Spinal osteomyelitis associated with urinary tract infections. *Radiology* 1955;**65**:334–42.
135. Leonard A, Comty CM, Shapiro FL, et al. Osteomyelitis in hemodialysis patients. *Ann Intern Med* 1973;**78**:651–8.
136. Lewis 2nd JS, Jorgensen JH. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? *Clin Infect Dis* 2005;**40**:280–5.
137. Lewis R, Gorbach S, Altner P. Spinal *Pseudomonas* chondro-osteomyelitis in heroin users. *N Engl J Med* 1972;**286**:1303.
138. Lewis RP, Sutter VL, Finegold SM. Bone infections involving anaerobic bacteria. *Medicine (Baltimore)* 1978;**57**:279–305.
139. Lifeso RM, Harder E, McCorkell SJ. Spinal brucellosis. *J Bone Joint Surg Br* 1985;**67**:345–51.
140. Littman ML, Walter JE. Cryptococcosis: current status. *Am J Med* 1968;**45**:922–32.
141. Liu PY. Cryptococcal osteomyelitis: case report and review. *Diagn Microbiol Infect Dis* 1998;**30**:33–5.
142. Longjohn DB, Zionts LE, Stott NS. Acute hematogenous osteomyelitis of the epiphysis. *Clin Orthop Relat Res* 1995;(316):227–34.
143. Lundy DW, Kehl DK. Increasing prevalence of *Kingella kingae* in osteoarticular infections in young children. *J Pediatr Orthop* 1998;**18**:262–7.
144. Majeed HA, Kalaawi M, Mohanty D, et al. Congenital dyserythropoietic anemia and chronic recurrent multifocal osteomyelitis in three related children and the association with Sweet syndrome in two siblings. *J Pediatr* 1989;**115**:730–4.
145. Makin M, Geller R, Jacobs J, et al. Non-toxic detergent irrigation of chronic infections. An experimental evaluation. *Clin Orthop Relat Res* 1973;(92)320–4.
146. Marshall GS, Mudido P, Rabalais GP, et al. Organism isolation and serum bactericidal titers in oral antibiotic therapy for pediatric osteomyelitis. *South Med J* 1996;**89**:68–70.
147. Marti-Bonmati L, Aparisi F, Poyatos C, et al. Brodie abscess: MR imaging appearance in 10 patients. *J Magn Reson Imaging* 1993;**3**:543–6.
148. Martinez-Aguilar G, Avalos-Mishaan A, Hulten K, et al. Community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* musculoskeletal infections in children. *Pediatr Infect Dis J* 2004;**23**:701–6.
149. Martinez-Aguilar G, Hammerman WA, Mason Jr EO, et al. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in children. *Pediatr Infect Dis J* 2003;**22**:593–8.
150. Masson AT, Gudnason T, Jonmundsson GK, et al. [Bacterial osteomyelitis and arthritis in Icelandic children 1996–2005]. *Laeknabladid* 2011;**97**:91–6.
151. Mazur JM, Ross G, Cummings J, et al. Usefulness of magnetic resonance imaging for the diagnosis of acute musculoskeletal infections in children. *J Pediatr Orthop* 1995;**15**:144–7.
152. McGregor JA, McFarren T. Neonatal cranial osteomyelitis: a complication of fetal monitoring. *Obstet Gynecol* 1989;**73**:490–2.
153. Medina F, Fuentes M, Jara LJ, et al. *Salmonella* pyomyositis in patients with the human immunodeficiency virus. *Br J Rheumatol* 1995;**34**:568–71.
154. Messina AF, Namtu K, Guild M, et al. Trimethoprim-sulfamethoxazole therapy for children with acute osteomyelitis. *Pediatr Infect Dis J* 2011;**30**:1019–21.
155. Miller DJ, Mejicano GC. Vertebral osteomyelitis due to *Candida* species: case report and literature review. *Clin Infect Dis* 2001;**33**:523–30.
156. Miller EH, Semian DW. Gram-negative osteomyelitis following puncture wounds of the foot. *Medicine (Baltimore)* 1978;**57**:279–05.
157. Modic MT, Feiglin DH, Piraino DW, et al. Vertebral osteomyelitis: assessment using MR. *Radiology* 1985;**157**:157–66.
158. Mok PM, Reilly BJ, Ash JM. Osteomyelitis in the neonate: clinical aspects and the role of radiography and scintigraphy in diagnosis and management. *Radiology* 1982;**145**:677–82.
159. Morgan A, Yates AK. The diagnosis of acute osteomyelitis of the pelvis. *Postgrad Med J* 1966;**42**:74–8.
160. Morrey BF, Bianco AJ, Rhodes KH. Hematogenous osteomyelitis at uncommon sites in children. *Mayo Clin Proc* 1978;**53**:707–13.
161. Morrey BF, Dunn JM, Heimbach RD, et al. Hyperbaric oxygen and chronic osteomyelitis. *Clin Orthop Relat Res* 1979;(144):121–7.
162. Murray MM, Welch DF, Kuhls TL. *Serratia* osteochondritis after puncture wounds of the foot. *Pediatr Infect Dis J* 1990;**9**:523–5.
163. Mustafa MM, Saez-Llorens X, McCracken Jr GH, et al. Acute hematogenous pelvic osteomyelitis in infants and children. *Pediatr Infect Dis J* 1990;**9**:416–21.
164. Nagal DA, Albright JA, Keggi KJ. Closer look at spinal lesions: open biopsy of vertebral lesions. *JAMA* 1965;**191**:975–8.
165. Nelson JD. Acute osteomyelitis in children. *Infect Dis Clin North Am* 1990;**4**:513–22.
166. Nelson JD. Toward simple but safe management of osteomyelitis. *Pediatrics* 1997;**99**:883–4.
167. Nelson JD, Bucholz RW, Kusmiesz H, et al. Benefits and risks of sequential parenteral-oral cephalosporin therapy for suppurative bone and joint infections. *J Pediatr Orthop* 1982;**2**:255–62.
168. Norden CW, Bryant R, Palmer D, et al. Chronic osteomyelitis caused by *Staphylococcus aureus:* controlled clinical trial of nafcillin therapy and nafcillin-rifampin therapy. *South Med J* 1986;**79**:947–51.
169. Pääkkönen M, Kallio PE, Kallio MJ, et al. Management of osteoarticular infections caused by *Staphylococcus aureus* is similar to that of other etiologies: analysis of 199 staphylococcal bone and joint infections. *Pediatr Infect Dis J* 2012;**31**:436–8.
170. Peltola H, Pääkkönen M, Kallio P, et al. Clindamycin vs. first-generation cephalosporins for acute osteoarticular infections of childhood—a prospective quasi-randomized controlled trial. *Clin Microbiol Infect* 2012;**18**:582–9.
171. Peltola H, Unkila-Kallio L, Kallio MJ. Simplified treatment of acute staphylococcal osteomyelitis of childhood. The Finnish Study Group. *Pediatrics* 1997;**99**:846–50.

172. Perlman MH, Patzakis MJ, Kumar PJ, et al. The incidence of joint involvement with adjacent osteomyelitis in pediatric patients. *J Pediatr Orthop* 2000;**20**:40–3.
173. Perry JW, Montgomerie JZ, Swank S, et al. Wound infections following spinal fusion with posterior segmental spinal instrumentation. *Clin Infect Dis* 1997;**24**:558–61.
174. Peters W, Irving J, Letts M. Long-term effects of neonatal bone and joint infection on adjacent growth plates. *J Pediatr Orthop* 1992;**12**:806–10.
175. Pichichero ME, Friesen HA. Polymicrobial osteomyelitis: report of three cases and review of the literature. *Rev Infect Dis* 1982;**4**:86–96.
176. Piehl FC, Davis RJ, Prugh SI. Osteomyelitis in sickle cell disease. *J Pediatr Orthop* 1993;**13**:225–7.
177. Poyhia T, Azouz EM. MR imaging evaluation of subacute and chronic bone abscesses in children. *Pediatr Radiol* 2000;**30**: 763–8.
178. Prober CG, Yeager AS. Use of the serum bactericidal titer to assess the adequacy of oral antibiotic therapy in the treatment of acute hematogenous osteomyelitis. *J Pediatr* 1979;**95**:131–5.
179. Rhangos WC, Chick EW. Mycotic infections of bone. *South Med J* 1964;**57**:664–74.
180. Robertson AJ, Firth GB, Truda C, et al. Epidemiology of acute osteoarticular sepsis in a setting with a high prevalence of pediatric HIV infection. *J Pediatr Orthop* 2012;**32**:215–9.
181. Robinson LG, Kourtis AP. Tale of a toothpick: *Eikenella corrodens* osteomyelitis. *Infection* 2000;**28**:332–3.
182. Rocco HD, Eyring EJ. Intervertebral disk infections in children. *Am J Dis Child* 1972;**123**:448–51.
183. Rodriguez W, Ross S, Khan W, et al. Clindamycin in the treatment of osteomyelitis in children: a report of 29 cases. *Am J Dis Child* 1977;**131**:1088–93.
184. Roine I, Faingezicht I, Arguedas A, et al. Serial serum C-reactive protein to monitor recovery from acute hematogenous osteomyelitis in children. *Pediatr Infect Dis J* 1995;**14**:40–4.
185. Roselle GA, Baird IM. *Aspergillus flavipes* group osteomyelitis. *Arch Intern Med* 1979;**139**:590–2.
186. Rubin HM, Eardley W, Nichols BL. *Shigella sonnei* osteomyelitis and sickle-cell anemia. *Am J Dis Child* 1968;**116**:83–7.
187. Rubin RC, Jacobs GB, Cooper PR, et al. Disc space infections in children. *Childs Brain* 1977;**3**:180–90.
188. Ruebner R, Keren R, Coffin S, et al. Complications of central venous catheters used for the treatment of acute hematogenous osteomyelitis. *Pediatrics* 2006;**117**:1210–15.
189. Sapico FL, Montgomerie JZ. Pyogenic vertebral osteomyelitis: report of nine cases and review of the literature. *Rev Infect Dis* 1979;**1**:754–76.
190. Schauwecker DS. Osteomyelitis: diagnosis with In-111-labeled leukocytes. *Radiology* 1989;**171**:141–6.
191. Schechter LS, Smith A, Pearl M. Intervertebral disk calcification in childhood. *Am J Dis Child* 1972;**123**:608–11.
192. Schultz C, Holterhus PM, Seidel A, et al. Chronic recurrent multifocal osteomyelitis in children. *Pediatr Infect Dis J* 1999; **18**:1008–13.
193. Scoles PV, Hilty MD, Sfakianakis GN. Bone scan patterns in acute osteomyelitis. *Clin Orthop Relat Res* 1980;(153)210–17.
194. Selby RC, Pillay KV. Osteomyelitis and disc infection secondary to *Pseudomonas aeruginosa* in heroin addiction: case report. *J Neurosurg* 1972;**37**:463–6.
195. Sfakianakis GN, Scoles P, Welch M, et al. Evolution of bone imaging findings in osteomyelitis. *J Nucl Med* 1978;**199**:706.
196. Smith CE, Beard RR, et al. Varieties of coccidioidal infection in relation to the epidemiology and control of the diseases. *Am J Public Health Nations Health* 1946;**36**:1394–402.
197. Smith RF, Taylor TK. Inflammatory lesions of intervertebral discs in children. *J Bone Joint Surg Am* 1967;**49**:1508–20.
198. Sorsdahl OA, Goodhart GL, Williams HT, et al. Quantitative bone gallium scintigraphy in osteomyelitis. *Skeletal Radiol* 1993; **22**:239–42.
199. Specht EE. Hemoglobinopathic *Salmonella* osteomyelitis: orthopedic aspects. *Clin Orthop Relat Res* 1971;(79)110–18.
200. Spiegel PG, Kengla KW, Isaacson AS, et al. Intervertebral disc-space inflammation in children. *J Bone Joint Surg Am* 1972;**54**:284–96.
201. Staab EV, McCartney WH. Role of gallium 67 in inflammatory disease. *Semin Nucl Med* 1978;**8**:219–34.
202. Starr CL. Acute hematogenous osteomyelitis. *Arch Surg* 1922;**4**: 567–87.
203. Steinbach LS, Tehranzadeh J, Fleckenstein JL, et al. Human immunodeficiency virus infection: musculoskeletal manifestations. *Radiology* 1993;**186**:833–8.
204. Stephens MM, MacAuley P. Brodie's abscess: a long-term review. *Clin Orthop Relat Res* 1988;(234):211–6.
205. Stone DB, Bonfiglio M. Pyogenic vertebral osteomyelitis. A diagnostic pitfall for the internist. *Arch Intern Med* 1963;**112**: 491–500.
206. Swick HM. Calcification of intervertebral discs in childhood. *J Pediatr* 1975;**86**:364–9.
207. Swiontkowski MF. Surgical approaches in osteomyelitis: use of laser Doppler flowmetry to determine nonviable bone. *Infect Dis Clin North Am* 1990;**4**:501–12.
208. Syrogiannopoulos GA, Nelson JD. Duration of antimicrobial therapy for acute suppurative osteoarticular infections. *Lancet* 1988;**1**:37–40.
209. Tang JS, Gold RH, Bassett LW, et al. Musculoskeletal infection of the extremities: evaluation with MR imaging. *Radiology* 1988;**166**:205–9.
210. Taplin D, Zaias N, Rebell G. Environmental influences on the microbiology of the skin. *Arch Environ Health* 1965;**11**:546–50.
211. Taylor JC, Fallon RJ. Osteomyelitis due to *Hemophilus influenzae*. *Lancet* 1966;**1**:715.
212. Taylor JR. Growth of human intervertebral discs and vertebral bodies. *J Anat* 1975;**120**:49–68.
213. Templeton 3rd WC, Wawrukiewicz A, Melo JC, et al. Anaerobic osteomyelitis of long bones. *Rev Infect Dis* 1983;**5**:692–712.
214. Tetsworth K, Cierny 3rd G. Osteomyelitis debridement techniques. *Clin Orthop Relat Res* 1999;(360)87–96.
215. Tetzlaff TR, McCracken Jr GH, Nelson JD. Oral antibiotic therapy for skeletal infections of children, part II: Therapy of osteomyelitis and suppurative arthritis. *J Pediatr* 1978;**92**:485–90.
216. Tilden RL, Jackson Jr J, Enneking WF, et al. ^{99m}Tc-polyphosphate: histological localization in human femurs by autoradiography. *J Nucl Med* 1973;**14**:576–8.
217. Torda AJ, Gottlieb T, Bradbury R. Pyogenic vertebral osteomyelitis: analysis of 20 cases and review. *Clin Infect Dis* 1995; **20**:320–8.
218. Treves S, Khettry J, Broker FH, et al. Osteomyelitis: early scintigraphic detection in children. *Pediatrics* 1976;**57**:173–86.
219. Trobs R, Moritz R, Buhligen U, et al. Changing pattern of osteomyelitis in infants and children. *Pediatr Surg Int* 1999;**15**: 363–72.
220. Trueta J. The three types of acute hematogenous osteomyelitis. *J Bone Joint Surg Am* 1959;**41**:671–80.
221. Tsukayama DT. Pathophysiology of posttraumatic osteomyelitis. *Clin Orthop Relat Res* 1999;(360):22–9.
222. Unger E, Moldofsky P, Gatenby R, et al. Diagnosis of osteomyelitis by MR imaging. *AJR Am J Roentgenol* 1988;**150**:605–10.
223. Unkila-Kallio L, Kallio MJ, Eskola J, et al. Serum C-reactive protein, erythrocyte sedimentation rate, and white blood cell count in acute hematogenous osteomyelitis of children. *Pediatrics* 1994;**93**:59–62.
224. Unkila-Kallio L, Kallio MJ, Peltola H. Acute haematogenous osteomyelitis in children in Finland. Finnish Study Group. *Ann Med* 1993;**25**:545–9.
225. Van Howe RS, Starshak RJ, Chusid MJ. Chronic, recurrent multifocal osteomyelitis: case report and review of the literature. *Clin Pediatr (Phila)* 1989;**28**:54–9.
226. Vaughan PA, Newman NM, Rosman MA. Acute hematogenous osteomyelitis in children. *J Pediatr Orthop* 1987;**7**:652–5.
227. Verdier I, Gayet-Ageron A, Ploton C, et al. Contribution of a broad range polymerase chain reaction to the diagnosis of osteoarticular infections caused by *Kingella kingae:* description of twenty-four recent pediatric diagnoses. *Pediatr Infect Dis J* 2005;**24**:692–6.
228. Waldvogel FA, Papageorgiou PS. Osteomyelitis: the past decade. *N Engl J Med* 1980;**303**:360–70.
229. Walker SH. Staphylococcal osteomyelitis in children: success with cephaloridine-cephalexin therapy. *Clin Pediatr (Phila)* 1973; **12**:98–100.
230. Watson FM, Whitesides Jr TE. Acute hematogenous osteomyelitis complicating closed fractures. *Clin Orthop Relat Res* 1976; (117):296–302.

231. Watson RJ, Burko H, Megas H, et al. The handfoot syndrome in sickle-cell disease in young children. *Pediatrics* 1963;**31**: 975–82.
232. Weissberg ED, Smith AL, Smith DH. Clinical features of neonatal osteomyelitis. *Pediatrics* 1974;**53**:505–10.
233. Weld PW. Osteomyelitis of the ilium: masquerading as acute appendicitis. *JAMA* 1960;**173**:634–6.
234. Widen AL, Cardon L. *Salmonella typhimurium* osteomyelitis with sickle cell-hemoglobin C disease: a review and case report. *Ann Intern Med* 1961;**54**:510–21.
235. Wiesseman GJ, Wood VE, Kroll LL, et al. *Pseudomonas* vertebral osteomyelitis in heroin addicts: report of five cases. *J Bone Joint Surg Am* 1973;**55**:1416–24.
236. Williamson JB, Galasko CS, Robinson MJ. Outcome after acute osteomyelitis in preterm infants. *Arch Dis Child* 1990;**65**:1060–2.
237. Winkelstein JA, Marino MC, Johnston Jr RB, et al. Chronic granulomatous disease: report on a national registry of 368 patients. *Medicine (Baltimore)* 2000;**79**:155–69.
238. Wong M, Isaacs D, Howman-Giles R, et al. Clinical and diagnostic features of osteomyelitis occurring in the first three months of life. *Pediatr Infect Dis J* 1995;**14**:1047–53.
239. Yagupsky P. *Kingella kingae:* from medical rarity to an emerging paediatric pathogen. *Lancet Infect Dis* 2004;**4**:358–67.
240. Yagupsky P. Antibiotic susceptibility of *Kingella kingae* isolates from children with skeletal system infections. *Pediatr Infect Dis J* 2012;**31**:212.
241. Yagupsky P, Howard CB, Einhorn M, et al. *Kingella kingae* osteomyelitis of the calcaneus in young children. *Pediatr Infect Dis J* 1993;**12**:540–1.
242. Yagupsky P, Porsch E, St Geme 3rd JW. *Kingella kingae:* an emerging pathogen in young children. *Pediatrics* 2011;**127**:557–65.
243. Zacharias J, Crosby LA. Sporotrichal arthritis of the knee. *Am J Knee Surg* 1997;**10**:171–4.
244. Zaoutis T, Localio AR, Leckerman K, et al. Prolonged intravenous therapy versus early transition to oral antimicrobial therapy for acute osteomyelitis in children. *Pediatrics* 2009;**123**:636–42.

PATHOPHYSIOLOGY

Synovial joints, also termed *diarthrodial joints*, are freely movable articulations containing synovia. Synovia is a transparent viscous fluid that lubricates the joint and nourishes the avascular articular cartilage. The synovium, a connective tissue layer interposed between the fibrous joint capsule and the fluid-filled synovial cavity, is responsible for formation of the joint fluid. The synovium contains a prominent capillary supply embedded in a connective tissue network containing at least two types of cells. One morphologic type (type A) seems to be related to mononuclear phagocytes; fibroblast-like type B cells seem to be responsible for the synthesis of hyaluronic acid. Joint fluid (synovia) is formed by filtration through the capillary network (i.e., the net balance of back-diffusion into the capillary bed and diffusion into the joint space). Diarthrodial joints normally contain small amounts of fluid (e.g., 0.5 to 3 mL in the knee),[93] with glucose and electrolyte concentrations equal to those in plasma. An oxygen partial pressure of 60 to 70 mm Hg, an albumin concentration of 10 to 20 g/L, and an IgG content of 500 mg/L are typical.[24,27,36,93]

Diffusion from the joint space is increased by any mechanism that increases pressure (distention with injected solution, active or passive motion, or external massage). Particulate material is removed from the joint space by synovial membrane macrophages and free monocytes (the latter usually are present in concentrations of $<60 \times 10^6$/L).[86] The viscosity of joint fluid is affected by the properties of hyaluronic acid; enzymatic depolymerization produces a viscosity approximately equivalent to that of water. With the loss of hyaluronidase from the synovia, the articular cartilage, with continued use, becomes eroded and sclerotic.[9] The vasculature of the synovial membrane is innervated, which has two consequences: joint pain is localized poorly because it results from stretching of the fibrous joint capsule, and inflammation within the joint cavity elicits an axon reflex leading to vasodilation and warmth over an infected joint. Lymphatic channels drain to the regional lymph nodes and are present in all the joint tissues except cartilage.

Microorganisms can enter the joint space by hematogenous spread, direct inoculation, or extension of a contiguous focus of infection. The synovial membrane has been shown to have circulatory perfusion similar to that of the brain (if one assumes that 1 g of synovial membrane is in an adult knee joint). Consequently, bacteria in blood are potentially delivered to the synovial membrane during transient bacteremia. A history of trauma often is cited as a predisposing factor for development of bacterial arthritis, but the significance of such a history is unclear in view of the great frequency of minor trauma in childhood.

Upper respiratory tract infections frequently precede the development of septic arthritis caused by *Haemophilus influenzae* type b and *Kingella kingae*, and oral ulcers are often noted prior to *Kingella* infections. Similar to traumatic injuries, they are presumed to increase the likelihood of bacteremia.[10,103] Septic arthritis also may develop after joint surgery and joint injections. Gram-negative organisms are the most frequent pathogens when septic arthritis occurs after surgery or instrumentation of the urinary or intestinal tract. *Salmonella* septic arthritis may develop during the course of *Salmonella* bacteremia in a normal host, but it occurs with increased frequency in patients with sickle-cell disease and related hemoglobinopathies. Although septic arthritis occurs in children and adults infected with human immunodeficiency virus (HIV),[68] as yet no data have substantiated that HIV increases the incidence of musculoskeletal infections in children. However, one recent report indicates that pneumococcal osteoarticular infections may be more common in HIV-positive children, compared with HIV-seronegative children.[85] Septic arthritis has been described during varicella, presumably caused by bacteremia resulting from infection of skin lesions. It must be differentiated from the apparent ability of varicella-zoster virus to cause joint inflammation on its own.[71,84] Arthritis also occurs during acute infection with other viruses (e.g., variola, Epstein-Barr virus, erythrovirus [parvovirus B19], mumps or measles viruses, and enteroviruses).

Inoculation arthritis occurs after invasion of the joint by a contaminated object. In one series, 5 of 35 cases of septic arthritis were caused by such a mechanism. A predilection for the knee exists; four of five cases cited in this study were secondary to kneeling on sewing needles.[88]

Aside from joint involvement during osteomyelitis, extension of a contiguous infection into the joint space rarely occurs. In one series, 10 of 77 patients with septic arthritis had disease originating from a contiguous focus.[20] None involved the joints of the foot, and eight occurred before the availability of many antibiotics (1951). This high frequency of septic arthritis caused by spread of infection from a contiguous focus has not been seen in more recent studies.

ETIOLOGY

Staphylococcus aureus is the most common agent causing septic arthritis, including methicillin-susceptible and methicillin-resistant *S. aureus* (MRSA).* Streptococci (especially group A beta-hemolytic organisms and pneumococci) have been responsible for most other gram-positive infections (Table 56-1). *K. kingae* has been recognized increasingly as a cause of septic arthritis,† perhaps because of improvements in laboratory methods.[105] In one series from Israel, *Kingella* was the most common bacterial isolate (48% of cases) and *S. aureus* was not found.[104] In neonates and sexually active adolescents with suspected septic arthritis, *Neisseria gonorrhoeae* should be considered.[41,58,60]

H. influenzae type b historically has been an important cause of septic arthritis in children younger than 2 years old but now is seen only rarely in areas with widespread immunization.[15,40,47,64] Arthritis caused by *Streptococcus pneumoniae* also generally occurs in children younger than 2 years old and may diminish in frequency with broader use of protein conjugate vaccines. In a few cases,

*References 3, 40, 64, 66, 69, 73, 78, 88.
†References 29, 45, 64, 67, 103, 104.

TABLE 56-1 **Bacterial Etiology of Septic Arthritis in Children**

	Gram-Positive Bacteria				*Gram-Negative Bacteria*							
Year of Report	***Staphylococcus aureus***	**Streptococci**	***Streptococcus pneumoniae***	**CNS**	***Haemophilus influenzae***	***Kingella kingae***	***Neisseria meningitidis***	***Salmonella***	**Non-*Salmonella* Enterobacteriaceae**	***Neisseria gonorrhoeae***	**Other**	**Total Cases**
1941[52]	50	45	2	10	0	0	0	0	0	14	0	121
1958[88]	18	8	5	2	3	0	0	0	2	0	1	38*
1972[73]	40	20	8	5	37	0	4	2	7	13	11	146*
1975[69]	37	10	0	5	14	0	0	0	2	0	7	75
1987[10]	40	8	5	0	20	0	4	0	2	2	9	90
1995[104]	0	2	3	0	8	19	1	1	1	0	5	40
1999[64]	10	5	2	2	1	3	3	0	4	2	1	33
Total	*195*	*98*	*25*	*24*	*83*	*22*	*12*	*3*	*18*	*31*	*34*	*545*
Percentage of all isolates	36	18	5	4	15	4	2	1	3	6	6	

*Two isolates in one case each.
CNS, coagulase-negative staphylococci.

TABLE 56-2 **Joints Involved in Septic Arthritis of Children**

Reference	Knee	Hip	Ankle	Wrist	Elbow	Shoulder	Small Diarthrodial Joints
Heberling[52]	40	50	13	3	8	9	2
Watkins et al.[100]	8	13	2	6	9	2	3
Samilson et al.[88]	8	19	2	0	6	3	0
Nelson[73]	103	48	38	12	35	10	4
Gillespie[37]	37	41	13	2	3	3	0
Yagupsky et al.[104]	16	6	13	1	2	3	1
Goergens et al.[40]	15	15	4	2	4	1	3
Total	*227*	*192*	*85*	*26*	*67*	*31*	*13*
Percentage of all cases	35	30	13	4	10	5	2

septic arthritis is seen with acute *Neisseria meningitidis* infection.

Salmonella spp. cause approximately 1 percent of the total cases of septic arthritis. Beyond the neonatal period, infections with other enteric gram-negative bacteria are rare occurrences in pediatric septic arthritis and often are associated with inoculation, instrumentation, or an immunocompromised state.[41] Infections with *Serratia*, *Aeromonas*, *Enterobacter*, *Bacteroides*, and *Campylobacter* generally occur in patients with malignancy who are immunosuppressed.* *Pseudomonas aeruginosa* infections are associated with arthritis in infants, with infection of puncture wounds, or with injectable drug use.[69,73,95] Discussion of Lyme arthritis is beyond the scope of this chapter, but intermittent, inflammatory arthritis is seen in many patients after *Borrelia burgdorferi* is transmitted by a tick bite.[56,92]

Unusual causes of septic arthritis include *Propionibacterium acnes*,[106] *Actinomyces* (formerly *Corynebacterium*) *pyogenes*,[77] and *Pasteurella multocida*.[44] *Streptobacillus moniliformis* infection of joints may become evident 2 to 3 days after a rat bite occurs; a macular rash commonly is present at initial evaluation. *Brucella*, mycobacteria (*Mycobacterium tuberculosis* and atypical species), and *Nocardia asteroides* may cause a chronic monarticular arthritis with a granulomatous reaction. Septic arthritis may also be caused during disseminated fungal infections, including histoplasmosis,[28] coccidioidomycosis,[13] blastomycosis,[73] and cryptococcosis.[91] The knee joint is most often involved in coccidioidal and cryptococcal arthritis.[13,91]

DIAGNOSIS

Clinical Findings

Almost all patients have fever and constitutional symptoms within the first few days of acquiring infection with the most common bacterial pathogens.[37] Table 56-2 lists the frequency of specific joint involvement in hematogenous septic arthritis of childhood. Lower extremity (knee, hip, and ankle) infections consistently account for approximately 80 percent of all cases.† Focal findings in the joint involved almost always are present.

In infants, in whom the hip is one of the most frequent joints involved, swelling, tenderness, and heat may be absent. Most commonly, the infant with septic arthritis lies with the involved leg abducted and externally rotated. Often, dislocation occurs.[75] When the capsule of the joint can be examined, swelling is noted; effusion was present in 22 of 24 cases in one series.[99] Because pain fibers are located in the capsule, any maneuver that increases intracapsular pressure also produces pain. In the hip, this pain can be elicited by compression of the head of the femur into the acetabulum. A portal of entry almost never is apparent, and bilateral hip joint infection may occur.[79] Pyogenic sacroiliitis often is accompanied by tenderness detected by pressure applied over the sacrum during a digital rectal examination and by pain experienced during simultaneous flexion, abduction, and external rotation at the hip.[2]

Gonococcal arthritis in neonates has nonspecific prodromal symptoms, including poor feeding, irritability, and fever. The exact portal of entry is unknown, and the joints below the hip usually are involved (knee, ankle, and metatarsal). During adolescence, gonococcal arthritis occurs as a manifestation of sepsis with fever, chills, rash, and multiple small joint involvement, often with tenosynovitis.[35] The illness frequently follows the onset of menses by a few days.

Radiologic Findings

Findings on plain radiography are due to capsular swelling. In the joints readily accessible to physical examination, radiographs add little to the diagnostic evaluation, but when septic arthritis of the hip is suspected in a child, they are a valuable adjunct and may identify other causes of hip pain, such as Legg-Calvé-Perthes disease, slipped capital femoral epiphysis, and fracture. Films of the hip should be made with the child in the frog-leg position and with the legs extended at the knee and slightly internally rotated. The early signs of septic arthritis are caused by swelling of the capsule, which displaces the fat lines. One of the oldest signs is the obturator sign: as the tendon of the obturator internus passes over the capsule of the hip joint, the margins of this muscle are displaced medially into the pelvis (Fig. 56-1).[53] With continued swelling of the hip joint capsule, the femoral head is displaced laterally and upward.[22]

*References 1, 6, 21, 41, 65, 73, 77.
†References 40, 52, 67, 69, 73, 88.

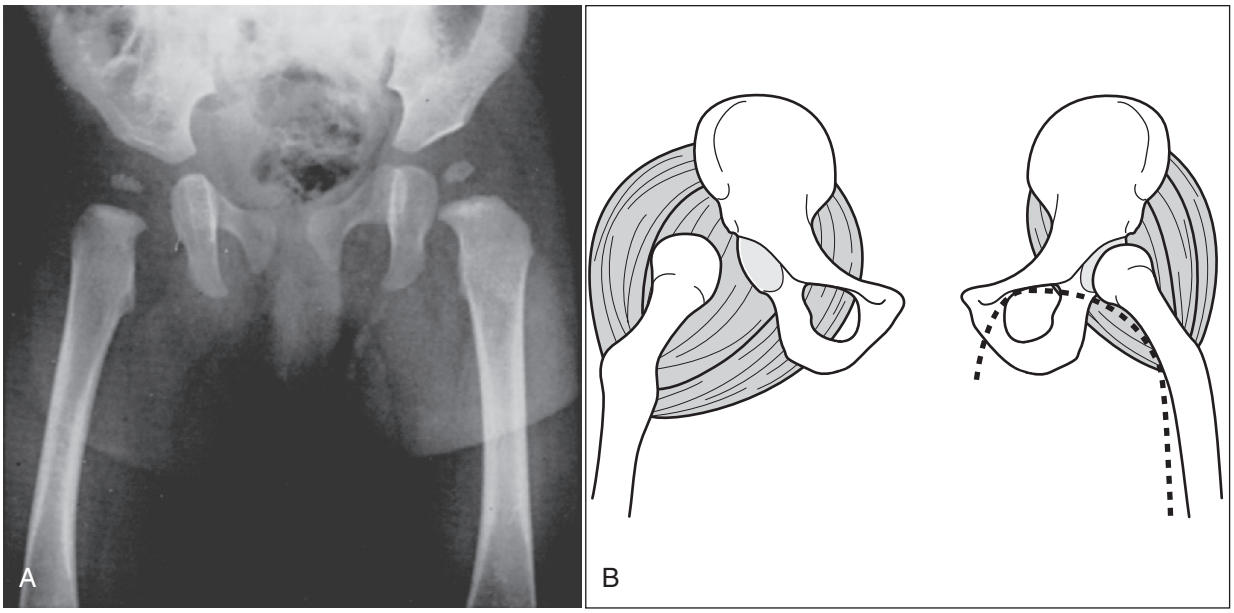

FIGURE 56-1 ■ The obturator sign in the hip is one of the oldest signs of septic arthritis. Another consistent finding is obliteration or lateral displacement of the gluteal fat lines and loss of continuity of the Shenton line. These findings are illustrated radiographically **(A)** and schematically **(B)**.

One of the most consistent findings is obliteration or lateral displacement of the gluteal fat lines (see Fig. 56-1).[101] Coincident with filling of the capsule with exudate, the femoral portion of the Shenton line is raised and its arc is widened.[101] If a technetium bone scan is performed, increased uptake on either side of the joint is seen during the "blood pool" phase of the scan.[98] Ultrasound evaluation has proved useful in evaluation of septic arthritis of the hip.[55,107] In a series of 96 patients, none of the 40 patients with normal ultrasound findings had septic arthritis.[107]

Bacterial infections causing pyogenic sacroiliitis may be particularly difficult to diagnose, because bone scintiscans and computed tomography initially may be negative. Magnetic resonance imaging seems to be the diagnostic radiologic method of choice.[2,31]

Laboratory Evaluation

Diagnostic evaluation for suspected septic arthritis generally includes determination of the erythrocyte sedimentation rate or C-reactive protein, and the peripheral blood leukocyte count and differential. These may be only mildly elevated in cases of proven infection, however.[30,107] Blood for culture always should be obtained because blood cultures sometimes may yield the pathogen when joint fluid cultures do not.[59]

Joint fluid should be collected in a heparinized syringe so that the large clot that usually forms in fluid obtained from patients with septic arthritis or juvenile rheumatoid arthritis does not preclude enumeration of leukocytes. A cell and differential count should be performed on aspirated joint fluid, and it must be Gram stained and cultured. Identification of organisms in joint fluid is the primary criterion for diagnosis of septic arthritis. Careful examination of a Gram-stained smear of joint fluid aspirates must be emphasized because joint fluid exerts a bacteriostatic effect on microorganisms and organisms that can be seen but may not grow in culture. Approximately 35 percent of joint aspirates are sterile in patients with other clinical and laboratory findings of a septic joint, including positive blood cultures.[10,73] Joint fluid should be cultured aerobically and under anaerobic conditions. The use of cell lysis culture bottles may enhance the recovery of *K. kingae* and other organisms.[105] *N. gonorrhoeae* is a highly fastidious organism, and oropharyngeal, rectal, and urogenital cultures or detection of gonococcal DNA in urine may be needed to confirm the diagnosis of gonococcal arthritis. Polymerase chain reaction analysis of joint fluid is likely to become a useful adjunct to the diagnosis of culture-negative disease, although it remains investigational.[70,96]

The median synovial fluid leukocyte count in bacterial arthritis in one study was 60.5×10^9 cells/L.[90] In this and other surveys,[86] polymorphonuclear leukocytes accounted for 75 to 90 percent of the white blood cells. Cell density generally is lower in fluid obtained from patients with acute rheumatic fever, juvenile rheumatoid arthritis, and other inflammatory causes of arthritis (Table 56-3).[8,86,90] The glucose concentration often is decreased in septic arthritis, but it may be normal.[90,99] It also may be depressed in rheumatoid arthritis and other conditions. A joint fluid leukocyte density of 5 to 8×10^9 cells/L has been found in joint fluid from patients ultimately proved to have septic arthritis.[86] Minimally turbid fluid with a seemingly low number of cells still should be processed for bacterial culture and Gram stain. Examination of the fluid for uric acid and other types of crystals should be considered in certain children (e.g., children with hyperuricemia).

TABLE 56-3 **Joint Fluid Findings in Childhood Arthritides**

Diagnosis	Spontaneous Clotting	Mucin Clot	Leukocytes/mL	% Polymorphonuclear Leukocytes	Glucose as Percentage of Blood Value
Septic arthritis	Rapid formation of large clot	Curdled milk	73,000	90	30
Rheumatic fever	Small to absent	Tightrope	18,000	60	75
Juvenile rheumatoid arthritis	Large clot	Small, friable masses	15,000	60	75

From Ropes M. Joint fluid findings in disease. Bull Rheum Dis *1957;***7***(Suppl.):21.*

DIFFERENTIAL DIAGNOSIS

Although bacterial infections are the most common cause of septic arthritis, other microorganisms, including viruses (varicella-zoster, erythrovirus [parvovirus B19], rubella, chikungunya[62] and other togaviruses, variola, vaccinia, certain enteroviruses, others), mycobacteria, and fungi (*Coccidioides* spp., *Sporothrix schenckii*, *Blastomyces dermatitidis*, *Candida* spp.), may be involved.

The differential diagnosis includes infectious and noninfectious entities. Local infections that mimic septic arthritis include obturator internus muscle abscess,[97] epiphyseal osteomyelitis, a deep cellulitis, and, rarely, septic (suppurative) bursitis. Septic bursitis may be particularly difficult to distinguish from septic arthritis. Children with septic bursitis frequently have a history of recent trauma and fever along with limitation of joint movement.[51,81] Careful physical examination and aspiration of bursal fluid allow the entities to be differentiated. Septic arthritis and arthralgia may occur during infectious endocarditis.

Toxic (or transient) synovitis (also referred to as irritable hip and reactive synovitis) is frequently seen in children and has milder presenting features than bacterial infections of the hip. The combination of fever, elevated white blood cell count, erythrocyte sedimentation rate (>40 mm/hr), C-reactive protein, and inability to bear weight permits septic arthritis to be distinguished from transient synovitis with high accuracy; greater than 90 percent sensitivity for the detection of septic arthritis has been reported when all of these factors are present.[17,30,59,107]

Other noninfectious entities to be considered in the differential diagnosis include traumatic arthritis, juvenile idiopathic arthritis, villonodular synovitis, leukemia, serum sickness, ulcerative colitis, granulomatous colitis, Schönlein-Henoch purpura, traumatic arthritis, fracture, Legg-Calvé-Perthes disease, slipped femoral capital epiphysis, and metabolic diseases affecting joints (e.g., ochronosis in adults with alkaptonuria).

TREATMENT

Surgical Treatment

In infants, septic arthritis in the hips or shoulders is a surgical emergency; these joints should be drained as soon as the diagnosis is apparent to prevent bony destruction.[79] In a study in adults, the outcome of septic arthritis was better in patients treated by repeated needle aspiration than in patients treated by surgical drainage.[42,43] Eighty percent of the patients treated by needle aspiration were thought to have a good outcome versus 47 percent treated by surgical drainage. All wrist joint infections in this series were treated only by needle aspiration; in treatment of septic arthritis of the knee, however, the outcome was almost equivalent. Similar findings have been reported by others.[11] The investigators suggested that surgical drainage should be performed in any joint whenever the presence of large amounts of fibrin, tissue debris, or loculation prevents adequate drainage by needle aspiration.

Antibiotic Therapy

Antibiotic treatment of septic arthritis should target the most common pathogens. In children beyond the neonatal period, *S. aureus* and other gram-positive organisms currently predominate.[15,64,73] Antistaphylococcal penicillins (e.g., oxacillin or nafcillin) are usually given, but vancomycin or clindamycin should be used as the empirical antistaphylococcal agent in areas where community-acquired MRSA is a concern.[3,66] Some authorities advocate that this be done when community-acquired MRSA represents more than 10 percent of *S. aureus* in a geographic area.[57] Vancomycin is preferred if clindamycin resistance is common among *S. aureus* isolates (>10%). At present, no regimen is clearly preferred for the treatment of penicillin-nonsusceptible *S. pneumoniae*, but ceftriaxone, cefotaxime, and clindamycin have been used successfully.[16]

The need for coverage for gram-negative pathogens should also be considered. Ceftriaxone and cefotaxime are appropriate for the treatment of gonococcal infection. In children below the age of 36 months,[32] adding empirical coverage for *Kingella* infection may also be indicated if Gram stain and culture have not identified an alternative pathogen; cefuroxime, cefotaxime, or ceftriaxone may be preferred.[102] Although the risk of acquiring invasive *H. influenzae* type b infection is low in areas with effective immunization, rare cases continue to be reported. Children younger than 2 years who have not been immunized against *H. influenzae* type b should be treated initially with a regimen that contains an antistaphylococcal agent, such as nafcillin, vancomycin, or clindamycin, and an agent active against this agent (e.g.,

cefotaxime). Cefuroxime, which is active against *S. aureus* (and other gram-positive pathogens) and *H. influenzae*, is a useful alternative.

All antibiotics that have been studied penetrate into joint fluid readily; soon after administration, at the time of peak serum levels, the joint fluid concentration averages 30 percent of the serum value.[5,7,72,83] The efflux of antibiotic from joint fluid back to serum is slow, however. Immediately before the next systemic dose is administered, joint fluid antibiotic concentrations frequently exceed the concentrations present in serum. Antibiotics, whether administered orally or parenterally, can achieve efficacious concentrations in joint fluid.

Injecting antibiotics into the joint space usually is unnecessary because of their excellent penetration. Many antibiotics (e.g., cephalothin) are capable of evoking an intense inflammatory reaction, much as they do if they are infiltrated beneath the skin. Tetzlaff and associates[94] have shown that septic arthritis can be treated for 1 week (or less) parenterally, with the balance of the drug given orally. These investigators considered the minimal duration of therapy to be 3 weeks, because some cases initially thought to reflect septic arthritis alone may actually have coincident bone infection.

The effectiveness of antibiotic therapy can be assessed by serial joint fluid examinations, leukocyte density, and culture results.[99] The time required for resolution of joint symptoms and the time for the synovia to become sterile are proportional to the duration of symptoms before the initiation of appropriate antibiotic therapy.[54] In one study, some patients still had cultures yielding bacteria after undergoing 1 week of therapy. In these patients, cellular density ranged from 25 to 253×10^9 cells/L (mean 109×10^9 cells/L) at the beginning of therapy; 92 percent of the cells were polymorphonuclear leukocytes. By the end of 2 weeks of therapy, all cultures were sterile, and leukocyte density ranged from 4.9 to 23×10^9 cells/L (mean of 12.3×10^9 cells/L).[99] Similar studies of the rate of resolution of indicators of inflammation in joint fluid have suggested that after receiving 9 days of treatment, patients who ultimately recovered completely had a density of 5×10^9 cells/L; however, in patients with recrudescent infection or a poor outcome, the density was 6×10^{10} cells/L.[41]

When an effusion reaccumulates, one should remove it by arthrocentesis, not only to make the patient more comfortable but also to permit serial assessment of therapy. Generally, disease caused by *S. aureus* and Enterobacteriaceae requires longer treatment than disease caused by *H. influenzae* or meningococci. Radiographs should be obtained during therapy to seek bone changes indicating that osteomyelitis may have been present. Concomitant osteomyelitis may require surgical intervention, and antibiotic therapy must continue beyond 21 days.

PROGNOSIS

Sequelae, including hip dislocation, are more likely to develop in infants and patients with symptoms of longer duration.[99,100] In one study, seven of eight patients in whom "permanent" hip dislocation developed had symptoms for 7 or more days before receiving treatment.[100] Similarly, all patients with spontaneous ankylosis had symptoms longer than 7 days.[100] Comparable data were obtained by Samilson and colleagues.[88] Eight of 10 patients with pathologic dislocation of the hip had symptoms for longer than 7 days.

A detailed analysis of factors affecting satisfactory outcomes in hematogenous septic arthritis of childhood showed no significant difference in the joint when arthrotomy, with or without irrigation, was compared with repeated aspiration.[69] Likewise, the specific antibiotic used had no effect on outcome as long as it was effective against the infecting organism. The literature does suggest that septic arthritis caused by Enterobacteriaceae is associated with more frequent sequelae than when caused by other pathogens.[43,74] *S. aureus* is more likely to cause sequelae than *H. influenzae*.[10]

A randomized, placebo-controlled study has examined the impact of a brief course of dexamethasone on the outcome of bacteriologically confirmed cases of septic arthritis of hematogenous origin in children. Children younger than 3 months were excluded from participation, and *H. influenzae* was responsible for 13 percent of cases among the 100 evaluable patients. After 1 year of follow-up, limping, joint pain, and restriction of movement were found in 26 percent of patients who received a placebo, but in only 1 of 50 patients treated with dexamethasone.[80] A second randomized trial involved 49 children (average age of 33 months) with septic arthritis to whom adjunctive dexamethasone was given during the first 4 days of antimicrobial therapy.[50] This treatment was associated with decreased duration of fever (1.7 vs. 2.8 days), pain (7.2 vs. 10.8 days), and parenteral antibiotics (9.9 vs. 12.6 days), but no difference in long-term outcome. At present, the benefits of dexamethasone therapy in septic arthritis remain unclear.

SPECIAL PROBLEMS

Neonatal Septic Arthritis

Neonatal septic arthritis is a problem that warrants special attention because of its subtle signs and symptoms,[23] its potential for catastrophic consequences of untreated disease,[78] and the unusual organisms occasionally seen. Any neonate who has swelling in the region of the thigh and the buttock and holds that leg flexed with slight abduction and external rotation at the hip should be suspected to have femoral-acetabular septic arthritis. It can occur 1 to 28 days after femoral venipuncture (in most neonates, 5 to 9 days) and should not be confused with femoral vein thrombosis.[4,19]

In most neonates, no toxemia, fever, or leukocytosis is present. More than one joint may be involved when initially evaluated. The progression of disease in neonates can be so indolent that the hip spontaneously drains along the obturator internus, and the condition is manifested as a lower abdominal mass just above the inguinal canal.[34] Problems in recognizing the disease in neonates undoubtedly contribute to the poor outcome. In one

series, the delay from onset to diagnosis was an average of 1 week; only two of the nine infants in this series had a normal hip examination at follow-up.[78]

In most series, the causative agents are staphylococci and streptococci,[4,14,19,23,78] but gram-negative organisms often are found. More importantly, arthritis caused by *Candida albicans* has been described,[26] and the gonococcus should not be forgotten. In gonococcal arthritis, the symptoms, which usually become apparent in infants 1 to 5 weeks of age, generally are polyarthritic (more than one joint involved).[39] As previously noted, other symptoms of neonatal gonococcal arthritis are no different from symptoms caused by other pathogens.

Initial antibiotic therapy for neonatal septic arthritis should be directed toward *S. aureus* and the nosocomial gram-negative bacteria that are prevalent in the nursery. Antibiotic therapy can be altered when the susceptibility of the causative bacterium is known. As noted earlier, the usual duration of therapy is 3 to 4 weeks, and radiography should be performed toward the end of treatment. Oral therapy has been used successfully to complete treatment of septic arthritis in neonates,[82] but absorption of antibiotics in this age range is unpredictable.[89]

Fungal Arthritis

Fungal arthritis is rare in children but may be seen with pathogens of endemic mycoses, such as *Histoplasma capsulatum*, *B. dermatitidis*,[73] and *Coccidioides* species, as well as with opportunistic fungal pathogens (e.g., *Candida* spp., *Cryptococcus neoformans*).[67,91] These pathogens usually manifest after an indolent course. The recommended medical treatment is generally identical to that required for other systemic infections caused by these organisms.

Joint Infections During Rheumatoid Arthritis

Joint infections that develop during a case of rheumatoid arthritis seem to occur more frequently in adults with rheumatoid arthritis than in children. In one series, only 2 of 17 patients were younger than age 10 years.[6] The notable features are that the hips generally are not involved and infection of multiple joints (17 of 44 patients had more than one joint involved) frequently occurs. Because of the preexisting joint disease, the diagnosis often is delayed and the outcome usually is poor. Septic arthritis should be considered if an unusual worsening of one joint occurs during a flare of rheumatoid arthritis.

Reactive Arthritis

An oligoarticular arthritis may occur after infection with *Shigella* spp.,[18,76] *Chlamydia trachomatis*, *Salmonella* spp.,[49] and certain *Yersinia* serotypes.[61] A link to infection with *Chlamydophila pneumoniae* (formerly *Chlamydia pneumoniae*) has also been described, and rare cases of septic arthritis may follow *Clostridium difficile* colitis in children.[12,25,33,63] This postinfectious joint inflammation seems to develop with greater frequency in individuals who express the histocompatibility antigen HLA-B*27, perhaps as a result of molecular similarities between bacterial antigens and the human protein. In addition, during the initial infection, bacterial antigens apparently may be deposited in the synovium and may persist, leading to pathogenic inflammation.[46,61]

Generally, the onset of joint symptoms occurs a few days to several weeks after a transient, and often mild episode of diarrhea or symptoms of urethritis. The arthritis may mimic rheumatic fever and is characterized by daily low-grade fever and an increased erythrocyte sedimentation rate. Classic Reiter syndrome, which includes ocular inflammation, oligoarticular arthritis, and sterile urethritis, may also occur.

Reactive arthritis generally can be distinguished from septic arthritis by analysis of synovial fluid; infectious arthritis usually is associated with an increased number of white blood cells in the synovial fluid.[8] Resolution of joint symptoms in cases of reactive arthritis usually occurs several weeks to months after onset. Anti-inflammatory agents are the cornerstone of therapy and may include nonsteroidal anti-inflammatory drugs, corticosteroids, or disease-modifying antirheumatic drugs.

Reactive arthritis also may occur after acute infections with *N. meningitidis*[48] and *H. influenzae*.[87] Symptoms and signs of joint inflammation often appear 1 week or more after the acute septic episode and resolve without sequelae.

NEW REFERENCES SINCE THE SIXTH EDITION

12. Birnbaum J, Bartlett JG, Gelber AC. *Clostridium difficile:* an under-recognized cause of reactive arthritis? *Clin Rheumatol* 2008;**27**:253–5.
25. Dacheux C, Pruvost I, Herbaux B, et al. [*Clostridium difficile* reactive arthritis in a 7-year-old child]. *Arch Pediatr* 2012;**19**: 607–11.
32. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
33. Durand CL, Miller PF. Severe *Clostridium difficile* colitis and reactive arthritis in a ten-year-old child. *Pediatr Infect Dis J* 2009;**28**:750–1.
50. Harel L, Prais D, Bar-On E, et al. Dexamethasone therapy for septic arthritis in children: results of a randomized double-blind placebo-controlled study. *J Pediatr Orthop* 2011;**31**:211–5.
62. Laoprasopwattana K, Kaewjungwad L, Jarumanokul R, et al. Differential diagnosis of Chikungunya, dengue viral infection and other acute febrile illnesses in children. *Pediatr Infect Dis J* 2012;**31**:459–63.
67. Masson AT, Gudnason T, Jonmundsson GK, et al. [Bacterial osteomyelitis and arthritis in Icelandic children 1996-2005]. *Laeknabladid* 2011;**97**:91–6.
85. Robertson AJ, Firth GB, Truda C, et al. Epidemiology of acute osteoarticular sepsis in a setting with a high prevalence of pediatric HIV infection. *J Pediatr Orthop* 2012;**32**:215–9.
102. Yagupsky P. Antibiotic susceptibility of *Kingella kingae* isolates from children with skeletal system infections. *Pediatr Infect Dis J* 2012;**31**:212.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ament ME, Gaal SA. *Bacteroides* arthritis. *Am J Dis Child* 1967;**114**:427–8.
2. Aprin H, Turen C. Pyogenic sacroiliitis in children. *Clin Orthop Relat Res* 1993;**287**:98–106.
3. Arnold SR, Elias D, Buckingham SC, et al. Changing patterns of acute hematogenous osteomyelitis and septic arthritis: emergence of community-associated methicillin-resistant *Staphylococcus aureus*. *J Pediatr Orthop* 2006;**26**:703–8.
4. Asnes RS, Arendar GM. Septic arthritis of the hip: a complication of femoral venipuncture. *Pediatrics* 1966;**38**:837–41.
5. Baciocco EA, Iles RL. Ampicillin and kanamycin concentrations in joint fluid. *Clin Pharmacol Ther* 1971;**12**:858–63.
6. Baitch A. Recent observations of acute suppurative arthritis. *Clin Orthop* 1962;**22**:157–66.
7. Balboni V, Shapiro IM, Kydd DM. The penetration of penicillin into joint fluid following intramuscular administration. *Am J Med Sci* 1945;**210**:588–91.
8. Baldassare AR, Chang F, Zuckner J. Markedly raised synovial fluid leucocyte counts not associated with infectious arthritis in children. *Ann Rheum Dis* 1978;**37**:404–9.
9. Barnett C, Davies DV, MacConcill MA. *Synovial joints*. Springfield, Ill; Charles C Thomas; 1961.
10. Barton LL, Dunkle LM, Habib FH. Septic arthritis in childhood: a 13-year review. *Am J Dis Child* 1987;**141**:898–900.
11. Betz RR, Cooperman DR, Wopperer JM, et al. Late sequelae of septic arthritis of the hip in infancy and childhood. *J Pediatr Orthop* 1990;**10**:365–72.
12. Birnbaum J, Bartlett JG, Gelber AC. *Clostridium difficile:* an under-recognized cause of reactive arthritis? *Clin Rheumatol* 2008;**27**:253–5.
13. Blair JE. State-of-the-art treatment of coccidioidomycosis skeletal infections. *Ann N Y Acad Sci* 2007;**1111**:422–33.
14. Bordanovitch A. Neonatal arthritis due to *Proteus vulgaris*. *Arch Dis Child* 1948;**23**:65–6.
15. Bowerman SG, Green NE, Mencio GA. Decline of bone and joint infections attributable to *Haemophilus influenzae* type b. *Clin Orthop Relat Res* 1997;**341**:128–33.
16. Bradley JS, Kaplan SL, Tan TQ, et al. Pediatric pneumococcal bone and joint infections. The Pediatric Multicenter Pneumococcal Surveillance Study Group (PMPSSG). *Pediatrics* 1998;**102**: 1376–82.
17. Caird MS, Flynn JM, Leung YL, et al. Factors distinguishing septic arthritis from transient synovitis of the hip in children: a prospective study. *J Bone Joint Surg Am* 2006;**88**:1251–7.
18. Calin A, Fries JF. An "experimental" epidemic of Reiter's syndrome revisited: follow-up evidence on genetic and environmental factors. *Ann Intern Med* 1976;**84**:564–6.
19. Chacha PB. Suppurative arthritis of the hip joint in infancy: a persistent diagnostic problem and possible complication of femoral venipuncture. *J Bone Joint Surg Am* 1971;**53**:538–44.
20. Chartier Y, Martin WJ, Kelly PJ. [Bacterial arthritis: experiences in the treatment of 77 patients]. *Ann Intern Med* 1959;**50**: 1462–74.
21. Chmel H, Armstrong D. Acute arthritis caused by *Aeromonas hydrophilia:* clinical and therapeutic aspects. *Arthritis Rheum* 1976;**19**:169–72.
22. Chont L. Roentgen sign of early suppurative arthritis of the hip in infancy. *Radiology* 1942;**38**:708–14.
23. Chung SM, Pollis RE. Diagnostic pitfalls in septic arthritis of the hip in infants and children. *Clin Pediatr (Phila)* 1975;**14**:758–61 passim.
24. Curtiss Jr PH. The pathophysiology of joint infections. *Clin Orthop Relat Res* 1973;(96):129–35.
25. Dacheux C, Pruvost I, Herbaux B, et al. [*Clostridium difficile* reactive arthritis in a 7-year-old child]. *Arch Pediatr* 2012;**19**: 607–11.
26. Dan M. Septic arthritis in young infants: clinical and microbiologic correlations and therapeutic implications. *Rev Infect Dis* 1984;**6**:147–55.
27. Daniel D, Akeson W, Amiel D, et al. Lavage of septic joints in rabbits: effects of chondrolysis. *J Bone Joint Surg Am* 1976;**58**: 393–5.
28. Darouiche RO, Cadle RM, Zenon GJ, et al. Articular histoplasmosis. *J Rheumatol* 1992;**19**:1991–3.
29. de Groot R, Glover D, Clausen C, et al. Bone and joint infections caused by *Kingella kingae:* six cases and review of the literature. *Rev Infect Dis* 1988;**10**:998–1004.
30. Del Beccaro MA, Champoux AN, Bockers T, et al. Septic arthritis versus transient synovitis of the hip: the value of screening laboratory tests. *Ann Emerg Med* 1992;**21**:1418–22.
31. Doita M, Yoshiya S, Nabeshima Y, et al. Acute pyogenic sacroiliitis without predisposing conditions. *Spine (Phila Pa 1976)* 2003; **28**:E384–9.
32. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
33. Durand CL, Miller PF. Severe *Clostridium difficile* colitis and reactive arthritis in a ten-year-old child. *Pediatr Infect Dis J* 2009;**28**:750–1.
34. Freiberg JA, Perlman R. Pelvic abscesses associated with acute purulent infection of the hip joint. *J Bone Joint Surg Am* 1936;**18**:417–27.
35. Garcia-Kutzbach A, Masi AT. Acute infectious agent arthritis (IAA): a detailed comparison of proved gonococcal and other blood-borne bacterial arthritis. *J Rheumatol* 1974;**1**:93–101.
36. Gardner ED. Physiology of joints. *J Bone Joint Surg Am* 1963;**45**:152–9.
37. Gillespie R. Septic arthritis of childhood. *Clin Orthop Relat Res* 1973;(96):152–9.
38. Gillespie WJ. Epidemiology in bone and joint infection. *Infect Dis Clin North Am* 1990;**4**:361–76.
39. Glaser S, Boxerbaum B, Kennell JH. Gonococcal arthritis in the newborn: report of a case and review of the literature. *Am J Dis Child* 1966;**112**:185–8.
40. Goergens ED, McEvoy A, Watson M, et al. Acute osteomyelitis and septic arthritis in children. *J Paediatr Child Health* 2005; **41**:59–62.
41. Goldenberg DL, Brandt KD, Cathcart ES, et al. Acute arthritis caused by gram-negative bacilli: a clinical characterization. *Medicine (Baltimore)* 1974;**53**:197–208.
42. Goldenberg DL, Brandt KD, Cohen AS, et al. Treatment of septic arthritis: comparison of needle aspiration and surgery as initial modes of joint drainage. *Arthritis Rheum* 1975;**18**:83–90.
43. Goldenberg DL, Cohen AS. Acute infectious arthritis: a review of patients with nongonococcal joint infections (with emphasis on therapy and prognosis). *Am J Med* 1976;**60**:369–77.
44. Gomez-Reino JJ, Shah M, Gorevic P, et al. *Pasteurella multocida* arthritis: case report. *J Bone Joint Surg Am* 1980;**62**:1212–3.
45. Goutzmanis JJ, Gonis G, Gilbert GL. *Kingella kingae* infection in children: ten cases and a review of the literature. *Pediatr Infect Dis J* 1991;**10**:677–83.
46. Granfors K, Jalkanen S, von Essen R, et al. *Yersinia* antigens in synovial-fluid cells from patients with reactive arthritis. *N Engl J Med* 1989;**320**:216–21.
47. Granoff DM, Sargent E, Jolivette D. *Haemophilus influenzae* type b osteomyelitis. *Am J Dis Child* 1976;**132**:488–90.
48. Greenwood BM, Whittle HC, Bryceson AD. Allergic complications of meningococcal disease, part II: immunological investigations. *BMJ* 1973;**2**:737–40.
49. Hakansson U, Low B, Eitrem R, et al. HLA-B27 and reactive arthritis in an outbreak of salmonellosis. *Tissue Antigens* 1975;**6**: 366–7.
50. Harel L, Prais D, Bar-On E, et al. Dexamethasone therapy for septic arthritis in children: results of a randomized double-blind placebo-controlled study. *J Pediatr Orthop* 2011;**31**:211–5.
51. Harwell JI, Fisher D. Pediatric septic bursitis: case report of retrocalcaneal infection and review of the literature. *Clin Infect Dis* 2001;**32**:E102–4.
52. Heberling JA. A review of two hundred and one cases of suppurative arthritis. *J Bone Joint Surg* 1941;**23**:917–21.
53. Hefke HW, Turner VC. The obturator sign as the earliest roentgenographic sign in the diagnosis of septic arthritis and tuberculosis of the hip. *J Bone Joint Surg* 1942;**24**:857–69.
54. Ho Jr G, Su EY. Therapy for septic arthritis. *JAMA* 1982; **247**:797–800.
55. Jamillo D, Treves ST, Kasser JR, et al. Osteomyelitis and septic arthritis in children: appropriate use of imaging to guide treatment. *A J R Am J Roentgenol* 1995;**165**:339–403.
56. Kalish R. Lyme disease. *Rheum Dis Clin North Am* 1993; **19**:399–426.

57. Kaplan SL. Osteomyelitis in children. *Infect Dis Clin North Am* 2005;**19**:787–97, vii.
58. Kleiman MB, Lamb GA. Gonococcal arthritis in a newborn infant. *Pediatrics* 1973;**52**:285–7.
59. Kocher MS, Zurakowski D, Kasser JR. Differentiating between septic arthritis and transient synovitis of the hip in children: an evidence-based clinical prediction algorithm. *J Bone Joint Surg Am* 1999;**81**:1662–70.
60. Kohen DP. Neonatal gonococcal arthritis: three cases and review of the literature. *Pediatrics* 1974;**53**:436–40.
61. Koopman WJ. Host factors in the pathogenesis of arthritis triggered by infectious organisms. Overview. *Rheum Dis Clin North Am* 1993;**19**:279–92.
62. Laoprasopwattana K, Kaewjungwad L, Jarumanokul R, et al. Differential diagnosis of Chikungunya, dengue viral infection and other acute febrile illnesses in children. *Pediatr Infect Dis J* 2012;**31**:459–63.
63. Loffler HA, Pron B, Mouy R, et al. *Clostridium difficile*-associated reactive arthritis in two children. *Joint Bone Spine* 2004;**71**:60–2.
64. Luhmann JD, Luhmann SJ. Etiology of septic arthritis in children: an update for the 1990s. *Pediatr Emerg Care* 1999;**15**:40–2.
65. Martin CM, Merrill RH, Barrett Jr O. Arthritis due to *Serratia*. *J Bone Joint Surg* 1970;**52**:1450–2.
66. Martinez-Aguilar G, Hammerman WA, Mason Jr EO, et al. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in children. *Pediatr Infect Dis J* 2003;**22**:593–8.
67. Masson AT, Gudnason T, Jonmundsson GK, et al. [Bacterial osteomyelitis and arthritis in Icelandic children 1996-2005]. *Laeknabladid* 2011;**97**:91–6.
68. Merchan EC, Magallon M, Manso F, et al. Septic arthritis in HIV positive haemophiliacs: four cases and a literature review. *Int Orthop* 1992;**16**:302–6.
69. Morrey BF, Bianco Jr AJ, Rhodes KH. Septic arthritis in children. *Orthop Clin North Am* 1975;**6**:923–34.
70. Moumile K, Merckx J, Glorion C, et al. Osteoarticular infections caused by *Kingella kingae* in children: contribution of polymerase chain reaction to the microbiologic diagnosis. *Pediatr Infect Dis J* 2003;**22**:837–9.
71. Mulhern LM, Friday GA, Perri JA. Arthritis complicating varicella infection. *Pediatrics* 1971;**48**:827–9.
72. Nelson JD. Antibiotic concentrations in septic joint effusions. *N Engl J Med* 1971;**284**:349–53.
73. Nelson JD. The bacterial etiology and antibiotic management of septic arthritis in infants and children. *Pediatrics* 1972;**50**: 437–40.
74. Newman JH. Review of septic arthritis throughout the antibiotic era. *Ann Rheum Dis* 1976;**35**:198–205.
75. Nicholson J. Pyogenic arthritis with pathologic dislocation of the hip in infants. *JAMA* 1949;**141**:826–31.
76. Noer HR. An "experimental" epidemic of Reiter's syndrome. *JAMA* 1966;**198**:693–8.
77. Norenberg DD, Bigley DV, Virata RL, et al. *Corynebacterium pyogenes* septic arthritis with plasma cell synovial infiltrate and monoclonal gammopathy. *Arch Intern Med* 1978;**138**:810–1.
78. Obletz BE. Acute suppurative arthritis of the hip in the neonatal period. *J Bone Joint Surg Am* 1960;**42**:23–30.
79. Obletz BE. Suppurative arthritis of the hip joint in infants. *Clin Orthop* 1962;**22**:27–33.
80. Odio CM, Ramirez T, Arias G, et al. Double blind, randomized, placebo-controlled study of dexamethasone therapy for hematogenous septic arthritis in children. *Pediatr Infect Dis J* 2003;**22**: 883–8.
81. Paisley JW. Septic bursitis in childhood. *J Pediatr Orthop* 1982;**2**:57–61.
82. Perkins MD, Edwards KM, Heller RM, et al. Neonatal group B streptococcal osteomyelitis and suppurative arthritis: outpatient therapy. *Clin Pediatr (Phila)* 1989;**28**:229–30.
83. Plott MA, Roth H. Penetration of clindamycin into synovial fluid. *Clin Pharmacol Ther* 1970;**11**:577–80.
84. Priest JR, Urick JJ, Groth KE, et al. Varicella arthritis documented by isolation of virus from joint fluid. *J Pediatr* 1978;**93**:990–2.
85. Robertson AJ, Firth GB, Truda C, et al. Epidemiology of acute osteoarticular sepsis in a setting with a high prevalence of pediatric HIV infection. *J Pediatr Orthop* 2012;**32**:215–9.
86. Ropes M. Joint fluid findings in disease. *Bull Rheum Dis* 1957;**7**(Suppl.):21.
87. Rush PJ, Shore A, Inman R, et al. Arthritis associated with *Haemophilus influenzae* meningitis: septic or reactive? *J Pediatr* 1986;**109**:412–5.
88. Samilson RL, Bersani FA, Watkins MB. Acute suppurative arthritis in infants and children; the importance of early diagnosis and surgical drainage. *Pediatrics* 1958;**21**:798–804.
89. Schwartz GJ, Hegyi T, Spitzer A. Subtherapeutic dicloxacillin levels in a neonate: possible mechanisms. *J Pediatr* 1976;**89**: 310–2.
90. Shmerling RH, Delbanco TL, Tosteson AN, et al. Synovial fluid tests: what should be ordered? *JAMA* 1990;**264**:1009–14.
91. Stead KJ, Klugman KP, Painter ML, et al. Septic arthritis due to *Cryptococcus neoformans*. *J Infect* 1988;**17**:139–45.
92. Steere AC. Lyme disease. *N Engl J Med* 2001;**345**:115–25.
93. Stravino VD. The synovial system. *Am J Phys Med* 1972;**51**:312–20.
94. Tetzlaff TR, McCracken Jr GH, Nelson JD. Oral antibiotic therapy for skeletal infections of children, part II: therapy of osteomyelitis and suppurative arthritis. *J Pediatr* 1978;**92**:485–90.
95. Tindel JR, Crowder JG. Septic arthritis due to *Pseudomonas aeruginosa*. *JAMA* 1971;**218**:559–61.
96. Verdier I, Gayet-Ageron A, Ploton C, et al. Contribution of a broad range polymerase chain reaction to the diagnosis of osteoarticular infections caused by *Kingella kingae:* description of twenty-four recent pediatric diagnoses. *Pediatr Infect Dis J* 2005;**24**:692–6.
97. Viani RM, Bromberg K, Bradley JS. Obturator internus muscle abscess in children: report of seven cases and review. *Clin Infect Dis* 1999;**28**:117–22.
98. Volberg FM, Sumner TE, Abramson JS, et al. Unreliability of radiographic diagnosis of septic hip in children. *Pediatrics* 1984;**74**:118–20.
99. Ward J, Cohen AS, Bauer W. The diagnosis and therapy of acute suppurative arthritis. *Arthritis Rheum* 1960;**3**:522–35.
100. Watkins MB, Samilson RL, Winter DM. Acute suppurative arthritis. *J Bone Joint Surg* 1956;**28**:1313–20.
101. White H. Roentgen findings of acute infectious disease of the hip in infants and children. *Clin Orthop* 1962;**22**:34–42.
102. Yagupsky P. Antibiotic susceptibility of *Kingella kingae* isolates from children with skeletal system infections. *Pediatr Infect Dis J* 2012;**31**:212.
103. Yagupsky P. *Kingella kingae:* from medical rarity to an emerging paediatric pathogen. *Lancet Infect Dis* 2004;**4**:358–67.
104. Yagupsky P, Bar-Ziv Y, Howard CB, et al. Epidemiology, etiology, and clinical features of septic arthritis in children younger than 24 months. *Arch Pediatr Adolesc* 1995;**149**:537–40.
105. Yagupsky P, Press J. Use of the isolator 1.5 microbial tube for culture of synovial fluid from patients with septic arthritis. *J Clin Microbiol* 1997;**35**:2410–2.
106. Yocum RC, McArthur J, Petty BG, et al. Septic arthritis caused by *Propionibacterium acnes*. *JAMA* 1982;**248**:1740–1.
107. Zawin JK, Hoffer FA, Rand FF, et al. Joint effusion in children with an irritable hip: US diagnosis and aspiration. *Radiology* 1993;**187**:459–63.

CHAPTER 57

Bacterial Myositis and Pyomyositis

Charles Grose

Myositis is not a common manifestation of bacterial infection, but when it occurs, the consequences to the patient may be severe or even fatal. *Staphylococcus aureus* and group A streptococci are the most likely causative organisms. Myositis also has been associated with several other infectious agents, including viruses, fungi, and parasites. These pathogens are listed in Table 57-1; they are discussed briefly in this chapter and more thoroughly in the chapters on the specific microorganisms. This chapter focuses on two forms of pyogenic myositis, sometimes differentiated as *acute bacterial myositis* and classical (or *tropical) pyomyositis*. The former is caused primarily by group A streptococci and the latter by *S. aureus*, including methicillin-resistant *S. aureus* (MRSA).

PYOMYOSITIS

The pathologic entity termed *spontaneous acute myositis* was recognized by Virchow in the mid-19th century, but the first clinical description of suppurative myositis generally is attributed to the Japanese surgeon Scriba.[21] In 1904, another Japanese surgeon, Miyake,[17] extensively reviewed the subject of skeletal muscle abscesses and added 33 more cases. As the British and French expanded their colonial empires at the turn of the 20th century, the disease was recognized with increasing frequency in the native populations and in the soldiers who lived in the tropical areas of Asia and Africa.[26] It acquired the name by which it now is known widely—*tropical pyomyositis*.[9,10]

The suitability of this designation was confirmed by an epidemiologic study in East Africa, which discovered that the disease was found commonly only in regions with a truly tropical climate (i.e., a fairly constant high temperature and high relative humidity) at an altitude below 4000 feet.[15] Pyomyositis has been described, however, in children from geographic regions of the United States as diverse as New England,[9] northern California,[2] Iowa,[16] and Texas.[11,19,22] Numerous reported cases within the continental United States have occurred in and around San Antonio, Texas.[5] In a 10-year chart review, one or two cases of pyomyositis per 4000 pediatric admissions occurred annually. In contrast, a review of consultations of pediatric infectious diseases at the University of Iowa Hospital disclosed fewer cases of pyomyositis among children younger than 16 years old.[7] Pyomyositis seems to occur more commonly in children who live in the southernmost regions of the United States (e.g., Texas) than in children who live in the northern regions (e.g., Iowa).

Pathophysiology

The etiologic agent of the skeletal muscle abscesses in more than 90 percent of cases is *S. aureus*. An increasing percentage are caused by community-associated MRSA.[28] A distant second bacteriologic isolate is *Streptococcus*, including group A and nonhemolytic strains.

Miyake[17] studied extensively the experimental conditions under which *Staphylococcus* spp. cause muscle abscesses. When healthy rabbits were given boluses of *Staphylococcus* intravenously, they occasionally developed small abscesses in the kidney, liver, or spleen but never in the skeletal muscles. When specific muscles were damaged by mechanical pinching or electric current 24 or 48 hours before the intravenous injection of bacteria was administered, small abscesses developed within 2 to 28 days at some of the injured sites in nearly half of the animals. Abscesses were not found in healthy muscle tissue.

The role of trauma was supported further by a study of pyomyositis in the British Army.[3] After physicians found this disease to be a common problem in Gurkha army recruits, they investigated 32 cases and made several observations. Two thirds of the men recalled having experienced trauma at the affected site, the incidence of abscesses increased as the severity of physical training increased, and the abscesses occurred three times more commonly on the dominant (right) side of the body. In an analysis of 78 cases in Uganda, abscesses also were found more commonly on the right side of the body.[15]

From experimental evidence and clinical observations, two conditions commonly are found when pyomyositis occurs: muscle injury and bacteremia, usually staphylococcal. A reported case is illustrative.[11] A 12-year-old girl caught her left foot in the wheel of a moving bicycle and tumbled to the ground. One week later, she developed a furuncle of the foot, and within the next 2 weeks she developed painful lumps in muscles of the thigh, shoulder, and chest wall (which had been injured during the original accident). Cultures from the furuncle and blood and from the incised muscle abscesses grew *S. aureus*. The initial episode of trauma resulted in a staphylococcal skin lesion and, presumably, a bacteremia that seeded sites of previously bruised muscle.

Seven children with pyomyositis are described in Table 57-2. An analysis of all seven cases illustrates the association of pyomyositis with trauma. The sources of muscle trauma have ranged from bicycle accidents to strenuous aerobic exercises. These cases also may explain the predilection of the disease to occur in warmer climates; concomitant skin infections and muscle trauma

are more likely to occur in a climate in which children can play or work outside wearing fewer clothes for most of the year. One predisposing disease not listed in Table 57-2 is varicella (chickenpox), in which muscle abscesses are secondary to bacterial invasion via the skin vesicles.[12]

In tropical countries, pyomyositis is said to occur in individuals who are malnourished and who have multiple parasitic infections. This association has not been confirmed, however, in children with pyomyositis seen in the United States or Australia.[5,7,13] The children have not been malnourished or vitamin-deficient, and they have not had parasitic infestation or marked eosinophilia. Extensive immunologic evaluations also have been normal; the tests included quantitative immunoglobulins, enumeration of T-lymphocyte subpopulations, total hemolytic complement levels, and leukocyte function as tested by reduction of nitroblue tetrazolium.

Clinical Presentation

Pyomyositis often is considered a disease of adolescents and young adults, even though it occurs in individuals of all ages, including infants and young children.[2,6,13] Boys are affected more often than girls. As more girls enter competitive sporting activities, however, pyomyositis is being reported in female athletes.[16] Most children with pyomyositis have a solitary lesion, but multiple lesions are common findings. The most common site of abscess formation is the thigh, followed by the calf, buttock, arm, scapula, and chest wall. The muscle lesions are firm or "woody" to palpation, with a well-defined border. The sign of fluctuation may be difficult to elicit. Erythema and warmth often are not apparent because of the deep location of the masses, although diffuse tenderness usually occurs. When a muscle in an extremity is involved, the entire limb may be swollen. Occasionally, pyomyositis also can occur in muscles of the pelvis, in which case pain may be transferred to the hip.[14,19]

In case reports with a clinical history, children with pyomyositis often had similar presenting complaints.[7,11] Many had incurred a recent accidental injury (often involving a leg) that usually was not considered serious. After a few days, the children developed low-grade fever, muscle pain, and, occasionally, an impaired gait. These symptoms persisted a few days to a few weeks until a mass appeared. When first examined, many of the patients were considered to have only a contusion or a hematoma; occasionally, a child was diagnosed as having a rhabdomyosarcoma. Although the disease usually occurs in individuals who are otherwise healthy, pyomyositis has been reported in patients with malignancy. Pyomyositis also may develop in children with acquired immunodeficiency syndrome or other immunodeficiency.[13] The pathophysiology of pyogenic muscle abscess may not be the same, however, in immunodeficient individuals with increased susceptibility to bacterial infection. Most children with pyomyositis have no definable immunologic abnormalities.

An unusual clinical presentation is acute abdominal pain. Beck and Grose[5] described two children with pyomyositis whose initial complaints were confined to the abdominal wall. One patient, a 6-year-old child, had been struck in the abdomen in a mock fistfight with an older sibling. One week later, he developed a low-grade fever

TABLE 57-1 **Infectious Causes of Myositis**

Bacterial
Tropical pyomyositis
Acute bacterial myositis

Viral
Influenza Myositis
Coxsackievirus Myositis

Fungal
Disseminated candidiasis

Parasitic
Trichinosis
Cysticercosis
Toxoplasmosis

TABLE 57-2 **Pyomyositis and Trauma**

Case*	Sex	Age (yr)	Source of Trauma	Circumstances of Trauma	Extent of Disease
1	F	12	Bicycle accident	Thrown from bicycle onto street after foot was caught in the wheel	Right deltoid, right chest wall, left thigh, right groin
2	M	3	Fall while running	Fell while running on street	Left calf, right scapula, right buttock
3	M	11	Hay bale accident	Struck in abdomen by bale of hay thrown from a hay baler	Abdominal wall musculature
4	M	6	Blunt trauma to abdomen	Struck in abdomen during mock fistfight with sibling	Abdominal wall musculature
5	F	17	Aerobic exercises	Injured while instructing others in aerobic exercises	Left thigh
6	M	7	Bicycle accident	Fell from fast-moving bicycle onto street	Left calf
7	F	13	Volleyball accident	Fell several times diving for volleyball during training exercises	Left iliopsoas

*Cases 1 and 2 from reference 11, cases 3 and 4 from reference 5, cases 5 and 6 from reference 7, and case 7 from reference 16.

and began to walk with a stoop; after another week, his mother detected a "knot" in his right mid-abdominal wall. The second case involved the 11-year-old son of a rancher; the boy was struck in the abdomen by a bale of hay tossed from a hay baler. When he subsequently developed symptoms of abdominal pain, the diagnosis of appendicitis was entertained. When a mass later became palpable in his abdominal wall, rhabdomyosarcoma was suspected. A correct diagnosis was made after the use of scintigraphy and sonography.[5] A review from Nigeria found muscle abscesses in the anterior abdominal wall to be common.[2]

Diagnosis

The diagnosis of pyomyositis should be considered in any child with fever and muscle pain, especially if a recent history of trauma exists. When a child has visible masses at commonly involved sites, such as the thigh, the diagnosis of pyomyositis can be made by needle aspiration of a mass. If a febrile child complains of myalgia in an extremity but has no palpable masses, the differential diagnosis must include more common inflammatory and infectious conditions of the bone or joint. A definitive diagnosis usually depends on one or more radiologic procedures. Plain films may show a soft tissue swelling or even a widened fascial plane suggestive of a mass lesion. A combination of plain radiography and magnetic resonance imaging (MRI) often can exclude osteomyelitis and pyoarthritis. If the diagnosis still is in question, scanning with a radioactive tracer can localize a muscle abscess precisely and visualize other intramuscular abscesses too small to palpate (Fig. 57-1). Alternatively, ultrasonography can detect muscle abscesses and may be preferable as an initial procedure because it avoids the radiation exposure from computed tomography (CT) or scintigrams (Fig. 57-2).[27]

MRI is very helpful in delineating the extent of a muscle abscess. In many cases, the abscess is much larger than suspected by physical signs and symptoms. The MRI scans of an illustrative case are presented in Figure 57-3. The patient was a 17-year-old girl with a swollen left lower thigh. She worked part-time as an attendant in an athletic club, where she participated in some of the vigorous exercise programs. Radiographs of the knee were normal, whereas a technetium bone scan showed slightly increased radionuclide uptake in the soft tissues around the distal left femur. MRI of the left and right thighs showed a large fluid collection extending from the middle to distal left femur. Surgical exploration identified extensive abscess formation around the posterior aspect of the femur; the culture grew *S. aureus*. The affected muscle groups included the vastus medialis, vastus intermedius, vastus lateralis, and biceps femoris. The bone was not involved. For the reasons described, MRI has become the preferred procedure for establishing the diagnosis of pyomyositis.[14,23,27]

Treatment

Because pyomyositis is an abscess of skeletal muscle, the treatment is surgical incision and drainage.[2] Several

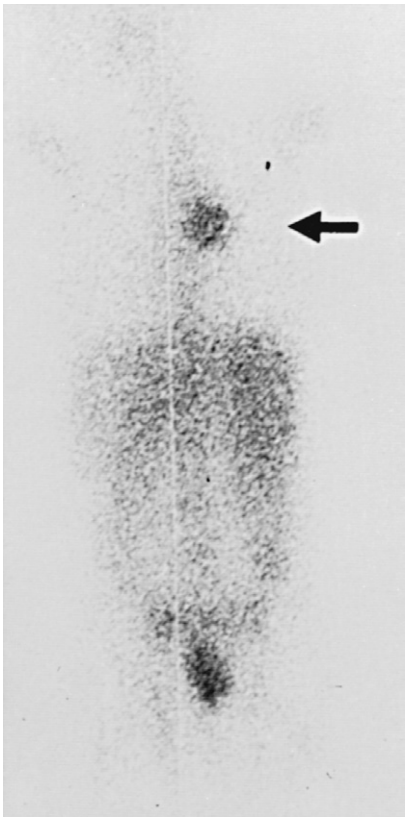

FIGURE 57-1 ■ Scintigram of a patient with pyomyositis. Radiopharmaceutical scan shows abnormally high uptake over the right scapula *(arrow),* where an abscess cavity was located within the muscle. Increased radioactivity also is observed in the bladder, which is a normal finding.

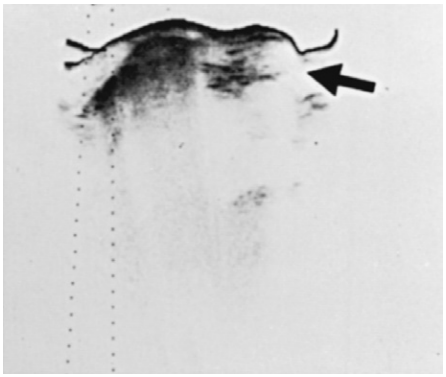

FIGURE 57-2 ■ Sonogram of the abdominal wall of a patient with pyomyositis. The transverse view of the abdomen shows an abscess cavity in the right belly of the rectus abdominis muscle *(arrow).*

physicians have seen that smaller lesions resolve spontaneously, even before the antibiotic era. An important role for systemic antibiotics is to prevent the formation of further muscle abscesses, especially in a patient with proven bacteremia. Because *S. aureus* is the most likely agent, a semisynthetic penicillinase-resistant penicillin is the preferred antibiotic in communities with relatively little MRSA. Usually, nafcillin or oxacillin 150 to 200 mg/kg is administered intravenously every 6 hours. When

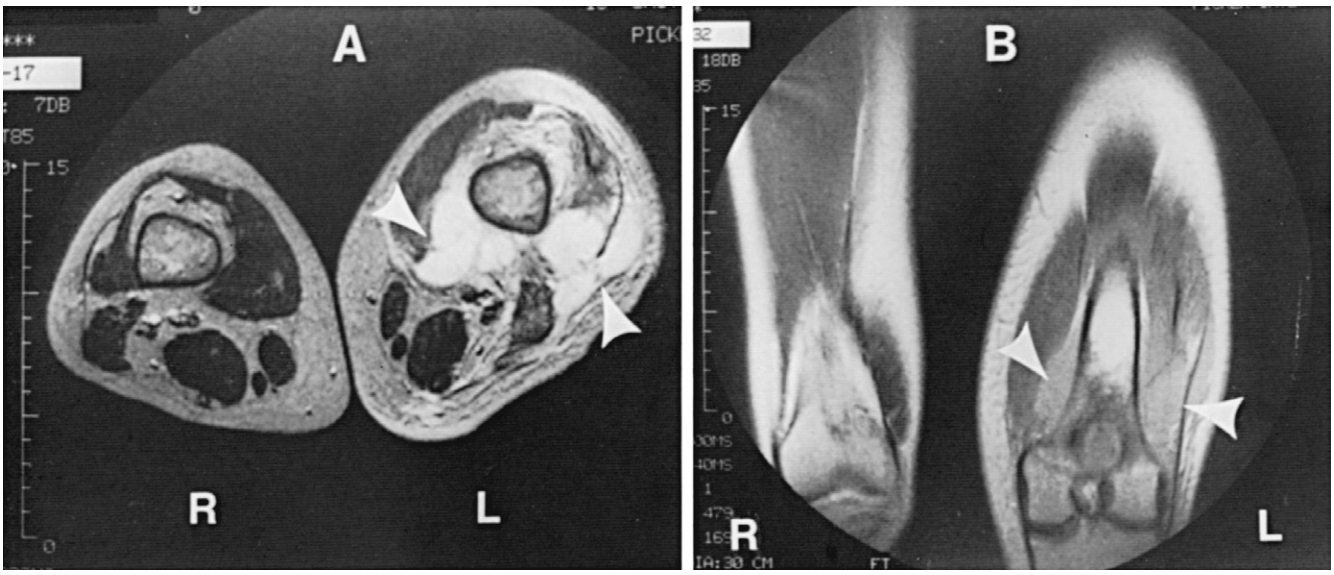

FIGURE 57-3 ■ Magnetic resonance imaging of the thighs of a patient with pyomyositis. **A,** Axial images of the right (*R*) and left *(L)* thighs. **B,** Coronal images around the distal femoral shafts and femoral condyles. The scans show several well-defined areas of extremely high intensity in the muscle bundles and fascial planes from the middle to distal left femur *(arrows).* The high signal intensity suggests a fluid collection (e.g., an abscess), rather than a neoplastic process. Edema of the subcutaneous tissues also is evident in the lateral aspect of the swollen left thigh. There are no abnormal signals within the bone.

pyomyositis occurs in a patient with penicillin hypersensitivity, clindamycin 40 mg/kg/day divided every 8 hours can be substituted because of its excellent coverage of gram-positive cocci. Parenteral therapy is continued until clinical improvement is evident, usually within a few days after surgical drainage. Thereafter, the antibiotics can be given orally for an additional 2 to 3 weeks.[20] A cephalosporin (cephalexin 100 mg/kg/day) or clindamycin 30 to 40 mg/kg/day is acceptable.

In many communities, MRSA is now a common agent.[28] In a clinical analysis of 182 patients with pyomyositis from Texas Children's Hospital in Houston, more than 50 percent of the isolated *S. aureus* strains were MRSA.[19] In these communities, initial antibiotic management must include either intravenous clindamycin or vancomycin 60 mg/kg/day divided every 6 hours. In adolescents the dosage of vancomycin is usually reduced to 45 mg/kg/day, divided every 8 hours. Clindamycin can be continued as an oral antibiotic. For MRSA isolates resistant to clindamycin, treatment with intravenous vancomycin can be followed by administration of oral linezolid 30 mg/kg/day divided every 8 hours for children younger than 12 years; for children 12 years and older, 20 mg/kg/day divided every 12 hours is given. Therapy of MRSA infection may require a longer duration, from 3 to 4 weeks. Before discontinuing antibiotics, the patient should have documented normal levels of C-reactive protein and erythrocyte sedimentation rate.

ACUTE BACTERIAL MYOSITIS

Far less common than classical (tropical) pyomyositis is acute bacterial myositis, usually caused by group A streptococci. In this condition, the bacterial infection often is not confined to distinct abscesses within the muscle, but instead it extends diffusely through one or more muscle groups. As with pyomyositis, the disease occurs more commonly in males than in females and usually is associated with prior physical exertion and perhaps minor trauma. In contrast to pyomyositis, most published cases of acute bacterial myositis have occurred in adults and not in children.[4] The disease has been divided by Svane[25] into the following four main types:

1. A malignant form with septicemia and a uniformly fatal outcome
2. An acute form with a more protracted clinical course and other foci, such as the bones, joints, or viscera
3. A subacute form with a better clinical prognosis
4. A benign type associated with more distinct muscle abscesses

The benign type of acute bacterial myositis by the Svane classification is the same disease process as described in the preceding section on pyomyositis.

Clinical Presentation

The main clinical difference between the acute myositis syndrome and the pyomyositis syndrome is the virulence of the former disease, especially the malignant type. A case in a San Antonio, Texas, native is illustrative. The patient was a 23-year-old jackhammer operator who was admitted because of high fever, malaise, and pains in his arms. On examination, both upper extremities were tender to palpation, and faint erythema was visible over the same areas. He soon became incoherent, and his general condition quickly deteriorated. Death followed within 24 hours. Blood cultures drawn before death grew group A streptococcus, and cultures of the biceps muscles obtained at autopsy grew the same organism. This case is similar to others described in the literature.[4,25] The

patient presumably would incur considerable minor trauma to the muscles of the upper arms and shoulders from his work as a jackhammer operator. Two reported cases of streptococcal myositis in children involved the left thigh and the left paravertebral muscles.[18] The first child developed septic shock and required intensive care for 27 days before a definitive diagnosis was made by CT.

A case of generalized myositis with staphylococcal septicemia has been described in a 15-year-old boy.[1] The patient presented with signs of fever and very diffuse muscle swelling and tenderness. All muscle groups of the extremities appeared to be inflamed and markedly tender; the creatine phosphokinase enzyme levels were markedly elevated. Culture of a muscle biopsy specimen grew *S. aureus*, as did previously drawn blood cultures. The patient experienced a stormy hospital course with severe hypotension and shock syndrome, requiring treatment with intravenous fluids, corticosteroids, dopamine, heparin, and assisted ventilation. He also received a total of 4 weeks of antistaphylococcal antibiotic therapy.

Diagnosis

Blood cultures are positive most often in patients with the most significant clinical symptoms. Cultures of muscle biopsy specimens may yield a profuse growth of bacteria. Rarely, myositis has occurred as a delayed complication of chickenpox, often together with fasciitis.[12,17,23] In this situation, bacteremia may not be documented because the bacterial process usually is the result of contiguous spread from a secondarily infected pock lesion. These streptococcal complications of chickenpox often occur in an extremity, which may become swollen and painful. Under these circumstances, MRI can be an extremely valuable diagnostic tool to gauge the depth and extent of inflammation.[27]

Treatment and the Eagle Effect

The treatment of acute streptococcal or staphylococcal myositis of the malignant type is a medical emergency. As soon as the diagnosis is suspected, bacteriologic cultures should be obtained and intravenous therapy should be initiated with a high-dose semisynthetic penicillin (e.g., nafcillin 50 mg/kg every 6 hours) and vancomycin. If the cultures yield group A streptococci rather than *Staphylococcus*, the antibiotic can be switched to penicillin G at an equivalent high dosage. Surgical consultation is required to evaluate the need for débridement and drainage. Obtaining MRI scans may be advisable to document the extent of the disease and the response to antibiotic therapy. In severe cases of bacterial myositis, the total duration of hospitalization can exceed 4 weeks.[1,18]

The apparent resurgence of serious streptococcal infection has led to an increased interest in what has been called the *Harry Eagle effect*, named after the scientist who first described the failure of penicillin to eradicate group A streptococcal infection in a mouse model.[8] Eagle inoculated mice intramuscularly with group A streptococci and observed a markedly retarded bactericidal action of penicillin on organisms in the older abscesses, even though the bacteria remained highly sensitive to the antibiotic. Even massive doses of penicillin 10,000 times greater than the minimum inhibitory concentration were not effective at eradicating the bacteria. Subsequent studies by other investigators confirmed the existence of the Eagle effect and disclosed that clindamycin showed superior efficacy to penicillin in treatment of streptococcal myositis in the mouse model.[24] These physicians observed that penicillin most likely fails to kill stationary-phase streptococci in muscle infections, an explanation for the Eagle effect. They suggested that streptococcal myositis or fasciitis be treated with intravenous clindamycin 40 mg/kg/day, if the condition fails to respond to penicillin therapy.

Miscellaneous Causes of Myositis

Viral infection occasionally leads to myositis (see Table 57-1). The most common example undoubtedly is myositis associated with influenza virus infection in children. Typically, school-aged children are affected. Muscle groups of the legs often are involved, associated with a marked elevation of serum creatine phosphokinase. Clinical symptoms generally last 2 to 4 days. Another, far less common myositis is caused by coxsackievirus B infection. This disease is termed *epidemic pleurodynia* because the myositis frequently involves the chest and upper abdomen. The enteroviral infection is known as *Bornholm disease*, named after the Danish island where it was first described.

Fungal infection (e.g., *Candida albicans*) can cause myositis. Candidal myositis is uncommon, however, and usually occurs in patients who are severely immunocompromised. Frequently, other sites also are involved by the disseminated fungal infection in addition to muscle.

Parasitic infection can cause myositis. The best known is trichinosis, caused by ingestion of the encysted larvae of *Trichinella spiralis*. The larvae typically are found in products containing pork or wild game, such as bear meat. The first signs of illness are fever and abdominal pain. Later, muscle pain is noted in the neck and chest. Eventually, calcifications form in the involved muscles. Treatment of the acute disease with mebendazole or albendazole may be beneficial. Another parasite more common in Central and South America is *Taenia solium*. Ingestion of eggs of this pork tapeworm leads to cysticercosis, with calcified densities in skeletal muscle. Finally, toxoplasmosis infection can lead to symptoms of myositis. During acute infection, *Toxoplasma gondii* can encyst in the skeletal muscle and lead to an inflammatory myopathy closely resembling dermatomyositis.

NEW REFERENCES SINCE THE SIXTH EDITION

20. Peltola H, Paakkonen M, Kallio P, et al. Clindamycin vs first generation cephalosporins for acute osteoarticular infections of childhood: a prospective quasi-randomized controlled trial. *Clin Microbiol Infect* 2011;**17**:1469–74.
28. Vanderhave K, Karmazyn B, Verma M, et al. Community-associated methicillin-resistant *Staphylococcus aureus* in acute musculoskeletal infection in children: a game changer. *J Pediatr Orthop* 2009; **29**:927–31.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adamski GB, Garin EH, Ballinger WE, et al. Generalized non-suppurative myositis with staphylococcal septicemia. *J Pediatr* 1980;**96**:694–7.
2. Ameh EA. Pyomyositis in children: analysis of 31 cases. *Ann Trop Paediatr* 1999;**19**:263–5.
3. Ashken MH, Cotton RE. Tropical skeletal muscle abscesses (pyomyositis tropicans). *Br J Surg* 1963;**50**:846–52.
4. Barrett AM, Gresham GA. Acute streptococcal myositis. *Lancet* 1958;**1**:347–51.
5. Beck W, Grose C. Pyomyositis presenting as acute abdominal pain. *Pediatr Infect Dis* 1984;**3**:445–8.
6. Chacha PB. Muscle abscesses in children. *Clin Orthop* 1970;**70**: 174–80.
7. Diamandakis V, Grose C. Bad consequences of bicycle accidents. *Pediatr Infect Dis J* 1994;**13**:422–5.
8. Eagle H. Experimental approach to the problem of treatment failure with penicillin. I. Group A streptococcal infection in mice. *Am J Med* 1952;**13**:389–99.
9. Echeverria P, Vaughn MC. Tropical pyomyositis: a diagnostic problem in temperate climates. *Am J Dis Child* 1975;**129**:856–7.
10. Goldberg JS, London WL, Nagel DM. Tropical pyomyositis: a case report and review. *Pediatrics* 1979;**63**:298–300.
11. Grose C. Staphylococcal pyomyositis in south Texas. *J Pediatr* 1978;**93**:457–8.
12. Grose C. Varicella-zoster virus infections: chickenpox, shingles, and varicella vaccine. In: Glaser R, Jones JF, editors. *Herpesvirus Infections*. New York: Marcel Dekker; 1994. p. 117–85.
13. Gubbay AJ, Issacs D. Pyomyositis in children. *Pediatr Infect Dis J* 2000;**19**:1009–12.
14. Karmazyn B, Kleiman MB, Buckwalter K, et al. Acute pyomyositis of the pelvis: the spectrum of clinical presentations and MR findings. *Pediatr Radiol* 2006;**36**:338–43.
15. Marcus RT, Foster WD. Observations on the clinical features, etiology and geographic distribution of pyomyositis in East Africa. *East Afr Med J* 1968;**45**:167–76.
16. Meehan J, Grose C, Soper RT, Kimura K. Pyomyositis in an adolescent female athlete. *J Pediatr Surg* 1995;**30**:127–8.
17. Miyake H. Beitrage zur kenntnis der sogenannten myositis infectiosa. *Mitt Grenageb Med Chir* 1904;**13**:155–98.
18. Moore DL, Delage G, Labelle H, et al. Peracute streptococcal pyomyositis: report of two cases and review of the literature. *J Pediatr Orthop* 1986;**6**:232–5.
19. Pannaraj PS, Hulten KG, Gonzalez BE, et al. Infective pyomyositis and myositis in children in the era of community acquired methicillin resistant *Staphylococcus aureus*. *Clin Infect Dis* 2006;**43**: 953–60.
20. Peltola H, Paakkonen M, Kallio P, et al. Clindamycin vs first generation cephalosporins for acute osteoarticular infections of childhood: a prospective quasi-randomized controlled trial. *Clin Microbiol Infect* 2011;**17**:1469–74.
21. Scriba J. Beitrag zur aetiologie der myositis acuta. *Dtsch Z Chir* 1885;**22**:497–502.
22. Sirinavin S, McCracken GH. Primary suppurative myositis in children. *Am J Dis Child* 1979;**133**:263–5.
23. Solar R, Rodriguez E, Aguiler C, Fernandez R. Magnetic resonance imaging of pyomyositis in 43 cases. *Eur J Radiol* 2000;**35**: 59–64.
24. Stevens DL, Gibbons AE, Bergstrom R, Winn V. The Eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *J Infect Dis* 1988;**158**: 23–8.
25. Svane S. Peracute spontaneous streptococcal myositis. *Acta Chir Scand* 1971;**137**:155–63.
26. Traquair RN. Pyomyositis. *J Trop Med Hyg* 1947;**50**:81–9.
27. Trusen A, Beissert M, Schultz G, Darge K. Ultrasound and MRI features of pyomyositis in children. *Eur Radiol* 2003;**13**:1050–5.
28. Vanderhave K, Karmazyn B, Verma M, et al. Community-associated methicillin-resistant *Staphylococcus aureus* in acute musculoskeletal infection in children: a game changer. *J Pediatr Orthop* 2009;**29**: 927–31.

SECTION X

SKIN INFECTIONS

CHAPTER 58

Cutaneous Manifestations of Systemic Infections

James D. Cherry

Many illnesses caused by infectious agents have associated cutaneous manifestations. In some cases, the exanthem may be the hallmark of the disease; in others, it may be only a vague indicator of a more significant underlying process. When an exanthem occurs, it often offers important clues to the etiology of a patient's illness. Although most exanthematous illnesses in children are benign, their differential diagnosis is critical because the early manifestations of potentially fatal bacterial and rickettsial diseases frequently have cutaneous findings.

HISTORY

Exanthematous manifestations of infectious illnesses have been important since medical antiquity. Major epidemics of both measles and smallpox occurred in the Roman Empire and in China at the beginning of the Christian era.[26,139] Scarlet fever was recognized as a distinct entity in the 17th century, and chickenpox and rubella were identified in the 18th and 19th centuries, respectively.[50]

In the writings of the early 20th century, maculopapular exanthematous illnesses of children frequently were referred to by number. Scarlet fever and measles historically were the first two classic maculopapular exanthems of childhood. Which one had the honor of being the "first disease" is unknown today. The "third disease" was rubella, which was recognized by the beginning of the 20th century as a distinct entity.[73,92,169,175,194-196] In 1900, Dukes[73] described an exanthematous illness with the characteristics of both rubella and scarlet fever, which he suggested was a "fourth disease." The general opinion today is that his disease was not a distinct entity. Shaw[196] suggested that Dukes' cases had mild atypical scarlet fever, and Powell[169] raised the possibility that the illness resulted from epidermolytic toxin–producing staphylococci. My opinion has been (see first edition of this textbook) that probably rubella and scarlet fever both were epidemic in the student population under Dr. Dukes' care; combined infections led to the confusion. In 1991, Morens and Katz[147] came to the same conclusion.

Erythema infectiosum (see Chapter 151) commonly is referred to as the *fifth disease*, and roseola infantum (see Chapter 59) qualifies as the *sixth disease*.[195]

During the last 60 years, interest in exanthematous diseases has been renewed because a large number of previously unknown viruses and other infectious agents that cause cutaneous manifestations have been discovered. In addition, the pattern of disease caused by classic exanthem-producing agents has changed; smallpox has been eradicated, the epidemiology of measles and rubella has been altered by immunization, and ecologic changes have resulted in differences in viral and bacterially induced rashes.

ETIOLOGIC AGENTS

Many different types of viruses, chlamydiae, rickettsiae, mycoplasmas, bacteria, fungi, and protozoan and metazoan agents cause illnesses with associated cutaneous manifestations. Although this chapter is devoted to systemic infectious diseases with cutaneous manifestations, the demarcation between exanthematous disease of systemic and local origin is not always readily apparent. For example, the recurrent cold sore caused by herpes simplex virus (HSV) infection frequently is considered a local problem, although its nature and pathogenesis involve central virus latency and host systemic immune functions. Similarly, superficial fungal diseases and other local infections, such as warts, may be quite dependent on more general immunologic functions of the host. The exanthems of enteroviral infections frequently are confused with those caused by insect bites and allergic problems.

Table 58-1 presents viruses that have cutaneous manifestations in humans. Erythema infectiosum is caused by human parvovirus B19.[7,222] This virus also is an important cause of the papular-purpuric gloves and socks syndrome that is an uncommon occurrence and mainly affects young adults.* Human parvovirus B19 also has been associated with a vesiculopustular exanthem, erythema multiforme, and other petechial and purpuric rashes. In one study, an erythematous maculopapular rash was noted in 9 percent of children with human bocavirus infections.[10] Adenovirus types 1, 2, 3, 4, 7, and 7a have been isolated from children and young adults with exanthem.[50,116,226,227] The overall clinical expression rate of exanthem in adenovirus infection rarely has been studied. Fukumi and associates[85] noted that rash occurred in 2 percent of adenoviral infections, Hope-Simpson and Higgins[103] indicated a rate of approximately 8 percent, and Esposito and associates[75] noted an occurrence rate of 5.7 percent.

*References 3, 6, 51, 83, 91, 96, 142, 200, 201.

Text continued on p. 749

TABLE 58-1 **Clinical Characteristics of Viral Infections with Cutaneous Manifestations**

Virus	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	Exanthem		Usual Duration (days)
					Lesions	Distribution	
Human parvovirus B19	Erythema infectiosum; gloves and socks syndrome	7-17	Winter and spring	Biphasic illness with mild prodromal period with headache and malaise for 2-3 days, then 7-day symptom-free period, followed by typical exanthema	Three-stage exanthema: Initially, rash on cheeks (slapped-cheek appearance) and then erythematous maculopapular rash on trunk and limbs; finally, rash develops a reticular pattern	Starts on face More prominent on extensor surfaces of extremities	7-21
Human bocavirus			Fall, winter, and spring	Fever, cough, coryza, respiratory distress (bronchitis, bronchiolitis, pneumonia)	Erythematous maculopapular	Mainly face, chest, and trunk	
Human papillomaviruses	Warts		Nonseasonal	Local cutaneous disease	Papular or nodular isolated lesions	Most common on extremities	100+
Adenovirus types 1, 2, 3, 4, 7, and 7a		6-9	Winter and spring	Fever and signs and symptoms of respiratory illness Occasionally, rash occurs after defervescence (roseola-like)	Most commonly erythematous, maculopapular, and discrete (rubelliform), but occasionally confluent (morbilliform) Rarely, erythema multiforme and Stevens-Johnson syndrome	Usually starts on face and spreads downward to trunk and extremities	3-5
Herpes simplex types 1 and 2	Cold sores, genital herpes, neonatal herpes, or other	2-12	Nonseasonal	Primary disease associated with fever and systemic symptoms Recurrent disease caused by exogenous and endogenous infections	Singular or grouped vesicular lesions varying in size from 2 to 10 mm, frequently on a mildly erythematous base Occasionally, erythema multiforme, Stevens-Johnson syndrome, and erythema nodosum	Lesions in primary infection with type 1 virus are mainly in and around the mouth Recurrent type 1 lesions usually perioral Primary and recurrent type 2 lesions usually on genitals	7-14
Human herpesvirus–6 (HHV-6)	Roseola infantum		Nonseasonal	Fever 3-5 days in duration, rapid defervescence, and then the appearance of rash	Erythematous macular or maculopapular	Most prominent on neck and trunk Face and extremities may be affected	1-2

Human herpesvirus–7 (HHV-7)	Roseola infantum		Nonseasonal	Fever 3-5 days in duration, rapid defervescence, and then the appearance of rash	Erythematous macular or maculopapular	Most prominent on neck and trunk Face and extremities may be affected	1-2
Human herpesvirus–8 (HHV-8)	Kaposi sarcoma	Months to years	Nonseasonal	Asymptomatic infection Most commonly noted in AIDS patients but occurs in other immunodeficiency states	Purple to blue nodular, raised lesions	Any epidermal or mucosal surface	Months to years
Varicella-zoster	Chickenpox (varicella)	12-20	Late fall, winter, and spring	Malaise and fever of 5-6 days duration	Basic lesion is vesicular, but lesions go through stages: macules, papules, vesicles, and crusts Lesions occur in crops	Lesions more profuse on trunk than on extremities Proximal end of extremities more involved than distal end	8-10
	Herpes zoster		Nonseasonal	Endogenous infection Pain and paresthesia with dermatome distribution	Basic lesion is vesicular, but lesions go through stages: macules, papules, vesicles, and crusts	Lesions localized to area of skin innervated by a single sensory ganglion	10-28
Epstein-Barr	Infectious mononucleosis	28-49	Nonseasonal	Fever, pharyngitis, and lymphadenopathy Exanthem occurs in 3%-13% of cases If ampicillin is administered, then exanthema in 50% of cases	Most commonly erythematous, macular, maculopapular, and discrete (rubelliform) In association with ampicillin administration, the rash may be more vivid Erythema multiforme and urticaria may occur	Mainly on trunk and proximal end of extremities	2-7
Cytomegalovirus	Cytomegalovirus mononucleosis		Nonseasonal	Acquired: mild febrile illness with lymphadenopathy Congenital: disseminated disease	Erythematous, maculopapular, and discrete Vesicular or petechial in congenital infection	Located mainly on trunk and proximal end of extremities	2-7

Continued on following page

TABLE 58-1 **Clinical Characteristics of Viral Infections with Cutaneous Manifestations** (Continued)

Virus	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	*Exanthem*		Usual Duration (days)
					Lesions	Distribution	
Vaccinia	Roseola vaccinatum, eczema vaccinatum, vaccination "take," or disseminated vaccinia		Nonseasonal	Illness caused by direct exposure via vaccination or exposure to a vaccinee	Vaccination and eczema vaccinatum lesions go through stages: papule, vesicle, pustule, and scab Roseola vaccinatum: erythematous maculopapular lesions Occasionally erythema multiforme Disseminated vaccinia: papular or vesicular lesions	Lesions in roseola vaccinatum, eczema vaccinatum, and disseminated vaccinia are generalized	7-14
Variola	Smallpox	8-17	Seasonal by geographic area	Abrupt onset of high fever, headache, and muscle and joint pain Rash appears 2-4 days after onset	Basic lesion is vesicular, but lesions go through stages: macules, papules, vesicles, pustules, and crusts	Most prominent on exposed body surfaces Starts on extremities and face Spreads centripetally	12-20
Monkeypox				Similar to mild smallpox Exposure to monkeys No human-to-human spread	Similar to mild smallpox	Similar to mild smallpox	
Orf	Ecthyma contagiosum	4-7	Spring	Disease of sheep acquired by humans	Initially erythematous papule Becomes umbilicated, nodular, and then vesicular Occasionally erythema multiforme	Solitary lesion, usually on hands	30-40
Molluscum contagiosum	Molluscum contagiosum			Local cutaneous disease	Umbilicated nodular lesions: singular or clusters	Most common on face, inner aspect of thigh, breasts, and genitalia	100+
Paravaccinia	Milker's nodules	4-7		Human infection acquired from infected calves	Nodular lesion Occasionally erythema multiforme	Solitary lesion, usually on hands	30-40
Tanapox				A virus of monkeys Human infection associated with fever and regional lymphadenopathy	Umbilicated vesicular lesion	Upper part of body Solitary lesion	35-56

Coxsackieviruses A2, A4, A5, A6, A7, A9, A10, and A16; coxsackieviruses B1-B5; echoviruses 1-7, 9, 11-14, 16-19, 24, 25, 30, and 33; enterovirus 71; parechoviruses 1 and 3		4-7	Summer and fall	Fever and mild to moderate pharyngitis Occasionally herpangina, meningitis, and other manifestations of systemic viral infection Exanthem occurs in 5%-50% of infections, depending on virus type Rash may occur during fever or after defervescence; hand, foot, and mouth syndrome	Most commonly erythematous, maculopapular, and discrete May have macular, petechial, vesicular, and urticarial components Rarely erythema multiforme	Usually starts on face and spreads downward to trunk and extremities May have peripheral distribution (hand, foot, and mouth syndrome)	3-7
Rhinoviruses (many types)		2-4	Fall, winter, and spring	Mild fever and signs and symptoms of respiratory illness Exanthem occurs in about 5% of cases	Erythematous or maculopapular and discrete	Starts on face and spreads downward to trunk and extremities	1-4
Foot and mouth		3-4		Direct animal contact Fever, sore mouth, and lymphadenopathy Vesicles and ulcers within the mouth	Vesicular lesions	Hands and feet	3-6
Colorado tick fever		3-5	Summer	Fever, chills, eye pain, myalgia, and headache Diphasic course Rash in only about 10% of cases	Occasionally maculopapular but usually petechial	Maculopapular rash is generalized Petechial rash most prominent on arms, legs, and trunk	2-7
Reovirus 2 and 3		4-7	Summer	Fever, mild pharyngitis, and cervical adenopathy	Erythematous or maculopapular Discrete or confluent Occasionally vesicular	Starts on face and spreads downward to trunk and extremities	3-9
Rotavirus	Gianotti-Crosti syndrome; infantile acute hemorrhagic edema	2-4	Fall, winter, and spring	Gastroenteritis	Petechial and morbilliform	Generalized	7-14

Continued on following page

TABLE 58-1 **Clinical Characteristics of Viral Infections with Cutaneous Manifestations** (Continued)

Virus	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	*Exanthem*		Usual Duration (days)
					Lesions	Distribution	
Chikungunya, o'nyong-nyong, Ross River, Sindbis			During periods of arthropod prevalence	Fever, headache, eye pain, and marked myalgia, arthralgia, and arthritis Geographically localized diseases	Rubelliform and morbilliform Frequently vesicular and petechial	Starts on face and spreads downward to trunk and extremities	
Rubella	Rubella (German measles)	15-21	Winter and spring	Mild symptoms with onset 1-5 days before rash Fever usually <38.5° C (101.5° F) Headache, malaise, and suboccipital and postauricular lymphadenopathy	Erythematous, maculopapular, and discrete	Starts on face and spreads downward to trunk and extremities	4-7
West Nile				Sudden onset of fever, chills, and drowsiness Rash may appear during or after fever Geographically localized disease	Erythematous, macular, and maculopapular	Starts on trunk and spreads to extremities	3-6
Dengue and Kunjin		7	During periods of specific arthropod prevalence	Sudden onset of high fever, then severe headache, myalgia, arthralgia, abdominal pain, and marked diaphoresis Fever lasts 5-6 days and ends by crisis Rash appears within 48 hours of onset of fever Geographically localized diseases	Initially macular, flushed appearance, then erythematous, maculopapular rash May be scarlatiniform Frequently becomes petechial and purpuric Small vesicles occur in Kunjin virus infection	Initial macular rash is more prominent centrally Maculopapular rash may start on hands and feet and spread to trunk	3-10
Influenza A and B		2-5	Fall, winter, and spring	Fever, cough, headache, and muscle aches and pains Usually in young children Rash an occasional occurrence	Erythematous, maculopapular, and discrete (rubelliform) Rarely erythema multiforme	Starts on face and trunk and spreads to extremities	1-3

Respiratory syncytial		2-5	Fall, winter, and spring	Fever, coryza, and respiratory distress (bronchitis, bronchiolitis, or pneumonia) Usually in children <2 years	Erythematous, maculopapular, and discrete (rubelliform)	Starts on face and trunk and spreads to extremities	1-3
Human metapneumovirus			Fall, winter, and spring	Fever, coryza, and respiratory distress (bronchitis, bronchiolitis, or pneumonia)	Erythematous, maculopapular		
Parainfluenza 1-3		2-5	Fall, winter, and spring	Fever, coryza, nasopharyngitis, croup, and bronchitis Usually in young children	Erythematous, maculopapular, and discrete (rubelliform)	Starts on face and trunk and spreads to extremities	1-3
Mumps	Mumps	14-21	Fall, winter, and spring	Fever, headache, and salivary gland swelling	Erythematous, maculopapular, and discrete; also, urticaria and vesicles; rarely, erythema multiforme	Most prominent on trunk	2-5
Measles	Measles	8-12	Winter and spring	Onset with fever, cough, coryza, and conjunctivitis			
				About 2 days after onset, appearance of enanthem (Koplik spots); and 2 days later, onset of exanthem	Erythematous, maculopapular, and confluent Develops a brownish appearance, and fine desquamation occurs	Starts behind ears and on forehead Spreads downward over body Confluence most prominent on face, trunk, and proximal end of extremities	5-7
Lassa	Lassa fever			Sudden onset of fever, chills, headache, and sore throat Progresses to pneumonia and renal failure Geographically localized outbreaks	Macular and sometimes petechial	Localized or general	

Continued on following page

TABLE 58-1 **Clinical Characteristics of Viral Infections with Cutaneous Manifestations** (Continued)

Virus	Disease or Syndrome	Incubation Period (days)	Main Season	Clinical Characteristics	*Exanthem*		Usual Duration (days)
					Lesions	Distribution	
Hepatitis B	Papular acrodermatitis of childhood	50-180		Insidious onset with arthralgia, arthritis, and rash occurring before jaundice	Maculopapular, macular, or urticarial In young children, papular (Gianotti-Crosti syndrome or papular acrodermatitis of childhood) Rarely, erythema multiforme	Generalized	4-10
Hepatitis C	Mixed cryoglobulinemia (not reported in children)	7-14	Nonseasonal	Acute hepatitis followed by chronic infection Skin findings occur late in disease	Palpable purpura	Mostly buttocks, lower extremities	Variable
Marburg		5-7		Headache, conjunctivitis, photophobia, myalgia, vomiting, diarrhea, and fever (biphasic) Exposure to vervet monkeys	Initially erythematous macular, then discrete maculopapular, and finally confluent maculopapular Exfoliation occurs Occasionally purpura	Generalized	2-14
Ebola	Hemorrhagic fever	5-10	Occurs in outbreaks	Febrile illness that progresses to hemorrhage, shock, and coma	Maculopapular rash that appears toward end of first week of illness	Lateral sides of trunk, groin, and axillae Can become generalized but spares the face	14-60
Hantavirus	Hemorrhagic fever with renal syndrome (nephropathia epidemica)		Spring and summer outbreaks	Febrile illness with hemorrhagic and renal manifestations	Flushing and petechial rash	Face (flushing), skinfolds (petechiae)	14-28
HIV		14-60	Nonseasonal	Fever, pharyngitis, myalgia, arthralgias, adenopathy, and rash	Macular	Mainly chest and abdomen	7
Human T-lymphotropic virus	Infective dermatitis		Nonseasonal	Acute onset of eczema	Severe exudative eczema with a crusting, generalized, fine papular rash	Scalp, eyelid margins, perinasal skin, retroauricular areas, axillae, and groin	Months to years

Data from references 1, 2, 4, 6, 7, 9-13, 17, 20, 23, 27, 37, 40, 43, 45, 47, 49-51, 52, 59, 60, 62, 64, 65, 67, 80-85, 91, 96, 100, 102, 103, 106, 108, 110, 112, 118, 122, 123, 125, 128-130, 135, 145, 148-151, 154-156, 162, 164, 170, 178, 179, 181, 182, 184-186, 190, 191, 200, 201, 206, 209, 214, 215, 219, 222, 223, 225-227, 234-236.

Eight species in the *Herpesvirus* genus have cutaneous manifestations associated with infection, but clinical expression rates vary greatly. Nearly all primary varicella infections are associated with exanthem, whereas exanthem with acquired cytomegalovirus infection is a rare manifestation.* The incidence of exanthem in Epstein-Barr virus (EBV) infection varies from 3 percent to nearly 100 percent, depending on whether concomitant ampicillin is administered.† EBV has been associated with a unilateral laterothoracic exanthem in a 1-year-old girl and a drug-induced hypersensitivity syndrome in an 8-year-old boy.[71,188] Although firm data are lacking, probably fewer than 10 percent of primary infections with HSV type 1 are associated with cutaneous manifestations. Erythema multiforme occasionally occurs with recurrent HSV infections.[37,80,113,154] Human herpesvirus–6 (HHV-6) is a major cause of roseola infantum[12,222,235] (see Chapter 59). HHV-7 also is a cause of roseola infantum[11]; in addition, some evidence suggests that this virus and HHV-6 may play a role in pityriasis rosea.[68,69,174] HHV-8 infection is necessary for the development of Kaposi sarcoma in patients with acquired immunodeficiency syndrome (AIDS) and other immunodeficiency states.[108,122,145]

At present, human illnesses with cutaneous manifestations caused by poxviruses rarely occur in the United States. Because smallpox as a disease has ceased to exist, the use of vaccinia virus for immunization has decreased dramatically. However, the terrorist events of 2001 raised concern about the possible use of smallpox virus as a terrorist weapon. Because of this potential danger, smallpox vaccines are being produced and used again. With the increased use of these vaccines, cutaneous complications of vaccinia virus infection can be expected. Monkeypox is a relatively common illness in areas of Africa (see Chapter 163). Outside of Africa, monkeypox, orf, and paravaccinia (milker's nodules) continue to occur as isolated events in exposed individuals.[186,226,227] Human infection with tanapox virus is a geographically related illness occurring in limited areas of Kenya.

In the present era, enteroviruses are the leading cause of infection-related exanthematous diseases.[54,56,115,226,227] Thirty-eight types have been associated with rash illnesses. The clinical expression rate varies greatly among the different types; it is as high as 50 percent in children with coxsackievirus A16 and echovirus 9 infections. Only approximately 15 percent of individuals infected with echovirus 4 have exanthem, and rash is a rare occurrence in echovirus 6 infection. Hope-Simpson and Higgins[103] noted exanthem in approximately 5 percent of patients with rhinoviral respiratory illness.

A young adult research worker had an influenza-like illness and a hand, foot, and mouth syndrome–like rash caused by infection with a calicivirus (San Miguel sea lion virus serotype 5) of oceanic origin.[199]

Two percent of patients with Colorado tick fever encephalitis have exanthem.[54] Although infection with reoviruses occurs commonly, exanthem has been noted on only nine occasions.[54,129] A morbilliform rash has been observed in one adult with a rotavirus infection, and a 4-year-old boy was noted to have a petechial rash in association with a rotaviral illness.[62,182] Di Lernia and Ricci[64] described three cases of Gianotti-Crosti syndrome and one child with infantile acute hemorrhagic edema associated with rotavirus infections.

Of the *Togaviridae* family of viruses, rubella virus is the most important as a worldwide cause of exanthematous disease. Several alphaviruses also frequently cause exanthems.[114,151,226,227] Each of these viruses has a marked geographic distribution. Similarly, flaviviruses also have exanthem as part of their clinical expression, and they too have specific geographic boundaries.[226,227] In the New York City area outbreak of West Nile virus infection in 1999, 19 percent of patients had exanthem.[148] The rash was erythematous macular, papular, or morbilliform.

Exanthem generally is not considered to be a manifestation of influenza virus infection, but Hope-Simpson and Higgins[103] noted exanthem in approximately 8 percent of patients from whom influenza B virus was isolated and in 1 or 2 percent of those infected with influenza A virus. The occurrence of Gianotti-Crosti syndrome was noted 1 week after live H1N1 influenza virus vaccination in a 9 year-old boy.[121] Measles virus is the most notable of the *Paramyxoviridae* family with an associated exanthem. However, exanthem occurs rather frequently in young children infected with parainfluenza virus types 1, 2, and 3 and also in those with respiratory syncytial virus (RSV) illnesses.[86,97,99,216,219] Hope-Simpson and Higgins[103] noted a 15 percent incidence of rash in RSV infection and an approximately 15 percent incidence in parainfluenza virus infection. Rash, which was not described further, was observed in four children with respiratory illnesses caused by human metapneumovirus infections.[170] Exanthem also has been noted on rare occasion with mumps virus infection.[50]

An outbreak involving 185 cases caused by Zika virus (a flavivirus) occurred on an island in Micronesia in 2007.[72] The patients (children and adults) had fever, macular or maculopapular exanthems, arthritis or arthralgia, and nonpurulent conjunctivitis.

Lassa fever virus, Marburg virus, Ebola virus, and hepatitis B virus all have been associated with exanthem on occasion.* Rash was noted in 3/5 children with coronavirus OC43 lower respiratory tract infections.[210] Hepatitis B virus is the main cause of papular acrodermatitis (Gianotti-Crosti syndrome) in children.[47,185,191] Chronic hepatitis C virus infection occasionally causes systemic vasculitis and cryoglobulinemia in adults, with purpuric lesions concentrated on the lower extremities.[2,101] Other cutaneous manifestations of chronic hepatitis C virus infection include urticaria, erythema nodosum, lichen planus, and nodular prurigo.[112,234]

Hantaviruses cause two major syndromes throughout the world: hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome.[4,40,162,190] Exanthem (facial flushing and petechial lesions in skinfolds) occurs in approximately 30 percent of patients with hemorrhagic

*References 19, 22, 50, 181, 206, 226.
†References 17, 29, 100, 110, 118, 158, 171, 214, 215.

*References 42, 46, 65, 79, 164, 226.

fever with renal syndrome, but rash is not reported in the hantavirus pulmonary syndrome. A macular rash has been noted in association with acute infection with human immunodeficiency virus type 1 (HIV-1).[8,148,150,205] Several reports have associated human T-lymphotropic virus type 1 (HTLV-1) with an atypical form of eczema termed *infective dermatitis*. This exanthem has an acute onset and is somewhat recalcitrant to treatment.[123,125,135]

Chlamydiae, rickettsiae, and mycoplasmas associated with cutaneous manifestations are listed in Table 58-2. Of the chlamydiae, only *Chlamydia psittaci* has been associated with exanthem. In contrast, all rickettsiae that infect humans, with the exception of *Coxiella burnetii*, usually display some cutaneous manifestations as part of their systemic disease.* Approximately 4 to 7 percent of adults with Q fever have exanthem.[40,203] Of the mycoplasmas that infect humans, only *Mycoplasma pneumoniae* is associated with exanthem.[14,53,54] In epidemics, exanthem occurs in approximately 15 percent of persons with respiratory illness.

In Table 58-3, bacterial agents for which cutaneous manifestations are part of the clinical illness are presented (see Chapter 60A). The clinical expression of exanthem varies tremendously among the different etiologic agents, as do the conditions associated with a specific infection. For example, infection with phage group 2 staphylococci usually results in cutaneous disease in young infants, whereas the same organisms rarely cause illness in adults. Symptomatic infection with *Streptococcus pneumoniae* is associated with cutaneous manifestations only occasionally; on the other hand, similar systemic disease with *Neisseria meningitidis* virtually always is associated with the characteristic petechial exanthem. Of the other bacterial agents listed in Table 58-3, exanthem is most important in *Neisseria gonorrhoeae*, *Salmonella typhi*, *Streptobacillus moniliformis*, *Spirillum minus*, *Pseudomonas aeruginosa*, and *Treponema pallidum*.

Fungal, protozoan, and metazoan agents associated with cutaneous manifestations in humans are listed in Tables 58-4, 58-5, and 58-6, respectively. These agents and their diseases, discussed more completely in other chapters, are included here for completeness of the differential diagnosis.

EPIDEMIOLOGY

Tables 58-1 through 58-6 clearly show that exanthematous disease has many possible etiologic agents; hence, no unified epidemiology exists. Epidemiologic events related to specific agents are considered in the appropriate sections throughout this text. Each agent with exanthem as a clinical manifestation has a unique epidemiologic pattern that, if understood, distinguishes it from many of the other agents that cause otherwise identical clinical illnesses. In the evaluation of all patients with rash, exposure, season, and incubation period are important aspects of the diagnostic process.

*References 32, 74, 90, 124, 131, 153, 159, 189.

PATHOPHYSIOLOGY AND PATHOLOGY OF EXANTHEMS

Even though the skin can respond in only a limited number of ways, what is obvious from the extensive number of etiologic agents is that multiple pathogenic mechanisms must occur. In many sections of this book, the pathology and pathophysiology of specific agents are presented in detail. An overview is presented here.

Small vessel vasculitis (leukocytoclastic vasculitis) is a leading event in most exanthematous illnesses caused by infectious agents.[202] The cutaneous manifestations of systemic diseases can be separated into three broad categories. The first category involves dissemination of infectious agents by blood (e.g., viremia and bacteremia), which results in secondary infection at the cutaneous site. The clinical cutaneous findings in this type of infection can be the direct result of infectious agents in the epidermis, dermis, or dermal capillary endothelium or can be the result of an immune response between the organism and antibody or cellular factors in the cutaneous location. The possible events in the skin with this type of infection are presented in Figure 58-1. Chickenpox, many enteroviral infections, and meningococcemia are examples of diseases in which infectious agents have reached the skin through the blood and are causing the cutaneous findings without the additional contribution of host immune factors. In illnesses such as measles, rubella, and gonococcemia, the timing, histologic picture, and difficulty of direct recovery of the agent by culture suggest both a direct effect and an immune-mediated response.

The second category of pathogenesis relates to the dissemination of known specific toxins of infectious agents. The infection is in a localized area of the body, but the toxin liberated by the infectious agents reaches the skin by blood-borne dissemination. Three examples of toxin-mediated exanthematous disease are streptococcal scarlet fever, staphylococcal scalded skin syndrome, and toxic shock syndrome.

The third category of pathogenesis in systemic disease with exanthem is poorly understood but appears to have an immunologic basis. Most important in this category are the clinical pictures of erythema multiforme, erythema multiforme exudativum (Stevens-Johnson syndrome), and erythema nodosum. In erythema multiforme associated with *M. pneumoniae* and HSV infection, the respective organisms have been isolated or identified at the skin site. In most instances, however, neither antigen localization nor disseminated toxin has been identified. The occurrence of erythema nodosum in a *Coccidioides immitis* infection indicates the development of cell-mediated immunity.

Important clinical aspects of exanthematous diseases are the distribution and progression of the lesions, yet little is known of the cause of these aspects. Differences in skin thickness, vascularity, proliferation rate, temperature, and metabolic activity are important in animal diseases with cutaneous manifestations.[54,77,133,144,166] In humans, similar factors must be important but obviously

Text continued on p. 759

TABLE 58-2 **Clinical Characteristics of Chlamydial, Rickettsial, and Mycoplasmal Infections with Cutaneous Manifestations**

					Exanthem		
Agent	**Disease or Syndrome**	**Incubation Period (days)**	**Main Season**	**Clinical Characteristics**	**Lesions**	**Distribution**	**Usual Duration (days)**
Chlamydia psittaci	Psittacosis	7-14	Nonseasonal	Fever, chills, headache, and cough Respiratory distress	Erythematous macules Occasionally erythema multiforme or erythema nodosum	Mainly on trunk	2-7
Rickettsia akari	Rickettsialpox	7-14	Nonseasonal	Fever, chills, headache, backache, and malaise 4-7 days after onset of primary lesion at site of mite bite Geographically localized disease	Initial lesion at site of mite bite is papular and then vesicular, and finally an eschar forms Two days after onset of fever, erythematous maculopapular discrete rash occurs Lesions progress to small vesicles and later to scabs	Most prominent on trunk and proximal end of extremities	7-10
Rickettsia typhi	Endemic, murine typhus	7-14	Nonseasonal	Fever and headache Rash appears on 4th-7th day Geographically localized disease	Initially discrete macules and then erythematous maculopapular May become purpuric	Initially upper part of trunk and axilla Progresses to entire body except face, palms, and soles	7-21
Rickettsia prowazekii	Epidemic typhus	10-14	Nonseasonal	Sudden onset of fever, chills, headache, and myalgias Rash appears on 4th-7th day Geographically localized disease	Initially discrete macules and then progresses to maculopapular and petechial lesions Sometimes purpuric	Appears first on trunk and spreads to extremities Spares palms and soles	7-14
Rickettsia tsutsugamushi	Scrub typhus	7-21	Nonseasonal	Sudden onset of chills, fever, and headache	Local lesion at site of chigger bite is present at onset of symptoms; characterized by vesicle, ulcer, and eschar Maculopapular rash occurs 5-8 days after onset of fever	Maculopapular rash first occurs on trunk and then becomes generalized	7-14
Rickettsia rickettsii	Rocky Mountain spotted fever	3-12	Summer	Abrupt onset of fever, chills, and headache Rash appears 2-4 days after onset	Early maculopapular, then petechial, and sometimes purpuric	Rash starts on distal end of extremities Rarely involves the trunk	7-14

Continued on following page

TABLE 58-2 **Clinical Characteristics of Chlamydial, Rickettsial, and Mycoplasmal Infections with Cutaneous Manifestations** (Continued)

					Exanthem		
Agent	**Disease or Syndrome**	**Incubation Period (days)**	**Main Season**	**Clinical Characteristics**	**Lesions**	**Distribution**	**Usual Duration (days)**
Other tick-borne rickettsiae			Tick seasons	Similar to mild Rocky Mountain spotted fever	Similar to Rocky Mountain spotted fever; eschar at site of tick bite	Similar to Rocky Mountain spotted fever	7-14
R. sibirica	North Asian tick-borne rickettsiosis						
R. australis	Queensland tick typhus						
R. conorii	Boutonneuse fever; Mediterranean spotted fever						
R. africae	African tick fever						
Coxiella burnetii	Q fever	20-40	Nonseasonal	Acute febrile illness with chills, headache, and myalgia	Fine discrete macular rash occurring during febrile illness Transient urticarial rash also noted	Mainly on trunk	2-7
Ehrlichia and *Anaplasma* spp.	Ehrlichiosis; anaplasmosis	14-28	Tick seasons	Similar to Rocky Mountain spotted fever, but rash usually not on palms and soles	Similar to endemic typhus	Similar to endemic typhus	7-14
Mycoplasma pneumoniae		21	All seasons	Gradual onset of fever, malaise, headache, and cough	Maculopapular rash occurs in 5%-15% of cases Vesicular and bullous lesions common (Stevens-Johnson syndrome); more common in males Papular, petechial, and urticarial lesions also noted Erythema multiforme common	Rash most prominent on trunk and proximal end of extremities	7-14

Data from references 14, 15, 19, 32, 36, 41, 53, 54, 58, 74, 90, 97, 117, 124, 131, 138, 146, 153, 159, 172, 189, 205, 228, 233.

TABLE 58-3 **Bacteria Associated with Cutaneous Manifestations**

Agent	Disease or Syndrome	Clinical Characteristics	*Exanthem*	
			Lesions	**Distribution**
Gram-Positive Cocci				
Staphylococcus aureus, exfoliative toxin-producing, mainly phage group 2	Bullous impetigo	Usually occurs in neonates May be epidemic	Rapid progression from vesicles to bullous lesions	Most common in diaper area
	Scalded skin syndrome Toxic epidermal necrolysis (Ritter disease in infants <4 mo; Lyell syndrome in older children)	Usually occurs in infants and children 1 mo–5 yr of age Mucopurulent nasal and eye discharge Fever	Scarlatiniform eruption with exfoliation Nikolsky sign present Crusty appearance around eyes and under nose	Generalized Most marked on trunk
	Staphylococcal scarlet fever or staphylococcal scarlatiniform eruption	Fever and staphylococcal infection in throat, but no evidence of pharyngitis	Scarlet fever–like rash with desquamation Pastia lines present	Generalized
Staphylococcus aureus, nonexfoliative toxin-producing	Septicemic disease	Severe septicemia with osteomyelitis, arthritis, endocarditis, or pneumonia	Diffuse, erythematous, confluent, and macular rash (flush) With endocarditis, may have petechiae and splinter hemorrhages, Osler nodes, Janeway spots	Trunk and proximal end of extremities
Staphylococcus aureus, toxin-I (TSST-1)-producing	Toxic shock syndrome	Fever, intense myalgias, vomiting, and diarrhea Mental confusion and hypotension	Erythematous, deep red (sunburn-like) rash Desquamation occurs	Generalized
Staphylococcus aureus, nonexfoliative toxin-producing	Folliculitis, furuncles, or carbuncles	See primary skin infections, Chapter 60A		
Streptococcus pyogenes	Scarlet fever	Fever, pharyngitis, and cervical lymphadenitis Rash onset within 2 days of first symptoms Incubation period 3-4 days	Diffuse erythematous and fine maculopapular (looks and feels like red sandpaper) Rash darker in skinfolds (Pastia lines) Desquamation occurs	Circumoral pallor Generalized rash, with trunk and proximal end of extremities being most involved
	Erysipelas	Fever, headache, and vomiting Localized infection	Circumscribed area that is raised and erythematous Advancing edge is irregular	Anywhere
	Impetigo	Localized superficial pyoderma See primary skin infections, Chapter 60A	Discrete and coalescent lesions of a vesicular nature Quickly becomes more pustular and then crusts over with a yellowish-brown appearance	Forearms, legs, and face
	Septicemia	Fever and systemic foci of infection	Petechiae	Diffuse
	Miscellaneous skin manifestations of *S. pyogenes* infections		Erythema multiforme, erythema nodosum, and erythema marginatum	

Continued on following page

TABLE 58-3 **Bacteria Associated with Cutaneous Manifestations** (Continued)

			Exanthem	
Agent	**Disease or Syndrome**	**Clinical Characteristics**	**Lesions**	**Distribution**
Streptococcus pneumoniae	Septicemia	Fever	Petechiae	Diffuse
Enterococcal and viridans group streptococci	Endocarditis	Endocarditis	Petechiae, splinter hemorrhages, Osier nodes, and Janeway spots	
Gram-Negative Cocci				
Neisseria gonorrhoeae	Gonococcemia	Fever and polyarthralgias	Papular, petechial purpuric, pustular, or necrotic lesions	Most common on extremities Extensor surfaces over joints
Neisseria meningitidis	Meningococcemia	Fever and pharyngitis Sudden onset of rash	Characteristic rash is petechial or purpuric Early lesions may be erythematous maculopapular, or urticarial	Generalized
Moraxella catarrhalis	Bacteremia	Fever and pharyngitis	Maculopapular and petechial	Generalized
Gram-Positive Bacilli				
Bacillus anthracis	Anthrax	Fever, headache, malaise, and joint pain	Initially, macular, pruritic lesion Later, a papule forms and then vesiculation Vesicles last 2-6 days, and then eschar forms	Usually, single lesion initially at point of exposure, secondary lesions in area develop later
Listeria monocytogenes	Listeriosis	Neonatal meningitis with hepatosplenomegaly	Maculopapular, discrete lesions Pustules	Trunk and legs
Erysipelothrix rhusiopathiae	Crab or fishnet dermatitis	Fever and local pain	Erysipeloid lesion (violet or red)	Hands
Corynebacterium diptheriae	Cutaneous diphtheria	Secondary infection in cutaneous wounds	Impetigo or ecthyma-like Rarely, erythema multiforme	Exposed surfaces
Arcanobacterium hemolyticum	Scarlet fever–like illness	Fever and pharyngitis	Scarlet fever–like rash Occasionally, rubelliform	Generalized rash with peripheral predominance
Enteric Gram-Negative Bacilli				
Salmonella typhi	Typhoid fever	Malaise, headache, and marked fever Rash onset 10 days after onset of fever	Rose spots, 2- to 4-mm macular lesions	Discrete lesions on abdomen
Other *Salmonella* spp.	Septicemic salmonellosis	Similar to mild typhoid fever	Similar to typhoid fever	Similar to typhoid fever
Shigella sonnei	Shigellosis	Diarrhea	Urticaria	Diffuse
Campylobacter species		Gastroenteritis	Skin pustules and erythema nodosum	Lower part of legs
Other Gram-Negative Bacilli				
Francisella tularensis	Tularemia	Chills, fever, headache, and localized lymphadenopathy	Initial papule that later ulcerates	Site of inoculation
Haemophilus ducreyi	Chancroid	Local pain and tenderness	Pustular lesions that ulcerate	External genitalia
Haemophilus influenzae	Septicemia	Fever	Petechiae	Diffuse
			Reddish purple cellulitis	Cellulitis mainly on cheeks and extremities

Streptobacillus moniliformis	Rat-bite fever	Fever, chills, malaise, headache, and polyarthritis	Erythematous, maculopapular rash that may become petechial	Most prominent on extremities, including palms and soles
Yersinia pestis	Septicemic plague	Sudden onset of fever	Initial generalized erythema followed by petechiae and purpura	Generalized
Yersinia pseudotuberculosis		Mesenteric lymphadenitis	Erythema nodosum and scarlatiniform eruption	Lower part of legs and generalized
Yersinia enterocolitica	Yersiniosis	Enterocolitis	Erythema nodosum and urticaria	Lower part of legs and generalized
Bartonella bacilliformis	Bartonellosis, Carrión disease, or Oroya fever	Initially intermittent fever, malaise, and myalgias 30-60 days after initial fever, exanthem appears	Erythematous maculopapular Later recurrent nodules	Face and extensor surface of extremities
Bartonella quintana	Trench fever	Usually mild fever, headache, chills, and tibial bone pain	Macular rash	Mainly on trunk
Calymmatobacterium granulomatis	Granuloma inguinale	See *Calymmatobacterium granulomatis,* Chapter 130	Nodular, ulcerovegetative, hypertrophic, or cicatricial lesions	Genitals
Pseudomonas aeruginosa	Ecthyma gangrenosa	Septicemia (usually in immunocompromised patients)	Initially vesicular and then hemorrhagic Become ulcerated with central black necrotic eschar	Anywhere
	Pseudomonas folliculitis (health spa dermatitis)	Headache, malaise, and fatigue	Papular and pustular	Generalized
Burkholderia mallei	Glanders, melioidosis	Fever, malaise, chills, arthralgia, and muscle pains	Nodule or ulcer at site of inoculation and then widespread papules, bullae, and pustules	Generalized
Brucella spp.	Brucellosis	Acute or subacute febrile illness Erythematous and maculopapular	Exanthem in 8% of cases Occasionally vesicles	Generalized
Legionella pneumophila	Legionnaires disease	Severe pneumonia	Maculopapular	Anterior of trunk
Bartonella henselae	Cat-scratch fever	Subacute regional lymphadenitis	Erythematous maculopapular, morbilliform, petechial, erythema nodosum, erythema multiforme, and erythema marginatum May be pruritic	Generalized

Continued on following page

TABLE 58-3 **Bacteria Associated with Cutaneous Manifestations** (Continued)

Agent	Disease or Syndrome	Clinical Characteristics	*Exanthem*	
			Lesions	**Distribution**
Acid-Fast Bacilli				
Mycobacterium tuberculosis	Lupus vulgaris	Usually associated with other manifestations of tuberculosis	Reddish-brown nodular or scaling lesions	Mainly on face and neck
	Papulonecrotic tuberculids	Associated with disseminated tuberculosis	Initially vesicular Become pustules, umbilical, and ulcerated and then form scabs and leave scars	Single or multiple lesions anywhere
Atypical mycobacteria			Granulomatous and ulcerative lesions at site of superficial injury	Usually on hands
Mycobacterium leprae	Erythema nodosum leprosum	General findings of lepromatous leprosy	Erythematous nodular lesions	Disseminated Most prominent on face and extremities
Spirochetes				
Treponema pallidum	Primary syphilis	Chancre	Large ulcers with indurated edges	Genitals
	Secondary syphilis		Erythematous maculopapules that frequently are scaly (psoriasiform)	Generalized, including palms and soles
Treponema pertenue	Yaws		Papular lesions at sites of inoculations Lesions ulcerate, leaving a wart-like appearance	Anywhere
Borrelia burgdorferi	Lyme disease (erythema chronicum migrans)	Skin, cardiac, neurologic, and joint abnormalities	Expanding erythematous, annular lesions	Thighs, buttocks, or axillae
Treponema carateum	Pinta		Initially, erythematous, papular lesions; increase in size during 1-month period and become scaly	Exposed surfaces of body
Spirillum minus	Rat-bite fever	Fever and chills	Discrete, macular rash	Trunk and extremities, including palms and soles
Leptospira spp.	Leptospirosis	Fever, conjunctivitis, and anorexia Rash rarely noted	Erythematous maculopapular rash	Mainly on trunk
Borrelia spp.	Relapsing fever	Relapsing fever, headache, myalgia, and photophobia	Morbilliform and petechial Erythema multiforme	Generalized

Data from references 5, 18, 22, 25, 30, 31, 33-35, 38, 39, 42, 55, 61, 66, 70, 78, 92, 93, 95, 104, 105, 107, 109, 111, 119, 120, 126, 127, 132, 134, 137, 141, 152, 157, 161, 163, 165, 167, 168, 173, 176, 183, 187, 193, 204, 206-208, 211-213, 214, 218, 221, 224, 229, 231, 232, 237.

TABLE 58-4 Fungi Associated with Cutaneous Manifestations

			Exanthem	
Agent	**Disease or Syndrome**	**Clinical Characteristics**	**Lesions**	**Distribution**
Dermatophytic fungi	Tinea capitis, tinea cruris, tinea pedis, or tinea circinata		Localized, brownish, maculopapular lesions that are scaly	
Erythema nodosum				
Candida albicans	Congenital cutaneous candidiasis	Congenital infection	Discrete vesicular lesions	Generalized
	Chronic mucocutaneous candidiasis	Immunodeficiency disease	Confluent, erythematous, and exudative lesions	Generalized, including scalp
	Acquired candidiasis		Confluent, fiery red lesions	Most common in diaper area
Candida spp.	Systemic candidiasis	Severe opportunistic infection	Erythematous nodular lesions	Generalized
Histoplasma capsulatum	Histoplasmosis	Primary respiratory tract infection	Erythema nodosum, erythema multiforme, and erythematous maculopapular	
Cryptococcus neoformans	Cryptococcosis	Primary respiratory tract infection	Erythema nodosum and acneiform eruptions	
Coccidioides immitis	Coccidioidomycosis	Primary respiratory tract infection	Initially, erythematous, maculopapular rash Later, erythema multiforme and erythema nodosum	Generalized maculopapular rash
Sporotrichum schenckii	Sporotrichosis	Cutaneous inoculation	Nodular lesions that ulcerate	Usually hands, arms, and legs
Blastomyces dermatitidis	Blastomycosis	Primary respiratory tract infection	Nodular lesions that ulcerate Erythema nodosum	
Scedosporium spp.	No specific syndrome	Severe opportunistic infection	Nodular or necrotic skin lesions	Generalized
Fusarium spp.	No specific syndrome	Severe opportunistic infection	Nodular skin lesions, abscesses	Generalized
Aspergillus spp.	No specific syndrome	Severe opportunistic infection	Nodular and purpuric lesions	Generalized

Data from references 15, 16, 24, 28, 44, 74, 77, 86, 88, 136, 140, 177, 198, 220, 230.

TABLE 58-5 Cutaneous Manifestations of Protozoan and Helminthic Infections

Agent	**Disease or Syndrome**	**Cutaneous Manifestations**
Plasmodium spp.	Malaria	Occasionally generalized urticaria in chronic infection
Toxoplasma gondii	Acquired toxoplasmosis	Occasionally generalized erythematous, maculopapular rash
	Congenital toxoplasmosis	Generalized petechial rash
Giardia lamblia	Giardiasis	Rarely urticaria
Entamoeba histolytica	Amebiasis	Rarely urticaria
Leishmania tropica	Oriental sore	Red nodular lesion that ulcerates; lasts 2-3 mo
Leishmania braziliensis and *mexicana*	American cutaneous leishmaniasis	Erythematous papular lesion that vesiculates and ulcerates
Trypanosoma gambiense	African trypanosomiasis	Red nodular lesion at site of bite, followed by generalized, pruritic, erythema multiforme–like rash
Trypanosoma cruzi	American trypanosomiasis or Chagas disease	Nodular lesion at site of bite; generalized recurrent erythematous, maculopapular rash
Trichomonas vaginalis	Vulvovaginalis	Rarely urticaria and erythema multiforme
Ascaris lumbricoides	Roundworm infestation	Erythema nodosum
Enterobius vermicularis	Pinworm infestation	Rarely urticaria
Necator americanus	Hookworm disease	Papules and papulovesicles on exposed surfaces (feet); generalized urticaria

Continued on following page

TABLE 58-5 **Cutaneous Manifestations of Protozoan and Helminthic Infections** (Continued)

Agent	Disease or Syndrome	Cutaneous Manifestations
Trichinella spiralis	Trichinosis	Urticaria common; also, generalized maculopapular rash may occur; petechiae frequently develop
Strongyloides stercoralis	Strongyloidiasis; also, creeping eruption (cutaneous larva migrans)	Erythematous, maculopapular lesions on feet; creeping eruption
Ancylostoma braziliense	Creeping eruption (cutaneous larva migrans)	Creeping eruption
Dermatobia hominis	Cutaneous myiasis	Creeping eruption, subacute draining lesions
Schistosoma haematobium, mansoni, and *japonicum*	Schistosomiasis	Pruritic papular eruption where exposed; generalized urticaria and granulomatous lesions
Trichobilharzia ocellata, physellae, and *stagnicolae*	Swimmer's itch or collector's itch	Initial erythema and urticaria followed by papules and vesiculation; pruritic
Wuchereria bancrofti	Filariasis	Localized erythema urticaria and erythema nodosum
Onchocerca volvulus	Onchocerciasis	Chronic, papular, scaly rash
Echinococcus granulosus and *multilocularis*	Echinococcosis	Frequent urticaria

Data from references 15, 21, 43, 57, 74, 76, 89, 94, 143.

TABLE 58-6 **Cutaneous Manifestations of Arthropod Bites and Stings**

Agent	Disease or Syndrome	Cutaneous Manifestations
Spiders		
Loxosceles rectus	Recluse spider bite or brown spider bite	Erythema followed by blister and necrosis
Ticks	Tick bite	Initial pruritus at site; becomes ulcerated and granulomatous
Mites		
Sarcoptes scabiei	Scabies	Pruritic burrows in body creases and generalized; become erythematous and then papular urticaria
Trombicula irritans	Chigger bite	Marked pruritus and then papular urticaria
Other mites: Food, grain, murine, and fowl		Marked pruritus and then papular urticaria
Lice		
Pediculus humanus	Body lice or pediculosis	Erythematous, maculopapular, pruritic lesions; sometimes urticaria
Phthirus pubis	Crabs	Pruritus and erythema under pubic hair
Bedbugs and kissing bugs		
Cimex lectularius	Bedbug bite	Pruritic papular urticaria
Triatoma sanguisuga	Kissing bug bite	Papular urticaria; occasionally hemorrhagic nodular lesions
Gypsy moth caterpillar		
Lymantria dispar	Gypsy moth rash	Pruritic blotchy erythema and maculopapular
Moths		
Hylesia alinda	Moth-associated dermatitis	Erythema and pruritus; feeling of warmth in area of rash; may have vesicular lesions
Ants		
Solenopsis saevissima	Fire ant bite	Painful papular urticarial lesions that become pustular and then nodular
Fleas		
Pulex irritans (human flea) and fleas of many animals	Flea bite	Papular urticaria
Flies and mosquitoes	Fly and mosquito bite	Papular, nodular, and urticarial lesions in sensitive persons

Data from references 46, 48, 63, 74, 101, 160, 180, 192, 197.

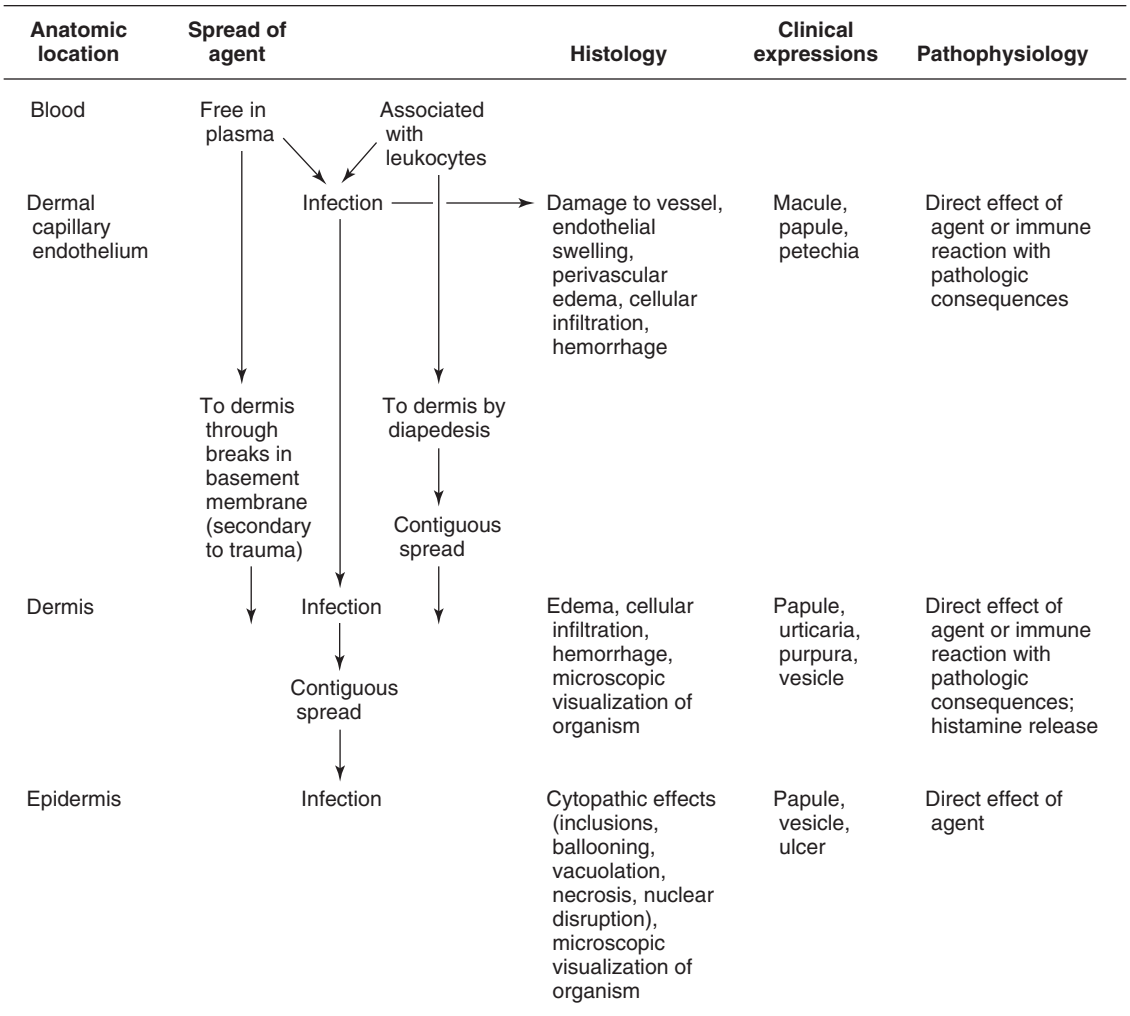

FIGURE 58-1 ■ Aspects of pathogenesis in exanthems associated with blood-borne dissemination of the infectious agent. (Modified from Cherry JD. Newer viral exanthems. Adv Pediatr 1969;**16**:233-86.)

affect the various etiologic agents differently (e.g., the more central exanthem of chickenpox vs. that of the hand, foot, and mouth syndrome of coxsackievirus A16 infection).

CLINICAL MANIFESTATIONS

The clinical findings in exanthematous diseases resulting from systemic infections are varied and depend on the inciting pathogens. By examination of skin alone, differentiating an exanthematous disease resulting from systemic infection (e.g., coxsackievirus A9, rubella virus infection) from primary cutaneous diseases of infectious and noninfectious origin (e.g., insect bites, acne, and contact with poison ivy) frequently is difficult. In Tables 58-1 through 58-6, the clinical characteristics of viral, chlamydial, rickettsial, bacterial, fungal, parasitic, and arthropod-induced illnesses with primary or secondary cutaneous manifestations are presented. In Tables 58-8 through 58-17, etiologic agents and clinical manifestations are presented on the basis of the more pronounced cutaneous manifestations or syndrome associations. The clinician must keep in mind that other aspects of an illness (e.g., exposure, season, incubation period, geographic location, patient age, and associated signs and symptoms) may be more important in determining the underlying etiologic agent. Clinical manifestations of specific exanthematous diseases are presented in greater detail in other chapters of this book.

Erythematous Macular Exanthems

When all infectious diseases with exanthems are taken into consideration, the occurrence of illnesses in which the lesions are just macular is rare. However, many important, severe diseases have a transitory erythematous macular rash early in their course, and recognition of this fact can be lifesaving. Infectious agents associated with illnesses in which macular exanthems have been observed are presented in Table 58-7.

TABLE 58-7 **Infectious Agents Associated with Illness in Which a Macular Exanthem Has Been Observed**

Infectious Agent	Illness
Human herpesvirus–6, 7	Roseola infantum
Epstein-Barr virus	Infectious mononucleosis
Coxsackieviruses BI, B2, B5	—
Echoviruses 2, 4, 5, 14, 17-19, 30	—
Enterovirus 71	—
Dengue virus	Dengue fever
Lassa virus	Lassa fever
Marburg virus	Marburg fever
Parvovirus	Erythema infectiosum
HIV-1	Manifestation of acute infection
Hantavirus	Hemorrhagic fever with renal syndrome
Chlamydia psittaci	Psittacosis
Rickettsia typhi	Murine typhus
Rickettsia prowazekii	Epidemic typhus
Rickettsia quintana	Trench fever
Coxiella burnetii	Q fever
Mycoplasma pneumoniae	—
Staphylococcus aureus	Septicemia and toxic shock syndrome
Streptococcus pyogenes	Scarlatina and septicemia
Bacillus anthracis	Anthrax
Salmonella typhi	Typhoid fever
Salmonella spp.	Septicemic salmonellosis
Spirillum minus	Rat-bite fever
Leptospira spp.	Leptospirosis
Yersinia pestis	Plague

The most common rash in infectious mononucleosis is erythematous and maculopapular, but rarely (most often in association with the administration of ampicillin) the exanthem is generalized, confluent, fiery red, and macular. Blotchy or diffuse erythematous macular rashes have been caused specifically by 12 different enterovirus types. Most of these descriptions involve neonates, other very young infants, and adults; children in the peak ages for enteroviral exanthematous diseases do not seem to have solely macular lesions. In neonates, enteroviral disease with a blotchy macular rash in association with fever and lethargy usually is confused with bacterial sepsis.

Patients with dengue, Lassa, and Marburg viral infections frequently have a macular, flushed appearance before other cutaneous manifestations develop. Similarly, in both murine and epidemic typhus, the initial skin manifestations are macular but progress rapidly to more pronounced findings.

Bacterial septicemia with both common and exotic organisms is associated frequently with a generalized flush. In staphylococcal disease, the rash is particularly apparent in endocarditis and osteomyelitis. The most famous disease with a macular rash is typhoid fever. Rose spots occur most commonly on the abdomen, but they also are seen on the chest and back. They are 2- to 4-mm erythematous, macular lesions. Lesions likewise have been noted in leptospirosis and psittacosis. In addition, rose spots are seen occasionally in septicemic illnesses caused by other *Salmonella* spp.

The slapped-cheek appearance in erythema infectiosum is caused by an erythematous macular flush of the cheeks. The full-blown rash in streptococcal scarlet fever is maculopapular, but frequently in mild cases and in those altered by antibiotic therapy, the exanthem is only macular (scarlatina).

Erythematous Maculopapular Exanthems

An erythematous maculopapular rash is the most common cutaneous manifestation of systemic infection. It also is an exceedingly common occurrence in allergic conditions. However, all too frequently, the rash of an infectious illness is ascribed to an allergic reaction to an administered drug rather than correctly to the disease process. The converse—an allergic rash illness attributed mistakenly to an infectious agent—rarely occurs. Infectious agents associated with illnesses in which maculopapular exanthems occur are presented in Table 58-8.

Both by the number of possible etiologic agents and by total infections, viruses account for the vast majority of illnesses with maculopapular eruptions. Although the distribution and progression of rashes are important aspects relating to the differential diagnosis, the single most important point is whether the lesions are discrete (rubelliform) or confluent (morbilliform). Adenoviruses are not uncommon causes of erythematous maculopapular eruptions. In most instances, signs and symptoms of upper respiratory tract infection are present. Most commonly, the lesions are discrete, but occasionally, a confluent morbilliform rash is present. A roseola infantum picture—occurrence of rash after the fever falls by crisis—frequently occurs. As a rule, the exanthem in adenoviral infections starts on the head and spreads to the trunk and extremities.

Enteroviruses account for the greatest number of erythematous maculopapular rash illnesses; 37 different serologic types have been implicated. The enteroviral types most commonly associated with maculopapular exanthems are coxsackieviruses A9 and B5 and echoviruses 4, 9, and 16. Echovirus 9 has been the most frequent cause of enteroviral exanthem for the last 35 years. Although morbilliform rashes do occur, the more usual cutaneous manifestation is one suggestive of rubella. The exanthem usually starts on the head and upper part of the trunk and spreads to the extremities.

Although they are not common manifestations of respiratory viruses (e.g., rhinoviruses, influenza A and B viruses, RSV, and parainfluenza viruses types 1 through 4), exanthems probably occur more often than is generally realized. Because children infected with these agents frequently are given antibiotics, confusion often occurs between an allergic and an infectious etiology. With all the respiratory viruses, the signs and symptoms of

TABLE 58-8 **Infectious Agents Associated with Illnesses in Which Maculopapular Exanthems Occur**

		Character of Rash	
Infectious Agent	**Illness**	**Discrete**	**Confluent**
Parvovirus	Erythema infectiosum	+++	+
Human bocavirus		++++	
Adenoviruses 1, 2, 3, 4, 7, 7a		+++	+
Human herpesvirus–6	Roseola infantum	+++	+
Epstein-Barr virus	Infectious mononucleosis	+++	+
Cytomegalovirus		++++	
Vaccinia virus	Roseola vaccinatum	+++	+
Coxsackieviruses A2, A4, A5, A6, A7, A9, A10, A16		+++	+
Coxsackieviruses B1-B5		+++	+
Echoviruses 1-7, 9, 11, 13, 14, 16-19, 22, 25, 30, 33		+++	+
Enterovirus 71		++++	
Rhinoviruses (many types)		++++	
Colorado tick fever virus	Colorado tick fever	++++	
Reoviruses 2, 3		++	++
Rotavirus	Gianotti-Crosti syndrome; infantile acute hemorrhagic edema	++++	
Alphaviruses: chikungunya, Sindbis, o'nyong-nyong fever, Ross River		++	++
Rubella virus	Rubella (German measles)	+++	+
Flavivirus: Dengue, Kunjin, West Nile	Dengue, Kunjin fever	++	++
Influenza viruses A, B		++++	
Respiratory syncytial virus		++++	
Parainfluenza viruses 1-4		++++	
Mumps virus	Mumps	++++	
Measles virus	Measles	+	+++
Hepatitis B virus		++++	
Marburg virus	Marburg fever	++	++
Ebola virus	Ebola hemorrhagic fever	+++	+
Rickettsia akari	Rickettsialpox	++++	
Rickettsia typhi	Murine typhus	+++	+
Rickettsia prowazekii	Epidemic typhus	+++	+
Rickettsia tsutsugamushi	Scrub typhus	+++	+
Rickettsia rickettsi	Rocky Mountain spotted fever		++++
Ehrlichia spp.	Ehrlichiosis	+++	+
Mycoplasma pneumoniae		+++	+
Staphylococcus aureus (exfoliative toxin producing)	Staphylococcal scarlet fever		++++
Streptococcus pyogenes	Scarlet fever		++++
Arcanobacterium hemolyticum		++	++
Neisseria meningitidis	Meningococcemia	++++	
Moraxella catarrhalis		++++	
Listeria monocytogenes	Listeriosis	++++	
Streptobacillus moniliformis	Rat-bite fever	+++	+
Yersinia pseudotuberculosis			++++
Bartonella bacilliformis	Bartonellosis	++++	
Brucella spp.	Brucellosis	++++	
Legionella pneumophila	Legionnaires' disease	++++	
Bartonella henselae	Cat-scratch fever	+++	+
Treponema pallidum	Secondary syphilis	+++	+
Leptospira spp.	Leptospirosis	++++	
Borrelia spp.	Relapsing fever		++++
Coccidioides immitis	Coccidioidomycosis	+++	+
Toxoplasma gondii	Toxoplasmosis	++++	
Strongyloides stercoralis	Strongyloidiasis	++++	

respiratory illness (e.g., cough, coryza, croup, and bronchiolitis) are prominent. The exanthems virtually are always discrete and rubelliform.

In dengue, the exanthem goes through several stages. Initially, it is macular, then erythematous maculopapular, and finally hemorrhagic. Similarly, the exanthems in the rickettsial diseases go through stages that vary in relation to the specific agent (see Table 58-2). In Rocky Mountain spotted fever, the rash starts on the distal ends of extremities. Although the hallmark of meningococcemia is a petechial or purpuric rash, in the initial stages, the exanthem may be erythematous and maculopapular. In addition, maculopapular eruptions are observed in chronic meningococcemia. The most notable cutaneous lesion in coccidioidomycosis is erythema nodosum, but a rubelliform rash early in infection is not an unusual manifestation.

Vesicular Exanthems

The three main categories of vesicular exanthems are single or localized lesions, generalized lesions in greatest concentration on the trunk and head, and generalized lesions with the greatest concentration on the extremities. Infectious agents associated with illnesses in which vesicular rashes develop are presented in Table 58-9. The exanthem in primary or recurrent HSV infection is localized, as it is in recurrent endogenous varicella-zoster infection (herpes zoster), ecthyma contagiosum, tanapox, scrub typhus, anthrax, and papulonecrotic tuberculids.

The vesicular exanthematous disease that occurs most commonly in children today is chickenpox. It should be a readily recognizable disease, but is all too frequently confused with enteroviral infections or insect bites and allergic conditions. Chickenpox has a long incubation period (16 days) and is associated with mild fever and an exanthem that starts on the head and upper part of the trunk and spreads to the extremities. The rash always is more prominent on the trunk than on the extremities. At any time during the first few days of the rash, lesions in all stages (i.e., macules, papules, and vesicles) can be seen. Individual lesions in chickenpox form scabs that persist for approximately 7 days.

In contrast to that of chickenpox, the exanthem in enteroviral infections frequently is peripheral in distribution, and the lesions generally heal without scabs. The incubation period (5 days) is much shorter than that of chickenpox. The hand, foot, and mouth syndrome is a common manifestation of enteroviral vesicular rash illnesses. Until relatively recently, the most frequent etiologic agent in the hand, foot, and mouth syndrome was coxsackievirus A16, but the syndrome also had been attributed to coxsackieviruses A5, A9, A10, B1, and B3 and enterovirus 71. In recent years, enterovirus 71 is the most common cause of this syndrome. Most recently coxsackievirus A6 has been noted in several outbreaks.[155,223]

Enteroviral infections with vesicular exanthems in which the hand, foot, and mouth distribution is not present quite frequently are diagnosed erroneously as insect bites or poison ivy.

TABLE 58-9 **Infectious Agents Associated with Illnesses in Which Vesicular Exanthems Occur**

Infectious Agent	Illness
Human parvovirus B19	
Herpes simplex virus types 1 and 2	Cold sores, genital herpes, or neonatal herpes
Varicella-zoster virus	Chickenpox (varicella) or herpes zoster
Vaccinia virus	Disseminated vaccinia or eczema vaccinatum
Variola virus	Smallpox
Monkeypox virus	
Orf virus	Ecthyma contagiosum
Tanapox virus	
Coxsackieviruses A4, A5, A6, A8, A10, A16	
Coxsackieviruses BI-B3	
Echoviruses 6, 9,11, 17	
Enterovirus 71	
Reovirus 2	
Calicivirus of oceanic origin	
Alphaviruses: Chikungunya, o'nyong-nyong fever, Ross River, Sindbis	
Kunjin virus	
Mumps virus	Mumps
Measles virus	Atypical measles
Rickettsia akari	Rickettsialpox
Rickettsia tsutsugamushi	
Mycoplasma pneumoniae	
Streptococcus pyogenes	Impetigo
Pseudomonas aeruginosa	
Brucella spp.	Brucellosis
Bacillus anthracis	Anthrax
Mycobacterium tuberculosis	Papulonecrotic tuberculids
Candida albicans	Congenital cutaneous candidiasis
Leishmania braziliensis	American cutaneous leishmaniasis
Necator americanus	Hookworm disease

Petechial and Purpuric Exanthems

A large number of infectious agents are associated with petechial and purpuric skin manifestations and are listed in Table 58-10. Infectious diseases with hemorrhagic rash can be fulminant fatal events or relatively benign illnesses. On a worldwide basis, meningococcemia is perhaps the most important and feared, although it is not the most prevalent of the petechial and purpuric exanthematous diseases. The relatively sudden onset of fever and a petechial rash must be considered and treated as meningococcemia unless another etiology can be established with absolute certainty. The most important of the differential diagnostic problems is exanthem caused by enteroviral infection. Many different enterovirus illnesses have a sudden onset with accompanying fever and petechial rash. In addition, the situation frequently is

TABLE 58-10 **Infectious Agents Associated with Illness in Which Petechial and Purpuric Exanthems Occur**

Infectious Agent	Illness
Human parvovirus B19	Glove and socks syndrome
Varicella-zoster virus	Hemorrhagic chickenpox
Cytomegalovirus	Congenital cytomegalovirus infection
Variola virus	Hemorrhagic smallpox
Coxsackieviruses A4, A9	
Coxsackieviruses B2-B4	
Echoviruses 4, 7, 9	
Colorado tick fever virus	Colorado tick fever
Rotavirus	
Alphaviruses: Chikungunya, o'nyong-nyong fever, Ross River, Sindbis	
Rubella virus	Rubella (German measles) or congenital rubella
Respiratory syncytial virus	
Measles virus	Hemorrhagic (black measles) or atypical measles
Lassa virus	Lassa fever
Marburg virus	
Hepatitis C virus	Mixed cryoglobulinemia
Hantavirus	Hemorrhagic fever with renal syndrome
Rickettsia typhi	Murine typhus
Rickettsia prowazekii	Epidemic typhus
Rickettsia rickettsii and other tick-borne rickettsiae	Rocky Mountain spotted fever
Ehrlichia species	Ehrlichiosis
Mycoplasma pneumoniae	
Streptococcus pyogenes	Scarlet fever or septicemia
Streptococcus pneumoniae	Pneumococcal septicemia
Enterococcal and viridans group streptococci	Endocarditis
Neisseria gonorrhoeae	Gonococcemia
Neisseria meningitidis	Meningococcemia
Moraxella catarrhalis	
Haemophilus influenzae	*H. influenzae* septicemia
Pseudomonas aeruginosa	Ecthyma gangrenosa
Streptobacillus moniliformis	
Yersinia pestis	Septicemic plague (black death)
Bartonella henselae	Cat-scratch fever
Treponema pallidum	Congenital syphilis
Borrelia species	Relapsing fever
Toxoplasma gondii	Congenital toxoplasmosis
Trichinella spiralis	Trichinosis

TABLE 58-11 **Infectious Agents Associated with Illness in Which Urticarial Exanthems Occur**

Infectious Agent	Illness
Epstein-Barr virus	Infectious mononucleosis
Coxsackieviruses A9, A16, B4, B5	
Echovirus 11	
Mumps virus	Mumps
Hepatitis B virus	
Hepatitis C virus	
Mycoplasma pneumoniae	
Neisseria meningitidis	Meningococcemia
Shigella sonnei	Shigellosis
Yersinia enterocolitica	Yersiniosis
Borrelia burgdorferi	Lyme disease
Plasmodium spp.	Malaria
Coxiella burnetii	Q fever
Giardia lamblia	Giardiasis
Entamoeba histolytica	Amebiasis
Trichomonas vaginalis	Vulvovaginalis
Enterobius vermicularis	Pinworm infestation
Necator americanus	Hookworm disease
Trichinella spiralis	Trichinosis
Schistosoma spp.	Schistosomiasis
Trichobilharzia spp.	Swimmer's itch or collector's itch
Wuchereria bancrofti	Filariasis
Echinococcus spp.	Echinococcosis
Sarcoptes scabiei	Scabies
Trombicula irritans	Chigger bites
Other mites	Mite bites
Pediculus humanus	Pediculosis
Bedbugs, kissing bugs, ants, fleas, flies, and mosquitoes	Bites and stings

complicated further by the occurrence of meningitis. The most important enterovirus in its ability to mimic meningococcemia is echovirus 9.

Purpuric and petechial lesions in infectious illnesses can result from a direct or indirect (immunologic) effect of the infectious agent at the cutaneous site or from the occurrence of thrombocytopenia. Thrombocytopenia is noted most commonly in acquired rubella virus infections.

Urticarial Exanthems

The occurrence of urticaria all too frequently leads the physician to suspect an allergic or dermatologic condition.[216,217] However, what has become quite evident is that when urticaria develops in association with an acute febrile illness, the cutaneous reaction is a direct effect of an infectious agent and its mediation does not require an allergic response. Listed in Table 58-11 are infectious agents associated with urticarial exanthems.

Papular urticaria occurs very commonly in children in the summer and fall and most frequently is the result of insect bites (see Table 58-6). However, virtually identical lesions occur in infections with coxsackievirus A as well as with other enteroviruses. The main point for differentiation is that fever regularly develops in the virus-induced exanthems but is not a characteristic associated with insect bites.

Early in the course of meningococcemia, the exanthem can be urticarial, so an illness of sudden onset with

TABLE 58-12 **Infectious Agents Associated with Papular, Nodular, and Ulcerative Lesions**

Agent	Illness
Wart virus	Warts (P and N)
Orf virus	Ecthyma contagiosum (N)
Molluscum contagiosum virus	Molluscum contagiosum (P and N)
Hepatitis B virus	Gianotti-Crosti syndrome (P)
Paravaccinia virus	Milker's nodules (N)
Francisella tularensis	Tularemia (U)
Haemophilus ducreyi	Chancroid (U)
Bartonella bacilliformis	Bartonellosis (N)
Calymmatobacterium granulomatis	Granuloma inguinale (N and U)
Pseudomonas aeruginosa	Ecthyma gangrenosa (U)
	Pseudomonas folliculitis (P)
Burkholderia mallei	Glanders (N and U)
Mycobacterium tuberculosis	Lupus vulgaris (N)
	Papulonecrotic tuberculids (U)
Atypical mycobacteria	(U)
Mycobacterium leprae	(N)
Treponema pallidum	Chancre (U)
Treponema pertenue	Yaws (P and U)
Sporotrichum schenckii	Sporotrichosis (U)
Blastomyces dermatitidis	Blastomycosis (N and U)
Fusarium species	Opportunistic infection (N)
Scedosporium spp.	Opportunistic infection (N)
Candida albicans	Systemic candidiasis (N)
Leishmania tropica	Oriental sore (N and U)
Leishmania braziliensis and *mexicana*	American cutaneous leishmaniasis (P and U)
Trypanosoma spp.	Trypanosomiasis (N)
Necator americanus	Hookworm disease (P)
Schistosoma spp.	Schistosomiasis (P)
Trichobilharzia spp.	Swimmer's itch or collector's itch (P)
Onchocerca volvulus	Onchocerciasis (P)
Loxosceles reclusa	Recluse spider bites (U)
Ticks	Tick bites (U)
Sarcoptes scabiei	Scabies (P)
Trombicula irritans	Chigger bites (P)
Other mites	Mite bites (P)
Cimex lectularius	Bedbug bites (P)
Triatoma sanguisuga	Kissing bug bites (P and N)
Solenopsis saevissima	Fire ant bites (P and N)
Fleas	Flea bites (P)
Flies and mosquitoes	Fly and mosquito bites (P)

N, Nodular; *P,* papular; *U,* ulcerative.

fever and this cutaneous manifestation never should be taken lightly.

Papular, Nodular, and Ulcerative Lesions

In many instances, the lesions in this category occur as single events at the site of primary inoculation. Specific illnesses and etiologic agents are listed in Table 58-12.

Distinctive Clinical Features or Syndromes

Erythema Multiforme

Erythema multiforme is a self-limited skin eruption that is erythematous and characterized by distinctive target or iris lesions or both. Small vesicles and urticarial areas also may develop. On occasion, the disease is severe and associated with mucosal involvement and genital lesions. In this latter illness—the Stevens-Johnson syndrome, bullous erythema multiforme, erythema multiforme exudativum major—severe ulcerative, oral, and genital lesions occur; generalized exanthems become bullous, and conjunctivitis is present. The illness is associated with fever and general distress.

Although the pathogenesis of erythema multiforme is unknown, what is clear is that multiple factors, including infectious agents, are responsible for its occurrence. Infectious agents associated with erythema multiforme are listed in Table 58-13. The single most important infectious cause of erythema multiforme and Stevens-Johnson syndrome is *M. pneumoniae*. When *M. pneumoniae* is the instigating agent, the patient nearly always has concomitant pneumonia.

HSV frequently has been recovered from the throats of persons with erythema multiforme, but the cause-and-effect relationship in many cases must be questioned. However, in a recent study, HSV DNA was found in the skin lesions of 11/31 patients with erythema multiforme.[60]

Erythema Nodosum

Erythema nodosum most commonly occurs on the anterior aspect of the lower part of the legs but may be seen anywhere on the body. The lesions are raised, erythematous, and painful to touch. Their usual size is approximately 2 to 4 cm, with a duration of 2 to 6 weeks.

Erythema nodosum occurs less commonly today than it did 4 decades ago, and the frequency of specific associated infectious agents also is different. In the past, streptococcal and mycobacterial infections were the agents most commonly related. Now, the exanthem most often is associated with respiratory tract infection with *Histoplasma capsulatum*, *Cryptococcus neoformans*, and *C. immitis*. Infectious agents associated with erythema nodosum are listed in Table 58-14.

Hand, Foot, and Mouth Syndrome

The hand, foot, and mouth syndrome is a clearly recognizable viral illness characterized by vesicular lesions in the anterior of the mouth and on the hands and feet in association with fever. Although several enteroviruses (i.e., coxsackieviruses A5, A6, A9, A10, A16, B1, and B3 and enterovirus 71) have been implicated, as have HSV and foot and mouth disease virus, most of these cases today are caused by enterovirus 71 and perhaps coxsackievirus A6.

TABLE 58-13 **Infectious Agents Associated with Erythema Multiforme**

Agent	Illness
Human parvovirus B19	Erythema infectiosum
Adenovirus 7	Respiratory tract infection
Herpes simplex virus type 1	Perioral or respiratory tract infection
Epstein-Barr virus	Infectious mononucleosis
Varicella virus	Chickenpox
Coxsackieviruses A10, A16, B5	Enterovirus syndrome
Echovirus 6	Enterovirus syndrome
Poliomyelitis virus	Poliomyelitis
Vaccinia virus	Smallpox vaccination
Variola virus	Smallpox
Orf virus	Ecthyma contagiosum
Paravaccinia virus	Milker's nodules
Influenza A virus	Influenza
Mumps	Mumps
Hepatitis B virus	Serum hepatitis
Chlamydia psittaci	Psittacosis
Chlamydia trachomatis	Lymphogranuloma venereum
Mycoplasma pneumoniae	Respiratory symptoms
Staphylococcus aureus	Septicemia
Streptococcus pyogenes	Respiratory symptoms
Neisseria gonorrhoeae	Gonorrhea
Corynebacterium diphtheriae	Diphtheria
Pseudomonas aeruginosa	Septicemia
Salmonella spp.	Gastroenteritis
Francisella tularensis	Tularemia
Yersinia spp.	Gastrointestinal symptoms
Vibrio parahaemolyticus	Gastroenteritis
Treponema pallidum	Syphilis
Bartonella henselae	Cat-scratch fever
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium leprae	Leprosy
Coccidioides immitis	Coccidioidomycosis
Histoplasma capsulatum	Histoplasmosis
Trichomonas vaginalis	Vulvovaginalis

TABLE 58-14 **Infectious Agents Associated with Erythema Nodosum**

Agent	Illness
Herpes simplex virus	Perioral or respiratory tract infection
Epstein-Barr virus	Infectious mononucleosis
Chlamydia psittaci	Psittacosis
Chlamydia trachomatis	Lymphogranuloma venereum
Streptococcus pyogenes	Respiratory tract infection
Neisseria meningitidis	Meningococcemia
Corynebacterium diphtheriae	Diphtheria
Campylobacter spp.	Gastroenteritis
Haemophilus ducreyi	Chancroid
Salmonella spp.	Salmonellosis
Yersinia spp.	Gastrointestinal symptoms
Brucella spp.	Brucellosis
Treponema pallidum	Syphilis
Bartonella henselae	Cat-scratch fever
Mycobacterium tuberculosis	Tuberculosis
Mycobacterium leprae	Leprosy
Trichophyton spp.	Kerion of scalp
Histoplasma capsulatum	Histoplasmosis
Cryptococcus neoformans	Cryptococcosis
Coccidioides immitis	Coccidioidomycosis
Blastomyces dermatitidis	Blastomycosis
Ascaris lumbricoides	Roundworm infestation
Wuchereria bancrofti	Filariasis

Roseola-like Illness

Roseola infantum is a classic pediatric illness characterized by fever of 3 to 5 days' duration, rapid defervescence, and then the appearance of an erythematous macular or maculopapular rash that persists for 1 to 2 days. Roseola is an age-related response to infection with many viruses. Recent studies suggest that a leading cause of roseola infantum is primary infection with HHV-6. The following other viruses have been noted in association with roseola: adenoviruses 1, 2, 3, and 14; coxsackieviruses A6, A9, B1, B2, B4, and B5; echoviruses 9, 11, 16, 25, 27, and 30; parainfluenza virus type 1; and measles vaccine virus.

Rocky Mountain Spotted Fever–Like Illness

Rocky Mountain spotted fever is a clinical illness characterized by fever and a petechial rash located mainly on the distal ends of extremities. The illness is caused by *Rickettsia rickettsii* and is prevalent in many areas of North America; the infectious agent is transmitted to humans by ticks. In other areas of the world, other tick-borne rickettsiae *(Rickettsia sibirica*, *Rickettsia australis*, *Rickettsia conorii)* produce similar human illness. Infection with *Ehrlichia* spp. also can cause an illness similar to Rocky Mountain spotted fever.

The most important illness that, in the past, was confused with Rocky Mountain spotted fever was atypical measles (see Chapter 179). This illness, which has both the constitutional symptoms of Rocky Mountain spotted fever and a rash most prominent on the extremities, occurs almost exclusively after exposure to measles virus in some persons previously immunized with inactivated (killed) measles vaccine.

Rat-bite fever caused by *S. moniliformis* also has been misdiagnosed as Rocky Mountain spotted fever.[168]

Exanthem and Meningitis

Aseptic and also bacterial meningitis frequently are characterized by both exanthem and symptoms and signs of neurologic involvement. Infectious agents associated with exanthem and meningitis are presented in Table 58-15. Of most importance in this category is the differential diagnosis of enteroviral syndromes and meningococcemia.

TABLE 58-15 **Infectious Agents Associated with Exanthem and Meningitis**

Agent	Illness
Herpes simplex virus type 2	Recurrent genital herpes
Coxsackieviruses A2, A9, B1, B4, B5	Enterovirus syndrome
Echoviruses 4, 6, 9, 11, 14, 17, 25, 33	Enterovirus syndrome
Colorado tick fever virus	Colorado tick fever
Reovirus 2	Respiratory tract infection
West Nile virus	Meningoencephalitis
Neisseria meningitidis	Meningococcemia
Borrelia burgdorferi	Lyme disease
Listeria monocytogenes	Listeriosis
Toxoplasma gondii	Toxoplasmosis

TABLE 58-16 **Infectious Agents Associated with Exanthem and Pulmonary Involvement**

Agent	Illness
Adenoviruses 7,7a	Respiratory tract infection
Herpes simplex virus type 1	Respiratory tract infection
Varicella-zoster virus	Chickenpox pneumonia
Epstein-Barr virus	Infectious mononucleosis
Coxsackievirus A9	Enterovirus syndrome
Echovirus 11	Enterovirus syndrome
Reovirus 3	Respiratory tract infection
Measles virus	Measles pneumonia and atypical measles
Chlamydia psittaci	Psittacosis
Mycoplasma pneumoniae	*M. pneumoniae* pneumonia
Neisseria meningitidis	Meningococcal pneumonia
Mycobacterium tuberculosis	Tuberculosis
Histoplasma capsulatum	Histoplasmosis
Cryptococcus neoformans	Cryptococcosis
Coccidioides immitis	Coccidioidomycosis

Exanthem and Pulmonary Involvement

Infectious agents associated with exanthem and pulmonary involvement are listed in Table 58-16. In patients older than 5 years of age, the leading cause of exanthem and pneumonia is *M. pneumoniae* infection. In younger children, adenoviruses are the most important etiologic agents. With the exception of enteroviral infections, which are more likely to involve young children, most of the illnesses listed in Table 58-16 occur in older children and young adults.

Gianotti-Crosti Syndrome (Papular Acrodermatitis)

Gianotti-Crosti syndrome is a distinct clinical entity characterized by a papular (lichenoid) exanthem, generalized lymphadenopathy, hepatomegaly, and acute anicteric hepatitis.[47,186,189] In most instances, this illness has been associated with hepatitis B virus infection. The syndrome also has been noted in association with EBV, cytomegalovirus, coxsackievirus B virus, and RSV infections.[66,118,187,209]

TABLE 58-17 **Important Aspects in the Diagnosis of Exanthematous Illness**

Exposure	Types of Rash
Season	Distribution of rash
Incubation period	Progression of rash
Age	Exanthem
Previous exanthems	Other associated symptoms
Relationship of rash to fever	Laboratory tests
Adenopathy	

From Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233-86.

Cutaneous Manifestations Associated with Infections in Immunocompromised Patients

All infectious agents that cause exanthems in immunologically normal children can cause infections in immunocompromised children. However, the clinical manifestations may be different. For example, measles virus infection in a child who is T-cell–deficient may be associated with a severe, progressive pneumonia but not the typical rash. Other viral exanthems that are self-limited in normal children, such as varicella, may be progressive and develop into hemorrhagic skin lesions with disseminated organ involvement in children with T-cell deficiency.

Of particular concern are bacterial and fungal infections, which are rarely a problem in normal children but are rapidly fatal in granulocytopenic children. These patients have characteristic skin lesions resulting from disseminated infections. Of importance are ecthyma gangrenosa resulting from *P. aeruginosa* septicemia and the nodular and purpuric lesions of disseminated fungal infections caused by *Aspergillus*, *Candida*, and other less common agents.

DIAGNOSIS

Differential Diagnosis

The diagnosis of infectious exanthems frequently is considered an impossible task by many physicians. Other physicians glibly call the first maculopapular exanthem of childhood *roseola* and the first vesicular rash *chickenpox*, without consideration of more appropriate choices. The hallmark of diagnosis in exanthematous disease is careful elicitation of historical data. Differential diagnosis requires the consideration of noninfectious etiologies as well as different infectious agents. Listed in Table 58-17

are the major considerations in the diagnosis of diseases with cutaneous manifestations.

A history of exposure is most important in making a differential diagnosis. For example, was the patient exposed to poison ivy, insects, or a person ill with a specific disease? In infectious illnesses with high clinical expression rates (e.g., measles, chickenpox, and rubella), proper questioning usually reveals a contact case or at least other cases in the community. On the other hand, in illnesses with low rates of clinical expression of exanthem, such as adenoviral and some enteroviral infections, the source may not be apparent.

Consideration of the seasonal occurrence of different infectious agents, as well as insects, is particularly useful in making a differential diagnosis. In temperate climates, enteroviral and arthropod-mediated diseases occur in the summer and fall. Exanthems with measles, varicella-zoster, and rubella viruses occur most often in the winter and spring. The diagnosis of rubella is important because of fetal consequences. All too frequently, rubella is overdiagnosed and underdiagnosed, both of which can be avoided if its seasonal prevalence is understood.

The incubation period is important in separating the exanthem caused by rubella, varicella-zoster, or measles viruses from rash illnesses caused by enteroviruses or common respiratory viruses. The former have long incubation periods, whereas in the others, the period from exposure to the onset of illness is less than 1 week. Age can be useful. Today in the United States, measles and rubella often are illnesses of adolescents and young adults. Enteroviral exanthem frequency is related inversely to age.

Questioning to obtain a pertinent history of previous exanthems can give useful information if it is done with care. For example, if patients are asked whether they had rubella, the answer is quite unreliable. However, if the past illness is documented by year, season, and symptoms, accurate information often is obtained. The relationship of rash to fever is most significant in the diagnosis of roseola. The presence or absence of fever is important in separating exanthems of infectious and noninfectious etiology. Frequently, insect bites are diagnosed as chickenpox by parents and physicians as well. Chickenpox rarely occurs without fever.

The type and distribution of exanthem obviously are important. They virtually are diagnostic in hand, foot, and mouth syndrome, Rocky Mountain spotted fever, and atypical measles. Enanthem can lead to a specific diagnosis (Koplik spots in measles) or a category diagnosis (e.g., herpangina in enteroviral infections). Other characteristics, such as those listed in Tables 58-8 through 58-17, obviously are useful in delineating a specific illness.

Specific Diagnosis

As with other infectious diseases, establishing specific diagnosis depends on the acquisition of proper cultures, serologic tests, and microscopic study of secretions or histologic or cytologic preparations. These techniques are discussed in other chapters of this book.

Vesicular lesions always should be scraped for cytologic study or direct antigen identification (e.g., varicella and herpes simplex), and, frequently, petechial lesions should be scraped and stained in a search for infectious agents (e.g., meningococci). The etiology of viral infections can be established by isolation of virus, direct antigen detection, or serologic methods. In most instances, a virus recovered from the throat indicates acute infection and is the probable cause of a particular illness. Serologic study without culture is useful in diagnosing rickettsial diseases, some viral infections, and a few illnesses of bacterial origin. Serologic study without virus isolation generally is not useful in diagnosing enteroviral illnesses.

TREATMENT, PROGNOSIS, AND PREVENTION

The treatment, prognosis, and prevention of exanthematous diseases are presented in appropriate chapters throughout this text.

NEW REFERENCES SINCE THE SIXTH EDITION

58. Colomba C, Saporito L, Polara VF, et al. Mediterranean spotted fever: clinical and laboratory characteristics of 415 Sicilian children. *BMC Infect Dis* 2006;**6**:60.
68. Drago F, Broccolo F, Rebora A. Pityriasis rosea: an update with a critical appraisal of its possible herpesviral etiology. *J Am Acad Dermatol* 2009;**61**:303–18.
71. Duarte AF, Cruz MJ, Baudrier T, et al. Unilateral laterothoracic exanthem and primary Epstein-Barr virus infection: case report. *Pediatr Infect Dis J* 2009;**28**:549–50.
72. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009; **360**:2536–43.
75. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**: 1337–42.
83. Fretzayas A, Douros K, Moustaki M, et al. Papular-purpuric gloves and socks syndrome in children and adolescents. *Pediatr Infect Dis J* 2009;**28**:250–2.
84. Fujimoto T, Iizuka S, Enomoto M, et al. Hand, foot, and mouth disease caused by coxsackievirus A6, Japan, 2011. *Emerg Infect Dis* 2012;**18**:337–9.
106. Ito M, Yamashita T, Tsuzuki H, et al. Detection of human parechoviruses from clinical stool samples in Aichi, Japan. *J Clin Microbiol* 2010;**48**:2683–8.
117. Kocianova E, Kost'anova Z, Stefanidesova K, et al. Serologic evidence of *Anaplasma phagocytophilum* infections in patients with a history of tick bite in central Slovakia. *Wien Klin Wochenschr* 2008;**120**:427–31.
121. Kroeskop A, Lewis AB, Barril FA, et al. Gianotti-Crosti syndrome after H1N1-influenza vaccine. *Pediatr Dermatol* 2011;**28**: 595–6.
129. Lee YS, Quek SC, Koay ES, et al. Chikungunya mimicking atypical Kawasaki disease in an infant. *Pediatr Infect Dis J* 2010;**29**: 275–7.
142. Meyer PM, Streuli JC, Schlumbom V, et al. A girl with a purpuric rash (case presentation). *Acta Paediatr* 2010;**99**:327–8, 475–6.
147. Morens DM, Katz AR. The "fourth disease" of childhood: reevaluation of a nonexistent disease. *Am J Epidemiol* 1991;**134**: 628–40.
155. Osterback R, Vuorinen T, Linna M, et al. Coxsackievirus A6 and hand, foot, and mouth disease, Finland. *Emerg Infect Dis* 2009;**15**: 1485–8.
156. Özdemir PG, Yazicioğlu M, Aylanç H, et al. EBV-induced erythema multiforme: a case report. *Allergol Immunopathol (Madr)* 2011;**39**:116–18.
174. Rebora A, Drago F, Broccolo F. Pityriasis rosea and herpesviruses: facts and controversies. *Clin Dermatol* 2010;**28**:497–501.

178. Rizzo C, Esposito S, Azzari C, et al. West Nile Virus infections in children: a disease pediatricians should think about. *Pediatr Infect Dis J* 2011;**30**:65–6.
188. Scagni P, Morello M, Ramus MV, et al. Drug-induced hypersensivity syndrome associated with Epstein-Barr virus infection: a pediatric case report. *Pediatr Dermatol* 2009;**26**:229–31.
210. Talbot HK, Shepherd BE, Crowe JE, Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J* 2009;**28**:682–7.
223. Wei SH, Huang YP, Liu MC, et al. An outbreak of coxsackievirus A6 hand, foot, and mouth disease associated with onychomadesis in Taiwan, 2010. *BMC Infect Dis* 2011;**11**:346.
236. Yee LC, Ng LC, Koh TH. Dermatological features of an imported case of chikungunya in an infant. *Singapore Med J* 2008;**49**:959–60.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 59

Roseola Infantum (Exanthem Subitum)

James D. Cherry

Roseola infantum (i.e., exanthem subitum, pseudorubella, exanthem criticum, sixth disease, or 3-day fever) is a common, acute illness of young children characterized by a fever of 3 to 5 days' duration, rapid defervescence, and then the appearance of an erythematous macular or maculopapular rash that persists for 1 to 2 days.

HISTORY

Zahorsky[81] generally is given credit for the original description of roseola infantum. However, in his writings, he pointed out that the syndrome was described in earlier pediatric and dermatology texts.[82-84] Altschuler[1] observed that a British dermatologist, Willan, presented a description of the illness in his 1809 book *On Cutaneous Diseases*. The descriptions in the older literature did not separate the syndrome from the known exanthematous diseases (i.e., measles, rubella, and scarlet fever), an omission that Zahorsky corrected.

In 1921, Veeder and Hempelmann[75] described the syndrome further and noted that leukopenia and relative lymphocytosis occurred. These investigators objected to the name *roseola infantum*, which in the past had been used to describe a large group of diseases with indefinite causes. They suggested the term *exanthem subitum* because it was "descriptive of the most striking clinical symptom, namely, the sudden, unexpected appearance of the eruption on the fourth day." Currently, the term *roseola* is used most commonly to describe the syndrome.

From 1920 through 1940, many excellent clinical descriptions of the syndrome were published.* From 1940 through 1988, articles relating to roseola were concerned with unusual manifestations and complications† and attempts to recover an etiologic agent.[26,30,40,45,64] In 1951 and 1954, Neva and associates[50-52] noted the association of a roseola-like illness and infection with echovirus 16. This association was noted again in 1974.[27] In 1988, Yamanishi and associates[79] identified human herpesvirus–6 (HHV-6) in the blood of infants with roseola, and since then the association between this virus and the disease has been confirmed on many occasions.‡ HHV-7 also has been found to be the cause of many cases of roseola.§

EPIDEMIOLOGY

In his original article, Zahorsky[81] reported that roseola occurred most commonly in the fall. In his second article, he observed a year-round incidence[82]; in 1925, he pointed out that most cases occurred in the spring, summer, and fall.[83] Breese[10] noted that the greatest number of cases occurred in the summer and early fall. In contrast, 55

*References 6, 7, 10, 17, 18, 20, 24, 25, 36, 83, 85.
†References 8, 11-13, 22, 32, 46, 48, 49, 53, 56, 63.
‡References 2-5, 9, 19, 21, 27, 28, 33-35, 39, 41-44, 54, 58, 60, 66-69, 77, 86.
§References 3, 14, 31, 68, 71, 72, 74.

REFERENCES

1. Africk JA, Halprin KM. Infectious mononucleosis presenting as urticaria. *JAMA* 1969;**209**:1524–5.
2. Agnello V, Abel G. Localization of hepatitis C virus in cutaneous vasculitic lesions in patients with type II cryoglobulinemia. *Arthr Rheum* 1997;**40**:2007–15.
3. Aguilar-Bernier M, Bassas-Vila J, Torne-Gutierrez JI, et al. Presence of perineuritis in a case of papular purpuric gloves and socks syndrome associated with mononeuritis multiplex attributable to B19 parvovirus. *J Am Acad Dermatol* 2006;**54**:896–9.
4. Ahlm C, Settergren B, Gothefors L, et al. Nephropathia epidemica (hemorrhagic fever with renal syndrome) in children: clinical characteristics. *Pediatr Infect Dis J* 1994;**13**:45–9.
5. Ahvonen P. Human yersiniosis in Finland. II. Clinical features. *Ann Clin Res* 1972;**4**:39–48.
6. Alfadley A, Aljubran A, Hainau B, et al. Papular-purpuric "gloves and socks" syndrome in a mother and daughter. *J Am Acad Dermatol* 2003;**48**:941–4.
7. Anderson MJ, Lewis E, Kidd IM, et al. An outbreak of erythema infectiosum associated with human parvovirus infection. *J Hyg* 1984;**93**:85–93.
8. Anonymous. Needlestick transmission of HTLV-III from a patient infected in Africa. *Lancet* 1984;**2**:1376–7.
9. Arkwright JA. Foot-and-mouth disease in man. *Lancet* 1928;**1**: 1191–2.
10. Arnold JC, Singh KK, Spector SA, et al. Human bocavirus: prevalence and clinical spectrum at a children's hospital. *Clin Infect Diseases* 2006;**43**:283–8.
11. Asano Y, Suga S, Yoshikawa T, et al. Clinical features and viral excretion in an infant with primary human herpesvirus 7 infection. *Pediatrics* 1995;**95**:187–90.
12. Asano Y, Yoshikawa T, Suga S, et al. Viremia and neutralizing antibody response in infants with exanthem subitum. *J Pediatr* 1989;**114**:535–9.
13. Athreya BH, Coriell LL. Erythema multiforme exudativum major: a review and report of four cases. *Clin Pediatr* 1964;**3**:68–74.
14. Azimi PH, Chase PA, Petru AM. Mycoplasmas: their role in pediatric disease. *Curr Prob Pediatr* 1984;**14**:7–46.
15. Baer RL. Perspective: erythema multiforme—1976. *Am J Med Sci* 1976;**271**:119–20.
16. Baley JE, Kliegman RM, Fanaroff AA. Disseminated fungal infections in very low-birth-weight infants: clinical manifestations and epidemiology. *Pediatrics* 1984;**73**:144–52.
17. Balfour HH, Jr, Forte FA, Simpson RB, et al. Penicillin-related exanthems in infectious mononucleosis identical to those associated with ampicillin. *Clin Pediatr* 1972;**11**:417–21.
18. Baron J, Shapiro ED. Unsuspected bacteremia caused by *Branhamella catarrhalis*. *Pediatr Infect Dis* 1985;**4**:100–1.
19. Barrett PK, Greenberg MJ. Outbreak of ornithosis. *Br Med J* 1966;**2**:206–7.
20. Baumgartner JD, Glauser MP, Burgo-Black AL, et al. Severe cytomegalovirus infection in multiply transfused, splenectomized, trauma patients. *Lancet* 1982;**2**:63–6.
21. Beall GN. Urticaria: a review of laboratory and clinical observations. *Medicine* 1964;**43**:131–51.
22. Benoit FL. Chronic meningococcemia: case report and review of the literature. *Am J Med* 1963;**35**:103–12.
23. Berant M, Naveh Y, Weissman I. Papular acrodermatitis with cytomegalovirus hepatitis. *Arch Dis Child* 1983;**58**:1024–5.
24. Berenguer J, Rodriguez-Tudela JL, Richard C, et al. Deep infections caused by *Scedosporium prolificans:* a report on 16 cases in Spain and a review of the literature. *Scedosporium Prolificans* Spanish Study Group. *Medicine* 1997;**76**:256–65.
25. Berger BW. Erythema chronicum migrans of Lyme disease. *Arch Dermatol* 1984;**120**:1017–21.
26. Black FL. Measles. In: Evans AS, editor. *Viral infections of humans: epidemiology and control*. New York: Plenum; 1976. p. 297–316.
27. Blatt J, Kastner O, Hodes DS. Cutaneous vesicles in congenital cytomegalovirus infection. *J Pediatr* 1978;**92**:509.
28. Bodey GP, Fainstein V. Systemic candidiasis. In: Bodey GP, Fainstein V, editors. *Candidiasis*. New York: Raven Press; 1985. p. 135–68.
29. Boughton CR. Glandular fever. A study of a hospital series in Sydney. *Med J Aust* 1970;**2**:529–35.
30. Bowmer EJ, McKiel JA, Cockcroft WH, et al. *Listeria monocytogenes* infections in Canada. *Can Med Assoc J* 1973;**109**:125–9 passim.
31. Bowmer MI, Leggat I, Barrowman JA. Disseminated gonococcal infection. *Can Med Assoc J* 1982;**126**:1188–90.
32. Bradford WD, Hawkins HK. Rocky Mountain spotted fever in childhood. *Am J Dis Child* 1977;**131**:1228–32.
33. Breese BB. Pharyngitis and scarlet fever. In: Breese BB, Hall CB, editors. *Beta hemolytic streptococcal diseases*. Boston: Houghton Mifflin; 1978. p. 65–78.
34. Breese BB. Streptococcal pharyngitis and scarlet fever. *Am J Dis Child* 1978;**132**:612–6.
35. Breese BB. Streptococcal skin infections. In: Breese BB, Hall CB, editors. *Beta hemolytic streptococcal diseases*: Boston: Houghton Mifflin; 1978. p. 176–211.
36. Brettman LR, Lewin S, Holzman RS, et al. Rickettsialpox: report of an outbreak and a contemporary review. *Medicine* 1981;**60**: 363–72.
37. Brody I. Topical treatment of recurrent herpes simplex and postherpetic erythema multiforme with low concentrations of zinc sulphate solution. *Br J Dermatol* 1981;**104**:191–4.
38. Browne SG. Mycobacterial diseases: leprosy. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1492–505.
39. Bruhn FW. Lyme disease. *Am J Dis Child* 1984;**138**:467–70.
40. Bruno P, Hassell LH, Brown J, et al. The protean manifestations of hemorrhagic fever with renal syndrome: a retrospective review of 26 cases from Korea. *Ann Intern Med* 1990;**113**:385–91.
41. Buckley B. Q fever epidemic in Victorian general practice. *Med J Aust* 1980;**1**:593–5.
42. Burnett JW. Uncommon bacterial infections of the skin. *Arch Dermatol* 1962;**86**:597–607.
43. Bwaka MA, Bonnet MJ, Calain P, et al. Ebola hemorrhagic fever in Kitwit, Democratic Republic of the Congo: clinical observations of 103 patients. *J Infect Dis* 1999;**179**:1–7.
44. Caputo RV, Solomon LM. Vascular reactive diseases. In: Solomon LM, Esterly NB, Loeffel ED, editors. *Adolescent dermatology*. Philadelphia: W. B. Saunders; 1978. p. 404–32.
45. Carlstrom G, Alden J, Belfrage S, et al. Acquired cytomegalovirus infection. *Brit Med J* 1968;**2**:521–5.
46. Carslaw RW. Skin infestation. *Practitioner* 1976;**216**:154–8.
47. Castellano A, Schweitzer R, Tong MJ, et al. Papular acrodermatitis of childhood and hepatitis B infection. *Arch Dermatol* 1978; **114**:1530–2.
48. Centers for Disease Control and Prevention. Moth-associated dermatitis: Cozumel, Mexico. *MMWR Morbid Mortal Wkly Rep* 1990;**39**:219–20.
49. Chanarin I, Walford DM. Thrombocytopenic purpura in cytomegalovirus mononucleosis. *Lancet* 1973;**2**:238–9.
50. Cherry JD. Newer viral exanthems. *Advances in pediatrics* 1969; **16**:233–86.
51. Cherry JD. Parvovirus infections in children and adults. *Adv Pediatr* 1999;**46**:245–69.
52. Cherry JD, Jahn CL. Exanthem and enanthem associated with mumps virus infection. *Arch Environ Health* 1966;**12**:518–21.
53. Cherry JD, Welliver RC. *Mycoplasma pneumoniae* infections of adults and children. *West J Med* 1976;**125**:47–55.
54. Cherry JD, Hurwitz ES, Welliver RC. *Mycoplasma pneumoniae* infections and exanthems. *J Pediatr* 1975;**87**:369–73.
55. Chesney PJ, Davis JP, Purdy WK, et al. Clinical manifestations of toxic shock syndrome. *JAMA* 1981;**246**:741–8.
56. Chonmaitree T, Menegus MA, Powell KR. The clinical relevance of 'CSF viral culture': a two-year experience with aseptic meningitis in Rochester, NY. *JAMA* 1982;**247**:1843–7.
57. Clark RF. Localized urticaria due to enterobius vermicularis. *Arch Dermatol* 1961;**84**:1026.
58. Colomba C, Saporito L, Polara VF, et al. Mediterranean spotted fever: clinical and laboratory characteristics of 415 Sicilian children. *BMC Infect Dis* 2006;**6**:60.
59. Cowdrey SC, Reynolds JS. Acute urticaria in infectious mononucleosis. *Ann Allergy* 1969;**27**:182–7.
60. Darragh TM, Egbert BM, Berger TG, et al. Identification of herpes simplex virus DNA in lesions of erythema multiforme by the polymerase chain reaction. *J Am Acad Dermatol* 1991;**24**: 23–6.

61. Daye S, McHenry JA, Roscelli JD. Pruritic rash associated with cat scratch disease. *Pediatrics* 1988;**81**:559–61.
62. Delage G, McLaughlin B, Berthiaume L. A clinical study of rotavirus gastroenteritis. *J Pediatr* 1978;**93**:455–7.
63. Derbes VJ. Arthropod bites and stings. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1656–68.
64. Di Lernia V, Ricci C. Skin manifestations with rotavirus infections. *Int J Dermatol* 2006;**45**:759–61.
65. Dienstag JL, Rhodes AR, Bhan AK, et al. Urticaria associated with acute viral hepatitis type B: studies of pathogenesis. *Ann Intern Med* 1978;**89**:34–40.
66. Dooley JR. Haemotropic bacteria in man. *Lancet* 1980;**2**:1237–9.
67. Draelos ZK, Hansen RC, James WD. Gianotti-Crosti syndrome associated with infections other than hepatitis B. *JAMA* 1986; **256**:2386–8.
68. Drago F, Broccolo F, Rebora A. Pityriasis rosea: an update with a critical appraisal of its possible herpesviral etiology. *J Am Acad Dermatol* 2009;**61**:303–18.
69. Drago F, Ranieri E, Malaguti F, et al. Human herpesvirus 7 in pityriasis rosea. *Lancet* 1997;**349**:1367–8.
70. Dryer RF, Goellner PG, Carney AS. Lyme arthritis in Wisconsin. *JAMA* 1979;**241**:498–9.
71. Duarte AF, Cruz MJ, Baudrier T, et al. Unilateral laterothoracic exanthem and primary Epstein-Barr virus infection: case report. *Pediatr Infect Dis J* 2009;**28**:549–50.
72. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. *N Engl J Med* 2009;**360**:2536–43.
73. Dukes C. On the confusion of two different diseases under the name of rubella (rose-rash). *Lancet* 1900;**2**:89–94.
74. Duncan WC. Cutaneous manifestations of infectious diseases. In: Hoeprich PD, editor. *Infectious diseases: a modern treatise of infectious processes*. Hagerstown, Md: Harper & Row; 1977. p. 68–74.
75. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**: 1337–42.
76. Farah FS. Protozoan and helminth infections. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1635–56.
77. Fass RJ, Saslaw S. Earth day histoplasmosis: a new type of urban pollution. *Arch Intern Med* 1971;**128**:588–90.
78. Fell HWK, Nagington J, Naylor GRE, et al. *Corynebacterium haemolyticum* infections in Cambridgeshire. *J Hyg (Lond)* 1977;**76**:269–75.
79. Fenner F. The clinical features and pathogenesis of mouse-pox (infectious ectromelia of mice). *J Pathol Bacteriol* 1948;**60**: 529–52.
80. Fiumara NJ, Solomon J. Recurrent herpes simplex virus infections and erythema multiforme: a report of three patients. *Sex Transm Dis* 1983;**10**:144–7.
81. Flaum A. Foot-and-mouth disease in man. *Acta Pathol Microbiol Scand* 1939;**16**:197–213.
82. Formenty P, Hatz C, Le Guenno B, et al. Human infection due to Ebola virus, subtype Cote d'Ivoire: clinical biologic presentation. *J Infect Dis* 1999;**179**:48–53.
83. Fretzayas A, Douros K, Moustaki M, et al. Papular-purpuric gloves and socks syndrome in children and adolescents. *Pediatr Infect Dis J* 2009;**28**:250–2.
84. Fujimoto T, Iizuka S, Enomoto M, et al. Hand, foot, and mouth disease caused by coxsackievirus A6, Japan, 2011. *Emerg Infect Dis* 2012;**18**:337–9.
85. Fukumi H, Nishikawa F, Kokubu Y, et al. Isolation of adenovirus from an exanthematous infection resembling roseola infantum. *Jpn J Med Sci Biol* 1957;**10**:87–91.
86. Game MA, Owen WC, Mitchell DK. Cutaneous manifestations of disseminated fungal infection in an immunocompromised child. *J Pediatr* 1998;**133**:466.
87. Gardner SD. The isolation of parainfluenza 4 subtypes A and B in England and serological studies of their prevalence. *J Hyg* 1969;**67**:545–50.
88. Goodwin RA, Loyd JE, Des Prez RM. Histoplasmosis in normal hosts. *Medicine* 1981;**60**:231–66.
89. Gordon PM, Hepburn NC, Williams AE, et al. Cutaneous myiasis due to *Dermatobia hominis:* a report of six cases. *Br J Dermatol* 1995;**132**:811–4.
90. Griffith GL, Luce EA. Massive skin necrosis in Rocky Mountain spotted fever. *South Med J* 1978;**71**:1337–40.
91. Grilli R, Izquierdo MJ, Farina MC, et al. Papular-purpuric "gloves and socks" syndrome: polymerase chain reaction demonstration of parvovirus B19 DNA in cutaneous lesions and sera. *J Am Acad Dermatol* 1999;**41**:793–6.
92. Gustafson TL, Band JD, Hutcheson RH, Jr, et al. *Pseudomonas* folliculitis: an outbreak and review. *Rev Infect Dis* 1983;**5**:1–8.
93. Halliday HL, Hirata T. Perinatal listeriosis: a review of twelve patients. *Am J Obstet Gynecol* 1979;**133**:405–10.
94. Hamrick HJ, Moore GW. Giardiasis causing urticaria in a child. *Am J Dis Child* 1983;**137**:761–3.
95. Hannuksela M, Ahvonen P. Skin manifestations in human yersiniosis. *Ann Clin Res* 1975;**7**:368–73.
96. Harel L, Straussberg I, Zeharia A, et al. Papular purpuric rash due to parvovirus B19 with distribution on the distal extremities and the face. *Clin Infect Dis* 2002;**35**:1558–61.
97. Harrison DL. A case of human ornithosis presenting as an obscure febrile illness associated with macular rash. *Practitioner* 1963;**190**: 245–6.
98. Herrmann EC, Jr, Hable KA. Experiences in laboratory diagnosis of parainfluenza viruses in routine medical practice. *Mayo Clin Proc* 1970;**45**:177–88.
99. Hilleman MR, Hamparian VV, Ketler A, et al. Acute respiratory illnesses among children and adults: field study of contemporary importance of several viruses and appraisal of the literature. *JAMA* 1962;**180**:445–53.
100. Hoagland RJ. The clinical manifestations of infectious mononucleosis: a report of two hundred cases. *Am J Med Sci* 1960;**240**: 55–63.
101. Honig PJ. Bites and parasites. *Pediatr Clin North Am* 1983;**30**: 563–81.
102. Hoofnagle JH. Hepatitis C: the clinical spectrum of disease. *Hepatology* 1997;**26**:15–20.
103. Hope-Simpson RE, Higgins PG. A respiratory virus study in Great Britain: review and evaluation. *Prog Med Virol [Fortschritte der medizinischen Virusforschung Prog Virol Med]* 1969;**11**:354–407.
104. Horton JM, Blaser MJ. The spectrum of relapsing fever in the Rocky Mountains. *Arch Intern Med* 1985;**145**:871–5.
105. Humphry T, Sanders S, Stadius M. Leptospirosis mimicking MLNS. *J Pediatr* 1977;**91**:853–4.
106. Ito M, Yamashita T, Tsuzuki H, et al. Detection of human parechoviruses from clinical stool samples in Aichi, Japan. *J Clin Microbiol* 2010;**48**:2683–8.
107. Jacobs RF, Hsi S, Wilson CB, et al. Apparent meningococcemia: clinical features of disease due to *Haemophilus influenzae* and *Neisseria meningitidis*. *Pediatrics* 1983;**72**:469–72.
108. Jaffe HW, Pellett PE. Human herpesvirus 8 and Kaposi's sarcoma: some answers, more questions. *N Engl J Med* 1999; **340**:1912–3.
109. Johnson RA. Atypical mycobacteria. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1505–8.
110. Joncas J, Chiasson JP, Turcotte J, et al. Studies on infectious mononucleosis. 3. Clinical data, serologic and epidemiologic findings. *Can Med Assoc J* 1968;**98**:848–54.
111. Kalis P, Le Frock JL, Smith W, et al. Listeriosis. *Am J Med Sci* 1976;**271**:159–69.
112. Kanazawa K, Yaoita H, Tsuda F, et al. Hepatitis C virus infection in patients with urticaria. *J Am Acad Dermatol* 1996;**35**:195–8.
113. Kazmierowski JA, Peizner DS, Wuepper KD. Herpes simplex antigen in immune complexes of patients with erythema multiforme: presence following recurrent herpes simplex infection. *JAMA* 1982;**247**:2547–50.
114. Kennedy AC, Fleming J, Solomon L. Chikungunya viral arthropathy: a clinical description. *J Rheumatol* 1980;**7**:231–6.
115. Kennett ML, Birch CJ, Lewis FA, et al. Enterovirus type 71 infection in Melbourne *Bull WHO* 1974;**51**:609–15.
116. Kiernan JP, Schanzlin DJ, Leveille AS. Stevens-Johnson syndrome associated with adenovirus conjunctivitis. *Am J Ophthalmol* 1981;**92**:543–5.
117. Kocianova E, Kost'anova Z, Stefanidesova K, et al. Serologic evidence of *Anaplasma phagocytophilum* infections in patients with a history of tick bite in central Slovakia. *Wien Klin Wochenschr* 2008;**120**:427–31.

118. Konno M, Kikuta H, Ishikawa N, et al. A possible association between hepatitis-B antigen-negative infantile papular acrodermatitis and Epstein-Barr virus infection. *J Pediatr* 1982;**101**: 222–4.
119. Krause VW, Embree JE, MacDonald SW, et al. Congenital listeriosis causing early neonatal death. *Can Med Assoc J* 1982;**127**: 36–8.
120. Krober MS, Bass JW, Barcia PJ. Scarlatiniform rash and pleural effusion in a patient with *Yersinia pseudotuberculosis* infection. *J Pediatr* 1983;**102**:879–81.
121. Kroeskop A, Lewis AB, Barril FA, et al. Gianotti-Crosti syndrome after H1N1-influenza vaccine. *Pediatr Dermatol* 2011;**28**:595–6.
122. Kusenbach G, Rubben A, Schneider EM, et al. Herpes virus (KSHV) associated Kaposi sarcoma in a 3-year-old child with non-HIV-induced immunodeficiency. *Eur J Pediatr* 1997;**156**: 440–3.
123. La Grenade L, Manns A, Fletcher V, et al. Clinical, pathologic, and immunologic features of human T-lymphotrophic virus type I-associated infective dermatitis in children. *Arch Dermatol* 1998; **134**:439–44.
124. Lackman DB. A review of information on ricketsialpox in the United States. *Clin Pediatr* 1963;**2**:296–301.
125. LaGrenade L, Hanchard B, Fletcher V, et al. Infective dermatitis of Jamaican children: a marker for HTLV-I infection. *Lancet* 1990;**336**:1345–7.
126. Lambie JA, Gustafson AA. Contagious ecthyma in man: report of two cases with laboratory studies. *Lancet* 1967;**87**:400–2.
127. Le CT. Tick-borne relapsing fever in children. *Pediatrics* 1980; **66**:963–6.
128. Leavell UW, Jr, Macnamara MJ, Meulling RJ. Ecthyma contagiosum (Orf). *South Med J* 1965;**58**:239–43.
129. Lee YS, Quek SC, Koay ES, et al. Chikungunya mimicking atypical Kawasaki disease in an infant. *Pediatr Infect Dis J* 2010;**29**: 275–7.
130. Lerner AM, Cherry JD, Klein JO, et al. Infections with reoviruses. *New Engl J Med* 1962;**267**:947–52.
131. Linnemann CC, Jr, Janson PJ. The clinical presentations of Rocky Mountain spotted fever: comments on recognition and management based on a study of 63 patients. *Clin Pediatr* 1978;**17**: 673–9.
132. Litt IF, Edberg SC, Finberg L. Gonorrhea in children and adolescents: a current review. *J Pediatr* 1974;**85**:595–607.
133. Liu C, Coffin DL. Studies of canine distemper infection by means of fluorescein-labeled antibody. I. The pathogenesis, pathology, and diagnosis of the disease in experimentally infected ferrets. *Virology* 1957;**3**:115–31.
134. Maki M, Vesikari T, Rantala I, et al. Yersiniosis in children. *Arch Dis Child* 1980;**55**:861–5.
135. Maloney EM, Hisada M, Palmer P, et al. Human T cell lymphotropic virus type I-associated infective dermatitis in Jamaica: a case report of clinical and biologic correlates. *Pediatr Infect Dis J* 2000;**19**:560–5.
136. Martinez-Roig A, Llorens-Terol J, Torres JM. Erythema nodosum and kerion of the scalp. *Am J Dis Child* 1982;**136**:440–2.
137. Mast WE, Burrows WM, Jr. Erythema chronicum migrans in the United States. *JAMA* 1976;**236**:859–60.
138. McDade JE. Ehrlichiosis: a disease of animals and humans. *J Infect Dis* 1990;**161**:609–17.
139. McNeill WH. *Plagues and peoples*. Garden City, NY: Anchor Press/ Doubleday; 1976. p. 105, 119.
140. Medeiros AA, Marty SD, Tosh FE, et al. Erythema nodosum and erythema multiforme as clinical manifestations of histoplasmosis in a community outbreak. *N Engl J Med* 1966;**274**:415–20.
141. Melish ME, Glasgow LA. Staphylococcal scalded skin syndrome: the expanded clinical syndrome. *J Pediatr* 1971;**78**:958–67.
142. Meyer PM, Streuli JC, Schlumbom V, et al. A girl with a purpuric rash (case presentation). *Acta Paediatr* 2010;**99**:327–8, 475–6.
143. Milder JE, Walzer PD, Kilgore G, et al. Clinical features of *Strongyloides stercoralis* infection in an endemic area of the United States. *Gastroenterology* 1981;**80**:1481–8.
144. Mims CA. Pathogenesis of rashes in virus diseases. *Bacteriol Rev* 1966;**30**:739–60.
145. Moore PS. The emergence of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8). *N Engl J Med* 2000;**343**:1411–13.
146. Moraga FA, Martinez-Roig A, Alonso JL, et al. Boutonneuse fever. *Arch Dis Child* 1982;**57**:149–51.
147. Morens DM, Katz AR. The "fourth disease" of childhood: reevaluation of a nonexistent disease. *Am J Epidemiol* 1991;**134**:628–40.
148. Myskowski PL, Ahkami R. Dermatologic complications of HIV infection. *Med Clin North Am* 1996;**80**:1415–35.
149. Nash D, Mostashari F, Fine A, et al. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 2001;**344**:1807–14.
150. Neisson-Vernant C, Arfi S, Mathez D, et al. Needlestick HIV seroconversion in a nurse. *Lancet* 1986;**2**:814.
151. Niklasson B, Espmark A, LeDuc JW, et al. Association of a Sindbis-like virus with Ockelbo disease in Sweden. *Am J Tropical Med Hyg* 1984;**33**:1212–7.
152. Nussbaum M, Scalettar H, Shenker IR. Gonococcal arthritis-dermatitis (GADS) as a complication of gonococcemia in adolescents. *Clin Pediatr* 1975;**14**:1037–40.
153. Older JJ. The epidemiology of murine typhus in Texas, 1969. *JAMA* 1970;**214**:2011–17.
154. Orton PW, Huff JC, Tonnesen MG, et al. Detection of a herpes simplex viral antigen in skin lesions of erythema multiforme. *Ann Intern Med* 1984;**101**:48–50.
155. Osterback R, Vuorinen T, Linna M, et al. Coxsackievirus A6 and hand, foot, and mouth disease, Finland. *Emerg Infect Dis* 2009; **15**:1485–8.
156. Özdemir PG, Yazicioğlu M, Aylanç H, et al. EBV-induced erythema multiforme: a case report. *Allergol Immunopathol (Madr)* 2011;**39**:116–18.
157. Patamasucon P, Schaad UB, Nelson JD. Melioidosis. *J Pediatr* 1982;**100**:175–82.
158. Patel BM. Skin rash with infectious mononucleosis and ampicillin. *Pediatrics* 1967;**40**:910–1.
159. Paterson PY, Taylor W. Rickettsialpox. *Bull NY Acad Med* 1966;**42**:579–87.
160. Perlman F. Arthropod sensitivity. In: Criep LH, editor. *Dermatologic allergy*. Philadelphia: W. B. Saunders; 1967. p. 222–44.
161. Peter G. Leptospirosis: a zoonosis of protean manifestations. *Pediatr Infect Dis* 1982;**1**:282–8.
162. Pether JV, Lloyd G. The clinical spectrum of human hantavirus infection in Somerset, UK. *Epidemiol Infect* 1993;**111**:171–5.
163. Pfister LE, Gallagher MV, Potterfield TG, et al. *Neisseria catarrhalis* bacteremia with meningitis. *JAMA* 1965;**193**:399–401.
164. Pittsley RA, Shearn MA, Kaufman L. Acute hepatitis B simulating dermatomyositis. *JAMA* 1978;**239**:959.
165. Place EH, Sutton LE. Erythema arthriticum epidemicum (Haverhill fever). *Arch Intern Med* 1934;**54**:659–84.
166. Platt H. The susceptibility of the skin and epidermoid mucous membranes to virus infection in man and other animals. *J Pathol Bacteriol* 1948;**76**:479–97.
167. Pollowitz JA. Acute urticaria associated with shigellosis: a case report. *Ann Allergy* 1980;**45**:302–3.
168. Portnoy BL, Satterwhite TK, Dyckman JD. Rat bite fever misdiagnosed as Rocky Mountain spotted fever. *South Med J* 1979; **72**:607–9.
169. Powell KR. Filatow-Dukes' disease. Epidermolytic toxin-producing staphylococci as the etiologic agent of the fourth childhood exanthem. *Am J Dis Child* 1979;**133**:88–91.
170. Principi N, Bosis S, Esposito S. Human metapneumovirus in paediatric patients. *Clin Microbiol Infect* 2006;**12**:301–8.
171. Pullen H, Wright N, Murdoch JM. Hypersensitivity reactions to antibacterial drugs in infectious mononucleosis. *Lancet* 1967;**2**: 1176–8.
172. Raoult D, Fournier PE, Fenollar F, et al. Rickettsia africae, a tick-borne pathogen in travelers to sub-Saharan Africa. *New Engl J Med* 2001;**344**:1504–10.
173. Rasmussen JE, Graves WH, 3rd. *Pseudomonas aeruginosa*, hot tubs, and skin infections. *Am J Dis Child* 1982;**136**:553–4.
174. Rebora A, Drago F, Broccolo F. Pityriasis rosea and herpesviruses: facts and controversies. *Clin Dermatol* 2010;**28**:497–501.
175. Reply ES. First, second, third, fourth and fifth diseases. *Am J Dis Child* 1964;**108**:440–1.
176. Resnick SD. Toxic shock syndrome: recent developments in pathogenesis. *J Pediatr* 1990;**116**:321–8.
177. Richter HS. Coccidioidomycosis: a report of 300 new cases. *GP* 1969;**39**:89–93.
178. Rizzo C, Esposito S, Azzari C, et al. West Nile Virus infections in children: a disease pediatricians should think about. *Pediatr Infect Dis J* 2011;**30**:65–6.

179. Robbins FC. Measles: clinical features: pathogenesis, pathology and complications. *Am J Dis Child* 1962;**103**:266–73.
180. Rook A. Papular urticaria. *Pediatr Clin North Am* 1961;**8**:817–33.
181. Rustgi VK, Sacher RA, O'Brien P, et al. Fatal disseminated cytomegalovirus infection in an apparently normal adult. *Arch Intern Med* 1983;**143**:372–3.
182. Ruzicka T, Rosendahl C, Braun-Falco O. A probable case of rotavirus exanthem. *Arch Dermatol* 1985;**121**:253–4.
183. Saari TN, Triplett DA. *Yersinia pseudotuberculosis* mesenteric adenitis. *J Pediatr* 1974;**85**:656–9.
184. Sabin AB. Research on dengue during World War II. *Am J Trop Med Hyg* 1952;**1**:30–50.
185. San Joaquin VH, Marks MI. Gianotti disease or Gianotti-Crosti syndrome? *J Pediatr* 1982;**101**:216–17.
186. Sanchez RL, Hebert A, Lucia H, et al. Orf: a case report with histologic, electron microscopic, and immunoperoxidase studies. *Arch Pathol Lab Med* 1985;**109**:166–70.
187. Saslaw S. Chronic meningococcemia: report of a case. *N Engl J Med* 1962;**266**:605–7.
188. Scagni P, Morello M, Ramus MV, et al. Drug-induced hypersensivity syndrome associated with Epstein-Barr virus infection: a pediatric case report. *Pediatr Dermatol* 2009;**26**:229–31.
189. Schaffner W. The rickettsioses. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1563–70.
190. Schmaljohn C, Hjelle B. Hantaviruses: a global disease problem. *Emerg Infect Dis* 1997;**3**:95–104.
191. Schneider JA, Poley JR, Millunchick EW, et al. Papular acrodermatitis (Gianotti-Crosti syndrome) in a child with anicteric hepatitis B, virus subtype adw. *J Pediatr* 1982;**101**:219–22.
192. Shama SK, Etkind PH, Odell TM, et al. Gypsy-moth-caterpillar dermatitis. *N Engl J Med* 1982;**306**:1300–1.
193. Shanson DC, Gazzard BG, Midgley J, et al. *Streptobacillus moniliformis* isolated from blood in four cases of Haverhill fever. *Lancet* 1983;**2**:92–4.
194. Shapiro L. The numbered diseases: first through sixth. *JAMA* 1965;**194**:210.
195. Shapiro L. On the numbered exanthemata. *Clin Pediatr (Phila)* 1967;**6**:611–12.
196. Shaw EB. Fifth disease. *Am J Dis Child* 1977;**131**:816.
197. Shelley ED, Shelley WB, Pula JF, et al. The diagnostic challenge of nonburrowing mite bites: *Cheyletiella yasguri*. *JAMA* 1984; **251**:2690–1.
198. Siewers CM, Cramblett HG. Cryptococcosis (torulosis) in children: a report of four cases. *Pediatrics* 1964;**34**:393–400.
199. Smith AW, Berry ES, Skilling DE, et al. In vitro isolation and characterization of a calicivirus causing a vesicular disease of the hands and feet. *Clin Infect Dis* 1998;**26**:434–9.
200. Smith PT, Landry ML, Carey H, et al. Papular-purpuric gloves and socks syndrome associated with acute parvovirus B19 infection: case report and review. *Clin Infect Dis* 1998;**27**:164–8.
201. Smith SB, Libow LF, Elston DM, et al. Gloves and socks syndrome: early and late histopathologic features. *J Am Acad Dermatol* 2002;**47**:749–54.
202. Somer T, Finegold SM. Vasculitides associated with infections, immunization, and antimicrobial drugs. *Clin Infect Dis* 1995;**20**: 1010–36.
203. Spelman DW. Q fever: a study of 111 consecutive cases. *Med J Aust* 1982;**1**:547–8, 551, 553.
204. Steere AC, Broderick TF, Malawista SE. Erythema chronicum migrans and Lyme arthritis. Epidemologic evidence for a tick vector. *Am J Epidemiol* 1978;**108**:312–21.
205. Stricof RL, Morse DL. HTLV-III/LAV seroconversion following a deep intramuscular needlestick injury. *N Engl J Med* 1986;**314**: 1115.
206. Strong WB. Petechiae and streptococcal pharyngitis. *Am J Dis Child* 1969;**117**:156–60.
207. Swartz MN, Weinberg AN. Infections due to gram-positive bacteria. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979: p. 1426–45.
208. Swartz MN, Weinberg AN. Miscellaneous bacterial infections with cutaneous manifestations. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1459–73.
209. Taieb A, Plantin P, Du Pasquier P, et al. Gianotti-Crosti syndrome: a study of 26 cases. *Br J Dermatol* 1986;**115**:49–59.
210. Talbot HK, Shepherd BE, Crowe JE, Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J* 2009;**28**:682–7.
211. Taylor PR, Weinstein WM, Bryner JH. *Campylobacter fetus* infection in human subjects: association with raw milk. *Am J Med* 1979;**66**:779–83.
212. Tertti R, Granfors K, Lehtonen OP, et al. An outbreak of *Yersinia pseudotuberculosis* infection. *J Infect Dis* 1984;**149**:245–50.
213. Thomas P, Moore M, Bell E, et al. *Pseudomonas* dermatitis associated with a swimming pool. *JAMA* 1985;**253**:1156–9.
214. Timar L, Budai J, Koller M. A prospective study on infectious mononucleosis in childhood: symptoms, serology, Epstein-Barr-Virus specific leukocyte migration inhibition. *Infection* 1982;**10**: 139–43.
215. Timar L, Budai J, Gero A, et al. Rare complications and unusual syndromes associated with Epstein-Barr virus. *Pediatr Infect Dis* 1985;**4**:212–3.
216. Toth M, Barna M, Voltay B. Aetiology of acute respiratory diseases in infants and children. *Acta Paediatr Hung* 1966;**6**:367–74.
217. Tudor RB. Urticaria in childhood. *Lancet* 1962;**82**:273–4.
218. Turner TW, Wilkinson DS. *Pasteurella pseudotuberculosis* as a cause of erythema nodosum. *Br J Dermatol* 1969;**81**:823–6.
219. Unger A, Tapia L, Minnich LL, et al. Atypical neonatal respiratory syncytial virus infection. *J Pediatr* 1982;**100**:762–4.
220. Utz JP, Shadomy HJ. Deep fungus infections. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1533–63.
221. Van Arsdall JA, 2nd Wunderlich HF, Melo JC, et al. The protean manifestations of Legionnaires' disease. *J Infect* 1983;7:51–62.
222. Ware R. Human parvovirus infection. *J Pediatr* 1989;**114**:343–8.
223. Wei SH, Huang YP, Liu MC, et al. An outbreak of coxsackievirus A6 hand, foot, and mouth disease associated with onychomadesis in Taiwan, 2010. *BMC Infect Dis* 2011;**11**:346.
224. Weinberg AN, Swartz MN. Gram-negative coccal and bacillary infections. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1445–59.
225. Weller TH. The cytomegaloviruses: ubiquitous agents with protean clinical manifestations. II. *N Engl J Med* 1971;**285**: 267–74.
226. Wenner HA. Virus diseases associated with cutaneous eruptions. *Prog Med Virol [Fortschritte der medizinischen Virusforschung]* 1973;**16**:269–336.
227. Wenner HA, Lou TY. Virus diseases associated with cutaneous eruptions. *Prog Med Virology [Fortschritte der medizinischen Virusforschung Progres en virologie medicale]* 1963;**5**:219–94.
228. Whiteford SF, Taylor JP, Dumler JS. Clinical, laboratory, and epidemiologic features of murine typhus in 97 Texas children. *Arch Pediatr Adolesc Med* 2001;**155**:396–400.
229. Wiesenthal AM, Todd JK. Toxic shock syndrome in children aged 10 years or less. *Pediatrics* 1984;**74**:112–17.
230. Winn WA, Levine HB, Broderick JE, et al. A localized epidemic of coccidioidal infection: primary coccidioidomycosis occurring in a group of ten children infected in a backyard playground in the San Joaquin Valley of California. *N Engl J Med* 1963; **268**:867–70.
231. Wolff K. Mycobacterial diseases: tuberculosis. In: Fitzpatrick TB, Eisen AZ, Wolff K, et al, editors. *Dermatology in general medicine*. New York: McGraw-Hill; 1979. p. 1473–92.
232. Wong ML, Kaplan S, Dunkle LM, et al. Leptospirosis: a childhood disease. *J Pediatr* 1977;**90**:532–7.
233. Woodward TE. Rocky Mountain spotted fever: epidemiological and early clinical signs are keys to treatment and reduced mortality. *J Infect Dis* 1984;**150**:465–8.
234. Yamamoto T, Yokoyama A. Hepatitis C virus infection and nodular prurigo. *Br J Dermatol* 1996;**135**:489–504.
235. Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988;**1**:1065–7.
236. Yee LC, Ng LC, Koh TH. Dermatological features of an imported case of chikungunya in an infant. *Singapore Med J* 2008;**49**: 959–60.
237. Young EJ. Human brucellosis. *Rev Infect Dis* 1983;**5**:821–42.

percent of Clemens' cases occurred in February, March, and April; 16 percent were seen in October.[17] In a review of 243 cases during a 10-year period, Juretic[38] observed that the peak month was May. Juretic also reviewed the seasonal incidence in 10 other studies and found only minor variations by month. Prevalence was greatest in March, April, and October and least in December. One epidemic of roseola in a maternity hospital occurred in the summer,[36] another epidemic in an infants' home occurred in the fall,[6] and a hospital outbreak occurred in the winter.[18]

Roseola predominantly is an illness of young children. It occurs rarely in infants younger than 3 months old or children older than 4 years. In a review of 1462 cases, the peak age range prevalence was 7 to 13 months of age; 55 percent of the cases occurred within the first year of life, and 90 percent occurred within the first 2 years of life.[38] Occasionally, cases have been seen in older children, adolescents, and young adults and in neonates and other infants younger than 6 months.[24,36]

Although Faber and Dickey[20] found twice as many girls as boys with the syndrome, the sex ratio in most large studies has been equal.[7,10,17,24,45] Although three epidemics have been reported, and cases frequently occur in groups by season, most cases occur sporadically without known exposure. The syndrome, when seen sporadically, generally is considered to be noncontagious, but secondary cases have been reported occasionally.[6,10,18,36] The incubation period range in epidemics is 5 to 15 days.[6,10,18]

The attack rate of roseola has not been well studied. Berenberg and associates[7] stated that roseola is the exanthem most commonly encountered in children younger than 2 years old. Breese[10] found that 16 percent of a group of infants he followed for the first 12 months of life had definite roseola. He estimated that 30 percent of children would have clinical roseola. Juretic[38] looked at the frequency of roseola in 6735 children; the yearly attack rate during a 10-year period ranged from 1 to 10 percent, with a mean of 3.3 percent.

ETIOLOGY

In 1941, Breese[10] reported vigorous attempts to isolate a filterable virus from three children with pre-eruptive roseola. These studies included extensive animal inoculations, but no viral agents were uncovered. In 1950, Kempe and associates[40] reported the passage of the illness to a 6-month-old susceptible infant by the intravenous injection of serum from an 18-month-old child with pre-eruptive roseola. Febrile illnesses without exanthem also were produced in monkeys with serum and throat washings from a child with the syndrome. In similar experiments, Hellstrom and Vahlquist[30] produced the syndrome in three children aged 6 to 9 days after the intramuscular administration of blood from typical roseola cases.

In electron microscopy studies, Reagan and associates[61] observed uniform viruslike particles (100 to 110 nm) in the blood of an 18-month-old child with the syndrome. Febrile illness was produced in two monkeys after concentrated virus-containing material was inoculated.

Since the advent of modern diagnostic virology in the early 1950s, numerous viral agents have been recovered from children with roseola. In 1951, Neva and associates[52] studied an epidemic exanthematous illness (i.e., Boston exanthem) caused by echovirus 16, in which many of the illnesses were characteristic of roseola. In 1954, Neva[50] observed additional cases of roseola-like illness associated with echovirus 16 infection. In 1974, Hall and colleagues[27] reported four additional echovirus 16 infections with clinical manifestations of roseola. The reporting of roseola in Rochester, New York, nearly doubled during the time of echovirus 16 activity in the area. Roseola-like illnesses that also have been associated with enteroviruses are caused by coxsackievirus A6, A9, B1, B2, B4, and B5 and echovirus 9, 11, 25, 27, and 30.[15,16,27,66,78] Outbreaks of roseola that occur in the summer and fall probably are caused by enteroviral infections.

In addition to enteroviruses, adenovirus types 1, 2, 3, and 14 and parainfluenza type 1 virus have been recovered from children with roseola.[23,37,51,78] Saitoh and associates[64] detected rotavirus capsomeres in fecal specimens of nine children with roseola. In contrast to these findings, Gurwith and colleagues[26] studied fecal specimens from five children with roseola, and in none were viral particles identified. One of 13 children in this study did develop antibody to rotavirus around the time of illness, however. In addition to the occurrence of roseola associated with numerous natural viral infections, its pattern (i.e., fever and then rash with defervescence) was observed frequently in recipients of Edmonton B measles vaccine.[15]

In 1988, Yamanishi and associates[79] isolated HHV-6 from four infants with roseola and all four had significant titer increases for this virus. Shortly after this finding was reported, several other investigators noted similar findings.* The implication from these studies, as suggested by the various investigators, is that HHV-6 is the cause of roseola. This viewpoint overlooks or ignores the past experience in which other viral agents have been associated with the clinical syndrome. Subsequent studies indicate that HHV-6 is a major cause of roseola and the cause of acute febrile illness without exanthem in infants.† Since 1993, HHV-7 has been accepted as an additional causative agent in roseola.‡

In a study of 1653 infants and young children with acute febrile illnesses, Hall and colleagues[27] found that 160 (9.7%) had primary HHV-6 infections; 27 (17%) of the children who were infected with HHV-6 had roseola. Zerr and associates[86] identified 80 children with primary HHV-6 infections, and of these, 30 percent had roseola. In the same population-based study, 3/80 (4%) children without primary HHV-6 infection also had roseola. In a study of clinical roseola, Okada and associates[54] found that 81 percent had serologic evidence of HHV-6 infection, and that 8 percent had an echovirus-18 infection. In a study of roseola in Italy, Braito and Uberti[9] found

*References 2, 5, 19, 33, 41, 69, 73.
†References 4, 9, 27, 34, 42, 44, 54, 58, 60, 67, 77, 86.
‡References 3, 15, 31, 68, 71, 77.

serologic evidence of HHV-6 infection in only 30 percent of the cases. In 33 percent of the remaining cases, they attributed the illnesses to another infectious agent. In 1994, Hidaka and associates[31] estimated that 73.5, 10.2, and 16.3 percent of their roseola cases were caused by HHV-6, HHV-7, and other viruses, respectively. In a study of HHV-6 infections in young Brazilian children with rashes it was noted that only 21 percent had typical roseola.[76] In summary, HHV-6 is an important cause of roseola, but not the only cause.

PATHOPHYSIOLOGY

The pathophysiology of roseola is unknown. Watson,[78] in the pre–HHV-6 era, suggested that roseola is not an infection caused by one particular pathogen, but is the result of an immunizing reaction against many different viruses. He also suggested that the rash is caused by the neutralization of virus in the skin at the end of the period of viremia.

Viremia is common in HHV-6, HHV-7, enteroviral, and adenoviral infections; thus, a reasonable conclusion (as originally suggested by Watson[78]) is that the rash in roseola is related to an immunologic event resulting from the virus that is localized in the skin. Why the pattern of fever and then rash with defervescence is so clearly age-dependent is unknown. Most of the viruses that in the past have been associated with roseola cause other exanthematous manifestations in older patients.[15]

CLINICAL PRESENTATION

The basic clinical pattern of roseola is a febrile period of 3 to 5 days, defervescence, and the appearance of a rash that persists for 1 to 2 days. Because the syndrome is caused by many different viruses, the illness apparently may be associated with numerous other signs and symptoms. The major manifestations have been reviewed elsewhere.*

Illness usually occurs with the apparent abrupt onset of fever. Slight irritability and malaise occur frequently, but more commonly, the child's temperature is taken because a parent notices that the child feels warm. The temperature usually is in the range of 38.9° to 40.6°C (102.2° to 105°F). Despite the high fever, the child usually is active, alert, and generally unfazed. The fever is constant or intermittent, with its greatest degree occurring in the early evening. Restlessness and irritability occur with higher temperatures. The usual duration of fever is 3 to 5 days, but it has persisted for 9 days. The temperature most often returns to normal by crisis, but in some cases, temperature "lysis" occurs over the course of 24 to 36 hours.

Mild cough and coryza are seen frequently in cases occurring in the winter and spring. Headache and abdominal pain are reported in older children, mainly in the summer and fall. Vomiting and diarrhea occur infrequently.

On initial physical examination during the febrile period, most children appear to be happy, alert, and playful. With high temperatures, some children are irritable; occasionally, a child appears to be sick, which suggests more serious illness, such as meningitis or septicemia. Examination within the oral cavity frequently reveals one or more abnormalities. Mild inflammation of the pharynx and tonsils occurs most commonly. Occasionally, small exudative follicular lesions are noted on the tonsils. In other cases, small ulcerative lesions on the soft palate, uvula, and tonsillar pillars are observed. Usually, the lesions on the soft palate consist of only erythematous macules and maculopapules, presumably because of lymphoid hyperplasia.

Mild injection of the tympanic membranes occurs commonly. Enlargement of the suboccipital, posterior cervical, and postauricular lymph nodes is a common finding, but the degree is not remarkable.

Berliner[8] noticed that children with roseola had palpebral edema. He suggested that the "heavy eyelids" or "droopy" or "sleepy" appearance resulting from this edema was diagnostic of the syndrome before the appearance of the rash. Bulging of the anterior fontanelle also has been observed in roseola.[56]

Appearance of the rash in roseola usually coincides with the subsidence of fever, but it may occur after an afebrile interlude of several hours to 2 days. When defervescence occurs by lysis, onset of the exanthem can occur before the temperature has returned entirely to normal. By definition, it is incorrect, however, to call an illness roseola if the fever and rash are truly concomitant.

Zahorsky[81,82] originally described the rash as morbilliform, but his use of *morbilliform* was not the same as is used today (i.e., measles-like, erythematous, maculopapular with confluence). The rash is erythematous and macular or maculopapular, and the lesions are discrete. The lesions are 2 to 5 mm in diameter and blanch on pressure. Frequently, individual lesions are surrounded by a whitish ring. The rash is most prominent on the neck and trunk, but the proximal extremities and the face also may be affected. Although they have been reported,[17,25] pruritus and desquamation usually do not occur. The rash usually persists for 24 to 48 hours. In occasional cases, well-documented rashes have been observed to appear and resolve within 2 to 4 hours. Yoshida and associates[80] described a 7-month-old boy with HHV-6 infection and typical roseola initially. On the ninth day of illness, vesicular lesions appeared on the face and limbs, however. These lesions persisted for 12 days.

Except for the white blood cell count, routine laboratory studies are of little use in roseola. The total white blood cell count usually is low. Early in the febrile period, high counts occasionally are found, however. The total count reaches its nadir by the third to sixth day of illness and then gradually returns to normal over the ensuing 7 to 10 days. During the same time frame, the percentage of lymphocytes increases from a normal value of about 50 percent to 60 to 80 percent on days 3 to 10 and then returns to normal over the next 7 days. Frequently,

*References 7, 10, 17, 24, 38, 82.

extreme counts in the range of 3000 cells/mm^3 with 90 percent lymphocytes are found, which raises the consideration of a granulocytic defect.

CLINICAL COMPLICATIONS

The most important complications of roseola are convulsions and other neurologic symptoms.* The incidence of convulsions has varied widely among reports. Juretic[38] did not find one instance of convulsions in the 243 cases in his study. Breese[10] did not report convulsions in any of 100 roseola attacks that he studied. In contrast, Greenthal[24] noted convulsions in 6 percent of his cases, and Faber and Dickey[20] found seizures in 8 of 26 cases of roseola. Möller[48] observed that 8 percent of children admitted to the hospital because of febrile convulsions eventually were diagnosed with roseola infantum.

Möller[48] also reported cerebrospinal fluid evaluations in 29 cases of roseola and febrile convulsions. In six instances, the pressure was elevated; in two, there were 5 white blood cells/mm^3; and in another instance, there were 9 white blood cells/mm^3. In most other cerebrospinal fluid examinations, the findings have been normal, but mild pleocytosis with mononuclear cells has been identified occasionally.[7,32] A surprising number of cases of encephalitis associated with roseola have been reported,[13,22,32,35] and residua have been common. Hemiplegia has occurred after illness,[13,22,59,62] and permanent paresis and mental retardation have occurred in some affected patients. The syndrome of inappropriate secretion of antidiuretic hormone has been reported in roseola associated with HHV-6 infection.[55,65] Facial nerve palsy and Guillain-Barré syndrome also have been noted after HHV-6 induced roseola.[47,57]

Thrombocytopenic purpura was noted in one report in five children with roseola; all of these patients recovered.[53] In a more recent study, Hashimoto and colleagues[29] noted five children with thrombocytopenia during the acute phase of roseola caused by HHV-6 infection. Their data suggested that the thrombocytopenia was due to bone marrow suppression, rather than immune-mediated peripheral consumption. A 14-month-old girl developed a generalized eruptive histiocytoma with rapid progression and then resolution after roseola.[70]

*References 2, 7, 11-13, 20, 22, 25, 32, 35, 43, 48, 59, 62, 63.

DIAGNOSIS

Although detecting leukopenia with relative lymphocytosis is fortuitous, the only necessity in establishing the diagnosis of roseola is to document the fever, defervescence, and exanthem pattern. Frequently, the first exanthematous illness that a child has is called roseola, regardless of whether the exanthem and the fever are concomitant, or the child has no febrile period at all.

The only problem in the differential diagnosis occurs when a febrile child is receiving antibiotics and a rash follows defervescence. This event occurs frequently, and the child usually is labeled allergic to the antibiotic, rather than suspected of having roseola. In most instances of drug allergy, the exanthem lasts longer than roseola does, and in allergic cases, pruritus and fever may accompany the rash.

TREATMENT AND PROGNOSIS

No specific treatment for roseola exists. When fever is a problem, it may be treated with acetaminophen. Acetaminophen can alter the temperature curve, possibly obscuring the correct diagnosis. Febrile seizures and other neurologic complications should be treated vigorously.

In most cases, the outlook is excellent. When encephalitis occurs, the prognosis must be guarded. Because roseola is the result of infection with multiple different viruses, no practical way to prevent it exists.

NEW REFERENCES SINCE THE SIXTH EDITION

76. Vianna RA, de Oliveira SA, Camacho LA, et al. Role of human herpesvirus 6 infection in young Brazilian children with rash illnesses. *Pediatr Infect Dis J* 2008;**27**:533–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Altschuler EL. Oldest description of roseola and implications for the antiquity of human herpesvirus 6. *Pediatr Infect Dis J* 2000; **19**:903.
2. Asano Y, Nakashima T, Yoshikawa T, et al. Severity of human herpesvirus-6 viremia and clinical findings in infants with exanthem subitum. *J Pediatr* 1991;**118**:891–5.
3. Asano Y, Suga S, Yoshikawa T, et al. Clinical features and viral excretion in an infant with primary human herpesvirus 7 infection. *Pediatrics* 1995;**95**:187–90.
4. Asano Y, Yoshikawa T, Suga S, et al. Viremia and neutralizing antibody response in infants with exanthem subitum. *J Pediatrics* 1989;**114**:535–9.
5. Asano Y, Yoshikawa T, Suga S, et al. Clinical features of infants with primary human herpesvirus 6 infection (exanthem subitum, roseola infantum). *Pediatrics* 1994;**93**:104–8.
6. Barenberg LH, Greenspan L. Exanthema subitum (roseola infantum). *Am J Dis Child* 1939;**58**:983–93.
7. Berenberg W, Wright S, Janeway CA. Roseola infantum (exanthem subitum). *N Engl J Med* 1949;**241**:253–9.
8. Berliner BC. A physical sign useful in diagnosis of roseola infantum before the rash. *Pediatrics* 1960;**25**:1034.
9. Braito A, Uberti M. Roseola infantum and its correlation with HHV6. *Eur J Pediatr* 1994;**153**:209.
10. Breese Jr BB. Roseola infantum (exanthem subitum). *N Y State J Med* 1941;**41**:1854–9.
11. Broberger O. [Exanthema subitum and febrile convulsions]. *Nordisk Med* 1958;**59**:523–5.
12. Brunner N. [172 Cases of exanthema subitum from practice and clinic]. *Helv Paediatr Acta* 1959;**14**:408–24.
13. Burnstine RC, Paine RS. Residual encephalopathy following roseola infantum. *J Dis Child* 1959;**98**:144–52.
14. Caserta MT, Hall CB, Schnabel K, et al. Primary human herpesvirus 7 infection: a comparison of human herpesvirus 7 and human herpesvirus 6 infections in children. *J Pediatr* 1998;**133**:386–9.
15. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.
16. Cherry JD, Lerner AM, Klein JO, et al. Coxsackie B5 infections with exanthems. *Pediatrics* 1963;**31**:455–62.
17. Clemens HH. Exanthem subitum (roseola infantum): report of eighty cases. *J Pediatr* 1945;**26**:66–77.
18. Cushing HB. An epidemic of roseola infantum. *Can Med Assoc J* 1927;**17**:905–6.
19. Enders G, Biber M, Meyer G, et al. Prevalence of antibodies to human herpesvirus 6 in different age groups, in children with exanthema subitum, other acute exanthematous childhood diseases, Kawasaki syndrome, and acute infections with other herpesviruses and HIV. *Infection* 1990;**18**:12–15.
20. Faber HK, Dickey LB. The symptomatology of exanthem subitum. *Arch Pediatr* 1927;**44**:491–6.
21. Fox JD, Ward P, Briggs M, et al. Production of IgM antibody to HHV6 in reactivation and primary infection. *Epidemiol Infect* 1990;**104**:289–96.
22. Friedman JH, Golomb J, Aronson L. Hemiplegia associated with roseola infantum (exanthum subitum). *N Y State J Med* 1950;**50**: 1749–50.
23. Fukumi H, Nishikawa F, Kokubu Y, et al. Isolation of adenovirus from an exanthematous infection resembling roseola infantum. *Japn J Med Sci Biol* 1957;**10**:87–91.
24. Greenthal RM. An unusual exanthem occurring in infants. *Am J Dis Child* 1922;**23**:63–5.
25. Greenthal RM. Roseola infantum (exanthem subitum). *Wisc Med J* 1941;**40**:25–7.
26. Gurwith M, Gurwith D, Wenman W, et al. Exanthem subitum not associated with rotavirus. *N Engl J Med* 1981;**305**:174–5.
27. Hall CB, Cherry JD, Hatch MH, et al. The return of Boston exanthem: echovirus 16 infections in 1974. *Am J Dis Child* 1977;**131**:323–6.
28. Hall CB, Long CE, Schnabel KC, et al. Human herpesvirus-6 infection in children: a prospective study of complications and reactivation. *N Engl J Med* 1994;**331**:432–8.
29. Hashimoto H, Maruyama H, Fujimoto K, et al. Hematologic findings associated with thrombocytopenia during the acute phase of exanthem subitum confirmed by primary human herpesvirus-6 infection. *J Pediatr Hematol Oncol* 2002;**24**:211–14.
30. Hellstrom B, Vahlquist B. Experimental inoculation of roseola infantum. *Acta Paediatr* 1951;**40**:189–97.
31. Hidaka Y, Okada K, Kusuhara K, et al. Exanthem subitum and human herpesvirus 7 infection. *Pediatr Infect Dis J* 1994;**13**: 1010–11.
32. Holliday Jr PB. Pre-eruptive neurological complications of the common contagious diseases, rubella, rubeola, roseola, and varicella. *J Pediatr* 1950;**36**:185–98.
33. Huang LM, Lee CY, Chen JY, et al. Primary human herpesvirus 6 infections in children: a prospective serologic study. *J Infect Dis* 1992;**165**:1163–4.
34. Irving WL, Chang J, Raymond DR, et al. Roseola infantum and other syndromes associated with acute HHV6 infection. *Arch Dis Child* 1990;**65**:1297–300.
35. Ishiguro N, Yamada S, Takahashi T, et al. Meningo-encephalitis associated with HHV-6 related exanthem subitum. *Acta Paediatr Scand* 1990;**79**:987–9.
36. James U, Freier A. Roseola infantum: an outbreak in a maternity hospital. *Arch Dis Child* 1948-1949;**23-24**:54–8.
37. Jansson E, Wager O, Forssell P, et al. An exanthema subitumlike rash in patients with adenovirus infection. *Ann Paediatr Fenn* 1961;**7**:3–11.
38. Juretic M. Exanthema subitum a review of 243 cases. *Helv Paediatr Acta* 1963;**18**:80–95.
39. Kawaguchi S, Suga S, Kozawa T, et al. Primary human herpesvirus-6 infection (exanthem subitum) in the newborn. *Pediatrics* 1992;**90**: 628–30.
40. Kempe CH, Shaw EB, Jackson JR, et al. Studies on the etiology of exanthema subitum (roseola infantum). *J Pediatr* 1950;**37**:561–8.
41. Kido S, Kondo K, Kondo T, et al. Detection of human herpesvirus 6 DNA in throat swabs by polymerase chain reaction. *J Med Virol* 1990;**32**:139–42.
42. Knowles WA, Gardner SD. High prevalence of antibody to human herpesvirus-6 and seroconversion associated with rash in two infants. *Lancet* 1988;**2**:912–13.
43. Kondo K, Nagafuji H, Hata A, et al. Association of human herpesvirus 6 infection of the central nervous system with recurrence of febrile convulsions. *J Infect Dis* 1993;**167**:1197–200.
44. Kusuhara K, Ueda K, Okada K, et al. Do second attacks of exanthema subitum result from human herpesvirus 6 reactivation or reinfection? *Pediatr Infect Dis J* 1991;**10**:468–70.
45. Letchner A. Roseola infantum: a review of fifty cases. *Lancet* 1955; **269**:1163–5.
46. McEnery JT. Postoccipital lymphadenopathy as a diagnostic sign in roseola infantum (exanthem subitum). *Clin Pediatr* 1970;**9**: 512–14.
47. Miyake F, Yoshikawa T, Suzuki K, et al. Guillain-Barré syndrome after exanthem subitum. *Pediatr Infect Dis J* 2002;**21**:569–70.
48. Möller KL. Exanthema subitum and febrile convulsions. *Acta Paediatr* 1956;**45**:534–40.
49. Moore Jr WF. Roseola infantum. *Hawaii Med J* 1963;**22**:431–4.
50. Neva FA. A second outbreak of Boston exanthem disease in Pittsburgh during 1954. *N Engl J Med* 1956;**254**:838–43.
51. Neva FA, Enders JF. Isolation of a cytopathogenic agent from an infant with a disease in certain respects resembling roseola infantum. *J Immunol* 1954;**72**:315–21.
52. Neva FA, Feemster RF, Gorbach IJ. Clinical and epidemiological features of an usual epidemic exanthem. *JAMA* 1954;**155**:544–8.
53. Nishimura K, Igarashi M. Thrombocytopenic purpura associated with exanthema subitum. *Pediatrics* 1977;**60**:260.
54. Okada K, Ueda K, Kusuhara K, et al. Exanthema subitum and human herpesvirus 6 infection: clinical observations in fifty-seven cases. *Pediatr Infect Dis J* 1993;**12**:204–8.
55. Okafuji T, Uchiyama H, Okabe N, et al. Syndrome of inappropriate secretion of antidiuretic hormone associated with exanthem subitum. *Pediatr Infect Dis J* 1997;**16**:532–3.
56. Oski FA. Roseola infantum: another cause of bulging fontanel. *Am J Dis Child* 1961;**101**:376–8.
57. Pitkaranta A, Lahdenne P, Piiparinen H. Facial nerve palsy after human herpesvirus 6 infection. *Pediatr Infect Dis J* 2004;**23**:688–9.
58. Portolani M, Cermelli C, Moroni A, et al. Human herpesvirus-6 infections in infants admitted to hospital. *J Med Virol* 1993;**39**: 146–51.
59. Posson DD. Exanthem subitum (roseola infantum) complicated by prolonged convulsions and hemiplegia. *J Pediatr* 1949;**35**:235.

60. Pruksananonda P, Hall CB, Insel RA, et al. Primary human herpesvirus 6 infection in young children. *N Engl J Med* 1992;**326**:1445–50.
61. Reagan RL, Chang SC, Moolten SE, et al. Electron microscopic studies of the roseola infantum (exanthem subitum) virus. *Tex Rep Biol Med* 1955;**13**:929–33.
62. Rosenblum J. Roseola infantum (exanthem subitum) complicated by hemiplegia. *Am J Dis Child* 1945;**69**:234–6.
63. Rothman PE, Naiditch MJ. Nervous complications of exanthem subitum. *Calif Med* 1958;**88**:39–44.
64. Saitoh Y, Matsuno S, Mukoyama A. Exanthem subitum and rotavirus. *N Engl J Med* 1981;**304**:845.
65. Shimura N, Kim H, Sugimoto H, et al. Syndrome of inappropriate secretion of antidiuretic hormone as a complication of human herpesvirus-6 infection. *Pediatr Int* 2004;**46**:497–8.
66. St Geme Jr JW, Prince JT, Scherer WF, et al. A clinical study of an exanthem due to ECHO virus type 9. *J Pediatr* 1959;**54**:459–67.
67. Suga S, Yazaki T, Kajita Y, et al. Detection of human herpesvirus 6 DNAs in samples from several body sites of patients with exanthem subitum and their mothers by polymerase chain reaction assay. *J Med Virol* 1995;**46**:52–5.
68. Suga S, Yoshikawa T, Nagai T, et al. Clinical features and virological findings in children with primary human herpesvirus 7 infection. *Pediatrics* 1997;**99**:E4.
69. Takahashi K, Sonoda S, Kawakami K, et al. Human herpesvirus 6 and exanthem subitum. *Lancet* 1988;**1**:1463.
70. Tamiya H, Tsuruta D, Takeda E, et al. Generalized eruptive histiocytoma with rapid progression and resolution following exanthema subitum. *Clin Exp Dermatol* 2005;**30**:294–307.
71. Tanaka K, Kondo T, Torigoe S, et al. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). *J Pediatr* 1994;**125**:1–5.
72. Torigoe S, Kumamoto T, Koide W, et al. Clinical manifestations associated with human herpesvirus 7 infection. *Arch Dis Child* 1995;**72**:518–19.
73. Ueda K, Kusuhara K, Hirose M, et al. Exanthem subitum and antibody to human herpesvirus-6. *J Infect Dis* 1989;**159**:750–2.
74. Ueda K, Kusuhara K, Okada K, et al. Primary human herpesvirus 7 infection and exanthema subitum. *Pediatr Infect Dis J* 1994;**13**:167–8.
75. Veeder BS, Hempelmann TC. A febrile exanthem occurring in childhood (exanthem subitum). *JAMA* 1921;**77**:1787–9.
76. Vianna RA, de Oliveira SA, Camacho LA, et al. Role of human herpesvirus 6 infection in young Brazilian children with rash illnesses. *Pediatr Infect Dis J* 2008;**27**:533–7.
77. Ward KN. The natural history and laboratory diagnosis of human herpesviruses-6 and -7 infections in the immunocompetent. *J Clin Virol* 2005;**32**:183–93.
78. Watson GI. The roseolar reaction (letter). *Br Med J* 1974;**4**:719–20.
79. Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988;**1**:1065–7.
80. Yoshida M, Fukui K, Orita T, et al. Exanthem subitum (roseola infantum) with vesicular lesions. *Br J Dermatol* 1995;**132**:614–6.
81. Zahorsky J. Roseola infantilis. *Pediatrics* 1910;**22**:60–4.
82. Zahorsky J. Roseola infantum. *JAMA* 1913;**61**:1446–50.
83. Zahorsky J. Roseola infantum: the rose rash of infants. *Arch Pediatr* 1925;**42**:610–13.
84. Zahorsky J. Roseola infantum: a critical survey of some recent literature. *Arch Pediatr* 1940;**57**:405–9.
85. Zahorsky J. Roseola infantum, a critical survey of recent literature. *Arch Pediatr* 1947;**64**:579–83.
86. Zerr DM, Meier AS, Selke SS, et al. A population-based study of primary human herpesvirus 6 infection. *N Engl J Med* 2005;**352**:768–76.

CHAPTER 60

Skin Infections

60A ■ BACTERIAL SKIN INFECTIONS

Mary Anne Jackson

NORMAL SKIN

Anatomy

The epidermal skin layer provides the primary barrier to invasion by microorganisms and an interface between the body and the environment. Hair follicles, sebaceous glands, nails, and sweat glands are considered epidermal appendages and as such may be involved in skin infection. A dermal layer composed of collagen and elastic fibers gives skin its elasticity; however, other cell elements that are present, including mast cells, blood and lymph vessels, and cutaneous nerves, may be involved in the inflammatory process in response to infection. The subcutaneous fat layer is just beneath the dermis and contributes primarily to thermal stability, but it also may be involved when infection extends beyond the epidermal–dermal layer.

Flora

Colonization is defined as the presence of a microorganism on the skin without either clinical signs or symptoms of infection at the time of isolation. Normal bacterial skin colonization is divided into resident and transient flora. Resident flora predominates and includes typical nonpathogens, such as *Staphylococcus epidermidis* and *Propionibacterium acnes*, in addition to other anaerobic diphtheroids and micrococci. Transient flora include pathogenic organisms, such as *Staphylococcus aureus*, streptococci, gram-negative enteric organisms, and *Candida albicans;* these pathogens usually are present in smaller numbers than the resident flora and may be removed by skin cleansing. Acutely or chronically damaged skin, contact with animate and inanimate environmental sources, and exposure to antimicrobial agents or indwelling devices can modify the skin flora and predispose to infection by resident or acquired transient flora.[78,88]

CUTANEOUS INFECTION AND DERMATOLOGIC MANIFESTATIONS OF SYSTEMIC DISEASE

Dermatologic manifestations of infection can occur when the skin is infected primarily or as a secondary phenomenon. Prompt diagnosis and treatment of certain systemic or disseminated diseases may be accomplished when the secondary dermatologic manifestations are recognized. Empirical treatment of systemic diseases such as endocarditis (septic emboli) or septicemia caused by bacterial pathogens, such as *Neisseria meningitidis* or *Pseudomonas aeruginosa*, is possible when the dermatologic manifestations (i.e., purpura fulminans, ecthyma gangrenosum) are noted. Generalized viral infections may be heralded by pathognomonic skin findings, such as occur in varicella or measles. Alternatively, skin manifestations may be mediated by toxin (staphylococcal scalded skin syndrome or toxic shock syndrome) or by immunologic mechanisms (gonococcemia).

The list of bacterial infectious agents associated with skin infections is extensive (Table 60A-1). This section focuses on the bacterial skin infections most frequently encountered by practicing clinicians. Viral, bacterial, and fungal systemic or disseminated diseases are categorized in Chapter 58 and are also presented in the specific chapters in Part III of this book.

IMPETIGO

Nonbullous or Simple Superficial Impetigo

The bacterial skin infection most commonly encountered in children is nonbullous impetigo, which accounts for more than 70 percent of impetigo cases in children. This superficial infection is seen predominantly in summer, with insect bites, cutaneous injuries, and primary dermatitis serving as the portal of entry.[45,60]

Nonbullous impetigo, sometimes called *thick crusted impetigo*, is characterized by the appearance of erythematous maculopapules that rapidly evolve from a vesicular to a pustular stage. Centrally crusted plaques range in size from a few millimeters to 1 cm and are surrounded by a distinct margin of erythema. The honey-colored crust is a classic feature, and removal of the crust results in the reaccumulation of fresh exudate. Regional lymphadenopathy can occur and often is the reason that the patient seeks medical attention. Spread to exposed areas, usually the face, neck, and limbs, occurs frequently. This form of pyoderma often is associated with a 2- to 3-week delay

TABLE 60A-1 Bacterial Infectious Agents Associated with Cutaneous Manifestations

Anthrax	*Bacillus anthracis*
Blistering dactylitis	*Streptococcus pyogenes*
	Streptococcus agalactiae
	Staphylococcus aureus
Cellulitis	*S. pyogenes*
	S. aureus
	Haemophilus influenzae type b
	Streptococcus pneumoniae
Chancroid	*Haemophilus ducreyi*
Diphtheria	*Corynebacterium diphtheriae*
Ecthyma gangrenosum	*Pseudomonas aeruginosa*
Erysipelas	*S. pyogenes*
	S. agalactiae; groups C, G streptococci
	S. pneumoniae
Erysipeloid	*Erysipelothrix rhusiopathiae*
Folliculitis	*S. aureus*
	Coagulase-negative staphylococci
	Klebsiella spp
	Enterobacter spp
	Escherichia coli
	P. aeruginosa
	Proteus spp
Erythrasma	*Corynebacterium minutissimum*
Furunculosis	*S. aureus*
Hidradenitis suppurativa	*S. aureus*
	Streptococcus milleri
	E. coli
	Anaerobic streptococci
Granuloma inguinale	*Calymmatobacterium granulomatis*
Impetigo	
Simple superficial	*S. aureus*
	S. pyogenes
Bullous	*S. aureus*
Lymphogranuloma venereum	*Chlamydia trachomatis*
Melioidosis	*Burkholderia pseudomallei*
Necrotizing fasciitis	*S. pyogenes*
	Polymicrobial
Nocardiosis	*Nocardia brasiliensis*
	Nocardia asteroides
Paronychia	Polymicrobial
Perianal dermatitis	*S. pyogenes*
Pitted keratolysis	Coryneform bacteria
Syphilis	*Treponema pallidum*

Entries in bold are discussed in the text.

in establishing the diagnosis because the lesions are slow to progress, only mildly tender at the site of the lesion, and generally not associated with systemic signs or symptoms.

Nonbullous impetigo classically has been associated with infection caused by group A β-hemolytic streptococcus. More recent data underscore the importance of *S. aureus*, however, which now accounts for most cases of nonbullous impetigo in the United States.[6,49]

Primarily a disease of children, nonbullous impetigo is spread within families and by close physical contact. It is prevalent during warm, humid seasons and is seen year-round in tropical regions. Endemic disease occurs in the southeastern United States and Hawaii.

Epidemics of streptococcal impetigo have been associated with postinfectious glomerulonephritis, and streptococcal strains, including types 2, 31, 49, 53, 55, 56, 57, and 60, have been implicated in such outbreaks.[66,106] Studies published in the 1950s and 1960s from the Red Lake Indian Reservation in Minnesota first confirmed the association of impetigo in school-aged children with a postinfectious nephritis that occurred 18 to 21 days after the onset of impetigo and implicated the so-called Red Lake strain, M-type 49.[5] Further studies in this population performed in the early 1970s found that group A β-hemolytic streptococcus was isolated from normal skin in 23/31 high-risk children a mean of 10 days before the development of impetigo.[33] Local trauma and other environmental factors seemed to explain the predilection of exposed skin to streptococcal infection, especially the skin of the legs, where 62 percent of the total lesions were noted. Secondary acquisition of streptococcal isolates in other family members occurred a mean of 5 days after the primary case, a time frame that was noted to be significantly shorter than that of secondary respiratory acquisition.[51] Rheumatic fever does not occur as a postinfectious sequela of streptococcal skin infection.

Cutaneous botryomycosis, an indolent infection reminiscent of crusted impetigo, usually is caused by *S. aureus*. Characterized by plaquelike lesions with superficial pustules and crusts, this entity has a predilection for patients with altered immune function.[22] Histologic examination may suggest the diagnosis of actinomycosis if a granulomatous lesion with granules resembling those seen with *Actinomyces* is noted. Successful treatment can be accomplished after the bacterial pathogen has been identified.

Bullous Impetigo

Bullous impetigo is diagnosed when the primary lesion begins as small vesicles and later appears as flaccid, painless bullae, generally measuring greater than 1 cm (Fig. 60A-1). Initially filled with clear fluid, the lesions eventually may exhibit a purulent fluid level. Rupture of the thin bullae usually reveals a moist, erythematous base that dries to a shiny lacquer-like appearance, sometimes described as a varnished finish (Fig. 60A-2). Systemic toxicity is not seen except in neonates, in whom disseminated disease may occur.

In contrast to thick, crusted impetigo, in virtually all cases of bullous impetigo, staphylococci are isolated in pure culture from aspirated bulla fluid. Other bullous dermatitides of childhood, such as pemphigus or Stevens-Johnson syndrome, may be excluded by isolation of the organism. Occasionally, biopsy is done in cases in which extensive bullae or an atypical clinical appearance is noted. Confirmation of a cleavage plane high in the

epidermis with gram-positive organisms and polymorphonuclear leukocytes present is a definitive diagnosis of bullous staphylococcal disease.

Infection generally is caused by phage group II strains, particularly phage type 71, but also 3A, 3C, and 55, which are noted to elaborate epidermolytic toxins A and B. Pathologically, these toxins act by disrupting the intercellular attachment of epidermal cells of the stratum granulosum. The toxin is thought to function as a protease in separating the upper layers of the epidermis of adult and infant human skin. Production of antibody to epidermolytic toxin occurs with age; however, it does not protect against the development of new bullous lesions during the localized impetiginous stage of this staphylococcal disease.

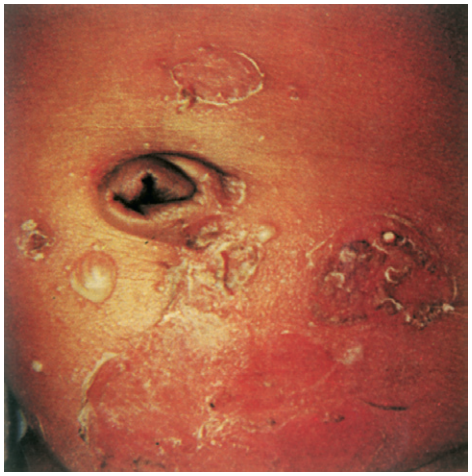

FIGURE 60A-1 ■ Bullous impetigo in a newborn infant caused by exfoliative toxin-producing *Staphylococcus aureus.* (See companion Expert Consult website for color version.)

Epidemiologically, large outbreaks of bullous impetigo have been traced most notably to hospital nurseries, where identification of infected infants always occurs within the first month of life but after the infant has been sent home. A more severe, generalized form of the epidermolytic toxin-mediated disease (Ritter disease) may be seen in a few infants during one of these outbreaks, underscoring the importance of infection control surveillance practices in recognizing such an outbreak (Fig. 60A-3).

Treatment of Impetigo

Topical mupirocin may be used in cases of nonbullous impetigo in which adequate coverage of the affected sites can be ensured.[17,32,97] In other cases, systemic treatment with an oral antistaphylococcal antimicrobial agent, such as cephalexin, should be employed.[37,38,44,108] As in other staphylococcal diseases, an increase in

FIGURE 60A-2 ■ Flaccid bullae and shiny lacquer base of staphylococcal impetigo.

FIGURE 60A-3 ■ Typical appearance of a neonate with Ritter disease.

community-acquired, methicillin-resistant *S. aureus* (MRSA) cases has been noted in the last decade.[61,62,71,123] In cases for which traditional antistaphylococcal agents are unsuccessful or in patients with recurrent disease, culture should be done to identify the bacterial strain and susceptibility pattern. Currently, the majority of community-acquired MRSA isolates are susceptible to clindamycin, but sensitivity patterns vary throughout the United States.[16,23]

PERIANAL STREPTOCOCCAL DERMATITIS

Formerly called *perianal cellulitis*, perianal streptococcal dermatitis, a commonly recognized superficial skin infection, is characterized by the presence of marked, well-demarcated, perirectal erythema with associated swelling, pruritus, and tenderness but an absence of systemic symptoms or progressive disease. Approximately half of patients complain of significant rectal pain on defecation, and a third note blood in their stools.[4,28,69,96]

Heavy growth of group A β-hemolytic streptococcus is seen on perianal culture, and in one study, isolation of a specific T-type *Streptococcus* (T 28) raised the question of whether certain streptococcal strains have tropism for the perineal region.[102] Asymptomatic patients were evaluated in two studies, and only sparse growth of group A β-hemolytic streptococcus was noted in 6 percent of cases.

Perianal streptococcal dermatitis is treated with oral penicillin agents. Topical mupirocin also has been used successfully. Recurrences are noted commonly, however. In one large series of patients, one third had recurrent disease.[85] Intrafamilial spread of disease frequently occurs and may provide a vector for recurrence. For patients with recurrent or persistent disease, clindamycin or a β-lactam agent plus rifampin may be used, and identification and treatment of other affected family members may be necessary.

BLISTERING DISTAL DACTYLITIS

Most commonly identified in school-aged children, blistering distal dactylitis is a distinctive superficial skin infection classically associated with group A β-hemolytic streptococcus.[12] Bullae 2 cm in diameter develop over the anterior fat pad of the distal phalanges, sometimes extending to involve the nail folds. Involvement of the proximal phalanges or the palms occasionally is noted. Frankly pustular lesions may occur, but the lesions themselves usually are asymptomatic or only mildly tender. A thin purulent exudate generally is apparent on incision and drainage.[70]

The diagnosis is confirmed by recovery of the etiologic agent on culture, most commonly group A β-hemolytic streptococcus, although group B streptococcus and *S. aureus* also have been noted.[57] Concurrent recovery of group A β-hemolytic streptococcus in the pharynx has been reported in a few cases. Treatment includes a 10-day course of an oral β-lactam agent, usually penicillin or amoxicillin, in addition to incision and drainage of any tense bullae.

ERYSIPELAS

The superficial cellulitis erysipelas, referred to as *St. Anthony's fire* in the Middle Ages, is characterized by the appearance of a bright erythematous plaque with a distinct, elevated border that sharply demarcates affected from unaffected skin. The lesion most often involves the face or lower extremity, although extensive involvement of the trunk has been noted. The involved skin is warm and tender and may have a peau d'orange appearance. Large tension bullae may be seen in the erythematous zone.[64] The patient generally appears toxic and is highly febrile, and rapid extension of the affected skin may occur over the course of hours.[30,65,135]

Histopathologic findings include intense edema with vascular dilation of the dermis and uppermost subcutaneous tissue. Involvement of lymphatic channels and tissue spaces with polymorphonuclear leukocytes is a typical finding.

Surgical wounds, the umbilicus of the neonate, or any break in the skin may serve as the portal of entry; however, the initial lesion may be inapparent. Localized edema, such as occurs from a renal or lymphatic source, is a predisposing factor, and antecedent respiratory tract infection often is reported. An increased risk for development of erysipelas has been noted in patients with hypogammaglobulinemia, certain malignancies such as lymphoma, or lymphedema complicating radiation therapy.

The diagnosis generally is recognized on clinical grounds, and group A β-hemolytic streptococcus traditionally has been isolated by aspiration of the advancing margin of the lesion. A few case reports have identified other streptococci (including groups B, C, and G), *Moraxella* spp., *Haemophilus influenzae*, and *Streptococcus pneumoniae* as etiologic agents.* A combination of intravenous penicillin and clindamycin should be used until the results of culture are available. Erysipelas has a classic clinical appearance, and appropriate diagnosis and therapy result in a prompt clinical response in most cases. Penicillin prophylaxis may be considered for patients with recurrent erysipelas, particularly patients with underlying risk factors.[15,39]

ECTHYMA

Ecthyma gangrenosa is a deep-seated infectious process that manifests as a necrotic ulcer covered by a black eschar. Usually, the initial lesion, a vesicopustule, sits on an erythematous base; it eventually erodes through the epidermis to the dermis, where it forms a crusted ulcer with heaped-up borders and then becomes frankly necrotic.[63]

This process rarely occurs in an otherwise healthy child, and if it does, an immunodeficiency workup should ensue. *Chromobacterium violaceum* has been reported to cause ecthyma and similarly should result in an immunologic evaluation focusing on neutrophil defects, including disorders such as chronic granulomatous disease.[21]

Ecthyma can occur as a primary cutaneous infection in an immunocompetent host, and other etiologic agents that have been confirmed include *S. aureus*, *Aeromonas hydrophila*, and group A β-hemolytic streptococcus.* A similar-appearing lesion is seen with cutaneous anthrax; however, extensive nonpitting edema of the surrounding soft tissues is an important clue to this diagnosis. Ecthymatous-like lesions have been seen in patients with herpes simplex infection.[83] Additionally, human orf infections result in ulcerative skin lesions that appear similar to lesions of ecthyma; the clue to establishing the diagnosis is contact with an infected animal, usually sheep or goats, or a contaminated fomite.[25]

When an ecthymatous lesion is noted in a febrile neutropenic host, it generally signals disseminated infection. *P. aeruginosa* is the etiologic pathogen identified most commonly in such cases, but other gram-negative pathogens and fungi, including *Enterobacter*, *Escherichia coli*, *Morganella*, *Pseudomonas cepacia*, *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Aspergillus*, *Mucor*, *Fusarium*, and *C. albicans*, have been implicated in ecthyma gangrenosum in compromised hosts.† Ecthyma has been seen as the heralding manifestation of acute lymphoblastic leukemia in children.[110] Empirical antimicrobial therapy for ecthyma gangrenosum in a neutropenic host should include intravenous therapy with an anti-*Pseudomonas* agent plus an aminoglycoside. Biopsy of the lesion may provide more specific etiologic information and allow for confirmation of antimicrobial susceptibility.

FOLLICULITIS, FURUNCULOSIS, AND CARBUNCLES

Folliculitis, furunculosis, and carbuncles represent a group of infections characterized by their origin in the hair follicles and the formation of abscesses. Virtually always caused by *S. aureus*, these infections were seen commonly in the 1950s in disease that often involved multiple family members. Outbreaks among athletes likewise have been reported.[129]

By definition, these infections involve sites where body hair is present, including the axilla, breast area, perineum, neck, and extremities. Lesions of folliculitis represent abscesses of a single hair follicle with limited surrounding tissue involvement. When deeper inflammatory nodules are associated with tissue edema, furunculosis is diagnosed. When several interconnecting furuncles are present, the lesion is referred to as a carbuncle. Generally, older children and adolescents are predisposed to the development of follicular infections, and individuals with diabetes mellitus, abnormal neutrophil chemotaxis, and impaired circulation may have recurrent disease.

Although *S. aureus* nearly always is the cause of folliculitis as in the past, outbreaks of so-called hot tub

*References 14, 29, 41, 86, 100, 122, 136.

*References 47, 56, 72, 82, 94, 103, 133.
†References 36, 50, 52, 95, 105, 111, 116, 121.

folliculitis have been described and almost always are caused by *P. aeruginosa;* rarely, other gram-negative organisms have been reported.[26,27] Folliculitis in an immunocompromised host often is caused by unusual fungal pathogens.[3,114,126] Although typically associated with hot tubs and whirlpools, a large outbreak of *Pseudomonas* folliculitis reported in 1984 involved 117 individuals after swimming in an indoor pool. An incubation period of 24 to 30 hours could be ascertained, and a typical follicular, pustular eruption was noted. The mean duration of the folliculitis, 15 days, is consistent with other reports, but some patients continued to complain of rash for weeks, and recurrent pustules appearing months later have been reported in other studies.[55]

A more recent report has identified four cases of *Mycobacterium fortuitum* complex furunculosis that developed after pedicures, suggesting this pathogen may be added to the differential diagnosis in such cases when a patient presents with nonhealing furuncles on the lower legs, especially when bacterial cultures have been negative, or the disease is unresponsive to antistaphylococcal therapy (including MRSA).[113] Early recognition and institution of appropriate therapy are essential, and the history of pedicures may be a clue to establishing the diagnosis. Cutaneous myiasis has been reported to manifest as a chronic boil or furuncle, but the diagnosis should be considered only in such cases in which an appropriate travel history is elicited.[31]

Recognition of the typical skin lesion usually is sufficient to establish the diagnosis. Unless the patient has a history of exposure to a hot tub or whirlpool, or recently has had a pedicure, a regimen of antistaphylococcal therapy should be sufficient. In the era of MRSA infection, local susceptibility profiles should be used to confirm appropriate therapy. Isolated boils resolve with drainage alone. Systemic therapy may be considered in cases in which lesions are large or multiple. Trimethoprim-sulfamethoxazole is a good choice for a nontoxic patient older than 2 months. Clindamycin is a good choice for most locales, although resistance may be increasing.[80,89] Because MRSA now accounts for 75 percent of cases in which children present with skin and soft tissue infection, treatment should be individualized, based on type of presentation, patient age, and underlying disease.[79] Systemic agents should be used for 7 to 10 days in patients who are toxic, who have extensive disease, or who have associated cellulitis. Large lesions, specifically larger than 5 cm, should be incised and drained, and culture should be done in all cases to confirm susceptibility testing. In some patients, hematogenous metastatic spread may occur, and a search for foci in the heart, bones, joints, deep tissues, or brain should be done in patients with significant systemic toxicity.

For patients in whom recurrent disease develops, chronic dermatoses, such as eczema, should be identified, and in obese adolescents, the diagnosis of diabetes mellitus should be considered.[7] The patient should be cautioned to refrain from sharing washcloths or towels, and skin trauma and use of irritants such as deodorants should be avoided.

The utility of decolonizing regimens with topical agents, such as mupirocin (nares and perianal area) and chlorhexidine or bleach baths (skin), may be considered in certain cases. *S. aureus* colonization of the nares, rectum, or skin can be detected by culture of these areas, but confirmation of carriage is likely necessary only in specific cases for which decolonization is desirable. The indications for and benefit of decolonization vary depending on host factors, underlying disease, and circumstances related to the health care setting.[125] Outbreaks of MRSA colonization or infection require a multifaceted approach, including attention given to hand washing, cohorting, barrier measures, and decolonization strategies. Examples of special circumstances include the following: (1) patients who are immunosuppressed and colonized and at risk for development of systemic infections; (2) patients who are more likely to spread the organisms, owing to behavior (e.g., mentally retarded patients); or (3) patients who have repeated infections caused by the MRSA strain they carry.

Most children with recurrent furunculosis are otherwise healthy, and no specific immunologic evaluation is necessary; however, cases of recurrent furunculosis in patients with hyper-IgE syndrome and common variable immunodeficiency have been reported, and an association with mannose-binding lectin deficiency was confirmed in a family.[76,81,124] Rarely, children with white blood cell defects may have recurrent staphylococcal skin abscesses, and tests of white blood cell function may be considered in specific patients. Data suggest that vitamin C may be beneficial in cases of recurrent folliculitis.[91,92]

HIDRADENITIS SUPPURATIVA

Hidradenitis suppurativa, a chronic, debilitating condition, is a disorder of the apocrine glands that involves primarily skin in the axilla and anogenital region, although scalp, umbilical, and breast involvement has been reported. Seen mainly in adolescents, this androgen-dependent condition is manifested by the development of multiple painful nodules and the formation of deep abscesses in the skin in areas where apocrine glands are present. The formation of fistulas, ulcers, and contracted scars may complicate the course, and recurrent relapses may be noted. Infection usually is polymicrobial, and pathogens to consider include *S. aureus*, gram-negative enteric organisms, and anaerobes. Drainage of abscesses and institution of systemic antimicrobial therapy may be necessary. When fistulas associated with anogenital disease develop, adjacent structures, including the urethra, bladder, and rectum, may be involved. Surgery usually is required for cure. Carbon dioxide laser treatment in some cases may be beneficial.[87]

CELLULITIS

The diagnosis of cellulitis is made when the subcutaneous tissues and dermis are involved in a clinical process manifested as localized edema, erythema, warmth, and tenderness of the tissues. The leading edge of the involved site may be notable, but it is not raised and well demarcated as in erysipelas.

Infection usually is caused by coagulase-positive staphylococci and group A β-hemolytic streptococcus; however, infection also is caused by group B streptococcus (neonates) and *S. pneumoniae*, and, in the past, *H. influenzae* type b cellulitis was described.[130] Streptococcal and staphylococcal cellulitis can involve patients of any age and any site, although the extremity is noted most often. Frequently, the patient has a history of antecedent trauma at the site of involvement, but the injury may not have appeared significant. Some researchers advocate culture of the cellulitic site, but in practice, it rarely is performed. Blood cultures are valuable in individuals with disease caused by group B streptococcus, *S. pneumoniae*, and *H. influenzae* type b.[74]

Group B streptococcal cellulitis occurs in neonates and generally is seen as part of invasive, late-onset disease. Unilateral involvement of the face or submandibular sites occurs most commonly, but inguinal, scrotal, and prepatellar involvement has been described.[10,68] When cellulitis occurs in an infant younger than 3 months old, group B streptococcal bacteremia should be suspected, even in the absence of other signs of systemic infection.

Pneumococcal soft tissue infections are common findings.[109] Patients with connective tissue disorders, such as systemic lupus erythematosus, seem especially prone, although these infections can occur in healthy infants and children. Sites of involvement include the head, neck, leg, and torso.

H. influenzae type b cellulitis often involves the face of infants (Fig. 60A-4). A violaceous hue of the cellulitic area, which some researchers thought to be pathognomonic, might be observed. This process nearly always was the result of hematogenous seeding by *H. influenzae* type b, and meningitis occurred in 15 to 20 percent of such patients.[67,128,139] As has been the case with other forms of *H. influenzae* type b disease, almost complete eradication has been achieved in the last decade with the use of conjugate vaccine.

FIGURE 60A-4 ■ Buccal involvement in an infant with invasive *Haemophilus influenzae* type b disease.

Treatment of simple cellulitis in patients with a clear-cut area of preceding trauma should include an agent that is active against *S. aureus* and group A β-hemolytic streptococcus, and in most locales, clindamycin remains a good choice for therapy, although resistance for *S. aureus* (10% to 15%) and group A β-hemolytic streptococcus (2% to 3%) is noted. The aforementioned caveats for treating staphylococcal skin infections should be followed. In infants with buccal or periorbital involvement or in infants with soft tissue involvement but without a clear-cut focus of infection, a third-generation cephalosporin, such as cefotaxime or ceftriaxone, should be included with clindamycin. Vancomycin always should be included in cases of a patient who is toxic and when metastatic suppurative disease is suspected.

NECROTIZING FASCIITIS

Necrotizing fasciitis is a rapidly progressive bacterial infection of the soft tissues associated with a fulminant course and a high mortality rate. This infection spreads rapidly in the plane between the subcutaneous tissue and superficial muscle fascia and causes widespread necrosis. Prompt and aggressive medical and surgical management is necessary to ensure a good outcome.

In children, necrotizing fasciitis usually is caused by group A β-hemolytic streptococcus; traumatic lesions involving the skin, including varicella, burns, or eczema, may predispose to this aggressive process.* An association has been noted among varicella, ibuprofen use, and invasive group A β-hemolytic streptococcus infection, although no data convincingly link this triad to necrotizing infection.[53,90,141] Patients with congenital or acquired immunodeficiencies are at greater risk for the development of necrotizing fasciitis, and in neonates, omphalitis and circumcision are predisposing conditions.

Clinical Manifestations

The child generally has a high fever and is fussy. In infants, the irritability may be profound and may not appear to be localized to an involved site, unless the clinician is meticulous in conducting the examination. An extremity most commonly is involved, and older infants and children often refuse to bear weight or move the affected extremity. Swelling of soft tissue usually is noted, but the erythema may be subtle. The hallmark tip-off on examination is the finding of intense pain on manipulation of the involved site that is out of proportion to the cutaneous signs. Skin changes that occur during the subsequent 24 to 48 hours include blistering with bleb formation, and a dusky appearance of the involved site is noted as vessels are thrombosed and cutaneous ischemia develops. Skin necrosis is a late sign and indicates a poor prognosis (Fig. 60A-5).[77,134]

*References 18, 46, 54, 58, 68, 107, 118, 119, 142.

FIGURE 60A-5 ■ Necrotizing fasciitis in a toddler with varicella.

Recognition of the manifestations of toxic shock syndrome (see Chapter 64) is crucial because mortality rates of 60 percent have been reported in patients with associated fasciitis. Multisystem complaints, including vomiting, diarrhea, and severe myalgia, are present when group A β-hemolytic streptococcus fasciitis is associated with streptococcal toxic shock syndrome. Tachycardia out of proportion to fever and altered mental status may be early signs of toxic shock syndrome. Renal and hepatic dysfunctions occur typically, and signs and symptoms of acute respiratory distress syndrome often are identified.

Diagnosis

The diagnosis cannot be based on the appearance of the involved site because none of the early findings of necrotizing fasciitis are pathognomonic. Plain radiographs usually are normal and of no value in establishing the diagnosis. Magnetic resonance imaging (MRI) is the preferred technique to detect soft tissue involvement. MRI permits visualization of the soft tissue edema infiltrating the fascial planes.[20,112,143] Although MRI may be helpful, it should not delay performing surgical intervention. Waiting for a radiographic procedure to be performed to confirm the diagnosis may serve only to delay implementing definitive surgical therapy and to increase the risk for development of systemic complications, contributing further to the increased morbidity and mortality rates. In the typical clinical scenario in which the index of suspicion for necrotizing fasciitis is high, surgical exploration is appropriate, even in the presence of "normal" MRI findings.

Laboratory manifestations of streptococcal toxic shock syndrome should be sought in any pediatric patient with fasciitis. Typically, the white blood cell count is normal; however, most patients have a significant increase in band forms (>50%) noted on the peripheral blood smear. Thrombocytopenia and evidence of coagulopathy are found commonly, and marked hypoalbuminemia with hypocalcemia is a typical finding. Laboratory findings associated with renal failure, myocardial dysfunction, and acute respiratory distress syndrome may develop during the first 48 to 72 hours.

A microbiologic diagnosis can be made by isolating bacteria from blood, tissue, or wound culture. In some cases, a polymicrobial etiology has been noted, particularly in patients with so-called Fournier gangrene or necrotizing fasciitis of the perineum.[48,73] In these cases, *S. aureus*, group A β-hemolytic streptococcus, and one or more anaerobes, including *Peptostreptococcus*, *Prevotella*, *Bacteroides fragilis*, and *Porphyromonas*, have been implicated in infection.[19] Fasciitis caused by *P. aeruginosa* or *Clostridium septicum* has been seen in neutropenic patients. In the last decade, group A β-hemolytic streptococcus has been reported widely as a single pathogen and is the etiologic agent in most cases of pediatric fasciitis. As in other cases of invasive disease caused by group A β-hemolytic streptococcus, virulence is related to certain structural characteristics of the organism and to its ability to produce biologically active substances, some of which facilitate invasion and spread of the pathogen.

Treatment

Surgical débridement of necrotic tissue is the key to managing necrotizing fasciitis, and increased mortality rates have been observed when débridement is delayed more than 24 hours.[104] Mandatory return to the operating room for examination and repeat débridement should occur during the following 24 to 48 hours. Careful management of fluids, attention to pain control, anticipation and management of multisystem organ failure, and administration of appropriate parenteral antimicrobial therapy should be initiated promptly. The use of intravenous immunoglobulin may be considered in cases of toxic shock syndrome–associated fasciitis.[24]

Appropriate therapy includes intravenous penicillin 150,000 U/kg/day divided into four to six doses, clindamycin 40 mg/kg/day divided into four doses, and vancomycin 40 mg/kg/day divided into three to four doses. The use of additional coverage with agents active against *P. aeruginosa* and gram-negative enteric organisms should be considered in neutropenic patients.

Response to therapy is assessed by careful serial examination. Control of pain is crucial in such patients, while keeping in mind that persistent, severe pain suggests ongoing tissue necrosis and may signal the need for further surgical intervention. Careful attention to nutritional support should be maintained throughout the child's hospital stay. Physical therapy is necessary for most patients, especially patients who require

amputation, skin grafting, or extensive reconstructive surgery, and providing for the psychosocial needs of the child and the family is imperative.

CONTAMINATED WOUNDS

Although staphylococci and streptococci are the most likely causes of infection after traumatic skin lesions, the list of causes may be extensive, depending on the nature of the injury. Specific pathogens should be considered when infections develop after human or animal bites, soil or water contamination, or various types of injury. Management of such infections depends on recognizing infection patterns and obtaining cultures for careful identification of the specific organism or organisms involved (Table 60A-2).

Human Bites

Two types of human bites are described: occlusional and clenched fist (see also Chapter 248). Occlusional bites are related most commonly to child abuse, although in a pediatric patient, a biting toddler may be the culprit. Accidental bites may occur during sporting activities and generally involve the face of a teammate. Clenched-fist injuries are associated with the most prevalent and severe infections that occur after human bites. Rapidly progressive infection may follow despite the patient receiving early medical attention.[11] When clenched-fist injuries are associated with bite wounds, laceration and puncture wounds commonly occur along the dorsal aspect of the third metacarpophalangeal joint, and bone, joint capsule, or tendon structures may be involved.

TABLE 60A-2 **Infections Associated with Animal Bites**

Nature of Injury	Pathogens Involved
Human bites	Staphylococci
	Anaerobic and aerobic streptococci
	Eikenella corrodens
Animal bites	
Dog and cat	*Pasteurella* spp
	Staphylococcus aureus
	Streptococci
	Anaerobes
	Capnocytophaga canimorsus, Capnocytophaga cynodegmi
	Moraxella spp
	Corynebacterium spp
	Neisseria spp
Reptile	Enteric gram-negatives
	Anaerobes
Horse and sheep	*Actinobacillus* spp, *Pasteurella* spp
Pig	*Flavobacterium* spp, *Actinobacillus* spp, *Pasteurella aerogenes*
Rat	*Streptobacillus moniliformis, S. aureus*

Polymicrobial infection is the usual finding, and in such cases, broad-spectrum empirical antimicrobial coverage should be initiated promptly, after adequate drainage has been performed.[117] In a multicenter study of infected human bites, the median number of isolates per wound was four, and aerobes and anaerobes were identified. The common pathogens were *Streptococcus anginosus*, *S. aureus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Prevotella melaninogenica*. *Candida* spp. were noted less commonly.[131] Many strains of *Prevotella* and *S. aureus* are β-lactamase producers, and *E. corrodens* is intrinsically resistant to clindamycin and cephalosporins. Ampicillin with clavulanate given orally (simple infections) or ampicillin-sulbactam given intravenously for more complicated infections may be used as a single agent. Unusual pathogens rarely occur. A report of a human bite infection caused by *Mycobacterium ulcerans* is noted; this pathogen is emerging rapidly in West African countries.[35] Antimicrobial prophylaxis should be considered for patients with human bites to the hands, feet, and skin overlying joints and for patients with bites that penetrate deeper than the epidermal layer.[115]

Animal Bites

Animal bite wounds occur commonly, with more than 4 million reported annually in the United States (see also Chapter 248). Children account for more than half of patients who go to emergency departments for care of bite wounds.[2] Although one survey revealed that rodents and lagomorphs are the biting animals most commonly reported, wounds related to these bites seldom are associated with infection. By contrast, cats and dogs together account for approximately 40 percent of bites and their bites are associated more frequently with morbidity. The etiology of animal bite infections often is related to the species of animal involved (see Table 60A-2).[40,43,75,93]

Approximately 1000 emergency department visits related to dog bites occur each day, at a cost of more than $100 million/yr.[127] The incidence of infection related to dog bites has been estimated at 3 to 17 percent.[9,34] Cat bites are most likely to become infected, probably because of the puncture-like nature of the injury. One study suggests that half of cat bites result in infection, prompting recommendations for prophylaxis of such bites, especially if they involve the face or hands. In the presence of infection, exploration plus débridement of devitalized tissue is necessary, and purulent collections should be drained.

Although *Pasteurella multocida* is implicated most frequently, numerous other pathogens, including *S. aureus*, *Capnocytophaga canimorsus*, and other aerobic and anaerobic bacteria, are associated with dog and cat bites. Depending on the depth of the wound's penetration, underlying structures such as bones, joints, and tendons may be involved in such infections.[59] The wound itself may not appear significant, particularly in the case of puncture wounds, but several clinical features should influence treatment decisions. The presence of tissue edema and point tenderness on palpation over the site should signal that deeper structures may be involved. Soft tissue imaging may be necessary, and appropriate

drainage or débridement of involved sites should be pursued.

As with human bites, oral amoxicillin-clavulanate may be used for prophylaxis or treatment of simple infections. Indications for prophylaxis of bite wounds include bites associated with a crush or puncture injury and bites involving the face, hands, feet, and genitalia. Wounds in immunocompromised, especially asplenic, individuals should be considered for prophylaxis. Intravenous ampicillin-sulbactam or ticarcillin-clavulanate can be used for more serious infections. For individuals who have a history of anaphylaxis with penicillin or cephalosporins, a combination of clindamycin plus trimethoprim-sulfamethoxazole or ciprofloxacin can be used.[112,114] Antimicrobial therapy may be modified further depending on the biting animal and suspected pathogen (see Table 60A-2).[13]

Appropriate prophylaxis against tetanus should be considered for all bites. Hepatitis B can be transmitted by human bites, and appropriate management should be ensured for susceptible patients. Rabies vaccine should be administered after bat, skunk, or raccoon bites; public health information should be accessed to decide whether rabies vaccine is indicated for other animal bites.

TABLE 60A-3 **Infections Associated with Soil-Contaminated or Water-Contaminated Wounds**

Soil-Contaminated Wounds
Staphylococcus aureus
Group A β-hemolytic streptococci
Many gram-negative enterics
Enterobacter cancerogenus
Anaerobes
Nocardia asteroides, Nocardia otitidiscaviarum
Mycobacterium fortuitum, Mycobacterium abscessus
Actinomyces
Aspergillus spp
Enterococcus spp
Water-Contaminated Wounds
Aeromonas hydrophila
Pseudomonas spp
Many gram-negative enteric organisms
Edwardsiella tarda (catfish injury)
Mycobacterium marinum

Soil-Contaminated and Water-Contaminated Wounds

Four factors that must be considered in the acute care of contaminated wounds include the (1) mechanism of injury, (2) the length of time that transpires from injury to treatment, (3) the type of pathogens that occur in the environment, and (4) the presence of underlying disease in the host.[42] When a traumatic wound becomes infected, a polymicrobial etiology is typical, and common pathogens such as *S. aureus*, gram-negative enteric organisms, and anaerobes characteristically are involved.[1] Unusual and rare organisms, such as nontuberculous mycobacteria; *Nocardia; Actinomyces;* fungi, including *Aspergillus* spp.; and unusual gram-negative organisms, occasionally may be encountered (Table 60A-3).[84,98,99,101,137] *A. hydrophila* has been implicated in infections associated with injuries contaminated by fresh water and may produce rapidly progressive wound infection with fascia, tendon, muscle, bone, or joint involvement.[120]

Managing wounds contaminated by soil or water is difficult, especially if the mechanism of injury is a catastrophic event with complex bone and soft tissue injuries. In the acute setting of such an event, complete exploration and thorough débridement with copious irrigation are performed primarily, and signs of infection generally are not present. Days later, the clinician often is faced with the dilemma of a patient who is receiving antimicrobial prophylaxis with broad-spectrum agents and in whom new signs or symptoms develop acutely. Separating infectious from noninfectious complications often is difficult; however, the onset of fever in such a patient, especially in the setting of an open fracture or dural tear, should prompt further evaluation. Careful serial examination of the site of injury is necessary, and more extensive evaluation generally is indicated. Such assessment may include radiographic imaging and specific evaluation of body fluids with appropriate cultures. Because open fractures are associated with an increased risk for development of infection, the utility of antibiotic-impregnated implants, which are biodegradable and osteoconductive, may prove beneficial in such cases.[132,138]

Infections associated with foreign bodies such as wood generally cannot be cured until the foreign body is identified and removed.[140] Puncture wounds should be explored carefully, and further débridement of necrotic tissue or drainage of involved sites such as joints may be necessary.[8]

Treatment of simple wound infections associated with soil or water contamination should include an agent such as ciprofloxacin, although deeper tissue infection may develop after a seemingly innocuous injury, and tissue débridement may be necessary. Determining the appropriate therapy for a patient with infection involving extensive soft tissue injury and open fractures is problematic. Administration of an empirical regimen with an agent such as piperacillin-tazobactam, imipenem, or a fluoroquinolone plus vancomycin may be reasonable after evaluation and appropriate culturing have been done.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abbott SL, Janda JM. *Enterobacter cancerogenus* ("*Enterobacter taylorae*") infections associated with severe trauma or crush injuries. *Am J Clin Pathol* 1997;**107**:359–61.
2. Aghabian RV, Conte JE Jr. Mammalian bite wounds. *Ann Emerg Med* 1980;**9**:79–83.
3. Alves EV, Martins JE, Ribeiro EB, et al. *Pityrosporum* folliculitis: renal transplantation case report. *J Dermatol* 2000;**27**:49–51.
4. Amren DP, Anderson AS, Wannamaker LW. Perianal cellulitis associated with group A streptococci. *Am J Dis Child* 1966;**112**: 546–52.
5. Anthony BF, Perlman LV, Wannamaker LW. Skin infections and acute nephritis in American Indian children. *Pediatrics* 1967; **39**:263–79.
6. Aranow H, Wood WB. Staphylococcal infections simulating scarlet fever. *JAMA* 1942;**119**:1491–5.
7. Aroni K, Aivaliotis M, Davaris P. Disseminated and recurrent infundibular folliculitis (D.R.I.F.): report of a case successfully treated with isotretinoin. *J Dermatol* 1998;**25**:51–3.
8. Ashford RU, Sargeant PD, Lum GD. Septic arthritis of the knee caused by *Edwardsiella tarda* after a catfish puncture wound. *Med J Aust* 1998;**168**:443–4.
9. Avner JR, Baker MD. Dog bites in urban children. *Pediatrics* 1991;**88**:55–7.
10. Baker CJ. Group B streptococcal cellulitis-adenitis in infants. *Am J Dis Child* 1982;**136**:631–3.
11. Baker MD, Moore SE. Human bites in children: a six-year experience. *Am J Dis Child* 1987;**141**:1285–90.
12. Barnett BO, Frieden IJ. Streptococcal skin diseases in children. *Semin Dermatol* 1992;**11**:3–10.
13. Benaoudia F, Escande F, Simonet M. Infection due to *Actinobacillus lignieresii* after a horse bite. *Eur J Clin Microbiol Infect Dis* 1994; **13**:439–40.
14. Binnick AN, Klein RB, Baughman RD. Recurrent erysipelas caused by group B streptococcus organisms. *Arch Dermatol* 1980; **116**:798–9.
15. Bitnun S. Prophylactic antibiotics in recurrent erysipelas. *Lancet* 1985;**1**:345.
16. Bratcher D. Methicillin-resistant *Staphylococcus aureus* in the community. *Pediatr Infect Dis J* 2001;**20**:1167–8.
17. Britton JW, Fajardo JE, Krafte-Jacobs B. Comparison of mupirocin and erythromycin in the treatment of impetigo. *J Pediatr* 1990;**117**:827–9.
18. Brogan TV, Nizet V, Waldhausen JH, et al. Group A streptococcal necrotizing fasciitis complicating primary varicella: a series of fourteen patients. *Pediatr Infect Dis J* 1995;**14**:588–94.
19. Brook I, Frazier EH. Clinical and microbiological features of necrotizing fasciitis. *J Clin Microbiol* 1995;**33**:2382–7.
20. Brothers TE, Tagge DU, Stutley JE, et al. Magnetic resonance imaging differentiates between necrotizing and non-necrotizing fasciitis of the lower extremity. *J Am Coll Surg* 1998;**187**: 416–21.
21. Brown KL, Stein A, Morrell DS. Ecthyma gangrenosum and septic shock syndrome secondary to *Chromobacterium violaceum*. *J Am Acad Dermatol* 2006;**54**:S224–8.
22. Buescher ES, Hebert A, Rapini RP. Staphylococcal botryomycosis in a patient with the hyperimmunoglobulin E-recurrent infection syndrome. *Pediatr Infect Dis J* 1988;**7**:431–3.
23. Bukharie HA, Abdelhadi MS, Saeed IA, et al. Emergence of methicillin-resistant *Staphylococcus aureus* as a community pathogen. *Diagn Microbiol Infect Dis* 2001;**40**:1–4.
24. Cawley MJ, Briggs M, Haith LR Jr, et al. Intravenous immunoglobulin as adjunctive treatment for streptococcal toxic shock syndrome associated with necrotizing fasciitis: case report and review. *Pharmacotherapy* 1999;**19**:1094–8.
25. Centers for Disease Control and Prevention. Orf virus infection in humans: New York, Illinois, California, and Tennessee, 2004-2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:65–8.
26. Chandrasekar PH, Rolston KV, Kannangara DW, et al. Hot tub-associated dermatitis due to *Pseudomonas aeruginosa:* case report and review of the literature. *Arch Dermatol* 1984;**120**:1337–40.
27. Chastain MA. A cycle: recurrent gram-negative folliculitis with *Citrobacter diversus (koseri)* following eradication of recurrent staphylococcal pyoderma. *Arch Dermatol* 2000;**136**:803.
28. Combs JT. Perianal streptococcal disease. *Clin Pediatr (Phila)* 2000;**39**:500.
29. Cox NH, Knowles MA, Porteus ID. Pre-septal cellulitis and facial erysipelas due to *Moraxella* species. *Clin Exp Dermatol* 1994;**19**: 321–3.
30. Crickx B, Chevron F, Sigal-Nahum M, et al. [Erysipelas: epidemiological, clinical and therapeutic data (111 cases)]. *Ann Dermatol Venereol* 1991;**118**:11–16.
31. Dada-Adegbola HO, Oluwatoba OA. Cutaneous myiasis presenting as chronic furunculosis: case report. *West Afr J Med* 2005;**24**: 346–7.
32. Dagan R, Bar-David Y. Double-blind study comparing erythromycin and mupirocin for treatment of impetigo in children: implications of a high prevalence of erythromycin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 1992;**36**:287–90.
33. Dajani AS, Ferrieri P, Wannamaker LW. Natural history of impetigo. II. Etiologic agents and bacterial interactions. *J Clin Invest* 1972;**51**:2863–71.
34. de Melker HE, de Melker RA. [Dog bites: publications on risk factors, infections, antibiotics and primary wound closure]. *Ned Tijdschr Geneeskd* 1996;**140**:709–13.
35. Debacker M, Zinsou C, Aguiar J, et al. First case of *Mycobacterium ulcerans* disease (Buruli ulcer) following a human bite. *Clin Infect Dis* 2003;**36**:e67–8.
36. Del Pozo J, Garcia-Silva J, Almagro M, et al. Ecthyma gangrenosum-like eruption associated with *Morganella morganii* infection. *Br J Dermatol* 1998;**139**:520–1.
37. Demidovich CW, Wittler RR, Ruff ME, et al. Impetigo: current etiology and comparison of penicillin, erythromycin, and cephalexin therapies. *Am J Dis Child* 1990;**144**:1313–15.
38. Dillon HC Jr. The treatment of streptococcal skin infections. *J Pediatr* 1970;**76**:676–84.
39. Duvanel T, Merot Y, Harms M, et al. Prophylactic antibiotics in erysipelas. *Lancet* 1985;**1**:1401.
40. Ejlertsen T, Gahrn-Hansen B, Sogaard P, et al. *Pasteurella aerogenes* isolated from ulcers or wounds in humans with occupational exposure to pigs: a report of 7 Danish cases. *Scand J Infect Dis* 1996;**28**:567–70.
41. Eriksson BK. Anal colonization of group G beta-hemolytic streptococci in relapsing erysipelas of the lower extremity. *Clin Infect Dis* 1999;**29**:1319–20.
42. Eron LJ. Targeting lurking pathogens in acute traumatic and chronic wounds. *J Emerg Med* 1999;**17**:189–95.
43. Escande F, Bailly A, Bone S, et al. *Actinobacillus suis* infection after a pig bite. *Lancet* 1996;**348**:888.
44. Esterly NB, Markowitz M. The treatment of pyoderma in children. *JAMA* 1970;**212**:1667–70.
45. Esterly NB, Nelson DB, Dunne WM Jr. Impetigo. *Am J Dis Child* 1991;**145**:125–6.
46. Falcone PA, Pricolo VE, Edstrom LE. Necrotizing fasciitis as a complication of chickenpox. *Clin Pediatr (Phila)* 1988;**27**:339–43.
47. Farber S, Vawter GF. Clinical pathological conference. The Children's Hospital Medical Center, Boston, Mass. *J Pediatr* 1966;**69**:485–9.
48. Farrell LD, Karl SR, Davis PK, et al. Postoperative necrotizing fasciitis in children. *Pediatrics* 1988;**82**:874–9.
49. Feldman CA. Staphylococcal scarlet fever. *N Engl J Med* 1962; **267**:877–8.
50. Fergie JE, Patrick CC, Lott L. Pseudomonas aeruginosa cellulitis and ecthyma gangrenosum in immunocompromised children. *Pediatr Infect Dis J* 1991;**10**:496–500.
51. Ferrieri P, Dajani AS, Wannamaker LW, et al. Natural history of impetigo. I. Site sequence of acquisition and familial patterns of spread of cutaneous streptococci. *J Clin Invest* 1972;**51**:2851–62.
52. Fine JD, Miller JA, Harrist TJ, et al. Cutaneous lesions in disseminated candidiasis mimicking ecthyma gangrenosum. *Am J Med* 1981;**70**:1133–5.
53. Forbes N, Rankin AP. Necrotizing fasciitis and non steroidal anti-inflammatory drugs: a case series and review of the literature. *N Z Med J* 2001;**114**:3–6.
54. Ford LM, Waksman J. Necrotizing fasciitis during primary varicella. *Pediatrics* 2000;**105**:1372–3; author reply 3-5.
55. Fox AB, Hambrick GW Jr. Recreationally associated *Pseudomonas aeruginosa* folliculitis: report of an epidemic. *Arch Dermatol* 1984; **120**:1304–7.

56. Francis YF, Richman S, Hussain S, et al. *Aeromonas hydrophila* infection: ecthyma gangrenosum with aplastic anemia. *N Y State J Med* 1982;**82**:1461–4.
57. Frieden IJ. Blistering dactylitis caused by group B streptococci. *Pediatr Dermatol* 1989;**6**:300–2.
58. Givner LB, Abramson JS, Wasilauskas B. Apparent increase in the incidence of invasive group A beta-hemolytic streptococcal disease in children. *J Pediatr* 1991;**118**:341–6.
59. Goldstein EJ. Current concepts on animal bites: bacteriology and therapy. *Curr Clin Top Infect Dis* 1999;**19**:99–111.
60. Gonzalez A, Schachner LA, Cleary T, et al. Pyoderma in childhood. *Adv Dermatol* 1989;**4**:127–41; discussion 42.
61. Gorak EJ, Yamada SM, Brown JD. Community-acquired methicillin-resistant *Staphylococcus aureus* in hospitalized adults and children without known risk factors. *Clin Infect Dis* 1999; **29**:797–800.
62. Gosbell IB, Mercer JL, Neville SA, et al. Non-multiresistant and multiresistant methicillin-resistant *Staphylococcus aureus* in community-acquired infections. *Med J Aust* 2001;**174**:627–30.
63. Greene SL, Su WP, Muller SA. Ecthyma gangrenosum: report of clinical, histopathologic, and bacteriologic aspects of eight cases. *J Am Acad Dermatol* 1984;**11**:781–7.
64. Guberman D, Gilead LT, Zlotogorski A, et al. Bullous erysipelas: a retrospective study of 26 patients. *J Am Acad Dermatol* 1999; **41**:733–7.
65. Gucluer H, Ergun T, Demircay Z. Ecthyma gangrenosum. *Int J Dermatol* 1999;**38**:299–302.
66. Hall WD, Blumberg RW, Moody MD. Studies in children with impetigo: bacteriology, serology, and incidence of glomerulonephritis. *Am J Dis Child* 1973;**125**:800–6.
67. Halperin SA. *Haemophilus influenzae* type B and its role in diseases of the head and neck. *J Otolaryngol* 1990;**19**:169–74.
68. Hauger SB. Facial cellulitis: an early indicator of group B streptococcal bacteremia. *Pediatrics* 1981;**67**:376–7.
69. Hayden GF. Skin diseases encountered in a pediatric clinic: a one-year prospective study. *Am J Dis Child* 1985;**139**:36–8.
70. Hays GC, Mullard JE. Blistering distal dactylitis: a clinically recognizable streptococcal infection. *Pediatrics* 1975;**56**:129–31.
71. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998;**279**:593–8.
72. Hewitt WD, Farrar WE. Bacteremia and ecthyma caused by *Streptococcus pyogenes* in a patient with acquired immunodeficiency syndrome. *Am J Med Sci* 1988;**295**:52–4.
73. Hirn M, Niinikoski J. Management of perineal necrotizing fasciitis (Fournier's gangrene). *Ann Chir Gynaecol* 1989;**78**: 277–81.
74. Ho PW, Pien FD, Hamburg D. Value of cultures in patients with acute cellulitis. *South Med J* 1979;**72**:1402–3.
75. Hsieh S, Babl FE. *Serratia marcescens* cellulitis following an iguana bite. *Clin Infect Dis* 1999;**28**:1181–2.
76. Hsu CT, Lin YT, Yang YH, et al. The hyperimmunoglobulin E syndrome. *J Microbiol Immunol Infect* 2004;**37**:121–3.
77. Jackson MA, Colombo J, Boldrey A. Streptococcal fasciitis with toxic shock syndrome in the pediatric patient. *Orthop Nurs* 2003; **22**:4–8.
78. Jarvis WR. The epidemiology of colonization. *Infect Control Hosp Epidemiol* 1996;**17**:47–52.
79. Kaplan SL. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in children. *Semin Pediatr Infect Dis* 2006;**17**:113–19.
80. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
81. Kars M, van Dijk H, Salimans MM, et al. Association of furunculosis and familial deficiency of mannose-binding lectin. *Eur J Clin Invest* 2005;**35**:531–4.
82. Kelly C, Taplin D, Allen AM. Streptococcal ecthyma: treatment with benzathine penicillin G. *Arch Dermatol* 1971;**103**:306–10.
83. Kimyai-Asadi A, Tausk FA, Nousari HC. Ecthyma secondary to herpes simplex virus infection. *Clin Infect Dis* 1999;**29**:454–5.
84. Kocher MS, Coombs CJ, Upton J 3rd. Actinomycetoma of the phalanx in an immunocompromised patient: a case report. *J Hand Surg Am* 1996;**21**:515–17.
85. Kokx NP, Comstock JA, Facklam RR. Streptococcal perianal disease in children. *Pediatrics* 1987;**80**:659–63.
86. Lacroix J, Cau D, Pascal C, et al. [A case of erysipelas caused by *Haemophilus influenzae*]. *Union Med Can* 1983;**112**:272.
87. Lapins J, Sartorius K, Emtestam L. Scanner-assisted carbon dioxide laser surgery: a retrospective follow-up study of patients with hidradenitis suppurativa. *J Am Acad Dermatol* 2002;**47**: 280–5.
88. Larson EL, Hughes CA, Pyrek JD, et al. Changes in bacterial flora associated with skin damage on hands of health care personnel. *Am J Infect Control* 1998;**26**:513–21.
89. Le J, Lieberman JM. Management of community-associated methicillin-resistant *Staphylococcus aureus* infections in children. *Pharmacotherapy* 2006;**26**:1758–70.
90. Lesko SM, O'Brien KL, Schwartz B, et al. Invasive group A streptococcal infection and nonsteroidal antiinflammatory drug use among children with primary varicella. *Pediatrics* 2001;**107**: 1108–15.
91. Levy R, Schlaeffer F. Successful treatment of a patient with recurrent furunculosis by vitamin C: improvement of clinical course and of impaired neutrophil functions. *Int J Dermatol* 1993;**32**: 832–4.
92. Levy R, Shriker O, Porath A, et al. Vitamin C for the treatment of recurrent furunculosis in patients with impaired neutrophil functions. *J Infect Dis* 1996;**173**:1502–5.
93. Lion C, Conroy MC, Dupuy ML, et al. Pasteurella "SP" group infection after a guinea pig bite. *Lancet* 1995;**346**:901–2.
94. Loebl EC, Marvin JA, Curreri PW, et al. Survival with ecthyma gangrenosum, a previously fatal complication of burns. *J Trauma* 1974;**14**:370–7.
95. Mandell IN, Feiner HD, Price NM, et al. *Pseudomonas cepacia* endocarditis and ecthyma gangrenosum. *Arch Dermatol* 1977;**113**: 199–202.
96. Marks VJ, Maksimak M. Perianal streptococcal cellulitis. *J Am Acad Dermatol* 1988;**18**:587–8.
97. McLinn S. A bacteriologically controlled, randomized study comparing the efficacy of 2% mupirocin ointment (Bactroban) with oral erythromycin in the treatment of patients with impetigo. *J Am Acad Dermatol* 1990;**22**:883–5.
98. Meredith FT, Sexton DJ. *Mycobacterium abscessus* osteomyelitis following a plantar puncture wound. *Clin Infect Dis* 1996;**23**:651–3.
99. Mereghetti L, van der Mee-Marquet N, Dubost AF, et al. *Nocardia otitidiscaviarum* infection of a traumatic skin wound. *Eur J Clin Microbiol Infect Dis* 1997;**16**:383–4.
100. Milstein P, Gleckman R. Pneumococcal erysipelas: a unique case in an adult. *Am J Med* 1975;**59**:293–6.
101. Miron D, El AL, Zuker M, et al. *Mycobacterium fortuitum* osteomyelitis of the cuboid after nail puncture wound. *Pediatr Infect Dis J* 2000;**19**:483–5.
102. Mogielnicki NP, Schwartzman JD, Elliott JA. Perineal group A streptococcal disease in a pediatric practice. *Pediatrics* 2000;**106**: 276–81.
103. Moyer CD, Sykes PA, Rayner JM. *Aeromonas hydrophila* septicaemia producing ecthyma gangrenosum in a child with leukaemia. *Scand J Infect Dis* 1977;**9**:151–3.
104. Murphy JJ, Granger R, Blair GK, et al. Necrotizing fasciitis in childhood. *J Pediatr Surg* 1995;**30**:1131–4.
105. Murphy O, Marsh PJ, Gray J, et al. Ecthyma gangrenosum occurring at sites of iatrogenic trauma in pediatric oncology patients. *Med Pediatr Oncol* 1996;**27**:62–3.
106. Nissenson AR, Baraff LJ, Fine RN, et al. Poststreptococcal acute glomerulonephritis: fact and controversy. *Ann Intern Med* 1979; **91**:76–86.
107. O'Brien K, Schwartz B, Lesko SM, et al. Necrotizing fasciitis during primary varicella. *Pediatrics* 2000;**105**:1373–5.
108. Ohana N, Keness J, Verner E, et al. Skin-isolated, community-acquired *Staphylococcus aureus:* in vitro resistance to methicillin and erythromycin. *J Am Acad Dermatol* 1989;**21**:544–6.
109. Patel M, Ahrens JC, Moyer DV, et al. Pneumococcal soft-tissue infections: a problem deserving more recognition. *Clin Infect Dis* 1994;**19**:149–51.
110. Pouryousefi A, Foland J, Michie C, et al. Ecthyma gangrenosum as a very early herald of acute lymphoblastic leukaemia. *J Paediatr Child Health* 1999;**35**:505–6.
111. Rabinowitz R, Lewin EB. Gangrene of the genitalia in children with pseudomonas sepsis. *J Urol* 1980;**124**:431–2.
112. Rahmouni A, Chosidow O, Mathieu D, et al. MR imaging in acute infectious cellulitis. *Radiology* 1994;**192**:493–6.

113. Redbord KP, Shearer DA, Gloster H, et al. Atypical *Mycobacterium* furunculosis occurring after pedicures. *J Am Acad Dermatol* 2006; **54**:520–4.
114. Rhie S, Turcios R, Buckley H, et al. Clinical features and treatment of *Malassezia folliculitis* with fluconazole in orthotopic heart transplant recipients. *J Heart Lung Transplant* 2000;**19**: 215–9.
115. Rittner AV, Fitzpatrick K, Corfield A. Best evidence topic report: are antibiotics indicated following human bites? *Emerg Med J* 2005;**22**:654.
116. Rodot S, Lacour JP, van Elslande L, et al. Ecthyma gangrenosum caused by *Klebsiella pneumoniae*. *Int J Dermatol* 1995;**34**:216–7.
117. Rolle U. *Haemophilus influenzae* cellulitis after bite injuries in children. *J Pediatr Surg* 2000;**35**:1408–9.
118. Roujeau JC. [Necrotizing fasciitis: clinical criteria and risk factors]. *Ann Dermatol Venereol* 2001;**128**:376–81.
119. Schwarz G, Sagy M, Barzilay Z. Multifocal necrotizing fasciitis in varicella. *Pediatr Emerg Care* 1989;**5**:31–3.
120. Semel JD, Trenholme G. *Aeromonas hydrophila* water-associated traumatic wound infections: a review. *J Trauma* 1990;**30**:324–7.
121. Sevinsky LD, Viecens C, Ballesteros DO, et al. Ecthyma gangrenosum: a cutaneous manifestation of *Pseudomonas aeruginosa* sepsis. *J Am Acad Dermatol* 1993;**29**:104–6.
122. Shama S, Calandra GB. Atypical erysipelas caused by group G streptococci in a patient with cured Hodgkin's disease. *Arch Dermatol* 1982;**118**:934–6.
123. Shopsin B, Mathema B, Martinez J, et al. Prevalence of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in the community. *J Infect Dis* 2000;**182**:359–62.
124. Sidwell RU, Ibrahim MA, Bunker CB. A case of common variable immunodeficiency presenting with furunculosis. *Br J Dermatol* 2002;**147**:364–7.
125. Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infect Dis* 2007;**44**:178–85.
126. Smith KJ, Neafie R, Yeager J, et al. Micrococcus folliculitis in HIV-1 disease. *Br J Dermatol* 1999;**141**:558–61.
127. Smith PF, Meadowcroft AM, May DB. Treating mammalian bite wounds. *J Clin Pharm Ther* 2000;**25**:85–99.
128. Sokol RJ, Bowden RA. An erysipelas-like scalp cellulitis due to *Haemophilus influenzae* type b. *J Pediatr* 1980;**96**:60–1.
129. Sosin DM, Gunn RA, Ford WL, et al. An outbreak of furunculosis among high school athletes. *Am J Sports Med* 1989;**17**:828–32.
130. Stone L, Codere F, Ma SA. Streptococcal lid necrosis in previously healthy children. *Can J Ophthalmol* 1991;**26**:386–90.
131. Talan DA, Abrahamian FM, Moran GJ, et al. Clinical presentation and bacteriologic analysis of infected human bites in patients presenting to emergency departments. *Clin Infect Dis* 2003;**37**: 1481–9.
132. Thomas DB, Brooks DE, Bice TG, et al. Tobramycin-impregnated calcium sulfate prevents infection in contaminated wounds. *Clin Orthop Relat Res* 2005;**441**:366–71.
133. Turnbull D, Parry MF. Ecthyma-like skin lesions caused by *Staphylococcus aureus*. *Arch Intern Med* 1981;**141**:689.
134. Umbert IJ, Winkelmann RK, Oliver GF, et al. Necrotizing fasciitis: a clinical, microbiologic, and histopathologic study of 14 patients. *J Am Acad Dermatol* 1989;**20**:774–81.
135. Vaillant L. [Diagnostic criteria for erysipelas]. *Ann Dermatol Venereol* 2001;**128**:326–33.
136. Varghese R, Melo JC, Chun CH, et al. Erysipelas-like syndrome caused by *Streptococcus pneumoniae*. *South Med J* 1979;**72**:757–8.
137. Wenger PN, Brown JM, McNeil MM, et al. *Nocardia farcinica* sternotomy site infections in patients following open heart surgery. *J Infect Dis* 1998;**178**:1539–43.
138. Wenke JC, Owens BD, Svoboda SJ, et al. Effectiveness of commercially-available antibiotic-impregnated implants. *J Bone Joint Surg Br* 2006;**88**:1102–4.
139. Wilfert CM. Epidemiology of *Haemophilus influenzae* type b infections. *Pediatrics* 1990;**85**:631–5.
140. Zentner J, Hassler W, Petersen D. A wooden foreign body penetrating the superior orbital fissure. *Neurochirurgia (Stuttg)* 1991; **34**:188–90.
141. Zerr DM, Rubens CE. NSAIDS and necrotizing fasciitis. *Pediatr Infect Dis J* 1999;**18**:724–5.
142. Zerr DM, Alexander ER, Duchin JS, et al. A case-control study of necrotizing fasciitis during primary varicella. *Pediatrics* 1999; **103**:783–90.
143. Zittergruen M, Grose C. Magnetic resonance imaging for early diagnosis of necrotizing fasciitis. *Pediatr Emerg Care* 1993;**9**: 26–8.

60B VIRAL AND FUNGAL SKIN INFECTIONS

Meena R. Julapalli • Moise L. Levy

VIRAL INFECTIONS

Cutaneous manifestations of viral infections are common. Exanthems often accompany acute viral infections. This chapter focuses on the common viral illnesses that manifest with cutaneous manifestations. More detailed information on each of these illnesses can be found in the chapters devoted to specific viral agents.

Viruses can invade the skin indirectly as a result of viremia or directly, as with warts or molluscum. Other mechanisms of producing exanthems include interactions between the infecting agent and humoral or cell-mediated factors or a systemic immune response in the absence of viral antigen in the skin.[63]

The type of exanthem encountered depends on the virus, the pathophysiologic mechanisms involved, the location of the eruption, and local and systemic immune factors. A single virus may manifest with a variety of cutaneous reactions within the same host. Clinicians caring for a patient with an exanthem usually are faced with a challenge to determine its cause. Most viral exanthems are benign and self-limited, but understanding the exact cause can be important when evaluating immunocompromised patients or in the setting of a fetus. Similarly, distinguishing a viral exanthem from skin eruptions caused by other infectious agents or drugs is important.

Generally, children presenting with fever and a rash cannot be diagnosed accurately by the presentation of the eruption.[29] The distribution and character of exanthems, such as the often characteristic reticulated erythema after "slapped cheek" exanthem typical of parvovirus B19 infections, are helpful; however, the virus can manifest with several other cutaneous findings.[10,45,48] Typical hand, foot, and mouth syndrome is caused by coxsackievirus, although it has been related more recently to enterovirus 71.[12] The concurrent administration of antibiotics in the setting of Epstein-Barr virus infection is important to know, as is the possible relationship between human herpesvirus infection and certain drugs resulting in drug rash with eosinophilia and systemic symptoms.[13]

Laboratory studies, in addition to specific viral cultures, polymerase chain reaction, and serologies, may be helpful in distinguishing between viral and bacterial diseases or drug eruptions. Coagulation studies may help clarify whether a purpuric eruption is caused by a primary coagulopathy or a viral process. Other laboratory tests, such as streptococcal screens or throat cultures or both, serum antibody titers, viral cultures, and antigen detection methods, are required for evaluating exanthems, depending on the state of the patient being evaluated.

WARTS

See Chapter 154.

MOLLUSCUM CONTAGIOSUM

Molluscum contagiosum represents a common viral infection of the skin (and sometimes mucous membranes) of children and adolescents. It is caused by a poxvirus and has not been grown in culture. The virus replicates within the cytoplasm[15] and is represented by four viral types based on DNA analysis: MCV-1 to MCV-4.[42] MCV-1 accounts for more than 90 percent of infections in the United States.[15,42] The subtypes do not show site specificity. MCV-2 was reported to be seen most commonly in patients infected with human immunodeficiency virus (HIV).[17,42]

Epidemiology

The disease is seen with increasing frequency in sexually active and immunodeficient individuals. Infection occurs at any age, with the highest frequency seen in patients younger than 5 to 10 years old.[9,15] The disease is two to three times more common in school-aged and sexually active males than in females. Transmission is via close contact. Spread is reported to occur via fomites. The incidence of disease is highest in warm climates and in areas of overcrowding.

Clinical Manifestations

The incubation period for molluscum contagiosum is 2 to 7 weeks, but it has been reported to occur at 1 week of age. Typical lesions are 1 to 5 mm, dome-shaped, skin-colored or pink papules with a distinctive central umbilication (Fig. 60B-1). Giant lesions 1 to 2 cm can be seen. According to an epidemiologic survey conducted at three pediatric dermatology centers, fewer than 15 lesions usually are seen.[15] In children, molluscum contagiosum is seen most frequently on the face, neck, trunk, and extremities but may be seen on any part of the body, including the mucous membranes.[9,15,41] Periocular lesions may lead to secondary keratoconjunctivitis or trachoma. In some cases, molluscum contagiosum is a sexually transmitted infection, raising the issue of sexual abuse when infection is seen in the genital area. The most common etiology of genital molluscum contagiosum is autoinoculation; however, if the lesions occur solely in the genital area, or a question exists about the patient's social situation, the possibility of abuse should be explored.

Atypical lesions of molluscum contagiosum are seen more commonly, particularly with the improved survival rates of patients with acquired immunodeficiency syndrome (AIDS) and other immunocompromised states.

FIGURE 60B-1 ■ Grouped dome-shaped papules of molluscum contagiosum on the skin of the abdomen. (See companion Expert Consult website for color version.)

FIGURE 60B-2 ■ Diffusely distributed, isolated, monomorphous skin-colored papules on the thigh of a child typical of Gianotti-Crosti syndrome.

The incidence of molluscum contagiosum in HIV infection is 5 to 18 percent. With improved antiretroviral therapies, molluscum contagiosum has decreased in this population.[11,15]

Molluscum contagiosum lesions in immunocompromised patients often are large, are situated more deeply in the epidermis, and may number in the hundreds. HIV-positive adults usually have molluscum contagiosum on the face, neck, and trunk. In a study of immunocompromised children, molluscum contagiosum was not considered to be common or severe, however. This study included only six patients, and two were disease-free and thought to be immunocompetent at the time of onset of the infection. This and other studies suggest that the presence and degree of cellular immunodeficiency are important in the presentation of molluscum contagiosum.

Patients with atopic dermatitis may be predisposed to develop more severe molluscum contagiosum infection. Available information is insufficient to know if this development is related to the dermatitis itself with its well-described barrier dysfunction or to the use of corticosteroids or other topical agents. The term *molluscum dermatitis* is used to describe an eczematous eruption, more commonly seen in patients with atopic dermatitis, which may occur around molluscum contagiosum lesions and is thought to represent a delayed-type hypersensitivity reaction to viral antigens in the dermis.

Molluscum contagiosum infections in healthy individuals usually resolve spontaneously over the course of several months to 3 to 5 years. For this reason, limited infections can be observed expectantly. More diffuse infections or infections that show continued extension can be considered for treatment. No definitive treatments are available for molluscum contagiosum. Most treatments, similar to those used for warts, are destructive. Immune enhancement for these infections has been tried, but its utility has not been proved. Multiple treatments have been used, although utility is uncertain in all but curettage.

PARVOVIRUS B19 INFECTIONS

See Chapter 151.

GIANOTTI-CROSTI SYNDROME (PAPULAR ACRODERMATITIS OF CHILDHOOD)

Gianotti-Crosti syndrome (papular acrodermatitis of childhood) is a viral exanthem first reported in 1955 by Gianotti,[26] who described a group of children who presented with predominately acrally located, skin-colored or slightly erythematous, lichenoid papules (Fig. 60B-2).[68] The flexures usually were spared. Involvement of the buttocks or trunk was seen in some cases. No involvement of mucous membranes occurred. Patients generally were healthy. The initial reports showed prior infection with hepatitis B. The eruption lasted 2 weeks to 2 months.

Since the initial report, similar cases have been associated with other infections, such as Epstein-Barr virus, coxsackievirus, echovirus, other viruses, and streptococcal disease.[47] Most cases are thought to be viral in origin and often caused by Epstein-Barr virus. Topical therapies do not hasten resolution of the exanthem.

ASYMMETRIC PERIFLEXURAL VIRAL EXANTHEM

Some children have been described with discrete or grouped lichenoid papules or urticarial papules beginning around flexures or the torso (Fig. 60B-3).[25] The eruption generalizes over several days and can appear as a more diffuse case of the papular acrodermatitis of childhood. The condition also has been described as the unilateral laterothoracic viral exanthem and seems to be associated with the same infectious agents as described in papular acrodermatitis of childhood.[7] The evolution of this condition is similar to that in the papular acrodermatitis of childhood.

FIGURE 60B-3 ■ Lichenoid papules involving the lateral trunk typical of the unilateral periflexural viral exanthem. (See companion Expert Consult website for color version.)

HAND, FOOT, AND MOUTH SYNDROME

See Chapter 165.

HERPES SIMPLEX VIRUS

See Chapter 157.

VARICELLA-ZOSTER VIRUS

See Chapter 161.

FUNGAL INFECTIONS

Superficial fungal infections, which include dermatophytes, yeast, or dematiaceous fungi, are encountered frequently in general pediatric practice. Particularly in an immunocompromised patient, fungal infections also may involve deeper cutaneous structures and other organ systems. This section focuses on the skin manifestations, diagnosis, and treatment of pediatric fungal infections.

SUPERFICIAL FUNGAL INFECTIONS

Dermatophyte Infections

Dermatophytes invade and grow in hair, nails, and the outer layer of the skin known as the *stratum corneum*. These lesions vary in appearance based on the site of infection and are named accordingly.

Tinea Capitis

Also known as *scalp ringworm*, tinea capitis is seen frequently in prepubertal children. The causative organisms vary according to country; in North America, tinea capitis is caused largely by *Trichophyton tonsurans* and less often by *Microsporum canis*.[16,56,70] *M. canis*, a zoophilic dermatophyte common to suburban and rural areas, can be transmitted to children who handle infected animals such as cats, dogs, and certain rodents; however, it does not spread between humans.[56] Thick, white patches of broken hair that fluoresce under Wood lamp examination characterize these lesions.

Human-to-human transmission of *T. tonsurans* occurs through the shedding of spores from infected skin scales and hair on items such as clothing, bed linen, combs, and hairbrushes. Lesions caused by *T. tonsurans* do not fluoresce under a Wood lamp and vary in appearance from diffuse scaling of the scalp to circumscribed areas of scaling and erythema with or without hair loss, and, in dark-haired individuals, to scaly patches of hair loss that may leave small dark hairs in the follicles (black-dot ringworm) (Fig. 60B-4). Tinea capitis lesions may be associated with suboccipital or posterior cervical lymphadenopathy and may produce more inflammatory lesions, including scaly, pustular areas of hair loss and formation of a kerion—a boggy, erythematous mass with follicular pustules that may lead to permanent hair loss and scarring if left untreated (Fig. 60B-5).[16,35,70] Another hypersensitivity response to the dermatophyte infection is a dermatophytid or id reaction, which involves papulovesicular

FIGURE 60B-4 ■ Nonscarring alopecia associated with tinea capitis. (See companion Expert Consult website for color version.)

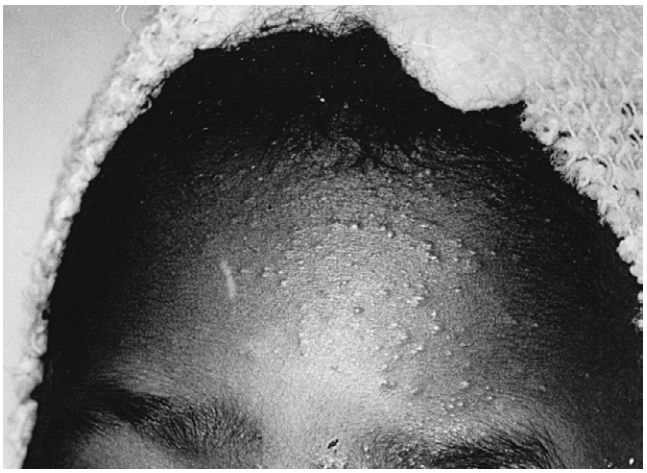

FIGURE 60B-6 ■ Multiple skin-colored papules and pustules on the forehead typical of an id reaction to tinea capitis.

FIGURE 60B-5 ■ Indurated tumor on the scalp typical of kerion. (See companion Expert Consult website for color version.)

FIGURE 60B-7 ■ Diffuse scaling plaques on the face caused by tinea faciei. (See companion Expert Consult website for color version.)

inflammatory eruptions adjacent to or distant from the site of the primary infection (Fig. 60B-6).[6]

Tinea capitis must be distinguished from alopecia areata, which consists of patches of total hair loss without scalp changes, and trichotillomania, which may be associated with excoriations, varying lengths of broken hairs, and ill-defined patterns of hair loss. Differential diagnosis also includes traction alopecia, seborrheic dermatitis, psoriasis, and pityriasis amiantacea. In addition, inflammatory lesions of tinea capitis may be confused with bacterial pyodermas of the scalp.[35,56,70]

Tinea Corporis

Tinea corporis is dermatophyte infection of the trunk or extremities. Lesions appear as erythematous patches or plaques with well-demarcated, annular borders that may be scaly, pustular, or vesicular.[35,56,70] The application of potent topical corticosteroids may obscure these characteristic borders, resulting in a manifestation termed *tinea incognito*.[56] The differential diagnosis includes seborrheic, atopic, and contact dermatitides; psoriasis; and granuloma annulare.[35,70]

Tinea Faciei

Dermatophyte infections of the face in children are characterized by erythematous, scaly plaques that may be unilateral or occur in a "butterfly" distribution, mimicking cutaneous findings of systemic lupus erythematosus and other collagen vascular diseases (Fig. 60B-7). As with

FIGURE 60B-8 ■ Annular scaling plaque on the upper inner thigh caused by tinea cruris. (See companion Expert Consult website for color version.)

tinea corporis, differential diagnosis includes seborrheic, atopic, and contact dermatitides.[35,70]

Tinea Pedis

Tinea pedis, which occurs more commonly in adolescents than in prepubertal children, is dermatophyte infection involving the feet. Findings vary and may be vesicles or erosions over the instep of the feet, fissuring between the toes with surrounding erythema and scaling, or diffuse scaling of the soles in a "moccasin-like" distribution. Differential diagnosis includes juvenile plantar dermatosis, dyshidrotic eczema, atopic dermatitis, contact dermatitis, granuloma annulare, scabies, psoriasis, and erythrasma.[35,56,70]

Tinea Cruris

Also predominately seen in adolescents, particularly boys, tinea cruris often occurs in association with tinea pedis and is transmitted through indirect or direct contact with infected skin scales or hair. Risk factors include obesity, friction, moisture, and tight clothing. The characteristic lesions are usually symmetric, well-demarcated, erythematous, scaly eruptions in the inguinal and inner thigh area with potential spread to the buttocks and perianal region. These lesions also may have a raised papular or pustular edge and often are very pruritic, leading to lichenification in chronic disease (Fig. 60B-8). The differential diagnosis includes seborrheic dermatitis, *Candida* intertrigo, flexural psoriasis, irritant dermatitis, contact dermatitis, and erythrasma.[35,56,70]

Tinea Unguium

The term *onychomycosis* refers to fungal invasion of the nail plate, which is termed *tinea unguium* when the infection is caused by dermatophytes. The lower incidence of tinea unguium in children compared with adults may be due to faster nail growth, decreased likelihood of nail trauma, and less exposure to tinea pedis, which often is seen concurrently with onychomycosis.[37,60] Clinical findings of infection include superficial white patches on the surface of the nail plate, white spots under the nail, and yellowing and thickening of the nail plate that usually extends from the distal to the proximal end (Figs. 60B-9 and 60B-10). The differential diagnosis includes psoriasis, lichen planus, hereditary onychodystrophy, and acquired trachyonychia, all of which may be distinguished from tinea unguium by their diffuse nail involvement as opposed to the more limited disease of a dermatophyte infection. Tinea unguium also may mimic *Candida* infection of the nails, but the latter disease differs in that it tends to spread distally from the proximal nail plate.*

FIGURE 60B-9 ■ Clinical photograph illustrating onychodystrophy and subungual hyperkeratosis involving the left thumb of a child with onychomycosis.

FIGURE 60B-10 ■ White superficial plaques typical of superficial white onychomycosis. (See companion Expert Consult website for color version.)

*References 16, 30, 35, 37, 60, 70.

Diagnosis

Light microscopy examination with potassium hydroxide should show spores in infected scalp hairs of tinea capitis and branching hyphae in affected nail samples or skin scrapings from advancing margins of lesions on the body (Fig. 60B-10). Fungal culture on Sabouraud dextrose agar treated with chloramphenicol and cycloheximide provides a growth medium selective for dermatophytes and may aid in differentiating various species. Rarely, biopsy may be required for definitive diagnosis.[16,35,56,70]

Treatment

Localized lesions of tinea corporis, faciei, pedis, and cruris generally respond to a 2- to 4-week course of topical antifungals, such as clotrimazole, tolnaftate, ciclopirox olamine, amorolfine, or terbinafine.[16] Severe or refractory disease may require oral antifungal medications. Recurrence of tinea pedis can be prevented by avoiding occlusive footwear and keeping the feet clean and dry.[56]

Griseofulvin is the only oral antifungal medication approved by the U.S. Food and Drug Administration (FDA) for use in children. Many agents, including terbinafine, itraconazole, and fluconazole, have been used successfully as off-label alternatives, however, despite limited data regarding their safety and efficacy in children.

Griseofulvin remains the gold standard for the treatment of tinea capitis in the United States. The approved dose of microsized griseofulvin is 11 mg/kg/day for 6 to 8 weeks; however, 20 to 25 mg/kg/day often is required to achieve an adequate response.[64] A 2- to 4-week course of terbinafine also has been shown to be as effective as griseofulvin, particularly in the treatment of tinea capitis caused by *Trichophyton* spp.[21,23,33,65] For *Microsporum* infections, higher doses or longer duration of therapy with terbinafine may be required.[21,23,33,43,65] Studies have shown that a regimen of fluconazole 6 mg/kg/day for 3 to 6 weeks has a cure rate comparable to that of griseofulvin.[22,33,65] Continuous or pulse itraconazole therapy of 5 mg/kg/day has been noted to result in significant improvement and even cure in *Trichophyton* and *Microsporum* tinea capitis by 6 weeks; however, infection with *Microsporum* may require a longer duration of treatment with itraconazole compared with effective courses for *Trichophyton* infection.[27,33,31] In addition, concomitant topical therapy with selenium sulfide 1 to 2.5 percent shampoo in affected individuals and household contacts reduces the number of viable spores and prevents the spread of infection.[28,56,59]

At present, no antifungal agent is approved by the FDA for treatment of onychomycosis in children, and data are limited regarding efficacy of topical and oral antifungal agents. Topical agents have poor penetration into the nail plate, usually leading to an inadequate response rate. Treating concurrent tinea pedis infections with topical therapy may help prevent recurrences of tinea unguium, however. Although griseofulvin is the drug of choice for treating other dermatophyte infections, it is not recommended for the treatment of onychomycosis because of the lengthy course (≤18 months), inadequate response, and high recurrence rate.

Terbinafine 3 to 6 mg/kg/day for 6 to 12 weeks, continuous or pulse itraconazole 5 mg/kg/day for 3 months, and fluconazole 3 to 6 mg/kg once a week for 12 to 26 weeks have been reported as safe, effective, and well-tolerated alternatives.[30,36,50,65] Because of their keratinophilic and lipophilic natures, these agents accumulate in the stratum corneum and persist at high concentrations in the nails for months, allowing for shorter courses of therapy.[30,31,36,37,65] Terbinafine may be crushed and taken with or without food. Optimal bioavailability with itraconazole capsules occurs when taken with fatty foods, whereas itraconazole solution should be taken on an empty stomach and may be associated with a higher incidence of gastrointestinal side effects than capsules. Studies regarding use of fluconazole for treatment of onychomycosis in children are limited, but successful therapy has been reported at a pulsed dose of 3 to 6 mg/kg once a week for 3 months.[30,36,65]

Candida

The most common cause of fungal infection in healthy and immunocompromised children is *Candida*, and *C. albicans* accounts for most cases. These yeast forms have a predilection for moist, warm areas of the body such as mucosal surfaces and intertriginous regions and often colonize these areas, becoming part of the normal flora. Disease is caused by overgrowth and infiltration into the epidermis. Superficial infections may be seen in healthy individuals and individuals with predisposing risk factors, such as prematurity; low-weight; diabetes mellitus; antibiotic, corticosteroid, or oral contraceptive therapy; and other immunocompromised states. These individuals also are more likely to develop systemic candidiasis.[16,24,70]

Thrush is characterized by superficial, sometimes tender, white plaques on oral mucosa that reveal denuded, erythematous bases when scraped off (Fig. 60B-11). These manifestations must be distinguished from other oral cavity lesions, such as aphthous stomatitis, epidermolysis bullosa, herpes simplex, hairy leukoplakia, lichen planus, geographic tongue, erythema multiforme, and burns.[70] Use of pacifiers in infants may increase the risk for development of thrush and colonization of the organism.[5] Oropharyngeal candidiasis may spread to the

FIGURE 60B-11 ■ White patches on buccal mucosa caused by *Candida albicans*. (See companion Expert Consult website for color version.)

FIGURE 60B-12 ■ Clinical photograph of an infant with *Candida* diaper dermatitis. Confluent and discrete erythematous papules and plaque involving the scrotum, penis, and superpubic and inguinal area.

FIGURE 60B-14 ■ Onychodystrophy involving the thumb and index finger, with erythema and edema of the paronychium. These features are typical of *Candida* paronychia.

FIGURE 60B-13 ■ Dense crusting overlying the flank and buttocks of a neonate with *Candida* dermatitis. (From Rowen JL, Atkins JT, Levy ML, et al. Invasive fungal dermatitis in the 1000-gram neonate. *Pediatrics* 1995;**95**:682–7.)

esophagus in immunocompromised individuals and lead to difficulty with feeding.

Diaper candidiasis appears as beefy, erythematous plaques that involve the inguinal creases and perianal region and often are associated with satellite (or surrounding) erythematous papules, plaques, or pustules (Figs. 60B-12 and 60B-13). These lesions may cause discomfort when the infant urinates onto affected skin. Superinfection with *Candida* should be suspected when irritant diaper dermatitis fails to improve within several days. Other lesions that may be confused with diaper candidiasis include psoriasis, Langerhans cell histiocytosis, and seborrheic dermatitis.[70]

Thumb-sucking or other trauma near nail beds may lead to nontender, erythematous swelling at the base of the nails termed *paronychia* (Fig. 60B-14). Some cases are complicated by *Candida* infection. Differential diagnosis of these lesions includes psoriasis, bacterial infection, lichen planus, and pachyonychia congenita. *Candida* also may produce onychomycosis, but its distribution usually involves the proximal nail plate, in contrast to dermatophytosis of the nails, which more commonly affects the distal nail plate.[70]

Adolescent girls may present with vulvovaginal candidiasis, which is characterized by thick, white discharge and white plaques on irritated, erythematous vaginal mucosa. These lesions may cause vaginal itching and dysuria.[70]

Diagnosis of localized *Candida* infection usually is made clinically. Microscopic examination of potassium hydroxide–prepared scrapings of skin and mucosal lesions reveals pseudohyphae and elongated budding yeast forms. Positive cultures of infected material on Sabouraud dextrose agar or cornmeal agar may signify infection or benign colonization. Biopsy rarely is required for establishing a definitive diagnosis.

Treatment of localized *Candida* infection depends on good hygiene practices and maintenance of a dry environment in the susceptible area. Localized infections usually respond well to topical antifungal therapy. Nystatin solution is the first-line agent used for thrush, but oral fluconazole or itraconazole and intravenous amphotericin B may be required to treat recurrent, refractory, or extensive disease, especially in immunocompromised children.[19] Nystatin or imidazole creams in conjunction with frequent diaper changes, barrier creams, and sometimes low-strength topical steroids are the recommended treatment for diaper candidiasis. Paronychia also is susceptible to nystatin and imidazole creams but may require oral therapy. Vulvovaginal candidiasis often can be treated successfully with topical azole medications, but oral azoles are recommended for refractory or recurrent cases. Adolescents can be treated with a single, 150-mg dose of oral fluconazole.[19,54]

Congenital candidiasis, a rare form of infection acquired in utero as a result of *Candida* chorioamnionitis, can manifest in full-term and premature neonates within the first few days of life as a diffuse, erythematous, papular eruption that progresses to formation of vesicles and

pustules, and subsequently crusting and desquamation. Palms and soles also may be affected. Term infants with congenital candidiasis rarely develop systemic manifestations, and the infection usually resolves spontaneously or with topical antifungal agents, such as nystatin, imidazoles, or allylamines. Premature neonates with very low birth-weight are at risk for developing ecchymosis and necrosis of the skin with dissemination of the *Candida* infection, requiring treatment with systemic antifungal medication. Congenital candidiasis in these premature infants may manifest as sepsis or pneumonia without cutaneous findings as well.[1,16,67]

Differential diagnosis of congenital candidiasis includes epidermolysis bullosa and infection with *Listeria monocytogenes*, *Staphylococcus*, herpesvirus, and syphilis.[14] Early diagnosis is established by histologic examination of the placenta and umbilical cord, which shows the characteristic pseudohyphae and spores and white microabscesses.[1,14] Microscopic examination and culture of the skin lesions can confirm the diagnosis.

FIGURE 60B-15 ■ Pustules and papules on the face and scalp owing to neonatal cephalic pustulosis. (See companion Expert Consult website for color version.)

Malassezia

Malassezia spp., most notably *Malassezia furfur*, are lipophilic yeast forms that are found more readily in humid, tropical environments and are part of the normal skin flora in humans. Colonization begins in the neonatal period and increases with age.[4] *Malassezia* is the organism thought to be responsible for neonatal cephalic pustulosis and tinea (or pityriasis) versicolor in older children, adolescents, and young adults. Invasion of the organism through indwelling catheters, particularly catheters being used for intralipid infusion in premature infants with very low birth-weight also may lead to sepsis and death.[3,58,67] *Malassezia* also has been implicated in the pathogenesis of seborrheic dermatitis, atopic dermatitis, and psoriasis, although this implication is controversial.*

Neonatal cephalic pustulosis is a self-limited disease characterized by scattered, erythematous papules and pustules in a head-and-neck distribution (Fig. 60B-15). Follicular accentuation and comedones are not observed. Diagnosis usually is made clinically, but may be confirmed by microscopic findings.[4,16,67]

Tinea versicolor is characterized by macules with fine scales that are distributed on the upper body and neck. The macules may appear hypopigmented in dark-skinned individuals or tan or dark brown in lighter skinned individuals (Fig. 60B-16). Differential diagnosis includes vitiligo, pityriasis alba, pityriasis rosea, postinflammatory hypopigmentation, melasma, seborrheic dermatitis, contact dermatitis, and tinea corporis. Lesions of tinea versicolor are fluorescent yellow to orange under Wood lamp. Microscopic examination of potassium hydroxide–prepared scrapings of the lesions shows a "spaghetti and meatball" appearance owing to the presence of short, curved hyphae and round spores. Biopsy of the lesions, which usually is unnecessary, reveals the characteristic hyphae and spores invading the stratum corneum.

The recommended treatment is application of 2.5 percent selenium sulfide or ketoconazole shampoo. Other topical agents, such as ciclopirox, terbinafine solutions, sodium hypochlorite, and other azoles, also may be effective. Short 1- to 5-day courses of oral itraconazole, fluconazole, and ketoconazole in single daily doses have been suggested as effective treatments; however, these agents are not approved by the FDA for treating tinea versicolor in children because of their limited safety and efficacy profiles.[6,18,55] It is important to advise patients that skin discolorations may take months to resolve and that recurrences are very common and may require retreatment.[16,35,55,67,70] *Malassezia* sepsis responds well to removal of indwelling catheters and rarely requires systemic antifungal therapy.[67]

FIGURE 60B-16 ■ Well-circumscribed annular scaling plaques on the neck typical of tinea versicolor. (See companion Expert Consult website for color version.)

Chromoblastomycosis

Fonsecaea pedrosoi, and less commonly *Cladophialophora carrionii*, and *Phialophora verrucosa*, are found in tropical

*References 3, 32, 35, 46, 49, 62, 69.

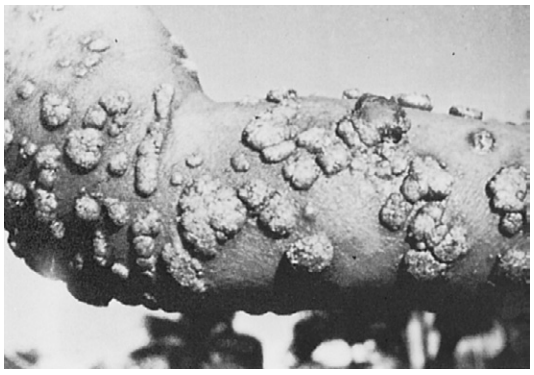

FIGURE 60B-17 ■ Chromoblastomycosis. The wart-like growth of epidermis and dermis is a result of traumatic implantation of the etiologic agent and subsequent autoinoculation from scratching. The agent was *Fonsecaea pedrosoi*.

FIGURE 60B-18 ■ Brown patch on the palm of the hand caused by tinea nigra palmaris. (See companion Expert Consult website for color version.)

climates and are organisms responsible for causing chromoblastomycosis, a chronic granulomatous disease affecting skin and subcutaneous tissue. Infection is acquired through traumatic inoculation into the skin. Cutaneous manifestations are described as pink papules that progressively enlarge to become tender, pruritic, hyperkeratotic nodules or verrucous plaques (Fig. 60B-17). Scratching may lead to secondary infection or result in autoinoculation of the organism to other areas of the body. Infection may lead to scarring of lymphatic vessels causing obstruction and lymphedema.[8,20,52,57]

Differential diagnosis includes tuberculosis, sporotrichosis, blastomycosis, leishmaniasis, and squamous cell carcinomas. Potassium hydroxide smears of skin scrapings reveal characteristic dark brown, round, sclerotic bodies with horizontal or vertical septa and thick, pigmented hyphae. Sclerotic bodies also are identified easily on hematoxylin and eosin stain. In addition, biopsy of the chromoblastomycosis lesions shows granulomatous inflammation with macrophages and neutrophils and a hyperkeratotic, hyperplastic epidermis with microabscesses.[8,20,57]

Treatment of localized lesions involves surgical excision, cryotherapy, or heat therapy. Chemotherapy with 5-fluorocytosine and antifungal agents such as azoles, amphotericin B, and terbinafine has been used with some success in more widespread disease.[8,20,66]

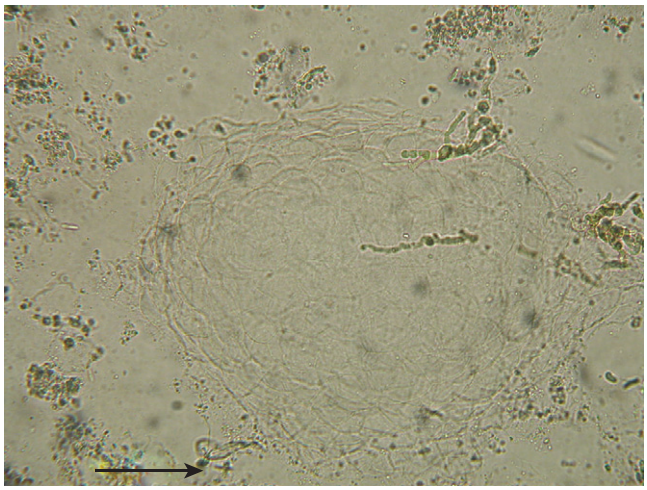

FIGURE 60B-19 ■ Septate hyphae seen by potassium hydroxide examination of scale from the patient in Figure 60B-18. (See companion Expert Consult website for color version.)

Tinea Nigra

Tinea nigra, caused by the organism *Hortaea werneckii*, is a superficial fungal infection seen primarily in children and adolescents.[35,44,51] The organism is found in soil, wood, sewage, salted dried fish, and vegetation, mainly in beach environments of Central and South America, Africa, Asia, and, less frequently, in the United States and Europe. Transmission occurs by direct contact.[44,51,53]

Tinea nigra usually is characterized by an asymptomatic, well-demarcated, single brown to black macule or patch primarily on the palm, but may involve other areas of the body as well (Fig. 60B-18). Lesions grow slowly and usually are nonscaly.[35,51] Tinea nigra lesions may mimic melanoma, lentigines, junctional nevi, and the hyperpigmented patches of Addison disease.[35,51,53] Under microscopic examination, potassium hydroxide–prepared scrapings of the lesion show yellow to light brown, branched, septate hyphae (Fig. 60B-19).[35,53] Diagnosis is confirmed further by culture, which grows oval to spindle-shaped, moist, shiny, black colonies on Sabouraud agar.[51]

Tinea nigra has been treated successfully with keratolytic ointments or solutions, which include salicylic acid and benzoic acid and azole and allylamine creams.[35,51,53] Scraping the lesion with a scalpel also can remove the discoloration.[53]

Trichosporonosis

Trichosporon spp., which are found in soil, vegetation, animal feces, and stagnant or fresh water, have a worldwide

distribution with a predilection for tropical climates. They also may colonize human skin and digestive, respiratory, and urinary tracts. In immunocompetent individuals, *Trichosporon* organisms produce superficial fungal infections of the hair shaft termed *white piedra* and, less commonly, are one of the causes of onychomycosis. Systemic disease, termed *trichosporonosis*, may develop in immunocompromised patients, particularly neutropenic patients with hematologic malignancies, and in premature infants with very low birth-weight with colonization of indwelling catheters.[16,38]

White piedra is described as soft white, tan, or reddish-green nodules encircling the hair shaft that may be associated with scalp hyperkeratosis, brittle hair, and alopecia. These concretions do not fluoresce under Wood lamp examination. Differential diagnosis includes tinea capitis, pediculosis, black piedra, monilethrix, trichomycosis axillaris or pubis, and trichorrhexis nodosa.[38,71] Skin manifestations of disseminated infection include desquamation and serous drainage, purpuric papules or nodules with central ulceration or necrosis, and white plaques.[16,61]

Fungal blood culture reveals germ tube–negative and urease-positive yeast. Potassium hydroxide mounts of affected hair show hyaline septate hyphae, pseudohyphae, rectangular arthroconidia, and spherical blastoconidia that circumscribe the hair shaft.[20,40,61] Culture on Sabouraud dextrose agar shows cream-colored colonies that become wrinkled in a few weeks.[39] Biopsy specimen of skin lesions shows perivascular inflammation with budding yeast cells.[34]

White piedra is a difficult disease to treat because of the high recurrence rate. Although the American Academy of Dermatology Guidelines Committee recommends shaving affected hair to eradicate white piedra,[15] cosmetic and social considerations render this approach an unfavorable option for most patients. Topical antifungal agents that have been tried with varying degrees of success include ciclopirox olamine, imidazoles, chlorhexidine solution, 2.5 percent sulfide shampoo, zinc pyrithione, Castellani paint, mercury bichloride solution, 5 percent sulfur ointment, 5 percent ammoniated mercury ointment, and amphotericin B lotion. Although topical treatments may clear hair shaft concretions, they are not as effective at preventing recurrence. Because of their ability to bind keratin and their excretion in sebum, oral antifungal agents such as itraconazole may achieve higher concentrations in hair follicles and lead to greater success in eliminating colonized *Trichosporon* organisms and preventing relapse.[39,40]

Disseminated trichosporonosis is associated with high morbidity and mortality rates. It has been treated with varying degrees of success using amphotericin B with or without 5-fluorocytosine and the newer azole drugs, such as voriconazole.[2,38]

DEEP FUNGAL INFECTIONS

Aspergillosis

See Chapter 197. See Figure 60B-20.

FIGURE 60B-20 ■ Eschar overlying eroded skin on a child with invasive aspergillosis. (See companion Expert Consult website for color version.)

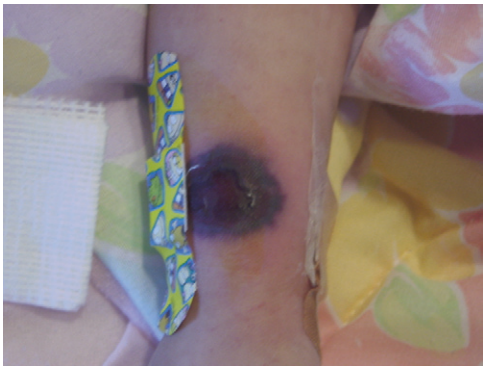

FIGURE 60B-21 ■ Purpura and ulceration in a patient with mucormycosis. (See companion Expert Consult website for color version.)

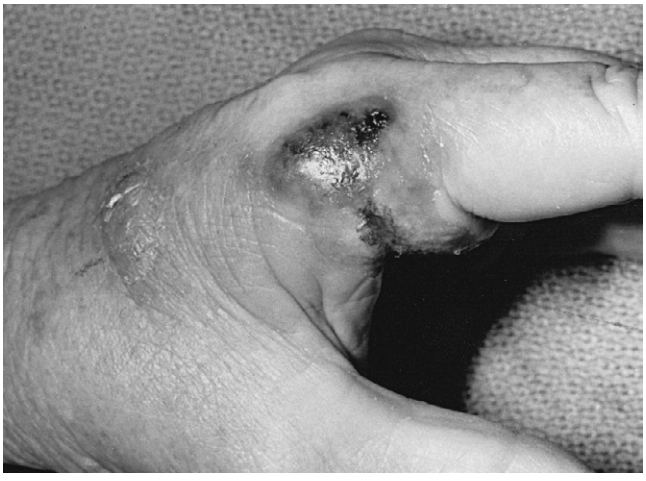

FIGURE 60B-22 ■ Two erythematous nodules on the dorsum of the left hand of a patient with sporotrichosis.

Blastomycosis

See Chapter 198.

Coccidioidomycosis

See Chapter 200.

Cryptococcosis

See Chapter 202.

Fusariosis

See Chapter 206.

Histoplasmosis

See Chapter 203.

Mucormycosis

See Chapter 205. See Figure 60B-21.

Sporotrichosis

See Chapter 204. See Figure 60B-22.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aldana-Valenzuela C, Morales-Marquec M, Castellanos-Martinez J, et al. Congenital candidiasis: a rare and unpredictable disease. *J Perinatol* 2005;**25**:680–2.
2. Antachopoulos C, Papakonstantinou E, Dotis J, et al. Fungemia due to *Trichosporon asahii* in a neutropenic child refractory to amphotericin B: clearance with voriconazole. *J Pediatr Hematol Oncol* 2005;**27**:283–5.
3. Ashbee HR, Evans EG. Immunology of diseases associated with *Malassezia* species. *Clin Microbiol Rev* 2002;**15**:21–57.
4. Bernier V, Weill FX, Hirigoyen V, et al. Skin colonization by *Malassezia* species in neonates: a prospective study and relationship with neonatal cephalic pustulosis. *Arch Dermatol* 2002;**138**:215–8.
5. Bessa CF, Santos PJ, Aguiar MC, et al. Prevalence of oral mucosal alterations in children from 0 to 12 years old. *J Oral Pathol Med* 2004;**33**:17–22.
6. Bhogal CS, Singal A, Baruah MC: Comparative efficacy of ketoconazole and fluconazole in the treatment of pityriasis versicolor: a one year follow-up study. *J Dermatol* 2001;**28**:535–9.
7. Bodemer C, de Prost Y. Unilateral laterothoracic exanthem in children: a new disease? *J Am Acad Dermatol* 1992;**27**:693–6.
8. Bonifaz A, Carrasco-Gerard E, Saul A. Chromoblastomycosis: clinical and mycologic experience of 51 cases. *Mycoses* 2001;**44**: 1–7.
9. Braue A, Ross G, Varigos G, et al. Epidemiology and impact of childhood molluscum contagiosum: a case series and critical review of the literature. *Pediatr Dermatol* 2005;**22**:287–94.
10. Butler GJ, Mendelsohn S, Franks A. Parvovirus B19 infection presenting as 'bathing trunk' erythema with pustules. *Australas J Dermatol* 2006;**47**:286–8.
11. Calista D, Boschini A, Landi G. Resolution of disseminated molluscum contagiosum with highly active anti-retroviral therapy (HAART) in patients with AIDS. *Eur J Dermatol* 1999;**9**:211–3.
12. Chen KT, Chang HL, Wang ST, et al. Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. *Pediatrics* 2007;**120**:e244–52.
13. Descamps V, Valance A, Edlinger C, et al. Association of human herpesvirus 6 infection with drug reaction with eosinophilia and systemic symptoms. *Arch Dermatol* 2001;**137**:301–4.
14. Diana A, Epiney M, Ecoffey M, et al. "White dots on the placenta and red dots on the baby": congenital cutaneous candidiasis—a rare disease of the neonate. *Acta Paediatr* 2004;**93**:996–9.
15. Dohil MA, Lin P, Lee J, et al. The epidemiology of molluscum contagiosum in children. *J Am Acad Dermatol* 2006;**54**:47–54.
16. Eichenfeld LF, Frieden IJ, Esterly NB. Fungal infections, infestations, and parasitic infections in neonates. In: *Textbook of Neonatal Dermatology*. Philadelphia: Saunders; 2001. p. 223–40.
17. Epstein WL. Molluscum contagiosum. *Semin Dermatol* 1992;**11**: 184–9.
18. Farschian M, Yaghoobi R, Samadi K. Fluconazole versus ketoconazole in the treatment of tinea versicolor. *J Dermatol Treat* 2002; **13**:73–6.
19. Knapp KM, Flynn PU. Candidiasis. In: Feigin RD, Cherry JD, Demmler GJ, et al, editors. *Textbook of Pediatric Infectious Diseases*. 5th ed. Philadelphia: Saunders; 2004. p. 2741–51.
20. Rowen JL. Miscellaneous mycoses. In: Feigin RD, Cherry JD, Demmler GJ, et al, editors. *Textbook of Pediatric Infectious Diseases*. 5th ed. Philadelphia: Saunders; 2004. p. 2821–751.
21. Fleece D, Gaughan JP, Aronoff SC. Griseofulvin versus terbinafine in the treatment of tinea capitis: a meta-analysis of randomized, clinical trials. *Pediatrics* 2004;**114**:1312–5.
22. Foster KW, Friedlander SF, Panzer H, et al. A randomized controlled trial assessing the efficacy of fluconazole in the treatment of pediatric tinea capitis. *J Am Acad Dermatol* 2005;**53**:798–809.
23. Friedlander SF, Aly R, Krafchik B, et al. Terbinafine in the treatment of *Trichophyton* tinea capitis: a randomized, double-blind, parallel-group, duration-finding study. *Pediatrics* 2002;**109**:602–7.
24. Fukushima C, Matsuse H, Tomari S, et al. Oral candidiasis associated with inhaled corticosteroid use: comparison of fluticasone and beclomethasone. *Ann Allergy Asthma Immunol* 2003;**90**: 646–51.
25. Gelmetti C, Caputo R. Asymmetric periflexural exanthem of childhood: who are you? *J Eur Acad Dermatol Venereol* 2001;**15**: 293–4.
26. Gianotti F. Papular acrodermatitis of childhood: an Australia antigen disease. *Arch Dis Child* 1973;**48**:794–9.
27. Ginter-Hanselmayer G, Smolle J, Gupta A. Itraconazole in the treatment of tinea capitis caused by *Microsporum canis:* experience in a large cohort. *Pediatr Dermatol* 2004;**21**:499–502.
28. Givens TG, Murray MM, Baker RC. Comparison of 1% and 2.5% selenium sulfide in the treatment of tinea capitis. *Arch Pediatr Adolesc Med* 1995;**149**:808–11.
29. Goodyear HM, Laidler PW, Price EH, et al. Acute infectious erythemas in children: a clinico-microbiological study. *Br J Dermatol* 1991;**124**:433–8.
30. Gupta AK, Skinner AR. Onychomycosis in children: a brief overview with treatment strategies. *Pediatr Dermatol* 2004;**21**:74–9.
31. Gupta AK, Cooper EA, Ginter G. Efficacy and safety of itraconazole use in children. *Dermatol Clin* 2003;**21**:521–35.
32. Gupta AK, Madzia SE, Batra R. Etiology and management of seborrheic dermatitis. *Dermatology* 2004;**208**:89–93.
33. Gupta AK, Adam P, Dlova N, et al. Therapeutic options for the treatment of tinea capitis caused by *Trichophyton* species: griseofulvin versus the new oral antifungal agents, terbinafine, itraconazole, and fluconazole. *Pediatr Dermatol* 2001;**18**:433–8.
34. Pierini A. Deep mycoses and opportunistic infections. In: Harper J, Orange A, Prose N. *Textbook of Pediatric Dermatology* Oxford: Blackwell; 2000.
35. Clayton Y. Superficial fungal infections. In: Harper J, Orange A, Prose N. *Textbook of Pediatric Dermatology* Oxford: Blackwell; 2000.
36. Heikkila H, Stubb S. Onychomycosis in children: treatment results of forty-seven patients. *Acta Derm Venereol* 2002;**82**:484–5.
37. Huang PH, Paller AS. Itraconazole pulse therapy for dermatophyte onychomycosis in children. *Arch Pediatr Adolesc Med* 2000;**154**: 614–8.
38. Kendirli T, Ciftci E, Ince E, et al. Successful treatment of *Trichosporon mucoides* infection with lipid complex amphotericin B and 5-fluorocytosine. *Mycoses* 2006;**49**:251–3.
39. Khandpur S, Reddy BS. Itraconazole therapy for white piedra affecting scalp hair. *J Am Acad Dermatol* 2002;**47**:415–8.
40. Kiken DA, Sekaran A, Antaya RJ, et al. White piedra in children. *J Am Acad Dermatol* 2006;**55**:956–61.
41. Kontoyiannis DP, Bodey GP, Hanna H, et al. Outcome determinants of fusariosis in a tertiary care cancer center: the impact of neutrophil recovery. *Leuk Lymphoma* 2004;**45**:139–41.
42. Laxmisha C, Thappa DM, Jaisankar TJ. Clinical profile of molluscum contagiosum in children versus adults. *Dermatol Online J* 2003;**9**:1.
43. Lipozencic J, Skerlev M, Orofino-Costa R, et al. A randomized, double-blind, parallel-group, duration-finding study of oral terbinafine and open-label, high-dose griseofulvin in children with tinea capitis due to *Microsporum* species. *Br J Dermatol* 2002;**146**: 816–23.

43a. Maronn M, Salm C, Lyon V, et al. One year experience with candida antigen immunotherapy for warts and molluscum. *Pedatir Dermatol* 2008;**25**:189–92.

44. McKinlay JR, Barrett TL, Ross EV. Picture of the month: tinea nigra. *Arch Pediatr Adolesc Med* 1999;**153**:305–6.
45. McNeely M, Friedman J, Pope E. Generalized petechial eruption induced by parvovirus B19 infection. *J Am Acad Dermatol* 2005;**52**:S109–13.
46. Nakabayashi A, Sei Y, Guillot J. Identification of *Malassezia* species isolated from patients with seborrhoeic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000;**38**:337–41.
47. Natarajan G, Lulic-Botica M, Rongkavilit C, et al. Experience with caspofungin in the treatment of persistent fungemia in neonates. *J Perinatol* 2005;**25**:770–7.
48. Nelson JS, Stone MS. Update on selected viral exanthems. *Curr Opin Pediatr* 2000;**12**:359–64.
49. Parry ME, Sharpe GR. Seborrhoeic dermatitis is not caused by an altered immune response to *Malassezia* yeast. *Br J Dermatol* 1998; **139**:254–63.
50. Partap R, Kaur I, Chakrabarti A, et al. Single-dose fluconazole versus itraconazole in pityriasis versicolor. *Dermatology* 2004;**208**: 55–9.
51. Pegas JR, Criado PR, Lucena SK, et al. Tinea nigra: report of two cases in infants. *Pediatr Dermatol* 2003;**20**:315–7.
52. Perez C, Colella MT, Olaizola C, et al. Tinea nigra: report of twelve cases in Venezuela. *Mycopathologia* 2005;**160**:235–8.

53. Perez-Blanco M, Hernandez Valles R, Garcia-Humbria L, et al. Chromoblastomycosis in children and adolescents in the endemic area of the Falcon State, Venezuela. *Med Mycol* 2006;**44**:467–71.
54. Pickering LK, Baker CJ, Long SS, et al. Candidiasis. In: *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 242–6.
55. Pickering LK, Baker CJ, Long SS, et al. Pityriasis versicolor. In: *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 522–3.
56. Pickering LK, Baker CJ, Long SS, et al. Tinea capitis, tinea corporis, tinea cruris, tinea pedis and tinea unguium. In: *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 654–60.
57. Pradhan SV, Talwar OP, Ghosh A, et al. Chromoblastomycosis in Nepal: a study of 13 cases. *Indian J Dermatol Venereol Leprol* 2007; **73**:176–8.
58. Prohic A, Ozegovic L. *Malassezia* species isolated from lesional and non-lesional skin in patients with pityriasis versicolor. *Mycoses* 2007;**50**:58–63.
59. Roberts BJ, Friedlander SF. Tinea capitis: a treatment update. *Pediatr Ann* 2005;**34**:191–200.
60. Romano C, Papini M, Ghilardi A, et al. Onychomycosis in children: a survey of 46 cases. *Mycoses* 2005;**48**:430–7.
61. Salazar GE, Campbell JR. Trichosporonosis: an unusual fungal infection in neonates. *Pediatr Infect Dis J* 2002;**21**:161–5.
62. Sandstrom Falk MH, Tengvall Linder M, Johansson C, et al. The prevalence of *Malessezia* yeasts in patients with atopic dermatitis, seborrhoeic dermatitis and healthy controls. *Acta Derm Venereol* 2005;**85**:17–23.
63. Mancini AJ, Wargon O. Viral infections. In: Schachner LA, Hansen RC, editors. *Pediatric Dermatology*. 4th ed. London: Mosby; 2003. p. 1425–69.
64. Scott LJ, Simpson D. Voriconazole: a review of its use in the management of invasive fungal infections. *Drugs* 2007;**67**: 269–98.
65. Sethi A, Antaya R. Systemic antifungal therapy for cutaneous infections in children. *Pediatr Infect Dis J* 2006;**25**:643–4.
66. Sharma NL, Sharma RC, Grover PS, et al. Chromoblastomycosis in India. *Int J Dermatol* 1999;**38**:846–51.
67. Smolinski KN, Shah SS, Honig PJ, et al. Neonatal cutaneous fungal infections. *Curr Opin Pediatr* 2005;**17**:486–93.
68. Taieb A, Plantin P, Du Pasquier P, et al. Gianotti-Crosti syndrome: a study of 26 cases. *Br J Dermatol* 1986;**115**:49–59.
69. Tollesson A, Frithz A, Stenlund K: Malassezia furfur in infantile seborrheic dermatitis. *Pediatr Dermatol* 1997;**14**:423–5.
70. Weston WL, Lane AT, Morelli JG. Fungal and yeast infections of the skin. In: Weston WL, Lane AT, Morelli JG, editors. *Color Textbook of Pediatric Dermatology*. 4th ed. London: Mosby; 2007. p. 81–96.
71. Youker SR, Andreozzi RJ, Appelbaum PC, et al. White piedra: further evidence of a synergistic infection. *J Am Acad Dermatol* 2003;**49**:746–9.

SECTION **XI**

OCULAR INFECTIOUS DISEASES

CHAPTER 61

Ocular Infections

Mohamed Hussein • Aaron M. Miller • Paul G. Steinkuller

The eye can be affected by infections and infestations manifested as primary diseases or as part of systemic processes. Some infections are vision-threatening, whereas others may have important implications for a generalized disease process. Ocular findings can help narrow the differential diagnosis list in some systemic diseases, such as congenital viral infections. A helpful approach in this regard is to use an anatomic scheme that reflects the primary site of ocular involvement. This chapter proceeds systematically from anterior to posterior, from infections involving the eyelids and superficial ocular surface to those involving deeper tissue and intraocular structures, and concludes with a discussion of infections involving the orbit. Some disease sites overlap, and duplications are therefore unavoidable.

Most infectious processes involving the eye can be diagnosed accurately by following the standard medical protocol: history, examination, laboratory analysis, differential diagnosis. The three simple basic tools—a visual acuity chart, a pen light, and a direct ophthalmoscope—facilitate establishing a diagnosis in most instances. In some cases, however, special ophthalmologic testing such as a slit-lamp examination, indirect ophthalmoscopy, ocular ultrasound, Gram stain and culture, or a biopsy are required. One of the cardinal rules in ophthalmology is *always* to measure the vision as the very first examination step.

INFECTIONS OF THE EYELIDS

The eyelids are composed of connective tissue, hair follicles, sweat and sebaceous glands, smooth and striated muscles, sensory and motor nerves, and vascular elements. The skin covering the eyelids is the thinnest of the entire body. The inner surface of the eyelid is covered by the palpebral conjunctiva. Bulbar conjunctiva covers the surface of the globe. Connecting these two conjunctival parts is the conjunctival fornix, which designates the most posterior limit of the conjunctiva. Sebaceous glands, the glands of Zeis, are associated with eyelid hair follicles. Meibomian glands, located within the tarsal plates of the eyelids, are also sebaceous and drain at the posterior aspect of the lid margin.[111]

Infection of the eyelids may be generalized or focal. Involvement of the skin by an infectious agent is referred to as *dermatoblepharitis*. *Staphylococcus aureus* and *Staphylococcus epidermidis* are the main infectious agents. The exception is angular blepharitis, inflammation at the lateral canthus, which is often caused by *Moraxella* spp. Impetigo or erysipelas of the eyelids may be caused by *Streptococcus pyogenes*. A variety of parasitic infestations, including *Demodex folliculorum*, *Sarcoptes scabiei* (scabies), *Pediculus capitis*, and the pubic louse, *Phthirus pubis*, may involve the eyelids.[155] Infection of the lid margin is more common in adults, but may occur in children. Infections of the anterior lid margin include bacterial blepharitis, molluscum contagiosum, and parasitic diseases. Infections of the posterior lid margin include those associated with chronic meibomian gland dysfunction. Herpes simplex virus (HSV) and herpes zoster virus may involve the lid and lid margin and are discussed elsewhere in this chapter.

Anterior Eyelid Infection

Staphylococcal Blepharitis

Staphylococcal eyelid infection may be acute or chronic. Typically, the chronic form of the disease manifests as erythema and crusting of the lid margins, especially on awakening. Thickening of the lid margin is a frequent manifestation. Mild chronic conjunctival infection often occurs as a spillover by local reaction to material secreted by the infecting organism. A child with staphylococcal blepharitis may be asymptomatic or may complain of ocular discomfort or a burning, itching, or foreign body sensation. Lid hygiene measures are frequently all that is required to treat the condition, consisting of eyelid scrubs with attention given to the lid margin. Warm tap water can be used, or, in refractory cases, the eyelids and lid margins can be cleansed once or twice daily with a 50:50 mixture of baby shampoo and warm water. This mixture is applied gently with a clean washcloth. Effective lid hygiene decreases the concentration of local bacterial flora and reduces or eliminates symptoms arising from chronic staphylococcal infection. A 2- to 4-week course of topical erythromycin, bacitracin, or azithromycin ophthalmic applied twice daily to the eyelid margin may hasten resolution and reduce recurrences. Ophthalmologic ointments may cause significant temporary blurring of vision; therefore, they often are used only at night before sleeping.

Molluscum Contagiosum Infection

Molluscum contagiosum is caused by members of the family *Poxviridae*. Infection of the eyelid may be unilateral or bilateral. Infection may be transmitted by skin-to-skin contact, through fomites, or by autoinoculation.

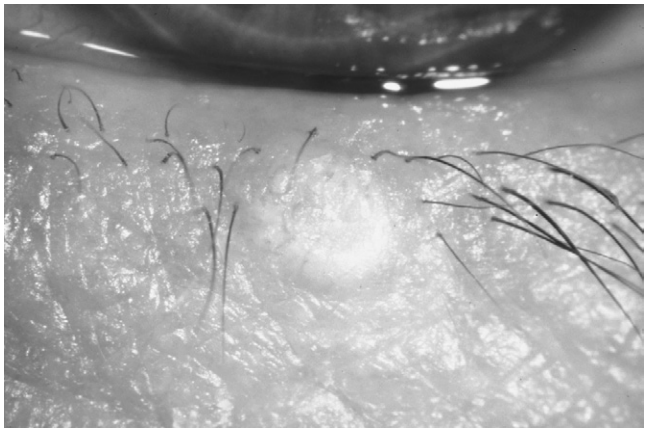

FIGURE 61-1 ■ *Molluscum contagiosum* typically is manifested as an isolated nodule or nodules approximately 1 to 3 mm in diameter. Older lesions often become umbilicated and develop a white or waxy-appearing core.

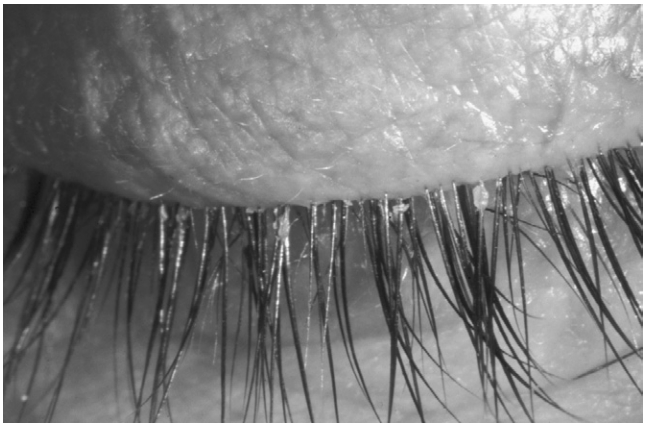

FIGURE 61-2 ■ Slit-lamp photo of *Phthirus pubis* infestation demonstrating adult lice firmly adherent to the eyelashes and egg cases (i.e., nits) attached to the proximal ends of the hair shafts.

Widespread development of lesions can occur as a result of autoinoculation. Epidemics in people who live in closed communities have been reported.[207] Eyelid manifestations typically include isolated nodules with mild surrounding inflammation (Fig. 61-1). The nodules are 1 to 3 mm in diameter. Older lesions frequently become umbilicated and develop a white or waxy-appearing core. Mild conjunctival injection frequently accompanies lesions on or near the margin of the eyelid. The associated conjunctivitis occasionally can be severe and chronic. In chronic cases, corneal epithelial disease may develop.

Molluscum infection is self-limited, with most lesions resolving spontaneously within a few months. However, the infection can be recalcitrant, especially in immunocompromised patients.[294,305] Chronic or atypical molluscum contagiosum lesions involving the eye have been reported in patients infected with human immunodeficiency virus (HIV).[183,245] In immunocompetent patients, treatment often is advocated to prevent autoinoculation, which can prolong the course of the disease. Treatment also may provide symptomatic relief. Mechanical treatment such as cryotherapy, expression or curettage of the central core, excision, and cautery may be effective.[54] Medical treatments effective against molluscum infection remote from the eye include 1 percent imiquimod cream[267] and podophyllotoxin.[266] These agents are not recommended for the treatment of periocular lesions, however, because of the possibility of injury to the eye.

Parasitic Eyelid Disease

Phthirus pubis *Infestation*

The crab louse *Phthirus pubis* is a tiny insect adapted to living in coarse, widely spaced hair. Infestation most commonly involves pubic, axillary, and body hair. The organism is transmitted by direct person-to-person contact, and perhaps by fomites.[255] Infestation of the eyelid often is a marker for sexually transmitted infections, especially in adult patients.[255] Affected patients typically have unilateral, chronic blepharoconjunctivitis; some patients may be asymptomatic. Although unmagnified ocular examination may reveal signs of the disease, diagnosis is facilitated by slit-lamp examination, which offers a magnified view of the eyelid margins and eyelashes (Fig. 61-2). Slit-lamp examination reveals adult lice firmly adherent to the eyelashes and egg cases (nits) attached to the proximal ends of the hair shafts. Reddish brown flecks of louse excreta may be found at the base of the cilia.[52] Treatment can be simple mechanical removal of the lice and nits under magnification (slit lamp or other devices.)

Medical treatment often is preferred for young children because of their inability to tolerate or permit mechanical removal. The organisms also can be smothered by the application of a bland ointment such as petrolatum jelly applied four times each day to the eyelids. Physostigmine ointment administered twice daily can be used. The agent inhibits nerve transmission in the insect and thus is directly toxic to the insect. It should be used with caution in infants and small children. If physostigmine comes into contact with the eye, it has several significant side effects, such as stimulation of accommodation, which can produce blurred distance vision that may last for several hours. Medical treatment should be continued for 2 weeks to ensure eradication of new lice that emerge from nits during the normal life cycle.[52] Lindane shampoo scrubs of the scalp, pubic hair, and body are recommended if infestation is found in these areas. Clothing and bed linen should be laundered, and family members should be examined and treated as necessary. Follow-up 4 to 6 weeks after treatment is recommended to detect reinfestation.

Demodex *Infection*

Demodex folliculorum and *Demodex brevis* are mites that frequently infest hair follicles in humans, including the hair follicles of the eyelids (Figure 61-3).[87,155] The organisms historically have been considered nonpathogenic parasites,[144] although it is thought they may cause increased hordeola formation as a result of obstruction of eyelid sebaceous glands.

Demodex is found in patients with rosacea, although a causal relationship is difficult to establish.[226] Rosacea-like

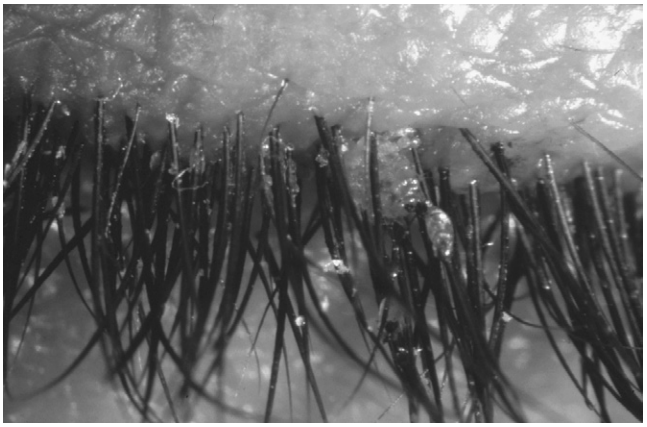

FIGURE 61-3 ■ *Demodex folliculorum* and *Demodex brevis* are mites that can infect the hair follicles of the eyelids and are found more commonly on eyelashes with cylindrical dandruff.

eruptions have been attributed to *Demodex*, and one pathologic report demonstrated a granulomatous dermal inflammation.[213] Treatment with topical pilocarpine gel may alleviate itching caused by *Demodex* infection.[97] Because of the frequency of *Demodex* infestation and the paucity of definitive disease caused by the organism, treatment often is unnecessary.

Posterior Eyelid Infection

Hordeolum

A hordeolum (i.e., stye) is an infection of the sebaceous glands in the eyelids. When the glands of Zeis are involved, the term *external hordeolum* is used. The lesion typically points to the skin surface. When the meibomian glands are involved, the term *internal hordeolum* is used. An internal hordeolum may point toward the skin or toward the palpebral conjunctiva. *S. aureus* is the most common causal agent of both internal and external hordeola.[159]

Patients with disease of the lid margin, such as those with chronic blepharitis, seborrhea, or rosacea, are prone to recurrent hordeola, especially during the first decade of life. Hordeola are manifested by erythematous, elevated, tender nodules. Nodules typically are 5 to 10 mm in diameter and usually solitary, although they may be multiple or bilateral. Patients with a history of recurrent hordeola may have them in various stages of evolution or resolution.

The lesions usually are self-limited and typically resolve within 5 to 7 days with spontaneous drainage of the abscess. Warm compresses may hasten resolution and improve comfort. Parents should be advised to place a clean washcloth soaked in warm tap water on the involved eyelid for 10 to 15 minutes several times each day. Parents should be advised to ensure that the water is not hot enough to cause a burn. For significant coexisting blepharitis, a topical antibiotic such as erythromycin ointment may prove useful by reducing the normal bacterial skin flora of the eyelid and decreasing the risk for recurrence. Systemic antibiotics rarely are indicated for acute hordeola. Children with frequent recurrences, however, may benefit from a short course of systemic antibiotics such as erythromycin (younger than 12 years) or tetracycline (older than 12 years). These agents further decrease the bacterial flora on the eyelid and may reduce the risk for recurrence even more. Lid hygiene efforts, as described for the treatment of staphylococcal blepharitis, should be instituted and maintained for children with recurrent hordeola. Surgical drainage of a hordeolum usually is unnecessary.

Chalazion

Cytologically, a chalazion may represent either a mixed-cell granulomatous inflammation or a suppurating granuloma.[69] The lesion occurs in a meibomian gland as a result of a foreign body reaction to secretions produced by the gland that have been extruded into surrounding tissue. A chalazion may develop after resolution of an internal hordeolum, in which case it is preceded by an acute stage, or it may develop primarily, without a preceding acute inflammatory phase. A typical chalazion appears as a round, nontender nodule within the substance of the eyelid. Multiple and bilateral chalazia may occur in susceptible patients. Recurrent lesions are not uncommon findings. They are typically 2 to 10 mm in diameter.[197]

Spontaneous resolution of smaller chalazia can be anticipated after a period of observation without treatment, sometimes as long as several months. Larger lesions, particularly those greater than 10 mm in diameter, frequently do not resolve without specific treatment. In patients with an acute or chronic inflammatory component, application of warm compresses may be beneficial. Surgical intervention may be warranted to treat medium to large chalazia that are cosmetically objectionable, produce astigmatism by pressing on the cornea, result in mechanical ptosis, or cause other symptoms. In rare circumstances, chalazia can result in vision loss from amblyopia.[76] The most common surgical treatment offered is incision and curettage through an internal incision on the palpebral conjunctival surface. Surgery is highly effective. It can be performed in the office, but general anesthesia is required for most young children. Because of the need for general anesthesia, we typically recommend deferring surgical treatment until several months have elapsed without spontaneous resolution. Earlier intervention may be recommended if the chalazion is particularly large, if pigmentary changes have developed in the overlying skin, or if astigmatism or ptosis is present in a young child at risk for the development of amblyopia.

Chalazia can be treated by intralesional steroid injection, surgical incision,[68] or both.[89] Dhaliwal and Bhatia[68] reported that incision plus curettage was the procedure of choice for lesions that have been present for 8.5 months or longer and for lesions 11.4 mm or larger in size, based on the results of a prospective study. The major potential drawback of intralesional steroid injection is the risk for complications developing with an otherwise relatively benign condition. Rarely, steroid injection can result in sterile abscess formation or eyelid necrosis. Intralesional

injections are done best with a chalazion clamp in place to protect the underlying globe from accidental needle trauma during injection. This device places a metal plate between the chalazion and the eye. If general anesthesia is required, incision and curettage is recommended over intralesional steroid injection.

Dacryoadenitis

Dacryoadenitis is an uncommon ophthalmic condition. Even in a busy ophthalmology practice, dacryoadenitis was diagnosed in approximately 1/10,000 patient visits.[230] The clinical manifestation is variable. Localized tenderness and swelling of the temporal aspect of the upper eyelid usually occur and often produce an "S"-shaped deformity of the lid margin. Pain is frequently a predominant feature. Associated signs and symptoms include fever, follicular conjunctivitis, mucopurulent discharge, limited extraocular movement, and proptosis.[230] Keratoconjunctivitis sicca has been reported as a consequence of Epstein-Barr virus (EBV)-associated dacryoadenitis in a child.[178]

Before the era of widespread immunization, mumps was a leading cause of dacryoadenitis. Bacteria such as staphylococci, streptococci, and gonococci also have been implicated. Exceedingly rare organisms such as *Brucella* are involved occasionally in bacterial cases.[16] EBV has been implicated as the etiologic agent in a large proportion of nonsuppurative cases. Marked regional lymphadenopathy may be a distinguishing feature of EBV dacryoadenitis.[230] Rare infectious causes include tuberculosis and Lyme disease.[198,240,243]

Appropriate laboratory evaluation includes Gram stain and culture of mucopurulent discharge from the eye. Neuroimaging is indicated when the patient has severe inflammation, proptosis, limitation of extraocular movement, or other orbital signs to rule out a more generalized orbital process. The condition easily can be confused clinically with orbital cellulitis when signs and symptoms are severe. Biopsy of the lacrimal gland may be required to confirm the diagnosis in patients not responding to standard medical treatment or when atypical features are identified on clinical examination or radiographic studies. Blood cultures are indicated for patients with signs of systemic toxicity.

Intravenous nafcillin or vancomycin is a reasonable initial antibiotic choice for severe dacryoadenitis caused by gram-positive organisms. Oral antistaphylococcal agents may be used for less severe cases. For gram-negative cases, ceftazidime or other similar agents should be considered. For suppurative cases without Gram-stain guidance, intravenous nafcillin or vancomycin should be considered as initial therapy for severe cases and oral antistaphylococcal agents for less acute cases.

Therapy in the form of warm compresses and oral analgesics is indicated for nonsuppurative dacryoadenitis. Serum testing for evidence of EBV infection should be considered in patients with regional lymphadenopathy.[230] Noninfectious causes of dacryoadenitis include sarcoidosis, Sjögren syndrome, leukemia, lymphoma, eosinophilic granuloma, autoimmune vasculitis (Churg-Strauss syndrome and Wegener granulomatosis), immunoglobulin (Ig)G4-related systemic disease, and orbital pseudotumor.[45,139,149] Noninfectious cases of dacryoadenitis sometimes can be difficult to distinguish from infectious cases. Lacrimal gland biopsy and neuroimaging usually are necessary to establish a diagnosis in noninfectious cases.[165] Intralesional steroid injection has been shown to be effective in the management of acute idiopathic dacryoadenitis; it results in marked improvement of symptoms within 7 days of treatment in most cases.[185]

Nasolacrimal Duct Obstruction

Simple membranous nasolacrimal duct obstruction occurs in an estimated 1 to 20 percent of the newborn population, and intermittent, mild, self-limited bacterial infection is a commonly associated feature.[92,110,164,200] It most commonly results from a blockage of the valve of Hasner, which is the opening from the lacrimal sac into the inferior nasal meatus. The signs of nasolacrimal duct obstruction consist of an increased tear lake, mucopurulent discharge, and epiphora. The periocular skin is sometimes mildly erythematous, with skin fissures in regions of pronounced irritation. The conjunctiva and sclera are usually white and are uninvolved. When pressure is applied over the lacrimal sac there is a reflux of mucopurulent material from the punctum.

More than 50 percent of congenital nasolacrimal duct obstructions resolve without surgical intervention.[196,211,215,221] Most cases can be observed with treatment limited to gentle lid hygiene and lacrimal sac massages. In more severe cases, medical treatment with a topical antibiotic is appropriate. The most common organisms identified in nasolacrimal duct obstruction cultures include *Streptococcus pneumoniae* and *Haemophilus influenzae*.[283] Most ophthalmologists prefer topical fluoroquinolones over aminoglycosides because they cause less ocular irritation and have better coverage of gram-positive organisms. When the condition fails to resolve, a probing procedure can be performed in the nasolacrimal duct system, which typically is successful in resolving the condition. This procedure is commonly performed in the operating room under general anesthesia but also can be performed in an office setting with child restraint when performed under 1 year of age.

Dacryocystitis

Dacryocystitis may result from congenital or acquired lacrimal outflow obstruction. More severe infection of the lacrimal sac results in dacryocystitis. This condition is manifested as acute erythema, pain, and swelling in the medial canthal region. The swelling typically is located below the medial canthal tendon. Marked epiphora generally is present, and a mucopurulent discharge frequently can be expressed through the lacrimal punctum if the proximal aspect of the lacrimal drainage system is not obstructed. If the infection has resulted in or from obstruction proximal and distal to the lacrimal sac, the overlying skin may become tense as the nasolacrimal sac distends in response to the infectious process. Formation of a fistula to the overlying skin may occur as a result of dacryocystitis, and surgical excision of the fistula tract

often is needed after the acute process has resolved. Dacryocystitis is a particularly common finding in neonates with a dacryocele, with dacryocystitis developing in as many as 60 percent of affected neonates.[212]

Aerobic and anaerobic bacteria and fungi may produce dacryocystitis.[34,36] In one study, *S. epidermidis* and *Pseudomonas* spp. were the aerobic organisms identified most frequently, and *Peptostreptococcus* spp. and *Propionibacterium* spp. were the anaerobes isolated most frequently. Less common agents include *Escherichia coli*, *Pseudomonas* spp., *H. influenzae*, *Pasteurella multocida*, and various anaerobes. Laboratory investigation should include aerobic and anaerobic culture of mucopurulent discharge from the lacrimal sac. Mild massage of the lacrimal sac may be used to facilitate expression of material for Gram stain and culture. A sepsis workup should be considered for children who are acutely ill and for young infants.

Intravenous nafcillin, vancomycin, or both are good initial therapeutic choices for serious gram-positive infections. Mild cases in older children can be treated with oral antibiotics. Intravenous ceftazidime is a reasonable initial antibiotic choice for gram-negative dacryocystitis. Oral ciprofloxacin is useful for less severe cases in adult patients, although the drug is not approved for use in children. Intravenous nafcillin or vancomycin typically provides good initial empirical therapy in patients when Gram-stain guidance is not available.

Ophthalmologic consultation should be requested in all cases of acute dacryocystitis. Decompression of the lacrimal sac by aspiration, incision, and drainage, or probing the proximal lacrimal drainage system may be needed to hasten resolution. Probing of the distal lacrimal system often is deferred until the acute infection has subsided, although probing during the acute infection has been reported to be a safe and effective adjunct to treatment.[219] Mucopurulent material obtained during surgical decompression should be sent for appropriate culture.

Preseptal (Periorbital) Cellulitis

The term *preseptal cellulitis* refers to an infectious process in the eyelids that is isolated to regions anterior to the orbital septum. The orbital septum is a thin layer of fascia that extends vertically from the periosteum of the orbital rim to the tarsal plate within the eyelids. Although it is penetrated by nerves and vascular structures, the septum provides a barrier that slows the spread of infectious agents into deeper orbital and retro-orbital structures.[262] Typical signs and symptoms of preseptal cellulitis include erythema and edema of the eyelids. Distinctively absent are signs of deeper orbital involvement such as restricted ocular motility, pain with eye movement, and proptosis. Preseptal cellulitis may occur after trauma or be caused by spread of infection from adjacent structures, such as skin and the upper respiratory system.[292]

Posttraumatic Preseptal Cellulitis

Posttraumatic preseptal cellulitis occurs after puncture wounds on the lids, face, or scalp. It also may occur after blunt trauma with no obvious entry wound. The etiologic agents most commonly identified are *S. aureus* and *S. pyogenes*, and polymicrobial infections can occur. Other bacterial causes include non–spore-forming anaerobes such as *Peptococcus*, *Peptostreptococcus*, and *Bacteroides*. Infection by aerobic gram-negative bacilli is an uncommon finding.[13] *P. multocida* is a common organism that produces posttraumatic preseptal cellulitis after dog and cat bites.[154,158] Cellulitis secondary to methicillin-resistant *S. aureus* (MRSA) is increasing in frequency and is a common cause of community-acquired cellulitis in some locations.

Clinical signs and symptoms are determined in large part by the severity of the injury, the interval since injury, and the infecting organisms. The involved lids are edematous, erythematous, and typically quite tender. Fluctuation of subcutaneous tissue may be seen if an abscess has developed. Swelling of the uninvolved contralateral eyelids may occur as a result of lymphedema. As with any form of isolated preseptal cellulitis, vision is unaffected and proptosis and eye movement disturbances are absent. On rare occasions, eyelid edema may be sufficiently severe to preclude adequate evaluation of the eye. Neuroimaging is required in such cases to assess the globe and rule out involvement of structures posterior to the orbital septum. Ophthalmologic consultation is critical in cases of severe posttraumatic preseptal cellulitis because of the potential for concurrent globe injury.

Laboratory analysis includes Gram stain and aerobic and anaerobic culture of mucopurulent material to aid in therapeutic decisions. Amoxicillin-clavulanate or a related agent is the drug of choice for treating posttraumatic cellulitis caused by a dog bite because of the high prevalence of *P. multocida*. Tetanus prophylaxis should be guided by standard recommendations. Surgical drainage of large abscesses may be required if a rapid response to antimicrobial therapy does not occur.

Nontraumatic Preseptal Cellulitis

The clinical features of nonsuppurative, nontraumatic preseptal cellulitis depend to a large degree on the causative agent. Erythema and swelling of the involved eyelids are typical and often are accompanied by pain. Signs of orbital infection, such as altered vision, proptosis, and eye movement abnormalities, are absent.

Before the advent of *H. influenzae* type b vaccine, this organism frequently was a cause of nonsuppurative preseptal cellulitis in children. *H. influenzae* type b is seen infrequently as a cause of preseptal cellulitis today.[73] It was a particularly dangerous agent because of a high risk for spread to the central nervous system (CNS), which occurred in as many as 2 to 3 percent of patients. Although rare today, *H. influenzae* type b infections still may be encountered. Like many infecting organisms, it gains access to subcutaneous tissue through infected nasal passages.

S. pneumoniae is now the most common bacterial cause of preseptal cellulitis in the pediatric age group.[73] It occurs in association with upper respiratory tract infection, although constitutional symptoms usually are less pronounced than those associated with *H. influenzae* type b infection. A variety of other bacterial agents may cause preseptal cellulitis, but they are seen less commonly.

Preseptal cellulitis occasionally has been reported to be due to a variety of other organisms, including *Trichophyton* (ringworm),[286] tuberculosis,[224] and anthrax.[9,41]

Adenovirus is another particularly common cause of preseptal cellulitis in children. Adenovirus is an important consideration in the differential diagnosis of childhood preseptal cellulitis because, despite being self-limited, the condition can occasionally mimic bacterial infection and prompt unnecessary treatment with antibiotics. Adenovirus can be recognized by its characteristic copious discharge, which may be serous. Swelling of the lid may be prominent, but erythema usually is minimal. Preauricular lymphadenopathy often occurs in older children, and marked conjunctival hyperemia with or without chemosis and subconjunctival hemorrhage may be present. Photophobia also may be noticed in cases of concurrent punctate keratopathy. A history of recent contact with other infected individuals frequently is noted and should be sought. Care should be taken to avoid spreading infection to family members, medical personnel, and others.

Hospital admission should be considered for children younger than 1 year of age with bacterial preseptal cellulitis. Hospitalization also is important for children with signs of systemic toxicity and those with inadequate *H. influenzae* immunization. A sepsis workup is indicated for children with signs of systemic toxicity and for extremely young children. Ophthalmologic consultation is recommended if orbital involvement is suspected or if clinical examination is inconclusive. Computed tomography (CT) usually is unnecessary for isolated preseptal cellulitis. Cultures of blood obtained from patients with preseptal cellulitis generally are negative but are more likely to be positive in children younger than 2 years. Culture of conjunctival discharge is done often but rarely has significant diagnostic benefit.

A severity index for scoring preseptal cellulitis in children has been reported to help guide treatment decisions.[289]

Antibacterial treatment should include intravenous agents for infants and those with signs of serious systemic infection. Intravenous cefuroxime or a combination of nafcillin plus cefotaxime or ceftriaxone frequently is recommended for empirical therapy. When MRSA preseptal cellulitis is suspected because of poor response to treatment or through cultures, clindamycin and trimethoprim-sulfamethoxazole are often the first-line agents. In situations in which MRSA preseptal cellulitis does not respond favorably to these medications, vancomycin or linezolid with rifampin may be effective treatments.[11,261] Outpatient treatment with intramuscular or oral antibiotics is reasonable for older, less acutely ill children. Intracranial infection has been associated with preseptal cellulitis in pediatric patients. Intracranial involvement should be suspected in any patient 7 years of age or older with preseptal or orbital cellulitis associated with an orbital subperiosteal abscess, Pott puffy tumor, concurrent sinusitis, complaints of headache, and persistent fever despite intravenous antibiotics.[229]

Systemic antibiotics should be continued for 7 to 10 days. Patients in whom intravenous antibiotics are started initially can switch to an oral antibiotic after they have been afebrile for at least 24 hours and otherwise have improved clinically, unless the possibility of development of sepsis or meningitis remains a concern. When antibiotic treatment fails, other noninfectious causes should be explored. Reports exist of conditions such as Langerhans histiocytosis, lipoblastomatosis, and dermoid cysts that can mimic preseptal cellulitis.[72,79,145]

Orbital Cellulitis

Bacterial infection of orbital structures posterior to the orbital septum is the most frequent cause of acute orbital inflammation. Orbital cellulitis occurs more commonly in children and more frequently during cold weather, when sinusitis is more prevalent.

Initial signs and symptoms can vary from mild inflammation to severe and fulminant orbital disease. Cardinal signs and symptoms of infectious orbital cellulitis include proptosis, limited eye movement (including total ophthalmoplegia), pain with eye movement, and an abnormal pupillary response. Decreased vision or even blindness can occur as the most serious ophthalmic complication.[239] Death can result from intracranial extension of the infection if appropriate treatment is not initiated. Elevated intraocular pressure and chemosis of the conjunctiva are common ancillary signs. Preseptal cellulitis often coexists with orbital cellulitis but is not a prerequisite.

Most cases of orbital cellulitis are caused by spread of infection from an adjacent infected sinus.[47,191] Ethmoid sinusitis is the most common predisposing factor.[184] Rare cases are due to penetrating orbital trauma or skin infection involving the face, with spread of organisms into the orbit. Orbital cellulitis may occur infrequently after orbital, ocular, or periocular surgery.[7,209,300] Orbital cellulitis and cavernous sinus thrombosis have been reported to occur after dental infections and dental surgery.[38]

Comprehensive evaluation of a patient with confirmed or suspected orbital cellulitis includes ophthalmologic and systemic examination. Assessment of visual acuity individually in each eye is important for excluding vision loss and establishing a baseline to aid in monitoring progression of the disease or the effects of therapy. Evaluation of optic nerve dysfunction by examining the pupils for an afferent pupillary defect (APD) is important before pupillary dilation is performed to assess the retina. Careful evaluation of extraocular movement should be performed in all extreme positions of gaze to identify limitation of ocular duction and elicit pain on eye movement. The presence or absence of proptosis can be assessed clinically by viewing the eyes from above (bird's-eye view) or below (worm's-eye view) and by comparing their relative positions within the orbits. In severe cases of orbital cellulitis, funduscopic examination may reveal dilation of the retinal venules and signs of compressive optic neuropathy, such as optic disk edema. In severe cases central retinal artery and vein occlusion has been reported.[58,135,205] Systemic evaluation includes determination of temperature, which usually is in the range of 39° to 40° C (102° to 104° F). Sinus examination, a screening neurologic examination, and evaluation for signs and symptoms of sepsis and meningitis should be performed.

In any case of clinically definite or suspected orbital cellulitis, CT of the orbit and brain is essential. CT can establish or confirm the diagnosis of orbital cellulitis and provides critical information needed to manage the patient. Imaging of the brain is important because orbital cellulitis can evolve into a brain abscess, meningitis, or cavernous sinus thrombosis. However, when a clinical response to treatment is noted, improvement in CT findings frequently is delayed. Therefore, recurrent CT scanning of a child with clinically improving findings is not indicated unless new signs or symptoms of concern develop.

Microbiologic studies often are acutely unhelpful for the routine patient with orbital cellulitis. Nonetheless, baseline studies remain important because critical information sometimes is acquired. Blood culture samples should be obtained at a minimum, and a lumbar puncture with culture of cerebrospinal fluid (CSF) should be considered in infants and those with signs of CNS infection. Culture of the ocular, nasal, and nasopharyngeal mucous membranes is of limited value and can be omitted. However, if surgical drainage is performed, samples of any material removed should be obtained for culture.

Optimal management of a child with orbital cellulitis requires a multidisciplinary approach. In addition to evaluation and management by an experienced pediatrician, ophthalmologic and otolaryngologic consultation should be obtained. Neurosurgical consultation is required when involvement of the CNS is diagnosed or suspected. In atypical cases and those not responding to treatment, consultation with an infectious disease specialist is warranted.

The most common offending etiologic bacteria are *S. aureus*, *Streptococcus* spp., and *Haemophilus* spp. (other than *H. influenzae*).[177] *S. pneumoniae* also is implicated frequently, and a variety of less common organisms have been reported. *H. influenzae* orbital cellulitis rarely has occurred since widespread use of the *H. influenzae* type b vaccine was implemented.[3,73] Fungal infection of the orbit occasionally is encountered, typically in immunocompromised individuals.[175]

The differential diagnosis of orbital inflammation in children includes a broad range of noninfectious conditions, including blunt trauma, cavernous sinus thrombosis, idiopathic inflammatory orbital pseudotumor, Wegener granulomatosis, sarcoidosis, leukemic infiltration, lymphoma, rhabdomyosarcoma, necrotic retinoblastoma, metastatic carcinoma, and histiocytosis X. Thyroid ophthalmopathy also can be manifested as an acute orbital inflammatory process, although usually its onset is slow and insidious.

Treatment of all patients with orbital cellulitis requires hospitalization and initiation of intravenous antibiotics as soon as possible. Infection with penicillin-resistant organisms is an increasingly common occurrence and must be considered during treatment.[43,257] Appropriate initial antibiotic therapy may include nafcillin, metronidazole, and cefotaxime as combination therapy. Given the increasing incidence of MRSA, initial treatment with vancomycin or clindamycin should be considered.[51,238,248] Other antimicrobial agents may be useful, and local susceptibility patterns should guide the choice of initial antimicrobial therapy. Antibiotic coverage should be modified according to the clinical course and culture results. If the patient fails to respond to antibiotic treatment within 24 to 48 hours, consultation with an infectious disease specialist and repeat CT to look for the development of an orbital abscess should be considered (Fig. 61-4). The mean hospital stay for uncomplicated cases of orbital cellulitis is 10 to 14 days, and oral antibiotics should be prescribed for 7 to 10 days after discharge. When the source of infection is believed to be from sinusitis, a nasal decongestant is commonly prescribed to aid in opening the sinus ostia, promoting drainage of the infected sinus, and speeding resolution. Nasal decongestants should be continued for 7 to 10 days after initiation.

FIGURE 61-4 ■ Failure of response of orbital cellulitis to antibiotic treatment may be a sign of development of a subperiosteal orbital abscess (seen here in the right medial orbit).

Although treatment of orbital cellulitis with steroids remains controversial, their use in the treatment of acute and chronic sinusitis has become increasingly common. Studies have shown that steroids can reduce levels of inflammatory cytokines in the sinonasal mucosa of individuals with sinusitis.[40,291] In a study on the use of steroids in children with orbital cellulitis, Yen and Yen[303] reported no adverse effects of this adjunct treatment with systemic antibiotics. Prospective studies on the use of steroids in orbital cellulitis have been proposed to determine whether they have any clinical benefit.

Children with orbital cellulitis require diligent follow-up while in the hospital. Vision should be assessed at the bedside daily; results may be more accurate if a single examiner routinely assesses vision for a given patient. Pupillary examination for an APD should be performed at each examination. Development of an APD indicates compromise of the optic nerve, warrants escalation of treatment, and usually requires urgent surgical

intervention. Reduction of vision, development of an APD, onset of CNS signs, or worsening of systemic status should prompt emergency repeat neuroimaging of the brain and orbit, with further intervention as dictated by results of the scan. The close anatomic relationship of the orbit to the brain, with the orbital venous system freely anastomosing with the facial venous plexus and cranial venous sinus system through a series of valveless veins, seriously increases the potential for spread of infection to contiguous structures, including the brain.

Acute surgical intervention to decompress the orbit or drain an orbital or subperiosteal abscess is indicated if vision loss or an APD is identified at any point during treatment. Immediate neurosurgical consideration is indicated if CNS involvement is documented. Most patients without evidence of vision loss, optic nerve dysfunction, or CNS involvement can be managed successfully medically. The surgical adage that all abscesses should be subjected to incision and drainage does not apply to abscesses associated with orbital cellulitis. Most subperiosteal abscesses located in the medial orbit can be managed successfully with medical therapy alone.[108,236] Formation of an abscess within the substance of the orbit (as opposed to a subperiosteal abscess) seldom occurs. Guidelines for surgical drainage are similar to those for subperiosteal abscess,[236] although such abscesses are more likely to require surgical intervention because of treatment failure or spread of infection to contiguous structures.[108] Provided that clinical improvement continues, no worrisome signs or symptoms of CNS involvement develop, and the patient's overall clinical status does not worsen, repeat neuroimaging of patients with an orbital abscess is unnecessary. Short-term resolution of an orbital abscess often is not obvious on CT. The radiographic appearance of the abscess typically remains unchanged on early follow-up scans, although it may no longer contain viable organisms.

Sinus drainage by an otolaryngologist frequently is required to hasten resolution. Formation of an abscess is not a prerequisite for surgical intervention, and orbital cellulitis without abscess formation may progress to the point of requiring surgery. Despite prompt and appropriate treatment, serious complications such as permanent vision loss and brain abscess can occur.[35,64,118,229,239] Most patients, however, can be treated effectively with no permanent sequelae.

Orbital cellulitis caused by fungal infection has a course and prognosis markedly different from those of bacterial orbital cellulitis, particularly cellulitis caused by mucormycosis and aspergillosis. Fungal orbital cellulitis typically occurs in patients who are immunocompromised or patients with metabolic acidosis, such as those with poorly controlled diabetes. It may be manifested as a subacute or chronic process. Orbital apex syndrome is considered to be the most severe form, with loss of function of all cranial nerves traversing the orbital apex into the orbit (i.e., cranial nerves II, III, IV, V, and VI). A black eschar-like lesion may form in the oropharynx or nasopharynx in cases of fungal infection.

Aspergillus orbital infections (most commonly caused by *Aspergillus flavus*, *Aspergillus fumigatus*, or *Aspergillus oryzae*) are rare findings in children and may take a slow, chronic course over a period of months or years. No clear predisposing factors exist, but it has a predilection for humid climates and most cases occur in otherwise healthy individuals. Signs and symptoms of orbital infection caused by *Aspergillus* spp. include loss of vision, constant dull pain, decreased or absent ocular motility, and proptosis with firm resistance to retropulsion. Palate and nasopharyngeal lesions occur but are rare. Biopsy is required for establishing the diagnosis.[107]

Effective treatment of orbital fungal infection requires correction of systemic and metabolic disturbances and administration of intravenous antifungal agents such as amphotericin B. Posaconazole has been shown to be effective as an alternative treatment in those who fail to respond to or cannot tolerate treatment with amphotericin B.[237] Surgical débridement of the orbit or adjacent infected sinuses frequently is required. Treatment often is unsuccessful, and fatalities are not uncommon, particularly with mucormycosis.

The larva of *Echinococcus granulosus* can produce a hydatid cyst in the orbit. The dog is the definitive host animal, although the organism also may live in the intestines of sheep, goats, cattle, pigs, and other animals. The disease is endemic in the Middle East, Africa, and Asia. Humans become infected by eating contaminated food, typically meat, and infection may occur in any age group. Affected patients have noninflammatory proptosis, decreased ocular motility, and dull orbital pain. Surgical excision is required for treatment. The cyst may be injected with hypertonic saline to kill the parasite, followed by excision.[105]

CONJUNCTIVAL INFECTIONS

The conjunctiva is the highly modified and specialized mucous membrane covering the inner surfaces of the eyelids and the anterior surface of the globe. The part that lines the inner surface of the eyelids is the *palpebral conjunctiva*, and the part covering the globe is the *bulbar conjunctiva*. The conjunctiva contains numerous small glands that produce two of the three parts of the tear film—a lower mucous layer acting as a wetting agent and a middle aqueous layer that makes up the bulk of the tear film; the third layer is oily and is produced by the sebaceous and meibomian glands of the eyelid and prevents rapid evaporation. The conjunctiva can be infected by many bacteria and viruses, and by noninfectious allergic and toxic agents.

Conjunctivitis can cause mild or significant symptoms—burning, itching, or a foreign body sensation. Itching commonly signifies an allergic or viral cause. The conjunctival redness (injection and erythema) of infectious conjunctivitis is usually more severe located away from the edge of the cornea, whereas conjunctival injection concentrated adjacent to the cornea (limbal, or ciliary flush) suggests inflammation of the cornea (keratitis), inflammation of the iris (iritis), inflammation of the iris and ciliary body (iridocyclitis), or occasionally glaucoma or a corneal foreign body. When the eye is very severely infected, however, it may be so intensely red that such a differentiating pattern is not discernible.

Conjunctivitis is often accompanied by a discharge with important diagnostic properties. Purulence suggests a bacterial cause, a mucoid discharge suggests a viral infection, and a serous discharge may be seen with viral or allergic causes. Patients with isolated conjunctivitis usually do not have significant blurring of the vision. Apparent conjunctivitis with poor vision warrants a search for another diagnosis.[101,174] A (very) general rule is red eye with good vision: probably conjunctivitis; red eye with bad vision: probably *not* conjunctivitis.[217]

Bacterial Conjunctivitis

Bacterial conjunctivitis is the most common form of infectious conjunctivitis in children. It is characterized by a purulent discharge and can be unilateral or bilateral. It is useful clinically to divide bacterial conjunctivitis into mild and severe forms because the treatments are different.[104,128]

Mild Bacterial Conjunctivitis

The agents most commonly causing mild bacterial conjunctivitis in children 5 years of age or older are *S. pneumoniae* and *Moraxella* spp.[101] *H. influenzae* was a prominent causative agent before the availability of *H. influenzae* type b vaccine. *S. aureus* conjunctivitis is seen most frequently after trauma or surgical manipulation. Conjunctival stains and cultures are usually not necessary, because the disease is almost invariably self-limited or responds rapidly to topical antibiotics.

Many topical antimicrobial agents for bacterial conjunctivitis are readily available, and include moxifloxacin, gatifloxacin, ciprofloxacin, ofloxacin, norfloxacin, tobramycin, gentamicin, erythromycin, sulfacetamide, and various combinations. Aminoglycoside-containing compounds such as neomycin can occasionally cause a dramatic allergic blepharoconjunctivitis that is worse than the original disease. The choice of using either drops or ointment is best left to the person who will be instilling the medication, because neither has any proven therapeutic advantage. With the emergence of multidrug-resistant organisms, the choice of antibiotic should be modified accordingly. Newer agents, such as the fourth-generation fluoroquinolones (moxifloxacin and gatifloxacin), have many advantages over older drugs, including broad-spectrum coverage, ease of dosing, and pH neutrality. A typical regimen for treatment of mild conjunctivitis is 1 drop or a ¼-inch bead of ointment placed into the inferior conjunctival fornix three to six times daily for 5 to 7 days, depending on the medication. Persistent infection calls for a prompt return to the physician for reconsideration of the diagnosis. Mild bacterial conjunctivitis generally resolves spontaneously in 7 to 14 days without treatment.[100,104]

Severe Bacterial Conjunctivitis

Severe bacterial conjunctivitis, characterized by ocular discomfort, pronounced redness, and copious purulent discharge, is usually caused by *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *S. aureus*, *S. pneumoniae*, and, in children younger than 5 years, *H. influenzae*. A hyperpurulent state in which the copious purulent discharge reaccumulates in a matter of minutes is characteristic of infection with *N. gonorrhoeae*.[117,258] Severe conjunctivitis calls for stains and cultures. Samples from both eyes should be stained and cultured separately, even if only one eye is involved, to allow the uninvolved eye to serve as a control.[269] Gram stains of conjunctival scrapings should be done at the time of culture. A useful culture technique includes the use of a cotton or calcium alginate swab (e.g., Calgiswab, Spectrum, or Houston) gently rubbed against the palpebral conjunctiva of the lower lid and inoculated onto blood and chocolate agar. If *N. gonorrhoeae* is suspected, a culture for chlamydia is also indicated, because concurrent infection with both organisms is common.[250,253]

The treatment of severe bacterial conjunctivitis is based initially on the results of the stains and later modified according to cultures and sensitivities. If *Neisseria* is strongly suspected, the patient should be treated for *Neisseria*, even if the laboratory results are not confirmatory. Ocular *N. gonorrhoeae* infection is a vision-threatening and life-threatening disease. This organism can penetrate the intact cornea and cause microbial keratitis (i.e., infection of the cornea), corneal perforation, and endophthalmitis (i.e., infection inside the eyeball), in addition to the conjunctivitis. *N. gonorrhoeae* conjunctivitis should be considered in three patient groups: neonates after passage through an infected birth canal, sexually active individuals, and possible victims of sexual abuse. Pediatric infection with *N. gonorrhoeae* requires hospital admission and administration of a systemic antibiotic. Because of the prevalence of penicillin-resistant strains, a broad-spectrum (third-generation) cephalosporin, such as ceftriaxone, is the most appropriate choice of antibiotic.[113] Adjunctive treatment of *N. gonorrhoeae* conjunctivitis with topical moxifloxacin and simple saline irrigation for 5 days can be helpful, but topical agents should never be used as isolated treatment.[117] Moxifloxacin is a particularly good antibiotic choice because it is effective against chlamydia, as well.

Conjunctivitis caused by gram-positive cocci can be treated with topical moxifloxacin, gatifloxacin, ciprofloxacin, or erythromycin. Systemic nafcillin, a second- or third-generation cephalosporin, or both can be added as needed for more extensive infections. Gram-negative bacterial conjunctivitis can be treated with topical erythromycin ointment or an aminoglycoside drip such as gentamicin or tobramycin. Systemic antibiotics can be added if needed.[269]

Viral Conjunctivitis

Adenoviral Conjunctivitis

Viruses are another common cause of infectious conjunctivitis in children. Many pediatric cases of viral conjunctivitis are caused by adenovirus. Serotypes 1, 2, 3, 4, 7, and 10 produce an acute form of conjunctivitis with prominent conjunctival follicles. Serotypes 3 and 7 also

may cause pharyngoconjunctival fever. This entity is characterized by conjunctivitis, fever, and pharyngitis.[25] Serotypes 8, 19, and 37 can cause epidemic keratoconjunctivitis.[269] The keratitis is characterized by a combination of a punctate epithelial keratitis (best viewed by slit-lamp examination) and an immune response that results in subepithelial (i.e., in the corneal stroma) infiltrates. Photophobia is a prominent feature of this condition, and visual acuity may be markedly decreased. Epidemic keratoconjunctivitis is often associated with pharyngitis and rhinitis, which may precede or be concurrent with the conjunctivitis.

Adenoviral conjunctivitis may be highly contagious. It is transmitted easily from an infected individual to others at home or at school. The incubation period is 5 to 10 days but may be as long as 21 days. The virus is shed from the infected conjunctiva for 7 to 12 days after the onset of infection. Frequently, a prodromal upper respiratory tract infection consisting of fever, pharyngitis, or otitis media occurs.[269] Ocular signs and symptoms include photophobia (with corneal involvement), foreign body sensation, epiphora (tearing), bulbar and palpebral conjunctival injection and chemosis (edema of the conjunctiva), and subconjunctival hemorrhage.[121] Formation of grayish-pink friable membranes on the palpebral conjunctiva is a hallmark of the disease and may result in bleeding when removed. Preauricular lymphadenopathy is a common feature in older children and adults. Keratitis may be prolonged, with mild to moderate reduction of vision lasting weeks to months.[121,173]

The diagnosis of adenoviral conjunctivitis is almost always clinical only. Only rarely is laboratory confirmation necessary. Viral cultures are possible when indicated, and a rapid enzyme immunoassay test is available.[296] Treatment of adenoviral conjunctivitis is supportive, aimed at decreasing the symptoms of irritation, photophobia, and blurred vision. Cool compresses and acetaminophen are helpful; removal of conjunctival membranes with a moistened cotton swab may relieve the foreign-body sensation. A small amount of bleeding often occurs after removal of the membranes, and they can recur after several days. If corneal subepithelial infiltrates cause significant discomfort or an unacceptable decrease in vision, treatment with a short course of topical steroids may provide symptomatic relief and speed resolution. The use of steroid preparations requires careful monitoring,[173,269] and they should be prescribed only by an ophthalmologist.[143,254]

The clinician should wear gloves when examining patients with suspected adenovirus infection. Careful hand washing is essential after direct contact. Instruments or equipment used in examining an infected patient should be cleaned with 10 percent sodium hypochlorite solution or other solutions known to eradicate the adenovirus.

Mini-epidemics of adenovirus-related conjunctivitis can originate in physicians' offices.[13,98] At home, families should exercise caution, and separate the towels and bed clothes of the patient from those of others in the household. Children of school age should be kept at home for 7 days or longer to reduce the risk for transmitting the infection to classmates.[269]

Herpes Simplex Virus Conjunctivitis and Complex Forms

HSV conjunctivitis may occur as a primary or secondary infection. Ocular infection usually is caused by HSV-1, except in newborns, in whom HSV-2 predominates.[49] Typical initial signs of HSV conjunctivitis include a serous discharge, scant conjunctival follicle formation on the inferior palpebral conjunctiva, and preauricular lymphadenopathy.[306] Eighty percent of cases are unilateral. Eyelid vesicles often occur in primary infections. Bulbar conjunctival ulceration is an unusual occurrence, but when present, it is virtually pathognomonic of primary HSV-1 infection. Keratitis occurs in as many as 50 percent of primary cases and is characterized by mild epithelial irregularities. Dendrites (branch-shaped lesions of the corneal epithelium) can occur in primary infections, but reactivated HSV keratitis frequently exhibits the characteristic dendritic pattern. Lid vesicles usually do not develop in cases of reactivated HSV infection.[142]

Primary HSV dermatoblepharitis is seen most commonly in children younger than 6 years, but it may occur at any age. The initial episode may be associated with an upper respiratory tract infection, and recurrences are common. Clinical signs include eyelid vesicular reaction, mild follicular conjunctivitis, preauricular lymphadenopathy, and, occasionally, atypical epithelial keratitis. Secondary bacterial infection can occur. Systemic acyclovir is a safe and effective treatment, although the disease itself typically is self-limited and does not require treatment.[142]

The diagnosis of HSV keratoconjunctivitis usually is made on the basis of the clinical appearance alone. Antigen detection tests or viral cultures can be used when the diagnosis is in question.[137] Viral cultures, when necessary, require special handling.[269] In culturing primary HSV cases, the skin surface is swabbed with an alcohol sponge, and a tuberculin syringe with a 30-gauge needle is used to aspirate fluid from an intact vesicle. If no vesicles are present, a Dacron swab is wiped on the palpebral conjunctiva of the lower lid. The viral transport medium is inoculated and taken to the laboratory for special handling.

Treatment of HSV conjunctivitis alone (in the absence of corneal epithelial disease) is controversial. Oral acyclovir may be used for severe cases of primary HSV infection.[246,265] Three topical antiviral agents are available: iododeoxyuridine, vidarabine, and trifluridine.

When epithelial HSV keratitis is reactivated and the typical dendritic (branched) epithelial lesions are seen, the cornea usually is hypoesthetic. Iritis may be associated with HSV keratitis and is characterized by miosis, photophobia, ocular pain, and foreign-body sensation. Vision may be decreased if the epithelial disease involves the visual axis.[269] Epithelial HSV may be atypical, may be more severe, and may be more complicated in patients receiving topical steroids and in immunocompromised patients.

Treatment with topical trifluridine or vidarabine is indicated in all cases of HSV keratitis. Topical steroids

are effective in decreasing corneal scarring caused by HSV stromal, but should be used by an ophthalmologist only. When using a topical steroid, coverage with a topical antiviral agent such as vidarabine always should be included to reduce the risk for recurrent active viral proliferation. Oral acyclovir is not effective for treating active HSV keratitis, but it may be useful in treating HSV uveitis.[269] Ancillary treatments include cycloplegic eye drops to dilate the pupil and pain medications as needed.

Although not helpful in treating acute epithelial disease, chronic suppression with oral acyclovir is effective in reducing the number of recurrences of HSV epithelial and stromal keratitis, especially in patients with stromal keratitis.[265,269] Interferon also significantly adds to the efficacy of topical antiviral therapy, but the adverse side effects of interferon must be weighed against its potential benefits.[265]

External Ocular Infections with Varicella-Zoster Virus

Childhood varicella infection (chickenpox) commonly is accompanied by conjunctivitis. Vesicles, ulcers, or both occur occasionally on the bulbar or palpebral conjunctiva. Conjunctivitis associated with varicella infection does not result in permanent visual sequelae. Corneal involvement rarely occurs and typically heals without sequelae. Occasionally, it may result in stromal scarring and produce irregular astigmatism and reduced vision.

After primary varicella infection occurs, the virus may persist in latent form in the trigeminal nerve ganglia. Herpes zoster ophthalmicus occurs when the ophthalmic division of the trigeminal nerve is affected by reactivation of the virus. The condition occurs more commonly in immunocompromised patients, and recurrences occur infrequently except in these patients.

The diagnosis of herpes zoster ophthalmicus is almost always made on the basis of the characteristic clinical feature of a painful, tender vesicular eruption of one V-1 dermatome. Ocular involvement may include keratitis and uveitis. Corneal epithelial lesions may appear dendritiform, with or without subepithelial infiltrates. Uveitis may occur with or without associated keratitis. Usually the uveitis is mild, but occasionally it may be severe, especially in immunocompromised patients, with the formation of a hypopyon (pus in the anterior chamber) or a hyphema. Immunofluorescence testing of vesicular base scrapings or viral cultures may be helpful if the manifestation is atypical or the diagnosis is in doubt.[156]

Treatment of herpes zoster ophthalmicus involves the administration of oral or intravenous acyclovir. Treatment is most effective when initiated within 72 hours of the appearance of vesicles. The keratitis and uveitis do not respond to topical antiviral therapy.[67] Occasionally, topical steroids may be helpful in treating varicella keratitis and uveitis, but such treatment should be administered under the direction of an ophthalmologist.[173]

Chlamydial Conjunctivitis and Trachoma

Chlamydia spp. can cause conjunctivitis and trachoma. *Chlamydia psittaci* rarely causes ocular disease in humans. *Chlamydia trachomatis*, however, has numerous serotypes that affect the eye. Serotypes A, B, Ba, and C cause trachoma, and serotypes B, C, D, Da, D–, E, F, G, H, I, Ia, J, and K cause inclusion conjunctivitis, including neonatal inclusion conjunctivitis. Neonatal chlamydial conjunctivitis is addressed in the discussion of neonatal conjunctivitis. Inclusion conjunctivitis in older children and adults is a subacute or chronic inflammation of the conjunctiva. The condition may be unilateral or bilateral. A mucopurulent discharge typically occurs, as do follicles on the bulbar and perilimbal conjunctiva. Preauricular lymphadenopathy is a common feature. Punctate epithelial keratitis with subepithelial infiltrates and a superior micropannus (i.e., vascular growth on the upper edge of the cornea) may develop.[61]

The differential diagnosis of inclusion conjunctivitis includes viral and bacterial conjunctivitis, molluscum contagiosum, and toxic keratoconjunctivitis from chronic administration of topical agents. Laboratory testing may be necessary to confirm the diagnosis. Giemsa staining of conjunctival scrapings may demonstrate the classic intracytoplasmic inclusions. A fluorescent antibody detection test or an enzyme immunoassay can be used for a rapid diagnosis.[269] Chlamydial cultures may be needed when the diagnosis remains in doubt.

Treatment consists of oral erythromycin or doxycycline if systemic infection is suspected. Topical erythromycin or tetracycline alone four times each day for 7 days is effective treatment of infection limited to the conjunctiva.[61,269] Moxifloxacin has been shown to be effective against chlamydia but is not yet the recommended treatment of choice.

Trachoma, a chronic blinding conjunctivitis, remains a prominent etiology of blindness worldwide, ranking 6th overall, causing 4 percent of all blindness. It is a disease of impoverished populations that is exacerbated by inadequate supplies of water and poor hygiene and sanitation. Trachoma seldom is seen in fully developed countries. In the United States, it is encountered on Native American reservations in the Southwest and in individuals from endemic foreign areas. Eye-seeking flies play an important role in transmission of *Chlamydia* from one person to another.[269]

Trachoma causes blindness by producing chronic inflammation of the palpebral conjunctiva of the upper eyelid with secondary scar formation. Contracture of the scars cause entropion of the eyelid and trichiasis (cilia rubbing on the cornea). After many years of infection and reinfection this leads to corneal neovascularization, opacification, and vision loss.[268,279] Less commonly, the cornea may be infected directly. The complete course of the disease from initial infection to blindness generally takes decades. Children do not become blind from trachoma, but they are the most significant constant chronic reservoir of the disease.

The diagnosis of trachoma usually is made on the basis of clinical findings alone. The disease passes through characteristic stages, from simple inflammation to scarring, entropion, and trichiasis. Multiple stages may coexist in various parts of the eyelids. The World Health Organization classifies the stages of trachoma as follows: TF, trachomatous follicular response; TI, diffuse

trachomatous conjunctival inflammation; TS, trachomatous scarring of the palpebral conjunctiva; TT, trachomatous trichiasis; and CO, trachomatous corneal opacification. In the pediatric population, physicians usually encounter only stages TI and TF.[279,302] The scars on the palpebral conjunctiva, referred to as *Arlt lines*, are linear and multidirectional. Slit-lamp examination may reveal a superior limbal corneal micropannus and Herbert pits (i.e., hollowed-out areas at the superior limbus), which represent sites of resolved follicles.

Treatment of trachoma now consists of periodic dosing of susceptible populations with azithromycin or, if azithromycin is not available, topical tetracycline or erythromycin ointment instilled twice daily for 2 months, as well as improved sanitation and hygiene to prevent recurrence.[269] Trachoma can be self-limited if hygiene alone is improved.[268] In children older than 8 years of age, doxycycline should be administered orally daily for 40 days. Erythromycin can be substituted in younger children or in patients intolerant of doxycycline.

Neonatal Conjunctivitis

Neonatal conjunctivitis, or ophthalmia neonatorum, is a common vision-threatening disorder throughout the world, although it has been relegated to a position of secondary importance in the fully industrialized world, where screening and prophylactic measures are widespread. It remains a significant cause of childhood ocular morbidity in poor countries.

Dilute topical silver nitrate solution instilled just after birth for prophylaxis against ophthalmia neonatorum was the first treatment used. In the 1880s it was responsible for reducing the prevalence of gonococcal ophthalmia neonatorum from 10 percent to 0.17 percent of live births in Europe.[269] Silver nitrate has been largely supplanted by other preparations; where it is used, a mild, self-limited chemical conjunctivitis can occur.[199] It lasts 2 to 4 days and resolves with no treatment. Topical erythromycin largely has replaced silver nitrate in developed countries because it is effective without producing a chemical conjunctivitis.[115] In the developing world, dilute povidone iodine solution, 2.5 percent, has proved to be a remarkably effective and inexpensive measure for prevention of *N. gonorrheae* neonatal conjunctivitis when instilled immediately after birth.[132]

In areas of regional conflicts, political instability, and burgeoning refugee populations, even such simple measures are often impossible.[96,115] Causes of neonatal conjunctivitis are highly variable among populations. In areas that use silver nitrate or povidone iodine drops for prophylaxis, chemical conjunctivitis is a common cause of neonatal conjunctivitis.[199] Infectious conjunctivitis occurs in 0.5 to 6.0 percent of live births in the United States. The leading infection is *C. trachomatis*.[18,120,251,269]

Neonatal infection is caused by ocular exposure to contaminated maternal discharge during normal birth, although it may occur in infants born by cesarean section, especially if the membranes rupture prematurely. Other important infectious agents that may cause ophthalmia neonatorum are *N. gonorrhoeae* and *S. aureus*.[95,258] Bacteria that normally reside in the vaginal or gastrointestinal tract occasionally are implicated in ophthalmia neonatorum.

Such bacteria include *Streptococcus* spp., *Haemophilus* spp., *Pseudomonas aeruginosa*, *Moraxella* spp., *Moraxella catarrhalis*, *N. meningitidis*, *E. coli*, and *Enterobacter cloacae*.[241,264] Viruses occasionally produce neonatal conjunctivitis. Rarely, HSV may cause keratoconjunctivitis.[96] It is associated with distinctive vesicular skin changes and systemic signs, and the diagnosis seldom is in doubt.[12]

Life-threatening HSV meningoencephalitis is a serious potential complication of HSV ophthalmia neonatorum.[192] Other viruses occasionally implicated include adenovirus, coxsackievirus A9, cytomegalovirus (CMV), and echovirus.[263]

Certain clinical features may help establish the specific etiologic diagnosis in cases of ophthalmia neonatorum (Table 61-1), but considerable overlap in findings exists, and physicians cannot rely on the history and physical examination alone to make a definitive diagnosis. Even when all appropriate investigative modalities are used, the cause may remain unknown in some cases.

Variations in the time of onset after birth, severity of inflammation, and character of the ocular discharge are common, and none is considered pathognomonic of a specific infectious process, but general considerations are worth reviewing.[241] Silver nitrate chemical conjunctivitis typically begins during the first 48 hours of life and produces a watery discharge with mild inflammation. It is self-limited and resolves within 48 to 72 hours of its appearance.[199] *C. trachomatis* conjunctivitis generally begins 1 to 21 days after birth and usually is apparent by day 7. A moderately copious mucopurulent discharge may be present but can be variable.[120,189] *S. aureus* conjunctivitis also begins during the first 3 weeks of life and can produce a moderately profuse mucopurulent discharge. Many other bacterial agents produce an overlapping clinical picture. Conjunctivitis caused by *N. gonorrhoeae* ordinarily begins somewhat earlier, usually by the third day of life, but it may appear as late as 3 weeks after birth. The hallmark is a copious, hyperpurulent discharge that can reaccumulate in a matter of minutes after it is removed. *N. gonorrhoeae* can penetrate an initially intact cornea and result in perforation of the eye and endophthalmitis.[117,244,263] Conjunctivitis caused by

TABLE 61-1 **Clinical Characteristics of Neonatal Conjunctivitis Caused by Various Agents**

Agent	Day of Life at Onset	Discharge
Silver nitrate	1 (0-2)	Serous
Chlamydia trachomatis	7 (1-21)	Mucopurulent
Staphylococcus aureus	5 (1-21)	Mucopurulent
Neisseria gonorrhoeae	3 (0-21)	Purulent
Other bacteria	7 (1-21)	Mucopurulent
Herpes simplex virus	5 (0-21)	Serosanguineous
Other viruses	Not established	Probably serous

gastrointestinal flora often begins within a few days of birth and can produce a copious purulent discharge.

Viral neonatal conjunctivitis is a rare occurrence. HSV infection may begin during the first 3 weeks of life and usually produces a serous or serosanguineous discharge.[96,192] Other viruses have highly variable characteristics, but a serous discharge is a common finding.

The differential diagnosis of neonatal conjunctivitis includes congenital glaucoma, dacryostenosis with or without dacryocystitis, keratitis, and uveitis. Dacryostenosis is manifested as epiphora and a watery or purulent discharge. Occasionally, conjunctivitis may develop. Because epiphora caused by congenital dacryostenosis ordinarily is not seen until the second or third week of life, it rarely is a serious consideration. Newborns with congenital glaucoma often are photophobic and irritable.[288] They may exhibit increased tearing, conjunctival injection, and corneal edema. Both keratitis and uveitis most often are accompanied by photophobia and pain. The discharge with keratitis can vary but with bacterial causes is purulent. The discharge with uveitis, if present, is watery.

Laboratory testing is important in establishing a specific diagnosis and should include Gram and Giemsa stains, a chlamydial immunoassay, and cultures for aerobic and anaerobic bacteria. Cotton-tipped swabs should be used to obtain material for culture from the conjunctival fornices. Viral and chlamydial cultures and immunoassays can be considered but usually are unnecessary.[17,272] Gonococcal immunoassay and HSV immunochemical tests are available but not in widespread use.

The initial treatment of neonatal conjunctivitis depends on the suspected infectious agent. Broad-spectrum treatment should be considered if the diagnostic possibilities cannot be narrowed. Silver nitrate–induced conjunctivitis is self-limited and does not require treatment. *C. trachomatis* conjunctivitis is treated with tetracycline (1%) or erythromycin (0.5%) ointment four times daily for 3 weeks and with systemic erythromycin for 2 to 3 weeks to prevent or to treat chlamydial pneumonia. Sulfonamides can be used to treat chlamydial conjunctivitis if erythromycin is not tolerated. Topical antibiotics alone are insufficient for the treatment of neonatal *Chlamydia* infection.[106,116,120] Staphylococcal conjunctivitis can be treated with erythromycin ointment or a variety of other topical antimicrobial agents every 4 to 6 hours for 3 to 7 days. Appropriate systemic antibiotic treatment should be considered if the infection is particularly severe.[269]

N. gonorrhoeae infection requires systemic treatment in all cases. Aqueous penicillin G can be considered if resistant strains are unlikely. However, one dose of intramuscular ceftriaxone is the treatment of choice in the United States because it is effective against all *N. gonorrhoeae* strains.[14,225] Eyes should be irrigated with saline every hour until the discharge clears and then in reduced frequency as necessary to reduce the risk for development of corneal infection. This interval usually is 24 to 48 hours after the initiation of systemic treatment.

Penicillin G drops are recommended by some ophthalmologists, but such drops do not appear to improve the overall prognosis or speed of recovery.

HSV neonatal conjunctivitis is treated with systemic acyclovir in appropriate doses for as long as 3 weeks.[96] Treatment should be instituted on an emergency basis because of the potential for development of serious permanent neurologic sequelae and death if treatment is delayed. Topical ophthalmic vidarabine or trifluridine also should be applied to the eyes four times daily for 2 to 3 weeks.

Prevention of ophthalmia neonatorum is simple and requires only instillation of an antibiotic or antiseptic agent within the first hour after birth. Silver nitrate drops (1%), erythromycin ointment (0.5%), and tetracycline ointment (1%) have been effective treatment against *N. gonorrhoeae* and *C. trachomatis*.[116,269] A 5 percent solution of povidone-iodine also has been effective and economical in prophylaxis against a variety of agents, including gonococcal neonatal conjunctivitis. It may be particularly useful in developing countries.[132] As with other sexually transmitted diseases, neonatal conjunctivitis caused by *N. gonorrhoeae*, *C. trachomatis*, or HSV should be addressed according to standard public health policies, with reporting, investigation, and case identification as required by local law.

KERATITIS: CORNEAL INFLAMMATION

The cornea, with its overlying tear film, is the major refracting component of the human eye. Keratitis means inflammation of the cornea. Microbial keratitis means inflammation of the cornea caused by infection with a microorganism.

Any irregularity of the corneal tear film, the surface of the cornea itself, or an opacity of the cornea involving the central visual axis may impair vision. The cornea is composed of five distinct layers: the epithelium, Bowman's layer, the stroma, Descemet's membrane, and the endothelium. The epithelium is several layers thick and, when healthy, can regenerate without scarring after an injury or other insult. The Bowman membrane lies beneath the epithelium. It does not regenerate, and it heals with a scar when injured. The stroma is the thickest part of the cornea. It is composed of regularly arranged collagen fibrils embedded in a matrix of mucoproteins and glycoproteins. Like the Bowman membrane, the stroma heals with scarring.

Descemet's membrane is the basement membrane of the corneal endothelium. It can regenerate after an insult and does not opacify. The innermost layer, the endothelium, is derived from neuroectoderm and consists of a single layer of cells that do not have significant regenerative capacity. The cornea is an avascular structure that is clear because of the regular arrangement of the collagen fibers in the stroma and the dehydrating pumping action of the endothelium. The cornea will become edematous and opacify if the endothelium is significantly damaged or diseased. The external corneal surface is protected from injury and exposure by the eyelids. Reflex tearing in response to mechanical irritation provides further protection. The blink response to threat and the antimicrobial properties of the tear film further protect the cornea from injury and infection.

Patients with keratitis usually have erythema of the perilimbal bulbar conjunctiva (limbal flush) and pain. Additional complaints may include decreased vision and photophobia. The diagnosis of keratitis depends on the presence of a corneal epithelial or stromal infiltrate. The infiltrate is verified by slit-lamp examination and can be extremely difficult to see in infants and uncooperative or frightened children. Ideally, the patient is examined with a slit-lamp; sometimes sedation is required for this. Stains and cultures are usually required to establish the diagnosis, preferably before topical antibiotics are started, so an ophthalmologist should be consulted promptly. Rarely, a biopsy of the cornea is needed to obtain material for culture. Even more rarely, a corneal transplantation is required to stop progression of an infection or to treat a corneal perforation. Exact etiologic diagnosis is based on clinical findings and on examination of stains and cultures.

Isolated Epithelial Keratitis

Keratitis can be classified according to the layer of cornea involved. Most infections involve the epithelium and/or the stroma. Isolated epithelial keratitis usually is caused by one of several viruses: HSV, varicella-zoster virus (VZV), adenovirus, EBV, and the (rubeola) measles virus. The first three organisms have been discussed in an earlier section.

EBV causes HSV-like dendritic corneal epithelial lesions; stromal disease also may occur. The lesions are self-limited and do not respond to antiviral therapy.[172] Measles keratitis usually consists of transient epithelial infiltrates, which resolve without permanent sequelae.[142] In malnourished children with vitamin A deficiency, measles represents a serious threat to life and to vision. Deep corneal ulcers may develop during the first few days of measles infection. Vitamin A deficiency remains the most common cause of pediatric blindness worldwide, almost exclusively involving children in developing countries. Measles is a common comorbidity factor. When a vitamin A–deficient child develops measles and keratitis, rapid progression leading to corneal perforation and ultimate loss of the eye may occur.[24,93] Whether the ulcers are caused by direct measles virus infection, by the keratomalacia (corneal melting) of vitamin A deficiency, or by some combination of these factors is still not entirely clear.

Measles immunization programs plus periodic mega vitamin A dosing programs, for susceptible children under 6 years of age, greatly reduces childhood blindness and childhood mortality in developing countries.

Stromal Keratitis

Syphilis and certain parasitic and viral infections can cause a nonsuppurative keratitis isolated to the corneal stroma. Congenital syphilis produces interstitial (stromal) keratitis in children, although the condition usually does not become apparent until late in the first decade of life. An indolent, peripheral corneal haze develops and slowly progresses centrally. Ghost vessels devoid of blood flow are seen in the corneal stroma on slit-lamp examination.[271] The condition is manifested as a bilateral stromal process[247] in 80 percent of cases and is accompanied by transient iritis, iridocyclitis, or scleritis at some point in the course of the disease. When ocular syphilis is diagnosed, systemic infection is present, by definition. The systemic disease must be treated according to accepted guidelines.

Bacterial Keratitis

Bacterial keratitis, usually involving a corneal ulcer, most often occurs after trauma that disrupts the normal integrity of the corneal epithelium. The trauma can be mild and occult, such as that caused by lens wear. *S. aureus*, *S. pneumoniae*, *P. aeruginosa*, and *Moraxella* spp. are the most common bacterial causes of severe necrotizing bacterial keratitis.[10,158,188,285] Other organisms, such as *S. epidermidis*, *Actinomyces* spp., viridans streptococci, and a variety of others, have been implicated in less severe cases. Accurate identification of the offending organism greatly facilitates effective treatment. A specimen is obtained by scraping the edge of the ulcer with a sterile platinum spatula. Smears should be fixed in 70 percent methanol, not by heat, and Gram stain for bacteria and acridine orange stain for fungi and *Acanthamoeba* are recommended. The yield of culture-positive cases is higher in laboratories skilled in handling corneal cultures. Material for culture should be inoculated onto fresh media such as blood and chocolate agar (aerobes and facultative anaerobes), Sabouraud agar (fungi), and thioglycolate broth (anaerobic bacteria). Thayer-Martin agar should be plated if *N. gonorrhoeae* is suspected. Initial treatment is based on the resulting stains (Table 61-2) and modified pending cultures (Table 61-3).[20,157]

Typically patients are started on frequent fortified tobramycin and ceftazidime drops until culture results become available. Medical treatment of bacterial keratitis includes fourth-generation fluoroquinolones, fortified topical antibiotics, and, occasionally, subconjunctival antibiotic injection (see Table 61-2).[44,202,269] Antibiotics are administered as often as every 15 to 30 minutes initially. Systemic antibiotics are required only for gonococcal, chlamydial, and onchocercal keratitis, as well as for actual or threatened perforation of the cornea, extension of infection to the sclera, or worsening of the process during topical or periocular treatment (or both). Topical steroids are useful if the resulting inflammation threatens to destroy the mechanical or optical integrity of the cornea, but steroids are never used as isolated treatment.[269]

Fungal Keratitis

The most common causes of fungal keratitis include *Candida*, *Aspergillus*, and *Fusarium*. Mycotic keratitis associated with the filamentous fungi *Aspergillus* and *Fusarium* is thought to occur with trauma, whereas keratitis caused by *Candida* is associated more commonly with a preexisting ocular (e.g., dry eye) or systemic (e.g., diabetes mellitus or immunosuppression) condition.[275] Fungal keratitis tends to be relatively indolent in contrast to bacterial keratitis. Typically, white stromal infiltrates

TABLE 61-2 Treatment of Keratitis Based on Smear Morphology

	Antibiotic	
Organism	**Ocular**	**Systemic***
Gram-positive cocci, gram-positive bacilli	Cefazolin†	Nafcillin, IV
Gram-positive filaments	Amikacin‡	Trimethoprim-sulfamethoxazole, IV
Gram-negative cocci	Ceftriaxone† or ciprofloxacin‡,§	Ceftriaxone, IV or IM
Gram-negative bacilli	Tobramycin†	Tobramycin, IV
Acid-fast bacilli	Amikacin‡	
Hyphal fragments	Natamycin,‡ fluconazole¶	Fluconazole, PO
Yeasts	Amphotericin B,‡ fluconazole¶	Fluconazole, PO
Cysts, trophozoites	Polyhexamethylene, biguanide,‡ paromomycin,‡ and propamidine isethionate‡	Itraconazole, PO

*Standard age-appropriate milligram-per-kilogram dosages.
†Topical and periocular use only.
‡Topical use only.
§Use in children 12 years of age or older.
¶Periocular use only.
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

TABLE 61-3 Treatment Based on Identification of Organisms

	Antibiotic	
Organism	**Ocular**	**Systemic**
Micrococcus, Staphylococcus (penicillin-resistant)	Cefazolin*	Nafcillin, IV
Micrococcus, Staphylococcus (methicillin-resistant)	Vancomycin*	Vancomycin, IV
Streptococcus	Penicillin G*	Penicillin G, IV
Enterococcus	Vancomycin* and gentamicin*	Vancomycin, IV, and gentamicin, IV
Anaerobic gram-positive coccus	Penicillin G*	Penicillin G, IV
Corynebacterium spp.	Penicillin G*	Penicillin G, IV
Mycobacterium fortuitum-chelonae	Amikacin†	Amikacin, IV,* or clarithromycin, PO
Nocardia	Amikacin†	Trimethoprim-sulfamethoxazole, IV
Neisseria gonorrhoeae	Ceftriaxone*	Ceftriaxone, IV or IM
Pseudomonas spp.	Ceftazidime* Tobramycin*	Ceftazidime, IV or IM
Other aerobic, gram-negative bacilli	Ceftazidime* Tobramycin*	Ceftazidime, IV or IM
Filamentous fungi	Natamycin† Fluconazole‡	Fluconazole, PO
Candida spp.	Amphotericin B† Fluconazole‡	Fluconazole, PO
Acanthamoeba	Polyhexamethylene biguanide,† paromomycin,† and propamidine isethionate†	Itraconazole, PO

*Topical and periocular use.
†Topical use only.
‡Periocular use only.
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

with irregular and indistinct borders are detected. Satellite lesions, separated from the main lesion, may be evident. Mild iritis is common. Severe iritis with hypopyon can occur but is an infrequent manifestation in fungal corneal keratitis.[203] For possible fungal keratitis, an acridine orange stain is recommended and culture on a Sabouraud agar plate is indicated. Hyphal fungi are treated with topical natamycin, topical fluconazole, systemic fluconazole, or some combination of these medications. Yeasts are treated with topical or systemic amphotericin B, fluconazole, or both agents.[94]

Protozoan Keratitis

Protozoan keratitis is a particularly serious vision-threatening process. It usually is caused by *Acanthamoeba*

and occurs more frequently in wearers of soft contact lenses. Use of homemade saline solution is a significant risk factor and should be discouraged.[37] Amoebae are ubiquitous organisms in soil, water, and air. They have been identified in hot tubs and in the feces of domestic animals.[172] People living in rural areas may be at special risk.

Acanthamoeba keratitis has been reported in children without a history of contact lens use or trauma.[62] *Acanthamoeba* corneal ulcers are pleomorphic.[131] Older lesions may exhibit a ringlike infiltrate around a central ulcer. Iritis or iridocyclitis may be intense, and severe pain is a hallmark of the disease. Acridine orange and calcofluor white stains aid in establishing an early diagnosis.[171] Tandem scanning confocal microscopy of corneal specimens may increase diagnostic accuracy, and the organism may grow on blood and chocolate agar.[37,167,216] Treatment of *Acanthamoeba* keratitis is complex and suboptimal. Topical application of polyhexamethylene biguanide (0.02%),[153] chlorhexidine (0.02%), paromomycin (0.1%), or propamidine isethionate (Brolene) drops may be effective. Oral itraconazole (Sporanox) has been used successfully in adults.[150,167,176] Cycloplegic agents are recommended for comfort, and pain medications are often required.

Corneal transplantation is done if the infection progresses despite treatment, if perforation occurs, or if significant corneal visual axis opacification remains after the active infection is eradicated.

Onchocerca volvulus is a filarial parasite that causes river blindness (i.e., onchocerciasis). The disease is endemic in sub-Saharan West Africa, in isolated areas of East and Central Africa, and in some areas of Central and Northern South America. The organism is transmitted by the bite of a blackfly of the family Simuliidae.[235] Larvae migrate to subcutaneous tissue and pass through several molts to become adult worms, which encapsulate in nodules and produce microfilariae that pass into blood, skin, and other organs, including the eyes. The conjunctiva, cornea, aqueous humor, vitreous, retina, uveal tract, sclera, and optic nerve all may be infested. Corneal involvement can lead to vision loss caused by corneal opacification, but most severe vision loss is caused by choroidal, retinal, and optic nerve disease.[114] Although children are often infected, blindness usually does not occur until they reach the third or fourth decade of life.[278,288]

Treatment of onchocercal infection is administration of ivermectin every 6 to 12 months to all individuals living in endemic areas. Ivermectin kills the microfilariae but not the adult worms, which live in subcutaneous nodules and remain until their death.[274] The adult worm may live 10 or more years. Because of the long life span, ivermectin should be taken every 6 months for at least 10 years to treat microfilariae as they develop.

Microsporidia are intracellular protozoa that represent a new emerging ocular pathogen. Interest in the organism has increased in the last decade because of its association with HIV and acquired immunodeficiency syndrome (AIDS). The ocular manifestation corresponds to both the immune status of the patient and the genus of microsporidia involved.[139] Typically, keratoconjunctivitis occurs in immunocompromised individuals and stromal keratitis is seen in immunocompetent individuals. Keratoconjunctivitis is manifested as bilateral conjunctival inflammation and epithelial keratopathy, which may lead to decreased vision. Stromal keratitis is manifested as an insidious diskiform keratitis with uveitis similar to that seen in HSV stromal keratitis and may require penetrating keratoplasty because of perforation or scarring. Potassium hydroxide plus calcofluor white and acid-fast stains are thought to be the most efficient means of establishing the diagnosis of microsporidial keratitis.[140] Transmission electron microscopy provides specificity in identification of the genus and possibly the species, but it may lack sensitivity and is laborious.[139] Knowledge of the genus and species may help guide management. Albendazole, fumagillin, itraconazole, metronidazole, and topical propamidine isethionate have been used as therapeutic agents.[51,139]

INFECTIONS PRIMARILY INVOLVING THE UVEA

The uveal tract is the vascular middle coat of the eye. It is situated between the sclera and the retina. Its major function is to provide nourishment for intraocular tissues, including the retina, lens, and cornea. The uveal tract is composed of the iris, ciliary body, and choroid.

Uveitis is a nonspecific term for inflammation of the uvea. If the inflammatory process primarily affects the iris, it is called *iritis*. If the ciliary body is involved, the process is called *cyclitis*. If these two structures together are involved, the process is called *iridocyclitis* (i.e., anterior uveitis). The term *intermediate uveitis* (i.e., pars planitis) applies to inflammation in the region of the ciliary body and peripheral retina. The term *posterior uveitis* usually applies to combined inflammation of the retina and choroids, also called *chorioretinitis*. If the choroid alone is involved, it is called *choroiditis;* inflammation of the retina alone is called *retinitis.* Because of the thinness and close apposition of these two tissue layers, inflammation in one layer frequently "spills over" into the other. The vitreous body occupies the central area of the eye behind the lens. It is composed of water, mucopolysaccharides, and collagen. Although transparent in a normal, healthy eye, the vitreous is subject to inflammation or vitritis, usually as a result of inflammation in adjacent retinal tissue or in the pars plana.

Causes of uveitis are numerous but can be categorized into two main groups: infectious and noninfectious. Infectious causes are discussed in this chapter. Uveitis in any area of the eye may result in pain, conjunctival or episcleral hyperemia, photophobia, lacrimation, and decreased vision, although these symptoms vary relative to the site and the aggressiveness of the inflammation.

With slit-lamp examination, the hallmark of anterior uveitis is the finding of hazy, proteinaceous aqueous humor, called *flare*, and the presence of leukocytes in the anterior chamber. The cell and flare components are graded independently on a 1+ to 4+ scale. If present in sufficiently great numbers, leukocytes can precipitate in the anterior chamber (with location dependent on gravity)

and form a mass called a *hypopyon*. The cells can aggregate on the back of the cornea and create fine, medium, and large lesions known as *keratic precipitates*. The conjunctiva usually is injected or hyperemic. With chronic inflammation, the border of the pupil often adheres to the anterior surface of the lens. Such adhesions are called *posterior synechiae* and may cause the pupil to have an irregular shape and size, as well as poor reactivity to light. Chronic or recurrent anterior segment inflammation may lead to the formation of a cataract. Iris nodules or atrophy may develop with long-standing inflammation. Anterior vitreous cells may occur as a spillover phenomenon in the setting of a prolonged or vigorous anterior chamber reaction. Although inflammatory effects on the ciliary body generally compromise its production of aqueous humor and thereby reduce intraocular pressure, cellular and proteinaceous debris can occlude aqueous humor outflow channels and lead to elevated intraocular pressure.

Inflammation primarily involving the posterior segment of the eye usually leads to decreased vision, which may be the symptom for which treatment initially is sought. Pain may be minimal or absent. Inflammatory lesions of the retina and choroid also may lead to the development of cellular debris in the vitreous and cause the patient to perceive "floaters." Initially, the borders of the retinal or choroidal inflammatory foci often are indistinct and cream-colored. In the healing phase, the borders of these lesions increasingly become distinct, and a defined, partially pigmented scar results. Inflammatory perivascular sheathing of the retinal vessels may occur, as well. Involvement of the macula with edema or exudates may result from inflammation of the posterior segment, and long-standing edema may evolve into a cystic configuration and cause loss of central visual acuity. The optic nerve may exhibit an inflammatory response; if the optic disk is so involved, the response is called *papillitis*. Inflammatory debris also may be found in the vitreous.[4,206,304]

Under normal circumstances, the amount of immune traffic within the eye, especially in the anterior chamber, is held to a minimum by a group of immunomodulatory pathways that together form anterior chamber–associated immune deviation (ACAID). Chief among these deviations is active suppression of delayed hypersensitivity; distinctive ocular antigen-presenting cells migrate to the spleen, where they generate an immune response deficient in $CD4^+$ cells but high in $CD8^+$ cells. These regulatory T cells return to the eye to suppress the $CD4^+$ populace, thereby minimizing the arm of inflammation most likely to impair visual clarity.[91,264]

Epidemiology

In the United States, most uveitis cases have noninfectious causes. Large samples demonstrate a predominance of anterior rather than posterior uveitis. The most common category of anterior uveitis is idiopathic; the most common infectious cause of anterior uveitis is herpetic keratouveitis (simplex and zoster). Posterior uveitis is caused most frequently by *Toxoplasma gondii*. In developing nations, infectious uveitis plays a larger role. In West Africa, *T. gondii* probably accounts for most cases of intraocular inflammation.[232,233]

Viral Uveitis

Herpes Simplex Virus

Most of the uveal inflammation associated with HSV (typically iridocyclitis) results from the corneal disease. Occasionally, a patient will have iritis as an isolated finding, but most patients with ocular HSV demonstrate conjunctivitis, keratitis, chorioretinitis, or retinal vasculitis.[227] The iritis may require treatment with topical steroids and topical antivirals as prophylaxis against the development of corneal epithelial disease.[284,304]

Varicella-Zoster Virus

Occasionally, varicella infection (i.e., chickenpox) may be associated with a transient iritis that requires no treatment. Although rare, cases of unifocal choroiditis causing visual loss but responsive to acyclovir in children with primary varicella have been reported.[186]

Herpes zoster virus may cause iridocyclitis during the acute stage of the disease; the anterior chamber reaction can persist or recur long after resolution of the cutaneous component of the condition. Herpes zoster always should be considered in the differential diagnosis of chronic unilateral iridocyclitis. Topical steroids are indicated for iritis, with the addition of oral or intravenous acyclovir for severe cases. Segmental iris atrophy is a characteristic sequela of herpes zoster uveitis. Glaucoma, hyphema, retinitis, vasculitis, and extraocular muscle palsy occasionally occur in patients with herpes zoster ophthalmicus. In a profoundly immunosuppressed patient, VZV can cause a devastating retinitis known as *progressive outer retinal necrosis*.[156]

VZV and HSV-2 have been implicated as causes of acute retinal necrosis syndrome. Patients in whom this syndrome is diagnosed range from 13 to 71 years of age, with an average age of 43 years. The syndrome typically occurs in healthy patients. The virus causes a triad of acute vitritis, retinal vasculitis, and peripheral necrotizing retinitis and is bilateral in 33 percent of patients. Treatment consists of intravenous acyclovir. Prophylactic laser photocoagulation may prevent retinal detachment. The visual prognosis is guarded.[26,156]

Epstein-Barr Virus

Ocular EBV involvement has been reported primarily in patients with infectious mononucleosis. Follicular conjunctivitis may be diagnosed in 2 to 40 percent of patients. Corneal stromal inflammation, iritis, episcleritis, optic neuritis, and chorioretinitis occur less commonly. Systemic corticosteroids and acyclovir may be useful in treating sight-threatening complications of chronic intraocular inflammation from EBV infection.[174] Postinfectious uveitis also has been described and is discussed later.

Enteroviruses

Coxsackievirus A24 and enterovirus 70 may cause a painful follicular conjunctivitis called acute hemorrhagic conjunctivitis. Rarely, chorioretinitis may be diagnosed. Treatment of these infections primarily is supportive.[206]

Rubella Virus

Rubella virus can cause congenital and acquired infections. Ocular manifestations of acquired rubella include conjunctivitis in 70 percent of patients, superficial keratitis, and iritis. Rarely, retinitis has been reported.

Congenital rubella syndrome can be manifested as cataracts, glaucoma, microphthalmos, and retinitis. Cataracts occur in 15 percent of patients and glaucoma in 10 percent. Retinal examination reveals a salt-and-pepper fundus caused by the alternating pattern of hypopigmentation and hyperpigmentation of the retinal pigment epithelium. The prognosis for patients with retinitis alone usually is good, with vision between 20/20 and 20/40. Glaucoma resulting from rubella commonly requires surgery, as do the cataracts. Iritis is reported less commonly.[138,174]

Mumps Virus

Ocular manifestations of mumps virus include dacryoadenitis, conjunctivitis, iritis, optic neuritis, and keratitis. Retinitis also has been reported. The prognosis for visual recovery from the retinitis is good, and sequelae of the iritis are rare occurrences.[94,174]

Measles Virus

Measles virus can cause congenital and acquired infections. In congenital infections, a salt-and-pepper retinopathy and cataract formation may occur, similar to that found in rubella. Ocular manifestations in acquired measles include conjunctivitis and, much less commonly, retinitis, retinal vasculitis, and optic nerve edema.[15,206]

Subacute Sclerosing Panencephalitis

Between 30 and 75 percent of patients with subacute sclerosing panencephalitis (SSPE or Dawson inclusion body encephalitis) have ocular findings. SSPE is caused by a variant of the measles virus; it differs from the wild-type virus by alteration or absence of viral M protein. Optic nerve edema, inflammation, and subsequent optic atrophy have been reported. Macular retinitis is a common finding, and the contiguous non-neural tissues (vitreous and choroid) almost never are involved. The visual prognosis of survivors is poor.[201,206]

Creutzfeldt-Jakob Disease

The most common ocular manifestation of Creutzfeldt-Jakob disease is cortical blindness. Optic atrophy may result from degeneration of the neurons of the optic nerve.[206]

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

As many as 75 percent of patients with advanced AIDS have ocular findings. Cotton-wool spots occur in more than 50 percent of patients and are bilateral in more than 80 percent. Cotton-wool patches represent focal infarctions of the neural layer of the retina and are the most common ocular finding in patients with AIDS. They generally produce no symptoms and do not decrease vision. These spots are white and fluffy and occur most commonly in the macular portion of the retina. They resolve in 4 to 6 weeks with no residual scars. Occasionally, flame-shaped hemorrhages are detected as well. The retina and choroid in these patients may become infected with HSV, CMV, VZV, syphilis, tuberculosis, ocular histoplasmosis, *Candida*, toxoplasmosis, and *Pneumocystis*, although in the highly active antiretroviral therapy (HAART) era, the incidence of CMV retinitis has declined and its advance in existing cases has been slowed or halted. Symptomatic anterior uveitis in the absence of the aforementioned pathogens occurs rarely in patients with AIDS and may be caused by HIV itself.[133]

Cytomegalovirus Infection

CMV infections may occur in preterm neonates and immunosuppressed patients, especially those with AIDS. In neonates with symptoms of CMV infection (i.e., low birth weight, microcephaly, jaundice, thrombocytopenia, hepatosplenomegaly, or petechial rash), congenital CMV infection[21] (i.e., prenatal transmission) may be manifested in the fundus as chorioretinal scars (21%) and optic atrophy (7%).[48]

CMV retinitis develops in approximately 30 percent of adult patients with AIDS, usually those with $CD4^+$ counts less than $50/mm^3$.[77] In the pediatric AIDS population, $CD4^+$ counts of less than $20/mm^3$ may be required for appearance of the retinitis.[75] The disease is less common in pediatric patients with AIDS. Patients with CMV retinitis have no external ocular signs but may complain of loss of vision. Children may not complain of such loss, and the problem may become apparent only after bilateral severe vision loss has occurred.[75] The retinal lesions of CMV typically are yellow-white and often are associated with hemorrhage. The retinitis generally follows a perivascular distribution. The retina becomes necrotic and eventually atrophies, with a gliotic scar. CMV optic neuritis also may develop. Treatment consists of intravenous or intravitreal ganciclovir, intravenous or intravitreal cidofovir, or intravenous foscarnet[78,304] and can often be discontinued when the $CD4^+$ count rises above $100/mm^3$.[277]

Parvovirus Infection

Bilateral panuveitis has been reported to be associated with parvovirus B19 infection.[166]

Human T-Cell Lymphotrophic Virus Infection

Human T-cell lymphotrophic virus is endemic in several regions of the globe: Japan, the Caribbean islands, and parts of central Africa. It probably is responsible for several cases of self-limited, occasionally recurrent uveitis in these regions.[193]

Lymphocytic Choriomeningitis Virus Infection

Lymphocytic choriomeningitis virus (LCMV) is an arenavirus endemic in mice; it also is reported in hamsters

and occasionally is transmitted to humans by direct contact with the rodent or through aerosolization of its feces or urine. Postnatal exposure results in asymptomatic seroconversion or aseptic meningitis, but intrauterine infection can have devastating consequences. Spontaneous abortion, congenital hydrocephalus, psychomotor retardation, and chorioretinitis have been documented. Diffuse chorioretinal scarring identified postnatally may bode a grim visual prognosis. LCMV may be an underdiagnosed cause of unexplained congenital chorioretinitis. The diagnosis is confirmed by elevated LCMV antibody titers. A survey of severely retarded, visually disabled children revealed immunologic evidence pointing to LCMV as the cause of the visual loss in approximately half of those surveyed.[179,181]

Viruses

Rift Valley Fever Virus. Rift Valley fever virus is an insect-borne virus. The arthropod vector directly inoculates the virus into the bloodstream of a host, resulting in end organ infections, including the retina. Cattle and sheep are the primary host, and mosquitoes are the vector for transmission. Outbreaks have been reported in eastern and sub-Saharan Africa and South Africa. Uveitis and conjunctivitis can occur in the acute phase of the disease.[67,252] A viral retinitis may occur 1 to 3 weeks after the illness. It may be unilateral or bilateral, affecting both the central and the peripheral fundus with hemorrhages, exudates, and retinal vasculitis.

Herpes B Virus. Herpes B is an α herpes virus within the herpes virinae family. Transmission to humans occurs via bites or scratches from infected monkeys where the virus is endemic. Infection may also occur via puncture wounds from contaminated objects.[194,234]

Herpes B virus has been associated with ocular complications in persons handling monkeys.[194,234] The reports suggest that the virus can cause retinal disease indistinguishable from acute retina necrosis syndrome, with multifocal necrotizing retinitis and panuveitis in an immunocompetent host.

Influenza A Virus. Influenza A virus is a pneumotropic virus with affinity to the branchial epithelial cells. It is transmitted by droplet infection.[23] Ocular manifestations in influenza include conjunctivitis, keratitis, iritis, and dacryoadenitis during the acute phase of the disease. Retinal involvement with influenza A virus has been reported. Retinitis of influenza A is typically bilateral and predominantly affects the macula with hemorrhages, exudates, and macular edema. Fluorescein angiography reveals diffuse vascular leakage. Optic disc edema may rarely be seen. The disease is typically self-limited, with return of vision to 20/20.[223]

Bacterial Uveitis

Syphilis

Syphilis *(Treponema pallidum)* should be considered as a possible cause in all cases of intraocular inflammation. Any patient with confirmed syphilitic uveitis should undergo a lumbar puncture to rule out asymptomatic neurosyphilis.

Ocular manifestations of congenital syphilis include interstitial keratitis, a mottled salt-and-pepper fundus, and chorioretinal scarring. Acute interstitial keratitis occurs as a late manifestation of congenital syphilis (5 to 25 years of age) and is thought to be a hypersensitivity response to treponemal antigen in the cornea. Patients complain of pain and photophobia and have a diffusely opaque cornea and anterior uveitis. Blood vessels invade the inflamed cornea and eventually are obliterated, with ghost vessels left in the corneal stroma. Glaucoma may occur. A unilateral manifestation of interstitial keratitis suggests postnatally acquired lues.

Secondary syphilis may involve any layer of the eye. Episcleritis, scleritis, iridocyclitis (acute, chronic, or recurrent), iris capillary dilation (i.e., iris roseata), vascular papules of the iris (i.e., iris papulosa), inflammatory nodules (i.e., iris nodosa), choroiditis, chorioretinitis, and retinal vasculitis all have been reported, as have optic neuritis and subsequent atrophy.

Tertiary syphilis may have associated gummata of the iris and an Argyll Robertson pupil (i.e., miotic, irregularly shaped pupil with loss of response to light but preservation of the near response). Intraocular inflammation seldom occurs at this stage.

Ocular inflammation secondary to syphilis should be treated as neurosyphilis. With the appropriate doses of penicillin G, the inflammation typically resolves rapidly. Occasionally, topical regional steroids are required to control local inflammation. Treatment of ocular syphilis may lead, at least initially, to a vigorous local and systemic self-limited, febrile response, presumably caused by liberation of spirochetal antigens or toxins. Topical oral corticosteroids may be required to quell this response, which is also known as a *Jarisch-Herxheimer reaction*.[4,170,206]

Lyme Disease

A mild follicular conjunctivitis occurs in 11 percent of patients with stage 1 Lyme disease, caused by *Borrelia burgdorferi*. During the second and third stages, neuro-ophthalmic manifestations, including cranial neuropathy (most often cranial nerves III, IV, VI, and VII), optic neuritis, bilateral keratitis, bilateral iridocyclitis, diffuse choroiditis, vasculitis, intermediate uveitis, and Parinaud oculoglandular syndrome, may be seen. The most frequent manifestation of late Lyme disease is arthritis. Ocular inflammation occurs in approximately 4 percent of children with Lyme arthritis.[130] Early, localized Lyme disease may be treated with doxycycline (8 years of age and older) or amoxicillin. More advanced or persistent disease is treated best with intravenous ceftriaxone.[6] Antibiotic treatment early in the course of the disease carries a better prognosis than does therapy initiated at later stages.[22]

Leptospirosis

Leptospirosis may cause an anterior uveitis that occurs months after the acute infection. Leptospirosis is identified as an important cause of epidemic panuveitis in

southern India; the most common posterior segment manifestations in this group are vasculitis and vitritis.[46]

Tuberculosis

Any structure of the eye may be affected by tuberculosis. Allergic and infectious processes have been implicated as important causes of tuberculous uveitis. Anterior uveitis with or without keratitis has been attributed to tuberculosis. Choroiditis, optic neuritis, and orbital infections have been detected in cases of miliary tuberculosis. The most frequent manifestations of ocular tuberculosis are choroidal nodules and scars; anterior uveitis is an uncommon occurrence.[30] Treatment should be undertaken with the appropriate antituberculous medications. Corticosteroids often are necessary in conjunction with antimicrobial therapy.

Leprosy

Because *Mycobacterium leprae*, the cause of leprosy, grows best at lower temperatures, corneal infections predominate. Corneal involvement is associated with prominence of the corneal nerves, interstitial keratitis, and corneal hypoesthesia, but corneal opacities are often peripheral and not visually significant.[56] Uveal involvement in leprosy frequently is silent and accounts for a large number of the ocular complications from the disease.[56,57]

Brucella *Infection*

Ocular manifestations of *Brucella* infection are rare but include iritis, focal nodular choroiditis, and panophthalmitis.[206]

Cat-Scratch Disease

Bartonella henselae is a gram-negative rod transmitted to humans by the bite or scratch of an infected animal, often a young cat or kitten. Regional lymphadenopathy is the predominant nonocular finding. A striking stellate neuroretinitis characterized by swelling of the optic nerves and lipid deposition in the retina is the most easily identifiable complication of ocular infection. Other manifestations include intermediate uveitis, optic disk swelling, multifocal choroiditis, and serous macular detachment. The discrete foci of multifocal choroiditis are the most common findings in the posterior segment. The role of antibiotics in this condition is debated.[146,259]

Fungal Uveitis

Histoplasmosis

The diagnosis of presumed ocular histoplasmosis syndrome is based on the clinical picture of disseminated punched-out retinal "histo spots," atrophic retinal changes around the optic nerve, and a clear vitreous.[220] Later in the course of the disease, subretinal hemorrhage and retinal detachment may occur. Ocular histoplasmosis often is bilateral and can result in legal blindness from the loss of macular vision. Presumed ocular histoplasmosis syndrome may occur after an episode of benign systemic histoplasmosis during childhood. Active inflammation and vitreous cells usually are not seen in this syndrome, although case series have documented active chorioretinal inflammation as new-onset lesions and as reactivation of previously quiescent lesions.[42,141] The hallmark histoplasmosis spots appear as white, punched-out, well-demarcated chorioretinal scars and represent healed fungal lesions. Generally, they first appear during adolescence, do not reduce vision, and do not require treatment. Macular disease, which may reduce vision, does not develop until after the patient reaches the second decade of life. Subretinal neovascularization may develop at the site of a macular histoplasmosis spot, with fluid, blood, and lipid accumulating in the subretinal space. This process plus local scarring can result in a marked reduction in central vision.

Macular neovascularization may be treated suitably with laser photocoagulation in an attempt to salvage the remaining central vision. Antifungal drugs may play a role in the unusual setting of demonstrated active histoplasmosis choroiditis.[240]

Candidiasis

Candida spp., including *Candida albicans*, are fungi with yeast and filamentous forms. Candidiasis is encountered in immunocompromised patients and in situations involving indwelling catheters, intravenous therapy, chronic antibiotic use, poorly controlled diabetes, and intravenous drug abuse. Candidal chorioretinitis can develop in approximately 9 percent of patients with blood cultures positive for the fungus,[75] although more recent studies have demonstrated that ocular involvement (either chorioretinitis or endophthalmitis) in children is a rare occurrence.[74]

In the eye, *Candida* infection usually begins in the choroid and eventually causes multifocal white chorioretinal lesions. If the fungus proliferates unchecked, it may break through the retina into the vitreous and produce the classic white snowball-like "fungus ball." Candidal infection that progresses to endophthalmitis is exceedingly rare if appropriate intravenous antifungals are started promptly on notice of a positive blood culture.

Intravenous amphotericin B is the drug of choice. Other antifungal agents such as fluconazole, flucytosine, and miconazole also may be effective, but none is dramatically so. Surgical treatment of intraocular *Candida* disease is discussed in the section on endophthalmitis.

Aspergillosis

Aspergillus spp. can infect the choroid, retina, and vitreous of immunocompromised individuals. One group of investigators found a high rate (7%) of these unusual infections on reviewing records of deceased liver transplant recipients.[129]

Coccidioidomycosis

Coccidioides spp. have yeast and filamentous forms. Ocular disease consists of a multifocal chorioretinitis that

develops during the course of systemic coccidioidomycosis. The lesions initially appear similar to those seen in histoplasmosis; in severe cases, endophthalmitis results, and vitrectomy may need to be performed. In less severe cases, the lesions may respond to intravenous amphotericin B. Occasionally, an isolated granulomatous iridocyclitis may develop.[187]

Cryptococcosis

Cryptococcus neoformans is a yeastlike fungus that can cause multifocal chorioretinitis and endophthalmitis. Most patients with cryptococcosis are severely immunocompromised, and many have AIDS. The CNS and eye are involved commonly, and elevated intracranial pressure may cause papilledema and sixth cranial nerve palsy. Intravenous amphotericin B in combination with oral flucytosine is the treatment regimen of choice.[6,147]

Sporotrichosis

The dimorphic fungus *Sporothrix schenckii* is encountered commonly in rotting vegetable matter, wood, and soil. The fungus gains access to the host by traumatic implantation or inhalation. It has been reported to be responsible for anterior uveitis in the setting of a suggestive lesion on a finger of the dominant hand, with presumed hand-to-eye transmission.[287]

Protozoal Uveitis

Leishmaniasis

Ocular leishmaniasis has been described. Manifestations include conjunctivitis, blepharitis, and anterior uveitis. This trio of findings responds to systemic treatment with sodium stibogluconate.[85]

Protozoal Infection

Amebiasis. Humans can be infected by ingestion of the cyst form of *Entamoeba histolytica* in contaminated water and food. The primary disease manifestation in *E. histolytica* infection is dysentery. Ocular involvement is rare and includes maculopathy in the form of a transparent cyst involving the central retina with surrounding retinal hemorrhages and anterior uveitis.[31,148]

Trypanosomiasis. Trypanosomiasis is a flagellated protozoa transmitted by tsetse flies. African trypanosomiasis is caused by *Trypanosoma brucei* and *Trypanosoma cruzi.* General manifestations include headache, general malaise related to fever, lymphadenopathy, and then CNS involvement with sleepiness, coma, and death. Reported ocular manifestations include urticarial swelling of the eyelids, interstitial keratitis, and, rarely, iritis or choroiditis.[231] In American trypanosomiasis, known as *Chagas disease*, intraocular disease has not been reported but eye involvement may occur in the form of eyelid swelling and periorbital edema spreading to the face.

Malaria. The most serious form of malaria is caused by *Plasmodium falciparum*. Hemolytic anemia and disseminated intravascular coagulation are presumed to lead to hypoxia of the brain and other tissues, resulting in the clinical manifestations of the disease. Ocular manifestations include subconjunctival hemorrhage, retinal hemorrhages, and cranial nerve palsies. Corneal involvement and uveitis are not caused directly by malaria.[148,161-163]

Giardiasis. Giardiasis is caused by *Giardia lamblia*, which can inhabit the human jejunum. Humans are infected through ingestion of water contaminated with human sewage. Reported ocular manifestations include retinal arteritis (vasculitis) and salt-and-pepper retinal pigmentary changes.[50,169]

Helminthic Uveitis

Toxocariasis

Toxocara canis causes visceral larva migrans, which is not associated with ocular disease. In the retina, larvae get trapped in small capillaries and burrow into surrounding tissue; the dead larvae incite an intense eosinophilic abscess. Ocular toxocariasis has three classic clinical manifestations, almost always involving one eye only. One form occurs in children 2 to 9 years of age and causes an indolent endophthalmitis and leukokoria (i.e., white pupil). Tractional retinal detachment may occur. The eye typically shows little or no external evidence of inflammation, and the patient experiences no pain.

A second form appears in children between 4 and 14 years of age. These patients have reduced vision but little or no external inflammation and no pain. The reduced vision may cause strabismus, which may be the first sign. An inflammatory granuloma is seen in the macula.

The third form of ocular toxocariasis occurs in patients between 6 and 40 years of age but may not be recognized until years later. Patients with this form of toxocariasis have good vision, but a peripheral retinal granuloma is seen on routine eye examination. Vision may be affected if a traction band from the granuloma distorts the macula.

Inactive *Toxocara* granulomas do not respond to medication. When active intraocular inflammation exists in such a magnitude that it poses a further threat to vision, administration of periocular or systemic steroids may be necessary. Anthelmintic agents eradicate migrating larvae, but their effect on "residing" larvae is questionable. When giving anthelmintics, one should combine them with a short course of corticosteroids because death of the larvae may incite vigorous inflammation. Cryotherapy and laser therapy may be useful when the *Toxocara* granuloma is located away from the macula and optic nerve. Vitrectomy may be helpful if significant traction on the retina occurs. Anthelmintic drugs should not be administered after the patient has undergone posterior segment surgery. Visual prognosis is poor if the macula is involved.[206,242,251,297]

Onchocerciasis

Onchocerciasis often causes a severe choroiditis with an overlying retinitis.[249] The various ocular manifestations of infestation with *O. volvulus* are discussed in the section on keratitis.

Loiasis

The *Loa loa* worm can migrate through the tissues of the eye and cause conjunctivitis, iridocyclitis, vitritis, and chorioretinitis. The disease is transmitted by the bite of a mango fly.[81] Vascular obstruction with intraretinal hemorrhage and retinal exudation may occur as well. Medical treatment with diethylcarbamazine can kill the adult worms and microfilariae. Adult worms also can be removed from the eye surgically.[206]

Cysticercosis

The tapeworm *Taenia solium* causes cysticercosis. When the larva gains access to the eye, cysts form in the vitreous or subretinal space in 13 to 46 percent of patients. The living worm may be seen undulating in these spaces. With death of the organism, severe panuveitis can occur. Orbital and subconjunctival involvement occur less commonly. Surgical removal of the intraocular cysts may prevent the severe inflammation that occurs on death of the worm. Praziquantel can kill the organism, but the ensuing increase in inflammation may be dramatic.[206]

Rare Causes of Parasitic Posterior Uveitis In Children

Schistosomiasis. Schistosomiasis is caused by platyhelminth, which belongs to the trematode class and has three recognized species: *Schistosoma haematobium*, *Shistosoma mansoni*, and *Shistosoma japonicum*. Humans are infected when the larval form (cercaria) emerges from its snail and pierces the skin to enter the dermal lymphatics and capillaries. Reported ocular lesions include generalized cutaneous edema of the eyelids in the acute phase, dacryoadenitis, choroiditis, and, rarely, retinal pigment epithelium changes.[71,134,218]

Hydatid disease. Hydatid disease is caused by *Ecchinococcus granulosus*, for which dogs are the primary host.[298] Reported ocular lesions include painless proptosis, subretinal cysts, and, rarely, involvement of the choroid and the vitreous.[105,298]

Coenurus infection is caused by *Multiceps multiceps*, which is a taenoid cystode responsible for coenurus infection. It is a parasite of dogs and related species with sheep acting as an intermediate host. Humans may occasionally become infected. Reported ocular lesions include conjunctival cysts, intraocular cysts, and subretinal involvement. Panophthalmitis and orbital involvement have been reported.[168,299]

Ascaris lumbricoides is a species of *Ascaris* found in humans. Humans are infected by ingestion of water contaminated with eggs containing infective larvae. Ocular lesions, which are rare, include iridocyclitis and chorioretinitis.[136]

Baylisascaris procyonis may cause ocular disease in humans. They are nematodes of the Ascaridida order and humans are infected by ingestion of material contaminated with ova.[280] *B. procyonis* is a presumptive cause of diffuse unilateral subacute neuroretinitis characterized by unilateral vitritis, papillitis, and recurrent grayish lesions affecting the outer retina and the retinal pigment epithelium.

Gnathostoma spinigerum. *Gnathostoma spinigerum* is a spiruroid nematode endemic in East Asia. The human is an accidental host, and infection occurs by eating infected food. Reported ocular lesions include eyelid swelling, orbital cellulites, uveitis, intraocular hemorrhage, central retinal artery occlusion, and tractional retinal detachment.[150,281]

Wuchereria bancrofti. *Wuchereria bancrofti* is prevalent in many tropical areas of the world. Larvae are ingested by suitable species of mosquito feeding on the skin. The larvae undergo further stages of development and become capable of infecting other individuals when they are disgorged in the saliva of the feeding insect. Reported ocular lesions include conjunctival edema, granulomatous iridocyclitis, and subretinal inflammatory lesions.[195]

Trichinosis is caused by *Trichinella spiralis*, which is a nematode parasite of carnivorous animals of widespread distribution. Human infection results from ingestion of infected undercooked pork or game. Reported ocular lesions include painful limitation of the eye movements associated with proptosis and chemosis, edema of the eyelids and the conjunctiva, and, rarely, retinal hemorrhages or papilloedema.[208,270]

Rickettsial disease. *Rickettsia* are obligate intracellular parasites, possessing both RNA and DNA. Unlike viral infections, they respond to antibiotics. They are transmitted by a vector such as ticks, mites, and fleas. The organisms causing the diseases in the following section have been rarely reported as causing posterior uveitis in humans.[80]

Typhus. Murine typhus is recognized in scattered pockets worldwide. It is caused by *Rickettsia mooseri* and is transmitted by the rat louse. Ocular manifestations include chorioretinitis, conjunctivitis, and uveitis.

Spotted Fever. Boutonneuse fever is recognized in the Mediterranean areas, Caspian and Black sea coastal regions, Africa, Southeast Asia, United States, Russia, and Korea. It is caused by *Rickettsia conorii* and is transmitted by ticks. Ocular manifestations include chorioretinitis, conjunctivitis, and iridocyclitis.

Q Fever. Q fever is caused by *Coxiella burnetii*, which is distributed worldwide and is transmitted by ticks. Ocular manifestations include conjunctivitis, episcleritis, uveitis, and chorioretinitis.

Trench Fever. Trench fever is caused by *Rochalimaea quintana* and is transmitted by the human body louse. Ocular manifestations include retinitis and conjunctivitis.

Uveitis Caused by Insect-Induced Disease

Ophthalmomyiasis is the ocular disorder caused by infestation with fly larvae, most commonly the larval form of the sheep botfly *Oestrus ovis*. Maggots may be seen in the conjunctival fornix (cul-de-sac) or inside the eye. Internal ophthalmomyiasis can be diagnosed by noting a motile larva in the anterior chamber, vitreous, or subretinal space. The maggot may leave trails ("railroad tracks") throughout the retina. A mild inflammatory response in the anterior chamber (e.g., iritis and iridocyclitis) or vitreous may occur. Treatment is surgical removal of the larva. Corticosteroids may be used to treat the accompanying intraocular inflammation.[82,206]

Postinfectious Uveitis

Increasingly, attention is being given to the role of bacterial and viral systemic illness in the eventual development of sterile intraocular inflammation. This type of uveitis is thought to be caused by an autoimmune response that occurs between sensitized lymphocytes and host tissues that bear some antigenic similarity to the recently cleared pathogen. Disruption of the ACAID is probably a prerequisite for the development of these postinfectious syndromes. Several reports exist of nongranulomatous anterior uveitis occurring weeks or months after streptococcal infection. These uveitides may be associated with other poststreptococcal findings (e.g., arthritis and glomerulonephritis) or may be the sole manifestation. Treatment with cycloplegics and topical corticosteroids is sufficient for the ocular manifestations.[19,282,301]

Uveitis, predominantly of the anterior type, has been reported to occur after illnesses caused by gram-negative enteric bacteria such as *Klebsiella*, *Salmonella*, and *Yersinia*. These gram-negative–induced uveitides are much more likely to occur in the setting of human leukocyte antigen (HLA)-B27 positivity. The ocular findings often parallel the development of arthritis, thus suggesting that parallel immunologic processes are occurring in both these mesenchymal cavities (i.e., the joint space and the anterior chamber). An association between recent EBV infection and acute tubulointerstitial nephritis and anterior uveitis has been described, with onset of the renal and ocular inflammation occurring several months after the characteristic acute EBV infection.[109]

INFECTIONS INVOLVING PRIMARILY THE RETINA

Eye Manifestations of Intrauterine Infections (TORCHS Complex)

The TORCHS complex is a group of congenital and perinatal infections that may cause severe systemic and ophthalmic abnormalities. The effect of infection with one of the TORCHS organisms—*T. gondii*, others (LCMV, EBV), rubella virus, CMV, herpesvirus, and syphilis—may be evident at birth or manifest later in childhood or adulthood. Congenital EBV infection (i.e., infectious mononucleosis) has been reported to be associated, possibly, with congenital cataracts.[103] All of these infections commonly cause either mild or no clinically evident disease in the mother.[180] The diagnosis cannot always be established on clinical grounds alone, and neonatal and maternal serologic tests must be performed to confirm the clinical suspicion.

Toxoplasmosis

T. gondii is an obligate intracellular parasite that has an affinity for the CNS and retina. The parasite has three forms: tachyzoite, bradyzoite, and sporozoite or oocyst. Human infection may be congenital or acquired. Acquired infection results from the ingestion of undercooked meat contaminated with oocysts or from exposure to the feces of an infected cat, the definitive host. The oocysts release tachyzoites, which multiply intracellularly and result in cell death. In adults, primary acquired infection usually is asymptomatic. The immune response then transforms the tachyzoite into a bradyzoite, which encysts and remains dormant in tissues for years. These cysts have the propensity to rupture sometime later and cause an inflammatory response resulting in recurrent infection.

Congenital infection is transmitted through the transplacental route. In the United States, the reported incidence of congenital toxoplasmosis is 1 case/1000 to 10,000 births.[12,55] Seventy percent of the obstetric population is negative for antibodies and therefore at risk for infection and transplacental transmission to the fetus.[180] Congenital infection is most severe when acquired in the first trimester and can result in chorioretinitis, intracranial calcifications, microcephaly, mental retardation, and deafness.[55,88,90] Symptomatic neonates with disseminated disease have hepatosplenomegaly, lymphadenopathy, jaundice, fever, anemia, pneumonitis, and a poor prognosis. Other ocular manifestations include cataracts, strabismus, microcornea, vitritis, retinal detachment, optic atrophy, microphthalmos, nystagmus, and ptosis.[180] In a prospective study, 15 percent of infected newborns had chorioretinal scars, indicative of infection in utero; 4 percent had active chorioretinitis; and, in 10 percent, retinal lesions developed by the time the children were 1 to 2 years of age. Long-term follow-up studies found that chorioretinal lesions develop in 82 to 85 percent of children with subclinical *Toxoplasma* infection, some with severe visual loss. Infants with asymptomatic toxoplasmosis should undergo regular ophthalmologic examination because retinal involvement can occur later in childhood and adulthood.[32,90,151] Some researchers suggest that all neonates with toxoplasmosis should receive drug therapy, even if they are asymptomatic.[90]

Toxoplasmosis is the leading cause of acquired necrotizing retinitis and, in many cases, represents reactivation of congenitally acquired infection. Clinically, an area of active retinochoroiditis is adjacent to the border of a chorioretinal scar.[90,228] Associated choroiditis and vitritis may be present. Primary acquired ocular toxoplasmosis manifested as retinochoroiditis is well documented also, and reports suggest that this route of infection may be more common than originally thought.[32,126] Recurrent

ocular disease with postnatally acquired toxoplasmosis has been reported.[29]

T. gondii causes a focal necrotizing retinitis with secondary choroiditis and vitritis. Patients may have floaters, blurred vision, and photophobia. Those with macular involvement can suffer significant visual loss. After the inflammation has resolved, a flat, pigmented chorioretinal scar develops. Visual loss depends on the location of the retinal lesion, with peripheral lesions resulting in little or no visual disturbance and macular lesions capable of producing profound visual loss.

Toxoplasma retinochoroiditis is an emerging problem in patients with AIDS and may be the initial manifestation of this syndrome.[293] The clinical appearance often is atypical.[256] Chronic suppressive therapy is necessary because infection recurs with discontinuation of treatment. A combination of pyrimethamine and clindamycin has been reported to be most effective as prophylaxis.[293]

The diagnosis of *Toxoplasma* retinochoroiditis usually is presumptive and is based on clinical appearance and serologic testing. Several serologic tests are available. The standard serologic diagnosis is based on the presence of anti-*Toxoplasma* IgM in any sample or demonstration of a significant rise in antibody titer in paired sera taken 4 to 6 weeks apart. However, in reactivated congenital infections, which many cases are, *Toxoplasma* IgM results are not positive. The antibody tests most commonly used are indirect immunofluorescent assay (IFA) and enzyme-linked immunosorbent assay for IgM and IgA.[103] False-positive results can occur with both tests in the presence of rheumatoid factor. If clinical infection is suspected and the initial testing results are negative, repeat testing or an alternative testing technique should be considered.

Standard treatment of *Toxoplasma* retinochoroiditis is triple therapy with sulfadiazine 100 to 200 mg/kg/day in four divided doses (maximum, 1.5 g), pyrimethamine 2 mg/kg/day for 3 days, then 1 mg/kg/day (maximum, 25 mg/day), and leucovorin (folinic acid) 10 to 25 mg given orally daily for 6 weeks. Folinic acid prevents the leukopenia and thrombocytopenia that may result from pyrimethamine therapy. Weekly complete blood counts and platelet counts are required to monitor toxicity from therapy. Alternative therapy with clindamycin 40 mg/kg/day in four divided doses (maximum, 2.4 g for 6 weeks) rather than sulfadiazine also has been effective.[273] This therapy is less toxic than triple therapy and therefore is better tolerated. In patients with severe intraocular inflammation, systemic corticosteroids can be used with concurrent antimicrobial therapy.

Preventive measures are twofold; first, avoidance of raw meat and cat feces during pregnancy, especially during the first trimester, and second, treatment of a mother known to have contracted the disease during pregnancy with spiramycin, because it has no known teratogenic effects.[53,65,66] In utero pyrimethamine and sulfadiazine treatment of a fetus known to be infected may be effective.[53,55,123]

Lymphocytic Choriomeningitis Infection

LCMV, an arenavirus discovered in 1933, was recognized as a cause of intrauterine infection in 1955 (also see the earlier section on viral uveitis).[182] The common house mouse *Mus musculus* is the natural host and reservoir of the virus, but laboratory mice and pet hamsters also may be infected.[8,122] LCMV may be transmitted to humans by airborne means, rodent bites, or food contaminated by rodent urine, feces, or saliva; the fetus may be infected by transplacental transmission.[103] The diagnosis may be confirmed by LCMV titers.

Systemic manifestations, often devastating, include hydrocephaly, microcephaly, periventricular calcifications, neonatal meningitis, hepatosplenomegaly, cerebral palsy, mental retardation, and seizures.[103] Ocular findings include chorioretinitis, optic atrophy, nystagmus, microphthalmos, strabismus, and cataracts.[182] The chorioretinitis may mimic congenital toxoplasmosis.[33] The most common ocular findings are peripheral chorioretinal scars, although macular involvement is a common occurrence as well. At present there is no effective treatment. Prevention involves avoidance of mice and hamsters by pregnant women.[103,182]

Rubella Infection

The rubella virion is an RNA virus of the family *Togaviridae* that causes a febrile exanthem. Before the advent of rubella vaccine in 1969, rubella, or German measles, epidemics occurred every 6 to 9 years. The last major outbreak in the United States took place in 1964.[14] With preschool immunization programs, most primary cases now reported occur in individuals between 15 and 24 years of age. Transmission occurs by inhalation of aerosolized droplets in the nasopharynx. Primary infection in adults results in a mild febrile illness associated with lymphadenopathy and a rash. The proportion of susceptible nonimmunized women of child-bearing age ranges from 10 to 25 percent. Fetal infection occurs transplacentally, and the likelihood of transmission from mother to fetus is highest (90%) in the first trimester; transmission at this stage can produce severe fetal damage and result in spontaneous abortion, stillbirth, or multiple congenital anomalies.

The classic congenital rubella syndrome, first described by Sir Norman McAlister in Australia in 1949,[182] is characterized by cardiac defects, ocular abnormalities, and hearing deficits. The incidence of ocular and cardiac defects is higher with exposure early in the first trimester; hearing deficits appear to be associated with exposure late in the first trimester. Givens and associates[102] reported that 88 percent had multiorgan involvement. Hearing loss is the most frequent nonocular manifestation.[182] The most common cardiac defects are patent ductus arteriosus, pulmonary artery stenosis, and pulmonary valve stenosis. Other features include microcephaly, thrombocytopenia, hepatosplenomegaly, and mental retardation.

Ocular involvement occurs in approximately 50 percent of infected infants.[99] Pigmentary retinopathy (i.e., salt-and-pepper retinopathy) affects 22 percent of infected infants. The retina has a mottled appearance, most frequently observed in the posterior pole; the optic nerve and vessels usually are normal unless the patient also has glaucoma.

Nuclear cataracts, which affect between 15 and 27 percent of patients, are the second most frequent ocular complication. Glaucoma is seen in approximately 10 percent of eyes; the combination of cataracts and glaucoma is an uncommon finding.[290] Microphthalmos (which occurs in 10 to 63 percent of affected individuals), iris atrophy, and iritis also have been reported.[27] Live virus may persist for years in the lens, so appropriate precautions must be taken during cataract surgery to prevent transmission.[103]

The retinopathy associated with rubella usually is asymptomatic and does not necessitate treatment. In some cases, it can be complicated by subretinal neovascularization, which may require laser or macular surgery. Cataracts cause most visual morbidity. Visual rehabilitation depends on early cataract extraction to prevent deprivation amblyopia, correction of aphakia with spectacles or contact lenses, and careful follow-up. Glaucoma in infants and children can be temporized with topical medications, but most cases require glaucoma surgery.

Congenital rubella has long-term consequences for all organs involved. In nearly two thirds of infants with no manifestations at birth, hearing loss or psychomotor deficits subsequently develop. From an ophthalmic standpoint, individuals with congenital rubella need continued follow-up.[102]

Cytomegalovirus Infection

CMV is an enveloped DNA virus of the family *Herpesviridae*. An estimated 80 percent of adults are infected by the time they reach 40 years of age.[260] In immunocompetent adults, infection is asymptomatic or can cause a mononucleosis syndrome. Individuals can shed virus in saliva, urine, and other body fluids for months to years after initial infection. Transmission occurs through exposure to body fluids, sexual contact, blood transfusion, or organ transplantation, or it can occur transplacentally. Primary infection acquired in the birth canal is not likely to result in serious disease.

Congenital CMV is the most common congenital infection in humans[261] and can result from exposure to the virus in utero or in the birth canal. In utero infection produces more serious disease. CMV infection occurs in 1 percent of all live births, and only 5 to 10 percent of infants with congenital CMV infection are symptomatic. Maternal infection may be primary or recurrent, but primary infection carries the greatest risk (30% to 40%) for transmission of symptomatic CMV disease to the newborn.[260] Blood transfusions from CMV antibody–positive donors also can result in severe CMV infection in the newborn. Systemic manifestations include intrauterine growth restriction (IUGR), thrombocytopenic purpura, microcephaly with periventricular calcifications, hepatosplenomegaly, pneumonia, and sensorineural deafness.[63] In symptomatic congenital CMV infection, the retina is the primary site of ocular involvement. Cytomegalic inclusion bodies are seen in all layers of the retina. Patchy white areas of necrotic retina with hemorrhage and vascular sheathing are seen in the peripheral portion of the retina, although the posterior pole can be affected as well.[160] Nonhemorrhagic retinitis also may be seen, and most affected infants do not have active retinitis at birth, although scars may be visible as evidence of previous disease.[48] Resolution of the retinitis results in an atrophic scar with areas of hyperpigmentation. If retinitis involves the retinal periphery, vision may be normal. However, visual loss may occur if the posterior pole is involved or if optic atrophy or retinal detachment occurs. CMV retinopathy develops in 5 to 30 percent of infants with clinically apparent disease.[83] Microphthalmos, optic nerve hypoplasia, optic atrophy, optic nerve colobomata, anophthalmia, corneal opacities, and anterior segment dysgenesis also have been associated with congenital CMV infection.[125] In addition, cyclopia and anophthalmia have been reported.[160]

The diagnosis of CMV retinitis is based on the clinical appearance and the constellation of systemic symptoms and signs. Recovery of the virus from urine, maternal cervical secretions, saliva, or aqueous humor can confirm the diagnosis. Complement fixation can identify IgM antibodies to CMV that do not cross react with other herpes viruses. Immunofluorescence techniques are more sensitive but less specific than complement fixation.[160]

Treatment of neonatal or pediatric CMV retinitis is based on the results of treatment of adults with CMV retinitis. Ganciclovir has been shown to stabilize and prevent spread of the disease in infants. Maintenance therapy is not required, but continued follow-up is necessary to detect recurrence. Granulocytopenia and thrombocytopenia can result. Retinal detachment requires vitrectomy, membrane peel, and silicone oil to tamponade the detached retina.[39,124]

Herpes Simplex Virus Infection

HSV is a double-stranded, enveloped DNA virus. Both subtypes, HSV-1 and HSV-2, can cause a vesicular skin eruption. HSV-1 typically is isolated from oral-facial infections; HSV-2 usually is isolated from genital infections. After primary infection occurs, HSV can maintain latency in neuronal ganglion cells and reactivate. Transmission occurs by exposure to infected body fluids such as saliva, and the risk for transmission is higher when the individual is symptomatic.[192]

Maternal-fetal transmission occurs through infected genital secretions in the birth canal (HSV-2) or exposure to infected individuals with oral-facial herpetic disease (HSV-1) in the postnatal period. In mothers with active genital disease, the risk for transmission to the neonate is 50 percent with vaginal delivery. Most series report that between 70 and 80 percent of neonatal HSV infection is caused by HSV-2. Neonatal HSV infection is life-threatening, and the mortality rate without treatment is 80 percent.[295] The diagnosis is suspected by the clinical findings—lethargy, respiratory distress, anorexia, vomiting, cyanosis, low birth weight, IUGR, microcephaly, seizures, intracranial calcifications, pneumonia, hepatosplenomegaly, and skin vesicles. The virus may be isolated from skin vesicles or from nasal or conjunctival secretions, CSF, or blood. The mortality rate of untreated neonatal HSV is 49 percent.[103]

Ocular sequelae develop in fewer than 1 percent of immunocompetent adults with HSV infection. In

contrast, 17 to 40 percent of infected neonates have ocular disease.[173,260] Thirteen percent of neonates with HSV have eye involvement. Ocular involvement can range from mild conjunctivitis to severe bilateral necrotizing retinitis and may be unilateral or bilateral. Conjunctivitis is the most common manifestation. Conjunctivitis, keratitis, and, occasionally, retinitis develop 2 to 14 days after birth. HSV retinitis causes punctate, white-yellow lesions in the periphery and the posterior pole accompanied by choroiditis, vascular sheathing, hemorrhage, and vitritis. Chorioretinal atrophic scars with variable amounts of pigmentation around the border result after resolution of acute infection.[127,224] Visual prognosis depends on the severity of disease, macular scarring, optic atrophy, or CNS involvement of the visual pathways. Severe chorioretinitis and cortical blindness are the usual sequelae of HSV infection acquired through transplantation, a rare mode of transmission.

El Azazi and colleagues[84] examined children with serologically proved HSV infection 1 to 15 years after neonatal exposure and found a higher prevalence of chorioretinal scars than noted in previous reports (28% vs. 4%). This finding suggests that HSV remains dormant in the retina and reactivates later in childhood or adulthood. Chorioretinitis, cataracts, optic atrophy, and microphthalmos have been reported.[112] Acute retinal necrosis from reactivation of HSV-2 has occurred as well.[276]

Ocular HSV infection can be seen in conjunction with a vesicular rash or disseminated disease. The differential diagnosis of ophthalmic complications from HSV includes infection with any of the TORCHS organisms. Identification of neonatal IgM antibody to HSV confirms the diagnosis of in utero infection. Demonstration of intranuclear inclusions and multinucleated giant cells in skin, conjunctiva, and oral and genital lesions may be diagnostic.

The drug of choice to treat neonatal HSV infection is intravenous acyclovir 60 mg/kg/day.[214] Conjunctival and corneal disease also can be treated by débridement and topical antivirals such as vidarabine. In neonatal HSV infection, systemic acyclovir should be given regardless of topical treatment. Early diagnosis and treatment can reduce ocular morbidity.

Varicella-Zoster Virus Infection

VZV is a DNA virus of the family *Herpesviridae;* enveloped virions are the infectious agents. Primary infection results in chickenpox, a highly communicable febrile illness with a vesicular rash that appears after 48 to 72 hours of incubation. VZV can remain dormant in sensory ganglion neurons and reactivate as herpes zoster, a painful rash in the dermatomal distribution of the sensory ganglion.

Congenital varicella syndrome is considered a rare entity. One prospective series reported a 24 percent incidence of congenital varicella with serologic or clinical confirmation of maternal infection during pregnancy. Mortality rates can be high if maternal infection develops 5 days before delivery to 2 days after delivery. Systemic complications of VZV infection include cranial nerve palsy, hemiparesis, cicatricial skin lesions, IUGR, developmental delay, seizures, neurogenic bladder, and learning difficulty.[152,210]

Ocular abnormalities in children with congenital VZV infection include chorioretinitis, cataracts, Horner syndrome, optic atrophy and optic nerve hypoplasia, retinal coloboma, and microphthalmos. The chorioretinal scars of VZV infection have a deeply pigmented center with depigmented borders or a gliotic white center with hyperpigmented edges. The neurotropic nature of VZV infection may explain the association of Horner syndrome. Ocular involvement can be unilateral or bilateral. VZV chorioretinitis affects the macula, periphery, or both.

Serologic testing for IgG and IgM antibodies to VZV, a history of maternal infection during pregnancy, and the constellation of systemic findings help establish the diagnosis. Because active infection may occur early in pregnancy, the neonate may have IgG but no detectable IgM antibodies to VZV by the time of delivery. The persistence of elevated IgG antibodies beyond 6 months, when passive immunity through maternal antibodies has waned, without evidence of primary VZV infection postnatally is a helpful indication of infection in utero.

Syphilis

Syphilis is caused by the spirochete *T. pallidum*. Acquired syphilis is a sexually transmitted chronic disease and has three stages of infection. Primary infection is characterized by painless, indurated chancres of the skin or mucous membranes at the site of inoculation. The secondary stage appears as a maculopapular rash, classically involving the palms and soles. Generalized lymphadenopathy, fever, malaise, sore throat, headache, and arthralgias can accompany the rash. Hypertrophic lesions (i.e., condyloma lata) occur in moist mucous membranes. Approximately a third of untreated cases progress to the tertiary stage, which occurs after a variable latent period that may have occasional recurrences of secondary syphilis. Transmission to the fetus can occur with any stage of maternal syphilis.[271]

Pigmentary retinopathy is the most common early ocular manifestation of congenital syphilis. Diffuse mottling in the periphery (i.e., salt-and-pepper retinopathy) is indicative of chorioretinitis in utero. Pigment clumping in the periphery usually has no effect on vision, but macular involvement can cause decreased vision. Retinal changes can appear later in adulthood, thus suggesting that inflammatory changes caused by congenital infection can occur after birth. Salt-and-pepper retinopathy is evidence of previous inflammation, and no treatment is required. Interstitial keratitis (see the discussion of stromal keratitis) is the hallmark of congenital syphilis and occurs in 75 percent of these patients.[271] It usually is not detected until late in the first decade of life. Other reported ocular manifestations include optic neuritis and iritis.

Testing the serum by nontreponemal and treponemal methods confirms the diagnosis of congenital infection. The American Academy of Pediatrics recommends physical examination, quantitative nontreponemal serologic

testing, a CSF Venereal Disease Research Laboratory (VDRL) test, long-bone radiographs, and antitreponemal IgM testing, as specified by the Centers for Disease Control and Prevention (CDC). The CDC states that congenital syphilis is presumptively diagnosed with a positive VDRL and at least one of the following: physical examination evidence of congenital syphilis, characteristic radiographic long-bone findings, VDRL-positive CSF findings, an otherwise unexplained elevated CSF protein or cell count, quantitative nontreponemal serologic titer four times greater than the mother's, or a positive fluorescent treponemal antibody absorbent FTA-(ABS) 19s IgM test.[5] Treatment is with intravenous penicillin G for 10 to 14 days.[6,170]

Endophthalmitis

Endophthalmitis is an infection within the eye that may arise endogenously during septicemia or exogenously from accidental or surgical trauma.[183,193] Sixty-two percent of cases occur after intraocular surgery, 20 percent after penetrating trauma, and 10 percent after glaucoma surgery; 8 percent result from metastatic infection.[75,222] The patient usually has severe ocular pain and visual loss at initial evaluation. Rarely, patients may be asymptomatic. Signs of endophthalmitis include conjunctival injection, vitritis, uveitis, hypopyon, and intraocular membrane formation.

Endophthalmitis is an ophthalmic emergency and necessitates immediate evaluation of aqueous and vitreous cultures and institution of intravitreal antibiotic therapy. Any significant delay in recognition and treatment of endophthalmitis can result in permanent vision loss.

Postsurgical endophthalmitis occurs in 0.086 percent of cataract operations.[2] The source of postsurgical infection may be from the eyelids, conjunctiva, contaminated instruments and irrigating solutions, or contamination by operating room personnel. The routine use of subconjunctival antibiotics after intraocular surgery does not prevent all cases of postoperative endophthalmitis. Vitreous loss, which may occur occasionally as a complication of cataract surgery, increases the risk for infection developing. Patients who have undergone glaucoma filtering surgery are particularly prone to endophthalmitis, primarily because sclerostomy is performed routinely as part of this procedure and the only remaining protection the eye has from infective organisms is a thin layer of conjunctiva overlying the sclerostomy. Local antimetabolites, often used in conjunction with glaucoma filtering procedures, predispose the conjunctiva to bleb leaks that allow direct access of microorganisms to the eye.

Endogenous (metastatic) endophthalmitis should be suspected when ocular inflammation occurs in a septicemic patient, particularly if the patient is immunocompromised or has an underlying systemic illness such as diabetes or leukemia.[28,190,204]

Posttraumatic endophthalmitis should be considered in any patient with a history of injury and visual loss. A history of a high-velocity projectile presents great concern for a penetrating injury, and apparently minor accidental ocular trauma may result in perforation of the globe with or without a retained foreign body. The likelihood of posttraumatic endophthalmitis occurring increases directly with the extent of the injury and the degree of intraocular contamination.

After the diagnosis is suspected, immediate referral to an ophthalmologist is necessary. Once the patient has been examined, a vitreous and aqueous aspirate is obtained under general anesthesia and material is evaluated with Gram and Giemsa stains. Staining with calcofluor white or acridine orange is performed if fungal infection is a concern. The material is cultured on blood and chocolate agar, thioglycolate broth, and Sabouraud agar. Vancomycin 1 mg to cover gram-positive organisms and ceftazidime 2.25 mg to cover gram-negative organisms are injected into the vitreous cavity. If fungal infection is suspected, intravitreal amphotericin B is given. Occasionally, the ophthalmologist uses intravitreal dexamethasone to control associated, intense intraocular inflammation. Gram stain results often are inconsistent with cultures.

The role of vitrectomy in postoperative endophthalmitis has been addressed in the Endophthalmitis Vitrectomy Study (EVS).[6] In eyes with better than light perception vision at initial evaluation, outcome measurements were equal between the vitrectomy group and the vitreous tap or biopsy group. In eyes with only light perception vision, the EVS found that patients who underwent immediate pars plana vitrectomy did significantly better than those who underwent vitreous tap or biopsy alone.

The most common infectious agents in endophthalmitis are *S. epidermidis*, *Bacillus* spp., *Streptococcus* spp., *S. aureus*, and various fungi. *Bacillus cereus* is isolated in 30 to 40 percent of cases and can cause severe ocular morbidity.[1,59,60,70,119] *S. epidermidis* is the predominant organism in postoperative cases, and *Streptococcus* spp. with filtering blebs and *B. cereus* are the organisms associated most frequently with penetrating trauma. Approximately 65 percent of cases are culture-positive.

Fungal endophthalmitis has become a relatively common form of endophthalmitis in childhood because of the prolonged hospital care required for severely ill immunocompromised children. The most common organism is *C. albicans*. Children with a central line catheter or receiving prolonged intravenous therapy of any type are particularly prone to infection. In patients with *Candida* endophthalmitis, the vitreous may be hazy and small, white "snowball" localizations of infected material may appear in the vitreous or on the surface of the retina. Daily careful observation may be required during intravenous amphotericin B therapy. If the endophthalmitis clears, no ocular surgical intervention is indicated. If the infection is not adequately controlled with intravenous therapy, vitrectomy with injection of intravitreal amphotericin may be required.[86]

NEW REFERENCES SINCE THE SIXTH EDITION

7. Armesto A, Ugrin MC. Orbital cellulitis after Faden operation on the medial rectus. *Strabismus* 2007;**15**:215–9.
9. Artac H, Silahli M, Keles S, et al. A rare cause of preseptal cellulitis: anthrax. *Pediatr Dermatol* 2007;**24**:330–1.
11. Bababeygy SR, Silva RA, Sun Y, et al. Rifampin and linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* preseptal cellulitis. *Ophthal Plast Reconstr Surg* 2009;**25**:227–8.

23. Betts RF, Douglas Jr RG. Comparative study of reactogenicity and immunogenicity of influenza A/New Jersey/8/76 (Hsw1N1) virus vaccines in normal volunteers. *J Infect Dis* 1977;**136**(suppl): S443–9.
31. Braley AE, Hamilton HE. Central serous choroiditis associated with amebiasis; a record of nine cases. *AMA Arch Ophthalmol* 1957;**58**:1–14.
35. Brook I. Microbiology and antimicrobial treatment of orbital and intracranial complications of sinusitis in children and their management. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1183–6.
45. Cheuk W, Yuen HK, Chan AC, et al. Ocular adnexal lymphoma associated with IgG4+ chronic sclerosing dacryoadenitis: a previously undescribed complication of IgG4-related sclerosing disease. *Am J Surg Pathol* 2008;**32**:1159–67.
50. Corsi A, Nucci C, Knafelz D, et al. Ocular changes associated with Giardia lamblia infection in children. *Br J Ophthalmol* 1998;**82**:59–62.
58. Das JK, Choudhury BD, Medhi J. Orbital abscess and hemiocclusion of the central retinal vein in a child. *Orbit* 2007; **26**:295–7.
64. DeMuri GP, Wald ER. Complications of acute bacterial sinusitis in children. *Pediatr Infect Dis J* 2011;**30**:701–2.
67. Deutman AF, Klomp HJ. Rift Valley fever retinitis. *Am J Ophthalmol* 1981;**92**:38–42.
71. Dickinson AJ, Rosenthal AR, Nicholson KG. Inflammation of the retinal pigment epithelium: a unique presentation of ocular schistosomiasis. *Br J Ophthalmol* 1990;**74**:440–2.
72. Dizon M, Ozturk A, Redett RJ, et al. Neuroimaging findings in a child with dumbbell-shaped frontosphenoidal dermoid cyst presenting as preseptal cellulitis. *Pediatr Radiol* 2009;**39**:850–3.
76. Donaldson MJ, Gole GA. Amblyopia due to inflamed chalazion in a 13-month old infant. *Clin Experiment Ophthalmol* 2005;**33**: 332–3.
79. Dutton JJ, Escaravage Jr GK, Fowler AM, et al. Lipoblastomatosis: case report and review of the literature. *Ophthal Plast Reconstr Surg* 2011;**27**:417–21.
80. Easty DL, William C. Viral and rickettsial disease. In: Garner A, Klintworth G, editors. *Pathobiology of ocular disease: A dynamic approach*. New York: Marcel Dekker, Inc; 1994. p. 227–62.
81. Edward JD. The ocular manifestations of trichinosis. *Trans Ophthalmol Soc UK* 1954;**74**:495–7.
92. Ffooks OO. Dacryocystitis in infancy. *Br J Ophthalmol* 1962;**46**:422–34.
104. Golde K, Gardiner MF. Bacterial conjunctivitis in children: a current review of pathogens and treatment. *Int Ophthalmol Clin* 2011;**51**:85–92.
105. Gomez Morales A, Croxatto JO, Crovetto L, et al. Hydatid cysts of the orbit: a review of 35 cases. *Ophthalmology* 1988;**95**: 1027–32.
110. Guerry D 3rd, Kendig Jr EL. Congenital impatency of the nasolacrimal duct. *Arch Ophthalmol* 1948;**39**:193–204.
111. Gupta N, Dhawan A, Beri S, et al. Clinical spectrum of pediatric blepharoconjunctivitis. *J AAPOS* 2010;**14**:527–9.
128. Hovding G. Acute bacterial conjunctivitis. *Acta Ophthalmol* 2008; **86**:5–17.
132. Isenberg SJ, Apt L, Wood M. A controlled trial of povidone-iodine as prophylaxis against ophthalmia neonatorum. *N Engl J Med* 1995;**332**:562–6.
134. Jakobiec FA, Gess L, Zimmerman LE. Granulomatous dacryoadenitis caused by Schistosoma haematobium. *Arch Ophthalmol* 1977;**95**:278–80.
136. Jeffrey MP. Ocular disease caused by nematodes. *Am J Ophthalmol* 1955;**40**:41–53.
138. Jordan N, Verma H, Ekbote A, et al. Dacryoadenitis and diffuse orbital inflammation: unusual first presentations of Churg-Strauss syndrome. *Orbit* 2011;**30**:160–1.
143. Kaufman HE. Adenovirus advances: new diagnostic and therapeutic options. *Curr Opin Ophthalmol* 2011;**22**:290–3.
144. Kempster R, Ang GS, Galloway G, et al. Langerhans cell histiocytosis mimicking preseptal cellulitis. *J Pediatr Ophthalmol Strabismus* 2009;**46**:108–11.
148. King RE, Praeger DL, Hallett JW. Amebic choroiditis. *Arch Ophthalmol* 1964;**72**:16–22.
149. Kiratli H, Sekeroğlu MA, Soylemezoğlu F. Unilateral dacryoadenitis as the sole presenting sign of Wegener's granulomatosis. *Orbit* 2008;**27**:157–60.
150. Kittiponghansa S, Prabriputaloong A, Pariyanonda S, et al. Intracameral gnathostomiasis: a cause of anterior uveitis and secondary glaucoma. *J Ophthalmol* 1987;**71**:618–22.
155. Liang L, Safran S, Gao Y, et al. Ocular demodicosis as a potential cause of pediatric blepharoconjunctivitis. *Cornea* 2010;**29**: 1386–91.
159. Lindsley L, Nichols JJ, Dickerson K. Interventions for acute internal hordeolum (review). *Cochrane Database Syst Rev* 2010;(9): CD007742.
161. Looareesuwan S, Charoenpan P, Ho M, et al. Fatal Plasmodium falciparum malaria after an inadequate response to quinine treatment. *J Infect Dis* 1990;**161**:577–80.
162. Looareesuwan S, Phillips RE, Karbwang J, et al. Plasmodium falciparum hyperparasitaemia: use of exchange transfusion in seven patients and a review of the literature. *Q J Med* 1990;**75**: 471–81.
163. Looareesuwan S, Warrell DA, White NJ, et al. Retinal hemorrhage, a common sign of prognostic significance in cerebral malaria. *Am J Trop Med Hyg* 1983;**32**:911–5.
164. MacEwen CJ, Young JD. Epiphora during the first year of life. *Eye (Lond)* 1991;**5**:596–600.
168. Manschot WA. Coenurus infestation of eye and orbit. *Arch Ophthalmol* 1976;**94**:961–4.
169. Mantovani MP, Guandalini S, Cosentini E, et al. ABO blood-group distribution and HLA typing in children with giardiasis. *Trans R Soc Trop Med Hyg* 1990;**84**:380–1.
177. McKinley SH, Yen MT, Miller AM, et al. Microbiology of pediatric orbital cellulitis. *Am J Ophthalmol* 2007;**144**:497–501.
184. Miller A, Castanes M, Yen M, et al. Infantile orbital cellulitis. *Ophthalmology* 2008;**115**:594.
194. Nanda M, Curtin VT, Hilliard JK, et al. Ocular histopathologic findings in a case of human herpes B virus infection. *Arch Ophthalmol* 1990;**108**:713–6.
195. Nelson GS. The pathology of filarial infections. *Helminthol Abstr* 1966;**35**:311–36.
196. Nelson LB, Calhoun JH, Menduke H. Medical management of congenital nasolacrimal duct obstruction. *Pediatrics* 1985;**76**: 172–5.
197. Nemet AY, Vinjer S, Kaiserman I. Associated morbidity of chalazia. *Cornea* 2011;**30**:1376–81.
198. Nieto JC, Kim N, Lucarelli MJ. Dacryoadenitis and orbital myositis associated with Lyme disease. *Arch Ophthalmol* 2008;**126**: 1165–6.
200. Noda S, Hayasaka S, Setogawa T. Congenital nasolacrimal duct obstruction in Japanese infants: its incidence and treatment with massage. *J Pediatr Ophthalmol Strabismus* 1991;**28**:20–2.
205. Okamoto Y, Hiraoka T, Okamoto F, et al. A case of subperiosteal abscess of the orbit with central retinal artery occlusion. *Eur J Ophthalmol* 2009;**19**:288–91.
208. Osuntokun O, Olurin O. Filarial worm (Loa loa) in the anterior chamber: report of two cases. *Br J Ophthalmol* 1975;**59**:166–7.
211. Paul TO, Shepherd R. Congenital nasolacrimal duct obstruction: natural history and the timing of optimal intervention. *J Pediatr Ophthalmol Strabismus* 1994;**31**:362–7.
215. Petersen RA, Robb RM. The natural course of congenital obstruction of the nasolacrimal duct. *J Pediatr Ophthalmol Strabismus* 1978;**15**:246–50.
217. Pichichero ME, Wagner RS, Granet DB. Pediatric conjunctivitis: clinical decision-making for optimal treatment. *Inf Dis Child May* 2011:1–17.
218. Pittella JE, Oréfice F. Schistosomotic choroiditis. II. Report of first case. *Br J Ophthalmol* 1985;**69**:300–2.
221. Price HW. Dacryostenosis. *J Pediatr* 1947;**30**:302–5.
223. Rabon RJ, Louis GJ, Zegarra H, et al. Acute bilateral posterior angiopathy with influenza A viral infection. *Am J Ophthalmol* 1987;**103**(3 pt 1):289–93.
231. Rodger FC. Eye disease. In: Manson-Bahr PE, Bell DR, editors. *Manson's tropical diseases*. London: Bailliere Tindall; 1987. p. 1133–82.
234. Roth AM, Purcell TW. Ocular findings associated with encephalomyelitis caused by Herpes virus simiae. *Am J Ophthalmol* 1977;**84**:345–8.
243. Schmoll C, Macrae M, Mulvihill A, et al. Tuberculous dacryoadenitis in a Scottish teenager. *Br J Ophthalmol* 2009;**93**:137–8, 274.

248. Seltz LB, Smith J, Durairaj VD, et al. Microbiology and antibiotic management of orbital cellulitis. *Pediatrics* 2011;**127:**e566–72.
249. Semba RD, Murphy RP, Newland HS, et al. Longitudinal study of lesions of the posterior segment in onchocerciasis. *Ophthalmology* 1990;**97**:1334–41.
250. Sheikh A, Hurwitz B. Topical antibiotics for acute bacterial conjunctivitis: Cochrane systematic review and meta-analysis update. *Br J Gen Practice* 2005;**55**:962–4.
252. Siam AL, Gharbawi KF, Meegan JM. Ocular complications of Rift Valley fever. *J Egypt Public Health Assoc* 1978;**53**:185–6.
253. Silver LH, Woodside AM, Montgomery DB. Clinical safety of moxifloxacin ophthalmic solution 0.5% (Vigamox) in pediatric and nonpediatric patients with bacterial conjunctivitis. *Surv Ophthalmol* 2005;**50**:(Suppl. 1):S55–63.
254. Skevaki CL, Galani IE, Pararas MV, et al. Treatment of viral conjunctivitis with antiviral drugs. *Drugs* 2011;**71**:331–47.
257. Smyth JD. In vitro studies and host-specificity in *Echinococcus*. *Bull World Health Organ* 1968;**39**:5–12.
270. Tabbara KF, Shoukrey N. Trichinosis. In: Gold DH, Weingeist TA, editors. *The eye in systemic disease*. Philadelphia: JB Lippincott; 1990. p. 191–2.
280. Toussaint D, Danis P. Retinopathy in generalized loa-loa filariasis: a clinicopathological study. *Arch Ophthalmol* 1965;**74**:470–6.
281. Tudor RC, Blair E. *Gnathostoma spinigerum:* an unusual cause of ocular nematodiasis in the Western Hemisphere. *Am J Ophthalmol* 1971;**30**(72):185–90.
283. Usha K, Smitha S, Shah N, Lalitha P, et al. Spectrum and the susceptibilities of microbial isolates in cases of congenital nasolacrimal duct obstruction. *J AAPOS* 2006;**10**:469–72.
298. Williams DF, Williams GA, Caya JG, et al. Intraocular *Echinococcus multilocularis*. *Arch Ophthalmol* 1987;**105**:1106–9.
299. Williams PH, Templeton AC. Infection of the eye by tapeworm coenurus. *Br J Ophthalmol* 1971;**55**:766–9.
302. World Health Organization. *Third report World Health organization technical report series 752*. Geneva: WHO; 1987.
306. Young RC, Hodge DO, Liesegang TJ, et al. Incidence, recurrence, and outcomes of Herpes simplex virus eye disease in Olmstead County, Minnesota, 1976-2007: the effect of oral prophylaxis. *Arch Ophthalmol* 2010;**128**:1178–83.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alfaro DV, Roth D, Liggett PE. Posttraumatic endophthalmitis: causative organisms, treatment, and prevention. *Retina* 1994;**14**: 206–11.
2. Allen HF, Mangiaracine AB. Bacterial endophthalmitis after cataract extraction. II. Incidence in 36,000 consecutive operations with special reference to preoperative topical antibiotics. *Arch Ophthalmol* 1974;**91**:3–7.
3. Ambati BK, Ambati J, Azar N, et al. Periorbital and orbital cellulitis before and after the advent of *Haemophilus influenzae* type B vaccination. *Ophthalmology* 2000;**107**:1450–3.
4. American Academy of Ophthalmology, Basic and Clinical Science Course. Section 9: *Intraocular inflammation and uveitis*. San Francisco: American Academy of Ophthalmology; 1995. p. 57–61.
5. American Academy of Ophthalmology Section 6. Pediatric ophthalmology and strabismus. In: Liesegang TJ, editor. *Basic and clinical science course 2006-2006*. San Francisco: American Academy of Ophthalmology; 2005. p. 203–9.
6. American Academy of Pediatrics. *1997 Red book: report of the Committee on Infectious Diseases*. 24th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 1997.
7. Armesto A, Ugrin MC. Orbital cellulitis after Faden operation on the medial rectus. *Strabismus* 2007;**15**:215–9.
8. Armstrong D, Fortner JG, Rowe WP, et al. Meningitis due to lymphocytic choriomeningitis virus endemic in a hamster colony. *JAMA* 1969;**209**:265–7.
9. Artac H, Silahli M, Keles S, et al. A rare cause of preseptal cellulitis: anthrax. *Pediatr Dermatol* 2007;**24**:330–1.
10. Asbell P, Stenson S. Ulcerative keratitis: survey of 30 years' laboratory experience. *Arch Ophthalmol* 1982;**100**:77–80.
11. Bababeygy SR, Silva RA, Sun Y, et al. Rifampin and linezolid in the treatment of methicillin-resistant *Staphylococcus aureus* preseptal cellulitis. *Ophthal Plast Reconstr Surg* 2009;**25**:227–8.
12. Bale Jr JF, Murph JR. Congenital infections and the nervous system. *Pediatr Clin North Am* 1992;**39**:669–90.
13. Barkin RM, Todd JK, Amer J. Periorbital cellulitis in children. *Pediatrics* 1978;**62**:390–2.
14. Bart KJ, Orenstein WA, Preblud SR, et al. Elimination of rubella and congenital rubella from the United States. *Pediatr Infect Dis* 1985;**4**:14–21.
15. Bedrossian RH. Neuroretinitis following measles. *J Pediatr* 1955;**46**:329–31.
16. Bekir NA, Gungor K, Namiduru M. *Brucella melitensis* dacryoadenitis: a case report. *Eur J Ophthalmol* 2000;**10**:259–61.
17. Bell TA, Kuo CC, Stamm WE, et al. Direct fluorescent monoclonal antibody stain for rapid detection of infant *Chlamydia trachomatis* infections. *Pediatrics* 1984;**74**:224–8.
18. Benezra D. *Ocular inflammation: basic and clinical concepts*. London Malden, Mass: Martin Dunitz; 1999.
19. Benjamin A, Tufail A, Holland GN. Uveitis as the only clinical manifestation of poststreptococcal syndrome. *Am J Ophthalmol* 1997;**123**:258–60.
20. Benson WH, Lanier JD. Current diagnosis and treatment of corneal ulcers. *Curr Opin Ophthalmol* 1998;**9**:45–9.
21. Berenberg W, Nankervis G. Long-term follow-up of cytomegalic inclusion disease of infancy. *Pediatrics* 1970;**46**:403–10.
22. Bertuch AW, Rocco E, Schwartz EG. Lyme disease: ocular manifestations. *Ann Ophthalmol* 1988;**20**:376–8.
23. Betts RF, Douglas Jr RG. Comparative study of reactogenicity and immunogenicity of influenza A/New Jersey/8/76 (Hsw1N1) virus vaccines in normal volunteers. *J Infect Dis* 1977;**136**(suppl): S443–9.
24. Bhaskaram P, Mathur R, Rao V, et al. Pathogenesis of corneal lesions in measles. *Hum Nutr Clin Nutr* 1986;**40**:197–204.
25. Birenbaum E, Linder N, Varsano N, et al. Adenovirus type 8 conjunctivitis outbreak in a neonatal intensive care unit. *Arch Dis Child* 1993;**68**(5 Spec No):610–1.
26. Blumenkranz M, Clarkson J, Culbertson WW, et al. Visual results and complications after retinal reattachment in the acute retinal necrosis syndrome: the influence of operative technique. *Retina* 1989;**9**:170–4.
27. Boniuk M, Zimmerman LE. Ocular pathology in the rubella syndrome. *Arch Ophthalmol* 1967;**77**:455–73.
28. Borne MJ, Shields JA, Shields CL, et al. Bilateral viral endophthalmitis as the presenting sign of severe combined immunodeficiency. *Arch Ophthalmol* 1994;**112**:1280–1.
29. Bosch-Driessen EH, Rothova A. Recurrent ocular disease in postnatally acquired toxoplasmosis. *Am J Ophthalmol* 1999;**128**: 421–5.
30. Bouza E, Merino P, Muñoz P, et al. Ocular tuberculosis: a prospective study in a general hospital. *Medicine (Baltimore)* 1997;**76**: 53–61.
31. Braley AE, Hamilton HE. Central serous choroiditis associated with amebiasis; a record of nine cases. *AMA Arch Ophthalmol* 1957; **58**:1–14.
32. Brezin AP, Kasner L, Thulliez P, et al. Ocular toxoplasmosis in the fetus: immunohistochemistry analysis and DNA amplification. *Retina* 1994;**14**:19–26.
33. Brezin AP, Thulliez P, Cisneros B, et al. Lymphocytic choriomeningitis virus chorioretinitis mimicking ocular toxoplasmosis in two otherwise normal children. *Am J Ophthalmol* 2000;**130**:245–7.
34. Brook I. Dacryocystitis caused by anaerobic bacteria in the newborn. *Pediatr Infect Dis J* 1998;**17**:172–3.
35. Brook I. Microbiology and antimicrobial treatment of orbital and intracranial complications of sinusitis in children and their management. *Int J Pediatr Otorhinolaryngol* 2009;**73**:1183–6.
36. Brook I, Frazier EH. Aerobic and anaerobic microbiology of dacryocystitis. *Am J Ophthalmol* 1998;**125**:552–4.
37. Buck SL, Rosenthal RA, Schlech BA. Methods used to evaluate the effectiveness of contact lens care solutions and other compounds against *Acanthamoeba:* a review of the literature. *CLAO J* 2000;**26**:72–84.
38. Bullock JD, Fleishman JA. Orbital cellulitis following dental extraction. *Trans Am Ophthalmol Soc* 1984;**82**:111–33.
39. Byrne PJ, Silver MM, Gilbert JM, et al. Cyclopia and congenital cytomegalovirus infection. *Am J Med Genet* 1987;**28**:61–5.
40. Cable BB, Wassmuth Z, Mann EA, et al. The effect of corticosteroids in the treatment of experimental sinusitis. *Am J Rhinol* 2000;**14**:217–22.
41. Caca I, Cakmak SS, Unlü K, et al. Cutaneous anthrax on eyelids. *Jpn J Ophthalmol* 2004;**48**:268–71.
42. Callanan D, Fish GE, Anand R. Reactivation of inflammatory lesions in ocular histoplasmosis. *Arch Ophthalmol* 1998;**116**: 470–4.
43. Chang CH, Lai YH, Wang HZ, et al. Antibiotic treatment of orbital cellulitis: an analysis of pathogenic bacteria and bacterial susceptibility. *J Ocul Pharmacol Ther* 2000;**16**:75–9.
44. Charukamnoetkanok P, Pineda R, 2nd. Controversies in management of bacterial keratitis. *Int Ophthalmol Clin* 2005;**45**:199–210.
45. Cheuk W, Yuen HK, Chan AC, et al. Ocular adnexal lymphoma associated with IgG4+ chronic sclerosing dacryoadenitis: a previously undescribed complication of IgG4-related sclerosing disease. *Am J Surg Pathol* 2008;**32**:1159–67.
46. Chu KM, Rathinam R, Namperumalsamy P, et al. Identification of *Leptospira* species in the pathogenesis of uveitis and determination of clinical ocular characteristics in south India. *J Infect Dis* 1998;**177**:1314–21.
47. Clary R, Weber AL, Eavey R, et al. Orbital cellulitis with abscess formation caused by sinusitis. *Ann Otol Rhinol Laryngol* 1988;**97**(2 pt 1):211–2.
48. Coats DK, Demmler GJ, Paysse EA, et al. Ophthalmologic findings in children with congenital cytomegalovirus infection. *J AAPOS* 2000;**4**:110–6.
49. Corey L. Herpes simplex viruses. In: Fauci AS, Braunwald E, Isselbacher KJ, et al, editors. *Harrison's principles of internal medicine*. 14th ed. New York: McGraw-Hill; 1998. p. 1080–5.
50. Corsi A, Nucci C, Knafelz D, et al. Ocular changes associated with Giardia lamblia infection in children. *Br J Ophthalmol* 1998;**82**: 59–62.
51. Costa SF, Weiss LM. Drug treatment of microsporidiosis. *Drug Resist Update* 2000;**3**:384–99.
52. Couch JM, Green WR, Hirst LW, et al. Diagnosing and treating *Phthirus pubis* palpebrarum. *Surv Ophthalmol* 1982;**26**:219–25.
53. Couvreur J, Thulliez P, Daffos F, et al. In utero treatment of toxoplasmic fetopathy with the combination pyrimethamine-sulfadiazine. *Fetal Diagn Ther* 1993;**8**:45–50.
54. Credo BV, Dyment PG. Molluscum contagiosum. *Adolesc Med* 1996;**7**:57–62.

55. Daffos F, Forestier F, Capella-Pavlovsky M, et al. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. *N Engl J Med* 1988;**318**:271–5.
56. Daniel E, Ebenezer GJ, Job CK. Pathology of iris in leprosy. *Br J Ophthalmol* 1997;**81**:490–2.
57. Daniel E, Ffytche TJ, Sundar Rao PS, et al. Incidence of ocular morbidity among multibacillary leprosy patients during a 2 year course of multidrug therapy. *Br J Ophthalmol* 2006;**90**:568–73.
58. Das JK, Choudhury BD, Medhi J. Orbital abscess and hemiocclusion of the central retinal vein in a child. *Orbit* 2007;**26**: 295–7.
59. Davey Jr RT, Tauber WB. Posttraumatic endophthalmitis: the emerging role of *Bacillus cereus* infection. *Rev Infect Dis* 1987;**9**: 110–23.
60. David DB, Kirkby GR, Noble BA. *Bacillus cereus* endophthalmitis. *Br J Ophthalmol* 1994;**78**:577–80.
61. Dawson DR, Sheppard JD. Follicular conjunctivitis. In: Tasman W, Jaeger EA, editors. *Duane's clinical ophthalmology*. Philadelphia: J. B. Lippincott; 1990. p. 16–8.
62. Demirci G, Ay GM, Karabas LV, et al. Acanthamoeba keratitis in a 5-year-old boy without a history of contact lens usage. *Cornea* 2006;**25**:356–8.
63. Demmler GJ. Congenital cytomegalovirus infection and disease. *Adv Pediatr Infect Dis* 1996;**11**:135–62.
64. DeMuri GP, Wald ER. Complications of acute bacterial sinusitis in children. *Pediatr Infect Dis J* 2011;**30**:701–2.
65. Desmonts G, Couvreur J. Congenital toxoplasmosis: a prospective study of 378 pregnancies. *N Engl J Med* 1974;**290**:1110–6.
66. Desmonts G, Couvreur J. [Congenital toxoplasmosis: prospective study of the outcome of pregnancy in 542 women with toxoplasmosis acquired during pregnancy]. *Ann Pediatr (Paris)* 1984;**31**: 805–9.
67. Deutman AF, Klomp HJ. Rift Valley fever retinitis. *Am J Ophthalmol* 1981;**92**:38–42.
68. Dhaliwal U, Bhatia A. A rationale for therapeutic decision-making in chalazia. *Orbit* 2005;**24**:227–30.
69. Dhaliwal U, Arora VK, Singh N, et al. Cytopathology of chalazia. *Diagn Cytopathol* 2004;**31**:118–22.
70. Diamond JG. Intraocular management of endophthalmitis: a systematic approach. *Arch Ophthalmol* 1981;**99**:96–9.
71. Dickinson AJ, Rosenthal AR, Nicholson KG. Inflammation of the retinal pigment epithelium: a unique presentation of ocular schistosomiasis. *Br J Ophthalmol* 1990;**74**:440–2.
72. Dizon M, Ozturk A, Redett RJ, et al. Neuroimaging findings in a child with dumbbell-shaped frontosphenoidal dermoid cyst presenting as preseptal cellulitis. *Pediatr Radiol* 2009;**39**:850–3.
73. Donahue SP, Schwartz G. Preseptal and orbital cellulitis in childhood: a changing microbiologic spectrum. *Ophthalmology* 1998;**105**:1902–5; discussion 1905–6.
74. Donahue SP, Hein E, Sinatra RB. Ocular involvement in children with candidemia. *Am J Ophthalmol* 2003;**135**:886–7.
75. Donahue SP, Greven CM, Zuravleff JJ, et al. Intraocular candidiasis in patients with candidemia: clinical implications derived from a prospective multicenter study. *Ophthalmology* 1994;**101**:1302–9.
76. Donaldson MJ, Gole GA. Amblyopia due to inflamed chalazion in a 13-month old infant. *Clin Experiment Ophthalmol* 2005;**33**: 332–3.
77. Drew WL. Cytomegalovirus infection in patients with AIDS. *J Infect Dis* 1988;**158**:449–56.
78. Du LT, Coats DK, Kline MW, et al. Incidence of presumed cytomegalovirus retinitis in HIV-infected pediatric patients. *J AAPOS* 1999;**3**:245–9.
79. Dutton JJ, Escaravage Jr GK, Fowler AM, et al. Lipoblastomatosis: case report and review of the literature. *Ophthal Plast Reconstr Surg* 2011;**27**:417–21.
80. Easty DL, William C. Viral and rickettsial disease. In: Garner A, Klintworth GK, editors. *Pathobiology of ocular disease: a dynamic approach*. New York: Marcel Dekker; 1994. p. 227–62.
81. Edward JD. The ocular manifestations of trichinosis. *Trans Ophthalmol Soc UK* 1954;**74**:495–7.
82. Edwards KM, Meredith TA, Hagler WS, et al. Ophthalmomyiasis interna causing visual loss. *Am J Ophthalmol* 1984;**97**:605–10.
83. Eichenwald HF, Shinefield HR. Viral infections of the fetus and of the premature and newborn infant. *Adv Pediatr* 1962;**12**: 249–305.
84. el Azazi M, Malm G, Forsgren M. Late ophthalmologic manifestations of neonatal herpes simplex virus infection. *Am J Ophthalmol* 1990;**109**:1–7.
85. El Hassan AM, Khalil EA, el Sheikh EA, et al. Post kala-azar ocular leishmaniasis. *Trans R Soc Trop Med Hyg* 1998;**92**:177–9.
86. Endophthalmitis Vitrectomy Study Group. Results of the Endophthalmitis Vitrectomy Study: a randomized trial of immediate vitrectomy and of intravenous antibiotics for the treatment of postoperative bacterial endophthalmitis. Endophthalmitis Vitrectomy Study Group. *Arch Ophthalmol* 1995;**113**:1479–96.
87. English FP, Nutting WB. Demodicosis of ophthalmic concern. *Am J Ophthalmol* 1981;**91**:362–72.
88. Engstrom Jr RE, Holland GN, Nussenblatt RB, et al. Current practices in the management of ocular toxoplasmosis. *Am J Ophthalmol* 1991;**111**:601–10.
89. Epstein GA, Putterman AM. Combined excision and drainage with intralesional corticosteroid injection in the treatment of chronic chalazia. *Arch Ophthalmol* 1988;**106**:514–6.
90. Fair JR. Clinical eye findings in congenital toxoplasmosis. *Surv Ophthalmol* 1961;**6**:923–35.
91. Feltkamp TE, Ringrose JH. Acute anterior uveitis and spondyloarthropathies. *Curr Opin Rheumatol* 1998;**10**:314–8.
92. Ffooks OO. Dacryocystitis in infancy. *Br J Ophthalmol* 1962; **46**:422–34.
93. Foster A, Sommer A. Corneal ulceration, measles, and childhood blindness in Tanzania. *Br J Ophthalmol* 1987;**71**:331–43.
94. Foster CS. Fungal keratitis. *Infect Dis Clin North Am* 1992; **6**:851–7.
95. Fox KR, Golomb HS. Staphylococcal ophthalmia neonatorum and the staphylococcal scalded skin syndrome. *Am J Ophthalmol* 1979;**88**:1052–5.
96. Friendly DS. Ophthalmia neonatorum. *Pediatr Clin North Am* 1983;**30**:1033–42.
97. Fulk GW, Murphy B, Robins MD. Pilocarpine gel for the treatment of demodicosis: a case series. *Optom Vis Sci* 1996;**73:** 742–5.
98. Gao YY, Di Pascuale MA, Li W, et al. High prevalence of Demodex in eyelashes with cylindrical dandruff. *Invest Ophthalmol Vis Sci* 2005;**46**:3089–94.
99. Geltzer AI, Guber D, Sears ML. Ocular manifestations of the 1964-65 rubella epidemic. *Am J Ophthalmol* 1967;**63**:221–9.
100. Gigliotti F, Hendley JO, Morgan J, et al. Efficacy of topical antibiotic therapy in acute conjunctivitis in children. *J Pediatr* 1984;**104**:623–6.
101. Gigliotti F, Williams WT, Hayden FG, et al. Etiology of acute conjunctivitis in children. *J Pediatr* 1981;**98**:531–6.
102. Givens KT, Lee DA, Jones T, et al. Congenital rubella syndrome: ophthalmic manifestations and associated systemic disorders. *Br J Ophthalmol* 1993;**77**:358–63.
103. Goldberg GN, Fulginiti VA, Ray CG, et al. In utero Epstein-Barr virus (infectious mononucleosis) infection. *JAMA* 1981;**246**: 1579–81.
104. Golde K, Gardiner MF. Bacterial conjunctivitis in children: a current review of pathogens and treatment. *Int Ophthalmol Clin* 2011;**51**:85–92.
105. Gomez Morales A, Croxatto JO, Crovetto L, et al. Hydatid cysts of the orbit: a review of 35 cases. *Ophthalmology* 1988;**95**:1027–32.
106. Goscienski PJ. Inclusion conjunctivitis in the newborn infant. *J Pediatr* 1970;**77**:19–26.
107. Green WR, Font RL, Zimmerman LE. Aspergillosis of the orbit: report of ten cases and review of the literature. *Arch Ophthalmol* 1969;**82**:302–13.
108. Greenberg MF, Pollard ZF. Medical treatment of pediatric subperiosteal orbital abscess secondary to sinusitis. *J AAPOS* 1998;**2**: 351–5.
109. Grefer J, Santer R, Ankermann T, et al. Tubulointerstitial nephritis and uveitis in association with Epstein-Barr virus infection. *Pediatr Nephrol* 1999;**13**:336–9.
110. Guerry D 3rd, Kendig Jr EL. Congenital impatency of the nasolacrimal duct. *Arch Ophthalmol* 1948;**39**:193–204.
111. Gupta N, Dhawan A, Beri S, et al. Clinical spectrum of pediatric blepharoconjunctivitis. *J AAPOS* 2010;**14**:527–9.
112. Hagler WS, Walters PV, Nahmias AJ. Ocular involvement in neonatal herpes simplex virus infection. *Arch Ophthalmol* 1969; **82**:169–76.

113. Haimovici R, Roussel TJ. Treatment of gonococcal conjunctivitis with single-dose intramuscular ceftriaxone. *Am J Ophthalmol* 1989;**107**:511–4.
114. Hall LR, Pearlman E. Pathogenesis of onchocercal keratitis (river blindness). *Clin Microbiol Rev* 1999;**12**:445–53.
115. Hammerschlag MR. Neonatal conjunctivitis. *Pediatr Ann* 1993; **22**:346–51.
116. Hammerschlag MR, Chandler JW, Alexander ER, et al. Erythromycin ointment for ocular prophylaxis of neonatal chlamydial infection. *JAMA* 1980;**244**:2291–3.
117. Hansen T, Burns RP, Allen A. Gonorrheal conjunctivitis an old disease returned. *JAMA* 1966;**195**:1156.
118. Hartstein ME, Steinvurzel MD, Cohen CP. Intracranial abscess as a complication of subperiosteal abscess of the orbit. *Ophthal Plast Reconstr Surg* 2001;**17**:398–403.
119. Hemady R, Zaltas M, Paton B, et al. Bacillus-induced endophthalmitis: new series of 10 cases and review of the literature. *Br J Ophthalmol* 1990;**74**:26–9.
120. Hess DL. Chlamydia in the neonate. *Neonatal Netw* 1993;**12**: 9–12.
121. Hierholzer JCH, Hatch M. Acute hemorrhagic conjunctivitis. In: Darnell RW, editor. *Viral diseases of the eye*. Philadelphia: Lea & Febiger; 1985. p. 165–96.
122. Hirsch MS, Moellering Jr RC, Pope HG, et al. Lymphocytic-choriomeningitis-virus infection traced to a pet hamster. *N Engl J Med* 1974;**291**:610–2.
123. Hohlfeld P, Daffos F, Thulliez P. Fetal toxoplasmosis: outcome of pregnancy and infant follow-up after in utero treatment. *J Pediatr* 1989;**115**(5 pt 1):765–9.
124. Holland GN. An update on AIDS-related cytomegalovirus retinitis. In: *Focal points: clinical modules for ophthalmologists*. Module 5. San Francisco: American Academy of Ophthalmology; 1991.
125. Holland GN. Infectious diseases. In: Isenberg S, editor. *The eye in infancy*. Chicago: Year Book; 1989.
126. Holland GN. Reconsidering the pathogenesis of ocular toxoplasmosis. *Am J Ophthalmol* 1999;**128**:502–5.
127. Honda Y, Nakazawa Y, Chihara E. Necrotizing chorioretinitis induced by herpes simplex virus infection in the neonate. *Metab Pediatr Syst Ophthalmol* 1983;**7**:147–52.
128. Hovding G. Acute bacterial conjunctivitis. *Acta Ophthalmol* 2008;**86**:5–17.
129. Hunt KE, Glasgow BJ. *Aspergillus* endophthalmitis: an unrecognized endemic disease in orthotopic liver transplantation. *Ophthalmology* 1996;**103**:757–67.
130. Huppertz HI, Munchmeier D, Lieb W. Ocular manifestations in children and adolescents with Lyme arthritis. *Br J Ophthalmol* 1999;**83**:1149–52.
131. Illingworth CD, Cook SD. Acanthamoeba keratitis. *Surv Ophthalmol* 1998;**42**:493–508.
132. Isenberg SJ, Apt L, Wood M. A controlled trial of povidone-iodine as prophylaxis against ophthalmia neonatorum. *N Engl J Med* 1995;**332**:562–6.
133. Jabs DA, Green WR, Fox R, et al. Ocular manifestations of AIDS. *Ophthalmology* 1989;**96**:1092–9.
134. Jakobiec FA, Gess L, Zimmerman LE. Granulomatous dacryoadenitis caused by *Schistosoma haematobium*. *Arch Ophthalmol* 1977; **95**:278–80.
135. Jarrett WH 2nd, Gutman FA. Ocular complications of infection in the paranasal sinuses. *Arch Ophthalmol* 1969;**8**:683–8.
136. Jeffrey MP. Ocular disease caused by nematodes. *Am J Ophthalmol* 1955;**40**:41–53.
137. Jones DB. Viral and chlamydial conjunctivitis. In: *Symposium on medical and surgical diseases of the cornea: transactions of the New Orleans Academy of Ophthalmology*. St. Louis: C. V. Mosby; 1980. p. 497–523.
138. Jordan N, Verma H, Ekbote A, et al. Dacryoadenitis and diffuse orbital inflammation: unusual first presentations of Churg-Strauss syndrome. *Orbit* 2011;**30**:160–1.
139. Joseph J, Vemuganti GK, Sharma S. Microsporidia: emerging ocular pathogens. *Indian J Med Microbiol* 2005;**23**:80–91.
140. Joseph J, Sridhar MS, Murthy S, et al. *Use of different stains for microscopic evaluation of corneal scrapings for diagnosis of microsporidial keratitis*. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1392708.
141. Katz BJ, Scott WE, Folk JC. Acute histoplasmosis choroiditis in 2 immunocompetent brothers. *Arch Ophthalmol* 1997;**115**: 1470–2.
142. Kaufman HE. Treatment of viral diseases of the cornea and external eye. *Prog Retinal Eye Res* 2000;**19**:69–85.
143. Kaufman HE. Adenovirus advances: new diagnostic and therapeutic options. *Curr Opin Ophthalmol* 2011;**22**:290–3.
144. Kemal M, Sümer Z, Toker MI, et al. The prevalence of *Demodex folliculorum* in blepharitis patients and the normal population. *Ophthal Epidemiol* 2005;**12**:287–90.
145. Kempster R, Ang GS, Galloway G, et al. Langerhans cell histiocytosis mimicking preseptal cellulitis. *J Pediatr Ophthalmol Strabismus* 2009;**46**:108–11.
146. Kerkhoff FT, Ossewaarde JM, de Loos WS, et al. Presumed ocular bartonellosis. *Br J Ophthalmol* 1999;**83**:270–5.
147. Kestelyn P, Taelman H, Bogaerts J, et al. Ophthalmic manifestations of infections with Cryptococcus neoformans in patients with the acquired immunodeficiency syndrome. *Am J Ophthalmol* 1993;**116**:721–7.
148. King RE, Praeger DL, Hallett JW. Amebic choroiditis. *Arch Ophthalmol* 1964;**72**:16–22.
149. Kiratli H, Sekeroğlu MA, Soylemezoğlu F. Unilateral dacryoadenitis as the sole presenting sign of Wegener's granulomatosis. *Orbit* 2008;**27**:157–60.
150. Kittiponghansa S, Prabriputaloong A, Pariyanonda S, et al. Intracameral gnathostomiasis: a cause of anterior uveitis and secondary glaucoma. *J Ophthalmol* 1987;**71**:618–22.
151. Koppe JG, Kloosterman GJ, de Roever-Bonnet H. Toxoplasmosis and pregnancy, with a long-term follow-up of the children. *Eur J Obstet Gynecol Reprod Biol* 1974;**4**:101–9.
152. Laforet EG, Lynch Jr CL. Multiple congenital defects following maternal varicella; report of a case. *N Engl J Med* 1947;**236:** 534–7.
153. Larkin DF, Kilvington S, Dart JK. Treatment of *Acanthamoeba* keratitis with polyhexamethylene biguanide. *Ophthalmology* 1992;**99**:185–91.
154. Lewis KT, Stiles M. Management of cat and dog bites. *Am Fam Physician* 1995;**52**:479–85, 489–90.
155. Liang L, Safran S, Gao Y, et al. Ocular demodicosis as a potential cause of pediatric blepharoconjunctivitis. *Cornea* 2010;**29**: 1386–91.
156. Liesegang TJ. Diagnosis and therapy of herpes zoster ophthalmicus. *Ophthalmology* 1991;**98**:1216–29.
157. Liesegang TJ. Bacterial keratitis. *Infect Dis Clin North Am* 1992; **6**:815–29.
158. Liesegang TJ, Forster RK. Spectrum of microbial keratitis in South Florida. *Am J Ophthalmol* 1980;**90**:38–47.
159. Lindsley L, Dickerson K. Interventions for acute internal hordeolum (review). *The Cochrane Database Syst Rev* 2010;(9):CD007742.
160. Lonn LI. Neonatal cytomegalic inclusion disease chorioretinitis. *Arch Ophthalmol* 1972;**88**:434–8.
161. Looareesuwan S, Charoenpan P, Ho M, et al. Fatal *Plasmodium falciparum* malaria after an inadequate response to quinine treatment. *J Infect Dis* 1990;**161**:577–80.
162. Looareesuwan S, Phillips RE, Karbwang J, et al. *Plasmodium falciparum* hyperparasitaemia: use of exchange transfusion in seven patients and a review of the literature. *Q J Med* 1990;**75**:471–81.
163. Looareesuwan S, Warrell DA, White NJ, et al. Retinal hemorrhage, a common sign of prognostic significance in cerebral malaria. *Am J Trop Med Hyg* 1983;**32**:911–5.
164. MacEwen CJ, Young JD. Epiphora during the first year of life. *Eye (Lond)* 1991;**5**:596–600.
165. Mafee MF, Edward DP, Koeller KK, et al. Lacrimal gland tumors and simulating lesions: clinicopathologic and MR imaging features. *Radiol Clin North Am* 1999;**37**:219–39, xii.
166. Maini R, Edelsten C. Uveitis associated with parvovirus infection. *Br J Ophthalmol* 1999;**83**:1403–4.
167. Mannis MJ, Tamaru R, Roth AM, et al. Acanthamoeba sclerokeratitis: determining diagnostic criteria. *Arch Ophthalmol* 1986;**104**: 1313–7.
168. Manschot WA. Coenurus infestation of eye and orbit. *Arch Ophthalmol* 1976;**94**:961–4.
169. Mantovani MP, Guandalini S, Cosentini E, et al. ABO blood-group distribution and HLA typing in children with giardiasis. *Trans R Soc Trop Med Hyg* 1990;**84**:380–1.

170. Margo CE, Hamed LM. Ocular syphilis. *Surv Ophthalmol* 1992;**37**:203–20.
171. Marines HM, Osato MS, Font RL. The value of calcofluor white in the diagnosis of mycotic and *Acanthamoeba* infections of the eye and ocular adnexa. *Ophthalmology* 1987;**94**:23–6.
172. Mathers WD, Sutphin JE, Folberg R, et al. Outbreak of keratitis presumed to be caused by Acanthamoeba. *Am J Ophthalmol* 1996;**121**:129–42.
173. Matoba A. Ocular viral infections. *Pediatr Infect Dis* 1984;**3**: 358–68.
174. Matoba AY. Ocular disease associated with Epstein-Barr virus infection. *Surv Ophthalmol* 1990;**35**:145–50.
175. McCarty ML, Wilson MW, Fleming JC, et al. Manifestations of fungal cellulitis of the orbit in children with neutropenia and fever. *Ophthal Plast Reconstr Surg* 2004;**20**:217–23.
176. McCulley JP, Alizadeh H, Niederkorn JY. The diagnosis and management of *Acanthamoeba* keratitis. *CLAO J* 2000;**26**:47–51.
177. McKinley SH, Yen MT, Miller AM, et al. Microbiology of pediatric orbital cellulitis. *Am J Ophthalmol* 2007;**144**:497–501.
178. Merayo-Lloves J, Baltatzis S, Foster CS. Epstein-Barr virus dacryoadenitis resulting in keratoconjunctivitis sicca in a child. *Am J Ophthalmol* 2001;**132**:922–3.
179. Mets MB. Childhood blindness and visual loss: an assessment at two institutions including a "new" cause. *Trans Am Ophthalmol Soc* 1999;**97**:653–96.
180. Mets MB. Eye manifestations of intrauterine infections. *Ophthalmol Clin North Am* 2001;**14**:521–31.
181. Mets MB, Bechtel RT, Haught KA. Lymphocytic choriomeningitis virus: a new addition to the TORCH evaluation. *Arch Ophthalmol* 1997;**115**:680–1.
182. Mets MB, Barton LL, Khan AS, et al. Lymphocytic choriomeningitis virus: an underdiagnosed cause of congenital chorioretinitis. *Am J Ophthalmol* 2000;**130**:209–15.
183. Meyer D. Eye signs that alert the clinician to a diagnosis of AIDS. *SADJ [Tydskrif van die Suid-Afrikaanse Tandheelkundige Vereniging]* 2005;**60**:386–7.
184. Miller A, Castanes M, Yen M, et al. Infantile orbital cellulitis. *Ophthalmology* 2008;**115**:594.
185. Mohammad Ael-N. Intralesional steroid injection for management of acute idiopathic dacryoadenitis: a preliminary result. *Ophthal Plast Reconstr Surg* 2005;**21**:138–41.
186. Moinfar N, Wagner DG, Chrousos GA, et al. Paediatric varicella choroiditis. *Br J Ophthalmol* 1998;**82**:1092–3.
187. Moorthy RS, Rao NA, Sidikaro Y, et al. Coccidioidomycosis iridocyclitis. *Ophthalmology* 1994;**101**:1923–8.
188. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med* 2006;**355**:666–74.
189. Mordhorst CH, Dawson C. Sequelae of neonatal inclusion conjunctivitis and associated disease in parents. *Am J Ophthalmol* 1971;**71**:861–7.
190. Nagelberg HP, Petashnick DE, To KW, et al. Group B streptococcal metastatic endophthalmitis. *Am J Ophthalmol* 1994;**117**: 498–500.
191. Nageswaran S, Woods CR, Benjamin Jr DK, et al. Orbital cellulitis in children. *Pediatr Infect Dis J* 2006;**25**:695–9.
192. Nahmias AJ, Visintine AM, Caldwell DR, et al. Eye infections with herpes simplex viruses in neonates. *Surv Ophthalmol* 1976;**21**: 100–5.
193. Nakao K, Ohba N, Nakagawa M, et al. Clinical course of HTLV-1-associated uveitis. *Jpn J Ophthalmol* 1999;**43**:404–9.
194. Nanda M, Curtin VT, Hilliard JK, et al. Ocular histopathologic findings in a case of human herpes B virus infection. *Arch Ophthalmol* 1990;**108**:713–6.
195. Nelson GS. The pathology of filarial infections. *Helminthol Abstr* 1966;**35**:311–36.
196. Nelson LB, Calhoun JH, Menduke H. Medical management of congenital nasolacrimal duct obstruction. *Pediatrics* 1985;**76**: 172–5.
197. Nemet AY, Vinjer S, Kaiserman I. Associated morbidity of chalazia. *Cornea* 2011;**30**:1376–81.
198. Nieto JC, Kim N, Lucarelli MJ. Dacryoadenitis and orbital myositis associated with Lyme disease. *Arch Ophthalmol* 2008;**126**: 1165–6.
199. Nishida H, Risemberg HM. Silver nitrate ophthalmic solution and chemical conjunctivitis. *Pediatrics* 1975;**56**:368–73.
200. Noda S, Hayasaka S, Setogawa T. Congenital nasolacrimal duct obstruction in Japanese infants: its incidence and treatment with massage. *J Pediatr Ophthalmol Strabismus* 1991;**28**:20–2.
201. Obenour LC. Subacute sclerosing panencephalitis. *Int Ophthalmol Clin* 1972;**12**:215–21.
202. O'Brien TP. Management of bacterial keratitis: beyond exorcism towards consideration of organism and host factors. *Eye (Lond)* 2003;**17**:957–74.
203. O'Day DM, Head WS. Advances in the management of keratomycosis and *Acanthamoeba* keratitis. *Cornea* 2000;**19**: 681–7.
204. Okada AA, Johnson RP, Liles WC, et al. Endogenous bacterial endophthalmitis: report of a ten-year retrospective study. *Ophthalmology* 1994;**101**:832–8.
205. Okamoto Y, Hiraoka T, Okamoto F, et al. A case of subperiosteal abscess of the orbit with central retinal artery occlusion. *Eur J Ophthalmol* 2009;**19**:288–91.
206. Opremcak EM. *Uveitis: a clinical manual for ocular inflammation*. New York: Springer-Verlag; 1995.
207. Oren B, Wende SO. An outbreak of molluscum contagiosum in a kibbutz. *Infection* 1991;**19**:159–61.
208. Osuntokun O, Olurin O. Filarial worm (Loa loa) in the anterior chamber: report of two cases. *Br J Ophthalmol* 1975;**59**:166–7.
209. Palamar M, Uretmen O, Kose S. Orbital cellulitis after strabismus surgery. *J AAPOS* 2005;**9**:602–3.
210. Paryani SG, Arvin AM. Intrauterine infection with varicella-zoster virus after maternal varicella. *N Engl J Med* 1986;**314**:1542–6.
211. Paul TO, Shepherd R. Congenital nasolacrimal duct obstruction: natural history and the timing of optimal intervention. *J Pediatr Ophthalmol Strabismus* 1994;**31**:362–7.
212. Paysse EA, Coats DK, Bernstein JM, et al. Management and complications of congenital dacryocele with concurrent intranasal mucocele. *J AAPOS* 2000;**4**:46–53.
213. Pena GP, Andrade Filho JS. Is Demodex really non-pathogenic? *Rev Inst Med Trop São Paulo* 2000;**42**:171–3.
214. Pepose JS, Holland GN, Wilhelmus KR. *Ocular infection & immunity*. St. Louis: Mosby; 1996.
215. Petersen RA, Robb RM. The natural course of congenital obstruction of the nasolacrimal duct. *J Pediatr Ophthalmol Strabismus* 1978;**15**:246–50.
216. Pfister DR, Cameron JD, Krachmer JH, et al. Confocal microscopy findings of *Acanthamoeba* keratitis. *Am J Ophthalmol* 1996; **121**:119–28.
217. Pichichero ME, Wagner RS, Granet DB. Pediatric conjunctivitis: clinical decision-making for optimal treatment. *Inf Dis Children May* 2011:1–17.
218. Pittella JE, Oréfice F. Schistosomotic choroiditis. II. Report of first case. *Br J Ophthalmol* 1985;**69**:300–2.
219. Pollard ZF. Treatment of acute dacryocystitis in neonates. *J Pediatr Ophthalmol Strabismus* 1991;**28**:341–3.
220. Prasad AG, Van Gelder RN. Presumed ocular histoplasmosis syndrome. *Curr Opin Ophthalmol* 2005;**16**:364–8.
221. Price HW. Dacryostenosis. *J Pediatr* 1947;**30**:302–5.
222. Puliafito CA, Baker AS, Haaf J, et al. Infectious endophthalmitis: review of 36 cases. *Ophthalmology* 1982;**89**:921–9.
223. Rabon RJ, Louis GJ, Zegarra H, et al. Acute bilateral posterior angiopathy with influenza A viral infection. *Am J Ophthalmol* 1987;**103**(3 Pt 1):289–93.
224. Raina UK, Jain S, Monga S, et al. Tubercular preseptal cellulitis in children: a presenting feature of underlying systemic tuberculosis. *Ophthalmology* 2004;**111**:291–6.
225. Raucher HS, Newton MJ, Stern RH. Ophthalmia neonatorum caused by penicillinase-producing *Neisseria gonorrhoeae*. *J Pediatr* 1982;**100**:925–6.
226. Rebora A. The management of rosacea. *Am J Clin Dermatol* 2002;**3**:489–96.
227. Reersted P, Hansen B. Chorioretinitis of the newborn with herpes simplex virus type 1: report of a case. *Acta Ophthalmol (Copenh)* 1979;**57**:1096–100.
228. Remington JS, Klein JO. *Infectious diseases of the fetus and newborn infant*. Philadelphia: Saunders; 1990.
229. Reynolds DJ, Kodsi SR, Rubin SE, et al. Intracranial infection associated with preseptal and orbital cellulitis in the pediatric patient. *J AAPOS* 2003;**7**:413–7.
230. Rhem MN, Wilhelmus KR, Jones DB. Epstein-Barr virus dacryoadenitis. *Am J Ophthalmol* 2000;**129**:372–5.

231. Rodger FC. Eye disease. In: Manson-Bahr PE, Bell DR, editors. *Manson's tropical diseases*. London: Bailliere Tindall; 1987. p. 1133–82.
232. Rodriguez A, Calonge M, Pedroza-Seres M, et al. Referral patterns of uveitis in a tertiary eye care center. *Arch Ophthalmol* 1996;**114**:593–9.
233. Ronday MJ, Stilma JS, Barbe RF, et al. Aetiology of uveitis in Sierra Leone, west Africa. *Br J Ophthalmol* 1996;**80**:956–61.
234. Roth AM, Purcell TW. Ocular findings associated with encephalomyelitis caused by herpes virus simiae. *Am J Ophthalmol* 1977;**84**:345–8.
235. Rowe SG, Durand M. Blackflies and whitewater: onchocerciasis and the eye. *Int Ophthalmol Clin* 1998;**38**:231–40.
236. Rubin SE, Rubin LG, Zito J, et al. Medical management of orbital subperiosteal abscess in children. *J Pediatr Ophthalmol Strabismus* 1989;**26**:21–7.
237. Rutar T, Cockerham KP. Periorbital zygomycosis (mucormycosis) treated with posaconazole. *Am J Ophthalmol* 2006;**142**:187–8.
238. Rutar T, Chambers HF, Crawford JB, et al. Ophthalmic manifestations of infections caused by the USA300 clone of community-associated methicillin-resistant *Staphylococcus aureus*. *Ophthalmology* 2006;**113**:1455–62.
239. Rutar T, Zwick OM, Cockerham KP, et al. Bilateral blindness from orbital cellulitis caused by community-acquired methicillin-resistant *Staphylococcus aureus*. *Am J Ophthalmol* 2005;**140**: 740–2.
240. Ryan Jr SJ. De novo subretinal neovascularization in the histoplasmosis syndrome. *Arch Ophthalmol* 1976;**94**:321–7.
241. Sandstrom KI, Bell TA, Chandler JW, et al. Diagnosis of neonatal purulent conjunctivitis caused by *Chlamydia trachomatis* and other organisms. In: Marah PA, Holmes KK, Oriel JD, et al, editors. *Chlamydial infections*. Amsterdam: Elsevier; 1982. p. 217–20.
242. Schantz PM, Glickman LT. Toxocaral visceral larva migrans. *N Engl J Med* 1978;**298**:436–9.
243. Schmoll C, Macrae M, Mulvihill A, et al. Tuberculous dacryoadenitis in a Scottish teenager. *Br J Ophthalmol* 2009;**93**:137–8, 274.
244. Schneider G. Silver nitrate prophylaxis. *Can Med Assoc J* 1984;**131**:193–6.
245. Schulz D, Sarra GM, Koerner UB, et al. Evolution of HIV-1-related conjunctival molluscum contagiosum under HAART: report of a bilaterally manifesting case and literature review. *Graefes Arch Clin Exp Ophthalmol* 2004;**242**:951–5.
246. Schwab IR. Oral acyclovir in the management of herpes simplex ocular infections. *Ophthalmology* 1988;**95**:423–30.
247. Schwartz GS, Harrison AR, Holland EJ. Etiology of immune stromal (interstitial) keratitis. *Cornea* 1998;**17**:278–81.
248. Seltz LB, Smith J, Durairaj VD, et al. Microbiology and antibiotic management of orbital cellulitis. *Pediatrics* 2011;**127**:e566–72.
249. Semba RD, Murphy RP, Newland HS, et al. Longitudinal study of lesions of the posterior segment in onchocerciasis. *Ophthalmology* 1990;**97**:1334–41.
250. Sheikh A, Hurwitz B. Topical antibiotics for acute bacterial conjunctivitis: Cochrane systematic review and meta-analysis update. *Br J Gen Pract* 2005;**55**:962–4.
251. Shields JA. Ocular toxocariasis: a review. *Surv Ophthalmol* 1984; **28**:361–81.
252. Siam AL, Gharbawi KF, Meegan JM. Ocular complications of Rift Valley fever. *J Egypt Public Health Assoc* 1978;**53**:185–6.
253. Silver LH, Woodside AM, Montgomery DB. Clinical safety of moxifloxacin ophthalmic solution 0.5% (Vigamox) in pediatric and nonpediatric patients with bacterial conjunctivitis. *Surv Ophthalmol* 2005;**50**(Suppl. 1):S55–63.
254. Skevaki CL, Galani IE, Pararas MV, et al. Treatment of viral conjunctivitis with antiviral drugs. *Drugs* 2011;**71**:331–47.
255. Skinner CJ, Viswalingam ND, Goh BT. *Phthirus pubis* infestation of the eyelids: a marker for sexually transmitted diseases. *Int J STD AIDS* 1995;**6**:451–2.
256. Smith RE. Toxoplasmic retinochoroiditis as an emerging problem in AIDS patients. *Am J Ophthalmol* 1988;**106**:738–9.
257. Smyth JD. In vitro studies and host-specificity in *Echinococcus*. *Bull World Health Organ* 1968;**39**:5–12.
258. Snowe RJ, Wilfert CM. Epidemic reappearance of gonococcal ophthalmia neonatorum. *Pediatrics* 1973;**51**:110–4.
259. Solley WA, Martin DF, Newman NJ, et al. Cat scratch disease: posterior segment manifestations. *Ophthalmology* 1999;**106**: 1546–53.
260. Stagno S. Cytomegalovirus. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 3rd ed. Philadelphia: W. B. Saunders; 1990.
261. Stagno S, Whitley RJ. Herpesvirus infections of pregnancy. I. Cytomegalovirus and Epstein-Barr virus infections. *N Engl J Med* 1985;**313**:1270–4.
262. Steinkuller PG, Jones DB. Microbial preseptal and orbital cellulitis. In: Duane TD, editor. *Clinical ophthalmology*. Hagerstown, Md: Harper & Row; 1997. p. 8.
263. Stenson S, Newman R, Fedukowicz H. Conjunctivitis in the newborn: observations on incidence, cause, and prophylaxis. *Ann Ophthalmol* 1981;**13**:329–34.
264. Streilein JW. Anterior chamber associated immune deviation: the privilege of immunity in the eye. *Surv Ophthalmol* 1990;**35**: 67–73.
265. Sudesh S, Laibson PR. The impact of the herpetic eye disease studies on the management of herpes simplex virus ocular infections. *Curr Opin Ophthalmol* 1999;**10**:230–3.
266. Syed TA, Lundin S, Ahmad M. Topical 0.3% and 0.5% podophyllotoxin cream for self-treatment of molluscum contagiosum in males: a placebo-controlled, double-blind study. *Dermatology* 1994;**189**:65–8.
267. Syed TA, Goswami J, Ahmadpour OA, et al. Treatment of molluscum contagiosum in males with an analog of imiquimod 1% in cream: a placebo-controlled, double-blind study. *J Dermatol* 1998;**25**:309–13.
268. Tabbara KF. Trachoma: have we advanced in the last 20 years? *Int Ophthalmol Clin* 1990;**30**:23–7.
269. Tabbara KF, Hyndiuk RA. *Infections of the eye*. 2nd ed. Boston: Little, Brown; 1986. p. 323–47, 361–85, 423–31, 433–78, 603–6.
270. Tabbara KF, Shoukrey N. Trichinosis. In: Gold DH, Weingeist TA, editors. *The eye in systemic disease*. Philadelphia: JB Lippincott; 1990. p. 191–2.
271. Taber LH. Syphilis. In: Kaplan SL, editor. *Current therapy in pediatric infectious disease*. St. Louis: C. V. Mosby; 1993. p. 243–5.
272. Talley AR, Garcia-Ferrer F, Laycock KA, et al. Comparative diagnosis of neonatal chlamydial conjunctivitis by polymerase chain reaction and McCoy cell culture. *Am J Ophthalmol* 1994; **117**:50–7.
273. Tate Jr GW, Martin RG. Clindamycin in the treatment of human ocular toxoplasmosis. *Can J Ophthalmol* 1977;**12**:188–95.
274. Taylor HR, Dax EM. Ocular onchocerciasis. In: Tabbara KF, Hyndiuk RA, editors. *Infections of the eye*. Boston: Little, Brown; 1996. p. 673–83.
275. Thomas PA. Fungal infections of the cornea. *Eye (Lond)* 2003; **17**:852–62.
276. Thompson WS, Culbertson WW, Smiddy WE, et al. Acute retinal necrosis caused by reactivation of herpes simplex virus type 2. *Am J Ophthalmol* 1994;**118**:205–11.
277. Thorne JE, Jabs DA, Kempen JH, et al. Incidence of and risk factors for visual acuity loss among patients with AIDS and cytomegalovirus retinitis in the era of highly active antiretroviral therapy. *Ophthalmology* 2006;**113**:1432–40.
278. Thylefors B. Onchocerciasis: an overview. *Int Ophthalmol Clin* 1990;**30**:21–2.
279. Thylefors B, Dawson CR, Jones BR, et al. A simple system for the assessment of trachoma and its complications. *Bull World Health Organ* 1987;**65**:477–83.
280. Toussaint D, Danis P. Retinopathy in generalized loa-loa filariasis: a clinicopathological study. *Arch Ophthalmol* 1965;**74**:470–6.
281. Tudor RC, Blair E. *Gnathostoma spinigerum:* an unusual cause of ocular nematodiasis in the western hemisphere. *Am J Ophthalmol* 1971;**30**:(72):185–90.
282. Ur Rehman S, Anand S, Reddy A, et al. Poststreptococcal syndrome uveitis: a descriptive case series and literature review. *Ophthalmology* 2006;**113**:701–6.
283. Usha K, Smitha S, Shah N, et al. Spectrum and the susceptibilities of microbial isolates in cases of congenital nasolacrimal duct obstruction. *J AAPOS* 2006;**10**:469–72.
284. Van der Lelij A, Ooijman FM, Kijlstra A, et al. Anterior uveitis with sectoral iris atrophy in the absence of keratitis: a distinct clinical entity among herpetic eye diseases. *Ophthalmology* 2000; **107**:1164–70.
285. Varaprasathan G, Miller K, Lietman T, et al. Trends in the etiology of infectious corneal ulcers at the F. I. Proctor Foundation. *Cornea* 2004;**23**:360–4.

286. Velazquez AJ, Goldstein MH, Driebe WT. Preseptal cellulitis caused by trichophyton (ringworm). *Cornea* 2002;**21**:312–4.
287. Vieira-Dias D, Sena CM, Oréfice F, et al. Ocular and concomitant cutaneous sporotrichosis. *Mycoses* 1997;**40**:197–201.
288. Von Noorden GK, Buck AA. Ocular onchocerciasis: an ophthalmological and epidemiological study in an African village. *Arch Ophthalmol* 1968;**80**:26–34.
289. Vu BL, Dick PT, Levin AV, et al. Development of a clinical severity score for preseptal cellulitis in children. *Pediatr Emerg Care* 2003;**19**:302–7.
290. Wagner RS. Glaucoma in children. *Pediatr Clin North Am* 1993;**40**:855–67.
291. Wallwork B, Coman W, Feron F, et al. Clarithromycin and prednisolone inhibit cytokine production in chronic rhinosinusitis. *Laryngoscope* 2002;**112**:1827–30.
292. Weiss A, Friendly D, Eglin K, et al. Bacterial periorbital and orbital cellulitis in childhood. *Ophthalmology* 1983;**90**:195–203.
293. Weiss A, Margo CE, Ledford DK, et al. Toxoplasmic retinochoroiditis as an initial manifestation of the acquired immune deficiency syndrome. *Am J Ophthalmol* 1986;**101**:248–9.
294. Wheaton AF, Timothy NH, Dossett JH, et al. The surgical treatment of *Molluscum contagiosum* in a pediatric AIDS patient. *Ann Plast Surg* 2000;**44**:651–5.
295. Whitley RJ. Herpes simplex virus infections. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 3rd ed. Philadelphia: W.B. Saunders; 1990.
296. Wiley L, Springer D, Kowalski RP, et al. Rapid diagnostic test for ocular adenovirus. *Ophthalmology* 1988;**95**:431–3.
297. Wilkinson CP, Welch RB. Intraocular toxocara. *Am J Ophthalmol* 1971;**71**:921–30.
298. Williams DF, Williams GA, Caya JG, et al. Intraocular *Echinococcus multilocularis*. *Arch Ophthalmol* 1987;**105**:1106–9.
299. Williams PH, Templeton AC. Infection of the eye by tapeworm Coenurus. *Br J Ophthalmol* 1971;**55**:766–9.
300. Wilson ME, Paul TO. Orbital cellulitis following strabismus surgery. *Ophthalmic Surg* 1987;**18**:92–4.
301. Wirostko WJ, Connor Jr TB, Wagner PF. Recurrent poststreptococcal uveitis. *Arch Ophthalmol* 1999;**117**:1649–50.
302. World Health Organization. *Third report World Health Organization technical report series 752*. Geneva: WHO; 1987.
303. Yen MT, Yen KG. Effect of corticosteroids in the acute management of pediatric orbital cellulitis with subperiosteal abscess. *Ophthal Plast Reconstr Surg* 2005;**21**:363–6; discussion 366-7.
304. Yoser SL, Forster DJ, Rao NA. Systemic viral infections and their retinal and choroidal manifestations. *Surv Ophthalmol* 1993;**37**: 313–52.
305. Yoshinaga IG, Conrado LA, Schainberg SC, et al. Recalcitrant *Molluscum contagiosum* in a patient with AIDS: combined treatment with CO(2) laser, trichloroacetic acid, and pulsed dye laser. *Lasers Surg Med* 2000;**27**:291–4.
306. Young RC, Hodge DO, Liesegang TJ, et al. Incidence, recurrence, and outcomes of Herpes simplex virus eye disease in Olmstead County, Minnesota, 1976-2007: the effect of oral prophylaxis. *Arch Ophthalmol* 2010;**128**:1178–83.

SECTION **XII**

SYSTEMIC INFECTIOUS DISEASES

CHAPTER 62

Bacteremia and Septic Shock

Sheldon L. Kaplan • Jesus G. Vallejo

One of the most serious and potentially life-threatening infectious diseases in childhood is a bacteremic illness. Bacteremia may be caused by a wide variety of gram-positive or gram-negative microorganisms, and it may or may not be associated with a specific focus of infection, such as pneumonia or meningitis. The bacteremias that are transient and self-limited are not discussed in this chapter.

The incidence of bacteremia in children has been studied in hospital and ambulatory settings. In otherwise normal children, beyond the neonatal age group, *Streptococcus pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, group A streptococcus, *Salmonella* spp., and *Neisseria meningitidis* are the most common microorganisms causing bacteremia.[92,221] The incidence of pneumococcal bacteremia has decreased substantially since the introduction of the conjugate pneumococcal vaccine.[115,163] In contrast, methicillin-resistant *S. aureus* (MRSA) bacteremia is on the rise in children in the United States.[77] Children with underlying illnesses that depress the host response to infection may develop bacteremia caused by these same microorganisms; however, in this population of children, especially when hospitalized, Enterobacteriaceae, *S. aureus*, coagulase-negative staphylococci, and fungi are the most important organisms commonly isolated from blood cultures.[5,157] Indwelling vascular lines, urinary catheters, endotracheal tubes, and other foreign material further predispose already compromised children to nosocomial infections. The incidence of diagnosed septicemia has increased over the years, partly owing to improved medical technology and the greater numbers of individuals with immunocompromising conditions who previously would not have survived.[146,147]

Gray and colleagues[85] reported that the incidence of bloodstream infections in a pediatric intensive care unit (PICU) during a 3-year period was 39 cases per 1000 admissions. Of the episodes, 64 percent were acquired in a PICU and 20.6 percent were community-acquired infections. Gram-positive and gram-negative organisms accounted for 62 percent and 31 percent of the isolates, respectively. Yeasts were isolated in 5.6 percent of episodes. The frequency of catheter-related bloodstream infections has been decreasing in many PICUs as a result of implementing insertion and maintenance bundles.[133] Children with acquired immunodeficiency syndrome or severe immunosuppression caused by human immunodeficiency virus infection also are at increased risk for developing bacteremias caused by gram-negative bacilli, especially *Pseudomonas aeruginosa*.[175]

Using a seven-state hospital discharge database, Watson and associates[221] estimated that the U.S. age-adjusted and sex-adjusted annual incidence of severe sepsis was 0.56 cases per 1000 children, or more than 42,000 cases per year. The highest age-specific incidence occurred in infants (5.16 cases per 1000), with the incidence declining to 0.20 cases per 1000 for children 10 to 14 years old. Half of the children had underlying comorbidity, with neuromuscular, cardiovascular, and respiratory disorders being the most common.

One potential consequence of bacteremia is septic shock, a state characterized by inadequate tissue perfusion that is associated frequently with endotoxemia. Although most children with septic shock have infections caused by gram-negative enteric bacteria, *P. aeruginosa*, or *N. meningitidis*, which are organisms with endotoxin or lipopolysaccharide (LPS) within cell walls, septic shock also is associated with disease caused by gram-positive bacteria (especially *S. aureus*, *Streptococcus pyogenes*, and viridans streptococci), viruses, rickettsiae, and fungi. Community-acquired methicillin-resistant *S. aureus* (CA-MRSA) in particular has been associated with severe sepsis and septic shock in young children and adolescents.[4,82] In adults, the frequency of septic shock continues to increase as the population ages, new treatment methods including more complicated surgery and immunosuppressive agents are developed, and antibiotic resistance grows.[178]

DuPont and Spink[59] reviewed the cases of 172 children, 30 days to 16 years of age, who were hospitalized at the University of Minnesota Medical Center with gram-negative bacteremia. Shock occurred in 25 percent of the children, 98 percent of whom died. In meningococcal infections, 11 to 40 percent of children developed hypotension.[105,216] During a 10-month study period, Naqvi and colleagues[144] reported that shock occurred in 5 of 39 (13%) episodes of gram-negative bacillary sepsis, with three deaths. Jacobs and associates[98] reviewed the admissions of previously normal children to a PICU in a large children's hospital for a 30-month period. Hypotension or evidence of peripheral hypoperfusion occurred in 143 children with confirmed bacterial sepsis, mostly *Haemophilus influenzae* type b, or apparent meningococcemia. Among 1058 consecutive admissions of 916 children to a PICU in Canada from July 1, 1991, to July 31, 1992, 25 episodes (2%) of septic shock occurred.[165] During a 12-month period, 140 episodes of septicemia (135 bacterial and 5 fungal) were documented in 100 pediatric hematology-oncology patients.[4,5] Septic shock occurred in 19 percent.

The organisms and case-fatality rates in the study by Watson and colleagues[221] are outlined in Table 62-1. *N. meningitidis* and fungi were associated with the highest

TABLE 62-1 **Occurrence and Case-Fatality Rates of Selected Pathogens Among Children with Severe Sepsis Based on Age***

	<1 Yr (N = 4643)		*1-10 Yr (N = 2724)*		*11-19 Yr (N = 2308)*	
Organism	**Cases (%)**	**Case-Fatality (%)**	**Cases (%)**	**Case-Fatality (%)**	**Cases (%)**	**Case-Fatality (%)**
Neisseria meningitidis	0.3	20	8	10.4	2.3	15.1
Haemophilus influenzae	1.6	4.2	2.4	1.6	1.9	6.8
Pseudomonas	3.6	14.6	7.7	12.4	6.9	9.4
Staphylococcus aureus	2.3	5.7	2.9	0	3.5	3.8
Group A streptococcus	0.3	0	0.7	5	0.2	0
Group B streptococcus	3.1	7.6	0.1	50	0.8	5.6
Fungus	10	10.8	13.3	16.8	10.4	11.6

*Represents data from a seven-state hospital discharge database in 1995.
Modified from Watson RS, Carcillo JA, Linde-Zwirble WT, et al. The epidemiology of severe sepsis in children in the United States. *Am J Respir Care Med* 2003;**167**:695–701.

TABLE 62-2 **Consensus Definitions of Systemic Inflammatory Response Syndrome (SIRS), Infection, Sepsis, Severe Sepsis, and Septic Shock**

SIRS

The presence of two or more of the following four criteria, one of which must be abnormal temperature or white blood cell count:

1. Core temperature (rectal, bladder, oral, or central catheter probe) >38.5°C (101.3°F) or <36°C (96.8°F)
2. Tachycardia defined as more than two standard deviations above normal for age in the absence of external factors or drugs *or* otherwise unexplained persistent elevation of a 0.5- to 4-hr time period *or* for children <1 year old, bradycardia, defined as a mean heart rate <10th percentile for age in the absence of external factors or drugs or otherwise unexplained persistent depression over a 0.5-hr period
3. Mean respiratory rate more than two standard deviations for age *or* mechanical ventilation for an acute process not related to an underlying neuromuscular disease or to general anesthesia
4. Peripheral white blood cell count elevated or depressed for age unrelated to medications or >10% immature neutrophils

Infection

A suspected or proven (by culture, tissue stain, polymerase chain reaction assay) infection caused by any pathogen *or* a clinical syndrome associated with a high probability of infection

Sepsis

SIRS in the presence of or caused by suspected or proven infection

Severe Sepsis

Sepsis plus one of the following: cardiovascular organ dysfunction, acute respiratory distress syndrome *or* two or more other instances of organ dysfunction as defined in the consensus statement

Septic Shock

Sepsis and cardiovascular organ dysfunction as defined in the consensus statement

Modified from Goldstein B, Giroir B, Randoph A, and the Members of the International Consensus Conference on Pediatric Sepsis. International Pediatric Sepsis Conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med* 2005;**6**:2–8.

mortality rates. Early-onset group B streptococcal infections in neonates and overwhelming *S. pneumoniae* infections in children with splenic dysfunction or asplenia are associated with shock in a high percentage of cases. *S. aureus* or group A streptococcus may cause hypotension in a child with or without other manifestations of toxic shock syndrome.[184]

Advances in understanding of the pathogenesis and pathophysiology of septic shock with respect to the host response to infection have required that more precise clinical definitions of sepsis and expanded syndromes be developed. Much of the impetus for this effort is related to the ability to identify more readily patients with infections who may benefit from administration of newer (expensive) adjunctive measures. An American College of Chest Physicians and Society of Critical Care Medicine Consensus Conference in 1991 developed new terminology to define sepsis and its sequelae.[25] This terminology has been modified for use in children by an international consensus panel of 20 experts in sepsis and clinical research (Table 62-2).[80]

PATHOPHYSIOLOGY

The pathophysiology of bacteremia is highly variable and depends on the specific microorganism isolated, the

immune status of the host, and other factors such as the locations of indwelling catheters. Highly encapsulated organisms, such as *S. pneumoniae*, *N. meningitidis*, and *H. influenzae* type b, normally may reside in the nasopharynx and, for reasons that are poorly understood, are capable of invading beyond mucosal barriers into the bloodstream. A preceding viral upper respiratory tract infection may play some role in alterations in local host defense mechanisms that result in bacteremia.[106,131]

Using human columnar nasopharyngeal tissue in organ cultures, Stephens and colleagues[189] showed that *N. meningitidis* organisms were ingested by the columnar cells, then found within phagocytic vacuoles, and later observed within subepithelial tissues, suggesting that the meningococci had penetrated the epithelial layer. In this same model, *H. influenzae* type b organisms attach to nonciliated columnar epithelial cells and subsequently are found in the intercellular spaces in association with a preceding disruption of the tight junctions of epithelial cells.[60] After passing the mucosal barriers, *H. influenzae* type b may enter the bloodstream directly through pharyngeal blood vessels.[177] Pneumococci adhere to specific ligands on respiratory cells. The inflammatory mediators generated during viral infections upregulate platelet-activating factor receptor on respiratory cells to which the pneumococci adhere more avidly and subsequently invade.[204] Pili or adhesins of gram-negative enteric organisms seem to be important in attachment and adherence of these microorganisms to specific receptors expressed on epithelial surfaces. Pili also have been shown to be important in the pathogenesis of some gram-positive infections, such as *S. pyogenes*, group B streptococcus, and *S. pneumoniae*.[14,140]

The placement of an endotracheal tube unmasks a greater number of these receptors, presumably through increased protease activity of secretions and decreased cell-bound fibronectin, and leads to colonization of the upper respiratory tract with gram-negative organisms, which are ubiquitous in the environment of an ICU.[232] Biofilm formation on the endotracheal tube surface may contribute to the persistence of colonization.[116] Altered host defense mechanisms allow these organisms to move beyond epithelial surfaces and cause bacteremia.

The gastrointestinal and genitourinary tracts are major sources of gram-negative organisms responsible for bacteremia. These organisms first may cause localized abscesses or peritonitis if intestinal perforation occurs, or they may translocate to the intestinal mucosa, particularly when the mucosa is affected by antineoplastic agents. Viridans streptococci can cause bacteremia in a neutropenic patient with severe mucositis that develops after the patient undergoes chemotherapy.[168] Microorganisms within the bladder may ascend the genitourinary tract and presumably enter the bloodstream through the kidneys. *S. aureus* and *S. pyogenes* are common inhabitants of the skin and skin structures. Any skin wound or foreign matter within the skin tissue renders the skin more susceptible to bacterial invasion. Staphylococci have a unique capability of adhering to solid surfaces, such as catheters, which may be an important prerequisite to colonization and subsequent catheter-related bacteremia.[160]

The pathophysiology of septic shock is very complex. Septic shock associated with gram-negative organisms has been studied most extensively, especially with respect to endotoxin or bacterial LPS, which has multiple biologic effects. Bacterial LPS has three basic components, as follows:

1. Terminal side chains consist of repeating oligosaccharides that differ from strain to strain and are responsible for the antigenic specificity of the O antigens.
2. A core LPS also consists of oligosaccharides but has less diversity in structure among strains than do the terminal side chains.
3. Lipid A is very similar among the different strains and is responsible for most of the biologic activity of endotoxin.

Endotoxin shock has been the subject of intensive animal research, and much of what is known about the pathogenesis of endotoxin shock has been derived from animal models. Although septic shock in humans is not simulated precisely in these animal models for a number of reasons, including the fact that animals do not have underlying host defense defects, much of what has been learned about endotoxin shock in animals has been corroborated in the human host.

Endotoxin Shock in Animals

Many animal models of endotoxin shock employ infusions of live gram-negative bacteria, usually *E. coli*, or purified endotoxin, after which observations are made. The effects of purified endotoxin depend partly on the species of animal being studied. Models employing cecal ligation and puncture are thought to be more relevant to human disease than direct infusion of bacteria or endotoxin.[235] The effects of endotoxin in animal models are summarized in Table 62-3.

Numerous mediators induced by endotoxin play pivotal roles in the pathogenesis of endotoxin shock. Tumor necrosis factor (TNF), a polypeptide hormone, is a key cytokine mediating septic shock.[200] The tissue injury induced by TNF largely is a result of other mediators that are induced by TNF, including interleukin (IL)-1β, IL-6, eicosanoids, and platelet-activating factor.[55,96,198,201] TNF is synthesized by a wide variety of cells (including monocytes/macrophages, natural killer cells, microglial cells, and hepatic Kupffer cells) after stimulation by LPS, C5a, viruses, and enterotoxins, among other agents. TNF initiates a cascade of events that leads to endothelial cell injury, an enhanced inflammatory response, and, ultimately, the characteristic findings of endotoxic shock.

Nitric oxide (i.e., endothelium-derived relaxing factor) is the final pathway by which endogenous vasodilators stimulated by endotoxin result in hypotension from altered control of microcirculation. LPS through the release of cytokines induces a form of the enzyme nitric oxide synthase II, which leads to increased production of nitric oxide.[138] Inhibitors of nitric oxide synthase, such as N^G-monomethyl-L-arginine, can reverse or prevent hypotension in animals challenged with LPS.[130]

TABLE 62-3 **Endotoxin Shock in Animal Models**

Effects	Mediators
Cardiovascular Effects	
Decreased peripheral vascular resistance[145]	Histamine, bradykinin,[132] serotonin, complement activation, prostaglandins, anaphylatoxins
Decreased cardiac output[6,10,167]	
Depressed myocardial function[128]	
Decreased systemic blood pressure[44]	
Metabolic Effects[167]	
Hyperglycemia[49]	Hypoinsulinemia
Hypoglycemia[64]	
Increased adrenocorticotropic hormone, growth hormone, and antidiuretic hormone[227]	
Decreased calcium[234]	
Increased triglycerides[107,200]	
Decreased iron, transferrin, and zinc	
Pulmonary Effects	
Congestive atelectasis[44,94]	Polymorphonuclear leukocytes
Increased capillary permeability[94]	Polymorphonuclear leukocytes
Vasoconstriction	Thromboxane, prostacyclin
Bronchoconstriction[30]	Leukotrienes
Central Nervous System Effects	
Decreased regional and total cerebral blood flow[166]	
Increased cerebral oxygen consumption[166]	

The pathophysiology of septic shock caused by gram-positive bacteria is similar to that described for gram-negative organisms.[187] Cell wall components, such as peptidoglycan and teichoic acid, promote proinflammatory activity but are less potent than endotoxin.

Endotoxin Shock in Humans

The pathophysiology of septic shock is highly complex and is related predominantly to actions of endogenous mediators released as part of the systemic inflammatory response to an infection. The cascade of events is intertwining, with production of one cytokine stimulating the synthesis of others; synergistic, in that the activities of certain cytokines act in concert; and sometimes antagonistic, with the production of other molecules to inhibit or compete with various cytokines. This complicated response to an infectious stimulus has been studied best for LPS, but a similar series of events occurs in response to gram-positive infections. Although the best-understood system is the one that recognizes bacterial LPS, others exist for sensing the presence of bacterial peptidoglycan, DNA, lipopeptides, flagella, viral double-stranded RNA, and other conserved microbial molecules.

The first host protein involved in the recognition of LPS is LPS-binding protein. LPS-binding protein is an acute-phase protein; its role is to bring LPS to the cell surface by binding to LPS and forming a ternary complex with the LPS receptor molecule, CD14.[222,233] Formation of the complex between LPS and CD14 facilitates the transfer of LPS to the LPS receptor complex, which is composed of Toll-like receptor 4 (TLR4) and MD2. Studies over the course of several years led to the discovery of the TLR4/MD2 receptor complex as the signaling entity for LPS (Fig. 62-1).[20] MD2 is a secreted glycoprotein that functions as an indispensable extracellular adapter molecule for LPS-initiated signaling events, perhaps by aiding in ligand recognition. The resulting signal promotes mononuclear phagocytes to produce reactive oxygen molecules, cytokines, and arachidonic acid metabolites, including prostaglandin and leukotrienes. A counterregulatory protein is a bactericidal, permeability-increasing protein that is stored in the granules of polymorphonuclear leukocytes and inhibits the effects of LPS.[72]

TNF is largely responsible for the biologic effects, including fever, shock, myocardial suppression, capillary leak (i.e., endothelial damage), coagulation alterations, and metabolic changes,[35,36,132,199,205] of LPS in humans. In children, including neonates, the role of cytokines in sepsis caused by a variety of organisms, but especially *N. meningitidis*, is well documented.[31,52,78,193,216] LPS and TNF each can induce the synthesis of other proinflammatory cytokines, such as IL-1β and IL-6.[56] IL-6 levels in plasma correlate with mortality. IL-8 plasma concentrations also are increased after infusion of LPS or IL-1β.[56,86] Serum IL-8 levels in children with septic shock were predictive of outcome in one study.[188]

The anti-inflammatory cytokine IL-10 is produced after LPS is injected and inhibits the production of TNF, IL-1β, and IL-6.[126] Naturally occurring inhibitors of TNF or IL-1β are present in serum samples of patients with the sepsis syndrome.[79,207,208] IL-1 receptor antagonist (IL-1a) binds competitively to the IL-1 receptor to block the action of IL-1. Soluble TNF receptors bind to circulating TNF, which prevents its proinflammatory actions (see Fig. 62-1).

In adults, gram-negative bacteremia is followed by a decrease in systemic vascular resistance and mean blood pressure and an increase in cardiac output.[21,224] Decreased systemic vascular resistance may be accompanied by activation of the complement and kinin systems.[134] After this early phase, the blood pressure decreases further without change in the central venous pressure. Certain patients, especially children, are able to maintain their cardiac output and cardiac index, and this ability may be associated with increased rates of survival. When peripheral resistance is measured within 12 to 24 hours of the onset of shock, its decrease is significant in patients who survive compared with patients who die.[147] In contrast, cardiac output is reduced significantly in other patients; this decrease is associated with increased concentration of

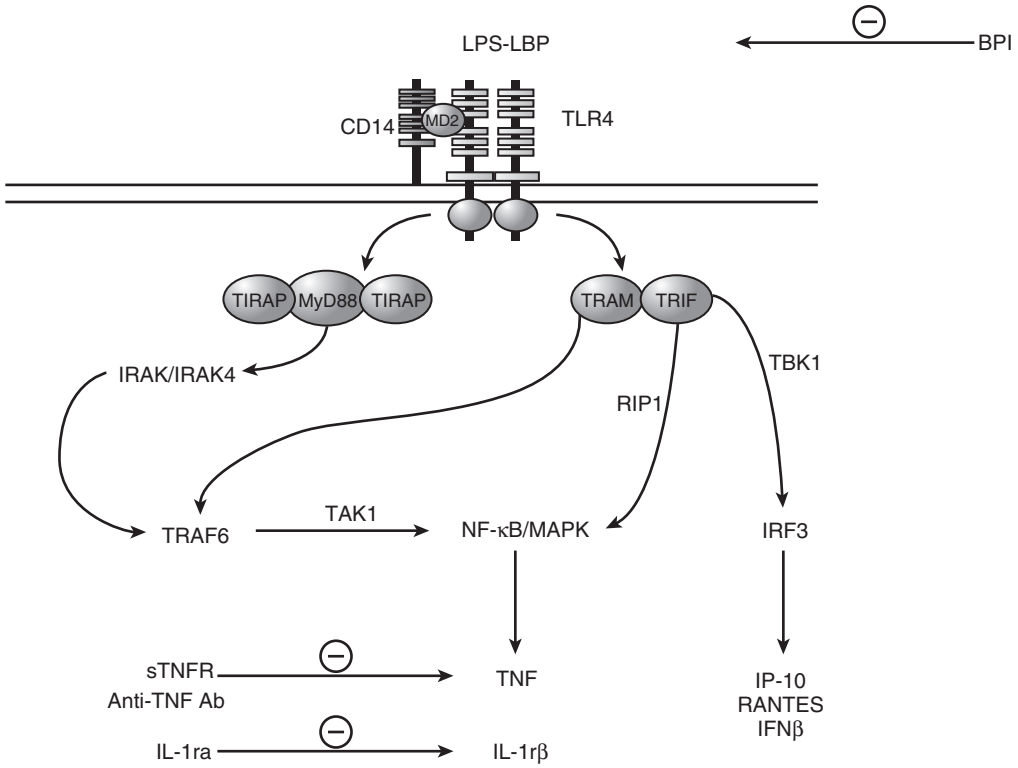

FIGURE 62-1 ■ Activation of the cytokine network by lipopolysaccharide (LPS). Circulating lipopolysaccharide-binding protein (LBP) recognizes LPS in the plasma and brings it to CD14. This aids the loading of LPS onto the LPS receptor complex, which is composed of dimerized Toll-like receptor 4 (TLR4) receptors and two molecules of the extracellular adapter MD-2. Signals activated by TLR4 can be subdivided into signals dependent on MyD88 (and Mal), which occur early (represented by the events illustrated on the left-hand side of the diagram), and signals independent of MyD88, which occur later and use the adapters TRIF and TRAM (depicted on the right). IL-1ra, interleukin-1 receptor antagonist; sTNFr, soluble tumor necrosis factor receptor.

blood lactate, decreased arterial blood pH, and decreased rates of survival.

In one small study, two distinct patterns of septic shock in children admitted to the PICU of a tertiary children's hospital were described.[29] Fifteen of 16 children with bacteremia related to central venous lines had a high cardiac index and low systemic vascular resistance. This pattern was seen in only 2 of 14 children with community-onset infections, in whom a normal or low cardiac index with variable systemic vascular resistance was typically noted.

Depression of myocardial function has been shown in adult and pediatric patients in septic shock.[63] These patients have a reduced ejection fraction, left ventricular dilation, and significantly altered ventricular performance in response to infusion of volume.[149] This depression of myocardial function is transient in survivors, however, reverting to normal within 1 to 4 days.[152] Parker and colleagues[153] found that patients who did not survive septic shock did not have left ventricular dilation or reduction in the ejection fraction. When the systemic vascular resistance index was averaged over the course of time, nonsurvivors had a significantly ($p < .05$) lower index than that of survivors of septic shock. This study included three children who were 9 to 17 years of age. Abraham and associates[1] sequentially monitored hemodynamic and oxygen transport measurements in 33 patients with septic shock. In the 24-hour period before the onset of hypotension, the survivors showed significantly greater cardiac index, left cardiac work index, oxygen delivery, and oxygen consumption than the nonsurvivors.

A circulating myocardial depressant factor in patients with septic shock was proposed more than 50 years ago, but myocardial dysfunction was not quantitatively linked to a serum factor until the late 1980s.[128,154,169] Kumar and colleagues[112] later reported that the myocardial depressant activity of sera obtained from patients with septic shock could be eliminated by the immunoprecipitation of TNF and IL-1β. Several investigators also have shown that TNF and IL-1β synergistically depress myocardial function in vitro and that this effect can be abolished by an inhibitor of nitric oxide.[34,44,151] Germane to this discussion is the observation that LPS-induced biosynthesis of TNF mRNA and protein is not strictly confined to peripheral mononuclear cells but also may occur within many different tissue compartments. Experimental studies have shown that the cardiac compartment can be a significant source of TNF during septic shock. Kapadia and colleagues[102] showed that administration of LPS leads to intramyocardial production of TNF mRNA and protein in vivo. These observations raise the possibility that the myocardial depression occurring in sepsis may develop directly in response to the compartmentalized production of TNF and other cytokines within the heart, as opposed to systemic production of these mediators by circulating mononuclear cells. TLR4 is expressed in the

heart, and investigators have suggested that it is involved in signaling the cytokine production induced by LPS within the heart.[17] The complex interactions leading to myocardial dysfunction or "septic cardiomyopathy" are incompletely understood but remain under active investigation.[70]

In children, the most comprehensive investigation of myocardial dysfunction has been in meningococcal septic shock. Thiru and coworkers[194] measured serum concentrations of the cardiac muscle–specific protein cardiac troponin I, which is released from injured cardiac myocytes, in 101 children with meningococcal septicemia. Minimum left ventricular ejection fraction was inversely related to peak cardiac troponin I levels. The degree of myocardial dysfunction, as determined by inotrope measurement, was related directly to peak cardiac troponin I concentrations. These researchers' results suggested that myocardial cell death might contribute, at least in part, to the cardiac dysfunction associated with meningococcal septic shock.

Hematologic changes, such as leukocytosis, leukopenia, and thrombocytopenia, have been observed in human volunteers after receiving an infusion of endotoxin. Thrombocytopenia commonly occurs in association with sepsis of any cause.[46] Septic shock is one of the most common causes of disseminated intravascular coagulation in children. Hageman factor (i.e., factor XII), which initiates the coagulation cascade, can be activated directly by LPS or through endothelial damage induced by bacteria.[122] In septic shock, concentrations of Hageman factor, prekallikrein, high-molecular-weight kininogen, and factor VII are decreased partly as a result of consumption.[48,101] Similarly, levels of inactivators of clotting factors, such as C1 esterase inhibitor, α_2-macroglobulin, and antithrombin III, also are diminished. Corrigan and Jordan[48] diagnosed disseminated intravascular coagulation in 24 of 26 children with septic shock and found that improvement in coagulation parameters seemed to be related most to restoration of blood pressure. A disseminated intravascular coagulation score using four components (platelet count, fibrinogen concentration, fibrin degradation products, and prothrombin time) was found to correlate with mortality in children with sepsis or septic shock.[108] Gram-negative bacteremia may be associated with a coagulopathy that is not disseminated intravascular coagulation but is characterized by prolongation of the prothrombin and partial thromboplastin times caused by a reduction in vitamin K–dependent coagulation factors.[47]

LPS or cell wall components of gram-positive organisms, through cytokine production, activate blood coagulation predominantly through the extrinsic pathway. The procoagulant state is enhanced further by decreased protein C activity, which is an important inhibitor of coagulation factors Va and VIIIa. The fibrinolytic system also is altered by endotoxemia and is mediated by plasminogen activator inhibitor–1-induced suppression. The coagulopathy associated with septic shock is characterized by a procoagulant state and inhibition of fibrinolysis.[117,119] Protein C has anti-inflammatory properties. The antithrombotic, profibrinolytic, and anti-inflammatory actions of activated protein C counteract the effects of cytokine activation, but a deficiency of protein C may be acquired during severe sepsis. Low levels of protein C have been associated with increased morbidity and mortality rates in patients with severe sepsis and septic shock.[67] For meningococcal disease in particular, dysfunction of the protein C activation pathway is a key factor in the development of the thrombosis associated with purpura fulminans. Downregulation of the endothelial thrombomodulin–endothelial protein C receptor pathway seems to be the mechanism for impaired activation of protein C during severe meningococcal sepsis.[61] Cytokine increases also lead to elevated serum ferritin levels during severe sepsis and septic shock; in one pediatric study levels above 500 ng/mL were associated with greater mortality.[74]

LPS can activate the complement cascade by the classic or the alternate pathway. Significantly depressed concentrations of C3 occur in patients with bacteremia and hypotension compared with normal individuals or with patients with uncomplicated bacteremia, and C3 is activated primarily by the alternate pathway.[62,101,124] In patients with bacteremia and hypotension, C1, C4, and C2 levels were not depressed significantly from values found in normal controls or in normotensive patients with bacteremia. In contrast, C3, C5, C6, C9, properdin, and factor B levels were decreased significantly ($p < .05$) in bacteremic patients with shock. In children with meningococcal disease, Tubbs[203] found a mean C3 concentration (as a percentage of normal values) of 132 ± 21 percent for survivors versus 91 ± 21 percent for nonsurvivors. The C3 levels did not correlate with endotoxin levels in sera.

Many metabolic alterations have been documented in the human host during endotoxin shock. Hyperglycemia followed by hypoglycemia can complicate the shock state induced by sepsis.[64,72,135,149] Whole-body use of glucose is increased during sepsis, which probably is cytokine mediated.[136,150] Glycolysis and gluconeogenesis are increased, but insulin resistance occurs in skeletal muscle. Children with underlying liver disease or with reduced glycogen stores are most likely to develop hypoglycemia during septic shock. Lactic acidosis develops as a result of poor tissue perfusion and cellular hypoxia. Lactic acid concentrations are increased in nonsurvivors and patients with poor or low-flow cardiac output during sepsis.

In clinical studies, Clowes and associates[43] identified a subgroup of patients with low-flow septic shock in whom concentrations of serum insulin were lower than concentrations in a control population. In children with meningococcal sepsis, van Waardenburg and colleagues[213] found higher blood glucose concentrations and significantly lower insulin levels on day 2 or 3 of hospitalization in children with shock compared with children without shock. Levels of plasma insulin and soluble TNF receptor–75 were inversely correlated in these children. Their findings were consistent with the inflammatory response inhibiting insulin secretion. In another study of meningococcal sepsis, both insulin resistance and beta cell dysfunction contributed to the hyperglycemia that occurred in a third of the children.[214]

Hypocalcemia and decreased serum ionized calcium concentrations occur frequently during bacterial sepsis.

In one study, 12 (20%) of 60 critically ill adults with bacterial sepsis had hypocalcemia.[234] The mortality rate in the hypocalcemic patients was 50 percent compared with 30 percent in the patients who were normocalcemic. Cardenas-Rivero and associates[38] studied calcium homeostasis in 145 children admitted to a PICU. Of 8 children with confirmed sepsis or meningitis (or both) not caused by *H. influenzae* type b, 7 had hypocalcemia and 6 of the 7 had ionized hypocalcemia. Five of the 6 children with ionized hypocalcemia had inappropriately normal concentrations of parathyroid hormone, which suggests that transient hypoparathyroidism occurs in some children with sepsis. Hypocalcemia also occurs commonly in patients with toxic shock syndrome.[226] In women with toxic shock syndrome and hypocalcemia, serum concentrations of calcitonin are elevated by unknown mechanisms.[40] Hypocalcemia and elevated calcitonin concentrations also have been documented in children with fulminant meningococcemia.[120]

Procalcitonin levels are elevated in several conditions associated with systemic inflammatory response syndrome, including sepsis, and have been proposed as adjunctive laboratory tests for the early detection of, and indicators for prognosis of, meningococcal disease and many other infections.[39] These changes in calcium levels are especially critical because the level of ionized calcium and cardiac output in septic shock can be correlated.[231] Other metabolic changes may occur during septic shock in humans, as follows:

1. Increased concentrations of cortisol and growth hormone (including in neonates)[196,217]
2. Relative adrenal insufficiency by adrenocorticotropin testing[90]
3. Depression of triiodothyronine and thyroxine levels related to poor nutrition[173]
4. Elevations in total concentrations of amino acid in plasma and the preferential use of branched-chain amino acids as an energy source for skeletal muscle[136]
5. Muscle proteolysis, possibly induced by one or more circulating agents in the plasma of patients with serious infections[42]
6. Elevations in concentrations of plasma thromboxane, which are observed in nonsurvivors of septic shock[171]
7. Elevations in concentrations of triglycerides and free fatty acid during gram-negative bacteremia[73,107]
8. Altered zinc homeostasis[229]

Liver dysfunction is an important aspect of endotoxin shock in adults. Banks and colleagues[12] found that clinical jaundice was apparent in 63 percent of their patients with septic shock, that it was found more commonly in nonsurvivors than in survivors, and that the degree of biochemical liver abnormalities was related to the duration of shock. Postmortem findings included focal liver necrosis, Kupffer cell hyperplasia, portal tract inflammation, venous congestion, and intrahepatic cholestasis.

Adult respiratory distress syndrome (ARDS), or shock lung, is a major complication of septic shock in children.[97,162] The lungs of children with ARDS have characteristic changes consisting of increased lung weight reflecting congestion and atelectasis, alveoli lined with hyaline membranes, microthrombi, hemorrhage, and interstitial edema.[97] Increased capillary permeability and intrapulmonary shunts have been documented in patients with ARDS.[50] C5a, a potent chemotactic factor, causes aggregation of polymorphonuclear neutrophils, is elevated in the sera of patients who ultimately develop ARDS, and is found in increased concentrations in bronchoalveolar lavage fluid obtained from patients with ARDS.[87,174] Leukocyte aggregates are thought to be trapped in lung tissue and may cause damage to the endothelium of the pulmonary microvasculature through the release of oxygen radicals, lysosomal enzymes, and products of arachidonic acid metabolism. Although neutrophils play a critical role in the pathogenesis of ARDS, other factors also are important, considering that ARDS can develop in patients who are neutropenic.[148,225] Thromboxane, platelet-activating factor, fibrin, and other substances contribute to the lung injury in ARDS.[176]

The effects of endotoxin shock on the central nervous system have not been studied carefully in humans.[84] The encephalopathy associated with sepsis seems to be caused partly by altered phenylalanine metabolism; concentrations of phenylalanine and its metabolite, phenylacetic acid, are increased in the sera and cerebrospinal fluid of septic adults who are stuporous or comatose.[137]

Endotoxin has been implicated in the pathogenesis of acute renal failure associated with sepsis. Wardle[218,219] showed that 12 of 16 patients with acute tubular necrosis had endotoxemia. Renal arterial blood flow and renal vascular resistance are decreased significantly in baboons 2 to 4 hours after infusion of endotoxin. Inadequate perfusion pressure was associated with renal ischemia and negligible urine output in these animals shortly after administration of endotoxin.[181] Pathologic examination of the kidneys revealed focal necrosis of the proximal tubular epithelium, eosinophilic casts within proximal and distal tubules, and microthrombi in the glomerular capillaries. Endothelin, a potent vasoconstrictor peptide produced by endothelial cells, is elevated in concentration in the plasma of patients with septic shock. Because endothelin contributes to the regulation of regional blood flow, elevated levels suggest that it may relate to renal vasoconstriction and dysfunction.[215]

Endotoxin can be measured in the plasma of patients with gram-negative bacteremia as well as in patients with other infections, even upper respiratory tract infections.[53] The presence of circulating endotoxin does not mean that bacteremia is present or ever has occurred because endotoxin presumably may be "absorbed or leak" into the circulation from the gastrointestinal tract.[118,192,202] Endotoxemia may be a valid indicator, however, of impending gram-negative septicemia in febrile patients.[209] Preformed antibody to LPS or lipid A is associated with protection against shock and death caused by gram-negative bacteremia in adults. McCartney and colleagues[126] detected endotoxin in the blood (after chloroform extraction) of patients with gram-negative septic shock; all 18 patients with persistently positive endotoxin assays died. In contrast, 9 patients who initially had endotoxemia but subsequently had negative assays survived. Other studies confirm the association between

endotoxemia and outcome.[125,210] Evidence exists that human endotoxin is cleared from the circulation by the liver and can be detoxified by neutrophil enzymes (i.e., acyloxacyl hydrolases).[123,141]

The sequence of events in the evolution of endotoxin shock has been outlined by several investigators.[145] Bacteria, endotoxin, or other bacterial products stimulate the production of TNF and other cytokines, which in concert with endotoxin set off a whole series of events. Potent mediators, including C3a, C5a, eicosanoids, platelet-activating factor, histamine, and myocardial depressant substance, are released. Potent vasodilators cause peripheral vasodilation, and decreased systemic peripheral resistance leads to pooling of blood and decreased venous return to the heart. Mean blood pressure may be low, or it may be normal if cardiac output increases sufficiently to compensate for these alterations despite depression of ventricular function. The central venous pressure, which partially depends on myocardial performance, may be low or in the normal range.

If intravascular volume is increased by the administration of sufficient fluids, shock may be prevented or corrected. Continued hypotension and diminished perfusion pressure may lead, however, to cellular hypoxia and increased production of lactic acid from pyruvate. The microcirculation is altered by local tissue acidosis. Capillary beds become congested, and intravascular fluid may leak into the interstitial spaces. Increased secretion of catecholamine leads to arteriolar and venular constriction and increased peripheral resistance. Pooling of blood is enhanced, which leads to a further diminution in venous return and a reduction of cardiac output. Oliguria, coagulation abnormalities, and additional metabolic alterations indicate multiple organ system failure and presage the death of the patient.

Wong et al.[228] have studied the genome level expression profiles of children with sepsis and septic shock. Multiple gene networks, primarily related to inflammation and immunity, were affected over time and were differentially regulated.[182] These investigations have led to the identification of three subclasses of distinct gene expression profiles; higher mortality is associated with one of these subclasses.[230] This type of classification scheme might be able to identify patients at the time of admission who are at greater risk for poor outcomes and who would be optimal candidates for studying newer interventions or strategies for treating children in septic shock.

CLINICAL PRESENTATION AND DIAGNOSIS

The signs and symptoms of bacteremia vary, greatly depending on the age and underlying disease of the patient, the duration of illness, and the specific microorganisms. Young, otherwise healthy children aged 3 months to 3 years may present with fever and evidence of an upper or lower respiratory tract infection or no focus of infection and yet have unsuspected bacteremia. Most studies indicated that the risk of developing bacteremia increases as the body temperature increases; and in the unimmunized child, almost 25 percent of these children may be bacteremic after the temperature exceeds 41°C [105.8°F].[15] In a previously healthy child, the persistence of irritability and the inability to console the child despite optimal environmental conditions have been proposed as key points in the physical examination that should alert the clinician to the possibility of a serious infection, such as bacteremia or meningitis.[125,185]

Underlying illnesses with splenic dysfunction place a child at increased risk for serious infections caused by encapsulated organisms, whereas children with leukemia or other immunosuppressive diseases or children in the ICU are more likely to be infected with gram-negative bacilli or *S. aureus*. A history of diarrhea may suggest *Salmonella* spp. as a possible cause of illness. Preceding skin infections or wounds are important clues to infection caused by *S. aureus* or group A streptococcus. An indwelling vascular catheter may precipitate overlying erythema in a patient with evidence of phlebitis proximally. Gram-positive cocci, gram-negative bacilli, and yeast can be associated with catheter-related sepsis. Toxic shock syndrome should be considered in a hypotensive girl or woman with a recent menstrual period and history of tampon use, although toxic shock also is associated with *S. aureus* sepsis in males and nonmenstruating females.[82,184] Evidence of osteomyelitis, with or without venous thrombosis, is a very common finding in staphylococcal sepsis. Intra-abdominal sources of infection increase the likelihood of developing anaerobic bacteremia.

Petechiae may be associated with many microorganisms, especially invasive disease caused by *N. meningitidis*.[211] Purpura is an ominous finding and frequently is associated with overwhelming infection caused by *N. meningitidis*, *S. pneumoniae*, and *H. influenzae* type b. *P. aeruginosa* is associated specifically with erythema gangrenosum.[57] Almost half of children presenting with erythroderma (diffuse erythema) either had shock at presentation or developed shock in one study.[33,38] Other skin and soft tissue manifestations of gram-negative sepsis include bullous lesions, cellulitis, fasciitis, thrombophlebitis, and symmetric peripheral gangrene with disseminated intravascular coagulation.[142] Signs of meningeal irritation or increased intracranial pressure are important because they may modify the approach to management of fluids in a child in shock.

The onset of bacteremia may be heralded by chills, fever, nausea, vomiting, diarrhea, rashes, and petechiae. Initially, the skin feels warm and appears flushed. A change or impairment in mental status may be the first clue to the presence of shock. Hyperventilation also may develop before the onset of clinical shock occurs, which can alert the physician to impending circulatory insufficiency.[22] In time, cold, clammy extremities; a weak pulse; tachycardia; tachypnea; hypotension; and oliguria may occur. The skin over the extremities, the tip of the nose, and the earlobes especially are prone to cyanosis. Auscultation of the lungs may reveal rales, indicating pneumonia or pulmonary edema. Abnormal distention or tenderness to palpation and guarding may be evidence of peritonitis. Costovertebral angle tenderness suggests acute pyelonephritis as a source of bacteremia.

The physician must distinguish among the following three main types of shock in children:

1. *Hypovolemic shock*, such as occurs with blood loss, fluid and electrolyte loss, adrenal insufficiency, or other causes
2. *Cardiogenic shock*, which is associated with drug intoxication, cardiac surgery, arrhythmias, and pericardial tamponade, among other causes
3. *Distributive shock*, which indicates abnormal distribution of blood flow leading to inadequate tissue perfusion (e.g., septic shock, anaphylaxis)

The laboratory evaluation of a child with bacteremia, septic shock, or both conditions should provide information concerning the cause and the data required for optimal supportive management. Several studies showed that a total white blood cell (WBC) count exceeding 15,000 cells/mm^3 in a 3- to 36-month-old child who has not been immunized with conjugate vaccines and with a temperature greater than 39° to 40° C [102.2° to 104° F] and without a focus of infection is an indication that the child is at increased risk for having bacteremia, especially pneumococcal bacteremia.[13,18] Since the introduction of the pneumococcal conjugate vaccine, pneumococcal bacteremia in this scenario has been greatly reduced.[92,180,191] Elevated erythrocyte sedimentation rates and C-reactive protein levels also have been suggested as useful screening tools for detecting serious bacterial infections. A low peripheral WBC count also may suggest septicemia and commonly is observed during episodes of overwhelming bacteremic illnesses.

Procalcitonin levels are elevated in bacteremic children and are related to the severity of illness, such as organ failure and even mortality.[88,113] Commercial kits for the rapid measurement of concentrations of procalcitonin are available. Hemoglobin and hematocrit results should help differentiate between septic and hemorrhagic shock. Examination of the peripheral smear may disclose evidence of splenic dysfunction (i.e., Howell-Jolly bodies) or fragmented red blood cells, as seen in disseminated intravascular coagulation. Thrombocytopenia, prolongation of prothrombin time and partial prothrombin time, and the presence of fibrin split products are consistent with disseminated intravascular coagulation.

Hyponatremia is a common finding. Concentrations of serum bicarbonate may be depressed, which may signify a state of metabolic acidosis. Elevated lactic acid concentrations result from inadequate tissue perfusion and in some reports have been significantly greater in nonsurvivors or patients with low-flow states than in survivors or patients with high-flow shock.[43] In pediatric studies, serial lactate levels showing normalization are associated with recovery.[58] Hyperglycemia or hypoglycemia may be encountered. In one study, serum glucose levels greater than 178 mg/dL were associated with a greater risk of death caused by septic shock.[27] Transaminase levels may be elevated and presumably reflect cellular injury. Serum calcium concentrations (preferably ionized calcium levels) should be checked periodically because hypocalcemia may interfere with optimal myocardial function.

A chest radiograph may reveal a pulmonary source of infection or show a secondary pulmonary manifestation of an invasive infection such as pneumonia or septic emboli in children with staphylococcal sepsis.[81] Arterial blood gases obtained early in endotoxin shock usually reveal hypocapnia and normal to elevated pH.[22,23] At this point, the patient has a mixed metabolic acidosis and respiratory alkalosis. If the shock state progresses, the metabolic acidosis becomes so severe that respiratory compensation is ineffective, and the patient becomes acidotic. In some patients, respiratory acidosis accompanies metabolic acidosis. In either case, decompensated metabolic acidosis in a patient with septic shock is associated with a grave prognosis. A major consequence of ARDS is hypoxemia. For patients with ARDS, the chest radiograph characteristically shows bilateral and diffuse hazy infiltrates; opacification of all lung fields usually is seen during the late phases of ARDS. Positive fluid balance contributes to the pulmonary dysfunction in patients with acute lung injury, including those related to sepsis.[69]

Concentrations of blood urea nitrogen and serum creatinine may be elevated. Jones and Weil[100] found that the ratio of urine to plasma osmolality was the most valuable indicator of renal impairment in adult patients with shock. When this ratio was greater than 1.5, the likelihood of developing progressive renal failure was remote. A urine osmolality value greater than 400 mOsm/kg also indicated adequate renal function. Gene microarray studies may identify novel biomarkers that identify a patient in septic shock who is at greater risk for acute kidney injury.[16] Many WBCs or WBC casts in the urine may suggest the genitourinary tract as the source of bacteremia. If one or more gram-negative rods are seen on the Gram stain of unspun urine, more than 10^5 colony-forming units/mL of bacteria are likely to be present.

Isolating the organism responsible for bacteremia or septic shock is important for documenting the infection and for providing optimal antimicrobial therapy. With instruments that continuously monitor growth in the blood culture bottles, growth can be detected sooner than if the bottles are inspected just once or twice daily. Before the use of the pneumococcal conjugate vaccine became routine, many authorities recommended that a blood culture be obtained in a 3- to 36-month-old child with temperature greater than 39° to 40° C [102.2° to 104° F] and a total WBC count greater than or equal to 15,000 cells/mm^3 and without a specific focus of infection. In this way, instances of "unsuspected" or "outpatient" bacteremia could be identified. In an outpatient setting, almost 90 percent of blood cultures growing true pathogens were positive within 24 hours of incubation using a continuously monitored system.[129] This approach is now less useful in the era of administration of pneumococcal conjugate vaccine to young infants.[99]

In an infant who has received three or more doses of the conjugate pneumococcal vaccine, the likelihood of developing invasive pneumococcal infection is reduced approximately 90 percent. The proportion of children with high fever without localizing findings and a WBC count of 15,000/mm^3 or greater who might have occult pneumococcal bacteremia may be less than 1 percent, a level that no longer justifies this approach. Most organisms isolated from blood cultures in these patients are

now more likely to be a contaminant than a true pathogen. Currently, the approach to a febrile child without a source has changed in many emergency departments such that more selective criteria for obtaining blood cultures are being developed.

When appropriate, cerebrospinal fluid, urine, and other pertinent sites should be cultured before initiating antibiotic therapy, if possible. When an intra-abdominal source of infection is likely, blood and other cultures should be processed anaerobically. Gram stain or acridine orange stain of a buffy coat smear of peripheral blood may reveal evidence of the causative microorganism, especially in an overwhelming infection.[110] Gram stain of material obtained from petechial or purpuric lesions may show gram-negative diplococci suggestive of meningococcus. Polymerase chain reaction for detecting *N. meningitidis* is available in selected laboratories and may be the only method by which infection is documented when cultures are sterile. Bacterial polysaccharide antigens can be detected rapidly in many body fluids by latex agglutination tests. Rapid diagnostic procedures for evaluating outpatients with suspected bacteremia are unreliable. Pneumococcal antigenuria is not detected readily when bacteremia occurs without a specific focus of infection. The value of assays for detecting circulating endotoxin is unclear.

TREATMENT

The initial selection of antibiotics for administration to a child with suspected bacteremia is based on the clinical situation (Table 62-4). If untreated initially, children with occult bacteremia are at risk for developing serious complications, such as meningitis or pneumonia.[121,143] Empirical antibiotic therapy for children who are selected carefully and observed seems reasonable.[13,15,68]

TABLE 62-4 **Empirical Antibiotic Regimens for Septic Shock in Infants and Children Under Selected Clinical Circumstances**

Circumstances	Antibiotics
Normal Child	
Skin findings suggesting meningococcemia or preceding skin trauma or varicella	Cefotaxime or ceftriaxone + vancomycin ± nafcillin
Urinary tract source	Cefotaxime or ceftriaxone + aminoglycoside
Intra-abdominal source	Clindamycin + gentamicin + piperacillin-tazobactam
Immunocompromised Child	
Malignancy or immunodeficiency or neutropenia or central line	Vancomycin + aminoglycoside + ticarcillin-clavulanate or piperacillin-tazobactam or ceftazidime
Asplenia or splenic dysfunction	Vancomycin + cefotaxime or ceftriaxone

In children who have not received more than two doses of the pneumococcal conjugate vaccine, one approach may be to obtain a blood culture from children aged 3 to 36 months with a temperature of 39° C [102.2° F] or greater who have no focal findings and whose peripheral WBC count is greater than or equal to 15,000/mm^3. In such patients, the decision to administer antibiotics expectantly may be based on several factors, especially the ability of the parents to observe the child and communicate this information back to the physician in a timely manner. Ceftriaxone given parenterally, an oral agent such as amoxicillin and amoxicillin-clavulanate, or other oral antibiotics can be administered. If treatment is initiated, a properly collected urine specimen for urinalysis or urine culture also should be obtained so that a urinary tract infection would not be treated inadvertently, because a urinary tract infection is now the most common serious bacterial infection in young children with fever without a source.[99] Occult bacteremia caused by *S. pneumoniae* intermediate or resistant to penicillin or intermediate to ceftriaxone in an otherwise normal child should resolve with any of the options noted earlier.[104] This approach probably is less useful in an infant or child who has received three or more doses of the conjugate pneumococcal vaccine.

Children who subsequently are determined to have *S. pneumoniae* bacteremia and who have been treated with antibiotics expectantly need to be reevaluated as soon as the results of the blood cultures are known. If the child appears well and has been afebrile for at least 24 hours and if the parents can observe the child carefully and are able to communicate frequently with the physician, outpatient management can be continued. For children who were not treated initially, who remain febrile, and who are called back to be reevaluated for a positive blood culture, a parenteral dose of antibiotics is recommended.[11] Close contact with the parents and patient is mandatory no matter how these children are managed initially. Hospitalization for intravenous antibiotics is indicated if the child appears "toxic" or has other signs suggesting a serious infection.

For children with suspected bacteremia who are ill and require admission to the hospital, antibiotics are selected to cover the most serious organisms causing that infection. In normal children aged 3 months or older, a combination of nafcillin 150 to 200 mg/kg/day or another semisynthetic antistaphylococcal penicillin plus cefotaxime 150 to 200 mg/kg/day or ceftriaxone 75 to 100 mg/kg/day covers most of the likely pathogens (e.g., *S. pneumoniae*, *S. aureus*, *S. pyogenes*, *N. meningitidis*, and *H. influenzae* type b). In areas of the world where CA-MRSA is a problem, an agent effective against MRSA should be included in the initial empirical regimen.[91,103] Vancomycin 45 to 60 mg/kg/day is the gold standard for treating infections caused by MRSA. Whether adding an aminoglycoside or rifampin to vancomycin is beneficial is unclear. Although most CA-MRSA isolates are susceptible to clindamycin, this agent is not generally recommended alone for bacteremic illness because it is not bactericidal. Some experts recommend adding clindamycin to vancomycin or nafcillin theoretically to reduce toxin production. This

reduction occurs in vitro, but no clinical data indicate that this approach leads to a decrease in the incidence of morbidity or mortality.[190]

Treatment of bacteremia associated with a genitourinary or gastrointestinal source requires antibiotics to which gram-negative enteric agents are susceptible. In such cases, initial therapy could consist of an aminoglycoside with an additional antibiotic active against anaerobes, such as clindamycin, metronidazole, ticarcillin-clavulanate, or piperacillin-tazobactam, for a gastrointestinal focus of infection. Extended-spectrum cephalosporins, such as cefotaxime or ceftriaxone, are the most commonly recommended drugs for treating serious gram-negative enteric infections. Optimal management of intra-abdominal or other abscesses usually requires surgical drainage, which should be undertaken as soon as the child's condition allows.

Immunosuppressed children or children in the ICU with serious illnesses require a different initial approach to suspected bacteremia. Gram-negative enteric agents, *P. aeruginosa*, *S. aureus*, and coagulase-negative staphylococcus are likely to be isolated from these patients. Empirical therapy for nosocomial infection is based on the current antibiotic susceptibility pattern within the hospital.[197] *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. are among the most common organisms causing bacteremia in patients in the PICU.[172] *E. coli* and *Klebsiella* spp. frequently produce β-lactamases that lead to resistance to ticarcillin or piperacillin. Generally, a combination of an antistaphylococcal semisynthetic penicillin or vancomycin (if a central line is present) or, if MRSA is a relatively common nosocomial pathogen, an aminoglycoside and an extended-spectrum penicillin (e.g., ticarcillin-clavulanate, piperacillin-tazobactam) or a third- or fourth-generation cephalosporin (cefotaxime, ceftazidime, cefepime) is administered initially until a specific pathogen is isolated. Broad-spectrum penicillins and aminoglycosides frequently exhibit synergy in vitro against gram-negative organisms, especially against *P. aeruginosa*.[183] In critically ill patients, a synergistic combination of antibiotics may be beneficial for treating bacteremia caused by *P. aeruginosa* or *Klebsiella* spp., although this subject remains controversial.[93,111,159] Combination therapy may be most helpful in the neutropenic patient with bacteremia, especially due to *P. aeruginosa*.

After a specific organism is identified and antibiotic susceptibilities are known, the most appropriate agent is selected. If *E. coli* or *Klebsiella* spp. are isolated, these organisms can produce extended-spectrum β-lactamases. With the current Clinical and Laboratory Standards Institute breakpoints, the extended-spectrum β-lactamase–producing bacteria should be detected.[41,156] If an organism is identified, treatment with an extended-spectrum cephalosporin (e.g., cefotaxime, ceftriaxone, ceftazidime) is not recommended.[158] In this case, a carbapenem, such as imipenem-cilastatin or meropenem, is advised.[158]

Enterobacter spp., *Citrobacter* spp., and *Serratia marcescens* may hyperproduce β-lactamase enzymes or be induced to hyperproduce these enzymes and are resistant or can become resistant to the extended-spectrum cephalosporins.[161] In many institutions, a high percentage of these isolates are resistant to ceftazidime.[32] Cefepime may be more active than is ceftazidime or cefotaxime against *Enterobacter* spp. and other Amp C, β-lactamase enzyme–producing, gram-negative bacilli.[179] A carbapenem with an aminoglycoside is the regimen recommended for treating infections caused by these isolates when resistant to the third-generation cephalosporins.

In these patients, rapidly achieving therapeutic aminoglycoside levels in the plasma is important.[139] Serum concentrations of the aminoglycoside should be measured within 24 hours of initiation of therapy to ensure that therapeutic concentrations have been reached. Higher doses of antibiotics than normally administered may be required in some patients.

Antibiotics should be administered as soon as the diagnosis of septic shock is suspected. The selection of antibiotics is the same as that for bacteremia. If *S. aureus* is suspected among the potential causes of septic shock, antibiotic therapy is complicated by two factors: MRSA may make up more than 50 percent of community-acquired isolates[103] and nafcillin or oxacillin is superior to vancomycin for the treatment of serious infections caused by MRSA.[83] Nafcillin or oxacillin plus an aminoglycoside may be synergistic against staphylococci, but adding an aminoglycoside increases the risk for nephrotoxicity to develop. Some authorities include nafcillin or oxacillin plus vancomycin plus gentamicin in the initial empirical therapy of patients with life-threatening infections possibly caused by *S. aureus*.[7] Combination empirical antibiotics appear to be advantageous because it is more likely that an antibiotic active against the causative pathogen will be administered compared with empirical monotherapy and not because of potential synergy.[54]

The management of septic shock is directed toward three main objectives: (1) control of the infectious process, (2) restoration of adequate tissue perfusion, and (3) maintenance of efficient respiratory function. Providing aggressive fluid resuscitation early in the emergency center proved to be one factor in decreasing the length of hospital stay in one study.[114] Details of management have been the subject of numerous reports.[37,109,178] The reader is referred to these reviews for expert detailed guidance in the fluid management, use of vasoactive agents, and respiratory treatments in children with septic shock. Providing aggressive fluid resuscitation early in the emergency center proved to be one factor in decreasing the length of hospital stay in one study.

The benefits of therapy with high doses of corticosteroids in the treatment of endotoxin shock remain unproved. Extensive experimental data document the salutary hemodynamic, metabolic, microcirculatory, and cellular effect of corticosteroids in laboratory models of endotoxin shock.[95,104] High-dose corticosteroids have never been found to be efficacious, however, in randomized controlled trials conducted in adults with severe sepsis or septic shock.[26,186]

On the basis of these studies, the routine use of high-dose corticosteroids in patients with severe sepsis or septic shock is not recommended. Results of randomized studies enrolling adults suggested that lower stress doses of hydrocortisone might be beneficial in managing patients with septic shock.[24,28] However, it is not clear if

TABLE 62-5 **Clinical Trials of Sepsis Medications in Adults**

Trial	Agent Studied	Total Patients (no.)	28-Day Mortality (%)	
			Medications	Controls
CHESS	HA-1A human monoclonal antibody[127,141]	1578	41	37
	Anti-TNF[45,51]	564	37.3	39.5
	Steroids[31]	1267	39	35
	IL-1ra[66,74]	893	31	35
	Soluble TNF receptor[65,73]	141	40	38
COMPASS	Platelet-activating factor acetylhydrolase[166]	1425	25	24
KyberSept	Antithrombin III[220]	2314	38.9	38.7
OPTIMIST	Tissue factor pathway inhibitor[2,3]	1754	34.2	33.9
PROWESS	Activated protein C[19,22]	1690	24.7	30.8
ADDRESS	Activated protein C[1]	2613	17	18.5

TNF, tumor necrosis factor.

there is a survival benefit at 28 days.[155] The role of low-dose hydrocortisone in children with septic shock remains controversial.[9,212] In one pediatric study the addition of fludrocortisone, a mineralocorticoid given enterally, to parenteral hydrocortisone was associated with shorter duration of norepinephrine support but more hypokalemia in children with sepsis.[89] Randomized studies are needed to clarify the role of stress doses of hydrocortisone in children with septic shock.

Every effort should be made to ensure an adequate airway, which may require periodic suctioning if the patient is unable to clear pooled secretions. Humidified oxygen in concentrations required to maintain an adequate partial pressure of oxygen should be provided early. The method of oxygen administration (e.g., mask, ventilator) depends on the clinical state of the patient. Intubation and assisted ventilation are indicated if the child shows evidence of impending respiratory failure.

ARDS, usually appearing within 2 days after the onset of shock, may complicate the respiratory and fluid management.[97] Pulmonary edema, atelectasis, decreased pulmonary compliance, and ventilation-perfusion abnormalities are some factors that can lead to inadequate oxygenation. Administration of excessive fluids may contribute to ARDS. The central venous pressure may remain normal, despite the presence of pulmonary edema. Positive end-expiratory pressure, oxygen, and careful attention to cardiovascular parameters are the mainstays of therapy for ARDS.[97,162,176]

The general supportive care of a child with septic shock includes attention to nutritional and metabolic requirements.[170] Parenteral alimentation may be the only means by which to provide nutrition, although the optimal amount and composition of elemental nutrients for children with septic shock are unknown. Hypocalcemia should be corrected. Platelet transfusions or fresh-frozen plasma may be necessary to correct coagulopathies. Continuous venovenous hemofiltration with or without dialysis may be instituted for complications of renal failure, such as fluid overload with pulmonary edema or hyperkalemia. Children who have acute renal failure complicating severe septic shock have a significantly higher mortality rate than children without acute renal failure.[164]

Investigative Therapies

Based on the evolving understanding of the pathophysiology of septic shock, many large, randomized, double-blind, multicenter trials have been conducted to assess the efficacy of agents that neutralize or counteract toxins or cytokines that are important in the evolution of septic shock. These immunomodulating strategies have been used on the basis of experimental data from animal studies. The conditions seen in experimental animal models of sepsis do not reflect the pathophysiologic situation seen in patients with severe sepsis or septic shock, however, which has led to many negative clinical trials (Table 62-5). One interesting newer approach is the development of endotoxin removal devices that are being evaluated to assess their potential as adjunctive measures to improve the outcome of patients with septic shock.[51]

Intravenous immunoglobulin is recommended for adjunctive therapy for patients with toxic shock syndrome caused by either group A streptococcus or *S. aureus* that is not responding to aggressive therapy after several hours or after appropriate drainage of focal infections.[8] The value of intravenous immunoglobulin in the treatment of septic shock caused by *S. aureus* infection but not associated with toxic shock syndrome has not been established.[76] Investigative antibodies such as tefibazumab (Aurexis), a monoclonal antibody directed against clumping factor A, an adhesin on the surface of *S. aureus*, have not been shown to improve the outcome of adults with *S. aureus* bacteremia.[223] Early phase II clinical trials of eritoran tetrasodium, a TLR4 antagonist, for the treatment of severe sepsis seemed promising; however, a recently completed phase III randomized trial did not improve the primary end point of 28-day all-cause mortality in 2000 adult patients with severe sepsis.[195*]

*http://www.ukmi.nhs.uk/applications/ndo/record_view_open.asp?newDrugID=4747

Plasmapheresis, exchange transfusions, and extracorporeal membrane oxygenation are heroic measures that seem to be beneficial in selected patients not responding to standard management.[18,75,206] These procedures may be considered for such patients when, in the opinion of experienced clinicians, their use is justified and they are the last hope for a successful outcome.[71]

PROGNOSIS

The morbidity and mortality rates for septic shock in children vary with age, the presence or absence of underlying diseases, and the specific microorganisms responsible for the septicemic state. DuPont and Spink reported a 98 percent mortality rate in their series of children with septic shock and gram-negative bacteremia. Jacobs and colleagues reported a 9.8 percent case-fatality rate for otherwise normal children with septic shock. In the pediatric HA-1A study, the overall mortality rate for severe sepsis or septic shock was 31 percent. The overall mortality rate for children with meningococcal infection was 8 percent in a 10-center surveillance study. For the seven-state sepsis cohort, the overall mortality rate was approximately 10 percent. Much progress has been made in the treatment of sepsis and septic shock since the report of Dupont and Spink was published. As with many infections, prevention is more desirable than is treatment. Careful attention given to sterile techniques for insertion and maintenance of intravascular or other lines and other procedures is crucial and may prevent some episodes of bacteremia and septic shock.

NEW REFERENCES SINCE THE SIXTH EDITION

8. American Academy of Pediatrics. Staphylococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *2009 Red Book: Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 601–16.
16. Basu RK, Standage SW, Cvijanovich NZ, et al. Identification of candidate serum biomarkers for severe septic shock-associated kidney injury via microarray. *Crit Care* 2011;**15**:R273.
29. Brierley J, Peters MJ. Distinct hemodynamic patterns of septic shock at presentation to pediatric intensive care. *Pediatrics* 2008;**122**:752–9.
41. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twentieth informational supplement (June 2010 update)*. CLSI Document M100-S20-U, 2010.
51. Davies B, Cohen J. Endotoxin removal devices for the treatment of sepsis and septic shock. *Lancet Infect Dis* 2011;**11**:65–71.
54. Dickinson JD, Kollef MH. Early and adequate antibiotic therapy in the treatment of severe sepsis and septic shock. *Curr Infect Dis Rep* 2011;**13**:399–405.
57. Doughty CB, Cruz AT, Kaplan SL. A bullous lesion in a neutropenic adolescent. *Pediatr Emerg Care* 2009;**25**:100–1.
69. Flori HR, Church G, Liu KD, et al. Positive fluid balance is associated with higher mortality and prolonged mechanical ventilation in pediatric patients with acute lung injury. *Crit Care Res Pract* 2011;**2011**:854142.
70. Flynn A, Chokkalingam Mani B, Mather PJ. Sepsis-induced cardiomyopathy: a review of pathophysiologic mechanisms. *Heart Fail Rev* 2010;**15**:605–11.
74. Garcia PC, Longhi F, Branco RG, et al. Ferritin levels in children with severe sepsis and septic shock. *Acta Paediatr* 2007;**96**:1829–31.
77. Gerber JS, Coffin SE, Smathers SA, Zaoutis TE. Trends in the incidence of methicillin-resistant *Staphylococcus aureus* infection in children's hospitals in the United States. *Clin Infect Dis* 2009;**49**:65–71.
89. Hebbar KB, Stockwell JA, Fortenberry JD. Clinical effects of adding fludrocortisone to a hydrocortisone-based shock protocol in hypotensive critically ill children. *Intensive Care Med* 2011;**37**:518–24.
90. Hebbar KB, Stockwell JA, Leong T, Fortenberry JD. Incidence of adrenal insufficiency and impact of corticosteroid supplementation in critically ill children with systemic inflammatory syndrome and vasopressor-dependent shock. *Crit Care Med* 2011;**39**:1145–50.
99. Jhaveri R, Byington CL, Klein JO, Shapiro ED. Management of the non–toxic-appearing acutely febrile child: a 21st century approach. *J Pediatr* 2010;**159**:181–5.
108. Khemani RG, Bart RD, Alonzo TA, et al. Disseminated intravascular coagulation score is associated with mortality for children with shock. *Intensive Care Med* 2009;**35**:327–33.
109. Kissoon N, Orr RA, Carcillo JA. Updated American College of Critical Care Medicine—pediatric advanced life support guidelines for management of pediatric and neonatal septic shock: relevance to the emergency care clinician. *Pediatr Emerg Care* 2010;**26**:867–9.
114. Larsen GY, Mecham N, Greenberg R. An emergency department septic shock protocol and care guideline for children initiated at triage. *Pediatrics* 2011;**127**:e1585–92.
115. Laupland KB, Gregson DB, Vanderkooi OG, et al. The changing burden of pediatric bloodstream infections in Calgary, Canada, 2000-2006. *Pediatr Infect Dis J* 2009;**28**:114–7.
116. Lee JM, Hashmi N, Bloom JD, et al. Biofilm accumulation on endotracheal tubes following prolonged intubation. *J Laryngol Otol* 2012;**126**:267–70.
133. Miller MR, Griswold M, Harris JM 2nd, et al. Decreasing PICU catheter-associated bloodstream infections: NACHRI's quality transformation efforts. *Pediatrics* 2010;**125**:206–13.
155. Patel GP, Balk RA. Systemic steroids in severe sepsis and septic shock. *Am J Respir Crit Care Med* 2012;**185**:133–9.
159. Paul M, Leibovici L. Combination antimicrobial treatment versus monotherapy: the contribution of meta-analyses. *Infect Dis Clin North Am* 2009;**23**:277–93.
163. Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.
188. Standage SW, Wong HR. Biomarkers for pediatric sepsis and septic shock. *Expert Rev Anti Infect Ther* 2011;**9**:71–9.
195. Tidswell M, Tillis W, Larosa SP, et al. Phase 2 trial of eritoran tetrasodium (E5564), a toll-like receptor 4 antagonist, in patients with severe sepsis. *Crit Care Med* 2010;**38**:72–83.
214. Verhoeven JJ, den Brinker M, Hokken-Koelega AC, et al. Pathophysiological aspects of hyperglycemia in children with meningococcal sepsis and septic shock: a prospective, observational cohort study. *Crit Care* 2011;**15**:R44.
228. Wong HR, Cvijanovich N, Allen GL, et al. Genomic expression profiling across the pediatric systemic inflammatory response syndrome, sepsis, and septic shock spectrum. *Crit Care Med* 2009;**37**:1558–66.
230. Wong HR, Wheeler DS, Tegtmeyer K, et al. Toward a clinically feasible gene expression-based subclassification strategy for septic shock: proof of concept. *Crit Care Med* 2010;**38**:1955–61.
235. Zanotti-Cavazzoni SL, Goldfarb RD. Animal models of sepsis. *Crit Care Clin* 2009;**25**:703–19, vii–viii.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abraham E, Bland RD, Cobo JC, Shoemaker WC. Sequential cardiorespiratory patterns associated with outcome in septic shock. *Chest* 1984;**85**:75–80.
2. Abraham E, Laterre PF, Garg R, et al. Drotrecogin alfa (activated) for adults with severe sepsis and a low risk of death. *N Engl J Med* 2005;**353**:1332–41.
3. Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. *JAMA* 2003;**290**:238–47.
4. Adem PV, Montgomery CP, Husain AN, et al. *Staphylococcus aureus* sepsis and the Waterhouse-Friderichsen syndrome in children. *N Engl J Med* 2005;**353**:1245–51.
5. Albano EA, Pizzo PA. Infectious complications in childhood acute leukemias. *Pediatr Clin North Am* 1988;**35**:873–901.
6. Aledo A, Heller G, Ren L, et al. Septicemia and septic shock in pediatric patients: 140 consecutive cases on a pediatric hematology-oncology service. *J Pediatr Hematol Oncol* 1998;**20**:215–21.
7. American Academy of Pediatrics. Toxic shock syndrome. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *2006 Red book: report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 650–5.
8. American Academy of Pediatrics. Staphylococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *2009 Red book: report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 601–16.
9. Aneja R, Carcillo JA. What is the rationale for hydrocortisone treatment in children with infection-related adrenal insufficiency and septic shock? *Arch Dis Child* 2007;**92**:165–9.
10. Archer LT, Benjamin BA, Beller-Todd BK, et al. Does LD100 *E coli* shock cause myocardial failure? *Circ Shock* 1982;**9**:7–16.
11. Bachur R, Harper MB. Reevaluation of outpatients with *Streptococcus pneumoniae* bacteremia. *Pediatrics* 2000;**105**:502–9.
12. Banks JG, Foulis AK, Ledingham IM, Macsween RN. Liver function in septic shock. *J Clin Pathol* 1982;**35**:1249–52.
13. Baraff LJ, Bass JW, Fleisher GR, et al. Practice guideline for the management of infants and children 0 to 36 months of age with fever without source. *Pediatrics* 1993;**92**:1–12.
14. Barocchi MA, Ries J, Zogaj X, et al. A pneumococcal pilus influences virulence and host inflammatory responses. *Proc Natl Acad Sci U S A* 2006;**103**:2857–62.
15. Bass JW, Steele RW, Wittler RR, et al. Antimicrobial treatment of occult bacteremia: a multicenter cooperative study. *Pediatr Infect Dis J* 1993;**12**:466–73.
16. Basu RK, Standage SW, Cvijanovich NZ, et al. Identification of candidate serum biomarkers for severe septic shock-associated kidney injury via microarray. *Crit Care* 2011;**15**:R273.
17. Baumgarten G, Knuefermann P, Nozaki N, et al. In vivo expression of proinflammatory mediators in the adult heart after endotoxin administration: the role of Toll-like receptor-4. *J Infect Dis* 2001;**183**:1617–24.
18. Beca J, Butt W. Extracorporeal membrane oxygenation for refractory septic shock in children. *Pediatrics* 1994;**93**:726–9.
19. Bernard GR, Vincent JL, Laterre PF, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;**344**:699–709.
20. Beutler B, Poltorak A. Sepsis and evolution of the innate immune response. *Crit Care Med* 2001;**29**:S2–6; discussion S6-7.
21. Blain CM, Anderson TO, Pietras RJ, Gunnar RM. Immediate hemodynamic effects of gram-negative vs gram-positive bacteremia in man. *Arch Intern Med* 1970;**126**:260–5.
22. Blair E. Hypocapnia and gram-negative bacteremic shock. *Am J Surg* 1970;**119**:433–9.
23. Blair E. Acid-base balance in bacteremic shock. *Arch Intern Med* 1971;**127**:731–9.
24. Bollaert PE, Charpentier C, Levy B, et al. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. *Crit Care Med* 1998;**26**:645–50.
25. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;**101**:1644–55.
26. Bone RC, Fisher CJ Jr, Clemmer TP, et al. A controlled clinical trial of high-dose methylprednisolone in the treatment of severe sepsis and septic shock. *N Engl J Med* 1987;**317**:653–8.
27. Branco RG, Garcia PC, Piva JP, et al. Glucose level and risk of mortality in pediatric septic shock. *Pediatr Crit Care Med* 2005;**6**:470–2.
28. Briegel J, Forst H, Haller M, et al. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. *Crit Care Med* 1999;**27**:723–32.
29. Brierley J, Peters MJ. Distinct hemodynamic patterns of septic shock at presentation to pediatric intensive care. *Pediatrics* 2008;**122**:752–9.
30. Brigham KL, Meyrick B. Endotoxin and lung injury. *Am Rev Respir Dis* 1986;**133**:913–27.
31. Buck C, Bundschu J, Gallati H, et al. Interleukin-6: a sensitive parameter for the early diagnosis of neonatal bacterial infection. *Pediatrics* 1994;**93**:54–8.
32. Burwen DR, Banerjee SN, Gaynes RP. Ceftazidime resistance among selected nosocomial gram-negative bacilli in the United States. National Nosocomial Infections Surveillance System. *J Infect Dis* 1994;**170**:1622–5.
33. Byer RL, Bachur RG. Clinical deterioration among patients with fever and erythroderma. *Pediatrics* 2006;**118**:2450–60.
34. Cain BS, Meldrum DR, Dinarello CA, et al. Tumor necrosis factor-alpha and interleukin-1beta synergistically depress human myocardial function. *Crit Care Med* 1999;**27**:1309–18.
35. Calandra T, Baumgartner JD, Grau GE, et al. Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-alpha, and interferon-gamma in the serum of patients with septic shock. Swiss-Dutch J5 Immunoglobulin Study Group. *J Infect Dis* 1990;**161**:982–7.
36. Cannon JG, Tompkins RG, Gelfand JA, et al. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 1990;**161**:79–84.
37. Carcillo JA, Fields AI. Clinical practice parameters for hemodynamic support of pediatric and neonatal patients in septic shock. *Crit Care Med* 2002;**30**:1365–78.
38. Cardenas-Rivero N, Chernow B, Stoiko MA, et al. Hypocalcemia in critically ill children. *J Pediatr* 1989;**114**:946–51.
39. Carrol ED, Newland P, Thomson AP, Hart CA. Prognostic value of procalcitonin in children with meningococcal sepsis. *Crit Care Med* 2005;**33**:224–5.
40. Chesney RW, McCarron DM, Haddad JG, et al. Pathogenic mechanisms of the hypocalcemia of the staphylococcal toxic-shock syndrome. *J Lab Clin Med* 1983;**101**:576–85.
41. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing; twentieth informational supplement (June 2010 update)*. CLSI Document M100-S20-U, 2010.
42. Clowes GH Jr, George BC, Villee CA Jr, Saravis CA. Muscle proteolysis induced by a circulating peptide in patients with sepsis or trauma. *N Engl J Med* 1983;**308**:545–52.
43. Clowes GH Jr, O'Donnell TF Jr, Ryan NT, Blackburn GL. Energy metabolism in sepsis: treatment based on different patterns in shock and high output stage. *Ann Surg* 1974;**179**:684–96.
44. Coalson JJ, Archer LT, Hall NK, et al. Prolonged shock in the monkey following live *E coli* organism infusion. *Circ Shock* 1979;**6**:343–55.
45. Cohen J, Carlet J. INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. *Crit Care Med* 1996;**24**:1431–40.
46. Corrigan JJ Jr. Thrombocytopenia: a laboratory sign of septicemia in infants and children. *J Pediatr* 1974;**85**:219–21.
47. Corrigan JJ Jr. Vitamin K–dependent coagulation factors in gram-negative septicemia. *Am J Dis Child* 1984;**138**:240–2.
48. Corrigan JJ Jr, Jordan CM. Heparin therapy in septicemia with disseminated intravascular coagulation. *N Engl J Med* 1970;**283**:778–82.
49. Cryer PE, Coran AG, Sode J, et al. Lethal *Escherichia coli* septicemia in the baboon: alpha-adrenergic inhibition of insulin secretion and its relationship to the duration of survival. *J Lab Clin Med* 1972;**79**:622–38.
50. Dantzker DR, Brook CJ, Dehart P, et al. Ventilation-perfusion distributions in the adult respiratory distress syndrome. *Am Rev Respir Dis* 1979;**120**:1039–52.

51. Davies B, Cohen J. Endotoxin removal devices for the treatment of sepsis and septic shock. *Lancet Infect Dis* 2011;**11**: 65–71.
52. DeBont ES, Martens A, van Raan J, et al. Tumor necrosis factor-alpha, interleukin-1beta, and interleukin-6 plasma levels in neonatal sepsis. *Pediatr Res* 1993;**33**:380–3.
53. Dholakia S, Inwald D, Betts H, Nadel S. Endotoxemia in pediatric critical illness—a pilot study. *Crit Care* 2011;**15**:R141.
54. Dickinson JD, Kollef MH. Early and adequate antibiotic therapy in the treatment of severe sepsis and septic shock. *Curr Infect Dis Rep* 2011;**13**:399–405.
55. Dinarello CA. Interleukin-1 and its biologically related cytokines. *Adv Immunol* 1989;**44**:153–205.
56. Dinarello CA, Wolff SM. The role of interleukin-1 in disease. *N Engl J Med* 1993;**328**:106–13.
57. Doughty CB, Cruz AT, Kaplan SL. A bullous lesion in a neutropenic adolescent. *Pediatr Emerg Care* 2009;**25**:100–1.
58. Dugas MA, Proulx F, de Jaeger A, et al. Markers of tissue hypoperfusion in pediatric septic shock. *Intensive Care Med* 2000; **26**:75–83.
59. DuPont HL, Spink WW. Infections due to gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958-1966. *Medicine (Baltimore)* 1969;**48**:307–32.
60. Farley MM, Stephens DS, Mulks MH, et al. Pathogenesis of IgA1 protease-producing and -nonproducing *Haemophilus influenzae* in human nasopharyngeal organ cultures. *J Infect Dis* 1986; **154**:752–9.
61. Faust SN, Levin M, Harrison OB, et al. Dysfunction of endothelial protein C activation in severe meningococcal sepsis. *N Engl J Med* 2001;**345**:408–16.
62. Fearson DT, Ruddy S, Schur PH, et al. Activation of the properdin pathway of complement in patients with gram-negative bacteremia. *N Engl J Med* 1975;**292**:937–40.
63. Feltes TF, Pignatelli R, Kleinert S, Mariscalco MM. Quantitated left ventricular systolic mechanics in children with septic shock utilizing noninvasive wall-stress analysis. *Crit Care Med* 1994; **22**:1647–58.
64. Filkins JP, Cornell RP. Depression of hepatic gluconeogenesis and the hypoglycemia of endotoxin shock. *Am J Physiol* 1974; **227**:778–81.
65. Fisher CJ Jr, Agosti JM, Opal SM, et al. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N Engl J Med* 1996;**334**:1697–702.
66. Fisher CJ Jr, Dhainaut JF, Opal SM, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome: results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA* 1995;**271**:1836–43.
67. Fisher CJ Jr, Yan SB. Protein C levels as a prognostic indicator of outcome in sepsis and related diseases. *Crit Care Med* 2000; **28**:S49–56.
68. Fleisher GR, Rosenberg N, Vinci R, et al. Intramuscular versus oral antibiotic therapy for the prevention of meningitis and other bacterial sequelae in young, febrile children at risk for occult bacteremia. *J Pediatr* 1994;**124**:504–12.
69. Flori HR, Church G, Liu KD, et al. Positive fluid balance is associated with higher mortality and prolonged mechanical ventilation in pediatric patients with acute lung injury. *Crit Care Res Pract* 2011;**2011**:854142.
70. Flynn A, Chokkalingam Mani B, Mather PJ. Sepsis-induced cardiomyopathy: a review of pathophysiologic mechanisms. *Heart Fail Rev* 2010;**15**:605–11.
71. Fortenberry JD, Paden ML. Extracorporeal therapies in the treatment of sepsis: experience and promise. *Semin Pediatr Infect Dis* 2006;**17**:72–9.
72. Froon AH, Dentener MA, Greve JW, et al. Lipopolysaccharide toxicity-regulating proteins in bacteremia. *J Infect Dis* 1995;**171**: 1250–7.
73. Gallin JI, Kaye D, O'Leary WM. Serum lipids in infection. *N Engl J Med* 1969;**281**:1081–6.
74. Garcia PC, Longhi F, Branco RG, et al. Ferritin levels in children with severe sepsis and septic shock. *Acta Paediatr* 2007;**96**: 1829–31.
75. Gardlund B, Sjolin J, Nilsson A, et al. Plasmapheresis in the treatment of primary septic shock in humans. *Scand J Infect Dis* 1993;**25**:757–61.
76. Gauduchon V, Cozon G, Vandenesch F, et al. Neutralization of *Staphylococcus aureus* Panton Valentine leukocidin by intravenous immunoglobulin in vitro. *J Infect Dis* 2004;**189**:346–53.
77. Gerber JS, Coffin SE, Smathers SA, Zaoutis TE. Trends in the incidence of methicillin-resistant *Staphylococcus aureus* infection in children's hospitals in the United States. *Clin Infect Dis* 2009;**49**:65–71.
78. Girardin E, Grau GE, Dayer JM, et al. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med* 1988;**319**:397–400.
79. Goldie AS, Fearon KC, Ross JA, et al. Natural cytokine antagonists and endogenous antiendotoxin core antibodies in sepsis syndrome. The Sepsis Intervention Group. *JAMA* 1995;**274**: 172–7.
80. Goldstein B, Giroir B, Randolph A. International Pediatric Sepsis Consensus Conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med* 2005;**6**:2–8.
81. Gonzalez BE, Hulten KG, Dishop MK, et al. Pulmonary manifestations in children with invasive community-acquired *Staphylococcus aureus* infection. *Clin Infect Dis* 2005;**41**:583–90.
82. Gonzalez BE, Martinez-Aguilar G, Hulten KG, et al. Severe staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant *Staphylococcus aureus. Pediatrics* 2005;**115**:642–8.
83. Gonzalez C, Rubio M, Romero-Vivas J, et al. Bacteremic pneumonia due to *Staphylococcus aureus:* a comparison of disease caused by methicillin-resistant and methicillin-susceptible organisms. *Clin Infect Dis* 1999;**29**:1171–7.
84. Graham DI, Behan PO, More IA. Brain damage complicating septic shock: acute haemorrhagic leucoencephalitis as a complication of the generalised Shwartzman reaction. *J Neurol Neurosurg Psychiatry* 1979;**42**:19–28.
85. Gray J, Gossain S, Morris K. Three-year survey of bacteremia and fungemia in a pediatric intensive care unit. *Pediatr Infect Dis J* 2001;**20**:416–21.
86. Hack CE, Hart M, van Schijndel RJ, et al. Interleukin-8 in sepsis: relation to shock and inflammatory mediators. *Infect Immun* 1992;**60**:2835–42.
87. Hammerschmidt DE, Weaver LJ, Hudson LD, et al. Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome: pathophysiological relevance and possible prognostic value. *Lancet* 1980;**1**:947–9.
88. Hatherill M, Tibby SM, Turner C, et al. Procalcitonin and cytokine levels: relationship to organ failure and mortality in pediatric septic shock. *Crit Care Med* 2000;**28**:2591–4.
89. Hebbar KB, Stockwell JA, Fortenberry JD. Clinical effects of adding fludrocortisone to a hydrocortisone-based shock protocol in hypotensive critically ill children. *Intensive Care Med* 2011; **37**:518–24.
90. Hebbar KB, Stockwell JA, Leong T, Fortenberry JD. Incidence of adrenal insufficiency and impact of corticosteroid supplementation in critically ill children with systemic inflammatory syndrome and vasopressor-dependent shock. *Crit Care Med* 2011;**39**:1145–50.
91. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998;**279**:593–8.
92. Herz AM, Greenhow TL, Alcantara J, et al. Changing epidemiology of outpatient bacteremia in 3- to 36-month-old children after the introduction of the heptavalent-conjugated pneumococcal vaccine. *Pediatr Infect Dis J* 2006;**25**:293–300.
93. Hilf M, Yu VL, Sharp J, et al. Antibiotic therapy for *Pseudomonas aeruginosa* bacteremia: outcome correlations in a prospective study of 200 patients. *Am J Med* 1989;**87**:540–6.
94. Hill SL, Elings VB, Lewis FR. Changes in lung water and capillary permeability following sepsis and fluid overload. *J Surg Res* 1980;**28**:140–50.
95. Hinshaw LB, Beller-Todd BK, Archer LT, et al. Effectiveness of steroid/antibiotic treatment in primates administered LD100 *Escherichia coli. Ann Surg* 1981;**194**:51–6.
96. Hinshaw LB, Tekamp-Olson P, Chang AC, et al. Survival of primates in LD100 septic shock following therapy with antibody

to tumor necrosis factor (TNF alpha). *Circ Shock* 1990;**30**: 279–92.
97. Holbrook PR, Taylor G, Pollack MM, Fields AI. Adult respiratory distress syndrome in children. *Pediatr Clin North Am* 1980; **27**:677–85.
98. Jacobs RF, Sowell MK, Moss MM, Fiser DH. Septic shock in children: bacterial etiologies and temporal relationships. *Pediatr Infect Dis J* 1990;**9**:196–200.
99. Jhaveri R, Byington CL, Klein JO, Shapiro ED. Management of the non–toxic-appearing acutely febrile child: a 21st century approach. *J Pediatr* 2010;**159**:181–5.
100. Jones LW, Weil MH. Water, creatinine and sodium excretion following circulatory shock with renal failure. *Am J Med* 1971;**51**: 314–8.
101. Kalter ES, Daha MR, ten Cate JW, et al. Activation and inhibition of Hageman factor–dependent pathways and the complement system in uncomplicated bacteremia or bacterial shock. *J Infect Dis* 1985;**151**:1019–27.
102. Kapadia S, Lee J, Torre-Amione G, et al. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. *J Clin Invest* 1995;**96**:1042–52.
103. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
104. Kaplan SL, Mason EO Jr, Barson WJ, et al. Outcome of invasive infections outside the central nervous system caused by *Streptococcus pneumoniae* isolates nonsusceptible to ceftriazone in children treated with beta-lactam antibiotics. *Pediatr Infect Dis J* 2001; **20**:392–6.
105. Kaplan SL, Schutze GE, Leake JA, et al. Multicenter surveillance of invasive meningococcal infections in children. *Pediatrics* 2006; **118**:e979–84.
106. Kaplan SL, Taber LH, Frank AL, Feigin RD. Nasopharyngeal viral isolates in children with *Haemophilus influenzae* type B meningitis. *J Pediatr* 1981;**99**:591–3.
107. Kaufmann RL, Matson CF, Beisel WR. Hypertriglyceridemia produced by endotoxin: role of impaired triglyceride disposal mechanisms. *J Infect Dis* 1976;**133**:548–55.
108. Khemani RG, Bart RD, Alonzo TA, et al. Disseminated intravascular coagulation score is associated with mortality for children with shock. *Intensive Care Med* 2009;**35**:327–33.
109. Kissoon N, Orr RA, Carcillo JA. Updated American College of Critical Care Medicine—pediatric advanced life support guidelines for management of pediatric and neonatal septic shock: relevance to the emergency care clinician. *Pediatr Emerg Care* 2010;**26**:867–9.
110. Kleiman MB, Reynolds JK, Schreiner RL, et al. Rapid diagnosis of neonatal bacteremia with acridine orange–stained buffy coat smears. *J Pediatr* 1984;**105**:419–21.
111. Korvick JA, Bryan CS, Farber B, et al. Prospective observational study of *Klebsiella* bacteremia in 230 patients: outcome for antibiotic combinations versus monotherapy. *Antimicrob Agents Chemother* 1992;**36**:2639–44.
112. Kumar A, Thota V, Dee L, et al. Tumor necrosis factor alpha and interleukin 1beta are responsible for in vitro myocardial cell depression induced by human septic shock serum. *J Exp Med* 1996;**183**:949–58.
113. Lacour AG, Gervaix A, Zamora SA, et al. Procalcitonin, IL-6, IL-8, IL-1 receptor antagonist and C-reactive protein as identificators of serious bacterial infections in children with fever without localising signs. *Eur J Pediatr* 2001;**160**:95–100.
114. Larsen GY, Mecham N, Greenberg R. An emergency department septic shock protocol and care guideline for children initiated at triage. *Pediatrics* 2011;**127**:e1585–92.
115. Laupland KB, Gregson DB, Vanderkooi OG, et al. The changing burden of pediatric bloodstream infections in Calgary, Canada, 2000-2006. *Pediatr Infect Dis J* 2009;**28**:114–7.
116. Lee JM, Hashmi N, Bloom JD, et al. Biofilm accumulation on endotracheal tubes following prolonged intubation. *J Laryngol Otol* 2012;**126**:267–70.
117. Levi M, ten Cate H, van der Poll T, van Deventer SJ. Pathogenesis of disseminated intravascular coagulation in sepsis. *JAMA* 1993; **270**:975–9.
118. Levin J, Poore TE, Young NS, et al. Gram-negative sepsis: detection of endotoxemia with the limulus test: with studies of associated changes in blood coagulation, serum lipids, and complement. *Ann Intern Med* 1972;**76**:1–7.
119. Lorente JA, Garcia-Frade LJ, Landin L, et al. Time course of hemostatic abnormalities in sepsis and its relation to outcome. *Chest* 1993;**103**:1536–42.
120. Mallet E, Lanse X, Devaux AM, et al. Hypercalcitoninaemia in fulminant meningococcaemia in children. *Lancet* 1983;**1**:294.
121. Marshall R, Teele DW, Klein JO. Unsuspected bacteremia due to *Haemophilus influenzae:* outcome in children not initially admitted to hospital. *J Pediatr* 1979;**95**:690–5.
122. Mason JW, Kleeberg U, Dolan P, Colman RW. Plasma kallikrein and Hageman factor in gram-negative bacteremia. *Ann Intern Med* 1970;**73**:545–51.
123. Matuschak GM, Rinaldo JE. Organ interactions in the adult respiratory distress syndrome during sepsis: role of the liver in host defense. *Chest* 1988;**94**:400–6.
124. McCabe WR. Serum complement levels in bacteremia due to gram-negative organisms. *N Engl J Med* 1973;**288**:21–3.
125. McCarthy PL. Controversies in pediatrics: what tests are indicated for the child under 2 with fever? *Pediatr Rev* 1979;**1**:51–6.
126. McCartney AC, Banks JG, Clements GB, et al. Endotoxaemia in septic shock: clinical and post mortem correlations. *Intensive Care Med* 1983;**9**:117–22.
127. McCloskey RV, Straube RC, Sanders C, et al. Treatment of septic shock with human monoclonal antibody HA-1A: a randomized, double-blind, placebo-controlled trial. CHESS Trial Study Group. *Ann Intern Med* 1994;**121**:1–5.
128. McConn R, Greineder JK, Wasserman F, Clowes GH Jr. Is there a humoral factor that depresses ventricular function in sepsis? *Circ Shock Suppl* 1979;**1**:9–22.
129. McGowan KL, Foster JA, Coffin SE. Outpatient pediatric blood cultures: time to positivity. *Pediatrics* 2000;**106**:251–5.
130. Meyer J, Traber LD, Nelson S, et al. Reversal of hyperdynamic response to continuous endotoxin administration by inhibition of NO synthesis. *J Appl Physiol* 1992;**73**:324–8.
131. Michaels RH, Myerowitz RL, Klaw R. Potentiation of experimental meningitis due to *Haemophilus influenzae* by influenza A virus. *J Infect Dis* 1977;**135**:641–5.
132. Michie HR, Manogue KR, Spriggs DR, et al. Detection of circulating tumor necrosis factor after endotoxin administration. *N Engl J Med* 1988;**318**:1481–6.
133. Miller MR, Griswold M, Harris JM 2nd, et al. Decreasing PICU catheter–associated bloodstream infections: NACHRI's quality transformation efforts. *Pediatrics* 2010;**125**:206–13.
134. Miller RL, Reichgott MJ, Melmon KL. Biochemical mechanisms of generation of bradykinin by endotoxin. *J Infect Dis* 1973; **128**(Suppl):144–56.
135. Miller SI, Wallace RJ Jr, Musher DM, et al. Hypoglycemia as a manifestation of sepsis. *Am J Med* 1980;**68**:649–54.
136. Mizock BA. Metabolic derangements in sepsis and septic shock. *Crit Care Clin* 2000;**16**:319–36, vii.
137. Mizock BA, Sabelli HC, Dubin A, et al. Septic encephalopathy: evidence for altered phenylalanine metabolism and comparison with hepatic encephalopathy. *Arch Intern Med* 1990; **150**:443–9.
138. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. *N Engl J Med* 1993;**329**:2002–12.
139. Moore RD, Smith CR, Lietman PS. The association of aminoglycoside plasma levels with mortality in patients with gram-negative bacteremia. *J Infect Dis* 1984;**199**:443–8.
140. Mora M, Bensi G, Capo S, et al. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. *Proc Natl Acad Sci U S A* 2005;**102**:15641–6.
141. Munford RS, Hall CL. Detoxification of bacterial lipopolysaccharides (endotoxins) by a human neutrophil enzyme. *Science* 1986;**234**:203–5.
142. Musher DM. Cutaneous and soft-tissue manifestations of sepsis due to gram-negative enteric bacilli. *Rev Infect Dis* 1980;**2**: 854–66.
143. Myers MG, Wright PF, Smith AL, Smith DH. Complications of occult pneumococcal bacteremia in children. *J Pediatr* 1974; **84**:656–60.
144. Naqvi SH, Chundu KR, Friedman AD. Shock in children with gram-negative bacillary sepsis and *Haemophilus influenzae* type b sepsis. *Pediatr Infect Dis* 1986;**5**:512–5.

145. Natanson C, Fink MP, Ballantyne HK, et al. Gram-negative bacteremia produces both severe systolic and diastolic cardiac dysfunction in a canine model that simulates human septic shock. *J Clin Invest* 1986;**78**:259–70.
146. National Center for Health Statistics. Increase in national hospital discharge survey rates for septicemia: United States, 1979-1987. *MMWR Morb Mortal Wkly Rep* 1990;**39**:31–4.
147. Nishijima H, Weil MH, Shubin H, Cavanilles J. Hemodynamic and metabolic studies on shock associated with gram negative bacteremia. *Medicine (Baltimore)* 1973;**52**:287–94.
148. Ognibene FP, Martin SE, Parker MM, et al. Adult respiratory distress syndrome in patients with severe neutropenia. *N Engl J Med* 1986;**315**:547–51.
149. Ognibene FP, Parker MM, Natanson C, et al. Depressed left ventricular performance: response to volume infusion in patients with sepsis and septic shock. *Chest* 1988;**93**:903–10.
150. Opal S, Laterre PF, Abraham E, et al. Recombinant human platelet-activating factor acetylhydrolase for treatment of severe sepsis: results of a phase III, multicenter, randomized, double-blind, placebo-controlled, clinical trial. *Crit Care Med* 2004; **32**:332–41.
151. Panas D, Khadour FH, Szabo C, Schulz R. Proinflammatory cytokines depress cardiac efficiency by a nitric oxide–dependent mechanism. *Am J Physiol* 1998;**275**:H1016–23.
152. Parker MM, Parrillo JE. Septic shock: hemodynamics and pathogenesis. *JAMA* 1983;**250**:3324–7.
153. Parker MM, Shelhamer JH, Bacharach SL, et al. Profound but reversible myocardial depression in patients with septic shock. *Ann Intern Med* 1984;**100**:483–90.
154. Parrillo JE, Burch C, Shelhamer JH, et al. A circulating myocardial depressant substance in humans with septic shock: septic shock patients with a reduced ejection fraction have a circulating factor that depresses in vitro myocardial cell performance. *J Clin Invest* 1985;**76**:1539–53.
155. Patel GP, Balk RA. Systemic steroids in severe sepsis and septic shock. *Am J Respir Crit Care Med* 2012;**185**:133–9.
156. Paterson DL, Yu VL. Extended-spectrum beta-lactamases: a call for improved detection and control. *Clin Infect Dis* 1999;**29**: 1419–22.
157. Patrick CC. Coagulase-negative staphylococci: pathogens with increasing clinical significance. *J Pediatr* 1990;**116**:497–507.
158. Patterson DL, Ko WC, Gottberg AV, et al. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical laboratory. *J Clin Microbiol* 2001;**39**: 2206–12.
159. Paul M, Leibovici L. Combination antimicrobial treatment versus monotherapy: the contribution of meta-analyses. *Infect Dis Clin North Am* 2009;**23**:277–93.
160. Peters G, Locci R, Pulverer G. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. *J Infect Dis* 1982;**146**:479–82.
161. Pfaller MA, Jones RN, Marshall SA, et al. Inducible amp C beta-lactamase producing gram-negative bacilli from blood stream infections: frequency, antimicrobial susceptibility, and molecular epidemiology in a national surveillance program (SCOPE). *Diagn Microbiol Infect Dis* 1997;**28**:211–9.
162. Pfenninger J, Gerber A, Tschappeler H, Zimmermann A. Adult respiratory distress syndrome in children. *J Pediatr* 1982; **101**:352–7.
163. Pilishvili T, Lexau C, Farley MM, et al. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.
164. Plotz FB, Hulst HE, Twisk JW, et al. Effect of acute renal failure on outcome in children with severe septic shock. *Pediatr Nephrol* 2005;**20**:1177–81.
165. Proulx F, Fayon M, Farrell CA, et al. Epidemiology of sepsis and multiple organ dysfunction syndrome in children. *Chest* 1996; **109**:1033–7.
166. Raymond RM, Emerson TE, Jr. Cerebral metabolism during endotoxin shock in the dog. *Circ Shock* 1978;**5**:407–14.
167. Reichgott MJ, Melmon KL, Forsyth RP, Greineder D. Cardiovascular and metabolic effects of whole or fractionated gram-negative bacterial endotoxin in the unanesthetized Rhesus monkey. *Circ Res* 1973;**33**:346–52.
168. Reilly AF, Lange BJ. Infections with viridans group streptococci in children with cancer. *Pediatr Blood Cancer* 2007;**49**:774–80.
169. Reilly JM, Cunnion RF, Burch-Whitman C, et al. A circulating myocardial depressant substance is associated with cardiac dysfunction and peripheral hypoperfusion (lactic acidemia) in patients with septic shock. *Chest* 1989;**95**:1072–80.
170. Reimer SL, Michener WM, Steiger E. Nutritional support of the critically ill child. *Pediatr Clin North Am* 1980;**27**:647–60.
171. Reines HD, Halushka PV, Cook JA, et al. Plasma thromboxane concentrations are raised in patients dying with septic shock. *Lancet* 1982;**2**:174–5.
172. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in pediatric intensive care units in the United States. National Nosocomial Infections Surveillance System. *Pediatrics* 1999;**103**:e39.
173. Richmand DA, Molitch ME, O'Donnell TF. Altered thyroid hormone levels in bacterial sepsis: the role of nutritional adequacy. *Metabolism* 1980;**29**:936–42.
174. Robbins RA, Russ WD, Rasmussen JK, Clayton MM. Activation of the complement system in the adult respiratory distress syndrome. *Am Rev Respir Dis* 1987;**135**:651–8.
175. Rongkavilit C, Rodriguez ZM, Gomez-Marin O, et al. Gram-negative bacillary bacteremia in human immunodeficiency virus type 1-infected children. *Pediatr Infect Dis J* 1998;**19**:122–8.
176. Royall JA, Levin DL. Adult respiratory distress syndrome in pediatric patients, part II: management. *J Pediatr* 1988;**112**: 335–47.
177. Rubin LG, Moxon ER. Pathogenesis of bloodstream invasion with *Haemophilus influenzae* type b. *Infect Immun* 1983;**41**:280–4.
178. Russell JA. Management of sepsis. *N Engl J Med* 2006;**355**: 1699–713.
179. Sanders CC. Cefepime: the next generation? *Clin Infect Dis* 1993;**17**:369–79.
180. Sard B, Bailey MC, Vinci R. An analysis of pediatric blood cultures in the postpneumococcal conjugate vaccine era in a community hospital emergency department. *Pediatr Emerg Care* 2006;**22**: 295–300.
181. Schmyer JP, Reynolds DG, Swan KG. Renal blood flow during endotoxin shock in the subhuman primate. *Surg Gynecol Obstet* 1973;**137**:3–6.
182. Shanley TP, Cvijanovich N, Lin R, et al. Genome-level longitudinal expression of signaling pathways and gene networks in pediatric septic shock. *Mol Med* 2007;**13**:495–508.
183. Shlaes DM, Bass SN. Combination antimicrobial therapy. *Pediatr Clin North Am* 1983;**30**:121–34.
184. Shulman ST, Ayoub EM. Severe staphylococcal sepsis in adolescents. *Pediatrics* 1976;**58**:59–66.
185. Smith AL. Commentary: the febrile infant. *Pediatr Rev* 1979;**1**: 35–6.
186. Sprung CL, Caralis PV, Marcial EH, et al. The effects of high-dose corticosteroids in patients with septic shock. A prospective, controlled study. *N Engl J Med* 1984;**311**:1137–43.
187. Sriskandan S, Cohen J. Gram-positive sepsis: mechanisms and differences from gram-negative sepsis. *Infect Dis Clin North Am* 1999;**13**:392–412.
188. Standage SW, Wong HR. Biomarkers for pediatric sepsis and septic shock. *Expert Rev Anti Infect Ther* 2011;**9**:71–9.
189. Stephens DS, Hoffman LH, McGee ZA. Interaction of *Neisseria meningitidis* with human nasopharyngeal mucosa: attachment and entry into columnar epithelial cells. *J Infect Dis* 1983;**148**: 369–76.
190. Stevens DL, Ma Y, Salmi DB, et al. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2007;**195**:202–11.
191. Stoll ML, Rubin LG. Incidence of occult bacteremia among highly febrile young children in the era of the pneumococcal conjugate vaccine: a study from a Children's Hospital Emergency Department and Urgent Care Center. *Arch Pediatr Adolesc Med* 2004;**158**:671–5.
192. Stumacher RJ, Kovnat MJ, McCabe WR. Limitations of the usefulness of the limulus assay for endotoxin. *N Engl J Med* 1973;**288**:1261–4.
193. Sullivan JS, Kilpatrick L, Costarino AT Jr, et al. Correlation of plasma cytokine elevations with mortality rate in children with sepsis. *J Pediatr* 1992;**120**:510–5.

194. Thiru Y, Pathan N, Bignall S, et al. A myocardial cytotoxic process is involved in the cardiac dysfunction of meningococcal septic shock. *Crit Care Med* 2000;**28**:2979–83.
195. Tidswell M, Tillis W, Larosa SP, et al. Phase 2 trial of eritoran tetrasodium (E5564), a toll-like receptor 4 antagonist, in patients with severe sepsis. *Crit Care Med* 2010;**38**:72–83.
196. Togari H, Sugiyama S, Ogino T, et al. Interactions of endotoxin with cortisol and acute phase proteins in septic shock neonates. *Acta Paediatr Scand* 1986;**75**:69–74.
197. Toltzis P, Blumer JL. Antibiotic-resistant gram-negative bacteria in the critical care setting. *Pediatr Clin North Am* 1995;**42**: 687–702.
198. Tracey KJ, Beutler B, Lowry SF, et al. Shock and tissue injury induced by recombinant human cachectin. *Science* 1986;**234**: 470–4.
199. Tracey KJ, Cerami A. Tumor necrosis factor: an updated review of its biology. *Crit Care Med* 1993;**21**:5415–22.
200. Tracey KJ, Lowry SF, Cerami A. Cachectin: a hormone that triggers acute shock and chronic cachexia. *J Infect Dis* 1988;**157**: 413–20.
201. Tracey KJ, Vlassara H, Cerami A. Cachectin/tumour necrosis factor. *Lancet* 1989;**1**:1122–6.
202. Triger DR, Boyer TD, Levin J. Portal and systemic bacteraemia and endotoxaemia in liver disease. *Gut* 1978;**19**:935–9.
203. Tubbs HR. Endotoxin in meningococcal infections. *Arch Dis Child* 1980;**55**:808–10.
204. Tuomanen EI. The biology of pneumococcal infection. *Pediatr Res* 1997;**42**:253–8.
205. van der Poll T, Buller HR, ten Cate H, et al. Activation of coagulation after administration of tumor necrosis factor to normal subjects. *N Engl J Med* 1990;**322**:1622–7.
206. van Deuren M, Santman FW, van Dalen R, et al. Plasma and whole blood exchange in meningococcal sepsis. *Clin Infect Dis* 1992;**15**:424–30.
207. van Deuren M, van der Ven-Jongekrijg J, Bartelink AK, et al. Correlation between proinflammatory cytokines and antiinflammatory mediators and the severity of disease in meningococcal infections. *J Infect Dis* 1995;**172**:433–9.
208. van Deuren M, van der Ven-Jongekrijg J, Demacker PN, et al. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. *J Infect Dis* 1994;**169**:157–61.
209. van Deventer SJ, Buller HR, ten Cate JW, et al. Endotoxaemia: an early predictor of septicaemia in febrile patients. *Lancet* 1988;**1**: 605–9.
210. van Langevelde P, Joop K, van Loon J, et al. Endotoxin, cytokines, and procalcitonin in febrile patients admitted to the hospital: identification of subjects at high risk of mortality. *Clin Infect Dis* 2000;**31**:1343–8.
211. Van Nguyen Q, Nguyen EA, Weiner LB. Incidence of invasive bacterial disease in children with fever and petechiae. *Pediatrics* 1984;**74**:77–80.
212. van Schaik SM. Do pediatric patients with septic shock benefit from steroid therapy? A critical appraisal of "low-dose hydrocortisone improves shock reversal and reduces cytokine levels in early hyperdynamic septic shock" by Oppert et al. (***Crit Care Med*** 2005; 33:2457-64). *Pediatr Crit Care Med* 2007;**8**:174–6.
213. van Waardenburg DA, Jansen TC, Vos GD, Buurman WA. Hyperglycemia in children with meningococcal sepsis and septic shock: the relation between plasma levels of insulin and inflammatory mediators. *J Clin Endocrinol Metab* 2006;**91**:3916–21.
214. Verhoeven JJ, den Brinker M, Hokken-Koelega AC, et al. Pathophysiological aspects of hyperglycemia in children with meningococcal sepsis and septic shock: a prospective, observational cohort study. *Crit Care* 2011;**15**:R44.
215. Voerman HJ, Stehouwer CD, van Kamp GJ, et al. Plasma endothelin levels are increased during septic shock. *Crit Care Med* 1992;**20**:1097–101.
216. Waage A, Brandtzaeg P, Halstensen A, et al. The complex pattern of cytokines in serum from patients with meningococcal septic shock. Association between interleukin 6, interleukin 1, and fatal outcome. *J Exp Med* 1989;**169**:333–8.
217. Wajchenberg B, Leme CE, Tambascia M, et al. The adrenal response to exogenous adrenocorticotrophin in patients with infections due to *Neisseria meningitidis*. *J Infect Dis* 1978;**138**: 387–91.
218. Wardle EN. Endotoxin and acute renal failure. *Nephron* 1975;**14**:321–32.
219. Wardle N. Acute renal failure in the 1980s: the importance of septic shock and of endotoxaemia. *Nephron* 1982;**30**:193–200.
220. Warren BL, Eid A, Singer P, et al. High-dose antithrombin III in severe sepsis: a randomized controlled trial. *JAMA* 2000;**286**: 1869–78.
221. Watson RS, Carcillo JA, Linde-Zwirble WT, et al. The epidemiology of severe sepsis in children in the United States. *Am J Respir Crit Care Med* 2003;**167**:695–701.
222. Watson RW, Redmond HP, Bouchier-Hayes D. Role of endotoxin in mononuclear phagocyte-mediated inflammatory responses. *J Leukoc Biol* 1994;**56**:95–103.
223. Weems JJ Jr, Steinberg JP, Filler S, et al. Phase II, randomized, double-blind, multicenter study comparing the safety and pharmacokinetics of tefibazumab to placebo for treatment of *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother* 2006; **50**:2751–5.
224. Weil MH, Nishjima H. Cardiac output in bacterial shock. *Am J Med* 1978;**64**:920–2.
225. Weiland JE, Davis WB, Holter JF, et al. Lung neutrophils in the adult respiratory distress syndrome: clinical and pathophysiologic significance. *Am Rev Respir Dis* 1986;**133**:218–25.
226. Wiesenthal AM, Todd JK. Toxic shock syndrome in children aged 10 years or less. *Pediatrics* 1984;**74**:112–7.
227. Wilson MF, Brackett DJ, Hinshaw LB, et al. Vasopressin release during sepsis and septic shock in baboons and dogs. *Surg Gynecol Obstet* 1981;**153**:869–72.
228. Wong HR, Cvijanovich N, Allen GL, et al. Genomic expression profiling across the pediatric systemic inflammatory response syndrome, sepsis, and septic shock spectrum. *Crit Care Med* 2009;**37**:1558–66.
229. Wong HR, Shanley TP, Sakthivel B, et al. Genome-level expression profiles in pediatric septic shock indicate a role for altered zinc homeostasis in poor outcome. *Physiol Genomics* 2007;**30**: 146–55.
230. Wong HR, Wheeler DS, Tegtmeyer K, et al. Toward a clinically feasible gene expression-based subclassification strategy for septic shock: proof of concept. *Crit Care Med* 2010;**38**:1955–61.
231. Woo P, Carpenter MA, Trunkey D. Ionized calcium: the effect of septic shock in the human. *J Surg Res* 1979;**26**:605–10.
232. Woods DE, Straus DC, Johanson WG Jr, Bass JA. Role of salivary protease activity in adherence of gram-negative bacilli to mammalian buccal epithelial cells in vivo. *J Clin Invest* 1981;**68**: 1435–40.
233. Wright SD, Ramos RA, Tobias PS, et al. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 1990;**249**:1431–3.
234. Zaloga GP, Chernow B. The multifactorial basis for hypocalcemia during sepsis: studies of the parathyroid hormone-vitamin D axis. *Ann Intern Med* 1987;**107**:36–41.
235. Zanotti-Cavazzoni SL, Goldfarb RD. Animal models of sepsis. *Crit Care Clin* 2009;**25**:703–19, vii-viii.

CHAPTER 63

Fever Without Source and Fever of Unknown Origin

Debra L. Palazzi

Petersdorf and Beeson[81] in 1961 proposed that the term *fever of unknown origin* (FUO) be reserved for persons with an illness persisting for 3 or more weeks and accompanied by temperatures higher than 38.4° C (101.2° F) on at least several occasions. They further specified that the cause of the fever should remain undetermined after at least 1 week of investigation in the hospital. Although this definition was arbitrary, it was useful at that time, when many of the diagnostic tests now in routine use were unknown. The purpose of their precise definition was to explore the cause of fever in this select group of adult patients and to permit comparison of data from different investigations. This exacting definition probably never was applied rigorously in pediatric practice. In children, the term *fever of unknown origin* should be reserved for fever of at least 8 days' duration and for which no diagnosis is apparent after the initial workup in the hospital or as an outpatient.

Many investigators prefer using the term *fever without source* (FWS) for fever of recent onset with no adequate explanation determined by the history or physical examination. The distinction between FUO and FWS is of more than academic interest for several reasons. First, although overlap exists, the differential diagnoses of these clinical conditions are distinct, and the most frequent causes of one can be different from the most frequent causes of the other. Second, a child with fever of recent onset generally warrants more immediate evaluation than does a child with FUO. The latter usually does not occur as an emergency and requires timely, but not urgent, diagnostic or therapeutic intervention. Third, although expectant antibiotic treatment of children with FUO generally is not indicated, expectant treatment of infants with FWS is recommended in most cases.

FEVER WITHOUT SOURCE

A convenient definition of FWS is the occurrence of fever for 1 week or less in a child in whom a careful history and physical examination fail to reveal a probable cause of the fever. An estimated 14 to 40 percent of children with fever have no localizing signs or symptoms.[9,13] Stein[93] found that the peak incidence occurs during the second year of life. On the basis of a review of private pediatric practices in upstate New York, Hoekelman and colleagues[46] predicted that every 4 to 5 days a practicing pediatrician would see one child between 1 and 24 months of age with FWS.

Most children with fever of recent onset have acute infectious diseases, the majority of which are self-limited.[108] A few of these patients have serious acute infectious diseases, including meningitis, bacteremia, and urinary tract infection, and a very few have acute noninfectious diseases or chronic disorders. For example, an occasional patient with FWS is discovered to have a disorder such as heat illness, drug poisoning, Kawasaki disease, malignancy, or connective tissue disease. However, these disorders occur infrequently. A physician faced with a child with FWS should consider the possibility of a noninfectious cause or the onset of a chronic disease, but unless a clinical clue suggests one of these entities, investigation in this direction is not warranted.

Many children with FWS are in the prodromal stages of an acute infectious illness, and evidence of a specific infection, such as pharyngitis, otitis media, or pneumonia, develops within hours to days of first being evaluated by a physician. Fever can precede the appearance of specific signs and symptoms by as long as 3 days, as in measles, Rocky Mountain spotted fever, and leptospirosis. In some infections, such as roseola, viral hepatitis, infectious mononucleosis, typhus, and typhoid fever, the interval between the onset of fever and the appearance of specific findings often is more than 3 days.

Occult Bacteremia

One major concern regarding a young child with FWS is the possibility that the child has occult bacteremia. The patient does not appear ill, is judged clinically well enough to be managed as an outpatient, and does not have an infection commonly associated with bacteremia, such as pneumonia, but the blood culture yields pathogenic bacteria such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Escherichia coli*, *Salmonella*, group A streptococcus, or *Staphylococcus aureus*. Before introduction of the pneumococcal conjugate vaccines, the incidence of occult bacteremia in children with FWS was approximately 3 to 5 percent.* More recent data report an incidence of less than 1 percent, although this figure may vary depending on vaccine coverage rates in the population studied.† A large prospective study of 3571 febrile children younger than 3 years of age found that no patients who had received at least 1 vaccination of heptavalent pneumococcal vaccine had

*References 7, 16, 26, 49, 60, 65, 74, 97, 108.
†References 2, 3, 32, 45, 84, 89, 95, 101, 106.

pneumococcal bacteremia.[27] With the introduction of the 13-valent pneumococcal conjugate vaccine, further decreases in occult bacteremia caused by pneumococci are anticipated.[28]

Historically, occult bacteremia has been found to occur more commonly in children with FWS than in febrile children of the same age with infections such as pharyngitis, otitis media, or upper respiratory tract infection. In the 1970s, investigators[65] reported the incidence of bacteremia in febrile children without an obvious source of infection to be 9.9 percent, as opposed to 3.3 percent in children with otitis media, upper respiratory tract infection, or a flulike syndrome. During the same era, Teele and colleagues[97] found a 3.9 percent incidence of bacteremia in children with FWS and a 1.5 percent incidence in comparably febrile children with otitis media or pharyngitis.

The risk for occult bacteremia developing in a child with FWS is age-related, with the greatest risk occurring in the first few months of life. Numerous studies have demonstrated a higher risk for bacteremia or other serious bacterial infections in febrile infants younger than 3 months old.[62,65,88,97] In a group of children with clinically unsuspected meningococcemia, all 12 patients who initially looked well enough to be treated as outpatients were younger than 24 months of age.[35] Bonadio and colleagues[22] found the incidence of positive bacterial cultures to be 12 percent in febrile infants younger than 4 weeks and 6 percent in those between 4 and 8 weeks of age.

The risk for having occult bacteremia and other serious bacterial infections increases with the severity of fever. In a prospective study of bacteremia in children seen in the outpatient department, McCarthy and colleagues[63] identified a small, but statistically significant, difference in the incidence of bacteremia between children with temperatures of 40° C (104° F) or higher and those with temperatures of 40.5° C (104.9° F) or higher. In a prospective study in which blood was obtained for culture from all febrile children younger than 2 years old seen in a walk-in clinic, no positive blood cultures were found in 44 children with FWS and rectal temperatures lower than 38.9° C (102° F), whereas five (3.9%) positive blood cultures were found in 129 children with FWS and rectal temperatures of 38.9° C (102° F) or higher.[86,97] Other studies have reported similar findings.[16,62,89] Several series of children with fevers of 41° C (105.8° F) or higher found a relatively high prevalence of bacteremia and other serious blood infections, especially meningitis and pneumonia.[21,62,86] However, even in this group of children with very high fever, most of those older than 2 or 3 months of age who looked well did not have a serious bacterial infection.

The white blood cell (WBC) count has been studied extensively as a potential tool in the diagnosis of occult bacteremia. On the basis of a study of hospitalized children, Todd[98] reported that the absolute number of polymorphonuclear leukocytes and the absolute number of nonsegmented polymorphonuclear leukocytes were more sensitive than was the total WBC count, the percentage of polymorphonuclear leukocytes, or the percentage of nonsegmented polymorphonuclear leukocytes. However, whether information based on hospitalized children—presumably all of whom had serious localized infections or looked ill enough to warrant hospitalization—can be applied to children with FWS who look well enough to be treated on an ambulatory basis is questionable.

Considerable debate continues over the usefulness of the WBC count in febrile children evaluated in the outpatient setting. McCarthy and associates[63] concluded that a WBC count of 15,000/mm^3 or greater was helpful in identifying patients at greatest risk for the development of bacteremia. Dershewitz[36] found a direct relationship between the total leukocyte count and the prevalence of bacteremia and stated that "knowledge of the count was a helpful but limited predictor of patients with positive blood cultures." Other investigators reported that the incidence of bacteremia increased with an increased WBC count[89] and that bacteremia most commonly occurred in patients with counts of 20,000/mm^3 or higher.[70]

Other studies have examined the utility of the WBC count specifically in children with FWS who look well enough to be treated on an outpatient basis. One such study found a sensitivity of 1.0 and a positive predictive value of 0.11 for a total WBC count of 15,000/mm^3.[97] Using a white blood cell count of 20,000/mm^3 would have decreased the sensitivity to 0.4 while increasing the positive predictive value to only 0.13. In another series, the sensitivity for a WBC count of 15,000/mm^3 was 0.87, with a specificity of 0.73.[16] Kline and coworkers[54] found that a WBC count of 15,000/mm^3 was more sensitive for *S. pneumoniae* bacteremia than for *H. influenzae* bacteremia. Although a total WBC count of 15,000/mm^3 does not accurately predict which child is or is not bacteremic, it is helpful in dividing the population of children with FWS into high- and low-risk groups.[17,45] However, the value of the 15,000/mm^3 level has been substantially reduced in the studies conducted since the pneumococcal conjugate vaccine was introduced.

Some investigators have found the erythrocyte sedimentation rate to be no more useful than the WBC count in predicting bacteremia in ambulatory febrile patients.[63] Others have reported that the serum concentration of C-reactive protein may be more accurate than the complete blood count or erythrocyte sedimentation rate in distinguishing bacterial from viral infections.[64,80] However, recent studies reported conflicting data regarding the utility of the C-reactive protein test in screening children 3 to 36 months old for occult bacteremia.[47,87] An elevated serum procalcitonin level has been found by some investigators to be at least as sensitive and specific as is C-reactive protein in predicting serious bacterial infection in children with fever and no localizing signs.[5,55,85]

Other hematologic findings that suggest bacteremia include thrombocytopenia,[31] Döhle inclusion bodies, toxic granulations, and vacuolization of neutrophils. In one study, peripheral blood smears of children younger than 24 months old with acute febrile illnesses were reviewed the following day by a single investigator to determine whether vacuolization and toxic granulations were present; when both abnormalities were present, the positive predictive value for bacteremia was 0.76.[57] The

presence of these findings should be considered when estimating the risk for bacteremia.[1,31,73]

Several studies have examined the response to acetaminophen and found no difference in the rate of reduction of temperature or improvement in clinical appearance between bacteremic and nonbacteremic children.[11,99,110] Mazur and associates,[61] however, found that febrile children aged 2 months to 6 years old who did not respond to a dose of acetaminophen by a reduction in temperature of at least 0.8° C in 2 hours had a statistically significant increased risk for having occult bacteremia in contrast to those who did respond.

The most important aspects of assessment of a febrile child are a careful history and physical examination. Laboratory data are secondary and should be ordered on the basis of the clinical assessment. By definition, a child with FWS has no localizing signs to explain the fever or indicate a site of infection. Many physicians suggest that a general impression can indicate whether the child has occult bacteremia. Some physicians have suggested that careful clinical judgment, based on extensive experience, can identify most, if not all, children with serious illnesses.[20,78] McCarthy and colleagues,[65-68] in a series of carefully designed studies, elucidated the variables of history and observation that were most useful in assessing febrile children. They found that observation of the variable *playfulness* had the strongest correlation with overall assessment.[67] However, they observed that even an experienced attending pediatrician could identify only 57 percent of seriously ill children by initial impression before performing a full physical examination. Dershewitz[36] found that private pediatricians were no more accurate than pediatric residents in identifying children with occult bacteremia and that in the private office, pediatricians were no better at predicting bacteremia in familiar patients than in first-time patients.

In a study of 292 consecutive febrile children seen in an emergency department, Waskerwitz and Berkelhammer[103] identified a subgroup of patients who had no localizing signs and who looked so well that they were predicted not to have bacteremia. The physicians were assisted in their assessment by a functional scale that gave 0 to 2 points for the child's eating, drinking, sleeping, and play activities, with a best possible score of 8. The group of patients who had functional scores of 5 or greater, with no localized infection and predicted clinically not to have bacteremia, were free of bacteremia, whereas 14 of 202 patients with functional scores of 4 or less were bacteremic. In this study, the physicians were not able to identify which patients had bacteremia and which did not; rather, they were able to identify one subgroup at high risk for having bacteremia and another at very low risk. Teach and Fleisher[96] found that although Yale Observation Scale scores were higher in patients with bacteremia than in those without, the difference was not clinically useful in detecting bacteremia in well-looking febrile children without a discernible focus of infection. The clinician's overall assessment of the degree of illness of the child appears to be a valuable, but not infallible, tool in estimating the risk for occult bacteremia in children with FWS.

Clinical Management of Fever Without Source

The clinician should not be dogmatic about the management of children with FWS. One reasonable approach, based on a careful history, thorough physical examination, and overall clinical impression, is to classify these children as being at low or high risk for the presence of occult bacteremia and other serious bacterial infections. For the low-risk group, no laboratory investigation is required routinely. For the high-risk group, a complete blood count and blood culture should be obtained, especially in young infants and children who are incompletely immunized. Many studies have shown that the most common serious bacterial infection in children with FWS is urinary tract infection.[4,101,104] Therefore, urinalysis and urine culture should be performed in febrile infants and children younger than 24 months of age. Lumbar puncture and chest radiography are considered on an individual basis. If the patient appears ill, admission to the hospital may be justified, even if all test results are negative. When high-risk children look well enough to be sent home, they are reasonable candidates for expectant antibiotic therapy, pending the outcome of blood and urine cultures. For patients clinically considered to be at moderate risk (not clearly high or low risk), the physician has the option of obtaining a WBC count and using the results to decide whether to obtain blood and urine for culture and prescribe antibiotics expectantly.

Table 63-1 lists risk factors for the development of occult bacteremia. Current information is not sufficient to warrant the use of scoring systems except as part of investigational series. In the final analysis, the clinician's judgment, taking into account all available clinical and laboratory data about each patient, is the guide to selecting which children require a diagnostic workup and expectant therapy with antibiotics.

If the physician elects to prescribe antibiotics while awaiting the results of blood culture, such antibiotic therapy should provide adequate coverage for *S. pneumoniae*, *N. meningitidis*, and *H. influenzae*, although the frequency of *H. influenzae* and *S. pneumoniae* has decreased dramatically with current immunization practice and *N. meningitidis* is uncommon. A single injection of ceftriaxone 50 to 75 mg/kg given while awaiting the results of blood culture has been successful in resolving fever, clearing bacteremia, and preventing meningitis and was found to be superior to oral regimens in several series.[14,18,41] Children with a positive blood culture should be recalled for reevaluation, even if they are afebrile.[8] Children who have been immunized against both *H. influenzae* and *S. pneumoniae* should be considered to be at relatively low risk for the development of occult bacteremia and may require less workup, unless they appear ill or have a very high fever.

Infants younger than 90 days old pose a special problem because they have an increased risk for developing a serious bacterial infection, clinical evaluation is more difficult, and a broader spectrum of invading organisms (e.g., group B streptococcus, *E. coli*, and *Listeria monocytogenes*) exists. Baker and coworkers[10] showed the safety of managing selected low-risk infants (i.e., normal WBC

TABLE 63-1 **Risk Factors for Occult Bacteremia**

Factor	High Risk	Low Risk
Age	≤3 mo	>3 mo
Immunization status*	Incomplete	Complete
Magnitude of fever	≥40° C (104° F)	≤39.4° C (103° F)
White blood cell count	≥15,000/mm^3	<15,000/mm^3
Peripheral blood smear	Toxic granulation or vacuolization of polymorphonuclear leukocytes, thrombocytopenia	Unremarkable
Underlying chronic disorder	Sickle-cell disease, immunodeficiency, malnutrition	None
History of contact with bacterial disease	Contact with *Neisseria meningitidis* or *Haemophilus influenzae*	None
Clinical appearance	Appears ill, toxic, or unhappy; inconsolable; irritable or lethargic; not eating or drinking enough	Looks well, playful, eating normally, not irritable

*Vaccination status is incomplete if the child has received fewer than 3 doses of *Haemophilus influenzae* vaccine and fewer than three doses of conjugated pneumococcal vaccine and is complete if the child received at least three doses of each vaccine.

count, urinalysis, lumbar puncture, and chest radiograph and, if diarrhea was present, negative smear for fecal leukocytes) 30 to 90 days of age on an outpatient basis without antibiotics. Jaskiewicz and coworkers[51] found the Rochester criteria (WBC count of 5000 to 15,000/mm^3, band count <1500/mm^3, spun urine specimen <10 WBCs/high-power field, stool specimen [if diarrhea] <5 WBCs/high-power field) to have a 98.9 percent negative predictive value in well-appearing, previously healthy infants younger than 90 days old with no focal infections. One reasonable practice guideline for managing infants with FWS is to hospitalize and treat all who appear toxic and all younger than 28 days. Those between 28 and 90 days of age can be managed as outpatients if they look well and the blood count, urinalysis, and cerebrospinal fluid analysis are within normal limits.[12,15]

FEVER OF UNKNOWN ORIGIN

The exact definition of FUO is a subject of considerable disagreement, and series in the pediatric literature differ in their criteria for inclusion. Brewis[23] defined FUO in children as a temperature of 38.3° C (101° F) or higher for 5 to 7 consecutive days without localizing signs or symptoms. In sharp contrast, McClung[69] and Lohr and Hendley[58] considered children with fever for at least 3 weeks on an outpatient basis or 1 week in the hospital to have FUO. Pizzo and associates,[83] however, required only that the fever be present for 2 weeks, with no distinction made between outpatient or in-hospital status. A reasonable working definition of FUO for clinical purposes is the presence of fever for 8 or more days in a child for whom a careful and thorough history, physical examination, and preliminary laboratory data fail to reveal a probable cause of the fever.

Most cases of FUO in children are caused by relatively common diseases.[39] In four series of FUO totaling 418 children, only 5 patients would be considered to have rare disorders (i.e., Behçet syndrome, ichthyosis, variant of "blue diaper" syndrome, diencephalic seizure disorder, and "possible chronic lead and/or arsenic intoxication").[23,58,69,83] The adage that FUO is more likely to be caused by an unusual manifestation of a common disorder than by a common manifestation of a rare disorder certainly is true in pediatrics. The three most common discernible causes of FUO in children, in order of decreasing frequency, are infectious diseases, connective tissue diseases, and neoplasms. In approximately 10 to 20 percent of cases, a definitive diagnosis never is established.

In the United States, the systemic infectious diseases diagnosed most frequently in children with FUO include tuberculosis, brucellosis, tularemia, salmonellosis, cat-scratch fever and infections caused by rickettsia, spirochetes (e.g., leptospirosis), Epstein-Barr virus, cytomegalic inclusion virus, human immunodeficiency virus (HIV), hepatitis viruses, and other viruses. The most common causes of localized infection are upper respiratory tract infections (e.g., sinusitis, otitis, tonsillitis), urinary tract infection, osteomyelitis, and occult abscesses, including hepatic and pelvic abscesses.

The connective tissue disease most commonly manifested as FUO in children is juvenile idiopathic arthritis, which accounts for more than 90 percent of connective tissue diseases in most series, followed by systemic lupus erythematosus, and then by undefined vasculitis.[58,69,72,83] Frequently, a definitive diagnosis of juvenile idiopathic arthritis can be made only after an extended period of observation because physical examination may yield few findings and the results of specific serologic studies generally are normal or negative.

Malignancy is a less frequent cause of FUO in children than in adults and usually is the third-largest group, after infectious diseases and connective tissue diseases. Malignancy accounted for 7 percent of FUO cases in the series reported by Pizzo and associates[83] and for 13 percent in the Lohr and Hendley[58] series. Leukemia and lymphoma are responsible for most cases of cancer manifested as FUO in children. Other tumors less commonly reported as causing FUO include neuroblastoma, hepatoma, sarcoma, and atrial myxoma.

The prognosis for children with FUO is better than that for adults, and most children with FUO have treatable or self-limited disease. Historically, mortality rates for children with FUO were 6 to 9 percent.[58,83] However, more contemporary studies indicate that mortality is quite low.[79]

Diagnostic Approach to a Child with Fever of Unknown Origin

A child with FUO is admitted to the hospital for more than simply laboratory investigation. Hospitalization provides an opportunity to observe the child, repeat the history and physical examination, analyze all available data, and investigate every potential diagnostic lead. In the Lohr and Hendley[58] series of 54 children with FUO, an incomplete history delayed establishing the diagnosis in 9 cases, and physical findings that were ignored delayed rendering the diagnosis in 4 cases. In McClung's report[69] of 99 pediatric cases of FUO, errors in the history or physical examination obscured the correct diagnosis for at least 10 patients. Failure to use existing laboratory data correctly is another common factor preventing early determination of the diagnosis in children with FUO.[58,83]

Clinical Evaluation

The first and most important step in the diagnostic workup of a child with FUO is obtaining a complete and detailed history and conducting a physical examination. The clinical evaluation must be thorough and careful, and it must be repeated frequently. Often, a patient or parent eventually recalls information that was omitted or forgotten when the initial history was obtained. Physical findings change, and abnormalities not originally present can appear subsequently. Lohr and Hendley[58] noted that in more than 25 percent of children admitted to the hospital with FUO, significant physical findings developed that were not present at the time of admission.

A detailed history should be obtained regarding contact with infected or otherwise ill persons and any exposure to animals, including pets and wild animals. The number of children with zoonotic infections is increasing each year. History of a cat scratch or exposure to kittens may be a clue for *Bartonella henselae*. Immunization of domestic animals such as the dog against leptospirosis can prevent canine disease, but it does not prevent carriage, excretion, and transmission of this infection. A history of travel extending back to birth must be elicited. Reemergence of histoplasmosis, coccidioidomycosis, blastomycosis, leishmaniasis, or malaria years after visiting or living in an endemic area can occur. Inquiring about prophylactic immunizations, precautions taken against the ingestion of contaminated food or water, and malarial prophylaxis is important. Questioning should include the possibility that rocks, soil, or artifacts from geographically distant regions may have been brought into the home, as well as the possibility that contact with persons who have visited distant countries has occurred. Even contact with insects can be important. Tick bites can be a clue to Rocky Mountain spotted fever or tick-borne relapsing fever. North American mosquitoes and some ticks carry a variety of arboviruses.

The physician should determine whether the patient has eaten game meat, raw meat, or raw shellfish. A history of pica should be sought routinely. Ingestion of dirt can suggest a diagnosis of visceral larva migrans, toxoplasmosis, or other infectious diseases. A detailed history regarding all medications, including topical agents and nonprescription items, must be elicited carefully. Any history of surgical procedures should be explored.

Questions designed to determine the genetic or ethnic background of the patient can reveal information that specifically suggests or largely excludes diagnoses such as nephrogenic diabetes insipidus (found in Ulster Scots), familial Mediterranean fever (found in Armenians, Arabs, and Sephardic Jews), familial dysautonomia (found in Jews), and Kikuchi-Fujimoto disease, a benign and self-limited histiocytic necrotizing lymphadenitis (found mostly in young Asian females and characterized by fever, lymphadenopathy, and malaise).

The history should be exacting regarding the duration, height, and pattern of the fever, as well as the circumstances under which temperature elevation occurs, whether the child appears ill or any signs or symptoms develop, and how well the fever responds to antipyretic drugs. A history of "fever" occurring only after exercise or late in the afternoon can indicate parental concern about normal variations in body temperature. A history of high fever occurring in the absence of malaise or other generalized signs can be a clue to factitious fever. The physician also should obtain a careful history regarding how well the fever has been documented. Has a thermometer been used, by whom, and in whose presence? A history of sweating and heat intolerance can indicate hyperthyroidism, whereas a history of heat intolerance with the absence of sweating can be a clue to ectodermal dysplasia.

Several investigators found that neither the pattern of fever nor its duration was useful in pointing to or establishing a diagnosis in children with FUO.[58,83] However, occasionally the character of the fever can be helpful. *Intermittent fever* is characterized by a return of temperature to normal at least once daily. If the peak of fever is high and the rate of defervescence quick, this pattern often is referred to as *hectic* or *spiking*. Intermittent fevers suggest pyogenic infections but also occur with tuberculosis, lymphoma, and juvenile idiopathic arthritis. In *remittent fever*, the temperature fluctuates but does not return to normal. A *sustained fever* pattern is characterized by persistent fever with little or no fluctuation and can occur in typhoid fever or typhus. Antipyretic agents can make a remittent or sustained fever appear intermittent. *Relapsing fever* refers to a pattern in which the patient is afebrile for 1 or more days between episodes of fever and can be seen with malaria, rat-bite fever, infection with *Borrelia*, and lymphoma. Recurrent episodes of fever of more than a year's duration can suggest metabolic defects, central nervous system abnormalities in temperature control, and immunodeficient states.

The general activity and appearance of the patient should be observed, vital signs checked, and growth parameters measured. Weight loss is an important, though nonspecific, finding. Impairment of linear growth or short stature can be a clue to inflammatory bowel disease, an intracranial lesion involving the pituitary gland, or a long-standing chronic disease. Examining the patient during an episode of fever to observe the presence or absence of sweating, the effect of the fever on the heart and respiratory rate, the presence or absence of malaise

or other symptoms, and the appearance of "toxicity" is helpful. The rash of juvenile idiopathic arthritis characteristically is evanescent and may be present only during periods of temperature elevation.

Some special aspects of the physical examination merit mention. Hypohidrosis, anomalous dentition, and sparse hair, particularly involving the eyebrows and eyelashes, suggest anhidrotic ectodermal dysplasia. Palpebral conjunctivitis can be a clue to the presence of infectious mononucleosis, Newcastle disease, or lupus erythematosus, whereas predominantly bulbar conjunctivitis can suggest leptospirosis or Kawasaki disease. Phlyctenular conjunctivitis can signal tuberculosis.

Absence of the pupillary constrictor response can be caused by a deficiency of the constrictor sphincter muscle of the eye. This muscle, derived from ectoderm rather than mesoderm, develops embryologically at the same time that hypothalamic structures and function are undergoing differentiation. Absence of this muscle can suggest that the elevation in temperature is the result of hypothalamic or autonomic dysfunction. Careful funduscopic examination can disclose evidence of miliary tuberculosis, vasculitis, or toxoplasmosis. Lack of tears, absence of corneal reflexes, and a smooth tongue with absence of the fungiform papillae suggest familial dysautonomia.

Purulent or persistent nasal discharge can be a sign of sinusitis. The physician should palpate for tenderness over the sinuses.

Hyperemia of the pharynx, even in the absence of exudate or specific symptoms, can be a clue to the diagnosis of infectious mononucleosis, cytomegalic inclusion disease, toxoplasmosis, tularemia, or leptospirosis. Gingival hypertrophy or inflammation and loosening or loss of teeth can indicate leukemia or Langerhans cell histiocytosis.

The bones and muscles should be palpated carefully. Tenderness over a bone can be found in cases of osteomyelitis or marrow invasion by neoplastic disease. The appreciation of a new heart murmur may result from infective endocarditis. Muscle tenderness can be associated with trichinosis, dermatomyositis, polyarteritis, or various arboviral infections.

The search for skin lesions and rash must be careful, extensive, and repeated. Petechiae can indicate endocarditis or other sources of bacteremia but also can occur with viral and rickettsial infections. A seborrheic rash can be a sign of histiocytosis. A small papule present for over a week may be the inoculation site for cat-scratch disease.

A careful rectal examination can reveal pararectal tenderness or a mass indicative of a pelvic abscess or tumor. A test for occult blood should be performed on stool. Examination of the external genitalia should be completed on patients of all ages, and sexually active adolescent females should undergo a pelvic examination.

Laboratory Evaluation

The extent of laboratory investigation depends on the age of the patient, duration of the fever, and history and physical examination findings. Laboratory studies should be directed, as much as possible, toward the most likely diagnostic possibilities. The tempo of the diagnostic evaluation should be adjusted to the severity of the illness. In a critically ill child, speedy evaluation is important. If the patient is less severely ill, however, the evaluation can proceed more slowly; sometimes the fever can disappear without apparent explanation before a definitive diagnosis can be established and any invasive diagnostic procedures have been undertaken.

A complete blood count and careful examination of the peripheral smear are indicated for all patients. Anemia, thrombocytosis, and thrombocytopenia should be noted. Although mild or moderate changes in the total WBC or differential count usually are of no help, in some series, children with more than 10,000 polymorphonuclear leukocytes or 500 nonsegmented neutrophils/mm^3 were found to have a greater likelihood of having a serious bacterial infection.[93,98] Atypical lymphocytes generally indicate viral infection, whereas bizarre or immature forms can suggest leukemia. Although the erythrocyte sedimentation rate and C-reactive protein are of no specific diagnostic value, they are a general indicator of inflammation and can help in ruling out factitious fever, determining the need for further evaluation, and monitoring the progress of the disease process.

Blood should be obtained from all patients for aerobic and anaerobic culture. In select cases, media appropriate for the isolation of *Francisella* organisms, *Leptospira*, and *Spirillum* also should be used.

Urine analysis and culture should be completed for all patients. In one series of FUO in children, failure to perform urinalysis and failure to investigate pyuria adequately were the most common laboratory errors.[69] Radiographic study of the urinary tract, however, should be performed only when indicated.

All patients should undergo radiographic examination of the chest. Diagnostic imaging of the nasal sinuses, mastoids, and gastrointestinal tract is performed initially only for specific indications but should be done eventually in all children whose fever persists without explanation for a long period. Persistent fever and elevation of the erythrocyte sedimentation rate or C-reactive protein, with or without anemia, abdominal complaints, anorexia, and weight loss, are sufficient indications for radiographic study to rule out inflammatory bowel disease.

All patients should have an intradermal tuberculin skin test. Control skin tests with antigens such as *Candida* are of limited value because the anergy may be specific for tuberculosis rather than universal for all skin-testing materials.[59,71,75,76] A positive control test result and negative tuberculin test result do not rule out tuberculosis.

Bone marrow examination is most useful in diagnosing cancer (especially leukemia), histiocytic disorders, and hemophagocytic disease. It is less useful in determining infection. Hayani and associates[44] reviewed the results of 414 bone marrow examinations for FUO in children. In only one case was an organism (*Salmonella* group D) recovered from the marrow that also was not recovered from blood or another source. Noninfectious causes of FUO were found in 8 percent of specimens: malignancy (6.7%), hemophagocytic lymphohistiocytosis (0.7%), histiocytosis (0.5%), and hypoplastic anemia (0.2%). In

most of these cases, the diagnosis had been suspected clinically before the bone marrow was examined.

Patients should undergo a serum test for HIV infection. Other appropriate serologic tests can help establish a diagnosis of cat-scratch disease, brucellosis, tularemia, Epstein-Barr virus infection, cytomegalovirus infection, other viral infections, toxoplasmosis, and certain fungal infections.

Hepatic enzymes and serum chemistry, including electrolytes, urea nitrogen, and creatinine, should be determined in all patients. Serum antinuclear antibody should be measured in those older than 5 years if family history is significant or clinical suspicion warrants it. Serum hepatitis antigens, electrocardiography, electroencephalography, echocardiography, and stool culture and examination for ova and parasites generally should be performed in selected cases. Other tests to be considered for individual patients include ophthalmologic examination by slit lamp, radiographic bone survey, technetium bone scan, abdominal imaging by ultrasonography, computed tomography (CT), or magnetic resonance imaging (MRI).[25,82] CT scanning and indium-111 scanning[40] can detect inflammatory lesions and tumors. Such scanning procedures offer a relatively noninvasive technique for screening patients with FUO for a variety of disorders. Although Steele and associates[92] found that radionucleotide scans seldom led to unsuspected diagnoses in children and suggested that they not be used indiscriminately, gallium scanning has been helpful in diagnosing adult patients with FUO,[43] but is not generally recommended in children. Lymph node biopsy, liver biopsy, and exploratory laparoscopy are reserved for patients with evidence of involvement of these organs.

In general, antibiotics or other medications should not be administered empirically as a diagnostic measure in children with FUO. Exceptions include the use of nonsteroidal agents in children with presumed juvenile idiopathic arthritis and the use of antituberculosis drugs in critically ill children thought to have disseminated tuberculosis. Empirical trials of broad-spectrum antibiotics generally do more to obscure than illuminate the etiology of FUO and can mask or delay establishing the diagnosis of infections such as meningitis, parameningeal infection, endocarditis, or osteomyelitis.

Examples of disorders that can be manifested as FUO in children are listed in Table 63-2. A few of these disorders are discussed briefly in the following sections.

TABLE 63-2 **Causes of Fever of Unknown Origin in Children**

Infectious Diseases

Bacterial
Bacterial endocarditis
Brucellosis
Cat-scratch disease
Leptospirosis
Liver abscess
Mastoiditis (chronic)
Osteomyelitis
Pelvic abscess
Perinephric abscess
Pyelonephritis
Salmonellosis
Sinusitis
Subdiaphragmatic abscess
Tuberculosis
Tularemia

Viral
Adenovirus
Arboviruses
Cytomegalovirus
Epstein-Barr virus (infectious mononucleosis)
Hepatitis viruses

Chlamydial
Lymphogranuloma venereum
Psittacosis

Rickettsial
Q fever
Rocky Mountain spotted fever

Fungal
Blastomycosis (nonpulmonary)
Histoplasmosis (disseminated)

Parasitic
Malaria
Toxoplasmosis
Visceral larva migrans

Unclassified
Sarcoidosis

Collagen Vascular Diseases
Juvenile rheumatoid arthritis
Polyarteritis nodosa
Systemic lupus erythematosus

Malignancies
Hodgkin disease
Leukemia and lymphoma
Neuroblastoma

Miscellaneous
Central diabetes insipidus
Drug fever
Ectodermal dysplasia
Factitious fever
Familial dysautonomia
Granulomatous colitis
Hemophagocytic lymphohistiocytosis
Infantile cortical hyperostosis
Kikuchi-Fujimoto disease
Nephrogenic diabetes insipidus
Pancreatitis
Periodic fever
Serum sickness
Thyrotoxicosis
Ulcerative colitis

Infectious Causes of Fever of Unknown Origin

Infectious causes of FUO can be divided into systemic and localized. Immunodeficient states may be considered under the general classification of infections.

Generalized Infections

Brucellosis. The manifestation of this disease as FUO is explained by the nonspecific symptoms that it engenders and by the chronicity of untreated infection. Many physicians, particularly in urban areas, tend to ignore the possibility of this disease and neglect to inquire about a history of exposure to animals or animal products, especially the consumption of unpasteurized goat's milk cheese (see Chapter 128).

Cat-Scratch Disease. During recent years, many children with FUO have proved to be infected with *B. henselae*. Cat-scratch disease is one of the most common causes of FUO in patients seen at the infectious disease service at Texas Children's Hospital in Houston.[6] Most of the children with this manifestation of cat-scratch disease have hepatosplenic involvement. Jacobs and Schutze[48] reported that *B. henselae* infection was the cause of 4.8 percent of all cases of FUO at the Arkansas Children's Hospital and 10.9 percent of the cases of FUO caused by infection. *B. henselae* infection is best diagnosed by serologic evaluation (i.e., immunofluorescence assay that detects serum antibody to *B. henselae*). Biopsy of lesions (e.g., lymph nodes, liver, bone marrow) may allow visualization of bacilli with the Warthin-Starry silver stain; however, this finding is not specific for *B. henselae*. Management of patients with cat-scratch disease is primarily symptomatic because the disease usually is self-limited. Antimicrobial therapy can be helpful in acutely or severely ill patients, especially those with hepatosplenic disease. Several oral antimicrobial regimens (rifampin, trimethoprim-sulfamethoxazole, and azithromycin) and parenteral gentamicin have been used successfully for the treatment of this disease. Specifically, rifampin 20 mg/kg/day in two divided doses for 14 days has been particularly efficacious.[6] However, the optimal duration of therapy is not known.

Leptospirosis. Leptospirosis is caused by a single family of organisms composed of multiple serotypes; it is one of the most widespread zoonoses in the world. Transmission of infection from animal to human can occur by direct contact with the blood, tissue, organs, or urine of infected animals or indirectly by exposure to an environment that has been contaminated by leptospires. The organism also can be acquired from soil or from fresh water after ingestion. Reports indicate that leptospirosis is not a rare disease, that many infections are not associated with occupational exposure, and that urban and suburban cases are becoming more prevalent.[111] Clinical manifestations of leptospirosis usually are not specific. A variety of laboratory aids are available, but specimens must be collected and handled properly. In some cases, establishing a definitive diagnosis may be impossible; negative cultures or failure to demonstrate a rise in antibody titer does not exclude the possibility that the patient has active infection because the organism may not be present in the specimens that have been cultured, the antibody titer may have peaked before an acute-phase specimen was collected, and antibiotic therapy may suppress the development of positive titers or delay their appearance (see Chapter 141).

Toxoplasmosis. Toxoplasmosis should be considered in any child with persistent fever. Cervical or supraclavicular adenopathy is present in most cases, but occasionally fever is the only manifestation. The diagnosis is established by demonstration of a rising serologic titer; antibody to *Toxoplasma gondii* is so prevalent that demonstration of a high titer alone is not diagnostic of acute infection. Demonstration of *Toxoplasma* in tissue sections or body fluid is highly suggestive, although the organism can persist in tissue for years. Isolation of the parasite is not absolutely diagnostic of recent infection (see Chapter 223).

Malaria. Malaria also should be considered in children with FUO. In addition to fever, splenomegaly usually is present. A history of travel to endemic areas should be sought, although malaria has occurred in patients who never traveled outside the United States. Disease can occur even in persons who have taken antimalarial drugs when they visited the endemic region. A delay of several months can occur between the development of infection and the onset of symptoms. The infection can be transmitted from a person who has visited an endemic area to one who has not when an appropriate mosquito vector is present. Malaria also can be acquired by blood transfusion or by the use of needles and syringes contaminated by the parasite. Demonstration of malarial organisms on appropriately stained thin or thick smears of blood is diagnostic (see Chapter 219).

Salmonellosis. *Salmonella* organisms are contaminants in many food products. In view of the nonspecific signs and symptoms with which salmonellosis can occur, its association with FUO in children is not surprising. Repetitive blood and stool cultures are most helpful in establishing a diagnosis (see Chapter 111).

Tuberculosis. Tuberculosis is an important cause of FUO in children, as well as in adults. Nonpulmonary tuberculosis is manifested as FUO more frequently than is pulmonary tuberculosis, which usually is evident on routine chest radiographs. FUO occurs most commonly with disseminated tuberculosis or infection of the liver, peritoneum, pericardium, or genitourinary tract. Active disseminated tuberculosis has been well documented in children with negative results on chest radiography and tuberculin skin tests.[76,94] Negative interferon-γ release assay results also may occur.[33] A high index of suspicion and a careful history of possible contacts are important to make the diagnosis. Funduscopic examination can reveal choroid tubercles. Liver and bone marrow

frequently are involved in children with miliary tuberculosis; liver biopsy specimens and bone marrow aspirates should be obtained and processed for morphologic evaluation and culture. If the chest radiograph yields abnormal results, cultures of gastric aspirates, sputum, or both should be obtained. Because nontuberculous mycobacteria (i.e., atypical organisms) are present in the gastric contents of normal individuals, demonstration of acid-fast organisms on smears of gastric secretion does not necessarily indicate disease. Rarely, a patient with tuberculous pericarditis has fever, weight loss, and weakness but no precordial pain or other specific cardiac complaints. Disseminated infection with atypical mycobacteria generally is seen in patients infected with HIV (see Chapter 96) or those with other immunodeficiencies.

Tularemia. Generally, failure to consider tularemia in children with FUO may be attributed to a lack of appreciation of the many sources of infection and the various routes of inoculation. The organism can be acquired from contact with a variety of animal species, as well as from ticks, mosquitoes, lice, fleas, flies, and contaminated water. The organism can penetrate mucous membranes and broken or unbroken skin, or it can be inhaled or swallowed. Patients and parents should be questioned about animal contact and the ingestion of rabbit or squirrel meat (see Chapter 132).

Viral Infections. Infection by most viruses produces an illness that is relatively brief. Exceptions to this rule can include infections by adenovirus, cytomegalovirus, Epstein-Barr virus, hepatitis viruses, and certain arboviruses. In all of these diseases, symptoms are extremely variable and signs and symptoms frequently are nonspecific. The diagnosis can be established by appropriate cultures and serologic studies (see Chapter 252).

Immunodeficiency. A variety of congenital and acquired immunodeficiency states can be manifested as FUO. Patients with immunoglobulin deficiencies (e.g., Bruton agammaglobulinemia) may have a long history of recurrent fever, with or without evident infection, whereas patients with abnormalities in lymphocyte function are more likely to have prolonged fever caused by persistent viral or parasitic infection.

Localized Infections

Bacterial Endocarditis. Infective endocarditis is an infrequent cause of FUO in children. Acute bacterial endocarditis tends to be fulminant, but the subacute form begins insidiously, generally at the site of a preexisting cardiac lesion. Subacute bacterial endocarditis is a rare occurrence in infants and increases in frequency with advancing age. The organisms most commonly encountered are viridans streptococci, enterococci, *S. aureus*, and *Staphylococcus epidermidis*. The absence of a cardiac murmur does not exclude the possibility of endocarditis, especially when the infection is limited to the right side of the heart. Endocarditis also can occur in the absence of positive blood cultures, particularly in association with the following factors: use of antibiotics for an undefined febrile illness, right-sided cardiac lesions, prolonged duration of disease, infection by unusual organisms such as *Brucella* or *Coxiella burnetii*, and inadequate culture methods for the detection of infection with anaerobic organisms. Frequently associated laboratory findings include anemia, leukocytosis, and an elevated erythrocyte sedimentation rate. Several blood cultures (aerobic and anaerobic) of adequate volume should be obtained before starting antibiotics. Echocardiography can reveal vegetations, but negative results do not rule out endocarditis (see Chapter 26).

Bone and Joint Infections. Infections of bones and joints usually can be diagnosed clinically but occasionally are manifested as FUO. This manifestation occurs commonly in young children who cannot explain where they hurt and is more likely to occur with osteomyelitis than with septic arthritis. Infection of the pelvic bones is implicated most often in this regard. Radioisotopic bone scan and MRI are more sensitive than are plain radiographs of the bones (see Chapters 55 and 56).

Intra-Abdominal Abscesses. Subphrenic, perinephric, and pelvic abscesses may be manifested as FUO. A history of previous intra-abdominal disease or abdominal surgery or a history of vague abdominal complaints should heighten suspicion of an intra-abdominal collection of pus. The organisms involved most commonly are *S. aureus*, streptococci, *E. coli*, and anaerobic flora. Fever may be the only sign of a pelvic, perinephric, or psoas abscess. Urinalysis generally yields normal results, but the mass can be demonstrated by ultrasound examination, CT, or MRI.

Liver Abscess and Other Hepatic Infections. Pyogenic liver abscesses are encountered most frequently in immunocompromised pediatric patients but can be seen in otherwise normal children.[53] In some patients, persistent fever is the only finding. Blood cultures usually are sterile, and serum levels of liver enzymes generally are close to or within normal limits. Many patients have hepatomegaly and right upper quadrant abdominal tenderness. The diagnosis can be established by examination of the liver by ultrasonography, radioisotope scanning, CT, or MRI. Bacterial hepatitis and bacterial cholangitis can occur in the absence of jaundice and other specific signs of liver dysfunction.[105,109] Granulomatous hepatitis is not a specific disease but rather a syndrome characterized by granuloma formation within the liver. A specific cause cannot be determined in every case. Although most reported cases have been in adults,[90] pediatric cases do occur, particularly with Epstein-Barr virus infection and with cat-scratch disease. The diagnosis can be made by ultrasound or other diagnostic imaging (see Chapter 49).

Upper Respiratory Tract Infections. Frequently, infections of the upper respiratory tract and related organs are manifested as FUO.[58,69,83] Although obvious signs or symptoms would be expected, the complaints

often appear trivial and may be ignored. Reported cases of FUO have occurred in children with mastoiditis, sinusitis, chronic or recurrent otitis media, chronic or recurrent pharyngitis, tonsillitis, peritonsillar abscess, and nonspecific upper respiratory tract infection. A parapharyngeal inflammatory pseudotumor manifested as FUO has been reported in a 3-year-old girl in whom anemia and weight loss also developed. The cause never was discerned, but the symptoms resolved after surgical removal of the inflammatory mass.[29]

Noninfectious Causes of Fever of Unknown Origin

Central Nervous System Dysfunction

Children with severe brain damage can have dysfunctional thermoregulation, and body temperature in some of these patients can remain elevated for months. Cases of otherwise neurologically normal children who have had fever as a result of central dysfunction also have been reported. Berger[19] discussed a 16-year-old child with recurrent episodes of fever that were thought to represent a form of epilepsy but disappeared when treatment with phenytoin was begun. Wolff and associates[107] reported a 14-year-old child with cyclic episodes of fever, nausea, vomiting, and emotional disturbance caused by a central nervous system lesion.

Diabetes Insipidus

Central and nephrogenic diabetes insipidus can cause FUO in infants and young children. Polyuria and polydipsia may not be appreciated during infancy. Hyperthermia, weight loss, and peripheral vascular collapse can ensue. Signs of dehydration or an increased serum concentration of sodium suggests the diagnosis. The diagnosis is established by simultaneous measurements of urine and serum electrolytes and osmolality during periods of normal hydration and after carefully controlled periods of water deprivation. Serum levels of antidiuretic hormone also can be measured by radioimmunoassay.

Drug Fever

Nearly any medication can be associated with an allergic reaction, including fever. The offending agent may be a prescribed drug, an over-the-counter preparation, or a street drug such as amphetamine or 1-[1-phenylcyclohexyl] piperidine (phencyclidine [PCP]). Atropine, whether taken systemically or used topically in the form of eye drops, can cause elevation of temperature. Phenothiazines and anticholinergic drugs can inhibit sweating and impair regulation of temperature. Epinephrine and related compounds can affect thermoregulatory control mechanisms and produce fever. Drug fever can be low-grade or high and spiking. Fever can be continuous or intermittent. Discontinuation of the drug generally is followed by disappearance of the fever within 48 to 72 hours, but it sometimes persists for as long as a month as a result of slow excretion of the offending agent.

Factitious Fever

A parent or patient may report the presence of fever that does not exist. The reading of the thermometer can be increased by immersing the bulb in hot liquid or by rinsing the mouth with hot liquid immediately before inserting the thermometer. Clues to factitious fever include absence of tachycardia, malaise, or discomfort despite a markedly elevated temperature; apparent rapid defervescence unaccompanied by diaphoresis; failure of the temperature curve to follow the normal diurnal variation of body temperature; hyperpyrexia; and normal temperature reading when the temperature is obtained rectally by someone who remains in attendance during the procedure. The presence of fever also can be confirmed or excluded by measuring the temperature of a freshly voided urine specimen. The current use of electronic thermometers in most hospitals decreases the possibility of factitious fever in that setting because the nurse or aide usually brings in the thermometer and stays in attendance during the relatively brief period of insertion. In more unusual cases, the patient or parent can induce fever by the injection of infective or foreign materials.

Familial Dysautonomia

Familial dysautonomia (Riley-Day syndrome), an autosomal recessive disorder, is characterized by autonomic and peripheral sensory nerve dysfunction. Eighty percent of patients are children of Jewish parentage, particularly Ashkenazi Jews. Defective regulation of temperature can result in hypothermia or hyperthermia.[34]

A careful history and physical examination can reveal poorly coordinated swallowing movements that lead to recurrent aspiration and pneumonia; recurrent episodes of vomiting; excessive salivation; excessive or diminished sweating; diminished formation of tears; periods of hypotension, hypertension, or both; and erythema or blanching of the skin. The fungiform papillae of the tongue are absent or diminished in number, and the sensation of taste is deficient.[91] Self-mutilation or multiple sites of skin trauma can reflect diminished or absent pain sensation peripherally. Deep tendon reflexes are diminished; corneal reflexes are impaired; and mental deficiency, dysarthria, and emotional lability are common findings.

Excretion of vanillylmandelic acid in urine can be diminished, and excretion of homovanillic acid can be increased. Administration of histamine intradermally can produce a wheal but no flare or pain at the site of injection. Placement of methacholine (2.5%) into the conjunctival sac produces pupillary constriction in children with familial dysautonomia but no response in normal children. Intravenous infusion of norepinephrine is followed by an exaggerated pressor response, and the hypotensive response to infusion of methacholine is increased.

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis is characterized by prolonged fever, hepatosplenomegaly, cytopenia, and

hyperferritinemia and hemophagocytosis in the bone marrow, liver, spleen, or lymph nodes.[50,77] It is a life-threatening and unusual disorder in which uncontrolled proliferation of activated lymphocytes and histiocytes results in unregulated hypersecretion of inflammatory cytokines. Hemophagocytic lymphohistiocytosis can be primarily a familial disease or can be manifested as a reactive process triggered by infection, malignancy, immunologic disease, or drugs.

The diagnosis of hemophagocytic lymphohistiocytosis is suggested by fever, hepatosplenomegaly, cytopenia in at least two cell lines, hypertriglyceridemia or hypofibrinogenemia, and an elevated ferritin level. Other laboratory abnormalities associated with hemophagocytic lymphohistiocytosis can include low or absent natural killer cell activity or elevated soluble CD25 (α chain of the interleukin-2 receptor), but these studies take time for results to return from reference laboratories.[52] Because this disorder can be manifested initially as FUO and progress to masquerade as overwhelming sepsis, a high index of suspicion is required for establishing the diagnosis. Further investigation should include evaluation of bone marrow or lymph nodes for the presence of hemophagocytosis. Therapy should include treatment of the underlying infection or trigger, if one exists, in addition to appropriate immune modulation therapies. A substantial proportion of hemophagocytic lymphohistiocytosis cases progress rapidly to death despite administration of appropriate chemotherapy.[50,52,77]

Inflammatory Bowel Disease

Fever is a prominent feature in many children with inflammatory bowel disease.[30,56,102] A greater percentage of children than adults with regional enteritis have fever. Appropriate contrast-enhanced radiographic studies of the intestines should be undertaken in children with prolonged FUO, even in the absence of findings specifically referable to the gastrointestinal tract, especially if the erythrocyte sedimentation rate is elevated and if the patient has anemia, weight loss, failure of linear growth, or a positive stool guaiac test.

Ulcerative colitis can be manifested as FUO, though less commonly than with regional enteritis. In patients with ulcerative colitis, symptoms referable to the gastrointestinal tract generally are present at the time that the patient is febrile.

Infantile Cortical Hyperostosis

The cause of infantile cortical hyperostosis (i.e., Caffey disease) is unknown. The decreased incidence in recent years suggests an infectious, possibly a viral, cause. Spontaneous hyperplasia of subperiosteal bone begins during infancy and is associated with swelling of the overlying tissues. The skull, mandible, clavicles, scapula, and ribs are affected most frequently, but in some children the long bones and even the metatarsal bones can be involved. Most patients have persistent fever, sometimes as high as 40° C (104° F). Tenderness over the affected regions, irritability, elevated erythrocyte sedimentation rate, and leukocytosis are common findings. The diagnosis is established by the clinical picture in conjunction with radiographically demonstrated periosteal involvement.

Juvenile Idiopathic (Rheumatoid) Arthritis

Juvenile idiopathic arthritis is a chronic inflammatory disorder that usually is manifested as one of three distinct syndromes: the systemic form, characterized by high, spiking temperatures (generally once or twice each day), evanescent rash, and lymphadenopathy; a polyarticular form; and a monarticular or pauciarticular form. Fever is associated with all three manifestations but occurs most commonly in the systemic form, in which case it is present in nearly 100 percent of patients. This form also is the one most likely to be manifested as FUO.[24] Arthritis may not develop for months to years after onset of the fever. The diagnosis often needs to be made by exclusion because no laboratory findings are specific for the diagnosis.

Periodic Fevers

The periodic fever disorders, also known as unprovoked inflammatory events that sometimes can occur without accompanying fever, are rare heritable disorders characterized by recurrent (periodic or irregular) attacks of fever and inflammation.[100] Between attacks, patients feel well. These disorders include familial Mediterranean fever and hyperimmunoglobulinemia D with periodic fever syndrome, which are autosomal recessive diseases. Familial Mediterranean fever is the most common periodic fever syndrome and is caused by a mutation in the *MEFV* gene, which encodes the protein pyrin. The illness is characterized by fever for 1 to 3 days accompanied by abdominal pain, pleurisy, and arthritis or arthralgia. Laboratory findings include peripheral leukocytosis and elevation of acute-phase reactants. The episodes occur irregularly. Persistent inflammation can lead to secondary amyloidosis. The disease occurs most commonly in Armenians, North Africans, Sephardic Jews, and Turks and sometimes Ashkenazi Jews, Italians, and Greeks or persons of other ethnicities. Hyperimmunoglobulinemia D with periodic fever syndrome is associated with mutations in the *MVK* gene that encodes mevalonate kinase. Disease commonly presents before the age of 1 year, with episodes of fever lasting 3 to 7 days accompanied by cervical lymphadenopathy, abdominal pain, and vomiting or diarrhea.[37,42] Patients also can have aphthous ulcers, rash, splenomegaly, and arthritis or arthralgias. Attacks may occur at irregular intervals and can be triggered by stress, vaccination, and viral infections. Immunoglobulin (Ig) D levels are elevated, and most patients also have elevated IgA. Secondary amyloidosis is rare.

Other periodic fever syndromes include tumor necrosis factor receptor–associated periodic syndrome; pyogenic sterile arthritis, pyoderma gangrenosum syndrome, and acne; and Blau syndrome. These are rare disorders that are autosomal dominant in transmission. The genes associated with these disorders encode proteins that share domains involved in innate immunity and apoptosis. Muckle-Wells syndrome, familial cold autoinflammatory syndrome, and neonatal-onset multisystem inflammatory

disorder are cryopyrin-associated periodic syndromes, a group of overlapping autoinflammatory disorders in which fever is an accompanying but not major feature of illness.

The syndrome of periodic fever with aphthous stomatitis, pharyngitis, and adenitis is a relatively common periodic fever syndrome. Symptoms such as fever, pharyngitis, cervical lymphadenopathy, and mild aphthous ulcers usually recur at 3- to 4-week intervals, generally beginning abruptly and resolving spontaneously in 3 to 5 days. Leukocytosis and elevation of inflammatory markers occur during the acute episode and normalize in between. The cause of this syndrome remains unknown.[38]

Acknowledgments

Debra L. Palazzi would like to acknowledge Martin I. Lorin and the late Ralph D. Feigin as the original authors of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION.

4. American Academy of Pediatrics, Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.
5. Andreola B, Bressan S, Callegaro S, et al. Procalcitonin and C-reactive protein as diagnostic markers of severe bacterial infections in febrile infants and children in the emergency department. *Pediatr Infect Dis J* 2007;**26**:672–7.
27. Carstairs KL, Tanen DA, Johnson AS, et al. Pneumococcal bacteremia in febrile infants presenting to the emergency department before and after the introduction of the heptavalent pneumococcal vaccine. *Ann Emerg Med* 2007;**49**:772–7.
28. Centers for Disease Control and Prevention, Advisory Committee on Immunization Practices. Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children, 2010. *MMWR Morb Mortal Wkly Rep* 2010; **59**:258–61.
32. Craig JC, Williams GJ, Jones M, et al. The accuracy of clinical symptoms and signs for the diagnosis of serious bacterial infection in young febrile children: prospective cohort study of 15 781 febrile illnesses. *BMJ* 2010;**340**:c1594.
33. Cruz AT, Geltemeyer AM, Starke JR, et al. Comparing the tuberculin skin test and T-SPOT TB blood test in children. *Pediatrics* 2011;**127**:e31–8.
52. Jordan MB, Allen CE, Weitzman S, et al. How I treat hemophagocytic lymphohistiocytosis. *Blood* 2011;**118**:4041–52.
79. Pasic S, Minic A, Djuric P, et al. Fever of unknown origin in 185 paediatric patients: a single-centre experience. *Acta Paediatr* 2006;**95**:463–6.
100. Tunca M, Ozdogan H. Molecular and genetic characteristics of hereditary autoinflammatory diseases. *Curr Drug Targets Inflamm Allergy* 2005;**4**:77–80.
101. Waddle E, Jhaveri R. Outcomes of febrile children without localising signs after pneumococcal conjugate vaccine. *Arch Dis Child* 2009;**94**:144–7.
104. Watt K, Waddle E, Jhaveri R. Changing epidemiology of serious bacterial infections in febrile infants without localizing signs. *PLoS One* 2010;**5**:e12448.
106. Wilkinson M, Bulloch B, Smith M. Prevalence of occult bacteremia in children aged 3 to 36 months presenting to the emergency department with fever in the postpneumococcal conjugate vaccine era. *Acad Emerg Med* 2009;**16**:220–5.
111. Yoder JS, Hlavsa MC, Craun GF, et al. Centers for Disease Control and Prevention. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events: United States, 2005–2006. *MMWR Surveill Summ* 2008;**57**:1–29.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 64

Toxic Shock Syndrome

Jeffrey Suen

Illnesses resembling toxic shock syndrome (TSS) have been reported since 1927,[7,102,115,295,318] but the initial description of TSS as a disease was published in 1978 by Dr. James K. Todd and colleagues.[305] The clinical illness is defined by criteria formulated for epidemiologic studies (Table 64-1). TSS has unique clinical manifestations not generally noted in septic shock, including diffuse erythroderma, delayed desquamation of the palms and soles, conjunctival and pharyngeal hyperemia, muscle injury, rapidly accelerated renal failure, and gastrointestinal symptoms. The capillary leak syndrome, or rapid and massive loss of fluid from capillaries into the interstitial space, loss of peripheral vascular resistance, and subsequent multisystem end-organ failure further characterize this entity.

When epidemic disease was first described, TSS had unique geographic, age, sex, and racial characteristics. It was associated with menses, particularly tampon use, as

REFERENCES

1. Adams KC, Dixon JH, Eichner ER. Clinical usefulness of polymorphonuclear leukocyte vacuolization in predicting septicemia in febrile children. *Pediatrics* 1978;**62**:67–70.
2. Alpern ER, Alessandrini EA, Bell LM, et al. Occult bacteremia from a pediatric emergency department: current prevalence, time to detection and outcome. *Pediatrics* 2000;**106**:505–11.
3. Alpern ER, Alessandrini EA, McGowan KL, et al. Serotype prevalence of occult pneumococcal bacteremia. *Pediatrics* 2001; **108**:e23.
4. American Academy of Pediatrics, Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.
5. Andreola B, Bressan S, Callegaro S, et al. Procalcitonin and C-reactive protein as diagnostic markers of severe bacterial infections in febrile infants and children in the emergency department. *Pediatr Infect Dis J* 2007;**26**:672–7.
6. Arisoy ES, Correa AG, Wagner ML, Kaplan SL. Hepatosplenic cat-scratch disease in children: selected clinical features and treatment. *Clin Infect Dis* 1999;**28**:778–84.
7. Ayus JC, Krothapalli RK, Arieff AI. Occult bacteremia in febrile children. *N Engl J Med* 1988;**318**:1338–9.
8. Bachur R, Harper M. Reevaluation of outpatients with *Streptococcus pneumoniae* bacteremia. *Pediatrics* 2000;**105**:502–9.
9. Baker MD, Bell LM, Avner JR. Outpatient management without antibiotics of fever in selected infants. *N Engl J Med* 1993;**329**: 1437–41.
10. Baker MD, Bell LM, Avner JR. The efficacy of routine outpatient management without antibiotics of fever in selected infants. *Pediatrics* 1999;**103**:627–31.
11. Baker RC, Tiller T, Bausher JC, et al. Severity of disease correlated with fever reduction in febrile infants. *Pediatrics* 1989;**83**: 1016–9.
12. Baraff LJ. Management of fever without source in infants and children. *Ann Emerg Med* 2000;**36**:602–14.
13. Baraff LJ, Lee SI. Fever without source: management of children 3 to 36 months of age. *Pediatr Infect Dis J* 1992;**11**:146–51.
14. Baraff LJ, Oslund S, Prather M. Effect of antibiotic therapy and etiologic microorganism on the risk of bacterial meningitis in children with occult bacteremia. *Pediatrics* 1993;**92**:140–3.
15. Baraff LJ, Bass JW, Fleisher GR, et al. Practice guidelines for the management of infants and children 0 to 36 months of age with fever without source. *Pediatrics* 1993;**92**:1–12.
16. Baron MA, Fink HD. Bacteremia in private pediatric practice. *Pediatrics* 1980;**66**:171–5.
17. Baron MA, Fink HD, Cicchetti DV. Blood cultures in private pediatric practice: An eleven-year experience. *Pediatr Infect Dis* 1989;**8**:2–7.
18. Bass JW, Steel RW, Wittler RR, et al. Antimicrobial treatment of occult bacteremia: a multicenter cooperative study. *Pediatr Infect Dis J* 1993;**12**:466–73.
19. Berger H. Fever: An unusual manifestation of epilepsy. *Postgrad Med* 1966;**40**:479–81.
20. Bloom HR. Must we teach clinical judgment? *Pediatrics* 1981; **67**:745–6.
21. Bonadio WA. Systemic bacterial infections in children with fever greater than 41° C. *Pediatr Infect Dis J* 1989;**8**:120–1.
22. Bonadio WA, Webster H, Wolfe A, Gorecki D. Correlating infectious outcome with clinical parameters of 1130 consecutive febrile infants aged zero to eight weeks. *Pediatr Emerg Care* 1993;**9**:84–6.
23. Brewis EC. Undiagnosed fever. *Br Med J* 1965;**1**:107–10.
24. Calabro JJ, Marchesano JM. Juvenile rheumatoid arthritis. *N Engl J Med* 1967;**277**:746–9.
25. Carey BM, Williams CE, Arthur RJ. Ultrasound demonstration of pericardial empyema in an infant with pyrexia of undetermined origin. *Pediatr Radiol* 1988;**18**:349–50.
26. Carroll WL, Farrell MK, Singer JI, et al. Treatment of occult bacteremia: a prospective randomized clinical trial. *Pediatrics* 1983;**72**:608–11.
27. Carstairs KL, Tanen DA, Johnson AS, et al. Pneumococcal bacteremia in febrile infants presenting to the emergency department before and after the introduction of the heptavalent pneumococcal vaccine. *Ann Emerg Med* 2007;**49**:772–7.
28. Centers for Disease Control and Prevention, Advisory Committee on Immunization Practices. Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**: 258–61.
29. Chan YF, Ma LT, Yeung LT, et al. Parapharyngeal inflammatory pseudotumor presenting as fever of unknown origin in a 3-year-old girl. *Pediatr Pathol* 1988;**8**:195–203.
30. Chron BB, Yarnis H. Continuous fever of intestinal origin. *Ann Intern Med* 1947;**26**:858–62.
31. Corrigan JJ. Thrombocytopenia: laboratory sign of septicemia in infants and children. *J Pediatr* 1974;**85**:219–23.
32. Craig JC, Williams GJ, Jones M, et al. The accuracy of clinical symptoms and signs for the diagnosis of serious bacterial infection in young febrile children: prospective cohort study of 15 781 febrile illnesses. *BMJ* 2010;**340**:c1594.
33. Cruz AT, Geltemeyer AM, Starke JR, et al. Comparing the tuberculin skin test and T-SPOT TB blood test in children. *Pediatrics* 2011;**127**:e31–8.
34. Dancis J, Smith AA. Familial dysautonomia. *N Engl J Med* 1966;**274**:207–9.
35. Dashefsky B, Teele DW, Klein JO. Unsuspected meningococcemia. *J Pediatr* 1983;**102**:69–72.
36. Dershewitz RA. A comparative study of the prevalence, outcome and prediction of bacteremia in children. *J Pediatr* 1983;**103**: 352–8.
37. Drenth JPH, Haagsma CJ, van Der Meer JWH. Hyperimmunoglobulinemia D and periodic fever syndrome. *Medicine (Baltimore)* 1994;**73**:133–44.
38. Feder HMJ, Bialecki CA. Periodic fever associated with aphthous stomatitis, pharyngitis and cervical adenitis. *Pediatr Infect Dis* 1989;**8**:186–9.
39. Feigin RD, Shearer WT. Fever of unknown origin in children. *Curr Probl Pediatr* 1976;**6**:2–57.
40. Fineman DS, Palestno CJ, Kim CK, et al. Detection of abnormalities in febrile AIDS patients with In-111–labelled leukocyte and Ga-67 scintigraphy. *Radiology* 1989;**170**:677–80.
41. Fleishner GR, Rosenberg N, Vinci R, et al. Intramuscular versus oral antibiotic therapy for the prevention of meningitis and other bacterial sequelae in young febrile children at risk for occult bacteremia. *J Pediatr* 1994;**124**:504–12.
42. Gross C, Schnetzer JR, Ferrante A, Vladutiu AO. Children with hyperimmunoglobulinemia D and periodic fever syndrome. *Pediatr Infect Dis J* 1996;**15**:72–7.
43. Habibian MR, Staab EV, Matthews HA. Gallium citrate Ga 67 scans in febrile patients. *JAMA* 1975;**233**:1073–6.
44. Hayani A, Mahoney DH, Fernback DJ. Role of bone marrow examination in the child with prolonged fever. *J Pediatr* 1990; **116**:919–20.
45. Herz AM, Greenhow TL, Alcantara J, et al. Changing epidemiology of outpatient bacteremia in 3- to 30-month-old children after the introduction of the heptavalent-conjugated pneumococcal vaccine. *Pediatr Infect Dis J* 2006;**25**:293–300.
46. Hoekelman R, Lewin EB, Shapira MD, et al. Potential bacteremia in pediatric practice. *Am J Dis Child* 1979;**133**:1017–9.
47. Isaacman DJ, Burke DL. Utility of the serum C-reactive protein for detection of occult bacterial infection in children. *Arch Pediatr Adolesc Med* 2002;**156**:905–9.
48. Jacobs RF, Schutze GE. *Bartonella henselae* as a cause of prolonged fever and fever of unknown origin in children. *Clin Infect Dis* 1988;**26**:80–4.
49. Jaffe DM, Tanz RR, Davis T, et al. Antibiotic administration to treat possible occult bacteremia in febrile children. *N Engl J Med* 1987;**317**:1175–80.
50. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Eur J Pediatr* 2007;**166**:95–109.
51. Jaskiewicz JA, McCarthy CA, Richardson AC, et al. Febrile infants at low risk for serious bacterial infection: an appraisal of the Rochester criteria and implications of management. *Pediatrics* 1994;**94**:390–6.
52. Jordan MB, Allen CE, Weitzman S, et al. How I treat hemophagocytic lymphohistiocytosis. *Blood* 2011;**118**:4041–52.

53. Kaplan SL, Feigin RD. Pyogenic liver abscess in normal children with fever of unknown origin. *Pediatrics* 1976;**58**:614–6.
54. Kline MW, Smith EO, Kaplan SL, et al. Effects of causative organism and presence or absence of meningitis on white blood cell counts in children with bacteremia. *J Emerg Med* 1988;**6**: 33–5.
55. Lacour AG, Gervaix A, Zamora SA, et al. Procalcitonin, IL-6, IL-8, IL-1 receptor antagonist and C-reactive protein as identificators of serious bacterial infections in children with fever without localising signs. *Eur J Pediatr* 2001;**160**:95–100.
56. Lee FI, Davies DM. Crohn's disease presenting as pyrexia of unknown origin. *Lancet* 1961;**1**:1205–6.
57. Liu C, Lehan C, Speer ME, et al. Early detection of bacteremia in an outpatient clinic. *Pediatrics* 1985;**75**:827–31.
58. Lohr JA, Hendley JO. Prolonged fever of unknown origin: record of experience with 54 childhood patients. *Clin Pediatr (Phila)* 1977;**16**:768–73.
59. Margolis MT. Specific anergy in tuberculosis. *N Engl J Med* 1983;**309**:1388.
60. Marshall R, Teele DW, Klein JO. Unsuspected bacteremia due to *Haemophilus influenzae*: outcome in children not initially admitted to hospital. *J Pediatr* 1979;**95**:690–5.
61. Mazur LJ, Jones T, Kozinetz CA. Temperature response to acetaminophen and risk of occult bacteremia: a case control study. *J Pediatr* 1989;**115**:888–91.
62. McCarthy PL, Dolan TF. Hyperpyrexia in children: eight-year emergency room experience. *Am J Dis Child* 1976;**130**:849–51.
63. McCarthy PL, Jekel JF, Dolan TF. Temperature greater than or equal to 40° C in children less than 24 months of age: a prospective study. *Pediatrics* 1977;**59**:663–8.
64. McCarthy PL, Frank AL, Ablow RC, et al. Value of the C-reactive protein test in the differentiation of bacterial and viral pneumonia. *J Pediatr* 1978;**92**:454–9.
65. McCarthy PL, Grundy GW, Spiesel SZ, et al. Bacteremia in children: an outpatient review. *Pediatrics* 1976;**57**:861–8.
66. McCarthy PL, Jekel JF, Stashwick CA, et al. History and observation variables in assessing febrile children. *Pediatrics* 1980;**65**: 1090–5.
67. McCarthy PL, Jekel JF, Stashwick CA, et al. Further definition of history and observation variables in assessing febrile children. *Pediatrics* 1981;**67**:687–93.
68. McCarthy PL, Sharpe MR, Spiesel SZ, et al. Observation scales to identify serious illness in febrile children. *Pediatrics* 1982; **70**:802–9.
69. McClung HJ. Prolonged fever of unknown origin in childhood. *Am J Dis Child* 1972;**124**:544–50.
70. McGowan JE, Bratton L, Klein JO, et al. Bacteremia in febrile children seen in a walk-in pediatric clinic. *N Engl J Med* 1973; **288**:1309–12.
71. McMurray DN, Echeverri A. Cell-mediated immunity in anergic patients with pulmonary tuberculosis. *Am Rev Respir Dis* 1978; **118**:827–34.
72. Miller ML, Szer I, Yogev R, Bernstein B. Fever of unknown origin. *Pediatr Clin North Am* 1995;**42**:999–1011.
73. Morens DW. WBC and differential: Value in predicting bacterial disease in children. *Am J Dis Child* 1979;**133**:25–7.
74. Murray DL, Zonana J, Seidel JS, et al. Relative importance of bacteremia and viremia in the course of acute fevers of unknown origin in outpatient children. *Pediatrics* 1981;**68**: 157–60.
75. Nash DR, Douglas JE. Anergy in active pulmonary tuberculosis: a comparison between positive and negative reactors and an evaluation of 5 TU and 250 TU skin test doses. *Chest* 1980;**77**: 32–7.
76. Ostrow JH. Tuberculin negative tuberculosis. *Am Rev Respir Dis* 1973;**107**:882–3.
77. Palazzi DL, McClain KL, Kaplan SL. Hemophagocytic syndrome in children: an important diagnostic consideration in fever of unknown origin. *Clin Infect Dis* 2003;**36**:306–12.
78. Pantell RH, Newman TB, Bernzweig J, et al. Management and outcomes of care of fever in early infancy. *JAMA* 2004;**291**: 1203–12.
79. Pasic S, Minic A, Djuric P, et al. Fever of unknown origin in 185 paediatric patients: a single-centre experience. *Acta Paediatr* 2006; **95**:463–6.
80. Peltola H. C-reactive protein in rapid differentiation of acute epiglottitis from spasmodic croup and acute laryngotracheitis: preliminary report. *J Pediatr* 1983;**102**:713–5.
81. Petersdorf RG, Beeson PB. Fever of unexplained origin: report on 100 cases. *Medicine (Baltimore)* 1961;**40**:1–30.
82. Picus D, Siegel MJ, Balfe DM. Abdominal computed tomography in children with unexplained prolonged fever. *J Comput Assist Tomogr* 1984;**8**:851–6.
83. Pizzo PA, Lovejoy FH, Smith DH. Prolonged fever in children: review of 100 cases. *Pediatrics* 1975;**55**:468–73.
84. Poehling KA, Talbot TR, Griffin MR, et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA* 2006;**295**:1668–74.
85. Prat C, Dominguez J, Rodrigo C, et al. Use of quantitative and semiquantitative procalcitonin measurements to identify children with sepsis and meningitis. *Eur J Clin Microbiol Infect Dis* 2004;**23**: 136–8.
86. Press S. Association of hyperpyrexia with serious disease in children. *Clin Pediatr (Phila)* 1994;**33**:19–25.
87. Pulliam PN, Attia MW, Cronan KM. C-reactive protein in febrile children 1 to 36 months of age with clinically undetectable serious bacterial infection. *Pediatrics* 2001;**108**:1275–9.
88. Roberts KB, Borzy MS. Fever in the first eight weeks of life. *Johns Hopkins Med J* 1977;**141**:9–13.
89. Sard B, Bailey MC, Vinci R. An analysis of pediatric blood cultures in the postpneumococcal conjugate vaccine era in a community hospital emergency department. *Pediatr Emerg Care* 2006;**22**: 295–300.
90. Simon HB, Wolff SM. Granulomatous hepatitis and prolonged fever of unknown origin: A study of 13 patients. *Medicine (Baltimore)* 1973;**52**:1–21.
91. Smith AA, Farbman A, Dancis J. Tongue in familial dysautonomia. *Am J Dis Child* 1965;**110**:152–3.
92. Steele RW, Jones SM, Lowe BA, et al. Usefulness of scanning procedures for diagnosis of fever of unknown origin in children. *J Pediatr* 1991;**119**:526–30.
93. Stein RC. The white blood cell count in fevers of unknown origin. *Am J Dis Child* 1972;**124**:60–3.
94. Steiner P, Portuguleza C. Tuberculous meningitis in children. *Am Rev Respir Dis* 1973;**107**:22–9.
95. Stoll MJ, Rubin LG. Incidence of occult bacteremia among highly febrile young children in the era of the pneumococcal conjugate vaccine. *Arch Pediatr Adolesc Med* 2004;**158**:671–5.
96. Teach SJ, Fleisher GR. Efficacy of an observation scale in detecting bacteremia in febrile children three to thirty six months of age, treated as outpatients. *J Pediatr* 1995;**126**:877–81.
97. Teele DW, Pelton SI, Grant MJ, et al. Bacteremia in febrile children under 2 years of age: results of cultures of blood of 600 consecutive febrile children in a "walk-in" clinic. *J Pediatr* 1975; **87**:227–30.
98. Todd JK. Childhood infections: diagnostic value of peripheral white blood cell and differential cell counts. *Am J Dis Child* 1974;**127**:810–6.
99. Torrey SB, Henretig F, Fleisher G, et al. Temperature response to antipyretic therapy in children. *Am J Emerg Med* 1985;**3**:190–2.
100. Tunca M, Ozdogan H. Molecular and genetic characteristics of hereditary autoinflammatory diseases. *Curr Drug Targets Inflamm Allergy* 2005;**4**:77–80.
101. Waddle E, Jhaveri R. Outcomes of febrile children without localising signs after pneumococcal conjugate vaccine. *Arch Dis Child* 2009;**94**:144–7.
102. Walker SH. Periodic fever in juvenile regional enteritis. *J Pediatr* 1962;**60**:561–5.
103. Waskerwitz S, Berkelhammer JE. Outpatient bacteremia: clinical findings in children under two years with initial temperatures of 39.5° C or higher. *J Pediatr* 1981;**99**:231–3.
104. Watt K, Waddle E, Jhaveri R. Changing epidemiology of serious bacterial infections in febrile infants without localizing signs. *PLoS One* 2010;**5**:e12448.
105. Weinstein L. Bacterial hepatitis: a case report on an unrecognized cause of fever of unknown origin. *N Engl J Med* 1978;**299**:1052–4.
106. Wilkinson M, Bulloch B, Smith M. Prevalence of occult bacteremia in children aged 3 to 36 months presenting to the emergency department with fever in the postpneumococcal conjugate vaccine era. *Acad Emerg Med* 2009;**16**:220–5.

107. Wolff SM, Ward SB, Landy M. A syndrome of periodic hypothalamic discharge. *Am J Med* 1964;**36**:956–66.
108. Wright PF, Thompson J, McKee KT, Jr, et al. Patterns of illness in the highly febrile young child: epidemiologic, clinical and laboratory correlates. *Pediatrics* 1981;**67**:694–700.
109. Wyllie R, Fitzgerald JF. Bacterial cholangitis in a 10-week-old infant with fever of undetermined origin. *Pediatrics* 1980;**65**: 164–7.
110. Yamamoto LT, Wigder HN, Fligner DJ, et al. Relationship of bacteremia to antipyretic therapy in febrile children. *Pediatr Emerg Care* 1987;**3**:223–6.
111. Yoder JS, Hlavsa MC, Craun GF, et al; Centers for Disease Control and Prevention. Surveillance for waterborne disease and outbreaks associated with recreational water use and other aquatic facility-associated health events: United States, 2005–2006. *MMWR Surveill Summ* 2008;**57**:1–29.

TABLE 64-1 Clinical Case Definition of Toxic Shock Syndrome

Clinical Findings

Fever: Temperature ≥38.9° C (102° F)

Rash: Diffuse macular erythroderma

Desquamation: 1-2 wk after onset of illness, particularly on palms, soles, fingers, and toes

Hypotension: Systolic blood pressure ≤90 mm Hg for adults; <5th percentile by age for children <16 yr old; orthostatic drop in diastolic blood pressure ≥15 mm Hg from lying to sitting; orthostatic syncope or orthostatic dizziness

Involvement of three or more of the following organ systems:
- Gastrointestinal: Vomiting or diarrhea at onset of illness
- Muscular: Severe myalgia or creatinine phosphokinase level greater than twice the upper limit of normal for the laboratory
- Mucous membrane: Vaginal, oropharyngeal, or conjunctival hyperemia
- Renal: BUN or serum creatinine greater than twice the upper limit of normal or ≥5 white blood cells per high-power field in the absence of a urinary tract infection
- Hepatic: Total bilirubin, AST, or ALT greater than twice the upper limit of normal for the laboratory
- Hematologic: platelets <100,000/mm^3
- Central nervous system: disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent

Negative results on the following tests, if obtained:
- Blood, throat, or cerebrospinal fluid cultures; blood culture may be positive for *Staphylococcus aureus*
- Serologic tests for Rocky Mountain spotted fever, leptospirosis, or measles

Case Classification

Probable: A case with 5 of the 6 clinical findings described above

Confirmed: A case with all 6 of the clinical findings described above, including desquamation, unless the patient dies before desquamation could occur

From Wharton M, Chorba TL, Vogt RL, et al. Case definitions for public health surveillance. *MMWR Recomm Rep* 1990;**39**(RR-13):1-43.

ALT, Alanine transaminase; *AST,* aspartate transaminase; *BUN,* blood urea nitrogen.

well as with a phenotypically distinctive type of *Staphylococcus aureus*. Patients with menses-associated TSS (menstrual TSS) had vaginal or cervical colonization with strains of *S. aureus*[21] that made a characteristic toxin initially called *staphylococcal enterotoxin F*[22] and *pyrogenic exotoxin C*[273] and now known as *TSST-I*. Cases of TSS not associated with menses (nonmenstrual TSS) were recognized with toxin-producing *S. aureus* isolated from a variety of sites, including burn and postsurgical wounds. Further, a toxic shocklike syndrome was recognized in patients with *Streptococcus pyogenes* infection.

TSST-I, staphylococcal enterotoxins, and streptococcal pyrogenic exotoxins A and B are now recognized to be "superantigens." These superantigens act as potent stimuli of T-cell proliferation by binding to selected V_β elements of the T-cell receptor and to major histocompatibility complex (MHC) class II receptors on antigen-processing cells. The resulting massive release of interleukin (IL)-1, IL-2, tumor necrosis factor-α (TNF-α), lymphotoxin (TNF-β), and interferon-γ mediates the manifestations of the disease.

EPIDEMIOLOGY

Surveillance and Incidence

The first 12 cases of epidemic TSS were identified in Wisconsin and Minnesota between July 1979 and January 1980 and were reported to the Centers for Disease Control and Prevention (CDC) in January 1980.[42,83,280] Surveillance for TSS began in Wisconsin[82] and Minnesota[234] in January 1980; in Utah in February 1980[180]; and in other states after the national communications about TSS in spring 1980. The National Center for Health Statistics recommended use of the International Classification of Diseases (ICD) code 040.89 for TSS, and a study found the sensitivity and specificity of using this code to identify cases was 85 and 95 percent, respectively.[142] In May 1980, the CDC reported findings of the first 55 cases,[42] 95 percent of which occurred in women. Of 40 patients for whom a menstrual history was obtained, 38 (95%) had onset during menstruation. By June 1980, case-control studies statistically linking the occurrence of menstrual TSS with tampons had been completed by the Wisconsin Division of Health[83] and the CDC,[280] and similar trends had been noted by the Utah Department of Health.[167] In September 1980, the CDC reported that TSS had been associated with many tampon brands, particularly Rely (Procter & Gamble). This brand was withdrawn with a documented decrease in reported cases.* In April 1985, two additional tampon manufacturers withdrew their superabsorbent tampons containing polyacrylate on the basis of an in vitro study[212] and a judicial decision.[226]

Between 1983 and 1994, the CDC received reports of 4192 cases of TSS through the National Electronic Telecommunications System for Surveillance (NETSS).[49] Of 2509 confirmed cases reported to the CDC through April 1984, 95 percent occurred in females,[253] 89 percent had an onset of TSS associated with menstruation, and 99 percent occurred in tampon users. In 1980 the reported rate of TSS was 2.4 to 12.3/100,000 women of menstruating age.[83,180,234,247] Since 1983, a continued downward trend in passively reported cases has been noted (Fig. 64-1).[323] The results of an active surveillance study conducted by the CDC in 1986 and 1987 confirmed the downward trend previously noted in the CDC passive surveillance system.[132] The incidence of menstrual TSS was found to be 1 case/100,000 women aged 15 to 44 years. Only 55 percent of the cases detected in the 1986 to 1987 study occurred in women, and only 45 percent of all cases were menstrual associated.[35,132] The TSS-related mortality rate for menstrual cases decreased from 5.5 percent in 1979 and 1980 to 2.8 percent in 1981 to 1986, then to 1.8 percent in 1987 to 1996.[138] The striking reduction in the incidence of menstrual TSS was attributed to a decrease in tampon absorbency, changes in

*References 23, 41, 43-45, 268.

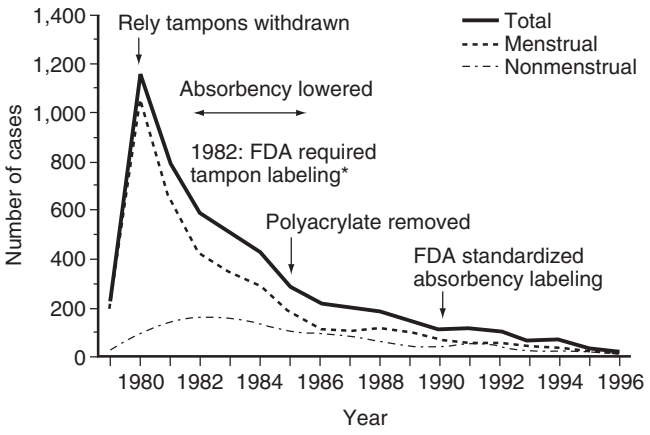

*FDA, Food and Drug Administration, including definite and probable toxic shock syndrome cases

FIGURE 64-1 ■ Menstrual and nonmenstrual toxic shock syndrome cases reported to the Centers for Disease Control and Prevention by year, 1979 to 1996. (From Hajjeh RA, Reingold A, Weil A, et al. Toxic-shock syndrome in the United States: surveillance update, 1979-1996. *Emerg Infect Dis* 1999;**5**:807–10.)

composition of tampons and patterns of use, and the impact of publicity on early recognition of symptoms.*

As the incidence of menstrual TSS declined, the incidence of nonmenstrual TSS in women (0.25 case/100,000 women) and men (0.16 case/100,000 men) remained stable from 1980 to 1987, with nonmenstrual TSS accounting for a greater proportion of overall TSS cases. The proportion of nonmenstrual cases reported after surgical procedures increased from 14 percent during 1979 to 1986 to 27 percent during 1987 to 1996.[138] In 1994, of 192 cases of TSS reported to the CDC, 42 percent were nonmenstrual.[49] Overall the case-fatality ratio for nonmenstrual cases has remained between 5.3 and 8.5 percent.[35,49]

Since the 1980s, a surveillance study conducted from 2000 to 2006 in the Minneapolis–St. Paul area found that the TSS incidence has remained stable with the average annual incidence per 100,000 of menstrual TSS 0.69 (95% confidence interval [CI], 0.39-1.16) and nonmenstrual TSS 0.32 (95% CI, 0.12-0.67). Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 7 percent of TSS cases.[94]

Although TSS cases in the United States have been reported in all 50 states, significant differences in incidence were noted among the states through 1983.[253] Through mid-1983, the five states of Wisconsin, Minnesota, Colorado, Utah, and California accounted for 44 percent of the total reported cases but represented only 16 percent of the U.S. population.[309] Although intensified surveillance may have been a factor, regional differences in the degree of immunity to TSS-associated *S. aureus* toxins and in the distribution of toxin-producing organisms may have been important factors.[243,317] Geographic differences in the occurrence of TSS continued even with the increase in the relative proportion of nonmenstrual cases.[35]

*References 48, 81, 82, 83, 118, 233, 276.

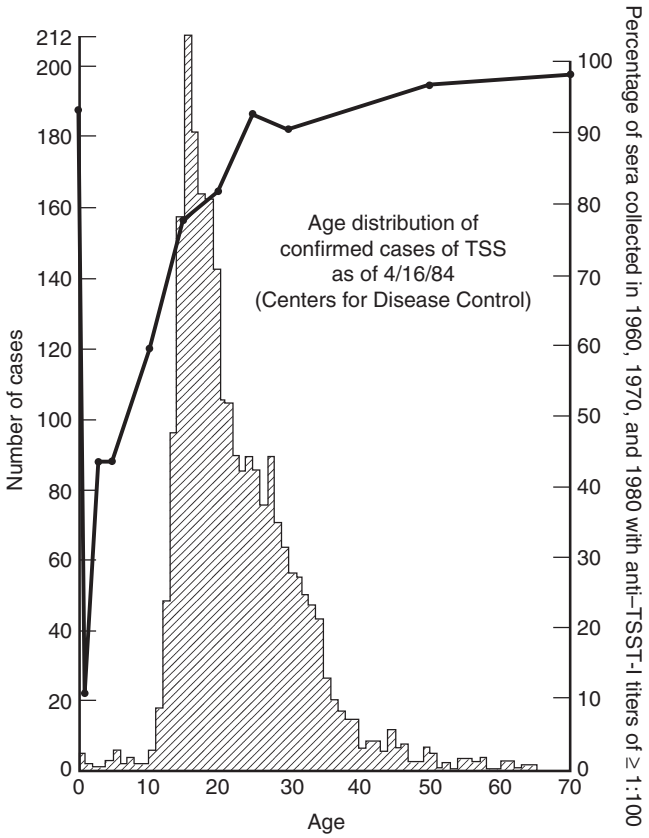

FIGURE 64-2 ■ Age distribution of patients with confirmed toxic shock syndrome (TSS) reported to the Centers for Disease Control and Prevention before April 16, 1984. The age-specific prevalence of antibodies to toxic shock syndrome toxin I (TSST-I) in a normal population in Wisconsin in the years 1960, 1970, and 1980 is indicated by the *solid connected line*. (Data from Vergeront JM, Stolz SJ, Crass BA, et al. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic shock syndrome. *J Infect Dis* 1983; **148**:692–8.)

Risk Factors for Menstrual Toxic Shock Syndrome

From 1980 through 1990, most reported cases of TSS occurred in previously healthy, young white menstruating women who were using tampons at the time of onset of the illness. The risk for developing menstrual TSS is highest in the ages 15 to 25 years (median age, 21 years, 1979 to 1980; 20 years, 1981 to 1986; 25 years, 1987 to 1996).[132,138,253] Roughly one third of cases occur in adolescents 15 to 19 years of age (Fig. 64-2).[317] The precise reason for the increased incidence of TSS in adolescent girls is not known, but a lower prevalence of antibody to TSST-I may increase susceptibility in this age group.[317]

A striking 97 percent of menstrual TSS cases have occurred in whites, who make up 83 percent of the U.S. population. The reasons for racial differences are only partially explained by differences in menses-related practices.[118,253,254] Racial differences in antibody to TSST-I may explain to some degree the differences in the distribution of cases.[317]

Menstruation and tampon use were established as clear risk factors for the development of TSS.* Although TSS occurs with all brands of tampons, a greater relative risk for menstrual TSS was found with brands with higher absorbency and particular compositions.[41,268] The Tri-State TSS Study and subsequent studies by the CDC established that tampons of increasing absorbency were associated with an increasing relative risk for the development of menstrual TSS; for each 1-g increase in absorbency, the risk for TSS increased by 34 to 37 percent.[23,122,232] In addition, the studies suggested that the effects of absorbency and chemical composition of tampons on TSS risk were independent.[35,232] Continuous use of tampons was associated with a greater risk for the development of TSS in one case-control study,[280] but this finding was not confirmed in a subsequent investigation.[232] No association between TSS and the frequency of changing tampons has been demonstrated.

On June 22, 1982, a U.S. Food and Drug Administration (FDA) regulation required that information on TSS and tampon-associated risks appear on tampon packages.[97] Since 1980, tampon composition has changed to cotton and rayon combinations with the removal of additives such as polyacrylate, polyester foam, cross-linked carboxymethylcellulose, and surfactants, including Pluronic L-92.[270] In 1980, products very high in absorbency were used by 42 percent of tampon users.[234] By 1983, this proportion had decreased to 18 percent and, by 1986, to 1 percent.[48] Overall, the absorbency of available tampon brands decreased from 10.3 to 20.5 g in 1980 to less than 6 to 15 g in 1990. In 1990, the FDA published the in vitro rating system to be used by manufacturers to inform the public about tampon absorbency.[187,221,314]

Risk Factors for Nonmenstrual Toxic Shock Syndrome

The mean age of patients with nonmenstrual TSS (27 years through 1982, 30 years in 1986) is significantly higher than that for menses-associated cases.[132,254] The number of confirmed cases of TSS reported in children younger than 10 years of age is surprisingly low, particularly in light of their demonstrated low antibody titers to TSST-I and the increased prevalence of nasal colonization (10%) with TSST-I–positive strains.[35,158,317,327] The racial distribution is less pronounced, with 87 percent occurring in whites, who make up 83 percent of the U.S. population, and the ratio of males to females with nonmenstrual TSS is closer to that in the general population.[253,254]

Data on risk factors (Table 64-2) was obtained for 559 nonmenstrual TSS cases reported to the CDC through 1986.[35] Associated infections or procedures included nonsurgical cutaneous and subcutaneous infections, 22 percent; childbirth or abortion, 15 percent; infections after a wide variety of surgical procedures, 15 percent; vaginal infections occurring at times other than during menses, 6 percent; vaginal contraceptive sponge use, 5 percent[78]; diaphragm use, 6 percent; and other or unknown sources of infection, 31 percent.

*References 35, 83, 132, 143, 167, 233, 268, 280.

TABLE 64-2 Risk Factors for Nonmenstrual Toxic Shock Syndrome

I. Colonization with toxin-producing *Staphylococcus aureus*
II. Absence of protective antitoxin antibody
III. Infected site
  A. Primary *S. aureus* infection
    Carbuncle
    Cellulitis
    Dental abscess
    Empyema
    Endocarditis
    Folliculitis
    Mastitis
    Osteomyelitis
    Peritonitis
    Peritonsillar abscess
    Pneumonia
    Pyarthrosis
    Pyomyositis
    Sinusitis
    Tracheitis
  B. After surgery: wound infection
    Abdominal
    Breast
    Cesarean section
    Dermatologic
    Ear, nose, and throat
    Genitourinary
    Neurosurgery
    Orthopedic
  C. Skin or mucous membrane disruption
    Burns (chemical, scald, etc.)
    Dermatitis
    Influenza
    Pharyngitis
    Postpartum (vaginal delivery)
    Superficial/penetrating trauma (insect bite, needle stick)
    Viral infection
    Varicella
  D. After surgical or nonsurgical foreign body placement
    Augmentation mammoplasty
    Catheters
    Diaphragm
    Sponge (contraceptive)
    Surgical prostheses/stents/packing material/sutures
  E. No obvious focus of infection (vaginal or pharyngeal colonization)

The three risk factors necessary for the acquisition of nonmenstrual disease include colonization (or acquisition) of a toxin-producing strain of *S. aureus*, absence of protective antitoxin antibody, and an infected site. TSS has been reported in association with *S. aureus* infections of almost every type, including primary staphylococcal infections and those occurring after surgery, infections associated with disruption of skin or a mucous membrane, and infections occurring after placement of a foreign body (see Table 64-2).[21] Numerous patients with

TSS have been reported for whom no obvious focus of infection was found.[35,254] Trauma or surgery in areas of the body frequently colonized with *S. aureus* (nose, skin, vagina) places individuals at enhanced risk for infection and subsequent TSS.

Host Risk Factors: General

Colonization with Exotoxin-Producing *Staphylococcus aureus*

For TSS to develop, an individual must be colonized with or acquire a strain of *S. aureus* that produces TSST-I or one of the staphylococcal enterotoxins. Evidence that staphylococcal enterotoxin B alone and, uncommonly, staphylococcal enterotoxin A or staphylococcal enterotoxin C alone, as well as TSST-I, may be responsible for the manifestations of TSS is compelling. Although many genotypically different strains of *S. aureus* possess the *tst*H gene, one clone has been isolated from 88 percent of menstrual cases and 54 percent of nonmenstrual cases.[218]

Absence of Protective Antibody Levels

A necessary factor for the development of TSS is the absence of protective antibody levels for the toxin (TSST-I or enterotoxin) produced by the isolate associated with TSS. Thus, more than 90 percent of women with menstrual TSS associated with TSST-I–positive strains have antibody titers of 1:10 or lower, whereas a titer of 1:100 or higher is considered protective.[21] Because most adults acquire these antibodies without the development of disease, absent antibody levels may be the result of lack of exposure to a toxin-producing organism or a lacunar inability to respond to the toxin. The failure of most patients, menstrual and nonmenstrual, to form antibody during convalescence may reflect this lacunar nonrecognition of staphylococcal protein antigen, or it may be a reflection of the superantigenic nature of these proteins and failure of the toxin proteins to be presented to the T-cell receptor as conventional antigens. In mice and one patient with TSS, in vivo TSST-I–induced proliferation was followed by hyporesponsiveness of TSST-I–responsive $V_\beta 2^+$ T cells.[186]

Interruption of a Mucosal or Skin Surface

Primary deep-tissue staphylococcal infections (e.g., osteomyelitis, pyarthrosis, pyomyositis,[4] endocarditis,[248,324] renal carbuncles, and bacteremia) rarely are associated with TSS. Most nonmenstrual cases occur in patients with an altered skin or mucosal surface. Examples of skin disruption associated with TSS include burns, insect bites, needle sticks, surgical incisions, and varicella. Cases of disruption of mucous membranes can be divided into those associated with respiratory mucosae and those associated with genital mucosae. Damage to respiratory mucosa may occur after nasal or other surgery, particularly with placement of stents or packing, in association with a viral respiratory tract infection such as influenza, or as a result of primary sinusitis, tracheitis, or a parapharyngeal abscess. Heavy colonization of the pharynx also has been associated with TSS. The vaginal mucosa may be damaged in numerous ways, including during placement of tampons or barrier contraceptives,[46,112,277] postpartum,[22,238] or after genital surgery or genital mucosal damage.[113,236,261] Heavy colonization of the vagina without any other apparent risk factor likewise has been associated with TSS.[254] These mucosal infections may provide the right conditions for production of TSST-I, including an aerobic environment, high carbon dioxide concentration, neutral pH, high protein and low glucose concentrations, and low to normal magnesium concentration.[212]

Presence of a Foreign Body

Tampons create an aerobic environment in the vagina, which normally is anaerobic. Tampons of three different types have been shown in humans to change the partial pressure of oxygen of the vaginal wall from an anaerobic environment to that of atmospheric air throughout the 90-minute interval after insertion.[319] Because oxygen is required for the production of TSST-I, researchers have suggested that tampons with enhanced absorbency allow the introduction of increasing concentrations of oxygen to enhance production of toxin.

Several alternative explanations for the role of tampons have been proposed. Tampons may remove vaginal substrates that normally inhibit the growth of *S. aureus*.[21] Another suggested role for tampons is that of inducing cervicovaginal ulceration. Such ulcers have been suggested to enhance bacterial growth or toxin absorption and expose submucosal fibronectin for binding of *S. aureus*.[73,75,322] Tampons can induce chronic cervicovaginal ulcers after long-term continuous use or superficial microulcerations after a brief insertion in otherwise healthy women. However, vaginal ulcerations of the type typically seen in TSS have been found during postmortem examination in women who had never used tampons. This finding suggests that vaginal ulceration, such as that seen in the esophagus and bladder in TSS, may be induced by TSST-I.[179]

Investigations to determine whether individual tampon components can induce the production of TSST-I in vitro have provided conflicting data, in part because no consensus exists on how best to test tampon components to determine their potential to increase risk for development of TSS. In one setting, although bacterial growth was unaffected, production of TSST-I varied from undetectable levels to 300 μg/mL, depending on the tampon studied.[21] Other investigators have found that most tampons tested were inhibitory to both bacterial growth and production of TSST-I and that none consistently increased the production of TSST-I.[21] Two studies clearly demonstrated enhanced production of TSST-I in vitro by the Rely tampon, which is composed of cross-linked carboxymethylcellulose and polyester foam.[241,272] The polyacrylate rayon and surfactant Pluronic L-92 included in some tampons brands increased the production of TSST-I under certain conditions.[211,272] Neither cotton nor rayon amplifies the production of TSST-I in vitro, nor do cotton tampons adsorb TSST-I or prevent its

production.[241,272] Thus, cotton tampons cannot be claimed to be safer than cotton and rayon combinations, although this contention has been disputed.[303]

Implanted foreign material, including sutures, central venous lines, metallic or polymeric implants, Teflon splints, and gauze packing, have been documented repeatedly to enhance the risk for acquiring bacterial infection.[21,160] These infections are characterized by limited spread beyond the tissues in immediate contact with the implants, poor response to antibiotics, and poor healing without removal of the foreign material. Two important differences between TSS and other *S. aureus* infections related to foreign material are the added ability of the organism to produce a toxin that readily disseminates and the limited ability of these strains to produce inflammation.[114] Researchers have suggested that the increased number of surgically associated TSS cases may be related to the number of implanted materials.[138]

The enhanced risk for acquiring infection by *S. aureus* in the presence of foreign material has been defined experimentally in animals. Rats remained asymptomatic after the subcutaneous injection of more than 1×10^6 colony-forming units of *S. aureus*, whereas 3×10^2 colony-forming units invariably led to infection in the presence of a suture. In addition, the ability of sutured tissue to resist infection varies with the kind of material implanted, particularly its physical or chemical configuration. For example, bacterial adherence is eightfold higher for braided sutures of silk, silicone-heated blue polyester, and absorbable polyglycolic acid than for monofilament nylon.[160]

Other Potential Host Risk Factors

Other factors have been examined in an attempt to identify individuals who may be at increased risk for the development of TSS.[86] Some of these factors have included human leukocyte antigen type,[21] neutrophil function,[145] adherence of *S. aureus* to vaginal epithelial cells,[20] alteration of the cervicovaginal flora,[21] hormonal factors, and personal hygiene practices.[86] Of all of these factors, those of potential importance included cervicovaginal flora and hormonal factors.

Vaginal co-colonization with *S. aureus* and Enterobacteriaceae has been postulated to enhance the risk for development of TSS.[62] Prospective examination of 495 healthy women revealed a 7 percent vaginal colonization rate for *S. aureus* TSST-I–positive strains. Women who were colonized with toxin-producing *S. aureus* had higher rates of colonization with *Escherichia coli* or other Enterobacteriaceae. *E. coli* isolation rates were 54 percent in women with TSST-I–positive isolates, 15 percent in women with TSST-I–negative isolates, and 11 percent in women with no *S. aureus*. Among the 14 patients with TSS monitored in this study, 9 had *E. coli* as well as TSST-I–positive *S. aureus* co-isolated during the acute TSS episode. The significance of these observations and their relationship to postulated roles for endotoxin in TSS are not clear.

The results of early case-control studies suggested that oral contraceptive steroids might have an effect on vaginal *S. aureus* organisms that may produce or release TSS-associated toxins[83,195,233] and that this effect was protective. However, one case-control study found no protective effect or enhanced risk associated with oral contraceptive use.[132] Hormonal control is known to be responsible for numerous cyclic changes in vaginal pH and flora, but the role of hormonal factors in the pathogenesis of TSS has not been examined well.

HISTOPATHOLOGY

The histopathologic findings on postmortem examination support the concept that TSS is a toxin-mediated disease.* Striking histopathologic similarities exist between patients with TSS and those with "scarlet fever" reported in 1936.[33] Typically, a total absence of tissue invasion by bacteria and minimal evidence of an inflammatory reaction in most organs are noted. Findings thought to be due to a direct effect of the toxin or mediators (or both) and unrelated to hypoperfusion include subepidermal ulcerations in the cervix, vagina, esophagus, and bladder; depletion of lymphocytes in lymph nodes; a subepidermal cleavage plane in the skin; and mild inflammatory changes in the kidney, liver, heart, and muscle.

Cervicovaginal ulcerations are the only characteristic lesions noted in the genital tract of patients with fatal menstrual TSS, and such ulcers have been found in a patient with menstrual TSS who had never used tampons.[179,238] The ulcerations are superficial. Capillary vasodilation and thrombosis with inflammation of the mucosa are present, but no deep-tissue bacterial invasion is seen. The layer of vacuolization and separation in the ulcers occurs beneath the basal layer. The same type of ulcer also has been found in the bladder and esophagus, which suggests that these ulcerations may be caused by the toxins or mediators and not by the use of tampons.

Although the myocardium was described as normal in one postmortem series of TSS, in another series of eight fatal cases, all patients had evidence of focal round-cell infiltration with variable degrees of congestion, edema, and hemorrhage.[179,235] Myxoid degeneration was found in all heart valves from four patients in one series. Sections of skeletal muscle have demonstrated only congestion, edema, focal hemorrhage or fiber necrosis, and a mild acute inflammatory infiltrate.

Varying degrees of triaditis or periportal lymphocytic inflammation have been the most consistent findings in the liver; centrilobular congestion with necrosis and mild cellular degeneration also has been described.[155] In the kidney, toxin-mediated mononuclear interstitial nephritis may result from perivasculitis of the adventitia of the renal venules, lesions that probably precede the development of hypotension-induced acute tubular necrosis. The most characteristic findings in the spleen and lymph nodes have been lymphocyte depletion; inactive hypocellular, hypoplastic lymphoid follicles with edema; marked histiocytosis in the interfollicular areas; and hemophagocytosis.

*References 1, 26, 33, 179, 235, 326.

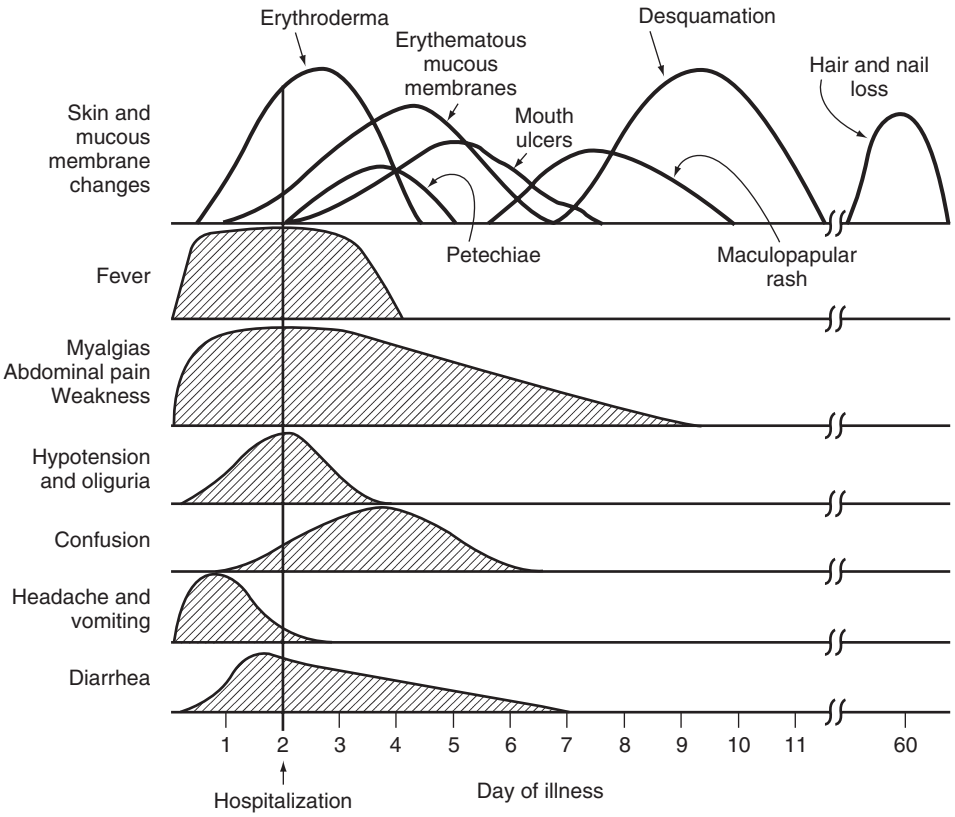

FIGURE 64-3 ■ Composite drawing of the major systemic skin and mucous membrane manifestations of toxic shock syndrome. (From Chesney PJ, Davis JP, Purdy WK, et al. The clinical manifestations of toxic shock syndrome. *JAMA* 1981;**246**:741–8. Copyright 1981, American Medical Association.)

Perivascular lymphocytic infiltrates and bullae that separate at the basement membrane are characteristic of the early skin changes in TSS.[8,10,145] No evidence of vasculitis has been reported.

CLINICAL SPECTRUM

Acute Phase: Moderate to Severe Disease*

The manifestations in TSS are the result of release of endogenous mediators after monocyte and T-cell activation by TSST-I or the staphylococcal enterotoxins. The physiologic changes induced by this dysregulation of the immune system are striking in their rapidity of onset and progression and the involvement of almost all body tissues and organs.

Multisystem end-organ damage secondary to loss of peripheral vascular resistance, loss of intravascular volume as a result of endothelial damage and the capillary leak syndrome, and interstitial edema constitute the most important mediator-induced changes of TSS. Prolonged hypotension, interstitial edema, and vascular congestion additionally may result in ischemic organ damage.

The onset of illness in patients with moderate to severe disease is abrupt, with signs and symptoms including fever, chills, malaise, headache, sore throat, myalgia, muscle tenderness, fatigue, vomiting, diarrhea, abdominal pain, and orthostatic dizziness or syncope (Figs. 64-3 and 64-4).

During the first 24 to 48 hours, diffuse erythroderma, severe vomiting and watery diarrhea (often with incontinence), decreased urine output, cyanosis, and edema of the extremities may be noted. Some patients may have purpura fulminans.[172] Cerebral ischemia and edema rapidly result in somnolence, confusion, irritability, agitation, and occasionally hallucinations, even in individuals without hypotension. Patients with TSS have had signs and symptoms of encephalopathy, cerebral infarction, meningismus,[15,25,140,184,285] and the cauda equina syndrome.[8]

On initial physical examination, fever, tachycardia, tachypnea, hypotension, erythroderma (generally not seen in patients with severe hypotension or in those without T cells)[164] (see Fig. 64-4A), and muscle tenderness are noted in conjunction with peripheral cyanosis and edema, conjunctival hyperemia, subconjunctival hemorrhages (see Fig. 64-4B), beefy red edematous mucous membranes, somnolence, disorientation, and agitation. In menstrual TSS, edema and erythema of the inner aspect of the thighs and the perineum may be noted in conjunction with normal findings on uterine and adnexal examination. In nonmenstrual cases, vaginitis or another focus of infection will be present. In most postoperative cases, the surgical wound is not inflamed. If

*References 57, 63, 84, 120, 122, 144, 196, 280, 305, 306.

FIGURE 64-4 ■ Skin and mucous membrane manifestations present at the onset of toxic shock syndrome (TSS). **A,** Diffuse erythroderma in a 7-year-old child with nonmenstrual TSS associated with osteomyelitis of the fibula. **B,** Bulbar conjunctival infection and subconjunctival hemorrhage in a 24-year-old woman with nonmenstrual TSS. (**B** from Bach MC. Dermatologic signs in toxic shock syndrome: clues to diagnosis. *J Am Acad Dermatol* 1983;**8**:343–7.)

erythroderma is present, it will be most intense surrounding the infected focus.

Surgical wounds and some abscesses colonized or infected with *S. aureus* and responsible for postoperative or nonmenstrual TSS may have minimal or no signs of inflammation.[6,17,100,254] The production of TNF-α by macrophages in response to TSST-I inhibits neutrophil mobilization in vitro,[114] which may provide an explanation for the absence of signs of inflammation. The incubation period for postoperative or postpartum TSS may be as short as 12 to 48 hours. Relatively few cases have been associated with deep-tissue infection.[21]

Laboratory Findings

Laboratory tests reflect the endogenous cytokine release, shock, and organ failure associated with TSS. Leukocytosis may not be present, but the proportion of neutrophils generally exceeds 90 percent. The proportion of immature neutrophils usually is 25 to 50 percent of the total number of neutrophils and is associated with absolute lymphopenia. Thrombocytopenia and anemia are present during the first few days and frequently are accompanied by prolonged prothrombin and partial thromboplastin times. Disseminated intravascular coagulation may be present. Sterile pyuria and cerebrospinal fluid pleocytosis are indicative of generalized involvement of the mucous membranes and serosal surfaces. Elevated blood urea nitrogen and creatinine levels reflect kidney damage,[57] and abnormalities in tests of liver function reflect liver damage and acute cholestasis.[135,155] Hypoproteinemia and hypoalbuminemia reflect capillary leak, and profound hypocalcemia may reflect both hypoproteinemia and high serum levels of a calcitonin-like material.[58,288] Muscle involvement is noted by an elevated creatine phosphokinase level, and the hypophosphatemia that occurs despite impaired renal function is unexplained.[14,58] Most of these test results will return to normal within 7 to 10 days of disease onset. *S. aureus* will be cultured from the cervix or vagina in more than 85 percent of patients with menstrual TSS and from the focus of infection in patients with nonmenstrual TSS. Positive blood culture results are rare findings.[74] Antibody to TSST-I or to the staphylococcal enterotoxins

will be absent at the onset of disease in more than 85 percent of patients.*

Diagnosis

Application of the case definition for a single episode[323] or recurrent episodes[84] to the patient's illness is currently the only way to confirm the diagnosis (see Table 64-1). Aggressive attempts to find the focus of *S. aureus* include culture of the cervix and vagina in patients with menses-associated illness and culture of other potentially infected sites in patients with nonmenstrual illness. *S. aureus* isolates can be examined for their ability to produce TSST-I, although such testing seldom is indicated. This test is of limited usefulness for nonmenstrual cases because TSST-I is produced by only 40 to 60 percent of *S. aureus* isolates from such patients.[131] Isolates from these patients can be examined for the presence of the other enterotoxins. Acute and convalescent sera can be tested for the presence of antibodies to TSST-I and the other enterotoxins.[149] Elevated levels of anti–TSST-I in the acute-phase serum of a patient with menstrual-associated TSS would be highly unusual.

In most instances, toxin detection tests are of value only for research or for patients with chronic recurrent or atypical disease. Genes for TSST-I and the enterotoxins have been detected in *S. aureus* strains by polymerase chain reaction† and hybridization techniques.[161,220,231] A noncompetitive enzyme-linked immunosorbent assay allows quantitation of TSST-I in clinical samples.[199,213] Reversed passive latex agglutination has been used in hospital laboratories to detect TSST-I and in research laboratories to detect enterotoxins.[89,111] Research laboratories also have developed techniques for detecting the selective expansion of $V_\beta 2$-positive T cells as evidence of host response to superantigenic toxins.[61,191]

Treatment

The four general principles of treatment of TSS (Table 64-3) are (1) identification and drainage of the focus of toxin production, (2) identification and susceptibility testing of the organism, (3) administration of antimicrobial therapy to block synthesis of the toxin and kill *S. aureus*, and (4) management of the systemic multiorgan actions of the toxins or mediators.‡

Location and Drainage of the Infected Site. The focus of infection should be identified rapidly, any foreign bodies should be removed, and the site should be drained or irrigated completely, even if it does not appear to be inflamed. Performing this procedure is of utmost importance because perpetuation of even a small, undrained focus of infection may result in serious clinical consequences. If TSS occurs in the immediate postoperative period, the wound should be assumed to be the source of infection, regardless of its benign appearance.

TABLE 64-3 **Therapeutic Principles for Management of Toxic Shock Syndrome**

1. Identify the focus of infection: Débride and irrigate extensively and remove any foreign material
2. Isolate the organism for antimicrobial susceptibility studies
3. Administer parenteral antimicrobial therapy
   Stop enzyme or toxin production with a protein synthesis inhibitor (e.g., clindamycin, erythromycin, gentamicin)
   and
   Eradicate the organism with a bacterial cell wall inhibitor (e.g., β-lactamase–resistant antistaphylococcal antimicrobial agent)
   Consider intravenous immunoglobulin to provide antitoxin antibodies for a subset of patients, including those with the following:
   - Disease refractory to initial fluid replacement and several hours of vasopressor support
   - A focus of infection that cannot be drained
   - Persistent oliguria despite massive fluid replacement and in the presence of pulmonary edema

   Consider additional measures, such as the following:
   - Methylprednisolone to suppress cytokine production and the inflammatory response
   - Fluid therapy to maintain adequate venous return and cardiac filling pressure and to prevent end-organ damage
4. Anticipate the management of multisystem organ failure

Identification and Susceptibility Testing of the Organism. The incidence of community-acquired MRSA infections has increased dramatically during the past 10 years.[134] These MRSA strains differ from the long-standing hospital-acquired MRSA strains in that they are acquired in the community by well individuals with no risk factors and are not multiresistant. Most strains are susceptible to clindamycin. Because MRSA strains are fully capable of producing TSST-I,[96,274] community-acquired menstrual and nonmenstrual cases of TSS may be caused by either community or multiresistant hospital-acquired strains of MRSA.[103] To date, vancomycin-resistant strains of *S. aureus* are rare findings.[50,148] Thus, every effort should be made to obtain the organism for susceptibility testing.

Administration of Antimicrobial Agents. Administration of antistaphylococcal antibiotics is indicated to eradicate organisms and prevent recurrence.[83,84] The infection may be a superficial or deep-tissue infection and may be associated with *S. aureus* bacteremia or bacteriuria. Antistaphylococcal antimicrobial agents should be administered intravenously at maximal doses for age as soon as possible. Once the patient is stable, high doses of an oral antimicrobial agent to which the organism is susceptible can be given to complete a total course of 10 to 14 days.

Subinhibitory concentrations of the protein synthesis inhibitors clindamycin, erythromycin, clarithromycin, kanamycin, gentamicin, tetracycline, and linezolid have been shown to suppress TSST-I production in

*References 22, 30, 71, 263, 283, 325.
†References 19, 162, 183, 198, 223, 275.
‡References 55, 63, 83, 84, 120, 122, 144, 196, 268, 290, 311.

vitro.[95,240,271,293] In one study, clindamycin concentrations 1/64 the minimal inhibitory concentration (MIC) were effective in totally blocking TSST-I production.[240] In a mouse model of myositis caused by *S. pyogenes*, another superantigen-producing organism, clindamycin and erythromycin were more efficacious than penicillin.[104,291,294]

Data suggest that subinhibitory concentrations of β-lactam antibiotics actually may *increase* TSST-I production by *S. aureus*. At a concentration of half the MIC, nafcillin can *increase* toxin production 10-fold more than under control conditions.[5] The effect is not seen with vancomycin, another cell wall–active drug, thus suggesting specificity beyond merely a cell wall effect. Coadministration of a protein synthesis inhibitor with a β-lactam antibiotic blocks the effect.[5]

The choice of initial empirical antimicrobial therapy has become more complex as a result of an increase in the number of community-acquired MRSA infections and the spread of multiresistant MRSA strains in hospitals.[166] In the past, the most effective initial empirical therapy was a combination of a β-lactamase–resistant penicillin and clindamycin. With recent concern for MRSA strains causing TSS, consideration should be given to initiating therapy with vancomycin and clindamycin. As an alternative for MRSA coverage, linezolid has been used successfully for treatment of staphylococcal TSS.[293] Once the results of susceptibility testing of the organism are available, therapy can be adjusted appropriately.

Management of Systemic Multiorgan Actions of the Toxins or Mediators

Intravenous Immunoglobulin and Toxin Inhibition. Given the high prevalence of antibodies to TSST-I in adults,[317] high levels of antibody to TSST-I are present in intravenous immunoglobulin (IVIG) preparations.[54,59,200,238,301] In animal models of TSS, human IVIG given at the time of inoculation of TSST-I–positive *S. aureus* prevented the development of TSS. When IVIG was administered 8 hours later, it decreased the mortality rate from 90 percent in control rabbits to 16 percent. When IVIG was given 29 hours after administration of TSST-I, the increase in survival rates in the IVIG-treated animals still was significant. No adverse reactions were noted in the treated animals, and no evidence was found of disease mediated by the formation of antigen-antibody complexes.[200-204] Monoclonal antibodies to TSST-I can prevent the development of manifestations of TSS in the rabbit model completely.[31,242,278]

High concentrations of antibodies to TSST-I and the staphylococcal enterotoxins (type A; type B; types C 1, 2, and 3; type D; and type E) have been demonstrated in pooled IVIG.[54,59,238,301] These antibodies may inhibit the binding of toxins to MHC class II antigen-processing cells or interfere with presentation of toxin by these cells to the T-cell receptor. As a result, production of TNF-α and TNF-β is inhibited by these antibodies in vitro in an apparent toxin-specific manner.[301]

The results of these in vitro studies have supported the use of IVIG for staphylococcal TSS.[257] Anecdotal case reports have indicated a beneficial effect on staphylococcal[16,59,67,202,225,238] TSS in humans. Because IVIG is expensive and most patients respond rapidly once standard therapeutic measures are initiated, some authors would reserve IVIG for patients with an inaccessible focus of infection or for those who continue to deteriorate after receiving fluid and vasopressor support for several hours (see Table 64-3). The dose most often used has been 400 mg/kg given as a single dose over the course of several hours. This dose results in a serum antibody titer of greater than 1:100, much higher than that appearing to provide immunity to TSST-I.[238] Some studies have used IVIG doses of up to 2 g/kg.[77] Because early administration of IVIG possibly could blunt the immune response to TSST-I or other toxins and increase the possibility of a recurrent episode, the potential risks and benefits of this form of therapy must be considered for each patient.

The role of endotoxin in the pathogenesis of TSS is unclear. The failure of polymyxin B and anti-J5 antiserum to alter the course of TSST-I–positive TSS in a rabbit model suggests that endotoxin may not be an important mediator of TSS.[203] Early and sporadic case reports of TSS suggested therapeutic benefit with naloxone,[65] calcium,[238] and exchange transfusion in severely ill patients unresponsive to the usual forms of therapy.

Corticosteroids. Short courses of methylprednisolone or dexamethasone, if given early in the course of the disease, have been associated with a reduction in the duration of fever and the severity of illness but no reduction in mortality rates.[307] In vitro, dexamethasone has been shown to downregulate TSST-I–induced cytokine production.[171] Because no controlled prospective study has demonstrated efficacy, the use of steroids probably should be restricted to hypotensive patients unresponsive to fluid resuscitation, antimicrobial agents, and IVIG.

Fluid Replacement. The most important aspect of the nonspecific treatment of symptomatic patients is fluid replacement.[304] Intravascular volume and cardiac filling pressure must be restored rapidly to achieve adequate tissue perfusion. Because of the ongoing capillary leakage, this fluid replacement may far exceed the estimated fluid requirements based on calculated maintenance and fluid deficit volumes. Some adults have required vasopressors and as much as 12 L of fluid during the first 24 hours to stabilize the circulating blood pressure. Pleural, pericardial, and peritoneal effusions and interstitial edema inevitably occur as a result of the continued vascular capillary fluid leak. Close monitoring in an intensive care unit will facilitate treatment of intravascular fluid loss, myocardial dysfunction, hemodynamic derangements, pulmonary edema, acute respiratory distress syndrome, acute renal failure, encephalopathy, and disseminated intravascular coagulation.

Subacute Phase: After Treatment Is Initiated

Once treatment is initiated, response usually is rapid, with defervescence within 48 hours. The hemodynamic changes are observed initially as tachycardia, decreased systemic vascular resistance, decreased central venous pressure, hypovolemia, normal pulmonary artery wedge pressure, and an increased cardiac index.[13,38,72,120,123] Once

aggressive fluid therapy has been initiated, myocardial edema and potential failure, along with pulmonary and cerebral edema in the face of renal failure, become the most critical management issues. The reasons for myocardial failure are unclear but probably are related to perivascular inflammation of the coronary vessels, edema, and postulated myocardial depressant factors.[179,235] TSST-I has been shown to inhibit systolic function in isolated rabbit atria, though at higher than usual circulating concentrations.[229] Arrhythmias may result from myocardial damage or electrolyte abnormalities.[197,259]

During the decompensated stage of myocardial dysfunction, the cardiac index falls and pulmonary wedge pressure increases, with both left atrial and ventricular and diastolic diameters being at the upper limits of normal.[120] Reversible electrocardiographic findings include sinus tachycardia, diffuse loss of voltage, flattened T waves, and diffuse nonspecific ST-T wave changes. If fatal arrhythmia does not occur during the decompensated stage, the toxic cardiomyopathy is reversible and rarely results in permanent changes. This process is similar to "stunned myocardium," a transient, postischemic myocardial dysfunctional state.[72]

Pulmonary edema and acute respiratory distress syndrome occur commonly in patients with severe disease when massive fluid replacement is necessary and the capillary leak syndrome continues in the lungs. Pulmonary edema appears rapidly once fluid replacement is initiated and often necessitates intubation and respirator management for several days.[304]

Forms of TSS-associated acute renal failure include prerenal azotemia and both nonoliguric and oliguric renal failure.[57] The type of renal failure manifested may depend on the degree of intravascular volume depletion. Unless severe acute tubular necrosis necessitates temporary hemodialysis, repletion of intravascular volume usually results in rapid restoration of renal function and, ultimately, diuresis. Permanent renal damage is an extremely rare event.[54]

The gastrointestinal, musculoskeletal, and hepatic changes resolve rapidly. Sequelae associated with these changes are rare, except for prolonged muscle weakness.[54,85] Joint manifestations generally are self-limited.[21,125]

Management of fluids, electrolytes, and metabolic acidosis in patients with TSS is complex. Although tetany is a rare occurrence, severe hypocalcemia may be life-threatening and should be corrected.[58,238,288] Most patients require potassium replacement and management of metabolic acidosis. The use of colloid for fluid replacement and removal of the toxin stimulus for capillary leak syndrome ultimately correct the hypoproteinemia.

The typical dermatologic manifestations follow a predictable sequence (see Fig. 64-3). A dandruff-like flaky desquamation begins on the trunk and extremities 5 to 7 days after the onset of symptoms. From days 10 to 12 and for as long as a month, the characteristic full-thickness desquamation of the fingers, toes, palms, and soles takes place (Fig. 64-5). A variety of atypical dermatologic manifestations, including petechiae and subepidermal bullae, have been described.[10,108,151]

Early in the acute phase, many patients exhibit desquamation of the mucous membranes, which is particularly painful when the oral mucous membranes are involved.[55] In addition, a small number of patients will have reactivated herpes simplex virus type 1 or 2 lesions with the acute illness.[55] A late-onset pruritic maculopapular rash with edema and low-grade fever probably unrelated to antimicrobial therapy occurs in more than 50 percent of menses-associated cases within 7 to 14 days of disease onset.[55,90] The cause of this late-onset rash is unknown.

Telogen effluvium, a common sequela, is a nonspecific response to trauma, sepsis, or stress that results in disturbed metabolism and keratinization of the hair follicles and nails. The hair follicles appear to transform prematurely from the growth, or anagen, phase to the telogen, or resting, phase. Hair and nail loss occurs 4 to 16 weeks after onset of the illness, with restoration taking place in 5 to 6 months.[21,24,55]

The hematologic system seldom is involved with major complications in TSS. Although disseminated

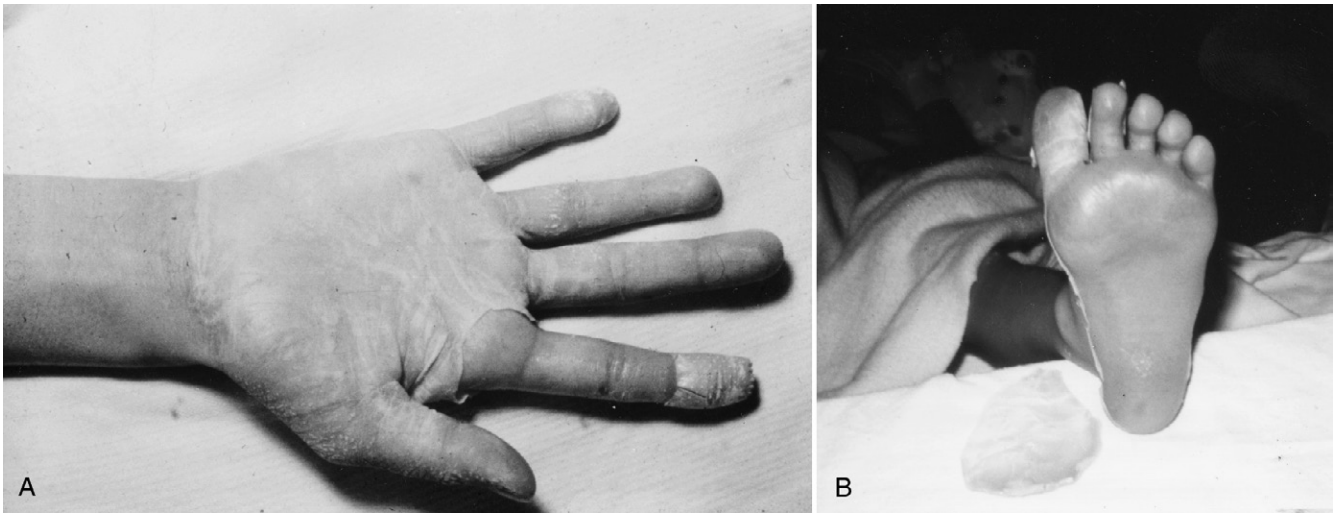

FIGURE 64-5 ■ Universal full-thickness desquamation of the hands **(A)** and feet **(B)** first noted 7 to 14 days after disease onset and persisting for up to 30 days.

intravascular coagulation may be present, gastrointestinal, uterine, or cerebral bleeding rarely occurs. Thrombocytopenia may be present initially in patients with disseminated intravascular coagulation; thrombocytosis is characteristic of the recovery phase. Mild to moderate normocytic, normochromic anemia, which probably is dilutional and a result of suppressed red blood cell synthesis, develops in virtually all moderately to severely ill patients with TSS and resolves during convalescence.[53,55] Hypoferrinemia occurs commonly.[53]

The relatively common toxic or ischemic encephalopathy, rarely complicated by seizures, resolves slowly during the first 4 to 5 days of hospitalization.

Outcome and Sequelae

Death associated with TSS usually takes place within the first few days of hospitalization, but it may occur as late as 15 days after admission. Fatalities have been attributed to refractory cardiac arrhythmias, cardiomyopathy, irreversible respiratory failure, and, rarely, bleeding caused by coagulation defects.[179,235] The duration of circulation of toxins and mediators and the associated hypotension may be the best predictors of the severity of the end-organ damage.

After being discharged, many patients experience prolonged fatigue, muscle weakness, and pain.[54] Sequelae that appear to be related to a prolonged period of hypotension have included chronic renal failure, gangrene, and telogen effluvium.[85,163,206,262,266] Other sequelae, such as neuropsychological abnormalities, prolonged myalgia and weakness, carpal tunnel syndrome, chronic dermatitis, Raynaud syndrome, and new allergies are explained less easily. Impaired memory and concentration have been found in patients who did not require any therapy other than intravenous fluids to restore their blood pressure.[262] In one center, patients with nonmenstrual TSS had more serious short- and long-term neuropsychological complications than did patients with menstrual TSS.[163] Sequelae related primarily to the neuromuscular system have resulted in speculation that the TSS-associated toxin may have a direct effect on nerve or muscle tissue.

One study compared the sequelae in 183 women with TSS and 366 control women hospitalized for appendicitis and appendectomy and matched for age, race, and duration of follow-up.[85] In contrast to controls, women with TSS were significantly more likely to report sequelae involving fatigue, hair loss and nail changes, problems with concentration and memory loss, emotional changes, and multiple organ systems (conditions involving the joint, cardiac, muscle, and genitourinary systems). Fertility patterns and pregnancy outcomes were similar in controls and women with TSS, both before and after the index illness.[85]

Recurrences

TSS carries a high rate of recurrence in patients with inadequately treated disease.* A small number of patients with menstrual disease and repeated tampon exposure have experienced as many as 6 to 12 recurrences. Recurrences associated with menstrual disease have no predictable pattern. In most patients the first episode is the most severe. However, asymptomatic menses may occur between symptomatic episodes, and the most severe episode may occur after one or more milder episodes.[84] Antistaphylococcal antimicrobial therapy for 10 to 14 days and discontinuation of tampon use reduces the rate of recurrence significantly.[84] Recurrences after nonmenstrual TSS are well described and usually are associated with inadequate initial therapy.[6,163,238]

The absent or delayed antibody response to TSST-I found in both menstrual and nonmenstrual TSS patients probably accounts for the continued susceptibility to TSS and high recurrence rate.* The fact that superantigenic toxins are not processed by antigen-processing cells and T lymphocytes as conventional antigens may provide an explanation for the poor convalescent antibody response to these antigens.[170]

Culture-negative, menses-associated recurrent episodes of TSS continue to be found in a small number of patients despite discontinuation of tampon use and administration of appropriate antimicrobial therapy during the acute episode.[84] Administration of an oral β-lactamase–resistant antistaphylococcal antimicrobial agent during menses has been tried in an attempt to prevent these recurrences but is not always successful. In recurrent cases resistant to this form of prophylaxis, consideration could be given to the untested and empirical use of rifampin, clindamycin, erythromycin, or IVIG (if the patient has no antibody to TSST-I) or to the use of an oral contraceptive.[195]

Atypical Manifestations

Mild Disease

Recognition of mild episodes of TSS is particularly important in patients with menstrual TSS because repeated use of tampons and the risk for recurrence are episodic.[56,92,310] Patients with mild or severe menstrual TSS typically do not form antibody to TSST-I during convalescence[21,30,263,283,325] and, without appropriate therapy, may have one or more recurrences. Such recurrences may be mild or severe.[83,84,196] Patients with nonmenstrual TSS also do not form antibodies in convalescence. A mild episode may be recognized only in retrospect, after desquamation, a recurrent episode, or both develop.[238]

The presence of any combination of fever, headache, sore throat, diarrhea, vomiting, orthostatic dizziness, syncope, and myalgia in a menstruating woman or an individual with a potential *S. aureus* infection should raise suspicion of TSS. A positive culture for *S. aureus* is helpful but not diagnostic because *S. aureus* may be cultured from the cervix or vagina in up to 33 percent of menstruating women.[190,245] Other laboratory data usually do not reflect multisystem involvement in mild disease, and assays for TSST-I in body fluids are investigational.[199,316] Support for the diagnosis of mild TSS depends on the

*References 6, 55, 83, 84, 195, 296.

*References 21, 30, 186, 263, 283, 296, 325.

constellation of findings present, including subsequent desquamation of the palms, soles, toes, or fingers; demonstration that *S. aureus* isolates from the site of infection produce TSST-I or an enterotoxin; absence of antibody to TSST-I or enterotoxins in acute-phase serum; and recurrent disease, if it develops.

Nonmenstrual Disease

The incidence of postoperative cases of TSS after all types of surgery has been estimated to be 3/100,000 population.[136] For ear, nose, and throat surgery, the incidence is higher (16.5/100,000 population).[157] A striking feature of most postoperative cases with rapid onset is the absence of any signs of wound infection.[17,136,251,264] The mean time from surgery to the onset of symptoms is 2 to 4 days.[17] Nosocomial acquisition of TSST-I–positive organisms rarely has been documented for postoperative cases.[9,164,207] A wide variety of types of surgery have been associated with TSS.*

Burn wounds provide a particularly rich environment for growth of *S. aureus* and the production of toxins.† In burn centers, TSS occurs predominantly in young children with small burns.[106] In one large pediatric burn center, *S. aureus* normally was not cultured from any site on admission.[59] However, it was acquired within a few days of admission and became the most common wound pathogen. Of all wound isolates of *S. aureus*, just 16 percent produced TSST-I. Only 50 percent of the children had antibodies to TSST-I on admission, which reflects the low prevalence of antibodies in this population.[317] Of blood products administered, 76 percent had antibodies to TSST-I, and seroconversion occurred in children receiving these products. A toxic shock–like syndrome developed in 13 percent (7/53) of children, only one of whom had TSST-I–producing *S. aureus* isolated from the wound. Staphylococcal enterotoxins A and B were produced by isolates from three patients. In burn patients, issues regarding the use of prophylactic antibiotics and occlusive dressings and enhancement of TSST-I production by topical antimicrobial agents are unsettled.[105] The mortality rate associated with TSS in children with burns may be as high as 57 percent.[127] Skin disrupted in any way, including disruption by varicella or a tattoo, also may be a focus for TSS.[21,34,70]

Patients whose anterior nares have been colonized by *S. aureus* are at particular risk for the development of TSS when the respiratory mucosa is disrupted by surgery, trauma, or a respiratory tract infection such as influenza.[21,47,69,185,312] TSS has been reported in association with sinusitis,[21,116] pharyngitis,[21,267] parapharyngeal abscesses,[267] tracheitis,[21,76,99,130,222] pneumonia,[21,79] rubeola,[298] and submandibular space abscesses. TSS occurring after ear, nose, and throat surgery has been associated with the use of nasal splints and packing materials and in part may be the result of interruption of the ciliary blanket.[3,21,126,211,332]

After orthopedic procedures or in association with bone and joint infections, the clinical manifestation of TSS may be confusing as a result of the intense and generalized myalgias associated with TSS, which may be misinterpreted as postoperative musculoskeletal symptoms. The wounds usually appear to be benign.[21,210,246,302] Infected abrasions under casts may be focal sites of TSST-I production.[287]

In one center, when cases of nonmenstrual TSS were compared with menstrual cases, patients with nonmenstrual disease were found to have a delayed onset of symptoms after the precipitating event, more frequent central nervous system manifestations, less frequent musculoskeletal involvement (myalgia and arthralgia), and a higher degree of anemia.[163] Recurrences occurred in both groups in untreated patients, and mortality rates were not different. Clinical differences between the two categories may be related to differences in the types of toxins produced. TSST-I was produced with comparable frequency in both groups. Staphylococcal enterotoxin A was produced less often by nonmenstrual isolates, and menstrual isolates more often produced both TSST-I and staphylococcal enterotoxin A. Staphylococcal enterotoxin B was produced more frequently by nonmenstrual isolates.

Recalcitrant Erythematous Desquamating Disorder

An atypical, subacute variant of TSS has been described in patients with acquired immunodeficiency syndrome (AIDS) and labeled recalcitrant erythematous desquamating disorder. *S. aureus* strains producing TSST-I or staphylococcal enterotoxins A or B have been isolated from patients with AIDS and prolonged erythema, extensive cutaneous desquamation, hypotension, tachycardia, and multiple and variable organ involvement.[67,98,169] The illness is recalcitrant, prolonged, and characterized by multiple recurrences. In one patient, elevated levels of TNF and IL-6 were found during severe episodes. When antitoxin antibodies have been measured during recurrence, they have been undetectable. Two patients responded well to IVIG.[67,169] Patients with the combined cellular and humoral immunodeficiencies of AIDS may be at particular risk for the development of severe, frequent, and prolonged recurrent episodes of TSS.[67,98,119,169,286] TSST-I and the enterotoxins may activate human immunodeficiency virus type 1 (HIV-1) gene expression in vivo, as has been observed in vitro.[128]

Neonatal Toxic Shock Syndrome–Like Exanthematous Disease

A toxic shock–like illness related to TSST-1–producing MRSA, neonatal toxic shock syndrome–like exanthematous disease (NTED), was initially described in neonates in Japan[300] and subsequently recognized in France.[315] These infants present in the first week of life with a combination of a generalized erythematous macular rash, thrombocytopenia, elevated acute-phase reactants, and fever. Patients are colonized with MRSA of a single clonal type that produces TSST-I and staphylococcal enterotoxin C.[167] Although no focus of infection is present and no exotoxins are detectable in serum, analysis

*References 21, 40, 215, 228, 256, 281, 284, 313.

†References 11, 59, 66, 105, 127, 129, 133, 147, 188, 192, 321.

of T cells shows selective expansion of $V_\beta 2$-positive T cells during the acute phase as a result of selective activation of TSST-I–reactive T cells.[299,300] Maternal IgG antibody to TSST-I plays a protective role in preventing NTED.[299] Although complications occur in premature neonates, term infants typically recover within 5 days without active treatment. The limited disease in contrast to TSS has been attributed to the minute amount of exotoxin from colonized sites and the high susceptibility to induction of anergy in the immature T cells of neonates.[299,300]

Streptococcal Toxic Shock Syndrome

An increase in the incidence and severity of *S. pyogenes* was recognized in the 1980s. These infections included septicemia, necrotizing fasciitis, and a streptococcal toxic shock–like syndrome. In 1993, the Working Group on Severe Streptococcal Infections proposed a consensus definition for streptococcal TSS with clinical criteria, including isolation of group A streptococci from a normally sterile site, hypotension, and two or more of the following: renal impairment, coagulopathy, liver dysfunction, acute respiratory distress syndrome, erythematous macular rash, and soft tissue necrosis.[329]

Since the 1990s, the rate of severe streptococcal infections has stabilized to 2 to 4 cases/100,000 per year. In adults, streptococcal TSS comprises 10 to 15 percent of severe streptococcal infections, with a mortality rate of 30 to 70 percent.[80,91,178] Streptococcal TSS is rare in children and carries a lower mortality rate than in adults. A multicenter study covering a U.S. population of 29.7 million from 2000 to 2004 identified 5400 cases of invasive group A streptococcal infection (3.5/100,000 per year), including 572 children younger than 10 years of age. Of the 572 children with severe streptococcal infection, 26 (4.6%) developed streptococcal TSS with a 7.2 percent case-fatality rate. [227]

Streptococcal TSS is similar to staphylococcal TSS in that it is mediated by superantigenic toxins and results in endothelial damage, hypotension, and multisystem organ involvement. It differs from staphylococcal TSS in numerous respects, including a slower onset over the course of several days; usual absence of vomiting, profuse diarrhea, and conjunctival infection; frequent absence of erythroderma or the presence of only a sandpaper-like rash; severe generalized hyperesthesia; and extreme pain at the site of skin involvement.[68,290,292] Both cell-associated and soluble streptococcal virulence factors, including M protein and pyrogenic exotoxins A and B, have been implicated as superantigens mediating the systemic effects of streptococcal TSS.[36,110,146]

Treatment follows the same principles as for staphylococcal TSS: identification of the focus of toxin production, identification and susceptibility testing of the organism, administration of antibiotic therapy to block synthesis of the toxin and to kill *S. pyogenes*, and management of the systemic multiorgan actions of the toxins. Although penicillin is the drug of choice for treatment of most group A streptococcal infections, the addition of clindamycin to inhibit protein synthesis is beneficial in cases of streptococcal TSS.[290]

IVIG as adjunctive therapy for streptococcal TSS has been supported by in vitro studies[258] and case reports in humans,[16,37,219,331] but clinical studies have been inconclusive. Downregulation of the lymphokine production induced by streptococcal pyrogenic exotoxin A by IVIG has been demonstrated in vitro.[219,283] A multicenter, randomized, double-blind, placebo-controlled trial to study IVIG for streptococcal TSS in adults showed 3.6-fold higher mortality in the placebo group. However, statistical significance was not achieved because the study identified only 10 IVIG recipients and 11 placebo patients before premature termination because of slow patient recruitment.[77] A multicenter, retrospective cohort study of children with streptococcal TSS from 2003 to 2007 identified 192 cases divided into 84 IVIG recipients and 108 nonrecipients. The study found no statistical difference in length of hospital stay or mortality, but there were only 3 deaths in either group.[279] As with staphylococcal TSS, experts recommend that IVIG be considered for cases of streptococcal TSS that are refractory to aggressive therapy or for patients with an inaccessible focus of infection.

Several patients with TSS have been reported to be simultaneously infected with *S. aureus* and *S. pyogenes*.[107] Determining which infection primarily was responsible for the manifestations or whether an amplified effect of exotoxins from both organisms was present was not possible.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of TSS includes clinical entities in which a rapid onset of fever, erythroderma-like rash, hypotension, and multisystem involvement are observed (Table 64-4).*

PREVENTION AND PROPHYLAXIS

To decrease the risk for development of menstrual TSS, in 1982, the Institute of Medicine Committee on TSS recommended that women, particularly adolescents, minimize their use of high-absorbency tampons.[154] The committee also recommended that women who have had TSS not use tampons. Although the frequency of changing tampons has not been associated with risk for TSS, limiting individual tampon use to less than 12 hours might decrease the risk for menstrual TSS. Women using intravaginal contraceptive devices also should be informed of the potential increase in risk for development of TSS.[277]

Although postoperative TSS is a rare event, it generally occurs in healthy people. As a result of the unexpected and potentially severe consequences of TSS, issues regarding surgical antimicrobial prophylaxis for prevention have been raised. Perioperative systemic antistaphylococcal antibiotics did not prevent TSS in four patients[32,264] and do not eradicate nasal carriage of *S. aureus*.[159] TSS with onset after 48 hours of amoxicillin

*References 39, 117, 205, 214, 223, 238, 249.

TABLE 64-4 **Differential Diagnosis of Toxic Shock Syndrome Based on Clinical Manifestations**

Diagnosis	Fever	Exanthem	Shock
Severe invasive *Streptococcus pyogenes* infection	+	+	+
Meningococcemia	+	+	+
Rocky Mountain spotted fever	+	+	±
Ehrlichiosis	+	+	±
Kawasaki disease	+	+	–
Staphylococcal scalded skin syndrome	+	+	–
Toxic epidermal necrolysis	+	+	–
Viral syndromes	+	+	–
Leptospirosis	+	+	–
Systemic lupus erythematosus	+	+	–
Erythema multiforme	+	+	–
Septic shock	+	–	+
Hantavirus pulmonary syndrome	+	–	+
Salmonella infections	+	–	±
Gastroenteritis	+	–	–
Urinary tract infection	+	–	–
Drug reactions			
Phenytoin (Dilantin)	+	+	±
Cocaine	+	+	±
Pseudoephedrine	+	+	±
Inhalational mercury	+	+	±
Quinidine	+	+	–
Sulfonamides	+	+	–
β-Lactam antibiotics	+	+	–
Quinolones	+	+	–

therapy was reported in two patients after endonasal sinus surgery.[2] Topical bacitracin ointment on nasal packing does not prevent the development of TSS.[32,93,157,159]

Most authors contend that the rare risk for development of postoperative TSS is comparable to the risk for having a severe antimicrobial reaction and that perioperative antimicrobial prophylaxis is not indicated for clean procedures of short duration.[136,251] Effort should be intensified to recognize postoperative cases early; to open, explore extensively, and irrigate wounds; and to provide immediate antimicrobial and supportive therapy for suspected TSS.

CHARACTERISTICS OF TOXIC SHOCK SYNDROME–ASSOCIATED *STAPHYLOCOCCUS AUREUS* ISOLATES

The ability to produce TSST-I, a previously uncharacterized protein, is the single most distinguishing characteristic of TSS-associated *S. aureus* strains. More than 90 percent of *S. aureus* isolates from patients with menstrual TSS[22,273] and 40 to 60 percent of isolates from patients with nonmenstrual disease[131] produce TSST-I, in contrast to less than 20 percent of non-TSS strains. *S. aureus* strains isolated from patients with TSS are phenotypically different from other strains of *S. aureus*.[21,306] They produce less β-hemolysis on sheep blood agar and less frequently harbor plasmids. Next to TSST-I production, production of protease is the most characteristic marker for these strains.[306] Unlike non-TSS *S. aureus* strains, most TSST-I–positive strains of *S. aureus* require tryptophan for growth and production.[64] Additional differences between TSS and non-TSS strains of *S. aureus* include the resistance of TSS strains to cadmium, arsenate, and penicillin, characteristics that usually are plasmid mediated but, in TSS strains, are chromosomally mediated.[21,306] TSST-I production is unrelated to methicillin susceptibility or resistance.[274]

ROLE OF STAPHYLOCOCCAL EXOTOXIN TOXIC SHOCK SYNDROME TOXIN–I AND THE ENTEROTOXINS IN TOXIC SHOCK SYNDROME

Role of Toxic Shock Syndrome Toxin–I

TSST-I appears to be an important toxin in TSS. Factors supporting this statement include (1) the observation that more than 90 percent of isolates from patients with TSS produce TSST-I, (2) the absence of acute-phase antibody to TSST-I in more than 90 percent of patients with menstrual TSS, (3) the increase in anti–TSST-I antibody during recovery in nonmenstrual cases, (4) absent or low levels of antibody in patients with recurrent menstrual TSS, (5) comparable illness inducible with TSST-I in in vivo animal models, and (6) neutralization of IL-1 stimulation and response to TSST-I by antibody to TSST-I.

Most convincing are experiments in which the ability to cause TSS followed bacteriophage-mediated transfer of the TSST-I–positive chromosomal segment into a recipient *S. aureus* strain without the segment.[88,238] Experiments demonstrating the effectiveness of monoclonal antibodies to TSST-I in reversing the effects of TSST-I provide additional strong support.[175]

Role of Enterotoxins

Because 40 to 60 percent of nonmenstrual TSS *S. aureus* isolates and 5 to 10 percent of menstrual TSS isolates do not produce TSST-I, a role for other staphylococcal exotoxins, including the enterotoxins, has been proposed.* Patients with nonmenstrual disease infected with TSST-I–negative strains may have a higher mortality rate.[131] Significant increases in the titer of antibody to the staphylococcal enterotoxins during convalescence strongly suggest a role for these proteins in the pathogenesis of TSS.[12]

In rabbit models, the morbidity and mortality occurring after the injection of TSST-I–negative TSS isolates

*References 21, 71, 182, 194, 239, 269, 330.

were comparable to those occurring after infection with TSST-I–positive strains and significantly greater than after inoculation with TSST-I–negative non-TSS isolates.[131] Administration of staphylococcal enterotoxins to animals results in many of the manifestations characteristic of TSS.[20,109]

On examination of isolates from patients with TSS, production of TSST-I alone is the most common toxin pattern.[21,71,189] Other patterns include production of TSST-I in combination with one or more of the identified enterotoxins, production of enterotoxins *only* (one or more), and nonproduction of TSST-I and enterotoxins. Of the TSS strains producing TSST-I, 60.6 percent also produce an enterotoxin.[71,189] A clone producing both TSST-I and staphylococcal enterotoxin A is associated with 88 percent of menstrual TSS cases and up to 54 percent of isolates from nonmenstrual TSS cases.[51] TSST-I and staphylococcal enterotoxin A have a common major histocompatibility complex (MHC) class II binding domain and share overlapping domains critical for superantigenic activities. A monoclonal antibody to TSST-I inhibits these staphylococcal enterotoxin A–induced activities.[176] The third most common pattern of toxin production is that of TSST-I plus staphylococcal enterotoxin C. These isolates have been associated with both menstrual and nonmenstrual cases and particularly with severe respiratory tract TSS-associated infections.[71] The fourth pattern is that of staphylococcal enterotoxin B alone. In one study, strains producing staphylococcal enterotoxin B alone accounted for 38 percent of all nonmenstrual TSS isolates.[182,269] This prevalence is significantly higher than that for isolates from non-TSS *S. aureus* infections (15%) or asymptomatic carriers (13%). Staphylococcal enterotoxin B never is coexpressed with TSST-I.[89] A TSS isolate producing no known toxins is the least common pattern. The identification of new enterotoxins[255,297] capable of producing TSS-like disease in rabbits may account for isolates previously identified as producing no toxins.

PREVALENCE OF EXOTOXIN-PRODUCING *STAPHYLOCOCCUS AUREUS* STRAINS AND ANTIBODY TO THE EXOTOXINS

Prevalence of Exotoxin-Producing Organisms

TSST-I–positive strains of *S. aureus* have been present since at least 1957, and antibody to TSST-I was as prevalent in the general population in 1960 as it was in 1999.[243,317] Thus, the increase in incidence of TSS from 1980 to 1985 is assumed to have been the result of newly introduced cofactors rather than an increase in the prevalence of TSST-I–positive organisms.

S. aureus is present in 20 to 40 percent of cultures from the anterior nasal vestibule of adults and in as many as 33 percent of nasal cultures from children.[156] A study of 3012 menstruating women between 13 and 40 years of age in North America from 1998 to 1999 showed that 26 percent were colonized with *S. aureus* in at least one of three body sites (nose, vagina, or anus) and 9 percent were colonized vaginally.[243] In women, the prevalence of *S. aureus* in vaginal cultures is higher during menses than at midcycle.[190,230] Vaginal carriage rates vary from 7 percent in premenarcheal and nonmenstruating women to 33 percent in menstruating women.[243,245] TSST-I–positive *S. aureus* is present in 1 to 5 percent of vaginal cultures in women, 7 percent of nasal cultures in hospitalized patients, and 18 percent of nasal cultures in children. In prospective studies of *S. aureus* isolates from healthy individuals and from specimens received for other purposes, 14 to 39 percent of all isolates produced TSST-I and 7 to 14 percent produced staphylococcal enterotoxin B.[92,238,243] Overall, between 1 and 7 percent of healthy individuals at any given time are colonized at a mucosal site with TSST-I–positive *S. aureus*.

Of 60 *S. aureus* isolates from blood cultures of patients who did not have TSS, 28 percent produced TSST-I.[54] Presumably, these patients had circulating antibody to TSST-I that prevented the development of TSS despite *S. aureus* infection. Adults and children with persistent nasal carriage of a TSST-I–positive strain have high levels of antibody to TSST-I.[243,258]

Evidence suggests that a single clone of *S. aureus* causes most cases of TSS.[51,218] Multilocus enzyme electrophoresis has demonstrated that the TSST-I gene *tst*H is distributed widely over the whole spectrum of *S. aureus* genotypes. Of 315 *S. aureus* strains collected from around the world, 88 percent of menstrual TSS strains and 53 percent of nonmenstrual strains were indistinguishable by multilocus enzyme electrophoresis. The same clone also was found in 28 percent of vaginal isolates from asymptomatic women. This phenomenon may reflect the unique ability of this clone to colonize the urogenital epithelium or the unique capability of this clone to cause disease. Although TSS strains are similar in gene content, indicative of a common ancestor, their considerable heterogeneity indicates that the common ancestor was not recent in evolutionary time.[124] Thus, the TSS outbreak was the result of a change in the host environment and not geographic dissemination of a new "hypervirulent" strain.[124]

Even though TSST-I–positive strains may cluster within families, living units, and hospital settings, the occurrence of TSS clusters is a rare event.[173,216] Probable TSS has developed within 24 hours in a husband and wife[121] and in two mother–daughter pairs. Nosocomial acquisition and transmission of TSST-I–positive organisms have been described.[9,173,216]

Prevalence of Antibody to Exotoxins

Antibody formation to exotoxins is thought to result from mucosal colonization with TSST-I–positive *S. aureus* strains.[9,258] Antibody titers were higher in women colonized with toxigenic *S. aureus* whether the colonization was persistent or transient.[244] Subclinical and mild, unrecognized disease may result in the formation of antibodies as well.[56] Patients in whom TSS develops have significantly lower levels of antibody to TSST-I and staphylococcal enterotoxins B and C than the general

population.[21,71] The antibody response to TSST-I is absent or delayed in both menstrual and nonmenstrual TSS.[238,296]

The prevalence of antibody to staphylococcal exotoxins increases with age. Antibody to TSST-I in 689 Wisconsin residents was found to be 47 percent at 1 year of age, 58 percent at 5 years, 70 percent at 10 years, 88 percent at 20 years, and 96 percent for ages 30 to 50 during the years 1960 to 1983 (see Fig. 64-2).[42] A study of 3012 menstruating women between 13 and 40 years of age across North America in 1998 and 1999 found similar results, with 85 percent overall and 81 percent of subjects aged 13 to 18 years having positive antibody to TSST-I.[243] The presence of transplacental antibody in more than 90 percent of infants also was demonstrated.[317] For enterotoxins, by the time that children reach 10 years of age, the proportion with antibody titers of 1:100 or higher is 15 percent for staphylococcal enterotoxin A, 65 percent for staphylococcal enterotoxin B, 30 percent for staphylococcal enterotoxin C, 5 percent for staphylococcal enterotoxin D, and 20 percent for staphylococcal enterotoxin E. By 22 years of age, the numbers increase to 55 percent for staphylococcal enterotoxin A, 77 percent for staphylococcal enterotoxin B, and 98 percent for staphylococcal enterotoxin C.[21]

Racial, sexual, and geographic differences in antibody prevalence have been noted. Among carriers of toxigenic *S. aureus*, a lower percentage of black women (89%) than white or Hispanic women (98% and 100%, respectively) had positive antibody titers. Sera randomly selected from 87 control women were seronegative more frequently for antibody to TSST-I (24%) than were those from 66 control men (9%), which led to the suggestion that women may be more susceptible than men to TSS.[263] A 2003 multicenter study of 3012 women from Ohio, New Jersey, Florida, Arizona, and Manitoba, Canada, found the prevalence of positive antibody to TSST-I (titer of ≥1:32) to be high in all geographic sites (82% to 89%). Although no regional differences were found in rates of *S. aureus* colonization, subjects from Manitoba were significantly more likely to have a positive antibody titer than were subjects from the other locations except Arizona.[243]

PHYSICOCHEMICAL AND BIOLOGIC CHARACTERISTICS OF TOXIC SHOCK SYNDROME TOXIN–I

Physicochemical Properties, Structure, and Function

Most bacterial superantigens are protease-, heat-, and acid-resistant, compact globular proteins of 20 to 30 kDa that share immunologic, biologic, and functional properties.[27] The mature secreted TSST-I protein is a single polypeptide chain with a molecular weight of 22,000 daltons. It is resistant to proteolytic digestion by trypsin but is hydrolyzed by pepsin at pH 4.5. In sterile solution at neutral pH, it is stable for months. TSST-I can be heated to 100° C (212° F) for longer than an hour without loss of biologic activity.[27] The molecule is folded into two closely associated domains that create two major grooves, the front and backside grooves, similar to the crystal structures of other superantigens.[193] Although they do not share sequence homology or obvious structural features, all of the secreted superantigens share a three-dimensional conformation that allows them to interact simultaneously with two different receptors on two different cell types.[27,170]

Efforts to understand the molecular actions of TSST-I have focused on separating regions that interact with the T-cell receptor from those required for lethality.[27,29,30,217] In animal models, lethality, induction of fever, and endotoxin susceptibility do not depend on T-cell proliferation as measured by superantigenic activity but must involve interaction of toxin with other host-cell receptors in the body.[27]

Regulation of Production

S. aureus is highly adaptable and able to live and grow in extremes of temperature, pH, and oxygen concentration. TSST-I production, however, is controlled tightly. TSST-I is not produced in unfavorable conditions, including an anaerobic environment, pH values lower than 6.0 or greater than 8.0, concentrations of clindamycin below the MIC of the organism, glucose concentrations greater than 3 percent, and temperatures lower than 37° C (98.6° F) or greater than 40° C (104° F).[21,27,308,328] The role of magnesium is unclear.[212]

Under optimal in vitro growth conditions, TSST-I production lags behind but parallels bacterial growth. Like other *S. aureus* exotoxins, TSST-I is made primarily during the postexponential phase of growth, when nutrients are scarce and cell density is great.[92] Levels of TSST-I remain stable throughout the stationary phase, even after the organisms begin to die. Under less than optimal conditions, synchronous production of TSST-I and growth of *S. aureus* do not occur. For example, clindamycin at concentrations below the MIC for the organism will inhibit the production of toxins without altering growth.[271]

The gene *tst*H is encoded by a transposon-like mobile genetic element.[64,89,152,174] The chromosomal fragment with the structural gene for production of TSST-I is a 10.6-kilobase (kb) unit that has been cloned in *E. coli*. *tst*H has been sequenced and encodes a 234–amino acid protein that is converted by the removal of a signal peptide of 40 amino acids to the mature exotoxin of 194 amino acids.[27,92,174] Production of TSST-I and other post–exponential-phase virulence factors is regulated by three separate genetic loci: the accessory gene regulator *(agr)*, the staphylococcal accessory regulator *(sar)*, and the extracellular protein regulator *(xpr)*.[12,27,92] All three regulators affect gene expression at the level of transcription during the late log phase of bacterial growth.

Glyceryl monolaurate, a surfactant and emulsifier used in food and cosmetic industries, inhibits the production of staphylococcal and streptococcal exotoxins at concentrations subinhibitory to growth.[250,270] As a lipophilic compound, the mechanism of action appears to be insertion into the cell membrane, with resulting interruption of signal transduction. Other surfactants used in the

tampon industry have been found to both inhibit and enhance[272] the production of TSST-I in vitro.

Kinetics of Distribution

Detecting TSST-I in body fluids is difficult. In humans, nanogram quantities have been detected by radioimmunoassay in the breast milk of a woman with TSS,[153,316] in serum early during illness in two of four patients,[199] in urine, and in vaginal washings in a small number of patients.[199] By means of the rabbit subcutaneous wiffle ball abscess model, TSST-I can be detected first in abscess fluid 4 hours after *S. aureus* has been inoculated into the wiffle ball and in urine 48 hours after inoculation.[199] When TSST-I radiolabeled with iodine-139 was injected intravenously into rabbits, measurement of plasma clearance demonstrated a half-life of 1.5 hours. Within 15 minutes of injection, TSST-I was concentrated fourfold in blood cells in contrast to plasma. Toxin persisted in the cellular compartment, and plasma concentrations fell. After 3 hours, most of the radiolabel was found in the spleen. TSST-I and staphylococcal enterotoxins A and B can cross epithelial membranes intact in a fully functional form.[139] In a cellular model of the blood-brain barrier, TSST-1 showed slow bidirectional movement consistent with restricted paracellular diffusion.[18]

Cellular Interactions

TSST-I has been shown to inhibit systolic function in isolated rabbit atria.[229] It also can bind directly to human and porcine endothelial cells and is cytotoxic to porcine endothelial cells because it permits leakage across endothelial cell monolayers.[177,181] However, concentrations of TSST-I needed to induce these and other diverse cytotoxic changes in vitro are higher than the usual tissue and serum levels. Interactions of TSST-I and the enterotoxins with T lymphocytes and antigen-processing cells are described in the next section.

Biologic Functions

Much information regarding pathogenesis has been obtained from the subcutaneous wiffle ball and vaginal tampon rabbit models and a primate model.[87,237] Rabbits and baboons exhibit clinical and laboratory changes most consistent with the human syndrome, including lethality.* The signs and symptoms observed in these animals after the intravenous injection of purified TSST-I include skin erythema, conjunctivitis, diarrhea, lethargy, tachypnea, respiratory distress, central nervous system changes, and increased capillary permeability.

The absence of in vitro cytotoxicity of TSST-I[21,237] despite potent in vivo biologic effects suggests an important role for endogenous mediators in TSS. The ability of staphylococcal enterotoxin B to induce dramatic T-cell proliferation was recognized a decade ago.[165,170] Subsequent work has shown it to be a feature of other bacterial exotoxins, including TSST-I, the staphylococcal enterotoxins, the streptococcal pyrogenic exotoxins A and C, a new streptococcal superantigen, and a streptococcal M surface protein.[170] The term *superantigen* was coined in 1989 to describe antigens that at concentrations lower than those of conventional antigens (picomolar concentrations) can stimulate proliferation of a large percentage of T cells bearing a T-cell receptor β-chain variable (V_β). Such activation of T cells results in profound alterations in immune system homeostasis by inducing the release of large quantities of monokines (IL-1, TNF-α) and lymphokines (IL-2, TNF-β).[170,282,334]

T cells are activated to produce lymphokines as a result of T-cell receptor–antigen binding and activation of signal transduction.[52,101,289] Antigens can bind to the T-cell receptor as conventional antigens or superantigens. Conventional antigens enter antigen-processing cells by endocytosis. They are broken into small peptides and form complexes with one of the allele-restricted MHC class II molecules.[282] This complex is transported to the cell surface, where it is recognized by a specific T-cell receptor. T-cell receptor gene rearrangement gives more than 10,000 possible combinations to recognize conventional antigenic peptides presented by antigen-processing cells.[282] Thus, one peptide will activate only 1 in 10^4 to 10^6 T cells, and only $CD4^+$ T cells respond to conventional antigens.

Superantigens are presented to T lymphocytes in a very different fashion. They bind as intact proteins to most allelic forms of MHC class II molecules in an unrestricted fashion at a site outside the peptide-binding groove. They then react with the T-cell receptor through the V_β element and will react with all T cells carrying that V_β element.[150] Because only 25 to 50 major families of V_β genes exist in humans, each superantigen can interact with 5 to 20 percent of resting T cells, depending on the frequency of T cells expressing that V_β family in each individual's repertoire. Both $CD4^+$ and $CD8^+$ T cells are activated. The fact that superantigens bind only to selected and specific V_β elements distinguishes them from the nondiscriminating mitogens.

Once the T cell has recognized the superantigen,[137] both antigen-processing cell and T-cell activation by signal transduction[52] result in release of cytokine by the antigen-processing cell (IL-1, TNF-α) and the lymphocyte (IL-2, TNF-β). In mice and possibly also in humans, the initial T-cell activation occurs in lymph nodes.[209] Within hours of T-cell activation by superantigens, cytokines are detected in vitro and in serum in vivo.

TSST-I binds only to the $V_\beta 2$ element.[260] In humans with TSS caused by TSST-I–secreting *S. aureus*, within 10 to 14 days 30 to 70 percent of the circulating T-cell population will be T cells bearing the $V_\beta 2$ element.[60,61,191] The number of circulating $V_\beta 2$ T cells does not return to normal for several months. In contrast, patients with TSS caused by *S. pyogenes* have a consistent pattern of depletion of certain V_β T-cell types after T-cell activation.[170,224,320] In mice, such depletion is thought to be the result of programmed cell death (apoptosis).[207,208] Thus, activation of T cells by superantigens may result in massive cytokine release with subsequent selected V_β T-cell expansion, T-cell deletion, or apoptosis, depending on the superantigen and species.

*References 29, 87, 88, 200, 203, 239, 252, 273, 278.

Evidence from the mouse model supports a central role for T-lymphocyte activation in superantigen-mediated disease. Mice that have received cyclosporine to block T-cell activation and lymphokine production or mice with severe combined immunodeficiency disease are protected against staphylococcal enterotoxin B–induced lethal shock. Repopulation with T cells results in susceptibility to staphylococcal enterotoxin B. The same mice are not protected against endotoxin-induced shock because lipopolysaccharide activates only monocytes.[207-209] Mononuclear production of IL-1 and TNF-α is central to the shock induced by an endotoxin. Production of these two cytokines in addition to production of the lymphokines IL-2, IFN-α, and TNF-β may explain the enhanced severity of superantigen-associated TSS.[28,137,170] If more than one superantigen is produced by an organism, different V_β specificities would result in an even greater number of T cells being activated, with the potential for development of disease with enhanced severity and mortality.

Once superantigens have resulted in monokine and lymphokine release, the subsequent pathophysiologic events appear to be related to the many and complex interactions of these cytokines,[28,141,333] with the ultimate result being profound endothelial damage. TNF-α and TNF-β and the complex interaction of cytokines, leukotrienes, prostaglandins, adhesion molecules, nitric oxide, platelet-activating factor, complement components, neutrophils, platelets, and endothelium-derived factors appear to be responsible for the extensive endothelial damage and resulting capillary fluid leakage and the decrease in peripheral vascular resistance.[28,141,265,333]

NEW REFERENCES SINCE THE SIXTH EDITION

80. Davies HD, McGeer A, Schwartz B, et al. Ontario Group A Streptococcal Study Group. Invasive group A streptococcal infections in Ontario, Canada. *N Engl J Med* 1996;**335**:547–54.
91. Demers B, Simor AE, Vellend H, et al. Severe invasive group A streptococcal infections in Ontario, Canada: 1987-1991. *Clin Infect Dis* 1993;**16**:792–800.
94. DeVries AS, Lesher L, Schlievert PM, et al. Staphylococcal toxic shock syndrome 2000-2006: epidemiology, clinical features and molecular characteristics. *PloS One* 2011;**6**:e22997.
178. Lamagni TL, Darenberg J, Luca-Harari B, et al. Epidemiology of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2008;**46**:2359–67.
227. O'Loughlin RE, Roberson A, Cieslak PR, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. *Clin Infect Dis* 2007;**45**:853–62.
244. Parsonnet J, Hansmann MA, Seymour JL, et al. Persistence survey of toxic shock syndrome toxin-1 producing *Staphylococcus aureus* and serum antibodies to this superantigen in five groups of menstruating women. *BMC Infect Dis* 2010;**10**:249.
279. Shah SS, Hall M, Srivastava R, et al. Intravenous immunoglobulin in children with streptococcal toxic shock syndrome. *Clin Infect Dis* 2009;**9**:1369–76.
290. Stevens DL. Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. *Emerg Infect Dis* 1995;**1**:69–78.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 65

Acute Respiratory Distress Syndrome in Children

Jan Hau Lee • Ira M. Cheifetz • David A. Turner

Acute respiratory distress syndrome (ARDS) is characterized by severe hypoxemia and diffuse infiltrates on radiologic examination. ARDS represents the final common pathway and clinical presentation of the most severe acute lung injury (ALI), which may be precipitated by various pulmonary (direct) or nonpulmonary (indirect) insults. Infections are an important cause of ARDS in children, with pneumonia and sepsis being most common.[39,116] ARDS is an important consideration in the setting of pediatric infectious disease, given the wide range of infections that may lead to this complication and its associated morbidity and mortality.[39,116] Morbidity, mortality, and resource usage related to ARDS also increase during outbreaks of infectious diseases, as seen during the 2009 to 2010 global influenza H1N1 pandemic.[36,87] This chapter will discuss the definition, pathophysiology, presentation, and clinical management of critically ill children with ARDS, with an emphasis on aspects that may be of particular interest to pediatric infectious disease specialists.

REFERENCES

1. Abdul-Karim FW, Lederman MM, Carter JR, et al. Toxic shock syndrome: clinicopathologic findings in a fatal case. *Hum Pathol* 1981;**12**:16–22.
2. Abram AC, Bellian KT, Giles WJ, et al. Toxic shock syndrome after functional endonasal sinus surgery: an all or none phenomenon? *Laryngoscope* 1994;**104**:927–31.
3. Allen ST, Liland JB, Nichols CG, et al. Toxic shock syndrome associated with use of latex nasal packing. *Arch Intern Med* 1990;**150**:2587–8.
4. Alsoub H. Toxic shock syndrome associated with pyomyositis. *Postgrad Med J* 1994;**70**:309–12.
5. Andrews MM, Giacobbe KD, Parsonnet J. Induction of toxic shock syndrome toxin-1 (TSST-1) and beta-lactamase by subinhibitory concentrations of beta-lactam antibiotics. Abstract N-IN-0011. Presented at Biomedicine '96, Washington, DC, May 3-6, 1996.
6. Andrews MM, Parent EM, Barry M, Parsonnet J. Recurrent nonmenstrual toxic shock syndrome: clinical manifestations, diagnosis, and treatment. *Clin Infect Dis* 2001;**32**:1470–9.
7. Aranow H Jr, Wood WB Jr. Staphylococcic infection simulating scarlet fever. *JAMA* 1942;**119**:1491–5.
8. Arend SM, Steenmeyer AV, Mosmans PCM, et al. Postoperative cauda syndrome caused by *Staphylococcus aureus*. *Infection* 1993;**21**: 248–50.
9. Arnow PM, Chou T, Weil D, et al. Spread of a toxic-shock syndrome–associated strain of *Staphylococcus aureus* and measurement of antibodies to staphylococcal enterotoxin F. *J Infect Dis* 1984;**149**:103–7.
10. Bach MC. Dermatologic signs in toxic shock syndrome: clues to diagnosis. *J Am Acad Dermatol* 1983;**8**:343–7.
11. Bacha EA, Sheridan RL, Donohue GA, et al. Staphylococcal toxic shock syndrome in a paediatric burn unit. *Burns* 1994;**20**: 499–502.
12. Balaban N, Novick RP. Autocrine regulation of toxin synthesis by *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 1995;**92**:1619–1623.
13. Bannister B, Platts AJ. Some cardiological findings in toxic shock syndrome. *J Infect* 1981;**3**:293–4.
14. Baracos V, Rodemann P, Dinarello CA, et al. Stimulation of muscle protein degradation and prostaglandin E_2 release by leucocytic pyrogen (interleukin-1). *N Engl J Med* 1983;**308**:553–8.
15. Barrett JA, Graham DR. TSS presenting as encephalopathy. *J Infect* 1986;**12**:276.
16. Barry W, Hudgins L, Donta ST, et al. Intravenous immunoglobulin therapy for toxic-shock syndrome. *JAMA* 1992;**267**:3315–17.
17. Bartlett P, Reingold AL, Graham DR, et al. Toxic shock syndrome associated with surgical wound infections. *JAMA* 1982;**247**: 1448–50.
18. Batisson M, Strazielle N, Hejmadi M, et al. Toxic shock syndrome toxin-1 challenges the neuroprotective functions of the choroidal epithelium and induces neurotoxicity. *J Infect Dis* 2006;**194**: 341–9.
19. Becker K, Roth R, Peters G. Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. *J Clin Microbiol* 1998;**36**:2548–53.
20. Beisel WR. Pathophysiology of staphylococcal enterotoxin, type B, (SEB) toxemia after the intravenous administration to monkeys. *Toxicon* 1972;**10**:433.
21. Bergdoll MS, Chesney PJ. *Toxic-shock syndrome*. Boca Raton, Fla: CRC Press; 1991.
22. Bergdoll MS, Crass BA, Reiser RF, et al. A new staphylococcal enterotoxin, enterotoxin F, associated with toxic-shock syndrome *Staphylococcus aureus* isolates. *Lancet* 1981;**1**:1017–21.
23. Berkley SF, Hightower AW, Broome CW, et al. The relationship of tampon characteristics to menstrual toxic-shock syndrome. *JAMA* 1987;**258**:917–20.
24. Bernstein GM, Crollick JS, Hassett JM Jr. Post febrile telogen effluvium in critically ill patients. *Crit Care Med* 1988;**16**:98–9.
25. Black DA, Maw DS. Toxic shock syndrome presenting as cerebral infarct. *Corres J Neurol Neurosurg Psychiatry* 1984;**47**:568.
26. Blair JD, Livingston DG, Vongsnichakul R. Tampon-related toxic-shock syndrome: histopathologic and clinical findings in a fatal case. *Am J Clin Pathol* 1982;**78**:372–6.
27. Bohach GA, Dinges MM, Mitchell DT, et al. Staphylococcal exotoxins. In: Leung DYM, Huber BT, Schlievert PM, editors. *Superantigens: Molecular biology, immunology and relevance to human disease*. New York: Marcel Dekker; 1997.
28. Bone RC. The pathogenesis of sepsis. *Ann Intern Med* 1991; **115**:457–69.
29. Bonventre PF, Heeg H, Cullen C, et al. Toxicity of recombinant toxic shock syndrome toxin 1 and mutant toxins produced by *Staphylococcus aureus* in a rabbit infection model of toxic shock syndrome. *Infect Immun* 1993;**61**:793–9.
30. Bonventre PF, Linnemann C, Weckback LS, et al. Antibody responses to toxic-shock-syndrome (TSS) toxin by patients with TSS and by healthy staphylococcal carriers. *J Infect Dis* 1984; **150**:662–6.
31. Bonventre PF, Thompson MR, Adinolfi LE, et al. Neutralization of TSST-1 by monoclonal antibodies in vitro and in vivo. *Infect Immun* 1987;**50**:135.
32. Breda SD, Jacobs JB, Lebowitz AS, et al. Toxic shock syndrome in nasal surgery: a physiochemical and microbiologic evaluation of Merocel and NuGauze nasal packing. *Laryngoscope* 1987;**97**: 1388.
33. Brody H, Smith LW. The visceral pathology in scarlet fever and related *Streptococcus* infections. *Am J Pathol* 1936;**12**:373.
34. Brook MG, Bannister BA. Staphylococcal enterotoxins in scarlet fever complicating chicken pox. *Postgrad Med J* 1991;**67**:1013–14.
35. Broome CV. Epidemiology of TSS in the United States: overview. *Rev Infect Dis* 1989;**11**(Suppl.):14–21.
36. Brown EJ. The molecular basis of streptococcal toxic shock syndrome. *N Engl J Med* 2004;**350**:2093–4.
37. Burnett AM, Domachowske JB. Therapeutic considerations for children with invasive group a streptococcal infections: a case series report and review of the literature. *Clin Pediatr* 2007;**46**: 550–5.
38. Burns JR, Menapace FJ. Acute reversible cardiomyopathy complicating toxic shock syndrome. *Arch Intern Med* 1982;**142**: 1032–4.
39. Cavanah DK, Ballas ZK. Pseudoephedrine reaction presenting as recurrent toxic shock syndrome. *Ann Intern Med* 1993;**119**: 302–3.
40. Cederna JP. Toxic shock syndrome after transverse rectus abdominis musculocutaneous flap breast reconstruction. *Ann Plast Surg* 1995;**34**:73–5.
41. Centers for Disease Control and Prevention. Follow-up on toxic-shock syndrome: United States. *MMWR Morb Mortal Wkly Rep* 1980;**29**:441–5.
42. Centers for Disease Control and Prevention. Toxic-shock syndrome: United States. *MMWR Morb Mortal Wkly Rep* 1980;**29**: 229–30.
43. Centers for Disease Control and Prevention. Toxic-shock syndrome: United States, 1970-1980. *MMWR Morb Mortal Wkly Rep* 1981;**30**:25–8, 33.
44. Centers for Disease Control and Prevention. Toxic-shock syndrome: United States, 1970-1982. *MMWR Morb Mortal Wkly Rep* 1982;**31**:201–4.
45. Centers for Disease Control and Prevention. Update: Toxic shock syndrome—United States. *MMWR Morb Mortal Wkly Rep* 1983; **32**:398–400.
46. Centers for Disease Control and Prevention. Toxic-shock syndrome and the vaginal contraceptive sponge. *MMWR Morb Mortal Wkly Rep* 1984;**33**:43–4, 49.
47. Centers for Disease Control and Prevention. Toxic shock syndrome following influenza: Oregon, United States; update on influenza activity: United States. *MMWR Morb Mortal Wkly Rep* 1987;**36**:64–5.
48. Centers for Disease Control and Prevention. Reduced incidence of menstrual toxic-shock syndrome: United States, 1980-1990. *MMWR Morb Mortal Wkly Rep* 1990;**39**:421–3.
49. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**:1–80.
50. Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin: United States, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:565–7.
51. Chang AH, Musser JM, Chow AW. A single clone which produces both TSST-1 and SEA causes the majority of menstrual toxic shock syndrome [Abstract]. *Clin Res* 1991;**39**:36.

52. Chatila T, Geha RS. Signal transduction by microbial superantigens via MHC class II molecules. *Immunol Rev* 1993;**131**: 43–59.
53. Chesney PJ, Zimmerman JJ. Hypoferrinemia in toxic-shock syndrome (TSS) [Abstract]. *Pediatr Res* 1986;**20**:306.
54. Chesney PJ, Crass BA, Polyak MB, et al. Toxic-shock syndrome: management and long-term sequelae. *Ann Intern Med* 1982;**96**: 847–51.
55. Chesney PJ, Davis JP, Purdy WK, et al. The clinical manifestations of toxic shock syndrome. *JAMA* 1981;**246**:741–8.
56. Chesney PJ, Slama SL, Hawkins RL, et al. Outpatient diagnosis and management of toxic-shock syndrome. *N Engl J Med* 1981; **304**:1426.
57. Chesney RW, Chesney PJ, Davis JP, et al. Renal manifestations of the staphylococcal toxic-shock syndrome. *Am J Med* 1981;**71**: 583–8.
58. Chesney RW, McCarren DM, Haddad JG, et al. Pathogenic mechanisms of the hypocalcemia of the staphylococcal toxic-shock syndrome. *J Lab Clin Med* 1983;**101**:576–85.
59. Childs C, Edwards-Jones V, Healthcote DM, et al. Patterns of *Staphylococcal aureus* colonization, toxin production, immunity and illness in burned children. *Burns* 1994;**20**:514–21.
60. Choi Y, Kotzin B, Herron L, et al. Interaction of *Staphylococcus aureus* toxin "superantigens" with human T cells. *Proc Natl Acad Sci U S A* 1989;**86**:8941–5.
61. Choi Y, Lafferty JA, Clements JR, et al. Selective expansion of T cells expressing V beta 2 in toxic-shock syndrome. *J Exp Med* 1990;**172**:981–4.
62. Chow A. Microbiology of toxic shock syndrome: overview. *Rev Infect Dis* 1989;**1**:55–60.
63. Chow AW, Wong CK, MacFarlane AM, et al. Toxic shock syndrome: clinical and laboratory findings in 30 patients. *Can Med Assoc J* 1984;**130**:425–30.
64. Chu MC, Kreiswirth BN, Pattee PA, et al. Association of toxic shock toxin-1 determinant with a heterologous insertion at multiple loci in the *Staphylococcus aureus* chromosome. *Infect Immun* 1988;**56**:2702–8.
65. Cohen KR, Emmons KM, Goldstein MF. Naloxone treatment of toxic shock syndrome. *Arch Intern Med* 1983;**143**:1072.
66. Cole RP, Shakespeare PG. Toxic-shock syndrome in scalded children. *Burns* 1990;**16**:221–4.
67. Cone LA, Woodward DR, Byrd RG, et al. A recalcitrant, erythematous, desquamating disorder associated with toxin-producing staphylococci in patients with AIDS. *J Infect Dis* 1992;**165**: 638–43.
68. Cone LA, Woodard DR, Schlievert PM, et al. Clinical and bacteriologic observations of a toxic-shock–like syndrome due to *Streptococcus pyogenes*. *N Engl J Med* 1987;**317**:146–9.
69. Conway EE Jr, Haber RS, Gumprecht J, et al. Toxic shock syndrome following influenza A in a child. *Crit Care Med* 1991;**19**: 123–5.
70. Cowan RK, Martens MG. Toxic shock syndrome mimicking pelvic inflammatory disease presumably resulting from tattoo. *South Med J* 1993;**86**:1427–31.
71. Crass BA, Bergdoll MS. Toxin involvement in toxic shock syndrome. *J Infect Dis* 1986;**153**:918–26.
72. Crews JR, Harrison JK, Corey GR, et al. Stunned myocardium in the toxic shock syndrome. *Ann Intern Med* 1992;**117**:912–13.
73. Crowder WE, Shannon FC. Colposcopic diagnosis of vaginal ulcerations in toxic-shock syndrome. *Obstet Gynecol* 1983;**61**: 505–35.
74. Crowther MA, Ralph ED. Menstrual toxic shock syndrome complicated by persistent bacteremia: case report and review. *Clin Infect Dis* 1993;**16**:288–9.
75. Danielson RW. Vaginal ulcers caused by tampons. *Am J Obstet Gynecol* 1983;**146**:547–9.
76. Dann EJ, Weinberger M, Gillis S, et al. Bacterial laryngotracheitis associated with toxic-shock syndrome in an adult. *Clin Infect Dis* 1994;**18**:437–9.
77. Darenberg J, Ihendyane N, Sjolin J, et al. Intravenous immunoglobulin G therapy in streptococcal toxic shock syndrome: a European randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2003;**37**:333–40.
78. Dart RC, Levitt MA. Toxic-shock syndrome associated with the use of the vaginal contraceptive sponge. *JAMA* 1985;**253**: 1877.
79. Davidson AC, Creach M, Cameron IR. Staphylococcal pneumonia, pneumatoceles, and the toxic shock syndrome. *Thorax* 1990; **45**:639–40.
80. Davies HD, McGeer A, Schwartz B, et al. Ontario Group A Streptococcal Study Group. Invasive group A streptococcal infections in Ontario, Canada. *N Engl J Med* 1996;**335**:547–54.
81. Davis JP, Vergeront JM. A review of toxic shock syndrome surveillance in Wisconsin: the effect of media publicity and laboratory services on reporting of illness. *Ann Intern Med* 1982;**96**:883–6.
82. Davis JP, Vergeront JM. The effect of publicity on the reporting of toxic-shock syndrome in Wisconsin. *J Infect Dis* 1982;**145**: 449–57.
83. Davis JP, Chesney PJ, Wand PJ, et al. Toxic shock syndrome: epidemiologic features, recurrences, risk factors and prevention. *N Engl J Med* 1980;**303**:1429–35.
84. Davis JP, Osterholm MT, Helms CM, et al. Tristate toxic-shock syndrome study. II. Clinical and laboratory findings. *J Infect Dis* 1982;**145**:441–8.
85. Davis JP, Vergeront JV, Amsterdam LE, et al. Long-term effects of TSS in women: sequelae, subsequent pregnancy, menstrual history, and long-term trends in catamenial product use. *Rev Infect Dis* 1989;**11**(Suppl.):50.
86. Davis JP, Vergeront JM, Chesney PJ. Possible host-defense mechanisms in toxic shock syndrome. *Ann Intern Med* 1982;**96**: 986–91.
87. de Azavedo JCS. Animal models for toxic-shock syndrome: overview. *Rev Infect Dis* 1989;**11**(suppl.):205–9.
88. de Azavedo JCS, Foster TJ, Hartigan J, et al. Expression of the cloned toxic shock syndrome toxin 1 gene *(tst)* in vivo with a rabbit uterine model. *Infect Immun* 1985;**50**:304–9.
89. De Boer ML, Chow AW. Toxic shock syndrome toxin 1–producing *Staphylococcus aureus* isolates contain the staphylococcal enterotoxin B genetic element but do not express staphylococcal enterotoxin B. *J Infect Dis* 1994;**170**:818–27.
90. Deetz TR, Reves R, Septimus E. Secondary rash in toxic shock syndrome. *N Engl J Med* 1981;**304**:174.
91. Demers B, Simor AE, Vellend H, et al. Severe invasive group A streptococcal infections in Ontario, Canada: 1987-1991. *Clin Infect Dis* 1993;**16**:792–800.
92. Deresiewicz RL. Staphylococcal toxic shock syndrome. In: Leung DYM, Huber BT, Schlievert PM, editors. *Superantigens: Molecular biology, immunology and relevance to human disease*. New York: Marcel Dekker; 1997.
93. deVries N, Vander Baan S. Toxic shock syndrome after nasal surgery: is prevention possible? A case report and review of literature. *Rhinology* 1989;**27**:125–8.
94. DeVries AS, Lesher L, Schlievert PM, et al. Staphylococcal toxic shock syndrome 2000-2006: epidemiology, clinical features and molecular characteristics. *PloS One* 2011;**6**:e22997.
95. Dickgiesser N, Wallach U. Toxic shock syndrome toxin-1 (TSST-1): influence of its production by subinhibitory antibiotic concentrations. *Infection* 1987;**15**:351–3.
96. Diep BA, Carleton HA, Chang RF, et al. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2006;**193**:1495–503.
97. Donawa ME, Schmid GR, Osterholm MT. Toxic shock syndrome: chronology of state and federal epidemiologic studies and regulatory decision-making. *Public Health Rep* 1984;**99**:342–50.
98. Dondorp AM, Veenstra J, vanderPoll T, et al. Activation of the cytokine network in a patient with AIDS and the recalcitrant erythematous desquamating disorder. *Clin Infect Dis* 1994;**18**: 942–5.
99. Donnelly BW, McMillan JA, Weiner LB. Bacterial tracheitis: report of eight new cases and a review. *Rev Infect Dis* 1990;**12**: 729–35.
100. Dornan KJ, Thompson DM, Conn AR, et al. Toxic shock syndrome in the postoperative patient. *Surg Gynecol Obstet* 1982;**154**:65–8.
101. Driessen C, Hirv K, Kirchner H, et al. Zinc regulates cytokine induction by superantigens and lipopolysaccharide. *Immunology* 1995;**84**:272–7.
102. Dunnet UB, Schallibaum EM. Scarlet fever–like illness due to staphylococcal infection. *Lancet* 1960;**2**:1227–9.
103. Durand G, Bes M, Meugnier H, et al. Detection of new methicillin-resistant *Staphylococcus aureus* clones containing the toxic shock

syndrome toxin 1 gene responsible for hospital- and community-acquired infections in France. *J Clin Microbiol* 2006;**44**:847–53.
104. Eagle H. Experimental approach to the problem of treatment failure with penicillin. I. Group A streptococcal infection in mice. *Am J Med* 1952;**13**:389–99.
105. Edwards-Jones V, Foster HA. The effect of topical antimicrobial agents on the production of toxic shock syndrome toxin-1. *J Med Microbiol* 1994;**41**:408–13.
106. Edwards-Jones V, Dawson MM, Childs C. A survey into toxic shock syndrome (TSS) in UK burn units. *Burns* 2000;**26**: 323–33.
107. Ejlertsen T, Porsborg PA. Toxic-shock syndrome related to simultaneous *Staphylococcus aureus* epiglottic abscess and group A streptococcal pharyngitis with bacteremia. *A P M I S* 1994;**102**: 956–9.
108. Elbaum DJ, Wood C, Abuabara F, et al. Bullae in a patient with toxic shock syndrome. *J Am Acad Dermatol* 1984;**10**:267–72.
109. Elsberry DD, Rhoda DA, Beisel WR. Hemodynamics of staphylococcal B enterotoxemia and other types of shock in monkeys. *J Appl Physiol* 1969;**27**:164–9.
110. Eriksson B, Anderson J, Bergholm AM, et al. Invasive group A streptococcal infections: T1M1 isolates expressing pyrogenic exotoxins A and B in combination with selective lack of toxin-neutralizing antibodies are associated with increased risk of streptococcal toxic shock syndrome. *J Infect Dis* 1999;**180**:410–18.
111. Espersen F, Baek L, Kjaeldgaard P, et al. Detection of staphylococcal toxic shock syndrome toxin 1 by a latex agglutination kit. *Scand J Infect Dis* 1988;**20**:449–50.
112. Faich G, Pearson K, Fleming D, et al. Toxic-shock syndrome and the vaginal contraceptive sponge. *JAMA* 1986;**255**:216–18.
113. Farley DE, Katz VL, Dotters DJ. Toxic shock syndrome associated with vulvar necrotizing fasciitis. *Obstet Gynecol* 1993;**82**: 660–2.
114. Fast DJ, Schlievert PM, Nelson RD. Nonpurulent response to toxic shock syndrome toxin-1–producing *Staphylococcus aureus*. *J Immunol* 1988;**140**:949–53.
115. Feldman CA. Staphylococcal scarlet fever. *N Engl J Med* 1962;**267**:877–88.
116. Ferguson MA, Todd JK. Toxic-shock syndrome associated with *Staphylococcus aureus* sinusitis in children. *J Infect Dis* 1990;**161**: 953–5.
117. Fichtenbaum CJ, Peterson LR, Weil GJ. Ehrlichiosis presenting as a life-threatening illness with features of the toxic shock syndrome. *Am J Med* 1993;**95**:351–7.
118. Finkelstein JW, von Eye A. Sanitary product use by white, black and Mexican American women. *Public Health Rep* 1990;**105**:491.
119. Finkelstein S, Hyland RH. Toxic shock syndrome as the AIDS defining diagnosis. *Chest* 1993;**104**:950–1.
120. Fisher CJ Jr, Horowitz Z, Albertson TE. Cardiorespiratory failure in toxic shock syndrome: effect of dobutamine. *Crit Care Med* 1985;**13**:160–5.
121. Fisher CJ Jr, Horowitz BZ, Nolan SM. The clinical spectrum of toxic shock syndrome. *West J Med* 1981;**15**:175–82.
122. Fisher RF, Goodpasture HC, Peterie JD, et al. Toxic shock syndrome in menstruating women. *Ann Intern Med* 1981;**94**: 156–63.
123. Fitz JD, Weeks KD, Duff P. Left ventricular dysfunction in a patient with toxic shock syndrome. *Am J Obstet Gynecol* 1983;**146**: 467–8.
124. Fitzgerald JR, Sturdevant DE, Mackie SM, et al. Evolutionary genomics of *Staphylococcus aureus*: insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proc Natl Acad Sci U S A* 2001;98:8821–8826.
125. Foley-Nolan D, Coughlan RJ, Sugrue D. Toxic shock syndrome associated arthropathy: *Staphylococcus aureus*—a further triggering event in reactive arthritis? *Ann Rheum Dis* 1989;**48**:331.
126. Fornadley JA, Gomez PJ, Crane RT, et al. Toxic shock syndrome following submandibular gland excision. *Head Neck* 1990;**12**: 66–8.
127. Frame JD, Eve MD, Hackett ME, et al. The toxic shock syndrome in burned children. *Burns* 1985;**11**:234–41.
128. Fuleihan R, Trede N, Chatila T, et al. Superantigens activate HIV-1 gene expression in monocytic cells. *Clin Immunol Immunopathol* 1994;**72**:357–61.
129. Galea P, Goel KM. Toxic shock syndrome (TSS) in children. *Scott Med J* 1987;**32**:28–9.
130. Gallagher PG, Myer CM. An approach to the diagnosis and treatment of membranous laryngotracheobronchitis in infants and children. *Pediatr Emerg Care* 1991;**7**:337–42.
131. Garbe PL, Arko RJ, Reingold AL, et al. *Staphylococcus aureus* isolates from patients with non-menstrual toxic shock syndrome: evidence for additional toxins. *JAMA* 1985;**253**:2538–42.
132. Gaventa S, Reingold AL, Hightower AW, et al. Active surveillance for TSS in the United States. *Rev Infect Dis* 1989;**11**(suppl.): 28–34.
133. Glazowski MJ, Ostergaard GZ, Arpi M, et al. Toxic shock syndrome: a case of a child with burns. *Ugeskr Laeger* 1992;**154**:868–9.
134. Gosbell IB. Epidemiology, clinical features and management of infections due to community methicillin-resistant *Staphylococcus aureus* (cMRSA). *Intern Med J* 2005;**35**(suppl. 2):S120–35.
135. Gourley GR, Chesney PJ, Davis JP, et al. Acute cholestasis in patients with toxic-shock syndrome. *Gastroenterology* 1981;**81**:928–31.
136. Graham DR, O'Brien M, Hayes JM, et al. Postoperative toxic shock syndrome. *Clin Infect Dis* 1995;**20**:895–9.
137. Hackett SP, Stevens DL. Superantigens associated with staphylococcal and streptococcal toxic shock syndrome are potent inducers of tumor necrosis factor β synthesis. *J Infect Dis* 1993;**168**: 232–235.
138. Hajjeh RA, Reingold A, Weil A, et al. Toxic shock syndrome in the United States: surveillance update, 1979-1996. *Emerg Infect Dis* 1999;**5**:807–10.
139. Hamad ARA, Marrach P, Kappler JW. Transcytosis of staphylococcal superantigen toxins. *J Exp Med* 1997;**185**:1447–54.
140. Hanafiah SR, Chong SKF. Toxic shock syndrome presenting as an acute encephalopathy and diarrhoea. *J R Soc Med* 1991;**84**:48–9.
141. Hauschildt S, Bessler WG, Scheipers P. Engagement of major histocompatibility complex class II molecules leads to nitrite production in bone marrow–derived macrophages. *Eur J Immunol* 1993;**23**:2988–92.
142. Hayward J, Vergeront JM, Stolz-LaVerriere SJ, et al. A hospital discharge code review of toxic shock syndrome in Wisconsin. *Am J Epidemiol* 1986;**123**:876–83.
143. Helgerson SD, Foster LR. Toxic shock syndrome in Oregon: epidemiologic findings. *Ann Intern Med* 1982;**96**:909–11.
144. Helms CM, Lengeling RW, Pinsky RL, et al. Toxic shock syndrome: a retrospective study of 25 cases from Iowa. *Am J Med Sci* 1981;**282**:50–60.
145. Hensler T, Koller M, Geoffroy C, et al. *Staphylococcus aureus* toxic shock syndrome toxin 1 and *Streptococcus pyogenes* erythrogenic toxin A modulate inflammatory mediator release from human neutrophils. *Infect Immun* 1993;**61**:1055–61.
146. Herwald H, Cramer H, Morgelin M, et al. M protein, a classical bacterial virulence determinant, forms complexes with fibrinogen that induce vascular leakage. *Cell* 2004;**116**:367–79.
147. Heywood AJ, al-Essa S. Toxic shock syndrome in child with only 2% burn. *Lancet* 1990;**335**:867.
148. Hiramatsu K. Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. *Lancet Infect Dis* 2001;**1**:147–55.
149. Hirose-Kumagai A, Whipple FH, Ikejima T, et al. A comparison of neutralizing and antigen-binding assays for human antibodies against toxic-shock-syndrome 1. *J Infect Dis* 1984;**150**:788.
150. Hurley JM, Shimonkevitz R, Hanagan A, et al. Identification of class II major histocompatibility complex and T cell receptor binding sites in the superantigen toxic shock syndrome toxin 1. *J Exp Med* 1995;**181**:2229.
151. Hurwitz RM, Ackerman AB. Cutaneous pathology of the toxic shock syndrome. *Am J Dermatopathol* 1985;**7**:563.
152. Iandolo JJ. Genetic analysis of extracellular toxins of *Staphylococcus aureus*. *Annu Rev Microbiol* 1989;**43**:375–402.
153. Ikejima T, Dinarello CA. Distribution of radiolabeled toxic shock syndrome toxin: implications for the pathogenesis of interleukin-1–mediated toxic shock syndrome. *J Leukoc Biol* 1985;**37**:714.
154. Institute of Medicine. *Prevention and recognition of TSS: Toxic shock syndrome: assessment of current information and future research needs*. Washington, DC: National Academy Press; 1982. p. 85–6.
155. Ishak KG, Rogers WA. Cryptogenic acute cholangitis: association with toxic shock syndrome. *Am J Clin Pathol* 1981;**76**:619–26.
156. Jacobson JA, Kasworm EM, Bolte RG, et al. Prevalence of nasal carriage of toxigenic *Staphylococcus aureus* and antibody to TSST-1 in Utah children. *Rev Infect Dis* 1989;**11**(suppl.):324–5.

157. Jacobson JA, Kasworm E, Daly JA. Risk of developing TSS associated with TSST-1 following nongenital staphylococcal infection. *Rev Infect Dis* 1989;**11**(suppl.):8–13.
158. Jacobson JA, Kasworm EM, Reiser RF, et al. Low incidence of toxic shock syndrome in children with staphylococcal infection. *Am J Med Sci* 1987;**294**:403–7.
159. Jacobson JA, Stevens MH, Kasworm EM. Evaluation of single-dose cefazol in prophylaxis for toxic-shock syndrome. *Arch Otolaryngol Head Neck Surg* 1988;**114**:326–7.
160. James RC, MacLeod CJ. Induction of staphylococcal infections in mice with small inocula introduced on sutures. *Br J Exp Pathol* 1961;**42**:266.
161. Jaulhac B, Bes M, Bornstein N, et al. Synthetic DNA probes for detection of genes for enterotoxins A, B, C, D, E and for TSST-1 in staphylococcal strains. *J Appl Bacteriol* 1992;**72**:386–92.
162. Johnson WM, Tyler SD, Ewan EP, et al. Detection of genes for enterotoxins, exfoliative toxins, and toxic shock syndrome toxin 1 in *Staphylococcus aureus* by the polymerase chain reaction. *J Clin Microbiol* 1991;**29**:426–30.
163. Kain KC, Schulzer M, Chow AW. Clinical spectrum of nonmenstrual toxic shock syndrome (TSS): comparison with menstrual TSS by multivariate discriminant analyses. *Clin Infect Dis* 1993;**16**:100–6.
164. Kamel NS, Banks MC, Dosik A, et al. Lack of mucocutaneous signs of toxic shock syndrome when T cells are absent: *S. aureus* shock in immunodeficient adults with multiple myeloma. *Clin Exp Immunol* 2002;**128**:131–9.
165. Kappler J, Kotzin B, Herron L, et al. V_β-specific stimulation of human T-cells by staphylococcal toxins. *Science* 1989;**244**:811–13.
166. Kato K, Tanaka T. MRSA infection and toxic shock syndrome in burn patients. *Jpn J Clin Med* 1992;**50**:1104–11.
167. Kehrberg MW, Latham RH, Haslam BT, et al. Risk factors for staphylococcal toxic-shock syndrome. *Am J Epidemiol* 1981;**114**:873–9.
168. Kikuchi K, Takahashi N, Piao C, et al. Molecular epidemiology of MRSA strains causing neonatal TSS-like exanthematous disease in neonatal and perinatal wards. *J Clin Microbiol* 2003;**41**:3001–6.
169. Kline MW, Dunkle LM. Toxic shock syndrome and the acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 1988;**7**:736–8.
170. Kotb M. Bacterial pyrogenic exotoxins as superantigens. *Clin Microbiol Rev* 1995;**8**:411–26.
171. Krakauer T. Inhibition of toxic shock syndrome toxin-1–induced cytokine production and T cell activation by interleukin-10, interleukin-4 and dexamethasone. *J Infect Dis* 1995;**172**:988–92.
172. Kravitz GR, Dries DJ, Peterson ML, et al. Purpura fulminans due to *Staphylococcus aureus*. *Clin Infect Dis* 2005;**40**:941–7.
173. Kreiswirth BN, Kravitz GR, Schlievert PM, et al. Nosocomial transmission of a strain of *Staphylococcus aureus* causing toxic shock syndrome. *Ann Intern Med* 1986;**105**:704.
174. Kreiswirth BN, Projan SJ, Schlievert PM, et al. Toxic shock syndrome toxin 1 is encoded by a variable genetic element. *Rev Infect Dis* 1989;**11**(suppl.):83–8.
175. Ku WWS, Chow AW. Monoclonal antibodies (MAb5 and MAb4) protect against the lethal effect of toxic shock syndrome toxin-1 in the D-galactosamine sensitized mouse mode [Abstract]. *J Invest Med* 1996;**110**:68.
176. Kum WW, Chow AW. Inhibition of staphylococcal enterotoxin A–induced superantigenic and lethal activities by a monoclonal antibody to toxic shock syndrome toxin-1. *J Infect Dis* 2001;**183**:1739–48.
177. Kushnaryov VM, MacDonald HS, Reiser RF, et al. Reaction of TSST-1 with endothelium of human umbilical cord vein. *Rev Infect Dis* 1989;**11**(suppl.):282–7.
178. Lamagni TL, Darenberg J, Luca-Harari B, et al. Epidemiology of severe *Streptococcus pyogenes* disease in Europe. *J Clin Microbiol* 2008;**46**:2359–67.
179. Larkin SM, Williams DN, Osterholm MT, et al. Toxic shock syndrome: clinical, laboratory, and pathologic findings in nine fatal cases. *Ann Intern Med* 1982;**96**:858–64.
180. Latham RH, Kehrberg MW, Jacobson JA, et al. Toxic shock syndrome in Utah: a case-control and surveillance study. *Ann Intern Med* 1982;**96**:906–8.
181. Lee PK, Vercellotti GM, Deringer JR, et al. Effects of staphylococcal toxic shock syndrome toxin 1 on aortic endothelial cells. *J Infect Dis* 1991;**164**:711–19.
182. Lee VTP, Chang AH, Chow AW. Detection of staphylococcal enterotoxin B among toxic-shock syndrome (TSS) and non-TSS associated *Staphylococcus aureus* isolates. *J Infect Dis* 1992;**166**:911–15.
183. Lovseth A, Loncarevic S, Berdal KG, et al. Modified multiplex PCR method for detection of pyrogenic exotoxin genes in staphylococcal isolates. *J Clin Microbiol* 2004;**42**:3869–72.
184. Lund L, Nielsen D, Anderson ES. Meningismus as the main symptom in toxic shock syndrome. *Acta Obstet Gynecol Scand* 1988;**67**:395.
185. MacDonald KL, Osterholm MT, Hedberg CW, et al. Toxic shock syndrome: a newly recognized complication of influenza and influenza like illness. *JAMA* 1987;**257**:1053–8.
186. Malknecht U, Hunter M, Hoffmann MK, et al. The toxic shock syndrome toxin-1 induces anergy in human T cells in vivo. *Hum Immunol* 1996;**45**:42–5.
187. Marlowe DE, Weigle RM, Stauffenberg RS. *Measurement of tampon absorbency: evaluation of tampon brands*. Rockville, MD: FDA Bureau of Biologics; 1981.
188. Marodi L, Kaposzta R, Rozgonyi F, et al. Staphylococcal enterotoxin A involvement in the illness of a 20-month-old burn patient. *Pediatr Infect Dis J* 1995;**14**:632–4.
189. Marples RR, Wienecke AA. Enterotoxins and toxic-shock syndrome-1 in non-enteric staphylococcal disease. *Epidemiol Infect* 1993;**110**:477–88.
190. Martin RR, Buttram V, Besch P, et al. Nasal and vaginal *Staphylococcus aureus* in young women: quantitative studies. *Ann Intern Med* 1982;**96**:951–3.
191. Matsuda Y, Kato H, Yamada R, et al. Early and definitive diagnosis of toxic shock syndrome by detection of marked expansion of T-cell-receptor $V_\beta 2$-positive T cells. *Emerg Infect Dis* 2003;**9**:387–9.
192. McAllister RM, Mercer NS, Morgan BD, et al. Early diagnosis of staphylococcal toxemia in burned children. *Burns* 1993;**19**:22–5.
193. McCormick JK, Yarwood JM, Schlievert PM. Toxic shock syndrome and bacterial superantigens: an update. *Annu Rev Microbiol* 2001;**55**:77–104.
194. McGann VG, Rollins JB, Mason DW. Evaluation of resistance to staphylococcal enterotoxin B: naturally acquired antibodies of man and monkey. *J Infect Dis* 1971;**124**:206–13.
195. McIvor ME, Levin ML. Treatment of recurrent toxic shock syndrome with oral contraceptive agents. *Md Med J* 1982;**31**:56–7.
196. McKenna UG, Meadows JA III, Brewer NS, et al. Toxic shock syndrome, a newly recognized disease entity: report of 11 cases. *Mayo Clin Proc* 1980;**55**:663–672.
197. McMahon WS, Patrenos ME, McConnell ME, et al. Complete heart block in toxic shock syndrome. *Am J Dis Child* 1990;**144**:748.
198. Mehrotra M, Wang G, Johnson WM, et al. Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin 1, and methicillin resistance. *J Clin Microbiol* 2000;**38**:1032–5.
199. Melish ME, Chen FS, Murata MS. Quantitative detection of toxic shock marker protein in human and experimental toxic shock syndrome [Abstract]. *Clin Res* 1983;**31**:122.
200. Melish ME, Frogner KS, Hirata SA, et al. Use of IVGG for therapy in the rabbit model of TSS [Abstract]. *Clin Res* 1987;**35**:220.
201. Melish ME, Murata S, Fukunaga C, et al. Corticosteroid and immunoglobulin therapy in toxic shock syndrome (TSS) [Abstract]. *Clin Res* 1988;**36**:781.
202. Melish ME, Murata S, Fukunaga C, et al. Corticosteroid and immunoglobulin therapy in TSS. *Rev Infect Dis* 1989;**11**(suppl.):332–3.
203. Melish ME, Murata S, Fukunaga C, et al. Endotoxin is not an essential mediator in TSS. *Rev Infect Dis* 1989;**11**(suppl.):219–28.
204. Melish ME, Murata S, Fukunaga C, et al. Vaginal tampon model for toxic shock syndrome. *Rev Infect Dis* 1989;**11**(suppl.):238–46.
205. Michels TC. Mucocutaneous lymph node syndrome in adults: differentiation from toxic shock syndrome. *Am J Med* 1986;**80**:724.
206. Michie CA, Davis T, MacAllister M. The sequelae of toxic shock syndrome [Abstract]. *Pediatr Res* 1996;**39**:179.
207. Miethke T, Duschek K, Wahl C, et al. Pathogenesis of the toxic shock syndrome: T-cell–mediated lethal shock caused by the superantigen TSST-1. *Eur J Immunol* 1993;**23**:1494–500.

208. Miethke T, Wahl IC, Heeg K, et al. T-cell–mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. *J Exp Med* 1992;**175**: 91–8.
209. Miethke T, Wahl C, Regele D, et al. *Staphylococcus aureus* mediated shock: a cytokine release syndrome. *Immunobiology* 1993;**189**: 270–84.
210. Miller SD. Postoperative toxic shock syndrome after lumbar laminectomy in a male patient. *Spine* 1994;**19**:1182–5.
211. Miller W, Stankiewicz JA. Delayed toxic shock syndrome in sinus surgery. *Otolaryngol Head Neck Surg* 1994;**111**:121–3.
212. Mills JT, Parsonnet J, Hickman RK, et al. Control of production of toxic-shock syndrome toxin-1 (TSST-1) by magnesium ion. *J Infect Dis* 1985;**151**:1158–61.
213. Miwa K, Fukuyama M, Kunitomo T, et al. Rapid assay for detection of toxic shock syndrome toxin 1 from human sera. *J Clin Microbiol* 1994;**32**:539–42.
214. Mohan SB, Tamilarasan A, Buhl M. Inhalational mercury poisoning masquerading as toxic shock syndrome. *Anaesth Intensive Care* 1994;**22**:305–6.
215. Mohsenipour M, Deusch E, Twerdy K, et al. Toxic shock syndrome in transsphenoidal neurosurgery. *Acta Neurochir* 1994;**128**: 169–70.
216. Moyer MA, Edwards LD, Bergdoll MS. Nosocomial toxic shock syndrome in two patients after knee surgery. *Am J Infect Control* 1983;**11**:83–7.
217. Murray DL, Earhart CA, Mitchell DT, et al. Localization of biologically important regions on toxic shock syndrome toxin-1. *Infect Immun* 1996;**64**:371–4.
218. Musser JM, Schlievert PM, Chow AW, et al. A single clone of *Staphylococcus aureus* causes the majority of cases of toxic-shock syndrome. *Proc Natl Acad Sci U S A* 1990;**87:**225–9.
219. Nadal D, Lauener RP, Braegger CP, et al. T-cell activation and cytokine release in streptococcal toxic shock–like syndrome. *J Pediatr* 1993;**122**:727–9.
220. Neill RJ, Fanning GR, Delahoz F, et al. Oligonucleotide probes for detection and differentiation of *Staphylococcus aureus* strains containing genes for enterotoxins A, B, and C and toxic shock syndrome toxin 1. *J Clin Microbiol* 1990;**28**:1514–18.
221. Nightingale SL. New requirements for tampon labeling. *Am Fam Physician* 1990;**41**:999.
222. Nijssen-Jordan C, Donaldson JD, Halperin SA. Bacterial tracheitis associated with respiratory syncytial virus infection and toxic-shock syndrome. *Can Med Assoc J* 1990;**142**:233–4.
223. Nishi J, Yoshinaga M, Miyanohara H, et al. An epidemiologic survey of methicillin-resistant *Staphylococcus aureus* by combined use of mec-HVR genotyping and toxin genotyping in a university hospital in Japan. *Infect Control Hosp Epidemiol* 2002;**23**:506–10.
224. Norrby-Teglund A, Pauksens K, Holm SE, et al. Relation between low capacity of human sera to inhibit streptococcal mitogens and serious manifestation of disease. *J Infect Dis* 1994;**170**: 585–91.
225. Ogawa M, Ueda S, Anzai N, et al. Toxic shock syndrome after staphylococcal pneumonia treated with intravenous immunoglobulin. *Vox Sang* 1995;**68**:59–60.
226. O'Gilvie vs. International Playtex, No. 83-1845, Vol. 37 (DC Kansas March 21, 1985, post-trial motions and court findings).
227. O'Loughlin RE, Roberson A, Cieslak PR, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. *Clin Infect Dis* 2007;**45**: 853–62.
228. Olesen LL, Ejlertsen T, Nielsen J. Toxic shock syndrome following insertion of breast prostheses. *Br J Surg* 1991;**78**:585–6.
229. Olson RD, Stevens DL, Melish ME. Direct effects of purified staphylococcal TSST-1 on myocardial function of isolated rabbit atria. *Rev Infect Dis* 1989;**11**(suppl.):313–15.
230. Onderdonk AB, Delaney ML, Zamarchi GR, et al. Normal vaginal microflora during use of various forms of catamenial protection. *Rev Infect Dis* 1989;**11**(suppl.):61–7.
231. Orden JA, Goyache J, Hernandez FJ, et al. Detection of staphylococcal enterotoxin and toxic shock syndrome toxin-1 (TSST-1) by immunoblot combined with a semiautomated electrophoresis system. *J Immunol Methods* 1991;**144**:197–202.
232. Osterholm MT, Davis JP, Gibson RW, et al. Toxic shock syndrome: relation to catamenial products, personal health and hygiene, and sexual practices. *Ann Intern Med* 1982;**96**:954–8.
233. Osterholm MT, Davis JP, Gibson RW, et al. Tri-state toxic-shock syndrome study. I. Epidemiologic findings. *J Infect Dis* 1982;**145**: 431–40.
234. Osterholm MT, Forfang JC. Toxic-shock syndrome in Minnesota: results of an active-passive surveillance system. *J Infect Dis* 1982;**145**:458–64.
235. Paris AL, Herwaldt LA, Blum D, et al. Pathologic findings in twelve fatal cases of toxic-shock syndrome. *Ann Intern Med* 1982;**96**:852–7.
236. Parkin DE. Fatal toxic shock syndrome following endometrial resection. *Br J Obstet Gynaecol* 1995;**102**:163–4.
237. Parsonnet J. Mediators in the pathogenesis of TSS: overview. *Rev Infect Dis* 1989;**11**(suppl.):263–9.
238. Parsonnet J. Nonmenstrual toxic shock syndrome: new insights into diagnosis, pathogenesis and treatment. *Curr Clin Top Infect Dis* 1996;**16**:1–20.
239. Parsonnet J, Gillis ZA, Pier GB. Induction of interleukin-1 by strains of *Staphylococcus aureus* from patients with non-menstrual toxic shock syndrome. *J Infect Dis* 1986;**154**:55–63.
240. Parsonnet J, Modern PA, Giacobbe K. Effect of subinhibitory concentrations of antibiotics on production of toxic shock syndrome toxin-1 (TSST-1). Abstract 29. Presented at the 32nd Annual Meeting of the Infectious Diseases Society of America, Washington, DC, 1994.
241. Parsonnet J, Modern PA, Giacobbe KD. Effect of tampon composition on production of toxic shock syndrome toxin-1 by *Staphylococcus aureus* in vitro. *J Infect Dis* 1996;**173**:98–103.
242. Parsonnet J, Gillis ZA, Thompson MR, et al. Effects of monoclonal antibody on biologic function of TSST-1 in vitro and in vivo. *Rev Infect Dis* 1989;**11**(suppl.):318–19.
243. Parsonnet J, Hansmann MA, Delaney ML, et al. Prevalence of toxic shock syndrome toxin 1–producing *Staphylococcus aureus* and the presence of antibodies to this superantigen in menstruating women. *J Clin Microbiol* 2005;**43**:4628–34.
244. Parsonnet J, Hansmann MA, Seymour JL, et al. Persistence survey of toxic shock syndrome toxin-1 producing *Staphylococcus aureus* and serum antibodies to this superantigen in five groups of menstruating women. *BMC Infect Dis* 2010;**10**:249.
245. Parsonnet J, Tosteson A, Modern P, et al. *Antibody to toxic shock syndrome toxin-1 (TSST-1) and vaginal colonization by TSST-1 producing* S. aureus *among adolescent women. Abstract 1327. Presented at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, DC: American Society for Microbiology; 1993.
246. Paterson MP, Hoffman EB, Roux P. Severe disseminated staphylococcal disease associated with osteitis and septic arthritis. *J Bone Joint Surg Br* 1990;**72**:94–7.
247. Petitti DB, Reingold A, Chin J. The incidence of toxic shock syndrome in northern California: 1972 through 1983. *JAMA* 1986;**255**:368–72.
248. Pokriefka R, Rabah M, Saravolatz L, et al. Toxic shock syndrome in an injection drug user with *Staphylococcus aureus* endocarditis. *Infect Med* 1994;**11**:34–6, 48–9.
249. Potter T, DiGregorio F, Stiff M, et al. Dilantin hypersensitivity syndrome imitating staphylococcal toxic shock. *Arch Dermatol* 1994;**130**:856–8.
250. Projan SJ, Brown-Skrobot S, Schlievert PM. Glycerol monolaurate inhibits the production of beta-lactamase, toxic shock toxin-1, and other staphylococcal exoproteins by interfering with signal transduction. *J Bacteriol* 1994;**176**:4204–9.
251. Raab MG, O'Brien M, Hayes JM, et al. Postoperative toxic shock syndrome. *Am J Orthop* 1995;**24**:130–6.
252. Rasheed JK, Arko RJ, Feeley JC, et al. Acquired ability of *Staphylococcus aureus* to produce toxic shock–associated protein and resulting illness in a rabbit model. *Infect Immun* 1985;**47**:598–604.
253. Reingold AL. Epidemiology of toxic-shock syndrome: United States, 1960-1984. *MMWR CDC Surveill Summ* 1984;**33**: 19SS–22SS.
254. Reingold AL, Hargrett NT, Dan BB, et al. Nonmenstrual toxic shock syndrome: a review of 130 cases. *Ann Intern Med* 1982; **96**:871–4.
255. Ren K, Bannan JD, Pancholi V, et al. Characterization and biological properties of a new staphylococcal exotoxin. *J Exp Med* 1994;**180**:1675–83.
256. Rhee CA, Smith RJ, Jackson IT. Toxic shock syndrome associated with suction-assisted lipectomy. *Aesthetic Plast Surg* 1994;**18**:161–3.

257. Rich RR. Intravenous IgG: Supertherapy for superantigens. *J Clin Invest* 1993;**91**:378.
258. Ritz HL, Kirkland JJ, Bond GG, et al. Association of high levels of serum antibody to staphylococcal toxic shock antigen with nasal carriage of toxic shock antigen–producing strains of *Staphylococcus aureus*. *Infect Immun* 1984;**43**:954–8.
259. Rolston RD, Yabek SM, Florman AL, et al. Severe cardiac conduction abnormalities associated with atypical toxic-shock syndrome. *J Pediatr* 1990;**117**:89.
260. Romagne F, Besnardeau L, Malissen B. A versatile method to produce antibodies to human T cell receptor V beta segments: frequency determination of human V beta 2+ T cells that react with toxic-shock syndrome toxin-1. *Eur J Immunol* 1992;**22**: 2749–52.
261. Rose PG, Wilson G. Advanced cervical carcinoma presenting with toxic shock syndrome. *Gynecol Oncol* 1994;**52**:264–6.
262. Rosene KA, Copass MK, Kastner LS, et al. Persistent neuropsychological sequelae of toxic shock syndrome. *Ann Intern Med* 1982;**96**:865–70.
263. Rosten PM, Bartlett KH, Chow AW. Serologic responses to toxic shock syndrome (TSS) toxin-1 in menstrual and nonmenstrual TSS. *Clin Invest Med* 1988;**11**:187–92.
264. Rovner RA, Baird RA, Malerich MM. Fatal toxic-shock syndrome as a complication of orthopedic surgery. *J Bone Joint Surg Am* 1984;**66**:952–4.
265. Royall JA, Berkow RL, Beckman JS, et al. Tumor necrosis factor and interleukin 1α increase vascular endothelial cell permeability. *Am J Physiol* 1989;**257**:L399–410.
266. Sahs AL, Helms CM, DuBois C. Carpal tunnel syndrome: complication of toxic shock syndrome. *Arch Neurol* 1983;**40**:414–15.
267. Sales JH, Kennedy KS, Galantich PT, et al. Toxic shock syndrome associated with pharyngitis and submandibular space abscess. *Ann Otol Rhinol Laryngol* 1991;**100**:540–3.
268. Schlech WF, III, Shands KN, Reingold AL, et al. Risk factors for development of toxic shock syndrome: association with a tampon brand. *JAMA* 1982;**7**:835–9.
269. Schlievert PM. Staphylococcal enterotoxin B and toxic-shock syndrome toxin-1 are significantly associated with non-menstrual TSS. *Lancet* 1986;**1**:1149.
270. Schlievert PM. Comparison of cotton and cotton/rayon tampons for effect on production of toxic shock syndrome toxin. *J Infect Dis* 1995;**172**:1112–14.
271. Schlievert PM, Kelly JA. Clindamycin-induced suppression of toxic-shock syndrome–associated exotoxin production. *J Infect Dis* 1984;**149**:471.
272. Schlievert PM, Deringer JR, Kim MH, et al. Effect of glycerol monolaurate on bacterial growth and toxin production. *Antimicrob Agents Chemother* 1992;**36**:626–32.
273. Schlievert PM, Shands KN, Dan BB, et al. Identification and characterization of exotoxin from *Staphylococcus aureus* associated with toxic shock syndrome. *J Infect Dis* 1981;**143**:509–16.
274. Schmitz FJ, Mackenzie CR, Geisel R, et al. Enterotoxin and toxic shock syndrome toxin-1 production of methicillin resistant and methicillin sensitive *Staphylococcus aureus* strains. *Eur J Epidemiol* 1997;**13**:699–708.
275. Schmitz FJ, Steiert M, Hofmann B, et al. Development of a multiplex-PCR for direct detection of the genes for enterotoxin B and C, and toxic shock syndrome toxin-1 in *Staphylococcus aureus* isolates. *J Med Microbiol* 1998;**47**:335–40.
276. Schuchat A, Broome CV. Toxic shock syndrome and tampons. *Epidemiol Rev* 1991;**13**:99–112.
277. Schwartz B, Gaventa S, Broome CV, et al. Nonmenstrual TSS associated with barrier contraceptives: report of a case-control study. *Rev Infect Dis* 1989;**11**(suppl.):43–8.
278. Scott DF, Best GK, Kling JM, et al. Passive protection of rabbits infected with TSS associated strains of *Staphylococcus aureus* by monoclonal antibody to TSST-1. *Rev Infect Dis* 1989;**11**(suppl.): 214–17.
279. Shah SS, Hall M, Srivastava R, et al. Intravenous immunoglobulin in children with streptococcal toxic shock syndrome. *Clin Infect Dis* 2009;**9**:1369–76.
280. Shands KN, Schmid GP, Dan BB, et al. Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. *N Engl J Med* 1980;**303**:1436–42.
281. Shlasko E, Harris MT, Benjamin E, et al. Toxic shock syndrome after pilonidal cystectomy: report of a case. *Dis Colon Rectum* 1991;**34**:502–5.
282. Sissons JG. Superantigens and infectious disease. *Lancet* 1993;**341**:1627–9.
283. Skansen-Saphir U, Andersson J, Bjork L, et al. Lymphokine production induced by streptococcal pyrogenic exotoxin A is selectively down regulated by pooled human IgG. *Eur J Immunol* 1994;**24**:916–22.
284. Slingluff CL Jr, Burns WW, Cooperberg C. Toxic shock syndrome after inguinal hernia repair: report of a case with patient survival. *Am Surg* 1990;**56**:610–12.
285. Smith DB, Gulinson J. Fatal cerebral edema complicating toxic shock syndrome. *Neurosurgery* 1988;**22**:598–9.
286. Sparano J, Ferranti E. The acquired immunodeficiency syndrome and non-menstrual toxic shock syndrome. *Ann Intern Med* 1986;**105**:300–1.
287. Spearman PW, Barson WJ. Toxic shock syndrome occurring in children with abrasive injuries beneath casts. *J Pediatr Orthop* 1992;**12**:169–72.
288. Sperber SJ, Blevins DD, Francis JB. Hypercalcitonemia, hypocalcemia, and toxic-shock syndrome. *Rev Infect Dis* 1990;**12**:736–9.
289. Spertini F, Spits H, Geha RS. Staphylococcal exotoxins deliver activation signals to human T-cell clones via major histocompatibility complex class II molecules. *Proc Natl Acad Sci U S A* 1991; **88**:7533–7.
290. Stevens DL. Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. *Emerg Infect Dis* 1995;**1**:69–78.
291. Stevens DL, Gibbons AE, Bergstrom R, et al. The eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *J Infect Dis* 1988;**158**: 23–8.
292. Stevens DL, Tanner MH, Winship J, et al. Severe group A streptococcal infections associated with a toxic shock–like syndrome and scarlet fever toxin A. *N Engl J Med* 1989;**321**:1–7.
293. Stevens DL, Wallace RJ, Hamilton SM, et al. Successful treatment of staphylococcal toxic shock syndrome with linezolid: a case report and in vitro evaluation of the production of toxic shock syndrome toxin type 1 in the presence of antibiotics. *Clin Infect Dis* 2006;**42**:729–30.
294. Stevens DL, Yan S, Bryant AE. Penicillin-binding protein expression at different growth stages determines penicillin efficacy in vitro and in vivo: an explanation for the inoculum effect. *J Infect Dis* 1993;**167**:1401–5.
295. Stevens FA. The occurrence of *Staphylococcus aureus* infection with a scarlatiniform rash. *JAMA* 1927;**18**:1957–8.
296. Stolz SJ, Davis JP, Vergeront JM, et al. Development of serum antibody to toxic shock toxin among individuals with toxic shock syndrome in Wisconsin. *J Infect Dis* 1985;**151**:883–9.
297. Su YC, Wong AC. Identification and purification of a new staphylococcal enterotoxin, H. *Appl Environ Microbiol* 1995;**61**: 1438–43.
298. Swift JD, Barruga MC, Perkin RM, et al. Respiratory failure complicating rubeola. *Chest* 1993;**104**:1786–7.
299. Takahashi N, Kato H, Imanishi K, et al. Immunopathophysiological aspects of an emerging neonatal infectious disease induced by a bacterial superantigen. *J Clin Invest* 2000;**106**:1409–15.
300. Takahashi N, Nishida H, Kato H, et al. Exanthematous disease induced by toxic shock syndrome toxin I in the early neonatal period. *Lancet* 1998;**351**:1614–19.
301. Takei S, Arora YK, Walker SM. Intravenous immunoglobulin contains specific antibodies inhibitory to activation of T cells by staphylococcal toxin superantigens. *J Clin Invest* 1993;**91**: 602–7.
302. Thompson TD, Friedman AL. Simultaneous occurrence of *Staphylococcus aureus*–associated septic arthritis and toxic shock syndrome. *Clin Pediatr (Phila)* 1994;**33**:243–5.
303. Tierno PM Jr, Hanna BA. Propensity of tampons and barrier contraceptives to amplify *Staphylococcus aureus* toxic shock syndrome toxin-1. *Infect Dis Obstet Gynecol* 1994;**2**:140–5.
304. Todd JK. Therapy of toxic shock syndrome. *Drugs* 1990;**39**: 856–61.
305. Todd JK, Fishaut M, Kapral F, et al. Toxic-shock syndrome associated with phage-group-1 staphylococci. *Lancet* 1978;**2**:1116–18.

306. Todd JK, Franco-Buff A, Lawellin DW, et al. Phenotypic distinctiveness of *Staphylococcus aureus* strains associated with toxic shock syndrome. *Infect Immun* 1984;**45**:339–44.
307. Todd JK, Ressman M, Caston SA, et al. Corticosteroid therapy for patients with toxic shock syndrome. *JAMA* 1984;**252**:3399–402.
308. Todd JK, Todd BH, Franco-Buff A, et al. Influence of focal growth conditions on the pathogenesis of toxic shock syndrome. *J Infect Dis* 1987;**155**:673–81.
309. Todd JK, Weisenthal AM, Ressman M, et al. Toxic shock syndrome. II. Estimated occurrence in Colorado as influenced by case ascertainment methods. *Am J Epidemiol* 1985;**22**:857–67.
310. Tofte RW, Williams DN. Toxic shock syndrome: evidence of a broad clinical spectrum. *JAMA* 1981;**246**:2163–7.
311. Tofte RW, Williams DN. Clinical and laboratory manifestations of toxic shock syndrome. *Ann Intern Med* 1982;**96**:843–7.
312. Tolan RW Jr. Toxic shock syndrome complicating influenza A in a child: case report and review. *Clin Infect Dis* 1993;**17**:43–5.
313. Turker R, Lubicky JP, Vogel LC. Toxic shock syndrome in patients with external fixators. *J Pediatr Orthop* 1992;**12**:658–62.
314. U.S. Food and Drug Administration. Tampon packages carry TSS information. *F D A Drug Bull* 1982;**3**:19–20.
315. Van der Mee-Marquet N, Lina G, Quentin R, et al. Staphylococcal exanthematous disease in a newborn due to a virulent methicillin-resistant *Staphylococcus aureus* strain containing the TSST-1 gene in Europe: an alert for neonatologists. *J Clin Microbiol* 2003;**41**:4883–4.
316. Vergeront JM, Evenson ML, Crass BA, et al. Recovery of staphylococcal enterotoxin F from the breast milk of a woman with toxic-shock syndrome. *J Infect Dis* 1982;**146**:456–9.
317. Vergeront JM, Stolz SJ, Crass BA, et al. Prevalence of serum antibody to staphylococcal enterotoxin F among Wisconsin residents: implications for toxic shock syndrome. *J Infect Dis* 1983;**148**:692–8.
318. Vic-Dupont MP, Duval P, Kamaliv SR. Scarlatiniform staphylococcal diseases. *Soc Med Hop Paris* 1965;**116**:51.
319. Wagner G, Bohr L, Wagner P, et al. Tampon-induced changes in vaginal oxygen and carbon dioxide tensions. *Am J Obstet Gynecol* 1984;**148**:147–50.
320. Watanabe-Ohnishi R, Low DE, McGeer A, et al. Selective depletion of V_β-bearing T cells in patients with severe invasive group A streptococcal infections and streptococcal toxic shock syndrome. *J Infect Dis* 1995;**171**:74–84.
321. Weinzweig J, Gottlich LJ, Krizek TJ. Toxic shock syndrome associated with the use of Biobrane in a scald burn victim. *Burns* 1994;**20**:180–1.
322. Weissberg SM, Dodson MG. Recurrent vaginal and cervical ulcers associated with tampon use. *JAMA* 1983;**250**:1430–1.
323. Wharton M, Chorba TL, Vogt RL, et al. Case definitions for public health surveillance. *MMWR Recomm Rep* 1990;**39**(RR-13): 1–43.
324. Whitby M, Fraser S, Gemmell CG, et al. Toxic shock syndrome and endocarditis. *Br Med J* 1983;**286**:1613.
325. Whiting JL, Rosten PM, Chow AW. Determination by Western blot (immunoblot) of seroconversions to toxic shock syndrome (TSS) toxin 1 and enterotoxin A, B, or C during infection with TSS- and non–TSS-associated *Staphylococcus aureus*. *Infect Immun* 1989;**57**:231–4.
326. Wick MR, Bahn RC, McKenna UG. Toxic shock syndrome: a fatal case with autopsy findings. *Mayo Clin Proc* 1982;**57**: 583–9.
327. Wiesenthal AM, Todd JK. Toxic shock syndrome in children aged 10 years or less. *Pediatrics* 1984;**74**:112–17.
328. Wong ACL, Bergdoll MS. Effect of environmental conditions on production of toxic shock syndrome toxin 1 by *Staphylococcus aureus*. *Infect Immun* 1990;**58**:1026–9.
329. Working Group on Severe Streptococcal Infections. Defining the group A streptococcal toxic shock syndrome: rationale and consensus definition. *JAMA* 1993;**269**:390–1.
330. Yagoob M, McClelland P, Murray AE, et al. Staphylococcal enterotoxins A and C causing toxic shock syndrome. *J Infect* 1990;**20**:176–8.
331. Yong JM. Necrotising fasciitis. *Lancet* 1994;**343**:1427.
332. Younis RT, Gross CW, Lazar RH. Toxic shock syndrome following functional endonasal sinus surgery: a case report. *Head Neck* 1991;**13**:247–8.
333. Zembowicz A, Vane JR. Induction of nitric oxide synthase activity by toxic shock syndrome toxin 1 in a macrophage-monocyte cell line. *Proc Natl Acad Sci U S A* 1992;**89**:2051–5.
334. Zumla A. Superantigens, T cells and microbes. *Clin Infect Dis* 1992;**15**:313–20.

TABLE 65-1 The Berlin Definition of Acute Respiratory Distress Syndrome 2011

	Acute Respiratory Distress Syndrome		
Criteria	**Mild**	**Moderate**	**Severe**
Time of onset	Within 1 week of known clinical insult or new or worsening respiratory symptoms		
Degree of hypoxemia	P/F ratio 201-300 with PEEP/CPAP ≥5	P/F ratio ≤200 with PEEP ≥5	P/F ratio ≤100 with PEEP ≥5
Origin of edema	Risk factors for ARDS must be present. Respiratory failure that is not fully explained by cardiac failure or fluid overload.*		
Radiologic findings+ (CXR or CT scan)	Bilateral opacities that are not fully explained by effusions, lobar or lung collapse, or nodules		

*If no risk factors are present, objective measures (e.g., echocardiography) are required to exclude hydrostatic edema.
ARDS, Acute respiratory distress syndrome; *CPAP,* continuous positive airway pressure; *CT,* computed tomography *CXR,* chest radiography; *PEEP,* positive end-expiratory pressure.

DEFINITION

Although first described by Ashbaugh and colleagues in 1967,[11] no formal definition of ARDS was developed for over 25 years. In 1994, the American-European Consensus Conference (AECC) defined ARDS as follows[15]:

1. Acute onset of respiratory symptoms
2. Frontal chest radiograph with bilateral infiltrates
3. Partial pressure of oxygen (Pao_2) to fraction of inspired oxygen (Fio_2) ratio (P/F ratio) 200 mm Hg or less
4. No clinical evidence of left atrial hypertension as defined by a pulmonary capillary wedge pressure less than 18 mm Hg (if measured)

The AECC also designated the term *acute lung injury* as a part of the spectrum of ARDS, and ALI is defined by a P/F ratio between 201 and 300 mm Hg. Despite the limitations,[111] these formal definitions have allowed for standardization of terminology and led to landmark clinical studies in ALI and ARDS that have contributed to improved understanding of this spectrum of conditions over the past 18 years.[20,41,104]

Recently, the Berlin Definition of ARDS was proposed by the European Society of Intensive Care Medicine (ESICM) in collaboration with the American Thoracic Society (ATS) at the ESICM Congress in October, 2011.[35,105] This new definition proposes that the term *ALI* be removed and ARDS be considered a single entity with three subgroups based on severity of hypoxemia (i.e., mild, moderate, and severe). The Berlin Definition of ARDS takes into account the timing of onset, degree of respiratory support (positive airway pressure), and overall fluid status. This new definition also allows for the use of either computed tomography (CT) of the chest or standard chest radiograph for determination of ARDS (Table 65-1).

ETIOLOGY

Numerous insults that both directly and indirectly affect the lung can lead to generation of inflammatory mediators that contribute to development of ARDS (Table 65-2). Direct pulmonary insults include pneumonia, aspiration, chest trauma, near drowning, and smoke inhalation. Indirect lung injury may be the result of generalized systemic conditions, such as sepsis, closed head injury, multisystem trauma, transfusion reactions, pancreatitis, and hemorrhagic shock.

TABLE 65-2 Causes of Acute Respiratory Distress Syndrome

Direct (Pulmonary) Causes	Indirect (Nonpulmonary) Causes
Pneumonia Bacterial Viral – H1N1, RSV Mycobacterium Fungal	Severe sepsis and septic shock Multiple trauma Severe hemorrhage Severe pancreatitis Transfusion reaction
Aspiration	Cardiopulmonary bypass
Near-drowning	
Smoke or chemical inhalation	
Pulmonary contusion	
Fat embolism (rare in children)	

Infective etiologies account for a large proportion of the causes for ARDS, with bacterial and viral infections being the most common infectious etiologies of ARDS.[14] *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* are common bacteria associated with severe pneumonia potentially leading to sepsis and, subsequently, ARDS.[14] Certain characteristics of these organisms have been shown to be instrumental in initiating this injury; for example, *P. aeruginosa* incites lung injury via the cytotoxic activity derived from the patatin-like phospholipase domain of ExoU toxin.[75]

Respiratory syncytial virus (RSV) infection is one of the most common indications for admission to the pediatric intensive care unit (PICU), especially during the winter months.[7] Although most critically ill children present as severe RSV bronchiolitis, a significant proportion (up to 27%) can present with ARDS; those with preexisting conditions are at the highest risk.[46] The influenza viruses are the most common cause of viral-induced ARDS, especially during an epidemic or pandemic.[36,87] Bacterial coinfection in patients with the severe pandemic

H1N1 viral infection has been shown to increase the need for mechanical ventilation, duration of intensive care unit (ICU) stay, and mortality.[90] Specifically, coinfection with methicillin-sensitive *Staphylococcus aureus* at admission was independently associated with almost a threefold increase in mortality.[90] There is a growing concern of community-acquired methicillin-resistant *S. aureus* (MRSA) infection leading to severe necrotizing pneumonia and ARDS, even with non-H1N1 influenza infection.[53,69,107] A less common cause of viral-induced ARDS is the coronavirus, such as the strain that caused the severe acute respiratory syndrome (SARS) in 2003 and affected adults more severely than children.[59,60]

In addition to bronchiolitis and pneumonia, severe sepsis is also a leading risk factor for ARDS in children.[117] In critically ill adults, sepsis is also associated with an approximately 40 percent risk for developing ARDS.[77] Multiorgan dysfunction and other illnesses associated with sepsis increase the risk for ARDS, and this risk is further compounded by the presence of chronic conditions or underlying comorbidities.[50] In addition, genetic differences, which regulate the immune responses of the lungs to these precipitating factors, account for differences in the degree of severity in ARDS in individual patients, even when similar risk profiles are present.[80] One such example of genetic polymorphisms is that related to nuclear factor-κB (NF-κB). Deletion polymorphism of the promoter region of this important transcription factor leads to inappropriate upregulation of proinflammatory genes, which has been shown to result in an increase in ARDS severity and mortality.[4,13]

Limited data exist on the impact of the underlying etiology of ARDS on clinical outcomes in children. In a study involving 736 adults with ARDS, patients with sepsis-related ARDS were more likely to have diabetes, longer pre-ICU stay, and higher APACHE III (a measure of severity of illness in critically ill adults) scores in contrast to those who had non–sepsis-related ARDS.[97] However, after accounting for differences in clinical characteristics, no difference was found in 60-day mortality between patients with sepsis-related ARDS and non–sepsis-related ARDS. Furthermore, no evidence exists to suggest that the source of sepsis is an important determinant of mortality in ARDS.[96]

INCIDENCE

In contrast to the incidence in adults, the incidence of ARDS in critically ill children is not as well described but does appear to be lower.[106] In a study conducted in the United States, 13 percent (828/6235) of patients who required mechanical ventilation had ARDS. This investigation reported an incidence of 16/100,000 person-years for those 15 to 19 years old and 306/100,000 person-years for adults 75 to 84 years old.[94] In a follow-up study involving children younger than 18 years, the incidence was 12.8/100,000 person-years.[117] Single-institution studies previously reported that ARDS accounted for 2.7 to 4.3 percent of PICU admissions.[28,43] In more recent data involving multiple centers, ARDS accounted for lower percentages (1.4% to 2.7%) of all PICU admissions,[49,116] making the conduct of clinical ARDS trials in critically ill children particularly challenging.

CLINICAL COURSE

The initial clinical course of ARDS begins with direct or indirect acute injury to the pulmonary parenchyma. In the initial stage, the clinical symptoms and physical findings vary depending on the etiology of the ALI. Children with pulmonary etiologies of ARDS will have predominantly respiratory symptoms; those with nonpulmonary etiologies of ARDS will have symptoms related to the initial insult (e.g., acute abdomen for pancreatitis). Early in the course of lung injury, patients may display mild tachypnea and dyspnea but tend to have normal radiographic findings.

After the inciting event and initial phase, a latent period follows that may last for a variable period. During this latent period, the patient may appear to be clinically stable, but early signs of pulmonary insufficiency develop, as manifested by hyperventilation with hypocarbia and respiratory alkalosis. The chest radiograph may remain clear or may begin to demonstrate a fine reticular pattern related to the development of pulmonary interstitial fluid (Fig. 65-1).[33] With progression of the lung injury, acute respiratory failure follows and is characterized by rapid onset of hypoxemia that is often refractory to supplemental oxygen administration. Diffuse pulmonary edema and worsening compliance cause significant atelectasis and intrapulmonary shunting. Clinically, patients develop rapid, shallow tachypnea with increased work of breathing. The physical signs of respiratory failure will vary with age and severity of illness, but usually include subcostal and supraclavicular retractions, grunting, and nasal flaring. Lung examination usually reveals diffuse crackles on auscultation. Radiographically, bilateral areas of consolidation with air bronchograms reflect alveolar filling and atelectasis (Fig. 65-2). A significant percentage of these children will require endotracheal intubation and

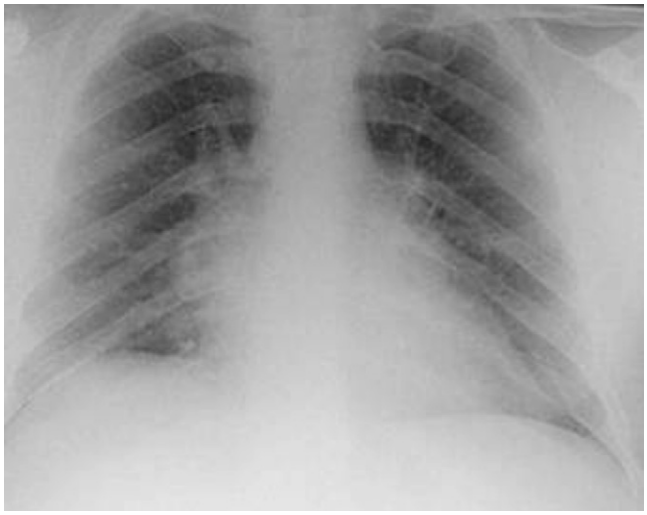

FIGURE 65-1 ■ Early stage of acute respiratory distress syndrome.

FIGURE 65-2 ■ Established acute respiratory distress syndrome.

mechanical ventilation with the application of positive end-expiratory pressure (PEEP). However, noninvasive ventilation may be an alternative for a subgroup of patients.[81]

Mortality

Most studies indicate that mortality associated with ARDS is due to nonrespiratory causes.[38,68] In most cases, early death (i.e., within 72 hours) is caused by the underlying illness or injury, whereas late death (i.e., beyond 72 hours) is caused by infection or multiorgan system failure. Reported mortality rates for children with ARDS vary greatly and range from 8 to 62 percent.* This variation is likely due to the myriad of underlying causes of ARDS, the differing degrees of ARDS severity, and the presence or absence of concomitant organ failures. Some of the factors that have been identified as predictors of mortality in pediatric ARDS include P/F ratio, oxygenation index ($[Fio_2 \times$ mean airway pressure$]/Pao_2$), pH, Pediatric Risk of Mortality (PRISM) score (a severity index score commonly used in PICUs), and presence of multiorgan failure.[84] Despite the large reported variation in mortality, it is generally accepted that the overall mortality rate of pediatric ARDS is lower than that of the adult population.[117]

Recent studies suggest that mortality from ARDS may be declining; however, the reason for this overall improvement is not completely clear. The trend toward decreasing ARDS mortality over time began over 20 years ago, before the publication and widespread adoption of low tidal volume ventilation strategy. Low tidal volume ventilation is the only mechanical ventilation strategy that has ever been clearly shown to decrease mortality in ARDS in adult patients in the setting of a randomized clinical study.[104] The extent that protective mechanical ventilation strategy has on mortality in actual clinical care remains to be determined, especially in the pediatric population.[2,66] Changes in organization of ICU care, improvement in supportive care, and improved monitoring capabilities may have accounted for the decreasing trend between the early 1990s and late 1990s (66% vs. 34% mortality, respectively).[2] More recent adult studies in the era of "low-volume ventilation" still report mortality rates of approximately 40 percent in adults with ARDS.[79,110] Similar trends are noted in pediatric patients, with mortality rates falling from 80 percent in the 1980s to approximately 20 percent in the early 2000s and potentially under 15 percent now.[117]

*References 16, 25, 28, 49, 84, 114.

PATHOLOGY AND ROLE OF IMMUNOMODULATORS

Pathology and Pathophysiology

The clinical stages of ARDS coincide with three pathologic stages: the exudative stage, the proliferative stage, and the fibrotic stage. The exudative stage is characterized by accumulation of protein-rich edema fluid into the alveoli. This fluid flux is secondary to diffuse injury to the alveolar-capillary membrane by either direct or indirect injury (see Table 65-1). Neutrophils play a predominant role in this stage of ARDS. In addition to influx of inflammatory cells and mediators, formation of microthrombi within the vasculature contributes further to the ALI and alters the pulmonary vascular tone. Pulmonary compliance is worsened by the presence of edema and can result in widespread atelectasis. Pulmonary compliance is further affected by the inactivation of surfactant that results from the presence of plasma proteins, such as fibrin, and inflammatory mediators, such as proteinases, in the alveolar space.[61,95] The development of microthrombi within the pulmonary vasculature together with the release of numerous vasoactive mediators from inflammatory cells and the activated endothelium contribute to the development of elevated pulmonary vascular resistance and further contribute to the ventilation/perfusion abnormalities characteristic of ARDS. The degree of epithelial injury and the subsequent ability to clear edema fluid, along with the reversibility of pulmonary hypertension, are important predictors of outcome in ARDS.[62,112]

The proliferative stage occurs 1 to 3 weeks after the initiation of injury and is characterized by attempted repair of the disrupted alveolar-capillary membrane. The mechanism of this repair requires not only the close coordination of numerous growth factors but also an intact basement membrane to provide a platform for cell adhesion and migration.[80]

The ability of the lung to recover depends on the presence of functional epithelium to clear the alveolar fluid and the body's ability to attenuate the inflammatory process. The ability of the alveolar epithelium to remove edema fluid depends on the degree of inflammation and injury in the exudative stage. After the initiation of the injury, anti-inflammatory processes take place to limit the degree of injury. Anti-inflammatory cytokines such as

interleukin (IL)-10 and lipid mediators have been shown to be important in the mitigation of this injury.[63] If lung injury and inflammation persist, the patient may develop severe physiologic abnormalities and may progress to the fibrotic stage of ARDS.

The fibrotic stage may be seen as early as 5 to 7 days after the onset of disease, although the presence of this injury becomes more clear after several weeks. Histologically, the alveolar space becomes filled with mesenchymal cells and lung tissue is replaced by collagenous tissue.[54] In addition, vascular changes in the fibrotic stage can lead to increased thickness of the pulmonary vasculature and even obliteration of small capillaries. Overall, these changes markedly decrease the available surface area for gas exchange and result in decreased effort tolerance in survivors of ARDS. In some patients, intractable respiratory failure or chronic lung disease result, necessitating prolonged ventilator support.

Immunomodulators

Numerous mediators of inflammation are implicated in the pathogenesis of ARDS.[88] Some of the more important mediators include tumor necrosis factor (TNF), ILs, and matrix metalloproteinases (MMPs).[36] The identification of these mediators potentially allows for development of biomarkers to predict the severity of ARDS. In a study involving 33 children with ARDS and 21 patients without ARDS who required mechanical ventilation, active MMP-9 in tracheal aspirates was significantly elevated in the ARDS patients. Furthermore, elevation of MMP-8 and MMP-9 at 48 hours predicts a longer duration of mechanical ventilation in children with ARDS, highlighting the potential use of MMPs as a biomarker in pediatric ARDS. The effects of mediators and inflammatory cells involved in ARDS, as well as regulatory molecules, are tightly woven and ultimately result in a balance between proinflammatory and anti-inflammatory and pro-edematous and anti-edematous factors.[37] Although inflammatory mediators could themselves disrupt the capillary-alveolar membrane, they also may exert effects to keep the inflammatory response in check. This may account for the lack of success of pharmacologic inhibitors to decrease the mortality associated with ARDS. Research efforts continue to investigate potential ways to more effectively modulate the inflammatory response during ARDS.[64,88]

Increasing evidence indicates that the genetic makeup of an individual influences the outcome of sepsis and ARDS.[109,115] One of the most commonly studied genetic polymorphisms in sepsis is that pertaining to TNF, which plays a central role in the "cytokine storm" in septic shock. Both pediatric and adult studies have shown that certain single nucleotide polymorphisms (SNPs) regulate the susceptibility of septic patients in developing ARDS.[12,44] A study of 490 children with sepsis found that changes in different SNPs in the TNF genotype affects the risk for developing ARDS (i.e., TNF-308 GA genotype was protective, and TNF-863 CA genotype increases the risk).[12] Genetic polymorphisms may potentially provide useful biomarkers for screening septic children who are at risk for developing ARDS and to identify a subgroup of patients who will benefit most from specific therapeutic strategies.

TREATMENT

The goal in the treatment of patients with ARDS is to treat the underlying disease (when possible), achieve adequate tissue oxygenation, and minimize ventilator-induced lung injury (VILI). In patients in whom ARDS is secondary to sepsis, prompt administration of appropriate antibiotics is essential.[29,58] The general antimicrobial approach depends on the local virulence and pattern of drug resistance in the community and within a given hospital. In the United States, where cases of infection with cephalosporin-resistant *S. pneumoniae* and MRSA are increasing, broad-spectrum gram-positive coverage (e.g., vancomycin) often is necessary.[29,53] The mainstay of support in the ARDS patient is the provision of supplemental oxygen and mechanical ventilation. In addition, nonpulmonary organ function must be meticulously maintained to optimize the clinical care of children with ARDS.

Pulmonary Management

Modes of Mechanical Ventilation

Multiple modes of mechanical ventilation are currently used in clinical practice to provide respiratory support for patients with ARDS. Conventional mechanical ventilation, which delivers tidal volume breaths, can be achieved effectively by either pressure-control or volume-control modes. In the former, the clinician controls the amount of inspiratory pressure in each breath delivered to the patient and, depending on the pulmonary compliance, the patient receives different tidal volumes. In volume-control modes, the clinician determines the amount of volume delivered to the patient and, with changing pulmonary compliance, the inspiratory pressure changes. Regardless of the conventional mechanical ventilation mode(s) used, it is paramount that VILI be minimized by paying meticulous attention to prevention of barotrauma and volutrauma. Prospective, randomized multicenter studies comparing one ventilatory mode to another in the setting of ARDS are limited and have not demonstrated a single ventilatory mode as superior to any other.[34,83]

In contrast to conventional mechanical ventilation, high-frequency oscillatory ventilation is an alternative mode that delivers extremely small-volume "breaths" (i.e., less than dead space volume) at high rates (5 to 15 Hz [equivalent to 300 to 900 breaths/min]). Although use of high-frequency oscillatory ventilation has become commonplace, the only definitive study of this method in pediatric ARDS is a crossover trial conducted nearly 20 years ago that showed improved oxygenation and a reduction in the need for supplemental oxygen at 30 days.[9] Despite the lack of robust evidence in support of high-frequency oscillatory ventilation, it has become a

standard technique in the management of ARDS in PICUs worldwide.[86]

Low Tidal Volume Ventilation

A landmark study of adults with ARDS published in the *New England Journal of Medicine* in 2000 showed that low tidal volume mechanical ventilation (6 mL/kg) decreased mortality by 22 percent and increased the number of ventilator-free days in contrast to a more traditional tidal volume (12 mL/kg).[104] The mortality rate was 31.0 percent in the low tidal volume group and 39.8 percent ($p = .007$) in the higher tidal volume group. Additionally, the plateau pressure (Pplat), a major factor in creating barotrauma in the lungs, was significantly decreased in the low tidal volume group (≤26 cm H_2O) in contrast to the control group (37 ± 9 cm H_2O). This study is the only investigation in either adult or pediatric ARDS that has demonstrated a mortality benefit associated with a specific ventilatory approach. Although still unproved, these results are likely applicable to infants and children with ARDS and this low tidal volume approach has become standard practice in the PICU setting.

Further data from other large mechanical ventilation studies in adults demonstrated that outcomes are improved when Pplat is limited to less than 32 to 35 cm H_2O.[6,18,100] No data exist in pediatric patients to guide practitioners in the establishment of peak inspiratory pressure or tidal volume, and ongoing investigation is needed. It is possible that the "critical" limit on plateau pressure for infants and children may be less than 32 to 35 cm H_2O, or there may be variation with patient age, but without further studies, clinicians must rely on available data on adults and individual clinical expertise. Without a similar large-scale, prospective, randomized trial in pediatrics, a ventilatory strategy should be undertaken that limits both tidal volume and inspiratory pressure.

Positive End-Expiratory Pressure

PEEP is the constant pressure applied to the airways and alveoli during exhalation and is an important component of the ventilator strategies employed in the management of ARDS. PEEP helps maintain alveolar patency and restore functional residual capacity. PEEP is typically titrated to a level that allows adequate oxygenation at an acceptable Fio_2 as described later in more detail.

The ARDS Network investigated the optimal PEEP-Fio_2 approach for adult patients with ARDS and showed that both "lower" and "higher" PEEP strategies produced similar survival rates.[20] The results of this study suggest that as long as adequate PEEP is applied, higher levels are not necessary but are acceptable. Thus, the balance between PEEP and Fio_2 (as long as alveolar collapse is minimized) can be left to the discretion of the clinician. PEEP- Fio_2 tables for adult patients are readily available[10]; however, no such generally accepted standards are available for infants and children. In summary, once appropriate PEEP is applied to maintain the lungs at an ideal volume, a further increase in PEEP in an attempt to reduce the Fio_2 does not lead to an improved outcome.[10]

In a follow-up meta-analysis of almost 2300 patients, Briel and colleagues[16] demonstrated that patients with pure ALI (i.e., recruitable lung) benefited from a higher PEEP strategy, whereas those without recruitable lung did not. Based on these data, a strategy that individualizes PEEP based on the specific clinical circumstances seems warranted.

It must be noted that an important consideration as PEEP is titrated is the impact of increasing mean intrathoracic pressure on systemic venous return and cardiac output. Increasing PEEP comes at a cost of increased mean intrathoracic pressure and potentially a decrease in systemic venous return and cardiac output.[67] As a result of these changes, patients with ARDS may need further intravascular volume loading and possibly inotropic support.[82] A comprehensive discussion of the cardiorespiratory effects of increasing PEEP is beyond the scope of this chapter.

Gas Exchange Goals

Every child with ARDS is hypoxemic by definition. Prolonged administration of high concentrations of oxygen can damage lungs, owing to the formation of oxygen free radicals. Human and animal studies suggest that a prolonged Fio_2 greater than 0.60 should be avoided to prevent oxygen-induced pulmonary damage.[52] However, the exact Fio_2 cutoff for oxygen toxicity in the ARDS patient, especially in children, remains unknown and may be less than 0.60. Increases in the set PEEP may allow for a reduction of the delivered Fio_2.

In the setting of ARDS, improved oxygenation has not been associated with improved outcomes. This finding was best demonstrated in the ARDS Network low tidal volume study, in which the control (12 mL/kg) group demonstrated improved oxygenation for the first 72 hours of mechanical ventilation but ultimately had a higher mortality in contrast to the lower tidal volume (6 mL/kg) group.[104] In a pediatric study of 470 children, no correlation was found between oxygenation or ventilation measured during the first 14 days of PICU admission in survivors or nonsurvivors of pediatric ALI.[106] Other studies in pediatric ARDS have demonstrated short-term improvements in oxygenation parameters with no difference in mortality.[30,31] The ideal target for oxygenation in ARDS remains controversial, but it is clinically reasonable to accept Sao_2 under 90 percent as long as tissue oxygen delivery is adequate, as described later in the discussion of permissive hypoxemia.

Permissive Hypercapnia

A consequence of low tidal volume ventilation is hypercapnia. Limiting the peak inspiratory pressure by reducing the tidal volume may decrease minute ventilation and result in hypercapnia. Limited evidence suggests that low-volume, pressure-limited ventilation with permissive hypercapnia may improve outcomes in patients with ARDS.[47,48] In a 10-year study, Milberg and colleagues[66]

reported a positive association between permissive hypercapnia and outcomes. Recent data from a laboratory model of ischemia-reperfusion ALI indicate that hypercapnic acidosis is protective and that buffering of the hypercapnic acidosis actually attenuates these protective effects.[56]

In addition, permissive hypercapnia has been increasingly studied in animal models with regard to its role in modulating sepsis-induced ARDS.[24,51] Permissive hypercapnia can potentially inhibit the immune response.[57] This inhibition of the immune system is of particular concern in patients with sepsis. However, recent animal studies reported a beneficial effect of hypercapnic acidosis in reducing ALI in early and prolonged systemic sepsis.[23] Earlier concerns that hypercapnic acidosis is harmful in patients with a pulmonary source of the sepsis (i.e., by increasing bacterial load and worsening lung injury) have been addressed by further studies that showed that administration of antibiotics prevented these deleterious effects.[22,73,74] These findings, along with data demonstrating worsening mortality with each hour in which antibiotics are delayed in septic patients,[55] reinforce the need for early administration of antibiotics in the management of pediatric sepsis and ARDS.[17] Despite these potential benefits and evidence suggesting that respiratory acidosis is not harmful, the exact degree of hypercapnia that can be safely tolerated remains controversial. Most of the undesirable effects of hypercapnia are reversible and minor when pH is greater than approximately 7.20.[37] We eagerly await future clinical studies looking at the role of permissive hypercapnia in sepsis-related lung injury given the increasing preclinical evidence of its potential therapeutic role.

Permissive Hypoxemia

In the situation of severe ARDS with elevated ventilator support, clinicians must carefully titrate a balance between avoiding toxic ventilator settings (e.g., elevated airway pressures and Fio_2) and accepting lower than normal arterial oxygen saturations.[1,84] This concept of accepting lower arterial oxygen saturations is termed *permissive hypoxemia*.[1,84] PEEP and other ventilatory parameters should be set so as to optimize oxygen delivery, with tolerance of lower oxygen saturations to allow for minimization of VILI. Because the minimal acceptable arterial oxygen saturation remains controversial the optimal oxygen saturation target for an individual patient remains unknown; however, the goal should be to provide adequate tissue/organ oxygenation while minimizing toxicity of the ventilator settings.

ADJUNCTIVE THERAPIES

Corticosteroids

Given that inflammation is the cornerstone of the pathophysiology of ARDS, corticosteroids have therapeutic potential as immunomodulatory or anti-inflammatory agents. There are, unfortunately, no studies of corticosteroids for treatment of ARDS in children. In the adult ARDS population, an inherent difficulty in summarizing the use of corticosteroids is the significant variability in the timing and dose of steroids used in the various studies. The timing of steroids in published clinical studies ranges from 72 hours to 4 weeks of onset of ARDS, and the doses of methylprednisolone (or its equivalent) range from 1 mg/kg/day to 120 mg/kg/day.[78] Meta-analyses of corticosteroid used within the first 2 weeks in adults with established ARDS demonstrated that steroids may reduce mortality and reduce total days of mechanical ventilation.[78,103] However, based on current data, corticosteroids do not appear to have a role in prevention of ARDS.[78] The use of steroids was not associated with an increase in secondary infections.[78,103]

Inhaled Nitric Oxide

Pulmonary vascular dysfunction has been associated with ARDS and is an important component of the pathophysiologic process.[21] This impact of ARDS on pulmonary vascular tone has led to the extensive investigation of inhaled nitric oxide, a potent vasodilator that works exclusively in the pulmonary vasculature, as a potential therapeutic option. Studies of inhaled nitric oxide for pediatric ARDS have demonstrated temporary improvement in oxygenation acutely but not improved survival.[3,30,31] This finding is similar in the adult population. The largest pediatric inhaled nitric oxide study to date involved 108 children.[31] Because of the crossover design, the study was not able to describe mortality results, but it did demonstrate that the groups of children who may acutely benefit most are immunocompromised patients and those with greater severity of hypoxemia.

A recent Cochrane Database Systematic Review looking at 14 randomized controlled trials concluded that inhaled nitric oxide cannot be recommended for either children or adults with ARDS because it resulted in transient improvement in oxygenation without mortality benefit.[5] Furthermore, it appeared that inhaled nitric oxide may increase the risk for renal impairment among adults with ARDS.

Surfactant Replacement

In ARDS, injury occurs to type II pneumocytes, resulting in decreased production of surfactant. This injury has prompted studies examining the possibility of the effectiveness of surfactant replacement in the management of ARDS. A recent study of 153 pediatric patients reported that exogenous surfactant acutely improved oxygenation and significantly decreased mortality in infants and children with ALI.[114] However, this study revealed no significant decrease in duration of mechanical ventilation, length of PICU admission, or length of hospital stay. The results of this study are controversial because a significantly higher number of immunocompromised patients (i.e., with a higher expected mortality) were included in the control group.[26] Follow-up studies have been completed, and data are currently being analyzed.

The evidence for benefit associated with surfactant therapy in adults also is limited. A large, randomized trial of nearly 450 patients did not show a mortality difference

with use of surfactant.[98] Other studies in adults also have not demonstrated a benefit. Unlike in the neonatal populations, current data do not demonstrate wide-scale benefits associated with surfactant use in pediatric or adult ALI, but there may be select circumstances in which this therapy is beneficial.

Prone Positioning

Through various mechanisms such as improvement in ventilation/perfusion matching and chest wall mechanics, prone positioning was first shown in a randomized controlled trial to improve oxygenation in patients with ARDS by Gattinoni and his Supine-Prone Study group.[41] Despite promising results of prone positioning from investigations in adults with ARDS, a study of prone positioning for pediatric ALI was closed for futility.[25] It is likely that not all children with ARDS will benefit from prone positioning. Experience from clinical studies in adults with ARDS showed that there is likely to be a subgroup of patients who benefit most from this maneuver.[101,102] Future pediatric studies should aim to identify this particular subset of patients.

Nonpulmonary Supportive Management

Fluid Balance

Initial aggressive fluid resuscitation is important in maintaining hemodynamics and improving clinical outcomes in patients with shock.[93] However, overhydration, once hemodynamics are restored, may lead to pulmonary edema. Fluids should be carefully titrated and fluid status monitored while normalizing the patient's intravascular volume status and maintaining adequate cardiac output.[8] The ARDS Network conducted a study to investigate conservative and liberal fluid management strategies in adult patients with ARDS.[113] In this study, a difference of 6000 mL was seen in the 7-day cumulative fluid balance between the two groups; with patients in the conservative fluid management group having improved lung function and shorter duration of mechanical ventilation and ICU length of stay. In critically ill children, the role of fluid balance is less conclusive. Although some studies demonstrate that fluid balance has an impact on oxygenation and morbidity,[8,49] other prospective pediatric studies have shown otherwise.[85] The Pediatric Acute Lung Injury and Sepsis Investigators (PALISI) Network investigators showed that cumulative fluid input and output did not affect the speed of weaning from mechanical ventilation or extubation outcomes.[85]

Sedation and Neuromuscular Blockade

Appropriate sedation and analgesia are important in children who are on mechanical ventilation. Prolonged sedation and neuromuscular blockage have been associated with worse clinical outcomes.[10,40] However, a recent study demonstrated that early neuromuscular blockage improved survival in adults with ARDS.[76] In this study of 340 adults with ARDS, the use of cisatracurium for 48 hours (vs. placebo) improved 90-day survival by nearly 1.5 times without an increase in muscle weakness. In contrast, growing evidence in adults indicates substantial benefits associated with sedation weaning and promotion of early mobilization.[42,70] Given the lack of consistent evidence in both adults and children, the use of neuromuscular blockade in children with ARDS remains controversial. Overall, use of these agents should be driven by a balance between maintaining adequate gas exchange and the potential adverse effects of critical illness weakness and myopathy.

Nutrition

As a general approach, adequate nutrition should be provided to optimize caloric intake and avoid a negative nitrogen balance. However, few studies examining the effects of nutrition in children with ARDS have been conducted. Clinical guidelines with regard to nutritional support of critically ill children are, at best, supported by small, randomized trials with reasonable risk for bias in their results.[65] Two studies in adults with a total of 1200 patients showed that a trophic feeding strategy did not increase the duration of mechanical ventilation or reduce mortality in contrast to full enteral feeding. The patients on trophic feeds, however, had fewer gastrointestinal side effects such as vomiting and constipation.[89,92]

Although a growing number of studies are investigating the potential utility of nutritional supplementation in ARDS, the results have not been consistent or encouraging. Using bronchoalveolar and plasma biomarkers as their end points, investigators examining the effect of omega-3 oil on adults with ARDS did not note any difference in IL-8, IL-6, and leukotriene B4 levels between patients with and without supplementation.[99] This was further supported by a recent clinical study that was stopped early for futility.[91] This study of twice-daily supplementation of omega-3 fatty acids within 48 hours of onset of ARDS showed a trend toward longer duration of mechanical ventilation and higher mortality in the treatment group.[91]

Patient Isolation

Even though infectious etiologies are the most common cause of ARDS in children, routine isolation of all ARDS patients is not warranted. In certain infective etiologies such as viruses (e.g., influenza, RSV, adenovirus), resistant bacteria, and mycobacteria (e.g., tuberculosis), appropriate isolation measures should be instituted.[111]

Rescue Therapies

Some patients with severe ARDS become refractory to maximal therapies and are unable to achieve acceptable therapeutic goals while avoiding oxygen toxicity or intolerably high airway pressures. In these circumstances, ECMO is becoming increasingly used as a rescue for these patients.

ECMO involves withdrawing blood from the patient by a mechanical pump and directing it to a membrane oxygenator, where oxygenation and removal of carbon dioxide occur before the blood is returned to the patient.

In refractory ARDS patients, venovenous ECMO is commonly used, which involves blood being withdrawn from the patient via a cannula placed in a central vein and returned to the venous system. Cardiac function must be sufficient to circulate this returned blood systemically to the body. ECMO has been used successfully in a wide range of clinical circumstances and is a viable treatment strategy in adults and children with refractory ARDS.[19,45] Traditionally, ECMO has been implemented in patients with an anticipated mortality that is extremely high, exceeding 80 to 90 percent in many circumstances, but reported survival from the Extracorporeal Life Support Organization (ELSO) registry for adults and children with ARDS is currently 50 and 65 percent, respectively.[19,32] In the past decade, use of ECMO has continued to increase, as evidenced by reports from the 2009 to 2010 H1N1 influenza A pandemic.[27,71,72,108] A more detailed discussion of ECMO support for pediatric and adult ARDS patients is beyond the scope of this chapter.

SUMMARY

ARDS represents a spectrum of clinical disease of varying pulmonary and nonpulmonary etiologies. Although much progress has been made with regard to understanding the pathophysiology of ARDS, the only ventilatory strategy that has been proved to improve clinical outcome is low tidal volume ventilation. It is anticipated that future clinical studies are likely to combine therapeutic strategies instead of simply focusing on a single intervention. Although meticulous balance is important in each organ system, limited data exist regarding the optimal supportive management strategies for children with ARDS. Current approaches largely represent clinical experience and extrapolation of the available data from the adult population. Without doubt, basic science and genomics studies will continue to be translated into clinical application at the bedside to better individualize treatment strategies for pediatric ALI.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdelsalam M, Cheifetz IM. Goal-directed therapy for severely hypoxic patients with acute respiratory distress syndrome: permissive hypoxemia. *Respir Care* 2010;**55**:1483–90.
5. Afshari A, Brok J, Moller AM, et al. Inhaled nitric oxide for acute respiratory distress syndrome (ARDS) and acute lung injury in children and adults. *Cochrane Database Syst Rev* 2010:CD002787.
8. Arikan AA, Zappitelli M, Goldstein SL, et al. Fluid overload is associated with impaired oxygenation and morbidity in critically ill children. *Pediatr Crit Care Med* 2012;**13**:253–8.
12. Azevedo ZM, Moore DB, Lima FC, et al. Tumor necrosis factor (TNF) and lymphotoxin-alpha (LTA) single nucleotide polymorphisms: importance in ARDS in septic pediatric critically ill patients. *Hum Immunol* 2012; **73**:661–7.
13. Bajwa EK, Cremer PC, Gong MN, et al. An NFKB1 promoter insertion/deletion polymorphism influences risk and outcome in acute respiratory distress syndrome among Caucasians. *PLoS One* 2011;**6**:e19469.
16. Briel M, Meade M, Mercat A, et al. Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis. *JAMA* 2010;**303**:865–73.
17. Brierley J, Carcillo JA, Choong K, et al. Clinical practice parameters for hemodynamic support of pediatric and neonatal septic shock: 2007 update from the American College of Critical Care Medicine. *Crit Care Med* 2009;**37**:666–88.
19. Brogan TV, Thiagarajan RR, Rycus PT, et al. Extracorporeal membrane oxygenation in adults with severe respiratory failure: a multi-center database. *Intensive Care Med* 2009;**35**:2105–14.
21. Bull TM, Clark B, McFann K, et al. Pulmonary vascular dysfunction is associated with poor outcomes in patients with acute lung injury. *Am J Respir Crit Care Med* 2010;**182**:1123–8.
22. Chonghaile MN, Higgins BD, Costello J, et al. Hypercapnic acidosis attenuates lung injury induced by established bacterial pneumonia. *Anesthesiology* 2008;**109**:837–48.
23. Costello J, Higgins B, Contreras M, et al. Hypercapnic acidosis attenuates shock and lung injury in early and prolonged systemic sepsis. *Crit Care Med* 2009;**37**:2412–20.
24. Curley G, Hayes M, Laffey JG, et al. Can 'permissive' hypercapnia modulate the severity of sepsis-induced ALI/ARDS? *Crit Care* 2011;**15**:212.
27. Davies A, Jones D, Bailey M, et al. Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) acute respiratory distress syndrome. *JAMA* 2009;**302**:1888–95.
29. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008;**36**:296–327.
32. Domico MB, Ridout DA, Bronicki R, et al. The impact of mechanical ventilation time before initiation of extracorporeal life support on survival in pediatric respiratory failure: a review of the Extracorporeal Life Support Registry. *Pediatr Crit Care Med* 2012;**13**:16–21.
36. Farias JA, Fernández A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. *Intensive Care Med* 2010;**36**:1015–22.
42. Girard TD, Kress JP, Fuchs BD, et al. Efficacy and safety of a paired sedation and ventilator weaning protocol for mechanically ventilated patients in intensive care (Awakening and Breathing Controlled trial): a randomised controlled trial. *Lancet* 2008;**371**: 126–34.
49. Hu X, Qian S, Xu F, et al. Incidence, management and mortality of acute hypoxemic respiratory failure and acute respiratory distress syndrome from a prospective study of Chinese paediatric intensive care network. *Acta Paediatr* 2010;**99**:715–21.
51. Ijland MM, Heunks LM, van der Hoeven JG, et al. Bench-to-bedside review: hypercapnic acidosis in lung injury: from 'permissive' to 'therapeutic'. *Crit Care* 2010;**14**:237.
53. Kallen AJ, Brunkard J, Moore Z, et al. *Staphylococcus aureus* community-acquired pneumonia during the 2006 to 2007 influenza season. *Ann Emerg Med* 2009;**53**:358–65.
63. Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol* 2011;**6**: 147–63.
65. Mehta NM, Compher C. A.S.P.E.N. Clinical Guidelines: nutrition support of the critically ill child. *JPEN J Parenter Enteral Nutr* 2009;**33**:260–76.
69. Napolitano LM, Brunsvold ME, Reddy RC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* pneumonia and ARDS: 1-year follow-up. *Chest* 2009;**136**:1407–12.
70. Needham DM, Korupolu R, Zanni JM, et al. Early physical medicine and rehabilitation for patients with acute respiratory failure: a quality improvement project. *Arch Phys Med Rehabil* 2010;**91**:536–42.
71. Noah MA, Peek GJ, Finney SJ, et al. Referral to an extracorporeal membrane oxygenation center and mortality among patients with severe 2009 influenza A(H1N1). *JAMA* 2011;**306**: 1659–68.
72. Norfolk SG, Hollingsworth CL, Wolfe CR, et al. Rescue therapy in adult and pediatric patients with pH1N1 influenza infection: a tertiary center intensive care unit experience from April to October 2009. *Crit Care Med* 2010;**38**:2103–7.
74. O'Croinin DF, Nichol AD, Hopkins N, et al. Sustained hypercapnic acidosis during pulmonary infection increases bacterial load and worsens lung injury. *Crit Care Med* 2008;**36**:2128–35.
76. Papazian L, Forel JM, Gacouin A, et al. Neuromuscular blockers in early acute respiratory distress syndrome. *N Engl J Med* 2010;**363**:1107–16.
78. Peter JV, John P, Graham PL, et al. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: meta-analysis. *BMJ* 2008;**336**:1006–9.

79. Phua J, Badia JR, Adhikari NK, et al. Has mortality from acute respiratory distress syndrome decreased over time? A systematic review. *Am J Respir Crit Care Med* 2009;**179**:220–7.
81. Piastra M, De Luca D, Marzano L, et al. The number of failing organs predicts non-invasive ventilation failure in children with ALI/ARDS. *Intensive Care Med* 2011;**37**:1510–6.
84. Randolph AG. Management of acute lung injury and acute respiratory distress syndrome in children. *Crit Care Med* 2009;**37**: 2448–54.
87. Randolph AG, Vaughn F, Sullivan R, et al. Critically ill children during the 2009-10 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
89. Rice TW, Mogan S, Hays MA, et al. Randomized trial of initial trophic versus full-energy enteral nutrition in mechanically ventilated patients with acute respiratory failure. *Crit Care Med* 2011;**39**:967–74.
90. Rice TW, Rubinson L, Uyeki TM, et al. Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. *Crit Care Med* 2012;**40**:1487–98.
91. Rice TW, Wheeler AP, Thompson BT, et al. Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA* 2011;**306**:1574–81.
92. Rice TW, Wheeler AP, Thompson BT, et al. Initial trophic vs full enteral feeding in patients with acute lung injury: the EDEN randomized trial. *JAMA* 2012;**307**:795–803.
96. Sevransky JE, Martin GS, Mendez-Tellez P, et al. Pulmonary vs nonpulmonary sepsis and mortality in acute lung injury. *Chest* 2008;**134**:534–8.
97. Sheu CC, Gong MN, Zhai R, et al. Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS. *Chest* 2010;**138**:559–67.
99. Stapleton RD, Martin TR, Weiss NS, et al. A phase II randomized placebo-controlled trial of omega-3 fatty acids for the treatment of acute lung injury. *Crit Care Med* 2011;**39**:1655–62.
101. Sud S, Friedrich JO, Taccone P, et al. Prone ventilation reduces mortality in patients with acute respiratory failure and severe hypoxemia: systematic review and meta-analysis. *Intensive Care Med* 2010;**36**:585–99.
102. Taccone P, Pesenti A, Latini R, et al. Prone positioning in patients with moderate and severe acute respiratory distress syndrome: a randomized controlled trial. *JAMA* 2009;**302**:1977–84.
103. Tang BM, Craig JC, Eslick G, et al. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. *Crit Care Med* 2009;**37**:1594–603.
105. The ARDS Task Force. Acute respiratory distress syndrome: The Berlin Definition. *JAMA* 2012;**307**:E1–E8.
108. Turner DA, Rehder KJ, Peterson-Carmichael SL, et al. Extracorporeal membrane oxygenation for severe refractory respiratory failure secondary to 2009 H1N1 influenza A. *Respir Care* 2011;**56**: 941–6.
110. Villar J, Blanco J, Añán JM, et al. The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. *Intensive Care Med* 2011;**37**: 1932–41.
111. Villar J, Blanco J, Kacmarek RM, et al. Acute respiratory distress syndrome definition: do we need a change? *Curr Opin Crit Care* 2011;**17**:13–17.
115. Wong HR. Genetics and genomics in pediatric septic shock. *Crit Care Med* 2012;**40**:1618–26.
116. Yu WL, Lu ZJ, Wang Y, et al. The epidemiology of acute respiratory distress syndrome in pediatric intensive care units in China. *Intensive Care Med* 2009;**35**:136–43.
117. Zimmerman JJ, Akhtar SR, Caldwell E, et al. Incidence and outcomes of pediatric acute lung injury. *Pediatrics* 2009;**124**:87–95.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdelsalam M, Cheifetz IM. Goal-directed therapy for severely hypoxic patients with acute respiratory distress syndrome: permissive hypoxemia. *Respir Care* 2010;**55**:1483–90.
2. Abel SJ, Finney SJ, Brett SJ, et al. Reduced mortality in association with the acute respiratory distress syndrome (ARDS). *Thorax* 1998;**53**:292–4.
3. Abman SH, Griebel JL, Parker DK, et al. Acute effects of inhaled nitric oxide in children with severe hypoxemic respiratory failure. *J Pediatr* 1994;**124**:881–8.
4. Adamzik M, Frey UH, Rieman K, et al. Insertion/deletion polymorphism in the promoter of NFKB1 influences severity but not mortality of acute respiratory distress syndrome. *Intensive Care Med* 2007;**33**:1199–203.
5. Afshari A, Brok J, Møller AM, et al. Inhaled nitric oxide for acute respiratory distress syndrome (ARDS) and acute lung injury in children and adults. *Cochrane Database Syst Rev* 2010;(7)CD002787.
6. Amato MB, Barbas CS, Medeiros DM, et al. Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med* 1998;**338**:347–54.
7. American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. *Pediatrics* 2006;**118**:1774–93.
8. Arikan AA, Zappitelli M, Goldstein SL, et al. Fluid overload is associated with impaired oxygenation and morbidity in critically ill children. *Pediatr Crit Care Med* 2012;**13**:253–8.
9. Arnold JH, Hanson JH, Toro-Figuero LO, et al. Prospective, randomized comparison of high-frequency oscillatory ventilation and conventional mechanical ventilation in pediatric respiratory failure. *Crit Care Med* 1994;**22**:1530–9.
10. Arroliga A, Frutos-Vivar F, Hall J, et al. Use of sedatives and neuromuscular blockers in a cohort of patients receiving mechanical ventilation. *Chest* 2005;**128**:496–506.
11. Ashbaugh DG, Bigelow DB, Petty TL, et al. Acute respiratory distress in adults. *Lancet* 1967;**2**:319–23.
12. Azevedo ZM, Moore DB, Lima FC, et al. Tumor necrosis factor (TNF) and lymphotoxin-alpha (LTA) single nucleotide polymorphisms: importance in ARDS in septic pediatric critically ill patients. *Hum Immunol* 2012;**73**:661–7.
13. Bajwa EK, Cremer PC, Gong MN, et al. An NFKB1 promoter insertion/deletion polymorphism influences risk and outcome in acute respiratory distress syndrome among Caucasians. *PLoS One* 2011;**6**:e19469.
14. Bauer TT, Ewig S, Rodloff AC, et al. Acute respiratory distress syndrome and pneumonia: a comprehensive review of clinical data. *Clin Infect Dis* 2006;**43**:748–56.
15. Bernard GR, Artigas A, Brigham KL, et al; The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. *Am J Respir Crit Care Med* 1994;**149**:818–24.
16. Briel M, Meade M, Mercat A, et al. Higher vs lower positive end-expiratory pressure in patients with acute lung injury and acute respiratory distress syndrome: systematic review and meta-analysis. *JAMA* 2010;**303**:865–73.
17. Brierley J, Carcillo JA, Choong K, et al. Clinical practice parameters for hemodynamic support of pediatric and neonatal septic shock: 2007 update from the American College of Critical Care Medicine. *Crit Care Med* 2009;**37**:666–88.
18. Brochard L, Roudot-Thoraval F, Roupie E, et al. Tidal volume reduction for prevention of ventilator-induced lung injury in acute respiratory distress syndrome. The Multicenter Trail Group on Tidal Volume reduction in ARDS. *Am J Respir Crit Care Med* 1998;**158**:1831–8.
19. Brogan TV, Thiagarajan RR, Rycus PT, et al. Extracorporeal membrane oxygenation in adults with severe respiratory failure: a multi-center database. *Intensive Care Med* 2009;**35**:2105–14.
20. Brower RG, Lanken PN, Mcintyre N, et al. Higher versus lower positive end-expiratory pressures in patients with the acute respiratory distress syndrome. *N Engl J Med* 2004;**351**:327–36.
21. Bull TM, Clark B, McFann K, et al. Pulmonary vascular dysfunction is associated with poor outcomes in patients with acute lung injury. *Am J Respir Crit Care Med* 2010;**182**:1123–8.
22. Chonghaile MN, Higgins BD, Costello J, et al. Hypercapnic acidosis attenuates lung injury induced by established bacterial pneumonia. *Anesthesiology* 2008;**109**:837–48.
23. Costello J, Higgins B, Contreras M, et al. Hypercapnic acidosis attenuates shock and lung injury in early and prolonged systemic sepsis. *Crit Care Med* 2009;**37**:2412–20.
24. Curley G, Hayes M, Laffey JG, et al. Can 'permissive' hypercapnia modulate the severity of sepsis-induced ALI/ARDS? *Crit Care* 2011;**15**:212.
25. Curley MA, Hibberd PL, Fineman LD, et al. Effect of prone positioning on clinical outcomes in children with acute lung injury: a randomized controlled trial. *JAMA* 2005;**294**:229–37.
26. Czaja AS. A critical appraisal of a randomized controlled trial: Willson et al: Effect of exogenous surfactant (calfactant) in pediatric acute lung injury (JAMA 2005, 293:470-6). *Pediatr Crit Care Med* 2007;**8**:50–3.
27. Davies A, Jones D, Bailey M, et al. Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) acute respiratory distress syndrome. *JAMA* 2009;**302**:1888–95.
28. Davis SL, Furman DP, Castarino AT Jr. Adult respiratory distress syndrome in children: associated disease, clinical course, and predictors of death. *J Pediatr* 1993;**123**:35–45.
29. Dellinger RP, Levy MM, Carlet JM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock: 2008. *Crit Care Med* 2008;**36**:296–327.
30. Dobyns EL, Anas NG, Fortinberry JD, et al. Interactive effects of high-frequency oscillatory ventilation and inhaled nitric oxide in acute hypoxemic respiratory failure in pediatrics. *Crit Care Med* 2002;**30**:2425–9.
31. Dobyns EL, Cornfield DN, Anas NG, et al. Multicenter randomized controlled trial of the effects of inhaled nitric oxide therapy on gas exchange in children with acute hypoxemic respiratory failure. *J Pediatr* 1999;**134**:406–12.
32. Domico MB, Ridout DA, Bronicki N, et al. The impact of mechanical ventilation time before initiation of extracorporeal life support on survival in pediatric respiratory failure: a review of the Extracorporeal Life Support Registry. *Pediatr Crit Care Med* 2012;**13**:16–21.
33. Effmann EL, Merten DF, Kirks DR, et al. Adult respiratory distress syndrome in children. *Radiology* 1985;**157**:69–74.
34. Esteban A, Alia I, Gordo F, et al. Prospective randomized trial comparing pressure-controlled ventilation and volume-controlled ventilation in ARDS. For the Spanish Lung Failure Collaborative Group. *Chest* 2000;**117**:1690–6.
35. European Society of Intensive Care Medicine. 2011 Available from: http://esicm.cyim.com/07-congresses/0A-annual-congress/webTv.asp.
36. Farias JA, Fernandez A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. *Intensive Care Med* 2010;**36**:1015–22.
37. Feihl F, Perret C. Permissive hypercapnia: how permissive should we be? *Am J Respir Crit Care Med* 1994;**150**:1722–37.
38. Ferring M, Vincent JL. Is outcome from ARDS related to the severity of respiratory failure? *Eur Respir J* 1997;**10**:1297–300.
39. Flori HR, Glidden DV, Rutherford GW, et al. Pediatric acute lung injury: prospective evaluation of risk factors associated with mortality. *Am J Respir Crit Care Med* 2005;**171**:995–1001.
40. Freire AX, Afessa B, Cawley P, et al. Characteristics associated with analgesia ordering in the intensive care unit and relationships with outcome. *Crit Care Med* 2002;**30**:2468–72.
41. Gattinoni L, Tognoni G, Pesenti A, et al. Effect of prone positioning on the survival of patients with acute respiratory failure. *N Engl J Med* 2001;**345**:568–73.
42. Girard TD, Kress JP, Fuchs BD, et al. Efficacy and safety of a paired sedation and ventilator weaning protocol for mechanically ventilated patients in intensive care (Awakening and Breathing Controlled trial): a randomised controlled trial. *Lancet* 2008;**371**:126–34.
43. Goh AY, Chan PW, Lum LC, et al. Incidence of acute respiratory distress syndrome: a comparison of two definitions. *Arch Dis Child* 1998;**79**:256–9.
44. Gong MN, Zhou W, Williams PL, et al. -308GA and TNFB polymorphisms in acute respiratory distress syndrome. *Eur Respir J* 2005;**26**:382–9.
45. Green TP, Timmons OD, Fackler JC, et al. The impact of extracorporeal membrane oxygenation on survival in pediatric patients with acute respiratory failure. Pediatric Critical Care Study Group. *Crit Care Med* 1996;**24**:323–9.

46. Hammer J, Numa A, Newth CJ. Acute respiratory distress syndrome caused by respiratory syncytial virus. *Pediatr Pulmonol* 1997;**23**:176–83.
47. Hickling KG, Henderson SJ, Jackson R. Low mortality associated with low volume pressure limited ventilation with permissive hypercapnia in severe adult respiratory distress syndrome. *Intensive Care Med* 1990;**16**:372–7.
48. Hickling KG, Walsh J, Henderson S, et al. Low mortality rate in adult respiratory distress syndrome using low-volume, pressure-limited ventilation with permissive hypercapnia: a prospective study. *Crit Care Med* 1994;**22**:1568–78.
49. Hu X, Qian S, Xu F, et al. Incidence, management and mortality of acute hypoxemic respiratory failure and acute respiratory distress syndrome from a prospective study of Chinese paediatric intensive care network. *Acta Paediatr* 2010;**99**:715–21.
50. Hudson LD, Milberg JA, Arnardi D, et al. Clinical risks for development of the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1995;**151**:293–301.
51. Ijland MM, Heunks LM, van der Hoeven JG, et al. Bench-to-bedside review: hypercapnic acidosis in lung injury: from 'permissive' to 'therapeutic'. *Crit Care* 2010;**14**:237.
52. Jenkinson SG. Oxygen toxicity. *New Horiz* 1993;**1**:504–11.
53. Kallen AJ, Brunkard J, Moore Z, et al. *Staphylococcus aureus* community-acquired pneumonia during the 2006 to 2007 influenza season. *Ann Emerg Med* 2009;**53**:358–65.
54. Kuhn C, 3rd, Boldt J, King TE Jr, et al. An immunohistochemical study of architectural remodeling and connective tissue synthesis in pulmonary fibrosis. *Am Rev Respir Dis* 1989;**140**:1693–703.
55. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006;**34**:1589–96.
56. Laffey JG, Kavanagh BP. Carbon dioxide and the critically ill: too little of a good thing? *Lancet* 1999;**354**:1283–6.
57. Laffey JG, Engelberts D, Kavanagh BP, et al. Buffering hypercapnic acidosis worsens acute lung injury. *Am J Respir Crit Care Med* 2000;**161**:141–6.
58. Leibovici L, Shraga I, Drucker M, et al. The benefit of appropriate empirical antibiotic treatment in patients with bloodstream infection. *J Intern Med* 1998;**244**:379–86.
59. Leung CW, Kwan YW, Ko PW, et al. Severe acute respiratory syndrome among children. *Pediatrics* 2004;**113**:e535–43.
60. Lew TW, Kwek TK, Tai D, et al. Acute respiratory distress syndrome in critically ill patients with severe acute respiratory syndrome. *JAMA* 2003;**290**:374–80.
61. Lewis JF, Veldhuizen R, Possmayer F, et al. Altered alveolar surfactant is an early marker of acute lung injury in septic adult sheep. *Am J Respir Crit Care Med* 1994;**150**:123–30.
62. Matthay MA, Wiener-Kronish JP. Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Dis* 1990;**142**:1250–7.
63. Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol* 2011;**6**:147–63.
64. Matthay MA, Zimmerman GA. Acute lung injury and the acute respiratory distress syndrome: four decades of inquiry into pathogenesis and rational management. *Am J Respir Cell Mol Biol* 2005;**33**:319–27.
65. Mehta NM, Compher C. A.S.P.E.N. Clinical guidelines: nutrition support of the critically ill child. *JPEN J Parenter Enteral Nutr* 2009;**33**:260–76.
66. Milberg JA, Davis DR, Steinberg KP, et al. Improved survival of patients with acute respiratory distress syndrome (ARDS): 1983-1993. *JAMA* 1995;**273**:306–9.
67. Mitaka C, Nagura T, Sakanishi N, et al. Two-dimensional echocardiographic evaluation of inferior vena cava, right ventricle, and left ventricle during positive-pressure ventilation with varying levels of positive end-expiratory pressure. *Crit Care Med* 1989;**17**:205–10.
68. Montgomery AB, Stager MA, Carrico CJ, et al. Causes of mortality in patients with the adult respiratory distress syndrome. *Am Rev Respir Dis* 1985;**132**:485–9.
69. Napolitano LM, Brunsvold ME, Reddy RC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* pneumonia and ARDS: 1-year follow-up. *Chest* 2009;**136**:1407–12.
70. Needham DM, Korupolu R, Zanni JM, et al. Early physical medicine and rehabilitation for patients with acute respiratory failure: a quality improvement project. *Arch Phys Med Rehabil* 2010;**91**:536–42.
71. Noah MA, Peek GJ, Finney SJ, et al. Referral to an extracorporeal membrane oxygenation center and mortality among patients with severe 2009 influenza A(H1N1). *JAMA* 2011;**306**:1659–68.
72. Wolfe CR, Norfolk SG, Hollingsworth CL, et al. Rescue therapy in adult and pediatric patients with pH1N1 influenza infection: a tertiary center intensive care unit experience from April to October 2009. *Crit Care Med* 2010;**38**:2103–7.
73. O'Croinin DF, Hopkins NO, Moore MM, et al. Hypercapnic acidosis does not modulate the severity of bacterial pneumonia-induced lung injury. *Crit Care Med* 2005;**33**:2606–12.
74. O'Croinin DF, Nichol AD, Hopkins N, et al. Sustained hypercapnic acidosis during pulmonary infection increases bacterial load and worsens lung injury. *Crit Care Med* 2008;**36**:2128–35.
75. Pankhaniya RR, Tamura M, Allmond LR, et al. Pseudomonas aeruginosa causes acute lung injury via the catalytic activity of the patatin-like phospholipase domain of ExoU. *Crit Care Med* 2004;**32**:2293–9.
76. Papazian L, Forel JM, Gacouin A, et al. Neuromuscular blockers in early acute respiratory distress syndrome. *N Engl J Med* 2010;**363**:1107–16.
77. Pepe PE, Potkin RT, Reus DH, et al. Clinical predictors of the adult respiratory distress syndrome. *Am J Surg* 1982;**144**:124–30.
78. Peter JV, John P, Graham PL, et al. Corticosteroids in the prevention and treatment of acute respiratory distress syndrome (ARDS) in adults: meta-analysis. *BMJ* 2008;**336**:1006–9.
79. Phua J, Badia JR, Adhikari NK, et al. Has mortality from acute respiratory distress syndrome decreased over time? A systematic review. *Am J Respir Crit Care Med* 2009;**179**:220–7.
80. Piantadosi CA, Schwartz DA. The acute respiratory distress syndrome. *Ann Intern Med* 2004;**141**:460–70.
81. Piastra M, De Luca D, Marzano L, et al. The number of failing organs predicts non-invasive ventilation failure in children with ALI/ARDS. *Intensive Care Med* 2011;**37**:1510–6.
82. Pollack MM, Fields AI, Holbrook RP. Cardiopulmonary parameters during high PEEP in children. *Crit Care Med* 1980;**8**:372–6.
83. Prella M, Feihl F, Domenighetti G, et al. Effects of short-term pressure-controlled ventilation on gas exchange, airway pressures, and gas distribution in patients with acute lung injury/ARDS: comparison with volume-controlled ventilation. *Chest* 2002;**122**:1382–8.
84. Randolph AG. Management of acute lung injury and acute respiratory distress syndrome in children. *Crit Care Med* 2009;**37**:2448–54.
85. Randolph AG, Forbes PW, Gedeit RG, et al. Cumulative fluid intake minus output is not associated with ventilator weaning duration or extubation outcomes in children. *Pediatr Crit Care Med* 2005;**6**:642–7.
86. Randolph AG, Meert KL, O'Neil ME, et al. The feasibility of conducting clinical trials in infants and children with acute respiratory failure. *Am J Respir Crit Care Med* 2003;**167**:1334–40.
87. Randolph AG, Vaughn F, Sullivan R, et al. Critically ill children during the 2009-2010 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
88. Ranieri VM, Suter PM, Tortorella C, et al. Effect of mechanical ventilation on inflammatory mediators in patients with acute respiratory distress syndrome: a randomized controlled trial. *JAMA* 1999;**282**:54–61.
89. Rice TW, Mogan S, Hays MA, et al. Randomized trial of initial trophic versus full-energy enteral nutrition in mechanically ventilated patients with acute respiratory failure. *Crit Care Med* 2011;**39**:967–74.
90. Rice TW, Rubinson L, Uyeki TM. et al. Critical illness from 2009 pandemic influenza A virus and bacterial coinfection in the United States. *Crit Care Med* 2012;**40**:1487–98.
91. Rice TW, Wheeler AP, Thompson BT, et al. Enteral omega-3 fatty acid, gamma-linolenic acid, and antioxidant supplementation in acute lung injury. *JAMA* 2011;**306**:1574–81.
92. Rice TW, Wheeler AP, Thompson BT, et al. Initial trophic vs full enteral feeding in patients with acute lung injury: the EDEN randomized trial. *JAMA* 2012;**307**:795–803.

93. Rivers E, Nguyen B, Havstad S. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *N Engl J Med* 2001;**345**:1368–77.
94. Rubenfeld GD, Caldwell E, Peabody E, et al. Incidence and outcomes of acute lung injury. *N Engl J Med* 2005;**353**:1685–93.
95. Seeger W, Gunther A, Walmrath HD, et al. Alveolar surfactant and adult respiratory distress syndrome: pathogenetic role and therapeutic prospects. *Clin Investig* 1993;**71**:177–90.
96. Sevransky JE, Martin GS, Mendez-Tellez P, et al. Pulmonary vs nonpulmonary sepsis and mortality in acute lung injury. *Chest* 2008;**134**:534–8.
97. Sheu CC, Gong MN, Zhai R, et al. Clinical characteristics and outcomes of sepsis-related vs non-sepsis-related ARDS. *Chest* 2010;**138**:559–67.
98. Spragg RG, Lewis JF, Walmrath HD, et al. Effect of recombinant surfactant protein C-based surfactant on the acute respiratory distress syndrome. *N Engl J Med* 2004;**351**:884–92.
99. Stapleton RD, Martin TR, Weiss NS, et al. A phase II randomized placebo-controlled trial of omega-3 fatty acids for the treatment of acute lung injury. *Crit Care Med* 2011;**39**:1655–62.
100. Stewart TE, Meade MO, Cook, DJ, et al. Evaluation of a ventilation strategy to prevent barotrauma in patients at high risk for acute respiratory distress syndrome. Pressure- and Volume-Limited Ventilation Strategy Group. *N Engl J Med* 1998;**338**: 355–61.
101. Sud S, Friedrich JO, Taccone P, et al. Prone ventilation reduces mortality in patients with acute respiratory failure and severe hypoxemia: systematic review and meta-analysis. *Intensive Care Med* 2010;**36**:585–99.
102. Taccone P, Pesenti A, Lattini R, et al. Prone positioning in patients with moderate and severe acute respiratory distress syndrome: a randomized controlled trial. *JAMA* 2009;**302**:1977–84.
103. Tang BM, Craig JC, Eslick GD, et al. Use of corticosteroids in acute lung injury and acute respiratory distress syndrome: a systematic review and meta-analysis. *Crit Care Med* 2009;**37**: 1594–603.
104. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. *N Engl J Med* 2000;**342**:1301–8.
105. The ARDS Task Force. Acute respiratory distress syndrome: the Berlin Definition. *JAMA* 2012;**307**:E1–E8.
106. Timmons OD, Havens PL, Fackler JC. Predicting death in pediatric patients with acute respiratory failure. Pediatric Critical Care Study Group. Extracorporeal Life Support Organization. *Chest* 1995;**108**:789–97.
107. Tseng MH, Wei BH, Lin WJ, et al. Fatal sepsis and necrotizing pneumonia in a child due to community-acquired methicillin-resistant *Staphylococcus aureus:* case report and literature review. *Scand J Infect Dis* 2005;**37**:504–7.
108. Turner DA, Rehder KJ, Peterson-Carmichael SL, et al. Extracorporeal membrane oxygenation for severe refractory respiratory failure secondary to 2009 H1N1 influenza A. *Respir Care* 2011;**56**: 941–6.
109. Villar J. Genetics and the pathogenesis of adult respiratory distress syndrome. *Curr Opin Crit Care* 2002;**8**:1–5.
110. Villar J, Blanco J, Añón JM, et al. The ALIEN study: incidence and outcome of acute respiratory distress syndrome in the era of lung protective ventilation. *Intensive Care Med* 2011;**37**: 1932–41.
111. Villar J, Blanco J, Kacmarek RM. Acute respiratory distress syndrome definition: do we need a change? *Curr Opin Crit Care* 2011;**17**:13–17.
112. Ware LB, Matthay MA. Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2001; **163**:1376–83.
113. Wiedemann HP, Wheeler AP, Bernard GR, et al. Comparison of two fluid-management strategies in acute lung injury. *N Engl J Med* 2006;**354**:2564–75.
114. Willson DF, Thomas NJ, Markovitz BP, et al. Effect of exogenous surfactant (calfactant) in pediatric acute lung injury: a randomized controlled trial. *JAMA* 2005;**293**:470–6.
115. Wong HR. Genetics and genomics in pediatric septic shock. *Crit Care Med* 2012;**40**:1618–26.
116. Yu WL, Lu ZJ, Wang Y, et al. The epidemiology of acute respiratory distress syndrome in pediatric intensive care units in China. *Intensive Care Med* 2009;**35**:136–43.
117. Zimmerman JJ, Akhtar SR, Caldwell E, et al. Incidence and outcomes of pediatric acute lung injury. *Pediatrics* 2009;**124**:87–95.

SECTION **XIII**

INFECTIONS OF THE FETUS AND NEWBORN

CHAPTER 66

Approach to Infections in the Fetus and Newborn

Gail J. Harrison

This chapter provides an overview and approach to the diagnosis and management of the more common infections that occur in the fetus and newborn infant. Details about how each organism affects the fetus and newborn, as well as information on diagnosis and management, are covered in the pathogen-specific and disease-specific chapters of this edition.

VIRAL INFECTIONS OF THE FETUS AND NEONATE

Viral infections are an important cause of fetal and neonatal morbidity and mortality. The cumulative frequency of viral infections in the fetus or newborn infant may be as high as 6 to 8 percent of all live births, whereas systemic bacterial disease occurs in only 1 to 2 percent of neonates. Viral infections of the fetus and newborn may be divided into congenital, perinatal or natal, and postnatal time periods of acquisition.

Pathogenesis

Congenital Viral Infections

Congenital viral infections are transmitted to the developing fetus during a maternal infection. Most congenital viral infections are from maternal primary infections, but some viruses (cytomegalovirus) may be transmitted to the fetus during a recurrent (reactivation or reinfection) maternal infection.[7] Congenital viral infections can result in resorption of the embryo; spontaneous abortion; stillbirth; congenital malformation; prematurity; intrauterine growth restriction (IUGR); or acute disease apparent in utero, at birth, or shortly thereafter. Some congenital infections, like most congenital cytomegalovirus (CMV) infections, are seemingly asymptomatic in the fetal or neonatal period, yet may be associated with persistent postnatal infection, sequelae later in life, or a normal infant with no apparent sequelae.

Most fetal infections are preceded by a systemic viral infection in the mother, with hematogenous spread of the virus to the placenta and subsequently to the fetus (Fig. 66-1). Ascending infection through amniotic membranes also may occur while the fetus is still in utero. With most viruses known to cause fetal infection—CMV, rubella virus, herpes simplex virus (HSV), varicella-zoster virus (VZV), and vaccinia virus—placental involvement by the virus also has been documented (Table 66-1). Viruses may reach the fetal circulation by (1) replication through the layers of the placenta; (2) production of virus-induced vascular lesions in the placenta, resulting in abnormal communications between the maternal and fetal circulation; or (3) diapedesis of virus-infected maternal leukocytes through the layers of the placenta to the fetal circulation.

Damage to the fetus also may occur in the absence of actual fetal viral infection as a result of severe systemic illness in the mother, such as measles or influenza, or alteration of placental function. Premature birth occurs in congenital viral infection, as well as small for gestational age as a result of IUGR.

Viruses and Congenital Malformation

Developmental malformations may result from infection of the fetus with certain viruses. Rubella virus is the classic known teratogen, causing disturbances in organogenesis, cardiac disease, and cataracts.[8] VZV has been shown to cause limb hypoplasia and developmental malformations of the eye. Other viruses that result in congenital abnormalities, such as CMV and HSV, usually cause inflammatory, destructive lesions of already developed organs. Type B coxsackieviruses have been associated with a variety of congenital malformations of the heart, and mumps has been linked to endocardial fibroelastosis.

Natal or Perinatal Viral Infections

Natal or perinatal infections are the result of exposure of the newborn to virus replicating in the maternal cervicovaginal tract (i.e., CMV, HSV, or hepatitis B virus) or perirectal area (e.g., enteroviruses). Natal or perinatal infections with some viruses can cause acute systemic illness leading to death, persistent infection with late sequelae, self-limited disease with no discernible damage, or asymptomatic infection (see Fig. 66-1 and Table 66-1). Because the incubation periods of HSV and enteroviruses are short, acute postnatal disease may appear in the neonate within 5 to 7 days of age. In contrast, the incubation periods of CMV and hepatitis B virus (HBV) are

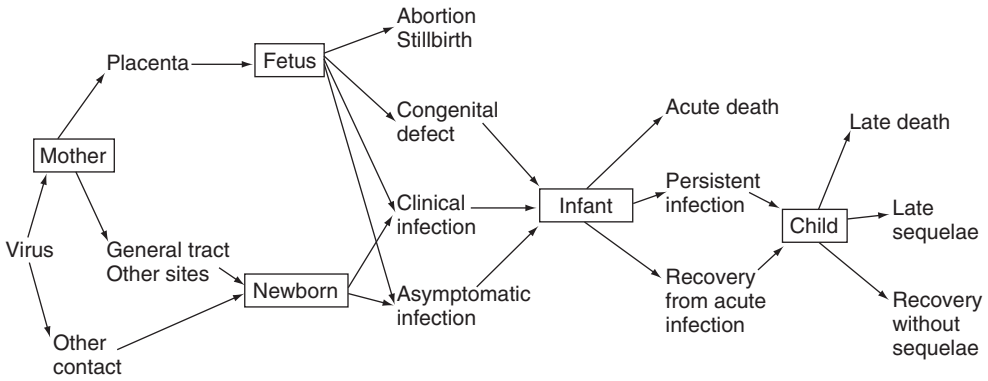

FIGURE 66-1 ■ Pathogenesis of viral infections in the fetus and newborn.

TABLE 66-1 **Period of Transmission of Selected Viruses to the Fetus or Newborn Infant**

Viruses	Congenital	Natal	Postnatal
Adenovirus	+	+	+
Cytomegalovirus	++	++	++
Echoviruses	+	+	+
Epstein-Barr	+	–	+
Hepatitis A	–	++	+
Hepatitis B	+	++	+
Hepatitis C	+	++	–
Herpes simplex	+	++	+
Herpesvirus-6	+	–	+
Human immunodeficiency virus	+	++	+
Human parvovirus B19	+	–	–
Influenza	(+)	–	+
Lymphocytic choriomeningitis virus	++	–	–
Measles	+	–	+
Mumps	+	–	–
Parechovirus	–	+	+
Polioviruses	+	+	+
Rubella	++	–	–
Smallpox	+	+	+
St. Louis encephalitis virus	(+)	–	(+)
Type B coxsackieviruses	+	+	+
Vaccinia	+	+	+
Varicella-zoster	++	+	+
West Nile virus	+	–	+
Western equine encephalitis	+	–	+

++, Major demonstrated route; +, minor demonstrated route; (+), suggested route, few supporting data; –, route not demonstrated.

longer, and clinically apparent disease from perinatal exposure, if it occurs, may not be observed for several weeks or even months after birth. Persistent postnatal infection can occur after perinatal infection with CMV, HSV, and HBV.

Postnatal Viral Infections

Postnatal infections in the neonate may be transmitted to the neonate from the mother, family members, caretakers in extended family, health care workers, outbreaks in neonatal care units, or community exposures (see Fig. 66-1). Neonatal infections and hospital-associated outbreaks of enterovirus and parechovirus, respiratory syncytial virus (RSV), rotavirus, adenovirus, rhinovirus, parainfluenza virus, human metapneumovirus, and influenza virus infection have occurred. In addition, health care–associated HSV infections have occurred in neonates (see Table 66-1). Although the maternal respiratory and gastrointestinal tracts are the most common sites from which virus can be transmitted to the neonate postnatally, other viruses, including human immunodeficiency virus (HIV), human T-cell leukemia virus types 1 and 2 (HTLV I and II), CMV, West Nile virus, rubella virus, hepatitis C virus (HCV), and HBV, have been recovered from breast milk. HSV and VZV lesions on the breast of a mother also may be a source of transmission of virus for the breast-feeding neonate, even though these viruses probably are not present in the breast milk. Finally, HIV, CMV, and HBV have been transmitted to newborn infants by blood transfusion. Although most postnatal infections are acute, self-limited processes, severe disease and fatalities have been reported, especially in preterm infants infected with CMV, enteroviruses, HSV, adenoviruses, or RSV, and persistent postnatal infection may occur with CMV and HBV.[4,5]

Approach to Diagnosis

Clinical Features in Fetus and Newborn

Fetal viral infection may be suspected if the mother is exposed to or experiences an infection with a virus known to transmit to the fetus. Abnormalities detected on routine fetal ultrasound also may suggest fetal infection. Fetal abnormalities detected during prenatal evaluation that may be caused by in utero virus infection include IUGR, microcephaly, cerebral ventriculomegaly or hydrocephalus, cataracts, hepatosplenomegaly, hepatic or intracranial calcifications, polymicrogyria, hydrocephalus, microcephaly, in utero central nervous system (CNS) vasculitis, echogenic bowel, fetal ascites, cardiomegaly,

congestive heart failure, fetal hydrops, enlarged or swollen placenta, or polyhydramnios or oligohydramnios. Intrauterine cardiac abnormalities, myocarditis, and heart failure may be associated with HIV, parvovirus B19, mumps virus, or adenovirus, and cardiac structural defects may be associated with rubella virus. Limb deformities or dysplasias, especially if they are associated with eye or CNS abnormalities, may be caused by intrauterine VZV or HSV infection. Consultation with a maternal-fetal medicine specialist is recommended if fetal viral infection is suspected because prenatal intervention strategies are now available for many of these infections (e.g., CMV and parvovirus B19), which may lessen the morbidity and mortality rates associated with viral infections in the fetus.

Icterus, petechiae, microcephaly, or hepatosplenomegaly at the time of birth or shortly thereafter in a small-for-gestational-age infant suggests a chronic in utero viral infection. On the other hand, acute viral infection in a neonate with suspected sepsis may be present when cultures of blood, spinal fluid, and urine fail to yield a bacterial or fungal agent. Postnatal infections with primarily respiratory virus pathogens may present with apnea, poor feeding, cough, and congestion. In some newborns, involvement of the CNS is the prominent presentation.

Evaluation of the Mother

A history of recent illness or exposures to the mother should be obtained to uncover circumstantial evidence for a viral infection. For example, the occurrence of maternal viral illness with an associated maculopapular rash suggests rubella or enterovirus infection in the neonate, exposure to mosquitoes suggests St. Louis encephalitis virus or West Nile virus, ulcerative genital lesions suggest HSV, and heterophile-negative infectious mononucleosis is suggestive of CMV. Maternal rash with arthralgias may be caused by parvovirus B19, especially if there is recent exposure to fifth disease in the community. One should note, however, that most maternal viral infections that lead to fetal or neonatal infection (almost all cases of CMV and HBV infection and half to two thirds of rubella virus and HSV infections) are asymptomatic in the mother. Therefore, the absence of a history of viral infection in the mother certainly does not rule out the possibility of a viral infection in the neonate.

Clinical Features in the Neonate

Certain clinical manifestations of viral disease in the neonate may provide helpful clues to the specific etiologic agent (Table 66-2). However, most viral infections are asymptomatic in neonates; more than 80 percent of CMV, 60 percent of rubella virus, and most HBV infections. In contrast, fewer than 1 percent of HSV infections in the neonate are subclinical. The clinical and laboratory manifestations of symptomatic disease in the neonate caused by numerous agents often have similar patterns.

Certain specific findings may be helpful in the differential diagnosis. The presence of cataracts, congenital heart disease, bone lesions, or microphthalmos is highly suggestive of rubella, whereas chorioretinitis, cerebral calcifications, and hydrocephaly are more common with congenital infection with CMV, lymphocytic choriomeningitis virus (LCMV), or HSV. The neonate may also experience acute systemic viral infections acquired perinatally or postnatally, most often caused by enteroviruses, HSV, or adenoviruses.[4,5] The features in neonates with these kinds of viral infection resemble those associated

TABLE 66-2 **Common Manifestations of Viral Infections in the Newborn Infant**

Asymptomatic Infection	
Chronic Infection (Early Gestation to Midgestation Congenital)	
GENERAL CHARACTERISTICS	
Manifestations present at birth or shortly thereafter	
Presence of congenital defects	
SPECIFIC FEATURES	
General	Small for gestational age
Central nervous system	Microcephaly, seizures, cerebral calcification, hypertonia or hypotonia, cerebrospinal fluid pleocytosis, encephalitis, hydrocephalus, hearing loss
Skin	Icterus, petechiae, purpura, vesicles, hypopigmentation
Eye	Chorioretinitis, cataracts, glaucoma, microphthalmos, optic atrophy
Heart	Patent ductus arteriosus, pulmonary artery stenosis
Abdomen	Hepatosplenomegaly, hepatitis
Lung	Pneumonitis
Musculoskeletal	Bone lesions, limb hypoplasia
Acute Infection (Late Gestation Congenital, Natal, or Postnatal)	
GENERAL CHARACTERISTICS	
Manifestations usually appear several days to weeks after birth	
Absence of congenital defects	
SPECIFIC FEATURES	
General	Hyperthermia or hypothermia, irritability, lethargy, jitters, poor feeding, vomiting
Central nervous system	Seizures, hypertonia or hypotonia, full fontanelle, meningitis, encephalitis, hearing loss
Skin	Icterus, petechiae, purpura, vesicles, maculopapular rash
Eye	Conjunctivitis, keratitis
Heart	Myocarditis
Abdomen	Hepatosplenomegaly, hepatitis
Lung	Pneumonitis, respiratory distress, cyanosis

with bacterial sepsis: lethargy or irritability, fever or hypothermia, respiratory distress, cyanosis, anorexia or vomiting, hepatomegaly, seizures, or abnormal movements. Skin lesions or rashes may not be present. Laboratory abnormalities also may show neutropenia, lymphocytosis, lymphopenia, thrombocytopenia, elevated liver transaminases, hyperbilirubinemia, and cerebrospinal fluid (CSF) pleocytosis. Neonatal viral infection should be included in the differential diagnosis and management plans of neonates with sepsis syndrome, especially if routine bacterial cultures are negative at 24 hours. Aseptic meningitis in the neonate may be caused by perinatal or postnatal infection with HSV 1 or 2, enteroviruses, or parechoviruses. In neonates with persistent severe viral infections, minimally responsive to antiviral therapy, primary immune deficiency disorder of T or B or natural killer (NK) cell function should be considered.

Differential Diagnosis

The differential diagnosis in a fetus or newborn infant with a suspected viral infection includes, but is not limited to, bacterial or fungal sepsis, toxoplasmosis, syphilis, erythroblastosis fetalis, metabolic inborn errors of metabolism, genetic chromosomal abnormalities, and other forms of congenital malformations.

Laboratory Diagnosis

The most direct, definitive, and preferred method of establishing the diagnosis of a congenital, perinatal, or postnatal viral infection is isolation or molecular detection by polymerase chain reaction (PCR) of the viral agent from an appropriate site in the fetus or neonate. Prenatal diagnosis of fetal viral infection may be established by testing amniotic fluid for viral culture and PCR and, rarely, by fetal blood sample or viral-specific immunoglobulin (Ig)M and PCR. If a congenital infection is suspected in the delivery room, the placenta should be sent for histopathologic studies.

In neonates, urine, saliva, and blood for CMV; throat, cataracts, and occasionally CSF or urine for rubella; skin vesicles, blood, CSF, conjunctivae, throat, stool, and urine for HSV; throat, stool, urine, CSF, or blood for enteroviruses; and skin vesicles and blood for VZV should be sent for virus isolation and virus detection by PCR. In addition, isolation or molecular detection by PCR of virus can be attempted from biopsy or autopsy specimens. Electron-microscopic examination of vesicle fluid or tissue can demonstrate typical herpesvirus particles in both HSV and VZV infection but cannot distinguish between the two. Dried blood spots archived after completion of newborn screening for metabolic and immunodeficiency disorder, may be retrieved through state newborn screening agencies and tested for presence of viral DNA.[2,3] Such approaches have reliably allowed the retrospective diagnosis of congenital CMV. Research evaluating urine and saliva as screening specimens for congenital CMV infection have also been conducted.[2,3,7]

Serologic methods should be used only when isolation or histopathologic or molecular detection by PCR of the virus organism is not possible. The TORCH serologic screen for antibodies (*Toxoplasma*, other, rubella, cytomegalovirus, and herpes simplex) was developed for this purpose and may be the diagnostic test available in rural or remote areas of the world. Maternal IgG, which contains antibody against viruses to which the mother has been exposed, passes transplacentally, beginning at midgestation. Peak levels are reached in fetal serum at the time of birth (umbilical cord blood); they decline to undetectable levels by the time that the infant reaches 6 to 12 months of age, depending on the virus. However, the use of more sensitive assays for IgG antibody detection, such as immunoblotting, has demonstrated that maternal IgG antibody may persist for as long as 15 to 18 months.

In contrast, maternal IgM antibody normally is not passed transplacentally.[17] Because the fetus is in a "protected" environment and usually does not receive an antigenic challenge in utero, fetal immunoglobulin levels remain low and do not begin to rise until after birth, when exposure to a variety of antigens occurs. However, the fetus is capable of mounting a humoral immune response when exposed to an antigen (a virus) in utero. Elevated levels of fetal immunoglobulins, therefore, can be detected at birth in umbilical cord blood. Because maternal IgG is present in such high concentration in cord blood serum, assays for IgM are performed. A fetus challenged in utero with a virus can have specific IgM antibodies against the viral agent, as well as elevated levels of the total IgM fraction. Often, a fetus with detectable virus-specific IgM will have severe in utero disease caused by the virus. Typically, three approaches can be used to make a serologic diagnosis of viral infection in a newborn infant: (1) assay of maternal serum and serum specimens from the infant at birth and at 5 to 6 months of age for antiviral antibody (predominantly IgG activity), (2) assay of neonatal serum for IgM antibody against a specific viral agent, and (3) assay of neonatal serum for quantitative IgM levels, a nonspecific indication of antigenic challenge in utero.

The presence of IgM antibodies in maternal, fetal, or neonatal serum against a specific virus usually is considered to be diagnostic of acute or recent infection with that agent. However, both false-positive and false-negative results can occur. False-positive findings can result from cross reaction between viruses, especially the herpesviruses,[4,5,8] and the presence of rheumatoid factor (i.e., IgM antibody that binds to the Fc portion of IgG) in serum. False-negative results can occur because of a poor or delayed humoral immune response in the fetus or neonate. For example, CMV IgM antibody was present in the serum of only 70 percent of congenitally infected infants, as demonstrated by isolation of virus from urine in the first few days of life.

The presence of IgM antibodies specific for HBsAg, CMV, parvo B-19, rubella or other viruses in maternal serum or isolation of HSV from the genital tract or an enterovirus from the stool at the time of delivery provides circumstantial evidence for the cause of the neonate's condition. Although the presence of specific IgM antibody against CMV, rubella virus, parvovirus B19, or HSV in a single maternal serum specimen is highly suggestive of recent infection, IgM tests may produce false-positive

results and need to be interpreted accordingly. Use of CMV IgG avidity testing may be helpful in some circumstances to determine the time of the maternal infection.[17]

Treatment

Intravenous acyclovir followed by long-term oral suppression with oral acyclovir is the mainstay for antiviral treatment of neonatal herpes simplex virus infection.[4,5,12] Congenital CMV disease, especially if it is severe or involves the CNS or sensory organs such as the eye and ear, may be treated with intravenous ganciclovir or oral valganciclovir.[7,11] Neonates with influenza virus may receive antiviral medications, such as oseltamivir, and ribavirin for severe RSV disease.

BACTERIAL DISEASES OF THE FETUS AND NEWBORN

Worldwide, millions of neonates die every year of bacterial infection.[13] In the United States, the infant mortality rate is approximately 6.85 infant deaths/1000 live births. Neonatal bacterial sepsis corresponds to the eighth leading cause of fatality. The incidence of neonatal sepsis ranges from 1 to 8/1000 live births, with the higher figures corresponding to developing countries. Low birth weight, male sex, and congenital malformation are important risk factors. Extreme prematurity is the greatest risk factor for early-onset sepsis and is associated with an increased risk for having adverse outcomes, including respiratory distress syndrome, bronchopulmonary dysplasia, severe intraventricular hemorrhage, and periventricular leukomalacia. The highest age-specific incidence of bacterial meningitis appears to occur during the first month of life.

Epidemiology and Pathogenesis

Throughout pregnancy and until the membranes rupture, the infant's environment usually is sterile. Not until delivery and the immediate neonatal period is the infant exposed to many microorganisms. The human birth canal is host to large numbers of aerobic and anaerobic bacteria, *Mycoplasma*, *Ureaplasma*, *Chlamydia*, fungi, yeast, and viruses. *Staphylococcus epidermidis*, lactobacilli, diphtheroids, and α-hemolytic streptococci are found in 50 to 100 percent of vaginal cultures of pregnant women and constitute the predominant aerobic flora. Significant but less common isolates include *Gardnerella vaginalis*, *Proteus*, and *Klebsiella* spp. and group B and D streptococci; *Staphylococcus aureus* and miscellaneous organisms, such as *Citrobacter*, *Acinetobacter*, and the *Campylobacter* group, are identified even less commonly. Approximately 85 percent of women with genital colonization by anaerobes harbor *Bacteroides* spp., including *Bacteroides fragilis* in a third of cases. Anaerobic streptococci, *Peptostreptococcus* and *Peptococcus*, are found in approximately 40 percent of women, and *Clostridium* is present in 20 percent. Uncommon anaerobic isolates include *Veillonella*, *Bifidobacterium*, and *Eubacterium*. Vaginal cultures of pregnant women yield mixed aerobic and anaerobic species, but the number of anaerobes decreases from early pregnancy to delivery.

Certain microorganisms are associated with the occurrence of stillbirths. Among these bacterial agents are *Escherichia coli*, group B streptococci (GBS), *Ureaplasma urealyticum*, *Listeria monocytogenes*, and *Treponema pallidum*. In countries with a high prevalence of syphilis, as many as half of all stillbirths are caused by this bacterium. In the United States, stillbirths occur in nearly 7/1000 births, and 10 to 25 percent of them appear to be caused by a maternal or fetal infection. In developing countries, the relative contribution of infection may be even greater.

During the process of delivery, encounters with some of these bacteria initiate colonization of the infant's respiratory and gastrointestinal tracts. In most infants, the microbial flora is established without incident; however, disease caused by one of these organisms develops occasionally in an infant. The conversion from colonization to disease produces fetal or neonatal sepsis or other bacterial illnesses.

A greater susceptibility of male infants is more evident in cases of sepsis caused by gram-negative enteric bacilli. The reasons behind this male preponderance are unknown but may be related to sex-linked factors in host susceptibility.

The bacterial cause of neonatal sepsis and meningitis varies from one geographic area to another, and has varied over time.[13] Although bacterial causes of neonatal sepsis in Western European countries are similar to those in the United States, a different pattern has been identified in Latin America, Asia, and Africa. In the latter sites, gram-negative rods are the most frequent pathogens, followed by *S. aureus*.

Streptococcus agalactiae, or GBS, is a major pathogen for the neonate, causing both early-onset sepsis and late-onset disease, usually meningitis, but also other manifestations (Table 66-3). The acute septicemic form of GBS disease can be caused by any of the group B types (B_I to B_{VIII}), and the specific B type causing disease in the infant

TABLE 66-3 **Common Bacterial Pathogens Causing Neonatal Sepsis**

Gram-Negative Organisms
Escherichia coli
Haemophilus influenza
Citrobacter
Enterobacter spp.
Pseudomonas spp.
Acinetobacter
Neisseria meningitidis
Gram-Positive Organisms
Group B streptococci *(Streptococcus agalactiae)*
Viridans streptococci
Enterococci
Streptococcus pneumoniae
Other streptococci
Listeria monocytogenes
Coagulase-negative staphylococci
Staphylococcus aureus

usually is found in the maternal vaginal tract. GBS meningitis is caused almost exclusively by the B_{III} organism. These organisms may be acquired from nonmaternal sites. Clusters of three or four cases of GBS meningitis have occurred in nurseries during short intervals, suggesting health care–associated outbreaks may occur. The administration of intrapartum antibiotics, such as ampicillin, has reduced the incidence of early-onset GBS disease in neonates. *E. coli* is the other important agent implicated in neonatal bacterial disease, including sepsis and meningitis and urinary tract infection, with an annual incidence of approximately 6.8/1000 live births, and it is the most common gram-negative bacterium causing meningitis during this period.[1] Sepsis caused by *E. coli* is associated with greater morbidity and mortality rates in some studies than is early-onset GBS infection. Ampicillin-resistant *E. coli* strains also may be emerging with more frequency as intrapartum prophylaxis becomes routine. Neonatal colonization with *E. coli* often results from maternal transmission during delivery. Thus, vaginal colonization, observed in 3 to 20 percent of pregnant women, seems to be an important step in neonatal infection. Vertical (mother-to-infant) and horizontal (nursery staff–to-infant, infant-to-infant) modes of transmission have been documented for *E. coli* K1 infections.

Klebsiella pneumoniae is one of the most important neonatal pathogens, causing sepsis and meningitis, in developing countries, with an incidence between 4.1 and 6.3/1000 live births and fatality rates of 18 to 68 percent. It is the second most frequent gram-negative organism associated with late-onset sepsis in developed nations. Although *Klebsiella* strains are part of the normal gastrointestinal and vaginal flora, the resistant nature of the hospital isolates indicates their presence in contaminated environmental reservoirs.

L. monocytogenes is a foodborne colonizer of the human gastrointestinal tract, and one of the host factors that increases the risk for *Listeria* infection and disease is pregnancy. Perinatal infection constituted 34 percent (470/1378) of cases of listeriosis. Transmission to the fetus in utero may produce granulomatous fetal disease, and an early-onset sepsis syndrome or a late-onset meningitis may occur in the neonate perinatally.

S. aureus is another important bacterial neonatal pathogen, causing sepsis, meningitis, osteomyelitis, and abscesses in newborns postnatally. Since the 1980s, epidemic and endemic colonization and disease of the newborn infant with community-acquired and hospital-acquired methicillin-sensitive and methicillin-resistant *S. aureus* (MSSA and MRSA) have been reported with increasing frequency in the United States and Europe. Epidemics of disease caused by MRSA have been reported in the United States and several other countries. Risk factors associated with the development of MRSA infection include lengthy hospitalization, previous antibiotic administration, overcrowding and understaffing, and the presence of predisposing factors such as indwelling central venous catheters, cerebrospinal shunts, mechanical ventilation, and prematurity. Potential reservoirs of MRSA in the hospital environment include colonized or infected neonates, hospital personnel, and the hospital inanimate environment. MRSA is spread by contact and by air and may circulate among patients, staff, and visitors for several months during an outbreak in hospitals, long-term care facilities, and the community. Although chronic nasal carriage of MRSA by hospital personnel has been implicated in several hospital outbreaks, it generally is an uncommon event and is not necessary for initiation or propagation of hospital outbreaks. Limited data suggest that the hospital inanimate environment may become contaminated with MRSA, thereby possibly sustaining outbreaks of infection. Colonized patients without clinical disease contribute substantially to the inpatient reservoir of MRSA. In recent years, an increase in the incidence of community-acquired MRSA infections has occurred in healthy patients without obvious risk factors.

S. epidermidis and other coagulase-negative staphylococci also have been found increasingly to be important neonatal pathogens and a common cause of late-onset sepsis and infections associated with indwelling devices. They are the most common species of the normal flora on the skin, nasal mucosa, and umbilicus of the newborn. With sensitive culture techniques, rates of colonization of the nose, umbilicus, gastrointestinal tract, and cutaneous areas of the neonate with coagulase-negative staphylococci can be as high as 83 percent at 4 days of age. The ubiquitous presence of the organisms and their tolerance to drying and temperature changes contribute to the increased presence of coagulase-negative staphylococci in neonates. Most studies have shown that infections with coagulase-negative staphylococci are not associated with significant mortality rates or morbidity in infected infants.

Enterococcus faecalis and *Enterococcus faecium* are members of the group D streptococci (enterococci), are normal inhabitants of the gastrointestinal tract, and can cause invasive disease in the neonate, usually urinary tract infections, sepsis, and meningitis. These bacteria are the third most frequent gram-positive organisms associated with late-onset sepsis. Outbreaks of bacteremia and meningitis caused by *E. faecium*, resistant to ampicillin and vancomycin, have also now been reported in neonates.

Sepsis Neonatorum

Sepsis neonatorum, or sepsis of the newborn, is a bacterial disease of infants 30 days of age or younger, most often in the first week of life.[15] It involves primarily the bloodstream, although spread to the meninges or other organs occurs in a substantial portion of affected infants. No obvious focus of infection of the bloodstream can be found in most cases.

In utero infection is identified by the presence of bacteria in blood obtained at delivery. Signs of fetal distress, such as tachycardia in second stage of labor, may be the first indication of infection, such as congenital sepsis or pneumonia, in the fetus.

The major signs and symptoms of sepsis in the newborn relate to disturbances in thermoregulation, respiration, heart rate, and gastrointestinal function, abnormalities in temperature regulation—hypothermia or hyperthermia—tachypnea, grunting respirations, cyanosis, intercostal and substernal retractions, and apnea. A heart rate persistently in excess of 160 beats per minute, or above

average for age and gestation, can be a sensitive indicator of early-onset neonatal sepsis. Other early signs of neonatal sepsis include poor feeding, regurgitation, vomiting, weak sucking, abdominal distention, diarrhea, jaundice, and, rarely, gallbladder distention.

Neonates may have cutaneous findings with onset of sepsis neonatorum, which may provide clinical clues for the cause of the condition. Such findings include cellulitis or abscesses (*Staphylococcus* or *Streptococcus*); papular (i.e., listeriosis), vascular, or bullous lesions (i.e., *Pseudomonas*); and exfoliative dermatitis (i.e., phage group II staphylococcal disease). Jaundice develops in approximately one third of infants with sepsis and can also occur in infants with urinary tract infection. Occasionally, jaundice is the only sign of infection and occurs in septic infants, regardless of the type of bacterial pathogen.

Bacterial sepsis in the newborn may be caused by almost any gram-positive or gram-negative organism.[1,15] The most commonly identified bacteria include GBS, group A streptococcus (GAS), and group D streptococcus (GDS), including the enterococci and viridans streptococci. In addition, *Streptococcus pneumoniae* may cause severe illness in the neonate. The gram-positive rod *L. monocytogenes* is also a well-recognized cause of neonatal sepsis. The staphylococci, including community-acquired and hospital-acquired MSSA and MRSA *S. aureus* and coagulase-negative staphylococci may cause sepsis in the newborn. Gram-negative enteric organisms, such as *E. coli* and *K. pneumoniae*, are common causes of neonatal sepsis. In addition, other gram-negative organisms, such as *Enterobacter cloacae* and *Acinetobacter* spp., may cause sepsis in the neonate. *Haemophilus influenzae* also has been isolated from neonates with sepsis.

Recovery of an organism in culture from a meaningful site, such as blood, CSF, urine, abscesses, pleural and peritoneal spaces, joints, bones, and middle ear cavities, substantiates the clinical impression of systemic bacterial disease in the neonate. Isolation of an organism from mucocutaneous sites, such as the skin, ear canal, nasopharynx, gastric aspirate, and rectum, usually does not reflect the microbiologic status of normally sterile body fluids or tissues.

Several sites to sample blood for culture give reliable results: peripheral vein, umbilical artery, and capillary blood. The preferred site is the peripheral vein. Venipuncture should be performed after the skin has been prepared properly by cleansing with an iodine-containing solution. A two-phase antisepsis procedure using 70 percent isopropyl alcohol followed by chlorhexidine or povidone-iodine has been shown to be superior to one using chlorhexidine or povidone-iodine alone in reducing skin colonization by *S. epidermidis*. The theoretical minimal amount of blood needed for detecting bacteremia is a function of the number of organisms circulating at any given time. Infants with *E. coli* sepsis have 5 to more than 1000 colony-forming units per milliliter of blood. Culturing as little as 0.2 mL of blood should be sufficient for detecting *E. coli* bacteremia in these patients. On the basis of experimental *E. coli* sepsis in rabbits, a cultured volume of blood of 0.2 mL is as sensitive as is 1 mL in detecting bacteremia at a threshold level of 5 organisms per milliliter of circulating blood. To improve culture sensitivity, a prudent procedure is to obtain at least 0.5 to 1 mL of blood for culture in infants aged birth to 2 months. Optimal results are obtained when the cultured volume of blood is 5 to 10 percent of the total amount of liquid growth medium to be inoculated.

Bacterial growth is seen in most blood cultures within 24 to 48 hours. Generally, experts recommend obtaining one or two blood cultures before initiating antibiotic therapy. Urine and CSF should be obtained for examination and culture before starting antibiotic therapy in most neonates.

Abnormalities in the white blood cell (WBC) count may be observed, with elevation, left shift with neutrophil predominance, leukemoid reaction, or neutropenia, with thrombocytopenia. The presence of severe thrombocytopenia, or less than 50,000 cells/mL, within 72 hours of birth is an uncommon occurrence and may be caused by early bacterial infection (e.g., GBS sepsis) or congenital viral infection (CMV or HSV). Late-onset thrombocytopenia is often caused by bacterial sepsis or necrotizing enterocolitis. In these cases, platelets fall rapidly, with a nadir at 24 to 48 hours, to counts less than 50,000 cells/mL and tend to persist until the infection is controlled. Slow recovery of platelet counts over a period of 1 to 2 weeks is common.

Elevation of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) also may occur.[16] The ESR in infected patients generally is elevated above the normal range of 1 to 2 mm/hr at 12 hours of age to 17 to 20 mm/hr at 14 days of age. Elevated rates usually are not observed until 24 to 48 hours after clinical signs of disease first occur. Other acute-phase reactants, such as CRP, haptoglobin, prealbumin, and transferrin, may be useful in establishing the diagnosis of neonatal sepsis and in monitoring the course of the infection. The most extensively studied of these acute-phase reactants is CRP. Concentrations of this protein increase significantly within a few hours of the onset of infection. In general, other perinatal events have little impact on neonatal CRP values. CRP concentrations decrease rapidly in infected neonates who respond to therapy and, conversely, are elevated persistently in neonates whose infections fail to respond to therapy. Reliance on a single CRP determination as an early indicator of neonatal bacterial infection is not recommended. Serial determinations are helpful, especially in combination with other hematologic tests, when making a decision to stop antimicrobial therapy safely.

When the diagnosis of sepsis is suspected and after the appropriate samples have been obtained for culture, antibiotic therapy should be instituted. Infants with suspected sepsis and no unusual risk factors usually should be treated with a combination that includes a penicillin class of antibiotic and an aminoglycoside. The choice of antibiotics must be based on the infectious history of the nursery, the antimicrobial susceptibilities of bacteria recently isolated from sick and healthy neonates, the probable etiologic agent based on clinical and epidemiologic clues, CSF penetration of antibiotics, and the infant's hepatic and renal function. For treating early-onset sepsis neonatorum, ampicillin and gentamicin are usually initiated. Ampicillin is effective in vitro and

clinically against GBS, *Listeria*, *Proteus*, most enterococci, and 15 to 30 percent of current *E. coli* strains. The aminoglycosides have broader antimicrobial activity against many Enterobacteriaceae, including most *E. coli*, *Klebsiella-Enterobacter*, and *Proteus* strains, and against *P. aeruginosa*. Although gentamicin frequently is used, the choice of aminoglycoside (e.g., gentamicin, amikacin, or tobramycin,) should be based on the antimicrobial susceptibility of nosocomial bacteria within individual nurseries. For infections caused by gentamicin-resistant coliforms, amikacin or third-generation cephalosporins such as cefotaxime and ceftazidime, or extended-spectrum penicillins and β-lactamase inhibitor, such as piperacillin-tazobactam or ticarcillin-clavulanate, should be used.

Cephalosporins are not active against *Listeria* or enterococci and should not be used without concomitant administration of ampicillin in most neonates. Moreover, when used with ampicillin, cephalosporins do not offer the advantage of synergism that aminoglycosides do against strains of enterococci. Staphylococci may cause neonatal sepsis, and antimicrobial therapy against them, with nafcillin or vancomycin, should be initiated if significant risk factors, such as indwelling vascular catheters, abscesses, or cellulitis, exist for staphylococcal infection. Therapeutic drug monitoring is recommended when aminoglycosides are used, especially in low-birth-weight neonates, and adjustment of the dosage is essential for infants with impaired renal function. Some experts recommend extended intervals for aminoglycoside administration in the neonatal period, and guidelines for drug monitoring in these circumstances have been developed.

Late-onset sepsis neonatorum may be caused by a variety of bacterial organisms.[1] In a previously healthy infant who already has been discharged from the hospital, ampicillin and an aminoglycoside or cefotaxime is usually recommended unless staphylococcal infection is highly suspected, in which case an antistaphylococcal agent (e.g., nafcillin or vancomycin) should replace ampicillin.

Selection of empirical antibiotic regimens can be more difficult for a septic premature infant who has had a prolonged hospitalization, previous antibiotic therapy, possible prolonged tracheal intubation, and placement of a central or peripheral intravascular catheter. In these patients, major pathogens include coagulase-negative and coagulase-positive staphylococci (including MRSA), aminoglycoside-resistant coliforms, highly resistant opportunistic organisms (e.g., *Pseudomonas*, *Serratia*), fungi, and possibly enterococci. As a result, empirical regimens in this situation should be individualized. Examples include ampicillin, amikacin, and clindamycin or piperacillin-tazobactam alone for suspected necrotizing enterocolitis, vancomycin and an aminoglycoside or cefotaxime for patients with indwelling central vascular lines, and nafcillin and an aminoglycoside for babies with skin infection suspected to be caused by susceptible staphylococci. In institutions with a high prevalence of MRSA infection, vancomycin generally is used as initial therapy for suspected staphylococcal disease. For infants who do not respond to antimicrobial therapy or have superficial cultures positive for *Candida albicans*, empirical use of amphotericin or fluconazole should be considered. Ceftazidime and cefotaxime usually should not be used routinely in neonatal units because of the potential for emergence of resistant *Enterobacter* and *Serratia* spp.

After culture and susceptibility studies are available, changes in therapy may be necessary. Ampicillin is preferred for susceptible enterococcal and *Listeria* infections, whereas ampicillin or penicillin can be used for GBS disease. Infants infected with these organisms usually receive a combination of ampicillin and an aminoglycoside for the first 3 to 5 days, followed by ampicillin for the balance of 7 to 10 days or longer. For *S. epidermidis* and MRSA infections, vancomycin is the drug of choice. In selected neonates, the addition of rifampin to vancomycin therapy may be beneficial in clearing persistent bacteremia or infections of the CNS. Central venous catheters or other foreign bodies frequently must be removed to eliminate the source of these organisms. If a gram-negative enteric isolate is susceptible to ampicillin and aminoglycosides, treatment with either antibiotic alone or with two antibiotics until the bloodstream is sterile, can be adequate. For *Pseudomonas* infections, combined therapy with ticarcillin, piperacillin, or ceftazidime and an aminoglycoside often is used for the duration of therapy; however, some experts recommend monotherapy once bloodstream infection has sterilized. In certain circumstances, antibiotics such as cefepime, imipenem or meropenem, and ciprofloxacin may be used to treat an infection caused by a multidrug-resistant gram-negative isolate. The duration of antimicrobial therapy for uncomplicated neonatal sepsis caused by a susceptible organism usually is at least 7 to 10 days or approximately 5 to 7 days after the clinical signs and symptoms, laboratory abnormalities, and positive cultures from infection have disappeared. Longer periods of treatment may be necessary in some neonates with complicated or persistent infections. Delayed clinical improvement or persistently positive blood cultures during therapy may indicate that antibiotics may need to be adjusted based on susceptibility of the organism isolated, or that occult sites of infection (e.g., endocarditis, abscesses, infected foreign bodies or indwelling catheters) exist.

For infants whose initial bacterial cultures are sterile after 48 to 72 hours of incubation, and the clinical suspicion for bacterial infection is low, antimicrobial therapy can be discontinued. If no pathogen has been isolated but bacterial sepsis cannot be excluded, a negative CRP test at 72 hours can help support the decision to discontinue antibiotics.

In addition to antibiotics, supportive care with careful attention to fluid and electrolyte balance; correcting hypoxia, acidosis, hypoglycemia, and other metabolic abnormalities; and providing nutritional support are necessary. Human immunoglobulin for intravenous use in septic neonates appears to be safe, but not convincingly proved to be beneficial in randomized clinical trials. In neonates with primary immunodeficiency syndromes involving B cell function that cause reduced levels of immunoglobulin, replacement therapy with immunoglobulin may be needed. Other supportive therapeutic options for critically ill neonates include extracorporeal membrane oxygenation and immunomodulating

strategies (e.g., anticytokine agents, steroids, pentoxifylline, and nitric oxide inhibitors).

Prevention of neonatal sepsis is a priority for clinicians and physician research scientists. Intrapartum chemoprophylaxis given to women at 35 to 37 weeks gestation with proved GBS colonization reliably prevents colonization of the newborn in the postpartum period and reduces early-onset sepsis caused by GBS. Therefore, universal prophylaxis of all pregnant women with GBS colonization is now recommended and is the current cornerstone for prevention of early-onset GBS disease.[1,20] Clinical trials evaluating maternal immunization with GBS as a way to prevent neonatal GBS disease are under way. Currently, no licensed vaccine against GBS exists; however, many experts feel this strategy, when available, will be the most effective and sustainable long-term preventive strategy for neonatal GBS disease.

Prevention of early sepsis caused by gram-negative bacilli by administration of intrapartum aminoglycosides is important in developing areas of the world where these organisms are prevalent, but no standardized guidelines exist.

Prevention of late-onset infection, as opposed to early-onset disease, in neonates by the administration of intravenous immunoglobulin to preterm babies has undergone intense clinical scrutiny, but consensus of summaries of clinical trial evidence does not support its routine use at this time.[20]

Bacterial Meningitis

As many as a fourth of neonates with bacterial sepsis have a simultaneous meningeal infection.[9,18] The incidence of neonatal meningitis varies greatly among institutions in North America. Rates are approximately 0.2 to 0.4 case/1000 live births but may be as high as 1/1000 live births in some nurseries. In general, group B β-hemolytic streptococci (GBS) and *E. coli* strains account for two thirds of all cases of neonatal meningitis in North America. Gram-negative enteric bacilli predominate in many developing areas of the world.

The early signs and symptoms of neonatal meningitis frequently are indistinguishable from those of septicemia and other disorders occurring in the neonatal period. The most frequent signs of neonatal meningitis are temperature instability, respiratory distress, irritability, lethargy, and poor feeding or vomiting. GBS occasionally has been reported to manifest as hydrocephalus without other signs of infection. Signs of meningeal involvement, such as a stiff neck, bulging fontanelle, seizures, and opisthotonos, usually are not present in neonates with meningitis. Therefore, newborns being evaluated for sepsis usually should undergo examination of CSF, especially if antimicrobial therapy is to be instituted.

Abnormal CSF parameters, with elevated WBC count with polymorphonuclear leukocyte predominance, hypoglycorrhachia (low glucose), elevated protein concentration, and positive Gram-stained smear with a positive bacterial culture are the classic and definitive methods to diagnose bacterial neonatal meningitis. However, bacterial meningitis may be present even in the absence of these abnormalities.

Interpretation of CSF values in newborn infants may be difficult. During the first several days of life, the mean WBC count is 15 ± 30 cells/mm^3 (95% confidence limit, 12 to 18 cells/mm^3) in the CSF of healthy or high-risk uninfected babies. Approximately 60 percent of these cells are polymorphonuclear leukocytes. During the first week of life, the cell count slowly diminishes in term infants and increases in premature infants. Cell counts in the range of 0 to 10 cells/mm^3 (median, 4 cells/mm^3) are observed in infants at approximately 2 to 4 weeks of age. The WBC count is unclear when bleeding occurs after the lumbar puncture has been performed. The fixed relationship between the number of WBCs in CSF and peripheral blood may be helpful, and a repeat lumbar puncture may be necessary in 12 to 24 hours to resolve the ambiguity of the traumatic lumbar puncture.

The mean cerebrospinal protein concentration in the first month of life is 64 ± 24 mg/dL, although individual values can be as great as 170 mg/dL, especially in low-birth-weight, premature infants. The ratio of cerebrospinal glucose to blood glucose is 60 to 70 percent and can be greater than 100 percent in term and preterm infants. The upper limits of normal for cellular and chemistry values are higher in uninfected infants with very low birth weight.

These data indicate that CSF values must be interpreted in relation to these normal findings for age and gestation if a diagnosis of neonatal meningitis is to be made early. Comparison of the results of initial CSF evaluations obtained from newborns with proved bacterial meningitis with those from normal or high-risk infants revealed considerable overlap in the findings. For example, approximately 30 percent of infants with GBS meningitis had normal spinal fluid leukocyte counts (<32 cells/mm^3), whereas only 4 percent of neonates with meningitis caused by gram-negative organisms had normal counts. The ratio of CSF to blood glucose was normal in 45 percent and 15 percent of patients with streptococcal and coliform meningitis, respectively. However, when the total CSF evaluation (including Gram-stained smears) was considered, fewer than 1 percent of babies with bacteriologically proved meningitis had totally normal CSF on initial lumbar tap. The likelihood of suppurative meningitis is diminished greatly but not impossible if evaluation of CSF discloses no abnormalities. In some patients in whom the diagnosis is obscured, a repeat CSF examination performed 4 to 6 hours after the initial tap (regardless of whether therapy has been instituted in the interim) may help establish the diagnosis. In premature infants with meningitis caused by *S. epidermidis*, the CSF analysis may be only mildly abnormal despite compatible clinical findings. Careful examination of the stained smears of CSF from every infant with suspected meningitis is important. Grossly clear fluid may contain few WBCs and many bacteria. The stained smears from approximately 20 percent of neonates with proved meningitis are interpreted as showing no bacteria. Because of the low concentrations of organisms, most Gram-stained smears of CSF from infants with *L. monocytogenes* meningitis do not reveal bacteria. The CSF findings in an infant with a brain abscess may include a pleocytosis of up to a few hundred

cells, a predominance of mononuclear cells, and an elevated protein concentration. Bacteria may not be seen on a Gram-stained smear of CSF if meningitis is not present.

Ventriculitis may complicate neonatal bacterial meningitis.[10,18] Obstructive ventriculitis is suspected when the head circumference increases dramatically, the anterior fontanelle is persistently full, CSF cultures are persistently positive, and examination of ventricular fluid shows elevation of WBCs with polymorphonuclear predominance, presence of bacteria on Gram-stained smear, and positive culture. Brain imaging by cranial ultrasound, computed tomography (CT), or magnetic resonance imaging (MRI), with contrast material, may show enhancement of the lining tissue of the ventricles or brain abscess formation.

Ampicillin and gentamicin or cefotaxime, administered in meningeal doses, are recommended for initial empirical treatment of neonatal meningitis for most neonates. If there is an indwelling device, such as a ventricular-to-peritoneal shunt in place, or a Gram-stained smear shows gram-positive cocci, staphylococci should be suspected and vancomycin administered. All neonates with bacterial meningitis should undergo repeat CSF examination and culture 24 to 48 hours after initiation of therapy. If organisms are observed on Gram-stained smears of fluid or cultures are still positive, modification of the therapeutic regimen should be considered, and repeat CSF examination should be performed until sterility is documented.

In all neonates with bacterial meningitis caused by *Citrobacter diversus* or *Cronobacter (Enterobacter) sakazakii*, cranial CT or brain MRI imaging should be obtained early because of the frequent association with brain abscess. The duration of antibiotic therapy should be extended up to 6 weeks or more, depending on clinical evolution and resolution of the lesion based on repeated CT. A neurosurgeon should be consulted early for needle aspiration of the abscesses to determine identification and susceptibility pattern of the organism. Aminoglycosides probably should not be used because of decreased activity in abscess cavities that have low pH and anaerobic conditions.

Third-generation cephalosporins possess attractive features, including lower MICs for gram-negative enteric bacilli than with aminoglycosides, good penetration into CSF in the presence of inflamed meninges, and a wide therapeutic index, for the treatment of bacterial meningitis in newborn infants. These agents are active against most streptococci but inactive against *L. monocytogenes* and enterococci. Only ceftazidime provides adequate activity against *Pseudomonas aeruginosa*. Among these agents, cefotaxime is preferred for the treatment of neonatal meningitis caused by susceptible gram-negative organisms, because of extensive experience with this drug in the neonatal period and because it does not alter the bowel flora substantially. Cefotaxime can be used singly or in combination with an aminoglycoside. Ceftazidime has been shown to be effective in treating patients with *P. aeruginosa* meningitis. Because of high biliary excretion and marked alteration of the normal intestinal flora and because of potential concern for displacement of bilirubin, routine use of ceftriaxone during the neonatal period is not recommended by most experts. After the pathogen has been identified and susceptibility studies are available, the single drug or combination of drugs that is most effective should be used. When MRSA or *S. epidermidis* is the cause of infection, vancomycin and amikacin or cefotaxime can be used initially, possibly with addition of rifampin systemically. Imipenem, meropenem, cefepime, and ciprofloxacin have been used to successfully treat neonatal meningitis caused by multiresistant gram-negative bacteria. For neonatal meningitis caused by *Flavobacterium meningosepticum* isolates, combined use of parenteral vancomycin and rifampin has been suggested as the best therapeutic option.

In general, penicillin G or ampicillin is preferred for GBS meningitis, ampicillin for *L. monocytogenes* and enterococci, ampicillin plus an aminoglycoside or cefotaxime for coliforms, and ceftazidime or ticarcillin-clavulanate and an aminoglycoside for *Pseudomonas* infections. The duration of systemic therapy in neonatal meningitis depends on the causative agent and the time necessary to sterilize CSF cultures. As a rule, therapy is given for approximately 2 weeks after bacteriologic cure has been achieved. For meningitis caused by GBS or *Listeria*, approximately 2 weeks of therapy usually is satisfactory. Because delayed sterilization is a common occurrence in infants with gram-negative enteric disease, systemic therapy is given for a minimum of 3 weeks and in some babies for many additional weeks. Final judgment about when to discontinue therapy must be based on the clinical course of the illness, CSF findings at the proposed end of treatment period, and brain imaging results to assess for abscess formation.

On the basis of the findings from the second Neonatal Meningitis Cooperative Study and summaries of recent clinical trials, intraventricular therapy cannot be recommended for the routine management of neonatal meningitis caused by gram-negative enteric bacilli.[3] However, on an individualized basis, intraventricular therapy may be of benefit in certain patients with obstructive ventriculitis caused by persistent or resistant organisms. The routine use of steroids in neonatal meningitis cannot be recommended. A prospective trial found lack of effectiveness of dexamethasone for neonatal meningitis in terms of mortality and sequelae rates in contrast to the nonsteroid group. Because this study was small (not sufficient statistical power), not blinded, and without a placebo group, additional studies are required to determine whether dexamethasone has a beneficial effect in neonates with meningitis.

Acute complications of bacterial meningitis include communicating and noncommunicating hydrocephalus, subdural effusions (~1% of patients), deafness, and blindness. Ventriculitis occurs in approximately 70 percent of neonates with coliform meningitis and usually is detected at the time of initial diagnosis. Brain abscess is an infrequent complication, except in infants with *C. diversus* or *C. (E). sakazakii* meningitis, in whom abscess develops approximately 70 percent of the time. A 30 to 50 percent incidence in neurologic sequelae has been reported in surviving infants. Although neurologic deficits may be obvious in some infants at discharge, most

babies appear "well" at this time. Only after prolonged and careful follow-up do the perceptual difficulties, behavioral problems, and other subtle neurologic signs become apparent. Within 4 to 6 weeks of recovering from meningitis, hearing should be evaluated by evoked response audiometry.

Case-fatality rates in neonates with meningitis range from 15 to greater than 30 percent.[17] A mortality rate of 30 percent was observed in 117 neonates with coliform meningitis enrolled in the first Neonatal Meningitis Cooperative Study. In this project, term infants had a significantly lower rate (18%) than that observed in low-birth-weight (<2500 g) babies (45%) and in infants older than 30 days (48%). Poor outcome after gram-negative enteric meningitis is correlated directly with the presence of ventriculitis, the persistence of positive CSF cultures, the presence and persistence of elevated endotoxin and interleukin-1β concentrations in CSF, a CSF cell count of greater than 10,000/mm^3, and a CSF protein concentration higher than 500 mg/dL. When meningitis is caused by *E. coli* K1, poor outcome is associated with persistence of large quantities of K1 capsular polysaccharide antigen in CSF.

Long-term follow-up studies of babies with coliform meningitis enrolled in the Neonatal Meningitis Cooperative Study have revealed that approximately 65 percent of survivors were normal 3 to 7 years after illness. An additional 30 percent were classified as having mild to moderate neurologic sequelae. Many of these latter patients had only slightly abnormal neurologic or psychological evaluations and were considered normal on routine physical examination. About 5 to 10 percent of survivors had severe neurologic or mental impairment requiring custodial care. Approximately 15 to 20 percent of survivors of GBS meningitis have major sequelae, including spastic quadriplegia, profound intellectual disabilities, hemiparesis, deafness, and blindness. Hydrocephalus develops in 11 percent, and 13 percent have a seizure disorder. The survivors without major sequelae on physical examination, however, appear to function within normal limits and comparably to their siblings. Experience with gram-negative enteric bacillary meningitis reported in 98 identified neonates managed from 1969 through 1989 revealed a case-fatality rate of 17 percent; 61 percent of survivors had long-term sequelae that included seizure disorders, hydrocephalus, physical disability, developmental delay, and hearing loss.

The outcomes for GBS meningitis have been reported. A 21 percent morbidity rate after GBS meningitis has been reported in one study. Of the survivors, 29 percent had severe neurologic sequelae, 21 percent had minor deficits, and 50 percent were functioning normally. Factors at initial evaluation associated with death or severe disability included coma, decreased perfusion, CSF protein greater than 300 mg/dL, an absolute neutrophil count of less than 1000, and a total leukocyte count of less than 5000/mm^3. A report from Canada noted that duration of seizures longer than 72 hours, presence of coma, use of inotropes, and leukopenia were the most important predictors of an adverse outcome.

Otitis Media

Otitis media is diagnosed infrequently in neonates because of the paucity of clinical findings and the difficulty of examining an infant's tympanic membrane. The external canal is narrow and tortuous and often is filled with debris. Because a healthy baby's membrane may appear thickened and dull, mobility of the drum determined by pneumatoscopy should be used as the single most reliable indicator of middle ear abnormalities.

Otitis media develops more commonly in premature than in term infants and occurs more often in bottle-fed babies. It is a frequent finding in neonates receiving intensive care, especially those with prolonged nasotracheal intubation. Meconium staining of amniotic fluid and prolonged rupture of membranes are two other risk factors for the subsequent development of middle ear disease. Neonates with cleft palate, Down syndrome, or maxillofacial anomalies are at high risk for the development of chronic middle ear disease. The onset of illness associated with otitis media is insidious, and the most common manifestations are rhinorrhea, irritability, and failure to thrive. Fever higher than 38°C (100.4°F) is a rare finding. The presence of lethargy, hypotonia, hypothermia, high fever, or jaundice suggests the otitis media is complicated by bacteremia or meningitis.

The cause of neonatal otitis media is similar to that observed in older infants and children. *S. pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis* account for more than 50 percent of cases. Approximately 10 to 15 percent of neonates have disease caused by coliforms, GBS, or *S. aureus*. Therefore, antibiotics that include those organisms in their spectrum of activity (i.e., second-generation cephalosporin or amoxicillin-clavulanate) may be preferable to the aminopenicillins for outpatient management of the neonate who otherwise appears well. The patient's condition should be reevaluated 8 to 72 hours after therapy is initiated to determine whether clinical improvement has occurred and whether the middle ear infection is resolving or if systemic disease is present. In patients demonstrating no improvement, tympanocentesis should be performed for examination of stained smears of middle ear fluid and for culture of the contents. Changes in therapy should be based on the findings of these examinations. If gram-negative organisms or staphylococci are observed, or if the neonate appears ill, the infant probably is managed best in the hospital with administration of parenteral antibiotics such as ampicillin and an aminoglycoside if coliforms are suspected or an antistaphylococcal penicillin or vancomycin for *S. aureus*.

Diarrheal Disease

Diarrhea can be a nonspecific symptom of sepsis or urinary tract infection in a newborn infant. Bacteria that may cause diarrhea in the neonate include enteropathogenic *E. coli*, *Vibrio cholerae*, some *E. coli* nonenteropathogenic strains, *Vibrio parahaemolyticus*, *Aeromonas*, *Shigella*, *Salmonella*, *Campylobacter*, and *Yersinia*. Some

diarrheal episodes in neonates probably can be caused by *Clostridium difficile* and may be a presenting symptom for Hirschsprung disease.

Although the cause of diarrhea in infants and children may be suspected on clinical grounds, usually this is not possible in newborn infants. As a general rule, diarrhea caused by enteropathogenic strains of *E. coli* is insidious in onset, is associated with 7 to 10 green watery stools daily, and generally is without blood or mucus. These infants do not appear to be acutely ill. Complications rarely occur and primarily are related to dehydration and electrolyte disturbances. Shigellosis in a newborn may occur as a diarrheic or dysenteric syndrome or may be evidenced only by a septic- or toxic-appearing infant. Suppurative complications are rare events, but dehydration and electrolyte disturbances are common and need immediate and constant attention. *Salmonella*, on the other hand, may cause sepsis and bacterial meningitis in the neonate. *Campylobacter jejuni* infection typically involves the gastrointestinal tract and produces watery diarrhea or a dysentery-like illness with fever and bloody mucoid stools. Extraintestinal infections related to *C. jejuni*, other than bacteremia, are rare occurrences but include cholecystitis, urinary tract infection, and meningitis. The clinical manifestations in neonates with *Campylobacter fetus* infection are similar to those caused by the common neonatal pathogens. *C. jejuni* has been reported to cause bloody diarrhea in otherwise asymptomatic neonates.

The most important aspect of therapy for diarrheal disease of newborn infants is maintenance of hydration and electrolyte balance. As a rule, oral or parenteral solutions containing appropriate electrolytes should be administered during the time of active diarrhea, and the infant should be examined and weighed frequently to ensure proper rehydration has been achieved and prevent the development of complications. Estimates of fluid loss from diarrhea and vomiting should be recorded carefully and used as a basis for replacement therapy.

Neonates and infants younger than 3 months with diarrhea caused by *Salmonella* should be carefully evaluated for systemic illness and treated with antibiotics. Neonates with shigellosis, more common in developing countries, also should receive orally administered antibiotics if the neonate otherwise appears well. Erythromycin, or other macrolides, is the preferred drug for treating symptomatic *C. jejuni* enteritis. Frequently, if erythromycin therapy is initiated within the first 4 days of illness, excretion of the organism is reduced and symptoms resolve rapidly. An aminoglycoside is the drug of choice for treating *C. fetus* infections; chloramphenicol is an alternative in developing countries.

Any infant with diarrhea must be isolated from other babies in the nursery. Surveillance of all infants in contact with the index case and institution of infection control measures are recommended.

Probiotics and synbiotics (generally strains of *Bifidobacterium*, *Lactobacillus*, or *Saccharomyces*) have been used for the prevention and treatment of acute gastroenteritis and neonatal sepsis with beneficial results, and their use in neonates remains under investigation.

Urinary Tract Infections

The incidence of bacteriuria and urinary tract infection in newborn infants ranges from 0.5 to 1 percent for term infants and is approximately 3 percent for premature infants. Most infants with significant bacteriuria are asymptomatic or have nonspecific signs and symptoms. The neonate may appear septic or may have decreased activity, feeding problems, and the other constitutional signs that are seen with infections of other organ systems. Jaundice, hepatomegaly, and thrombocytopenia may be observed in a few infants with urinary tract infection; these findings are associated with septicemia or cholestatic hepatitis in some babies. Localizing signs suggesting urinary tract involvement are unusual findings. When present, they usually consist of a weak urinary stream on voiding or an abdominal tumor from bladder distention, hydronephrosis, or both.

E. coli is the most common etiologic agent of urinary tract infections and accounts for approximately 90 percent of acute infections and 70 to 80 percent of recurrent disease. *Proteus*, *Klebsiella*, and *Pseudomonas* spp. are usually encountered in patients with recurrent disease, particularly those receiving prolonged antimicrobial prophylaxis. Gram-positive bacteria, with the exception of enterococci, rarely are encountered as pathogens for the urinary tract. Only a few neonatal cases of renal abscess have been reported in the literature. *S. aureus* and coliforms were the predominant etiologic agents.

The diagnosis of urinary tract infection is confirmed by examination and culture of urine. The results of these tests depend largely on the method of urine collection. Most pediatricians obtain urine with a sterile, plastic receptacle applied to the cleansed perineum. However, urine obtained by this method may have an elevated cell count because of recent circumcision, vaginal reflux of urine, or contamination from the perineum. Neonatal asphyxia also may increase the urine cell count. WBCs must be differentiated from round epithelial cells, which appear in the urine in significant numbers during the early days of life. Although pyuria commonly accompanies significant bacteriuria, cells can be few or absent. Direct microscopic examination of uncentrifuged, fresh urine is useful. If bacteria are seen readily in each high-power field, they generally number greater than 10^5 cells/mL.

Quantitative urine cultures from infants with documented disease usually contain more than 100,000 colonies/mL of a single bacterial species. Any number of bacteria in a urine specimen obtained by percutaneous needle puncture of the bladder should be considered significant. The procedure should not be performed in infants who are dehydrated, are distended, have abnormal bladder or urinary system anatomy, or have bleeding problems. If a "bagged urine specimen" contains fewer than 100,000 colonies/mL of a single species of bacteria or if the culture yields a mixed bacterial population, a repeat urine specimen should be obtained by suprapubic bladder aspiration or catheterization for culture.

Blood and urine should be obtained for culture from all newborn infants with suspected or proved urinary tract infection before antimicrobial therapy is initiated.

Antibiotics initially are administered parenterally because sepsis is associated with urinary tract infection in 20 to 30 percent of infants and absorption of antibiotics after oral administration is erratic in some babies. Therapy should be initiated with an aminoglycoside and ampicillin in most neonates to provide antibacterial coverage for the anticipated coliforms, enterococci, and GBS. If the patient has renal impairment, ampicillin and cefotaxime constitute an alternative empirical regimen.

A repeat urine culture performed 48 to 72 hours after initiation of appropriate therapy should be sterile or show a substantial reduction in the bacterial count. Infants with persistent bacteriuria should be evaluated for the possibility of resistant organism, obstruction, or perinephric abscess. In uncomplicated disease, therapy usually is continued for a period of 7 to 10 days. Approximately 1 week after discontinuance of therapy, a repeat urine culture is performed.

Neonates with documented infection should undergo radiologic evaluation of the urinary tract. Renal sonogram may be performed to evaluate the possibility of gross congenital abnormalities of the urinary system. Congenital malformations are unusual findings in the fetus or in the first week of life in the neonate, but may be present in a significant proportion of infants with urinary tract infections after this age. If obstruction is demonstrated, urologic procedures to ensure proper drainage are mandatory if therapy is to be successful. Infants found to have anatomic abnormalities (e.g., significant vesicoureteral reflux) may be protected, for a period of time, from reinfection by prophylactic suppressive administration of antibiotics. Routine circumcision of the newborn protects against urinary tract infections during early infancy.

Suppurative Arthritis and Osteomyelitis

Osteomyelitis and suppurative arthritis are unusual in the first 4 weeks of life. Bone and joint infections can be difficult to detect in neonates and young infants. Early establishment of the diagnosis and appropriate management are important steps to reduce orthopedic abnormalities later in life.

The infecting organisms in osteomyelitis and septic arthritis vary, but the predominant ones are *S. aureus* (both MSSA and MRSA), GBS, and gram-negative enteric organisms such as *Klebsiella*, *Proteus*, and *E. coli*. Gonococcal arthritis and tenosynovitis were common occurrences in neonates in previous decades and still may be seen in developing countries. Other bacteria associated infrequently with newborn infection in the bones and joints are *Salmonella* and *Pseudomonas*.

Osteomyelitis and arthritis have been reported in newborns after several invasive procedures, including heel puncture, femoral venipuncture, exchange transfusions, fetal monitoring with electrodes, serial lumbar puncture, and umbilical artery catheterization. Osteomyelitis of cranial bones has complicated infected cephalohematomas. The use of peripheral and central intravascular catheters in neonates has been associated with the development of bacterial osteomyelitis. Septic embolization from catheter tip thrombi, together with local hypoxia from partial occlusion of the vessel by the catheter, may explain this association. During the infant's first month of life, the epiphyseal plate is traversed by multiple small transepiphyseal vessels that provide direct communication between the articular space and the metaphysis of the long bones. As a result, infection of the metaphysis (i.e., osteomyelitis) can spread across the growth plate and penetrate the epiphysis or enter the joint space. The large vascular spaces and thin spongy structure of the metaphyseal cortex in infants permit early decompression of the primary abscess into the subperiosteal space. The abscess then dissects rapidly between the loosely attached periosteum. As pressure increases from the accumulating pus, a subcutaneous abscess can develop that may point and drain spontaneously through the skin and form a sinus tract.

Two distinct clinical syndromes that may be associated with osteomyelitis in the newborn period have been described. The first form presents as a local infection, in which the earliest sign of bone and joint infection in newborns is failure to move an extremity spontaneously or apparent pain on movement without systemic evidence of infection. Swelling, erythema, and heat localized to the affected part are late findings. Multiple bones or joints can be involved, especially when disease is caused by *S. aureus*. The second form of presentation is characterized by disseminated, systemic manifestations of sepsis; only later are multiple sites of bone and visceral involvement noted.

Another unique clinical presentation of osteomyelitis in the newborn is maxillary bone involvement by osteomyelitis, usually caused by GBS or *S. aureus*. Maxillitis or osteomyelitis of the superior maxilla is an unusual form of bone infection in newborn infants. More than 85 percent of all maxillary infections in infants take place in the first 3 months of life, with the peak incidence occurring in the second to fourth weeks. Swelling of the cheek, associated with unilateral nasal discharge of pus, and swelling of the alveolar ridge of the maxilla should alert the physician to this entity.

Conventional radiography may establish the diagnosis in patients with suspected suppurative arthritis; radiographs may be normal or show enlargement of the joint space. Later in the course of disease, subluxation and destruction of the joint are common findings. In early osteomyelitis, the normal radiographic water markings of tissues adjacent to the affected bone may be obliterated, because of deep-tissue inflammation and swelling. Lifting of the periosteum also may be observed, but cortical bone destruction seldom occurs before the second week of illness. A complete skeletal survey should be performed because of the frequent involvement of multiple sites in neonates, especially if bacteremia or sepsis is present. MRI also is recommended for establishing the diagnosis of osteomyelitis in the newborn period.

Blood should be obtained for culture from all infants with osteomyelitis or septic arthritis. A recommended sample is percutaneous needle aspiration of intra-articular pus in patients with suspected suppurative arthritis or aspiration of sequestrum or abscess or lytic bone lesion in those with suspected osteomyelitis. If pus or tissue is obtained, the material should be Gram stained and cultured.

Selection of initial antimicrobial therapy is guided by preliminary identification of the pathogen from stained smears of material obtained by needle aspiration. If no microorganisms are seen, initiating treatment with two drugs, a penicillinase-resistant penicillin (e.g., nafcillin) for treatment of *S. aureus*, and cefotaxime, to provide treatment for both enteric gram-negative organisms and GBS, is advisable. Vancomycin should be added initially, if MRSA is a possibility.

Surgical removal of infected material is an integral part of treatment, even in the neonate. Open drainage is essential for managing septic hip disease. The inflammation in osteomyelitis or septic arthritis can occupy the epiphyseal and metaphyseal sides of the growth plate and result in ischemia and necrosis of the plate and permanent orthopedic damage. For other joints, repeated daily evacuation of fluid with a needle and syringe usually is adequate. In patients with osteomyelitis, the subperiosteal space and the metaphysis should be drained if pus is obtained during diagnostic aspiration. If only a small amount of bloody material is obtained at aspiration, surgery does not need to be performed immediately and the patient generally can be managed with antibiotic therapy alone as long as the local and systemic signs resolve. If improvement is unsatisfactory, repeat aspiration or open surgical drainage may need to be performed.

Antimicrobial treatment of neonatal musculoskeletal infections caused by *S. aureus* or gram-negative organisms is continued for a minimum of 4 to 6 weeks, depending on clinical response. Intravenous treatment may be followed by long-term suppressive treatment in patients with complicated, recurrent, or chronic bone disease. GBS infection of bones and joints is usually treated with penicillin G or ampicillin for at least 2 weeks, longer in complicated cases. Ten days of treatment usually is adequate for treatment of gonococcal disease of the bones or joints in most neonates.

Conjunctivitis and Orbital Cellulitis

Infections of the eye of the newborn can be caused by a variety of microorganisms, including *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *S. aureus* (MSSA or MRSA), and *P. aeruginosa*. Other less frequently encountered organisms included *M. catarrhalis*, *Pasteurella multocida*, and *Neisseria meningitides*. Nonbacterial organisms, including *Chlamydia* and herpes simplex virus, should be considered in the differential diagnosis of neonatal conjunctivitis. Any infant with a conjunctival discharge should be evaluated carefully to determine the cause. Three tests should be performed: Gram and methylene blue stain of the exudate, culture of the exudate, and Giemsa stain and culture for *Chlamydia*, if available, of scrapings made from the lower palpebral conjunctiva after the exudate has been removed. The results of the stained smears and cultures determine the appropriate therapy.

Funisitis and Omphalitis

If inflammation of the cord alone is present, funisitis is diagnosed. It is characterized by wet, malodorous umbilical cord and stump, without surrounding cellulitis. The umbilical cord may be colonized with numerous different potential bacterial pathogens, some of which, like GAS, may not only cause inflammation in the cord of the neonate but also produce nursery outbreaks of disease.

Omphalitis, or infection of the umbilicus, has many causes and occurs more frequently in low-birth-weight infants and those with complicated deliveries. The incidence is estimated to be approximately 2 percent, and symptoms start at an average age of 3 days. Culture and susceptibility test results are necessary for the selection of an appropriate antibiotic. *S. aureus* is the most frequent pathogen isolated, followed by GAS, GBS, and gram-negative rods. Some of the complications secondary to omphalitis reported in the literature are bacterial sepsis, spontaneous evisceration of the small bowel through the umbilical cicatrix, necrotizing fasciitis, small bowel obstruction, peritonitis, and superficial, retroperitoneal, or hepatic abscesses.

Breast Abscess

Breast abscesses are encountered most frequently during the second or third week of life and are more common findings in girls, particularly those older than 2 weeks. The disease does not appear to occur in premature infants, presumably because of underdevelopment of the mammary glands in these infants. Bilateral disease is a rare event.

The major clinical finding is swelling of the affected breast with or without accompanying erythema and warmth. Systemic manifestations are uncommon occurrences, and only a fourth of patients have low-grade fever. The disease sometimes can progress rapidly and involve breast tissue and the entire subcutaneous tissue beyond the breast's anatomic area. This condition is associated with considerable toxicity and systemic signs and symptoms. *S. aureus* is the major pathogen, but coliform bacteria, such as *E. coli* and *Salmonella*, and GBS have become more common causes in the past decade. Mixed infection rarely occurs. Breast abscess is diagnosed by examination of stained purulent material obtained from gentle manipulation of the nipple or by needle aspiration of the abscess. If gram-positive cocci are identified, nafcillin or another penicillinase-resistant penicillin is administered. For gram-negative bacilli, an aminoglycoside or cefotaxime is appropriate. When no organisms are seen, nafcillin and an aminoglycoside or cefotaxime should be used initially until results of culture are available. Blood cultures should be obtained, but bacteremia is a rare finding in this condition. If the patient has only mild cellulitis and no discernible fluctuance, antibiotic treatment alone may suffice. Fluctuant abscesses should be drained.

Suppurative Parotitis

Suppurative parotitis of the newborn usually is easy to recognize, although occasionally it is confused with infection of a preauricular or superior anterior cervical lymph node. Infection occurs more commonly in low-birth-weight infants than term infants and in boys.

Dehydration predisposes to stasis of parotid secretions and subsequent infection. Bilateral infection is a rare event.

Although *S. aureus* (MSSA and MRSA) accounts for most cases, disease may be caused by coliform bacteria, *Pseudomonas*, pneumococci, and GAS. The clinical manifestations include fever, anorexia, irritability, and failure to gain weight. Erythema, swelling, and tenderness over the involved gland may occur. The diagnosis can be confirmed by expressing pus through the parotid duct or by needle aspiration of a fluctuant area. Gram staining of this material helps identify the causative agent. Selection of antimicrobial therapy should be based on interpretation of the Gram-stained smear of expressed pus. If gram-positive cocci are seen, nafcillin or another penicillinase-resistant penicillin should be used. Vancomycin should be added if MRSA is a possibility. Gram-negative organisms are treated best with an aminoglycoside, cefotaxime, or ceftazidime to cover both coliform bacteria and *Pseudomonas*. If no organisms are seen in the purulent material, a combination of nafcillin and gentamicin or cefotaxime should be used until the results of culture are available. In most cases, antibiotic therapy alone suffices, and surgical incision and drainage are unnecessary. The gland should not be extirpated. Response to therapy generally is rapid. Most patients require only 7 to 10 days of therapy until healing is complete.

Scalp Abscess

Scalp abscesses usually are a complication of fetal monitoring with scalp electrodes. The number of vaginal examinations, use of more than one electrode, and fetal scalp blood sampling are risk factors for the development of scalp abscesses. Pathogens incriminated include staphylococci, gonococci, and gram-negative enteric bacteria. A polymicrobial flora, including anaerobic organisms, frequently is isolated in scalp abscesses caused by electronic fetal monitoring electrodes. Incision and drainage of the infected site along with antibiotics for 7 days usually is sufficient for most term neonates with uncomplicated scalp abscesses caused by susceptible bacteria. Scalp lesions associated with scalp electrodes that do not show bacteria on Gram stain or culture may be caused by herpes simplex virus, and should be evaluated with viral culture of lesions and surfaces, examined by HSV DNA PCR of blood and CSF, and treated with intravenous acyclovir.

Pneumonia

Neonatal lower respiratory tract bacterial infections may be acquired congenitally or postnatally. Congenital or perinatal infection results from transplacental transfer of the agent (i.e., congenital infection) or from inhalation of infected amniotic fluid (usually associated with prolonged rupture of membranes) or infected vaginal secretions during delivery.

Common bacterial pathogens causing postnatally acquired pneumonia are *S. aureus*, coliform bacilli, and *Pseudomonas*. These infections occur sporadically or epidemically and may be acquired from the community in otherwise healthy neonates or health care associated in hospitalized neonates. They may be rapidly progressive, necrotic pneumonias that result in pyogenic complications (e.g., empyema and pulmonary abscesses) and metastatic disease to the bones or meninges. The early signs of respiratory disease in neonates frequently are nonspecific and include change in feeding status, listlessness or irritability, and poor color. More specific findings that may not be present at the onset of illness are tachypnea, dyspnea, cyanosis, alteration of temperature (i.e., hypothermia or fever), coughing, and grunting. Accentuation of the normal irregularity of breathing and "head bobbing" also may be present.

The physical findings of pneumonia are variable. Flaring of the alae nasi, rapid respirations, and sternal and subcostal retractions are common findings. Coughing indicates lower respiratory tract involvement; brassy coughing is found frequently in viral disease. Dullness to percussion is difficult to demonstrate, but when present, it indicates consolidation or effusion. Breath sounds also may be diminished over the affected area. Crackles or wheezes usually can be heard on deep inspiration (or when the baby is crying) but may be absent early in disease. The clinician frequently is surprised by the meager clinical signs in the face of clearly demonstrable and sometimes extensive radiographic findings of pneumonitis.

Cultures of blood and material from the trachea frequently help in defining the etiologic agent of neonatal pneumonia. A chest radiograph should be obtained for all babies with suspected lower respiratory tract disease. Radiographic evidence of pneumonia may exist in the absence of specific physical findings. Although the cause of neonatal pneumonia usually can be determined from a radiograph, certain radiologic patterns are associated with specific diseases. With acute-onset GBS disease, the radiograph may mimic one showing hyaline membrane disease. A consolidating bronchopneumonia with pneumatoceles, with or without empyema, suggests staphylococcal disease. When a lobar infiltrate is associated with expansion of the lobe, *K. pneumoniae* infection should be considered. A miliary type of bronchopneumonia in a septic neonate is characteristic of listeriosis or, less commonly, mycobacterial disease.

Pertussis is also an important cause of respiratory disease and pneumonia in the neonate. The onset of disease generally is in the second to sixth weeks of life; the earliest onset reported was at 10 days of age. Most young infants do not have a characteristic whoop. Pertussis should be suspected when an infant has a paroxysmal cough with excessive mucus. Because apneic spells are common occurrences in these infants, all infants with pertussis are admitted to the hospital for management. PCR-based methods are currently recommended, as well as fluorescent antibody testing, to confirm the diagnosis. The greatest hazards to an infant with pertussis are asphyxia and secondary bacterial pneumonia. Supportive care is essential. Excessive mucus must be suctioned, and equipment for emergency airway intubation should be at hand. Administration of fluid therapy is necessary, and maintaining adequate nutritional intake may present

the greatest challenge. Infants with pertussis should not be placed in mist tents. Mist therapy provides no substantial amelioration of paroxysmal coughing episodes, and it increases the risk for development of secondary infection with *P. aeruginosa* (or with other commensals). Atelectasis caused by mucus plugs is a common complication of pertussis in small babies. After the infant has passed the stage of severe paroxysms, chest physiotherapy can be initiated. In almost all cases the atelectasis resolves within 2 to 3 weeks.

Initial antibiotic treatment of suspected bacterial pneumonia should consist of ampicillin or nafcillin or vancomycin and an aminoglycoside or cefotaxime. The most suitable combination of these drugs depends on the clinical features of the illness and the recent experience with bacterial diseases in the local nursery or community. For patients in whom the cause never is defined and staphylococcal disease cannot be ruled out, therapy with nafcillin or vancomycin and an aminoglycoside or cefotaxime is indicated. If the organism is identified, the single most effective drug should be used. Penicillin G or ampicillin is effective against GBS and penicillin-susceptible staphylococci, ampicillin against *Listeria*, and nafcillin or another suitable antistaphylococcal penicillin against penicillinase-producing *S. aureus.* Vancomycin should be used for disease caused by MRSA or coagulase-negative staphylococci. For pneumonia caused by gram-negative bacilli (e.g., *K. pneumoniae* and others), an aminoglycoside or a third-generation cephalosporin should be used. *Pseudomonas* pneumonia is treated best with ticarcillin or ceftazidime in combination, sometimes also with an aminoglycoside. Therapy is continued for 10 to 14 days for disease caused by GBS and *Listeria* and for a minimum of 3 weeks for pneumonia caused by staphylococci or gram-negative bacilli.

Health care–associated or ventilator-associated pneumonia may occur in mechanically ventilated premature infants and can be caused by a vast array of microorganisms. Accordingly, treatment with an empirical regimen of broad-spectrum antibiotics is advised until microbial identification is made. Nonetheless, the physician should realize that isolation of bacteria from tracheal aspirates usually represents airway colonization and not necessarily the etiologic agents. For a reliable etiologic approach, a combination of clinical, radiographic, laboratory, and microbiologic findings is recommended. An important matter to recognize is that antimicrobial therapy commonly fails to eradicate colonizing microorganisms from the airway of ventilated infants despite clinical and radiographic resolution of pneumonia.

Empyema is managed best by closed drainage and use of chest tubes with the largest possible caliber. Generally, placement of one tube high and anteriorly and a second tube low and posterolaterally is necessary to achieve optimal drainage. Pyopneumothorax is another indication for immediate insertion of a catheter into the pleural space. After the infant has improved clinically and the amount of drainage is minimal, the tubes should be removed. In general, they should not remain in the chest for more than 5 to 7 days. Therapy for pertussis consists of azithromycin or erythromycin administered orally or intravenously. Antibiotic therapy may lessen the symptoms of pertussis if administered early in the paroxysmal stage and is valuable for rendering the patient noncontagious. Associations between bronchopulmonary dysplasia and respiratory colonization by *U. urealyticum*, *C. trachomatis*, *Mycoplasma hominis*, and adenovirus have been shown in different studies.

YEAST AND FUNGAL INFECTIONS OF THE FETUS AND NEONATE

The improved survival of preterm infants since the early 1980s has resulted in the emergence of yeasts and fungi as significant neonatal pathogens. *C. albicans* remains the most frequently isolated pathogenic yeast overall, but other *Candida* spp., especially *Candida parapsilosis* and *Candida tropicalis*, and less common pathogenic yeasts, such as *Malassezia*, *Trichosporon*, *Rhodotorula*, and *Pichia* (formerly *Hansenula*), have increased in frequency. The incidence of invasive fungal disease, often with fatal outcomes, caused by *Aspergillus* and Zygomycetes (*Mucor*; *Rhizopus*, *Rhizomucor*; and *Absidia*) also has increased in neonates, especially infants who were born premature or with an inherited metabolic disorder or primary immunodeficiency.

Neonatal Candidiasis

Candida spp. account for approximately 9 percent of all health care–associated infections in the neonatal intensive care unit (NICU). *Candida* spp. infect approximately 1 to 6 percent of infants with a birth weight less than 1500 g (very low birth weight [VLBW]) and 5 to 10 percent of infants with a birth weight less than 1000 g (extremely low birth weight [ELBW]) (Fig. 66-2).

The species isolated most frequently from neonates is *C. albicans*, which represents approximately 40 to 60 percent of isolates from neonates with systemic disease and is the most common *Candida* spp. associated with NICU outbreaks.[19] *Candida* spp. other than *C. albicans* have become increasingly more prevalent, however, and in some NICUs may be isolated more frequently than *C. albicans*. Among these organisms, *C. parapsilosis* is the most common, followed by *C. tropicalis;* both of them have caused outbreaks in NICUs. *Candida glabrata*, *Candida guilliermondii*, *Candida lusitaniae*, and *Candida krusei* also have caused invasive disease in neonates. Most recently, *Candida haemulonii* has emerged as a neonatal pathogen and caused an outbreak in an NICU. Several risk factors consistently have been associated with systemic candidiasis, the most common being prematurity and VLBW and ELBW, with attendant impairment of host defense mechanisms; indwelling intravenous catheters; and prolonged use of broad-spectrum antimicrobial therapy, in particular, third-generation cephalosporins, which suppress normal gastrointestinal flora. Neonatal candidiasis may be congenital or acquired and ranges from commonly encountered benign oral and cutaneous candidiasis to systemic infection with candidemia to more severe and often fatal disseminated candidiasis.

Oral candidiasis is the most common form of infection with *Candida* spp. Lesions on the mucous membranes of

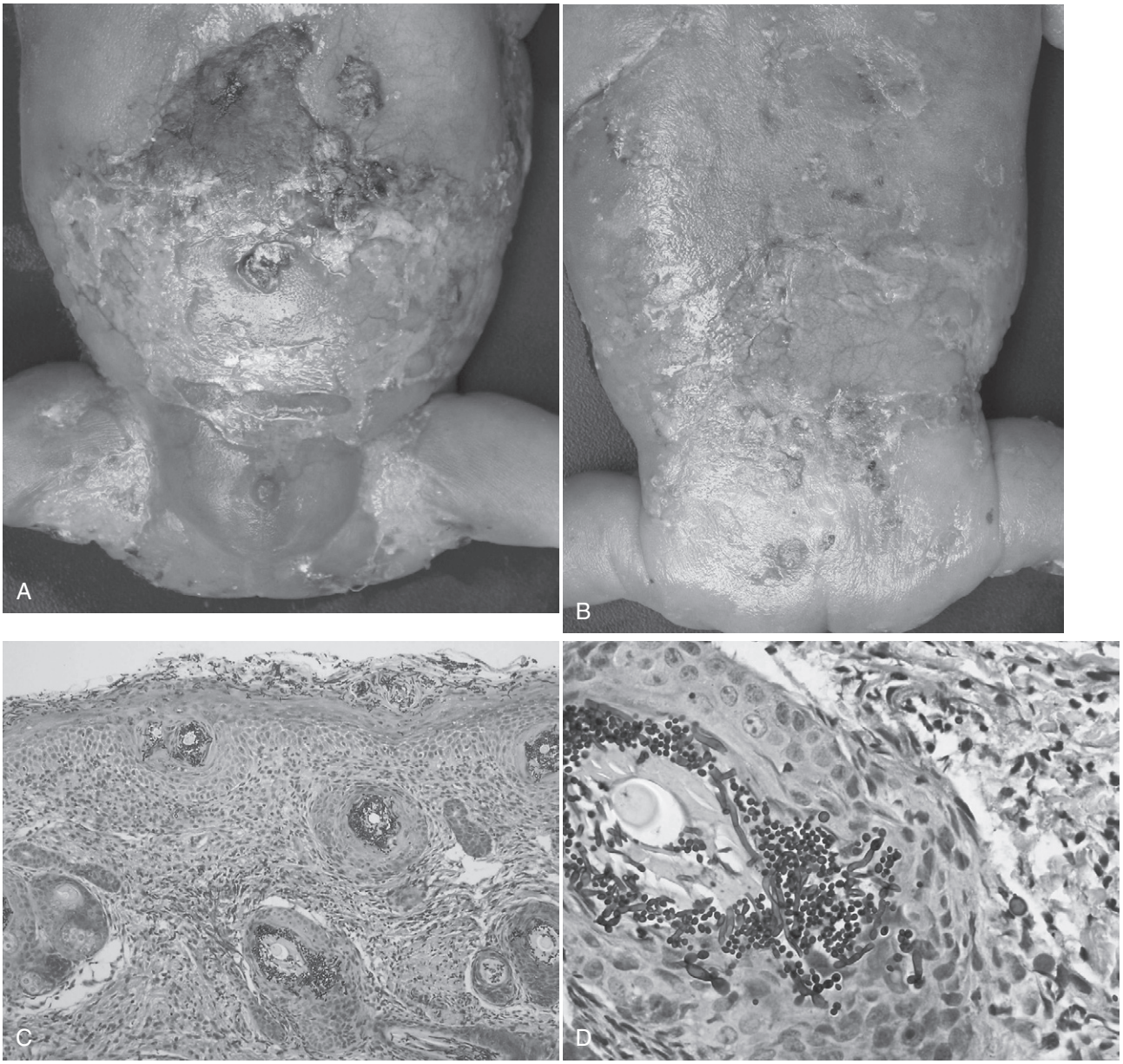

FIGURE 66-2 ■ Invasive cutaneous candidiasis. A 3-week-old, former 24-$\frac{2}{7}$ weeks of gestation premature infant with fatal invasive candidiasis. The skin on the abdomen **(A)** and back **(B)** showed extensive cutaneous ulceration and exudates caused by *Candida* infection. Autopsy examination showed disseminated candidiasis with thromboemboli, infarction, and microabscesses involving the brain, lungs, and kidneys. Skin biopsy specimen showed numerous ovoid yeasts and pseudohyphae infiltrating the stratum corneum and within hair follicles (periodic acid–Schiff stain, 100× original magnification) **(C)** and infiltration of yeasts deep into the dermal connective tissue (periodic acid–Schiff stain, 400× original magnification) **(D).** (Courtesy Dr. Megan K. Dishop, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, Tex.)

the mouth and oropharynx usually appear on the 7th to 10th day of life as whitish-gray plaques that can be scraped easily from the mucosa to expose an erythematous base. Persistent or recurrent infection may be caused by the continued use of bottle nipples and pacifiers that harbor *Candida* on their surface. Primary and acquired immunodeficiency states, including infection with HIV, should be considered if oral thrush fails to clear with appropriate therapy.

Cutaneous candidiasis typically is manifested by erythematous, vesiculopustular lesions found primarily on the skin of the perineum, axilla, and intertriginous areas. The periumbilical area also can be involved. Benign diaper dermatitis with so-called satellite lesions is a frequent occurrence in an otherwise healthy infant, and the peak incidence occurs at 3 to 4 months of age. In VLBW and ELBW premature infants, seemingly benign mucocutaneous lesions, including lesions in the perineum or diaper area, may progress rapidly to invasive dermatitis, producing cutaneous scales, crusting, erosions, ulcerations, or extensive maceration of skin, and result in a potentially lethal systemic candidiasis (see Fig. 66-2).

Scattered, faint, erythematous maculopapular lesions of the skin may be an expression of a serious disseminated candidiasis. Chronic mucocutaneous candidiasis may be diagnosed in an otherwise well-appearing infant, if *Candida* infections of the skin, mucous membranes, and nails persist or recur, despite administration of antifungal therapy. These neonates have a congenital defect in T-lymphocyte function specific to *Candida* spp.

Congenital candidiasis may cause fetal infection with onset of premature labor or intrauterine demise; it may be evident at birth, or it may appear within the first 24 to 48 hours of life. Term and preterm neonates may be affected. Maternal risk factors include a history during pregnancy of *Candida* vaginitis, use of antibiotics, retained intrauterine contraceptive device, cervical cerclage, and prolonged rupture of fetal membranes, although none of these factors may be present in the history. Poor prognostic factors for neonates include the presence of pneumonia or sepsis, prematurity, and early-onset neutropenia at presentation. Congenital candidiasis usually is caused by *C. albicans*, but other *Candida* spp., including *C. tropicalis* and *C. glabrata*, also have been reported to cause congenital candidiasis. Congenital cutaneous candidiasis most often manifests as a widespread, mucocutaneous infection, with erythematous maculopapular or vesiculopustular rash. Congenital pneumonia also may be a part of the disease, with or without the mucocutaneous or systemic signs. Congenital systemic candidiasis may manifest as life-threatening, early-onset sepsis syndrome, especially in premature neonates with septic shock, pneumonia, or meningitis; it also may be accompanied by leukocytosis or neutropenia, or it may mimic congenital leukemia. Twins may be affected, sometimes with different *Candida* spp., manifestations, and outcomes for each twin. Prenatal diagnosis of intrauterine infection with *Candida* may be determined by amniocentesis. A presumptive diagnosis in a neonate can be made by identifying budding yeast organisms on potassium hydroxide preparations or Gram-stained smears of the pustular or vesicular skin lesions, blood buffy coat, or gastric fluid aspirate. Growth of *Candida* in culture provides confirmatory evidence. Term newborns with congenital candidiasis usually have infection limited to the skin, mucous membranes, lungs, or gastrointestinal tract; *Candida* rarely is isolated from blood or other sterile body sites. In contrast, premature neonates with congenital candidiasis often have more severe disease, with positive blood cultures and evidence of disseminated disease, including meningitis. The umbilical cord from a fetus or newborn with congenital candidiasis may show *Candida* funisitis with yellow-white nodules and fungal microabscesses; the placenta also may have nodules and shows chorioamnionitis on histologic examination.

Acquired systemic candidiasis includes (1) catheter-associated sepsis, in which *Candida* is isolated from the blood of infants who have central venous catheters, but no evidence of focal infection or disseminated disease; (2) disseminated candidiasis, in which *Candida* is isolated from the bloodstream in association with other foci of infection, such as skin or intestine, regardless of whether the infant has a central venous catheter; and (3) focal disease of an organ, such as the lung or kidney or brain or disseminated disease caused by *Candida* spp., without a positive blood culture. No significant differences in presentation of systemic candidiasis caused by different *Candida* spp. have been apparent. The clinical and laboratory signs of systemic candidiasis usually are nonspecific and resemble signs seen with bacterial sepsis. Knowledge of prior colonization with *Candida* spp. or presence of cutaneous crusting or erosions, dermatitis, or skin maceration provides clinical clues that a sepsis syndrome in a premature neonate may be caused by invasive *Candida* spp. Signs and symptoms most commonly reported in neonates with systemic candidiasis include respiratory deterioration (74%), apnea and bradycardia (60%), carbohydrate intolerance (56%), skin manifestations (53%), abdominal distention (49%), temperature instability (35%), guaiac-positive stools (26%), and hypotension (21%). The sites most frequently involved in systemic neonatal candidiasis include the blood (60% to 80%), CNS (meningoencephalitis, 25% to 60%), lungs (pneumonia, 70%), kidneys (renal candidiasis, 60%), liver and spleen (19%), the eyes (endophthalmitis, 30%). End-organ disease is accompanied most often by positive blood or urine cultures, especially if cultures are persistently positive for more than 5 days. Not all neonates with end-organ disease with *Candida* spp. have positive blood or urine cultures, however.

The organ involved most frequently in systemic candidiasis seems to be the kidney. *Candida* may be isolated from the urine of 73 percent of neonates with systemic candidiasis with positive blood cultures, but only 5 to 15 percent of such neonates have documented *Candida* pyelonephritis with renal infiltration, mycetoma, or renal abscess. The kidney seems to be particularly vulnerable to the formation of renal cortical abscesses and obstructive masses, which usually occur at the ureteropelvic junction. A neonate with persistently positive urine or blood cultures, hypertension, renal insufficiency or acute renal failure, or oliguria or anuria should undergo ultrasound imaging to determine the presence of a bezoar-associated obstructive uropathy, which requires immediate attention. Urinary catheters or nephrostomy tubes may become colonized with *Candida* and result in secondary infections that may range from asymptomatic to invasive disease with obstruction.

CNS involvement occurs in 3 to 23 percent of neonates with systemic candidiasis; percentages of 54 to 64 percent have been reported in a few series. In addition to causing meningitis, *Candida* may invade the brain tissue, through hematogenous dissemination, producing focal nodular encephalitis, with minimal inflammation of the meninges. The diagnosis of candidal meningoencephalitis requires a high index of suspicion, followed by thoughtful and careful evaluation.

Candida meningitis may occur in the absence of positive blood cultures; CSF pleocytosis may be minimal or absent, and elevated protein or hypoglycorrhachia is an inconsistent finding. Gram-stained smears of CSF rarely reveal budding yeast, even when CSF cultures are positive for *Candida*. More recent reviews determined that meningitis with *Candida* isolated from the CSF occurred in 8 percent of ELBW infants, but only 37 to 52 percent

had *Candida*-positive blood cultures. CSF findings in neonates diagnosed with *Candida* meningitis also may vary. Of neonates with *Candida* meningitis, 43 percent may have normal CSF. Fernandez and associates,[9] in a 10-year review of candidal meningitis in 23 infants in an NICU, reported that pleocytosis occurred in only 39 percent of infants and hypoglycorrhachia occurred in 25 percent, despite a positive CSF culture for *Candida* in 74 percent of infants. The remaining neonates are diagnosed when a positive blood culture occurs with an abnormal, but culture-negative, CSF. *Candida* also may colonize and infect external ventricular drains placed for management of hydrocephalus.

Ventriculitis, brain abscesses, and brain calcifications are unusual complications of *Candida* meningitis that can be diagnosed with brain imaging procedures. Mortality or neurodevelopmental disabilities in survivors may occur in 73 percent of neonates with *Candida* meningitis.

Endocarditis is estimated to occur in 5 to 15 percent of neonates with systemic candidiasis. It is a serious and potentially fatal complication of neonatal systemic candidiasis, with reported fatality rates of 25 to 73 percent. *C. albicans* has been isolated most often, followed by *C. parapsilosis*. *Candida* endocarditis may occur in neonates with normal hearts and neonates with structural heart disease and as a postoperative complication of cardiac surgery. *Candida* endocarditis in neonates may differ in presentation from *Candida* endocarditis in older children and adults. It usually occurs in premature neonates with persistent candidemia associated with an indwelling vascular catheter. Most neonates with *Candida* endocarditis have structurally normal hearts, however, and not all reported cases in neonates have been associated with vascular devices. The presentation of *Candida* endocarditis in neonates may be acute and associated with an episode of persistent candidemia, or late onset, often discovered weeks or months after a presumably silent or resolved episode of candidemia. Cardiac murmurs may be absent. Early literature reports consisted mostly of autopsy diagnoses; however, more recently, echocardiography has proved to be a valuable tool in establishing the diagnosis of *Candida* endocarditis. Intracardiac vegetations on the mitral, tricuspid, and aortic valves, or the presence of a right atrial mass, in association with persistently positive blood cultures for *Candida*, strongly supports the diagnosis of endocarditis.

Eye disease may occur in 3 to 11 percent of neonates with systemic candidiasis; figures as high as 45 percent have been reported. Candidal eye disease may complicate systemic candidiasis in term, near-term, and preterm infants. More recent reports suggest, however, that the prevalence of ophthalmologic complications in hospitalized infants and children, including premature VLBW infants who are treated aggressively and survive candidemia, may be decreasing. Eye disease may occur on the first day of systemic candidiasis but more often is associated with prolonged candidemia. Advanced eye disease may be visible on routine examination; however, establishing the diagnosis of endophthalmitis early requires indirect ophthalmoscopy by an ophthalmologist. Most lesions involve the retina and choroids; characteristic lesions include unilateral or bilateral yellow-white fluffy, retinal, or free-floating vitreal opacities or balls, often accompanied by hemorrhage or inflammatory vitreous haze. The iris and lens of the eye also may be involved. Lens abscesses manifesting as lens opacities or cataracts also may occur, and they may be a nidus of ongoing infection poorly reached by systemic antifungal therapy and a source for recurrent disease. Untreated, *Candida* endophthalmitis also may progress and produce corneal thinning and perforation of the globe. Rarely, *Candida* endophthalmitis may mimic retinoblastoma. In addition, VLBW infants who experience candidemia may have concomitant endophthalmitis and retinopathy of prematurity or they may develop more severe retinopathy of prematurity (stage 3 or beyond) or retinal detachment and require laser therapy. Premature, high-risk, VLBW infants with candidemia should be monitored closely for *Candida* eye disease and for progression of retinopathy of prematurity.

Osteoarthritis is a rare manifestation of systemic candidiasis in neonates. It may occur temporarily associated with persistent candidemia or as a late complication, weeks to months later, after apparent resolution of an episode of candidemia. In addition to premature infants, newborns with underlying inherited disorders of metabolism also may be at increased risk. Clinical features of candidal osteoarthritis in neonates are similar to the features of osteoarthritis caused by bacterial pathogens and include warmth, pain, tenderness, and swelling of the extremity and diminished range of motion. Imaging may show swelling of soft tissue and joint and cortical bone erosion. Culture of aspirated joint fluid or biopsy of bone tissue positive for *Candida* spp. confirms the diagnosis.

Intra-abdominal infections, including enteritis, necrotizing enterocolitis (NEC), and peritonitis, and intra-abdominal and hepatosplenic abscesses are estimated to occur in 8 percent of neonates with invasive systemic candidiasis. *Candida* peritonitis and sepsis may complicate surgical procedures performed on the intestine for congenital bowel atresias or bowel perforation associated with NEC. Distinct from NEC is idiopathic spontaneous focal intestinal perforation (SFIP), which occurs rarely in VLBW infants and has been associated with systemic candidiasis in half of cases. SFIP is approximately 12-fold less common than NEC in preterm neonates and has a distinct combination of clinical and laboratory features that help distinguish it from NEC. Prognosis and management differ, so it is important to understand the difference between these two clinical entities. Neonates with SFIP are more likely than neonates with NEC to be smaller at birth, have lower Apgar scores, experience early hypotension or hypothermia, and require more intensive neonatal resuscitation. Neonates with SFIP present acutely in the second or third week of life (mean, 16.7 days of life), with distention and a bluish discolored abdomen. Radiographs in SFIP reveal a gasless abdomen without pneumatosis intestinalis or portal venous gas commonly associated with NEC. SFIP frequently is an easily resectable focal area of necrosis associated with a single perforation less than 1 cm in size, often located in the terminal ileum, but also may involve the colon or stomach. The remaining

bowel may be covered with serosal exudate, but otherwise it appears normal. In contrast, NEC often involves large areas of transmural bowel necrosis that are difficult to resect completely in many patients; *Candida* and other organisms may be seen in multiple sections of resected bowel. Histologic findings in SFIP show focal hemorrhagic necrosis, often with *Candida* invading the bowel wall at the site of perforation only, and otherwise healthy bowel. *Candida* spp. and *S. epidermidis* or coagulase-negative staphylococci are the predominant pathogens isolated in SFIP, whereas Enterobacteriaceae predominate in peritonitis caused by NEC-associated perforations, with *Candida* spp. isolated in approximately 10 percent of cases of NEC. A peritoneal culture should be obtained in all neonates with intestinal perforation, regardless of cause, because results of cultures determine the most appropriate antimicrobial therapy for bacteria and *Candida* spp.

Acquired pulmonary candidiasis with pneumonia may occur in 8 to 10 percent of ventilated premature neonates and must be differentiated clinically and radiographically from asymptomatic *Candida* colonization and pneumonia caused by other pathogens.

Diagnosis

Congenital or acquired cutaneous candidiasis may be diagnosed by the presence of budding yeast organisms in scrapings of skin lesions, with the species involved established by culture. Invasive dermatitis caused by *Candida* can be differentiated from benign cutaneous dermatitis by skin biopsy. Histopathologic evidence of organisms beyond the stratum corneum is evidence of more serious invasive dermatitis that leads to potentially fatal systemic candidiasis.

Congenital or acquired systemic candidiasis in a fetus or neonate is established by isolation of *Candida* spp. in culture from a normally sterile body fluid, such as amniotic fluid, urine, blood, or CSF, or from an involved site, such as joint or bone or from abscess. The presence of characteristic yeast or fungal elements in tissue specimens from the placenta or the umbilical cord supports diagnosis of congenital candidiasis. In the evaluation of an infant for possible candidemia, blood for culture should be obtained from a peripheral vein or artery and through all intravascular catheters in place at the time. In addition, at least one blood culture should be obtained after removal of a colonized catheter to determine if candidemia persists after the catheter is removed. Infants whose urine, blood, or CSF cultures yield *Candida*, especially if persistently positive, should have further evaluation done to detect any evidence of dissemination that would influence the prognosis and duration of antifungal therapy. Culture of CSF should be performed if candidemia is documented, even if CSF indices are normal. Urine obtained by suprapubic bladder aspiration, often facilitated by ultrasound guidance, is an excellent source for recovery of *Candida*. If the urine grows *Candida*, renal or systemic infection is suggested. Bag specimens of urine are unreliable for establishing the diagnosis because of the high rate of perineal candidal colonization in infants. Catheterized urine specimens, although preferable to specimens obtained by bag, may be contaminated from contact with perineum and penis. Urine obtained from an indwelling urinary catheter may represent colonization of the catheter; a repeat specimen obtained using sterile technique, at least 24 hours after removal of the catheter, is recommended. Fungal culture of urine may yield candidal growth in a premature neonate with disseminated candidiasis, even when other body fluids, such as blood and CSF, do not. Microscopic analysis of urine, Gram-stained smear of urine, or potassium hydroxide preparation of freshly obtained urine may show evidence of yeast or fungi, providing evidence for a presumptive diagnosis and early initiation of antifungal therapy. In addition to cultures of blood, CSF, and urine, cultures of all clinically involved sites, such as joints, bones, and abscesses, should be performed. Respiratory cultures for *Candida* are associated with pneumonia, and invasive pulmonary candidiasis may be present. Routine laboratory evaluations may support the diagnosis of systemic or invasive candidiasis. Persistent thrombocytopenia is a frequent early finding in infants with systemic candidiasis. Additional laboratory tests, such as a complete blood cell count, liver function tests, and determination of serum glucose, blood urea nitrogen, and creatinine, may be helpful in assessing the degree of systemic involvement.

Treatment

Oral candidiasis or thrush in an otherwise healthy infant is treated with oral nystatin suspension or gentian violet. Superficial cutaneous candidiasis in an otherwise healthy neonate is treated with topical nystatin cream or powder. When the diaper area is extensively involved, oral nystatin therapy may be administered as well in an attempt to eliminate the yeast from the gastrointestinal tract. Cutaneous candidiasis that persists or evolves into invasive candidiasis requires systemic antifungal therapy, usually with amphotericin B or fluconazole.

Congenital candidiasis usually requires only topical antifungal therapy in an otherwise healthy newborn; neonates who have pulmonary involvement or who are preterm should receive systemic antifungal therapy because they are at greater risk for dissemination and a poor outcome. In these instances, amphotericin B or fluconazole may be used for approximately 10 to 25 days, until clinical signs and symptoms have resolved.

Prompt systemic antifungal therapy, usually with amphotericin B or fluconazole, is indicated if even a single blood culture from a neonate is positive for *Candida* spp. In addition to removal of the catheter, systemic antifungal therapy should be administered for at least 10 to 14 days. If the peripheral blood culture also is positive for *Candida* spp., or end-organ disease is present, dissemination via the bloodstream may have occurred, and most experts recommend duration of antifungal therapy to be at least 25 to 30 days.

Isolation of *Candida* spp. from urine obtained from an indwelling catheter suggests possible colonization of the catheter, requiring removal of the catheter if the infection is to be eradicated. Isolation of *Candida* spp. from a urine specimen obtained by sterile technique denotes invasive

renal or systemic disease and requires further evaluation and systemic antifungal therapy. In some patients, medical therapy alone may eradicate the *Candida* infection, even if partial obstruction is present. Some neonates may require an urgent surgical or endoscopic procedure, however, to relieve the obstruction and restore renal function. Duration of antifungal therapy required for cure may range from 25 to 42 days or longer and should be guided by microbiologic cure and resolution of lesions by imaging studies.

Treatment of meningoencephalitis caused by *Candida* spp. in a neonate usually requires systemic therapy for at least 30 days; longer courses of treatment may be required if ventriculitis or brain abscesses or nodules are present. Repeat CSF examination and culture before discontinuing antifungal therapy is recommended to document sterilization and improved CSF parameters of white cells, glucose, and protein. If *Candida* ventriculitis is associated with an indwelling ventricular drain or shunt, prompt removal of the device also is indicated. Repeat CSF examination and culture should be done every 24 to 48 hours until sterile, and treatment should be continued when, and for at least a week after, the drain or shunt is replaced.

Treatment of endocarditis or septic thrombophlebitis caused by *Candida* spp. requires prolonged antifungal treatment, at least 30 days and usually 42 to 60 days, depending on clearance of candidemia, clinical progress, and resolution of intracardiac vegetations or lesions as documented by echocardiography. Consultation with a cardiologist or cardiovascular surgeon is recommended. If septic thrombophlebitis with vascular abscess is present, or an enlarging valvular mass obstructs blood flow, surgical intervention may be considered. Reports of resolution of endocarditis with medical therapy alone suggest, however, that it may be a valid option to consider, especially for neonates who are hemodynamically stable, with right-sided, nonvalvular lesions.

Treatment of eye disease, such as endophthalmitis, requires systemic antifungal therapy for 25 to 42 days, and consultation with an ophthalmologist is recommended to assess the extent of disease, presence or progression of comorbid retinopathy of prematurity, and response to therapy. If an abscess of the lens is present, surgical drainage or lensectomy may be necessary. Long-term follow-up of neonates with *Candida* endophthalmitis suggests that early medical therapy results in cure for most neonates who survive systemic candidiasis and that invasive procedures usually performed in adults with *Candida* eye disease, such as vitreous taps or intravitreal injections of medications, are unnecessary in most neonates. Neonates with invasive candidiasis should be observed closely, however, for progression of retinopathy of prematurity. Renal disease caused by invasive *Candida* spp. usually responds to systemic antifungal therapy, the duration of which usually is at least 25 days. Longer duration of therapy may be required to eradicate renal mycetomas, abscesses, or masses ("fungal balls"). If obstructive uropathy is present, urologic consultation is recommended to determine if nephrostomy or other diverting procedure is indicated to relieve the obstruction temporarily.

Treatment of osteoarthritis caused by *Candida* spp. usually requires prolonged duration of systemic antifungal therapy (≥42 days). Aspiration or surgical drainage by an orthopedic surgeon may be necessary if there is an infected joint or bone abscess.

Peritonitis associated with NEC or SFIP usually requires at least 25 days of systemic antifungal therapy, but longer duration may be required if intra-abdominal abscesses develop. Surgical management of SFIP also differs from that of NEC; simple surgical procedures, such as suturing or resection with primary anastomosis, may be all that is required for treatment of SFIP, whereas NEC may require extensive bowel resection.

Duration of systemic antifungal treatment for pulmonary candidiasis is not well established. Treatment should be guided by clinical and radiographic improvement; however, 25 days is likely to be adequate for most patients.

Prevention

The high frequency and significant morbidity and mortality rates associated with *Candida* infections in VLBW neonates have been the impetus behind animal models and clinical studies of preventive strategies using antifungal agents. Single-center and cohort studies and a large, multicenter, randomized, double-blind, placebo-controlled trial have shown that fluconazole prophylaxis (administered daily, every other day, or twice weekly either orally or intravenously for 4 to 6 weeks after delivery) reduces *Candida* colonization, invasive candidiasis, or mortality rates in high-risk, VLBW premature neonates weighing less than 1500 g.[6] Emergence of fluconazole resistant *Candida* spp., including *C. krusei* and *C. parapsilosis*, may occur. Also disease caused by invasive yeasts other than *Candida* spp. may occur. An outbreak of *Rhodotorula mucilaginosa*, a fluconazole-resistant yeast, occurred more recently in an NICU that routinely administered prophylactic fluconazole.

Invasive Yeasts Other Than Candida

Congenital and perinatal infections with the encapsulated yeast *Cryptococcus neoformans* have been reported. Fetal demise may occur, especially in pregnant women with untreated or severe disease. Neonates also may aspirate the organism during delivery and have fever and respiratory symptoms; meningitis, gastrointestinal involvement, and disseminated disease also may occur. Neonates and young infants with disseminated *C. neoformans* also may have a congenital immune disorder, such as severe combined immunodeficiency. Systemic antifungal treatment with amphotericin B is preferred, used in combination with 5-fluorocytosine (5-FC). Although *C. neoformans* seems to be susceptible to triazole agents, experience using these compounds to treat neonates with cryptococcosis is limited.

The *Malassezia* genus of yeasts includes seven species. The six lipophilic species are *Malassezia furfur*, *Malassezia globosa*, *Malassezia obtusa*, *Malassezia restricta*, *Malassezia*

slooffiae, and *Malassezia sympodialis*, and one species, *Malassezia pachydermatis*, is not strictly lipophilic. *Malassezia* spp. frequently colonize the skin of neonates. By the time they are 1 week old, 5 percent of neonates have positive skin cultures for *Malassezia* spp.; by 2 weeks of age, 37 to 51 percent of VLBW premature neonates have positive skin cultures. By the time they reach 3 to 6 months of age, healthy newborns have been observed to have colonization rates of 48 to 91 percent, as have preterm neonates in the NICU. *Malassezia* spp. can be introduced into the NICU on the hands of health care workers and transmitted to neonates; it also seems to persist on environmental surfaces such as incubators. The species most frequently associated with neonatal infections and NICU outbreaks include *M. furfur*, *M. pachydermatis*, *M. sympodialis*, and *M. globosa*. Skin infections with *M. furfur* and *M. sympodialis* in neonates have been associated with nonfollicular pustulosis skin conditions, such as neonatal cephalic pustulosis and neonatal acne. Skin scrapings of the pustules may show neutrophilic cells and fungal or yeast organisms when examined under the microscope, and fungal culture confirms the organism. Neonatal cephalic pustulosis usually is a benign, self-limited condition of the skin and responds to topical antifungal therapy. It must be differentiated, however, from more invasive forms of yeast and fungal diseases. Skin infections with *M. globosa* and, to a lesser extent, *M. sympodialis* are associated with pityriasis versicolor. Intravascular catheters may become colonized with *M. furfur* or *M. pachydermatis* and cause bloodstream infections, often with serious complications in VLBW preterm neonates. Risks for development of invasive disease include previous treatment with broad-spectrum antibiotics and prolonged use of intravascular catheters and intravenous fat emulsions or lipid infusions. Neonates may have systemic signs of sepsis, pulmonary decompensation, and thrombocytopenia. Complications of catheter-associated bloodstream infections with *M. furfur* include peripheral thrombophlebitis, thromboembolism, catheter occlusion, adhesion of the vascular catheter to the vein wall, and endocarditis with intracardiac mass and thromboembolic phenomena. Neonatal meningitis and urinary tract infections associated with *M. furfur* and *M. pachydermatis* also have been reported. Cultures of blood, catheter tip, urine, CSF, and involved tissue should be obtained. The organism also has been seen on peripheral blood smear. Diagnosis may be elusive, however, because recovery of *Malassezia* spp. in routine cultures is rare; if the diagnosis is suspected, special fungal cultures on Sabouraud dextrose agar overlaid with sterile olive oil or media containing long-chain fatty acids may enhance recovery of lipophilic *Malassezia* spp.

Pichia (formerly *Hansenula*) *anomala* and *Pichia ohmeri* are emerging yeasts that may cause infection in preterm VLBW neonates and infants with severe combined immunodeficiency. In some NICUs, 17 percent of preterm neonates may be colonized, and single cases and NICU outbreaks associated with indwelling catheters, administration of total parenteral nutrition solutions, and abdominal surgery have been described. Invasive diseases in preterm neonates include bloodstream infections, sepsis syndrome, abscesses, and meningitis with ventriculitis. Management includes removal of indwelling catheters and systemic antifungal therapy.

Trichosporon spp. colonize the hair, skin, mucous membranes, and gastrointestinal tract. *Trichosporon beigelii* has caused invasive dermatitis, late-onset sepsis, pulmonary infiltrates, and urinary tract infections in VLBW preterm neonates with intravascular catheters. It also may cause endophthalmitis and endocarditis. *Trichosporon asahii* infection in neonates has been reported more recently, suggesting that this species has emerged as a neonatal pathogen. *Trichosporon* spp. have been isolated from blood, urine, tracheal aspirates, skin lesions, and catheter tip cultures from neonates. The organism may be identified preliminarily as a germ-tube negative yeast and be confused initially with *Candida* spp. Breakthrough *Trichosporon* infections in patients receiving antifungal agents for candidemia have been reported. The clinician may erroneously attribute persistently positive cultures to a resistant *Candida* spp. rather than *Trichosporon* spp. *Trichosporon* spp. also share heat-stable antigens with *C. neoformans* and produce false-positive cryptococcal antigen tests. A real-time PCR assay to detect *T. asahii* DNA in sera has been developed and may help facilitate rapid establishment of the diagnosis of this potentially fatal infection. The organism has unpredictable susceptibility to antifungal agents, and there are no standardized recommendations for management of neonates with invasive *Trichosporon* infection. Most neonates have been treated with amphotericin B deoxycholate or liposomal amphotericin B combined with voriconazole.

Rhodotorula spp. are another emerging opportunistic yeast that has caused catheter-associated bloodstream infections in neonates.

Invasive Fungal Diseases

Congenital and Perinatal Transmission

Fetal demise, congenital infection, and perinatal transmission associated with the endemic dimorphic fungi *Coccidioides immitis* and *Blastomyces dermatiditis* have been described. Published case reports are very rare, however. Third-trimester acute infection with *C. immitis* seems to carry increased risk for developing severe illness for the mother and fetus. Perinatal transmission for both organisms likely occurs from neonatal aspiration of infected cervicovaginal secretions from a chronically infected maternal genitourinary tract. Cultures of urine, respiratory secretions, and lung tissue are most likely to yield the organisms. If coccidioidomycosis or blastomycosis is diagnosed in a neonate, treatment with systemic amphotericin B is recommended.

Acquired Invasive Fungal Disease

Invasive fungal disease acquired by VLBW or ELBW premature neonates involves primarily the skin, lungs, or gastrointestinal tract. Because many of the invasive fungi that affect neonates also are angioinvasive, sepsis syndrome with hematogenous dissemination to lung, liver, spleen, CNS, and eye may occur rapidly.

Aspergillus. Aspergillus spp. include *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus glaucus*, *Aspergillus nidulans*, *Aspergillus niger*, and *Aspergillus terreus*. *Aspergillus* spp. frequently are found in the environment and the hospital. Infection in neonates and outbreaks in NICUs have been associated with nearby construction or renovation. Diseases associated with *Aspergillus* infection of the neonate include primary cutaneous aspergillosis or invasive dermatitis, pulmonary aspergillosis, and disseminated aspergillosis. Disseminated disease may involve the heart, liver, spleen, lungs, CNS, or eye.

Neonatal aspergillosis usually manifests in the second week of life. Risk factors include extreme prematurity and VLBW or ELBW, administration of steroids and broad-spectrum antibiotics, hyperglycemia, and trauma of the extremely vulnerable skin of the premature infant. The delicate skin of VLBW infants can be macerated easily by adhesive tape, traumatized at the site of insertion of an indwelling intravascular catheter or by surgical procedures, or damaged by pressure sores, providing a portal of entry for the fungus. Invasive fungal dermatitis then occurs. *Aspergillus* spp. also may gain entry through the lungs and cause invasive pulmonary aspergillosis. Because the fungus has a predilection for invasion of blood vessels, it can disseminate quickly in the bloodstream and occlude vessels, causing thrombosis, infarction, and necrosis of tissue. Establishment of the diagnosis requires a high index of suspicion and documentation of the fungus by histopathologic examination and culture of the involved tissue or organ. Suspicious skin lesions may appear early as erythematous plaques, vesicles, scales, or persistent maceration; advanced disease may show dark, necrotic skin lesions; skin biopsy that shows invasion below the stratum corneum establishes the diagnosis of invasive fungal dermatitis. *Aspergillus* also may be recovered from tracheal aspirate cultures, ascites, and CSF. No studies have evaluated the performance of galactomannan antigen tests for the early diagnosis of invasive aspergillosis in neonates.

Early diagnosis followed by prompt and aggressive treatment of a neonate with invasive aspergillosis is important to minimize the usually high morbidity and mortality rates associated with this disease. Traditional treatment has included prolonged, high-dose amphotericin B deoxycholate or lipid complex and surgical excision of accessible tissue, such as the skin and soft tissue, if local disease progresses despite administration of systemic antifungal therapy. Treatment failures frequently occur. More recently, case reports of premature neonates with primary cutaneous aspergillosis who were treated successfully with voriconazole alone or in combination with amphotericin B lipid complex and micafungin have been published.

Zygomycetes (Absidia, Rhizopus, Mucor, and Rhizomucor). Zygomycosis, a term preferred now over mucormycosis, usually is caused by the following species of fungi: *Absidia (Absidia corymbifera)*, *Rhizopus (Rhizopus arrhizus*, *Rhizopus microsporus)*, *Mucor (Mucor amphibiorum*, *Mucor circinelloides*, *Mucor hiemalis*, *Mucor indicus*, *Mucor racemosus*, *Mucor ramosissimus)*, and *Rhizomucor (Rhizomucor pusillus*, *Rhizomucor miehei*, *Rhizomucor variabilis)*. Zygomycosis in a neonate usually manifests during the second and third weeks of life. Health care–associated outbreaks have been linked to contaminated dressings, bandages, or armboards. Risk factors for acquisition of infection include VLBW; extreme prematurity; indwelling intravascular catheters; exposure to elastic bandages, adhesive dressings and tapes, and armboards or tongue depressors used to splint extremities; and host factors such as congenital immunodeficiency or genetic disorders of metabolism that cause metabolic acidosis.

The Zygomycetes are angiotrophic and aggressively invade blood vessels very early in the disease process. Invasion of blood vessels leads to thrombosis, infarction, and tissue necrosis; destruction of local tissue can be extensive, and the fungus may disseminate throughout the body and CNS. The most common presentation of invasive zygomycosis in neonates is primary cutaneous disease. Cutaneous zygomycosis usually begins at an area of minor skin trauma or at the site of intravenous catheter insertion that has come in contact with adhesive tape or bandages, first appearing as a cluster of necrotic vesicles or scales, which then rapidly progresses to extensive necrotizing cellulitis. It usually involves the skin and soft tissues of the upper extremities, but the lower extremities, abdominal wall, or face also may be presenting sites.

Gastrointestinal zygomycosis in a premature neonate often mimics NEC clinically, but without the characteristic radiographic bowel patterns; rarely, it has presented as Hirschsprung disease. Intestinal perforation, of small or large bowel or appendix, with secondary peritonitis, may occur. Rhinocerebral zygomycosis classically manifests in diabetics with ketoacidosis, but it also may affect neonates with conditions associated with metabolic acidosis and premature infants. The fungus enters via the nasal passages and invades the sinus cavities and orbits, spreading to the CNS by fungal thrombosis of cavernous sinus and internal carotid artery. Pulmonary zygomycosis also may occur, either as a primary pneumonia with consolidation and necrosis or secondary to vascular dissemination from a cutaneous or gastrointestinal source. Disseminated zygomycosis is a fatal fungal sepsis syndrome, usually involving all major organ systems and the CNS.

Diagnosis is established by histopathologic examination of tissue, in which characteristic fungal elements invading the tissue and blood vessels can be seen. Fungal culture confirms the identification of the fungus. Successful management includes early diagnosis and aggressive surgical débridement and resection of involved tissues, to clear margins of resection. Amputation or extensive skin grafts may be necessary in some patients. Aggressive surgery is important because the fungus is resistant to most currently available antifungal agents, including the echinocandins and first-generation and second-generation triazoles. The fungus may be inhibited by amphotericin B given in high doses (1.5 mg/kg/day of amphotericin B deoxycholate or ≥5 mg/kg of lipid formulations), which should be administered as soon as the diagnosis is suspected. The newest triazole compound, posaconazole, seems to have activity against zygomycetes, but experience in administering this new agent to neonates is unavailable.

Treatment

Invasive disease of the neonate caused by fungi is treated with antifungal agents. The choice of antifungal agents available to treat invasive fungal disease of the neonate include the traditional polyene antibiotics, nystatin and amphotericin B deoxycholate, and the pyrimidine analog 5-FC, as well as lipid formulations of amphotericin B, first-generation triazoles (fluconazole and itraconazole), second-generation triazoles (posaconazole, ravuconazole, and voriconazole), and echinocandins (caspofungin, micafungin, and anidulafungin). The choice of which antifungal agent to administer, and the duration of treatment, should be guided by the reported susceptibility pattern of the fungus, and dosages should be based on available pharmacokinetic information for neonates.

CONGENITAL TOXOPLASMOSIS

Congenital toxoplasmosis results from placental infection that develops after primary maternal infection with *Toxoplasma gondii* and subsequent hematogenous spread to the fetus (Table 66-4).[14]

The clinical manifestations of congenital toxoplasmosis in newborns generally are indistinguishable from the manifestations associated with other agents of congenital infection, such as CMV and *T. pallidum*. Many neonates with congenital toxoplasmosis are seemingly asymptomatic at birth. The most characteristic clinical findings, frequently referred to as the *classic triad of congenital toxoplasmosis*, are chorioretinitis, intracranial calcifications, and hydrocephalus. They are seen in approximately 86 percent (chorioretinitis), 37 percent (intracranial calcifications), and 20 percent (hydrocephalus) of symptomatic infants. These conditions often are accompanied by a combination of signs and symptoms, including anemia (59%), jaundice (43%), splenomegaly (41%), seizures (41%), fever (40%), hepatomegaly (34%), lymphadenopathy (32%), microcephaly (9%), and eosinophilia (9%).

TABLE 66-4 **Vertical Transmission and Severity of Disease for *Toxoplasma gondii* by the Timing of Maternal Infection During Pregnancy**

	Trimester of Maternal Infection		
	First	**Second**	**Third**
Overall transmission rate (%)	15	30	60
Proportion of infected children with specific disease severity			
Stillborn/perinatal death (%)	35	7	0
Subclinical (asymptomatic) (%)	18	67	90
Mild disease (%)	6	18	10
Severe disease (%)	41	8	0

The diagnosis of congenital toxoplasmosis can be established by isolating the organism from infected body fluids and tissues, such as the placenta, amniotic fluid, fetal blood obtained by cordocentesis, umbilical cord blood, infant blood, and CSF. Alternatively, a diagnosis can be established by histopathologic examination of the placenta in which tachyzoites are revealed. PCR has been used successfully to detect *Toxoplasma* DNA in amniotic fluid, placenta, CSF, and fetal and infant blood. The most practical and widely used method of establishing the diagnosis is by testing maternal and newborn blood for serologic evidence of *Toxoplasma* infection by measuring *T. gondii*–specific IgG and IgM antibodies. Consultation with a recognized reference laboratory* for toxoplasmosis testing is recommended.

Treatment with spiramycin of an acutely infected woman during pregnancy may prevent transmission of the infection to the fetus. Treatment during the first year of life with pyrimethamine, sulfadiazine, and leucovorin is effective treatment for acute disease and improves long-term outcome in congenital toxoplasmosis.[14]

Routine serologic screening of women during pregnancy has been an effective means of prevention in France and Austria, and it has been advocated in other areas where the incidence of congenital toxoplasmosis remains high. Another focus of prevention should be on educating women of child-bearing age to avoid cat feces in litter boxes and outdoor sandboxes and gardens and avoid ingestion of raw meat and mutton.

CHLAMYDIA, MYCOPLASMA, AND UREAPLASMA INFECTIONS IN THE NEONATE

C. trachomatis is a cause of neonatal conjunctivitis and perinatally acquired infant pneumonia. The introduction of systematic screening and treatment of pregnant women has resulted in a dramatic decrease in the number of perinatal chlamydial infections in developed countries. However, in countries in which pregnant women are not screened routinely, including many developing countries, *C. trachomatis* remains the most frequent cause of neonatal conjunctivitis. The incubation period of *C. trachomatis* conjunctivitis is 5 to 14 days after delivery—or earlier if premature rupture of membranes has occurred. The manifestation is extremely variable and ranges from mild conjunctivitis with scant mucoid discharge to severe bilateral conjunctivitis with copious purulent discharge, chemosis, friable mucosa, and pseudomembrane formation. Eyelid erythema and edema frequently are present. A Gram-stained conjunctival smear initially may reveal a predominance of polymorphonuclear leukocytes. Chlamydial conjunctivitis needs to be differentiated from gonococcal ophthalmia or other bacterial and viral causes of conjunctivitis in the neonate. Infants with pneumonia will have symptoms appear between the ages of 2 weeks and 12 weeks of age, with cough, congestion, tachypnea,

*Toxoplasmosis Serology Laboratory, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301; 415-326-8120.

and rales. They may have eosinophilia and elevated immunoglobulins. *Chlamydia* pneumonia should be differentiated from pneumonia in neonates and young infants caused by other perinatally acquired viral, bacterial, and mycoplasma organisms. Laboratory diagnosis with detection of the organism through culture, direct fluorescent antibody antigen detection, or molecular PCR methods will establish the diagnosis. Treatment with macrolide antibiotic (i.e., erythromycin, clarithromycin, or azithromycin) is recommended. Vertical transmission of *Ureaplasma* spp. and *M. hominis* from a colonized mother to her newborn occurs in utero or during delivery.

M. hominis, *U. urealyticum*, and *Ureaplasma parvum* are the genital mycoplasmas with clinical significance in neonatal disease. Vertical transmission of *Ureaplasma* spp. and *M. hominis* from a colonized mother to her newborn may occur in utero or during delivery. Isolation of *Ureaplasma* spp. from endotracheal secretions, the nasopharynx, the throat, or gastric aspirates (or any combination of these sites) has been associated with acute pneumonia and chronic lung disease of prematurity. *M. hominis* and *Ureaplasma* spp. have been recovered from the lungs, brain, heart, and viscera of aborted fetuses and stillborn infants, with histologic findings of bronchopneumonia present in the lungs. The roles of *Ureaplasma* spp. and *M. hominis* in neonatal disease continue to be investigated and defined.

Acknowledgment

The author acknowledges the contributions of Pablo J. Sánchez, Margaret Hammerschlag, Vladana Milisavljevic, James Cherry, James McAuley, Kenneth Boyer, Dora Estripeaut, and Xavier Saez-Laurens to the contents of this chapter from the previous editions of this textbook. For detailed information provided by these authors, as well as detailed references, the reader is referred to the 6th edition.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Bizzarro MJ, Dembry L, Baltimore R, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008;**121**:689–96.
2. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. *JAMA* 2010;**303**:1375–82.
3. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. *N Engl J Med* 2011;**364**:2111–18.
4. Caviness A, Demmler G, Selwyn B. Clinical and laboratory features of neonatal herpes simplex virus infection: a case control study. *Pediatr Infect Dis J* 2008;**27**:425–30.
5. Caviness A, Demmler G, Almendarez Y, et al. The prevalence of neonatal herpes simplex virus infection compared with serious bacterial illness in hospitalized neonates. *J Pediatr* 2008;**153**:164–9.
6. Clerihew L, Austin N, McGuire W. Prophylactic systemic antifungal agents to prevent mortality and morbidity in very low birth weight infants. *Cochrane Database Syst Rev* 2007;(4):CD003850.
7. Demmler-Harrison G. Congenital cytomegalovirus: public health action towards awareness, prevention, and treatment. *J Clin Virol* 2009;**46**(suppl 4):S1–4.
8. Dewan P, Gupta P. Burden of congenital rubella syndrome (CRS) in India: a systematic review. *Indian Pediatr* 2012;**49**:377–99.
10. Gaschignard J, Levy C, Romain O, et al. Neonatal bacterial meningitis: 444 cases in 7 years. *Pediatr Infect Dis J* 2011;**30**:212–17.
11. Kimberlin D, Acosta E, Sanchez P, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. *J Infect Dis* 2008;**197**:836–45.
12. Kimberlin D, Whitley R, Wan W, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N Engl J Med* 2011; **365**:1284–92.
13. Laving A, Musoke R, Wasunna A, et al. Neonatal bacterial meningitis at the newborn unit of Kenyatta National Hospital. *East Afr Med J* 2003;**90**:456–62.
14. McLeod R, Boyer K, Karrison T, et al. Outcome of treatment for congenital toxoplasmosis, 1981-2004: the National Collaborative Chicago-based, Congenital Toxoplasmosis Study. *Clin Infect Dis* 2006;**42**:1383–94.
15. Mukhopadhyay S, Puopolo K. Risk assessment in neonatal early onset sepsis. *Semin Perinatol* 2012;**36**:408–15.
16. Mussap M, Noto A, Cibecchini F, et al. The importance of biomarkers in neonatology. *Semin Fetal Neonatal Med* 2013;**18**: 56–64.
17. Rajasekariah H, Scott G, Robertson P, et al. Improving diagnosis of primary cytomegalovirus infection in pregnant women using immunoblots. *J Med Virol* 2013;**85**:315–19.
18. Shah S, Ohlsson A, Shah V. Intraventricular antibiotics for bacterial meningitis in neonates. *Cochrane Database Syst Rev* 2012;(7): CD004496.
19. Steinbach W, Roilides E, Berman D, et al. Results from a prospective, international epidemiology study of invasive candidiasis in children and neonates. *Pediatr Infect Dis J* 2012;**31**:1252–7.
20. Verani J, McGee L, Schrag S. Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease-revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2010;**59**(RR-10):1–36.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Bizzarro MJ, Dembry L, Baltimore R, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008;**121**: 689–96.
2. Boppana SB, Ross SA, Novak Z, et al. Dried blood spot real-time polymerase chain reaction assays to screen newborns for congenital cytomegalovirus infection. *JAMA* 2010;**303**:1375–82.
3. Boppana SB, Ross SA, Shimamura M, et al. Saliva polymerase-chain-reaction assay for cytomegalovirus screening in newborns. *N Engl J Med* 2011;**364**:2111–18.
4. Caviness A, Demmler G, Selwyn B. Clinical and laboratory features of neonatal herpes simplex virus infection: a case control study. *Pediatr Infect Dis J* 2008;**27**:425–30.
5. Caviness A, Demmler G, Almendarez Y, et al. The prevalence of neonatal herpes simplex virus infection compared with serious bacterial illness in hospitalized neonates. *J Pediatr* 2008;**153**:164–9.
6. Clerihew L, Austin N, McGuire W. Prophylactic systemic antifungal agents to prevent mortality and morbidity in very low birth weight infants. *Cochrane Database Syst Rev* 2007;(4):CD003850.
7. Demmler-Harrison G. Congenital cytomegalovirus: public health action towards awareness, prevention, and treatment. *J Clin Virol* 2009;**46**(suppl 4):S1–4.
8. Dewan P, Gupta P. Burden of congenital rubella syndrome (CRS) in India: a systematic review. *Indian Pediatr* 2012;**49**:377–99.
9. Fernandez M, Moylett EH, Noyola DE, Baker CJ. Candidal meningitis in neonates: a 10-year review. *Clin Infect Dis* 2000; **31**:458–63.
10. Gaschignard J, Levy C, Romain O, et al. Neonatal bacterial meningitis: 444 cases in 7 years. *Pediatr Infect Dis J* 2011;**30**:212–17.
11. Kimberlin D, Acosta E, Sanchez P, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. *J Infect Dis* 2008;**197**:836–45.
12. Kimberlin D, Whitley R, Wan W, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N Engl J Med* 2011;**365**:1284–92.
13. Laving A, Musoke R, Wasunna A, et al. Neonatal bacterial meningitis at the newborn unit of Kenyatta National Hospital. *East Afr Med J* 2003;**90**:456–62.
14. McLeod R, Boyer K, Karrison T, et al. Outcome of treatment for congenital toxoplasmosis, 1981-2004: the National Collaborative Chicago-based, Congenital Toxoplasmosis Study. *Clin Infect Dis* 2006;**42**:1383–94.
15. Mukhopadhyay S, Puopolo K. Risk assessment in neonatal early onset sepsis. *Semin Perinatol* 2012;**36**:408–15.
16. Mussap M, Noto A, Cibecchini F, et al. The importance of biomarkers in neonatology. *Semin Fetal Neonatal Med* 2013;**18**: 56–64.
17. Rajasekariah H, Scott G, Robertson P, et al. Improving diagnosis of primary cytomegalovirus infection in pregnant women using immunoblots. *J Med Virol* 2013;**85**:315–19.
18. Shah S, Ohlsson A, Shah V. Intraventricular antibiotics for bacterial meningitis in neonates. *Cochrane Database Syst Rev* 2012;(7): CD004496.
19. Steinbach W, Roilides E, Berman D, et al. Results from a prospective, international epidemiology study of invasive candidiasis in children and neonates. *Pediatr Infect Dis J* 2012;**31**:1252–7.
20. Verani J, McGee L, Schrag S. Division of Bacterial Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease-revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2010;**59**(RR-10):1–36.

SECTION **XIV**

INFECTIONS OF THE COMPROMISED HOST

CHAPTER 67

Primary Immunodeficiencies

Javier Chinen • Mark W. Kline • William T. Shearer

Primary immunodeficiencies can be defined as congenital and hereditary disorders caused by an intrinsic molecular defect affecting the development or function of the immune system; and, as a consequence, patients with these conditions have increased susceptibility to infections, neoplasia, and autoimmunity. More than 165 primary immunodeficiency syndromes have been described, and the gene defects responsible for the immunologic phenotype have been identified in most of them.[2] Previously considered to be uncommon, primary immunodeficiency disorders are estimated to occur with a minimum incidence of 1 in 10,000 live births. A survey in the United States done in 2007 using a sample of 10,000 households reported a prevalence of 1 in 1,200 people with a diagnosis of primary immunodeficiency,[14] suggesting that these conditions are more common than reported in the literature.

In contrast to primary immunodeficiencies, secondary immunodeficiencies are acquired disorders, with immune dysfunction occurring as a result of exogenous factors or along with some other nonimmune primary disease processes. They are far more commonly encountered than primary immunodeficiencies. Causes of secondary immunodeficiency include infection (e.g., human immunodeficiency virus [HIV]), drugs (e.g., corticosteroids), malnutrition, and neoplastic or metabolic diseases (e.g., Hodgkin disease, diabetes mellitus). These factors may also affect the fetus while developing in utero and present in the neonatal period and should be considered in the differential diagnosis of immunodeficiencies at this age.

An immunologic evaluation is indicated when children develop unusually frequent or severe infections and when infections are caused by uncommon organisms (Table 67-1). Most of these patients are immunocompetent and may have risk factors for increased frequency of infections, such as allergic disorders, anatomic abnormalities, or other clinical conditions that secondarily produce increased susceptibility to infections, as just discussed. However, some of the evaluated children will indeed have a known primary immunodeficiency and will benefit from early diagnosis and management.

The focus of this chapter is on the elements of the medical history and physical examination that are helpful for the diagnosis of a primary immunodeficiency. Screening laboratory tests that assess clinically significant immune dysfunction are described. The most representative antibody, cellular immunity, complement, and phagocyte deficiencies are discussed. Also, a few rare, but distinctive conditions are reviewed, such as ataxia-telangiectasia and leukocyte adhesion deficiency (LAD).

INITIAL EVALUATION FOR SUSPECTED IMMUNODEFICIENCY

Medical History

A comprehensive medical history is essential in identifying children with a defective immune response. Children with immunodeficiency often have a history of frequent and severe infections (e.g., pneumonia, meningitis, septicemia, osteomyelitis, abscesses of soft tissue or an internal organ). Although infections might occur commonly throughout childhood, epidemiologic studies have established a range for the average number of infections that a normal child may suffer per year. For example, otherwise healthy children younger than 5 years of age average between three and eight episodes of upper respiratory tract infections annually.[32,39,40] By 1 year of age, 62 percent of children have had at least one episode of acute otitis media and 17 percent have had three or more episodes.[81] By 3 years of age, more than 80 percent of children have had at least one episode of acute otitis media and 46 percent have had three or more episodes. The incidence of gastroenteritis among children in the United States is approximately two to three episodes per child-year, with rates as high as five episodes per child-year among children attending daycare centers.[10,33] Occurrence of these infections during infancy or early childhood in normal children is modified by several potential factors, including immunologic immaturity or naïveté (lack of prior exposure to infectious agents), poor hygiene, mouthing behavior, allergic disease, exposure to tobacco smoking, and frequent exposure to ill contacts in the home, school, or daycare settings.

Pediatricians might also suspect immunodeficiency when the course of an infection in a child is unusually prolonged or associated with unexpected complications (e.g., lung abscess in a child with pneumonia or skull bone osteomyelitis as a complication of sinusitis). In general, infections over time at multiple body sites are more suggestive of a defective immune response than infections occurring at only one site (e.g., recurrent otitis media). In the latter circumstance, a mechanical or anatomic explanation for the infections (e.g., foreign body or occult tracheoesophageal fistula in the child with recurrent pneumonia, allergic inflammation, congenital fistulous tract to the middle ear in the child with recurrent bacterial meningitis) should be considered first.

The nature of the infection could suggest a defect in a particular compartment of the immune system. Children with primary antibody deficiencies typically experience

TABLE 67-1 When to Suspect an Immunodeficiency Disorder

- Frequency of infectious illnesses higher than expected
- Unusual severity or duration of infectious disease
- Poor response to conventional antibiotic therapy
- Unusual organisms (opportunistic infections) causing disease
- Failure to thrive, secondary to frequent illnesses
- Poor wound healing
- "Cold" *Staphylococcus* spp. skin infection (absence of pus)
- Recurrent periodontitis
- Low granulocyte or lymphocyte count

TABLE 67-2 Common Pathogens in Children with Primary Immunodeficiency

Immunodeficiency	Common Pathogens
Antibody deficiencies	*Streptococcus pneumoniae, Haemophilus influenzae, Staphylococcus aureus, Pseudomonas aeruginosa, Mycoplasma, Salmonella, Shigella, Campylobacter,* rotavirus, enteroviruses, *Giardia*
Cellular and combined immunodeficiencies	*Mycobacteria, S. pneumoniae, P. aeruginosa, Candida, Pneumocystis jiroveci,* herpesviruses, adenoviruses
Complement deficiencies	*S. pneumoniae, H. influenzae, Neisseria*
Phagocyte deficiencies	*S. aureus, Nocardia* spp., *P. aeruginosa, Serratia,* enteric gram-negative bacilli, *Candida, Aspergillus*

TABLE 67-3 X-linked Primary Immunodeficiency Disorders

- X-linked agammaglobulinemia (Bruton disease)
- Immunodeficiency with hyper-IgM (CD40 ligand deficiency)
- X-linked ectodermal dysplasia with immunodeficiency (NEMO defect)
- Immunodeficiency, polyendocrinopathy, enteropathy, X-linked (IPEX)
- X-linked lymphoproliferative syndrome
- Severe combined immunodeficiency (common γ-chain deficiency)
- Properdin deficiency
- Wiskott-Aldrich syndrome
- X-linked chronic granulomatous disease

infections caused by extracellular bacteria with polysaccharide capsules (e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae* type b). In contrast, children with primary cellular immunodeficiencies often have infections with unusual or opportunistic viruses, fungi, protozoa, and mycobacteria (Table 67-2). Because of the impairment of T cell–dependent antibody responses, infections with common bacteria also may be observed. Children with primary deficiencies of complement components might have recurrent neisserial infections, and those with phagocyte deficiencies may have invasive infections with a variety of catalase-positive bacterial (e.g., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*) and fungal (e.g., *Candida* spp., *Aspergillus* spp.) organisms. Severe or repeated infections with atypical mycobacteria suggest a defect in interleukin (IL)-12–mediated or interferon (IFN)-γ–mediated responses.

In addition to the microbial cause of infections, other clues from the clinical history may help to define the risk for and possible nature of a primary immunodeficiency. Because young infants are afforded some protection against infections by the presence of maternal immunoglobulin (Ig) G, children with primary antibody deficiencies generally have a variable onset of infections occurring between 3 and 18 months of age. In contrast, children with severe T-cell, complement, or phagocyte deficiencies may have onset of infections in the first days or weeks of life.

Omphalitis, resulting in delayed detachment of the umbilical cord stump, and poor wound healing suggest phagocyte deficiency. Hypocalcemic seizures in the neonatal period and congenital heart disease are clues for the diagnosis of DiGeorge syndrome. Severe disease caused by administration of live vaccines (e.g., bacille Calmette-Guérin [BCG] lymphadenitis, measles, poliomyelitis, varicella) justifies screening for immune response defects.

Exploring family history can further support the suspicion of a primary immunodeficiency. A history of parental consanguinity or deaths from infection or from unexplained causes during infancy or early childhood suggests a genetic defect. Many of the immunodeficiency syndromes have X-linked inheritance patterns (Table 67-3), which explains the male-to-female ratio of 5 : 1 observed in series of children with primary immunodeficiencies.

Physical Examination

In the absence of infections, the physical examination in most children with primary immunodeficiencies provides few clues pointing to an immune defect, especially when patients are very young and have not been exposed to potential pathogens.

Short stature with wasting or failure to thrive is a relatively common feature of severe T-cell deficiencies, likely secondary to recurrent infections or other chronic conditions, such as chronic or frequent diarrhea with intestinal malabsorption. Omenn syndrome is recognized by the severe rash during the newborn period. A paucity of lymphoid tissues (e.g., tonsils, lymph nodes) suggests impaired lymphocyte development. Oral candidiasis, omphalitis, and multiple skin abscesses may indicate an underlying immune disorder. Other examples of helpful physical stigmata include the characteristic facial features (Fig. 67-1) and cardiac malformations in children with DiGeorge syndrome. Telangiectasia of the

FIGURE 67-1 ■ Typical facial appearance of a child with DiGeorge anomaly. Notice the microstomia, hypertelorism, upturned nose, and posteriorly rotated and small, low-set ears. (See companion Expert Consult website for color version.)

FIGURE 67-2 ■ Telangiectases of the bulbar conjunctivae in a child with ataxia-telangiectasia. (See companion Expert Consult website for color version.)

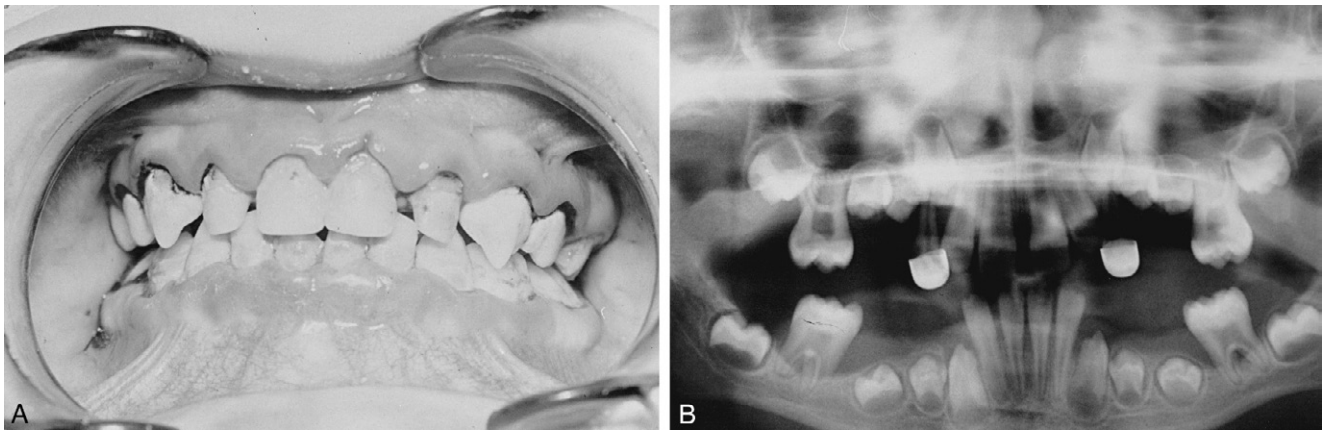

FIGURE 67-3 ■ **A,** Chronic periodontitis in a boy with leukocyte adhesion deficiency. **B,** Radiograph of the same patient shows extensive alveolar bone loss. (Courtesy Dr. Bruce Carter, Texas Children's Hospital, Houston, TX.)

bulbar conjunctivae (Fig. 67-2), nasal bridge, ears, and flexor surfaces of the extremities, with or without ataxia, suggest ataxia-telangiectasia. Chronic eczema is observed in the hyperimmunoglobulinemia E (hyper-IgE) and Wiskott-Aldrich syndromes, and severe gingivitis with secondary loss of alveolar bone and dentition can occur in children with LAD (Fig. 67-3).

Laboratory Tests

Commonly available, relatively inexpensive laboratory tests such as a complete blood cell count with differential can be used in the initial screening for immunodeficiency to exclude most severe disorders (Table 67-4). This evaluation should be targeted to the type of immunodeficiency (e.g., antibody deficiency vs. T-cell defect) suggested by the medical history and physical examination findings. Laboratory test results must be interpreted in the context of the child's age, because average normal serum immunoglobulin levels, T-cell counts, and other immune parameters physiologically change with age.[56] Because 50 to 70 percent of circulating lymphocytes are T cells, children with severe cellular (e.g., complete DiGeorge syndrome) or combined (e.g., severe combined immunodeficiency [SCID]) immunodeficiencies have absolute lymphopenia. Children with Wiskott-Aldrich syndrome typically show reduced numbers of platelets, which are small (decreased mean platelet

TABLE 67-4 **Screening Tests for Suspected Primary Immunodeficiency**

Immunodeficiency	Screening Tests
All types	Complete blood cell count (CBC)
	Peripheral blood smear, for percentage of leukocyte types
Antibody deficiency	Quantitative serum immunoglobulins: IgG, IgA, IgM
	Postimmunization specific IgG antibody titers
	Isohemagglutinins
Cellular immunodeficiency	Delayed hypersensitivity skin tests
	Chest imaging studies for thymus size
	Lymphocyte subset phenotyping
Complement deficiency	Total hemolytic complement (CH50) assay
Phagocyte deficiency	Dihydrorhodamine (DHR)-1,2,3 reduction by flow cytometry
	Neutrophil CD11a, CD11b, CD11c, CD18 expression

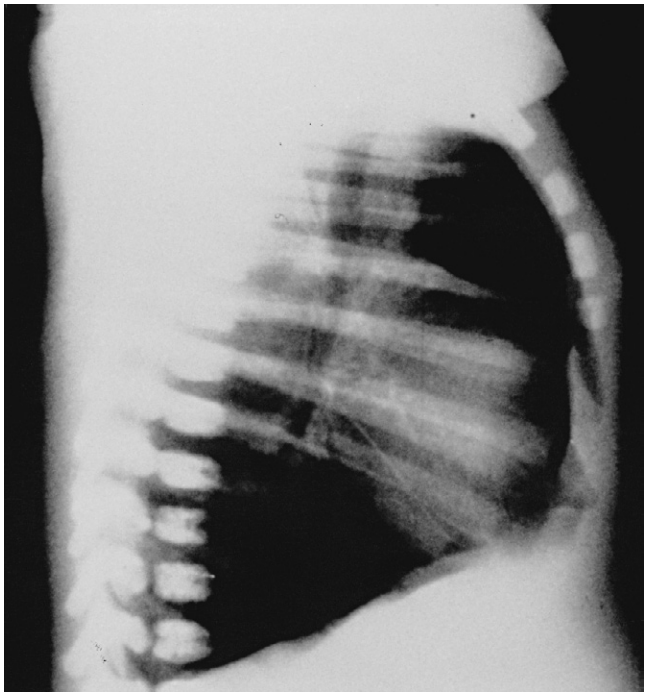

FIGURE 67-4 ■ Lateral chest radiograph of an infant with severe combined immunodeficiency. Notice absence of the normal thymic shadow.

volume), a characteristic that is specific for this syndrome and the related X-linked thrombocytopenia. Large neutrophil cytoplasmic granules, noted in blood smears, are observed in children with Chédiak-Higashi syndrome.

A complete blood cell count is also useful in excluding congenital neutropenia. Children with LAD often have the opposite, with markedly increased neutrophil and total white blood cell counts. The presence of Howell-Jolly bodies, with or without thrombocytosis, suggests anatomic or functional asplenia. Tests to rule out HIV infection, malnutrition, or metabolic disorders as causes of secondary immunodeficiency should be considered.

Evaluation of Humoral Immunity

Screening evaluation of the child with suspected antibody deficiency should include quantitative measurement of serum immunoglobulins and functional assessment of specific antibody responses. Except for the rare deficiency of antibody response to specific antigens, children with other primary antibody deficiencies have abnormalities of serum immunoglobulin concentrations. Measurement of serum IgG, IgA, and IgM concentrations identify children with panhypogammaglobulinemia and those with deficiency of a particular immunoglobulin isotype, such as selective IgA deficiency.

Functional antibody production is usually evaluated by measuring antibody titers generated in response to immunization with vaccines, such as diphtheria and tetanus toxoids. Antibody responses to polysaccharide antigens are evaluated separately using the pneumococcal polysaccharide vaccine (Pneumovax 23) after 24 months of age. Younger infants are thought to have a functional immaturity in the ability to respond to this class of antigens. Alternatively, because ABO blood group antigens are polysaccharides and cross-reacting environmental antigen epitopes are ubiquitous, antipolysaccharide antibody production can be assessed by measuring serum isohemagglutinin titers, taking into consideration that children with blood type AB do not form isohemagglutinins. The conjugated pneumococcal (Prevnar 13) and *H. influenzae* type b vaccines are not suitable for the assessment of antipolysaccharide antibody responses because they are not pure polysaccharides. Patients vaccinated with the conjugated pneumococcal vaccine can be evaluated for antipolysaccharide antibody production because the pneumococcal polysaccharide vaccine contains serotype antigens against which the conjugated vaccine does not induce protective antibodies.

Measurement of serum IgG subclasses may be indicated in children with apparent abnormalities in functional antibody production. Further evaluation of children whose screening tests indicate significant quantitative and functional antibody abnormalities should include enumeration of T- and B-cell subsets in the peripheral blood and in vitro studies of mitogen- or antigen-induced lymphocyte cell proliferation.

Evaluation of Cellular Immunity

The screening evaluation for cellular immunodeficiency starts with delayed hypersensitivity skin tests and, in the young infant, posteroanterior and lateral chest radiographs for the assessment of the size of the thymus (Fig. 67-4). If available, chest computed tomography (CT), magnetic resonance imaging (MRI), or ultrasound evaluation also may be helpful.

Delayed hypersensitivity skin tests are performed using vaccine or microbial antigens to which the child has had prior exposure. Commonly used antigens include tetanus toxoid and *Candida albicans*. Standard initial dilutions are 1:100 for both antigens, but a *C. albicans* dilution of 1:10 may be more appropriate for children 5 years of age or younger because they are less likely to have been exposed to this antigen. The diluted antigen is administered intradermally, and the skin reaction is read at 24 and 48 hours for the presence of wheal formation. Delayed hypersensitivity skin test responses depend on previous exposure to the antigen tested and often are absent in children younger than 2 years of age. Therefore, enumeration of peripheral blood T cells, T-cell subset phenotyping (i.e., $CD4^+$ or $CD8^+$ lymphocyte counts), and mitogen- and antigen-induced lymphocyte proliferation studies may be needed for a reliable assessment of cellular immune function.[56]

Evaluation of the Complement System

Deficiencies of components of the classic complement pathway can be detected by the total serum hemolytic complement (CH50) assay. This test measures the ability of proteins contained in the fresh patient serum to lyse antibody-coated sheep erythrocytes and reflects the activity of all components of the classic complement pathway from C1 through C9. Complete deficiency of any of these components results in very low levels of CH50. Mildly abnormal CH50 values might be obtained because of inadequate sample handling; single complement component levels and function testing should be pursued when the CH50 value is zero or near zero.[94] Testing of complement components C3 and C4 is available in most clinical laboratories and may be considered when complement deficiency is suspected. The alternate complement pathway is assessed with a similar assay (AH50).

Evaluation of Phagocyte Function

Few assays are available for the assessment of phagocyte function. The nitroblue tetrazolium (NBT) dye test uses the reduction of NBT to formazan by activated phagocytes to measure the oxidative metabolic responses that accompany phagocytosis. Children with chronic granulomatous disease (CGD) show reduced dye reduction (<10% of cells are formazan positive); carriers of X-linked CGD typically have between 20 and 90 percent formazan-positive cells. The specificity and sensitivity of this test largely depend on the expertise of the performing laboratory. Flow cytometry that detects hydrogen peroxide production in phagocytes using dihydrorhodamine-1,2,3 (DHR) as the fluorescent indicator has proven to be more reliable and sensitive than the NBT test.[56] More sophisticated tests available in research laboratories include assays of chemotaxis, phagocytosis, and bactericidal activity, which may be performed when the NBT or DHR test results are normal and a high clinical suspicion of a phagocyte disorder exists. Enumeration of CD11a, CD11b, CD11c, and CD18 white blood cells is indicated when diagnosis of LAD is suspected.

TABLE 67-5 **Examples of Gene Defects in Primary Immunodeficiencies**

Immunodeficiency	Gene Defect	Chromosome
Antibody Deficiency		
Agammaglobulinemia	Heavy-chain immunoglobulin	14q23
	Bruton tyrosine kinase	Xq22
Hyper-IgM syndrome	Activation-induced deaminase	12p13
	CD40 ligand *(CD40LG)*	Xq26
Cellular Immunodeficiency		
DiGeorge syndrome	Unknown, *TBX1*?	22q11.2, 10p13
Severe combined immunodeficiency	*RAG1, RAG2*	11p13
	JAK3	19p13.1
	Adenosine deaminase	20q13.11
	Common γ chain	Xq13.1
	IL-7 receptor	5p13
Ataxia-telangiectasia	ATM, DNA kinase	11q22.3
Wiskott-Aldrich syndrome	*WAS,* cytoskeleton protein	Xp11.23
Phagocyte Deficiency		
Chronic granulomatous disease	gp67phox	1q25
	gp47phox	7q11.23
	gp22phox	16q24
	gp91phox	Xp21.1
Leukocyte adhesion deficiency type I	*ITGB2*	21q22

Genetic Testing

More than 165 gene defects responsible for immunodeficiency disorders have been identified (Table 67-5). Currently, gene sequence testing for these conditions is available only in a few specialized laboratories but should be pursued when a primary immunodeficiency with a known genetic defect is suspected. Genetic studies for immunodeficiencies are used most often to confirm the diagnosis of DiGeorge syndrome, in which a microdeletion in chromosome 22 is identified by fluorescent in situ hybridization (FISH) in approximately 95 percent of patients affected.[80] Genetic testing is recommended for affected patients who are ill with infections and have abnormal immune function, for prenatal diagnosis of the unborn child with a sibling affected with a primary immunodeficiency, and for an individual who may have inherited or is a carrier of a known immunodeficiency gene defect. For some of the conditions, knowing the specific gene defect may help assessing prognosis and therapeutic options. For example, it has been reported that bone marrow transplantation for X-linked SCID (*IL2RG* gene defect) is more successful than for other forms of SCID.[17] Knowledge of the specific gene defect is also useful for genetic counseling, most often to answer the concern of parents regarding the probability of having another child with a similar condition.

Neonatal Screening for T-Cell Deficiencies

In June 2010, the U.S. Department of Health and Human Services approved the addition of SCID to the list of diseases recommended for universal neonatal screening.[77] The technique was pioneered in Wisconsin and gradually implemented in several U.S. states, including Massachusetts, California, New York, Colorado, Connecticut, Delaware, Michigan, Mississippi, and Texas, and the territory of Puerto Rico. Children with severe T-cell deficiencies, such as SCID, are born with absence of T-cell receptor excision circles (TRECs, DNA recombination by-products), which can be tested in blood spots from Guthrie cards. This program has been successful to diagnose children with severe immunodeficiency before devastating infections occur, with significant improvement of treatment outcomes.[63] It has also allowed for the estimation of the actual incidence of severe T-cell defects in different states; for example, this estimate approaches 1 in 25,000 live births in California. A similar approach for the diagnosis of B-cell deficiencies in the newborn is being developed.[12]

Management

The immunodeficient patient requires specialized care according to the specific diagnosis and patient condition. For example, hematopoietic cell transplantation is indicated for the most severe immunodeficiencies and needs careful individualized assessment of risks and benefits to provide a treatment protocol that ensures the highest survival chance.[17]

One common therapeutic measure is administration of intravenous or subcutaneous immunoglobulins (IVIG or SCIG, respectively) for patients with deficiency of IgG responses, both when presenting alone or in combination with cellular immunity defects. Other recommendations specific to patients with immunodeficiency include the avoidance of contact with infectious illness and avoidance of live vaccines[6]; the use of irradiated, leukocyte-depleted, cytomegalovirus-negative blood products when needed; antibiotic prophylaxis; and the prompt diagnosis and treatment of infections. Except for patients with severe T-cell deficiency waiting for hematopoietic cell transplants, isolation in sterile environments is not recommended because the risk for infection needs to be balanced with the severe impact in psychosocial development induced by isolation. The infection risk is also moderated with advances in the treatment and prevention of community-acquired infections (Table 67-6).

TABLE 67-6 Key Concepts in the Management of Primary Immunodeficiencies

1. *Immune function.* T- and B-cell number and function should be assessed periodically.
2. *IgG replacement therapy:* for patients with low IgG levels and poor antibody function.
3. *Immunizations.* Live vaccines should not be administered to immunodeficient patients, except to those with complement deficiency and selective IgA deficiency. Patients with complement deficiency may benefit from receiving the pneumococcal and meningococcal vaccines because of their increased susceptibility to encapsulated organisms. Household contacts of children with immunodeficiency should not receive oral poliovirus vaccines because of the risk for transmission to the immunodeficient child. Other live vaccines (BCG, MMRV) may be administered to the household contact. If the vaccine recipient develops a rash, contact with the immunodeficient child should be avoided.[7]
4. *Blood products.* When needed, patients with immunodeficiencies should receive only irradiated, cytomegalovirus-negative, leukocyte-depleted blood products.
5. *Antibiotic prophylaxis.* T cell–deficient patients should take antibiotic prophylaxis for *Pneumocystis jiroveci.* Antibiotic prophylaxis is recommended for dental and surgical procedures and should be considered for patients with recurrent infections.
6. *Infectious diseases.* Infections should be recognized promptly, and unusual pathogens should be considered. Antibiotic therapy should be started early and discontinued cautiously.
7. *Diet and activity.* Patients with immunodeficiency should have a regular diet and lifestyle but should be instructed to avoid eating raw food and playing in environments potentially highly contaminated with pathogens, including daycare centers. Strict hand washing precautions and reverse isolation may be indicated for patients with poor T-cell function.

SELECTED PRIMARY ANTIBODY DEFICIENCIES

X-Linked Agammaglobulinemia

Clinical Features

Boys with X-linked agammaglobulinemia (XLA, Bruton disease) often are healthy during the first months of life because of the protective presence of transplacentally acquired maternal IgG. As maternal immunoglobulin disappears from the infant, chronic or recurrent infections develop.[21,22,62,95] Frequent otitis media, sinusitis, pneumonia, and diarrhea are the most common findings, but infections are not limited to mucosal surfaces and bacteremia, meningitis, and osteomyelitis also may occur. The mean age at diagnosis in a retrospective study of 201 patients with XLA was 2.5 years in patients with a family history of the disease and 3.5 years when patients did not have family history.[95] *S. pneumoniae*, *H. influenzae* type b, *S. aureus*, and *P. aeruginosa* are the most common bacterial pathogens observed in the setting of XLA. *Mycoplasma* spp. infections also occur with increased frequency and have been implicated as a cause of a subacute, destructive arthritis. Gastrointestinal infections may be caused by *Salmonella*, *Shigella*, *Campylobacter*, or rotavirus. Chronic giardiasis with intestinal malabsorption has been reported with increased frequency.

Pathogenesis

The defective gene *(BTK)* maps to the midportion of the long arm of the X chromosome. The gene encodes a

cytoplasmic protein-tyrosine kinase, a signal transducer that is necessary for expansion of B-cell populations during their maturation.[85] As a consequence, blood, lymph nodes, and bone marrow contain markedly diminished numbers of B cells and plasma cells, resulting in hypogammaglobulinemia.

Diagnosis

Because of the presence of transplacentally acquired maternal IgG, the determination of serum immunoglobulin for diagnosis of XLA in the first 6 months of life does not reflect the infant's immunoglobulin production. Infants with XLA also have low concentrations of other Ig isotypes (e.g., IgA, IgM), but defining values that clearly differentiate between infants with the disease and normal infants has been difficult. Definitive diagnosis during early infancy generally relies on immunophenotyping by flow cytometry to demonstrate absence of B cells in peripheral blood. After an infant with XLA reaches 6 months of age, serum IgG concentrations usually are less than 100 mg/dL and concentrations of other immunoglobulin isotypes are low or undetectable. Isohemagglutinins are absent, and specific antibodies are not produced in response to immunization or natural infection. Recurrent infections in male family members may suggest an X-linked condition. Definite diagnosis is obtained by finding inactivating mutations in the *BTK* gene sequence.

Treatment and Prognosis

Lifetime immunoglobulin replacement therapy is indicated for all patients with XLA.[57] It decreases the frequency of serious infections, reduces the need for hospitalization, and may help to prevent the development of chronic lung disease with progressive decrease of lung function. The dose and frequency of administration of immunoglobulins are adjusted to produce serum IgG trough concentrations of at least 500 mg/dL. For most patients, IVIG is required at a dose of 400 to 600 mg/kg every 3 or 4 weeks. Equivalent doses of SCIG might be used alternatively.

Acute infections should be promptly treated in patients with XLA. Minor middle ear, sinus, and skin infections usually respond to oral antibiotics. Pneumonia and other serious focal or systemic infections should be treated initially with intravenously administered antibiotics. Empirical antibiotic therapy is directed against common bacterial pathogens, including *S. pneumoniae*, *H. influenzae*, and *S. aureus*. If possible, etiologic diagnosis should be obtained, particularly in cases of severe or chronic infection. Because serum IgG concentrations often decrease during acute infection, the serum IgG concentration should be measured and additional doses of immunoglobulins may be indicated. Chronic pulmonary disease with bronchiectasis is an important cause of death of patients with XLA. Long-term antibiotic therapy, similar to that employed in patients with cystic fibrosis, may be helpful in individual cases.

Immunoglobulin Deficiency with Increased IgM

Clinical Features

About 70 percent of cases of immunoglobulin deficiency with increased IgM (i.e., hyper-IgM syndrome) are associated with X-linked inheritance, owing to *CD40LG* gene defects, and the rest of patients show an autosomal recessive inheritance pattern.[23,43,46,52] Patients with this disorder develop recurrent pyogenic infections during infancy as transplacentally acquired maternal IgG wanes. Respiratory tract infections and chronic diarrhea with failure to thrive are common; septicemia, meningitis, and other serious systemic infections also occur. Opportunistic infections, such as *Pneumocystis jiroveci* pneumonia, are relatively common in this syndrome when the defective protein is CD40LG.

One half of patients with hyper-IgM syndrome have neutropenia. It can be intermittent but lacks the precise periodicity of cyclic neutropenia. Aphthous ulcers occur commonly. Perirectal ulcers and abscesses have also been reported. Hyperplasia of superficial and deep lymph nodes is usually found, and intestinal nodular lymphoid hyperplasia may lead to malabsorption and protein-losing enteropathy. The incidence of lymphoreticular malignancies is increased in these patients. Autoimmune conditions, including arthritis and nephritis, have been described with increased frequency compared with other children.

Pathogenesis

Males with hyper-IgM syndrome may have mutations of a gene on the X chromosome that encodes the T-cell ligand *(CD40LG)* for the B-cell surface molecule CD40.[4,8] Several patients with normal expression of CD40LG but defects in the B cell CD40 signaling pathway also have been described.[23] Interaction of CD40 with the CD40 ligand is essential for B-cell proliferation, isotype switching, and terminal differentiation of B cells to antibody-secreting plasma cells. Mutations in the activation-induced cytidine deaminase *(AICDA)* and the uracil DNA glycosylase *(UNG)* genes are other defects found in patients with the autosomal recessive form of the hyper-IgM syndrome.[52]

Diagnosis

Patients with hyper-IgM syndrome may have a characteristic increase in serum IgM concentrations in association with low to absent concentrations of serum IgA and IgG. Serum IgM concentrations may exceed 1000 mg/dL; however, they can be within normal range. Circulating B cells expressing surface IgM are found in normal numbers, but there are only a few cells bearing surface IgA or IgG. Antibody responses to immunization often are present but consist predominantly or exclusively of IgM because isotype switching does not occur. Over half of the patients are diagnosed before 1 year of age, and 90 percent of patients are diagnosed by age 4 years. Evaluation of boys with suspected immunoglobulin deficiency

with increased IgM should include a determination of CD40LG expression in activated T cells done by flow cytometry.[8]

Treatment and Prognosis

Immunoglobulin replacement therapy and antibiotic prophylaxis are indicated.[57] CD40L-deficient patients have increased morbidity and mortality compared with patients with autosomal forms of hyper-IgM syndrome, likely secondary to the X-linked condition resulting from a T-cell defect rather than a B-cell defect. Causes of mortality without definitive treatment include infections other than those affecting the respiratory tract, sclerosing cholangitis with progressive liver disease, often secondary to *Corynebacterium parvum* infection, and malignancy.[46] Human leukocyte antigen (HLA)-identical bone marrow transplantation has been successful in this condition and should be considered early after diagnosis, before severe complications occur. Administration of recombinant CD40LG has been reported to improve immune responses in these patients.[36]

Common Variable Immunodeficiency

Clinical Features

Common variable immunodeficiency (CVID) includes a heterogeneous group of disorders with similar clinical manifestations characterized by hypogammaglobulinemia with low or normal numbers of B cells, abnormal antibody production, recurrent infections, and a propensity for autoimmune conditions. Onset can occur at any time from infancy[97] to old age, but symptoms frequently begin during the second or third decades of life.[65]

The clinical manifestations of CVID are similar to those of XLA, with recurrent infections of the respiratory tract, bacteremia, meningitis, osteomyelitis, and septic arthritis. Frequently, patients develop bronchiectasis and chronic lung disease. The most common infectious organisms are *S. pneumoniae*, *H. influenzae*, and *S. aureus*.[65] Many individuals with CVID suffer from chronic diarrhea and intestinal malabsorption that have an infectious or autoimmune basis. The inflammation induced by these conditions may result in protein-losing enteropathy and may exacerbate the underlying hypogammaglobulinemia. Organisms implicated in these gastrointestinal infections include *Giardia*, *Campylobacter*, *Salmonella*, and *Cryptosporidium*. Patients with CVID have an increased frequency of ulcerative colitis, Crohn disease, atrophic gastritis with achlorhydria, viral or autoimmune chronic active hepatitis, and cholelithiasis. Approximately 22 percent of individuals with CVID develop autoimmune conditions, which include a chronic arthritis resembling juvenile rheumatoid arthritis; scleroderma, a lupus-like syndrome; hypothyroidism; and autoimmune neutropenia, anemia, and thrombocytopenia. Malignancies, such as lymphomas and gastric carcinoma, are common findings among adults with CVID. A pseudolymphoma syndrome with lymphoid hyperplasia of the lung (i.e., lymphoid interstitial pneumonia) and intestine (i.e., nodular lymphoid hyperplasia), massive splenomegaly, and mediastinal adenopathy may be seen in patients with CVID.

Pathogenesis

The pathogenesis of CVID is attributed to several different genetic defects that result in loss of antibody secretion. Bone marrow B-cell maturation is intact, and normal or low numbers of B cells are present in peripheral blood and lymph nodes. Decreased or absent memory B cells is associated with disease severity and development of autoimmune disorders and malignancies.[3,19,68] Patients with CVID appear to have an intrinsic B-cell defect that impairs the ability of these cells to differentiate into immunoglobulin-secreting plasma cells, but a variety of T-cell functional abnormalities also have been described. They include decreased lymphocyte proliferation to mitogens and antigens and reduced expression of cytokines.

Mutations in the *TNFRSF13B*, *ICOS*, *MSH5*, *TNFRSF13C*, *CD27*, *CD81*, *MS4A1*, and *CD19* genes have been individually found linked to disease in a few families with several members affected with CVID and IgA deficiency, providing a link between these two clinical syndromes and supporting the concept of multiple causes for CVID.[41,73,89,90,93]

Diagnosis

Quantitative immunoglobulin determination generally reveals a serum IgG concentration persistently less than 250 mg/dL, or less than two standard deviations below the normal range for age, with comparable decreases in other immunoglobulin isotypes. Immunophenotyping of peripheral blood lymphocytes demonstrates the presence of mature B cells expressing surface immunoglobulins, with a subset of patients having reduced counts of switched memory B cells. Antibody responses to immunizations are subnormal or absent. Because CVID usually becomes evident in adulthood, it is important to rule out conditions that might result in hypogammaglobulinemia, such as protein-losing enteropathies and B-cell lymphomas.

Treatment and Prognosis

Treatment of patients with CVID is similar to that of patients with XLA. Immunoglobulin replacement therapy is indicated and has been shown to decrease the frequency of respiratory tract infections.[47] Antibiotic prophylaxis may be indicated as an adjunctive measure in patients who continue to have infections despite adequate serum IgG levels. Because of enteric protein loss, patients with CVID who have enteropathy may require unusually large immunoglobulin doses to maintain protective serum IgG concentrations. Chronic lung disease with bronchiectasis resulting from multiple occurrences of pneumonia is the most frequent cause of morbidity and mortality.[65] CVID patients are at higher risk for developing lymphoma, splenomegaly, and autoimmune complications.

IgA Deficiency

Clinical Features

IgA deficiency is estimated to occur with a frequency of 1 in 500 people and is considered the most common immunodeficiency, although the frequency might vary widely among different ethnicities.[26] Most individuals with IgA deficiency are clinically asymptomatic.[71,76] Some patients have frequent, noninvasive viral and bacterial infections of the respiratory tract. Chronic diarrhea occurs with increased frequency, and *Giardia* is a pathogen commonly isolated from stool samples. Infections in patients with IgA deficiency usually are less severe than those observed in patients with XLA or CVID. Individuals with more severe or chronic infections often have another associated immune deficiency, such as IgG subclass deficiency[74] or, rarely, ataxia-telangiectasia.

Patients with IgA deficiency have a higher incidence of atopy than does the general population. Autoimmune disorders, including systemic lupus erythematosus, rheumatoid arthritis, and pernicious anemia, and lymphoid and gastrointestinal malignancies are more prevalent than in the general population.

Pathogenesis

The pathogenesis of IgA deficiency has been paralleled with that of CVID. The fundamental defect in both disorders appears to be a failure of B cells to differentiate into immunoglobulin-secreting plasma cells. Rare alleles of complement genes within major histocompatibility complex III on chromosome 6 have been associated strongly with the development of selective IgA deficiency and CVID, suggesting that these two disorders may be related.[92] Some individuals with selective IgA deficiency subsequently develop CVID. Inactivating mutations in *TNFRSF13B* and *ICOS* genes have been found in families with members with either of these conditions.[19]

Diagnosis

Selective IgA deficiency is diagnosed by a quantitative serum immunoglobulin determination showing subnormal concentration or absence of circulating IgA in the presence of normal amounts of the other immunoglobulins. In interpreting test results, an important consideration is that serum IgA concentrations can be undetectable in normal infants younger than 6 to 9 months of age, possibly because of a maturational delay. This diagnosis is reliable only after the child reaches 4 years of age and the IgA deficiency is persistent.

Treatment and Prognosis

Immunoglobulin replacement is generally not recommended in the management of patients with selective IgA deficiency. Although rarely reported, IgA-deficient patients are at risk for developing IgE-mediated anaphylactic reactions if the patient is sensitized to IgA and receives blood products containing IgA.[64]

IgA-deficient patients respond adequately to vaccines unless they have concomitant IgG2 deficiency. Sinopulmonary infections in individuals with IgA deficiency often require unusually long courses of antibiotic therapy. Antibiotic prophylactic for respiratory tract infections may be recommended if infections are recurrent. Parenteral antibiotic therapy sometimes is necessary for refractory cases of sinusitis or pneumonia.

IgG Subclass Deficiency

Clinical Features

The most common IgG subclass deficiency reported is IgG2, sometimes occurring in association with deficiency of IgG4 or IgA. The second most common is IgG3 deficiency, which may occur in association with IgG1 deficiency.[1,74] Many individuals with IgG subclass deficiencies are asymptomatic; others have recurrent or chronic bacterial infections, usually of the respiratory tract. Commonly implicated pathogens include *S. pneumoniae*, *H. influenzae* type b, and other encapsulated bacteria. Children with selective IgG subclass deficiency do not usually have problems with intestinal malabsorption or autoimmunity.

Pathogenesis

The pathogenesis of IgG subclass deficiency is still not well defined. T cell immunity has been reported to be intact in patients with IgG subclass deficiencies.

Diagnosis

A diagnosis of IgG subclass deficiency is supported by finding a marked decrease from age-adjusted normal values in the serum concentrations of one or more IgG subclasses, in addition to evidence of a functional impairment in antibody responses to immunizations. The total serum IgG concentration may be normal, decreased, or even elevated as a result of a compensatory increase in production of unaffected IgG subclasses.

Children with low IgG2 subclass levels may respond poorly to polysaccharide vaccines.[1,86] Responses to protein antigens (e.g., tetanus toxoid) and protein-conjugated polysaccharide vaccines (e.g., *H. influenzae* type b conjugate vaccines) may also be abnormal. Assessment of the patient's antibody responses to vaccination with polysaccharide and protein antigens can help to establish the functional significance of an IgG subclass deficiency. Because of the age-dependent development of antipolysaccharide antibody responses, such testing is not reliable in children younger than 2 years of age.

Treatment and Prognosis

Antibiotic prophylaxis of recurrent sinopulmonary infections and early and prompt treatment of infections form the cornerstone of management for symptomatic IgG subclass deficiency. Immunoglobulin replacement therapy is reserved for patients with abnormal antibody responses and a demonstrated propensity for frequent or chronic infections.[57] Because the clinical benefit of this measure in the long term has not been demonstrated, the

effectiveness and continued need for immunoglobulins should be reassessed periodically.

Transient Hypogammaglobulinemia of Infancy

Clinical Features

Infants with transient hypogammaglobulinemia come to medical attention because of recurrent respiratory tract infections (e.g., otitis media, sinusitis) and serum immunoglobulin levels that are lower than laboratory normal ranges. Septicemia, meningitis, and other serious systemic infections are rare.[59]

Pathogenesis

Transient hypogammaglobulinemia of infancy is a developmental disorder with a delay in the physiologic maturation of immunoglobulin synthesis, resulting in prolongation of the relative hypogammaglobulinemia observed in most normal infants at 4 or 5 months of age, when most maternal immunoglobulins have cleared from the child circulation. Patients with transient hypogammaglobulinemia of infancy do not appear to have any inherent defects of B-cell maturation or function or defects of specific antibody responses.

Diagnosis

The diagnosis can be made with certainty only in retrospect. Serum immunoglobulin concentrations may remain low in children until they are up to several years of age in some cases.[59] Circulating mature B-cell numbers are normal and generate normal antibody responses to diphtheria, tetanus, and pertussis vaccines.

Treatment and Prognosis

Infants with suspected transient hypogammaglobulinemia should be followed clinically, and serial serum immunoglobulin concentration measurements should be performed. Some of these children eventually are diagnosed as having CVID, selective IgA deficiency, or another secondary immunodeficiency disorder. Because it is self-limited and specific antibody production usually is normal, immunoglobulin replacement therapy is not indicated. Antibiotic prophylaxis for respiratory tract infections is indicated in selected cases with recurrent episodes of infection.

SELECTED PRIMARY CELLULAR AND COMBINED IMMUNE DEFICIENCIES

DiGeorge Syndrome

Clinical Features

DiGeorge syndrome is a relatively common genetic disorder that includes facial, cardiac, parathyroid, and thymic abnormalities.[80] It may present early in infancy as findings unrelated to immunodeficiency. Congenital heart disease, particularly truncus arteriosus and interrupted aortic arch, is usually detected in the first few weeks of life. Neonatal hypoparathyroidism occurs in almost all infants with DiGeorge syndrome, and hypocalcemic seizures or tetany is a common presenting feature in about 30 percent of cases. Characteristic facial features include microstomia and micrognathia; hypertelorism; upturned nose; arched palate; posteriorly rotated and small, low-set ears with notched pinnae; and antimongoloid slant of the eyes (see Fig. 67-1). Hypothyroidism, esophageal atresia, tracheoesophageal fistula, and bifid uvula are other abnormalities that have been described.

According to the degree of immunodeficiency, clinical manifestations may include predisposition to a wide variety of common and opportunistic infectious diseases. Common findings are recurrent or chronic pneumonia, chronic diarrhea, and candidiasis of the skin and mucosae. Recurrent or severe herpesvirus infections (e.g., herpes simplex virus, cytomegalovirus), *P. jiroveci* pneumonia, and other opportunistic viral, fungal, protozoal, and mycobacterial infections occasionally are observed when immunodeficiency is severe. Fatal graft-versus-host disease may occur if infants receive blood products containing viable lymphocytes, often during surgical correction of their heart defects. Although DiGeorge syndrome is the most common of the cellular immunodeficiencies, with an incidence of 1 in 3000 live births, only a minority of patients (<1%) with DiGeorge syndrome have severe T-cell deficiency (complete DiGeorge syndrome).[80] Most patients develop adequate T-cell numbers and function by 1 year of age and do not have an increased incidence of opportunistic infections (partial DiGeorge syndrome). At Texas Children's Hospital, 20 percent of patients with DiGeorge syndrome and mild T-cell deficiency have an increased incidence of respiratory tract infections.[20]

Pathogenesis

The syndrome is classified as a developmental defect resulting from faulty embryologic development of the third and fourth branchial arches and their derivatives, including parathyroid glands, aortic arch structures, and the thymus gland.[80] Microdeletions of chromosome 22q11 can be demonstrated in more than 90 percent of patients. A few patients have been reported with a deletion in chromosome 10p. Three groups of investigators simultaneously reported that they reproduced all the characteristic features of DiGeorge syndrome, working with mice models bearing the deletion of *Tbx1*, a gene located within the chromosome 22 critical region.[96]

Diagnosis

Hypocalcemia, congenital heart disease, and characteristic facies may lead to suspicion of DiGeorge syndrome in the neonatal period. The microdeletion in chromosome 22q is detected by FISH analysis. Lymphopenia is variable, as well as T-cell deficiency. Determination of naïve T-cell number and a proliferative response to

mitogens allows a better assessment of the degree of immunodeficiency. Quantitative serum immunoglobulin determinations often are normal, but antibody responses after immunization might be decreased. Imaging studies may show absence of the thymic shadow. It should be taken into consideration that a significant group of patients with DiGeorge syndrome are totally or partially thymectomized while heart surgery is performed for repair of their characteristic conotruncal cardiac malformations.

Treatment and Prognosis

Initial management should focus on the treatment of hypocalcemia and the surgical correction of congenital heart disease. Immunoglobulin replacement therapy and antibiotic prophylaxis should be considered for patients with recurrent infections. Children with very low $CD4^+$ T-cell counts or evidence of significant cellular immune dysfunction should receive prophylaxis for *P. jiroveci* pneumonia. Infections should be recognized early and treated promptly. Only irradiated, cytomegalovirus-negative blood products and inactivated vaccines should be administered. Several small retrospective studies have suggested that children with partial DiGeorge syndrome and normal or only mildly decreased $CD4^+$ T-cell counts may receive live vaccines without serious adverse events.[9,53,60] However, the safety of live vaccination has not been well established and is currently not recommended. Approximately 8 percent of patients develop autoimmunity, frequently cytopenias and hypothyroidism. Therapeutic options for DiGeorge syndrome patients with severe immunodeficiency are bone marrow transplantation and, experimentally, thymus transplantation.[48]

Wiskott-Aldrich Syndrome

Clinical Features

Wiskott-Aldrich syndrome is an X-linked disorder classically characterized by recurrent infection, bleeding, and eczema. However, only a minority of patients have this classic triad of features, and some patients only have infectious manifestations.[54,55] At presentation, patients have a hemorrhagic diathesis, followed by recurrent otitis media or pneumonia caused by encapsulated bacteria (e.g., *S. pneumoniae*, *H. influenzae*). Septicemia, meningitis, and other serious systemic bacterial infections also may occur. Common opportunistic pathogens include *Candida*, cytomegalovirus, other herpesviruses, and *P. jiroveci*.

Individuals with Wiskott-Aldrich syndrome often have low platelet counts. A unique feature is the presence of small platelets (low mean platelet volume). As a consequence, thrombocytopenia leads to bruises and gastrointestinal bleeding in the first months of life. Life-threatening gastrointestinal or intracranial hemorrhage also may occur. Eczematous lesions are generalized and prone to bacterial superinfection.

Patients with Wiskott-Aldrich syndrome have a high incidence of autoimmune disorders.[54] Hemolytic anemia, a juvenile rheumatoid arthritis-like condition, and large- or small-vessel vasculitis have been reported. Some patients develop autoimmune thrombocytopenia as their disease progresses. Lymphoid malignancies, especially non-Hodgkin lymphomas involving the brain, have been reported.

Pathogenesis

The *WAS* gene is mutated in individuals with Wiskott-Aldrich syndrome[24] and maps to the X chromosome. This gene encodes a protein that is expressed in lymphocytes and platelets and is involved in cytoskeletal organization and formation of pseudopodia.[83] The immunologic synapse is not well formed as a result of a defective WAS protein, resulting in poor cognate interaction between T cell and B cell. X-linked thrombocytopenia without or with only mild immunodeficiency and eczema has been found to be caused by *WAS* mutations that allow partial protein expression.[91]

Diagnosis

Wiskott-Aldrich syndrome should be suspected in an infant with thrombocytopenia and small volume platelets, which is best assessed by examining a peripheral blood smear, because automatic hematology cell counters report average values. The presence of eczema supports the diagnosis. Serum immunoglobulin concentrations are variable, with the most typical pattern showing normal IgG, increased IgA and IgE, and decreased IgM. Antibody responses to protein antigens (e.g., tetanus toxoid) usually are normal, but responses to polysaccharides (e.g., *S. pneumoniae*, *H. influenzae* type b, isohemagglutinins) are absent.

Patients with Wiskott-Aldrich syndrome are anergic on delayed hypersensitivity skin testing. They have near-normal numbers of circulating T cells, and in vitro proliferative responses to mitogens could be normal. However, responses to specific antigens are decreased. Monocytes exhibit abnormal chemotaxis and poor antibody-dependent cellular cytotoxicity.

The determination of WAS protein expression and the sequencing of the *WAS* gene is available in specialized research laboratories.

Treatment and Prognosis

Hematopoietic stem cell transplantation commonly results in normalization of cellular immunity, specific antibody responses, and platelet count. Splenectomy should be considered for severe thrombocytopenia when bone marrow transplantation is not available and may improve quality of life. Daily antibiotic prophylaxis directed against *S. pneumoniae* and *H. influenzae* should be indicated for patients who have undergone splenectomy. Acute infections should be treated aggressively. Immunoglobulin replacement therapy is indicated for patients with recurrent bacterial infections.

The prognosis of Wiskott-Aldrich syndrome has improved in recent years, with some patients surviving to adulthood. Infection, malignancy, and hemorrhage are the leading causes of death.[54]

Ataxia-Telangiectasia

Clinical Features

Ataxia-telangiectasia is characterized by cerebellar ataxia, oculocutaneous telangiectases, variable immunodeficiency with frequent infections, and a high incidence of malignancy.[51] Neurologic signs and symptoms dominate the clinical picture. Ataxia usually becomes evident when the child is approximately 1 year of age. Progressive choreoathetosis, myoclonic jerking movements, and oculomotor abnormalities subsequently develop, resulting in severe disability. Telangiectases appear on the bulbar conjunctivae, usually in patients between 2 and 5 years of age (see Fig. 67-2). They subsequently appear on the nasal bridge, ears, and other areas of sun exposure or trauma. Other cutaneous manifestations include café-au-lait spots, vitiligo, and prematurely gray hair. Recurrent infections are a major feature of ataxia-telangiectasia in most patients. Sinopulmonary infections predominate; however, systemic infections are rare in spite of the immunologic abnormalities. Organisms commonly implicated include *S. pneumoniae* and *H. influenzae*. Over 15 percent of patients with ataxia-telangiectasia develop neoplasias, from which non-Hodgkin lymphomas and leiomyomas occur most frequently. Carcinomas (especially of the stomach) occur commonly among adults with ataxia-telangiectasia.[51]

Pathogenesis

Ataxia-telangiectasia is inherited in an autosomal recessive manner. The defective gene *(ATM)* responsible for the disorder maps to chromosome 11.[69] One domain of the ATM protein appears to be important in numerous cellular responses, including cytokine signaling. Another region of the protein is involved in DNA repair. Disease manifestations result from a major defect in one or more DNA repair mechanisms. Impairment of normal mechanisms of DNA rearrangements required for expression of the T-cell receptor genes and immunoglobulin heavy-chain genes on chromosomes 7 and 14 explains the observed immunodeficiency.

Diagnosis

A clinical diagnosis of ataxia-telangiectasia is possible when the disease is fully manifested. Increased serum α-fetoprotein concentrations are observed in essentially all patients older than 6 months of age. Most patients have deficiencies in serum IgA and IgE. Specific antibody responses usually decline as the patient ages. Most patients have serum IgM in a monomeric 7S form rather than the usual pentameric 19S molecule. Common manifestations of immunodeficiency include delayed hypersensitivity skin test anergy and decreased lymphocyte proliferative responses to mitogens and antigens. The sequencing of the *ATM* gene is available in specialized laboratories.

Treatment and Prognosis

No specific treatment is available for ataxia-telangiectasia because of the pathologic processes involving multiple body systems. Infections should be treated promptly with oral or parenteral antibiotics. Continuous prophylactic antibiotic therapy may be of benefit to individual patients. Immunoglobulin replacement therapy is reserved for patients with recurrent infections and abnormal specific antibody responses.

The clinical course and prognosis of ataxia-telangiectasia are variable. Death from chronic pulmonary disease or malignancy occurs commonly during the second or third decade of life.

Severe Combined Immunodeficiency

Clinical Features

Infants with SCID are usually diagnosed during their first few months of life because of recurrent or severe infections.[79] In the neonatal period they may have a rash produced by a graft-versus-host reaction induced by maternal lymphocytes. Recurrent pneumonia and persistent or severe viral infections caused by respiratory syncytial virus, parainfluenza virus, or rhinovirus are clues that an infant may have SCID. Infants with SCID may also have severe, disseminated infection with cytomegalovirus, especially if accompanied by very high plasma viral load and hemolytic anemia. In addition, unusual reactions to live virus vaccines, including disseminated varicella after Varivax or measles-mumps-rubella-varicella (MMRV) vaccine administration, and persistent infection with vaccine strains of rubella and measles virus may occur in some patients with SCID. Disseminated infection with BCG after BCG vaccine also may occur in infants with SCID. In addition, persistent oral and cutaneous candidiasis are common occurrences, as are chronic diarrhea and failure to thrive. Septicemia and other serious systemic bacterial infections also occur. Causative organisms include routine pathogens (e.g., *S. pneumoniae*, *H. influenzae* type b) and more unusual organisms. Life-threatening opportunistic infections, including *P. jiroveci* pneumonia, frequently occur early in infancy.

Pathogenesis

SCID represents a heterogeneous group of genetic disorders having in common a profound immunodeficiency with failure of both cellular and humoral immune function. Various types of SCID have been defined on the basis of enzymatic, genetic, and immunologic criteria (Table 67-7), including reticular dysgenesis, with impaired lymphoid, myeloid, and erythroid differentiation; absence of T-cell and B-cell differentiation; selective defects of T-cell differentiation; and purine metabolism defects (e.g., adenosine deaminase deficiency). Autosomal and X-linked recessive inheritance patterns have been recognized. Individuals with X-linked SCID have a defect in the γ chain of the IL-2 receptor, and it is the genetic defect identified in about half of SCID patients.[88] This protein is a functional component of the receptors for IL-4, IL-7, IL-9, IL-15, and IL-21 and is essential for development of T cells and natural killer cells.[45]

TABLE 67-7 **Types of Severe Combined Immunodeficiency (SCID)**

Type	Defect
Reticular dysgenesis	Defective stem cell maturation
RAG1, RAG2 deficiency, Omenn syndrome	Rearrangement of B- and T-cell receptor genes
Artemis deficiency	Rearrangement of B- and T-cell receptor genes
X-linked SCID (common γ-chain)	Signaling for IL-2, IL-4, IL-7, IL-15, and IL-21
JAK3 deficiency	Cytokine signaling
IL-7 receptor deficiency	Signaling for IL-7
CD3D or *CD3E* deficiency	*CD3D* or *CD3E* signaling
PTPRC (*or CD45*) deficiency	Signaling for *PTPRC,* cell activation
Coronin 1A	Defective thymic egress of T cells
Adenosine deaminase deficiency	Metabolite (dATP) toxicity to lymphocytes
MHC class I deficiency	Defect in transporter proteins TAP-1, TAP-2
MHC class II deficiency	Defects in transcription factors RFXAP, RFX5, RFXANK, and transactivator CIITA

MHC, major histocompatibility complex.

Diagnosis

Infants with SCID typically lack palpable lymph nodes, visible tonsils, and radiographic evidence of a thymus gland. Lymphopenia is often identified.[88] Some patients have panhypogammaglobulinemia, whereas others have depressed concentrations of only one or two immunoglobulin isotypes. Antibody responses almost always are profoundly impaired or absent. Lymphocyte monoclonal phenotyping may reveal the presence of circulating mature nonfunctional B cells. Particularly in boys with X-linked SCID and JAK3-deficient SCID, B cells may account for all circulating lymphocytes. Circulating T-cell counts are diminished (<10% of normal range) in most patients with SCID. Delayed hypersensitivity skin test anergy is found, and lymphocyte proliferative responses to mitogens and antigens are severely depressed. Testing for a specific genetic defect should be pursued, and a genetic evaluation of immediate relatives for carrier status is recommended for genetic counseling purposes. Biochemical tests for adenosine deaminase and purine nucleoside phosphorylase levels and enzymatic activities are available in specialized laboratories.

Treatment and Prognosis

Hematopoietic stem cell transplantation (HSCT) is the treatment of choice for most patients with SCID.[18] Prognosis is poor for patients without definitive therapy. HSCT performed with HLA-matched related donors have a survival rate of over 95 percent. However, most patients do not have such a donor available and receive an HLA-haploidentical transplant. Survival rates with these donors are variable but have increased significantly with advances in antimicrobial therapy and intensive care. This survival rate can increase to over 95 percent if the SCID is detected early and HSCT is performed before 3.5 months of age, before infections and organ damage occur. HLA-matched unrelated donors and HLA-matched cord blood have been increasingly used for different forms of SCID, accompanied with different chemotherapy regimens for myeloablation. As an alternative due to the high mortality and morbidity associated with HSCT, European investigators have reported success in experimental gene therapy trials for X-linked SCID. Using autologous $CD34^+$ stem cells carrying the correct version of the gene, they were able to restore humoral and cellular immunity in 12 of 14 affected patients.[34] Unfortunately, 4 patients developed leukemia that was in part caused by the gene therapy vector. Three of the 4 patients survived after receiving conventional antileukemia therapy. New developments to reduce the risk for leukemia in gene therapy are underway.[28] Enzyme replacement using bovine-derived pegylated adenosine deaminase and gene therapy[18,29] have been used for treatment of SCID resulting from adenosine deaminase deficiency. Pegylated adenosine deaminase therapy has demonstrated efficacy to increase lymphocyte counts and provide adequate immunity in most affected patients. Some of them ultimately develop "resistance" to the drug in the form of eliciting neutralizing anti–adenosine deaminase antibodies or simply demonstrating a decrease of lymphocyte counts. Positive results of experimental gene therapy trials for SCID caused by adenosine deaminase deficiency with the restoration of both cellular and humoral immunity have been reported in more than 30 patients using a protocol that included withdrawal of exogenous adenosine deaminase and reduced myeloablation.

Miscellaneous Cellular Immune Deficiencies

Many other disorders of cellular immunity have been described. Particularly noteworthy are disorders characterized for dysregulation of the immune system with autoimmune inflammation. The X-linked lymphoproliferative (XLP) syndrome represents a defect in control of Epstein-Barr virus infection. Mutations in the SLAM-associated protein (SAP), a factor involved in the signal transduction of T cells, have been identified to cause XLP syndrome. The absence of this protein affects the interaction of T and B cells, leading to an inability to control B-cell proliferation caused by Epstein-Barr virus infection.[30,66,70] At presentation, patients may have severe and often fatal infectious mononucleosis or B-cell lymphoma.

Chronic mucocutaneous candidiasis is a condition characterized by chronic, severe infection with *Candida* spp. It affects the mucous membranes, skin, and nails

and often is associated with autoimmune polyendocrinopathy, in which case it is named autoimmune polyendocrinopathy ectodermal dystrophy (APECED).[38] T-cell numbers and function are usually normal, but most patients do not manifest delayed hypersensitivity skin test responses to *Candida*, and their lymphocytes fail to proliferate in response to *Candida* antigen in vitro. In cases with APECED, deleterious mutations have been found in the autoimmune regulator *(AIRE)* gene. This gene promotes the expression of many self-antigens in the thymus, facilitating the development of immune tolerance during lymphocyte differentiation. Another autoimmune endocrinopathy with immunodeficiency is IPEX (immunodeficiency, polyendocrinopathy, enteropathy, X-linked). IPEX results from mutations in the *FOXP3* gene and is characterized by lack of T regulatory cells and clinically by eczema, chronic diarrhea, diabetes mellitus, and other hormone deficiencies due to autoimmunity and decreased immunologic tolerance.[42]

Mutations in the IFN-γ receptor genes have been demonstrated in patients with severe infections caused by atypical mycobacteria.[37,67] Patients with a mutation in *IL12RB2* or in *IL12B* have also shown increased susceptibility to mycobacterial disease and disseminated nontuberculous mycobacterial infection.[5]

NEMO deficiency is a primary immunodeficiency caused by specific missense mutations in the *IKBKG* (also known as nuclear factor kappa B [NFκB]-enhancing modulator or NEMO) gene, located on chromosome Xq28.[78,98] The gene participates in signal transduction mediated by NFκB. Most mutations in *IKBKG* are lethal for male fetuses, and female carriers of these mutations may have incontinentia pigmenti. The surviving male patients with specific missense mutations have anhidrotic ectodermal dysplasia, conical teeth (Fig. 67-5), low IgG, low IgA, normal or increased IgM levels, and decreased T-cell proliferative responses to mitogen and antigen stimuli.[61]

FIGURE 67-5 ■ Conical teeth characteristic of incontinentia pigmentosa with immunodeficiency due to defects in the *IKBKB* gene (NEMO defect). (See companion Expert Consult website for color version.)

PRIMARY COMPLEMENT DEFICIENCIES

Clinical Features

Three types of complement function deficiency are associated with increased susceptibility to infections: deficiency of opsonic activity of complement proteins, deficiency of the complement lytic proteins, and deficiency of the mannose-binding lectin pathway.[13,75] Patients with deficiencies of early-acting complement components (e.g., C3, C1, C4, C2) are particularly susceptible to infection with encapsulated bacteria, including *S. pneumoniae* and *H. influenzae;* in addition, they have an increased risk for developing autoimmune disorders such as systemic lupus erythematosus.[13] *Neisseria meningitidis* is the most important bacterial pathogen observed in patients with deficiencies of terminal complement components (C5 through C9).[7] Recurrent episodes of septicemia and meningitis are especially common. Although patients with deficiencies of late-acting complement components are at increased risk for developing meningococcal septicemia and meningitis, they appear to suffer lower rates of morbidity and mortality than immunologically normal individuals with systemic meningococcal infection. The frequency of primary complement deficiencies among patients with invasive meningococcal disease is 5 to 10 percent[7,50]; however, the likelihood of having complement deficiency increases dramatically (31%) among individuals who have had more than one episode of invasive disease. A deficiency of opsonization in children with recurrent pyogenic infections and failure to thrive led to the recognition of the mannose-binding lectin pathway deficiencies. The clinical characteristic of these patients is the increased frequency of pyogenic infections; however, the essential role of the mannose-binding lectin pathway is controversial owing to the high proportion of healthy individuals with mannose-binding lectin deficiency.[35]

Pathogenesis

Various gene defects are responsible for the many distinct primary complement deficiencies. The particular bacteria that causes infection may indicate the missing component in host defense. For example, C3b, the major cleavage product of C3, is an important opsonic ligand that promotes ingestion and killing of bacteria. As a consequence, patients with C3 deficiency have increased susceptibility to bacterial infections (e.g., *S. pneumoniae*, *H. influenzae*), for which opsonization is the primary mechanism of host defense. Individuals with deficiencies of C1, C4, or C2 also have increased susceptibility to these encapsulated bacteria because these components are necessary for activation of C3 through the classical pathway. Activation of terminal complement components C5, C6, C7, C8, and C9 results in assembly of the membrane attack complex C5b-9, a multicomponent macromolecule capable of

bactericidal activity. Only gram-negative bacteria are susceptible to this bactericidal effect. Infections in patients with deficiencies of terminal complement components are therefore limited to gram-negative bacteria, such as *N. meningitidis*.

Diagnosis

When the history of infections is suggestive, the screening test used for complement deficiencies is the CH50 assay, which depends on the activity of all numbered components of the classical complement pathway from C1 through C9. Specific immunochemical and functional testing can be performed for identifying the deficient component, when CH50 test results suggest a complement component deficiency.

Treatment and Prognosis

No specific therapy exists for primary complement deficiencies. Meningococcal vaccine is recommended for children with terminal complement component deficiencies. Antibiotic prophylaxis and vaccines for other encapsulated organisms may be beneficial.

PRIMARY PHAGOCYTE DEFICIENCIES

The primary phagocyte deficiencies are a heterogeneous group of disorders having in common a susceptibility for frequent infections, resulting from a decreased number of phagocytic cells (e.g., neutropenia) or because of impaired adhesion, chemotaxis, opsonization and phagocytosis, or intracellular killing (Table 67-8). Infections resulting from quantitative or qualitative phagocyte deficiencies tend to be prolonged and recurrent, with a response to antibiotic therapy that is slower than expected. Common pathogens include *S. aureus*, *P. aeruginosa*, enteric gram-negative bacteria, and certain fungi (e.g., *Candida* spp., *Aspergillus* spp.).

TABLE 67-8 **Primary Phagocyte Deficiencies**

Quantitative Defects
Infantile agranulocytosis
Familial granulocytopenia
Cyclic neutropenia
Qualitative Defects
Adhesion Defects
Leukocyte adhesion deficiency
Type I, β-integrin deficiency
Type II, fucose-transporter deficiency
Type III, *FERMT3* deficiency
Intracellular Killing Defects
Chronic granulomatous disease
X-linked
Autosomal recessive
Glucose-6-phosphate dehydrogenase deficiency
Myeloperoxidase deficiency
Chédiak-Higashi syndrome
Griscelli syndrome

Quantitative Phagocyte Abnormalities

Primary quantitative phagocyte deficiencies can be observed as solitary defects or in association with other disorders (e.g., Shwachman syndrome). Infantile agranulocytosis (i.e., Kostmann syndrome) is an autosomal recessive disorder characterized by arrest of granulocyte maturation, markedly decreased numbers of circulating granulocytes, and severe infection. The more benign familial granulocytopenia syndrome manifests at various ages from infancy to adulthood, usually with indolent infections of the skin and soft tissues.

Cyclic neutropenia is an autosomal dominant defect of myelopoiesis due to mutation in the leukocyte elastase *(ELANE)* gene in which periodic disappearance of granulocytes from the circulation occurs.[15] Early granulocyte precursors are present in the bone marrow during periods of granulocytopenia, suggesting transient maturation arrest. Periods of granulocytopenia usually last 5 to 7 days and occur at 14- to 35-day intervals. The length of the cycle generally is constant for any given individual. Fever, malaise, aphthous stomatitis, and skin and soft tissue infections often are observed during periods of granulocytopenia.

Some of the primary quantitative phagocyte deficiencies, particularly familial granulocytopenia and cyclic neutropenia, respond to therapy with recombinant granulocyte colony-stimulating factor.[27]

Chronic Granulomatous Disease

Clinical Features

Patients with CGD have infections that begin during infancy and recur throughout their lives. Occasionally, patients have a relatively mild disease course and come to medical attention only during adolescence or adulthood. A history of suppurative lymphadenitis, soft tissue abscesses, pneumonia, lung abscess, hepatic abscess, perirectal abscesses, and osteomyelitis suggests CGD.[72] Infections in patients with CGD may progress with few symptoms, and the erythrocyte sedimentation rate and C-reactive protein value may be useful to detect active infection.

Patients with CGD generally have infections caused by catalase-positive bacteria and fungi. *S. aureus* accounts for almost one third of all infections of determined cause. *Nocardia*, *S. marcescens*, *Pseudomonas* spp., *Burkholderia cepacia*, and certain enteric gram-negative bacilli also are common findings. Fungi, especially *Aspergillus* spp., account for nearly one fifth of all defined infections in patients with CGD (Fig. 67-6).[11]

Patients with CGD often have poor wound healing. Granulomatous obstructive lesions of the urinary and gastrointestinal tracts have been reported. Granulomatous bowel involvement resembles Crohn disease. Corticosteroid therapy may be effective in relieving these sometimes life-threatening lesions.[44] A small percentage of boys with X-linked CGD have the McLeod blood

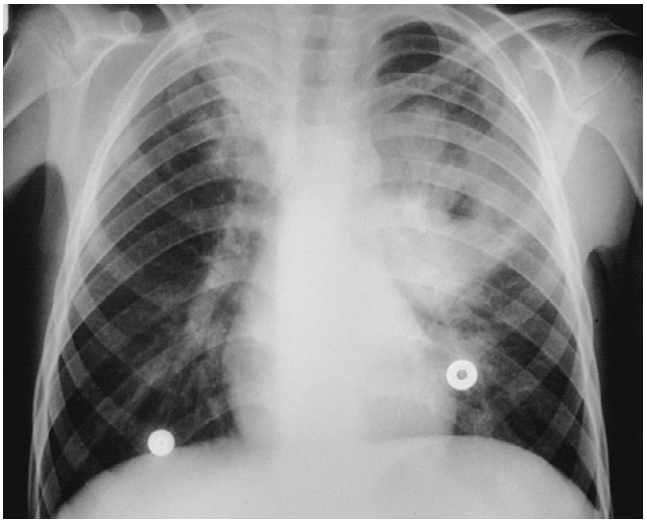

FIGURE 67-6 ■ Chest radiograph of a 10-year-old boy with chronic granulomatous disease shows a left-sided pulmonary infiltrate and cavitary lung lesion. Biopsy revealed an *Aspergillus fumigatus* infection.

phenotype, characterized by absence of the Kell antigen in red blood cells as a consequence of a contiguous gene deletion, which results in difficult crossmatching for blood transfusion and the potential for hemolytic transfusion reactions.[16]

Pathogenesis

X-linked and autosomal recessive forms of CGD have been described. The various gene defects result in abnormalities of membrane or cytosolic components of the nicotinamide adenine dinucleotide phosphate oxidase complex system. Despite the genetic heterogeneity, all individuals with CGD have in common the failure of the cellular respiratory burst, which ordinarily accompanies phagocytosis and various soluble stimuli. As a consequence, oxygen-derived microbicidal factors are not formed, resulting in impairment of intracellular killing of phagocytized microorganisms.

Diagnosis

The diagnosis of CGD has historically been made with the NBT dye test.[51] Neutrophils from normal individuals show 100 percent reduction of NBT, asymptomatic carriers show 20 to 90 percent reduction, and patients with CGD show essentially no dye reduction. In patients with mild forms of the disease, the NBT test result may be normal. A more sensitive method to measure hydrogen peroxide production is based on chemiluminescence of DHR on activation, detected by flow cytometry and increasingly used as a confirmatory test of the disease, replacing the NBT dye test.

Treatment and Prognosis

Specific microbiologic diagnosis of infections should be established whenever possible. Empirical therapy is directed against the organisms known to be common causes of infection: *S. aureus*, *Pseudomonas* spp., and enteric gram-negative bacilli. Surgical drainage or débridement of sites of infection often is required. Anecdotal reports suggest beneficial effects of granulocyte transfusions in patients with CGD and serious bacterial or fungal infections. HSCT has been used successfully,[17,49] and gene therapy trials for X-linked CGD in humans have resulted in gene-corrected, autologous granulocytes detectable for more than 30 months after treatment, with resolution of chronic infections.[58] However, the oxidase activity of the gene-corrected cells had progressively decreased, by mechanisms of "gene silencing."

Antibiotic prophylaxis with cotrimoxazole reduces the frequency of bacterial infections in patients with CGD.[72] Itraconazole and other antifungal agents given as prophylaxis may be effective for the prevention of *Aspergillus* infections, which are usually severe and difficult to eradicate. The administration of IFN-γ, given by subcutaneous injection three times weekly, reduces the incidence of serious infections in CGD by as much as two thirds without causing major side effects. Patients should be educated about not performing activities that expose themselves to environments containing mold spores, such as mulching or raking leaves. The prognosis of CGD has improved remarkably during the past several decades and now most patients survive to adulthood.

Leukocyte Adhesion Deficiency

Clinical Features

Patients with LAD suffer severe and recurrent bacterial infections of the skin and soft tissues, mucosal surfaces, and gastrointestinal tract, often beginning during early infancy. Common pathogenic microorganisms include staphylococci and *Pseudomonas* spp. Infants with the severe phenotype may have delayed separation of the umbilical cord secondary to omphalitis. Cutaneous infections show as inflamed tissues but without pus, owing to a defect in leukocyte extravasation, and may become necrotic, resembling ecthyma gangrenosum or pyoderma gangrenosum. Poor wound healing also is observed. Individuals surviving infancy typically develop severe gingivitis and periodontitis with progressive alveolar bone loss (see Fig. 67-3).

Marked granulocytosis is a hallmark of LAD. Circulating granulocyte counts may range between 15,000/mm^3 and 75,000/mm^3, even in the absence of infection; and counts of 100,000/mm^3 or greater are common findings during episodes of infection.

Pathogenesis

LAD type I is an autosomal recessive disorder that maps to chromosome 21. Neutrophils from individuals with LAD are defective in their expression of several surface glycoproteins known as the leukocyte-integrin complex. These molecules are critical for adhesion-dependent functions, and their absence is responsible for defects in

leukocyte adherence, chemotaxis, and phagocytosis. The severity of infectious complications of LAD is related directly to the degree of CD18 expression.[84] Patients with the severe clinical phenotype have undetectable expression of these complexes on their phagocytes, whereas individuals with the moderate phenotype generally have 2 to 8 percent expression, explained by missense gene mutations allowing surface expression of a defective protein.

A second form of LAD was described in patients with craniofacial dysmorphism, neurologic deficits, recurrent respiratory tract infections, and marked granulocytosis.[25] Unlike those of patients with LAD type I, neutrophils from patients with LAD type II had normal surface expression of CD18. The molecular basis for the phagocyte dysfunction is a defect in the fucose transporter influencing the glycosylation of sialyl-Lewis X, a carbohydrate ligand for the endothelial adhesion molecules E-selectin and P-selectin. A third form of LAD has been described, characterized for a deficiency of all β integrins, owing to a deficiency of FERMT3, a signal transducer necessary for integrin stabilization.

Diagnosis

The diagnosis of LAD type I is made by flow cytometry using fluorescence-labeled monoclonal anti-CD11/CD18 antibody.[84] In vitro studies reveal abnormalities of phagocyte adherence, chemotaxis, and phagocytosis. Individuals with LAD type II have similar phagocyte function abnormalities, with normal expression of CD18 and downregulation of selectins. LAD type III is characterized by altered expression of all β integrins.

Treatment and Prognosis

Intercurrent bacterial infections in patients with LAD must be treated aggressively with prolonged courses of parenteral antibiotics. Antibiotic prophylaxis and universal hygiene measures are recommended. Although survival to adulthood is described, almost one half of affected individuals die before reaching the second decade of life. Administration of fucose normalizes leukocyte function in LAD type II. Bone marrow transplantation offers the best hope for long-term survival.[82]

Other Primary Phagocyte Deficiencies

A variety of other defects of phagocyte chemotaxis, phagocytosis, and intracellular killing have been described.[87] The Chédiak-Higashi syndrome is a rare autosomal recessive disorder characterized by partial oculocutaneous albinism, rotatory nystagmus, and peripheral neuropathy. Affected individuals develop recurrent serious or life-threatening infections caused by a wide variety of bacteria. In the accelerated phase of the illness, hepatosplenomegaly, lymphadenopathy, lymphocytic infiltration of multiple organs, and unexplained febrile illnesses are common occurrences. Giant granules are found in numerous different cell types, including leukocytes, in the body. Neutrophils exhibit defective chemotaxis and abnormal bactericidal activity.

Hyperimmunoglobulinemia E (hyper-IgE syndrome, Job syndrome, Buckley syndrome) is characterized by markedly elevated serum IgE levels, eczema, recurrent staphylococcal abscesses, sinusitis, and otitis media. Coarse facial features are common findings. In addition to staphylococcal abscesses, recurrent pneumonia with bronchiectasis and pneumatoceles and mucocutaneous candidiasis are common occurrences. Other observed features include delay of shedding of primary dentition, recurrent bone fractures, scoliosis, and joint hyperextensibility. Hyper-IgE syndrome presents most often as autosomal dominant inheritance and can be diagnosed when the patient or family members have the skeletal manifestations. Mutations in the *STAT3* gene are responsible for this syndrome.[31]

CONCLUSION

Primary immunodeficiencies, once considered rare diseases, are increasingly being diagnosed to explain the high frequency of infections in children. Deleterious mutations in more than 165 genes have been identified to be responsible for primary immunodeficiencies, and several advances have clarified the immunopathogenesis of these conditions. The severity of the immunologic defects ranges from an increase of sinorespiratory tract infections seen in IgG subclass deficiencies to increased susceptibility to opportunistic infections during the first weeks of life, such as in SCID.

Pediatricians should be alert that these diseases should be suspected and investigated in children with infections of unusual severity, frequency, or etiologic organisms, as well as unexplained general symptoms, such as fever or failure to thrive. The initial immunologic screening consists of simple laboratory tests, and prompt referral to the clinical immunologist is warranted when an immunodeficiency is suspected. The management for these disorders has advanced over recent years to remarkably improved care, reflected in the majority of immunodeficient patients surviving to adulthood. Universal neonatal screening for T-cell deficiencies is a public health directive to identify severe defects before damaging infections occur and has proven to help patients avoid potential complications. Curative treatment is available for severe immunodeficiencies in the form of allogeneic HSCT; however, it is not without considerable mortality risk, and cure is not always optimal. Although still experimental, gene therapy has reached the stage of clinical trials for X-linked SCID, adenosine deaminase–deficient SCID, Wiskott-Aldrich syndrome, and CGD, with successful and promising results.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abrahamian F, Agrawal S, Gupta S. Immunological and clinical profile of adult patients with selective immunoglobulin subclass deficiency: response to intravenous immunoglobulin therapy. *Clin Exp Immunol* 2010;**159**:344–50.
2. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Front Immunol* 2011;**2**:54–66.

3. Al Kindi M, Mundy J, Sullivan T, et al. Utility of peripheral blood B cell subsets analysis in common variable immunodeficiency. *Clin Exp Immunol* 2012;**167**:275–81.
6. American Academy of Pediatrics. Active and passive immunization: immunocompromised children. In: Pickering LK, editor. *2009 Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 72–86.
7. American Academy of Pediatrics. Meningococcal infections. In: Pickering LK, editor. *2009 Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 452–60.
11. Blumental S, Mouy R, Mahlaoui N, et al. Invasive mold infections in chronic granulomatous disease: a 25-year retrospective survey. *Clin Infect Dis* 2011;**53**:e159–69.
12. Borte S, Wang N, Oskarsdóttir S, et al. Newborn screening for primary immunodeficiencies: beyond SCID and XLA. *Ann N Y Acad Sci* 2011;**1246**:118–30.
13. Botto M, Kirschfink M, Macor P, et al. Ross complement in human diseases: lessons from complement deficiencies. *Mol Immunol* 2009;**46**:2774–83.
15. Boztug K, Klein C. Genetic etiologies of severe congenital neutropenia. *Curr Opin Pediatr* 2011;**23**:21–6.
17. Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: long-term outcomes. *Immunol Res* 2011;**49**:25–43.
18. Cappelli B, Aiuti A. Gene therapy for adenosine deaminase deficiency. *Immunol Allergy Clin North Am* 2010;**30**:249–60.
23. Davies EG, Thrasher AJ. Update on the hyper immunoglobulin M syndromes. *Br J Haematol* 2010;**149**:167–80.
26. Feng ML, Zhao YL, Shen T, et al. Prevalence of immunoglobulin A deficiency in Chinese blood donors and evaluation of anaphylactic transfusion reaction risk. *Transfus Med* 2011;**21**:338–43.
27. Fioredda F, Calvillo M, Lanciotti M, et al. Pegfilgrastim in children with severe congenital neutropenia. *Pediatr Blood Cancer* 2010;**54**:465–7.
28. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. Strategies for retrovirus-based correction of severe, combined immunodeficiency (SCID). *Methods Enzymol* 2012;**507**:15–27.
29. Gaspar HB, Aiuti A, Porta F, et al. How I treat ADA deficiency. *Blood* 2009;**114**:3524–32.
34. Hacein-Bey-Abina S, Hauer J, Lim A, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2010;**363**:355–64.
35. Heitzeneder S, Seidel M, Förster-Waldl E, Heitger A. Mannan-binding lectin deficiency—good news, bad news, doesn't matter? *Clin Immunol* 2012;**143**:22–38.
36. Jain A, Kovacs JA, Nelson DL, et al. Partial immune reconstitution of X-linked hyper IgM syndrome with recombinant CD40 ligand. *Blood* 2011;**118**:3811–7.
38. Kisand K, Peterson P. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. *Ann N Y Acad Sci* 2011;**1246**:77–91.
39. Koch A, Homøe P, Pipper C, et al. Chronic suppurative otitis media in a birth cohort of children in Greenland: population-based study of incidence and risk factors. *Pediatr Infect Dis J* 2011;**30**:25–9.
40. Kozer E, Lotem Z, Elgarushe M, et al. RCT of montelukast as prophylaxis for upper respiratory tract infections in children. *Pediatrics* 2012;**129**:e285–90.
41. Kuijpers TW, Bende RJ, Baars PA, et al. CD20 deficiency in humans results in impaired T cell–independent antibody responses. *J Clin Invest* 2010;**120**:214–22.
44. Leiding JW, Freeman AF, Marciano BE, et al. Corticosteroid therapy for liver abscess in chronic granulomatous disease. *Clin Infect Dis* 2012;**54**:694–700.
47. Lucas M, Lee M, Lortan J, et al. Infection outcomes in patients with common variable immunodeficiency disorders: relationship to immunoglobulin therapy over 22 years. *J Allergy Clin Immunol* 2010;**125**:1354–60.
48. Markert ML, Devlin BH, Chinn IK, McCarthy EA. Thymus transplantation in complete DiGeorge anomaly. *Immunol Res* 2009;**44**:61–70.
49. Martinez CA, Shah S, Shearer WT, et al. Excellent survival after sibling or unrelated donor stem cell transplantation for chronic granulomatous disease. *J Allergy Clin Immunol* 2012;**129**:176–83.
51. Micol R, Ben Slama L, Suarez F, et al. Morbidity and mortality from ataxia-telangiectasia are associated with ATM genotype. *J Allergy Clin Immunol* 2011;**128**:382–9.
54. Ochs HD, Filipovich AH, Veys P, et al. Wiskott-Aldrich syndrome: diagnosis, clinical and laboratory manifestations, and treatment. *Biol Blood Marrow Transplant* 2009;**15**(1 Suppl):84–90.
56. Oliveira JB, Fleisher TA. Laboratory evaluation of primary immunodeficiencies. *J Allergy Clin Immunol* 2010;**125**(2 Suppl 2): S297–305.
59. Ozen A, Baris S, Karakoc-Aydiner E, et al. Outcome of hypogammaglobulinemia in children: immunoglobulin levels as predictors. *Clin Immunol* 2010;**137**:374–83.
61. Picard C, Casanova JL, Puel A. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. *Clin Microbiol Rev* 2011;**24**:490–7.
63. Puck JM. The case for newborn screening for severe combined immunodeficiency and related disorders. *Ann N Y Acad Sci* 2011;**1246**:108–17.
64. Rachid R, Bonilla FA. The role of anti-IgA antibodies in causing adverse reactions to gamma globulin infusion in immunodeficient patients: a comprehensive review of the literature. *J Allergy Clin Immunol* 2012;**129**:628–34.
65. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood* 2012;**119**:1650–7.
66. Rezaei N, Hedayat M, Aghamohammadi A, Nichols KE. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol* 2011;**127**:1329–41.
75. Shearer WT. Screening for severe combined immunodeficiency in newborns. *J Allergy Clin Immunol* 2012;**129**:619–21.
76. Shkalim V, Monselize Y, Segal N, et al. Selective IgA deficiency in children in Israel. *J Clin Immunol* 2010;**30**:761–5.
77. Skattum L, van Deuren M, van der Poll T, Truedsson L. Complement deficiency states and associated infections. *Mol Immunol* 2011;**48**:1643–55.
83. Thrasher AJ, Burns SO. WASP: a key immunological multitasker. *Nat Rev Immunol* 2010;**10**:182–92.
87. van de Vosse E, van Dissel JT, Ottenhoff TH. Genetic deficiencies of innate immune signalling in human infectious disease. *Lancet Infect Dis* 2009;**9**:688–98.
88. van der Burg M, Gennery AR. The expanding clinical and immunological spectrum of severe combined immunodeficiency. *Eur J Pediatr* 2011;**170**:561–71.
90. van Zelm MC, Smet J, Adams B, et al. *CD81* gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *J Clin Invest* 2010;**120**:1265–74.
93. Warnatz K, Salzer U, Rizzi M, et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc Natl Acad Sci U S A* 2009;**106**: 13945–50.
97. Yong PL, Orange JS, Sullivan KE. Pediatric common variable immunodeficiency: immunologic and phenotypic associations with switched memory B cells. *Pediatr Allergy Immunol* 2010;**21**:852–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abrahamian F, Agrawal S, Gupta S. Immunological and clinical profile of adult patients with selective immunoglobulin subclass deficiency: response to intravenous immunoglobulin therapy. *Clin Exp Immunol* 2010;**159**:344–50.
2. Al-Herz W, Bousfiha A, Casanova JL, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency. *Front Immunol* 2011;**2**:54–66.
3. Al Kindi M, Mundy J, Sullivan T, et al. Utility of peripheral blood B cell subsets analysis in common variable immunodeficiency. *Clin Exp Immunol* 2012;**167**:275–81.
4. Allen RC, Armitage RJ, Conley ME, et al. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science* 1993;**259**:990–93.
5. Altare F, Durandy A, Lammas D, et al. Impairment of mycobacterial immunity in human interleukin-12 receptor deficiency. *Science* 1998;**280**:1432–35.
6. American Academy of Pediatrics. Active and passive immunization: immunocompromised children. In: Pickering LK, editor. *2009 Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 72–86.
7. American Academy of Pediatrics. Meningococcal infections. In: Pickering LK, editor. *2009 Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 452–60.
8. Aruffo A, Farrington M, Hollenbaugh D, et al. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell* 1993;**72**:291–300.
9. Azzari C, Gambineri E, Resti M, et al. Safety and immunogenicity of measles-mumps-rubella vaccine in children with congenital immunodeficiency (DiGeorge syndrome). *Vaccine* 2005;**23**:1668–71.
10. Bartlett AV, Moore M, Gary GW, et al. Diarrheal illness among infants and toddlers in day care centers, part II: comparison with day care homes and households. *J Pediatr* 1985;**107**:503–9.
11. Blumental S, Mouy R, Mahlaoui N, et al. Invasive mold infections in chronic granulomatous disease: a 25-year retrospective survey. *Clin Infect Dis* 2011;**53**:e159–69.
12. Borte S, Wang N, Oskarsdóttir S, et al. Newborn screening for primary immunodeficiencies: beyond SCID and XLA. *Ann N Y Acad Sci* 2011;**1246**:118–30.
13. Botto M, Kirschfink M, Macor P, et al. Ross complement in human diseases: lessons from complement deficiencies. *Mol Immunol* 2009;**46**:2774–83.
14. Boyle JM, Buckley RH. Population prevalence of diagnosed primary immunodeficiency diseases in the United States. *J Clin Immunol* 2007;**27**:497–502.
15. Boztug K, Klein C. Genetic etiologies of severe congenital neutropenia. *Curr Opin Pediatr* 2011;**23**:21–6.
16. Brzica SM Jr, Pineda AA, Taswell HF, et al. Chronic granulomatous disease and the McLeod phenotype: successful treatment of infection with granulocyte transfusions resulting in subsequent hemolytic transfusion reaction. *Mayo Clin Proc* 1977;**52**:153–6.
17. Buckley RH. Transplantation of hematopoietic stem cells in human severe combined immunodeficiency: long-term outcomes. *Immunol Res* 2011;**49**:25–43.
18. Cappelli B, Aiuti A. Gene therapy for adenosine deaminase deficiency. *Immunol Allergy Clin North Am* 2010;**30**:249–60.
19. Castigli E, Geha RS. Molecular basis of common variable immunodeficiency. *J Allergy Clin Immunol* 2006;**117**:740–6.
20. Chinen J, Rosenblatt HM, Smith EO, et al. Long-term assessment of T cell populations in DiGeorge syndrome. *J Allergy Clin Immunol* 2003;**111**:573–9.
21. Conley ME, Howard V. Clinical findings leading to the diagnosis of X-linked agammaglobulinemia. *J Pediatr* 2002;**141**:566–71.
22. Conley ME, Larche M, Bonagura VR, et al. Hyper IgM syndrome associated with defective CD40-mediated B cell activation. *J Clin Invest* 1994;**94**:1404–9.
23. Davies EG, Thrasher AJ. Update on the hyper immunoglobulin M syndromes. *Br J Haematol* 2010;**149**:167–80.
24. Derry JMJ, Ochs H, Francke U. Isolation of a novel gene mutated in Wiskott-Aldrich syndrome. *Cell* 1994;**78**:635–44.
25. Etzioni A, Sturla L, Antonellis A, et al. Leukocyte adhesion deficiency (LAD) type II/carbohydrate deficient glycoprotein (CDG) II c founder effect in genotype/phenotype correlation. *Am J Med Genet* 2002;**110**:131–5.
26. Feng ML, Zhao YL, Shen T, et al. Prevalence of immunoglobulin A deficiency in Chinese blood donors and evaluation of anaphylactic transfusion reaction risk. *Transfus Med* 2011;**21**:338–43.
27. Fioredda F, Calvillo M, Lanciotti M, et al. Pegfilgrastim in children with severe congenital neutropenia. *Pediatr Blood Cancer* 2010;**54**:465–7.
28. Fischer A, Hacein-Bey-Abina S, Cavazzana-Calvo M. Strategies for retrovirus-based correction of severe, combined immunodeficiency (SCID). *Methods Enzymol* 2012;**507**:15–27.
29. Gaspar HB, Aiuti A, Porta F, et al. How I treat ADA deficiency. *Blood* 2009;**114**:3524–32.
30. Grierson H, Purtilo DT. Epstein-Barr virus infections in males with the X-linked proliferative syndrome. *Ann Intern Med* 1987;**106**:538–45.
31. Grimbacher B, Holland SM, Puck JM. Hyper-IgE syndromes. *Immunol Rev* 2005;**203**:244–50.
32. Grüber C, Keil T, Kulig M, et al; MAS-90 Study Group. History of respiratory infections in the first 12 yr among children from a birth cohort. *Pediatr Allergy Immunol* 2008;**19**:505–12.
33. Guerrant RL, Lohr JA, Williams EK. Acute infectious diarrhea, part I: epidemiology, etiology, and pathogenesis. *Pediatr Infect Dis* 1986;**5**:353–59.
34. Hacein-Bey-Abina S, Hauer J, Lim A, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2010;**363**:355–64.
35. Heitzeneder S, Seidel M, Förster-Waldl E, Heitger A. Mannan-binding lectin deficiency—good news, bad news, doesn't matter? *Clin Immunol* 2012;**143**:22–38.
36. Jain A, Kovacs JA, Nelson DL, et al. Partial immune reconstitution of X-linked hyper IgM syndrome with recombinant CD40 ligand. *Blood* 2011;**118**:3811–7.
37. Jouanguy E, Döffinger R, Dupuis S, et al. IL-12 and IFN-gamma in host defense against mycobacteria and salmonella in mice and men. *Curr Opin Immunol* 1999;**11**:346–51.
38. Kisand K, Peterson P. Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy: known and novel aspects of the syndrome. *Ann N Y Acad Sci* 2011;**1246**:77–91.
39. Koch A, Homøe P, Pipper C, et al. Chronic suppurative otitis media in a birth cohort of children in Greenland: population-based study of incidence and risk factors. *Pediatr Infect Dis J* 2011;**30**:25–9.
40. Kozer E, Lotem Z, Elgarushe M, et al. RCT of montelukast as prophylaxis for upper respiratory tract infections in children. *Pediatrics* 2012;**129**:e285–90.
41. Kuijpers TW, Bende RJ, Baars PA, et al. CD20 deficiency in humans results in impaired T cell–independent antibody responses. *J Clin Invest* 2010;**120**:214–22.
42. LeBras S, Geha RS. IPEX and the role of FoxP3 in the development and function of human Tregs. *J Clin Invest* 2005;**116**:1473–75.
43. Lee WI, Torgerson TR, Schumacher MJ, et al. Molecular analysis of a large cohort of patients with the hyper immunoglobulin M (IgM) syndrome. *Blood* 2005;**105**:1881–90.
44. Leiding JW, Freeman AF, Marciano BE, et al. Corticosteroid therapy for liver abscess in chronic granulomatous disease. *Clin Infect Dis* 2012;**54**:694–700.
45. Leonard WJ, Noguchi IM, Russell SM, et al. The molecular basis of X-linked severe combined immunodeficiency: the role of interleukin-2 receptor gamma chain is a common gamma chain. *Immunol Rev* 1994;**138**:61–4.
46. Levy J, Espanol-Boren T, Thomas C, et al. The clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr* 1997;**131**:47–54.
47. Lucas M, Lee M, Lortan J, et al. Infection outcomes in patients with common variable immunodeficiency disorders: relationship to immunoglobulin therapy over 22 years. *J Allergy Clin Immunol* 2010;**125**:1354–60.
48. Markert ML, Devlin BH, Chinn IK, McCarthy EA. Thymus transplantation in complete DiGeorge anomaly. *Immunol Res* 2009;**44**:61–70.
49. Martinez CA, Shah S, Shearer WT, et al. Excellent survival after sibling or unrelated donor stem cell transplantation for chronic granulomatous disease. *J Allergy Clin Immunol* 2012;**129**:176–83.
50. Merino J, Rodriguez-Valverde V, Lamelas JA, et al. Prevalence of deficits of complement components in patients with recurrent meningococcal infections. *J Infect Dis* 1983;**148**:331.

51. Micol R, Ben Slama L, Suarez F, et al. Morbidity and mortality from ataxia-telangiectasia are associated with ATM genotype. *J Allergy Clin Immunol* 2011;**128**:382–9.
52. Minegishi Y, Lavoie A, Cunningham-Rundles C, et al. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. *Clin Immunol* 2000;**97**:203–10.
53. Moylett EH, Wasan AN, Noroski LM, et al. Live viral vaccines in patients with partial DiGeorge syndrome: clinical experience and cellular immunity. *Clin Immunol* 2004;**112**:106–12.
54. Ochs HD, Filipovich AH, Veys P, et al. Wiskott-Aldrich syndrome: diagnosis, clinical and laboratory manifestations, and treatment. *Biol Blood Marrow Transplant* 2009;**15**(1 Suppl):84–90.
55. Ochs HD, Thrasher AJ. The Wiskott-Aldrich syndrome. *J Allergy Clin Immunol* 2006;**117**:725–38.
56. Oliveira JB, Fleisher TA. Laboratory evaluation of primary immunodeficiencies. *J Allergy Clin Immunol* 2010;**125**(2 Suppl 2): S297–305.
57. Orange JS, Hossny EM, Weiler CR, et al. Use of intravenous immunoglobulin in human disease: a review of the evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. *J Allergy Clin Immunol* 2006;**117**:S525–53.
58. Ott MG, Schmidt M, Schwarzwaelder K, et al. Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of *MDS1-EVI1*, *PRDM16* or *SETBP1*. *Nat Med* 2006;**12**:401–9.
59. Ozen A, Baris S, Karakoc-Aydiner E, et al. Outcome of hypogammaglobulinemia in children: immunoglobulin levels as predictors. *Clin Immunol* 2010;**137**:374–83.
60. Perez EE, Bokszzanin A, McDonald-McGinn D, et al. Safety of live viral vaccines in patients with chromosome 22q11.2 deletion syndrome (DiGeorge syndrome/velocardiofacial syndrome). *Pediatrics* 2003;**112**:e325.
61. Picard C, Casanova JL, Puel A. Infectious diseases in patients with IRAK-4, MyD88, NEMO, or IκBα deficiency. *Clin Microbiol Rev* 2011;**24**:490–7.
62. Plebani A, Soresina A, Rondelli R, et al. Clinical, immunological and molecular analysis of a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. *Clin Immunol* 2002;**104**:221–30.
63. Puck JM. The case for newborn screening for severe combined immunodeficiency and related disorders. *Ann N Y Acad Sci* 2011;**1246**:108–17.
64. Rachid R, Bonilla FA. The role of anti-IgA antibodies in causing adverse reactions to gamma globulin infusion in immunodeficient patients: a comprehensive review of the literature. *J Allergy Clin Immunol* 2012;**129**:628–34.
65. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. *Blood* 2012;**119**:1650–7.
66. Rezaei N, Hedayat M, Aghamohammadi A, Nichols KE. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol* 2011;**127**:1329–41.
67. Rosenzweig SD, Dorman SE, Uzel G, et al. A novel mutation in IFN-gamma receptor 2 with dominant negative activity: biological consequences of homozygous and heterozygous states. *J Immunol* 2004;**173**:4000–8.
68. Sánchez-Ramón S, Radigan L, Yu JE, et al. Memory B cells in common variable immunodeficiency: clinical associations and sex differences. *Clin Immunol* 2008;**128**:314–21.
69. Savitsky K, Bar-Shira A, Gilad S, et al. A single ataxia-telangiectasia gene with a product similar to PI-3 kinase. *Science* 1995;**268**: 1749–53.
70. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative disease gene product SAP regulates signal induced through the coreceptor SLAM. *Nature* 1998;**395**:462–69.
71. Schaffer FM, Monteiro RC, Volanakis JE, et al. IgA deficiency. *Immunodefic Rev* 1991;**3**:15–44.
72. Seger RA. Modern management of chronic granulomatous disease. *Br J Haematol* 2008;**140**:255–66.
73. Sekine H, Ferreira RC, Pan-Hammarstrom Q, et al. Role for Msh5 in the regulation of Ig class switch recombination. *Proc Natl Acad Sci U S A* 2007;**104**:7193–98.
74. Shackelford PG, Granoff DM, Polmar SH, et al. Subnormal serum concentrations of IgG2 in children with frequent infections associated with varied patterns of immunologic dysfunction. *J Pediatr* 1990;**116**:529–38.
75. Shearer WT. Screening for severe combined immunodeficiency in newborns. *J Allergy Clin Immunol* 2012;**129**:619–21.
76. Shkalim V, Monselize Y, Segal N, et al. Selective IgA deficiency in children in Israel. *J Clin Immunol* 2010;**30**:761–5.
77. Skattum L, van Deuren M, van der Poll T, Truedsson L. Complement deficiency states and associated infections. *Mol Immunol* 2011;**48**:1643–55.
78. Smahi A, Courtois G, Vabres P, et al. Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. The International Incontinentia Pigmenti (IP) Consortium. *Nature* 2000;**405**:466–72.
79. Stephan JL, Vlekova V, Le Deist F, et al. Severe combined immunodeficiency: a retrospective single-center study of clinical presentation and outcome in 117 cases. *J Pediatr* 1993;**123**:564–72.
80. Sullivan KE. The clinical, immunological, and molecular spectrum of chromosome 22q11.2 deletion syndrome and DiGeorge syndrome. *Curr Opin Allergy Clin Immunol* 2004;**4**:505–12.
81. Teele DW, Klein JO, Rosner B, et al. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective cohort study. *J Infect Dis* 1989;**160**:83–94.
82. Thomas C, Le Deist F, Cavazzana-Calvo M, et al. Results of allogeneic bone marrow transplantation in patients with leukocyte adhesion deficiency. *Blood* 1995;**86**:1629–35.
83. Thrasher AJ, Burns SO. WASP: a key immunological multitasker. *Nat Rev Immunol* 2010;**10**:182–92.
84. Todd RF III, Freyer DR. The CD11/18 leukocyte glycoprotein deficiency. *Hematol Oncol Clin North Am* 1988;**2**:13–31.
85. Tsukada S, Saffran DC, Rawlings DJ, et al. Deficient expression of a B cell cytoplasmic tyrosine kinase in human X-linked agammaglobulinemia. *Cell* 1993;**72**:279–90.
86. Umetsu DT, Ambrosino DM, Quinti I, et al. Recurrent sinopulmonary infection and impaired antibody response to bacterial capsular polysaccharide antigen in children with selective IgG-subclass deficiency. *N Engl J Med* 1985;**313**:1247–51.
87. van de Vosse E, van Dissel JT, Ottenhoff TH. Genetic deficiencies of innate immune signalling in human infectious disease. *Lancet Infect Dis* 2009;**9**:688–98.
88. van der Burg M, Gennery AR. The expanding clinical and immunological spectrum of severe combined immunodeficiency. *Eur J Pediatr* 2011;**170**:561–71.
89. van Zelm MC, Reisli I, van der Burg M, et al. An antibody-deficiency syndrome due to mutations in the *CD19* gene. *N Engl J Med* 2006;**354**:1901–12.
90. van Zelm MC, Smet J, Adams B, et al. *CD81* gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *J Clin Invest* 2010;**120**:1265–74.
91. Villa A, Notarangelo L, Mach P, et al. X-linked thrombocytopenia and Wiskott-Aldrich syndrome are allelic diseases with mutations in the *WASP* gene. *Nat Genet* 1995;**9**:414–17.
92. Volanakis JE, Zhu ZB, Schaffer FM, et al. Major histocompatibility complex class III genes and susceptibility to immunoglobulin A deficiency and common variable immunodeficiency. *J Clin Invest* 1992;**89**:1914–22.
93. Warnatz K, Salzer U, Rizzi M, et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proc Natl Acad Sci U S A* 2009;**106**: 13945–50.
94. Wen L, Atkinson JP, Giclas PC. Clinical and laboratory evaluation of complement deficiency. *J Allergy Clin Immunol* 2004;**113**: 585–93.
95. Winkelstein JA, Marino MC, Lederman HM, et al. X-linked agammaglobulinemia: report on a United States registry of 201 patients. *Medicine (Baltimore)* 2006;**85**:193–202.
96. Yagi H, Furutani Y, Hamada H, et al. Role of *TBX1* in human del22q11.2 syndrome. *Lancet* 2003;**362**:1366–73.
97. Yong PL, Orange JS, Sullivan KE. Pediatric common variable immunodeficiency: immunologic and phenotypic associations with switched memory B cells. *Pediatr Allergy Immunol* 2010;**21**: 852–8.
98. Zonana J, Elder ME, Schneider LC, et al. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). *Am J Hum Genet* 2000;**67**:1555–62.

CHAPTER 68

The Febrile Neutropenic Patient

Brian T. Fisher • Lillian Sung

Since chemotherapeutic agents were first administered for the treatment of malignancy, infection has been a cause of significant morbidity and mortality. Initial reports documented an infection-related mortality rate among patients with acute leukemia that was greater than 70 percent.[56,60] In the last 5 decades, chemotherapeutic regimens have been refined, resulting in improved pediatric cancer survival rates.[106,141] However, the immunosuppressive consequences of chemotherapy continue to exist, and infections still account for more than 60 percent of deaths in children receiving intensely myelosuppressive chemotherapy.[29,127]

For many decades, researchers have sought to identify risk factors for infection and to devise risk-stratified therapeutic approaches to reduce infectious mortality rates in patients receiving chemotherapy. Early descriptive pediatric studies confirmed that the depth and duration of neutropenia was a risk factor for developing infection. Further, fever was a frequent and sometimes the only clinical sign indicating the presence of infection in this at-risk population.[60] In these early years, fever in patients with neutropenia prompted an infectious evaluation but empirical antibiotic therapy was not considered the standard of care. Because infection was the most common cause of fever in neutropenic patients and because some patients with fever and neutropenia had rapid progression to death, particularly resulting from *Pseudomonas aeruginosa* bacteremia, empirical therapy was explored. In 1971, Schimpff and colleagues[117] published one of the sentinel experiences of combination empirical therapy with carbenicillin and gentamicin in a series of patients with fever and neutropenia.[117] Their efforts revealed a reduction in infectious mortality with this approach relative to historical controls. Specifically, in the historical control period, when empirical antibiotic therapy was not used, 20 of 22 *P. aeruginosa* infections ultimately resulted in death. This compared to only 4 deaths out of 21 *P. aeruginosa* infections during the study period. Subsequent landmark trials by Pizzo and colleagues[99,101] supported the continuation of empirical antibiotics through the duration of neutropenia and prompted the addition of empirical antifungal therapy in the setting of prolonged fever and neutropenia despite broad-spectrum antibiotic therapy.

In the decades that followed, empirical antibacterial and antifungal therapy has evolved into the standard of care for patients with fever and neutropenia. Clinical practice guidelines from various societies have better standardized the approach to this clinical scenario.[31,46,73,84] Although these guidelines have been useful, they have primarily focused on adult patient populations. This has left pediatricians to apply guidelines that have not accounted for important differences between children and adults presenting with fever and neutropenia.[126] Fortunately, some of the important differences between pediatric and adult fever and neutropenia have been addressed in recent international pediatric fever and neutropenia guidelines.[76] Nonetheless, as noted in the pediatric guidelines, many questions remain surrounding the diagnostic and therapeutic approach to fever and neutropenia. For example, are there ways to improve diagnostic approaches to identify the presence or absence of bacterial and fungal infections, and is prophylactic antimicrobial therapy a useful approach to reducing infection?

This chapter will focus on the epidemiology of fever and neutropenia and approaches for risk stratification that are pertinent to pediatric patients. Additionally, advances in modalities for diagnosing bacterial and fungal infections will be explored. Finally, empirical, preemptive, and prophylactic therapeutic interventions will be reviewed. At relevant points, areas for necessary future investigation will be highlighted. Many other etiologies of neutropenia (e.g., congenital neutropenia) exist,[107] and such patients also will incur periods of fever and neutropenia. However, the importance of fever in these settings varies substantially and thus the focus of this chapter will be on fever in the setting of chemotherapy-induced neutropenia.

EPIDEMIOLOGY

Children receiving chemotherapy often are confronted with at least one episode of fever and neutropenia during the course of their cancer care. The frequency of fever and neutropenia varies depending on factors such as type and extent of malignancy, intensity of chemotherapy, and duration of neutropenia. An estimated 10 to 50 percent of patients with solid tumors will suffer at least one episode of fever and neutropenia. The incidence of fever and neutropenia increases to 80 percent in patients with hematologic malignancy and approaches 100 percent in some higher-risk populations.[46,131]

Bacterial Pathogens

Fever in the setting of neutropenia often is the initial and potentially the only clinical sign of a bacterial, fungal, or

viral infection. Of patients in whom a pathogen is identified, bacteria predominate. In a landmark study evaluating 793 pediatric cancer patients with fever and neutropenia, Pizzo and colleagues[100] found that 87 percent of patients with a microbiologically documented infection suffered from a bacterial infection. Recent epidemiologic studies specific to pediatric fever and neutropenia have suggested a reduction in the proportion of episodes attributable to bacteria. However, despite the reduction, bacterial pathogens still accounted for the majority of all identified pathogens.[21,54]

Early reports on the epidemiology of these bacterial infections highlighted gram-negative pathogens as the predominant cause of infection in neutropenic children and adults. *Escherichia coli*, *P. aeruginosa*, and *Klebsiella* spp. were the three most commonly isolated pathogens.[120] Subsequently, toward the end of the 1980s, gram-positive pathogens were being increasingly recognized as an important source of fever of neutropenia[34,139] and soon thereafter gram-positive pathogens were found to be more common than gram-negative organisms.[145] This shift in pathogens has been attributed to increased use of central venous lines, a greater prevalence of mucositis, and prophylactic regimens with predominantly gram-negative activity. Additionally, escalation to more intensive chemotherapy regimens, such as those containing high-dose cytarabine, have resulted in more impressive mucositis, which has contributed to increases in specific pathogens such as viridans group streptococci.[127]

The spectrum of bacterial pathogens depends on specific chemotherapy, use of antimicrobial prophylaxis, and setting. Table 68-1 displays the distribution of bacterial infections across various international pediatric oncology cohorts.[11,21,54,75] Variation in the frequency in isolating a bacterial pathogen is apparent. This is likely secondary to variation in patient makeup of each cohort and variation in diagnostic testing practices. With the exception of the Malaysian cohort, the predominance of gram-positive pathogens has persisted in pediatric oncology cohorts, consistent with data published toward the end of the previous century. Among the gram-positive pathogens, viridans group streptococci, *Staphylococcus aureus*, and coagulase-negative staphylococci are predominant. As for gram-negative pathogens, *E. coli*, *P. aeruginosa*, and *Klebsiella* spp. are still most commonly identified.[11,21,54,75]

Fungal Pathogens

The likelihood of an invasive fungal infection during an episode of fever and neutropenia varies depending on risk factors, as discussed in more detail later. Generally, fungal pathogens are considered the etiology of fever and neutropenia when these two clinical signs are concurrently prolonged or when fever is recurrent during the same episode of neutropenia. Defining the epidemiology of an invasive fungal infection in specific patient populations can be challenging. Published guidelines on classifying the likelihood of an invasive fungal infection have been helpful in standardizing reporting of such infections[32]; however, limitations in diagnostic modalities for identifying an invasive fungal infection persist, and thus published infection rates are likely underestimated. Despite these challenges, prospective multicenter data have documented an invasive fungal infection rate nearing 6 percent of children hospitalized with fever and neutropenia.[138] *Candida* spp. are the most commonly isolated fungal pathogens, likely related to the frequent existence of *Candida* as a commensal organism on the skin and mucosal surfaces.[39] Compromise of these important anatomic barriers (e.g., mucositis) can result in invasive candidiasis. Additionally, *Candida* spp. can infect central venous catheters, which are commonly present in children with cancer. Although mortality data specific to candidiasis in pediatric oncology patients are limited, the attributable mortality of invasive candidiasis in all pediatric patients has been estimated to be 10 percent.[143]

Invasive mold infections are less common but still extremely important pathogens given the poor outcomes related to these infections. In a contemporary pediatric oncology single center observational cohort, only 54 percent of patients with invasive mold infections responded to therapy in the first 12 weeks from diagnosis and 31 percent of these infections resulted in death within the same time period.[49] Among the mold pathogens, *Aspergillus* spp. are most common, followed by *Mucorales* and *Fusarium* spp.[49,127]

Viral Pathogens

Owing to advancement in viral diagnostic methodologies, multiple publications have attempted to delineate

TABLE 68-1 **Distribution of Bacterial Pathogens in Selected Pediatric Oncology Cohorts**

Author/Year	Ariffin[11] 2002	Lehrnbecher[75] 2004	Castagnola[21] 2007	Hakim[54] 2009
Study location	Malaysia	Germany	Italy	United States
Patient type	Any malignancy	AML	Leukemia, solid tumor, or allogeneic HSCT	Any malignancy
Clinical scenario	Fever and neutropenia	Fever with and without neutropenia	Fever and neutropenia	Fever and neutropenia
Infection episodes (total patients)	762 (513)	855 (304)	614 (NA)	337 (337)
Bacterial pathogen isolation N (%)	270 (35.4%)	252 (29.5%)	97 (15.8%)	54 (16%)
Gram positive pathogens n (%)	103 (38.1%)	203 (80.6%)	57 (58.8%)	31* (57%)
Gram negative pathogens n (%)	167 (61.9%)	49 (19.4%)	40 (41.2%)	23 (43%)

* Includes seven episodes of *Clostridium difficile* infection.
AML, Acute myeloid leukemia; *HSCT,* hematopoietic stem cell transplantation; *N/A,* not available.

TABLE 68-2 **Frequency of Viral Respiratory Pathogens at Presentation for Fever and Neutropenia**

Author/Year	Long[77] 1987	Arola[13] 1995	Christensen[24] 2005	Koskenvuo[71] 2008
Study duration	5 yr	17 mo	12 mo	5.5 yr
Patient type	Leukemia, solid tumors	Any malignancy	Any malignancy	Leukemia
Clinical scenario	Suspicion of virus	Fever	Fever	Fever
Total patients (episodes)	200 (not reported)	32 (75)	66 (250)	51 (138)
Testing methods	Culture, immunofluorescence	Culture, antigen, and antibodies	PCR	Culture, antigen, and PCR
Respiratory virus isolation rate	148 (N/A)	28 (37%)	19 (8%)*	61 (59%)
Bacteremia plus virus isolation	Not reported	None	Not reported	13%

*Mouth swabs and not nasopharyngeal swabs performed on a majority of patients.
N/A, not available; *PCR,* polymerase chain reaction.

the attribution of fever and neutropenia events to viral pathogens. Table 68-2 displays the frequency of recovered viral upper respiratory tract pathogens in pediatric patients with fever with or without symptoms of a viral infection.[13,24,71,77] Although these studies were not limited to patients with fever and neutropenia, a majority of the patients had concomitant neutropenia. The frequency of laboratory-confirmed viral respiratory tract infection ranged from 8 to 59 percent. Although some authors suggest routine comprehensive viral testing at the time of presentation,[24] the utility of routine viral testing is not clear. First, ideally the identification of a viral pathogen should allow for discontinuation of broad-spectrum antibiotics in the febrile and neutropenic patient. However, bacterial and viral infections are not mutually exclusive, with as many as 13 percent of patients with a viral pathogen suffering a simultaneous bacteremia.[71] Second, the evolution in the availability of viral polymerase chain reaction testing may result in detection of virus well after symptom resolution. Finally, only a few antiviral agents are available for the treatment of some of these viral pathogens, and their effectiveness is not clearly delineated. Viral testing should be limited to patients in whom positive results would result in a change in management such as de-escalation of antibiotic therapy, initiation of an appropriate antiviral therapy (e.g., neuraminidase inhibitor for influenza), or institution of appropriate isolation precautions.

Fever and Neutropenia of Unknown Origin

Despite evaluation, many patients have negative cultures and do not have a clinically evident site of infection. This presentation is often referred to as *fever of unknown origin (FUO).* Although children with FUO can have an occult infection, frequently an infectious pathogen or clinical infection is not present. In a combined pediatric and young adult cohort, Pizzo and colleagues described 306 consecutive episodes of fever and neutropenia in patients cared for at the National Institutes of Health. They found that only 54 percent of patients had an identifiable infection within 7 days from presentation.[101] Despite advances in infectious diagnostic modalities (e.g., more sensitive viral tests), the rates of unidentified etiology of fever and neutropenia in children have remained above 50 percent in the past decade.[11,21,54,75]

RISK STRATIFICATION

Risk Stratification at Initial Presentation

The risk for infection and serious morbidity is highly heterogeneous among patients with fever and neutropenia, and this heterogeneity has resulted in efforts to risk stratify patients. The primary purpose for risk stratification is to guide physicians in deciding when patients can safely be managed with less aggressive interventions such as outpatient management, early discontinuation of antibiotics, and oral therapy and when to intensify diagnostic investigations and therapy for a potential infection. In adults, the predominant risk stratification schema is the Multinational Association for Supportive Care in Cancer Scoring System. Unfortunately, this system incorporates parameters that are not applicable to children, such as lack of chronic obstructive pulmonary disease and age younger than 60 years to determine low-risk status. The inclusion of these parameters limits the applicability of this scoring system to children.[126]

Both retrospective and prospective pediatric cohort studies have been performed to evaluate various clinical prediction rules for designating children as low risk for infection in the setting of fever and neutropenia. These studies have been summarized in two separate meta-analyses.[96,97] Considered collectively, data from these studies support the potential success of implementing a risk stratification protocol. However, each trial used varied patient-relevant factors (e.g., age and malignancy), clinical factors (e.g., timing of chemotherapy, degree of fever, and presence of hypotension), and laboratory parameters (e.g., duration and depth of neutropenia, C-reactive protein value, and platelet count) and had various outcome definitions (e.g., bacteremia, serious infection, and death). Furthermore, attempts to validate six of the pediatric risk stratification schemas in separate populations have resulted in suboptimal performance in contrast to the original study.* Thus, clinicians

*References 5-7, 14, 68, 78, 79, 87, 96, 97, 105, 111, 114, 115.

at individual institutions are left with the challenge of identifying the prediction model that is most consistent with their patient population (rules tend to perform less favorably when applied in different settings) and is most feasible to implement.

The advent of serum biomarkers has resulted in a renewed interest in trying to improve current prediction rules. Investigators have studied the clinical utility of more than 170 different biomarkers, including cytokines, cell surface markers, coagulation system molecules, receptors in various inflammatory cascades, and acute-phase reactants in various pediatric and adult clinical settings.[98] Some biomarkers have emerged as potentially useful tools to guide therapeutic approaches for fever and neutropenia. However, although some biomarkers are often elevated in patients with a bacterial infection, an inability to identify a threshold value for a single biomarker to accurately discriminate between infected and noninfected patients has limited their clinical applicability. Future investigations combining multiple biomarkers with traditional laboratory values and clinical findings may improve the discriminating ability of a pediatric risk prediction rule.

Risk Stratification for Invasive Fungal Infection

The risk stratification prediction rules discussed earlier are specific to risk for outcomes, including bacterial infection at the time of presentation for fever and neutropenia. However, the likelihood of an invasive fungal infection increases as the duration of fever and neutropenia increases, depending on various patient, laboratory, and clinical factors. Children with persistent fever and neutropenia should be further stratified relative to their risk for invasive fungal infection. Although no studies have been specifically dedicated to deriving an invasive fungal infection risk prediction rule in a pediatric cancer cohort, some observational studies have identified important risk factors for invasive fungal infection that can provide general guidelines for risk.[37] Patient populations with reported invasive fungal infection incidence greater than 10 percent include children with acute myeloid leukemia (AML),[52,127,128] relapsed acute lymphoblastic leukemia (ALL),[72] and recipients of an allogeneic stem cell transplantation.[15,37,58,69,134] Additionally, prolonged neutropenia (absolute neutrophil count $\leq 500/mm^3$) or low monocyte counts ($\leq 100/mm^3$) and increased C-reactive protein level of 90 mg/L or greater on the fourth day of fever are laboratory values that have each been associated with invasive fungal infection.[138] Finally, recent prolonged steroid exposure (>10 days) has been repeatedly identified as a risk factor for invasive fungal infection.[37,51,58] Based on these pediatric data, adult and pediatric fever and neutropenia guidelines[47,76] and guidelines for defining invasive fungal infection[32] recommend that patients with fever and neutropenia should be considered at high risk for invasive fungal infection if they have recently received intensive chemotherapy (e.g., AML or relapsed ALL patient) with expected prolonged neutropenia (>7 to 10 days) or recent exposure to prolonged courses of steroids. All other patients should be considered at low risk for invasive fungal infection. This risk stratification is a general guide, and it should be noted that invasive fungal infection has been described in patients who would fall in the low-risk classification.[38] Additional work is necessary to establish specific prediction rules to more accurately discriminate between invasive fungal infection high-risk and low-risk patients.

HISTORY AND PHYSICAL EXAMINATION

Before initiating routine diagnostic testing, a complete history and physical examination should be performed. A comprehensive history can identify important exposures that could alert the physician to consider less common opportunistic infections and direct early additional diagnostic testing. The history should include details about symptoms other than fever, adherence to current medications (especially prophylactic antimicrobials), and most recent chemotherapy regimen. Additionally, the clinician should question about sick contacts at home; updates on recent travel; daycare, school, and occupational exposures; animal exposures (e.g., new domestic pets such as cats or birds and farm animals); and dietary exposures (e.g., unpasteurized cheeses). On physical examination, specific attention should be focused on areas sometimes overlooked, such as the nasal passages and oropharynx, middle ears, genitalia, perineum (in particular, the perianal area), and scalp. Central venous line exit sites also are important areas for inspection.

INITIAL DIAGNOSTIC EVALUATION

Regardless of the risk designation of a pediatric patient presenting with fever and neutropenia, an infectious diagnostic evaluation is warranted in all such patients. Various testing modalities may be employed depending on the status of the patient. In all patients, routine blood cultures should be performed before initiation of antibiotics. Because most patients have central venous access, this would include culturing samples from all lumens of the central venous catheter.

Performing a peripheral blood culture in addition to central venous cultures is controversial. Multiple studies of paired cultures taken from pediatric and adult patients with underlying malignancy suggest a small but consistent probability of detecting true bacteremia from the peripheral culture when the central culture is negative.[1,22,35,104,116] A recent meta-analysis determined that 13 percent of bacteremias were identified only with a peripheral culture.[109] These data suggest that a peripheral blood collection should be considered concurrent with cultures from the central line.

Although no pediatric or adult study has documented the yield of daily blood cultures in an otherwise clinically stable patient with persistent fever and neutropenia, it is unlikely that continued daily cultures will identify a true pathogen if initial cultures were negative and the child is

clinically stable. Therefore, it is reasonable to follow the adult guideline recommendations, which suggest daily blood cultures for only the first 3 days of fever and neutropenia.[47] Repeat blood cultures are appropriate in patients with a significant clinical change (e.g., hypotension, tachypnea) or in those whose fever initially resolved but then recurred.

A urine culture is warranted if the patient presents with signs or symptoms of a urinary tract infection (UTI). The importance of routine urine cultures for children presenting with fever and neutropenia without signs or symptoms of a UTI is controversial. In a typical pediatric population, UTI is a common source of FUO.[23] Asymptomatic UTIs are likely to be more common in neutropenic children given the lack of an appropriate inflammatory response. This is evidenced by the fact that only 4 percent of oncology patients with a urine culture consistent with a UTI pathogen will have pyuria on urinalysis.[67]

Of the various observational trials that have prospectively evaluated patients with fever and neutropenia,[21,54,75,100] only two have commented on the impact of UTI. Hakim and colleagues[54] noted that of 337 patients presenting with fever and neutropenia, two (0.6%) had a positive urine culture. Urine culture was not a component of the routine diagnostic evaluation, and it is possible that there were more patients with a UTI as 53 percent of the cohort did not have an identified source of fever. Data from Santolaya and associates suggest that UTIs are more common, as they identified a urinary pathogen in 4.6 percent of fever and neutropenia episodes.[114] Again, urine culture was not performed as a routine diagnostic test so 4.6 percent may also be an underestimate of the true frequency of UTI in the setting of fever and neutropenia.

Given these data, routine urinalysis and urine culture in patients presenting with fever and neutropenia should be obtained if a midstream or clean catch specimen is feasible. In patients who require urinary catheterization for specimen collection, the risks of the intervention should be weighed against the benefit of potentially identifying the source of the fever. However, antibiotic administration should never be delayed if a patient is unable to provide a timely urine specimen.

In children with focal respiratory signs (e.g., tachypnea or auscultatory changes) or symptoms (e.g., cough or increased work of breathing) chest radiography should be a component of the initial diagnostic workup. Additionally, in children who are capable of producing an adequate sputum sample, one should be collected and sent for routine culture. The utility of routine chest radiography has been examined in children presenting with fever and neutropenia. Together, these studies evaluated 487 episodes of pediatric fever and neutropenia; only five chest radiographs revealed pneumonia in the absence of any respiratory signs or symptoms.[43,65,70,108] These data do not support the routine use of chest radiography in children with fever and neutropenia in the absence of respiratory symptomatology.

Finally, important findings noted on history or physical examination should dictate additional testing for less common sources of infection. For example, a patient with fever and neutropenia and a reported history of a lovebird in the home should prompt additional testing for cryptococcosis early in the evaluation period.[133] Likewise, identification of black cutaneous lesion(s) in a patient with antecedent neutropenia should prompt consideration for immediate biopsy over concern for a cutaneous mold infection. In the setting of exotic exposures or important clinical findings, consultation with an infectious disease physician with experience in immunocompromised patients may be warranted early in the hospitalization, because early identification of less common opportunistic infections will likely translate into improved patient care.

INITIAL THERAPY FOR FEVER AND NEUTROPENIA

Traditionally, the standard of care for fever and neutropenia has been hospital admission with administration of intravenous broad-spectrum antibiotics. However, employing a risk stratification rule offers the opportunity to differentially manage children at low and high risk for infectious outcomes. Children at high risk should be admitted for initiation of a parenteral antibiotic regimen with broad-spectrum gram-negative coverage, including *P. aeruginosa* and reasonable gram-positive coverage for organisms such as the viridans group streptococci. Antibiotic options that meet these criteria include an antipseudomonal penicillin (e.g., piperacillin-tazobactam), a cephalosporin (e.g., cefepime), or a carbapenem (e.g., meropenem or imipenem-cilastatin). Two separate pediatric meta-analyses compared antipseudomonal penicillins to cephalosporins[81] and antipseudomonal penicillins to carbapenems[82] and concluded similar effectiveness of each of these options for empirical therapy.

It is important to note that a meta-analysis of predominantly adult randomized controlled trials identified a significant increased risk for all-cause mortality in patients receiving cefepime in contrast to those receiving other β-lactam agents for fever and neutropenia.[94,142] However, in a subsequent analysis, the U.S. Food and Drug Administration (FDA) supplemented the available published data with unpublished mortality data specific to febrile neutropenic patients and found no mortality difference among the antibiotic options. The FDA specifically reviewed the available case report forms from the fever and neutropenia trials and could not identify a biologically plausible reason for the previously identified association of cefepime and death.[66] An additional meta-analysis of fever and neutropenia trials and a retrospective investigation of pediatric AML patients did not reveal a difference in mortality risk between those exposed to cefepime in contrast to other antibiotics commonly used for fever and neutropenia.[44,132] Based on evaluation of the available data, cefepime should remain a viable option for empirical therapy for febrile neutropenia.

Because gram-positive organisms are an increasing cause of fever and neutropenia episodes, the utility of adding additional broad gram-positive coverage (e.g., vancomycin) to the empirical regimen has been questioned.

Although pediatric-specific data do not exist, multiple adult randomized controlled trials have investigated the efficacy of adding a glycopeptide to various β-lactam–containing regimens. These trials were summarized in two separate meta-analyses, each of which failed to identify a significant benefit relative to all-cause mortality in patients receiving empirical glycopeptide therapy.[93,136] Therefore, routine empirical glycopeptides are not recommended for fever and neutropenia.

In general, for a child presenting with fever and neutropenia who is otherwise stable, monotherapy with an antipseudomonal penicillin, an antipseudomonal fourth-generation cephalosporin, or an antipseudomonal carbapenem is appropriate. There is a paucity of published data that report on the importance of certain patient-level factors in determining an appropriate antibiotic regimen. However, as suggested by the adult fever and neutropenia guidelines,[47] certain patient specific factors should be evaluated when managing antibiotic regimens. For instance, in patients with or developing hemodynamic instability, the addition of a second gram-negative agent (e.g., an aminoglycoside) and broader gram-positive therapy (e.g., vancomycin) may be necessary. Additionally, broad gram-positive therapy should be considered in patients with evidence of a focal gram-positive infection (e.g., cellulitis or central access tunnel infection). Finally, a history of an invasive infection with a resistant organism (e.g., methicillin-resistant *S. aureus*) should inform empirical antibiotic choices during subsequent fever and neutropenia episodes. Likewise, each institution's choice of empirical therapy should be guided by local active surveillance of the resistance profiles of pathogens identified among oncology unit patients. In certain institutions, an identified change in the resistance profiles may warrant a switch from one antimicrobial agent to another or from empirical monotherapy to empirical multiagent therapy.

For low-risk patients, outpatient management with either oral or parenteral therapy for fever and neutropenia has garnered much support in recent years. Some pediatric-specific randomized trials have noted similar safety for early hospital discharge on intravenous therapy versus continued hospitalization,[3,112] early hospital discharge on oral therapy versus continued hospitalization,[17] inpatient intravenous versus inpatient oral therapy,[19] and outpatient intravenous versus outpatient oral therapy.[53,90,92,95] Although each of these studies was limited by small sample sizes, a pediatric stratified analysis of randomized trials and a pediatric-specific meta-analysis of prospective studies support the safety of outpatient management and oral antibiotic administration in low-risk children with fever and neutropenia.[83,130] In addition, outpatient management of low-risk febrile neutropenic patients is associated with a large reduction in health care costs in contrast to inpatient management.[112,129] Of interest, although patients and parents both report that the home environment has a positive impact on physical activity, self-esteem, and family activities,[123] it seems that some parents may prefer inpatient management for fever and neutropenia episodes given the perception of reduced effectiveness of outpatient management.[17,36,103] As the standard of care for low-risk fever and neutropenia transitions from the inpatient to outpatient setting, additional clinical efforts will be necessary to support families and evaluate the effectiveness of these approaches outside of carefully monitored clinical trials.

DIAGNOSTIC EVALUATION FOR PERSISTENT FEVER AND NEUTROPENIA

A primary concern in the patient with prolonged fever and neutropenia (>96 hours) despite broad-spectrum antibiotics is the possibility of an invasive fungal infection, especially in patients considered to be at high risk for it. No specific clinical signs suggest an invasive fungal infection diagnosis, and routine cultures have limited yield for yeasts such as *Candida* spp. and extremely poor yield for molds such as *Aspergillus* spp.[10] Thus, focus has increased on improving diagnostic modalities for detecting invasive fungal infection in these patients. Two serum fungal biomarkers have received significant attention, galactomannan and β-D-glucan. Galactomannan, developed to detect a cell wall component of *Aspergillus* spp., has been well evaluated in pediatric cohorts. Table 68-3 displays the operating characteristics of these pediatric studies.* The data suggest that galactomannan testing can be useful in identifying patients with actual invasive aspergillosis. Of importance, the false positive rate of the assay has improved with recent analyses.

The appeal of β-D-glucan is that it has the potential to detect a wide variety of invasive fungi, with the exception of *Cryptococcus* and molds of the Mucorales order.[85] Large adult prospective trials describing the operating characteristics of β-D-glucan have been compelling[41,91] and a positive β-D-glucan result is considered as supportive evidence for probable invasive fungal infection according to the most recent European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group definitions for invasive fungal infection.[32] However, to date, data on the β-D-glucan assay in children have been limited to a cohort of healthy patients[122] and a smaller number of immunocompromised children and thus it should not be used clinically in children with fever and neutropenia until additional studies are performed.[88,144]

PCR testing represents a third novel approach to detect an invasive fungal infection. A pan-fungal PCR approach has been evaluated in both pediatric[40] and adult patients[61] with neutropenia with and without fever. The operating characteristics of this technique were reasonable but did not prove to be better than galactomannan testing. PCR testing has promise but this approach is limited by the need for release of DNA outside of the fungal cell wall and the potential for sample contamination.[10]

Lastly, radiographic testing such as systematic computed tomography (CT) imaging has been proposed as a

*References 12, 45, 55, 59, 110, 124, 125.

TABLE 68-3 **Operating Characteristics of Serum Galactomannan Testing for Diagnosis of Proven or Probable Aspergillosis* in Pediatric Patients**

Author Year	Total No. Patients/Episodes in Study	Proven or Probable Aspergillosis	Sensitivity (%)	Specificity (%)	TP	FP	TN	FN	False-Positive Rate (%)†
Hayden[55] 2008‡	56	17	65	87	11	5	34	6	12.8
Steinbach[124] (2007)	64	1	0	87	0	8	55	1	12.7§
Hovi[59] (2007)	117	1	100	93	1	11	105	0	9.5
Sulahian[125] (2001)	347	9	100	89.9	9	34	304	0	10.1
Rohrlich[110] (1996)	37	10	100	92.6	10	2	25	0	7.4
Armenian[12] (2009)	78	3¶	100	98.7	3¶	1	74	0	1.3
Fisher[45] (2012)	195	1	0	95	0	10	184	1	5.2

Modified from Fisher BT, Zaoutis TE, Park JR, et al. Galactomannan antigen testing for diagnosis of invasive aspergillosis in pediatric hematology patients. *J Pediatr Infect Dis Soc* 2012;**1**:109.
* The definition of proven or probable aspergillosis varied depending on the publication.
†Defined as the number of false-positive results per total number of episodes/patients without invasive aspergillosis.
‡Case-control study design may inflate the calculated false-positive rate for this study.
§After accounting for piperacillin-tazobactam exposure the false-positive rate was 8.5%.
¶All three probable cases required inclusion of positive galactomannan enzyme immunoassay results to qualify for this designation
FN, False-negative; *FP,* false-positive; *TN,* true-negative; *TP,* true-positive.

method to detect an infectious etiology of persistent fever and neutropenia. Evaluation of adult patients early in the course of prolonged fever and neutropenia suggests that high-resolution CT scan of the chest with subsequent diagnostic intervention (e.g., bronchoalveolar lavage) has the potential to direct additional specific antimicrobial therapy[57] and to reduce the time to diagnosis of invasive pulmonary aspergillosis.[20] Pediatric data on the utility of systematic CT imaging of the sinuses, chest, abdomen, and pelvis in prolonged fever and neutropenia are limited to a retrospective case series of 68 pediatric episodes. In just two of the cases, chest CT scan results altered the course of therapy.[2]

Of the previously mentioned diagnostic tests, only the data on galactomannan testing support its use as an adjunctive diagnostic tool in the setting of pediatric prolonged fever and neutropenia. Nonetheless, additional investigation is necessary to better define the ideal galactomannan testing approach. Regardless of the diagnostic modality, the challenge with interpreting the data on these adjunctive tools in the setting of prolonged fever and neutropenia is that in previous studies significant variation existed relative to patient populations, interpretations of positive assay threshold, type of test used, timing and frequency of testing, and definitions for invasive fungal infection. It appears that no single diagnostic modality has proven ideal for directing the care of the pediatric patient with prolonged fever and neutropenia. It may be that a combination of diagnostic tests is necessary to best inform clinical practices. In a randomized, controlled trial in adults, a preemptive antifungal therapy approach guided by clinical assessment, laboratory testing (e.g., galactomannan testing), and chest imaging results was shown to be as effective as empirical antifungal therapy.[27] Future studies are necessary to confirm this as an appropriate approach for prolonged fever and neutropenia in children.

THERAPEUTIC ADJUSTMENTS FOR PROLONGED FEVER AND NEUTROPENIA

Adjustment of Empirical Antibiotic Therapy

Few data exist to guide recommendations for adjusting antibiotic therapy in patients with prolonged fever and neutropenia with or without an identified source. One randomized, controlled trial in adults found that the addition of vancomycin in patients with persistent fever and neutropenia receiving piperacillin-tazobactam monotherapy failed to provide benefit relative to fever resolution.[26] Guidelines for both adult and pediatric patients agree that the persistence of fever alone is not an indication for expanding or modifying antibiotic coverage, assuming the patient remains clinically stable. In this setting, close monitoring is necessary to evaluate for changes in clinical status.[47,76] Signs of clinical deterioration, for example, hypotension or respiratory distress, should prompt expansion of the antimicrobial regimen. The nature of the clinical change should dictate the specific addition or modification to the antimicrobial regimen.

An important question is the optimal time to discontinue antibiotics when fever has resolved but neutropenia persists. Retrospective[8,9,63,74] and prospective observational[25,89] and randomized[113] studies support the safety of antibiotic cessation after fever resolution with evidence of bone marrow recovery. It also may be safe to stop antibiotics irrespective of bone marrow recovery on day 3 in low-risk patients.[113] However, the optimal approach in high-risk patients without bone marrow recovery is less clear. Pizzo and colleagues[101] showed that if empirical antibiotics are stopped at 7 days in patients with

resolution of fever but persistent neutropenia, a significant risk exists for return of fever and potential for death. Future research is necessary to better define the appropriate timing for antibiotic cessation in high-risk children with persistent neutropenia.

In patients in whom an organism is thought to be the source of fever, the antimicrobial regimen should be modified to target the identified organism. It is not clear whether it is necessary to retain the same broad-spectrum coverage appropriate for initial presentation of high-risk fever and neutropenia or whether it is possible to focus the antibiotic regimen while the patient is still neutropenic. A reasonable approach would be to maintain broad-spectrum coverage if empirical therapy would otherwise be continued and to narrow therapy if criteria for cessation of empirical therapy are met. Future investigation of narrowing therapy in high-risk patients with an identified pathogen is necessary to determine whether this approach is safe.

Initiation of Antifungal Therapy

Initial prospective randomized data highlighted the success of empirical antifungal therapy in children with persistent fever and neutropenia despite broad-spectrum antibiotics.[100] As previously discussed, considerable attention has since focused on defining patients at high risk for invasive fungal infection. Current guidelines for adults support the initiation of empirical antimold therapy in patients with persistent or recurrent fever lasting 4 to 7 days when the duration of neutropenia is anticipated to persist beyond 7 days.[47] Recently published pediatric-specific guidelines have supported the initiation of empirical antifungal therapy in patients at high risk for invasive fungal infection after 96 hours of fever and neutropenia that is not responsive to broad-spectrum antibiotics when the duration of neutropenia is anticipated to last longer than 10 days.[75] In patients at low risk for invasive fungal infection it is reasonable to withhold empirical antimold therapy.

When initiating an empirical antimold agent, options include an amphotericin product (amphotericin B deoxycholate, lipid amphotericin, or liposomal amphotericin), an echinocandin (anidulafungin, caspofungin, or micafungin), or an azole (voriconazole or posaconazole). Pediatric prospective randomized trials have compared caspofungin to liposomal amphotericin[80] and the latter to amphotericin B deoxycholate.[102] Although similar relative to efficacy outcomes, significantly fewer side effects were associated with liposomal amphotericin in contrast to amphotericin B deoxycholate. Safety and efficacy comparisons showed no difference between caspofungin and liposomal amphotericin. Given these data, caspofungin or liposomal amphotericin are both appropriate first-line agents for empirical antifungal therapy. Second-generation triazoles represent alternative options for empirical mold therapy; however, the lack of pediatric-specific data for these agents limits the feasibility of their use. Although voriconazole dosing recommendations are available for children 2 years of age and older, the optimal dosing regimen has not yet been defined and intrapatient and interpatient variability in voriconazole pharmacokinetics among the pediatric population is significant.[64,118] Dosing recommendations for posaconazole do not exist for those younger than 13 years of age. Finally, neither voriconazole nor posaconazole have been specifically studied as an empirical antimold therapeutic agent in children.

The empirical antifungal approach for prolonged fever and neutropenia has been challenged because of the lack of specificity of prolonged fever and neutropenia as a marker for invasive fungal infection, the potential to unnecessarily expose patients to antifungal agents who do not have an invasive fungal infection, and the increased cost in administering these agents for prolonged periods.[33,84] Although designation of high- and low-risk invasive fungal infection status has reduced some unnecessary empirical therapy, it has not eliminated it. As noted previously, an alternative approach to empirical antifungal therapy is a preemptive strategy. In this approach, the results of a combination of diagnostic studies (e.g., fungal biomarkers and CT scans) are used to inform the initiation of antifungal therapy. In a randomized trial of high-risk adult patients with fever and neutropenia, Cordonnier and colleagues[27] showed that a preemptive approach reduced the cost associated with antifungal use without a change in mortality. However, the preemptive approach was associated with a higher rate of invasive fungal infection. Further refinement of a preemptive approach with a focus on pediatric cohorts is necessary before such a strategy can be adopted in children.

The appropriate time to discontinue empirical antifungal therapy has not been well defined. A conservative approach is to continue until resolution of neutropenia; this approach is supported by a recently published pediatric guideline.[76] Some clinicians perform CT scans at the time of neutropenia resolution and use this information to inform cessation of antifungal therapy. However, no data exist to document the clinical impact of this approach. Future research should focus on identifying the optimal criteria for cessation of antifungal treatment in the empirical setting.

PREVENTION MEASURES

Antibacterial Prophylaxis

Much effort has focused on determining the efficacy and safety of antibacterial prophylaxis in patients with cancer. In 2005, two large, randomized, placebo-controlled trials evaluated the efficacy of levofloxacin for preventing fever in patients with anticipated short and long periods of neutropenia.[18,30] These trials discovered that levofloxacin prophylaxis reduced the rates of fever and bacteremia during periods of anticipated prolonged neutropenia[18] and similarly reduced the rate of fever and infection during anticipated short periods of neutropenia.[30] Further, a meta-analysis of 95 randomized trials revealed a significant reduction in mortality when antibiotic prophylaxis was administered to neutropenic patients.[48] Based on these data, multiple guidelines for adults support the use of fluoroquinolone prophylaxis.[47,73] However, the limited pediatric data regarding the utility of antibacterial

prophylaxis[4] has precluded a similar pediatric recommendation. Furthermore, potential negative consequences associated with prophylaxis, such as an increase in resistance among colonizing organisms, or precipitation of *Clostridium difficile* infection, were not adequately evaluated in the aforementioned large trials in adults. Currently, the Children's Oncology Group is conducting a trial to investigate the benefits of levofloxacin prophylaxis in high-risk children with neutropenia and intends to evaluate some of these important secondary outcomes.

Antifungal Prophylaxis

Prophylactic antifungal therapy also has been explored as a mechanism to reduce invasive fungal infection in patients with neutropenia. The earliest trials in adults showed fluconazole was efficacious in contrast to placebo in reducing systemic and superficial fungal infections in bone marrow transplant recipients.[50,121] A subsequent meta-analysis suggested that the benefit of prophylactic fluconazole was realized in the setting of increased risk (>15%) for systemic fungal infection.[62] This meta-analysis appropriately highlighted the need to consider the prevalence of invasive fungal infection when considering the impact of antifungal prophylaxis in a specific patient population. More recently, agents with antimold activity have been evaluated in the prophylactic setting. Vehreschild and colleagues[137] showed, in a small cohort of adult AML patients, that voriconazole in contrast to placebo was efficacious in reducing the rate of pulmonary infiltrates.[137] In additional clinical trials involving predominately adults, fluconazole has been compared to posaconazole in patients with prolonged neutropenia,[28] to voriconazole in allogeneic stem cell transplant recipients,[140] and to micafungin in stem cell transplant recipients.[135] The former study revealed that posaconazole was efficacious at reducing invasive aspergillosis, whereas the latter studies suggested that voriconazole and micafungin prophylaxis trended toward improved protection against invasive aspergillosis in contrast to fluconazole. Collectively, these data support guideline recommendations in adults to administer antifungal prophylaxis in patients considered at high risk for invasive fungal infection. However, the guidelines for adults were not consistent as to when to administer fluconazole and when to escalate to antimold prophylaxis.[47,73]

Pediatric-specific data for antifungal prophylaxis from randomized clinical trials are not available. Based on the available data in adults, most pediatric experts would support the initiation of fluconazole prophylaxis in patients at high risk for invasive fungal infection.[38] The benefits of broadening prophylaxis to include antimold coverage in a pediatric population are not yet well defined. Currently, a randomized trial sponsored by the Children's Oncology Group is actively enrolling patients to compare the efficacy of caspofungin to fluconazole prophylaxis in pediatric patients with AML. The outcome of this study should inform the importance of antimold activity in a fungal prophylaxis regimen for high-risk pediatric patients.

Hospital Infection Control Practices

Children receiving chemotherapy represent a patient population at high risk for hospital-acquired infection (HAI) because they often require prolonged hospital admissions during which they are immunosuppressed. Recently, McCullers and colleagues[86] documented the impact of HAIs at a single institution. Not unexpectedly depth (<100/mm^3) and duration of neutropenia, as well as prolonged length of stay were identified as risk factors for HAI. Over the 26-year study period they documented a decrease in the rate of HAIs that correlated with the implementation of various infection control initiatives.

These data strongly support the potential impact of infection control practices in reducing HAI even in high-risk patient populations. Important infection control practices often focus on interrupting the transmission of infectious pathogens from health care workers to their patients. Such practices include programs to promote high compliance of hand hygiene, enforcement of work restriction at times of health care worker illness, and optimization of health care worker influenza vaccination.[16,42,119] Enacting a mandatory vaccination policy has proved to result in near complete health care worker vaccine coverage at a large children's hospital.[42] Finally, use of a protocol to screen visitors before entering the patient room can reduce unnecessary patient exposures.

Despite the importance of infection control practices, significant variation likely exists in policies across institutions and in enforcement of policies within institutions. Future efforts are needed to establish goals for reducing HAIs and guidelines for standardized infection control practices specific to pediatric oncology patients to achieve these goals. Ultimately, the institution of any standardized infection control practice should be accompanied by an institutional-level surveillance of HAIs to monitor the effectiveness of each intervention.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Adamkiewicz TV, Lorenzana A, Doyle J, Richardson S. Peripheral vs. central blood cultures in patients admitted to a pediatric oncology ward. *Pediatr Infect Dis J* 1999;**18**:556–8.
2. Agrawal AK, Saini N, Gildengorin G, Feusner JH. Is routine computed tomographic scanning justified in the first week of persistent febrile neutropenia in children with malignancies? *Pediatr Blood Cancer* 2011;**57**:620–4.
3. Ahmed N, El-Mahallawy HA, Ahmed IA, et al. Early hospital discharge versus continued hospitalization in febrile pediatric cancer patients with prolonged neutropenia: a randomized, prospective study. *Pediatr Blood Cancer* 2007;**49**:786–92.
4. Alexander S, Wade KC, Hibberd PL, Parsons SK. Evaluation of risk prediction criteria for episodes of febrile neutropenia in children with cancer. *J Ped Hematol Oncol* 2002;**24**:38–42.
5. Alexander S, Nieder M, Zerr DM, et al. Prevention of bacterial infection in pediatric oncology: What do we know, what can we learn? *Pediatr Blood Cancer* 2012;**59**:16–20.
6. Ammann RA, Hirt A, Lüthy AR, Aebi C. Identification of children presenting with fever in chemotherapy-induced neutropenia at low risk for severe bacterial infection. *Med Pediatr Oncol* 2003; **41**:436–43.
7. Ammann RA, Bodmer N, Hirt A, et al. Predicting adverse events in children with fever and chemotherapy-induced neutropenia: the prospective multicenter SPOG 2003 FN study. *J Clin Oncol* 2010;**28**:2008–14.
8. Aquino VM, Tkaczewski I, Buchanan GR. Early discharge of low-risk febrile neutropenic children and adolescents with cancer. *Clin Infect Dis* 1997;**25**:74–8.

9. Aquino VM, Buchanan GR, Tkaczewski I, Mustafa MM. Safety of early hospital discharge of selected febrile children and adolescents with cancer with prolonged neutropenia. *Med Pediatr Oncol* 1997;**28**:191–5.
10. Arendrup MC, Fisher BT, Zaoutis TE. Invasive fungal infections in the paediatric and neonatal population: diagnostics and management issues. *Clin Microbiol Infect* 2009;**15**:613–24.
11. Ariffin H, Navaratnam P, Lin HP. Surveillance study of bacteraemic episodes in febrile neutropenic children. *Int J Clin Pract* 2002;**56**:237–40.
12. Armenian SH, Nash KA, Kapoor N, et al. Prospective monitoring for invasive aspergillosis using galactomannan and polymerase chain reaction in high risk pediatric patients. *J Pediatr Hematol Oncol* 2009;**31**:920–6.
13. Arola M, Ruuskanen O, Ziegler T, Salmi TT. Respiratory virus infections during anticancer treatment in children. *Pediatr Infect Dis J* 1995;**14**:690–4.
14. Baorto EP, Aquino VM, Mullen CA, Buchanan GR, DeBaun MR. Clinical parameters associated with low bacteremia risk in 1100 pediatric oncology patients with fever and neutropenia. *Cancer* 2001;**92**:909–13.
15. Benjamin DK, Miller WC, Bayliff S, et al. Infections diagnosed in the first year after pediatric stem cell transplantation. *Pediatr Infect Dis J* 2002;**21**:227–34.
16. Bolyard EA, Tablan OC, Williams WW, et al. Guideline for infection control in healthcare personnel, 1998: Hospital Infection Control Practices Advisory Committee. *Infect Control Hosp Epidemiol* 1998;**19**:407–63.
17. Brack E, Bodmer N, Simon A, et al. First-day step-down to oral outpatient treatment versus continued standard treatment in children with cancer and low-risk fever in neutropenia: a randomized controlled trial within the multicenter SPOG 2003 FN study. *Pediatr Blood Cancer* 2012;**59**:423–30.
18. Bucaneve G, Micozzi A, Menichetti F, et al. Levofloxacin to prevent bacterial infection in patients with cancer and neutropenia. *N Engl J Med* 2005;**353**:977–87.
19. Cagol AR, Castro Junior CG de, Martins MC, et al. Oral vs. intravenous empirical antimicrobial therapy in febrile neutropenic patients receiving childhood cancer chemotherapy. *J Pediatr (Rio J)* 2009;**85**:531–5.
20. Caillot D, Casasnovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* 1997;**15**:139–47.
21. Castagnola E, Fontana V, Caviglia I, et al. A prospective study on the epidemiology of febrile episodes during chemotherapy-induced neutropenia in children with cancer or after hemopoietic stem cell transplantation. *Clin Infect Dis* 2007;**45**:1296–304.
22. Chen W-T, Liu T-M, Wu S-H, et al. Improving diagnosis of central venous catheter-related bloodstream infection by using differential time to positivity as a hospital-wide approach at a cancer hospital. *J Infect* 2009;**59**:317–23.
23. Chow A, Robinson JL. Fever of unknown origin in children: a systematic review. *World J Pediatr* 2011;**7**:5–10.
24. Christensen MS, Nielsen LP, Hasle H. Few but severe viral infections in children with cancer: a prospective RT-PCR and PCR-based 12-month study. *Pediatr Blood Cancer* 2005;**45**:945–51.
25. Cohen KJ, Leamer K, Odom L, Greffe B, Stork L. Cessation of antibiotics regardless of ANC is safe in children with febrile neutropenia. A preliminary prospective trial. *J Pediatr Hematol Oncol* 1995;**17**:325–30.
26. Cometta A, Kern WV, De Bock R, et al. Vancomycin versus placebo for treating persistent fever in patients with neutropenic cancer receiving piperacillin-tazobactam monotherapy. *Clin Infect Dis* 2003;**37**:382–9.
27. Cordonnier C, Pautas C, Maury S, et al. Empirical versus preemptive antifungal therapy for high-risk, febrile, neutropenic patients: a randomized, controlled trial. *Clin Infect Dis* 2009;**48**:1042–51.
28. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007;**356**:348–59.
29. Creutzig U, Zimmermann M, Reinhardt D, et al. Early deaths and treatment-related mortality in children undergoing therapy for acute myeloid leukemia: analysis of the multicenter clinical trials AML-BFM 93 and AML-BFM 98. *J Clin Oncol* 2004;**22**:4384–93.
30. Cullen M, Steven N, Billingham L, et al. Antibacterial prophylaxis after chemotherapy for solid tumors and lymphomas. *N Engl J Med* 2005;**353**:988–98.
31. De Naurois J, Novitzky-Basso I, Gill M. Management of febrile neutropenia: ESMO clinical practice guidelines. *Ann Oncol* 2010;**21**(suppl 5):v252–6.
32. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**:1813–21.
33. de Pauw BE. Between over- and undertreatment of invasive fungal disease. *Clin Infect Dis* 2005;**41**:1251–3.
34. Del Favero A, Menichetti F, Bucaneve G, Minotti V, Pauluzzi S. Septicaemia due to gram-positive cocci in cancer patients. *J Antimicrob Chemother* 1988;**21**(suppl C):157–65.
35. DesJardin JA, Falagas ME, Ruthazer R, et al. Clinical utility of blood cultures drawn from indwelling central venous catheters in hospitalized patients with cancer. *Ann Intern Med* 1999;**131**:641–7.
36. Diorio C, Martino J, Boydell KM, et al. Parental perspectives on inpatient versus outpatient management of pediatric febrile neutropenia. *J Pediatr Oncol Nurs* 2011;**28**:355–62.
37. Dvorak CC, Steinbach WJ, Brown JMY, Agarwal R. Risks and outcomes of invasive fungal infections in pediatric patients undergoing allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant* 2005;**36**:621–9.
38. Dvorak CC, Fisher BT, Sung L, et al. Antifungal prophylaxis in pediatric hematology/oncology: new choices & new data. *Pediatr Blood Cancer* 2012;**59**:21–6.
39. El-Mahallawy HA, Attia I, Ali-el-Din NH, et al. A prospective study on fungal infection in children with cancer. *J Med Microbiol* 2002;**51**:601–5.
40. El-Mahallawy HA, Shaker HH, Ali Helmy H, et al. Evaluation of pan-fungal PCR assay and *Aspergillus* antigen detection in the diagnosis of invasive fungal infections in high risk paediatric cancer patients. *Med Mycol* 2006;**44**:733–9.
41. Ellis M, Al-Ramadi B, Finkelman M, et al. Assessment of the clinical utility of serial beta-D-glucan concentrations in patients with persistent neutropenic fever. *J Med Microbiol* 2008;**57**(Pt 3):287–95.
42. Feemster KA, Prasad P, Smith MJ, et al. Employee designation and health care worker support of an influenza vaccine mandate at a large pediatric tertiary care hospital. *Vaccine* 2011;**29**:1762–9.
43. Feusner J, Cohen R, O'Leary M, Beach B. Use of routine chest radiography in the evaluation of fever in neutropenic pediatric oncology patients. *J Clin Oncol* 1988;**6**:1699–702.
44. Fisher BT, Aplenc R, Localio R, et al. Cefepime and mortality in pediatric acute myelogenous leukemia: a retrospective cohort study. *Pediatr Infect Dis J* 2009;**28**:971–5.
45. Fisher BT, Zaoutis TE, Park JR, et al. Galactomannan antigen testing for diagnosis of invasive aspergillosis in pediatric hematology patients. *J Pediatr Infect Dis Soc* 2012;**1**:103–11.
46. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**52**:e56–93.
47. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**52**:427–31.
48. Gafter-Gvili A, Fraser A, Paul M, Leibovici L. Meta-analysis: antibiotic prophylaxis reduces mortality in neutropenic patients. *Ann Intern Med* 2005;**142**(12 Pt 1):979–95.
49. Georgiadou SP, Pongas G, Fitzgerald NE, et al. Invasive mold infections in pediatric cancer patients reflect heterogeneity in etiology, presentation, and outcome: a 10-year, single-institution, retrospective study. *J Pediatr Infect Dis Soc* 2012;**1**:125–35.
50. Goodman JL, Winston DJ, Greenfield RA, et al. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992;**326**:845–51.
51. Grigull L, Beier R, Schrauder A, et al. Invasive fungal infections are responsible for one-fifth of the infectious deaths in children with ALL. *Mycoses* 2003;**46**:441–6.

52. Groll AH, Kurz M, Schneider W, et al. Five-year-survey of invasive aspergillosis in a paediatric cancer centre: epidemiology, management and long-term survival. *Mycoses* 1999;**42**:431–42.
53. Gupta A, Swaroop C, Agarwala S, et al. Randomized controlled trial comparing oral amoxicillin-clavulanate and ofloxacin with intravenous ceftriaxone and amikacin as outpatient therapy in pediatric low-risk febrile neutropenia. *J Pediatr Hematol Oncol* 2009;**31**:635–41.
54. Hakim H, Flynn PM, Knapp KM, et al. Etiology and clinical course of febrile neutropenia in children with cancer. *J Pediatr Hematol Oncol* 2009;**31**:623–9.
55. Hayden R, Pounds S, Knapp K, et al. Galactomannan antigenemia in pediatric oncology patients with invasive aspergillosis. *Pediatr Infect Dis J* 2008;**27**:815–19.
56. Hersh EM, Bodey GP, Nies BA, Freireich EJ. Causes of death in acute leukemia: a ten-year study of 414 patients from 1954–1963. *JAMA* 1965;**193**:105–9.
57. Heussel CP, Kauczor HU, Heussel GE, et al. Pneumonia in febrile neutropenic patients and in bone marrow and blood stem-cell transplant recipients: use of high-resolution computed tomography. *J Clin Oncol* 1999;**17**:796–805.
58. Hovi L, Saarinen-Pihkala UM, Vettenranta K, Saxen H. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. *Bone Marrow Transplant* 2000;**26**:999–1004.
59. Hovi L, Saxen H, Saarinen-Pihkala UM, et al. Prevention and monitoring of invasive fungal infections in pediatric patients with cancer and hematologic disorders. *Pediatr Blood Cancer* 2007;**48**:28–34.
60. Hughes WT. Fatal infections in childhood leukemia. *Am J Dis Child* 1971;**122**:283–7.
61. Jordanides NE, Allan EK, McLintock LA, et al. A prospective study of real-time panfungal PCR for the early diagnosis of invasive fungal infection in haemato-oncology patients. *Bone Marrow Transplant* 2005;**35**:389–95.
62. Kanda Y, Yamamoto R, Chizuka A, et al. Prophylactic action of oral fluconazole against fungal infection in neutropenic patients: a meta-analysis of 16 randomized, controlled trials. *Cancer* 2000;**89**:1611–25.
63. Kaplan AH, Weber DJ, Davis L, et al. Short courses of antibiotics in selected febrile neutropenic patients. *Am J Med Sci* 1991;**302**:353–4.
64. Karlsson MO, Lutsar I, Milligan PA. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.
65. Katz JA, Bash R, Rollins N, Cash J, Buchanan GR. The yield of routine chest radiography in children with cancer hospitalized for fever and neutropenia. *Cancer* 1991;**68**:940–3.
66. Kim PW, Wu Y-T, Cooper C, et al. Meta-analysis of a possible signal of increased mortality associated with cefepime use. *Clin Infect Dis* 2010;**51**:381–9.
67. Klaassen ILM, de Haas V, van Wijk JAE, et al. Pyuria is absent during urinary tract infections in neutropenic patients. *Pediatr Blood Cancer* 2011;**56**:868–70.
68. Klaassen RJ, Goodman TR, Pham B, Doyle JJ. "Low-risk" prediction rule for pediatric oncology patients presenting with fever and neutropenia. *J Clin Oncol* 2000;**18**:1012–19.
69. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis* 2010;**50**:1091–100.
70. Korones DN, Hussong MR, Gullace MA. Routine chest radiography of children with cancer hospitalized for fever and neutropenia: is it really necessary? *Cancer* 1997;**80**:1160–4.
71. Koskenvuo M, Möttönen M, Rahiala J, et al. Respiratory viral infections in children with leukemia. *Pediatr Infect Dis J* 2008;**27**:974–80.
72. Leahey AM, Bunin NJ, Belasco JB, et al. Novel multiagent chemotherapy for bone marrow relapse of pediatric acute lymphoblastic leukemia. *Med Pediatr Oncol* 2000;**34**:313–18.
73. Lee D-G, Kim S-H, Kim SY, et al. Evidence-based guidelines for empirical therapy of neutropenic fever in Korea. *Korean J Intern Med* 2011;**26**:220–52.
74. Lehrnbecher T, Stanescu A, Kühl J. Short courses of intravenous empirical antibiotic treatment in selected febrile neutropenic children with cancer. *Infection* 2002;**30**:17–21.
75. Lehrnbecher T, Varwig D, Kaiser J, et al. Infectious complications in pediatric acute myeloid leukemia: analysis of the prospective multi-institutional clinical trial AML-BFM 93. *Leukemia* 2004;**18**:72–7.
76. Lehrnbecher T, Phillips RS, Alexander S, et al. Guidelines for the management of fever and neutropenia in children with cancer and/or undergoing hematopoietic stem cell transplantation. *J Clin Oncol* 2012;**30**:4427–38.
77. Long DR, Craft AW, Kernahan J, et al. Virus infections in childhood malignant disease. *Pediatr Hematol Oncol* 1987;**4**:283–92.
78. Macher E, Dubos F, Garnier N, et al. Predicting the risk of severe bacterial infection in children with chemotherapy-induced febrile neutropenia. *Pediatr Blood Cancer* 2010;**55**:662–7.
79. Madsen K, Rosenman M, Hui S, Breitfeld PP. Value of electronic data for model validation and refinement: bacteremia risk in children with fever and neutropenia. *J Pediatr Hematol Oncol* 2002;**24**:256–62.
80. Maertens JA, Madero L, Reilly AF, et al. A randomized, double-blind, multicenter study of caspofungin versus liposomal amphotericin b for empiric antifungal therapy in pediatric patients with persistent fever and neutropenia. *Pediatr Infect Dis J* 2010;**29**:415–20.
81. Manji A, Lehrnbecher T, Dupuis LL, et al. A meta-analysis of anti-pseudomonal penicillins and cephalosporins in pediatric patients with fever and neutropenia. *Pediatr Infect Dis J* 2011;**31**:353–8.
82. Manji A, Lehrnbecher T, Dupuis LL, et al. A systematic review and meta-analysis of anti-pseudomonal penicillins and carbapenems in pediatric febrile neutropenia. *Support Care Cancer* 2012;**20**:2295–304.
83. Manji A, Beyene J, Dupuis LL, et al. Outpatient and oral antibiotic management of low-risk febrile neutropenia are effective in children-a systematic review of prospective trials. *Support Care Cancer* 2012;**20**:1135–45.
84. Marchetti O, Cordonnier C, Calandra T. Empirical antifungal therapy in neutropaenic cancer patients with persistent fever. *Eur J Cancer Suppl* 2007;**5**:32–42.
85. Marty FM, Koo S. Role of (1–>3)-beta-D-glucan in the diagnosis of invasive aspergillosis. *Med Mycol* 2009;**47**(suppl 1):S233–40.
86. McCullers JA, Williams BF, Wu S, et al. Healthcare-associated infections at a children's cancer hospital, 1983–2008. *J Pediatr Infect Dis Soc* 2012;**1**:26–34.
87. Miedema KG, de Bont ES, Oude Nijhuis CS, et al. Validation of a new risk assessment model for predicting adverse events in children with fever and chemotherapy-induced neutropenia. *J Clin Oncol* 2011;**29**:e182–4, author reply e185.
88. Mularoni A, Furfaro E, Faraci M, et al. High levels of beta-D-glucan in immunocompromised children with proven invasive fungal disease. *Clin Vaccine Immunol* 2010;**17**:882–3.
89. Mullen CA, Buchanan GR. Early hospital discharge of children with cancer treated for fever and neutropenia: identification and management of the low-risk patient. *J Clin Oncol* 1990;**8**:1998–2004.
90. Mullen CA, Petropoulos D, Roberts WM, et al. Outpatient treatment of fever and neutropenia for low risk pediatric cancer patients. *Cancer* 1999;**86**:126–34.
91. Odabasi Z, Mattiuzzi G, Estey E, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004;**39**:199–205.
92. Paganini H, Gómez S, Ruvinsky S, et al. Outpatient, sequential, parenteral-oral antibiotic therapy for lower risk febrile neutropenia in children with malignant disease: a single-center, randomized, controlled trial in Argentina. *Cancer* 2003;**97**:1775–80.
93. Paul M, Borok S, Fraser A, et al. Empirical antibiotics against Gram-positive infections for febrile neutropenia: systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother* 2005;**55**:436–44.
94. Paul M, Yahav D, Bivas A, et al. Anti-pseudomonal beta-lactams for the initial, empirical, treatment of febrile neutropenia:

comparison of beta-lactams. *Cochrane Database Syst Rev* 2010;(1):CD005197.
95. Petrilli AS, Dantas LS, Campos MC, et al. Oral ciprofloxacin vs. intravenous ceftriaxone administered in an outpatient setting for fever and neutropenia in low-risk pediatric oncology patients: randomized prospective trial. *Med Pediatr Oncol* 2000;**34**:87–91.
96. Phillips B, Wade R, Stewart LA, Sutton AJ. Systematic review and meta-analysis of the discriminatory performance of risk prediction rules in febrile neutropaenic episodes in children and young people. *Eur J Cancer* 2010;**46**:2950–64.
97. Phillips RS, Lehrnbecher T, Alexander S, Sung L. Updated systematic review and meta-analysis of the performance of risk prediction rules in children and young people with febrile neutropenia. *PLoS ONE* 2012;**7**:e38300.
98. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. *Crit Care* 2010;**14**:R15.
99. Pizzo PA, Robichaud KJ, Gill FA, Witebsky FG. Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am J Med* 1982;**72**:101–11.
100. Pizzo PA, Robichaud KJ, Wesley R, Commers JR. Fever in the pediatric and young adult patient with cancer: a prospective study of 1001 episodes. *Medicine* 1982;**61**:153–65.
101. Pizzo PA, Robichaud KJ, Gill FA, et al. Duration of empiric antibiotic therapy in granulocytopenic patients with cancer. *Am J Med* 1979;**67**:194–200.
102. Prentice HG, Hann IM, Herbrecht R, et al. A randomized comparison of liposomal versus conventional amphotericin B for the treatment of pyrexia of unknown origin in neutropenic patients. *Br J Haematol* 1997;**98**:711–18.
103. Quezada G, Sunderland T, Chan K-W, et al. Medical and nonmedical barriers to outpatient treatment of fever and neutropenia in children with cancer. *Pediatr Blood Cancer* 2007;**48**:273–7.
104. Raad I, Hanna HA, Alakech B, et al. Differential time to positivity: a useful method for diagnosing catheter-related bloodstream infections. *Ann Intern Med* 2004;**140**:18–25.
105. Rackoff WR, Gonin R, Robinson C, Kreissman SG, Breitfeld PB. Predicting the risk of bacteremia in children with fever and neutropenia. *J Clin Oncol* 1996;**14**:919–24.
106. Ravindranath Y. Recent advances in pediatric acute lymphoblastic and myeloid leukemia. *Curr Opin Oncol* 2003;**15**:23–35.
107. Reagan JL, Castillo JJ. Why is my patient neutropenic? *Hematol Oncol Clin North Am* 2012;**26**:253–66.
108. Renoult E, Buteau C, Turgeon N, et al. Is routine chest radiography necessary for the initial evaluation of fever in neutropenic children with cancer? *Pediatr Blood Cancer* 2004;**43**:224–8.
109. Rodríguez L, Ethier M-C, Phillips B, et al. Utility of peripheral blood cultures in patients with cancer and suspected blood stream infections: a systematic review. *Support Care Cancer* 2012;**28**:326–7.
110. Rohrlich P, Sarfati J, Mariani P, et al. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. *Pediatr Infect Dis J* 1996;**15**:232–7.
111. Rondinelli PIP, Ribeiro K de CB, de Camargo B. A proposed score for predicting severe infection complications in children with chemotherapy-induced febrile neutropenia. *J Pediatr Hematol Oncol* 2006;**28**:665–70.
112. Santolaya ME, Alvarez AM, Avilés CL, et al. Early hospital discharge followed by outpatient management versus continued hospitalization of children with cancer, fever, and neutropenia at low risk for invasive bacterial infection. *J Clin Oncol* 2004;**22**: 3784–89.
113. Santolaya ME, Alvarez AM, Avilés CL, et al. Prospective evaluation of a model of prediction of invasive bacterial infection risk among children with cancer, fever, and neutropenia. *Clin Infect Dis* 2002;**35**:678–83.
114. Santolaya ME, Alvarez AM, Becker A, et al. Prospective, multicenter evaluation of risk factors associated with invasive bacterial infection in children with cancer, neutropenia, and fever. *J Clin Oncol* 2001;**19**:3415–21.
115. Santolaya ME, Villarroel M, Avendaño LF, Cofré J. Discontinuation of antimicrobial therapy for febrile, neutropenic children with cancer: a prospective study. *Clin Infect Dis* 1997;**25**:92–7.
116. Scheinemann K, Ethier M-C, Dupuis LL, et al. Utility of peripheral blood cultures in bacteremic pediatric cancer patients with a central line. *Support Care Cancer* 2010;**18**:913–19.
117. Schimpff S, Satterlee W, Young VM, Serpick A. Empiric therapy with carbenicillin and gentamicin for febrile patients with cancer and granulocytopenia. *N Engl J Med* 1971;**284**:1061–5.
118. Shima H, Miharu M, Osumi T, et al. Differences in voriconazole trough plasma concentrations per oral dosages between children younger and older than 3 years of age. *Pediatr Blood Cancer* 2010;**54**:1050–2.
119. Siegel JH, Korniewicz DM. Keeping patients safe: an interventional hand hygiene study at an oncology center. *Clin J Oncol Nurs* 2007;**11**:643–6.
120. Singer C, Kaplan MH, Armstrong D. Bacteremia and fungemia complicating neoplastic disease: a study of 364 cases. *Am J Med* 1977;**62**:731–42.
121. Slavin MA, Osborne B, Adams R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation: a prospective, randomized, double-blind study. *J Infect Dis* 1995;**171**:1545–52.
122. Smith PB, Benjamin DK, Alexander BD, et al. Quantification of 1,3-beta-D-glucan levels in children: preliminary data for diagnostic use of the beta-glucan assay in a pediatric setting. *Clin Vaccine Immunol* 2007;**14**:924–5.
123. Speyer E, Herbinet A, Vuillemin A, et al. Agreement between children with cancer and their parents in reporting the child's health-related quality of life during a stay at the hospital and at home. *Child Care Health Dev* 2009;**35**:489–95.
124. Steinbach WJ, Addison RM, McLaughlin L, et al. Prospective *Aspergillus* galactomannan antigen testing in pediatric hematopoietic stem cell transplant recipients. *Pediatr Infect Dis J* 2007;**26**:558–64.
125. Sulahian A, Boutboul F, Ribaud P, et al. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* 2001;**91**:311–8.
126. Sung L, Phillips R, Lehrnbecher T. Time for paediatric febrile neutropenia guidelines—children are not little adults. *Eur J Cancer* 2011;**47**:811–13.
127. Sung L, Lange BJ, Gerbing RB, et al. Microbiologically documented infections and infection-related mortality in children with acute myeloid leukemia. *Blood* 2007;**110**:3532–9.
128. Sung L, Gamis A, Alonzo TA, et al. Infections and association with different intensity of chemotherapy in children with acute myeloid leukemia. *Cancer* 2009;**115**:1100–8.
129. Teuffel O, Amir E, Alibhai SMH, et al. Cost-effectiveness of outpatient management for febrile neutropenia in children with cancer. *Pediatrics* 2011;**127**:e279–86.
130. Teuffel O, Ethier MC, Alibhai SMH, et al. Outpatient management of cancer patients with febrile neutropenia: a systematic review and meta-analysis. *Ann Oncol* 2011;**22**:2358–65.
131. Thomson B, Park JR, Felgenhauer J, et al. Toxicity and efficacy of intensive chemotherapy for children with acute lymphoblastic leukemia (ALL) after first bone marrow or extramedullary relapse. *Pediatr Blood Cancer* 2004;**43**:571–9.
132. Towne TG, Lewis JS, Echevarria K. Efficacy and safety of cefepime. *Lancet Infect Dis* 2009;**9**:4–6; author reply 6–7.
133. Urbini B, Castellini C, Rondelli R, et al. Cryptococcal meningitis during front-line chemotherapy for acute lymphoblastic leukemia. *Haematologica* 2000;**85**:1103–4.
134. van Burik J-AH, Carter SL, Freifeld AG, et al. Higher risk of cytomegalovirus and *Aspergillus* infections in recipients of T cell-depleted unrelated bone marrow: analysis of infectious complications in patients treated with T cell depletion versus immunosuppressive therapy to prevent graft-versus-host disease. *Biol Blood Marrow Transplant* 2007;**13**:1487–98.
135. van Burik J-AH, Ratanatharathorn V, Stepan DE, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. *Clin Infect Dis* 2004;**39**:1407–16.
136. Vardakas KZ, Samonis G, Chrysanthopoulou SA, et al. Role of glycopeptides as part of initial empirical treatment of febrile neutropenic patients: a meta-analysis of randomised controlled trials. *Lancet Infect Dis* 2005;**5**:431–9.
137. Vehreschild JJ, Böhme A, Buchheidt D, et al. A double-blind trial on prophylactic voriconazole (VRC) or placebo during induction chemotherapy for acute myelogenous leukaemia (AML). *J Infect* 2007;**55**:445–9.

138. Villarroel M, Avilés CL, Silva P, et al. Risk factors associated with invasive fungal disease in children with cancer and febrile neutropenia. *Pediatr Infect Dis J* 2010;**29**:816–21.
139. Viscoli C, Van der Auwera P, Meunier F. Gram-positive infections in granulocytopenic patients: an important issue? *J Antimicrob Chemother* 1988;**21**(suppl C):149–56.
140. Wingard JR, Carter SL, Walsh TJ, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood* 2010;**116**:5111–18.
141. Woods WG. Curing childhood acute myeloid leukemia (AML) at the half-way point: promises to keep and miles to go before we sleep. *Pediatr Blood Cancer* 2006;**46**:565–9.
142. Yahav D, Paul M, Fraser A, et al. Efficacy and safety of cefepime: a systematic review and meta-analysis. *Lancet Infect Dis* 2007;**7**:338–48.
143. Zaoutis TE, Argon J, Chu J, et al. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin Infect Dis* 2005; **41**:1232–9.
144. Zhao L, Tang J-Y, Wang Y, et al. [Value of plasma beta-glucan in early diagnosis of invasive fungal infection in children]. *Zhongguo Dang Dai Er Ke Za Zhi* 2009;**11**:905–8.
145. Zinner SH. Changing epidemiology of infections in patients with neutropenia and cancer: emphasis on gram-positive and resistant bacteria. *Clin Infect Dis* 1999;**29**:490–4.

CHAPTER 69

Opportunistic Infections in Hematopoietic Stem Cell Transplantation

Christopher C. Dvorak • William J. Steinbach

Advances in supportive care have led to considerable improvements in hematopoietic stem cell transplant (HSCT) outcomes over the last decade. The 1-year transplant-related mortality rate for children with acute leukemia and unrelated donor HSCT performed before 1995 approached 40 percent. More recently, rates from 2003 to 2006 fell by more than half to approximately 15 percent.[106] This has led to significantly more alternative donor HSCTs performed for a wide variety of nonmalignant diseases, including T-cell and phagocyte primary immunodeficiencies, hemoglobinopathies, bone marrow failure syndromes, and metabolic syndromes. However, these children often have relatively intact immune systems at the time of HSCT and thus require increased pre-HSCT immunoablation to prevent graft rejection. This often leads to delays in post-HSCT immune reconstitution, which in turn is associated with significant opportunistic infections. In addition to causing significant morbidity and prolonged hospitalizations, opportunistic infections cause a staggering 37 to 40 percent of transplant-related mortality following allogeneic HSCT.[135]

Evidence indicates that certain opportunistic infections may play a role in the triggering of alloreactivity and graft-versus-host disease (GVHD). For example, animal models suggest that administration of probiotics may alter the intestinal microflora and decrease inflammation in mesenteric lymph nodes, thereby abrogating GVHD.[59] A randomized trial of fluconazole versus placebo demonstrated less severe GVHD of the intestinal tract in the fluconazole recipients, possibly as a result of decreased intestinal antigenic stimulation.[111] Some data suggest that both cytomegalovirus (CMV) and human herpesvirus–6 (HHV-6) reactivations can trigger the development of GVHD.[105,141] Because GVHD is another significant component of transplant-related mortality after allogeneic HSCT, further understanding of the interplay between opportunistic infections and initiation of GVHD may provide another avenue toward making HSCT a safer procedure with improved overall outcomes.

EPIDEMIOLOGY

Although opportunistic infections do occur after autologous HSCT, the much more profound and ongoing

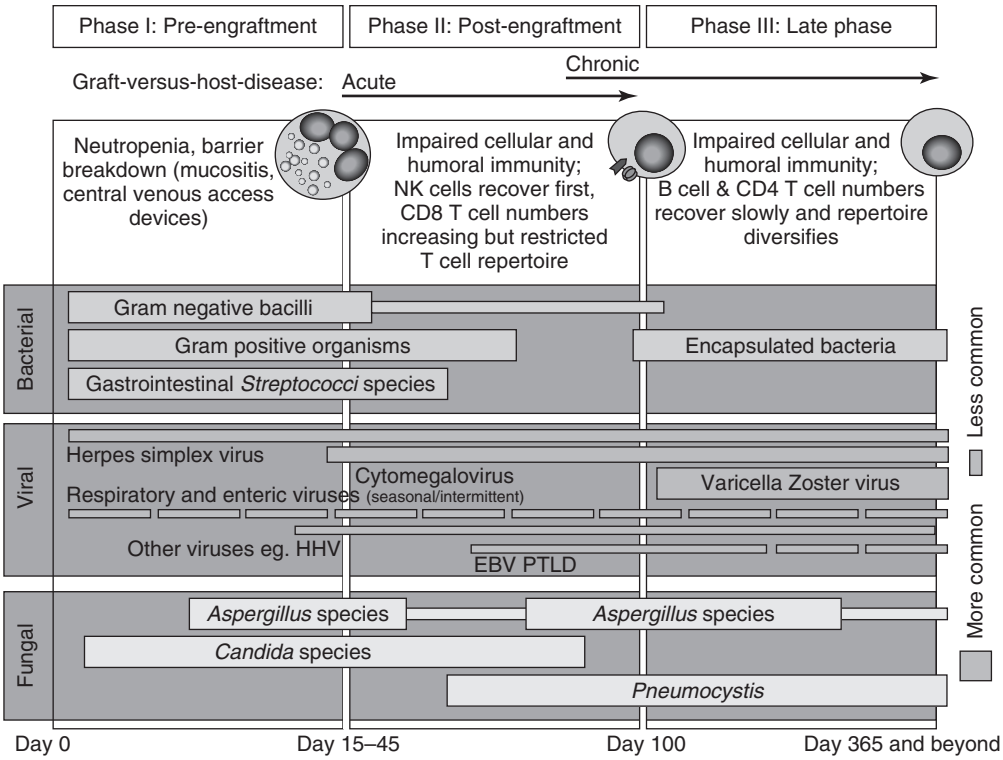

FIGURE 69-1 ■ The phases of opportunistic infections after allogeneic HSCT. *EBV,* Epstein-Barr virus; *HHV,* human herpesvirus; *NK,* natural killer; *PTLD,* posttransplant lymphoproliferative disease. (From Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009;**15**:1143–238. Used with permission from the publisher.)

T-cell dysfunction that occurs before and after allogeneic HSCT makes opportunistic infections far more likely to occur in this population. Multiple therapy-induced alterations of host defenses contribute to this risk.[99] The three major contributors to the development of an opportunistic infection are (1) breakdown in natural barriers (e.g., indwelling central venous catheters and mucositis), (2) defects in cell-mediated immunity (e.g., lymphopenia from corticosteroids and other anti–T-cell cytotoxic agents), and (3) deficient numbers of phagocytes (e.g., as a result of myeloablative chemotherapy). Classically, three phases of risk for opportunistic infections occur after HSCT, as shown in Figure 69-1.[175]

Phase I: Pre-Engraftment (<30 Days)

Infections in phase I are similar to those seen with other forms of profoundly myelosuppressive chemotherapy, and include gram-positive and gram-negative bacteremia, candidemia, invasive aspergillosis, and reactivation of oral herpes simplex virus contributing to mucositis. Administration of granulocyte-colony stimulating factor (G-CSF) during this period will shorten the time to recovery of neutrophils by several days and may decrease rates of documented infections.[46] However, less evidence exists that G-CSF improves infection-related or transplant-related mortality, and it may increase the incidence of acute GVHD.[178] Therefore, only approximately 60 percent of HSCT centers use G-CSF routinely.[96]

Phase II: Early Post-Engraftment (30 to 100 Days)

Some infections in phase II are caused by a combination of persistent defects in the function of the patients' phagocytes (partly from the administration of corticosteroids to treat acute GVHD) and retention of central venous catheters, which can lead to gram-positive bacteremia and candidemia. Other infections are due to the ongoing relative and functional lymphopenia and involve reactivations of CMV and other double-stranded DNA (dsDNA) viruses, *Pneumocystis jiroveci*, or certain parasitic infections.

Phase III: Late Post-Engraftment

Infections in phase III are generally caused by ongoing immunosuppressive therapy for the treatment of chronic GVHD, which also is associated with functional asplenia. Thus, patients can develop infections with encapsulated bacteria, *P. jiroveci*, and ongoing problems with dsDNA viruses. There is also a second important peak of invasive aspergillosis during this time.

Knowledge of the timing of the variety of infections that can occur after HSCT allows clinicians to develop rational approaches to antimicrobial prophylaxis, diagnostic monitoring for infections, and earlier treatment of proved infections.

MAJOR TYPES OF OPPORTUNISTIC INFECTIONS AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION

Bacterial

Classic Gram-Positive and Gram-Negative Bacteria

Bacterial infections after HSCT most commonly divide into gram-positive organisms originating from the skin or gastrointestinal tract, and gram-negative organisms translocating from the gastrointestinal tract. The period of highest risk for bacterial bloodstream infections, especially with enteric gram-negative rods, is the pre-engraftment period,[27] during which the incidence of bacteremia can range from 21 to 34 percent and 21 to 58 percent for patients undergoing autologous and allogeneic HSCT, respectively, although some studies report no difference between the two groups.[24,25,28,29,126] In HSCT patients, bloodstream infections before engraftment are a significant independent predictor of mortality.[1,144] Several studies have demonstrated that either host or donor polymorphisms in genes responsible for immunity may contribute to the risk for bacterial infections.*

Given the significant risk for developing bacteremia, it is surprising that there is little evidence that routine antibacterial prophylaxis plays a role in pediatric HSCT recipients. In adult patients undergoing HSCT, general consensus exists regarding the utility of a prophylactic quinolone with antipseudomonal activity.[175] This derives from data generated by a large contemporary trial of levofloxacin prophylaxis in which 45 percent of enrolled patients were undergoing autologous HSCT. The rates of fever and bacteremia were significantly reduced in patients receiving levofloxacin, but prophylaxis did not have an impact on mortality.[22] Similar results have been reported using ciprofloxacin and vancomycin as a prophylactic regimen for autologous HSCT patients.[54] Unlike autologous HSCT, no contemporary large randomized trials of the use of antibiotic prophylaxis versus no prophylaxis in allogeneic HSCT patients have been conducted. Most existing studies in HSCT patients have included small patient numbers and have compared two prophylactic regimens, as opposed to prophylaxis versus no prophylaxis, and have given rise to variable results.[68,92,138,163,168] All of these studies were done in adult patients; therefore, caution must be used in applying the specific results to pediatric HSCT recipients.

Other approaches to antibacterial prophylaxis use nonsystemic treatments to potentially spare medication toxicities and the development of resistant organisms. Chlorhexidine gluconate is an antiseptic bactericidal to gram-positive and gram-negative bacteria, including multidrug resistant organisms. The mechanism of action involves bacterial membrane disruption; its onset is relatively rapid, and the effect is persistent. A 2 percent chlorhexidine gluconate–impregnated cloth product has been studied as a skin cleansing product in the adult critical care setting, where it has been shown to decrease risk for central line–associated infections by at least 50 percent and new acquisition of multidrug-resistant organisms by 30 to 50 percent.[14,40,142,189] Currently, no peer-reviewed published studies report on the effect of chlorhexidine gluconate bathing on prophylaxis of bloodstream infections in children undergoing HSCT. Similarly, because many cases of bacteremia are seen in the setting of central venous catheters, the use of ethanol lock solutions may potentially decrease central venous catheter colonization without the concern of resistance from antibiotic locks. A randomized, double-blind study in adults receiving chemotherapy or HSCT demonstrated the benefit of ethanol locks versus heparin.[154] Studies of prophylactic ethanol locks in pediatric HSCT recipients are currently lacking.

When patients develop a fever early after transplant, empirical antibacterial treatment is usually started. Although a variety of different regimens exist, they are generally tailored to cover *Streptococcus* spp. and *Staphylococcus* spp., plus enteric gram-negative organisms, based on local susceptibility patterns.

Clostridium difficile

Although hardly unique to the pediatric HSCT population, because of the widespread use of broad-spectrum antibiotics for empirical therapy of febrile neutropenia *Clostridium difficile* associated diarrhea is not uncommon. In adults, up to 15 percent of patients may develop this complication within 30 days of HSCT,[3] although pediatric-specific data are relatively lacking. Prevention of *C. difficile* may potentially be accomplished through the administration of certain strains of probiotics.[117] It is not yet clear that this practice is safe in the immediate post-HSCT phase, during which a combination of neutropenia and compromised intestinal integrity could theoretically lead to the development of bacteremia from the ingested strain.[70] Treatment of *C. difficile*–associated diarrhea in the pediatric HSCT patient is similar to that of the general population, with metronidazole or oral vancomycin, although caution must be used during the conditioning phase of HSCT when metronidazole can enhance toxicities from radiation or busulfan.[67]

Encapsulated Organisms

Patients with chronic GVHD appear to have defects in their splenic function, and thus encapsulated bacteria, such as *Streptococcus pneumoniae*, have been noted to cause significant mortality. Prophylaxis with penicillin appears to diminish this risk,[93] although the optimal duration of treatment is not yet defined. In patients with chronic GVHD, serum immunoglobulin (Ig)G levels should be monitored and, for patients with levels less than 400 mg/dL, administration of intravenous gammaglobulin should be considered.[175]

Mycobacteria

Although less common than invasive fungal infections, infections with mycobacteria must always be considered in the differential diagnosis of pulmonary nodules after

*References 7, 35, 95, 119, 150, 186.

HSCT. In developed countries, classic *Mycobacterium tuberculosis* infections are very rare in children undergoing HSCT, although it always should be considered in patients whose households have recently immigrated.[58] More commonly encountered are environmental atypical nontuberculous mycobacteria (NTM), which can cause infections in the lungs, skin, lymph nodes, and bloodstream. Because of the relative rarity of NTM infections, a general paucity of literature exists in the HSCT population. NTM infections have been reported to occur in as many as 9.7 percent of T-cell depleted and 7 percent of conventional allogeneic HSCTs in adults, although the mortality rate was low.[193] Pediatric HSCT recipients have a somewhat lower rate of NTM infections (3.8%), which occur at a median of 115 days (range, 14 to 269 days) after HSCT.[181]

The exact risk factors associated with the development of NTM infections after allogeneic HSCT are not well characterized. The protective immunity against NTM infections appears to be primarily driven by interferon (IFN)-γ production by T-cells, as evidenced by the high rate of NTM infections in patients with IFN-γ deficiency.[72] Thus, T-cell depletion (ex vivo or with T-cell–depleting antibodies) or treatment of GVHD with immunosuppressants that block IFN-γ might be expected to be risk factors for the development of NTM infections. Similarly, patients with an underlying T-cell immunodeficiency might be colonized with an NTM organism pre-HSCT that puts them at risk for developing NTM disease after HSCT. The optimal treatment of NTM infections is not clear. A wide variety of traditional antibiotics and antituberculosis agents have some activity against NTM organisms, but reports vary on the number of agents that must be used in combination and the duration of optimal therapy. In patients with human immunodeficiency virus (HIV) infection, the general recommendation is to treat most cases of NTM infections for 12 months after establishment of negative cultures.[66] It is not clear that such a prolonged duration is required for HSCT patients who, in the absence of chronic GVHD, tend to have improvement in, and even normalization of, their T-cell function over time.

Fungal

Although bacteria represent the most common infection after HSCT, invasive fungal infections account for a significant amount of posttransplant mortality. Several retrospective reports on the development of invasive fungal infections in pediatric HSCT patients have been published, with a 1-year incidence as high as 13 to 20 percent and a 58 to 83 percent mortality.[12,49,79,152] The most commonly identified invasive fungal organisms after HSCT are *Candida* and *Aspergillus* spp. Patients who are considered to be at the highest risk for developing an invasive fungal infection after HSCT are those who undergo transplant from either an unrelated donor (including umbilical cord blood) or a partially matched related donor or for treatment of a malignancy, bone marrow failure syndrome, or congenital immunodeficiency.*

Currently, use of antifungal prophylaxis is nearly universal in HSCT patients. Empirical therapy directed against resistant *Candida* or molds generally commences after approximately 72 hours of prolonged fevers despite administration of broad-spectrum antibacterials. One surprising feature to note from the original prophylactic fluconazole studies is that, even in the placebo arm, more than 80 percent of HSCT patients did not develop an invasive fungal infection, although these studies did include lower-risk autologous HSCT recipients.[63,164] This suggests that other explanations must exist for the development of invasive fungal infection after HSCT. Researchers are now finding that either host or donor polymorphisms in genes responsible for immunity appear to play a significant role in invasive fungal infections risk.* Most of these proposed genetic risk factors still require validation in a prospective multicenter cohort, but the future possibility of having different prophylactic strategies based on an a priori risk for developing an invasive fungal infection is promising.

Candida

Invasive candidiasis tends to occur during the neutropenic period immediately after HSCT, although later cases can occur in the setting of GVHD and prolonged immunosuppression, especially when central venous catheters are still in place. Typically, invasive candidiasis originates from endogenous *Candida* spp. colonizing patients' gastrointestinal tracts.[42] Pediatric patients appear to have relatively more invasive infections caused by *Candida parapsilosis* and fewer infections caused by *Candida glabrata*, in contrast to adults.[107] Two placebo-controlled trials from the early 1990s, performed in patients older than 12 years of age undergoing autologous or allogeneic HSCT, demonstrated that prophylactic administration of fluconazole significantly decreased invasive fungal infections.[63,163] Long-term follow-up of allogeneic HSCT patients given fluconazole prophylaxis supports a survival benefit (mortality 20% in the fluconazole arm vs. 35% in the placebo arm; $p = .004$), postulated to be at least partly due to less severe GVHD of the gastrointestinal tract in the fluconazole recipients from decreased intestinal antigenic stimulation.[111] The classic duration of fluconazole prophylaxis administration is during the high-risk period until 75 days after HSCT.[110,111] Because fluconazole does not cover *Candida krusei* and has variable activity against *C. glabrata*, alternative agents should be considered for patients known to be colonized with these species. Reasonable options include extended-spectrum triazoles, echinocandins, and lipid-formulations of amphotericin B (LFAB), all of which also have some coverage of *Aspergillus* spp.

When patients develop invasive candidiasis after HSCT, the most common site of infection is the bloodstream. In addition to initiation of appropriate antifungal agents, the standard practice is to discontinue all central venous catheters in patients with candidemia.[145] Less common, but more perplexing, disseminated *Candida* infections of the liver, spleen, or lungs can often be blood

*References 23, 49, 91, 111, 122, 175.

*References 15, 45, 65, 88, 121, 159, 198.

culture negative and may require tissue biopsy to establish a diagnosis. Serum β-glucan levels will be very useful for identifying cases of possible invasive candidiasis once the assay has been optimized for use in children.[167] Furthermore, in patients with disseminated candidiasis, a formal ophthalmologic evaluation is recommended to rule out *Candida* endophthalmitis, which may require intravitreal injection of antifungal agents to preserve vision.[149] For treatment of cases of invasive candidiasis, especially those who develop on fluconazole prophylaxis, the echinocandins are a class of antifungal agents that target β-(1,3)-D-glucan synthase and interrupt biosynthesis of the glucan polymers that make up fungal cell walls. Because mammalian cells do not possess cell walls, echinocandin administration to human patients has generally resulted in limited toxicity. Echinocandins possess fungicidal activity against *Candida* spp.[5] In some settings, the echinocandins may be superior to fluconazole[147] or amphotericin B[125] for treatment of invasive candidiasis.

Aspergillus

In adult HSCT recipients, *Aspergillus* spp. have a bimodal distribution, with a first peak at a median of 16 days and the second at a median of 96 days after allogeneic HSCT.[183,184] This second peak may be less pronounced in children.[28] Invasive aspergillosis can be one of the most devastating infections to occur after allogeneic HSCT. In a multivariate analysis of risk factors for mortality among a modern cohort of pediatric patients with invasive aspergillosis, the major risk factor for death was the development of invasive aspergillosis after an allogeneic HSCT.[23] Although treatment options for invasive aspergillosis have increased in recent years, significant attention also has been paid to preventing invasive aspergillosis infections. Although fluconazole prophylaxis has been shown to reduce the risk for invasive fungal infection relative to placebo, fluconazole lacks activity against *Aspergillus* spp. Given this lack of antimold activity, several trials have compared fluconazole to mold-active agents in hope of decreasing rates of invasive aspergillosis. The first of these trials compared fluconazole to low-dose conventional deoxycholate amphotericin B (D-AMB).[197] However, D-AMB did not show improvement over fluconazole and resulted in a higher adverse event rate. Several trials, including one in children,[151] have evaluated the LFAB (often given only three times per week) for antifungal prophylaxis in HSCT and acute leukemia patients.[87,116,137] However, like D-AMB, LFAB has not been shown superior to fluconazole in overall success and typically demonstrates increased side effects.

Extended-spectrum triazoles such as itraconazole, voriconazole, and posaconazole do possess anti-*Aspergillus* activity[5]; however, clear limitations exist to each as a potential prophylactic agent. Several trials of itraconazole versus fluconazole have been performed, and a meta-analysis showed significantly less invasive fungal infection,[187] but because of its common gastrointestinal side effects, greater drug interactions, and poor tolerability,[110] itraconazole prophylaxis has generally been abandoned in children. The results of a multicenter, double-blinded trial showed that voriconazole was not superior to fluconazole in the prevention of invasive fungal infection, although the safety profile was similar.[194] Given voriconazole's broader spectrum of activity, this result was surprising but may have been due to an incomplete understanding of the complex pharmacokinetics of voriconazole. In adults and children over the age of 12 years, voriconazole has nonlinear pharmacokinetics with relatively well-established dosing regimens. Even in adults, however, recent studies have questioned standard dosing regimens and have proposed dosing based on serum drug levels,[134,166,177] although the optimal goal serum voriconazole level is still unclear. Part of this variability may be due to allelic polymorphisms of the gene encoding for cytochrome (CYP)2C19, which can result in an increase or decrease in voriconazole metabolism.[134] In children, the situation is further complicated by linear voriconazole kinetics, so that younger children may need significantly higher dosages of voriconazole.[57,85,128] Voriconazole also has significant drug interactions with commonly used agents in a pediatric HSCT population. Voriconazole is a substrate of CYP2CP (major), 2C19 (major), and 3A4 (minor) and an inhibitor of 2C9 (moderate), 2C19 (weak), and 3A4 (moderate).[44] Proton pump inhibitors increase voriconazole levels, while voriconazole increases serum levels and toxicity of corticosteroids, imatinib, and many other medications.[44] The approved voriconazole label reports that concomitant use of voriconazole can cause a 1.7- to 3-fold increase in cyclosporine or tacrolimus levels, and recommends that the dosing of cyclosporine be decreased by 50 percent and the dosing of tacrolimus be decreased by 66 percent of the normal dose. A recent study demonstrated that in a cohort of 27 adult HSCT patients receiving voriconazole, 100 percent of patients required multiple tacrolimus dose reductions to achieve a safe target level.[176] Furthermore, the use of voriconazole with sirolimus is officially contraindicated, and when its use has been reported, investigators have recommended dropping the levels of sirolimus to 90 percent of original dosing at the time of initiation of voriconazole.[113]

Posaconazole is a triazole with broad coverage of most fungi, including mucormycosis (previously called zygomycosis).[5] In a trial of adult patients with neutropenia, posaconazole prophylaxis was superior to fluconazole or itraconazole, but was also associated with an increased risk for serious adverse events.[43] In a trial of teenagers and adults receiving treatment for acute or chronic GVHD, posaconazole was superior to fluconazole at preventing breakthrough and death from invasive fungal infection, with similar rates of toxicity.[180] Unfortunately, this did not translate to improved overall survival. Factors limiting the use of posaconazole in children include the lack of pharmacokinetic data in children younger than 13 years of age, the current lack of an intravenous formulation (because these children typically have nausea, mucositis, and poor oral intake during the highest risk period), and unreliable absorption in the setting of limited oral intake. Finally, posaconazole shares many of the same enzymatic pathways, and therefore drug interactions, as voriconazole. Because of the CYP interactions, azole prophylaxis is generally started after the conditioning regimen is complete. For patients who enter the HSCT period being treated for a preexisting invasive fungal

infection, discontinuation of azoles 72 hours before the start of conditioning chemotherapy is often done to allow the CYP enzymes to return to baseline function, although some data suggest that fluconazole coadministration may actually help with cyclophosphamide metabolism and decrease toxicity.[183]

Echinocandins possess fungistatic activity against *Aspergillus* spp.[5] In a prophylactic antifungal trial, micafungin demonstrated reduced need for empirical antifungal therapy and an improved safety profile in contrast to fluconazole.[185] However, the number of pediatric subjects enrolled was small (n = 84), and a reduction in the incidence of proved or probable invasive fungal infection was not demonstrated. The lack of impact on invasive fungal infection may have been because the incidence of breakthrough invasive fungal infections in both groups was very low, likely as a result of the inclusion of low-risk patients (46% autologous HSCT recipients) and very few patients undergoing umbilical cord blood transplant (n = 30). Caspofungin has been shown to be at least equivalent to itraconazole in the setting of antifungal prophylaxis,[115] with few caspofungin-related adverse events,[39] although prospective trials in this population are lacking. The major disadvantages to widespread echinocandin use are cost and the lack of an oral formulation.

If prophylaxis fails, the typical locations where invasive aspergillosis develops after an allogeneic HSCT are the lungs and sinuses, with not uncommon seeding of the brain. It should be noted that children do not always display the classic radiographic findings seen in adults with invasive aspergillosis, such as the air crescent or halo signs,[23] and a high index of suspicion must be maintained. Much attention has been focused on developing noninvasive tests for diagnosing invasive aspergillosis. Galactomannan is a polysaccharide cell-wall component released by *Aspergillus* during growth. The Platelia EIA *Aspergillus* galactomannan assay was approved by the U.S. Food and Drug Administration in 2003 for use in adult patients.[140] Early large-scale clinical testing included few children, but available data suggest that detection values for adult patients can be extrapolated to children.[73,172] Serum β-D-glucan (found in all fungi except *Cryptococcus* spp. and mucormycosis) can be detected using an approved diagnostic serum assay and has been found to have high specificity and high positive predictive values for the detection of invasive fungal infection in adults.[132] However, data on the performance of this assay in children are limited.[167] Ultimately, obtaining cultures through bronchial alveolar washings or biopsy is the best way to be certain of what organism is being treated. Speciation of the *Aspergillus* is also important if polyene therapy is planned, because of the intrinsic resistance of *Aspergillus terreus* to amphotericin B.[47]

Based on a pivotal trial performed in teenagers and adults, voriconazole has become the standard therapy for patients with invasive aspergillosis.[75] Because of the high mortality rate of invasive aspergillosis in post-HSCT patients, there has been great enthusiasm for potentially synergistic combination therapies, such as with a triazole and an echinocandin.[108] If amenable to a surgical approach, strong consideration should be made for resection of cases of invasive aspergillosis, which has been shown to improve mortality if disease is precariously located near a major vessel.[23] During the neutropenic period in patients who develop invasive aspergillosis, transfusions of irradiated random donor granulocytes have been proposed as a method to improve outcomes, although conclusive proof that this laborious procedure is beneficial is lacking.[158] Finally, exciting work has demonstrated the importance of adaptive immunity to fighting invasive aspergillosis.[74] Based on this, work has been done on creating *Aspergillus*-specific T cells for use in adoptive immunotherapy. It has been demonstrated that a cohort of patients who developed invasive aspergillosis after haploidentical HSCT and who received *Aspergillus*-specific T cells had significantly better resolution of the infection than a similar cohort not given the specific T cells.[139]

Rare Fungi

Less frequently, infections occur with the agents of mucormycosis (*Mucor*, *Rhizopus*, and *Absidia* spp.), *Trichosporon* spp., *Fusarium* spp., and other saprophytic fungi.[109] For these rare fungi, the key is to obtain culture identification, by biopsy if necessary, so that the optimal antifungal agent may be initiated. Posaconazole is a triazole with broad coverage of most fungi, including mucormycoses,[5] and thus may be the optimal treatment for many of these organisms, despite all of the caveats mentioned earlier regarding this agent. Recent work has demonstrated that Mucorales-specific T cells are generated after an infection with these organisms and thus might be useful as a diagnostic marker,[143] but also suggesting that generation of similar cells for treatment of Mucorales infections one day may be possible.

Pneumocystis jiroveci

Previously referred to as *Pneumocystis carinii*, that term now refers only to pneumocystis that infects rodents and *Pneumocystis jiroveci* refers to the distinct species that infects humans. The abbreviation PCP (or PJP) is often used to refer to pneumocystis pneumonia. PCP reactivations or infections are generally thought to be preventable after HSCT with administration of classic prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX). However, in the setting of alternative prophylaxis agents or TMP-SMX noncompliance, episodes of PCP do still occur beginning about 2 months after HSCT and continuing through recovery of T-cell functional immunity. TMP acts by interference with the bacterial dihydrofolate reductase, inhibiting synthesis of tetrahydrofolic acid and thus nucleic acid synthesis. Because of concerns about bone marrow toxicity, TMP-SMX is often held for several weeks after HSCT until evidence of neutrophil recovery, although this practice has been challenged.[56] The necessary amount of TMP-SMX required to prevent PCP has not been well studied, and a variety of dosing regimens exist, with administration 2 or 3 days per week being the most common. Generally, TMP-SMX is continued for at least 6 months after an allogeneic HSCT, although this should be lengthened for patients receiving ongoing immunosuppressive therapy.[179] In addition to possible bone marrow suppression, many patients have allergic or other reactions to TMP-SMX that induce

clinicians to prematurely discontinue its use. However, the optimal second-line prophylactic agent is not well defined and all options appear to be potentially less effective than TMP-SMX. Options that have been used include oral dapsone, intravenous or inhaled pentamidine, and oral atovaquone. Dapsone is inexpensive but has a high incidence of adverse events, especially in patients with glucose-6-phosphate deficiency.[155] Intravenous pentamidine given every 4 weeks also has been used, although inadequate protection has been noted in children under 2 years of age and those undergoing HSCT,[89] who may need more frequent dosing. Aerosolized pentamidine is generally well-tolerated, other than occasional bronchospasm, but its effectiveness has been questioned.[188] Atovaquone is generally well-tolerated, but absorption can be limited in patients not eating diets containing fatty foods. In vitro, the echinocandin class of antifungal agents appear to have some activity against the cyst form of *P. jiroveci*. To date, no studies have evaluated the use of echinocandins as a solitary prophylaxis agent; however, a few case reports have described its potential utility in combination with TMP-SMX for the treatment of PCP.[2,11]

PCP must be suspected in HSCT patients presenting with hypoxemia (often disproportionally more severe than the degree of hypercapnia), dyspnea, cough, fever, and bilateral infiltrates,[162,179] especially if noncompliance is suspected or alternative prophylaxis agents have been used. Although it is highly sensitive in patients with HIV infection, measuring the serum lactate dehydrogenase level is less useful in other immunocompromised patients because of low levels of sensitivity and specificity.[190] Serum β-D-glucan levels could potentially be very useful for identifying cases of PCP once the assay has been optimized for use in children.[167] Until then, the definitive diagnosis of PCP still requires identification of the organism on special stains or polymerase chain reaction (PCR) of induced sputum or bronchoalveolar lavage fluid.[162] Once PCP is identified, the treatment of choice is high-dose TMP-SMX (15 to 20 mg TMP/kg/day divided every 6 hours).[163] This high-dose TMP-SMX may be difficult to administer to a patient recovering from HSCT with marginal bone marrow reserves. Therefore, some physicians have used folinic acid "rescue," analogous to what is used after methotrexate administration, with the hypothesis that bacteria are unable to take up the folinic acid from the environment and thus only the host bone marrow cells are helped. Although no data exist in children undergoing HSCT, caution should be taken with this approach because it has been associated with increased treatment failures in patients with HIV infection.[153] Although counterintuitive in a patient with poor T-cell function, TMP-SMX has been combined with a short course of corticosteroids, which appears to be beneficial in preventing temporary worsening of hypoxemia resulting from inflammation from dying organisms. The recommended dose is prednisone 1 mg/kg/dose twice daily (maximum 40 mg twice daily) (or the equivalent dose of methylprednisolone) tapered in half after 5 days and then again in another 5 days, then discontinued after 21 days.[84] It should be noted that these data derive solely from the HIV literature in adults, and it is unclear whether this approach is safe or beneficial in pediatric HSCT patients.

Viral

Viral infections after pediatric HSCT can be divided into two broad categories: DNA viruses that occur as a result of reactivation of a previous infection (and occasionally as a de novo infection) and community-acquired respiratory viruses. Other than donor and recipient serostatus, the major risk factor for reactivation of a dsDNA viral infection after HSCT is the use of T-cell depletion, either ex vivo or in vivo, with serotherapy.[41,127,133] Recently, a few polymorphisms in host immune response genes also have been shown to possibly contribute to the development of dsDNA viral infections after HSCT.[19,20,81]

Herpes Family Viruses

Herpes Simplex Virus. Herpes simplex virus–1 (HSV-1) and HSV-2 infections after HSCT usually occur as a result of reactivations from a previously acquired latent virus. Thus, pretransplant serologies are very useful in identifying at-risk patients. Because acquisition of HSV occurs as patients age, many pediatric HSCT patients will be seronegative. In the era before routine prophylaxis of seropositive patients, reactivation rates were approximately 70 to 80 percent and significantly contributed to post-HSCT mucositis.[156] Dissemination of HSV to the lungs, liver, or brain is also possible. Routine acyclovir prophylaxis is usually given during the period of mucositis, and with this, HSV reactivations are relatively rare.[61] However, severely immunocompromised individuals can continue to develop reactivations for many months after HSCT and thus some centers will continue prophylaxis for up to 6 months,[104] a typical period for development of T-cell reconstitution. This is not needed for patients who receive ganciclovir for CMV prophylaxis, because ganciclovir also has activity against HSV.[62] In addition, some strains of HSV have become acyclovir-resistant, and thus patients with mucositis more severe than anticipated should have a lesion swabbed for HSV culture and resistant strains treated with either foscarnet or cidofovir.[175]

Routine acyclovir prophylaxis does not appear to have a role in HSV-seronegative patients, although consideration of varicella-zoster virus (VZV) serostatus also must be taken into account. Furthermore, patients with B-cell immunodeficiencies may be unable to mount an antibody response before HSV infection and should be considered for prophylaxis, especially if close contacts such as the parents or siblings have a history of HSV infections.

Cytomegalovirus. Historically, one of the most severe complications of allogeneic HSCT was CMV infection. In the modern era, the use of PCR-based detection strategies has significantly reduced the incidence of CMV disease. More than 50 percent of healthy adults in the United States are CMV seropositive, and the incidence increases with age, with 36 percent of children 6 to 11 years of age already exposed.[171] With the use of CMV-negative blood products, the rate of de novo infection in

CMV-seronegative donor and recipient (D–R–) pairs after HSCT is as low as 2 percent.[129] Thus, most infections occur as a result of reactivations, likely of host CMV, because infections are much less common in donor-positive, recipient-negative (D+R–) transplants than in those in which the recipient is positive (D+R+ or D–R+).[129] Other factors can alter the risk for reactivation in a CMV-seropositive HSCT recipient. After autologous HSCT, CMV reactivations are not uncommon, but CMV disease is rare,[195] except in the setting of CD34 selection. T-cell depletion of donor cells in the allogeneic HSCT setting is a major risk factor for reactivation, which may occur even before neutrophil engraftment.[76,184] GVHD and its treatment with corticosteroids and poor T-cell reconstitution, even in the absence of GVHD, are risk factors for CMV reactivation.[18] Of interest, even in the absence of serotherapy, the choice of GVHD prophylaxis regimen may alter the risk for CMV reactivation. Sirolimus, an inhibitor of mammalian (or mechanistic) target of rapamycin (mTOR), appears to provide a protective effect against CMV when used in combination with tacrolimus and in place of other agents.[114] It remains to be determined if this effect is due to direct inhibition of CMV viral replication from sirolimus or enhanced reconstitution of anti-CMV immunity.[114] Overall, as many as 65 percent of seropositive allogeneic HSCT recipients will develop CMV reactivation if not given specific prophylaxis.[51]

Several agents have been used for CMV prophylaxis in at-risk patients (D–R– patients are typically excluded). Because acyclovir has some in vitro activity against CMV, high-dose acyclovir (500 mg/m^2/dose three times daily) has been shown to prevent CMV.[120] Because of its improved absorption, valacyclovir may be superior to high-dose acyclovir for the long-term prevention of CMV reactivation.[103] Ganciclovir is the most commonly used CMV prophylactic agent.[175] As a result of its marrow-suppressive qualities, it is typically started after neutrophil engraftment,[16,196] although some have suggested a role for a therapeutic window during the pre-HSCT conditioning.[124] In addition, ganciclovir use may partially inhibit recovery of both normal and CMV-specific T-cell function.[9] In patients at high risk for early CMV reactivation before neutrophil recovery, foscarnet has been used because of its lack of significant marrow suppression.[8] If prophylaxis is to be used, it is typically continued for 100 days after HSCT,[175] but longer courses may be indicated for patients with significantly impaired immune systems. All of these prophylactic strategies are generally used in combination with screening for reactivation by serum PCR.[175]

Because all of the anti-CMV agents have some degree of toxicity, the alternative strategy to prophylaxis is preemptive treatment based on detection of CMV viremia. A randomized trial comparing prophylaxis versus preemptive management of CMV showed similar outcomes.[16] Patients are screened at least once weekly for CMV DNA and specific antiviral therapy begun once a threshold amount of virus has been detected. Ganciclovir is the most common first-line agent used,[175] although foscarnet can be considered in patients with poor marrow reserve.[148] Even in patients with good marrow function, ganciclovir can cause neutropenia and its use requires monitoring of neutrophil counts and often support with G-CSF.[175] Typically, ganciclovir is given twice daily for a minimum of 2 weeks, or longer for patients whose viremia is still detectable after 2 weeks.[16] Daily maintenance ganciclovir therapy also has been used in patients at high risk for a second reactivation because of poor immune recovery. The duration of maintenance therapy can vary from 4 weeks after first negative PCR result or up to day 100. Oral valganciclovir has become more widely used for preemptive therapy, either as a first-line agent or to replace intravenous ganciclovir at the time of switching to maintenance therapy.[52]

Early in the treatment course, CMV viral loads may rise, but in antiviral-naïve patients this is typically not a sign of resistance. However, continuing increases in viral loads after 2 weeks of therapy or signs of CMV disease should raise suspicion for a resistant strain of CMV. Mutations in the *CMV UL97* phosphotransferase gene typically cause resistance only to ganciclovir, so foscarnet and cidofovir remain alternative agents.[30,48] Mutations in the UL54 viral DNA polymerase will cause resistance to ganciclovir, and cidofovir, and occasionally foscarnet.[48] For the patient who appears be to failing or intolerant of all three drugs, leflunomide is a third-line alternative.[50] Leflunomide is an immunomodulatory agent that inhibits pyrimidine synthesis and results in both antiproliferative and anti-inflammatory effects. The active metabolite appears to inhibit replication of both CMV and human polyomavirus type I, commonly referred to as *BK virus.*

The role of CMV-specific antibodies, either in conventional gammaglobulin products or high-titer products, is of debatable efficacy. On the other hand, efforts to produce donor-derived CMV-specific T cells have shown great promise in treating CMV disease.[53] Given the low rates of GVHD associated with these cells, they may be an upcoming preventive strategy.[139] Furthermore, the techniques that enable generation of CMV-specific T cells also can be used to generate T cells active against Epstein-Barr virus (EBV) and adenoviruses.[71] These so-called trivirus-specific cells represent an exciting avenue of adoptive immunotherapy in severely immunocompromised post-HSCT patients.

Epstein-Barr Virus. EBV infection after HSCT usually results from the reactivation of latent EBV in either residual host B cells or passively transferred donor B cells. As with other herpes family viruses, pre-HSCT serologies can help identify at-risk patients, but because approximately 95 percent of adults are seropositive[13] virtually all patients undergoing adult unrelated-donor HSCT are potentially susceptible. Unlike other herpes family viruses, no clearly effective antiviral agent exists against EBV and thus no post-HSCT prophylaxis strategy exists. Fortunately, the rate of EBV reactivation in patients receiving T-replete grafts is quite low. Conversely, patients who receive ex vivo T cell–depleted stem cells, anti–T-cell serotherapy, and umbilical cord blood grafts have an elevated risk.[60] EBV reactivation can lead to the development of posttransplant lymphoproliferative disease, signs and symptoms of which can include fever, lymphadenopathy, fulminate sepsis, or mass lesions in

lymph nodes, spleen, or central nervous system. Several histologic subtypes exist, and all generally occur within the first 3 to 6 months of the posttransplant period. The first step to be undertaken once an EBV infection is suspected is to reduce immunosuppression when possible.[173] If that fails, because EBV proliferates primarily within B cells, administration of rituximab often is successful at eliminating the infection.[191] Copy numbers of EBV in the blood can be easily monitored after HSCT by PCR, and routine monitoring of high-risk patients is recommended.[175] Less clear, however, is exactly what threshold level should be used for initiation of preemptive therapy, because some patients will have transient self-limited EBV viremia after HSCT.[191] Nevertheless, evidence suggests that preemptive rituximab for treatment of EBV viremia is more effective than initiation once posttransplant proliferative disease is established.[173] Some groups have established methods for enriching for EBV-cytotoxic T lymphocytes, which can both prevent and treat EBV posttransplant proliferative disease in high-risk patients, with little risk for causing GVHD.[118] For centers without access to specific donor T cells, the fact that most donors are EBV seropositive allows the use of unmanipulated donor lymphocyte infusion, which is often effective, albeit with a high risk for inducing GVHD.[77,131]

Varicella-Zoster Virus. With the advent of routine vaccination against VZV at 1 year of age, the majority of immunocompetent patients entering HSCT are seropositive for VZV. In the absence of prophylaxis, reactivations occur in approximately 30 percent of patients after HSCT.[17] A double-blinded trial of acyclovir prophylaxis given for 1 year after HSCT demonstrated a significant reduction in VZV reactivations, although this duration of treatment was inadequate in patients who continued on immunosuppressive therapy for treatment of chronic GVHD.[17] An unresolved question is whether pediatric HSCT recipients who are VZV seropositive before HSCT as a result of prior live-attenuated vaccination, rather than exposure to wild-type VZV, are at a high enough risk for reactivation to warrant long-term prophylaxis. Several case reports suggest that the live-attenuated strain is capable of causing zoster and even disseminated disease in immunocompromised children.[33,101] Therefore, until new data are available, the recommendation for acyclovir prophylaxis should be applied to all VZV-seropositive patients.

For post-HSCT patients who are exposed to an individual with active wild-type VZV infection (including shingles) and are not receiving prophylactic acyclovir or intravenous immunoglobulin (IVIG), passive immunization with varicella-zoster immunoglobulin should be initiated within 96 hours of exposure.[175] There does not appear to be a significant rate of transmission of live-attenuated vaccine strain VZV to immunocompromised individuals, and thus household contacts of HSCT recipients are allowed to receive the vaccine, although optimally it should be given before the HSCT when possible.[175]

According to the 2000 Centers for Disease Control and Prevention recommendations, patients who underwent HSCT were instructed to not be vaccinated with the live-attenuated strains of VZV, thereby placing previously naïve patients at long-term risk for developing a potentially serious infection. However, two reports have since demonstrated the safety and seroconversion rates of the live-attenuated VZV vaccine when given to this patient population after first demonstrating adequate T-cell immune reconstitution.[38,94]

Human Herpesvirus–6. HHV-6 is a ubiquitous pathogen that has infected the majority of people by the age of 2 years and has been reported to reactivate in approximately 50 percent of HSCT recipients according to PCR-based monitoring.[199] The majority of these episodes of viremia appear to be self-limited and asymptomatic, significantly limiting recommendations regarding preemptive therapy. However, a variety of reports have linked HHV-6 reactivations to post-HSCT fevers, rashes, hepatitis, pneumonia, and delayed engraftment.[175] HHV-6 also appears to be a rare cause of post-HSCT encephalitis, typically manifested as memory loss, seizures, hyponatremia, cerebrospinal fluid pleocytosis, and magnetic resonance imaging (MRI) abnormalities in the mesial temporal lobe.[64,157] Patients manifesting these symptoms should have their cerebrospinal fluid examined for HHV-6 by PCR. Recently, HHV-6 replication has been implicated in the initiation of GVHD.[141] When HHV-6–associated disease is diagnosed, ganciclovir is typically the first-line agent, although both foscarnet and cidofovir also have activity against HHV-6.[200] If the HHV-6 viremia does not appear to be resolving with antiviral treatment, the possibility of chromosomally integrated virus must be considered.[82] This can be determined by paired serum and whole blood PCRs and demonstrating that the copy numbers in the whole blood sample are significantly higher and the serum copy numbers are generally very low.

Other Double-Stranded DNA Viruses

Adenovirus. A large number of serotypes of adenovirus have been implicated in causing human disease, and by the age of 5 years most individuals have been exposed to at least one serotype, thereby allowing latent virus to enter the system. Thereafter, reactivation during a period of intense immunosuppression results in the majority of cases of adenoviral viremia after HSCT.[21] The major risk factor for adenoviral reactivation is the degree of immunosuppression experienced by the patient, especially among recipients of T cell–depleted grafts or grafts from unrelated donors and those being treated for GVHD.[32,127] Of interest, for reasons that are not entirely clear, younger age also appears to be a risk factor.[21]

With the advent of PCR-based testing of blood and other body fluids, it has been demonstrated that adenoviral reactivations occur in approximately 27 to 32 percent of pediatric HSCT recipients.[102,127] Because of this high incidence, some centers have now adopted routine screening of blood by PCR for early detection of adenoviral reactivations in high-risk patients.[102] When adenovirus viremia progresses to invasive disease, manifestations include pneumonia, hepatitis, enteritis, cystitis, and nephritis.[21] Adenoviral disease after HSCT has been

associated with mortality rates as high as 78 percent.[127] The only currently available agent for treatment of adenoviral viremia or disease is intravenous cidofovir, typically given on a schedule of three times per week.[78] However, intravenous cidofovir has potentially significant toxicities to both the kidneys and bone marrow. This makes its use as a preemptive agent problematic, especially when some patients appear to spontaneously clear the viremia without treatment.[127,192] An experimental oral liposomal formulation of cidofovir (CMX-001) may potentially be less toxic than the intravenous formulation, and is currently being tested to determine whether it can serve as a preemptive agent. Furthermore, because the major risk factor for the development of adenoviral infection is profound immunosuppression, rapid tapering or withdrawal of immunosuppression, when possible, may potentially play a role in the treatment.[32] For patients who develop adenoviral infection after T cell–depleted HSCT, researchers are working on developing adenovirus-specific T cells for adoptive immunotherapy.[97]

Human Polyomavirus Type I. Human polyomavirus type I, typically referred to as *BK virus*, is a common asymptomatic infection in adults, 90 percent of whom are seropositive.[90] Like other dsDNA viruses, it can reactivate during periods of intense immunosuppression, and between 50 and 80 percent of HSCT recipients will be found to shed BKV in their urine in the first several months after HSCT.[4,147] Much of the time, this viruria is asymptomatic, but BK virus reactivation can be associated with the development of hemorrhagic cystitis (HC) in approximately 5 to 15 percent of HSCT recipients, typically 3 to 6 weeks after HSCT.[10] The patients at highest risk for developing BK virus–associated HC appear to be those who received cyclophosphamide-based conditioning regimens and T cell–depleted grafts.[36] Furthermore, BK virus can be found in the blood of some patients after HSCT and high levels have been associated with the development of BK virus–associated nephropathy,[69] which can be definitively diagnosed only by renal biopsy. Of interest, fluoroquinolone antibiotics inhibit BK viral replication in vitro, and some data suggest that their use as antibacterial prophylaxis after HSCT also may decrease rates of BK virus–associated HC.[100] Decreasing immunosuppression, whenever possible, is the first step toward treating BK virus disease. If the BK virus disease persists, cidofovir is the most commonly used antiviral agent, although leflunomide also appears to have some efficacy[146] and may potentially be better tolerated than cidofovir in patients with significant renal dysfunction.

Respiratory Viruses

Influenza. During seasonal outbreaks, HSCT recipients are at risk for developing influenza infections from close contacts. Although upper respiratory tract symptoms are common, systemic symptoms such as fever or myalgias may be absent.[136] Thus, a high index of suspicion must be maintained during the peak season, because 20 to 50 percent of influenza infections can rapidly progress to lower respiratory tract disease with hypoxemia and a risk for mechanical ventilation and death.[37] Bronchoalveolar lavage may be needed in patients with evidence of lower respiratory tract symptoms, because highly immunosuppressed patients are at risk for harboring multiple pathogens. Preemptive treatment with neuraminidase inhibitors (the choice depending on that year's circulating strain) appears to be relatively efficacious at preventing progress to lower respiratory tract disease.[26] Although the data are limited, combination regimens have been employed for patients with resistant strains or with respiratory failure. No parenteral agent is commercially available in the United States, but several are under investigation or available through emergency use authorization.[2] The use of moderate dosages of corticosteroids to reduce excessive inflammation is controversial,[26] and other forms of immunosuppression should be lowered when possible.

Prevention strategies for influenza center around strict infection control practices, including hand washing, isolation, and eliminating potential exposures via universal vaccination of health care workers, patient family members, and caregivers. If a recent (<2 years or still immunocompromised) HSCT recipient has a significant exposure to a confirmed case of influenza, prophylactic administration of a neuraminidase inhibitor is recommended. This strategy also could be considered if a nosocomial outbreak was to occur on an HSCT unit. Vaccination of HSCT recipients (with the inactivated strain) is highly recommended to occur for several years beginning at 6 months after HSCT, before which time the majority of adult patients are unlikely to mount a response.[26] However, pediatric data are limited, and a fixed 6-month rule fails to take into account the significant variation in immunologic recovery times that occurs among patients depending on stem cell source and presence of GVHD. It is not unreasonable to allow earlier vaccination for patients on tapering doses of immunosuppression,[175] even without documented B-cell function, because a T-cell response to influenza vaccine also may provide some protection.[6] Finally, efforts are under way to create influenza-specific T cells that can be given in a prophylactic fashion following HSCT.[170]

Respiratory Syncytial Virus. During seasonal outbreaks, upper respiratory tract infections from the RNA virus respiratory syncytial virus (RSV) are common in HSCT recipients. Approximately 50 percent of those infected will progress to involve the lower respiratory tract, especially in those with GVHD or severe lymphopenia.[160] Untreated RSV pneumonia in HSCT recipients has a mortality rate approaching 80 percent.[160] Ribavirin, a guanosine analog, is available in aerosolized form for the treatment of RSV. Several potential regimens exist, including a continuous 18-hour infusion and a 2- to 3-hour infusion given three times per day. Because of concerns about potential teratogenicity, it is usually administered in a scavenging tent. Respiratory side effects from inhaled ribavirin are common and include potential bronchoconstriction. Despite its problems and costs, a comprehensive review of inhaled ribavirin treatment in adult HSCT recipients suggests that initiation of ribavirin at the time of diagnosis of RSV upper respiratory tract infection can significantly decrease the risk for progression to lower respiratory tract disease and in those with

lower respiratory tract disease can decrease the risk for death.[160] An oral formulation of ribavirin (used for treating hepatitis C) and an intravenous formulation (not available in the United States) have also been used for treating RSV. However, the data are not conclusive that these formulations are as effective as the inhaled ribavirin.

Palivizumab, an RSV-specific monoclonal antibody, is effective at preventing RSV infections in very young high-risk children. Another potential immunomodulator is IVIG, which often contains anti-RSV antibodies and may decrease RSV-mediated cytokine-induced pulmonary inflammation. A review of the available studies of immunomodulation plus inhaled ribavirin suggests that the combination is superior at preventing progression to lower respiratory tract disease and RSV-related death, than inhaled ribavirin alone.[160] However, because of the small numbers, the report was unable to separate out those who specifically received palivizumab. As a result of this and the lack of any controlled trial, the use of the expensive palivizumab has been questioned. Even more controversial is the prophylactic use of palivizumab. Fortunately, the incidence of RSV infection in the first 100 days after HSCT is still relatively low, at 5.8 percent.[136] However, based on decision-tree analysis, in children undergoing HSCT, it has been estimated that the use of prophylactic palivizumab may increase the absolute survival rate from 83 to 92 percent, suggesting that 12 children would need to be treated to prevent one RSV-related death.[174] Clinical confirmation of this concept is lacking.

Even with "optimal" therapy of inhaled ribavirin plus an immunomodulator, the RSV-attributable mortality in HSCT recipients with lower respiratory tract disease is approximately 25 percent. Therefore, improved treatments are desperately needed and work is under way on higher-affinity monoclonal antibodies, high-titer RSV immune globulin, and antisense compounds.[160]

Other Respiratory Viruses

Other respiratory viruses, including parainfluenza virus,[169] human metapneumovirus,[55] and rhinovirus[123] have been implicated in causing lower respiratory tract disease in HSCT recipients. Risk factors for progression from upper respiratory tract infection to viral pneumonia include acquiring the infection early after HSCT, a low absolute lymphocyte count, and corticosteroid usage.[169] There is no FDA-approved antiviral agent with proved efficacy against these three viruses. However, identifying one of these viruses from the nasopharynx or bronchial washings can be useful in limiting empirical usage of potentially toxic agents to treat other infections, although these viruses often show up as copathogens. Some uncontrolled reports have suggested that inhaled ribavirin may be useful against parainfluenza[31]; however, others have questioned its utility.[130] Recently, a case report demonstrated possible efficacy of DAS18, an inhaled sialidase fusion protein, in an HSCT recipient with severe parainfluenza infection.[34] Ribavirin has in vitro activity against human metapneumovirus, and a recent report suggests that ribavirin may play a role in helping resolve lower respiratory tract disease,[161] although controlled comparisons are lacking. Clearly, the best strategy for dealing with respiratory viral infections after HSCT is to avoid them through careful respiratory isolation.

Protozoa

Toxoplasma gondii

The majority of HSCT programs have dietary counseling in place that eliminates most undercooked meat; thus, toxoplasmosis after HSCT is generally caused by reactivation of previously acquired cysts. Therefore, serologic screening of HSCT candidates can identify most patients at potential risk (excepting those with deficient antibody production),[112] although reports of transmission from white blood cells do exist.[83] In seropositive patients, PCP prophylaxis with TMP-SMX also provides excellent coverage against toxoplasmosis.[112] Patients who cannot tolerate TMP-SMX need careful attention to the possibility of toxoplasmosis, because alternative PCP prophylaxis agents do not appear to provide sufficient protection.[175] For these patients, clindamycin or pyrimethamine combinations can be used as either prophylaxis or preemptive treatment based on PCR for reactivation.[175]

When toxoplasmosis occurs in a post-HSCT patient, the general time frame is 2 to 6 months after HSCT. Because the brain is the most commonly affected organ, fever plus focal neurologic signs, headaches, seizures, or altered mental status, are the typical symptoms that should prompt evaluation for toxoplasmosis. Brain MRI may not always show the characteristic ring-enhancing lesions; therefore, cerebrospinal fluid confirmation should be obtained.[80]

Strongyloides stercoralis

Strongyloides stercoralis is endemic to the tropics and subtropics, including the southeastern United States. Transmission is usually via penetration of the larvae into exposed skin, from which it migrates into the intestines and possibly the lungs. HSCT candidates with intact immune systems may be asymptomatic or have only eosinophilia, and stool testing should be considered for anyone entering HSCT with elevated eosinophil counts.[86] If positive, treatment with ivermectin is generally effective. Infected individuals may begin to show evidence of disseminated disease within 1 to 3 months after HSCT. This can be manifested by diffuse pulmonary infiltrates, often with blood-tinged sputum, or colonic wall penetration, which leads to superinfection with gram-negative enteric organisms.

Cryptosporidium parvum *and* Cryptosporidium hominis

Cryptosporidium spp. are enteric protozoa acquired from contaminated water supplies or livestock that infect intestinal epithelial cells. It causes seasonal transient nonbloody diarrhea and occasional systemic symptoms in healthy hosts and is frequently mistaken for a viral gastroenteritis. In immunocompromised HSCT recipients, however, cryptosporidiosis can cause a chronic severe diarrhea, plus rare biliary tract disease. Multicenter

incidence studies have never been performed, but based on one single-center prospective study, it may be as high as 4 percent of all HSCT recipients and 10 percent of those with diarrhea.[98] Patients with B-cell deficiencies appear to be especially high risk.[98] In HSCT recipients, the symptoms of a *Cryptosporidium* infection can easily be confused with that of intestinal GVHD, and testing for *Cryptosporidium* oocysts should be considered before initiation of additional immunosuppressants.[98] Therapy for *Cryptosporidium* is with paromomycin, azithromycin, or nitazoxanide. HSCT recipients may need combination therapy, and decreases in systemic immunosuppression should be performed when possible.[98]

VACCINATIONS AFTER HEMATOPOIETIC STEM CELL TRANSPLANT

HSCT, especially from an allogeneic donor, involves the creation of a new immune system that lacks protective immunity in regard to vaccines received before the HSCT. Although some memory T and B cells may be passively transferred from the donor at the time of the cell infusion, the majority of the long-term immune system derives from newly generated naïve T and B cells. Therefore, all HSCT recipients require revaccination after recovery of immune function. The major HSCT and infectious disease groups, plus the Centers for Disease Control and Prevention, have published guidelines regarding the timing of vaccine administration,[175] but it has been argued that a "one-size-fits-all" approach, as used in generally healthy infants, is insufficient to produce protective immunity in many HSCT recipients and that the vaccine schedule should be tailored to the individual kinetics of a patient's T and B cell recovery and followed by titers to demonstrate adequate response.[165]

NEW REFERENCES SINCE THE SIXTH EDITION

13. Bertrand KA, Birmann BM, Chang ET, et al. A prospective study of Epstein-Barr virus antibodies and risk of non-Hodgkin lymphoma. *Blood* 2010;**116**:3547–53.
26. Casper C, Englund J, Boeckh M. How I treat influenza in patients with hematologic malignancies. *Blood* 2010;**115**:1331–42.
34. Chen Y-B, Driscoll JP, McAfee SL, et al. Treatment of parainfluenza 3 infection with DAS181 in a patient after allogeneic stem cell transplantation. *Clin Infect Dis* 2011;**53**:e77–e80.
37. Choi S, Boudreault A, Xie H, et al. Differences in clinical outcomes after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. *Blood* 2011;**117**:5050–6.
38. Chou J, Kernan N, Prockop S, et al. Safety and immunogenicity of the live attenuated varicella vaccine following T replete or T cell-depleted related and unrelated allogeneic hematopoietic cell transplantation (alloHCT). *Biol Blood Marrow Transplant* 2011;**17**:1708–13.
40. Climo MW, Sepkowitz KA, Zuccotti G, et al. The effect of daily bathing with chlorhexidine on the acquisition of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and healthcare-associated bloodstream infections: results of a quasi-experimental multicenter trial. *Crit Care Med* 2009;**37**:1858–65.
44. Cronin S, Chandrasekar P. Safety of triazole antifungal drugs in patients with cancer. *Antimicrob Chemother* 2010;**65**:410–16.
45. Cunha C, Di Ianni M, Bozza S, et al. Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* 2010;**116**:5394–402.
48. Drew WL. Cytomegalovirus resistance testing: pitfalls and problems for the clinician. *Clin Infect Dis* 2010;**50**:733–6.
54. Eleutherakis-Papaiakovou E, Kostis E, Migkou M, et al. Prophylactic antibiotics for the prevention of neutropenic fever in patients undergoing autologous stem-cell transplantation: results of a single institution, randomized phase 2 trial. *Am J Hematol* 2010;**85**:863–7.
56. Fontanet A, Chalandon Y, Roosnek E, et al. Cotrimoxazole myelotoxicity in hematopoietic SCT recipients: time for reappraisal. *Bone Marrow Transplant* 2011;**46**:1272–3.
57. Friberg L, Ravva P, Karlsson M, et al. Integrated population pharmacokinetics of voriconazole in children, adolescents and adults. *Antimicrob Agents Chemother* 2012;**56**:3032–42.
67. Gulbis A, Culotta K, Jones R, et al. Busulfan and metronidazole: an often forgotten but significant drug interaction. *Ann Pharmacother* 2011;**45**:e39.
68. Guthrie K, Yong M, Frieze D, et al. The impact of a change in antibacterial prophylaxis from ceftazidime to levofloxacin in allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant* 2010;**45**:675–81.
69. Haines HL, Laskin BL, Goebel J, et al. Blood, and not urine, BK viral load predicts renal outcome in children with hemorrhagic cystitis following hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**:1512–19.
71. Hanley P, Cruz C, Savoldo B, et al. Functionally active virus-specific T cells that target CMV, adenovirus, and EBV can be expanded from naive T-cell populations in cord blood and will target a range of viral epitopes. *Blood* 2009;**114**:1958–67.
81. Jaskula E, Dlubek D, Duda D, et al. Interferon gamma 13-CA-repeat homozygous genotype and a low proportion of CD4(+) lymphocytes are independent risk factors for cytomegalovirus reactivation with a high number of copies in hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2009;**15**:1296–305.
82. Jeulin H, Salmon A, Gautheret-Dejean A, et al. Contribution of human herpesvirus 6 (HHV-6) viral load in whole blood and serum to investigate integrated HHV-6 transmission after haematopoietic stem cell transplantation. *J Clin Virol* 2009;**45**:43–6.
84. Kaplan J, Benson C, Holmes K, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009;**58**(RR-4):1–207.
85. Karlsson M, Lutsar I, Milligan P. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.
91. Kontoyiannis D, Marr K, Park B, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis* 2010;**50**:1091–100.
94. Kussmaul S, Horn B, Dvorak C, et al. Safety of the live, attenuated varicella vaccine in pediatric recipients of hematopoietic SCTs. *Bone Marrow Transplant* 2010;**45**:1602–6.
97. Leen A, Christin A, Myers G, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood* 2009;**114**:4283–92.
118. Melenhorst J, Leen A, Bollard C, et al. Allogeneic virus-specific T cells with HLA alloreactivity do not produce GVHD in human subjects. *Blood* 2010;**116**:4700–2.
119. Mensah N, Peterlongo P, Steinherz P, et al. Toll-like receptor 4 polymorphisms and risk of gram-negative bacteremia after allogeneic stem cell transplantation: a prospective pilot study. *Biol Blood Marrow Transplant* 2009;**15**:1130–3.
122. Mikulska M, Raiola A, Bruno B, et al. Risk factors for invasive aspergillosis and related mortality in recipients of allogeneic SCT from alternative donors: an analysis of 306 patients. *Bone Marrow Transplant* 2009;**44**:361–70.
123. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;**115**:2088–94.

124. Milano F, Pergam SA, Xie H, et al. Intensive strategy to prevent CMV disease in seropositive umbilical cord blood transplant recipients. *Blood* 2011;**118**:5689–96.
128. Neely M, Rushing T, Kovacs A, et al. Voriconazole pharmacokinetics and pharmacodynamics in children. *Clin Infect Dis* 2010; **50**:27–36.
133. Park S, Choi S, Lee D, et al. Infectious complications associated with alemtuzumab use for allogeneic hematopoietic stem cell transplantation: comparison with anti-thymocyte globulin. *Transpl Infect Dis* 2009;**11**:413–23.
135. Pasquini M, Wang Z. Current use and outcome of hematopoietic stem cell transplantation. *CIBMTR Summary Slides* 2010; Available at: http://www.cibmtr.org.
141. Pichereau C, Desseaux K, Janin A, et al. The complex relationship between human herpesvirus 6 and acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2012;**18**:141–4.
142. Popovich KJ, Hota B, Hayes R, et al. Effectiveness of routine patient cleansing with chlorhexidine gluconate for infection prevention in the medical intensive care unit. *Infect Control Hosp Epidemiol* 2009;**30**:959–63.
143. Potenza L, Vallerini D, Barozzi P, et al. Mucorales-specific T cells emerge in the course of invasive mucormycosis and may be used as a surrogate diagnostic marker in high-risk patients. *Blood* 2011;**118**:5416–19.
146. Raval M, Gulbis A, Bollard C, et al. Evaluation and management of BK virus-associated nephropathy following allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**: 1589–93.
149. Riddell J, Comer G, Kauffman C. Treatment of endogenous fungal endophthalmitis: focus on new antifungal agents. *Clin Infect Dis* 2011;**52**:648–53.
158. Seidel M, Peters C, Wacker A, et al. Randomized phase III study of granulocyte transfusions in neutropenic patients. *Bone Marrow Transplant* 2008;**42**:679–84.
160. Shah J, Chemaly R. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011; **117**:2755–63.
161. Shahda S, Carlos WG, Kiel PJ, et al. The human metapneumovirus: a case series and review of the literature. *Transplant Infect Dis* 2011;**13**:324–8.
165. Small T, Cowan M. Immunization of hematopoietic stem cell transplant recipients against vaccine-preventable diseases. *Expert Rev Clin Immunol* 2011;**7**:193–203.
169. Srinivasan A, Wang C, Yang J, et al. Symptomatic parainfluenza virus infections in children undergoing hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**:1520–7.
170. Stadtmauer E, Vogl D, Luning Prak E, et al. Transfer of influenza vaccine-primed costimulated autologous T cells after stem cell transplantation for multiple myeloma leads to reconstitution of influenza immunity: results of a randomized clinical trial. *Blood* 2011;**117**:63–71.
173. Styczynski J, Einsele H, Gil L, et al. Outcome of treatment of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in hematopoietic stem cell recipients: a comprehensive review of reported cases. *Transpl Infect Dis* 2009;**11**:383–92.
175. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009;**15**:1143–238.
176. Trifilio S, Scheetz M, Pi J, et al. Tacrolimus use in adult allogeneic stem cell transplant recipients receiving voriconazole: preemptive dose modification and therapeutic drug monitoring. *Bone Marrow Transplant* 2010;**45**:1352–6.
178. Trivedi M, Martinez S, Corringham S, et al. Optimal use of G-CSF administration after hematopoietic SCT. *Bone Marrow Transplant* 2009;**43**:895–908.
186. van der Velden W, Blijlevens N, Maas F, et al. NOD2 polymorphisms predict severe acute graft-versus-host and treatment-related mortality in T-cell-depleted haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2009;**44**:243–8.
190. Vogel M, Weissgerber P, Goeppert B, et al. Accuracy of serum LDH elevation for the diagnosis of *Pneumocystis jiroveci* pneumonia. *Swiss Med Wkly* 2011;**141**:w13184.
194. Wingard JR, Carter SL, Walsh TJ, et al. Randomized double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection (IFI) after allo hematopoietic cell transplantation (HCT). *Blood* 2010;**116**:5111–18.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 70

Infections in Pediatric Heart Transplantation

Sheldon L. Kaplan

Each year in the United States, more than 450 children undergo heart transplantation,[75] and infection is an important cause of morbidity and some mortality in these patients. The Registry of the International Society for Heart and Lung Transplantation found that for children who underwent heart transplantation between 1998 and 2010, infection caused by an organism other than cytomegalovirus (CMV) was the cause of mortality in 12.4 percent of patients in the first 30 days after receiving transplants, peaked at 13 percent for 31 days to 1 year after transplantation, and thereafter accounted for 4.1 to 8.6 percent of deaths until more than 10 years after

REFERENCES

1. Almyroudis NG, Fuller A, Jakubowski A, et al. Pre- and post-engraftment bloodstream infection rates and associated mortality in allogeneic hematopoietic stem cell transplant recipients. *Transpl Infect Dis* 2005;**7**:11–17.
2. Annaloro C, Della Volpe A, Usardi P, et al. Caspofungin treatment of *Pneumocystis* pneumonia during conditioning for bone marrow transplantation. *Eur J Clin Microbiol Infect Dis* 2006;**25**:52–4.
3. Arango J, Restrepo A, Schneider D, et al. Incidence of *Clostridium difficile*-associated diarrhea before and after autologous peripheral blood stem cell transplantation for lymphoma and multiple myeloma. *Bone Marrow Transplant* 2006;**37**:517–21.
4. Arthur R, Shah K, Charache P, et al. BK and JC virus infections in recipients of bone marrow transplants. *J Infect Dis* 1988;**158**: 563–9.
5. Ashley ESD, Lewis R, Lewis JS, et al. Pharmacology of systemic antifungal agents. *Clin Infect Dis* 2006;**43**:28–39.
6. Avetisyan G, Aschan J, Hassan M, et al. Evaluation of immune responses to seasonal influenza vaccination in healthy volunteers and in patients after stem cell transplantation. *Transplantation* 2008;**86**:257–63.
7. Azarian M, Busson M, Rocha V, et al. The PTPN22 R620W polymorphism is associated with severe bacterial infections after human leukocyte antigen geno-identical haematopoietic stem-cell transplantations. *Transplantation* 2008;**85**:1859–62.
8. Bacigalupo A, Tedone E, Van Lint M, et al. CMV prophylaxis with foscarnet in allogeneic bone marrow transplant recipients at high risk of developing CMV infections. *Bone Marrow Transplant* 1994; **13**:783–8.
9. Battiwalla M, Wu Y, Bajwa R, et al. Ganciclovir inhibits lymphocyte proliferation by impairing DNA synthesis. *Biol Blood Marrow Transplant* 2007;**13**:765–70.
10. Bedi A, Miller C, Hanson J, et al. Association of BK virus with failure of prophylaxis against hemorrhagic cystitis following bone marrow transplantation. *J Clin Oncol* 1995;**13**:1103–9.
11. Beltz K, Kramm C, Laws H, et al. Combined trimethoprim and caspofungin treatment for severe *Pneumocystis jiroveci* pneumonia in a five year old boy with acute lymphoblastic leukemia. *Klin Padiatr* 2006;**218**:177–9.
12. Benjamin DK, Miller WC, Bayliff S, et al. Infections diagnosed in the first year after pediatric stem cell transplantation. *Pediatr Infect Dis J* 2002;**21**:227–34.
13. Bertrand KA, Birmann BM, Chang ET, et al. A prospective study of Epstein-Barr virus antibodies and risk of non-Hodgkin lymphoma. *Blood* 2010;**116**:3547–53.
14. Bleasdale SC, Trick WE, Gonzalez IM, et al. Effectiveness of chlorhexidine bathing to reduce catheter-associated bloodstream infections in medical intensive care unit patients. *Arch Intern Med* 2007;**167**:2073–9.
15. Bochud P-Y, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med* 2008;**359**:1766–77.
16. Boeckh M, Gooley T, Myerson D, et al. Cytomegalovirus pp65 antigenemia-guided early treatment with ganciclovir versus ganciclovir at engraftment after allogeneic marrow transplantation: a randomized double-blind study. *Blood* 1996;**88**:4063–71.
17. Boeckh M, Kim HW, Flowers MED, et al. Long-term acyclovir for prevention of varicella zoster virus disease after allogeneic hematopoietic cell transplantation: a randomized double-blind placebo-controlled study. *Blood* 2006;**107**:1800–5.
18. Boeckh M, Leisenring W, Riddell SR, et al. Late cytomegalovirus disease and mortality in recipients of allogeneic hematopoietic stem cell transplants: importance of viral load and T-cell immunity. *Blood* 2003;**10**:407–14.
19. Bogunia-Kubik K, Jaskula E, Lange A. The presence of functional CCR5 and EBV reactivation after allogeneic haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2007;**40**:145–50.
20. Bogunia-Kubik K, Mlynarczewska A, Jaskula E, et al. The presence of IFNG 3/3 genotype in the recipient associates with increased risk for Epstein-Barr virus reactivation after allogeneic haematopoietic stem cell transplantation. *Br J Haematol* 2006;**132**: 326–32.
21. Bruno B, Gooley T, Hackman R, et al. Adenovirus infection in hematopoietic stem cell transplantation: effect of ganciclovir and impact on survival. *Biol Blood Marrow Transplant* 2003;**9**:341–52.
22. Bucaneve G, Micozzi A, Menichetti F, et al. Levofloxacin to prevent bacterial infection in patients with cancer and neutropenia. *N Engl J Med* 2005;**353**:977–87.
23. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics* 2008;**121**:e1286–94.
24. Busca A, Saroglia EM, Giacchino M, et al. Analysis of early infectious complications in pediatric patients undergoing bone marrow transplantation. *Support Care Cancer* 1999;**7**:253–9.
25. Cappellano P, Viscoli C, Bruzzi P, et al. Epidemiology and risk factors for bloodstream infections after allogeneic hematopoietic stem cell transplantion. *New Microbiol* 2007;**30**:89–99.
26. Casper C, Englund J, Boeckh M. How I treat influenza in patients with hematologic malignancies. *Blood* 2010;**115**:1331–42.
27. Castagnola E, Faraci M. Management of bacteremia in patients undergoing hematopoietic stem cell transplantation. *Expert Rev Anti Infect Ther* 2009;**7**:607–21.
28. Castagnola E, Bagnasco F, Faraci M, et al. Incidence of bacteremias and invasive mycoses in children undergoing allogeneic hematopoietic stem cell transplantation: a single center experience. *Bone Marrow Transplant* 2008;**41**:339–47.
29. Castagnola E, Faraci M, Moroni C, et al. Bacteremias in children receiving hemopoietic SCT. *Bone Marrow Transplant* 2008;**41** (suppl 2):S104–6.
30. Cesaro S, Zhou X, Manzardo C, et al. Cidofovir for cytomegalovirus reactivation in pediatric patients after hematopoietic stem cell transplantation. *J Clin Virol* 2005;**34**:129–32.
31. Chakrabarti S, Collingham K, Holder K, et al. Parainfluenza virus type 3 infections in hematopoetic stem cell transplant recipients: response to ribavirin therapy. *Clin Infect Dis* 2000;**31**:1516–18.
32. Chakrabarti S, Mautner V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood* 2002;**100**:1619–27.
33. Chan Y, Smith D, Sadlon T, et al. Herpes zoster due to Oka vaccine strain of varicella zoster virus in an immunosuppressed child post cord blood transplant. *J Paediatr Child Health* 2007; **43**:713–15.
34. Chen Y-B, Driscoll JP, McAfee SL, et al. Treatment of parainfluenza 3 infection with DAS181 in a patient after allogeneic stem cell transplantation. *Clin Infect Dis* 2011;**53**:e77–e80.
35. Chien J, Boeckh M, Hansen J, et al. Lipopolysaccharide binding protein promoter variants influence the risk for Gram-negative bacteremia and mortality after allogeneic hematopoietic cell transplantation. *Blood* 2008;**111**:2462–9.
36. Childs R, Sanchez C, Engler H, et al. High incidence of adeno- and polyomavirus-induced hemorrhagic cystitis in bone marrow allotransplantation for hematological malignancy following T cell depletion and cyclosporine. *Bone Marrow Transplant* 1998;**22**: 889–93.
37. Choi S, Boudreault A, Xie H, et al. Differences in clinical outcomes after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. *Blood* 2011;**117**:5050–6.
38. Chou J, Kernan N, Prockop S, et al. Safety and immunogenicity of the live attenuated varicella vaccine following T replete or T cell-depleted related and unrelated allogeneic hematopoietic cell transplantation (alloHCT). *Biol Blood Marrow Transplant* 2011;**17**:1708–13.
39. Chou L, Lewis R, Ippoliti C, et al. Caspofungin as primary antifungal prophylaxis in stem cell transplant recipients. *Pharmacotherapy* 2007;**27**:1644–50.
40. Climo MW, Sepkowitz KA, Zuccotti G, et al. The effect of daily bathing with chlorhexidine on the acquisition of methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, and healthcare-associated bloodstream infections: results of a quasi-experimental multicenter trial. *Crit Care Med* 2009;**37**: 1858–65.
41. Cohen J, Gandhi M, Naik P, et al. Increased incidence of EBV-related disease following paediatric stem cell transplantation with reduced-intensity conditioning. *Br J Haematol* 2005;**129**:229–39.
42. Cole G, Halawa A, Anaissie E. The role of the gastrointestinal tract in hematogenous candidiasis: from the laboratory to the bedside. *Clin Infect Dis* 1996;**22**(suppl 2):S73–88.
43. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007;**356**:348–59.

44. Cronin S, Chandrasekar P. Safety of triazole antifungal drugs in patients with cancer. *Antimicrob Chemother* 2010;**65**:410–16.
45. Cunha C, Di Ianni M, Bozza S, et al. Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient- and donor-dependent mechanisms of antifungal immunity. *Blood* 2010;**116**:5394–402.
46. Dekker A, Bulley S, Beyene J, et al. Meta-analysis of randomized controlled trials of prophylactic granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor after autologous and allogeneic stem cell transplantation. *J Clin Oncol* 2006;**24**:5207–15.
47. Dodds Ashley E, Lewis R, Lewis J, et al. Pharmacology of systemic antifungal agents. *Clin Infect Dis* 2006;**43**(suppl 1):S28–39.
48. Drew WL. Cytomegalovirus resistance testing: pitfalls and problems for the clinician. *Clin Infect Dis* 2010;**50**:733–6.
49. Dvorak C, Steinbach W, Brown J, et al. Risks and outcomes of invasive fungal infections in pediatric patients undergoing allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant* 2005;**36**:621–9.
50. Ehlert K, Groll A, Kuehn J, et al. Treatment of refractory CMV-infection following hematopoietic stem cell transplantation with the combination of foscarnet and leflunomide. *Klin Padiatr* 2006;**218**:180–4.
51. Einsele H, Hebart H, Kauffmann-Schneider C, et al. Risk factors for treatment failures in patients receiving PCR-based preemptive therapy for CMV infection. *Bone Marrow Transplant* 2000;**25**: 757–63.
52. Einsele H, Reusser P, Bornhäuser M, et al. Oral valganciclovir leads to higher exposure to ganciclovir than intravenous ganciclovir in patients following allogeneic stem cell transplantation. *Blood* 2006;**107**:3002–8.
53. Einsele H, Roosnek E, Rufer N, et al. Infusion of cytomegalovirus (CMV)-specific T cells for the treatment of CMV infection not responding to antiviral chemotherapy. *Blood* 2002;**99**:3916–22.
54. Eleutherakis-Papaiakovou E, Kostis E, Migkou M, et al. Prophylactic antibiotics for the prevention of neutropenic fever in patients undergoing autologous stem-cell transplantation: results of a single institution, randomized phase 2 trial. *Am J Hematol* 2010; **85**:863–7.
55. Englund J, Boeckh M, Kuypers J, et al. Brief communication: fatal human metapneumovirus infection in stem-cell transplant recipients. *Ann Intern Med* 2006;**144**:344–9.
56. Fontanet A, Chalandon Y, Roosnek E, et al. Cotrimoxazole myelotoxicity in hematopoietic SCT recipients: time for reappraisal. *Bone Marrow Transplant* 2011;**46**:1272–3.
57. Friberg L, Ravva P, Karlsson M, et al. Integrated population pharmacokinetics of voriconazole in children, adolescents and adults. *Antimicrob Agents Chemother* 2012;**56**:3032–42.
58. Garces Ambrossi G, Jakubowski A, Feinstein M, et al. Active tuberculosis limited to foreign-born patients after allogeneic hematopoietic stem cell transplant. *Bone Marrow Transplant* 2005; **36**:741–3.
59. Gerbitz A, Schultz M, Wilke A, et al. Probiotic effects on experimental graft-versus-host disease: let them eat yogurt. *Blood* 2004;**103**:4365–7.
60. Gerritsen E, Stam E, Hermans J, et al. Risk factors for developing EBV-related B cell lymphoproliferative disorders (BLPD) after non-HLA-identical BMT in children. *Bone Marrow Transplant* 1996;**18**:377–82.
61. Gluckman E, Lotsberg J, Devergie A, et al. Prophylaxis of herpes infections after bone-marrow transplantation by oral acyclovir. *Lancet* 1983;**2**:406–8.
62. Goodrich J, Bowden RFL, Keller C, et al. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. *Ann Intern Med* 1993;**118**:173–8.
63. Goodman J, Winston D, Greenfield R, et al. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992;**326**:845–51.
64. Gorniak R, Young G, Wiese D, et al. MR imaging of human herpesvirus-6-associated encephalitis in 4 patients with anterograde amnesia after allogeneic hematopoietic stem-cell transplantation. *AJNR Am J Neuroradiol* 2006;**27**:887–91.
65. Granell M, Urbano-Ispizua A, Suarez B, et al. Mannan-binding lectin pathway deficiencies and invasive fungal infections following allogeneic stem cell transplantation. *Exp Hematol* 2006;**34**:1435–41.
66. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
67. Gulbis A, Culotta K, Jones R, et al. Busulfan and metronidazole: an often forgotten but significant drug interaction. *Ann Pharmacother* 2011;**45**:e39.
68. Guthrie K, Yong M, Frieze D, et al. The impact of a change in antibacterial prophylaxis from ceftazidime to levofloxacin in allogeneic hematopoietic cell transplantation. *Bone Marrow Transplant* 2010;**45**:675–81.
69. Haines HL, Laskin BL, Goebel J, et al. Blood, and not urine, BK viral load predicts renal outcome in children with hemorrhagic cystitis following hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**:1512–19.
70. Hammerman C, Bin-Nun A, Kaplan M. Safety of probiotics: comparison of two popular strains. *BMJ* 2006;**333**:1006–8.
71. Hanley P, Cruz C, Savoldo B, et al. Functionally active virus-specific T cells that target CMV, adenovirus, and EBV can be expanded from naive T-cell populations in cord blood and will target a range of viral epitopes. *Blood* 2009;**114**:1958–67.
72. Haverkamp M, van Dissel J, Holland S. Human host genetic factors in nontuberculous mycobacterial infection: lessons from single gene disorders affecting innate and adaptive immunity and lessons from molecular defects in interferon-gamma-dependent signaling. *Microbes Infect* 2006;**8**:1157–66.
73. Hayden R, Pounds S, Knapp K, et al. Galactomannan antigenemia in pediatric oncology patients with invasive aspergillosis. *Pediatr Infect Dis J* 2008;**27**:815–19.
74. Hebart H, Bollinger C, Fisch P, et al. Analysis of T-cell responses to *Aspergillus fumigatus* antigens in healthy individuals and patients with hematologic malignancies. *Blood* 2002;**100**:4521–8.
75. Herbrecht R, Denning D, Patterson T, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002;**347**:408–15.
76. Hertenstein B, Hampl W, Bunjes D, et al. In vivo/ex vivo T cell depletion for GVHD prophylaxis influences onset and course of active cytomegalovirus infection and disease after BMT. *Bone Marrow Transplant* 1995;**15**:387–93.
77. Heslop H, Brenner M, Rooney C. Donor T cells to treat EBV-associated lymphoma. *N Engl J Med* 1994;**331**:679–80.
78. Hoffman J, Shah A, Ross L, et al. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001;**7**:388–94.
79. Hovi L, Saarinen-Pihkala UM, Vettenranta K, et al. Invasive fungal infections in pediatric bone marrow transplant recipients: single center experience of 10 years. *Bone Marrow Transplant* 2000;**26**:999–1004.
80. Ionita C, Wasay M, Balos L, et al. MR imaging in toxoplasmosis encephalitis after bone marrow transplantation: paucity of enhancement despite fulminant disease. *AJNR Am J Neuroradiol* 2004;**25**:270–3.
81. Jaskula E, Dlubek D, Duda D, et al. Interferon gamma 13-CA-repeat homozygous genotype and a low proportion of CD4(+) lymphocytes are independent risk factors for cytomegalovirus reactivation with a high number of copies in hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2009;**15**:1296–305.
82. Jeulin H, Salmon A, Gautheret-Dejean A, et al. Contribution of human herpesvirus 6 (HHV-6) viral load in whole blood and serum to investigate integrated HHV-6 transmission after haematopoietic stem cell transplantation. *J Clin Virol* 2009;**45**:43–6.
83. Jurges E, Young Y, Eltumi M, et al. Transmission of toxoplasmosis by bone marrow transplant associated with Campath-1G. *Bone Marrow Transplant* 1992;**9**:65–6.
84. Kaplan J, Benson C, Holmes K, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009;**58**(RR-4):1–207.
85. Karlsson M, Lutsar I, Milligan P. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.

86. Keiser P, Nutman T. *Strongyloides stercoralis* in the immunocompromised population. *Clin Microbiol Rev* 2004;**17**:208–17.
87. Kelsey S, Goldman J, McCann S, et al. Liposomal amphotericin (AmBisome) in the prophylaxis of fungal infections in neutropenic patients: a randomised, double-blind, placebo-controlled study. *Bone Marrow Transplant* 1999;**23**:163–8.
88. Kesh S, Mensah N, Peterlongo P, et al. TLR1 and TLR6 polymorphisms are associated with susceptibility to invasive aspergillosis after allogeneic stem cell transplantation. *Ann N Y Acad Sci* 2005;**1062**:95–103.
89. Kim S, Dabb A, Glenn D, et al. Intravenous pentamidine is effective as second line Pneumocystis pneumonia prophylaxis in pediatric oncology patients. *Pediatr Blood Cancer* 2008;**50**: 779–83.
90. Knowles W, Pipkin P, Andrews N, et al. Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. *J Med Virol* 2003;**71**:115–23.
91. Kontoyiannis D, Marr K, Park B, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) Database. *Clin Infect Dis* 2010;**50**:1091–100.
92. Kroschinsky F, Wichmann G, Bornhauser M, et al. Efficacy and tolerability of prophylactic treatment with intravenous piperacillin/tazabactam in patients undergoing hematopoietic stem cell transplantation. *Transpl Infect Dis* 2002;**2**:132–6.
93. Kulkarni SPR, Treleaven J, Riley U, et al. Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants. *Blood* 2000; **95**:3683–6.
94. Kussmaul S, Horn B, Dvorak C, et al. Safety of the live, attenuated varicella vaccine in pediatric recipients of hematopoietic SCTs. *Bone Marrow Transplant* 2010;**45**:1602–6.
95. Lee K, Park S, Kim I, et al. P2X7 receptor polymorphism and clinical outcomes in HLA-matched sibling allogeneic hematopoietic stem cell transplantation. *Haematologica* 2007;**92**:651–7.
96. Lee S, Astigarraga C, Eapen M, et al. Variation in supportive care practices in hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2008;**14**:1231–8.
97. Leen A, Christin A, Myers G, et al. Cytotoxic T lymphocyte therapy with donor T cells prevents and treats adenovirus and Epstein-Barr virus infections after haploidentical and matched unrelated stem cell transplantation. *Blood* 2009;**114**:4283–92.
98. Legrand F, Grenouillet F, Larosa F, et al. Diagnosis and treatment of digestive cryptosporidiosis in allogeneic haematopoietic stem cell transplant recipients: a prospective single centre study. *Bone Marrow Transplant* 2011;**46**:858–62.
99. Lehrnbecher T, Foster C, Vázquez N, et al. Therapy-induced alterations in host defense in children receiving therapy for cancer. *J Pediatr Hematol Oncol* 1997;**19**:399–417.
100. Leung A, Chan M, Yuen K, et al. Ciprofloxacin decreased polyoma BK virus load in patients who underwent allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2005;**40**:528–37.
101. Levin M, Dahl K, Weinberg A, et al. Development of resistance to acyclovir during chronic infection with the Oka vaccine strain of varicella-zoster virus, in an immunosuppressed child. *J Infect Dis* 2003;**188**:954–9.
102. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood* 2003;**102**:1114–20.
103. Ljungman P, de la Camara R, Milpied N, et al. Randomized study of valacyclovir as prophylaxis against cytomegalovirus reactivation in recipients of allogeneic bone marrow transplants. *Blood* 2002; **99**:3050–6.
104. Ljungman P, Wilczek H, Gahrton G, et al. Long-term acyclovir prophylaxis in bone marrow transplant recipients and lymphocyte proliferation responses to herpes virus antigens in vitro. *Bone Marrow Transplant* 1986;**1**:185–92.
105. Lönnqvist B, Ringdén O, Wahren B, et al. Cytomegalovirus infection associated with and preceding chronic graft-versus-host disease. *Transplantation* 1984;**38**:465–8.
106. MacMillan M, Davies S, Nelson G, et al. Twenty years of unrelated donor bone marrow transplantation for pediatric acute leukemia facilitated by the National Marrow Donor Program. *Biol Blood Marrow Transplant* 2008;**14**:16–22.
107. Malani P, Bradley S, Little R, et al. Trends in species causing fungaemia in a tertiary care medical centre over 12 years. *Mycoses* 2001;**44**:446–9.
108. Marr K, Boeckh M, Carter R, et al. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004;**39**:797–802.
109. Marr K, Carter R, Crippa F, et al. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:909–17.
110. Marr KA, Crippa F, Leisenring W, et al. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. *Blood* 2004;**103**:1527–33.
111. Marr KA, Seidel K, Slavin MA, et al. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000;**96**:2055–61.
112. Martino R, Bretagne S, Einsele H, et al. Early detection of *Toxoplasma* infection by molecular monitoring of *Toxoplasma gondii* in peripheral blood samples after allogeneic stem cell transplantation. *Clin Infect Dis* 2005;**40**:67–78.
113. Marty F, Lowry C, Cutler C, et al. Voriconazole and sirolimus coadministration after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006;**12**:552–9.
114. Marty FM, Bryar J, Browne SK, et al. Sirolimus-based graft-versus-host disease prophylaxis protects against cytomegalovirus reactivation after allogeneic hematopoietic stem cell transplantation: a cohort analysis. *Blood* 2007;**110**:490–500.
115. Mattiuzzi G, Alvarado G, Giles F, et al. Open-label, randomized comparison of itraconazole versus caspofungin for prophylaxis in patients with hematologic malignancies. *Antimicrob Agents Chemother* 2006;**50**:143–7.
116. Mattiuzzi G, Estey E, Raad I, et al. Liposomal amphotericin B versus the combination of fluconazole and itraconazole as prophylaxis for invasive fungal infections during induction chemotherapy for patients with acute myelogenous leukemia and myelodysplastic syndrome. *Cancer* 2003;**97**:450–6.
117. McFarland L. Meta-analysis of probiotics for the prevention of antibiotic associated diarrhea and the treatment of *Clostridium difficile* disease. *Am J Gastroenterol* 2006;**101**:812–22.
118. Melenhorst J, Leen A, Bollard C, et al. Allogeneic virus-specific T cells with HLA alloreactivity do not produce GVHD in human subjects. *Blood* 2010;**116**:4700–2.
119. Mensah N, Peterlongo P, Steinherz P, et al. Toll-like receptor 4 polymorphisms and risk of gram-negative bacteremia after allogeneic stem cell transplantation: a prospective pilot study. *Biol Blood Marrow Transplant* 2009;**15**:1130–3.
120. Meyers J, Reed E, Shepp D, et al. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N Engl J Med* 1988;**318**:70–5.
121. Mezger M, Steffens M, Beyer M, et al. Polymorphisms in the chemokine (C-X-C motif) ligand 10 are associated with invasive aspergillosis after allogeneic stem-cell transplantation and influence CXCL10 expression in monocyte-derived dendritic cells. *Blood* 2008;**111**:534–6.
122. Mikulska M, Raiola A, Bruno B, et al. Risk factors for invasive aspergillosis and related mortality in recipients of allogeneic SCT from alternative donors: an analysis of 306 patients. *Bone Marrow Transplant* 2009;**44**:361–70.
123. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;**115**:2088–94.
124. Milano F, Pergam SA, Xie H, et al. Intensive strategy to prevent CMV disease in seropositive umbilical cord blood transplant recipients. *Blood* 2011;**118**:5689–96.
125. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med* 2002;**347**:2020–9.
126. Mullen CA, Nair J, Sandesh S, et al. Fever and neutropenia in pediatric hematopoietic stem cell transplant patients. *Bone Marrow Transplant* 2000;**25**:59–65.
127. Myers G, Krance R, Weiss H, et al. Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). *Bone Marrow Transplant* 2005;**36**: 1001–8.

128. Neely M, Rushing T, Kovacs A, et al. Voriconazole pharmacokinetics and pharmacodynamics in children. *Clin Infect Dis* 2010; **50**:27–36.
129. Nichols W, Corey L, Gooley T, et al. High risk of death due to bacterial and fungal infection among cytomegalovirus (CMV)-seronegative recipients of stem cell transplants from seropositive donors: evidence for indirect effects of primary CMV infection. *J Infect Dis* 2002;**185**:273–82.
130. Nichols WG, Corey L, Gooley T, et al. Parainfluenza virus infections after hematopoietic stem cell transplantation: risk factors, response to antiviral therapy, and effect on transplant outcome. *Blood* 2001;**98**:573–8.
131. O'Reilly R, Small T, Papadopoulos E, et al. Biology and adoptive cell therapy of Epstein-Barr virus-associated lymphoproliferative disorders in recipients of marrow allografts. *Immunol Rev* 1997; **157**:195–216.
132. Ostrosky-Zeichner L, Alexander B, Kett D, et al. Multicenter clinical evaluation of the (1–>3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin Infect Dis* 2005;**41**: 654–9.
133. Park S, Choi S, Lee D, et al. Infectious complications associated with alemtuzumab use for allogeneic hematopoietic stem cell transplantation: comparison with anti-thymocyte globulin. *Transpl Infect Dis* 2009;**11**:413–23.
134. Pascual A, Calandra T, Bolay S, et al. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. *Clin Infect Dis* 2008;**46**:201–11.
135. Pasquini M, Wang Z. Current use and outcome of hematopoietic stem cell transplantation. *CIBMTR Summary Slides* 2010; Available at: http://www.cibmtr.org.
136. Peck A, Englund J, Kuypers J, et al. Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. *Blood* 2007;**110**:1681–8.
137. Penack O, Schwartz S, Martus P, et al. Low-dose liposomal amphotericin B in the prevention of invasive fungal infections in patients with prolonged neutropenia: results from a randomized, single-center trial. *Ann Oncol* 2006;**17**:1306–12.
138. Perez-Simon J, Garcia-Escobar I, Martinez J, et al. Antibiotic prophylaxis with meropenem after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2004;**33**:183–7.
139. Perruccio K, Tosti A, Burchielli E, et al. Transferring functional immune responses to pathogens after haploidentical hematopoietic transplantation. *Blood* 2005;**106**:4397–406.
140. Pfeiffer C, Fine J, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006;**42**:1417–27.
141. Pichereau C, Desseaux K, Janin A, et al. The complex relationship between human herpesvirus 6 and acute graft-versus-host disease. *Biol Blood Marrow Transplant* 2012;**18**:141–4.
142. Popovich KJ, Hota B, Hayes R, et al. Effectiveness of routine patient cleansing with chlorhexidine gluconate for infection prevention in the medical intensive care unit. *Infect Control Hosp Epidemiol* 2009;**30**:959–63.
143. Potenza L, Vallerini D, Barozzi P, et al. Mucorales-specific T cells emerge in the course of invasive mucormycosis and may be used as a surrogate diagnostic marker in high-risk patients. *Blood* 2011;**118**:5416–19.
144. Poutsiaka DD, Price LL, Ucuzian A, et al. Blood stream infection after hematopoietic stem cell transplantation is associated with increased mortality. *Bone Marrow Transplant* 2007;**40**:63–70.
145. Raad I, Hanna H, Boktour M, et al. Management of central venous catheters in patients with cancer and candidemia. *Clin Infect Dis* 2004;**38**:1119–27.
146. Raval M, Gulbis A, Bollard C, et al. Evaluation and management of BK virus-associated nephropathy following allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**: 1589–93.
147. Reboli A, Rotstein C, Pappas P, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med* 2007;**356**:2472–82.
148. Reusser P, Einsele H, Lee J, et al. Randomized multicenter trial of foscarnet versus ganciclovir for preemptive therapy of cytomegalovirus infection after allogeneic stem cell transplantation. *Blood* 2002;**99**:1159–64.
149. Riddell J, Comer G, Kauffman C. Treatment of endogenous fungal endophthalmitis: focus on new antifungal agents. *Clin Infect Dis* 2011;**52**:648–53.
150. Rocha V, Franco R, Porcher R, et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 2002; **100**:3908–18.
151. Roman E, Osunkwo I, Militano O, et al. Liposomal amphotericin B prophylaxis of invasive mold infections in children post allogeneic stem cell transplantation. *Pediatr Blood Cancer* 2008;**50**: 325–30.
152. Safdar A, Rodriguez G, De Lima M, et al. Infections in 100 cord blood transplantations: spectrum of early and late posttransplant infections in adult and pediatric patients 1996-2005. *Medicine (Baltimore)* 2007;**86**:324–33.
153. Safrin S, Lee B, Sande M. Adjunctive folinic acid with trimethoprim-sulfamethoxazole for *Pneumocystis carinii* pneumonia in AIDS patients is associated with an increased risk of therapeutic failure and death. *J Infect Dis* 1994;**170**:912–17.
154. Sanders J Pithie A, Ganly P, et al. A prospective double-blind randomized trial comparing intraluminal ethanol with heparinized saline for the prevention of catheter-associated bloodstream infection in immunosuppressed haematology patients. *J Antimicrob Chemother* 2008;**62**:809–15.
155. Sangiolo D, Storer B, Nash R, et al. Toxicity and efficacy of daily dapsone as *Pneumocystis jiroveci* prophylaxis after hematopoietic stem cell transplantation: a case-control study. *Biol Blood Marrow Transplant* 2005;**11**:521–9.
156. Saral R, Burns W, Laskin O, et al. Acyclovir prophylaxis of herpes-simplex-virus infections. *N Engl J Med* 1981;**305**:63–7.
157. Seeley W, Marty F, Holmes T, et al. Post-transplant acute limbic encephalitis: clinical features and relationship to HHV6. *Neurology* 2007;**69**:156–65.
158. Seidel M, Peters C, Wacker A, et al. Randomized phase III study of granulocyte transfusions in neutropenic patients. *Bone Marrow Transplant* 2008;**42**:679–84.
159. Seo K, Kim D, Sohn S, et al. Protective role of interleukin-10 promoter gene polymorphism in the pathogenesis of invasive pulmonary aspergillosis after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2005;**36**:1089–95.
160. Shah J, Chemaly R. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011; **117**:2755–63.
161. Shahda S, Carlos WG, Kiel PJ, et al. The human metapneumovirus: a case series and review of the literature. *Transplant Infectious Disease* 2011;**13**:324–8.
162. Shankar S, Nania J. Management of *Pneumocystis jiroveci* pneumonia in children receiving chemotherapy. *Paediatr Drugs* 2007;**9**: 301–9.
163. Slavin M, Grigg A, Schwarer A, et al. A randomized comparison of empiric or pre-emptive antibiotic therapy after hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2007;**40**: 1571–63.
164. Slavin M, Osborne B, Adams R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation: a prospective, randomized, double-blind study. *J Infect Dis* 1995;**171**:1545–52.
165. Small T, Cowan M. Immunization of hematopoietic stem cell transplant recipients against vaccine-preventable diseases. *Expert Rev Clin Immunol* 2011;**7**:193–203.
166. Smith J, Safdar N, Knasinski V, et al. Voriconazole therapeutic drug monitoring. *Antimicrob Agents Chemother* 2006;**50**:1570–2.
167. Smith P, Benjamin DJ, Alexander B, et al. Quantification of 1,3-beta-D-glucan levels in children: preliminary data for diagnostic use of the beta-glucan assay in a pediatric setting. *Clin Vaccine Immunol* 2007;**14**:924–5.
168. Solano C, Gutierrez A, Martinez F, et al. Prophylaxis of early bacterial infections after autologous peripheral blood stem cell transplantation (PBSCT): a matched pair study comparing oral fluoroquinolones and intravenous pipericillin-tazobactam. *Bone Marrow Tranplant* 2005;**36**:59–65.
169. Srinivasan A, Wang C, Yang J, et al. Symptomatic parainfluenza virus infections in children undergoing hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**:1520–7.
170. Stadtmauer E, Vogl D, Luning Prak E, et al. Transfer of influenza vaccine-primed costimulated autologous T cells after stem cell transplantation for multiple myeloma leads to reconstitution of influenza immunity: results of a randomized clinical trial. *Blood* 2011;**117**:63–71.

171. Staras SAS, Dollard SC, Radford KW, et al. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. *Clin Infect Dis* 2006;**43**:1143–51.
172. Steinbach W, Addison R, McLaughlin L, et al. Prospective *Aspergillus* galactomannan antigen testing in pediatric hematopoietic stem cell transplant recipients. *Pediatr Infect Dis J* 2007;**26**: 558–64.
173. Styczynski J, Einsele H, Gil L, et al. Outcome of treatment of Epstein-Barr virus-related post-transplant lymphoproliferative disorder in hematopoietic stem cell recipients: a comprehensive review of reported cases. *Transpl Infect Dis* 2009;**11**:383–92.
174. Thomas N, Hollenbeak C, Ceneviva G, et al. Palivizumab prophylaxis to prevent respiratory syncytial virus mortality after pediatric bone marrow transplantation: a decision analysis model. *J Pediatr Hematol Oncol* 2007;**29**:227–32.
175. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009;**15**:1143–238.
176. Trifilio S, Scheetz M, Pi J, et al. Tacrolimus use in adult allogeneic stem cell transplant recipients receiving voriconazole: preemptive dose modification and therapeutic drug monitoring. *Bone Marrow Transplant* 2010;**45**:1352–6.
177. Trifilio S, Singhal S, Williams S, et al. Breakthrough fungal infections after allogeneic hematopoietic stem cell transplantation in patients on prophylactic voriconazole. *Bone Marrow Transplant* 2007;**40**:451–6.
178. Trivedi M, Martinez S, Corringham S, et al. Optimal use of G-CSF administration after hematopoietic SCT. *Bone Marrow Transplant* 2009;**43**:895–908.
179. Tuan I, Dennison D, Weisdorf D. *Pneumocystis carinii* pneumonitis following bone marrow transplantation. *Bone Marrow Transplant* 1992;**10**:267–72.
180. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *New Engl J Med* 2007;**356**:335–47.
181. Unal E, Yen C, Saiman L, et al. A low incidence of nontuberculous mycobacterial infections in pediatric hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2006;**12**: 1188–97.
182. Upton A, McCune J, Kirby K, et al. Fluconazole coadministration concurrent with cyclophosphamide conditioning may reduce regimen-related toxicity postmyeloablative hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2007;**13**:760–4.
183. van Burik J, Weisdorf D. Infections in recipients of blood and marrow transplantation. *Hematol Oncol Clin North Am* 1999;**13**: 1065–89.
184. van Burik J, Carter S, Freifeld A, et al. Higher risk of cytomegalovirus and aspergillus infections in recipients of T cell-depleted unrelated bone marrow: analysis of infectious complications in patients treated with T cell depletion versus immunosuppressive therapy to prevent graft-versus-host disease. *Biol Blood Marrow Transplant* 2007;**13**:1487–98.
185. van Burik J, Ratanatharathorn V, Stepan D, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. *Clin Infect Dis* 2004;**39**:1407–16.
186. van der Velden W, Blijlevens N, Maas F, et al. NOD2 polymorphisms predict severe acute graft-versus-host and treatment-related mortality in T-cell-depleted haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2009;**44**:243–8.
187. Vardakas K, Michalopoulos A, Falagas M. Fluconazole versus itraconazole for antifungal prophylaxis in neutropenic patients with haematological malignancies: a meta-analysis of randomised-controlled trials. *Br J Haematol* 2005;**131**:22–8.
188. Vasconcelles M, Bernardo M, King C, et al. Aerosolized pentamidine as pneumocystis prophylaxis after bone marrow transplantation is inferior to other regimens and is associated with decreased survival and an increased risk of other infections. *Biol Blood Marrow Transplant* 2000;**6**:35–43.
189. Vernon MO, Hayden MK, Trick WE, et al. Chlorhexidine gluconate to cleanse patients in a medical intensive care unit: the effectiveness of source control to reduce the bioburden of vancomycin-resistant enterococci. *Arch Intern Med* 2006;**166**: 306–12.
190. Vogel M, Weissgerber P, Goeppert B, et al. Accuracy of serum LDH elevation for the diagnosis of *Pneumocystis jiroveci* pneumonia. *Swiss Med Wkly* 2011;**141**:w13184.
191. Wagner H-J, Cheng YC, Huls MH, et al. Prompt versus preemptive intervention for EBV lymphoproliferative disease. *Blood* 2004;**103**:3979–81.
192. Walls T, Hawrami K, Ushiro-Lumb I, et al. Adenovirus infection after pediatric bone marrow transplantation: is treatment always necessary? *Clin Infect Dis* 2005;**40**:1244–9.
193. Weinstock D, Feinstein M, Sepkowitz K, et al. High rates of infection and colonization by nontuberculous mycobacteria after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2003;**31**:1015–21.
194. Wingard JR, Carter SL, Walsh TJ, et al. Randomized double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection (IFI) after allo hematopoietic cell transplantation (HCT). *Blood* 2010;**116**:5111–18.
195. Wingard J, Sostrin M, Vriesendorp H, et al. Interstitial pneumonitis following autologous bone marrow transplantation. *Transplantation* 1988;**43**:61–5.
196. Winston D, Ho W, Bartoni K, et al. Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients: results of a placebo-controlled, double-blind trial. *Ann Intern Med* 1993;**118**:179–84.
197. Wolff S, Fay J, Stevens D, et al. Fluconazole vs low-dose amphotericin B for the prevention of fungal infections in patients undergoing bone marrow transplantation: a study of the North American Marrow Transplant Group. *Bone Marrow Transplant* 2000;**25**: 853–9.
198. Zaas A, Liao G, Chien J, et al. Plasminogen alleles influence susceptibility to invasive aspergillosis. *PLoS Genet* 2008;4:e1000101.
199. Zerr DM, Corey L, Kim HW, et al. Clinical outcomes of human herpesvirus 6 reactivation after hematopoietic stem cell transplantation. *Clin Infect Dis* 2005;**40**:932–40.
200. Zerr D, Gupta D, Huang M, et al. Effect of antivirals on human herpesvirus 6 replication in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:309–17.

TABLE 70-1 **Types of Infections Encountered in 332 Children After Heart Transplantation in a Multi-institutional Study***

Type	Number
Bacterial (total)	164
Coagulase-negative staphylococci	25
Enterobacter species	21
Pseudomonas aeruginosa	16
Viral	
Cytomegalovirus	51
Varicella-zoster	11
Respiratory syncytial virus	10
Herpes simplex	6
Other viruses	8
Fungal	19
Candida species	12
Pneumocystis jiroveci	7

*Two hundred seventy-six infections in 136 patients.
Data from Schowengerdt KO, Naftel D, Seib PM, et al. Infection after pediatric heart transplantation; results of a multi-institutional study. *J Heart Lung Transplant* 1997;**16**:1207–16.

transplantation. The Pediatric Heart Transplant Study Group prospectively collected data from 22 pediatric centers in the United States from January 1993 to December 1994 on 332 children younger than 18 years (mean age, 5.5 years) who had undergone heart transplantation.[121] One or more infections (276 total) occurred in 41 percent of the patients (mean follow-up time, 11.8 months) for an average of 0.84 infections per patient; 22 percent had one infection, 8 percent had two infections, and 11 percent had three or more infections during the study period (Table 70-1). In a similar multicenter study in adults who had undergone heart transplantation between January 1990 and June 1991, infections developed in 31 percent of 814 patients, with 22 percent having one infection and 9 percent having two or more infections.[96] Bacterial infections were the most common type of infection occurring after transplantation in pediatric and adult patients.

Immunosuppressive therapy for children who have undergone heart transplantation usually consists of some combination of cyclosporine or tacrolimus, mycophenolate mofetil or azathioprine, and corticosteroids. Sirolimus, an mTOR inhibitor, has been administered to about 10 percent of pediatric heart transplant recipients.[75] Induction immunosuppression may include interleukin-2 receptor antagonists or antithymocyte globulin.[76] Cyclosporine and tacrolimus predominantly block the effect of interleukin-2 on T cells, an action resulting in a diminished T-cell response to mitogen stimulation.[54] The infections seen in heart transplant patients outside the postoperative period generally are a result of this block in T-cell function. Because the types of infections seen in these patients vary with the time elapsed since transplantation, the following discussion is organized in such a manner.

TABLE 70-2 **Evaluation of Children Before Heart Transplantation**

Serology
Cytomegalovirus
Epstein-Barr virus
Toxoplasma gondii
Human immunodeficiency virus
Hepatitis A, B, and C
Rubeola, mumps, rubella, varicella*
Cultures
Nasopharyngeal, stool, or tracheal aspirates†
Skin tests
Purified protein derivative
Freezing of an extra aliquot of serum
Review of the child's immunization status

*For children who are older than 12 months and previously immunized.
†See text for explanation.

PRETRANSPLANTATION EVALUATION

Several infectious agents may be transmitted to the patient via the transplanted organ or can become "reactivated" after transplantation. Determining the antibody status of the recipient and the donor against selected microorganisms (CMV, Epstein-Barr virus [EBV], *Toxoplasma gondii*) helps physicians anticipate or diagnose infections that develop after transplantation. A reasonable pretransplant evaluation for children is outlined in Table 70-2. The child's immunization status is documented, and vaccinations are completed when possible (i.e., hepatitis B vaccine, *Haemophilus influenzae* type b, or *Streptococcus pneumoniae*). The Committee on Infectious Diseases of the American Academy of Pediatrics recommends for immunized children older than age 12 months who are scheduled to undergo solid-organ transplantation that serologic tests be performed for rubeola, mumps, rubella, and varicella to determine whether protective titers are present; if they are not, then vaccination is repeated.[3] If possible, appropriate vaccines should be administered at least 1 month before the patient undergoes transplantation. Evidence of selected active infections is a contraindication for transplantation. Chemoprophylaxis should be considered strongly for children with a positive tuberculin skin test (purified protein derivative). Dental status also is assessed.

In addition to having the routine pretransplant evaluation as outlined in Table 70-2, each patient should be screened carefully for selected infections appropriate to the individual circumstances. If surgery is planned during the respiratory disease season and the patient has respiratory symptoms just before having surgery, screening for influenza virus or respiratory syncytial virus (RSV) by rapid techniques may facilitate prescribing antiviral therapy postoperatively. In areas where community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of infection, performing surface cultures to detect MRSA colonization may modify the choice of antibiotics used for surgical prophylaxis.[72] Applying mupirocin intranasally may influence the rate of postoperative MRSA infections.[101] In

areas where coccidioidomycosis is endemic, pretransplant serology for *Coccidioides immitis* is recommended; if the result is positive, antifungal prophylaxis is provided.[149] A similar approach for histoplasmosis should be considered.[29] Children from resource-poor countries may harbor *Salmonella* or intestinal parasites asymptomatically, and these organisms can cause serious infection after the child undergoes transplantation. Preoperative stool cultures for enteropathogens and examination of the stool for parasites may alert the clinician that these pathogens are present and could be the cause of postoperative infections.

If the patient is being mechanically ventilated before undergoing transplantation, review of recent tracheal aspirate cultures may help in the selection of empirical antibiotics for initial treatment of suspected nosocomial sepsis or pneumonia. Pretransplant infections associated with procedures such as implantation of ventricular assist devices may require prolonged antibiotic therapy after transplantation is performed.[88,130] These infections typically are caused by common nosocomial pathogens and are not a contraindication to undergoing heart transplantation.

SURGICAL PROPHYLACTIC ANTIBIOTICS

Prophylactic antibiotics typically are administered to patients undergoing heart transplantation surgery. For each institution, selection of prophylactic antibiotics should be based partly on the organisms isolated from postoperative wound infections in that center and the antimicrobial susceptibility of these organisms. Cefazolin generally is a reasonable choice for prophylaxis, unless MRSA is a nosocomial pathogen of concern, in which case vancomycin is suggested.[20] In areas where MRSA is a common community pathogen, vancomycin or some other antibiotic active against MRSA might be considered instead of cefazolin, as determined by preoperative surveillance cultures. Routine use of extended-spectrum cephalosporins is discouraged because it may lead to colonization of the patient by antibiotic-resistant, gram-negative organisms that hyperproduce β-lactamase, such as *Enterobacter cloacae*. Recommendations for the duration of prophylactic antibiotic treatment in these patients are not definite, but some centers continue prophylactic antibiotics for 2 to 5 days or more postoperatively, or until all lines and chest tubes have been removed.

IMMEDIATE POSTOPERATIVE INFECTIONS

Common Infections

During the month after the patient undergoes heart transplantation, the types of infections encountered are the same as those complicating major thoracic surgery. Pneumonia and bacteremia are the most common postoperative infections. The frequency of bacteremia and the distribution of organisms are similar in children and adults after undergoing heart transplantation.[96,121] In a multi-institutional pediatric study, the risk of development of any infection was 25 percent 1 month after transplantation.[121] Overall, 60 episodes of bacteremia occurred in the 136 patients who became infected, and the bloodstream was the most common site of bacterial infection.[122] Lung abscesses and mediastinitis are seen less frequently. Familiarity with the organisms and the antimicrobial susceptibility of isolates recovered from other children in the intensive care units (ICUs) in which these patients receive care helps direct the initial empirical selection of antibiotics.

Postoperative bacteremia is related predominantly to the indwelling lines required for monitoring and infusion of medication. *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp., and gram-negative enteric organisms such as *Enterobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp., and *Escherichia coli* are the most common causes of nosocomial bacteremia in the pediatric ICU.[67,113] Other foci of infection, such as pneumonia or mediastinitis, also may result in bacteremia.[150] Vancomycin plus an aminoglycoside is a typical antibiotic combination for empirical use for treatment of suggested bacteremia in patients with central lines in place and without focal evidence of infection. Vancomycin therapy should be discontinued as soon as possible if an organism requiring the administration of vancomycin is not isolated.[110] A bacterial line infection may be eradicated successfully without removing the line, but the line should be removed if blood cultures remain positive or the patient's clinical condition deteriorates.[94] Optimally, central lines are removed for *S. aureus* central line–related bloodstream infections.[25] Fungemia, generally with *Candida albicans* or another *Candida* spp., also may be associated with central line–related infections. Central lines complicated by fungemia should be removed immediately.[39,94] Centrally placed lines must be removed as soon as practical so that catheter-associated infections can be prevented.

As in other critically ill children, pneumonia is a particularly common occurrence in heart transplant patients because of the operative site and requirements for intubation and mechanical ventilation. During the first postoperative week, definite bacterial pneumonia developed in 3 of 22 children (14%) in an early study from the University of Pittsburgh Medical Center (UPMC).[58] In the multi-institutional Pediatric Heart Transplant Study Group study, 56 bacterial lung infections were identified, 24 of which developed in patients maintained on a ventilator at the time of transplantation.[121] Nosocomial pneumonia caused by gram-negative bacilli such as *Pseudomonas* and *Enterobacter* or *S. aureus* is especially common in this setting.[113] Daily chest radiographs taken until the patient is released from the ICU may identify pneumonitis before it is clinically suspected. Gram stain and culture of a tracheal aspirate can help guide therapy for lobar pneumonia.

A broad-spectrum combination of antibiotics, such as an extended-spectrum penicillin with a β-lactamase inhibitor (i.e., piperacillin-tazobactam) plus an aminoglycoside, usually is initiated until a pathogen or pathogens are identified. Empirical therapy should be based on the antibiotic susceptibility patterns of the common nosocomial pathogens in the specific ICU in which the

patient is receiving care. Vancomycin also should be considered if MRSA is part of the resident flora in the ICU. Computed tomography (CT) of the chest may detect basilar and retrocardiac pneumonia, which may not be visualized readily by conventional chest radiographs. CT or ultrasonography generally is helpful in assessing the size or characteristics of pleural effusions that may require drainage.

If interstitial pneumonitis is encountered, a more aggressive approach to determining a cause is warranted. Bronchoscopy with bronchoalveolar lavage should be considered strongly. In children who have pulmonary infiltrates after undergoing heart transplantation, flexible bronchoscopy is more useful for establishing a fungal or viral etiology as opposed to a bacterial cause of pneumonia because most patients have received broad-spectrum antibiotics before undergoing the procedure.[140] Lavage fluid is pooled and processed for bacteria including mycobacteria, viruses, fungi, and protozoa by using culture techniques and special stains. Mini bronchoalveolar lavages are commonly employed in some pediatric centers, but how their results compare to a formal bronchoalveolar lavage procedure is not clear and has not been well studied in children.[45] *Legionella* may be an important consideration in some centers.[19,111] Noninfectious causes of pulmonary infiltrates in these children include pulmonary edema, atelectasis, hemorrhage, and adult respiratory distress syndrome.

Urinary tract infections (UTIs) also are common occurrences in the month after undergoing heart transplantation. Urinary catheterization and the immunosuppressive agents contribute to the risk for developing a UTI. Gram-negative enteric organisms (*E. coli*, *Klebsiella pneumoniae*, *P. aeruginosa*, and *Enterobacter* spp.), enterococci, and *Candida* spp. are isolated most commonly. Removal of the catheter as soon as possible minimizes the potential for development of a UTI, which has occurred in approximately 10 percent of adults. In the multi-institutional Pediatric Heart Transplant Study Group study, the urinary tract was the site of 16 bacterial infections.[121] In addition, UTIs developed in three children (14%) at UPMC during the 2 to 3 weeks after undergoing transplantation.[58]

The broad-spectrum antibiotics used to treat the bacterial complications of transplantation promote candidal infection of the urinary tract. Along with removal of the urinary catheter, a short course of intravenous amphotericin B for 10 days or less or of fluconazole with careful dosing because of drug interactions with cyclosporine and tacrolimus is an option for treating candidal cystitis.[105] In some patients, a urine culture positive for *Candida* is a clue that a disseminated candidal infection is present and that further investigation is necessary to exclude the involvement of other organs, especially the kidneys.

Risk factors for early infection in the multi-institutional Pediatric Heart Transplant Study Group study were younger recipient age (particularly <6 months), mechanical ventilation at the time of transplantation, positive donor CMV serology with a CMV-negative recipient, and longer donor ischemic time.[121]

Sternal Wounds and Mediastinitis

Sternal wound infections and mediastinitis occur in less than 5 percent of adult patients receiving modern immunosuppressive therapy for heart transplantation.[73,96,97] Most of these infections occur during the first postoperative month, usually within the first 2 weeks, and are superficial. Almost all are caused by bacteria. Staphylococci and other gram-positive bacteria generally are responsible for 50 percent of cases, and the remainder are caused by a variety of gram-negative bacilli.[97,99] Surgical wound infections developed in eight children in the multi-institutional Pediatric Heart Transplant Study Group study, although the site of the infection was not noted.[121] Over a 15-year period, 15 (0.2%) children at Texas Children's Hospital, Houston, Texas, developed mediastinitis after undergoing cardiac surgery; 2 children had undergone heart transplantation.[143] At another children's hospital, between 1995 and 2003, 3 percent (5 of 165) of children undergoing heart and lung transplantation developed mediastinitis.[83]

Postoperative bleeding requiring reexploration is a risk factor for development of mediastinitis. Fever, incisional pain, and an unstable sternum suggest mediastinitis; however, patients may have no specific evidence of infection, including fever. The white blood cell count may be elevated. A pericardial effusion frequently is detected with the development of mediastinitis, and pericardiocentesis may yield purulent material. CT of the chest may show a fluid collection or abscess within the mediastinum and can detect sternal osteomyelitis. Most cases of mediastinitis are caused by *S. aureus*, coagulase-negative staphylococci, or gram-negative bacteria. Median sternotomy wound infections after repair of a congenital heart lesion occur in less than 1 percent of children in large centers. Mediastinitis caused by gram-negative bacilli in association with pneumonia and bacteremia developed in 3 of the 22 children in the UPMC series; each occurred within 2 weeks postoperatively.[58] Two of the three patients died. *Candida* spp. caused both cases of mediastinitis in the 2 children after heart transplantation. Long and colleagues[83,84] reported that the mediastinitis that developed in the five patients who had undergone heart and lung transplantation was caused by *E. coli*, *Torulopsis glabrata*, *Aspergillus fumigatus*, *Burkholderia cepacia*, and vancomycin-resistant enterococcus.

A superficial median sternotomy wound infection not associated with an unstable sternum can be treated by local drainage of the infected subcutaneous tissue and administration of appropriate antibiotics.[35] Vacuum-assisted closure may be an important aid in addition to antibiotics.[1] A more aggressive approach is required for more serious infections associated with an unstable sternum, mediastinitis, or osteomyelitis of the sternum.[24,35,73] Adequate drainage and débridement of the area are crucial, and any involved wires should be removed. Mediastinal drains usually are kept in place for several days. Some authorities recommend irrigating the drains with povidone-iodine, but the duration of irrigation is uncertain. A reoperation after the initial drainage procedure may be necessary. Pending culture

results, antibiotic therapy is directed against *S. aureus* and gram-negative bacilli. A 4- to 6-week course of antibiotics usually is recommended. Antifungal therapy is initiated if a yeast or fungus is noted on Gram or special stains or isolated from cultures. Careful attention given to surgical technique to minimize postoperative bleeding and early withdrawal of chest and mediastinal tubes placed intraoperatively decrease the incidence of these potentially fatal infections.

Other Infections Encountered During the First Postoperative Month

Herpes Simplex

Herpes simplex infections of the oral mucosa and other superficial surfaces are common after heart transplantation. Oral herpes simplex was observed in 21 percent (11 of 53) of children undergoing transplantation at Stanford University Medical Center.[14] In the multi-institutional Pediatric Heart Transplant Study Group study, only six episodes of herpes simplex infection were noted.[121] Visceral involvement is unusual, although it may develop.[80] Herpes simplex infections typically occur approximately 13 days (range, 0 to 4 months) after transplantation and are reactivation processes, not newly acquired infections.[109] A decrease in lymphocyte transformation in response to viral antigen in vitro may explain the increased rate of infection that occurs in the first 12 weeks post transplantation. In a large randomized study comparing azathioprine with mycophenolate mofetil in adult heart transplant recipients who also were receiving cyclosporine and corticosteroids, herpes simplex infection occurred more commonly in the group treated with mycophenolate mofetil (23% vs. 16%, $p < .05$).[36] Institution of antiviral therapy for herpes simplex infection is warranted in these immunocompromised patients. The most common antiviral agent for herpes simplex is acyclovir, which can be administered orally or intravenously. Valacyclovir dosing in children has been established for children older than age 2 years, but a commercial suspension is unavailable.

Prophylactic acyclovir is recommended by some experts for heart transplant recipients who are seropositive for herpes simplex.[53] Acyclovir is given intravenously during the perioperative period and then orally for 3 to 6 months after transplantation. Other physicians suggest that because labial or oral herpes simplex is treated so easily, a prophylactic approach is not warranted. In view of concern related to toxicity, drug interactions, and the possibility of resistance developing, I favor the approach that targets treatment after a mucocutaneous lesion is noted.

Legionella pneumophila

Legionella pneumophila infection should be included in the differential diagnosis for fever, respiratory symptoms, and pulmonary infiltrates that develop after heart transplantation.[27] *Legionella* pneumonia can develop during the first postoperative month, but the frequency with which this infection occurs varies among transplant centers. Although legionnaires' disease is an uncommon occurrence in children, nosocomial infections have been documented in a children's hospital and immunosuppression is a risk factor.[19,23] Appropriate cultures and direct fluorescent antibody stains for *Legionella* should be performed on sputum, other respiratory secretions (obtained by invasive techniques), pleural fluid, or lung tissue to detect this pathogen in a timely fashion. A *Legionella* urinary antigen test is available for serogroup 1 antigens and is quite sensitive.[141]

Macrolides should be considered for empirical therapy if *Legionella* is a serious consideration in children with nosocomial pneumonia. Erythromycin or azithromycin is provided for 2 weeks to complete therapy. Macrolides interact with many of the immunosuppressive agents administered to these patients, however. Quinolones also are quite active against *Legionella* and avoid many of these interactions; in adult transplant patients, they have become the agents of choice.[141] The use of quinolones should be considered for pediatric organ transplant recipients in whom infection with *L. pneumophila* is suspected. In hospitals caring for patients with transplants, routine surveillance culture of the hospital water supply should be considered.

Respiratory Syncytial Virus

Ten episodes of RSV infection were noted in the multi-institutional study by the Pediatric Heart Transplant Study Group.[121] RSV can be acquired in the hospital soon after undergoing transplantation or can be acquired in the community before undergoing surgery or after discharge. Too few patients in whom RSV infection developed after they underwent heart transplantation have been described to comment on the clinical features. Two children in the early UPMC study[58] were noted to be infected with RSV, with both infections occurring on postoperative day 10. Rapidly progressive patchy infiltrates on chest radiographs developed in one child after undergoing heart-lung transplantation, and the second child had only mild upper respiratory symptoms. Tachypnea, cough, fever, wheezing, and the use of accessory muscles occurred commonly in the 18 pediatric liver transplant recipients from the UPMC with RSV infection.[108] Radiographic changes included interstitial and lobar infiltrates, atelectasis, and pleural effusion in 12 patients. Two patients required mechanical ventilation after the onset of symptoms related to RSV infection occurred; three others were intubated before acquiring RSV infection and subsequently had complicated courses.

Morbidity and mortality rates related to RSV infection are increased in otherwise immunocompetent children with congenital heart disease, especially when associated with pulmonary hypertension.[86] Because RSV can be acquired in the hospital, RSV infection should be considered in a young transplant patient with respiratory symptoms and fever, especially during the colder months.[60] RSV infection is documented by culture or by rapid detection of RSV infection by enzyme-linked immunosorbent assay or fluorescent antibody testing of respiratory secretions.

The decision to administer ribavirin to these patients is based primarily on the severity of the illness. In a small group of children with underlying bronchopulmonary dysplasia or congenital heart disease, aerosolized ribavirin seemed to be associated with more rapid improvement than that in patients given placebo.[61] Administration of aerosolized ribavirin to a heart transplant recipient with proved or suspected moderate to severe RSV infection is reasonable. As is the case with children requiring mechanical ventilation because of severe RSV lower respiratory tract infection, however, the efficacy of ribavirin in this situation is unknown.[135] The 2009 *Red Book* states, "Ribavirin … may be considered for use in select patients with documented, potentially life-threatening RSV infection."[6]

The monoclonal antibody palivizumab was found to be safe, well tolerated, and effective in preventing serious RSV infections in young children with hemodynamically significant congenital heart disease in a large multicenter randomized, double-blind, placebo-controlled trial.[41] Although no specific recommendations have been made regarding the use of palivizumab in children who have undergone heart or other organ transplantation, it would be reasonable to administer it to children 24 months or younger after they undergo heart transplantation and especially postoperatively when the patient is stable.[7] The combination of ribavirin and RSV immunoglobulin has been administered to pediatric bone marrow transplant recipients with RSV lower respiratory tract infection.[31] The outcome in these patients was improved over that of historical controls, but no randomized trials have been conducted. RSV immunoglobulin was not efficacious in treating RSV lower respiratory tract infection in children with congenital heart disease who were younger than 2 years.[114] Although palivizumab reduced the concentration of RSV in the tracheal aspirates of children with respiratory failure caused by RSV, its efficacy in treatment is unknown.[68,89]

INFECTIONS BETWEEN THE FIRST AND SIXTH POSTOPERATIVE MONTHS

Cytomegalovirus

CMV is the virus that most frequently infects immunosuppressed cardiac transplant patients. Asymptomatic or symptomatic infections are noted most often between the first and sixth months after undergoing transplantation and rarely after the seventh month. In adult series, 12 to 90 percent of patients had evidence of CMV infection postoperatively.[34,56,96] In the multi-institutional Pediatric Heart Transplant Study Group study,[121] 51 episodes of CMV infection occurred in 332 patients and accounted for 60 percent of the viral infections, with a peak occurrence in the second month after transplantation. CMV infection occurred more frequently in older children than in infants. In another study, infants younger than 120 days old had CMV infection and disease less often than did infants older than 120 days after undergoing heart transplantation.[46] Maternal antibody to CMV may have been protective in the younger infants. In the UPMC series, seven children (32%) had CMV infections, with onset occurring at a mean of 33 days after transplant (range, 23 to 43 days).[58]

CMV infection in transplant recipients occurs in three or four possible settings. In a seronegative recipient, primary CMV infection is acquired through the transplanted heart, through blood transfusions from seropositive donors, or from community exposure. Seropositive recipients can have reactivation of latent CMV infection or be reinfected with a second strain of CMV from the heart or from blood products derived from seropositive donors.[26,27] The exact site within the donor heart where CMV may reside in a latent form is unknown, but it may be either cardiac cells or leukocytes that remain within the donor heart. When primary CMV infection is acquired from the donor organ, CMV disease tends to be more severe than if CMV infection is acquired from blood or blood products.[155,156]

Several risk factors for development of CMV infection after undergoing organ transplantation are recognized. Donor and recipient serologic status and the immunosuppressive regimen are the most significant risk factors for acquiring CMV infection after undergoing heart transplantation. Gorensek and colleagues[56] found that positive recipient CMV serology before transplantation and a larger than average dose of corticosteroids were significant risk factors for acquiring CMV infection. Among the group of patients with CMV infection, positive recipient serology was associated with asymptomatic infection and excessive corticosteroid dosing was a risk factor for acquiring symptomatic CMV infection. CMV tissue invasion occurred more commonly in patients receiving mycophenolate mofetil compared with azathioprine.

The clinical manifestations of CMV infection vary. Patients may seroconvert, or a latent infection may be reactivated, as determined by positive cultures, but these patients have no symptoms attributable to the CMV infection. Fever, leukopenia, and thrombocytopenia are common postoperative manifestations of systemic CMV infection. Patients may complain of arthralgias, myalgias, and nonspecific abdominal pain. Atypical lymphocytes are noted more often in adult than pediatric patients. CMV infection can cause hepatitis, pneumonitis, retinitis, myocarditis, and gastrointestinal disease, including colitis.* The retinitis may be asymptomatic or associated with complaints such as floaters or scotomas. Ophthalmologic screening for CMV retinitis is recommended by some authorities for all patients 3 to 4 months after cardiac transplantation.[42]

Of the tissues invaded by CMV, involvement of the lung leads to the greatest mortality rate—13 percent in one study.[76] CMV pneumonitis is characterized by fever, hypoxemia, and, usually, diffuse interstitial infiltrates, although lobar consolidation may occur.[128] Pulmonary infections with other viruses or with bacteria or *Pneumocystis jiroveci* and other pathologic processes (infarction) may coexist with CMV pneumonitis. Gastritis, gastric ulceration, duodenitis, esophagitis, pyloric perforation, and colonic hemorrhage can be documented by

*References 34, 40, 42, 55, 56, 71, 129.

endoscopy. In the multi-institutional Pediatric Heart Transplant Study Group study, the lung or gastrointestinal tract was the site of CMV infection in 13 (lung) and 6 (gastrointestinal tract) episodes.[58] Death related to CMV in the pediatric study occurred in 6 percent. In the heart transplant registry spanning 1998 to 2010, CMV was considered the cause of death in 2.7, 0.4, and 0.4 percent of patients 31 days to 1 year, after 3 to 5 years, and after 10 years post transplantation, respectively.[75] In another analysis using a multi-institutional registry of pediatric heart transplantation patients, clinical CMV infection was uncommon in the setting of antiviral and/or CMV antibody prophylaxis.[87]

The diagnosis of CMV infection can be based on changes in antibody titer to CMV in paired sera run in parallel with the use of established tests or on changes in CMV IgM results. Isolation of CMV or detection of CMV DNA from a variety of sources, such as urine, blood, bronchial washings, or tissues, also establishes that CMV infection is present. In a seronegative recipient, the possibility of active CMV infection can be anticipated by periodic monitoring of CMV serology, quantitative blood PCR, or cultures. Whether the CMV infection is causing a symptomatic or invasive illness is more difficult to establish. Histopathologic evidence of CMV infection, such as typical viral inclusions or detection of antigen in tissue by special stains, is required to confirm organ involvement by CMV, although this criterion often is not considered a requirement for clinical trials of preventive measures for CMV disease. Cultures of the buffy coat are positive more frequently in patients with symptomatic than in patients with asymptomatic CMV infection; in patients with primary CMV infection and lung involvement, CMV cultures tend to be positive earlier in the postoperative period than in patients who do not have lung involvement.[56] CMV antigen–positive leukocytes detected by monoclonal antibodies to the early antigen of CMV were found 10 to 28 days before increases in CMV antibody occurred in five patients with active CMV infection.[148] Polymerase chain reaction (PCR) can detect DNA from CMV in blood and other tissues readily and with great sensitivity.[44,48] The antigen and PCR techniques have been used for early detection of CMV infection so that preemptive antiviral therapy can be initiated.[36]

In addition to the CMV infection syndromes, CMV infection itself seems to affect the transplant recipient adversely in other ways. Symptomatic or asymptomatic CMV infection is associated with a higher rate of graft rejection or graft loss, a greater risk of development of fungal infection, more frequent and earlier graft atherosclerosis, and a significantly lower survival rate than occur in patients who do not have CMV infection in some studies.[44,116] In the multi-institutional study, CMV-positive donor serology in conjunction with CMV-negative recipient serology was a risk factor for the acquisition of earlier infection with any organism.[121] More recent studies have not found an association between pretransplant CMV seropositivity and death or coronary artery vasculopathy.[87]

CMV infection of the wide variety of cells that it invades leads to the activation of protein synthesis and the production of multiple immunologically active molecules, including cytokines, especially tumor necrosis factor-α, which adds to the immune deficits induced by the immunosuppressive agents.[44,77] Allograft injury or rejection may be associated with CMV infection of the transplanted organ itself.[44]

Successful treatment or suppression of visceral CMV disease by ganciclovir, a nucleoside analog active in vitro against CMV, requires a timely diagnosis. Ganciclovir has been shown to alter CMV disease favorably in heart transplant patients, along with allowing a reduction in immunosuppressive therapy, when possible.[74] The standard approach for treating symptomatic CMV infection is administration of 2 to 3 weeks of intravenous ganciclovir, although the optimal duration of this therapy and the need for maintenance oral doses are unclear.[117] The dosage is 5 mg/kg every 12 hours with careful monitoring of hematologic parameters and renal function if renal function initially is normal. Modification of the dose is necessary if renal function is impaired. Viremia should be cleared before discontinuing therapy.[44] The most common adverse reactions to ganciclovir are neutropenia, thrombocytopenia, impaired renal function, seizures, and other central nervous system (CNS) abnormalities.

The role of CMV hyperimmunoglobulin in treating CMV infection in these patients requires further study. After bone marrow transplantation, the addition of CMV immunoglobulin to ganciclovir may be superior to ganciclovir alone in treating CMV pneumonia.[38,112]

In one pediatric study, symptomatic CMV disease developed in five children after they had undergone heart transplantation; each had blood cultures and PCR positive for CMV.[51] Four were treated with ganciclovir for 14 days; one received ganciclovir for 30 days. All received CMV-IgG (150 mg/kg) weekly for 3 weeks. Symptomatic CMV disease was treated successfully in each case.

If possible, prevention of CMV infection in heart transplant recipients is optimal. Only seronegative blood should be used for transfusions when the recipient and the donor are seronegative. Careful control of immunosuppressive therapy, especially with corticosteroids, may help avoid the acquisition of some infections. In the situation of a seronegative recipient of a heart from a seropositive donor, prophylactic administration of CMV immunoglobulin may be useful. In one study involving the prevention of CMV disease, ganciclovir was compared with CMV immunoglobulin (misoprostol [Cytotec]) in 31 CMV-seropositive heart transplant recipients in whom OKT3 monoclonal antibody was used for early immunoprophylaxis.[2] CMV disease and visceral involvement occurred more frequently in the group given CMV immunoglobulin (40%) than in the group given ganciclovir (6%; $p = .03$). CMV-IgG would be expected to be more beneficial in recipients who are seronegative to CMV.

In one large randomized double-blind, placebo-controlled trial of CMV-seropositive transplant recipients, ganciclovir significantly reduced the incidence of CMV illness during the first 120 days after heart transplantation (9% vs. 46% in controls, $p < .001$).[93] No differences were noted between the study groups for seronegative recipients. Combining ganciclovir and CMV-IgG for

prophylaxis of high-risk seronegative recipients of hearts from seropositive donors has resulted in mixed findings. Avery[11] found that the combination was not particularly effective; symptomatic CMV syndrome developed in 50 percent of patients. Garjarski and associates[48] provided CMV-IgG 150 mg/kg intravenously at weeks 0, 2, 4, 6, and 8, and 100 mg/kg intravenously at weeks 12 and 16 plus ganciclovir 5 mg/kg every 12 hours intravenously for weeks 1 and 2 and 6 mg/kg/day intravenously at weeks 3 and 4 to 19 children who were recipients of heart transplants from CMV-seropositive donors. CMV disease occurred in 3 of the 10 children who were CMV seronegative and in 1 of the 10 recipients who were seropositive. Adverse effects of these agents were not reported.

In the study from Stanford University Medical Center, high-risk recipients received CMV-IgG immediately after undergoing transplantation (150 mg/kg administered within 72 hours after transplantation, followed by 100 mg/kg at weeks 2, 4, 6, and 8, and 50 mg/kg at weeks 12 and 16).[146,147] In addition, ganciclovir was administered intravenously immediately after transplantation at a dose of 5 mg/kg every 12 hours for 14 days, followed by 6 mg/kg/day for the next 2 weeks. These patients were compared with a historical control group at the same institution who received ganciclovir in the 2 to 3 years before CMV-IgG was used. The 27 recipients treated prophylactically with ganciclovir and CMV-IgG had a higher disease-free incidence of CMV, a lower incidence of rejection, and a higher survival rate than those of the historical cohort treated with ganciclovir alone. Data from the Scientific Registry of Transplant Recipients indicated that for children undergoing heart transplantation, CMV-IgG with or without ganciclovir and ganciclovir with or without CMV-IgG were associated with significant reduction in graft loss and deaths versus no prophylaxis.[136] The combination looks promising for prevention of CMV disease in high-risk heart transplant recipients but a randomized trial is required before the combination can be recommended routinely.

CMV resistant to ganciclovir may emerge as a result of ganciclovir prophylaxis or treatment.[13,16] Foscarnet or cidofovir is an alternative agent in this situation.

In a meta-analysis of CMV prevention strategies for posttransplantation CMV disease, Small and associates[134,136] concluded that universal prophylaxis and preemptive approaches are equally effective in reducing the incidence of CMV disease.

Epstein-Barr Virus

EBV may cause a spectrum of diseases, including a mononucleosis-like syndrome, polyclonal lymphoproliferation, or monoclonal lymphoproliferation, usually of B cells, in pediatric heart transplant recipients. The transplanted organ is thought to be the most frequent source of EBV. Posttransplantation lymphoproliferative disorders (PTLDs) refer to B-cell expansion that may be localized, nodal, extranodal, or widely disseminated. The largest series of children who have undergone heart transplantation is from the UPMC; in this series, PTLD developed in 7.7 percent (6 of 78) of pediatric heart transplant recipients.[18] A major risk factor for the subsequent development of PTLD was being seronegative for EBV before undergoing transplantation. PTLD developed in one third of seronegative recipients of thoracic organs who acquired primary EBV infection (10 of 30). PTLD developed in none of the children who were seropositive before transplantation. Almost all these cases occurred within 1 year of transplantation.

In another series, 19 children were EBV seropositive and 31 were EBV seronegative before undergoing heart transplantation. PTLD developed in 1 of 19 patients who were seropositive before undergoing transplantation and in 12 of 19 who became seropositive after transplantation.[158] It did not develop in any of the 12 recipients who remained EBV seronegative. In contrast to the UPMC experience, the mean time to confirmation of PTLD was 29 months (range 3 to 72 months).

Webber and associates[153,154] reviewed the experience with PTLD after heart transplantation among 1184 primary organ recipients in 19 centers from 1993 to 2002. Fifty-six patients (5%) developed PTLD a mean of 23.8 months post transplantation. In a study from Germany, 12 of 147 (8.2%) developed PTLD a mean of 3.2 ± 2.2 years post transplantation.[123] Among 173 heart transplant recipients at the University of Toronto Hospital, 23 (13.3%) developed PTLD within a median of 4 years post transplantation.[90] Higher maximum EBV viral load and longer duration of induction therapy were associated with increased risk of PTLD. In the largest study among a multicenter group, PTLD developed in about 5 percent of 2374 children.[47] Pretransplant seropositivity for EBV and the receipt of induction treatment except OKT3 was associated with a reduced risk of developing PTLD.

Symptoms of PTLD may include fever, malaise, sore throat, and lymphadenopathy. Some children may have splenomegaly, CNS symptoms such as lethargy or seizures, or gastrointestinal complaints.[16,18] Concurrent opportunistic infections are common. Nodules in the lung may be noted on chest radiographs.

The diagnosis of PTLD requires biopsy of involved tissue showing lymphoid proliferation with an immunoblastic component. Molecular techniques typically detect EBV nucleic acids in tissue. EBV serology also is helpful. Quantitative measurement of EBV DNA and RNA in peripheral blood is used to detect primary infection or reactivation at a very early time point and to monitor viral loads serially over time to detect PTLD in the most timely manner.[115] When reported as EBV copies per 10^5 peripheral blood lymphocytes, patients with PTLD typically have viral loads between 500 and 5000, which are much greater than the loads detected in normal latency.

Management generally involves decreasing the immunosuppressive regimen or discontinuing it temporarily. Anti-CD20 antibody (rituximab) is a commercially available monoclonal antibody that specifically binds to the CD20 antigen of normal and malignant B cells and results in antibody-dependent and complement-dependent cytotoxicity. Rituximab has been administered with success to children and adults with PTLD after they have undergone solid-organ transplantation.[37,123,125,126] Antiviral therapy with acyclovir or ganciclovir also is

administered. Some centers administer ganciclovir, acyclovir, or intravenous gamma-globulin for prevention of PTLD, although no prospective studies have confirmed that such an approach is efficacious.[57]

Toxoplasma gondii

An increased incidence of toxoplasmosis is apparent in recipients of heart transplants compared with other organ transplants, although it remains an uncommon infection after heart transplantation.[138] In the multi-institutional study by the Pediatric Heart Transplant Study Group,[121] toxoplasmosis was not mentioned. *T. gondii* has a predilection for muscle and can be transmitted to the recipient from the heart of a seropositive donor. Active *T. gondii* infections may occur in donor hearts.[118] Less commonly, reactivation of old infection occurs in the recipient. The greatest risk for acquisition of toxoplasmosis occurs in a seronegative recipient of a heart from a seropositive donor. Clinical symptoms usually develop after the first postoperative month and generally within 3 months of transplantation.[62,85,95] Fever alone may be the only clinical manifestation. Dissemination of the parasite to the CNS may lead to signs and symptoms of meningoencephalitis, such as lethargy, seizures, coma, and hemiparesis. Chorioretinitis may result in diminished visual acuity. A sepsis-like picture, pneumonia, and cutaneous lesions are unusual manifestations.[10]

CT or magnetic resonance imaging of the brain may detect ring-enhancing mass lesions, which typically are multiple and in periventricular locations. Definitive diagnosis of CNS toxoplasmosis requires the demonstration of tachyzoites or cysts in tissue by biopsy or at necropsy. Serologic tests help monitor seronegative patients for seroconversion and allow a more aggressive approach to be taken to the early diagnosis of toxoplasmosis.[133] *T. gondii* has been seen on endomyocardial biopsy specimens routinely obtained to monitor for rejection.[84]

Therapy for toxoplasmosis with pyrimethamine and sulfadiazine may lead to recovery; these drugs also should be administered if the patient seroconverts.[49] Prophylactic administration of pyrimethamine to seronegative recipients of hearts from seropositive donors is recommended.[157] Prophylactic trimethoprim-sulfamethoxazole also seems to be protective in this high-risk situation.[12,99] Spiramycin is not a useful prophylactic agent.[133]

Aspergillus fumigatus

A. fumigatus is the non-*Candida* fungal infection most commonly reported outside the immediate postoperative period in most series and may be noted first at necropsy.[106] In a report from Stanford University Medical Center,[57] *Aspergillus* infections developed in 620 consecutive heart transplant recipients between 1980 and 1996.[99] Most commonly, *Aspergillus* infections were in the lung (31 recipients) or were disseminated (17 recipients). The median time to onset was 52 days. Disseminated aspergillosis was the most common infectious episode responsible for the highest mortality rates in this series. One child in Pittsburgh had disseminated aspergillosis.[58] In the multi-institutional study by the Pediatric Heart Transplant Study Group, seven non-*Candida* fungal infections occurred: *Aspergillus* spp., two; *Cryptococcus*, *Rhizopus*, and *Rhizomucor*, one each; and unspecified, two.[121] All patients with disseminated infection died. Among 1854 children in the Pediatric Heart Transplant Study, 18 had infection due to *Aspergillus* spp. (including 9 with pulmonary and 5 with cutaneous infections).[159] Risk was highest in the first 6 months after transplantation; prior surgery and the need for mechanical ventilation at transplant were independent risk factors for invasive fungal infection.

CNS invasion occurs in many patients with pulmonary aspergillosis, and aspergillosis is the most common cause of brain abscess in organ transplant recipients.[62,98,132] Alterations in mental status occur most frequently, and seizures may occur in 40 percent of cases. Multifocal lesions are seen commonly on CT of head and show a predilection for the junction of the gray and white matter. Mediastinitis and endocarditis are other manifestations of invasive aspergillosis.[81,126] Although isolation of *Aspergillus* from respiratory secretions in a patient with pneumonitis does not establish a diagnosis, aspergillosis is so difficult to establish firmly and is so frequently fatal that treatment should be considered seriously based on this culture alone. Serial measurement of serum galactomannan levels may help in recognizing this infection. Measuring the galactomannan in the bronchoalveolar lavage fluid may also detect evidence of invasive pulmonary aspergillosis in solid-organ transplant patients, perhaps leading to earlier administration of antifungal therapy.[28]

Voriconazole is the agent of choice for invasive aspergillosis.[64,151] The reader is referred to Chapter 197 on aspergillosis and the Infectious Diseases Society of America clinical guidelines for recommendations regarding therapy.[151]

INFECTIONS AFTER THE SIXTH POSTOPERATIVE MONTH

Nocardia asteroides

A dry cough, fever, and the presence of a solitary pulmonary nodule or abscess on a chest radiograph are characteristic of infection with *N. asteroides*.[79,131] Although pulmonary nodules are characteristic of *Nocardia*, *Aspergillus* and CMV can be associated with nodules as well.[99] Some patients are asymptomatic despite having an abnormal chest radiograph. Infection with *Nocardia* was noted in only 3 percent of cyclosporine-treated patients in the early Stanford University Medical Center series.[65] The median time to the onset of infection was 225 days. A similar incidence of lung nodules or masses secondary to *Nocardia* was noted in a series from New York.[63] In the follow-up Stanford series, 23 episodes of *Nocardia* infection (3.7%) occurred, with 19 in the lung.[99] The median time of onset of infection in the second series was 147 days. *Nocardia* infections are rare in pediatric heart transplant recipients.

Nocardia is isolated best from direct lung tissue specimens, but it also may be cultured from bone, skin, or

other sites of involvement. If a cutaneous skin lesion of *Nocardia* is recognized, other sites should be evaluated promptly for involvement.[52,54] The formation of single or multiple abscesses in the CNS may result from hematogenous dissemination.[64] Seizures may develop after invasion of brain parenchyma.

The drug of choice for treating *N. asteroides* infection is a sulfonamide. Prolonged administration (average 10 months) generally leads to clearing of the pulmonary lesions. Minocycline is an alternative therapeutic agent.[107] The incidence of *Nocardia* infection has declined with the introduction of routine prophylaxis with trimethoprim-sulfamethoxazole.

Pneumocystis jiroveci

P. jiroveci (formerly *P. carinii*) pneumonia has been reported in 3 to 4 percent of heart transplantation recipients during the postcyclosporine era.[43,59,65] In the multiinstitutional study of the Pediatric Heart Transplant Study Group, *P. carinii* was noted in seven children (2.1%).[121] In the Pediatric Heart Transplant Study, 18 of 1854 patients (1%) had *P. jiroveci* infection. Ninety-five percent of these infections occurred between 2 months and 2 years after transplantation.[100] Fever, a nonproductive cough, and tachypnea are typical symptoms; hypoxemia is characteristic.[159] The chest radiograph classically shows a diffuse interstitial infiltrate that can progress rapidly. The most expeditious method of documenting *P. jiroveci* pneumonia in children is with methenamine silver or specific antibody staining of fluid or tissue obtained by bronchoalveolar lavage or lung biopsy.

Elevation in serum β-D-glucan concentration can be a clue to this infection. Coinfection with CMV or other pathogens occurs commonly.

Treatment of *Pneumocystis* pneumonia in a pediatric heart transplant recipient is identical to that for other immunocompromised children. Use of trimethoprim-sulfamethoxazole prophylaxis for at least 4 months after transplantation has decreased markedly the incidence of this infection in recipients of heart transplants.[103] Only one patient died in the Pediatric Heart Transplant Study Group study as a result of *Pneumocystis* pneumonia.[100]

Streptococcus pneumoniae

S. pneumoniae is an important community-acquired pathogen in heart transplant recipients, who are at increased risk for acquiring this organism.[8] In the multiinstitutional Pediatric Heart Transplant Study Group study, two episodes of pneumococcal infection were recorded.[121] During a 10-year period, 9 (11%) of 80 cardiac transplant patients in Little Rock, Arkansas, had 12 episodes of pneumococcal bacteremia.[142] Over an 11-year period, 4 of 105 children undergoing heart transplantation in Toronto had systemic pneumococcal infections.[144] In the follow-up series from Stanford University Medical Center, seven pulmonary infections with *S. pneumoniae* were reported.[99] In an eight-center pediatric surveillance study spanning 5 years, pneumococcal infection developed in 10 patients after a median time from transplantation of 17 months (range, 5 to 76 months).[124] Three of the 10 patients had two episodes, and one patient had three episodes of pneumococcal infection. The median age of the 10 patients at the time of the first pneumococcal infection was 26 months (range, 15 to 89 months). The pneumococcal serotypes of the isolates from these patients were the same as noted in healthy children and generally would be covered by the 23-valent pneumococcal vaccine or the 13-valent pneumococcal conjugate vaccine.

Other Viruses

Influenza and parainfluenza viruses can cause serious infections at any time after transplantation, but especially in the immediate postoperative period.[9,91] Additional risk factors for development of severe disease leading to death are young age and augmentation of immunosuppression. Fever, cough, rhinorrhea, and pharyngitis are typical symptoms of upper respiratory tract infections. More serious manifestations include adult respiratory distress syndrome, a requirement for intubation and mechanical ventilation, a sepsis-like picture, or CNS symptoms such as headache, photophobia, and lethargy. Viral infection may enhance the likelihood of allograft rejection.

For influenza A infection, oseltamivir, amantadine, or rimantadine should be considered in younger children older than 12 months of age with a heart transplant because of their enhanced predisposition for severe or complicated influenza infection.[8] In older children, zanamivir or oseltamivir is indicated. Recommendations vary depending on the susceptibility of the circulating influenza strains, and thus the treating physician needs to be aware of this information.

Parvovirus B19 infection is recognized to cause severe anemia associated with low or no reticulocytes in recipients of heart transplants, similar to the red blood cell suppression in other immunosuppressed patients.[102] In one child, severe pneumonia developed in association with fever and a blanching maculopapular rash involving the face, trunk, and extremities.[69] The parvovirus B19 genome also has been detected in myocardial biopsy specimens of children experiencing cardiac allograft rejection. Of six children described in one report, one had a diffuse rash and two had persistent rejection despite receiving aggressive therapy.[122] In a series of children from Loma Linda University Medical Center who underwent myocardial biopsy for possible rejection, parvovirus genome was detected in 5 of 553 biopsy samples taken from 149 children.[127] The genome parvovirus B19 was the most commonly detected viral genome in endomyocardial biopsy specimens from 99 children in one study.[21] The detection of parvovirus B19 genome did not correlate with cellular rejection, but if it was present chronically there was an association with transplant coronary artery disease. Intravenous immunoglobulin is beneficial for the treatment of anemia related to parvovirus B19, but its efficacy in treating pneumonia or possible allograft rejection is unknown.[70]

Varicella virus infection remains a common childhood illness that can cause life-threatening disease in

immunocompromised children. In a group of 28 children younger than 10 years old at the time of undergoing heart transplantation who had been monitored for at least 1 year between 1986 and 1999, 14 cases of primary infection with varicella-zoster virus were identified.[33] The mean time after transplant was 3 years (range, 9 months to 7.5 years). All children were seronegative at the time of transplantation. These children were treated successfully with either parenteral therapy followed by oral acyclovir or oral valacyclovir (mean dose, 77 mg/kg/day) for 7 days. Only one child had recurrent varicella, and none had zoster. Routine administration of varicella vaccine to young children helps to decrease most concerns about varicella developing after organ transplantation because of herd protection. Some patients do lose serologically measured immunity to varicella after undergoing transplantation, especially those children who undergo a transplant in the first 2 years of life.[145,152]

Adenovirus has been associated with serious infections after solid-organ transplantation. Among 28 children with solid-organ transplants (9 with heart transplants) and adenoviral infections, the most common symptoms were fever, diarrhea, vomiting, and abdominal pain.[30] Infection occurred a median of 1.6 months after transplantation. Only 2 of these children received antiviral therapy, and all survived the infection. Cidofovir may be beneficial in some patients with disseminated infection, especially of the lungs, or increasing viral loads determined by PCR.[33,66]

IMMUNOSUPPRESSIVE AGENTS AND ANTIBIOTICS

Cyclosporine and tacrolimus have improved the success of organ transplantation considerably. Generally, the incidence of infection seems to be less since the introduction of cyclosporine than with the earlier immunosuppressive regimens, although morbidity rates remain high in heart transplant recipients. Cyclosporine serum levels are monitored carefully to ensure that concentrations associated with optimal immunosuppression and minimal adverse effects are maintained. Some antibiotics interfere with the pharmacokinetics of cyclosporine or tacrolimus, which may lead to an increase or decrease in their levels.* These interactions are outlined in Table 70-3. Because cyclosporine and tacrolimus are nephrotoxic, the antimicrobial agents (amphotericin B, aminoglycosides, acyclovir, ceftazidime) administered to heart transplant recipients may be additive; renal function must be monitored carefully.

TABLE 70-3 **Effect of Various Antibiotics on Cyclosporine/Tacrolimus Levels**

Increased Level	Decreased Level
Clarithromycin	Sulfadiazine
Azithromycin	Rifampin
Erythromycin	Trimethoprim-sulfamethoxazole
Ketoconazole	? Nafcillin
Fluconazole	? Isoniazid
Itraconazole	? Ciprofloxacin (counteracts immunosuppression)
Posaconazole	
Voriconazole	
Caspofungin	

IMMUNIZATIONS

No specific guidelines exist for the immunization of children after they have received a heart transplant, although studies examining the immunogenicity of selected vaccines in this population are being conducted. A prudent approach is to follow the recommendations that the Committee on Infectious Diseases of the American Academy of Pediatrics has developed for immunizing immunosuppressed children.[4] Ideally, the patient will have received the recommended routine vaccines before undergoing transplantation.

Children who have received transplants before reaching 2 to 3 years of age do not respond to the pneumococcal polysaccharide vaccine as well as do children who are older when they receive a transplanted heart, even though the vaccine is administered several years after they receive the transplant.[51] An impairment in immunoglobulin isotype switching from IgM to IgG and especially IgG2 seems to result from the immunosuppressive therapy that these children are receiving.[50] In one study, the antibody response of children aged 2 to 18 months old after solid-organ transplantation was compared with age-matched children to the 7-valent pneumococcal conjugate vaccine (PCV7) followed by the 23-valent polysaccharide vaccine (PV23) 2 months later.[82] The antibody levels achieved were significantly higher in the controls, and a second dose of PCV7 given 2 months after the administration of the first PCV7 dose did not lead to higher antibody levels.

For previously unimmunized transplant recipients aged 2 to 18 years old, conjugate pneumococcal vaccine is recommended, followed by the 23-valent pneumococcal vaccine at least 2 to 12 months later. Ideally, children will have completed their PCV7 series before undergoing transplantation.[5] Ongoing immunosuppression seems to prevent maturation of the response to polysaccharide antigens in younger children, which it is hoped that the conjugate vaccine can overcome. The PV23 is less immunogenic in adults after they have undergone heart transplantation than it is in healthy controls.

Antibodies to the influenza vaccine develop in most children, but previous exposure predicts a better response.[92] Annual administration of influenza vaccine seems to be safe and immunogenic in children after they have undergone solid-organ transplantation. In one study, low-level histologic rejection occurred after the administration of influenza vaccine.[17] In a later study, the administration of inactivated influenza vaccine was not

*References 15, 21, 78, 104, 119, 120, 137, 139.

associated with an increased risk of rejection.[155] Depending on the time of year and the approximate date of transplantation, influenza vaccination is appropriate for the patient and all household contacts and health care workers to whom the patient might be exposed. The live-attenuated influenza vaccine is not recommended for individuals receiving immunosuppressive therapy or their close contacts.

After transplantation has been performed and after immunosuppressive therapy has been initiated, administration of live viral vaccines is contraindicated. The enhanced inactivated polio vaccine should be given to the child and to normal siblings. Measles, mumps, rubella, and varicella vaccines can be given to the siblings. Diphtheria, tetanus, and acellular pertussis inactivated vaccines should be given at the routine booster schedule, although antibody responses may not be equivalent to those observed in normal children. Varicella vaccine should be administered before transplantation is performed, if varicella antibody is not detected at the pretransplant evaluation in a child aged 12 months or older. Whether the antibody response data generated in children with leukemia during maintenance chemotherapy can be applied to heart transplant recipients who must continue daily immunosuppressive therapy is unclear.

NEW REFERENCES SINCE THE SIXTH EDITION

4. American Academy of Pediatrics. Immunization in special clinical circumstances. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 72–86.
5. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 524–35.
6. American Academy of Pediatrics. Respiratory syncytial virus. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 560–9.
7. American Academy of Pediatrics. Policy statements—modified recommendations for use of palivizumab for prevention of respiratory syncytial virus infections. *Pediatrics* 2009;**124**:1694–701.
15. Berge M, Chevalier P, Benammar M, et al. Safe management of tacrolimus together with posaconazole in lung transplant patients with cystic fibrosis. *Ther Drug Monit* 2009;**31**:396–9.
21. Breinholt JP, Moulik M, Dreyer WJ, et al. Viral epidemiologic shift in inflammatory heart disease: the increasing involvement of parvovirus B19 in the myocardium of pediatric cardiac transplant patients. *J Heart Lung Transplant* 2010;**29**:739–46.
25. Carrillo-Marquez MA, Hulten KG, Mason EO, Kaplan SL. Clinical and molecular epidemiology of *Staphylococcus aureus* catheter-related bacteremia in children. *Pediatr Infect Dis J* 2010;**29**:410–14.
29. Cuellar-Rodriguez J, Avery RK, Lard M, et al. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. *Clin Infect Dis* 2009;**49**:710–16.
47. Gajarski RJ, Blume ED, Urschel S, et al. Infection and malignancy after pediatric heart transplantation: the role of induction therapy. *J Heart Lung Transplant* 2011;**30**:299–308.
67. Hsu RB, Chang CI, Fang CT, et al. Bloodstream infection in heart transplant recipients: 12-year experience at a university hospital in Taiwan. *Eur J Cardiothorac Surg* 2011;**40**:1362–7.
68. Hynicka LM, Ensor CR. Prophylaxis and treatment of respiratory syncytial virus in adult immunocompromised patients. *Ann Pharmacother* 2012;**46**:558–66.
70. Jordan SC, Toyoda M, Kahwaji J, Vo AA. Clinical aspects of intravenous immunoglobulin use in solid organ transplant recipients. *Am J Transplant* 2011;**11**:196–202.
75. Kirk R, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth pediatric heart transplantation report—2011. *J Heart Lung Transplant* 2011;**30**:1095–103.
87. Mahle WT, Fourshee MT, Naftel DM, et al. Does cytomegalovirus serology impact outcome after pediatric heart transplantation? *J Heart Lung Transplant* 2009;**28**:1299–305.
90. Manlhiot C, Pollock-Barziv SM, Holmes C, et al. Post-transplant lymphoproliferative disorder in pediatric heart transplant recipients. *J Heart Lung Transplant* 2010;**29**:648–57.
94. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.
100. Ng B, Dipchand A, Naftel D, et al. Outcomes of *Pneumocystis jiroveci* pneumonia infections in pediatric heart transplant recipients. *Pediatr Transplant* 2011;**15**:844–8.
105. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
123. Schubert S, Abdul-Khaliq H, Lehmkuhl HB, et al. Diagnosis and treatment of post-transplantation lymphoproliferative disorder in pediatric heart transplant patients. *Pediatr Transplant* 2009;**13**:54–62.
145. Urschel S, Cremer S, Birnbaum J, et al. Lack of serologic immunity against vaccine-preventable diseases in children after thoracic transplantation. *Transpl Int* 2009;**23**:619–27.
149. Vikram HR, Blair JE. Coccidioidomycosis in transplant recipients: a primer for clinicians in nonendemic areas. *Curr Opin Organ Transplant* 2009;**14**:606–12.
151. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
159. Zaoutis TE, Webber S, Naftel DC, et al. Invasive fungal infections in pediatric heart transplant recipients: incidence, risk factors, and outcomes. *Pediatr Transplant* 2011;**15**:465–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agarwal JP, Ogilvie M, Wu LC, et al. Vacuum-assisted closure for sternal wounds: a first-line therapeutic management approach. *Plast Reconstr Surg* 2005;**116**:1035–40; discussion 1041–3.
2. Aguado JM, Gomez-Sanchez MA, Lumbreras C, et al. Prospective randomized trial of efficacy of ganciclovir versus that of anti-cytomegalovirus (CMV) immunoglobulin to prevent CMV disease in CMV-seropositive heart transplant recipients treated with OKT3. *Antimicrob Agents Chemother* 1995;**39**:1643–5.
3. American Academy of Pediatrics. Immunization in special clinical circumstances. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 80–1.
4. American Academy of Pediatrics. Immunization in special clinical circumstances. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 72–86.
5. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 524–35.
6. American Academy of Pediatrics. Respiratory syncytial virus. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 560–69.
7. American Academy of Pediatrics. Policy statements—modified recommendations for use of palivizumab for prevention of respiratory syncytial virus infections. *Pediatrics* 2009;**124**:1694–701.
8. Amber IJ, Gilbert EM, Schiffman G, Jacobson JA. Increased risk of pneumococcal infections in cardiac transplant recipients. *Transplantation* 1990;**49**:122–5.
9. Apalsch AM, Green M, Ledesma-Medina J, et al. Parainfluenza and influenza virus infections in pediatric organ transplant recipients. *Clin Infect Dis* 1995;**20**:394–9.
10. Arnold SJ, Kinney MC, McCormick MS, et al. Disseminated toxoplasmosis: unusual presentations in the immunocompromised host. *Arch Pathol Lab Med* 1997;**121**:869–73.
11. Avery RK. Prevention and treatment of cytomegalovirus infection and disease in heart transplant recipients. *Curr Opin Cardiol* 1998;**13**:122–9.
12. Baden LR, Katz JT, Franck L, et al. Successful toxoplasmosis prophylaxis after orthotopic cardiac transplantation with trimethoprim-sulfamethoxazole. *Transplantation* 2003;**75**:339–43.
13. Baldanti F, Simoncini L, Sarasini A, et al. Ganciclovir resistance as a result of oral ganciclovir in a heart transplant recipient with multiple human cytomegalovirus strains in blood. *Transplantation* 1998;**66**:324–9.
14. Baum D, Bernstein D, Starnes VA, et al. Pediatric heart transplantation at Stanford: results of a 15-year experience. *Pediatrics* 1991;**88**:203–14.
15. Berge M, Chevalier P, Benammar M, et al. Safe management of tacrolimus together with posaconazole in lung transplant patients with cystic fibrosis. *Ther Drug Monit* 2009;**31**:396–9.
16. Bernstein D, Baum D, Berry G, et al. Neoplastic disorders after pediatric heart transplantation. *Circulation* 1993;**88**:230–7.
17. Blumberg EA, Fitzpatrick J, Stutman PC, et al. Safety of influenza vaccine in heart transplant recipients. *J Heart Lung Transplant* 1998;**17**:1075–80.
18. Boyle GJ, Michaels MG, Webber SA, et al. Transplantation lymphoproliferative disorders in pediatric thoracic organ recipients. *J Pediatr* 1997;**131**:309–13.
19. Brady MT. Nosocomial legionnaires disease in a children's hospital. *J Pediatr* 1989;**115**:46–50.
20. Bratzler DW, Houck PM. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Clin Infect Dis* 2004;**38**:1706–15.
21. Breinholt JP, Moulik M, Dreyer WJ, et al. Viral epidemiologic shift in inflammatory heart disease: the increasing involvement of parvovirus B19 in the myocardium of pediatric cardiac transplant patients. *J Heart Lung Transplant* 2010;**29**:739–46.
22. Campana C, Regazzi MB, Buggia I, Molinaro M. Clinically significant drug interactions with cyclosporin: an update. *Clin Pharmacokinet* 1996;**30**:141–79.
23. Carlson NC, Kuskie MR, Dobyns EL, et al. Legionellosis in children: an expanding spectrum. *Pediatr Infect Dis J* 1990;**9**:133–7.
24. Carrier M, Hudon G, Paquet E, et al. Mediastinal and pericardial complications after heart transplantation: not-so-unusual postoperative problems? *Cardiovasc Surg* 1994;**2**:395–7.
25. Carrillo-Marquez MA, Hulten KG, Mason EO, Kaplan SL. Clinical and molecular epidemiology of *Staphylococcus aureus* catheter-related bacteremia in children. *Pediatr Infect Dis J* 2010;**29**:410–14.
26. Chou SW. Cytomegalovirus infection and reinfection transmitted by heart transplantation. *J Infect Dis* 1987;**155**:1054–6.
27. Chow JW, Yu VL. *Legionella:* a major opportunistic pathogen in transplant recipients. *Semin Respir Infect* 1998;**13**:132–9.
28. Clancy CJ, Jaber RA, Leather HL, et al. Bronchoalveolar lavage galactomannan in diagnosis of invasive pulmonary aspergillosis among solid-organ transplant recipients. *J Clin Microbiol* 2007;**45**:1759–65.
29. Cuellar-Rodriguez J, Avery RK, Lard M, et al. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. *Clin Infect Dis* 2009;**49**:710–16.
30. de Mezerville MH, Tellier R, Richardson S, et al. Adenoviral infections in pediatric transplant recipients: a hospital-based study. *Pediatr Infect Dis J* 2006;**25**:815–18.
31. DeVincenzo JP, Hirsch RL, Fuentes RJ, Top FH Jr. Respiratory syncytial virus immune globulin treatment of lower respiratory tract infection in pediatric patients undergoing bone marrow transplantation—a compassionate use experience. *Bone Marrow Transplant* 2000;**25**:161–5.
32. Doan ML, Mallory GB, Kaplan SL, et al. Treatment of adenovirus pneumonia with cidofovir in pediatric lung transplant recipients. *J Heart Lung Transplant* 2007;**26**:883–9.
33. Dodd DA, Burger J, Edwards KM, Dummer JS. Varicella in a pediatric heart transplant population on nonsteroid maintenance immunosuppression. *Pediatrics* 2001;**108**:E80.
34. Dummer JS, White LT, Ho M, et al. Morbidity of cytomegalovirus infection in recipients of heart or heart-lung transplants who received cyclosporine. *J Infect Dis* 1985;**152**:1182–91.
35. Edwards MS, Baker CJ. Median sternotomy wound infections in children. *Pediatr Infect Dis* 1983;**2**:105–9.
36. Egan JJ, Lomax J, Barber L, et al. Preemptive treatment for the prevention of cytomegalovirus disease: in lung and heart transplant recipients. *Transplantation* 1998;**65**:747–52.
37. Elstrom RL, Andreadis C, Aqui NA, et al. Treatment of PTLD with rituximab or chemotherapy. *Am J Transplant* 2006;**6**:569–76.
38. Emanuel D, Cunningham I, Jules-Elysee K, et al. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. *Ann Intern Med* 1988;**109**:777–82.
39. Eppes SC, Troutman JL, Gutman LT. Outcome of treatment of candidemia in children whose central catheters were removed or retained. *Pediatr Infect Dis J* 1989;**8**:99–104.
40. Etheridge SP, Bolman RM 3rd, Braunlin EA. Cytomegalovirus colitis in a pediatric heart transplant patient. *Clin Transplant* 1994;**8**:409–12.
41. Feltes TF, Cabalka AK, Meissner HC, et al. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. *J Pediatr* 2003;**143**:532–40.
42. Fishburne BC, Mitrani AA, Davis JL. Cytomegalovirus retinitis after cardiac transplantation. *Am J Ophthalmol* 1998;**125**:104–6.
43. Fishman JA. *Pneumocystis carinii* and parasitic infections in transplantation. *Infect Dis Clin North Am* 1995;**9**:1005–44.
44. Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998;**338**:1741–51.
45. Fujitani S, Yu VL. Diagnosis of ventilator-associated pneumonia: focus on nonbronchoscopic techniques (nonbronchoscopic bronchoalveolar lavage, including mini-BAL, blinded protected specimen brush, and blinded bronchial sampling) and endotracheal aspirates. *J Intensive Care Med* 2006;**21**:17–21.
46. Fukushima N, Gundry SR, Razzouk AJ, Bailey LL. Cytomegalovirus infection in pediatric heart transplantation. *Transplant Proc* 1993;**25**:1423–5.

47. Gajarski RJ, Blume ED, Urschel S, et al. Infection and malignancy after pediatric heart transplantation: the role of induction therapy. *J Heart Lung Transplant* 2011;**30**:299–308.
48. Gajarski RJ, Rosenblatt HM, Denfield SW, et al. Outcomes among pediatric heart transplant recipients. *Tex Heart Inst J* 1997;**24**:97–104.
49. Gallino A, Maggiorini M, Kiowski W, et al. Toxoplasmosis in heart transplant recipients. *Eur J Clin Microbiol Infect Dis* 1996;**15**:389–93.
50. Gennery AR, Cant AJ, Baldwin CI, Calvert JE. Characterization of the impaired antipneumococcal polysaccharide antibody production in immunosuppressed pediatric patients following cardiac transplantation. *J Clin Immunol* 2001;**21**:43–50.
51. Gennery AR, Cant AJ, Spickett GP, et al. Effect of immunosuppression after cardiac transplantation in early childhood on antibody response to polysaccharide antigen. *Lancet* 1998;**351**: 1778–81.
52. Gentry LO, Zeluff B, Kielhofner MA. Dermatologic manifestations of infectious diseases in cardiac transplant patients. *Infect Dis Clin North Am* 1994;**8**:637–54.
53. Gold D, Corey L. Acyclovir prophylaxis for herpes simplex virus infection. *Antimicrob Agents Chemother* 1987;**31**:361–7.
54. Goldman MH, Barnhart G, Mohanakumar T, et al. Cyclosporine in cardiac transplantation. *Surg Clin North Am* 1985;**65**:637–59.
55. Gonwa TA, Capehart JE, Pilcher JW, Alivizatos PA. Cytomegalovirus myocarditis as a cause of cardiac dysfunction in a heart transplant recipient. *Transplantation* 1989;**47**:197–9.
56. Gorensek MJ, Stewart RW, Keys TF, et al. A multivariate analysis of the risk of cytomegalovirus infection in heart transplant recipients. *J Infect Dis* 1988;**157**:515–22.
57. Green M, Reyes J, Webber S, Rowe D. The role of antiviral and immunoglobulin therapy in the prevention of Epstein-Barr virus infection and post-transplant lymphoproliferative disease following solid organ transplantation. *Transpl Infect Dis* 2001;**3**:97–103.
58. Green M, Wald ER, Fricker FJ, et al. Infections in pediatric orthotopic heart transplant recipients. *Pediatr Infect Dis J* 1989;**8**: 87–93.
59. Gryzan S, Paradis IL, Zeevi A, et al. Unexpectedly high incidence of *Pneumocystis carinii* infection after lung-heart transplantation: implications for lung defense and allograft survival. *Am Rev Respir Dis* 1988;**137**:1268–74.
60. Hall CB, Douglas RG Jr, Geiman JM, Messner MK. Nosocomial respiratory syncytial virus infections. *N Engl J Med* 1975;**293**: 1343–6.
61. Hall CB, McBride JT, Gala CL, et al. Ribavirin treatment of respiratory syncytial viral infection in infants with underlying cardiopulmonary disease. *JAMA* 1985;**254**:3047–51.
62. Hall WA, Martinez AJ, Dummer JS, et al. Central nervous system infections in heart and heart-lung transplant recipients. *Arch Neurol* 1989;**46**:173–7.
63. Haramati LB, Schulman LL, Austin JH. Lung nodules and masses after cardiac transplantation. *Radiology* 1993;**188**:491–7.
64. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002;**347**:408–15.
65. Hofflin JM, Potasman I, Baldwin JC, et al. Infectious complications in heart transplant recipients receiving cyclosporine and corticosteroids. *Ann Intern Med* 1987;**106**:209–16.
66. Hoffman JA. Adenoviral disease in pediatric solid organ transplant recipients. *Pediatr Transplant* 2006;**10**:17–25.
67. Hsu RB, Chang CI, Fang CT, et al. Bloodstream infection in heart transplant recipients: 12-year experience at a university hospital in Taiwan. *Eur J Cardiothorac Surg* 2011;**40**:1362–7.
68. Hynicka LM, Ensor CR. Prophylaxis and treatment of respiratory syncytial virus in adult immunocompromised patients. *Ann Pharmacother* 2012;**46**:558–66.
69. Janner D, Bork J, Baum M, Chinnock R. Severe pneumonia after heart transplantation as a result of human parvovirus B19. *J Heart Lung Transplant* 1994;**13**:336–8.
70. Jordan SC, Toyoda M, Kahwaji J, Vo AA. Clinical aspects of intravenous immunoglobulin use in solid organ transplant recipients. *Am J Transplant* 2011;**11**:196–202.
71. Kaplan CS, Petersen EA, Icenogle TB, et al. Gastrointestinal cytomegalovirus infection in heart and heart-lung transplant recipients. *Arch Intern Med* 1989;**149**:2095–100.
72. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
73. Karwande SV, Renlund DG, Olsen SL, et al. Mediastinitis in heart transplantation. *Ann Thorac Surg* 1992;**54**:1039–45.
74. Keay S, Petersen E, Icenogle T, et al. Ganciclovir treatment of serious cytomegalovirus infection in heart and heart-lung transplant recipients. *Rev Infect Dis* 1988;**10**:563–72.
75. Kirk R, Edwards LB, Kucheryavaya AY, et al. The Registry of the International Society for Heart and Lung Transplantation: Fourteenth pediatric heart transplantation report—2011. *J Heart Lung Transplant* 2011;**30**:1095–103.
76. Kirklin JK, Naftel DC, Levine TB, et al. Cytomegalovirus after heart transplantation: risk factors for infection and death: a multiinstitutional study. The Cardiac Transplant Research Database Group. *J Heart Lung Transplant* 1994;**13**:394–404.
77. Koskinen PK, Kallio EA, Tikkanen JM, et al. Cytomegalovirus infection and cardiac allograft vasculopathy. *Transpl Infect Dis* 1999;**1**:115–26.
78. Kramer MR, Marshall SE, Denning DW, et al. Cyclosporine and itraconazole interaction in heart and lung transplant recipients. *Ann Intern Med* 1990;**113**:327–9.
79. Krick JA, Stinson EB, Remington JS. *Nocardia* infection in heart transplant patients. *Ann Intern Med* 1975;**82**:18–26.
80. Kusne S, Schwartz M, Breinig MK, et al. Herpes simplex virus hepatitis after solid organ transplantation in adults. *J Infect Dis* 1991;**163**:1001–7.
81. Levin T, Suh B, Beltramo D, Samuel R. *Aspergillus* mediastinitis following orthotopic heart transplantation: case report and review of the literature. *Transpl Infect Dis* 2004;**6**:129–31.
82. Lin PL, Michaels MG, Green M, et al. Safety and immunogenicity of the American Academy of Pediatrics—recommended sequential pneumococcal conjugate and polysaccharide vaccine schedule in pediatric solid organ transplant recipients. *Pediatrics* 2005;**116**:160–7.
83. Long CB, Shah SS, Lautenbach E, et al. Postoperative mediastinitis in children: epidemiology, microbiology and risk factors for Gram-negative pathogens. *Pediatr Infect Dis J* 2005;**24**: 315–19.
84. Luft BJ, Billingham M, Remington JS. Endomyocardial biopsy in the diagnosis of toxoplasmic myocarditis. *Transplant Proc* 1986; **18**:1871–3.
85. Luft BJ, Naot Y, Araujo FG, et al. Primary and reactivated *Toxoplasma* infection in patients with cardiac transplants: clinical spectrum and problems in diagnosis in a defined population. *Ann Intern Med* 1983;**99**:27–31.
86. MacDonald NE, Hall CB, Suffin SC, et al. Respiratory syncytial viral infection in infants with congenital heart disease. *N Engl J Med* 1982;**307**:397–400.
87 Mahle WT, Fourshee MT, Naftel DM, et al. Does cytomegalovirus serology impact outcome after pediatric heart transplantation? *J Heart Lung Transplant* 2009;**28**:1299–305.
88. Malani PN, Dyke DB, Pagani FD, Chenoweth CE. Nosocomial infections in left ventricular assist device recipients. *Clin Infect Dis* 2002;**34**:1295–300.
89. Malley R, DeVincenzo J, Ramilo O, et al. Reduction of respiratory syncytial virus (RSV) in tracheal aspirates in intubated infants by use of humanized monoclonal antibody to RSV F protein. *J Infect Dis* 1998;**178**:1555–61.
90. Manlhiot C, Pollock-Barziv SM, Holmes C, et al. Post-transplant lymphoproliferative disorder in pediatric heart transplant recipients. *J Heart Lung Transplant* 2010;**29**:648–57.
91. Mauch TJ, Bratton S, Myers T, et al. Influenza B virus infection in pediatric solid organ transplant recipients. *Pediatrics* 1994;**94**: 225–9.
92. Mauch TJ, Crouch NA, Freese DK, et al. Antibody response of pediatric solid organ transplant recipients to immunization against influenza virus. *J Pediatr* 1995;**127**:957–60.
93. Merigan TC, Renlund DG, Keay S, et al. A controlled trial of ganciclovir to prevent cytomegalovirus disease after heart transplantation. *N Engl J Med* 1992;**326**:1182–6.
94. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.

95. Michaels MG, Wald ER, Fricker FJ, et al. Toxoplasmosis in pediatric recipients of heart transplants. *Clin Infect Dis* 1992;**14**: 847–51.
96. Miller LW, Naftel DC, Bourge RC, et al. Infection after heart transplantation: a multi-institutional study. *J Heart Lung Transplant* 1994;**13**:981–93.
97. Miller R, Ruder J, Karwande SV, Burton NA. Treatment of mediastinitis after heart transplantation. *J Heart Transplant* 1986; **5**:477–9.
98. Montero CG, Martinez AJ. Neuropathology of heart transplantation: 23 cases. *Neurology* 1986;**36**:1149–54.
99. Montoya JG, Giraldo LF, Efron B, et al. Infectious complications among 620 consecutive heart transplant patients at Stanford University Medical Center. *Clin Infect Dis* 2001;**33**:629–40.
100. Ng B, Dipchand A, Naftel D, et al. Outcomes of *Pneumocystis jiroveci* pneumonia infections in pediatric heart transplant recipients. *Pediatr Transplant* 2011;**15**:844–8.
101. Nicholson MR, Huesman LA. Controlling the usage of intranasal mupirocin does impact the rate of *Staphylococcus aureus* deep sternal wound infections in cardiac surgery patients. *Am J Infect Control* 2006;**34**:44–8.
102. Nour B, Green M, Michaels M, et al. Parvovirus B19 infection in pediatric transplant patients. *Transplantation* 1993;**56**:835–8.
103. Olsen SL, Renlund DG, O'Connell JB, et al. Prevention of *Pneumocystis carinii* pneumonia in cardiac transplant recipients by trimethoprim sulfamethoxazole. *Transplantation* 1993;**56**:359–62.
104. Osowski CL, Dix SP, Lin LS, et al. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. *Transplantation* 1996;**61**:1268–72.
105. Pappas PG, Kauffman CA, Andes DG, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**:503–35.
106. Paya CV. Fungal infections in solid-organ transplantation. *Clin Infect Dis* 1993;**16**:677–88.
107. Petersen EA, Nash ML, Mammana RB, Copeland JG. Minocycline treatment of pulmonary nocardiosis. *JAMA* 1983;**250**: 930–2.
108. Pohl C, Green M, Wald ER, Ledesma-Medina J. Respiratory syncytial virus infections in pediatric liver transplant recipients. *J Infect Dis* 1992;**165**:166–9.
109. Pollard RB, Arvin AM, Gamberg P, et al. Specific cell-mediated immunity and infections with herpes viruses in cardiac transplant recipients. *Am J Med* 1982;**73**:679–87.
110. Recommendation for preventing the spread of vancomycin resistance. Hospital Infection Control Practices Advisory Committee (PICPAC). *Infect Control Hosp Epidemiol* 1995;**16**:105–13.
111. Redd SC, Schuster DM, Quan J, et al. Legionellosis in cardiac transplant recipients: results of a nationwide survey. *J Infect Dis* 1988;**158**:651–3.
112. Reed EC, Bowden RA, Dandliker PS, et al. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann Intern Med* 1988;**109**:783–8.
113. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in pediatric intensive care units in the United States. National Nosocomial Infections Surveillance System. *Pediatrics* 1999;**103**:e39.
114. Rodriguez WJ, Gruber WC, Welliver RC, et al. Respiratory syncytial virus (RSV) immune globulin intravenous therapy for RSV lower respiratory tract infection in infants and young children at high risk for severe RSV infections: Respiratory Syncytial Virus Immune Globulin Study Group. *Pediatrics* 1997;**99**:454–61.
115. Rowe DT, Webber S, Schauer EM, et al. Epstein-Barr virus load monitoring: its role in the prevention and management of post-transplant lymphoproliferative disease. *Transpl Infect Dis* 2001;**3**: 79–87.
116. Rubin RH. The indirect effects of cytomegalovirus infection on the outcome of organ transplantation. *JAMA* 1989;**261**:3607–9.
117. Rubin RH. Prevention and treatment of cytomegalovirus disease in heart transplant patients. *J Heart Lung Transplant* 2000;**19**:731–5.
118. Ryning FW, McLeod R, Maddox JC, et al. Probable transmission of *Toxoplasma gondii* by organ transplantation. *Ann Intern Med* 1979;**90**:47–9.
119. Sadaba B, Lopez de Ocariz A, Azanza JR, et al. Concurrent clarithromycin and cyclosporin A treatment. *J Antimicrob Chemother* 1998;**42**:393–5.
120. Sands M, Brown RB. Interactions of cyclosporine with antimicrobial agents. *Rev Infect Dis* 1989;**11**:691–7.
121. Schowengerdt KO, Naftel DC, Seib PM, et al. Infection after pediatric heart transplantation: results of a multiinstitutional study. The Pediatric Heart Transplant Study Group. *J Heart Lung Transplant* 1997;**16**:1207–16.
122. Schowengerdt KO, Ni J, Denfield SW, et al. Association of parvovirus B19 genome in children with myocarditis and cardiac allograft rejection: diagnosis using the polymerase chain reaction. *Circulation* 1997;**96**:3549–54.
123. Schubert S, Abdul-Khaliq H, Lehmkuhl HB, et al. Diagnosis and treatment of post-transplantation lymphoproliferative disorder in pediatric heart transplant patients. *Pediatr Transplant* 2009;**13**: 54–62.
124. Schutze GE, Mason EO Jr, Wald ER, et al. Pneumococcal infections in children after transplantation. *Clin Infect Dis* 2001; **33**:16–21.
125. Serinet MO, Jacquemin E, Habes D, et al. Anti-CD20 monoclonal antibody (Rituximab) treatment for Epstein-Barr virus-associated, B-cell lymphoproliferative disease in pediatric liver transplant recipients. *J Pediatr Gastroenterol Nutr* 2002;**34**: 389–93.
126. Sherman-Weber S, Axelrod P, Suh B, et al. Infective endocarditis following orthotopic heart transplantation: 10 cases and a review of the literature. *Transpl Infect Dis* 2004;**6**:165–70.
127. Shirali GS, Ni J, Chinnock RE, et al. Association of viral genome with graft loss in children after cardiac transplantation. *N Engl J Med* 2001;**344**:1498–503.
128. Shulman LL. Cytomegalovirus pneumonitis and lobar consolidation. *Chest* 1978;**91**:558–601.
129. Shuster LD, Cox G, Bhatia P, Miner PB Jr. Gastric mucosal nodules due to cytomegalovirus infection. *Dig Dis Sci* 1989;**34**: 103–7.
130. Simon D, Fischer S, Grossman A, et al. Left ventricular assist device-related infection: treatment and outcome. *Clin Infect Dis* 2005;**40**:1108–15.
131. Simpson GL, Stinson EB, Egger MJ, Remington JS. Nocardial infections in the immunocompromised host: a detailed study in a defined population. *Rev Infect Dis* 1981;**3**:492–507.
132. Singh N, Husain S. Infections of the central nervous system in transplant recipients. *Transpl Infect Dis* 2000;**2**:101–11.
133. Sluiters JF, Balk AH, Essed CE, et al. Indirect enzyme-linked immunosorbent assay for immunoglobulin G and four immunoassays for immunoglobulin M to *Toxoplasma gondii* in a series of heart transplant recipients. *J Clin Microbiol* 1989;**27**:529–35.
134. Small LN, Lau J, Snydman DR. Preventing post-organ transplantation cytomegalovirus disease with ganciclovir: a meta-analysis comparing prophylactic and preemptive therapies. *Clin Infect Dis* 2006;**43**:869–80.
135. Smith DW, Frankel LR, Mathers LH, et al. A controlled trial of aerosolized ribavirin in infants receiving mechanical ventilation for severe respiratory syncytial virus infection. *N Engl J Med* 1991;**325**:24–9.
136. Snydman DR, Kistler KD, Ulsh P, Morris J. Cytomegalovirus prevention and long-term recipient and graft survival in pediatric heart transplant recipients. *Transplantation* 2010;**90**:1432–8.
137. Spes CH, Angermann CE, Stempfle HU, et al. Sulfadiazine therapy for toxoplasmosis in heart transplant recipients decreases cyclosporine concentration. *Clin Invest* 1992;**70**:752–4.
138. Spiers GE, Hakim M, Calne RY, Wreghitt TG. Relative risk of donor-transmitted *Toxoplasma gondii* infection in heart, liver and kidney transplant recipients. *Clin Transplant* 1988;**2**: 257–60.
139. Stamatakis MK, Richards JG. Interaction between quinupristin/dalfopristin and cyclosporine. *Ann Pharmacother* 1997;**31**:576–8.
140. Stokes DC, Shenep JL, Parham D, et al. Role of flexible bronchoscopy in the diagnosis of pulmonary infiltrates in pediatric patients with cancer. *J Pediatr* 1989;**115**:561–7.
141. Stout JE, Yu VL. Legionellosis. *N Engl J Med* 1997;**337**:682–7.
142. Stovall SH, Ainley KA, Mason EO Jr, et al. Invasive pneumococcal infections in pediatric cardiac transplant patients. *Pediatr Infect Dis J* 2001;**20**:946–50.

143. Tortoriello TA, Friedman JD, McKenzie ED, et al. Mediastinitis after pediatric cardiac surgery: a 15-year experience at a single institution. *Ann Thorac Surg* 2003;**76**:1655–60.
144. Tran L, Hebert D, Dipchand A, et al. Invasive pneumococcal disease in pediatric organ transplant recipients: a high-risk population. *Pediatr Transplant* 2005;**9**:183–6.
145. Urschel S, Cremer S, Birnbaum J, et al. Lack of serologic immunity against vaccine-preventable diseases in children after thoracic transplantation. *Transpl Int* 2009;**23**:619–27.
146. Valantine HA. Prevention and treatment of cytomegalovirus disease in thoracic organ transplant patients: evidence for a beneficial effect of hyperimmune globulin. *Transplant Proc* 1995;**27**: 49–57.
147. Valantine HA, Luikart H, Doyle RJ, et al. Impact of cytomegalovirus hyperimmune globulin on outcome after cardiothoracic transplantation: a comparative study of combined prophylaxis with CMV hyperimmune globulin plus ganciclovir versus ganciclovir alone. *Transplantation* 2001;**72**:1647–52.
148. Van der Bij W, Van Dijk RB, Van Son WJ, et al. Antigen test for early diagnosis of active cytomegalovirus infection in heart transplant recipients. *J Heart Transplant* 1988;**7**:106–9.
149. Vikram HR, Blair JE. Coccidioidomycosis in transplant recipients: a primer for clinicians in nonendemic areas. *Curr Opin Organ Transplant* 2009;**14**:606–12.
150. Wagener MM, Yu VL. Bacteremia in transplant recipients: a prospective study of demographics, etiologic agents, risk factors, and outcomes. *Am J Infect Control* 1992;**20**:239–47.
151. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
152. Warmington L, Lee BE, Robinson JL. Loss of antibodies to measles and varicella following solid organ transplantation in children. *Pediatr Transplant* 2005;**9**:311–14.
153. Webber SA, McCurry K, Zeevi A. Heart and lung transplantation in children. *Lancet* 2006;**368**:53–69.
154. Webber SA, Naftel DC, Fricker FJ, et al. Lymphoproliferative disorders after paediatric heart transplantation: a multiinstitutional study. *Lancet* 2006;**367**:233–9.
155. White-Williams C, Brown R, Kirklin J, et al. Improving clinical practice: should we give influenza vaccinations to heart transplant patients? *J Heart Lung Transplant* 2006;**25**:320–3.
156. Wreghitt T. Cytomegalovirus infections in heart and heart-lung transplant recipients. *J Antimicrob Chemother* 1989;**23**(Suppl E): 49–60.
157. Wreghitt TG, Gray JJ, Pavel P, et al. Efficacy of pyrimethamine for the prevention of donor-acquired *Toxoplasma gondii* infection in heart and heart-lung transplant patients. *Transpl Int* 1992;**5**:197–200.
158. Zangwill SD, Hsu DT, Kichuk MR, et al. Incidence and outcome of primary Epstein-Barr virus infection and lymphoproliferative disease in pediatric heart transplant recipients. *J Heart Lung Transplant* 1998;**17**:1161–6.
159. Zaoutis TE, Webber S, Naftel DC, et al. Invasive fungal infections in pediatric heart transplant recipients: incidence, risk factors, and outcomes. *Pediatr Transplant* 2011;**15**:465–9.

CHAPTER 71

Infections in Pediatric Lung Transplantation

Jill A. Hoffman

The first pediatric lung transplant was reported to the International Society for Heart and Lung Transplantation (ISHLT) in 1986; and by the end of 2009, a total of 1664 transplants had been reported to the registry, with 127 performed in 2009.[57] Although this represents an increase over time worldwide, there has been a decrease in these transplant procedures in the United States from 42 in 1998 to 20 in 2009.[511] The reasons for this decline are likely multifactorial, but a primary one is the increased survival of persons with cystic fibrosis (CF), which is the leading indication for pediatric lung transplantation; life expectancies for these persons are now into the fifth and sixth decades for infants born in 2000.[136,186] This reflects improvements in therapy and the effect of widespread neonatal screening.[264,639] In addition, some controversy exists on the survival benefit of transplantation for patients with CF.[7,218] Transplantation is not a cure for the lung disease of persons with CF, and the factors that predict optimal timing of transplant to maximize survival and quality of life are not entirely defined. Living lobar transplantation (LLT) has been performed at some centers as a way to address the shortage of available organs,[645,665,714,738]; the number of these procedures peaked in 1999 and all of the pediatric lung transplantation procedures performed in the United States in 2007 to 2009 used lobes from deceased donors. At Children's Hospital Los Angeles (CHLA), the first pediatric LLT was performed in 1993 on a 13-year-old boy with CF. He received lobes from each of his parents and survived for 5 years, unfortunately succumbing to renal failure while waiting for a kidney transplant.[738] Fifty-five LLT procedures (49% of 113 total-lung and 12 heart-lung transplants) have been performed on pediatric patients at CHLA to date.[300]

The primary indication for lung transplantation in the pediatric population remains CF—20 percent in the United States (2007-2009), followed by 13 percent each for primary pulmonary hypertension and obliterative bronchiolitis.[511] From 2000 to 2010 in the United States, 55 percent of transplants were in patients with CF, compared with 75 percent in Europe. Worldwide, 56 percent of 6- to 11-year-olds and 72 percent of 12- to 17-year-olds underwent transplants for this indication. Most pediatric transplant recipients are adolescents (>70%).[57,433] For younger children, the most common diagnoses were surfactant B deficiency and complex congenital heart disease in infants younger than age 1 year undergoing lung transplantation and primary pulmonary hypertension in children aged 1 to 5 years.[57,85]

Infectious morbidity and mortality remain high for lung transplant recipients.[17] This incidence results from constant communication of the lungs and fresh bronchial anastomosis with the environment and from the high preoperative microbial burden of patients with CF. Denervation of the transplanted lung, interruption of the bronchial and lymphatic circulation, abnormal cough reflexes, and impaired mucociliary clearance also probably play roles.[17] In addition, these patients receive relatively high-dose, long-term immunosuppression; at 5 years, most patients still are maintained on mycophenolate mofetil (55%), tacrolimus (70%), and corticosteroids (98%).[57] In the first year after lung transplantation at CHLA, the overall incidence of infection was 0.24 episode per patient per month, or 2.88 infections per patient per year.[394] Fifty-five percent of all infections occurred in the first month after transplantation, with infections becoming much less frequent thereafter (Table 71-1).[394] A summary of the causes of infection in this cohort during the first year after transplantation is presented in Tables 71-2 and 71-3.[394]

The additional indirect impact of infections on the outcome of lung transplant recipients is also recognized. Cytomegalovirus (CMV) and other viruses, as well as bacterial infections, have been implicated in immunologically mediated processes leading to bronchiolitis obliterans syndrome or chronic graft dysfunction, acute rejection, graft loss, and death (see later discussion).[17,57]

The overall survival of pediatric lung transplant recipients (2002-2009, n = 653) as reported to the ISHLT is 84, 65, 53, and 45 percent at 1, 3, 5, and 7 years, respectively. This survival is similar to that of adult recipients, with a transplant half-life (50% mortality in recipients) of 5.5 years.[57] There is no difference in survival according to donor lung source (i.e., living vs. deceased).[52,665,714] Infection is the single most common cause of death from 1 month to 1 year after transplantation (46%), and it remains a significant cause of death in all periods through 5 years after transplantation.[57] Bronchiolitis obliterans syndrome causes approximately 40 percent of deaths after the first year. Graft failure accounts for a third of deaths in the first month and 13 to 25 percent thereafter.[4] In the series of 75 pediatric transplants at CHLA (12 heart-lung, 18 cadaveric, 45 LLTs), the mortality rate was 18.6 percent at 1 year, 50 percent of which was attributed to infection.[394]

TABLE 71-1 **Etiology of Infections in 75 Pediatric Lung Transplant Recipients in the First Postoperative Month at Children's Hospital Los Angeles**

Etiology	Number of Episodes
Pseudomonas, respiratory tract	22
Candida, respiratory tract	13
Herpes stomatitis	12
Cytomegalovirus infection	10
Aspergillus infection of the respiratory tract, bronchial anastomosis	4

TABLE 71-2 **Etiology of Infections in 75 Pediatric Lung Transplant Recipients in the First Year at Children's Hospital Los Angeles**

Etiology	% of Total Infections
Bacterial infections	55
Pseudomonas spp.	18 (35% of bacterial infections)
Viral	27
Cytomegalovirus	12 (42% of viral infections)
Fungal	16
Candida	10
Aspergillus	4

TABLE 71-3 **Timetable of Infections for Pediatric Lung Transplant Recipients**

Time of Initial Evaluation	Infection/Pathogen	Comments
0-1 Month	Wound, respiratory tract, line/bloodstream, and urinary tract infections	Related to the surgical procedure: *Candida, Staphylococcus* spp., *Pseudomonas* spp.
	HSV stomatitis	Reactivation
	CMV infection	
	HHV-6?	
	Bronchial anastomosis *(Aspergillus)*	
	Other: rabies, West Nile virus, lymphocytic choriomeningitis virus, endemic mycoses	Unusual pathogens preexisting in the recipient or in the donor graft
1-6 Months	CMV disease	Onset of opportunistic infections from immunosuppression
	EBV	
	Aspergillus	
	Mycobacterium tuberculosis	
	Pneumocystis jiroveci	
	Endemic mycoses	
	HHV-6	
	Respiratory tract, line/bloodstream, and urinary tract infections	Continued bacterial *(Pseudomonas)* and candidal infections common through 3-4 months
	Respiratory viruses	Community-acquired infections
>6 Months	Respiratory viruses	Community-acquired infections
	Persistent *Pseudomonas, Burkholderia cepacia,* and *Aspergillus* infections	Persistence of organisms from cystic fibrosis in the proximal airways and sinuses
	Opportunistic infections:	Patients receiving continuous high-level immunosuppression or therapy for steroid-resistant rejection, CMV infection
	Pneumocystis jiroveci	
	Cryptococcosis	
	Nontuberculous mycobacteria	
	EBV/PTLD	
	Herpes zoster	

CMV, cytomegalovirus; *EBV,* Epstein-Barr virus; *HHV,* human herpesvirus; *HSV,* herpes simplex virus; *PTLD,* posttransplant lymphoproliferative disease.

Modified from Rubin RH, Ikonen T, Gummert JF, Morris RE. The therapeutic prescription for the organ transplant recipient: the linkage of immunosuppression and antimicrobial strategies. *Transpl Infect Dis* 1999;**1**:39.

IMMUNOSUPPRESSION AND TIMING OF INFECTION

An understanding of the mechanisms and effects of immunosuppressive therapy is fundamental to anticipating the timing and types of infections that occur after transplantation, and several excellent reviews on these subjects are available.[135,576,580] The predictability of certain infections has facilitated establishing more timely diagnosis and determining strategies for preventive antimicrobial therapy.[212] Historically, the mainstay of immunosuppression has included corticosteroids and combinations of the following medications: azathioprine, the calcineurin inhibitors cyclosporine and tacrolimus, sirolimus, mycophenolate mofetil (MMF), and polyclonal and monoclonal antibodies. In recent years, substitution

TABLE 71-4 **Timing of Infections in 75 Pediatric Lung Transplant Recipients at Children's Hospital Los Angeles**

Months After Transplantation	% of Episodes
0-1	55
1-3	18
3-6	13
6-9	8
9-12	7

of MMF and tacrolimus for azathioprine and cyclosporine, respectively, has become standard and "corticosteroid-sparing therapy" also is gaining favor. Newer regimens may provide a superior cost-benefit ratio in terms of prevention of rejection, toxicity, and risk for infection.[135] A general time line of the occurrence of specific infectious agents in pediatric lung transplant recipients is presented in Table 71-4.

OVERVIEW OF INFECTIONS AND ANTIBIOTIC USE IN SOLID-ORGAN TRANSPLANTATION

The "therapeutic prescription," as described by Rubin and colleagues,[580] for successful organ transplantation requires a careful balance between immunosuppression and the use of antimicrobial agents therapeutically, prophylactically, or preemptively to manage the risk for rejection versus infection. A transplant recipient's risk for developing infection is determined by multiple factors, including (1) the technical skill of the surgeon; (2) the quality of perioperative care; (3) the "net state of immunosuppression"; (4) the patient's infectious exposure, including that from the community (past and present), hospital, and donor grafts; (5) the underlying condition of the patient (e.g., microbial colonization, organ dysfunction); and (6) the virulence of the organisms. Preventive/prophylactic strategies can alter these patterns, most notably for CMV and fungal infections, and delay their appearance past the expected time periods.[577] In sum, these considerations indicate that although a standard set of antimicrobial agents can be prescribed, they must be tailored closely to the risks, exposure, and immunosuppression of each individual.[581]

General principles of infection and therapy have been proposed by Rubin and Marty[581] and include the recognition that a transplant recipient is likely to have a greater microbial load and more advanced infection at the time of diagnosis, requiring longer therapy with greater potential for drug toxicity. Early establishment of the diagnosis and provision of therapy are paramount to a successful outcome. Furthermore, a broader range of organisms must be considered. Although a common practice in medicine is to assume a single diagnosis for a given manifestation, a recognized caveat is that transplant recipients may have "simultaneous and sequential" infections. In fact, many infectious processes are influenced directly by proceeding or concurrent events. The prototype of this interaction is the immunomodulating effect of CMV infection such that alterations in the cytokine/chemokine milieu, T-lymphocyte subsets and function, neutrophil activity, and activation of endothelial cells emerge. The result of these derangements is further immunosuppression, enhanced production of virus, and subsequent increased susceptibility to additional infections. Other putative immunomodulating viruses include human herpesvirus–6 (HHV-6), Epstein-Barr virus (EBV), and the hepatitis viruses.[581,613]

Length of therapy depends on consideration of the degree of current immunosuppression and location of the infection. In general, experts suggest that treatment be continued until all signs and symptoms of infection are gone, followed by a "buffer period" of weeks to months, depending on the infection,[581] but controlled trials on specific length of therapy for most infections are not available. Finally, drug interactions and toxicities, which may be synergistic, must be taken into consideration when prescribing medications for these patients.[440,580]

SITES OF INFECTION

Thoracic Cavity: Respiratory Tract Infections, Including Pneumonia and Anastomotic Site Infections

The vast majority, up to 80 percent, of infections in lung transplant recipients occur in the thorax-lung, mediastinum, and pleural space,[222] largely presenting as pneumonia.[9,17] In the CHLA cohort of patients during the first 12 months after transplantation, 62 percent of all infections consisted of pneumonia.[394] Organisms most frequently recovered include *Pseudomonas aeruginosa*, CMV, *Candida*, and *Aspergillus*, reflecting the high percentage of patients with CF in the transplant population. The onset of pneumonia in lung transplant recipients appears in a bimodal pattern, with most cases occurring in the first postoperative month. A second, albeit smaller group has late (6 to 12 months) recurrent episodes of gram-negative pneumonia associated with a poor outcome. The immunologic consequences of these infections in some patients may include bronchiolitis obliterans with organizing pneumonia (BOOP), which is characterized by inflammation and fibrotic granulation tissue of the small airways extending into the alveoli. The mortality rate of patients with BOOP is high (50%), and those who survive appear to be at an increased risk for developing bronchiolitis obliterans syndrome (41%).[17,118,222,246]

Unique to lung transplantation are bronchial anastomotic infections, most of which are fungal and caused by *Aspergillus* and *Candida*.[268,291,362,497,521] The bronchial anastomosis is susceptible to such infections for a variety of reasons. Anatomically, the posttransplant bronchus is relatively devascularized, with the blood supply flowing retrograde from the pulmonary arterial circulation. Neovascularization from collateral vessels takes as long as 1

month to occur, and, in the interim, the bronchus may experience ischemia and subsequent necrosis and sloughing of bronchial epithelium. Evolving surgical techniques have diminished this risk for ischemia in recent years. This environment is optimal for developing infection caused by saprophytic flora, such as *Aspergillus*, present in ambient air to which the airways are in direct contact. In addition, local reaction to suture material, postoperative corticosteroid use, and immunosuppressive therapy have a negative impact on healing and increase the patient's susceptibility to infection. As a result of this early tenuous anatomy, infections at this site are most likely to occur within the first 2 to 3 months after surgery.[127,268,291,499] Bronchial anastomotic infections rarely can lead to early catastrophic complications, such as dehiscence and hemorrhage. They are linked to substantial late bronchial complications, including dehiscence, bronchial stenosis, malacia, and retransplantation.[482,497]

Although some studies have demonstrated that pretransplant isolation of *Aspergillus* spp. has not been found to be a risk factor for the development of *Aspergillus* infection after transplantation,[335,499,529] others suggest that patients with CF and perioperative *Aspergillus* colonization may be at heightened risk for the development of early bronchial anastomotic infection. Although the numbers are small, the authors speculate that the presence of *Aspergillus* at the site of the fresh, devascularized anastomosis renders it more vulnerable.[499] Postoperative isolation of *Aspergillus* from airways appears to identify patients at risk for the later development of bronchial anastomotic abnormalities.[127,293,484,499] The overall incidence of airway infections and/or complications ranges from 5 to 25 percent.[127,268,293,482,497] The mortality rate associated with bronchial anastomotic infection and its complications is reported to be 2 to 7 percent.[291,497] Finally, one small study evaluating the effect of early sirolimus-based immunosuppression on the rate of rejection described poor wound healing and anastomotic complications in an unexpectedly high number of patients treated with sirolimus, which resulted in changes in immunosuppression strategies at that institution.[261]

A 2010 ISHLT consensus statement has formulated standardization of thoracic site infections in cardiothoracic transplant recipients, including pneumonia, tracheobronchitis, bronchial anastomotic infections, and viral/CMV and fungal respiratory tract infections.[314] The use of standard definitions going forward will better define the epidemiology of these infections and advance the quality of research aimed at therapeutic and prophylactic interventions.

Bloodstream Infections

The second most common site of infection is the bloodstream. Data are emerging regarding the epidemiology of bloodstream infections in pediatric and adult lung transplant recipients.[158,311,517] Bloodstream infections occur in 12 to 26 percent of transplant recipients, with staphylococci, *Pseudomonas aeruginosa*, and *Candida* spp. being the most common organisms identified. Late infections consist of a wide range of gram-negative organisms. Posttransplant bloodstream infections, especially early (<30 days) infection in children and candidal infection in both populations, were associated significantly with mortality. Infections may be related to pulmonary or vascular sites. In the pediatric population, most bloodstream infections were related to central venous catheters and were not secondary to other sites of infection (5% with pneumonia, sternal wound, ruptured subglottic cysts). These infections were most likely to occur in the first 7 days after transplantation. In adult transplant recipients, bloodstream infections largely were associated with pulmonary infections. Specifically, *P. aeruginosa* bacteremia was more likely to develop in adult patients with CF (20% of the transplant cohort) than in patients with other indications for transplantation. In this study, bloodstream infections with *Burkholderia cepacia* and *Staphylococcus aureus* were seen in similar frequency in non-CF patients, the former possibly related to a nosocomial outbreak.[517] In addition, bloodstream infections in patients with CF were associated with a decreased risk for death when compared with other lung transplant recipients with similar infections, possibly because antimicrobial agents active against resistant gram-negative organisms were being administered at the time that the infection developed. In children, *Pseudomonas* bloodstream infection was not associated with the diagnosis of CF (45% of the transplant cohort), nor with any outcome measures in this study. There exists an increasing concern for the impact of multidrug-resistant organisms, which were recovered in 57 percent of gram-negative–resistant bacteremias in one study (see later discussion).[311]

Empirical therapy in patients with suspected bloodstream infections should include agents active against staphylococcal and *Pseudomonas* spp. and be based on the patient's previous microbiologic findings, with a low threshold for adding antifungal therapy. Furthermore, the need for indwelling central catheters should be assessed routinely and catheters should be removed as soon as clinically feasible.

SELECTED PATHOGENS

Bacteria

Pseudomonas aeruginosa *and* Burkholderia cepacia *Complex*

P. aeruginosa and *B. cepacia* play prominent roles in postoperative infections in patients with CF. Despite the removal of colonized lung tissue with the procedure, the proximal airways and sinuses remain colonized and a source of infection postoperatively in immunosuppressed transplant recipients.[425] In patients with multidrug-resistant organisms this concern is heightened. Gram-negative multidrug-resistant organisms have been defined as those resistant to all agents in two or more classes of effective drugs[587] (e.g., resistance to all aminoglycosides, β-lactams, fluoroquinolones). *B. cepacia* complex organisms are resistant to aminoglycosides intrinsically, having high rates of resistance to β-lactams, inducible resistance to fluoroquinolones, and resistance to colistin and polymyxin.[93] In addition, these bacteria exist in a biofilm in

the airways of patients with CF, which greatly decreases therapeutic efficacy.[144,629]

As lung transplantation was emerging as a treatment option, patients infected with multidrug-resistant organisms generally were excluded.[499] Such decisions were based on data suggesting that pretransplant infection with multidrug-resistant *P. aeruginosa* and *B. cepacia* complex diminished the chance of survival and, therefore, was considered a contraindication to transplantation.[356,426,634] More recent data have documented that the presence of preoperative multidrug-resistant *P. aeruginosa* may not have an impact on survival; these patients may be more likely to acquire postoperative infections, but they generally respond to treatment without increases in short-term mortality rates, as recorded in the United Network for Organ Sharing (UNOS) database, so as not to prohibit transplantation.* The most recent ISHLT guidelines for selection of lung transplant recipients list "colonization with highly resistant or highly virulent bacteria, fungi, or mycobacteria" as a relative contraindication.[510] Additionally, these guidelines acknowledge that although "certain resistant pathogens may increase risk for poor outcome … it is not possible currently to identify absolute contraindications based on either the type of organisms or the pattern of antibiotic resistance." Despite the status of the immunocompromised host, the relatively low virulence of some of these organisms, the new healthy lung epithelium and improved airway function of the transplanted lungs, the aggressive use of antimicrobial agents during and after the procedure, and discordance between in vitro susceptibilities and in vivo efficacy may account for the diminished influence that multidrug-resistant pseudomonads have on the outcomes of transplant recipients.[510]

B. cepacia complex represents a group of nine related species that possess variable pathogenic potential in abnormal hosts.[397,398] Misidentification of *B. cepacia* complex organisms at the genus (*Alcaligenes*, *Stenotrophomonas*, and *Pseudomonas* most commonly) and species levels can occur.[397,447] This information is critical to evaluating patients considering transplantation and, in postoperative care, to assessing their risk for developing invasive disease.[397] There are species-specific differences in antimicrobial resistance and virulence. Several putative virulence/transmissibility factors that have been identified for *B. cenocepacia* (formerly genomovar III, ET-12 strain)[397] include quorum sensing,[636] cable pili (*cbl*A) for enhanced binding to respiratory epithelium,[589] increased neutrophil recruitment with suboptimal activation of polymorphonuclear neutrophils and macrophages,[588] and production of exopolysaccharides that inhibit neutrophil chemotaxis and scavenge reactive oxygen species.[97]

In the United States there has been a decline in *B. cepacia* complex in the CF population, from 3.6 percent in 1995 to 2.5 percent in 2010.[150,560] In Canada, this decline has also been documented from 15 percent, and as many as 80 percent involving *B. cenocepacia/genomovar III*,[174] to 4.6 percent in 2010.[105] This reassuring decrease in *B. cepacia* organisms is likely multifactorial, reflecting better microbiologic techniques for identification and infection control practices.[560] Controversy continues regarding performing transplantation on patients who harbor *B. cenocepacia*. Data demonstrate increased morbidity and mortality rates associated with this organism in nontransplanted patients with CF, including the occurrence of fatal cepacia syndrome.[323,331,380,381,424] Multiple studies of lung transplant recipients demonstrate an increase in mortality associated with *B. cenocepacia*, including late deaths as a result of infection associated with bronchiolitis obliterans syndrome and possibly *Burkholderia gladiola* but no other *Burkholderia* spp. It remains a contraindication for transplantation in some centers,* although successful transplantation has been accomplished with aggressive multidrug antimicrobial therapy, perhaps guided by multiple combination bactericidal testing, and a reduction in immunosuppressive therapy.[119,174,394,483]

Optimal therapy for infections due to multidrug-resistant *Pseudomonas* and *Burkholderia* spp. is not known. Therapy should be tailored to susceptibility studies, although in vitro susceptibilities may not correlate with in vivo activity and outcome.[631] In vitro synergy or multiple combination bactericidal testing for multidrug-resistant or pan-resistant gram-negative organisms can identify combinations of antimicrobial agents that inhibit bacterial growth and may facilitate the use of effective multidrug regimens.[1,2,93,373,587] A biofilm grown model of in vitro multiple combination bactericidal testing may better inform our therapeutic choices for these multidrug-resistant organisms and has identified a combination of high-dose tobramycin (inhaled), plus meropenem and a third antimicrobial agent (piperacillin/tazobactam, sulfamethoxazole/trimethoprim, amikacin, or ceftazidime) that inhibited the growth of most isolates.[151] How these data translate to patient care, however, has yet to be fully demonstrated. Published data did not reveal better outcomes, specifically the time to the next exacerbation, when using combination therapy to treat CF patients.[2] In this report, study patients received two-drug combinations based on multiple combination bactericidal testing, but these patients did not necessarily have multidrug-resistant/pan-resistant organisms, nor had they failed conventional therapy. Extrapolating these results to lung transplant recipients with multidrug-resistant organisms is difficult. The CHLA transplant team has been using multiple combination bactericidal testing from the University of Ottawa since 2001; our experience suggests that this approach, with three or four drugs used in combination, is helpful for managing preoperative and postoperative patients with multidrug-resistant/pan-resistant organisms, including *B. cepacia* complex.[394] Clearly, additional data are needed to assess this approach for lung transplant recipients infected with multidrug-resistant organisms.

For lung transplant recipients with susceptible *P. aeruginosa* infection, combination therapy is recommended, including an extended-spectrum, antipseudomonal β-lactam and an aminoglycoside, if renal status allows. For *B. cepacia* complex, combinations that include ceftazidime, meropenem, TMP-SMX, tetracyclines, quinolones, and

*References 34, 182, 214, 269, 335, 498.

*References 14, 35, 75, 119, 173, 175, 397, 479, 483.

even chloramphenicol have been used.[317] Aerosolized antibiotics (tobramycin, colistin, or aztreonam) can deliver drug levels above the minimal inhibitory concentrations of resistant organisms directly to the lungs and thereby reduce the bacterial burden with minimal systemic toxicity.[205,235,239,456] As a rule, aerosolized antibiotics should not be used as single therapy for infection in lung transplant recipients, who are at risk for dissemination. A prophylactic protocol utilizing aerosolized aztreonam has been employed at CHLA.[739] Therapy is initiated in patients identified with *B. cepacia* complex, preoperatively if possible, and continued through the first year after transplantation. Preliminary data suggest that outcomes are similar to those of patients who are colonized with susceptible *Pseudomonas* organisms. Further investigation is needed in this promising area of therapy. Colistin aerosols may also be useful for the treatment of pan-resistant organisms.[39,53] Although historically, renal toxicity often precluded its systemic use, recent studies suggest that intravenous colistin can be used successfully as well.[152,202,213] Finally, strategies that employ extended or continuous infusions of β-lactam antibiotics may optimize the pharmacokinetic/pharmacodynamic properties of these drugs and may improve outcome.[466,472,566] The duration of therapy requires individualization but generally is not less than 2 weeks, and treatment should be continued for a significant period after the signs and symptoms of infection have resolved.

β-*Lactamase–Producing Multidrug-Drug Resistant Organisms (Enterobacteriaceae,* Acinetobacter*)*

Multidrug-resistant, β-lactamase–producing Enterobacteriaceae *(Klebsiella pneumoniae*, *Escherichia coli)* and *Acinetobacter baumannii* are being increasingly recognized as causes of infection worldwide; as many as 1000 β-lactamases have been identified, including cephalosporinases extended-spectrum β-lactamases (ESBL), K. pneumonia carbapenemases (KPC) , AmpC β-lactamases, and metallo β-lactamases (MBL), rendering them resistant to a wide range of β-lactamase antimicrobial agents.[427,434,683] The extent of their impact on transplant recipients in general and in pediatric lung transplant recipients is unknown and limited to case reports and small surveys, mostly in adult transplant populations,[36,58,532] but has been described in pediatric liver or intestinal transplantation and, notably, in outbreak situations.[250,561] Infections may originate from the recipient or donor.[242,437] Increased risk related to antimicrobial exposure, infection control practices, invasive procedures, and prolonged hospitalization, especially associated with intensive care units, is likely.[476] Carbapenems (meropenem, imipenem) are generally considered the drugs of choice for extended-spectrum β-lactamase–producing organisms, especially for empirical therapy,[427,736] although β-lactam/β-lactam inhibitor combinations may be appropriate based on in vitro susceptibilities, especially at high doses.[537,568] Carbapenemase-producing organisms provide additional challenges to therapy, because the number of available effective agents is limited and few new options are in development; therapy should be guided by susceptibilities and perhaps multiple combination bactericidal testing. Drug combinations including colistin, tigecycline, doripenem, and ceftobiprole may be considered.[329,434,532,683] The transplant community continues to discuss the implications these organisms may have on transplant recipient and donor candidacy.[608]

Mycobacterium tuberculosis

Worldwide, *Mycobacterium tuberculosis* (MTB) remains an important pathogen in solid-organ transplant recipients, and the rate of infection is associated with the endemicity of infection. As noted by many authors, MTB behaves as an opportunistic infection in these patients in that its frequency in solid-organ transplant recipients is greatly increased in comparison with the normal population (up to 74-fold) and presents as diverse manifestations, including high rates of extrapulmonary disease and increased mortality rates.* Most cases of infection with MTB in adult transplant recipients arise from reactivation of latent infection from recipient or donor; acquired cases are seen, especially in pediatric patients.[91,445,470,690] Multiple cases of donor-derived MTB infection, including pulmonary MTB infection in lung transplant recipients[565] and extrapulmonary MTB infection in renal and liver transplant patients, have been documented.[248,565,737] In addition, nosocomial transmission to transplant recipients has occurred.[328]

Among solid-organ transplant recipients, MTB infections occur most frequently after lung transplants (1% to 6.5%).[626,675] Studies of MTB infection in solid-organ transplant recipients, including small numbers of children, suggest that most cases occur within the first post-transplant year, with a median time of 9 months for all solid-organ transplant recipients and 3.5 months for lung transplant recipients. Risk factors for MTB infection include allograft rejection; receipt of OKT3 or anti–T-cell antibodies, which also predict dissemination; previous exposure to MTB, with radiographic evidence of old infection or a history of a positive tuberculin skin test; renal failure/hemodialysis; liver disease; diabetes mellitus; and older age.[91,675] Coinfection with CMV, mycoses, *Pneumocystis jiroveci*, and *Nocardia* has been associated with the development of disease.[470,477] As would be expected, most manifestations include pulmonary disease (>70%), with interstitial infiltrates, nodules, effusion, and cavities seen on imaging studies.[626,675] Presentations with extrapulmonary disease (15%) involve the gastrointestinal tract, skin, musculoskeletal system, genitourinary system, lymph nodes, and central nervous system (CNS). Disseminated disease has been demonstrated in up to a third of patients, 91 percent of whom were febrile as compared with 64 percent of those with localized disease.[626] Attributable mortality from MTB infection in solid-organ transplant recipients is reported to be 9.5 to 29 percent. Significant predictors of mortality are disseminated disease, previous rejection, and receipt of OKT3 or anti–T-cell antibodies.[626]

Several studies have investigated mycobacterial infections specifically in lung transplant recipients.[81,340,428,470] Two of the studies were conducted in regions with a low

*References 91, 477, 578, 626, 656, 675.

endemicity of MTB infection—Australia and North America (affecting 5 to 15/100,000 population)—and two in Spain (25 to 49/100,000 population). Rates of MTB infection in lung transplant recipients in the two former studies were less than 1 percent, although still well above the national average,[340,428] but they were 2.6 percent and 6.4 percent (500-fold higher than the national average) in the Spanish studies.[81,470] Indeed, nontuberculous mycobacterial infections were more common than were MTB infections in the first two reports, whereas nontuberculous mycobacterial infection was encountered only once in the Spanish study. Mortality ranged from 0 to 43 percent.[81,340,470,626]

Few data exist on MTB infections in pediatric solid-organ transplant recipients, and none exist in lung transplant recipients. Although children have an age-related, lower accumulated risk for being infected, even immunocompetent children with MTB infection are more likely to have progression to dissemination and mortality, especially those younger than 5 years of age and during adolescence.[147] This pattern appears to be mirrored in the transplant population. Small, published series of MTB infection in solid-organ transplant recipients most often describe fever and pulmonary findings, with high rates of dissemination (50%); mortality ranged from 0 to 30 percent.[91,445,687,690] Pediatric patients presented later after transplant than adults (median of 8 months vs. 4 months) as a result of differences in acquisition of disease. Tuberculin skin test results were negative or minimal in all patients. Furthermore, screening of family members was very useful in both series of pediatric patients, thus suggesting that, as for immunocompetent children, the index case of exposure is in the home. Invasive diagnostic procedures were needed to confirm the diagnosis in many patients. An additional case report of MTB complex disease in a pediatric renal transplant recipient highlights another potential difference between pediatric and adult cases.[124] *Mycobacterium bovis* disease, which can be indistinguishable from MTB infection but often presents as abdominal disease, developed in this child. It is usually acquired from the ingestion of unpasteurized milk products from infected cows. It appears to be distinctly more common in children and, in the United States, is seen most often in children who have immigrated from or visited countries where *M. bovis* is endemic in cattle, such as Mexico.[154] The importance of recognizing this infection is its inherent resistance to pyrazinamide and the implications for therapy.

A screening risk assessment with history, including exposures, travel, receipt of bacille Calmette-Guérin (BCG) vaccine, and physical examination, as well as performing a tuberculin skin test should be documented in all pediatric transplant candidates.[91,656] A 5-mm induration should be considered positive in an immunocompromised child, because positive results require intact T-cell function; false-negative results may be secondary to nutritional status, organ failure, or medication-related immunodeficient states.[28,91] Screening of household contacts should also be considered. Interferon-gamma (IFN-γ) release assays have emerged as valuable tools for the screening/diagnosis of latent tuberculosis infection and include the "QuantiFERON-TB Gold (QFT-G) and in tube" (QFT-GIT), a whole-blood enzyme-linked immunosorbent assay (ELISA), and "T-SPOT.TB," an ex-vivo enzyme-linked immunospot (ELISpot) assay.[442,443,563,597] Both assays rely on interferon production from peripheral blood mononuclear cells in response to specific MTB antigens. The advantage of these tests is the discrimination of MTB from nontuberculous mycobacterial infections, as well as BCG vaccine response. These tests have higher specificity than the tuberculin skin test, especially with regard to the BCG vaccine, and may be most useful in low-endemic, high-resource settings.[442,514,644] In addition, positive and negative controls allow for better assessment of anergic responses. Clear differences in the sensitivities of the tuberculin skin test and the available IFN-γ release assays are difficult to demonstrate.[443,516,551] The T-SPOT.TB may be more sensitive in immunocompromised patients.[443,551,564] However, the use of IFN-γ release assays in young children (<5 years) and immunocompromised patients is still being evaluated.* The use of both the tuberculin skin test and the IFN-γ release assays may increase the sensitivity of detection of infection, because discordant results in children and immunocompromised populations have been well documented.[6,91]

Diagnosis of active MTB infection in the transplant setting can be challenging because symptoms may be nonspecific, such as fever of unknown origin, or unusual, such as abdominal manifestations; coinfections may also modify the signs and symptoms, and immune response–based diagnostic methods lack sensitivity. Consequently, a high index of suspicion is required, and, frequently, aggressive diagnostic techniques, such as bronchoscopy, laparoscopy, and tissue biopsy, are needed.[445,690] The American Thoracic Society and the Centers for Disease Control and Prevention (CDC) have published recommendations for diagnosis of MTB disease.[29,108,597] Several new methods are available for culture-based diagnosis. Broth-based culture systems coupled with DNA probes allow more rapid establishment of a diagnosis.[515,597] Two commercially available kits are available in the United States for diagnosis based on nucleic acid amplification assays.[515] They are approved for smear-positive and smear-negative samples and may provide results within 24 to 48 hours. Although there is still some debate on the optimal use of these tests, CDC guidelines state that nucleic acid amplification assays should be performed on at least one specimen for all patients in whom the diagnosis of tuberculosis is being considered but not established.[110] Although culture-based, indirect diagnostics are the most widely available methods by which to assess susceptibility, these methods have long turnaround times. Technologies for the rapid detection of multidrug-resistant organisms are evolving.[38,597,669] Newer modalities such as the microscopic-observation drug susceptibility, nitrate reductase assay, and molecular methods such as GenoType MTBDR assays may allow rapid, inexpensive diagnosis and susceptibility testing even in countries with limited resources.[96,113,322,469,515]

Because pediatric lung transplant recipients are at heightened risk for development of progressive

*References 414, 443, 514, 551, 564, 644, 670.

tuberculous infection, expert opinion supports the use of 9 months of isoniazid (INH) chemoprophylaxis for pediatric candidates or recipients with latent tuberculosis. The morbidity and mortality from MTB is high enough to warrant the risk for potential hepatotoxicity in most cases, unless severe liver disease is also present.[91,477,626,656] Rifampin alone may also be an acceptable regimen, although drug-drug interactions are common (see later discussion).[656] Completing therapy prior to transplant is optimal, but it can be accomplished after transplantation. An alternative strategy is active surveillance, with initiation of INH only if additional risk factors are present, such as recent conversion, exposure to active MTB, a recipient or donor with a history of MTB infection without adequate therapy, or the existence of significant abnormalities on chest radiographs.[578] All family and close contacts with latent or active tuberculosis should also be treated.[91]

Optimal therapy for pediatric lung transplant recipients with active tuberculosis has not been defined, but an empirical multidrug regimen such as those recommended for active disease in other populations, modified by susceptibilities as necessary, should be initiated.[25,71,656] Complicating factors include liver toxicity and drug interactions. INH, especially in combination with other first-line drugs, such as rifampin and pyrazinamide, can lead to toxicity, requiring discontinuation of all or some of these drugs and the need to institute new regimens.[477,626] Drug toxicity is most notable in adult liver transplant recipients. Liver biopsy may be necessary to distinguish drug toxicity from rejection or hepatic granuloma from MTB infection.[477,578,626] Pediatric patients generally tolerate first-line therapy, with occasional need for reduced dosage and rarely discontinuation.[25,71,124,690] For patients with suspect or proven multidrug-resistant tuberculosis, close consultation with public health services and specialists in treating this disease should be employed for optimal management.

The use of rifampin in solid-organ transplant recipients deserves special comment. Although rifampin-containing regimens may portend the best microbiologic outcome, significant morbidity from graft dysfunction and loss can be due to the simultaneous use of rifampin with immunosuppressant agents. Rifampin lowers serum levels of cyclosporine and tacrolimus considerably, as well as decreases corticosteroid levels somewhat, via induction of cytochrome P-450.[183] Current recommendations suggest that rifampin can be used successfully with increased calcineurin inhibitor dosing and careful monitoring of calcineurin inhibitor serum levels.[428,690] Rifabutin may offer equivalent efficacy with less effect on drug interactions, as has been documented in tuberculosis/human immunodeficiency virus (HIV)–coinfected individuals on antiretroviral therapy.[91,109,656] Others suggest that a longer duration of therapy (up to 50%) with rifampin-free/INH-containing regimens has a comparable outcome without the risk for rejection and potential graft loss.[81,477] Most authors agree that regimens should contain INH if at all possible.* If rifampin-free regimens are used, therapy should be prolonged to a minimum of 12 to 18 months.[81,626] Ethambutol and second-line therapies (aminoglycosides and quinolones) may be considered if a first-line drug cannot be used.[477,578] If both INH and rifampin are not used, because of toxicity, drug interactions, or resistance, therapy should be continued for a prolonged period, possibly 2 to 3 years.[91,578]

*References 81, 91, 470, 477, 626, 656.

Nontuberculous Mycobacterial Infections

Nontuberculous mycobacterial infections are more common in lung transplant recipients than MTB infections, in areas where MTB is not endemic, including the United States.[189] There are currently more than 125 species of nontuberculous mycobacterial organisms; recent advances in molecular methods of taxonomy have better informed our understanding of the epidemiology, outcome, and treatment of these different species.[258] The clinically relevant species are divided into slowly growing species—*Mycobacterium avium* complex (MAC), *Mycobacterium kansasii*, *Mycobacterium marinum*, and *Mycobacterium ulcerans*—and rapid growers—*Mycobacterium fortuitum* group, *Mycobacterium chelonae*, and *Mycobacterium abscessus*.[26] *M. abscessus* has now been subdivided as well into *M. abscessus* subsp. *abscessus*, *M. abscessus* subsp. *bolletii*, and a third subspecies awaiting further taxonomic clarification.[256] Patients with chronic lung disease and CF in particular have a high incidence of nontuberculous mycobacterial colonization. More recent data suggest that 6 to 24 percent of patients with CF are colonized with nontuberculous mycobacterial organisms, most commonly *M. abscessus* or MAC.* The contribution of these organisms to progression of lung disease in pretransplant patients is still being defined,† but the impact on the lung transplant recipient, especially from infections due to *M. abscessus*, deserves emphasis.

Clinical manifestations of nontuberculous mycobacterial infection in lung transplantation vary by organism and include pulmonary, surgical site infections, and cutaneous and disseminated disease.[189] In a survey of 31 transplant centers regarding *M. abscessus* infection in lung transplant recipients, 17 patients were identified (0.33%).[125] The majority had pulmonary infections, followed by skin involvement; several patients had both. Disease occurred at a median of 18.5 months after transplantation (1 to 111 months). Only 2 were preoperatively colonized. Pulmonary disease may be manifested as chronic cough, sputum production, and fatigue. Skin infections may involve surgical sites (sternal wounds) and extremities.[115,428] Painful, erythematous cutaneous or subcutaneous nodules may develop into abscesses and ulcerate.[125] In two additional single-center retrospective studies involving adult lung transplant recipients, most of whom underwent transplants because of chronic obstructive pulmonary disease, the rate of posttransplant nontuberculous mycobacterial infection was approximately 20 percent, with progression to disease in 25 percent (pulmonary infection) and 11 percent (pulmonary and surgical site infections) of those colonized.[303,348] Nontuberculous mycobacterial infection, colonization, or disease was

*References 115, 201, 255, 389, 399, 505, 508, 546, 556, 610.
†References 149, 201, 253, 280, 505-507.

associated with increased mortality in one study, which showed with a higher rate of *M. abscessus* infection.[303] A study of end-stage CF patients referred for transplantation revealed 20 percent of patients were colonized with nontuberculous mycobacterial organisms (equal numbers of MAC and *M. abscessus*); after the transplant, nontuberculous mycobacteria were isolated from 14 percent of CF lung transplant recipients. The prevalence of posttransplant invasive disease was approximately 3 percent (25% of those colonized), in both pulmonary and surgical site infections, the majority being caused by *M. abscessus*.[115] Pretransplant colonization with *M. abscessus* was associated with posttransplant disease. All patients in this series who were ill from infection with this organism were successfully treated. Emerging data support the association between preoperative colonization and posttransplant infections with *M. abscessus* and document rare, yet serious and sometimes fatal posttransplant infections.*

Diagnosis requires a high level of suspicion. Cutaneous lesions should be sampled for histology, special stains, and culture. The differential diagnosis includes fungal and nocardial infections. These organisms are ubiquitous, and differentiating respiratory tract colonization from infection may be difficult. The diagnosis of nontuberculous mycobacterial infection should be entertained, with a low threshold for performing bronchoalveolar lavage (BAL) or biopsy for any unexplained or unresolving pleuropulmonary disease.[189,258] The American Thoracic Society/Infectious Diseases Society of America (ATS/IDSA) has published diagnostic criteria that include signs and symptoms present after the treatment of other possible diseases, such as radiologic evidence of progressive pulmonary disease, including infiltrates, cavities, nodules, or bronchiectasis, and bacteriologic evidence of nontuberculous mycobacterial infection, generally on multiple respiratory or biopsy specimens.[158]

Providing therapy for nontuberculous mycobacterial infection may be difficult, depends on the species isolated, and should be determined in consultation with experts and with adherence to the ATS/IDSA guidelines.[258] Despite the use of seemingly adequate therapy, failures and recurrences are common.[256-258] Therapy for infection with MAC includes a macrolide, ethambutol, and rifampin, with or without an aminoglycoside, depending on the severity of disease. Susceptibility to macrolides should be performed; it is the only class of drug for MAC in which there is evidence to support an in vitro and in vivo response.[256,258] Therapy for infection with *M. abscessus* is undefined because this organism is notoriously resistant to many antimicrobial agents. Even regimens based on in vitro susceptibilities may not produce a cure, which may be in part explained by the discovery of the presence of an inducible macrolide resistance gene *erm(41)* in many clinical isolates, which can produce in vivo resistance despite demonstration of susceptible minimal inhibitory concentrations.[484] Combinations that include a macrolide, amikacin, and cefoxitin or a carbapenem, based on susceptibility testing, are general recommendations. Acquired resistance mutations to clarithromycin and amikacin can occur.[258] The duration of therapy is prolonged and depends on the patient's clinical, radiologic, and microbiologic response, but the suggested minimum duration is 12 to 18 months. Reduction of immunosuppression, surgical excision or débridement, and treatment of coinfections may improve the outcome.[125,256] Relapses are not uncommon. Consideration of lifelong suppression is recommended by some experts for patients with a high burden of disease and persistently high levels of immunosuppression.[109] Most patients who can complete long-term therapy improve (32% cleared their infection, 36% improved); minimal response to therapy was seen in 23 percent of lung transplant recipients.[196] Fatal infections occur rarely.[125,591,667] Drug interactions between macrolides and rifampin and between calcineurin inhibitors and sirolimus occur. Both can lower serum levels of immunosuppressive drugs and trigger rejection.

Most centers do not consider the presence of nontuberculous mycobacterial infection an exclusion to transplantation, although the optimal management of these patients is debated. Clearly, patients often remain colonized in respiratory tree sites above the anastomosis. The hilar lymph nodes in two patients were positive for *M. abscessus*, thus suggesting other potential reservoirs.[115] Given the concern for emergence of resistance toxicity and drug-drug interactions related to long-term therapy, prolonged antimycobacterial therapy cannot be recommended for all patients. Some centers opt for pretransplant or peritransplant prophylaxis, whereas others recommend observation for evidence of disease.[189]

Fungal Infections

Aspergillus

Aspergillus infections remain a major challenge to the success of lung transplantation. Most common presentations involve the respiratory tree (up to 90%), with manifestations ranging from semi-invasive tracheobronchitis and bronchial anastomotic infections to invasive pulmonary disease and, rarely, dissemination to extrapulmonary sites, including the CNS (10%).[232,619] The published rates of invasive *Aspergillus* infections vary considerably and probably reflect differences in dates of publication, patient populations, underlying disease such as CF, net state of immunosuppression, use of prophylactic measures, local issues of exposure (e.g., construction), and rates of rejection and viral infection, among others. In addition, distinguishing between isolation of *Aspergillus* from airway cultures versus true invasive disease may be difficult. Despite these caveats, rates of invasive *Aspergillus* infection in lung transplant recipients are the highest among solid-organ transplant recipients. Studies suggest that invasive disease occurs in 3 to 33 percent of lung transplant recipients, although rates of colonization/isolation of *Aspergillus* may be considerably higher. In most cases, colonization appears to be transient and does not lead to invasive disease.*

*References 115, 125, 189, 340, 428, 591, 667, 744.

*References 98, 159, 232, 244, 288, 402, 448, 461, 467, 486, 499, 526, 619.

A bimodal pattern of the development of invasive *Aspergillus* infection in transplant recipients has been recognized. Most infections develop 1 to 6 months after transplantation and are a major cause of death during this time period,* but late infections longer than 1 year post transplant occur as well.[232,402,486,617] Infections that develop less than 3 months after transplantation are more likely to be tracheobronchitis or bronchial anastomosis site infections; patients who require retransplantation or have bronchial anastomotic infection may present in the first month.[127,268,291,461] Invasive pulmonary or disseminated aspergillosis may occur at any time.[159,402,486,619,637] Risk factors for early disease in lung transplant recipients include *Aspergillus* colonization in the 6 months before transplantation or early posttransplant colonization, a complicated postoperative period, A2 or repeated rejection, and CMV disease or receipt of CMV prophylaxis as a marker for donor/recipient seropositivity.† Corroboration of the importance of CMV is the observation that a significant decrease in invasive *Aspergillus* was associated with the use of prophylactic ganciclovir in heart transplant recipients, reflecting the immunomodulating effects of the virus on host immune status.[574,704] Adult patients in whom invasive *Aspergillus* infection developed later after transplantation were older and "overimmunosuppressed" secondary to chronic or refractory graft rejection or dysfunction.[232,621] In both the early and late groups, invasive *Aspergillus* was associated with renal failure and repeated bacterial infections.

Aspergillus fumigatus causes the majority of disease, although non–*A. fumigatus* species are becoming more common.[98,448] Such species include *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus nodularis*, *Aspergillus ustus*, and *Aspergillus terreus*. Non–*A. fumigatus* species have a recognized potential for differences in antifungal susceptibility. For example, *A. terreus* is innately resistant to amphotericin B and *A. ustus* has diminished susceptibility to the azoles.[51]

Clinical findings in patients with pulmonary disease are largely nonspecific and may include cough, shortness of breath, and hypoxia, although chest pain and occasionally hemoptysis may raise clinical suspicion of infection with invasive *Aspergillus*.[165] Symptoms are seen more commonly in patients with diffuse disease than in those with focal or nodular disease. Although fever is a common early manifestation of infection with invasive *Aspergillus* in neutropenic patients with a hematologic disorder, it occurred in only 15 percent of lung transplant recipients with *Aspergillus* infection and usually more often in patients with disseminated (50%) or invasive pulmonary disease (20%) than in those with tracheobronchial or bronchial anastomotic infections (0%).[619] Lack of fever also has been documented in other studies of nonneutropenic patients with invasive *Aspergillus* infection. Patients with disseminated disease may have involvement of the sinuses, orbits, musculoskeletal system (osteomyelitis), ophthalmologic structures, cutaneous sites, and CNS. Symptoms of CNS disease may include headache, sinus pain, cranial nerve abnormalities, seizures, changes in mental status, or other focal neurologic findings.[51,165,244]

The radiologic manifestations of invasive *Aspergillus* infection in lung transplant recipients are highly variable.[165,178,606,673] Chest radiographs are insensitive and nonspecific. Computed tomography (CT) of the chest has become an integral part of early diagnostic strategies and has been incorporated into recent definitions of invasive fungal infections in immunocompromised hosts.[40,168] On chest CT scans of neutropenic adults, large nodules and cavitary lesions are common findings.[40,100,254] In addition, much has been made of the early "halo sign," a macronodule surrounded by a perimeter of "ground-glass" opacity (edema and hemorrhage), and the later "air crescent sign," a crescent-shaped air interface between viable lung and infarcted tissue at the periphery of a nodule that is seen with recovery and return of neutrophils. These findings are considered highly suggestive, although not pathognomonic, of invasive *Aspergillus* infection. Unfortunately, these findings are seen less commonly in lung transplant recipients and in children in general. Multiple ill-defined pulmonary nodules were the most common findings in adult lung transplant recipients, with approximately half demonstrating halo signs. Cavitation was rarely seen, and no air crescent signs were observed.[178] In children, radiographic findings were highly variable and included consolidations and small nodular masses and effusions, with little evidence of cavitation or halo or crescent signs.[92,673]

The gold standard for diagnosis of invasive *Aspergillus* infection is the demonstration of small hyaline, septate, dichotomously branched hyphae in tissue with evidence of associated tissue damage or positive cultures from a normally sterile body site, in the presence of signs and symptoms of invasive disease.[40,51,165,168,298] Blood cultures are rarely positive. Obtaining adequate tissue specimens in a timely manner frequently is not possible. An immunocompromised patient with neutropenia or who is on corticosteroid therapy may not exhibit obvious signs or symptoms of invasive disease until late in the course of infection.[15] Additionally, these patients are often deemed too ill to undergo the invasive procedures necessary to obtain tissue for microbiologic and histopathologic evaluation.[627] Therefore, the diagnosis is established late in the course of the disease or at autopsy. Indeed, frequently it is not established but is based on radiologic and host factors.

Early diagnosis is thought to be critical to improving the outcome of patients with invasive *Aspergillus* infection, especially now with the availability of more active therapeutic agents. In addition to early CT in high-risk patients, especially those who do not respond to broad-spectrum antibacterial therapy, there have been several recent advances in molecular/non–culture-based approaches to establishing a diagnosis. The most widely used and well characterized of these modalities is the Platelia *Aspergillus* serum galactomannan antigen, detected by double-sandwich ELISA.* This test detects very small amounts of a polysaccharide cell wall

*References 159, 362, 448, 461, 486, 619.
†References 98, 159, 232, 288, 321, 402, 467, 499, 617.

*References 451, 452, 654, 665, 722, 724.

component of *Aspergillus* and other fungal species released by invading hyphal elements. A repeated serum level of more than 0.5 is considered positive. This galactomannan antigen has been shown to be a useful tool for screening high-risk patients, triggering obtaining CT scans, thus establishing the probable diagnosis of invasive *Aspergillus* infection[95,420,569] and allowing for early therapy.[421-423,429] The use of this assay has been incorporated into the definitions for invasive fungal infections from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) consensus group.[40,168] It has also proved useful in measuring response to therapy, and correlations have been made with progressive disease seen with rising levels of galactomannan antigen and improved survival with decreasing levels.[112,416] It may also be informative if the diagnosis of immune reconstitution inflammatory syndrome (IRIS) is being considered (see later discussion).[267,454]

False-positive tests have been documented, notably in patients receiving piperacillin-tazobactam, which has been found to contain galactomannan antigen,* likely related to cross reactions to *Penicillium* spp.–derived semisynthetic penicillins.[655] More recent analysis for galactomannan antigen in patients receiving piperacillin-tazobactam and of vials of currently available brand piperacillin-tazobactam did not demonstrate this association.[457] In general, false-positive tests result in transient and low-positive values and may be differentiated from true-positive results, which tend to be repeatedly positive.[451,722] Initial pediatric data suggested that children had higher rates of false positivity[290,569,657]; more recent pediatric studies have not confirmed this observation.[37,208,301]

In solid-organ transplant recipients and nonneutropenic patients, galactomannan antigen testing of blood appears to be less sensitive for the detection of invasive *Aspergillus*.[215,313,372,543,691] In addition, antifungal therapy has been shown to decrease the sensitivity of the assay.[429,432,569] However, its use in BAL fluid may have a higher diagnostic yield and has been recently approved by the U.S. Food and Drug Administration (FDA) for this use.† Several studies in lung transplant recipients suggest that galactomannan antigen levels greater than 1.0 from BAL fluid evaluation have good sensitivity (60% to 100%) and specificity (90% to 98%) for determination of invasive *Aspergillus* infection.[273,312,316,531] Galactomannan antigen values between 0.5 and 1.0 should be interpreted in clinical context.

The Fungitell (1→3)-β-D-glucan assay measures a cell wall component found in many fungi, including *Aspergillus* and *Candida* spp. and *Pneumocystis*, but like galactomannan antigen does not detect Zygomycetes.[338,465,500,512,535] It is approved by the FDA to aid in the diagnosis of invasive fungal infections and has been incorporated into the definitions for these infections.[168] Cross-reacting glucan-containing material occurs commonly in the environment, and false-positive tests have been demonstrated in dialysis patients, those exposed to gauze, and those with some gram-positive bacteremia. In addition, false-positive results are associated with the use of some antimicrobial agents and with sample manipulation, thus suggesting that laboratory contamination is possible.* A study in lung transplant recipients suggests it has a low positive predictive value, thus limiting it use as a screening tool, and false-positive findings occurred with mold colonization and hemodialysis. Unlike serum galactomannan antigen, it does not appear to correlate with response to therapy or have prognostic value, because it declines slowly, even with clinical improvement.[16,355]

The *Aspergillus* polymerase chain reaction (PCR) assay remains nonstandardized, but most assays rely on detection of fungal sequences (panfungal or genera/species specific) of 18S or 28S rRNA or mitochondrial genes in whole blood, plasma, serum, or BAL fluid. More recent protocols have used real-time PCR, which allows rapid, automated, quantitative, and reliable results, with less chance of contamination.† Optimization of nucleic acid extraction, design of primer pairs and probes, and patient sample site (e.g., blood, serum BAL fluid) as well as the development of newer techniques that can identify resistance genes are underway. [361] While clinical information is accumulating, standardization and validation from large studies remains lacking, although some studies suggest that PCR can detect *Aspergillus* before galactomannan antigen appears in blood and may be effective when applied to nonblood samples such as BAL fluid, CSF, and tissue.[495,630,729,731] Studies comparing the use of molecular methods of diagnosis are ongoing.‡ Several authors suggest that combining two methodologies may improve diagnostic sensitivity.§

Despite historically poor outcomes of invasive *Aspergillus* infection in immunocompromised patients, new mold-active therapies appear to be associated with improving mortality figures.[495,658,682] The Infectious Diseases Society of America (IDSA) practice guidelines for *Aspergillus* infections, published in 2008, recommends therapy with voriconazole as primary therapy for most patients, because it has demonstrated outcome superiority.[46,168,172,710] Additionally, voriconazole has shown promise for treatment of CNS disease, which historically has been uniformly fatal.[329,599,719] For patients refractory to or intolerant of primary therapy, lipid formulations of amphotericin B deoxycholate (AmBd), itraconazole, and caspofungin are FDA approved for treatment of invasive *Aspergillus* infection.

Data are now emerging on the use of voriconazole for invasive *Aspergillus* infection in pediatric patients and solid-organ transplant recipients. The optimal dosing for pediatric patients remains undefined at present but data suggest that much higher doses than currently recommended are tolerated and beneficial.¶ Several studies

*References 5, 41, 412, 419, 420, 625, 658, 701, 712.
†References 55, 170, 347, 413, 480, 592, 702.

*References 180, 298, 439, 535, 544, 724.
†References 12, 15, 89, 90, 116, 197, 276, 282, 298, 332-334, 338, 376, 406, 459, 642, 682, 728, 730, 734.
‡References 82, 83, 90, 338, 407, 480, 535, 592.
§References 82, 141, 142, 188, 459, 480, 535, 592.
¶References 30, 63, 87, 88, 184, 464, 485, 530, 632, 638, 646, 651, 709.

support the importance of therapeutic drug monitoring of voriconazole as it relates to improved outcome, especially in children and other patient populations, including those with CF.[30,59,88,185,485,638] Reproducible data suggest trough levels between 1 and 5 μg/mL are correlated with improved outcome and decreased toxicity. Toxicities include hepatotoxicity and CNS and visual disturbances (photopsia) and are generally dose related and reversible, although fatal liver failure has been reported.* Cutaneous photosensitivity, which can be chronic, and the development of skin malignancies have also been documented but are likely related to duration of therapy and not serum levels.[60,146,751] Voriconazole is available in intravenous and oral formulations, which allow for transitioning to oral dosage when the patient's condition has improved and he or she can tolerate oral medication and is at home. However, there appear to be multiple factors that affect the "bioavailability," most notably in children, but also some adults and CF and other high-risk patients, such that therapeutic drug monitoring should be done before all oral dosing to evaluate absorption, food intake, and polymorphisms in metabolism of CYP2C19 P-450 enzyme.[30] Concern over potential renal toxicity associated with the accumulation of the intravenous solvent vehicle sulfobutylether-β-cyclodextrin, as seen in animal models, has led to a contraindication of the intravenous administration of voriconazole in patients with renal failure on standard renal replacement therapy.[3,270,487,703] This remains an area of investigation.

Several important drug interactions occur with medications routinely used in solid-organ transplant recipients. The most important of these interactions involves tacrolimus and sirolimus, in which concomitant use with voriconazole can lead to decreased metabolism, via cytochrome P-450, and result in greatly increased serum levels of the immunosuppressant.[582] The use of tacrolimus can be controlled safely by reducing the dose by 50 percent and monitoring daily levels.[582,688] The effect of voriconazole on sirolimus levels is even more pronounced, and it is labeled as an absolute contraindication by the manufacturer.[582,583] Several case reports and small series describe the use of these two drugs together, with significant dose reduction of sirolimus (up to 90%) and close follow-up of serum sirolimus levels.[438,441,583] The use of rifampin and voriconazole should be avoided if possible, because voriconazole levels may be significantly decreased in this setting.[234] Posaconazole, the newest and most broad-spectrum azole, with activity against many non-*Aspergillus* molds and Zygomycetes is recommended as an alternative for treatment of invasive *Aspergillus* infection in patients refractory or intolerant of other therapy, including voriconazole.[13,360,600,708,711] It is also licensed for prophylaxis in patients 13 years of age and older with fever and neutropenia or graft-versus-host disease.[139,679] Its safety and drug interaction profile is similar to that of voriconazole. On the other hand, optimal dosing remains an issue, especially in children for whom no specific dose recommendations exist,[718] and it is only available in oral formulation, which limits its utility in critically ill patients. Absorption is improved with fatty foods and lower stomach pH. Therapeutic drug monitoring, with steady-state serum targets of greater than 0.5 to 0.7 μg/mL, have been suggested to correlate with improved outcome, although this remains an area of active investigation.[187,302,605] The use of posaconazole as primary therapy for invasive *Aspergillus* infection is also currently being studied.

The polyene amphotericin B deoxycholate (AmBd) and its lipid formulation (LFAB) are active against *Aspergillus* spp., except for *A. terreus*, and exert their activity on the cell membrane. AmBd was the gold standard for serious fungal infections for almost 40 years but now has been supplanted by newer, more effective and less toxic agents. Lipid formulations of amphotericin B can be recommended as alternative therapy for patients unable to tolerate or refractory to voriconazole. The recent consensus suggests that LFAB may be more effective than AmBd in certain settings and is clearly less toxic.[185,708]

Echinocandins have a unique mode of activity; they inhibit the biosynthesis of (1→3)-β-D-glucan in the fungal cell wall, which is absent in mammalian cells, thus offering a high therapeutic index, and additionally have minimal drug interactions.[185,296,431] There are three echinocandins licensed in the United States: caspofungin, micafungin, and anidulafungin. There do not appear to be clinically significant differences among these drugs in spectrum, efficacy, or toxicity. Both caspofungin and micafungin have been investigated for treatment of invasive *Aspergillus* infection, although only caspofungin has been indicated for the treatment of invasive *Aspergillus* infection in patients refractory to or intolerant of other therapies.* Several studies have addressed the safety, efficacy, and dosing of echinocandins in pediatric patients.† All studies have found them to be safe and well tolerated in children, even neonates. Extrapolation of pediatric dosing from adult dosing led to underdosing, which has been revised on the basis of pediatric pharmacokinetic data.[296,297,602,707] Randomized, controlled data on the use of echinocandins for primary treatment of invasive *Aspergillus* infection are lacking.

Combination therapy remains controversial. Possible advantages of combination therapy include synergistic interaction of two drugs with different targets, lower doses leading to less toxicity, greater spectrum of activity, and decreased potential for the development of resistance.[390] Potential disadvantages include antagonistic antifungal activity, increased overall drug interactions, enhanced toxicity, and great increases in cost.[330,475,700] Preclinical data from in vitro and animal studies investigating the use of combinations of antifungal agents against *Aspergillus* are generally promising.[330,475,647,649,700] Complexities and differences in methodologies used to determine in vitro efficacy, experimental design, and animal models confound interpretation of the data and render extrapolating to patients difficult.[475,647,649,723] Clinical data are emerging for patient populations including solid-organ transplant recipients, which demonstrate safety and some promise. Most studies are small, retrospective

*References 30, 63, 87, 88, 184, 185, 289, 408, 464, 485, 530, 632, 638.

*References 117, 166, 260, 262, 349, 354, 418, 686, 708, 713, 735.
†References 217, 262, 296, 453, 501, 646, 707.

studies, often for salvage or intolerance not primary therapy, and with the use of historical controls with LFAB as the comparator to combination therapy, not voriconazole, such that success is hard to define.* Combinations include azoles (voriconazole or posaconazole) with echinocandins or LFAB. Data from prospective, randomized trials in which voriconazole is compared with combinations that include voriconazole are needed to evaluate first-line therapy. Many authors recommend caution in using combination therapy before the results of controlled, prospective investigations are available, even though they acknowledge that widespread use of combination therapy already is evident.† Indeed a survey of invasive *Aspergillus* infection in transplant recipients documented primary combination therapy was used in 35 percent of patients.[44] The most recent IDSA guidelines on the treatment of invasive *Aspergillus* infection conclude that "there are insufficient clinical data to support combination therapy as routine primary treatment…."[708]

No definitive data are available on how long to treat invasive *Aspergillus* infection, although the natural history of disease, assessment of risk factors, and the state and length of immunosuppression, along with investigations on response to therapy and animal studies, inform us on regimens most likely to be effective. Clearly, one must balance the risk for incomplete therapy and recurrent disease with the dangers of increased toxicity and expense from prolonged therapy. One thoughtful approach, based on these many variables, suggests use of the "most effective therapy first for 10 to 12 weeks, or for at least 4 to 6 weeks beyond resolution of all clinical and radiographic abnormalities, whichever is longer."[612] Length of therapy should be individualized, and factors such as recovery from neutropenia or other immunocompromised state, extent of disease, presence of graft-versus-host disease, and coinfections (e.g., with CMV) predict poorer outcome and slower response and probably will prolong duration of therapy for patients with these comorbid findings.

Adjunctive surgical therapy may be recommended for cutaneous and soft tissue infections,[286] for lesions on the great vessels or major airways to prevent massive hemoptysis,[61,99,101,513] in patients with endophthalmitis[717] and osteomyelitis,[344] and for removal of residual lesions before hematopoietic stem cell transplantation is performed. Immunomodulatory strategies to improve host immune response, in theory, are attractive adjuvant therapies. Alveolar macrophages, polymorphonuclear neutrophils, and pulmonary dendritic cells are critical first-line defenses against invasive *Aspergillus* infection.[571] Therefore, modalities that improve the number or function of these cells may improve outcome. Decreasing the use of immunosuppression and corticosteroids as much as possible should be the goal in transplant recipients with invasive *Aspergillus* infection. Additional strategies, including enhancement of T-helper type 1 (Th1) immune responses, exogenous administration of colony-stimulating factors (granulocyte colony-stimulating factor [G-CSF], granulocyte-macrophage colony-stimulating factor [GM-CSF]) or cytokines (IFN-γ) to activate or recruit phagocytes (or both), and G-CSF donor-primed white blood cell transfusions have been investigated and may be used in select cases.* Finally, augmentation of innate pathogen recognition pathways (Toll-like receptors, pentraxins), vaccine strategies, and adoptive immunotherapy have shown promise in animal models of invasive *Aspergillus* and await further refinement and clinical trials.†

When *Aspergillus* causes invasive disease, mortality rates are high (30% to 100%) and notably so for invasive and disseminated or CNS forms of the infection.[98,232,448,637] In a review of English language articles on *Aspergillus* infections in adult lung transplant recipients, the overall mortality rate was 53 percent (59/112); it was 24 percent (9/38) for tracheobronchial or bronchial anastomotic infections, 82 percent (18/22) in patients with invasive pulmonary disease, and 67 percent (2/3) for disseminated invasive infection.[619] These published data reflect the limited therapeutic options previously available (before the advent of extended-spectrum azoles and echinocandins). Several more recent studies suggest improvement in mortality rate (40%) since the widespread use of voriconazole and therapeutic drug monitoring.[43,493,680]

Despite the lack of controlled trials, several surveys document that most lung transplant centers use prophylactic or preemptive systemic antifungal agents (azoles, echinocandins, or amphotericin formulations), aerosolized amphotericin formulations, or both in patients with preoperative or postoperative isolation of airway *Aspergillus*, and almost 60 percent of the centers employed universal prophylaxis.[167,190,191,320,488] Because *Aspergillus* infections are acquired through the lungs and most infections are pulmonary, attaining high pulmonary drug levels via aerosolization and limiting systemic toxicity are attractive options for prevention.[191,627] Multiple trials have suggested a reduction in invasive *Aspergillus* infections with the use of aerosolized amphotericin formulations.‡ A single retrospective study incorporating the use of universal voriconazole prophylaxis with or without inhaled amphotericin compared with targeted itraconazole in lung transplant recipients decreased the rate of invasive *Aspergillus* infection at 1 year from 23.5 to 1.5 percent.[315] Abnormalities in liver enzymes were common, necessitating discontinuation of voriconazole in 14 percent versus 7 percent of the comparator. This study was performed before widespread use of voriconazole therapeutic drug monitoring, the use of which may have precluded some of this toxicity. Based on two phase 2 studies, posaconazole is FDA approved for prophylaxis of invasive fungal infections in certain high-risk patient populations 13 years or older.[139,680] The generalizability of these findings to solid-organ transplant recipients has not been examined. Further randomized, multicenter studies are needed to clarify which agents and prophylactic strategies are most effective.

*References 166, 351, 352, 354, 417, 430, 478, 494, 523, 620, 649, 672, 735.
†References 49, 330, 475, 647, 649, 700.

*References 181, 570, 584, 585, 603, 648, 651, 708.
†References 77-79, 107, 229, 233, 601.
‡References 73, 102, 191, 192, 200, 291, 461, 468, 518, 562.

Prevention of invasive *Aspergillus* infection through environmental controls also is important.[522,534,558,671] Infections with this fungus in transplant recipients can be nosocomial or community acquired. Most germane to prevention is air control, inasmuch as both epidemic invasive *Aspergillus* and sporadic invasive *Aspergillus* correlate with concentrations of *Aspergillus* in the air.[11] A protective hospital environment should consist of high-efficiency particulate air (HEPA) filtration, positive air pressure, high air exchange rate, properly sealed rooms, and removal of carpets, plants, and water-damaged ceiling and floor tiles.[140,195,275,534] Patients should be wearing "fit-tested" masks (N95) when leaving protected environments, efforts should be made to have construction barriers in place, and patient transport routes should be adjusted accordingly if construction is occurring in the hospital and clinics.[522] In addition, monitoring the environment and air for changes in particulates and fungal spores may be considered based on local epidemiology, rates of invasive *Aspergillus*, and infection control practices. When patients are discharged home, they should be counseled to avoid certain high-risk exposure to agents such as excessive dust and molds if possible.[534]

Candida

Although *Candida* infections are the most common cause of fungal infections in solid-organ transplant recipients, the patients at highest risk are abdominal transplant recipients (liver, small bowel and pancreas), with lung transplant recipients at intermediate risk and renal transplants at the least risk.[486,528] The overall rate of *Candida* infections in lung transplant recipients has decreased over time, in one study from 20 percent (1980-1986) to 1.8 percent (2001-2004), as has attributable mortality from 39 to 15 percent.[595] *Candida* infections in lung transplant recipients generally occur early, within the first month after transplantation, associated with long-term broad-spectrum antibiotic therapy, the presence of central venous access and other indwelling foreign bodies, surgical complications, CMV infection, and renal replacement therapy.[362,524,528] Infections present in the form of bloodstream infections are often catheter-associated urinary tract infections, especially if the Foley catheter remains in situ, and respiratory tract infections (tracheitis, pneumonia, and rarely bronchial anastomosis site infections).[528,595,685] Although colonization of the respiratory tract is a common occurrence, primary invasive candidal pneumonia is not. Invasion usually occurs when comorbid conditions are present.[362] In patients with a fungemia workup for metastatic sites, including ophthalmologic examination, echocardiography and imaging may be necessary. Rarely, disseminated disease of abdominal organs and the CNS occurs in solid-organ transplant recipients.[524] The source of infection usually is endogenous, although contributions from donor organs and nosocomial transmission may also occur. Studies have demonstrated changes in species distribution, with a decline in *Candida albicans* infections and increase in non–*C. albicans* species.[524,540] Mirroring these changes, the Transplant Associated Infections Surveillance Network documented an increase in non–*C. albicans* candidal species, which was the driving force increasing azole resistance in *Candida* species (overall 16%), especially for *Candida glabrata* and *Candida krusei*.[405,527] Of interest, the azole resistance of *C. albicans*, *Candida tropicalis*, and *Candida parapsilosis* remained relatively low (1%). In solid-organ transplant recipients, fluconazole resistance was associated with any fluconazole use in the 3 months before infection.

The diagnosis of *Candida* infections can be challenging and requires differentiating colonization from infection because *Candida* spp. are ubiquitous, especially in the upper gastrointestinal and respiratory tracts. In addition, although the diagnosis is often made by culture, these techniques are insensitive especially for invasive disease and, except for blood culture, nonspecific. If recovery of *Candida* spp. from BAL fluid is associated with respiratory symptoms or deterioration of lung function (e.g., increased sputum production), treatment should be considered, although other causes such as rejection and coinfections should be explored as well. Organisms should be identified to the species level because of species-specific susceptibility profiles.[405] Early germ tube determination can confirm the presence of *Candida albicans*, and a negative test can alert the physician to the presence of non–*C. albicans* species, which may have an impact on the choice of antifungal agent.[524] Newer molecular probes, such as peptide nucleic acid fluorescence in situ hybridization (PNA FISH), can identify *C. albicans* rapidly from positive blood cultures.[12]

The Fungitell (1→3)-β-D-glucan assay has been FDA approved as an adjunct for the diagnosis of invasive *Candida*,[528] and its role in the early diagnosis of these infections is still under investigation; it may be especially useful for the diagnosis of deep-seated candidiasis, when blood cultures are known to be insensitive.[492,524] Use of the PCR techniques shows promise, but these techniques are still experimental and their impact on clinical outcome unknown.[42,224,346,492,524] A meta-analysis of PCR on blood samples for invasive candidiasis demonstrated good sensitivity and specificity for candidemia and invasive disease.[42] Whole blood assays using panfungal DNA targets, with subsequent species identification and in vitro sensitivity of at least 10 colony-forming units per milliliter may allow for earlier and more sensitive diagnosis in high-risk patients. The usefulness of these tests in lung transplant recipients with candidal infections is currently unknown. Although galactomannan antigen is also produced by *Candida* spp., this assay is not FDA approved for detection of *Candida* infections.

Although candidemia should always be treated with an antifungal agent, the issue of removal of indwelling catheters has been controversial. The IDSA guidelines for the management of candidiasis and catheter-related bloodstream infections recommend a "qualified yes" regarding the early removal of catheters and suggest that decisions to do so should be individualized, in the presence of the lack of randomized controlled data designed to answer this question.[4,503,527] Two large reviews, performing subgroup analysis from previous controlled trials of patients with invasive candidiasis and candidemia, suggest a nuanced approach as well.[31,80,131,496] Nucci and associates found that the survival benefit demonstrated in univariate analysis was lost in multivariate analysis, which

did not find any clinical benefit to early line removal in 842 adults with candidemia.[496] The accompanying editorial points out that the wide confidence intervals for the odds ratio for survival and the point estimates greater than 1 associated with catheter removal suggest that "clinically meaningful results cannot be excluded … and illustrates a lack of power for evidence against catheter removal."[80] Andes and associates performed a patient-level quantitative review of 1915 adults from seven trials with invasive candidiasis, including 1349 with bloodstream infections. This group did demonstrate a survival benefit from catheter removal.[31] As these authors and the accompanying editorial note, the lack of randomization for catheter removal, standardization of timing of removal and antifungal therapy, lack of data on important high-risk subgroups, and the 15-year time period spanned during the studies are limitations of the data but do lend support to the negative impact of catheter retention.[31,131]

The choice of initial antifungal therapy should be based on azole exposure, dominant species and their susceptibility profiles at the institution caring for the patient, comorbid conditions, sites of involvement, and the use of other medications that might have significant drug-drug interactions.[527] The IDSA guidelines for management of candidiasis recommend fluconazole or an echinocandin in most settings, the latter especially in ill patients with recent azole exposure, with transition to fluconazole when stable.[527] In the study by Andes and associates, treatment of candidemia/invasive candidiasis with an echinocandin was associated with decreased mortality and supports the ascendancy of echinocandins as drugs of choice for most *Candida* infections.[31,131,177] For infections due to *C. glabrata*, an echinocandin is preferred and transition to fluconazole is done only if confirmed results of susceptibility are available. Treatment failures have been noted with the use of echinocandin therapy for *C. parapsilosis*, which has an increased minimal inhibitory concentration to echinocandins, owing to a naturally occurring proline to alanine amino acid change in the *Fks1p* gene, thus leading to a glucan synthase that is 1 to 2 logs less sensitive to echinocandins than *C. albicans*.[228,296] Although this is a common pathogen in neonates, the widespread clinical relevance of this observation is unclear.[31] Fluconazole and AmBd formulations are the drugs of choice, although success with echinocandins is also well documented. Voriconazole or AmBd formulations may be used for selected cases of *C. krusei* or *C. glabrata*, depending on susceptibilities. AmBd formulations with or without flucytosine are still recommended for CNS infections, including meningitis and endophthalmitis, and should be considered for cardiovascular infections (endocarditis, pericarditis, suppurative thrombophlebitis) especially in critically ill patients. Duration of therapy, as with other infections, should be individualized, but treatment should be continued for a minimum of 2 weeks after the last positive culture has been obtained and the signs and symptoms have resolved.[525,527] As noted earlier for *Aspergillus* infections, issues of dosing in pediatrics with the newer antifungal agents and drug-drug interactions should be considered. Several excellent resources are available for detailed treatment algorithms.[524,525,640] Susceptibility testing should be considered on non–*C. albicans* spp. and for *C. albicans* infections that are refractory to conventional therapy. Interpretive breakpoints are available for most common antifungal agents including echinocandins.[15,524,541,542]

Cryptococcus neoformans

Although relatively rare, cryptococcosis is the third most common fungal infection seen in solid-organ transplant recipients, after *Aspergillus* and *Candida*, with an overall incidence of 2.8 percent (0.3% to 5%).[618,663] Available data suggest infection is less common in pediatric patients than in adults.[618,628,696] This infection usually occurs late, after the first year post transplant, but may be earlier in lung transplant recipients.[618,622,663] Disease can be newly acquired but most often in adults is reactivated from latent disease, and cases of donor-derived infection are documented.[243,319,618,663,742] In the latter two scenarios, presentation may be within the first month after transplantation, the occurrence of which is most common in liver transplant recipients.[45,661,663] The overall incidence and time to onset has not changed over many decades, but there are notable shifts in the recent epidemiology. Patients with cryptococcosis are more likely to be lung transplant recipients or multivisceral transplant recipients as opposed to renal recipients. Although CNS involvement and dissemination, with or without fungemia, remains the most common presentation, compared with a prior cohort the presentation of isolated pulmonary infection has become more common in solid-organ transplant recipients. This is especially true since the almost universal use of calcineurin inhibitors.* These immunosuppressants target the fungal homologs of calcineurin, resulting in synergistic interactions with antifungal therapy and increased anticryptococcal activity and thus protecting against disseminated disease.[245,319,353,615,616] Pulmonary infections may have a variety of presentations, including single or multiple nodules, infiltrates, consolidation, and pleural effusion; isolated pulmonary disease may be asymptomatic and detected as an incidental finding. Cutaneous disease manifests as nodular, maculopapular, ulcers or pustules, or cellulitis and most commonly indicates disseminated disease; this finding requires a full evaluation of extent of infection including CNS involvement.[660]

The diagnosis can be made by culture, visualization of encapsulated yeast with India ink or other special stains, and testing for cryptococcal capsular polysaccharide antigen in serum or CSF.[123] Although the serum cryptococcal antigen test is most reliable for CNS and disseminated disease, and a positive test should prompt a full evaluation, it is known to have decreased sensitivity for isolated pulmonary infection; indeed, among solid-organ transplant recipients, lung transplant recipients with isolated pulmonary disease appear most likely to have negative cryptococcal serum antigen.[660,696,699]

The IDSA updated the guidelines for management for cryptococcal disease in 2010.[538] The mainstay of therapy for severe, disseminated disease with CNS involvement

*References 162, 319, 615, 663, 696, 699, 742.

is a combination of an LFAB plus flucytosine, which confers a survival advantage over conventional AmBd—11 percent versus 40 percent mortality in solid-organ transplant recipients.[622,659] Induction therapy should continue for a minimum of 2 weeks and should last 4 to 6 weeks if flucytosine is not used. An extended period of fluconazole maintenance therapy (6 to 12 months) is recommended because relapses are well documented, especially in HIV-infected patients. The risk for relapse in solid-organ transplant recipients and the optimal length of therapy are not known. Duration of treatment less than 6 months has been associated with an increased rate of relapse.[538,614,622] Management of elevated intracranial pressure is essential and may require repeated, sometimes daily lumbar punctures and, rarely, placement of a ventriculoperitoneal shunt.[123,538] For less severe disease localized to the lungs, fluconazole alone may be adequate.[538,622] Mortality rates for solid-organ transplant recipients have improved over time: the overall mortality rate for cryptococcal disease is reported as 11 to 30 percent and varies by site of infection, treatment regimen, type of transplant, and possibly immunosuppressive regimen. The highest rates are seen in liver and kidney transplant recipients with disseminated disease or fungemia, or both (20% to 70%). The lowest mortality rates (3% to 13%) are seen in localized pulmonary disease.*

Immune reconstitution inflammatory syndrome (IRIS) is well described in cryptococcosis in solid-organ transplant recipients and correlates with treatment and the evolving host response to infection, with improvement in cellular immunity as Th1/Th17 responses are resto red.[538,614,623,624,662] IRIS presents in solid-organ transplant recipients a mean of 6 weeks after initiation of therapy and correlates with reduction of immunosuppression. This remains a cornerstone to therapy but should be done in a gradual, stepwise fashion to avoid rapid reconstitution. Manifestations of IRIS include fever, lymphadenopathy, and progression of pulmonary and CNS symptoms and lesions, without documentation of increased titers or positive cultures. Based on observations including the potential benefit of calcineurin inhibitors to antifungal activity and the lack of association of the reduction of corticosteroids on the risk for developing IRIS, consideration should be given initially to tapering corticosteroids, followed by reduction but not discontinuation of calcineurin inhibitors. There is no established therapy for IRIS. Minor manifestations may resolve over time, but IRIS may be life threatening in severe cases and can lead to graft loss.[624] Increasing doses of corticosteroids have been used. Investigational therapies include statins to promote Th2/Tregs over Th1/17 pathways and tumor necrosis factor-α inhibitors.[614,662]

Pneumocystis jiroveci *(Formerly* Pneumocystis carinii*)*

Pneumocystis pneumonia (PCP) is caused by the renamed fungal pathogen *Pneumocystis jiroveci*.[653] PCP has been associated notably with defects in T-cell immunity, as in HIV infection and in patients with leukemia, and with the immunosuppression used in solid-organ transplant recipients. It also is described in patients with other immune defects, including those associated with corticosteroid use and radiation therapy, neutropenia, and congenital immunodeficiency.[436,567] Serologic data suggest universal, albeit asymptomatic, infection occurs by 4 years of age. In most cases, symptomatic PCP in an immunocompromised host is thought to be reactivation of latent infection, although putative nosocomial person-to-person transmission linked by molecular evidence is increasingly observed.[153,163,384,549,598]

Heart-lung recipients and lung transplant recipients are at increased risk for developing infection, with reported attack rates of 6.5 to 43 percent without prophylaxis, up to a 10-fold increase as seen in heart transplants alone.[568] Higher rates of infection in solid-organ transplant recipients, including lung transplant recipients, have been associated with CMV coinfection, use of cyclosporine and corticosteroids, and receipt of therapy for rejection; cases can occur many years after transplantation.[474,549,598,715] Therefore, guidelines recommend a benefit of long-term and perhaps lifelong prophylaxis, especially if additional risk factors are ongoing.[568,598,715] The drug of choice for prophylaxis remains TMP-SMX, and its use is associated with dramatic decreases in the incidence of PCP infection. Additional benefits of this drug include prevention of many community-acquired respiratory, gastrointestinal, and urinary tract pathogens and protection against most *Toxoplasma gondii* and *Nocardia* infections. Multiple regimens exist, and dosing for 3 days per week appears to be as effective as is daily dosing in all populations studied. Toxicity remains an issue for some patients and includes bone marrow suppression, decreased renal function, hepatitis, and rash (Stevens-Johnson syndrome), which may be severe. Alternative regimens exist for patients intolerant of TMP-SMX, but they should be considered second line because of breakthrough PCP infection or diminished coverage of non-PCP pathogens. Such alternatives include pentamidine, dapsone, atovaquone, and clindamycin/pyrimethamine. Details of these regimens are reviewed elsewhere.[567,598]

PCP infection is manifested as progressive dyspnea, cough, tachypnea, chest pain, and cyanosis over the course of days to weeks. In addition, fevers, sweats, and flulike symptoms may be prominent. Hypoxia and shortness of breath, in the context of normal or minimal findings on chest radiographs, are common findings. Corticosteroids and calcineurin inhibitors may alter the signs and symptoms and render early diagnosis more difficult to establish. The diagnosis is confirmed by identification of *P. jiroveci* in a respiratory sample or lung tissue. Immunofluorescent staining of organisms with monoclonal antibodies had been the technique of choice; Gomori methenamine silver nitrate, which stains cyst forms only (5% to 10% of the total organisms), is the most reliable staining method.[567] BAL fluid and tissue obtained by open-lung biopsy have the highest diagnostic yield.[567] Recently, PCR assays on BAL specimens have been recognized as highly sensitive and specific diagnostic techniques and are now commercially available. This assay may allow the distinction between colonization and low

*References 162, 614, 615, 622, 659, 696, 742.

fungal burden infection.* (1→3)-β-D-Glucan is a component of the *P. jiroveci* cell wall and can be detected in the serum of patients with PCP.[143,171,355,594] This assay appears to be a reliable marker of infection, with the most convincing results shown in patients with HIV infection.[171] At this time, it cannot be recommended to supplant appropriate testing of respiratory samples, but it may be considered as an adjunct for diagnosis, especially in patients too ill to undergo invasive procedures.[473]

The mainstay of therapy for severe PCP infection remains high-dose TMP-SMX for 14 to 21 days.[209,436] Therapy can be continued through mild adverse events, such as rash and minimal perturbations in aminotransferase levels and complete blood cell counts. Dose reduction, desensitization, or both may be helpful, and renal dosing should be implemented for renal dysfunction. Corticosteroids are generally recommended for severe disease, including a 5- to 7-day course with a slow taper over 7 to 14 days. For patients intolerant of TMP-SMX or those less seriously ill, alternative therapies can be considered, including dapsone with trimethoprim, atovaquone, intravenous pentamidine, trimetrexate, clindamycin/primaquine, and pyrimethamine/sulfadiazine.[209,436] Relapse in patients without HIV infection is an uncommon occurrence with TMP-SMX therapy if a reduction in immunosuppression can be accomplished and coinfections such as with CMV do not occur.

Endemic Mycoses

Endemic mycoses, or the dimorphic fungal infections histoplasmosis, blastomycosis, and coccidioidomycosis, are rare but important causes of disease in solid-organ transplant recipients.[555] Spores are inhaled from the environment and convert to noncontagious yeast forms at 37° C. Infection may be caused by latent reactivated disease in the recipient or donor or may be newly acquired. Because of the nonspecific findings, latency of these organisms, increased worldwide travel, and organ procurement strategies, infections in transplant recipients require a high index of suspicion, especially if they occur in nonendemic areas.

Coccidioidomycosis, caused by *Coccidioides immitis* and *Coccidioides posadasii*, is the endemic mycosis most commonly seen after transplantation. It occurs in up to 9 percent of solid-organ transplant recipients in endemic areas of the southwestern United States (especially California and Arizona), northern Mexico, and Central America.[68,69,692] Unlike histoplasmosis and blastomycosis, pretransplant infection poses an increased risk for development of serious posttransplant disease. Transplant candidates should be screened for a history of coccidioidomycosis and environmental exposure, which should prompt serologic testing for *Coccidioides*. Secondary prophylaxis is very effective and should be given to all patients with a history of *Coccidioides* infection; without its use, the mortality rate is very high (up to 70%).[68,339,555,692] Pulmonary infections may be accompanied by an acute onset of respiratory symptoms and fever and progress rapidly to respiratory failure. Nonspecific symptoms of anorexia, weight loss, and fatigue may herald extrapulmonary disease. Dissemination to bones, skin, and the CNS occurs commonly when infection occurs in transplant recipients. The time of highest risk appears to be in the first 3 months after transplantation, with the majority of infections occurring by 1 year. Donor-derived coccidioidomycosis has been documented in lung transplant recipients.[70,458,555,676,740] Recipients did not have evidence of pretransplant coccidioidomycosis, nor did they live or travel to endemic areas. Several of the donors had documented epidemiologic risk factors. Commonly these lung transplant recipients had fulminant pulmonary or disseminated infections within the first 3 weeks after transplantation, the majority of which were fatal.

The diagnosis of coccidioidomycosis can be made definitively by culture, usually from respiratory samples or urine and rarely from blood or CSF.[32] Histopathologic evaluation may reveal large spherules with the presence of endospores. Serologic tests include tube-precipitin antigen (IgM), detected early in disease, and complementation fixation or immunodiffusion (IgG), which can be titered and is helpful in monitoring response to therapy.[32,68] Immunocompromised patients may have negative serologic studies. Skin testing for delayed hypersensitivity no longer is available in the United States. The optimal therapy for solid-organ transplant recipients with coccidioidomycosis is unknown but generally follows published guidelines.[226,555] For mild pulmonary disease, fluconazole or itraconazole is appropriate in most cases. Most experts suggest that patients with rapidly progressive, nonmeningeal disease should receive LFAB until their condition is stabilized, followed by a prolonged course of azole therapy (fluconazole, itraconazole, possibly voriconazole or posaconazole). For meningitis, high-dose azoles are indicated, probably lifelong. Patients with evidence of previous infection should receive secondary prophylaxis with azole therapy for a minimum of 6 to 12 months after undergoing transplantation.[32,68,226,555] Echinocandins should not be used for endemic fungal infections.

Histoplasma capsulatum infection is a rare occurrence after transplantation, even in patients with presumed pretransplant infection. Cases in transplant recipients have been observed in endemic areas and in conjunction with outbreaks in areas hyperendemic for histoplasmosis, the Mississippi and Ohio River valleys, and Central America.[148,259,681] Two cases of histoplasmosis in renal transplant recipients were linked by molecular typing to donor-derived disease.[393] A 10-year experience of 3436 solid-organ transplant recipients in Ohio observed 14 cases of posttransplant infection, all with dissemination, but with good response to prolonged therapy.[148] Median time to diagnosis was 17 months after transplant, and time from onset of symptoms to diagnosis was 3 weeks, reflecting the protean nature of initial presentation. Fourteen additional patients had evidence of histoplasmosis in explant or donor tissues; all received antifungal prophylaxis and none developed active infection.[725] Manifestations of infection include pulmonary, mediastinal, inflammatory, and disseminated syndromes with or without CNS involvement. The diagnosis can be made

*References 10, 74, 193, 279, 395, 509, 590.

by culture, histopathology, serology, or antigen detection.[725] In immunosuppressed patients with disseminated disease, culture of appropriately obtained specimen, and antigen detection (urine, serum, BAL) appear to be the most useful (sensitivity of 80% to 100% using third-generation assays).[259,271,274,721] Cross reactivity with *Aspergillus* and *Blastocystis* can result in false-positive *Histoplasma* antigen tests with these infections.[689] In addition, positive galactomannan antigen assays have been reported in solid-organ transplant recipients with histoplasmosis.[689] Guidelines recommend long-term therapy (3 to 4 months) with LFAB for immunocompromised patients with disseminated or CNS involvement, followed by 6 to 12 months of itraconazole.[555,726] Voriconazole and posaconazole have good in vitro activity and have been successfully used as well.[219,220] Monitoring antigen levels is beneficial to assess response to therapy and for relapse.[272,721,726]

Blastomycosis dermatitidis, the causative agent of blastomycosis, appears to be the least common endemic fungal infection in the posttransplant setting.[231] These infections are rare events in series of fungal infections in immunocompromised patients, including patients with the acquired immunodeficiency syndrome, even in endemic areas of the south central and north central United States, particularly in the Ohio and Mississippi River valleys and around the Great Lakes.[555] Cases of posttransplant disease most commonly present as pulmonary disease, which may lead to acute respiratory distress syndrome and a high mortality rate. Diseases of the skin and CNS are also common.[259,336,603,689] Diagnosis is made by histopathology, culture, and antigen detection.[121,138,555] Guidelines for therapy recommend immunocompromised patients should receive LFAB until stabilized (4 to 6 weeks), followed by a prolonged course (6 to 12 months) of an azole.[120,121,555] Most data are available for itraconazole, although voriconazole and posaconazole also may be effective.[121,336]

*Emerging Non-*Aspergillus *Mycelial Fungi*

The impact of non-*Aspergillus* mycelial fungi is currently unknown for lung transplant recipients. Published reports suggest that these mold infections are becoming increasingly common and are recognized pathogens in lung transplant recipients and other solid-organ transplant recipients.* These organisms include non-*Aspergillus* hyalohyphomycetes or hyaline hyphae without pigment (*Scedosporium apiospermum*, *Fusarium* spp.); phaeohyphomycetes, or dematiaceous pigmented molds (*Cladophialophora bantiana*, *Scedosporium prolificans*, *Exophiala jeanselmei*, *Pyrenochaeta romeroi*, *Cladosporium* spp.); and Zygomycetes, or nonseptated hyphae (*Rhizopus* spp., *Mucor* spp.).[363] Many of these organisms have unique susceptibility profiles, which include the newer azoles, voriconazole and posaconazole, as well as LFAB and terbinafine. Frequent poor outcome has prompted the use of combination therapy, although without conclusive data.[185,363,641] Indeed, these infections may be more likely to be disseminated and fatal than those caused by *Aspergillus* spp.[310] Surgical débridement may play an important role in therapy in addition to medical therapy.[363]

*References 310, 363, 374, 462, 586, 604, 609, 650.

Viral Infections

Cytomegalovirus (CMV)

The epidemiology of CMV infection in solid-organ transplant recipients has changed in recent years. Advances in diagnosis and in preemptive and prophylactic strategies and therapy have decreased the impact of early CMV infection in transplant populations. However, once antiviral therapy is discontinued, CMV infection is common in high-risk patients and it remains one of the most important pathogens in solid-organ transplant recipients, with wide-ranging effects on morbidity and mortality.[160,307,308,463] Thus, continued efforts to optimize preventative strategies are paramount.[463,520,533] The source of infection can be endogenous reactivation or from the donor graft or leukocyte-containing blood products and cause primary infection or reinfection. Rarely, primary infection may be acquired from the community, especially in young children.

The effects of CMV infection have both profound direct and indirect consequences. As per published definitions, direct effects of the virus range from "asymptomatic" CMV infection, which can be detected by viremia (culture positive), antigenemia (pp65 in leukocytes), and DNAemia or RNAemia (generally by quantitative PCR methods); CMV syndrome with viremia, fever, neutropenia, thrombocytopenia; to true end-organ disease, which is often found primarily in the allograft but may disseminate. The diagnosis of end-organ disease, such as pneumonitis, enteritis/colitis, hepatitis, and encephalitis/meningitis, requires signs or symptoms of disease at that site, with detection of CMV by culture, histopathology, immunohistochemical staining, or in situ hybridization. Except for CNS disease, detection by PCR is not sufficient for establishing the diagnosis because it is probably too sensitive and may signify transient viral shedding. In addition, the identification of co-pathogens is important and may confound the diagnosis.*

The indirect, immunomodulatory effects of CMV have broad-reaching consequences and can be divided into three categories: (1) immunomodulation as it contributes to the net state of immunosuppression and increased risk for opportunistic superinfections (e.g., aspergillosis, PCP); (2) increased risk for oncogenesis, such as seen with EBV-related lymphoproliferative diseases; and (3) allograft injury and dysfunction, as seen in classic rejection and vasculopathy of the allograft. In lung transplant recipients, bronchiolitis obliterans syndrome, a severe form of chronic rejection (small airways disease), has been associated with CMV infection.[403,579,746] Of interest, in a retrospective, multicenter study of almost 600 pediatric lung transplant recipients, development of bronchiolitis obliterans syndrome in the first year (14%) was not associated with CMV infection.[160]

The greatest risk factor for developing CMV infection in children, as in adults, is donor/recipient (D/R)

*References 160, 309, 358, 403, 573,745,746.

seropositivity, most notably D+/R– mismatch, followed by D–/R+ mismatch. Infection can occur in D+/R+ and rarely in D–/R– matches.[155,160] Other risks include the use of induction therapy, antilymphocyte antibody, and blood product transfusion (performed previously).[746,747] In pediatric lung transplant recipients, the risk for CMV infection has also been associated with living donor transplant, older age at transplant, and multiple episodes of A2 rejection before CMV.[160] The incidence of CMV infection in lung transplant recipients is higher than that in other solid-organ transplant recipients. The reasons are multifactorial but include long-term, intense immunosuppression and high levels of CMV latency, viral load, and reactivation associated with the lung and its transplantation.[50,747] The incidence of infection and disease is reported to be 54 to 92 percent without prophylaxis.[746,747] The rates of CMV infection, and its consequences, have been considerably decreased by utilizing various preventative strategies (see later discussion), although different study design, end points, and follow-up periods point to the difficulty in identifying the optimal regimen and time course.[160,520,533,746]

A combination of approaches are employed to reduce the incidence of CMV infection. The use of CMV-negative or leukocyte-free blood products for CMV-negative recipients is standard practice in most transplant centers and has been shown to reduce infection rates. Matching of D/R serologic status probably would reduce infection rates but also would limit the donor pool for recipient-negative recipients significantly and is not considered to be indicated. The final strategies use different regimens of antivirals to suppress replication with or without immunoglobulin products for enhanced passive immunity.[579,746] These regimens can be prophylactic, in which all patients at risk are treated for a certain period, or preemptive, in which highly sensitive screening techniques, such as antigenemia (pp65) or DNAemia, are used to identify patients needing therapy. The rationale of this approach is to limit drug exposure, thereby decreasing toxicity and viral resistance. To complicate matters, regimens can include multiple antiviral agents, such as intravenous ganciclovir or acyclovir and/or oral ganciclovir, acyclovir, or valganciclovir for various periods. Several surveys of lung transplant centers (adult and pediatric) show a preponderance of the use of universal prophylaxis, based on serostatus stratification.[156,749] Centers used a variety of antiviral agents, although intravenous ganciclovir and/or valganciclovir were most commonly used, and the duration of therapy ranged from 3.5 weeks to indefinitely, but most used 12 weeks. In addition, many responding centers used CMV intravenous immunoglobulin (IVIG) with variable schedules, although the benefits of its use need further study.[72,557,745,748] Methods and regimens for viral monitoring also varied greatly. These surveys highlight the need for additional data, some of which is now available, including a randomized controlled trial comparing 3 versus 12 months of valganciclovir in adult lung transplant recipients.[465] This study and others provide evidence that for high-risk lung transplant recipients, universal prophylaxis and extending prophylaxis beyond 3 months to 12 months and possibly "indefinitely" has an overall beneficial effect in terms of CMV infection and its consequences.* In adult lung transplant recipients receiving valganciclovir prophylaxis, drug-resistant CMV infections, including some that resulted in death of the patient, were documented, although drug-resistant strains were not observed at higher rates than previously documented for lung transplant recipients.[635]

Although increasing the length of prophylactic antiviral therapy may decrease the incidence of CMV infection, additional complexity relates to the resultant immature T-cell response associated with long-term and potent antiviral suppression, whereas some "escape" may allow a protective T-cell response after therapy is discontinued.[579] In addition, the concern remains for the creation of drug-resistant CMV infection in the presence of long-term antiviral exposure. Rates of resistant CMV remain relatively low, in spite of broad use of antiviral agents for prophylaxis, although they are highest in lung transplant recipients among the solid-organ transplant recipients; this is believed to be secondary to intense immunosuppression and high viral load associated with lung tissue.[520,732] A study in high-risk adult renal transplant recipients documents a higher incidence of drug-resistant isolates in preemptive strategies, designed to decrease drug exposure.[145] This supposed contradiction may be explained because the higher viral loads were seen in these patients, because by definition they were viremic before antiviral agents were initiated and because of the use of oral valganciclovir, rather than intravenous therapy, during periods of high replication; both are risk factors for resistance.[210]

Several evidence-based consensus guidelines by expert advisory groups on the management of CMV infection in solid-organ transplant recipients have been published and are summarized here[309,358,747]:

1. All D+ or R+ lung transplant recipients should be considered for prophylaxis.

2a. Prophylaxis should continue for a minimum of 180 days, although some pediatric experts would limit this to 3 months, given the risk for catheter-associated infection if intravenous ganciclovir is used.

2b. The drug of choice for prophylaxis is intravenous ganciclovir or oral valganciclovir.

2c. Combination prophylaxis with CMV IVIG may be considered, if available, because some evidence suggests that its use may reduce infection rates further.

3a. Monitoring should be performed every 2 weeks for the first 6 months after transplantation to assess for breakthrough viremia and disease and then periodically for 8 to 12 weeks after completion of prophylaxis.

3b. Whole-blood quantitative PCR assay is the monitoring method of choice and should be validated at each center.

3c. If a preemptive approach is being used, CMV monitoring should be performed more frequently.

4. Breakthrough disease should be treated with ganciclovir 5 mg/kg administered intravenously every

*References 160, 236, 307, 463, 520, 533, 732, 748.

12 hours for up to 21 days, until the viral load is below detection. Immunosuppression should be reduced if possible.

5. Infection that occurs after the initial prophylaxis has been stopped should be treated with ganciclovir or valganciclovir until the viral load is below detection.

6a. Resistance should be considered in patients with breakthrough viremia, recurrent infections, or poor response to therapy. Genotypic analysis for common mutations (UL97 [viral kinase] and UL54 [DNA polymerase]) should be performed on isolates recovered from these patients.

6b. Foscarnet is the drug of choice, with or without ganciclovir, for possible resistant strains.

7. Reinitiation of prophylaxis should be considered in patients requiring antilymphocyte antibodies or high doses of corticosteroids for rejection.

Of note, valganciclovir is not FDA approved for prophylaxis in lung transplant recipients, although it is for other solid-organ transplant recipients, and available data suggest that it is safe and effective in lung transplant recipients as well.[307,463,520,732,747] Data in pediatric patients for the safety and efficacy of valganciclovir for prophylaxis and treatment are emerging. Some preliminary data on formulations and pharmacokinetics are available and support concerns for underdosing, leading to resistance and some hematologic toxicity.[103,435]

Epstein-Barr Virus/Posttransplant Lymphoproliferative Disorder

The disease manifestations of primary EBV infection in solid-organ transplant recipients range from uncomplicated mononucleosis (fever, malaise, exudative pharyngitis, lymphadenopathy, hepatosplenomegaly, atypical lymphocytosis) to conditions indistinguishable from malignant lymphoma. Organ-specific disease may include pneumonitis, hepatitis, as well as gastrointestinal, CNS, and hematologic involvement. Early lesions of EBV-driven lymphoproliferation, such as plasmacytic hyperplasia, are seen in mononucleosis-like syndromes, within the context of normal tissue architecture. The term *posttransplant lymphoproliferative disease* (PTLD) is generally reserved for proliferation of EBV-positive immunoblasts and atypical lymphocytes associated with effacement or destruction of normal tissue architecture. These disorders can be classified further into polymorphic or monomorphic PTLD and finally malignant lymphomas that may contain clonal chromosomal abnormalities. These tumors are characterized by rapid and progressive growth despite a reduction in immunosuppression. Fatal non-PTLD viral syndromes can occur in solid-organ transplant recipients as well. The reader is referred to several excellent, in-depth reviews and guidelines.[18,253,552,553,666]

The manifestation of EBV/PTLD is varied and depends on the site of involvement, which may be intrathoracic, extrathoracic (abdominal, head and neck, CNS), or disseminated and intranodal or extranodal.[392] Often, PTLD is seen in the allograft, as with lung, liver, and intestinal transplantation.[253] Signs and symptoms of PTLD can be nonspecific, such as fever of unknown origin, malaise, weight loss, and sore throat.[76] Abdominal pain, gastrointestinal bleeding, intestinal obstruction and perforation, allograft dysfunction such as changes in respiratory status in lung transplant recipients, diffuse lymphadenopathy on physical examination or CT, or hepatosplenomegaly should raise the index of suspicion for PTLD. Focal neurologic findings also can be seen with CNS disease. An additional challenge is to differentiate allograft rejection from PTLD; both entities in lung transplant recipients may be characterized by allograft dysfunction with diffuse consolidation on imaging, without obvious lymphadenopathy or a mass. Because the therapies for these two conditions are diametrically opposed, increasing or decreasing immunosuppression, this distinction obviously is critical.[253,553]

Risk factors for the development of PTLD have been identified.[18,76,134] Pretransplant EBV seronegativity (as a surrogate marker for the risk for development of posttransplant primary EBV infection) is probably the most important predisposing factor for the development of PTLD. Because primary EBV infection occurs almost universally by the time of adulthood, older individuals are already immune and young children undergoing transplantation are therefore at greatest risk. Presumably, it is the lytic phase of the primary EBV infection that sets the stage for PTLD, because the delayed EBV-specific cytotoxic T lymphocyte (CTL) response observed in immunocompromised children allows for high viral loads and uncontrolled B-cell infection, latency, and reactivation.[18] In addition, concomitant CMV infection or CMV mismatch before transplantation increases the risk for developing PTLD, contributing to the "net state of immunosuppression." Certain immunosuppressive regimens, such as the use of OKT3 and polyclonal antilymphocyte antibodies and possibly tacrolimus in pediatric patients, are linked to the development of PTLD, although it is likely that the overall intensity of immunosuppression, not a specific agent, is most important in defining the risk for development of PTLD.

Patients at highest risk by type of transplant are small bowel recipients, followed by recipients of hearts and lungs and livers. This risk is probably multifactorial and includes more intense immunosuppressive regimens and transplantation of large amounts of lymphoid tissue with the graft, thereby increasing recipients' potential exposure to donor-derived EBV. The published incidence of PTLD in pediatric lung transplant recipients ranges from 7.7 to 26.3 percent.[76,134,545] PTLD most often occurs within 12 months after transplantation, with median onset of primary infection at 6 weeks and symptoms presenting in the 2- to 3-month range.[18,76] When late PTLD occurs, it is associated with older recipients, long duration of immunosuppression, EBV-negative disease, and poorer prognosis.[134,199,379]

The diagnosis of PTLD requires a high index of suspicion. The gold standard is histopathologic examination of tissue. Specimens should be processed by pathologists familiar with the morphologic classifications (e.g., phenotype, lineage, clonality) and ancillary tests that may be helpful, such as tissue staining for EBV-encoded RNA

2 by in situ hybridization (EBERS), which is more sensitive than in situ targeting viral DNA or the use of immunohistochemical staining of latent antigens (EBNA-1 and 2, LMP-1) and more specific than EBV DNA amplification; the expression of CD20, which has implications for therapy, should also be determined.[18,552] Imaging also can be used for presumptive diagnosis, to guide biopsy, and for follow-up.[545,607] In lung transplant recipients, CT of the chest may reveal discrete nodules, air space consolidation, or mediastinal lymphadenopathy. Extrathoracic involvement is less common but may include abdominal lymphadenopathy, liver and spleen lesions, and a thickened bowel wall. Head and neck imaging may reveal involvement of cervical lymph nodes, pharynx, orbit, sinus, and rarely the brain. Fluorodeoxyglucose-labeled positron emission tomography combined with CT (FDG-PET/CT) in adult and pediatric solid-organ transplant recipients with PTLD may offer a more nuanced approach to diagnosis and staging and may be particularly helpful in management to distinguish between active versus necrotic/fibrotic lesions.[62,66,415]

Viral load determination (quantitative PCR) from blood should be sought in patients with a concern for PTLD. Although an elevated load may support the diagnosis, the value of a single measurement is uncertain; no diagnostic threshold level has been identified. In addition, these assays are not standardized, and many questions remain regarding their use, including which compartment is most revealing and should be sampled (peripheral blood lymphocytes, whole blood, or serum), extraction methods, thresholds for initiation of therapy, and the effect of serostatus on the sensitivity and specificity of viral loads.* Because of significant interlaboratory variability, longitudinal testing of patient samples should be performed at a single site. Finally, not all patients with EBV/PTLD have elevated viral loads and viral loads may be elevated without evidence of disease; therefore, PCR assays lack sensitivity and specificity for PTLD.[18,65] Although these assays are now widely used at transplant centers, further controlled studies are necessary to optimize their use in immunocompromised patients.

Patients at the highest risk for PTLD and mortality are those in whom primary EBV infection develops early after transplantation; therefore, every effort should be made to identify these patients. Pretransplant serologic assessment of donors and recipients for both EBV and CMV can alert physicians to most of these high-risk patients, because donor/recipient mismatch of either virus appears to play an important role in risk stratification.[76,134] Once identified, these patients should be monitored by PCR assay for evidence of primary infection, and careful examination and use of CT for early diagnosis of disease should be included. Several strategies have been used for the prevention of EBV/PTLD in high-risk patients.[253,553] The use of EBV-negative donors, although probably effective, would reduce the donor pool substantially and generally is not advocated. The use of prophylactic antiviral therapy has not been proved to prevent the development of PTLD. Although these agents are active against the lytic viral infection that precedes PTLD, they are not active against the latent viral phase that characterizes PTLD. Theoretically, antiviral agents may decrease the number of newly infected cells, thus impacting viral loads. Acyclovir and ganciclovir are often used for prevention of CMV, and comparisons of the incidence of PTLD in certain high-risk patients receiving these antiviral agents suggest some benefit; however, PTLD has developed in some patients while receiving these agents.[161,223] A small prospective controlled study of high-risk (D+/R–) pediatric renal transplant recipients found valganciclovir prophylaxis to significantly reduce the incidence of primary EBV infection, lower viral loads in those who became infected, and less symptomatic disease associated with infection but did not investigate the link to PTLD.[292] The use of IVIG or CMV IVIG is similarly a potential, but unproven strategy.[251,253,306] Given insufficient evidence, universal and routine use of antiviral agents and IVIG products for prophylaxis and preemption has not been endorsed by the American Transplant Society.[18]

Preemptive strategies aimed at identifying primary infection by blood PCR monitoring have received significant attention.* In these protocols, high-risk patients undergo EBV quantitative viral load monitoring at frequent intervals (usually weekly) to identify increases associated with infection, which is a prerequisite for the development of EBV/PTLD. Patients with a persistently high viral load should be evaluated aggressively for the presence of PTLD on examination and imaging, and suspicious lesions should be sampled. When the diagnosis of PTLD is made, immunosuppression should be decreased as much as feasible, the optimal duration of which is uncertain.[18,383] Most patients with nonmalignant lesions will respond to this maneuver alone. Return to higher levels of immunosuppression is usually prompted by rejection or clear evidence of clinical and virologic response. The use of antiviral agents in this setting has become routine, although without proven benefit.[18] Surgical resection and local radiation therapy may be beneficial for localized disease and in the event of gastrointestinal involvement. Characterization and outcome of patients with chronic high viral loads, without evidence of PTLD, is emerging. These studies suggest that up to 45 percent of patients will progress to PTLD up to 8 years after transplant.[65] Efforts to immunologically define and identify this high-risk group are ongoing.[410,411] Risk factors for poor outcomes include poor performance status, multisite disease, CNS involvement, T or NK cell– and EBV-negative PTLD, monoclonal disease, and presence of certain oncogenes or tumor suppressor genes.[18]

Monoclonal B-cell antibody therapy has become an attractive option for $CD20^+$ lesions not responsive to a reduction in immunosuppression.[18,553] The currently available product is an anti-CD20 antibody (rituximab). Reports document initial response rates of 40 to 60 percent, although relapses are not uncommon.† A

*References 18, 249, 252, 337, 377, 552.

*References 18, 48, 56, 198, 249, 253, 552, 572, 652, 678.
†References 67, 128, 129, 249, 460, 502.

PTLD-specific prognostic index identified high-risk patients with a 0 percent 2-year survival, in contrast to 50 percent in intermediate and 88 percent in low-risk adult patients receiving rituximab for PTLD; this highlights the potential heterogeneity of the patients and outcomes.[129] These high-risk patients all required additional chemotherapy, and 55 percent died of PTLD. Although generally well tolerated, long-term hypogammaglobulinemia is a common occurrence in recipients, and viral complications, such as severe CMV and hepatitis B and C infections, as well as parvovirus B19–induced aplasia and enteroviral meningoencephalitis, have been associated with its use.[553] For refractory disease, cytotoxic chemotherapy may be indicated, in consultation with oncology colleagues. The preliminary data on the use of adoptive immunotherapy (autologous and allogeneic EBV-specific cytotoxic T lymphocytes) show promising expansion, persistence, and efficacy of cytotoxic lymphocytes.[277,593] The generation of calcineurin-resistant T-cell clones is expected to improve the performance of cytotoxic lymphocyte therapy in the presence of ongoing immunosuppression.[84] Other investigational therapies include immunomodulatory therapy (anti–interleukin-6, Th1 response with IFN-α), interruption of viral signaling pathways (LMP1, LMP2a), and vaccine technology.[18,249,278,553]

Other Herpesviruses: Herpes Simplex Viruses Types 1 and 2, Varicella-Zoster Virus, Human Herpesviruses Types 6, 7, and 8

Infections with herpes simplex virus (HSV) types 1 and 2 are common occurrences in solid-organ transplant recipients; and in older children and adults, HSV usually is a reactivation disease manifested as orolabial or genital disease. Primary disease occurs in young children who contract the infection from viral shedding of close contacts. Serostatus of transplant candidates should be determined because symptomatic reactivation occurs in up to 68 percent of seropositive solid-organ transplant recipients not receiving prophylaxis and naïve recipients are at risk for severe and prolonged primary infections.[750] In lung transplant recipients, serious or disseminated disease such as esophagitis, hepatitis, and pneumonitis can occur.[633] Infections tend to occur early, within the first month after transplantation, unless prophylaxis is used. As with other herpesviruses, concomitant infection with CMV is not an uncommon event. The diagnosis can be made clinically when typical lesions are present. Culture and PCR technology can confirm the diagnosis and be helpful for unusual manifestations. PCR has advantages of rapidity, increased sensitivity, and identifying HSV in blood and other body fluids. Prophylactic strategies with acyclovir or ganciclovir used for CMV (3 to 6 months) can prevent HSV. Generally, prophylaxis for HSV infection does not need to be continued past the early transplant period unless there are frequent clinical recurrences, in which case suppressive antiviral therapy should be continued until reduction of immunosuppression can occur. Occasionally, lifelong suppression may be necessary. Intravenous acyclovir and oral valacyclovir or famciclovir are effective therapies for localized mucocutaneous disease. More severe disease should be treated with intravenous formulations until resolution of symptoms. Resistance of HSV in solid-organ transplant recipients is rare, but when it is suspected in instances of clinical failure, culture and susceptibility should be performed. Most resistance results from changes in the viral thymidine kinase gene, which is necessary to initiate the triphosphorylization for active antiviral activity (inhibition of DNA chain elongation).[548] Foscarnet, which does not require phosphorylization, is usually the drug of choice. Less frequent mutations with altered DNA polymerase may require cidofovir. A new lipid ester of cidofovir, CMX001, which has broad and potent antiviral activity and less toxicity, is currently in phase 3 trials for treatment of several DNA virus infections.[554]

Primary varicella-zoster virus (VZV) infection can occur in pediatric lung transplant recipients, as can reactivation of previous disease leading to herpes zoster. The rates of VZV infections in solid-organ transplant recipients are not known, but presumably the infection rate after transplantation is low, since the widespread use of varicella vaccine (Varivax), as it is in the general community.[27] Because VZV infection is a vaccine-preventable disease and complications of primary infection can be life threatening, all patients should be screened for evidence of immunity, and seronegative transplant candidates should receive varicella vaccine.[4,157,539] Available data demonstrate that this vaccine is safe and effective in this setting, although seroconversion rates may be reduced. Optimally, two doses 4 to 6 weeks apart and a minimum of 2 to 4 weeks before transplant should be administered. If the vaccine does not prevent disease, it should mitigate development of severe disease. In addition, susceptible family members also should receive the vaccine. The zoster vaccine (Zostavax) has a 14-fold higher virus titer and theoretically could pose a higher risk for complications in immunocompromised patients.[130,157] It is indicated for patients older than 60 years of age with a history of VZV infection. It may be considered in adult transplant candidates with serologic evidence of VZV at least 2 months before transplant; there is no indication for pediatric patients. Exposure in posttransplant patients should be treated with a high-titer varicella immunoglobulin preparation if available (VZIG is no longer manufactured; VariZIG remains unlicensed and available through IND application only); IVIG is recommended based on the "best judgment of experts" if high titer preparations are not available, although no clinical data document its efficacy. The window for benefit of administration of immunoglobulin preparations has been extended from 4 to 10 days after exposure.[27,111] Additionally, some experts recommend the use of prophylactic acyclovir if exposure such as household or intimate contact is likely to lead to development of disease or is revealed past the window for immunoglobulin prophylaxis to be effective. Varicella vaccine, which is an attenuated live viral product, generally is not recommended in posttransplant recipients at this time. Limited data suggest that it is safe and may ameliorate disease in some immunocompromised patient populations, such as those with leukemia, HIV infection, or renal transplants.[94] VZV infection in lung transplant recipients should be

treated with intravenous acyclovir until all lesions are crusted and the patient's symptoms have lessened.[539] Patients taking corticosteroids, especially during the incubation period, are at increased risk for the development of severe disease. Hemorrhagic and disseminated disease, including encephalitis, hepatitis, and pneumonitis, can occur in immunocompromised patients. All patients admitted to the hospital with VZV infections should be placed in combined airborne precautions; immunocompromised patients may shed virus for a prolonged period. Hospitalized lung transplant recipients who have received immunoglobulin postexposure prophylaxis should remain in isolation from day 10 to day 28.[539]

Herpes zoster is a common event in adult transplant recipients; rates in children have not been published for solid-organ transplant recipients. A study of solid-organ transplant recipients aged 16 to 74 years found a 15 percent incidence of herpes zoster in lung transplant recipients, with a mean time to onset of 14 months and a median of 9 months (9 days to 5.8 years).[247] The diagnosis is made clinically and with the use of viral culture or PCR techniques if necessary. Infections can become disseminated, as evidenced by multiple dermatomal spread, and should be treated with intravenous acyclovir and pain control. The use of corticosteroids should be decreased, if possible.

HHV-6, variants A and B, the latter of which is most commonly implicated in human disease, is an emerging opportunistic viral pathogen. Seropositivity reaches nearly 100 percent in early childhood, and HHV-6 is responsible for febrile illnesses with or without gastrointestinal or upper respiratory tract symptoms, febrile seizures, roseola (exanthema subitum, sixth disease), and asymptomatic infection in normal infants and young children.[19] Therefore, disease in all but the very youngest transplant recipients probably represents reactivation. Several studies have documented HHV-6 by PCR assay or culture in a majority (66% to 90%) of apparently asymptomatic adult lung transplant recipients.[327,386] Infections occur early after transplantation (median of 6 and 18 days) and can occur in the setting of CMV antiviral prophylaxis. Most are without obvious clinical manifestations that could be ascribed to HHV-6 alone.[559] HHV-6 DNA can be recovered from BAL fluid in 20 percent of lung transplant recipients and often is found with other pathogens, including other herpesviruses.[378] In immunocompromised patients, HHV-6 may exert direct and indirect effects on the host. It probably is responsible for rare cases of encephalitis, hepatitis, pneumonitis, febrile illnesses, and bone marrow suppression.[386] The presence of HHV-6 alone or as a cofactor to CMV may augment the immunomodulatory effects and increase the risk associated with fungal and other infections, as well as allograft rejection, bronchiolitis obliterans syndrome, and mortality.[327,378,450,490,559]

Many questions regarding HHV-6 in this population remain unanswered. PCR assay of peripheral blood lymphocytes is the most sensitive method for detection of virus, but it cannot differentiate between latent and active virus. Routine monitoring of asymptomatic patients is not recommended. Data are insufficient to recommend prophylactic or preemptive therapies, although the virus is susceptible to achievable levels of ganciclovir, foscarnet, and cidofovir. Treatment with these drugs may be considered in patients with compatible syndromes if other causes are eliminated.[559] Whether asymptomatic patients with documented HHV-6 infection would benefit from therapy with regard to the indirect effects of the virus remains unknown. Finally, HHV-6, unique in the herpesvirus family, can rarely be chromosomally integrated as a mechanism of latency (CIHHV-6).[674] It is estimated to occur in 0.8 percent of the normal population. Because the viral DNA integrates into every nucleated human cell, CIHHV-6 is characterized by extremely high viral loads (>1 million copies/mL blood), which may not indicate active infection. This has been described in solid-organ transplant recipients, although the significance of this occurrence is unclear.[382] Although some patients received antiviral therapy because of the concern of high titers, none had perceivable decline in their viral loads and those who did not receive therapy remained clinically asymptomatic. CIHHV-6 may lead to unnecessary antiviral therapy.

The role of HHV-7 and HHV-8 in solid-organ transplant recipients is undefined. HHV-7 behaves in a similar manner as HHV-6 in normal hosts, although it appears to be less prevalent and occurs later in life. Symptomatic disease is less well characterized, but it may be responsible for similar syndromes seen with HHV-6 in normal children and transplant recipients.[559] Evidence of HHV-8 infection in normal children is rare. Seroprevalence rates have wide geographic variation from 0 to 5 percent in North America to more than 50 percent in parts of Africa and thus may have increased relevance in transplant recipients from certain countries.[471] It is associated with Kaposi sarcoma in immunocompromised patients, as well as body cavity lymphomas, PTLD, and Castleman disease.[20,559] It also may be associated with fever and bone marrow suppressive syndromes of donor origin in transplant recipients.[409]

Community-Acquired Respiratory Viruses: Respiratory Syncytial Virus, Parainfluenza Virus, Human Metapneumovirus, Influenza, and Adenovirus

The paramyxoviruses respiratory syncytial virus (RSV), parainfluenza virus (PIV), and human metapneumovirus (hMPV) are common causes of upper and lower respiratory tract disease in normal children; symptoms range from congestion and rhinorrhea to laryngeal tracheobronchitis (croup), bronchiolitis, and pneumonia.[22-24] Similar clinical syndromes can be caused by a variety of viruses, and lung transplant recipients may have atypical presentation; thus testing should remain broad until a diagnosis is made.[324] Data on the impact of these viruses on lung transplant recipients are emerging. Respiratory viral illness is encountered commonly after pediatric lung transplantation and can be severe and persistent. Coinfection and sequential infection with other respiratory viruses, CMV, and fungal pathogens occurs.[40,237] A decreased 1-year survival was associated with respiratory viral illness, but not at 2 years in retrospective studies of

pediatric lung transplant recipients.[400,401] Neither study found an association with bronchiolitis obliterans syndrome, although others have.[347] Risk factors for respiratory viral illness included young age and CMV infection. Transmission occurs person to person, from exposure to infected nasopharyngeal secretions, and from fomites. Consideration should be given to screen recipients for incubating respiratory viruses before they undergo transplantation, especially if high levels of circulating virus are present in the community or illness is present in family members. Immunocompromised patients can shed infectious virus for long periods. Strict adherence to hospital infection control practices is most important in reducing nosocomially acquired disease from infected patients, visitors, and health care workers.[324] Patients and their families should be counseled on avoidance of exposure and on good hand washing practices when discharged from the hospital.

RSV can cause severe lower respiratory tract disease in lung transplant recipients. Although most infections resolve, with or without therapy, fatal cases have been reported, as have long-term declines in pulmonary function.[64,237,444,719] The diagnosis can be made from nasal washings by rapid antigen-detection kits, standard viral culture, or more rapid shell vial culture methods, although PCR-based assays, which provide sensitive detection of a broad range of viral pathogens, are now considered the preferred method.[324] Risk factors for poor outcome in immunocompromised patients include neutropenia, lymphopenia, age younger than 1 year, underlying lung disease, and augmented immunosuppression. High mortality rates in hematopoietic stem cell transplant recipients have led to prevention and treatment strategies that can serve as models for care in solid-organ transplant recipients,[176,238,727] although they are not without controversy.[64,241] Supportive care and reduction in immunosuppression remain the mainstay of therapy. For children with upper tract disease and risk factors or lower tract disease, aerosolized ribavirin should be considered. In addition, because outcomes, even in treated hematopoietic stem cell transplant recipients, are poor with ribavirin alone, some experts suggest combination therapy with RSV IVIG or monoclonal antibody (palivizumab) for immunocompromised patients with significant infections.[64,664] More recently, published reports using oral ribavirin, which is considerably easier and less expensive to use, suggest successful treatment.[391,536] Effective prophylaxis with palivizumab and RSV IVIG has been achieved in young infants with lung disease.[263,449] Care must be taken when extrapolating to other patient populations, given the unexpected poor outcomes of some infants with complex congenital heart disease who were administered RSV IVIG[611] but not pavilizumab.[204] Some experts would consider the use of immunoprophylaxis in young infants undergoing organ transplantation during the RSV season.[324]

hMPV was described in healthy young children in 2001.[684] It appears to have signs and symptoms similar to those of RSV, and almost universal infection probably occurs by 5 years of age.[733] The impact of hMPV on immunocompromised patients is as yet uncharacterized, in part because diagnostic tests have only recently become widely available. The virus can be cultured, but reverse transcriptase PCR appears to be the test of choice and is available as part of a PCR respiratory viral panel in most centers. Antigen assays are not yet available.[22] Studies on adult lung transplant recipients demonstrate that hMPV respiratory viral illnesses are common, often with coinfections and associated with graft dysfunction/acute rejection, but less likely than RSV infections to be associated with chronic graft rejection and bronchiolitis obliterans syndrome.[237,299,375,716] There is no established therapy for hMPV infections, outside of supportive care and treatment of co-pathogens, although ribavirin and IVIG are sometimes utilized.[299,324,723]

PIV (types 1 to 4) is isolated frequently as a cause of upper respiratory tract infections and croup in normal children.[23] PIV can cause severe lower tract infection in immunocompromised patients, including respiratory failure in lung transplant recipients, most notably caused by serotype 3.[33,694,697,720] Although rarely fatal in adults, persistent declines in pulmonary function and acute allograft rejection are important consequences of PIV infection.[444,694,720] A retrospective study on PIV in pediatric solid-organ transplant recipients (after liver, small bowel, lung, heart, kidney transplants) found significant age-related morbidity and mortality.[33] Although 44 percent of all patients with PIV infection had upper respiratory tract infections, only 11 percent of children younger than 1 year old had limited disease. A 16 percent mortality rate was associated with PIV, and predictors of mortality included age younger than 6 months, infection occurring less than 3 months after transplantation, and augmented immunosuppression. Infection in these children occurred in the context of community outbreaks. The diagnosis can be made by PCR assays and antigen detection; culture isolation can take weeks. Respiratory secretions obtained from nasal washings or BAL fluid can be used.[64] No proven therapy for PIV in lung transplant recipients is available. Intravenous and aerosolized ribavirin has been used in immunocompromised hosts, but without randomized controlled data or clear evidence of efficacy.[64,324,444]

Despite available immunoprophylaxis and chemoprophylaxis, influenza viruses A and B remain an important cause of morbidity in the normal population and lead to a considerable rate of mortality in the very young and the elderly.[21] In addition to the usual symptoms of acute onset of malaise, fever, myalgias, and respiratory symptoms, gastrointestinal symptoms also may be prominent.[227] Transplant recipients are at risk for acquiring community-acquired infection during the yearly epidemics, as well as nosocomial infection from visitors, hospital workers, and other transplant recipients as a result of infections occurring in the inpatient setting.[426,575,695] Seasonal influenza infection is relatively rare in solid-organ transplant recipients—4.2 percent of adult lung transplant recipients over the course of a 10-year period and 2.6 percent of pediatric solid-organ transplant recipients over a similar period[33,64]—but lung transplant recipients appear to be at a uniquely high risk for acquiring infection. In the adult study, the incidence was 41.8 cases per 1000

person-years for adult lung transplant recipients versus 2.8 and 4.3 per 1000 person-years in liver and kidney transplant recipients, respectively.[698] The authors speculate that optimal protection from influenza infection afforded by serum and secretory antibodies in the respiratory tract are altered in lung transplant recipients, thereby leading to higher attack rates. In addition, risk for disease and complications are high for pediatric transplant recipients in particular.[324] Previous data suggested that upper respiratory tract symptoms were the most common presentation of pediatric solid-organ transplant recipients with influenza, although 3 of 13 patients (23%) died.[33] In these children, corticosteroid bolus and OKT3 therapy given within a week of diagnosis of infection were associated with mortality. Transplant recipients may be at increased risk for acquiring secondary bacterial infections and nonpulmonary complications such as hepatitis, myocarditis, and aseptic meningitis.[64,698] Influenza was the causative agent in 8.9 percent of 101 respiratory viral illnesses documented in a series of pediatric lung transplant recipients observed between 1998 and 2007.[401]

Although we have an incomplete picture of the current epidemiology of influenza infections in pediatric lung transplant recipients, the H1N1 epidemic of 2009-2010 has added to our understanding of its impact. Risk factors for hospitalization and severe disease included age younger than 5 years and immunosuppression.[741] Two studies on adult lung transplant recipients suggest the majority of patients did well, but with a wide range of clinical impact from upper respiratory tract symptoms, association with progression to bronchiolitis obliterans syndrome, mechanical ventilation, and mortality (0%, 21%).[216,491] In a retrospective series of solid-organ transplant recipients (adults and children) with H1N1 infection, children were more likely to have fever, rhinorrhea, sore throat, and headache and less likely to have chest radiographic evidence of lower tract disease.[368] Of note, almost 50 percent of all patients had gastrointestinal complaints. In the pediatric cohort, mortality was 0 percent versus 7 percent in adults, with similar rates of admission (66%, 73%) and intensive care unit care (12%, 17.5%). Children were more likely to receive antiviral therapy within 48 hours.

Infection with the influenza virus is unique among respiratory virus infections in that both vaccine and chemoprophylactic strategies are available. Unfortunately, immunosuppressed patients may not respond well to influenza vaccine and, if disease is not recognized promptly, the effectiveness of therapy for established disease may not be optimal. Studies on the immunogenicity of influenza vaccine in pediatric solid-organ transplant recipients are more encouraging than in adult reports, which have documented poor response, especially when associated with mycophenolate mofetil or sirolimus.[94,281] In pediatric renal transplant recipients, response was similar to that of normal controls, without evidence of rejection,[196,225] although in pediatric heart transplant recipients protective antibody responses were not achieved until three doses of vaccine were administered.[8] No conclusive evidence documents a link between immunization and graft dysfunction with the intramuscular trivalent inactivated influenza vaccines, which have been used routinely for decades.[364] The current recommendation is that influenza vaccine be administered yearly to all solid-organ transplant recipients older than 6 months of age and 3 to 6 months after transplantation and to those awaiting transplantation, as well as to household contacts and caretakers.[21,94,364] During the 2009 H1N1 pandemic, recommendations for immunization were reduced to 1 month after transplant, with the acknowledgment that only partial protection was likely.[369] Children younger than 9 years of age receiving influenza vaccine for the first time should have two doses 4 weeks apart. The use of live attenuated influenza vaccine and intradermal and adjuvanted vaccines is not recommended. Oseltamivir chemoprophylaxis can be administered to patients who have not been vaccinated or who have not had sufficient time to respond to vaccination, especially if influenza is circulating in the community or if there is a known exposure. Dosing for young children and infants is now available.[325,343,369] Other agents approved for prophylaxis of influenza A in children older than 1 year include amantadine and rimantadine (M2 inhibitors), although resistance of influenza A is high and they are not active against influenza B strains.[21,695]

The diagnosis of influenza should be made by real-time PCR or other nucleic acid–based detection whenever possible. Multiplex PCR assays for respiratory viruses are now routinely available at most centers.[324,369] Rapid antigen testing is also available, but performance characteristics, especially during the 2009-2010 H1N1 pandemic, suggest this method is less reliable.*

Most experts would recommend empirical therapy with antiviral agents in a lung transplant recipient with suspected (community epidemic with compatible symptoms) or proven influenza, regardless of duration of symptoms (e.g., >48 hours).[64,326,365] During the 2009-2010 H1N1 pandemic, early therapy was associated with improved outcome (decreased hospitalization, intensive care unit, mortality) in high-risk patients as well as normal hosts.[368,741] The choice of agent depends on the type of virus (A or B) and information about resistance. The neuraminidase inhibitors oseltamivir and zanamivir are currently favored because of their broad spectrum of activity and less reported resistance.[324,695] Oseltamivir-resistant H1N1 retained susceptibility to zanamivir.[369] In addition, higher doses and prolonged therapy may be necessary, because the viral burden and length of shedding are increased in transplant recipients and children. Optimal length of therapy is not known; consideration for continuation of therapy until PCR testing is negative must be countered by the concern for emergence of viral resistance. Two intravenous antiviral agents, peramivir and zanamivir, as well as combination therapies are undergoing investigation and/or available for compassionate use from the FDA.[369] Choice of antiviral therapy should be made after consultation with the CDC and public health officials.

With improved management of herpesvirus infections and better diagnostic techniques, the impact of

*References 122, 137, 221, 240, 367, 371, 668.

adenoviral infections in transplant recipients is increasingly recognized. Adenoviral infections in solid-organ transplant recipients follow the epidemiology of infection in the general population, such that infections are more common in young pediatric than adult transplant recipients, who may remain naïve to many of the 52 recognized serotypes.[164,294,455,504] Other risk factors for infection include exposure to lytic antibodies, high levels of maintenance immunosuppression, and adenovirus serologic mismatch. Establishment of latency in lymphoid tissue has been documented, and numerous immune evasion genes have been identified.[294] Adenoviral infections have multiple putative sources, including the donor organ, reactivation from latency in host tissues, and infection acquired from the community or nosocomially.*

Infections in lung transplant recipients may be asymptomatic, lead to recognizable syndromes of the respiratory and/or gastrointestinal tracts, or cause disseminated disease, defined as involvement in two or more organs. Often disease is most pronounced in the allograft (e.g., pneumonitis, hepatitis, enteritis, hemorrhagic cystitis).[294] Cases of acute, disseminated, and fatal disease have been described in cardiothoracic transplant recipients. In lung transplant recipients, infections occur in the early post-transplant period and often cause disease in the graft in the form of necrotizing pneumonia.[86,504] Adenoviral infection has been associated with graft dysfunction, bronchiolitis obliterans syndrome, retransplantation, and death.[86]

Available diagnostic methods for the detection of adenovirus include culture, direct identification of antigens, and serology, as well as histologic examination of tissues for the presence of adenoviral inclusions and immunohistochemical staining.[126,294] Viral isolation by culture can be expedited by centrifugation in shell vial assays and immunofluorescent staining with adenoviral monoclonal antibodies, although viral serotyping cannot be done with this method. Direct identification of adenoviral antigens, usually performed on respiratory specimens, can be achieved by radioimmunoassay, immunofluorescence, or ELISA techniques, which are rapid and specific but less sensitive than culture.

Detection of virus with culture techniques and direct antigen detection are insensitive in identifying patients at risk for the development of disseminated disease. In addition, cultures may take a week or longer, and neither method may detect low levels of circulating virus. PCR assay has emerged as a powerful tool for detection of adenovirus in body fluids and tissues and for serotyping.† The virus's ability to establish latency can render interpreting the presence of virus or viral DNA in clinical specimens a challenge.[230,388] A single determination of the presence of adenovirus by PCR assay in immunocompromised patients may be nonspecific and perhaps misleading and should be correlated with histopathology if available and appropriate clinical syndromes to differentiate between asymptomatic infection and disease. As with other important viruses that establish latency, quantitative viral load patterns appear to be more informative with regard to the pathogenic role of adenovirus in these patient populations. No threshold has been identified to predict progression of infection or outcome. Studies in pediatric hematopoietic stem cell transplant recipients support the use of blood PCR surveillance as a method to identify patients at risk for disseminated disease.* Although routine surveillance is not recommended for lung transplant recipients, its use for early detection and monitoring in certain high-risk patients may be advocated.

The optimal therapy for adenovirus infection has not been determined. Supportive care and reduction of immunosuppression are the mainstays of therapy.[294] The role of immune recovery should not be underestimated, of which adenovirus-specific cytotoxic T cells are particularly critical.[114,284] Cidofovir, an acyclic nucleoside phosphonate, inhibits viral DNA polymerase with activity against all serotypes. It may be beneficial in severely ill patients with disseminated disease and rising viral loads.† Use of this agent may be associated with significant renal toxicity,[283,385,550] and use of modified dosing regimens has been investigated with some success.[106,295,481,705] CMX001 is an oral lipid ester of CDV with broad-spectrum antiviral activity that achieves very high intracellular antiviral activity and improved toxicity profile. It has been used for salvage therapy for severe adenoviral infection in immunocompromised patients and appears promising.[213] Strategies involving adoptive immunotherapy, such as infusion of adenovirus-specific cytotoxic lymphocytes that has been investigated in adenoviral infections in hematopoietic stem cell transplant recipients, may also be feasible,[206] with proof of concept for cytotoxic lymphocyte therapy published for solid-organ transplant recipients and EBV infections.[84,277,593]

Although a detailed discussion is beyond the scope of this chapter, the link between respiratory viruses and bronchiolitis obliterans syndrome or chronic allograft dysfunction should be addressed briefly. Bronchiolitis obliterans syndrome is a progressive condition, without good treatment options, and the greatest impediment to long-term survival for lung transplant recipients. It affects 50 percent of patients alive 5 years after transplantation and is the leading cause of death in adult and pediatric lung transplant recipients 1 year after transplantation.[677,714] Although the exact mechanisms are still being determined, the final common pathway is epithelial injury and intraluminal proliferation of fibroblasts, leading to airflow obstruction.[318,341,693] Stimulation of T cells and release of cytokines and chemokines by viruses are leading candidates for the initiation of these pathways. Many studies have linked viral infections, such as those caused by CMV, adenovirus, RSV, PIV, and others, often working in concert, to the development of bronchiolitis obliterans syndrome.‡ The role of these agents has yet to be proved, although it is biologically plausible.[318] Reduction of bronchiolitis obliterans syndrome remains an incentive for the continued development of preventive strategies against these viral infections.

*References 104, 294, 350, 446, 455, 547.
†References 194, 266, 285, 294, 305, 387, 398, 596.

*References 133, 194, 266, 285, 294, 387, 396, 596, 706.
†References 106, 293, 295, 385, 404, 705, 743.
‡References 21, 203, 366, 519, 694, 697.

Zoonoses and Donor-Derived Infections: Rabies, West Nile Virus, Lymphocytic Choriomeningitis Virus, and *Bordetella*

Isolation of unusual pathogens often is a sign of an unexpected exposure.[47,580] This statement is epitomized by the recent reports of solid-organ transplant recipients with uncommon zoonotic infections.[359] In the case of donor-derived rabies, lymphocytic choriomeningitis virus, and West Nile virus infections, multiple cases were traced to a donor or donors with retrospectively assessed exposures and risk factors.[207,287,326,643] West Nile virus in solid-organ transplant recipients also has been community acquired,[345,370] and cases of infection with *Bordetella bronchiseptica* in transplant recipients have been traced to sick pet dogs.[54,132,491] These cases are humbling in multiple respects. Many of these infections have no established therapy and, as expected, are associated with increased morbidity and mortality in solid-organ transplant recipients.[169,207,345,370,489] Therefore, prevention is of utmost importance. These cases advise us to counsel our post-transplant recipients on avoidance of potentially infectious exposures, such as travel, sick pets, and arthropod vectors of diseases.[357,365,489] Undeniably, they remind us of the almost limitless pathogens that may befall solid-organ transplant recipients, including those not yet described. These cases have newly informed the policies for reporting suspected donor transmitted conditions and opened a new dialogue on how screening procedures of organ and blood donors are conducted.*

NEW REFERENCES SINCE THE SIXTH EDITION

3. Abel S, Allan R, Gandelman K, et al. Pharmacokinetics, safety and tolerance of voriconazole in renally impaired subjects. *Clin Drug Invest* 2008;**28**(7):409–20.
4. Abuali MM, Arnon R, Posada R. An update on immunizations before and after transplantation in the pediatric solid organ transplant recipient. *Pediatr Transplant* 2011;**15**:770–7.
6. Adetifa IMO, Ota MOC, Jeffries DJ, et al. Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent *Mycobacterium tuberculosis* infection in childhood contacts in the Gambia. *Pediatr Infect Dis J* 2010;**29**(5):439–43.
7. Adler FR, Aurora P, Baker DH, et al. Lung transplantation for cystic fibrosis. *Proc Am Thorac Soc* 2009;**6**:619–33.
10. Alanio A, Desoubeaux G, Sarfati C, et al. Real-time PCR assay-based strategy for differentiation between active *Pneumocystis jirovecii* pneumonia and colonization in immunocompromised patients. *Clin Microbiol Infect* 2011;**17**:1531–7.
13. Alexander BD, Perfect JR, Daly JS, et al. Posaconazole as salvage therapy in patients with invasive fungal infections after solid organ transplant. *Transplantation* 2008;**86**(6):791–6.
14. Alexander BD, Petzold EW, Reller LB, et al. Survival after lung transplantation of cystic fibrosis patients infected with *Burkholderia cepacia* complex. *Am J Transplant* 2008;**8**:1025–30.
16. Alexander BD, Smith PB, Davis D, et al. The (1,3)-β-D-glucan test as an aid to early diagnosis of invasive fungal infections following lung transplantation. *J Clin Microbiol* 2010;**48**(11):4083–8.
18. Allen U, Preiksaitis J; The AST Infectious Diseases Community of Practice. Epstein-Barr virus and posttransplant lymphoproliferative disorder in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s87–96.
30. Andes D, Lepak A. Editorial commentary: Antifungal therapeutic drug monitoring progress: getting it right the first time. *Clin Infect Dis* 2012;**55**:391–3.
31. Andes DR, Safdar N, Hoffman J, Baddley JW. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clin Infect Dis* 2012;**54**(8):1110–22.
36. Ariza-Heredia EJ, Patel R, Blumberg RC, et al. Outcomes of transplantation using organs from a donor infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*. *Transplant Infect Dis* 2012;**14**:229.
37. Armenian SH, Nash KA, Kapoor N, et al. Prospective monitoring for invasive aspergillosis using galactomannan and polymerase chain reaction in high risk pediatric patients. *J Pediatr Hematol Oncol* 2009;**31**(12):920–6.
39. Arnold HM, Sawyer AM, Kollef MH. Use of adjunctive aerosolized antimicrobial therapy in the treatment of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ventilator-associated pneumonia. *Respir Care* 2012;**57**:1226.
41. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. *Clin Microbiol* 2011;**49**(2):665–70.
43. Baddley JW, Andes DR, Marr KA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. *Clin Infect Dis* 2010;**50**(12):1559–67.
44. Baddley JW, Andes DR, Marr KA, et al. Antifungal therapy and length of hospitalization in transplant patients with invasive aspergillosis. *Med Mycol* 2013;**51**(2):128–35.
45. Baddley JW, Schain DC, Gupte AA, et al. Transmission of *Cryptococcus* neoformans by organ transplantation. *Clin Infect Dis* 2011;**52**(4):e94–e98.
57. Benden C, Aurora P, Edward LB, et al. The Registry of the International Society for Heart and Lung Transplantation Report: Fourteenth Pediatric Lung and Heart-Lung Transplantation–2011. *J Heart Lung Transplant* 2011;**30**(10):1123–32.
58. Bergamasco MD, Barroso M, de Oliveira Garcia D, et al. Infection with Klebsiella pneumoniae carbapenemase (KPC)-producing *K. pneumoniae* in solid organ transplantation. *Transpl Infect Dis* 2012;**14**(2):198–205.
59. Berge M, Guillemain R, Boussaud V, et al. Voriconazole pharmacokinetic variability in cystic fibrosis lung transplant patients. *Transpl Infect Dis* 2009;**11**:211–9.
60. Bernhard S, Kernland Lang K, Ammann RA, et al. Voriconazole-induced phototoxicity in children. *Pediatr Infect Dis J* 2012;**31**(7):769–71.
62. Bianchi E, Pascual M, Nicod M, et al. Clinical usefulness of FDG-PET/CT scan imaging in the management of post-transplant lymphoproliferative disease. *Transplantation* 2008;**85**:707–12.
63. Billaud EM, Guillemain R, Berge M, et al. Pharmacological considerations for azole antifungal drug management in cystic fibrosis lung transplant patients. *Med Mycol* 2010;**48**(Suppl 1):S52–9.
65. Bingler MA, Feingold B, Miller SA, et al. Chronic high Epstein-Barr viral load state and risk for late-onset post-transplant lymphoproliferative disease/lymphoma in children. *Am J Transplant* 2008;**8**:442–5.
66. Blaes AH, Cioc AM, Froelich JW, et al. Positron emission tomography scanning in the setting of post-transplant lymphoproliferative disorders. *Clin Transplant* 2009;**23**:794–9.
68. Blair J. Approach to the solid organ transplant patient with latent infection and disease caused by *Coccidioides* species. *Curr Opin Infect Dis* 2008;**21**:415–20.
70. Blodget E, Geiseler PJ, Larsen RA, et al. Donor-derived *Coccidioides immitis* fungemia in solid organ transplant recipients. *Transpl Infect Dis* 2012;**14**(3):305–10.
72. Bonaros N, Mayer B, Schachner T, et al. CMV-hyperimmune globulin for preventing cytomegalovirus infection and disease in solid organ transplant recipients: a meta-analysis. *Clin Transplant* 2008;**22**:89–97.
73. Borro JM, Sole A, de la Torree M, et al. Efficiency and safety of inhaled amphotericin B lipid complex (Abelcet) in the prophylaxis of invasive fungal infections following lung transplantation. *Transplant Proc* 2008;**40**:3090–8.
74. Botterel F, Cabaret O, Foulet F, et al. Clinical significance of quantifying *Pneumocystis jirovecii* DNA by using real-time PCR in

*References 179, 207, 211, 265, 304, 342.

bronchoalveolar lavage fluid from immunocompromised patients. *J Clin Microbiol* 2012;**50**(2):277–31.
75. Boussaud V, Guillemain R, Grenet D, et al. Clinical outcome following lung transplantation in patients with cystic fibrosis colonized with *Burkholderia cepacia* complex: results from two French centuries. *Thorax* 2008;**63**:732–7.
80. Brass EP, Edwards JE. Should the guidelines for management of central venous catheters in patients with candidemia be changed now? *Clin Infect Dis* 2010;**51**(3):304–6.
84. Brewin J, Mancao C, Straathof K, et al. Generation of EBV-specific cytotoxic T cells that are resistant to calcineurin inhibitors for the treatment of posttransplantation lymphoproliferative disease. *Blood* 2011;**114**(23):4792–803.
87. Bruggemann RJM, Donnelly JP, Aarnoutse RE, et al. Therapeutic drug monitoring of voriconazole. *Ther Drug Monit* 2008;**30**(4): 403–11.
91. Bumbacea D, Arend SM, Eyuboglu F, et al. The risk of tuberculosis in transplant candidates and recipients: a TBNET consensus statement. *Eur Respir Soc* 2012;**40**(4):990–1013.
92. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics* 2008;**121**:e1286–e1294.
96. Bwanga F, Hoffner S, Haile M, et al. Direct susceptibility testing for multi-drug resistant tuberculosis: a meta-analysis. *BMC Infect Dis* 2009;**9**:67.
103. Camacho-Gonzalez AF, Gutman J, et al. 24 weeks of valganciclovir prophylaxis in children after renal transplantation: a 4-year experience. *Transplantation* 2011;**91**(2):245–50.
105. *Canadian Cystic Fibrosis Patient Data Registry Report 2010-Cystic Fibrosis Canada*. 2010. Available at www.cysticfibrosis.ca.
110. Centers for Disease Control and Prevention. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *MMWR Morb Mortal Wkly Rep* 2009;**58**(1):7–10.
111. Centers for Disease Control and Prevention. FDA approval of an extended period for administering VariZIG for postexposure prophylaxis of varicella. *MMWR Morb Mortal Wkly Rep* 2012; **61**(12):212.
112. Chai LYA, Kullberg BJ, Johnson EM, et al. Early serum galactomannan trend as a predictor of outcome of invasive aspergillosis. *J Clin Microbiol* 2012;**50**(7):2330–6.
113. Chaiyasirinroje B, Nyein Aung M, Moolphate S, et al. Prospective evaluation of simply modified MODS assay: an effective tool for TB diagnosis and detection of MDR-TB. *Infect Drug Resist* 2012;**5**:79–88.
121. Chapman SW, Dismukes WE, Proia LA, et al. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2008; **46**:1801–12.
122. Chartrand C, Leefland MM, Minion JB, et al. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med* 2012; **156**(7):500–11.
130. Chow J, Golan Y. Vaccination of solid-organ transplantation candidates. *Clin Infect Dis* 2009;**49**:1550–6.
131. Clancy CJ, Hong Nguyen M. The end of an era in defining the optimal treatment of invasive candidiasis. *Clin Infect Dis* 2012; **54**(8):1123–5.
135. Coelho T, Tredger M, Dhawan A. Current status of immunosuppressive agents for solid organ transplantation in children. *Pediatr Transplant* 2012;**16**:106–22.
136. Cohen-Cymberknoh M, Shoseyov D, Kerem E. Managing cystic fibrosis; strategies that increase life expectancy and improve quality of life. *Am J Respir Crit Care Med* 2011;**183**:1463–71.
138. Connolly P, Hage CA, Bariola JR, et al. *Blastomyces dermatitidis* antigen detection by quantitative enzyme immunoassay. *Clin Vacc Immunol* 2012;**19**(1):53–6.
143. Costa J-M, Botterel F, Cabaret O, et al. Association between circulating DNA, serum (1-3) β-D-glucan, and pulmonary fungal burden in *Pneumocystis* pneumonia. *Clin Infect Dis* 2012;**55**(2): e5–e8.
145. Couzi L, Helou S, Bachelet T, et al. High incidence of anticytomegalovirus drug resistance among D+R– kidney transplant recipients receiving preemptive therapy. *Am J Transplant* 2012; **12**:202–9.
146. Cowen EW, Nguyen JC, Miler DD, et al. Chronic phototoxicity and aggressive squamous cell carcinoma of the skin in children and adults during treatment with voriconazole. *J Am Acad Dermatol* 2010;**62**(1):31–7.
148. Cuellar-Rodriquez J, Avery RK, Budev M, et al. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. *Clin Infect Dis* 2009;**49**:710–6.
150. Cystic Fibrosis Foundation. *Patient Registry Annual Data Report* 2010. Available at www.cff.org.
151. Dales L, Ferris W, Vandermheen K, et al. Combination antibiotic susceptibility of biofilm-grown *Burkholderia cepacia* and *Pseudomonas aeruginosa* isolated from patients with pulmonary exacerbations of cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1275–9.
152. Dalfino L, Puntillo F, Mosca A, et al. High-dose, extended-interval colistin administration in critically ill patients: is this the right dosing strategy? A preliminary study. *Clin Infect Dis* 2012;**54**(12):1720–6.
153. Damiani C, Choukri F, Le Gal S, et al. Possible nosocomial transmission of *Pneumocystis jirovecii*. *Emerg Infect Dis* 2012;**18**: 877–8.
157. Danziger-Isakov L, Kumar D; AST Infectious Disease Community of Practice. Guidelines for vaccination of solid organ transplant candidates and recipients. *Am J Transpl* 2009;**9**(Suppl 4): s258–62.
159. Danziger-Isakov LA, Worley S, Arrigain A, et al. Increased mortality after pulmonary fungal infection within the first year after pediatric lung transplantation. *J Heart Lung Transpl* 2008;**27**(6): 655–61.
160. Danziger-Isakov LA, Worley S, Michael MG, et al. The risk, prevention and outcome of cytomegalovirus after pediatric lung transplantation. *Transplantation* 2009;**87**(10):1541–8.
162. Davis JA, Horn DL, Marr KA, et al. Central nervous system involvement in cryptococcal infection in individuals after solid organ transplantation or with AIDS. *Transpl Infect Dis* 2009;**11**: 432–7.
168. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG). *Clin Infect Dis* 2008;**46**(12):1813–21.
170. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009;**28**(4):283–6.
171. Desmet S, Van Wijngaerden E, Maertens J, et al. Serum (1-3) β-D-glucan as a tool for diagnosis of *Pneumocystis jirovecii* pneumonia in patients with human immunodeficiency virus infection or hematological malignancy. *J Clin Microbiol* 2009;**47**(12): 3871–4.
173. De Soyza, Meachery G, Hester KLM, et al. Lung transplantation for patients with cystic fibrosis and *Burkholderia cepacia* complex infection: a single-center experience. *J Heart Lung Transplant* 2010;**29**(12):1395–404.
183. Doblas A, Torre-Cisneros J. The current state of tuberculosis in solid organ transplantation: three principles for optimal management. *Am J Transplant* 2011;**11**:1769–70.
184. Doby EH, Benjamin DK Jr, Baschke AJ, et al. Therapeutic monitoring of voriconazole in children less than 3 years of age: a case report and summary of voriconazole concentrations for 10 children. *Pediatr Infect Dis* 2012;**31**:632.
187. Dolton MJ, Ray JE, Marriott D. Posaconazole exposure-response relationship: evaluating the utility of therapeutic drug monitoring. *Antimicrob Agents Chemother* 2012;**56**(6):2806–13.
200. Eriksson M, Lemstrom K, Soujaranta-Ylinen R, et al. Control of early *Aspergillus* mortality after lung transplantation: outcome and risk factors. *Transplant Proc* 2010;**42**:4459–64.
202. Falagas M, Sideri G, Vouloumanou EV, et al. Intravenous colistimethate (Colistin) use in critically ill children without cystic fibrosis. *Pediatr Infect Dis J* 2009;**28**(2):123–7.
208. Fisher BT, Zaoutis TE, Park JR, et al. Galactomannan antigen testing for diagnosis of invasive aspergillosis in pediatric hematology patients. *Pediatr Infect Dis J* 2012;**1**(2):103–11.
210. Fishman JA. Prophylaxis, preemption and drug resistance in CMV infection: too little, too much or just right? *Am J Transplant* 2012;**12**:13–4.

211. Fishman JA, Greenwald MA, Grossi PA. Transmission of infection with human allografts: essential considerations in donor screening. *Clin Infect Dis* 2012;**55**:720.
213. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012;**18**:732–8.
216. Fox BD, Raviv Y, Rozengarten D, et al. Pandemic influenza (H1N1): impact on lung transplant recipients and candidates. *J Heart Lung Transplant* 2010;**29**:1034–8.
219. Freifeld A, Proia L, Andes D. Voriconazole use for endemic fungal infections. *Antimicrob Agents Chemother* 2009;**53**(4):1648–51.
220. Freifeld AG, Wheat LJ, Kaul DR. Histoplasmosis in solid organ transplant recipients: early diagnosis and treatment. *Curr Opin Organ Transplant* 2009;**14**(6):601–5.
228. Garcia-Effron G, Katiyar SK, Park S, et al. A naturally occurring proline-to-alanine amino acid change in Fks1p in *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*. *Antimicrob Agents Chemother* 2008;**52**(7):2305–12.
240. Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J Clin Virol* 2009; **45**(3):191–5.
242. Goldberg E, Bishara J, Lev S, et al. Organ transplantation from a donor colonized with a multidrug-resistant organism: a case report. *Transpl Infect Dis* 2011;**14**:296–9.
246. Gottlieb J, Mattner F, Weissbrodt H, et al. Impact of graft colonization with gram-negative bacteria after lung transplantation on the development of bronchiolitis obliterans syndrome in recipients with cystic fibrosis. *Respir Med* 2008;**103**:743–9.
256. Griffith DE. Nontuberculous mycobacterial lung disease. *Curr Opin Infect Dis* 2010;**23**:185–90.
257. Griffith DE, Aksamit TR. Therapy of refractory nontuberculous mycobacterial lung disease. *Curr Opin Infect Dis* 2012;**25**(2): 218–27.
259. Grim SA, Proia L, Miller R, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. *Transpl Infect Dis* 2012;**14**:17–23.
260. Groetzner J, Kaczmarek I, Wittwer T, et al. Caspofungin as first-line therapy for the treatment of invasive aspergillosis after thoracic organ transplantation. *J Heart Lung Transplant* 2008; **27**(1):1–6.
265. Grossi PA, Fishman JA; AST Infectious Disease Community of Practice. Donor-derived infections in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s19–26.
267. Gupta A, Singh N. Immune reconstitution syndrome and fungal infections. *Curr Opin Infect Dis* 2011;**24**:527–33.
270. Hafner V, Czock D, Burhenne J, et al. Pharmacokinetics of sulfobutylether-beta-cyclodextrin and voriconazole in patients with end-stage renal failure during treatment with two hemodialysis systems and hemodiafiltration. *Antimicrob Agents Chemother* 2010;**54**(6):2596–602.
271. Hage CA, Davis TE, Fuller D, et al. Diagnosis of histoplasmosis by antigen detection on BAL fluid. *Chest* 2010;**137**(3):623–8.
272. Hage CA, Kirsch EJ, Stump TE, et al. *Histoplasma* antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. *Clin Vaccine Immunol* 2011;**18**(4):661–6.
274. Hage CA, Ribes JA, Wengenack NL, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin Infect Dis* 2011;**53**(5):448–54.
278. Hatton O, Martinez OM, Esquivel CO. Emerging therapeutic strategies for Epstein-Barr virus + post-transplant lymphoproliferative disorder. *Pediatr Transpl* 2012;**16**:220–9.
279. Hauser PM, Bille J, Lass-Florl C, et al. Multicenter, prospective clinical evaluation of respiratory samples from subjects at risk for *Pneumocystis jirovecii* infection by use of a commercial real-time PCR assay. *J Clin Microbiol* 2011;**49**(5):1872–8.
292. Höcker B, Böhm S, Fickenscher H, et al. (Val-)Ganciclovir prophylaxis reduces Epstein-Barr virus primary infection in pediatric renal transplantation. *Transpl Int* 2012;**25**(7):723–31.
294. Hoffman JA. Adenovirus infections in solid organ transplant recipients. *Curr Opin Organ Transplant* 2009;**14**:625–33.
296. Hoffman J, Walsh T. Echinocandins in children. *Pediatr Infect Dis J* 2011;**30**(6):508–9.
299. Hopkins P, McNeil K, Kermeen F, et al. Human metapneumovirus in lung transplant recipients and comparison to respiratory syncytial virus. *Am J Respir Crit Care Med* 2008;**178**:876–81.
300. Horn M, Children's Hospital Los Angeles Cardiothoracic Transplant coordinator, personal communication, May 29, 2012.
302. Howard SJ, Felton TW, Gomez-Lopez A, et al. Posaconazole: the case for therapeutic drug monitoring. *Ther Drug Monit* 2012;**34** (1):72–6.
303. Huang HC, Weigt S, Derhovanessian A, et al. Non-tuberculous *Mycobacterium* infection after lung transplantation is associated with increased mortality. *J Heart Lung Transplant* 2011;**30**(7): 790–8.
308. Humar A, Lebrancu Y, Blumberg EA, et al. The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in high-risk kidney transplant recipients. *Am J Transplant* 2010; **10**:1228–37.
309. Humar A, Snydman D; AST Infectious Disease Community of Practice. Cytomegalovirus in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s78–86.
312. Husain S, Clancy CJ, Nguyen MH, et al. Performance characteristics of the Platelia *Aspergillus* enzyme immunoassay for detection of *Aspergillus* galactomannan antigen in bronchoalveolar lavage fluid. *Clin Vaccine Immunol* 2008;**15**(12):1760–3.
314. Husain S, Mooney ML, Danziger-Isakov L, et al. A 2010 working formulation for the standardization of definitions of infections in cardiothoracic transplant recipients. *J Heart Lung Transplant* 2011;**30**(4):361–74.
324. Ison MG, Michaels MG; AST Infectious Disease Community of Practice. RNA respiratory viral infections in solid organs transplant recipients. *Am J Transpl* 2009;**9**(suppl 4):s166–72.
325. Ison MG, Szakaly P, Shapira MY, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**(6):955–64.
329. Jernigan MG, Press EG, Hong Nguyen M, et al. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2012;**56**(6):3395–8.
337. Kasztelewicz B, Jankowska I, Pawlowska J, et al. Epstein-Barr virus DNA load in peripheral blood mononuclear cells and whole blood from pediatric transplant recipients. *Transpl Infect Dis* 2011;**13**:471–9.
339. Keckich DW, Blair JE, Vikram HR, et al. Reactivation of coccidioidomycosis despite antifungal prophylaxis in solid organ transplant recipients. *Transplantation* 2011;**92**(1):88–93.
343. Kimberlin DW, Shalabi M, Abzug MJ, et al. Safety of oseltamivir compared with the adamantanes in children less than 12 months of age. *Pediatr Infect Dis J* 2010;**29**(3):195–8.
348. Knoll BM, Kappagoda S, Gill RR, et al. Non-tuberculous mycobacterial infection among lung transplant recipients: a 15-year cohort study. *Transpl Infect Dis* 2012;**14**:452–60.
353. Kontoyiannis DP, Lewis RE, Alexander BD, et al. Calcineurin inhibitor agents interact synergistically with antifungal agents in vitro against *Cryptococcus neoformans* isolates: correlation with outcome in solid organ transplant recipients with cryptococcosis. *Antimicrob Agents Chemother* 2008;**52**(2):735–8.
354. Kontoyiannis DP, Ratanatharathorn V, Young J-A, et al. Micafungin alone or in combination with other systemic antifungal therapies in hematopoietic stem cell transplant recipients with invasive aspergillosis. *Trans Infect Dis* 2009;**11**:89–93.
355. Koo S, Baden LR, Marty FM. Post-diagnostic kinetics of the (1→3)-β-D-glucan assay in invasive aspergillosis, invasive candidiasis and *Pneumocystis jirovecii* pneumonia. *Clin Microbiol Infect* 2012;**18**:e122–e7.
358. Kotton CN, Kumar D, Caliendo AM, et al. International Consensus Guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010;**89**(7):779–95.
359. Kourkoumpetis TK, Burgwyn Fuchs B, Coleman JJ, et al. Polymerase chain reaction-based assays for the diagnosis of invasive fungal infection. *Clin Infect Dis* 2012;**54**(9):1322–31.
363. Kubak BM, Huprikar SS; AST Infectious Disease Community of Practice. Emerging & rare fungal infections in solid organ transplant recipients. *Transplantation* 2009;**9**(Suppl 4):s208–26.
364. Kumar D, Blumberg EA, Danziger-Isakov L, et al. Influenza vaccination in the organ transplant recipient: review and summary recommendations. *Am J Transplant* 2011;**11**:2020–30.

367. Kumar S, Henrickson J. Update in influenza diagnostics: lessons from the novel H1N1 influenza A pandemic. *Clin Microbiol Rev* 2012;**25**(2):344–61.
368. Kumar D, Michaels MG, Morris M. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplant: a multicenter cohort study. *Lancet Infect Dis* 2010;**10**: 521–6.
369. Kumar D, Morris MI, Kotton CN. Guidance on novel influenza A/H1N1 in solid organ transplant recipients. *Am J Transplant* 2010;**10**:18–25.
374. Lanternier F, Sun H-Y, Ribaud P, et al. Mucormycosis in organ and stem cell transplant recipients. *Clin Infect Dis* 2012;**54**(11): 1629–36.
377. Laus S, Kingsley LA, Green M, et al. Comparison of QIAsymphony automated and QIAamp manual DNA extraction systems for measuring Epstein-Barr virus DNA load in whole blood using real-time PCR. *J Mol Diagn* 2011;**13**(6):695–700.
378. Lautenschlager I, Razonable RR. Human herpesvirus–6 infections in kidney, liver, lung and heart transplantation: review. *Transpl Int* 2012;**25**:493–502.
382. Lee SO, Brown RA, Razonable RR, et al. Chromosomally integrated human herpesvirus-6 in transplant recipients. *Transpl Infect Dis* 2012;**14**:346.
384. LeGal S, Damiani C, Rouille A, et al. A cluster of *Pneumocystis* infections among renal transplant recipients: molecular evidence of colonized patients as potential infectious sources of *Pneumocystis jirovecii*. *Clin Infect Dis* 2012;**54**(7):e62–71.
389. Levy I, Grisaru-Soen G, Lerner-Geva L, et al. Multicenter cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients, Israel. *Emerg Infect Dis* 2008;**14**(3): 378–84.
391. Li L, Avery R, Budev M, et al. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. *J Heart Lung Transplant* 2012;**31**:839.
399. LiPuma JJ. The changing microbial epidemiology in cystic fibrosis: *Clin Microbiol Rev* 2010;**23**(2):299–323.
400. Liu M, Mallory GB, Schecter MG, et al. Long-term impact of respiratory viral infection after pediatric lung transplantation. *Pediatr Transplant* 2010;**14**:431–6.
401. Liu M, Worley S, Arrigain S, et al. Respiratory viral infections within one year after pediatric lung transplant. *Transpl Infect Dis* 2009;**11**:304–12.
402. Liu M, Worley S, Mallory GB, et al. Fungal infections in pediatric lung transplant recipients: colonization and invasive disease. *J Heart Lung Transplant* 2009;**28**(11):1226–30.
405. Lockhart SR, Wagner D, Iqbal N, et al. Comparison of in vitro susceptibility characteristics of *Candida* species from cases of invasive candidiasis in solid organ and stem cell transplant recipients: Transplant-Associated Infections Surveillance Network (TRANSNET), 2001 to 2006. *Clin Microbiol* 2011;**49**(7): 2404–10.
407. Luong ML, Clancy CJ, Vadnerkar A, et al. Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. *Clin Infect Dis* 2011;**52**(10):1218–26.
408. Luong ML, Hosseini-Moghaddam SM, Singer LG, et al. Risk factors for voriconazole hepatotoxicity at 12 weeks in lung transplant recipients. *Am J Transplant* 2012;**12**(7):1929–35.
410. Macedo C, Webber SA, Donnenberg AD, et al. EBV-specific CD8+ T cells from asymptomatic pediatric thoracic transplant patients carrying chronic high EBV loads display contrasting features: activated phenotype and exhausted function. *J Immunol* 2011;**186**:5854–62.
411. Macedo C, Zeevi A, Bentlejewski C, et al. The impact of EBV load on T cell immunity in pediatric thoracic transplant recipients. *Transplantation* 2009;**88**(1):123–8.
414. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children. *Pediatr Infect Dis J* 2011;**30**(8):694–700.
416. Maertens J, Buve K, Theunissen K, et al. Galactomannan serves as a surrogate endpoint for outcome of pulmonary invasive aspergillosis in neutropenic hematology patients. *Cancer* 2008; **115**:355–62.
425. Mainz JG, Hentschel J, Schien C, et al. Sinonasal persistence of *Pseudomonas aeruginosa* after lung transplantation. *J Cyst Fibros* 2012;**11**:158–61.
427. Malloy AMW, Campos JM. Extended-spectrum beta-lactamases—a brief clinical update. *Pediatr Infect Dis* 2011;**30**(12):1092–3.
434. Marsik FJ, Nambiar S. Review of carbapenemases and ampC-beta lactamases. *Pediatr Infect Dis* 2011;**30**(12):1094–5.
435. Martin M, Goyette N, Ives J, et al. Incidence and characterization of cytomegalovirus resistance mutation among pediatric solid organ transplant patients who received valganciclovir prophylaxis. *J Clin Virol* 2010;**47**:321–4.
436. Martin SI, Fishman JA; AST Infectious Disease Community of Practice. *Pneumocystis* pneumonia in solid organ transplant recipients. *Transplantation* 2009;**9**(Suppl 4):s227–33.
437. Martins N, Martins IS, de Freitas JA, et al. Severe infection in a lung transplant recipient caused by donor-transmitted carbapenem-resistant *Acinetobacter baumannii*. *Transpl Infect Dis* 2011;**14**: 316–20.
443. Mazurek GH, Jereb J, Vernon A, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States 2010. *MMWR Recomm Rep* 2010;**59**(RR05):1–25.
456. Michalopoulos A, Papadakis E. Inhaled anti-infective agents: emphasis on colistin. *Infection* 2010;**38**:81–8.
457. Mikulska M, Furfaro E, Del Bono V, et al. Piperacillin/tazobactam (Tazocin) seems to be no longer responsible for false-positive results of the galactomannan assay. *J Antimicrob Chemother* 2012; **67**:1746–8.
462. Miraldi F, Anile M, Ruberto F, et al. *Scedosporium apiospermum* atrial mycetomas after lung transplantation for cystic fibrosis. *Transpl Infect Dis* 2012;**14**(2):188–91.
463. Mitsani D, Hong Nguyen H, Kwak EJ, et al. Cytomegalovirus disease among donor-positive/recipient-negative lung transplant recipients in the era of valganciclovir prophylaxis. *Heart Lung Transplant* 2010;**29**:1014–20.
464. Mitsani D, Nguyen MH, Shields RK, et al. Prospective, observational study of voriconazole therapeutic drug monitoring among lung transplant recipients receiving prophylaxis: factors impacting levels of and associations between serum, troughs, efficacy, and toxicity. *Antimicrob Agents Chemother* 2012;**56**(5): 2371–7.
466. Mohd Hafiz AA, Staatz CE, Kirkpatrick CMJ, et al. Continuous infusion vs. bolus dosing: implications for beta-lactam antibiotics. *Minerva Anestesiol* 2011;**77**(4):1–11.
468. Monforte V, Ussetti P, Gavalda J, et al. Feasibility, tolerability, and outcomes of nebulized liposomal amphotericin B for *Aspergillus* infection prevention in lung transplantation. *J Heart Lung Transplant* 2010;**29**:523–20.
471. Moore PS. The emergence of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8). *N Engl J Med* 2012;**343**(19): 1411–3.
472. Moriyama B, Henning S, Childs R, et al. High-dose continuous infusion β-lactam antibiotics for the treatment of resistant *Pseudomonas aeruginosa* infections in immunocompromised patients. *Pharmacotherapy* 2010;**44**:929–35.
473. Morris AM, Masur H. A serologic test to diagnose *Pneumocystis* pneumonia: are we there yet? *Clin Infect Dis* 2011;**53**(2): 203–4.
474. Muhammad Iqbal AH, Lim SK, Ng KP, et al. *Pneumocystis jirovecii* pneumonia 13 years post renal transplant following a recurrent cytomegalovirus infection. *Transpl Infect Dis* 2012;**14**(4): E23–6.
479. Murray S, Charbeneau J, Marshall BC, et al. Impact of *Burkholderia* infection on lung transplantation in cystic fibrosis. *Am J Respir Crit Care Med* 2008;**178**:363–71.
483. Nash EF, Coonar A, Kremer R, et al. Survival of *Burkholderia cepacia* sepsis following lung transplantation in recipients with cystic fibrosis. *Transplant Infect Dis* 2010;**12**:551–4.
484. Nash KA, Brown-Elliott BA, Wallace Jr RJ. A novel gene, *erm*(41), confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. *Antimicrob Agents Chemother* 2009;**53**(4):1367–76.
485. Neely M, Rushing T, Kovacs A, et al. Voriconazole pharmacokinetics and pharmacodynamics in children. *Clin Infect Dis* 2010; **50**:27–36.

486. Neofytos D, Fishman JA, Horn D, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. *Transpl Infect Dis* 2010;**12**:220–9.
487. Neofytos D, Lombardi LR, Shields RK, et al. Administration of voriconazole in patients with renal dysfunction. *Clin Infect Dis* 2012;**54**(7):913–21.
488. Neoh CF, Snell GI, Kotsimbos T, et al. Antifungal prophylaxis in lung transplantation—a world-wide survey. *Transplant J* 2011;**11**: 361–6.
491. Ng BJH, Glanville AR, Snell G, et al. The impact of pandemic influenza A H1N1 2009 on Australian lung transplant recipients. *Am J Transplant* 2011;**11**:568–74.
492. Nguyen HM, Wissel MC, Shields RK, et al. Performance of *Candida* real-time polymerase chain reaction, β-D-glucan assay, and blood cultures in the diagnosis in invasive candidiasis. *Clin Infect Dis* 2012;**54**(9):1240–8.
493. Nivoix Y, Velten M, Letscher-Bru V, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. *Clin Infect Dis* 2008;**47**:1176–98.
495. Nouer SA, Nucci M, Sanath Kumar N, et al. Earlier response assessment in invasive aspergillosis based on the kinetics of serum *Aspergillus* galactomannan: proposal for a new definition. *Clin Infect Dis* 2011;**54**(7):671–6.
496. Nucci M, Anaissie E, Betts RF, et al. Early removal of central venous catheter in patients with candidemia does not improve outcome: analysis of 842 patients from 2 randomized clinical trials. *Clin Infect Dis* 2010;**51**(3):295–303.
503. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis* 2011;**52**:1087–99.
509. Oren I, Hardak E, Finkelstein R, et al. Polymerase chain reaction-based detection of *Pneumocystis jirovecii* in bronchoalveolar lavage fluid for the diagnosis of pneumocystis pneumonia. *J Med Sci* 2011;**342**(3):182–5.
511. Organ Procurement Transplant Network and Scientific Registry of Transplant Recipients. *Annual data report* 2010. Available at http://optn.transplant.hrsa.gov/.
516. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008;**149**(3):177–84.
520. Palmer SM, Limaye AP, Banks M, et al. Extended valganciclovir prophylaxis to prevent cytomegalovirus after lung transplantation. *Ann Intern Med* 2010;**152**(12):761–9.
526. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**(8):1101–11.
527. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009; **48**:503–35.
528. Pappas PG, Silveria FP; AST Infectious Disease Community of Practice. *Candida* in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s173–9.
530. Pascual A, Calandra T, Bolay S, et al. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. *Clin Infect Dis* 2008;**46**: 201–11.
531. Pasqualotto AC, Xavier MO, Sanchez LB, et al. Diagnosis of invasive aspergillosis in lung transplant recipients by detection of galactomannan in the bronchoalveolar lavage fluid. *Transplantation* 2010;**90**(3):306–11.
532. Patel G, Perez F, Bonomo RA. Carbapenem-resistant Enterobacteriaceae and *Acinetobacter baumannii*: assessing their impact on organ transplantation. *Curr Opin Organ Transplant* 2010;**15**: 676–82.
533. Patel N, Snyder LD, Copeland CAF, et al. Is prevention the best treatment? CMV after lung transplantation. *Transplantation* 2012; **12**:539–44.
536. Pelaez AD, Lyon GM, Force SD, et al. Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. *J Heart Lung Transplant* 2009;**28**: 67–71.
537. Perez F, Bonomo R. Can we really use β-lactam/β-lactam inhibitor combinations for the treatment of infections caused by extended-spectrum β-lactamase-producing bacteria? *Clin Infect Dis* 2012;**54**(2):175–7.
538. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
539. Pergam SA, Limaye AP; AST Infectious Disease Community of Practice. Varicella zoster virus (VZV) in solid organ transplant recipients. *Am J Transpl* 2009;**9**(Suppl 4):s108–15.
542. Pfaller MA, Diekema DJ, Ostrosky-Zeichner L, et al. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. *Clin Microbiol* 2008;**46**(8):2620–9.
548. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrob Agents Chemother* 2011;**55**(2):459–72.
549. Pliquett RU, Asbe-Vollkopf A, Hauser PM, et al. A *Pneumocystis jirovecii* pneumonia outbreak in a single kidney-transplant center: role of cytomegalovirus co-infection. *Eur J Clin Microbiol Infect Dis* 2012;**91**:905–16.
551. Pong A, Moser KS, Park SM, et al. Evaluation of an interferon gamma release assay to detect tuberculosis infection in children in San Diego, California. *Pediatr Infect Dis Soc* 2012;**1**(1):74–7.
554. Prichard MN, Kern ER, Hartline CB. CMX001 potentiates the efficacy of acyclovir in herpes simplex virus infections. *Antimicrob Agents Chemother* 2011;**55**(10):4728–34.
555. Proia L, Miller R; AST Infectious Disease Community of Practice. Endemic fungal infections in solid organ transplant recipients. *J Transplantation* 2009;**9**(Suppl 4):s199–207.
556. Radhakrishnan DK, Yau Y, Corey M, et al. Non-tuberculous mycobacteria in children with cystic fibrosis: isolation, prevalence, and predictors. *Pediatr Pulmonol* 2009;**44**:1100–6.
557. Ranganathan K, Worley S, Michaels MG, et al. CMVIG decreases the risk of cytomegalovirus infection but not disease after pediatric lung transplantation. *Heart Lung Transplant* 2009; **28**(10):1050–6.
559. Razonable RR, Zerr DM; AST Infectious Disease Community of Practice. HHV-6, HHV-7 and HHV-8 in Solid Organ Transplant Recipients. *Am J Transpl* 2009;**9**(Suppl 4): s97–100.
560. Razvi S, Quitell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the US 1995-2005. *Chest* 2009; **136**(6):1554–60.
564. Richeldi L, Losi M, D'Amico R, et al. Performance of tests for latent tuberculosis in different groups of immunocompromised patients. *Chest* 2009;**136**:198–204.
566. Roberts JA, Webb S, Paterson D, et al. A systematic review on clinical benefits of continuous administration of β-lactam antibiotics. *Crit Care Med* 2009;**37**(6):2071–8.
568. Rodriguez-Bano J, Navarro MD, Retamar P, et al. β-Lactam/β-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum β-lactamase-producing *Escherichia coli:* a post hoc analysis of prospective cohorts. *Clin Infect Dis* 2012;**54**(2):167–74.
590. Samuel CM, Whitelaw A, Corcoran C, et al. Improved detection of *Pneumocystis jirovecii* in upper and lower respiratory tract specimens from children with suspected *Pneumocystis* pneumonia using real-time PCR: a prospective study. *BMC Infect Dis* 2011; **11**:329.
594. Sax PE, Komarow L, Finkelsman MA, et al. Blood (1→3)-β-D-glucan as a diagnostic test for HIV-related *Pneumocystis jirovecii* pneumonia. *Clin Infect Dis* 2011;**53**(2):197–202.
595. Scaenman JM, Rosso F, Austin JM, et al. Trends in invasive disease due to *Candida* species following heart and lung transplantation. *Transpl Infect Dis* 2009;**11**:112–21.
598. Schmoldt S, Schuhegger R, Wendler T, et al. Molecular evidence of nosocomial *Pneumocystis jirovecii* transmission among 16 patients after kidney transplantation. *J Clin Microbiol* 2008;**46**(3):966–71.
604. Sheu R, Bricker AO, Sahi H, et al. *Pseudallescheria boydii* (*Scedosporium* species) in 3 lung transplant recipients: computed tomography findings and literature review. *Comput Assist Tomogr* 2009; **33**(2):247–52.
605. Shields RK, Clancy CJ, Vandnerkar A, et al. Posaconazole serum concentrations among cardiothoracic transplant recipients: factors impacting trough levels and correlation with clinical response to therapy. *Antimicrob Agents Chemother* 2011;**55**(3):1308–11.

608. Sifri CD, Ison MG. Highly resistant bacteria and donor-derived infections: treading in uncharted territory. *Transpl Infect Dis* 2012;**14**:223–8.
609. Silveira FP, Kwak EJ, Paterson DL, et al. Post-transplant colonization with non-*Aspergillus* molds and risk of development of invasive fungal disease in lung transplant recipients. *J Heart Lung Transpl* 2008;**27**(8):850–5.
610. Simmon KE, Brown-Elliott BA, Ridge PG, et al. *Mycobacterium chelonae*-abscessus complex associated with sinopulmonary disease, Northeastern USA. *Emerg Infect Dis* 2011;**17**(9):1692–700.
614. Singh N. How I treat cryptococcosis on organ transplant recipients. *Transplantation* 2012;**93**(1):17–21.
616. Singh N, Alexander BD, Lortholary O, et al. Pulmonary cryptococcosis in solid organ transplant recipients: Clinical relevance of serum cryptococcal antigen. *Clin Infect Dis* 2008;**46**:e12–e18.
618. Singh N, Forrest G; AST Infectious Disease Community of Practice. Cryptococcosis in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s192–8.
632. Smith J, Andes D. Therapeutic drug monitoring of antifungals: pharmacokinetic and pharmacodynamic considerations. *Ther Drug Monit* 2008;**30**(2):167–72.
635. Snydman DR, Limaye AP, Potena L, et al. Update and review: state-of-the-art management of cytomegalovirus infection and disease following thoracic organ transplantation. *Transplant Proc* 2011;**43**(Suppl):s1–s17.
638. Soler-Palacin P, Frick MA, Martin-Nalda A, et al. Voriconazole drug monitoring in the management of invasive fungal infection in immunocompromised children: a prospective study. *J Antimicrob Chemother* 2012;**67**:700–6.
639. Southern KW, Mérelle M, Dankert-Roelse JE, Nagelkerke AD. Newborn screening for cystic fibrosis (review). *Cochrane Database Syst Rev* 2009;(1):CD001402.
641. Spellberg B, Ibrahim A, Roilides E, et al. Combination therapy for mucormycosis: why, what, and how? *Clin Infect Dis* 2012;**54** (Suppl 1):s73–8.
650. Stelzmueller I, Lass-Floerl, Geltner C, et al. Zygomycosis and other rare filamentous fungal infections in solid organ transplant recipients. *Transpl Int* 2008;**21**:534–46.
656. Subramanian A, Dorman S; AST Infectious Disease Community of Practice. *Mycobacterium tuberculosis* in solid organ transplant recipients. *Am J Transpl* 2009;**9**(Suppl 4):S57–61.
659. Sun HY, Alexander BD, Lortholary O, et al. Lipid formulations of amphotericin B significantly improve outcome in solid organ transplant recipients with central nervous system cryptococcosis. *Clin Infect Dis* 2009;**49**:1721–8.
660. Sun HY, Alexander BD, Lortholary O, et al. Cutaneous cryptococcosis in solid organ transplant recipients. *Med Mycol* 2010; **48**(6):785–91.
661. Sun HY, Alexander BD, Lortholary O. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. *Clin Infect Dis* 2010;**51**(9):1062–9.
662. Sun HY, Singh N. Opportunistic infection-associated immune reconstitution syndrome in transplant recipients. *Clin Infect Dis* 2011;**53**(2):168–76.
663. Sun HY, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cells, and tissue transplant recipients: Evidence-based evolving trends. *Clin Infect Dis* 2009;**48**: 1566–78.
670. Theodoropoulos N, Lanternier F, Rassiwala J, et al. Use of the QuantiFERON-TB Gold interferon-gamma release assay for screening transplant candidates: a single-center retrospective study. *Transpl Infect Dis* 2012;**14**(1):1–8.
672. Thomas A, Korb V, Guillemain R, et al. Clinical outcomes of lung-transplant recipients treated by voriconazole and caspofungin combination in aspergillosis. *Clin Pharm Ther J* 2010;**35**: 49–53.
675. Torre-Cisneros J, Doblas A, Aguado JM, et al. Tuberculosis after solid-organ transplant: incidence, risk factors, and clinical characteristics in the RESITRA (Spanish Network of Infection in Transplantation) cohort. *Clin Infect Dis* 2009;**48**:1657–65.
683. van Delden C, Blumberg E; AST Infectious Disease Community of Practice. Multidrug resistant gram-negative bacteria in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4): S27–34.
685. van Hal SJ, Marriott DJE, Chen SCA, et al. Candidemia following solid organ transplantation in the era of antifungal prophylaxis: the Australian experience. *Transpl Infect Dis* 2009;**11**:122–7.
687. Vecino R, Santiago B, Baquero-Artigao F, et al. Tuberculosis in pediatric solid organ and hematopoietic stem cell transplant recipients. *Pediatr Infect Dis J* 2012;**31**(7):774–7.
689. Vergidis P, Walker RC, Kaul DR, et al. False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis. *Transpl Infect Dis* 2012;**14**(2):213–17.
692. Vikram HR, Dosanjh A, Blair JE. Coccidioidomycosis and lung transplantation. *Transplantation* 2011;**92**(7):717–21.
715. Wang EH, Partovi N, Levy RD, et al. *Pneumocystis* pneumonia in solid organ transplant recipients: not yet an infection of the past. *Transpl Infect Dis* 2012;**14**(5):519–25.
716. Weinberg A, Lyu DM, Marquesen J, et al. Incidence and morbidity of human metapneumovirus and other community-acquired respiratory viruses in lung transplant recipients. *Transpl Infect Dis* 2010;**12**:330–5.
718. Welzen MEB, Bruggemann RJM, Van Den Berg JH, et al. A twice daily posaconazole dosing algorithm for children with chronic granulomatous disease. *Pediatr Infect Dis J* 2011;**30**(9): 794–7.
729. White PL, Bretagne S, Klingspor L, et al. *Aspergillus* PCR: one step closer to standardization. *J Clin Microbiol* 2010;**48**(4): 1231–40.
731. White LP, Mengoli C, Bretagne S, et al. Evaluation of *Aspergillus* PCR protocols for testing serum specimens. *J Clin Microbiol* 2011;**49**(11):3842–8.
732. Wiita AP, Roubinian N, Khan Y, et al. Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis. *Transplant Infect Dis* 2012; **14**:248–58.
735. Winkler M, Pratschke J, Schulz U, et al. Caspofungin for post solid organ transplant invasive fungal disease: results of a retrospective observational study. *Transpl Infect Dis* 2010;**12**: 230–7.
736. Winters H, Parbhoo R, Schafer JJ, et al. Extended-spectrum β-lactamase-producing bacterial infections in adult solid organ transplant recipients. *Pharmacotherapy* 2011;**45**:309–16.
741. Writing Committee of the WHO Consultation. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med* 2012;**362**:1708–19.
744. Zaidi S, Elidemir O, Heinle JS, et al. *Mycobacterium abscessus* in cystic fibrosis lung transplant recipients: report of 2 cases and risk for recurrence. *Transpl Infect Dis* 2009;**11**:243–8.
749. Zuk DM, Humar A, Weinkauf JG, et al. An international survey of cytomegalovirus management practices in lung transplantation. *Transplantation* 2010;**90**:672–6.
750. Zukerman R, Wald A; AST Infectious Disease Community of Practice. Herpes simplex virus infections in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s104–7.
751. Zwald FO, Spratt M, Lemos, et al. Duration of voriconazole exposure: an independent risk factor for skin cancer after lung transplantation. *Dermatol Surg* 2012;**38**(8):1369–74.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aaron SD, Ferris W, Henry DA, et al. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with *Burkholderia cepacia*. *Am J Respir Crit Care Med* 2000;**161**:1206.
2. Aaron SD, Vandemheen KL, Ferris W, et al. Combination antibiotic susceptibility testing to treat exacerbations of cystic fibrosis associated with multiresistant bacteria: a randomised, double-blind, controlled clinical trial. *Lancet* 2005;**366**:463.
3. Abel S, Allan R, Gandelman K, et al. Pharmacokinetics, safety and tolerance of voriconazole in renally impaired subjects. *Clin Drug Invest* 2008;**28**(7):409–20.
4. Abuali MM, Arnon R, Posada R. An update on immunizations before and after transplantation in the pediatric solid organ transplant recipient. *Pediatr Transplant* 2011;**15**:770–7.
5. Adam O, Auperin A, Wilquin F, et al. Treatment with piperacillin-tazobactam and false-positive *Aspergillus* galactomannan antigen test results for patients with hematological malignancies. *Clin Infect Dis* 2004;**38**:917.
6. Adetifa IMO, Ota MOC, Jeffries DJ, et al. Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent *Mycobacterium tuberculosis* infection in childhood contacts in the Gambia. *Pediatr Infect Dis J* 2010;**29**(5): 439–43.
7. Adler FR, Aurora P, Baker DH, et al. Lung transplantation for cystic fibrosis. *Proc Am Thorac Soc* 2009;**6**:619–33.
8. Admon D, Engelhard D, Strauss N, et al. Antibody response to influenza immunization in patients after heart transplantation. *Vaccine* 1997;**15**:1518.
9. Aguilar-Guisado M, Givalda J, Ussetti P, et al. Pneumonia after lung transplantation in the RESITRA cohort: a multicenter prospective study. *Am J Transplant* 2007;**7**:1989–96.
10. Alanio A, Desoubeaux G, Sarfati C, et al. Real-time PCR assay-based strategy for differentiation between active *Pneumocystis jirovecii* pneumonia and colonization in immunocompromised patients. *Clin Microbiol Infect* 2011;**17**:1531–7.
11. Alberti C, Bouakline A, Ribaud P, et al. Relationship between environmental fungal contamination and the incidence of invasive aspergillosis in haematology patients. *J Hosp Infect* 2001;**48**:198.
12. Alexander BD. Diagnosis of fungal infection: new technologies for the mycology laboratory. *Transpl Infect Dis* 2002;**4**(Suppl. 3):32.
13. Alexander BD, Perfect JR, Daly JS, et al. Posaconazole as salvage therapy in patients with invasive fungal infections after solid organ transplant. *Transplantation* 2008;**86**(6):791–6.
14. Alexander BD, Petzold EW, Reller LB, et al. Survival after lung transplantation of cystic fibrosis patients infected with *Burkholderia cepacia* complex. *Am J Transplant* 2008;**8**:1025–30.
15. Alexander B, Pfaller MA. Contemporary tools for the diagnosis and management of invasive mycoses. *Clin Infect Dis* 2006;**43**:S15.
16. Alexander BD, Smith PB, Davis D, et al. The (1,3) β-D-glucan test as an aid to early diagnosis of invasive fungal infections following lung transplantation. *J Clin Microbiol* 2010;**48**(11); 4083–8.
17. Alexander BD, Tapson VF. Infectious complications of lung transplantation. *Transpl Infect Dis* 2001;**3**:128.
18. Allen U, Preiksaitis J; AST Infectious Disease Community of Practice. Epstein-Barr virus and posttransplant lymphoproliferative disorder in solid organ transplant recipients. *Am J Transplant* 2009;**9** (Suppl 4):s87–96.
19. American Academy of Pediatrics. Human herpesvirus 6 and 7. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 414.
20. American Academy of Pediatrics. Human herpesvirus 8. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors: *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 416.
21. American Academy of Pediatrics. Influenza. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 439.
22. American Academy of Pediatrics. Human metapneumovirus. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 509.
23. American Academy of Pediatrics. Parainfluenza viral infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS., editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 533.
24. American Academy of Pediatrics. Respiratory syncytial virus. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 609.
25. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 739.
26. American Academy of Pediatrics. Diseases caused by nontuberculous mycobacteria. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 759.
27. American Academy of Pediatrics. Varicella-zoster infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 774.
28. American Academy of Pediatrics Committee on Infectious Diseases. Screening for tuberculosis in infants and children. *Pediatrics* 1994;**93**:131.
29. American Thoracic Society. Rapid diagnostic tests for tuberculosis: what is the appropriate use? American Thoracic Society Workshop. *Am J Respir Crit Care Med* 1997;**155**:1804.
30. Andes D, Lepak A. Editorial Commentary: Antifungal therapeutic drug monitoring progress: getting it right the first time. *Clin Infect Dis* 2012;**55**:391–3.
31. Andes DR, Safdar N, Hoffman J, Baddley JW. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clin Infect Dis* 2012;**54**(8):1110–22.
32. Anstead GM, Graybill JR. Coccidioidomycosis. *Infect Dis Clin North Am* 2006;**20**:621.
33. Apalsch AM, Green M, Ledesma-Medina J, et al. Parainfluenza and influenza virus infections in pediatric organ transplant recipients. *Clin Infect Dis* 1995;**20**:394.
34. Aris RM, Gilligan PH, Neuringer IP, et al. The effects of panresistant bacteria in cystic fibrosis patients on lung transplant outcome. *Am J Respir Crit Care Med* 1997;**155**:1699.
35. Aris RM, Routh JC, LiPuma JJ, et al. Lung transplantation for cystic fibrosis patients with *Burkholderia cepacia* complex: survival linked to genomovar type. *Am J Respir Crit Care Med* 2001;**164**:2102.
36. Ariza-Heredia EJ, Patel R, Blumberg RC, et al. Outcomes of transplantation using organs from a donor infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*. *Transplant Infect Dis* 2012;**14**:229.
37. Armenian SH, Nash KA, Kapoor N, et al. Prospective monitoring for invasive aspergillosis using galactomannan and polymerase chain reaction in high risk pediatric patients. *J Pediatr Hematol Oncol* 2009;**31**(12):920–6.
38. Arnold C, Westland L, Mowat G, et al. Single-nucleotide polymorphism–based differentiation and drug resistance detection in *Mycobacterium tuberculosis* from isolates or directly from sputum. *Clin Microbiol Infect* 2005;**11**:122.
39. Arnold HM, Sawyer AM, Kollef MH. Use of adjunctive aerosolized antimicrobial therapy in the treatment of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ventilator-associated pneumonia. *Respir Care* 2012;**57**:1226.
40. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002;**34**:7.
41. Aubry A, Porche R, Bottero J, et al. Occurrence and kinetics of false-positive *Aspergillus* galactomannan test results following treatment with beta-lactam antibiotics in patients with hematological disorders. *J Clin Microbiol* 2006;**44**:389.
42. Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: systematic review and meta-analysis. *Clin Microbiol* 2011; **49**(2):665–70.
43. Baddley JW, Andes DR, Marr KA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. *Clin Infect Dis* 2010;**50**(12):1559–67.

44. Baddley JW, Andes DR, Marr KA, et al. Antifungal therapy and length of hospitalization in transplant patients with invasive aspergillosis. *Med Mycol* 2013;**51**(2):128–35.
45. Baddley JW, Schain DC, Gupte AA, et al. Transmission of Cryptococcus neoformans by organ transplantation. *Clin Infect Dis* 2011;**52**(4):e94–e98.
46. Baden LR, Katz JT, Fishman JA, et al. Salvage therapy with voriconazole for invasive fungal infections in patients failing or intolerant to standard antifungal therapy. *Transplantation* 2003; **76**:1632.
47. Baden LR, Rubin RH. The sentinel chicken revisited: the impact of West Nile virus infection on transplant patients. *Transplantation* 2004;**77**:356.
48. Bakker NA, van Imhoff GW, Verschuuren EA, et al. Presentation and early detection of post-transplant lymphoproliferative disorder after solid organ transplantation. *Transpl Int* 2007;**20**:207.
49. Bal AM. Combination antifungal therapy in solid organ transplant recipients. *Transplantation* 2006;**82**:291.
50. Balthesen M, Messerle M, Reddehase MJ. Lungs are a major organ site of cytomegalovirus latency and recurrence. *J Virol* 1993;**67**:5360.
51. Barnes PD, Marr KA. Aspergillosis: spectrum of disease, diagnosis, and treatment. *Infect Dis Clin North Am* 2006;**20**:545.
52. Barr ML, Schenkel FA, Bowdish ME, et al. Living donor lobar lung transplantation: current status and future directions. *Transplant Proc* 2005;**37**:3983.
53. Bauldoff GS, Nunley DR, Manzetti JD, et al. Use of aerosolized colistin sodium in cystic fibrosis patients awaiting lung transplantation. *Transplantation* 1997;**64**(5):748–52.
54. Bauwens JE, Spach DH, Schacker TW, et al. *Bordetella bronchiseptica* pneumonia and bacteremia following bone marrow transplantation. *J Clin Microbiol* 1992;**30**:2474.
55. Becker MJ, Lugtenburg EJ, Cornelissen JJ, et al. Galactomannan detection in computerized tomography–based bronchoalveolar lavage fluid and serum in haematological patients at risk for invasive pulmonary aspergillosis. *Br J Haematol* 2003;**121**:448.
56. Benden C, Aurora P, Burch M, et al. Monitoring of Epstein-Barr viral load in pediatric heart and lung transplant recipients by real-time polymerase chain reaction. *J Heart Lung Transplant* 2005;**24**: 2103.
57. Benden C, Aurora P, Edward LB, et al. The Registry of the International Society for Heart and Lung Transplantation Report. Fourteenth Pediatric Lung and Heart-Lung Transplantation–2011. *J Heart Lung Transplant* 2011;**30**(10):1123–32.
58. Bergamasco MD, Barbosa M, de Oliveira Garcia D, et al. Infection with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* in solid organ transplantation. *Transpl Infect Dis* 2012;**14**(2):198–205.
59. Berge M, Guillemain R, Boussaud V, et al. Voriconazole pharmacokinetic variability in cystic fibrosis lung transplant patients. *Transplant Infect Dis* 2009;**11**:211–9.
60. Bernhard S, Kernland Lang K, Ammann RA, et al. Voriconazole-induced phototoxicity in children. *Pediatr Infect Dis J* 2012; **31**(7):769–71.
61. Bernard A, Caillot D, Couaillier JF, et al. Surgical management of invasive pulmonary aspergillosis in neutropenic patients. *Ann Thorac Surg* 1997;**64**:1441.
62. Bianchi E, Pascual M, Nicod M, et al. Clinical usefulness of FDG-PET/CT scan imaging in the management of post-transplant lymphoproliferative disease. *Transplantation* 2008;**85**: 707–12.
63. Billaud EM, Guillemain R, Berge M, et al. Pharmacological considerations for azole antifungal drug management in cystic fibrosis lung transplant patients. *Med Mycol* 2010;**48** (Suppl 1):S52–9.
64. Billings JL, Hertz MI, Wendt CH. Community respiratory virus infections following lung transplantation. *Transpl Infect Dis* 2001; **3**:138.
65. Bingler MA, Feingold B, Miller SA, et al. Chronic high Epstein-Barr viral load state and risk for late-onset post-transplant lymphoproliferative disease/lymphoma in children. *Am J Transplant* 2008;**8**:442–5.
66. Blaes AH, Cioc AM, Froelich JW, et al. Positron emission tomography scanning in the setting of post-transplant lymphoproliferative disorders. *Clin Transplant* 2009;**23**:794–9.
67. Blaes AH, Peterson BA, Bartlett N, et al. Rituximab therapy is effective for posttransplant lymphoproliferative disorders after solid organ transplantation: results of a phase II trial. *Cancer* 2005;**104**:1661.
68. Blair J. Approach to the solid organ transplant patient with latent infection and disease caused by *Coccidioides* species. *Curr Opin Infect Dis* 2008;**21**:415–20.
69. Blair JE, Logan JL. Coccidioidomycosis in solid organ transplantation. *Clin Infect Dis* 2001;**33**:1536.
70. Blodget E, Geiseler PJ, Larsen RA, et al. Donor-derived *Coccidioides immitis* fungemia in solid organ transplant recipients. *Transpl Infect Dis* 2012;**14**(3):305–10.
71. Blumberg HM, Burman WJ, Chaisson RE, et al. American Thoracic Society/Centers for Disease Control and Prevention/ Infectious Diseases Society of America: Treatment of tuberculosis. *Am J Respir Crit Care Med* 2003;**167**:603.
72. Bonaros N, Mayer B, Schachner T, et al. CMV-hyperimmune globulin for preventing cytomegalovirus infection and disease in solid organ transplant recipients: a meta-analysis. *Clin Transplant* 2008;**22**:89–97.
73. Borro JM, Sole A, de la Torree M, et al. Efficiency and safety of inhaled amphotericin b lipid complex (Abelcet) in the prophylaxis of invasive fungal infections following lung transplantation. *Transplant Proc* 2008;**40**:3090–8.
74. Botterel F, Cabaret O, Foulet F, et al. Clinical significance of quantifying *Pneumocystis jirovecii* DNA by using real-time PCR in bronchoalveolar lavage fluid from immunocompromised patients. *J Clin Microbiol* 2012;**50**(2):277–31.
75. Boussaud V, Guillemain R, Grenet D, et al. Clinical outcome following lung transplantation in patients with cystic fibrosis colonized with *Burkholderia cepacia* complex: results from two French centuries. *Thorax* 2008;**63**:732–7.
76. Boyle GJ, Michaels MG, Webber SA, et al. Posttransplantation lymphoproliferative disorders in pediatric thoracic organ recipients. *J Pediatr* 1997;**131**:309.
77. Bozza S, Gaziano R, Lipford GB, et al. Vaccination of mice against invasive aspergillosis with recombinant *Aspergillus* proteins and CpG oligodeoxynucleotides as adjuvants. *Microbes Infect* 2002;**4**:1281.
78. Bozza S, Montagnoli C, Gaziano R, et al. Dendritic cell–based vaccination against opportunistic fungi. *Vaccine* 2004;**22**:857.
79. Bozza S, Perruccio K, Montagnoli C, et al. A dendritic cell vaccine against invasive aspergillosis in allogeneic hematopoietic transplantation. *Blood* 2003;**102**:3807.
80. Brass EP, Edwards JE. Should the guidelines for management of central venous catheters in patients with candidemia be changed now? *Clin Infect Dis* 2010;**51**(3):304–6.
81. Bravo C, Roldan J, Roman A, et al. Tuberculosis in lung transplant recipients. *Transplantation* 2005;**79**:59.
82. Bretagne S, Costa JM. Towards a molecular diagnosis of invasive aspergillosis and disseminated candidosis. *FEMS Immunol Med Microbiol* 2005;**45**:361.
83. Bretagne S, Costa JM, Bart-Delabesse E, et al. Comparison of serum galactomannan antigen detection and competitive polymerase chain reaction for diagnosing invasive aspergillosis. *Clin Infect Dis* 1998;**26**:1407.
84. Brewin J, Mancao C, Straathof K, et al. Generation of EBV-specific cytotoxic T cells that are resistant to calcineurin inhibitors for the treatment of posttransplantation lymphoproliferative disease. *Blood* 2011;**114**(23):4792–803.
85. Bridges ND, Mallory GB, Huddleston CB, et al. Lung transplantation in infancy and early childhood. *J Heart Lung Transplant* 1996;**15**:895.
86. Bridges ND, Spray TL, Collins MH, et al. Adenovirus infection in the lung results in graft failure after lung transplantation. *J Thorac Cardiovasc Surg* 1998;**116**:617.
87. Bruggemann RJM, Donnelly JP, Aarnoutse RE, et al. Therapeutic drug monitoring of voriconazole. *Ther Drug Monit* 2008;**30**(4): 403–11.
88. Bruggemann RJM, van der Linden JWM, Verweij PE, et al. Impact of therapeutic drug monitoring of voriconazole in a pediatric population. *Pediatr Infect Dis J* 2003;**30**(6):533–4.
89. Buchheidt D, Baust C, Skladny H, et al. Detection of *Aspergillus* species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. *Clin Infect Dis* 2001;**33**:428.
90. Buchheidt D, Hummel M, Schleiermacher D, et al. Prospective clinical evaluation of a LightCycler-mediated polymerase chain

reaction assay, a nested-PCR assay and a galactomannan enzyme-linked immunosorbent assay for detection of invasive aspergillosis in neutropenic cancer patients and haematological stem cell transplant recipients. *Br J Haematol* 2004;**125**:196.
91. Bumbacea D, Arend SM, Eyuboglu F, et al. The risk of tuberculosis in transplant candidates and recipients: a TBNET consensus statement. *Eur Respir Soc* 2012;**40**(4):990–1013.
92. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics* 2008;**121**:e1286–e1294.
93. Burns JL, Saiman L. *Burkholderia cepacia* infections in cystic fibrosis. *Pediatr Infect Dis J* 1999;**18**:155.
94. Burroughs M, Moscona A. Immunization of pediatric solid organ transplant candidates and recipients. *Clin Infect Dis* 2000; **30**:857.
95. Busca A, Locatelli F, Barbui A, et al. Usefulness of sequential *Aspergillus* galactomannan antigen detection combined with early radiologic evaluation for diagnosis of invasive pulmonary aspergillosis in patients undergoing allogeneic stem cell transplantation. *Transplant Proc* 2006;**38**:1610.
96. Bwanga F, Hoffner S, Haile M, et al. Direct susceptibility testing for multi-drug resistant tuberculosis: a meta-analysis. *BMC Infect Dis* 2009;**9**:67.
97. Bylund J, Burgess LA, Cescutti P, et al. Exopolysaccharides from *Burkholderia cenocepacia* inhibit neutrophil chemotaxis and scavenge reactive oxygen species. *J Biol Chem* 2006;**281**:2526.
98. Cahill BC, Hibbs JR, Savik K, et al. *Aspergillus* airway colonization and invasive disease after lung transplantation. *Chest* 1997;**112**: 1160.
99. Caillot D, Casasnovas O, Bernard A, et al. Improved management of invasive pulmonary aspergillosis in neutropenic patients using early thoracic computed tomographic scan and surgery. *J Clin Oncol* 1997;**15**:139.
100. Caillot D, Couaillier JF, Bernard A, et al. Increasing volume and changing characteristics of invasive pulmonary aspergillosis on sequential thoracic computed tomography scans in patients with neutropenia. *J Clin Oncol* 2001;**19**:253.
101. Caillot D, Mannone L, Cuisenier B, et al. Role of early diagnosis and aggressive surgery in the management of invasive pulmonary aspergillosis in neutropenic patients. *Clin Microbiol Infect* 2001; **7**(Suppl. 2):54.
102. Calvo V, Borro JM, Morales P, et al. Antifungal prophylaxis during the early postoperative period of lung transplantation. Valencia Lung Transplant Group. *Chest* 1999;**115**:1301.
103. Camacho-Gonzalez AF, Gutman J, Hymes LC, et al. 24 weeks of valganciclovir prophylaxis in children after renal transplantation: a 4-year experience. *Transplantation* 2011;**91**(2):245–50.
104. Cames B, Rahier J, Burtomboy G, et al. Acute adenovirus hepatitis in liver transplant recipients. *J Pediatr* 1992;**120**:33.
105. *Canadian Cystic Fibrosis Patient Data Registry Report 2010–Cystic Fibrosis Canada*. 2010. Available at www.cysticfibrosis.ca.
106. Carter BA, Karpen SJ, Quiros-Tejeira RE, et al. Intravenous cidofovir therapy for disseminated adenovirus in a pediatric liver transplant recipient. *Transplantation* 2002;**74**:1050.
107. Cenci E, Mencacci A, Bacci A, et al. T cell vaccination in mice with invasive pulmonary aspergillosis. *J Immunol* 2000;**165**:381.
108. Centers for Disease Control and Prevention. Update: Nucleic acid amplification tests for tuberculosis. *MMWR Morb Mortal Wkly Rep* 2000;**49**(26):593.
109. Centers for Disease Control and Prevention. Notice to Readers: Updated guidelines for the use of rifabutin or rifampin for treatment among HIV-infected patients taking protease inhibitors or nonnucleoside reverse transcriptase inhibitors. *MMWR Morb Mortal Wkly Rep* 2000;**49**(09):185–9.
110. Centers for Disease Control and Prevention. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberulosis. *MMWR Morb Mortal Wkly Rep* 2009;**58**(1):7–10.
111. Centers for Disease Control and Prevention. FDA approval of an extended period for administering VariZIG for postexposure prophylaxis of varicella. *MMWR Morb Mortal Wkly Rep* 2012; **61**(12):212.
112. Chai LYA, Kullberg BJ, Johnson EM, et al. Early serum galactomannan trend as a predictor of outcome of invasive aspergillosis. *J Clin Microbiol* 2012;**50**(7):2330–6.
113. Chaiyasirinroje B, Nyein Aung M, Moolphate S, et al. Prospective evaluation of simply modified MODS assay: an effective tool for TB diagnosis and detection of MDR-TB. *Infect Drug Resist* 2012;**5**:79–88.
114. Chakrabarti S, Mautner V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation; incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood* 2002;**100**(5):1619–27.
115. Chalermskulrat W, Sood N, Neuringer IP, et al. Non-tuberculous mycobacteria in end stage cystic fibrosis: implications for lung transplantation. *Thorax* 2006;**61**:507.
116. Challier S, Boyer S, Abachin E, et al. Development of a serum-based Taqman real-time PCR assay for diagnosis of invasive aspergillosis. *J Clin Microbiol* 2004;**42**:844.
117. Chandrasekar PH, Sobel JD. Micafungin: a new echinocandin. *Clin Infect Dis* 2006;**42**:1171.
118. Chaparro C, Chamberlain D, Maurer J, et al. Bronchiolitis obliterans organizing pneumonia (BOOP) in lung transplant recipients. *Chest* 1996;**110**:1150.
119. Chaparro C, Maurer J, Gutierrez C, et al. Infection with *Burkholderia cepacia* in cystic fibrosis: outcome following lung transplantation. *Am J Respir Crit Care Med* 2001;**163**:43.
120. Chapman SW, Bradsher RW Jr, Campbell GD Jr, et al. Practice guidelines for the management of patients with blastomycosis. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:679.
121. Chapman SW, Dismukes WE, Proia LA, et al. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:1801–12.
122. Chartrand C, Leefland MM, Minion JB, et al. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med* 2012; **156**(7):500–11.
123. Chayakulkeeree M, Perfect JR. Cryptococcosis. *Infect Dis Clin North Am* 2006;**20**:507.
124. Chen SF, Gutierrez K. *Mycobacterium bovis* disease in a pediatric renal transplant patient. *Pediatr Infect Dis J* 2006;**25**:564.
125. Chernenko SM, Humar A, Hutcheon M, et al. *Mycobacterium abscessus* infections in lung transplant recipients: the international experience. *J Heart Lung Transplant* 2006;**25**(12):1447.
126. Cherry JD. Adenoviruses. In: Feigin RD, Cherry JD, Demmler GJ, Kaplan SL, editors. *Textbook of pediatric infectious diseases*. 6th ed. Philadelphia: WB Saunders; 2009. vol. 2, p. 1949–72.
127. Choong CK, Swee SC, Zoole JB, et al. Bronchial airway anastomotic complications after pediatric lung transplantation: incidence, cause, management, and outcome. *J Thorac Cardiovasc Surg* 2006;**131**:198.
128. Choquet S, Leblond V, Herbrecht R, et al. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. *Blood* 2006;**107**(8):3053–7.
129. Choquet S, Oertel S, LeBlond V, et al. Rituximab in the management of post-transplantation lymphoproliferative disorder after solid organ transplantation: proceed with caution. *Ann Hematol* 2007;**85**:599–607.
130. Chow J, Golan Y. Vaccination of solid-organ transplantation candidates. *Clin Infect Dis* 2009;**49**:1550–6.
131. Clancy CJ, Hong Nguyen M. The end of an era in defining the optimal treatment of invasive candidiasis. *Clin Infect Dis* 2012;**54**(8):1123–5.
132. Choy KW, Wulffraat NM, Wolfs TF, et al. *Bordetella bronchiseptica* respiratory infection in a child after bone marrow transplantation. *Pediatr Infect Dis J* 1999;**18**:481.
133. Claas EC, Schilham MW, de Brouwer CS, et al. Internally controlled real-time PCR monitoring of adenovirus DNA load in serum or plasma of transplant recipients. *J Clin Microbiol* 2005; **43**:1738.
134. Cockfield SM. Identifying the patient at risk for post-transplant lymphoproliferative disorder. *Transpl Infect Dis* 2001;**3**:70.
135. Coelho T, Tredger M, Dhawan A. Current status of immunosuppressive agents for solid organ transplantation in children. *Pediatr Transplant* 2012;**16**:106–22.
136. Cohen-Cymberknoh M, Shoseyov D, Kerem E. Managing cystic fibrosis; strategies that increase life expectancy and improve quality of life. *Am J Respir Crit Care Med* 2011;**183**:1463–71.
137. Coiras MT, Aguilar JC, Garcia ML, et al. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. *J Med Virol* 2004;**72**:484.

138. Connolly P, Hage CA, Bariola JR, et al. *Blastomyces dermatitidis* antigen detection by quantitative enzyme immunoassay. *Clin Vaccine Immunol* 2012;**19**(1):53–6.
139. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007;**356**:348.
140. Cornet M, Levy V, Fleury L, et al. Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against *Aspergillus* airborne contamination during hospital renovation. *Infect Control Hosp Epidemiol* 1999;**20**:508.
141. Costa C, Costa JM, Desterke C, et al. Real-time PCR coupled with automated DNA extraction and detection of galactomannan antigen in serum by enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis. *J Clin Microbiol* 2002;**40**:2224.
142. Costa C, Vidaud D, Olivi M, et al. Development of two real-time quantitative TaqMan PCR assays to detect circulating *Aspergillus fumigatus* DNA in serum. *J Microbiol Methods* 2001;**44**:263.
143. Costa J-M, Botterel F, Cabaret O, et al. Association between circulating DNA, serum (1-3) β-D-glucan, and pulmonary fungal burden in *Pneumocystis* pneumonia. *Clin Infect Dis* 2012;**55**(2): e5–e8.
144. Costerton JW, Stewart P, Greenbert EP. Bacterial biofilms: a common cause of persistent infections. *Science* 1999;**284**: 1318–22.
145. Couzi L, Helou S, Bachelet T, et al. High incidence of anticytomegalovirus drug resistance among D+R– kidney transplant recipients receiving preemptive therapy. *Am J Transplant* 2012; **12**:202–9.
146. Cowen EW, Nguyen JC, Miler DD, et al. Chronic phototoxicity and aggressive squamous cell carcinoma of the skin in children and adults during treatment with voriconazole. *J Am Acad Dermatol* 2010;**62**(1):31–7.
147. Crus AT, Starke JR. Clinical manifestations of tuberculosis in children. *Paediatr Respir Rev* 2007;**8**(2):107–17.
148. Cuellar-Rodriquez J, Avery RK, Budev M, et al. Histoplasmosis in solid organ transplant recipients: 10 years of experience at a large transplant center in an endemic area. *Clin Infect Dis* 2009;**49**:710–6.
149. Cullen AR, Cannon CL, Mark EJ, et al. *Mycobacterium abscessus* infection in cystic fibrosis. Colonization or infection? *Am J Respir Crit Care Med* 2000;**161**:641.
150. Cystic Fibrosis Foundation. *Patient Registry Annual Data Report* 2010. Available at www.cff.org.
151. Dales L, Ferris W, Vandermheen K, et al. Combination antibiotic susceptibility of biofilm-grown *Burkholderia cepacia* and *Pseudomonas aeruginosa* isolated from patients with pulmonary exacerbations of cystic fibrosis. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1275–9.
152. Dalfino L, Puntillo F, Mosca A, et al. High-dose, extended-interval colistin administration in critically ill patients: is this the right dosing strategy? A preliminary study. *Clin Infect Dis* 2012; **54**(12):1720–6.
153. Damiani C, Choukri F, Le Gal S, et al. Possible nosocomial transmission of *Pneumocystis jirovecii*. *Emerg Infect Dis* 2012;**18**:877–8.
154. Dankner WM, Davis CE. *Mycobacterium bovis* as a significant cause of tuberculosis in children residing along the United States–Mexico border in the Baja California region. *Pediatrics* 2000;**105**: E79.
155. Danziger-Isakov LA, DelaMorena M, Hayashi RJ, et al. Cytomegalovirus viremia associated with death or retransplantation in pediatric lung-transplant recipients. *Transplantation* 2003;**75**: 1538.
156. Danziger-Isakov LA, Faro A, Sweet S, et al. Variability in standard care for cytomegalovirus prevention and detection in pediatric lung transplantation: survey of eight pediatric lung transplant programs. *Pediatr Transplant* 2003;**7**:469.
157. Danziger-Isakov LA, Kumar D; AST Infectious Disease Community of Practice. Guidelines for vaccination of solid organ transplant candidates and recipients. *Am J Transpl* 2009;**9** (Suppl 4):s258–62.
158. Danziger-Isakov LA, Sweet S, DelaMorena M, et al. Epidemiology of bloodstream infections in the first year after pediatric lung transplantation. *Pediatr Infect Dis J* 2005;**24**:324.
159. Danziger-Isakov LA, Worley S, Arrigain A, et al. Increased mortality after pulmonary fungal infection within the first year after pediatric lung transplantation. *J Heart Lung Transpl* 2008;**27**(6): 655–61.
160. Danziger-Isakov LA, Worley S, Michael MG, et al. The risk, prevention and outcome of cytomegalovirus after pediatric lung transplantation. *Transplantation* 2009;**87**(10):1541–8.
161. Darenkov IA, Marcarelli MA, Basadonna GP, et al. Reduced incidence of Epstein-Barr virus–associated posttransplant lymphoproliferative disorder using preemptive antiviral therapy. *Transplantation* 1997;**64**:848.
162. Davis JA, Horn DL, Marr KA, et al. Central nervous system involvement in cryptococcal infection in individuals after solid organ transplantation or with AIDS. *Transpl Infect Dis* 2009;**11**: 432–7.
163. de Boer M, Bruijnesteijn van Coppenraet L, Gaasbeek A, et al. An outbreak of *Pneumocystis jiroveci* pneumonia with 1 predominant genotype among renal transplant recipients: interhuman transmission or a common environmental source? *Clin Infect Dis* 2007;**44**:1143.
164. de Mezerville MH, Tellier R, Richardson S, et al. Adenoviral infections in pediatric transplant recipients: a hospital-based study. *Pediatr Infect Dis J* 2006;**25**:815.
165. Denning DW. Invasive aspergillosis. *Clin Infect Dis* 1998;**26**:781.
166. Denning DW, Marr KA, Lau WM, et al. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. *J Infect* 2006;**53**:337.
167. De Pauw BE, Donnelly JP. Prophylaxis and aspergillosis—has the principle been proven? *N Engl J Med* 2007;**356**:409.
168. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG). *Clin Infect Dis* 2008;**46**(12):1813–21.
169. DeSalvo D, Roy-Chaudhury P, Peddi R, et al. West Nile virus encephalitis in organ transplant recipients: another high-risk group for meningoencephalitis and death. *Transplantation* 2004; **77**:466.
170. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009;**28**(4):283–6.
171. Desmet S, Van Wijngaerden E, Maertens J, et al. Serum (1-3) β-D-glucan as a tool for diagnosis of *Pneumocystis jirovecii* pneumonia in patients with human immunodeficiency virus infection or hematological malignancy. *J Clin Microbiol* 2009;**47**(12):3871–4.
172. De Soyza A, Corris PA. Lung transplantation and the *Burkholderia cepacia* complex. *J Heart Lung Transplant* 2003;**22**:954.
173. De Soyza, Meachery G, Hester KLM, et al. Lung transplantation for patients with cystic fibrosis and *Burkholderia cepacia* complex infection: a single-center experience. *J Heart Lung Transplant* 2010;**29**(12):1395–404.
174. De Soyza A, McDowell A, Archer L, et al. *Burkholderia cepacia* complex genomovars and pulmonary transplantation outcomes in patients with cystic fibrosis. *Lancet* 2001;**358**:1780.
175. De Soyza A, Morris K, McDowell A, et al. Prevalence and clonality of *Burkholderia cepacia* complex genomovars in UK patients with cystic fibrosis referred for lung transplantation. *Thorax* 2004;**59**:526.
176. DeVincenzo JP, Hirsch RL, Fuentes RJ, et al. Respiratory syncytial virus immune globulin treatment of lower respiratory tract infection in pediatric patients undergoing bone marrow transplantation—a compassionate use experience. *Bone Marrow Transplant* 2000;**25**:161.
177. de Wet N, Llanos-Cuentas A, Suleiman J, et al. A randomized, double-blind, parallel-group, dose-response study of micafungin compared with fluconazole for the treatment of esophageal candidiasis in HIV-positive patients. *Clin Infect Dis* 2004;**39**:842.
178. Diederich S, Scadeng M, Dennis C, et al. *Aspergillus* infection of the respiratory tract after lung transplantation: chest radiographic and CT findings. *Eur Radiol* 1998;**8**:306.
179. Dietzschold B, Koprowski H. Rabies transmission from organ transplants in the USA. *Lancet* 2004;**364**:648.
180. Digby J, Kalbfleisch J, Glenn A, et al. Serum glucan levels are not specific for presence of fungal infections in intensive care unit patients. *Clin Diagn Lab Immunol* 2003;**10**:882.
181. Dignani MC, Rex JH, Chan K-W, et al. Immunomodulation with interferon-gamma and colony-stimulating factors for refractory fungal infections in patients with leukemia. *Cancer* 2005;**104**(1): 199–204.

182. Dobbin C, Maley M, Harkness J, et al. The impact of pan-resistant bacterial pathogens on survival after lung transplantation in cystic fibrosis: results from a single large referral centre. *J Hosp Infect* 2004;**56**:277.
183. Doblas A, Torre-Cisneros J. The current state of tuberculosis in solid organ transplantation: three principles for optimal management. *Am J Transplant* 2011;**11**:1769–70.
184. Doby EH, Benjamin DK Jr, Baschke AJ, et al. Therapeutic monitoring of voriconazole in children less than 3 years of age: a case report and summary of voriconazole concentrations for 10 children. *Pediatr Infect Dis* 2012;**31**:632.
185. Dodds Ashley E, Lewis R, Lewis JS, et al. Pharmacology of systemic antifungal agents. *Clin Infect Dis* 2006;**43**:S28.
186. Dodge JA, Lewis PA, Wilsher J. Cystic fibrosis mortality and survival in the UK: 1947-2003. *Eur Respir J* 2007;**29**(3):522–6.
187. Dolton MJ, Ray JE, Marriott D. Posaconazole exposure-response relationship: evaluating the utility of therapeutic drug monitoring. *Antimicrob Agents Chemother* 2012;**56**(6):2806–13.
188. Donnelly JP. Polymerase chain reaction for diagnosing invasive aspergillosis: getting closer but still a ways to go. *Clin Infect Dis* 2006;**42**:487.
189. Doucette K, Fishman JA. Nontuberculous mycobacterial infection in hematopoietic stem cell and solid organ transplant recipients. *Clin Infect Dis* 2004;**38**:1428.
190. Drew R. Potential role of aerosolized amphotericin B formulations in the prevention and adjunctive treatment of invasive fungal infections. *Int J Antimicrob Agents* 2006;**27**(Suppl. 1):36.
191. Drew RH, Dodds Ashley E, Benjamin DK, Jr., et al. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. *Transplantation* 2004;**77**:232.
192. Dummer JS, Lazariashvilli N, Barnes J, et al. A survey of antifungal management in lung transplantation. *J Heart Lung Transplant* 2004;**23**:1376.
193. Durand-Joly I, Chabe M, Soula F, et al. Molecular diagnosis of *Pneumocystis pneumonia*. *FEMS Immunol Med Microbiol* 2005; **45**:405.
194. Echavarria M, Forman M, van Tol MJ, et al. Prediction of severe disseminated adenovirus infection by serum PCR. *Lancet* 2001; **358**:384.
195. Eckmanns T, Ruden H, Gastmeier P. The influence of high-efficiency particulate air filtration on mortality and fungal infection among highly immunosuppressed patients: a systematic review. *J Infect Dis* 2006;**193**:1408.
196. Edvardsson VO, Flynn JT, Deforest A, et al. Effective immunization against influenza in pediatric renal transplant recipients. *Clin Transplant* 1996;**10**:556.
197. Einsele H, Hebart H, Roller G, et al. Detection and identification of fungal pathogens in blood by using molecular probes. *J Clin Microbiol* 1997;**35**:1353.
198. Elstrom RL, Andreadis C, Aqui NA, et al. Treatment of PTLD with rituximab or chemotherapy. *Am J Transplant* 2006;**6**:569.
199. Epstein-Barr virus and lymphoproliferative disorders after transplantation. *Am J Transplant* 2004;**4**(Suppl. 10):59.
200. Eriksson M, Lemstrom K, Soujaranta-Ylinen R, et al. Control of early *Aspergillus* mortality after lung transplantation: outcome and risk factors. *Transplant Proc* 2010;**42**:4459–64.
201. Esther CR Jr, Henry MM, Molina PL, et al. Nontuberculous mycobacterial infection in young children with cystic fibrosis. *Pediatr Pulmonol* 2005;**40**:39.
202. Falagas M, Sideri G, Vouloumanou EV, et al. Intravenous colistimethate (Colistin) use in critically ill children without cystic fibrosis. *Pediatr Infect Dis J* 2009;**28**(2):123–7.
203. Faul JL, Akindipe OA, Berry GJ, et al. Influenza pneumonia in a paediatric lung transplant recipient. *Transpl Int* 2000;**13**:79.
204. Feltes TF, Cabalka AK, Meissner HC, et al. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. *J Pediatr* 2003;**143**:532.
205. Fernandez DJ, Torronters SR, Pineda MM, et al. Inhaled aztreonam therapy in patients with cystic fibrosis colonized with *Pseudomonas aeruginosa*. *Spanish Ann Pediatr* 1994;**40**.
206. Feuchtinger T, Matthes-Martin S, Lion T, et al. Safe adoptive transfer of virus-specific T-cell for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. *Br J Haematol* 2006;**134**(1):64–76.
207. Fischer SA, Graham MB, Kuehnert MJ, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation. *N Engl J Med* 2006;**354**:2235.
208. Fisher BT, Zaoutis TE, Park JR, et al. Galactomannan antigen testing for diagnosis of invasive aspergillosis in pediatric hematology patients. *Pediatr Infect Dis J* 2012;**1**(2):103–11.
209. Fishman JA. Treatment of infection due to *Pneumocystis carinii*. *Antimicrob Agents Chemother* 1998;**42**:1309.
210. Fishman JA. Prophylaxis, preemption and drug resistance in CMV infection: too little, too much or just right? *Am J Transplant* 2012;**12**:13–14.
211. Fishman JA, Greenwald MA, Grossi PA. Transmission of infection with human allografts: essential considerations in donor screening. *Clin Infect Dis* 2012;**55**:720.
212. Fishman JA, Rubin RH. Infection in organ-transplant recipients. *N Engl J Med* 1998;**338**:1741.
213. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012;**18**:732–8.
214. Flume PA, Egan TM, Paradowski LJ, et al. Infectious complications of lung transplantation: impact of cystic fibrosis. *Am J Respir Crit Care Med* 1994;**149**:1601.
215. Fortun J, Martin-Davila P, Alvarez ME, et al. *Aspergillus* antigenemia sandwich-enzyme immunoassay test as a serodiagnostic method for invasive aspergillosis in liver transplant recipients. *Transplantation* 2001;**71**:145.
216. Fox BD, Raviv Y, Rozengarten D, et al. Pandemic influenza (H1N1): Impact on lung transplant recipients and candidates. *J Heart Lung Transplant* 2010;**29**:1034–8.
217. Franklin JA, McCormick J, Flynn PM. Retrospective study of the safety of caspofungin in immunocompromised pediatric patients. *Pediatr Infect Dis J* 2003;**22**:747.
218. Frederick R, Aurora P, Hoffman J, et al. Lung transplantation for cystic fibrosis. *Proc Am Thorac Soc* 2009;**6**:619.
219. Freifeld A, Proia L, Andes D. Voriconazole use for endemic fungal infections. *Antimicrob Agents Chemother* 2009;**53**(4):1648–51.
220. Freifeld AG, Wheat LJ, Kaul DR. Histoplasmosis in solid organ transplant recipients: early diagnosis and treatment. *Curr Opin Organ Transplant* 2009;**14**(6):601–5.
221. Freymuth F, Vabret A, Cuvillon-Nimal D, et al. Comparison of multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to hospital with an acute respiratory illness. *J Med Virol* 2006; **78**:1498.
222. Frost AE. Role of infections, pathogenesis, and management in lung transplantation. *Transplant Proc* 1999;**31**:175.
223. Funch DP, Walker AM, Schneider G, et al. Ganciclovir and acyclovir reduce the risk of post-transplant lymphoproliferative disorder in renal transplant recipients. *Am J Transpl* 2005;**5**: 2894–900.
224. Fungal infections. *Am J Transplant* 2004;**4**(Suppl. 10):110.
225. Furth SL, Neu AM, McColley SA, et al. Immune response to influenza vaccination in children with renal disease. *Pediatr Nephrol* 1995;**9**:566.
226. Galgiani JN, Ampel NM, Blair JE, et al: *Coccidioidomycosis. Clin Infect Dis* 2005;**41**:1217.
227. Garantziotis S, Howell DN, McAdams HP, et al. Influenza pneumonia in lung transplant recipients: clinical features and association with bronchiolitis obliterans syndrome. *Chest* 2001; **119**:1277.
228. Garcia-Effron G, Katiyar SK, Park S, et al. A naturally occurring proline-to-alanine amino acid change in Fks1p in *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*. *Antimicrob Agents Chemother* 2008;**52**(7):2305–12.
229. Garlanda C, Hirsch E, Bozza S, et al. Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature* 2002;**420**:182.
230. Garnett CT, Erdman D, Xu W, et al. Prevalence and quantitation of species C adenovirus DNA in human mucosal lymphocytes. *J Virol* 2002;**76**:10608.
231. Gauthier GM, Safdar N, Klein BS, et al. Blastomycosis in solid organ transplant recipients. *Transplant Infect Dis* 2007;**9**:310–7.
232. Gavalda J, Len O, San Juan R, et al. Risk factors for invasive aspergillosis in solid-organ transplant recipients: a case-control study. *Clin Infect Dis* 2005;**41**:52.

233. Gaziano R, Bozza S, Bellocchio S, et al. Anti–*Aspergillus fumigatus* efficacy of pentraxin 3 alone and in combination with antifungals. *Antimicrob Agents Chemother* 2004;**48**:4414.
234. Geist NJP, Egerer G, Burhenne J, et al. Induction of voriconazole metabolism by rifampin in a patient with acute myeloid leukemia: importance of interdisciplinary communication to prevent treatment errors with complex medications. *Antimicrob Agents Chemother* 2007;**51**(9):3455–6.
235. Geller DE, Pitlick WH, Nardella PA, et al. Pharmacokinetics and bioavailability of aerosolized tobramycin in cystic fibrosis. *Chest* 2002;**122**:219.
236. Gerbase MW, Dubois D, Rothmeier C, et al. Costs and outcomes of prolonged cytomegalovirus prophylaxis to cover the enhanced immunosuppression phase following lung transplantation. *Chest* 1999;**116**:1265.
237. Gerna G, Vitulo P, Rovida F, et al. Impact of human metapneumovirus and human cytomegalovirus versus other respiratory viruses on the lower respiratory tract infections of lung transplant recipients. *J Med Virol* 2006;**78**:408.
238. Ghosh S, Champlin RE, Englund J, et al. Respiratory syncytial virus upper respiratory tract illnesses in adult blood and marrow transplant recipients: combination therapy with aerosolized ribavirin and intravenous immunoglobulin. *Bone Marrow Transplant* 2000;**25**:751.
239. Gibson RL, Retsch-Bogart GZ, Oermann C, et al. Microbiology, safety, and pharmacokinetics of aztreonam lysinate for inhalation in patients with cystic fibrosis. *Pediatr Pulmonol* 2006;**41**:656.
240. Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J Clin Virol* 2009;**45**(3):191–5.
241. Glanville AR, Scott AI, Morton JM, et al. Intravenous ribavirin is a safe and cost-effective treatment for respiratory syncytial virus infection after lung transplantation. *J Heart Lung Transplant* 2005;**24**:2114.
242. Goldberg E, Bishara J, Lev S, et al. Organ transplantation from a donor colonized with a multidrug-resistant organism: a case report. *Transpl Infect Dis* 2011;**14**:296–9.
243. Goldman DL, Khine H, Abadi J, et al. Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 2001;**107**:E66.
244. Gordon SM, Avery RK. Aspergillosis in lung transplantation: incidence, risk factors, and prophylactic strategies. *Transpl Infect Dis* 2001;**3**:161.
245. Gorlach J, Fox DS, Cutler NS, et al. Identification and characterization of a highly conserved calcineurin binding protein, CBP1/calcipressin, in *Crypotococcus neoformans*. *EMBO J* 2000;**19**(14):3618–29.
246. Gottlieb J, Mattner F, Weissbrodt H, et al. Impact of graft colonization with gram-negative bacteria after lung transplantation on the development of bronchiolitis obliterans syndrome in recipients with cystic fibrosis. *Respir Med* 2009;**103**(5):743–9.
247. Gourishankar S, McDermid JC, Jhangri GS, et al. Herpes zoster infection following solid organ transplantation: incidence, risk factors and outcomes in the current immunosuppressive era. *Am J Transplant* 2004;**4**:108.
248. Graham JC, Kearns AM, Magee JG, et al. Tuberculosis transmitted through transplantation. *J Infect* 2001;**43**:251.
249. Green M. Management of Epstein-Barr virus–induced posttransplant lymphoproliferative disease in recipients of solid organ transplantation. *Am J Transplant* 2001;**1**:103.
250. Green M, Barbadora K. Recovery of ceftazidime-resistant *Klebsiella pneumoniae* from pediatric liver and intestinal transplant recipients. *Pediatr Transplant* 1998;**2**:224.
251. Green M, Michaels MG, Katz BZ, et al. CMV-IVIG for prevention of Epstein Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. *Am J Transpl* 2006;**6**:1906–12.
252. Green M, Webber SA. EBV viral load monitoring: unanswered questions. *Am J Transplant* 2002;**2**:894.
253. Green M, Webber S. Posttransplantation lymphoproliferative disorders. *Pediatr Clin North Am* 2003;**50**:1471.
254. Greene RE, Schlamm HT, Oestmann JW, et al. Imaging findings in acute invasive pulmonary aspergillosis: clinical significance of the halo sign. *Clin Infect Dis* 2007;**44**:373.
255. Griffith DE. Emergence of nontuberculous mycobacteria as pathogens in cystic fibrosis. *Am J Respir Crit Care Med* 2003;**167**:810.
256. Griffith DE. Nontuberculous mycobacterial lung disease. *Curr Opin Infect Dis* 2010;**23**:185–90.
257. Griffith DE, Aksamit TR. Therapy of refractory nontuberculous mycobacterial lung disease. *Curr Opin Infect Dis* 2012;**25**(2):218–27.
258. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367.
259. Grim SA, Proia L, Miller R, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. *Transpl Infect Dis* 2012;**14**:17–23.
260. Groetzner J, Kaczmarek I, Wittwer T, et al. Caspofungin as first-line therapy for the treatment of invasive aspergillosis after thoracic organ transplantation. *J Heart Lung Transplant* 2008;**27**(1):1–6.
261. Groetzner J, Kur F, Spelsberg F, et al. Airway anastomosis complications in de novo lung transplantation with sirolimus-based immunosuppression. *J Heart Lung Transplant* 2004;**23**:632.
262. Groll AH, Attarbaschi A, Schuster FR, et al. Treatment with caspofungin in immunocompromised paediatric patients: a multicentre survey. *J Antimicrob Chemother* 2006;**57**:527.
263. Groothuis JR. Role of antibody and use of respiratory syncytial virus (RSV) immune globulin to prevent severe RSV disease in high-risk children. *J Pediatr* 1994;**124**:S28.
264. Grosse SD, Rosenfeld M, Devine OJ, et al. Potential impact of newborn screening for cystic fibrosis on child survival: a systematic review and analysis. *J Pediatr* 2006;**149**:362–6.
265. Grossi PA, Fishman JA; AST Infectious Disease Community of Practice. Donor-derived infections in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s19–26.
266. Gu Z, Belzer SW, Gibson CS, et al. Multiplexed, real-time PCR for quantitative detection of human adenovirus. *J Clin Microbiol* 2003;**41**:4636.
267. Gupta A, Singh N. Immune reconstitution syndrome and fungal infections. *Curr Opin Infect Dis* 2011;**24**:527–33.
268. Hadjiliadis D, Howell DN, Davis RD, et al. Anastomotic infections in lung transplant recipients. *Ann Transplant* 2000;**5**:13.
269. Hadjiliadis D, Steele M, Chaparro C, et al. Survival of lung transplant patients with cystic fibrosis harboring panresistant bacteria other than *Burkholderia cepacia*, compared with patients harboring sensitive bacteria. *J Heart Lung Transplant* 2007;**26**(8):834–8.
270. Hafner V, Czock D, Burhenne J, et al. Pharmacokinetics of sulfobutylether-beta-cyclodextrin and voriconazole in patients with end-stage renal failure during treatment with two hemodialysis systems and hemodiafiltration. *Antimicrob Agents Chemother* 2010;**54**(6):2596–602.
271. Hage CA, Davis TE, Fuller D, et al. Diagnosis of histoplasmosis by antigen detection on BAL fluid. *Chest* 2010;**137**(3):623–8.
272. Hage CA, Kirsch EJ, Stump TE, et al. *Histoplasma* antigen clearance during treatment of histoplasmosis in patients with AIDS determined by a quantitative antigen enzyme immunoassay. *Clin Vaccine Immunol* 2011;**18**(4):661–6.
273. Hage CA, Reynolds JM, Durkin M, et al. Plasmalyte as a cause of false positive *Aspergillus* galactomannan in bronchoalveolar lavage fluid. *J Clin Microbiol* 2007;**45**:676–7.
274. Hage CA, Ribes JA, Wengenack NL, et al. A multicenter evaluation of tests for diagnosis of histoplasmosis. *Clin Infect Dis* 2011;**53**(5):448–54.
275. Hahn T, Cummings KM, Michalek AM, et al. Efficacy of high-efficiency particulate air filtration in preventing aspergillosis in immunocompromised patients with hematologic malignancies. *Infect Control Hosp Epidemiol* 2002;**23**:525.
276. Halliday C, Hoile R, Sorrell T, et al. Role of prospective screening of blood for invasive aspergillosis by polymerase chain reaction in febrile neutropenic recipients of haematopoietic stem cell transplants and patients with acute leukaemia. *Br J Haematol* 2006;**132**:478.
277. Haque T, Wilkie GM, Jones MM, et al. Allogeneic cytotoxic T-cell therapy for EBV-positive posttransplantation lymphoproliferative disease: results of a phase 2 multicenter clinical trial. *Blood* 2007;**110**(4):1123–31.

278. Hatton O, Martinez OM, Esquivel CO. Emerging therapeutic strategies for Epstein-Barr virus + post-transplant lymphoproliferative disorder. *Pediatr Transpl* 2012;**16**:220–9.
279. Hauser PM, Bille J, Lass-Florl C, et al. Multicenter, prospective clinical evaluation of respiratory samples from subjects at risk for *Pneumocystis jiroveci* infection by use of a commercial real-time PCR assay. *J Clin Microbiol* 2011;**49**(5):1872–8.
280. Hayes D, Jr. *Mycobacterium abscessus* and other nontuberculous mycobacteria: evolving respiratory pathogens in cystic fibrosis: a case report and review. *South Med J* 2005;**98**:657.
281. Hayney MS, Welter DL, Francois M, et al. Influenza vaccine antibody responses in lung transplant recipients. *Prog Transplant* 2004;**14**:346.
282. Hebart H, Loffler J, Reitze H, et al. Prospective screening by a panfungal polymerase chain reaction assay in patients at risk for fungal infections: implications for the management of febrile neutropenia. *Br J Haematol* 2000;**111**:635.
283. Hedderwick SA, Greenson JK, McGaughy VR, et al. Adenovirus cholecystitis in a patient with AIDS. *Clin Infect Dis* 1998; **26**:997.
284. Heemskerk B, Lankester AC, van Vreeswijk T. Immune reconstitution and clearance of human adenovirus viremia in pediatric stem-cell recipients. *J Infect Dis* 2005;**191**:520–30.
285. Heim A, Ebnet C, Harste G, et al. Rapid and quantitative detection of human adenovirus DNA by real-time PCR. *J Med Virol* 2003;**70**:228.
286. Heinz T, Perfect J, Schell W, et al. Soft-tissue fungal infections: surgical management of 12 immunocompromised patients. *Plast Reconstr Surg* 1996;**97**:1391.
287. Hellenbrand W, Meyer C, Rasch G, et al. Cases of rabies in Germany following organ transplantation. *Euro Surveill* 2005;**10**: E050224.6.
288. Helmi M, Love RB, Welter D, et al. *Aspergillus* infection in lung transplant recipients with cystic fibrosis: risk factors and outcomes comparison to other types of transplant recipients. *Chest* 2003; **123**:800.
289. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002;**347**:408.
290. Herbrecht R, Letscher-Bru V, Oprea C, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002;**20**:1898.
291. Herrera JM, McNeil KD, Higgins RS, et al. Airway complications after lung transplantation: treatment and long-term outcome. *Ann Thorac Surg* 2001;**71**:989.
292. Höcker B, Böhm S, Fickenscher H, et al. (Val-)Ganciclovir prophylaxis reduces Epstein-Barr Virus primary infection in pediatric renal transplantation. *Transpl Int* 2012;**25**(7):723–31.
293. Hoffman JA. Adenoviral disease in pediatric solid organ transplant recipients. *Pediatr Transplant* 2006;**10**:17.
294. Hoffman JA. Adenovirus infections in solid organ transplant recipients. *Curr Opin Organ Transplant* 2009;**14**:625–33.
295. Hoffman JA, Shah AJ, Ross LA, et al. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001;**7**:388.
296. Hoffman J, Walsh T. Echinocandins in children. *Pediatr Infect Dis J* 2011;**30**(6):508–9.
297. Hope WW, Seibel NL, Schwartz CL, et al. Population pharmacokinetics of micafungin in pediatric patients and implications for antifungal dosing. *Antimicrob Agents Chemother* 2007;**51**(10): 3714–9.
298. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. *Lancet Infect Dis* 2005;**5**:609.
299. Hopkins P, McNeil K, Kermeen F, et al. Human metapneumovirus in lung transplant recipients and comparison to respiratory syncytial virus. *Am J Respir Crit Care Med* 2008;**178**:876–81.
300. Horn M, Children's Hospital Los Angeles Cardiothoracic Transplant coordinator, personal communication, May 29, 2012.
301. Hovi L, Saxen H, Saarine-Pihkala UM, et al. Prevention and monitoring of invasive fungal infections in pediatric patients with cancer and hematologic disorders. *Pediatr Blood Cancer* 2007;**48**(1):28–34.
302. Howard SJ, Felton TW, Gomez-Lopez A, et al. Posaconazole: the case for therapeutic drug monitoring. *Ther Drug Monit* 2012; **34**(1):72–6.
303. Huang HC, Weigt S, Derhovanessian A, et al. Non-tuberculous *Mycobacterium* infection after lung transplantation is associated with increased mortality. *J Heart Lung Transplant* 2011;**30**(7): 790–8.
304. Humar A. Screening for West Nile virus: more uncertainty. *Am J Transplant* 2004;**4**:1217.
305. Humar A, Doucette K, Kumar D, et al. Assessment of adenovirus infection in adult lung transplant recipients using molecular surveillance. *J Heart Lung Transplant* 2006;**25**:1441.
306. Humar A, Hebert D, Davies HD, et al. A randomized trial of ganciclovir versus ganciclovir plus immune globulin for prophylaxis against Epstein-Barr virus related posttransplant lymphoproliferative disorder. *Transplantation* 2006;**27**(6):856–61.
307. Humar A, Kumar D, Preiksaitis J, et al. A trial of valganciclovir prophylaxis for cytomegalovirus prevention in lung transplant recipients. *Am J Transplant* 2005;**5**:1462–8.
308. Humar A, Lebrancu Y, Blumberg EA, et al. The efficacy and safety of 200 days valganciclovir cytomegalovirus prophylaxis in high-risk kidney transplant recipients. *Am J Transplant* 2010;**10**: 1228–37.
309. Humar A, Snydman D; AST Infectious Disease Community of Practice. Cytomegalovirus in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s78–86.
310. Husain S, Alexander BD, Munoz P, et al. Opportunistic mycelial fungal infections in organ transplant recipients: emerging importance of non-*Aspergillus* mycelial fungi. *Clin Infect Dis* 2003;**37**:221.
311. Husain S, Chan KM, Palmer SM, et al. Bacteremia in lung transplant recipients in the current era. *Am J Transplant* 2006;**6**: 3000–7.
312. Husain S, Clancy CJ, Nguyen MH, et al. Performance characteristics of the Platelia *Aspergillus* enzyme immunoassay for detection of *Aspergillus* galactomannan antigen in bronchoalveolar lavage fluid. *Clin Vaccine Immunol* 2008;**15**(12):1760–3.
313. Husain S, Kwak EJ, Obman A, et al. Prospective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. *Am J Transplant* 2004;**4**:796.
314. Husain S, Mooney ML, Danziger-Isakov L, et al. A 2010 working formulation for the standardization of definitions of infections in cardiothoracic transplant recipients. *J Heart Lung Transplant* 2011;**30**(4):361–74.
315. Husain S, Paterson DL, Studer S, et al. Voriconazole prophylaxis in lung transplant recipients. *Am J Transplant* 2006;**6**:3008.
316. Husain S, Paterson DL, Studer SM, et al. *Aspergillus* galactomannan antigen in the bronchoalveolar lavage fluid for the diagnosis of invasive aspergillosis in lung transplant recipients. *Transplantation* 2007;**83**(10):1330–6.
317. Husain S, Singh N. *Burkholderia cepacia* infection and lung transplantation. *Semin Respir Infect* 2002;**17**:284.
318. Husain S, Singh N. Bronchiolitis obliterans and lung transplantation: evidence for an infectious etiology. *Semin Respir Infect* 2002;**17**:310.
319. Husain S, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis* 2001;**7**:375.
320. Husain S, Zaldonis D, Kusne S, et al. Variation in antifungal prophylaxis strategies in lung transplantation. *Transpl Infect Dis* 2006;**8**:213.
321. Husni RN, Gordon SM, Longworth DL, et al. Cytomegalovirus infection is a risk factor for invasive aspergillosis in lung transplant recipients. *Clin Infect Dis* 1998;**26**:753.
322. Iseman MD, Heifets LB. Rapid detection of tuberculosis and drug-resistant tuberculosis. *N Engl J Med* 2006;**355**:1606.
323. Isles A, Maclusky I, Corey M, et al. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* 1984;**104**(2): 206–10.
324. Ison MG, Michaels MG; AST Infectious Disease Community of Practice. RNA respiratory viral infections in solid organs transplant recipients. *Am J Transpl* 2009;**9**(Suppl 4):s166–72.
325. Ison MG, Szakaly P, Shapira MY, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**(6):955–64.
326. Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Engl J Med* 2003;**348**:2196.

327. Jacobs F, Knoop C, Brancart F, et al. Human herpesvirus-6 infection after lung and heart-lung transplantation: a prospective longitudinal study. *Transplantation* 2003;**75**:1996.
328. Jereb JA, Burwen DR, Dooley SW, et al. Nosocomial outbreak of tuberculosis in a renal transplant unit: application of a new technique for restriction fragment length polymorphism analysis of *Mycobacterium tuberculosis* isolates. *J Infect Dis* 1993;**168**:1219.
329. Jernigan MG, Press EG, Hong Nguyen M, et al. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2012;**56**(6):3395–8.
330. Johnson MD, MacDougall C, Ostrosky-Zeichner L, et al. Combination antifungal therapy. *Antimicrob Agents Chemother* 2004; **48**:693.
331. Jones AM, Dodd ME, Govan JR, et al. *Burkholderia cenocepacia* and *Burkholderia multivorans*: influence on survival in cystic fibrosis. *Thorax* 2004;**59**:948.
332. Jones ME, Fox AJ, Barnes AJ, et al. PCR-ELISA for the early diagnosis of invasive pulmonary *Aspergillus* infection in neutropenic patients. *J Clin Pathol* 1998;**51**:652.
333. Jordanides NE, Allan EK, McLintock LA, et al. A prospective study of real-time panfungal PCR for the early diagnosis of invasive fungal infection in haemato-oncology patients. *Bone Marrow Transplant* 2005;**35**:389.
334. Kami M, Fukui T, Ogawa S, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis* 2001;**33**:1504.
335. Kanj SS, Tapson V, Davis RD, et al. Infections in patients with cystic fibrosis following lung transplantation. *Chest* 1997;**112**:924.
336. Kauffman CA. Endemic mycoses: blastomycosis, histoplasmosis, and sporotrichosis. *Infect Dis Clin North Am* 2006;**20**:645.
337. Kasztelewicz B, Jankowska I, Pawlowska J, et al. Epstein-Barr virus DNA load in peripheral blood mononuclear cells and whole blood from pediatric transplant recipients. *Transpl Infect Dis* 2011;**13**:471–9.
338. Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004;**42**:2733.
339. Keckich DW, Blair JE, Vikram HR, et al. Reactivation of coccidioidomycosis despite antifungal prophylaxis in solid organ transplant recipients. *Transplantation* 2011;**92**(1):88–93.
340. Kesten S, Chaparro C. Mycobacterial infections in lung transplant recipients. *Chest* 1999;**115**:741.
341. Khalifah AP, Hachem RR, Chakinala MM, et al. Respiratory viral infections are a distinct risk for bronchiolitis obliterans syndrome and death. *Am J Respir Crit Care Med* 2004;**170**:181.
342. Kiberd BA, Forward K. Screening for West Nile virus in organ transplantation: a medical decision analysis. *Am J Transplant* 2004; **4**:1296.
343. Kimberlin DW, Shalabi M, Abzug MJ, et al. Safety of oseltamivir compared with the adamantanes in children less than 12 months of age. *Pediatr Infect Dis J* 2010;**29**(3):195–8.
344. Kirby A, Hassan I, Burnie J. Recommendations for managing *Aspergillus* osteomyelitis and joint infections based on a review of the literature. *J Infect* 2006;**52**:405.
345. Kleinschmidt-DeMasters BK, Marder BA, Levi ME, et al. Naturally acquired West Nile virus encephalomyelitis in transplant recipients: clinical, laboratory, diagnostic, and neuropathological features. *Arch Neurol* 2004;**61**:1210.
346. Klingspor L, Jalal S. Molecular detection and identification of *Candida* and *Aspergillus* spp. from clinical samples using real-time PCR. *Clin Microbiol Infect* 2006;**12**:745.
347. Klont RR, Mennink-Kersten MA, Verweij PE. Utility of *Aspergillus* antigen detection in specimens other than serum specimens. *Clin Infect Dis* 2004;**39**:1467.
348. Knoll BM, Kappagoda S, Gill RR, et al. Non-tuberculous mycobacterial infection among lung transplant recipients: a 15-year cohort study. *Transpl Infect Dis* 2012;**14**:452–60.
349. Kohno S, Masaoka T, Yamaguchi H, et al. A multicenter, open-label clinical study of micafungin (FK463) in the treatment of deep-seated mycosis in Japan. *Scand J Infect Dis* 2004;**36**:372.
350. Koneru B, Jaffe R, Esquivel CO, et al. Adenoviral infections in pediatric liver transplant recipients. *JAMA* 1987;**258**:489.
351. Kontoyiannis DP, Boktour M, Hanna H, et al. Itraconazole added to a lipid formulation of amphotericin B does not improve outcome of primary treatment of invasive aspergillosis. *Cancer* 2005;**103**:2334.
352. Kontoyiannis DP, Hachem R, Lewis RE, et al. Efficacy and toxicity of caspofungin in combination with liposomal amphotericin B as primary or salvage treatment of invasive aspergillosis in patients with hematologic malignancies. *Cancer* 2003;**98**:292.
353. Kontoyiannis DP, Lewis RE, Alexander BD, et al. Calcineurin inhibitor agents interact synergistically with antifungal agents in vitro against *Cryptococcus neoformans* isolates: correlation with outcome in solid organ transplant recipients with cryptococcosis. *Antimicrob Agents Chemother* 2008;**52**(2):735–8.
354. Kontoyiannis DP, Ratanatharathorn V, Young J-A, et al. Micafungin alone or in combination with other systemic antifungal therapies in hematopoietic stem cell transplant recipients with invasive aspergillosis. *Trans Infect Dis* 2009;**11**:89–93.
355. Koo S, Baden LR, Marty FM. Post-diagnostic kinetics of the (1→3)-β-D-glucan assay in invasive aspergillosis, invasive candidiasis and *Pneumocystis jirovecii* pneumonia. *Clin Microbiol Infect* 2012;**18**:e122–e127.
356. Kotloff RM, Zuckerman JB. Lung transplantation for cystic fibrosis: special considerations. *Chest* 1996;**109**:787.
357. Kotton CN. Zoonoses in solid-organ and hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2007;**44**:857.
358. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010;**89**(7):779–95.
359. Kourkoumpetis TK, Burgwyn Fuchs B, Coleman JJ, et al. Polymerase chain reaction-based assays for the diagnosis of invasive fungal infection. *Clin Infect Dis* 2012;**54**(9):1322–31.
360. Krishna G, Sansone-Parsons A, Martinho M, et al. Posaconazole plasma concentrations in juvenile patients with invasive fungal infection. *Antimicrob Agents Chemother* 2007;**51**:812.
361. Kshettry VR, Kroshus TJ, Hertz MI, et al. Early and late airway complications after lung transplantation: incidence and management. *Ann Thorac Surg* 1997;**63**:1576.
362. Kubak BM. Fungal infection in lung transplantation. *Transpl Infect Dis* 2002;**4**(Suppl. 3):24.
363. Kubak BM, Huprikar SS; AST Infectious Disease Community of Practice. Emerging & rare fungal infections in solid organ transplant recipients. *Transplantation* 2009;**9**(Suppl 4):s208–26.
364. Kumar D, Blumberg EA, Danziger-Isakov L, et al. Influenza vaccination in the organ transplant recipient: review and summary recommendations. *Am J Transplant* 2011;**11**:2020–30.
365. Kumar D, Drebot MA, Wong SJ, et al. A seroprevalence study of West Nile virus infection in solid organ transplant recipients. *Am J Transplant* 2004;**4**:1883.
366. Kumar D, Erdman D, Keshavjee S, et al. Clinical impact of community-acquired respiratory viruses on bronchiolitis obliterans after lung transplant. *Am J Transplant* 2005;**5**:2031.
367. Kumar S, Henrickson J. Update in influenza diagnostics: lessons from the novel H1N1 influenza A pandemic. *Clin Microbiol Rev* 2012;**25**(2):344–61.
368. Kumar D, Michaels MG, Morris M. Outcomes from pandemic influenza A H1N1 infection in recipients of solid-organ transplant: a multicenter cohort study. *Lancet Infect Dis* 2010;**10**: 521–6.
369. Kumar D, Morris MI, Kotton CN. Guidance on novel influenza A/H1N1 in solid organ transplant recipients. *Am J Transplant* 2010;**10**:18–25.
370. Kumar D, Prasad GV, Zaltzman J, et al. Community-acquired West Nile virus infection in solid-organ transplant recipients. *Transplantation* 2004;**77**:399.
371. Kuypers J, Wright N, Ferrenberg J, et al. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. *J Clin Microbiol* 2006; **44**:2382.
372. Kwak EJ, Husain S, Obman A, et al. Efficacy of galactomannan antigen in the Platelia *Aspergillus* enzyme immunoassay for diagnosis of invasive aspergillosis in liver transplant recipients. *J Clin Microbiol* 2004;**42**:435.
373. Lang BJ, Aaron SD, Ferris W, et al. Multiple combination bactericidal antibiotic testing for patients with cystic fibrosis infected with multiresistant strains of *Pseudomonas aeruginosa*. *Am J Respir Crit Care Med* 2000;**162**:2241.

374. Lanternier F, Sun HY, Ribaud P, et al. Mucormycosis in organ and stem cell transplant recipients. *Clin Infect Dis* 2012;**54**(11): 1629–36.
375. Larcher C, Geltner C, Fischer H, et al. Human metapneumovirus infection in lung transplant recipients: clinical presentation and epidemiology. *J Heart Lung Transplant* 2005;**24**:1891.
376. Lass-Florl C, Aigner J, Gunsilius E, et al. Screening for *Aspergillus* spp. using polymerase chain reaction of whole blood samples from patients with haematological malignancies. *Br J Haematol* 2001;**113**:180.
377. Laus S, Kingsley LA, Green M, et al. Comparison of QIAsymphony automated and QIAamp manual DNA extraction systems for measuring Epstein-Barr virus DNA load in whole blood using real-time PCR. *J Mol Diagn* 2011;**13**(6):695–700.
378. Lautenschlager I, Razonable RR. Human herpesvirus–6 infections in kidney, liver, lung and heart transplantation: review. *Transpl Int* 2012;**25**:493–502.
379. Leblond V, Davi F, Charlotte F, et al. Posttransplant lymphoproliferative disorders not associated with Epstein-Barr virus: a distinct entity? *J Clin Oncol* 1998;**16**:2052.
380. Ledson MJ, Gallagher MJ, Corkill JE, et al. Cross infection between cystic fibrosis patients colonised with *Burkholderia cepacia*. *Thorax* 1998;**53**:432.
381. Ledson MJ, Gallagher MJ, Jackson M, et al. Outcome of *Burkholderia cepacia* colonisation in an adult cystic fibrosis centre. *Thorax* 2002;**57**:142.
382. Lee SO, Brown RA, Razonable RR, et al. Chromosomally integrated human herpesvirus–6 in transplant recipients. *Transpl Infect Dis* 2012;**14**:346.
383. Lee TC, Savoldo B, Rooney CM, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. *Am J Transplant* 2005;**5**(9):2222–8.
384. LeGal S, Damiani C, Rouille A, et al. A cluster of *Pneumocystis* infections among renal transplant recipients: molecular evidence of colonized patients as potential infectious sources of *Pneumocystis jirovecii*. *Clin Infect Dis* 2012;**54**(7):e62–71.
385. Legrand F, Berrebi D, Houhou N, et al. Early diagnosis of adenovirus infection and treatment with cidofovir after bone marrow transplantation in children. *Bone Marrow Transplant* 2001;**27**:621.
386. Lehto JT, Halme M, Tukiainen P, et al. Human herpesvirus–6 and –7 after lung and heart-lung transplantation. *J Heart Lung Transplant* 2007;**26**:41.
387. Leruez-Ville M, Minard V, Lacaille F, et al. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. *Clin Infect Dis* 2004;**38**:45.
388. Leung AY, Chan M, Cheng VC, et al. Quantification of adenovirus in the lower respiratory tract of patients without clinical adenovirus-related respiratory disease. *Clin Infect Dis* 2005;**40**:1541.
389. Levy I, Grisaru-Soen G, Lerner-Geva L, et al. Multicenter cross-sectional study of nontuberculous mycobacterial infections among cystic fibrosis patients, Israel. *Emerg Infect Dis* 2008; **14**(3):378–84.
390. Lewis RE, Kontoyiannis DP. Rationale for combination antifungal therapy. *Pharmacotherapy* 2001;**21**:149S.
391. Li L, Avery R, Budev M, et al. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. *J Heart Lung Transplant* 2012;**31**:839.
392. Lim GY, Newman B, Kurland G, et al. Posttransplantation lymphoproliferative disorder: manifestations in pediatric thoracic organ recipients. *Radiology* 2002;**222**:699.
393. Limaye AP, Connolly PA, Sagar M, et al. Transmission of *Histoplasma capsulatum* by organ transplantation. *N Engl J Med* 2000; **343**:1163.
394. Linsangan LC, Hoffman JA, Woo MS, MacLaughlin EF, et al. *Infectious complications in pediatric lung and heart lung transplantation, including living donors.* From Los Angeles, Childrens Hospital Los Angeles, Keck School of Medicine, University of Southern California. Unpublished data. 2006.
395. Linssen CF, Jacobs JA, Beckers P, et al. Inter-laboratory comparison of three different real-time PCR assays for the detection of *Pneumocystis jiroveci* in bronchoalveolar lavage fluid samples. *J Med Microbiol* 2006;**55**:1229.
396. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood* 2003;**102**:1114.
397. LiPuma JJ. *Burkholderia cepacia* complex: a contraindication to lung transplantation in cystic fibrosis? *Transpl Infect Dis* 2001;**3**:149.
398. LiPuma JJ. Update on the *Burkholderia cepacia* complex. *Curr Opin Pulm Med* 2005;**11**:528.
399. LiPuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;**23**(2):299–323.
400. Liu M, Mallory GB, Schecter MG, et al. Long-term impact of respiratory viral infection after pediatric lung transplantation. *Pediatr Transplant* 2010;**14**:431–6.
401. Liu M, Worley S, Arrigain S, et al. Respiratory viral infections within one year after pediatric lung transplant. *Transpl Infect Dis* 2009;**11**:304–12.
402. Liu M, Worley S, Mallory GB, et al. Fungal infections in pediatric lung transplant recipients: colonization and invasive disease. *J Heart Lung Transplant* 2009;**28**(11):1226–30.
403. Ljungman P, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis* 2002;**34**:1094.
404. Ljungman P, Ribaud P, Eyrich M, et al. Cidofovir for adenovirus infections after allogeneic hematopoietic stem cell transplantation: a survey by the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2003;**31**:481.
405. Lockhart SR, Wagner D, Iqbal N, et al. Comparison of in vitro susceptibility characteristics of *Candida* species from cases of invasive candidiasis in solid organ and stem cell transplant recipients: Transplant-Associated Infections Surveillance Network (TRANSNET), 2001 to 2006. *Clin Microbiol* 2011;**49**(7):2404–10.
406. Loeffler J, Hebart H, Brauchle U, et al. Comparison between plasma and whole blood specimens for detection of *Aspergillus* DNA by PCR. *J Clin Microbiol* 2000;**38**:3830.
407. Luong ML, Clancy CJ, Vadnerkar A, et al. Comparison of an *Aspergillus* real-time polymerase chain reaction assay with galactomannan testing of bronchoalveolar lavage fluid for the diagnosis of invasive pulmonary aspergillosis in lung transplant recipients. *Clin Infect Dis* 2011;**52**(10):1218–26.
408. Luong ML, Hosseini-Moghaddam SM, Singer LG, et al. Risk factors for voriconazole hepatotoxicity at 12 weeks in lung transplant recipients. *Am J Transplant* 2012;**12**(7):1929–35.
409. Luppi M, Barozzi P, Schulz TF, et al. Bone marrow failure associated with human herpesvirus 8 infection after transplantation. *N Engl J Med* 2000;**343**:1378.
410. Macedo C, Webber SA, Donnenberg AD, et al. EBV-specific $CD8^+$ T cells from asymptomatic pediatric thoracic transplant patients carrying chronic high EBV loads display contrasting features: activated phenotype and exhausted function. *J Immunol* 2011;**186**:5854–62.
411. Macedo C, Zeevi A, Bentlejewski C, et al. The impact of EBV load on T cell immunity in pediatric thoracic transplant recipients. *Transplantation* 2009;**88**(1):123–8.
412. Machetti M, Furfaro E, Viscoli C. Galactomannan in piperacillin-tazobactam: how much and to what extent? *Antimicrob Agents Chemother* 2005;**49**:3984.
413. Machetti M, Zotti M, Veroni L, et al. Antigen detection in the diagnosis and management of a patient with probable cerebral aspergillosis treated with voriconazole. *Transpl Infect Dis* 2000; **2**:140.
414. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children. *Pediatr Infect Dis J* 2011;**30**(8):694–700.
415. Maecker B, Schirg E, et al. Post transplant lymphoproliferative disease in pediatric solid organ transplant patients: a possible role for [^{18}F]-FDG-PET(/CT) in initial staging and therapy monitoring. *Eur J Radiol* 2007;**63**(3):427–35.
416. Maertens J, Buve K, Theunissen K, et al. Galactomannan serves as a surrogate endpoint for outcome of pulmonary invasive aspergillosis in neutropenic hematology patients. *Cancer* 2008;**115**: 355–62.
417. Maertens J, Glasmacher A, Herbrecht R, et al. Multicenter, noncomparative study of caspofungin in combination with other antifungals as salvage therapy in adults with invasive aspergillosis. *Cancer* 2006;**107**:2888.

418. Maertens J, Raad I, Petrikkos G, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004;**39**:1563.
419. Maertens J, Theunissen K, Verhoef G, et al. False-positive *Aspergillus* galactomannan antigen test results. *Clin Infect Dis* 2004; **39**:289.
420. Maertens J, Theunissen K, Verhoef G, et al. Galactomannan and computed tomography–based preemptive antifungal therapy in neutropenic patients at high risk for invasive fungal infection: a prospective feasibility study. *Clin Infect Dis* 2005;**41**:1242.
421. Maertens J, Van Eldere J, Verhaegen J, et al. Use of circulating galactomannan screening for early diagnosis of invasive aspergillosis in allogeneic stem cell transplant recipients. *J Infect Dis* 2002;**186**:1297.
422. Maertens J, Verhaegen J, Demuynck H, et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. *J Clin Microbiol* 1999;**37**:3223.
423. Maertens J, Verhaegen J, Lagrou K, et al. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001;**97**:1604.
424. Mahenthiralingam E, Vandamme P, Campbell ME, et al. Infection with *Burkholderia cepacia* complex genomovars in patients with cystic fibrosis: virulent transmissible strains of genomovar III can replace *Burkholderia multivorans*. *Clin Infect Dis* 2001;**33**:1469.
425. Mainz JG, Hentschel J, Schien C, et al. Sinonasal persistence of *Pseudomonas aeruginosa* after lung transplantation. *J Cyst Fibros* 2012;**11**:158–61.
426. Malavaud S, Malavaud B, Sandres K, et al. Nosocomial outbreak of influenza virus A (H3N2) infection in a solid organ transplant department. *Transplantation* 2001;**72**:535.
427. Malloy AM, Campos JM. Extended-spectrum beta-lactamases—a brief clinical update. *Pediatr Infect Dis* 2011;**30**(12):1092–3.
428. Malouf MA, Glanville AR. The spectrum of mycobacterial infection after lung transplantation. *Am J Respir Crit Care Med* 1999;**160**:1611.
429. Marr KA, Balajee SA, McLaughlin L, et al. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004;**190**:641.
430. Marr KA, Boeckh M, Carter RA, et al. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004;**39**:797.
431. Marr KA, Hachem R, Papanicolaou G, et al. Retrospective study of the hepatic safety profile of patients concomitantly treated with caspofungin and cyclosporin A. *Transpl Infect Dis* 2004;**6**:110.
432. Marr KA, Laverdiere M, Gugel A, et al. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassay. *Clin Infect Dis* 2005;**40**:1762.
433. Marshall SE, Kramer MR, Lewiston NJ, et al. Selection and evaluation of recipients for heart-lung and lung transplantation. *Chest* 1990;**98**:1488.
434. Marsik FJ, Nambiar S. Review of carbapenemases and AmpC-beta lactamases. *Pediatr Infect Dis* 2011;**30**(12):1094–5.
435. Martin M, Goyette N, Ives J, et al. Incidence and characterization of cytomegalovirus resistance mutation among pediatric solid organ transplant patients who received valganciclovir prophylaxis. *J Clin Virol* 2010;**47**:321–4.
436. Martin SI, Fishman JA; AST Infectious Disease Community of Practice. *Pneumocystis* pneumonia in solid organ transplant recipients. *Transplantation* 2009;**9**(Suppl 4):s227–33.
437. Martins N, Martins IS, de Freitas JA, et al. Severe infection in a lung transplant recipient caused by donor-transmitted carbapenem-resistant *Acinetobacter baumannii*. *Transpl Infect Dis* 2011;**14**: 316–20.
438. Marty FM, Lowry CM, Cutler CS, et al. Voriconazole and sirolimus coadministration after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006;**12**:552.
439. Marty FM, Lowry CM, Lempitski SJ, et al. Reactivity of (1→3)-beta-D-glucan assay with commonly used intravenous antimicrobials. *Antimicrob Agents Chemother* 2006;**50**:3450.
440. Marty FM, Rubin RH. The prevention of infection post-transplant: the role of prophylaxis, preemptive and empiric therapy. *Transpl Int* 2006;**19**:2.
441. Mathis AS, Shah NK, Friedman GS. Combined use of sirolimus and voriconazole in renal transplantation: a report of two cases. *Transplant Proc* 2004;**36:**2708.
442. Mazurek GH, Jereb J, Lobue P, et al. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep* 2005;**54** (RR-15):49.
443. Mazurek GH, Jereb J, Vernon A, et al. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States 2010. *MMWR Recomm Rep* 2010;**59**(RR-5):1–25.
444. McCurdy LH, Milstone A, Dummer S. Clinical features and outcomes of paramyxoviral infection in lung transplant recipients treated with ribavirin. *J Heart Lung Transplant* 2003;**22**:745.
445. McDiarmid SV, Blumberg DA, Remotti H, et al. Mycobacterial infections after pediatric liver transplantation: a report of three cases and review of the literature. *J Pediatr Gastroenterol Nutr* 1995;**20**:425.
446. McLaughlin GE, Delis S, Kashimawo L, et al. Adenovirus infection in pediatric liver and intestinal transplant recipients: utility of DNA detection by PCR. *Am J Transplant* 2003;**3**:224.
447. McMenamin JD, Zaccone TM, Coenye T, et al. Misidentification of *Burkholderia cepacia* in US cystic fibrosis treatment centers: an analysis of 1,051 recent sputum isolates. *Chest* 2000;**117**:1661.
448. Mehrad B, Paciocco G, Martinez FJ, et al. Spectrum of *Aspergillus* infection in lung transplant recipients: case series and review of the literature. *Chest* 2001;**119**:169.
449. Meissner HC, Welliver RC, Chartrand SA, et al. Immunoprophylaxis with palivizumab, a humanized respiratory syncytial virus monoclonal antibody, for prevention of respiratory syncytial virus infection in high risk infants: a consensus opinion. *Pediatr Infect Dis J* 1999;**18**:223.
450. Mendez JC, Dockrell DH, Espy MJ, et al. Human beta-herpesvirus interactions in solid organ transplant recipients. *J Infect Dis* 2001;**183**:179.
451. Mennink-Kersten MA, Donnelly JP, Verweij PE. Detection of circulating galactomannan for the diagnosis and management of invasive aspergillosis. *Lancet Infect Dis* 2004;**4**:349.
452. Mennink-Kersten MA, Verweij PE. Non–culture-based diagnostics for opportunistic fungi. *Infect Dis Clin North Am* 2006; **20**:711.
453. Merlin E, Galambrun C, Ribaud P, et al. Efficacy and safety of caspofungin therapy in children with invasive fungal infections. *Pediatr Infect Dis J* 2006;**25**:1186.
454. Miceli MH, Maertens J, Buve K, et al. Immune reconstitution inflammatory syndrome in cancer patients with pulmonary aspergillosis recovering from neutropenia: proof of principle, description, and clinical and research implications. *Cancer* 2007;**110**(1): 112–20.
455. Michaels MG, Green M, Wald ER, et al. Adenovirus infection in pediatric liver transplant recipients. *J Infect Dis* 1992;**165**:170.
456. Michalopoulos A, Papadakis E. Inhaled anti-infective agents: emphasis on colistin. *Infection* 2010;**38**:81–8.
457. Mikulska M, Furfaro E, Del Bono V, et al. Piperacillin/tazobactam (Tazocin) seems to be no longer responsible for false-positive results of the galactomannan assay. *J Antimicrob Chemother* 2012; **67**:1746–8.
458. Miller MB, Hendren R, Gilligan PH. Posttransplantation disseminated coccidioidomycosis acquired from donor lungs. *J Clin Microbiol* 2004;**42**:2347.
459. Millon L, Piarroux R, Deconinck E, et al. Use of real-time PCR to process the first galactomannan-positive serum sample in diagnosing invasive aspergillosis. *J Clin Microbiol* 2005;**43**:5097.
460. Milpied N, Vasseur B, Parquet N, et al. Humanized anti-CD20 monoclonal antibody (rituximab) in post transplant B-lymphoproliferative disorder: a retrospective analysis on 32 patients. *Ann Oncol* 2000;**11**(Suppl. 1):113.
461. Minari A, Husni R, Avery RK, et al. The incidence of invasive aspergillosis among solid organ transplant recipients and implications for prophylaxis in lung transplants. *Transpl Infect Dis* 2002;**4**:195.
462. Miraldi F, Anile M, Ruberto F, et al. *Scedosporium apiospermum* atrial mycetomas after lung transplantation for cystic fibrosis. *Transpl Infect Dis* 2012;**14**(2):188–9.
463. Mitsani D, Hong Nguyen H, Kwak EJ, et al. Cytomegalovirus disease among donor-positive/recipient-negative lung transplant

recipients in the era of valganciclovir prophylaxis. *Heart Lung Transplant* 2010;**29**:1014–20.
464. Mitsani D, Nguyen MH, Shields RK, et al. Prospective, observational study of voriconazole therapeutic drug monitoring among lung transplant recipients receiving prophylaxis: factors impacting levels of and associations between serum, troughs, efficacy, and toxicity. *Antimicrob Agents Chemother* 2012;**56**(5): 2371–7.
465. Miyazaki T, Kohno S, Mitsutake K, et al. Plasma (1→3)-beta-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. *J Clin Microbiol* 1995;**33**:3115.
466. Mohd Hafiz AA, Staatz CE, Kirkpatrick CMJ, et al. Continuous infusion vs. bolus dosing: implications for beta-lactam antibiotics. *Minerva Anestesiol* 2011;**77**(4):1–11.
467. Monforte V, Roman A, Gavalda J, et al. Nebulized amphotericin B prophylaxis for *Aspergillus* infection in lung transplantation: study of risk factors. *J Heart Lung Transplant* 2001;**20**:1274.
468. Monforte V, Ussetti P, Gavalda J, et al. Feasibility, tolerability, and outcomes of nebulized liposomal amphotericin B for *Aspergillus* infection prevention in lung transplantation. *J Heart Lung Transplant* 2010;**29**:523–20.
469. Moore DA, Evans CA, Gilman RH, et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *N Engl J Med* 2006;**355**:1539.
470. Morales P, Briones A, Torres JJ, et al. Pulmonary tuberculosis in lung and heart-lung transplantation: fifteen years of experience in a single center in Spain. *Transplant Proc* 2005;**37**:4050.
471. Moore PS. The emergence of Kaposi's sarcoma-associated herpesvirus (human herpesvirus 8). *N Engl J Med* 2012;**343**(19): 1411–13.
472. Moriyama B, Henning S, Childs R, et al. High-dose continuous infusion β-lactam antibiotics for the treatment of resistant *Pseudomonas aeruginosa* infections in immunocompromised patients. *Pharmacotherapy* 2010;**44**:929–35.
473. Morris AM, Masur H. A serologic test to diagnose *Pneumocystis* pneumonia: Are we there yet? *Clin Infect Dis* 2011;**53**(2):203–4.
474. Muhammad Iqbal AH, Lim SK, Ng KP, et al. *Pneumocystis jirovecii* pneumonia 13 years post renal transplant following a recurrent cytomegalovirus infection. *Transpl Infect Dis* 2012;**14**(4):E23–6.
475. Mukherjee PK, Sheehan DJ, Hitchcock CA, et al. Combination treatment of invasive fungal infections. *Clin Microbiol Rev* 2005; **18**:163.
476. Multiply antibiotic-resistant gram-negative bacteria. *Am J Transplant* 2004;**4**(Suppl. 10):21.
477. Munoz P, Rodriguez C, Bouza E. *Mycobacterium tuberculosis* infection in recipients of solid organ transplants. *Clin Infect Dis* 2005; **40**:581.
478. Munoz P, Singh N, Bouza E. Treatment of solid organ transplant patients with invasive fungal infections: should a combination of antifungal drugs be used? *Curr Opin Infect Dis* 2006;**19**:365.
479. Murray S, Charbeneau J, Marshall BC, et al. Impact of *Burkholderia* infection on lung transplantation in cystic fibrosis. *Am J Respir Crit Care Med* 2008;**178**:363–71.
480. Musher B, Fredricks D, Leisenring W, et al. *Aspergillus* galactomannan enzyme immunoassay and quantitative PCR for diagnosis of invasive aspergillosis with bronchoalveolar lavage fluid. *J Clin Microbiol* 2004;**42**:5517.
481. Nagafuji K, Aoki K, Henzan H, et al. Cidofovir for treating adenoviral hemorrhagic cystitis in hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2004;**34**:909.
482. Nathan SD, Shorr AF, Schmidt ME, et al. *Aspergillus* and endobronchial abnormalities in lung transplant recipients. *Chest* 2000;**118**:403.
483. Nash EF, Coonar A, Kremer R, et al. Survival of *Burkholderia cepacia* sepsis following lung transplantation in recipients with cystic fibrosis. *Transplant Infect Dis* 2010;**12**:551–4.
484. Nash KA, Brown-Elliott BA, Wallace Jr RJ. A novel gene, *erm*(41), confers inducible macrolide resistance to clinical isolates of *Mycobacterium abscessus* but is absent from *Mycobacterium chelonae*. *Antimicrob Agents Chemother* 2009;**53**(4):1367–76.
485. Neely M, Rushing T, Kovacs A, et al. Voriconazole pharmacokinetics and pharmacodynamics in children. *Clin Infect Dis* 2010; **50**:27–36.
486. Neofytos D, Fishman JA, Horn D, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. *Transpl Infect Dis* 2010;**12**:220–9.
487. Neofytos D, Lombardi LR, Shields RK, et al. Administration of voriconazole in patients with renal dysfunction. *Clin Infect Dis* 2012;**54**(7):913–21.
488. Neoh CF, Snell GI, Kotsimbos T, et al. Antifungal prophylaxis in lung transplantation—a world-wide survey. *Transplantation J* 2011;**11**:361–6.
489. Ner Z, Ross LA, Horn MV, et al. *Bordetella bronchiseptica* infection in pediatric lung transplant recipients. *Pediatr Transplant* 2003; **7**:413.
490. Neurohr C, Huppmann P, Leuchte H, et al. Human herpesvirus–6 in bronchoalveolar lavage fluid after lung transplantation: a risk factor for bronchiolitis obliterans syndrome? *Am J Transplant* 2005;**5**:2982.
491. Ng BJH, Glanville AR, Snell G, et al. The impact of pandemic influenza A H1N1 2009 on Australian lung transplant recipients. *Am J Transplant* 2011;**11**:568–74.
492. Nguyen HM, Wissel MC, Shields RK, et al. Performance of *Candida* real-time polymerase chain reaction, β-D-glucan assay, and blood cultures in the diagnosis in invasive candidiasis. *Clin Infect Dis* 2012;**54**(9):1240–8.
493. Nivoix Y, Velten M, Letscher-Bru V, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. *Clin Infect Dis* 2008;**47**:1176–98.
494. Nivoix Y, Zamfir A, Lutun P, et al. Combination of caspofungin and an azole or an amphotericin B formulation in invasive fungal infections. *J Infect* 2006;**52**:67.
495. Nouer SA, Nucci M, Sanath Kumar N, et al. Earlier response assessment in invasive aspergillosis based on the kinetics of serum *Aspergillus* galactomannan: proposal for a new definition. *Clin Infect Dis* 2011;**54**(7):671–6.
496. Nucci M, Anaissie E, Betts RF, et al. Early removal of central venous catheter in patients with candidemia does not improve outcome: analysis of 842 patients from 2 randomized clinical trials. *Clin Infect Dis* 2010;**51**(3):295–303.
497. Nunley DR, Gal AA, Vega JD, et al. Saprophytic fungal infections and complications involving the bronchial anastomosis following human lung transplantation. *Chest* 2002;**122**:1185.
498. Nunley DR, Grgurich W, Iacono AT, et al. Allograft colonization and infections with *Pseudomonas* in cystic fibrosis lung transplant recipients. *Chest* 1998;**113**:1235.
499. Nunley DR, Ohori P, Grgurich WF, et al. Pulmonary aspergillosis in cystic fibrosis lung transplant recipients. *Chest* 1998;**114**:1321.
500. Odabasi Z, Mattiuzzi G, Estey E, et al. Beta-D-glucan as a diagnostic adjunct for invasive fungal infections: validation, cutoff development, and performance in patients with acute myelogenous leukemia and myelodysplastic syndrome. *Clin Infect Dis* 2004;**39**:199.
501. Odio CM, Araya R, Pinto LE, et al. Caspofungin therapy of neonates with invasive candidiasis. *Pediatr Infect Dis J* 2004; **23**:1093.
502. Oertel SH, Verschuuren E, Reinke P, et al. Effect of anti-CD 20 antibody rituximab in patients with post-transplant lymphoproliferative disorder (PTLD). *Am J Transplant* 2005;**5**:2901.
503. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis* 2011;**52**:1087–99.
504. Ohori NP, Michaels MG, Jaffe R, et al. Adenovirus pneumonia in lung transplant recipients. *Hum Pathol* 1995;**26**:1073.
505. Oliver A, Maiz L, Canton R, et al. Nontuberculous mycobacteria in patients with cystic fibrosis. *Clin Infect Dis* 2001;**32**:1298.
506. Olivier KN. The natural history of nontuberculous mycobacteria in patients with cystic fibrosis. *Paediatr Respir Rev* 2004; **5**(Suppl. A):S213.
507. Olivier KN, Weber DJ, Lee JH, et al. Nontuberculous mycobacteria, part II: nested-cohort study of impact on cystic fibrosis lung disease. *Am J Respir Crit Care Med* 2003;**167**:835.
508. Olivier KN, Weber DJ, Wallace RJ, Jr., et al. Nontuberculous mycobacteria, part I: multicenter prevalence study in cystic fibrosis. *Am J Respir Crit Care Med* 2003;**167**:828.
509. Oren I, Hardak E, Finkelstein R, et al. Polymerase chain reaction-based detection of *Pneumocystis jirovecii* in bronchoalveolar lavage fluid for the diagnosis of *Pneumocystis* pneumonia. *J Med Sci* 2011;**342**(3):182–5.
510. Orens JB, Estenne M, Arcasoy S, et al. International guidelines for the selection of lung transplant candidates: 2006 update—a consensus report from the Pulmonary Scientific Council of the

International Society for Heart and Lung Transplantation. *J Heart Lung Transplant* 2006;**25**:745.
511. Organ Procurement Transplant Network and Scientific Registry of Transplant Recipients. *Annual Data Report* 2010. Available at http://optn.transplant.hrsa.gov/.
512. Ostrosky-Zeichner L, Alexander BD, Kett DH, et al. Multicenter clinical evaluation of the (1→3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. *Clin Infect Dis* 2005; **41**:654.
513. Pagano L, Ricci P, Nosari A, et al. Fatal haemoptysis in pulmonary filamentous mycosis: an underevaluated cause of death in patients with acute leukaemia in haematological complete remission. A retrospective study and review of the literature. Gimema Infection Program (Gruppo Italiano Malattie Ematologiche dell'Adulto). *Br J Haematol* 1995;**89**:500.
514. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis, part I: latent tuberculosis. *Expert Rev Mol Diagn* 2006;**6**:413.
515. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis, part II: active tuberculosis and drug resistance. *Expert Rev Mol Diagn* 2006;**6**:423.
516. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med* 2008;**149**(3):177–84.
517. Palmer SM, Alexander BD, Sanders LL, et al. Significance of blood stream infection after lung transplantation: analysis in 176 consecutive patients. *Transplantation* 2000;**69**:2360.
518. Palmer SM, Drew RH, Whitehouse JD, et al. Safety of aerosolized amphotericin B lipid complex in lung transplant recipients. *Transplantation* 2001;**72**:545.
519. Palmer SM, Jr., Henshaw NG, Howell DN, et al. Community respiratory viral infection in adult lung transplant recipients. *Chest* 1998;**113**:944.
520. Palmer SM, Limaye AP, Banks M, et al. Extended valganciclovir prophylaxis to prevent cytomegalovirus after lung transplantation. *Ann Intern Med* 2010;**152**(12):761–9.
521. Palmer SM, Perfect JR, Howell DN, et al. Candidal anastomotic infection in lung transplant recipients: successful treatment with a combination of systemic and inhaled antifungal agents. *J Heart Lung Transplant* 1998;**17**:1029.
522. Panackal AA, Dahlman A, Keil KT, et al. Outbreak of invasive aspergillosis among renal transplant recipients. *Transplantation* 2003;**75**:1050.
523. Pancham S, Hemmaway C, New H, et al. Caspofungin for invasive fungal infections: combination treatment with liposomal amphotericin B in children undergoing hemopoietic stem cell transplantation. *Pediatr Transplant* 2005;**9**:254.
524. Pappas PG. Invasive candidiasis. *Infect Dis Clin North Am* 2006;**20**:485.
525. Pappas PG, Rex JH, Sobel JD, et al. Guidelines for treatment of candidiasis. *Clin Infect Dis* 2004;**38**:161.
526. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**(8):1101–11.
527. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
528. Pappas PG, Silveria FP; AST Infectious Disease Community of Practice. *Candida* in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s173–9.
529. Paradowski LJ. Saprophytic fungal infections and lung transplantation—revisited. *J Heart Lung Transplant* 1997;**16**: 524.
530. Pascual A, Calandra T, Bolay S, et al. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. *Clin Infect Dis* 2008;**46**:201–11.
531. Pasqualotto AC, Xavier MO, Sanchez LB, et al. Diagnosis of invasive aspergillosis in lung transplant recipients by detection of galactomannan in the bronchoalveolar lavage fluid. *Transplantation* 2010;**90**(3):306–11.
532. Patel G, Perez F, Bonomo RA. Carbapenem-resistant Enterobacteriaceae and *Acinetobacter baumannii*: assessing their impact on organ transplantation. *Curr Opin Organ Transplant* 2010;**15**: 676–82.
533. Patel N, Snyder LD, Copeland CAF, et al. Is prevention the best treatment? CMV after lung transplantation. *Transplantation* 2012; **12**:539–44.
534. Patterson JE, Peters J, Calhoon JH, et al. Investigation and control of aspergillosis and other filamentous fungal infections in solid organ transplant recipients. *Transpl Infect Dis* 2000;**2**:22.
535. Pazos C, Ponton J, Del Palacio A. Contribution of (1→3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005;**43**:299.
536. Pelaez AD, Lyon GM, Force SD, et al. Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. *J Heart Lung Transplant* 2009;**28**: 67–71.
537. Perez F, Bonomo R. Can we really use β-lactam/β-lactam inhibitor combinations for the treatment of infections caused by extended-spectrum β-lactamase-producing bacteria? *Clin Infect Dis* 2012;**54**(2):175–7.
538. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
539. Pergam SA, Limaye AP; AST Infectious Disease Community of Practice. Varicella zoster virus (VZV) in solid organ transplant recipients. *Am J Transpl* 2009;**9**(Suppl 4):s108–15.
540. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; **20**:133.
541. Pfaller MA, Diekema DJ, Andes D, et al. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Update* 2011;**14**(3):164–76.
542. Pfaller MA, Diekema DJ, Ostrosky-Zeichner L, et al. Correlation of MIC with outcome for *Candida* species tested against caspofungin, anidulafungin, and micafungin: analysis and proposal for interpretive MIC breakpoints. *Clin Microbiol* 2008;**46**(8):2620–9.
543. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006;**42**:1417.
544. Pickering JW, Sant HW, Bowles CA, et al. Evaluation of a (1→3)-beta-D-glucan assay for diagnosis of invasive fungal infections. *J Clin Microbiol* 2005;**43**:5957.
545. Pickhardt PJ, Siegel MJ, Hayashi RJ, et al. Posttransplantation lymphoproliferative disorder in children: clinical, histopathologic, and imaging features. *Radiology* 2000;**217**:16.
546. Pierre-Audigier C, Ferroni A, Sermet-Gaudelus I, et al. Age-related prevalence and distribution of nontuberculous mycobacterial species among patients with cystic fibrosis. *J Clin Microbiol* 2005;**43**:3467.
547. Pinchoff RJ, Kaufman SS, Magid MS, et al. Adenovirus infection in pediatric small bowel transplantation recipients. *Transplantation* 2003;**76**:183.
548. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrobial Agents Chemother* 2011;**55**(2):459–72.
549. Pliquett RU, Asbe-Vollkopf A, Hauser PM, et al. A *Pneumocystis jirovecii* pneumonia outbreak in a single kidney-transplant center: role of cytomegalovirus co-infection. *Eur J Clin Microbiol Infect Dis* 2012;**91**:905–16.
550. Polis MA, Spooner KM, Baird BF, et al. Anticytomegaloviral activity and safety of cidofovir in patients with human immunodeficiency virus infection and cytomegalovirus viruria. *Antimicrob Agents Chemother* 1995;**39**:882.
551. Pong A, Moser KS, Park SM, et al. Evaluation of an interferon gamma release assay to detect tuberculosis infection in children in San Diego, California. *Pediatr Infect Dis Soc* 2012;**1**(1):74–7.
552. Preiksaitis JK. New developments in the diagnosis and management of posttransplantation lymphoproliferative disorders in solid organ transplant recipients. *Clin Infect Dis* 2004;**39**:1016.
553. Preiksaitis JK, Keay S. Diagnosis and management of posttransplant lymphoproliferative disorder in solid-organ transplant recipients. *Clin Infect Dis* 2001;**33**(Suppl. 1):S38.
554. Prichard MN, Kern ER, Hartline CB. CMX001 potentiates the efficacy of acyclovir in herpes simplex virus infections. *Antimicrob Agents Chemother* 2011;**55**(10):4728–34.

555. Proia L, Miller R; AST Infectious Disease Community of Practice. Endemic fungal infections in solid organ transplant recipients. *J Transplantation* 2009;**9**(Suppl 4):s199–207.
556. Radhakrishnan DK, Yau Y, Corey M, et al. Non-tuberculous mycobacteria in children with cystic fibrosis: isolation, prevalence, and predictors. *Pediatr Pulmonol* 2009;**44**:1100–6.
557. Ranganathan K, Worley S, Michaels MG, et al. CMVIG decreases the risk of cytomegalovirus infection but not disease after pediatric lung transplantation. *Heart Lung Transplant* 2009; **28**(10):1050–6.
558. Raviv Y, Kramer MR, Amital A, et al. Outbreak of aspergillosis infections among lung transplant recipients. *Transpl Int* 2007; **20**:135.
559. Razonable RR, Zerr DM; AST Infectious Disease Community of Practice. HHV-6, HHV-7 and HHV-8 in solid organ transplant recipients. *Am J Transpl* 2009;**9**(Suppl 4): s97–100.
560. Razvi S, Quitell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the US 1995-2005. *Chest* 2009; **136**(6):1554–60.
561. Rebuck JA, Olsen KM, Fey PD, et al. Characterization of an outbreak due to extended-spectrum beta-lactamase–producing *Klebsiella pneumoniae* in a pediatric intensive care unit transplant population. *Clin Infect Dis* 2000;**31**:1368.
562. Reichenspurner H, Gamberg P, Nitschke M, et al. Significant reduction in the number of fungal infections after lung, heart-lung, and heart transplantation using aerosolized amphotericin B prophylaxis. *Transplant Proc* 1997;**29**:627.
563. Richeldi L. Rapid identification of *Mycobacterium tuberculosis* infection. *Clin Microbiol Infect* 2006;**12**(Suppl. 9):34.
564. Richeldi L, Losi M, D'Amico R, et al. Performance of tests for latent tuberculosis in different groups of immunocompromised patients. *Chest* 2009;**136**:198–204.
565. Ridgeway AL, Warner GS, Phillips P, et al. Transmission of *Mycobacterium tuberculosis* to recipients of single lung transplants from the same donor. *Am J Respir Crit Care Med* 1996;**153**:1166.
566. Roberts JA, Webb S, Paterson D, et al. A systematic review on clinical benefits of continuous administration of β-lactam antibiotics. *Crit Care Med* 2009;**37**(6):2071–8.
567. Rodriguez M, Fishman JA. Prevention of infection due to *Pneumocystis* spp. in human immunodeficiency virus–negative immunocompromised patients. *Clin Microbiol Rev* 2004;**17**:770.
568. Rodriguez-Bano J, Navarro MD, Retamar P, et al. β-lactam/β-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum β-lactamase-producing *Escherichia coli:* a post hoc analysis of prospective cohorts. *Clin Infect Dis* 2012; **54**(2):167–174.
569. Rohrlich P, Sarfati J, Mariani P, et al. Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. *Pediatr Infect Dis J* 1996;**15**:232.
570. Roilides E, Lyman CA, Panagopoulou P, et al. Immunomodulation of invasive fungal infections. *Infect Dis Clin North Am* 2003; **17**:193.
571. Romani L. Immunity to fungal infections. *Nat Rev Immunol* 2004;**4**:1.
572. Rowe DT, Webber S, Schauer EM, et al. Epstein-Barr virus load monitoring: its role in the prevention and management of post-transplant lymphoproliferative disease. *Transpl Infect Dis* 2001;**3**:79.
573. Rubin RH. Cytomegalovirus in solid organ transplantation. *Transpl Infect Dis* 2001;**3**(Suppl. 2):1–5.
574. Rubin RH. Overview: pathogenesis of fungal infections in the organ transplant recipient. *Transpl Infect Dis* 2002;**4**(Suppl. 3):12.
575. Rubin RH. Influenza and the transplant recipient. *Transpl Infect Dis* 2002;**4**:175.
576. Rubin RH. Infections in the organ transplant patient. In: Rubin RH, Young LS, editors. *Clinical approach to infection in the compromised host*. 4th ed. New York: Kluwer Academic/Plenum Publishers; 2002. p. 573.
577. Rubin RH. Temporal aspects of transplant infectious disease. *Transpl Infect Dis* 2003;**5**:63.
578. Rubin RH. Management of tuberculosis in the transplant recipient. *Am J Transplant* 2005;**5**:2599.
579. Rubin RH. The pathogenesis and clinical management of cytomegalovirus infection in the organ transplant recipient: the end of the "silo hypothesis." *Curr Opin Infect Dis* 2007;**20**:399–407.
580. Rubin RH, Ikonen T, Gummert JF, et al. The therapeutic prescription for the organ transplant recipient: the linkage of immunosuppression and antimicrobial strategies. *Transpl Infect Dis* 1999;**1**:29.
581. Rubin RH, Marty FM. Principles of antimicrobial therapy in the transplant patient. *Transpl Infect Dis* 2004;**6**:97.
582. Saad AH, DePestel DD, Carver PL. Factors influencing the magnitude and clinical significance of drug interactions between azole antifungals and select immunosuppressants. *Pharmacotherapy* 2006;**26**:1730.
583. Sadaba B, Campanero MA, Quetglas EG, et al. Clinical relevance of sirolimus drug interactions in transplant patients. *Transplant Proc* 2004;**36**:3226.
584. Safdar A, Rodriguez GH, Lichtiger B, et al. Recombinant interferon γ1b immune enhancement in 20 patients with hematologic malignancies and systemic opportunistic infections treated with donor granulocyte transfusions. *Cancer* 2006;**106**(12):2664–71.
585. Safdar A, Rodriguez G, Ohmagari N, et al. The safety of interferon–γ-1b therapy for invasive fungal infections after hematopoietic stem cell transplantation. *Cancer* 2005;**103**(4):731–9.
586. Sahi H, Avery RK, Minai OA, et al. *Scedosporium apiospermum (Pseudallescheria boydii)* infection in lung transplant recipients. *J Heart Lung Transpl* 2007;**26**(4):350–6.
587. Saiman L, Mehar F, Niu WW, et al. Antibiotic susceptibility of multiply resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, including candidates for transplantation. *Clin Infect Dis* 1996;**23**:532.
588. Sajjan U, Thanassoulis G, Cherapanov V, et al. Enhanced susceptibility to pulmonary infection with *Burkholderia cepacia* in Cftr(–/–) mice. *Infect Immun* 2001;**69**:5138.
589. Sajjan U, Wu Y, Kent G, et al. Preferential adherence of cablepiliated *Burkholderia cepacia* to respiratory epithelia of CF knockout mice and human cystic fibrosis lung explants. *J Med Microbiol* 2000;**49**:875.
590. Samuel CM, Whitelaw A, Corcoran C, et al. Improved detection of *Pneumocystis jirovecii* in upper and lower respiratory tract specimens from children with suspected *Pneumocystis* pneumonia using real-time PCR: a prospective study. *BMC Infect Dis* 2011;**11**:329.
591. Sanguinetti M, Ardito F, Fiscarelli E, et al. Fatal pulmonary infection due to multidrug-resistant *Mycobacterium abscessus* in a patient with cystic fibrosis. *J Clin Microbiol* 2001;**39**:816.
592. Sanguinetti M, Posteraro B, Pagano L, et al. Comparison of real-time PCR, conventional PCR, and galactomannan antigen detection by enzyme-linked immunosorbent assay using bronchoalveolar lavage fluid samples from hematology patients for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol* 2003;**41**:3922.
593. Savoldo B, Goss JA, Hammer MM, et al. Treatment of solid organ transplant recipients with autologous Epstein Barr virus–specific cytotoxic T lymphocytes (CTLs). *Blood* 2006;**108**(9):2942–9.
594. Sax PE, Komarow L, Finkelsman MA, et al. Blood (1→3)-β-D-glucan as a diagnostic test for HIV-related *Pneumocystis jirovecii* pneumonia. *Clin Infect Dis* 2011;**53**(2):197–202.
595. Scaenman JM, Rosso F, Austin JM, et al. Trends in invasive disease due to *Candida* species following heart and lung transplantation. *Transpl Infect Dis* 2009;**11**:112–21.
596. Schilham MW, Claas EC, van Zaane W, et al. High levels of adenovirus DNA in serum correlate with fatal outcome of adenovirus infection in children after allogeneic stem-cell transplantation. *Clin Infect Dis* 2002;**35**:526.
597. Schluger NW. Changing approaches to the diagnosis of tuberculosis. *Am J Respir Crit Care Med* 2001;**164**:2020.
598. Schmoldt S, Schuhegger R, Wendler T, et al. Molecular evidence of nosocomial *Pneumocystis jirovecii* transmission among 16 patients after kidney transplantation. *J Clin Microbiol* 2008;**46**(3):966–71.
599. Schwartz S, Ruhnke M, Ribaud P, et al. Improved outcome in central nervous system aspergillosis, using voriconazole treatment. *Blood* 2005;**106**:2641.
600. Segal BH, Barnhart LA, Anderson VL, et al. Posaconazole as salvage therapy in patients with chronic granulomatous disease and invasive filamentous fungal infection. *Clin Infect Dis* 2005;**40**:1684.
601. Segal BH, Kwon-Chung J, Walsh TJ, et al. Immunotherapy for fungal infections. *Clin Infect Dis* 2006;**42**:507.
602. Seibel NL, Schwartz C, Arrieta A, et al. Safety, tolerability, and pharmacokinetics of micafungin (FK463) in febrile neutropenic pediatric patients. *Antimicrob Agents Chemother* 2005;**49**:3317.

603. Serody JS, Mill MR, Detterbeck FC, et al. Blastomycosis in transplant recipients: report of a case and review. *Clin Infect Dis* 1993;**16**:54.
604. Sheu R, Bricker AO, Sahi H, et al. *Pseudallescheria boydii* (*Scedosporium* species) in 3 lung transplant recipients: computed tomography findings and literature review. *Comput Assist Tomogr* 2009; **33**(2):247–52.
605. Shields RK, Clancy CJ, Vandnerkar A, et al. Posaconazole serum concentrations among cardiothoracic transplant recipients: factors impacting trough levels and correlation with clinical response to therapy. *Antimicrob Agents Chemother* 2011;**55**(3): 1308–11.
606. Shreeniwas R, Schulman LL, Berkmen YM, et al. Opportunistic bronchopulmonary infections after lung transplantation: clinical and radiographic findings. *Radiology* 1996;**200**:349.
607. Siegel MJ, Lee EY, Sweet SC, et al. CT of posttransplantation lymphoproliferative disorder in pediatric recipients of lung allograft. *AJR Am J Roentgenol* 2003;**181**:1125.
608. Sifri CD, Ison MG. Highly resistant bacteria and donor-derived infections: treading in uncharted territory. *Transpl Infect Dis* 2012;**14**:223–8.
609. Silveira FP, Kwak EJ, Paterson DL, et al. Post-transplant colonization with non-*Aspergillus* molds and risk of development of invasive fungal disease in lung transplant recipients. *J Heart Lung Transpl* 2008;**27**(8):850–5.
610. Simmon KE, Brown-Elliott BA, Ridge PG, et al. *Mycobacterium chelonae-abscessus* complex associated with sinopulmonary disease, northeastern USA. *Emerg Infect Dis* 2011;**17**(9):1692–700.
611. Simoes EA, Sondheimer HM, Top FH, Jr., et al. Respiratory syncytial virus immune globulin for prophylaxis against respiratory syncytial virus disease in infants and children with congenital heart disease. The Cardiac Study Group. *J Pediatr* 1998;**133**:492.
612. Singh N. Treatment of opportunistic mycoses: how long is long enough? *Lancet Infect Dis* 2003;**3**:703.
613. Singh N. Interactions between viruses in transplant recipients. *Clin Infect Dis* 2005;**40**:430.
614. Singh N. How I treat cryptococcosis on organ transplant recipients. *Transplantation* 2012;**93**(1):17–21.
615. Singh N, Alexander BD, Lortholary O, et al. *Cryptococcus neoformans* in organ transplant recipients: impact of calcineurin-inhibitor agents on mortality. *J Infect Dis* 2007;**195**:756.
616. Singh N, Alexander BD, Lortholary O, et al. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. *Clin Infect Dis* 2008;**46**:e12–e18.
617. Singh N, Avery RK, Munoz P, et al. Trends in risk profiles for and mortality associated with invasive aspergillosis among liver transplant recipients. *Clin Infect Dis* 2003;**36**:46.
618. Singh N, Forrest G; AST Infectious Disease Community of Practice. Cryptococcosis in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s192–8.
619. Singh N, Husain S. *Aspergillus* infections after lung transplantation: clinical differences in type of transplant and implications for management. *J Heart Lung Transplant* 2003;**22**:258.
620. Singh N, Limaye AP, Forrest G, et al. Combination of voriconazole and caspofungin as primary therapy for invasive aspergillosis in solid organ transplant recipients: a prospective, multicenter, observational study. *Transplantation* 2006;**81**:320.
621. Singh N, Limaye AP, Forrest G, et al. Late-onset invasive aspergillosis in organ transplant recipients in the current era. *Med Mycol* 2006;**44**:445.
622. Singh N, Lortholary O, Alexander BD, et al. Antifungal management practices and evolution of infection in organ transplant recipients with *Cryptococcus neoformans* infection. *Transplantation* 2005;**80**:1033.
623. Singh N, Lortholary O, Alexander BD, et al. Allograft loss in renal transplant recipients with *Cryptococcus neoformans* associated immune reconstitution syndrome. *Transplantation* 2005;**80**:1131.
624. Singh N, Lortholary O, Alexander BD, et al. An immune reconstitution syndrome–like illness associated with *Cryptococcus neoformans* infection in organ transplant recipients. *Clin Infect Dis* 2005;**40**:1756.
625. Singh N, Obman A, Husain S, et al. Reactivity of Platelia *Aspergillus* galactomannan antigen with piperacillin-tazobactam: clinical implications based on achievable concentrations in serum. *Antimicrob Agents Chemother* 2004;**48**:1989.
626. Singh N, Paterson DL. *Mycobacterium tuberculosis* infection in solid-organ transplant recipients: impact and implications for management. *Clin Infect Dis* 1998;**27**:1266.
627. Singh N, Paterson DL. *Aspergillus* infections in transplant recipients. *Clin Microbiol Rev* 2005;**18**:44.
628. Singh N, Rihs JD, Gayowski T, et al. Cutaneous cryptococcosis mimicking bacterial cellulitis in a liver transplant recipient: case report and review in solid organ transplant recipients. *Clin Transplant* 1994;**8**:365.
629. Singh PK, Schaefer AL, Parsek MR, et al. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 2000;**407**:762–4.
630. Skladny H, Buchheidt D, Baust C, et al. Specific detection of *Aspergillus* species in blood and bronchoalveolar lavage samples of immunocompromised patients by two-step PCR. *J Clin Microbiol* 1999;**37**:3865.
631. Smith AL, Fiel SB, Mayer-Hamblett N, et al. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration; lack of association in cystic fibrosis. *Chest* 2003;**123**:1495–502.
632. Smith J, Andes D. Therapeutic drug monitoring of antifungals: pharmacokinetic and pharmacodynamic considerations. *Ther Drug Monit* 2008;**30**(2):167–72.
633. Smyth RL, Higenbottam TW, Scott JP, et al. Herpes simplex virus infection in heart-lung transplant recipients. *Transplantation* 1990;**49**:735.
634. Snell GI, de Hoyos A, Krajden M, et al. *Pseudomonas cepacia* in lung transplant recipients with cystic fibrosis. *Chest* 1993;**103**:466.
635. Snydman DR, Limaye AP, Potena L, et al. Update and review: state-of-the-art management of cytomegalovirus infection and disease following thoracic organ transplantation. *Transplant Proc* 2011;**43**(Suppl):s1–s17.
636. Sokol PA, Sajjan U, Visser MB, et al. The CepIR quorum-sensing system contributes to the virulence of *Burkholderia cenocepacia* respiratory infections. *Microbiology* 2003;**149**:3649.
637. Sole A, Morant P, Salavert M, et al. *Aspergillus* infections in lung transplant recipients: risk factors and outcome. *Clin Microbiol Infect* 2005;**11**:359.
638. Soler-Palacin P, Frick MA, Martin-Nalda A, et al. Voriconazole drug monitoring in the management of invasive fungal infection in immunocompromised children: a prospective study. *J Antimicrob Chemother* 2012;**67**:700–6.
639. Southern KW, Mérelle M, Dankert-Roelse JE, Nagelkerke AD. Newborn screening for cystic fibrosis (review). *Cochrane Database Syst Rev* 2009;(1):CD001402.
640. Spellberg BJ, Filler SG, Edwards JE, Jr. Current treatment strategies for disseminated candidiasis. *Clin Infect Dis* 2006;**42**:244.
641. Spellberg B, Ibrahim A, Roilides E, et al. Combination therapy for mucormycosis: why, what, and how? *Clin Infect Dis* 2012; **54**(Suppl 1):s73–8.
642. Spiess B, Buchheidt D, Baust C, et al. Development of a LightCycler PCR assay for detection and quantification of *Aspergillus fumigatus* DNA in clinical samples from neutropenic patients. *J Clin Microbiol* 2003;**41**:1811.
643. Srinivasan A, Burton EC, Kuehnert MJ, et al. Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med* 2005;**352**:1103.
644. Starke JR. Interferon-gamma release assays for diagnosis of tuberculosis infection in children. *Pediatr Infect Dis J* 2006;**25**:941.
645. Starnes VA, Bowdish ME, Woo MS, et al. A decade of living lobar lung transplantation: recipient outcomes. *J Thorac Cardiovasc Surg* 2004;**127**:114.
646. Steinbach WJ. Pediatric aspergillosis: disease and treatment differences in children. *Pediatr Infect Dis J* 2005;**24**:358.
647. Steinbach WJ. Combination antifungal therapy for invasive aspergillosis—is it indicated? *Med Mycol* 2006;**44**(Suppl.):373.
648. Steinbach WJ, Stevens DA. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. *Clin Infect Dis* 2003;**37**(Suppl. 3):S157.
649. Steinbach WJ, Stevens DA, Denning DW. Combination and sequential antifungal therapy for invasive aspergillosis: review of published in vitro and in vivo interactions and 6281 clinical cases from 1966 to 2001. *Clin Infect Dis* 2003;**37**(Suppl. 3):S188.
650. Stelzmueller I, Lass-Floerl, Geltner C, et al. Zygomycosis and other rare filamentous fungal infections in solid organ transplant recipients. *Transpl Int* 2008;**21**:534–46.

651. Stevens DA, Kan VL, Judson MA, et al. Practice guidelines for diseases caused by *Aspergillus*. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:696.
652. Stevens SJ, Verschuuren EA, Pronk I, et al. Frequent monitoring of Epstein-Barr virus DNA load in unfractionated whole blood is essential for early detection of posttransplant lymphoproliferative disease in high-risk patients. *Blood* 2001;**97**:1165.
653. Stringer JR, Beard CB, Miller RF, et al. A new name *(Pneumocystis jiroveci)* for *Pneumocystis* from humans. *Emerg Infect Dis* 2002; **8**:891.
654. Stynen D, Goris A, Sarfati J, et al. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. *J Clin Microbiol* 1995;**33**:497.
655. Stynen D, Sarfati J, Goris A, et al. Rat monoclonal antibodies against *Aspergillus* galactomannan. *Infect Immun* 1992;**60**:2237.
656. Subramanian A, Dorman S; AST Infectious Disease Community of Practice. *Mycobacterium tuberculosis* in solid organ transplant recipients. *Am J Transpl* 2009;**9**(Suppl 4):S57–61.
657. Sulahian A, Boutboul F, Ribaud P, et al. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* 2001;**91**:311.
658. Sulahian A, Touratier S, Ribaud P. False positive test for *Aspergillus* antigenemia related to concomitant administration of piperacillin and tazobactam. *N Engl J Med* 2003;**349**:2366.
659. Sun HY, Alexander BD, Lortholary O, et al. Lipid formulations of amphotericin B significantly improve outcome in solid organ transplant recipients with central nervous system cryptococcosis. *Clin Infect Dis* 2009;**49**:1721–8.
660. Sun HY, Alexander BD, Lortholary O, et al. Cutaneous cryptococcosis in solid organ transplant recipients. *Med Mycol* 2010; **48**(6):785–91.
661. Sun HY, Alexander BD, Lortholary O. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. *Clin Infect Dis* 2010;**51**(9):1062–9.
662. Sun HY, Singh N. Opportunistic infection-associated immune reconstitution syndrome in transplant recipients. *Clin Infect Dis* 2011;**53**(2):168–76.
663. Sun HY, Wagener MM, Singh N. Cryptococcosis in solid-organ, hematopoietic stem cells, and tissue transplant recipients: evidence-based evolving trends. *Clin Infect Dis* 2009;**48**:1566–78.
664. Swedish Consensus Group. Management of infections caused by respiratory syncytial virus. *Scand J Infect Dis* 2001;**33**:323.
665. Sweet SC. Pediatric living donor lobar lung transplantation. *Pediatr Transplant* 2006;**10**:861.
666. Taylor AL, Marcus R, Bradley JA. Post-transplant lymphoproliferative disorders (PTLD) after solid organ transplantation. *Crit Rev Oncol Hematol* 2005;**56**:155.
667. Taylor JL, Palmer SM. *Mycobacterium abscessus* chest wall and pulmonary infection in a cystic fibrosis lung transplant recipient. *J Heart Lung Transplant* 2006;**25**:985.
668. Templeton KE, Scheltinga SA, Beersma MF, et al. Rapid and sensitive method using multiplex real-time PCR for diagnosis of infections by influenza A and influenza B viruses, respiratory syncytial virus, and parainfluenza viruses 1, 2, 3, and 4. *J Clin Microbiol* 2004;**42**:1564.
669. Tenover FC. Rapid detection and identification of bacterial pathogens using novel molecular technologies: infection control and beyond. *Clin Infect Dis* 2007;**44**:418.
670. Theodoropoulos N, Lanternier F, Rassiwala J, et al. Use of the QuantiFERON-TB Gold interferon-gamma release assay for screening transplant candidates: a single-center retrospective study. *Transpl Infect Dis* 2012;**14**(1):1–8.
671. Thio CL, Smith D, Merz WG, et al. Refinements of environmental assessment during an outbreak investigation of invasive aspergillosis in a leukemia and bone marrow transplant unit. *Infect Control Hosp Epidemiol* 2000;**21**:18.
672. Thomas A, Korb V, Guillemain R, et al. Clinical outcomes of lung-transplant recipients treated by voriconazole and caspofungin combination in aspergillosis. *Clin Pharm Ther J* 2010;**35**: 49–53.
673. Thomas KE, Owens CM, Veys PA, et al. The radiological spectrum of invasive aspergillosis in children: a 10-year review. *Pediatr Radiol* 2003;**33**:453.
674. Torelli G, Barozzi P, Marasca R, et al. Targeted integration of human herpesvirus 6 in the p arm of chromosome 17 of human peripheral blood mononuclear cells in vivo. *J Med Virol* 1995; **45**(3):178–88.
675. Torre-Cisneros J, Doblas A, Aguado JM, et al. Tuberculosis after solid-organ transplant: incidence, risk factors, and clinical characteristics in the RESITRA (Spanish Network of Infection in Transplantation) cohort. *Clin Infect Dis* 2009;**48**:1657–65.
676. Tripathy U, Yung GL, Kriett JM, et al. Donor transfer of pulmonary coccidioidomycosis in lung transplantation. *Ann Thorac Surg* 2002;**73**:306.
677. Trulock EP, Edwards LB, Taylor DO, et al. Registry of the International Society for Heart and Lung Transplantation: twenty-third official adult lung and heart-lung transplantation report—2006. *J Heart Lung Transplant* 2006;**25**:880.
678. Tsai DE, Nearey M, Hardy CL, et al. Use of EBV PCR for the diagnosis and monitoring of post-transplant lymphoproliferative disorder in adult solid organ transplant patients. *Am J Transplant* 2002;**2**:946.
679. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007;**356**:335.
680. Upton A, Kirby KA, Carpenter P, et al. Invasive aspergillosis following hematopoietic cell transplantation: outcomes and prognostic factors associated with mortality. *Clin Infect Dis* 2007; **44**:531–40.
681. Vail GM, Young RS, Wheat LJ, et al. Incidence of histoplasmosis following allogeneic bone marrow transplant or solid organ transplant in a hyperendemic area. *Transpl Infect Dis* 2002;**4**:148.
682. Van Burik JA, Myerson D, Schreckhise RW, et al. Panfungal PCR assay for detection of fungal infection in human blood specimens. *J Clin Microbiol* 1998;**36**:1169.
683. van Delden C, Blumberg E; AST Infectious Disease Community of Practice. Multidrug resistant gram-negative bacteria in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4): S27–34.
684. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;**7**:719.
685. van Hal SJ, Marriott DJE, Chen SCA, et al. Candidemia following solid organ transplantation in the era of antifungal prophylaxis: the Australian experience. *Transpl Infect Dis* 2009;**11**:122–7.
686. Vazquez JA, Sobel JD. Anidulafungin: a novel echinocandin. *Clin Infect Dis* 2006;**43**:215.
687. Vecino R, Santiago B, Baquero-Artigao F, et al. Tuberculosis in pediatric solid organ and hematopoietic stem cell transplant recipients. *Pediatr Infect Dis J* 2012;**31**(7):774–7.
688. Venkataramanan R, Zang S, Gayowski T, et al. Voriconazole inhibition of the metabolism of tacrolimus in a liver transplant recipient and in human liver microsomes. *Antimicrob Agents Chemother* 2002;**46**:3091.
689. Vergidis P, Walker RC, Kaul DR, et al. False-positive *Aspergillus* galactomannan assay in solid organ transplant recipients with histoplasmosis. *Transpl Infect Dis* 2012;**14**(2):213–17.
690. Verma A, Dhawan A, Wade JJ, et al. *Mycobacterium tuberculosis* infection in pediatric liver transplant recipients. *Pediatr Infect Dis J* 2000;**19**:625.
691. Verweij PE, Weemaes CM, Curfs JH, et al. Failure to detect circulating *Aspergillus* markers in a patient with chronic granulomatous disease and invasive aspergillosis. *J Clin Microbiol* 2000; **38**:3900.
692. Vikram HR, Dosanjh A, Blair JE. Coccidioidomycosis and lung transplantation. *Transplantation* 2011;**92**(7):717–21.
693. Vilchez RA, Dauber J, Kusne S. Infectious etiology of bronchiolitis obliterans: the respiratory viruses connection—myth or reality? *Am J Transplant* 2003;**3**:245.
694. Vilchez RA, Dauber J, McCurry K, et al. Parainfluenza virus infection in adult lung transplant recipients: an emergent clinical syndrome with implications on allograft function. *Am J Transplant* 2003;**3**:116.
695. Vilchez RA, Fung J, Kusne S. The pathogenesis and management of influenza virus infection in organ transplant recipients. *Transpl Infect Dis* 2002;**4**:177.
696. Vilchez RA, Fung J, Kusne S. Cryptococcosis in organ transplant recipients: an overview. *Am J Transplant* 2002;**2**:575.
697. Vilchez RA, McCurry K, Dauber J, et al. The epidemiology of parainfluenza virus infection in lung transplant recipients. *Clin Infect Dis* 2001;**33**:2004.

698. Vilchez RA, McCurry K, Dauber J, et al. Influenza virus infection in adult solid organ transplant recipients. *Am J Transplant* 2002; **2**:287.
699. Vilchez R, Shapiro R, McCurry K, et al. Longitudinal study of cryptococcosis in adult solid-organ transplant recipients. *Transpl Int* 2003;**16**:336.
700. Viscoli C. Combination therapy for invasive aspergillosis. *Clin Infect Dis* 2004;**39**:803.
701. Viscoli C, Machetti M, Cappellano P, et al. False-positive galactomannan Platelia *Aspergillus* test results for patients receiving piperacillin-tazobactam. *Clin Infect Dis* 2004;**38**:913.
702. Viscoli C, Machetti M, Gazzola P, et al. *Aspergillus* galactomannan antigen in the cerebrospinal fluid of bone marrow transplant recipients with probable cerebral aspergillosis. *J Clin Microbiol* 2002;**40**:1496.
703. von Mach MA, Burhenne J, Weilemann LS. Accumulation of the solvent vehicle sulphobutylether beta cyclodextrin sodium in critically ill patients treated with intravenous voriconazole under renal replacement therapy. *BMC Clin Pharmacol* 2006;**6**:6.
704. Wagner JA, Ross H, Hunt S, et al. Prophylactic ganciclovir treatment reduces fungal as well as cytomegalovirus infections after heart transplantation. *Transplantation* 1995;**60**:1473.
705. Wallot MA, Dohna-Schwake C, Auth M, et al. Disseminated adenovirus infection with respiratory failure in pediatric liver transplant recipients: impact of intravenous cidofovir and inhaled nitric oxide. *Pediatr Transplant* 2006;**10**:121.
706. Walls T, Hawrami K, Shingadia D, et al. Polymerase chain reaction in the management of disseminated adenovirus infection after stem cell transplantation. *Pediatr Infect Dis J* 2004;**23**:1167.
707. Walsh TJ, Adamson PC, Seibel NL, et al. Pharmacokinetics, safety, and tolerability of caspofungin in children and adolescents. *Antimicrob Agents Chemother* 2005;**49**:4536.
708. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of *Aspergillus*: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
709. Walsh TJ, Karlsson MO, Driscoll T, et al. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. *Antimicrob Agents Chemother* 2004; **48**:2166.
710. Walsh TJ, Lutsar I, Driscoll T, et al. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *Pediatr Infect Dis J* 2002;**21**:240.
711. Walsh TJ, Raad I, Patterson TF, et al. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. *Clin Infect Dis* 2007;**44**:2.
712. Walsh TJ, Shoham S, Petraitiene R, et al. Detection of galactomannan antigenemia in patients receiving piperacillin-tazobactam and correlations between in vitro, in vivo, and clinical properties of the drug-antigen interaction. *J Clin Microbiol* 2004;**42**:4744.
713. Walsh TJ, Teppler H, Donowitz GR, et al. Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. *N Engl J Med* 2004;**351**:1391.
714. Waltz DA, Boucek MM, Edwards LB, et al. Registry of the International Society for Heart and Lung Transplantation: Ninth official pediatric lung and heart-lung transplantation report—2006. *J Heart Lung Transplant* 2006;**25**:904.
715. Wang EH, Partovi N, Levy RD, et al. *Pneumocystis* pneumonia in solid organ transplant recipients: not yet an infection of the past. *Transpl Infect Dis* 2012;**14**(5):519–25.
716. Weinberg A, Lyu DM, Marquesen J, et al. Incidence and morbidity of human metapneumovirus and other community-acquired respiratory viruses in lung transplant recipients. *Transpl Infect Dis* 2010;**12**:330–5.
717. Weishaar PD, Flynn HW, Jr., Murray TG, et al. Endogenous *Aspergillus* endophthalmitis: clinical features and treatment outcomes. *Ophthalmology* 1998;**105**:57.
718. Welzen MEB, Bruggemann RJM, Van Den Berg JH, et al. A twice daily posaconazole dosing algorithm for children with chronic granulomatous disease. *Pediatr Infect Dis J* 2011;**30**(9):794–7.
719. Wendt CH. Community respiratory viruses: organ transplant recipients. *Am J Med* 1997;**102**:31.
720. Wendt CH, Fox JM, Hertz MI. Paramyxovirus infection in lung transplant recipients. *J Heart Lung Transplant* 1995;**14**:479.
721. Wheat L. Current diagnosis of histoplasmosis. *Trends Microbiol* 2003;**11**:488.
722. Wheat LJ. Rapid diagnosis of invasive aspergillosis by antigen detection. *Transpl Infect Dis* 2003;**5**:158.
723. Wheat LJ. Combination therapy for aspergillosis: is it needed, and which combination? *J Infect Dis* 2003;**187**:1831.
724. Wheat LJ. Antigen detection, serology, and molecular diagnosis of invasive mycoses in the immunocompromised host. *Transpl Infect Dis* 2006;**8**:128.
725. Wheat LJ. Histoplasmosis: a review for clinicians from non-endemic areas. *Mycoses* 2006;**49**:274.
726. Wheat LJ, Freifeld AG, Kleimann MB. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:807–25.
727. Whimbey E, Champlin RE, Englund JA, et al. Combination therapy with aerosolized ribavirin and intravenous immunoglobulin for respiratory syncytial virus disease in adult bone marrow transplant recipients. *Bone Marrow Transplant* 1995;**16**:393.
728. White PL, Barton R, Guiver M, et al. A consensus on fungal polymerase chain reaction diagnosis? A United Kingdom–Ireland evaluation of polymerase chain reaction methods for detection of systemic fungal infections. *J Mol Diagn* 2006;**8**:376.
729. White LP, Bretagne S, Klingspor L, et al. *Aspergillus* PCR: one step closer to standardization. *J Clin Microbiol* 2010;**48**(4): 1231–40.
730. White PL, Linton CJ, Perry MD, et al. The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. *Clin Infect Dis* 2006;**42**:479.
731. White PL, Mengoli C, Bretagne S, et al. Evaluation of aspergillus PCR protocols for testing serum specimens. *J Clin Microbiol* 2011;**49**(11):3842–8.
732. Wiita AP, Roubinian N, Khan Y, et al. Cytomegalovirus disease and infection in lung transplant recipients in the setting of planned indefinite valganciclovir prophylaxis. *Transplant Infect Dis* 2012;**14**:248–58.
733. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004;**350**:443.
734. Williamson EC, Leeming JP, Palmer HM, et al. Diagnosis of invasive aspergillosis in bone marrow transplant recipients by polymerase chain reaction. *Br J Haematol* 2000;**108**:132.
735. Winkler M, Pratschke J, Schulz U, et al. Caspofungin for post solid organ transplant invasive fungal disease: results of a retrospective observational study. *Transpl Infect Dis* 2010;**12**: 230–7.
736. Winters H, Parbhoo R, Schafer JJ, et al. Extended-spectrum β-lactamase-producing bacterial infections in adult solid organ transplant recipients. *Pharmacotherapy* 2011;**45**:309–16.
737. Winthrop KL, Kubak BM, Pegues DA, et al. Transmission of *Mycobacterium tuberculosis* via lung transplantation. *Am J Transpl.* 2004;**4**:1529–33.
738. Woo MS, MacLaughlin EF, Horn MV, et al. Living donor lobar lung transplantation: the pediatric experience. *Pediatr Transplant* 1998;**2**:185.
739. Woo MS, Perez E, Horn MV, et al. Postoperative use of aerosolized aztreonam in cystic fibrosis patients colonized with *Burkholderia cepacia* prior to lung transplantation. *J Heart Lung Transplant* 2004;**23**:S78.
740. Wright PW, Pappagianis D, Wilson M, et al. Donor-related coccidioidomycosis in organ transplant recipients. *Clin Infect Dis* 2003;**37**:1265.
741. Writing Committee of the WHO Consultation. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med* 2012;**362**:1708–19.
742. Wu G, Vilchez RA, Eidelman B, et al. Cryptococcal meningitis: an analysis among 5,521 consecutive organ transplant recipients. *Transpl Infect Dis* 2002;**4**:183.
743. Yusuf U, Hale GA, Carr J, et al. Cidofovir for the treatment of adenoviral infection in pediatric hematopoietic stem cell transplant patients. *Transplantation* 2006;**81**:1398.
744. Zaidi S, Elidemir O, Heinle JS, et al. *Mycobacterium abscessus* in cystic fibrosis lung transplant recipients: report of 2 cases and risk for recurrence. *Transpl Infect Dis* 2009;**11**:243–8.

745. Zamora MR. Use of cytomegalovirus immune globulin and ganciclovir for the prevention of cytomegalovirus disease in lung transplantation. *Transpl Infect Dis* 2001;**3**(Suppl. 2): 49–52.
746. Zamora MR. Cytomegalovirus and lung transplantation. *Am J Transplant* 2004;**4**:1219.
747. Zamora MR, Davis RD, Leonard C. Management of cytomegalovirus infection in lung transplant recipients: evidence-based recommendations. *Transplantation* 2005;**80**:157.
748. Zamora MR, Nicolls MR, Hodges TN, et al. Following universal prophylaxis with intravenous ganciclovir and cytomegalovirus immune globulin, valganciclovir is safe and effective for prevention of CMV infection following lung transplantation. *Am J Transplant* 2004;**4**:1635.
749. Zuk DM, Humar A, Weinkauf JG, et al. An international survey of cytomegalovirus management practices in lung transplantation. *Transplantation* 2010;**90**:672–6.
750. Zukerman R, Wald A; AST Infectious Disease Community of Practice. Herpes simplex virus infections in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl 4):s104–7.
751. Zwald FO, Spratt M, Lemos BD, et al. Duration of voriconazole exposure: an independent risk factor for skin cancer after lung transplantation. *Dermatol Surg* 2012;**38**(8):1369–74.

CHAPTER 72

Opportunistic Infections in Liver and Intestinal Transplantation

Michael Green • Marian G. Michaels

Liver transplantation has been established as an effective treatment for children with end-stage liver disease for more than 25 years. Improving surgical techniques and the availability of new and more potent immunosuppressive regimens have led to enhanced survival rates, with 3-year survival rates approaching or exceeding 80 percent.[103] In response to these excellent results, more children are being referred for liver transplantation and increasing numbers of pediatric centers routinely perform this procedure. Intestinal transplantation, performed as an isolated procedure or in combination with the liver or other organs, has also gained expanded acceptance as a treatment for children with refractory intestinal failure with steadily improving survival rates over time.[103] This is particularly true for those children who experience progressive liver failure due to hyperalimentation-induced liver disease. Although this newer procedure has been routinely performed at only a limited number of centers, an increasing number of transplant programs are beginning to offer intestinal transplantation.

Infectious complications have been a significant cause of morbidity and mortality in children undergoing liver transplantation since this procedure gained its initial acceptance in the 1980s. However, improvements in the treatment options for, and management of, immune suppression along with the increasing availability of new antimicrobial agents and diagnostic tools have resulted in reductions in the impact of infectious complications on these children. Intestinal transplantation is associated with many of the same infectious complications seen after liver transplantation, as well as a number of infectious issues that appear to be unique to this procedure. Although development of increasingly effective treatment strategies for infectious complications is of great benefit to the children undergoing these procedures, emphasis has increasingly shifted to the development of strategies aimed at the prevention of infectious complications in children undergoing abdominal transplantation. A general overview of the problem of infections after liver and intestinal transplantation in children is presented in this chapter.

PREDISPOSING FACTORS

Liver and intestinal transplantation are associated with a set of technical and medical conditions that predispose to a unique set of infectious complications. The abdomen is a common site of infection in patients undergoing both of these procedures.[27,82] This is almost certainly due to the occurrence of local ischemic injury and bleeding, as well as potential soilage with contaminated material.[61] Additional factors predisposing to infection can be divided into those that exist before a transplant and those secondary to intraoperative and post-transplant activities.

Pretransplant Factors

The underlying illnesses leading to transplantation may be associated with intrinsic risk factors for infection. Some disorders may have required palliative surgery, which increases the technical difficulty of the transplant and may be associated with an enhanced risk for developing post-transplant infections.[23] For example, children undergoing liver transplantation for biliary atresia may have previously undergone a Kasai procedure (choledochojejunostomy), which may predispose to recurrent episodes of bacterial cholangitis before transplantation, increasing the likelihood of colonization with multidrug-resistant bacteria that can cause infection after transplantation. Similarly, children undergoing liver transplantation for cystic fibrosis may have an increased risk for developing invasive aspergillosis if they were colonized with this pathogen before transplantation. Complications of end-stage liver disease (as part of a primary liver disease or as a consequence of hyperalimentation in patients with intestinal insufficiency) may also predispose to infection after a transplant. A history of one or more episodes of spontaneous bacterial peritonitis before transplantation in patients with ascites has been associated with an increased rate of bacterial infections after liver transplantation[129] and could be expected in patients with liver disease associated with intestinal insufficiency. Finally, children undergoing intestinal transplantation experience frequent episodes of gastrointestinal-associated bloodstream infections. Recurrent exposure to antimicrobial agents to treat these episodes of bacteremia increases the likelihood of colonization and disease with multidrug-resistant bacterial and fungal pathogens after transplantation. Risks for bacterial translocation after intestinal transplantation with resultant bloodstream infection include the presence of a colon graft,[20] hospitalization before transplantation, and treatment with mycophenolate mofetil (MMF).[82]

Age, another important pretransplant factor, is a major determinant of susceptibility to certain pathogens, severity of expression of infection, and immune maturation.[1] Young children undergoing abdominal transplantation may experience moderate to severe infection with certain viral (e.g., respiratory syncytial virus [RSV]) or bacterial (coagulase-negative staphylococci) pathogens compared with more mild illness experienced by adult recipients infected with these pathogens. In contrast, certain pathogens, such as *Cryptococcus neoformans*, uncommonly manifest infection before young adulthood.[132] Age is also an important factor governing clinical expression of infection with cytomegalovirus (CMV) and Epstein-Barr virus (EBV). When transplants are performed in young patients there is a high likelihood that they will be seronegative for CMV and EBV and therefore susceptible to primary infections, which are more severe than infections due to reactivation.[10,62,105]

Donor-related issues represent another set of pretransplant factors. Transplant recipients are at risk for acquiring infections that may be active or latent within the donor at the time of organ harvesting. The most common examples of donor-associated infections are CMV and EBV. [9,11,18,105] Infections caused by CMV and EBV have been more severe after intestinal transplant compared with other organs. This may be because the intestine is an organ rich in lymphoid tissue, which may result in bringing a larger viral load of viruses from the donor compared with other graft types. Similarly, adenovirus has been isolated more commonly from pediatric recipients of intestinal transplants compared with other organs, which may also be related to donor transmission in the accompanying lymphoid tissue.[31,89,99] In contrast to CMV and EBV for which donor-derived transmission is expected, increasing attention has focused on the unexpected transmission of other pathogens such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Although the systematic use of donor screening has led to a decreased frequency of unexpected transmission of HIV, HBV, and HCV,[4,28,71] recent evidence now demonstrates donor-associated transmission of West Nile virus, rabies, as well as a number of other uncommonly transmitted pathogens.[70-72,121] Because organs from a single donor often go to disparate sites it is important for the recipient center to report back to the United Network for Organ Sharing (UNOS) any unusual infections that might possibly have come from the donor.

Intraoperative Factors

Operative factors unique to liver transplantation may predispose to infectious complications. For example, liver transplant recipients undergoing Roux-en-Y choledochoduodenostomy experience more infectious episodes than those who undergo a choledochocholedochostomy with T-tube drainage.[76,110] However, only the former option is usually performed in children undergoing liver transplantation because of the small size of their bile ducts. For combined liver-intestinal transplantation, evolution to en bloc replacement of the liver and intestine avoids the need for additional biliary anastomosis and minimizes the risk for infection related to biliary complications. Prolonged operative time (>12 hours) during the initial transplant has been associated with an increased risk for infection after transplant[38,76] and is likely a surrogate marker for the technical difficulty of the surgery. Intraoperative events, such as contamination of the operative field, also predispose to postoperative infections. Finally, the inability to close the abdomen after the transplantation due to size discrepancy or intraoperative complications appears to increase the risk for postoperative infections.

Post-Transplant Factors

Technical problems, immunosuppression, presence of indwelling cannulas, and nosocomial exposures are major postoperative risk factors for infectious complications. Thrombosis of the hepatic artery is the most serious technical problem after liver transplantation and predisposes to areas of necrotic liver and development of hepatic abscesses and bacteremia.[108,110] Bile duct strictures, developing as a sequelae of thrombosed hepatic artery and ischemia or due to technical difficulties, may predispose to cholangitis.[110] Retransplantation represents a high risk for intra-abdominal infection after intestinal transplantation.[82]

Immunosuppression is the critical postoperative factor predisposing to infection in all transplant recipients. Immunosuppressive regimens have evolved in an attempt to achieve more specific control of rejection with the least impairment of immunity. Thus, this evolution is aimed not only at improved control of rejection but also at decreased morbidity and mortality from infections. The use of cyclosporine-based regimens resulted in a decreased incidence of infections in renal and cardiac transplant recipients.[27,63,95] The introduction of tacrolimus has allowed many patients to be managed without corticosteroids.[51,127] Although reported rates of infection have been similar in liver transplant recipients treated with tacrolimus compared with those receiving cyclosporine, an apparent decrease in morbidity and mortality, especially from viral pathogens, has been noted with tacrolimus.[51] In contrast to these results, some centers have reported an increased rate of EBV-associated post-transplant lymphoproliferative disease (PTLD) in patients receiving tacrolimus.[22] However, data from the University of Pittsburgh suggests that the short and long-term incidence of EBV-associated PTLD appears to be similar in pediatric liver transplant recipients treated with either cyclosporine or tacrolimus.[14] More recently, induction immunotherapy has been used in combination with corticosteroid-free or corticosteroid-sparing immunosuppressive regimens whereas newer agents also are being explored. Potential infectious risks will need to be assessed for these and other evolving immunosuppressive regimens.

Children undergoing intestinal transplantation require an increased baseline level of immunosuppression compared with patients undergoing most other solid-organ transplant procedures.[105] With this increased level of immunosuppression has come an increased risk for development of infection. In an effort to overcome this, a

number of alternative immunosuppressive strategies have been explored. Looking at one such alternative strategy, Loinaz and colleagues found an increased risk for bacterial infections with both MMF and daclizumab after intestinal transplantation.[82] Another alternative approach evaluated the use of alemtuzumab induction in pediatric intestinal transplant recipients, which was associated with a marked increased incidence of EBV-associated post-transplant lymphoproliferative disease (PTLD) in one study[112] but not another.[73]

The treatment of rejection with additional or higher doses of immunosuppressant agents increases the risk for invasive and potentially fatal infection. Of particular concern is the use of antilymphocyte preparations, which are often indispensable in the management of corticosteroid-resistant rejection.[9,38,76]

The prolonged use of indwelling cannulas at any site is an important cause of infection throughout the post-operative course. The presence of central venous catheters is a cause of bacteremia after transplantation. This is particularly important for children undergoing intestinal transplantation where maintenance of long-term central venous access has been required for prolonged periods of time after transplantation.[34] The development of urinary tract infections and bacterial pneumonia are associated with the use of urethral catheters and prolonged nasotracheal or endotracheal intubation, respectively.[38,76]

Nosocomial exposures constitute the final group of postoperative risk factors in the hospital setting. Transplant recipients, especially children, may be exposed to many common viral pathogens (e.g., rotavirus, RSV, or influenza) while in hospital. Finally, the presence of resistant bacteria in the hospital increases the risk for nosocomial transmission and disease. Attention to infection control policies aimed at local epidemiology of circulating infections is paramount.

Finally, the presence in the hospital of heavy areas of contamination with pathogenic fungi, such as *Aspergillus*, may increase the risk for invasive fungal disease in these patients. The rate of fungal colonization increases during times of hospital construction. Infection control policies aimed at local epidemiology of circulating infections is paramount.

TABLE 72-1 **Differential Diagnosis of Infectious Complications During the Early Period (0 to 30 Days) After Pediatric Liver and Intestinal Transplantation**

Clinical Syndrome	Associated Pathogens
Wound infection	
Superficial	*Staphylococcus aureus* Enterococci
Deep	Enterobacteriaceae *Candida* spp.
Intra-abdominal infection	
Peritonitis	Enterobacteriaceae
Intra-abdominal abscess	Enterococci
Intrahepatic abscess (isolated liver and liver-intestine transplants) ± bacteremia	*Candida* spp.
Bloodstream infection, associated with:	
Central venous catheters	Coagulase-negative staphylococci Enterococci *S. aureus* *Candida* spp.
Hepatic artery thrombosis (isolated liver transplant only)	Enterobacteriaceae Enterococci *Candida* spp.
Intestinal rejection (intestine only)	Enterobacteriaceae Enterococci *Candida* spp.
Bacterial cholangitis (isolated liver transplant only)	Enterobacteriaceae Enterococci *Candida* spp.
Urinary tract infection	Enterobacteriaceae Enterococci *Candida* spp.
Ventilator-associated pneumonia	Enterobacteriaceae Enterococci *S. aureus*
Nosocomial acquisition of common community pathogens	Respiratory syncytial virus Parainfluenza virus Influenza virus Rotavirus
Noninfectious etiologies	Rejection Drug fever

TIMING OF INFECTIONS

The time of onset of infection with various pathogens after transplantation tends to be predictable. The majority of clinically important infections occur within the first 180 days after transplantation,[48,76] although infections continue to occur quite frequently beyond this time after intestinal transplantation.[73] The timing of infections can be divided into three intervals: early (0 to 30 days after transplantation), intermediate (30 to 180 days after transplantation), and late (>180 days after transplantation). In addition, some infections may occur throughout the postoperative course. These divisions should be viewed with flexibility because they may be modified by prophylaxis strategies and are somewhat less applicable to recipients of intestinal transplantation. Despite this, they remain useful, giving a general approach to a patient with fever after transplantation and can be used as a guide to differential diagnosis. An overview of the infectious complications occurring during each of these time periods is provided in Tables 72-1 through 72-3 and is summarized in the following sections.

Early Infections (0 to 30 Days) (Table 72-1)

Early infections tend to be associated with preexisting conditions and surgical manipulation. In general, they are caused by either bacteria or yeast. Bacterial infections are particularly common after intestinal transplantation, being reported in up to 90 percent of recipients.[82,125] As many as half of these early infectious complications may develop in the first 2 weeks after abdominal

TABLE 72-2 Differential Diagnosis of Infectious Complications During the Intermediate Period (31 to 180 Days) After Pediatric Liver and Intestinal Transplantation

Clinical Syndrome	Associated Pathogens
Viral syndrome	CMV
Fever, leukopenia, thrombocytopenia ± atypical lymphocytosis	EBV
Hepatitis	CMV EBV Adenovirus Hepatitis B Hepatitis C
Enteritis	CMV EBV Rotavirus Adenovirus *Clostridium difficile*
PTLD	EBV
Bacterial cholangitis*	Enterobacteriaceae Enterococci *Candida* spp.
Pneumonia	*Streptococcus pneumoniae* CMV Adenovirus RSV Parainfluenza virus Influenza virus Human metapneumovirus *Pneumocystis jiroveci* *Aspergillus fumigatus*
Bacteremia†	Enterobacteriaceae *Enterococcus* Coagulase-negative staphylococci (with central venous catheter) *Candida* spp.
Adenopathy	EBV/PTLD
Pulmonary nodules	EBV/PTLD *Aspergillus fumigatus*

*Typically only seen in isolated liver transplant recipients and usually associated with the presence of technical complication (e.g. biliary stricture).
†Seen in intestinal transplant recipients in association with presence of central venous catheters, intestinal rejection or PTLD involving intestine.
CMV, cytomegalovirus; EBV, Epstein-Barr virus; PTLD, post-transplant lymphoproliferative disease.

TABLE 72-3 Differential Diagnosis of Infectious Complications During the Late Period (>180 Days) After Pediatric Liver and Intestinal Transplantation

Clinical Syndrome	Associated Pathogens
Bacterial cholangitis*	Enterobacteriaceae Enterococci *Candida* spp.
PTLD	EBV
Bacteremia†	Enterobacteriaceae *Enterococcus* *Candida* spp.
Varicella-zoster	Varicella-zoster virus
Respiratory tract infections	Community-acquired viruses‡

*Usually associated with the presence of technical complication (e.g., biliary stricture).
†Seen in intestinal transplant recipients in association with presence of central venous catheters, intestinal rejection, or PTLD involving intestine.
‡Respiratory syncytial virus, parainfluenza virus, influenza virus A and B, human metapneumovirus, adenovirus, rhinovirus, and enterovirus.
EBV, Epstein-Barr virus; PTLD, Post-transplant lymphoproliferative disease.

transplantation.[8] Cholangitis or spontaneous bacterial peritonitis presenting at or near the time of liver transplantation may lead to intra-abdominal infection after the transplant. Herpes simplex infection can also reactivate and cause early symptomatic disease,[76] although this is uncommon in children. Technical difficulties (e.g., thrombosis of the hepatic artery or portal vein and biliary strictures) predispose to early bacterial infections. Likewise, development of bile leaks and bowel perforations are associated with polymicrobial intra-abdominal infections, primarily consisting of enteric bacteria and *Candida* spp., in the first month after transplantation.[40] Early bacteremia is exceptionally common in intestinal transplant recipients and is usually associated with the presence of central venous catheters.[34] Likewise early bacteremia with gram-negative pathogens in these children has been associated with intestinal rejection.[34,43] Re-exploration of the abdomen is associated with increased rates of fungal infection.[76]

Intermediate Period (31 to 180 Days) (Table 72-2)

The intermediate period is the typical time of onset of infections associated with nonbacterial donor-associated transmission (either organ or blood products), reactivated viruses, and opportunistic infections. Without the use of prophylaxis, CMV peaks in incidence during this time.[9,76] The intermediate period is also when many patients begin to present with EBV-associated PTLD,[13,62] and *Pneumocystis jiroveci* (formerly *P. carinii*) pneumonia.[76] However, the use of prophylaxis against CMV and *P. jiroveci* can modify the time of presentation so that the occurrence of disease from these organisms may be after 180 days. Finally, bacteremia continues to be a frequent occurrence in pediatric intestinal transplant recipients with as many as 50 percent of all bloodstream infections occurring in this population being identified in this time period.[34]

Late Infections (Greater than 180 Days) (Table 72-3)

Late infections after abdominal transplantation are less well characterized than other periods because patients have usually been discharged from the transplant center

to their respective homes, which often are quite far away. This makes the accurate accumulation of data on these late infections difficult. Nonetheless, problems such as recurrent episodes of bacterial cholangitis in liver transplant recipients (typically associated with underlying problems of the biliary tree) and bacteremia associated with ongoing requirement for a central venous catheter, intestinal graft rejection, and/or PTLD in intestinal transplant recipients[34,82] continue to occur in this time period. In addition, children who are at high risk for CMV infection and have been kept on prophylaxis may delay the onset of CMV infection to the late period.[16]

Infections Occurring Throughout the Postoperative Course

Iatrogenic factors are an important cause of bacterial and fungal infections at all times but predominate in the early transplant period. Central venous lines are maintained for a variable time; the risk for infection persists for the entire period that the catheter remains in place. This is a particularly important problem for recipients of intestinal transplants who maintain central line access until the graft is fully functional. Similarly, the presence of urethral catheters and endotracheal tubes increases the risk for infections whenever they are in use.

Nosocomial acquisition of community viruses, such as RSV, rotavirus, and influenza A or B can occur at any time after transplant. These viruses spread easily in hospital environments from personnel or other hospitalized patients to transplant recipients. It is therefore important to modify diagnostic considerations according to local epidemiologic considerations.

BACTERIAL AND FUNGAL INFECTIONS

Bacterial and fungal pathogens are important causes of morbidity and occasional mortality in children undergoing liver and/or intestinal transplantation. With the exception of infections related to the use of indwelling catheters, sites of bacterial infection tend to occur at or near the transplanted organ. Accordingly, the intra-abdominal space is an important site of infection after any of the abdominal transplant procedures. Adding to the complexity of the management of bacterial infections in children undergoing abdominal transplantation is the fact that recovery of multidrug-resistant bacteria is increasingly common. Outbreaks of colonization and disease due to vancomycin-resistant *Enterococcus faecium* and extended-spectrum β-lactamase (ESBL)–producing *Klebsiella pneumoniae* have been reported among pediatric liver and intestinal transplant recipients.[42,44] These multidrug-resistant bacteria have been transmitted from patient to patient, prompting the imposition of strict infection control procedures. Reports of multidrug-resistant strains of *Enterobacter cloacae* associated with de-repression of a chromosomally located broad-spectrum β-lactamase enzyme have identified this very resistant organism as an important pathogen after liver transplantation.[19] Isolates of *E. cloacae* identified to also be carrying ESBL enzymes have also been recovered from intestinal transplant candidates and recipients, further limiting antimicrobial treatments. In some cases, effective antimicrobial agents may be unavailable to treat these complications. Knowledge of results of previous cultures and antimicrobial resistance patterns locally as well as from the home institution of the patient are critical components in choosing initial empirical antibiotic therapy for these patients to maximize their outcomes.

Liver Transplantation

Bacterial and fungal infections are a common early problem after liver transplantation.[21,59,64,131,137] Rates for bacterial infection of 40 to 70 percent have been reported from multiple series.[51,76,110] Bacteremia often occurs in association with intra-abdominal infection or with use of central venous catheters, but it can occur without an obvious source. Enteric gram-negative organisms account for more than one half of episodes. Bacterial infections involving the abdomen or surgical wound are common in most series. Infectious complications of the transplanted liver also occur. The most important complication is hepatic abscess, associated with hepatic artery or portal vein thrombosis, which often is accompanied by persistent bacteremia. However, the introduction of frequent surveillance Doppler studies early after transplant to monitor for development of thrombosis, coupled with the use of operative thrombectomy and thrombolysis, have essentially eliminated the development of hepatic abscesses in this population.

Ascending cholangitis is common after liver transplantation and is usually associated with biliary abnormalities. This diagnosis typically is made on clinical grounds in a patient with fever and biochemical evidence of biliary inflammation. Enteric gram-negative bacteria and enterococcal species predominate. However, this clinical picture can be identical to that of acute graft rejection; liver biopsy should be performed to differentiate these processes. A cholangiogram is performed to assess the status of the biliary tract for patients with proven cholangitis.

As many as 40 percent of children undergoing liver transplantation may develop a fungal infection during the first year following this procedure.[131] *Candida* spp. are the most common fungal pathogens, and infection usually is associated with an intra-abdominal focus or indwelling catheter. Infections due to *Candida* spp. are most commonly recognized in the first month after transplantation with candidal peritonitis most likely presenting in the first 2 weeks after liver transplantation in association with a bile leak or bowel perforation. Other risk factors associated with the development of candidal infection include prolonged duration of intubation after transplant, hepatic artery thrombosis, volume of blood transfused, and exposure to corticosteroids within the 3 months before the transplant. The recovery of *Candida* from a Jackson-Pratt drain in the early postoperative period may be the first indication of either of these two technical complications and may occur before the onset of clinical symptoms of intra-abdominal infection.[42] Accordingly, recovery of

Candida, alone or in combination with enteric bacteria, should prompt initiation of antimicrobial therapy and an aggressive evaluation for the presence of these complications. Early initiation of treatment is particularly important given an attributable mortality rate of up to 33 percent for candidal infections in pediatric liver transplant recipients.[42] The availability of fluconazole and echinocandin antifungal agents (e.g., caspofungin and micafungin) increases the number of therapeutic options for the treatment of candidal infections. However, acquired or inherent resistance to the azoles is an increasing concern, as are drug-drug interactions between azoles with both cyclosporine and tacrolimus and echinocandins with cyclosporine.

Episodes of invasive aspergillosis are uncommon but can be fatal.[52,56] Children undergoing liver transplantation for cystic fibrosis are at particular risk for developing infection due to *Aspergillus*.[130] Early disseminated disease in children with cystic fibrosis undergoing liver transplantation at Children's Hospital of Pittsburgh was observed in the early era of transplantation, prompting the use of perioperative antifungal prophylaxis in liver recipients with cystic fibrosis and a history of recovery of *Aspergillus* before transplant. Because data defining the precise duration of prophylaxis necessary to protect against this complication are not available, prophylactic treatment has ranged from 1 month of intravenous amphotericin to prolonged use of an oral azole agent (e.g., itraconazole or voriconazole). The availability of newer antifungal agents, including the advanced-generation azoles (voriconazole and posaconazole) as well as the echinocandins, has increased the number and complexity of therapeutic options for treatment of aspergillosis in children undergoing liver transplantation.[55] A summary of a suggested approach to the diagnosis and management of fungal infections after liver transplantation in children is provided in Table 72-4.

Intestinal Transplantation

Although increasing, the numbers of children having undergone intestinal transplantation remain relatively small. Many have received combined liver and intestine or multivisceral transplants. Bacterial infection occurs frequently in these patients.[43,116] One series reported that as many as 92 percent of children undergoing intestinal transplantation experienced an average of 2.9 episodes of bacterial infection per patient.[82] More than 80 percent of these patients experienced their first bacterial infection during the first 2 months after transplant. Bacteremia, which can be explained in part by disruption of the mucosal barrier associated with harvest injury or intestinal allograft rejection, is a common finding,[43,116] with one center reporting that 69 percent of pediatric intestinal transplant recipients experienced at least one episode of bloodstream infection.[34] Coagulase-negative staphylococci, enterococci, and gram-negative enteric bacilli account for most episodes. As noted earlier, antibiotic resistance is commonly seen in recovered pathogens. In the Children's Hospital of Pittsburgh experience, episodes of gut-associated bacteremia frequently secondarily infect central venous catheters, producing persistent positive cultures even after resolution of clinical symptoms. Accordingly, treatment strategies aimed at preserving the catheter (e.g., antibiotic lock therapy) may need to be used in combination with systemic antibiotics to accomplish a sustained clinical cure.[91]

Intra-abdominal and wound infections also are seen commonly in this population, occurring in more than one third of patients and are typically detected during the first month after transplantation. Gram-negative enteric pathogens, which frequently demonstrate multiple-antibiotic resistance as well as enterococci (often exhibiting vancomycin resistance) are the most common organisms associated with these complications. Recurrent laparotomies have been identified as a risk factor for intra-abdominal infection. One unique aspect of intestinal transplantation is the potential inability to achieve abdominal wall closure. Although failure to close the abdominal wall is an obvious risk for the development of intra-abdominal infection, the use of abdominal mesh as part of efforts to resolve difficult abdominal wall closures has also been associated with the development of superficial and deep wound infection.[26]

Another important site of infection is the intestine itself. *Clostridium difficile* enteritis can present as a pattern of fever, abdominal pain, and diarrhea that can easily be mistaken for graft rejection or viral infection due to CMV, EBV, and adenovirus. Accordingly, the diagnosis of *C. difficile* enteritis must be considered in any child in whom fever and changes in stool output are noted. In one small series, *C. difficile* enteritis was diagnosed in nearly 10 percent of children undergoing intestinal transplantation.[136] The frequent exposure to antibiotics and prolonged hospital stays that children undergoing intestinal transplantation experience are major risk factors for the development of this complication. Standard treatment with oral metronidazole is the recommended first-line therapy. However, use of prolonged therapy or oral vancomycin might be necessary for patients who experience relapse or recurrent episodes after primary treatment.

Invasive candidiasis and candidemia occur relatively frequently after intestinal transplantation in children, with one center reporting an incidence of approximately 23 percent in a series of 98 pediatric intestinal transplant recipients.[35] Candidemia accounted for 50 percent of the episodes of infection in this series, while nearly 40 percent involved the intra-abdominal space. Although the majority of episodes of candidemia infection occur in the first 3 to 6 months after transplant, later episodes may occur. Intra-abdominal infection with *Candida* is also observed, typically as part of a polymicrobial infection related to technical problems occurring during the initial transplant surgery or subsequent laparotomies. Specific risk factors associated with invasive candidiasis and candidemia include ongoing requirement for total parenteral nutrition and antibiotic treatment in the 7 days before diagnosis.[35]

Invasive mycoses due to fungal pathogens other than *Candida* are rarely observed. A recent report on fungal infections from the University of Nebraska identified four cases of invasive aspergillosis in 98 children undergoing intestinal transplantation,[32] whereas our experience at Children's Hospital of Pittsburgh has identified rare

TABLE 72-4 **Overview of Diagnosis and Management of Fungal Infections After Liver and Intestinal Transplantation in Children**

	***Candida*—Noninvasive (Mucositis, Dermatitis and Cystitis)**	***Candida*—Invasive**	***Aspergillus***	***Cryptococcus***	**Others (*Histoplasma, Mucor, Fusarium, Blastomycetes, Alternaria*, etc.)**
Frequency	Common*	Common*	Uncommon*	Rare*	Rare*
Diagnostic Tests	Clinical examination Culture Gram stain	Culture Gram stain Histology	Culture Gram stain Histology Radiographic staging**	Culture Antigen test India ink stain Histology CSF examination	Culture Histology Antigen testing (when appropriate)
Treatment					
Primary	Nystatin Clotrimazole	Lipid formulation of amphotericin B§ Fluconazole‡‖	Lipid formulation of amphotericin B§	Lipid formulation of amphotericin B§ Fluconazole‡‖	Lipid formulation of amphotericin B§
Secondary	Topical amphotericin B† Fluconazole‡**	Echinocandin therapy§§ Flucytosine¶	Echinocandin therapy§§ Itraconazole‡ †† ‡‡ Voriconazole‡‖‖ Flucytosine¶	Flucytosine¶	Azole therapy (for susceptible organisms)‡
Adjunctive		Removal of central lines	Surgical resection		Surgical débridement
Duration of Therapy	Dependent on rate of clearance	Dependent on the rate of clearance: minimum of 14 days	Dependent on the rate of clearance: minimum of 4 weeks, usually 8-12 weeks	Minimum of 6-8 weeks Many would continue with fluconazole indefinitely	Dependent on rate of clearance
Follow-up	Clinical examination Repeat urine analysis/cultures	Dependent on clinical scenario	Dependent on clinical scenario	Clinical examination Antigen testing Repeat culture of appropriate source (sputum, CSF, urine) Radiographs if relevant	Clinical examination Antigen testing Repeat culture of appropriate source (sputum, CSF, urine) Radiographs if relevant

*Common: >5%; uncommon 1-5%; rare <1%.
†Topical amphotericin B for bladder wash for noninvasive candiduria; ultrasonography of kidneys recommended to confirm absence of invasive disease.
‡Azole use must be accompanied by close follow-up of levels of cyclosporine or tacrolimus. In general, tacrolimus dosing should be cut in half when using a standard dose of fluconazole.
§Dose varies according to specific agent.
‖Fluconazole is alternative first-line drug for invasive disease if the species is known to be sensitive to fluconazole and the patient is clinically stable. Dosage is 6 to 12 mg/kg/day based on severity of infection.
¶Flucytosine should not be used alone but is synergistic when used in conjunction with amphotericin B. Dosage is 100 to 150 mg/kg/day divided every 6 hours.
**Radiographic staging includes computed tomography of head, chest, and abdomen.
††Itraconazole can be used long-term for patients who have been treated for invasive *Aspergillus* but in general is not recommended as first-line therapy.
‡‡Itraconazole absorption can be erratic. Accordingly, monitoring of itraconazole levels is recommended. Itraconazole is dosed at 3 to 5 mg/kg/day as a single dose. Dosing adjustment based on monitoring of levels is recommended. Adjustment of cyclosporine or tacrolimus dosing should be individualized.
§§The use of either of the approved echinocandins (caspofungin or micafungin) may be an appropriate alternative for treatment of invasive candidiasis, candidemia, and aspergillosis. Dose adjustments may be necessary in the presence of impaired liver function. Monitoring of tacrolimus levels are indicated because these agents may decrease tacrolimus levels.
‖‖‖Voriconazole levels should be checked for patients treated with oral therapy.
CSF, cerebrospinal fluid.

infections with *Aspergillus*, *Alternaria*, and *Scedosporium* in this population. In general, these children have been on high levels of immune suppression or have other complications such as PTLD and outcome of these infections has been poor. Guidelines for the diagnosis and management of fungal infection in children undergoing intestinal transplantation are provided in Table 72-4.

VIRAL INFECTIONS

Viral pathogens, especially herpesviruses, are a major source of morbidity and mortality after solid-organ transplantation. Patterns of disease associated with individual viral pathogens generally are similar among all transplant recipients. However, frequency, mode of presentation,

and relative severity can differ, according to the type of organ transplanted and the serologic status of the recipient.

Cytomegalovirus

CMV continues to be the most common and one of the most important viral pathogens after organ transplantation in children. CMV infection can be asymptomatic or symptomatic and may be due to primary infection (either from the donor graft or blood products), reactivation of latent infection, or superinfection with a different CMV strain in a previously seropositive child. Prior to the use of prophylaxis, the incidence of symptomatic CMV infection was reported to be as high as 22 percent in adult[56] and 40 percent in pediatric[9] liver transplant recipients. Use of ganciclovir prophylaxis or viral monitoring and preemptive treatment has resulted in a decreased rate and severity of CMV disease.[45] Intestinal transplantation was introduced after the availability of ganciclovir, and therefore has been able to take advantage of using ganciclovir both as prophylaxis as well as treatment. Despite this, it is clear that CMV disease can be very severe after intestinal transplantation and has a high rate of recurrence.[11,77] More recent reports identify decreased the incidence and severity of CMV disease in this population, although these patients continued to experience high recurrence rates of CMV infection.[33,85] Primary CMV infection, typically acquired from the donor organ (or passenger donor leukocytes that accompany the organ), is associated with the greatest degree of morbidity and mortality.[41,45] Accordingly, CMV-seronegative recipients of organs from CMV-seropositive donors are considered at high risk for developing CMV disease. Reactivation of, or superinfection with CMV, tends to result in milder illness after liver transplantation but can still be severe after intestinal transplantation.[10,12] CMV disease appears more likely to develop in CMV-seropositive recipients of CMV-seropositive donors than in seropositive recipients of seronegative donors.[41] Patients treated with unusually high doses of immunosuppressants, especially antilymphocyte antibody preparations, experience an increased rate of CMV disease regardless of previous immunity.[9,76]

Symptomatic CMV disease classically presented between 1 and 3 months after transplantation. It is important to note that the use of prophylactic or preemptive monitoring regimens may delay onset of CMV disease.[33] When present, a characteristic constellation of fever (which may be high grade, prolonged, and hectic) and hematologic abnormalities (including leukopenia, atypical lymphocytosis, and thrombocytopenia) is frequently seen. This "CMV syndrome" occurs in 25 to 50 percent of patients with symptomatic CMV infection. Invasive CMV disease is manifest by visceral organ involvement; common sites include the gastrointestinal tract, liver, and lungs. CMV hepatitis appears to be the most common site among liver transplant recipients whereas CMV enteritis is a frequent finding among intestinal transplant recipients. CMV chorioretinitis is rare in organ transplant recipients.

Diagnosis of CMV disease may be confirmed by detecting the presence of CMV DNA or pp65 antigen in the blood of a patient with a compatible clinical syndrome.[77,126] However, clinicians must be aware that results of viral cultures of the urine and even bronchoalveolar lavage specimens are difficult to interpret in previously infected patients because CMV is frequently shed asymptomatically in these secretions. Similarly, the presence of pp65 antigen and CMV DNA in the blood can be misleading, because these assays are often positive in asymptomatic patients. The specificity of this approach may be improved by quantitative determination of the pp65 antigen or CMV DNA. Because of the lack of specificity of these assays, histologic examination of involved organs to confirm the presence of CMV is critical when the diagnosis of invasive CMV is being entertained.

Antiviral agents with activity against CMV (e.g., ganciclovir, foscarnet, and cidofovir) have improved the survival of transplant recipients with CMV disease. Fatal, disseminated CMV disease occurred in 19 percent of infected children[9] and 5 percent of infected adults undergoing liver transplantation in the era before ganciclovir was introduced.[114] For clinical CMV disease, ganciclovir therapy is given in conjunction with reduction of immunosuppression unless evidence of rejection is present.[74] Clinical response usually occurs 5 to 7 days after initiation of therapy. Baseline immunosuppression levels are typically restored at the time of initial clinical response or on recognition of rejection. Data support the use of serial monitoring of CMV load in the peripheral blood as a guide to duration of treatment of CMV disease.[24,68,112] Valganciclovir was equally efficacious in treating adult kidney transplant recipients with CMV disease but not those who had undergone liver transplantation.[6] Controlled studies in children are lacking, but many centers use valganciclovir in children once they are discharged from the hospital. The role of CMV hyperimmune globulin in combination with ganciclovir in the treatment of CMV disease is controversial, although some evidence for improved outcome has been reported in the treatment of CMV pneumonia in adult liver transplant recipients.[39,68] Finally, because of the relatively high rates of nephrotoxicity associated with their use, foscarnet or cidofovir should be restricted to patients with apparent or proven resistance to ganciclovir, which should be suspected in patients who either fail to clinically improve or have persistent or rising CMV viral loads after 2 weeks of ganciclovir therapy.[68]

Historically, approximately 25 percent of patients treated with ganciclovir for an initial episode of symptomatic CMV will develop one or more episodes of recurrent CMV disease,[112,123] although rates of recurrent disease may have fallen in the presence of longer initial treatment and the use of secondary prophylaxis.[68] When present, recurrences are observed about 1 month after the initial infectious episode and may be associated with invasive disease. More commonly, however, these recurrent episodes tend to be milder than the initial episode. Risk factors identified with an increased risk for developing recurrent CMV disease include being a CMV-seronegative recipient of a CMV-seropositive donor, having disseminated CMV disease, and having a history of multiple treatment courses for rejection and persistent elevations of the CMV load at the end of therapy.[112]

TABLE 72-5 **Overview of Diagnosis and Management of Viral Infections After Liver and Intestinal Transplantation in Children**

	Cytomegalovirus	Epstein-Barr Virus	Respiratory Syncytial Virus	Influenza	Parainfluenza	Adenovirus
Frequency	Common*	Common	Uncommon	Uncommon	Uncommon	Liver: uncommon† Intestine: common*
Diagnostic Tests	Culture Quantitative CMV PCR (blood) pp65 Antigen Histology	Quantitative EBV PCR (blood) Histology Serology	NP aspirate for antigen detection or PCR	NP aspirate for antigen detection, PCR and/or culture	NP aspirate for culture or PCR	Viral culture or PCR Histology
Treatment						
Primary	Ganciclovir IV (5 mg/kg bid)	Decrease immune suppression	Supportive care	Supportive care	Supportive care	Decrease immune suppression
Secondary	Foscarnet§ Cidofovir§	Rituximab Cytoxan/ prednisone	Aerosolized ribavirin	Zanamivir Oseltamivir Amantadine Rimantadine		IV ribavirin
Adjunctive	Decrease immune suppression CMV-IVIG	Ganciclovir IVIG	Palivizumab Decrease immune suppression	Decrease immune suppression	Decrease immune suppression	IVIG§
Duration of Therapy	Site dependent	Individualized	Individualized	Individualized	Individualized	Individualized
Follow-up	Monitor quantitative CMV PCR or pp65 (treat until negative)	Monitor quantitative EBV PCR Repeat imaging studies if positive at outset	None	None	None	None

*Common: frequency ~>5%.
†Uncommon: frequency ~1-5%.
‡Rare: frequency <1%
§Foscarnet or cidofovir used for CMV infection when ganciclovir resistance is suspected or proven. Experience from HIV-infected patients suggests that a synergistic benefit will be obtained from the combined use of both of these agents when ganciclovir resistance is present.

Although current guidelines recommend continuing antiviral therapy until the CMV viral load becomes negative, further research is needed on duration.[68] A summary of our suggested approach to the diagnosis and management of CMV infection is provided in Table 72-5.

Epstein-Barr Virus

EBV infection, including EBV-associated PTLD, is an important cause of morbidity and mortality after liver transplantation and intestinal transplantation.* This is particularly true among children undergoing intestinal transplantation, who experience the highest rates of EBV-related disease than any of the other organ transplant recipients.[97]

Symptomatic EBV infection in general and PTLD in particular most commonly occur among transplant recipients experiencing primary EBV infection, particularly those who receive organs from seropositive donors. Accordingly, children undergoing transplantation are disproportionately affected by EBV compared with their adult counterparts.[53] As many as 80 percent of children who are EBV seronegative before liver transplantation will develop a primary EBV infection after this procedure.[117,120] Although primary infection occurs in the vast majority of seronegative patients, clinical disease develops in less than one third of these children.[117,120] In one study, 4 percent of children undergoing solid-organ transplantation and 10 percent of children with primary EBV infection developed PTLD between 1 month and 5 years after transplant[62]; 75 percent of cases occurred during the first postoperative year in patients undergoing cyclosporine-based immune suppression. Historically, cumulative occurrence rates reached as high as 12 to 20 percent by 7 to 12 years after pediatric liver transplantation.[83,94] Current rates of PTLD in this cohort appear lower, with an incidence of 4.7 percent at 5 years after transplant, likely owing to improvements in immune suppression and the use of preventive monitoring strategies.[103]

Pediatric recipients of intestinal transplantation appear to behave differently than children undergoing other types of organ transplantation in that the rate of EBV disease, including PTLD, occur irrespective of EBV

*References 3, 13, 62, 122, 128, 135.

serostatus before transplantation. Rates of EBV disease and PTLD after intestinal transplantation as high as 30 to 40 percent were reported during the initial experiences with intestinal transplantation. More recently, these rates have declined to approximately 11 percent,[85] reflecting improved immune suppression regimens and EBV-monitoring protocols. However, these rates still remain higher than those seen among other organ recipients.

A wide spectrum of EBV disease is recognized that includes nonspecific viral illness, mononucleosis, and PTLD (including lymphoma). Histologic evaluation is important in differentiating among these categories; manifestations can evolve in individual patients. Asymptomatic seroconversion also occurs. Variation in severity and extent of disease is related to the degree of immunosuppression and adequacy of the host immune response. Although EBV disease and PTLD may present as involvement of many different clinical sites, there is a tendency for EBV disease to involve the transplanted organ. Thus, EBV hepatitis and PTLD of the liver are observed more commonly among liver transplant recipients. Similarly, the intestine is the most frequently observed site of involvement of EBV disease among intestinal transplant recipients. Of interest, involvement of sites beyond the gastrointestinal tract is uncommon in intestinal transplant recipients.

Onset of viral syndrome, mononucleosis, and PTLD occur primarily within the first year, whereas lymphoma tends to present later. Immunosuppressive regimens based on the use of tacrolimus appear to have affected a shift in the timing of PTLD; only rare cases occur longer than 18 months after transplant.[15,135] This pattern of timing of EBV disease and PTLD appears to apply to all pediatric organ recipients, including children undergoing liver or intestinal transplantation. The impact of newer immunosuppressive agents and regimens on EBV disease needs to be constantly reevaluated because unexpectedly high rates of PTLD have occurred with some new regimens.[54,113]

The diagnosis of EBV-associated PTLD is made on the basis of clinical, laboratory, and histopathologic examination and should be suspected in patients with protracted fever, exudative tonsillitis, lymphadenopathy, organomegaly, leukopenia, or atypical lymphocytosis.[47,97] Gastrointestinal involvement should be suspected in patients with persistent fever and diarrhea. Accordingly, EBV must be considered in the differential diagnosis of rejection among intestinal transplant recipients. Serologic diagnosis is often confounded by the presence of passive antibody acquired at the time of transplantation or during subsequent transfusions. The detection of increased EBV viral load in peripheral blood identified by PCR has gained wide acceptance as an assay to predict risk for or presence of EBV or PTLD.[50,74,97,106,109] Although extremely sensitive, these assays are limited by both a lack of uniformity across different laboratories[101] and their lack of specificity; they are often elevated in asymptomatic patients.[1,2] Accordingly, every effort should be made to histologically confirm the diagnosis of EBV or PTLD. Occult sites of PTLD are assessed by performance of computed tomography of chest and abdomen. Palpable nodes or lesions (or both) identified by radiographic surveillance should be sampled. Endoscopic evaluation should be considered in patients with diarrheal illnesses and elevated viral loads.[17] Histologic evaluation for typical features may be augmented through the use of the Epstein-Barr–encoded RNA (EBER) probe.[104] The use of the EBER probe may be particularly useful in differentiating between the presence of rejection and EBV infection among intestinal transplant recipients.

Management of patients with PTLD is controversial.[15,48,97] Reduction of immunosuppression is recommended widely. Antiviral agents typically are used,[57,107] although their role has not been studied formally. Reduction of immunosuppression, alone or in combination with antiviral agents, results in an approximate 67 percent cure rate of EBV disease and PTLD. The potential impact of monoclonal antibodies,[30] interferon,[111] and chemotherapy[37] awaits formal clinical trials. Resection of tumor also may be of value for patients with lymphoma. Recent experience has focused on several novel approaches to the management of EBV disease and PTLD. These newer strategies have typically been used for patients who fail to respond to reduction of immune suppression (with or without the use of antiviral agents). Rituximab, an anti-CD20 antibody, has been increasingly used for the treatment of EBV disease. Experience to date suggests that as many as two thirds of patients who fail initial withdrawal of immunosuppression will respond to a 4-week course of this agent.[49] However, relapse of EBV disease has been observed in 20 to 25 percent of treated patients 6 to 8 months after completion of therapy at the time the rituximab is no longer present in the body. An alternate, chemotherapy-based approach for patients who fail to respond to initial reduction or withdrawal of immunosuppression has also been proposed.[56] This strategy, which uses modified doses of cyclophosphamide and prednisone, has also achieved success in approximately two thirds of treated patients. Unfortunately, as with rituximab, relapse of PTLD has been seen in 22 percent of treated patients and outright treatment failures have occurred in patients presenting with fulminant disease. Definitive studies comparing these two second-line therapies are needed to define the best option for those children who fail to respond to initial therapeutic modification of immunosuppression. A summary of our suggested approach and management of EBV/PTLD is provided in Table 72-5.

Other Herpesviruses

Other herpesviruses can also be hazardous after transplantation. Herpes simplex can reactivate early after surgery or after augmentation of immunosuppression. Prophylaxis with acyclovir (or ganciclovir) has been beneficial in these situations. A summary of the suggested approach to the diagnosis and management of HSV is provided in Table 72-5. Primary varicella infection (chickenpox) in transplant recipients can lead to disseminated, fatal disease[88] and should be treated early and aggressively with intravenous acyclovir. Some centers have used valacyclovir rather than intravenous medication, but further studies are needed. Reactivation disease

as shingles is more commonly seen in adults and can be prevented with antiviral prophylaxis early in the transplant period.

Interest has also focused on determining what role, if any, human herpesvirus–6 (HHV-6) and 7 (HHV-7) may play in causing disease in organ transplant recipients in general and abdominal transplant recipients in particular. Several groups of investigators have identified a potential interaction between the development of HHV-6 and HHV-7 and CMV infection in organ transplant recipients.[67,90] Reactivation of HHV-6 infection after liver transplantation has been associated with the development of an increased CMV viral load in the peripheral blood as well as a greater likelihood of developing symptomatic CMV disease.[67] In addition, it has been suggested that some or all of the symptoms typically associated with the CMV syndrome (e.g., fever, leukopenia) in patients with proven CMV infection may be attributable in part to HHV-6 or HHV-7. Studies in children have suggested that infection due to HHV-6 alone is a relatively common cause of unexplained fever in pediatric liver transplant recipients.[133,134] Interest also exists in the role, if any, that human herpesvirus–8 (HHV-8) may have in causing infection and disease in organ transplant recipients. This is particularly true for recipients who come from countries with moderate to high rates of HHV-8 prevalence such as Africa, the Middle East, and the Caribbean.[93] The full spectrum of disease of these more recently recognized herpesviruses and their potential therapies remain to be determined.

Adenovirus

Adenovirus has been reported to be the third most important virus affecting pediatric liver transplant recipients, occurring in 10 percent of one series of 484 children undergoing liver transplantation under cyclosporine-based immunosuppression.[92] Symptomatic disease (ranging from self-limited fever, gastroenteritis, or cystitis to devastating illness with necrotizing hepatitis or pneumonia) occurred in over 60 percent of infected patients. Infection was most commonly diagnosed within the first 3 months after transplantation.[46] The frequency of invasive adenovirus infection after pediatric liver transplantation appears to have decreased markedly with the use of tacrolimus-based immunosuppression.[51] In a more recent report, McLaughlin and colleagues found that only 4.2 percent of pediatric liver recipients using tacrolimus-based therapy had adenovirus disease.[89] This is in contrast to a significantly higher incidence of 20.8 percent of adenovirus infection in pediatric intestinal transplant recipients at the same institution. Florescu and colleagues reported a rate of adenovirus infection of 39 percent in their cohort of pediatric intestinal transplant recipients, although only 60 percent of infected patients were diagnosed to have adenovirus disease.[31] Finally, Pinchoff and colleagues found adenovirus in all 14 of their pediatric intestinal transplant recipients.[99] However, these exceptionally high rates might be attributable to the fact that viral cultures and PCRs were performed as part of routine screening of graft biopsies and not all of the patients were symptomatic. In all of these studies, high stool output, alone or in the presence of fever, was the most common sign found in these patients.

It is very difficult to presumptively diagnose infection due to adenovirus in pediatric abdominal transplant recipients, because fever, hepatitis, and pneumonia may be caused by a variety of other pathogens and high stool output after intestinal transplantation is nonspecific and can also occur with rejection. The presence of high-grade fevers and symptoms suggestive of adenovirus infection should prompt serial cultures for viruses (including adenovirus) or PCR investigation and evaluation of graft biopsies. Unexplained elevations in hepatocellular enzymes suggestive of hepatitis should warrant consideration of a liver biopsy. Similarly, an increase in stool output, with or without fever, should prompt endoscopic evaluation of the intestinal allograft. Histologic examination for the presence of adenoviral inclusions as well as the use of immunohistochemical stains of biopsies specimens from either site should be undertaken to help confirm this diagnosis.

Definitive evidence evaluating potential treatments for adenoviral disease is not available at this time. The most important component of therapy is supportive care along with a decrease in immunosuppression. The role of antiviral agents is unproven[7,46,49] but increasing data support the use of cidofovir in bone marrow or solid-organ transplant recipients.[45,58,105] Current available guidelines support consideration of use of this agent for patients with proven disease.[69] Ongoing studies are investigating the potential role of a lipid ester of cidofovir (CMX-001), which is currently in trial in bone marrow transplant recipients.[69] This drug, which is orally bioavailable, has been associated with less nephrotoxicity and may actually be more potent than the parent compound. Finally, a single case report also raised the possible role of intravenous immunoglobulin as treatment for adenovirus infection.[25] A summary of a suggested approach to the diagnosis and management of adenovirus infection is provided in Table 72-5.

Common Community-Acquired Viruses

Although the course of illness has been poorly documented, most children who undergo liver and intestinal transplantation experience the usual respiratory viruses and gastrointestinal illness without significant problems. However, infections due to influenza virus, parainfluenza virus, or RSV led to more severe disease in young children, especially if infection occurs soon after transplant and during periods of maximal immunosuppression.[5,29,71,100] Likewise, transplant recipients may have prolonged viral shedding even after resolution of symptoms. A summary of suggested strategies for the diagnosis and management of these community-acquired viruses can be found in Table 72-5.

Other Viruses

Other viruses, including both donor-associated viral infections (e.g., hepatitis B and hepatitis C virus) as well as community-acquired viral pathogens (e.g., enterovirus, rotavirus) are relatively uncommon causes of clinically

significant infection or disease after abdominal transplantation. Although this may be explained in part by the paucity of data describing infectious complications late after transplant, infection owing to these agents is clearly uncommon. For those patients for whom infection due to these pathogens is recognized, consultation with the primary transplant center is strongly recommended. Suggested approaches to the diagnosis and management of each of these viral pathogens are provided in Table 72-5.

OPPORTUNISTIC INFECTIONS

P. jiroveci is a well-documented cause of pneumonia in immunocompromised patients, including liver and intestinal transplant recipients. Prophylactic trimethoprim-sulfamethoxazole is safe, inexpensive, and effective.[65,66] The use of this strategy has eliminated *P. jiroveci* pneumonia in these patients at Children's Hospital of Pittsburgh, with rare breakthroughs occurring in patients who discontinued prophylaxis. Alternative prophylactic regimens for the sulfa-allergic patient include aerosolized pentamidine (for patients >5 years of age)[80] or dapsone.[69]

Tuberculosis (TB) is a particular concern in immunosuppressed hosts, including recipients of liver and intestinal transplantation. The incidence of TB after liver transplantation in Europe and the United States has been reported to range from 0.9 to 2.3 percent, with most reported cases in adults.[115,130] In contrast, up to 15 percent of organ transplant recipients in areas of high-level endemicity may develop TB.[115] However, development of TB after pediatric liver transplantation is extremely uncommon, with less than 20 cases reported to date.[86,115,130] To date, no cases have been reported among recipients of intestinal transplantation. Development of TB among solid-organ transplant recipients is associated with mortality rates ranging from 25 to 40 percent, with additional morbidity and mortality associated with development of rejection in patients receiving anti-TB therapy.[84,115,130] Diagnosis of TB in transplant recipients is complicated by the fact that extrapulmonary disease occurs frequently and purified protein derivative (PPD) testing is likely to be unreliable after transplant. Management of TB in liver transplant recipients is difficult both because of the side effects of anti-TB agents and their potential interactions with immunosuppressive agents.[115,130] Limited published experience in pediatric liver transplant recipients suggests that the majority of infections due to *Mycobacterium tuberculosis* are most likely due to a primary infection often associated with family contacts who have positive skin tests.[84,130] In contrast, experience with adult transplant recipients suggests that development of TB is more likely due to reactivation of latent TB.[60,115,130] Although only limited published information is available describing TB in these patients, transplant recipients known to have a positive PPD or who come from an area endemic for TB appear to be at increased risk for symptomatic reactivation after transplantation.[60,130] Similar data are not available for recipients of intestinal transplantation. Additional factors predisposing to the development of TB after transplantation include severe hepatic failure at the time of transplant, aggressive antirejection therapy, and/or concurrent HIV infection.[60,124] Experience among adult renal transplant recipients suggests that although the risk appears greatest in patients who received inadequate or no prior TB therapy,[81,84] it can also occur in patients who received appropriate anti-TB therapy in the pretransplant period.[81,84,102] Although TB has only rarely been encountered among pediatric liver transplant recipients,[130] and not among intestinal recipients, screening for TB by history and a PPD test along with review of a chest radiograph for lesions consistent with healed TB is highly recommended. Patients with a positive TB history and/or a positive PPD result should receive isoniazid for 6 to 12 months after transplant, although some experts recommend continuing this agent indefinitely while patients remain on immunosuppression. Attempts at a more definitive diagnosis are indicated for patients from endemic areas with a negative PPD result but suggestive chest radiograph. Careful evaluation for evidence of side effects, particularly hepatotoxicity, is recommended, and isoniazid is discontinued if unacceptable toxicity is identified.

Additional potential opportunistic infections include cryptococcosis, coccidioidomycosis, and histoplasmosis, although the pathogens of these infections have not been frequently reported among pediatric liver or intestinal transplant recipients. Prior infection with these pathogens is common in geographic areas where they are endemic. Because patients often travel to transplant centers distant to their homes, it is imperative that transplant physicians be aware of the local environmental risks for each patient. Experience with coccidioidomycosis in transplant recipients suggests that a minimum of 4 months of antifungal therapy, such as fluconazole, should be given in transplant recipients with this history.[56] Similarities between coccidioidomycosis and other fungal pathogens suggests that similar strategies may be necessary for patients with a positive history of prior fungal infection with pathogens known to recur after resolution of primary infection.

MANAGEMENT

Pretransplant Evaluation

A pretransplant evaluation is helpful in the management of infectious complications in liver and intestinal transplant recipients. A complete history and physical examination should be performed with particular attention to previous infections, immunizations, and drug allergies. Attention should be paid to a history of infection with multidrug-resistant bacteria, which may provide guidance for future empirical treatment that may be required after transplantation. An intermediate-strength tuberculin skin test should be performed on all patients. Alternatively, for children older than 5 five years of age, the interferon-γ release assays can be used.

The Children's Hospital of Pittsburgh pretransplant protocol includes obtaining serology for CMV, EBV, varicella, herpes simplex virus, hepatitis A, B (surface antibody, surface antigen, and core antibody), and C, and

HIV for all candidates. In addition, serology to measles, mumps, and rubella is obtained to ensure that the patients have a measurable antibody response to prior immunizations. Serologic tests on the donor should include HIV, hepatitis B and C, CMV, and EBV. Donors positive for HIV should be excluded whereas those positive for hepatitis B or C virus would be contraindicated except in the circumstance in which the recipient is also positive for hepatitis B or C, respectively. Knowledge of donor and recipient status for these viruses allows one to anticipate infection, identifying patients who might benefit from prophylactic regimens and guiding in the diagnostic evaluation of fever.

Prophylactic Regimens

Prophylactic regimens vary among transplant centers. These strategies have been divided into perioperative and long-term prophylaxis and often evolve to reflect the infectious complications seen at individual institutions.

Perioperative prophylaxis is used to prevent intraoperative sepsis and wound infection. It is based on individual patient characteristics and expected normal flora. At Children's Hospital of Pittsburgh, piperacillin-tazobactam is used as the standard agent for perioperative prophylaxis. This is modified if the recipient is known to harbor resistant bacteria. If sepsis is suspected in the donor, antibiotics are chosen to cover those organisms identified from the donor and treatment is usually extended to a therapeutic course of 10 to 14 days. In the absence of proven or suspected infection in the donor, perioperative prophylaxis is usually limited to the first 48 hours after transplant.

Considerations regarding long-term prophylaxis against infections occurring beyond the perioperative period include the risk and severity of infection as well as the toxicity, cost, and efficacy of a given prophylactic strategy. Nystatin is recommended for all pediatric transplant recipients in an effort to prevent oropharyngeal candidiasis until the patients are on less than 5 mg/day of prednisone. Trimethoprim-sulfamethoxazole is used to prevent *P. jiroveci* pneumonia. Although some centers recommend using trimethoprim-sulfamethoxazole for only the first 6 months after liver transplantation, anecdotal experience with patients presenting with PCP long after transplant and the relative safety of this agent have led us to recommend its use indefinitely after liver and intestinal transplantation in children.

The frequency and severity of CMV infection in transplant recipients prompts consideration of prophylactic strategies; optimizing the target population and timing of interventions require further study.[24,78] Potential roles exist for intravenous and oral ganciclovir[36] and oral valganciclovir.[96] Currently the Children's Hospital of Pittsburgh protocols use intravenous ganciclovir alone (for varying durations) for liver and low-risk intestinal transplant recipients (recipient CMV positive before transplant or donor and recipient seronegative) and ganciclovir plus intravenously administered immune globulin containing a high titer of antibody against CMV[118,119] for high-risk (donor CMV positive and recipient CMV negative) intestinal transplant recipients if available. At the present time, the use of oral valganciclovir is an alternative to prophylaxis with intravenous ganciclovir for adolescents, although the use of this medication is not approved for liver transplant recipients. This is because a higher rate of tissue invasive disease was observed in liver transplant recipients receiving valganciclovir compared with oral ganciclovir in the pivotal trial evaluating this agent. Nonetheless, published guidelines support the use of valganciclovir for adult liver transplant recipients.[68,98] Pharmacokinetic studies for dosing oral valganciclovir suspension in children after transplantation adjusted for body surface area and renal function suggest that ganciclovir exposures similar to those established as safe and effective in adults can be achieved.

Serial monitoring of blood CMV viral load using quantitative CMV PCR or the pp65 antigen assay as an indicator for the use of preemptive antiviral therapy has been proposed as an alternative to these chemoprophylactic and immunoprophylactic strategies.[47,68,75] In this approach, only patients demonstrating increased risk because of increased viral load are treated with intravenous or oral valganciclovir. Although this strategy has gained acceptance at some centers, experience in pediatric transplant recipients in general and in abdominal transplant recipients in particular remains limited. A combined hybrid strategy using viral load monitoring after completion of chemoprophylaxis is gaining increasing acceptance.[75]

Similar to CMV, there is an interest in the prevention of EBV infections and PTLD. A number of different strategies are used, including immunoprophylaxis, viral load monitoring, and preemptive therapy[49,87]; the efficacy of these approaches has not been established. The use of viral load monitoring to inform preemptive reductions in immunosuppression appears to be the most promising of these strategies.[3,79,87] Because reduction of immune suppression is not always possible for intestinal transplant recipients with a rising EBV load, preemptive use of intravenous ganciclovir and intravenous immune globulin has been used in addition to reduction of immune suppression (where possible) for this cohort of patients. Although prospective comparative data are not available, the use of this strategy appears to have resulted in decreased risk for disease and improved outcome compared with historical controls that were not followed with this strategy.[43] The use of anti-CD20 monoclonal therapy has also been used by some centers when decreased immunosuppression is not possible.

SUMMARY

Infections remain an important problem after liver and intestinal transplantation. Knowledge of the type, timing, and predisposing risk factors for these infectious complications allows for their timely and appropriate diagnosis and management.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Allen U, Green M. Prevention and treatment of infectious complications after solid organ transplantation in children. *Pediatr Clin North Am* 2010;**57**(2):459–79.

3. Allen U, Preiksaitis J, and the AST Infectious Diseases Community of Practice. Epstein-Barr virus and post-transplant lymphoproliferative disorder in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S87–96.
31. Florescu DF, Islam MK, Mercer DF, et al. Adenovirus infections in pediatric small bowel transplant recipients. *Transplantation* 2010;**90**:198–204.
32. Florescu DF, Islam KM, Grant W, et al. Incidence and outcome of fungal infections in pediatric small bowel transplant recipients. *Transplant Infect Dis* 2010;**12**:497–504.
33. Florescu DF, Langnas AN, Grant W, et al. Incidence, risk factors, and outcomes associated with cytomegalovirus disease in small bowel transplant recipients. *Pediatr Transplant* 2011;**16**:294–301.
34. Florescu DF, Qiu F, Langnas AN, et al. Bloodstream infections during the first year after pediatric small bowel transplantation. *Pediatr Infect Dis J* 2012;**31**(7):700–4.
35. Florescu DF, Qui F, Mercer DF, et al. Risk factors for systemic Candida infections in pediatric small bowel transplant recipients. *Pediatr Infect Dis J* 2012;**31**:120–3.
54. Grinyo J, Charpentier B, Pestana JM, et al. An integrated safety profile analysis of belatacept in kidney transplant recipients. *Transplantation* 2010;**90**:1521–7.
55. Groetzner J, Kaczmarek J, Wittwer T, et al. Caspofungin as first-line therapy for the treatment of invasive aspergillosis after thoracic organ transplantation. *J Heart Lung Transplant* 2008;**27**:1–6.
68. Humar A, Snydman D, and the AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S78–86.
69. Ison MG, Green M, and the AST Infectious Diseases Community of Practice. Adenovirus in solid organ transplantation. *Am J Transplant* 2009;**9**(Suppl. 4):S161–5.
70. Ison MG, Hager J, Blumberg E, et al. Donor-derived disease transmission events in the United States: Data reviewed by the OPTN/UNOS Disease Transmission Advisory Committee. *Am J Transplant* 2009;**9**:1929–35.
75. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010;**89**:779–95.
85. Mazareigos GV. Intestinal transplantation: current outcomes and opportunities. *Curr Opin Organ Transplant* 2009;**14**:515–21.
91. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.
101. Preiksaitis JK, Pang XL, Fox JN, et al. Interlaboratory comparison of Epstein-Barr virus viral load assays. *Am J Transplant* 2009;**9**:269–79.
103. Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR) 2010 Annual Data Report. Department of Health and Human Services. Health Care Resources and Services Administration Health Care Systems Bureau. Division of Transplantation. *Am J Transplant* 2012;**12**(Suppl. 1).
113. Sindhi R, Ashokkumar C, Higgs BW, et al. Allospecific CD154+ T-cytotoxic memory cells as potential surrogate for rejection risk in pediatric intestine transplantation. *Pediatr Transplant* 2012;**16**(1):83–91.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 73

Opportunistic Infections in Kidney Transplantation

Gail J. Harrison

Renal transplantation is the therapy of choice for end-stage renal disease (ESRD) in children and adolescents.[154] It is successful in 90 percent of recipients and allows most children the best opportunities for normal growth and development and an almost normal lifestyle.[82,157,239] Despite the overall success of renal transplantation, infection remains the major cause of morbidity, graft loss, and mortality in renal transplant recipients.[57,99,150,232] Newer and more effective immunosuppressive regimens have reduced the rates and hospitalizations for graft rejection after renal transplantation, but rates and hospitalizations for bacterial and viral infections seem to be unchanged or increasing.[60,158,202] Prednisone-free maintenance immunosuppressive combinations with tacrolimus, mycophenolate mofetil, and sirolimus help maintain graft function, but they also may increase the risk for development of posttransplantation lymphoproliferative disorders (PTLD) and invasive fungal diseases.[116]

These infections can be managed successfully if detected early and treated appropriately.[116] In this chapter a general approach to renal transplant recipients is presented from the perspective of the infectious disease

REFERENCES

1. Allen U, Green M. Prevention and treatment of infectious complications after solid organ transplantation in children. *Pediatr Clin North Am* 2010;**57**(2):459–79.
2. Allen U, Hebert D, Petric M, et al. Utility of semiquantitative polymerase chain reaction for Epstein-Barr virus to measure virus load in pediatric organ transplant recipients with and without posttransplant lymphoproliferative disease. *Clin Infect Dis* 2001; **33**:145–50.
3. Allen U, Preiksaitis J, and the AST Infectious Diseases Community of Practice. Epstein-Barr virus and post-transplant lymphoproliferative disorder in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S87–96.
4. Angelis M, Cooper JT, Freeman RB. Impact of donor infections on orthotopic liver transplantation. *Liver Transplant* 2003;**9**: 451–62.
5. Apalsch AM, Green M. Influenza and parainfluenza virus infections in pediatric organ transplant recipients. *Clin Infect Dis* 1995; **20**:394–9.
6. Asberg A, Humar A, Rollag H, et al. Oral valganciclovir is noninferior to intravenous ganciclovir for the treatment of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant* 2007;**7**:2106–13.
7. Boger-Arav R, Echavarria M, Forman M, et al. Clearance of adenoviral hepatitis with ribavirin therapy in a pediatric liver transplant recipient. *Pediatr Infect Dis J* 2000;**19**:1097–100.
8. Bouchut JC, Stamm D, Boillot O, et al. Postoperative infectious complications in paediatric liver transplantation: a study of 48 transplants. *Paediatr Anaesth* 2001;**11**:93–8.
9. Bowman JS, Green M, Scantlebury VP, et al. OKT3 and viral disease in pediatric liver transplant recipients. *Clin Transplant* 1991;**5**:294–300.
10. Breinig MK, Zitelli B, Starzl TE, Ho M. Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. *J Infect Dis* 1987;**156**:273–9.
11. Bueno J, Green M, Kocoshis S, et al. Cytomegalovirus infection after intestinal transplantation in children. *Clin Infect Dis* 1997;**25**: 1078–83.
12. Burroughs M, Sobanjo A, Florman S, et al. Cytomegalovirus matching does not predict symptomatic disease in intestinal transplantation. *Transplant Proc* 2002;**34**:946–7.
13. Cacciarelli TV, Green M, Jaffe R, et al. Management of posttransplant lymphoproliferative disease in pediatric liver transplant recipients receiving primary tacrolimus (FK506) therapy. *Transplantation* 1998;**66**:1047–52.
14. Cacciarelli TV, Jaffe R, Green M, et al. A decreased incidence of post-transplant lymphoproliferative disorder (PTLD) in pediatric liver transplant recipients under primary tacrolimus (FK506) therapy. Program and abstracts of the 16th annual meeting of the Scientific Committee of the American Society for Transplant Physicians. 1997, abstract 289, p. 157.
15. Cacciarelli TV, Reyes J, Jaffe R, et al. Primary tacrolimus (FK 506) therapy and the long-term risk of posttransplant lymphoproliferative disease in pediatric liver transplant recipients. *Pediatr Transplant* 2001;**5**:359–64.
16. Campbell AL, Herold BC. Strategies for the prevention of cytomegalovirus infection and disease in pediatric liver transplantation recipients. *Pediatr Transplant* 2004;**8**:619–27.
17. Cao S, Cox K, Esquivel CO, et al. Posttransplant lymphoproliferative disorders and gastrointestinal manifestations of Epstein-Barr virus infection in children following liver transplantation. *Transplantation* 1998;**66**:851–6.
18. Cen H, Breinig MC, Atchinson RW, et al. Epstein-Barr virus transmission via the donor-organ in solid-organ transplantation: polymerase chain reaction and restriction fragment length polymorphism analogue of IR2, IR3, IR4. *J Virol* 1991;**65**:976–80.
19. Chow JW, Fine MJ, Shlaes DM, et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* 1991;**115**:585–90.
20. Cicalese L, Sileri P, Green M, et al. Bacterial translocation in clinical intestinal transplantation. *Transplantation* 2001;**71**:1414–7.
21. Colonna JO, Winston DJ, Brill JE, et al. Infectious complications in liver transplantation. *Arch Surg* 1988;**123**:360–4.
22. Cox KL, Lawrence-Miyasaki LS, Garcia-Kennedy R, et al. An increased incidence of Epstein-Barr virus infection and lymphoproliferative disorder in young children on FK506 after pediatric liver transplantation. *Transplantation* 1995;**59**:524–9.
23. Cuervas-Mons V, Rimola A, Van Thiel DH, et al. Does previous abdominal surgery alter the outcome of pediatric patients subjected to orthotopic liver transplantation? *Gastroenterology* 1986; **90**:853–7.
24. Cytomegalovirus. In: Green M, Avery RK, Preiksaittis J, editors. Guidelines for the prevention and management of infectious complications of solid organ transplantation. *Am J Transplant* 2004;**4**(Suppl. 10):51–8.
25. Dagan R, Schwartz RH, Insel RA, Menegua MA. Severe diffuse adenovirus 7a pneumonia in a child with combined immunodeficiency: possible therapeutic effect of human immune serum containing specific neutralizing antibodies. *Pediatr Infect Dis J* 1984; **3**:246–51.
26. Di Benedetto F, Lauro A, Masetti M, et al. Use of prosthetic mesh in difficult abdominal wall closure after small bowel transplantation in adults. *Transplant Proc* 2005;**37**:2272–4.
27. Dummer JS, Hardy A, Poorsattar A, Ho M. Early infections in kidney, heart and liver transplant recipients on cyclosporine. *Transplantation* 1983;**36**:259–67.
28. Dummer JS, Siegfried E, Breinig MK, et al. Infection with human immunodeficiency virus in the Pittsburgh transplant population. *Transplantation* 1989;**47**:134–9.
29. Englund JE, Whimbey EE. Community-acquired respiratory viruses after hematopoietic stem cell or solid organ transplantation. In: Bowden RA, Ljungman P, Paya CV, editors. *Transplant infections*. 2nd ed. Baltimore: Lippincott Williams & Wilkins; 2003. p. 367–98.
30. Fischer A, Blanche S, Le Bidols, et al. Anti-B-cell monoclonal antibody in the treatment of severe B-cell lymphoproliferative syndrome following bone marrow and solid-organ transplantation. *N Engl J Med* 1991;**324**:1451–6.
31. Florescu DF, Islam MK, Mercer DF, et al. Adenovirus infections in pediatric small bowel transplant recipients. *Transplantation* 2010;**90**:198–204.
32. Florescu DF, Islam KM, Grant W, et al. Incidence and outcome of fungal infections in pediatric small bowel transplant recipients. *Transplant Infect Dis* 2010;**12**:497–504.
33. Florescu DF, Langnas AN, Grant W, et al. Incidence, risk factors, and outcomes associated with cytomegalovirus disease in small bowel transplant recipients. *Pediatr Transplant* 2011;**16**:294–301.
34. Florescu DF, Qiu F, Langnas AN, et al. Bloodstream infections during the first year after pediatric small bowel transplantation. *Pediatr Infect Dis J* 2012;**31**(7):700–4.
35. Florescu DF, Qui F, Mercer DF, et al. Risk factors for systemic *Candida* infections in pediatric small bowel transplant recipients. *Pediatr Infect Dis J* 2012;**31**:120–3.
36. Gane E, Saliba F, Valdecasas GJ, et al. Randomized trial of efficacy and safety of oral ganciclovir in prevention of cytomegalovirus disease in liver-transplant recipients. *Lancet* 1997;**350**:1729–33.
37. Garrett TJ, Chadburn A, Barr ML, et al. Posttransplantation lymphoproliferative disorders treated with cyclophosphamide-doxorubicin-vincristine-prednisone chemotherapy. *Cancer* 1993; **72**:2782–5.
38. George DL, Arnow PM, Fox AS, et al. Bacterial infection as a complication of liver transplantation: epidemiology and risk factors. *Rev Infect Dis* 1991;**13**:387–96.
39. George MJ, Snydman DR, Werner BG, et al. Use of ganciclovir plus cytomegalovirus immune globulin to treat CMV pneumonia in orthotopic liver transplant recipients. *Transplant Proc* 1993;**25** (Suppl. 4):22–4.
40. Gladdy RA, Richardson SE, Davies HD, Superina RA. *Candida* infection in pediatric liver transplant recipients. *Liver Transplant Surg* 1999;**5**:16–24.
41. Green M. Vancomycin-resistant enterorocci: impact and management in pediatrics. *Adv Pediatr Infect Dis* 1998;**13**:257–77.
42. Green M, Barbadora K. Recovery of ceftazidime-resistant *Klebsiella pneumoniae* from pediatric liver transplant recipients. *Pediatr Transplant* 1998;**2**:224–30.
43. Green M, Bueno J, Sigurdsson L, et al. Unique aspects of the infectious complications of intestinal transplantation. *Curr Opin Organ Transplant* 1999;**4**:361–7.
44. Green M, Kaufmann M, Wilson J, Reyes J. Comparison of intravenous ganciclovir followed by oral acyclovir with intravenous ganciclovir alone for prevention of cytomegalovirus and

Epstein-Barr virus disease after liver transplantation in children. *Clin Infect Dis* 1997;**25**:1344–9.
45. Green M, Michaels M. Infectious complications after solid-organ transplantation. *Adv Pediatr Infect Dis* 1992;**7**:181–204.
46. Green M, Michaels M. Adenovirus, parvovirus B19, and papillomavirus. In: Bowden RA, Ljungman P, Paya C, editors. *Transplant infections*. Philadelphia: Lippincott-Raven; 1998. p. 287–94.
47. Green M, Michaels M. Preemptive therapy of cytomegalovirus disease in pediatric transplant recipients. *Pediatr Infect Dis J* 2000;**19**:875–7.
48 Green M, Michaels MG, Webber SA, et al. The management of Epstein-Barr virus–associated post-transplant lymphoproliferative disorders in pediatric solid-organ transplant recipients. *Pediatr Transplant* 1999;**3**:271–81.
49. Green M, Reye J, Rowe D. New strategies in the prevention and management of Epstein-Barr virus infection and posttransplant lymphoproliferative disease following solid organ transplantation. *Curr Opin Organ Transplant* 1998;**3**:143–7.
50. Green M, Reyes J, Webber S, et al. The role of viral load in the diagnosis, management, and possible prevention of Epstein-Barr virus–associated posttransplant lymphoproliferative disease following solid organ transplantation. *Curr Opin Organ Transplant* 1999;**4**:292–6.
51. Green M, Tzakis A, Reyes J, et al. Infectious complications of pediatric liver transplantation under FK 506. *Transplant Proc* 1991;**23**:3038–9.
52. Green M, Wald ER, Tzakis A, et al. Aspergillosis of the central nervous system in a pediatric liver transplant recipient and review of the literature. *Rev Infect Dis* 1991;**13**:653–7.
53. Green M, Weinfeld A, Mazariegos G, Reyes J. Short-course intravenous ganciclovir prophylaxis against cytomegalovirus disease following liver transplantation in children. *Pediatr Transplant* 1998;**2**(Suppl 1):75.
54. Grinyo J, Charpentier B, Pestana JM, et al. An integrated safety profile analysis of belatacept in kidney transplant recipients. *Transplantation* 2010;**90**:1521–7.
55. Groetzner J, Kaczmarek J, Wittwer T, et al. Caspofungin as first-line therapy for the treatment of invasive aspergillosis after thoracic organ transplantation. *J Heart Lung Transplant* 2008;**27**:1–6.
56. Gross TG, Bucuvalas JC, Park JR, et al. Low-dose chemotherapy for Epstein-Barr virus–positive post-transplantation lymphoproliferative disease in children after solid organ transplantation. *J Clin Oncol* 2005;**23**:6481–8.
57. Hanto DW, Frizzera G, Gajl-Peczalska KJ, et al. Epstein-Barr virus–induced B-cell lymphoma after renal transplantation: acyclovir therapy and transition from polyclonal to monoclonal B-cell proliferation. *N Engl J Med* 1982;**306**:913–8.
58. Hedderwick SA, Greenson JK, McGaughy VR, et al. Adenovirus cholecystitis in a patient with AIDS. *Clin Infect Dis* 1998;**28**:997–9.
59. Hiatt JR, Ament ME, Berquist WJ, et al. Pediatric liver transplantation at UCLA. *Transplant Proc* 1987;**19**:3282–8.
60. Higgins R, Kusne S, Reyes J, et al. *Mycobacterium tuberculosis* after liver transplantation: management and guidelines for prevention. *Clin Transplant* 1992;**6**:81–90.
61. Ho M, Dummer JS. Risk factors and approaches to infection in transplant recipients. In: Mandell GL, Douglas Jr RG, Bennett JE, editors. *Principles and practice of infectious diseases*. 3rd ed. Edinburgh: Churchill Livingstone; 1990.
62. Ho M, Jaffe R, Miller G, et al. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation* 1988;**45**:719–27.
63. Hofflin JM, Potasman I, Baldwin JC, et al. Infectious complication in heart transplant recipients receiving cyclosporine and corticosteroids. *Ann Intern Med* 1987;**106**:209–16.
64. Hollenbeak CS, Alfrey EJ, Sheridan K, et al. Surgical site infections following pediatric liver transplantation: risks and costs. *Transpl Infect Dis* 2003;**5**:72–8.
65. Hughes WT, Kennedy W, Dugdale M, et al. Prevention of *Pneumocystis carinii* pneumonitis in AIDS patients with weekly dapsone. *Lancet* 1990;**ii**:1066.
66. Hughes, WT, Rivera GK, Schell MJ, et al. Successful intermittent chemoprophylaxis for *Pneumocystis carinii* pneumonitis. *N Engl J Med* 1987;**316**:1627–32.
67. Humar A, Malkan G, Moussa G, et al. Human herpesvirus-6 is associated with cytomegalovirus reactivation in liver transplant recipients. *J Infect Dis* 2000;**181**:1450–3.
68. Humar A, Snydman D, and the AST Infectious Diseases Community of Practice. Cytomegalovirus in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S78–86.
69. Ison MG, Green M, and the AST Infectious Diseases Community of Practice. Adenovirus in solid organ transplantation. *Am J Transplant* 2009;**9**(Suppl. 4):S161–5.
70. Ison MG, Hager J, Blumberg E, et al. Donor-derived disease transmission events in the United States: Data reviewed by the OPTN/UNOS Disease Transmission Advisory Committee. *Am J Transplant* 2009;**9**:1929–35.
71. Ison MG, Hayden FG. Viral infections in immunocompromised patients: what's new with respiratory viruses? *Curr Opin Infect Dis* 2002;**15**:355–67.
72. Iwamoto M, Jernigan DB, Guashch A, et al. West Nile Virus in Transplant Recipients Investigation Team. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Engl J Med* 2003;**348**:2196–203.
73. Kato T, Tzakis AG, Selvaggio G, et al. Intestinal and multivisceral transplantation in children. *Ann Surg* 2006;**243**:756–66.
74. Kenagy DN, Schlessinger Y, Weck K, et al. Epstein-Barr virus DNA in peripheral blood leukocytes of patients with post-transplant lymphoproliferative disease. *Transplantation* 1995;**60**:547–54.
75. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010;**89**:779–95.
76. Kusne S, Dummer JS, Singh N, et al. Infection after liver transplantation: an analysis of 101 consecutive cases. *Medicine* 1988;**67**:132–43.
77. Kusne S, Manez R, Frye BL, et al. Use of DNA amplification for diagnosis of cytomegalovirus enteritis after intestinal transplantation. *Gastroenterology* 1997;**112**:1121–8.
78. Kusne S, Shapiro R, Fung J. Prevention and treatment of cytomegalovirus infection in organ transplant recipients. *Transplant Infect Dis* 1999;**1**:187–203.
79. Lee TC, Savoldo B, Rooney CM, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. *Am J Transplant* 2005;**5**:2222–8.
80. Leoung GS, Feigal DW, Montgomery B, et al. Aerosolized pentamidine for prophylaxis against *Pneumocystis carinii* pneumonia. *N Engl J Med* 1990;**323**:769–75.
81. Lichenstein IH, MacGregor RR. Mycobacterial infections in renal transplant recipients: report of five cases and review of the literature. *Rev Infect Dis* 1983;**5**:216–26.
82. Loinaz C, Kato T, Nishida S, et al. Bacterial infections after intestine and multivisceral transplantation: the experience of the University of Miami (1994–2001). *Hepatogastroenterology* 2006;**53**:234–42.
83. Malatack JJ, Gartner JC, Urbach AH, Zitelli BJ. Orthotopic liver transplantation, Epstein-Barr virus, cyclosporine and lymphoproliferative syndrome—a growing concern. *J Pediatr* 1991;**118**:667–75.
84. Malhorta KK, Dash SC, Dhawan IK, et al. Tuberculosis and renal transplantation—observations from an endemic area. *Postgrad Med J* 1986;**62**:359–62.
85. Mazareigos GV. Intestinal transplantation: current outcomes and opportunities. *Curr Opin Organ Transplant* 2009;**14**:515–21.
86. McDiarmid SV, Blumberg DA, Remotti H, et al. Mycobacterial infections after pediatric liver transplantation: a report of three cases and review of the literature. *J Pediatr Gastroenterol Nutr* 1995;**20**:425–31.
87. McDiarmid SV, Jordan S, Lee GS, et al. Prevention and preemptive therapy of post-transplant lymphoproliferative disease in pediatric liver recipients. *Transplantation* 1998;**66**:1604–11.
88. McGregor RS, Zitelli BJ, Urbach AH, et al. Varicella in pediatric orthotopic liver transplant recipients. *Pediatr* 1989;**83**:256–61.
89. McLaughlin GE, Delis S, Kashimawo L, et al. Adenovirus infection in pediatric liver and intestinal transplant recipients: utility of DNA detection by PCR. *Am J Transplant* 2003;**3**:224–8.
90. Mendez JC, Dockrell DH, Espy MJ, et al. Human beta-herpesvirus interactions in solid-organ transplant recipients. *J Infect Dis* 2001;**183**:179–84.
91. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.

92. Michaels M, Green M, Wald ER, Starzl TE. Adenovirus infection in pediatric orthotopic liver transplant recipients. *J Infect Dis* 1992;**165**:170–4.
93. Michaels M, Jenkins F. Human herpesvirus 8: is it time for routine surveillance in pediatric solid organ transplant recipients to prevent the development of Kaposi's sarcoma? *Pediatr Transplant* 2003;**7**:1–3.
94. Migliazza L, Lopez SM, Murcia J, et al. Long-term survival expectancy after liver transplantation in children. *J Pediatr Surg* 2000; **35**:5–7.
95. Najarian JS, Fryd DS, Strand M, et al. A single institution, randomized, prospective trial of cyclosporine versus azathioprine-antilymphocyte globulin for immunosuppression in renal allograft recipients. *Ann Surg* 1985;**201**:142–57.
96. Paya C. Prevention of cytomegalovirus disease in recipients of solid-organ transplants. *Clin Infect Dis* 2001;**32**:596–603.
97. Paya C, Fung JJ, Nalesnik MA, et al. Epstein-Barr virus–induced posttransplant lymphoproliferative disorders. *Transplantation* 1999;**68**:1517–25.
98. Paya C, Humar A, Dominguez E, et al. Efficacy and safety of valganciclovir vs. oral ganciclovir for prevention of cytomegalovirus disease in solid organ transplant recipients. *Am J Transplant* 2004;**4**:611–20.
99. Pinchoff RJ, Kaufman SS, Magid MS, et al. Adenovirus infection in pediatric small bowel transplantation recipients. *Transplantation* 2003;**76**:183–9.
100. Pohl C, Green M, Wald ER. RSV infections in pediatric liver transplant recipients. *J Infect Dis* 1992;**165**:166–9.
101. Preiksaitis JK, Pang XL, Fox JN, et al. Interlaboratory comparison of Epstein-Barr virus viral load assays. *Am J Transplant* 2009;**9**: 269–79.
102. Quinibi W, Al-Sibai MB, Taher S, et al. Mycobacterial infection after renal transplantation: report of 14 cases and review of the literature. *Q J Med* 1990;**282**:1039–60.
103. Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR) 2010 Annual Data Report. Department of Health and Human Services. Health Care Resources and Services Administration Health Care Systems Bureau. Division of Transplantation. *Am J Transplant* 2012;**12**(Suppl. 1).
104. Randhawa PS, Jaffe R, Demetris AJ, et al. Expression of Epstein-Barr virus–encoded small RNA (by the *EBER-1* gene) in liver specimens from transplant recipients with post-transplantation lymphoproliferative disease. *N Engl J Med* 1992;**327**:1710–4.
105. Reyes J, Mazariegos GV, Bond GM, et al. Pediatric intestinal transplantation: historical notes, principles and controversies. *Pediatr Transplant* 2002;**6**:193–207.
106. Ribaud P, Scieux C, Freymuth F, et al. Successful treatment of adenovirus disease with intravenous cidofovir in an unrelated stem-cell transplant recipient. *Clin Infect Dis* 1999;**28**:690–1.
107. Riddler SA, Breinig MC, McKnight JLC. Increased levels of circulating Epstein-Barr virus–infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. *Blood* 1994;**84**:972–84.
108. Rollins NK, Andrews WS, Currino G, et al. Infected bile lakes following pediatric liver transplantation: non-surgical management. *Radiology* 1988;**166**:169–71.
109. Rowe DT, Qu L, Reyes J, et al. Use of quantitative competitive PCR to measure Epstein-Barr virus genome load in peripheral blood of pediatric transplant recipients with lymphoproliferative disorders. *J Clin Microbiol* 1997;**35**:1612–5.
110. Schroter GPJ, Hoelscher M, Putnam CW, et al. Infections complicating orthotopic liver transplantation. *Arch Surg* 1976;**111**: 1337–47.
111. Shapiro RS, Chauvenet A, McGuire W, et al. Treatment of B-cell lymphoproliferative disorders with interferon alpha and intravenous gamma globulin. *N Engl J Med* 1988;**318**:1334.
112. Sia IG, Wilson JA, Groettum CM, et al. Cytomegalovirus (CMV) DNA load predicts relasping CMV infection after solid organ transplantation. *J Infect Dis* 2000;**181**:717–20.
113. Sindhi R, Ashokkumar C, Higgs BW, et al. Allospecific CD154+ T-cytotoxic memory cells as potential surrogate for rejection risk in pediatric intestine transplantation. *Pediatr Transplant* 2012;**16**(1):83–91.
114. Singh N, Dummer S, Kusne S, et al. Infections with cytomegalovirus and other herpesviruses in 121 liver transplant recipients: transmission by donated organ and the effect of OKT3 antibodies. *J Infect Dis* 1988;**155**:202–6.
115. Singh N, Paterson DL. *Mycobacterium tuberculosis* infection in solid-organ transplant recipients: impact and implications for management. *Clin Infect Dis* 1998;**27**:1266–77.
116. Sigurdsson L, Reyes J, Kocoshis SA, et al. Bacteremia after intestinal transplantation in children correlates temporally with rejection or gastrointestinal lymphoproliferative disease. *Transplantation* 2000;**70**:302–5.
117. Smets F, Bodeus M, Goubau P, et al. Characteristics of Epstein-Barr virus primary infection in pediatric liver transplant recipients. *J Hepatol* 2000;**32**:100–4.
118. Snydman DR, Werner BG, Dougherty NN, et al. Cytomegalovirus immune globulin prophylaxis in liver transplantation. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1993;**119**:984–91.
119. Snydman DR, Werner BG, Heinze-Lacey B, et al. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. *N Engl J Med* 1987;**317**: 1049–54.
120. Sokal EM, Antunes H, Beguin C, et al. Early signs and risk factors for the increased incidence of Epstein-Barr virus–related posttransplant lymphoproliferative diseases in pediatric liver transplant recipients treated with tacrolimus. *Transplantation* 1997;**64**: 1438–42.
121. Srinivasan A, Burton EC, Kuehnert MJ, et al. Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med* 2005;**352**:1103–11.
122. Starzl TE, Porter KA, Iwatsuki S, et al. Reversibility of lymphomas and lymphoproliferative lesions developing under cyclosporin-steroid therapy. *Lancet* 1984;**1**:583–7.
123. Stratta RJ, Shaefer MS, Markin RS, et al. Clinical patterns of cytomegalovirus disease after liver transplantation. *Arch Surg* 1989;**124**:1443–50.
124. Strernecik M, Ferrell S, Asher N, et al. Mycobacterial infection after liver transplantation: a report of three cases and review of the literature. *Clin Transplant* 1992;**6**:55–61.
125. Sudan DL, Chinnakotla S, Horslen S, et al. Basiliximab decreases the incidence of acute rejection in intestinal transplantation. *Transplant Proc* 2002;**34**:940–1.
126. The TH, van der Ploeg M, van der Berg A, et al. Direct detection of cytomegalovirus in peripheral blood leukocytes—a review of the antigenemia assay and polymerase chain reaction. *Transplantation* 1992;**54**:193–8.
127. Todo S, Fung JJ, Starzl TE, et al. Liver, kidney, and thoracic organ transplantation under FK 506. *Ann Surg* 1990;**212**;295–307.
128. Touraine JL, Bosi E, El Yafi MS, et al. The infectious lymphoproliferative syndrome in transplant recipients under immunosuppressive treatment. *Transpl Proc* 1985;**17**:96–8.
129. Ukah FO, Merhave H, Kramer D. Early outcome of liver transplantation in patients with a history of spontaneous bacterial peritonitis. *Transplant Proc* 1993;**25**:1113–5.
130. Verma A, Dhawan A, Wade JJ, et al. *Mycobacterium tuberculosis* infections in pediatric liver transplant recipients. *Pediatr Infect Dis J* 2000;**19**:625–30.
131. Verma A, Wade JJ, Cheeseman P, et al. Risk factors for fungal infection in paediatric liver transplant recipients. *Pediatr Transplant* 2005;**9**(2):220–5.
132. Wittner M. Cryptococcosis. In: Feigin RD, Cherry JD, editors. *Textbook of Pediatric infectious diseases*. 2nd ed. Philadelphia: WB Saunders; 1987.
133. Yoshikawa T, Ihira M, Furukawa H, et al. Four cases of human herpesvirus 6 variant B infection after pediatric liver transplantation. *Transplantation* 1998;**65**:1266–9.
134. Yoshikawa T, Ihira M, Suzuki K, et al. Human herpesvirus 6 infection after living related liver transplantation. *J Med Virol* 2000;**62**:52–9.
135. Younes BS, McDiarmid SV, Hargas JH, et al. The effect of immunosuppression on posttransplant lymphoproliferative disease in pediatric liver transplant patients. *Transplantation* 2000;**70**:94–9.
136. Ziring D, Tran R, Edelstein S, et al. Infectious enteritis after intestinal transplantation: incidence, timing, and outcome. *Transplantation* 2005;**79**:702–9.
137. Zitelli BJ, Gartner JC, Malatach JJ, et al. Pediatric liver transplantation: patient evaluation and selection, infectious complications, and life-style after transplantation. *Transpl Proc* 1987;**19**: 3309–16.

specialist. Specific, detailed information regarding the diagnosis and management of each particular pathogen can be found in the respective chapters in the section of this textbook dedicated to infections with specific microorganisms.

PRETRANSPLANT EVALUATION

The role of the pediatric infectious disease specialist in the care of a renal transplant recipient ideally begins during the pretransplantation period.[185,186] Before the patient undergoes transplantation, a thorough history and physical examination should be performed, with a focus on evaluation for evidence of an active infection that may require immediate therapy or, rarely, preclude transplantation (Table 73-1). The history should be comprehensive but focus on the details of any history of previous infections that may reemerge during the posttransplant period, including urinary tract infections (UTIs); mucocutaneous diseases such as herpes simplex virus (HSV) infection; systemic illnesses such as tuberculosis; chronic infections such as those caused by hepatitis B virus (HBV), hepatitis C virus (HCV), or human immunodeficiency virus (HIV); and diarrheal diseases. Infection with HIV previously was regarded as a reason to exclude a potential recipient from undergoing renal transplantation. More recent successes with highly active antiretroviral therapy have improved long-term survival in HIV-infected pediatric patients, however, and have led clinicians, research scientists, and family members themselves to investigate the role of renal transplantation as a treatment for ESRD.[68,184]

TABLE 73-1 **Guidelines for Pretransplant Evaluation in Pediatric Kidney Transplant Candidates**

History
Past infectious diseases
Routine childhood illnesses
Travel to or birth or residence in areas endemic for fungal or parasitic diseases
Tuberculosis exposure
Animal exposure
Diet preferences and water resources
Vaccinations
Reactions or allergies to antimicrobial agents
Current or past immunosuppression
Physical Examination
Search for active or latent focus of infection
Nutritional status
Laboratory and Other Tests
Purified protein derivative
Chest radiograph
Urinalysis and urine culture
Viral serology for HSV, CMV, EBV, VZV, human erythrovirus (formerly human parvovirus B19), HAV, HBV, HCV, HIV, BK virus, WNV, and others depending on the history
Baseline HSV, CMV and EBV DNA PCR or CMV antigenemia, erythrovirus B19, HCV, BK virus if seropositive or posttransplant monitoring is anticipated
Fungal and parasitic testing if travel or exposure history positive; sputum or stool tests as indicated
Anticipatory Guidance
Update vaccines
Counsel regarding measures to reduce infection risk
Consider antimicrobial prophylaxis if at risk

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HSV, herpes simplex virus; PCR, polymerase chain reaction; VZV, varicella-zoster virus; WNV, West Nile virus.

Renal transplant candidates undergo peritoneal dialysis or hemodialysis, so a history of previous dialysis catheter–associated infections should be documented and any current infection should be treated and eliminated before transplantation is performed. Another focus of the history should include an exposure history for the patient's country of origin or foreign travel, especially to areas endemic for organisms such as *Strongyloides stercoralis*, *Coccidioides immitis*, *Blastomyces dermatitidis*, or *Histoplasma capsulatum* and for diseases such as malaria and tuberculosis.[95,202] Other important exposures include blood product transfusions; exposure to animals or plants; well water as a source of drinking water; and dietary habits, especially consumption of raw or undercooked eggs or meat or unpasteurized dairy products.[185,186] Patients waiting for transplantation may become colonized with resistant organisms, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus*, gram-negative–resistant bacteria, fluconazole-resistant *Candida* species, and *Clostridium difficile*. In children who are unimmunized, a careful history of routine childhood illnesses, including varicella, measles, mumps, and rubella, should be documented.[119]

The immunization history of the patient should be documented carefully and, if needed or indicated, updated before the patient undergoes transplantation. Ideally, children with chronic renal disease should be fully immunized before progression to ESRD, for optimal immune response and protection during outbreaks of vaccine-preventable diseases.[47] Vaccinations against tetanus, pertussis, and diphtheria; polysaccharide vaccines, such as those against pneumococcus and *Haemophilus influenzae* type B; inactivated vaccines against polio and hepatitis A; and recombinant vaccines, such as those for hepatitis B, may be given or updated at any time before transplantation is performed.[33,226,238] Meningococcal vaccine is indicated routinely now for specific age groups and for special outbreak situations. The live varicella vaccine is recommended for renal transplant candidates who have not had varicella; it should be given at least 2 to 4 weeks before transplantation is performed.[257] The measles-mumps-rubella vaccine should be administered even earlier, preferably months before the patient undergoes transplantation. In addition, annual influenza vaccine and a tetanus booster every 10 years are recommended for patients who are renal transplant candidates or recipients.

If the child is unable to be fully immunized before undergoing transplantation, the routine immunization

schedule for the inactivated vaccines may be reinstituted after immunosuppression is decreased, which usually occurs 6 to 12 months after an uncomplicated transplant procedure.[33,226,238] Live virus vaccines should be avoided in the posttransplant period in most instances. Reports of safe and effective vaccination with varicella vaccine in selected posttransplant patients have been published, however.[31,39,79,178,179] Close contacts and family members of renal transplant candidates and recipients also should be fully immunized and should receive the annual influenza vaccine.[11] Other important points in the pretransplant evaluation history include allergies or reactions to medications, especially antibiotics, and the use of immunosuppressive agents.

Pretransplant laboratory and diagnostic imaging evaluations for most renal transplant candidates should include a chest radiograph, urinalysis, and urine culture for bacteria. Baseline renal and liver function tests also should be performed.

Serologic screening of the transplant recipient's status regarding organisms that may reactivate in the recipient or infect the recipient via the donor organ should be performed; such screening should include tests for HSV; cytomegalovirus (CMV); varicella-zoster virus (VZV); Epstein-Barr virus (EBV); human erythrovirus (formerly human parvovirus B19); *Treponema pallidum; Toxoplasma gondii;* hepatitis A, B, and C viruses; and HIV. Transplant recipients who are seropositive for HSV or CMV usually receive antiviral prophylaxis during the peritransplant period, whereas recipients seropositive for EBV may undergo posttransplant monitoring. It may be helpful to save an aliquot of the recipient's (and donor's) serum in case unusual circumstances occur, such as infection with West Nile virus (WNV). In CMV-seropositive transplant candidates, baseline CMV antigenemia or quantitative DNA polymerase chain reaction (PCR) may be useful; and in EBV-seropositive candidates, detection of EBV DNA by quantitative PCR may be helpful to document pretransplant viral load, especially if posttransplant virologic monitoring is to be performed.

Evaluation for active or latent tuberculosis infection should also be performed before the transplant. This evaluation should include a history of risk factors, including BCG vaccination, close contacts, and country of origin, and a chest radiograph. In addition, a tuberculin skin test (TST) and interferon-γ release assay (IGR) (QuantiFERON-TB gold, QuantiFERON-TB gold in tube, or T-SPOT.TB) should be performed. Positive IGR assay results predict subsequent development of tuberculosis in adult kidney transplant recipients in whom latent tuberculosis infection cannot be detected by TST.[124] General reviews of IGR assay results in adults with ESRD and in children also suggest IGR assays are useful, but caution should be used when interpreting IGR test results in young children, especially when results are discordant with TST.[41,204] The pretransplant evaluation also is an opportunity for the infectious disease specialist to counsel the patient and family about measures that may reduce the transplant recipient's risk for development of infectious disease complications after the transplant. Patients who have an exposure, or even a suspected exposure, to varicella (chickenpox) or zoster (shingles) should contact their physicians immediately to see whether passive immunoprophylaxis with varicella-zoster immunoglobulin or postexposure antiviral therapy with acyclovir or valacyclovir is indicated. Plans for foreign travel to remote areas also should be discussed with the physician. The transplant recipient should consume only thoroughly cooked meat and seafood and thoroughly washed fresh fruits and vegetables. In addition, drinking water should be pure. Transplant recipients should avoid, if possible, changing cat litter boxes, aquariums, and birdcages and should avoid close contact with people who have viral respiratory tract illnesses. Finally, medical attention should be sought if fever occurs, especially if it is significant or persistent.

POSTTRANSPLANT INFECTIOUS COMPLICATIONS

Infections occurring in the posttransplant period can be grouped into three main time frames: the first month post transplant (early), 2 to 6 months post transplant (middle), and 6 months post transplant and onward (late).[205,222] Although almost any organism or pathogen can infect a transplant recipient at any time, these time periods provide the clinician with a guide to the organisms and disease processes most commonly encountered (Table 73-2).

Infections Occurring During the Early Posttransplant Period

Infections occurring during the first month after renal transplantation has been performed usually are bacterial.[211] Common sites of early infections include the wound, urinary tract, lungs, and indwelling intravascular catheters.[191]

Wound Infections

As with any surgical procedure, wound infections may develop in a renal transplant recipient. They occur in approximately 2 percent of renal transplant recipients and range in severity from a superficial wound infection, easily treated with wound care and antimicrobial therapy, to deep perinephric abscesses that may be difficult to treat and result in transplant nephrectomy.[206,211] Wound infections are more likely to occur in patients with technical problems associated with the transplant surgery, including urinary leaks, vesicoureteral reflux, wound hematomas, or lymphoceles.[163,258] Malnutrition during the pretransplant period may impair wound healing and predispose the patient to development of wound infection.

Open Penrose drains may increase the likelihood of introducing microorganisms into the wound, whereas closed suction drainage, such as with a Jackson-Pratt drain, may reduce this risk. In addition, prompt removal of all drains, usually within 5 days in most uncomplicated cases, and administration of prophylaxis with perioperative antibiotics may decrease the incidence of wound infections; such measures are performed routinely in most renal transplant centers. The regimen usually is

TABLE 73-2 Timetable for the Occurrence of Common Infections and Usual Pathogens After Kidney Transplantation

Early Period
Wound infections
Urinary tract infection
Bacteremia and sepsis syndrome
Pneumonia
Herpes simplex virus
Hepatitis B virus
Human erythrovirus*
West Nile virus
Seasonal viruses
Drug reactions

Middle Period
Herpesviruses
  Cytomegalovirus
  Epstein-Barr virus
  Varicella-zoster virus
  Herpesvirus types 6, 7, and 8
Polyomaviruses
  JC virus
  BK virus
Papillomaviruses
Adenoviruses
Human erythrovirus*
Listeria monocytogenes
Mycobacterium tuberculosis
Atypical mycobacteria
Nocardia
Fungal diseases
Pneumocystis jiroveci
Parasitic diseases

Late Period
Community-acquired respiratory viruses and bacterial pathogens
Urinary tract infection
Streptococcus pneumoniae
Cytomegalovirus
Epstein-Barr virus
Varicella-zoster virus
Hepatitis B virus
Hepatitis C virus
Human immunodeficiency virus/acquired immunodeficiency syndrome

*Formerly human parvovirus B19.

aimed at uropathogens and staphylococci. One dose generally is given before the transplant, and the regimen is continued for only 24 hours after the transplant.

The diagnosis of a wound infection should be suspected if erythema, warmth, or discharge is present at the wound site or if an unexplained fever develops. Fluid may drain from the wound persistently, or a fluid collection or abscess may be seen on imaging of the deeper operative sites. The patient will be receiving immunosuppressive agents, however, and the findings may be unusual or the symptoms may be blunted. Any fluid or pus obtained should be stained and cultured for bacterial, mycobacterial, and fungal organisms. Organisms most likely to be identified as causes of wound infection include staphylococci (*Staphylococcus aureus*, especially methicillin-resistant *S. aureus*, but also coagulase-negative staphylococci), streptococci, and gram-negative enteric organisms. Unusual, multidrug-resistant, or health care–associated bacterial pathogens and yeast, such as *Candida albicans*, also may cause wound infections after the transplant. In addition, case reports of wound and perinephric fluid collections infected with *Mycoplasma hominis* have been published.[224]

Appropriate antimicrobial therapy, initially broad-spectrum and then ultimately tailored to the isolated organism and its susceptibility pattern, should be administered. The duration of appropriate antimicrobial therapy usually is 10 to 14 days, or until the wound infection has resolved and the patient has been afebrile for 3 to 5 days. Deep abscesses or unusual organisms may require longer therapy.

Urinary Tract Infections

UTIs are common developments after renal transplantation and may affect 35 to 79 percent of renal transplant recipients.[129,258] Posttransplant UTIs are associated with acute and chronic graft dysfunction and may threaten renal graft survival.* They may occur during the early, middle, or late posttransplant period and should be differentiated as afebrile and febrile.[116] A UTI that is febrile and occurs during the early or early/middle posttransplant period often is a severe illness complicated by pyelonephritis, urosepsis, metastatic foci of infection, allograft dysfunction, rejection, and relapse.[169,207] UTIs occurring in the early posttransplant period are associated more often with graft loss than are UTIs that occur later.[55]

The risk for developing an invasive UTI after undergoing renal transplantation seems to be increased in patients with prolonged bladder catheterization (most catheters can be removed during the first few days after transplant), dysfunctional bladder, ureterovesical disorders with reflux, malnutrition, underlying disorders, renal stones, obstructive uropathy, or a contaminated cadaveric kidney.[2,185] The inability of the bladder to empty completely or surgical complications, such as hematoma, reflux, or obstruction at the urinary anastomosis, are associated with UTIs. In addition, young infants, especially infants who have vesicoureteral reflux, seem to have a high incidence of complicated UTIs after a transplant procedure.[169] Procedures that address the etiology and correct underlying physiologic and anatomic abnormalities of the bladder and ureters should be performed before transplantation to avoid or lessen development of complications associated with these problems.[2,14,35,231] Newer surgical techniques allow children with reconstructed bladders to undergo successful renal transplantation with minimal risk for posttransplant development of UTIs.[163,207]

The organisms most commonly isolated from patients with early-onset UTIs include not only the typical

*References 2, 14, 35, 55, 118, 167, 231.

gram-negative enteric bacteria but also enterococci, staphylococci, and *Pseudomonas aeruginosa*. Unusual organisms, such as *Streptococcus mitis*, *Serratia marcescens*, and *Corynebacterium urealyticum*, also can be found. Antimicrobial therapy should be tailored to the susceptibility pattern of the organism isolated from the urine. Because 30 percent of patients experiencing a UTI during the early posttransplant period may have recurrent UTIs, a prolonged 6-week course of antibiotics usually is recommended to reduce the risk for developing a relapsing kidney infection.[163,207] Antimicrobial prophylaxis may reduce the risk for developing a UTI. Trimethoprim-sulfamethoxazole administered for the first 4 months after renal transplantation is effective in preventing most UTIs and can provide cross-cover prophylaxis against *Pneumocystis jiroveci* pneumonia and other diseases.[77]

Pneumonia

Pneumonia can occur during the first month after renal transplantation and often is associated with prolonged endotracheal intubation. Gram-positive and gram-negative bacterial pathogens acquired from normal oropharyngeal flora and unusual or health care–associated multidrug-resistant organisms predominate during the early posttransplant period.[186] *Legionella pneumophila* and unusual *Legionella* spp., such as *L. micdadei*, *L. bozemanae*, and *L. dumoffii*, also have caused outbreaks of serious, life-threatening pneumonia in transplant recipients in some centers.[34,45,128,186] *P. jiroveci* can cause pneumonia in a transplant recipient during the early posttransplant period, but more often it is associated with disease after the first month post transplant.[98] Rare or unusual pathogens, such as *Rhodococcus equi*, may cause pneumonia in these patients.[142]

Patients with pneumonia usually have fever, chills, chest pain, malaise, change in tracheal secretions, cough, dyspnea, tachypnea, change in ventilatory status, rales or rhonchi on auscultation of the lungs, and pulmonary infiltrates evident on a chest radiograph. Pneumonia may be complicated by pleural effusion, empyema, or pulmonary abscess, and death may occur if pneumonia is severe and not diagnosed and treated promptly. Treatment with antibiotics effective against the bacterial pathogens isolated from culture of tracheal aspirates, bronchoalveolar lavage fluid, or lung tissue is appropriate.

Bacteremia, Fungemia, and Sepsis

Bacteremia, fungemia, and sepsis occurring during the early posttransplant period often are associated with indwelling catheters.[184] The urinary tract, surgical wound, or transplanted or native kidney also may be a source.[153] Usual bacterial organisms such as coagulase-negative staphylococci or *S. aureus*, unusual bacterial organisms, and yeasts such as *C. albicans* and other *Candida* spp. may be involved. *Listeria monocytogenes* also may cause primary bacteremia or sepsis at any time after a kidney transplantation has been performed, but the greatest risk occurs during the early period. Complications include meningitis, and 10 percent of these patients may die.[166,217] Renal transplant recipients also are at increased risk for development of bacteremia with *Salmonella* nontyphoidal species.[53,110,184]

Complications such as UTI, graft infection, peritonitis, abscesses, and meningitis can occur, and recurrences are common. Therapy for bacteremia, sepsis, or its complications is tailored to the susceptibility pattern of the organism isolated from the patient's blood. The duration of therapy for uncomplicated bacteremia usually is 10 to 14 days, but a longer course of treatment may be indicated if abscesses occur or an unusual organism is isolated. Removal of the indwelling catheter may be necessary to clear persistent bacteremia, and abscesses and other foci of infection should be drained.

Other Bacterial Diseases

Pediatric renal transplant recipients also seem to be at increased risk for development of antibiotic-associated colitis caused by *Clostridium difficile*.[250] Infection of a lymphocele with *Pasteurella multocida* has been reported, as has systemic infection with *Bartonella henselae*.[3,43,59,146,184] A high index of suspicion always must be maintained when evaluating a transplant recipient for infection because the immunosuppression required to maintain the transplanted kidney predisposes the recipient to development of infection with unusual organisms.

Viral Infections

Herpes Simplex Virus. HSV is the most common virus encountered during the early posttransplant period, although antiviral prophylaxis significantly reduces the risk for development of this infection.[64,65] It occurs less frequently in pediatric (8%) than in adult renal transplant recipients (30%).[184,185] Most HSV infections encountered post transplant are due to reactivation of the recipient's strain; however, primary or recurrent infection acquired from the renal allograft may occur and primary infection from person-to-person transmission has been documented.[64,184,185,207]

Posttransplant HSV infection may be asymptomatic or associated with disease, most frequently oral ulcers in pediatric patients.[91] Genital and perianal ulcers may occur in adolescents and adults. Rarely, HSV may cause disseminated cutaneous lesions or zosteriform eruptions. HSV esophagitis may be manifested as dysphagia, refusal to eat, irritability, and substernal chest pain, and it may complicate oral HSV disease, especially if the oral mucosa has been traumatized by orogastric or nasogastric tubes. Acute, severe hepatitis with hepatic necrosis, often accompanied by hypotension and disseminated intravascular coagulation, also can occur. Tracheobronchitis and pneumonitis may occur as a result of HSV, primarily in patients with pneumonia caused by another pathogen and whose mucosa has been traumatized by endotracheal intubation. It is often severe and life threatening, even with appropriate supportive care and antiviral therapy.[184] Encephalitis also has been reported in renal transplant recipients.[87]

The diagnosis is made by isolation of HSV in cell culture or detection of viral antigen by immunofluorescence or viral DNA by PCR in the end-organ involved.

Detection or isolation of HSV in body secretions may represent asymptomatic shedding or disease and should be correlated clinically. If HSV DNA by PCR is detected in blood, viremia is likely present and should be treated. Treatment with acyclovir is recommended for patients with mucocutaneous lesions, viremia, or end-organ disease, and HSV-seropositive transplant recipients should receive acyclovir or valacyclovir prophylaxis during the peritransplant period to prevent development of HSV infection. Most patients receiving ganciclovir, valganciclovir, foscarnet, or cidofovir for CMV prophylaxis also are protected against HSV.

Other Viruses. Seasonal viruses, especially winter respiratory viruses such as respiratory syncytial virus, influenza viruses, parainfluenza viruses, and adenoviruses; winter diarrhea viruses, such as rotavirus; and late summer/early fall viruses, such as the enteroviruses, can infect the transplant recipient during the early posttransplant period and cause disease. Such infections may be acquired from the family or the community, or they may occur from health care–associated exposures. Transplant recipients who may not be adequately protected by routine vaccination are at risk for severe influenza and its complications. In 2012, results of a study on oseltamivir prophylaxis with once-daily dosing for 12 weeks during periods of local influenza circulation showed that it was well tolerated and reduced influenza in adult and pediatric kidney and other solid-organ transplant recipients.[113] Oseltamivir-resistant influenza A was detected during the study period.[113]

Renal transplant recipients who are chronically infected with HBV or HCV may experience liver dysfunction during the early transplant period, but the late posttransplant period, beyond the first year, carries the greatest risk for progression of liver disease to cirrhosis. If a renal transplant recipient acquires HBV soon after undergoing transplantation, acute hepatitis can develop, often with death from liver failure.[52,184]

Noninfectious Causes of Fever

The most common noninfectious cause of fever in the first posttransplant month is allograft rejection.[206] Fever often is the first sign of rejection, especially in children, and rejection should be considered if an infectious source of the fever is not identified. Another common noninfectious cause of fever early after transplantation is antilymphocyte antibody therapy (OKT3). The first two or three doses of OKT3 produce a release of cytokines, which cause fever and chills. In most patients, these symptoms resolve after the third dose. Other noninfectious causes of fever during this period include drug reactions and pulmonary emboli.

Infections Occurring During the Middle Posttransplant Period

The cumulative effects of immunosuppression begin to be revealed during the period 2 to 6 months after transplantation. If a significant amount of antirejection therapy is required for multiple episodes of rejection, the effects may be more pronounced. Such immunosuppression allows classic opportunistic pathogens, such as CMV, *P. jiroveci*, *Toxoplasma gondii*, *L. monocytogenes*, *Aspergillus*, and *Nocardia*, to evade immune surveillance and cause disease.[98,184,249,251] Reactivation of organisms previously infecting the transplant recipient or the donor allograft, including *Mycobacterium tuberculosis*, HBV, HCV, HIV, *H. capsulatum*, and *C. immitis*, also may cause disease. In addition, an occult bacterial focus of infection that was not adequately identified and treated before the transplant may become apparent at this time and cause significant disease.[206]

Herpesviruses

The Herpesviridae (HSV types 1 and 2, CMV, EBV, VZV, and human herpesvirus [HHV] types 6, 7, and 8) share the biologic properties of latency, reactivation, cell association, and oncogenicity, which renders them the most important group of pathogens that affect renal transplant recipients.[184,212] HSV is more important during the early posttransplant period, whereas the other herpesviruses are important causes of morbidity and mortality during the middle and late posttransplant periods.[99]

Cytomegalovirus. CMV may cause primary infection in a CMV-seronegative transplant recipient through a renal allograft or blood product transfusion from a seropositive donor.[20,51,203] Person-to-person transmission within the family or close community also is possible. Recurrent CMV infection develops in seropositive transplant recipients if the recipient's CMV strain becomes reactivated. The renal allograft from a seropositive donor also can be a source of reinfection to the recipient and produce active CMV infection or disease.[42,92] CMV infection in renal transplant recipients may cause silent or asymptomatic infection; end-organ diseases such as hepatitis, esophagitis, colitis, encephalitis, vasculitis, and retinitis; and systematic disease with persistent fever and leukopenia. A case report also linked CMV infection with posttransplant occurrence of atypical hemolytic-uremic syndrome that resolved with plasma exchange and ganciclovir antiviral therapy.[177] It has been linked in some studies to allograft dysfunction and nephropathy.

CMV also causes depressed cell-mediated immunity and impaired alveolar macrophage function, rendering the host more vulnerable to other opportunistic infections, such as fungal disease and *P. jiroveci* pneumonia, and it serves as a cofactor for other viruses, such as EBV and HHV-6 and HHV-7.[123,180,192,234,235] In addition, CMV is associated with acute and chronic rejection and allograft nephropathy and decreased long-term patient survival.[1,66,134,184,203]

Disease caused by CMV can be documented by isolation of CMV from blood or tissue; detection of CMV antigen pp65 in circulating leukocytes; or detection of CMV DNA by PCR or similar assays in blood, bronchoalveolar fluid, or tissue.[26,27,188,193,203] Isolation of CMV in urine or saliva documents active infection but has little significance in predicting disease; serologic tests may document seroconversion in primary infections but generally should be reserved for pretransplant screening

only. Most transplant patients at risk for acquiring CMV disease (i.e., CMV-seropositive recipients [D^+/R^+] or CMV-seronegative recipients [D^+/R^-] who received a renal allograft from a seropositive donor) should be monitored by viral surveillance, usually by testing blood weekly for CMV antigen pp65 or for quantitative CMV DNA by PCR.[25,104]

Detection of significant levels of the virus by quantitative or semiquantitative assay, or an increase from baseline levels, usually predicts CMV disease.[235] Reduction of immunosuppression, along with preemptive antiviral therapy, generally is indicated and results in a decrease or resolution of CMV levels detected in blood.* Despite receiving adequate antiviral therapy, some patients have persistently positive CMV DNA by PCR, however, which should be interpreted within the clinical context.[26] In some patients who otherwise appear well, the DNA may be fragmented and nonreplicating, whereas in other patients with persistent symptoms, a strain of CMV resistant to one or more antiviral agents may be the cause.

The antiviral agents currently available for the treatment of CMV disease and preemptive therapy for positive CMV markers include ganciclovir, valganciclovir, foscarnet, and cidofovir.[248] For renal transplant patients with moderate to severe CMV-associated disease, 2 to 3 weeks of therapy with intravenous ganciclovir usually is adequate to treat disease; however, patients with CMV retinitis or repeat episodes of rejection may require maintenance therapy, usually with oral valganciclovir.[111,148,170] Asymptomatic or mild CMV infections may respond to oral valganciclovir administered for 1 to 3 months.[148,164] Preemptive therapy should be continued throughout the period of immunosuppression in patients who are severely immunosuppressed and at risk for development of CMV disease and recurrence (D^+/R^-).[89,104,149,164] Foscarnet and cidofovir have significant renal toxicity and should be used with caution in renal transplant recipients. Prophylaxis for CMV disease is indicated in high-risk transplant recipients (D^+/R^-); options include intravenous CMV immune globulin and oral valganciclovir.†

Epstein-Barr Virus. EBV infection in children who have received a renal transplant may be asymptomatic or may be associated with a variety of different syndromes, including a nonspecific viral syndrome, mononucleosis, smooth muscle tumors (leiomyoma), post-transplant PTLD, and lymphoma.‡ Infection and disease may occur after primary and recurrent EBV infection, but primary infection is more likely to occur and produce PTLD in younger children than in adults who have received solid-organ transplants.[35,96] EBV disease may affect 10 percent of pediatric renal transplant recipients and may develop 1 month to 5 or more years post transplant, with the risk accumulating every year of post-transplant survival. Recurrence of post-transplant PTLD may also occur years after transplant and may involve unusual sites.[13,57,218]

Knowledge about post-transplant EBV infection and disease is evolving; as graft survival has improved, with more intense immunosuppressive regimens that preserve graft function, and other opportunistic infections such as HSV, CMV, and *P. jiroveci* being successfully managed, EBV has emerged as a formidable obstacle to successful solid-organ transplantation in children.* The estimated overall risk for development of serious life-threatening EBV-associated illness is at least 4 percent and increases to 10 percent in children who experience a primary infection with EBV after undergoing renal transplantation.[56,106] Other risk factors for PTLD in children who have received solid-organ transplants include receipt of antilymphocyte therapy such as OKT3 for rejection; receipt of tacrolimus, sirolimus, or mycophenolate mofetil, rather than cyclosporine, for immunosuppression; and CMV or EBV donor/recipient (D^+/R^-) mismatch.[58,115,116]

Nonspecific viral syndromes and mononucleosis occur during the earlier posttransplant period, whereas lymphoma and leiomyoma are more likely to be a manifestation during the late posttransplant period.[30,61,78,94] Uncomplicated posttransplant mononucleosis is characterized by the self-limited illness of fever, pharyngitis, cervical adenopathy, and splenomegaly. The signs and symptoms of PTLD vary but often include persistent fever, weight loss, and generalized adenopathy.[106] The disease frequently is multisystemic and progressive, and involvement of the neck, chest, lungs, gastrointestinal tract, liver, spleen, eyes, and brain, as well as lesions, may be detected by computed tomography of the head, neck, chest, and abdomen.[4,5,185,213] The renal allograft also may be involved and show dysfunction. Lymphoma may be manifested as solid tumors in the renal allograft, lung, liver, spleen, brain, and soft tissues.[78,96] Smooth muscle tumors (leiomyoma) frequently are multicentric and multifocal; they often occur in lung, liver, and spleen but also may occur in unusual locations, including the brain and renal allograft.[28,94,105]

Laboratory diagnosis of posttransplant, EBV-associated disease is based on detection by PCR of EBV DNA in circulating lymphocytes.[5,49,70] Quantitative or semiquantitative PCR assays may show increasing or persistently high copies of EBV DNA in the circulating lymphocytes of patients who are at risk for developing PTLD.[5,93,201] If end-organ disease is observed, the diagnosis can be confirmed histopathologically by detection by PCR of EBV DNA in tissue or by in situ hybridization with an EBV-encoded RNA probe.[4,235]

The diseases associated with EBV also may be classified in the laboratory as polyclonal or monoclonal. Polyclonal illnesses seem to be more benign than are monoclonal diseases, which often are associated with chromosomal abnormalities and malignant transformation.[185] The virus cannot be cultivated by routine means; when studied by special culture techniques, EBV has been found frequently in the oropharyngeal secretions of seropositive transplant recipients and is not predictive of EBV-associated disease.[185,201] Similarly, serologic approaches to establishing the diagnosis of EBV infection after a transplant procedure also are nonspecific and difficult to interpret in most patients, unless the recipient

*References 7, 27, 46, 49, 85, 89, 143.
†References 30, 74, 85, 137, 170, 216, 223, 244.
‡References 28, 29, 61, 94, 96, 106.

*References 28, 56, 58, 61, 94, 121, 218.

clearly has seroconverted during the posttransplant period.

Aggressive or intense treatment of uncomplicated EBV-associated viral syndrome or mononucleosis usually is unnecessary because these illnesses seem to be self-limited in most patients. Immunosuppression may be reduced, and acyclovir may be administered; the patient should be monitored carefully. If symptoms in the patient or EBV DNA levels in the blood persist or increase, PTLD should be suspected and the diagnosis should be confirmed.[229,236]

Treatment of established PTLD is challenging.[90] Mortality rates are high, and the best results seem to occur if the disease is diagnosed by quantitative PCR viral-load monitoring and serial imaging studies and treated aggressively.[235] Preemptive therapy instituted when viral surveillance monitoring detects an increase in EBV DNA in circulating lymphocytes before end-organ disease is evident also may be helpful in some patients.[4,49,148] Reduction in immunosuppression remains the most widely recommended strategy, but a variety of regimens have been studied as well.

Antiviral agents such as acyclovir and ganciclovir seem to reduce EBV replication early in the course of the disease process and may halt the progression of disease in some patients.[58,186,248] Antiviral agents are ineffective, however, against latent EBV or cells that have been transformed by the virus. Interferon-α, immunoglobulin, and anti–CD20 monoclonal antibody preparations such as rituximab have been used successfully in patients with established PTLD. Experimental protocols evaluating adoptive immunotherapy in transplant recipients are ongoing. In addition, some experts suggest that administration of intense anti-CMV therapy with ganciclovir and CMV hyperimmune globulin may improve chances of survival in certain patients with PTLD because CMV may serve as a cofactor in progression of disease caused by other members of the Herpesviridae.* Treatment of patients with lymphoma or leiomyoma includes a reduction in immunosuppression, chemotherapy, radiation therapy, and surgical resection of tumors.

Varicella-Zoster Virus. VZV can cause primary (varicella, chickenpox) or reactivation (zoster, shingles) disease in a renal transplant recipient.[185,222] Infection with VZV can occur at any time but does so most often during the middle posttransplant period. Patients have fever and painful or pruritic vesicular skin lesions.[170] Hepatitis, encephalitis, acute or chronic recurrent cerebral vasculitis and vasculopathy, and pneumonia also may occur, even in the absence of skin lesions, especially in an immunocompromised host.[107] Varicella occurs more frequently in children, and zoster occurs more frequently in adolescent and adult transplant recipients.[40]

In contrast to other herpesviruses, VZV almost always is transmitted person to person or by aerosol in health care, family, and community settings; it rarely, if ever, has been linked to the transplanted allograft.[156,185] Before the advent of routine immunization and antiviral therapy, infection with VZV was a major cause of morbidity and mortality in children receiving solid-organ transplants.[40,72,207] Untreated primary varicella may continue for several weeks and result in visceral dissemination, pneumonitis, hepatic necrosis, encephalitis, vasculitis with stroke, disseminated intravascular coagulation, hemorrhagic skin lesions, and death.[72,186,212] Zoster in solid-organ transplant recipients may remain localized to a dermatome, but it often disseminates beyond the dermatomal distribution and produces widespread skin lesions and even visceral dissemination.

The diagnosis of VZV disease often is clinical, but it should be confirmed by isolation of VZV from fresh, vesicular skin lesions or detection of viral antigen by direct immunofluorescence assay on cells obtained by scraping the base of the skin lesion.[186] Tzanck smear may show multinucleated giant cells, suggesting a viral infection, but it cannot differentiate VZV from HSV. Other pathogens, especially HSV, may mimic VZV disease, so establishing an accurate viral diagnosis is important. VZV also may be detected by PCR-based DNA detection methods, which also may distinguish wild-type strains from the OKA vaccine strain of VZV; qualitative and quantitative VZV PCR tests may be performed by reference and research laboratories. Establishing a serologic diagnosis of active VZV infection is difficult. Routine serologic screening performed in the pretransplant period identifies patients who are seronegative and at risk for developing primary infection with VZV.

Treatment of established VZV disease should be instituted as early as possible during the course of the illness because survival is improved if treatment begins before the fifth day of illness.[72] Acyclovir is recommended for most renal transplant recipients experiencing either primary infection with varicella or zoster with dissemination; it is administered intravenously in high doses (500 mg/m^2 per dose every 8 hours if renal function is normal or adjusted for renal function as needed) for 5 to 10 days, or until new lesions have ceased to occur for 24 to 48 hours, old lesions have crusted, the fever has resolved, and disease has abated.[31,178,185,248] Uncomplicated zoster or very mild primary varicella may be treated with oral acyclovir, famciclovir, or valacyclovir, provided that the patient is monitored carefully for clinical response. Ganciclovir, foscarnet, or cidofovir also provide cross-cover protection for most VZV strains, if these antiviral agents are being administered for difficult CMV infections. If acyclovir-resistant varicella develops, then treatment with foscarnet or a combination of two or three antiviral agents may be necessary to control the infection.

Prevention of posttransplant VZV disease can be accomplished by several effective strategies, which should be discussed during the pretransplant evaluation. Transplant recipients who were seronegative for VZV during the pretransplant evaluation should receive varicella vaccine before undergoing transplantation, if possible.[79,125,257] Pretransplant varicella vaccination is safe, beneficial, and cost effective when administered to pediatric renal transplant candidates and in selected renal transplant recipients after a transplant procedure.[31,39,79,80,178]

Seronegative, unimmunized transplant recipients who are exposed to varicella or zoster during the posttransplant period should receive passive immunoprophylaxis with varicella-zoster immunoglobulin (VariZig).

*References 1, 20, 61, 189, 221, 234.

Preemptive therapy with oral acyclovir also is recommended by some experts in this situation because varicella-zoster immunoglobulin does not prevent, but only attenuates, the postexposure disease process. Finally, acyclovir, valacyclovir, ganciclovir, and valganciclovir administered prophylactically to transplant recipients who are seropositive for HSV or CMV also may provide protection against VZV disease.[137,185]

Human Herpesviruses Types 6, 7, and 8. HHV-6, HHV-7, and HHV-8 also infect renal transplant recipients by primary infection and by reactivation.* Infection with these viruses becomes evident during the middle posttransplant period, but their roles in specific disease processes are unclear.[186,215,230] HHV-6 and HHV-7 may act as cofactors in the progression of a disease, especially diseases caused by CMV and EBV.[69,123,170,180,234] HHV-8 is associated with Kaposi sarcoma after a transplant procedure in severely immunosuppressed adult renal transplant recipients, but to date it has not been appreciated as a major opportunistic pathogen in pediatric renal transplant recipients.† HHV-8 may be transmitted through the renal allograft or by blood product transfusion, or it can become reactivated in the recipient after transplant surgery.[198]

Polyomaviruses and Papillomaviruses

Polyomaviruses. Human polyomaviruses (JC, BK, and SV40) frequently infect children and are present in urine and stool.[242,243] They can be detected in the urine of 33 to 58 percent of renal transplant recipients and detected serologically in 56 percent, and they are found histopathologically and by PCR in blood, body fluids, and tissue in 8 to 13 percent.‡ Knowledge about the roles that these viruses play in the outcome of renal transplant patients is evolving.

The JC and BK polyomaviruses seem to have a significant impact on adult and pediatric renal transplant recipients.§ BK virus has been implicated in various syndromes, including ureteral stenosis, hemorrhagic or chronic cystitis, interstitial nephritis with graft failure, allograft nephropathy (BK-associated nephropathy [BKAN]), and rejection, in renal transplant recipients.|| Disease associated with these viruses most often occurs during the middle and late post-transplant periods. BK virus may be asymptomatic or cause increasing serum creatinine levels, cystitis, tubular necrosis, graft dysfunction, or allograft rejection.[181,196] Risk factors for BKAN seem to include BK serostatus at the time of transplant, greater immunosuppression with use of mycophenolate mofetil, and concurrent CMV infection.¶

Ciprofloxacin prophylaxis in kidney transplant recipients has been shown in one recent study to reduce BK virus infection and BKAN 3 months post transplant, but not at 1 year post transplant.[252] BK virus historically was detected in urine by the presence of "decoy cells," which are cells containing intranuclear viral inclusions. Currently, quantitative PCR assays that detect and quantify BK viral DNA are used for detection. BK viruria precedes BK viremia, which precedes BK nephropathy.[15,103] Patients with BK-associated nephropathy may have BK viral DNA detected in the urine, blood, or plasma, and renal biopsy specimens may show characteristic viral inclusions. Most recently, quantitative PCR viral surveillance in blood or plasma has been shown in renal transplant recipients to be useful in predicting the development of BK nephropathy and in guiding preemptive antiviral and immunosuppressive strategies.

Pediatric kidney transplant recipients who have increasing BK viremia levels should be managed preemptively with carefully monitored reduction of immunosuppression to prevent development of more serious complications of BKAN and graft rejection.[194] This strategy usually results in reduction and then clearance of BK viremia and development of BK-specific cellular immunity coincident with BK virus clearance.[140] Treatment of established BKAN includes a reduction in immunosuppression. Cidofovir and fluoroquinolones have activity against BK virus, and both have been used to treat BK virus infection and treat severe disease caused by BKAN.[103,248] Intravenous immune globulin may also be of benefit; one report resulted in decrease of BK viremia and histologic resolution of BKAN.[8]

JC virus causes a rare syndrome called *progressive multifocal leukoencephalopathy*. It may be diagnosed by viral DNA detection using PCR-based assays available in reference and research laboratories or histopathologically by brain biopsy or autopsy examination. Successful treatment options for progressive multifocal leukoencephalopathy caused by JC virus are very limited, and most, if not all, patients die of progressive encephalopathy.

Papillomaviruses. Human papillomaviruses are common viruses that infect healthy children, adolescents, and adults. Adult renal transplant recipients are at increased risk for development of human papillomavirus–associated disease, such as cervical cancer and anogenital papillomas, whereas pediatric patients may develop numerous disfiguring warts post transplant, especially if they require severe immunosuppression.[175,185,200] Malignant transformation of cutaneous warts caused by high-risk types has been documented.[200] Treatment options are limited but include physical removal of papillomas with laser and cryotherapy and careful reduction of immunosuppression. Information on the use of human papillomavirus vaccines in patients with ESRD or renal transplant recipients has not been published.

Adenoviruses

Adenoviruses may infect pediatric renal transplant recipients at any time, but they are most likely to cause significant disease during the middle posttransplant period. They continue to emerge as a significant pathogen in pediatrics, including transplant recipients. They do not seem to play as important a role in renal transplant

*References 37, 38, 43, 69, 102, 205, 206, 221.
†References 37, 40, 71, 185, 198, 211.
‡References 6, 63, 67, 75, 76, 81, 83, 84, 101, 103, 105, 108, 140, 162, 171-173, 181, 183, 193, 194, 208, 237.
§References 6, 23, 81, 147, 162, 171, 173, 199, 208.
||References 23, 44, 54, 63, 75, 76, 105, 108, 172, 183, 193, 200, 208.
¶References 67, 83, 84, 101, 140, 237.

outcome, however, as do other viruses, such as the herpesviruses, and they currently are not as prominent in renal transplant recipients as they are in other transplant recipients, such as recipients of liver, lung, bone marrow, or stem cell transplants.[88,152,176,185,186] Nonetheless, infections with adenoviruses in pediatric renal transplant recipients, when they occur, can be serious and include hemorrhagic cystitis, diarrhea, allograft nephropathy, pneumonia, hepatitis, and disseminated disease with multisystemic involvement.*

The diagnosis is made by isolation or detection by PCR of the virus in respiratory secretions, stool, urine, blood, or tissue. Rapid diagnosis may be accomplished within 30 to 60 minutes, using a rapid immunochromatographic assay that detects adenovirus antigens in eye secretions, respiratory samples, urine, and stool. Viral DNA also may be detected by PCR or quantitative PCR available in reference or research laboratories, and characteristic changes may be seen by histopathology in tissue. Adenovirus serotyping by neutralization assays and genotyping by PCR sequence-based assays are available in reference laboratories.[88] Treatment of serious adenoviral disease primarily is supportive. Immunosuppression also may be reduced, when possible. Antiviral agents, such as ribavirin, ganciclovir, and cidofovir, have activity against adenoviruses. Clinical reports of use of cidofovir in traditional weekly dosing and intermittent renal sparing dosing is safe and well tolerated in pediatric transplant recipients with adenovirus disease.[21,36,62]

Human Erythrovirus

Human erythrovirus (formerly human parvovirus B19) has been reported to cause acute and chronic infection, manifesting as chronic anemia, red blood cell aplasia, and pancytopenia in renal transplant recipients.† It also has been implicated in collapsing glomerulopathy and thrombotic microangiopathy in renal transplant recipients.[246] Unusual manifestations, such as hepatic necrosis or central nervous system infection with vasculitis, also have been reported.[19,22,131,132] Transmission most likely is person to person; however, some reports indicate that the virus may be transmitted by blood product transfusion or in the renal allograft.[18,19,130] More recent reports suggest that the role that this virus plays in the outcome of renal transplant recipients may be more important than previously recognized, but systemic studies in pediatric patients have not been done.[18]

The diagnosis of acute infection with human erythrovirus is supported by erythrovirus-specific IgG seroconversion; detection of erythrovirus-specific IgM antibodies in serum; or detection of viral DNA in plasma, blood, body fluids, bone marrow, or tissue. The virus is not cultivatable in clinical virology laboratories that use routine cell cultures, but laboratories using special erythroid precursor cell lines may be able to cultivate erythrovirus for research purposes. No specific antiviral therapy is available, but anecdotal experience and case reports support a beneficial role for intravenous immunoglobulin and carefully monitored reduction in immunosuppression as management strategies to resolve erythrovirus viremia and associated end-organ disease.[159]

West Nile Virus

WNV is a single-stranded RNA flavivirus that has emerged as an important pathogen worldwide. It usually is transmitted by mosquitoes and causes a febrile illness, viral meningoencephalomyelitis, and acute flaccid paralysis in the normal host. Renal transplant recipients may become infected with WNV that is transmitted by the organ donor, by blood product transfusions, or naturally from mosquitoes.* Routine screening of solid-organ donors for WNV infection has been discussed but currently is not routinely performed.[112,168] Infection with WNV may manifest in the early posttransplant period if it is transmitted by the graft or blood products and, because of immunosuppression, produce severe febrile illnesses, seizures with status epilepticus, encephalitis, paralysis, and movement disorders. Death also may occur.[50] A high index of suspicion is required; and if WNV is suspected, serum and cerebrospinal fluid should be sent for WNV-specific IgG and IgM antibody testing. The virus also may be isolated in special cell cultures and detected by reverse transcriptase PCR-based assays. Immunohistochemical staining may show WNV proteins in tissues from biopsy or autopsy examination. Outcome in renal transplant recipients with WNV disease improves with early establishment of the diagnosis and carefully managed reduction of immunosuppression.[197]

Mycoplasma

Mycoplasma spp. commonly infect school-aged children. Children and adolescents who undergo renal transplantation also may experience respiratory tract infections with *M. pneumoniae* and *M. hominis*. The infection also may disseminate to extrapulmonary sites, causing hepatitis, septic arthritis, or perinephric fluid collections in pediatric renal transplant recipients.[120,151,224] *Mycoplasma* spp. may be detected using special biphasic culture media or PCR-based assays. Serologic diagnosis is difficult to accomplish, owing to cross-reactivity of antigens producing the possibility of false-positive reactions in some patients. Antimicrobial therapy with macrolide antibiotics, along with carefully managed reduction of immunosuppression, may be beneficial in selected patients.[120,151]

Bacterial and Mycobacterial Diseases

Bacteria that commonly produce disease in renal transplant recipients during the middle posttransplant period include *L. monocytogenes*, which often causes sepsis or meningitis.[185,217] Routine bacterial illnesses that were not identified and properly treated during the pretransplant period also may emerge at this time and cause abscesses, sepsis syndrome, and death.

*References 24, 122, 206, 232, 241, 251, 254.
†References 10, 18, 19, 22, 131, 139, 141, 145, 159, 182, 227, 246, 253.
*References 50, 112, 114, 126, 197, 245.

Mycobacterial disease caused by *M. tuberculosis* may occur at any time after transplantation, but it occurs most frequently during the middle posttransplant period.[135,138,185,195] *M. tuberculosis* causes disease in approximately 1 percent of renal transplant recipients in the developed continents, such as North America and Europe, and in 15 percent of renal transplant recipients in developing countries with a high prevalence of tuberculosis, such as India or Pakistan.* Tuberculosis may develop in renal transplant recipients as a result of primary and reactivation infection in almost any site, and transmission from the renal allograft and reactivation in the native kidney have occurred.[127,160,161,202] *M. tuberculosis* may cause various diseases, including pulmonary infiltrates, cavitary lesions, adenopathy, cutaneous lesions, bone and joint disease, liver or spleen granulomas, and meningitis. Fever frequently is noted, and miliary or disseminated disease may occur, especially in young children.

Because the TST is negative in most renal transplant recipients with active tuberculosis, the diagnosis is determined best by IGR assays, detection of acid-fast bacilli in smears from tissue or sputum, and isolation of *M. tuberculosis* in cultures of gastric aspirates, sputum, tracheal secretions, spinal fluid, or tissue.[138,185] The presence of granulomas in tissue also suggests the diagnosis of tuberculosis.

Treatment of tuberculosis in a renal transplant recipient usually includes isoniazid, rifampin, and pyrazinamide for 1 year, although shorter courses may be acceptable in some patients.[135,185] Other antituberculous drugs may be added to or substituted for this standard regimen, depending on the disease process, the susceptibility pattern of the organism, and potential drug interactions. Because many antituberculosis drugs are excreted by the kidney, doses may need to be adjusted in a renal transplant recipient. Because rifampin may interact with cyclosporine, levels of cyclosporine should be monitored closely to avoid rejection of the allograft.[174] If a history of tuberculosis exposure is elicited during the pretransplant evaluation, the transplant candidate should be evaluated for tuberculosis, including having a TST, IGR assay on blood, and chest radiograph.[160,161,195] All close contacts also should be investigated for evidence of having tuberculosis. Treatment is recommended if disease is discovered, and prophylaxis with isoniazid is indicated for most patients who have a recently positive result of a purified protein derivative (PPD) test on pretransplant evaluation.[135,185,186,210,256] A transplant recipient who receives an allograft from a donor with a history of tuberculosis or a positive TST or IGR assay also may be a candidate for prophylaxis with isoniazid after a transplant procedure.[135,185,186]

Atypical mycobacteria are ubiquitous nontuberculous mycobacteria that can infect and produce disease in renal transplant recipients. They can cause disease during the middle posttransplant period and so are included here. Their effects usually do not become evident, however, until many years after the patient has undergone transplantation, during the late posttransplant period.[48,161,187] Atypical mycobacteria that have been documented to cause disease in solid-organ transplant recipients include *M. kansasii*, *M. avium-intracellulare*, *M. fortuitum*, *M. xenopi*, *M. haemophilum*, *M. marinum*, *M. chelonae*, *M. abscessus*, *M. gastri*, *M. scrofulaceum*, and *M. thermoresistibile*.[117,185,186]

A high index of suspicion is necessary to detect these elusive pathogens. They should be considered as the cause of disease in patients with persistent cutaneous ulcers, abscesses, adenopathy, pulmonary nodules, chronic wound infections, or bone and joint disease after negative routine bacterial cultures have been obtained and the patient has failed to respond to standard antimicrobial therapy.[206] Disseminated, multisystemic disease also can occur. Environmental sources of atypical mycobacteria include contaminated dialysis equipment, soil, and contaminated water in aquariums and pools. The diagnosis is established by isolation of nontuberculous mycobacteria in fluid or tissue. Granulomas are not observed consistently in tissue, and acid-fast stains may be negative.[206] Atypical nontuberculous mycobacteria are difficult to treat, and treatment must be individualized to each patient. Strategies include reduction of immunosuppression and surgical débridement of localized disease. Antimicrobial therapy based on in vitro susceptibility testing of the isolate also should be administered, often for a prolonged period.

Nocardia

Nocardia asteroides is the most common *Nocardia* spp. causing illness in renal transplant recipients; however, other, more unusual *Nocardia* spp., including *N. transvalensis*, *N. brasiliensis*, *N. nova*, *N. otitidiscaviarum*, and *N. farcinica*, also have been shown to cause disease in solid-organ transplant recipients.[185,186,251] The most common manifestations of nocardial disease in transplant recipients are fever, cough, and pulmonary infiltrates.[251] Pleural effusion, pulmonary nodules, and cavitary lesions also may occur. Cutaneous infection, adenitis, arthritis, meningitis with brain abscesses, and infection of the renal allograft also may occur. *Nocardia* spp. can be seen on Gram and modified acid-fast stains of sputum, bronchoalveolar lavage fluid, abscess fluid, and tissue. They can be isolated on routine media but may take longer than conventional bacteria to grow. Prolonged treatment with sulfonamides, alone or in combination with trimethoprim, is recommended. Amikacin and other antimicrobial agents may be added in selected patients with severe disease, provided that renal function is monitored closely.[206] Trimethoprim-sulfamethoxazole prophylaxis for UTI and *P. jiroveci* also may be effective in preventing disease caused by *Nocardia*.

Fungal Diseases

Fungal infections occur infrequently in renal transplant recipients relative to other solid-organ transplant recipients.[12,185,186,206] This lower rate of infection probably is related to technical procedures performed at the time of transplantation and the lower level of immunosuppression required to maintain most renal allografts.[206] When they do occur, however, fungal infections often are serious and life threatening.[95,99,109] Fungal disease in a renal transplant recipient usually is manifested in one of two ways:

*References 117, 185, 195, 202, 209, 210.

(1) pulmonary or disseminated disease caused by one of the environmental mycoses, such as *H. capsulatum* or *C. immitis*, or (2) opportunistic infection with fungi that rarely cause disease in a normal host, such as *Candida* spp., *P. jiroveci*, *Aspergillus* spp., *Cryptococcus neoformans*, *Nocardia* spp., and others.[95,219]

Travel to endemic areas increases a transplant recipient's risk for development of histoplasmosis and coccidioidomycosis and may be identified during the pretransplant evaluation. Factors that increase a transplant recipient's risk for acquisition of opportunistic fungal disease include underlying conditions such as diabetes mellitus, repeated episodes of rejection, long-term administration of corticosteroids, prolonged use of antibiotics, and CMV infection. Similar to tuberculosis, invasive fungal disease can be a result of primary infection or reactivation infection with secondary dissemination.[206] *P. jiroveci*, currently classified as a fungus, may cause pneumonia in 10 percent of solid-organ transplant recipients, most often during the first 6 months after a transplant procedure.[9,185,186] It seems to occur more commonly in children than in adults and usually causes fever, dyspnea, tachypnea, hypoxemia, and a nonproductive cough.[64,185] Interstitial pulmonary infiltrates are typical findings, but almost any radiographic picture can be observed, and pneumothorax is common in severe disease. The diagnosis can be suspected clinically but is documented best by organisms being shown in lung biopsy specimens; bronchoalveolar lavage may provide the diagnosis in some patients.

Treatment with high-dose oral or intravenous trimethoprim-sulfamethoxazole for 14 to 21 days is used most often. Intravenous pentamidine may be administered to selected patients, but renal function should be monitored carefully.[206] Corticosteroids may be helpful in treating severe disease if given early in the course. Low-dose oral trimethoprim-sulfamethoxazole provides effective prophylaxis against *P. jiroveci* pneumonia and UTIs and usually is administered to renal transplant recipients for at least 6 months post transplant.[77] Transplant recipients experiencing repeated episodes of rejection, allograft dysfunction, or CMV disease may require a longer period of prophylaxis.[185,186] Aerosolized pentamidine is an alternative prophylaxis strategy for some patients.[206]

Candida spp., especially *C. albicans*, are the opportunistic fungi most frequently isolated in renal transplant recipients.[185,186] Other *Candida* spp. isolated include *C. krusei*, *C. glabrata*, and *C. tropicalis*. Most fungal infections caused by *Candida* spp. occur during the first 2 months after transplant surgery, usually at the site of indwelling intravascular and urinary catheters.[206] Esophagitis, abscesses, and arthritis also can develop, and endocarditis with metastatic foci can occur if the fungemia persists. Other opportunistic fungi such as *Aspergillus* most often cause sinusitis and pulmonary disease, and, because of its angioinvasive nature, *Aspergillus* often disseminates and causes lesions in the liver, spleen, and brain.[185,186,219,220]

A thorough search for metastatic foci always should be undertaken when a primary focus of invasive fungal disease is documented. *C. neoformans* usually causes cutaneous lesions or abscesses and pneumonia with pleural effusion, but meningitis, arthritis, and pyelonephritis also can occur.[185,186,214] Exposure to soil or bird droppings provides an epidemiologic clue to the diagnosis. In addition, the Zygomycetes, including *Rhizopus*, *Mucor*, *Rhizomucor*, and *Absidia* spp., can cause disease in solid-organ transplant recipients.[219,220] They most often cause cutaneous, rhinocerebral, or pulmonary disease or brain abscesses in renal transplant patients. Cutaneous and soft tissue infections, wound infections, and gastrointestinal disease with perforation also have been described. The Zygomycetes, similar to *Aspergillus*, are angioinvasive and disseminate via the bloodstream to cause disease, which often is severe. A variety of unusual fungi, such as *Paecilomyces*, *Fusarium*, and *Bipolaris*, have been shown to cause cutaneous infection, usually at the site of indwelling catheters, and *Hansenula anomala* reportedly has caused UTI in a renal transplant recipient.[86]

The endemic dimorphic fungi include *H. capsulatum*, *C. immitis*, *B. dermatitidis*, and *Paracoccidioides brasiliensis*. Infection with these organisms can occur at any time after the patient has undergone transplantation but usually occurs during the intermediate posttransplant period.[233] These organisms usually are noted in renal transplant recipients who reside in endemic areas. Histoplasmosis is endemic in the central part of the United States and many foreign countries, and nosocomial outbreaks have occurred during hospital construction projects. Fever, chills, and cough are the usual initial signs. Skin lesions, hepatosplenomegaly, and meningitis also can occur. Pancytopenia often is present as well, and the organism frequently is found in the bone marrow of patients with disseminated disease. Coccidioidomycosis is endemic in the southwestern portion of the United States and northern Mexico. It usually is manifested as fever, cough, and pulmonary infiltrates, but extrapulmonary dissemination frequently occurs. Blastomycosis is endemic in the southern United States, along the Mississippi and Ohio River valleys, and in the Great Lakes area. It is a rare development after renal transplantation, but most often causes lung and skin lesions. Paracoccidioidomycosis rarely has been reported in renal transplant recipients.[228]

The diagnosis of invasive fungal disease is established best by isolating the fungus from sputum or tracheal aspirate, bone marrow, tissue, or fluid. Serum tests and bronchoalveolar lavage for galactomannan and 1-3-β-D-glucan levels support the diagnosis of invasive fungal disease, and DNA PCR tests on serum and bronchoalveolar lavage fluid also may now be performed to detect *Candida* spp. and other fungi.[12]

C. neoformans also may be isolated from urine. Fungal specific serology and tests for cryptococcal antigen may support the diagnosis of cryptococcal infection.[228]

Fungal identification and susceptibility testing now can be performed in clinical and reference laboratories and should be used to guide therapy whenever possible. Amphotericin B usually is used to treat most invasive fungal disease in transplant recipients, and the deoxycholate, lipid complex, or liposomal forms can be used, provided that renal function is monitored closely.[136] Flucytosine may have additive or synergistic effects against many yeasts and fungi. The azole antifungal agents are not nephrotoxic and are used in renal transplant recipients whenever possible. Many *Candida* spp. are susceptible to fluconazole, although resistance is emerging in all *Candida* spp. and is present in a high percentage of *C. krusei* and *C. glabrata*. Itraconazole and

voriconazole may be effective against other fungi, such as *Aspergillus*.[12] Voriconazole is the treatment of choice for most *Aspergillus* infections. Itraconazole should be used with caution because it has variable oral absorption and may interact with cyclosporine.[233] Caspofungin, an echinocandin, also has activity against most *Candida* spp. and a variety of opportunistic fungi, including some species of *Aspergillus*. The triazole posaconazole and the echinocandin micafungin have also been used successfully in pediatric renal transplant recipients with invasive fungal disease.

Surgical resection or drainage of abscesses, avascular cavities, or effusions may be required to treat some forms of invasive fungal disease of the lungs, liver, or spleen. Carefully monitored reduction in immunosuppression also may help the host recover from invasive fungal disease.

Parasitic Infections

The parasites most commonly encountered in renal transplant recipients during the intermediate posttransplant period are *T. gondii*, *S. stercoralis*, and *Trypanosoma cruzi*.[73,185] These infections occur most often in adults. Children also may be infected with routine parasites such as *Enterobius vermicularis*, *Ascaris*, *Giardia*, and others before a transplant operation. Other parasitic infections that may be found in renal transplant recipients include leishmaniasis, schistosomiasis, and malaria. Renal transplant recipients seem to be at much lower risk for acquisition of parasitic diseases than are other solid-organ transplant recipients.

Infection with *T. gondii* usually occurs as a result of reactivation of latent disease in the donor allograft or recipient.[144] It is encountered most frequently in heart transplant recipients, but any solid-organ recipient, including renal transplant patients, may be affected. Clinical manifestations of infection with *T. gondii* include focal meningoencephalitis, brain abscesses, pneumonia, myocarditis, pericarditis, hepatitis, and retinochoroiditis.[155,225,233,240] The diagnosis may be made by demonstration of the organism in tissue by histopathology, detection of DNA by PCR in body fluids or tissue, or serologically by detection of high titers of IgG and specific IgM antibody to *T. gondii*. Treatment with pyrimethamine, sulfadiazine, and folinic acid usually is recommended; however, some patients also may respond to clindamycin. Routine prophylaxis with trimethoprim-sulfamethoxazole for UTI and *P. jiroveci* infection may help prevent disease with *T. gondii*.

S. stercoralis is an important pathogen in adult transplant recipients, but it is rarely, if ever, a significant problem in pediatric patients. Because *S. stercoralis* can be maintained in the human intestinal tract for decades, it can disseminate and cause serious disease in transplant recipients who were infected before the procedure. A complete blood cell count for eosinophilia should be obtained, and stool and other specimens should be examined for rhabditiform larvae if the pretransplant evaluation reveals travel to endemic areas. Thiabendazole should be given before transplantation is performed if *S. stercoralis* infection has been documented or is suspected. *T. cruzi*, the cause of Chagas disease, rarely has been transmitted by renal transplantation.[73,133]

Infections Occurring During the Late Posttransplant Period

Infections occurring 6 months or more after the patient has undergone renal transplantation usually are less severe than infections experienced in the earlier periods, especially if the level of immunosuppressive therapy is low and the allograft is functioning well.[205] Constant vigilance for health care–associated and community-acquired viral and bacterial diseases and unusual infections should continue, however.

Chronic rejection or repeated episodes of acute rejection complicated by allograft dysfunction predispose the patient to development of more serious opportunistic infections, similar to infections encountered during the first 6 months after a transplant. UTIs that develop during this period, in contrast to UTIs in the early posttransplant period, usually are benign, especially if the patient is afebrile, and may be treated with conventional antimicrobial therapy in most instances, if they do not seem to threaten the function of the allograft or the patient's survival. Patients also may experience community-acquired infections with respiratory viruses, such as influenza virus and respiratory syncytial virus, and with bacteria, especially *Streptococcus pneumoniae* (pneumococcus) or *S. aureus*. These common community-acquired bacterial pathogens may be resistant to usual β-lactam antibiotics.[226,238] In addition, patients vaccinated with 7-valent or 23-valent pneumococcal vaccines still may acquire invasive pneumococcal disease with emerging, nonvaccine serotypes, such as 19A and 3A. These serotypes often are also multidrug-resistant. Opportunistic viral infections encountered during this period are zoster or, rarely, CMV retinitis.[164] The risk for developing PTLD and BKAN also persists during this late posttransplant period.

Renal transplant recipients may be chronically infected with HBV or HCV before a transplant is performed. Increased risk for mortality from fulminant hepatitis with hepatic failure may occur during the early pretransplant period, and chronic liver disease, cirrhosis, and liver failure may be seen 10 years or more after renal transplantation has been performed in hepatitis B surface antigen (HBsAg)–positive recipients.[255] All transplant recipients who are not immune to HBV should receive HBV vaccine before transplantation.[247] Most experts agree that renal allografts from HBsAg-positive donors should not be used, but the decision to perform renal transplantation in recipients who are HBsAg-positive is controversial and must be made on an individualized basis.

Renal transplant recipients who are infected with HCV before the transplant usually do well during the early posttransplant period, but long-term survival is poorer than in recipients who are not infected with HCV. Not only can chronic liver disease develop, but also membranoproliferative glomerulonephritis has been reported in renal transplant recipients infected with HCV.[32] Despite these risks, however, most experts do not

consider infection with HCV a contraindication for renal transplantation in patients with ESRD.[190] Hepatitis G virus also has been detected in the serum of renal transplant recipients, and at least one association with membranous glomerulonephritis has been reported.[16,17] The role of HGV in clinical disease in renal transplant recipients is not established, however.

HIV may be transmitted by kidney transplantation despite routine screening of donors of blood and organs.[130,200] A transplant recipient also may become infected with HIV after undergoing transplantation.[185] Shortly after a patient has undergone transplantation, HIV infection may cause fever and a mononucleosis-like syndrome. Complications of acquired immunodeficiency syndrome may develop during the late transplant period. HIV-infected patients with ESRD are no longer excluded from receiving renal transplantation because recent studies have shown successful outcomes.[97,100,165,185]

NEW REFERENCES SINCE THE SIXTH EDITION

8. Anyaegbu EI, Almond PS, Milligan T, et al. Intravenous immune globulin treatment of BK viremia and nephropathy in pediatric renal transplant recipients. *Pediatr Transplant* 2012;**16**(1):E19–24.
12. Badiee P, Alborzi A. Invasive fungal infection in renal transplant recipients. *Exp Clin Transplant* 2011;**9**(66):355–62.
13. Banks CA, Meier JD, Stallworth CR, White DR. Recurrent post-transplant lymphoproliferative disorder involving the larynx and trachea: case report and review of the literature. *Ann Otol Rhinol Laryngol* 2012;**121**(5):291–5.
15. Bechert CJ, Schnadig VJ, Payne DA, Dong J. Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. *Am J Clin Pathol* 2010;**133**(2):242–50.
21. Bhadri VA, Lee-Horn L, Shaw PJ. Safety and tolerability of cidofovir in high-risk pediatric patients. *Transpl Infect Dis* 2009; **11**(4):373–9.
41. Chiappii E, Bonsignori F, Accetta G, et al. Interferon-gamma release assays for the diagnosis of *Mycobacterium tuberculosis* infection in children: a literature review. *Int J Immunopathol Pharmacol* 2012;**25**(2):335–43.
54. Dharnidharka V, Abdulnour HA. The BK virus in renal transplant recipients—review of pathology, diagnosis and treatment. *Pediatr Nephrol* 2011;**26**(10):1763–74.
97. Harbell J, Fung J, Nissen N, et al. Surgical complications in 275 HIV-infected liver and/or kidney transplant recipients. *Surgery* 2012;**152**(3):376–81.
100. Hayes, K, VanSickels N, Buell J, et al. Successful transplantation of HIV patients: the Louisiana experience. *J La State Med Soc* 2012;**164**(4):191–3.
112. Inojosa WO, Scotton PG, Fuser R, et al. West Nile virus transmission through organ transplantation in north-eastern Italy: a case report and implications for pre-procurement screening. *Infection* 2012;**40**(5):557–62.
113. Ison MG, Szakaly P, Shapira MY, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**(6):955–64.
124. Kim SH, Lee SO, Park JB, et al. A prospective longitudinal study evaluating the usefulness of a T-cell–based assay for latent tuberculosis infection in renal transplant recipients. *Am J Transplant* 2011;**11**(9):1927–35.
150. Mencarelli F, Marks SD. Non-viral infections in children after renal transplantation. *Pediatr Nephrol* 2012;**27**:1465–76.
165. Muzuecos A, Rodriguez Benot A, Moreno A, et al. Renal replacement treatment in patients with HIV infection in a European region: outcomes following renal transplantation. *Transplant Proc* 2012;**44**(7):2053–6.
168. Nett RJ, Kuehnert MJ, Ison MG, et al. Current practices and evaluation of screening solid organ donors for West Nile virus. *Transpl Infect Dis* 2012;**14**(3):268–77.
204. Rogerson TE, Chen S, Kok J, et al. Tests for latent tuberculosis in people with end stage renal disease: a systematic review. *Am J Kidney Dis* 2013;**61**(1):33–43.
252. Wojciechowski D, Chanda R, Chandran S, et al. Ciprofloxacin prophylaxis in kidney transplant recipients reduces BK virus infection at 3 months but not at 1 year. *Transplantation* 2012; **94**(11):1117–23.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 74

Infections Related to Prosthetic or Artificial Devices

Ram Yogev • Tina Q. Tan

The development of biomaterials used in the manufacturing of temporary or permanent implantable prosthetic devices has been one of the greatest advances in modern medicine.[90,114,115] These devices have become an integral and important part of the current practice of medicine and have improved considerably the lives and health of countless patients. In the United States, the number and types of permanent prosthetic devices implanted to replace diseased or damaged body parts has increased substantially during the last several decades. An estimated

REFERENCES

1. Acott P, Lee S, Bitter-Suermann H, et al. Infection concomitant with pediatric renal allograft rejection. *Transplantation* 1996; **62**:689.
2. Adams J, Mehls O, Wiesel M. Pediatric renal transplantation and the dysfunctional bladder. *Transpl Int* 2004;**17**:596.
3. Ahsan N, Holman M, Riley T, et al. Peliosis hepatis due to *Bartonella henselae* in transplantation: a hemato-hepato-renal syndrome. *Transplantation* 1998;**65**:1000.
4. Al-Atlar L, Berrocal A, Warman R, et al. Diagnosis by PCR of ocular post transplant lymphoproliferative disease after pediatric renal transplantation. *Am J Ophthalmol* 2004;**137**:569.
5. Allen U, Herbert D, Pedric M, et al. Utility of semiquantitative polymerase chain reaction for Epstein-Barr virus to measure virus load in pediatric organ transplant recipients with and without post-transplant lymphoproliferative disease. *Clin Infect Dis* 2001; **33**:145.
6. Andrews S, Shas K, Daniel R, et al. A serologic investigation of BK virus and JC virus infections in recipients of renal allografts. *J Infect Dis* 1988;**158**:176.
7. Anglicheau D, Lautrette A, Scieux C, et al. Efficacy and safety of lowering immunosuppression to treat CMV infection in renal transplant recipients with valacyclovir prophylaxis: a pilot study. *Nephrol Dial Transplant* 2003;**18**:1654.
8. Anyaegbu EI, Almond PS, Milligan T, et al. Intravenous immune globulin treatment of BK viremia and nephropathy in pediatric renal transplant recipients. *Pediatr Transplant* 2012;**16**(1):E19–24.
9. Arend S, Westendrop R, Kroon F, et al. Rejection treatment and cytomegalovirus infection as risk factors for *Pneumocystis carinii* pneumonia in renal transplant recipients. *Clin Infect Dis* 1996; **22**:920.
10. Ashan N, Holman M, Gocke C, et al. Pure red cell aplasia due to parvovirus B19 infection in solid organ transplantation. *Clin Transplant* 1997;**11**:265–70.
11. Avery R. Infections and immunizations in organ transplant recipients: a preventive approach. *Cleve Clin J Med* 1994;**61**:386.
12. Badiee P, Alborzi A. Invasive fungal infection in renal transplant recipients. *Exp Clin Transplant* 2011;**9**(66):355–62.
13. Banks CA, Meier JD, Stallworth CR, White DR. Recurrent post-transplant lymphoproliferative disorder involving the larynx and trachea: case report and review of the literature. *Ann Otol Rhinol Laryngol* 2012;**121**(5):291–5.
14. Barrero R, Fij J, Fernandez-Hurtado M, et al. Vesicoureteral reflux after kidney transplantation in children. *Pediatr Transpl* 2007;**11**:498.
15. Bechert CJ, Schnadig VJ, Payne DA, Dong J. Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. *Am J Clin Pathol* 2010;**133**(2):242–50.
16. Berthoux P, Dejean C, Cecillon S, et al. High prevalence of hepatitis G (HGV) infection in renal transplantation. *Nephrol Dial Transplant* 1998;**13**:2909.
17. Berthoux P, Laurent B, Cecillon S, et al. Membrane proliferative glomerulonephritis and subendothelial deposits (type 1) associated with hepatitis G virus infection. *Am J Nephrol* 1999;**19**:513.
18. Bertoni E, Rosati A, Zanazzi M, et al. Aplastic anemia due to B19 parvovirus infection in cadaveric renal transplant recipients: an underestimated infectious disease in the immune compromised host. *J Nephrol* 1997;**10**:152.
19. Bertoni E, Rosati A, Zanazzi M, et al. Unusual incidence of aplastic anemia due to B-19 parvovirus infection in renal transplant recipients. *Transplant Proc* 1997;**29**:818.
20. Betts R, Freeman R, Douglas R, et al. Clinical manifestations of renal allograft derived primary cytomegalovirus infection. *Am J Dis Child* 1977;**131**:759.
21. Bhadri VA, Lee-Horn L, Shaw PJ. Safety and tolerability of cidofovir in high-risk pediatric patients. *Transpl Infect Dis* 2009; **11**(4):373–9.
22. Bilge I, Sadkoglu B, Emre S, et al. Central nervous system vasculitis secondary to parvovirus B19 infection in a pediatric renal transplant patient. *Pediatr Nephrol* 2005;**20**:529.
23. Binet I, Nickeleit V, Hirsch H, et al. Polyomavirus disease under new immunosuppressive drugs: a cause of renal graft dysfunction and graft loss. *Transplantation* 1999;**67**:918.
24. Blohme L, Nyberg S, Jeansson S, et al. Adenovirus infection in a renal transplant patient. *Transplant Proc* 1992;**24**:295.
25. Boland G, deGast G, Hene R, et al. Early detection of active cytomegalovirus (CMV) infection after heart and kidney transplantation by testing for immediate early antigenemia and influence of cellular immunity on the occurrence of CMV infection. *J Clin Microbiol* 1990;**28**:2069.
26. Boom R, Sol C, Schuurman T, et al. Human cytomegalovirus DNA in plasma and serum specimens of renal transplant recipients is highly fragmented. *J Clin Microbiol* 2002;**40**:4105.
27. Boom R, Sol C, Weel J, et al. A highly sensitive assay for the detection and quantitation of human cytomegalovirus DNA in serum and plasma by PCR and electrochemiluminescence. *J Clin Microbiol* 2000;**37**:1489.
28. Boudjemaa S, Boman F, Guigonis V, et al. Brain involvement in multicentric Epstein-Barr virus–associated smooth muscle tumors in a child after kidney transplantation. *Virchows Arch* 2004;**444**:387.
29. Brenig M, Zitelli B, Starzl T, et al. Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. *J Infect Dis* 1987;**156**:273.
30. Brennam D, Garlock K, Singer G, et al. Prophylactic oral ganciclovir compared with deferred therapy for control of cytomegalovirus in renal transplant recipients. *Transplantation* 1997;**64**:1843.
31. Breyer M, Tete M, Guest G, et al. Varicella-zoster in children after kidney transplantation: long-term results of vaccination. *Pediatrics* 1997;**99**:35.
32. Brunkhorst R, Kliem V, Koch K. Recurrence of membranoproliferative glomerulonephritis after renal transplantation in a patient with chronic hepatitis C. *Nephron* 1996;**72**:465.
33. Burroughs M, Moscona A. Immunization of pediatric solid organ transplant candidates and recipients. *Clin Infect Dis* 2000;**30**:857.
34. Campins M, Ferrer A, Callis L, et al. Nosocomial legionnaire's disease in a children's hospital. *Pediatr Infect Dis J* 2000;**19**:228.
35. Capizzi A, Zanon G, Zachello G, et al. Kidney transplantation in children with reconstructed bladders. *Transplantation* 2004; **15**:1113.
36. Carter B, Karpen S, Quiros-Tejeiro R, et al. Intravenous cidofovir treatment for disseminated adenovirus in a pediatric liver transplant recipient. *Transplant* 2002;**74**:1050.
37. Caterino-de-Araujo A, Magri M, Santos-Fortuna E, et al. Human herpesvirus–8 infection in hemodialysis patients from São Paulo, Brazil: preliminary results. *Transplant Proc* 2007;**39**:3044.
38. Cattani P, Capuano M, Graffeo R, et al. Kaposi's sarcoma associated with previous human herpesvirus 8 infection in kidney transplant recipients. *J Clin Microbiol* 2001;**39**:506.
39. Chaves-Ido S, Lopes M, deSouza V, et al. Seroprevalence of antibody against varicella zoster virus and response to the varicella vaccine in pediatric renal transplant patients. *Pediatr Transplant* 2005;**9**:192.
40. Chavez B, Gillingham K, Mata A. Complications by age in primary pediatric renal transplant recipients. *Pediatr Nephrol* 1997;**11**:399.
41. Chiappii E, Bonsignori F, Accetta G, et al. Interferon-gamma release assays for the diagnosis of *Mycobacterium tuberculosis* infection in children: a literature review. *Int J Immunopathol Pharmacol* 2012;**25**(2):335–43.
42. Chou S. Acquisition of donor strains of cytomegalovirus by renal transplant recipients. *N Engl J Med* 1986;**314**:1418.
43. Cline M, Cummings O, Goldman M, et al. Bacillary angiomatosis in a renal transplant recipient. *Transplantation* 1999;**67**:296.
44. Coleman D, MacKenzie E, Gardner S, et al. Human polyomavirus (BK) infection and ureteric stenosis in renal allograft recipients. *J Clin Pathol* 1978;**31**:338.
45. Colombo J, Sammut P, Langnas A, et al. The spectrum of *Pneumocystis carinii* infection after liver transplant in children. *Transplantation* 1992;**54**:621.
46. Conti D, Freed B, Singh T, et al. Preemptive ganciclovir therapy in cytomegalovirus-positive renal transplant recipients. *Arch Surg* 1995;**130**:1217.
47. Cortesa M, Jordan HT, Cums AT, et al. Mumps vaccine performance among university students during a mumps outbreak. *Clin Infect Dis* 2008;**46**:1172.
48. Costa J, Meyers A, Botha J, et al. Mycobacterial infections in recipients of kidney allografts: a seventeen-year experience. *Acta Med Port* 1988;**1**:51.
49. Crompton C, Cheung C, Conjon I, et al. Epstein-Barr virus surveillance after renal transplantation. *Transplantation* 1994;**57**:1182.

50. Cushing M, Brat D, Mosunijac M, et al. Fatal West Nile virus encephalitis in a renal transplant recipient. *Am J Clin Pathol* 2004;**121**:26.
51. Danziger-Isakov L, Storch G. Prevention and treatment of cytomegalovirus infections in solid organ transplant recipients. *Pediatr Infect Dis J* 2002;**21**:431.
52. Davis C, Gretch D, Carithers R. Hepatitis B and transplantation. *Infect Dis Clin North Am* 1995;**9**:925.
53. Dhar J, Al-Khader A, Al-Sulaiman M, et al. Non-typhoidal *Salmonella* in renal transplant recipients: a report of twenty cases and review of the literature. *Q J Med* 1991;**78**:235.
54. Dharnidharka V, Abdulnour HA, Araya CE. The BK virus in renal transplant recipients-review of pathology, diagnosis and treatment. *Pediatr Nephrol* 2011;**26**(10):1763–74.
55. Dharnidharka V, Agodoa L, Abbott K. Effects of urinary tract infection on outcomes after renal transplantation in children. *Clin J Am Soc Nephrol* 2007;**2**:100.
56. Dharnidharka V, Araya C. Post-transplant lymphoproliferative disease. *Pediatr Nephrol* 2009;**24**:731–6.
57. Dharnidharka V, Harmon W. Management of pediatric postrenal transplantation infections. *Semin Nephrol* 2001;**21**:521.
58. Dharnidharka V, Ho P, Stablein D, et al. Mycophenolate, tacrolimus, and post-transplant lymphoproliferative disorder: a report of the North American Pediatric Renal Transplant Cooperative Study. *Pediatr Transplant* 2002;**6**:396.
59. Dharnidharka V, Richard G, Neiberger R, et al. Cat scratch disease and acute rejection after pediatric renal transplantation. *Pediatr Transplant* 2002;**6**:327.
60. Dharnidharka V, Stablein D, Harmon W. Post-transplant infections now exceed acute rejection as cause for hospitalization: a report of the NAPRTCS. *Am J Transplant* 2004;**4**:384.
61. Dionne J, Carter J, Matsell D, et al. Renal leiomyoma associated with EBV in a pediatric transplant patient. *Am J Kidney Dis* 2005;**46**:351.
62. Doan ML, Mallory GB, Kaplan SL, et al. Treatment of adenovirus pneumonia with cidofovir in pediatric lung transplant recipients. *J Heart Lung Transplant* 2007;**26**(9):883–9.
63. Drachenberg C, Beskow C, Cangno C, et al. Human polyomavirus in renal allograft biopsies: morphological findings and correlation with urine cytology. *Hum Pathol* 1999;**30**:970.
64. Dummer J, Armstrong J, Somers J, et al. Transmission of infection with herpes simplex virus by renal transplantation. *J Infect Dis* 1987;**155**:202.
65. Dummer J, Hardy A, Poorsattar A, et al. Early infections in kidney, heart and liver transplant recipients on cyclosporine. *Transplantation* 1983;**36**:259.
66. Durlik M, Siennicka J, Litwinska B, et al. Clinical manifestations and diagnosis of cytomegalovirus infection in renal allograft recipients. *Transplant Proc* 2001;**33**:1237.
67. Elldemir O, Chang I, Schecter M, et al. BK-virus associated hemorrhagic cystitis in a pediatric lung transplant recipient. *Pediatr Transplant* 2007;**11**:807.
68. El Sayegh S, Keller M, Huprikar S, et al. Solid organ transplantation in HIV-infected recipients. *Pediatr Transplant* 2004;**8**:214.
69. Emery V. Human herpesviruses 6 and 7 in solid organ transplant recipients. *Clin Infect Dis* 2001;**32**:1357.
70. Falco D, Nepomuceno R, Karm S, et al. Identification of Epstein-Barr virus-specific $CD8^+$ T lymphocytes in the circulation of pediatric transplant recipients. *Transplantation* 2002;**74**:501.
71. Fargie D, Lebbe C, Marjanovic Z, et al. Human herpesvirus–8 and other risk factors for Kaposi's sarcoma in kidney transplant recipients. *Transplantation* 1999;**67**:1236.
72. Feldhoff C, Balfour H, Simmons R, et al. Varicella in children with renal transplants. *J Pediatr* 1981;**98**:25.
73. Ferraz A, Figueiredo J. Transmission of Chagas disease through transplanted kidney: occurrence of the acute form of the disease in two recipients from the same donor. *Rev Inst Med Trop São Paulo* 1993;**35**:461.
74. Flechner A, Avery R, Fisher R, et al. A randomized prospective controlled trial of oral acyclovir versus oral ganciclovir for cytomegalovirus prophylaxis in high-risk kidney transplant recipients. *Transplantation* 1998;**66**:1682.
75. Fogazzi G, Cantu M, Saglembeni L. Decoy cells in the urine due to polyomavirus BK infection easily seen by phase-contrast microscopy. *Nephrol Dial Transplant* 2001;**16**:1496.
76. Fogeda M, Munoz P, Luque A, et al. Cross-sectional study of BK-virus infection in pediatric kidney transplant recipients. *Pediatr Transplant* 2007;**11**:394.
77. Fox B, Sollinger H, Belzer F, et al. A prospective, randomized, double-blind study of trimethoprim-sulfamethoxazole for prophylaxis of infection in renal transplantation: clinical efficacy, absorption, trimethoprim-sulfamethoxazole effects on the microflora, and the cost-benefit of prophylaxis. *Am J Med* 1990;**89**:255.
78. Frias C, Lauzurica R, Vaquero M, et al. Determination of Epstein-Barr virus in posttransplant T-cell lymphoma in a kidney transplant recipient: case report and review. *Clin Infect Dis* 2006; **30**:576.
79. Furth S, Fivush B. Varicella vaccination in pediatric kidney transplant candidates. *Pediatr Transplant* 2002;**6**:97.
80. Furth S, Hogg R, Tarver J, et al. Varicella vaccination in children with chronic renal failure: a report of the Southwest Pediatric Nephrology Study Group. *Pediatr Nephrol* 2003;**18**:33.
81. Gardner S, MacKenzie E, Smith C, et al. Prospective study of the human polyoma viruses BK and JC and cytomegalovirus in renal transplant recipients. *J Clin Pathol* 1984;**37**:578.
82. Giessling M, Muller D, Winkelman B, et al. Kidney transplantation in children and adolescents. *Transplant Proc* 2007;**39**:2197.
83. Ginevri F, Azzi A, Hirsch H, et al. Prospective monitoring of polyomavirus BK replication and impact on pre-emptive intervention in pediatric kidney transplant recipients. *Am J Transplant* 2007;**7**:2727.
84. Ginevri F, DeSantis R, Comoli P, et al. Polyomavirus BK infection in pediatric kidney-allograft recipients: a single center analysis of incidence, risk factors and novel therapeutic approaches. *Transplantation* 2003;**75**:1266.
85. Ginevri F, Losurdo G, Fontana I, et al. Acyclovir plus CMV immunoglobulin prophylaxis and early therapy with ganciclovir are effective and safe in CMV high-risk renal transplant pediatric recipients. *Transpl Int* 1998;**11**(Suppl. 1):130.
86. Girardi M, Glusac E, Imaedo S. Subcutaneous *Fusarium* foot abscess in a renal transplant patient. *Cutis* 1999;**63**:67.
87. Gomez E, Melon S, Aguado S, et al. Herpes simplex virus encephalitis in a renal transplant patient: diagnosis by polymerase chain reaction detection of HSV DNA. *Am J Kidney Dis* 1997;**30**:423.
88. Gray G, McCarthy T, Lebeck M, et al. Genotype prevalence and risk factors for severe clinical adenovirus infections, United States 2004–2006. *Clin Infect Dis* 2007;**45**:1120.
89. Green M, Michaels M. Pre-emptive therapy of CMV disease in pediatric transplant recipients. *Pediatr Infect Dis J* 2000;**19**:875.
90. Green M, Michaels M, Webber S, et al. The management of Epstein-Barr virus associated post-transplant lymphoproliferative disorders in pediatric solid-organ transplant recipients. *Pediatr Transplant* 1999;**3**:271.
91. Greenberg M, Friedman H, Cohen S, et al. A comparative study of herpes simplex infections in renal transplant and leukemia patients. *J Infect Dis* 1987;**156**:280.
92. Grundy J, Super M, Sweney P, et al. Symptomatic cytomegalovirus infection in seropositive kidney recipients: reinfection with donor virus rather than reactivation of recipient virus. *Lancet* 1988;**2**:132.
93. Gupta M, Filler G, Kovesi T, et al. Quantitative tissue polymerase chain reaction for Epstein-Barr virus in pediatric solid organ recipients. *Am J Kidney Dis* 2003;**41**:212.
94. Haddu T, Andre J, Bourguard R, et al. Long-term followup of Epstein-Barr virus viremia in pediatric recipients of renal transplants. *Pediatr Nephrol* 2005;**20**:76.
95. Hall K, Copeland J, Zuloski C, et al. Markers of coccidioidomycosis prior to cardiac or renal transplant and risk of recurrent disease. *Transplantation* 1993;**55**:1422.
96. Hanto D, Frizzera G, Gajl-Peczaiska K, et al. Epstein-Barr virus induced B-cell lymphoma after renal transplantation. *N Engl J Med* 1982;**306**:913.
97. Harbell J, Fung J, Nissen N, et al. Surgical complications in 275 HIV-infected liver and/or kidney transplant recipients. *Surgery* 2012;**152**(3):376–81.
98. Hardy A, Wajszczuk C, Suffredini F, et al. *Pneumocystis carinii* pneumonia in renal transplant recipients treated with cyclosporine and steroids. *J Infect Dis* 1984;**149**:143.
99. Harman W. Opportunistic infection in children following renal transplantation. *J Pediatr Nephrol* 1991;**5**:118.

100. Hayes K, VanSickels N, Buell J, et al. Successful transplantation of HIV patients: the Louisiana experience. *J La State Med Soc* 2012;**164**(4):191–3.
101. Haysom L, Rosenberg A, Kainer G, et al. BK virus infection in an Australian pediatric renal transplant population. *Pediatr Transplant* 2004;**8**:480.
102. Herbein G, Strasswimmer J, Alteiri M, et al. Longitudinal study of human herpesvirus 6 infection in organ transplant recipients. *Clin Infect Dis* 1996;**22**:171.
103. Herman J, van Ranst M, Snoeck R, et al. Polyomavirus infection in pediatric renal transplant recipients: evaluation using a quantitative real-time PCR technique. *Pediatr Transplant* 2004;**8**:485.
104. Hibberd P, Tolkoff-Rubin N, Conti D, et al. Pre-emptive ganciclovir therapy to prevent cytomegalovirus disease in cytomegalovirus antibody-positive renal transplant recipients: a randomized controlled trial. *Ann Intern Med* 1995;**123**:18.
105. Hirsch H. Polyomavirus BK nephropathy: A (re-) emerging complication in renal transplantation. *Am J Transplant* 2002;**2**:25.
106. Ho M, Jaffe R, Miller G, et al. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation* 1988;**45**:719.
107. Hovens M, Vaessen N, Sijpkens Y, et al. Unusual presentation of central nervous system manifestations of varicella-zoster virus vasculopathy in renal transplant recipients. *Transpl Infect Dis* 2007;**9**:237.
108. Howard D, Smith S, Butterly M, et al. Diagnosis and management of BK polyomavirus interstitial nephritis in renal transplant recipients. *Transplantation* 1999;**68**:1279.
109. Howard R, Simmons R, Najarian J. Fungal infections in renal transplant recipients. *Ann Surg* 1978;**188**:598.
110. Huang J, Huang C, Lai M, et al. *Salmonella* infection in renal transplant recipients. *Transplant Proc* 1994;**26**:2147.
111. Humar A, Gillingham K, Payne W, et al. Association between cytomegalovirus disease and chronic rejection in kidney transplant recipients. *Transplantation* 1999;**68**:1879.
112. Inojosa WO, Scotton PG, Fuser R, et al. West Nile virus transmission through organ transplantation in northeastern Italy: a case report and implications for pre-procurement screening. *Infection* 2012;**40**(5):557–62.
113. Ison MG, Szakaly P, Shapira MY, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**(6):955–64.
114. Iwamoto M, Jernigan D, Guasch A, et al. Transmission of West Nile virus from an organ to four transplant recipients. *N Engl J Med* 2003;**348**:2196.
115. Jamil B, Nicholls K, Becker G, et al. Influence of anti-rejection therapy on the timing of cytomegalovirus disease and other infections in renal transplant recipients. *Clin Transplant* 2000;**114**:14.
116. Jensen S, Jackson E, Riley L, et al. Tacrolimus-based immunosuppression with steroid withdrawal in pediatric kidney transplantation—4 year experience at a moderate volume center. *Pediatr Transplant* 2003;**7**:11.
117. Jie T, Matas A, Gillingham K, et al. Mycobacterial infection after kidney transplant. *Transplant Proc* 2005;**37**:937–9.
118. John U, Kemper M. Urinary tract infections in children after renal transplantation. *Pediatr Nephrol* 2008;**23**:16.
119. Kalman S, Bakkaloglu S, Ozkaya O, et al. Measles: a rare communicable disease in a child with renal transplantation. *Pediatr Transplant* 2002;**6**:432.
120. Kalman S, Kurekci A, Gak F, et al. *Mycoplasma pneumoniae* infection in a child after renal transplant. *Pediatr Transplant* 2004; **18**:493.
121. Karakayali H, Emiroglu R, Arslan G, et al. Major infectious complications after kidney transplantation. *Transplant Proc* 2001; **33**:1816.
122. Keswani M, Moudgli A. Adenovirus-associated hemorrhagic cystitis in a pediatric renal transplant recipient. *Pediatr Transplant* 2007;**11**:568.
123. Kidd I, Clark D, Sabin C, et al. Prospective study of human betaherpesviruses following renal transplantation: association with HHV-7 and CMV co-infection with CMV disease and increased rejection. *Transplantation* 2000;**69**:2400.
124. Kim SH, Lee SO, Park JB, et al. A prospective longitudinal study evaluating the usefulness of a T-cell based assay for latent tuberculosis infection in renal transplant recipients. *Am J Transplant* 2011;**11**(9):1927–35.
125. Kitai I, King S, Gafni A, et al. An economic evaluation of varicella vaccine for pediatric liver and kidney transplant recipients. *Clin Infect Dis* 1993;**17**:441.
126. Kleinschmidt-Demasters B, Marder B, Levi M, et al. Naturally acquired West Nile virus encephalomyelitis in transplant recipients: clinical, laboratory, diagnostic and neuropathologic features. *Arch Neurol* 2004;**61**:1210.
127. Klemperer J, Wang J, Hartman B, Stubenbord W. *Mycobacterium tuberculosis* infection of a native polycystic kidney following renal transplantation. *Transplantation* 1998;**66**:118.
128. Knirsch C, Jakob K, Schoonmaker D, et al. An outbreak of *Legionella micdadei* pneumonia in transplant patients: evaluation, molecular epidemiology and control. *Am J Med* 2000;**108**:290.
129. Krieger J, Brem A, Kaplan M. Urinary tract infection in pediatric renal transplantation. *Urology* 1980;**15**:362.
130. Kumar P, Pearson J, Martin D, et al. Transmission of human immunodeficiency virus by transplantation of a renal allograft with development of the acquired immunodeficiency syndrome. *Ann Intern Med* 1987;**106**:244.
131. Laurenz M, Winkelman B, Roigas J, et al. Severe parvovirus B 19 encephalopathy after renal transplantation. *Pediatr Transplant* 2006;**10**:978.
132. Lee P, Hung C, Lei H, et al. Parvovirus B 19-related acute hepatitis in an immunosuppressed kidney transplant. *Nephrol Dial Transplant* 2000;**15**:1486.
133. Leiguarda R, Roncoroni A, Taratuto L, et al. Acute central nervous system infection by *Trypanosoma cruzi* (Chagas disease) in immunosuppressed patients. *Neurology* 1990;**40**:830.
134. Lewis R, Johnson P, Golden D, et al. The adverse impact of cytomegalovirus infection on clinical outcome in cyclosporine-prednisone treated renal allograft recipients. *Transplantation* 1988; **45**:353.
135. Lichenstein I, MacGregor R. Mycobacterial infections in renal transplant recipients: report of five cases and review of the literature. *Rev Infect Dis* 1983;**5**:216.
136. Linden P, Williams P, Chann K. Efficacy and safety of amphotericin B lipid complex (ABLC) in solid-organ transplant recipients with invasive fungal infections. *Clin Transplant* 2000;**14**:329.
137. Lowance D, Neumayer H-H, Legendre C, et al. Valacyclovir for the prevention of cytomegalovirus disease after renal transplantation. *N Engl J Med* 1999;**340**:1462.
138. Malhorta K, Dash S, Dhawan I, et al. Tuberculosis and renal transplantation: observations from an endemic area. *Postgrad Med J* 1986;**62**:359.
139. Marchand S, Tchernia G, Hiesse C, et al. Human parvovirus B19 infection in organ transplant recipients. *Clin Transplant* 1999;**13**:17.
140. Marinelli K, Bagnarelli P, Gaffi G, et al. PCR real time assays for the early detection of BK virus-DNA in immune compromised patients. *New Microbiol* 2007;**30**:275.
141. Masutani K, Shapiro R, Basu A, et al. The Banff 2009 working proposal for polyomavirus nephropathy: a critical evaluation of its utility as a determinant of clinical outcome. *Am J Transplant* 2012;**12**(4):907–18.
142. Marsh H, Bowler I, Watson C. Successful treatment of *Rhodococcus equi* pulmonary infection in a renal transplant recipient. *Ann R Coll Surg Engl* 2000;**82**:107.
143. Mas V, Alvarellos T, Albano S, et al. Utility of cytomegalovirus viral load in renal transplant patients in Argentina. *Transplantation* 2000;**67**:1050.
144. Mason J, Ordelheide K, Grames G, et al. Toxoplasmosis in two renal transplant recipients from a single donor. *Transplantation* 1987;**44**:588.
145. Mathias R. Chronic anemia as a complication of parvovirus B 19 infection in a pediatric kidney transplant recipient. *Pediatr Nephrol* 1997;**11**:355.
146. Mayo R, Lipschultz D. An interesting case of failed renal transplant complicated by a lymphocoele infected with *Pasteurella multocida* and a review of the literature. *Am J Nephrol* 1996; **16**:361.
147. McCormick W, Schochet S, Sailes H, et al. Progressive multifocal leukoencephalopathy in renal transplant recipients. *Arch Intern Med* 1976;**136**:829.

148. McDiarmid S, Jordan S, Geoffrey S, et al. Prevention and preemptive therapy of post-transplant lymphoproliferative disease following solid organ transplantation. *Transplantation* 1998;**68**: 1604–11.
149. Melgosa-Hinojosa M, Garcia-Mesegher C, Pena-Garcia F, et al. Pre-emptive treatment with oral ganciclovir for pediatric renal transplantation. *Clin Nephrol* 2004;**61**:246.
150. Mencarelli F, Marks SD. Non-viral infections in children after renal transplantation. *Pediatr Nephrol* 2012;**27**:1465–76.
151. Mian A, Farney A, Mendley S. *Mycoplasma hominis* septic arthritis in a pediatric renal transplant recipient: case report and review of the literature. *Am J Transplant* 2005;**5**:103.
152. Michaels M, Green M, Wald E. Adenovirus infection in pediatric orthotopic liver transplant recipients. *J Infect Dis* 1992;**165**:170.
153. Miemois-Foley J, Paunio M, Lyytikainen O. Bacteremia among kidney transplant recipients: a case-control study of risk factors and short-term outcome. *Scand J Infect Dis* 2000;**32**:69.
154. Mir S, Erdogan H, Serdaroglu E, et al. Pediatric renal transplantation: single center experience. *Pediatr Transplant* 2005;**9**:56.
155. Mocelin A, Brandina P, Gordan J, et al. Immunosuppression and circulating *Trypanosoma cruzi* in a kidney transplant recipient. *Transplantation* 1977;**23**:163.
156. Molyneaux P, Parker S, Khan I, et al. Use of genomic analysis of varicella zoster virus to investigate suspected varicella-zoster transmission within a renal unit. *J Clin Virol* 2006;**36**:76.
157. Morris P. Transplantation—a medical miracle of the 20th century. *N Engl J Med* 2004;**23**:2678.
158. Moudgil A, Puliyanda D. Induction therapy in pediatric renal transplant recipients: an overview. *Paediatr Drugs* 2007;**9**:323.
159. Moudgil A, Snidban H, Nast C, et al. Parvovirus B19 infection-related complications in renal transplant recipients: treatment with intravenous immune globulin. *Transplantation* 1997;**64**:1847.
160. Mourad G, Soulillou J, Chong G, et al. Transmission of *Mycobacterium tuberculosis* with renal allografts. *Nephron* 1985;**41**:82.
161. Mrowka C, Heintz B, Reul J, et al. Cerebral tuberculoma 11 years after renal transplantation. *Am J Nephrol* 1998;**18**:557.
162. Muller A, Beck B, Theilemann K, et al. Detection of polyomaviruses BK and JC in children with kidney disease and renal transplant recipients. *Pediatr Infect Dis J* 2005;**24**:778.
163. Munoz P. Management of urinary tract infections and lymphocele in renal transplant recipients. *Clin Infect Dis* 2001;**33**(Suppl. 1):53.
164. Murray B, Subramaniam J. Late cytomegalovirus infection after oral ganciclovir prophylaxis in renal transplant recipients. *Transpl Infect Dis* 2004;**6**:3.
165. Muzuecos A, Rodriguez Benot A, Moreno A, et al. Renal replacement treatment in patients with HIV infection in a European region: outcomes following renal transplantation. *Transplant Proc* 2012;**44**(7):2053–6.
166. Mylonakis E, Hohmann E, Calderwood S. Central nervous system infection with *Listeria monocytogenes:* 33 years experience at a general hospital and review of 776 episodes from the literature. *Medicine (Baltimore)* 1998;**77**:313.
167. Nahas T, Antonopoulos I. Comparison of renal transplant outcomes in children with or without bladder dysfunction: a customized approach equals the difference. *J Urol* 2008;**179**:712.
168. Nett RJ, Kuehnert MJ, Ison MG, et al. Current practices and evaluation of screening solid organ donors for West Nile virus. *Transpl Infect Dis* 2012;**14**(3):268–77.
169. Neuhaus T, Schwobel M, Schlumpf R, et al. Pyelonephritis and vesiculoureteral reflux after renal transplant in young children. *J Virol* 1997;**157**:1400.
170. Nichols J, Becker G, Walker R. Influence of anti-rejection therapy on the timing of cytomegalovirus disease and other infections in renal transplant recipients. *Clin Transplant* 2000;**14**:14.
171. Nickeleit V, Hirsch H, Binet I, et al. Polyomavirus infection of renal allograft recipients from latent infection to manifestations of disease. *J Am Soc Nephrol* 1999;**10**:1080.
172. Nickeleit V, Hirsch H, Zeiler M, et al. BK-virus nephropathy in renal transplant-tubular necrosis, MHC-class II expression, and rejection in a puzzling game. *Nephrol Dial Transplant* 2000;**15**:324.
173. Nickeleit V, Klimkait T, Binet I, et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. *N Engl J Med* 2000;**342**:1309.
174. Offermann G, Keller F, Molzahn M. Low cyclosporin A blood levels and acute graft rejection in a renal transplant recipient during rifampin treatment. *Am J Nephrol* 1985;**5**:385.
175. Ogunbiyi O, Scholefield J, Raftery A, et al. Prevalence of anal human papillomavirus infection and intraepithelial neoplasia in renal allograft recipients. *Br J Surg* 1994;**81**:365.
176. Ohori N, Michaels M, Jaffe R, et al. Adenovirus pneumonia in lung transplant recipients. *Hum Pathol* 1995;**26**:1073.
177. Olie K, Goodshop T, Verlaak R, et al. Posttransplantation CMV-induced recurrence of atypical hemolytic uremic syndrome associated with a factor H mutation: successful treatment with intensive plasma exchanges and ganciclovir. *Am J Kidney Dis* 2005;**45**:e12.
178. Olson A, Shope T, Flynn J. Pre-transplant varicella vaccination is cost-effective in pediatric renal transplantation. *Pediatr Transplant* 2001;**5**:44.
179. Ortega-Sanchez IR. Economics of an adolescent meningococcal conjugate vaccination catch-up campaign in the United States. *Clin Infect Dis* 2008;**46**:1–13.
180. Osman H, Peiris J, Taylor C, et al. Cytomegalovirus disease in renal allograft recipients: is human herpesvirus 7 a co-factor for disease progression? *J Med Virol* 1996;**48**:295–301.
181. Pahari A, Rees L. BK virus-associated renal problems. *Pediatr Nephrol* 2003;**18**:643.
182. Pamidi S, Friedman K, Kampalath B, et al. Human parvovirus B 19 infection presenting as persistent anemia in renal transplant recipients. *Transplantation* 2000;**69**:2666.
183. Pappo O, Demetris A, Raikow R, et al. Human polyoma virus infection of renal allografts: histopathologic diagnosis, clinical significance, and literature review. *Mod Pathol* 1996;**9**:105.
184. Patel K, Heman M, Williams P, et al. Long-term effectiveness of highly active antiretroviral therapy on the survival of children and adolescents with HIV infection: a 10-year perspective. *Clin Infect Dis* 2008;**46**:1751.
185. Patel R. Infections in recipients of kidney transplants. *Infect Dis Clin North Am* 2001;**15**:1.
186. Patel R, Paya C. Infections in solid-organ transplant recipients. *Clin Microbiol Rev* 1997;**10**:86.
187. Patel R, Roberts G, Keating M, et al. Infections due to nontuberculous mycobacteria in kidney, heart and liver transplant recipients. *Clin Infect Dis* 1994;**19**:263.
188. Patel R, Smith T, Espy M, et al. Detection of cytomegalovirus DNA in sera of liver transplant recipients. *J Clin Microbiol* 1994; **32**:1431.
189. Pellegrin I, Garrigue I, Ekouevi E. New molecular assays to predict occurrence of CMV disease in renal transplant recipients. *J Infect Dis* 2000;**182**:36.
190. Pereira B, Natov S, Bouthot B, et al. Effects of hepatitis C infection and renal transplantation on survival in end-stage renal disease. The New England Organ Bank Hepatitis C Study Group. *Kidney Int* 1998;**53**:1374.
191. Peterson P, Balfour H, Fryd D, et al. Fever in renal transplant recipients: causes, prognostic significance and changing patterns at the University of Minnesota Hospital. *Am J Med* 1981;**71**:345.
192. Pirsch J. Cytomegalovirus infection and posttransplant lymphoproliferative disease in renal transplant recipients: results of the U.S. multicenter FK506 Kidney Transplant Study Group. *Transplantation* 1999;**68**:1203.
193. Priftakis P, Bogdanovic G, Tyden G, et al. Polyomavirury in renal transplant patients is not correlated to the cold ischemia period or to rejection episodes. *J Clin Microbiol* 2000;**38**:406.
194. Puliyanda D, Toyoda M, Traum A, et al. Outcome of management strategies for BK virus replication in pediatric renal transplant recipients. *Pediatr Transplant* 2008;**12**:180.
195. Quinibi W, Al-Sibai M, Taher S, et al. Mycobacterial infection after renal transplant: report of 14 cases and review of the literature. *Q J Med* 1991;**77**:1039.
196. Randhawa P, Finkelstein S, Scantlebury V, et al. Human polyomavirus–associated interstitial nephritis in the allograft kidney. *Transplantation* 1999;**67**:103.
197. Ravindra K, Freifeld A, Kalil A, et al. West Nile–associated encephalitis in recipients of renal and pancreas transplants: case series and literature review. *Clin Infect Dis* 2004;**38**:1257.
198. Regamey N, Tamm M, Wernli M, et al. Transmission of human herpesvirus 8 infection from renal-transplant donors to recipients. *N Engl J Med* 1998;**339**:1358.
199. Reznick M, Halleux J, Urbain E, et al. Two cases of progressive multifocal leukoencephalopathy after renal transplantation. *Acta Neuropathol Suppl* 1981;**7**:189.

200. Ribot S, Eslami H. HIV infection in kidney transplant recipients. *N Engl J Med* 1992;**39**:597.
201. Riddler S, Brenig M, McKnight J. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of post transplant lymphoproliferative disease in solid-organ transplant recipients. *Blood* 1994;**3**:972.
202. Rizvi S, Naqvi S, Hussain Z, et al. Living-related pediatric renal transplantation: a single-center experience from a developing country. *Pediatr Transplant* 2002;**6**:101.
203. Robinson L, Hilinski J, Graham F, et al. Predictors of cytomegalovirus disease among pediatric transplant recipients within one year of renal transplantation. *Pediatr Transplant* 2002;**6**:111.
204. Rogerson TE, Chen S, Kok J, et al. Tests for latent tuberculosis in people with end stage renal disease: a systematic review. *Am J Kidney Dis* 2013;**61**(1):33–43.
205. Rubin R. Infectious disease complications of renal transplantation. *Kidney Int* 1993;**44**:221.
206. Rubin R. Infection in the organ transplant recipient. In: Rubin RH, Young LS, editors. *Clinical approach to infection in the compromised host*. 3rd ed. New York: Plenum Medical; 1994. p. 629–705.
207. Rubin R, Wolfson J, Cosimi A, Tolkoff-Rubin N. Infection in the renal transplant recipient. *Am J Med* 1981;**70**:405.
208. Saitoh K, Sugae N, Koike N, et al. Diagnosis of childhood BK virus cystitis by electron microscopy and PCR. *J Clin Pathol* 1993;**46**:773.
209. Sakhuja V, Jha V, Varma P, et al. The high incidence of tuberculosis among renal transplant recipients in India. *Transplantation* 1996;**61**:211.
210. Sayiner A, Ece T, Duman S, et al. Tuberculosis in renal transplant recipients. *Transplantation* 1999;**68**:1268.
211. Schmaldienst S, Horl W. Bacterial infection after renal transplantation. *Nephron* 1997;**75**:140.
212. Scroggs M, Wolfe J, Bollinger R, et al. Causes of death in renal transplant recipients. *Arch Pathol Lab Med* 1987;**111**:983.
213. Sebire N, Malone M, Risdon R, et al. Epstein-Barr virus–associated lymphoproliferative disease presenting as gastrointestinal lesions in childhood. *Pediatr Dev Pathol* 2005;**8**:88.
214. Shaariah W, Morad Z, Suleiman A. Cryptococcosis in renal transplant recipients. *Transplant Proc* 1992;**24**:1898.
215. Sheldon J, Henry S, Mourad M, et al. Human herpes virus 8 infection in kidney transplant patients in Belgium. *Nephrol Dial Transplant* 2000;**15**:1443.
216. Shen G, Alfrey E, Knoppel C, et al. Eradication of cytomegalovirus reactivation disease using high-dose acyclovir and targeted intravenous ganciclovir in kidney and kidney/pancreas transplantation. *Transplantation* 1997;**64**:931.
217. Shorter G, Weil R. *Listeria monocytogenes* infection after renal transplantation. *Arch Intern Med* 1977;**137**:1395.
218. Shroft R, Ross L. The post-transplantation lymphoproliferative disorders: a literature review. *Pediatr Nephrol* 2004;**19**:369.
219. Singh N. Invasive mycoses in organ transplant recipients: controversies in prophylaxis and management. *J Antimicrob Agents Chemother* 2000;**45**:749.
220. Singh N, Gayowski T, Singh T, et al. Invasive gastrointestinal zygomycosis in a liver transplant recipient: case report and review of zygomycosis in solid-organ transplant recipients. *Clin Infect Dis* 1995;**20**:617.
221. Smith S, Butterly D, Alexander B, et al. Viral infections after renal transplantation. *Am J Kidney Dis* 2001;**37**:659.
222. Snydman D. Epidemiology of infections after solid-organ transplantation. *Clin Infect Dis* 2001;**33**(Suppl. 1):5.
223. Snydman D, Werner B, Heinze-Lacey B, et al. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. *N Engl J Med* 1987;**317**:1049.
224. Souweine B, Mathevon T, Bret L, et al. Successful treatment of infection due to *Mycoplasma hominis* with streptogramins in a renal transplant patient: case report and review. *Clin Infect Dis* 1998; **26**:1233.
225. Speirs G, Hakim M, Wreghitt T. Relative risk of donor-transmitted *Toxoplasma gondii* infection in heart, liver, and kidney transplant recipients. *Clin Transplant* 1988;**2**:257.
226. Stovall S, Ainley K, Mason E, et al. Invasive pneumococcal infection in pediatric cardiology transplant patients. *Pediatr Infect Dis J* 2001;**20**:946.
227. Sturm I, Watschinger B, Geissler K, et al. Chronic parvovirus B 19 infection-associated pure red cell anemia in a kidney transplant recipient. *Nephrol Dial Transplant* 1996;**11**:1367.
228. Sugar A, Restrepo A, Stevens D. Paracoccidioidomycosis in the immune suppressed host: report of a case and review of the literature. *Am Rev Respir Dis* 1984;**129**:340.
229. Suzuki T, Karzum I, Okubo Y, et al. Epstein-Barr virus DNA load and seroconversion in pediatric renal transplantation with tacrolimus immunosuppression. *Pediatr Transplant* 2002;**11**:749.
230. Szende B, Toth A, Perner F, et al. Clinicopathologic aspects of 8 Kaposi's sarcomas among 1009 renal transplant patients. *Gen Diagn Pathol* 1997;**143**:209.
231. Taghizadeh A, Desai D, Lederman S, et al. Renal transplantation or bladder augmentation first? A comparison of complications and outcomes in children. *B J U Int* 2007;**100**:1365.
232. Their M, Holmberg C, Lautenschlager I, et al. Infections in pediatric kidney and liver patients after perioperative hospitalization. *Transplantation* 2000;**69**:1617.
233. Tolkoff-Rubin N, Rubin R. Opportunistic fungal and bacterial infections in the renal transplant recipient. *J Am Soc Nephrol* 1992;**2**(Suppl. 12):264.
234. Tong C, Bakran A, Williams H, et al. Association of human herpesvirus 7 with cytomegalovirus disease in renal transplant recipients. *Transplantation* 2000;**70**:213.
235. Tong C, Cuevas L, Williams H, et al. Use of laboratory assays to predict cytomegalovirus disease in renal transplant recipients. *J Clin Microbiol* 1998;**36**:2681.
236. Toyoda M, Moudgil A, Warady B, et al. Clinical significance of peripheral blood Epstein-Barr viral load monitoring using PCR in renal transplant recipients. *Pediatr Transplant* 2008; **12**:778.
237. Toyoda M, Puliyanda D, Amat N, et al. Co-infection of polyomavirus BK and cytomegalovirus (CMV) in renal transplant recipients. *Transplantation* 2005;**80**:198.
238. Tran L, Hebert D, Dipchand A, et al. Invasive pneumococcal disease in pediatric solid organ transplant recipients: a high risk population. *Pediatr Transplant* 2005;**9**:183.
239. Travis L, Kalia A. Renal transplantation in children: experience of 23 years at the Children's Renal Center of the University of Texas Medical Branch at Galveston. *J Tex Med* 1991;**87**:50.
240. Tsanaclis A, deMorais C. Cerebral toxoplasmosis after renal transplantation: case report. *Pathol Res Proc* 1986;**181**:339.
241. Umekawa T, Kurita T. Acute glomerulonephritis by adenovirus type 11 with and without type 37 after kidney transplantation. *Urol Int* 1996;**56**:114.
242. Vanchiere J, Nicome R, Greer J, et al. Frequent detection of polyomaviruses in stool samples from hospitalized children. *J Infect Dis* 2005;**192**:658.
243. Vanchiere J, White Z, Butel J. Detection of BK virus and Simian virus 40 in the urine of healthy children. *J Med Virol* 2005; **75**:447.
244. Varga M, Remport A, Hidvegi M, et al. Comparing CMV prophylaxis in renal transplantation: single center experience. *Transpl Infect Dis* 2005;**7**:63.
245. Wadie H, Alangaden G, Sillix D, et al. West Nile virus encephalitis: an emerging disease in renal transplant recipients. *Clin Transplant* 2004;**18**:753.
246. Waldman M, Kopp J. Parvovirus B-19–associated complications in renal transplant recipients. *Nat Clin Pract Nephrol* 2007;**3**:540.
247. Watkins S, Alexander S, Brewer E, et al. Response to recombinant hepatitis B vaccine in children and adolescents with chronic renal failure. *Am J Kidney Dis* 2002;**40**:365.
248. Waugh S, Pillay D, Carrington D, et al. Antiviral prophylaxis and treatment (excluding HIV therapy). *J Clin Microbiol* 2002; **25**:241.
249. Weiland D, Ferguson R, Peterson P, et al. Aspergillosis in 25 renal transplant patients. *Ann Surg* 1983;**198**:622.
250. West M, Pirenne J, Chavers B, et al. *Clostridium difficile* colitis after kidney and kidney-pancreas transplantation. *Clin Transplant* 1999;**13**:318.
251. Wilson J, Turner H, Kirchner K, et al. Nocardial infections in renal transplant recipients. *Medicine (Baltimore)* 1989;**68**:38.
252. Wojciechowski D, Chanda R, Chandran S, et al. Ciprofloxacin prophylaxis in kidney transplant recipients reduces BK virus infection at 3 months but not at 1 year. *Transplantation* 2012;**94**(11): 1117–23.

253. Wong T, Chan P, Leung C, et al. Parvovirus B19 infection causing red cell aplasia in renal transplantation on tacrolimus. *Am J Kidney Dis* 1999;**34**:1132.
254. Yagisawa T, Takahashi K, Yamaguchi Y, et al. Adenovirus induced nephropathy in kidney transplant recipients. *Transplant Proc* 1989;**21**:2097.
255. Yagisawa T, Toma H, Tanabe K, et al. Long-term outcome of renal transplantation in hepatitis B surface antigen-positive patients in cyclosporine era. *Am J Nephrol* 1997;**17**:440.
256. Yildiz A, Sever M, Turkman A, et al. Tuberculosis after renal transplant: experience of one Turkish center. *Nephrol Dial Transplant* 1998;**13**:1872.
257. Zamora I, Simon J, DaSilva M, et al. Attenuated varicella vaccine in children with renal transplants. *Pediatr Nephrol* 1994;**8**:190.
258. Zaontz M, Hatch D, Firlit C. Urological complications in pediatric renal transplantation: management and prevention. *J Urol* 1988;**140**:1123.

TABLE 74-1 **Partial List of Implantable Devices Prone to Infection**

Central nervous system shunts
Intracranial pressure monitors
Intrathecal pump infusion
Orthopedic prostheses (artificial hip, knee, and other joints; screws, pins, plates, and rods)
Intracardiac pacemakers and defibrillator
Left ventricular assist devices
Extracorporeal membrane oxygenation circuits
Ocular prostheses (artificial globes, intraocular lenses, ocular explants)
Cochlear implant devices
Tissue expander devices

3 million or more people in the United States currently have some type of long-term biomedical implant.[105,402,478] Table 74-1 lists implantable prosthetic devices discussed in this chapter.

One of the major medical complications associated with the use of implantable prosthetic devices is infection, which may result in serious tissue destruction and dysfunction of the prosthetic device or in local and systemic consequences that may be life-threatening. In most cases, these infections are very difficult to cure with antimicrobial agents alone, and removal of the device usually is required for resolution of the infection.

INTERACTION OF THE HOST WITH A PROSTHETIC DEVICE

The prosthetic devices currently in use are composed of a variety of biomaterials, including cobalt-chromium-molybdenum alloy, titanium alloy, and complex polymers such as polytetrafluoroethylene, silicone, and polyethylene, which in general are chosen for their inert, nonreactive, and nontoxic qualities. The interplay of both implant and host factors determines the risk for acquiring and the severity of infection. The human body has numerous well-developed defense mechanisms to protect it against possible invasion by various microorganisms. Such mechanisms include cellular and humoral immune systems, anatomic barriers, and an elaborate network of cells that phagocytize and destroy invading organisms. The presence of a foreign body may compromise one or more of these defenses and elicit a complex acute or chronic inflammatory response (or both) from the host. Many of these devices breach cutaneous and mucosal barriers, thereby creating a direct route by which bacteria and fungi may invade. In addition, implanted devices may alter the local immunity of the host directly or indirectly.[114,402,478,504]

Shortly after being implanted, hydrophobic polymeric materials such as polyethylene, Dacron, polydimethylsiloxane, and polyether urethanes become coated with a layer of host proteins such as plasma and interstitial fluid proteins (i.e., fibronectin, albumin, laminin, collagen, immunoglobulin G, fibrinogen) that bind to and are absorbed readily into the surface of the implant.[32,363,479] This protein layer (especially fibrinogen) has a major influence on the body's response to and the biocompatibility of the implant. The presence of fibrinogen attracts a large number of phagocytic cells (i.e., neutrophils, monocytes, and macrophages) to the implant; these cells interact with the implant surface and initiate an acute inflammatory response.[114,478] Some of these host proteins also serve as a receptor for various colonizing microorganisms. Collagen, laminin, and fibrinogen have been reported to play a role in adherence of bacteria,[509] and fibronectin has been found to be the major receptor for gram-positive cocci, especially *Staphylococcus aureus*.[392,487]

Chronic inflammatory responses, also known as *foreign body reactions*, are seen around many types of biomaterial implants and arise from interactions among the protein-coated surfaces of the implant and host tissues and adhering phagocytic cells such as macrophages and foreign body giant cells. These interactions may result in degradation and damage to the implant from the continuous generation of toxic catabolites and release of inflammatory mediators such as hydrolases, activated complement components, tumor necrosis factor (TNF), interleukins, prostaglandins, coagulation factors, and plasminogen activator by the phagocytic cells.[474,478,504]

INTERACTION OF MICROORGANISMS WITH A PROSTHETIC DEVICE

Once a prosthetic device is implanted, the surface of the device provides a potential area for adherence and multiplication of bacteria. Adherence is a complex process that involves electrostatic attachment of the bacteria to the surface of the implant, bacterial mechanisms that function specifically in attachment, and host-derived substances that coat the prosthetic device and serve as receptors for various bacteria. Bacteria arrive at the surface of the implant by many different routes; they may be inoculated at the time of implantation, the patient may have episodes of transient bacteremia, the device may be exposed by local trauma or infection, or the device may be implanted within an area in which the organism is part of the normal flora.[105,114]

Adherence of bacteria to the surface of an implant is influenced by numerous different factors, including the material used to make the device, the source of the device material (adherence is greater with synthetic material than with biomaterial), the surface of the device (i.e., irregular more than regular, textured more than smooth, hydrophobic more than hydrophilic), and the shape of the device.[105] Cell surface molecules or structures known as *adhesins* also play a role in adherence by attaching or binding an organism to specific receptors on implant surfaces; different bacteria use different adhesins to attach to and colonize medical implants. For example, *Staphylococcus epidermidis* uses proteinaceous autolysin and capsular polysaccharide intercellular adhesin for initial adherence to the implant surface and for adherence of bacteria to each other. These bacteria also produce a biofilm that increases cell-to-cell association and allows accumulation of bacteria.[409] *Streptococcus pyogenes* uses lipoteichoic acid as its adhesin, whereas *S. aureus* uses

both lipoteichoic acid and host-tissue ligands (e.g., fibronectin, fibrinogen, and collagen) for adherence. Binding of *S. aureus* to host-tissue ligands is mediated by genetically defined microbial surface proteins known as *microbial surface components recognizing adhesive matrix molecules* (MSCRAMM).[106] *Escherichia coli* and other bacteria use fimbriae as an adhesin to mediate binding to receptors on the surfaces of target cells.

Bacteria also can protect themselves from host defenses by synthesizing and excreting numerous complex polysaccharides, known as *glycocalyces*, that function either as part of the bacterial capsule or as the slime layer. This slime layer is known to play a major role in keeping an organism attached to an implant surface by coalescing with the polysaccharides of other bacteria and with host products to produce a thick, adherent, and somewhat impenetrable biofilm.[505] The biofilm functions by trapping nutrients and protecting the organism from phagocytosis, antimicrobial agents, and competing microflora. It also plays a role in inhibiting the response to chemotactic stimuli, increases both *N*-formyl-methionyl-leucyl-phenylalanine (FMLP)-induced superoxide generation and release of specific granules, and impairs natural killer cell function while also altering the composition of T-lymphocyte cell subpopulations.[170,172,227,505] Therefore, the biofilm aids bacteria in evading host cellular and humoral defense mechanisms and thereby allows the organism to colonize and infect an implanted device effectively.

Substantial progress has been made in our understanding of the pathogenesis, prevention, and treatment of foreign body infections; this increased knowledge has resulted in a dramatic decrease in the morbidity associated with these infections, as well as subsequent improvement in the patient's quality of life. The following sections discuss specific device-related infections and the suggested treatment and management of these infections.

TISSUE EXPANDERS

The use of soft tissue expansion in reconstructive surgery was reported in the literature first in 1957, and since that time it has been used widely for the correction of multiple problems in plastic and reconstructive surgery in the adult and pediatric populations.[19,27] These expanders consist of an alloplastic prosthesis with a filling port that is implanted into a subcutaneous pocket. The expander is filled with saline through the filling port at various intervals to create adequate expansion of the skin. Subsequent improvements of this technique led to the development of osmotic tissue expanders. The Osmed osmotic tissue expander is made of an osmotically active hydrogel (vinyl pyrrolidone and methyl methacrylate) in a silicone construct. When implanted, water is drawn from the surrounding tissues into the device, which can spontaneously expand up to 10 times its original volume. In children, tissue expansion is used most commonly to provide coverage for skin defects caused by burns, trauma, hemangiomas, and other congenital deformities.*

*References 57, 87, 307, 351, 476, 534.

The most common complication of tissue expansion is infection of the subcutaneous expander pocket, usually with skin organisms introduced during insertion of the expander. Cellulitis of the overlying skin and hematogenous or lymphatic seeding of the expander pocket are seen as well but occur much less frequently.* In several pediatric case series, the infection rate ranged from 3.4 to 11 percent.[1,159,307] *S. aureus*, *S. epidermidis*, and group A streptococci are the microorganisms recovered most commonly from these infections.[159,215,302,307] Less commonly, nontypable *Haemophilus influenzae*,[307] *Pseudomonas aeruginosa*, *E. coli*, and *Actinomyces* spp.[327] have been isolated from infections of expander pockets.[302]

In cases of infection of subcutaneous pockets, treatment consists of removal of the tissue expander, débridement and drainage of the subcutaneous pocket, and administration of intravenous antibiotic therapy tailored to the organism isolated. For cellulitis of the overlying skin of an expander pocket, treatment with intravenous antibiotics but without removal of the tissue expander has been shown to be successful.[19]

The most common empirical antimicrobial therapeutic regimen consists of a first-generation cephalosporin or an extended-spectrum, penicillinase-resistant penicillin. For all of the infections of prosthetic devices discussed in the chapter, empirical antistaphylococcal coverage should include agents effective against community strains of methicillin-resistant *S. aureus* (MRSA) if warranted by their frequency in the area.

Therapy is tailored once the organism has been identified and its antibiotic susceptibility has been determined.

COCHLEAR IMPLANTS

During the last several decades, cochlear implantation has emerged as one of the best methods of providing auditory rehabilitation for the profoundly deaf (congenital or acquired). The goal of this surgery in young children is to provide hearing that is adequate to facilitate the development of receptive and expressive language. The surgical technique involves the creation of a C-shaped flap in the postauricular and parietal-occipital scalp skin areas, elevation of the flap, implantation of a multichannel prosthesis, and insertion of an electrode array into the cochlea through openings drilled into the temporal bone.[95,98,295]

The most common infectious complications associated with these implants are cellulitis of the overlying skin flap, meningitis, otitis media, and delayed cochlear implant infections leading to extrusion of the implant.† Rates of infection range from 0.3 to 0.5 percent for meningitis, 2 to 12 percent for cellulitis of the skin flap and delayed cochlear implant infections, and to 36 percent for otitis media. The reported cases of meningitis have occurred in association with leakage of cerebrospinal fluid (CSF) in persons with a malformed cochlea who

*References 19, 149, 159, 215, 300, 307, 343.
†References 45, 96, 97, 102, 108, 162, 183, 189, 198, 199, 209, 218, 220, 288, 323, 344, 361, 527.

undergo cochlear implantation, as a consequence of intracranial spread of a developing middle ear infection along the electrode pathway, or via pneumococcal bacteremia with hematogenous seeding of the cochlea—for example, at a site of tissue necrosis related to the electrode or positioner with contiguous spread to the CSF and meninges. In June 2002, the U.S. Food and Drug Administration received numerous reports of bacterial meningitis in children with cochlear implants who were younger than 6 years old when they received the implants. The most common causative organism identified was *Streptococcus pneumoniae*, followed by nontypable and type b *H. influenzae*. Other less common organisms that have been reported include *S. pyogenes*, *Acinetobacter baumannii*, *Escherichia coli*, and *Enterococcus* spp.[401] The incidence of pneumococcal meningitis in this group of patients was calculated to be 138.2 cases/100,000 person-years—more than 30 times the incidence in the same-aged cohort in the general population. This increased incidence of meningitis was found to be associated strongly with the use of a cochlear implant with a positioner (a wedge-shaped insert that facilitates transmission of the electrical signal by pushing the electrode against the medial wall of the cochlea) in conjunction with the presence of radiographic evidence of a malformation of the inner ear and leakage of CSF. Cochlear implants with a positioner were voluntarily recalled in the United States in July 2002, although removal of existing implants containing a positioner was not recommended; use of appropriate vaccination against *S. pneumoniae* and type b *H. influenzae* was strongly recommended.[26,48,168,401] On February 24, 2010, a 13-valent pneumococcal conjugate vaccine (PCV13) was licensed by the U.S. Food and Drug Administration. This vaccine contains polysaccharides of the seven serotypes in PCV7 and polysaccharides from 6 additional serotypes. This vaccine replaces PCV7 for all scheduled doses of PCV7 in infants. A supplemental dose of PCV13 is recommended for children 14 months through 18 years of age with a cochlear implant.[2] Children with cochlear implants may have a higher risk for middle ear infections because of several factors, including the naturally high incidence of acute otitis media in this population, the presence of a foreign body in the area of the infection, and the potential for spread of the infection into the cochlea along the electrode pathway. In a study of 50 children who received cochlear implants between 1991 and 1995, researchers found that children prone to the development of otitis media before undergoing implantation were at higher risk for developing postimplantation acute otitis media but responded well to routine oral antimicrobial therapy. The overall prevalence and the severity of acute otitis media were not found to be increased in children with cochlear implants.[292]

In cases of cellulitis of the skin flap, intravenous antimicrobial therapy should include empirical broad-spectrum antimicrobial agents effective against community strains of MRSA, methicillin-susceptible strains of *S. aureus*, and group A streptococci. Empirical antimicrobial therapy for meningitis usually consists of vancomycin and a third-generation cephalosporin that is tailored to the organism isolated. An exception is for children with the onset of meningitis during the first 2 weeks after cochlear implantation; in these instances, causal bacteria may include a broader range of pathogens, including gram-negative bacilli and gram-positive bacteria. Selection of a combination of antimicrobial agents that provides broader-spectrum coverage against gram-negative bacilli should be chosen. For delayed development of cochlear implant infections, therapy consists of removal of the implant and administration of intravenous antibiotics.[402,406]

OCULAR PROSTHESES

The ocular prosthetic devices include artificial globes used primarily for cosmetic purposes, orbital implants, ocular explants, intraocular lenses, and contact lenses.

Orbital Implants

Orbital implants are made of hydroxyapatite or porous polyethylene and frequently are used in orbital reconstruction after enucleation or evisceration surgery. Infection of these implants is a rare event, with only a handful of cases reported in the literature.* Patients most commonly report anophthalmic socket pain or discomfort, pain with eye movement, and excessive conjunctival edema and irritation while wearing an artificial globe.[229] Papillary conjunctivitis of the socket with exudate and sometimes dehiscence of the overlying conjunctiva also may be seen.[160,337] Infection may develop months to years after placement of the implant, and severity ranges from cellulitis to the development of an abscess around the implant itself, with implant extrusion. In a retrospective clinical case series of 103 patients who underwent hydroxyapatite implant insertion at one institution, patients who had a freestanding polycarbonate peg placed to enhance the motility of their ocular prostheses had a significantly higher rate of infection than those patients with nonpegged hydroxyapatite implants, at 42.9 versus 19.5 percent.[135]

Radiographic studies that can aid in the detection of these types of infection include technetium 99m–labeled leukocyte scintigraphy, which is most useful in detecting early low-grade graft infection,[228] and computed tomography (CT) and magnetic resonance imaging (MRI), which are useful later in the course of the infection to detect the presence of abscesses and structural tissue changes. Gram-positive cocci, primarily *S. aureus*, and coagulase-negative staphylococci are the organisms associated most commonly with these infections; however, *H. influenzae*, *S. pneumoniae*, α-hemolytic streptococci, *Capnocytophaga*, *Pseudomonas*, and *Aspergillus fumigatus* have been cultured as well.† To cure the infection effectively, treatment involves both removal of the implant and institution of topical and parenteral antibiotic therapy directed against the organism isolated. Empirical therapy directed against gram-positive organisms may be started initially

*References 5, 134, 161, 208, 231, 238, 545.
†References 88, 401, 408, 436, 464, 538, 546.

until the results of culture and sensitivity testing are available. This should include antimicrobial agents effective against community strains of MRSA. In addition, other common empirical regimens include a first-generation or second-generation cephalosporin or an extended-spectrum, penicillinase-resistant penicillin.

Intraocular Lenses

Insertion of polymethyl methacrylate (PMMA) intraocular lenses at the time of removal of a cataract is the standard surgical therapy for this disorder. Even though the rate of postoperative infection of intraoperative lenses is low (0.10% to 0.30%), the infection usually is serious and results in endophthalmitis and permanent loss of vision.[231,258] The predisposing factor for infection is bacterial adhesion to intraocular lenses during insertion. After adhesion is accomplished, the bacteria replicate, congregate, and form multiple layers of microcolonies that represent a biofilm in which the bacteria are embedded in a layer of slime. The major pathogens associated with this infection are *S. aureus* and *S. epidermidis*, which account for 90 percent of all isolates, although α-hemolytic *Streptococcus* spp., gram-negative bacilli, *Pseudomonas*, fungi, *Chlamydia trachomatis*, *Nocardia* spp., and rapidly growing mycobacteria are isolated on occasion.* Intraocular lens–associated endophthalmitis is a serious infection that is difficult to diagnose and treat.[34,394,477,492,530] In a few cases, use of topical and systemic antibiotics alone has been successful in eradicating the infection, but in most cases surgical débridement and systemic antibiotics are required for cure. Empirical therapy for these infections consists of an extended-spectrum, penicillinase-resistant penicillin, a second-generation cephalosporin, or, in some instances, clindamycin or vancomycin. In cases of fungal endophthalmitis, treatment with the later-generation azoles alone and in combination with an echinocandin has been shown to have clinical success.[121] In rapidly growing mycobacterial endophthalmitis combinations of topical and intravenous antibiotics to which the organism is susceptible have been used for treatment.[311]

Contact Lenses

Primarily three different types of contact lenses are available. Hard lenses are made of PMMA, a substance that is impermeable to water and gas. This type of lens is designed to be worn only during waking hours because it limits oxygen flow to the cornea to that present in tears. Gas-permeable hard lenses are composed of silicone, cellulose acetate butyrate, or PMMA-silicone copolymers; they allow gas but not fluid to pass through the lens. Hydrophilic or soft lenses are made of a cross-linked hydrogel polymer or copolymer and consist of 38 to 85 percent water by weight; these lenses are permeable to both gas and water and allow the user to wear them continuously. However, for all these types of lenses, infection may result in damage to the corneal epithelium.

The two main infections that occur in association with contact lenses are conjunctivitis and keratitis.[77] The causative bacteria seen most commonly with these infections are *S. aureus*, streptococci, *Pseudomonas* spp. (found in improperly stored cleansing solutions), and fungi (including *Candida* spp., *Alternaria*, *Paecilomyces*, *Fusarium*, *Curvularia*, and other filamentous fungi, and *Acremonium*). Improper cleaning of soft or hydrophilic lenses may cause them to become a source of infection when bacteria penetrate the lens matrix. Risk factors for fungal keratitis include use of contaminated contact lens solution,[8,79,499,549] trauma, contact lens wear, ocular surface disease, systemic diseases affecting the immune system, diabetes, use of systemic steroids, and recent chemotherapy. Fungal keratitis frequently is a severe disease in which diagnosis may be difficult, response to medical therapy is generally slow, and the clinical outcome may be poor. Corneal perforation is 5 to 6 times more likely with fungal keratitis than in bacterial keratitis.[542] In a retrospective multicenter case series of 733 cases of fungal keratitis that looked at the epidemiology and microbiologic characteristics of fungal keratitis at tertiary eye care centers in the United States, it was found that filamentous fungi were more commonly found as the cause of keratitis among refractive contact lens wearers while yeasts, especially *Candida* spp., were the most common causative organisms in patients with ocular surface disease and ocular trauma.[240,548] Treatment of keratitis usually consists of removal of the lens and application of topical antibiotics or antifungals. The most commonly used topical antifungal regimen is a combination of natamycin 5 percent and amphotericin B 0.15 percent. Twenty-five percent of patients also may require systemic antifungal therapy in addition to the topical therapy. In fungal keratitis, 16 to 26 percent of patients require surgical intervention with therapeutic keratoplasty in addition to medical therapy.[219,240] To prevent the development of infections associated with contact lenses, users should adhere strictly to the manufacturer's suggested guidelines for wearing and cleaning the lenses.

A rare and often devastating infection associated with the use of contact lenses is infection with the fresh-water protozoan *Acanthamoeba*.[93,195,337,467] This organism contaminates the lens when sterility of the cleansing solutions is not maintained; it has been associated with users who prepare their own solutions and use of contaminated contact lens disinfection solution.[107,508,551] *Acanthamoeba* is very difficult to eradicate because it is not susceptible to standard antiparasitic agents, and it produces a chronic keratitis that can be complicated by corneal perforation and loss of the eye. Corneal transplantation may be necessary in many cases to restore vision.[195,467] In a study by Kaiserman and colleagues to assess the prognostic factors influencing visual prognosis and length of treatment after acanthamoeba keratitis, infection acquired by swimming or related to contact lenses, having neuritis and pseudodendrites, the absence of an epithelial defect, having been treated with chlorhexidine, and not having received steroids were factors that were associated with better final best spectacle-corrected visual acuity (BCVA); having an epithelial defect on presentation and having been treated with topical steroid were associated with worse BCVA.[230]

*References 11, 121, 258, 311, 371, 372, 435, 547.

LEFT VENTRICULAR ASSIST DEVICES

The development plus use of mechanical circulatory assist devices has grown very rapidly in the last several decades, especially with the shortage of available donor hearts. Such devices have improved considerably the hemodynamic status and quality of life of patients with heart failure who are awaiting cardiac transplantation. Figure 74-1 shows a left ventricular assist device (LVAD), a pneumatically driven pump located outside the heart that draws blood from an inflow cannula in the left ventricular apex and ejects the blood through an outlet into the ascending aorta. Within the pump are several sections of Dacron graft material and two trileaflet porcine valves in the inflow and outflow positions to ensure that blood flow is unidirectional. The pump is encased in titanium and implanted via an extended median sternotomy into the left rectus sheath. A percutaneous driveline connects to an exterior power pack for venting or for pneumatic actuation and exits the body after passing through a subcutaneous tunnel, in the left lower quadrant. The interior surface of the pump is textured to prevent the formation of thrombi and encourage the deposition of a biologic pseudointimal lining. Most LVADs are surgically implanted through a median sternotomy, with pump placement primarily in the intra-abdominal or preperitoneal space. Ventricular assist device designs primarily fall into two major categories: pulsatile and continuous flow devices. Pulsatile devices are first-generation devices that were designed to mimic nature by providing pulsatile circulation, but have many moving parts, including a diaphragm to hold and eject blood, valves to maintain directionality, and an air vent and conduit for cooling of the working parts. These requirements have limited the durability and size of these devices. Because of the multiple moving parts, these devices begin to wear out within the first 2 years of use and the large size necessitates placement in the preperitoneal space or intraperitoneal space. The second-generation continuous flow devices are smaller and have fewer moving parts. These devices primarily consist of inflow and outflow pumps and a rotor, with a smaller conduit to the power source that does not include an air vent. Since January of 2010, 98 percent of LVADs implanted are of continuous flow design.[249] Once the device is implanted, in most cases it cannot be removed without performing concurrent cardiac transplantation.[191,239,316,315,359]

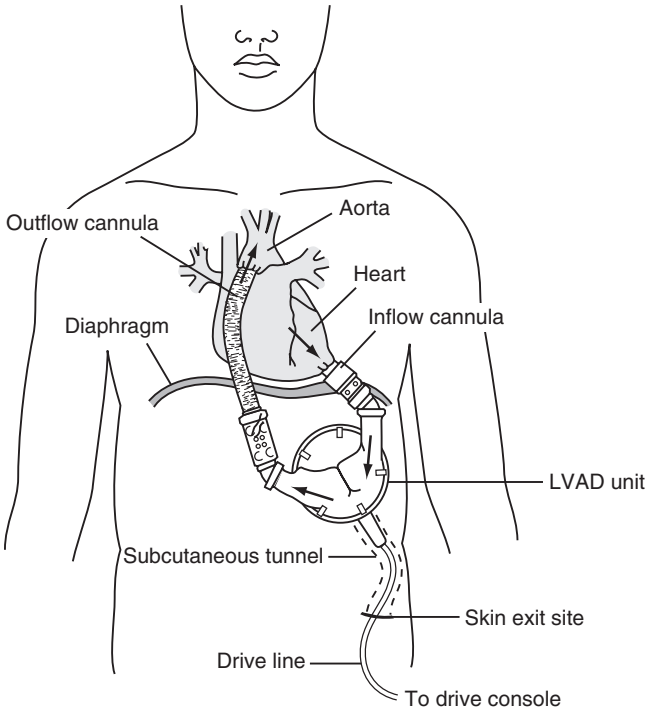

FIGURE 74-1 ■ **Left ventricular assist device.** (Modified from Fisher SA, Trenholme GM, Costanzo MR, Piccione W. Infectious complications in left ventricular assist device recipients. *Clin Infect Dis* 1997;**24**:18–23.)

Infection of an LVAD can involve any portion of the LVAD, including the surgical site, driveline, pocket, and pump. Infections occur most commonly within the first 3 months of device implantation, with the risk for severe infection and sepsis peaking at 1 month. The cumulative risk for device infection increases the longer the device remains in place; the incidence of infection significantly decreases after 3 months.[72,203,205,236] Based on data from the Interagency Registry for Mechanical Circulatory Support (INTERMACS), overall infection rates were 17.46 infections/100 patient-months during the first 12 months after implantation.[250,447,465] The rates of infection have been shown to vary among generations of device, with 28.9 infections/100 months in the pulsatile devices (n = 406) and 11.8 infections/100 months in the continuous-flow devices (n = 548) (p <.0001). It was also found that infection accounted for 16 percent of deaths associated with LVAD implantation.[203,249,419,426]

The approach taken by many centers for infection of an LVAD includes both preventive and interventional steps that are instituted before, during, and after implantation. Preventive strategies are focused on prevention of infections related primarily to the driveline and device pocket through the use of clean implantation techniques and limited traffic in the operating room in which the procedure is being performed. Antibiotic prophylaxis usually is given for 48 hours near the time of implantation of the LVAD and may include intravenous trimethoprim-sulfamethoxazole, rifampin, and fluconazole, along with the application of mupirocin ointment to the nares. Additional preventive measures include soaking the surfaces of the LVAD in vancomycin and gentamicin for 30 minutes before placing it, irrigating the device pocket with povidone-iodine (Betadine), and meticulously placing the subcutaneous tunnel in the appropriate position. Postoperative care with sterile and semisterile dressing changes around the driveline is emphasized.*

Despite the preventive measures taken, infection, thromboembolism, and hemorrhage at the time of implantation remain the most common complications associated with the use of mechanical circulatory support devices. Of these complications, infection has the most significant impact on morbidity and mortality.[153] Predisposing factors for development of device-related infections in these patients include a variety of host, device,

*References 16, 140, 204, 390, 446, 521.

and operative characteristics. Device-related risk factors are correlated with increased size and surface area of the device, turbulence of flow, contact of blood with the prosthetic surfaces, device-related pockets and cavities, transcutaneous drivelines, and power cables.[343,360] Operative and environmental risk factors include extent of surgery necessary for implantation hemorrhage, reoperation, frequent occurrence of multiple organ dysfunction or failure, duration of mechanical circulatory support, duration of hospitalization and intensive care unit stay, use of indwelling lines and catheters, parenteral nutrition, operative technique, and time.[283,391] The incidence of infection in patients on LVAD support for longer than 60 days is reported to be two times higher than that in patients with an LVAD in place for less than 30 days; initial episodes of infection after 90 days are rare.[342,384,443] With regard to patient-related risk factors, the severity of heart failure symptoms and presence of poor health and comorbid conditions, including diabetes, hyperglycemia, obesity, alcohol use, and renal disease, has been associated with increased risk for infection.[47,166,335,399,443] In addition, some evidence indicates that implantation of an LVAD itself may lead to defects in cellular immunity secondary to an aberrant state of T-cell activation that predisposes LVAD recipients to the development of candidal and other systemic infections.[18,164,217,248,384] The incidence of infection after implantation of an LVAD is reported to range between 13 and 80 percent, with most studies documenting an infection rate of 30 to 50 percent.* Most series report that the device pocket and drivelines are the device-related sites that account for most of the infections. Placement of the LVAD in the abdominal cavity instead of the preperitoneal area has been shown to decrease the chance for development of infection.[342]

Driveline infections are thought to result from a lack of integration of tissue around the driveline that allows the driveline to move and irritate surrounding tissues, which can result in an infection in the exit site and, in some cases, bacteremia. Such infections generally are defined by pain, erythema, warmth, drainage, or purulent discharge at the driveline exit site in the presence of a positive culture. Bacteremia developing after implantation of a pump and infections of the pump itself are additional reported complications.[22,317] In a study of 205 patients who underwent placement of LVADs at the Cleveland Clinic, positive blood cultures were noted in 52 percent, infections of the driveline site occurred in 22 percent, and infections of the pump pocket developed in 9.2 percent.[239] Sun and colleagues[472] reported their experience with 95 patients who underwent insertion of an LVAD. Twenty-six (27%) of these patients experienced device-related infections involving the driveline, device pocket, or blood-contacting surfaces; 15 of the 26 (57.7%) had infections of the driveline site. Persistent bacteremia and progression of infection of the device pocket and driveline site can lead to the serious infection of LVAD endocarditis, which mimics prosthetic valve endocarditis, is very difficult to treat, and is associated with a high mortality rate. LVAD endocarditis is defined as positive cultures of the LVAD surface in conjunction with clinical signs and symptoms of infection during LVAD support. Manifestations of this condition are varied and range from persistent fever and bacteremia or fungemia to cerebral thromboembolism, LVAD inlet obstruction with hemorrhage, or LVAD outflow graft rupture.[22] In a report by Weyland and associates[531] of 27 patients who underwent insertion of LVADs, infections of the driveline developed in 8 (30%) of the 27 recipients, and LVAD endocarditis, defined as positive cultures from the pump chamber, was found in 12 (44%) of the patients; 8 of the 12 (67%) died of endocarditis.

Despite a high potential for the development of serious morbidity after placement of an LVAD, studies have shown that the presence of an infection after insertion of an LVAD does not seem to have very much influence on eventual cardiac transplantation and posttransplant outcome. A study by Argenziano found that infection subsequently developed in 29 of 60 (48%) patients who underwent insertion of an LVAD.[22] The most frequent sites of infection were the blood (27%), LVAD driveline (13%), LVAD surface (13%), and central venous catheter (10%); these sites thus represented 63 percent of all infections. The overall mortality rate, the rate of successful cardiac transplantation, and the rate of infection after transplantation were not influenced by the presence of infection during LVAD support.[22] In another study, Sinha and colleagues[446] reviewed their experience with 86 patients who received LVADs; device-related infections developed in 6 patients, 5 (83%) of whom had infections of the pocket. This study also found that the presence of infection during LVAD support did not influence successful cardiac transplantation or patient survival after transplantation. Over a 6-year period, Schulman and colleagues[427] reviewed their experience with 149 patients who received LVADs as a bridge to heart transplantation and sought to determine the impact of device-related infections on posttransplant survival and posttransplant infection. Of the 149 patients, 110 were successfully bridged to cardiac transplantation. Pretransplant device-related infections (e.g., pocket infections, wound infections, and postimplant bacteremia) had no negative effect on bridging to transplantation. However, patients with pretransplant sepsis were less often successfully bridged to cardiac transplantation. The presence of a pretransplant device-related infection was found to have no effect on posttransplant 1-year survival rates, although it was associated with an increased rate of posttransplant infection, especially if the patient had a driveline infection during LVAD support. Specifically, having a driveline infection during LVAD support predisposed for having an infection in the former LVAD pocket or driveline site after cardiac transplantation, but did not predispose to having sternal wound infections, bacteremia, or sepsis in the posttransplant period. Diagnosing an infection early, determining the location and the extent of the infection, and assessing therapeutic efficacy can be a challenge in patients with LVADs. Anatomic imaging modalities such as CT and MRI may have limited value because of the presence of metallic CT artifacts and contraindications to using MRI. New imaging modalities, such as radiolabeled leukocyte single-photon emission computed tomography (SPECT/CT), are being studied to assess

*References 22, 140, 148, 203, 315, 317, 376, 472, 531.

the accuracy of these modalities in revealing both anatomic location and extent of infection in patients with an LVAD. Preliminary results of leukocyte SPECT/CT have shown it to be very accurate in diagnosing an LVAD-related infection.[68,284]

Microbiology of Left Ventricular Assist Device Infections

Numerous organisms have been isolated from LVAD infections. In most series, *S. aureus* (including methicillin-resistant strains), *S. epidermidis* and other coagulase-negative staphylococci, and *Enterococcus* spp. are the organisms most commonly isolated from these infections, usually within the first 4 weeks after implantation. Other organisms such as *Enterobacter* spp., *P. aeruginosa*, *Serratia marcescens*, *Klebsiella* spp., *Stenotrophomonas maltophilia*, *Citrobacter koseri*, other gram-negative organisms, and polymicrobial organisms were seen more commonly later in the clinical course.* Fungal infections (e.g., *Candida albicans*, *Candida parapsilosis*, *Aspergillus* spp., and *Mucor* spp.) have been reported or observed as well and were responsible for approximately 16 percent of the infections.† However, colonization with fungi occurs in 35 to 39 percent of patients with LVADs.[164] Investigators suggested that treatment with broad-spectrum antibiotics (common in these patients) rendered them more susceptible to the development of fungal infection. When a fungal LVAD infection is suspected, signs of thrush, cutaneous rashes, esophagitis, retinal changes, peripheral embolization, and unexplained decrease in LVAD output should be investigated. The presence of yeast on Gram stain of the fluid surrounding the device and ultrasound showing increasing amounts of fluid around the device can be used to help confirm the diagnosis.[164]

Management of these infections, especially LVAD endocarditis, presents a major challenge, given that the device usually cannot be removed unless simultaneous heart transplantation is performed; moreover, if transplantation is accomplished, multiple issues regarding the immunosuppressed condition of the patient are raised. In most cases, management involves a prolonged course (i.e., usually 4 to 6 weeks) of aggressive antimicrobial or antifungal therapy (or both) appropriate for the organism or organisms isolated, with meticulous attention given to skin care around the driveline exit site; débridement, drainage, surgical revision, and irrigation with povidone-iodine of infected wound sites; or in certain instances, replacement of the LVAD unit or cardiac transplantation before completing antibiotic therapy if a donor heart becomes available and all blood culture results are negative.[140,204,317]

EXTRACORPOREAL MEMBRANE OXYGENATION CIRCUITS

Extracorporeal membrane oxygenation (ECMO) is a life support device used most commonly for treating severe cardiac and pulmonary failure in neonates and children. Almost 1 percent of all children treated by open heart surgery will undergo ECMO. In addition to these post-cardiotomy patients, a growing number of children receive ECMO for acute decompensation of myocarditis or cardiomyopathy. ECMO also is used to support children after sudden cardiac arrest when conventional closed or open heart massage is unsuccessful. In children, perfusion through the neck venoarterial vessels is the preferred method, but the trans-sternal approach is used when direct decompression of the distended left ventricle is required. Although the overall survival rate of children managed by ECMO has improved in recent years, it still is only 50 to 70 percent.[35,525,536] Multiple cardiac and noncardiac factors are associated with increased morbidity and mortality rates. Patients with a single ventricle or residual cardiac defect after surgery have a less favorable ECMO outcome.[50,104,255] Renal dysfunction, multiple organ system failure, initiation of ECMO in the operating room, blood product transfusion, mediastinal bleeding, ECMO circuit problems, and duration of ECMO for longer than 10 days also were predictors of increased risk for mortality.[255,336] The infection rate for patients on ECMO ranges from 6 percent to more than 40 percent. Recent reports suggested a higher infection rate in older patients with culture-proven infections, at 6.1 to 10.1 percent in neonatal patients, 18.7 to 20.8 percent in pediatric patients, and 20.5 to 30.6 percent in adults.[49,536] Multiple risk factors for acquiring infection while on ECMO were identified, including indwelling vascular or urinary catheters,[336] duration of ECMO (>7 days),[214,468] procedures before or during ECMO,[355] ECMO complications,[64] and changes in the humoral and cellular immunity.[503] Patients with infections usually will need a longer bypass support[355] and will have a delay in post-ECMO lung recovery and increased mortality.[49,259,336]

Earlier reports suggested that gram-positive cocci (e.g., *S. aureus* and coagulase-negative staphylococci) are the most common etiologic agents. However, gram-negative bacteria (both lactose fermenting and non–lactose fermenting[214]) and fungi[193] are becoming more commonly reported. *Candida* spp. were found to be the most frequent pathogens in children and adults.[49,473] The distribution of these pathogens is similar to their distribution in other patients with infections treated in the intensive care unit.[193] The signs and symptoms of infection often are subtle and nonspecific. Fever develops in only half of these patients. The value of tachycardia and elevated cardiac output also is limited because these patients frequently are tachycardic, with increased cardiac output caused by a systemic inflammatory response (e.g., complement; cytokines such as TNF, interleukin [IL]-1, and IL-6), which is characteristic of cardiopulmonary bypass procedures.[67,332,468] For the same reason, leukocytosis (unless developing acutely) and an elevated erythrocyte sedimentation rate (ESR) should be interpreted with caution. In addition, physical examination and radiologic tests may be difficult to interpret.[67] Consequently, a high index of suspicion with careful evaluation of potential infection source (e.g., surgical wound and insertion site of catheter) and surveillance of blood and urine cultures is needed to identify these infections.

*References 22, 116, 140, 167, 204, 314, 317, 446, 486, 531.
†References 30, 112, 116, 204, 314, 366, 438.

Most patients undergoing ECMO are treated with multiple broad-spectrum antibiotics. This practice probably is one of the reasons for the increased incidence of multidrug-resistant, gram-positive (e.g., *Enterococcus*) and gram-negative bacteria, as well as fungal (e.g., *Candida*), infections. In addition, many of these patients will be colonized with these same bacteria. Therefore, if a deep bronchial suction or urine culture (performed routinely) becomes positive but without supporting evidence of infection (e.g., increased white blood cell [WBC] count, fever), serious effort to distinguish between colonization and infection should be made before initiating an unnecessary change in antimicrobial therapy. If a decision to treat is made, appropriate coverage for both gram-positive and gram-negative organisms should be chosen. If blood, urine, and bronchial cultures remain negative and the patient's condition is not improving or the culture becomes positive for a fungus, antifungal therapy should be added.

Most ECMO centers administer antibiotic prophylaxis; however, the optimal spectrum and duration of this practice are unknown. Some studies suggested that use of prophylaxis (for 24 hours before the ECMO is started) was effective in reducing infections,[65,468] others found no correlation between antibiotic prophylaxis and reduction in infection rate.[214] Of interest, despite advances in critical care for ECMO patients (including antibiotic prophylaxis) no significant decrease has occurred in infection rate during the last two decades.[64] The lack of specific guidelines and evidence from randomized trials evaluating different durations and spectrums of antibiotic contribute to the confusion and controversy in this area.[235] If prophylaxis is considered, antifungal prophylaxis (e.g., fluconazole) should be added because it was shown to decrease the incidence of fungal infections, which are increasing.[154]

PERMANENT CARDIAC PACEMAKER AND IMPLANTABLE CARDIOVERTER-DEFIBRILLATOR INFECTIONS

Permanent Cardiac Pacemaker Infections

Cardiac pacemakers have been widely used since the 1980s and the American Heart Association (AHA) has recently expanded the indications for their use.[129] Two types of permanent pacemaker generators are in use; those with transvenous electrodes and those with epicardial electrodes. Both types are implanted in either the chest or the abdominal wall; pacemakers with epicardial electrodes are the ones more commonly used in the pediatric population.

Infection is one of the most common medical complications of implantation of permanent pacemakers, with reported rates ranging from 0.2 to 5.8 percent and a high mortality rate.[70,86,518] An estimated 25 percent of cardiac pacemaker–associated infections develop within 1 to 2 months of placement of the device, but delays of up to 12 months may occur before diagnosis is established. Several studies suggested that the relative rate of infection associated with permanent pacemakers has increased despite improved surgical techniques and better design of the devices themselves,[70,502,518] but a recent study demonstrated a fairly constant rate of infection over 20 years.[226] Studies have shown that infections of permanent cardiac pacemakers seem to occur more commonly in patients with both local and systemic underlying medical problems, especially diabetes mellitus or an underlying renal insufficiency, or in those who are undergoing treatment with corticosteroids, anticoagulants, or other types of immunosuppressive therapy.[51,452,518] Other well-characterized independent risk factors include surgery related to any part of the pacemaker (especially replacement of a battery or upgrade of the pacemaker),* the presence of a hematoma after implantation,[71,347,451] temporary transvenous pacing[194] and epicardial leads.[451] Multiple (two or more) pacemaker insertions and physician inexperience with insertion also represent significant risks for development of infection.[185,347] The presence of a central venous line also increases the risk for systemic infection.[76] Multivariable analysis of the largest reported pediatric pacemaker infections identified only presence of Down syndrome and manipulations of the pacing system during revisions as risk factors.[94]

Infections of permanent cardiac pacemakers are subdivided into groups depending on the specific site of involvement and whether they are early infections (<1 to 2 months after implantation) or late infections (>2 months after implantation). The groups are (1) local inflammation, infection, and the formation of an abscess in the generator pocket or subcutaneous portion of the lead, or in both sites; (2) secondary infection involving either the generator or the electrodes (including pacemaker endocarditis)[71,124]; (3) fever plus associated bacteremia, with or without concomitant infective endocarditis, in a patient without any apparent focus of infection[124]; and (4) mediastinitis, pericarditis, bronchopleural cutaneous fistulas, and mixed infections.[124]

The generator pocket and the subcutaneous portions of the leads (i.e., transvenous and epicardial) are the most common sites of infection. Infections of the pocket often are difficult to diagnose and may develop at any time after implantation; however, most tend to develop early and frequently are the result of contamination by skin flora at the time of the procedure.[333] Late infection usually occurs as a consequence of erosion of the device through the subcutaneous tissue and skin. The infection may remain localized to the pocket or may spread to the adjacent electrodes and lead to the development of bacteremia. Infection of transvenous pacemaker leads may occur in as many as 17 percent of patients who receive permanent pacemakers. These infections often are not apparent initially but have been associated with significant morbidity and mortality.[175] Infection generally develops along the subcutaneous portion of the leads and, if unrecognized, may progress centrally and result in sustained bacteremia, endocarditis, or both. Infections of the leads tend to occur later than infections of the pocket, with the median time of occurrence being 7 to 8 months after implantation of the pacemaker. Endocarditis as a

*References 76, 185, 194, 226, 265, 347.

complication of unrecognized infected transvenous leads can develop years (2 to 3 years) after placement of the pacemaker. Infections of permanent epicardial leads generally occur as a complication of infections of the generator pocket, skin erosion, or direct contamination at the time of placement. However, in contrast to infections of the transvenous leads, infections of the epicardial leads usually result in only local symptoms; rarely, they may lead to more severe disseminated disease such as pericarditis, mediastinitis, bronchopleural cutaneous fistulas, bacteremia, or sepsis.

Microbiology

Staphylococci (*S. aureus* and coagulase-negative staphylococci) are the most common causes of pacemaker-related infections, accounting for more than 85 percent of such infections; however, a wide variety of organisms have been isolated.* An increasing number of the isolated staphylococci are methicillin-resistant. Other gram-positive organisms such as viridans streptococci, other streptococci, enterococci, *Listeria*, and *Corynebacteria* have been isolated as well, but each causes fewer than 1 percent of cases. Gram-negative organisms such as *E. coli*, *Proteus*, *Enterobacter cloacae*, *P. aeruginosa*, *Klebsiella*, and *S. marcescens* are isolated from 5 to 20 percent of device-related infections. In addition, *Candida*, *Aspergillus*, *Pseudallescheria*, and other fungi have been isolated rarely as well as *Mycobacterium* spp.; however, infections with these organisms generally are associated with poor clinical outcomes.†

Clinical and Laboratory Findings

Diagnosing a pacemaker-associated infection can be difficult because of its nonspecific symptomatology, and months may elapse after the onset of symptoms before the diagnosis is established. Symptoms may be confined to a local area or may be more widespread, with the development of bacteremia or other systemic effects. Rarely, the only sign of infection is persistent pain at the pocket site.[254] Fever (84% to 100%) and chills (75% to 84%) are considered the most common systemic symptoms and are indicators of local infection, especially if they occur after the second postoperative day in association with other signs; however, they may be the only clinical manifestation in more than a third of patients.[71,253,334,353,535] Early infections are more likely to be accompanied by both local and systemic clinical findings and are manifested more commonly as infections of the generator pocket, bacteremia, or septicemia[71,294,557]; late infections, on the other hand, typically cause vague symptoms that evolve over the course of time and usually are lead-related infections and endocarditis.[265,513]

Infections of the generator pocket typically cause local swelling (21%), erythema (34%), pain (32%), drainage (through an incompletely healed incision or fistulous tract) over or adjacent to the generator pocket (25%), and warmth (11.5%).[86] Sterile breakdown of the pacemaker pocket develops in an estimated 5 percent of patients with permanent pacemakers, and skin or soft tissue erosion over the electrodes occurs in an additional 2 to 4 percent. Because these complications are associated with a high risk for the subsequent development of infections, the presence of any erosion is considered a potential indicator of device-related infection, especially if the erosion occurs more than 24 months after placement of the pacemaker, at which time the infection rate may be as high as 80 percent.[46,174] Pacemaker-related endocarditis is a relatively rare complication that occurs in 0.05 to 0.5 percent of cases after implantation of a pacemaker. Such endocarditis is associated with a mortality rate as high as 34 percent in some series.[71,152,253] It tends to be a late complication; only 27 to 36 percent of patients are seen within 6 to 12 weeks after the last procedure at the pacemaker implant site, with symptoms developing in most patients a mean of 25 months after the last procedure at the implant site. The diagnosis of pacemaker-related endocarditis is difficult to establish, and usually a delay in making the diagnosis, with a mean interval of 5 to 8 months after the onset of symptoms, occurs.[71,253] Pulmonary symptoms are found in 20 to 45 percent of patients with pacemaker-related infections and may consist of bronchitis, lung abscess, pneumonia, or pulmonary embolism; these symptoms are seen more commonly in late-onset infection and in patients with intravenous lead–related infection.[71,253]

A variety of laboratory and imaging studies may be performed to aid in establishing the diagnosis of a pacemaker-related infection. A recent update from the AHA suggested two sets of cultures from blood, generator pocket site, and lead tips when infection is suspected.[29] An elevated ESR is found in 82 to 97 percent of patients, and peripheral leukocytosis is seen in 50 to 66 percent. The presence of a collection of fluid around the device as seen on ultrasound is suggestive of device-related infection. Gallium scanning may be performed in an attempt to determine the nature of the fluid (i.e., inflammatory vs. infectious). Transesophageal echocardiography (TEE) can be helpful in identifying endocarditis (especially when blood culture results are negative). TEE has emerged as a major tool in diagnosis; studies have shown that this test was able to demonstrate vegetations and other lesions on the electrodes, ventricular endocardium, and tricuspid valve in 90 to 96 percent of patients with pacemaker-related infections.[71,253,513] Only the combination of negative TEE and ECHO should be used to rule out endocarditis.[453]

Management and Treatment of Infection

Appropriate therapy for pacemaker-related infections is tailored to the specific clinical situation and depends on several factors: (1) whether the infection is limited to the generator pocket or subcutaneous electrodes, without bacteremia; (2) whether bacteremic pacemaker-related endocarditis is present with or without involvement of the subcutaneous electrodes; and (3) what organism is involved.

*References 71, 76, 309, 347, 451, 453, 513.
†References 10, 71, 82, 85, 237, 264, 270, 301, 453, 513, 535.

If the infection is limited to the generator pocket or subcutaneous electrodes, optimal treatment consists of both medical and surgical intervention in which all of the parts of the infected device are removed. Studies have shown that failure to remove completely all portions of the infected device results not only in failure to cure the infection but also in higher mortality rates.[29,71,517] In addition, infection relapse is higher.[86,305,452] The patient is given parenteral antimicrobial therapy, and an exchange of the pacing system with total removal of the infected device and simultaneous insertion of a new pacemaker at a different site is performed surgically. The infected subcutaneous pocket is drained, débrided, and packed open, and local wound care is instituted. Initial antibiotic therapy should provide coverage for both staphylococci and gram-negative bacteria; therapy is tailored once the organism is identified and its antibiotic susceptibility is known. Initial regimens include vancomycin plus an aminoglycoside. Oxacillin or nafcillin plus an aminoglycoside may be used if MRSA is rare in the community. In certain instances, a new pacemaker cannot be placed at the time of removal of the infected device; in such situations, a period of temporary transvenous pacing is instituted until a new pacemaker can be implanted. For local pocket infections, antimicrobial therapy targeted at the causative organism should be administered for 10 to 14 days.

The consensus approach to the management of pacemaker-related endocarditis and bacteremia involves removal of both the generator unit and the electrodes in conjunction with prolonged administration of parenteral antibiotic therapy designed to treat endocarditis (usual duration, 4 weeks).[29,452] In cases of MRSA, duration of treatment should be extended to 6 weeks.[285] A longer duration may be considered in patients with other sites of infection (e.g., osteomyelitis or septic thrombophlebitis). Vancomycin should be the initial antimicrobial treatment of choice before the etiologic agent is identified, because the majority of infections are due to staphylococci species. More specific antibiotic regimens can be used when the pathogen is identified and in vitro susceptibility is available. Mortality rates may be as high as 50 percent for patients in whom total removal of the pacemaker unit is not performed.[71,253,375] Percutaneous removal of electrodes that have lead-associated vegetations smaller than 20 mm is acceptable,[29,139] but extraction of the electrodes with vegetations larger than 20 mm may be difficult and risky to perform, and surgical removal by cardiotomy may be necessary.[29] In patients who require a pacemaker, temporary transvenous pacing may be implemented until a new permanent pacemaker can be placed. Sterility of blood cultures should be ensured before a new pacemaker generator and electrode are placed.

Infection of Implantable Cardioverter-Defibrillators

The first implantable cardioverter-defibrillator (ICD) was placed in 1980, and during the last several decades the device has become a successful therapeutic modality for treatment of patients (both adults and children) with life-threatening ventricular arrhythmias. Older devices required surgical placement of a pulse generator, extrapericardial or epicardial defibrillation patches, and a transvenous rate-sensing electrode. The systems placed today use a rate-sensing lead that is implanted transvenously, a superior vena cava coil electrode, and additional subcutaneous or epicardial electrodes all connected to a pulse generator that is placed subcutaneously or submuscularly.[241,461,539]

Infection is the most serious complication of placement of an ICD.[71,267] Infection rates associated with placement of ICDs ranged from 1 to 7 percent.[462,490] However, infection rates have decreased substantially with the advent of transvenous non–thoracotomy-placed systems, with rates ranging from 0.8 to 1.5 percent.* Cost associated with hospital admission, length of stay, and mortality are substantially increased in patients with infection in contrast to those without it.[450] Several risk factors that have been identified as increasing the likelihood for development of infection include steroid use, diabetes, heart failure, and renal failure.[51] Previous generator replacement, use of anticoagulants, abdominal implantation, postoperative hematoma, and surgeon experience also were reported as contributing to increased risk for infection.[51,275,501] Most ICD-related infections are clinically apparent within 3 to 6 months after placement, with infections of the generator pocket, subcutaneous patch wound site, epicardial patches, or bacteremia and endocarditis being the most common manifestations. Some infections can be indolent, and only a high index of suspicion will initiate an investigation. An increased WBC count and ESR, anemia, and microscopic hematuria are the most common laboratory findings. As with pacemaker infections, TEE is an effective test to exclude endocarditis because echocardiography is helpful in only half of the cases.[146] Infection of the generator pocket or subcutaneous patch wound site usually causes local findings of pain, erythema, and collection and drainage of fluid from the site. Occasionally, these patients also may be bacteremic or hypotensive. In contrast, infection of the epicardial patches generally results in more systemic symptoms, bacteremia, or pericarditis.[357] At least two blood cultures should be obtained to rule out bacteremia. Cultures of the generator pocket and lead tips after removal of the device are essential for identifying the pathogen. Sonication of the extracted device increases the yield of positive cultures.[308]

Microbiology

S. aureus and coagulase-negative streptococci are the major pathogens seen with ICD-related infections and account for 60 to 80 percent of cases.[267,357,416,462,515] However, a broad spectrum of other gram-positive, gram-negative, and fungal organisms, including *P. aeruginosa*, *Corynebacterium*, streptococci, *E. coli*, *Klebsiella*, *Bacteroides fragilis*, *Propionibacterium acnes*, atypical mycobacteria, and *Candida* spp., have been isolated from ICD-related infections. Polymicrobial infections may occur in the generator pocket.[267,416,449]

*References 163, 326, 357, 449, 501, 529.

Management

The same approach as was previously described for pacemaker infection should be applied to ICD infection. To eliminate ICD-related infections effectively, optimal management involves a combination of medical and surgical interventions, especially if the patient is bacteremic or has systemic findings or if the causative organism is *S. aureus*. Several studies have suggested that conservative management (i.e., antibiotic therapy without removal of the hardware) may be sufficient. However, such an approach should be tried only in cases with superficial or incisional infection and in rare occasions when only the generator pocket is infected or in patients whose condition prevents removal of the entire device. In these cases, the management should consist of administration of parenteral antibiotics, wound care, and removal of only the generator portion of the device with implantation of another generator at a different site.[357,497] The optimal management of any other ICD-related infection is parenteral antibiotics and complete removal of the entire ICD system, followed by reimplantation (after the infection is eradicated) at another site.[86,267,416,537] Although no data exist on the optimal duration of appropriate antibiotic therapy, at least 2 weeks should be considered, and if blood cultures continue to be positive 24 hours after removal of the ICD, a longer period (e.g., 4 weeks) should be considered. For infections limited to the pocket site, 7 to 10 days of appropriate antibiotics may be sufficient.

PROSTHETIC JOINT AND ORTHOPEDIC IMPLANT INFECTIONS

The implantation of prosthetic joints along with the use of other implantable orthopedic devices (e.g., pins, screws, plates, rods, external fixators, or Ilizarov apparatus) has improved quality of life greatly and restored function to patients suffering from debilitating bone and joint disease, tumor, or injury. Based on conservative estimates, millions of people worldwide have some form of prosthetic joint or other implantable orthopedic device. Of the possible complications associated with implantation, infection is the second most common cause of prosthetic joint failure.[17] It occurs in 0.6 to 2.4 percent of total joint replacement[123] and results in postoperative prosthesis failure, chronic pain, immobility, and, in some cases, loss of the affected limb or, in the worst-case scenario, loss of life.[4,42,119,467] The health care cost in the United States for treating a single infection of a prosthetic joint is estimated to be more than $280 million per year nationwide.[190] The number of prosthetic joint infections is increasing.[263]

Prosthetic joints and implantable orthopedic devices may become infected by two major mechanisms: (1) the prosthetic device may be contaminated by microorganisms at the time of implantation, either as a result of airborne contamination in the operating room or by direct inoculation at the time of surgery; or (2) the prosthetic device may become infected as a result of hematogenous seeding from bacteremia or by direct contiguous spread from an infection adjacent to the prosthesis. Of infections of prosthetic joints, 20 to 40 percent arise by hematogenous seeding, with the remainder occurring as a result of airborne or direct inoculation.[3,281,282] Infections may remain asymptomatic for years before symptoms become apparent, and usually a long delay occurs between onset of the infection and the appearance of symptoms and confirmation of the diagnosis. The overall rate of infection of prosthetic joints has been shown to be highest in the first 6 months postoperatively, with a steady decline occurring after this time. The incidence rate for infection of total hip and total knee arthroplasties during the first 2 years postoperatively is reported to be 5.9 infections/1000 joint-years; in contrast, during postoperative years 2 to 10, it is 2.3 infections/1000 joint-years.[466] The risk for acquiring infection and the incidence of infection depend on the anatomic location of the implanted orthopedic device or prosthetic joint, with the hips having the highest risk, followed in descending order by the knees, elbows, shoulders, wrists, and ankles.[91,395,469] For implantable orthopedic devices, rates of infection range from 2 to 30 percent.[299,511]

Risk Factors

Numerous factors have been identified as increasing a patient's risk for developing infection of a prosthetic joint or orthopedic implant, including rheumatoid arthritis, diabetes mellitus, obesity, poor nutritional status, use of steroids, immunocompromised status, psoriasis, hemophilia, sickle-cell hemoglobinopathy, solid-organ transplant, dialysis-dependent renal failure, joint dislocation, and extremes of age.[42,110,184,364,536] In addition, procedure-related factors such as previous surgery at the site of the prosthesis or implant, extended operation time (>2.5 hours), postoperative bleeding, and hematoma also increase the risk for acquiring an infection.[110,368,544] The relative risk for the development of prosthesis-related infections in patients with poor healing and wound complications increases from 13- to 20-fold after total-knee replacement and from 22- to 52-fold after total-hip replacement.[544] in addition, bacteremia from any source increases the risk for prosthesis infection.[556] For example, prosthesis-related infection developed in 34 percent of patients with *S. aureus* bacteremia who were followed prospectively.[340]

The implanted metal prosthetic device and the PMMA cement that binds the prosthetic device to adjacent bone also predispose the joint space and bone to infectious processes, given that both are foreign bodies. In vitro studies have shown that the unpolymerized form of PMMA cement predisposes to infection by inhibiting phagocytic, lymphocytic, and complement function; the risk for acquiring infection seems to be enhanced further once the cement has polymerized in the body.[379,380] Cementless prostheses have been designed in an attempt to overcome the problem of infection associated with the PMMA cement. For certain orthopedic implants, the integrity of the skin is compromised chronically, thus providing ready access to organisms from the external environment.

Microbiology

Although any microorganism can cause prosthetic joint infections, more than half of the infections are caused by *S. aureus* or coagulase-negative staphylococci.[110,556] Other gram-positive bacteria (e.g., β-hemolytic streptococci, viridans streptococci, and enterococci) cause 20 to 25 percent of infections. Antibiotic resistance in these organisms is increasing, and multiple strains of staphylococci may be present in a single prosthetic joint infection.[223,299,417,511,540] Less commonly, aerobic gram-negative bacilli, including *E. coli*, *Proteus mirabilis*, *Klebsiella* spp., *Salmonella* spp., *S. marcescens*, other Enterobacteriaceae, and *P. aeruginosa*, may cause infection. In addition, 4 to 10 percent of infections are caused by anaerobic organisms such as peptostreptococci and *Bacteroides* spp.; polymicrobial infections occur in approximately 10 percent of cases, with *S. aureus* and anaerobes the most frequently found.[304] Infections with fungi, particularly *Candida*, *Aspergillus*, and *Penicillium* spp., or with mycobacteria (i.e., *Mycobacterium tuberculosis*, *Mycobacterium avium* complex [MAC], and other rapidly growing mycobacteria) also have been described.[28,41,302,495] Rarely, a wide spectrum of other organisms, including *Corynebacterium*, *Propionibacterium*, *Bacillus* spp., and *Mycoplasma hominis*, have been reported to cause infection. Zoonotic bacteria such as *Brucella* spp., *Yersinia* spp., and *Pasteurella* spp. rarely can cause infection and should be considered in the correct epidemiologic setting.[302] Approximately 10 percent of infections are culture negative.

Certain clinical situations may predispose a patient to particular organisms as the cause of infection. Pyogenic skin infections commonly result in staphylococcal and streptococcal infections of prosthetic joints, whereas infections of the teeth and gums are frequent causes of viridans streptococcal and anaerobic infections in prostheses. Genitourinary and gastrointestinal tract procedures or infections frequently are associated with enterococcal and gram-negative bacillary infections of prostheses. The increased use of TNF-α blockers in patients with joint prosthesis predisposes them to infections with unusual pathogens (e.g., fungi or mycobacteria).

Clinical Manifestations

The clinical findings and severity of symptoms seen with infections of prosthetic joints are highly variable and determined primarily by three factors: (1) the route of infection—the hematogenous route versus direct inoculation; (2) the virulence of the infecting pathogen—*S. aureus* and, to a lesser extent, β-hemolytic streptococci and gram-negative bacilli seem to be particularly virulent pathogens capable of producing a fulminant clinical picture, whereas infection with organisms such as coagulase-negative streptococci is associated with a more chronic, indolent course; and (3) the nature of the tissue in which the microorganism proliferates—hematomas, seromas, ischemic wounds, and the tissues of diabetic patients and those receiving steroids all enhance the ability of the bacteria to proliferate and spread, thereby promoting the development of a more deep-seated fulminant infection.

The most common initial symptom is joint pain, which occurs in 95 percent of cases. Such pain can range from an acute fulminant illness with erythema, severe joint pain, swelling (38%), high fever (43%), and systemic symptoms to a chronic, slowly increasing pain in the joint without typical signs of inflammation that may be associated with the formation of a cutaneous draining sinus (32%) but no systemic symptoms.[110,556] The presence of constant joint pain is more indicative of infection than is the presence of pain occurring only with movement or weight bearing, which is more common in aseptic and mechanical complications.

For implantable orthopedic devices, the most common initial symptom of infection is erythema, swelling, pain, or drainage from the area around and adjacent to the implant. Local symptoms also may be associated with fever, especially if the device is extensive or deep-seated.

Diagnostic Studies

Laboratory screening tests commonly used to diagnose infection of a prosthetic joint or implanted orthopedic device such as peripheral WBC count, C-reactive protein (CRP), and ESR are neither specific nor sensitive (sensitivity 60% to 96% and specificity 55% to 92%).[110,459] Of these tests, CRP seems to be the better test. These test results may be normal, especially in patients with chronic, slowly progressing infection. Several biomarkers (e.g., procalcitonin, TNF, and IL-6[43,434]) have been suggested as diagnostic tests. Molecular techniques such as polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) and immunofluorescent microscopy were reported to have a sensitivity of 63 to 100 percent in detecting bacteria in prosthetic joint infections[196,306] but were not studied widely.[12,320]

The principal radiologic studies used for detection of an infected prosthetic joint include plain radiographs, arthrograms or sinograms, and radioisotope scans (indium and technetium diphosphate). Abnormalities that may be suggestive of infection and can be seen on plain radiographs include radiolucency at the bone–cement interface, motion and changes in position of the prosthetic components, evidence of osteomyelitis, cement fractures, and periosteal reaction. Intra-articular injection of dye (arthrogram or sinogram—in the presence of a sinus tract) may demonstrate abnormal communication between the joint space and the bone–cement interface. These radiographic abnormalities are present in approximately 50 percent of infected prostheses.[101,297] Nuclear scans with indium-111–labeled WBC or technetium-labeled sulfur colloid (^{99m}Tc) may be used to detect periprosthetic inflammation. Scanning techniques have a sensitivity of 80 to 86 percent and a specificity of 94 to 100 percent.[362,481] Of note, increased uptake can be seen around uninfected prostheses within the first 6 to 12 months after implantation. Therefore, positive findings after this time are abnormal and reflect inflammation (which could be due to a variety of causes) but not specifically infection.[122,200,234,358] Of interest, ^{99m}Tc-labeled ciprofloxacin (which probably binds to live bacteria at the infection site) was shown to be 94 percent sensitive and

83 percent specific for diagnosis of chronic infection.[456] Use of this technique allowed 11/12 infections of prosthetic joints to be identified, whereas ^{99m}Tc-WBC scintigraphy was positive in only seven patients.[457] Combining antigranulocyte antibody (^{99m}Tc sulesomab) and ^{99m}Tc collodial rhenium sulfide (nanocolloids) was shown to be an acceptable alternative.[458] Positron emission tomography with fludeoxyglucose F-18 (18 FP-FDG PET) was also evaluated as a diagnostic technique.[110,266,471] CT and MRI are not used routinely in the evaluation of patients with suspected infection of a prosthetic implant because of the large amount of imaging artifact created by prosthetic devices.[58,331]

Aspiration of joint fluid for culture and culture of tissue obtained intraoperatively are the optimal ways to establish a specific diagnosis of infection of a prosthetic joint. Unfortunately, the yield is only 50 to 90 percent. To optimize the yield, antibiotics should be stopped 2 to 3 days before aspiration or operative cultures are taken.[556] Although joint fluid findings indicative of an infectious process are a high leukocyte count (consisting mainly of polymorphonuclear leukocytes [PMNs]), a high protein content, and a low glucose concentration, these changes are not specific for bacterial infection and are present in only some patients. Using a cutoff value of 1700 WBC/μL with at least 65 percent PMN cells gave excellent results.[488] The fluid obtained should be cultured for a variety of organisms; Gram stain is positive in a third of cases, and a causative pathogen can be identified in only two thirds of patients.[375,460] Intraoperative cultures should include, if possible, any purulent discharge, devitalized bone, and tissue from the bone–cement interface. Vortexing, bath sonication, or both of the explanted prosthesis may increase the yield.[369] Histopathologic examination of this tissue usually reveals an infiltration of PMNs consistent with an acute inflammatory reaction but not specific for infection.[176,489] More rapid, specific, and sensitive tests (e.g., FISH, PCR, immunofluorescent microscopy) are needed to differentiate between noninfectious inflammation and bacterial infection and identify the pathogen in patients already receiving antibiotic or infected with fastidious pathogens.

Treatment

Successful treatment of an infected prosthetic joint involves extensive surgical débridement of all devitalized bone and tissue, removal of the prosthesis and all associated cement, and prolonged parenteral antibiotic therapy.[33,89] For optimal results, the débridement should be performed within 1 to 3 weeks.[110,271,303] Microbiologic cure has been found to correlate well with the extent of débridement and the completeness of removal of all residual methylmethacrylate cement. Historically, attempts at simple surgical débridement without removal of the prosthetic device in conjunction with parenteral antibiotic therapy had variable success, with relapse rates being as high as 88 percent by 2 to 4 years after therapy.[141,422,537] High failure rate was reported in *S. aureus* infections,[60,514] but in patients with a short duration of prosthetic joint infection caused by penicillin-susceptible streptococci, débridement and antibiotic therapy alone appear to be sufficient, with a low risk for relapse.[325]

Two protocols have been used for the treatment of these infections. A two-stage surgical procedure with prolonged parenteral antibiotic therapy has been shown to be one of the most successful treatment regimens with the best functional results. The first stage involves complete removal of the prosthesis and cement followed by a 6-week regimen of antibiotic therapy empirically chosen to cover the most likely organism and then tailored once the organism has been identified and its antibiotic susceptibility is known. The second stage involves reimplantation of a new prosthetic device at the end of the antibiotic course. Success rates with this procedure range from 87 to 100 percent.[138,278] An alternative method of therapy involves a one-stage exchange operation in which the infected prosthetic device and cement are removed, all devitalized tissue and bone are débrided, and a new prosthesis is reimplanted immediately, followed by 6 weeks of parenteral antibiotic therapy. Antibiotic-impregnated (i.e., tobramycin or gentamicin) methylmethacrylate cement is used in these situations, and success rates of more than 75 percent were reported.* This procedure is appropriate only for patients with infections caused by less virulent microorganisms because of the high failure rates seen when a more virulent organism such as *S. aureus* or a gram-negative bacillus is the cause of the infection. The Ilizarov method allows simultaneous treatment of infection, bone and joint deformities, bone loss, and shortening of the limb. This device includes proximal and distal circular external rings with wires passing through the bone and soft tissue from one side of the limb to the other. Although this method is used more often for the treatment of bone nonunion or bone loss with or without infection, David and colleagues[109] used this technique successfully to treat 12 patients who failed total-knee arthroplasty because of infection. Wound infection and chronic osteomyelitis caused by infection of the wire tract occur infrequently and should be treated by débridement of the infected soft tissue and curettage of the infected bone.

The two-stage prosthetic removal-reimplantation procedure coupled with the incorporation of antibiotic-impregnated cement during reinsertion of the implant, in combination with a 6-week antibiotic regimen, is the mainstay of therapy for infections of prosthetic joints. Selection of antibiotics should take into account the recent increase in methicillin resistance among staphylococci for the following reasons: MRSA infections of the prosthetic joint result in a higher incidence of treatment failure than do infections with methicillin-susceptible *S. aureus*, and the former result in longer durations of hospitalization and a low survival rate free of treatment failure.[412] Thus, vancomycin (with or without rifampin) should be the initial drug of choice for gram-positive bacteria until the susceptibility of the infecting organism is known.[457] In chronic and hematogenous infections (in which gram-negative bacteria are more common) carbapenems, aminoglycosides, or fluoroquinolones should be considered in addition to vancomycin until the

*References 74, 126, 127, 184, 373, 398, 500, 556.

microbiology results are available. Reports of treatment failures (even with vancomycin susceptibility within the treatment range [i.e., 2 to 4 μg/mL])[213,482] suggest that other antibiotics (e.g., trimethoprim-sulfamethoxazole or linezolid) should be considered. Several reports have shown that linezolid is a reasonable alternative that results in control of the infection.[328,510]

For infected implanted orthopedic devices, the success of therapy is based on total removal of the device together with parenteral antibiotic therapy. In cases in which the foreign body cannot be removed, extended parenteral antibiotic therapy should be instituted and continued until the device can be removed. The duration of therapy varies with the severity of the infection and ranges from several weeks to several years.

Prevention

Like other surgical procedures, prevention efforts should be focused on reducing the ability of the pathogen to reach the surgical site.[16] Patients should be screened for *S. aureus* colonization, and efforts should be made to decolonize those found to be carriers. The success of intranasal mupiricin or bathing with chlorhexidine in reducing the rate of surgical site infections suggests that such measures should be considered for patients receiving prosthetic joint or other implantable orthopedic devices.[53,377]

The use of prophylactic antibiotics for dental procedures in patients with an orthopedic device is controversial. Although no evidence supports such prophylaxis,[44] the American Academy of Orthopedic Surgery recommended it for all patients with joint implants.[13] The practice of using antimicrobial-loaded bone cements for prophylaxis is also controversial. Retrospective studies found it to be effective, but prospective studies found no extra benefit compared to systemic antibiotic prophylaxis.

CENTRAL NERVOUS SYSTEM SHUNTS

Infection is a major cause of morbidity in children who undergo CSF shunting procedures.[243,260,444] Such procedures are performed to divert CSF in symptomatic hydrocephalic patients and are used commonly in patients with anatomic abnormalities (e.g., meningomyelocele and Chiari malformations), in premature newborns with intraventricular hemorrhage or intracranial infections (e.g., congenital cytomegalovirus, congenital toxoplasmosis, and bacterial meningitis), and in patients with central nervous system (CNS) tumors or head trauma. Usually, the proximal end of the shunt is placed in the frontal or fourth ventricle and the distal end is inserted into the peritoneal cavity (i.e., ventriculoperitoneal shunt). Other compartments such as the right atrium (i.e., ventriculoatrial shunt), the pleural cavity, or the gallbladder can be used to place the distal end. CSF shunts are prone to complications, with a 10-year failure rate greater than 50 percent.[117] The most common complication is mechanical (such as obstruction or overdrainage from siphoning), followed by shunt-related infection. The incidence of shunt-related infection varies considerably, from 0.3 to 26 percent.[66,99,132,182,243,260,444] These infections increase morbidity rates and, in some cases, significantly affect patient outcomes. In addition, the increased morbidity caused additional expenses and resulted in higher costs to the health care system.[496] Understanding some of the factors that contribute to the development of these infections has helped reduce their incidence.[136,526]

Epidemiology

Almost two thirds of shunt-related infections occur within 1 month after placement of the shunt, and 90 percent of infections are manifested within 6 months.[181,257,321,428] The incidence of shunt-related infections is significantly higher in infants in the first 6 months of life than in older children or adults.[128,269,382,516] The infection rate is even higher in newborns with intraventricular hemorrhage who undergo shunting in the first week of life[14] and in premature infants.[260,322] Reasons for the increased incidence of shunt-related infection in very young patients are multifactorial. Several mechanisms that have been suggested include delayed wound healing, higher skin density of bacteria that are more resistant to antibiotics and more adherent to the shunt than in older children, longer duration of hospitalization, surgical technique, and increased exposure to antibiotics just before the shunt is placed.

No significant difference in infection rate is seen in patients with ventriculoperitoneal or ventriculoatrial shunts,[354,441] but a lower infection rate was noted in those with lumboperitoneal or cholecystic shunts. An increased risk for development of infection was reported for shunts placed immediately after removal of a previously infected shunt, probably because of incomplete eradication of bacteria.[131,246] Several other factors, including the underlying cause of the hydrocephalus,[128] the surgeon's experience,[92,242] previous shunt infection,[315] the duration of surgery, the number of people in the operating room, operative technique (e.g., prophylactic antibiotics, skin preparation, and shaveless operation),[210] operative time, open surgery to insert the abdominal catheter versus direct puncture of the abdominal wall with a trocar, postoperative CSF leakage, and others,[260,444] have been reported to be associated with an increased incidence of shunt-related infection. Although a trend toward more shunt-related infections has been reported with these factors, it has not been demonstrated consistently. A few studies suggest that tapping the shunt may result in an infection, with an incidence ranging from negligible to greater than 30 percent in premature infants with shunts tapped multiple times.[66] Reinfection rate was estimated to be around 25 percent.[243,261,493] Multiple factors were suggested as contributing to reinfection, including treatment duration of the first infection, age younger than 6 months, male sex, nonsurgical management, intraventricular hemorrhage (IVH) of prematurity, intracranial cysts, or aqueductal stenosis.[322,444,493] The median time to reinfection (i.e., second episode) ranged from 65 to 90 days.[261,493] Almost 60 percent of children with second infection developed a third reinfection, and 47 percent of

TABLE 74-2 **Pathogens Causing Cerebrospinal Fluid Shunt Infection**

Pathogens	Incidence (%)
Gram-Positive Bacteria	
Staphylococcus, coagulase-negative (e.g., *Staphylococcus epidermidis, Staphylococcus capitis, Staphylococcus hominis, Staphylococcus warneri, Staphylococcus lugdunensis, Staphylococcus haemolyticus*)	25-70
Staphylococcus aureus	10-40
Streptococci (e.g., *Streptococcus pyogenes,* group B or C streptococci, *Enterococcus, Streptococcus pneumoniae*)	3-7
Propionibacterium spp.	Rare
Corynebacterium spp.	1-2
Gram-Negative Bacteria	
Escherichia coli	5-25
Klebsiella spp.	5-10
Proteus spp.	2-6
Pseudomonas spp.	2-4
Acinetobacter spp.	1-3
Other gram-negative bacteria (e.g., *Neisseria* spp., *Haemophilus influenzae, Pasteurella*)	<1
Fungi	<1
Candida spp.	
Histoplasma	
Cryptococcus	
Torulopsis	

them developed a fourth infection.[494] The median time to the second infection was 65 days, for the third infection 477 days, and for the fourth infection 2137 days.[493]

Etiology

Staphylococcal species are the most common cause of shunt-related infection, with coagulase-negative staphylococci (e.g., *S. epidermidis*, *Staphylococcus capitis*, *Staphylococcus hominis*, and *Staphylococcus lugdunensis*)[125] being isolated in 25 to 70 percent of cases (Table 74-2).[125,254,269,441] *S. aureus*, the second most common gram-positive bacterium, is responsible for 10 to 40 percent of cases. Streptococci (e.g., viridans, group B or C, *S. pyogenes*, *S. pneumoniae*, enterococci) are identified less commonly (3% to 7%). Other gram-positive bacteria such as *Propionibacterium*[269,485] and *Corynebacterium* (diphtheroids)[23,173] are isolated as well. The seemingly increased incidence of shunt-related infections caused by these two groups of bacteria probably is the result of poor culture technique (e.g., failure to use anaerobic culture media, less than 5 to 7 days' incubation period), misinterpretation of culture results (e.g., culture contamination) leading to underreporting, or both.

Gram-negative bacteria (e.g., *E. coli*, *Klebsiella* spp., and *Proteus* spp.) together are the cause of 5 to 25 percent of shunt-related infections.[464] *Pseudomonas* spp. and *Acinetobacter* spp. are reported as well, but less frequently. Shunt-related infections caused by gram-negative bacteria occur more commonly in patients with myelomeningocele and those in whom the distal part of the ventriculoperitoneal shunt is inserted into the peritoneal cavity via a percutaneous trocar (i.e., with an inadvertently perforated intestinal tract). Many other etiologic agents, including fungi[82] (e.g., *Candida*,[31,158] *Histoplasma*,[429] *Cryptococcus*,[216] *Torulopsis*[523]), *Pasteurella multocida*,[272] *Neisseria* spp.,[211,470] *S. marcescens*,[63] *Listeria monocytogenes*,[276] nontuberculous mycobacteria,[78] and others, have been reported less commonly as causing shunt-related infections. With the increase in the number of patients who are immunocompromised for various reasons (e.g., neutropenia, chronic intravenous catheters, prolonged administration of broad-spectrum antibiotics, and hyperalimentation), the incidence of these rare infections will increase. Bacteria that traditionally cause meningitis, such as *H. influenzae*,[440,516] *S. pneumoniae*,[354,441,517] and *Neisseria meningitidis*,[273] were reported as causing shunt-related infections. Whether these cases were isolated shunt-related infections or extension of meningitis into the ventricular system (i.e., ventriculitis) is not clear. Therefore, if such bacteria are isolated from a suspected shunt and the patient has a communicating hydrocephalus, lumbar puncture should be performed to rule out meningitis. Of interest, most children with *H. influenzae* or *S. pneumoniae* infections recovered with only antibiotic therapy and without shunt removal.[440]

Pathogenesis

Several observations suggest that most CNS shunt–related infections are caused by inoculation of the organism during surgery or bacterial colonization during the early phase of wound healing.[483] Contamination of the device by ward personnel during manipulation also was suggested.[380,522] These observations include the facts that common skin flora (e.g., coagulase-negative staphylococci and *S. aureus*) are the pathogens most frequently encountered, most of the infections occur within the first few weeks after surgery, and irrigation of the system is a risk factor for the development of infection. Another common mechanism (occurring with gram-negative bacterial infections) is retrograde progression of bacteria from the gastrointestinal tract (i.e., bowel perforation)[206,407] or from the urinary tract (in the case of a ventriculoureteral shunt).[156] Other mechanisms by which shunts become infected include (1) hematogenous infection in which a distant site of infection produces bacteremia leading to a shunt infection (this type of infection occurs quite rarely and in most cases represents meningitis with secondary infection of the shunt, such as *S. pneumoniae* or *N. meningitidis* CNS infection in patients with a shunt) and (2) wound or skin infection (e.g., cellulitis or decubitus ulcer), with direct extension from the infection site to the shunt.

The predominant role of coagulase-negative staphylococci and *S. aureus* in CNS shunt–related infection is the result of their being the major constituents of normal cutaneous flora, especially in young children,[279,433] and

their having the ability to adhere directly to the shunt (e.g., *S. epidermidis*) or to host proteins covering the shunt (e.g., *S. aureus*). In addition, coagulase-negative staphylococci (and some *S. aureus* strains) produce large amounts of extracellular slime (i.e., biofilm) that completely covers the organism.[62] More than 60 percent of staphylococci isolated from infected shunts produce biofilm.[113,133,177] The biofilm of *S. epidermidis* is a mixture of teichoic acid and protein. Production of biofilm also was reported in corynebacterial infection, which may explain the increasing importance of this bacterium in CNS shunt–related infections.[36] The biofilm facilitates attachment of these organisms to the surface of the shunt and protects the bacteria from the host's immune defenses (i.e., reduces phagocytosis). Once the organisms are attached to the shunt material, they are extremely difficult to remove except by completely replacing the shunt. In addition, penetration of antibiotic into the biofilm is variable, and the biofilm antagonizes the antimicrobial activity of some antibiotics (e.g., vancomycin).[118,137] The importance of production of biofilm for the establishment of shunt-related infection caused by coagulase-negative staphylococci was shown by Younger and colleagues,[553] who found that 88 percent of the coagulase-negative staphylococci strains isolated from true shunt-related infections produced slime. Moreover, infections caused by nonadherent organisms were significantly more likely to be cured by antibiotics alone (without removal of the colonized shunt) than were infections caused by adherent organisms. Similarly, Diaz-Mitoma and coworkers[113] found that both obstruction of ventriculoperitoneal shunts and failure to cure the infection with antibiotics alone occurred more frequently when infectious episodes were caused by biofilm-producing coagulase-negative staphylococci. Therefore, complete removal of the shunt should be considered in patients with coagulase-negative staphylococci or *S. aureus* infection because biofilm-producing organisms may not be treated effectively when the shunt is in situ.

S. aureus infection is established primarily by the production of adhesin proteins. The most important proteins are fibronectin-binding (finb A and finb B) and fibrinogen-binding (Clf A and Clf B) proteins.[144] The ability to bind to fibronectin is very common in isolated strains of *S. aureus*, and its efficiency depends on the amount of finb A and finb B expressed on the cell surface of the individual isolate. The two fibrinogen-binding proteins attach to different parts of the host ligand, which suggests that they are acting synergistically or allow the bacteria to adhere to the ligand even during unfavorable conditions (e.g., antibodies against one of them).

The immature humoral immune system of young infants is not likely to explain the increased incidence of shunt-related infections in patients younger than 6 months old because these infants mount antistaphylococcal antibody responses that are comparable to those of older children.[389] Although levels of immunoglobulins and complement proteins are lower in this young group, levels of these proteins normally are very low in the CSF of older individuals (CSF levels of IgG and IgA are between 0.25% and 0.5% of those in serum). In addition, the types of bacteria causing CNS shunt–related infections are not associated commonly with humoral immunodeficiency states, thus suggesting that humoral protection is less important in CNS shunt–related infections. Little is known about the possible role of reduced tissue immunity in these infections.

The foreign body nature of the shunt apparatus plays an important role in the local host defense defect.[55] Electron-microscopic findings demonstrate irregularities in catheters that allow microorganisms to be buried in the catheter. In addition, the function of neutrophils is suboptimal because phagocytic and bactericidal activities are reduced as a result of the loss of lysosomal contents. Therefore, even when pathogens are phagocytized, they may not be killed and are protected from antibiotics that do not penetrate the cell membrane.

Other mechanisms that may contribute to shunt-related infections include (1) abnormal CSF flow (i.e., not being absorbed by the venous sinuses, thought to be important for prevention of infection in the CNS) and (2) interruption of the blood-brain barrier by the shunt catheter, with the creation of a direct tract between the subcutaneous tissues and the ventricles resulting in significant compromise in host defenses.[522]

Clinical Manifestations

The initial signs and symptoms of most patients with shunt-related infections are nonspecific and include mild to moderate fever, malaise, irritability, nausea, vomiting, vague abdominal pain, and headache. With such nonspecific findings, the physician must be careful to differentiate between the possibility of a shunt-related infection and an intercurrent viral or bacterial infection of the upper respiratory, urinary, or gastrointestinal tract. Examination of the CSF (from a shunt tap) may be of help (see the discussion of diagnosis). Only a minority of patients have the classic signs and symptoms of CNS inflammation, such as a stiff neck, bulging fontanelle, change in mental status, cranial nerve palsy, or papilledema. In some patients the shunt tract may be infected, with evidence of cellulitis, dehiscence, or both of the surgical wounds. Tenderness, edema, or erythema along the tract itself may be the only sign. Patients with known seizures (e.g., epilepsy) frequently develop seizures with malfunctioning shunt (inducing infection).[251]

The type of shunt affects the nature of the infection. For example, ventriculoperitoneal shunt–related infections may cause symptoms and signs confined to the abdominal cavity, such as abdominal pain, tenderness (with or without guarding), intestinal obstruction,[403] or spontaneous bacterial peritonitis.[157,330] Rarely, a distal shunt-related infection will be manifested as frank ascites as a result of CSF malabsorption.[262] A relatively common complication of ventriculoperitoneal shunts is an inflammatory peritoneal exudate that may lead to CSF loculation and the subsequent formation of a peritoneal pseudocyst.[15,404] These pseudocysts often are palpable and can be visualized by ultrasonography or CT. Bacteria are isolated in a third of cases, suggesting that infection may play a role in the pathogenesis of pseudocysts. In most cases, however, a high index of suspicion is required because the initial symptoms frequently are abdominal,

with or without food intolerance and with no other signs of shunt malfunction.

A unique complication of patients with ventriculoatrial shunts is the development of immune complex disease such as shunt nephritis (a form of acute glomerulonephritis), arthritis, or rash.[414] In most of these cases, the infecting organism is *S. epidermidis*, but other bacteria such as *Corynebacterium* can cause this complication.[54] In the case of shunt nephritis, the patient has fever, edema, malaise, hepatosplenomegaly, hypocomplementemia, anemia, azotemia, hematuria, and proteinuria.[103] Pathologic findings consist of mesangial hypercellularity and granular deposits of immunoglobulins and complement along the glomerular membrane. Rarely, arthritis may be the initial symptom because it may develop by the same immunologic mechanisms that cause nephritis.[274]

Diagnosis

Although shunt-related infections are not common occurrences, the nonspecific signs and symptoms and the insidious onset in many cases render establishing a diagnosis very difficult. Therefore, any patient with a CNS shunt and fever without an obvious source should be suspected of having a shunt-related infection, especially if the symptoms continue for longer than a week. A higher index of suspicion for shunt-related infection is needed in young patients, in whom fever develops within 3 to 6 months after placement of the shunt. The only definitive diagnostic test is direct observation and culture of CSF. Tapping the shunt or sampling fluid in direct contact with the shunt should be performed if no signs or symptoms of increased intracranial pressure are noted. CT of the head is recommended before the tap is done if such symptoms exist. The shunt tap should be performed with utmost attention given to sterile technique. The tap should be done by a neurosurgeon or physician who is familiar with the technique and the underlying hardware.

When percutaneous needle aspiration is performed, the area around the shunt reservoir should be scrubbed with antiseptic soap and the surrounding hair shaved (2 inches in each direction). The scalp area should be prepared by repeated application (at least three times) of povidone-iodine solution followed by alcohol. A 21- or 23-gauge butterfly needle is placed into the reservoir (or valve if no reservoir is present). Measurement of opening pressure can help diagnose a distal malfunction (i.e., increased pressure) or proximal shunt obstruction (i.e., less than expected pressure). CSF sample aliquots then should be allowed to drip into sterile vials. Gentle aspiration of CSF sometimes is performed if no fluid returns spontaneously. If only a few drops of CSF can be obtained, the more important tests, Gram stain and culture, should be performed first. Culturing the shunt wound, blood, or CSF obtained by lumbar puncture (which usually is not communicating with the ventricular fluid) often is unrevealing, misleading, or both. Although bacteremia frequently is present in patients with ventriculoatrial shunts and may help diagnose the etiologic agent, blood cultures generally are negative in patients with all other shunts (e.g., ventriculoperitoneal or pleural shunts). In addition, coagulase-negative staphylococci are the most common contaminants of blood cultures, and, therefore, interpreting a positive blood culture in these patients would be difficult.

CSF should be tested for glucose concentration, differential cell count, Gram stain, and culture. Protein concentration is requested often but is of very limited help in evaluating the presence or absence of an infection because high protein levels are found in many patients with shunt malfunction and no infection. In contrast, normal protein levels have been reported in many patients with shunt-related infections. A low glucose level suggests an infection, but one should confirm that the CSF sample was not diluted before the test was performed. Some physicians use saline to get a better flow of CSF (because of an occluded tube), which may affect the biochemical results. Of importance is to note that in many cases of shunt-related infection, the glucose level is within the normal range. Usually, pleocytosis with a predominance of PMNs is indicative of a shunt-related infection. A count of 100 cells/μL or more is highly suggestive of infection.[268,277,319] A WBC count greater than 100 cells/μL with more than 10 percent PMN cells in febrile patients is 82 percent sensitive and 99 percent specific for shunt infection, with a positive predictive value of 93 percent.[319] In contrast, if the PMNs are less than 10 percent, the negative predictive value (i.e., no infection) is 95 percent. A recent review suggests that infection with gram-negative bacteria resulted in a higher initial and peak WBC count, with a greater percentage of PMNs than gram-positive bacteria.[151] In contrast, *P. acnes* was associated with the lowest WBC and PMN percentage. The possibility that these conclusions were affected by a higher percentage of shunt colonization or culture contamination with gram-positive bacteria and *Propionibacterium* spp. should be kept in mind when interpreting these results. Although in some cases the finding of a positive CSF culture is interpreted as a shunt-related infection despite a normal WBC count (<10 WBCs/mm^3), the absence of clinical symptoms (e.g., fever) in many of these patients suggests that the positive culture probably represents colonization or contamination. Other cells, such as mononuclear cells[257,464] or eosinophils[319,532] may predominate during an infection. If eosinophilia is the predominant cellular response, an allergy to the shunt (e.g., silicone[225]) or the materials used for sterilization (e.g., ethylene oxide[386]) or use of an antibiotic-impregnated catheter[494] or intraventricular administration of antibiotics (e.g., gentamicin[329] and vancomycin[171]) should be considered. Other conditions should also increase the eosinophil count in the CSF.[440] It is important to note that eosinophilia (without these causes, including infection) is relatively common (but transient) in children with CNS shunt.[150,188] Leakage of CSF under the skin or blood in the CSF are two possible reasons for this phenomenon.

Interpretation of the WBC count should be done cautiously if the red blood cell count is high because the increased number of WBCs can be the result of blood spilling into the CSF without any infection or be part of the inflammatory response to the presence of blood (i.e., chemical ventriculitis).[381] A positive Gram stain with an increased CSF WBC count, reduced glucose level, or both is helpful in making the diagnosis of a shunt-related infection. A negative Gram stain result

does not exclude an infection, and one should wait for the results of culture. Ventricular fluid always should be cultured anaerobically as well as aerobically. Although most bacteria causing shunt-related infections grow within 48 to 78 hours, cultures should be held for 7 days (if still negative) for fastidious organisms such as *Propionibacterium*.[25] The possibility of contamination or colonization of the shunt without infection should be considered when the culture is positive but other CSF parameters are normal. If such a scenario occurs and bacteria are growing only from one sample (e.g., a shunt tap) and not from follow-up cultures (i.e., from extraventricular drainage), a shorter course of therapy (see later discussion) may be sufficient.

Blood cultures, a peripheral complete blood count (CBC), and ultrasound of the abdomen (for ventriculoperitoneal shunts) are of limited value. For example, although 90 percent of patients with ventriculoatrial shunt–related infection will have a positive blood culture, fewer than 10 percent of patients with other shunt-related infections will have a positive culture. In addition, in more than a third of patients with shunt-related infections, no elevation in the peripheral WBC count was found. Some investigators suggest that blood but not CSF CRP levels may be helpful in establishing the diagnosis of shunt-related infection when other concurrent infections (e.g., sinusitis, pneumonia) were excluded.[268,425] One suggestion is that if the CRP level is less than 7 mg/L, the shunt should not be removed because no infection is present. More data are needed to verify this observation. In patients with a ventriculoatrial shunt–related infection, measurement of serum antistaphylococcal antibodies or the C3 and C4 components of the complement cascade may aid in establishing the diagnosis.[37,414] The triad of fever, abnormal CSF WBC count, and greater than 5 percent eosinophilia is highly predictive of a shunt-related infection.[319]

Treatment

A variety of medical and surgical approaches to treatment of an infected shunt have been suggested.[532] Regimens include (1) the use of antibiotics alone (systemically, with or without intraventricular administration) without replacement of the shunt; (2) removal of the infected shunt followed by immediate insertion of a new shunt and the administration of systemic or intraventricular antibiotics or both[344]; (3) removal of the infected shunt and insertion of an extraventricular device (EVD) to monitor the patient's response to the accompanying antibiotic therapy, with a new shunt inserted only when the ventricular system is sterilized; (4) removal of the infected shunt followed by a stereotactic third ventriculostomy and administration of antibiotics; and (5) externalization of only the distal (e.g., peritoneal) catheter, along with the administration of systemic or intraventricular antibiotics or both.

The use of antibiotics alone without surgery was justified by the need to maintain CSF drainage and avoid costly operations and lengthy duration of hospital stay. The low success rate of this approach (33%) and the higher mortality rate associated with it suggest that it should not be used (Table 74-3). Of interest, the failure rate was much higher with infections caused by slime-producing organisms than with infections caused by non–slime-producing bacteria.[113] Only in shunted patients

TABLE 74-3 **Shunt Infection Cure Rates in Relation to the Therapeutic Approach**

First Author	Antibiotics Alone*	Antibiotics and Immediate Replacement with a New Shunt	Antibiotics, Removal of the Shunt, and Insertion of an EVD
Schoenbaum[423]	5/30†		25/26
Nelson[345]	10/13		46/46
Salmon[413]		5/10	
Sells[432]	1/8	1/6	9/9
James[222]	3/10		9/10
Venes[507]		6/9	3/3
James[221]	4/11	11/13	16/17
Wald[520]	15/20		
Mates[310]	7/8		
Shurtleff[439]	2/27	6/20	19/19
Morrice[338]	4/14	19/23	14/18
Frame[147]	8/11	21/27	
Forward[143]	8/15	2/2	13/13
Luthardt[293]	1/17		
O'Brien[352]	11/11	15/19	9/9
Walters[522]	13/92	11/21	44/71
Swayne[475]			19/20
Ronan[405]	3/4	4/7	21/22
Stamos[463]			23/23
Younger[553]	4/11	42/46	
Total (success rate)	**99/302 (32.8%)**	**143/203 (70.4%)**	**270/306 (88.2%)**

*With and without intraventricular antibiotics.
†Number cured/number treated.
EVD, Extraventricular device.

with purulent meningitis caused by *S. pneumoniae*, *N. meningitidis*, or *H. influenzae* did the administration of systemic antibiotics alone without removal of the shunt seem to be an acceptable option.[273,359]

Combining immediate replacement of the infected shunt with a new shunt and antibiotic therapy has a higher rate of success (70%, see Table 74-3) than does the use of antibiotics alone. Nonetheless, it is less effective than removal of the infected shunt accompanied by insertion of an EVD and administration of antibiotic therapy (88% success rate, see Table 74-3). A decision analysis of 17 studies reached the same conclusion and suggested that "this treatment option has the highest cure rate and the lowest failure and mortality rates."[424] In addition, lack of the ability to monitor when the ventricular fluid is sterilized results in a longer period of systemic antibiotic therapy (e.g., 4 to 8 weeks), which may lead to an increase in iatrogenic infections and cost. Some surgeons suggest removing the shunt and delaying replacement (i.e., a few days after removal to allow sterilization of the shunt's tract), but this approach is associated with increased morbidity.[143,524]

For infection that involves only the distal part of the shunt (e.g., pseudocyst, appendicitis,[370] erythema or swelling along the shunt tract, or surgical wound infection), externalization of only the distal end of the shunt along with the administration of antibiotic therapy is recommended by some neurosurgeons. Potential advantages of this technique include (1) diversion of CSF from an infected area to avoid ascending infection, (2) maintenance of CSF flow to prevent increased intracranial pressure (ICP), (3) the ability to perform frequent CSF sampling, and (4) the capability of monitoring therapy. The disadvantage is that early infection or colonization of the proximal portion of the shunt may be obscured by the antibiotic treatment and become active after discontinuation of therapy and reinsertion of the distal part. In patients with *P. acne*–related distal shunt infection, catheter removal without (or very short) antibiotic treatment is sufficient.[25]

Internal shunting by a third ventriculostomy with avoidance of a prosthetic device during the infected CSF shunt removal was investigated as another option to reduce reinfection. Although this procedure was shown to be effective in managing patients with refractory shunt-related infections who have a noncommunicating hydrocephalus, patent subarachnoid space, and adequate CSF absorption, the preventive effect for reinfection was marginal.[143,313,346,437,441] Yet, it seems that the reinserted ventriculoperitoneal shunts after a third ventriculostomy have a better longevity than those inserted without this procedure.[437] Shunt independence for extended periods was documented in patients without myelomeningocele. The success rate is lower in those with myelomeningocele or hemorrhage or after meningitis. Disadvantages of this technique include increased morbidity (e.g., hypothalamic injury and subarachnoid hemorrhage), technical difficulty in younger children, and, if the stereotactic technique is used, cost and availability of the necessary equipment.[130]

The most effective treatment of shunt-related infections is to remove the entire infected shunt and insert an EVD to control ICP and monitor the infection (i.e., provide CSF access). After antibiotic therapy has been successful, a new shunt is placed. With this approach, treatment success is very high (see Table 74-3), with more rapid clearance of the infection and a shorter duration of therapy. The choice of intravenous antibiotic depends on local patterns of antimicrobial susceptibility and the ability of the antibiotic to penetrate the blood-brain barrier. With the increasing rate of methicillin-resistant staphylococci, vancomycin should be used as initial therapy while awaiting bacteriologic identification and the results of antibiotic sensitivity testing. Effort should be made to discontinue vancomycin as soon as the infecting bacteria are found to be sensitive to the semisynthetic penicillins so that the chance of the bacteria becoming resistant to vancomycin is reduced. In addition, even if in vitro data suggest that the bacteria are highly sensitive to the first-generation cephalosporins (e.g., cephalothin, cefazolin, and cephapirin), they should not be used because they penetrate the blood-brain barrier poorly. Linezolid has been used successfully in cases of shunt infection.[52,75,350,512,550] This drug has a good CSF penetration, with concentrations above the minimal inhibitory concentration (MIC) of most gram-positive bacteria, including MRSA.[38,552]

It is also effective against bacteria living in biofilms. The limited data available suggest that linezolid should be considered cautiously when vancomycin fails or cannot be given.

To achieve more consistent and efficient eradication of bacteria, the CSF drug level should be at least 10 times higher than the MIC of the pathogen. Therefore, the dosage of antibiotic or antibiotics and the dosing interval should be maximized (i.e., "meningeal schedule"). If the selected antibiotic does not clear the infection within 2 to 3 days and no improvement occurs in CSF biochemical and WBC parameters (i.e., seemingly no control of the infection), measurement of the bactericidal titer of ventricular fluid should be considered. To determine bactericidal titer, 1 mL of CSF (if CSF production is >5 mL/hr) at the expected peak antibiotic level (i.e., 2 to 3 hours after the antibiotic is given parenterally) is diluted serially with culture media to produce dilutions of 1:2, 1:4, 1:8, and 1:16. To these dilutions, an equal amount of medium with 10^5 colonies per milliliter of the offending bacteria is added (the final CSF dilution is 1:4, 1:8, 1:16, and 1:32). After 24 hours of incubation, the tubes are observed for turbidity, which reflects the growth of bacteria. If no turbidity is seen in the 1:8 and 1:16 or higher dilution tubes, the CSF level of the antibiotic probably is sufficient, and continued growth of bacteria from CSF may be caused by colonization of the EVD or contamination. On the other hand, if turbidity is noted in the tube with less than a 1:8 dilution (i.e., a 1:4 dilution), the CSF level of antibiotic may not be sufficient to combat the infection and the addition of another antibiotic or change to a different antibiotic is warranted.

In vitro synergy studies can help determine the best drug combination. Rifampin should be considered as one of the drugs in the combination for gram-positive bacteria for three reasons. First, most staphylococci are still sensitive to this antibiotic and their MIC usually is 10-fold

lower (i.e., 0.05 μg/mL) than that of other antistaphylococcal drugs (e.g., vancomycin, 0.5 μg/mL). Unfortunately, the use of rifampin alone may be followed rapidly by rifampin-resistant variants because they already are present in small numbers in any staphylococcal population. Second, rifampin penetrates CSF well and easily achieves a greater than 10-fold level over the MIC of most staphylococci. Third, rifampin has demonstrated good bactericidal activity even when staphylococci were embedded in slime. In contrast, staphylococcal slime inhibits the antimicrobial activity of vancomycin.[137]

Selection of the initial antibiotic before culture results are known can be based on the patient's clinical and CSF findings. An algorithm for initial antibiotic therapy before CSF culture results are available is presented in Figure 74-2. This algorithm should not be used for neonates or immunocompromised patients with suspected shunt-related infections because they often have less severe clinical symptoms and CSF response. The decision-making process starts with assessment of the patient's clinical condition. Usually, patients with infection in the CNS are only mildly symptomatic, whereas patients with *S. aureus* or gram-negative bacterial infection often are more seriously ill.

The next step is evaluation of the Gram stain. If the Gram stain result is positive for gram-positive bacteria (e.g., *Staphylococcus* or *Streptococcus*), the drug of choice is vancomycin. If the Gram stain shows gram-negative bacteria, the drug of choice is cefotaxime or ceftriaxone. Treatment with an aminoglycoside is acceptable, but the outcome seems to be less favorable because of poorer penetration into CSF.[363] If the Gram stain result is negative, other CSF parameters (e.g., WBC count and glucose) should be examined. If either is abnormal and the patient is not severely sick, vancomycin alone should be started because the chance of having a gram-negative infection is low.[463]

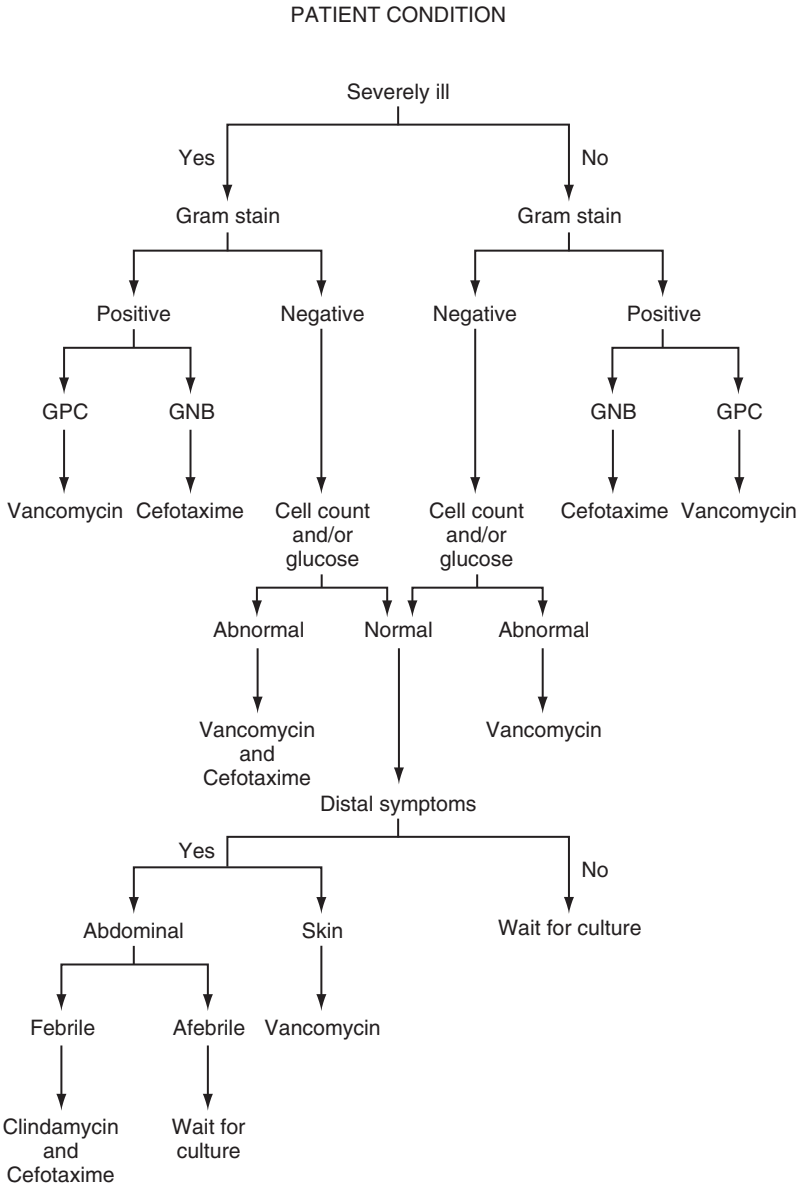

FIGURE 74-2 ■ Algorithm for selection of antibiotic therapy before culture results are known. *GNB,* Gram-negative bacillus; *GPC,* gram-positive coccus.

In contrast, if the patient is more seriously ill, coverage for gram-negative bacteria should be added (e.g., cefotaxime or ceftriaxone). In nontoxic patients with normal CSF parameters and no distal symptoms (e.g., peritonitis or wound or tract infection), antibiotic therapy can be withheld until the results of culture are known. On the other hand, if distal signs or symptoms exist, therapy is tailored according to the site. In patients with skin involvement, vancomycin is the drug of choice, whereas in febrile patients with abdominal symptoms, the combination of cefotaxime (or ceftriaxone) and clindamycin (to cover both gram-positive and anaerobic bacteria) is preferred.

Direct instillation of antibiotics into the ventricular system to increase their levels is suggested by some experts.[555] Such treatment may be considered in patients with persistently positive cultures despite intravenous therapy or multidrug-resistant organisms requiring specific antibiotics with inadequate CSF penetration. Several studies have reported promising results using combination of intravenous and intraventricular therapy.[127,287,479] Unfortunately, the suggested doses for intraventricular treatment have been determined empirically on only a small number of patients, and their pharmacokinetics and pharmacodynamics have not been studied well. This therapy is not without hazard, especially when the recommended doses often are much higher than those found to cause neurotoxicity.[252,448,528] Pleocytosis and eosinophilia also have occurred in patients receiving intraventricular vancomycin[290] or gentamicin.[329] In addition, preservative-containing preparations should be checked for appropriateness for intraventricular instillation. Limited pharmacokinetic data suggest that clearance of the instilled intraventricular antibiotic is sufficiently slow to allow once-daily administration. If possible, the EVD should be closed for 30 to 60 minutes after the drug is administered. If the EVD cannot be closed and the amount of CSF drainage exceeds 7 to 10 mL/hr, the frequency of intraventricular antibiotic instillation should be increased to twice daily.

Antibiotics commonly recommended for intravenous and intraventricular use according to the etiologic agent are shown in Table 74-4. A small study in adults showed that administration of vancomycin 10 mg intraventricularly once daily was safe and achieved levels greater than 5 μg/mL (the recommended CSF trough level needed to achieve cure) for up to 21 hours.[383] Because of the potential toxicity of the empirically recommended intraventricular antibiotic doses and the unpredictability of CSF levels, irrigation of the ventricular system with a known concentration of an antibiotic solution is preferred when systemic antibiotics fail to eradicate the bacteria. To achieve irrigation, two EVDs must be inserted to produce a continuous flow of solution. The concentration of antibiotic in the solution should be equal to the highest safe plasma level when the drug is given intravenously. For example, for treating gram-negative bacteria sensitive to amikacin, amikacin at a dose of 30 to 40 mg/L of saline solution (producing a concentration of 30 to 40 μg/mL) is recommended. Gentamicin 10 to 12 mg/L (using a special intrathecal preparation) is an acceptable alternative when the gentamicin MIC for the bacteria is less than 1 μg/mL. The antibiotic solution is administered through one EVD at a rate of 10 mL/hr, and the second EVD is left open to drain the fluid and to remove debris and pus from the ventricles.

When antibiotic therapy is completed, abnormal CSF findings such as low glucose or mildly elevated protein or cell counts should not delay reshunting. The duration of treatment is empirical and depends on the etiologic agent, the CSF parameters at initial evaluation, and the time to sterilization. In our institution, we found the following schedule to be successful. If CSF parameters are *normal* but culture yields coagulase-negative staphylococci only from the operating room (i.e., the initial sample), therapy should be given for only 3 to 4 days. If subsequent cultures also are positive, therapy should continue until negative cultures have been obtained for 7 days. If CSF parameters are *abnormal* in the operating room and culture is positive *only* from that specimen, therapy should continue for 7 days. If subsequent samples show abnormal CSF findings and positive cultures, therapy should be extended until negative cultures have been obtained for 10 days. This protocol was adopted by the Infectious Diseases Society of America guidelines for the management of CSF shunt infections.

A longer duration of antibiotic therapy is recommended with other bacteria (e.g., *S. aureus*, gram-negative bacteria). If culture is positive *only* on samples from the *operating room* and CSF findings are normal, treatment should be given until negative cultures have been obtained for 7 days. In all other situations, therapy should continue until negative cultures have been achieved for 10 days. Reshunting should take place immediately at the end of treatment. No benefit is found in observing the patient for a time without antibiotics for relapse of the infection.[526]

Complications

Shunt-related infections are associated with increased morbidity and mortality rates. Patients with shunt-related infection have an increase in shunt-related operations, which contributes to the increased morbidity and cost. In addition, these patients have been shown to have an increase in mortality rates in contrast to patients without shunt-related infection.[448] Even when the original infection is treated successfully, secondary infection (or contamination) of the EVD occurs in 5 to 10 percent of cases. To minimize the risk for development of a secondary infection, a sterile closed-drainage system should be maintained carefully. Only trained personnel should be allowed to drain CSF samples from the system, and injections into the system should be avoided. Continuous external drainage of CSF also causes loss of electrolytes and fluid. Therefore, routine assessment of serum electrolytes once or twice weekly is recommended, and the total daily amount of drained CSF should be replaced. Recurrent shunt-related infection after completion of treatment is common, with two thirds of such infections being caused by the same organism.[243] Unusual complications of ventricular shunt infections include brain abscess and subdural empyema.[132,519]

TABLE 74-4 **Recommended Intravenous and Intraventricular Antibiotic Therapy for Shunt Infection According to Etiologic Agent**

Etiologic Agent	Antibiotic	Intravenous Dose (mg/kg/day)	Intraventricular Dose (mg/day, 1 Dose)
Bacteria			
Staphylococcus aureus or coagulase-negative staphylococci			
Methicillin-sensitive	Oxacillin*,†	200 (every 8 hr)	NA
	or		
	Nafcillin*,†	200 (every 8 hr)	50-75
Methicillin-resistant	Vancomycin†	60 (every 8 hr)	5-20
	or		
	Linezolid	30 (every 8 hr)	
Streptococcal species	Penicillin*	200,000 U (every 6 hr)	NA
or	*or*		
Diphtheroids	Ampicillin*	400 (every 6 hr)	10-25
Enterococcus faecalis	Ampicillin*	Doses as above	
	or		
	Penicillin*		NA
	plus		
	Aminoglycoside‡		
Anaerobic bacteria	Metronidazole	22.5-30 (every 8 hr)	NA
Escherichia coli, Klebsiella, Proteus, Enterobacter	Cefotaxime	200-300 (every 6 hr)	NA
	or		
	Ceftriaxone	100 (every 12 hr)	NA
	or		
	Amikacin§	22.5 (every 8 hr)	5-30
	or		
	Tobramycin§	7.5 (every 8 hr)	5-20
	or		
	Gentamicin§	7.5 (every 8 hr)	1-8
Pseudomonas spp.	Ceftazidime	200 (every 8 hr)	NA
	or		
	Aminoglycoside		
	plus		
	Broad-spectrum penicillin		
Fungi			
Candida spp.	Amphotericin B¶	1 (every 24 hr)	0.1-0.5
	AmBisome or Amphotec	5 (every 24 hr)	

*In patients allergic to penicillin, use vancomycin.
†If cerebrospinal fluid levels are not sufficient and the bacteria are sensitive to rifampin, add rifampin, 20 mg/kg/day divided every 12 hours.
‡See doses (for the specific aminoglycoside) for treatment of *E. coli* below.
§The addition of a broad-spectrum penicillin (e.g., piperacillin or ticarcillin, 300 to 400 mg/kg/day divided every 6 hours) may add to the bactericidal activity.
¶If ventricular fluid remains positive after 5 to 7 days of therapy with amphotericin, add flucytosine (150 mg/kg/day divided every 6 hr).
NA, Not available.

Prognosis

Complete removal of the shunt system results in better prognosis.[444] Long-term morbidity occurring after a shunt-related infection includes seizures, psychomotor retardation, and cognitive deficiency. The intelligence quotient (IQ) scores of children with myelomeningocele who had a shunt-related infection were found to be significantly lower (mean IQ, 72) than the scores of children with shunts but no infection (mean IQ, 95).[324] A trend was observed in which younger children with shunt-related infections had a lower IQ than older children with shunt-related infections, especially if the infection was caused by gram-negative bacteria. In addition, shunt-related infection adversely affected school performance.[516] With appropriate combined medical and surgical therapy, the mortality rate from shunt-related infection is low, but any episode of shunt-related infection appears to increase the probability of another episode developing.

Prevention

Standardized care protocols have been shown to reduce infections.[207,244,385,456] Preventive measures to reduce the incidence of shunt-related infection include attention to preoperative and intraoperative technique and the prophylactic use of antibiotics. Bactericidal shampoos (e.g., chlorhexidine) should be used to reduce the bacterial density of the scalp before surgery is performed. Shaving the scalp seems to be a risk factor, so shaveless surgery should be considered.[210] During the operation, only essential personnel should be present, and the skin should be cleaned with a fat solvent, followed by solutions that reduce the number of bacteria (elemental tincture of iodine, 20 ppm, is preferred over 10% povidone-iodine, which contains <1 ppm free iodine)[83] or their ability to adhere to the shunt (bacitracin A).[170] Careful attention should be paid to surgical technique, with contact between the shunt and the skin avoided. A detailed preoperative protocol developed by the Hydrocephalus Clinical Research Network (HCRN) has reduced the infection rate from 8.8 to 5.7 percent.[244] Other investigators were able to reduce the infection rate by using measures similar to those suggested by the HCRN.[84] A single-center study showed that infection rate was reduced from 21 to 4.3 percent when nonantimicrobial impregnated sutures versus impregnated sutures were used, respectively[445] and other studies suggested that use of an antibiotic-impregnated shunt system significantly reduces the incidence of shunt infections in contrast to the use of a standard shunt.[169,367,371,430,431] Yet, no significant reduction in the overall shunt-related infection rate was observed in other studies.[187,233] In addition, the antibiotic-impregnated catheter may increase the rate of bacteria resistant to its antibiotic components.[111] Large prospective, randomized studies are needed to evaluate the real contribution of an antibiotic-impregnated shunt to the reduction in infection rate.

Multiple studies have examined the effect of prophylactic antibiotics for the shunt insertion procedure on reducing the infection rate.[61,269,396,397] Major variations in study design and the number of patients in each study preclude arriving at definitive conclusions. Previous meta-analysis of well-designed studies suggests that the use of prophylactic antibiotics is associated with a significant reduction in the incidence of infections *if* the baseline rate of shunt-related infections is greater than 5 percent.[180] If the infection rate is lower than 5 percent, prophylactic antibiotics are *not* recommended. A recent meta-analysis of 2134 patients (in 17 trials) found a statistically significant benefit for either systemic antibiotic prophylaxis (15 studies) or antibiotic-impregnated catheters (2 studies) in reducing the incidence of shunt-related infections, and some experts suggested to add intraventricular antibiotic administration (e.g., gentamicin or vancomycin) during the procedure.[393,396] The choice of antibiotics should be based on the local antibiotic sensitivity pattern of the pathogens commonly causing shunt-related infections in that area. Although many experts recommend giving the first dose 30 to 60 minutes before skin incision, prophylaxis should be considered to start 8 to 12 hours before surgery to allow higher levels of drug to accumulate in skin tissue than would do so if treatment were given just before the operation. The duration of prophylaxis should not exceed 24 to 36 hours. Further studies are needed to identify factors important in the development of shunt-related infections so that better techniques can be developed to further reduce or even eliminate this devastating complication of CNS shunting.

INTRACRANIAL PRESSURE MONITORS

Monitoring of ICP has become an important part of the evaluation, treatment, and management of children with a variety of intracranial pathologic processes,[291] including congenital anomalies (e.g., cranial or craniofacial dysostosis), metabolic diseases (e.g., Reye syndrome), trauma, intraventricular or subarachnoid hemorrhage, intracranial infections (e.g., encephalitis), and other ischemic or hypoxic insults.[365] Several studies have demonstrated the therapeutic and prognostic benefit of monitoring ICP in children with high ICP[145,224,280,484]; nonetheless, this procedure is not without complications such as hematoma, bleeding, leakage of CSF, and infection. Infections for which monitoring of ICP is used include ventriculitis, meningitis, brain abscess, subdural empyema, skin infection, and cranial bone infections.[253,290,541]

Several methods are available for monitoring ICP. Intraventricular placement of a catheter with the distal end connected to a pressure transducer is the method used most frequently because of its accuracy and ease of calibration. This procedure also allows drainage of CSF for biochemical or dynamic testing. Disadvantages of ventriculostomy are penetration of the meninges and brain, technical difficulties if the ventricles are small, and greater risk for the development of infection. Other methods used to monitor ICP include a subdural bolt or catheter (only the meninges are penetrated, but the brain remains intact), an epidural transducer (the dura remains intact), and a continuous intraparenchymal monitor (i.e., a fiberoptic cable is inserted through the dura into the brain parenchyma, not intraventricularly). Although both the epidural transducer and the intraparenchymal monitor have fewer complications (e.g., infection) than the intraventricular devices, they are prone to inaccuracy, which limits their use. Selection of the appropriate ICP-monitoring method depends on the patient's condition and the risk associated with the procedure.[298]

Epidemiology

Assessing the true infection rate of ICP monitoring devices is very difficult, mainly because the methods used to define infection (i.e., inclusion and exclusion criteria), the indication for ICP placement, and antibiotic use vary widely among reported studies. The incidence of infection ranged from less than 1 percent to 45 percent, with an average infection rate of 9.5 percent.[247,289] A much higher rate of infection was reported in patients who had devices that penetrated the meninges[202] than in those who had parenchymal monitors.[390]

TABLE 74-5 **Risk Factors for Infection of Devices for Monitoring Intracranial Pressure**

Intraventricular hemorrhage
Subarachnoid hemorrhage
Neurosurgical procedure or operation
Perforation of the dura
Duration of catheterization
Cerebrospinal fluid leak from fracture or intracranial pressure site
Open head trauma or fracture
Intracranial pressure >20 mm Hg
Blockage
Disconnections
Frequent manipulation of the intracranial pressure device Dressing changes or irrigation Sample collection Device component changes

Multiple factors were suggested as increasing the risk for development of ICP device–related infection (Table 74-5).[192] Patients with intracerebral hemorrhage usually do not have a higher risk for acquiring infection than those without such hemorrhage. In contrast, if the bleeding is intraventricular, the incidence of infection increases dramatically. The rate of infection also is higher in patients with open head trauma than in those with closed head trauma or intracranial malignancy.[212] Neurosurgical operations contributed significantly to the risk for acquiring infection,[241,373] and the same was noted if ICP was greater than 20 mm Hg.

Interruption of the monitoring system's integrity (e.g., CSF leak, number of times that the system was open, malfunction, and irrigation) was identified by several investigators as increasing patient risk for acquiring infection.[81,197,296] The effect of the duration of monitoring on the risk for infection is controversial, especially in patients with ventriculostomy catheters. Although several studies found a correlation between the length of time that the monitoring device was in place and the infection rate,[247,289] some investigators found no relationship with the duration of monitoring.* The different conclusions probably were the result of differences in the populations studied, the types of devices used to measure ICP, the definitions of infection, and analyses of the data. A critical review of the literature evaluating 23 major studies found a correlation between the duration of ICP monitoring and the rate of infections.[289]

The need to replace the device at a certain time has been challenged. Analysis of the outcome in 584 patients (receiving 712 ventriculostomy catheters) who had 61 infections showed a steady increase in the daily incidence of infection that peaked at day 10 with an average time to onset of infection 6.8 days.[202] Only one infection occurred after day 10. These data suggest that the majority of infections occurred early after the ICP insertion and, therefore, ICP monitoring devices should be removed as quickly as possible, but if "prolonged monitoring is required, there appears to be no benefit from or need for catheter exchange."[202]

*References 21, 81, 197, 286, 420, 421, 492.

Etiology

In general, the microorganisms that cause infections related to ICP monitoring devices are the same as those causing CNS shunt–related infections (see Table 74-2). The major difference is that gram-negative bacteria are isolated more often in ICP monitor–related infection than in CNS shunt–related infections.[286,296,418] The more common bacteria include *Enterobacter* spp., *Klebsiella* spp., *S. marcescens*, and *Acinetobacter* spp. These bacteria are found in water and cause widespread colonization of hospitalized patients. Colonization of the respiratory tract is an especially common occurrence in patients in the intensive care unit and in those with serious underlying disease. Thus, these organisms are occurring increasingly frequently in ICP monitor–related infections. Few infections are caused by *Corynebacterium*, *Propionibacterium*, and fungi.[179,420]

Clinical Manifestations

ICP monitor–related infections often occur in patients with an altered sensorium; therefore, the signs and symptoms of meningeal irritation usually are not present. The clinical diagnosis is complicated further by the fact that these patients frequently are critically ill and their signs and symptoms are caused by the underlying condition and are not a result of the ICP monitor–related infection. In addition, they may be receiving multiple antibiotics for other sources of infection (e.g., pneumonia, bacteremia, or urinary tract infection), both nosocomial and non-nosocomial. Although fever is the most frequent indication of infection, the presence of infections or inflammatory processes at other body sites causes the predictive value of fever to be low. Therefore, providing close follow-up, having a high index of suspicion, and obtaining frequent cultures of CSF when available (e.g., ventriculostomy device) are recommended.

Diagnosis

The predictive value of the peripheral WBC count and differential count are very low, and the diagnostic specificity of the CSF parameters is questionable.[383,418] CSF biochemistry (i.e., protein and glucose) has a very low predictive value. Although a low glucose level may be of help in establishing the diagnosis, a normal level does not exclude the possibility of an infection. Calculation of the ratio of leukocytes to erythrocytes in CSF versus their ratio in peripheral blood was suggested as a potential diagnostic tool for early infection, but it was not validated in a subsequent study.[383,418] Most patients with ICP monitor–related infection will have an increased CSF WBC count with a predominance of PMNs, but in cases with low growth of bacteria, no elevation in CSF WBC counts (<10/mm^3) was reported. Most of these cases were caused by coagulation-negative staphylococci. In such cases, if no other indicators of infection (e.g., clinical

symptoms or low glucose) are present, the possibility that the positive cultures represent colonization or contamination should be considered before treatment is initiated.

Treatment and Prophylaxis

Only a few control trials have evaluated treatment strategies for ICP infection; therefore, the same principles used for the management of CNS shunt–related infections can be applied to infections associated with ICP monitoring devices. The use of antibiotics alone without removal of the device may be adequate for the treatment of infections associated with devices placed in the subdural or epidural space, unless an abscess or empyema has formed. In the case of ventriculostomy, the infected device should be removed and appropriate antibiotic therapy instituted. Because gram-negative bacteria are also causing ICP device–related infections, initial antibiotic therapy should include both vancomycin and cefotaxime or ceftazidime if the Gram stain is negative. When the etiologic agent is identified, the specific antibiotic or antibiotics can be chosen from those listed in Table 74-4. The optimal treatment for fungal infections is unknown. Clinical experience with amphotericin B suggests that it is effective despite the fact that it achieves low levels in the CSF. Because fluconazole and voriconazole are known to achieve the best CSF levels, they should be considered as the first drugs of choice.[245] The duration of therapy depends on the etiologic agent and the CSF parameters. In patients with positive CSF culture but minimal CSF pleocytosis, if the CSF culture becomes negative immediately after removal of the catheter, therapy should be given for only 3 or 4 days because the cultures before removal of the catheter reflect colonization and not infection. If subsequent cultures are positive, therapy should continue for 10 days of negative cultures.

Although removal of the ICP monitor (if longer than 5 days) and reinsertion at an alternative site was recommended as a prophylactic approach to reduce the infection rate, this practice is controversial, and several studies showed that routine changes of the ICP monitor did not reduce the risk for development of infection.[289,543]

Many physicians use prophylactic antibiotics for the duration of ICP monitoring in the hope of preventing infection. Unfortunately, the studies that have evaluated the utility of antimicrobial agents in preventing such infections reported contradictory results.[400] A decreased rate of ICP monitor infection was reported in patients who received prophylaxis for the duration of the ICP monitoring.[387] Yet, other studies showed no benefit of either preprocedural only or combined with prolonged antibiotic therapy (until the device was removed).[9,73] In contrast, Poon and colleagues[387] reported that prolonged use of antibiotics reduced the infection rate but had an increase in MRSA and *Candida* infections. In addition, broad-spectrum antibiotic prophylaxis was associated with shifting to resistant gram-negative pathogens.[312] Observational studies suggested that use of antibiotic-coated devices significantly reduced infection rate in contrast to historical control[178,340] and the same results were seen in a longitudinal study comparing infection rates in patients with and without antibiotic-coated catheter.[186] The investigators observed a rate of 6.7 percent reduced to 1 percent when coated catheters were used and climbing to 7.6 percent when standard catheters were reintroduced. When coated catheters were again reintroduced, the infection rate dropped to 0.9 percent. In contrast, a retrospective analysis of patients who developed ventriculitis after placement of either antibiotic-coated EVD or non–antibiotic-impregnated EVD found no major advantage for the impregnated device.[454] A recent systematic review of all studies evaluating systemic prophylactic antibiotic throughout the duration of the external ventricular drainage or placement of antibiotic-coated devices concluded that there is probably some benefit to using either of them.[455] The varying quality of the reviewed studies (most nonrandomized) suggests that cautious interpretation and further research is needed to confirm these findings. In the meantime, antibiotic-impregnated catheters (with or without systemic antibiotics) should be considered for prophylaxis.

INTRATHECAL PUMP INFUSION DEVICES

The intrathecal pump infusion system currently is used to infuse morphine to treat refractory pain,[356] deliver baclofen (a γ-aminobutyric acid agonist), or treat spasticity of spinal or cerebral origin.[7,24,375] In addition, patients with generalized dystonia also benefited from intrathecal baclofen.[6] More than 50,000 patients are being treated with implanted intrathecal pumps for pain or spasticity. Several pump systems are used. The simpler systems consist of an externalized catheter system similar to the Hickman catheter[118] or a subcutaneous reservoir system similar to an intravascular implanted port. The more sophisticated systems include a programmable pump that usually is implanted in an abdominal subcutaneous pocket and connected via a subcutaneous catheter to the subarachnoid space.[39] An optional sideport in some of these implanted pumps allows aspiration of CSF from the subarachnoid space.

Complications of the intrathecal pump system are relatively rare events[165,506] and include mechanical problems with the catheter, such as breaking, kinking, dislodging, or leaking.[480] Skin necrosis also has been reported.[155,480] Complications (especially wound complications such as CSF fistula, dehiscence, granuloma formation, and infection) occur more frequently in children than in adults, probably because of their decreased muscle and subcutaneous tissue.[9,142,506] The pump itself is very durable and only very rarely causes a mechanical problem. Overdose that may result in coma, respiratory depression, apnea, cardiac conduction abnormalities, hypotension or hypertension, and abnormalities of the pupils was reported as well.[378] Intrathecal baclofen can induce both recurrent and new-onset seizures.[24,256] In addition, acute withdrawal syndrome was reported when treatment was stopped.[442] Infectious complications included suppuration at the exit site,[69,120,201] along the subcutaneous catheter (tunnel infection),[39,69,120,201] or around the pump.[39,69,80]

Furthermore, more severe infections such as epidural abscess and meningitis also were reported.*

Epidemiology

The infection rate of intrathecal pump infusion devices ranges from 1.2 to 6.1 percent.[20,56] Of note, in general the infection rate was lower in studies that included a larger number of patients. Systemic review of the literature identified 10 studies with 821 patients in whom 20 catheter-related infections were found—10 superficial (rate of 2.3%) and 10 deep (rate of 1.4%).[20] A more accurate description of the incidence would be the infection rate per 1000 catheter-days, which ranged from 0 to 2.5. The infection rate was higher in patients with an externalized catheter (0.65/1000 catheter-days) than in those who had an implanted system (0.48/1000 catheter-days). A meta-analysis of epidural catheters showed that 1/35 patients having the catheter for an average of 74 days developed epidural abscess.[409] Most of the infections occurred at the exit site, along the catheter, or in the subcutaneous pocket of the pump, but the infection in as many as 16 percent of cases involved the epidural space, the meninges, or both. The incidence of infections can be reduced by using the subfascial pump implementation technique and perioperative antibiotic prophylaxis.[56,339]

Infections occur more often within 2 to 4 weeks after insertion of the catheter, which suggests that the initial surgery to place the intrathecal pump system may be a risk factor. Byers and coworkers[69] found that the only risk factor associated with infection during this surgery was a prolonged duration of the procedure (i.e., >100 minutes). Multiple other factors, such as patient age, underlying diseases, immune deficiency, other concurrent infections (e.g., pneumonia), previous intrathecal catheter, number of pump refills, intraspinal anesthetics, surgeon or anesthesiologist experience with the procedure, and operative complications (e.g., operative loss of blood), were not associated significantly with an increased risk for the acquisition of infection.[69] Very thin or malnourished patients may be prone to skin break or wound dehiscence, which may increase their vulnerability to infection. In addition, patients with narrowed intervertebral spaces tended to have operations of longer duration, which puts them at risk for the development of infection. A recent study reported an increased infection frequency in children with a percutaneous endoscopic gastrostomy (PEG) tube or urinary or fecal incontinence.[142]

Etiology

The most common bacteria causing intrathecal device–related infection are from the skin flora. Coagulase-negative staphylococci and *S. aureus* lead the list, but *Streptococcus mitis*,[69,349] *Streptococcus* group A or G,[69] *Corynebacterium striatum*,[69] and *Enterococcus faecalis*[151] infections are observed as well. In addition, several studies have reported gram-negative bacteria as the cause of the pump-related infection. Such bacteria include *P. aeruginosa*,[69,151] *Pseudomonas paucimobilis*,[375] *E. coli*,[120] and *Klebsiella pneumoniae*.[374] One case each of *C. albicans*,[120] *Mycobacterium* spp.,[120] and *Acinetobacter baumanii*[498] also has been reported. A rare case of baclofen vial contamination with the fungus *Wangiella* that caused infection was reported as well.[322,375]

Clinical Manifestations

Clinical findings depend on the site of the infection. Patients with more severe infections (i.e., epidural abscess or meningitis) may have symptoms of these infections (e.g., fever, irritability, headache, nerve root pain, or meningeal signs). In some patients, pain experienced during the injection may be the clue, but in many patients, a high level of awareness for possible infection is important because they will have nonspecific signs and symptoms.

Exit-site and superficial catheter-related infections usually cause local inflammation (e.g., swelling, redness), tenderness, and drainage. The more serious deep-tract (tunnel) infections are more difficult to assess. Visible inflammation along the tract or a soft fluctuant fluid collection around it may be the only clue. Fever is not always present.

Diagnosis

Performing a complete physical and neurologic examination may be helpful in some cases. A routine CBC is relatively unhelpful because leukocytosis is not always present. MRI may be useful for diagnosing an epidural abscess and evaluating its extent. The only definitive diagnostic tests are Gram stain and culture of exudate or drainage from the exit site (if available) or an aspirate of the fluid collection along the catheter tract if tunnel infection is suspected or CSF for suspected meningitis. Irrigation of the epidural space with 1 or 2 mL of saline after removal of the pump filter (which should be sent for culture) may help in diagnosing an epidural abscess or empyema.

Treatment and Prophylaxis

If infection has occurred only at the exit site and the superficial catheter tract, local drainage with aggressive cleansing (i.e., topical antibiotics and antiseptics) but without removal of the catheter was suggested.[120] Unfortunately, more severe infections developed (e.g., deep-tunnel and epidural abscess) in some of these patients. In addition, failure of "catheter-sparing" treatment also was reported.[201] Therefore, removal of the catheter should be considered even for mild infections if they do not respond promptly to therapy. In patients with pocket, epidural, and meningeal infections, the intrathecal pump system should be removed as soon as possible and systemic antibiotic therapy initiated (for selection of drugs and doses, see Table 74-4). With the increasing rate of multidrug-resistant staphylococci and the low CSF levels of vancomycin (only 20% to 30% of serum levels), linezolid should be considered as an alternative. Linezolid has good CSF penetration (60% to 70% of the serum level)[552] and has been shown effective in treating severe CNS

*References 40, 69, 100, 120, 344, 415, 554.

infections,[411] including intrathecal infusion pump infection.[232] Although a few studies have reported clinical improvement (and sometimes cure) of tunnel infection or meningitis with the administration of intravenous antibiotics and retention of the catheter, in many cases, the infection recurred when the treatment was stopped.[69,120] A few reports suggest successful treatment of severe pump infections (including meningitis) without removal of the pump system.[59,152,414,506,554] In some of these cases, the pathogen was considered to be of low virulence (e.g., coagulase-negative staphylococci) or colonization, which may be the reason for the success. In most of these cases, however, instillation of antibiotics into the reservoir with cautious flushing of the catheter or repetitive local application of antibiotic-impregnated fleece[374] was combined with systemic antibiotics. In one protocol, "intrapump therapy is started by filling the pump to the total reservoir volume with a 5-mg vancomycin/185-µg baclofen/mL solution and programming the pump to deliver an initial volume of 1 mL/day, and oral baclofen therapy is initiated to prevent withdrawal. Intravenous vancomycin therapy is used concomitantly and serum trough levels are drawn to optimize the dose and avoid toxicity."[506] The main reason for the intrathecal administration of antibiotics is to increase the level of drug in the CSF. Before antibiotics are added to the baclofen infusion, their compatibility with baclofen should be verified or tested (vancomycin is compatible and can be used safely according to Zed and associates).[554]

No guidelines exist on how to treat severe infections of intrathecal pump systems, but in most cases, removal of the pump system while systemic antibiotics are given and then reimplantation appear to be the treatment of choice. In rare cases in which removal of the pump will be detrimental to the patient, a trial of intrathecal administration of antibiotics may be justified, with close observation to ensure that the patient is not getting worse.

Although many physicians use perioperative (or longer) antibiotics for prophylaxis, their efficacy in preventing infection has not been proved. Therefore, prophylactic antibiotics should not be used or, if given, should not be administered for longer than 24 hours after the operation. Because the catheter hub is the most common site for bacteria entry, change of bacterial filters every 2 months is advisable.[20]

NEW REFERENCES SINCE THE SIXTH EDITION

13. American Academy of Orthopaedic Surgeons. *Information statement: antibiotic prophylaxis for bacteremia in patients with joint replacement*. 2011. http://www.aaos.org/about/papers/advistmt/1033.asp.
49. Bizzarro MJ, Conrad SA, Kaufman DA, et al. Infections acquired during extracorporeal membrane oxygenation in neonates, children, and adults. *Pediatr Crit Care Med* 2011;**12**:277–81.
57. Bottcher-Haberzeth S, Kapoor S, Meuli M, et al. Osmotic expanders in children: no filling-no control-no problem? *Eur J Pediatr Surg* 2011;**21**:163–7.
72. Califano S, Pagani FD, Malani PN. Left ventricular assist device-associated infections. *Infect Dis Clin North Am* 2012;**26**:77–87.
73. Camacho EF, Boszczowski I, Basso M, et al. Infection rate and risk factors associated with infections related to external ventricular drain. *Infection* 2011;**39**:47–51.
89. Cobo J, Miguel LG, Euba G, et al. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. *Clin Micro Infect* 2011;**17**:1632–7.
111. Demetriades AK, Bassi S. Antibiotic resistant infections with antibiotic-impregnated Bactiseal catheters for ventriculoperitoneal shunts. *Br J Neurosurg* 2011;**25**:671–3.
151. Fulkerson DH, Sivaganesan A, Hill JD, et al. Progression of cerebrospinal fluid cell count and differential over a treatment course of shunt infection. *J Neurosurg Pediatr* 2011;**8**:613–19.
154. Gardner AH, Prodhan P, Stovall SH, et al. Fungal infections and antifungal prophylaxis in pediatric cardiac extracorporeal life support. *J Thoracic Cardio Surg* 2012;**143**:689–95.
162. Gluth MB, Singh R, Atlas MD. Prevention and management of cochlear implant infections. *Cochlear Implants Int* 2011;**12**: 223–7.
226. Johansen JB, Jorgensen OD, Moller M, et al. Infection after pacemaker implantation: infection rates and risk factors associated with infection in a population-based cohort study of 46299 consecutive patients. *Eur Heart J* 2011;**32**:991–8.
230. Kaiserman I, Bahar I, McAllum P, et al. Prognostic factors in *Acanthamoeba* keratitis. *Can J Ophthalmol* 2012;**47**:312–17.
235. Kao LS, Fleming GM, Escamilla RJ, et al. Antimicrobial prophylaxis and infection surveillance in extracorporeal membrane oxygenation patients: a multi-institutional survey of practice patterns. *ASAIO J* 2011;**57**:231–8.
237. Karnam S, Alla VM, Kwon J, et al. *Mycobacterium phlei*, a previously unreported cause of pacemaker infection: thinking outside the box in cardiac device infections. *Cardiol J* 2011;**18**:687–90.
240. Keay LJ, Gower EW, Iovieno A, et al. Clinical and microbiological characteristics of fungal keratitis in the United States, 2001–2007: a multicenter study. *Ophthalmology* 2011;**118**:920–6.
244. Kestle JR, Riva-Cambrin J, Wellons JC, et al. A standardized protocol to reduce cerebrospinal fluid shunt infection: the hydrocephalus clinical research network quality improvement initiative. *J Neurosurg Pediatr* 2011;**8**:22–9.
247. Kim JH, Desai NS, Ricci J, et al. Factors contributing to ventriculostomy infection. *World Neurosurg* 2012;**77**:135–40.
276. Le Monnier A, Blanot S, Abachin E, et al. *Listeria monocytogenes*: a rare complication of ventriculoperitoneal shunt in children. *J Clin Microbiol* 2011;**49**:3924–7.
285. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:e18–55.
308. Mason PK, DiMarco JP, Ferguson JD, et al. Sonication of explanted cardiac rhythm management devices for the diagnosis of pocket infections and asymptomatic bacterial colonization. *Pacing Clin Electrophysiol* 2011;**34**:143–9.
394. Ramchandran R, DiLoreto DA, Chung MM, et al. Infectious endophthalmitis in adult eyes receiving Boston type 1 keratoprosthesis. *Ophthalmology* 2012;**119**:674–81.
399. Raymond AL, Kfoury AG, Bishop CJ, et al. Obesity and left ventricular assist device driveline exit site infection. *ASAIO J* 2010;**56**:57–60.
406. Rubin LG, Papsin B, and the Committee on Infectious Diseases and Section on Otolaryngology-Head and Neck Surgery. Cochlear implants in children: surgical site infections and the prevention and treatment of acute otitis media and meningitis. *Pediatrics* 2010;**126**:381–91.
419. Schaffer JM, Allen JG, Weiss ES, et al. Infectious complications after pulsatile-flow and continuous-flow left ventricular assist device implantation. *J Heart Lung Transplant* 2011;**30**:164–74.
420. Scheithauer S, Burgel U, Bickenbach J, et al. External ventricular and lumbar drainage-associated meningoventriculitis: prospective analysis of time-dependent infection rates and risk factor analysis. *Infection* 2010;**38**:205–9.
437. Shimizu T, Luciano MG, Fukuhara T. Role of endoscopic third ventriculostomy at infected cerebrospinal fluid shunt removal. *J Neurosurg Pediatr* 2012;**9**:320–6.
444. Simon TD, Hall M, Dean JM, et al. Reinfection following initial cerebrospinal fluid shunt infection. *J Neurosurg Pediatr* 2010;**6**: 277–85.
450. Sohail MR, Henrikson CA, Braid-Forbes MJ, et al. Mortality and cost associated with cardiovascular implantable electronic device infections. *Arch Intern Med* 2011;**171**:1821–8.
451. Sohail MR, Hussain S, Le KY, et al. Risk factors associated with early-versus-late-onset implantable cardioverter-defibrillator infections. *J Interv Card Electrophysiol* 2011;**31**:171–83.

454. Soleman J, Marbacher S, Fandino J, Fathi AR. Is the use of antibiotic-impregnated external ventricular drainage beneficial in the management of iatrogenic ventriculitis? *Acta Neurochir* 2012; **154**:161–4.
455. Sonabend AM, Korenfeld Y, Crisman C, et al. Prevention of ventriculostomy-related infections with prophylactic antibiotics and antibiotic-coated external ventricular drains: a systematic review. *Neurosurgery* 2011;**68**:996–1005.
457. Sousa R, Pereira A, Massada M, et al. Empirical antibiotic therapy in prosthetic joint infections. *Acta Orthopaed Belgica* 2010;**76**:254–9.
458. Sousa R, Massada M, Pereira A, et al. Diagnostic accuracy of combined 99m-Tc-sulesomab and 99mTc-nanocolloid bone marrow imaging in detecting prosthetic joint infection. *Nuclear Med Commun* 2011;**32**:834–9.
465. Starling RC, Naka Y, Boyle AJ, et al. Results of the post-U.S. Food and Drug Administration-approval study with a continuous flow left ventricular assist device as a bridge to heart transplantation: a prospective study using the INTERMACS. *J Am Coll Cardiol* 2011;**57**:1890–8.
473. Sun HY, Ko WJ, Tsai PR, et al. Infections occurring during extracorporeal membrane oxygenation use in adult patients. *J Thorac Cardiovasc Surg* 2010;**140**:1125e–32e.
479. Tangden T, Enblad P, Ullberg M, et al. Neurosurgical gram-negative bacillary ventriculitis and meningitis: a retrospective study evaluating the efficacy of intraventricular gentamicin therapy in 31 consecutive cases. *Clin Infect Dis* 2011;**52**:1310–16.
486. Topkara VK, Kondareddy S, Malik F, et al. Infectious complications in patients with left ventricular assist device: etiology and outcomes in the continuous-flow era. *Ann Thorac Surg* 2010;**90**: 1270–7.
491 Tse T, Cheng K, Wong K, et al. Ventriculostomy and infection: a 4-year review in a local hospital. *Surg Neuro Int* 2010;**1**:47–52.
492. Tsui I, Uslan DZ, Hubschman JP, et al. *Nocardia farcinica* infection of a Baerveldt implant and enophthalmitis in a patient with a Boston type 1 keratoprosthesis. *J Glaucoma* 2010;**19**: 339–40.
493. Tuan TJ, Thorell EA, Mayer-Hamblett N. Treatment and microbiology of repeated cerebrospinal fluid shunt infections in children. *Ped Infect Dis J* 2011;**30**:731–5.
494. Tubbs RS, Muhleman M, Loukas M, Cohen-Gadol AA. Ventriculoperitoneal shunt malfunction from cerebrospinal fluid eosinophilia in children: case-based update. *Childs Nerv Syst* 2012;**28**: 345–8.
501. Uslan DZ, Gleva MJ, Warren DK, et al. Cardiovascular implantable electronic device replacement infections and prevention: results from the REPLACE registry. *PACE* 2012;**35**:81–7.
514. Vilchez F, Martinez-Pastor JC, Garcia-Ramiro S, et al. Efficacy of debridement in hematogenous and early post-surgical prosthetic joint infections. *Intl J Artificial Organs* 2011;**34**:863–9.
521. Walker PC, Depestel DD, Miles NA, et al. Surgical infection prophylaxis practices for left ventricular assist device implantation. *J Card Surg* 2011;**26**:440–3.
529. Wazni O, Epstein LM, Carrillo RG, et al. Lead extraction in the contemporary setting: the LExICon study: an observational retrospective study of consecutive laser lead extractions. *J Am Coll Cardiol* 2010;**55**:579–86.
548. Yildiz EH, Abdalla YF, Elsahn AF, et al. Update on fungal keratitis from 1999 to 2008. *Cornea* 2010;**29**:1406–11.
549. Yildiz EH, Ailani H, Hammersmith KM, et al. *Alternaria* and *Paecilomyces* keratitis associated with soft contact lens wear. *Cornea* 2010;**29**:564–8.
550. Yilmaz A, Dalgic N, Musluman M, et al. Linezolid treatment of shunt-related cerebrospinal fluid infections in children. *J Neurosurg Pediatr* 2010;**5**:443–8.
551. Yoder JS, Verani J, Heidman N, et al. Acanthamoeba keratitis: the persistence of cases following a multistate outbreak. *Ophthalmic Epidemiol* 2012;**19**:221–5.
555. Ziai WC, Lewin III JJ. Improving the role of intraventricular antimicrobial agents in the management of meningitis. *Curr Opin Neurol* 2009;**22**:277–82.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 75

Infections Related to Craniofacial Surgical Procedures

Marc A. Mazade

Craniofacial surgeons are operating more frequently in recent years in the management of traumatic injury, neoplasia, and syndromic and nonsyndromic craniofacial dysostosis, owing in part to the earthquakes in Haiti and Chile in 2010 that contributed to the need for craniofacial reconstructive procedures.[18] The incidence of associated craniofacial surgical infections was 14.7 percent in one study and 8.1 percent in another, but it varies greatly and may range from 3 to 45 percent, depending on the number of procedures undertaken during a single anesthesia, surgical duration, and the structures involved.[9,11] A review published in 2005 demonstrated a rate of 3.2 infections per 100 craniofacial surgical procedures and found that surgical duration longer than 7 hours, closure of skin under tension, use of bovine pericardium, and the presence of more than four surgeons in the operating

REFERENCES

1. Adler N, Dorafshar AH, Bauer BS, et al. Tissue expander infections in pediatric patients: management and outcomes. *Plast Reconstr Surg* 2009;**124**:484–9.
2. Advisory Committee on Immunization Practices. Licensure of a 13-valent pneumococcal conjugate vaccine (PCV13) and recommendations for use among children. *MMWR Morb Mortal Wkly Rep* 2010;**59**:258–61.
3. Ahlberg A, Carlsson AS, Lindberg L. Hematogenous infection in total joint replacement. *Clin Orthop Relat Res* 1978;**137**:69–75.
4. Ahnfelt L, Herberts P, Malchau H, Andersson GB. Prognosis of total hip replacement: a Swedish multicenter study of 4,664 revisions. *Acta Orthop Scand Suppl* 1990;**238**:1–26.
5. Ainbinder DJ, Haik BG, Tellado M. Hydroxyapatite orbital implant abscess: histological correlation of an infected implant following evisceration. *Ophthal Plast Reconstr Surg* 1994;**10**: 267–70.
6. Albright AL. Baclofen in the treatment of cerebral palsy. *J Child Neurol* 1996;**11**:77–83.
7. Albright AL, Ferson S. Intrathecal baclofen therapy in children. *Neurosurg Focus* 2006;**21**:E3.
8. Alfonso EC, Cantu-Dibildox J, Munir WM, et al. Insurgence of *Fusarium* keratitis associated with contact lens wear. *Arch Ophthalmol* 2006;**124**:941–7.
9. Alleyne CH Jr, Hassan M, Zabramski JM. The efficacy and cost of prophylactic and periprodecural antibiotics in patients with external ventricular drains. *Neurosurg* 2000;**47**:1124–7.
10. Al Soub H, Al Maslamani M, Al Khuwaiter J, et al. Myocardial abscess and bacteremia complicating *Mycobacterium fortuitum* pacemaker infection: case report and review of the literature. *Pediatr Infect Dis J* 2009;**28**:1032–4.
11. Altiparmak UE, Ozer PA, Ozkuyumcu C, et al. Postoperative endophthalmitis caused by *Bacillus cereus* and *Chlamydia trachomatis*. *J Cataract Refract Surg* 2007;**33**:1284–7.
12. Amann R, Fuchs BM. Single-cell identification in microbial communities by improved fluorescence in situ hybridization technique. *Nat Rev Microbiol* 2008;**6**:339–48.
13. American Academy of Orthopaedic Surgeons. *Information statement: antibiotic prophylaxis for bacteremia in patients with joint replacement*. 2011. http://www.aaos.org/about/papers/advistmt/1033.asp.
14. Ammirati M, Raimondi AJ. Cerebrospinal fluid shunt infections in children. *Childs Nerv Syst* 1987;**3**:106–9.
15. Anderson CM, Sorrells DL, Kerby JD. Intra-abdominal pseudocysts as a complication of ventriculoperitoneal shunts: a case report and review of the literature. *Curr Surg* 2003;**60**:338–40.
16. Anderson DJ, Kaye KS, Classen D, et al. Strategies to prevent surgical site infections in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**(suppl. 1):S51–61.
17. Anguita-Alonso P, Hanssen AD, Patel R. Prosthetic joint infection. *Expert Rev Antiinfect Ther* 2005;**3**:797–804.
18. Ankersmit HJ, Tugulea S, Spanier T, et al. Activation-induced T-cell death and immune dysfunction after implantation of left-ventricular assist device. *Lancet* 1999;**354**:550–5.
19. Antonyshyn O, Gruss JS, Mackinnon SE, Zuker R. Complications of soft tissue expansion. *Br J Plast Surg* 1988;**41**:239–50.
20. Aprili D, Bandschapp O, Rochlitz C, et al. Serious complications associated with external intrathecal catheters used in cancer pain patients. *Anesthesiol* 2009;**111**:1346–55.
21. Arabi Y, Memish ZA, Balkhy HH, et al. Ventriculostomy-associated infections: incidence and risk factors. *Am J Infect Control* 2005;**33**:137–43.
22. Argenziano M, Catanese KA, Moazami N, et al. The influence of infection on survival and successful transplantation in patients with left ventricular assist devices. *J Heart Lung Transplant* 1997;**16**:822–31.
23. Arisoy ES, Demmler GJ. *Corynebacterium xerosis* ventriculoperitoneal shunt infection in an infant: report of a case and review of the literature. *Pediatr Infect Dis J* 1993;**12**:536–8.
24. Armstrong RW, Steinbok P, Cochrane DD, et al. Intrathecally administered baclofen for treatment of children with spasticity of cerebral origin. *J Neurosurg* 1997;**87**:409–14.
25. Arnell K, Cesarini K, Lagerqvist-Widh A, et al. Cerebrospinal fluid shunt infections in children over a 13-year period: anaerobic cultures and comparison of clinical signs of infection with *Propionibacterium acnes* and with other bacteria. *J Neurosurg Pediatr* 2008;**1**:366–72.
26. Arnold W, Bredberg G, Gstottner W, et al. Meningitis following cochlear implantation: pathomechanisms, clinical symptoms, conservative and surgical treatments. *ORL J Otorhinolaryngol Relat Spec* 2002;**64**:382–9.
27. Austad ED. Evolution of the concept of tissue expansion. *Facial Plast Surg* 1988;**5**:277–9.
28. Austin KS, Testa NN, Luntz RK, et al. *Aspergillus* infection of total knee arthroplasty presenting as a popliteal cyst: case report and review of the literature. *J Arthroplasty* 1992;**7**:311–4.
29. Baddour LM, Epstein AE, Erickson CC, et al. Update on cardiovascular implantable electronic device infections and their management: a scientific statement from the American Heart Association. *Circulation* 2010;**121**:458–77.
30. Bagdasarian NG, Malani AN, Pagani FD, et al. Fungemia associated with left ventricular assist device support. *J Card Surg* 2009;**24**:763–5.
31. Bagheri F, Cervellione KL, Maruf M, et al. *Candida parapsilosis* meningitis associated with shunt infection in an adult male. *Clin Neurol Neurosurg* 2010;**112**:248–51.
32. Baier RE, Dutton RC. Initial events in interactions of blood with a foreign surface. *J Biomed Mater Res* 1969;**3**:191–206.
33. Barberan J. Management of infections of osteoarticular prosthesis. *Clin Microbiol Infect* 2006;**12**(suppl. 3):93–101.
34. Barnes SD, Dohlman CH, Durand ML. Fungal colonization and infection in Boston keratoprosthesis. *Cornea* 2007;**26**:9–15.
35. Bartlett RH, Roloff DW, Custer JR, et al. Extracorporeal life support: the University of Michigan experience. *JAMA* 2000; **283**:904–8.
36. Bayston R, Rodgers J. Role of serological tests in the diagnosis of immune complex disease in infection of ventriculoatrial shunts for hydrocephalus. *Eur J Clin Microbiol Infect Dis* 1994;**13**: 417–20.
37. Bayston R, Compton C, Richards K. Production of extracellular slime by coryneforms colonizing hydrocephalus shunts. *J Clin Microbiol* 1994;**32**:1705–9.
38. Beer R, Engelhardt KW, Pfausler B, et al. Pharmacokinetics of intravenous linezolid in cerebrospinal fluid and plasma in neurointensive care patients and staphylococcal ventriculitis associated with external ventricular drains. *Antimicrob Agents Chemother* 2007;**51**:379–82.
39. Benedetti C, McDonald JS, Lingam R, Seitz M. Efficacy of a Port-a-Cath epidural system for cancer pain management [abstract]. *Anesthesiology* 1992;**77**:841.
40. Bennett MI, Tai YMA, Symonds JM. Staphylococcal meningitis following Synchromed intrathecal pump implant: a case report. *Pain* 1994;**56**:243–4.
41. Berbari E, Hanssen A, Duffy M, et al. Prosthetic joint infection due to *Mycobacterium tuberculosis*: A case series and review of the literature. *Am J Orthop* 1998;**27**:219–27.
42. Berbari EF, Hanssen AD, Duffy MC, et al. Risk factors for prosthetic joint infection: case-control study. *Clin Infect Dis* 1998;**27**: 1247–54.
43. Berbari E, Mabry T, Tsaras G, et al. Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systemic review and meta-analysis. *J Bone Joint Surg Am* 2010;**92**:2102–9.
44. Berbari EF, Osmon DR, Carr A, et al. Dental procedures as risk factors for prosthetic hip or knee infection: a hospital-based prospective case-control study. *Clin Infect Dis* 2010;**50**:8–16.
45. Berkowitz RG, Franz BKH, Shepherd RK, et al. Pneumococcal middle ear infections and cochlear implantation. *Ann Otol Rhinol Laryngol Suppl* 1987;**128**:55–6.
46. Bernstein AD, Parsonnet V. Survey of cardiac pacing and defibrillation in the United States in 1993. *Am J Cardiol* 1996;**78**: 187–96.
47. Bhama JK, Rayappa S, Zaldonis D, et al. Impact of abdominal complications on outcome after mechanical circulatory support. *Ann Thorac Surg* 2010;**89**:522–8.
48. Biernath KR, Reefhuis J, Whitney CG, et al. Bacterial meningitis among children with cochlear implants beyond 24 months after implantation. *Pediatrics* 2006;**117**:284–9.
49. Bizzarro MJ, Conrad SA, Kaufman DA, et al. Infections acquired during extracorporeal membrane oxygenation in neonates, children, and adults. *Pediatr Crit Care Med* 2011;**12**:277–81.

50. Black MD, Coles JG, Williams WG, et al. Determinants of success in pediatric cardiac patients undergoing extracorporeal membrane oxygenation. *Ann Thorac Surg* 1995;**60**:133–8.
51. Bloom H, Heeke B, Leon A, et al. Renal insufficiency and the risk of infection from pacemaker or defibrillator surgery. *Pacing Clin Electrophysiol* 2006;**29**:142–5.
52. Boak LM, Li J, Spelman D, et al. Successful treatment and cerebrospinal fluid penetration of oral linezolid in a patient with coagulase-negative *Staphylococcus* ventriculitis. *Ann Pharmacother* 2006;**40**:1451–5.
53. Bode LG, Kluytmans JA, Wertheim HF, et al. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. *N Engl J Med* 2010;**362**:9–17.
54. Bolton WK, Sande MA, Normansell DE, et al. Ventriculojugular shunt nephritis with *Corynebacterium bovis*. *Am J Med* 1975;**59**: 417–23.
55. Borges LF. Host defenses. *Neurosurg Clin North Am* 1992;**3**: 275–8.
56. Borowski A, Littleton AG, Borkhuu B. Complications of intrathecal baclofen pump therapy in pediatric patients. *J Pediatr Orthop* 2010;**30**:76–81.
57. Bottcher-Haberzeth S, Kapoor S, Meuli M, et al. Osmotic expanders in children: no filling-no control-no problem? *Eur J Pediatr Surg* 2011;**21**:163–7.
58. Boutin R, Brossman J, Sartoris D, et al. Update on imaging of orthopedic infections. *Orthop Clin North Am* 1998;**29**:41–6.
59. Boviatsis EJ, Kouyialis AT, Boutsikakis I, et al. Infected CNS infusion pumps: is there a chance for treatment without removal? *Acta Neurochir (Wien)* 2004;**146**:463–7.
60. Brandt CM, Sistrunk WW, Duffy MC, et al. *Staphylococcus aureus* prosthetic joint infection treated with debridement and prosthesis retention. *Clin Infect Dis* 1997;**24**:914–19.
61. Bratzler DW, Houck PM. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Am J Surg* 2005;**189**:395–404.
62. Braxton Jr EE, Ehrlich GD, Hall-Stoodley L, et al. Role of biofilms in neurosurgical device-related infections. *Neurosurg Rev* 2005;**28**:249–55.
63. Bremer AA, Darouiche RO. Ventriculoperitoneal shunt infection due to *Serratia marcescens*. *J Infect* 2005;**50**:138–41.
64. Brogan TV, Thiagarajan RR, Rycus PT, et al. Extracorporeal membrane oxygenation in adults with severe respiratory failure: a multi-center database. *Intensive Care Med* 2009;**35**:2105–14.
65. Brown KL, Ridout DA, Shaw M, et al. Healthcare-associated infection in pediatric patients on extracorporeal life support: the role of multidisciplinary surveillance. *Pediatr Crit Med* 2006;**7**: 546–50.
66. Bruinsma N, Stobberingh EE, Herpers MJ, et al. Subcutaneous ventricular catheter reservoir and ventriculoperitoneal drain–related infection in preterm infants and young children. *Clin Microbiol Infect* 2000;**6**:202–6.
67. Burket JS, Bartlett RH, Vander Hyde K, Chenoweth CE. Nosocomial infections in adult patients undergoing extracorporeal membrane oxygenation. *Clin Infect Dis* 1999;**28**:828–33.
68. Burroni L, D'Alessandria C, Signore A. Diagnosis of vascular prosthesis infection: PET or SPECT? *J Nucl Med* 2007;**48**: 1227–9.
69. Byers K, Axelrod P, Michael S, Rosen S. Infections complicating tunneled intraspinal catheter systems used to treat chronic pain. *Clin Infect Dis* 1995;**21**:403–8.
70. Cabell CH, Heidenreich PA, Chu VH, et al. Increasing rates of cardiac device infections among Medicare beneficiaries: 1990–1999. *Am Heart J* 2004;**147**:582–6.
71. Cacoub P, Leprince P, Nataf P, et al. Pacemaker infective endocarditis. *Am J Cardiol* 1998;**82**:480–4.
72. Califano S, Pagani FD, Malani PN. Left ventricular assist device-associated infections. *Infect Dis Clin North Am* 2012;**26**:77–87.
73. Camacho EF, Boszczowski I, Basso M, et al. Infection rate and risk factors associated with infections related to external ventricular drain. *Infection* 2011;**39**:47–51.
74. Carlsson AS, Josefsson G, Lindberg L. Revision with gentamicin-impregnated cement for deep infection in total hip arthroplasties. *J Bone Joint Surg Am* 1978;**60**:1059–64.
75. Castro P, Soriano A, Escrich C, et al. Linezolid treatment of ventriculoperitoneal shunt infection without implant removal. *Eur J Clin Microbiol Infect Dis* 2005;**24**:603–6.
76. Cengiz M, Okutucu S, Ascioglu S, et al. Permanent pacemaker and implantable cardioverter defibrillator infections: seven years of diagnostic and therapeutic experience of a single center. *Clin Cardiol* 2010;**33**:406–11.
77. Chahipa E, Swarhrick HA, Holden BA, Sjöstrand J. Severe corneal infections associated with contact lens wear. *Ophthalmology* 1987;**94**:17–22.
78. Chan KH, Mann KS, Seto WH. Infection of a shunt by *Mycobacterium fortuitum*: case report. *Neurosurgery* 1991;**29**:472–4.
79. Chang DC, Grant GB, O'Donnell K, et al. Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* 2006;**296**:953–63.
80. Cherry DA, Gourlay GK, Counsins MJ, Gannon BJ. A technique for the insertion of an implantable portal system for the long-term epidural administration of opioids in the treatment of cancer pain. *Anaesth Intensive Care* 1985;**13**:145–52.
81. Chi H, Chang KY, Chang HC, et al. Infections associated with indwelling ventriculostomy catheters in a teaching hospital. *Int J Infect Dis* 2010;**14** e216–19.
82. Chiou CC, Wong TT, Lin HH, et al. Fungal infection of ventriculoperitoneal shunts in children. *Clin Infect Dis* 1994;**19**: 1049–53.
83. Choi SH, McComb JG, Levy ML, et al. Use of elemental iodine for shunt infection prophylaxis. *Neurosurgery* 2003;**52**:908–13.
84. Choksey MS, Malik IA. Zero tolerance to shunt infections: can it be achieved? *J Neurol Neurosurg Psychiatry* 2004;**75**:87–91.
85. Chrissoheris MP, Kadakia H, Marieb M, et al. Pacemaker pocket infection due to *Mycobacterium goodii*: case report and review of the literature. *Conn Med* 2008;**72**:75–7.
86. Chua JD, Wilkoff BL, Lee I, et al. Diagnosis and management of infections involving implantable electrophysiologic cardiac devices. *Ann Intern Med* 2000;**133**:604–8.
87. Chummun S, Addison P, Stewart KJ. The osmotic tissue expander: a 5 year experience. *J Plas Reconstr Aesthet Surg* 2010;**63**: 2128–32.
88. Chuo JY, Dolman PJ, Ng TL, et al. Clinical and histopathologic review of 18 explanted porous polyethylene orbital implants. *Ophthalmology* 2009;**116**:349–54.
89. Cobo J, Miguel LG, Euba G, et al. Early prosthetic joint infection: outcomes with debridement and implant retention followed by antibiotic therapy. *Clin Microbiol Infect* 2011;**17**:1632–7.
90. Cobb DK, High KP, Sawyer RG, et al. A controlled trial of scheduled replacement of central venous and pulmonary-artery catheters. *N Engl J Med* 1992;**327**:1062–7.
91. Cobb TK, Beckenbaugh RD. Biaxial total wrist arthroplasty. *J Hand Surg Am* 1996;**21**:1011–21.
92. Cochrane DD, Kestle JRW. The influence of surgical operative experience on the duration of first ventriculoperitoneal shunt infection. *Pediatr Neurosurg* 2003;**38**:295–301.
93. Cohen EJ, Buchanan HW, Laughrea PA, et al. Diagnosis and management of *Acanthamoeba* keratitis. *Am J Ophthalmol* 1985; **100**:389–95.
94. Cohen MI, Bush DM, Gaynor JW, et al. Pediatric pacemaker infections: twenty years of experience. *J Thorac Cardiovasc Surg* 2002;**124**:821–7.
95. Cohen NL. Cochlear implant soft surgery: fact or fantasy? *Otolaryngol Head Neck Surg* 1997;**117**:214–6.
96. Cohen NL, Hoffman RA. Complications of cochlear implant surgery in adults and children. *Ann Otol Rhinol Laryngol* 1991; **100**:708–11.
97. Cohen NL, Hoffman RA. Surgical complications of multichannel cochlear implants in North America. *Adv Otorhinolaryngol* 1993;**48**:70–4.
98. Cohen NL, Hoffman RA, Stroschein M. Medical or surgical complications related to the nucleus multichannel cochlear implant. *Ann Otol Rhinol Laryngol* 1988;**97**:8–13.
99. Cotton MF, Hartzenberg B, Donald PR, Burger PJ. Ventriculoperitoneal shunt infections in children: a 6-year study. *S Afr Med J* 1991;**79**:139–42.
100. Crawford ME, Andersen H, Augustenborg GA, et al. Pain treatment on outpatient basis utilizing extradural opiates: a Danish multicentre study comprising 105 patients. *Pain* 1983; **16**:41–7.
101. Cuckler JM, Star AM, Alavi A, Noto RB. Diagnosis and management of the infected total joint arthroplasty. *Orthop Clin North Am* 1991;**22**:512–30.

102. Cunningham CD 3rd, Slattery WH 3rd, Luxford WM. Postoperative infection in cochlear implant patients. *Otolaryngol Head Neck Surg* 2004;**131**:109–14.
103. Da Costa A, Lelievre H, Kirkorian GM, et al. Role of preaxillary flora in pacemaker infections: a prospective study. *Circulation* 1998;**97**:1791–5.
104. Dalton HJ, Siewers RD, Fuhrman BP, et al. Extracorporeal membrane oxygenation for cardiac rescue in children with severe myocardial dysfunction. *Crit Care Med* 1993;**21**:1020–8.
105. Darouiche RO. Device-associated infections: a macroproblem that starts with microadherence. *Clin Infect Dis* 2001;**33**: 1567–72.
106. Darouiche RO, Landon GC, Patti GM, et al. Role of *Staphylococcus aureus* surface adhesions in orthopaedic device infections. *J Med Microbiol* 1997;**46**:75–9.
107. Dart JK, Saw VP, Kilvington S. Acanthamoeba keratitis: diagnosis and treatment update. *Am J Ophthalmol* 2009;**148**:487–99.
108. Daspit CP. Meningitis as a result of cochlear implant: case report. *Otolaryngol Head Neck Surg* 1991;**105**:115–16.
109. David R, Shtarker H, Horesh Z, et al. Arthrodesis with the Ilizarov device after failed knee arthroplasty. *Orthopedics* 2001; **24**:33–6.
110. Del Pozo JL, Patel R. Clinical practice: infection associated with prosthetic joints. *N Engl J Med* 2009;**361**:787–94.
111. Demetriades AK, Bassi S. Antibiotic resistant infections with antibiotic-impregnated Bactiseal catheters for ventriculoperitoneal shunts. *Brit J Neurosurg* 2011;**25**:671–3.
112. de Repentigny L, St-Germain G, Charest H, et al. Fatal zygomycosis caused by *Mucor indicus* in a child with an implantable left ventricular assist device. *Pediatr Infect Dis J* 2008;**27**:365–9.
113. Diaz-Mitoma F, Hardin GKM, Hoban DJ, et al. Clinical significance of a test for slime production in ventriculoperitoneal shunt infections caused by coagulase-negative staphylococci. *J Infect Dis* 1987;**156**:555–60.
114. Dickinson GM, Bisno AL. Infections associated with indwelling devices: concepts of pathogenesis; infections associated with intravascular devices. *Antimicrob Agents Chemother* 1989;**33**:597–601.
115. Dickinson GM, Bisno AL. Infections associated with indwelling devices: infections related to extravascular devices. *Antimicrob Agents Chemother* 1989;**33**:602–7.
116. Didisheim P, Olsen DB, Farrar DJ, et al. Infections and thromboembolism with implantable cardiovascular devices. *Trans Am Soc Artif Intern Organs* 1994;**35**:54–70.
117. Di Rocco C, Marchese E, Velardi F. A survey of the first complication of newly implanted CSF shunt devices for the treatment of nontumoral hydrocephalus. *Childs Nerv Syst* 1994;**10**:321–5.
118. Downing JE, Busch EH, Stedman PM. Epidural morphine delivered by a percutaneous epidural catheter for outpatient treatment of cancer pain. *Anesth Analg* 1988;**67**:1159–61.
119. Duggan JM, Georgiadis GM, Kleshinski JF. Management of prosthetic joint infections. *Infect Med* 2001;**18**:534–41.
120. Du Pen SL, Peterson DG, Williams A, Bogosian AJ. Infection during chronic epidural catheterization: diagnosis and treatment. *Anesthesiology* 1990;**73**:905–9.
121. Durand ML, Kim IK, D'Amico DJ, et al. Successful treatment of *Fusarium* endophthalmitis with voriconazole and *Aspergillus* endophthalmitis with voriconazole plus caspofungin. *Am J Ophthalmol* 2005;**140**:552–4.
122. Duus BR, Boeckstyns M, Stadeager C. The natural course of radionuclide bone scanning in the evaluation of total knee replacement: a 2 year prospective study. *Clin Radiol* 1990;**41**:341–3.
123. Edwards JR, Peterson KD, Mu Y, et al. National Healthcare Safety Network (NHSN) report: data summary for 2006 through 2008, issued December 2009. *Am J Infect Control* 2009;**37**: 783–805.
124. Eggimann P, Waldvogel R. Pacemaker and defibrillator infections. In: Waldvogel FA, Bisno AL, editors: *Infections associated with indwelling medical devices*. 3rd ed. Washington, DC: A.S.M, Press; 2000. p. 247–64.
125. Elliott SP, Yogev R, Shulman ST. *Staphylococcus lugdunensis*: an emerging cause of ventriculoperitoneal shunt infections. *Pediatr Neurosurg* 2001;**35**:128–30.
126. Elson R. One-stage exchange in the treatment of the infected total hip arthroplasty. *Semin Arthroplasty* 1994;**5**:137–41.
127. Elvy J, Porter D, Brown E. Treatment of external ventricular drain-associated ventriculitis caused by *Enterococcus faecalis* with intraventricular daptomycin. *J Antimicrob Chemother* 2008;**61**: 461–2.
128. Enger PO, Svendsen F, Wester K. CSF shunt infections in children: experiences from a population-based study. *Acta Neurochir* 2003;**145**:243–8.
129. Epstein AE, DiMarco JP, Ellenbogen KA, et al. ACC/AHA/HRS 2008 guidelines for device-based therapy of cardiac rhythm abnormalities: a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines developed in collaboration with the American Association for Thoracic Surgery and Society of Thoracic Surgeons. *Circulation* 2008;**117**:e350–408.
130. Ersahin Y, Arslan D. Complications of endoscopic third ventriculostomy. *Childs Nerv Syst* 2008;**24**:943–8.
131. Ersahin Y, Yurtseven T. Rare complications of shunt infection. *Pediatr Neurosurg* 2004;**40**:90–2.
132. Ersahin Y, McLone DG, Storrs BB, et al. Review of 3,017 procedures for the management of hydrocephalus in children. *Concepts Pediatr Neurosurg* 1989;**9**:21–33.
133. Etienne J, Charpin B, Grando J, et al. Characterization of clinically significant isolates of *Staphylococcus epidermidis* from patients with cerebrospinal fluid shunt infections. *Epidemiol Infect* 1991; **106**:467–75.
134. Extracorporeal Life Support Organization Registry Report: International Summary, January 2010.
135. Fahim DK, Frueh BR, Musch DC, Nelson CC. Complications of pegged and non-pegged hydroxyapatite orbital implants. *Ophthalm Plast Reconstr Surg* 2007;**23**:206–10.
136. Faillace WJ. A no-touch technique protocol to diminish cerebrospinal fluid shunt infection. *Surg Neurol* 1995;**43**:344–50.
137. Farber BF, Kaplan MH, Clogston AG. *Staphylococcus epidermidis* extracted slime inhibits the antimicrobial action of glycopeptide antibiotics. *J Infect Dis* 1988;**161**:37–40.
138. Fehring TK, Calton TF, Griffin WL. Cementless fixation in 2-stage reimplantation for periprosthetic sepsis. *J Arthroplasty* 1999;**14**:175–81.
139. Field ME, Jones SO, Epstein LM. How to select patients for lead extraction. *Heart Rhythm* 2007;**4**:978–85.
140. Fischer SA, Trenholme GM, Costanzo MR, Piccione W. Infectious complications in left ventricular assist device recipients. *Clin Infect Dis* 1997;**24**:18–23.
141. Fitzgerald RH, Nolan DR, Ilstrup DM, et al. Deep wound sepsis following total hip arthroplasty. *J Bone Joint Surg Am* 1977;**59**: 847–55.
142. Fjelstad AB, Hommelstad J, Sorteberg A. Infections related to intrathecal baclofen therapy in children and adults: frequency and risk factors. *J Neurosurg Pediatr* 2009;**4**:487–93.
143. Forward KR, Fewer HD, Stiver HG. Cerebrospinal fluid shunt infections: a review of 35 infections in 32 patients. *J Neurosurg* 1983;**59**:389–94.
144. Foster TJ, Hook M. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol* 1998;**6**:484–8.
145. Fouyas IP, Case ATH, Thompson D, et al. Use of intracranial pressure monitoring in the management of childhood hydrocephalus and shunt-related problems. *Neurosurgery* 1996;**38**:726–32.
146. Fowler VG, Li J, Corey GR, et al. Role of echocardiography in evaluation of patients with *Staphylococcus aureus* bacteremia: experience in 103 patients. *J Am Coll Cardiol* 1997;**30**:1072–8.
147. Frame PT, McLaurin RL. Treatment of CSF shunt infections with intrashunt plus oral antibiotic therapy. *J Neurosurg* 1984; **60**:354–60.
148. Frazier OH, Rose EA, Macmanus Q, et al. Multicenter clinical evaluation of the HeartMate 1000 IP left ventricular assist device. *Ann Thorac Surg* 1992;**53**:1080–90.
149. Friedman RM, Ingram Jr AE, Rohrich RJ, et al. Risk factors for complications in pediatric tissue expansion. *Plast Reconstr Surg* 1996;**98**:1242–6.
150. Fulkerson DH, Boaz JC. Cerebrospinal fluid eosinophilia in children with ventricular shunts. *J Neurosurg Pediatr* 2008;**1**: 288–95.
151. Fulkerson DH, Sivaganesan A, Hill JD, et al. Progression of cerebrospinal fluid cell count and differential over a treatment course of shunt infection. *J Neurosurg Pediatr* 2011;**8**:613–19.
152. Galloway A, Falope FZ. *Pseudomonas aeruginosa* infection in an intrathecal baclofen pump: successful treatment with adjunct intra-reservoir gentamicin. *Spinal Cord* 2000;**38**:126–8.

153. Gandelman G, Frishman WH, Wiese C, et al. Intravascular device infections. *Cardiol Rev* 2007;**15**:13–23.
154. Gardner AH, Prodhan P, Stovall SH, et al. Fungal infections and antifungal prophylaxis in pediatric cardiac extracorporeal life support. *J Thoracic Cardio Surg* 2012;**143**:689–95.
155. Gardner B. Intrathecal baclofen: a multicentre clinical comparison of the Medtronics Programmable, Cordis Sector, and constant infusion Infusaid drug delivery systems. *Paraplegia* 1995;**33**: 551–4.
156. Gardner P, Leipzig T, Phillips P. Infections of central nervous system shunts: symposium on infections of the central nervous system. *Med Clin North Am* 1985;**69**:297–314.
157. Gaskill SJ, Marlin AE. Spontaneous bacterial peritonitis in patients with ventriculoperitoneal shunts. *Pediatr Neurosurg* 1997;**26**:115–19.
158. Geers TA, Gordon SM. Clinical significance of *Candida* species isolated from cerebrospinal fluid following neurosurgery. *Clin Infect Dis* 1999;**28**:1139–47.
159. Gibstein LA, Abramson DL, Bartlett RA, et al. Tissue expansion in children: a retrospective study of complications. *Ann Plast Surg* 1997;**38**:358–64.
160. Glasgow BJ, Weinberg DA, Shorr N, Goldberg RA. Draining cutaneous fistula associated with infections of hydroxyapatite orbital implant. *Ophthal Plast Reconstr Surg* 1996;**12**:131–5.
161. Glatt HJ, Googe PB, Powers T, Apple DJ. Anophthalmic socket pain. *Am J Ophthalmol* 1993;**116**:357–62.
162. Gluth MB, Singh R, Atlas MD. Prevention and management of cochlear implant infections. *Cochlear Implants Int* 2011;**12**: 223–7.
163. Gold MR, Peters RW, Johnson JW. Complications associated with pectoral implantation of cardioverter defibrillators. *Pacing Clin Electrophysiol* 1997;**20**:208–11.
164. Goldberg SP, Baddley JW, Aaron MF, et al. Fungal infections in ventricular assist devices. *ASAIO J* 2000;**46**:S37–40.
165. Gooch JL, Oberg WA, Grams B, et al. Complications of intrathecal baclofen pumps in children. *Pediatr Neurosurg* 2003; **39**:1–6.
166. Gordon RJ, Quagliarello B, Lowy FD. Ventricular assist device-related infections. *Lancet Infect Dis* 2006;**7**:426–37.
167. Gordon SM, Schmitt SK, Jacobs M, et al. Nosocomial bloodstream infections in patients with implantable left ventricular assist devices. *Ann Thorac Surg* 2001;**72**:725–30.
168. Govaerts PJ, De Beukelaer C, Daemers K, et al. Outcome of cochlear implantation at different ages from 0 to 6 years. *Otol Neurol* 2002;**23**:885–90.
169. Govender ST, Med M, Nathoo N, Van Dellen JR. Evaluation of an antibiotic-impregnated shunt system for the treatment of hydrocephalus. *J Neurosurg* 2003;**99**:831–9.
170. Gower DJ, Gower VC, Richardson SH, Kelly DL. Reduced bacterial adherence to silicone plastic neurosurgical prosthesis. *Pediatr Neurosci* 1985;**12**:127–33.
171. Grabb PA, Albright AI. Intraventricular vancomycin–induced cerebrospinal fluid eosinophilia: report of two patients. *Neurosurgery* 1992;**30**:630–5.
172. Gray ED, Peters G, Verstegen M, Regelmann WE. Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. *Lancet* 1984;**1**:365–7.
173. Greene KA, Clark RJ, Zabramski JM. Ventricular CSF shunt infections associated with *Corynebacterium jeikeium*: report of three cases and review. *Clin Infect Dis* 1993;**16**:139–41.
174. Griffith MJ, Mounsey JP, Bexton RS, Holden MP. Mechanical, but not infective, pacemaker erosion may be successfully managed by re-implantation of pacemakers. *Br Heart J* 1994;**71**:202–5.
175. Grogler FM, Frank G, Greven G, et al. Complications of permanent transvenous cardiac pacing. *J Thorac Cardiovasc Surg* 1975; **69**:895–904.
176. Gruninger RP. Diagnostic microbiology in bone and joint infections. In: Gustilo RB, editor. *Orthopaedic infection: Diagnosis and treatment*. Philadelphia: W. B. Saunders; 1989. p. 42–51.
177. Guevara JA, Zuccaro G, Trevisan BS, Denoya CD. Bacterial adhesion to cerebrospinal fluid shunts. *J Neurosurg* 1987;**67**: 438–45.
178. Gutierez-Gonzalez R, Boto GR, Fernandez-Perez C, et al. Protective effect of rifampicin and clindamycin impregnated devices against *Staphylococcus* spp infection after cerebrospinal fluid diversion procedures. *BMC Neurol* 2010;**10**:93.
179. Hader WJ, Steinbok P. The value of routine cultures of the cerebrospinal fluid in patients with external ventricular drains. *Neurosurgery* 2000;**46**:1149–55.
180. Haines SJ, Walters BC. Antibiotic prophylaxis for cerebrospinal fluid shunts: a metaanalysis. *Neurosurgery* 1994;**34**:87–92.
181. Hanekom W, Yogev R. Diagnosis and management of CSF shunt infections. *Adv Pediatr Infect Dis* 1995;**11**:29–54.
182. Hanlo PW, Cinalli G, Vandertop WP, et al. Treatment of hydrocephalus determined by the European Orbis Sigma Valve II survey: a multicenter prospective 5 year shunt survival study in children and adults in whom a flow-regulating shunt was used. *J Neurosurg* 2003;**99**:52–7.
183. Hansen S, Anthonsen K, Stangerup SE, et al. Unexpected findings and surgical complications in 505 consecutive cochlear implantations: a proposal for reporting consensus. *Acta Otolaryngologica* 2010;**130**:540–9.
184. Hanssen AD, Rand JA. Evaluation and treatment of infection at the site of a total hip or knee arthroplasty. *J Bone Joint Surg Am* 1998;**80**:910–22.
185. Harcombe AA, Newell SA, Ludman PF, et al. Late complications following permanent pacemaker implantation or elective unit replacement. *Heart* 1998;**80**:240–4.
186. Harrop JS, Sharan AD, Ratliff J, et al. Impact of a standardized protocol and antibiotic-impregnated catheters on ventriculostomy infection rates in cerebrovascular patients. *Neurosurgery* 2010;**67**: 187–91.
187. Hayhurst C, Cooke R, Williams D, et al. The impact of antibiotic-impregnated catheters on shunt infection in children and neonates. *Childs Nerv Syst* on line, Oct. 26, 2007.
188. Heidemann SM, Fiore M, Sood S, Ham S. Eosinophil activation in the cerebrospinal fluid of children with shunt obstruction. *Pediatr Neurosurg* 2010;**46**:255–8.
189. Hellingman CA, Dunnebier EA. Cochlear implantation in patients with acute or chronic middle ear infectious disease: a review of the literature. *Eur Arch Otorhinolaryngol* 2009;**266**:171–6.
190. Hellman M, Mehta SD, Bishai DM, et al. The estimated magnitude and direct hospital costs of prosthetic joint infections in the United States, 1997 to 2004. *J Arthroplasty* 2010;**25**: 766–71.
191. Helman DN, Morales DL, Edwards NM, et al. Left ventricular assist device bridge-to-transplant network improves survival after failed cardiotomy. *Ann Thorac Surg* 1999;**68**:1187–94.
192. Hickman KM, Mayer BL, Muwaswes M. Intracranial pressure monitoring: review of risk factors associated with infection. *Heart Lung* 1990;**19**:84–90.
193. Hidron AI, Edwards JR, Patel J, et al. National Healthcare Safety Network Team; Participating National Healthcare Safety Network Facilities. NHSN annual update: Antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. *Infect Control Hosp Epidemiol* 2008;**29**:996–1011.
194. Hildick-Smith DJ, Lowe MD, Newell SA, et al. Ventricular pacemaker upgrade: experience, complications and recommendations. *Heart* 1998;**79**:383–7.
195. Hirst LW, Green WR, Merz W, et al. Management of *Acanthamoeba* keratitis: a case report and a review of the literature. *Ophthalmology* 1984;**91**:1105–11.
196. Hoeffel DP, Hinrichs SH, Garvin KL. Molecular diagnostics for the detection of musculoskeletal infection. *Clin Orthop Relat Res* 1999;**360**:37–46.
197. Hoefnagel D, Dammers R, Ter Laak-Poort MP, et al. Risk factors for infections related to external ventricular drainage. *Acta Neurochir (Wien)* 2008;**150**:209–14.
198. Hoffman RA. Cochlear implant in the child under two years of age: skull growth, otitis media, and selection. *Otolaryngol Head Neck Surg* 1997;**117**:217–19.
199. Hoffman RA, Cohen NL. Complications of cochlear implant surgery. *Ann Otol Rhinol Laryngol* 1995;**104**(suppl. 166):420–2.
200. Hofmann AA, Wyatt RW, Daniels A, et al. Bone scans after total knee arthroplasty in asymptomatic patients: cemented versus cementless. *Clin Orthop Relat Res* 1990;**251**:183–8.
201. Hogan Q, Haddox JD, Abram S, et al. Epidural opiates and local anesthetics for the management of cancer pain. *Pain* 1991;**46**: 271–9.

202. Holloway KL, Barnes T, Choi S, et al. Ventriculostomy infections: the effect of monitoring duration and catheter exchange in 584 patients. *J Neurosurg* 1996;**85**:419–24.
203. Holman WL, Kirklin JK, Naftel DC, et al. Infection after implantation of pulsatile mechanical circulatory support devices. *J Thorac Cardiovasc Surg* 2010;**139**:632–6.
204. Holman WL, Murrah CP, Ferguson ER, et al. Infections during extended circulatory support: University of Alabama at Birmingham experience. *Ann Thorac Surg* 1996;**61**:366–71.
205. Holman WL, Park SJ, Long JW, et al. Infection in permanent circulatory support: experience from the REMATCH trial. *J Heart Lung Transplant* 2004;**23**:1359–65.
206. Holt RJ. Bacteriological studies on colonised ventriculoatrial shunts. *Dev Med Child Neurol* 1970;**12**(suppl. 22):83–7.
207. Honda H, Jones JC, Craighead MC, et al. Reducing the incidence of intraventricular catheter-related ventriculitis in the neurology-neurosurgical intensive care unit at a tertiary care center in St. Louis, Missouri: an 8-year follow-up study. *Infect Control Hosp Epidemiol* 2010;**31**:1078–81.
208. Hong SW, Paik JS, Kim SY, et al. A case of orbital abscess following porous orbital implant infection. *Korean J Ophthalmol* 2006;**20**:234–7.
209. Hopfenspirger MT, Levine SC, Rimell FL. Infectious complications in pediatric cochlear implants. *Laryngoscope* 2007;**117**: 1825–9.
210. Horgan MA, Piatt Jr JH. Shaving of the scalp may increase the rate of infection in CSF shunt surgery. *Pediatr Neurosurg* 1997;**26**:180–4.
211. Hornyik G, Piatt Jr JH. Cerebrospinal fluid shunt infection by *Neisseria sicca*. *Pediatr Neurosurg* 1994;**21**:189–91.
212. House WF, Luxford WM, Courtney B. Otitis media in children following cochlear implant. *Ear Hear* 1985;**6**(suppl.):24–6.
213. Howden BP, Johnson PD, Charles PG, Grayson ML. Failure of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* infections. *Clin Infect Dis* 2004;**39**:1544–5.
214. Hsu MS, Chiu KM, Huang YT, et al. Risk factors for nosocomial infection during extracorporeal membrane oxygenation. *J Hosp Infect* 2009;**73**:210–6.
215. Iconomou TG, Michelow BJ, Zuker RM. Tissue expansion in the pediatric patient. *Ann Plast Surg* 1993;**31**:134–40.
216. Ingram CW, Haywood HB III, Morris VM, et al. Cryptococcal ventricular-peritoneal shunt infection: clinical and epidemiological evaluation of two closely associated cases. *Infect Control Hosp Epidemiol* 1993;**14**:719–22.
217. Itescu S, Ankersmit JH, Kocher AA, et al. Immunobiology of left ventricular assist devices. *Prog Cardiovasc Dis* 2000;**43**: 67–80.
218. Ito J, Fujino K, Okumura T, et al. Surgical difficulties and postoperative problems associated with cochlear implants. *Ann Otol Rhinol Laryngol* 1995;**104**(suppl. 166):425–6.
219. Iyer SA, Tuli SS, Wagoner RC. Fungal keratitis: emerging trends and treatment outcomes. *Eye Contact Lens* 2006;**32**: 267–71.
220. Jackler RK, O'Donoghue GM, Schindler RA. Cochlear implantation: strategies to protect the implanted cochlea from middle ear infection. *Ann Otol Rhinol Laryngol* 1986;**95**:66–70.
221. James HE, Walsh JW, Wilson HD, Connor JD. Management of CSF shunt infection: a clinical experience. *Monogr Neurol Sci* 1982;**8**:75–7.
222. James HE, Walsh JW, Wilson HD, et al. A prospective randomized study of therapy in cerebrospinal fluid shunt infection. *Neurosurgery* 1980;**7**:459–63.
223. James PJ, Butcher IA, Gardner ER, Hamblen DL. Methicillin-resistant *Staphylococcus epidermidis* in infection in hip arthroplasties. *J Bone Joint Surg Br* 1994;**76**:725–7.
224. Jenkins JG, Glasgow JFT, Black GW, et al. Reye's syndrome: assessment of ICP monitoring. *B M J* 1987;**294**:337–8.
225. Jiminez DF, Keating R, Goodrich JT. Silicone allergy in ventriculoperitoneal shunts. *Childs Nerv Syst* 1994;**10**:59–63.
226. Johansen JB, Jorgensen OD, Moller M, et al. Infection after pacemaker implantation: infection rates and risk factors associated with infection in a population-based cohort study of 46299 consecutive patients. *Eur Heart J* 2011;**32**:991–8.
227. Johnson GM, Lee DA, Regelmann WE, et al. Interference with granulocyte function by *Staphylococcus epidermidis* slime. *Infect Immun* 1986;**54**:13–20.
228. Jordan DR, Brownstein S, Shivinder SJ. Abscessed hydroxyapatite orbital implants: a report of two cases. *Ophthalmology* 1996;**103**: 1784–7.
229. Jordan DR, Gilberg S, Bawazeer A. Coralline hydroxyapatite orbital implant (Bio-Eye): experience with 158 patients. *Ophthal Plast Reconstr Surg* 2004;**20**:69–74.
230. Kaiserman I, Bahar I, McAllum P, et al. Prognostic factors in *Acanthamoeba* keratitis. *Can J Ophthalmol* 2012;**47**:312–17.
231. Kaitreider SA, Newman SA. Prevention and management of complications associated with the hydroxyapatite implant. *Ophthal Plast Reconstr Surg* 1996;**12**:18–31.
232. Kallweit U, Harzheim M, Markleni G, et al. Successful treatment of methicillin-resistant *Staphylococcus aureus* meningitis using linezolid without removal of intrathecal infusion pump. *J Neurosurg* 2007;**107**:651–3.
233. Kan P, Kestle J. Lack of efficacy of antibiotic-impregnated shunt systems in preventing shunt infections in children. *Childs Nerv Syst* 2007;**23**:773–7.
234. Kantor SG, Schneider R, Insall JN, Becker MW. Radionuclide imaging of asymptomatic versus symptomatic total knee arthroplasties. *Clin Orthop Relat Res* 1990;**260**:118–23.
235. Kao LS, Fleming GM, Escamilla RJ, et al. Antimicrobial prophylaxis and infection surveillance in extracorporeal membrane oxygenation patients: a multi-institutional survey of practice patterns. *ASAIO J* 2011;**57**:231–8.
236. Karchmer AW, Longworth DL. Infections of intracardiac devices. *Infect Dis Clin North Am* 2002;**16**:477–505.
237. Karnam S, Alla VM, Kwon J, et al. *Mycobacterium phlei*, a previously unreported cause of pacemaker infection: thinking outside the box in cardiac device infections. *Cardiol J* 2011;**18**:687–90.
238. Karsloglu S, Serin D, Simsek I, et al. Implant infection in porous orbital implants. *Ophthal Plast Reconstr Surg* 2006;**22**:461–6.
239. Kasirajan V, McCarthy PM, Hoercher KJ, et al. Clinical experience with long-term use of implantable left ventricular assist devices: indications, implantation, and outcomes. *Semin Thorac Cardiovasc Surg* 2000;**12**:229–37.
240. Keay LJ, Gower EW, Iovieno A, et al. Clinical and microbiological characteristics of fungal keratitis in the United States, 2001–2007: a multicenter study. *Ophthalmology* 2011;**118**: 920–6.
241. Kennergren C. Impact of implant techniques on complications with current implantable cardioverter-defibrillator systems. *Am J Cardiol* 1996;**78**(suppl. 5A):15–20.
242. Kestle JRW, Cochrane DD, Drake JM. Shunt insertion in the summer: is it safe? *Neurosurg Pediatr* 2006;**105**:165–8.
243. Kestle JRW, Garton HJL, Whitehead WE, et al. Management of shunt infections: a multicenter pilot study. *J Neurosurg* 2006;**105**(suppl. 3):177–81.
244. Kestle JR, Riva-Cambrin J, Wellons JC, et al. A standardized protocol to reduce cerebrospinal fluid shunt infection: the hydrocephalus clinical research network quality improvement initiative. *J Neurosurg Pediatr* 2011;**8**:22–9.
245. Kethireddy S, Andes D. CNS pharmacokinetics of antifungal agents. *Expert Opin Drug Metab Toxicol* 2007;**3**:573–81.
246. Ketoff J, Klein RL, Maukkassa KF. Ventricular cholecystic shunts in children. *J Pediatr Surg* 1997;**32**:181–3.
247. Kim JH, Desai NS, Ricci J, et al. Factors contributing to ventriculostomy infection. *World Neurosurg* 2012;**77**:135–40.
248. Kimball PM, Flattery M, McDougan F, et al. Cellular immunity impaired among patients on left ventricular assist device for 6 months. *Ann Thorac Surg* 2008;**85**:1656–61.
249. Kirklin JK, Naftel DC, Kormos RL, et al. Second INTERMACS annual report: more than 1000 primary left ventricular assist device implants. *J Heart Lung Transplant* 2010;**29**:1–10.
250. Kirklin JK, Naftel DC, Kormos RL, et al. Third INTERMACS annual report: the evolution of destination therapy in the United States. *J Heart Lung Transplant* 2010;**30**:115–23.
251. Klepper J, Busse M, Strabury HM, Sorensen N. Epilepsy in shunt-treated hydrocephalus. *Dev Med Child Neurol* 1998;**40**: 731–6.
252. Klibanov OM, Filicko JE, DeSimone Jr JA, Tice DS. Sensorineural hearing loss associated with intrathecal vancomycin. *Ann Pharmacother* 2003;**37**:61–5.
253. Klug D, Lacroix D, Savoye C, et al. Systemic infection related to endocarditis on pacemaker leads: clinical presentation and management. *Circulation* 1997;**95**:2098–107.

254. Klug D, Wallet F, Lacroix D, et al. Local symptoms at the site of the pacemaker implantation indicate latent systemic infection. *Heart* 2004;**90**:882–6.
255. Kocis KC. Pediatric cardiac extracorporeal membrane oxygenation: supporting life or prolonging death? *Crit Care Med* 2000; **28**:594–5.
256. Kofler M, Kronenberg MF, Rifici C, et al. Epileptic seizures associated with intrathecal baclofen application. *Neurology* 1994; **44**:25–7.
257. Kontny U, Höfling B, Gutjahr P. CSF shunt infections in children. *Infection* 1993;**21**:89–95.
258. Kristinsson JK, Sigurdsson H, Sigfusson A, et al. Detection of orbital implant infection with technetium 99m–labeled leukocytes. *Ophthal Plast Reconstr Surg* 1997;**13**:256–8.
259. Kulik TJ, Moler FW, Palmisano JM, et al. Outcome-associated factors in pediatric patients treated with extracorporeal membrane oxygenator after cardiac surgery. *Circulation* 1996;**94**(suppl. II): 63–8.
260. Kulkarni AV, Drake JM, Lamberti-Pasculli M. Cerebrospinal fluid shunt infection: a prospective study of risk factors. *J Neurosurg* 2001;**94**:195–201.
261. Kulkarni AV, Rabin D, Lamberti-Pasculli M, et al. Repeat cerebrospinal fluid shunt infection in children. *Pediatr Neurosurg* 2001;**35**:66–71.
262. Kumar R, Sahay S, Gaur B, Singh V. Ascites in ventriculoperitoneal shunt. *Indian J Pediatr* 2003;**70**:859–64.
263. Kurtz SM, Lau E, Schmier J, et al. Infection burden for hip and knee arthroscopy in the United States. *Arthroplasty* 2008;**23**: 984–91.
264. Kurup A, Janardhan MN, Seng TY. *Candida tropicalis* pacemaker endocarditis. *J Infect* 2000;**41**:275–6.
265. Kusumoto FM, Goldschlager N. Cardiac pacing. *N Engl J Med* 1996;**334**:89–97.
266. Kwee TC, Kwee RM, Alavi A. FDG-PET for diagnosing prosthetic joint infection: systematic review and meta analysis. *Eur J Nucl Med Mol Imaging* 2008;**35**:2122–32.
267. Lai KK, Fontecchio SA. Infections associated with implantable cardioverter-defibrillators placed transvenously and via thoracotomies: epidemiology, infection control, and management. *Clin Infect Dis* 1998;**27**:265–9.
268. Lan CC, Wong TT, Chen SJ, et al. Early diagnosis of ventriculoperitoneal shunt infections and malfunctions in children with hydrocephalus. *J Microbiol Immunol Infect* 2003;**36**:47–50.
269. Langley JM, Gravel D, Moore D, et al. Study of cerebrospinal fluid shunt-associated infections in the first year following placement, by the Canadian Nosocomial Infection Surveillance Program. *Infect Control Hosp Epidemiol* 2009;**30**:285–8.
270. Laurini JA, Carter JE, Kahn AG. Tricuspid valve and pacemaker endocarditis due to *Pseudallescheria boydii (Scedosporium apiospermum)*. *South Med J* 2009;**102**:515–17.
271. Lee SH, Oh JH, Lee KS, et al. Infection after prosthetic reconstruction in limb salvage surgery. *Int Orthop* 2002;**26**:179–84.
272. Lee T, Kerr RS, Adams CB. *Pasteurella multocida*: a rare case of shunt infection. *Br J Neurosurg* 1990;**4**:237–9.
273. Leggiadro RJ, Atluru VL, Katz SP. Meningococcal meningitis associated with cerebrospinal fluid shunts. *Pediatr Infect Dis J* 1984;**3**:489–90.
274. Legoupil N, Ronco P, Berenbaum F. Arthritis-related shunt nephritis in an adult. *Rheumatology* 2003;**42**:698–9.
275. Lekkerkerker JC, van Nieuwkoop C, Trines SA, et al. Risk factors and time delay associated with cardiac device infections: Leiden device registry. *Heart* 2009;**95**:715–20.
276. Le Monnier A, Blanot S, Abachin E, et al. *Listeria monocytogenes*: a rare complication of ventriculoperitoneal shunt in children. *J Clin Microbiol* 2011;**49**:3924–7.
277. Lenfestey RW, Smith PB, Moody MA, et al. Predictive value of cerebrospinal fluid parameters in neonates with intraventricular drainage devices. *J Neurosurg* 2007;**107**(suppl. 3):209–12.
278. Lentino JR. Prosthetic joint infections: bane of orthopedists, challenge for infectious disease specialists. *Clin Infect Dis* 2003; **36**:1157–61.
279. Leyden JJ, McGinley KJ, Mills OH, Kligman AM. Age-related changes in the resident bacterial flora of the human face. *J Invest Dermatol* 1975;**65**:379–81.
280. Lidofsky SP, Bass NM, Prage MC, et al. Intracranial pressure monitoring and liver transplantation for fulminant hepatic failure. *Hepatology* 1992;**16**:1–4.
281. Lidwell OM, Lowbury EJL, Whyte W, et al. Effect of ultraclean air in operating rooms on deep sepsis in the joint after total hip or knee replacement: a randomized study. *Br Med J* 1982;**250**: 99–102.
282. Lidwell OM, Lowbury EJL, Whyte W, et al. Airborne contamination of wounds in joint replacement operations: the relationship to sepsis rates. *J Hosp Infect* 1983;**4**:111–31.
283. Lietz K, Long JW, Kfoury AG, et al. Outcomes of left ventricular assist device implantation as destination therapy in the post-REMATCH era: implications for patient selection. *Circulation* 2007;**116**:497–505.
284. Litzler PY, Manrique A, Etienne M, et al. Leukocyte SPECT/CT for detecting infection of left-ventricular-assist devices: preliminary results. *J Nucl Med* 2010;**51**:1044–8.
285. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:e18–55.
286. Lo CH, Spelman D, Bailey M, et al. External ventricular drain infections are independent of drain duration: an argument against elective revision. *J Neurosurg* 2007;**106**:378–83.
287. Lopez-Alvarez B, Martin-Laez R, Farinas MC, et al. Multidrug-resistant *Acinetobacter baumannii* ventriculitis: successful treatment with intraventricular colistin. *Acta Neurochir (Wien)* 2009;**151**: 1465–72.
288. Loundon N, Blanchard M, Roger G, et al. Medical and surgical complications in pediatric cochlear implantation. *Arch Otolaryngol Head Neck Surg* 2010;**136**:12–5.
289. Lozier AP, Sciacca RR, Romagnoli MF, Connolly ES. Ventriculostomy-related infections: a critical review of the literature. *Neurosurgery* 2002;**51**:170–82.
290. Luer MS, Hatton J. Vancomycin administration into the cerebrospinal fluid: a review. *Ann Pharmacother* 1993;**27**:912–21.
291. Luerssen TG. Intracranial pressure: current status in monitoring and management. *Semin Pediatr Neurol* 1997;**4**:146–53.
292. Luntz M, Hodges AV, Balkany T, et al. Otitis media in children with cochlear implants. *Laryngoscope* 1995;**106**:1403–5.
293. Luthardt T. Bacterial infections in ventriculo-auricular shunt systems. *Dev Med Child Neurol* 1970;**12**(suppl. 22):105–9.
294. Lutwick LI, Vaghjimal A, Connolly MW. Postcardiac surgery infections. *Crit Care Clin* 1998;**14**:221–50.
295. Luxford WM, House WF. House 3M cochlear implant: surgical considerations. In: Clark GM, Busby PA, editors. International Cochlear Implant Symposium and Workshop, Melbourne, 1985. *Ann Otol Rhinol Laryngol* 1987;**96**(suppl. 128):12–4.
296. Lyke KE, Obasanjo OO, Williams MA, et al. Ventriculitis complicating use of intraventricular catheters in adult neurosurgical patients. *Clin Infect Dis* 2001;**33**:2028–33.
297. Lyons CW, Berquist TH, Lyons JC, et al. Evaluation of radiographic findings in painful hip arthroplasties. *Clin Orthop Relat Res* 1985;**195**:239–51.
298. Lyons MK, Meyer FB. Cerebrospinal fluid physiology and the management of increased intracranial pressure. *Mayo Clin Proc* 1990;**65**:684–707.
299. Mahan J, Selgison D, Henry SL, et al. Factors in pin tract infections. *Orthopedics* 1991;**14**:305–8.
300. Manders EK, Schenden MJ, Furrey JA, et al. Soft-tissue expansion: concepts and complications. *Plast Reconstr Surg* 1984;**74**: 493–507.
301. Marchena-Yglesias PJ, Rodriguez JA, Garcia GA, et al. Pacemaker lead infection caused by *Aspergillus fumigatus*. *Eur J Intern Med* 2006;**17**:209–10.
302. Marculescu CE, Berbari EF, Cockerill FR III, Osmon DR. Fungi, mycobacteria, zoonotic and other organisms in prosthetic joint infection. *Clin Orthop Relat Res* 2006;**451**:64–72.
303. Marculescu CE, Berbari EF, Hanssen AD, et al. Outcome of prosthetic joint infections treated with debridement and retention of components. *Clin Infect Dis* 2006;**42**:471–8.
304. Marculescu CE, Cantey JR. Polymicrobial prosthetic joint infections: risk factors and outcome. *Orthop Relate Res* 2008;**466**: 1397–404.
305. Margey R, McCann H, Blacke G, et al. Contemporary management of and outcomes from cardiac device related infections. *Europace* 2010;**12**:64–70.
306. Mariani BD, Martin DS, Levine MJ, et al. Polymerase chain reaction detection of bacterial infection in total knee arthroscopy. *Clin Orthop Relat Res* 1996;**331**:11–22.

307. Mason AC, Davison SP, Manders EK. Tissue expander infections in children: look beyond the expander pocket. *Ann Plast Surg* 1999;**43**:539–41.
308. Mason PK, DiMarco JP, Ferguson JD, et al. Sonication of explanted cardiac rhythm management devices for the diagnosis of pocket infections and asymptomatic bacterial colonization. *Pacing Clin Electrophysiol* 2011;**34**:143–9.
309. Massoure PL, Reuter S, Lafitte S, et al. Pacemaker endocarditis: clinical features and management of 60 consecutive cases. *Pacing Clin Electrophysiol* 2007;**30**:12–19.
310. Mates S, Glaser J, Shapiro K. Treatment of cerebrospinal fluid shunt infections with medical therapy alone. *Neurosurgery* 1982; **11**:781–3.
311. Matieli LC, De Freitas D, Samparo J, et al. *Mycobacterium abscessus* endophthalmitis: treatment dilemma and review of the literature. *Retina* 2006;**26**:826–9.
312. May AK, Fleming SB, Carpenter RO, et al. Influence of broad-spectrum antibiotic prophylaxis on intracranial pressure monitor infections and subsequent infectious complications in head-injured patients. *Surg Infect* 2006;**7**:409–17.
313. Mayhall CG, Archer NH, Lamb VA, et al. Ventriculostomy-related infections: a prospective epidemiologic study. *N Engl J Med* 1984;**310**:553–9.
314. McBride LR, Ruzevich SA, Pennington DG, et al. Infectious complications associated with ventricular assist device support. *Trans Am Soc Artif Intern Organs* 1994;**33**:201–2.
315. McCarthy PM, Wang N, Vargo R. Preperitoneal insertion of the HeartMate 1000 IP implantable left ventricular assist device. *Ann Thorac Surg* 1994;**57**:634–8.
316. McCarthy PM, Portner PM, Oyer PE, et al. Clinical experience with the Novacor ventricular assist system: bridge-to-transplant and the transition to chronic application. *J Thorac Cardiovasc Surg* 1991;**102**:578–87.
317. McCarthy PM, Schmitt SK, Vargo RL, et al. Implantable LVAD infections: implications for permanent use of the device. *Ann Thorac Surg* 1996;**61**:359–65.
318. McCarthy PM, Smedira NO, Vargo RL, et al. One hundred patients with the HeartMate left ventricular assist device: evolving concepts and technology. *J Thorac Cardiovasc Surg* 1998;**115**: 904–12.
319. McClinton D, Carraccio C, Englander R. Predictors of ventriculoperitoneal shunt pathology. *Pediatr Infect Dis J* 2001;**20**: 593–7.
320. McDowell A, Patrick S. Evaluation of nonculture methods for the detection of prosthetic hip biofilms. *Clin Orthop Relat Res* 2005;**437**:74–82.
321. McGirt MJ, Leveque JC, Wellons JC 3rd, et al. Cerebrospinal fluid shunt survival and etiology of failures: a seven year institutional experience. *Pediatr Neurosurg* 2002;**36**:248–55.
322. McGirt MJ, Zaas A, Fuchs HE, et al. Risk factors for pediatric ventriculoperitoneal shunt infection and predictors of infectious pathogens. *Clin Infect Dis* 2003;**36**:858–62.
323. McJunkin J, Jeyakumar A. Complications in pediatric cochlear implants. *Am J Otolaryngol Head Neck Med Surg* 2010;**31**:110–13.
324. McLone DG, Czyzewski D, Raimondi AJ, Sommers RC. Central nervous system infections as a limiting factor in the intelligence of children with myelomeningocoele. *Pediatrics* 1982;**70**:338–42.
325. Meehan AM, Osmon DR, Duffy CT, et al. Outcome of penicillin-susceptible streptococcal prosthetic joint infection treated with debridement and retention of the prosthesis. *Clin Infect Dis* 2003;**36**:845–9.
326. Mela T, McGovern BA, Garan H, et al. Long-term infection rates associated with the pectoral versus abdominal approach to caridoverter-defibrillator implants. *Am J Cardiol* 2001;**88**:750–3.
327. Menderes A, Ayden OE, Vayvada H, et al. Aerobic *Actinomyces* spp. infection with a silicone tissue expander: a case report. *J Plast Reconstr Aesthet Surg* 2006;**59**:560–1.
328. Merino P, Arribi A, Gestoso I, et al. Linezolid treatment of a prosthetic joint infection with *Staphylococcus lugdunensis* in a patient with multiple myeloma. *Intl J Antimicrob Agents* 2010;**35**: 203–4.
329. Mine S, Sato A, Yamaura A, et al. Eosinophilia of the cerebrospinal fluid in a case of shunt infection: case report. *Neurosurgery* 1986;**19**:835–6.
330. Mobley LW 3rd, Doran SE, Hellbusch LC. Abdominal pseudocyst: predisposing factors and treatment algorithm. *Pediatr Neurosurg* 2005;**41**:77–83.
331. Modic M, Pflanze W, Feiglin D, Belhobek G. Magnetic resonance imaging of musculoskeletal infections. *Radiol Clin North Am* 1986;**24**:247–58.
332. Mojcik CF, Levy JH. Aprotinin and the systemic inflammatory response after cardiopulmonary bypass. *Ann Thorac Surg* 2001; **71**:745–54.
333. Mokaddem A, Bachraoui K, Sdiri W, et al. Pacemaker infections. *Tunis Med* 2002;**80**:509–14.
334. Molina JE. Undertreatment and overtreatment of patients with infected antiarrhythmic implantable devices. *Ann Thorac Surg* 1997;**63**:504–9.
335. Monkowshi DH, Axelrod P, Fekete T, et al. Infections associated with ventricular assist devices: epidemiology and effect on prognosis after transplantation. *Transpl Infect Dis* 2007;**9**:114–20.
336. Montgomery VL, Strotman JM, Ross MP. Impact of multiple organ system dysfunction and nosocomial infections on survival of children treated with extracorporeal membrane oxygenation after heart surgery. *Crit Care Med* 2000;**28**:526–31.
337. Moore MB, McCully JP, Luckenback M, et al. *Acanthamoeba* keratitis associated with soft contact lenses. *Am J Ophthalmol* 1985; **100**:396–403.
338. Morrice JJ, Young DG. Bacterial colonisation of Holter valves: a ten-year survey. *Dev Med Child Neurol* 1974;**16**(suppl. 32):85–90.
339. Motta F, Buonaguro V, Stignani C. The use of intrathecal baclofen pump implants in children and adolescents: safety and complications in 200 consecutive cases. *J Neurosurg* 2007;**107** (suppl. 1):32–5.
340. Murdoch DR, Roberts SA, Fowler Jr VG, et al. Infection of orthopedic prostheses after *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2001;**32**:647–9.
341. Muttaiyah S, Ritchie S, John S, et al. Efficacy of antibiotic-impregnated external ventricular drain catheters. *J Clin Neurosci* 2010;**17**:296–8.
342. Myers TJ, Khan T, Frazier OH. Infectious complications associated with ventricular assist systems. *ASAIO J* 2000;**46**:S28–36.
343. Nahabedran MY, Tsangans R, Momen B, et al. Infectious complications following breast reconstruction with expanders and implants. *Plast Reconstr Surg* 2003;**112**:467–76.
344. National Institutes of Health consensus conference. Cochlear implants in adults and children. *JAMA* 1995;**274**:1955–61.
345. Naveira FA, Speight KL, Rauck RL, et al. Meningitis after injection of intrathecal baclofen. *Anesth Analg* 1996;**82**:1297–9.
346. Nelson JD. Cerebrospinal fluid shunt infections. *Pediatr Infect Dis* 1984;**3**(suppl):30–2.
347. Nery PB, Fernandes R, Nair GM, et al. Device-related infection among patients with pacemakers and implantable defibrillators: incidence, risk factors, and consequences. *J Cardiovasc Electrophysiol* 2010;**21**:786–90.
348. Nicholas JL, Kamal IM, Eckstein HB. Immediate shunt replacement in the treatment of bacterial colonisation of Holter valves. *Dev Med Child Neurol* 1970;**12**(suppl. 22):110–3.
349. Nitescu P, Hultman E, Appelgren L, et al. Bacteriology, drug stability and exchange of percutaneous delivery systems and antibacterial filters in long-term intrathecal infusion of opioid drugs and bupivacaine in "refractory" pain. *Clin J Pain* 1992; **8**:324–37.
350. Ntziora F, Falagas ME. Linezolid for the treatment of patients with central nervous system infection. *Ann Pharmacother* 2007; **41**:296–308.
351. Obdeijn MC, J-Phillip N, Werker PMN. The osmotic tissue expander: a three-year clinical experience. *J Plast Reconstr Aesthet Surg* 2009;**62**:1219–22.
352. O'Brien M, Parent A, Davis B. Management of ventricular shunt infections. *Childs Brain* 1979;**5**:304–9.
353. O'Keeffe PT, Bayston R. Pneumococcal meningitis in a child with a ventriculoperitoneal shunt. *J Infect* 1991;**22**:77–9.
354. Olsen L, Frykberg T. Complications in the treatment of hydrocephalus in children. *Acta Paediatr Scand* 1983;**72**:385–90.
355. O'Neil JM, Shutze GE, Heulit MJ, et al. Nosocomial infections during extracorporeal membrane oxygenation. *Intensive Care Med* 2001;**27**:1247–53.
356. Onofrio BM, Yaksh TL. Long-term pain relief produced by intrathecal morphine infusion in 53 patients. *J Neurosurg* 1992; **72**:200–4.
357. O'Nunain S, Perez I, Roelke M, et al. The treatment of patients with infected implantable cardioverter-defibrillator systems. *J Thorac Cardiovasc Surg* 1997;**113**:121–9.

358. Owen RJ, Harper WM, Finlay DB, Belton IP. Isotope bone scans in patients with painful knee replacements: do they alter management? *Br J Radiol* 1995;**68**:1204–7.
359. Oz MC, Argenziano M, Catanese KA, et al. Bridge experience with long-term implantable left ventricular assist devices: are they an alternative to transplantation? *Circulation* 1997;**95**: 1844–52.
360. Pae WE, Connell JM, Adelowo A, et al. Does total implantability reduce infection with the use of a left ventricular assist device? the LionHeart experience in Europe. *J Heart Lung Transplant* 2007; **26**:219–29.
361. Page EL, Eby TL. Meningitis after cochlear implantation in Mondini malformation. *Otolaryngol Head Neck Surg* 1997;**116**: 104–6.
362. Palestro CJ, Swyer AJ, Kim CK, et al. Infected knee prosthesis: diagnosis with In-111 leukocyte, Tc-99m sulfur colloid, and the Tc-99m MDP imaging. *Radiology* 1991;**179**:645–8.
363. Pankowsky DA, Ziats NP, Topham NS, et al. Morphologic characteristics of absorbed human plasma proteins on vascular grafts and biomaterials. *J Vasc Surg* 1990;**11**:599–606.
364. Papagelopoulos PJ, Hay JE, Galanis E, Morrey BF. Infection around joint replacements in patients who have a renal or liver transplantation. *J Bone Joint Surg Am* 1998;**80**:607–8.
365. Papo I, Caruselli G. Long-term intracranial pressure monitoring in comatose patients suffering from head injuries: a critical survey. *Acta Neurochir (Wien)* 1977;**39**:187–200.
366. Pappas PG, Kauffman CA, Andes D. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
367. Parker SL, Attenello FJ, Sciubba DM, et al. Comparison of shunt infection incidence in high-risk subgroups receiving antibiotic-impregnated versus standard shunts. *Childs Nerv Syst* 2009;**25**:77–83.
368. Parvizi J, Ghanem E, Joshi A, et al. Does "excessive" anticoagulation predispose to periprosthetic infection? *J Arthroplasty* 2007;**22**(6 suppl 2):24–8.
369. Patel R, Osmon DR, Hanssen AD. The diagnosis of prosthetic joint infection: current techniques and emerging technologies. *Clin Orthop Relat Res* 2005;**437**:55–8.
370. Patrick D, Marcotte P, Garber GE. Acute abdomen in the patient with a ventriculoperitoneal shunt. *Can J Surg* 1990;**33**:37–40.
371. Pattavilakom A, Xenos C, Bradfield O, Danks A. Reduction in shunt infection using antibiotic impregnated CSF shunt catheters: an Australian prospective study. *J Clin Neurosci* 2007;**14**:526–31.
372. Pagan-Langston D, Dohlman CH. Boston keratoprosthesis treatment of herpes zoster neurotrophic keratopathy. *Ophthalmology* 2008;**115**:S21–3.
373. Pavoni GL, Giannella M, Falcone M, et al. Conservative medical therapy of prosthetic joint infections: retrospective analysis of an 8-year experience. *Clin Microbiol Infect* 2004;**10**:831–7.
374. Peerdeman SM, de Groot V, Feller RE. In situ treatment of an infected intrathecal baclofen pump implant with gentamicin-impregnated collagen fleece. *J Neurosurg* 2010;**112**:1308–10.
375. Penn RA. Intrathecal baclofen for spasticity of spinal origin: seven years of experience. *J Neurosurg* 1992;**77**:236–40.
376. Pennington DG, McBride LR, Peigh PS, et al. Eight years' experience with bridging to cardiac transplantation. *J Thorac Cardiovasc Surg* 1994;**107**:472–81.
377. Perl TM, Cullen JJ, Wenzel RP, et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. *N Engl J Med* 2002;**346**:1871–7.
378. Perry HE, Wright RO, Shannon MW, et al. Baclofen overdose: drug experimentation in a group of adolescents. *Pediatrics* 1998; **101**:1045–8.
379. Petty W. The effect of methylmethacrylate on bacterial inhibiting properties of normal human serum. *Clin Orthop Relat Res* 1978; **132**:266–77.
380. Petty W. The effect of methylmethacrylate on bacterial phagocytosis and killing by human polymorphonuclear leukocytes. *J Bone Joint Surg Am* 1978;**60**:752–7.
381. Pfausler B, Beer R, Engelhardt K, et al. Cell index: a new parameter for the early diagnosis of ventriculostomy (external ventricular drainage)-related ventriculitis in patients with intraventricular hemorrhage? *Acta Neurochir (Wien)* 2004;**146**:477–81.
382. Pfausler B, Spiss H, Beer R, et al. Treatment of staphylococcal ventriculitis associated with external cerebrospinal fluid drains: a prospective randomized trial of intravenous compared with intraventricular vancomycin therapy. *J Neurosurg* 2003;**98**: 1040–4.
383. Pfisterer W, Muhlbauer M, Czech T, Reinprecht A. Early diagnosis of external ventricular drainage infection: results of a prospective study. *J Neurol Neurosurg Psychother* 2003;**74**: 929–32.
384. Piccione W. Left ventricular assist device implantation: short and long-term surgical complications. *J Heart Lung Transplant* 2000;**19**:S89–94.
385. Pirotte BJM, Lubansu A, Bruneau M, et al. Sterile surgical technique for shunt placement reduces the shunt infection rate in children: preliminary analysis of a prospective protocol in 115 consecutive procedures. *Childs Nerv Syst* 2007;**23**:1251–61.
386. Pittman T, Williams D, Rathore M, et al. The role of ethylene oxide allergy in sterile shunt malfunctions. *Br J Neurosurg* 1994;**8**:41–3.
387. Poon WS, Ng S, Wai S. CSF antibiotic prophylaxis for neurosurgical patients with ventriculostomy: a randomized study. *Acta Neurochir Suppl* 1998;**71**:146–8.
388. Pople IK, Bayston R, Hayward RD. Infection of cerebrospinal fluid shunts in infants: a study of etiological factors. *J Neurosurg* 1992;**77**:29–36.
389. Pople IK, Mulbauer MS, Sanford RA, et al. Results and complications of intracranial pressure monitoring in 303 children. *Pediatr Neurosurg* 1995;**23**:64–9.
390. Popovich KJ, Hota B, Hayes R, et al. Effectiveness of routine patient cleansing with chlorhexidine gluconate for infection prevention in the medical intensive care unit. *Infect Control Hosp Epidemiol* 2009;**30**:959–63.
391. Poston RS, Husain S, Sorce D, et al. LVAD bloodstream infections: therapeutic rationale for transplantation after LVAD infection. *J Heart Lung Transplant* 2003;**22**:914–21.
392. Proctor RA. The staphylococcal fibronectin receptor: evidence for its importance in invasive infections. *Rev Infect Dis* 1987; **9**(suppl.):335–40.
393. Ragel BT, Browd SR, Schmidt RH. Surgical shunt infection: significant reduction when using intraventricular and systemic antibiotic agents. *J Neurosurg* 2006;**105**:242–7.
394. Ramchandran R, DiLoreto DA, Chung MM, et al. Infectious endophthalmitis in adult eyes receiving Boston type 1 keratoprosthesis. *Ophthalmology* 2012;**119**:674–81.
395. Rand JA, Morrey BF, Bryan RS. Management of the infected total joint arthroplasty. *Orthop Clin North Am* 1984;**15**:491–504.
396. Ratilal B, Costa J, Sampaio C. Antibiotic prophylaxis for surgical introduction of intracranial ventricular shunts. *Cochrane Database Syst Rev* 2006;3:CD005365.
397. Ratilal B, Costa J, Sampaio C. Antibiotic prophylaxis for surgical introduction of intracranial ventricular shunts: a systematic review. *J Neurosurg Pediatrics* 2008;**1**:48–56.
398. Raut VV, Orth MS, Orth MC, et al. One stage revision arthroplasty of the hip for deep gram negative infection. *Int Orthop* 1996;**20**:12–14.
399. Raymond AL, Kfoury AG, Bishop CJ, et al. Obesity and left ventricular assist device driveline exit site infection. *ASAIO J* 2010;**56**:57–60.
400. Rebuck JA, Murray KR, Rhoney DH, et al. Infection related to intracranial pressure monitors in adults: analysis of risk factors and antibiotic prophylaxis. *J Neurol Neurosurg Psychiatry* 2000; **69**:381–4.
401. Reefhuis J, Honein MA, Whitney CG, et al. Risk of bacterial meningitis in children with cochlear implants. *N Engl J Med* 2003;**349**:435–45.
402. Reid G. Bacterial colonization of prosthetic devices and measures to prevent infection. *New Horizons* 1998;**6**(suppl. 2):58–63.
403. Reynolds M, Sherman JO, McLone DG. Ventriculoperitoneal shunt infection masquerading as an acute surgical abdomen. *J Pediatr Surg* 1983;**18**:951–4.
404. Roitberg BZ, Tomita T, McLone DG. Abdominal cerebrospinal fluid pseudocyst: a complication of ventriculoperitoneal shunt in children. *Pediatr Neurosurg* 1998;**29**:267–73.
405. Ronan A, Hogg GG, Klug GL. Cerebrospinal fluid shunt infections in children. *Pediatr Infect Dis J* 1995;**14**:782–6.
406. Rubin LG, Papsin B, and the Committee on Infectious Diseases and Section on Otolaryngology-Head and Neck Surgery. Cochlear implants in children: surgical site infections and the prevention

and treatment of acute otitis media and meningitis. *Pediatrics* 2010;**126**:381–91.
407. Rubin RC, Ghatak NR, Kisudhipan P. Asymptomatic perforated viscus and gram-negative ventriculitis as a complication of valve-regulated ventriculoperitoneal shunts. *J Neurosurg* 1972; **37**:616–8.
408. Rubinstein JT, Gantz FJ, Parkinson WS. Management of cochlear implant infections. *Am J Otol* 1999;**20**:46–9.
409. Rupp ME, Ulphani JS, Fey PD, et al. Characterization of the importance of polysaccharide intercellular/hemagglutinin of *Staphylococcus epidermidis* in the pathogenesis of biomaterial-based infection in a mouse foreign body infection model. *Infect Immun* 1999;**67**:2627–32.
410. Ruppen W, Derry S, McQuary HJ, Moore RA. Infection rates associated with epidural indwelling catheters for seven days or longer: systemic review and meta-analysis. *BMC Palliat Care* 2007;**6**:3.
411. Rupprecht TA, Pfister HW. Clinical experience with linezolid for the treatment of central nervous system infections. *Eur J Neurol* 2005;**12**:536–42.
412. Salgado CD, Dash S, Cantey JR, Marculescu CE. Higher risk of failure of methicillin-resistant *Staphylococcus aureus* prosthetic joint infections. *Clin Orthop Relat Res* 2007;**461**:48–53.
413. Salmon JH. Adult hydrocephalus: evaluation of shunt therapy in 80 patients. *J Neurosurg* 1972;**37**:423–8.
414. Samtleben W, Bauriedel G, Bosch T, et al. Renal complications of infected ventriculoatrial shunts. *Artif Organs* 1993;**17**: 695–701.
415. Samuel M, Finnerty GT, Rudge P. Intrathecal baclofen pump infection treated by adjuvant intrareservoir antibiotic instillation. *J Neurol Neurosurg Psychiatry* 1994;**57**:1146–7.
416. Samuels LE, Samuels FL, Kaufman MS, et al. Management of infected implantable cardiac defibrillators. *Ann Thorac Surg* 1997; **64**:1702–6.
417. Sanderson PJ. Infection in orthopaedic implants. *J Hosp Infect* 1991;**18**(suppl. A):367–75.
418. Schade RP, Schinkel J, Roelandse FW, et al. Lack of value of routine analysis of cerebrospinal fluid for prediction and diagnosis of external drainage-related bacterial meningitis. *J Neurosurg* 2006;**104**:101–8.
419. Schaffer JM, Allen JG, Weiss ES, et al. Infectious complications after pulsatile-flow and continuous-flow left ventricular assist device implantation. *J Heart Lung Transplant* 2011;**30**:164–74.
420. Scheithauer S, Burgel U, Bickenbach J, et al. External ventricular and lumbar drainage-associated meningoventriculitis: prospective analysis of time-dependent infection rates and risk factor analysis. *Infection* 2010;**38**:205–9.
421. Scheithauer S, Burgel U, Ryang YM, et al. Prospective surveillance of drain associated meningitis/ventriculitis in a neurosurgery and neurological intensive care unit. *J Neurol Neurosurg Psychiatry* 2009;**80**:1381–5.
422. Schoifet SD, Morrey BF. Treatment of infection after total knee arthroplasty by débridement with retention of the components. *J Bone Joint Surg Am* 1990;**72**:1383–90.
423. Schoenbaum SC, Gardner P, Shillito J. Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. *J Infect Dis* 1975;**131**:543–52.
424. Schreffler RT, Schreffler AJ, Wittler RR. Treatment of cerebrospinal fluid shunt infections: a decision analysis. *Pediatr Infect Dis J* 2002;**21**:632–6.
425. Schuhmann MU, Ostrowski KR, Draper EJ, et al. The value of C-reactive protein in the management of shunt infections. *J Neurosurg* 2005;**103**(3 suppl.):223–30.
426. Schulman AR, Martens TP, Christos PJ, et al. Comparisons of infection complications between continuous flow and pulsatile flow left ventricular assist devices. *J Thorac Cardiovasc Surg* 2007;**133**:841–2.
427. Schulman AR, Martens TP, Russo MJ, et al. Effect of left ventricular assist device infection on post-transplant outcomes. *J Heart Lung Transplant* 2009;**28**:237–42.
428. Schultz M, Moore K, Foote AW. Bacterial ventriculitis and duration of ventriculostomy catheter insertion. *J Neurosci Nurs* 1993;**25**:158–64.
429. Schwartz JG, Tio FO, Fetchick RJ. Filamentous *Histoplasma capsulatum* involving a ventriculoatrial shunt. *Neurosurgery* 1986; **18**:487–90.
430. Sciubba DM, Morgan S, Metthew BA. Effect of antibiotic-impregnated shunt catheters in decreasing the incidence of shunt infection in the treatment of hydrocephalus. *J Neurosurg* 2005;**103**(suppl. 2):131–6.
431. Sciubba DM, Lin LM, Woodworth GF, et al. Factors contributing to the medical costs of cerebrospinal fluid shunt infection treatment in pediatric patients with standard shunt components compared with those in patients with antibiotic impregnated components. *Neurosurg Focus* 2007;**22**:E9.
432. Sells CJ, Shurtleff DB, Loeser JD. Gram-negative cerebrospinal fluid shunt–associated infections. *Pediatrics* 1977;**59**: 614–18.
433. Selwyn S, Ellis H. Skin bacteria and skin disinfection reconsidered. *Br Med J* 1972;**1**:136–40.
434. Shah K, Mohammed A, Patil S, et al. Circulating cytokines after hip and knee arthroplasty: a preliminary study. *Clin Orthop Relat Res* 2009;**467**:946–51.
435. Sharma NS, Ooi JL, Downie JA, et al. Corneal perforation and intraocular lens prolapse in *Serratia marcescens* endophthalmitis. *Clin Exp Ophthalmol* 2007;**35**:381–2.
436. Sharma NS, Ooi JL, Maloof TC, et al. Culture-proven *Aspergillus fumigatus* infection in a primary hydroxyapatite orbital implant. *Clin Exp Ophthalmol* 2007;**35**:294–5.
437. Shimizu T, Luciano MG, Fukuhara T. Role of endoscopic third ventriculostomy at infected cerebrospinal fluid shunt removal. *J Neurosurg Pediatr* 2012;**9**:320–6.
438. Shoman S, Shaffer R, Sweet L, et al. Candidemia in patients with ventricular assist devices. *Clin Infect Dis* 2007;**44**:e9–12.
439. Shurtleff DB, Stuntz JT, Hayden PW. Experience with 1201 cerebrospinal fluid shunt procedures. *Pediatr Neurosci* 1986;**12**: 49–57.
440. Shurtleff DB, Foltz EL, Weeks RD, Loeser J. Therapy of *Staphylococcus epidermidis:* infections associated with cerebrospinal fluid shunts. *Pediatrics* 1974;**53**:55–62.
441. Shurtleff DB, Loeser JD, Avellino AM, et al. *Haemophilus influenzae* and *Streptococcus pneumoniae* infections in children with cerebrospinal fluid shunts. *Pediatr Neurosurg* 2009;**45**:276–80.
442. Siegfried RN, Jacobson L, Chabal C. Development of an acute withdrawal syndrome following the cessation of intrathecal baclofen in a patient with spasticity. *Anesthesiology* 1992;**77**: 1048–50.
443. Simon D, Fischer S, Grossman A, et al. Left ventricular assist device–related infection: treatment and outcome. *Clin Infect Dis* 2005;**40**:1108–15.
444. Simon TD, Hall M, Dean JM, et al. Reinfection following initial cerebrospinal fluid shunt infection. *J Neurosurg Pediatr* 2010;**6**:277–85.
445. Simon TD, Hall M, Riva-Cambrin J, et al. Infection rates following initial cerebrospinal fluid shunt placement across pediatric hospitals in the United States. *J Neurosurg Pediatr* 2009;**4**: 156–65.
446. Sinha P, Chen JM, Flannery M, et al. Infections during left ventricular assist device support do not affect posttransplant outcomes. *Circulation* 2000;**102**(suppl. III):194–9.
447. Slaughter MS, Rogers JG, Milano CA, et al. Advanced heart failure treated with continuous-flow left ventricular assist device. *N Engl J Med* 2009;**361**:2241–51.
448. Smith E, Butler W, Barker FG 2nd. In-hospital mortality rates after ventriculoperitoneal shunt procedures in the United States, 1998 to 2000: relation to hospital and surgeon volume of care. *J Neurosurg* 2004;**100**(suppl. 2):90–7.
449. Smith PN, Vidaillet HJ, Hayes JJ, et al. Infections with nonthoracotomy implantable cardioverter defibrillators: can these be prevented? *Pacing Clin Electrophysiol* 1998;**21**:42–55.
450. Sohail MR, Henrikson CA, Braid-Forbes MJ, et al. Mortality and cost associated with cardiovascular implantable electronic device infections. *Arch Intern Med* 2011;**171**:1821–8.
451. Sohail MR, Hussain S, Le KY, et al. Risk factors associated with early-versus-late-onset implantable cardioverter-defibrillator infections. *J Interv Card Electrophysiol* 2011;**31**:171–83.
452. Sohail MR, Uslan DZ, Kahn AH, et al. Management and outcome of permanent pacemaker and implantable cardioverter-defibrillator infections. *J Am Coll Cardiol* 2007;**49**:1851–9.
453. Sohail MR, Uslan DZ, Khan AH, et al. Infective endocarditis complicating permanent pacemaker and implantable cardioverter-defibrillator infection. *Mayo Clin Proc* 2008;**83**:46–53.

454. Soleman J, Marbacher S, Fandino J, Fathi AR. Is the use of antibiotic-impregnated external ventricular drainage beneficial in the management of iatrogenic ventriculitis? *Acta Neurochir* 2012;**154**:161–4.
455. Sonabend AM, Korenfeld Y, Crisman C, et al. Prevention of ventriculostomy-related infections with prophylactic antibiotics and antibiotic-coated external ventricular drains: a systematic review. *Neurosurgery* 2011;**68**:996–1005.
456. Sonmezoglu K, Sonmezoglu M, Halac M, et al. Usefulness of ^{99m}Tc-ciprofloxacin (Infecton) scan in diagnosis of chronic orthopedic infections: comparative study with ^{99m}Tc-HMPAO leukocyte scintigraphy. *J Nucl Med* 2001;**42**:567–74.
457. Sousa R, Pereira A, Massada M, et al. Empirical antibiotic therapy in prosthetic joint infections. *Acta Orthopaed Belgica* 2010;**76**:254–9.
458. Sousa R, Massada M, Pereira A, et al. Diagnostic accuracy of combined 99m-Tc-sulesomab and 99mTc-nanocolloid bone marrow imaging in detecting prosthetic joint infection. *Nuclear Med Commun* 2011;**32**:834–9.
459. Spangehl M, Masri B, O'Connell J, Duncan C. Prospective analysis of preoperative and intraoperative investigations for the diagnosis of infection at the sites of 202 revision total hip arthroplasties. *J Bone Joint Surg Am* 1999;**81**:672–83.
460. Spangehl MJ, Younger ASE, Masri BA, et al. Diagnosis of infection following total hip arthroplasty. *J Bone Joint Surg Am* 1997;**79**:1578–88.
461. Spencer M, Bird G. Device-related nonintravascular infections. *Crit Care Nurs Clin North Am* 1995;**7**:685–93.
462. Spinler SA, Nawarskas JJ, Foote EF, et al. Clinical presentation and analysis of risk factors for infectious complications of implantable cardioverter-defibrillator implantations at a university medical center. *Clin Infect Dis* 1998;**26**:1111–16.
463. Stamos JK, Kaufman BA, Yogev R. Ventriculoperitoneal shunt infections with gram-negative bacteria. *Neurosurgery* 1993;**33**: 858–62.
464. Stark WJ, Worthen DM, Holladay JT, et al. The FDA report on intraocular lenses. *Ophthalmology* 1983;**90**:311–17.
465. Starling RC, Naka Y, Boyle AJ, et al. Results of the post-U.S. Food and Drug Administration-approval study with a continuous flow left ventricular assist device as a bridge to heart transplantation: a prospective study using the INTERMACS. *J Am Coll Cardiol* 2011;**57**:1890–8.
466. Steekelberg JM, Osmon DR. Prosthetic joint infections. In: Waldvogel FA, Bisno AL, editors. *Infections associated with indwelling medical devices*. 3rd ed. Washington, DC: ASM Press; 2000. p. 173–209.
467. Stehr-Green J, Bailey TM, Brandt FH, et al. *Acanthamoeba* keratitis in soft contact lens wearers. *JAMA* 1987;**258**:57–60.
468. Steiner CK, Stewart DL, Bond SJ, et al. Predictors of acquiring a nosocomial bloodstream infection on extracorporeal membrane oxygenation. *J Pediatr Surg* 2001;**36**:487–92.
469. Stewart MPM, Kelly IG. Total shoulder replacement in rheumatoid disease: 7–13 year followup of 37 joints. *J Bone Joint Surg Br* 1997;**79**:68–72.
470. Stotka JL, Rupp ME, Meier FA, et al. Meningitis due to *Neisseria mucosa*: case report and review. *Rev Infect Dis* 1991;**13**: 837–41.
471. Stumpe KD, Romero J, Ziegler O, et al. The value of FDG-PET in patients with painful total knee arthroplasty. *Eur J Nucl Med Mol Imaging* 2006;**33**:1218–25.
472. Sun BC, Catanese KA, Spanier TB, et al. 100 Long-term implantable left ventricular assist devices: the Columbia Presbyterian interim experience. *Ann Thorac Surg* 1999;**68**:688–94.
473. Sun HY, Ko WJ, Tsai PR, et al. Infections occurring during extracorporeal membrane oxygenation use in adult patients. *J Thorac Cardiovasc Surg* 2010;**140**:1125e–32e.
474. Sutherland K, Mahoney JR II, Coury AJ, Eaton JW. Degradation of biomaterials by phagocyte-derived oxidants. *J Clin Invest* 1993;**92**:2360–7.
475. Swayne R, Rampling A, Newsom SWB. Intraventricular vancomycin for treatment of shunt-associated ventriculitis. *J Antimicrob Chemother* 1987;**19**:249–53.
476. Swan MC, Goodacre TE, Czernuszka JR, et al. Cleft palate repair with the use of osmotic tissue expanders: a response. *J Plast Reconstr Aesthet Surg* 2008;**61**:220–1.
477. Tang CW, Cheng CK, Lee TS. Community-acquired bleb-related endophthalmitis caused by vancomycin-resistant enterococci. *Can J Ophthalmol* 2007;**42**:477–8.
478. Tang L, Eaton JW. Inflammatory responses to biomaterials. *Am J Clin Pathol* 1995;**103**:466–71.
479. Tangden T, Enblad P, Ullberg M, et al. Neurosurgical gram-negative bacillary ventriculitis and meningitis: a retrospective study evaluating the efficacy of intraventricular gentamicin therapy in 31 consecutive cases. *Clin Infect Dis* 2011;**52**:1310–16.
480. Teddy P, Jamous A, Gardner B, et al. Complications of intrathecal baclofen delivery. *Br J Neurosurg* 1992;**6**:115–18.
481. Teller RE, Christie MJ, Martin W, et al. Sequential indium-labeled leukocyte and bone scans to diagnose prosthetic joint infection. *Clin Orthop Relat Res* 2000;**373**:241–7.
482. Tenover FC, McDonald LC. Vancomycin-resistant staphylococci and enterococci: epidemiology and control. *Curr Opin Infect Dis* 2005;**18**:300–5.
483. Thompson DN, Hartley JC, Hayward RD. Shunt infection: is there a near-miss scenario? *J Neurosurg* 2007;**106**(suppl):15–19.
484. Thompson DN, Malcolm GP, Jones BM, et al. Intracranial pressure in single-suture craniosynostosis. *Pediatr Neurosurg* 1995;**22**: 235–9.
485. Thompson TP, Albright AL. *Propionibacterium acnes* infections of cerebrospinal fluid shunts. *Childs Nerv Syst* 1998;**14**:378–80.
486. Topkara VK, Kondareddy S, Malik F, et al. Infectious complications in patients with left ventricular assist device: etiology and outcomes in the continuous-flow era. *Ann Thorac Surg* 2010; **90**:1270–7.
487. Toy PTCY, Lai LW, Drake TA, Sande MA. Effect of fibronectin on adherence of *Staphylococcus aureus* to fibrin thrombi in vitro. *Infect Immun* 1985;**48**:83–6.
488. Trampuz A, Hanssen AD, Osmon DR, et al. Synovial fluid leukocyte count and differential for the diagnosis of prosthetic knee infection. *Am J Med* 2004;**117**:556–62.
489. Trampuz A, Piper KE, Jacobson MJ, et al. Sonication of removed hip and knee prostheses for diagnosis of infection. *N Engl J Med* 2007;**357**:654–63.
490. Trappe HJ, Pfitzner P, Klein H. Infections after cardioverter-defibrillator implantation: observations in 335 patients over 10 years. *Br Heart J* 1995;**73**:20–4.
491. Tse T, Cheng K, Wong K, et al. Ventriculostomy and infection: a 4-year review in a local hospital. *Surg Neuro Int* 2010;**1**: 47–52.
492. Tsui I, Uslan DZ, Hubschman JP, et al. *Nocardia farcinica* infection of a Baerveldt implant and enophthalmitis in a patient with a Boston type 1 keratoprosthesis. *J Glaucoma* 2010;**19**: 339–40.
493. Tuan TJ, Thorell EA, Mayer-Hamblett N. Treatment and microbiology of repeated cerebrospinal fluid shunt infections in children. *Ped Infect Dis J* 2011;**30**:731–5.
494. Tubbs RS, Muhleman M, Loukas M, Cohen-Gadol AA. Ventriculoperitoneal shunt malfunction from cerebrospinal fluid eosinophilia in children: case-based update. *Childs Nerv Syst* 2012;**28**: 345–8.
495. Tunkel AR, Thomas CY, Wispelwey B. *Candida* prosthetic arthritis: report of a case treated with fluconazole and review of the literature. *Am J Med* 1993;**94**:100–3.
496. Turgut M, Alabaz D, Erbey F. Cerebrospinal fluid shunt infections in children. *Pediatr Neurosurg* 2005;**41**:131–6.
497. Turkisher V, Priel I, Dan M. Successful management of an infected implantable cardioverter defibrillator with oral antibiotics and without removal of the device. *Pacing Clin Electrophysiol* 1997;**20**: 2268–70.
498. Ubogu EE, Lindenberg JR, Werz MA. Transverse myelitis associated with *Acinetobacter baumanii* intrathecal pump catheter–related infection. *Reg Anesth Pain Med* 2003;**28**:470–4.
499. Update: *Fusarium* keratitis: United States, 2005–2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:563–4.
500. Ure KJ, Amstutz HC, Nasser S, Schmalzried TP. Direct-exchange arthroplasty for the treatment of infection after total hip replacement. An average ten-year follow-up. *J Bone Joint Surg Am* 1998;**80**:961–8.
501. Uslan DZ, Gleva MJ, Warren DK, et al. Cardiovascular implantable electronic device replacement infections and prevention: results from the REPLACE registry. *PACE* 2012;**35**:81–7.

502. Uslan DZ, Sohail MR, St. Sauver JL, et al. Permanent pacemaker and implantable cardioverter defibrillator infection: a population-based study. *Arch Intern Med* 2007;**167**:669–75.
503. Van Meurs K, Lally KP, Peek G, et al. *ECMO extracorporeal cardiopulmonary support in critical care*. 3rd ed. Ann Arbor (Mich): Extracorporeal Life Support Organization; 2005.
504. Vaudaux PE, Francois P, Lew DP, Waldvogel FA. Host factors predisposing to and influencing therapy of foreign body infections. In: Waldvogel FA, Bisno AL, editors. *Infections associated with indwelling medical devices*. 3rd ed. Washington, DC.: A. S. M. Press; 2000. p. 1–26.
505. Vaudaux PE, Zulian G, Huggler E, Waldvogel FA. Attachment of *Staphylococcus aureus* to polymethylmethacrylate increases its resistance to phagocytosis in foreign body infection. *Infect Immun* 1985;**50**:472–7.
506. Vender JR, Hester S, Waller JL, et al. Identification and management of intrathecal baclofen pump complications: a comparison of pediatric and adult patients. *J Neurosurg* 2006;**104**:9–15.
507. Venes JL. Control of shunt infection: report of 150 consecutive cases. *J Neurosurg* 1976;**45**:311–14.
508. Verani JR, Lorick SA, Yoder JS, et al. National outbreak of *Acanthamoeba* keratitis associated with use of a contact lens solution, United States. *Emerg Infect Dis* 2009;**15**:1236–42.
509. Vercellotti GM, McCarthy JD, Lindholm P, et al. Extracellular matrix proteins (fibronectin, laminin and type IV collagen) bind and aggregate bacteria. *Am J Pathol* 1985;**120**:13–21.
510. Vercillo M, Patzakis MJ, Holtom P, Zalavras CG. Linezolid in the treatment of implant-related chronic osteomyelitis. *Clin Orthop Relat Res* 2007;**461**:40–3.
511. Vertullo CJ, Duke PF, Askin GN. Pin-site complications of the halo thoracic brace with routine pin re-tightening. *Spine* 1997; **22**:2514–16.
512. Viale P, Pagani L, Cristini F, et al. Linezolid for the treatment of central nervous system infections in neurosurgical patients. *Scand J Infect Dis* 2002;**34**:456–9.
513. Victor F, DePlace C, Camus C, et al. Pacemaker lead infection: echocardiographic features, management, and outcome. *Heart* 1999;**81**:82–7.
514. Vilchez F, Martinez-Pastor JC, Garcia-Ramiro S, et al. Efficacy of debridement in hematogenous and early post-surgical prosthetic joint infections. *Intl J Artificial Organs* 2011;**34**:863–9.
515. Villamil CL, Rodriguez FM, Van den Eynde CA, et al. Permanent transvenous pacemaker infections: an analysis of 59 cases. *Eur J Intern Med* 2007;**18**:484–8.
516. Vinchon M, Dhellemmes P. Cerebrospinal fluid shunt infection: risk factors and long-term follow-up. *Childs Nerv Syst* 2006; **22**:692–7.
517. Vogt PR, Sagdic K, Lachat M, et al. Surgical management of infected permanent transvenous pacemaker systems: ten year experience. *J Card Surg* 1996;**11**:180–6.
518. Voigt A, Shalaby A, Saba S. Rising rates of cardiac rhythm management device infections in the United States: 1996 through 2003. *J Am Coll Cardiol* 2006;**48**:590–1.
519. Vougioukas VI, Feuerhake F, Hubbe U, et al. Latent abscess formation adjacent to a non-functioning intraventricular catheter. *Childs Nerv Syst* 2003;**19**:119–21.
520. Wald SL, McLaurin RL. Cerebrospinal fluid antibiotic levels during treatment of shunt infections. *J Neurosurg* 1980;**52**:41–6.
521. Walker PC, Depestel DD, Miles NA, et al. Surgical infection prophylaxis practices for left ventricular assist device implantation. *J Card Surg* 2011;**26**:440–3.
522. Walters BC. Cerebrospinal fluid shunt infection. *Neurosurg Clin North Am* 1992;**3**:387–401.
523. Walters BC, Hoffman HJ, Hendrick EB, Humphreys RP. Cerebrospinal fluid shunt infection: influences on initial management and subsequent outcome. *J Neurosurg* 1984;**60**:1014–21.
524. Walters Jr EB, Gingras JL, McKinney Jr RE. Systemic *Torulopsis glabrata* infection in a neonate. *South Med J* 1990;**83**:837–8.
525. Walters HL III, Hakimi M, Rice MD, et al. Pediatric cardiac surgical ECMO: multivariate analysis of risk factors for hospital death. *Ann Thorac Surg* 1995;**60**:329–37.
526. Wang KC, Lee HJ, Sung JN, Cho BK. Cerebrospinal fluid shunt infection in children: efficiency of management protocol, rate of persistent shunt colonization, and significance of "off-antibiotics" trial. *Childs Nerv Syst* 1999;**15**:38–44.
527. Wang RC, Parisier SC, Weiss MH, et al. Cochlear implant flap complications. *Ann Otol Rhinol Laryngol* 1990;**99**:791–5.
528. Watanabe I, Hodges GR, Dworzack DL, et al. Neurotoxicity of intrathecal gentamicin: a case report and experimental study. *Ann Neurol* 1978;**4**:564–72.
529. Wazni O, Epstein LM, Carrillo RG, et al. Lead extraction in the contemporary setting: the LExICon study: an observational retrospective study of consecutive laser lead extractions. *J Am Coll Cardiol* 2010;**55**:579–86.
530. Weber DJ, Hofman KL, Thoft RA, Baker AS. Endophthalmitis following intraocular lens implantation. *Rev Infect Dis* 1986;**8**: 12–80.
531. Weyland M, Hermann M, Kondruweit M, et al. Clinical impact of infections of left ventricular assist device recipients: the importance of site and organism. *Transplant Proc* 1997;**29**:3327–9.
532. Whitehead WE, Kestle JRW. The treatment of cerebrospinal fluid shunt infections. *Pediatr Neurosurg* 2001;**35**:205–10.
533. Wiersbitzky SK, Ahrens N, Becker T, et al. The diagnostic importance of eosinophil granulocytes in the CSF of children with ventricular-peritoneal shunt systems. *Acta Neurol Scand* 1998;**97**: 201–3.
534. Wiese KG, Vogel M, Guthoff R, et al. Treatment of congenital anophthalmos with self-inflating polymer expanders: a new method. *J Craniomaxillofac Surg* 1999;**27**:72–6.
535. Wilhelm MJ, Schmid C, Hammel D, et al. Cardiac pacemaker infection: surgical management with and without extracorporeal circulation. *Ann Thorac Surg* 1997;**64**:1707–12.
536. Wilkoff BL, Lowe CJ, Byrd CL, et al. Transvenous lead extraction: Heart Rhythm Society expert consensus on facilities, training, indications, and patient management: *Heart Rhythm* 2009; **6**:1085–104.
537. Wilson MG, Kelley K, Thornhill TS. Infection as a complication of total knee-replacement arthroplasty: risk factors and treatment in sixty-seven cases. *J Bone Joint Surg Am* 1990;**72**: 878–83.
538. Wilson MW, Wobig JL, Dailey RA. Infection of a porous polyethylene orbital implant with *Capnocytophaga*. *Ophthal Plast Reconstr Surg* 1998;**14**:398–402.
539. Wilson WR, Greer GE, Grubb BP. Implantable cardioverter-defibrillators in children: a single-institutional experience. *Ann Thorac Surg* 1998;**65**:775–81.
540. Wimmer C, Gluch H, Franzreb M, Ogon M. Predisposing factors for infection in spine surgery: a survey of 850 spinal procedures. *J Spinal Disord* 1998;**11**:124–8.
541. Winn HR, Dacey RG, Jane JA. Intracranial subarachnoid pressure recording: experience with 650 patients. *Surg Neurol* 1977;**8**: 41–7.
542. Wong TY, Ng TP, Fong KS, et al. Risk factors and clinical outcomes between fungal and bacterial keratitis: a comparative study. *CLAO J* 1997;**23**:275–81.
543. Wong GKC, Poon WS, Wai S, et al. Failure of regular external ventricular drain exchange to reduce cerebrospinal fluid infection: result of a randomized controlled trial. *J Neurol Neurosurg Psychother* 2002;**73**:759–61.
544. Wymenga AB, van Horn JR, Theeuwes A, et al. Perioperative factors associated with septic arthritis after arthroplasty: prospective multicenter study of 362 knee and 2,651 hip operations. *Acta Orthop Scand* 1992;**63**:665–71.
545. Yar JR, Seo JH, Kim YH, et al. Six cases of bacterial infection in porous orbital implants. *Jpn J Ophthalmol* 2003;**47**:512–18.
546. Yeh LK, Kao SC, Tsai CC, et al. [Delayed-onset of *Pseudomonas* infection in a hydroxyapatite orbital implant: a case report.] *Chung Hua I Hsueh Tsa Chih [Chinese Medical Journal]* 1999;**62**: 832–7.
547. Yildiran ST, Mutlu FM, Sarachi MA, et al. Fungal endophthalmitis caused by *Aspergillus ustus* in a patient following cataract surgery. *Med Mycol* 2006;**44**:665–9.
548. Yildiz EH, Abdalla YF, Elsahn AF, et al. Update on fungal keratitis from 1999 to 2008. *Cornea* 2010;**29**:1406–11.
549. Yildiz EH, Ailani H, Hammersmith KM, et al. *Alternaria* and *Paecilomyces* keratitis associated with soft contact lens wear. *Cornea* 2010;**29**:564–8.
550. Yilmaz A, Dalgic N, Musluman M, et al. Linezolid treatment of shunt-related cerebrospinal fluid infections in children. *J Neurosurg Pediatr* 2010;**5**:443–8.

551. Yoder JS, Verani J, Heidman N, et al. *Acanthamoeba* keratitis: the persistence of cases following a multistate outbreak. *Ophthalmic Epidemiol* 2012;**19**:221–5.
552. Yogev R, Damle B, Levy G, Nachman S. Pharmocokinetics and distribution of linezolid in cerebrospinal fluid in children and adolescents. *Pediatr Infect Dis J* 2010;**29**:827–30.
553. Younger JJ, Christensen GD, Bartley DL, et al. Coagulase-negative staphylococci isolated from cerebrospinal fluid shunts: importance of slime production, species identification, and shunt removal to clinical outcome. *J Infect Dis* 1987;**156**: 548–54.
554. Zed PJ, Stiver G, Devonshire V, et al. Continuous intrathecal pump infusion of baclofen with antibiotic drugs for treatment of pump-associated meningitis. *J Neurosurg* 2000;**92**:347–9.
555. Ziai WC, Lewin III JJ. Improving the role of intraventricular antimicrobial agents in the management of meningitis. *Curr Opin Neurol* 2009;**22**:277–82.
556. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004;**351**:1645–54.
557. Zipes DP, Roberts D. Results of the international study of the implantable pacemaker cardioverter-defibrillator: a comparison of epicardial and endocardial lead systems. *Circulation* 1995;**92**:59–65.

suite were common risks for development of infection.[28] Determining when an infection has occurred is difficult. The overall facial appearance of the child may be so altered by the surgical intervention and postoperative swelling that even parents may not recognize that infection has occurred. Diagnostic needle aspiration may be helpful. Because infections tend to be caused by bacteria that reside in the sites through which incisions and osteotomies are performed, the main culprits are skin, paranasal sinus, and oropharyngeal flora. Each additional site that is surgically violated increases the risk for development of an infection. Infections include cellulitis and dehiscence of the wound, infection of subgaleal fluid, osteomyelitis, focal soft tissue abscesses, epidural abscesses, and septicemia.[9,27,28] Exposure of harvested bone and use of metal plates, special cements, distraction pin systems, and wires render infection treatment decisions difficult, so follow-up plays a greater role in management of related infections than in management of simple surgical site infections. Routes of antibiotic administration and duration of antibiotic therapy are poorly established. Most procedures have required extensive preoperative planning, and what is done may not be so easily undone. The infectious diseases consultant must be prepared to engage in an ongoing dialogue with the surgeon and the patient's family, determining what can be removed and at what cost, providing objective feedback about the success of the treatment to that point, and declaring, when needed, that a failure of medical therapy has occurred. Stating dogmatically that all devitalized bone and foreign material must be removed will, in all likelihood, cause the surgeon to delay requesting consultation when infectious diseases services are again needed.

PROCEDURES AND INFECTIONS

Some children require palatal reconstruction, others need distraction osteogenesis by mandibular distraction pin device systems for mandibular hypoplasia.[16] Some require advancement of large portions of the face. One less commonly performed procedure to correct midfacial retrusion is the frontofacial monobloc advancement.[20] This procedure involves detachment and advancement of the entire facial bony mask and commonly requires a large frontal craniotomy, the bone from which may be shaped to complete the repair or used as needed to form a suitable foundation to which the advanced monobloc can be secured. Osteotomies are performed along the lower portion of the frontal bone and posteriorly along the roof of the orbits, along the lateral and medial inferior walls of the orbits, and through the zygomatic arches, with subsequent pterygomaxillary disjunction. A frontoethmoidal osteotomy divides the posterior portion of the nasal septum to free the monobloc so that it can be advanced and secured in place by wiring it anteriorly to a slightly fore-tilted strip of frontal bone and stabilizing it laterally with wired-in strips of calvarial bone grafts to bridge gaps in the zygomatic arches. Bone blocks in the pterygomaxillary area help to hold the maxillary portion forward. The temporary dead space created in the anterior cranial fossa is problematic in some patients.[27] This procedure is associated with an inherent risk for developing meningitis, which is associated less frequently with the extracranial procedures described later, although meningitis still may occur.[24] An alternative procedure that seeks to avoid entering sinus cavities and the cranium is the subcranial Le Fort III osteotomy.[12,20] Exposure is provided through a coronal incision with distraction of the soft tissues of the forehead. Osteotomies free part of the medial, lateral, and inferior walls of the orbit and the nasal bridge, and the remainder of the midface bloc is freed by pterygopalatine disjunction and osteotomies of the zygomatic arch and posterior nasal septum. Thus, only the midface bloc is brought forward, and interposition grafts, hydroxyapatite, microplates, and wires secure the bloc in the advanced position. As in the frontofacial advancement, split calvarial bone grafts bridge the space in the zygomatic arches. A Le Fort I osteotomy is an isolated maxillary advancement procedure that might additionally be used to change the position of the upper teeth.[12,15,20]

Some cases of Treacher Collins syndrome require the Integrale (simultaneous midfacial and mandibular osteotomies).[20] In this complicated procedure requiring a tracheostomy, the midface osteotomies exclude the temporal, lateral walls of the orbits from the advanced bloc. In addition, osteotomies shaped like a C or inverted V through the mandibular rami with placement of the interposition bone grafts and wiring provide advancement of the mandible. Split calvarial bone grafts subsequently reestablish the zygomatic arches.

Reconstructive materials include bone autograft and solvent-treated allograft, titanium plates, and alloplasts such as polymethyl methacrylate and hydroxyapatite cements, some of which readily accept bone ingrowth and wires. New materials are in development. In an effort to limit postoperative infection, antibiotics such as tobramycin may be mixed into hydroxyapatite cements, which are released into the surrounding field within the first 24 hours of placement.[17]

In many instances, infection permeates the entire operative field.[11] Donor bone graft sites may be involved in some instances. Bacteria may infect the wound or soft tissue area, may spread by contiguous extension through cortical bone during long periods of contact, or may extend through osteotomies that have disrupted the integrity of the periosteum and cortical bone barriers. Additionally, surgical alteration of the local blood supply to the cranial bones through disruption of medullary channels and removal of the adherent periosteum, as well as the presence of hardware required to secure the bony structures in their new locations, can render infection difficult to treat. Repeated or prolonged hospitalizations for previous surgical procedures may predispose to subsequent postoperative infection with multidrug-resistant bacteria. Attempts to reduce the incidence of infection have made the use of parenteral prophylactic perioperative antibiotic regimens for 2 to 5 days commonplace.

The development of meningitis is a concern with many craniofacial procedures; however, the dura provides a significant barrier to infection when painstaking neurosurgical technique is applied during repair. The presence of a cerebrospinal fluid leak is a predisposing factor, and

meningitis may be manifested years after the procedure in the case of a leak.[24] No cases of meningitis were identified from a combined report of complications after 567 procedures spanning 6.5 years at Medical City Dallas Hospital and at the Division of Plastic Surgery at the University of Pennsylvania in Philadelphia, primarily for cranial vault remodeling.[10] Similarly, meningitis was not identified from an earlier report of 170 transcranial operations spanning 10 years at the South Australian Cranio-Facial Unit in which 53 accidental dural tears occurred and 32 planned dural openings were performed.[8] Nonetheless, meningitis has been documented to occur in association with a frontal abscess with osteomyelitis, subgaleal fluid infection, contamination of cerebrospinal fluid drains, and monobloc advancement.[11,24]

As with many procedures, longer operative times result in more infections. Staging of surgeries for complicated problems has helped manage the risk for infection. The Australian group just mentioned documented an average operative time of 10.5 hours for patients in whom a postoperative infection developed, which was 2.5 hours longer than operations on patients in whom an infection did not develop.[8] Longer and more complicated monobloc advancement procedures have been associated with infection rates as high as 45 percent,[11] whereas anterior cranial vault remodeling may be associated with infection rates as low as 2.5 percent.[10] The dura encountered during primary craniofacial procedures is manipulated more easily and has better vascularization than does the scarred dura encountered in more complicated and laborious secondary procedures, which are associated with higher rates of infection.[9,10] Additionally, craniofacial postoperative infections develop in infants far less frequently than in adults.[8,10] Of the common procedures, infection remains a relatively rare complication of the less complicated Le Fort I maxillary osteotomy. In a large review of outcomes of 1000 such procedures, only 1.1 percent developed abscess or related infection.[13] Infections complicating distraction osteogenesis have been reported to occur in 9.5 percent of cases; however, osteomyelitis or deep infection requiring removal of instrumentation is less common, occurring in 0.5 to 0.9 percent of cases.[16]

As experience in craniofacial surgery has grown, the reduction in the frequency of infection has been attributed to shortened operative time, attempts to avoid entrance into the contaminated sinus cavities (an easier task in young children, in whom the sinuses often are still poorly developed), and mucosal repair at the end of surgery.[26] The mean time to diagnosis of infection is approximately 10 days.[11]

MICROBIOLOGY

Organisms causing infection include flora of the skin such as *Candida albicans*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, beta-hemolytic streptococci, and *Propionibacterium acnes*. Resident flora of the oropharynx such as *Bacteroides* spp., *Corynebacterium* spp., various α-hemolytic streptococci, *Streptococcus pneumoniae*, *Morganella morganii*, *Eikenella corrodens*, *Haemophilus influenzae*, and *H. parainfluenzae* can be causative, as can *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* spp., and *Acinetobacter calcoaceticus*.[6,10,28] Infections are often polymicrobial. Procedures that do not violate the mouth or paranasal sinuses are usually the result of skin flora. The frequency of infection with *Candida* spp. may rival the frequency of infection with more common bacteria.[10,27,28]

PREOPERATIVE PREPARATION, INTRAOPERATIVE IRRIGATION, AND PERIOPERATIVE ANTIBIOTIC THERAPY

Because wounds created during craniofacial surgery fall between the clean-contaminated category and the contaminated category,[6,7] screening for and treating dental caries, periodontal disease, and acute sinusitis is prudent before embarking on procedures involving intraoral incisions and trans-sinus osteotomies.[25] Vaccination of undervaccinated or unvaccinated children with pneumococcal vaccines during the planning process may be wise.[24] Some surgeons make use of intraoperative antibiotic-containing irrigants to help reduce the incidence of infection. These irrigants may be flushed over the intact cranium after subperiosteal exposure and again before skin closure. Other surgeons prefer to use a solution of one part povidone-iodine to four parts saline for intraoperative irrigation.[25] No strong evidence supports any one approach over another for reducing infection.[11]

Continuation of antibiotics beyond the intraoperative period is similar to the approach used in treatment of an open fracture and not very much like antibiotic prophylaxis in the traditional sense. The choice and duration of perioperative antibiotics vary among surgical centers and their surgical teams. Antibiotic regimens may include penicillin, ampicillin-sulbactam, a cephalosporin, clindamycin, or any two of these drugs in combination.[6,7,10,11,13] Erythromycin is used in penicillin-allergic patients in some centers.[1]

The optimal duration of perioperative antibiotic therapy is not clear. Higher infection rates occurred in some centers that tried restricting antibiotics to intraoperative use only.[1] Reports from one orthognathic surgical center showed a 10-fold reduction in infection rates with a 5-day antibiotic regimen in comparison with a single-day regimen.[2] Longer durations of treatment with postoperative antibiotics may be needed for patients with more heavily contaminated wounds, such as those found to have sinusitis.[6] Some advocates of a shorter duration of treatment with perioperative antibiotics suggested that longer administration of perioperative antibiotics contributes to infection with gram-negative organisms or may result in infection with more resistant gram-negative bacilli such as *P. aeruginosa* or other pathogens such as *C. albicans*.[7-11]

EVALUATION

Signs of postoperative craniofacial surgical infection include fever, warmth, tenderness, and erythema; and

rapid recurrence of swelling.[5] Yet, fever is a normal physiologic response to craniofacial surgery, occurring up to 5 days postoperatively.[9,21] Periorbital swelling may persist for some time and vary with sleeping position. Violaceous discoloration and bruising of the overlying skin are common. Distraction pins may dislodge as the integrity of the bone is compromised by infection.[23]

Purulent or seropurulent wound drainage may indicate underlying soft tissue and possibly bone infection. Wound drainage, pooling, and lack of wound healing at one location should prompt exploration for undiscovered infection elsewhere.[5] Laboratory abnormalities may be subtle. Leukocytosis may be present. The erythrocyte sedimentation rate has been shown to peak approximately 5 days after major orthopedic surgery and to decline slowly and irregularly for a period of 3 to 9 weeks, but it provides little diagnostic value initially.[3,14,22] C-reactive protein (CRP) levels peak within 2 to 3 days but usually normalize within 3 weeks.[3,14,19,22] Because an abrupt increase in CRP may be indicative of infection, some orthopedic centers monitor CRP levels to assist in early detection of infection after elective procedures such as total joint replacement and spinal surgery.[14,22] There may be value in following the CRP level in craniofacial patients postoperatively.

Needle aspiration of underlying fluid collections is the best primary means of diagnosing infection, especially if significant residual postoperative swelling has rendered clinical assessment of a particular area difficult. Diagnostic imaging such as computed tomography with contrast enhancement or magnetic resonance imaging may identify occult fluid collections, but diagnosing bone infection, especially in the flat bones of the cranium, is difficult with these modalities. Surgical exploration of suspected areas of infection remains the most helpful method to provide diagnostic information regarding infection.

TREATMENT

Management of infections complicating craniofacial procedures is primarily surgical. Open débridement, inspection and scraping of devitalized and contiguous bone, and copious pressurized saline irrigation may be curative. Removal of all hardware, while desirable, is often impractical. Successful treatment of these infections has been reported in some surgical centers in which the initial bony débridement was limited to areas of visibly apparent osteitis.[10] The value of adding antibiotics to the irrigant is questionable. Some physicians argue that irrigation with povidone-iodine may devitalize tissues that participate in the healing process and should not be used, whereas other physicians avoid using povidone-iodine irrigation because of concern about systemic iodine absorption.[10]

Placement of several drains prevents accumulation of sequestered focal soft tissue fluid collections. Resolution of drainage and subsequent wound healing can be a sign of cure. Continuous subgaleal flow-through irrigation with saline at a rate of 15 to 30 mL/hr (with or without antibiotic additives) is an adjunctive measure instituted by some surgeons.[10]

In infections acquired during cranioplasty, some patient groups may fare well with attempted preservation of a bone flap with minor surgical débridement and systemic antibiotic therapy. Within a small group of five patients who had undergone craniotomy without a history of previous craniotomy, radiation therapy, or skull base surgery, operative débridement accompanied by culture and susceptibility data–driven systemic antibiotic therapy was successful in treating infection with a mean of 35 ± 20 months of follow-up. Several other patients required second-look operations with more débridement, but they did not require removal of the bone flap and were cured. In the same study, the two other subjects had undergone more extensive craniofacial surgery and had recurrent infection requiring removal of the bone flap at 2 months after presentation in one patient and at 29 months in the other one, respectively; this finding bears out the concern that contamination of the bone flap can result in persistent, long-standing infection.[4]

For infections that complicate procedures not involving the oropharynx or sinus cavities, an antistaphylococcal antibiotic such as vancomycin, cefazolin, nafcillin, or clindamycin may be an appropriate empirical antibiotic choice pending the results of intraoperative drainage and débridement cultures. A third-generation cephalosporin with or without an aminoglycoside may be considered if the risk for developing an infection with a nosocomial pathogen is high because of prolonged hospitalization or persistent tracheal colonization with gram-negative bacilli such as *Pseudomonas*[8] or if infection of the central nervous system threatens. Infections complicating procedures involving the oropharynx or sinuses often are caused by organisms resistant to the perioperative antibiotic chosen.[6] Thus, it may be reasonable to consider antibiotic therapy with clindamycin or metronidazole when a cephalosporin has been used or treatment with a second- or third-generation cephalosporin or ampicillin-sulbactam when clindamycin has been used prophylactically. The duration of antibiotic therapy must be individualized to each case. A period of 6 weeks of antibiotic therapy has been suggested for patients with suspected osteomyelitis.[21] Some would reserve oral antibiotic suppression for patients who live long distances from the craniofacial surgery center who cannot be observed closely.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Esparza J, Hinojosa J. Complications in the surgical treatment of craniosynostosis and craniofacial syndromes: apropos of 306 transcranial procedures. *Childs Nerv Syst* 2008;**24**:1421–30.
16. Master DL, Hanson PR, Gosain AK. Complications of mandibular distraction osteogenesis. *J Craniofac Surg* 2010;**21**:1565–70.
18. Ray JM, Lindsay RW, Kumar AR. Treatment of earthquake-related craniofacial injuries aboard the USNS Comfort during Operation Unified Response. *Plast Reconstr Surg* 2010;**126**:2102–8.
24. Watanabe Y, Akizuki T. Prevention and treatment of penicillin-resistant *Streptococcus pneumoniae* meningitis after intracranial surgery with distraction osteogenesis. *J Craniofac Surg* 2008;**19**:1542–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Acebal-Bianco F, Vuylsteke PL, Mommaerts MY, De Clercq CA. Perioperative complications in corrective facial orthopedic surgery: a 5-year retrospective study. *J Oral Maxillofac Surg* 2000;**58**:754–60.
2. Bentley KC, Head TW, Aiello GA. Antibiotic prophylaxis in orthognathic surgery: a 1-day versus 5-day regimen. *J Oral Maxillofac Surg* 1999;**57**:226–30.
3. Bilgen O, Atici T, Durak K, et al. C-reactive protein values and erythrocyte sedimentation rates after total hip and total knee arthroplasty. *J Int Med Res* 2001;**29**:7–12.
4. Bruce JN, Bruce SS. Preservation of bone flaps in patients with postcraniotomy infections. *J Neurosurg* 2003;**98**:1203–7.
5. Carson BS, Dufresne CR. Surgical complications. In: Dufresne CR, Carson BS, Zinreich SJ, editors. *Complex craniofacial problems: a guide to analysis and treatment*. New York: Churchill Livingstone; 1992. p. 467–87.
6. Clayman GL, Raad II, Hankins PD, Weber RS. Bacteriologic profile of surgical infection after antibiotic prophylaxis. *Head Neck* 1993;**15**:526–31.
7. Conover MA, Kaban LB, Mulliken JB. Antibiotic prophylaxis for major maxillocraniofacial surgery. *J Oral Maxillofac Surg* 1985;**43**: 865–70.
8. David DJ, Cooter RD. Craniofacial infection in 10 years of transcranial surgery. *Plast Reconstr Surg* 1987;**80**:213–25.
9. Esparza J, Hinojosa J. Complications in the surgical treatment of craniosynostosis and craniofacial syndromes: apropos of 306 transcranial procedures. *Childs Nerv Syst* 2008;**24**:1421–30.
10. Fearon JA, Yu J, Bartlett SP, et al. Infections in craniofacial surgery: a combined report of 567 procedures from two centers. *Plast Reconstr Surg* 1997;**100**:862–8.
11. Israele V, Seigel JD. Infectious complications of craniofacial surgery in children. *Rev Infect Dis* 1989;**11**:9–15.
12. Kawamoto Jr HK, Cohen SR. Aesthetic Le Fort I, II, and III. In: Ousterhout DK, editor. *Aesthetic contouring of the craniofacial skeleton*. Boston: Little, Brown; 1991. p. 487–99.
13. Kramer FJ, Baethge C, Swennen G, et al. Intra- and perioperative complications of Le Fort I osteotomy: a prospective evaluation of 1000 patients. *J Craniofac Surg* 2004;**15**:971–7.
14. Larsson S, Thelander U, Friberg S. C-reactive protein (CRP) levels after elective orthopedic surgery. *Clin Orthop Relat Res* 1992;**275**: 237–42.
15. Mason RM, Georgiade NG. Facial osteotomies. In: Georgiade, NG, Georgiade GS, Riefkohl R, Barwick W, editors. *Essentials of plastic, maxillofacial, and reconstructive surgery*. Baltimore: Williams & Wilkins; 1987. p. 332–40.
16. Master DL, Hanson PR, Gosain AK. Complications of mandibular distraction osteogenesis. *J Craniofac Surg* 2010;**21**:1565–70.
17. Pietrzak WS, Eppley BL. Antibiotic elution from hydroxyapatite cement cranioplasty materials. *J Craniofac Surg* 2005;**16**:228–33.
18. Ray JM, Lindsay RW, Kumar AR. Treatment of earthquake-related craniofacial injuries aboard the USNS Comfort during Operation Unified Response. *Plast Reconstr Surg* 2010;**126**:2102–8.
19. Rosahl SK, Gharabaghi A, Zink PM, Samii M. Monitoring of blood parameters following anterior cervical fusion. *J Neurosurg* 2000;**92**(Suppl.):169–74.
20. Stratoudakis AC. An outline of craniofacial anomalies and principles of their correction. In: Georgiade NG, Georgiade GS, Riefkohl R, Barwick W, editors. *Essentials of plastic, maxillofacial, and reconstructive surgery*. Baltimore: Williams & Wilkins; 1987. p. 299–331.
21. Takagi S, Anderson PJ, David DJ. Pyrexia after transcranial surgery. *J Craniofac Surg* 2006;**17**:202–4.
22. Thelander U, Larsson S. Quantitation of C-reactive protein levels and erythrocyte sedimentation rate after spinal surgery. *Spine* 1992; **17**:400–4.
23. Van der Meulen J, Wolvius E, Van der Wal K, et al. Prevention of halo pin complications in post-cranioplasty patients. *J Craniomaxillofac Surg* 2005;**33**:145–9.
24. Watanabe Y, Akizuki T. Prevention and treatment of penicillin-resistant *Streptococcus pneumoniae* meningitis after intracranial surgery with distraction osteogenesis. *J Craniofac Surg* 2008;**19**: 1542–8.
25. Whitaker LA. Problems and complications in craniofacial surgery. In: Goldwyn RM, editor. *The unfavorable result in plastic surgery: avoidance and treatment*. 2nd ed. Boston: Little, Brown; 1984. p. 229–50.
26. Whitaker LA, Munro IR, Salyer KE, et al. Combined report of problems and complications in 793 craniofacial operations. *Plast Reconstr Surg* 1979;**64**:198–203.
27. Wolfe SA. The monoblock frontofacial advancement: outcome after retrofrontal epidural abscess. In: *Craniofacial surgery. Proceedings of the Sixth International Congress of the International Society of Cranio-Facial Surgery*. Bologna, Italy: Munduzzi; 1995. p. 211–13.
28. Yeung LC, Cunningham ML, Allpress AL, et al. Surgical site infections after pediatric intracranial surgery for craniofacial malformation: frequency and risk factors. *Neurosurgery* 2005;**56**: 733–8.

CHAPTER 76

Infections in Burn Patients

Janak A. Patel • Natalie Williams-Bouyer

According to the American Burn Association, approximately 450,000 persons were treated for burns in 2011 in the United States; of these, approximately 45,000 were hospitalized and approximately 3,500 died of the burn injury.[4] About 75 percent of the deaths occurred before arrival at the hospital. For those who are hospitalized, mortality peaks 20 days after hospital admission.[75] Improvement in the rate of burn-associated mortality is a direct result of advancement in burn care, comprising developments in fluid resuscitation, wound care, early excision and grafting, nutritional support, infection control, and antimicrobial therapy. The mortality rates and lengths of stay of children with burn injury have been reduced greatly in the past several decades. In the 1960s, the likelihood of survival was only 50 percent for pediatric patients with burns covering 35 to 44 percent of the total body surface area (TBSA), and few children with burns covering more than 45 percent of TBSA survived. The average length of stay was 103 days. In 2011, the LA_{50} (lethal burn size for 50% of patients) for children younger than age 16 years exceeded 90 percent of TBSA.[4] The overall mortality rate for children is less than 1 percent, which is much lower than that of adults, who have mortality rates in the range of 2 to 26 percent.[4] In a recent autopsy study at the Shriners Hospitals for Children–Galveston, sepsis was found to be the leading cause of death and accounted for 47 percent of deaths.[139]

BURN WOUND

Burn Wound Depth

Burn wounds are categorized by their depth (Fig. 76-1).[48] Accurate assessment of the depth of burn wounds is important in planning the early definitive care of an individual patient. However, the majority of burn wounds are evaluated by clinical examination only. In this respect, laser Doppler imaging is an important advance in noninvasive techniques for predicting burn wound depth.[72]

First-degree burns consist of epidermal damage only. These wounds are painful and erythematous as a result of local vasodilation. They heal spontaneously, usually without forming scars, within 7 days.

Second-degree burns are partial-thickness injuries and are further categorized as superficial or deep. The epidermis and superficial portions of the dermis are injured in superficial second-degree burns. These wounds are painful and often result in formation of blisters. Healing occurs through epithelial migration from the wound edges, hair follicles, and sebaceous glands. Relatively little scarring occurs, and reepithelialization occurs within 2 weeks. Deep second-degree burns are much more serious. The majority of the dermis is destroyed, leaving the bases of the epidermal appendages spared. The nerve endings also are destroyed, rendering the wound insensate. Blisters usually are not present, owing to the thicker formation of eschar. These wounds are treated as full-thickness injuries. Reepithelialization is tenuous and slow. The protracted inflammatory phase often results in excessive deposition of collagen and extensive scarring.

Third-degree burns are full-thickness injuries to the skin. Healing occurs by contraction and reepithelialization from the edges of the wound. As with deep second-degree burns, these wounds are insensate and without blistering. Infants and young children have a much thinner dermal layer to their skin, resulting in increased propensity for deeper burn injury. Treatment of third-degree burns is excision and skin grafting.

Fourth-degree burns extend into the deep tissue, which includes muscle, bone, and viscera. Treatment is débridement and possible amputation. Closure of these wounds may vary from primary closure after amputation to skin grafting and possibly flap reconstruction.

Cytologic Findings

The effects of extreme heat on the skin lead to cellular and subcellular impairment. The determining factors of how severe a burn will be are the temperature, the length of exposure, and the actual burning agent. Moritz and Henrique showed that the skin is able to withstand temperatures up to 40° C (104° F) for relatively long periods of time before an injury becomes apparent.[95] Increase in temperature leads to cell membrane dysfunction as ion channels are disrupted, resulting in sodium and water intake. As temperatures exceed 45° C (113 °F), protein denaturation supersedes the cell's reparative capabilities and oxygen radicals are liberated. Plasma membrane necrosis has been observed in cells exposed to 45° C for 1 hour. Other cytologic findings in thermal injury include the redistribution of solid and fluid components of the cell nuclei. Imbibition of fluid results in nuclear swelling, rupture of membranes, and pyknosis. As denaturation proceeds, vital cellular metabolic processes are injured. If enzyme activity is decreased to less than 50 percent of its normal level, cell death occurs. In lesser degrees of enzyme impairment, cell recovery may be possible.

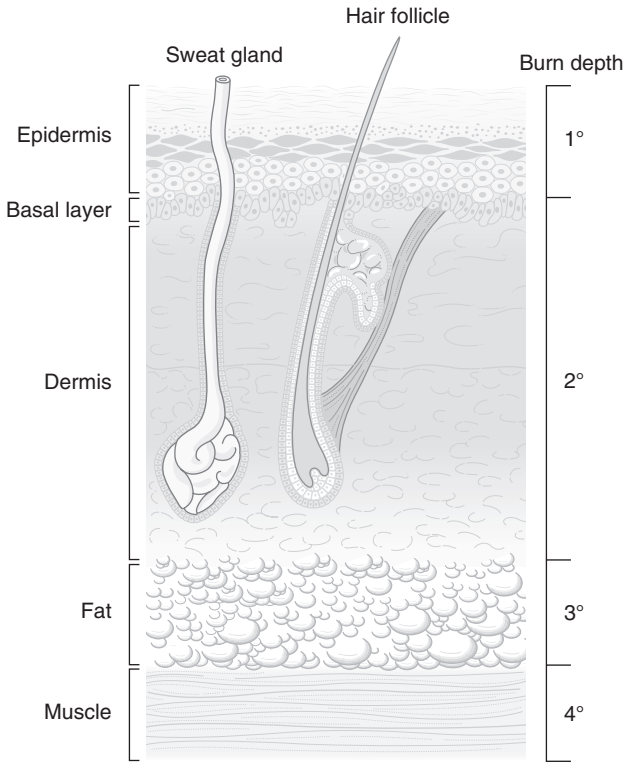

FIGURE 76-1 ■ **The burn wound depth.** (Reprinted with permission from Greenhalgh D. Wound healing. In: Herndon DN, editor. *Total burn care*. Philadelphia: WB Saunders; 2007. p. 579.)

Local Tissue Changes

Local burn injury is classically described by Jackson in three concentric zones. As temperature increases, protein denaturation results in coagulation.[61] The protein architecture is destroyed and new aberrant macromolecules are formed. The central area of a burn wound is that which is in direct contact with the source of heat. Cell necrosis is complete and is called the *zone of coagulation*. Cellular recovery is impossible, and the severity of injury decreases from the surface to the deeper levels. This zone is called the *burn eschar*. At the peripheral margins of the zone of coagulation, a less injured zone is present. The cells in this *zone of stasis* show direct injury from the heat, but the damage is not lethal. However, blood flow becomes progressively impaired to this area. Ischemia to the already compromised cells may lead to necrosis and conversion to dead eschar. Circulatory impairment occurs via adherence of neutrophils to the vessel wall, deposition of fibrin, formation of platelet microthrombus, vasoconstriction, and endothelial swelling. Heat-compromised erythrocytes lose their ability to deform, and their passage through microvessels is impeded. The circulatory embarrassment may be delayed for up to 24 hours, and the ischemia may progress for up to 48 hours after the burn has occurred. If stasis conditions are minimal, the injury may be halted and cell recovery may occur within 1 week. However, this tissue is fragile and further insults such as infection, hypovolemia, pressure, and overresuscitation can lead to further necrosis. Finally, the *zone of hyperemia* lies peripheral to the zone of stasis. This zone sustains minimal injury and often recovers within 7 to 10 days. Notable vasodilation is caused by potent vasoactive mediators secondary to the inflammatory response. Complete recovery is expected in this zone, barring further trauma or infection.

Burn Inflammation

Many of the just mentioned processes are either part of or result from the inflammatory process. Cellular infiltration, initiated by local inflammatory mediators such as prostanoids and leukotrienes, as well as proinflammatory cytokines from the burn wound, begins with the arrival of neutrophils at 4 to 5 days post burn, followed by macrophages.[121] The neutrophils further mediate damage by releasing oxygen free radicals.[42] Reestablishment of blood flow in the zone of stasis is yet another setting in which oxygen free radicals are produced, leading to further injury. This phenomenon of ischemia-reperfusion injury occurs as oxygen is restored to the tissues.[66] Inflammation becomes prominent at 7 to 10 days. Consequently, blood flow is maximal at this stage, creating a troublesome and hazardous setting for surgical excision of the eschar. Along with local inflammatory responses, several systemic responses occur with burns of more than 15 percent of the TBSA.

Inhalation Injury

Burn victims, especially those trapped in enclosed areas, injure the respiratory tract on inhalation of toxic gases from surrounding burning materials. An actual thermal airway injury is quite rare. The upper airway is rather effective in cooling and warming inspired air. Also, air has a very low heat capacity. To cause a direct injury to the airway, the flames must come into direct contact with them. Injury to the oropharynx after inhalation of toxic gases resembles thermal injury elsewhere in the body.[1] Protein denaturation, release of inflammatory mediators, and increased cellular and microvascular permeability all occur, leading to airway edema and consequent obstruction of the airway.

The chemical injury from inhalation of toxic gases can damage the tracheobronchial tree. First, separation of ciliated epithelial cells from the basement membrane occurs.[12] Next, the circulation of blood to the lung, as well as to the bronchial tree, is increased owing to vasodilation. Shortly thereafter, edema is evident. The inflammatory phase is followed by an exudative phase.[58] Furthermore, the protein component of this fluid is composed of lung lymph and induces bronchoconstriction. As postburn time increases, fibrin casts are formed from the exudates, resulting in obstruction of the airway. As the epithelium sloughs and formation of fibrin casts increases, susceptibility to infection also increases. Pneumonia leading to sepsis and death are well-known sequelae at this stage. Finally, formation of pseudomembranes proceeds and then squamous metaplasia follows.[106] Healing may take weeks to initiate, and permanent damage to the airway (e.g., stenosis and formation of tracheal granulomas) may occur.

INFLAMMATORY AND IMMUNE RESPONSES IN BURNS

Intact human skin is vital for preservation of the host's protection against infection. A combination of impaired local and systemic host defenses and loss of the skin barrier are major factors responsible for the increased susceptibility to infections in patients with burns. The major elements initially contributing to the inflammatory response that occurs after burns are incurred include the plasma proteins, mast cells, tissue macrophages, and systemically recruited neutrophils and monocytes.

Alterations in the host defenses include induction of local and systemic cytokine synthesis, decreased immunoglobulin levels, changes in the concentration and activity of both the classical and alternative complement pathways, reduced levels of circulating plasma fibronectin, depressed serum opsonic activity, and impairment of the macrophages, lymphocytes, neutrophils, and the reticuloendothelial system. However, many mechanisms of immune alterations remain unknown; for example, an association of the volume of blood transfusions with increased mortality rates and infectious episodes in patients with major burns was observed in two reports.[63,106] The immunologic status of the burn patient has a measurable impact on survival, death, and major morbidity.

The Cytokine Response

After burn injury occurs, numerous cytokines are induced rapidly. Many cytokines correlate with the severity of the burn injury and the prognosis. Recent studies at Shriners Hospitals for Children–Galveston have shown a specific pattern of systemic cytokine responses in children with thermal injury. Compared with unburned healthy children, children with burns covering more than 40 percent of TBSA had significant increases in serum levels of 15 cytokines and immunoregulatory molecules during the first week after incurring the thermal injury: interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-17, interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), macrophage inflammatory protein (MIP)-1α, and granulocyte colony-stimulating factor (G-CSF).[37,75] The amount of granulocyte-macrophage colony-stimulating factor (GM-CSF) was significantly increased during the second week after burn occurred. Within 5 weeks, the serum concentrations of most cytokines decreased, approaching normal levels. In another study of children, serum IL-6, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, tumor necrosis factor-α, MIP-1β, MCP-1, G-CSF, and GM-CSF values were significantly increased in large burns.[36,75] In general, there is a clear increase in these cytokines when the affected TBSA is greater than 60 percent when compared with burns affecting a lesser TBSA.[75]

In children with inhalation injury, the serum cytokine studies at enrollment showed significant reduction in level of IL-7 and elevation of IL-12p70, as compared with children without inhalation injury.[36] However, 5 to 7 days later, the levels were comparable. Host genetic factors also may affect the cytokine response in patients with burns. For example, single nucleotide polymorphisms for TNF-α (–308G), Toll-like receptor 4 (+896G), IL-6 (–174C), and CD14 (–159C) were significantly associated with an increased risk for severe sepsis after burns.[8] In another study, children with IL-10 (–1082GG) genotype, which is associated with high production of IL-10, were found to have an increased risk for sepsis.[107]

Complex cytokine pathways lead to several effects throughout the body. Among these, TNF-α may be the most important cytokine during acute inflammatory injury of burns because it induces production of C-reactive protein and IL-6, migration of and phagocytosis by neutrophils and macrophages, hormone regulatory processes, particularly hypothalamic thermoregulation, and insulin hypersensitivity.

Neutrophils

Thermal injury induces neutropenia and myeloid maturation arrest despite elevated G-CSF levels.[123] The degree of neutropenia correlates with the reduction in bone marrow G-CSF receptor expression. The neutrophils of patients with burns also are functionally altered[96]: The expression of the Fc receptor is decreased, and intracellular killing capacity is depressed (this is a differential suppression, more for some organisms than others) and is accompanied by a brief increase in neutrophil respiratory burst response. Failure of initial alkalization of the phagolysosome and alteration of subsequent kinetics of acidification also occur.[16] This depression of oxygen-independent bactericidal mechanism may impair the capacity of the neutrophil for intracellular killing after a thermal injury occurs. Expression of CD16 (Fc receptor [FcR], Fc immunoglobulin G [IgG] receptor) and CD11 (adhesion molecule) on neutrophils is impaired after a major injury occurs; this reduction appears to be related directly to the appearance of bacteremia or pneumonia.[6] These changes in expression of adhesion molecules, which is closely related to chemotaxis, may play a part in the failure of delivery of neutrophils in adequate numbers to the local site of a burn. Furthermore, a defect is present in actin polymerization in the neutrophils of patients with burns; as a basic mechanism of chemotaxis, it also may contribute to a failure of motility.[132]

Generation of leukotrienes from the neutrophils of severely burned patients also is impaired and appears to be based on the availability, or lack of availability, of the metabolizable substrate-free arachidonic acid.[71] Because leukotriene B also is a potent neutrophil chemotactic agent, this impairment may further contribute to the failure of neutrophil function.

Complements

The fluid of the burn blister shows much lower opsonic activity for bacteria such as *Pseudomonas aeruginosa* than the patient's own serum.[102] A mild impairment of production of C3 and release by macrophages of burn patients in vitro also occurs. Systemically, both the classical and the alternative pathways are depleted, but the alternative pathway is more profoundly perturbed. After

the development of bacteremia, additional complement activation and depletion occur.[130]

Macrophages

Suppression of the ability of the reticuloendothelial system to take up particulate material was among the original observations of burn immunology made in the 1960s. More recent reports, however, have described the demonstration of a differentially increased uptake of colloid in alveolar macrophages compared with other organs, perhaps indicating alveolar macrophage activation. Macrophages and monocytes appear to be activated in a fashion similar to that of lymphocytes after thermal injury occurs. Activation of macrophages, as measured by the serum neopterin level, is increased after thermal injury occurs.[7] This activation is confirmed by increased expression of the monocyte cell surface antigens C3b and iC3b.[94] At the same time, expression of human leukocyte antigen (HLA)-DR, HLA-DQ, and HLA-DP by monocytes is reduced, and these class II antigens are obligatory for many cell-mediated immunologic processes, thereby implying that a possible loss of function of monocytes occurs after thermal injury.[45] Production of C3 by macrophages is suppressed in patients with burns, but the synthetic ability for key cytokines such as IL-6 is increased.[143]

Peripheral blood monocytes are superstimulated to produce large amounts of IL-1, leading to exhaustion of the function on monocytes.[83] Reduced production of IL-1 by monocytes was found in patients with complicated organ injury, multiorgan failure, and systemic infection. Blood monocytes of patients with burns produce significantly more IL-10 at 7 to 10 days after burn injury, which correlates significantly with subsequent septic events.[68]

T Lymphocytes and Cell-Mediated Immunity

Early studies of T cells in patients with burns showed a variety of changes: impairment in mitogenic and antigenic responsiveness of lymphocytes, suppression of graft-versus-host reactivity related to the size of the burn, suppression of delayed cutaneous sensitivity tests, and diminution in both numbers of peripheral lymphocytes and concentration of thoracic duct lymphocytes. Whether the failure of T cell functions is due to an intracellular defect related to thermal injury, the result of "overuse," or indirectly the result of downregulation by the cytokine cascade or other products of the inflammatory reaction remains controversial. A study has shown that severe burn injury could result in activation and maturation of regulatory T cells leading to immunosuppression.[60] The elevation of levels of cytokines produced by regulatory T cells and the activation markers on the surface of these cells correlate with burn size and are higher in those with sepsis than those without. However, among septic patients, the regulatory T cell parameters in the survival group were markedly lower than those with fatal outcome, suggesting that persistence of a pronounced immunoparalysis induced by regulatory T cells after severe sepsis is associated with poor outcome after burns.

Analysis of peripheral blood T cells supports the theory that, rather than an absolute reduction in CD4 and an increase in CD8 cells, a redistribution of lymphocyte traffic may occur.[104] Suppression in the numbers of the total population of lymphocytes is the only consistent overall change. Furthermore, not only does lymphocyte traffic between stores of central lymphocytes and the peripheral blood occur after a thermal injury, but the responsiveness of these populations of lymphocytes also varies according to the site; for example, splenic lymphocytes of experimentally burned animals remain most profoundly depressed in response to antigenic stimulation, compared with the peripheral blood and other organs.[29] In addition, in peripheral blood, the appearance of "activation" antigens on CD4 and CD8 cells (HLA-DR, IL-2 receptor [IL-2R], and transferrin receptor) is depressed significantly as early as 1 day after burn injury occurs.[87]

Addition of recombinant IL-2 does not appear to reverse the suppression of the appearance of surface markers such as IL-2R in burn patients, although it does not improve the response of natural killer (NK) cells to stimulation.[40] In experimental preparations, at least some of the observed T-cell suppression can be alleviated by early removal of the burn wound, thereby creating one further argument for promptly closing the burn wound.[51]

B Lymphocytes and Humoral Immunity

The function of B cells after the occurrence of a thermal injury is less well documented than is that of macrophages or T cells. The expression of this major histocompatibility complex is impaired and, therefore, some diminution of B-cell function can be expected as a result of diminished recognition of antigenic presentation.[100] Under the influence of stress-induced corticosteroids, the number of circulating B cells is relatively increased compared with T cells in peripheral blood.[65] Spontaneous cytokine (IL-4 and IL-2)-induced expression of the activation antigen CD23 is reduced significantly during the second to fifth week after burn injury occurs.[118]

If the products of B-cell activation, namely, the immunoglobulins, are measured in vivo, the results are somewhat difficult to interpret because of the increased catabolism of protein and the leakage through the burn wound. Briefly, marked diminution of serum IgG concentration (total and all subclasses) is present; these levels return to normal between 10 and 14 days after the burn injury has occurred. Extremely low levels of IgG on admission (300 to 400 mg/dL) are predictors of a poor prognosis. Levels of IgM and IgA appear to be relatively unaffected. Overall, the defective production of immunoglobulin after a thermal injury occurs appears to be a factor of macrophage/lymphocyte interaction rather than a failure of intrinsic activity by B cells.[35]

BURN WOUND MICROBIOLOGY

A working knowledge of the common flora of burn wounds is essential to appropriately tailor therapy.

TABLE 76-1 **Tissue Association of Microorganisms Most Commonly Found in Burn Wound Infection**

Organism	Soft Tissue Skin	Upper Respiratory Tract	Lower Respiratory Tract	Endocardial	Gastrointestinal	Urogenital	Bone and Joint
Staphylococcus aureus	NF, P	P, NF	P	P	P	NF, P	P
Staphylococcus epidermidis	NF, P	NF, P	P	P	P	NF, P	P
Other staphylococci	NF	NF, P	P	P	NF, P	NF, P	P
Streptococcus pyogenes	P, NF	P, NF	P	P	P	P	P
Other streptococci	NF	NF	P	P	NF	NF, P	P
Enterococcus spp.	P	P	P	P	NF, P	NF, P	P
Escherichia coli	NF, P	NF, P	P	P	NF, P	NF, P	P
Klebsiella pneumoniae	NF, P	NF, P	P	P	NF, P	NF, P	P
Enterobacter cloacae	NF, P	NF, P	P	P	NF, P	NF, P	P
Enterobacter aerogenes	NF, P	NF, P	P	P	NF, P	NF, P	P
Proteus spp.	NF, P	P	P	P	NF, P	NF, P	P
Serratia marcescens	NF, P	(–)	P	P	P	P	P
Other enterics	NF, P	NF, P	P	P	NF, P	P	P
Pseudomonas aeruginosa	P	NF, P	P	P	NF, P	P	P
Acinetobacter spp.	NF	NF	P	(–)	NF	NF	P
Candida albicans	NF	NF	P	P	NF	P, NF	P

NF, normal flora; *P,* pathogens; (–), normally not found, but these organisms should not be ignored when encountered.
Modified from Heggers J. Microbiology for surgeons. In: Kerstein MD, editor. *Management of surgical infections.* Mt. Kisco, NY: Futura Publishing, 1980. p. 27-55.

Pathogens peculiar to thermal injuries are basically no different from the normal flora of the environment. Table 76-1 shows the types of microorganisms found in various body tissues and secretions as either normal flora or as pathogens. However, the organisms causing infections change over the course of burn wound treatment. Gram-positive organisms prevail in the early postburn period and then are replaced by gram-negative bacteria and fungi.[33] Many of these organisms produce biofilms in burn wounds. The biofilms consist of organisms surrounded by a matrix consisting of various proteins, polymers, complex carbohydrates, and water. Biofilms are associated with development of antibiotic resistance because antibiotics do not penetrate through the matrix in sufficient concentration. Biofilm formation also inhibits effective local immune response as well as wound healing. Using electron microscopy, Kennedy and colleagues showed that in both ulcerated and escharotomy sites, evidence of biofilm was seen as early as 7 days post injury.[69] Formation of biofilm is best prevented by early excision and coverage of the wound with topical antimicrobial agents.

The organisms that predominate as causative agents of burn wound infection in any burn treatment facility change over time. For example, at Shriners Hospitals for Children–Galveston, from 1989 to 1999, only 42 percent of children died of sepsis due to multidrug-resistant organisms and in 25 percent of these children *Pseudomonas* was the responsible organism. From 1999 to 2009, 86 percent of patients died of sepsis due to multidrug-resistant organisms and in 64 percent of these children again the cause was *Pseudomonas* infection. In this latter period, *Acinetobacter* also emerged as a major pathogen of death due to sepsis while the role of *Klebsiella* declined.

Gram-Positive Bacteria

The gram-positive predominance is consistent with the normal inhabitants of the skin before the thermal injury. *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp., *Pediococcus* spp., and *Enterococcus* spp. are gram-positive cocci commonly encountered in burn wounds. Erol and colleagues demonstrated that coagulase-negative *Staphylococcus* and *Staphylococcus aureus* were the most prevalent isolates in admission cultures, followed by diphtheroids.[33] These organisms can be life threatening as invasive infections or simply be locally colonized. The distribution of gram-positive bacteria at Shriners Hospitals for Children–Galveston, which receives pediatric burn patients from all over the world, is shown in Table 76-2. For the year 2011, the gram-positive cocci accounted for 55 percent of bacterial isolates. Within this group, staphylococci are more prevalent (52%) than enterococci (22%). Among *S. aureus* isolates, the rates of methicillin-resistant (MRSA) and methicillin-sensitive (MSSA) isolates were equal (about 10%). Interestingly, the rate of MRSA in the burn patient population is lower than the community rate of MRSA, which is about 60 percent of *S. aureus* isolates in the region around our facility.

Because of such resistance patterns, Cook stresses the importance of microbial surveillance and epidemiologic studies.[24] This approach is thought to reduce the prevalence of MRSA, yet it may be inadequate for eradicating or preventing outbreaks. Minimizing transmission and infection is emphasized. However, Reardon and associates suggest that this process is time consuming and requires extensive resources for little gain.[110] Colonization with MSSA and MRSA in 86 patients was studied and found to have no significant changes on length of

TABLE 76-2 **Bacteria Isolated from 168 Inpatient Acute Care Pediatric Burn Patients Admitted to Shriners Hospitals for Children–Galveston (January 2011 to December 2011)**

	No. (%) of Isolates
Gram-Positive Organisms	
Methicillin-resistant *Staphylococcus epidermidis*	86 (19.4)
Enterococcus faecalis	57 (12.8)
Methicillin-sensitive *Staphylococcus aureus*	48 (10.8)
Methicillin-resistant *Staphylococcus aureus*	46 (10.3)
Methicillin-sensitive *Staphylococcus epidermidis*	43 (9.6)
Enterococcus faecium	39 (8.7)
Staphylococcus lugdunensis	10 (2.2)
Other Gram-positive organisms*	117 (26.2)
Total No. of Gram-Positive Isolates	***446 (55.1)***
Gram-Negative Organisms	
Enterobacteriaceae (Enterics)	
Escherichia coli	84 (23.1)
Enterobacter cloacae	34 (9.3)
Escherichia coli (ESBL)	32 (8.8)
Klebsiella pneumoniae	29 (8.0)
Klebsiella pneumoniae (ESBL)	12 (3.3)
Other Enterobacteriaceae†	41 (11.2)
Non-Enterobacteriaceae	
Pseudomonas aeruginosa	64 (17.6)
Acinetobacter spp.	44 (12.1)
Stenotrophomonas maltophilia	6 (1.6)
Other non-Enterobacteriaceae‡	18 (5.0)
Total No. of Gram-Negative Isolates	***364 (44.9)***
Total No. of Bacterial Isolates	**810**

*Includes *Aerococcus* spp. (n = 3); other *Staphylococcus* spp. (n = 75); other *Enterococcus* spp. (n = 18); *Micrococcus* spp. (n = 8); *Rhodococcus* spp. (n = 2); and *Streptococcus* spp. (n = 11).
†Includes other *Enterobacter* spp. (n = 12); *Klebsiella oxytoca* (n = 6); *Serratia marcescens* (n = 4); *Proteus* spp. (n = 8); *Citrobacter* spp. (n = 8); *Hafnia alvei* (n = 1); *Morganella morganii* (n = 1); *Providencia stuartii* (n = 1).
‡Includes *Aeromonas hydrophila* (n = 3); *Alcaligenes* spp. (n = 1); *Burkholderia cepacia* (n = 5); *Ochrobactrum anthropi* (n = 1); other *Pseudomonas* spp. (n = 7); *Shewanella putrefaciens* (n = 1).
ESBL, extended spectrum β-lactamase.

stay, number of operations, or mortality between these two organisms. However, the presence of either type of *S. aureus* significantly increased the number of surgical procedures performed and the lengths of stay. Many burn units report frequent colonization of burn patients with toxic shock toxin (TSS-1)–producing strains of staphylococci; however, their presence does not correlate well with increased morbidity or mortality rates.[23]

In the past, group A β-hemolytic streptococcus frequently was the cause of epidemics in burn units, but it seldom is encountered today because of the frequent empirical use of antibiotics for manipulations of burn wounds. Other β-hemolytic streptococci belonging to groups B, C, E, F, and G can be encountered as well.[81] Significant infection-control vigilance still is necessary, because occasional clusters of outbreaks of group A streptococcal infection continue to be reported.[48,113] Fortunately, group A streptococcus remains uniformly sensitive to penicillins; hence, prophylaxis and treatment are easily accomplished.

Although enterococcal infections (*Enterococcus faecalis* and *Enterococcus faecium*) account for only 22 percent of burn-wound infections caused by gram-positive bacteria (see Table 76-2), a significant cause for concern is the emergence of vancomycin-resistant *Enterococcus* (VRE) in burn units.[77,79] Although additional morbidity associated with VRE itself is not clear, when it occurs as a polymicrobial bacteremia, a mortality rate as high as 20 percent has been noted.[79]

Other gram-positive bacilli include the aerobic *Corynebacterium* spp. and *Listeria* spp., as well as the spore-forming *Bacillus* spp. (aerobe) and *Clostridium* spp. (anaerobe). *Bacillus* spp. and *Clostridium* spp. are associated with burn wounds that have come in contact with contaminated soil. In avascular muscle injuries (e.g., electrical injuries or crush injuries combined with burns), the risk for developing tetanus (from infection with *Clostridium tetani*) is high and has led to the practice of using tetanus immunoprophylaxis and booster vaccines.[78,120]

Gram-Negative Bacteria

The presence of the gram-negative bacteria in burn wounds is due in part to translocation of the bacteria from the gastrointestinal tract of the patients.[9] In a study of children with burns at Shriners Hospitals for Children–Galveston, patients with large wounds (>50% TBSA) were found to have significantly higher colonization with their fecal gram-negative bacteria than were those with smaller wounds.[38] *P. aeruginosa* bacteremia is predicted by isolation of *P. aeruginosa* from another site (wound, urine, or sputum), and the prior nonblood isolates of *P. aeruginosa* can correctly predict the antimicrobial sensitivity pattern in 75 percent of patients.[86] At Shriners Hospitals for Children–Galveston, gram-negative bacteria account for 45 percent of the total bacterial isolates of the wound (see Table 76-2), and most of these bacteria produce extended spectrum β-lactamase (ESBL) enzyme. *Escherichia coli* and *Klebsiella pneumoniae* are the predominant ESBL-producing bacteria. Bennett and coworkers have shown that infections caused by ESBL-producing *K. pneumoniae* are predictive of death when occurring in older persons.[15]

Although the carbohydrate-fermenting Enterobacteriaceae account for 64 percent of the gram-negative isolates as a whole, *P. aeruginosa* and other nonfermenters are also important in burn patients. Other important gram-negative bacteria include the Enterobacteriaceae such as *E. coli*, *Enterobacter cloacae*, *K. pneumoniae*, and *Serratia marcescens*. Enterobacteriaceae also are encountered as a cause of nosocomial pneumonia in patients with inhalation injury who are on ventilators, and they are a

cause of urinary tract infection in patients with indwelling urinary catheters. *Acinetobacter*, an increasingly more common cause of gram-negative infections, has been found more frequently in burn patients with glucose intolerance or preexisting diabetes mellitus[39] and in patients with more severe burns and comorbidities.[3]

Even without invasive infections, gram-negative bacteria have been implicated in systemic inflammatory diseases, including shock and disseminated intravascular coagulation, secondary to the circulation of bacterial endotoxin from the gut and the burn wound.[74,84] Often gut decontamination is instituted to reduce the incidence of endotoxin-mediated disease.

Fungi

Until the advent of topical antimicrobial agents and systemic antibiotics, fungal infections were not common developments in patients with burns. The burn wound is the site most commonly infected, although fungemia and dissemination to the respiratory tract in patients on ventilators and to the urinary tract in patients with indwelling catheters are encountered frequently. *Candida* spp. are the most common fungal colonizers of the wound (Table 76-3); however, less than 20 percent of patients develop widespread candidiasis. Overall, the rate of candidemia in the burn patient population is 3 to 5 percent, and burn wound invasion has a comparable rate. A study of children with burn injury at Shriners Hospitals for Children–Galveston showed that those developing candidemia did so during the first week post burn and 7 days after excision of burn eschar.[28] One hypothesis is that massive burns with immunosuppression are further suppressed by repeated surgical intervention, anesthesia, and perioperative use of broad-spectrum antibiotics, further predisposing these patients to early development of *Candida* septicemia. In one study, the attributable mortality with candidemia was 15 percent.[93] With early recognition of invasion of burn wounds by routine biopsies, wound swabs, and early amphotericin therapy, the mortality rate has been reduced to less than 10 percent, as compared with 60 to 90 percent reported in earlier series.[119]

Unlike *Candida*, true fungal infections caused by *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, *Rhizomucor*, *Fusarium*, and *Curvularia* occur early in the hospital course, specifically in those exposed to the spores on the ground or in water at the time the injury occurred. Once colonized, broad nonbranching hyphae extend into subcutaneous tissue and stimulate an inflammatory response. Vascular invasion occurs frequently and often is accompanied by thrombosis and avascular necrosis, which is clinically observed as rapidly advancing dark discolorations of the wound margin. Systemic dissemination occurs with invasion of the vasculature.

TABLE 76-3 Medically Important Fungi Isolated from Acute Care Pediatric Burn Patients Admitted to Shriners Hospitals for Children–Galveston (January 2011 to December 2011)

Organism	No. (%) of Isolates
Candida spp.*	75 (94.0)
Aspergillus spp.	1 (1.2)
Curvularia spp.	1 (1.2)
Fusarium spp.	1 (1.2)
Trichosporon beigelii	1 (1.2)
Scedosporium prolificans	1 (1.2)
Total No. of Fungal Isolates	***80***

*Includes *C. albicans/dubliniensis* (n = 39); *C. glabrata* (n = 11); *C. parapsilosis* (n = 9); *C. tropicalis* (n = 5); *C. rugosa* (n = 3); *C. zeylaroides* (n = 1); *C. stellatoidea* (n = 3); *C. krusei* (n = 3); and *C. paratropicalis* (n = 1).

Viruses

Linnemann and MacMillan performed a retrospective survey of serum for viral antibodies in pediatric burn patients; 22 percent had fourfold increases in antibodies to cytomegalovirus (CMV), 8 percent had increases to herpes simplex virus (HSV) and to Epstein-Barr virus, and 5 percent had increases in antibodies to varicella-zoster virus (VZV).[82] None of the patients had evidence of adenovirus or hepatitis B virus infection. On the basis of these observations, a prospective study of viral infections, using both serologic and viral culture techniques, was performed. This study showed that CMV infection developed in 33 percent of the children, HSV infection occurred in 25 percent, and adenovirus infection was noted in 17 percent. CMV infections developed in all of the most severely burned children, and both primary and reactivation infections were observed. Most primary CMV infections that develop during treatment for burns are likely to occur from transfusion of blood products. CMV infection typically occurs approximately 1 month after the burn occurs and clinically presents as fever of unknown origin with lymphocytosis; however, it rarely alters the patient's clinical course.[27] Kealey and colleagues[67] have shown that 56 percent of burn patients who initially were seropositive for CMV had a fourfold or greater rise in CMV antibodies as evidence of CMV reactivation. These patients tended to be younger, to have a larger burn area, and to have a longer hospital stay. No patient who experienced CMV infection, whether primary or reactivated, had serious complications attributable to CMV. On the basis of these observations, despite the availability of anti-CMV agents such as ganciclovir and valganciclovir, treatment of CMV infection remains controversial.[111]

Since the screening of blood began, hepatitis C virus (HCV) has been an important risk factor. Coursaget and colleagues screened 45 burn patients for anti-HCV antibodies at the time of burn injury and more than 6 months post burn.[25] HCV infection was detected in 18 percent of these patients as a consequence of the numerous transfusions of blood or blood derivatives used during the postburn treatment. Five patients displayed evidence of anti-C100, anti-C33c, and anti-core antibodies together; two patients had only anti-C100 and anti-C33c antibodies, and the last one showed only anti-core antibodies.

Chronic hepatitis was observed in 83 percent of HCV infections. Kinetics of appearance of anti-HCV antibodies varied among patients. Anti-core antibodies generally are the first to be detected at high levels; however, in at least one case, they were detected only 2.5 months after C100 and C33c antibodies were detected. The incidence of HCV using polymerase chain reaction (PCR) assay to detect the viral genome has not been evaluated in burn patients. Nonetheless, the current procedures used at blood banks have decreased the transmission of HCV by blood products.

Another transfusion-related agent is human immunodeficiency virus (HIV), which has become extremely rare as a result of the screening of donors that began in 1987 in the United States. However, significant risk existed prior to that period. A retrospective review of children with burn injury at Shriners Hospital for Children–Galveston who had received blood or blood products between 1978 and 1985 identified 52 patients at risk for developing HIV infection.[115] More than 50 percent of the identified population had received three or more units of blood or blood products during their acute hospital stay. A total of 214 patients (36.8%) were tested for HIV seroconversions: 5 tested HIV-positive by enzyme-linked immunosorbent assay (ELISA) and 4 were confirmed by Western blot, yielding a 1.9 percent incidence. The 4 confirmed patients received two to nine total-body blood volume turnovers during their postburn period in the hospital. HIV may affect the outcome of burn wound injury. A study of Malawian children showed that with burns affecting 11 to 30 percent of the body surface area, HIV-positive children had a mortality rate approximately twice that of HIV-negative children.[62]

HSV is of significant concern in burn units because it is a dermatopathologic virus. A review of the literature suggests that patients younger than 10 years of age were at greater risk for acquiring an HSV infection when the size of the burn wound was greater than 15 percent of TBSA.[52] However, the role of HSV in the healing of wounds is unclear. Bourdarias and associates showed that in 11 patients with burns, local areas of active epidermal regeneration were affected most often.[17] Acyclovir therapy was not used, and the duration of hospitalization was normal when compared with that for other children. Nonetheless, HSV in lungs may worsen morbidity. Byers and coworkers showed that the relative risk for developing HSV infection was higher for patients with adult respiratory distress syndrome but not for those with pneumonia.[20] Disseminated HSV infection also can be fatal.[20]

Another dermatopathologic virus is VZV. Miniepidemics of VZV have occurred within pediatric burn units.[138] With the routine VZV vaccination of young children (at 1 year of age) in the United States, the outbreaks of varicella in burn units are now uncommon. The characteristic fluid-filled lesions appear in partial-thickness burns that are healed or healing, as well as in uninjured epithelium and mucous membranes. The vesicles are much more destructive in the injured than uninjured skin and may present as hemorrhagic, oozing pock marks that are prone to development of secondary infection and subsequent scarring. Neovascularized skin grafts may be lost; therefore, further grafting procedures should be delayed until the lesions are quiescent.

The morbidity due to respiratory virus infections, particularly in those with inhalation injury, has not been studied well. We tested more than 100 pediatric burn patients for respiratory syncytial virus (RSV) during the winter seasons of 1995 and 1996. Only 6 patients were found to be positive for RSV, with one death (1% mortality rate).

Parasites

Parasitic infestation also is seen especially in children from the developing world. Because we see many patients who originate from Mexico, where such infestation is endemic, parasitemia has been found to complicate burn injuries. Parasites that are asymptomatic in sites such as the intestinal tract and the respiratory tract can become symptomatic as a result of the stress of a burn injury. At the Shriners Hospital for Children–Galveston, we have described three cases of *Ascaris* pneumonitis that exacerbated the smoke-induced lung injury.[53] In 2006, the parasites isolated most frequently were *Giardia lamblia* and *Blastocystis hominis*.

CLINICAL MANIFESTATIONS

Local Signs

An open burn wound is a favorable target for bacterial colonization. The progression from simple eschar colonization to the invasive process is favored by a series of factors related to the patient, such as extension and depth of the burn, age, presence of previous disease, and local conditions of the wound; to the microorganism, such as density, motility, toxins, and antimicrobial resistance; to iatrogenic causes such as prosthetic devices; and to the nosocomial spread of bacteria. It is essential to recognize the early signs of infection of a local burn wound by examining the wound at least once a day.

The local signs of burn wound infection include black or dark brown focal areas of discoloration, conversion of partial-thickness injury to full-thickness necrosis, hemorrhagic discoloration of subcutaneous tissue, enhanced sloughing of burned tissue or eschar, and purplish discoloration or edema of skin around the margins of the wound. Presence of *Pseudomonas* infection can lead to ecthyma gangrenosa and green pigmentation of subcutaneous fat. In fungal infection, centrifugal advance of subcutaneous edema with central ischemic necrosis and hemorrhagic saponification of subcutaneous fat in fungal infection can be seen. In viral infection, vesicular lesions in healing or healed partial-thickness burns and crusted serrated margins of partial-thickness burns may be observed.

Systemic Signs

Progression from local to systemic invasion can occur rapidly, which correlates with the size of the burn wound, the extent of environmental contamination, and the

TABLE 76-4 **Signs and Symptoms of Progression from Local Invasion to Systemic Illness**

Gram-Negative Sepsis	Gram-Positive Sepsis
Burn wound biopsy >10^5 organisms/g tissue and/or histologic tissue invasion	Same
Rapid onset, well to ill in 8-12 hr	Gradual
Temp. 37-39°C (98.6-102.2°F), can be normal, followed by hypothermia (34-35°C [93.2-95°F]), plus decrease in WBC	Temp. >40°C
WBC may be elevated	WBC 20-50 × 10^3, hematocrit decrease
Ileus	Same
Decreased blood pressure and urinary output	Same
Wounds develop focal gangrene, satellite lesions away from burn wound	Macerated wounds, ropy and tenacious exudate
Mental obtundation	Anorexic and irrational

Note: Five or more signs or symptoms are definitive diagnostic parameters.
WBC, white blood cell count.
Modified from Gallagher JJ, Williams-Bouyer N, Villarreal C, et al.: Treatment of infections in burns. In: Herndon DN, editor. *Total burn care.* Philadelphia: WB Saunders; 2007. p. 143.

surgical procedures. Early recognition of systemic invasion is critical to avoid the high rates of mortality. Many of the signs of sepsis resemble complications of the burn itself; for example, fevers, tachycardia, shock, and elevated or depressed neutrophil count can occur in burn patients with or without infection. However, certain patterns of clinical signs and symptoms may help recognize the systemic bacterial invasion (Table 76-4).[41]

Biomarkers of Infection and Sepsis

A rise in C-reactive protein serum levels has been found useful in predicting systemic infection, although increases in the first 2 days after the burn or the day after surgery may occur without infection.[99]

Complications of Infection

Other than the primary infection of the burned skin, several types of infectious complications in burn patients have been recognized. Bacteremia is a frequent complication. When sepsis occurs, it is preceded by increased C-reactive protein about 2 days before the patient exhibits signs of clinical sepsis. Another important biomarker is serum procalcitonin. Its relatively early rise within 4 hours of response to infection or trauma, with a long plateau of up to 24 hours, makes this marker very valuable in early assessment of sepsis. Nonetheless, the lack of widespread availability of procalcitonin assay limits its usefulness in clinical practice. Elevated levels of certain cytokines may also be useful biomarkers of infection, but they largely remain research tools. Burn wound manipulations are responsible for development of bacteremias in approximately 50 percent of cases, but routine instrumentation and intravascular catheter devices also can cause bacteremia.[14,50] The risk for developing bacteremia also correlates with the TBSA affected by burns; Sasaki and associates showed that those patients with a positive blood culture had an average TBSA injury of 47 percent, whereas those with a 26 percent injury had negative blood cultures.[116] Bloodstream infection has been associated with longer duration of hospitalization and mechanical ventilation but not with mortality.[19] Toxic shock syndrome caused by TSS toxin–producing strains of *S. aureus* has been identified in acutely burned children. Childs and coworkers found that 13 percent of children developed a toxic shock–like illness; however, its effect on overall burn mortality was not clear.[23]

Subacute bacterial endocarditis is a risk associated with persistent bacteremia caused by any cause, including repeated instrumentation, surgical intervention, and placement of central venous catheters.[13,21] *S. aureus* and gram-negative bacilli are the most frequent cause. In most cases, the antemortem diagnosis rarely is suspected in children with burn injury.[2,13] In addition to causing local valvular damage, infected vegetations may dislodge septic emboli. Suppurative thrombophlebitis occurs at the site of the insertion of the catheter. It may occur in as many as 5 percent of patients with burns covering 20 percent of the TBSA.[124]

Suppurative chondritis occurs in patients with full-thickness burn of the ear.[91] Because of the auricle's relatively low level of blood supply, chondritis frequently follows the progression of tissue ischemia, usually 3 to 5 weeks after burn injury occurs.[92] *P. aeruginosa* and *S. aureus* are the most common pathogens. However, with use of mafenide acetate as a topical agent, the incidence of suppurative chondritis has decreased significantly.

Suppurative sinusitis is seen in patients with long-term nasotracheal intubation. In one study, 8 percent of patients with burns who had nasotracheal intubation for more than 7 days developed sinusitis.[18] Pneumonia may occur with or without inhalation injury, although those with inhalation injury have a substantially higher risk.[122] Bronchopneumonia is the most common type of pulmonary infection, usually occurring in the second week of burn injury. Predisposing factors are size of the burn wound (hematogenous spread), aspiration, presence of tracheostomy or nasotracheal tube (nosocomial spread from burn wound), existence of inhalation injury, and disturbances of fluid and electrolyte balances. Causative organisms include gram-positive bacteria, especially *S. aureus;* gram-negative organisms such as *P. aeruginosa*, *E. coli*, and *K. pneumoniae;* and viruses, including HSV and CMV. Currently, most infection-related deaths in burn patients are caused by pneumonia or bloodstream infections, rather than wound infection.[122] Fatal viral pneumonia due to CMV and HSV has been described as well.[26]

Urinary tract infection occurs in association with prolonged and often unnecessary catherization.[117] Osteomyelitis can occur when bones are exposed by the burn

or by open fracture accompanying the burn; from extension of infection from a septic joint, introduction of organisms along traction pins, and internal fracture fixation devices; or by bacteremia. However, clinically significant osteomyelitis in burn patients is rare.[34] Septic arthritis occurs when a joint is exposed by a burn or by removal of burn eschar.[34] The joints most frequently exposed are the knee, the elbow, the proximal interphalangeal joints of the hand, and the metacarpophalangeal joints on the dorsal surfaces of the hand. The incidence of septic arthritis is obscured by its frequent association with signs and symptoms of severe burns that rarely are separable. Rarely in burns, a joint may become infected from adjacent metaphysical osteomyelitis. In children, most joints can be salvaged. Adult joints are less resilient.

Central nervous system infections in burn patients include meningitis, microabscesses, and septic infarcts. In one review, *Candida* spp., *S. aureus*, and *P. aeruginosa* caused almost 80 percent of infections, occurring most frequently in patients with extensive burns with wound infection or endocarditis.[140]

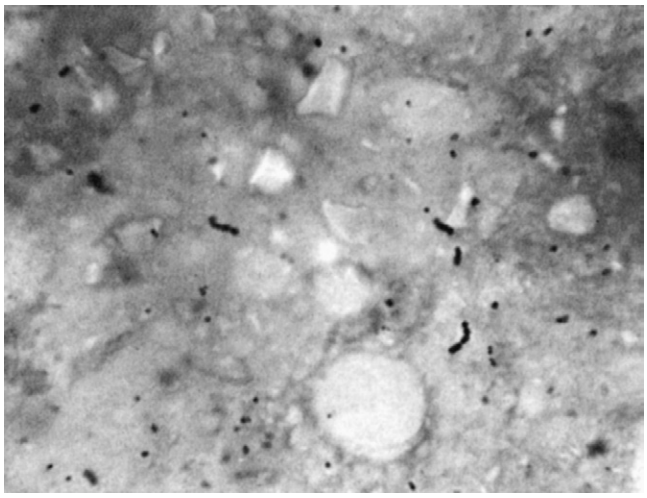

FIGURE 76-2 ■ Photomicrograph of a homogenized biopsy specimen stained with Gram stain. Gram-positive cocci are seen (cultures grew group A streptococcus at greater than 10^5 colony-forming units/gram tissue). (Reprinted with permission from Hunsicker L, Heggers JP, Patel JA. Infections in burn patients. In: Patrick CC, editor. *Clinical management of infections in immunocompromised infants and children.* 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 338.)

MICROBIOLOGIC INVESTIGATIONS

The three major approaches to determine burn wound infection are (1) quantitative burn wound cultures, (2) histologic assessment of bacterial invasion, and (3) bronchoalveolar lavage (BAL).

Quantitative Burn Wound Cultures by Biopsy

Teplitz demonstrated that quantitative bacterial counts of burn wound cultures correlated with histologic specimens showing invasion or colonization.[127] Burn wound infection (often referred to as "burn wound sepsis" in surgical literature) is suspected when proliferating microorganisms exceed 10^5 per gram of tissue and when invasion of subjacent unburned tissue has occurred (Fig. 76-2). The presence of microorganisms within the necrotic eschar cannot be considered evidence of burn wound infection. Furthermore, although a bacterial count of 10^5 per gram of tissue is likely to indicate bacterial invasion, this is not invariably true. Only the histologic sections can indicate the level of infection. Therefore, burn wound cultures always should be accompanied by histologic sections from the same area.[108,109]

At the time that surgery is performed, the potentially infected tissues should be excised with a punch biopsy (Fig. 76-3) and divided into equal aliquots.[55] One aliquot should be placed into saline and delivered to the microbiology section for quantitative assessment. These biopsy samples are weighed aseptically and homogenized in a sterile tube in 3 mL of sterile saline. Known dilutions of the homogenate then are plated using precalibrated loops (10 μL) onto blood agar, colistin-neomycin agar, MacConkey agar, and Sabouraud agar for identification in initial dilutions of 0.1 mL with a 1-mL sterile pipette. After 24 hours, the number of colonies are counted and

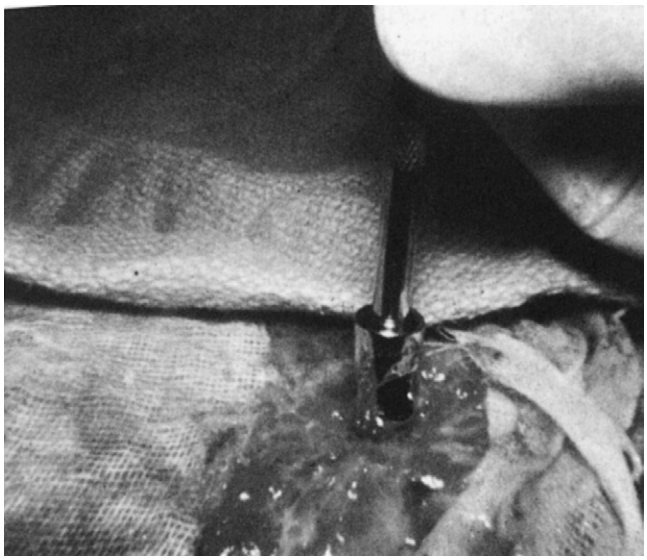

FIGURE 76-3 ■ Photograph showing the use of a 6-mm punch for collecting a skin biopsy specimen for tissue histology and quantitative culture. (Reprinted with permission from Hunsicker L, Heggers JP, Patel JA. Infections in burn patients. In: Patrick CC, editor. *Clinical management of infections in immunocompromised infants and children.* 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 339.)

the quantitative wound culture is calculated according to the following formula:

$$\text{Colony-forming unit (CFU)} \times \text{g of tissue} \times \text{number of colonies} \times \text{volume} \times \text{dilution weight of biopsy in grams}$$

Despite the dependence on quantitative tissue cultures, reliability of this procedure has been questioned because of the high degree of variability in quantitative

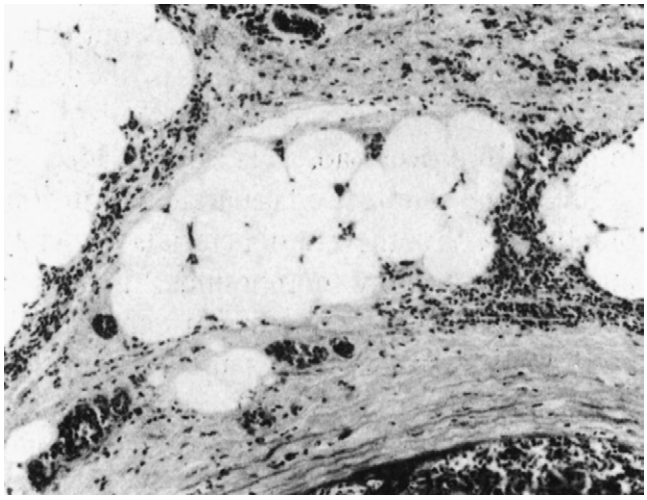

FIGURE 76-4 ■ Photomicrograph of a cross-section of tissue stained with hematoxylin and eosin showing invasion of *Pseudomonas aeruginosa.* (Reprinted with permission from Hunsicker L, Heggers JP, Patel JA. Infections in burn patients. In: Patrick CC, editor. *Clinical management of infections in immunocompromised infants and children.* 2nd ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 338.)

counts. Woolfrey and colleagues found that when biopsy samples were divided and cultured separately, only 38 percent of paired quantitative results agreed within the same $\log_{10}$ unit, whereas 44 percent differed by 2 $\log_{10}$ units or more.[141]

Histologic Procedures

Standard histologic procedures, as well as cryostat examination, are necessary.[109] The tissues should be examined for morphologic changes and for the presence of pathogens. Many bacterial and fungal pathogens can be identified by staining with Gram stain and Gomori methenamine silver stain (Fig. 76-4). More specialized stains may be necessary to identify other fastidious bacteria and fungi. Tzanck stain can be used to identify inclusion bodies suggestive of invasion of HSV or VZV. Viral-specific antibodies also can be used to detect these viruses by immunohistochemistry. The microbial status of the burn wound (i.e., colonization or invasion), as assessed by histologic examination of a biopsy specimen, can be graded on the basis of the density and depth of penetration of microorganisms (Table 76-5).[109]

Bronchoalveolar Lavage

BAL fluid needs to be collected by personnel experienced in the evaluation of patients with inhalation injury who are at risk for developing airway edema and obstruction. Ventilator-associated pneumonia can be diagnosed if 10^4 or more organisms per milliliter are cultured. In a study reported by Wahl and colleagues, BAL eliminated the unnecessary antibiotic treatment of 21 percent of patients.[133] In winter months, BAL fluids also can be processed for prevalent respiratory viruses such as respiratory syncytial virus (RSV), influenza A and B viruses, and adenoviruses by rapid antigenic detection by immunoassays that are within the capability of most laboratories. Other respiratory viruses, as well as herpesviruses such as HSV and CMV in the respiratory tract, may be detected by viral culture or molecular diagnostics performed by a specialized virology laboratory or reference laboratory.

TABLE 76-5 **Staging of Microbial Status of the Burn Wound by Biopsy Histology**

Stage I: Colonization

A. Superficial: microorganisms present only on burn wound surface
B. Penetrating: variable depth of microbial penetration of eschar
C. Proliferating: variable level of microbial proliferation at nonviable-viable tissue interphase (subeschar space)

Stage II: Invasion

A. Microinvasion: microorganisms present in viable tissue immediately subjacent to subeschar space
B. Deep invasion: penetration of microorganisms to variable depth and expanse within viable subcutaneous tissue
C. Microvascular involvement: microorganisms within small blood vessels and lymphatics (thrombosis of vessels common)

Modified from Pruitt BA, Foley FD. The use of biopsies in burn patient care. *Surgery* 1973;**73**:887-97.

Local and Systemic Viral Infection

Viral culture or molecular testing using qualitative or quantitative PCR of the wound surface obtained by swab or by tissue obtained by biopsy will detect the presence of HSV or VZV. Disseminated viral infection with HSV, VZV, CMV, and other viruses may be evaluated by sending whole blood or plasma for specific viral DNA detection and quantification by PCR.

PREVENTION AND TREATMENT OF INFECTION

Care of the wound is the mainstay of burn therapy. The goal is to minimize infection and facilitate healing of the burn wound. Wound care addresses both partial- and full-thickness burn injuries until all wounds are closed.

Wound Dressing

The superficial epidermal layer provides a barrier to microorganisms while the deeper lipid epidermal layer provides protection against loss of water vapor. In full-thickness burns, the eschar may extend beyond the skin into the subcutaneous fat and muscle. Closure of the wound cannot occur until the eschar is removed. Bacterial proteases lead to eschar pseudoseparation and are slowed with antiseptic therapies. Then, the eschar is allowed to fragment and slough, with resultant decreased size. This process is called *wound contraction*. This can also be excised and skin grafted. Full-thickness skin grafts have the least contraction rates. Deep dermal wounds

allowed to heal spontaneously lead to hypertrophic scarring. Compression garments are used for prevention and treatment.

When approaching burn wound care, a plan must first be decided. Outer burn dressings provide comfort, metabolic enhancement, and protection. First, superficial burns are exquisitely sensitive to air currents, as are deeper burns after some healing. Also, dressings provide splinting and drainage containment. Next, occlusive dressings help eliminate shivering, cold stress, and evaporative heat loss. In open, granulating wounds, loss of water vapor is maximal. Finally, the protective component deals with the control of topical organisms. Supplies consist of large 9 × 9-inch burn gauze, Kerlix wraps, Ace wraps, and topical antimicrobial agents. Gentle cleansing of the wound and daily débridement also are important. Buttock burn wounds should be examined carefully and frequently for the presence of deep stool staining, an ominous predictor of burn wound sepsis and death.[112] Such wounds should be emergently excised.

Topical Antimicrobial Agents

Topical antimicrobial agents are used extensively in burn patients. Most patients with stage I and stage II A and B wounds (see Table 76-5) can be treated with topical or subeschar antimicrobial agents alone.

Silver Sulfadiazine (Silvadene, SSD, Thermazene, Flamazine, Burnazine)

Silver sulfadiazine is a 1 percent water-soluble cream combining sulfadiazine with silver (Ag^+). The Ag^+ ion binds with the DNA of an organism and consequently releases the sulfonamide that interferes with the intermediary metabolic pathway of the microbe. It is most effective against *P. aeruginosa*, the gram-negative enteric bacteria, and *C. albicans*. *S. aureus* and some strains of *Klebsiella* spp. have been less effectively controlled.[41] Caution is advised with use of sulfadiazine products because cases of *P. aeruginosa* resistance to sulfadiazine have been reported.[54] When compared with collagenase ointment, topical silver sulfadiazine has been shown to be associated with lower rates of wound infection.[105] Antimicrobial effectiveness has been observed to last for up to 24 hours. More frequent changes are required if a creamy exudate forms on the wound. The benefits of this topical agent are its ease of use and its ability to reduce pain. It has some tissue-penetrating ability, but it is limited to the epidermis.

Silver sulfadiazine can be used separately or in combination with other antibacterial agents or enzymatic escharotomy compounds. It can be combined with nystatin, which enhances the antifungal activity of this agent. By itself, silver sulfadiazine has been shown to retard wound healing; however, in conjunction with nystatin or aloe vera, the wound-retardant effect is reversed. When topical silver sulfadiazine was compared with the more expensive collagenase ointment for treatment of partial thickness burns in children, there was no significant difference in clinical course, outcome, or need for skin grafting.[105]

Cerium Nitrate–Silver Sulfadiazine (Flammacerium)

The lanthanide salt cerium nitrate has been added to silver sulfadiazine since the 1970s and has been shown to have increased bacteriostatic effect in large burn wounds. The antimicrobial spectrum is similar to that of silver nitrate. Methemoglobinemia has been seen only rarely, and no associated electrolyte disturbances occur. Only minimal cerium absorption has been noted in patients with large burns treated for weeks. No complications were noted. Its use is somewhat limited because it is not commercially available in the United States. It is, however, available in several western European countries. Koller and Orsag studied the effects of cerium sulfadiazine in 20 burn patients and found it to be safe and effective in the treatment of deep, extensive burn wounds.[73]

Silver Nitrate

Silver nitrate is available as a 0.5 percent solution that does not injure the regenerating epithelium in the wound and provides bacteriostatic effect against *S. aureus*, *E. coli*, and *P. aeruginosa*.[41] Silver nitrate is most effective when the wound is cleansed carefully of all emollients and other debris. Multilayered coarse mesh dressings should be placed over the wound and saturated with the silver nitrate solution. Like silver sulfadiazine, silver nitrate has limited penetration because the Ag^+ ion is bound rapidly to the body's natural anions such as Cl^-. Because it is hypotonic, it can cause osmolar dilution, resulting in hyponatremia and hypochloremia. Serum electrolyte levels should be monitored very carefully.

Notable detriments to the use of silver nitrate include its high expense and light sensitivity. Furthermore, it also requires special handling because if it is allowed to dry or if it is covered with an impervious dressing hyperpyrexia could occur. *Klebsiella*, *Providencia*, and other Enterobacteriaceae are not as susceptible to it as are other bacteria. *E. cloacae* and other nitrate-positive bacteria can cause methemoglobinemia by converting nitrite to nitrate.

Mafenide Acetate (Sulfamylon)

Mafenide acetate is available both in a 10 percent water-soluble cream or a 5 percent solution and has more substantial bacteriologic data to support its efficacy than do any of the other topical antimicrobial agents.[76,89] It has been shown to be effective against a broad range of microorganisms, especially against all strains of *P. aeruginosa* and *Clostridium* spp.[116] After a wound has been cleansed of debris, mafenide acetate is applied to the wound like "butter." The treated burn surface is left exposed for maximal antimicrobial potency. The cream is applied a minimum of twice a day and can be reapplied as needed. The 5 percent solution is applied every 8 hours.

Additionally, mafenide acetate has the ability to permeate burn eschar and thereby reduce the risk for bacterial colonization of a deep burn wound, and it is especially effective after the dead tissue is removed from the

granulating bed. Unfortunately, several detrimental aspects are associated with the use of mafenide acetate. Protracted use with the low environmental pH favors the growth of *C. albicans.* Mafenide acetate is converted to *p*-sulfamoyl benzoic acid by monamine oxidase, which is a carbonic anhydrase inhibitor; it subsequently causes metabolic acidosis in the patient. It also is painful when applied to superficial, partial-thickness burns with intact free nerve endings, and its requirement to remain uncovered for antimicrobial activity may be considered a disadvantage when a dressing is required.

Membrane Dressings

Acticoat and Aquacel are silver-coated dressings that have a broad antibacterial activity. Compared with silver nitrate or silver sulfadiazine use, Acticoat and Aquacel have been found easier to apply, were associated with less pain on removal, and have faster rates of reepithelialization.[22,128] In one clinical study, Acticoat was found to have better antibacterial activity than silver sulfadiazine.[70] Acticoat is also better than hydrogel (Solosite) and paraffin-embedded woven cotton fabric (Jelonet) in protection against wound colonization/infection.[134] Mepitel is another grid-like, silicone-coated, nylon dressing used in treatment of partial-thickness burn wounds. Gotschall and associates found this product to decrease the pain experienced during dressing change, the time needed for the wound to heal, and the overall duration of the hospital stay as compared with silver sulfadiazine.[46] This study did not find any difference in the incidence of infections between Mepitel and silver sulfadiazine treatments.

Topical Antibiotics

Several topical antibiotics have been tried in the management of burn infections. The use of these agents is highly discouraged in a burn unit because of rapid emergence of resistance:

1. *Gentamicin sulfate:* Available as a 0.1 percent water-soluble cream, this antibiotic is chemically similar to the other aminoglycosides, such as kanamycin and neomycin. It has a broad spectrum of antimicrobial activity. It is used for its activity against *P. aeruginosa;* however, its topical use is highly limited because of the high level of gentamicin resistance in burn units.
2. *Bacitracin/polymyxin:* This antibiotic combination has little or no effect on localized infections of burn wounds.
3. *Nitrofurantoin:* This agent is used topically as adjunctive therapy in patients with second- and third-degree burns. With good eschar penetration, it can be used in the treatment of invasive burn wound infections with sensitive agents. The drug presents some advantages for the ambulatory patient. Tissue granulation begins sooner and crusts separate more rapidly.
4. *Mupirocin (Bactroban):* Studies at Shriners Hospitals for Children–Galveston have shown mupirocin to be superior to silver sulfadiazine in treating MSSA or MRSA infection.[125] Although mupirocin is weaker against gram-negative bacteria, it is also comparable to silver sulfadiazine and mafenide acetate against *P. aeruginosa*, *E. coli*, and *K. pneumoniae.*

Nystatin (Mycostatin, Nilstat)

Studies have shown that combining nystatin and silver sulfadiazine or nitrofurantoin results in effective prevention of local and systemic *Candida* infections, as well as burn wound sepsis.[56] However, in combination therapy with silver sulfadiazine and nystatin, mafenide acetate actually loses its antimicrobial activity. Therefore, use of nystatin with mafenide acetate is discouraged. Concentrated nystatin powder has also been studied for its effects on angioinvasive fungi (e.g., *Fusarium*, *Aspergillus*) refractory to systemic amphotericin B and serial excisions, including amputations.[11] A concentrated form of nystatin (6 million units/g) was used as dry aerosol every 6 hours and wet to dry dressings laid on top. Within 14 days, in all four of the children studied eradication occurred of their invasive fungal infections. Also, all areas previously autografted underneath the nystatin powder healed nicely.

Sodium Hypochlorite (0.025% Heggers Solution)

Currently, the most effective topical antibacterial agent for cleansing a wound is sodium hypochlorite (NaClO). It transcends the topical antimicrobial effects and tissue toxicity of such products as povidone-iodine, acetic acid, and hydrogen peroxide. The efficacy of NaClO has been determined to be at a concentration of 0.025 percent, which is bactericidal, is nontoxic to fibroblasts, and does not inhibit wound healing, provided buffers are used.[57] It is a broad-spectrum antiseptic and is bactericidal for *P. aeruginosa*, *S. aureus* (MRSA and MSSA), enterococci, and other gram-negative and gram-positive organisms.[116]

Povidone-Iodine (Betadine)

The active antimicrobial component in this compound is the iodine. It has a broad spectrum of antibacterial and antifungal activities. However, there are disadvantages that limit its use in a burn center. It is painful when applied and is inactivated by wound exudates. Renal dysfunction and acidosis have been noted in association with systemic absorption when applied to open wounds.

Chlorhexidine

Chlorhexidine when combined with 0.5 percent silver nitrate has efficacy similar to that of silver sulfadiazine.[80] Some variants of this product have broader antimicrobial activities, but pain experienced by patients on application has limited its use. Unlike the sulfonamides, plasma-mediated resistance has not occurred.

Citric Acid

In a study from India,[98] citric acid was formulated into a 3 percent gel and was compared with silver sulfadiazine. Citric acid gel application was better in wound healing. However, large-scale studies are lacking.

Subeschar Antibiotics

Moncrief has described the technique of subeschar antibiotic infusion.[92] It is used for microbial invasion into unburned tissue, when topical therapies have not been effective, or in cases in which treatment has been delayed. The most common drugs used are tobramycin, gentamicin, and kanamycin. However, development of antimicrobial resistance is likely to be substantial, so subeschar infusion of antibiotics should be used infrequently. The antibiotic solution is administered via multiple needle infusions by subcutaneous lysis. The results of this technique are more rapid separation of eschar. The fluid infusion limit is 2000 mL (adult dose) and should be accounted for in a patient's fluid requirements. The infusion fluid should consist of 0.25 to 0.45 N saline to avoid salt overload. Use of lactated Ringer solution should be avoided because some of the drugs are incompatible with calcium. Erythromycin has been studied but is too painful to use. Colistin, novobiocin, and cephaloridine have been used but found to be ineffective.

Systemic Anti-infective Agents

Drug pharmacokinetics are significantly altered in the burn patient and show significant interpatient and intrapatient variation.[137] Dolton and coworkers showed that vancomycin clearance is significantly higher in burn patients, but there is no significant difference in the volume of distribution.[31] Doh and associates also showed that meropenem clearance and volume of distribution were higher.[30] These studies suggest that higher dosing of vancomycin and meropenem may be needed in burn patients. Despite these studies, however, a review of the currently available literature shows that for many drugs there is a paucity of information to support current dosage recommendations, specifically in children of various age groups. In addition, many reports are based on small numbers of patients, and even in larger studies, no standardization of the study population exists with regard to the important variables known to affect drug handling. For the subpopulation of burn patients who eliminate drugs extremely rapidly, a concern exists over the adequacy of antibiotic dosing. Researchers have suggested that antibiotic serum concentrations be measured, when available, in every patient to ascertain whether a significant problem exists with dosing.

Whereas stage I and stage II A and B wounds (see Table 76-5) can be treated with topical or subeschar antimicrobials alone, stage II C wounds require the immediate institution of systemic anti-infective agents. Systemic agents also are indicated for the treatment of various systemic infectious complications (see earlier discussion). Detailed information on selection of the appropriate systemic antimicrobial agents for specific bacterial, fungal, and viral pathogens is available elsewhere in this text. Nonetheless, the following general principles can help guide the use of antimicrobial therapy:

1. Each antimicrobial agent must be selected for its specificity for the microbe present.
2. Such decisions should be made on appropriately collected culture and susceptibility data.
3. Colonizing flora should be distinguished from those responsible for inflammation and invasion.
4. The time, dosage, route of administration, and duration of treatment should be in accordance with what is required to make the organism nonpathogenic.
5. The need for broad-spectrum antibiotics should be balanced with the risk for promoting fungal infections.

Treatment of Multidrug-Resistant, Gram-Negative Bacteria

This issue deserves specific mention because it most severely affects the burn units. In many centers, panresistant *P. aeruginosa* strains and *Acinetobacter* spp. are identified frequently. In these cases, colistin and polymyxin B often remain the only susceptible antimicrobial agents. In a recent study, Goverman and coworkers summarized their experience in 14 children treated with intravenous colistin: a favorable response was obtained in 79 percent, and the overall mortality rate was 14 percent.[47] Both colistin and polymyxin B are associated with significant elevation in serum creatinine value or with renal failure in 15 to 25 percent of recipients. Neurotoxicity in children is an infrequent occurrence. Intravenous and/or aerosol polymyxin B, doxycycline, and ampicillin/sulbactam (the active component is thought to be sulbactam) have been tried in multidrug-resistant *Acinetobacter* infections as well.[101] Other studies have demonstrated in vitro susceptibility of multidrug-resistant *Acinetobacter* to various synergistic combinations of antimicrobial agents, including carbapenems, colistin, rifampin, tigecycline, and ampicillin-sulbactam.[44,59,142] The clinical utility of these combinations against panresistant *Acinetobacter* remains to be determined.

Treatment of Viral Infections

If herpes simplex virus or varicella zoster virus infection of the wound surface, lungs, or disseminated viral infection with viremia is present, then systemic antiviral treatment with acyclovir is indicated. Pneumonitis and systemic infection with significant viremia with CMV should be treated with ganciclovir. If viral resistance to first-line antiviral agents occurs, then foscarnet or cidofovir may be administered. Respiratory tract infection, including pneumonia, associated with influenza A or B virus may be treated with oseltamivir, which can be orally administered if the patient is able to absorb enterally administered medications.

Antibiotic Prophylaxis

Prophylactic use of antibiotics remains a highly controversial topic. Penicillin prophylaxis is used commonly in many burn centers during outbreaks of group A streptococcal infections.[49,113] Prophylactic antibiotics also are used before surgical manipulation or instrumentation because the risk for developing bacteremia is as high as 50 percent. The choice of perioperative antibiotics is dependent on the knowledge of existing microorganisms,

which are present not only on injured skin but also in the wound that is ready for surgery. Nonetheless, carefully controlled studies are necessary to identify the value of prophylactic antibiotics. In a placebo-controlled study of cefazolin use in children with burn injury, researchers found that in children with burns of less than 35 percent TBSA, cefazolin was not necessary, and in those with burns of 35 percent or more, it was not effective.[114] In a retrospective review study, antibiotic prophylaxis was not associated with any reduction in the rate of wound infection; instead, the duration of hospitalization was found to be longer.[32] A recent meta-analysis of studies of prophylactic antibiotics found that most of the studies were weak.[5] The most important finding was that staphylococcal infection rates declined with prophylaxis but resistance to prophylactic antibiotics increased.

Wound Excision and Grafting

Studies have shown that early excision of wounds leads to significantly decreased rates of bacterial colonization and wound infection.[10] In a meta-analysis of six trials it was found that early excision of burns is beneficial in reducing mortality (in patients without inhalational injury) and length of hospital stay. The only drawback is the greater volume of blood loss.[103]

Gut Support and Decontamination

The gut microflora have been implicated in the development of multiorgan failure when the gut barrier fails. Hence, one theory is that early enteral feeding of the gut, which promotes function of the gut barrier, is important for preventing multiorgan failure. A decontaminated gut with nonabsorbable, broad-spectrum antibiotics might diminish the impact of failure of the gastrointestinal barrier. Although the suggestion has been made that the rate of pneumonia may be decreased by such maneuvers, no apparent impact on mortality rates has been noted.[85] Overall, convincing human data on the beneficial effect of enteral feeding and decontamination of the gut on sepsis and multiorgan failure are lacking.

Immunomodulators

In view of the intricate relationship between altered immune defenses in burn patients and increased susceptibility to infections, various types of immunotherapies have been tried. In an Italian study, treatment with intravenous pooled immunoglobulin (IVIG) was found to have a beneficial effect on septic phenomena and recovery.[90] However, in two U.S. studies, no change in rates of infection or mortality was shown. Overall, the beneficial role of IVIG as prophylaxis or treatment remains unproven.[97,135] In a German study, prophylaxis with intravenous anti-*Pseudomonas* immunoglobulin did not appear to be beneficial to burn patients in general; however, it was shown to be effective in burn patients with inhalation injury.[126] In a South African study, prophylaxis with high-titer anti-lipopolysaccharide immunoglobulin G reduced the incidence of burn wound infection but did not affect mortality.[64] Benefits of plasmapheresis or fresh frozen plasma infusion have not been proven.

INFECTION CONTROL

Hospital-acquired infections in burn patients are common occurrences. In pediatric burn patients, wound infections, ventilator-related pulmonary infections, central-line bacteremias, and catheter-associated urinary tract infections are the most common nosocomial infections.[117,136] The rates of infections may be lower than those seen in adults, but urinary tract infections occur more frequently in children.[117] In a German study of children with burns, the overall infection rate was 59.7 nosocomial infections per 1000 inpatient-days and the device-associated infection rates per 1000 device-days were 55.2 for pneumonia, 8.9 for primary bloodstream infections, and 41.7 for urinary tract infections.[43] The incident density of burn wound infections was 18.5 per 1000 inpatient-days, which was associated with the percentage of TBSA of the burn wound. On the other hand, the rate of device-associated infections was not associated with the percentage of TBSA.

Surveillance of infection in a burn patient is performed to implement prompt treatment on the basis of surveillance cultures and antimicrobial sensitivities at the earliest sign of invasion. Such surveillance requires cultures of sputum, urine, and wounds about three times weekly; however, the need for such cultures and the frequency of monitoring remain controversial. Additional infection control measures involve surveillance of spread of pathogens among burn patients in a burn unit. In dealing with the burn wound, strict infectious disease precautions must be maintained to prevent contamination and worsening infection in these already immunocompromised patients.

In the past, poor hand washing and shared hydrotherapy tubs were the source of many infections, some of which were life threatening. Strict enforcement of hand washing and use of gowns, gloves, and masks have led to decreased rates of patient contamination. Also, disposable hydrotherapy tub liners are used without the risk for patient-to-patient transmission of infections.[129] Besides this, isolation of burn patients in a single room compared with placing them in an open ward has dramatically affected infection rates. McManus and associates studied this measure in 2519 patients and found significantly lower incidence and mortality rates associated with gram-negative bacteremia in the isolated patients.[88] Also, the patients in an open ward with bacterial isolates showed significantly more antimicrobial resistance compared with patients in isolation.

Additional infection control measures include the daily use of germicidal solutions to clean all of the hardware, such as intravenous poles and pumps, monitoring equipment, bedside tables, and beds in patients' rooms. On discharge of the patient, everything in the room, including floors, walls, ceilings, and mattresses, needs to be cleaned with germicidal solutions. Air filters should be monitored repeatedly for fungal and bacterial growth.

Lai and colleagues studied the effects of strict isolation of patients with vancomycin-resistant enterococci, the use of vancomycin, and the cost-to-benefit analysis of barrier precautions.[77] The study showed that pharyngeal swabs were poor for surveillance but that rectal swabs were more useful. Also, guidelines for use of vancomycin were adhered to by 85 percent of the staff. The overall cost for these implementations, including the barrier supplies and cleaning protocols, was $11,000. Nonetheless, vancomycin-resistant enterococci were not eradicated. On the other hand, van Rijn and colleagues[131] have shown that isolation in a quarantine unit was highly effective in preventing outbreaks of multidrug-resistant bacteria.

NEW REFERENCES SINCE THE SIXTH EDITION

4. *American Burn Association National Burn Repository (2011 Report)*. Available at http://ameriburn.org/2011NBRAnnualReport.pdf. Accessed July 1, 2012.
5. Avni T, Levcovich A, Ad-El DD, et al. Prophylactic antibiotics for burns patients: systematic review and meta-analysis. *BMJ* 2010;**340**:c241.
15. Bennett JW, Robertson JL, Hospenthal DR, et al. Impact of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* infections in severely burned patients. *J Am Coll Surg* 2010;**211**: 391–9.
19. Brusselaers N, Monstrey S, Snoeij T, et al. Morbidity and mortality of bloodstream infections in patients with severe burn injury. *Am J Crit Care* 2010;**19**:e81–7.
26. D'Avignon LC, Hogan BK, Murray CK, et al. Contribution of bacterial and viral infections to attributable mortality in patients with severe burns: an autopsy series. *Burns* 2010;**36**:773–9.
30. Doh K, Woo H, Hur J, et al. Population pharmacokinetics of meropenem in burn patients. *J Antimicrob Chemother* 2010;**65**: 2428–35.
31. Dolton M, Xu H, Cheong E, et al. Vancomycin pharmacokinetics in patients with severe burn injuries. *Burns* 2010;**36**:469–76.
60. Huang LF, Yao YM, Dong N, et al. Association between regulatory T cell activity and sepsis and outcome of severely burned patients: a prospective, observational study. *Crit Care* 2010;**14**(1):R3.
69. Kennedy P, Brammah S, Wills E. Burns, biofilm and a new appraisal of burn wound sepsis. *Burns* 2010;**36**:49–56.
70. Khundkar R, Malic C, Burge T. Use of Acticoat dressings in burns: what is the evidence? *Burns* 2010;**36**:751–8.
72. Kim LH, Ward D, Lam L, Holland AJ. The impact of laser Doppler imaging on time to grafting decisions in pediatric burns. *J Burn Care Res* 2010;**31**:328–32.
75. Kraft R, Herndon DN, Al-Mousawi AM, et al. Burn size and survival probability in paediatric patients in modern burn care: a prospective observational cohort study. *Lancet* 2012;**379**:1013–21.
86. Mahar P, Padiglione AA, Cleland H, et al. *Pseudomonas aeruginosa* bacteraemia in burns patients: risk factors and outcomes. *Burns* 2010;**36**:1228–33.
93. Moore EC, Padiglione AA, Wasiak J, et al. *Candida* in burns: risk factors and outcomes. *J Burn Care Res* 2010;**31**:257–63.
98. Nagoba BS, Gandhi RC, Hartalkar AR, et al. Simple, effective and affordable approach for the treatment of burns infections. *Burns* 2010;**36**:1242–7.
105. Ostlie DJ, Juang D, Aguayo P, et al. Topical silver sulfadiazine vs collagenase ointment for the treatment of partial thickness burns in children: a prospective randomized trial. *J Pediatr Surg* 2012;**47**:1204–7.
107. Palumbo A, Forte GI, Pileri D, et al. Analysis of IL-6, IL-10 and IL-17 genetic polymorphisms as risk factors for sepsis development in burned patients. *Burns* 2012;**38**:208–13.
134. Wang XQ, Kravchuk O, Kimble RM. A retrospective review of burn dressings on a porcine burn model. *Burns* 2010;**36**:680–7.
139. Williams FN, Herndon DN, Hawkins HK, et al. The leading causes of death after burn injury in a single pediatric burn center. *Crit Care* 2009;**13**(6):R183.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdi S, Herndon DN, McGuire J, et al. Time course of alterations in lung lymph and bronchial blood flows after inhalation injury. *J Burn Care Rehabil* 1990;**11**:510–5.
2. Albertson S, Greenhalgh DG, Breeden MP, et al. Cardiac abnormalities in children with burns: an autopsy analysis. *J Burn Care Rehabil* 1994;**15**:401–4.
3. Albrecht MC, Griffith ME, Murray CK, et al. Impact of *Acinetobacter* infection on the mortality of burn patients. *J Am Coll Surg* 2006;**203**:546–50.
4. *American Burn Association National Burn Repository (2011 Report)*. Available at http://ameriburn.org/2011NBRAnnualReport.pdf. Accessed July 1, 2012.
5. Avni T, Levcovich A, Ad-El DD, et al. Prophylactic antibiotics for burns patients: systematic review and meta-analysis. *BMJ* 2010;**340**:c241.
6. Babcock GF, Alexander JW, Warden GD. Flow cytometric analysis of neutrophil subsets in thermally injured patients developing infection. *Clin Exp Immunol* 1990;**54**:117–25.
7. Balogh D, Lammer H, Kornberger E, et al. Neopterin plasma levels in burn patients. *Burns* 1992;**18**:185–8.
8. Barber RC, Chang LY, Arnoldo BD, et al. Innate immunity SNPs are associated with risk for severe sepsis after burn injury. *Clin Med Res* 2006;**4**:250–5.
9. Baron P, Traber LD, Traber DL, et al. Gut failure and translocation following burn and sepsis. *J Surg Res* 1994;**57**:197–204.
10. Barret JP, Herndon DN. Effects of burn wound excision on bacterial colonization and invasion. *Plast Reconstr Surg* 2003;**111**:744–50.
11. Barret JP, Ramzy PI, Heggers JP, et al. Topical nystatin powder in severe burns: a new treatment for angioinvasive fungal infections refractory to other topical and systemic agents. *Burns* 1999;**25**:505–8.
12. Barro RE, Wang CZ, Cox RA, et al. Cellular sequence of tracheal repair in sheep post smoke inhalation injury. *Lung* 1992;**170**:331–8.
13. Baskin TW, Rosenthal A, Pruitt BA. Acute bacterial endocarditis: a silent source of sepsis in the burn patient. *Ann Surg* 1976;**184**:618.
14. Beard CH, Ribeiro CD, Jones DM. The bacteremia associated with burns surgery. *Br J Surg* 1975;**62**:638–41.
15. Bennett JW, Robertson JL, Hospenthal DR, et al. Impact of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* infections in severely burned patients. *J Am Coll Surg* 2010;**211**:391–9.
16. Bjerknes R, Vindenes H. Neutrophil dysfunction after thermal injury—alteration of phagolysosomal acidification in patients with large burns. *J Trauma* 1989;**15**:71–81.
17. Bourdarias B, Perro G, Cutillas M, et al. Herpes simplex virus infection in burned patients: epidemiology of 11 cases. *Burns* 1996;**22**:287–90.
18. Bowers BL, Purdue GF, Hunt JL. Paranasal sinusitis in burn patients following nasotracheal intubation. *Arch Surg* 1991;**126**:1411–2.
19. Brusselaers N, Monstrey S, Snoeij T, et al. Morbidity and mortality of bloodstream infections in patients with severe burn injury. *Am J Crit Care* 2010;**19**:e81–7.
20. Byers RJ, Hasleton PS, Quigley A, et al. Pulmonary herpes simplex in burns patients. *Eur Respir J* 1996;**9**:2313–7.
21. Cartotto RC, Macdonald DB, Wasan SM. Acute bacterial endocarditis following burns: case report and review. *Burns* 1998;**24**:363–73.
22. Caruso DM, Foster KN, Blome-Eberwein SA, et al. Randomized clinical study of Hydrofiber dressing with silver or silver sulfadiazine in the management of partial-thickness burns. *J Burn Care Rehabil* 2006;**27**:298–309.
23. Childs C, Edwards-Jones V, Heathcote DM, et al. Patterns of *Staphylococcus aureus* colonization, toxin production, immunity and illness in burned children. *Burns* 1994;**20**:514–21.
24. Cook N. Methicillin-resistant *Staphylococcus aureus* versus the burn patient. *Burns* 1998;**24**:91–8.
25. Coursaget P, Lesage G, Simpson B, et al. Incidence of hepatitis C virus infection in burn patients: detection of anti-C100, anti-C33c and anti-core antibodies. *Biomed Pharmacother* 1991;**45**:445–9.
26. D'Avignon LC, Hogan BK, Murray CK, et al. Contribution of bacterial and viral infections to attributable mortality in patients with severe burns: an autopsy series. *Burns* 2010;**36**:773–9.
27. Deepe Jr GS, MacMillan BC, Linnemann Jr CC. Unexplained fever in burned patients due to cytomegalovirus infection. *JAMA* 1982;**248**:2299–301.
28. Desai MH, Herndon DN, Abston S. *Candida* infection in massively burned patients. *J Trauma* 1987;**27**:1186–8.
29. Dietich EA, Xu D, Qi L. Different lymphocyte compartments respond differently to mitogenic stimulation after thermal injury. *Ann Surg* 1990;**211**:72–7.
30. Doh K, Woo H, Hur J, et al. Population pharmacokinetics of meropenem in burn patients. *J Antimicrob Chemother* 2010;**65**:2428–35.
31. Dolton M, Xu H, Cheong E, et al. Vancomycin pharmacokinetics in patients with severe burn injuries. *Burns* 2010;**36**:469–76.
32. Ergün O, Celik A, Ergün G, et al. Prophylactic antibiotic use in pediatric burn units. *Eur J Pediatr Surg* 2004;**14**:422–6.
33. Erol S, Altoparlak U, Akcay MN, et al. Changes in microbial flora and wound colonization in burned patients. *Burns* 2004;**30**:357–61.
34. Evans EB. Musculoskeletal changes secondary to thermal burns. In: Herndon DN, editor. *Total burn care*. London: WB Saunders; 2007. p. 652–66.
35. Faist E, Ertel W, Baker CC, et al. Terminal B-cell maturation and immunoglobulin (Ig) synthesis in vitro in patients with major injury. *J Trauma* 1989;**29**:2–9.
36. Finnerty CC, Herndon DN, Jeschke MG. Inhalation injury in severely burned children does not augment the systemic inflammatory response. *Crit Care* 2007;**11**:R22.
37. Finnerty CC, Herndon DN, Przkora R, et al. Cytokine expression profile over time in severely burned pediatric patients. *Shock* 2006;**26**:13–9.
38. Fleming RY, Zeigler ST, Walton MA, et al. Influence of burn size on the incidence of contamination of burn wounds by fecal organisms. *J Burn Rehabil* 1991;**12**:510–5.
39. Furniss D, Gore S, Azadian B, et al. *Acinetobacter* is associated with acquired glucose intolerance in burn patients. *J Burn Care Rehabil* 2005;**26**:405–8.
40. Gadd MA, Hansbrough JF, Hoyt DB, et al. Defective T-cell surface antigen expression after mitogen stimulation: an index of lymphocyte dysfunction after controlled murine injury. *Ann Surg* 1989;**29**:112–8.
41. Gallagher JJ, Williams-Bouyer N, Villarreal C, et al. Treatment of infections in burns. In: Herndon DN, editor. *Total burn care*. Philadelphia: WB Saunders; 2007. p. 143.
42. Gasser H, Paul E, Redl H, et al. Loss of plasma antioxidants after burn injury [abstract]. *Circ Shock* 1991;**34**:13.
43. Gastmeier P, Weigt O, Sohr D, Rüden H. Comparison of hospital-acquired infection rates in paediatric burn patients. *J Hosp Infect* 2002;**52**:161–5.
44. Giamarellos-Bourboulis EJ, Xirouchaki E, Giamarellou H. Interactions of colistin and rifampin on multidrug-resistant *Acinetobacter baumannii*. *Diagn Microbiol Infect Dis* 2001;**40**:117–20.
45. Gibbons RA, Martinez OM, Lim RC, et al. Reduction in HLA-DR, HLA-DQ, and HLA-DP expression by Leu-M3+ cells. *Clin Exp Immunol* 1989;**75**:371–5.
46. Gotschall CS, Morrison MI, Eichelberger MR. Prospective, randomized study of the efficacy of Mepitel on children with partial-thickness scalds. *J Burn Care Rehabil* 1998;**19**:279–83.
47. Goverman J, Weber JM, Keaney TJ, et al. Intravenous colistin for the treatment of multi-drug resistant, gram-negative infection in the pediatric burn population. *J Burn Care Res* 2007;**28**:421–6.
48. Greenhalgh D. Wound healing. In: Herndon DN, editor. *Total burn care*. Philadelphia: WB Saunders; 2007. p. 578–9.
49. Gruteke P, van Belkum A, Schouls LM, et al. Outbreak of group A streptococci in a burn center: use of pheno- and genotypic procedures for strain tracking. *J Clin Microbiol* 1996;**34**:114–8.
50. Hamory BH. Nosocomial sepsis related to intravascular access. *Crit Care Nurs J* 1989;**11**:58–65.
51. Hansbrough JF, Zapata-Sirvent R, Hoyt D. Postburn immune suppression: an inflammatory response to the burn wound? *J Trauma* 1990;**30**:671–5.
52. Hayden FG, Himmel HN, Heggers JP. Herpes virus infections in burn patients. *Chest* 1994;**106**(Suppl):S15–21.

53. Heggers JP, Muller MJ, Elwood E, Herndon DN. *Ascariasis* pneumonitis: a potentially fatal complication in smoke inhalation injury. *Burns* 1995;**21**:149–51.
54. Heggers JP, Robson MC. The emergence of silver sulfadiazine resistant *Pseudomonas aeruginosa*. *Burns* 1978;**5**:184–7.
55. Heggers JP, Robson MC, editors. *Quantitative bacteriology: its role in the armamentarium of the surgeon*. Boca Raton, Fla.: CRC Press; 1991. p. 1–139.
56. Heggers JP, Robson MC, Herndon DN, Desai MH. The efficacy of nystatin combined with topical microbial agents in the treatment of burn wound sepsis. *J Burn Care Rehab* 1989;**10**:508–11.
57. Heggers JP, Sazy JA, Stenberg BD, et al. Bactericidal and wound healing properties of sodium hypochlorite. The 1991 Lindberg Award. *J Burn Care Rehab* 1991;**12**:420–4.
58. Herndon DN, Traber LD, Linares H, et al. Etiology of the pulmonary pathophysiology associated with inhalation injury. *Resuscitation* 1986;**14**:43–59.
59. Higgins PG, Wisplinghoff H, Stefanik D, et al. In vitro activities of the beta-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam alone or in combination with beta-lactams against epidemiologically characterized multidrug-resistant *Acinetobacter baumannii* strains. *Antimicrob Agents Chemother* 2004;**48**:1586–92.
60. Huang LF, Yao YM, Dong N, et al. Association between regulatory T cell activity and sepsis and outcome of severely burned patients: a prospective, observational study. *Crit Care* 2010; **14**(1):R3.
61. Jackson DM. The diagnosis of the depth of burning. *Br J Surg* 1953;**40**:588–96.
62. James J, Hofland HW, Borgstein ES, et al. The prevalence of HIV infection among burn patients in a burn unit in Malawi and its influence on outcome. *Burns* 2003;**29**:55–60.
63. Jeschke MG, Mlcak RP, Finnerty CC, et al. Burn size determines the inflammatory and hypermetabolic response. *Crit Care* 2007; **11**:R90.
64. Jones EB. Prophylactic anti-lipopolysaccharide freeze-dried plasma in major burns: a double blind controlled trial. *Burns* 1995; **21**:267–72.
65. Kagan RJ, Bratescu A, Jonason O, et al. The relationship between the percentage of circulating B cells, corticosteroid levels, and other immunologic parameters in thermally injured patients. *J Trauma* 1989;**29**:208–13.
66. Kaufman T, Neuman RA, Weinberg A. Is postburn dermal ischemia enhanced by oxygen free radicals? *Burns* 1989;**15**:291–4.
67. Kealey GP, Bale JF, Strauss RG, et al. Cytomegalovirus infection in burn patients. *J Burn Care Rehabil* 1987;**8**:543–5.
68. Kelly JL, Lyons A, Soberg CC, et al. Anti-interleukin-10 antibody restores burn-induced defects in T-cell function. *Surgery* 1997;**122**: 146–52.
69. Kennedy P, Brammah S, Wills E. Burns, biofilm and a new appraisal of burn wound sepsis. *Burns* 2010;**36**:49–56.
70. Khundkar R, Malic C, Burge T. Use of Acticoat dressings in burns: what is the evidence? *Burns* 2010;**36**:751–8.
71. Killer M, Konig W, Brom J, et al. Studies on the mechanisms of granulocyte dysfunctions in severely burned patients—evidence for altered leukotriene generation. *J Trauma* 1989;**29**:435:45.
72. Kim LH, Ward D, Lam L, Holland AJ. The impact of laser Doppler imaging on time to grafting decisions in pediatric burns. *J Burn Care Res* 2010;**31**:328–32.
73. Koller J, Orsag M. Our experience with the use of cerium sulphadiazine in the treatment of extensive burns. *Acta Chir Plast* 1998; **40**:73–5.
74. Konigova R, Konickova Z, Bouska I. Clinical forms of endotoxin in burns. *Scand J Plast Reconstr Surg* 1979;**13**:61–2.
75. Kraft R, Herndon DN, Al-Mousawi AM, et al. Burn size and survival probability in paediatric patients in modern burn care: a prospective observational cohort study. *Lancet* 2012;**379**: 1013–21.
76. Kucan JO, Smoot EC. Five percent mafenide acetate solution in the treatment of thermal injuries. *J Burn Care Rehabil* 1993;**14**: 158–3.
77. Lai KK, Kelley AL, Melvin ZS, et al. Failure to eradicate vancomycin-resistant enterococci in a university hospital and the cost of barrier precautions. *Infect Control Hosp Epidemiol* 1998;**19**: 647–52.
78. Larkin JM, Moylan JA. Tetanus following a minor burn. *J Trauma* 1975;**15**:546–8.
79. Law EJ, Blecher K, Still JM. Enterococcal infection as cause of morbidity and mortality in patients with burns. *J Burn Care Rehabil* 1994;**15**:236–9.
80. Lawrence JC, Cason JS, Kidson A. Evaluation of phenoxetol-chlorhexidine cream as prophylactic agent in burns. *Lancet* 1982; **24**:524–5.
81. Lesseva M, Girgitzova BP, Bojadjiev C. Beta-hemolytic streptococcal infections in burned patients. *Burns* 1994;**20**:422–5.
82. Linnemann Jr CC, MacMillan BG. Viral infections in pediatric burn patients. *Am J Dis Child* 1981;**135**:750–3.
83. Liu XS, Yang ZC, Luo ZH, et al. Clinical significance of the change in blood monocyte interleukin-1 production in vitro in severely burned patients. *Burns* 1994;**20**:302–6.
84. Ljunghusen O, Lundahl J, Nettelblad H, et al. Endotoxemia and complement activation after severe burn injuries—effect on leukocytes, soluble selectins, and inflammatory cytokines. *Inflammation* 1996;**20**:229–41.
85. Mackie DP, van Hertum WA, Schumburg T, et al. Prevention of infection in burns: preliminary experience with selective decontamination of the digestive tract in patients with extensive injuries. *J Trauma* 1992;**32**:570–5.
86. Mahar P, Padiglione AA, Cleland H, et al. *Pseudomonas aeruginosa* bacteraemia in burns patients: risk factors and outcomes. *Burns* 2010;**36**:1228–33.
87. Maldonado MD, Venturoli A, Franco A, et al. Specific changes in peripheral blood lymphocyte phenotype from burn patients: probable origin of the thermal injury–related lymphocytopenia. *Burns* 1991;**17**:188–92.
88. McManus AT, Mason Jr AD, McManus WF, Pruitt Jr BA. A decade of reduced gram-negative infections and mortality associated with improved isolation of burned patients. *Arch Surg* 1994;**129**:1306–9.
89. Mendelson JA. The management of burns under conditions of limited resources using topical aqueous Sulfamylon (mafenide) hydrochloride spray. *J Burn Care Rehabil* 1997;**18**:238–44.
90. Mian EU, Gianfaldoni R, Mian M. The use of intravenous immunoglobulins in patients with severe burns. *Clin Ther* 1992; **141**:75–81.
91. Mills II DC, Roberts LW, Mason Jr AD, et al. Suppurative chondritis: its incidence, prevention, and treatment in burn patients. *Plast Reconstr Surg* 1988;**82**:267–76.
92. Moncrief JA. Topical antibacterial treatment of the burn wound. In: Artz CP, Moncrief JA, Pruitt Jr BA, editors. *Burns: a team approach*. Philadelphia: W. B. Saunders; 1979. p. 250–69.
93. Moore EC, Padiglione AA, Wasiak J, et al. *Candida* in burns: risk factors and outcomes. *J Burn Care Res* 2010;**31**:257–63.
94. Moore Jr FD, Davis CF. Monocyte activation after burns and endotoxemia. *J Surg Res* 1989;**46**:350–4.
95. Moritz AR, Henrique Jr FC. Studies of thermal injury: the relative importance of time and surface temperature in the causation of cutaneous burns. *Am J Pathol* 1947;**23**:695–720.
96. Munster AM. Alterations of the host defense mechanism in burns. *Surg Clin North Am* 1970;**50**:1217–25.
97. Munster AM, Moran KT, Thupari J, et al. Prophylactic intravenous immunoglobulin replacement in high-risk burn patients. *J Burn Care Rehabil* 1987;**8**:376–80.
98. Nagoba BS, Gandhi RC, Hartalkar AR, et al. Simple, effective and affordable approach for the treatment of burns infections. *Burns* 2010;**36**:1242–7.
99. Neely AN, Smith WL, Warden GD. Efficacy of a rise in C-reactive protein serum levels as an early indicator of sepsis in burned children. *J Burn Care Rehabil* 1998;**19**:102–5.
100. Noelle RJ, Snow EC. T helper cell–dependent B cell activation. *FASEB J* 1991;**5**:2770–6.
101. Obritsch MD, Fish DN, MacLaren R, et al. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. *Pharmacotherapy* 2005;**25**: 1353–64.
102. Ono Y, Kunii O, Suzuki H, et al. Opsonic activity of sera and blister fluid from severely burned patients evaluated by a chemiluminescence method. *Microbiol Immunol* 1994;**38**:373–7.
103. Ong YS, Samuel M, Song C. Meta-analysis of early excision of burns. *Burns* 2006;**32**:145–50.
104. Organ BC, Antonacci AC, Chio J, et al. Changes in lymphocyte number and phenotype in seven lymphoid compartments after thermal injury. *Ann Surg* 1988;**210**:78–89.

105. Ostlie DJ, Juang D, Aguayo P, et al. Topical silver sulfadiazine vs collagenase ointment for the treatment of partial thickness burns in children: a prospective randomized trial. *J Pediatr Surg* 2012; **47**:1204–7.
106. Palmieri TL, Caruso DM, Foster KN, et al. Effect of blood transfusion on outcome after major burn injury: a multicenter study. *Crit Care Med* 2006;**34**:1062–7.
107. Palumbo A, Forte GI, Pileri D, et al. Analysis of IL-6, IL-10 and IL-17 genetic polymorphisms as risk factors for sepsis development in burned patients. *Burns* 2012;**38**:208–13.
108. Parks DH, Linares HA, Thompson PD. Surgical management of burn wound sepsis. *Surg Gynecol Obstet* 1982;**153**:374–6.
109. Pruitt BA, Foley FD. The use of biopsies in burn patient care. *Surgery* 1973;**73**:887–97.
110. Reardon CM, Brown TP, Stephenson AJ, et al. Methicillin-resistant *Staphylococcus aureus* in burns patients—why all the fuss? *Burns* 1998;**24**:393–7.
111. Rennekampff HO, Hamprecht K. Cytomegalovirus infection in burns: a review. *J Med Microbiol* 2006;**55**:483–7.
112. Renz BM, Sherman R. Exposure of buttock burn wounds to stool in scald-abused infants and children: stool-staining of eschar and burn wound sepsis. *Am Surg* 1993;**59**:379–83.
113. Ridgway EJ, Allen KD. Clustering of group A streptococcal infections on a burn unit: important lessons in outbreak management. *J Hosp Infect* 1993;**25**:173–82.
114. Rodgers GL, Fisher MC, Lo A, et al. Study of antibiotic prophylaxis during burn wound débridement in children. *J Burn Care Rehabil* 1997;**18**:342–6.
115. Rutan RL, Bjarnason DL, Desai MH, et al. Incidence of HIV seroconversion in paediatric burn patients. *Burns* 1992;**18**:216–9.
116. Sasaki TM, Welch OW, Herndon DN, et al. Burn wound manipulation induced bacteremia. *J Trauma* 1979;**19**:46–8.
117. Schlager T, Sadler J, Weber D, et al. Hospital-acquired infections in pediatric burn patients. *South Med J* 1994;**87**:481–4.
118. Schluter B, Konig W, Koller M, et al. Differential regulation of T- and B-lymphocyte activation in severely burned patients. *J Trauma* 1991;**31**:239–46.
119. Sheridan RL, Weber JM, Budkevich LG, et al. Candidemia in pediatric patients with burns. *J Burn Care Rehabil* 1995;**16**: 440–3.
120. Sherman RT. The prevention and treatment of tetanus in the burn patient. *Surg Clin North Am* 1970;**50**:1277–81.
121. Shilling JA. Burn healing. *Physiol Rev* 1968;**48**:374–423.
122. Shiranin KZ, Pruitt Jr BA, Mason Jr AD. The influence of inhalation injury and pneumonia on burn mortality. *Ann Surg* 1987; **205**:82–7.
123. Shoup M, Weisenberger JM, Wang JL, et al. Mechanisms of neutropenia involving myeloid maturation arrest in burn sepsis. *Ann Surg* 1998;**228**:112–22.
124. Stein JM, Pruitt Jr BA. Suppurative thrombophlebitis: a lethal iatrogenic disease. *N Engl J Med* 1970;**282**:1452–5.
125. Strock LL, Lee M, Rutan RL, et al. Topical bactroban (Mupirocin): efficacy in treating MRSA wounds infected with methicillin-resistant staphylococci. *J Burn Care Rehabil* 1990;**11**:454–9.
126. Stuttmann R, Hebebrand D, Hartert M, et al. [Prevention with pseudomonas immune globulin in burn injury patients with inhalation trauma: Does it have an effect on lung function and outcome]? *Klin Wochenschr* 1991;**69**(Suppl. 26):S168–77.
127. Teplitz C. The pathology of burns and the fundamentals of burn wound sepsis. In: Artz CP, Moncrief JA, Pruitt Jr BA, editors. *Burns—a team approach*. Philadelphia: WB Saunders; 1979. p. 45.
128. Tredget EE, Shankowsky HA, Groeneveld A, et al. A matched-pair, randomized study evaluating the efficacy and safety of Acticoat silver-coated dressing for the treatment of burn wounds. *J Burn Care Rehabil* 1998;**19**:531–7.
129. Tredget EE, Shankowsky HA, Joffe AM, et al. Epidemiology of infections with *Pseudomonas aeruginosa* in burn patients: the role of hydrotherapy. *Clin Infect Dis* 1992;**15**:941–9.
130. Utoh J, Utsunomiya T, Imamura T, et al. Complement activation and neutrophil dysfunction in burned patients with sepsis—a study of two cases. *Jpn J Surg* 1989;**19**:462–7.
131. van Rijn RR, Kuijper EC, Kreis RW. Seven-year experience with a "quarantine and isolation unit" for patients with burns: a retrospective analysis. *Burns* 1997;**23**:345–8.
132. Vindenes HA, Bjerknes R. Impaired actin polymerization and depolymerization in neutrophils from patients with thermal injury. *Burns* 1997;**23**:131–6.
133. Wahl WL, Ahrns KS, Brandt MM, et al. Bronchoalveolar lavage in diagnosis of ventilator-associated pneumonia in patients with burns. *J Burn Care Rehabil* 2005;**26**:57–61.
134. Wang XQ, Kravchuk O, Kimble RM. A retrospective review of burn dressings on a porcine burn model. *Burns* 2010;**36**:680–7.
135. Waymack JP, Jenkins ME, Alexander JW, et al. A prospective trial of prophylactic intravenous immune globulin for the prevention of infections in severely burned patients. *Burns* 1989;**15**:71–6.
136. Weber JM, Sheridan RL, Pasternack MS, et al. Nosocomial infections in pediatric patients with burns. *Am J Infect Control* 1997; **25**:195–201.
137. Weinbren MJ. Review: Pharmacokinetics of antibiotics in burn patients. *J Antimicrob Chemother* 1999;**44**:319–27.
138. Weintrub WH, Lilly AB, Randolph JG. A chickenpox epidemic in a pediatric burn unit. *Surgery* 1974;**76**:490–4.
139. Williams FN, Herndon DN, Hawkins HK, et al. The leading causes of death after burn injury in a single pediatric burn center. *Crit Care* 2009;**13**(6):R183.
140. Winkelman MD, Galloway PG. Central nervous system complications of thermal burns: a postmortem study of 139 patients. *Medicine (Baltimore)* 1992;**71**:271–83.
141. Woolfrey BF, Fox JM, Quall CO. An evaluation of burn wound quantitative microscopy, part I: quantitative eschar cultures. *Am J Pathol* 1981;**75**:532–7.
142. Yoon J, Urban C, Terzian C, et al. In vitro double and triple synergistic activities of Polymyxin B, imipenem, and rifampin against multidrug-resistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2004;**48**:753–7.
143. Zhou D, Munster AM, Winchurch RA. Inhibitory effects of interleukin 6 on immunity: possible implications in burns. *Arch Surg* 1992;**127**:65–8.

SECTION XV

UNCLASSIFIED INFECTIOUS DISEASES

CHAPTER 77

Kawasaki Disease

Stanford T. Shulman

Kawasaki disease is a multisystem acute febrile vasculitic syndrome of presumably infectious origin that affects predominantly infants and young children. The diagnosis is based on characteristic clinical features (Table 77-1). Serious complications include coronary arteritis, coronary artery aneurysms and stenoses, coronary thrombosis leading to myocardial infarction, and, very rarely, rupture of a coronary aneurysm. Kawasaki disease has become the leading cause of acquired heart disease in children in most developed countries including the United States and Japan.[386,387] It has been reported in children of all racial groups and from all continents. Although the etiology of Kawasaki disease remains unknown and a specific diagnostic test is lacking, establishing a timely diagnosis is very important because administration of intravenous immunoglobulin (IVIG) and aspirin before the 10th day of illness generally has a dramatic effect on the clinical manifestations and markedly reduces the likelihood of development of coronary abnormalities (see the later sections on treatment).*

Synonyms for Kawasaki disease include *Kawasaki syndrome* and *mucocutaneous lymph node syndrome* (MCLS, MLNS, or MCLNS). The disease also has been referred to as *lymphomucocutaneous syndrome*. An earlier term used in autopsy reports was *infantile periarteritis nodosa* (IPAN), which is pathologically indistinguishable from fatal Kawasaki disease. The International Classification of Diseases (ICD-9) designates the condition as both Kawasaki disease and mucocutaneous lymph node syndrome (acute) (febrile) (infantile) under rubric 446.1. Before 1983, the National Library of Medicine listed Kawasaki disease publications under various subject headings, particularly "Lymphatic Diseases." Since 1984, publications have been listed under "Mucocutaneous Lymph Node Syndrome," a name that has been replaced by Kawasaki disease.

HISTORY

The illness now bearing his name was first recognized as a clinical entity in 1961 by Dr. Tomisaku Kawasaki, who subsequently became chairman of the Department of Pediatrics at Tokyo's Japan Red Cross Medical Center. Between 1961 and 1967, Kawasaki identified 50 infants and young children who manifested a distinctive constellation of signs that included prolonged high fever, unilateral cervical lymphadenopathy, bilateral conjunctival injection, a polymorphous erythematous rash, changes of the mucosa of the upper respiratory tract, and edema and erythema of the extremities, with subsequent desquamation of the finger and toes. Although the syndrome was distinctive, its signs were nonspecific. Laboratory tests ruled out other disorders. A series of the first seven cases was presented by Kawasaki at the 61st Chiba General Meeting of the Japan Pediatric Society in 1962.[163] Convinced that he was observing a distinct clinical syndrome, Kawasaki published a report of his experience with 50 cases of "febrile oculo-oro-cutaneo-acrodesquamatous syndrome with or without acute nonsuppurative cervical lymphadenitis" in 1967.[164,165,167] Other Japanese physicians quickly recognized the syndrome after Kawasaki's report, although considerable discussion ensued about whether it was a distinct entity or an illness related to Stevens-Johnson syndrome.[166]

Cardiac involvement in this illness was suspected first in 1968, when Yamamoto and Kimura reported an infant with Kawasaki disease who had transient tachycardia with a gallop rhythm, cardiomegaly, and minor electrocardiographic (ECG) abnormalities.[422] In 1970, Kawasaki obtained funding to establish the Research Committee of Mucocutaneous Lymph Node Syndrome, sponsored by the Japanese Ministry of Health and Welfare, which was organized with Dr. Fumio Kosaki as chair.[167] In the first national survey of this committee, four autopsied and six nonautopsied cases of children who had died of coronary artery complications after apparent Kawasaki disease were identified.[182] These children were predominantly younger than 2 years of age and had died suddenly within 30 days of onset of disease, with evidence of coronary aneurysms and acute thrombosis. The first biennial Japanese national epidemiologic survey was conducted by this committee in 1970 under the leadership of Dr. I. Shigematsu and was published in 1972.[336] By this time, it had become well established that some patients who recovered apparently uneventfully from this acute illness were at risk for sudden cardiac death, with findings of acute myocardial infarction secondary to thrombosis within coronary arteries damaged by a severe vasculitic process.[182]

In 1971, physicians at the University of Hawaii who were unaware of the Japanese experience began to recognize patients with an unusual Reiter syndrome–like illness. When information about Kawasaki disease was published in the English-language literature,[167] the illness in Hawaii was recognized clearly as Kawasaki disease. Information exchanged between Japanese and U.S. investigators led to the 1974 publication of English-language articles by both groups[167,228,229] and triggered worldwide recognition of cases. In the early 1970s, death

*References 104, 247, 257, 260-262, 341, 389.

TABLE 77-1 Diagnostic Criteria for Classic or Typical Kawasaki Disease

Fever for at least 5 days*
plus
Presence of four of the following features:
Bilateral conjunctival injection
Polymorphous exanthem
Changes in the lips and oral cavity (erythema, cracking of lips; oropharyngeal erythema; strawberry tongue)
Peripheral extremity changes (erythema and swelling of hands and feet; later periungual desquamation, Beau lines)
Cervical lymphadenopathy (≥1.5 cm in diameter)
Exclusion of other diseases with similar features

Note: The finding of fever plus three criteria in the presence of coronary abnormalities qualifies.
*In the presence of classic features, experienced clinicians may be able to establish the diagnosis before the fifth day of illness.

from myocardial infarction was reported to occur in approximately 2 percent of cases of Kawasaki disease; more recent data reflect much lower mortality rates of less than 0.02 percent.[124,251,408,428] Seven deaths were recorded in Japan among 47,067 patients in 2007 to 2010 (0.014%). With the availability of echocardiography in the late 1970s, researchers determined that 20 to 25 percent of patients developed evidence of coronary artery abnormalities.[153]

In the decades before Kawasaki recognized the clinical features of the illness, many individual reports of fatal coronary arteritis in children (usually labeled IPAN) were published in the non-Japanese pediatric and pathology literature.* Clinical details of these cases generally are highly suggestive of Kawasaki disease, and the pathologic features of IPAN are indistinguishable from those of Kawasaki disease, as demonstrated conclusively by Landing and Larson in 1977.[189] Almost 100 years before Kawasaki's description was published, Samuel Gee of St. Bartholomew's Hospital in London in 1871 reported the case of a 7-year-old boy who at death ("following scarlatinal dropsy") had three coronary aneurysms, each filled with a fresh clot; histologic examination of that patient's cardiac tissue is compatible with inactive Kawasaki disease with extensive coronary myointimal proliferation and fibrosis.[108,345] Shibuya and colleagues retrospectively identified cases of illnesses compatible with Kawasaki disease that occurred in Japan up to 2 decades before Kawasaki's description was published.[335] Likely, patients with Kawasaki disease in previous decades were misdiagnosed as having measles, scarlet fever, rubella, or other once common conditions, and reductions in the numbers of cases of those illnesses helped to facilitate recognition of Kawasaki disease.[166]

Kawasaki's clinical description of the syndrome has remained the foundation of diagnosis and the basis of the clinical and epidemiologic case definitions in use today (see Table 77-1). In 2004, the American Heart Association Committee on Rheumatic Fever, Bacterial Endocarditis, and Kawasaki Disease published guidelines for the management of patients with incomplete (or atypical) Kawasaki disease (see later).[262]

*References 55, 83, 242, 284, 298, 305.

EPIDEMIOLOGY

Sources of Epidemiologic Data

As with most notifiable diseases, passive reporting of Kawasaki disease cases is incomplete. Passive surveillance data may help to monitor secular trends and to identify epidemics, but these data are of little value in estimating the true incidence of disease. Outbreak investigations are more sensitive in determining local disease incidence, and they enable investigators to study potential risk factors.

In the absence of a confirmatory diagnostic test, the epidemiologic case definitions of Kawasaki disease are relatively strict and exclude from surveillance data other exanthematous conditions that could dilute "true" cases and thus could obscure secular trends. However, the original epidemiologic case definitions were not intended for clinical application, a very important point since effective treatment became available. Thus, less strict application of clinical case criteria is appropriate for management of patients. Clinicians must be aware that children often present with clinical illnesses that do not completely fulfill the diagnostic criteria for Kawasaki disease but who are nonetheless at risk for developing coronary artery sequelae and therefore warrant therapy. These patients generally are considered to have *incomplete* or *atypical* Kawasaki disease.[28,96,262,317] Incomplete presentations of Kawasaki disease are particularly common occurrences in young infants in whom clinical signs often are subtle or fleeting but who are at the highest risk for development of coronary artery abnormalities.[42,309] In the United States, the Centers for Disease Control and Prevention (CDC) case definition usually is used for epidemiologic purposes and the American Heart Association published an algorithm to aid in the diagnosis of incomplete (atypical) Kawasaki disease.[262] The current Japanese diagnostic guidelines were revised for the fifth time and also reflect the importance of incomplete cases.[17]

Incidence Rates

The incidence of Kawasaki disease varies throughout the world and reflects primarily the racial composition of various countries. Rates in Japan have climbed steadily, with an annual rate of 239.6 per 100,000 children younger than 5 years of age in 2010 (21st national survey).[408] The highest reported rate in Korea was 118 per 100,000 children younger than 5 years old in 2007.[134] In countries with predominantly non-Asian populations, the rate is 15 to 20 per 100,000 children younger than 5 years of age.[133]

Gender

In virtually all population-based studies in many countries, the ratio of male-to-female patients with Kawasaki disease approximates 1.5 : 1.[408,428,430] In addition, serious

and fatal complications also are significantly more common findings among male patients with Kawasaki disease compared with female patients.[251,429] Examination of fatal Japanese cases indicated almost three times as many Kawasaki-related fatalities among male patients compared with female patients, with a higher ratio in infancy.[251,408] The basis for the preponderance of Kawasaki disease in male patients and for the even greater predominance of serious coronary artery disease in male patients with Kawasaki disease remains unclear. Of interest is that a male predominance is observed in many infectious diseases.

Race or Ethnic Background

The first cases of Kawasaki disease were recognized in Japanese children and in Hawaiian children of predominantly Japanese ethnicity, and subsequent data consistently supported higher rates in those of Asian background. Annual incidence rates in Japan have climbed steadily to 239.6 cases per 100,000 children younger than 5 years of age in 2010,[408] from 102.6 in 1995 and 184.6 in 2005 to 2006,[249] and are the highest in the world, approached only by rates for Japanese-American children in Hawaii (210.5/100,000 < age 5 years).[132] More than 12,700 cases of Kawasaki disease were reported in Japan in 2010, with local clusters rather than nationwide epidemics, as were seen in 1979, 1982, and 1985 to 1986.[408] In epidemic years in Japan, the annual age-specific incidence rates reached or exceeded 200 per 100,000 children younger than 5 years of age.[424] Incidence rates in white children in many communities are much lower, most often approximating 10 to 20 per 100,000 children younger than 5 years.[64,133,348] Surveys in countries with almost exclusively white populations often yield rates of 5 to 10 cases per 100,000 children younger than 5 years of age.* In Washington state, ethnic group–specific incidence rates per 100,000 children younger than 5 years of age were estimated to be 33.3 for Asian Americans, 23.4 for blacks, and 12.7 for whites.[64] In Hawaii, with its complex racial-ethnic makeup, the overall annual incidence is about 45 per 100,000 children younger than 5 years of age.[132] The yearly incidence for Japanese children in Hawaii approaches 200 cases per 100,000 children, and for whites it is 35 per 100,000, with intermediate rates for those of native Hawaiian and Chinese, Filipino, and other Asian ancestry.[132] Extrapolation of data from surveys of U.S. hospitals with large children's services led to estimates of approximately 2500 cases per year in the United States from 1984 to 1993,[386,387] with more recent estimates as high as 5000 cases annually.[35] A study of Kawasaki disease in the United States identified approximately 4200 hospitalizations in the year 2000, with highest rates among Asian and Pacific Islanders, lowest rates in whites, and intermediate rates in blacks and Hispanics.[133]

Age

Kawasaki disease occurs almost exclusively in children. In the United States and Japan, adult cases are quite rare, although some reports of adults diagnosed by accepted diagnostic criteria have been published.[195] Many of these adult patients have been infected with human immunodeficiency virus (HIV).* Most of the early reported adult cases, however, probably actually represented toxic shock syndrome or drug hypersensitivity reactions. Perhaps the best-documented adult case (confirmed by Dr. Kawasaki) was that of a 31-year-old Japanese man who later developed bilateral coronary artery aneurysms.[365] Because the signs and symptoms are nonspecific, adults suspected to have Kawasaki disease should be evaluated carefully for infectious, toxic, and other possible causes of illness.

The distribution of Kawasaki disease by age in childhood is highly characteristic. The disease occurs most frequently in young children: 50 percent are younger than 2 years of age, 80 percent are younger than 5 years of age, and cases seldom occur in those older than 12 years of age.[133,357,428] In 2009 to 2010, 0.7 percent of Japanese patients with Kawasaki disease were 10 years old or older.[408] Infants in the first 3 to 5 months of life have a relatively low incidence of Kawasaki disease, but the incidence rises rapidly from that point.[408] In the United States, the peak age is 15 to 18 months, whereas more recent data from Japan identified 25 percent as younger than 1 year old, 88 percent as younger than 5 years, and the peak age incidence as 6 to 11 months of age.[408] In Hawaii, 29 percent of cases are in infants younger than 1 year old and 85 percent of patients are younger than 5 years.[133] The lower peak age in Japan and perhaps in Hawaii may reflect better recognition of Kawasaki disease in infants, in whom Kawasaki disease may be more difficult to diagnose, or it may indicate a biologic difference. The age-incidence curve may be helpful in elucidating risk factors for developing Kawasaki disease. Such a pattern is compatible with highly transmissible infectious agents, particularly respiratory agents, and suggests possible transplacental immunity. The features of 28 patients with Kawasaki disease who were aged 8 years and older at the time of diagnosis at Children's Memorial Hospital in Chicago have been reported.[357] Delays in establishing the diagnosis and in providing treatment were common occurrences and were at least partially related to the prominence of arthritic and gastrointestinal symptoms in this population. I recently cared for a 19-year-old woman with classic Kawasaki disease.

Earlier Japanese mortality data suggested that fatality rates are approximately three times higher in children younger than 1 year old at the time of onset of disease, compared with older children, and that fatalities occur predominantly in the first several months after onset of Kawasaki disease.[427] The overall mortality rate in Japan has dropped from the initial report of approximately 2 percent to more recent Japanese estimates of approximately 0.01 percent.[408] Male patients account for a disproportionate number of deaths in infants and older children.[124,253,408,427] Long-term mortality rates in a large Japanese Kawasaki disease cohort study were increased over background rates, particularly during and shortly after the acute stage of illness.[251,253]

*References 29, 32, 47, 125, 290, 354.

*References 12, 43, 46, 82, 113, 148, 233, 293, 297, 331.

A Taiwanese series of 25 infants who developed Kawasaki disease at 3 months or younger included 19 males and 6 females. Only 6 infants met Kawasaki disease diagnostic criteria, with the other 19 classified as incomplete. Coronary artery involvement was noted in 80 percent, although only one infant had persistent abnormalities at 1 year follow-up.[52]

Recurrent Kawasaki Disease

A recurrence of Kawasaki disease is defined generally as a new episode of illness meeting clinical criteria for Kawasaki disease that begins at least 3 months after the initial episode and after inflammatory markers such as the erythrocyte sedimentation rate or serum C-reactive protein level have normalized. The frequency of recurrences after first cases of Kawasaki disease in Japan was estimated to be approximately 1.9 percent during a 3-year follow-up period, with approximately 0.07 percent of patients experiencing a third episode.[250] This corresponds to a rate of 5.21 per 1000 person-years for one or more recurrences.[250] With longer follow-up, recurrence rates in Japan may approach 3 percent.[429] Data from the United States suggest a recurrence rate of 1 to 2 percent.[23] Recurrences occur most frequently within the first 2 years after the initial episode, especially in male patients and in children who have their initial episode before reaching their second birthday.[250] The frequency of recurrences in Chicago appears to be approximately 1 percent, occurring more often in Asian children, and recurrences were documented in about 2 percent of Hawaiian cases from 1996 to 2000,[132] a finding likely reflecting a racial difference. The true recurrence rate will be determined only when a specific diagnostic test becomes available for Kawasaki disease and thus minimizes recognition bias.

Family Cases

Simultaneous or sequential cases of Kawasaki disease in siblings, twins, or other family contacts also have been reported, particularly during outbreaks in Japan.[91,139,221,430] Japanese epidemiologists have documented secondary sibling cases in approximately 1 percent of cases, a rate that is approximately 10 times greater than that in the general child population.[91] However, such figures are difficult to interpret because they may be influenced by recognition and reporting biases. Sibling cases are reported to occur more frequently in twins than in nontwins. Only three sibling pairs were recognized in approximately 1900 Chicago patients, and none were reported in 400 children in Los Angeles.[219] A report from two U.S. medical centers noted 18 families with multiple affected Kawasaki disease patients, including 9 families with 2 affected siblings and 9 with Kawasaki disease in two generations or in multiple affected members.[68]

Epidemics and Outbreaks

Japanese investigators noted large nationwide outbreaks of Kawasaki disease in 1979, 1982, and 1985 to 1986, with wavelike spread occurring from one prefecture to the next, suggesting an infectious origin.[252,423,425] The 1982 Japanese epidemic started simultaneously in four areas and spread outward from each region, similar to the way that epidemic influenza spreads in Europe and America.[252,423] In the 1985 to 1986 Japanese epidemic, investigators identified epidemic "waves" that spread outward from an initial focus in the Tokyo metropolitan area and extended simultaneously northward and southward to involve most of the country within approximately 4 months.[252,423,425] A similar but less distinctive pattern of interprefectural progression in waves was noted in the 1982 Japanese epidemic. Within the northern Tohoku District, for example, Kawasaki disease spread from prefecture to prefecture over a period of approximately 7 months.[423] Korean epidemics were detected 7 and 15 months after the Japanese epidemics of 1979 and 1985 to 1986, respectively.[194,423] More localized outbreaks also have been observed. In the United States and elsewhere, community-wide outbreaks were documented beginning in 1977.[24,398] Investigation of outbreaks provides opportunities to study potential risk and etiologic factors. Clustering of cases within families, schools, or neighborhoods is quite unusual, even during large-scale epidemics. Japanese investigators have associated epidemics with a significantly increased likelihood of second cases occurring in families, fatalities, and recurrent cases, but the implications of these findings are uncertain. It is striking that since 1986 no further nationwide Japanese outbreaks have been identified, suggesting that the epidemiology of Kawasaki disease may have changed.[327,432]

Geography

Kawasaki disease has been diagnosed throughout the United States and Japan and in virtually all developed and many developing countries on all continents, including temperate and tropical zones.* No striking rural-urban differences have been noted. Elevation, longitude, and latitude have not been implicated. Travel histories of patients are unremarkable, although an anecdotal report of a 7-month-old infant in Australia documented Kawasaki disease onset 17 days after leaving Japan during that country's 1982 epidemic.[354]

Seasonality

In Japan, Kawasaki disease occurs year round but is most prevalent in the winter, with peaks usually occurring in December or January, with a lower peak in June, and the lowest number of cases in October.[36,409,426,429] In Korea, peaks were observed in summer and winter,[288] in Taiwan in summer,[138] in Beijing and Shanghai China in spring and summer,[74] and in spring in Sichuan and Hong Kong.[211,263] In the United States and other temperate areas, the number of cases peaks in the winter and early spring and is lowest in late summer; nonetheless, cases occur throughout the year.[23,25,133,219,299] No clear seasonality has been seen in Hawaii.[132] Winter predominance has been observed in at least some Southern Hemisphere countries. That sporadic cases are recognized year round

*References 32, 47, 84, 215, 294, 323, 325, 397.

is somewhat different from the pattern usually observed with highly transmissible respiratory viral diseases, incidences of which peak sharply in the winter and spring (e.g., measles, rubella, influenza).

Communicability

Although little direct evidence exists that Kawasaki disease is transmissible from person to person, considerable circumstantial evidence supports an infectious origin. Secondary or co-primary cases in families occur but are not common. Several outbreak investigations found a higher rate of antecedent respiratory tract illness in patients with Kawasaki disease compared with matched controls.[24,375] This finding is of particular interest in view of the finding that immunoglobulin A (IgA) plasma cells infiltrate the proximal respiratory tract and that viral-like inclusion bodies are observed in bronchial epithelial cells in fatal cases of Kawasaki disease, and it strongly supports a respiratory mode of spread of an inciting agent (see later).[134,318] Japanese family data suggest that sibling cases cluster either on the same day as the index case or 7 days later.[91] Because these results are based on questionnaire data, however, the possibility of ascertainment bias is great; thus, determining the degree to which Japanese familial cases represent co-primary or secondary cases is difficult.

Other Risk Factors

In addition to demographic risk factors for Kawasaki disease, specific exposures that could be related to an etiologic agent have been linked to the disorder by older epidemiologic investigations. History of more frequent recent antecedent respiratory illnesses in cases compared with controls was documented in the early 1980s.[24] Because many viral agents are prevalent in the winter and spring, when Kawasaki disease is most prevalent, some "background" isolation of various viruses from patients and from matched controls is expected.

Other past reported Kawasaki disease associations include exposure to recent carpet cleaning or shampooing, exposure to house dust mites, and residence near bodies of water; very few recent reports support these associations. The association of Kawasaki disease with exposure to recently cleaned or shampooed carpets was observed in some reports* but not in others.† The significance of a possible association with shampoo is unclear, because it is absent entirely in many well-studied outbreaks and because carpets are relatively uncommon in Japan.[62,238] Conceivably, rug shampooing could lead to aerosolization of a microbial or sensitizing agent present in the carpet, such as mites or microbial agents. A possible link between Kawasaki disease and house dust mites (chiefly *Dermatophagoides farinae* and *D. pteronyssinus*) was initially proposed as an allergic hypothesis.[105] Some investigators suggested that Kawasaki disease could result from an infectious agent in dust mites.[90] However, counts of dust mites were not significantly different in the houses of case and control patients,[114,141] and no evidence of increased anti-mite IgE, IgG, or antibodies to *D. pteronyssinus* was found in patients with Kawasaki disease.[114,149]

In a small number of outbreaks, investigators from the CDC found that patients with Kawasaki disease lived closer to "bodies of water" compared with control patients.[300,301] However, other studies, including one in Washington State, did not find living in proximity to water to be a risk factor.[64]

ETIOLOGY

The origin of Kawasaki disease remains unknown. However, clinical and epidemiologic features strongly suggest that the disease has an infectious cause. A self-limited, usually nonrecurring illness manifested by fever, rash, enanthem, conjunctival injection, swollen and red hands and feet, and cervical adenitis fits well with an infectious cause. The epidemiologic features noted earlier, including the striking age distribution (Fig. 77-1), winter-spring seasonality in most areas, occurrence of community outbreaks with wavelike geographic spread, and apparent epidemic cycles, resemble those of a transmissible disease of childhood. The laboratory features, including leukocytosis with a "left shift," elevated acute-phase reactants, and pyuria, also suggest infection. Vasculitis with inflammatory cell infiltration (including IgA plasma cells)[318] may be primarily infectious or may represent an immune reaction to infection.

A very attractive hypothesis is that Kawasaki disease is caused by a ubiquitous infectious agent that produces clinically apparent disease only in selected, genetically predisposed individuals, with particular predilection for Asians. Its rarity in the first few months of life and in older children and adults suggests an agent to which virtually all adults are immune and from which very young infants are protected to some degree by passive maternal antibody. Consistent with this hypothesis is the paucity of evidence of person-to-person spread of Kawasaki disease, because most infections are likely asymptomatic, and only very few of those infected likely develop clinical features of Kawasaki disease. This would be analogous to acute poliomyelitis, the relatively uncommon complication of poliovirus infection that occurs in only approximately 1 in 200 of those infected. However, efforts to identify an infectious agent of Kawasaki disease using conventional bacterial and viral culture and serologic methods, as well as inoculation of primates, mice, and guinea pigs, have failed to yield an infectious cause.[321,346]

Two major theories to explain the etiology of Kawasaki disease have gained prominence in the past decade: the specific respiratory pathogen theory[311] and the superantigen theory.[205] A very long list of microorganisms, including bacteria, leptospires, spirochetes, fungi, chlamydia, rickettsiae, and viruses, have been suggested at one time or another to cause Kawasaki disease, without confirmation.[321] *Yersinia*, particularly *Y. pseudotuberculosis*, causes a systemic illness resembling Kawasaki disease in selected areas of Japan,[328] but convincing evidence of an etiologic link between *Yersinia* and Kawasaki disease is lacking, despite the use of molecular tools.[378]

*References 27, 85, 114, 137, 274, 289, 299, 300.
†References 62, 214, 238, 299, 307, 308, 388.

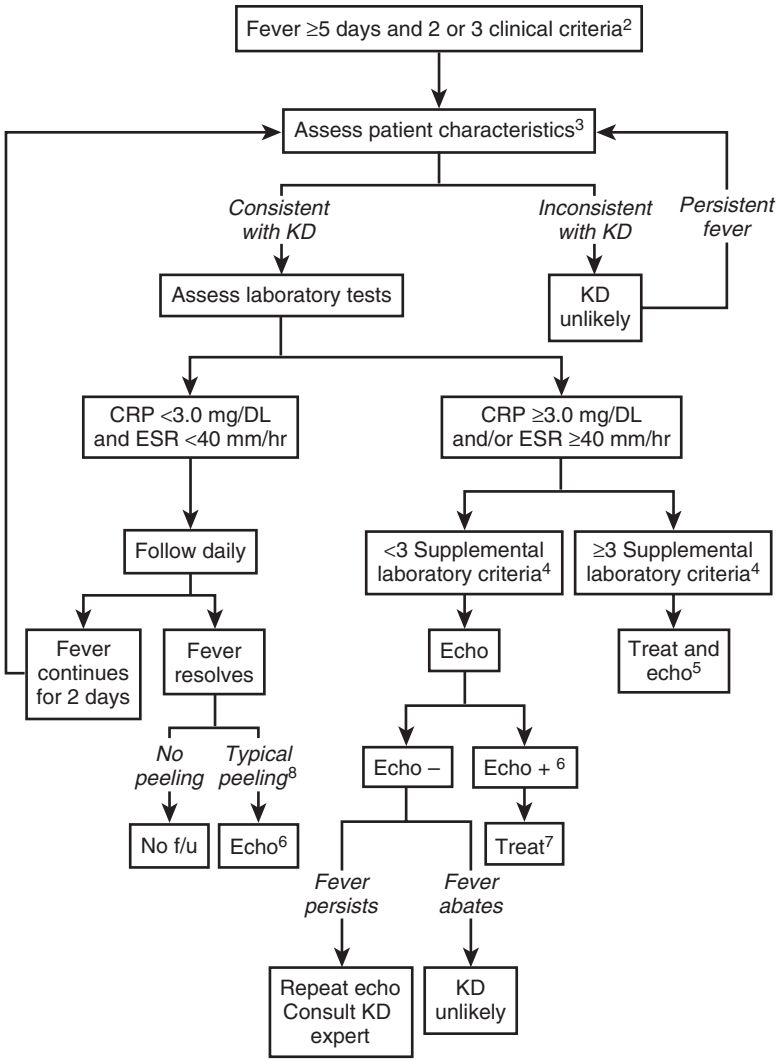

FIGURE 77-1 ■ Evaluation of suspected incomplete Kawasaki disease (KD). *1,* In the absence of a gold standard for diagnosis, this algorithm cannot be evidence based but rather represents the informed opinion of an expert committee. Consultation with an expert should be sought anytime assistance is needed. *2,* Infants up to 6 months old on day 7 or later of fever without any other explanation should undergo laboratory testing and, if evidence of systemic inflammation is found, an echocardiogram, even if the infants have no clinical criteria. *3,* Patient characteristics suggesting Kawasaki disease are listed in Table 77-1. Characteristics suggesting disease other than Kawasaki disease include exudative conjunctivitis, exudative pharyngitis, discrete intraoral lesions, bullous or vesicular rash, or generalized adenopathy. Consider alternative diagnoses (see Table 77-2). *4,* Supplemental laboratory criteria include albumin less than or equal to 3.0 g/dL, anemia for age, elevation of alanine aminotransferase value, platelet count after seventh day 450,000/mm^3 or greater, white blood cell count 15,000/mm^3 or higher, and urine specimen showing 10 or more white blood cells per high-power field. *5,* The patient can be treated before an echocardiogram is performed. *6,* Echocardiogram is considered positive for purposes of this algorithm if any of three conditions are met: Z score of left anterior descending (LAD) or right coronary artery (RCA) equal to 2.5 or greater, coronary arteries meet Japanese Ministry of Health criteria for aneurysms, or three or more other suggestive features exist, including perivascular brightness, lack of tapering, decreased left ventricular function, mitral regurgitation, pericardial effusion, or Z scores in LAD or RCA equal to 2.0 to 2.5. *7,* If the echocardiogram is positive, treatment should be given to children within 10 days of fever onset and to those beyond day 10 with clinical and laboratory signs (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) of ongoing inflammation. *8,* Typical peeling begins under nail bed of fingers and then toes. See the text for further details. f/u, follow-up. (From Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease. American Heart Association Scientific Statement. *Circulation* 2004;**110**:2747–71.)

That Kawasaki disease may be caused by a novel retrovirus was suggested when two groups reported reverse transcriptase activity in cultured peripheral blood mononuclear cells from patients with acute Kawasaki disease.[37,344] Other studies failed to confirm this finding,[230,278,319] although features resembling Kawasaki disease have been described in adults with HIV infection.[148]

The hypothesis that Kawasaki disease may be related to a bacterial superantigenic toxin was suggested because of possible selective expansion of $V\beta_2$ and $V\beta_8$ T-cell receptor families among circulating mononuclear cells.[1] Superantigens such as bacterial toxins bind to major histocompatibility complex (MHC) class II molecules on monocytes and B cells and to the T-cell receptor, with resultant activation of large numbers of immunoreactive

cells with release of inflammatory cytokines. Superantigens potentially cause expansion of autoreactive T cells.[127,183] Features of immune cell activation characterize the acute phase of Kawasaki disease. Toxic shock syndrome and Kawasaki disease share the features of fever, rash, conjunctival injection, and convalescent desquamation to some degree, but differences are present, such as the rarity of hypotension in Kawasaki disease and the absence of maculopapular or erythema multiforme–like rashes in toxic shock syndrome.[121,292] Reports of selective expansion of the $V\beta_2$ and $V\beta_8$ T-cell receptor families in acute Kawasaki disease,[1,2,58] were not confirmed by others.[3,266,295,400] Although 11 of 16 patients with Kawasaki disease and only 1 of 15 controls were colonized with toxic shock syndrome toxin I (TSST-I)–producing staphylococci,[205] *Staphylococcus aureus* colonization rates, including by TSST-I–producing staphylococci, were not significantly different among patients with Kawasaki disease and controls in other studies.[206,217,392] That most studies failed to show *any* disturbance of Vβ T-cell receptor expression in Kawasaki disease substantially challenges the superantigen hypothesis.[65]

More recent investigations indicate that the immune response in Kawasaki disease is oligoclonal (antigen driven, i.e., a response to a conventional antigen) rather than polyclonal (as typical in superantigen-driven responses), with both B-cell and T-cell oligoclonality demonstrated.[51,320] In a series of studies, Rowley and colleagues[314,318] demonstrated that IgA plasma cells infiltrate peribronchial, coronary arterial, and other tissues in patients who died of Kawasaki disease in the acute stage and that the IgA plasma cells are oligoclonal,[320] findings very strongly suggesting an immune response driven by a conventional agent. Using the techniques of modern molecular biology, Rowley and colleagues[311] synthesized oligoclonal antibodies from the IgA genes from a fatal acute-stage case of Kawasaki disease and used those antibodies to identify an antigen in acute Kawasaki disease (but not control) bronchial epithelial cells and in a subset of macrophages in inflamed acute Kawasaki disease tissues, including coronary arteries. This antigen was localized to distinctive perinuclear, primarily apical, intracytoplasmic "spheroidal bodies" in acute Kawasaki disease ciliated bronchial epithelium. Light microscopic and transmission electron microscopic studies showed that the bodies are homogeneous electron-dense perinuclear inclusion bodies up to 1.4 μg in diameter that contain both protein and RNA and are consistent with aggregates of viral proteins and RNA, possibly related to the etiologic agent of Kawasaki disease.[312] Electron microscopic studies strongly suggest the presence of viral-like particles associated with the inclusion bodies, and work is ongoing to identify the particles.[313,314]

Reports of an association between a novel human coronavirus (dubbed HCoV-NH but later shown to be HCoV-NL63) in 2005[81] were not confirmed in several additional studies from four countries.[22,48,71,78,337] Efforts to associate Kawasaki disease with exposure to drugs[135] or to such environmental pollutants as toxins, pesticides, chemicals, and heavy metals have failed. However, clinical similarities between Kawasaki disease and acrodynia (mercury poisoning) are notable.[16]

Some have suggested that Kawasaki disease may be an immunologic response triggered by any of several different microbial agents. Consistent with this hypothesis is documented infection by different microorganisms in different individual cases, failure to detect a single microbiologic or environmental agent after 4 decades of study, and analogies to other multifactorial syndromes (e.g., aseptic meningitis). However, this hypothesis is difficult to reconcile with the distinctive clinical and laboratory pictures of Kawasaki disease, with its epidemiologic features such as epidemics and wavelike geographic spread, and with the studies of Rowley and colleagues in which a single monoclonal antibody reacts with almost all tissues tested from Kawasaki disease patients.[311,312]

A new hypothesis suggests that the agent of Kawasaki disease may be carried from central Asia to Japan, Hawaii, and San Diego on dust particles, based on analysis of wind patterns and Kawasaki disease time-space clustering and seasonal occurrence.[306] This awaits supporting data.

Clearly, conventional culture and serologic methodologies have failed to yield the causative agent of Kawasaki disease. In addition, establishing the diagnosis can be difficult, particularly in young infants and in those with incomplete or atypical presentations.[28,42,317] A useful diagnostic test probably cannot be developed unless it is based on the etiologic agent, and more specific therapy and preventive efforts also await discovery of the cause of this disease. The hope is that the use of modern molecular biology techniques[311,312,316,320,343] will finally clarify the origin of Kawasaki disease, as well as provide insights into the mechanisms of disease pathogenesis.

Genetic Susceptibility

The higher rates of Kawasaki disease in those of Japanese and some other Asian backgrounds suggest a genetic basis rather than environmental factors, as supported by increased rates among third- and fourth-generation immigrants from Japan to Hawaii.[132] The increased incidence of Kawasaki disease among siblings and parents of patients also supports a genetic predisposition.[410,427] The genetic basis is complex, and no single human leukocyte antigen (HLA) or MHC class II gene is common to most patients with Kawasaki disease. Immunoglobulin allotypic markers were studied as possible genetic markers for Kawasaki disease, and differences between patients and race-matched controls were found in Japanese and whites, supporting a complex genetic basis for susceptibility to Kawasaki disease.[349] Transmission disequilibrium studies involving children with Kawasaki disease and their parents demonstrated evidence of the genetic basis of susceptibility, with Onouchi and colleagues identifying functional polymorphisms in the *inositol triphosphate kinase-3 (ITPKC)* gene on chromosome 19 and in *CASP3* on chromosome 4, which confer risk in Japanese and white children.[41,279,280]

Genome-wide association studies to define Kawasaki disease susceptibility risk loci have identified additional loci on chromosome 8 and other chromosomes.[33,281,418] Davila and colleagues, also in a genome-wide association study, identified a polymorphism in the *FCGR2A* gene on chromosome 1 related to immune activation as a

Kawasaki disease risk marker.[63] Several additional loci have been identified in the Taiwanese population.[418] The picture is emerging that there are more than 6 to 10 genetic susceptibility markers for Kawasaki disease, each of which is associated with a 30 to 40 percent increased risk for Kawasaki disease compared with controls. This is an area of intense research activity.

PATHOLOGY AND PATHOGENESIS

Relationship with Infantile Periarteritis Nodosa

When the first Japanese survey of Kawasaki disease in 1970 yielded reports of 10 deaths, the pathologic diagnosis was thought to be IPAN.[145,168,182] Japanese and Western investigators quickly recognized the pathologic similarities between fatal Kawasaki disease and IPAN, long known to have an unusual predilection for the coronary arteries.[4,20,21,381-384] In the most definitive study, Landing and Larson[189,193] showed clearly that IPAN and fatal Kawasaki disease were indistinguishable pathologically. Published case reports of autopsies of children with IPAN frequently contain clinical histories that include many or all features of acute Kawasaki disease and the development of severe vasculitis affecting primarily the coronary arteries.[305] The failure of some reported patients with IPAN to meet Kawasaki disease case criteria applied retrospectively is likely related to documentation bias and to the young age of many of the patients. Young infants with Kawasaki disease are less likely to have a classic presentation than are older children (see later). When pathologic and clinical criteria are combined, the two diseases appear indistinguishable. Adult-type periarteritis nodosa was described first in 1866 by Kussmaul and Maier,[188] and it differs from IPAN primarily by the presence of hypertension and involvement of small and medium muscular arteries, especially in the lung, kidney, and intestines[92,189,383,384] An early recorded case of possible IPAN dates from 1899.[185] In 1959, Munro-Faure[242] very clearly delineated a syndrome of infantile necrotizing arteritis with coronary artery involvement, fever, rash, conjunctival and pharyngeal infection, and cervical adenitis, distinct from classic periarteritis nodosa. Roberts and Fetterman[305] and others expanded on these observations to define a distinct clinicopathologic syndrome of IPAN shortly before Kawasaki recognized the clinical syndrome.[164,165]

Coronary artery aneurysms were described as early as the early 20th century, with a male-to-female ratio of roughly 3:1, including a male preponderance in childhood cases. Childhood death from multiple coronary artery aneurysms was known to occur at least as early as 1871, as reported by Samuel Gee.[108] The cardiac specimen from Gee's case, formalin fixed for more than 120 years in the pathology museum at St. Bartholomew's Hospital, London, recently was sectioned and examined histologically. The coronary arteries showed the characteristic histologic findings of inactive Kawasaki disease and IPAN.[345] Whether these early cases with childhood coronary artery aneurysms truly represented early examples of Kawasaki disease is not certain; details of the clinical histories usually were scant.[108,298] What is clear, however, is that most of these cases greatly resemble (and very likely do, in fact, represent) Kawasaki disease.[55,83,108,242,298] From the features of many such early case reports, a reasonably accurate picture of Kawasaki disease as recognized today emerges. It is highly likely that in the decades before the introduction of the measles vaccine in the 1960s, cases of Kawasaki disease were misdiagnosed as measles, scarlet fever, drug hypersensitivity, or other common conditions.

Pathologic Features of Kawasaki Disease

Cardiac death in Kawasaki disease generally occurs in the subacute or convalescent stages of illness but also can occur earlier.[251,253] Autopsy findings usually reveal evidence of active or inactive vasculitis, which is most severe in the medium-sized arteries, with a marked predilection for the coronary arteries.* Small arterioles, larger arteries, capillaries, and veins also are affected to a lesser extent.[9,92] In more than 80 percent of fatal cases in the acute stage, the immediate cause of death is acute thrombosis of inflamed coronary arteries, with resultant myocardial infarction. In a few early deaths, pancarditis with inflammation of the atrioventricular conduction system apparently caused a fatal arrhythmia or intractable congestive heart failure, whereas small numbers of other deaths have been associated with acute coronary rupture, usually within a few weeks of the onset of illness. Deaths that occur months to years after the acute episode of Kawasaki disease often are secondary to coronary stenosis, with or without acute thrombosis, resulting in myocardial ischemia. In some patients with coronary artery abnormalities (usually in those who also have quite severe coronary disease), aneurysms of other major medium-sized arteries, including the brachial, renal, and iliac arteries, also may be present. Although phlebitis may be found, vascular inflammation more typically and more severely affects medium-sized musculoelastic arteries in their extraparenchymal portions. In the acute stage of Kawasaki disease, systemic inflammatory changes also are evident in many other organs, including myocardium, pericardium, cardiac valves, cerebrospinal fluid, lung, lymph nodes, pancreas, spleen, joints, and liver.[10,92,236]

A recent extensive study by Orenstein and associates of hearts from 32 patients who died and 8 who received heart transplants sheds new light on the pathologic features of severely affected Kawasaki disease patients.[282] Three characteristic vasculopathic processes were identified by light and transmission electron microscopy: (1) acute self-limited necrotizing arteritis (NA), (2) subacute/chronic (SA/C) vasculitis, and (3) luminal myofibroblastic proliferation (LMP). NA is a neutrophilic process that starts at the endothelial lining of vessels only in the first 2 weeks of illness, progressively destroying the vessel wall and causing development of saccular aneurysms, which can thrombose or rupture. SA/C vasculitis begins within the first 2 weeks of illness in the adventitia/perivascular

*References 8, 9, 94, 189, 374, 383, 384.

tissue and damages the vessel wall during its progression toward the lumen. This can lead to fusiform and saccular aneurysms that can thrombose, and it is associated with transition of medial and adventitial smooth muscle cells into smooth muscle cell–derived myofibroblasts which with LMP leads to stenosing luminal lesions (SA/C-LMP). LMP can also occur in some areas without local SA/C vasculitis.[313] NA is the only self-limiting of these three processes, is responsible for the earliest morbidity and mortality, and is consistent with acute viral infection. SA/C vasculitis can begin early but can persist for months or even years, whereas LMP causes progressive arterial stenosis and thrombosis and is composed of unique smooth muscle cell–derived activated myofibroblasts.

In time, the vessel wall may become stenotic, and occlusion by stenosis or by superimposed thrombus can result. Calcification of thrombi may occur,[282] and intraluminal thrombus may become organized, remodeled, and recanalized.[8,255,327,370] Several groups investigated the mechanisms of vascular remodeling in Kawasaki disease. Suzuki and colleagues[371] reported that immunohistochemistry studies indicate that active remodeling continues for at least several years after the onset of Kawasaki disease, with luxuriant intimal proliferation and neoangiogenesis. Extensive expression of vascular growth factors, including vascular endothelial growth factor (VEGF) within smooth muscle cells, was evident particularly in newly formed microvessels in the intima.[371] Gavin and colleagues[107] showed the prominence of matrix metalloproteinases in this process, particularly within the intima and media. Suzuki and associates[372] found evidence of inflammatory changes in the intact coronary artery of a child who died 13 months after having Kawasaki disease and who had no evidence of coronary artery involvement during the acute stage.

Fujiwara and associates[93,95] identified significant atrioventricular conduction system lesions in 5 of 10 autopsy specimens, with a strong correlation found between ECG findings, especially PQ-segment prolongation, and acute inflammation of the atrioventricular conduction system. Severe acute changes were most pronounced at 21 to 31 days after onset. In a unique study of right ventricular endomyocardial biopsy specimens in 201 patients with uncomplicated acute Kawasaki disease, Yutani and associates[435,436] noted some degree of myocarditis and cellular infiltration, ranging from very mild (subclinical) to quite severe myocardial inflammation, in all of the patients studied.

A histologic-immunochemical study examined the intercostal arteries to compare the nature and developmental processes of lesions with those of the coronary arteries.[220] Although similar lesions were seen, the lesions appeared somewhat later in the intercostal arteries.

The precise nature of the inflammatory infiltrate in the vessel wall or myocardium has been studied. Terai and colleagues[390] demonstrated helper (CD4) T cells and monocytes/macrophages in the arterial wall of one patient with fatal acute Kawasaki disease, as well as expression of class II MHC antigen (an activation marker) in coronary vascular endothelium, findings supporting the importance of immune responses in this form of vasculitis. Terai and colleagues[393] also reported evidence of eosinophils in epicardial microvessel lesions in acute Kawasaki disease leading to death.

Rowley and associates[316] demonstrated the presence of many plasma cells of the IgA isotype in the vascular walls of patients who died in the acute stage. They also showed that the IgA plasma cells are oligoclonal (i.e., they appear to be responding in an antigen-driven process)[320] and that they can be observed also in peribronchial, pancreatic, and renal tissues.[318] A predominance of CD8 cytotoxic/suppressor T cells over CD4 helper T cells in seven of eight acute-stage deaths also was observed in my colleagues and my laboratory by immunohistochemistry.[31] Takahashi[375] reported $CD68^+$ monocytes and macrophages to be most prominent in acute Kawasaki disease, but $CD3^+$ cells (T cells), $CD20^+$ cells (B cells), and neutrophils also were present in autopsy specimens of children who died in the first month of the illness.

Other less commonly reported pathologic findings include renal infarcts and glomerular histologic changes, possible evidence of immune complex deposition,[322] including mesangial deposition of IgM and C3, and multifocal periglomerular infiltration of lymphocytes and IgA plasma cells.[318] Changes have been noted in noncoronary arteries, the thymus, and lymph nodes. Tanaka[381] described thymic atrophy and nondiagnostic lymph node changes at autopsy (although most patients likely had been treated with corticosteroids). Naoe and colleagues[256] reported that cervical lymph node biopsy specimens showed thrombotic arteriolitis, severe lymphadenitis with necrosis, and postcapillary venules with endothelial cell and reticular cell hyperplasia. Other reports described early lymph node biopsy specimens showing multiple foci of necrosis and fibrin thrombi within the microvasculature, as well as T-zone hyperplasia, B-zone macrophage infiltration, and immunoblast proliferation.[112] Rowley and associates[318] identified IgA plasma cells not only in vascular and renal tissues but also in pancreatic and peribronchial locations. Miura and colleagues[236] found prominent macrophage infiltration of pancreatic acini and islets in 3 of 10 fatal cases.

CLINICAL MANIFESTATIONS

Clinical Phases of Illness

The clinical course of Kawasaki disease can be divided into acute, subacute, and late or convalescent phases. The *acute* febrile phase begins with fever, rash, conjunctival injection, "strawberry" tongue, red swollen lips, edema and erythema of the hands and feet, cervical lymphadenitis, and sometimes aseptic meningitis and mild hepatic dysfunction. Young children often are quite irritable. Evidence of myocarditis, rarely including congestive heart failure or arrhythmias, may develop during this time. Pericardial effusion, mitral regurgitation, or depressed myocardial function may be detected by echocardiogram. Without IVIG and aspirin treatment, the acute phase generally lasts 8 to 30 days (mean, 11 days).

After defervescence, the physical findings rapidly disappear, but during this *subacute* phase, the child may remain irritable and anorectic, with decreased activity.

Some conjunctival injection may persist. Arthritis or arthralgia, mainly of larger joints, may develop in the subacute phase. Desquamation of fingers and toes, typically beginning in the periungual region, and thrombocytosis are very common manifestations during this period. The subacute phase persists until the child has returned to his or her normal state of health at 3 to 6 weeks after the onset of fever, with normalization of inflammatory markers. The time of greatest risk for sudden death occurring from acute coronary artery thrombosis in patients with coronary lesions is during the subacute phase and the early convalescent phase. The *convalescent* phase begins when all clinical signs and symptoms have disappeared and inflammatory markers are normal, usually at 6 to 8 weeks after onset.

When manifested completely, Kawasaki disease is a distinctive clinical entity with a fairly predictable course.[59,262] The principal clinical diagnostic criteria are presented in Table 77-1. A diagnosis of typical or classic Kawasaki disease, according to these accepted clinical criteria, is made in patients with fever and at least four of the five clinical criteria and with exclusion of other illnesses that mimic Kawasaki disease. Each of the five clinical features is present in 80 to 90 percent of patients with typical cases, except cervical lymphadenopathy, which is present in approximately 50 percent of patients. However, children who do not fulfill the classic criteria for diagnosis of Kawasaki disease, in fact, may have the illness and are at risk for developing complications, particularly coronary artery disease (see section on incomplete or atypical Kawasaki disease, later).[262,317] Kawasaki disease should be considered in the differential diagnosis of infants and children with fever for at least 5 days associated with two or more of the five classic features: (1) generalized polymorphous erythematous rash, (2) conjunctival injection, (3) characteristic changes of the lips and mouth, (4) bilateral redness and swelling of the hands and feet, and (5) unilateral nonfluctuant cervical lymph node enlargement greater than 1.5 cm.[262] All features are not necessarily present at the same time. The most commonly encountered diseases to be excluded are febrile exanthems, presumably viral, including measles; acute streptococcal and staphylococcal infections; and drug hypersensitivity reactions. Japanese diagnostic guidelines for Kawasaki disease are similar to the U.S. guidelines with rare exception: they consider fever and the other five major features to be six equal criteria and require at least five of those six criteria for diagnosis.[17] Thus, a child could have no fever but all five other criteria and be considered a typical case by the Japanese criteria.[16] The Japanese criteria also accept a course of fever lasting less than 5 days if it is shortened by early IVIG treatment.[17]

Certain scoring systems using clinical and laboratory features have been developed, primarily in Japan.[14,123,254] The initial goal of these systems was to identify those patients with Kawasaki disease at highest risk for development of coronary abnormalities and who, therefore, would benefit most from receiving IVIG therapy, as well as those at low risk for coronary changes who could be spared IVIG treatment. This practice has decreased in Japan because no such scoring system appears sufficiently sensitive and specific to enable selective therapy (i.e., to allow nontreatment of patients predicted to be at low risk for development of coronary abnormalities). More recent scoring systems have focused on identifying patients at high risk for IVIG treatment failure and coronary abnormalities who may benefit from more aggressive primary therapy.[178,179] Patients who are at greatest risk are as follows: those younger than 1 year old; male patients; those with prolonged or recurrent fever; and those with anemia, hypoalbuminemia, hyponatremia, and thrombocytopenia. The Kobayashi scoring system is not effective in predicting treatment failure in multiracial populations as seen in the United States.[178,352] All patients diagnosed with Kawasaki disease within the first 10 days after onset of disease and those diagnosed later who are still manifesting significant inflammation should be treated with IVIG and aspirin.[262]

In Kawasaki disease, fever typically is high spiking and remittent, with peak temperatures generally exceeding 39° C (102.2° F) and in many cases exceeding 40° C (104° F). Unless treatment is initiated with IVIG and aspirin, fever persists for a mean of 11 days,[129] but it may continue for 3 to 4 weeks and rarely longer. In patients whose disease is treated with 80 to 100 mg/kg/day of aspirin and a single 2 g/kg dose of IVIG, fever generally resolves within 1 to 2 days.[257,261]

Bilateral painless vascular injection of the bulbar conjunctivae, clearly more severe than injection of the palpebral conjunctivae and sparing the limbic region around the cornea, generally is seen in the first week of illness, usually beginning shortly after onset of fever. It generally is not associated with exudate, conjunctival edema, or corneal ulceration, thus distinguishing the eye findings of Kawasaki disease from purulent conjunctivitis and from Stevens-Johnson syndrome. Mild acute iridocyclitis or anterior uveitis, which may be noted by slit lamp examination, resolves rapidly and only rarely is associated with photophobia or eye pain.[34,109,192,275,353] Less common ocular findings include superficial punctate keratitis, vitreous opacities, vitreous and chorioretinal inflammation, lateral rectus palsy, periorbital vasculitis, and papilledema and other optic disk changes.[11,86,143,419]

Changes of the mouth and lips consist of the following: (1) erythema, dryness, fissuring, peeling, cracking, and bleeding of the lips; (2) a strawberry tongue indistinguishable from that associated with streptococcal scarlet fever, with erythema and prominent papillae; and (3) diffuse erythema of the oropharyngeal mucosae. Oral ulcerations, pharyngeal exudates, and Koplik spots are rarely, if ever, found in Kawasaki disease and when present help to exclude the diagnosis.

Changes in the extremities are among the most distinctive features of Kawasaki disease. The hands and feet become indurated and swollen with stretched, shiny skin, sometimes with painful induration. The palms and soles become erythematous, often with an abrupt change to normal skin at the wrist and ankle. Infants and young children frequently refuse to hold objects or to bear weight. In the subacute phase, a distinctive pattern of periungual desquamation of fingers and toes may occur from 2 weeks to 2 months after onset of Kawasaki disease in 50 to 70 percent of affected patients. Beau lines, which

are transverse grooves across the nails, may appear at the nail base 1 to 2 months after a case of acute Kawasaki disease and may grow out over several months. Peripheral gangrene is a rare complication (see later).

The erythematous rash associated with Kawasaki disease may take many forms. Most common is a nonspecific, diffuse, maculopapular, primarily truncal erythematous rash. Occasionally, diffuse scarlatiniform erythroderma, urticaria, or an erythema multiforme–like rash with target lesions develops. Vesicles and bullae are not seen, although very fine pustules occur rarely. Perineal erythema and then desquamation are quite common manifestations in diapered as well as toilet-trained children in the acute stage of illness. Although rashes in Kawasaki disease tend to be most prominent on the trunk, with perineal accentuation they frequently also involve the face and extremities.

Cervical lymphadenopathy is the least common of the five principal diagnostic criteria, but it sometimes is the dominant clinical feature along with fever.[356] It usually is unilateral and confined to the anterior cervical triangle. To fulfill diagnostic criteria, the enlarged node or mass of nodes exceeds 1.5 cm, is not fluctuant, usually is not associated with erythema of the overlying skin, and is not tender or only moderately tender. Lymphadenopathy generally is benign and transient. Clinicians should be aware that children with suspected acute bacterial cervical adenitis that is unresponsive to antibiotic therapy may have Kawasaki disease. Because other features of Kawasaki disease often are present but overlooked, Kawasaki disease should be considered in such febrile children without an alternate diagnosis.[356] The ultrasound appearance of lymph nodes in Kawasaki disease has been described to be similar to that of acute Epstein-Barr virus infection and distinct from bacterial adenitis.[385] The absence of changes suggesting suppuration on imaging (e.g., ultrasonography or computed tomography [CT]) of such lesions should strengthen the suspicion of possible Kawasaki disease in this setting. Impressive cervical adenopathy in patients with Kawasaki disease generally resolves remarkably promptly after administration of appropriate therapy.

The associated features of Kawasaki disease reflect its multisystemic nature (Table 77-2). Sterile pyuria as a manifestation of urethritis, occasionally with meatitis, is found in approximately half of patients. Arthritis appearing during the first week of illness can be polyarticular or oligoarticular, including the small interphalangeal joints as well as large weight-bearing joints, with a reported prevalence of approximately 7.5 percent.[115] Arthrocentesis during this early phase yields purulent-appearing fluid, with a mean white blood cell count of 125,000 to 300,000/mm^3, normal glucose levels, and negative Gram stain and bacterial cultures. Arthritis developing after the 10th day of illness has a predilection for large weight-bearing joints, especially the knees and ankles, with a somewhat lower synovial fluid white blood cell count.[129] Gastrointestinal complaints occur in approximately one third of patients, especially in older patients; these complaints may be severe, even leading to laparotomy, and they include nausea, abdominal pain, and some diarrhea. These findings may be related to a hydropic gallbladder, pancreatitis, or appendicular vasculitis.[15,437] Obstructive jaundice and acute hydrops of the gallbladder are not uncommon findings, whereas mild to moderate elevations of serum aminotransferases occur in almost half of patients. Central nervous system involvement including aseptic meningitis occurs in almost half of patients.[66] Transient unilateral lower motor neuron facial nerve palsy occurs rarely,[296] as does sensorineural hearing loss.[176] Characteristic marked irritability is very common, especially in young infants. Reactivation of inflammation at the site of a previous bacillus Calmette-Guérin (BCG) vaccination coincident with acute Kawasaki disease is a common finding in Japan, where BCG is used widely,[351] and Terp and associates observed a child in whom both a BCG site and a purified protein derivative (PPD) test site reactivated with acute Kawasaki disease.[394] Some patients experience a flare of atopic dermatitis or psoriasis during or after experiencing acute Kawasaki disease.[30,77] Various pulmonary manifestations of Kawasaki disease, including isolated pulmonary nodules,[88] pleural effusions, acute respiratory distress syndrome,[286] and pulmonary infiltrates,[411] all of which are interesting in view of the increased numbers of IgA plasma cells found in respiratory tract tissue,[316,318] have been observed.

By far, the most important associated feature of Kawasaki disease is *cardiac involvement*. Cardiac manifestations can be prominent in acute Kawasaki disease and are the major cause of long-term morbidity and mortality. In addition to the coronary artery abnormalities that

TABLE 77-2 **Associated Noncardiac Features of Kawasaki Disease**

Musculoskeletal System
Arthritis or arthralgia
Central Nervous System
Aseptic meningitis
Facial nerve palsy
Marked irritability
Sensorineural hearing loss
Gastrointestinal System
Hydrops of gallbladder
Abdominal pain, diarrhea
Hepatic dysfunction, obstructive jaundice
Pancreatitis
Genitourinary System
Urethritis, meatitis
Respiratory System
Pulmonary infiltrates or nodules
Preceding respiratory illness
Other
Erythema and induration of bacille Calmette-Guérin vaccine site
Anterior uveitis (mild)
Desquamating groin rash
Flare of atopic dermatitis or psoriasis
Peripheral gangrene (young infants)

develop in 20 to 25 percent of untreated children, pericardial effusion and myocarditis with congestive heart failure, tachycardia, gallop rhythm, nonspecific changes on the electrocardiogram, or arrhythmia may occur.[153,156,158,262] An imperfect correlation exists between clinically apparent cardiac involvement and echocardiographic evidence of coronary abnormalities, although in the pre-IVIG era, echocardiographic evidence of mitral regurgitation, impaired left ventricular function, or pericardial effusion in the acute stage was shown to be predictive of subsequent coronary abnormalities.[110] Echocardiographic evidence of perivascular brightness of coronary arteries was suggested as a criterion for early diagnosis of Kawasaki disease, but a recent report did not confirm the reliability of this finding as a useful sign of incomplete Kawasaki disease.[434] Acute Kawasaki disease may involve pericardium, myocardium, endocardium, coronary arteries, and cardiac valves. Clinical and auscultatory features may include a hyperdynamic precordium, tachycardia out of proportion for the child's age and temperature, a gallop rhythm, and a flow murmur. Some infants may manifest very low cardiac output, and ECG changes (ST-segment and T-wave changes, prolonged PR interval, and arrhythmias) may be present.

A relatively recently recognized unusual presentation of acute Kawasaki disease is hypotension or shock.[72,151] Approximately 3.5 percent of patients may require admission to an intensive care unit for suspected septic shock or toxic shock but are ultimately recognized to have features of Kawasaki disease. In the few small series of cases, females and older patients have predominated, laboratory studies reveal more dramatically elevated markers of inflammation, and patients were more refractory to therapy compared with control subjects with Kawasaki disease. Hyponatremia was reported recently in a group of these patients, suggesting a possible association with the syndrome of inappropriate secretion of antidiuretic hormone.[350]

INCOMPLETE OR ATYPICAL KAWASAKI DISEASE

A substantial subset of children have illnesses that do not completely fulfill diagnostic criteria for Kawasaki disease but that include at least 5 days of fever and one, two, or three features of the disease. Incomplete or atypical Kawasaki disease (*incomplete* is the preferred term) is associated with a substantial risk for development of coronary artery aneurysms,[28,96,209,262,317] but it can be very difficult to diagnose. Incomplete Kawasaki disease occurs most frequently in young infants, who unfortunately are at greatest risk for developing coronary disease with Kawasaki disease[42,309,317]; and fatalities have occurred. The laboratory profile of incomplete cases is similar to that of classic cases, and laboratory results can increase or decrease the likelihood of the presence of Kawasaki disease in a particular patient.[262] Echocardiographic findings in the acute stage, including perivascular brightness, coronary ectasia, decreased myocardial contractility, pericardial effusion, and mild mitral regurgitation, can support the diagnosis of Kawasaki disease. Individual manifestations of Kawasaki disease in young infants tend to be more subtle than those in older children and can be fleeting.[222,309] Kawasaki disease should be considered in the differential diagnosis of prolonged fever in infants because patients are described in whom such fevers are virtually the sole manifestation of Kawasaki disease.

A committee of the American Heart Association developed a valuable algorithm to assist in the evaluation of patients with suspected incomplete Kawasaki disease (see Fig. 77-1), with emphasis placed on clinical assessment and measurement of acute-phase reactants (C-reactive protein [CRP], erythrocyte sedimentation rate [ESR]) in patients with 5 or more days of fever and two or three features of Kawasaki disease or in infants 6 months old or younger with 7 days or more of fever without other explanation.[262] In addition to elevated ESR (≥40 mm/hr) and/or CRP (≥3.0 mg/dL), a set of six supplementary laboratory criteria that can be useful in this regard is as follows: albumin, 3.0 g/dL or less; anemia for age; increased alanine aminotransferase; platelets after day 7 of more than 450,000/mm^3; white blood cell count of 15,000/mm^3 or more; and 10 or more white blood cells/high-power field in the urine sample.[262] Hyponatremia is also useful in this regard.

Retrospective diagnosis of Kawasaki disease often is based on finding coronary abnormalities on an echocardiogram, although the clinician's goal is to identify patients with incomplete Kawasaki disease before coronary changes have occurred. The existence of these patients again emphasizes the need to identify the etiologic agent of Kawasaki disease so that a diagnostic test can be developed. When possible, patients with illnesses suggesting incomplete Kawasaki disease should be referred to physicians with considerable experience in making the diagnosis.

LABORATORY FINDINGS

A specific diagnostic test for Kawasaki disease is not available and awaits discovery of the etiologic agent of the illness (Table 77-3). The laboratory features of Kawasaki

TABLE 77-3 **Laboratory Features of Kawasaki Disease**

Leukocytosis with neutrophilia
Elevated erythrocyte sedimentation rate
Elevated C-reactive protein (and other acute-phase reactants)
Anemia
Thrombocytosis after week 1
Sterile pyuria
Hypoalbuminemia
Hyponatremia
Elevated serum levels of aminotransferases and γ-glutamyltransferase
Plasma lipid abnormalities
Cerebrospinal fluid pleocytosis
Synovial fluid pleocytosis

disease, although quite nonspecific, are nonetheless characteristic of the illness. Leukocytosis, especially with neutrophilia, is typical in the acute stage, with a predominance of immature and mature granulocytes. White blood cell counts in excess of 30,000/mm^3 occur rarely, and those in excess of 15,000/mm^3 occur in approximately 50 percent of patients. Leukopenia is unusual. Toxic granulations and Döhle bodies occasionally are seen on peripheral blood smear.[26] Anemia may develop, usually with normocytic red blood cell indices, particularly in patients with more prolonged duration of active inflammation. Severe hemolytic anemia requiring transfusions occurs but is unusual and usually is related to IVIG therapy.[54,342] Curiously, Kawasaki's first patient in 1961 (and only rare subsequent patients) manifested Coombs-positive hemolytic anemia.[164,345]

Elevation of acute-phase reactants such as ESR, CRP, and α_1-antitrypsin is nearly universal in Kawasaki disease. CRP values rise and fall much more quickly than do ESR values. Additionally, IVIG therapy per se leads to elevation of the ESR (but not CRP) for several weeks, and, thus, ESR is of little or no value in assessing the degree of inflammatory activity in IVIG-treated patients; CRP or other acute-phase reactants clearly are superior for this purpose.[13]

A very characteristic feature of the subacute phases of illness is thrombocytosis, with platelet counts ranging from 500,000 to more than 1.5 million/mm^3. Thrombocytosis rarely is present in the first week of illness, usually appears in the second week, and peaks in the third week, with a gradual return to normal by 4 to 8 weeks after onset in uncomplicated cases. The mean peak platelet count is approximately 700,000/mm^3. In one study, infants younger than 1 year old with fever without a source who had platelet counts greater than 800,000/mm^3 were 17 times more likely to be diagnosed with Kawasaki disease ultimately than were infants with platelet counts lower than 800,000/mm^3.[265] No difference exists in chromium-65–labeled autologous platelet survival between cases and controls, and little correlation exists between thrombocytosis and increased platelet aggregation. The latter situation has been detected in patients with Kawasaki disease from a few days until a year after onset.[161,420] The rare patients with thrombocytopenia in the acute stage of Kawasaki disease, most often young patients, are at increased risk for development of coronary artery disease and myocardial infarction.[267,269] The mechanism of thrombocytopenia appears to be low-grade consumptive coagulopathy.[269]

Plasma lipids are markedly perturbed in acute Kawasaki disease, with depression of plasma cholesterol, high-density lipoprotein (HDL) cholesterol, and apolipoprotein A-I (apoA-I).[44,258,326] Similar changes are observed in other conditions associated with an acute-phase response.[44] Marked appearance of serum amyloid A (SAA) protein in plasma, associated with HDL3-like lipoprotein particles, is seen acutely.[44] Cabana and colleagues[44] also showed that total cholesterol, HDL cholesterol, apoA-I, and triglyceride levels normalize over the course of several weeks and that SAA disappears from plasma. The core composition of HDL normalizes more slowly than do plasma HDL cholesterol and apoA-I levels, a finding suggesting that Kawasaki disease has a profound effect on the lipoprotein profile acutely and a more subtle sustained effect on HDL composition.

Mild to moderate elevations in serum aminotransferase levels are present in as many as 40 percent of patients, and mild hyperbilirubinemia occurs in approximately 10 percent.[39] Plasma gamma-glutamyl transpeptidase levels are elevated in most patients.[395] Hypoalbuminemia and hyponatremia are associated with more prolonged and more severe disease.[123,190,350,413] Urinalysis reveals intermittent mild to moderate sterile pyuria in approximately one third of patients, although suprapubic urine generally does not show pyuria, a finding suggesting urethritis.[67,228] In those children who undergo lumbar puncture, evidence of aseptic meningitis, with a predominance of mononuclear cells, normal glucose, and normal to mildly elevated protein levels, is a common finding.[66]

Laboratory tests, even though nonspecific, can provide diagnostic support in patients with clinical features that are suggestive, but not diagnostic, of Kawasaki disease[262] and may aid in prediction of nonresponder patients, at least in Japanese patients.[178] A moderately to markedly elevated CRP (>3.0 mg/dL) or ESR (>40 mm/hr), almost universal in Kawasaki disease, is an uncommon finding in viral exanthems and hypersensitivity reactions. Platelet counts higher than 450,000 mm^3 usually are present in patients with Kawasaki disease after the seventh day of illness. In cases of incomplete Kawasaki disease associated with coronary abnormalities, thrombocytosis and elevated ESR are very common events in the acute stage. Clinical experience suggests that Kawasaki disease is unlikely if platelet counts and a full panel of acute-phase inflammatory reactants (e.g., ESR, CRP) are normal after the seventh day of illness.

Immunologic Findings

Studies of children with acute Kawasaki disease reveal widespread immune perturbations[347] and have focused on immune activation, endothelial activation, and vascular infiltration with a variety of immune cells. Marked immune activation is present and is reflected by increased levels of many proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interferon-γ, interleukin (IL)-1, IL-2, IL-4, IL-6, IL-8, IL-10, and soluble IL-2 receptor.* Increased chemokine and selectin activity also has been reported in acute Kawasaki disease.[380,416] The most intense immune activation and cytokine production occur during the acute and early subacute phases, the period of most intense vascular inflammation and of aneurysm formation. The immune perturbations subside as the illness resolves either spontaneously[201] or more promptly in response to anti-inflammatory therapy, and the benefit of therapy appears to result from immune modulating effects, although precise details are lacking.[198,340]

In acute Kawasaki disease, serum IgG levels are lower than normal for age,[261] whereas in the subacute stage, polyclonal elevation of all immunoglobulin classes is often found.[208] Serum IgE and IgM levels particularly are frequently elevated.[184,186] The increase in serum

*References 18, 76, 99, 131, 172, 191,212, 213, 224, 225.

immunoglobulins is associated with a very high proportion of circulating activated B cells,[173] which is reversed by aspirin/IVIG treatment.[200] Shingadia and colleagues[339] found decreased numbers of circulating B lymphocytes and plasma cells with surface or cytoplasmic IgA in the acute and subacute stages of Kawasaki disease, thus complementing the finding of IgA plasma cell infiltration in tissues.[316,318]

Circulating immune complexes can be detected in the subacute and convalescent (but not acute) stages; they appear unrelated to the development of coronary abnormalities by virtue of their time course of detection* and to be unaffected by IVGG infusions.[218] Some investigators have detected IgA in these immune complexes.[276] Most circulating IgG immune complexes in Kawasaki disease contain IgG1 and IgG3,[210] with Fc portions that bind to monocytes and platelets. Immune complexes in Kawasaki disease may promote aggregation of platelets, with release of vasoactive factors.[210]

Several autoantibodies have been found in Kawasaki disease sera, but antinuclear antibody and rheumatoid factor are very uncommon findings. Antibodies to type III collagen have been detected,[177] but without clear relation to coronary complications. Some acute-phase sera contain IgM antimyosin antibodies directed against epitopes that differ from those reactive with acute rheumatic fever sera, a finding suggesting a possible relationship with myocarditis in Kawasaki disease.[57] Both IgM and IgG antineutrophil cytoplasmic antibodies (ANCAs)[303,330,355] and antimyeloperoxidase antibodies[303] have been reported in Kawasaki disease. Several reports have noted IgM or IgG antiendothelial cell antibodies, which may injure endothelial cells directly or participate in complement-mediated injury or in antibody-mediated cytotoxic reactions.[152,199,202-204] Among the endothelial cell antigens of interest are adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), E-selectin, P-selectin, and endothelin, all of which may be upregulated in Kawasaki disease.[38,98,171,240,272] The precise role of immunologic reactions to endothelial cells in the pathogenesis of Kawasaki disease is unclear; Miura and colleagues[237] found focal but not widespread expression of VCAM-1 or E-selectin on endothelial cells of acute Kawasaki disease coronary arteries, especially in areas of neovascularization. However, the temporal correlation of immune activation and endothelial cell activation with secretion of monocyte chemoattractant protein-1 (MCP-1)[38,204,390] and the development of coronary abnormalities and their suppression by IVIG are consistent with an immune stimulant that triggers an immune response leading to vascular damage in part through cytokine-induced exposure of endothelial epitopes, inflammatory cell infiltration, myointimal proliferation, matrix metalloproteinase release, and other mechanisms resulting in vascular wall damage.[107,333] An animal model of Kawasaki disease induced by injected cell wall fragments of *Lactobacillus casei* has been reported and is used, but its relevance to Kawasaki disease is unclear.[197] Schulte and coworkers presented data that macrophages and dendritic cells (innate immunity) collaborate with T cells but not B cells (adaptive immunity) to result in coronary arteritis in this model.[332]

Investigation of the distribution of circulating T cells in acute and subacute Kawasaki disease has yielded conflicting results that range from no significant change in distribution of CD3, CD4, CD8, and CD19 cells at any stage of illness[51] to significant decreases in circulating CD8[207,391] or CD4[391] numbers acutely. An immunohistochemistry study of eight autopsied acute-stage Kawasaki hearts found activated T cells, a predominance of CD8 cells and macrophages, but no B cells,[31] whereas Terai and colleagues' autopsy study on a child showed predominance of activated CD4 cells and macrophages with activated coronary endothelial cells.[390] The presence of activated T cells in peripheral blood in acute Kawasaki disease is controversial. Jia and colleagues reported that Th17 helper cells and expression levels of IL-17A, IL-6, and IL-23 were significantly upregulated in children with acute Kawasaki disease whereas regulatory T cells and the transcription factor FoxP3 were significantly downregulated. These changes were more dramatic in IVIG-resistant patients.[146]

Although expansion of Vβ2 and Vβ8 T-cell receptor families in the acute stage of Kawasaki disease was reported,[1,2,58] this has not been reproduced by other investigators.* Such expansion suggests the possible role of a superantigen or could reflect clonal expansion of certain Vβ families in response to a conventional antigen. However, clonal expansion of CD8 T cells in acute Kawasaki disease supports the hypothesis that a conventional antigen rather than a superantigen is involved in the pathogenesis,[51,346] and compelling evidence of an oligoclonal IgA response in the vascular wall also strongly supports this hypothesis.[320]

Evidence for activation of neutrophils in Kawasaki disease is of interest because they, with monocytes and macrophages, are postulated to mediate endothelial cell damage by production of oxygen radicals.[268,375,407] Widespread activation of monocytes and macrophages,[100-102] T lymphocytes,[103,329] and B lymphocytes is also present.[173] Jejunal mucosal changes similar to delayed-type hypersensitivity in the epithelium and lamina propria, including decreases in $CD8^+$ cells and increases in $CD4^+$ and $HLA\text{-}DR^+$ cells, have been detected.[248] Delayed hypersensitivity skin test responsiveness to *Candida*, streptokinase/streptodornase, phytohemagglutinin, and PPD (tuberculin) is suppressed during the acute stage of Kawasaki disease but normalizes within 1 to 2 months.[431] Acute Kawasaki disease is associated with inflammatory reactivation of previous BCG vaccination sites, apparently reflecting both cellular and humoral responses.[433] Terp and colleagues cared for a patient with acute Kawasaki disease and reactivation of both a BCG site and the site of a recent tuberculin skin test.[394] Plasma fibronectin levels are reported to be decreased early in illness but to rebound to high levels by the fourth week of illness.[338]

Circulating complement components C3 and C4 commonly are elevated, probably as acute reactants in the first several weeks of illness, and then normalize.[304]

*References 128, 208, 210, 218, 276, 283, 324, 414.

*References 3, 217, 231, 266, 295, 392, 400.

Nonetheless, complement appears to be activated through the classical pathway,[180] with high plasma C4D levels.[246]

Production of the chemoattractant leukotriene B_4 by polymorphonuclear cells is increased from 13 to 29 days after onset of Kawasaki disease,[120] and it may attract more inflammatory cells to the site. Leukotriene E_4 and prostaglandin E_2 also are elevated in Kawasaki disease,[97,196,226] as are increased platelet synthesis of thromboxane A_2 and plasma levels of thromboxane B_2.[130]

The strongest evidence of the importance of immunologic factors in the pathogenesis of vasculitis in Kawasaki disease is provided by the remarkably beneficial effect of IVIG on the acute febrile illness and the marked reduction in development of coronary aneurysms.[247,257,261,273,340] This correlates with the effect of IVIG to reduce B-cell activation, more rapidly normalize T-cell activity, downregulate cytokine secretion, and induce disappearance of endothelial cell activation antigens.[198,204] Potential mechanisms of the beneficial action of IVIG in Kawasaki disease include modulation of cytokine production, suppression of endothelial cell activation, inhibition of immunoglobulin production by a negative feedback mechanism, specific neutralization of an unknown etiologic agent or toxin, nonspecific blockade of a receptor for immune complexes, and augmentation of T-cell suppressor activity.[340]

TABLE 77-4 **Treatment of Kawasaki Disease**

Acute and Subacute Stages
IVIG 2 g/kg infusion over 10 to 12 hours
Plus
Aspirin 80 to 100 mg/kg/day in four divided doses (until 14th illness day *and* patient afebrile at least 3 to 4 days), then 3 to 5 mg/kg once daily for 6 to 8 weeks.
IVIG may be repeated if fever persists or recurs together with at least one classic sign of Kawasaki disease and/or elevated C-reactive protein level (see text for other alternative "rescue therapies")
Convalescent Stage
No coronary abnormalities: no therapy
Transient coronary abnormalities: aspirin 3 to 5 mg/kg once daily at least until resolution of coronary abnormalities
Persistent small to medium coronary aneurysms: aspirin 3 to 5 mg/kg once daily
Giant or multiple small coronary aneurysms: aspirin 3 to 5 mg/kg once daily, with or without clopidogrel 1 mg/kg/day, with warfarin or low-molecular-weight heparin for most patients
Coronary obstruction: thrombolytic therapy, surgical or interventional procedures

IVIG, intravenous immunoglobulin.

MANAGEMENT

Treatment During the Acute Stage

Patients diagnosed with Kawasaki disease should be admitted to hospital, undergo a baseline echocardiogram, and receive IVIG 2 g/kg over the course of 10 to 12 hours, with high-dose aspirin at 80 to 100 mg/kg/day in four divided doses as soon as practical (Table 77-4).[59,262] When administered by the 10th illness day (onset of fever defines first day) this regimen is highly effective in reducing the risk for development of coronary abnormalities.[257] Because few data exist regarding management of patients treated later than the 10th illness day, the goal is to treat by illness day 10 whenever possible. Patients who are diagnosed after the 10th illness day and who are still febrile may benefit from therapy, but the ability to prevent coronary changes is less certain. IVIG and aspirin have been shown to prevent the development of giant coronary aneurysms[53,315] and to have direct benefits on cardiac function.[259] The mechanism of action of IVIG in Kawasaki disease remains unknown. The single high-dose schedule is superior to the earlier regimen of 400 mg/kg/day IVIG for 4 days with high-dose aspirin with respect to rapidity of defervescence and normalization of acute-phase reactants, as well as in preventing development of coronary artery abnormalities.[257,261,262,273] The large single-dose infusion generally is well tolerated, even in patients with decreased myocardial function.[259] Patients should generally remain hospitalized until they have been afebrile for at least 24 hours, to ensure that they are available for re-treatment if necessary.

Whether treatment with IVIG and aspirin earlier than the fifth illness day leads to better or poorer outcomes remains unclear. Tse and colleagues[402] compared 89 children treated on or before the fifth day of illness to control subjects treated on illness day 6 to 9.[402] These investigators found significantly fewer patients with coronary ectasia at 1 year after Kawasaki disease and shorter total fever duration in the former group, without differences in frequency of recrudescence, need for additional therapy, need for corticosteroids, length of hospitalization, or development of coronary aneurysms within 3 months.[402] Zhang and associates also concluded that patients treated before the sixth illness day had a significantly lower risk for developing cardiac sequelae compared with patients treated later than the sixth day.[438] In contrast, Muta and colleagues evaluated 4731 patients treated on illness days 1 to 4 and 4020 treated on days 5 to 9 and found that patients treated earlier were somewhat more likely to require additional therapy (odds ratio = 1.12; 95% confidence interval [CI], 1.10 to 1.16); no significant differences in cardiac sequelae were found between groups.[243] Another study also found that treatment before illness day 5 was associated with increased need for re-treatment but no difference in coronary outcome.[87]

Single infusions of IVIG at doses less than 2 g/kg have not been demonstrated to be as effective as 2 g/kg.[19,80] Terai comprehensive meta-analysis of all Japanese and North American IVIG treatment trials showed that the coronary artery outcome is correlated directly with the *total* dose per kilogram of IVIG, with 2 g/kg superior to 1.6 g/kg, which is superior to 1.2 and 1.0 g/kg; the initial dose of aspirin did not appear to influence coronary outcome.[389] Another study confirmed this finding.[75]

Patients diagnosed after the 10th illness day who are still febrile and/or who manifest other signs of active disease may benefit from IVIG and aspirin therapy because this treatment may result in prompt clinical improvement, with subsidence of fever and other signs of

inflammation. Little evidence supports that this approach results in lower rates of development of coronary abnormalities, however. Patients beyond the 10th to 12th illness day who have become afebrile and have resolved their clinical features of Kawasaki disease without therapy are unlikely to benefit from IVIG. Such children should be treated instead with low doses of aspirin, 3 to 5 mg/kg once daily, and should be evaluated carefully by serial echocardiograms. In patients who have already developed coronary aneurysms and whose acute manifestations of illness have resolved, there is no convincing evidence of a beneficial effect of IVIG.

Various preparations of IVIG differ in manufacturing processes and, therefore, in composition (e.g., the proportion of IgG monomers and the presence of proteins other than IgG). Although adverse reaction rates differ among products, clinical efficacy does not seem to differ.[310] An exception is a report that showed IVIG prepared with β-propiolactone, which can affect the biologic activity of the Fc portion of IgG, to be less effective in Kawasaki disease.[401] Immunization with live virus vaccines such as for measles and varicella should be deferred for 9 to 11 months after administration of high-dose IVIG because of impaired immune responses.

The aspirin dosage for acute Kawasaki disease that is most thoroughly studied in the United States is 80 to 100 mg/kg/day in four divided doses. I recommend that this therapy be maintained until approximately the 14th day of illness (longer if necessary to ensure that the patient has been afebrile for at least 3 to 4 days) and then reduced to a daily dose of 3 to 5 mg/kg for antiplatelet activity. The lower dose should be continued until 2 to 3 months after onset of illness in those who have not developed coronary abnormalities.[262] Patients who develop coronary abnormalities should continue to take low-dose aspirin. High-dose aspirin is used for its anti-inflammatory activity, whereas the much lower dose inhibits platelet aggregation. Japanese clinicians generally use an intermediate anti-inflammatory dose of 30 to 50 mg/kg/day because of perceived higher rates of untoward effects in Japan.[17,187] Difficulty in achieving standard therapeutic anti-inflammatory serum salicylate levels during the acute phase of illness (20 to 25 mg/dL) occasionally may complicate aspirin treatment, and selected patients with rare refractory disease may require salicylate doses in excess of 100 mg/kg/day to achieve anti-inflammatory benefit.[144] This probably reflects impaired absorption and bioavailability and enhanced salicylate clearance in acute Kawasaki disease.[181] An important note is that patients with Kawasaki disease who are receiving very high aspirin doses can suddenly increase their salicylate absorption with clinical improvement and then are at risk for toxicity. Serum salicylate levels should be monitored if symptoms of vomiting, hyperpnea, tinnitus, lethargy, or striking liver function abnormalities develop in children receiving aspirin.

A study that randomized U.S. patients to receive salicylates at 80 to 100 mg/kg/day or at 3 to 5 mg/kg/day for initial therapy (each regimen with 2 g/kg of IVIG) concluded that no difference in coronary outcome occurred, but a more prompt clinical anti-inflammatory benefit was noted in the high-dose aspirin group.[232] In the absence of IVIG, aspirin therapy does not decrease the frequency of coronary abnormalities.[75] Reye syndrome has been reported rarely in children taking high-dose aspirin for Kawasaki disease, but there are few data to suggest that low-dose aspirin poses this risk.[377]

The potential value of adding corticosteroid therapy to IVIG or aspirin for primary therapy has been addressed in several trials.[179,260,271,302] For example, Jibiki and colleagues compared 3 days of intravenous dexamethasone 0.3 mg/kg/day plus heparin and IVIG, with subsequent low-dose aspirin, to IVIG plus higher-dose aspirin and found more prompt decreases in fever and inflammatory markers in the dexamethasone-treated group but no difference in rates of coronary abnormalities.[147] Okada and associates showed that the addition of intravenous prednisolone to IVIG and aspirin followed by a long taper of oral prednisolone was associated with shorter duration of fever and more prompt fall in CRP and circulating IL-2, IL-6, IL-8, and IL-10 levels.[277] Inoue and colleagues[140] compared 88 patients treated with IVIG and aspirin to 90 patients who received in addition intravenous prednisolone 2 mg/kg/day in three divided doses until afebrile and then orally until the CRP value normalized, with a subsequent taper over 15 days, for a median of 23 days of corticosteroids. The results indicated fewer coronary abnormalities, shorter durations of fever, more rapid decline in CRP level, and fewer initial treatment failures in the steroid recipients. Caution must be exercised in the interpretation of the findings of these Japanese trials because the IVIG regimens and aspirin doses usually differ from those used in the United States and echocardiograms were not interpreted by investigators blinded to the treatment group. A well-designed U.S. trial in this regard showed little if any direct benefit of the addition of a single dose of intravenous methylprednisolone 30 mg/kg to the current IVIG and aspirin regimen, with the possible exception of the subset of patients who failed to respond to standard therapy and who required re-treatment of the disease.[260]

A recent Japanese multicenter, randomized, open-label, blinded end points trial (the RAISE study) assessed the benefit of adjunctive corticosteroid primary therapy for Kawasaki disease patients considered at high risk to be refractory to initial therapy.[179] Using the Kobayashi score[178] to identify patients at high risk for nonresponse to IVIG and aspirin, high-risk subjects were randomized to IVIG and aspirin (30 mg/kg/d until afebrile, then 3-5 mg/kg) or to IVIG, aspirin, and intravenous prednisolone 2 mg/kg/d for 5 days, converting to oral dosage once free of fever and then a 15-day corticosteroid taper beginning when the CRP level normalized. Significantly fewer recipients of corticosteroids developed coronary changes (3% vs. 13% at 4 weeks), and coronary z-scores were significantly lower in corticosteroid recipients. In another recent Japanese study,[271] Ogata and colleagues evaluated corticosteroids with IVIG and aspirin 30 to 50 mg/kg/day in those patients with disease predicted to be refractory. Subjects were randomized to IVIG and aspirin alone or with a single 30 mg/kg dose of intravenously administered methylprednisolone and heparin from 2 hours before to 24 hours after the corticosteroid was given. Corticosteroid recipients had more rapid defervescence and decline in CRP levels with somewhat lower mean coronary z-scores ($z \geq 2.5$ in 9% of

corticosteroid group, 38.5% in the noncorticosteroid group, $p = .04$), with transient adverse events only in the corticosteroid group.

Unfortunately the Japanese scoring systems to identify high-risk patients are not useful in ethnically diverse populations as seen in the United States,[352,399] and it is thus difficult to know how to target adjunctive therapy outside Japan.

Prediction of Treatment Failures

Several attempts have been made in Japan to develop scoring systems to predict, at the time of initial presentation, those patients who are at increased risk for failure to respond to standard therapy[79,178] and/or to develop coronary lesions after therapy.[239] In a retrospective analysis of 193 patients, Mori and colleagues found elevated white blood cell and neutrophil counts and CRP levels after IVIG treatment predicted increased risk for subsequent development of coronary lesions.[239] Egami and associates compared 279 patients who responded to initial standard treatment to 41 patients who were treatment resistant.[79] With their scoring system giving one point each for age younger than 6 months, treatment before 4 days of illness, platelet count less than or equal to 300,000/mm^3, and CRP 8 mg/dL or higher and two points for alanine aminotransferase level 80 IU/L or higher, a total score of three points or higher identified the IVIG-resistant group with 78 percent sensitivity and 76 percent specificity.[79] A similar predictive scoring system for IVIG unresponsiveness was developed by Kobayashi and colleagues,[178] who gave two points each for initial treatment on illness day 1 to 4, serum sodium level less than 133 mmol/L, aspartate aminotransferase value 100 IU/L or higher, and neutrophils 80 percent or greater and two points each for CRP 10 mg/dL or higher, platelet count up to 300,000/mm^3, and age 12 months or younger; this yielded 86 percent sensitivity and 67 to 68 percent specificity for patients with four or more points. The Kobayashi score was used to select patients at high risk for treatment failure in a randomized corticosteroid trial, as discussed earlier.[179]

Rescue Therapy for Treatment Failures

Most patients with acute Kawasaki disease respond promptly to treatment with IVIG and aspirin, with defervescence and subsidence of inflammatory manifestations occurring within 48 hours.[257,262,341] A subgroup of 10 to 20 percent, however, fails to show significant clinical response; these patients remain febrile 36 to 48 hours after receiving IVIG or they manifest only transient improvement, with recurrent fever and clinical evidence of inflammatory signs. These patients need additional anti-inflammatory therapy, and specific guidelines or controlled treatment trials do not exist. Of course, when treating apparently treatment-refractory patients, it is also prudent to reconfirm the initial diagnosis of Kawasaki disease.

In these patients, administration of a second dose of 2 g/kg of IVIG generally is effective in suppressing disease activity.[89,262,366] In a retrospective study of 179 patients with Kawasaki disease, 89 percent responded to the first dose of IVIG and 67 percent of the nonresponders responded to a second IVIG dose; thus, only 3 to 4 percent of all patients failed to respond after a second dose of IVIG.[122] Some recommend a course (usually 3 days) of intravenous pulse methylprednisolone at 30 mg/kg/day instead of a second dose of IVIG.[417] I generally prefer to reserve pulse corticosteroid therapy for the rare patient with highly refractory acute disease who has failed to respond to at least two 2-g/kg doses of IVIG, because early Japanese data suggested that corticosteroids may predispose to development of coronary disease and because most patients respond well to a second IVIG dose.[122,161,235] In addition, a more recent study noted coronary dilation in three of nine treatment-refractory patients shortly after pulse corticosteroids were administered.[126] No randomized comparison between repeat IVIG dosing and corticosteroids for refractory patients has been performed. In a few particularly treatment-refractory patients, I have employed a slow oral corticosteroid taper over the course of several months once inflammatory activity appeared to have been controlled.

Infliximab (Remicade), a monoclonal anti–TNF-α antibody, has been reported in an open-label experience to be effective in most patients with acute Kawasaki disease who are refractory to standard therapy[40,223]; this agent is being studied in a randomized trial of patients with refractory Kawasaki disease. Even less published experience exists regarding other therapies for IVIG-refractory Kawasaki disease. Cyclophosphamide with or without methotrexate has been used in a very small number of patients, as has plasmapheresis or plasma exchange.[412] Cyclosporine has been reported to be promising in this regard,[373] as has single-dose methotrexate.[170]

Sequelae

The major (and virtually the only) sequelae of Kawasaki disease are cardiovascular, particularly coronary artery abnormalities. Therefore, appropriate cardiac imaging is critical for the evaluation of patients suspected to have acute Kawasaki disease and for their subsequent follow-up. Echocardiography has been considered the ideal imaging modality because it is noninvasive and has high sensitivity and specificity for detection of abnormalities of the proximal left main, left anterior descending, circumflex, and right coronary arteries.[262] This procedure should be performed under the supervision of an echocardiographer experienced with evaluating children, and internal arterial diameters should be measured and compared with normal standards for body surface area, expressed as z-scores.[69,216,262] The American Heart Association classifies coronary aneurysms as small (<5 mm), medium (5 to 8 mm), or giant (>8 mm); and de Zorzi and colleagues[69] and more recently Manlhiot and coworkers[216] showed that adjusting coronary dimensions for body surface area may identify more accurately those patients with enlarged coronary arteries. Giant coronary aneurysms, defined as having 8 mm or larger internal diameter, are associated with worse prognosis, and these patients require particularly close follow-up.[53,262] The sensitivity and specificity

of echocardiography to identify coronary thrombi and coronary stenosis are uncertain, and visualization of coronary vessels is more difficult as body size increases. Therefore, angiography, magnetic resonance angiography (MRA),[117,379] and ultrafast CT angiography (CTA)[45,291] have been useful for selected patients with Kawasaki disease.[262,334]

Management Beyond the Acute Stage

Patients with Kawasaki disease should be reevaluated within 2 weeks after hospital discharge and again 6 to 8 weeks after onset of illness because echocardiography at these time points is most likely to detect coronary artery aneurysms should they develop. If a baseline study and these two follow-up echocardiograms fail to detect evidence of coronary abnormality, performing further echocardiograms probably is unnecessary,[406] although a 6- to 12-month follow-up echocardiogram is performed at many centers.[60,262] Low-dose aspirin therapy (3 to 5 mg/kg/day) can be discontinued after the 6- to 8-week follow-up echocardiogram unless evidence of coronary abnormalities is present. To reduce the theoretical (and extremely low) risk for Reye syndrome in patients receiving low-dose aspirin, clopidogrel 1 mg/kg/day can be substituted for aspirin for a brief time in patients who develop varicella or influenza. Clopidogrel also can be used in the rare patient who is allergic to, or intolerant of, aspirin.

COMPLICATIONS

Myocardial Infarction

Myocardial infarction is the most common cause of death in Kawasaki disease (Table 77-5). In a cooperative Japanese study of 195 cases, the first myocardial infarction usually occurred in the first year after onset of disease and was fatal in 22 percent and asymptomatic in 37 percent of these patients. Major symptoms were shock, vomiting, and abdominal pain, with chest pain complaints only in children older than 4 years of age. Of those patients who survived a first infarct, 16 percent had a second myocardial infarct.[155,157] Patients with giant (>8 mm or z-score >10) coronary aneurysms are at greatest risk for having infarcts, particularly related to thrombi and/or to stenotic areas adjacent to a giant aneurysm. Most fatal infarctions are the result of obstruction of the left main coronary artery or both the right coronary and left anterior descending coronary arteries; survivors are most likely to have isolated right coronary involvement. Approximately half the survivors of acute myocardial infarction had one or more complications, including ventricular dysfunction, mitral regurgitation, and arrhythmias. Parents of all children with Kawasaki disease with significant coronary abnormalities should be instructed to seek emergency medical care if chest pain, dyspnea, lethargy, or syncope develops. Prompt fibrinolytic therapy should be attempted at a tertiary care center if acute coronary thrombosis is diagnosed.[154,262] The degree of reversibility of coronary thrombosis in children with Kawasaki disease may be somewhat less than that in adults with atherosclerotic disease. Late cardiac sequelae of Kawasaki disease may not manifest until adulthood.[160] A recent Japanese study assessed long-term outcomes of 60 Kawasaki disease patients who had a myocardial infarct between 1976 and 2007, with median follow-up of 16 years. The 30-year survival rate was 63 percent (95% CI, 45% to 78%) and the 25-year ventricular tachycardia-free survival rate was 29 percent (CI, 15% to 46%). Development of ventricular tachycardia was related to degree of nonviable myocardium.[403]

TABLE 77-5 Cardiac Abnormalities in Kawasaki Disease

Acute Stage
Pericardial effusion
Decreased myocardial function
Mitral regurgitation
Brightness of coronary artery wall (controversial)
Enlargement (ectasia) of coronary arteries
Arrhythmia (rare)
Subacute Stage
Coronary aneurysms, irregularity, ectasia
Significant mitral or aortic regurgitation, or both (rare)
Coronary aneurysm rupture (very rare)
Myocardial infarction (rare)
Convalescent Stage
Persistent coronary aneurysms
Regressed coronary aneurysms (residual fibrosis)
Coronary artery stenosis
Coronary aneurysm rupture (very rare)
Myocardial infarction (rare)

Other Cardiovascular Complications

Other cardiac complications include myocardial fibrosis, valvulitis, and coronary rupture. Some researchers have suggested that valvular disease occurs in as many as 1 percent of patients with Kawasaki disease; most of these cases result in mitral regurgitation.[5,175] Patients with well-documented aortic regurgitation also have been observed. At least one patient with Kawasaki disease developed severe aortic and mitral regurgitation that necessitated double valve replacement.[111] In an autopsy study, coronary rupture was a rare finding but was noted more often among older children who died of Kawasaki disease within the first months after disease onset, whereas myocardial infarction was seen more often in younger fatal cases.[318]

Peripheral artery aneurysms develop in fewer than 1 percent of patients with Kawasaki disease, virtually always in those who also have significant coronary abnormalities.[162,369] These abnormalities generally involve medium-sized muscular arteries, such as subclavian, brachial, axillary, iliac, or femoral arteries and occasionally the hepatic or renal arteries or the abdominal aorta. In patients with Kawasaki disease who are undergoing their

first coronary arteriography, abdominal aortography and subclavian arteriography are therefore recommended.[262]

Peripheral Gangrene

A rare but very serious complication in the acute febrile stage of Kawasaki disease is severe peripheral ischemia and dry gangrene of distal extremities.[396] Virtually all such patients have been young infants up to approximately 7 months of age with giant coronary aneurysms, and some have developed peripheral (especially axillary or iliac) arterial aneurysms as well. This complication is virtually unknown in Japan[166] and has been reported primarily in non-Asian children in North America.[73,396] Possible pathogenic mechanisms of peripheral gangrene include the following: severe arteritis of digital or other small peripheral arteries; arteriospasm of peripheral arteries, perhaps in association with severe vasculitis; thrombosis of inflamed or spastic arteries as a result of stasis and damaged endothelium; thrombosis of a more proximal aneurysm (especially axillary) with embolism distally; rarely, cardiogenic shock; and, most likely, a combination of these factors.[373,388] This process has led to amputations in a small number of infants. Therapy has been empirical, primarily because the precise mechanisms of disease are unclear, and has included aggressive use of anti-inflammatory agents, prostaglandin infusion, and antiplatelet, anticoagulant, and vasodilation therapies.[73,396,415]

Nonvascular Complications

As many as 5 to 10 percent of patients with Kawasaki disease develop painful arthritis or arthralgia in the acute stage of disease, often in the ankles or other lower extremity joints, and they may benefit from treatment with a nonsteroidal anti-inflammatory agent. Earlier-onset arthritis (≤10 days of illness) tends to be polyarticular, whereas later-onset arthritis involves primarily larger weight-bearing joints. I have used naproxen, usually 10 to 15 mg/kg/day divided into two or three doses, for several weeks with considerable success.

Abdominal pain and diarrhea in the early acute stage usually respond to intravenous hydration and supportive care. Acalculous distention (hydrops) of the gallbladder manifests clinically as right upper quadrant tenderness or a mass with or without obstructive jaundice and can be confirmed by ultrasonography.[262,359] Performing a cholecystectomy is not necessary. Hepatic involvement appears to be entirely self-limited and has not been associated with chronic liver disease.

Rare patients with Kawasaki disease develop hemophagocytic syndrome, also known as *macrophage activation syndrome*, as a complication.[56,241,287] This syndrome manifests as persistent fever associated with cytopenias, hepatosplenomegaly, hepatic dysfunction, often hyperferritinemia, elevated serum lactate dehydrogenase, hypofibrinogenemia, and hypertriglyceridemia. Therapy with high doses of prednisone or other immune modifiers is indicated for this rare but serious complication.

Rare events that occur in association with Kawasaki disease include sensorineural hearing loss,[176] transient unilateral facial nerve palsy,[106,296] and pneumonitis or pulmonary nodules.[88] I have cared for two older children who had sufficient abdominal findings to warrant performing appendectomies before establishing the diagnosis of Kawasaki disease. Consultation with a center that treats large numbers of patients with Kawasaki disease should be sought by the physician faced with patients with rare or serious complications.

LONG-TERM FOLLOW-UP AND PROGNOSIS

Kawasaki disease normally is an acute and self-limited illness. However, cardiac abnormalities that develop when the disease is active may be progressive, and prognosis clearly is related to the coronary artery status. Twenty to 25 percent of patients not treated with IVIG develop coronary abnormalities that are detectable by two-dimensional echocardiography or angiography. The risk for development of coronary aneurysms is reduced to 2 to 3 percent overall when IVIG is given in the first 10 days of illness.[257,261] However, the rates for coronary abnormalities for young infants are somewhat higher, even with timely IVIG therapy. Patients who develop moderate to severe coronary abnormalities are at risk for myocardial ischemia, myocardial infarction, and even sudden death for many years after onset of illness and into adulthood.[157,227]

Children without apparent cardiac sequelae during the first month after onset of Kawasaki disease appear to return to their previous states of health without cardiac signs or symptoms. However, some reports suggest the possibility of generalized endothelial dysfunction in patients with Kawasaki disease who never had coronary abnormalities. These dysfunctions include altered lipid metabolism,[44] increased brachial-radial artery mean pulse wave velocity,[50] lower myocardial flow reserve,[245] and abnormal endothelium-dependent brachial artery reactivity,[70,234,264,421] but the data conflict somewhat, and additional studies are needed.

Regression of small and medium aneurysms as seen on an echocardiogram is a common occurrence. Overall, approximately half of all children with coronary aneurysms at 4 to 8 weeks after onset demonstrate regression by 1 to 2 years, with apparently normal vessels on angiography or echocardiography.[155,158] The likelihood that an aneurysm will appear to regress by echocardiography or angiography is higher with smaller aneurysms, age younger than 1 year at the onset of Kawasaki disease, fusiform rather than saccular morphology, and involvement of a more distal coronary segment.[7,376] Regression of the internal diameter of the vessel to normal may occur by luminal smooth muscle cell–derived myofibroblastic proliferation or by thrombus organization and recanalization.[94,282,327] Intravascular ultrasound studies have been interpreted to suggest that thickened intima and media sometimes with calcifications are present in areas of apparently regressed coronary aneurysms.[119,362] However, direct correlation of intravascular findings and histology have not been performed. A very recent histologic study of 41 heart specimens demonstrated that calcification was found only in areas of organized thrombus not in vascular media or intima.[282] Regressed aneurysmal

segments are histologically abnormal and have abnormal functional responses with decreased vascular reactivity in response to exercise or pharmacologic agents such as isosorbide dinitrate or acetylcholine.[70,138,327,361] Only a limited number of regressed aneurysms progress to stenosis.[159,361,368,370]

Functional abnormalities of coronary vessel endothelium relaxation years after the onset of Kawasaki disease have been reported[70,138,234] and warrant further investigation. Newer imaging methods have demonstrated vascular wall changes sometimes even in patients with no history of abnormalities in the acute phase. The meaning of these findings in patients thought to have escaped coronary abnormalities with acute Kawasaki disease and their long-term significance are uncertain.

Patients with giant coronary aneurysms are at the greatest risk for the development of significant stenosis with resultant myocardial ischemia.[162,358] The risk for significant stenosis, usually developing at the inlet or outlet of a moderate to large coronary aneurysm, shows a steady rise over 15 to 20 years of observation.[150,162,358,363,368] These markedly abnormal vessels are subject to calcification and thrombosis, which may lead to myocardial ischemia or infarction. A recent analysis of 76 subjects with giant aneurysms from one Japanese center with median follow-up of 19 years showed 95 percent, 88 percent, and 88 percent 10-, 20-, and 30-year survival rates. Catheter and surgical coronary interventions resulted in 28 percent, 43 percent, and 59 percent cumulative coronary intervention rates at 5-, 15-, and 25-year follow-up.[358]

In 10- to 20-year follow-up studies of patients with Kawasaki disease, the arteries most likely to develop stenosis are the right main and left anterior descending coronary arteries.[150,162,358,363,368] A limited number of studies of young adults with ischemic heart disease and history of diagnosed Kawasaki disease or a compatible clinical illness have been performed.[61,160] A survey of Japanese adult cardiologists identified 130 adult patients with coronary aneurysms detected by angiography to evaluate myocardial infarction or ischemia.[160] Twenty-one of these patients (mean age, 34 years; range, 20 to 63 years) had a history compatible with Kawasaki disease in childhood. These patients had severe coronary disease with myocardial infarction, angina pectoris, mitral regurgitation, arrhythmias, congestive heart failure, and need for coronary bypass grafting. This study indicates that the coronary artery sequelae of Kawasaki disease likely are important causes of ischemic heart disease in young adults. Daniels and coworkers evaluated 261 adults younger than age 40 years who underwent angiography for suspected myocardial ischemia and found 16 with coronary aneurysms, 13 of whom had definite or presumed Kawasaki disease as the cause.[61] Fatty deposits and advanced changes resembling atherosclerotic disease sometimes have been found at autopsy of children with a history of Kawasaki disease who died of other causes.[374] This finding raises the important but unanswered question of whether patients with Kawasaki disease are at increased risk for developing premature or accelerated atherosclerosis. Gupta-Malhotra and colleagues reported higher levels of total cholesterol and apolipoprotein B but not increased carotid intimal-medial thickness or arterial stiffness by tonometry in patients who had Kawasaki disease 7 to 20 years earlier compared with control subjects.[118]

Long-Term Management

The risk for coronary artery thrombosis or stenosis that may result in myocardial ischemia and infarction remains the most important long-term clinical problem in the subset of patients with Kawasaki disease who develop significant coronary abnormalities (see Table 77-5). Patients have traditionally been classified as having small (<6 mm), medium (6 to 8 mm), and giant (>8 mm) aneurysms, and the latter two categories are at substantial risk for development of stenosis years after having the acute illness, compared with patients with small aneurysms or no aneurysmal changes.* Patients with giant aneurysms are at the highest risk for thrombosis.[262] Manlhiot and colleagues[216] documented that the American Heart Association classification scheme noted earlier underestimated the severity of coronary abnormalities and showed the following optimal definitions: small aneurysm, z-score of 2.5 to less than 5.0; large aneurysm, z-score greater than or equal to 5.0 to less than 10.0; and giant aneurysm, z-score greater than or equal to 10.0. Echocardiography and electrocardiography often are not sufficiently sensitive to detect stenotic lesions. Various stress tests for detection of reversible myocardial ischemia, including nuclear perfusion scans with exercise, exercise echocardiography, and stress echocardiography with agents such as dobutamine, dipyridamole, or adenosine, have been used in children to detect stenosis.[262,270,285] Newer techniques, including stress magnetic resonance imaging (stress MRI), MR angiography (MRA), and CT angiography (CTA) are being utilized.[45,117,291,379] Coronary arteriography has been the most definitive method to determine the degree of stenosis and the adequacy of collateral circulation but is invasive. Intravascular ultrasonography is an effective method to evaluate vascular wall morphology in selected patients during angiography.[364,367] All patients with evidence of myocardial ischemia or infarction should be studied by angiography to determine the need for intervention.[262] Most experts agree that patients with moderate to severe coronary artery aneurysms, a single large aneurysm, or multiple aneurysms should have their coronary anatomy defined by angiography or MRA or CTA at least once after the acute stage of illness (after the inflammatory process has subsided) to define fully the extent of involvement and to identify potential sites of thrombotic or stenotic complications.[262] Because aneurysms can also occur in noncoronary arteries, especially the subclavian, brachial, axillary, iliac, and femoral arteries, and occasionally in the abdominal aorta and renal arteries[369] (almost always in patients with coronary abnormalities), abdominal aortography and subclavian arteriography are recommended for patients with Kawasaki disease who are undergoing coronary angiography for the first time.[262]

Patients who have had Kawasaki disease are divided into five risk levels for long-term management by the American Heart Association, as outlined here.[262]

*References 7, 150, 155, 158, 162, 369, 404.

Patients with No Evidence of Coronary Artery Abnormalities at Any Time (Risk Level I)

Patients who have never manifested coronary artery abnormalities have no need for aspirin or other antiplatelet medication beyond 6 to 8 weeks after onset of illness or for restriction of physical activities in the convalescent stage. Only routine pediatric follow-up beyond 1 year, with routine cardiovascular risk assessment, is indicated.

Patients with Transient Coronary Ectasia or Dilation (Risk Level II)

Patients with transient coronary artery abnormalities that resolve by 6 to 8 weeks should be treated with aspirin 3 to 5 mg/kg/day until resolution of abnormalities. No restrictions are indicated after 6 to 8 weeks, angiography is not indicated, and risk assessment and counseling are recommended at 3- to 5-year intervals.[360]

Patients with Isolated (Solitary) Small to Medium (3- to 6-mm) Coronary Aneurysm in One or More Coronary Arteries (Risk Level III)

Patients with solitary small to medium coronary artery aneurysms should be maintained on daily low-dose aspirin 3 to 5 mg/kg at least until apparent regression is documented with annual echocardiographic follow-up.[360] For patients younger than 11 years old, no restriction on physical activity is indicated, but for those 11 years old and older, physical activity should be guided by a biennial stress test or myocardial perfusion test. Angiography should be performed if stenosis or ischemia is suggested.

Patients with One or More Large (>6-mm) or Giant (>8-mm) Coronary Aneurysm, or Multiple (Segmented) Smaller or Complex Aneurysms Without Obstruction (Risk Level IV)

Long-term antiplatelet therapy with aspirin 3 to 5 mg/kg once daily or clopidogrel 1 mg/kg/day up to adult dose of 75 mg is indicated for these children and should be continued indefinitely. Anticoagulant therapy with warfarin, with the international normalized ratio (INR) maintained at 2.0 to 2.5, or daily subcutaneous low-molecular-weight heparin should be added for patients with giant aneurysms because these patients are at substantial risk for coronary thrombosis. All such patients should be under the care of a pediatric cardiologist with experience in managing patients with Kawasaki disease. Cardiac evaluation with an echocardiogram and an electrocardiogram should be performed approximately every 6 months, with stress testing performed approximately annually. Angiography should be performed 6 to 12 months after the patient has recovered from the acute stage of disease to define the coronary anatomy, and it should be repeated whenever symptoms or stress tests suggest the presence of myocardial ischemia. Physical activity should be regulated on the basis of annual stress test results and level of anticoagulation, and participation in strenuous or contact athletics should be discouraged.

Patients with Coronary Artery Obstruction (Risk Level V)

Patients with obstructive lesions or signs of myocardial ischemia should be evaluated urgently for possible intervention. Balloon angioplasty, rotablator angioplasty, coronary artery bypass grafting, stent placement, and even cardiac transplantation all have been employed for patients with Kawasaki disease who have particularly serious coronary artery disease.[6,49,116,142,244,405] Arterial bypass grafts are superior to venous grafts in these patients.[174] Balloon angioplasty procedures have been associated with high rates of recurrent stenosis in patients with Kawasaki disease and coronary stenosis.[6]

NEW REFERENCES SINCE THE SIXTH EDITION

13. Anderson MS, Dominguez SR, Eladawy M, et al. Failure of CRP to fall predicts IVIG resistance in Kawasaki disease. Presented at 2011 meeting, Pediatric Academic Societies, Denver, Colorado, May 2011.
33. Burgner D, Davila S, Breunis WB, et al. A genome-wide association study identifies novel and functionally related susceptibility loci for Kawasaki disease. *PLoS Genet* 2009;**5**(1):e1000319.
45. Carbone I, Cannata D, Algeri E, et al. Adolescent Kawasaki disease: usefulness of 64-slice CT coronary angiography for follow-up investigation. *Pediatr Radiol* 2011;**41**:1165–73.
56. Cummings C, McCarthy P, van Hoff J, et al. Kawasaki disease associated with reactive hemophagocytic lymphohistiocytosis. *Pediatr Infect Dis J* 2008;**27**:116–18.
61. Daniels LB, Tjajadi MS, Walford HH, et al. Prevalence of Kawasaki disease in young adults with suspected myocardial ischemia. *Circulation* 2012;**125**:2447–53.
63. Davila S, Khor CC, Breunis WB, et al. A genome-wide association study identifies a new genetic susceptibility factor for Kawasaki disease. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
72. Dominguez S, Friedman K, Seewald R, et al. Kawasaki disease in a pediatric intensive care unit. *Pediatr* 2008;**122**:e786–90.
118. Gupta-Malhotra M, Gruber D, Abraham SS, et al. Atherosclerosis in survivors of Kawasaki disease. *J Pediatr* 2009;**155**:572–7.
132. Holman RC, Christensen KY, Belay ED, et al. Racial and ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. *Hawaii Med J* 2010;**69**:194–7.
134. Hong YM. Epidemiology of Kawasaki diseases in Korea. Presented at 10th International Kawasaki disease Symposium. Kyoto, Japan, February 2012.
136. Huang WC, Huang LM, Chang IS, et al. Epedimiologic features of Kawasaki disease in Taiwan 2003–6. *Pediatrics* 2009;**123**:e401–5.
146. Jia S, Li C, Wang G, et al. T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. *Clin Exp Immunol* 2010;**162**:131–7.
151. Kanegaye JT, Wilder MS, Molkara D, et al. Recognition of a Kawasaki disease shock syndrome. *Pediatr* 2009;**123**:e783–9.
170. Kim DS. Methotrexate in IVIG-resistant Kawasaki disease. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
179. Kobayashi T, Saji T, Otani T, et al. Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomised, open-label, blinded-endpoints trial. *Lancet* 2012;**379**:1613–20.
211. Li X, Li X, Li H, et al. Epidemiological study of Kawasaki disease in Sichuan, China. *J Trop Pediatr* 2008;**52**:133–6.
216. Manlhiot C, Millar K, Golding F, McCrindle BW. Improved classification of coronary artery abnormalities based only on coronary artery z-scores after Kawasaki disease. *Pediatr Cardiol* 2010;**31**:242–9.
223. Matsuura H, Nakagawa M, Ogino H, et al. Safety and effectiveness of infliximab in patients with IVIG-resistant acute Kawasaki disease. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
227. McCrindle BW. Kawasaki disease: a childhood disease with important consequences into adulthood. *Circulation* 2009;**120**:6–8.

235. Miura M, Fukushima N, Tamame T, et al. Steroid pulse therapy for Kawasaki disease unresponsive to additional immunoglobulin therapy. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
244. Muta H, Ishii M. Percutaneous coronary intervention versus coronary bypass grafting for stenotic lesions after Kawasaki disease. *J Pediatr* 2010;**157**:120–6.
249. Nakamura Y. Forty-one years after the 1st report of Kawasaki disease in Japan. Proceedings of Ninth International Kawasaki Disease Symposium. Taipei, April 2008.
264. Niboshi A, Hamaoka K, Sakata K, et al. Endothelial dysfunction in adult patients with history of Kawasaki disease. *Eur J Pediatr* 2008;**167**:189–96.
271. Ogata S, Ogihara Y, Honda T, et al. Corticosteroid pulse combination therapy for refractory Kawasaki disease: a randomized trial. *Pediatrics* 2012;**129**:1–7.
279. Onouchi Y, Gunji T, Burns JC, et al. ITPKC functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. *Nat Genet* 2008;**40**:35–42.
280. Onouchi Y, Ozaki K, Burns JC, et al. Common variants in *CASP3* confer susceptibility to Kawasaki disease. *Hum Mol Genet* 2010; **19**:2895–906.
281. Onouchi Y, Ozaki K, Burns JC, et al. A genome-wide association study identifies three new loci for Kawasaki disease. *Nat Genet* 2012;**44**:517–21.
282. Orenstein J, Shulman ST, Fox LM, et al. Three linked vasculopathic processes characterize Kawasaki disease: a light and transmission electron microscopic study. *PLoS One* 2012;**7**(6): e38998.
288. Park YW, Han JW, Hong VM, et al. Epidemiologic features of Kawasaki disease in Korea, 2006–08. *Pediatr Int* 2011;**53**:36–9.
291. Peng Y, Zeng J, Du Z, et al. Usefulness of 64 slice MDCT for Kawasaki disease. *Eur J Radiol* 2009;**69**:500–9.
302. Reindel R, Shulman ST. Corticosteroids as primary therapy in Kawasaki disease. *Nat Rev Rheumatol* 2012;**8**:373–4.
306. Rodo X, Ballester J, Cayan D, et al. Association of Kawasaki disease with tropospheric wind patterns. *Sci Rep* 2011;**1**:152.
313. Rowley AH, Baker SC, Shulman ST, et al. RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after Kawasaki disease. *PLoS One* 2008;**3**(2):e1582.
314. Rowley AH, Baker SC, Shulman ST, et al. Ultrastructural, immunofluorescence and RNA evidence support the hypothesis of a "new" virus associated with Kawasaki disease. *J Infect Dis* 2011; **203**(7):1021–30.
332. Schulte DJ, Yilmaz A, Shimada K, et al. Involvement of innate and adaptive immunity in a murine model of coronary arteritis mimicking Kawasaki disease. *J Immun* 2009;**183**:5311–18.
334. Senzaki H. Long-term outcome of Kawasaki disease. *Circulation* 2008;**118**:2769–72.
350. Shulman ST, Schuster JE, Innocentini N, Rowley AH. Hyponatremia is a feature of Kawasaki shock syndrome. Presentation at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
352. Sleeper LA, Minich LL, McCrindle BM, et al. Evaluation of Kawasaki disease risk-scoring systems for IVIG resistance. *J Pediatr* 2011;**158**:831–5.
358. Suda K, Iemura M, Nishiono H, et al. Long-term prognosis of Kawasaki disease complicated by giant coronary aneurysms. *Circulation* 2011;**123**:1836–42.
360. Sugahara Y, Ishii M, Muta H, et al. Warfarin therapy for giant aneurysm prevents myocardial infarction in Kawasaki disease. *Pediatr Cardiol* 2008;**29**:398–401.
373. Suzuki H. Cyclosporine A treatment for patients with Kawasaki disease resistant to IVIG. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
399. Tremoulet A, Best BM, Song S, et al. Resistance to IVIG in children with Kawasaki disease. *J Pediatr* 2008;**153**:117–21.
403. Tsuda E, Hirata T, Matsuo O, et al. The 30 year outcome for patients after myocardial infarction due to coronary artery lesions of Kawasaki disease. *Pediatr Cardiol* 2011;**32**:176–82.
408. Uehara R. Epidemiologic features of Kawasaki disease in Japan, 2007–10. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
409. Uehara R, Belay ED. Epidemiology of Kawasaki disease in Asia, Europe, and the U.S. *J Epidemiol* 2012;**22**(2):79–85.
434. Yu JJ, Jang WS, Ko HK, et al. Perivascular brightness of coronary arteries in Kawasaki disease. *J Pediatr* 2011;**159**:454–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abe J, Kotzin BL, Jujo K, et al. Selective expansion of T cells expressing T-cell receptor variable regions V beta 2 and V beta 8 in Kawasaki disease. *Proc Natl Acad Sci U S A* 1992;**89**:4066–70.
2. Abe J, Kotzin BL, Meissner C, et al. Characterization of T cell repertoire changes in acute Kawasaki disease. *J Exp Med* 1993; **177**:791–6.
3. Abe J, Takeda T, Ito Y, et al. TCR-V specificity of *Staphylococcus aureus* isolated from acute patients with Kawasaki syndrome. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 127–32.
4. Ahlström H, Lundström NR, Mortensson W, et al. Infantile periarteritis nodosa or mucocutaneous lymph node syndrome: a report of four cases and diagnostic considerations. *Acta Paediatr Scand* 1977;**66**:193–8.
5. Akagi T, Kato H, Inoue O, et al. Valvular heart disease in Kawasaki syndrome: incidence and natural history. *Am Heart J* 1990;**120**: 366–72.
6. Akagi T, Ogawa S, Ino T, et al. Catheter interventional therapy in Kawasaki disease. *J Pediatr* 2000;**137**:181–6.
7. Akagi T, Rose V, Benson LN, et al. Outcome of coronary artery aneurysms after Kawasaki disease. *J Pediatr* 1992;**121**:689–94.
8. Amano S, Hazama F, Hamashima Y. Pathology of Kawasaki disease, part I: pathology and morphogenesis of the vascular changes. *Jpn Circ J* 1979;**43**:633–43.
9. Amano S, Hazama F, Hamashima Y. Pathology of Kawasaki disease, part II: distribution and incidence of the vascular lesions. *Jpn Circ J* 1979;**43**:741–8.
10. Amano S, Hazama F, Kubagawa H, et al. General pathology of Kawasaki disease. *Acta Pathol Jpn* 1980;**30**:681–94.
11. Anand S, Yang YC. Optic disc changes in Kawasaki disease. *J Pediatr Ophthalmol Strabismus* 2004;**41**:177–9.
12. Anderson LJ, Morens DM, Hurwitz ES. Kawasaki disease in a young adult. *Arch Intern Med* 1980;**140**:280–1.
13. Anderson MS, Dominguez SR, Eladawy M, et al. Failure of CRP to fall predicts IVIG resistance in Kawasaki disease. Presented at 2011 meeting, Pediatric Academic Societies, Denver, Colorado, May 2011.
14. Asai T. Evaluation method for the degree of seriousness in Kawasaki disease. *Acta Paediatr Jpn* 1983;**25**:170–5.
15. Asano T, Sasaki N, Yashiro K, et al. Acute pancreatitis with Kawasaki disease. *Eur J Pediatr* 2005;**164**:180–1.
16. Aschner M, Aschner JL. Mucocutaneous lymph node syndrome: is there a relationship to mercury exposure? *Am J Dis Child* 1989;**143**:1133–4.
17. Ayusawa M, Sonobe T, Uemura S, et al. Revision of diagnostic guidelines for Kawasaki disease (5th revised edition). *Pediatr Int* 2005;**47**:232–4.
18. Barron KS, Montalvo JF, Joseph AK, et al. Soluble interleukin-2 receptors in children with Kawasaki syndrome. *Arthritis Rheum* 1990;**33**:1371–7.
19. Barron KS, Murphy DJ, Silverman ED, et al. Treatment of Kawasaki syndrome: a comparison of two dosage regimens of intravenously administered globulins. *J Pediatr* 1990;**117**:638–44.
20. Becker AE. Kawasaki disease. *Lancet* 1976;**1**:864.
21. Becker AE, Beekman RP, van der Hal AL. De infantiele polyarteritis nodosa en de ziekte van Kawasaki ("muco-cutaneous lymph node syndrome"): Twee verschillende ziekten of uitingen van een zelfde ziekteproces? *Ned Tijdschr Geneeskd* 1976;**120**: 2147–51.
22. Belay ED, Erdman DD, Anderson LJ, et al. Kawasaki disease and human coronavirus. *J Infect Dis* 2005;**192**:352–3.
23. Belay ED, Maddox RA, Holman RC, et al. Kawasaki syndrome and risk factors for coronary artery abnormalities: United States, 1994–2003. *Pediatr Infect Dis J* 2006;**25**:245–9.
24. Bell DM, Brink EW, Nitzkin J, et al. Kawasaki syndrome: description of two outbreaks in the United States. *N Engl J Med* 1981; **304**:1568–75.
25. Bell DM, Morens DM, Holman RC, et al. Kawasaki syndrome in the United States. *Am J Dis Child* 1983;**137**:211–24.
26. Birdi N, Klassen T, Quinlan A, et al. Role of the toxic neutrophil count in the early diagnosis of Kawasaki disease. *J Rheumatol* 1999;**26**:904–8.
27. Blum-Hoffmann E, Hoffman GF, Wessel A, et al. Kawasaki syndrome: association mit der exposition von teppichshampoo und erfolgreiche therapie mit immunoglobulinen in der zweiten krankheitswoche. *Monatsschr Kinderheilkd* 1992;**140**:273–6.
28. Boven K, De Fraeff-Meeder ER, Spliet W, et al. Atypical Kawasaki disease: an often missed diagnosis. *Eur J Pediatr* 1992;**151**: 577–80.
29. Bronstein DE, Dille AN, Austin JP, et al. Relationship of climate, ethnicity, and socioeconomic status to Kawasaki disease in San Diego County, 1994–1998. *Pediatr Infect Dis J* 2000;**19**:1087–91.
30. Brosius CL, Newburger JW, Burns JC, et al. Increased prevalence of atopic dermatitis in Kawasaki disease. *Pediatr Infect Dis J* 1988; **7**:863–6.
31. Brown TJ, Crawford SE, Shulman ST, et al. CD8 positive cells and macrophages infiltrate coronary artery aneurysms in acute Kawasaki disease. *Pediatr Res* 2001;**49**:13A.
32. Bülow SL, Hansen US, Hansen D, et al. Kawasaki's syndrom: Forekomst i Danmark i perioden 1981–1990. *Ugeskr Laeger* 1994; **156**:4813–16.
33. Burgner D, Davila S, Breunis WB, et al. A genome-wide association study identifies novel and functionally related susceptibility loci for Kawasaki disease. *PLoS Genet* 2009;**5**(1):e1000319.
34. Burke MJ, Rennebohm RM. Eye involvement in Kawasaki disease. *J Pediatr Ophthalmol Strabismus* 1981;**18**:7–11.
35. Burns JC. The riddle of Kawasaki disease. *N Engl J Med* 2007; **356**:659–61.
36. Burns JC, Cayan DR, Tong G, et al. Seasonality and temporal clustering of Kawasaki syndrome. *Epidemiology* 2005;**16**:220–5.
37. Burns JC, Geha RS, Schneeberger EE, et al. Polymerase activity in lymphocyte culture supernatants from patients with Kawasaki disease. *Nature* 1987;**323**:814–16.
38. Burns JC, Glodé MP. Kawasaki syndrome. *Lancet* 2004;**364**: 533–44.
39. Burns JC, Mason WH, Glodé MP, et al. Clinical and epidemiologic characteristics of patients referred for evaluation of possible Kawasaki disease. *J Pediatr* 1991;**118**:680–6.
40. Burns JC, Mason WH, Hauger SB, et al. Infliximab treatment for refractory Kawasaki syndrome. *J Pediatr* 2005;**146**:662–7.
41. Burns JC, Shimizu C, Gonzalez E, et al. Genetic variations in the receptor-ligand pair CCR5 and CCL31 are important determinants of susceptibility to Kawasaki disease. *J Infect Dis* 2005;**192**: 344–9.
42. Burns JC, Wiggins JW, Toews WH, et al. Clinical spectrum of Kawasaki syndrome in infants younger than 6 months of age. *J Pediatr* 1986;**109**:759–63.
43. Butler DF, Hough DR, Friedman SJ, et al. Adult Kawasaki syndrome. *Arch Dermatol* 1987;**123**:1356–61.
44. Cabana VG, Gidding SS, Getz GS, et al. Serum amyloid A and high density lipoprotein participate in the acute phase response of Kawasaki disease. *Pediatr Res* 1997;**42**:651–5.
45. Carbone I, Cannata D, Algeri E, et al. Adolescent Kawasaki disease: usefulness of 64-slice CT coronary angiography for follow-up investigation. *Pediatr Radiol* 2011;**41**:1165–73.
46. Caron GA. Kawasaki disease in an adult. *JAMA* 1980;**243**:430.
47. Casey F, Craig B, Shanks D, et al. Kawasaki disease: the Northern Ireland experience. *Ir J Med Sci* 1993;**162**:397–400.
48. Chang L-Y, Chiang B-L, Kao C-L, et al. Lack of association between infection with a novel coronavirus and Kawasaki disease in Taiwan. *J Infect Dis* 2006;**193**:283–6.
49. Checci PA, Pahl E, Shaddy R, et al. Cardiac transplantation for Kawasaki disease. *Pediatrics* 1997;**100**:695–9.
50. Cheung YF, Yung TC, Tam SC, et al. Novel and traditional cardiovascular risk factors in children after Kawasaki disease. *J Am Coll Cardiol* 2004;**43**:120–4.
51. Choi I, Chwae Y, Shim W, et al. Clonal expansion of CD8 positive T cells in Kawasaki disease. *J Immunol* 1997;**159**:481–6.
52. Chuang CH, Hsiao MH, Chiu CH, et al. Kawasaki disease in infants three months or younger. *J Microb Immunol Infect* 2006; **39**:387–91.
53. Chung KJ, and U.S. Multicenter Kawasaki Study Group. Incidence and prognosis of giant coronary artery aneurysms in Kawasaki disease. *Circulation* 1989;**80**(Suppl. II):II-282.
54. Comenzo RL, Malachowski SE, Meissner HC, et al. Immune hemolysis, DIC, and serum sickness after large doses of IVIG for Kawasaki disease. *J Pediatr* 1992;**120**:926–8.
55. Crocker DW, Sobin S, Thomas WC. Aneurysms of the coronary arteries: report of three cases in infants and review of the literature. *Am J Pathol* 1957;**33**:819–43.

56. Cummings C, McCarthy P, van Hoff J, et al. Kawasaki disease associated with reactive hemophagocyctic lymphohistiocytosis. *Pediatr Infect Dis J* 2008;**27**:116–18.
57. Cunningham MW, Meissner HC, Heuser JS, et al. Anti-human cardiac myosin autoantibodies in Kawasaki disease. *J Immunol* 1999;**163**:1060–5.
58. Curtis N, Zheng R, Lamb JR, et al. Evidence for a superantigen-mediated process in Kawasaki disease. *Arch Dis Child* 1995;**72**: 308–11.
59. Dajani AS, Taubert KA, Gerber MA, et al. Diagnosis and therapy of Kawasaki disease in children. *Circulation* 1993;**87**:1776–80.
60. Dajani AS, Taubert KA, Takahashi M, et al. Guidelines for long-term management of patients with Kawasaki disease. *Circulation* 1994;**89**:916–22.
61. Daniels LB, Tjajadi MS, Walford HH, et al. Prevalence of Kawasaki disease in young adults with suspected myocardial ischemia. *Circulation* 2012;**125**:2447–53.
62. Daniels SR, Specker B. Association of rug shampooing and Kawasaki disease. *J Pediatr* 1991;**118**:485–8.
63. Davila S, Khor CC, Breunis WB, et al. A genome-wide association study identifies a new genetic susceptibility factor for Kawasaki disease. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
64. Davis RL, Waller PL, Mueller BA, et al. Kawasaki syndrome in Washington state: race-specific incidence rates and residential proximity to water. *Arch Pediatr Adolesc Med* 1995;**149**:66–9.
65. DeInocencio J, Hirsch R. The role of T cells in Kawasaki disease. *Crit Rev Immunol* 1995;**15**:349–57.
66. Dengler LD, Capparelli EV, Bastian JF, et al. Cerebrospinal fluid profile in acute Kawasaki disease. *Pediatr Infect Dis J* 1998;**17**: 478–81.
67. Dennis MK, Ayoub EM, Graham T, et al. Mucocutaneous lymph node syndrome in Florida. *J Fla Med Assoc* 1977;**64**:21–6.
68. Dergun M, Kao A, Hauger S, et al. Familial occurrence of Kawasaki syndrome in North America. *Arch Pediatr Adolesc Med* 2005;**159**:876–81.
69. de Zorzi A, Colan SD, Gauvreau K, et al. Coronary artery dimensions may be misclassified as normal in Kawasaki disease. *J Pediatr* 1998;**133**:354–8.
70. Dhillon R, Clarkson P, Donald SE, et al. Endothelial dysfunction late after Kawasaki disease. *Circulation* 1996;**94**:2103–6.
71. Dominguez S, Anderson MS, Glodé MP, et al. Blinded case-control study of the relationship between human coronavirus NL63 and Kawasaki disease. *J Infect Dis* 2006;**194**:1697–701.
72. Dominguez S, Friedman K, Seewald R, et al. Kawasaki disease in a pediatric intensive care unit. *Pediatr* 2008;**122**:e786–90.
73. Durall AL, Phillips JR, Weisse ME, et al. Infantile Kawasaki disease and peripheral gangrene. *J Pediatr* 2006;**149**:131–3.
74. Du ZD, Zhao D, Du J, et al. Epidemiologic study on Kawasaki disease in Beijing from 2000 through 2004. *Pediatr Infect Dis J* 2007;**26**:449–51.
75. Durongpisitkul K, Gururaj VJ, Park JM, et al. The prevention of coronary artery aneurysm in Kawasaki disease. *Pediatrics* 1995;**96**: 1057–61.
76. Eberhard BA, Anderson U, Laxer RM, et al. Evaluation of the cytokine response in Kawasaki disease. *Pediatr Infect Dis J* 1995;**14**:199–203.
77. Eberhard BA, Sundel RP, Newburger JW, et al. Psoriatic eruption in Kawasaki disease. *J Pediatr* 2000;**137**:578–80.
78. Ebihara T, Endo R, Ma X, et al. Lack of association between New Haven coronavirus and Kawasaki disease. *J Infect Dis* 2005;**192**:351.
79. Egami K, Muta H, Ishii M, et al. Prediction of resistance to IVIG treatment in patients with Kawasaki disease. *J Pediatr* 2006;**149**: 237–40.
80. Engle MA, Fatica NS, Bussel JB, et al. Clinical trial of single dose intravenous gamma globulin in Kawasaki disease: preliminary report. *Am J Dis Child* 1989;**143**:1300–4.
81. Esper F, Shapiro ED, Weibel C, et al. Association between a novel human coronavirus and Kawasaki disease. *J Infect Dis* 2005;**191**: 499–502.
82. Everett ED. Mucocutaneous lymph node syndrome (Kawasaki's disease) in adults. *JAMA* 1979;**242**:542–3.
83. Fager DB, Bigler JA, Simonds JP. Polyarteritis nodosa in infancy and childhood. *J Pediatr* 1951;**39**:65–79.
84. Falcini F, Cimaz R, Calabri GB, et al. Kawasaki disease in northern Italy. *Clin Exp Rheumatol* 2002;**20**:421–6.
85. Fatica NS, Ichida F, Engle MA, et al. Rug shampoo and Kawasaki disease. *Pediatrics* 1989;**84**:231–4.
86. Felz M, Patni A, Brooks S, et al. Periorbital vasculitis complicating Kawasaki syndrome in an infant. *Pediatrics* 1998;**101**:e9.
87. Fong NC, Hui YW, Li CK, et al. Evaluation of the efficacy of treatment of Kawasaki disease before day 5 of illness. *Pediatr Cardiol* 2004;**25**:31–4.
88. Freeman AF, Crawford SE, Finn LS, et al. Inflammatory pulmonary nodules in Kawasaki disease. *Pediatr Pulmonol* 2003;**36**: 102–6.
89. Freeman AF, Shulman ST. Refractory Kawasaki disease. *Pediatr Infect Dis J* 2004;**23**:463.
90. Fujimoto T, Kato H, Ichiose E, et al. Immune complex and mite antigen in Kawasaki disease. *Lancet* 1982;**2**:980–1.
91. Fujita Y, Nakamura Y, Sakata K, et al. Kawasaki disease in families. *Pediatrics* 1989;**84**:666–9.
92. Fujiwara H, Fujiwara T, Kao TC, et al. Pathology of Kawasaki disease in the healed stage: relationships between typical and atypical cases of Kawasaki disease. *Acta Pathol Jpn* 1986;**36**: 857–67.
93. Fujiwara T, Fujiwara H, Nakano H. Pathological features of coronary arteries in children with Kawasaki disease in which coronary arterial aneurysm was absent at autopsy: quantitative analysis. *Circulation* 1988;**78**:345–50.
94. Fujiwara H, Hamashima Y. Pathology of the heart in Kawasaki disease. *Pediatrics* 1978;**61**:100–7.
95. Fujiwara H, Kawai C, Hamashima Y. Clinico-pathologic study of the conduction systems in 10 patients with Kawasaki's disease (mucocutaneous lymph node syndrome). *Am Heart J* 1978;**96**: 744–50.
96. Fukushige J, Takahashi N, Ueda Y. Incidence and clinical features of incomplete Kawasaki disease. *Acta Paediatr* 1994;**83**:1057–60.
97. Fulton DR. Effects of current therapy of Kawasaki disease on eicosanoid metabolism. *Am J Cardiol* 1988;**61**:1323–7.
98. Furukawa S, Imai K, Matsubara T, et al. Increased levels of ICAM-1 in Kawasaki disease. *Arthritis Rheum* 1992;**35**:672–7.
99. Furukawa S, Matsubara T, Jujoh K, et al. Peripheral blood monocyte/macrophages and serum tumor necrosis factor in Kawasaki disease. *Clin Immunol Immunopathol* 1988;**48**:247–51.
100. Furukawa S, Matsubara T, Jujoh K, et al. Reduction of peripheral blood macrophages/monocytes in Kawasaki disease by intravenous gamma globulin. *Eur J Pediatr* 1990;**150**:43–7.
101. Furukawa S, Matsubara T, Motohashi T, et al. Expression of Fc,R2/CD23 on peripheral blood macrophages/monocytes in Kawasaki disease. *Clin Immunol Immunopathol* 1990;**56**:280–6.
102. Furukawa S, Matsubara T, Yabuta K. Mononuclear cell subsets and coronary artery lesions in Kawasaki disease. *Arch Dis Child* 1992;**67**:706–8.
103. Furumoto H, Sakano T, Tanabe A, et al. Serum soluble CD8 antigen level is not elevated in mucocutaneous lymph node syndrome (Kawasaki disease) in spite of an increase in serum soluble interleukin 2 receptors. *Eur J Pediatr* 1990;**149**:448–9.
104. Furusho K, Kamiya T, Nakano H, et al. High-dose intravenous gamma globulin for Kawasaki disease. *Lancet* 1984;**2**:1055–8.
105. Furusho K, Ohba T, Soeda T, et al. Possible role for mite antigen in Kawasaki disease. *Lancet* 1981;**2**:194–5.
106. Gallagher PG. Facial nerve paralysis and Kawasaki disease. *Rev Infect Dis* 1990;**12**:403–5.
107. Gavin PJ, Crawford SE, Shulman ST, et al. Systemic arterial expression of matrix metalloproteinases-2 and -9 in acute Kawasaki disease. *Arterioscl Thromb Vasc Biol* 2003;**23**:576–81.
108. Gee SJ. Aneurysms of the coronary arteries in a boy. *St Barth Hosp Rep Lond* 1871;**7**:148.
109. Germain BF, Moroney JD, Guggino GS, et al. Anterior uveitis in Kawasaki disease. *J Pediatr* 1980;**97**:780–1.
110. Gidding SS, Duffy CE, Pajcic S, et al. Usefulness of echocardiographic evidence of pericardial effusion and mitral regurgitation in predicting coronary artery aneurysms. *Am J Cardiol* 1987;**60**: 76–9.
111. Gidding SS, Shulman ST, Ilbawi M, et al. Mucocutaneous lymph node syndrome (Kawasaki disease): delayed aortic and mitral insufficiency secondary to active valvulitis. *J Am Coll Cardiol* 1986;**7**:894–7.
112. Giesker DW, Krause PJ, Pastuszak WT, et al. Lymph node biopsy for early diagnosis in Kawasaki disease. *Am J Surg Pathol* 1982;**6**: 493–501.

113. Glanzer JM, Galbraith WB, Jacobs JP. Kawasaki disease in a 28-year-old man. *JAMA* 1980;**244**:1604–6.
114. Glode MP, Brogden R, Joffe LS, et al. Kawasaki syndrome and house dust mite exposure. *Pediatr Infect Dis J* 1986;**5**:644–8.
115. Gong GWK, McCrindle BW, Ching JC, et al. Arthritis presenting during the acute phase of Kawasaki disease. *J Pediatr* 2006;**148**: 800–5.
116. Gotteiner N, Mavroudis C, Backer CL, et al. Coronary artery bypass grafting for Kawasaki disease. *Pediatr Cardiol* 2002;**23**: 62–7.
117. Greil GF, Seeger A, Miller S, et al. Coronary magnetic resonance angiography and vessel wall imaging in children with Kawasaki disease. *Pediatr Radiol* 2007;**37**:666–73.
118. Gupta-Malhotra M, Gruber D, Abraham SS, et al. Atherosclerosis in survivors of Kawasaki disease. *J Pediatr* 2009;**155**:572–7.
119. Hamada R, Uehara R, Fuyama Y. CT detection of coronary calcification in Kawasaki disease. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 598–602.
120. Hamasaki Y, Miyazaki S. Leukotriene B_4 and Kawasaki disease. *Acta Paediatr Jpn* 1991;**33**:771–7.
121. Hansen RC. Staphylococcal scaled skin syndrome, toxic shock syndrome, and Kawasaki disease. *Pediatr Clin North Am* 1983;**30**: 533–44.
122. Han RK, Silverman ED, Newman A, et al. Management and outcome of persistent or recurrent fever after initial intravenous gamma globulin therapy in acute Kawasaki disease. *Arch Pediatr Adolesc Med* 2000;**154**:694–9.
123. Harada K, Yamaguchi H, Kato H, et al. Indication for IVGG in Kawasaki disease. In: Takahashi M, Taubert KA, editors. *Proceedings of Fourth International Symposium on Kawasaki Disease*. Dallas: American Heart Association; 1993. p. 459–62.
124. Hayasaka S, Nakamura Y, Yashiro M, et al. Analyses of fatal cases of Kawasaki disease in Japan. *J Epidemiol* 2003;**13**:246–50.
125. Harnden A, Alves B, Sheikh A. Rising incidence of Kawasaki disease in England: analysis of hospital admission data. *BMJ* 2002;**324**:1424–5.
126. Hashino K, Ishii M, Iemura M, et al. Retreatment for immune globulin resistant Kawasaki disease. *Pediatr Int* 2001;**43**:211–17.
127. Herman A, Kappler JW, Marrack P, et al. Superantigens: mechanism of T cell stimulation and role in immunologic responses. *Annu Rev Immunol* 1991;**9**:745–72.
128. Herold BC, Davis AT, Arroyave CM, et al. Cryoprecipitates in Kawasaki syndrome: association with coronary artery aneurysms. *Pediatr Infect Dis J* 1988;**7**:255–7.
129. Hicks RV, Melish ME. Kawasaki syndrome: rheumatic complaints and analysis of salicylate therapy. *Arthritis Rheum* 1979;**22**:621–2.
130. Hidaka T, Nakano M, Ueta T, et al. Increased synthesis of thromboxane A_2 by platelets from patients with Kawasaki disease. *J Pediatr* 1983;**102**:94–6.
131. Hirao J, Hibi S, Andoh T, et al. High levels of circulating IL-4 and IL-10 in Kawasaki disease. *Int Arch Allergy Immunol* 1997; **112**:152–6.
132. Holman RC, Christensen KY, Belay ED, et al. Racial and ethnic differences in the incidence of Kawasaki syndrome among children in Hawaii. *Hawaii Med J* 2010;**69**:194–7.
133. Holman RC, Curns AT, Belay ED, et al. Kawasaki syndrome hospitalizations in the United States, 1997 and 2000. *Pediatrics* 2003;**112**:495–501.
134. Hong YM. Epidemiology of Kawasaki diseases in Korea. Presented at 10th International Kawasaki Disease Symposium. Kyoto, Japan, February 2012.
135. Hurvitz H, Branski D, Gross-Kieselstein E, et al. Acetaminophen hypersensitivity resembling Kawasaki disease. *Isr J Med Sci* 1984; **20**:145–7.
136. Huang WC, Huang LM, Chang IS, et al. Epidemiologic features of Kawasaki disease in Taiwan, 2003–2006. *Pediatrics* 2009;**123**: e401–5.
137. Ichida F, Fatica NS, O'Loughlin JE, et al. Epidemiologic aspects of Kawasaki disease in a Manhattan hospital. *Pediatrics* 1989;**84**: 235–41.
138. Iemura M, Ishii M, Sugimura T, et al. Long-term consequences of regressed coronary aneurysms after Kawasaki disease: vascular wall morphology and function. *Heart* 2000;**83**:3047–11.
139. Imada Y, Kawasaki T, Nakamura Y. Cousin cases of Kawasaki disease suggesting person-to-person transmission. *Pediatrics* 1990; **85**:1127.
140. Inoue Y, Okada Y, Shinohara M, et al. A multicenter prospective randomized trial of corticosteroids in primary therapy for Kawasaki disease. *J Pediatr* 2006;**149**:336–41.
141. Ishii A, Yatani T, Kato H, et al. Mite fauna, house dust, and Kawasaki disease. *Lancet* 1983;**2**:102–3.
142. Ishii M, Ueno T, Akagi T, et al. Guidelines for catheter intervention in coronary artery lesion in Kawasaki disease. *Pediatr Int* 2001;**43**:558–62.
143. Jacob JL, Polomeno RC, Chad Z, et al. Ocular manifestations of Kawasaki disease (mucocutaneous lymph node syndrome). *Can J Ophthalmol* 1982;**17**:199–202.
144. Jacobs JC. Salicylate treatment of epidemic Kawasaki disease in New York City. *Ther Drug Monit* 1979;**1**:123–30.
145. Japanese Kawasaki Disease Research Committee. Shonika Rinsho. *Jpn J Pediatr* 1971;**24**:2545–59.
146. Jia S, Li C, Wang G, et al. T helper type 17/regulatory T cell imbalance in patients with acute Kawasaki disease. *Clin Exp Immunol* 2010;**162**:131–7.
147. Jibiki T, Terai M, Kurosaki T, et al. Efficacy of IVIG therapy combined with dexamethasone for the initial treatment of acute Kawasaki disease. *Eur J Pediatr* 2004;**163**:229–33.
148. Johnson RM. Kawasaki-like syndromes associated with HIV infection. *Clin Infect Dis* 2001;**32**:1628–34.
149. Jordan SC, Platts-Mills TA, Mason W, et al. Lack of evidence for mite-antigen–mediated pathogenesis in Kawasaki disease. *Lancet* 1983;**1**:931.
150. Kamiya T, Suzuki A, Ono Y, et al. Angiographic follow-up study of coronary artery lesion in the cases with a history of Kawasaki disease: with a focus on the follow-up more than 10 years after the onset of the disease. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 569–73.
151. Kanegaye JT, Wilder MS, Molkara D, et al. Recognition of a Kawasaki disease shock syndrome. *Pediatr* 2009;**123**:e783–9.
152. Kaneko K, Savage CO, Pottinger BE, et al. Antiendothelial cell antibodies can be cytotoxic to endothelial cells without cytokine pre-stimulation and correlate with ELISA antibody measurement in Kawasaki disease. *Clin Exp Immunol* 1994;**98**:264–9.
153. Kato H. Natural history of Kawasaki disease. In: Shiokawa Y, editor. *Vascular lesions of collagen diseases and related conditions*. Baltimore: University Park Press; 1977. p. 281–6.
154. Kato H. Intracoronary thrombolytic therapy in Kawasaki disease: treatment and prevention of acute myocardial infarction. *Prog Clin Biol Res* 1987;**250**:445–54.
155. Kato H. Long-term consequences of Kawasaki disease: pediatrics to adults. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 557–66.
156. Kato H, Ichinose E. Cardiovascular involvement in Kawasaki disease. *Acta Paediatr Jpn* 1984;**26**:132–45.
157. Kato H, Ichinose E, Kawasaki T. Myocardial infarction in Kawasaki disease. *J Pediatr* 1986;**108**:923–8.
158. Kato H, Ichinose E, Yoshioka F, et al. Fate of coronary aneurysms in Kawasaki disease: serial coronary angiography and long-term follow-up study. *Am J Cardiol* 1982;**49**:1758–66.
159. Kato H, Inoue O, Akagi T. Kawasaki disease: cardiac problems and management. *Pediatr Rev* 1988;**9**:209–17.
160. Kato H, Inoue O, Kawasaki T, et al. Adult coronary artery disease probably due to childhood Kawasaki disease. *Lancet* 1992;**340**: 1127–9.
161. Kato H, Koike S, Yokoyama T. Kawasaki disease: effect of treatment of coronary artery involvement. *Pediatrics* 1979;**63**:175–9.
162. Kato H, Sugimura T, Akagi T, et al. Long-term consequences of Kawasaki disease. *Circulation* 1996;**94**:1279–85.
163. Kawasaki T. Non–scarlet fever syndrome with desquamation. *Chiba Igakukai Zasshi* 1962;**38**:279.
164. Kawasaki T. Acute febrile mucocutaneous syndrome with lymphoid involvement with specific desquamation of the fingers and toes in children. *Jpn J Allergy* 1967;**16**:178–222. [English translation, *Pediatr Infect Dis J* 2002;**21**:993–5].
165. Kawasaki T. Kawasaki disease. *Asian Med J* 1989;**32**:497–506.
166. Kawasaki T. Personal communication, March 2001.
167. Kawasaki T, Kosaki F, Okawa S, et al. A new infantile acute febrile mucocutaneous lymph node syndrome (MLNS) prevailing in Japan. *Pediatrics* 1974;**54**:271–6.
168. Kawasaki T, Kubo N, Sakata G, et al. Two autopsy cases of infantile periarteritis nodosa and their clinical findings: in relation to Kawasaki disease. *Chiryo J Ther* 1970;**52**:633–44.

169. Kim DS. Serum interleukin-6 in Kawasaki disease. *Yonsei Med J* 1992;**33**:183–8.
170. Kim DS. Methotrexate in IVIG-resistant Kawasaki disease. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
171. Kim DS, Lee KY. Serum soluble E-selectin levels in Kawasaki disease. *Scand J Rheumatol* 1995;**23**:283–6.
172. Kim DS, Lee HK, Noh GW, et al. Increased serum IL-10 in Kawasaki disease. *Yonsei Med J* 1996;**37**:125–30.
173. Kisimoto T. B-cell stimulatory factors (BSFs): molecular structure, biological function, and regulation of expression. *J Clin Immunol* 1987;**7**:343–55.
174. Kitamura S, Kamedo Y, Sekit S, et al. Long-term outcome of myocardial revascularization in Kawasaki disease. *J Thorac Cardiovasc Surg* 1994;**197**:663–74.
175. Kitamura S, Kawashima Y, Kawachi K, et al. Severe mitral regurgitation due to coronary arteritis of mucocutaneous lymph node syndrome. *J Thorac Cardiovasc Surg* 1980;**80**:629–36.
176. Knott PD, Orloff L, Harris JP, et al. Sensorineural hearing loss and Kawasaki disease. *Am J Otolaryngol* 2001;**22**:343–8.
177. Kobayashi S, Wada N, Kubo M. Antibodies to native type III collagen in the serum of patients with Kawasaki disease. *Eur J Pediatr* 1992;**151**:183–7.
178. Kobayashi T, Inoue Y, Takeuchi K, et al. Prediction of IVIG unresponsiveness in patients with Kawasaki disease. *Circulation* 2006;**113**:2606–12.
179. Kobayashi T, Saji T, Otani T, et al. Efficacy of immunoglobulin plus prednisolone for prevention of coronary artery abnormalities in severe Kawasaki disease (RAISE study): a randomised, open-label, blinded-endpoints trial. *Lancet* 2012;**379**:1613–20.
180. Kohsaka T, Abe J, Asahina T, et al. Classical pathway complement activation in Kawasaki syndrome. *J Allergy Clin Immunol* 1994; **93**:520–5.
181. Koren G, Schaffer F, Silverman ED, et al. Determinants of low serum salicylates in patients with Kawasaki disease. *J Pediatr* 1988;**112**:663–7.
182. Kosaki F, Kawasaki T, Okawa S, et al. Clinicopathological conference on 10 fatal cases with acute febrile mucocutaneous lymph node syndrome. *Shonika Rinsho (Jpn J Pediatr)* 1971;**24**: 2545–59.
183. Kotb M. Superantigens: A possible link between infection and autoimmunity. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 111–19.
184. Krous HF, Clausen CR, Ray CG. Elevated immunoglobulin E in infantile polyarteritis nodosa. *Pediatrics* 1974;**84**:841–5.
185. Krzyszkowski J. Periarteritis nodosa. *Przegl Post Nauk Lek (Warsaw)* 1899;**38**:30, 45, 58.
186. Kusakawa S, Heiner DC. Elevated levels of immunoglobulin E in the acute febrile mucocutaneous lymph node syndrome. *Pediatr Res* 1976;**10**:108–11.
187. Kusakawa S, Tatara K. Efficacies and risks of aspirin in the treatment of Kawasaki disease. *Prog Clin Biol Res* 1987;**20**:401–13.
188. Kussmaul A, Maier R. *Dtsch Arch Klin Med* 1866;**1**:484.
189. Landing BH, Larson EJ. Are infantile periarteritis nodosa with coronary artery involvement and fatal mucocutaneous lymph node syndrome the same? Comparison of 20 patients from North America with patients from Hawaii and Japan. *Pediatrics* 1977; **59**:651–62.
190. Lang BA, Silverman ED, Laxer RM, et al. Spontaneous tumor necrosis factor production in Kawasaki disease. *J Pediatr* 1989; **115**:939–43.
191. Lang BA, Silverman ED, Laxer RM, et al. Serum-soluble interleukin-2 receptor levels in Kawasaki disease. *J Pediatr* 1990; **116**:592–6.
192. Lapointe N, Chad Z, Lacroix J, et al. Kawasaki disease: association with uveitis in seven patients. *Pediatrics* 1982;**69**:376–9.
193. Larson EJ. Comparison of pathology of infantile periarteritis nodosa (IPN) with coronary artery disease in North America with Kawasaki disease (MCLS) in Hawaii and Japan. In: Japan Medical Research Foundation, editor. *Vascular lesions of collagen diseases and related conditions*. Tokyo: University of Tokyo Press; 1977. p. 322–34.
194. Lee DB. Epidemiological survey of Kawasaki syndrome in Korea (1976–1984). *J Cath Med Coll* 1985;**38**:13–19.
195. Lee T, Vaughan D. Mucocutaneous lymph node syndrome in a young adult. *Arch Intern Med* 1979;**139**:104–5.
196. Lee T, Furukawa S, Fukuda Y, et al. Plasma prostaglandin E_2 level in Kawasaki disease. *Prostaglandins Leukot Essent Fatty Acids* 1988; **31**:53–7.
197. Lehman TJA, Warren R, Gietl D, et al. Variable expression of *Lactobacillus casei* cell-wall–induced coronary arteritis: an animal model of Kawasaki's disease in selected inbred mouse strains. *Clin Immunol Immunopathol* 1988;**48**:108–18.
198. Leung DYM. Immunomodulation by intravenous immune globulin in Kawasaki disease. *J Allergy Clin Immunol* 1989;**84**: 588–94.
199. Leung DYM. The potential role of cytokine-mediated vascular endothelial cell activation in the pathogenesis of Kawasaki disease. *Acta Paediatr Jpn* 1991;**33**:739–44.
200. Leung DYM, Burns JC, Newburger JW, et al. Reversal of lymphocyte activation in vivo in the Kawasaki syndrome by intravenous gammaglobulin. *J Clin Invest* 1987;**79**:468–72.
201. Leung DYM, Chu ET, Wood N, et al. Immunoregulatory T cell abnormalities in mucocutaneous lymph node syndrome. *J Immunol* 1983;**130**:2002–4.
202. Leung DYM, Collins T, LaPierre LA, et al. Immunoglobulin M antibodies present in the acute phase of Kawasaki syndrome lyse cultured vascular endothelial cells stimulated by gamma interferon. *J Clin Invest* 1986;**77**:1428–35.
203. Leung DYM, Geha R, Newberger J. Two monokines, interleukin-1 and tumor necrosis factor, render cultured vascular endothelial cells susceptible to lysis by antibodies circulating during Kawasaki syndrome. *J Exp Med* 1986;**164**:1958–72.
204. Leung DYM, Kurt-Jones E, Newberger JW, et al. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet* 1989;**2**:1298–302.
205. Leung DYM, Meissner HC, Fulton DR, et al. Toxic shock syndrome toxin-secreting *Staphylococcus aureus* in Kawasaki syndrome. *Lancet* 1993;**342**:1385–8.
206. Leung DYM, Meissner HC, Shulman ST, et al. Prevalence of superantigen-secreting bacteria in patients with Kawasaki disease. *J Pediatr* 2002;**140**:742–6.
207. Leung DYM, Seigel RL, Grady S, et al. Immunoregulatory abnormalities in mucocutaneous lymph node syndrome. *Clin Immunol Immunopathol* 1982;**23**:100–12.
208. Levinsky R, Marshall WC. Circulating immune complexes in mucocutaneous lymph node syndrome. *Arch Dis Child* 1979;**54**: 240–5.
209. Levy M, Koren G. Atypical Kawasaki disease: analysis of clinical presentation and diagnostic clues. *Pediatr Infect Dis J* 1990;**9**: 122–6.
210. Li CR, Yang XQ, Shen J, et al. Immunoglobulin G subclasses in serum and circulating immune complexes in patients with Kawasaki syndrome. *Pediatr Infect Dis J* 1990;**9**:544–7.
211. Li X, Li X, Li H, et al. Epidemiological study of Kawasaki disease in Sichuan, China. *J Trop Pediatr* 2008;**52**:133–6.
212. Lin CY, Lin CC, Hwang B, et al. Serial changes of serum interleukin-6, interleukin-8, and tumor necrosis factor alpha among patients with Kawasaki disease. *J Pediatr* 1992;**121**: 924–6.
213. Lin CY, Lin CC, Hwang B, et al. Cytokines predict coronary aneurysm formation in Kawasaki disease patients. *Eur J Pediatr* 1993;**152**:309–12.
214. Lin FYC, Bailowitz A, Koslowe P, et al. Kawasaki syndrome: a case-control study during an outbreak in Maryland. *Am J Dis Child* 1985;**139**:277–9.
215. Lynch M, Holman RC, Mulligan A, et al. Kawasaki syndrome hospitalizations in Ireland, 1996–2000. *Pediatr Infect Dis J* 2003; **22**:959–62.
216. Manlhiot C, Millar K, Golding F, McCrindle BW. Improved classification of coronary artery abnormalities based only on coronary artery z-scores after Kawasaki disease. *Pediatr Cardiol* 2010;**31**: 242–9.
217. Marchette NJ, Cao Y, Kihara S, et al. Staphylococcal toxic shock syndrome toxin-1, one possible cause of Kawasaki syndrome. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 149–55.
218. Mason W, Jordan S, Sakai R, et al. Lack of effect of gammaglobulin infusion on circulating immune complexes in patients with Kawasaki syndrome. *Pediatr Infect Dis J* 1988;**7**:94–9.
219. Mason W, Schneider T, Takahashi M. The epidemiology and etiology of Kawasaki disease. *Cardiol Young* 1991;**1**:196–205.

220. Masuda H, Shozawa T, Naoe S, et al. The intercostal artery in Kawasaki disease: a pathologic study of 17 autopsy cases. *Arch Pathol Lab Med* 1986;**110**:1136–42.
221. Matsubara T, Furukawa S, Ino T, et al. A sibship with recurrent Kawasaki disease and coronary artery lesion. *Acta Paediatr* 1994; **83**:1002–4.
222. Matsubara T, Furukawa S, Yabuta K. Serum levels of tumor necrosis factor, interleukin-2 receptor, and interferon-gamma in Kawasaki disease involved coronary artery lesions. *Clin Immunol Immunopathol* 1990;**56**:29–36.
223. Matsuura H, Nakagawa M, Ogino H, et al. Safety and effectiveness of infliximab in patients with IVIG-resistant acute Kawasaki disease. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
224. Maury CP, Salo E, Pelkonen P. Circulating interleukin-1 beta in patients with Kawasaki disease. *N Engl J Med* 1988;**312**: 1670–1.
225. Maury CP, Salo E, Pelkonen P. Elevated circulating tumor necrosis factor-alpha in patients with Kawasaki disease. *J Lab Clin Med* 1989;**113**:651–4.
226. Mayatepek E, Lehmann WD. Increased generation of cysteinyl leukotrienes in Kawasaki disease. *Arch Dis Child* 1995;**72**:526–7.
227. McCrindle BW. Kawasaki disease: a childhood disease with important consequences into adulthood. *Circulation* 2009;**120**: 6–8.
228. Melish ME, Hicks RM, Larson EJ. Mucocutaneous lymph node syndrome (MCLS) in the U.S. *Pediatr Res* 1974;**8**:427.
229. Melish ME, Hicks RM, Larson EJ. Mucocutaneous lymph node syndrome in the United States. *Am J Dis Child* 1976;**130**: 599–607.
230. Melish ME, Marchette NJ, Kaplan JC, et al. Absence of significant RNA-dependent DNA polymerase activity in lymphocytes from patients with Kawasaki syndrome. *Nature* 1989;**337**:288–90.
231. Melish ME, Parsonett J, Marchette NJ. Kawasaki syndrome is not caused by toxic shock syndrome toxin-1 (TSST-1) positive staphylococci. *Pediatr Res* 1994;**35**:187A.
232. Melish ME, Takahashi M, Shulman ST, et al. Comparison of low dose aspirin versus high dose aspirin as an adjunct to IVGG for Kawasaki syndrome. *Pediatr Res* 1992;**31**:170A.
233. Milgrom H, Palmer EL, Slovin SF, et al. Kawasaki disease in a healthy young adult. *Ann Intern Med* 1980;**92**:467–70.
234. Mitani Y, Okada Y, Inoue M, et al. Impaired endothelium dependent relaxation of angiographically normal coronary arteries in patients after Kawasaki disease in the long-term follow-up period. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 587–91.
235. Miura M, Fukushima N, Tamame T, et al. Steroid pulse therapy for Kawasaki disease unresponsive to additional immunoglobulin therapy. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
236. Miura M, Garcia F, Crawford SE, et al. Macrophage infiltration of pancreatic acini and islets in acute Kawasaki disease. *Pediatr Infect Dis J* 2003;**22**:1106–8.
237. Miura M, Garcia FL, Crawford SE, et al. Cell adhesion molecule expression in coronary artery aneurysms in acute Kawasaki disease. *Pediatr Infect Dis J* 2004;**23**:931–6.
238. Morens DM. Kawasaki disease and rug shampooing. *J Pediatr* 1992;**120**:333–4.
239. Mori M, Imagawa T, Yasui K, et al. Predictors of coronary artery lesions after IVGG treatment in Kawasaki disease. *J Pediatr* 2000; **137**:177–80.
240. Morise T, Takeuchi Y, Takeda R, et al. Increased plasma endothelin levels in Kawasaki disease: a possible marker for Kawasaki disease. *Angiology* 1993;**44**:719–23.
241. Muise A, Tallett SE, Silverman ED. Are children with Kawasaki disease and prolonged fever at risk for macrophage activation syndrome? *Pediatrics* 2003;**12**:e495–7.
242. Munro-Faure H. Necrotizing arteritis of the coronary vessels in infancy: case report and review of the literature. *Pediatrics* 1959;**23**:914–26.
243. Muta H, Ishii M, Egami K, et al. Early IVGG treatment for Kawasaki disease: the nationwide surveys in Japan. *J Pediatr* 2004;**144**:496–9.
244. Muta H, Ishii M. Percutaneous coronary intervention versus coronary bypass grafting for stenotic lesions after Kawasaki disease. *J Pediatr* 2010;**157**:120–6.
245. Muzik O, Paridon SM, Singh TP, et al. Quantification of myocardial blood flow and flow reserve in children with a history of Kawasaki disease and normal coronary arteries. *J Am Coll Cardiol* 1996;**28**:757–62.
246. Myones B, Tomita S, Shulman ST. Intravenous IgG administration is associated with a decrease in classical pathway activation products in Kawasaki disease. *Arthritis Rheum* 1991;**34**(Suppl.): S44.
247. Nagashima M, Matsushima M, Matsuoka H, et al. High-dose gamma globulin therapy for Kawasaki disease. *J Pediatr* 1987; **110**:710–12.
248. Nagata S, Yamashiro Y, Maeda M, et al. Immunohistochemical studies on small intestinal mucosa in Kawasaki disease. *Pediatr Res* 1993;**33**:557–63.
249. Nakamura Y. Forty-one years after the 1st report of Kawasaki disease in Japan. Proceedings of Ninth International Kawasaki Disease Symposium. Taipei, April 2008.
250. Nakamura Y, Hirose K, Yanagawa H, et al. Incidence rate of recurrent Kawasaki disease in Japan. *Acta Paediatr* 1994;**83**:1061–4.
251. Nakamura Y, Yanagawa H, Harada K, et al. Mortality among persons with a history of Kawasaki disease in Japan: the fifth look. *Arch Pediatr Adolesc Med* 2002;**156**:162–5.
252. Nakamura Y, Yanagawa H, Kawasaki T. Temporal and geographical clustering of Kawasaki disease in Japan. *Prog Clin Biol Res* 1987; **250**:19–32.
253. Nakamura Y, Yanagawa H, Kawasaki T. Mortality among children with Kawasaki disease in Japan. *N Engl J Med* 1992;**326**:1246–9.
254. Nakano H, Ueda K, Saito A, et al. Scoring method for identifying patients with Kawasaki disease at high risk of coronary artery aneurysms. *Am J Cardiol* 1986;**58**:739–42.
255. Naoe S, Shibuya K, Takahashi M, et al. Pathological observations concerning the cardiovascular lesions in Kawasaki disease. *Cardiol Young* 1991;**1**:212–20.
256. Naoe S, Takahashi K, Masuda H. Kawasaki disease with particular emphasis on arterial lesions. *Acta Pathol Jpn* 1991;**41**:785–97.
257. Newburger JW, Takahashi M, Beiser AS, et al. A single intravenous infusion of gamma globulin as compared with four infusions in the treatment of acute Kawasaki syndrome. *N Engl J Med* 1991;**324**:1633–9.
258. Newburger JW, Burns JC, Beiser AS, et al. Altered lipid profile after Kawasaki disease. *Circulation* 1991;**85**:625–31.
259. Newburger JW, Sanders SP, Burns JC, et al. Left ventricular contractility and function in Kawasaki disease. *Circulation* 1989; **79**:1237–46.
260. Newburger JW, Sleeper LA, McCrindle BW, et al. Randomized trial of pulse steroid therapy for primary treatment of Kawasaki disease. *N Engl J Med* 2007;**356**:663–75.
261. Newburger JW, Takahashi M, Burns JC, et al. The treatment of Kawasaki syndrome with intravenous gamma globulin. *N Engl J Med* 1986;**315**:341–7.
262. Newburger JW, Takahashi M, Gerber MA, et al. Diagnosis, treatment, and long-term management of Kawasaki disease. American Heart Association Scientific Statement. *Circulation* 2004;**110**: 2747–71.
263. Ng YM, Sung RY, So LF, et al. Kawasaki disease in Hong Kong, 1994–2000. *Hong Kong Med J* 2005;**11**:331–5.
264. Niboshi A, Hamaoka K, Sakata K, et al. Endothelial dysfunction in adult patients with history of Kawasaki disease. *Eur J Pediatr* 2008;**167**:189–96.
265. Nigrovic LE, Nigrovic PA, Harper MB, et al. Extreme thrombocytosis predicts Kawasaki disease in infants. *Clin Pediatr* 2006; **45**:446–52.
266. Nishiyori A, Sakaguchi M, Kato H, et al. Toxic shock syndrome toxin 1 and V2 expression on T cells in Kawasaki disease. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 139–43.
267. Niwa K, Aotsuka M, Karasawa K, et al. Thrombocytopenia: a risk factor for acute myocardial infarction during acute Kawasaki disease. *Coron Artery Dis* 1995;**6**:857–64.
268. Niwa Y, Sohmiya K. Enhanced neutrophilic functions in mucocutaneous lymph node syndrome, with special reference to the possible role of increased oxygen intermediate generation in the pathogenesis of coronary thromboarteritis. *J Pediatr* 1984;**104**: 56–60.
269. Nofech-Mozes Y, Garty B-Z. Thrombocytopenia in Kawasaki disease. *Pediatr Hematol Oncol* 2003;**20**:597–601.

270. Noto N, Ayusawa M, Karasawa K, et al. Dobutamine stress echocardiography for detection of coronary artery stenosis in children with Kawasaki disease. *J Am Coll Cardiol* 1996;**27**:1251–6.
271. Ogata S, Ogihara Y, Honda T, et al. Corticosteroid pulse combination therapy for refractory Kawasaki disease: a randomized trial. *Pediatrics* 2012;**129**:1–7.
272. Ogawa S, Zhang J, Yuge K, et al. Increased plasma endothelin-1 concentration in Kawasaki disease. *J Cardiovasc Pharmacol* 1993; **22**(Suppl. 8):S364–6.
273. Ogino H, Ogawa M, Harima Y, et al. Clinical evaluation of gamma-globulin preparations for the treatment of Kawasaki disease. *Prog Clin Biol Res* 1987;**250**:555–6.
274. Ohga K, Yamanaha R, Kinumaki H, et al. Kawasaki disease and rug shampoo. *Lancet* 1983;**1**:930.
275. Ohno S, Miyajima T, Higuchi M, et al. Ocular manifestations of Kawasaki disease (mucocutaneous lymph node syndrome). *Am J Ophthalmol* 1982;**93**:713–17.
276. Ohshio G, Furukawa F, Khine M, et al. High levels of IgA-containing circulating immune complex and secretory IgA in Kawasaki disease. *Microbiol Immunol* 1987;**31**:891–8.
277. Okada Y, Shinohara M, Kobayashi T, et al. Effect of corticosteroids in addition to IVIG on serum cytokine levels in acute Kawasaki disease. *J Pediatr* 2003;**143**:363–7.
278. Okamoto T, Kuwabara H, Shimotohno K, et al. Lack of evidence of retroviral involvement in Kawasaki disease. *Pediatrics* 1988; **81**:599.
279. Onouchi Y, Gunji T, Burns JC, et al. *ITPKC* functional polymorphism associated with Kawasaki disease susceptibility and formation of coronary artery aneurysms. *Nat Genet* 2008;**40**:35–42.
280. Onouchi Y, Ozaki K, Burns JC, et al. Common variants in *CASP3* confer susceptibility to Kawasaki disease. *Hum Mol Genet* 2010;**19**: 2895–906.
281. Onouchi Y, Ozaki K, Burns JC, et al. A genome-wide association study identifies three new loci for Kawasaki disease. *Nat Genet* 2012;**44**:517–21.
282. Orenstein J, Shulman ST, Fox LM, et al. Three linked vasculopathic processes characterize Kawasaki disease: a light and transmission electron microscopic study. *PLoS One* 2012;7(6):e38998.
283. Pachman LM, Herold BC, Davis AT. Immune complexes in Kawasaki syndrome: a review. *Prog Clin Biol Res* 1987;**250**: 193–207.
284. Packard M, Wechsler HF. Aneurysm of the coronary arteries. *Arch Intern Med* 1929;**43**:1–14.
285. Pahl E, Sehgal R, Chrystof D, et al. Feasibility of exercise stress echocardiography for follow-up of children with coronary involvement secondary to Kawasaki disease. *Circulation* 1995;**91**:122–8.
286. Palmer AL, Walker T, Smith JC. Acute respiratory distress syndrome in a child with Kawasaki disease. *South Med J* 2005;**98**: 1031–3.
287. Palazzi DL, McClain KL, Kaplan SL. Hemophagocytic syndrome after Kawasaki disease. *Pediatr Infect Dis J* 2003;**22**:663–6.
288. Park YW, Han JW, Hong VM, et al. Epidemiologic features of Kawasaki disease in Korea, 2006–08. *Pediatr Int* 2011;**53**:36–9.
289. Patriarca PA, Rogers MF, Morens DM, et al. Kawasaki syndrome: association with the application of rug shampoo. *Lancet* 1982;**2**: 578–80.
290. Pelkonen P, Salo E. Epidemiology of Kawasaki disease. *Clin Exp Rheumatol* 1994;**12**(Suppl. 10):S83–5.
291. Peng Y, Zeng J, Du Z, et al. Usefulness of 64 slice MDCT for Kawasaki disease. *Eur J Radiol* 2009;**69**:500–9.
292. Person JR. Kawasaki disease and staphylococcal exotoxins. *Arch Dermatol* 1980;**116**:986.
293. Phillips WG, Marsden JR. Adult Kawasaki syndrome. *Br J Dermatol* 1993;**129**:330–3.
294. Pierre R, Sue-Ho R, Watson D. Kawasaki syndrome in Jamaica. *Pediatr Infect Dis J* 2000;**9**:539–43.
295. Pietra BA, De Inocencio J, Giannini EH, et al. TCR V family repertoire and T cell activation markers in Kawasaki disease. *J Immunol* 1994;**153**:1881–8.
296. Poon LK, Lun KS, Ng YM. Facial nerve palsy and Kawasaki disease (a review). *Hong Kong Med J* 2000;**6**:224–6.
297. Porneuf M, Sotto A, Barbuat C, et al. Kawasaki syndrome in an adult AIDS patient. *Int J Dermatol* 1996;**35**:292–4.
298. Rae MV. Coronary aneurysms with thrombosis in rheumatic carditis: unusual occurrence accompanied by hyperleukocytosis in a child. *Arch Pathol* 1937;**24**:369–76.
299. Rauch AM. Kawasaki syndrome: critical review of U.S. epidemiology. *Prog Clin Biol Res* 1987;**250**:33–44.
300. Rauch AM, Glode MP, Wiggins JW, et al. Outbreak of Kawasaki syndrome in Denver, Colorado: association with rug and carpet cleaning. *Pediatrics* 1991;**87**:663–9.
301. Rauch AM, Kaplan SL, Nihill MR, et al. Kawasaki syndrome clusters in Harris County, Texas, and eastern North Carolina. *Am J Dis Child* 1988;**142**:441–4.
302. Reindel R, Shulman ST. Corticosteroids as primary therapy in Kawasaki disease. *Nat Rev Rheumatol* 2012;**8**:373–4.
303. Rider LG, Wener MH, French J, et al. Autoantibody production in Kawasaki syndrome. *Clin Exp Rheumatol* 1993;**11**:445–9.
304. Rieger CH, Kawasaki T, Yanare Y, et al. Complement and protease inhibitors in Kawasaki disease. *Eur J Pediatr* 1983;**140**:92–7.
305. Roberts FB, Fetterman GH. Polyarteritis nodosa in infancy. *J Pediatr* 1963;**63**:519–29.
306. Rodo X, Ballester J, Cayan D, et al. Association of Kawasaki disease with tropospheric wind patterns. *Sci Rep* 2011;**1**:152.
307. Rogers MF. Kawasaki syndrome. *Am J Dis Child* 1986;**140**:191.
308. Rogers MF, Kochel RL, Hurwitz ES, et al. Kawasaki syndrome: is exposure to rug shampoo important? *Am J Dis Child* 1985; **139**:777–9.
309. Rosenfeld EA, Corydon KE, Shulman ST. Kawasaki disease in infants less than one year of age. *J Pediatr* 1995;**126**:524–9.
310. Rosenfeld EA, Shulman ST, Corydon KE, et al. Comparative safety and efficacy of two IVIG products in Kawasaki disease. *J Pediatr* 1995;**126**:1000–3.
311. Rowley AH, Baker SC, Shulman ST, et al. Detection of antigen in bronchial epithelium and macrophages in acute Kawasaki disease by use of synthetic antibody. *J Infect Dis* 2004;**190**: 856–65.
312. Rowley AH, Baker SC, Shulman ST, et al. Cytoplasmic inclusion bodies are detected by synthetic antibody in ciliated bronchial epithelium during acute Kawasaki disease. *J Infect Dis* 2005;**192**: 1757–66.
313. Rowley AH, Baker SC, Shulman ST, et al. RNA-containing cytoplasmic inclusion bodies in ciliated bronchial epithelium months to years after Kawasaki disease. *PLoS One* 2008;**3**(2):e1582.
314. Rowley AH, Baker SC, Shulman ST, et al. Ultrastructural, immunofluorescence and RNA evidence support the hypothesis of a "new" virus associated with Kawasaki disease. *J Infect Dis* 2011;**203**(7):1021–30.
315. Rowley AH, Duffy E, Shulman ST. Prevention of giant aneurysms in Kawasaki disease by intravenous gamma globulin therapy. *J Pediatr* 1988;**113**:290–4.
316. Rowley AH, Eckerley CA, Jack HM, et al. IgA plasma cells in vascular tissue of patients with Kawasaki syndrome. *J Immunol* 1997;**159**:5946–55.
317. Rowley AH, Gonzalez-Crussi F, Gidding SS, et al. Incomplete Kawasaki disease with coronary artery involvement. *J Pediatr* 1987;**110**:409–13.
318. Rowley AH, Shulman ST, Mask CA, et al. IgA plasma cell infiltration in proximal respiratory tract, pancreas, kidney and coronary artery in acute Kawasaki disease. *J Infect Dis* 2000; **182**:1183–91.
319. Rowley AH, Shulman ST, Preble OT, et al. Serum interferon concentrations and retroviral serology in Kawasaki syndrome. *Pediatr Infect Dis J* 1988;**7**:663–5.
320. Rowley AH, Shulman ST, Spike BT, et al. Oligoclonal IgA response in the vascular wall in acute Kawasaki disease. *J Immunol* 2001;**166**:1334–43.
321. Rowley AH, Wolinsky SM, Relman DA, et al. Search for highly conserved viral and bacterial nucleic acid sequences corresponding to an etiologic agent of Kawasaki disease. *Pediatr Res* 1994;**36**: 567–71.
322. Salcedo JR, Greenberg L, Kapur S. Renal histology of mucocutaneous lymph node syndrome. *Clin Nephrol* 1988;**29**:47–51.
323. Salo E. Kawasaki disease in Finland in 1982–1983. *Scand J Infect Dis* 1993;**25**:497–502.
324. Salo E, Kekomaki R, Pelkonen P, et al. Kawasaki disease: Monitoring of circulating immune complexes. *Eur J Pediatr* 1988;**147**: 377–80.
325. Salo E, Pelkonen P, Pettay O. Outbreak of Kawasaki syndrome in Finland. *Acta Paediatr Scand* 1986;**75**:75–80.
326. Salo E, Pesonen E, Viikari J. Serum cholesterol levels during and after Kawasaki disease. *J Pediatr* 1991;**119**:557–61.

327. Sasaguri Y, Kato H. Regression of aneurysms in Kawasaki disease: a pathologic study. *Pediatrics* 1982;**100**:225–31.
328. Sato K, Ouichi K, Taki M. *Yersinia pseudotuberculosis* infection in children, resembling Izumi fever and Kawasaki syndrome. *Pediatr Infect Dis J* 1983;**2**:123–6.
329. Sato N, Sagawa K, Sasaguri Y, et al. Immunopathology and cytokine detection in the skin lesions of patients with Kawasaki disease. *J Pediatr* 1993;**122**:198–203.
330. Savage COS, Tizard J, Jayne D, et al. Antineutrophil cytoplasm antibodies in Kawasaki disease. *Arch Dis Child* 1989;**64**:360–3.
331. Saxe N, Horak K, Goldblatt J. Mucocutaneous lymph node syndrome in a young adult. *S Afr Med J* 1980;**68**:1011–13.
332. Schulte DJ, Yilmaz A, Shimada K, et al. Involvement of innate and adaptive immunity in a murine model of coronary arteritis mimicking Kawasaki disease. *J Immun* 2009;**183**:5311–18.
333. Senzaki H. The pathophysiology of coronary artery aneurysms in Kawasaki disease: role of matrix metalloproteinases. *Arch Dis Child* 2006;**91**:847–51.
334. Senzaki H. Long-term outcome of Kawasaki disease. *Circulation* 2008;**118**:2769–72.
335. Shibuya N, Shibuya K, Kato H, et al. Kawasaki disease before Kawasaki at Tokyo University Hospital. *Pediatrics* 2002; **110**:e17.
336. Shigematsu I. [Epidemiology of mucocutaneous lymph node syndrome]. *Acta Paediatr Jpn* 1972;**76**:696. In Japanese.
337. Shimizu C, Shike H, Baker SC, et al. Human coronavirus NL63 is not detected in the respiratory tracts of children with acute Kawasaki disease. *J Infect Dis* 2005;**192**:1767–71.
338. Shimizu S, Kuratsuji T, Ojima T. Plasma fibronectin concentrations in mucocutaneous lymph node syndrome. *Arch Dis Child* 1986;**61**:72–4.
339. Shingadia D, O'Gorman M, Rowley AH, Shulman ST. Surface and cytoplasmic immunoglobulin expression in circulating B-lymphocytes in acute Kawasaki disease. *Pediatr Res* 2001;**50**: 538–43.
340. Shulman ST. IVGG therapy in Kawasaki disease: mechanism(s) of action. *Clin Immunol Immunopathol* 1989;**53**:5141–6.
341. Shulman ST. Management of Kawasaki syndrome: a consensus statement prepared by North American participants of the Third International Kawasaki Disease Symposium: Tokyo, Japan, December, 1988. *Pediatr Infect Dis J* 1989;**8**:663–7.
342. Shulman ST. Hemolysis in Kawasaki disease [letter]. *Transfusion* 1991;**31**:572.
343. Shulman ST. A commentary on disease mechanism. In: Takahashi M, Taubert K, editors. *Proceedings of the Fourth International Symposium on Kawasaki Disease*. Dallas: American Heart Association; 1993.
344. Shulman ST, Rowley AH. Does Kawasaki disease have a retroviral etiology? *Lancet* 1986;**2**:545–6.
345. Shulman ST, Rowley AH. Kawasaki disease before the 1967 description by T. Kawasaki [abstract]. *Pediatr Res* 2000;**47**:277A.
346. Shulman ST, Rowley AH. Advances in Kawasaki disease. *Eur J Pediatr* 2004;**163**:285.
347. Shulman ST, DeInocencio J, Hirsch R. Kawasaki disease. *Pediatr Clin North Am* 1995;**42**:1205–22.
348. Shulman ST, McAuley JB, Pachman LM, et al. Risk of coronary abnormalities due to Kawasaki disease in an urban area with a small Asian population. *Am J Dis Child* 1987;**141**:420–5.
349. Shulman ST, Melish M, Inoue O, et al. Immunoglobulin allotypic markers in Kawasaki disease. *J Pediatr* 1993;**122**:84–6.
350. Shulman ST, Schuster JE, Innocentini N, Rowley AH. Hyponatremia is a feature of Kawasaki shock syndrome. Presentation at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
351. Sinha R, Balakumar T. BCG reactivation: a useful diagnostic tool even for incomplete Kawasaki disease. *Arch Dis Child* 2005;**90**:891.
352. Sleeper LA, Minich LL, McCrindle BM, et al. Evaluation of Kawasaki disease risk-scoring systems for IVIG resistance. *J Pediatr* 2011;**158**:831–5.
353. Smith LB, Newburger JW, Burns JC. Kawasaki syndrome and the eye. *Pediatr Infect Dis J* 1989;**8**:116–18.
354. Smith PK, Goldwater PN. Kawasaki disease in Adelaide: a review. *J Paediatr Child Health* 1993;**29**:126–31.
355. Soppi E, Salo E, Pelkonen P. Antibodies against neutrophil cytoplasmic components in Kawasaki disease. *APMIS* 1992;**100**: 269–72.
356. Stamos JK, Corydon K, Donaldson J, et al. Lymphadenitis as the dominant manifestation of Kawasaki disease. *Pediatrics* 1994;**93**: 525–8.
357. Stockheim JA, Innocentini N, Shulman ST. Kawasaki disease in older children and adolescents. *J Pediatr* 2000;**137**:250–2.
358. Suda K, Iemura M, Nishiono H, et al. Long-term prognosis of Kawasaki disease complicated by giant coronary aneurysms. *Circulation* 2011;**123**:1836–42.
359. Suddleson EA, Reid B, Woolley MM, et al. Hydrops of the gall bladder associated with Kawasaki syndrome. *J Pediatr Surg* 1987; **22**:956–9.
360. Sugahara Y, Ishii M, Muta H, et al. Warfarin therapy for giant aneurysm prevents myocardial infarction in Kawasaki disease. *Pediatr Cardiol* 2008;**29**:398–401.
361. Sugimura T, Kato H, Inoue O, et al. Vasodilatory response of the coronary arteries after Kawasaki disease: evaluation by intracoronary injection of isosorbide dinitrate. *Pediatrics* 1992;**121**: 684–8.
362. Sugimura T, Kato H, Inoue O, et al. Intravascular ultrasound of coronary arteries in children: assessment of the wall morphology and the lumen after Kawasaki disease. *Circulation* 1994;**89**: 258–65.
363. Sugimura T, Kato H, Inoue O, et al. Long-term consequences of Kawasaki disease: serial coronary angiography and 10–20 years follow-up study. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 574–9.
364. Sugimura T, Kato H, Yokoi H, et al. Intravascular ultrasound study in Kawasaki disease: assessment of coronary and systemic arterial pathology and application for coronary intervention. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 460.
365. Sugiura K, Sakurai K, Takamoto T, et al. [Kawasaki disease in an adult.] *Intern Med* 1981;**47**:857–60. In Japanese.
366. Sundel R, Burns JC, Baker A, et al. Gamma globulin re-treatment in Kawasaki disease. *J Pediatr* 1998;**123**:657–9.
367. Suzuki A, Arakaki Y, Sugiyama H, et al. Observation of coronary arterial lesion due to Kawasaki disease by intravascular ultrasound. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 451–9.
368. Suzuki A, Kamiya T, Arakaki Y, et al. Fate of coronary aneurysms in Kawasaki disease. *Am J Cardiol* 1994;**74**:822–4.
369. Suzuki A, Kamiya T, Kuwahara N, et al. Coronary arterial lesions of Kawasaki disease: cardiac catheterization findings of 1100 cases. *Pediatr Cardiol* 1986;**7**:3–9.
370. Suzuki A, Kamiya T, Ono Y, et al. Functional behavior and morphology of coronary artery wall in Kawasaki disease. *J Am Coll Cardiol* 1996;**27**:291–6.
371. Suzuki A, Miyagawa-Tomita S, Komatsu K, et al. Active remodeling of the coronary arterial lesions in the late phase of Kawasaki disease. *Circulation* 2000;**101**:2935–41.
372. Suzuki A, Miyagawa-Tomita S, Komatsu K, et al. Immunohistochemical study of apparently intact coronary artery in a child after Kawasaki disease. *Pediatr Int* 2004;**46**:590–6.
373. Suzuki H. Cyclosporine A treatment for patients with Kawasaki disease resistant to IVIG. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
374. Takahashi K, Naoe S, Wakayama M, et al. Pathologic study of coronary aneurysms in adults who had Kawasaki disease in childhood. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 592–7.
375. Takahashi K, Oharaseki T, Naoe S, et al. Neutrophilic involvement in the damage to coronary arteries in acute stage of Kawasaki disease. *Pediatr Int* 2005;**47**:305–10.
376. Takahashi M, Mason W, Lewis AB. Regression of coronary aneurysms in patients with Kawasaki syndrome. *Circulation* 1987;**75**: 387–94.
377. Takahashi M, Mason W, Thomas D, et al. Reye syndrome following Kawasaki syndrome confirmed by liver histopathology. In: Kato H, editor. *Kawasaki disease.* Proceedings of 5th International Kawasaki Disease Symposium, Fukuoka, Japan, 1995. New York: Elsevier; 1995. p. 436–44.
378. Takeda T, Abe J, Yoshino K, et al. Establishment of a novel superantigen produced by *Yersinia pseudotuberculosis* and its association with systemic Kawasaki disease–like symptoms. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 193–9.

379. Takemura A, Suzuki A, Inaba R, et al. Utility of coronary MR angiography in children with Kawasaki disease. *Am J Radiol* 2007;**188**:W534–9.
380. Takeshita S, Dobashi H, Nakatani K, et al. Circulating soluble selectins in Kawasaki disease. *Clin Exp Immunol* 1997;**108**: 446–50.
381. Tanaka N. Kawasaki disease (acute febrile mucocutaneous lymph node syndrome) in Japan: relationship with infantile periarteritis nodosa. *Pathol Microbiol* 1975;**43**:204–18.
382. Tanaka N, Naoe S, Kawasaki T. Pathological study of autopsy cases of MCLS: relationship with infantile periarteritis nodosa. *J Jpn Red Cross Cen Hosp* 1971;**2**:85–94.
383. Tanaka N, Sekimoto K, Fukushima T, et al. Pathological study of fatal MCLS cases of Kawasaki disease: relationship with infantile polyarteritis nodosa. In: Shiokawa Y, editor. *Vascular lesions of collagen diseases and related conditions*. Baltimore: University Park Press; 1977. p. 44.
384. Tanaka N, Sekimoto K, Naoe S. Kawasaki disease: relationship with infantile periarteritis nodosa. *Arch Pathol Lab Med* 1976; **100**:81–6.
385. Tashiro N, Matsubara T, Uchida M, et al. Ultrasonographic evaluation of cervical nodes in Kawasaki disease. *Pediatrics* 2002;**109**:e77.
386. Taubert KA, Rowley AH, Shulman ST. Seven-year national survey of Kawasaki disease and acute rheumatic fever. *Pediatr Infect Dis J* 1994;**13**:704–8.
387. Taubert KA, Rowley AH, Shulman ST. A 10-year (1984–1993) United States hospital survey of Kawasaki disease. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 34–8.
388. Teixeira OHP, Quinn A. Kawasaki syndrome. *Am J Dis Child* 1986;**140**:190–1.
389. Terai M, Shulman ST. Prevalence of coronary artery abnormalities in Kawasaki disease is highly dependent on gammaglobulin dose but independent of salicylate dose. *J Pediatr* 1997;**131**:888–93.
390. Terai M, Kohno Y, Namba M, et al. Class II major histocompatibility antigen expression on coronary arterial endothelium in a patient with Kawasaki disease. *Hum Pathol* 1990;**21**:231–4.
391. Terai M, Kohno Y, Niwa K, et al. Imbalance among T-cell subsets in patients with coronary arterial aneurysms in Kawasaki disease. *Am J Cardiol* 1987;**60**:555–9.
392. Terai M, Miwa K, Williams T, et al. The absence of evidence of staphylococcal toxin involvement in the pathogenesis of Kawasaki disease. *J Infect Dis* 1995;**172**:558–61.
393. Terai M, Yasukawa K, Honda T, et al. Peripheral blood eosinophilia and eosinophil accumulation in coronary microvessels in acute Kawasaki disease. *Pediatr Infect Dis J* 2002;**21**:777–80.
394. Terp S, Bar-Meir M, Tan T. Inflammatory reaction at the site of a BCG vaccination scar in an adopted child. *Clin Infect Dis* 2007;**45**:487, 518–19.
395. Ting EC, Capparelli EV, Billman GF, et al. Elevated gamma-GT concentrations in acute Kawasaki disease. *Pediatr Infect Dis J* 1998;**17**:431–2.
396. Tomita S, Chung K, Mas M, et al. Peripheral gangrene associated with Kawasaki disease. *Clin Infect Dis* 1992;**14**:121–6.
397. Tourneux P, Dufillot D, Belloy M, et al. Epidemiologie de la maladie de Kawasaki en Guadeloupe. *Presse Med* 2005;**34**:25–8.
398. Treadwell TA, Maddox RA, Holman RC, et al. Investigation of Kawasaki syndrome risk factors in Colorado. *Pediatr Infect Dis J* 2002;**21**:976–8.
399. Tremoulet A, Best BM, Song S, et al. Resistance to IVIG in children with Kawasaki disease. *J Pediatr* 2008;**153**:117–21.
400. Tristani-Fironzi M, Kamango-Sollo ED, Sun S, et al. TCR V gene family repertoire and humoral immunity in Kawasaki syndrome. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 200–5.
401. Tsai M-H, Huang Y-C, Yen M-H, et al. Clinical responses of patients with Kawasaki disease to different brands of intravenous immunoglobulin. *J Pediatr* 2006;**148**:38–43.
402. Tse SML, Silverman ED, McCrindle BW, et al. Early treatment with IVIG in Kawasaki disease. *J Pediatr* 2002;**140**:450–5.
403. Tsuda E, Hirata T, Matsuo O, et al. The 30 year outcome for patients after myocardial infarction due to coronary artery lesions of Kawasaki disease. *Pediatr Cardiol* 2011;**32**:176–82.
404. Tsuda E, Kamiya T, Ono Y, et al. Incidence of stenotic lesions predicted by acute phase changes in coronary arterial diameter during Kawasaki disease. *Pediatr Cardiol* 2005;**26**:73–9.
405. Tsuda E, Kitamura S. National survey of coronary artery bypass grafting for coronary stenosis caused by Kawasaki disease in Japan. *Circulation* 2004;**110**(Suppl. II):II61–6.
406. Tuohy AMM, Tani LY, Cetta F, et al. How many echocardiograms are necessary for follow-up evaluation of patients with Kawasaki disease? *Am J Cardiol* 2001;**88**:328–30.
407. Uchida N, Asayama K, Dobashi K, et al. Antioxidant enzymes and lipoperoxide in blood in patients with Kawasaki disease: comparison with the changes in acute infections. *Acta Paediatr Jpn* 1990;**32**:242–8.
408. Uehara R. Epidemiologic features of Kawasaki disease in Japan, 2007–10. Presented at 10th International Kawasaki Disease Symposium, Kyoto, Japan, February 2012.
409. Uehara R, Belay ED. Epidemiology of Kawasaki disease in Asia, Europe, and the U.S. *J Epidemiol* 2012;**22**(2):79–85.
410. Uehara R, Yashiro M, Nakamura H, et al. Kawasaki disease in parents and children. *Acta Paediatr* 2003;**92**:694–7.
411. Uziel Y, Hashkes PJ, Kassem E, et al. "Unresolving pneumonia" as the main manifestation of atypical Kawasaki disease. *Arch Dis Child* 2003;**88**:940–2.
412. Wallace CA, French JW, Kahn SJ, et al. Initial IVIG treatment failure in Kawasaki disease. *Pediatrics* 2000;**43**:211–17.
413. Watanabe T, Abe Y, Sato S, et al. Hyponatremia in Kawasaki disease. *Pediatr Nephrol* 2006;**21**:778–81.
414. Weindling AM, Levinsky RJ, Marshall WC. Circulating immune complexes in mucocutaneous lymph node syndrome (Kawasaki disease). *Arch Dis Child* 1979;**54**:241–2.
415. Westphalen MA, McGrath MA, Kelly W, et al. Kawasaki disease with severe peripheral ischemia: treatment with prostaglandin E_1 infusion. *J Pediatr* 1988;**112**:431–3.
416. Wong M, Silverman ED, Fish EN. Evidence for RANTES, MCP-1, and MIP-β in Kawasaki disease. *J Rheumatol* 1997;**24**: 1179–85.
417. Wright DA, Newberger JW, Baker A, et al. Treatment of immune globulin–resistant Kawasaki disease with pulsed doses of corticosteroids. *J Pediatr* 1996;**128**:146–9.
418. Wu SF, Chang J-S, Wan L, et al. Association of IL-1Ra gene polymorphisms, but no association of IL-1β and IL-4 gene polymorphisms, with Kawasaki disease. *J Clin Lab Anal* 2005;**19**:99–102.
419. Wurzburger BJ, Avner JR. Lateral rectus palsy in Kawasaki disease. *Pediatr Infect Dis J* 1999;**18**:1029–30.
420. Yamada K, Fukumoto T, Shinkai A, et al. The platelet function in acute febrile mucocutaneous lymph node syndrome and a trial of prevention for thrombosis by antiplatelet agent. *Acta Hematol Jpn* 1978;**41**:791–802.
421. Yamakawa R, Ishii M, Sugimura T, et al. Coronary endothelial dysfunction after Kawasaki disease. *J Am Coll Cardiol* 1998;**31**: 1074–80.
422. Yamamoto T, Kimura J. [Acute mucocutaneous lymph node syndrome: a case associated with carditis]. *Jpn J Pediatr* 1968;**21**:336–9. In Japanese.
423. Yanagawa H. Epidemiology of Kawasaki disease in Japan. *Prog Clin Biol Res* 1987;**250**:5–17.
424. Yanagawa H, Kawasaki T, Shigematsu I. Nationwide survey on Kawasaki disease in Japan. *Pediatrics* 1987;**80**:58–62.
425. Yanagawa H, Nakamura Y, Kawasaki T, et al. Nationwide epidemic of Kawasaki disease in Japan during winter of 1985–86. *Lancet* 1986;**2**:1138–9.
426. Yanagawa H, Nakamura Y, Yashiro M, et al. A nationwide incidence survey of Kawasaki disease in 1985–1986 in Japan. *J Infect Dis* 1988;**158**:1296–301.
427. Yanagawa H, Nakamura Y, Yashiro M, et al. Results of the nationwide epidemiologic survey of Kawasaki disease in 1995 and 1996 in Japan. *Pediatrics* 1998;**102**:e65.
428. Yanagawa H, Nakamura Y, Yashiro M, et al. Incidence of Kawasaki disease in Japan: the nationwide surveys of 1999–2002. *Pediatr Int* 2006;**48**:356–61.
429. Yanagawa H, Yashiro M, Nakamura Y, et al. Epidemiologic pictures of Kawasaki disease in Japan: from the nationwide incidence survey in 1991 and 1992. *Pediatrics* 1995;**95**:475–9.
430. Yanagawa H, Yashiro M, Nakamura Y, et al. Results of 12 nationwide epidemiological incidence surveys of Kawasaki disease in Japan. *Arch Pediatr Adolesc Med* 1995;**149**:779–83.
431. Yanase Y, Takayama J, Aso S, et al. Studies on serum immunoglobulins and delayed skin tests in patients with MCLS. *Acta Paediatr Jpn* 1982;**24**:408–9.

432. Yashiro M, Nakamura Y, Hirose K, et al. Surveillance of Kawasaki disease in Japan, 1984–1994. In: Kato H, editor. *Kawasaki disease*. Amsterdam: Elsevier Science; 1995. p. 15–21.
433. Yokota S. Heat shock protein as a predisposing and immunopotentiating factor in Kawasaki disease. *Acta Pediatr Jpn* 1991;**33**:756–64.
434. Yu JJ, Jang WS, Ko HK, et al. Perivascular brightness of coronary arteries in Kawasaki disease. *J Pediatr* 2011;**159**:454–7.
435. Yutani C, Go S, Kamiya F, et al. Cardiac biopsy of Kawasaki disease. *Arch Pathol Lab Med* 1981;**105**:470–3.
436. Yutani C, Okano K, Kamiya T, et al. Histopathological study on right endomyocardial biopsy of Kawasaki disease. *Br Heart J* 1980;**43**:589–92.
437. Zalian F, Falcini F, Zancan L, et al. Acute surgical abdomen as presenting manifestation of Kawasaki disease. *J Pediatr* 2003;**142**:731–5.
438. Zhang T, Yanagawa H, Oki I, et al. Factors related to the cardiac sequelae of Kawasaki disease one month after initial onset. *Acta Paediatr* 2002;**91**:517–20.

TABLE 78-1 Centers for Disease Control and Prevention 1994 Workshop Case Definition of Chronic Fatigue Syndrome

1. Fatigue (persistent or relapsing) that has new or definite onset, is of >6 months' duration, and leads to a substantial (>50%) reduction in level of activity
   plus
2. At least four of the following:
   Impaired memory or concentration
   Sore throat
   Tender lymph nodes: cervical or axillary
   Myalgias
   Arthralgias (multiple joints, without swelling or erythema)
   New onset (or in severity) of headaches
   Unrefreshing sleep (hypersomnia or insomnia)
   Postexertional fatigue lasting >24 hours
   and
3. The absence of another diagnosis for the individual's signs and symptoms

Modified from Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* 1994;**121**:953–9.

despite similar arrays of signs and symptoms, patients with CFS likely represent a heterogeneous population. Still, evaluation of groups of patients with this symptom-complex has provided information about pathophysiologic changes occurring in patients with CFS and potentially beneficial, although not curative, approaches to therapy for affected individuals have been developed.

In an attempt to provide a degree of uniformity as a basis for research into the evaluation of such patients, the U.S. Centers for Disease Control and Prevention (CDC) developed a definition of CFS in 1988.[47] These criteria were revised in 1994, with physical signs removed from the definition because they appeared to be unreliably documented in studies, and the required number of symptoms for the diagnosis of CFS decreased from 8 of a list of 11 to 4 of 8 components (Table 78-1).[38] These changes, being less restrictive, may serve to increase the sensitivity of the diagnosis, but they also decrease the specificity. However, these criteria are purely speculative in the absence of any gold standard or definitive diagnostic test for CFS. These 1994 criteria also suggest subdivisions of patients with CFS for research purposes and provide guidelines for nonresearch clinicians as well. Other international groups have generated similar definitions of CFS for evaluating this condition.[16,65,93]

The primary manifestations of CFS are severe fatigue of more than 6 months' duration that limits activity to less than 50 percent of premorbid function and the association of multiple other symptoms, as outlined in the case definitions of CFS.[38,47,65,93] These symptoms include new or more intense headaches, decreased ability to focus or concentrate, recurrent sore throats, a sensation of tender cervical or axillary lymph nodes, low-grade temperature elevations, myalgias and arthralgias, postexertional fatigue lasting longer than 24 hours, and sleep disturbances (hypersomnia or insomnia). The severity and persistence of these findings vary among individual patients with CFS. In addition, although not included in the case definitions, many patients report dizziness, especially with changes in position, feeling hot when others are cold or vice versa, chronic costochondritis, and a Raynaud-like phenomenon.

HISTORICAL OVERVIEW

In all likelihood, CFS is not a new illness. Numerous conditions with features comparable to those of CFS have appeared in the medical literature during the past several centuries.[107] Many of these descriptions attempted to associate an illness characterized by prolonged debilitating fatigue and numerous other symptoms with an infectious agent. They have included chronic brucellosis,[100] chronic enteroviral syndrome,[21,25,42] chronic candidiasis,[87] myalgic encephalomyelitis,[2] chronic mononucleosis (Epstein-Barr virus [EBV] infection),[4,49,112,113] human herpesvirus–6 infection,[11,120] human herpesvirus–7 infection,[30] chronic Lyme disease,[101] parvovirus B19 infection,[55] and a new retroviral infection.[27] Noninfectious conditions described with similarities to CFS include total-allergy syndrome, hypoglycemia, neurasthenia, Iceland disease, Royal Free disease, and fibromyalgia rheumatica.[59] In addition to clinical similarity to CFS, all these conditions, at least at the time they were described, lacked a diagnostic test to confirm a definitive causative agent and, in their acute form, manifested as fatigue in association with multiple other complaints.

A review of the experience in the mid 1980s associated groups of patients with CFS-like illness with patients with chronic EBV infection.[4,49,112] These reports described elevated and aberrant patterns of EBV antibody responses in individuals with prolonged fatigue and multiple symptoms consistent with CFS. Acute infectious mononucleosis also often manifests as fatigue, fever, malaise, sore throat, lymphadenitis, and multiple systemic complaints, although typically in a more pronounced manner. Subsequent studies, however, revealed that the elevation or pattern of EBV antibody responses in such patients was not consistently different from findings in others who resolved symptoms of acute EBV infection.[73] Additionally, shedding of EBV in secretions was not increased in these individuals and no association was found between viral shedding and severity of symptoms.[114] Furthermore, no response to the antiviral drug acyclovir occurred in a group of such patients in a placebo-controlled, double-blind crossover study in terms of improvement of clinical symptoms.[109] After these observations were made, the name *chronic fatigue syndrome* was chosen to define this illness,[47] at least until a specific cause or marker for this illness is identified.

Cameron and colleagues reported on a cohort of patients with prolonged fatigue after having infectious mononucleosis. No difference in EBV load was demonstrated in eight patients with 6 months or more of disabling symptoms after the diagnosis of infectious mononucleosis was established, and again there was a lack of correlation between persistent symptoms and

viremia or altered host responses to EBV.[14] Despite the lack of association of ongoing EBV infection with CFS, Katz and colleagues observed that infectious mononucleosis may be an antecedent risk factor for CFS in adolescents.[54]

Recently increased detection of nucleic acid of a xenotropic murine leukemia virus (XMRV), a newly discovered retrovirus, was reported in two different series of CFS patients.[67,68] Subsequent studies failed to confirm these observations, including two reports that utilized samples from the same patients reported in the two positive studies just cited.[5,46,96,116] Contamination of the assays in the positive studies may have been responsible for the positive results, and both of those studies were subsequently retracted.[3,43,95]

EPIDEMIOLOGY

The definitions of CFS allow for attempts to characterize the prevalence and demographic features of this condition. Still, given the vagaries of the symptom-complex, possible variations in application of the diagnosis by different health care providers, and the potential differences within groups of people to seek medical attention for this condition, data reported on these issues may not be a complete representation of the epidemiology of this condition. CFS has been reported in all age groups, including children as young as 5 years of age, and in all ethnic, racial, and socioeconomic groups, but most CFS cases have been reported to occur in middle- to upper-class white women with a median age of 35 to 40 years.[58] Based on a study of physician-diagnosed patients from four cities using the 1988 case definition of CFS,[1] the CDC estimates a minimum prevalence rate of CFS of 2 to 10 cases per 100,000, or higher, in adults aged 18 years and older in the United States.[12,70,84] A population-based descriptive epidemiologic study conducted by the CDC in Kansas estimated the prevalence of CFS to be 235 per 100,000 persons and a 1-year incidence to be 180 per 100,000 persons.[88] Studies from outside the United States have reported similar or higher prevalence rates of CFS in adults.[31,65]

Although the CDC definition does not include age criteria for the diagnosis of CFS, the prevalence of CFS in the pediatric age range has not been studied well. Some researchers have suggested that the diagnosis of CFS should not be used in children, to avoid potential delay in making an alternative medical or psychological diagnosis.[83] However, many referral centers have reported groups of pediatric patients, primarily adolescents, with features and a clinical course similar to those reported in adults.* Furthermore, in these studies, missed or alternative diagnoses were not found, despite years of follow-up. Rimes and colleagues reported prevalence rates of CFS in an adolescent population similar to those reported in adults.[89]

In these pediatric studies, a predominance of female patients with a median age of 14 years at the time of diagnosis was noted. Additionally, these patients were from predominately middle- to upper-class families, and, as discussed later, their signs and symptoms at presentation (Table 78-2) and their course of illness were similar to those reported in adults with CFS. CDC studies suggest that the prevalence of CFS in adolescents approaches that reported in adults, but cases in children younger than 12 years of age occur much less often.[38] One suggestion is that a shorter duration of symptoms (3 to 4 months vs. 6 months) may be appropriate for establishing the diagnosis of CFS in pediatric patients.[38,50] Multiple family members with CFS may be seen as well, but, to date, evidence that CFS is contagious does not exist.

TABLE 78-2 **Symptoms Reported by 58 Children and Adolescents Evaluated for Chronic Fatigue Syndrome (1989 to 1994)**

Symptom	No. Patients	Percentage (%)
Fatigue	58	100
Headache	43	74
Sore throat	34	59
Abdominal pain	28	48
Fever	21	36
Impaired cognition	19	33
Myalgia	18	31
Diarrhea	17	29
Adenopathy	17	29
Anorexia	16	28
Nausea/vomiting	15	26
Congestion	13	22
Dizziness	10	17
Arthralgia	10	17
Otitis	6	10
Cough	6	10
Rash	5	9
Sweats	5	9
Chills	4	7
Depression	4	7

From Krilov LR, Fisher M, Friedman SB, et al. Course and outcome of chronic fatigue in children and adolescents. *Pediatrics* 1998;**102**:360–6.

ETIOLOGY AND PATHOGENESIS

Infection

As noted previously, an infectious origin of CFS has not been demonstrated and a single microorganism as the cause of CFS is unlikely. However, an acute infection appears possibly to play a role in precipitating CFS because most (as many as two thirds) patients with CFS relate a sudden onset of their symptoms to an acute infection, most often infectious mononucleosis. Lyme disease[69,101] and an influenza-like illness[48] also have been reported in association with the onset of CFS.

*References 7, 17, 32, 61, 71, 99.

Immunologic Dysfunction

A role for immunologic dysfunction in CFS has been suggested, based on numerous studies demonstrating abnormalities in lymphocyte function or cytokine production.* However, these findings have not been reproducible in different groups of patients with CFS. Some evidence suggests that nonspecific elevation of antibody titers and an increase in allergic symptoms occur in patients with CFS.[110] However, these findings are mild, and similar immunologic changes may be seen in other conditions, including depression. Individuals with CFS may report an increased frequency and duration of infections compared with their pre-CFS state, but they tend to be mostly routine viral illnesses. Neither unusual nor opportunistic infection nor increased risk for developing malignant disease has been observed in these individuals. Intravenous immunoglobulin infusions have not demonstrated long-term clinical benefit when they are administered to patients with CFS and may cause significant adverse reactions.[66,82,106]

Genetic Components

A number of recent studies of gene expression from peripheral blood samples have identified potential gene patterns associated with neuroendocrine function, stress reactions, and immune responses that may be associated with CFS.[45,56] Further definition of such genes may help further establish a biologic basis for development of CFS and eventually lead to diagnostic tests for CFS and targeted approaches to therapy.

Some studies have focused on potential genetic markers of CFS using DNA microarray technology.[102] An analysis of cytokine gene polymorphisms in 80 Italian patients with a diagnosis of CFS assessed using the 1994 CDC definition reported significant difference in tumor necrosis factor and interferon-γ phenotypes compared with controls. The authors hypothesized a potential altered immunologic or inflammatory response based on these differences as potentially contributing to the pathogenesis of CFS.[10,15,35] An analysis of prolonged postinfectious fatigue in patients who had had mononucleosis identified certain genes involved in immune responses, and hormonal and neurologic pathways that were altered in a subset of patients with mononucleosis were associated with the development of prolonged fatigue.[14,41,98] If reproducible gene expression markers are confirmed, they could potentially be used as diagnostic tools for CFS.

CLINICAL MANIFESTATIONS

Neurologic Factors

Many patients with CFS report cognitive impairment manifest as a decreased ability to concentrate and focus, difficulty in processing information, and trouble with word recall. Complaints of headache (new onset or altered pattern) and other neurologic symptoms, such as paresthesias and dysequilibrium, also are reported commonly by patients with CFS.

Despite these complaints, physical examination does not reveal abnormal neurologic signs. Formal neuropsychometric testing also may not reveal objective abnormalities to the extent reported by the patient. Whether this discrepancy reflects a problem with testing methods or altered perception on the part of the individual is uncertain at this time.[11,79]

Magnetic resonance imaging (MRI) has been reported to show an increase in cerebral white matter abnormalities in patients with CFS.[11,78,91] Similarly, in many studies, single photon emission computed tomography (SPECT) has demonstrated changes in perfusion in certain areas of the brain in patients with CFS.[23,34,92] In other studies, however, abnormalities specific to CFS have not been observed consistently and methodological concerns have been raised regarding the interpretation of abnormal findings cited previously.[22,44] An abnormality at the base of the fourth ventricle detected on focal computed tomography (CT) of the base of the brain and responding to neurosurgical intervention was reported in a group of patients with CFS,[40] but this defect is not seen on routine CT or MRI and requires confirmation by other researchers before determining its significance for these patients. At present, imaging of the central nervous system is not indicated routinely in the evaluation of a patient with CFS.

Endocrinologic Factors

Subtle defects in the hypothalamic-pituitary-adrenal axis have been described in cohorts of patients with CFS. These abnormalities include decreased free urinary cortisol levels and exaggerated adrenal responsiveness to corticotropin infusion.[29] These changes are quantitatively minor, and mean values are still within normal for age and sex, thus rendering these values unreliable as a diagnostic test for CFS. Similar changes have been described in patients with fibromyalgia and post-traumatic stress disorder.

Cardiovascular Factors

In 1995, investigators at Johns Hopkins Hospital in Baltimore described a cohort of seven adolescents with chronic fatigue and autonomic dysfunction as assessed by tilt-table testing.[90] Furthermore, these patients reported improvement or resolution of symptoms, including fatigue, with treatment of their orthostatic intolerance. Further studies from this group demonstrated some component of orthostatic hypotension in 92 percent of patients with CFS.[9] Studies in groups of adults, in contrast, showed tilt-table test abnormalities in only 25 to 40 percent of patients.[36,97] Whether this finding reflects true age-related differences in this phenomenon in patients with CFS is uncertain, given potential methodological differences in the performance of the tests.

The pattern of orthostatic intolerance among adolescents with CFS was characterized further by Stewart and colleagues and is consistent with the postural orthostatic

*References 13, 57, 64, 74, 108, 111, 115.

tachycardia syndrome (POTS).[103-105] Symptoms of POTS may develop after an acute infectious illness or with severe deconditioning and include fatigue, lightheadedness, impaired cognition, inappropriate sweating, headache, palpitations, nausea, vomiting, and tremulousness. These authors suggest that CFS may be an extreme expression of POTS in at least some cases.

The importance of these cardiovascular findings in CFS remains unsettled. Subjective components may contribute to defining a tilt-table test as abnormal because these studies are not performed blinded. Some healthy individuals also may experience hypotension during these tests. Further studies defining the effect of treatment of POTS on the course of CFS should help to answer this question. In this context, evaluation of, and therapy for, orthostatic changes, especially if dizziness is a significant component of the patient's complaints, may be indicated.[128]

Sleep Physiology

Sleep disturbances have been described in selected groups of patients with CFS, and some of these conditions may be amenable to therapy.[63,86] However, no single pattern of sleep abnormality has been reported for these patients, and, in our experience, the pattern of hypersomnia or insomnia tends to improve as the patient recovers.[117]

Psychological Factors

Psychological factors have been considered in the origin and perpetuation of CFS. Adults with CFS have demonstrated a higher frequency of depression and other psychiatric disorders before the onset of their CFS compared with age-matched controls.[1,62] Certainly, many of the symptoms reported in CFS also are reported commonly in depression. They may include sleep disturbances, loss of energy, difficulty in concentrating, changes in appetite, and musculoskeletal complaints. Studies in children and adolescents with CFS also have shown significant psychological features, especially depression and somatization, compared with both healthy controls and those with other chronic illnesses (e.g., juvenile idiopathic arthritis, cancer, cystic fibrosis).[17,18,50,80,122] Still, certain features of CFS argue against its being solely a variant of depression. Individuals with CFS do not have the mood-related symptoms reported in patients with clinical depression. These mood-related symptoms include negative affect, anhedonia, low self-esteem, and suicidal ideation. Furthermore, patients with CFS and their families have a firm belief that an infectious, immunologic, or other medical cause for their symptoms exists, and the patients desire a return to normal activities.

In considering the possible link between depression or psychological stress and CFS, several possible relationships may exist. Preexisting depression or stress may create a psychological vulnerability that allows for the development of CFS in combination with any of numerous other factors discussed previously. Studies have demonstrated a role for psychological factors predicting the response to mononucleosis and influenza.[48,52] Similar factors may be involved in the development of CFS. Alternatively, depression may be a physiologic consequence of the central nervous system changes that occur in those who develop CFS, just as decreased concentration and memory occur as part of the syndrome.[50,122] Furthermore, reactive depression may occur in these patients in response to the inability to participate in their usual activities and to absence from school and separation from friends. Finally, CFS may be, at least in some cases, a manifestation of separation anxiety or school phobia in which secondary gain, as a conversion reaction, is playing a major role. Certainly, at least a subset of adolescents and children with CFS in our experience does not appear eager to return to school or activities, and secondary gain may play a role in the perpetuation of their illness.[48]

In summary, each of these potential links between depression or stress and CFS likely plays a role, with the relative contribution of each feature varying for different people. This suggestion is consistent with the overall hypothesis that many different factors appear to contribute to the development and perpetuation of CFS. The relative contribution of these different features may vary from individual to individual and even for the same patient over the course of the illness. The assessment and management of the patient with CFS should attempt to consider these issues and their relative importance for that individual.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of CFS is one of exclusion and requires a comprehensive history. In the pediatric population, this information is obtained best from the patient and parents. Additionally, we request that families bring prior medical records, test results, and pertinent school records to facilitate a complete evaluation. This process may be lengthy because the details are complicated, of long standing, and often a source of debate between the patient and parents. The history should focus on the onset of illness, the duration and severity of symptoms, prior evaluations (often multiple) as well as medical history before the illness, family history, academic performance, and social history. The physical examination of the individual with suspected CFS almost always is essentially normal despite the multitude of symptoms. Findings of mild pharyngeal erythema and cervical adenopathy commonly are reported. However, finding fever, weight loss, significant adenopathy, or organomegaly should alert the clinician to the possibility of an alternative diagnosis.

DeMeirleir and colleagues reported increased detection of a 37-kDa, 2-5A-synthetase binding protein in peripheral blood mononuclear cells from patients with CFS compared with healthy controls.[28] These binding proteins are related to the ribonuclease L antiviral pathway of these cells. This finding requires further confirmation before it can be considered a potential diagnostic test for CFS. At present, no specific tests for diagnosing CFS exist and laboratory testing is aimed primarily at eliminating other possible diagnoses. Most patients have undergone multiple laboratory tests before a diagnosis of

CFS has been considered, but these tests may be repeated or completed as part of the initial CFS evaluation. Additionally, interpreting previously performed tests may be part of the initial patient assessment. Screening studies may include a complete blood cell count with differential and platelets, erythrocyte sedimentation rate, hepatic and renal function studies, urinalysis, and thyroid function tests. Additional tests that may be indicated based on history and physical examination may include toxicology screening, human immunodeficiency virus serology, antinuclear antibody, rheumatoid factor, tuberculin skin test, and cortisol level. Serologic evaluations for EBV, Lyme disease, and group A streptococcal infection may be requested based on history and physical infection. In most instances, these tests are not indicated; however, they often are obtained before CFS is suspected, and they need to be appropriately reviewed or repeated as part of the initial evaluation for CFS. Screening radiographic studies may include a chest radiograph or imaging of the paranasal sinuses based on the patient's symptoms.

Elimination of every possible disorder that may cause a patient to experience prolonged fatigue is impossible; but when guided by history, physical examination, and laboratory screening tests as outlined previously, the clinician can make a reliable diagnosis of CFS. Follow-up for periods of 4 to 13 years for pediatric patients diagnosed with CFS have not identified cases of missed or alternative diagnoses for their complaints.[6,61] In general, the longer the duration and the greater the number of symptoms, the less the need for extensive laboratory evaluations to suggest the diagnosis of CFS. Conversely, alternative diagnoses should be considered if a single symptom dominates the clinical presentation or if physical examination or laboratory tests reveal significant abnormalities.

Psychosocial assessment is indicated for all children and adolescents presenting for CFS evaluation. The extent of such evaluation may be limited to assessment by primary care personnel or may include collaboration with a social worker, psychologist, or psychiatrist, based on the individual's needs and the comfort level of the examiner. Cardiopulmonary and neurologic evaluations may be used in some cases, both to consider possible alternative diagnoses and to assist in assessing factors that may be contributing to the symptoms of CFS.

MANAGEMENT

One of the essential qualities of the clinician is interest in humanity, for the secret of care of the patient is in caring for the patient.

DR. FRANCIS PEABODY, 1926 (WALL PLAQUE, LOBBY OF THE MASSACHUSETTS GENERAL HOSPITAL, BOSTON)

Management of patients with CFS is aimed at providing a combination of supportive treatment and emotional support (Table 78-3).[37] This process can be initiated during the initial evaluation, with a discussion of the diagnostic criteria for CFS and a review of the results of previously obtained laboratory tests. The relationship between physical and psychological symptoms can be explained, and the patient and family can be reassured that symptoms are real, even if the symptoms have psychiatric components. Additionally, the patient and family can be advised that most children and adolescents with CFS do well over time[6,32,60,61] and have a better long-term outlook than that described for adults with CFS.[51,127] Furthermore, emphasizing the frequent ups and downs in symptoms that generally characterize the course of CFS is important in assisting the patient and family to develop coping skills. This assistance may include guidance on how to modify lifestyle most appropriately and how to set realistic schedules and goals. Studies from the United Kingdom suggest that formal cognitive-behavioral therapy[26,85,94,126] or graded exercise programs, or both, may be beneficial for patients with CFS.[19,39,123,125]

Many other therapies have been advocated by different groups for patients with CFS. These treatments may include supplements, such as essential fatty acids,[121] magnesium,[24] liver extract injections,[53] and vitamin and nutritional supplements,[72] as well as pharmacologic treatments, such as corticosteroids,[20,75,81] reduced nicotinamide adenine dinucleotide,[118] antidepressants,[77,119] and growth hormone.[76] A recent brief observation report suggested that methylphenidate may be beneficial in relieving fatigue and improving concentration in some patients with CFS.[8] Homeopathic therapies, osteopathy, and massage therapy[33] also have been reported to be beneficial in the management of CFS. Most of these approaches have not been studied adequately to allow definitive comment on their potential benefit for a given patient. Still, the clinician should be aware of them, to help guide patients who are likely to hear about them from other patients or outside sources, including the internet.[40,124,126]

TABLE 78-3 **Approach to Management of Chronic Fatigue Syndrome in Children and Adolescents**

- Evaluation and explanation of the diagnosis, including overview of multifactorial components
- Reassurance that symptoms are real
- Anticipatory guidance regarding secondary problems, up-and-down course of syndrome, secondary gain
- Coping skills: lifestyle modification, decreased stress, and realistic expectations and schedule
- Cognitive behavioral approaches: gradual increases in activity, an exercise program, attention to sleep patterns, attention to nutrition
- Psychological support for individual and family
- Educational issues: return to classes, home tutors, neuropsychiatric testing as indicated
- Relationship issues: friends and family
- Follow-up plan: monitoring of physical symptoms and psychological issues, ongoing guidance and reassurance (follow-up visits every 4 to 6 weeks)
- Minimize shopping for a doctor, unnecessary testing, family strain, and unconventional, unproven, or experimental therapies

Modified from Krilov LR, Fisher M: Chronic fatigue syndrome in children and adolescents. *Contemp Pediatr* 2002;**19**:61–8.

PROGNOSIS AND FUTURE DIRECTIONS

CFS likely affects heterogeneous groups of patients, and a single cause or definitive treatment modality most likely will not be uncovered. Nonetheless, studying groups of such patients yields helpful information on the pathophysiology of this condition, and useful steps to address and alleviate symptoms for patients have been reported. In recognition of the significance of this entity, the CDC launched an awareness campaign, including the development of an internet site for health care professionals and the public.* In addition, long-term follow-up data demonstrating improvement over the course of time, especially in children and adolescents, without emergence of significant other conditions, are encouraging for patients, families, and clinicians caring for such individuals.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Alberts B. Retraction. *Science* 2011;**334**:1636.
5. Barnes E, Flanagan P, Brown A, et al. Failure to detect xenotropic murine leukemia virus–related virus in blood of individuals at high risk of blood-borne viral infections. *J Infect Dis* 2010;**202**:1482–5.
8. Blockmans D, Persons P, Van Houdenhove B, Bobbaers H. Does methylphenidate reduce the symptoms of chronic fatigue syndrome? *Am J Med* 2006;**119**:167–8.
10. Brenu EW, Van Driel ML, Staines DR, et al. Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis. *J Transl Med* 2011;**9**:81–90. Available at http://www.translational-medicine.com/content/9/1/81.
35. Fletcher MA, Zeng XR, Maher K, et al. Biomarkers in chronic fatigue syndrome: evaluation of natural killer cell function and dipeptidyl peptidase IV/CD26. *PLoS* 2010;**5**:e10817.
41. Goertzel BN, Pennachin C, de Souza Coelho L, et al. Combinations of single nucleotide polymorphisms in neuroendocrine effector and receptor genes predict chronic fatigue syndrome. *Pharmacogenomics* 2006;**7**:475–83.
43. Groom CT, Bishop KN. The tale of xenotropic murine leukemia virus–related virus. *J Gen Virol* 2012;**93**:915–24.
45. Hampton T. Researchers find genetic clues to chronic fatigue syndrome. *JAMA* 2006;**295**:2466–7.
46. Henrich TJ, Li JZ, Felsenstein D, et al. Xenotropic murine leukemia virus–related virus prevalence in chronic fatigue syndrome or chronic immunomodulatory conditions. *J Infect Dis* 2010;**202**:1478–81.
54. Katz BZ, Shiraishi Y, Mears CJ, et al. Chronic fatigue syndrome after infectious mononucleosis in adolescents. *Pediatrics* 2009;**124**:189–93.
56. Kerr JR, Petty R, Burke B, et al. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. *J Infect Dis* 2008;**197**:1171–84.
67. Lo SC, Pripuzova N, Li B, et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. *Proc Natl Acad Sci U S A* 2010;**107**:15874–9.
68. Lombardi VC, Ruscetti FW, Das Gupta J, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 2009;**326**:585–9.
95. Silverman RH, Das Gupta J, Lombardi VC, et al. Partial retraction. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 2011;**334**:176.
96. Simmons G, Glynn SA, Komaroff AL, et al. Failure to confirm XRMV/MLVs in the blood of patients with chronic fatigue syndrome: a multi-laboratory study. *Science* 2011;**334**:814–17.
116. Switzer WM, Jia H, Hohn O, et al. Absence of evidence of xenotropic murine leukemia virus–related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. *Retrovirology* 2010;**7**:57. Available at http://www.retrovirology.com/content/7/1/57.
117. Togo F, Natelson BH, Cherniack NS, et al. Sleep structure and sleepiness in chronic fatigue syndrome with or without coexisting fibromyalgia. *Arthritis Res Ther* 2008;**10**:R56. Available at http://arthritis-research.com/content/10/3/R56.
125. White PD, Goldsmith KA, Johnson AL, et al. Comparison of adaptive pacing therapy, cognitive behavior therapy, graded exercise therapy, and specialist care for chronic fatigue syndrome (PACE): a randomized trial. *Lancet* 2011;**377**:823–7.

The full reference list for this chapter is available at expertconsult.com.

*http://www.cdc.gov/cfs/.

REFERENCES

1. Abbey SE, Garfinkel PE. Chronic fatigue syndrome and the psychiatrist. *Can J Psychiatry* 1990;**35**:625–33.
2. Acheson ED. The clinical syndrome variously called benign myalgic encephalomyelitis, Iceland disease and epidemic neuromyasthenia. *Am J Med* 1959;**26**:569–95.
3. Alberts B. Retraction. *Science* 2011;**334**:1636.
4. Barnes DM. Mystery disease at Lake Tahoe challenges virologists and clinicians. *Science* 1986;**234**:541–2.
5. Barnes E, Flanagan P, Brown A, et al. Failure to detect xenotropic murine leukemia virus–related virus in blood of individuals at high risk of blood-borne viral infections. *J Infect Dis* 2010;**202**:1482–5.
6. Bell DS, Jordan K, Robinson M. Thirteen-year follow-up of children and adolescents with chronic fatigue syndrome. *Pediatrics* 2001;**107**:994–8.
7. Bell KM, Cookfair D, Bell DS, et al. Risk factors associated with chronic fatigue syndrome in a cluster of pediatric cases. *Rev Infect Dis* 1991;**13**(Suppl.):S320–38.
8. Blockmans D, Persons P, Van Houdenhove B, Bobbaers H. Does methylphenidate reduce the symptoms of chronic fatigue syndrome? *Am J Med* 2006;**119**:167–8.
9. Bou-Holaigah I, Rowe PC, Kan J, Calkins H. The relationship between neurally mediated hypotension and the chronic fatigue syndrome. *JAMA* 1995;**274**:961–7.
10. Brenu EW, Van Driel ML, Staines DR, et al. Immunological abnormalities as potential biomarkers in chronic fatigue syndrome/myalgic encephalomyelitis. *J Transl Med* 2011;**9**:81–90. Available at http://www.translational-medicine.com/content/9/1/81.
11. Buchwald D, Cheney PR, Peterson DL, et al. A chronic illness characterized by fatigue, neurologic and immunologic disorders, and active human herpesvirus type 6 infection. *Ann Intern Med* 1992;**116**:103–13.
12. Buchwald D, Umali P, Umali J, et al. Chronic fatigue and the chronic fatigue syndrome: prevalence in a Pacific Northwest health care system. *Ann Intern Med* 1995;**123**:81–8.
13. Caligiuri M, Murray C, Buchwald D, et al. Phenotypic and functional deficiency of natural killer cells of patients with chronic fatigue syndrome. *J Immunol* 1987;**139**:3306–13.
14. Cameron B, Galbraith S, Zhang Y, et al. Gene expression correlates of post-infective fatigue syndrome after infectious mononucleosis. *J Infect Dis* 2007;**196**:56–66.
15. Carlo-Stella N, Badulli C, DeSilvestri A, et al. A first study of cytokine genomic polymorphisms in CFS: positive association of TNF-857 and IFNγ 874 rare alleles. *Clin Exp Rheumatol* 2006;**24**:179–82.
16. Carruthers BM, Jain AK, DeMeirleir KL, et al. Myalgic encephalomyelitis/chronic fatigue syndrome: clinical working definition, diagnostic and treatment protocols. *J Chronic Fatigue Syndr* 2003;**11**:7–36.
17. Carter BD, Edwards JF, Kronenberger WG, et al. Case control study of chronic fatigue in pediatric patients. *Pediatrics* 1995;**95**:179–86.
18. Carter BD, Kronenberger WG, Edwards JF, et al. Differential diagnosis of chronic fatigue in children: behavioral and emotional dimensions. *Dev Behav Pediatr* 1996;**17**:16–21.
19. Chambers D, Bagnall AM, Hempel S, Forbes C. Interventions for the treatment, management and rehabilitation of patients with chronic fatigue syndrome/myalgic encephalitis: an updated systematic review. *J R Soc Med* 2006;**99**:506–20.
20. Cleare AJ, Heap E, Malhi GS, Wessely S, et al. Low-dose hydrocortisone in chronic fatigue syndrome: a randomized crossover trial. *Lancet* 1999;**353**:455–8.
21. Clements GB, McGarry F, Nairn C, Galbraith DN. Detection of enterovirus-specific RNA in serum: the relationship to chronic fatigue syndrome. *J Med Virol* 1995;**45**:156–61.
22. Cope H, David AS. Neuroimaging in chronic fatigue. *J Neurol Neurosurg Psychiatry* 1996;**60**:471–3.
23. Costa DC, Tannock C, Brostoff J. Brainstem perfusion is impaired in chronic fatigue syndrome. *Q J Med* 1995;**88**:767–73.
24. Cox IM, Campell MJ, Dowson D. Red blood cell magnesium and chronic fatigue syndrome. *Lancet* 1991;**337**:757–60.
25. Cunningham L, Bowles NE, Lane RJM, et al. Persistence of enteroviral RNA in chronic fatigue syndrome is associated with abnormal production of equal amounts of positive and negative strands of enteroviral RNA. *J Gen Virol* 1990;**71**:1399–402.
26. Deale A, Chalder T, Marks I, Wessely S. Cognitive behavior therapy for chronic fatigue syndrome: a randomized controlled trial. *Am J Psychiatry* 1997;**154**:408–14.
27. DeFritas E, Hilliard B, Cheney PR, et al. Retroviral sequences related to human T-lymphotropic virus type II in patients with chronic fatigue immune dysfunction syndrome. *Proc Natl Acad Sci U S A* 1991;**88**:2922–6.
28. DeMeirleir K, Bisbal C, Campine I, et al. A 37 kDa 2–5A binding protein as a potential biochemical marker for chronic fatigue syndrome. *Am J Med* 2000;**108**:99–105.
29. Demitrack MA, Dale JK, Straus SE, et al. Evidence for impaired activation of the hypothalamic-pituitary-adrenal axis in patients with chronic fatigue syndrome. *J Clin Endocrinol Metab* 1991;**73**:1224–34.
30. DiLuca D, Zorzenon M, Mirandola P, et al. Human herpesvirus 6 and human herpesvirus 7 in chronic fatigue syndrome. *J Clin Microbiol* 1995;**33**:1660–1.
31. Dowsett EG, Colby J. Long-term sickness absence due to ME/CFS in UK schools: an epidemiological study with medical and educational implications. *J Chronic Fatigue Syndr* 1997;**3**:29–42.
32. Feder HM, Dworkin PH, Orkin C. Outcome of 48 pediatric patients with chronic fatigue: a clinical experience. *Arch Fam Med* 1994;**3**:1049–55.
33. Field TM, Sunshine W, Hernandez-Reif M, et al. Massage therapy effects on depression and somatic symptoms in chronic fatigue syndrome. *J Chronic Fatigue Syndr* 1997;**3**:43–51.
34. Fischler B, D'Haenen H, Cluydts R, et al. Comparison of 99mTc HMPAO SPECT scan between chronic fatigue syndrome, major depression and healthy controls: An exploratory study of clinical correlates of regional cerebral blood flow. *Neuropsychobiology* 1996;**34**:175–83.
35. Fletcher MA, Zeng XR, Maher K, et al. Biomarkers in chronic fatigue syndrome: evaluation of natural killer cell function and dipeptidyl peptidase IV/CD26. *PLoS* 2010;**5**:e10817.
36. Freeman R, Komaroff AL. Does the chronic fatigue syndrome involve the autonomic system? *Am J Med* 1997;**102**:357–62.
37. Fukuda K, Gantz NM. Management strategies for chronic fatigue syndrome. *Fed Pract* 1995;**12**:12–27.
38. Fukuda K, Straus SE, Hickie I, et al. The chronic fatigue syndrome: a comprehensive approach to its definition and study. *Ann Intern Med* 1994;**121**:953–9.
39. Fulcher KY, White PD. Randomised, double-blind, placebo-controlled trial of graded exercise in patients with chronic fatigue syndrome. *BMJ* 1997;**314**:1647–52.
40. Gantz NM, Coldsmith EE. Chronic fatigue syndrome and fibromyalgia resources on the World Wide Web: a descriptive journey. *Clin Infect Dis* 2001;**32**:938–48.
41. Goertzel BN, Pennachin C, de Souza Coelho L, et al. Combinations of single nucleotide polymorphisms in neuroendocrine effector and receptor genes predict chronic fatigue syndrome. *Pharmacogenomics* 2006;**7**:475–83.
42. Gow JW, Behan WMH, Simpson K, et al. Studies on enterovirus in patients with chronic fatigue syndrome. *Clin Infect Dis* 1994;**18**(Suppl. 1):S126–9.
43. Groom CT, Bishop KN. The tale of xenotropic murine leukemia virus-related virus. *J Gen Virol* 2012;**93**:915–24.
44. Greco A, Tannock C, Brostoff J, Costa DC. Brain MR in chronic fatigue syndrome. *AJNR Am J Neuroradiol* 1997;**18**:1265–9.
45. Hampton T. Researchers find genetic clues to chronic fatigue syndrome. *JAMA* 2006;**295**:2466–7.
46. Henrich TJ, Li JZ, Felsenstein D, et al. Xenotropic murine leukemia virus–related virus prevalence in chronic fatigue syndrome or chronic immunomodulatory conditions. *J Infect Dis* 2010;**202**:1478–81.
47. Holmes GP, Kaplan JE, Gantz NM, et al. Chronic fatigue syndrome: a working case definition. *Ann Intern Med* 1988;**108**:387–9.
48. Imboden JB, Canter A, Cluff LE. Convalescence from influenza. *Arch Intern Med* 1961;**108**:115–21.
49. Jones JF, Ray CG, Minnich LL, et al. Evidence for Epstein-Barr virus infection in patients with persistent, unexplained illnesses: elevated anti-early antigen antibodies. *Ann Intern Med* 1985;**102**:1–7.

50. Joyce E, Blumentahl S, Wessely S. Memory, attention, and executive function in chronic fatigue syndrome. *J Neurol Neurosurg Psychiatry* 1996;**60**:495–503.
51. Joyce J, Hotopf M, Wessely S. The prognosis of chronic fatigue and chronic fatigue syndrome: a systematic review. *Q J Med* 1997;**90**:223–33.
52. Kasl SV, Evans AS, Niederman JC. Psychosocial risk factors in the development of infectious mononucleosis. *Psychosom Med* 1979;**41**:445–66.
53. Kaslow JE, Rucker L, Onishi R. Liver extract–folic acid–cyanocobalamin vs. placebo for chronic fatigue syndrome. *Arch Intern Med* 1989;**149**:2501–3.
54. Katz BZ, Shiraishi Y, Mears CJ, et al. Chronic fatigue syndrome after infectious mononucleosis in adolescents. *Pediatrics* 2009;**124**: 189–93.
55. Kerr JR, Bracewell J, Laing I, et al. Chronic fatigue syndrome and arthralgia following parvovirus B19 infection. *J Rheumatol* 2002; **29**:595–602.
56. Kerr JR, Petty R, Burke B, et al. Gene expression subtypes in patients with chronic fatigue syndrome/myalgic encephalomyelitis. *J Infect Dis* 2008;**197**:1171–84.
57. Klimas NG, Salvato FR, Morgan R, Fletcher MA. Immunologic abnormalities in chronic fatigue syndrome. *J Clin Microbiol* 1990; **28**:1403–10.
58. Komaroff AL, Fagioli LR, Geiger AM, et al. An examination of the working case definition of chronic fatigue syndrome. *Am J Med* 1996;**100**:56–64.
59. Krilov LR. Chronic fatigue syndrome. *Pediatr Ann* 1995;**24**: 290–4.
60. Krilov LR, Fisher M. Chronic fatigue syndrome in children and adolescents. *Contemp Pediatr* 2002;**19**:61–8.
61. Krilov LR, Fisher M, Friedman SB, et al. Course and outcome of chronic fatigue in children and adolescents. *Pediatrics* 1998; **102**:360–6.
62. Kruesi D, Dale J, Straus SE. Psychiatric diagnoses in patients who have chronic fatigue syndrome. *J Clin Psychiatry* 1989;**50**: 53–6.
63. Krupp LB, Jandorf L, Coyle PK, Mendelson WB. Sleep disturbance in chronic fatigue syndrome. *J Psychosom Res* 1993;**37**: 325–31.
64. Landay AL, Jessop C, Lennette ET, Levy JA. Chronic fatigue syndrome: clinical condition associated with immune activation. *Lancet* 1991;**338**:707–21.
65. Lloyd A, Hickie I, Boughton CR, et al. Prevalence of chronic fatigue syndrome in an Australian population. *Med J Aust* 1990; **153**:522–8.
66. Lloyd A, Hickie I, Wakefield D, et al. A double-blind, placebo-controlled trial of intravenous immunoglobulin therapy in patients with chronic fatigue syndrome. *Am J Med* 1990;**89**:561–8.
67. Lo SC, Pripuzova N, Li B, et al. Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. *Proc Natl Acad Sci U S A* 2010;**107**:15874–9.
68. Lombardi VC, Ruscetti FW, Das Gupta J, et al. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 2009;**326**:585–9.
69. MacDonald KL, Osterholm MT, LeDell KH, et al. A case-controlled study to assess possible triggers and cofactors in chronic fatigue syndrome. *Am J Med* 1996;**100**:548–54.
70. Marshall GS. Report of a workshop on the epidemiology, natural history, and pathogenesis of chronic fatigue syndrome in adolescents. *J Pediatr* 1999;**134**:395–405.
71. Marshall GS, Gesser RM, Yamanishi K, Starr SE. Chronic fatigue in children: clinical features, Epstein-Barr virus and human herpesvirus 6 serology and long-term follow-up. *Pediatr Infect Dis J* 1991;**10**:287–90.
72. Martin RWY, Ogston SA, Evans JR. Effects of vitamin and mineral supplementations on symptoms associated with chronic fatigue syndrome with coxsackie B antibodies. *J Nutr Med* 1994;**4**:11–23.
73. Mawle AC, Nisenbaum R, Dobbins JG, et al. Seroepidemiology of chronic fatigue syndrome: a case-control study. *Clin Infect Dis* 1995;**21**:1386–9.
74. Mawle AC, Nisenbaum R, Dobbins JG, et al. Immune responses associated with chronic fatigue syndrome: a case-control study. *J Infect Dis* 1997;**175**:136–41.
75. McKenzie R, O'Fallon A, Dale J, et al. Low-dose hydrocortisone for treatment of chronic fatigue syndrome: a randomized controlled trial. *JAMA* 1998;**280**:1061–6.
76. Moorkens G, Wynants H, Abs R. Effect of growth hormone treatment in patients with chronic fatigue syndrome. *Growth Horm IGF Res* 1998;**8**:131–3.
77. Natelson BH, Cheu J, Pareja J, et al. Randomized, double-blind, controlled placebo-phase in trial of low dose phenelzine in the chronic fatigue syndrome. *Psychopharmacology* 1996;**124**:226–30.
78. Natelson BH, Cohen JM, Brassloff I, et al. A controlled study of brain magnetic resonance imaging in patients with the chronic fatigue syndrome. *J Neurol Sci* 1993;**120**:213–17.
79. National Institutes of Allergy Infectious Diseases. *CFS*. Available at http://www3.niaid.gov/topics/womensHealth.pdf.
80. Pelcovitz D, Septimus A, Friedman SB, et al. Psychosocial correlates of chronic fatigue syndrome in adolescent girls. *Dev Behav Pediatr* 1995;**16**:333–8.
81. Peterson PK, Pheley A, Schreoppel J, et al. A preliminary placebo-controlled crossover trial of fludrocortisone for chronic fatigue syndrome. *Arch Intern Med* 1998;**158**:908–14.
82. Peterson PK, Shepard J, Macres M, et al. A controlled trial of intravenous immunoglobulin G in chronic fatigue syndrome. *Am J Med* 1990;**89**:554–60.
83. Plioplys AV. Chronic fatigue syndrome should not be diagnosed in children. *Pediatrics* 1997;**100**:270–1.
84. Price PK, North CS, Wessely S, Fraser VJ. Estimating the prevalence of chronic fatigue syndrome and associated symptoms in the community. *Public Health Rep* 1992;**107**:514–22.
85. Prins J, Bleijenberg G, Bazelmans E, et al. Cognitive behaviour therapy for chronic fatigue syndrome: a multicentre randomized controlled trial. *Lancet* 2001;**357**:841–7.
86. Regestein QR, Monk TH. Delayed sleep phase syndrome: a review of its clinical aspects. *Am J Psychiatry* 1995;**152**:602–8.
87. Renfro L, Feder HM, Lane TJ, et al. Yeast connection among 100 patients with chronic fatigue. *Am J Med* 1989;**86**:165–8.
88. Reyes M, Nisenbsaum R, Hoaglin DC. Prevalence and incidence of chronic fatigue syndrome in Wichita, Kansas. *Arch Intern Med* 2003;**163**:1530–6.
89. Rimes KA, Goodman R, Hotopf M, et al. Incidence, prognosis, and risk factors for fatigue and chronic fatigue syndrome in adolescents: a prospective community study. *Pediatrics* 2007;**119**: e603–9.
90. Rowe PC, Bou-Holaigah I, Kan JS, Calkins H. Is neurally mediated hypotension an unrecognized cause of chronic fatigue? *Lancet* 1995;**345**:623–4.
91. Schwartz RB, Garada BM, Komaroff AL, et al. Detection of intracranial abnormalities in patients with chronic fatigue syndrome: comparison of MR imaging and SPECT. *AJR Am J Roentgenol* 1994;**162**:935–41.
92. Schwartz RB, Komaroff AL, Garada BM, et al. SPECT imaging of the brain: comparison of findings in patients with chronic fatigue syndrome, AIDS dementia complex, and major unipolar depression. *AJR Am J Roentgenol* 1994;**162**:943–51.
93. Sharpe M. A report—chronic fatigue syndrome: guidelines for research. *J R Soc Med* 1991;**84**:118–21.
94. Sharpe M, Hawton K, Simkin S, et al. Cognitive behaviour therapy for the chronic fatigue syndrome: a randomized controlled trial. *BMJ* 1996;**312**:22–6.
95. Silverman RH, Das Gupta J, Lombardi VC, et al. Partial retraction. Detection of an infectious retrovirus, XMRV, in blood cells of patients with chronic fatigue syndrome. *Science* 2011;**334**:176.
96. Simmons G, Glynn SA, Komaroff AL, et al. Failure to confirm XRMV/MLVs in the blood of patients with chronic fatigue syndrome: a multi-laboratory study. *Science* 2011;**334**:814–17.
97. Sisto SA, Tapp W, Dristal S, et al. Vagal tone is reduced during paced breathing in patients with the chronic fatigue syndrome. *Clin Auton Res* 1995;**5**:139–43.
98. Smith AK, White PD, Aslakson E, et al. Polymorphisms in genes regulating the HPA axis associated with empirically delineated classes of unexplained chronic fatigue. *Pharmacogenomics* 2006;**7**: 387–94.
99. Smith MS, Mitchell J, Corey L, et al. Chronic fatigue in adolescents. *Pediatrics* 1991;**88**:195–202.
100. Spink WW. What is chronic brucellosis? *Ann Intern Med* 1951; **35**:358–74.

101. Steere AC, Taylor E, McHugh GL, Logigian EL. The overdiagnosis of Lyme disease. *JAMA* 1993;**269**:1812–16.
102. Steinau M, Unger E, Vernon S, et al. Differential display PCR of peripheral blood for biomarker discovery in chronic fatigue syndrome. *J Mol Med* 2004;**82**:750–5.
103. Stewart J. Autonomic nervous system dysfunction in adolescents with postural orthostatic tachycardia syndrome and chronic fatigue syndrome is characterized by attenuated vagal baroreflex and potentiated sympathetic vasomotion. *Pediatr Res* 2000;**48**: 218–26.
104. Stewart JM, Gewitz MH, Weldon A, Munoz J. Patterns of orthostatic intolerance: the orthostatic tachycardia syndrome and adolescent chronic fatigue. *J Pediatr* 1999;**135**:218–25.
105. Stewart JM, Gewitz MH, Weldon A, et al. Orthostatic intolerance in adolescent chronic fatigue syndrome. *Pediatrics* 1999;**103**: 116–21.
106. Straus SE. Intravenous immunoglobulin treatment for the chronic fatigue syndrome. *Am J Med* 1990;**89**:551–3.
107. Straus SE. History of chronic fatigue syndrome. *Rev Infect Dis* 1991;**13**(Suppl. 1):S2–7.
108. Straus SE, Dale JK, Peter JB, Dinarello CA. Circulating lymphokine levels in the chronic fatigue syndrome. *J Infect Dis* 1989; **160**:1085–6.
109. Straus SE, Dale DK, Tobi M, et al. Acyclovir treatment of the chronic fatigue syndrome. *N Engl J Med* 1988;**319**:1692–8.
110. Straus SE, Dale JK, Wright R, Metcalfe DD. Allergy and the chronic fatigue syndrome. *J Allergy Clin Immunol* 1988;**81**: 791–5.
111. Straus SE, Fritz S, Dale JK, et al. Lymphocyte phenotype and function in the chronic fatigue syndrome. *J Clin Immunol* 1993; **13**:30–40.
112. Straus SE, Tosato G, Armstrong G, et al. Persisting illness in adults with evidence of Epstein-Barr infection. *Ann Intern Med* 1985;**102**:7–16.
113. Sumaya CV. Serologic and virologic epidemiology of Epstein-Barr virus: relevance to chronic fatigue syndrome. *Rev Infect Dis* 1991; **13**(Suppl. 1):S19–25.
114. Swanink CM, Van der Meer JW, Vercoulen JH, et al. Epstein-Barr virus (EBV) and the chronic fatigue syndrome: normal virus load in blood and normal immunologic reactivity in the EBV regression assay. *Clin Infect Dis* 1995;**20**:1390–2.
115. Swanink CM, Vercoulen JH, Galama JM, et al. Lymphocyte subsets, apoptosis, and cytokines in patients with chronic fatigue syndrome. *J Infect Dis* 1996;**173**:460–3.
116. Switzer WM, Jia H, Hohn O, et al. Absence of evidence of xenotropic murine leukemia virus–related virus infection in persons with chronic fatigue syndrome and healthy controls in the United States. *Retrovirology* 2010;**7**:57. Available at http://www.retrovirology.com/content/7/1/57.
117. Togo F, Natelson BH, Cherniack NS, et al. Sleep structure and sleepiness in chronic fatigue syndrome with or without coexisting fibromyalgia. *Arthritis Res Ther* 2008;**10**:R56. Available at http://arthritis-research.com/content/10/3/R56.
118. Vollmer-Conna U, Lloyd A, Hickie I, Wakefield D. Chronic fatigue syndrome: an immunological perspective. *Aust N Z J Psychiatry* 1998;**32**:523–7.
119. Vercoulen J, Swanink CM, Zitman FG, et al. Randomised, double-blind, placebo-controlled study of fluoxetine in chronic fatigue syndrome. *Lancet* 1996;**347**:858–61.
120. Wakefield D, Lloyd A, Dwyer J, et al. Human herpesvirus 6 and myalgic encephalomyelitis. *Lancet* 1988;**1**:1059.
121. Warren G, McKendrick M, Peet M. The role of essential fatty acids in chronic fatigue syndrome: a case-controlled study of red-cell membrane essential fatty acids (EFA) and a placebo-controlled treatment study with high-dose of EFA. *Acta Neurol Scand* 1999; **99**:112–16.
122. Weardon AJ, Appleby L. Research on cognitive complaints and cognitive functioning in patients with chronic fatigue syndrome (CFS): what conclusions can we draw? *J Psychosom Res* 1996; **41**:197–211.
123. Wearden AJ, Morriss RK, Mullis R, et al. Randomised, double-blind, placebo-controlled treatment trial of fluoxetine and graded exercise for chronic fatigue syndrome. *Br J Psychiatry* 1998;**172**: 485–92.
124. Wessely S. Chronic fatigue syndrome: trials and tribulations. *JAMA* 2001;**286**:1378–9.
125. White PD, Goldsmith KA, Johnson AL, et al. Comparison of adaptive pacing therapy, cognitive behavior therapy, graded exercise therapy, and specialist care for chronic fatigue syndrome (PACE): a randomized trial. *Lancet* 2011;**377**:823–7.
126. Whiting P, Bagnall AM, Sowden AJ, et al. Interventions for the treatment and management of chronic fatigue syndrome: A systematic review. *JAMA* 2001;**286**:1360–8.
127. Wilson A, Hickie L, Lloyd A, et al. Longitudinal study of outcome of chronic fatigue syndrome. *BMJ* 1994;**308**:756–9.
128. Wyller VB, Thaulow E, Amlie JP. Treatment of chronic fatigue and orthostatic intolerance with propranolol. *J Pediatr* 2007; **150**:654–5.

This page intentionally left blank

PART III

INFECTIONS WITH SPECIFIC MICROORGANISMS

This page intentionally left blank

SECTION **XVI**

BACTERIAL INFECTIONS

CHAPTER 79

Nomenclature for Aerobic and Anaerobic Bacteria

David A. Bruckner

Table 79-1 represents an update of the current nomenclature, taxonomy, and classification of various microbial agents and is based on the organism's morphologic and stain characteristics. Organisms included are those that often are associated with pathologic processes or that are medically significant. The current names are those either officially recognized or proposed for recognition and currently used in the literature. Some organisms listed have been characterized, but have never been cultured.

Taxonomic ranks for naming bacterial organisms include kingdom, division, class, order, family, genus, species, and subspecies. All of these ranks have official standing in nomenclature. Ranks below subspecies have no official standing but are used to indicate groups of strains or isolates that can be distinguished by some special characteristics (Table 79-2). Each taxonomic name should be represented by a nomenclature type. The species is represented by a type strain that is deposited in recognized culture collections.[4] Nomenclature priorities for bacteriologic names date back to May, 1753. Because of difficulties in searching the literature and limited available information on described species, approved lists of bacterial names were published in the *International Journal of Systematic Bacteriology* in 1980. Names not included on those lists have lost all standing in nomenclature status.

Historically, bacterial classification has been based on phenotypic characteristics. Multivariate analysis has played a large role in classification since the 1950s. This analysis used biochemical, cultural, and morphologic characteristics and susceptibilities to antibiotics and inorganic compounds to define the degrees of similarities among organisms. More recently, molecular techniques (e.g., DNA hybridization, rRNA-DNA hybridization, gene sequence analysis, and multilocus sequence analysis) have played a major role in determining phylogenetic relationships. The DNA molecular weight for most bacteria is 1×10^9 to 8×10^9 D, enough to specify 1500 to 6000 genes. Using nucleic acid analysis, researchers have developed numerous parameters to determine taxonomic relationships. By correlating phenotypic results with DNA homology and rRNA sequence analysis, researchers have been able to select phenotypic tests that can be used more accurately to identify organisms belonging to specific groups.[3,7] Molecular methods have helped to define groupings of organisms and have led to considerable changes in bacterial nomenclature. The primary purpose of nomenclature is to permit us to know as exactly as possible what another clinician, microbiologist, epidemiologist, or investigator is referring to when describing an organism responsible for infecting individuals or for causing an outbreak. The *International Code of Nomenclature of Bacteria*[5] includes rules on how to name bacteria and use the name. The most comprehensive taxonomic information available for bacteriologic classification can be found in *Bergey's Manual of Systematic Bacteriology*,[1,2] volumes 1 through 4. Names are validated only when they are included in the validation lists regularly published by the *International Journal of Systematic and Evolutionary Microbiology*.[4,6] An overview of validly published names can be obtained at http://www.bacterio.cict.fr or http://www.dsmz.de/microorganisms/bacterial_nomenclature.php.

Text continued on p. 1112

TABLE 79-1 Current Bacterial Nomenclature, Taxonomy, and Classification

I. Aerobic Gram-Positive Cocci

Characteristics: Aerotolerant anaerobes that can occur singly or in pairs, tetrads, chains, or clusters; they can be catalase-positive or catalase-negative. Organisms positive for coagulase or clumping factor include *Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus lugdunensis,* and *Staphylococcus schleiferi* subspecies *coagulans*.

Current Name	Synonym
Catalase-Positive Organisms	
Alloiococcus otitidis	*Alloiococcus otitis*
Auritidibacter ignavus	
Kocuria carniphila	
Kocuria kristinae	*Micrococcus kristinae*
Kocuria rhizophila	
Kocuria rosea	*Micrococcus roseus*
Kocuria varians	*Micrococcus varians*
Kytococcus schroeteri	
Kytococcus sedentarius	*Micrococcus sedentarius*
Micrococcus luteus	
Micrococcus lylae	
Staphylococcus arlettae	
Staphylococcus aureus subspecies *anaerobius*	
Staphylococcus aureus subspecies *aureus*	
Staphylococcus auricularis	
Staphylococcus capitis subspecies *capitis*	
Staphylococcus capitis subspecies *ureolyticus*	
Staphylococcus caprae	
Staphylococcus carnosus subspecies *carnosus*	
Staphylococcus carnosus subspecies *utilis*	
Staphylococcus chromogenes	*Staphylococcus hyicus* subspecies *chromogenes*
Staphylococcus cohnii subspecies *cohnii*	
Staphylococcus cohnii subspecies *urealyticus*	
Staphylococcus condimenti	
Staphylococcus delphini	
Staphylococcus epidermidis	*Staphylococcus albus*
Staphylococcus equorum subspecies *equorum*	
Staphylococcus equorum subspecies *linens*	
Staphylococcus felis	
Staphylococcus fleurettii	
Staphylococcus gallinarum	
Staphylococcus haemolyticus	
Staphylococcus hominis subspecies *hominis*	
Staphylococcus hominis subspecies *novobiosepticus*	
Staphylococcus hyicus	
Staphylococcus intermedius	
Staphylococcus kloosii	
Staphylococcus lentus	*Staphylococcus sciuri* subspecies *lentus*
Staphylococcus lugdunensis	
Staphylococcus lutrae	
Staphylococcus muscae	
Staphylococcus nepalensis	
Staphylococcus pasteuri	
Staphylococcus pettenkoferi	
Staphylococcus piscifermentans	
Staphylococcus pseudintermedius	
Staphylococcus saccharolyticus	*Peptococcus saccharolyticus*
Staphylococcus saprophyticus subspecies *bovis*	*Micrococcus* subgroup 3
Staphylococcus saprophyticus subspecies *saprophyticus*	
Staphylococcus schleiferi subspecies *coagulans*	
Staphylococcus schleiferi subspecies *schleiferi*	
Staphylococcus sciuri subspecies *carnaticus*	
Staphylococcus sciuri subspecies *rodentium*	
Staphylococcus sciuri subspecies *sciuri*	
Staphylococcus simiae	
Staphylococcus simulans	
Staphylococcus succinus subspecies *casei*	
Staphylococcus succinus subspecies *succinus*	
Staphylococcus warneri	
Staphylococcus xylosus	
Catalase-Negative Organisms	
Abiotrophia defectiva	*Streptococcus defectivus* Nutritionally variant streptococci
Aerococcus christensenii	
Aerococcus sanguinicola	*Aerococcus sanguicola*
Aerococcus urinae	
Aerococcus urinaehominis	
Aerococcus viridans	
Dolosicoccus paucivorans	
Dolosigranulum pigrum	
Enterococcus avium	*Streptococcus avium* Group D *Enterococcus*
Enterococcus caccae	
Enterococcus casseliflavus	*Enterococcus flavescens* *Streptococcus casseliflavus*
Enterococcus cecorum	*Streptococcus cecorum*
Enterococcus dispar	
Enterococcus durans	*Streptococcus durans* Group D *Enterococcus*

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Enterococcus faecalis	*Streptococcus faecalis* Group D *Enterococcus*
Enterococcus faecium	*Streptococcus faecium* Group D *Enterococcus*
Enterococcus gallinarum	*Streptococcus gallinarum*
Enterococcus gilvus	
Enterococcus hawaiiensis	
Enterococcus hirae	
Enterococcus italicus	*Enterococcus saccharominimus*
Enterococcus malodoratus	
Enterococcus mundtii	
Enterococcus pallens	
Enterococcus pseudoavium	
Enterococcus raffinosus	
Enterococcus sanguinicola	
Facklamia hominis	
Facklamia ignava	
Facklamia languida	
Facklamia sourekii	
Gemella bergeri	*Gemella bergeriae*
Gemella haemolysans	*Neisseria haemolysans*
Gemella morbillorum	*Streptococcus morbillorum* *Peptostreptococcus morbillorum*
Gemella sanguinis	
Globicatella sanguinis	Salt-tolerant viridans streptococci
Granulicatella adiacens	*Abiotrophia adiacens* *Streptococcus adiacens* Nutritionally variant streptococci
Granulicatella elegans	*Abiotrophia elegans* Nutritionally variant streptococci
Granulicatella para-adiacens	
Helcococcus kunzii	
Helcococcus pyogenica	
Helcococcus sueciensis	
Ignavigranum ruoffiae	
Lactococcus garvieae	*Streptococcus garvieae* Lancefield group N
Lactococcus lactis	
Leuconostoc citreum	
Leuconostoc dextranicum	
Leuconostoc lactis	
Leuconostoc mesenteroides	
Leuconostoc pseudomesenteroides	
Oenococcus oeni	*Leuconostoc oenos*
Pediococcus acidilactici	
Pediococcus damnosus	
Pediococcus dextrinicus	
Pediococcus equinus	*Streptococcus equinus*
Pediococcus parvulus	
Pediococcus pentosaceus	
Streptococcus acidominimus	
Streptococcus bovis group	Group D streptococci
Streptococcus alactolyticus	
Streptococcus bovis	
Streptococcus equinus	
Streptococcus gallolyticus subspecies *gallolyticus*	*S. bovis* biotype I
Streptococcus gallolyticus subspecies *macedonicus*	*S. bovis* biotype II.1
Streptococcus gallolyticus subspecies *pasteurianus*	*S. bovis* biotype II.2
Streptococcus infantarius subspecies *coli*	
Streptococcus infantarius subspecies *infantarius*	
Streptococcus anginosis group	Viridans streptococci
Streptococcus anginosus	
Streptococcus constellatus subspecies *pharyngis*	
Streptococcus intermedius	
***Streptococcus mitis* group**	Viridans streptococci
Streptococcus australis	
Streptococcus cristatus	
Streptococcus gordonii	
Streptococcus infantis	
Streptococcus mitis	*Streptococcus mitior* *Streptococcus sanguis* II
Streptococcus oligfermentans	
Streptococcus oralis	
Streptococcus orisratti	
Streptococcus parasanguinis	
Streptococcus peroris	
Streptococcus pneumoniae	*Diplococcus pneumoniae*
Streptococcus pseudopneumoniae	
Streptococcus sanguinis	
Streptococcus sinensis	
***Streptococcus mutans* group**	*Viridans streptococci*
Streptococcus cricetus	
Streptococcus mutans	
Streptococcus ratti	
Streptococcus sobrinus	
***Streptococcus pyogenes* group**	
Streptococcus agalactiae	Group B streptococci
Streptococcus canis	
Streptococcus dysgalactiae subspecies *equisimilis*	Group C streptococci
Streptococcus equi subspecies *zooepidermidis*	*Streptococcus equi* *Streptococcus equisimilis*
Streptococcus iniae	Group G streptococci *Streptococcus shiloi*
Streptococcus porcinus	
Streptococcus pyogenes	Group A streptococci

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Streptococcus salivarius group	Viridans streptococci
Streptococcus salivarius	
Streptococcus thermophilus	
Streptococcus vestibularis	
Streptococcus suis	
Tetragenococcus halophilus	*Pediococcus halophilus*
Tetragenococcus solitarius	*Enterococcus solitarius*
Vagococcus fluvialis	
Weissella paramesenteroides	*Leuconostoc paramesenteroides*
II. Anaerobic Gram-Positive Cocci	
Characteristics: Occur singly or in pairs, chains, or clumps.	
Anaerococcus hydrogenalis	*Peptostreptococcus hydrogenalis*
Anaerococcus lactolyticus	*Peptostreptococcus lactolyticus*
Anaerococcus murdochii	
Anaerococcus octavius	*Peptostreptococcus octavius*
Anaerococcus prevotii	*Peptostreptococcus prevotii*
Anaerococcus tetradius	*Peptostreptococcus tetradius*
Anaerococcus vaginalis	
Blautica productus	*Peptostreptococcus productus*
Blautica wexlerae	
Fingoldia magna	*Ruminococcus productus*
Parvimonas micra	*Peptococcus magnus*
	Peptostreptococcus micros
	Micromonas micros
Peptococcus niger	*Micrococcus niger*
Peptoniphilus asaccharolyticus	*Peptostreptococcus asaccharolyticus*
Peptoniphilus gorbachii	
Peptoniphilus harei	*Peptostreptococcus harei*
Peptoniphilus indolicus	
Peptoniphilus ivorii	*Peptostreptococcus ivorii*
Peptoniphilus lacrimalis	*Peptostreptococcus lacrimalis*
Peptoniphilus olsenii	
Peptostreptococcus anaerobius	
Peptostreptococcus indolicus	*Peptococcus indolicus*
Peptostreptococcus magnus	*Peptococcus magnus*
	Peptococcus variabilis
Peptostreptococcus massiliae	
Peptostreptococcus stomatis	
Peptostreptococcus trisimilis	
Ruminococcus faecis	
Ruminococcus gauvreauii	*Streptococcus hansenii*
Slackia heliotrinireducens corrig	*Peptostreptococcus heliotrinireducens*
III. Aerobic Gram-Negative Coccii	
Characteristics: Occur singly or in pairs or clumps; catalase and oxidase positive.	
Lautropia mirabilis	*Sarcina mirabilis*
Neisseria bacilliformis	
Neisseria canis	
Neisseria cinerea	*Micrococcus cinereus*
	Neisseria pharyngis
Neisseria elongata subspecies *elongata*	*Neisseria elongata*
Neisseria elongata subspecies *glycolytica*	*Neisseria elongata*
Neisseria elongata subspecies *nitroreducens*	*Neisseria elongata*
	CDC group M-6
Neisseria flavescens	
Neisseria gonorrhoeae	
Neisseria lactamica	*Neisseria lactamicus*
Neisseria meningitidis	
Neisseria mucosa	
Neisseria parelongata	
Neisseria polysaccharea	
Neisseria shayeganii	
Neisseria sicca	
Neisseria subflava biovar *flava*	*Neisseria subflava*
Neisseria subflava biovar *perflava*	*Neisseria subflava*
Neisseria subflava biovar *subflava*	*Neisseria subflava*
Neisseria wadsworthii	
Neisseria weaveri	Moraxella sp. M-5
	CDC group M-5
IV. Anaerobic Gram-Negative Cocci	
Characteristics: Occur in pairs or clumps.	
Acidaminococcus fermentans	
Acidaminococcus intestini	
Anaeroglobus geminatus	
Megasphaera elsdenii	*Peptostreptococcus elsdenii*
Megasphaera micronuciformis	
Veillonella atypica	
Veillonella denticariosi	
Veillonella dispar	
Veillonella montpellierensis	
Veillonella parvula	
V. Aerobic Gram-Positive Bacilli	
Characteristics: Rodlike; catalase-negative or catalase-positive; some are acid-fast stain–positive, and some have branching. Only *Bacillus, Brevibacillus,* and *Brevibacterium* spp produce spores.	
Actinomadura latina	
Actinomadura madurae	
Actinomadura pelletieri	
Arcanobacterium bernardiae	*Actinomyces bernardiae*
	CDC coryneform group 2
Arcanobacterium haemolyticum	*Corynebacterium haemolyticum*
Arcanobacterium pyogenes	*Actinomyces pyogenes*
	Corynebacterium pyogenes
Arthrobacter albus	
Arthrobacter cumminsii	
Arthrobacter oxydans	
Bacillus anthracis	

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Bacillus cereus	
Bacillus circulans	
Bacillus coagulans	
Bacillus firmis	
Bacillus idriensis	
Bacillus infantis	
Bacillus lentus	
Bacillus licheniformis	
Bacillus megaterium	
Bacillus mycoides	
Bacillus pumilus	
Bacillus sphaericus	
Bacillus subtilis	
Bacillus thuringiensis	
Brevibacillus agri	*Bacillus agri*
Brevibacillus brevis	*Bacillus brevis*
Brevibacillus centrosporus	
Brevibacillus laterosporus	*Bacillus laterosporus*
Brevibacillus parabrevis	CDC coryneform groups B-1 and B-3
Brevibacterium casei	
Brevibacterium epidermidis	
Brevibacterium mcbrellneri	
Brevibacterium sanguinis	
Cellulomonas denverensis	CDC coryneform group A-3
Cellulomonas hominis	
Cellulosimicrobium cellulans	*Cellulomonas cellulans*
	Oerskovia xanthineolytica
	CDC coryneform groups A-1 and A-2
Corynebacterium accolens	
Corynebacterium afermentans subspecies *afermentans*	CDC coryneform group ANF-1
Corynebacterium afermentans subspecies *lipophilum*	CDC coryneform group ANF-1
Corynebacterium amycolatum	*Corynebacterium xerosis*
	Corynebacterium minutissimum
	Corynebacterium striatum
	CDC coryneform groups F-2 and I-2
Corynebacterium aquaticum	
Corynebacterium argentoratense	
Corynebacterium atypicum	
Corynebacterium aurimucosum	CDC fermentive coryneform group 4
Corynebacterium auris	*Corynebacterium nigricans*
	CDC coryneform group ANF-1
Corynebacterium bovis	
Corynebacterium canis	
Corynebacterium confusum	
Corynebacterium coyleae	
Corynebacterium diphtheriae	
Corynebacterium durum	
Corynebacterium falsenii	
Corynebacterium freiburgense	
Corynebacteriun freneyi	
Corynebacterium glucuronolyticum	
Corynebacterium hansenii	
Corynebacterium imitans	
Corynebacterium jeikeium	*Corynebacterium* group JK
	CDC coryneform group JK
Corynebacterium kroppenstedtii	
Corynebacterium lipophiloflavum	
Corynebacterium macginleyi	CDC coryneform group G-1
Corynebacterium massiliense	
Corynebacterium matruchotii	*Bacterionema matruchotii*
Corynebacterium minutissimum	
Corynebacterium mucifaciens	
Corynebacterium pilbarense	
Corynebacterium pilosum	
Corynebacterium propinquum	CDC coryneform group ANF-3
Corynebacterium pseudodiphtheriticum	*Corynebacterium hofmannii*
Corynebacterium pseudotuberculosis	
Corynebacterium resistens	
Corynebacterium riegelii	
Corynebacterium sanguinis	
Corynebacterium simulans	
Corynebacterium singulare	
Corynebacterium statienis	
Corynebacterium striatum	
Corynebacterium sundsvallense	*Corynebacterium thomssenii*
	Rothia dentocariosa
Corynebacterium thomssenii	
Corynebacterium timonense	
Corynebacterium tuberculostearicum	
Corynebacterium tuscania	
Corynebacterium ulcerans	
Corynebacterium urealyticum	*Corynebacterium* group D2
	CDC coryneform group D2
Corynebacterium ureicelerivorans	
Corynebacterium xerosis	
Curtobacterium spp	
Dermabacter hominis	CDC fermentative coryneform groups 3 and 5
Dermatophilus congolensis	*Actinomyces congolensis*
Dietzia aurantiaca	
Dietzia cinnamea	
Erysipelothrix rhusiopathiae	*Erysipelothrix insidiosa*
Exiguobacterium acetylicum	*Brevibacterium acetylicum*
Exiguobacterium aurantiacum	*Brevibacterium aurantiacum*
Gardnerella vaginalis	*Haemophilus vaginalis*
	Corynebacterium vaginalis
Gordonia bronchialis	*Rhodococcus bronchialis*
Gordonia polyisoprenivorans	
Gordonia rubropertincta	*Rhodococcus rubiopertincta*

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Gordonia sputi	*Rhodococcus sputi*
	Rhodococcus chubuensis
Gordonia terrae	*Rhodococcus terrae*
Listeria grayi subspecies *grayi*	*Listeria grayi*
Listeria ivanovii subspecies *ivanovii*	*Listeria monocytogenes* serovar 5
Listeria ivanovii subspecies *londoniensis*	
Listeria monocytogenes	
Lysinibacillus massiliensis	*Bacillus massiliensis*
Lysinibacillus sphaericus	
Microbacterium foliorum	
Microbacterium oxydans	
Mycobacterium africanum subtype I	
Mycobacterium africanum subtype II	
Mycobacterium agri	
Mycobacterium aichiense	
Mycobacterium alvei	
Mycobacterium aromaticivorans	
Mycobacterium arosiense	
Mycobacterium arupense	
Mycobacterium asiaticum	
Mycobacterium aurum	
Mycobacterium avium subspecies *avium*	
Mycobacterium avium subspecies *paratuberculosis*	
Mycobacterium barassie	
Mycobacterium boenickei	
Mycobacterium bohemicum	
Mycobacterium botniense	
Mycobacterium bouchedurhonense	
Mycobacterium bovis	
Mycobacterium branderi	
Mycobacterium brumae	
Mycobacterium canariassense	
Mycobacterium caprae	
Mycobacterium celatum	
***Mycobacterium chelonae/ abscessus* group**	
Mycobacterium abscessus subspecies *abscessus*	
Mycobacterium abscessus subspecies *bolletii*	
Mycobacterium chelonae	*Mycobacterium chelonei*
Mycobacterium immunogenum	
Mycobacterium salmoniphilum	
Mycobacterium chimaera	
Mycobacterium chitae	
Mycobacterium chlorophenolicum	
Mycobacterium chubuense	
Mycobacterium colombiense	
Mycobacterium conceptionense	
Mycobacterium confluentis	
Mycobacterium conspicuum	
Mycobacterium cookii	
Mycobacterium cosmeticum	
Mycobacterium crocinum	
Mycobacterium diernhoferi	
Mycobacterium doricum	
Mycobacterium duvalii	
Mycobacterium elephantis	
Mycobacterium europaeum	
Mycobacterium fallax	
Mycobacterium farcinogenes	
Mycobacterium flavescens	
Mycobacterium florentinum	
Mycobacterium fluoranthenivorans	
***Mycobacterium fortuitum* group**	
Mycobacterium boenickei	
Mycobacterium brisbanense	
Mycobacterium fortuitum	
Mycobacterium houstonense	
Mycobacterium neworleansense	
Mycobacterium peregrimum	
Mycobacterium porcinum	
Mycobacterium senegalense	
Mycobacterium septicum	
Mycobacterium setense	
Mycobacterium gastri	
Mycobacterium genavense	
Mycobacterium gordonae	*Mycobacterium aquae*
Mycobacterium haemophilum	
Mycobacterium heckeshornense	
Mycobacterium heidelbergense	
Mycobacterium hiberniae	
Mycobacterium interjectum	
Mycobacterium intermedium	
Mycobacterium intracellulare	
Mycobacterium jacuzzi	
Mycobacterium kansasii	
Mycobacterium kubicae	
Mycobacterium lentiflavum	
Mycobacterium leprae	
Mycobacterium lepraemurium	
***Mycobacterium mageritense/ wolinskyi* group**	
Mycobacterium mageritense	
Mycobacterium wolinskyi	
Mycobacterium malmoense	
Mycobacterium mantenii	*Mycobacterium balnei*
Mycobacterium marinum	
Mycobacterium marseillense	
Mycobacterium microti	
Mycobacterium monacense	
Mycobacterium monteofiorense	
***Mycobacterium mucogenicum* group**	
Mycobacterium aubagnense	
Mycobacterium mucogenicum	
Mycobacterium phocaicum	
Mycobacterium moriokaense	

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Mycobacterium nebraskense	
Mycobacterium neoaurum	
Mycobacterium neworleansene	
Mycobacterium nonchromogenicum	
Mycobacterium noviomagense	
Mycobacterium novocastrense	
Mycobacterium palustre	
Mycobacterium paraffinicum	
Mycobacterium parascrofulaceum	
Mycobacterium paraseoulense	
Mycobacterium parmense	
Mycobacterium pseudoshottsii	
Mycobacterium pulveris	
Mycobacterium riyadhense	
Mycobacterium saskatchewanense	
Mycobacterium scrofulaceum	
Mycobacterium senuense	
Mycobacterium seoulense	
Mycobacterium sherrisii	
Mycobacterium shimoidei	
Mycobacterium shinjukuense	
Mycobacterium shottsii	
Mycobacterium simiae	*Mycobacterium habana*
***Mycobacterium smegmatis* group**	
Mycobacterium goodi	
Mycobacterium smegmatis	
Mycobacterium stomatepiae	
Mycobacterium szulgai	
Mycobacterium terrae	
Mycobacterium timonense	
Mycobacterium triplex	
Mycobacterium triviale	
Mycobacterium tuberculosis	
Mycobacterium tusciae	
Mycobacterium ulcerans	*Mycobacterium buruli*
Mycobacterium xenopi	
Nocardia abscessus	
Nocardia africana	
Nocardia anaemiae	
Nocardia aobensis	
Nocardia araoensis	
Nocardia arthritidis	
Nocardia asiatica	
Nocardia asteroides type IV	
Nocardia asteroides type VI	
Nocardia beijingensis	
Nocardia blacklockiae	
Nocardia brasiliensis	
Nocardia brevicatena	*Micropolyspora brevicatena*
Nocardia carnea	
Nocardia concava	
Nocardia corynebacteroides	
Nocardia cyriacigeorgica	*Nocardia cyriacigeorgici*
Nocardia elegans	
Nocardia exalbida	
Nocardia farcinica	
Nocardia higoensis	
Nocardia ignorata	
Nocardia inohanensis	
Nocardia kruczakiae	
Nocardia mexicana	
Nocardia neocaledoniensis	
Nocardia nigatensis	
Nocardia niniae	
Nocardia niwae	
Nocardia nova	
Nocardia orientalis	
Nocardia otitidiscaviarum	*Nocardia caviae*
Nocardia paucivorans	
Nocardia pneumoniae	
Nocardia pseudobrasiliensis	
Nocardia puris	
Nocardia shimofusensis	
Nocardia sienata	*Nocardia senatus*
Nocardia takadensii	
Nocardia terpenica	
Nocardia thailandica	
Nocardia transvalensis	
Nocardia vermiculata	
Nocardia veterana	
Nocardia vinacea	
Nocardia wallacei	
Nocardia yamanashiensis	
Nocardiopsis dassonvillei	*Actionmadura dassonvillei*
	Nocardia dassonvillei
Oerskovia turbata	
Paenibacillus alvei	*Bacillus alvei*
Paenibacillus konsidensis	
Paenibacillus macerans	*Bacillus mascerans*
Paenibacillus massiliensis	
Paenibacillus polymyxa	*Bacillus polymyxa*
Paenibacillus popiliae	
Paenibacillus sanguinis	
Paenibacillus timonensis	
Rhodococcus equi	*Corynebacterium equi*
Rothia aeria	
Rothia dentocariosa	*Nocardia dentocariosus*
Rothia mucilaginosa	*Stomatococcus mucilaginosus*
Streptomyces albus	
Streptomyces bikiniensis	
Streptomyces cacaoi subspecies *cacaoi*	
Streptomyces thermovulgaris	
Tropheryma whipplei	*Tropheryma whippelii*
Tsukamurella inchonensis	
Tsukamurella paurometabola	*Gordona aurantiaca*
Tsukamurella pulmonis	
Tsukamurella stranjordii	
Tsukamurella tyrosinosolvens	
Turicella otitidis	*Rhodococcus aurantiacus*
Williamsia deligens	
Williamsia muralis	

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
VI. Anaerobic Gram-Positive Bacilli (Non–Spore-Forming)	
Characteristics: May be long-branching bacilli or pleomorphic coccobacilli.	
Actinobaculum massiliae	
Actinobaculum schaalii	
Actinobaculum suis	*Actinomyces suis*
Actinobaculum timonae	
Actinobaculum urinale	
Actinomyces cardiffensis	
Actinomyces dentalis	
Actinomyces europaeus	
Actinomyces funkei	
Actinomyces georgiae	*Actinomyces* DO8
Actinomyces gerencseriae	*Actinomyces israelii* serotype II
Actinomyces graevenitzii	
Actinomyces hongkongensis	
Actinomyces israelii	
Actinomyces johnsonii	
Actinomyces massiliensis	
Actinomyces meyeri	
Actinomyces naeslundii	
Actinomyces nasicola	
Actinomyces neuii subspecies *anitratus*	
Actinomyces neuii subspecies *neuii*	CDC coryneform group 1
Actinomyces odontolyticus	
Actinomyces oricola	
Actinomyces radicidentis	
Actinomyces radingae	CDC coryneform group E; APL1
Actinomyces turicensis	CDC coryneform group E; APL10
Actinomyces urogenitalis	
Actinomyces viscosus	
Anaerofustis stercorihominis	
Atopobium fossor	*Eubacterium fossor*
Atopobium minutum	*Lactobacillus minutus*
Atopobium parvulum	*Streptococcus parvulus* *Peptostreptococcus parvulus*
Atopobium rimae	*Lactobacillus rimae*
Atopobium vaginae	
Bifidobacterium adolescentis	
Bifidobacterium angulatum	
Bifidobacterium bifidum	
Bifidobacterium breve	
Bifidobacterium catenulatum	
Bifidobacterium dentium	*Bifidobacterium appendicitis*
Bifidobacterium gallicum	
Bifidobacterium longum subspecies *infantis*	*Bifidobacterium infantis*
Bifidobacterium longum subspecies *longum*	*Bifidobacterium longum*
Bifidobacterium pseudocatenulatum	
Bifidobacterium scardovii	
Bifidobacterium subtile	
Collinsella aerofaciens	*Eubacterium aerofaciens*
Collinsella intestinalis	
Collinsella stercoralis	
Cryptobacterium curtum	
Dorea formicigenerans	*Eubacterium formicigenerans*
Dorea longicatena	
Eggerthella hongkongensis	
Eggerthella lenta	*Eubacterium lentum*
Eggerthella sinensis	
Eggerthia catenaformis	*Lactobacillus catenaformis*
Eubacterium brachy	
Eubacterium combesii	
Eubacterium contortum	
Eubacterium infirmum	
Eubacterium limosum	
Eubacterium minutum	*Eubacterium tardum*
Eubacterium moniliforme	
Eubacterium nitritogenes	
Eubacterium nodatum	
Eubacterium rectale	
Eubacterium saburreum	
Eubacterium saphenum	
Eubacterium sulci	*Fusobacterium sulci*
Eubacterium tenue	
Eubacterium timidum	
Eubacterium yurii subspecies *margaretiae*	
Eubacterium yurii subspecies *yurii*	
Holdemania filiformis	*Eubacterium* S14
Kandleria vitulinus	*Lactobacillus vitulinus*
Lactobacillus acidophilus	
Lactobacillus brevis	
Lactobacillus casei	
Lactobacillus colehominis	
Lactobacillus confusa	
Lactobacillus crispatus	
Lactobacillus fermentum	
Lactobacillus gasseri	
Lactobacillus iners	
Lactobacillus jensenii	
Lactobacillus johnsonii	
Lactobacillus plantarum	
Lactobacillus rhamnosus	*Lactobacillus* GG
Lactobacillus salivarius	
Lactobacillus ultunensis	
Mobiluncus curtisii subspecies *curtisii*	
Mobiluncus curtisii subspecies *holmesii*	
Mobiluncus mulieris	*Falcivibrio grandis*
Mogibacterium timidum	
Mogibacterium vescum	
Olsenella profusa	
Olsenella uli	*Lactobacillus uli*
Parascardovia denticolens	*Bifidobacterium denticolens*

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Propionibacterium acidifaciens	
Propionibacterium acnes	
Propionibacterium avidum	
Propionibacterium granulosum	
Propionibacterium lymphophilum	
Propionibacterium propionicum	*Propionibacterium propionicum*
	Arachnia propionica
	Actinomyces propionicus
Pseudoramibacter alactolyticus	*Eubacterium alactolyticum*
Scardovia inopinata	*Bifidobacterium inopinatum*
	Bifidobacterium dentium
Scardovia wiggsiae	
Shuttleworthia satelles	
Slackia exigua	*Eubacterium exigaum*
Solobacterium moorei	
Varibaculum cambriense	
VII. Anaerobic Gram-Positive Bacilli (Spore-Forming)	
Characteristics: Broad, short bacilli with blunt ends. Most organisms readily produce spores, except *Clostridium perfringens* and *Tissierella praeacuta*.	
Clostridium aldenense	
Clostridium amygdalinum	
Clostridium argentinense	*Clostridium botulinum* group G
	Clostridium subterminale
	Clostridium hastiforme
Clostridium baratii	*Clostridium barati*
	Clostridium paraperfringens
	Clostridium perenne
Clostridium bartlettii	
Clostridium bifermentans	
Clostridium bolteae	
Clostridium botulinum	*Clostridium putrificum*
Clostridium butyricum	*Clostridium pseudotetanicum*
Clostridium cadaveris	
Clostridium carnis	
Clostridium celerecrescens	
Clostridium citroniae	
Clostridium clostridioforme	*Clostridium clostridioforme*
Clostridium cocleatum	
Clostridium difficile	*Clostridium difficilis*
Clostridium disporicum	
Clostridium fallax	*Clostridium pseudofallax*
Clostridium ghonii	*Clostridium ghoni*
Clostridium glycolicum	
Clostridium haemolyticum	*Clostridium novyi*
Clostridium hastiforme	
Clostridium hathewayi	
Clostridium hiranonis	
Clostridium histolyticum	
Clostridium indolis	
Clostridium innocuum	
Clostridium intestinale	
Clostridium irregulare	*Clostridium irregularis*
Clostridium leptum	
Clostridium limosum	CDC group P-1
Clostridium malenominatum	
Clostridium neonatale	
Clostridium novyi	
Clostridium orbiscindens	
Clostridium oroticum	*Zymobacterium oroticum*
Clostridium paraputrificum	
Clostridium perfringens	*Clostridium welchii*
	Welchia perfringens
Clostridium piliforme	*Bacillus piliformis*
Clostridium putrefaciens	
Clostridium putrificum	
Clostridium ramosum	*Eubacterium filamentosum*
	Ramibacterium ramosum
	Actinomyces ramosus
	Eubacterium ramosum
Clostridium scindens	
Clostridium septicum	
Clostridium sordellii	
Clostridium sphenoides	
Clostridium spiroforme	
Clostridium sporogenes	Nontoxigenic *Clostridium botulinum*
Clostridium subterminale	
Clostridium symbiosum	*Fusobacterium symbiosum*
	Fusobacterium biacutus
	Bacteroides symbiosus
Clostridium tertium	
Clostridium tetani	
Filifactor alocis	*Fusobacterium alocis*
Filifactor villosus	*Clostridium villosum*
Tissierella praeacuta	*Bacteroides praeacutus*
	Clostridium hastiforme
VIII. Aerobic Gram-Negative Bacilli: Enterobacteriaceae	
Characteristics: Ferment sugars; are oxidase-negative; most reduce nitrate to nitrite. Diagnostic laboratories may report *Salmonella* serovars by name (e.g., *Salmonella typhi* or *Salmonella* serovar *typhi*).	
Buttiauxella gaviniae	
Buttiauxella noackiae	CDC enteric group 59
Cedecea davisae	CDC enteric group 15
Cedecea lapagei	
Cedecea neteri	
Citrobacter amalonaticus	*Levinea amalonatica*
Citrobacter braakii	*Citrobacter freundii*
Citrobacter farmeri	*Citrobacter amalonaticus* biogroup 1
Citrobacter freundii	*Colobactrum freundii*
Citrobacter gillenii	*Citrobacter genomospecies* 10
	Citrobacter freundii
Citrobacter koseri	*Citrobacter diversus*
	Levinea malonatica
Citrobacter murliniae	*Citrobacter genomospecies* 11
	Citrobacter freundii

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Citrobacter rodentium	*Citrobacter genomospecies* 9
	Citrobacter freundii
Citrobacter sedlakii	*Citrobacter genomospecies* 8
	Citrobacter freundii
Citrobacter werkmanii	*Citrobacter genomospecies* 7
	Citrobacter freundii
Citrobacter youngae	*Citrobacter genomospecies* 5
	Citrobacter freundii
Cronobacter dublinensis subspecies *dublinensis*	
Cronobacter malonaticus	
Cronobacter muytjensii	
Cronobacter sakazakii	*Enterobacter sakazakii*
Cronobacter turicensis	
Edwardsiella hoshinae	
Edwardsiella tarda	
Enterobacter aerogenes	*Aerobacter aerogenes*
Enterobacter agglomerans group	
Enterobacter amnigenus	
Enterobacter asburiae	CDC enteric group 17
Enterobacter cancerogenus	*Enterobacter cancerogenus*
	Erwinia cancerogena
	CDC enteric group 19
Enterobacter cloacae	
Enterobacter cowanii	
Enterobacter agglomerans group	*Enterobacter agglomerans* group
Enterobacter agglomerans group	*Enterobacter agglomerans* group
Enterobacter hormaechei subspecies *oharae*	
Enterobacter hormaechei subspecies *steigerwaltii*	
Enterobacter kobei	
Enterobacter ludwisii	
Erwinia persicinus	
Escherichia albertii	
Escherichia blattae	
Escherichia coli	
Escherichia fergusonii	CDC enteric group 10
Escherichia hermannii	CDC enteric group 11
Escherichia vulneris	CDC enteric group 1
Ewingella americana	CDC enteric group 40
Hafnia alvei	*Enterobacter hafniae*
Klebsiella granulomatis	*Calymmatobacterium granulomatis*
Klebsiella ornithinolytica	*Klebsiella oxytoca* ornithine positive
Klebsiella oxytoca	
Klebsiella pneumoniae subspecies *ozaenae*	*Klebsiella ozaenae*
Klebsiella pneumoniae subspecies *pneumoniae*	*Klebsiella pneumoniae*
Klebsiella pneumoniae subspecies *rhinoscleromatis*	
Kluyvera ascorbata	CDC enteric group 8
Kluyvera cryocrescens	
Kluyvera georgiana	CDC enteric group 36/37
	Kluyvera spp group 3
Kluyvera intermedia	*Enterobacter intermedius*
Leclercia adecarboxylata	*Escherichia adecarboxylata*
	CDC enteric group 41
Leminorella grimontii	CDC enteric group 57
Leminorella richardii	
Moellerella wisconsensis	CDC enteric group 46
Morganella morganii subspecies *morganii*	*Proteus morganii*
Morganella morganii subspecies *sibonii*	*Proteus morganii*
Morganella psychrotolerans	
Pantoea agglomerans	*Enterobacter agglomerans*
Pantoea ananatis	
Pantoea dispersa	
Photorhabdus asymbiotica	
Photorhabdus luminescens subspecies *kleinii*	
Photorhabdus temperate subspecies *stackebrandtii*	
Pragia fontium	
Proteus hauseri	*Proteus vulgaris* biogroup 3
Proteus mirabilis	
Proteus penneri	*Proteus vulgaris* biogroup 1
Proteus vulgaris	*Proteus vulgaris* biogroup 2
Providencia alcalifaciens	*Proteus inconstans*
Providencia heimbachae	
Providencia rettgeri	*Proteus rettgeri*
Providencia rustigianii	*Providencia alcalifaciens* biogroup 3
Providencia stuartii	*Proteus inconstans*
Rahnella aquatilis	
Raoultella ornithinolytica	*Klebsiella ornithinolytica*
Raoultella planticola	*Klebsiella planticola*
Raoultella terrigena	*Klebsiella terrigena*
Salmonella bongori	*Salmonella* subspecies V
Salmonella enterica subspecies *arizonae*	*Salmonella choleraesuis* subspecies *arizonae*
	Salmonella subspecies IIIa
Salmonella enterica subspecies *diarizonae*	*Salmonella choleraesuis* subspecies *diarizonae*
	Salmonella subspecies IIIb
Salmonella enterica subspecies *enterica*	*Salmonella choleraesuis* subspecies *choleraesuis*
	Salmonella subspecies I
Salmonella enterica subspecies *houtenae*	*Salmonella choleraesuis* subspecies *houtenae*
	Salmonella subspecies IV
Salmonella enterica subspecies *indica*	*Salmonella choleraesuis* subspecies *indica*
	Salmonella subspecies VI
Salmonella enterica subspecies *salamae*	*Salmonella choleraesuis* subspecies *salamae*
	Salmonella subspecies II

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Serratia ficaria	
Serratia fonticola	
Serratia grimesii	
Serratia liquefaciens	*Serratia liquefaciens*
	Enterobacter liquefaciens
Serratia marcescens	
Serratia odorifera	
Serratia plymuthica	
Serratia rubidaea	
Shigella boydii	*Shigella* biogroup C
Shigella dysenteriae	*Shigella* biogroup A
Shigella flexneri	*Shigella* biogroup B
Shigella sonnei	*Shigella* biogroup D
Tatumella ptyseos	CDC group EF-9
Yersinia aldovae	
Yersinia bercovieri	
Yersinia enterocolitica	*Yersinia enterocolitica* biogroup 3b
	Pasteurella enterocolitica
Yersinia frederiksenii	
Yersinia intermedia	
Yersinia kristensenii	
Yersinia mollaretii	*Yersinia enterocolitica* biogroup 3a
Yersinia pestis	*Pasteurella pestis*
Yersinia pseudotuberculosis	*Pasteurella pseudotuberculosis*
Yersinia rohdei	
Yersinia ruckeri	
Yokenella regensburgei	*Koserella trabulsii*
	CDC enteric group 45
IX. Aerobic Gram-Negative Bacilli: Nonenterobacteriaceae; Fermentative	
Characteristics: Ferment sugars; are oxidase positive.	
Aeromonas allosaccharophila	
Aeromonas caviae	
Aeromonas enteropelogenes	
Aeromonas hydrophila subspecies *dhakensis*	*Pseudomonas hydrophila*
Aeromonas hydrophila subspecies *hydrophila*	
Aeromonas jandaei	
Aeromonas popoffii	
Aeromonas salmonicida	
Aeromonas schubertii	
Aeromonas tecta	
Aeromonas trota	
Aeromonas veronii biotype *haemolyticum*	
Aeromonas veronii biotype *sobria*	
Aeromonas veronii biotype veronii	
Chromobacterium violaceum	*Bacillus violaceus*
Grimontia hollisae	*Vibrio hollisae*
Pasteurella aerogenes	
Pasteurella bettyae	CDC group HB-5
Pasteurella canis	*Pasteurella multocida* biotype 6
Pasteurella dagmatis	*Pasteurella* new sp 1
	Pasteurella "gas"
Pasteurella gallinarum	
Pasteurella multocida subspecies *gallicida*	
Pasteurella multocida subspecies *multocida*	
Pasteurella multocida subspecies *septica*	*Pasteurella septica*
Pasteurella pneumotropica	
Pasteurella stomatis	
Photobacterium damselae	
Plesiomonas shigelloides	*Aeromonas shigelloides*
Vibrio alginolyticus	*Vibrio parahaemolyticus* biotype 2
Vibrio carchariae	
Vibrio cholerae	*Vibrio comma*
Vibrio cincinnatiensis	
Vibrio damsela	CDC group EF-5
Vibrio fluvialis	CDC group EF-6
Vibrio furnissii	*Vibrio fluvialis* biogroup 2
Vibrio metschnikovii	CDC enteric group 16
	Vibrio cholerae biovar *proteus*
Vibrio mimicus	*Vibrio cholerae* sucrose negative
Vibrio parahaemolyticus	
Vibrio vulnificus	CDC group EF-3
	Beneckea vulnifica
X. Aerobic Gram-Negative Bacilli: Nonenterobacteriaceae; Nonfermentative	
Characteristics: May or may not oxidize sugars; are catalase positive; are oxidase variable.	
Achromobacter denitrificans	*Alcaligenes denitrificans*
	Alcaligenes xylosoxidans subspecies *denitrificans*
Achromobacter insolitus	
Achromobacter piechaudii	*Alcaligenes piechaudii*
Achromobacter spanius	
Achromobacter xylosoxidans	*Alcaligenes xylosoxidans*
	Alcaligenes xylosoxidans subspecies *xylosoxidans*
	Alcaligenes denitrificans subspecies *xylosoxidans*
	Achromobacter xylosoxidans
	CDC groups IIIa and IIIb
Acidovorax delafieldii	*Pseudomonas delafieldii*
Acidovorax facilis	*Pseudomonas facilis*
Acidovorax temperans	*Pseudomonas temperans*
Acinetobacter baumannii	*Acinetobacter anitratus*
Acinetobacter beijerinckii	
Acinetobacter bereziniae	
Acinetobacter calcoaceticus	*Acinetobacter anitratus*
	Acinetobacter calcoaceticus subspecies *calcoaceticus*
Acinetobacter guillouiae	

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Acinetobacter gyllenbergii	
Acinetobacter haemolyticus	*Acinetobacter anitratus*
Acinetobacter johnsonii	
Acinetobacter junii	*Acinetobacter anitratus*
	Acinetobacter grimontii
Acinetobacter lwoffii	*Acinetobacter calcoaceticus* subspecies *lwoffi*
Acinetobacter nosocomialis	*Acinetobacter* genomic species 13TU
Acinetobacter parvus	
Acinetobacter pittii	*Acinetobacter* genomic species 3
Acinetobacter radioresistens	
Acinetobacter schindleri	
Acinetobacter ursingii	
Acinetobacter venetianus	
Advenella incenata	
Agrobacterium radiobacter	*Agrobacterium tumefaciens*
	CDC group Vd-3
Alcaligenes aquatilis	
Alcaligenes faecalis subspecies *faecalis*	*Alcaligenes odorans*
	Pseudomonas odorans
	CDC group VI
Asaia bogorensis	
Azospirillum brasilense	*Roseomonas fauriae*
Balneatrix alpica	
Bergeyella zoohelcum	*Weeksella zoohelcum*
	CDC group IIj
Brevundimonas diminuta	*Pseudomonas diminuta*
	CDC group Ia
Brevundimonas vesicularis	*Pseudomonas vesicularis*
	Corynebacterium vesiculare
Burkholderia ambifaria	
Burkholderia anthina	
Burkholderia arboris	
Burkholderia cenocepacia	
Burkholderia cepacia	*Pseudomonas cepacia*
	Pseudomonas multivorans
	Pseudomonas kingae
	CDC group EO-1
Burkholderia contaminians	
Burkholderia diffusa	
Burkholderia dolosa	
Burkholderia gladioli	*Pseudomonas gladioli*
	Pseudomonas marginata
Burkholderia lata	
Burkholderia latens	*Pseudomonas mallei*
Burkholderia mallei	
	Actinobacillus mallei
Burkholderia metallica	
Burkholderia multivorans	*Burkholderia cepacia* genomovar II
Burkholderia oklahomensis	
Burkholderia pseudomallei	*Pseudomonas pseudomallei*
Burkholderia pyrrocinia	
Burkholderia seminalis	
Burkholderia stabilis	*Burkholderia cepacia* genomovar IV
Burkholderia thailandensis	
Burkholderia ubonensis	*Burkholderia cepacia* genomovar V
Burkholderia vietnamiensis	
Chryseobacterium anthropic	
Chryseobacterium caeni	
Chryseobacterium gleum	*Flavobacterium gleum*
Chryseobacterium hominis	
Chryseobacterium indologenes	*Flavobacterium indologenes*
Chryseobacterium massiliae	CDC group IIb
Comamonas aquatic	
Comamonas kerstersii	CDC group EF-19
Comamonas terrigena	
Comamonas testosteroni	*Pseudomonas testosteroni*
Delftia acidovorans	*Comamonas acidovorans*
	Pseudomonas acidovorans
Elizabethkingia meningoseptica	*Chryseobacterium meningosepticum*
	Flavobacterium meningosepticum
	CDC group IIa
Elizabethkingia miricola	*Chryseobacterium miricola*
Empedobacter brevis	*Flavobacterium breve*
Granulibacter bethesdensis	
Inquilinus limosus	
Kerstersia gyiorum	
Laribacter hongkongensis	
Massilia oculi	
Massilia timonae	
Methylobacterium aminovarans	
Methylobacterium extorquens	
Methylobacterium fujisawaense	
Methylobacterium mesophilicum	
Methylobacterium organophilum	
Methylobacterium podarium	
Methylobacterium radiotolerans	
Methylobacterium rhodesianum	
Methylobacterium rhodinum	
Methylobacterium zatmanii	
Moraxella atlantae	CDC group M-3
Moraxella catarrhalis	*Branhamella catarrhalis*
	Neisseria catarrhalis
Moraxella lacunata	*Moraxella liquefaciens*
Moraxella lincolnii	
Moraxella nonliquefaciens	
Moraxella osloensis	
Myroides odoratimimus	
Myroides odoratus	
Chryseobacterium odoratum	*Flavobacterium odoratum*
	CDC group M-4f

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Ochrobactrum anthropi	*Achromobacter* spp. biotypes 1 and 2
	CDC groups Vd-1, Vd-2
Ochrobactrum intermedium	
Oligella ureolytica	CDC group Ive
Oligella urethralis	*Moraxella urethralis*
	CDC group M-4
Pandoraea apista	
Pandoraea norimbergensis	
Pandoraea pnomenusa	
Pandoraea pulmonicola	
Pandoraea sputorum	
Paracoccus yeei	*Paracoccus yeeii*
	CDC group EO-2
Photorhabdus asymbiotica	*Xenorhabdus luminescens*
Pseudomonas aeruginosa	*Pseudomonas pyocyanea*
	Bacterium aeruginosum
Pseudomonas alcaligenes	
Pseudomonas chlororaphis	*Pseudomonas aureofaciens*
Pseudomonas fluorescens	
Pseudomonas luteola	*Chryseomonas luteola*
	CDC group Ve-1
Pseudomonas mendocina	CDC group Vb-2
Pseudomonas monteilii	
Pseudomonas mosselii	
Pseudomonas oryzihabitans	*Flavimonas oryzihabitans*
	CDC group Ve-2
Pseudomonas otitidis	
Pseudomonas pseudoalcaligenes	*Pseudomonas alcaligenes* biotype B
Pseudomonas putida	
Pseudomonas stutzeri	CDC group Vb-1
Psychrobacter faecalis	*Micrococcus cryophilus*
Psychrobacter immobilis	
Psychrobacter phenylpyruvicus	*Moraxella phenylpyruvicus*
	CDC group M-2
Psychrobacter pulmonis	
Ralstonia mannitolilytica	
Ralstonia pickettii	*Burkholderia pickettii*
	Pseudomonas pickettii
	CDC groups Va-1, Va-2, Va-3
	Pseudomonas thomasii
Rhizobium radiobacter	*Agrobacterium radiobacter*
Roseomonas cervicalis	CDC "pink coccoid" group
Roseomonas genomospecies 4	CDC "pink coccoid" group
Roseomonas genomospecies 5	CDC "pink coccoid" group
Roseomonas gilardii subspecies *gilardii*	
Roseomonas gilardii subspecies *rosea*	
Roseomonas mucosa	
Shewanella algae	
Shewanella putrefaciens	*Alteromonas putrefaciens*
	Pseudomonas putrefaciens
	CDC groups Ib-1, Ib-2
Sphingobacterium mizutaii	*Flavobacterium mizutaii*
Sphingobacterium multivorum	*Flavobacterium multivorum*
	CDC group IIk-2
Sphingobacterium spiritivorum	*Flavobacterium spiritivorum*
	Sphingobacterium versatilis
	CDC group IIk-3
Sphingomonas parapaucimobilis	
Sphingomonas paucimobilis	*Pseudomonas paucimobilis*
	CDC group IIk-1
Stenotrophomonas maltophilia	*Xanthomonas maltophilia*
	Pseudomonas maltophilia
	Stenotrophomonas africana
Wautersiella falsenii	
Weeksella virosa	*Flavobacterium genitale*
	CDC group IIf
XI. Anaerobic Gram-Negative Bacilli	
Characteristics: May appear as rods with rounded ends, curved rods, coccobacilli, or slender, spindle-shaped rods with tapered ends. *Dialister* and *Johnsonella* belong to the Clostridium subphylum.	
Alistipes finegoldii	
Alistipes indistinctus	
Alistipes onderdonkii	
Alistipes shahii	
Anaerobiospirillum succiniciproducens	
Anaerobiospirillum thomasii	
Anaerorhabdus furcosus	*Bacteroides furcosus*
Anaerostipes cocae	
Bacteroides caccae	
Bacteroides capillosus	
Bacteroides cellulosilyticus	
Bacteroides clarus	
Bacteroides coagulans	
Bacteroides coprophilus	
Bacteroides dorei	
Bacteroides faecis	
Bacteroides finegoldii	
Bacteroides fluxus	
Bacteroides forsythus	
Bacteroides fragilis	
Bacteroides fragilis group	True Bacteroides
Bacteroides intestinalis	*Bacteroides putredinis*
	Alistipes putredinis
	Bacteroides furcosus
Bacteroides massiliae	
Bacteroides nordii	
Bacteroides oleiciplenus	
Bacteroides ovatus	
Bacteroides putredinis	
Bacteroides pyogenes	*Bacteroides tectus*
	Bacteroides tectum
Bacteroides salyersae	*Baceroides suis*
Bacteroides splanchnicus	

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym	Current Name	Synonym
Bacteroides stercoris		*Parabacteroides goldsteinii*	*Bacteroides goldsteinii*
Bacteroides thetaiotaomicron	*Bacteroides corrodens*	*Parabacteroides gordonii*	
Bacteroides uniformis		*Parabacteroides johnsonii*	*Bacteroides merdae*
Bacteroides ureolyticus		*Parabacteroides merdae*	
Bacteroides vulgatus			*Bacteroides fragilis* T4-1
Bacteroides xylanisolvens		*Paraprevotella clara*	
Barnesiella intestinihominis		*Paraprevotella xylaniphila*	
Bilophila wadsworthia		*Parasutterella excrementihominis*	
Butyrivibrio fibrisolvens			
Catonella morbi		*Parasutterella secunda*	
Centipeda periodontii		*Phocaeicola abscessus*	
Cetobacterium somerae		*Porphyromonas asaccharolytica*	*Bacteroides asaccharolyticus*
Desulfomicrobium orale			
Desulfovibrio desulfuricans			*Bacteroides melaninogenicus* subspecies *asaccharolyticus*
Desulfovibrio fairfieldensis			
Desulfovibrio piger			
Desulfovibrio salexigens			
Desulfovibrio vulgaris		*Porphyromonas bennonis*	
Dialister invisus		*Porphyromonas cangingivalis*	
Dialister pneumosintes	*Bacteroides pneumosintes*	*Porphyromonas canoris*	
Dialister propionicifaciens		*Porphyromonas cansulci*	
Dialister succinatiphilus	*Bacteroides nodosus*	*Porphyromonas catoniae*	*Oribaculum catoniae*
Fusobacterium canifelinum		*Porphyromonas circumdentaria*	
Fusobacterium gonidiaformans		*Porphyromonas crevioricanis*	
Fusobacterium mortiferum		*Porphyromonas endodontalis*	*Bacteroides endodontalis*
Fusobacterium naviforme		*Porphyromonas gingivalis*	*Bacteroides gingivalis*
Fusobacterium necrogenes		*Porphyromonas gingivicanis*	
Fusobacterium necrophorum		*Porphyromonas gulae*	
subspecies *funduliforme*		*Porphyromonas levii*	*Bacteroides levii*
Fusobacterium necrophorum			*Bacteroides melaninogenicus*
subspecies *necrophorum*			subspecies *levii*
Fusobacterium nucleatum			
subspecies *fusiforme*		*Porphyromonas macacae*	*Bacteroides macacae*
Fusobacterium nucleatum		*Porphyromonas somerae*	
subspecies *nucleatum*		*Prevotella annii*	
Fusobacterium nucleatum		*Prevotella aurantiaca*	
subspecies *polymorphum*		*Prevotella baroniae*	
Fusobacterium nucleatum		*Prevotella bergensis*	
subspecies *vincentii*		*Prevotella bivia*	*Bacteroides bivius*
Fusobacterium perfoetens		*Prevotella buccae*	*Bacteroides buccae*
Fusobacterium periodonticum			*Bacteroides ruminicola*
Fusobacterium russii			subspecies *brevis*
Fusobacterium simiae			*Bacteroides capillus*
Fusobacterium ulcerans			*Bacteroides pentosaceus*
Fusobacterium varium	*Fusobacterium pseudonecrophorum*	*Prevotella buccalis*	*Bacteroides buccalis*
		Prevotella copri	
Johnsonella ignava		*Prevotella corporis*	*Bacteroides corporis*
Jonquetella anthropi		*Prevotella dentalis*	*Mitsuokella dentalis*
Leptotrichia buccalis			*Hallella seregens*
Leptotrichia goodfellowii		*Prevotella denticola*	*Bacteroides denticola*
Leptotrichia hofstadii		*Prevotella disiens*	*Bacteroides disiens*
Leptotrichia hongkongensis		*Prevotella enoeca*	
Leptotrichia shahii		*Prevotella fusca*	*Bacteroides heparinolyticus*
Leptotrichia trevisanii		*Prevotella heparinolytica*	
Leptotrichia wadei		*Prevotella histicola*	
Meganonas funiformis		*Prevotella intermedia*	*Bacteroides intermedius*
Odoribacter laneus			*Bacteroides melaninogenicus*
Odoribacter splanchnicus			subspecies *intermedius*
Parabacteroides distasonis	*Bacteroides distasonis*		

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Prevotella loescheii	*Bacteroides loescheii*
Prevotella maculosa	
Prevotella marshii	
Prevotella massiliensis	
Prevotella melaninogenica	*Bacteroides melaninogenicus*
	Bacteroides melaninogenicus subspecies *melaninogenicus*
Prevotella micans	*Prevotella intermedia*
Prevotella multiformis	
Prevotella multisaccharivorax	
Prevotella nanceiensis	
Prevotella nigrescens	
Prevotella oralis	*Bacteroides oralis*
Prevotella oris	*Bacteroides oris*
	Bacteroides ruminicola subspecies *brevis*
Prevotella oulorum	*Bacteroides oulorum*
	Prevotella oulora
Prevotella pallens	
Prevotella pleuritidis	
Prevotella saccharolytica	
Prevotella salivae	
Prevotella scopos	
Prevotella shahii	
Prevotella stercorea	
Prevotella tannerae	
Prevotella timonensis	
Prevotella veroralis	*Bacteroides veroralis*
Prevotella zoogleoformans	*Bacteroides zoogleoformans*
Pyramidobacter piscolens	
Selenomonas artemidis	
Selenomonas dianae	
Selenomonas flueggei	
Selenomonas infelix	
Selenomonas noxia	
Selenomonas sputigena	
Sneathia sanguinegens	*Leptotrichia sanguinegens*
Sutterella parvirubra	
Sutterella wadsworthensis	
Synergistes cluster I	
Synergistes cluster II	
Tannerella forsythia	*Bacteroides forsythus*
	Tannerella forsythensis
XII. Aerobic Gram-Negative Fastidious Coccobacilli	
Characteristics: Small, curved or straight gram-negative bacilli or coccobacilli. They may require carbon dioxide and enriched media or special conditions for adequate growth.	
Actinobacillus equuii	
Actinobacillus hominis	
Actinobacillus lignieresii	
Actinobacillus suis	
Actinobacillus ureae	*Pasteurella ureae*
Afipia broomeae	
Afipia clevelandensis	
Afipia felis	
Aggregatibacter actinomycetemcomitans	*Actinobacillus actinomycetemcomitans*
	CDC groups HB-3, HB-4
Aggregatibacter aphrophilus	*Haemophilus aphrophilus*
	CDC group HB-2
Aggregatibacter paraphrophilus	*Haemophilus paraphrophilus*
Aggregatibacter segnis	*Haemophilus segnis*
Anaplasma phagocytophilum	*Ehrlichia egui*
Arcobacter butzleri	*Ehrlichia phagocytophila* HGE agent
	Campylobacter butzleri
Arcobacter cryaerophilus	*Campylobacter cryaerophila*
Arcobacter skirrowii	
Bartonella alsatica	
Bartonella bacilliformis	
Bartonella bovis	
Bartonella clarridgeiae	
Bartonella doshiae	
Bartonella elizabethae	*Rochalimaea elizabethae*
Bartonella grahamii	
Bartonella henselae	*Rochalimaea henselae*
Bartonella koehlerae	
Bartonella quintana	*Rochalimaea quintana*
Bartonella vinsonii subspecies *arupensis*	*Rochalimaea vinsonii*
Bartonella vinsonii subspecies *berkhoffii*	
Bartonella shoenbuchensis	
Bordetella avium	CDC group IVa
Bordetella bronchiseptica	
Bordetella hinzii	
Bordetella holmesii	CDC group NO-2
Bordetella parapertussis	
Bordetella pertussis	
Bordetella petrii	
Bordetella trematum	
Brucella abortus	
Brucella canis	
Brucella melitensis	
Brucella suis	
Campylobacter coli	
Campylobacter concisus	CDC group EF-22
Campylobacter curvus	*Wolinella curva*
Campylobacter fetus subspecies *fetus*	*Vibrio fetus*
Campylobacter gracilis	*Bacteroides gracilis*
Campylobacter hominis	
Campylobacter hyointestinalis subspecies *hyointestinalis*	
Campylobacter jejuni subspecies *doylei*	
Campylobacter jejuni subspecies *jejuni*	
Campylobacter rectus	*Wolinella recta*
Campylobacter showae	
Campylobacter sputorum subspecies *sputorum*	
Campylobacter upsaliensis	
Capnocytophaga canimorsus	CDC group DF-2
Capnocytophaga cynodegmi	CDC group DF-2

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
Capnocytophaga gingivalis	CDC group DF-1
Capnocytophaga granulosa	
Capnocytophaga haemolytica	
Capnocytophaga ochracea	CDC group DF-1
Capnocytophaga sputigena	CDC group DF-1
Cardiobacterium hominis	CDC group IId
Cardiobacterium valvarum	
Chlamydia pneumoniae	Taiwan acute respiratory agent (TWAR)
	Chlamydophila pneumoniae
Chlamydia psittaci	*Chlamydophila psittaci*
Chlamydia trachomatis	*Chlamydophila trachomatis*
Chromobacterium haemolyticum	
Chromobacterium violaceum	
Coxiella burnetii	
Dysgonomonas capnocytophagoides	CDC group DF-3
Dysgonomonas gadei	
Dysgonomonas mossii	
Ehrlichia canis	Human granulocytic ehrlichiosis
Ehrlichia chaffeensis	Human monocytic ehrlichiosis
Ehrlichia ewingii	
Eikenella corrodens	
Francisella novicida	
Francisella philomiragia	CDC group HB-1
Francisella tularensis subspecies *mediaasiatica*	*Yersinia philomiragia*
Francisella tularensis subspecies *holarctica*	
Francisella tularensis subspecies *tularensis*	*Francisella tularensis* type A
	Pasteurella tularensis
	Bacterium tularense
Haemophilus actinomycetemcomitans	
Haemophilus aegyptis	
Haemophilus ducreyi	
Haemophilus haemolyticus	
Haemophilus influenzae	
Haemophilus parahaemolyticus	
Haemophilus parainfluenzae	
Haemophilus pittmaniae	
Helicobacter bilis	
Helicobacter bovis	
Helicobacter cinaedi	*Campylobacter cinaedi*
Helicobacter canadensis	
Helicobacter felis	
Helicobacter fennelliae	*Campylobacter fennelliae*
Helicobacter ganmani	*Gastrospirillum hominis*
Helicobacter heilmannii	
Helicobacter pullorum	
Helicobacter pylori	*Campylobacter pylori*
Helicobacter salomonis	
Helicobacter suis	
Helicobacter winghamensis	
Kingella denitrificans	CDC group TM-1
Kingella kingae	*Moraxella kingae*
	Moraxella kingii
Kingella oralis	
Kingella potus	
Legionella anisa	
Legionella birminghamensis	
Legionella bozemanii	*Fluoribacter bozemanae*
Legionella cincinnatiensis	
Legionella dumoffii	*Fluoribacter dumoffii*
Legionella feeleii	
Legionella gormanii	
Legionella hackeliae	
Legionella jordanis	
Legionella lansingensis	
Legionella longbeachae	
Legionella lytica	
Legionella maceachernii	
Legionella micdadei	*Tatlockia micdadei*
Legionella nagasakiensis	
Legionella oakridgensis	
Legionella parisiensis	
Legionella pneumophila	
Legionella sainthelensi	
Legionella tucsonensis	
Legionella wadsworthii	
Neorickettsia sennetsu	*Ehrlichia sennetsu*
Orientia tsutsugamushi	*Rickettsia tsutsugamushi*
Rickettsia aeschlimannii	
Rickettsia africae	
Rickettsia akari	
Rickettsia australis	
Rickettsia conorii subspecies *caspia*	
Rickettsia conorii subspecies *conorii*	
Rickettsia conorii subspecies *indica*	
Rickettsia conorii subspecies *israelensis*	
Rickettsia felis	
Rickettsia heilongjiangensis	
Rickettsia honei	
Rickettsia japonica	
Rickettsia marmionii	
Rickettsia massiliae	
Rickettsia monacensis	
Rickettsia mongoolotimonae	
Rickettsia parkeri	
Rickettsia prowazekii	
Rickettsia rickettsii	
Rickettsia sibirica subspecies *sibirica*	
Rickettsia slovaca	
Rickettsia texiana	
Rickettsia typhi	
Streptobacillus moniliformis	*Haverhillia multiformis*
Suttonella indologenes	*Kingella indologenes*

Continued on following page

TABLE 79-1 **Current Bacterial Nomenclature, Taxonomy, and Classification** (Continued)

Current Name	Synonym
XIII. Mycoplasma (Pleuropneumonia-Like Organisms)	
Characteristics: Small, highly pleomorphic organisms that are difficult to observe with routine stains; require complex medium for growth.	
Acholeplasma laidlawii	
Mycoplasma amphoriforme	
Mycoplasma buccale	
Mycoplasma faucium	
Mycoplasma fermentans	*Mycoplasma incognitus*
Mycoplasma gallisepticum	
Mycoplasma genitalium	
Mycoplasma hominis	
Mycoplasma lipophilum	
Mycoplasma orale	
Mycoplasma penetrans	
Mycoplasma pirum	
Mycoplasma pneumoniae	
Mycoplasma primatum	
Mycoplasma salivarium	
Mycoplasma spermatophilum	
Mycoplasma synoviae	
Ureaplasma parvum	
Ureaplasma urealyticum	T-mycoplasma
XIV. Treponemataceae (Spiral Organisms)	
Characteristics: Filamentous, spiral organisms that may or may not stain with usual laboratory stains; require complex media or animal host for growth.	
Borrelia afzelii	
Borrelia burgdorferi	
Borrelia caucasica	
Borrelia crocidurae	
Borrelia duttoni	
Borrelia garinii	
Borrelia hermsii	
Borrelia hispanica	
Borrelia lusitaniae	
Borrelia mazzottii	
Borrelia parkeri	
Borrelia persica	
Borrelia recurrentis	
Borrelia spielmanii	*Borrelia spielmani*
Borrelia turicatae	
Borrelia valaisiana	
Borrelia venezuelensis	
Brachyspira aalborgi	
Brachyspira pilosicoli	*Serpulina pilosicoli*
Leptospira alexandria	
Leptospira borgpetersenii	
Leptospira broomii	
Leptospira fainei	
Leptospira inadai	
Leptospira interrogans	
Leptospira kirschneri	
Leptospira kmetyi	
Leptospira licerasiae	
Leptospira meyeri	
Leptospira noguchii	
Leptospira santarosai	
Leptospira weilii	
Spirillum minus	*Spirillum minor*
Spirillum wolffii	
Treponema amylovorum	
Treponema carateum	
Treponema denticola	
Treponema maltophilum	
Treponema minutum	
Treponema pallidum subspecies *endemicum*	*Treponema pallidum*
Treponema pallidum subspecies *pallidum*	*Treponema pallidum*
Treponema pallidum subspecies *pertenue*	*Treponema pertenue*
Treponema parvum	
Treponema pectinovorum	
Treponema phagedenis	
Treponema putidum	
Treponema refringens	
Treponema vincentii	

CDC, Centers for Disease Control and Prevention.

TABLE 79-2 **Bacterial Ranks Below Subspecies**

Preferred Name	Synonym	When Applied
Biovar	Biotype	Special biochemical or physiologic features
Genomovar	Genomotype	No distinct phenotypic differences
Morphovar	Morphotype	Special morphologic features
Pathovar	Pathotype	Host-specific pathogenic features
Phagovar	Phagotype	Lysis by distinct bacteriophages
Serovar	Serotype	Distinct antigenic features

NEW REFERENCES SINCE THE SIXTH EDITION

3. Kämpfer P. The characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;**60**:7.
4. Kämpfer P. Certificates of deposit: a key element of the bacteriological code and an indispensable prerequisite for comparative taxonomic research—report of a case of falsification and a reply to the letter to the editor by Tindall (2008). *Int J Syst Evol Microbiol* 2010;**60**:475–7.
6. Stackebrandt E, Frederiksen W, Garrity GM, et al. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* 2002;**52**:1043–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Garrity GM, editor. *Bergey's Manual of Systematic Bacteriology*, Vols: 1 and 2. New York: Springer; 2001 and 2005.
2. Holt JG, editor. *Bergey's Manual of Systematic Bacteriology*, Vols: 3 and 4. Baltimore: Williams & Wilkins; 1989.
3. Kämpfer P. The characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* 2010;**60**:7.
4. Kämpfer P. Certificates of deposit: a key element of the bacteriological code and an indispensable prerequisite for comparative taxonomic research—report of a case of falsification and a reply to the letter to the editor by Tindall (2008). *Int J Syst Evol Microbiol* 2010;**60**:475–7.
5. Sneath PHA. *International Code of Nomenclature of Bacteria: Bacteriological Code, 1990 Revision*. Washington, DC: American Society of Microbiology; 1992.
6. Stackebrandt E, Frederiksen W, Garrity GM, et al. Report of the ad hoc committee for the re-evaluation of the species definition in bacteriology. *Int J Syst Evol Microbiol* 2002;**52**:1043–7.
7. Stackebrandt E, Goebel BM. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* 1994;**44**:846–9.

SUBSECTION 1 *GRAM-POSITIVE COCCI*

CHAPTER 80

Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

Sheldon L. Kaplan • Kristina G. Hulten • Edward O. Mason

Staphylococcus aureus is a gram-positive coccus that occurs in pairs, chains, and grapelike clusters (Fig. 80-1). *S. aureus* is ubiquitous in nature and can be pathogenic for humans and animals. Staphylococci are nonmotile, are aerobic or facultative anaerobic, and are readily cultivated on routine laboratory media. These organisms are part of the normal human flora. *S. aureus* is responsible for an impressive variety of diseases ranging from minor skin and soft tissue infections to major life-threatening and fatal infections such as bacteremia, endocarditis, pericarditis, pneumonia, empyema, osteomyelitis, myositis, and septic arthritis. The focus of this chapter is on epidemiology, virulence factors, pathogenesis, diagnosis, and treatment of infections caused by *S. aureus*. The reader is referred to the many chapters in this textbook that address the specific infections noted.

On blood agar, *S. aureus* forms round, convex, shiny opaque colonies 1 to 4 mm in diameter, often with a zone of clear β-hemolysis (Fig. 80-2) surrounding the colony. Pigment production is variable, with strains exhibiting a yellow or golden pigment on primary isolation; yellow pigment is actually an *S. aureus* virulence factor.[176] *S. aureus* secretes free coagulase, and this is the basis for the most definitive and most accepted method for identification of pathogenic staphylococci associated with human and animal infection. Free coagulase reacts with coagulase activator in plasma and converts fibrinogen to fibrin with the formation of a fibrin clot. Coagulase also can be evaluated by testing for bound coagulase or clumping factor in a rapid slide test (Fig. 80-3). Clumping factor bound to the organism acts directly on fibrinogen and converts it to fibrin; it is detected by visible clumping or agglutination when a suspension is incubated with plasma. The bound coagulase test is used commonly in clinical laboratories and is more rapid than the tube coagulase test. However, the slide test for clumping factor may be falsely negative in 10 to 15 percent of cases. Thus, a negative slide coagulase test with an isolate that is highly suggestive of being *S. aureus* should be confirmed with a tube coagulase test for free coagulase.[162,234] A limited number of biochemical reactions can differentiate *S. aureus* from other staphylococci (Table 80-1). Commercially available molecular techniques can rapidly detect *S. aureus* from blood cultures with gram-positive cocci in clusters. By including gene targets both for an *S. aureus*–specific gene and a sequence near the insertion site of the staphylococcal cassette chromosome *mec* element (SCC*mec*) in the assay, it is possible to detect *S. aureus* and distinguish methicillin-susceptible (MSSA) isolates from methicillin-resistant (MRSA) isolates simultaneously. If and how the rapid detection of *S. aureus* using these molecular tests impact patient care needs to be determined.[90]

STRUCTURE

Capsule

Capsule production often is considered a virulence factor and frequently is used by bacteria to hinder phagocytosis by the host.[214] Karakawa and colleagues described a scheme of 8 *S. aureus* capsular types in 1982.[148] Subsequently, Sompolinsky and associates described 3 more serotypes, bringing the total number to 11.[265] Most *S. aureus* organisms are encapsulated to a varying degree (Fig. 80-4). Two serotypes (1 and 2) produce mucoid colonies on agar media but are rare among clinical isolates.[265] Serotypes 5 and 8 account for about 75 percent of strains recovered from infections in humans, including children.[56,244,274] In practice, strains that do not belong to serotypes 1, 2, 5, or 8 are referred to as *nontypeable* (see pulsotypes, later).[123] The role of capsule polysaccharide in virulence comes primarily from studies of CP5 and CP8 and is not completely clear. These studies show that inhibition of phagocytosis by capsule can result in persistence on surfaces but modulates adherence to endothelial surfaces.[214] A protein conjugate vaccine composed of CP5 and CP8 conjugated to nontoxic recombinant *Pseudomonas aeruginosa* exotoxin A (StaphVax) initially appeared

promising in prevention of *S. aureus* bacteremia in hemodialysis patients.[257] However, in a larger study this vaccine was not found to be efficacious.[258] The major clone of MRSA associated with community-onset infections, USA300, does not express a capsular polysaccharide, further casting doubt on the contribution of the capsule to virulence of the organism.[274]

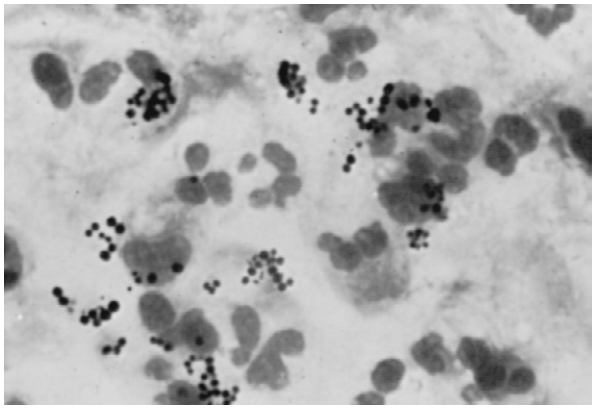

FIGURE 80-1 ■ Staphylococci in pus. The organisms tend to form clusters, are round, and stain purple with Gram stain (positive), similar to bunches of grapes.

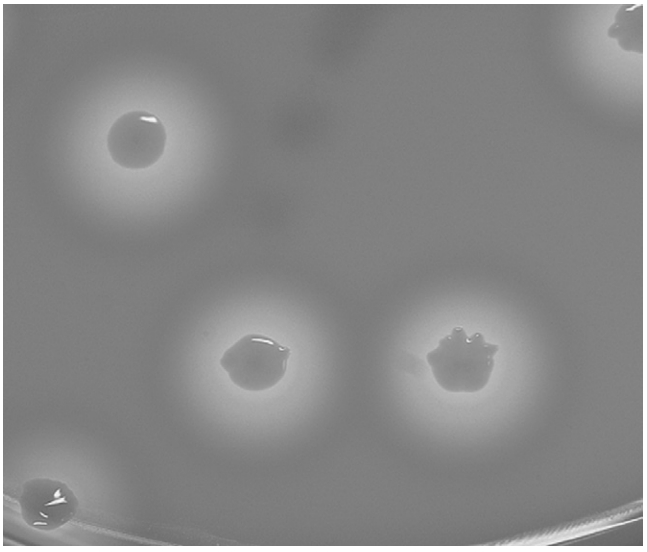

FIGURE 80-2 ■ Typical hemolysis produced by *Staphylococcus aureus* on sheep blood agar.

Protein A

Staphylococcal protein A, a major component of the cell wall of coagulase-positive staphylococci, has been found to bind to the Fc portion of immunoglobulin (Ig) G (IgG). This binding is nonimmune. It differs from specific antigen-antibody reactions in that the reaction is nonspecific, although both precipitation and agglutination are observed. Protein A binds the IgG of many mammalian species. This property has rendered protein A a major reagent in many immune assays. Protein A has also been found to bind to platelets through the platelet gC1qr, a 33-kDa cell protein,[212] as well as to von Willebrand factor,[114] both of which may be important in the pathogenesis of endovascular infections caused by *S. aureus*. Protein A plays a central role in the pathogenesis of staphylococcal pneumonia in that TNFR1, a receptor for tumor necrosis factor-α, is also receptor for protein A, and thus protein A is a principal staphylococcal proinflammatory factor in the lung.[99,222] The complex interactions of protein A, as well as other virulence factors, and the host in the pathogenesis of *S. aureus* pneumonia was reviewed by Parker and Prince.[222] The gene encoding for protein A, *spa*, contains a polymorphic repeat region, which is the target for *spa* typing, a molecular method frequently used to characterize *S. aureus*.[256]

Extracellular Products

S. aureus elaborates a wide variety of extracellular toxins, many of which have potent biologic effects in the isolated

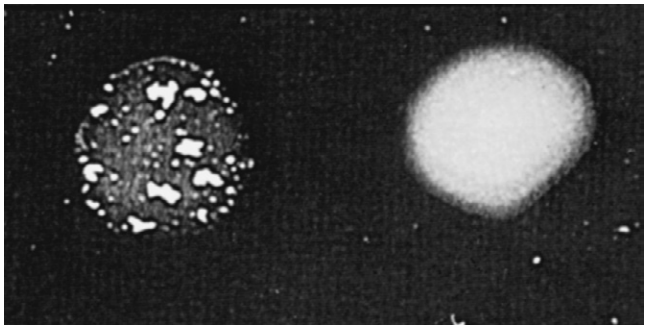

FIGURE 80-3 ■ Bound (slide) coagulase test. A suspension of organisms is mixed with plasma. Immediate clumping (reaction on the *left*) indicates both the presence of bound coagulase and the fact that the organism is coagulase-positive.

TABLE 80-1 **Identification of Staphylococci**

Characteristics	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	–	–
Acid aerobically from:			
Sucrose	+	+	+
Trehalose	+	–	+
Mannitol	+	–	+
Phosphatase	+	+	–
Novobiocin	Sensitive	Sensitive	Resistant

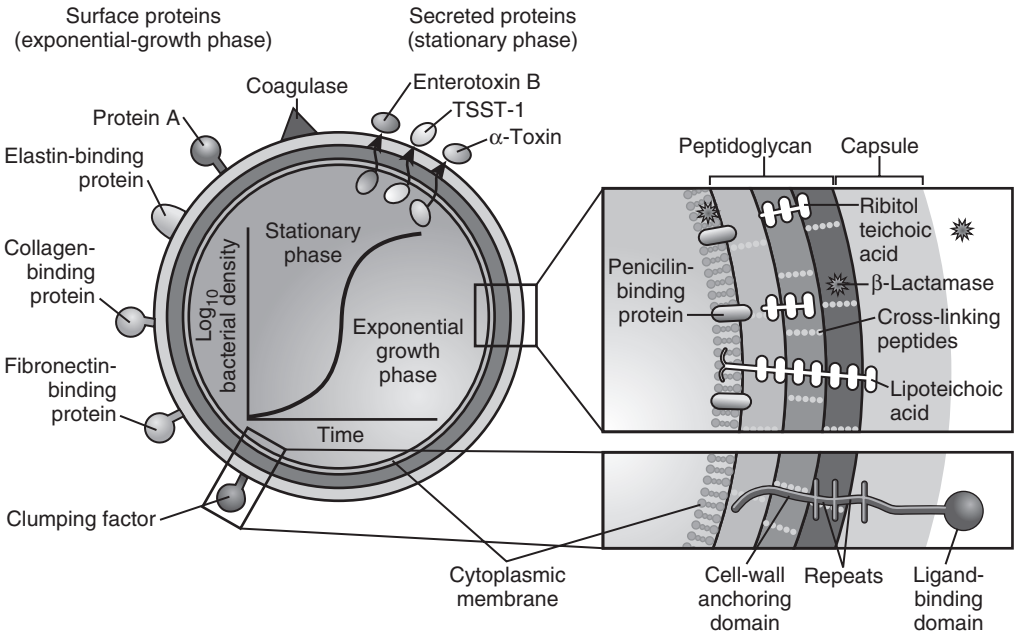

FIGURE 80-4 ■ Structure of *Staphylococcus aureus.* TSST-1, toxic shock syndrome toxin type I. (From Lowy, F. D.: Medical progress: Staphylococcus aureus infections. *N Engl J Med* 339:520–532, 1998.)

state on intact animals, tissues, cells, and membranes. These toxins generally are thought to be responsible for the virulence of *S. aureus*, and the role of several of these products in staphylococcal infections has been proved definitively in humans. The important staphylococcal extracellular products include α-, β-, γ- and δ-hemolysins, coagulases, leukocidin, hyaluronidase, staphylokinase, bacteriocins, the epidermolytic toxins, toxic shock syndrome toxin type 1 (TSST-1), and the enterotoxins. In recent years a group of cytolytic peptides, the phenol-soluble modulins, have gained attention for their moderate cytolytic capacity, although the precise function of these molecules remains unclear.[156] The pore-forming toxins include α hemolysin, γ hemolysin, Panton-Valentine leukocidin (PVL), LukED, and LukAB (also referred to as LukGH).[66,288]

Almost half of the known toxins and virulence genes in *S. aureus* are found in pathogenicity islands. These genetic clusters, commonly of 15 to 20 kb, also contain genetic elements such as integrases and transposases. A high genetic instability is associated with many of these islands, and mosaic structures are common findings. Seven pathogenicity islands (νSa) have been associated with *S. aureus*.[93] Table 80-2 lists some of the features described for these islands, although allelic variations in gene content occur. All sequenced strains of *S. aureus* contain variations of the νSaα, νSaβ, and νSaγ.[60,93,124] In addition to the pathogenicity islands, virulence genes are also located on prophages (e.g., *luk*S-PV and *luk*F-PV on ΦSa2).

TABLE 80-2 **Pathogenicity Islands of *Staphylococcus aureus***

Island	Alleles	Genes	Reference
νSa1	SaPI1, SaPI3	*seb, tst, sek, seq, ear*	173
νSa2	SaPIbov	*tst, sec*	77
νSa3	SaPI3	Type I: *fhuD;* Type II: *sel2, sec4, ear*	306
νSa4	SaPI2	Type I: *sel, sec3, tst*; Type II: 4 unknown ORFs	172
νSaα		Type I: *set* 6-15, *lpl* 1-9; Type II, *set* 16-26, *lpl* 10-14; Type III: *set* 1-5, *lpl* 2, 7, 8, 11, 13	93, 309
νSaβ		Type I: *spl* A-F, *lukDE, ear,* epidermin gene cluster; Type II: *spl* A-F, *lukDE, ear, seg, sen, sei, sem, seo,* epidermin gene cluster	93
νSaγ		*set* 1,3,4, *eta,* phenol-soluble modulin	93

Enterotoxins: *seb, sec, seg, sei, sel, sem, sen, seo;* Exotoxins: *set* 1-26, toxic shock toxin TSST-1 *(tst)*: β-lactamase *(ear),* tandem lipoprotein *(lpl),* ferric hydroxamate uptake *(fhuD),* leukocidin *(lukDE),* serine protease (IA-F), exfoliative toxin A.
ORF, open reading frame.

Hemolysins and Leukocidins

Encoded by *hla*, α hemolysin is produced by most *S. aureus* isolates and is a classic pore-forming bacterial toxin. The purified toxin has impressive hemolytic, dermonecrotic, and lethal properties. Advances in protein purification have allowed the production of pure toxin; this development, in turn, has led to a better understanding of its properties.

The protein interacts with and damages a variety of cell membranes, releases hemoglobin from erythrocytes of various mammalian and avian species, and is cytotoxic to a number of cell lines in tissue culture. It lyses rabbit and human platelets and disrupts lysosomes. It causes contraction in skeletal and vascular smooth muscle, with the latter action perhaps explaining its property of causing localized dermal necrosis. In a rat model, α toxin damaged the air-blood barrier in vivo during *S. aureus* pneumonia.[193] The α toxin forms pores in the membranes of endothelial cells, leading to vasoconstriction and increasing vascular permeability, and also has effects that lead to apoptosis of a number of different cells.[62] Damage from the α toxin to endothelial cells is mediated via its binding to the receptor for A-disintegrin and metalloprotease 10 (ADAM10).[229] This toxin also is an important activator of caspase 1/calpain signaling, which leads to pyroptosis, a form of inflammatory death, of human keratinocytes in vitro, which may allow *S. aureus* to penetrate across the skin to cause infection.[266]

Injection of α toxin is lethal to mammals and reptiles, with death occurring within 2 to 5 minutes. Human death has been attributed to preformed α toxin on at least one occasion. Anti–α toxin can be demonstrated in normal individuals,[47] and antibody to α hemolysin was protective in a mouse model of *S. aureus* pneumonia.[237] However, because the level of anti–α hemolysin is high in approximately 70 percent of patients with staphylococcal osteomyelitis and the antibody is also present in children with acute osteomyelitis at the time of admission to hospital, there is doubt regarding a protective role, at least in patients with osteomyelitis.[39,276]

The other hemolytic toxins, γ, β, and δ hemolysin, also possess hemolytic and cytotoxic activities. Highly cytotoxic, γ hemolysin lyses human neutrophils; and β toxin produces lethal and dermonecrotic effects. Eighty to 100 percent of adults possess antibody to β hemolysin. LukAB/GH has a reduced cytolytic action on neutrophils compared with γ hemolysin and PVL.[66] The biologic role of LukED remains unknown, although a variant of this molecule was highly toxic against human neutrophils.[206]

Despite evidence that these toxins are produced during infection, their roles in production of the typical staphylococcal tissue lesion remain unclear.[22,62] Although the toxins may play important roles in the establishment of infection by interacting with each other and with other biologically active staphylococcal products, this theory has not been proven; human infection is not prevented by antitoxin antibody, and virulent staphylococci that lack one or more of these toxins are encountered.[263]

An in vitro study showed that α toxin was present in culture supernatants at sevenfold to ninefold greater concentrations in the USA300 strain compared with the USA400 strain when grown at midexponential and stationary phases of growth.[34] Further studies on how the strain-specific expression of virulence factors during the establishment and course of infection influence the pathology and host response are warranted.

Even though certain hemolysins are toxic to various leukocytes, PVL is the only known extracellular toxin that attacks the leukocyte exclusively.[221] It consists of two protein components, LukS-PV and LukF-PV, encoded by the genes *lukS-PV* and *lukF-PV* carried on a bacteriophage. LukS-PV and LukF-PV assemble to form octameric β-barrel pores in polymorphonuclear leukocytes and macrophages.[27] Leukocidin injected into rabbits causes a striking fall in levels of circulating and bone marrow leukocytes, followed by marked granulocytosis; these changes occur without death of the rabbits. Leukocidin interacts with the membrane phospholipid and causes depolarization, increased permeability, and cell death. Local secretion of leukocidin might appear to confer an advantage to the staphylococcus by killing leukocytes and thereby preventing phagocytosis and intracellular killing. Levels of anti-leukocidin rise rapidly in the course of infection,[96,135] and some evidence indicates that infants and mothers with high levels of anti-leukocidin antibody are less likely to contract staphylococcal disease in high-risk epidemiologic situations.[18] In one study, a history of furunculosis, suggesting a skin infection caused by PVL-producing *S. aureus* isolates, was associated with a decrease in complications and mortality for patients with *S. aureus* PVL-associated necrotizing pneumonia.[238] Brown and colleagues noted that children who had invasive staphylococcal infections such as osteomyelitis or pneumonia had higher antibody concentrations to PVL at the time of admission to the hospital than children with skin and soft tissue infections, suggesting that PVL antibodies were not protective against invasive *S. aureus* infections.[30] Similarly, high levels of PVL antibody were not found to prevent initial or recurrent skin community-acquired methicillin-resistant *S. aureus* (CA-MRSA) infections.[117]

In early studies, only a fraction of *S. aureus* isolates recovered from humans carried the PVL genes.[230] Interest in PVL greatly increased after reports that *S. aureus* isolates carrying PVL genes were associated with severe furunculosis[51] and particularly necrotizing pneumonia in children and adolescents that frequently was fatal.[72,95,170] Furthermore, the independent emergence worldwide of CA-MRSA clones that generally carry the PVL genes led to further studies of the role of PVL in the pathogenesis of *S. aureus* infections.[286] The USA300 CA-MRSA clone has been especially linked to PVL[202,204] Using polymerase chain reaction (PCR) the PVL genes have been increasingly detected (*pvl*$^+$) in CA-MSSA strains in the United States as well.[31,127] In children with invasive *S. aureus* osteomyelitis, the presence of the PVL genes has been associated with greater local and systemic inflammation, longer extent of fever, greater frequency of positive blood cultures, and more frequent complications such as the development of chronic osteomyelitis or venous thrombosis.[25,63,189] Pulmonary manifestations were also more frequently observed among children with invasive *S. aureus* infections caused by *pvl*$^+$ isolates[100]; *pvl*$^+$ strains were isolated almost exclusively among a group of children with staphylococcal pneumonia in one study.[36] In adults with staphylococcal pneumonia, *pvl*$^+$ isolates were associated with increased rates of mortality in one study but were not found to a significant factor in a larger study of hospital-acquired or ventilator-associated pneumonia.[182,225] In a multicenter international study, *pvl*$^+$ did not

predict outcome of *S. aureus* skin and soft tissue infections in adults.[282] Thus, the presence of PVL genes may be a marker for isolates capable of causing more severe disease in some populations of patients but not in others.[73]

The role of PVL in the pathogenesis of infection in experimental models also remains controversial. The contribution of PVL to severity of *S. aureus* infections has been examined in mice using isogenic *pvl*$^+$ or *pvl*$^-$ isolates. One study found no difference in lethality for sepsis or abscess volume in a skin abscess model,[291] as well as no difference in the destruction of human neutrophils by the isogenic strains. The investigators concluded that PVL was not a major virulence determinant of CA-MRSA. The other study found *pvl*$^+$ isolates caused much greater lung inflammation and necrosis in a mouse acute pneumonia model.[160] PVL was detected in the infected lung tissues. These investigators also discovered that staphylococcal protein A is more highly expressed in *pvl*$^+$ strains and postulated that PVL coupled with increased staphylococcal protein A could result in greater tissue inflammation than what is found with *pvl*$^-$ strains. A clear role for PVL has been shown in the pathogenesis of necrotizing pneumonia and necrotizing skin infections in a rabbit model.[59,174] In a rabbit model of osteomyelitis using an isogenic PVL genes negative derivative of a USA300 CA-MRSA isolate (LAC), PVL appeared to play a role in the persistence and extension of infection.[50] The discrepancy in these studies may be explained by use of different *S. aureus* isolates as well as different models of infection, especially the differential activity of PVL on neutrophils from humans or rabbits compared with murine or simian cells.[181]

Although the exact role of PVL in *S. aureus* infections has not been fully established, treatment measures directed against PVL have been proposed. These measures include the preferred use of protein-inhibiting antibiotics rather than, or in addition to, bactericidal antibiotics to decrease PVL production,[65,269] as well as adjunctive administration of intravenous immunoglobulin (IVIG) preparations containing PVL antibody.[92] However, currently there is no evidence that neutralizing PVL or decreasing PVL production affects outcomes.

Enzymes

Staphylococci elaborate a variety of enzymes, including hyaluronidase, nuclease, proteases, lipase, catalase, lysozyme, and lactate dehydrogenase, that might play a role in the spread of infection in local tissues or the establishment of a nidus of infection.

Other biologically active extracellular products, as yet unidentified, undoubtedly are produced by the staphylococcus. The potency and number of these weapons in the armamentarium of the coagulase-positive staphylococcus, in contrast to the small number of extracellular products associated with the much less virulent coagulase-negative staphylococcus, suggest that the synergistic action of these toxins and enzymes may explain the superior ability of coagulase-positive staphylococci to establish infection and cause tissue necrosis. Several extracellular toxins have been proved to have specific roles in staphylococcal infection or disease: the epidermolytic toxins, TSST-1, and the enterotoxins.

Epidermolytic Toxins

Two biochemically and immunologically distinct exotoxins, epidermolytic toxins A and B (epidermolysins, exfoliatins), can separate adjacent cell layers within the epidermis and cause the various skin manifestations of the staphylococcal scalded skin syndrome (SSSS). The toxin acts extracellularly and does not induce cell death directly or lysis of the cell membrane or elicit an inflammatory response. It does not damage any organ or cell, except those of the upper epidermis.[69] These low-molecular-weight (~24,000 daltons) protein exotoxins are now known to hydrolyze desmoglein-1, an important structural skin protein.[10] Exfoliatin toxin D has been associated with bullous impetigo but not SSSS.[256,305]

Toxic Shock Syndrome Toxin Type 1

TSST-1 was discovered independently in 1981 by two research groups. It was documented to be an excellent marker for staphylococci associated with vaginal toxic shock syndrome (TSS), in which it correctly identified more than 90 percent of strains in blinded testing. In experimental animal models, purified TSST-1 can induce the major physiologic changes of TSS: fever, mucous membrane suffusion, renal impairment, hepatic damage, hypocalcemia, lymphocytopenia, and hypotension. TSST-1 can be detected in blood, pus, urine, and tissues during human and experimental model TSS. USA300, the major CA-MRSA clone in the United States, does not carry the gene encoding TSST-1.[204] Toxic shock syndrome and the role of TSST-1 as a superantigen are discussed in Chapter 64.

Enterotoxins

Over 20 different staphylococcal enterotoxins (SEA to SE*l*V) have been identified, most of which cause emesis in primates.[62,116] The toxins are heat stable and resist boiling. Therefore, once sufficient toxin has formed within food, even heating or boiling will not inactivate the toxin. Foods involved in outbreaks of food poisoning frequently have been inoculated by a lesion on the hand of a food handler in situations in which the food is then held at temperatures that allow bacterial growth (between 25° and 60° C) for some time before the food is served. Foods implicated in particular are ham, salads with starch and mayonnaise, salami, poultry, cream sauces, pastries, and dairy products. The mode of action of enterotoxins is not well understood in part because of the lack of a convenient animal model.[185] Intravenous injection of purified enterotoxins in laboratory animals causes hypotension, cardiovascular collapse, and death. The significance of these properties of enterotoxins in naturally occurring human infections is unknown, although they may be linked to clinical syndromes, including TSS. Enterotoxin A often is elaborated with TSST-1 in menstrual-associated TSS, but it also is elaborated

without TSST-1 in patients with septicemia in whom TSS does not develop. Staphylococcal enterotoxin B, most often isolated in phage group V organisms, is associated with local infections and pneumonia.[188] USA300 strains typically do not contain the genes encoding most enterotoxins but frequently contain molecular variants of enterotoxins Q and K.[58] In contrast, USA400 isolates may carry many of the enterotoxin genes, including enterotoxin H, which is unique among the other *S. aureus* genomes.[14,210]

Staphylococcus aureus Microbial Surface Components Recognizing Adhesive Matrix Molecules

Bacterial adherence to host tissues is mediated by a number of bacterial surface components or adhesins that recognize and bind to host extracellular matrix and cell surface molecules.[134] The subfamily of adhesins that bind to the extracellular matrix are known as microbial surface components recognizing adhesive matrix molecules (MSCRAMMs). For *S. aureus*, important MSCRAMMs are protein A, fibronectin-binding proteins (A and B), clumping factors A and B (fibrinogen-binding proteins), and collagen-binding protein (Cna).[81] Many other less well-characterized adhesins also have been identified from the *S. aureus* genome.[243] The MSCRAMMs are linked to bacterial wall peptidoglycan after sortase cleavage of the LPXTG (LeuProXThrGly) motif that is a conserved C-terminal cell wall sorting signal among the adhesins.[42] Fibronectin-binding proteins play a role in *S. aureus* invasion into endothelial cells.[25,134] Collagen adhesin protein may contribute to the pathogenesis of *S. aureus* musculoskeletal infections,[68,219] although there are conflicting animal studies as well as clinical studies in this area.[136,248] USA300 CA-MRSA isolates do not carry the Cna gene, *cna*, yet osteomyelitis and septic arthritis are common CA-MRSA invasive infections in children.[35,189]

Clumping factor A has been the target for protective immunity in the prevention of *S. aureus* infections, and this approach has been successful in experimental models.[289] The safety and pharmacokinetics of an intravenous immunoglobulin preparation enriched for antibody to clumping factor A were established in low-birth-weight infants; however, a large randomized trial did not document efficacy in prevention of staphylococcal infections in this population.[24] A monoclonal antibody (tefibazumab [Aurexis]) directed against clumping factor A has undergone preliminary evaluation as adjunctive therapy for *S. aureus* bacteremia in adults, but further development of the product is doubtful.[296]

Regulation

Bacterial pathogenesis is reliant on cell population, density-dependent gene expression. This quorum sensing acts to adjust the production of accessory genes (e.g., virulence genes), depending on the needs of the cell. Studies have shown that in low-density populations, when the bacterial cells are multiplying, accessory genes are either turned off or expressed at a low level to the advantage of other genes, such as genes involved in cell wall formation. In high-density bacterial populations, expression of the accessory genes is increased. The accessory gene regulator, *agr*, is genus specific, and the effector molecule is a regulatory RNA molecule, named RNAIII. Many staphylococcal toxins and virulence factors are under the regulation of *agr*, and *agr* dysfunction is associated with reduced clearance of MRSA infection by vancomycin.[2,13,29,84,203] The staphylococcal accessory regulator, sar*A*, is another global regulator in *S. aureus*. The *sar* locus produces three overlapping transcriptional units (*sarB*, *sarC*, and *sarA*), and the effector peptide, SarA, is a DNA-binding protein that functions as a transcriptional regulator. In animal models of infection, both RNAIII and SarA have been shown to affect virulence. In addition to *agr* and *sar*A, several other factors have been described that affect accessory gene regulation.[23] Thus, the complex network of regulatory operons that act on each other and/or directly on specific genes is not completely understood.

Staphylococcal L-Forms and Small Colony Variants

L-forms are staphylococcal variants with impaired or absent cell walls. L-forms can be induced in vitro by growing staphylococci in hypertonic media, in the presence of the muralytic enzyme lysostaphin, or with the addition of antibiotics that inhibit the formation of cell walls, such as the penicillin-methicillin group, cycloserine, and vancomycin. These L-forms, or protoplasts, require hypertonic environments for survival, do not accept Gram stain, and are resistant to cell wall–inhibiting antibiotics. Under favorable conditions, L-forms revert to complete bacteria with cell walls.

Clinical interest in L-forms has arisen because of concern that antibiotics might induce these forms in vivo. L-forms then could persist in the host in a latent or less virulent phase and revert to a fully virulent state at some time when antibiotics no longer are present. Identification of L-forms in specimens from human infection or in infected animals treated with antibiotics has been reported exceedingly rarely, despite considerable effort.[293] Despite extensive laboratory and clinical investigation, no evidence has demonstrated that L-forms are important causes of bacterial persistence in clinical infections.

Small colony variants of *S. aureus* are isolates with atypical colony morphology, decreased hemolysis and pigmentation, decreased growth rate, and unusual biochemical characteristics that result in difficulty isolating and identifying these organisms.[287] Small colony variants also have been implicated in persistent and recurrent *S. aureus* infections, including osteomyelitis,[232] brain abscesses, and pulmonary infections in patients with cystic fibrosis.[141,254] Small colony variants can persist intracellularly, are often more resistant to antibiotics than the parent strain and antibiotics that penetrate into cells (e.g., rifampin), and do not activate innate host systems to the same degree as wild-type phenotypes, which helps to explain their successful intracellular persistence.[233,283]

Staphylococcal Cassette Chromosome *mec*

The staphylococcal cassette chromosome mec (SCC*mec*) is a region spanning between 20 and 70 kb located approximately 30 kb downstream of the chromosomal origin of replication. SCC*mec* contains the *mec*A gene encoding a penicillin-binding protein (PBP2a or 2′) with poor affinity to β-lactam antibiotics, allowing essential functions of cell wall formation to proceed in the presence of β-lactam antibiotics. The *mec*A gene is regulated by *mecR1* and *mec*I where activated *mec*R1 (signal transducer) inactivates *mec*I (repressor) through site-specific proteolytic cleavage, thereby initiating production of PBP2′. In vitro studies have suggested that the β-lactamase regulators *bla*R1 and *bla*I may also be effective regulators of *mec*A.[196]

The SCC*mec* classification systems are based on several genetic elements including the class of *mec* and the *ccr* genes, which are recombination genes involved in the mobility of the genetic cassette. Other elements that may be present are IS431, IS1272, Tn554, pUB110, and pT181. SCC*mec* types II and III also contain other antibiotic resistance genes such as *tet* (tetracycline resistance). All strains contain a J(unk) region of variable size and composition. Several schemes for SCC*mec* typing have been published.[154,216,217] Historically, nosocomial MRSA isolates contained the SCC*mec* types I to III; and when community isolates were first described, three new types—IV, V, and VI—were introduced. Two more SCC*mec* types have been described, tentatively named VII and VIII. The smaller size (<30 kb) of SCC*mec* IV to VI is thought to explain an increased mobility between strains. Because the community strains have been brought into the health care setting, SCC*mec* typing cannot be used to predict whether an infection is health care or community related.[102,115,255]

TYPING METHODS

Staphylococcal strains were historically characterized by bacteriophage typing (World Health Organization method).[19] *S. aureus* isolates are susceptible to lysis when exposed to bacteriophages. Bacteriophage typing has been used primarily as an epidemiologic tool for identifying related strains in epidemics. An international system for strain identification by bacteriophage typing has been established but is not widely used. Staphylococci of phage group 80/81 were widespread during the pandemic of staphylococcal disease in the late 1950s. At present, a wide variety of phage types are involved in staphylococcal disease.[242]

In the 1990s pulsed-field gel electrophoresis (PFGE) became the gold standard for strain typing utilized by both reference and research laboratories.[277] The method is reliable and reproducible, and the use of digital imaging and band analysis has enhanced the quality of inter-gel comparisons. The Centers for Disease Control and Prevention (CDC) used the PFGE method to define and interrelate *S. aureus* clones and designated 11 staphylococcal clones circulating in the United States as USA100 to USA1100.[192*]

Multilocus sequence typing (MLST) is a nucleotide sequence-based method adapted from multilocus enzyme electrophoresis (MLEE). In MLST typing the alleles at each locus of seven housekeeping genes (*arc*C, *aro*E, *glp*F, *gmk*, *pta*, *tpi*, and *yqi*L) are determined through PCR amplification, nucleotide sequencing, and submission of sequence data via computer website to the MLST database (http://www.mlst.net).[70] In this database, strains can be compared with strains submitted by other investigators and within-laboratory comparisons with control strains thus have become obsolete.

spa typing is a single-locus sequence method, based on the polymorphic X or the short sequence repeat (SSR) region of the protein A gene.[260] *spa* typing has some advantages over PFGE in terms of speed, interpretation, and exportability, the main limitation being the single-locus–based design.[110] Less commonly used methods include repetitive-element PCR (rep-PCR), restriction fragment length polymorphism (RFLP), and toxin typing.

Staphylococcus aureus Clones in the Hospital and in the Community

Of the methods mentioned earlier, MLST has been especially useful in dividing *S. aureus* strains into lineages (or clonal clusters). Frequently, these clones are described in the literature by their sequence type followed by the SCC*mec* type (e.g., ST8-IV). Most human *S. aureus* strains belong to 1 of 10 independent lineages. Five major clonal clusters (CC) that have been described to contain a majority of the human MRSA strains are CC5, CC8, CC22, CC30, and CC45. Of these, MRSA clones within the CC5 and CC8 clusters have been recognized as the main causes of hospital outbreaks worldwide.[46] Examples of widespread hospital MRSA clones are EMRSA15-ST22, EMRSA16 (ST36-II), the Archaic (ST250-I or 247-I), the Pediatric (ST5-IV), and the Brazilian (ST239-III) clones.[98]

Although a few hospital MRSA clones have circulated around the world, genetically distinct community clones have been reported from different geographic areas. Commonly, these strains contain the smaller SCC*mec* elements, type IV or V.[241] Why these different clones emerged in parallel at this point in time is unknown.

In the United States, the two most commonly described community MRSA clones are USA300-ST8 and USA400-ST1. USA400, originally described as MRSA strain MW2 which caused four pediatric deaths in Minnesota and North Dakota, was more commonly observed in the midwestern states.[89] USA300 is the most frequently reported strain causing community-acquired disease in both pediatric and adult settings in the United States.[56] Like many other epidemic strains, it has been associated with a spectrum of disease presentations ranging from simple skin and soft tissue infections[142,146] to severe sepsis syndrome,[101] sometimes with fatal outcome. USA300 is a highly virulent strain that has been distributed over the

*Available through the Network on Antimicrobial Resistance of *Staphylococcus aureus* (NARSA) at http://www.narsa.net.

continent and has become the most prevalent cause of community-acquired and community-onset health care–associated infections in less than a decade in the United States as well as in many other countries on different continents.[125,204,213] It has also become the most common source of hospital-acquired *S. aureus* infection in many hospitals, where it has acquired one or more antibiotic resistance markers.[57,102,126,255]

MLST typing has enabled the ancestry of many of these clones to be traced phylogenetically. For example, phage type 80/81 (ST30-MSSA) was epidemic in the 1960s and is the likely ancestor to the widely circulated Southwest Pacific clone ST30-MRSA-IV, which has been successful both as a colonizer and as a cause of diseases ranging from skin and soft tissue infections to invasive manifestations.[242] Similarly, the USA300 (ST8-MRSA-IV) clone is postulated to have descended from an archaic ST8-MSSA.[71]

GENOMES

The list of current or completed *S. aureus* genome sequencing projects, submitted to the National Institute of Health (NIH) genomes database (www.ncbi.nlm.nih.gov) contains more than a hundred isolates and is continuously increasing. The sequenced genomes include representatives of the widespread CA-MRSA clones from the United States (e.g., MW2[14], FPR3757,[60] and TCH 1516[120]). Among the sequenced hospital-associated MRSA strains, one of the first reported MRSA clones, *S. aureus* COL,[93] as well as EMRSA-16,[137] N315, and Mu50,[159] have been sequenced. In addition, methicillin-susceptible isolates such as strains 476[124] and NCTC 8325[94] have been well defined.

Genome sizes range from 2.7 to 3.1 Mb, and the structures of the chromosomes are conserved. The core genome (the part of the genome that is the same in all strains) represents approximately 75 percent of the genome.[171] The biologic function remains unknown for at least a third of the predicted proteins. In the future the knowledge gained from these ongoing sequencing projects and analyses of the resulting large datasets will provide a deeper insight into the genetic differences of *S. aureus* strains and may give clues into the strain-specific causes of disease versus colonization, suggest candidate genes for potential vaccine or drug targets, or provide information into the acquisition of antibiotic resistance or virulence factors. For example, a comparative genome project of vancomycin-resistant strains provided proof that the vancomycin-resistant isolates reported to date in the United States all represented new acquisition of transposon Tn1546 from *Enterococcus* spp. and also identified a change in a gene important for uptake of foreign DNA, which may have facilitated the genetic exchange.[155]

EPIDEMIOLOGY

S. aureus isolates are responsible for both sporadic infections and epidemics of varying extent ranging from the intrafamily outbreaks of staphylococcal disease commonly encountered to large and often prolonged hospital-associated outbreaks, such as those emanating from a neonatal nursery or a surgical service. Epidemic spread of staphylococci of phage type 80/81 was so widespread worldwide in the period encompassing the mid 1950s through the early 1960s that it constituted a pandemic. Researchers have suggested that a few "epidemiologically virulent" strains, such as 80/81, are particularly capable of spreading widely and causing disease. Robinson and colleagues[242] concluded that descendants of 80/81 have acquired methicillin resistance to emerge as a CA-MRSA clone, closely related to the southwest Pacific ST30 clone. Because we are largely ignorant of the factors responsible for pandemic spread, we cannot prevent or predict recurrence.

Staphylococci may be transmitted by multiple routes, including contact with infected persons, contact with asymptomatic carriers, airborne spread, and contact with contaminated objects. Of these mechanisms, contact with a person who has a staphylococcal lesion appears to be particularly important in the spread of staphylococci. Persons with open draining lesions disseminate organisms into their environment and to others via direct contact. In a hospital, staphylococci may be spread from an infected patient to another on the hands of caretakers, such as physicians or nurses. Hospital personnel with mild or unapparent lesions, such as sties, furuncles, or paronychia, may themselves spread organisms. In family and small community outbreaks, multiple secondary cases can occur and frequently can be traced to an individual with a draining lesion. Secondary cases tend to appear for months after the initiating case within these small epidemiologic units.[267] Staphylococci also may be spread by asymptomatic carriers who have staphylococci in one or more body sites, including the nose, skin, hair, nails, axillae, and perineum.

At any one time, up to 30 percent of individuals are colonized in their anterior nose with *S. aureus*.[152] The rate of nasal carriage of MRSA appears to have increased. Nasal carriage of MRSA was 0.8 percent in 2001[211] and 9 percent in 2004[49] for children seen in an emergency center in Nashville, Tennessee. For children admitted to a hospital in Corpus Christi, Texas, in 2005, the rate of MRSA colonization was 22 percent.[7] In 2004 to 2005, an average of 2.6 percent (range, 0% to 9%) of children in 11 different practices in the St. Louis metropolitan area was colonized with MRSA in the nose.[86] Why nasal colonization of children by CA-MRSA has increased over the past decade is unclear but is likely related to unique properties of the major CA-MRSA clone USA300.[60] Actually for children with skin and soft tissue infections, CA-MRSA colonization of the groin is more commonly detected than is nasal carriage.[88]

Detailed studies have been performed to delineate factors regulating the carrier state and its establishment and perpetuation, as well as factors responsible for dispersion of organisms from carriers. Asymptomatic carriers may be a source of disease for themselves and others. For example, a wound infection appears to be more likely to develop in a hospitalized patient who becomes a carrier

than in a noncarrier.[297] Much attention has been devoted to detecting and attempting to treat nasal carriers of staphylococci; however, studies of nursery outbreaks have indicated that nasal carriage is not as important as is hand transmission in the dispersal of staphylococci.[298] Transmission by hand contact can be minimized by effective hand washing.[97] The problem of hair carriage of staphylococci in operating room personnel has been minimized by using improved head coverings.

Staphylococci are widespread in the environment and can be cultured from clothing, carpets, toiletries (e.g., hairbrushes, razors), and virtually all environmental surfaces. Airborne dissemination of organisms is possible, particularly in operating rooms with poor ventilation and heavy traffic; improved methods of ventilating operating rooms may have reduced the sepsis or colonization rate. Environmental staphylococci may serve as an important reservoir, but direct human-to-human transfer probably is a much more important means of transmission in epidemic situations than is airborne spread or contact with contaminated objects. This means of transmission is particularly true for spread of CA-MRSA among athletes.[150] Furthermore, USA300 appears to be especially able to spread among household members, in part related to increased contamination of environmental surfaces.[200,284] Molecular methods of typing offer adjunctive information useful in characterizing strains involved in outbreaks.[61,70]

The neonatal nursery has been an area of particular concern in the transmission of *S. aureus*. During the pandemic of strain 80/81 disease, outbreaks of serious neonatal disease were commonplace, high colonization rates were found in infants on discharge from the nursery, and the subsequent incidence of disease in some outbreaks was as high as 50 to 70 percent. Skin disease and infant and maternal mastitis usually appear within 1 to 4 weeks after discharge. Staphylococcal pneumonia, far more common during that period than at present, may not be seen for months after delivery, even though the infecting strain was acquired in a hospital. During the experience with strain 80/81 disease, a particularly high staphylococcal attack rate was seen in the families of colonized infants. The same epidemiology has been noted for the USA300 CA-MRSA clone that has been associated with staphylococcal infections in otherwise healthy newborns up through 30 days of life.[79] One study reported that 20 percent of infants with a *S. aureus* infection had a mother with a concomitant (proven or presumed) infection.[79] In a study of infants up to 30 days of age with community-onset *S. aureus* infections, the infecting strain in the neonate was the same as a nasal *S. aureus* strain recovered from the mother in 8/34 (24%) mother-neonate pairs.[78] This suggests that maternal nasal colonization is not the sole source of *S. aureus* isolates causing infection in their otherwise healthy full-term neonate. In other studies, *S. aureus* colonization of infants was more likely to be related to horizontal than vertical transmission from the mother.[132]

Various control measures were used successfully to terminate individual outbreaks during the 1980s. Measures to protect the infant from colonization and subsequent development of disease by establishing a barrier at the site of initial colonization included hexachlorophene bathing, application of antibiotic ointment to the umbilicus and circumcision site, and application of an antiseptic dye to the umbilicus. Another approach, deliberate colonization of the umbilicus with an interfering "avirulent" strain, was successful in controlling several epidemics but itself has caused skin disease and, in one case, fatal septicemia.[259] Hexachlorophene bathing became a widespread practice during the period in which nosocomial staphylococcal infections were declining.[97,227] After approximately a decade of using hexachlorophene, researchers recognized that cutaneous absorption of hexachlorophene could result in potentially toxic hexachlorophene blood levels in infants, particularly premature ones, subjected to repeated daily baths over time. Brain stem abnormalities associated with the use of hexachlorophene have been demonstrated in premature infants. For these reasons, controls were placed on the sale of hexachlorophene, and its routine use was discouraged in 1973. In one neonatal nursery outbreak of MRSA, the use of 0.3 percent triclosan (Bacti-Stat) was associated with cessation of an outbreak.[307]

Patterns of crowding of infants and understaffing contribute to outbreaks. Active clinical and bacteriologic surveillance and cohorting of infants may be effective in reducing outbreaks.[16,109]

S. aureus bacteremia related to intravenous catheters and *S. aureus* infections of prosthetic devices are common.[37,186,187] In particular, methicillin-resistant staphylococci are responsible for widespread nosocomial outbreaks of disease in hospitals.*

HOST DEFENSES

S. aureus is ubiquitous in the environment, and approximately 30 percent of the population are carriers. A major defense against staphylococcal infection is intact skin. Minor wounds frequently become colonized and infected and serve as portals to deeper, more significant staphylococcal infection. In some cases, the integumentary infection may be of major importance; in others, minor skin punctures may serve to introduce infection to distant internal sites. Burns, varicella virus and cutaneous herpesvirus infections, insect bites, minor lacerations or abrasions, primary skin diseases (e.g., atopic eczema), epidermolysis bullosa, and surgical wounds are important portals of entry for staphylococci. A deficiency in peptides (defensins and cathelicidins) important for innate immunity of skin against *S. aureus* has been shown in skin specimens from patients with atopic dermatitis and likely contributes to the increased susceptibility of the skin to *S. aureus* infections in these patients.[153,218] In hospitalized patients, intravenous needles and catheters may be sources of staphylococcal infection.[186,187]

Foreign bodies reduce local resistance to staphylococcal engraftment and are important in the pathogenesis and perpetuation of infection. Noteworthy foreign bodies

*References 26, 32, 48, 53, 73, 151, 251, 271, 295.

are cerebrospinal fluid shunts, prosthetic cardiac valves, nonabsorbable sutures, vascular prostheses (including arteriovenous shunts for hemodialysis), orthopedic prostheses, nails, and wires. In neonates, the umbilicus and circumcision sites, which may be colonized within the first few hours of life and from which both local and distant infections may be established, are important portals of entry.

Viral respiratory diseases such as measles and influenza[108,228] predispose to development of pulmonary infection, again predominantly by damaging the integrity of the barrier at the portal of entry. In an animal model, differences in the ability of *S. aureus* strains to cause secondary bacterial pneumonia after influenza can be demonstrated.[129] Disruption of the respiratory epithelium and impairment of ciliary motion and other local defenses may allow secondary staphylococcal invasion.

Once the integumental barrier has been breached, the polymorphonuclear leukocyte appears to be the most important line of defense. Successful phagocytosis involves chemotaxis, opsonization, and intracellular killing. The incidence of difficulty with staphylococcal infection is highest in patients with defects in this area of host defense.[80,126,303] Granulocytopenia of any origin predisposes to the development of infection, particularly with the host's endogenous bacteria, including staphylococci.[15] Krishna and Miller have extensively reviewed the innate and adaptive immune response of the skin to *S. aureus* infections.[157]

Once the staphylococcus and the leukocyte are close to one another, opsonization of the bacterium must proceed for phagocytosis to occur. Two systems for opsonization of staphylococci have been described. Serum from normal adults has good opsonic activity when unheated but is generally inactive after heating at 56° C for 1 hour. The heat lability of normal opsonin and the observation of normal or only slightly decreased opsonic activity in unheated sera from patients with agammaglobulinemia[301] indicate that the major opsonin is complement. A few sera, generally from patients convalescing from serious staphylococcal disease, contain heat-stable opsonins, presumably antibody directed at the staphylococcal cell wall components.[163] Therefore, either complement or antibody can provide opsonins for staphylococci; specific antibody is helpful but not required. The clinical correlate to these observations may be found in patients with defective defenses; patients with agammaglobulinemia and defective antibody generally have more severe problems with organisms other than staphylococci, whereas patients with deficiencies in complement components have been reported to have repeated and severe staphylococcal infections.*

Once the bacterium has been ingested, intracellular killing proceeds normally. Coagulase-positive staphylococci can survive within polymorphonuclear leukocytes for a considerably longer period than can coagulase-negative organisms.[198] Prolonged intracellular survival may be an important virulence factor separating coagulase-negative from coagulase-positive strains and may provide a mechanism whereby surviving organisms may be carried to distant body sites to establish metastatic foci of infection. A number of *S. aureus* exotoxins outlined earlier, such as PVL, contribute to the ability of *S. aureus* to evade human neutrophils. In addition, many *S. aureus* genes are regulated upward or downward after exposure to human neutrophils and there are differences in gene expression among various strains in response to human innate host response that might account for differences in the ability of the isolate to cause infection.[290]

Patients with chronic granulomatous disease of childhood have an inborn error in intraleukocytic killing of catalase-positive bacteria and fungi. These patients have an early onset of recurrent purulent infections of the skin, subcutaneous tissue, lungs, and reticuloendothelial organs, particularly the liver, that often are caused by *S. aureus*.[236] Staphylococcal infections are also characteristics of hyper-IgE syndrome. Neutrophil disorders are discussed in detail in Chapters 2 and 67.

In contrast to the primacy of polymorphonuclear defense against staphylococci, evidence for an important role of specific humoral and cellular immunity in host defense against staphylococci is either lacking or contradictory. Specific antibody is not required for opsonization of unencapsulated strains of staphylococci. The opsonic activity of serum from patients recovering from staphylococcal endocarditis or other serious disease is greater than that of otherwise normal persons.[163,300] Despite the presence of humoral antibodies to cell wall teichoic acids and to various toxins and enzymes, which are found regularly in those convalescing from serious staphylococcal infection, the patient remains susceptible to recurrence of infection with the same strain of staphylococci. Staphylococcal infections generally occur and progress in the presence of some degree of humoral immunity. Specific antibody to one or more staphylococcal components or products does not protect against infection.

Clinical evidence from patients with deficient cell-mediated immunity (combined immunodeficiency, thymic aplasia, Nezelof syndrome) suggests that staphylococcal infections are not among the most important pathogens in these patients. However, in an animal model, interleukin-17 production by local T cells is important for host defense against *S. aureus* cutaneous infections.[45]

Present evidence, therefore, suggests that intact local skin and mucous membrane barriers are the most important defense against the establishment of staphylococcal infection. Once infection is established, an intact polymorphonuclear response is essential for containment of the infection and clearance of the organisms.

Genomic studies are leading to a greater understanding of the host response to *S. aureus* and may lead to novel treatment or prevention modalities. Using microarray techniques to describe the host immune transcriptional profile in children with primarily invasive *S. aureus* infections, Banchereau and colleagues[17] found an overexpression of innate immunity and hematopoiesis genes and an underexpression of genes associated with adaptive immunity. Differences in gene expression were seen between children with musculoskeletal infections and those with pneumonia. Of interest, patients with

*References 4, 8, 9, 82, 201, 224.

osteoarticular infections displayed overexpression of transcripts linked to coagulation of blood that might relate to the venous thromboses that may occur with *S. aureus* osteomyelitis.[103]

PATHOGENESIS

Staphylococci cause disease by two mechanisms: direct invasion of tissues with liberation of toxins, which may have effects at sites distant from the focus of infection, and colonization. The hallmark of a staphylococcal lesion is the abscess. Local tissue destruction at the site of inoculation is followed rapidly by hyperemia and a vigorous inflammatory response marked by the accumulation of large numbers of polymorphonuclear leukocytes. Tissue necrosis in the center of the lesion occurs next. At the site of intensive hyperemia surrounding the lesion, a fibrin wall is formed. Liquefaction necrosis occurs centrally; the mature lesion consists of a fibrin wall surrounded by inflamed tissues enclosing a central core of pus consisting of organisms and leukocytes. Live bacteria may persist within these lesions for a considerable period of time. As pus accumulates, it may drain toward the skin surface or into adjacent tissues, where it forms sinus tracts and secondary abscesses. In the presence of an intact host inflammatory response, this type of reaction may be seen in diverse areas, including the skin and subcutaneous tissues, lymph nodes, joints, kidney, liver, parotid glands, muscles, lungs, and long bones.

In addition to local extension, *S. aureus* may disseminate hematogenously from this focus of infection, even from abscesses that are trivial in size. Hematogenous dissemination may result in infection of bones, joints, and heart valves. Given the ubiquitous nature of staphylococci, skin and wounds, which are the usual ports of entry, appear to be remarkably resistant to infection. This natural resistance is affected dramatically by the presence of foreign bodies within the wound, such as sutures and bits of soil or gravel. Natural resistance also is affected by the tissue compromise that occurs after ecchymosis or hemorrhage and by vascular insufficiency. Poor personal hygiene likewise predisposes to development of staphylococcal skin infection. Moist, macerated skin is invaded more easily and thus contributes to the increased frequency of staphylococcal skin infection in intertriginous areas and in tropical climates.

Toxigenic staphylococcal disease includes staphylococcal scalded skin syndrome, TSS, and staphylococcal food poisoning. Toxin can be elaborated from *S. aureus* isolates that are colonizing sites without a specific site of infection but leading to TSS. The major manifestations of these diseases are caused by the effects of specific toxins. Clinical manifestations of infections caused by *S. aureus* in children are discussed in multiple chapters, and the reader is referred to the specific infection of interest.

DIAGNOSIS

The diagnosis of significant staphylococcal infections should be pursued with vigor. Collections of pus, whether superficial or deep, should be aspirated or drained surgically for diagnostic and therapeutic purposes. Gram stain and culture should be performed. An aggressive approach to the diagnosis of osteomyelitis by bone aspiration and bone biopsy provides an etiologic security that is helpful during the prolonged treatment phase that necessarily follows. When infection is associated with a foreign body, such as an intravenous catheter or suture, removal and culture of the foreign body help in determining the etiology and removal is almost always required for successful resolution of infection.

When possible, at least two blood cultures should be obtained before starting therapy for all serious infections. One need not wait for fever spikes or delay therapy to obtain specimens. Blood cultures frequently are negative in serious staphylococcal infection, a fact that demonstrates the need for performing other cultures. Blood cultures are positive in most cases of staphylococcal endocarditis, approximately half the cases of osteomyelitis and septic arthritis, and less than half the cases of pneumonia and deep tissue abscesses. A number of molecular tests are available to rapidly identify MRSA once isolated (or growing in blood culture bottles) in the microbiology laboratory as well as to detect MRSA colonization in patients at the time of hospital admission.[285]

Measurement of nonspecific indicators of inflammation (e.g., erythrocyte sedimentation rate and C-reactive protein), although of limited value in diagnosis, can be helpful in monitoring the clinical course of infection and response to intervention. Accurate, sensitive serologic methods for making a diagnosis of serious staphylococcal infection are not available.

Enterotoxins can be identified by a variety of methods. Immunoassay is used routinely but may lack the sensitivity required to detect levels seen in staphylococcal food poisoning. DNA oligonucleotide probes are highly sensitive, but their clinical utility is limited by the identification of nonexpressed genes.[215]

TREATMENT

Successful treatment of staphylococcal infection depends on adequate drainage of collections of pus and the rational use of antibiotic therapy. Strategies for clinical management of MRSA infections, including those in children, are outlined in guidelines published by the Infectious Diseases Society of America.[175] Staphylococcal infections have a particular tendency to persist and recur; for these reasons, prolonged antibiotic therapy usually is required for all but minor infections. Surgical drainage is extremely important and, in some patients with minor superficial abscesses, may be all that is required.[44,67] In one study an abscess less than 5 cm in diameter was associated with successful outcome after incision and drainage regardless of whether the antibiotic administered was active against the isolated pathogen, which was CA-MRSA.[167] In other studies the size of the abscess was not associated with outcome.[45] But for some infections, a period of antibiotic therapy after surgical drainage better ensures that the infection has been contained. In an adult study, antibiotics active against CA-MRSA were associated with

significantly greater cures than agents not active against CA-MRSA in patients who had undergone incision and drainage of skin and soft tissue infections.[246] Failure to provide surgical drainage is an important reason for persistence or recurrence of organisms. Antibiotics cannot be expected to penetrate into the avascular center of abscess cavities. When abscess cavities are undrained or when antibiotic therapy is discontinued before an area is sterilized, live bacteria may persist and disseminate to cause later recurrence at that site or distant sites. After incision and drainage, adjunctive antibiotics are recommended for patients with underlying conditions or with immunosuppression; multiple sites of infection; rapidly progressive lesions or lesions associated with cellulitis; abscesses located on the face, hand, or perineum; young children (<1 to 2 years old); or those patients whose condition has not improved with incision and drainage alone.[175]

For moderate to severe staphylococcal infection, the patient should be hospitalized initially for intravenous therapy. This strategy ensures peak antibiotic levels, which may allow greater penetration into relatively avascular areas. Once the patient is afebrile, has a negative blood culture, and there is definite evidence of clinical improvement, therapy can be completed with home administration of antibiotics or with oral agents, as deemed appropriate by the treating physicians.

Antibiotics for *Staphylococcus aureus* Infections

Since the mid 1960s, the vast majority of *S. aureus* isolates from most sections of North America and Europe have been penicillinase producers and, therefore, penicillin resistant. When *S. aureus* is likely to be the cause of infection, the most appropriate agents to administer for empirical treatment of infections with onset in the community in normal children are based on the relative frequency of CA-MRSA isolates in the particular community. In areas where CA-MRSA isolates are not a concern, treatment with a penicillinase-resistant penicillin or cephalosporin should be initiated before isolation of bacteria and susceptibility testing (Tables 80-3 and 80-4). Methicillin-resistant organisms cannot be treated

TABLE 80-3 **Therapy for Staphylococcal infections in Infants and Children (Excluding Neonates)**

Drug	*Oral (Mild–Moderate Infection)*		*Parenteral (Moderate–Severe Infection)*	
	CHILDREN <40 KG	CHILDREN >40 KG AND ADULTS	CHILDREN <40 KG	CHILDREN >40 KG AND ADULTS
Penicillins				
Oxacillin			150-200 mg/kg/24 hr in 4-6 doses q4–6hr IV	4-8 g/24 hr in 4-6 doses q4–6hr IV
Nafcillin			150-200 mg/kg/24 hr in 4-6 doses q4–6hr IV	4-8 g/24 hr in 4-6 doses q4–6hr IV
Cloxacillin	50-100 mg/kg/24 hr in 4 doses	2-4 g/24 hr in 4 doses		
Dicloxacillin	25-75 mg/kg/24 hr in 4 doses	1-2 g/24 hr in 4 doses		
Cephalosporins				
Cefazolin (Ancef, Kefzol)			75-100 mg/kg/24 hr in 3 doses q8hr	4-6 g/24 hr in 3 doses
Cephalexin (Keflex)	25-100 mg/kg/24 hr in 4 doses	1-4 g/24 hr in 4 doses		
Cefadroxil (Duricef, Ultracef)	30 mg/kg/24 hr in 2 doses	1-2 g/24 hr in 2 doses		
Other Agents				
Erythromycin	35-50 mg/kg/24 hr in 4 doses	1-2 g/24 hr in 4 doses		
Clindamycin	25-40 mg/kg/24 hr in 3 doses	600-1200 mg/24 hr in 3-4 doses	30-40 mg/kg/24 hr q6–8h IV	600-3000 mg/24 hr q6–8h IV
Vancomycin			40-60 mg/kg/24 hr q6h drip over 1 hr or by continuous IV drip	1-2 g/24 hr q6h over 1 hr or by drip continuous drip
Linezolid	10 mg/kg/dose q8hr*	10 mg/kg/dose q12hr†	10 mg/kg/dose q8hr*	10 mg/kg/dose q12hr†
Doxycycline	2-4 mg/kg/24 hr 1or 2 doses for children >7 yr of age	100-200 mg/24 hr		
Trimethoprim-sulfamethoxazole	8-12 mg/kg/24 hr of TMP in 2 doses	160 mg of TMP q12hr		

*The dose for children younger than 12 years.
†The dose for children ≥ 12 years of age, not to exceed 600 mg/dose.

TABLE 80-4 **Anti-staphylococcal Therapy in Neonates with Moderate to Severe Infection ≤ 28 Postnatal Days of Age**

Drug	*Body Weight <2 kg*		*Body Weight > 2 kg*	
	<1 WEEK	1-4 WEEKS	<1 WEEK	1-4 WEEKS
Oxacillin or Nafcillin IV	25 mg/kg/dose q12hr	25 mg/kg/dose q8hr	25mg/kg/dose q8hr	25 mg/kg/dose q6hr
Linezolid IV	10 mg/kg q12hr	10 mg/kg/dose q8hr	10 mg/kg/dose q8hr	10 mg/kg/dose q8hr
Vancomycin IV	Dose based on serum creatinine*			

*Vancomycin Dose Recommendations (modified from American Academy of Pediatrics. *Red Book: 2012 Report of the Committee on Infectious Diseases*. Pickering LK, ed. 29th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2012.

Creatinine Concentration (mg/dL)†	Vancomycin Dose
<0.7	15 mg/kg q12hr
0.7-0.9	20 mg/kg q24hr
1-1.2	15 mg/kg q24hr
1.3-1.6	10 mg/kg q24hr
>1.6	15 mg/kg q48hr

†Add 0.2 mg to serum creatinine concentrations for these dosing recommendations for neonates ≤ 28 weeks' gestational age.

adequately with β-lactam antibiotics, including cephalosporins. In some locations, methicillin resistance is widespread in community isolates and thus alternative agents (see later) are indicated for empirical treatment. When a penicillin-resistant organism that is susceptible to oxacillin is isolated (MSSA), the semisynthetic penicillinase-resistant penicillins are the drugs of choice. Oxacillin and nafcillin are available for parenteral use. Dicloxacillin and cephalexin are the preferred oral agents.

If clinical response appears to be slow, nothing is to be gained by switching to another antibiotic within the same category; instead, microbiologic data should be reviewed and the dose of antibiotic rechecked to be certain it is correct, the patient is reassessed to be certain all sites requiring drainage have been addressed, and compliance with therapy is assured, especially if the antibiotics have been administered orally.

The cephalosporin antibiotics are active against penicillinase-producing staphylococci and cause less irritation of veins with intravenous infusion than do penicillinase-resistant penicillins. A potential disadvantage lies in their broader spectrum of activity, which may promote superinfection with cephalosporin-resistant, gram-negative organisms in a debilitated patient with serious staphylococcal disease. Cephalosporins have been advocated widely for use in patients allergic to penicillin, but because of considerable cross-reactivity, they should be used extremely cautiously, if at all, in patients with a clear history of serious penicillin allergy or anaphylaxis. Among this group of antibiotics, cefazolin is the agent of choice for parenteral use.[226] It is well tolerated and can be administered every 8 hours intravenously. Serum concentrations of cefazolin are higher, and effective tissue levels appear to be easier to attain. Cefazolin also is associated with less bone marrow suppression than nafcillin or oxacillin. The efficacy of the second- and third-generation cephalosporins against *S. aureus* is reduced. Therefore, these drugs, especially cefotaxime, ceftriaxone, and cefuroxime, should be given in addition to specific antistaphylococcal agents if *S. aureus* is strongly suspected. Clindamycin and trimethoprim-sulfamethoxazole are alternative agents for patients who are seriously allergic to or intolerant of β-lactam antibiotics.[12,74,147] Clindamycin is an important option for treating invasive *S. aureus* infections but should only be considered if or when blood cultures are negative and endovascular infection, such as infective endocarditis, is not a concern because clindamycin is a bacteriostatic agent. In one study using a large insurance database for assessing outcome of large numbers of children with skin and skin structure infections (not *S. aureus*–specific infections), trimethoprim-sulfamethoxazole and cephalexin were more likely than clindamycin to be associated with treatment failures and recurrences.[299]

The β-lactam resistance of MRSA is caused by the production of a novel penicillin-binding protein (PBP) designated PBP-2′, which unlike the intrinsic set of PBPs (PBP-1 to PBP-4) of *S. aureus* has remarkably reduced binding affinities to β-lactam antibiotics. Despite the presence of otherwise inhibitory concentrations of β-lactam antibiotics, MRSA can continue to synthesize cell walls solely through the uninhibited activity of PBP-2′ οr 2α.[149,191] PBP-2′ is encoded by the *mec*A gene, which is carried by a unique mobile genetic element integrated into the *S. aureus* chromosome designated SCC*mec*.[122]

Some cases of MRSA actually may be caused by infection with *S. aureus* that lacks the *mec*A gene responsible for true methicillin resistance. The mechanism of resistance in these cases may be hyperproduction of β-lactamase.[139] Frequently in these cases, methicillin resistance is borderline, with minimal inhibitory concentrations (MICs) of 8 μg/mL or less. The clinical significance of these isolates is unknown.

Management of Methicillin-Resistant *Staphylococcus aureus*

MRSA first was described within 1 year of the introduction of penicillinase-resistant penicillins.[131] Initial reports of infection appeared in England in the early 1960s and

subsequently were followed by reports from other European countries.[161,270] In the United States, only sporadic cases were observed initially[151,161] and not until 1968 was the first nosocomial outbreak described.[20] Since then, the prevalence of MRSA in the hospital setting has increased steadily. In a survey of U.S. hospitals performed in 1989, 97 percent reported the presence of MRSA.[26] The SCOPE prospective surveillance project conducted between 1995 and 2001 identified over 3400 episodes of nosocomial bacteremias in children. *S. aureus* accounted for 9 percent of isolates.[302] The proportion of *S. aureus* isolates that were MRSA increased from 10 percent in 1995 to 29 percent in 2001. Risk factors associated with infection or colonization with MRSA include recent or prolonged hospitalization, exposure to antibiotics, and stay in an intensive care unit.[177] Nosocomially acquired MRSA appears to be fully virulent, with in vitro characteristics similar to those of methicillin-susceptible staphylococci, and equivalent virulence in studies of experimental infection in mice. Clinical studies confirm comparable mortality rates.[119,281] These strains characteristically are multiresistant and usually show little or no susceptibility to cephalosporins, aminoglycosides, erythromycin, clindamycin, and tetracyclines.[5,294] The clinical efficacy of cephalosporins against methicillin-resistant strains has been poor.[294]

Epidemic outbreaks of nosocomially acquired MRSA have been reported. In these outbreaks, nasopharyngeal colonization with MRSA often occurs before infection. A high rate of nasal and hand carriage has been observed in health care workers associated with units that have MRSA outbreaks.[207] The usual approach to outbreaks has been to emphasize hand washing between examining and caring for patients. Strict isolation in a private room generally is advocated.[208] Single-room isolation may be impractical in neonatal and some pediatric intensive care units, where isolation facilities are in short supply and a single MRSA-colonized patient may occupy a room for months. Strict adherence to universal precautions (body substance isolation) with all moist body fluids and strict hand washing between seeing patients appear to be rational alternatives to "strict isolation."[183] Intranasal application of mupirocin ointment has been found to be capable of eliminating nasal and hand carriage of both colonized patients and hospital staff.[240] Interventions to control outbreaks of MRSA in intensive care or other units are multifaceted, and the reader is referred to major guidelines for recommendations.[208,262]

Occasional cases of MRSA infection apparently acquired in the community were observed sporadically in the 1970s. However, these infections were from patients who were chronically ill, and many gave a history of nursing home residence, recent admission to acute or chronic health care facilities, previous receipt of antibiotics, or intravenous drug abuse.[168,195,250,273] Hence, in these cases, infections usually were traceable to the hospital setting. By contrast, since the 1980s, cases of true CA-MRSA infection in patients without identified risk factors have appeared in the literature. The first reports in children arose from small MRSA outbreak investigations.[111] Subsequently, Rathore and Kline described three patients with deep-seated infections caused by MRSA acquired in the community.[239] In the 1990s, reports of CA-MRSA in patients without known risk factors continued to appear sporadically in the literature. However, most of the infections described occurred in adults.[105,164,205] Four pediatric deaths in Minnesota and North Dakota in the period 1997 to 1999 demonstrated the potential for severe disease from CA-MRSA infections.[89] The landmark study describing the changing epidemiology of CA-MRSA in children was published in 1998.[118] Herold and colleagues performed a retrospective review of medical records and compared the frequency of *S. aureus* isolation in hospitalized children during two time periods: between 1988 and 1990 and between 1993 and 1995. The prevalence of CA-MRSA in children without identified risk factors was 25.9 times higher in 1993 to 1995 than in 1988 to 1990.[118] After the publication of this article, several reports of CA-MRSA in children without risk factors from different parts of the United States and many regions of the world have appeared.* This explosive increase in reporting that occurred in just a few years suggests that clones of CA-MRSA have unique properties that allow rapid spread once they are introduced into the community.[143]

All reports of the clinical characteristics of CA-MRSA infection in children without risk factors document a predominance of superficial infections, including subcutaneous abscesses and cellulitis.[31,88,108,175] However, a large proportion of children with abscesses require admission to the hospital for incision and drainage under anesthesia.[146] Recurrent skin infections are also very common. Invasive disease with CA-MRSA, including severe, life-threatening infection, has been increasing and accounted for about 5 percent of cases in children seeking care in an emergency center of a large children's hospital in Houston.[6,89,100,101,146]

Unlike hospital-acquired MRSA, CA-MRSA isolates usually are susceptible to most non–β-lactam antibiotics, including clindamycin, gentamicin, trimethoprim-sulfamethoxazole, and doxycycline or minocycline, in addition to vancomycin, daptomycin, and linezolid.[142] Erythromycin susceptibility is somewhat more variable, with the proportion of susceptible isolates varying from 29 to 80 percent in various studies.[106,118,128,209] Macrolide (erythromycin, azithromycin, clarithromycin)-resistant strains remain susceptible to clindamycin if the macrolide resistance is caused by an efflux pump (MEF). Clindamycin, lincosamide, and streptogramin B resistance can be constitutive (MLS_B) or inducible ($iMLS_B$). Both mechanisms can be detected with molecular methods but can be detected reliably in the routine laboratory by the "D test" (Fig. 80-5).[75] An important note is that in vitro as well as some in vivo evidence indicates that in the presence of erythromycin resistance *S. aureus* could become resistant to clindamycin during therapy with this antibiotic.[64,194,220,292] This phenomenon, inducible macrolide-lincosamide-streptogramin B (MLS_B) resistance, which consists of modification of the target rRNA of *S. aureus*, is mediated by the presence of the resistance-conferring *erm* (erythromycin resistance methylase) gene, which

*References 33, 43, 140, 146, 235, 253, 308.

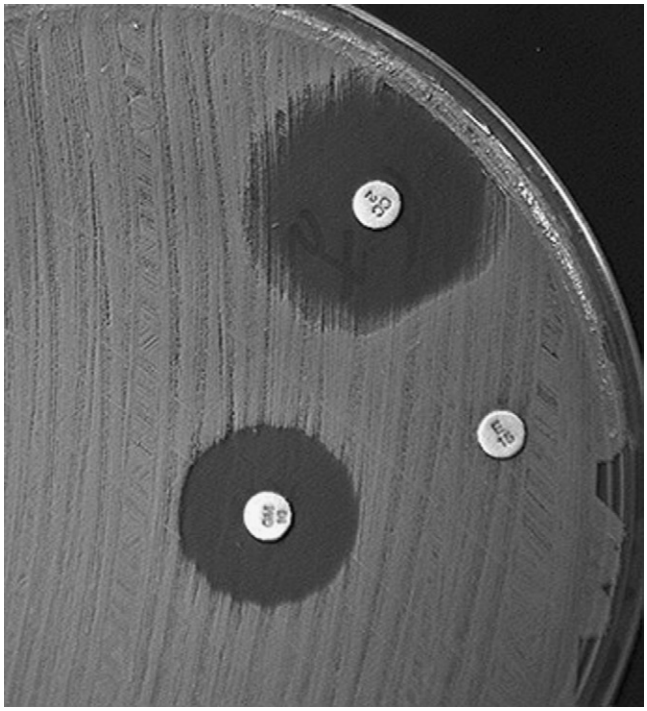

FIGURE 80-5 ■ D test.

encodes a 23S rRNA methylase.[130,165] Although 14-membered ring macrolides such as erythromycin are the most potent inducers, lincosamides such as clindamycin also can act as weaker inducers.[165] This ability may have clinical relevance in the setting of infections for which the bacteria are not eliminated quickly and may be exposed to subinhibitory concentrations of clindamycin for any amount of time. Examples include therapy for undrained deep-seated abscesses or treatment of osteoarticular infections. In patients infected with a strain having the inducible MLS_B resistance genotype and expressing the erythromycin-resistant, clindamycin-susceptible phenotype, cases of clindamycin therapeutic failure have been reported.[85,169,261] Therefore, for MRSA isolates that are erythromycin resistant and clindamycin susceptible, detection of the presence of inducible MLS_B resistance should be performed by the disk approximation method, in which clindamycin- and erythromycin-impregnated disks are set 15 to 20 mm apart over Mueller-Hinton agar containing a standard inoculum of bacteria. After a 24-hour incubation period, if the zone of inhibition around the clindamycin disk is flattened or blunted ("D shaped"; see Fig. 80-5) on the side facing the erythromycin disk, the isolate is classified as having an inducible MLS_B resistance phenotype.[165]

Vancomycin is the drug of choice for serious MRSA infections. There is no evidence that addition of an aminoglycoside or rifampin to vancomycin is beneficial for treatment of infections that do not involve prosthetic material.[223] Linezolid, an oxazolidinone antibiotic, has demonstrated significant activity against MRSA, vancomycin-resistant enterococci, and penicillin-resistant *Streptococcus pneumoniae*. Linezolid is an important option for treatment of MRSA infections due to clindamycin-resistant isolates and may be a drug of choice for treating MRSA pneumonia.[145,304] It is available in both oral and intravenous formulations. Quinupristin-dalfopristin is an intravenous streptogramin antibiotic combination that has been found to be effective in the treatment of MRSA and vancomycin-resistant *Enterococcus faecium* infections in patients intolerant of or failing previous therapy. The frequent development of phlebitis with parenteral administration through a peripheral vein mandates the use of central venous access whenever possible. Both linezolid and quinupristin-dalfopristin are expensive, so their use should be restricted to situations for which other alternatives are not feasible. Quinupristin-dalfopristin has only been studied in children in compassionate use trials, and the development of other agents has limited its use in pediatrics.[180] Daptomycin has rapidly bactericidal activity in vitro against MRSA and is an effective agent for treating MRSA infections, including bacteremia and right-sided endocarditis in adults.[28,83] Pharmacokinetic studies in children indicate a larger dose per kilogram is required to obtain the same drug exposure in adults that resulted in effective treatment of MRSA infections.[1] The safety and efficacy of daptomycin for treating *S. aureus* infections in children are being studied. Ceftaroline is a fifth-generation cephalosporin that is approved for treatment of MRSA skin and soft tissue infections in adults, and studies are being conducted to determine the pharmacokinetics, safety, and efficacy of this agent in children.[252]

For severe infections caused by MRSA, the antibiotic of choice is vancomycin. In areas where CA-MRSA has been isolated from children without identified risk factors, severe, life-threatening infections suspected to be caused by *S. aureus* should be treated empirically with both nafcillin and vancomycin because nafcillin is a more active antibiotic than is vancomycin for the treatment of methicillin-susceptible isolates.[104] Antibiotic therapy can be adjusted subsequently after antibiotic susceptibility testing results are available. Clindamycin and linezolid are options for therapy depending on the susceptibility of the isolate, the desired route of administration, and the absence of endovascular involvement. Daptomycin is approved for serious *S. aureus* infections including bacteremia in adults but is not yet approved for children.[11,83] Tigecycline is another agent most useful for polymicrobial infections including MRSA in adults but has not been studied in children.[268] The role of parenteral trimethoprim-sulfamethoxazole in treating invasive MRSA infections is unclear owing to the paucity of reports.[199] Nafcillin, oxacillin, and cefazolin are the recommended agents for treatment of severe MSSA infections.

For mild to moderate skin and soft tissue infections caused by CA-MRSA, empirical treatment may include other antibiotics such as clindamycin or trimethoprim-sulfamethoxazole. Doxycycline and minocycline are options for children older than 8 years old.[245] If more than 90 percent or so of community *S. aureus* isolates are susceptible to clindamycin, clindamycin can be used for empirical treatment of moderately invasive infections such as in children with osteomyelitis or pneumonia and empyema who do not require intensive care and in whom bacteremia is not thought to be likely. This approach is

not recommended when the clindamycin rate exceeds 10 percent of the CA-MRSA and CA-MSSA isolates combined.[55] Vancomycin or linezolid would be considerations in such circumstances.[145] Once an organism is isolated, therapy is modified based on susceptibility patterns. For moderate MSSA infections, nafcillin, oxacillin, and cefazolin are the preferred parenteral agents and dicloxacillin and cephalexin are the preferred oral agents.

The origin of CA-MRSA is not known. The absence of health care exposure in patients harboring the bacterium, the unique antibiotic susceptibility characteristics of these isolates, and distinctive pulsed-field electrophoresis patterns that are different from the patterns of hospital-acquired MRSA isolates for a given institution suggest that the origin may be the community and that these isolates were not merely transferred from the hospital setting.[3,107] The unique combination of the gene complex and the much smaller SCC*mec* size than identified in the SCC*mec* of hospital-acquired MRSA also suggests a different origin.[184] Whether the origin is the hospital or the community, the changing epidemiology of MRSA is remarkably similar to the emergence of penicillin-resistant *S. aureus* that occurred in the 1940s and 1950s.[40] Finally, the increasing rate of isolation of MRSA will mandate the use of alternative antibiotics, which in turn may promote the development of additional resistance to other antibiotic classes, the one of most concern being resistance to glycopeptide antibiotics such as vancomycin. Although vancomycin is the antibiotic of choice for severe MRSA infection, its use must remain monitored and controlled because clinical isolates with decreased susceptibility to this antibiotic—vancomycin- or glycopeptide-intermediate *S. aureus* (VISA or GISA)—have been reported.[264]

In June 2002, the first documented infection by vancomycin-resistant *S. aureus* in the United States was reported.[38] The isolate, obtained from a catheter exit site of a 40-year-old diabetic patient undergoing chronic dialysis, had high MICs for vancomycin (1024 μg/mL) and oxacillin (>16 μg/mL) but was susceptible to chloramphenicol, linezolid, minocycline, quinupristin-dalfopristin, and trimethoprim-sulfamethoxazole.[38,41] Since then, six additional isolates have been reported from Michigan, Pennsylvania, and New York.[39,279,280] All of the vancomycin-resistant *S. aureus* strains are resistant to the glycopeptides by virtue of the *van*A gene found in the enterococci. VISA strains (MIC of 4 or 8 μg/mL) do not harbor the *van*A gene and are intermediately resistant to vancomycin because of a thickened cell wall containing vancomycin-binding dipeptides.[52,112,121] Vancomycin (and other glycopeptides)-intermediate resistance is not detected by disk diffusion techniques and may not be consistently detected by automated systems.[278] In response to Clinical and Laboratory Standards Institute guidelines, microbiology laboratories have lowered the breakpoints for vancomycin susceptibility to 2 μg/mL or less, for intermediate resistance to 4 to 8 μg/mL, and for resistance to 16 μg/mL or more. In actuality, very few *S. aureus* have MICs greater than 4 μg/mL.[279]

Several studies in adults have reported worse outcomes for vancomycin treatment of MRSA infections, especially bacteremia or ventilator-associated pneumonia, when isolates have a vancomycin MIC greater than 1.0 μg/mL.[113,178,249] The relative increase in vancomycin MIC has been referred to as "vancomycin creep," but the MIC values are dependent on the method used to determine the MIC.[138] MIC results generally are higher using the E-test, but outcome of treatment may correlate better with E-test results compared with other methods.[158] As a result, dosing vancomycin such that the trough concentration remains between 15 and 20 μg/mL to achieve optimal drug exposure is recommended for adults with serious MRSA infection by experts.[175,247] However, "vancomycin creep" does not appear to be a problem in MRSA isolates recovered from children, and higher vancomycin troughs are associated with nephrotoxicity and thus the need to target vancomycin trough levels at 15 to 20 μg/mL is unclear in children.[91,133,179,190,310] Furthermore, doses of vancomycin much higher than the conventional dose (60 mg/kg/day) are often required in children to achieve this trough target. One approach would be to target this trough value for treatment of serious infections when initiating vancomycin but to reduce the dose to 60 mg/kg/day once the patient has clearly improved clinically and blood cultures are negative. Daptomycin and perhaps ceftaroline will likely become the primary options for treating serious invasive infections in children caused by MRSA strains with reduced susceptibility to vancomycin after the optimal doses and safety profiles of these agents have been established in children.

PREVENTION

Staphylococcal infections are so common that virtually everyone has had at least some minor encounters. Skin infections occur more commonly in tropical climates or during warm, humid weather in temperate areas and are likely to arise in moist areas of the body such as the axillae and skin creases. High standards of personal hygiene, careful cleaning, and adequate protection of abrasions and minor lacerations reduce the likelihood of a skin infection developing.[275] Early attention to minor infection in these small wounds with careful cleaning and antibiotic ointment may help prevent more serious or invasive infection. Even minor cutaneous infections can precede the development of more serious staphylococcal infections such as osteomyelitis, pneumonia, and endocarditis.

Person-to-person spread from an overt lesion is a major route for dissemination of infection within families, in hospitals, and in schools. A person with an infected, purulent wound should receive prompt treatment and be excused from school and from such occupations as hospital worker or food handler while the infection is open or draining. At home, special precautions should be taken in care and dressing of the wound. Disposable gauze pads should be used to wash and dry it, and towels and washcloths should not be shared with other members of the family. For children with recurrent infections or for families with multiple family members with infection,

in addition to routine hygienic measures, Fritz and associates[87] found that a 5-day regimen of twice-daily intranasal mupirocin and daily chlorhexidine body washes for everyone in the household reduced the frequency of subsequent skin and soft tissue infections in the child as well as other household members. The Infectious Diseases Society of America guidelines also include mupirocin along with chlorhexidine as part of a strategy to decolonize patients with recurrent skin and soft tissue infections.[175] Resistance to mupirocin or chlorhexidine is likely to increase as these agents are used more frequently in the community for prevention programs.[166,197] Thus, monitoring for resistance to mupirocin and chlorhexidine among *S. aureus* isolates should be more routinely performed. Dilute bleach kills *S. aureus* in vitro very quickly and has been used by dermatologists for years as part of a strategy to prevent staphylococcal skin infections.[76,144] We have found that having the patients take a bath for 15 minutes twice a week in water to which bleach (1 teaspoon/gallon of bath water) has been added decreases the frequency of recurrences, and we are evaluating this approach in a randomized trial.

To date no vaccine targeting polysaccharide or adhesion proteins has been successfully developed for the prevention of *S. aureus* infections.[54,231] One trial using immune globulin enriched for antibody to clumping factor A failed at preventing staphylococcal infections in low-birth-weight infants,[24] and a monoclonal antibody directed against clumping factor A was investigated preliminarily in adults with *S. aureus* bacteremia.[296] A polyclonal IgG preparation containing high levels of antibody to *S. aureus* capsular polysaccharides 5 and 8 has been developed by collecting plasma from healthy donors immunized with StaphVAX.[21] The pharmacokinetics and safety of this product in very-low-birth-weight infants were established in a phase 2 trial, but further development of this antibody preparation is questionable. The safety and pharmacokinetics of human chimeric monoclonal antibody directed against the lipoteichoic acid, an integral component of the cell wall of gram-positive organisms, including *S. aureus*, has been evaluated in a phase 2 study in very-low-birth-weight-infants.[24] This antibody (pagibaximab) also did not appear to be efficacious in preventing invasive *S. aureus* infections in very-low-birth-weight infants.

Perhaps taking advantage of the *S. aureus* genome with a reverse vaccinology approach will lead to a safe and efficacious vaccine.[272] But until such time, simple measures such as frequent hand washing, attention to skin breaks, and avoiding contact of obviously infected skin lesions are the most effective measures to prevent *S. aureus* infections.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdel-Rahman SM, Chandorkar G, Akins RL, et al. Single-dose pharmacokinetics and tolerability of daptomycin 8 to 10 mg/kg in children aged 2 to 6 years with suspected or proved gram-positive infections. *Pediatr Infect Dis J* 2011;**30**:712–4.
17. Banchereau R, Jordan-Villegas A, Ardura M, et al. Host immune transcriptional profiles reflect the variability in clinical disease manifestations in patients with *Staphylococcus aureus* infections. *PLoS One* 2012;**7**:e34390.
30. Brown EL, Bowden MG, Bryson RS, et al. Pediatric antibody response to community-acquired *Staphylococcus aureus* infection is directed to Panton-Valentine leukocidin. *Clin Vaccine Immunol* 2009;**16**:139–41.
31. Brown ML, O'Hara FP, Close NM, et al. Prevalence and sequence variation of panton-valentine leukocidin in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in the United States. *J Clin Microbiol* 2012;**50**:86–90.
35. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. USA300 is the predominant genotype causing *Staphylococcus aureus* septic arthritis in children. *Pediatr Infect Dis J* 2009;**28**:1076–80.
36. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
37. Carrillo-Marquez MA, Hulten KG, Mason EO, Kaplan SL. Clinical and molecular epidemiology of *Staphylococcus aureus* catheter-related bacteremia in children. *Pediatr Infect Dis J* 2010;**29**:410–4.
45. Cho JS, Pietras EM, Garcia NC, et al. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *J Clin Invest* 2010;**120**:1762–73.
50. Cremieux AC, Dumitrescu O, Lina G, et al. Panton-Valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. *PLoS One* 2009;**4**:e7204.
54. Daum RS, Spellberg B. Progress toward a *Staphylococcus aureus* vaccine. *Clin Infect Dis* 2012;**54**:560–7.
56. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus:* epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 2010;**23**:616–87.
59. Diep BA, Chan L, Tattevin P, et al. Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. *Proc Natl Acad Sci U S A* 2010;**107**:5587–92.
66. Dumont AL, Nygaard TK, Watkins RL, et al. Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. *Mol Microbiol* 2011;**79**:814–25.
67. Duong M, Markwell S, Peter J, Barenkamp S. Randomized, controlled trial of antibiotics in the management of community-acquired skin abscesses in the pediatric patient. *Ann Emerg Med* 2010;**55**:401–7.
78. Fortunov RM, Hulten KG, Allen CH, et al. Nasal *Staphylococcus aureus* colonization among mothers of term and late preterm previously healthy neonates with community-acquired *Staphylococcus aureus* infections. *Pediatr Infect Dis J* 2011;**30**:74–6.
87. Fritz SA, Hogan PG, Hayek G, et al. Household versus individual approaches to eradication of community-associated *Staphylococcus aureus* in children: a randomized trial. *Clin Infect Dis* 2012;**54**:743–51.
88. Fritz SA, Hogan PG, Hayek G, et al. *Staphylococcus aureus* colonization in children with community-associated *Staphylococcus aureus* skin infections and their household contacts. *Arch Pediatr Adolesc Med* 2012;**166**:551–7.
90. Frye AM, Baker CA, Rustvold DL, et al. Clinical impact of a real-time PCR assay for rapid identification of staphylococcal bacteremia. *J Clin Microbiol* 2012;**50**:127–33.
91. Frymoyer A, Guglielmo BJ, Wilson SD, et al. Impact of a hospitalwide increase in empiric pediatric vancomycin dosing on initial trough concentrations. *Pharmacotherapy* 2011;**31**:871–6.
113. Haque NZ, Zuniga LC, Peyrani P, et al. Relationship of vancomycin minimum inhibitory concentration to mortality in patients with methicillin-resistant *Staphylococcus aureus* hospital-acquired, ventilator-associated, or health-care-associated pneumonia. *Chest* 2010;**138**:1356–62.
116. Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev* 2011;**36**:815–36.
117. Hermos CR, Yoong P, Pier GB. High levels of antibody to Panton-Valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clin Infect Dis* 2010;**51**:1138–46.
126. Hulten KG, Kaplan SL, Lamberth LB, et al. Hospital-acquired *Staphylococcus aureus* infections at Texas Children's Hospital, 2001–2007. *Infect Control Hosp Epidemiol* 2010;**31**:183–90.

127. Hulten KG, Mason EO, Lamberth LB, et al. Increasing invasive methicillin-susceptible *Staphylococcus aureus* infections at Texas Children's Hospital, 2007–10. Presented before the 49th annual meeting of the Infectious Diseases Society of America. Boston, Mass., 2011.
129. Iverson AR, Boyd KL, McAuley JL, et al. Influenza virus primes mice for pneumonia from *Staphylococcus aureus*. *J Infect Dis* 2011;**203**:880–8.
132. Jimenez-Truque N, Tedeschi S, Saye EJ, et al. Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Pediatrics* 2012;**129**:e1252–9.
133. Jimenez-Truque N, Thomsen I, Saye E, Creech CB. Should higher vancomycin trough levels be targeted for invasive community-acquired methicillin-resistant *Staphylococcus aureus* infections in children? *Pediatr Infect Dis J* 2010;**29**:368–70.
155. Kos VN, Desjardins CA, Griggs A, et al. Comparative genomics of vancomycin-resistant *Staphylococcus aureus* strains and their positions within the clade most commonly associated with methicillin-resistant *S. aureus* hospital-acquired infection in the United States. *MBio* 2012;**3**(3). pii: e00112–12.
156. Kretschmer D, Gleske AK, Rautenberg M, et al. Human formyl peptide receptor 2 senses highly pathogenic *Staphylococcus aureus*. *Cell Host Microbe* 2010;**7**:463–73.
157. Krishna S, Miller LS. Innate and adaptive immune responses against *Staphylococcus aureus* skin infections. *Semin Immunopathol* 2012;**34**:261–80.
158. Kullar R, Davis SL, Levine DP, Rybak MJ. Impact of vancomycin exposure on outcomes in patients with methicillin-resistant *Staphylococcus aureus* bacteremia: support for consensus guidelines suggested targets. *Clin Infect Dis* 2011;**52**:975–81.
166. Lee AS, Macedo-Vinas M, Francois P, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant *Staphylococcus aureus* carriage after decolonization therapy: a case-control study. *Clin Infect Dis* 2011;**52**:1422–30.
174. Lipinska U, Hermans K, Meulemans L, et al. Panton-Valentine leukocidin does play a role in the early stage of *Staphylococcus aureus* skin infections: a rabbit model. *PLoS One* 2011;**6**:e22864.
175. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011;**52**:285–92.
178. Lodise TP, Graves J, Evans A, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. *Antimicrob Agents Chemother* 2008;**52**:3315–20.
179. Lodise TP, Patel N, Lomaestro BM, et al. Relationship between initial vancomycin concentration-time profile and nephrotoxicity among hospitalized patients. *Clin Infect Dis* 2009;**49**:507–14.
181. Loffler B, Hussain M, Grundmeier M, et al. *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathog* 2010;**6**:e1000715.
185. Maina EK, Hu DL, Tsuji T, et al. Staphylococcal enterotoxin A has potent superantigenic and emetic activities but not diarrheagenic activity. *Int J Med Microbiol* 2012;**302**:88–95.
190. Mason EO, Lamberth LB, Hammerman WA, et al. Vancomycin MICs for *Staphylococcus aureus* vary by detection method and have subtly increased in a pediatric population since 2005. *J Clin Microbiol* 2009;**47**:1628–30.
197. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin resistance in Staphylococcus aureus causing recurrent skin and soft tissue infections in children. *Antimicrob Agents Chemother* 2011; **55**:2431–3.
199. Messina AF, Namtu K, Guild M, et al. Trimethoprim-sulfamethoxazole therapy for children with acute osteomyelitis. *Pediatr Infect Dis J* 2011;**30**:1019–21.
200. Miller LG, Eells SJ, Taylor AR, et al. *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. *Clin Infect Dis* 2012;**54**:1523–35.
203. Moise PA, Forrest A, Bayer AS, et al. Factors influencing time to vancomycin-induced clearance of nonendocarditis methicillin-resistant *Staphylococcus aureus* bacteremia: role of platelet microbicidal protein killing and *agr* genotypes. *J Infect Dis* 2010;**201**:233–40.
222. Parker D, Prince A. Immunopathogenesis of *Staphylococcus aureus* pulmonary infection. *Semin Immunopathol* 2012;**34**:281–97.
223. Perlroth J, Kuo M, Tan J, et al. Adjunctive use of rifampin for the treatment of *Staphylococcus aureus* infections: a systematic review of the literature. *Arch Intern Med* 2008;**168**:805–19.
225. Peyrani P, Allen M, Wiemken TL, et al. Severity of disease and clinical outcomes in patients with hospital-acquired pneumonia due to methicillin-resistant *Staphylococcus aureus* strains not influenced by the presence of the Panton-Valentine leukocidin gene. *Clin Infect Dis* 2011;**53**:766–71.
229. Powers ME, Kim HK, Wang Y, Bubeck Wardenburg J. *ADAM10* mediates vascular injury induced by *Staphylococcus aureus* alpha-hemolysin. *J Infect Dis* 2012;**206**(3):352–6;
231. Proctor RA. Challenges for a universal *Staphylococcus aureus* vaccine. *Clin Infect Dis* 2012;**54**:1179–86.
237. Ragle BE, Bubeck Wardenburg J. Anti-alpha-hemolysin monoclonal antibodies mediate protection against *Staphylococcus aureus* pneumonia. *Infect Immun* 2009;**77**:2712–8.
238. Rasigade JP, Sicot N, Laurent F, et al. A history of Panton-Valentine leukocidin (PVL)-associated infection protects against death in PVL-associated pneumonia. *Vaccine* 2011;**29**:4185–6.
247. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am J Health Syst Pharm* 2009;**66**:82–98.
252. Saravolatz LD, Stein GE, Johnson LB. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2011;**52**:1156–63.
256. Shi D, Higuchi W, Takano T, et al. Bullous impetigo in children infected with methicillin-resistant *Staphylococcus aureus* alone or in combination with methicillin-susceptible *S. aureus:* analysis of genetic characteristics, including assessment of exfoliative toxin gene carriage. *J Clin Microbiol* 2011;**49**:1972–4.
266. Soong G, Chun J, Parker D, Prince A. *Staphylococcus aureus* activation of caspase 1/calpain signaling mediates invasion through human keratinocytes. *J Infect Dis* 2012;**205**:1571–9.
274. Sutter DE, Summers AM, Keys CE, et al. Capsular serotype of *Staphylococcus aureus* in the era of community-acquired MRSA. *FEMS Immunol Med Microbiol* 2011;**63**:16–24.
282. Tong A, Tong SY, Zhang Y, et al. Panton-Valentine leukocidin is not the primary determinant of outcome for *Staphylococcus aureus* skin infections: evaluation from the CANVAS studies. *PLoS One* 2012;**7**:e37212.
284. Uhlemann AC, Knox J, Miller M, et al. The environment as an unrecognized reservoir for community-associated methicillin resistant *Staphylococcus aureus* USA300: a case-control study. *PLoS One* 2011;**6**:e22407.
288. Ventura CL, Malachowa N, Hammer CH, et al. Identification of a novel *Staphylococcus aureus* two-component leukotoxin using cell surface proteomics. *PLoS One* 2010;**5**:e11634.
299. Williams DJ, Cooper WO, Kaltenbach LA, et al. Comparative effectiveness of antibiotic treatment strategies for pediatric skin and soft-tissue infections. *Pediatrics* 2011;**128**:e479–87.
304. Wunderink RG, Niederman MS, Kollef MH, et al. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis* 2012;**54**: 621–9.
310. Zheng X, Qi C, Arrieta M, et al. Lack of increase in vancomycin resistance of pediatric methicillin-resistant *Staphylococcus aureus* isolates from 2000 to 2007. *Pediatr Infect Dis J* 2010;**29**:882–4.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdel-Rahman SM, Chandorkar G, Akins RL, et al. Single-dose pharmacokinetics and tolerability of daptomycin 8 to 10 mg/kg in children aged 2 to 6 years with suspected or proved gram-positive infections. *Pediatr Infect Dis J* 2011;**30**:712–4.
2. Abdelnour A, Arvidson A, Bremell T, et al. The accessory gene regulator (agr) controls *Staphylococcus aureus* virulence in a murine arthritis model. *Infect Immun* 1993;**61**:3879–85.
3. Abi-Hanna P, Frank AL, Quinn JP, et al. Clonal features of community-acquired methicillin-resistant *Staphylococcus aureus* in children. *Clin Infect Dis* 2000;**30**:630–1.
4. Abramson N, Alper CA, Lachmann PJ, et al. Deficiency of C3 inactivator in man. *JImmunol* 1971;**107**:19–27.
5. Acar JF, Courvalin P, Chabbert YA. Methicillin-resistant staphylococcemia: bacteriological failure of treatment with cephalosporins. *Antimicrobial Agents Chemother (Bethesda)* 1970;**10**:280–5.
6. Adem PV, Montgomery CP, Husain AN, et al. *Staphylococcus aureus* sepsis and the Waterhouse-Friderichsen syndrome in children. *N Engl J Med* 2005;**353**:1245–51.
7. Alfaro C, Mascher-Denen M, Fergie J, Purcell K. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in patients admitted to Driscoll Children's Hospital. *Pediatr Infect Dis J* 2006;**25**:459–61.
8. Alper CA, Abramson N, Johnston Jr RB, et al. Increased susceptibility to infection associated with abnormalities of complement-mediated functions and of the third component of complement (C3). *N Engl J Med* 1970;**282**:350–4.
9. Alper CA, Colten HR, Rosen FS, et al. Homozygous deficiency of C3 in a patient with repeated infections. *Lancet* 1972;**2**: 1179–81.
10. Amagai M, Matsuyoshi N, Wang ZH, et al. Toxin in bullous impetigo and staphylococcal scalded-skin syndrome targets desmoglein 1. *Nat Med* 2000;**6**:1275–7.
11. Arbeit RD, Maki D, Tally FP, Campanaro E, Eisenstein BI. The safety and efficacy of daptomycin for the treatment of complicated skin and skin-structure infections. *Clin Infect Dis* 2004; **38**:1673–81.
12. Ardati KO, Thirumoorthi MC, Dajani AS. Intravenous trimethoprim-sulfamethoxazole in the treatment of serious infections in children. *J Pediatr* 1979;**95**:801–6.
13. Arvidson S, Tegmark K. Regulation of virulence determinants in *Staphylococcus aureus*. *Int J Med Microbiol* 2001;**291**:159–70.
14. Baba T, Takeuchi F, Kuroda M, et al. Genome and virulence determinants of high virulence community-acquired MRSA. *Lancet* 2002;**359**:1819–27.
15. Baehner RL. Neutrophil dysfunction associated with states of chronic and recurrent infection. *Pediatr Clin North Am* 1980;**27**: 377–401.
16. Baker CJ. Nosocomial septicemia and meningitis in neonates. *Am J Med* 1981;**70**:698–701.
17. Banchereau R, Jordan-Villegas A, Ardura M, et al. Host immune transcriptional profiles reflect the variability in clinical disease manifestations in patients with *Staphylococcus aureus* infections. *PLoS One* 2012;**7**:e34390.
18. Banffer JR. Anti-leucocidin and mastitis puerperalis. *BMJ* 1962; **5314**:1224–26.
19. Bannerman TL, Hancock GA, Tenover FC, Miller JM. Pulsed-field gel electrophoresis as a replacement for bacteriophage typing of *Staphylococcus aureus*. *J Clin Microbiol* 1995;**33**:551–5.
20. Barrett FF, McGehee Jr RF, Finland M. Methicillin-resistant *Staphylococcus aureus* at Boston City Hospital: bacteriologic and epidemiologic observations. *N Engl J Med* 1968;**279**:441–8.
21. Benjamin DK, Schelonka R, White R, et al. A blinded, randomized, multicenter study of an intravenous *Staphylococcus aureus* immune globulin. *J Perinatol* 2006;**26**:290–5.
22. Bhakdi S, Tranum-Jensen J. Alpha-toxin of *Staphylococcus aureus*. *Microbiol Rev* 1991;**55**:733–51.
23. Blevins JS, Elasri MO, Allmendinger SD, et al. Role of *sarA* in the pathogenesis of *Staphylococcus aureus* musculoskeletal infection. *Infect Immun* 2003;**71**:516–23.
24. Bloom B, Schelonka R, Kueser T, et al. Multicenter study to assess safety and efficacy of INH-A21, a donor-selected human staphylococcal immunoglobulin, for prevention of nosocomial infections in very low birth weight infants. *Pediatr Infect Dis J* 2005;**24**:858–66.
25. Bocchini CE, Hulten KG, Mason Jr EO, et al. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. *Pediatrics* 2006;**117**:433–40.
26. Boyce JM. Methicillin-resistant *Staphylococcus aureus:* detection, epidemiology, and control measures. *Infect Dis Clin North Am* 1989;**3**:901–13.
27. Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus:* the role of Panton-Valentine leukocidin. *Lab Invest* 2007;**87**:3–9.
28. Bradley JS. Newer antistaphylococcal agents. *Curr Opin Pediatr* 2005;**17**:71–7.
29. Bronner S, Monteil H, Prevost G. Regulation of virulence determinants in *Staphylococcus aureus:* complexity and applications. *FEMS Microbiol Rev* 2004;**28**:183–200.
30. Brown EL, Bowden MG, Bryson RS, et al. Pediatric antibody response to community-acquired *Staphylococcus aureus* infection is directed to Panton-Valentine leukocidin. *Clin Vaccine Immunol* 2009;**16**:139–41.
31. Brown ML, O'Hara FP, Close NM, et al. Prevalence and sequence variation of panton-valentine leukocidin in methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in the United States. *J Clin Microbiol* 2012;**50**:86–90.
32. Brumfitt W, Hamilton-Miller J. Methicillin-resistant *Staphylococcus aureus*. *N Engl J Med* 1989;**320**:1188–96.
33. Buckingham SC, McDougal LK, Cathey LD, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* at a Memphis, Tennessee Children's Hospital. *Pediatr Infect Dis J* 2004;**23**:619–24.
34. Burlak C, Hammer CH, Robinson MA, et al. Global analysis of community-associated methicillin-resistant *Staphylococcus aureus* exoproteins reveals molecules produced in vitro and during infection. *Cell Microbiol*, 2007;**9**(5):1172–90.
35. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. USA300 is the predominant genotype causing *Staphylococcus aureus* septic arthritis in children. *Pediatr Infect Dis J* 2009;**28**:1076–80.
36. Carrillo-Marquez MA, Hulten KG, Hammerman W, et al. *Staphylococcus aureus* pneumonia in children in the era of community-acquired methicillin-resistance at Texas Children's Hospital. *Pediatr Infect Dis J* 2011;**30**:545–50.
37. Carrillo-Marquez MA, Hulten KG, Mason EO, Kaplan SL. Clinical and molecular epidemiology of *Staphylococcus aureus* catheter-related bacteremia in children. *Pediatr Infect Dis J* 2010;**29**: 410–4.
38. Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin—United States, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:565–7.
39. Centers for Disease Control and Prevention. Vancomycin-resistant *Staphylococcus aureus*—New York, 2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:322–3.
40. Chambers HF. The changing epidemiology of *Staphylococcus aureus? Emerg Infect Dis* 2001;**7**:178–82.
41. Chang S, Sievert DM, Hageman JC, et al. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. *N Engl J Med* 2003;**348**:1342–7.
42. Chavakis T, Wiechmann K, Preissner KT, Herrmann M. *Staphylococcus aureus* interactions with the endothelium: the role of bacterial "secretable expanded repertoire adhesive molecules" (SERAM) in disturbing host defense systems. *Thromb Haemost* 2005;**94**: 278–85.
43. Chavez-Bueno S, Bozdogan B, Katz K, et al. Inducible clindamycin resistance and molecular epidemiologic trends of pediatric community-acquired methicillin-resistant *Staphylococcus aureus* in Dallas, Texas. *Antimicrob Agents Chemother* 2005;**49**:2283–8.
44. Chen AE, Carroll KC, Diener-West M, et al. Randomized controlled trial of cephalexin versus clindamycin for uncomplicated pediatric skin infections. *Pediatrics* 2011;**127**:e573–80.
45. Cho JS, Pietras EM, Garcia NC, et al. IL-17 is essential for host defense against cutaneous *Staphylococcus aureus* infection in mice. *J Clin Invest* 2010;**120**:1762–73.
46. Cockfield JD, Pathak S, Edgeworth JD, Lindsay JA. Rapid determination of hospital-acquired methicillin-resistant *Staphylococcus aureus* lineages. *J Med Microbiol* 2007;**56**:614–9.
47. Colque-Navarro P, Jacobsson G, Andersson R, et al. Levels of antibody against 11 *Staphylococcus aureus* antigens in a healthy population. *Clin Vaccine Immunol* 2010;**17**:1117–23.

48. Coovadia YM, Bhana RH, Johnson AP, et al. A laboratory-confirmed outbreak of rifampicin-methicillin resistant *Staphylococcus aureus* (RMRSA) in a newborn nursery. *J Hosp Infect* 1989; **14**:303–12.
49. Creech CB, Kernodle DS, Alsentzer A, et al. Increasing rates of nasal carriage of methicillin-resistant *Staphylococcus aureus* in healthy children. *Pediatr Infect Dis J* 2005;**24**:617–21.
50. Cremieux AC, Dumitrescu O, Lina G, et al. Panton-Valentine leukocidin enhances the severity of community-associated methicillin-resistant *Staphylococcus aureus* rabbit osteomyelitis. *PLoS One* 2009;**4**:e7204.
51. Cribier B, Prevost G, Couppie P, et al. *Staphylococcus aureus* leukocidin: a new virulence factor in cutaneous infections? An epidemiological and experimental study. *Dermatology* 1992;**185**: 175–80.
52. Cui L, Murakami H, Kuwahara-Arai K, et al. Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. *Antimicrob Agents Chemother* 2000;**44**:2276–85.
53. Dacre J, Emmerson AM, Jenner EA. Gentamicin-methicillin–resistant *Staphylococcus aureus:* epidemiology and containment of an outbreak. *J Hosp Infect* 1986;**7**:130–6.
54. Daum RS, Spellberg B. Progress toward a *Staphylococcus aureus* vaccine. *Clin Infect Dis* 2012;**54**:560–7.
55. David MZ, Crawford SE, Boyle-Vavra S, et al. Contrasting pediatric and adult methicillin-resistant *Staphylococcus aureus* isolates. *Emerg Infect Dis* 2006;**12**:631–7.
56. David MZ, Daum RS. Community-associated methicillin-resistant *Staphylococcus aureus:* epidemiology and clinical consequences of an emerging epidemic. *Clin Microbiol Rev* 2010;**23**:616–87.
57. Davis SL, Rybak MJ, Amjad M, et al. Characteristics of patients with healthcare-associated infection due to SCCmec type IV methicillin-resistant *Staphylococcus aureus*. *Infect Control Hosp Epidemiol* 2006;**27**:1025–31.
58. Diep BA, Carleton HA, Chang RF, et al. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2006;**193**:1495–503.
59. Diep BA, Chan L, Tattevin P, et al. Polymorphonuclear leukocytes mediate *Staphylococcus aureus* Panton-Valentine leukocidin-induced lung inflammation and injury. *Proc Natl Acad Sci U S A* 2010; **107**:5587–92.
60. Diep BA, Gill SR, Chang RF, et al. Complete genome sequence of USA300, an epidemic clone of community-acquired meticillin-resistant *Staphylococcus aureus*. *Lancet* 2006;**367**:731–9.
61. Diep BA, Perdreau-Remington F, Sensabaugh GF. Clonal characterization of *Staphylococcus aureus* by multilocus restriction fragment typing, a rapid screening approach for molecular epidemiology. *J Clin Microbiol* 2003;**41**:4559–64.
62. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of *Staphylococcus aureus*. *Clin Microbiol Rev* 2000;**13**:16–34, table.
63. Dohin B, Gillet Y, Kohler R, et al. Pediatric bone and joint infections caused by Panton-Valentine leukocidin-positive *Staphylococcus aureus*. *Pediatr Infect Dis J* 2007;**26**:1042–8.
64. Drinkovic D, Fuller ER, Shore KP, et al. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J Antimicrob Chemother* 2001;**48**:315–6.
65. Dumitrescu O, Boisset S, Badiou C, et al. Effect of antibiotics on *Staphylococcus aureus* producing Panton-Valentine leukocidin. *Antimicrob Agents Chemother* 2007;**51**:1515–9.
66. Dumont AL, Nygaard TK, Watkins RL, et al. Characterization of a new cytotoxin that contributes to *Staphylococcus aureus* pathogenesis. *Mol Microbiol* 2011;**79**:814–25.
67. Duong M, Markwell S, Peter J, Barenkamp S. Randomized, controlled trial of antibiotics in the management of community-acquired skin abscesses in the pediatric patient. *Ann Emerg Med* 2010;**55**:401–7.
68. Elasri MO, Thomas JR, Skinner RA, et al. *Staphylococcus aureus* collagen adhesin contributes to the pathogenesis of osteomyelitis. *Bone* 2002;**30**:275–80.
69. Elias PM, Fritsch P, Dahl MV, Wolff K. Staphylococcal toxic epidermal necrolysis: pathogenesis and studies on the subcellular site of action of exfoliatin. *J Invest Dermatol* 1975;**65**: 501–12.
70. Enright MC, Day NP, Davies CE, et al. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* 2000;**38**:1008–15.
71. Enright MC, Robinson DA, Randle G, et al. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci U S A* 2002;**99**:7687–92.
72. Etienne J. Panton-Valentine leukocidin: a marker of severity for *Staphylococcus aureus* infection? *Clin Infect Dis* 2005;**41**:591–3.
73. Eykyn SJ. Staphylococcal sepsis: the changing pattern of disease and therapy. *Lancet* 1988;**1**:100–4.
74. Feigin RD, Pickering LK, Anderson D, et al. Clindamycin treatment of osteomyelitis and septic arthritis in children. *Pediatrics* 1975;**55**:213–23.
75. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol* 2003;**41**:4740–4.
76. Fisher RG, Chain RL, Hair PS, Cunnion KM. Hypochlorite killing of community-associated methicillin-resistant *Staphylococcus aureus*. *Pediatr Infect Dis J* 2008;**27**:934–5.
77. Fitzgerald JR, Monday SR, Foster TJ, et al. Characterization of a putative pathogenicity island from bovine *Staphylococcus aureus* encoding multiple superantigens. *J Bacteriol* 2001;**183**:63–70.
78. Fortunov RM, Hulten KG, Allen CH, et al. Nasal *Staphylococcus aureus* colonization among mothers of term and late preterm previously healthy neonates with community-acquired *Staphylococcus aureus* infections. *Pediatr Infect Dis J* 2011;**30**:74–6.
79. Fortunov RM, Hulten KG, Hammerman WA, et al. Community-acquired *Staphylococcus aureus* infections in term and near-term previously healthy neonates. *Pediatrics* 2006;**118**:874–81.
80. Foster TJ. Immune evasion by staphylococci. *Nat Rev Microbiol* 2005;**3**:948–58.
81. Foster TJ, Hook M. Surface protein adhesins of *Staphylococcus aureus*. *Trends Microbiol* 1998;**6**:484–8.
82. Fournier B, Philpott DJ. Recognition of *Staphylococcus aureus* by the innate immune system. *Clin Microbiol Rev* 2005;**18**:521–40.
83. Fowler Jr VG, Boucher HW, Corey GR, et al. Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *N Engl J Med* 2006;**355**:653–65.
84. Fowler Jr VG, Sakoulas G, McIntyre LM, et al. Persistent bacteremia due to methicillin-resistant *Staphylococcus aureus* infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. *J Infect Dis* 2004;**190**:1140–9.
85. Frank AL, Marcinak JF, Mangat PD, et al. Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2002;**21**:530–4.
86. Fritz SA, Garbutt J, Elward A, et al. Prevalence of and risk factors for community-acquired methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* colonization in children seen in a practice-based research network. *Pediatrics* 2008;**121**:1090–8.
87. Fritz SA, Hogan PG, Hayek G, et al. Household versus individual approaches to eradication of community-associated *Staphylococcus aureus* in children: a randomized trial. *Clin Infect Dis* 2012;**54**: 743–51.
88. Fritz SA, Hogan PG, Hayek G, et al. *Staphylococcus aureus* colonization in children with community-associated *Staphylococcus aureus* skin infections and their household contacts. *Arch Pediatr Adolesc Med* 2012;**166**:551–7.
89. From the Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997–1999. *JAMA* 1999;**282**:1123–1125.
90. Frye AM, Baker CA, Rustvold DL, et al. Clinical impact of a real-time PCR assay for rapid identification of staphylococcal bacteremia. *J Clin Microbiol* 2012;**50**:127–33.
91. Frymoyer A, Guglielmo BJ, Wilson SD, et al. Impact of a hospitalwide increase in empiric pediatric vancomycin dosing on initial trough concentrations. *Pharmacotherapy* 2011;**31**:871–6.
92. Gauduchon V, Cozon G, Vandenesch F, et al. Neutralization of *Staphylococcus aureus* Panton-Valentine leukocidin by intravenous immunoglobulin in vitro. *J Infect Dis* 2004;**189**:346–53.
93. Gill SR, Fouts DE, Archer GL, et al. Insights on evolution of virulence and resistance from the complete genome analysis of an early methicillin-resistant *Staphylococcus aureus* strain and a biofilm-producing methicillin-resistant *Staphylococcus epidermidis* strain. *J Bacteriol* 2005;**187**:2426–38.

94. Gillaspy AF, Worrell V, Orvis J, et al. *The Staphylococcus aureus NCTC 8325 genome*. 2nd ed. Washington, D.C.: ASM Press; 2006.
95. Gillet Y, Issartel B, Vanhems P, et al. Association between *Staphylococcus aureus* strains carrying gene for Panton-Valentine leukocidin and highly lethal necrotising pneumonia in young immunocompetent patients. *Lancet* 2002;**359**:753–9.
96. Gladstone GP, Mudd S, Hochstein HD, Lenhart NA. The assay of anti-staphylococcal leucocidal components (F and S) in human serum. *Br J Exp Pathol* 1962;**43**:295–312.
97. Gluck L, Wood HF. Effect of an antiseptic skin-care regimen in reducing staphylococcal colonization in newborn infants. *N Engl J Med* 1961;**265**:1177–81.
98. Gomes AR, Vinga S, Zavolan M, de Lencastre H. Analysis of the genetic variability of virulence-related loci in epidemic clones of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2005;**49**:366–79.
99. Gomez MI, Lee A, Reddy B, et al. *Staphylococcus aureus* protein A induces airway epithelial inflammatory responses by activating TNFR1. *Nat Med* 2004;**10**:842–8.
100. Gonzalez BE, Hulten KG, Dishop MK, et al. Pulmonary manifestations in children with invasive community-acquired *Staphylococcus aureus* infection. *Clin Infect Dis* 2005;**41**:583–90.
101. Gonzalez BE, Martinez-Aguilar G, Hulten KG, et al. Severe staphylococcal sepsis in adolescents in the era of community-acquired methicillin-resistant *Staphylococcus aureus*. *Pediatrics* 2005;**115**:642–8.
102. Gonzalez BE, Rueda AM, Shelburne SA III, et al. Community-associated strains of methicillin-resistant *Staphylococccus aureus* as the cause of healthcare-associated infection. *Infect Control Hosp Epidemiol* 2006;**27**:1051–6.
103. Gonzalez BE, Teruya J, Mahoney Jr DH, et al. Venous thrombosis associated with staphylococcal osteomyelitis in children. *Pediatrics* 2006;**117**:1673–9.
104. Gonzalez C, Rubio M, Romero-Vivas J, et al. Bacteremic pneumonia due to *Staphylococcus aureus:* a comparison of disease caused by methicillin-resistant and methicillin-susceptible organisms. *Clin Infect Dis* 1999;**29**:1171–7.
105. Gosbell IB, Barbagiannakos T, Neville SA, et al. Non-multiresistant methicillin-resistant *Staphylococcus aureus* bacteraemia in Sydney, Australia: emergence of EMRSA-15, Oceania, Queensland and Western Australian MRSA strains. *Pathology* 2006;**38**:239–44.
106. Gottlieb RD, Shah MK, Perlman DC, Kimmelman CP. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in otolaryngology. *Otolaryngol Head Neck Surg* 1992; **107**:434–7.
107. Groom AV, Wolsey DH, Naimi TS, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. *JAMA* 2001;**286**:1201–5.
108. Hageman JC, Uyeki TM, Francis JS, et al. Severe community-acquired pneumonia due to *Staphylococcus aureus*, 2003–04 influenza season. *Emerg Infect Dis* 2006;**12**:894–9.
109. Haley RW, Bregman DA. The role of understaffing and overcrowding in recurrent outbreaks of staphylococcal infection in a neonatal special-care unit. *J Infect Dis* 1982;**145**:875–85.
110. Hallin M, Deplano A, Denis O, et al. Validation of pulsed-field gel electrophoresis and spa typing for long-term, nationwide epidemiological surveillance studies of *Staphylococcus aureus* infections. *J Clin Microbiol* 2007;**45**:127–33.
111. Hamoudi AC, Palmer RN, King TL. Nafcillin resistant *Staphylococcus aureus:* a possible community origin. *Infect Control* 1983;**4**: 153–7.
112. Hanaki H, Kuwahara-Arai K, Boyle-Vavra S, et al. Activated cell-wall synthesis is associated with vancomycin resistance in methicillin-resistant *Staphylococcus aureus* clinical strains Mu3 and Mu50. *J Antimicrob Chemother* 1998;**42**:199–209.
113. Haque NZ, Zuniga LC, Peyrani P, et al. Relationship of vancomycin minimum inhibitory concentration to mortality in patients with methicillin-resistant *Staphylococcus aureus* hospital-acquired, ventilator-associated, or health-care-associated pneumonia. *Chest* 2010;**138**:1356–62.
114. Hartleib J, Kohler N, Dickinson RB, et al. Protein A is the von Willebrand factor binding protein on *Staphylococcus aureus*. *Blood* 2000;**96**:2149–56.
115. Healy CM, Hulten KG, Palazzi DL, et al. Emergence of new strains of methicillin-resistant *Staphylococcus aureus* in a neonatal intensive care unit. *Clin Infect Dis* 2004;**39**:1460–6.
116. Hennekinne JA, De Buyser ML, Dragacci S. *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. *FEMS Microbiol Rev* 2011;**36**:815–36.
117. Hermos CR, Yoong P, Pier GB. High levels of antibody to Panton-Valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clin Infect Dis* 2010;**51**:1138–46.
118. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998;**279**:593–8.
119. Hershow RC, Khayr WF, Smith NL. A comparison of clinical virulence of nosocomially acquired methicillin-resistant and methicillin-sensitive *Staphylococcus aureus* infections in a university hospital. *Infect Control Hosp Epidemiol* 1992;**13**:587–93.
120. Highlander SK, Hulten KG, Qin X, et al. Subtle genetic changes enhance virulence of methicillin-resistant and sensitive *Staphylococcus aureus*. *BMC Microbiol* 2007;**7**:99.
121. Hiramatsu K, Hanaki H, Ino T, et al. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother* 1997;**40**:135–6.
122. Hiramatsu K, Katayama Y, Yuzawa H, Ito T. Molecular genetics of methicillin-resistant *Staphylococcus aureus*. *Int J Med Microbiol* 2002;**292**:67–74.
123. Hochkeppel HK, Braun DG, Vischer W, et al. Serotyping and electron microscopy studies of *Staphylococcus aureus* clinical isolates with monoclonal antibodies to capsular polysaccharide types 5 and 8. *J Clin Microbiol* 1987;**25**:526–30.
124. Holden MT, Feil EJ, Lindsay JA, et al. Complete genomes of two clinical *Staphylococcus aureus* strains: evidence for the rapid evolution of virulence and drug resistance. *Proc Natl Acad Sci U S A* 2004;**101**:9786–91.
125. Hulten KG, Kaplan SL, Gonzalez BE, et al. Three-year surveillance of community onset health care-associated *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2006;**25**:349–53.
126. Hulten KG, Kaplan SL, Lamberth LB, et al. Hospital-acquired *Staphylococcus aureus* infections at Texas Children's Hospital, 2001–2007. *Infect Control Hosp Epidemiol* 2010;**31**:183–90.
127. Hulten KG, Mason EO, Lamberth LB, et al. Increasing invasive methicillin-susceptible *Staphylococcus aureus* infections at Texas Children's Hospital, 2007–10. Presented before the 49th annual meeting of the Infectious Diseases Society of America. Boston, Mass., 2011.
128. Hussain FM, Boyle-Vavra S, Bethel CD, Daum RS. Current trends in community-acquired methicillin-resistant *Staphylococcus aureus* at a tertiary care pediatric facility. *Pediatr Infect Dis J* 2000;**19**:1163–6.
129. Iverson AR, Boyd KL, McAuley JL, et al. Influenza virus primes mice for pneumonia from *Staphylococcus aureus*. *J Infect Dis* 2011; **203**:880–8.
130. Jenssen WD, Thakker-Varia S, Dubin DT, Weinstein MP. Prevalence of macrolides-lincosamides-streptogramin B resistance and *erm* gene classes among clinical strains of staphylococci and streptococci. *Antimicrob Agents Chemother* 1987;**31**:883–8.
131. Jevons MP. "Celbenin"- resistant Staphylococci. *Br Med J* 1961;**1**:124–125.
132. Jimenez-Truque N, Tedeschi S, Saye EJ, et al. Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Pediatrics* 2012;**129**:e1252–9.
133. Jimenez-Truque N, Thomsen I, Saye E, Creech CB. Should higher vancomycin trough levels be targeted for invasive community-acquired methicillin-resistant *Staphylococcus aureus* infections in children? *Pediatr Infect Dis J* 2010;**29**:368–70.
134. Joh D, Wann ER, Kreikemeyer B, et al. Role of fibronectin-binding MSCRAMMs in bacterial adherence and entry into mammalian cells. *Matrix Biol* 1999;**18**:211–23.
135. Johanovsky J. [Importance of antileukocidin and antitoxin in immunity against staphylococcal infections.]. *Z Immunitatsforsch Allerg Klin Immunol* 1958;**116**:318–28, In German.
136. Johansson A, Flock JI, Svensson O. Collagen and fibronectin binding in experimental staphylococcal osteomyelitis. *Clin Orthop Relat Res* 2001;(382):241–6.
137. Johnson AP, Aucken HM, Cavendish S, et al. Dominance of EMRSA-15 and -16 among MRSA causing nosocomial bacteraemia in the UK: analysis of isolates from the European Antimicrobial Resistance Surveillance System (EARSS). *J Antimicrob Chemother* 2001;**48**:143–4.

138. Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. *Clin Infect Dis* 2006;**42**(Suppl. 1): S13–24.
139. Jorgensen JH. Mechanisms of methicillin resistance in *Staphylococcus aureus* and methods for laboratory detection. *Infect Control Hosp Epidemiol* 1991;**12**:14–9.
140. Jungk J, Como-Sabetti K, Stinchfield P, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* at a pediatric healthcare system, 1991–2003. *Pediatr Infect Dis J* 2007;**26**:339–44.
141. Kahl B, Herrmann M, Everding AS, et al. Persistent infection with small colony variant strains of *Staphylococcus aureus* in patients with cystic fibrosis. *J Infect Dis* 1998;**177**:1023–9.
142. Kaplan SL. Implications of methicillin-resistant *Staphylococcus aureus* as a community-acquired pathogen in pediatric patients. *Infect Dis Clin North Am* 2005;**19**:747–57.
143. Kaplan SL. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in children. *Semin Pediatr Infect Dis* 2006; **17**:113–9.
144. Kaplan SL. Commentary: prevention of recurrent staphylococcal infections. *Pediatr Infect Dis J* 2008;**27**:935–7.
145. Kaplan SL, Afghani B, Lopez P, et al. Linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2003;**22**:S178–85.
146. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
147. Kaplan SL, Mason Jr EO, Feigin RD. Clindamycin versus nafcillin or methicillin in the treatment of *Staphylococcus aureus* osteomyelitis in children. *South Med J* 1982;**75**:138–42.
148. Karakawa WW, Vann WF. Capsular polysaccharides of *Staphylococcus aureus*. *Semin Infect Dis* 1982;**4**:285–93.
149. Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2000;**44**:1549–55.
150. Kazakova SV, Hageman JC, Matava M, et al. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. *N Engl J Med* 2005;**352**:468–75.
151. Klimek JJ, Marsik FJ, Bartlett RC, et al. Clinical, epidemiologic and bacteriologic observations of an outbreak of methicillin-resistant *Staphylococcus aureus* at a large community hospital. *Am J Med* 1976;**61**:340–5.
152. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. *Clin Microbiol Rev* 1997;**10**:505–20.
153. Komatsuzawa H, Ouhara K, Yamada S, et al. Innate defences against methicillin-resistant *Staphylococcus aureus* (MRSA) infection. *J Pathol* 2006;**208**:249–60.
154. Kondo Y, Ito T, Ma XX, et al. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. *Antimicrob Agents Chemother* 2007;**51**: 264–74.
155. Kos VN, Desjardins CA, Griggs A, et al. Comparative genomics of vancomycin-resistant *Staphylococcus aureus* strains and their positions within the clade most commonly associated with methicillin-resistant *S. aureus* hospital-acquired infection in the United States. *MBio* 2012;**3**(3). pii: e00112–12.
156. Kretschmer D, Gleske AK, Rautenberg M, et al. Human formyl peptide receptor 2 senses highly pathogenic *Staphylococcus aureus*. *Cell Host Microbe* 2010;**7**:463–73.
157. Krishna S, Miller LS. Innate and adaptive immune responses against *Staphylococcus aureus* skin infections. *Semin Immunopathol* 2012;**34**:261–80.
158. Kullar R, Davis SL, Levine DP, Rybak MJ. Impact of vancomycin exposure on outcomes in patients with methicillin-resistant *Staphylococcus aureus* bacteremia: support for consensus guidelines suggested targets. *Clin Infect Dis* 2011;**52**:975–81.
159. Kuroda M, Ohta T, Uchiyama I, et al. Whole genome sequencing of methicillin-resistant *Staphylococcus aureus*. *Lancet* 2001;**357**: 1225–40.
160. Labandeira-Rey M, Couzon F, Boisset S, et al. *Staphylococcus aureus* Panton-Valentine leukocidin causes necrotizing pneumonia. *Science* 2007;**315**:1130–3.
161. Lacey RW. Genetic basis, epidemiology, and future significance of antibiotic resistance in *Staphylococcus aureus:* a review. *J Clin Pathol* 1973;**26**:899–913.
162. Lairscey R, Buck GE. Performance of four slide agglutination methods for identification of *Staphylococcus aureus* when testing methicillin-resistant staphylococci. *J Clin Microbiol* 1987;**25**: 181–2.
163. Laxdal T, Messner RP, Williams Jr RC, Quie PG. Opsonic, agglutinating, and complement-fixing antibodies in patients with subacute bacterial endocarditis. *J Lab Clin Med* 1968;**71**: 638–53.
164. Layton MC, Hierholzer Jr WJ, Patterson JE. The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. *Infect Control Hosp Epidemiol* 1995;**16**:12–7.
165. Leclercq R, Courvalin P. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. *Antimicrob Agents Chemother* 1991;**35**:1267–72.
166. Lee AS, Macedo-Vinas M, Francois P, et al. Impact of combined low-level mupirocin and genotypic chlorhexidine resistance on persistent methicillin-resistant *Staphylococcus aureus* carriage after decolonization therapy: a case-control study. *Clin Infect Dis* 2011;**52**:1422–30.
167. Lee MC, Rios AM, Aten MF, et al. Management and outcome of children with skin and soft tissue abscesses caused by community-acquired methicillin-resistant *Staphylococcus aureus*. *Pediatr Infect Dis J* 2004;**23**:123–7.
168. Levine DP, Cushing RD, Jui J, Brown WJ. Community-acquired methicillin-resistant *Staphylococcus aureus* endocarditis in the Detroit Medical Center. *Ann Intern Med* 1982;**97**:330–8.
169. Lewis JS, Jorgensen JH. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? *Clin Infect Dis* 2005;**40**:280–5.
170. Lina G, Piemont Y, Godail-Gamot F, et al. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia. *Clin Infect Dis* 1999;**29**: 1128–32.
171. Lindsay JA, Holden MT. *Staphylococcus aureus:* superbug, super genome? *Trends Microbiol* 2004;**12**:378–85.
172. Lindsay JA, Moore CE, Day NP, et al. Microarrays reveal that each of the ten dominant lineages of *Staphylococcus aureus* has a unique combination of surface-associated and regulatory genes. *J Bacteriol* 2006;**188**:669–76.
173. Lindsay JA, Ruzin A, Ross HF, et al. The gene for toxic shock toxin is carried by a family of mobile pathogenicity islands in *Staphylococcus aureus*. *Mol Microbiol* 1998;**29**:527–43.
174. Lipinska U, Hermans K, Meulemans L, et al. Panton-Valentine leukocidin does play a role in the early stage of *Staphylococcus aureus* skin infections: a rabbit model. *PLoS One* 2011;**6**:e22864.
175. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011;**52**:285–92.
176. Liu GY, Essex A, Buchanan JT, et al. *Staphylococcus aureus* golden pigment impairs neutrophil killing and promotes virulence through its antioxidant activity. *J Exp Med* 2005;**202**:209–15.
177. Locksley RM, Cohen ML, Quinn TC, et al. Multiply antibiotic-resistant *Staphylococcus aureus:* introduction, transmission, and evolution of nosocomial infection. *Ann Intern Med* 1982;**97**: 317–24.
178. Lodise TP, Graves J, Evans A, et al. Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. *Antimicrob Agents Chemother* 2008;**52**:3315–20.
179. Lodise TP, Patel N, Lomaestro BM, et al. Relationship between initial vancomycin concentration-time profile and nephrotoxicity among hospitalized patients. *Clin Infect Dis* 2009;**49**:507–14.
180. Loeffler AM, Drew RH, Perfect JR, et al. Safety and efficacy of quinupristin/dalfopristin for treatment of invasive gram-positive infections in pediatric patients. *Pediatr Infect Dis J* 2002;**21**: 950–6.
181. Loffler B, Hussain M, Grundmeier M, et al. *Staphylococcus aureus* Panton-Valentine leukocidin is a very potent cytotoxic factor for human neutrophils. *PLoS Pathog* 2010;**6**:e1000715.
182. Lopez-Aguilar C, Perez-Roth E, Mendez-Alvarez S, et al. Association between the presence of the Panton-Valentine leukocidin-encoding gene and a lower rate of survival among hospitalized

pulmonary patients with staphylococcal disease. *J Clin Microbiol* 2007;**45**:274–6.
183. Lynch P, Cummings MJ, Roberts PL, et al. Implementing and evaluating a system of generic infection precautions: body substance isolation. *Am J Infect Control* 1990;**18**:1–12.
184. Ma XX, Ito T, Tiensasitorn C, et al. Novel type of staphylococcal cassette chromosome *mec* identified in community-acquired methicillin-resistant *Staphylococcus aureus* strains. *Antimicrob Agents Chemother* 2002;**46**:1147–52.
185. Maina EK, Hu DL, Tsuji T, et al. Staphylococcal enterotoxin A has potent superantigenic and emetic activities but not diarrheagenic activity. *Int J Med Microbiol* 2012;**302**:88–95.
186. Maki DG. Nosocomial bacteremia: an epidemiologic overview. *Am J Med* 1981;**70**:719–32.
187. Maki DG, Goldman DA, Rhame FS. Infection control in intravenous therapy. *Ann Intern Med* 1973;**79**:867–87.
188. Marples RR, Wieneke AA. Enterotoxins and toxic-shock syndrome toxin-1 in non-enteric staphylococcal disease. *Epidemiol Infect* 1993;**110**:477–88.
189. Martinez-Aguilar G, Avalos-Mishaan A, Hulten K, et al. Community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* musculoskeletal infections in children. *Pediatr Infect Dis J* 2004;**23**:701–6.
190. Mason EO, Lamberth LB, Hammerman WA, et al. Vancomycin MICs for *Staphylococcus aureus* vary by detection method and have subtly increased in a pediatric population since 2005. *J Clin Microbiol* 2009;**47**:1628–30.
191. Matthews P, Tomasz A. Insertional inactivation of the *mec* gene in a transposon mutant of a methicillin-resistant clinical isolate of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1990;**34**:1777–9.
192. McDougal LK, Steward CD, Killgore GE, et al. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J Clin Microbiol* 2003;**41**:5113–20.
193. McElroy MC, Harty HR, Hosford GE, et al. Alpha-toxin damages the air-blood barrier of the lung in a rat model of *Staphylococcus aureus*-induced pneumonia. *Infect Immun* 1999;**67**:5541–4.
194. McGehee RF, Barre FF, Finland M. Resistance of *Staphylococcus aureus* to lincomycin, clinimycin, and erythromycin. *Antimicrob Agents Chemother (Bethesda)* 1968;**8**:392–7.
195. McGowan JE. The impact of changing pathogens of serious infections in hospitalized patients. *Clin Infect Dis* 2000;**31** (Suppl 4):S124–30.
196. McKinney TK, Sharma VK, Craig WA, Archer GL. Transcription of the gene mediating methicillin resistance in *Staphylococcus aureus (mecA)* is corepressed but not coinduced by cognate *mecA* and beta-lactamase regulators. *J Bacteriol* 2001;**183**:6862–8.
197. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin resistance in *Staphylococcus aureus* causing recurrent skin and soft tissue infections in children. *Antimicrob Agents Chemother* 2011;**55**:2431–3.
198. Melly MA, Thomison JB, Rogers DE. Fate of staphylococci within human leukocytes. *J Exp Med* 1960;**112**:1121–30.
199. Messina AF, Namtu K, Guild M, et al. Trimethoprim-sulfamethoxazole therapy for children with acute osteomyelitis. *Pediatr Infect Dis J* 2011;**30**:1019–21.
200. Miller LG, Eells SJ, Taylor AR, et al. *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. *Clin Infect Dis* 2012;**54**:1523–35.
201. Miller ME, Nilsson UR. A familial deficiency of the phagocytosis-enhancing activity of serum related to a dysfunction of the fifth component of complement (C5). *N Engl J Med* 1970;**282**:354–8.
202. Mishaan AM, Mason Jr EO, Martinez-Aguilar G, et al. Emergence of a predominant clone of community-acquired *Staphylococcus aureus* among children in Houston, Texas. *Pediatr Infect Dis J* 2005;**24**:201–6.
203. Moise PA, Forrest A, Bayer AS, et al. Factors influencing time to vancomycin-induced clearance of nonendocarditis methicillin-resistant *Staphylococcus aureus* bacteremia: role of platelet microbicidal protein killing and *agr* genotypes. *J Infect Dis* 2010;**201**:233–40.
204. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med* 2006;**355**:666–74.
205. Moreno F, Crisp C, Jorgensen JH, Patterson JE. Methicillin-resistant *Staphylococcus aureus* as a community organism. *Clin Infect Dis* 1995;**21**:1308–12.
206. Morinaga N, Kaihou Y, Noda M. Purification, cloning and characterization of variant LukE-LukD with strong leukocidal activity of staphylococcal bi-component leukotoxin family. *Microbiol Immunol* 2003;**47**:81–90.
207. Muder RR, Brennen C, Wagener MM, et al. Methicillin-resistant staphylococcal colonization and infection in a long-term care facility. *Ann Intern Med* 1991;**114**:107–12.
208. Muto CA, Jernigan JA, Ostrowsky BE, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and enterococcus. *Infect Control Hosp Epidemiol* 2003;**24**:362–86.
209. Naimi TS, LeDell KH, Boxrud DJ, et al. Epidemiology and clonality of community-acquired methicillin-resistant *Staphylococcus aureus* in Minnesota, 1996–1998. *Clin Infect Dis* 2001;**33**:990–6.
210. Naimi TS, LeDell KH, Como-Sabetti K, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. *JAMA* 2003;**290**:2976–84.
211. Nakamura MM, Rohling KL, Shashaty M, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* nasal carriage in the community pediatric population. *Pediatr Infect Dis J* 2002;**21**:917–22.
212. Nguyen T, Ghebrehiwet B, Peerschke EI. *Staphylococcus aureus* protein A recognizes platelet gC1qR/p33: a novel mechanism for staphylococcal interactions with platelets. *Infect Immun* 2000;**68**:2061–8.
213. Nimmo GR. USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2012;**18**(8):725–34;
214. O'Riordan K, Lee JC. *Staphylococcus aureus* capsular polysaccharides. *Clin Microbiol Rev* 2004;**17**:218–34.
215. Okoji CN, Inglis B, Stewart PR. Potential problems in the use of oligonucleotide probes for staphylococcal enterotoxin genes. *J Appl Bacteriol* 1993;**74**:637–44.
216. Okuma K, Iwakawa K, Turnidge JD, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002;**40**:4289–94.
217. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the *mec* element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002;**46**:2155–61.
218. Ong PY, Ohtake T, Brandt C, et al. Endogenous antimicrobial peptides and skin infections in atopic dermatitis. *N Engl J Med* 2002;**347**:1151–60.
219. Palmqvist N, Foster T, Fitzgerald JR, et al. Fibronectin-binding proteins and fibrinogen-binding clumping factors play distinct roles in staphylococcal arthritis and systemic inflammation. *J Infect Dis* 2005;**191**:791–8.
220. Panagea S, Perry JD, Gould FK. Should clindamycin be used as treatment of patients with infections caused by erythromycin-resistant staphylococci? *J Antimicrob Chemother* 1999;**44**:581–2.
221. Panton PN, Valentine FCO. Staphylococcal toxin. *Lancet* 1932;**222**:506–8.
222. Parker D, Prince A. Immunopathogenesis of *Staphylococcus aureus* pulmonary infection. *Semin Immunopathol* 2012;**34**:281–97.
223. Perlroth J, Kuo M, Tan J, et al. Adjunctive use of rifampin for the treatment of *Staphylococcus aureus* infections: a systematic review of the literature. *Arch Intern Med* 2008;**168**:805–19.
224. Peterson PK, Quie PG, Kim Y, et al. Recognition of *Staphylococcus aureus* by human phagocytes: signals and disguises of the bacterial surface. *Scand J Infect Dis Suppl* 1983;**41**:67–78.
225. Peyrani P, Allen M, Wiemken TL, et al. Severity of disease and clinical outcomes in patients with hospital-acquired pneumonia due to methicillin-resistant *Staphylococcus aureus* strains not influenced by the presence of the Panton-Valentine leukocidin gene. *Clin Infect Dis* 2011;**53**:766–71.
226. Pickering LK, O'Connor DM, Anderson D, et al. Comparative evaluation of cefazolin and cephalothin in children. *J Pediatr* 1974;**85**:842–7.
227. Plueckhahn VD. Hexachlorophene and the control of staphylococcal sepsis in a maternity unit in Geelong, Australia. *Pediatrics* 1973;**51**:368–82.

228. Podewils LJ, Liedtke LA, McDonald LC, et al. A national survey of severe influenza-associated complications among children and adults, 2003–2004. *Clin Infect Dis* 2005;**40**:1693–6.
229. Powers ME, Kim HK, Wang Y, Bubeck Wardenburg J. *ADAM10* mediates vascular injury induced by *Staphylococcus aureus* alpha-hemolysin. *J Infect Dis* 2012;**206**(3):352–6;
230. Prevost G, Couppie P, Prevost P, et al. Epidemiological data on *Staphylococcus aureus* strains producing synergohymenotropic toxins. *J Med Microbiol* 1995;**42**:237–45.
231. Proctor RA. Challenges for a universal *Staphylococcus aureus* vaccine. *Clin Infect Dis* 2012;**54**:1179–86.
232. Proctor RA, van Langevelde P, Kristjansson M, et al. Persistent and relapsing infections associated with small-colony variants of *Staphylococcus aureus*. *Clin Infect Dis* 1995;**20**:95–102.
233. Proctor RA, von Eiff C, Kahl BC, et al. Small colony variants: a pathogenic form of bacteria that facilitates persistent and recurrent infections. *Nat Rev Microbiol* 2006;**4**:295–305.
234. Punsalang Jr A, Migneault PC, Nolte FS. Reliability of latex agglutination tests for identification of *Staphylococcus aureus* resistant to oxacillin. *J Clin Microbiol* 1986;**24**:1104–6.
235. Purcell K, Fergie J. Epidemic of community-acquired methicillin-resistant *Staphylococcus aureus* infections: a 14-year study at Driscoll Children's Hospital. *Arch Pediatr Adolesc Med* 2005;**159**:980–5.
236. Quie PG. Chronic granulomatous disease of childhood. *Adv Pediatr* 1969;**16**:287–300.
237. Ragle BE, Bubeck Wardenburg J. Anti-alpha-hemolysin monoclonal antibodies mediate protection against *Staphylococcus aureus* pneumonia. *Infect Immun* 2009;**77**:2712–18.
238. Rasigade JP, Sicot N, Laurent F, et al. A history of Panton-Valentine leukocidin (PVL)-associated infection protects against death in PVL-associated pneumonia. *Vaccine* 2011;**29**:4185–6.
239. Rathore MH, Kline MW. Community-acquired methicillin-resistant *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 1989;**8**:645–7.
240. Reagan DR, Doebbeling BN, Pfaller MA, et al. Elimination of coincident *Staphylococcus aureus* nasal and hand carriage with intranasal application of mupirocin calcium ointment. *Ann Intern Med* 1991;**114**:101–6.
241. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2003;**47**:3926–34.
242. Robinson DA, Kearns AM, Holmes A, et al. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. *Lancet* 2005;**365**:1256–8.
243. Roche FM, Massey R, Peacock SJ, et al. Characterization of novel LPXTG-containing proteins of *Staphylococcus aureus* identified from genome sequences. *Microbiology* 2003;**149**:643–54.
244. Roghmann M, Taylor KL, Gupte A, et al. Epidemiology of capsular and surface polysaccharide in *Staphylococcus aureus* infections complicated by bacteraemia. *J Hosp Infect* 2005;**59**:27–32.
245. Ruhe JJ, Monson T, Bradsher RW, Menon A. Use of long-acting tetracyclines for methicillin-resistant *Staphylococcus aureus* infections: case series and review of the literature. *Clin Infect Dis* 2005;**40**:1429–34.
246. Ruhe JJ, Smith N, Bradsher RW, Menon A. Community-onset methicillin-resistant *Staphylococcus aureus* skin and soft-tissue infections: impact of antimicrobial therapy on outcome. *Clin Infect Dis* 2007;**44**:777–84.
247. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am J Health Syst Pharm* 2009;**66**:82–98.
248. Ryding U, Flock JI, Flock M, et al. Expression of collagen-binding protein and types 5 and 8 capsular polysaccharide in clinical isolates of *Staphylococcus aureus*. *J Infect Dis* 1997;**176**:1096–9.
249. Sakoulas G, Moise-Broder PA, Schentag J, et al. Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *J Clin Microbiol* 2004;**42**:2398–402.
250. Saravolatz LD, Markowitz N, Arking L, et al. Methicillin-resistant *Staphylococcus aureus:* epidemiologic observations during a community-acquired outbreak. *Ann Intern Med* 1982;**96**:11–16.
251. Saravolatz LD, Pohlod DJ, Arking LM. Community-acquired methicillin-resistant *Staphylococcus aureus* infections: a new source for nosocomial outbreaks. *Ann Intern Med* 1982;**97**:325–9.
252. Saravolatz LD, Stein GE, Johnson LB. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2011;**52**:1156–63.
253. Sattler CA, Mason Jr EO, Kaplan SL. Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible *Staphylococcus aureus* infection in children. *Pediatr Infect Dis J* 2002;**21**:910–17.
254. Sendi P, Rohrbach M, Graber P, et al. *Staphylococcus aureus* small colony variants in prosthetic joint infection. *Clin Infect Dis* 2006;**43**:961–7.
255. Seybold U, Kourbatova EV, Johnson JG, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. *Clin Infect Dis* 2006;**42**:647–56.
256. Shi D, Higuchi W, Takano T, et al. Bullous impetigo in children infected with methicillin-resistant *Staphylococcus aureus* alone or in combination with methicillin-susceptible *S. aureus:* analysis of genetic characteristics, including assessment of exfoliative toxin gene carriage. *J Clin Microbiol* 2011;**49**:1972–4.
257. Shinefield H, Black S, Fattom A, et al. Use of a *Staphylococcus aureus* conjugate vaccine in patients receiving hemodialysis. *N Engl J Med* 2002;**346**:491–6.
258. Shinefield HR. Use of a conjugate polysaccharide vaccine in the prevention of invasive staphylococcal disease: is an additional vaccine needed or possible? *Vaccine* 2006;**24**(Suppl 2):S2–9.
259. Shinefield HR, Ribble JC, Eichenwald HF, et al. Bacterial interference: its effect on nursery-acquired infection with *Staphylococcus aureus*, part V: an analysis and interpretation. *Am J Dis Child* 1963;**105**:683–8.
260. Shopsin B, Gomez M, Montgomery SO, et al. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* 1999;**37**:3556–63.
261. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. *Clin Infect Dis* 2003;**37**:1257–60.
262. Siegel JD, Rhinehart E, Jackson M, Chiarello L; the Healthcare Infection Control Practices Advisory Committee. Management of multidrug-resistant organisms in healthcare settings. *Am J Infect Control* 2007;**35**(10 Suppl 2):S165–93.
263. Smeltzer MS, Hart ME, Iandolo JJ. Phenotypic characterization of xpr, a global regulator of extracellular virulence factors in *Staphylococcus aureus*. *Infect Immun* 1993;**61**:919–25.
264. Smith TL, Pearson ML, Wilcox KR, et al. Emergence of vancomycin resistance in *Staphylococcus aureus.* Glycopeptide-Intermediate *Staphylococcus aureus* Working Group. *N Engl J Med* 1999;**340**:493–501.
265. Sompolinsky D, Samra Z, Karakawa WW, et al. Encapsulation and capsular types in isolates of *Staphylococcus aureus* from different sources and relationship to phage types. *J Clin Microbiol* 1985;**22**:828–34.
266. Soong G, Chun J, Parker D, Prince A. *Staphylococcus aureus* activation of caspase 1/calpain signaling mediates invasion through human keratinocytes. *J Infect Dis* 2012;**205**:1571–9.
267. Steele RW, Ashcraft EW, Payton TS, Eisenach KD. Recurrent staphylococcal infection in a pediatric residential care facility. *Am J Infect Control* 1983;**11**:217–20.
268. Stein GE, Craig WA. Tigecycline: a critical analysis. *Clin Infect Dis* 2006;**43**:518–24.
269. Stevens DL, Ma Y, Salmi DB, et al. Impact of antibiotics on expression of virulence-associated exotoxin genes in methicillin-sensitive and methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2007;**195**:202–11.
270. Stewart GT, Holt RJ. Evolution of natural resistance to the newer penicillins. *BMJ* 1963;**5326**:308–11.
271. Storch GA, Rajagopalan L. Methicillin-resistant *Staphylococcus aureus* bacteremia in children. *Pediatr Infect Dis* 1986;**5**:59–67.
272. Stranger-Jones YK, Bae T, Schneewind O. Vaccine assembly from surface proteins of *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 2006;**103**:16942–7.
273. Strausbaugh LJ, Jacobson C, Sewell DL, et al. Methicillin-resistant *Staphylococcus aureus* in extended-care facilities: experiences in a Veterans' Affairs nursing home and a review of the literature. *Infect Control Hosp Epidemiol* 1991;**12**:36–45.

274. Sutter DE, Summers AM, Keys CE, et al. Capsular serotype of *Staphylococcus aureus* in the era of community-acquired MRSA. *FEMS Immunol Med Microbiol* 2011;**63**:16–24.
275. Taplin D, Lansdell L, Allen AM, et al. Prevalence of streptococcal pyoderma in relation to climate and hygiene. *Lancet* 1973;**1**: 501–3.
276. Taylor AG, Cook J, Fincham WJ, Millard FJ. Serological tests in the differentiation of staphylococcal and tuberculous bone disease. *J Clin Pathol* 1975;**28**:284–8.
277. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**:2233–9.
278. Tenover FC, Lancaster MV, Hill BC, et al. Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *J Clin Microbiol* 1998;**36**:1020–7.
279. Tenover FC, Moellering Jr RC. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clin Infect Dis* 2007;**44**:1208–15.
280. Tenover FC, Weigel LM, Appelbaum PC, et al. Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. *Antimicrob Agents Chemother* 2004;**48**:275–80.
281. Thompson RL, Cabezudo I, Wenzel RP. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. *Ann Intern Med* 1982;**97**:309–17.
282. Tong A, Tong SY, Zhang Y, et al. Panton-Valentine leukocidin is not the primary determinant of outcome for *Staphylococcus aureus* skin infections: evaluation from the CANVAS studies. *PLoS One* 2012;**7**:e37212.
283. Tuchscherr L, Heitmann V, Hussain M, et al. *Staphylococcus aureus* small-colony variants are adapted phenotypes for intracellular persistence. *J Infect Dis* 2010;**202**:1031–40.
284. Uhlemann AC, Knox J, Miller M, et al. The environment as an unrecognized reservoir for community-associated methicillin-resistant *Staphylococcus aureus* USA300: a case-control study. *PLoS One* 2011;**6**:e22407.
285. van Hal SJ, Stark D, Lockwood B, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) detection: comparison of two molecular methods (IDI-MRSA PCR assay and GenoTypeMRSA Direct PCR assay) with three selective MRSA agars (MRSA ID, MRSASelect and CHROMagar MRSA) for infection control swabs. *J Clin Microbiol* 2007;**45**(8):2486–90.
286. Vandenesch F, Naimi T, Enright MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. *Emerg Infect Dis* 2003;**9**:978–84.
287. Vaudaux P, Kelley WL, Lew DP. *Staphylococcus aureus* small colony variants: difficult to diagnose and difficult to treat. *Clin Infect Dis* 2006;**43**:968–70.
288. Ventura CL, Malachowa N, Hammer CH, et al. Identification of a novel *Staphylococcus aureus* two-component leukotoxin using cell surface proteomics. *PLoS One* 2010;**5**:e11634.
289. Vernachio JH, Bayer AS, Ames B, et al. Human immunoglobulin G recognizing fibrinogen-binding surface proteins is protective against both *Staphylococcus aureus* and *Staphylococcus epidermidis* infections in vivo. *Antimicrob Agents Chemother* 2006;**50**:511–18.
290. Voyich JM, Braughton KR, Sturdevant DE, et al. Insights into mechanisms used by *Staphylococcus aureus* to avoid destruction by human neutrophils. *J Immunol* 2005;**175**:3907–19.
291. Voyich JM, Otto M, Mathema B, et al. Is Panton-Valentine leukocidin the major virulence determinant in community-associated methicillin-resistant *Staphylococcus aureus* disease? *J Infect Dis* 2006;**194**:1761–70.
292. Watanakunakorn C. Clindamycin therapy of *Staphylococcus aureus* endocarditis: clinical relapse and development of resistance to clindamycin, lincomycin and erythromycin. *Am J Med* 1976; **60**:419–25.
293. Watanakunakorn C. Changing epidemiology and newer aspects of infective endocarditis. *Adv Intern Med* 1977;**22**:21–47.
294. Watanakunakorn C. Treatment of infections due to methicillin-resistant *Staphylococcus aureus*. *Ann Intern Med* 1982;**97**:376–8.
295. Webster J, Faoagali JL. Endemic methicillin-resistant *Staphylococcus aureus* in a special care baby unit: a 2-year review. *J Paediatr Child Health* 1990;**26**:160–3.
296. Weems Jr JJ, Steinberg JP, Filler S, et al. Phase II, randomized, double-blind, multicenter study comparing the safety and pharmacokinetics of tefibazumab to placebo for treatment of *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother* 2006;**50**: 2751–5.
297. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *Lancet Infect Dis* 2005;**5**:751–62.
298. Williams CP, Oliver Jr TL. Nursery routines and staphylococcal colonization of the newborn. *Pediatrics* 1969;**44**:640–6.
299. Williams DJ, Cooper WO, Kaltenbach LA, et al. Comparative effectiveness of antibiotic treatment strategies for pediatric skin and soft-tissue infections. *Pediatrics* 2011;**128**:e479–87.
300. Williams Jr RC, Dossett JH, Quie PG. Comparative studies of immunoglobulin opsonins in osteomyelitis and other established infections. *Immunology* 1969;**17**:249–65.
301. Williams Jr RC, Quie PG. Opsonic activity of agammaglobulinemic human sera. *J Immunol* 1971;**106**:51–5.
302. Wisplinghoff H, Seifert H, Tallent SM, et al. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. *Pediatr Infect Dis J* 2003;**22**:686–91.
303. Wolff SM. The Chédiak-Higashi syndrome: studies of host defenses. *Ann Intern Med* 1972;**76**:293–306.
304. Wunderink RG, Niederman MS, Kollef MH, et al. Linezolid in methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia: a randomized, controlled study. *Clin Infect Dis* 2012;**54**:621–9.
305. Yamasaki O, Tristan A, Yamaguchi T, et al. Distribution of the exfoliative toxin D gene in clinical *Staphylococcus aureus* isolates in France. *Clin Microbiol Infect* 2006;**12**:585–8.
306. Yarwood JM, McCormick JK, Paustian ML, et al. Characterization and expression analysis of *Staphylococcus aureus* pathogenicity island, part 3: implications for the evolution of staphylococcal pathogenicity islands. *J Biol Chem* 2002;**277**:13138–47.
307. Zafar AB, Butler RC, Reese DJ, et al. Use of 0.3% triclosan (Bacti-Stat) to eradicate an outbreak of methicillin-resistant *Staphylococcus aureus* in a neonatal nursery. *Am J Infect Control* 1995; **23**:200–8.
308. Zaoutis TE, Toltzis P, Chu J, et al. Clinical and molecular epidemiology of community-acquired methicillin-resistant *Staphylococcus aureus* infections among children with risk factors for health care-associated infection: 2001–2003. *Pediatr Infect Dis J* 2006; **25**:343–8.
309. Zheng WF, Tan RX, Yang L, Liu ZL. Two flavones from *Artemisia giraldii* and their antimicrobial activity. *Planta Med* 1996;**62**: 160–2.
310. Zheng X, Qi C, Arrieta M, et al. Lack of increase in vancomycin resistance of pediatric methicillin-resistant *Staphylococcus aureus* isolates from 2000 to 2007. *Pediatr Infect Dis J* 2010;**29**:882–4.

CHAPTER 81

Coagulase-Negative Staphylococcal Infections

David Y. Hyun

Coagulase-negative staphylococci are some of the most common pathogens of nosocomial infections. They are also some of the most frequently isolated organisms in the microbiology laboratory, in part owing to their commensal nature with human skin and mucosal membranes. Neonates, immunocompromised patients, and patients with indwelling medical devices are at higher risk for acquiring coagulase-negative staphylococcal infections.[174,191,219,267] Diagnostic challenges remain despite advances in molecular epidemiologic tools, owing to the difficulty of accurately differentiating true infections from contaminated clinical samples. Infections are usually more indolent compared with other gram-positive cocci. Effective therapy can be difficult to achieve because of the high prevalence of resistance to multiple antibiotics. The discussion presented here is focused on the microbiology, epidemiology, pathogenesis, clinical manifestations, diagnoses, and treatment of infections caused by these ubiquitous organisms.

HISTORICAL BACKGROUND

Frederick Julius Rosenbach is credited with identifying *Staphylococcus epidermidis* in 1884, which at the time he named *S. albus* to denote the white colonies and to differentiate it from *S. aureus*, which was observed to have yellow colonies. The *S. albus* term was subsequently used as the descriptive terminology for all coagulase-negative staphylococci until the 1960s. During the 1960s, coagulase-negative staphylococci began gaining recognition as significant infectious pathogens in certain patient populations and were identified as etiologic agents of infections in neonates with sepsis and in adult patients with atrioventricular shunts and peritoneal catheters.[31,33,146,234] As a result, efforts to further differentiate and delineate species of coagulase-negative staphylococci led to the new nomenclature of *S. epidermidis* supplanting *S. albus* as well as to the identification and recognition of *S. saprophyticus* as a pathogen of the urinary tract. Descriptions and identifications of other species followed. Today, there are more than 40 known species of coagulase-negative staphylococci.

MICROBIOLOGY

Staphylococci are gram-positive, nonmotile, non–spore forming, and catalase-positive bacteria. They are members of the family Micrococcaceae along with *Micrococcus*, *Planococcus*, and *Stomatococcus*. Members of the genus *Staphylococcus* have a low DNA G + C content (30 to 39 mol%), whereas members of the genus *Micrococcus* have a G + C content within the range of 66 to 75 mol percent.[134] Staphylococci grow in irregular clusters, which are often described as "grapelike" when viewed microscopically. The cell wall is composed primarily of peptidoglycan with teichoic acid and an assortment of interspersed proteins, many of which are exposed on the surface and interact with host cells.[193] The teichoic acid has a glycerol backbone, in contrast to the ribitol of *S. aureus*.[181] Staphylococci are further divided by their ability to produce coagulase, the enzyme responsible for coagulation of rabbit plasma. *S. aureus* and *S. intermedius* are the two known coagulase-positive species. Most clinical laboratories utilize an alternative agglutination method that targets antigens specific to *S. aureus* to differentiate it from coagulase-negative staphylococci.[111] Fermentation of mannitol is another feature of *S. aureus* that differentiates it from most coagulase-negative staphylococci.

Species identification for coagulase-negative staphylococci requires assessment of a combination of features, including colony morphology, oxygen requirements, novobiocin resistance, aerobic acid production from carbohydrates, and selected liability to enzymatic activities.[132,133] Resistance to novobiocin is observed in *S. saprophyticus*, *S. cohnii*, and *S. saprophyticus*.[200] Commercial kits utilizing rapid biochemical reactions are available to identify species of coagulase-negative staphylococci. Although these kits have varying degrees of confidence and accuracy depending on the species, most systems can reliably identify species most commonly associated with human disease, such as *S. epidermidis*, *S. haemolyticus*, and *S. saprophyticus*.[203] *S. lugdunensis*, which has recently emerged as a significant pathogen in humans, can be differentiated from other coagulase-negative staphylococci by a negative tube coagulase test, a positive pyrrolidonyl arylamidase (PYR) reaction, and positive ornithine decarboxylase activity.[75]

EPIDEMIOLOGY

Coagulase-negative staphylococci are primarily isolated from human skin and mucosal membranes (Table 81-1). The number of commensal coagulase-negative staphylococci on skin can vary from 10 to 10^6 colony-forming units per square centimeter depending on the anatomic

TABLE 81-1 **Coagulase-Negative Staphylococci Commensal to Human Skin and Mucosal Membranes**

Species	Anatomic Site	Pathogenic Potential
S. epidermidis	Nares, axillae, skin	Common
S. saprophyticus	Occasionally skin	Common
S. haemolyticus	Skin of head, arms, and legs	Uncommon
S. hominis	Axillae, skin of head, arms, and legs	Uncommon
S. lugdunensis	Widely distributed on body	Uncommon
S. simulans	Occasionally skin	Uncommon
S. cohnii	Occasionally skin	Uncommon
S. warneri	Occasionally skin	Uncommon
S. saccharolyticus	Rarely skin	Rare
S. caprae	Occasionally skin	Rare
S. capitis	Skin of head, face, ears, and arms	Rare
S. auricularis	Ears	Rare
S. schleiferi	Skin	Rare
S. xylosus	Occasionally skin	Rare

Modified from Pfaller MA, Herwaldt LA. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. *Clin Microbiol Rev* 1988;**1**:281–99.

TABLE 81-2 **Methods of Epidemiologic Analysis of Coagulase-Negative Staphylococci**

Conventional	Molecular
Biotyping[103,187]	Multilocus enzyme electrophoresis[180,255,267,284]
Colony morphology[134]	Plasmid analysis[8,134,185,186,230,231,252,272]
Antibiotic susceptibility[99,100,134,145,160,168]	Chromosomal analysis[21,84,114]
Serology[1,196,281]	Polymerase chain reaction amplification[134]
Polypeptide analysis[32,34,48,64,255]	
Biofilm production[44,51]	
Phage typing[43,55,187,188,245,250]	
Mass spectrometry[77]	

location, with some species demonstrating a predilection for colonizing specific body sites.[135] Colonization can begin as early as the neonatal period. Two studies addressed the colonization of *S. epidermidis* in low-birth-weight neonates,[50,98] with both reports describing rapid colonization, in which 75 percent of neonates were colonized by 2 weeks of age. Although most infants acquire coagulase-negative staphylococci from environmental sources, including hospital personnel, a few are colonized by vertical transmission.[23,97,194]

Eighteen species have been isolated from humans. *Staphylococcus epidermidis* continues to be the predominant species identified among commensal and clinical isolates.[275] Most coagulase-negative staphylococci cause nosocomial infections frequently associated with indwelling prosthetic devices such as central venous catheters. As a result, the increase in use of these medical devices has led to a proportional increase in the rates of infections.[219] *S. saprophyticus* and *S. lugdunensis* are notable exceptions that have been associated with community-acquired urinary tract infections and native-valve endocarditis, respectively.[12,144]

Epidemiologic typing methods are used in coagulase-negative staphylococcal infections when investigating a common-source outbreak or repeated infections within an individual (Table 81-2).[180,203,267] Older phenotypic typing tools such as antibiotic susceptibility pattern analysis, biochemical reactivity, slime production detection, and serologic typing lacked the power to differentiate closely related strains and were hampered by poor sensitivities and specificities.[246,275] In contrast, molecular genotyping methods have demonstrated higher discriminatory power with pulse-field gel electrophoresis (PFGE) becoming the method of choice when investigating patterns of transmission and sources of outbreaks.[27,143,169,254,267] Random amplification of polymorphic DNA (RAPD) by polymerase chain reaction (PCR) also has been successful in identifying clonal outbreaks and transmissions. Although it is less discriminatory than is PFGE, RAPD is less costly and time consuming.[24,210] Multilocus sequence typing (MLST) can be used to delineate clonal relationships between strains and has been used to identify predominant clones from a group of clinical isolates.[154,283]

PATHOGENESIS

Risk factors for coagulase-negative staphylococcal infection are associated with nosocomial interventions that modify or bypass the host defense and immune mechanisms. They include immunosuppression,[71,126] prior antibiotic therapies,[127,235] and breakdown of mucocutaneous barriers, which is most commonly seen with the presence of prosthetic devices such as central venous catheters, cerebrospinal fluid (CSF) shunts, and peritoneal dialysis catheters.[13,20,66,149]

Biofilms (Fig. 81-1) are major virulence factors for coagulase-negative staphylococci especially in infections associated with prosthetic devices.* They are agglomerates of bacteria embedded in extracellular material and provide a mechanism of evasion against host defense and antibiotic activities.† They act as mechanical barriers to most immune cells, interfere with phagocytic activity, and limit antibiotic diffusion. Biofilm formation occurs in steps, with initial attachment followed by accumulation and detachment.

The initial attachment of *S. epidermidis* to polystyrene and other surfaces[154,155] is primarily mediated by several microbial surface components recognizing adhesive matrix molecules (MSCRAMMs).[107,112,220,256,270] MSCRAMMs facilitate binding to specific human matrix proteins,

*References 5, 43, 45, 57-59, 63, 154, 257, 265.
†References 35, 67, 87, 119, 139, 237, 238, 266, 270, 271.

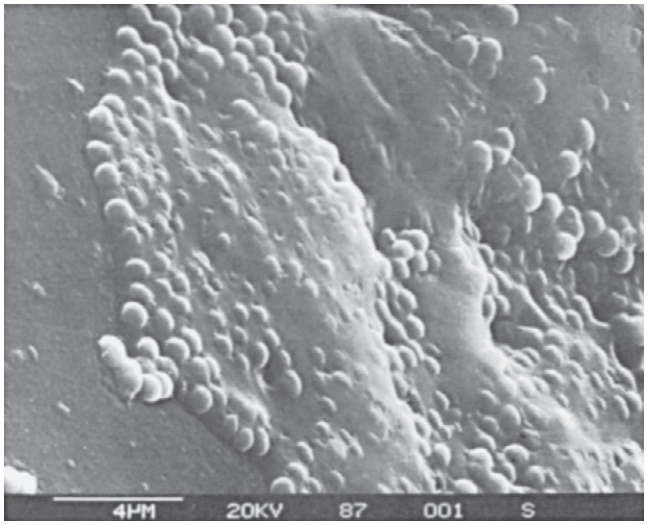

FIGURE 81-1 ■ Scanning electron microscopy of slime-producing coagulase-negative staphylococci. (From Peters G, Locci R, Pulverer G. Adherence and growth of coagulase-negative staphylococci on surfaces of intravenous catheters. *J Infect Dis* 1982;**146**: 479-82.)

including fibrinogen, fibronectin, vitronectin, and collagen. Nonspecific electrostatic and hydrophobic interactions[104,109,258] and teichoic acids[112] also play roles in adhesion. Initial adhesion is followed by intercellular aggregation and accumulation of biofilms mediated by production of polysaccharide intercellular adhesion (PIA),[106,221] which acts as the primary component for the extracellular matrix of *S. epidermidis*. In addition to its role in biofilm formation, PIA also acts as a hemaglutinin[154,218] and provides protection against phagocytosis and killing by human polymorphonuclear leukocytes.[239,271] The *ica* operon encodes enzymes necessary for PIA production.[106]

In numerous epidemiologic studies, the presence of the *ica* operon has been associated more frequently with *S. epidermidis* strains isolated from hospitalized patients with prosthetic device infections than with community strains isolated from healthy individuals. Eighty-one to 89 percent of isolates from hospitalized patients have been reported to carry the *ica* operon. In contrast, only 6 to 38 percent of community-acquired isolates were found to carry the *ica* operon.[76,137,259] However, utilizing *ica* operon detection to differentiate between invasive and commensal strains of *S. epidermidis* has proven to be unreliable. Investigations of clinical strains obtained from patients in neonatal intensive care units, bone marrow transplant units, and oncology units have shown that although both invasive and commensal strains demonstrated high carriage rates of the *ica* operon, no significant difference was found between the two groups.[28,56,197,214] Higher proportions of invasive strains demonstrated biofilm production in quantitative assays compared with commensal strains.[28,214] Most of the commensal strains were biofilm-negative despite possessing the *ica* operon, a finding suggesting that regulation of gene expression plays a large role in virulence. Clinical strains of *S. epidermidis* producing biofilms independently of PIA have also been described.[136,213,214]

Unlike *S. aureus*, which is capable of producing various cytolytic and superantigenic toxins, *S. epidermidis* produces very few toxins.[269] More recently, phenol-soluble modulins (PSMs) have been identified and characterized as a family of peptides produced by *S. epidermidis* with cytolytic and proinflammatory capacities.[41,165] *Delta* toxin is part of the PSM complex and is an enteropathogenic toxin encoded by the *hld* component of the regulatory system agr that has been linked to necrotizing enterocolitis in infants.[232] *S. epidermidis* also produces antibacterial peptides called *lantibiotics* that may play a role in bacterial interference on skin and mucous membranes and in the creation of an ecologic niche for *S. epidermidis*.[141] Numerous extracellular enzymes are also produced by coagulase-negative staphylococci; an extracellular metalloprotease with elastase activity; a cysteine protease that degrades human secretory immunoglobulin A, immunoglobulin M, serum albumin, fibrinogen, and fibronectin; and an extracellular serine protease involved in epidermin processing.[105,269,273] Two lipases have been postulated to facilitate skin colonization.[105,269]

S. saprophyticus can cause urinary tract infections in the absence of indwelling catheters.[141] Several virulence factors have been described to explain its pathogenic potential to adhere and persistently grow in the urinary tract. A surface-exposed protein with hemagglutinin and adhesive properties[83] and a surface fibrillar protein,[82] designated *ssp*, are associated with attachment to urinary tract epithelium. Urease production also has been associated with invasiveness of this organism by causing damage to bladder tissues.[80,81] The whole genome of *S. saprophyticus* has been sequenced and compared with *S. aureus* and *S. epidermidis*.[141] Analysis revealed a single, unique cell wall–anchored protein with properties of hemagglutination and adherence to human bladder cells. A high population of ionic transport systems capable of providing osmotolerance in the highly variant ionic environment of urine also was demonstrated.

CLINICAL MANIFESTATIONS

Coagulase-negative staphylococci have been implicated in a variety of clinical infections, which are mostly nosocomial in immunocompetent and immunocompromised patients (Table 81-3).[133,191,222] Because of the commensal nature of these organisms, separating true infections from contaminated culture results continues to be a challenge. This is further compounded by the variability among clinical studies when defining the criteria for clinically significant cultures.[61] Repeated isolation of the same phenotypic or genotypic strains of coagulase-negative staphylococci can facilitate interpretation. Quantitative cultures can also add value in the diagnosis of catheter-associated bacteremia by demonstrating a five- to tenfold increase in the number of colony-forming units in cultures obtained from a catheter compared with cultures from a peripheral vessel. The Committee on Infectious Diseases of the American Academy of Pediatrics suggested the following considerations to distinguish pathogenic from contaminant coagulase-negative staphylococci: (1) two or more positive blood cultures from

TABLE 81-3 **Clinically Significant Coagulase-Negative Staphylococcal Infections**

Bacteremia
Neonates
Patients with leukemia and lymphoma
Bone marrow transplant recipients
Infections in Patients with Medical Devices
Intravascular catheters
Cerebrospinal fluid shunts
Peritoneal dialysis catheter
Prosthetic valves
Other Infections
Prosthetic joints
Vascular grafts and prostheses
Hemodialysis catheter
Pacemaker
Scalp electrodes
Native Valve Endocarditis
Urinary Tract Infections
Miscellaneous Infections
Endophthalmitis
Postoperative wound infections
Osteomyelitis
Toxic shock syndrome

Modified from Patrick CC. Coagulase-negative staphylococci: pathogens with increasing clinical significance. *J Pediatr* 1990;116:497–507.

different sites, (2) a positive culture from blood and another usually sterile site with identical or nearly identical antimicrobial susceptibility patterns, (3) growth in continuously monitored blood culture system within 15 hours of incubation, (4) clinical findings of infection, (5) an intravascular catheter that has been in place for 3 days or more, and (6) similar or identical genotypes among all isolates.[4]

Clinical presentation markedly differs from that of *S. aureus* infection. In general, infections caused by coagulase-negative staphylococci are more indolent and can have a subacute or even chronic course. They occur mostly as health care–associated bacteremia and infections of prosthetic medical devices.

Bacteremia

Coagulase-negative staphylococci, particularly *S. epidermidis*, are the most common pathogens in nosocomial bloodstream infections and have become the major nosocomial pathogens in most studies.[61,212] An analysis of 6,290 nosocomial infections in 110,709 patients from 61 pediatric intensive care units in the United States during 1992 to 1997 showed that coagulase-negative staphylococci were the causative agents in 38 percent (717 of 1887) of nosocomial bloodstream infections.[212] In another large surveillance study between 1995 and 2001 among 49 hospitals in the United States, coagulase-negative staphylococci were identified in 43 percent of nosocomial bacteremias in pediatric patients.[277] Among oncologic immunocompromised pediatric patients, coagulase-negative staphylococci accounted for up to 35 percent of all positive blood culture isolates.[2,11,142] A significant majority of all coagulase-negative bacteremia occurs with the use of indwelling vascular catheters.

Neonatal Bacteremia

Coagulase-negative staphylococci are the most frequent cause of late-onset septicemia and bloodstream infections among premature infants.* These infections are found predominantly in premature infants with a gestational age of less than 34 weeks and those with low birthweights,[88,102,116,192] owing to their higher likelihood of prolonged use of a central catheter and parenteral nutrition and their immature immune system, including quantitative and qualitative neutrophil deficiencies.[36,242,277] The presence of indwelling peripheral or umbilical catheters has been implicated in approximately half of neonatal coagulase-negative staphylococcal bacteremias.[17,96,178,187] The salient features from six large studies are listed in Table 81-4.†

Clinical findings of coagulase-negative staphylococcal bacteremia are mostly indolent and subtle and include bradycardia, apnea, and temperature instability (see Table 81-4). The frequency of fulminant late-onset sepsis caused by coagulase-negative staphylococcal bacteremia is very low (1%).[125] Mortality rates associated with coagulase-negative staphylococcal infections in neonates are much lower compared with those with other pathogens.[156,249] However, neonates with prolonged and persistent bacteremia have higher mortality rates that are comparable to those of other pathogens.[39,116]

Laboratory studies need to be interpreted within clinical contexts and risk factors consistent with coagulase-negative staphylococcal bacteremia.[102,110,161,175] Leukocytosis may not be present, and there have been conflicting findings in regard to the utility of biofilm detection as a marker for infection.[96,192] Use of quantitative blood cultures correlated with clinical criteria may have a role in identifying the true pathogen.[248] The U.S. Centers for Disease Control and Prevention (CDC) defines laboratory-confirmed bloodstream infections for common skin contaminants including coagulase-negative staphylococci in patients 1 year or younger as (1) at least one finding of fever, hypotension, or chills; (2) signs, symptoms, and laboratory findings not consistent with infection at another site; and (3) isolation of an organism from two or more blood cultures drawn on separate occasions.[110]

Persistence of coagulase-negative staphylococcal bacteremia despite administration of adequate antibiotic therapy has been described in neonates.[60,130,192] Mean duration of persistent bacteremia is 10 to 14 days. This persistence was observed in patients with and without central venous catheters and in patients with normal findings on cardiac echocardiography. Neonates with

*References 17, 72, 88, 95, 96, 179, 192.
†References 72, 96, 122, 123, 172, 192.

TABLE 81-4 Clinical Characteristics of Coagulase-Negative Staphylococcal Bacteremia in Neonates

Characteristics	Munson et al., 1982[172] (n = 27)	Fleer et al., 1983[72] (n = 30)	Hall et al., 1987[96] (n = 29)	Patrick et al., 1989[192] (n = 32)	Kacica et al., 1994[122] (n = 47)	Kallman et al., 1997[123] (n = 27)
Patient						
Mean birth-weight (g)	1130	1564	1607	1172	1259	NR
Gestational age (wk)	28.9	32.1	31	28.6	29	<30
Central lines (%)	85	NR	34	19	NR	NR
TPN (%)	78	77	NR	91	81	NR
Clinical						
Apnea or bradycardia (%)	>50	100	62	78	NR	NR
Temperature instability (%)	<50	70	7	22	NR	NR
Tachycardia (%)	>50	100	NR	6	NR	NR
Mortality (%)	0	0	0	0	NR	7.4
Laboratory						
Biofilm production (%)	NR	NR	79	54	65	NR
Antibiotic susceptibility	100% S to cephalothin	100% S to cephalothin	83% S to methicillin; 100% S to vancomycin	100% S to vancomycin	48% S to oxacillin; 100% S to vancomycin	86% S to methicillin* 55% S to methicillin†

*Period: 1981-1986.
†Period: 1987-1994.
NR, not reported; S, susceptible; TPN, total parenteral nutrition.

TABLE 81-5 Clinical Characteristics of Coagulase-Negative Staphylococcal Bacteremia in Pediatric Patients with Cancer

Characteristics	Friedman et al., 1984[78] (n = 150)	Langley and Gold, 1988[142] (n = 100)	Aledo et al., 1998[2] (n = 140)	Auletta et al., 1999[11] (n = 102)
Coagulase-negative staphylococci among total bacteremias (%)	12.7	35	31.4	35
Patients with central venous catheters (%)	32	53	95	95
Mortality (%)	10.5	38	<5	19
Patient isolates susceptible to:				
Methicillin (%)	17	38	NR	NR
Vancomycin (%)	100	100	NR	NR

NR, not reported.

persistent bacteremia had a significantly higher incidence of severe thrombocytopenia requiring platelet transfusions than did those with nonpersistent bacteremia.[60,130]

Rarely, coagulase-negative staphylococcal meningitis in the absence of intraventricular devices has been reported in low-birth-weight neonates with normal CSF white blood cell counts.[93] CSF examination may be considered in neonates with coagulase-negative staphylococcal bacteremia because abnormal CSF findings or a positive CSF culture may influence the duration of therapy. Coagulase-negative staphylococci also have been implicated as etiologic agents of necrotizing enterocolitis,[92,232] although one report showed no association.[217]

Bacteremia in Immunocompromised Patients

Immunocompromised patients with leukemia or lymphoma are at risk for coagulase-negative staphylococcal bacteremia (Table 81-5).* In several reports, coagulase-negative staphylococci were the isolates most commonly

*References 2, 11, 78, 142, 204, 205, 236.

causing bacteremia in pediatric patients with cancer and accounted for 35 to 50 percent of all initial isolates.[2,11,142,195] One third of these patients did not have central venous catheters at the time of their bacteremia.[78] Risk factors for this patient population include indwelling catheters, malnutrition, and myelosuppression, especially neutropenia after chemotherapy. In addition to catheter-associated bacteremias, translocation of pathogens through inflamed mucosal surfaces secondary to chemotherapy, including the gastrointestinal tract, is a prominent mechanism of bacteremia. One study utilizing molecular epidemiologic tools, including RAPD and PFGE analysis of clinical isolates obtained from adult neutropenic oncology patients, showed that skin flora was the likely source for 75 percent of coagulase-negative staphylococcal catheter-associated infections.[180]

Coagulase-negative staphylococci have also become the primary pathogens for bacteremia among hematopoietic stem cell transplant recipients.[32,167] These episodes occur most often during periods of agranulocytosis before marrow engraftment,[32] and they can be fatal.[19] Most bacteremic episodes are due to use of central venous catheters and broad-spectrum antibiotics.

Significant morbidity from coagulase-negative staphylococcal bacteremia can be attributed to the organism's acquisition of multiple antibiotic resistance that requires the use of antimicrobial agents with a higher risk for toxicity, such as vancomycin or linezolid.* Mortality rates have varied from 0 to 11 percent.[78,142]

Indwelling Medical Devices

Intravascular Catheter-Related Infections

Coagulase-negative staphylococci are the most common pathogens for catheter-related infections.[74,166,278] Risk for infections varies among the different types of catheters. Short-term central venous catheters (CVCs), defined as those in place for fewer than 14 days, account for the majority of catheter-related infections. Peripherally inserted central catheters (PICCs) carry similar risks of infection to those of short-term CVCs when used in intensive care units.[166] However, the frequency of catheter-related infections for PICCs used for outpatient parenteral therapies appears to be significantly lower.[148,263] Long-term CVCs that are surgically implanted with a tunneled portion and a Dacron cuff just inside the skin exit site[30,108] also have lower infection rates at 0.14 per 100 catheter-days,[111] with the catheter hub being the major source of infection.[208] Finally, totally implanted vascular devices with subcutaneous ports or reservoirs carry the lowest infection risk, with rates of 0 to 0.04 per 100 catheter-days.[111,216] Infections at the catheter insertion sites also can lead to bacteremia or septic thrombophlebitis, with further complications caused by metastatic spread.[207] Overall, infectious complications involving central venous catheters have a variable reported frequency of 2.7 to 47 percent,† with coagulase-negative staphylococci identified as the pathogens in approximately half of these cases. The wide range of frequency is attributed to the intended use of the catheters, types of catheters, frequency of catheter access, characteristics of patients, and use of preventive strategies.[166]

As with other coagulase-negative staphylococcal infections, *S. epidermidis* is the predominant species associated with catheter-related infections, accounting for approximately 70 percent of cases.[20,101] This likely correlates to the high prevalence of the organisms colonizing the human skin.[135] Other implicated coagulase-negative staphylococcal species include *S. haemolyticus*, *S. warneri*, and *S. hominis*.[101]

Establishing accurate diagnoses of catheter-related coagulase-negative staphylococcal infections remains a challenge owing to the difficulty of differentiating true infections from contaminated specimens and depends on the patient's clinical status and the isolation of identical strains from repeated cultures. In addition, it is important to distinguish catheter-related bacteremia from bacteremia caused by infections at other sites. The use of quantitative blood cultures has shown that catheter-related bacteremia has a 5- to 10-fold difference in bacterial concentration in blood cultures drawn from the catheter when compared with the concentration in peripheral cultures.[73,211] When a catheter is removed because of a suggested catheter-related infection, a semiquantitative culture of the distal 5 cm of the catheter is recommended.[166] This can be accomplished by rolling the distal portion of the catheter on a culture plate. Growth of more than 15 colony-forming units is indicative of catheter colonization.[157]

Duration of antimicrobial therapy for uncomplicated coagulase-negative staphylococcal catheter-related infections depends on whether the catheter is removed or retained.[166] When the catheter is no longer needed, it should be removed and the patient should be given 5 to 7 days of antimicrobial therapy. If the catheter is retained, the duration of antimicrobial therapy should be 10 to 14 days and accompanied by antibiotic lock therapy. Therapy with retention of a catheter carries a higher risk for recurrence.[209] Complicated catheter-related infections, including those with tunnel infections, port abscesses, septic thrombosis, endocarditis, and osteomyelitis, are indications for catheter removal. *S. lugdunensis* infections should be treated more conservatively in a manner similar to *S. aureus* infections owing to the higher virulence of this organism compared with other coagulase-negative staphylococci.

Central Nervous System Shunts

Coagulase-negative staphylococci are the predominant pathogens for infections of central nervous system shunts that are used to relieve hydrocephalus by diverting CSF infections.[129] They account for 60 to 75 percent of all bacterial causes of shunt infections.[129,176,240] Ventriculoperitoneal shunts that divert CSF into the peritoneal cavity have primarily been used since the 1960s because of their lower rate of mechanical complications.[129,173,182] Ventriculoatrial shunts that divert CSF into the right atrium are used infrequently because of higher

*References 78, 126, 142, 152, 153, 206, 229.
†References 20, 85, 101, 151, 170, 244, 268.

complication rates.[182] However, the incidences of infection involving both ventriculoperitoneal and the ventriculoatrial shunts are comparable.[129,182,240] Infection rates are higher in neonates.

Shunt infections can begin at the insertion site by contamination of the catheter from the patient's skin flora[240] or during subsequent revisions. Short-term infection rates are reported to be 13 percent, and 10-year infection rates are 27 percent.[215] Seventy percent of ventriculoperitoneal shunt infections occur within the first 2 months after placement.[176]

CSF examination can be helpful in patients with signs and symptoms consistent with a shunt infection. Gram stain of ventricular fluid is often negative, but culture is sensitive. Subtle changes are noted in CSF and ventricular fluid cell counts or cytochemical findings.[280] Patients with ventriculoatrial shunts often have signs and symptoms compatible with septicemia. In patients with ventriculoperitoneal shunts, an intra-abdominal cyst may develop at the distal end of the catheter. Dysfunctions of the shunt secondary to development of infections often lead to signs and symptoms consistent with increased intracranial pressure. Standard therapy for ventriculoperitoneal shunt infections has been removal of the shunt system and administration of systemic antibiotics.[240,280,282]

Peritoneal Dialysis Catheters

S. epidermidis is the bacterial pathogen most commonly isolated in peritoneal dialysis catheter infections,[91,201] accounting for up to 50 percent of infections.[201] These include catheter exit site infections as well as peritonitis.[201] The prevalence of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis is approximately 60 percent. Signs of peritonitis include fever, abdominal pain, and cloudy peritoneal dialysis fluid. Removal of the catheter usually is not necessary for successful therapy but may be required in refractory cases or when the catheter malfunctions. Antibiotics can be administered systemically as well as intraperitoneally through the dialysis fluid.[69]

Prosthetic Valves

Coagulase-negative staphylococci are frequent pathogens causing prosthetic valve endocarditis, accounting for approximately 17 percent of contemporary cases.[52,274] This is in contrast to native valve endocarditis for which coagulase-negative staphylococci are rarely isolated. Although many cases of coagulase-negative staphylococcal prosthetic valve endocarditis occur within 60 days of device implantation,[147] they can also occur as late as 1 year after surgery, with inoculation of the organism at the time of surgery followed by abscess formation of the valvular ring being the likely mechanism.[27] Signs and symptoms can vary, but fever is the most common finding. Typical findings of endocarditis caused by other pathogens such as peripheral emboli can be mostly absent in coagulase-negative staphylococcal endocarditis, owing to the organism's indolent nature. Anemia is the abnormality identified most often by laboratory testing. Evaluation should include multiple blood cultures and echocardiography. The antimicrobial regimen for prosthetic valve endocarditis caused by coagulase-negative staphylococci usually involves vancomycin and rifampin for a minimum of 6 weeks along with gentamicin for the first 2 weeks because the majority of organisms are methicillin resistant.[14] Surgery may be indicated when infection is complicated by perivalvular or myocardial abscesses.

Other Indwelling Medical Devices

Coagulase-negative staphylococci are involved in an expanded spectrum of infections involving indwelling medical devices.[57,89] They include infections of prosthetic joints,[70,124] vascular grafts,[15,62] hemodialysis shunts,[189,233,234] and pacemaker pockets.[42,115,158,199] Additionally, osteomyelitis secondary to *S. epidermidis* has been reported to occur after hemodialysis and in neonates after the use of a monitoring scalp electrode.[183]

Native Valve Endocarditis

Native valve endocarditis along with *S. saprophyticus* urinary tract infection are the only non-nosocomial infections caused by coagulase-negative staphylococci.[118] Approximately 7 percent of cases of native valve endocarditis are caused by coagulase-negative staphylococci, and *S. epidermidis* is the most frequently isolated species.[46] These infections usually are subacute and arise from transient bacteremia. Pathogenesis is thought to involve seeding of a previously damaged valve or endocarditis that had not been previously identified.[6,18]

S. lugdunensis can cause acute, severe, and destructive endocarditis similar to *S. aureus* endocarditis,[261] with most favorable outcomes occurring in patients who undergo valve replacement.[53] Most patients with *S. lugdunensis* endocarditis have been older than 50 years of age. Mortality rates are higher compared with infections by other species.[12] Native aortic or mitral valves are involved most frequently.[260] The perineum appears to have a high colonization rate of *S. lugdunensis.*[120]

Surgical Site Infections

Coagulase-negative staphylococci are the second most common cause of postoperative surgical site infections, according to National Nosocomial Infections Surveillance Report survey data.[38] Most infections are likely caused by the patient's own endogenous skin flora, but outbreaks originating from operating room personnel have been reported.[27] Outbreaks of *S. epidermidis* surgical site infections, such as mediastinitis and endocarditis, among patients who have undergone cardiac valve replacements also have been reported.[26,210]

Urinary Tract Infections

S. saprophyticus is the most common coagulase-negative staphylococcal species that causes infections in both the upper and the lower urinary tracts.[144] These infections occur predominantly in young, healthy, sexually active women,[121,144,161] during late summer and fall, with a

pattern similar to that of sexually transmitted diseases.[80] Recent sexual intercourse, outdoor swimming, and occupational meat processing have been identified as risk factors.[80] *S. epidermidis* and other coagulase-negative staphylococci rarely cause urinary tract infections, but they have been noted to produce disease in older adults with urinary tract complications.[94,150,177] McDonald and Lohr[163] and Hall and Snitzer[95] described healthy children with pyelonephritis caused by *S. epidermidis*.

Miscellaneous Infections

Endophthalmitis secondary to *S. epidermidis* has been reported in patients undergoing ocular surgery or trauma.[16,29,202] One center reported coagulase-negative staphylococci as being the most common organisms isolated from patients with culture-positive endophthalmitis over a 20-year period, accounting for 38 percent of the cases.[40] Postoperative mediastinitis that develops after median sternotomy for open heart surgery can be caused by *S. epidermidis*.[25,65,90] Primary osteomyelitis secondary to coagulase-negative staphylococci is a rare occurrence in healthy children.[184] *S. caprae*[241] and *S. lugdunensis*[227] have been linked to bone and joint infections. Coagulase-negative staphylococci also have been implicated as a possible cause of toxic shock syndrome.[49]

TREATMENT

Vancomycin is the drug of choice for most coagulase-negative staphylococcal infections, owing to the high prevalence of methicillin resistance especially among nosocomial strains.[3,224,243] When susceptibility data are not available, vancomycin should be used for empirical therapy. If the coagulase-negative staphylococcus is known to be susceptible to methicillin, oxacillin or nafcillin is the preferred therapeutic agent.[203,225]

Most coagulase-negative staphylococci, particularly *S. epidermidis* and *S. haemolyticus*, are resistant to multiple antibiotics, including methicillin.* Cross-resistance exists between penicillinase-resistant penicillins and cephalosporins. Therefore, methicillin-resistant coagulase-negative staphylococci should be considered as cephalosporin resistant even if routine susceptibility testing indicates susceptibility to cephalosporins.[91,117,145,224] Methicillin-resistant coagulase-negative staphylococci are often resistant to other antibiotics, such as clindamycin, erythromycin, and gentamicin.[7] The *mecA* gene is responsible for methicillin-resistance because it encodes a penicillin-binding protein (PBP2a) that interferes with the binding of β-lactams to the bacterial cell wall.[105,113] The same mechanism accounts for methicillin resistance in *S. aureus*. Despite the understanding of the mechanism, consistent detection of methicillin resistance for coagulase-negative staphylococci can be a challenging process in the clinical laboratory owing to heterogeneous expression of the *mecA* phenotype in minimal inhibitory concentration or disk diffusion testing with only a minority of the bacterial population expressing the resistant phenotype.[7,113,224,279] To facilitate more reliable detection, the Clinical and Laboratory Standards Institute revised specific breakpoints for coagulase-negative staphylococci in 2007 (Table 81-6).[47] In addition, to enhance phenotypic expression of methicillin resistance, the use of lower incubation temperatures of 30° to 35° C for prolonged periods and of increased sodium chloride content in culture medium is recommended.[159,164,223] Molecular detection of the *mecA* gene is an accurate method to predict methicillin resistance.[113]

TABLE 81-6 **Breakpoints for Antibiotic Susceptibility for Coagulase-Negative Staphylococci**

	Oxacillin-Susceptible	Oxacillin-Intermediate	Oxacillin-Resistant
MIC			
CoNS	≤0.25 μg/mL		≥0.5 μg/mL
S. aureus	≤2 μg/mL		≥4 μg/mL
Zone Size			
CoNS	≥18 mm	No intermediate zone	≤17 mm
S. aureus	≥13 mm	11-12 mm	≤10 mm

CoNS, coagulase-negative staphylococci; MIC, minimal inhibitory concentration.
Data from Clinical and Laboratory Standards Institute. Performance testing for antimicrobial susceptibility testing, 17th informational supplement M100-S17, 2007.

Coagulase-negative staphylococci were the first organisms in which glycopeptide resistance was recognized in 1986.[235] A prolonged course of vancomycin is a well-recognized risk factor for the emergence of strains with decreased susceptibility to glycopeptides.[22,279] Although they are uncommon occurrences, infections caused by these strains have been described and may become more common. To prevent or delay development of resistance, the CDC published recommendations for the prudent use of vancomycin.[37] Decreased susceptibility occurs more frequently to teicoplanin than to vancomycin.[9,140,276] The minimal inhibitory concentrations of teicoplanin usually fall over a wide range, whereas vancomycin minimal inhibitory concentrations tend to remain more stable over a narrower range within the limits of susceptibility.[22] Intermediate resistance to vancomycin in coagulase-negative staphylococci has been rarely described.[228,235,264] Thickened cell walls, as seen by electron microscopy and altered cell metabolism that affects vancomycin binding, may be associated with glycopeptide resistance mechanisms.[105]

Gentamicin and rifampin are active against coagulase-negative staphylococci, but rapid emergence of resistance has limited their use as single-drug regimens.[43,162,225] However, these two antibiotics have been shown to be synergistic with vancomycin against methicillin-resistant, coagulase-negative staphylococci.[152] Additionally, rifampin

*References 7, 9, 43, 54, 68, 86, 235, 247, 276.

has been used in neonates with persistent bacteremia to eradicate the organisms.[251,262] Combination therapies utilizing gentamicin or rifampin are frequently used for prosthetic device infections including endocarditis and CSF shunt infections.

Quinupristin-dalfopristin,[10] linezolid,[222] daptomycin,[138,226] and tigecycline[79,138] have all demonstrated significant in vitro activities against coagulase-negative staphylococci and may have a role in the treatment of multidrug-resistant strains. However, there is a paucity of clinical efficacy data, especially compared with the number of studies available for the treatment of methicillin-resistant *S. aureus*. Linezolid resistance in coagulase-negative staphylococci has been described and linked to increased utilization.[128,171]

PREVENTION

Preventing the development of coagulase-negative staphylococcal infection is difficult because of the ubiquitous and commensal nature of these organisms. A 3-year molecular analysis of coagulase-negative staphylococcal nosocomial infections in a neonatal intensive care unit showed that many of the infections were caused by clonal dissemination and potentially were preventable by hand washing, thus reducing the transmission rate from staff to patient or from patient to patient.[267]

Developing catheters that are both inert and resistant to bacterial colonization adherence has been only marginally successful.[131,190,198] The technique of impregnating the surface of catheters with antibiotics or disinfectants appears promising.[253] Prophylactic antibiotics are administered routinely for surgical procedures involving placement of a prosthetic device or material. However, the antimicrobial of choice to prevent infections caused by coagulase-negative staphylococci varies among institutions.[3] Vancomycin often is recommended for placement of a CSF shunt or prosthetic valve.

Infection control policies such as standardizing or "bundling" procedures for aseptic intravascular catheter placement and care is the single most important factor in preventing catheter-associated infections. Standard precautions should be used for methicillin-resistant, coagulase-negative staphylococci. For vancomycin-intermediate, coagulase-negative staphylococci, contact transmission precautions for multidrug-resistant organisms should be used.[4]

CONCLUSION

Coagulase-negative staphylococci, particularly *S. epidermidis*, are a major source of nosocomial infection in a variety of clinical situations. Most infections occur in patients who have indwelling medical devices. Epidemiologic studies have been limited by the commensal nature of the organisms, but newer molecular tools may help investigators to gain further insight and to differentiate true infections from contaminants. Therapy can be complex because of the usual presence of an indwelling medical device and the multiple antimicrobial resistances of the organisms.

NEW REFERENCES SINCE THE SIXTH EDITION

12. Babu E, Oropello J. *Staphylococcus lugdunensis*: the coagulase-negative staphylococcus you don't want to ignore. *Expert Rev Anti Infect Ther* 2011;**9**:901–7.
23. Bjorkqvist M, Liljedahl M, Zimmermann J, et al. Colonization pattern of coagulase-negative staphylococci in preterm neonates and the relation to bacteremia. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1085–93.
41. Cheung GY, Rigby K, Wang R, et al. *Staphylococcus epidermidis* strategies to avoid killing by human neutrophils. *PLoS Pathog* 2010;**6**:e1001133.
59. Dickinson GM, Bisno AL. Infections associated with prosthetic devices: clinical considerations. *Int J Artif Organs* 1993;**16**: 749–54.
60. Dimitriou G, Fouzas S, Giormeszis N, et al. Clinical and microbiological profile of persistent coagulase-negative staphylococcal bacteraemia. *Clin Microbiol Infect* 2011;**17**:1684–90.
74. Fontela PS, Platt RW, Rocher I, et al. Epidemiology of central line–associated bloodstream infections in Quebec intensive care units: a 6-year review. *Am J Infect Control* 2012;**40**:221–6.
75. Frank KL, Del Pozo JL, Patel R. From clinical microbiology to infection pathogenesis: how daring to be different works for *Staphylococcus lugdunensis*. *Clin Microbiol Rev* 2008;**21**:111–33.
116. Jean-Baptiste N, Benjamin DK, Cohen-Wolkowiez M, et al. Coagulase-negative staphylococcal infections in the neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:679–86.
128. Kelly S, Collins J, Maguire M, et al. An outbreak of colonization with linezolid-resistant *Staphylococcus epidermidis* in an intensive therapy unit. *J Antimicrob Chemother* 2008;**61**:901.
140. Kristof K, Kocsis E, Szabo D, et al. Significance of methicillin-teicoplanin–resistant *Staphylococcus haemolyticus* in bloodstream infections in patients of the Semmelweis University hospitals in Hungary. *Eur J Clin Microbiol Infect Dis* 2011;**30**:691–9.
148. Levy I, Bendet M, Samra Z, et al. Infectious complications of peripherally inserted central venous catheters in children. *Pediatr Infect Dis J* 2010;**29**:426–9.
166. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.
171. Mulanovich VE, Huband MD, McCurdy SP, et al. Emergence of linezolid-resistant coagulase-negative *Staphylococcus* in a cancer centre linked to increased linezolid utilization. *J Antimicrob Chemother* 2010;**65**:2001–4.
209. Raad I, Kassar R, Ghannam D, et al. Management of the catheter in documented catheter-related coagulase-negative staphylococcal bacteremia: remove or retain? *Clin Infect Dis* 2009;**49**:1187–94.
213. Rohde H, Burandt EC, Siemssen N, et al. Polysaccharide intercellular adhesion or protein factors in biofilm accumulation of *Staphylococcus epidermidis* and *Staphylococcus aureus* isolated from prosthetic hip and knee joint infections. *Biomaterials* 2007;**28**: 1711–20.
249. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 2001;**110**:285–91.
262. van der Lugt NM, Steggerda SJ, Walther FJ. Use of rifampin in persistent coagulase-negative staphylococcal bacteremia in neonates. *BMC Pediatr* 2010;**10**:84.
263. Van Winkle P, Whiffen T, Liu IL. Experience using peripherally inserted central venous catheters for outpatient parenteral antibiotic therapy in children at a community hospital. *Pediatr Infect Dis J* 2008;**27**:1069–72.
275. Widerstrom M, Wistrom J, Sjostedt A, Monsen T. Coagulase-negative staphylococci: update on the molecular epidemiology and clinical presentation with a focus on *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. *Eur J Clin Microbiol Infect Dis* 2012;**31**:7–20.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aasen J, Oeding P. Antigenic studies on *Staphylococcus epidermidis*. *Acta Pathol Microbiol Scand* 1972;**79**:827–34.
2. Aledo A, Hellr G, Ren L, et al. Septicemia and septic shock in pediatric patients: 140 consecutive cases on a pediatric hematology-oncology service. *J Pediatr Hematol Oncol* 1998;**20**:215–21.
3. American Academy of Pediatrics. Antimicrobial prophylaxis. In: Pickering LK, Baker CJ, Long SS, McMillan JA, editors. *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 829–36.
4. American Academy of Pediatrics. Staphylococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 26th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009. p. 602–3.
5. Ammendiola MG, Di Rosa R, Montanaro L. Slime production and expression of the slime-associated antigen by staphylococcal clinical isolates. *J Clin Microbiol* 1999;**37**:3235–8.
6. Archer GL. *Staphylococcus epidermidis* and other coagulase-negative staphylococci. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 5th ed. New York: Churchill Livingstone; 2000. p. 2092–100.
7. Archer GL, Climo MW. Antimicrobial susceptibility of coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1994;**38**: 2231–7.
8. Archer GL, Karchmer AW, Vishniavsky N, et al. Plasmid-pattern analysis for the differentiation of infecting from noninfecting *Staphylococcus epidermidis*. *J Infect Dis* 1984;**149**:913–20.
9. Arioli V, Pallanza R. Teicoplanin-resistant coagulase-negative staphylococci. *Lancet* 1987;**1**:39.
10. Auckenthaler R, Courvalin P, Feer C, et al. In vitro activity of quinupristin/dalfopristin in comparison with five antibiotics against worldwide clinical isolates of staphylococci. *Clin Microbiol Infect* 2000;**6**:608–12.
11. Auletta J, Riordan MA, Nieder ML. Infections in children with cancer: a continued need for the comprehensive physical examination. *J Pediatr Hematol Oncol* 1999;**21**:501–8.
12. Babu E, Oropello J. *Staphylococcus lugdunensis*: the coagulase-negative staphylococcus you don't want to ignore. *Expert Rev Anti Infect Ther* 2011;**9**:901–7.
13. Baddour LM, Smalley DL, Kraus AP, Jr, et al. Comparison of microbiologic characteristics of pathogenic and saprophytic coagulase-negative staphylococci from patients on continuous ambulatory peritoneal dialysis. *Diagn Microbiol Infect Dis* 1986; **5**:197–205.
14. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association. *Circulation* 2005;**111**: 394–434.
15. Bandyk DF, Berni GA, Thiele BL, et al. Aortofemoral graft infection due to *Staphylococcus epidermidis*. *Arch Surg* 1984;**119**: 102–8.
16. Baum JL. Current concepts in ophthalmology: ocular infections. *N Engl J Med* 1978;**299**:28–31.
17. Baumgart S, Hall SE, Campos JM, et al. Sepsis with coagulase-negative staphylococci in critically ill newborns. *Am J Dis Child* 1983;**137**:461–3.
18. Belik J, Finn G, Rivera G, et al. Successful management of bacterial endocarditis of the mitral valve due to *Staphylococcus epidermidis* in an immunocompromised host. *Acta Paediatr Scand* 1980;**69**: 731–4.
19. Bender JW, Hughes WT. Fatal *Staphylococcus epidermidis* sepsis following bone marrow transplantation. *Johns Hopkins Med J* 1980;**146**:13–15.
20. Benezra D, Kiehn TE, Gold JW, et al. Prospective study of infections in indwelling central venous catheters using quantitative blood cultures. *Am J Med* 1988;**85**:495–8.
21. Bialkowska-Hobrzanska H, Jaskor D, Hammerberg O. Evaluation of restriction endonuclease fingerprinting of chromosomal DNA and plasmid profile analysis for characterization of multiresistant coagulase-negative staphylococci in bacteremic neonates. *J Clin Microbiol* 1990;**28**:269–75.
22. Biavasco F, Vignaroli C, Varaldo PE. Glycopeptide resistance in coagulase-negative staphylococci. *Eur J Clin Microbiol Infect Dis* 2000;**19**:403–17.
23. Bjorkqvist M, Liljedahl M, Zimmermann J, et al. Colonization pattern of coagulase-negative staphylococci in preterm neonates and the relation to bacteremia. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1085–93.
24. Bogado I, Limansky A, Sutich E, et al. Molecular characterization of methicillin-resistant coagulase-negative staphylococci from a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2002; **23**:447–51.
25. Bor DH, Rose RM, Modlin JF. Mediastinitis after cardiovascular surgery. *Rev Infect Dis* 1983;**5**:885–96.
26. Bou R, Peris M, Perpinan J, et al. A protracted outbreak of *Staphylococcus epidermidis* infections among patients undergoing valve replacement. *Infect Control Hosp Epidemiol* 2004;**25**:498–503.
27. Boyce JM, Potter-Bynoe G, Opal SM. A common source outbreak of *Staphylococcus epidermidis* infections among patients undergoing cardiac surgery. *J Infect Dis* 1990;**161**:493–9.
28. Bradford R, Abdul Manan R, Daley AJ, et al. Coagulase-negative staphylococci in very-low-birth-weight infants: inability of genetic markers to distinguish invasive strains from blood culture contaminants. *Eur J Clin Microbiol Infect Dis* 2006;**25**:283–90.
29. Brinton GS, Topping TM, Hyndiuk RA. Posttraumatic endophthalmitis. *Arch Ophthalmol* 1984;**102**:547–50.
30. Broviac JW, Cole JJ, Scribner BH. A silicone rubber atrial catheter for prolonged parenteral alimentation. *Surg Gynecol Obstet* 1973; **136**:602–6.
31. Bruce AM, Lorber J, Shedden WIH, et al. Persistent bacteremia following ventriculo-caval shunt operations for hydrocephalus in infants. *Dev Med Child Neurol* 1963;**5**:461–70.
32. Buckner CD, Clift RA, Sanders JE, et al. Protective environment for bone marrow transplant recipients: a prospective study. *Ann Intern Med* 1978;**89**:893–901.
33. Buetow KC, Klein SW, Lane RB. Septicemia in premature infants. *Am J Dis Child* 1965;**110**:29–41.
34. Burnie JP, Lee W, Matthews RC, et al. Immunoblot fingerprinting of coagulase-negative staphylococci. *J Clin Pathol* 1988;**41**: 103–10.
35. Bykowska K, Ludwicka A, Wegrzynowicz Z. Anticoagulant properties of extracellular slime substance produced by *Staphylococcus epidermidis*. *Thromb Haemost* 1985;**54**:853–6.
36. Cairo MS. Neonatal neutrophil host defense: Prospects for immunologic enhancement during neonatal sepsis. *Am J Dis Child* 1989;**143**:40–6.
37. Centers for Disease Control and Prevention, NNIS System. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1997. *Am J Infect Control* 1997;**25**:477–87.
38. Centers for Disease Control and Prevention, Hospital Infection Control Practices Advisory Committee (HICPAC). Recommendations for preventing the spread of vancomycin resistance. *MMWR Morb Mortal Wkly Rep* 1995;**44**:1–13.
39. Chapman RL, Faix RG. Persistent bacteremia and outcome in late onset infection among infants in a neonatal intensive care unit. *Pediatr Infect Dis J* 2003;**22**:17–21.
40. Chen X, Adelman RA. Microbial spectrum and resistance patterns in endophthalmitis: a 21-year (1988-2008) review in Northeast United States. *J Ocul Pharmacol Ther* 2012;**28**:329–34.
41. Cheung GY, Rigby K, Wang R, et al. *Staphylococcus epidermidis* strategies to avoid killing by human neutrophils. *PLoS Pathog* 2010;**6**:e1001133.
42. Choo MH, Holmes Jr DR, Gersh BJ, et al. Permanent pacemaker infections: characterization and management. *Am J Cardiol* 1981;**48**:559–64.
43. Christensen GD, Parisi JT, Bisno AL, et al. Characterization of clinically significant strains of coagulase-negative staphylococci. *J Clin Microbiol* 1983;**18**:258–69.
44. Christensen GD, Simpson WA, Bisno AL, et al. Adherence of slime-producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect Immun* 1982;**37**:318–26.
45. Christensen GD, Simpson WA, Bisno AL, et al. Experimental foreign body infections in mice challenged with slime-producing *Staphylococcus epidermidis*. *Infect Immun* 1983;**40**:407–10.

46. Chu V, Cabell C, Abrutyn E, et al. Native valve endocarditis due to coagulase-negative staphylococci: report of 99 episodes from the International Collaboration on Endocarditis Merged Database. *Clin Infect Dis* 2004;**39**:1527–30.
47. Clinical and Laboratory Standards Institute. Performance testing for antimicrobial susceptibility testing, 17th informational supplement. M100-S17, 2007.
48. Clink J, Pennington TH. Staphylococcal whole-cell polypeptide analysis: evaluation as a taxonomic and typing tool. *J Med Microbiol* 1987;**23**:41–4.
49. Crass BA, Bergdoll MS. Involvement of coagulase-negative staphylococci in toxic shock syndrome. *J Clin Microbiol* 1986;**23**: 43–5.
50. D'Angio CT, McGowan KL, Baumgart S. Surface colonization with coagulase-negative staphylococci in premature neonates. *J Pediatr* 1989;**114**:1029–34.
51. Davenport DS, Massanari RM, Pfaller MA, et al. Usefulness of a test for slime production as a marker for clinically significant infections with coagulase-negative staphylococci. *J Infect Dis* 1986;**153**:332–9.
52. Day MD, Gauvreau K, Shulman S, et al. Characteristics of children hospitalized with infective endocarditis. *Circulation* 2009; **119**:865–70.
53. De Hondt G, Ieven M, Vandermersch C. Destructive endocarditis caused by *Staphylococcus lugdunensis*: case report and review of the literature. *Acta Clin Belg* 1997;**52**:27–30.
54. Del Bene VE, John Jr JF, Twitty JA, et al. Antistaphylococcal activity of teicoplanin, vancomycin, and other antimicrobial agents: the significance of methicillin resistance. *J Infect Dis* 1986;**154**:349–52.
55. de Saxe MJ, Crees-Morris JA, Marples RR, et al. Evaluation of current phage-typing systems for coagulase-negative staphylococci. In: Jeljaszewicz J, editor. *Staphylococci and staphylococcal infections*. Stuttgart: Gustave Fischer; 1981. p. 197–204.
56. de Silva GD, Kantzanou M, Justice A, et al. The *ica* operon and biofilm production in coagulase-negative staphylococci associated with carriage and disease in a neonatal intensive care unit. *J Clin Microbiol* 2002;**40**:382–8.
57. Dickinson GM, Bisno AL. Infections associated with indwelling devices: concepts of pathogenesis; infections associated with intravascular devices. *Antimicrob Agents Chemother* 1989;**33**:597–601.
58. Dickinson GM, Bisno AL. Infections associated with indwelling devices: infections related to extravascular devices. *Antimicrob Agents Chemother* 1989;**33**:602–7.
59. Dickinson GM, Bisno AL. Infections associated with prosthetic devices: clinical considerations. *Int J Artif Organs* 1993;**16**: 749–54.
60. Dimitriou G, Fouzas S, Giormeszis N, et al. Clinical and microbiological profile of persistent coagulase-negative staphylococcal bacteraemia. *Clin Microbiol Infect* 2011;**17**:1684–90.
61. Donowitz LC, Haley CE, Gregory WW, et al. Neonatal intensive care unit bacteremia: emergence of gram-positive bacteria as major pathogens. *Am J Infect Control* 1987;**15**:141–7.
62. Dougherty SH, Simmons RL. Infections in bionic man: the pathobiology of infections in prosthetic devices, parts I and II. *Curr Probl Surg* 1982;**19**:217–319.
63. Drewry DT, Galbraith L, Wilkinson BJ, et al. Staphylococcal slime: a cautionary tale. *J Clin Microbiol* 1990;**28**:1292–6.
64. Dryden MS, Talsania HG, Martin S, et al. Evaluation of methods for typing coagulase-negative staphylococci. *J Med Microbiol* 1992;**37**:109–17.
65. Edwards MS, Baker CJ. Median sternotomy wound infections in children. *Pediatr Infect Dis J* 1987;**2**:105–9.
66. Etienne J, Brun Y, El Solh N, et al. Characterization of clinically significant isolates of *Staphylococcus epidermidis* from patients with endocarditis. *J Clin Microbiol* 1988;**26**:613–7.
67. Farber BF, Kaplan MH, Clogston AG. *Staphylococcus epidermidis* extracted slime inhibits the antimicrobial action of glycopeptide antibiotics. *J Infect Dis* 1990;**161**:27–40.
68. Fass RJ, Helsel VL, Barnishan J, et al. In vitro susceptibilities of four species of coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1986;**30**:545–52.
69. Fine RN, Salusky IB, Hall T, et al. Peritonitis in children undergoing continuous ambulatory peritoneal dialysis. *Pediatrics* 1983; **71**:806–9.
70. Fitzgerald Jr RH, Nolan DR, Ilstrup DM, et al. Deep wound sepsis following total hip arthroplasty. *J Bone Joint Surg Am* 1977;**59**:847–55.
71. Fleer A, Gerards LJ, Aerts P, et al. Opsonic defense to *Staphylococcus epidermidis* in the premature neonate. *J Infect Dis* 1985;**152**: 930–7.
72. Fleer A, Senders RC, Visser MR, et al. Septicemia due to coagulase-negative staphylococci in a neonatal intensive care unit: clinical and bacteriological features and contaminated parenteral fluids as a source of sepsis. *Pediatr Infect Dis J* 1983;**2**:426–31.
73. Flynn PM, Shenep JL, Stokes DC, et al. In situ management of confirmed central venous catheter-related bacteremia. *Pediatr Infect Dis J* 1987;**6**:729–34.
74. Fontela PS, Platt RW, Rocher I, et al. Epidemiology of central line-associated bloodstream infections in Quebec intensive care units: a 6-year review. *Am J Infect Control* 2012;**40**:221–6.
75. Frank KL, Del Pozo JL, Patel R. From clinical microbiology to infection pathogenesis: how daring to be different works for *Staphylococcus lugdunensis*. *Clin Microbiol Rev* 2008;**21**:111–33.
76. Frebourg NB, Lefebvre S, Baert S, Lemeland JF. PCR-based assay for discrimination between invasive and contaminating *Staphylococcus epidermidis* strains. *J Clin Microbiol* 2000;**38**:877–80.
77. Freeman R, Goodfellow M, Ward AC, et al. Epidemiological typing of coagulase-negative staphylococci by pyrolysis mass spectrometry. *J Med Microbiol* 1991;**34**:245–8.
78. Friedman LE, Brown AE, Miller DR, et al. *Staphylococcus epidermidis* septicemia in children with leukemia and lymphoma. *Am J Dis Child* 1984;**138**:715–19.
79. Fritsche TR, Kirby JT, Jones RN. In vitro activity of tigecycline (GAR-936) tested against 11,859 recent clinical isolates associated with community-acquired respiratory tract and gram-positive cutaneous infections. *Diagn Microbiol Infect Dis* 2004;**49**:201.
80. Gatermann S, Crossley KB. Urinary tract infections. In: Crossley KB, Archer GL, editors. *The staphylococci in human disease*. New York: Churchill Livingstone; 1997. p. 493–508.
81. Gatermann S, John J, Marre R. *Staphylococcus saprophyticus* urease: characterization and contribution to uropathogenicity in unobstructed urinary tract infections of rats. *Infect Immun* 1989;**57**: 110–6.
82. Gatermann S, Kreft B, Marre R, et al. Identification and characterization of a surface-protein (Ssp) of *Staphylococcus saprophyticus*. *Infect Immun* 1992;**60**:1055–60.
83. Gatermann S, Meyer HG, Wanner G. *Staphylococcus saprophyticus* hemagglutinin is a 160-kilodalton surface protein. *Infect Immun* 1992;**65**:4127–32.
84. Goering RV, Winters MA. Rapid method for epidemiologic evaluation of gram-positive cocci by field inversion gel electrophoresis. *J Clin Microbiol* 1992;**30**:577–80.
85. Goldman DA, Pier GB. Pathogenesis of infections relapsed to intravascular catheterization. *Clin Microbiol Rev* 1993;**6**:176–92.
86. Graninger W, Wenish C, Hasenhandl M. Treatment of staphylococcal infections. *Curr Opin Infect Dis* 1995;**8**(Suppl. 1):520–8.
87. Gray ED, Peters G, Verstegen M, et al. Effect of extracellular slime substance from *Staphylococcus epidermidis* on the human cellular immune response. *Lancet* 1984;**1**:365–7.
88. Gray JE, Richardson DK, McCormick MC, et al. Coagulase-negative staphylococcal bacteremia among very low birth weight infants: relation to admission illness severity, resource use, and outcome. *Pediatrics* 1995;**95**:225–30.
89. Gristina AG. Biomaterial-centered infection: microbial adhesion versus tissue integration. *Science* 1987;**237**:1588–95.
90. Grossi EA, Culliford AT, Krieger KH, et al. A survey of 77 major infectious complications of median sternotomy: a review of 7,949 consecutive operative procedures. *Ann Thorac Surg* 1985;**40**: 214–23.
91. Gruer LD, Bartlett R, Ayliffe GAJ. Species identification and antibiotic sensitivity of coagulase-negative staphylococci from CAPD peritonitis. *J Antimicrob Chemother* 1984;**13**:577–83.
92. Gruskay JA, Abbasi S, Anday E, et al. *Staphylococcus epidermidis*–associated enterocolitis. *J Pediatr* 1986;**109**:520–4.
93. Gruskay J, Harris MC, Costarino AT, et al. Neonatal *Staphylococcus epidermidis* meningitis with unremarkable CSF examination results. *Am J Dis Child* 1989;**143**:580–2.
94. Gunn BA, Davis Jr CE. *Staphylococcus haemolyticus* urinary tract infection in a male patient. *J Clin Microbiol* 1988;**26**:1055–7.

95. Hall DE, Snitzer JA, III. *Staphylococcus epidermidis* as a cause of urinary tract infections in children. *J Pediatr* 1994;**124**:437–8.
96. Hall RT, Hall SL, Barnes WG, et al. Characteristics of coagulase-negative staphylococci from infants with bacteremia. *Pediatr Infect Dis J* 1987;**6**:377–83.
97. Hall SL, Hall RT, Barnes WG, et al. Relationship of maternal to neonatal colonization with coagulase-negative staphylococci. *Am J Perinatol* 1990;**7**:384–8.
98. Hall SL, Riddell SW, Barnes WG, et al. Evaluation of coagulase-negative staphylococcal isolates from serial nasopharyngeal cultures of premature infants. *Diagn Microbiol Infect Dis* 1990;**13**: 17–23.
99. Hamilton-Miller JMT, Iliffe A. Antimicrobial resistance in coagulase negative staphylococci. *J Med Microbiol* 1985;**19**:217–26.
100. Hamory BH, Parisi JT, Hutton JP. *Staphylococcus epidermidis*: a significant nosocomial pathogen. *Am J Infect Control* 1987;**15**: 59–74.
101. Haslett TM, Isenberg HD, Hilton E, et al. Microbiology of indwelling central intravascular catheters. *J Clin Microbiol* 1988; **26**:696–701.
102. Healy CM, Baker CJ, Palazzi DL, et al. Distinguishing true coagulase-negative *Staphylococcus* infections from contaminants in the neonatal intensive care unit. *J Perinatol* 2012 Apr 12 [Epub ahead of print].
103. Hebert GA, Cooksey RC, Clark NC, et al. Biotyping coagulase-negative staphylococci. *J Clin Microbiol* 1988;**26**:1950–6.
104. Heidrich C, Hantke K, Bierbaum G, et al. Identification and analysis of a gene encoding a Fur-like protein of *Staphylococcus epidermidis*. *FEMS Microbiol Lett* 1996;**140**:253–9.
105. Heilmann C, Peters G. Biology and pathogenesis of *Staphylococcus epidermidis*. In: Fischetti VA, Novick RP, Ferretti JJ, et al, editors. *Gram-positive pathogens*. Washington, D.C.: ASM Press; 2000. p. 442–9.
106. Heilmann C, Schweitzer O, Gerke C, et al. Molecular basis of intercellular adhesion in the biofilm-forming *Staphylococcus epidermidis*. *Mol Microbiol* 1996;**20**:1083–91.
107. Heilmann C, Thumm G, Chhatwal GS, et al. Identification and characterization of a novel autolysin (Aae) with adhesive properties from *Staphylococcus epidermidis*. *Microbiology* 2003;**149**: 2769–78.
108. Hickman RO, Buckner CD, Clift RA, et al. A modified right atrial catheter for access to the venous system in marrow transplant recipients. *Surg Gynecol Obstet* 1979;**148**:871–5.
109. Hogt AH, Dankert J, Hulstaert CE, et al. Cell surface characteristics of coagulase-negative staphylococci and their adherence to fluorinated poly(ethyleneprophylene). *Infect Immun* 1986;**51**: 294–301.
110. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**:309–32.
111. Huebner J, Goldman DA. Coagulase negative staphylococci: role as pathogens. *Annu Rev Med* 2000;**50**:223–36.
112. Hussain M, Heilmann C, Peters G, Herrmann M. Teichoic acid enhances adhesion of *Staphylococcus epidermidis* to immobilized fibronectin. *Microb Pathog* 2001;**31**:261–70.
113. Hussain Z, Stoakes L, Garrow S, et al. Rapid detection of *mecA*-positive and *mecA*-negative coagulase-negative staphylococci by an anti-penicillin binding protein 2a slide latex agglutination test. *J Clin Microbiol* 2000;**38**:2051–4.
114. Izard NC, Hachler H, Grehn M, et al. Ribotyping of coagulase-negative staphylococci with special emphasis on intraspecific typing of *Staphylococcus epidermidis*. *J Clin Microbiol* 1992;**30**: 817–23.
115. Jara FM, Toledo-Pereyra L, Lewis JW, Jr, et al. The infected pacemaker pocket. *J Thorac Cardiovasc Surg* 1979;**78**:298–300.
116. Jean-Baptiste N, Benjamin DK, Cohen-Wolkowiez M, et al. Coagulase-negative staphylococcal infections in the neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:679–86.
117. John JF, McNeill WF. Activity of cephalosporins against methicillin-susceptible and methicillin-resistant, coagulase-negative staphylococci: minimal effect of beta-lactamases. *Antimicrob Agents Chemother* 1980;**17**:179–83.
118. Johnson CM, Rhodes KH. Pediatric endocarditis. *Mayo Clin Proc* 1982;**57**:86–94.
119. Johnson GM, Lee DA, Regelmann WE, et al. Interference with granulocyte function by *Staphylococcus epidermidis* slime. *Infect Immun* 1986;**54**:13–20.
120. Jones RM, Jackson MA, Ong C, Lofland GK. Endocarditis caused by *Staphylococcus lugdunensis*. *Pediatr Infect Dis J* 2002;**21**:265–8.
121. Jordan PA, Iravani A, Richard GA, et al. Urinary tract infection caused by *Staphylococcus saprophyticus*. *J Infect Dis* 1980;**142**: 510–15.
122. Kacica MA, Horgan MJ, Preston KE, et al. Relatedness of coagulase-negative staphylococci causing bacteremia in low-birthweight infants. *Infect Control Hosp Epidemiol* 1994;**15**: 658–62.
123. Kallman J, Kihlstrom E, Schollin J. Increase of staphylococci in neonatal septicaemia: A fourteen-year study. *Acta Paediatr* 1997;**86**:533–8.
124. Kamme C, Lindberg L. Aerobic and anaerobic bacteria in deep infections after total hip arthroplasty: differential diagnosis between infectious and non-infectious loosening. *Clin Orthop Relat Res* 1981;**154**:201–7.
125. Karlowicz M, Buescher S, Surka A. Fulminant late-onset sepsis in a neonatal intensive care unit, 1988-1997, and the impact of avoiding empirical vancomycin therapy. *Pediatrics* 2000;**106**: 1387–90.
126. Karp JE, Dick JD, Angelopulos C, et al. Empiric use of vancomycin during prolonged treatment-induced granulocytopenia: randomized, double-blind, placebo-controlled clinical trial in patients with acute leukemia. *Am J Med* 1986;**81**:237–42.
127. Keane WF, Comty CM, Verbrugh HA, et al. Opsonic deficiency of peritoneal dialysis effluent in continuous ambulatory peritoneal dialysis. *Kidney Int* 1984;**25**:539–43.
128. Kelly S, Collins J, Maguire M, et al. An outbreak of colonization with linezolid-resistant *Staphylococcus epidermidis* in an intensive therapy unit. *J Antimicrob Chemother* 2008;**61**:901.
129. Keucher TR, Mealey J, Jr. Long-term results after ventriculoatrial and ventriculoperitoneal shunting for infantile hydrocephalus. *J Neurosurg* 1979;**50**:79–186.
130. Khashu M, Osiovich H, Henry D. et al. Persistent bacteremia and severe thrombocytopenia caused by coagulase-negative *Staphylococcus* in a neonatal intensive care unit. *Pediatrics* 2000;**117**: 340–8.
131. Kingston D, Seal D, Hill ID. Self-disinfecting plastics for intravenous catheters and prosthetic inserts. *J Hyg* 1986;**96**:185–98.
132. Kloos WE, Ballard DN, George CG, et al. Delimiting the genus *Staphylococcus* through description of *Micrococcus caseolyticus* gen. nov., comb. nov. and the new species *Macrococcus equipercicus* sp. nov., *Macrococcus bovicus* sp. nov., and *Macrococcus carouselicus* sp. nov. *Int J Syst Bacteriol* 1998;**48**:859–77.
133. Kloos WE, Bannerman TL. Update of clinical significance of coagulase-negative staphylococci. *Clin Microbiol Rev* 1994;**7**: 117–40.
134. Kloos WE, Bannerman TL. *Staphylococcus* and *Micrococcus*. In: Murray PR, Baron EJ, Pfaller A, et al, editors. *Manual of clinical microbiology*. 7th ed. Washington, D.C.: American Society for Microbiology; 1999. p. 264–82.
135. Kloos WE, Musselwhite MS. Distribution and persistence of *Staphylococcus* and *Micrococcus* species and other aerobic bacteria on human skin. *Appl Microbiol* 1975;**30**:381–5.
136. Kogan G, Sadovskaya I, Chaignon P, et al. Biofilms of clinical strains of *Staphylococcus* that do not contain polysaccharide intercellular adhesion. *FEMS Microbiol Lett* 2006;**255**:11–16.
137. Kozitskaya S, Olson ME, Fey PD, et al. Clonal analysis of *Staphylococcus epidermidis* isolates carrying or lacking biofilm-mediating genes by multilocus sequence typing. *J Clin Microbiol* 2005;**43**: 4751–7.
138. Kratzer C, Rabitsch W, Hirschl AM, et al. In vitro activity of daptomycin and tigecycline against coagulase-negative staphylococcus blood isolates from bone marrow transplant recipients. *Eur J Haematol* 2007;**79**:405.
139. Kristinsson KG, Hastings JG, Spencer RC. The role of extracellular slime in opsonophagocytosis of *Staphylococcus epidermidis*. *J Med Microbiol* 1988;**27**:207–13.
140. Kristof K, Kocsis E, Szabo D, et al. Significance of methicillin-teicoplanin–resistant *Staphylococcus haemolyticus* in bloodstream infections in patients of the Semmelweis University hospitals in Hungary. *Eur J Clin Microbiol Infect Dis* 2011;**30**:691–9.

141. Kuroda M, Yamashita A, Hirakawa H, et al. Whole genome sequence of *Staphylococcus saprophyticus* reveals the pathogenesis of uncomplicated urinary tract infection. *Proc Natl Acad Sci U S A* 2005;**102**:13272–7.
142. Langley J, Gold R. Sepsis in febrile neutropenic children with cancer. *Pediatr Infect Dis J* 1988;**7**:34–7.
143. Lark RL, VanderHyde K, Deeb GM, et al. An outbreak of coagulase-negative staphylococcal surgical-site infections following aortic valve replacement. *Infect Control Hosp Epidemiol* 2001; **22**:618–23.
144. Latham RH, Running K, Stamm WE. Urinary tract infections in young adult women caused by *Staphylococcus saprophyticus*. *JAMA* 1983;**250**:3063–6.
145. Laverdiere M, Peterson PK, Verhoef J. In vitro activity of cephalosporins against methicillin-resistant coagulase-negative staphylococci. *J Infect Dis* 1978;**137**:245–50.
146. Levison ME, Bush LM. Peritonitis and other intra-abdominal infections. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. New York: Churchill Livingstone; 2000. p. 821–56.
147. Levison ME, Pallasch TJ, Gage TW, et al. Unique features of infective endocarditis in childhood. *Pediatrics* 2002;**109**:931.
148. Levy I, Bendet M, Samra Z, et al. Infectious complications of peripherally inserted central venous catheters in children. *Pediatr Infect Dis J* 2010;**29**:426–9.
149. Lew DP. Physiopathology of foreign body infections. *Eur J Cancer Clin Oncol* 1989;**25**:1379–82.
150. Lewis JF, Brake SR, Anderson DJ, et al. Urinary tract infection due to coagulase-negative Staphylococcus. *Am J Clin Pathol* 1982;**77**:736–9.
151. Lowder JN, Lazarus HM, Herzig RH. Bacteremias and fungemias in oncologic patients with central venous catheters: changing spectrum of infection. *Arch Intern Med* 1982;**142**:1456–9.
152. Lowy FD, Chang DS, Lash PR. Synergy of combinations of vancomycin, gentamicin, and rifampin against methicillin-resistant, coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1983;**23**:932–4.
153. Lowy FD, Walsh JA, Mayers MM. Antibiotic activity in-vitro against methicillin-resistant *Staphylococcus epidermidis* and therapy of an experimental infection. *Antimicrob Agents Chemother* 1979; **16**:314–21.
154. Mack D, Davies AP, Harris LG, et al. Microbial interactions in *Staphylococcus epidermidis* biofilms. *Anal Bioanal Chem* 2007;**387**: 399–408.
155. Mack D, Siemssen N, Laufs R. Parallel induction by glucose of adherence and a polysaccharide antigen specific for plastic-adherent *Staphylococcus epidermidis*: evidence for functional relation to intracellular adhesion. *Infect Immun* 1992;**60**:2048–57.
156. Makhoul IR, Sujov P, Smolkin T, et al. Epidemiological, clinical, and microbiological characteristics of late onset sepsis among very low birth weight infants in Israel: a national survey. *Pediatrics* 2002;**109**:34–9.
157. Maki D, Weise CE, Safarin HW. A semi-quantitative culture method for identifying intravenous-catheter–related infection. *N Engl J Med* 1977;**296**:1305–9.
158. Mansour KA, Kauten JR, Hatcher CR, Jr. Management of the infected pacemaker: explanation, sterilization, and reimplantation. *Ann Thorac Surg* 1985;**40**:617–19.
159. Maranan MC, Moreira B, Boyle-Vavra S, Daum RS. Antimicrobial resistance in staphylococci: epidemiology, molecular mechanisms, and clinical relevance. *Infect Dis Clin North Am* 1997;**11**: 813.
160. Marples RR. Laboratory assessment in the epidemiology of infections caused by coagulase-negative staphylococci. *J Med Microbiol* 1986;**22**:285–7.
161. Marrie TJ, Kwan C, Noble A. *Staphylococcus saprophyticus* as a cause of urinary tract infections. *J Clin Microbiol* 1982;**16**:427–31.
162. Marsik FJ, Brake S. Species identification and susceptibility to 17 antibiotics of coagulase-negative staphylococci isolated from clinical specimens. *J Clin Microbiol* 1982;**15**:640–5.
163. McDonald JA, Lohr JA. *Staphylococcus epidermidis* pyelonephritis in a previously healthy child. *Pediatr Infect Dis J* 1994;**13**: 1155–6.
164. McDougal LK, Thornsberry C. New recommendations for disk diffusion antimicrobial susceptibility tests for methicillin-resistant (hetero-resistant) staphylococci. *J Clin Microbiol* 1984;**19**:482–8.
165. Mehlin C, Headley CM, Klebanoff SJ. An inflammatory polypeptide complex from *Staphylococcus epidermidis*: isolation and characterization. *J Exp Med* 1999;**189**:907–18.
166. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.
167. Meyers JD. Infection in bone marrow transplant recipients. *Am J Med* 1986;**81**(Suppl. 1A):27–38.
168. Mickelsen PA, Plorde JJ, Gordon KP, et al. Instability of antibiotic resistance in a strain of *Staphylococcus epidermidis* isolated from an outbreak of prosthetic valve endocarditis. *J Infect Dis* 1985; **152**:50–8.
169. Milisavljevic V, Wu F, Cimmotti J, et al. Genetic relatedness of *Staphylococcus epidermidis* from infected infants and staff in the neonatal intensive care unit. *Am J Infect Control* 2005;**33**:341–7.
170. Mirro J, Jr Rao BN, Stokes DC, et al. A prospective study of Hickman/Broviac catheters and implantable ports in pediatric oncology patients. *J Clin Oncol* 1989;**7**:214–22.
171. Mulanovich VE, Huband MD, McCurdy SP, et al. Emergence of linezolid-resistant coagulase-negative *Staphylococcus* in a cancer centre linked to increased linezolid utilization. *J Antimicrob Chemother* 2010;**65**:2001–4.
172. Munson DP, Thompson TR, Johnson DE, et al. Coagulase-negative staphylococci septicemia: experience in a newborn intensive care unit. *J Pediatr* 1982;**101**:602–5.
173. Murtagh F, Lehman R. Peritoneal shunts in the management of hydrocephalus. *JAMA* 1967;**202**:1010–14.
174. Nafziger DA, Wenzel RP. Coagulase-negative staphylococci: epidemiology, evaluation, and therapy. *Infect Dis Clin North Am* 1989;**3**:915–28.
175. Nataro JP, Corcoran L, Zirin S, et al. Prospective analysis of coagulase-negative staphylococcal infections in hospitalized infants. *J Pediatr* 1994;**125**:798–804.
176. Nelson JD. Cerebrospinal fluid shunt infections. *Pediatr Infect Dis J* 1984;**3**(Suppl.):S30–2.
177. Nicolle LE, Hoban SA, Harding GKM. Characterization of coagulase-negative staphylococci from urinary tract infections. *J Clin Microbiol* 1983;**17**:267–71.
178. Noel GJ, Edelson PJ. *Staphylococcus epidermidis* bacteremia in neonates: Further observations and the occurrence of focal infection. *Pediatrics* 1984;**74**:832–7.
179. Noel GJ, O'Loughlin JE, Edelson PJ. Neonatal *Staphylococcus epidermidis* right-sided endocarditis: description of five catheterized infants. *Pediatrics* 1988;**82**:234–9.
180. Nouwen JL, van Belkum A, de Marie S, et al. Clonal expansion of *Staphylococcus epidermidis* strains causing Hickman catheter–related infections in a hemato-oncologic department. *J Clin Microbiol* 1998;**36**:2696–702.
181. Oeding P. Genus *Staphylococci*. In: Gergan T, Norris JR, editors. *Methods in microbiology*, vol. 12. New York: Academic Press; 1978. p. 130–3.
182. Olsen L, Frykberg T. Complications in the treatment of hydrocephalus in children: a comparison of ventriculoatrial and ventriculoperitoneal shunts in a 20-year material. *Acta Paediatr Scand* 1983;**72**:385–90.
183. Overturf GD, Balfour G. Osteomyelitis and sepsis: severe complications of fetal monitoring. *Pediatrics* 1975;**55**:244–7.
184. Paley D, Moseley CF, Armstrong P, et al. Primary osteomyelitis caused by coagulase-negative staphylococci. *J Pediatr Orthop* 1986;**6**:622–6.
185. Parisi JT. Coagulase-negative staphylococci and the epidemiological typing of *Staphylococcus epidermidis*. *Microbiol Rev* 1985;**49**: 126–39.
186. Parisi JT, Hecht DW. Plasmid profiles in epidemiologic studies of infections by *Staphylococcus epidermidis*. *J Infect Dis* 1980;**141**: 637–43.
187. Parisi JT, Lampson BC, Hoover DL, et al. Comparison of epidemiologic markers for *Staphylococcus epidermidis*. *J Clin Microbiol* 1986;**24**:56–60.
188. Parisi JT, Talbot Jr HW, Skahan JM. Development of a phage typing set for *Staphylococcus epidermidis* in the United States. *Zentralbl Bakteriol [A]* 1978;**241**:60–7.
189. Parker MA, Tuazon CU. Cervical osteomyelitis: infection due to *Staphylococcus epidermidis* in hemodialysis patients. *JAMA* 1978; **240**:50–1.

190. Pascual A, Fleer A, Westerdaal NAC, et al. Modulation of adherence of coagulase-negative staphylococci to Teflon catheters in vitro. *Eur J Clin Microbiol* 1986;**5**:518–22.
191. Patrick CC. Coagulase-negative staphylococci: pathogens with increasing clinical significance. *J Pediatr* 1990;**116**:497–507.
192. Patrick CC, Kaplan SL, Baker CJ, et al. Persistent bacteremia due to coagulase-negative staphylococci in low birth weight neonates. *Pediatrics* 1989;**84**:977–85.
193. Patrick CC, Plaunt MR, Sweet SM, et al. Defining *Staphylococcus epidermidis* cell wall proteins. *J Clin Microbiol* 1990;**28**:2757–60.
194. Patrick CH, John JF, Levkoff AH, et al. Relatedness of strains of methicillin-resistant coagulase-negative *Staphylococcus* colonizing hospital personnel and producing bacteremias in a neonatal intensive care unit. *Pediatr Infect Dis J* 1992;**11**:935–40.
195. Paulus SC, van Saene HK, Hemsworth S, et al. A prospective study of septicaemia on a paediatric oncology unit: a three-year experience at The Royal Liverpool Children's Hospital, Alder Hey, UK. *Eur J Cancer* 2005;**41**:2132–40.
196. Pereira AT. Coagulase-negative strains of *Staphylococcus* possessing antigen 51 as agents of urinary tract infection. *J Clin Pathol* 1962;**15**:252–3.
197. Persson L, Strid H, Tidefelt U, Soderquist B. Phenotypic and genotypic characterization of coagulase-negative staphylococci isolated in blood cultures from patients with haematological malignancies. *Eur J Clin Microbiol Infect Dis* 2006;**25**:299–309.
198. Peters G, Pulverer G. Pathogenesis and management of *Staphylococcus epidermidis* "plastic" foreign body infections. *J Antimicrob Chemother* 1984;**14**(Suppl. D):67–71.
199. Peters G, Saborowski F, Locci R, et al. Investigations on staphylococcal infection of transvenous endocardial pacemaker electrodes. *Am Heart J* 1984;**108**:359–65.
200. Peters G, von Eiff C, Herrmann M. The changing pattern of coagulase-negative staphylococci as infectious pathogens. *Curr Opin Infect Dis* 1995;**8**(Suppl. 1):512–9.
201. Peterson PK, Matzke G, Keane WF. Current concepts in the management of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. *Rev Infect Dis* 1987;**9**:604–12.
202. Peyman GA, Carroll CP, Raichand M. Prevention and management of traumatic endophthalmitis. *Ophthalmology* 1980;**87**:320–4.
203. Pfaller MA, Herwaldt LA. Laboratory, clinical, and epidemiological aspects of coagulase-negative staphylococci. *Clin Microbiol Rev* 1988;**1**:281–99.
204. Pizzo PA, Hathorn JW, Hiemenz J, et al. A randomized trial comparing ceftazidime alone with combination antibiotic therapy in cancer patients with fever and neutropenia. *N Engl J Med* 1986;**315**:552–8.
205. Pizzo PA, Ladisch S, Simon RM, et al. Increasing incidence of gram-positive sepsis in cancer patients. *Med Pediatr Oncol* 1978;**5**:241–4.
206. Ponce De Leon S, Wenzel RP. Hospital-acquired bloodstream infections with *Staphylococcus epidermidis*: Review of 100 cases. *Am J Med* 1984;**77**:639–44.
207. Power J, Wing EJ, Talamo TS, et al. Fatal bacterial endocarditis as a complication of permanent indwelling catheters: Report of two cases. *Am J Med* 1986;**81**:166–8.
208. Raad I, Costerton W, Sabharwal U, Sacilowski M, Anaissie E, Bodey GP. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. *J Infect Dis* 1993;**168**:400–7.
209. Raad I, Kassar R, Ghannam D, et al. Management of the catheter in documented catheter-related coagulase-negative staphylococcal bacteremia: remove or retain? *Clin Infect Dis* 2009;**49**:1187–94.
210. Raimundo O, Heussler H, Bruhn JB, et al. Molecular epidemiology of coagulase-negative staphylococcal bacteraemia in a newborn intensive care unit. *J Hosp Infect* 2002;**51**:33–42.
211. Raucher HS, Hyatt AC, Barzilai A. Quantitative blood cultures in the evaluation of septicemia in children with Broviac catheters. *J Pediatr* 1984;**104**:29–33.
212. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in pediatric intensive care units in the United States. *Pediatrics* 1999;**103**:e39.
213. Rohde H, Burandt EC, Siemssen N, et al. Polysaccharide intercellular adhesion or protein factors in biofilm accumulation of *Staphylococcus epidermidis* and *Staphylococcus aureus* isolated from prosthetic hip and knee joint infections. *Biomaterials* 2007;**28**:1711–20.
214. Rohde H, Kalitzky M, Kroger N, et al. Detection of virulence-associated genes not useful for discriminating between invasive and commensal *Staphylococcus epidermidis* strains from a bone marrow transplant unit. *J Clin Microbiol* 2004;**42**:5614–9.
215. Roos KL, Scheld WM. Central nervous system infections. In: Crossley KB, Archer GL, editors. *The Staphylococci in Human Disease*. New York: Churchill Livingstone; 1997. p. 413–40.
216. Ross MN, Haase GM, Poole MA. Comparison of totally implanted reservoirs with external catheters as venous access devices in pediatric oncologic patients. *Surg Gynecol Obstet* 1988;**167**:141.
217. Rotbart HA, Johnson ZT, Reller LB. Analysis of enteric coagulase-negative staphylococci from neonates with necrotizing enterocolitis. *Pediatr Infect Dis J* 1989;**8**:140–2.
218. Rupp ME, Archer G. Hemagglutination and adherence to plastic by *Staphylococcus epidermidis*. *Infect Immun* 1992;**60**:1055–60.
219. Rupp ME, Archer GL. Coagulase-negative staphylococci: pathogens associated with medical progress. *Clin Infect Dis* 1994;**19**:231–45.
220. Rupp ME, Fey PD, Heilmann C, Gotz F. Characterization of the importance of *Staphylococcus epidermidis* autolysin and polysaccharide inter-cellular adhesin in the pathogenesis of intravascular catheter-associated infection in a rat model. *J Infect Dis* 2001;**183**:1038–42.
221. Rupp ME, Ulphani JS, Fey PD, Mack D. Characterization of *Staphylococcus epidermidis* polysaccharide intercellular adhesin/hemagglutinin in the pathogenesis of intravascular catheter-associated infection in a rat model. *Infect Immun* 1999;**67**:2656–9.
222. Ryback MJ, Cappelletty DM, Moldovan T, et al. Comparative in vitro activities and postantibiotic effects of the oxazolidinone compounds eperezolid (PNU-100592) and linezolid (PNU-100766) versus vancomycin against *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterococcus faecalis*, and *Enterococcus faecium*. *Antimicrob Agents Chemother* 1998;**42**:712–24.
223. Sabath LD. Chemical and physical factors influencing methicillin-resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *J Antimicrob Chemother* 1977;**3**:S47–51.
224. Sabath LD. Reappraisal of the antistaphylococcal activities of first-generation (narrow-spectrum) and second-generation (expanded-spectrum) cephalosporins. *Antimicrob Agents Chemother* 1989;**33**:407–11.
225. Sabath LD, Garner C, Wilcox C, et al. Susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to 65 antibiotics. *Antimicrob Agents Chemother* 1976;**9**:962–9.
226. Sader HS, Streit JM, Fritsche TR, Jones RN. Antimicrobial susceptibility of gram-positive bacteria isolated from European medical centers: results of the Daptomycin Surveillance Programme (2002-2004). *Clin Microbiol Infect* 2006;**12**:844–52.
227. Sampathkumar P, Osmon DR, Cockerill FR. Prosthetic joint infection due to *Staphylococcus lugdunensis*. *Mayo Clin Proc* 2000;**75**:511–512.
228. Sanyal D, Johnson AP, George RC, et al. Peritonitis due to vancomycin-resistant *Staphylococcus epidermidis*. *Lancet* 1991;**337**:54.
229. Sattler FR, Foderaro JB, Aber RC. *Staphylococcus epidermidis* bacteremia associated with vascular catheters: an important cause of febrile morbidity in hospitalized patients. *Infect Control* 1984;**5**:279–83.
230. Schaberg DR, Zervos MJ. Intergeneric and interspecies gene exchange in gram-positive cocci. *Antimicrob Agents Chemother* 1986;**30**:817–22.
231. Schaberg DR, Zervos M. Plasmid analysis in the study of the epidemiology of nosocomial gram-positive cocci. *Rev Infect Dis* 1986;**8**:705–12.
232. Scheifele DW, Bjornson GL, Dyer RA, et al. Delta-like toxin produced by coagulase-negative staphylococci is associated with neonatal necrotizing enterocolitis. *Infect Immun* 1987;**55**:2268–73.
233. Scheretz RJ, Falk RJ, Huffman KA, et al. Infections associated with subclavian Udall catheters. *Arch Intern Med* 1983;**143**:52–6.
234. Schimke RT, Black PH, Mark VH, et al. Indolent *Staphylococcus albus* or *aureus* bacteremia after ventriculoatriostomy: role of foreign body in its initiation and perpetuation. *N Engl J Med* 1961;**264**:264–70.

235. Schwalbe RS, Stapleton JT, Gilligan PH. Emergence of vancomycin resistance in coagulase-negative staphylococci. *N Engl J Med* 1987;**316**:927–31.
236. Shenep JL, Hughes WT, Robertson PK, et al. Vancomycin, ticarcillin and amikacin compared with ticarcillin-clavulanate and amikacin in the empirical treatment of febrile, neutropenic children with cancer. *N Engl J Med* 1988;**319**:1053–8.
237. Sheth NK, Franson TR, Rose HD, et al. Colonization of bacteria on polyvinyl chloride and Teflon intravascular catheters in hospitalized patients. *J Clin Microbiol* 1983;**18**:1061–3.
238. Sheth NK, Franson TR, Sohnle PG. Influence of bacterial adherence to intravascular catheters on in vitro antibiotic susceptibility. *Lancet* 1985;**2**:1266–8.
239. Shiro H, Muller E, Gutierrez N, et al. Transposon mutants or *Staphylococcus epidermidis* deficient in elaboration of capsular polysaccharide/adhesion and slime are avirulent in a rabbit model or endocarditis. *J Infect Dis* 1994;**169**:1042–9.
240. Shoenbaum SC, Gardner P, Shillito J. Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. *J Infect Dis* 1975;**131**:543–52.
241. Shuttleworth R, Behme RJ, McNabb A, et al. Human isolates of bone and joint infections. *J Clin Microbiol* 1997;**35**:2537–41.
242. Schutze GE, Hall MA, Baker CJ, et al. Role of neutrophil receptors in opsonophagocytosis of coagulase-negative staphylococci. *Infect Immun* 1991;**59**:2573–8.
243. Siebert WT, Moreland N, Williams Jr TW. Methicillin-resistant *Staphylococcus epidermidis*. *South Med J* 1978;**71**:1353–5.
244. Sitges-Serra A, Puig P, Jaurrieta E. Catheter sepsis due to *Staphylococcus epidermidis* during parenteral nutrition. *Surg Gynecol Obstet* 1980;**151**:481–3.
245. Skahan JM, Parisi JT. Development of a bacteriophage-typing set for *Staphylococcus epidermidis*. *J Clin Microbiol* 1977;**6**:16–18.
246. Sloos JH, Dijkshoorn L, Vogel L, van Boven CP. Performance of phenotypic and genotypic methods to determine the clinical relevance of serial blood isolates of *Staphylococcus epidermidis* in patients with septicemia. *J Clin Microbiol* 2000;**38**:2488–93.
247. Smith JA, Henry DA, Bourgault A, et al. Comparison of agar disk diffusion, microdilution broth, and agar dilution for testing antimicrobial susceptibility of coagulase-negative staphylococci. *J Clin Microbiol* 1987;**25**:1741–6.
248. St. Geme JW, III, Bell LM, Baumgart S, et al. Distinguishing sepsis from blood culture contamination in young infants with blood cultures growing coagulase-negative staphylococci. *Pediatrics* 1990;**86**:157–62.
249. Stoll BJ, Hansen N, Fanaroff AA, et al. Late-onset sepsis in very low birth weight neonates: the experience of the NICHD Neonatal Research Network. *Pediatrics* 2001;**110**:285–91.
250. Talbot Jr HW, Parisi JT. Phage typing of *Staphylococcus epidermidis*. *J Clin Microbiol* 1976;**3**:519–23.
251. Tan TQ, Mason Jr EO, Ou CN, et al. Use of intravenous rifampin in neonates with persistent staphylococcal bacteremia. *Antimicrob Agents Chemother* 1993;**37**:2401–6.
252. Tan TQ, Musser JM, Shulman RJ, et al. Molecular epidemiology of coagulase-negative *Staphylococcus* blood isolated from neonates with persistent bacteremia and children with central venous catheter infections. *J Infect Dis* 1994;**169**:1393–7.
253. Tebbs SE, Elliott TSJ. Modification of central venous catheter polymers to prevent in vitro microbial colonization. *Eur J Clin Microbiol Infect Dis* 1994;**13**:111–7.
254. Tenover FC, Jones RN, Swenson B, et al. Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. *J Clin Microbiol* 1999;**37**:4051–8.
255. Thomson-Carter FM, Pennington TH. Characterization of coagulase-negative staphylococci by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot analysis. *J Clin Microbiol* 1989;**27**:2199–203.
256. Timmermann CP, Fleer A, Besnier JM, et al. Characterization of a proteinaceous adhesion of *Staphylococcus epidermidis* which mediates attachment to polystyrene. *Infect Immun* 1991;**59**:4187–92.
257. Tojo M, Yamashita N, Goldmann DA, et al. Isolation and characterization of a capsular polysaccharide adhesion from *Staphylococcus epidermidis*. *J Infect Dis* 1988;**157**:713–22.
258. Van Bronswijk H, Verbrugh HA, Heezius CJM, et al. Heterogeneity in opsonic requirements of *Staphylococcus epidermidis*: relative importance of surface hydrophobicity, capsules and slime. *Immunology* 1989;**67**:81–6.
259. Vandecasteele SJ, Peetermans WE, Merckx R, et al. Reliability of the *ica*, *aap* and *atlE* genes in the discrimination between invasive, colonizing and contaminant *Staphylococcus epidermidis* isolates in the diagnosis of catheter-related infections. *Clin Microbiol Infect* 2003;**9**:114–19.
260. Vandenesch F, Etienne J, Reverdy ME, Eykyn SJ. Endocarditis due to *Staphylococcus lugdunensis*: report of 11 cases and review. *Clin Infect Dis* 1993;**5**:871–6.
261. Vandenesch F, Eykyn SJ, Etienne J. Infections caused by newly-described species of coagulase-negative staphylococci. *Rev Med Microbiol* 1995;**6**:94–100.
262. van der Lugt NM, Steggerda SJ, Walther FJ. Use of rifampin in persistent coagulase-negative staphylococcal bacteremia in neonates. *BMC Pediatr* 2010;**10**:84.
263. Van Winkle P, Whiffen T, Liu IL. Experience using peripherally inserted central venous catheters for outpatient parenteral antibiotic therapy in children at a community hospital. *Pediatr Infect Dis J* 2008;**27**:1069–72.
264. Veach LA, Pfaller MA, Barrett M, et al. Vancomycin resistance in *Staphylococcus haemolyticus* causing colonization and bloodstream infection. *J Clin Microbiol* 1990;**28**:2064.
265. Veenstra GJ, Cremers FFM, van Dijk H, et al. Ultrastructural organization and regulation of a biomaterial adhesion of *Staphylococcus epidermidis*. *J Bacteriol* 1996;**178**:537–41.
266. Venditti M, Santini C, Serra P, et al. Comparative in vitro activities of new fluorinated quinolones and other antibiotics against coagulase-negative *Staphylococcus* blood isolates from neutropenic patients, and relationship between susceptibility and slime production. *Antimicrob Agents Chemother* 1989;**33**:209–11.
267. Villari P, Sarnataro C, Iacuzio L. Molecular epidemiology of *Staphylococcus epidermidis* in a neonatal intensive care unit over a three-year period. *J Clin Microbiol* 2000;**38**:1740–6.
268. Viscoli C, Garaventa A, Boni L, et al. Role of Broviac catheters in infections in children with cancer. *Pediatr Infect Dis J* 1988;**7**:556–60.
269. von Eiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. *Lancet Infect Dis* 2002;**2**:677–85.
270. Vuong C, Otto M. *Staphylococcus epidermidis* infections. *Microbes Infect* 2002;**4**:481–9.
271. Vuong C, Voyich JM, Fischer ER, et al. Polysaccharide intercellular adhesin (PIA) protects *Staphylococcus epidermidis* against major components of the human innate immune system. *Cell Microbiol* 2004;**6**:269–75.
272. Wachsmuth K. Molecular epidemiology of bacterial infections: examples of methodology and investigations of outbreaks. *Rev Infect Dis* 1986;**8**:682–92.
273. Wadstrom T, Rozgonyi F. Virulence determinants of coagulase-negative staphylococci. In: Mardh PA, Schleifer KH, editors. *Coagulase-negative staphylococci*. Stockholm: Almquist and Wiksel International; 1986. p. 123–30.
274. Wang A, Athan E, Pappas PA, et al. Contemporary clinical profile and outcome of prosthetic valve endocarditis. *JAMA* 2007;**297**:1354–61.
275. Widerstrom M, Wistrom J, Sjostedt A, Monsen T. Coagulase-negative staphylococci: update on the molecular epidemiology and clinical presentation with a focus on *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. *Eur J Clin Microbiol Infect Dis* 2012;**31**:7–20.
276. Wilson APR, O'Hare MD, Felmingham D, et al. Teicoplanin-resistant coagulase-negative *Staphylococcus*. *Lancet* 1986;**2**:973.
277. Wilson CB. Immunologic basis for increased susceptibility of the neonate to infection. *J Pediatr* 1986;**108**:1–12.
278. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24, 179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004;**39**:309–17.
279. Wong SS, Ho PL, Woo PC, et al. Bacteremia caused by staphylococci with inducible vancomycin heteroresistance. *Clin Infect Dis* 1999;**29**:760–7.
280. Yogev R. Cerebrospinal fluid shunt infections: a personal view. *Pediatr Infect Dis J* 1985;**4**:113–18.
281. Yoshida K, Umeda A, Ichiman T, et al. Cross protection between a strain of *Staphylococcus epidermidis* and eight other species of

coagulase-negative staphylococci. *Can J Microbiol* 1988;**34**: 913–5.
282. Younger JJ, Christensen GD, Bartley DL, et al. Coagulase-negative staphylococci isolated from cerebrospinal fluid shunts: Importance of slime production, species identification and shunt removal to clinical outcome. *J Infect Dis* 1987;**156**:548–54.
283. Ziebuhr W, Hennig S, Eckart M, et al. Nosocomial infections by *Staphylococcus epidermidis*: how a commensal bacterium turns into a pathogen. *Int J Antimicrob Agents* 2006;**28**(Suppl. 1):S14–20.
284. Zimmerman RJ, Kloos WE. Comparative zone electrophoresis of esterases of *Staphylococcus* species isolated from mammalian skin. *Can J Microbiol* 1976;**22**:771–9.

CHAPTER 82

Group A, Group C, Group G Beta Hemolytic Streptococcal Infections

Linette Sande • Anthony R. Flores

Streptococcus pyogenes is the only organism within the group A β-hemolytic streptococci (GAS). GAS are among the most common pathogens that infect children and adolescents and are capable of causing a wide range of infections, including relatively benign pharyngitis and life-threatening invasive infections. Despite their uniform susceptibility to penicillin, GAS remain significant clinical and public health problems in this age group. Worldwide, it is estimated that GAS are responsible for more than 500,000 deaths, the majority of which occur in underdeveloped countries.[27] GAS is unique among pyogenic streptococci in its ability to produce postinfectious sequelae such as acute rheumatic fever and acute poststreptococcal glomerular nephritis (APSGN). Infections caused by GAS were brought to the forefront in the United States in the late 1990s with a resurgence of acute rheumatic fever[97,152] and the appearance of serotype M1 and M3 strains causing severe morbidity and mortality.[9,150]

ORGANISM

GAS are gram-positive cocci, generally less than 2 μm, and tend to grow in chains in liquid media. GAS produce clear zones of hemolysis (β) on blood agar, differentiating them from streptococci producing partial (α) and nonhemolytic (γ) streptococci. Hemolysis is best observed when GAS are grown on agar containing 5 percent sheep blood. Some strains of GAS may appear to produce only partial hemolysis on blood agar plates. However, clear hemolysis can more easily be observed when grown anaerobically or by making a short cut or stab into the agar at the time of inoculation.[76] GAS are rarely nonhemolytic.[72,74] They can be differentiated from other β-hemolytic streptococci by their group-specific polysaccharide in the cell wall, the basis for latex agglutination and enzyme immunoassays, or by inhibition of growth by bacitracin.

GAS are frequently categorized or typed based on serologically distinct surface proteins, the M proteins, or by sequencing the gene encoding the M protein, *emm*.[43,44,76,86] The M protein is a major virulence factor of GAS. Acquired immunity to GAS is based, at least in part, on specific opsonic antibodies targeted against the M protein.[85] N-terminal sequence variation in the gene encoding the M protein has led to the description of approximately 200 different *emm*-types,[13] and many more are being added to a curated database publicly available through the Centers for Disease Control and Prevention (CDC).[31] Certain GAS M serotypes have been associated with specific diseases and can be classified as skin or pharyngitis "specialists"; however, some M serotypes have the capacity to cause both types of infection.[15]

The GAS cell is complex and contains many antigenic substances. Freshly isolated clinical strains are typically covered with a hyaluronic acid capsule that is structurally similar to that found in mammals, including humans, and thus is nonimmunogenic.[19,49,80] The GAS capsule gives colonies a mucoid appearance. The M protein is a major constituent of the cell surface, and proteins similar to M protein have been identified. Genes encoding M and M-like proteins (e.g., *enn* and *mrp*) are part of an *emm* gene superfamily. Also found on the cell surface, protruding through the hyaluronic acid capsule, are fimbriae composed of lipoteichoic acid[12] that are frequently associated with M protein.[48] The surface proteins T and R and serum opacity factor are proteins of interest in part because of classification schemes based on each. The T protein is part of the GAS pilus structure,[98] and T-agglutination patterns are generally associated with specific M serotypes.[73] In strains that produce serum opacity factor, the serologically specific serum opacity factor proteins are closely correlated with a particular M serotype.[14]

The GAS cell surface contains a polysaccharide polymer of rhamnose with side-chains of N-acetyl-glucosamine. The carbohydrate is found in sufficient quantities for antibody detection and forms the basis for group specificity originally described by Lancefield.[85] Rigidity of the cell is provided by peptidoglycan consisting of alternating residues of N-acetyl-muramic acid and N-acetyl-glucosamine linked by a peptide bond. Lysozyme produced on mucosal surfaces and by phagocytic cells hydrolyzes the carbohydrate backbone, leading to lysis of the cell and release of bacterial fragments. Detection of the bacterial fragments by host pattern recognition receptors leads to initiation of the host immune response to infection. Some streptococci are able to modify their peptidoglycan, leading to lysozyme resistance,[39] and alter the host immune response. Whether GAS has this capability is unknown.

The innermost layer of the cell wall consists of the cell membrane composed of lipoprotein or lipid–protein complexes. Wall-less forms of streptococci, termed *L forms*, are osmotically fragile and lack the outermost constituents of the GAS cell wall. GAS L forms are of interest because of their resistance to penicillin[52] and their potential role in persistence.[96,97]

In addition to cell surface components, GAS produces many extracellular products. Among these are two distinct hemolysins: streptolysin O and streptolysin S. Streptolysin O, designated so because of its oxygen lability, is toxic to erythrocytes and many other cell types, including phagocytes. Streptolysin O is produced by almost all GAS strains, is antigenic, and measurement of anti–streptolysin O (ASO) antibodies is used as an indicator of GAS infection. Streptolysin S is stable in the presence of oxygen, heat labile, and not antigenic. Hemolysis on the surface of blood agar is the result of streptolysin S; streptolysin O results in hemolysis in the subsurface or in anaerobic conditions.

Several extracellular proteins allow the spread of GAS through tissue. Deoxyribonucleases (DNAses A, B, C, and D) break down extracellular DNA and are believed to facilitate escape from neutrophil extracellular traps consisting in large part of DNA.[24] Spread through connective tissue is achieved through the action of hyaluronidases that enzymatically degrade hyaluronic acid, a major component of connective tissue. GAS streptokinase dissolves clots through conversion of plasminogen to plasmin. GAS produces a C5a peptidase that specifically cleaves C5a, inhibiting phagocyte recruitment. Originally thought to be a GAS superantigen, streptococcal pyrogenic exotoxin B (SpeB) is a potent protease critical in the pathogenesis of severe invasive disease.[28]

The streptococcal pyrogenic exotoxins (Spe) constitute a family of bacterial superantigens believed to be associated with streptococcal toxic shock syndrome and the pathogenesis of severe infections. SpeA and SpeC are bacteriophage-encoded and are associated with scarlet fever (i.e., scarlatinal toxins). Outbreaks of streptococcal toxic shock syndrome in the United States have been associated with strains producing SpeA.[34] Development of streptococcal toxic shock syndrome is multifactorial, and susceptibility may be associated with specific human leukocyte antigen haplotypes.[82]

TRANSMISSION AND EPIDEMIOLOGY

GAS are found almost exclusively in humans and rarely occur in other species.[86] Distinct and significant differences exist in the epidemiology and transmission of GAS based on the site of infection.[118,156]

Streptococcal pharyngitis is one of the most common infections of childhood. Whereas GAS cause the majority of infections, strains of other serogroups, including groups C and G, may instead be involved. GAS pharyngitis occurs primarily in children aged 5 to 15 years, with the peak incidence occurring during the first school years. Severe epidemics may occur in conditions of overcrowding, including daycare centers,[1] residential facilities,[117] and military barracks.[155] The effect of crowding may explain, at least in part, the seasonal occurrence in temperate and cold climates. Contaminated foods (e.g., milk and egg salad) may result in outbreaks of GAS pharyngitis.[69,91] Fomite contamination and airborne routes are unlikely to play any role in the spread of GAS pharyngitis.[30]

The mechanism of spread is facilitated by close personal contact. Early studies demonstrated that large droplets or direct transfer of respiratory secretions containing large amounts of infectious bacteria is required.[65,66] Experimental nonhuman primate studies[153] have elucidated the mechanisms of interpersonal GAS spread. The infection cycle consists of colonization, acute infection, and asymptomatic carrier phases of infection. During acute infection, GAS are present in large amounts in both the anterior nares and throat.[66] Although the acute symptomatic phase lasts only a few days, left untreated, the organism can persist for weeks in the absence of symptoms. During the asymptomatic carrier phase, bacterial burden decreases in the anterior nares and persists at lower numbers in the throat. Early studies also demonstrated that GAS in the asymptomatic carrier phase possess less hyaluronic acid capsule[126] and M protein.[123] The end result is that asymptomatic carriers are less likely to transmit the organism to close contacts than are those acutely infected. Antibiotic therapy rapidly reduces the bacterial burden and in most cases eradicates GAS from the throat. Thus, most patients are considerably less contagious within 24 hours of initiation of antibiotic therapy, reducing the risk for spread to contacts.[138]

The epidemiology and transmission of GAS skin infections is markedly different compared to throat infections. Prevalence of GAS skin infections appears to be influenced most by climate and level of hygiene. In contrast to throat infections, the peak age of incidence in impetigo and pyoderma is in children 2 to 5 years of age (i.e., preschool aged). GAS skin infections occur more frequently in hot or tropical climates[26] and, in temperate climates, infections are more common during the summer months.[46] Infection causing impetigo and pyoderma appears to be facilitated by disruption of the skin by trauma, insect bites, or preexisting skin disorders (e.g., eczema). The role of fomites and environmental contamination in the spread of GAS skin infections remains undefined.

Prospective studies on the transmission of streptococcal impetigo demonstrated that GAS initially colonize unbroken skin.[46] GAS skin colonization preceded development of impetiginous lesions by an average of 10 days.

Measures to prevent spread of GAS infections vary in their effectiveness. Spread of throat or skin infection within a family often occurs before the index case is identified and isolated or treated. In epidemic situations, especially situations involving cases of rheumatic fever or acute nephritis, a culture survey with treatment of all individuals with positive cultures (mass prophylaxis) may be indicated. Reduction of crowding, especially in sleeping quarters, seems to be an effective long-term method of minimizing the incidence of transmission of streptococcal sore throat among some groups.

In families in which persistence or recurrence of streptococcal infection is a problem, simultaneous throat culture and culture of skin lesions of all members and treatment of all who have positive results have been successful in eradicating the organism. Control of environmental contamination would be expected to have little or no influence on the spread of GAS respiratory infections,

although it possibly has an effect in controlling skin or wound infections. It also is suggested that family toothbrushes may have a role in the intrafamilial transmission of GAS. More recent guidelines have suggested, however, that performing cultures of all family members in instances in which invasive GAS infections occur is not always necessary. Because of reported instances in which secondary cases have occurred, this guideline remains controversial.[30,120]

PATHOGENESIS

Despite the numerous studies focusing on the pathogenesis of GAS infections, we understand little of mechanisms that give GAS predilection for certain body sites or why certain M serotypes are more frequently found in pharyngitis and yet others in skin infections. Infection begins with successful colonization of skin or pharyngeal epithelial surfaces. Initial interaction of GAS with epithelial surfaces is mediated by fimbriae and pili, believed to overcome the negative electrostatic forces between bacterial and host cell surfaces. More specific binding occurs through pili, M protein, hyaluronic acid capsule, and numerous fibronectin binding proteins. The complement of fibronectin binding proteins identified in GAS include proteins F1 (Prtf1) and F2 (Prtf2), SfbI, SfbII, FBP54, and others.[106]

Steps in initial colonization also require successful competition between resident flora on either the skin or pharyngeal epithelium. α-Hemolytic or viridans streptococci may interfere with throat colonization, and GAS elaborates bacteriocin-like substances that may facilitate competition.[106] Colonization of skin surfaces appears to be enhanced by physical removal of local bacterial flora.[4]

Several GAS virulence determinants participate in tissue invasion. Immune evasion is facilitated by expression of the antiphagocytic hyaluronic acid capsule, M protein, and IdeS (also known as Mac, which cleaves immunoglobulin [Ig]G bound to GAS); breakdown of neutrophil extracellular traps and host tissues by DNAses, hyaluronidases, and the cysteine protease SpeB; breakdown of chemoattractants by *S. pyogenes* cell envelope protease (SpyCEP) (cleaves IL-8), C5a peptidase (cleaves C5a); and others, including streptococcal inhibitor of complement (SIC), streptolysin O, and streptokinase. Together, these virulence factors subvert immune defenses and facilitate spread through tissues.

Recently, researchers have shed light on the in vivo molecular events that lead to invasive GAS infections. Many of the GAS virulence factors required for invasive infection are normally repressed by the CovRS two-component regulatory system. During invasive infection of serotype M1, mutations in the genes encoding the CovRS proteins result in de-repression (i.e., increased expression) of virulence factors, leading to more severe disease.[42,142] Molecular epidemiologic studies of serotype M3 GAS isolates from human pharyngitis and invasive infections demonstrated that mutations in *covS* occur at a significantly higher frequency in isolates from invasive disease in contrast to pharyngitis.[129] Whether similar de-repressing mutations occur in other serotypes is unknown.

We understand little of the mechanisms leading to early host defense against GAS infection. Although specific antibody targeted against the M protein promotes phagocytosis, it usually does not develop until 6 to 8 weeks after initial infection.[41] Rather than act during initial infection, type-specific antibody against the M protein more likely prevents reinfection by the same serologic type. Thus, in the early stages of infection, protection via phagocytosis by monocytes and polymorphonuclear leukocytes appears to be the primary mechanism of defense.

The rash and other toxic manifestations of scarlet fever have been attributed to the development of hypersensitivity to the pyrogenic toxins.[18] Toxic manifestations that have been noted in GAS toxic shock syndrome also may result from a direct influence of the pyrogenic exotoxins on lymphokines, such as tumor necrosis factor.[141] Hypersensitivity to other streptococcal products also may contribute to the manifestations of streptococcal disease.

Theories abound about the pathogenetic mechanism leading to the development of the nonsuppurative complications of streptococcal infections, acute rheumatic fever, and acute glomerulonephritis. Most of these hypotheses invoke immunologic processes in one way or another.[70,151] A major impediment toward clarifying the pathogenetic mechanism responsible for the development of nonsuppurative sequelae has been the lack of an appropriate animal model for study.

CLINICAL MANIFESTATIONS

Pharyngitis or tonsillitis caused by GAS has a brief incubation period of 12 hours to 4 days. Acute illness is characterized by abrupt onset of sore throat, malaise, fever, and headache. Children may present with nausea, vomiting, and abdominal pain. Severity can vary greatly from subclinical to a toxic appearance. Common physical findings include fever, erythema, edema, and lymphoid hyperplasia of the posterior pharynx, enlarged, hyperemic tonsils, exudate on the pharyngeal and tonsillar surfaces, and enlarged, tender lymph nodes at the mandibular angle.

Clinical manifestations generally subside spontaneously in 3 to 5 days in the absence of suppurative complications (e.g., otitis media, sinusitis, and peritonsillar abscess). Prompt antibiotic therapy (e.g., penicillin) shortens the period of fever, toxicity, and infectivity.[83,104,119] After acute streptococcal infection of the upper respiratory tract, the average latent period for glomerulonephritis is 10 days and the latent period for acute rheumatic fever is 18 days.[156]

Localized pharyngeal infection is less common in younger children, especially infants. An infantile form of streptococcal infection, streptococcal childhood fever, may be more prolonged, with persistent low-grade fever, generalized lymphadenopathy, and persistent serous nasal discharge. The term *streptococcosis* more correctly indicates the changing clinical picture with age analogous to that of tuberculosis.[116]

Infection with strains that elaborate streptococcal pyrogenic exotoxins may manifest as scarlet fever. Most commonly, scarlet fever is associated with pharyngeal

infection; however, it may also follow infection of wounds or puerperal sepsis. The characteristic rash is erythematous and finely punctate, with centrifugal spread beginning on the upper chest or trunk. The rash usually appears on the second day of illness, blanches with pressure, and spreads to cover the entire body within several hours to days. Lines of deeper red may be noted in the skinfolds of the neck, axillae, elbows, groin, and knees (Pastia's lines). The tongue may take on a strawberry appearance and become swollen and red. The skin may take on a sandpaper texture because of the occlusion of sweat glands, a particularly useful feature in dark-skinned people. Severe forms may be associated with local and hematogenous spread of the organism (i.e., septic scarlet fever) or toxemia (i.e., toxic scarlet fever). Rarely, scarlet fever may be complicated by arthritis, jaundice, and hydrops of the gallbladder. Severe manifestations are less frequent with the use of antibiotics.

Impetigo caused by GAS develops days or weeks after colonization on normal skin with an average latent period of 10 days.[46] The infection is usually painless in contrast to pharyngitis. Vesicles with minimal surrounding erythema rapidly develop into pustules and then into a thick, honey-colored crust that may persist for days or weeks. GAS impetigo does not involve the dermis, and depigmentation may be seen on healing but does not scar.

Strains of GAS that cause skin infections differ from those associated with tonsillitis and pharyngitis. The serologic types associated with skin infection tend to be higher numbered M-types because of their more recent discovery. A small number of serotypes are capable of causing both pharyngitis and skin infections.

APSGN may develop after cutaneous infection by a nephritogenic strain. Whereas rheumatic fever has not been proved to develop after skin infections with GAS, a clear association exists between GAS skin infections and rheumatic heart disease especially in Pacific populations.[109] The latent period for APSGN after skin infection is longer, 3 weeks on average, than is seen after pharyngitis (10 days).[78]

Erysipelas is a superficial streptococcal infection of the skin and sometimes adjacent mucous membranes. Three features distinguish erysipelas clinically from other forms of skin infection: (1) lesions are raised above the level of the surrounding skin, (2) a clear line of demarcation distinguishes infected from uninfected areas, and (3) lesions are a brilliant salmon-red color. Erysipelas is more common in infants and young children and most often involves the face. Lesions may be associated with wounds, an area of dermatosis (e.g., eczema), or the umbilical stump of a newborn. The cutaneous inflammation of erysipelas is often accompanied by systemic manifestations of toxicity observed in other forms of streptococcal infection.

If nothing else, GAS is notable for its ability to cause a wide range of infections. In addition to those already mentioned, suppurative complications of upper respiratory tract infection include otitis media, retropharyngeal or peritonsillar abscess, sinusitis, mastoiditis, pneumonia, and empyema. GAS are frequently isolated in cultures from peritonsillar abscesses[143] and likely act in concert with anaerobic bacteria to produce this entity.[22] Classically, acute puerperal sepsis has been associated with GAS. Infection of the skin and subcutaneous tissues (streptococcal cellulitis) may complicate burns, wounds, surgical incisions, or seemingly mild trauma. More severe infections of the deeper subcutaneous tissues and fascia with rapid spread and necrosis (necrotizing fasciitis or streptococcal gangrene) also are associated with GAS. These bacteria are a common cause of perianal cellulitis and vaginitis in children.[7,81] Subpectoral abscesses and empyemas may develop as complications of streptococcal infections of the thumb and index finger as a result of the lymphatic drainage of that part of the hand.[7] Septic complications of varicella, including varicella gangrenosa,[25,137] osteomyelitis (especially in infants),[63] hand and foot syndrome,[64] blistering distal dactylitis,[68] necrotizing fasciitis, and toxic shock syndrome, are associated with β-hemolytic streptococci.

STREPTOCOCCAL UPPER RESPIRATORY TRACT CARRIER STATE

In addition to causing a wide range of diseases, asymptomatic carriage of GAS is a common occurrence among children and adolescents. Depending on the population studied, rates of GAS carriage range from 5 to 15 percent among children,[128] a rate greatly exceeding that of any disease caused by GAS. Very early studies demonstrated that pharyngeal carriers of GAS are less dangerous to others because they harbor fewer organisms and rarely spread the organism to close contacts.[65,66] However, multiple epidemiologic studies have shown that close contacts may harbor virulent organisms in the absence of symptoms.[35,99] Thus, carriage represents a diagnostic and therapeutic dilemma for the clinician. The epidemiologic and immunologic reasons for the establishment and continuation of the carrier state are an active area of investigation.

A combination of host and bacterial factors are believed to contribute to the GAS carrier state. As opposed to true infection, in which an individual has the presence of the organism and evidence for an immunologic response, GAS carriers may harbor the organism for long periods in the absence of a host immune response.[76] Few studies have examined the bacterial or host factors that lead to persistent colonization by GAS. Bacterial studies suggest that GAS are able to internalize into epithelial cells and that internalization is associated with the presence of certain fibronectin-binding proteins.[11,102] Because penicillin does not effectively penetrate the epithelial cell, intriguingly, some have suggested that internalization of GAS into pharyngeal epithelial cells contributes to persistence despite antibiotic therapy.[77]

PEDIATRIC AUTOIMMUNE NEUROPSYCHIATRIC DISORDERS ASSOCIATED WITH STREPTOCOCCAL INFECTIONS

Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections, referred to by the acronym PANDAS, is autoimmune disorder with

obsessive-compulsive disorder or tic disorder manifestations associated with GAS. Initially described as pediatric infection-triggered autoimmune neuropsychiatric disorders (PITANDS) by Allen and colleagues[3] in a four-patient case series and later termed PANDAS by Swedo and associates[147] in a larger study involving 50 patients, establishing the diagnostic criteria, which include the following[147]:

1. Presence of obsessive-compulsive disorder and/or a tic disorder meeting lifetime *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) diagnostic criteria
2. Age at onset between 3 years of age and the beginning of puberty
3. Clinical course characterized by the abrupt onset of symptoms or by dramatic symptom exacerbations
4. Temporal association with GAS infection, diagnosed by positive throat culture and/or elevated anti-GAS antibody titers
5. Association with neurologic abnormalities such as choreiform movements or tics

This proposed disorder is currently a hypothesis, and it remains a controversial topic. Several authors believe in this disorder as a separate entity and recommend treatment and prophylaxis. Others argue that it is only a hypothesis and that further evidence with double-blind studies is needed before recommending treatment and prevention for children given a diagnosis of PANDAS.

Proposed Pathogenesis

The hypothesis for PANDAS involves several factors, including pathologic strains of GAS bacteria, host susceptibility (e.g., genetics, development, or other), and abnormal immune response.[148] A pathogenesis model has been proposed to describe the disorder (Figure 82-1).[144] Ongoing research is being conducted to understand the pathologic mechanisms involved in the PANDAS subgroup.

As part of the diagnostic criteria, temporal association with GAS infection and neuropsychiatric exacerbation is very important. It is essential to distinguish exacerbations of PANDAS resulting from GAS infection from the waxing and waning course seen in Tourette disorder and some cases of childhood-onset obsessive-compulsive disorder.[144]

According to Swedo,[144] host susceptibility is thought to result from genetic, developmental, and immunologic factors. Children in the PANDAS subgroup may have similar genetic susceptibility for poststreptococcal sequelae, as children with Sydenham chorea. Development also seems to play a role in the pathogenesis of PANDAS, because the peak age of onset of symptoms is 6 to 7 years and prepubertal onset is characteristic. PANDAS is described as an autoimmune process, and many think there are antineuronal cross-reactive antibodies similar to the autoantibodies found in Sydenham chorea.[144] These antibodies are meant to react against GAS, but in the case of PANDAS cross-react with cells in the basal ganglia.[59] Studies using MRI volumetric scans have shown bilateral enlargements of the caudate, putamen, and globus pallidus among patients in the PANDAS subgroup.[58,59]

Diagnosis

The diagnostic criteria defined by Swedo and colleagues during the description of the first 50 cases of PANDAS aimed to define a homogeneous subgroup of patients with obsessive-compulsive and tic disorders who had dramatic acute exacerbations usually following a GAS infection.[147]

These criteria as further described by Swedo and coworkers[148] are as follows:

1. *The presence of a tic disorder and/or obsessive-compulsive disorder.* The symptoms and severity required for making a diagnosis of a tic disorder and/or obsessive-compulsive disorder have been defined in the DSM-IV. The authors who established the diagnostic criteria for PANDAS limited the disorder to only tics and obsessive-compulsive symptoms to have a homogeneous patient cohort.[146] However, in the initial cohort, other psychiatric comorbidities were also found frequently, particularly attention-deficit/hyperactivity disorder (ADHD), affective disorders, and anxiety disorders. More recently, other authors propose the criteria be expanded to include related disorders such as ADHD[111] and anorexia.[140]

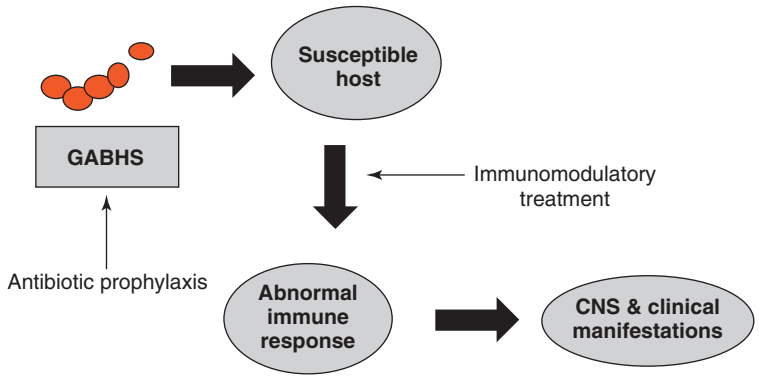

FIGURE 82-1 ■ Model of pathogenesis for PANDAS. *GABHS,* group A β-hemolytic streptococci. (See companion Expert Consult website for color version.) (Swedo SE. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections [PANDAS]. *Mol Psychiatry.* 2002;**7**[suppl 2]:S24.)

2. *Prepubertal age at onset, usually between 3 and 12 years of age.* The authors established this criterion based on historical data showing that rheumatic fever and other poststreptococcal sequelae are rare before the age of 3 years and after the age of 12 years. Protective serum antibodies against streptococcal infections were found in 98 percent of healthy 12-year-old controls in a study by Fischetti.[48]
3. *Abrupt symptom onset and/or episodic course of symptom severity.* Obsessive-compulsive disorder related to PANDAS usually can be distinguished from other forms of obsessive-compulsive disorder because children in the PANDAS subgroup typically have a sudden onset of symptoms, reaching a peak in 24 to 48 hours, whereas in non-PANDAS obsessive-compulsive disorder, a more gradual onset of obsessions and compulsions occurs. In a similar manner, children with PANDAS who have tics have more intense, frequent motor and vocal tics than those with a simple isolated, intermittent tic not associated with PANDAS.[146]
4. *Temporal association between symptom exacerbations and streptococcal infections.* Based on observational clinical studies, exacerbations of neuropsychiatric symptoms begin within 7 to 14 days after an infection with GAS and usually occur simultaneously.[146] However, because streptococcal infections are so common during childhood, a single positive throat culture or elevated antistreptococcal antibody titer is not enough evidence to say that a child's neuropsychiatric symptoms are associated with a streptococcal infection. For a child to fit this diagnostic criterion, prospective evaluation and documentation of at least two episodes of neuropsychiatric symptoms that correlate with streptococcal infections should be done, as well as documentation of evidence of negative throat cultures or antibody titers when the patient goes into remission of neuropsychiatric symptoms.
5. *Presence of neurologic abnormalities during periods of symptom exacerbation.* Choreiform movements seen in PANDAS patients are small, jerky movements that occur in an irregular and arrhythmic pattern in different muscles,[147] described as fine piano-playing movements of the fingers. These movements are distinct from the movements of Sydenham chorea because choreiform movements are not present at rest and must be elicited through stressed posture, and in the case of chorea the movements are constantly present and increase with unrelated voluntary movements.[146] Patients with a diagnosis of rheumatic fever, including chorea, should be excluded from the PANDAS subgroup.[147] Children considered to have possible PANDAS syndrome who have symptoms suggestive of streptococcal infection should undergo evaluation for GAS infection. Rapid streptococcal antigen tests backed by throat culture if negative should be obtained. In patients who have negative throat cultures, ASO and anti–DNAse B titers could be obtained if the onset of PANDAS was at least 4 to 6 weeks earlier. With acute exacerbations of tics or obsessive-compulsive disorder symptoms, patients should be reevaluated for an acute infection resulting from GAS.[145]

A diagnostic marker present in children with rheumatic fever—D8/17—has been postulated to also identify children with PANDAS,[148] but its use in diagnosis of PANDAS remains controversial.[131]

Treatment

All patients who have an acute GAS infection should receive appropriate antibiotic therapy, usually with penicillin, because it remains the drug of choice. Treatment of the streptococcal infection has been noted by some authors to improve or cure the neuropsychiatric symptoms of PANDAS. A small prospective study[101] of 12 patients showed resolution of symptoms of new-onset obsessive-compulsive disorder, anxiety, and tics within an average of 14 days after starting treatment with an antibiotic (i.e., penicillin, amoxicillin, amoxicillin/clavulanate, or a cephalosporin). Every recurrent episode of PANDAS was associated with a GAS infection, and all patients with recurrences showed improvement after treatment with antibiotics.[101] However, this was a small study, not a double-blind, randomized controlled trial.

Children with a significant tic disorder or obsessive-compulsive disorder should receive appropriate psychiatric medications as necessary. Medications used in the treatment of tics include α agonists. For the treatment of obsessive-compulsive disorder, medications used include classic or atypical antipsychotics and selective serotonin reuptake inhibitors. Cognitive behavioral therapy also may be beneficial in the treatment of obsessive-compulsive disorder. These treatments are commonly used in tics and obsessive-compulsive disorder not related to PANDAS and also have been used for treatment of neuropsychiatric symptoms in patients given a diagnosis of PANDAS.[84]

Immunomodulators have been used in therapeutic trials based on the proposed model of immunologic dysfunction. One study reported benefits in patients fulfilling criteria for PANDAS in the group treated with plasma exchange and in the group treated with intravenous immunoglobulin (IVIG).[110] However, this study was limited by small sample size, highly selective recruitment, and limited comparisons with controls. In addition, nearly two thirds of patients receiving active treatment experienced adverse effects.[110] Currently, the National Institute of Mental Health recommends that plasma exchange and IVIG be reserved only for patients involved in approved research protocols.[103]

Prevention

The pathogenesis of PANDAS has been proposed to be an autoimmune process similar to rheumatic fever and Sydenham chorea.[144] Penicillin is recommended to prevent recurrences of the latter two conditions. A few clinical trials have been performed to determine whether antibiotic prophylaxis to prevent GAS infection can help reduce the neuropsychiatric morbidity seen in PANDAS and prevent future exacerbations. In a placebo-controlled,

double-blind crossover study involving 37 children with PANDAS published in 1999, penicillin failed to prevent streptococcal infections and no differences were found in the severity of obsessive-compulsive disorder and tics between the two phases.[53] A double-blind, randomized controlled trial of 23 children published in 2005 compared penicillin (as "active placebo") and azithromycin in the prevention of GAS infection and exacerbation of PANDAS. Both penicillin and azithromycin prophylaxis were found to be effective in the prevention of recurrent GAS infections and decreased the neuropsychiatric symptom exacerbations in the PANDAS subgroup.[139] However, this study had a small sample size and lacked an inactive placebo group. At present, antibiotics are recommended only to treat the acute GAS infection and are not indicated for the prophylaxis of PANDAS recurrences.[145,146]

Arguments Against PANDAS

Considerable controversy still exists surrounding the topic of PANDAS.[92,135] At this moment, it remains a hypothesis, and many argue that sufficient evidence is lacking to support PANDAS as a diagnostic entity.[132,133] For instance, a recent prospective longitudinal study comparing PANDAS and non-PANDAS cases of Tourette syndrome and obsessive-compulsive disorder did not show a temporal relation between exacerbation of neuropsychiatric symptoms and a GAS infection in the PANDAS group (a few exacerbations of symptoms were associated with a GAS infection in the control group).[87] An argument has even been proposed that the initial cases with choreiform movements could represent missed cases of chorea.[84] The authors in a study showing failure of immune markers to correlate with clinical exacerbations in PANDAS question the autoimmune pathogenesis of the disorder.[134] Similar concerns were raised by a study that failed to show differences in serum autoantibodies between PANDAS and Tourette syndrome (controls).[100]

Further research is needed to establish PANDAS as a clinical entity, to further describe the pathogenesis of the disease, demonstrate disease in animal models, and determine its appropriate treatment and prevention in randomized double-blind, placebo-controlled trials.

Updates to PANDAS Definition

Because the concept of PANDAS has generated much controversy and conflict regarding the scientific evidence to support this hypothesis, many researchers and clinicians have recently proposed elimination of the diagnosis of PANDAS in favor of a broader diagnostic category.[135] Many other infectious agents in addition to GAS have been suggested as possible causes of acute neuropsychiatric symptoms, including *Borrelia burgdorferi*, herpes simplex virus, varicella-zoster virus, and *Mycoplasma pneumoniae*.[135,149] Singer and colleagues[135] proposed moving away from PANDAS and using the term *childhood-onset acute neuropsychiatric symptoms (CANS)*. This definition requires only an acute, dramatic onset of symptoms, but the authors emphasize the importance of a thorough history, physical examination, and differential diagnosis to search for a specific etiology and treat accordingly.

Even though debate has continued about the role of GAS in the pathogenesis of PANDAS, experts on either side still agree on the existence of a subgroup of children with obsessive-compulsive disorder with an abrupt onset of symptoms.[149] To the PANDAS controversy, a group of clinicians and scientists met in July, 2010 for a workshop on PANDAS. During this workshop, modifications to the PANDAS criteria were made to broaden the spectrum of acute-onset obsessive-compulsive disorder and related neuropsychiatric disorders. It was decided that the new syndrome should not exclude cases with postpubertal onset. One of the names proposed was CANS, but it was not favored because childhood onset would exclude adolescents.[149] The consensus of the workshop members was to use the term *pediatric acute-onset neuropsychiatric syndrome (PANS)* because it would extend the age to at least 18 years (and 21 years by some definitions).[149] The three PANS criteria, as defined by Swedo and colleagues,[149] are (1) abrupt, dramatic onset of obsessive-compulsive disorder or severely restricted food intake; (2) concurrent presence of additional neuropsychiatric symptoms, with similarly severe and acute onset, from at least two of the following seven categories: (a) anxiety; (b) emotional lability and/or depression; (c) irritability, aggression, and/or severely oppositional behaviors; (d) behavioral (developmental) regression; (e) deterioration in school performance; (f) sensory or motor abnormalities; and (g) somatic signs and symptoms, including sleep disturbances, enuresis, or urinary frequency; and (3) symptoms not better explained by a known neurologic or medical disorder, such as Sydenham chorea, systemic lupus erythematosus, Tourette disorder, or others. Patients thought to have PANS should undergo a comprehensive workup, which may include magnetic resonance imaging, lumbar puncture, or electroencephalogram, if necessary, to rule out other neuropsychiatric disorders.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

A combination of clinical appearance, identification of the organism, and epidemiologic findings are used to confirm the probability of pharyngitis or tonsillitis caused by GAS. Multiple etiologic agents may be responsible for pharyngitis, compounding the difficulty in the diagnosis of GAS as the cause.[2] Pharyngitis, characterized by the triad of fever, sore throat, and pharyngeal inflammation, may be caused by multiple viruses, *Corynebacterium diphtheriae*, *Neisseria gonorrhoeae*, *Fusobacterium necrophorum*, and groups C and G streptococci, in addition to GAS. The clinical syndrome associated with *Arcanobacterium hemolyticus* can be very similar to that associated with GAS.

Multiple clinical prediction rules have been developed to aid in the diagnosis of GAS pharyngitis. Acute pharyngitis attributable to GAS may be aided by the use of clinical scoring systems.[32,94,95] These prediction rules are limited because the signs and symptoms of many viral causes of acute pharyngitis overlap with infection caused

by GAS. The rules are best at identifying patients with a low probability for GAS infection. Thus, the guidelines from the Infectious Diseases Society of America (IDSA), the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP), and the American Heart Association recommend confirmation of GAS infection by rapid antigen detection testing, throat culture, or both.[5,16,38] Clinical manifestations that are most suggestive of GAS pharyngitis include the scarlatiniform rash, excoriated nares (especially in infants), tender (may or may not be enlarged) anterior cervical lymph nodes, and a history of close contact with a known case of GAS infection. Cough, hoarseness, or conjunctivitis make the diagnosis of streptococcal pharyngitis far less likely. Exudative pharyngitis in infants usually is nonstreptococcal in etiology.

Empirical therapy based on the use of predication rules has been implicated in the overuse of antibiotics for the treatment of pharyngitis.[95] A study on the effectiveness of these strategies showed that empirical treatment is a reasonable strategy for those least likely to have GAS pharyngitis but microbiologic confirmation was the most effective and least expensive when considering all factors.[105] In addition, in 78 percent of cases, physicians did not adhere to any guideline, leading to overuse of antibiotics for the treatment of pharyngitis.[88] More recently, it was shown that incorporating local GAS prevalence rates into prediction rules reduced the number of missed cases and the misuse of antibiotics where they were unnecessary.[47]

Cultures may yield invalid results unless they are obtained and processed carefully. Assaying for the presence of GAS by throat culture on sheep blood agar plate, as first described by Breese and Disney,[20] has been accepted practice for diagnosing streptococcal pharyngitis for over 50 years. Ideally, specimens should be obtained from bilateral tonsillar surfaces and the posterior pharynx while avoiding the mouth and other surfaces of the pharynx. Impetiginous lesions should be cleansed with alcohol, and the vesicle should be punctured or the crust lifted by a sterile needle so that the moist base or purulent material can be touched with the swab.

Numerous rapid techniques for direct identification of GAS from the upper respiratory tract are available commercially. Rapid antigen detection testing has become more readily available and in some instances has been reported to equal or exceed the sensitivity and specificity of throat culture. Rapid testing may lead to more timely treatment and, in so doing, can reduce the spread of GAS, reduce time missed from school or work, and minimize suppurative and nonsuppurative sequelae of GAS. The original rapid antigen detection tests were based on the detection of GAS cell wall carbohydrate antigen by enzyme immunoassays. Subsequently, optical immunoassays became commercially available and initially were thought to provide greater sensitivity than the previous generation of tests. Owing to differences in the manufacturer, test, and study design, the sensitivities and specificities of all rapid antigen detection tests are essentially the same.[55] Newer assays employ molecular biology methods to detect DNA specific to GAS by chemiluminescence or real-time polymerase chain reaction, but are restricted because of the need to batch specimens and the specialized equipment required. The sensitivity of rapid antigen detection tests is 70 to 90 percent, in contrast to blood agar plate culture, with a specificity of approximately 95 percent.[57] Of importance, rapid antigen detection test specimens must be collected in a manner similar to specimen collection for blood agar plate culture. Swabs obtained from the mouth and subjected to rapid antigen detection testing have a sensitivity of less than 20 versus 80 percent for those obtained properly from the posterior pharynx and tonsils.[50] The current recommendation is that a negative rapid antigen detection test be confirmed by blood agar plate culture for all children.[5,16,38]

In addition to microbiologic and rapid antigen detection tests, immunologic tests for the presence of GAS frequently are used to assist in the diagnosis of GAS infection. The ASO test is used most frequently.[130] However, streptolysin O is also produced by groups C and G streptococci; thus, the test is not specific for GAS infection. Patients with skin infections caused by GAS may have a feeble response to streptolysin O.[79] In contrast, anti-DNAse B and antihyaluronidase are more reliable after either skin or throat infections.[79]

Streptozyme agglutination, another antibody test, is based on antibody agglutination of erythrocytes coated with a mixture of streptococcal extracellular antigens. Peak titers for an immune response as measured by the Streptozyme test appear within the first 7 to 10 days after infection,[107] whereas ASO (3 to 6 weeks) or anti-DNAse B (6 to 8 weeks) do not peak until later. Recent studies indicate that the initial titer of either may not be indicative of infection and that elevation after measurement of sequential titers is more accurate.[75] Given the delay in antibody response and need for sequential measurement, ASO anti-DNAse B should not be used to determine acute infection.

TREATMENT

GAS remain universally susceptible to penicillin in vitro. Oral penicillin V and intramuscular benzathine penicillin are the drugs of choice for the treatment of GAS, except in patients with a penicillin allergy.[57] The advantages of penicillin include a narrow spectrum of activity, proved efficacy, and low cost.[57]

Initial studies on the efficacy of penicillin for the primary prevention of acute rheumatic fever were conducted with military recruits in the 1950s.[40,157,158] Initial studies involved the use of injectable penicillin suspended in either sesame oil or peanut oil, and they showed therapeutic penicillin concentrations in serum for 9 to 10 days.[158] Later studies found an equal bacterial eradication rate of 10 days with either oral penicillin G or injectable benzathine penicillin.[157] Oral penicillin remains the first-line therapy because benzathine penicillin G is associated with pain and increased risk for allergic reactions.[112] However, in certain situations in which adherence to regimen or follow-up is not possible, intramuscular benzathine penicillin is preferred.

The established regimen is 10 days of penicillin V treatment or a single dose of benzathine penicillin to

TABLE 82-1 **Primary Prevention of Rheumatic Fever (Treatment of Streptococcal Tonsillopharyngitis)***

Agent	Dose	Mode	Duration	Rating
Penicillins				
Penicillin V (phenoxymethyl penicillin)	Children: 250 mg 2-3 times per day for ≤27 kg (60 lb); children >27 kg (60 lb), adolescents, and adults: 500 mg 2-3 times per day	Oral	10 days	IB
	or			
Amoxicillin	50 mg/kg/day (maximum 1 g)	Oral	10 days	IB
	or			
Benzathine penicillin G	600,000 U for patients ≤27 kg (60 lb); 1,200,000 U for patients >27 kg (60 lb)	Intramuscular	Once	IB
For individuals allergic to penicillin				
Narrow-spectrum cephalosporin† (cephalexin, cefadroxil)	Variable	Oral	10 days	IB
	or			
Clindamycin	20 mg/kg/day divided in 3 doses (maximum 1.8 g/day)	Oral	10 days	IIaB
	or			
Azithromycin	12 mg/kg/day (maximum 500 mg)	Oral	5 days	IIaB
	or			
Clarithromycin	15 mg/kg/day divided in 2 doses (maximum 250 mg bid)	Oral	10 days	IIaB

From Gerber MA, Baltimore RS, Eaton CB, et al: Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research—endorsed by the American Academy of Pediatrics. *Circulation* 119:1544, 2009.

Rating indicates classification of recommendation and level of evidence (LOE) (e.g., IB indicates class I, LOE B)

*For other acceptable alternatives, see text. The following are not acceptable: sulfonamides, trimethoprim, tetracyclines, and fluoroquinolones.

†To be avoided in those with immediate (type I) hypersensitivity to penicillin.

prevent acute rheumatic fever.[17,57] The most recent American Heart Association guidelines for the prevention of rheumatic fever and treatment of acute streptococcal pharyngitis[57] recommend dosages of penicillin V 40 mg/kg (not to exceed 750 mg for those weighing <27 kg) per 24 hours given in three equally divided doses for 10 days. The dose recommended for most children is 250 mg twice per day, and the dose usually recommended for adolescents and adults is 500 mg two or three times per day (Table 82-1).[57] Penicillin V is preferred to penicillin G because it is more resistant to gastric acid. Although penicillin V is the recommended drug, many physicians prescribe amoxicillin because of its improved taste. Recently, an oral time-released formulation of amoxicillin was approved by the U.S. Food and Drug Administration (FDA) for once-daily therapy for patients 12 years of age and older.[57]

For patients with a penicillin allergy, a 10-day oral course of a narrow-spectrum cephalosporin (e.g., cefadroxil or cephalexin) is recommended. Some studies have shown that 10 days of an oral cephalosporin is superior to a 10 day-course of oral penicillin.[61,114] Other studies suggest that treatment with 5 days of an oral broad-spectrum cephalosporin is comparable to treatment with 10 days of oral penicillin in the eradication of GAS tonsillopharyngitis.[57,115] However, at present these shorter regimens have not been approved by the FDA. In the past, erythromycin was the drug of choice for penicillin-allergic patients, but it has been replaced by newer medications because they have lower rates of gastrointestinal side effects and easier administration regimens.[33,51,57] In patients with an immediate hypersensitivity (anaphylactic type) to penicillin, cephalosporins should be avoided, and macrolides (i.e., erythromycin or clarithromycin), azithromycin (an azalide), or clindamycin (a lincosamide) should be used. Recommended doses are shown in Table 82-1.

Macrolide resistance has been documented in many countries and remains an ongoing concern for the treatment of GAS infections.[51,89] Although erythromycin-resistant organisms were found in the 1950s, it was not until the 1970s that clinically significant macrolide resistance was noted.[51] Resistance to macrolides occurs by one of two mechanisms: an active efflux pump (M phenotype) related to enzymes encoded for by *mef* genes or ribosomal target site modification by methylation (*erm* genes), which causes coresistance to macrolide, lincosamide, and streptogramin (MLS) antibiotics (MLS phenotype).[89] Countries such as Japan, Finland, Italy, and the United States have reported treatment failures resulting from macrolide resistance.[51] A study in Finland[127] reported a decrease in the use of macrolide antibiotics from 2.40 defined daily doses per 1000 inhabitants per day in 1991 to 1.38 in 1992, after national recommendations were

made to reduce the number of macrolides prescribed for respiratory and skin infections. This resulted in a decrease in macrolide resistance from 16.5 percent in 1992 to 8.6 percent in 1996.[127] Although the rate of resistance in the United States is relatively low in contrast to much of the rest of the world, a recent U.S. multicenter surveillance study[62] found an overall adjusted rate of macrolide resistance in GAS of 5.2 percent. This represents a near twofold increase from rates reported in the United States in the 1990s. In recent years, U.S. macrolide resistance rates have ranged between 5 and 8 percent.[5] To avoid further increases in macrolide resistance that may result in treatment failures and acute rheumatic fever, some experts recommend that macrolide antibiotics be reserved for the treatment of GAS infections in patients with the anaphylactic type of penicillin allergy.[89]

Certain broad-spectrum antibiotics should not be used against GAS. Tetracycline has a high prevalence of resistance and thus is not recommended. Sulfonamides and trimethoprim-sulfamethoxazole should be avoided because they do not eradicate GAS from the pharynx. Ciprofloxacin has limited activity against GAS and therefore should not be used. Newer fluoroquinolones, such as levofloxacin and moxifloxacin, have shown in vitro activity against GAS but should not be used because they are expensive and provide an excessive broad-spectrum activity.[37,54,57]

Even though penicillin remains the drug of choice in the treatment of GAS, penicillin treatment failures of 20 to 40 percent or higher have been reported in the literature.[113] It has been documented more often after oral penicillin than after benzathine penicillin.[45] Possible explanations for penicillin treatment failures include lack of compliance, penicillin tolerance, and carrier state.[57,113] Other considerations that have been proposed include copathogens in the pharynx[60,113,114,136] and antibiotic suppression of immunity.[21,57,159] Some authors have suggested that certain β-lactamase organisms such as *Staphylococcus aureus*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and anaerobes may be present in the pharynx during a GAS infection and inactivate penicillin. They argue that this may be one of the reasons for the superiority of β-lactamase stable antibiotics in cases of penicillin failures.[115] Studies from the 1940s and 1950s documented that antibiotics given shortly after onset of symptoms (within 24 to 48 hours) could suppress the immune response to the infection.[21,159] Other studies did not show a difference whether treatment was started early or delayed for 48 hours.[56] It has been shown that penicillin therapy is effective in preventing rheumatic fever when started within 9 days after the onset of illness.[29,57] Delaying therapy for 24 to 48 hours until culture results are known does not increase the risk for rheumatic fever.[57] However, therapy should not be delayed in patients who appear toxic or are severely ill.

At present, recommendations in the management of GAS pharyngitis in children include obtaining a streptococcal rapid antigen detection test, with initiation of treatment when positive. Negative rapid antigen detection tests should be backed by a throat culture. Follow-up cultures in patients treated for GAS are no longer routinely recommended and should be performed only in those who remain symptomatic, have recurrence of symptoms, or have a history of rheumatic fever.[17,57] Children with recurrence of GAS pharyngitis symptoms after completing a 10-day course of a recommended oral antimicrobial agent can be retreated with the same antimicrobial agent, given an alternative oral drug, or given an intramuscular dose of penicillin G benzathine, especially if noncompliance is suspected. Alternative drugs include a narrow-spectrum cephalosporin, amoxicillin-clavulanate, clindamycin, a macrolide, azalide, or penicillin plus rifampin.[5,54]

Individuals found to have positive rapid antigen detection test or throat cultures without clinical evidence of infection or rise in antistreptococcal titers are considered chronic streptococcal carriers and do not need to be treated with antibiotics. However, because it may be difficult to distinguish a carrier state from true GAS infection when a viral upper respiratory tract infection develops in a carrier, appropriate antibiotics should be prescribed to any patient with acute pharyngitis and evidence of GAS from a positive rapid antigen detection test or throat culture.[57]

Although guidelines from the American Heart Association exist for the treatment of GAS tonsillopharyngitis, no official guidelines exist for the treatment of streptococcal impetigo. For patients with a few lesions, topical therapy with mupirocin or retapamulin ointment may be useful for limiting person-to-person spread and for eradicating localized disease.[5] In more severe cases with multiple lesions or with nonbullous impetigo in multiple family members, child care groups, or athletic teams, impetigo should be treated with systemic antimicrobials.[5] Oral or parenteral penicillin may be used in the same doses as those for streptococcal infections. Regimens with first-generation cephalosporins or macrolides should be used for penicillin-allergic patients. With the increase in skin and soft tissue infections resulting in methicillin-resistant *Staphylococcus aureus* (MRSA), obtaining appropriate cultures and treating with antibiotics to cover this possible pathogen also must be considered.

Although treatment with penicillin has been shown to prevent the development of rheumatic fever, no definitive evidence exists that antimicrobial therapy after treatment of streptococcal skin infections prevents acute poststreptococcal glomerulonephritis.[5]

Otitis media and cervical adenitis resulting from GAS can be treated with the same regimens used for the treatment of streptococcal pharyngitis. Systemic parenteral antimicrobial therapy is indicated for severe infections, such as septicemia, pneumonia, endocarditis, meningitis, arthritis, osteomyelitis, erysipelas, necrotizing fasciitis, neonatal omphalitis, and streptococcal toxic shock syndrome. Treatment often is prolonged (2 to 6 weeks).[5] In the case of toxic shock syndrome, clindamycin is also recommended because it is a protein-synthesis inhibitor and can neutralize bacterial toxins. Drainage of any lesions is warranted. IVIG can be considered as adjunctive therapy for GAS-related toxic shock syndrome or necrotizing fasciitis if the patient is severely ill.[5]

PREVENTION

Treatment of GAS pharyngitis with antimicrobials aims to prevent rheumatic fever through primary prevention. Prevention of recurrence of rheumatic fever (secondary prevention) requires continuous antimicrobial prophylaxis (see Chapter 29). A meta-analysis of 10 trials on primary prevention of rheumatic fever that were conducted in the United States during 1950 to 1961 revealed that, although the methodology of the studies was poor, administration of antibiotics in patients with GAS infection reduced the risk for acute rheumatic fever by nearly 70 percent.[121] A subgroup analysis showed that the risk reduction was 80 percent when treated with intramuscular penicillin.[121]

Although the incidence of acute rheumatic fever has decreased in the developing world for the past 50 years, outbreaks in the community and the military continue to occur, and acute rheumatic fever remains a major cause of morbidity and mortality in the developing world.[36] Current strategies for disease prevention include infection-control measures such as isolation, appropriate hygiene measures for postpartum and postsurgical patients, and establishing nationwide surveillance systems.[36] During episodes of epidemic GAS infections, mass prophylaxis with penicillin, particularly intramuscular benzathine penicillin G, has been shown effective.

Children in the outpatient setting being treated for a GAS infection may return to daycare or school after 24 hours of treatment. For hospitalized children, droplet precautions are recommended for GAS pharyngitis or pneumonia until 24 hours after initiation of appropriate antimicrobial therapy. In the case of burns with secondary GAS infection and extensive or draining cutaneous infections that cannot be covered by dressings, patients should remain in contact precautions for at least 24 hours after the start of appropriate therapy.[5] Asymptomatic household contacts do not require diagnostic evaluation or chemoprophylaxis, unless exposure occurs during outbreaks or the contacts are at higher risk for developing sequelae of infection.[5]

In certain cases of GAS pharyngitis refractory to treatment, tonsillectomy has been performed, but at present, data are insufficient to recommend routine tonsillectomy as part of the management of GAS pharyngeal infections.

The persistence of acute rheumatic fever and its associated morbidity and mortality, especially in the developing world, despite the use of antimicrobials, has led to intensive research in vaccine development against GAS. However, despite more than 70 years of research, a commercial vaccine is still not available.[36] One of the main concerns for development of vaccines based on the M protein was the possibility of causing autoantibodies that could cross-react with the heart, skeletal muscle, brain, and glomerular basement membrane tissues.[33,36] Early studies in the 1960s found a higher incidence of acute rheumatic fever in those vaccinated than in the general population.[33] Two vaccines have been evaluated in clinical trials and found to be well tolerated and safe. One of these is a 26-valent vaccine including 86 percent of the serotypes causing GAS pharyngitis, which could prevent 49 to 63 percent of invasive GAS infections among children and 43 to 50 percent among elderly individuals, with similar decreases in the number of deaths.[108] It was tested in 30 healthy adults in the United States and was found to be immunogenic and safe.[36] However, because of the geographic and temporal differences in M-types between countries, this vaccine may not be as effective in other countries, such as Japan, Ethiopia, or New Zealand.[36,124] Other components of GAS have been under investigation as potential candidates in the development of a vaccine. These include carbohydrate antigens, adhesins, C5a peptidase, and the GAS pilus.[33] Mucosal vaccines to GAS are also under investigation because mouse studies have found induction of specific IgA production during intranasal administration of vaccine, along with IgG production in serum.[33] Further research is needed in GAS vaccine development, with a goal of vaccines that may be useful globally.

GROUP C AND GROUP G STREPTOCOCAL INFECTIONS

Organisms

The β-hemolytic streptococci include GAS, *Streptococcus agalactiae* (group B streptococcus), and groups C, F, and G streptococci, based on the β-hemolysis they produce on blood agar plates. In addition, *Streptococcus milleri* organisms (*Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*) may have variable hemolysis, and approximately one third have group A, C, F, or G antigens.[6] Depending on their colony size, strains with either the group C or group G Lancefield carbohydrate antigens can be divided into small or large colony–forming strains. The small colony–forming strains (<0.5 mm in diameter) belong to the *S. anginosus (S. milleri)* group, which are considered part of the viridans group streptococci. Large colony–forming strains (>0.5) are considered true group C or group G streptococci (GCS and GGS, respectively).[23]

Lancefield group carbohydrate antigen and hemolytic reaction have been classically used for the identification of streptococci. However, streptococcal taxonomy has been significantly revised recently because of overlap between species. Currently, three species of groups C and G have been described that are pathogenic in humans: *Streptococcus dysgalactiae*, *Streptococcus equi* subsp. *zooepidemicus*, and *Streptococcus canis*.[23]

Group C streptococci (GCS) are catalase-negative, aerobic, facultatively anaerobic, coprophilic organisms. They are usually β-hemolytic, although all types of hemolysis have been seen with GCS. The group C antigenic determinant in their cell wall is rhamnose-*N*-acetylgalactosamine. Four species that possess this antigenic determinant were defined based on their ability to ferment various carbohydrates (*Streptococcus equisimilis*, *S. zooepidemicus*, *S. equi*, and *S. dysgalactiae*). Revision of the taxonomy after new research based on genetic studies (such as 16S RNA analysis) has shown that there are actually two GCS species, S. *dysgalactiae* and *S. equi*, each with two subspecies.

Group G streptococci (GGS) typically show β-hemolysis on blood agar plates. They are facultative anaerobes. The group G antigenic determinant in their cell wall is L-rhamnose. The majority of GGS infections in humans are due to *S. dysgalactiae* subsp. *equisimilis*, but a few infections with *S. canis* have been reported.

Some of the same virulent factors found in GAS have been shown to be present in GCS and GGS, such as M protein, streptolysin, hyaluronidase, and C5a peptidase.[67,71,90,125]

Epidemiology

GCS and GGS in humans may colonize the skin, oropharynx, gastrointestinal tract, and vagina.[6] In addition, GCS have been cultured from the umbilicus of asymptomatic newborns and from routine puerperal vaginal samples. Classically, these streptococcal groups have been thought to be a rare cause of infection in humans. They are common causes of animal infections, and many humans infected with these streptococci report animal contact. GCS and GGS have been known to cause foodborne outbreaks of pharyngitis.[6]

The classification and nomenclature of GCS and GGS has changed significantly, however, and clinical specimens are rarely identified to the species level by clinical laboratories, making it difficult to know the true burden of these infections. The first population-based study of the epidemiology and burden of invasive disease resulting from non–group A or B β-hemolytic streptococci (NABS) in the United States over a 2-year period was published in 2009.[23] According to this study, the majority of patients (87%) with invasive disease had underlying comorbid conditions, as has been previously reported.[67,90] *S. dysgalactiae* subsp. *equisimilis* was the most common organism (80%) among patients with invasive disease. However, the data for individuals younger than 20 years of age (6% of the patients) was different. Only 23 percent of the younger patients had underlying conditions, with asthma as the most common comorbidity. Isolates from pediatric patients were most often group F (40%), followed by group G (36%) and group C (24%). One very important finding of this study is that the burden of NABS infection in the United States during the study period was similar to that caused by invasive GAS infection: 3.2 cases/100,000 population and 2.89 cases/100,000, respectively.[23] A 6-month surveillance study published in Argentina in 2005 reported similar findings that most of the GCS and GGS *S. dysgalactiae* subsp. *equisimilis* infections occurred in adults and that diabetes was frequently seen as an underlying condition.[90]

Clinical Manifestations

GCS and GGS may cause invasive disease in infants, older children, and adults. The principal clinical manifestations of these organisms are upper and lower respiratory tract infections, septicemia, pneumonitis, skin and soft tissue infections, septic arthritis, osteomyelitis, meningitis with a parameningeal focus, brain abscess, puerperal and neonatal sepsis, peritonitis, and endocarditis.[6,8,154]

Pharyngitis caused by GCS and GGS has symptoms similar to those seen with GAS pharyngitis. GCS are a relatively common cause of acute pharyngitis among college students and among adults seen in the emergency department.[57] Between 1 and 18 percent of asymptomatic pharyngeal carriers of GCS may be found in temperate climates, with higher percentages in the tropics. Similarly, between 1 and 23 percent of asymptomatic pharyngeal carriers of GGS may live in temperate climates. In addition, foodborne episodes of pharyngitis caused by GCS and GGS have been documented.[6] These epidemic outbreaks may occur after consumption of contaminated products, such as unpasteurized cow's milk.

Acute rheumatic fever has not been documented as a complication of either GCS- or GGS-associated pharyngitis.[57] Acute glomerulonephritis has been reported as a complication of GCS-associated pharyngitis, but a causal relationship has not been established.

Rare neonatal GGS and GCS infections have been reported, with symptoms similar to those of early-onset GBS sepsis.

GCS and GGS have been reported as a rare cause of skin and soft tissue infections. A report reviewing all cases of septic arthritis caused by β-hemolytic streptococci at Stanford University Medical Center in California from 1985 through 1996 found that 40 percent of cases were due to GAS, 30 percent to GBS, and 30 percent to GGS. No cases caused by GCS were reported during that time. Presence of infected prosthetic implants was found in the majority of patients with septic arthritis caused by GGS.[125] Rare cases of deep soft tissue infection resulting from GGS, such as necrotizing fasciitis, localized pyomyositis, and even myositis with subsequent fatal streptococcal toxic shock syndrome have been reported in the literature.[154]

Streptococcal toxic shock syndrome typically has been associated with GAS, but recent case reports have shown cases resulting from GCS and GGS infection. Most of the cases have been due to *S. dysgalactiae* subsp. *equisimilis*, but a recent study from Japan[67] reported the first case of streptococcal toxic shock syndrome caused by *S. equi* subsp. *zooepidemicus*. Because most clinical isolates of GCS and GGS are β-hemolytic and some isolates are susceptible to bacitracin, some GCS and GGS may have been misidentified as GAS if Lancefield serologic testing was not performed.[67] Therefore, the true rates of these diseases may be underestimated. Patients with GAS-related streptococcal toxic shock syndrome usually are previously healthy, whereas patients with infections caused by GCS and GGS, including streptococcal toxic shock syndrome, usually have underlying conditions such as diabetes mellitus, cardiopulmonary disease, malignancy, collagen disease, liver failure, or renal failure.[67]

Although rare, some cases of bacterial endocarditis may be caused by GCS and GGS infections. The mortality rates for these infections are high, reported as 41 percent for GCS and 35 percent for GGS, although the overall mortality rate of infective endocarditis by β-hemolytic streptococci was much lower (12.9%) in a questionnaire study by Baddour and the IDSA's Emerging Infections Network (EIN) members.[10]

Treatment

Penicillin remains the treatment of choice for GCS and GGS infections.[8,122] Penicillin-tolerant strains have been reported in laboratory findings, but the clinical significance is not known.[8,122] For most GCS and GGS infections, treatment with penicillin G alone is adequate. Other antimicrobial regimens that have been shown to work in vitro include penicillin with gentamicin, other β-lactam agents such as cephalosporins, vancomycin, and vancomycin with rifampin.[6] Resistance to macrolides, tetracycline, and chloramphenicol have been reported in the literature.[23,67,90] A few isolates of GCS and GGS *S. dysgalactiae* subsp. *equisimilis* have shown high-level resistance to aminoglycosides.[23,90]

Guidelines for the treatment of endocarditis caused by GCS and GGS have not been established because these infections are infrequent. Usually, high-dose penicillin G is recommended for 4 to 6 weeks. Some experts recommend the addition of gentamicin to penicillin for at least the first 2 weeks of therapy.[10]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Aguero J, Ortega-Mendi M, Eliecer Cano M, et al. Outbreak of invasive group A streptococcal disease among children attending a day-care center. *Pediatr Infect Dis J* 2008;**27**:602–4.
3. Allen AJ, Leonard HL, Swedo SE. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. *J Am Acad Child Adolesc Psychiatry* 1995;**34**:307–11.
5. American Academy of Pediatrics. Group A streptococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, editors. *Red Book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2009. p. 616–28.
6. American Academy of P. Non-Group A or B streptococcal and enterococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 634–6.
9. Aziz RK, Kotb M. Rise and persistence of global M1T1 clone of Streptococcus pyogenes. *Emerg Infect Dis* 2008;**14**:1511–17.
10. Baddour LM. Infective endocarditis caused by beta-hemolytic streptococci. The Infectious Diseases Society of America's Emerging Infections Network. *Clin Infect Dis* 1998;**26**:66–71.
23. Broyles LN, Van Beneden C, Beall B, et al. Population-based study of invasive disease due to beta-hemolytic streptococci of groups other than A and B. *Clin Infect Dis* 2009;**48**:706–12.
28. Carroll RK, Musser JM. From transcription to activation: how group A Streptococcus, the flesh-eating pathogen, regulates SpeB cysteine protease production. *Mol Microbiol* 2011;**81**:588–601.
31. Centers for Disease Control and Prevention. *Streptococcus pyogenes emm sequence database*. http://www.cdc.gov/ncidod/biotech/strep/m-proteingene_typing.htm. 2012. Accessed April 28, 2012.
33. Chang C. Cutting edge issues in rheumatic fever. *Clin Rev Allergy Immunol* 2012;**42**:213–37.
39. Davis KM, Weiser JN. Modifications to the peptidoglycan backbone help bacteria to establish infection. *Infect Immun* 2011; **79**:562–70.
47. Fine AM, Nizet V, Mandl KD. Improved diagnostic accuracy of group A streptococcal pharyngitis with use of real-time biosurveillance. *Ann Intern Med* 2011;**155**:345–52.
50. Fox JW, Marcon MJ, Bonsu BK. Diagnosis of streptococcal pharyngitis by detection of Streptococcus pyogenes in posterior pharyngeal versus oral cavity specimens. *J Clin Microbiol* 2006; **44**:2593–4.
57. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research—endorsed by the American Academy of Pediatrics. *Circulation* 2009;**119**:1541–51.
75. Johnson DR, Kurlan R, Leckman J, Kaplan EL. The human immune response to streptococcal extracellular antigens: clinical, diagnostic, and potential pathogenetic implications. *Clin Infect Dis* 2010;**50**:481–90.
87. Leckman JF, King RA, Gilbert DL, et al. Streptococcal upper respiratory tract infections and exacerbations of tic and obsessive-compulsive symptoms: a prospective longitudinal study. *J Am Acad Child Adolesc Psychiatry* 2011;**50**:108–18 e103.
89. Logan LK, McAuley JB, Shulman ST. Macrolide treatment failure in streptococcal pharyngitis resulting in acute rheumatic fever. *Pediatrics* 2012;**129**:e798–802.
92. Martino D, Defazio G, Giovannoni G. The PANDAS subgroup of tic disorders and childhood-onset obsessive-compulsive disorder. *J Psychosom Res* 2009;**67**:547–57.
100. Morris CM, Pardo-Villamizar C, Gause CD, Singer HS. Serum autoantibodies measured by immunofluorescence confirm a failure to differentiate PANDAS and Tourette syndrome fr m controls. *J Neurol Sci* 2009;**276**:45–8.
106. Nobbs AH, Lamont RJ, Jenkinson HF. Streptococcus adherence and colonization. *Microbiol Mol Biol Rev* 2009;**73**:407–50.
109. Parks T, Smeesters PR, Steer AC. Streptococcal skin infection and rheumatic heart disease. *Curr Opin Infect Dis* 2012;**25**:145–53.
117. Rainbow J, Jewell B, Danila RN, et al. Invasive group a streptococcal disease in nursing homes, Minnesota, 1995-2006. *Emerg Infect Dis* 2008;**14**:772–7.
124. Safar A, Lennon D, Stewart J, et al. Invasive group A streptococcal infection and vaccine implications, Auckland, New Zealand. *Emerg Infect Dis* 2011;**17**:983–9.
128. Shaikh N, Leonard E, Martin JM. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. *Pediatrics* 2010;**126**:e557–564.
129. Shea PR, Beres SB, Flores AR, et al. Distinct signatures of diversifying selection revealed by full-genome analysis of the population structure of upper respiratory tract and invasive bacterial strains. *Proc Natl Acad Sci U S A* 2011;**108**:5039–44.
132. Shulman ST. Pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS): update. *Curr Opin Pediatr* 2009;**21**:127–30.
134. Singer HS, Gause C, Morris C, Lopez P. Serial immune markers do not correlate with clinical exacerbations in pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Pediatrics* 2008;**121**:1198–205.
135. Singer HS Gilbert DL, Wolf D, et al. Moving from PANDAS to CANS. *J Pediatr* 2012;**160**:725–31.
143. Sunnergren O, Swanberg J, Molstad S. Incidence, microbiology and clinical history of peritonsillar abscesses. *Scand J Infect Dis* 2008;**40**:752–5.
149. Swedo SE, Leckman JF, Rose NR. From research subgroup to clinical syndrome: modifying the PANDAS criteria to describe PANS (pediatric acute-onset neuropsychiatric syndrome). *Pediatr Therapeut* 2012;**2**:1–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aguero J, Ortega-Mendi M, Eliecer Cano M, et al. Outbreak of invasive group A streptococcal disease among children attending a day-care center. *Pediatr Infect Dis J* 2008;**27**:602–4.
2. Alcaide ML, Bisno AL. Pharyngitis and epiglottitis. *Infect Dis Clin North Am* 2007;**21**:449–69, vii.
3. Allen AJ, Leonard HL, Swedo SE. Case study: a new infection-triggered, autoimmune subtype of pediatric OCD and Tourette's syndrome. *J Am Acad Child Adolesc Psychiatry* 1995;**34**:307–11.
4. Aly R, Maibach HI, Shinefield HR, Strauss WG. Survival of pathogenic microorganisms on human skin. *J Invest Dermatol* 1972;**58**:205–10.
5. American Academy of Pediatrics. Group A streptococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 616–28.
6. American Academy of Pediatrics. Non-group A or B streptococcal and enterococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 634–6.
7. Amren DP. Unusual forms of streptococcal disease. In: Wannamaker LW, Matsen JM, editors. *Streptococci and streptococcal diseases: recognition, understanding, and management*. New York: Academic Press; 1972. p. 545–56.
8. Arditi M, Shulman ST, Davis AT, Yogev R. Group C beta-hemolytic streptococcal infections in children: nine pediatric cases and review. *Rev Infect Dis* 1989;**11**:34–45.
9. Aziz RK, Kotb M. Rise and persistence of global M1T1 clone of Streptococcus pyogenes. *Emerg Infect Dis* 2008;**14**:1511–17.
10. Baddour LM. Infective endocarditis caused by beta-hemolytic streptococci. The Infectious Diseases Society of America's Emerging Infections Network. *Clin Infect Dis* 1998;**26**:66–71.
11. Baldassarri L, Creti R, Imperi M, et al. Detection of genes encoding internalization-associated proteins in Streptococcus pyogenes isolates from patients with invasive diseases and asymptomatic carriers. *J Clin Microbiol* 2007;**45**:1284–7.
12. Beachey EH, Ofek I. Epithelial cell binding of group A streptococci by lipoteichoic acid on fimbriae denuded of M protein. *J Exp Med* 1976;**143**:759–71.
13. Beall B, Facklam R, Thompson T. Sequencing emm-specific PCR products for routine and accurate typing of group A streptococci. *J Clin Microbiol* 1996;**34**:953–8.
14. Beall B, Gherardi G, Lovgren M, et al. emm and sof gene sequence variation in relation to serological typing of opacity-factor-positive group A streptococci. *Microbiology* 2000;**146**(Pt 5): 1195–209.
15. Bessen DE, Carapetis JR, Beall B, et al. Contrasting molecular epidemiology of group A streptococci causing tropical and non-tropical infections of the skin and throat. *J Infect Dis* 2000;**182**: 1109–16.
16. Bisno AL, Gerber MA, Gwaltney Jr JM, Kaplan EL, Schwartz RH. Practice guidelines for the diagnosis and management of group A streptococcal pharyngitis. Infectious Diseases Society of America. *Clin Infect Dis* 2002;**35**:113–25.
17. Bisno AL, Gerber MA, Gwaltney Jr JM, et al. Group A streptococcal pharyngitis: diagnosis and management—a practice guideline. Infectious Diseases Society of America. *Clin Infect Dis* 2002;**35**: 113–25.
18. Bohach GA, Fast DJ, Nelson RD, Schlievert PM. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. *Crit Rev Microbiol* 1990;**17**:251–72.
19. Bordet J. *A contribution to the study of antistreptococcal serum*, vol. 108. New York: John Wiley and Sons; 1907.
20. Breese BB, Disney FA. The accuracy of diagnosis of beta streptococcal infections on clinical grounds. *J Pediatr* 1954;**44**:670–3.
21. Breese BB, Disney FA, Talpey WB. The prevention of type specific immunity to streptococcal infections due to the therapeutic use of penicillin: occurrence of second attacks due to the same type of group A hemolytic streptococci. *Am J Dis Child* 1960; **100**:353–9.
22. Brook I. Microbiology and management of peritonsillar, retropharyngeal, and parapharyngeal abscesses. *J Oral Maxillofac Surg* 2004;**62**:1545–50.
23. Broyles LN, Van Beneden C, Beall B, et al. Population-based study of invasive disease due to beta-hemolytic streptococci of groups other than A and B. *Clin Infect Dis* 2009;**48**:706–12.
24. Buchanan JT, Simpson AJ, Aziz RK, et al. DNase expression allows the pathogen group A Streptococcus to escape killing in neutrophil extracellular traps. *Curr Biol* 2006;**16**:396–400.
25. Bullowa JGM, Wishik SM. Complications of varicella: their occurrence among 2,534 patients. *Am J Dis Child* 1935;**49**: 923–6.
26. Carapetis JR, Currie BJ, Kaplan EL. Epidemiology and prevention of group A streptococcal infections: acute respiratory tract infections, skin infections, and their sequelae at the close of the twentieth century. *Clin Infect Dis* 1999;**28**:205–10.
27. Carapetis JR, Steer, Andrew C, et al. The global burden of group A streptococcal diseases. *Lancet Infect Dis* 2005;**5**:685–94.
28. Carroll RK, Musser JM. From transcription to activation: how group A Streptococcus, the flesh-eating pathogen, regulates SpeB cysteine protease production. *Mol Microbiol* 2011;**81**:588–601.
29. Catanzaro FJ, Stetson CA, Morris AJ, et al. The role of the streptococcus in the pathogenesis of rheumatic fever. *Am J Med* 1954;**17**:749–56.
30. Centers for Disease Control and Prevention. Prevention of invasive group A streptococcal disease among household contacts of case patients and among postpartum and postsurgical patients: recommendations from the Centers for Disease Control and Prevention. *Clin Infect Dis* 2002;**35**:950–9.
31. Centers for Disease Control and Prevention. *Streptococcus pyogenes emm sequence database*. http://www.cdc.gov/ncidod/biotech/strep/m-proteingene_typing.htm. 2012. Accessed April 28, 2012.
32. Centor RM, Witherspoon JM, Dalton HP, et al. The diagnosis of strep throat in adults in the emergency room. *Med Decis Making* 1981;**1**:239–46.
33. Chang C. Cutting edge issues in rheumatic fever. *Clin Rev Allergy Immunol* 2012;**42**:213–37.
34. Cleary PP, Kaplan EL, Handley JP, et al. Clonal basis for resurgence of serious Streptococcus pyogenes disease in the 1980s. *Lancet* 1992;**339**:518–21.
35. Cockerill 3rd FR, MacDonald KL, Thompson RL, et al. An outbreak of invasive group A streptococcal disease associated with high carriage rates of the invasive clone among school-aged children. *JAMA* 1997;**277**:38–43.
36. Cohen-Poradosu R, Kasper DL. Group A Streptococcus epidemiology and vaccine implications. *Clin Infect Dis* 2007;**45**: 863–5.
37. Coonan KM, Kaplan EL. In vitro susceptibility of recent North American group A streptococcal isolates to eleven oral antibiotics. *Pediatr Infect Dis J* 1994;**13**:630–5.
38. Dajani A, Taubert K, Ferrieri P, Peter G, Shulman S. Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young, the American Heart Association. *Pediatrics* 1995;**96**:758–64.
39. Davis KM, Weiser JN. Modifications to the peptidoglycan backbone help bacteria to establish infection. *Infect Immun* 2011;**79**: 562–70.
40. Denny Jr FW, Perry WD, Wannamaker LW. Type-specific streptococcal antibody. *J Clin Invest* 1957;**36**:1092–100.
41. Denny FW, Wannamaker LW, Brink WR, Rammelkamp Jr CH, Custer EA. Prevention of rheumatic fever; treatment of the preceding streptococcic infection. *J Am Med Assoc* 1950;**143**:151–3.
42. Engleberg NC, Heath A, Miller A, Rivera C, DiRita VJ. Spontaneous mutations in the CsrRS two-component regulatory system of Streptococcus pyogenes result in enhanced virulence in a murine model of skin and soft tissue infection. *J Infect Dis* 2001; **183**:1043–54.
43. Facklam R. What happened to the streptococci: overview of taxonomic and nomenclature changes. *Clin Microbiol Rev* 2002;**15**: 613–30.
44. Facklam RF, Martin DR, Lovgren M, et al. Extension of the Lancefield classification for group A streptococci by addition of 22 new M protein gene sequence types from clinical isolates: emm103 to emm124. *Clin Infect Dis* 2002;**34**:28–38.
45. Feinstein AR, Wood HF, Epstein JA, et al. A controlled study of three methods of prophylaxis against streptococcal infection in a population of rheumatic children. II. Results of the first three

years of the study, including methods for evaluating the maintenance of oral prophylaxis. *N Engl J Med* 1959;**260**:697–702.
46. Ferrieri P, Dajani AS, Wannamaker LW, Chapman SS. Natural history of impetigo. I. Site sequence of acquisition and familial patterns of spread of cutaneous streptococci. *J Clin Invest* 1972; **51**:2851–62.
47. Fine AM, Nizet V, Mandl KD. Improved diagnostic accuracy of group A streptococcal pharyngitis with use of real-time biosurveillance. *Ann Intern Med* 2011;**155**:345–52.
48. Fischetti VA. Streptococcal M protein: molecular design and biological behavior. *Clin Microbiol Rev* 1989;**2**:285–314.
49. Foley MJ, Wood Jr WB. Studies on the pathogenicity of group A streptococci. II. The antiphagocytic effects of the M protein and the capsular gel. *J Exp Med* 1959;**110**:617–28.
50. Fox JW, Marcon MJ, Bonsu BK. Diagnosis of streptococcal pharyngitis by detection of Streptococcus pyogenes in posterior pharyngeal versus oral cavity specimens. *J Clin Microbiol* 2006;**44**: 2593–4.
51. Freeman AF, Shulman ST. Macrolide resistance in group A Streptococcus. *Pediatr Infect Dis J* 2002;**21**:1158–60.
52. Freimer EH. Studies on L forms and protoplasts of group A streptococci. II. Chemical and immunological properties of the cell membrane. *J Exp Med* 1963;**117**:377–99.
53. Garvey MA, Perlmutter SJ, Allen AJ, et al. A pilot study of penicillin prophylaxis for neuropsychiatric exacerbations triggered by streptococcal infections. *Biol Psychiatry* 1999;**45**:1564–71.
54. Gerber MA. Antibiotic resistance in group A streptococci. *Pediatr Clin North Am* 1995;**42**:539–51.
55. Gerber MA, Shulman ST. Rapid diagnosis of pharyngitis caused by group A streptococci. *Clin Microbiol Rev* 2004;**17**:571–80.
56. Gerber MA, Randolph MF, DeMeo KK, Kaplan EL. Lack of impact of early antibiotic therapy for streptococcal pharyngitis on recurrence rates. *J Pediatr* 1990;**117**:853–8.
57. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis: a scientific statement from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee of the Council on Cardiovascular Disease in the Young, the Interdisciplinary Council on Functional Genomics and Translational Biology, and the Interdisciplinary Council on Quality of Care and Outcomes Research—endorsed by the American Academy of Pediatrics. *Circulation* 2009;**119**:1541–51.
58. Giedd JN, Rapoport JL, Garvey MA, Perlmutter S, Swedo SE. MRI assessment of children with obsessive-compulsive disorder or tics associated with streptococcal infection. *Am J Psychiatry* 2000;**157**:281–3.
59. Giedd JN, Rapoport JL, Leonard HL, Richter D, Swedo SE. Case study: acute basal ganglia enlargement and obsessive-compulsive symptoms in an adolescent boy. *J Am Acad Child Adolesc Psychiatry* 1996;**35**:913–5.
60. Gooch 3rd WM. Alternatives to penicillin in the management of group A streptococcal pharyngitis. *Pediatr Ann* 1992;**21**:810–5.
61. Gooch 3rd WM, McLinn SE, Aronovitz GH, et al. Efficacy of cefuroxime axetil suspension compared with that of penicillin V suspension in children with group A streptococcal pharyngitis. *Antimicrob Agents Chemother* 1993;**37**:159–63.
62. Green MD, Beall B, Marcon MJ, et al. Multicentre surveillance of the prevalence and molecular epidemiology of macrolide resistance among pharyngeal isolates of group A streptococci in the USA. *J Antimicrob Chemother* 2006;**57**:1240–3.
63. Hall JE, Silverstein EA. Acute hematogenous osteomyelitis. *Pediatrics* 1963;**31**:1033–8.
64. Haltalin KC, Nelson JD. Hand-foot syndrome due to streptococcal infection. *Am J Dis Child* 1965;**109**:156–9.
65. Hamburger M, Green MJ, Hamburger VG. The problem of the dangerous carrier of hemolytic streptococci. II. Spread of infection by individuals with strongly positive nose cultures who expelled large numbers of hemolytic streptococci. *J Infect Dis* 1945;**79**:68–81.
66. Hamburger Jr M, Green MJ, Hamburger VG. The problem of the "dangerous carrier" of hemolytic streptococci. I. Number of hemolytic streptococci expelled by carriers with positive and negative nose cultures. *J Infect Dis* 1945;**77**:68–81.
67. Hashikawa S, Iinuma Y, Furushita M, et al. Characterization of group C and G streptococcal strains that cause streptococcal toxic shock syndrome. *J Clin Microbiol* 2004;**42**:186–92.
68. Hays GC, Mullard JE. Blistering distal dactylitis: a clinically recognizable streptococcal infection. *Pediatrics* 1975;**56**:129–31.
69. Hill HR, Zimmerman RA, Reid GV, Wilson E, Kilton RM. Foodborne epidemic of streptoccal pharyngitis at the United States Air Force Academy. *N Engl J Med* 1969;**280**:917–21.
70. Hosein B, McCarty M, Fischetti VA. Amino acid sequence and physicochemical similarities between streptococcal M protein and mammalian tropomyosin. *Proc Natl Acad Sci U S A* 1979;**76**: 3765–8.
71. Humar D, Datta V, Bast DJ, et al. Streptolysin S and necrotising infections produced by group G Streptococcus. *Lancet* 2002;**359**: 124–9.
72. James L, McFarland RB. An epidemic of pharyngitis due to a nonhemolytic group A Streptococcus at Lowry Air Force Base. *N Engl J Med* 1971;**284**:750–2.
73. Johnson DR, Kaplan EL. A review of the correlation of T-agglutination patterns and M-protein typing and opacity factor production in the identification of group A streptococci. *J Med Microbiol* 1993;**38**:311–15.
74. Johnson DR, Kaplan EL, Ferrieri P. Pharyngitis-associated M-12 group A Streptococcus satellite strain: association with Neisseria subflava. *Pediatr Infect Dis J* 1989;**8**:800–2.
75. Johnson DR, Kurlan R, Leckman J, Kaplan EL. The human immune response to streptococcal extracellular antigens: clinical, diagnostic, and potential pathogenetic implications. *Clin Infect Dis* 2010;**50**:481–90.
76. Johnson DR, Kaplan EL, Sramek J, Motlova J, Bicova R. *Laboratory diagnosis of group A streptococcal infections: A laboratory manual*. Geneva, Switzerland: World Health Organization; 1996.
77. Kaplan EL, Johnson DR. Unexplained reduced microbiological efficacy of intramuscular benzathine penicillin G and of oral penicillin V in eradication of group a streptococci from children with acute pharyngitis. *Pediatrics* 2001;**108**:1180–6.
78. Kaplan EL, Anthony BF, Chapman SS, Wannamaker LW. Epidemic acute glomerulonephritis associated with type 49 streptococcal pyoderma. I. Clinical and laboratory findings. *Am J Med* 1970;**48**:9–27.
79. Kaplan EL, Anthony BF, Chapman SS, Ayoub EM, Wannamaker LW. The influence of the site of infection on the immune response to group A streptococci. *J Clin Invest* 1970;**49**:1405–14.
80. Kass EH, Seastone CV. The role of the mucoid polysaccharide (hyaluronic acid) in the virulence of group a hemolytic streptococci. *J Exp Med* 1944;**79**:319–30.
81. Kokx NP, Comstock JA, Facklam RR. Streptococcal perianal disease in children. *Pediatrics* 1987;**80**:659–63.
82. Kotb M, Norrby-Teglund A, McGeer A, et al. An immunogenetic and molecular basis for differences in outcomes of invasive group A streptococcal infections. *Nat Med* 2002;**8**:1398–404.
83. Krober MS, Bass JW, Michels GN. Streptococcal pharyngitis. Placebo-controlled double-blind evaluation of clinical response to penicillin therapy. *JAMA* 1985;**253**:1271–4.
84. Kurlan R, Kaplan EL. The pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS) etiology for tics and obsessive-compulsive symptoms: hypothesis or entity? Practical considerations for the clinician. *Pediatrics* 2004;**113**:883–6.
85. Lancefield RC. Current knowledge of type-specific M antigens of group A streptococci. *J Immunol* 1962;**89**:307–13.
86. Lancefield RC. Group A streptococcal infections in animals: Natural and experimental. In: Wannamaker LW, Matsen JM, editors. *Streptococci and streptococcal diseases: recognition, understanding, and management*. New York: Academic Press; 1972. p. 313–26.
87. Leckman JF, King RA, Gilbert DL, et al. Streptococcal upper respiratory tract infections and exacerbations of tic and obsessive-compulsive symptoms: a prospective longitudinal study. *J Am Acad Child Adolesc Psychiatry* 2011;**50**:108–18 e103.
88. Linder JA, Chan JC, Bates DW. Evaluation and treatment of pharyngitis in primary care practice: the difference between guidelines is largely academic. *Arch Intern Med* 2006;**166**:1374–9.
89. Logan LK, McAuley JB, Shulman ST. Macrolide treatment failure in streptococcal pharyngitis resulting in acute rheumatic fever. *Pediatrics* 2012;**129**:e798–802.
90. Lopardo HA, Vidal P, Sparo M, et al. Six-month multicenter study on invasive infections due to Streptococcus pyogenes and

Streptococcus dysgalactiae subsp. equisimilis in Argentina. *J Clin Microbiol* 2005;**43**:802–7.
91. Lossos IS, Felsenstein I, Breuer R, Engelhard D. Food-borne outbreak of group A beta-hemolytic streptococcal pharyngitis. *Arch Intern Med* 1992;**152**:853–5.
92. Martino D, Defazio G, Giovannoni G. The PANDAS subgroup of tic disorders and childhood-onset obsessive-compulsive disorder. *J Psychosom Res* 2009;**67**:547–57.
93. McCarty M. The streptococcal cell wall. *Harvey Lect* 1971;**65**: 73–96.
94. McIsaac WJ, White D, Tannenbaum D, Low DE. A clinical score to reduce unnecessary antibiotic use in patients with sore throat. *CMAJ* 1998;**158**:75–83.
95. McIsaac WJ, Kellner JD, Aufricht P, Vanjaka A, Low DE. Empirical validation of guidelines for the management of pharyngitis in children and adults. *JAMA* 2004;**291**:1587–95.
96. Michailova L, Markova N, Radoucheva T, et al. Atypical behaviour and survival of Streptococcus pyogenes L forms during intraperitoneal infection in rats. *FEMS Immunol Med Microbiol* 2000; **28**:55–65.
97. Miner LJ, Petheram SJ, Daly JA, et al. Molecular characterization of Streptococcus pyogenes isolates collected during periods of increased acute rheumatic fever activity in Utah. *Pediatr Infect Dis J* 2004;**23**:56–61.
98. Mora M, Bensi G, Capo S, et al. Group A Streptococcus produce pilus-like structures containing protective antigens and Lancefield T antigens. *Proc Natl Acad Sci U S A* 2005;**102**:15641–6.
99. Morita JY, Kahn E, Thompson T, et al. Impact of azithromycin on oropharyngeal carriage of group A Streptococcus and nasopharyngeal carriage of macrolide-resistant Streptococcus pneumoniae. *Pediatr Infect Dis J* 2000;**19**:41–6.
100. Morris CM, Pardo-Villamizar C, Gause CD, Singer HS. Serum autoantibodies measured by immunofluorescence confirm a failure to differentiate PANDAS and Tourette syndrome from controls. *J Neurol Sci* 2009;**276**:45–8.
101. Murphy ML, Pichichero ME. Prospective identification and treatment of children with pediatric autoimmune neuropsychiatric disorder associated with group A streptococcal infection (PANDAS). *Arch Pediatr Adolesc Med* 2002;**156**:356–61.
102. Musumeci R, Bue CL, Milazzo I, et al. Internalization-associated proteins among Streptococcus pyogenes isolated from asymptomatic carriers and children with pharyngitis. *Clin Infect Dis* 2003; **37**:173–9.
103. National Institute of Mental Health. *Plasma exchange and intravenous immunoglobulin lack proven benefit and carry risk for children with PANDAS, Tourette syndrome or OCD*. 2000. http://www.nimh.nih.gov.
104. Nelson JD. The effect of penicillin therapy on the symptoms and signs of streptococcal pharyngitis. *Pediatr Infect Dis* 1984;**3**:10–13.
105. Neuner JM, Hamel MB, Phillips RS, Bona K, Aronson MD. Diagnosis and management of adults with pharyngitis: a cost-effectiveness analysis. *Ann Intern Med* 2003;**139**:113–22.
106. Nobbs AH, Lamont RJ, Jenkinson HF. Streptococcus adherence and colonization. *Microbiol Mol Biol Rev* 2009;**73**:407–50.
107. Ofek I, Kaplan O, Bergner-Rabinowitz S, et al. Antibody tests in streptococcal pharyngitis: Streptozyme versus conventional methods. *Clin Pediatr (Phila)* 1973;**12**:341–4.
108. O'Loughlin RE, Roberson A, Cieslak PR, et al. The epidemiology of invasive group A streptococcal infection and potential vaccine implications: United States, 2000-2004. *Clin Infect Dis* 2007;**45**: 853–62.
109. Parks T, Smeesters PR, Steer AC. Streptococcal skin infection and rheumatic heart disease. *Curr Opin Infect Dis* 2012;**25**:145–53.
110. Perlmutter SJ, Leitman SF, Garvey MA, et al. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *Lancet* 1999; **354**:1153–8.
111. Peterson BS, Leckman JF, Tucker D, et al. Preliminary findings of antistreptococcal antibody titers and basal ganglia volumes in tic, obsessive-compulsive, and attention deficit/hyperactivity disorders. *Arch Gen Psychiatry* 2000;**57**:364–72.
112. Pichichero ME. Group A streptococcal tonsillopharyngitis: cost-effective diagnosis and treatment. *Ann Emerg Med* 1995;**25**:390–403.
113. Pichichero ME, Casey JR. Systematic review of factors contributing to penicillin treatment failure in Streptococcus pyogenes pharyngitis. *Otolaryngol Head Neck Surg* 2007;**137**:851–7.
114. Pichichero ME, Margolis PA. A comparison of cephalosporins and penicillins in the treatment of group A beta-hemolytic streptococcal pharyngitis: a meta-analysis supporting the concept of microbial copathogenicity. *Pediatr Infect Dis J* 1991;**10**:275–81.
115. Pichichero ME, Gooch WM, Rodriguez W, et al. Effective short-course treatment of acute group A beta-hemolytic streptococcal tonsillopharyngitis: ten days of penicillin V vs 5 days or 10 days of cefpodoxime therapy in children. *Arch Pediatr Adolesc Med* 1994;**148**:1053–60.
116. Powers GF, Boisvert PL. Tuberculosis and streptococcosis. *Yale J Biol Med* 1943;**15**:517–30.
117. Rainbow J, Jewell B, Danila RN, et al. Invasive group a streptococcal disease in nursing homes, Minnesota, 1995-2006. *Emerg Infect Dis* 2008;**14**:772–7.
118. Rammelkamp CH. Epidemiology of streptococcal infections. *Harvey Lect* 1957;**51**:113–42.
119. Randolph MF, Gerber MA, DeMeo KK, Wright L. Effect of antibiotic therapy on the clinical course of streptococcal pharyngitis. *J Pediatr* 1985;**106**:870–5.
120. Recco RA, Zaman MM, Cortes H, et al. Intra-familial transmission of life-threatening group A streptococcal infection. *Epidemiol Infect* 2002;**129**:303–6.
121. Robertson KA, Volmink JA, Mayosi BM. Antibiotics for the primary prevention of acute rheumatic fever: a meta-analysis. *BMC Cardiovasc Disord* 2005;**5**:11.
122. Rolston KV, LeFrock JL, Schell RF. Activity of nine antimicrobial agents against Lancefield group C and group G streptococci. *Antimicrob Agents Chemother* 1982;**22**:930–2.
123. Rothbard S, Watson RF. Variation occurring in group A streptococci during human infection: progressive loss of M substance correlated with increasing susceptibility to bacteriostasis. *J Exp Med* 1948;**87**:521–33.
124. Safar A, Lennon D, Stewart J, et al. Invasive group A streptococcal infection and vaccine implications, Auckland, New Zealand. *Emerg Infect Dis* 2011;**17**:983–9.
125. Schattner A, Vosti KL. Bacterial arthritis due to beta-hemolytic streptococci of serogroups A, B, C, F, and G: analysis of 23 cases and a review of the literature. *Medicine (Baltimore)* 1998;**77**: 122–39.
126. Seastone CV. The occurrence of mucoid polysaccharide in hemolytic streptococci of human origin. *J Exp Med* 1943;**77**:21–8.
127. Seppala H, Klaukka T, Vuopio-Varkila J, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. *N Engl J Med* 1997;**337**: 441–6.
128. Shaikh N, Leonard E, Martin JM. Prevalence of streptococcal pharyngitis and streptococcal carriage in children: a meta-analysis. *Pediatrics* 2010;**126**:e557–564.
129. Shea PR, Beres SB, Flores AR, et al. Distinct signatures of diversifying selection revealed by full-genome analysis of the population structure of upper respiratory tract and invasive bacterial strains. *Proc Natl Acad Sci U S A* In Press, 2011.
130. Shet A, Kaplan EL. Clinical use and interpretation of group A streptococcal antibody tests: a practical approach for the pediatrician or primary care physician. *Pediatr Infect Dis J* 2002;**21**:420–6; quiz 427-430.
131. Shulman ST. Pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS). *Pediatr Infect Dis J* 1999; **18**:281–2.
132. Shulman ST. Pediatric autoimmune neuropsychiatric disorders associated with streptococci (PANDAS): update. *Curr Opin Pediatr* 2009;**21**:127–30.
133. Singer HS, Loiselle C. PANDAS: a commentary. *J Psychosom Res* 2003;**55**:31–9.
134. Singer HS, Gause C, Morris C, Lopez P. Serial immune markers do not correlate with clinical exacerbations in pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Pediatrics* 2008;**121**:1198–205.
135. Singer HS, Gilbert DL, Wolf D, et al. Moving from PANDAS to CANS. *J Pediatr* 2012;**160**:725–31.
136. Sinkinson CAPM. The compromises of managing acute pharyngitis. *Emerg Med Rep* 1988;**9**:161–8.
137. Smith EW, Garson Jr A, Boyleston JA, Katz SL, Wilfert CM. Varicella gangrenosa due to group A beta-hemolytic Streptococcus. *Pediatrics* 1976;**57**:306–10.

138. Snellman LW, Stang HJ, Stang JM, Johnson DR, Kaplan EL. Duration of positive throat cultures for group A streptococci after initiation of antibiotic therapy. *Pediatrics* 1993;**91**:1166–70.
139. Snider LA, Lougee L, Slattery M, Grant P, Swedo SE. Antibiotic prophylaxis with azithromycin or penicillin for childhood-onset neuropsychiatric disorders. *Biol Psychiatry* 2005;**57**:788–92.
140. Sokol MS. Infection-triggered anorexia nervosa in children: clinical description of four cases. *J Child Adolesc Psychopharmacol* 2000;**10**:133–45.
141. Stevens DL. Streptococcal toxic-shock syndrome: spectrum of disease, pathogenesis, and new concepts in treatment. *Emerg Infect Dis* 1995;**1**:69–78.
142. Sumby P, Whitney AR, Graviss EA, DeLeo FR, Musser JM. Genome-wide analysis of group a streptococci reveals a mutation that modulates global phenotype and disease specificity. *PLoS Pathog* 2006;**2**:e5.
143. Sunnergren O, Swanberg J, Molstad S. Incidence, microbiology and clinical history of peritonsillar abscesses. *Scand J Infect Dis* 2008;**40**:752–5.
144. Swedo SE. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS). *Mol Psychiatry* 2002;**7**(suppl 2):S24–25.
145. Swedo SE, Grant PJ. Annotation: PANDAS: a model for human autoimmune disease. *J Child Psychol Psychiatry* 2005;**46**: 227–34.
146. Swedo SE, Leonard HL, Rapoport JL. The pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection (PANDAS) subgroup: separating fact from fiction. *Pediatrics* 2004; **113**:907–11.
147. Swedo SE, Leonard HL, Garvey M, et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *Am J Psychiatry* 1998;**155**:264–71.
148. Swedo SE, Leonard HL, Mittleman BB, et al. Identification of children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections by a marker associated with rheumatic fever. *Am J Psychiatry* 1997;**154**:110–2.
149. Swedo SE, Leckman JF, Rose NR. From research subgroup to clinical syndrome: modifying the PANDAS criteria to describe PANS (pediatric acute-onset neuropsychiatric syndrome). *Pediatr Therapeut* 2012;**2**:1–8.
150. Tart AH, Walker MJ, Musser JM. New understanding of the group A Streptococcus pathogenesis cycle. *Trends Microbiol* 2007; **15**:318–25.
151. Veasy LG, Hill HR. Immunologic and clinical correlations in rheumatic fever and rheumatic heart disease. *Pediatr Infect Dis J* 1997;**16**:400–7.
152. Veasy LG, Tani LY, Daly JA, et al. Temporal association of the appearance of mucoid strains of Streptococcus pyogenes with a continuing high incidence of rheumatic fever in Utah. *Pediatrics* 2004;**113**:e168–172.
153. Virtaneva K, Porcella SF, Graham MR, et al. Longitudinal analysis of the group A Streptococcus transcriptome in experimental pharyngitis in cynomolgus macaques. *Proc Natl Acad Sci U S A* 2005;**102**:9014–9.
154. Wagner JG, Schlievert PM, Assimacopoulos AP, et al. Acute group G streptococcal myositis associated with streptococcal toxic shock syndrome: case report and review. *Clin Infect Dis* 1996; **23**:1159–61.
155. Wannamaker LW. The epidemiology of streptococcal infection. In: McCarty M, editor. *Streptococcal infections*. New York: Columbia University Press; 1953. p. 157–75.
156. Wannamaker LW. Differences between streptococcal infections of the throat and of the skin. I. *N Engl J Med* 1970;**282**:23–31.
157. Wannamaker LW, Denny FW, Perry WD, et al. The effect of penicillin prophylaxis on streptococcal disease rates and the carrier state. *N Engl J Med* 1953;**249**:1–7.
158. Wannamaker LW, Rammelkamp Jr CH, Denny FW, et al. Prophylaxis of acute rheumatic fever by treatment of the preceding streptococcal infection with various amounts of depot penicillin. *Am J Med* 1951;**10**:673–95.
159. Weinstein L, Tsao CC. Effect of types of treatment on development of antistreptolysin in patients with scarlet fever. *Proc Soc Exp Biol Med* 1946;**63**:449.

CHAPTER 83

Group B Streptococcal Infections

Pia S. Pannaraj • Carol J. Baker

HISTORY

Group B *Streptococcus*, or *Streptococcus agalactiae*, was isolated first by Nocard in 1887[252] and for decades was recognized as a cause of bovine mastitis[241] but not human infection. Serologic techniques for differentiating β-hemolytic streptococci were developed by Lancefield,[191] who also described isolation of group B streptococci (GBS) from parturient women in 1935.[193] In that same year, Congdon[84] included one fatal puerperal case of GBS sepsis and pneumonia in a report of streptococcal infections associated with childbirth. The significance of this organism as a human pathogen was reported first by Fry[127] in 1938, who described three cases of fatal puerperal sepsis. GBS infections continued to be reported sporadically until the 1960s, when maternal and neonatal infections increasingly were ascribed to this pathogen.[69,109,161] In the 1970s, GBS emerged as the predominant organism causing bacteremia and meningitis in neonates.[13,31,125,164,262] The incidence of neonatal infection remained stable, with reported attack rates ranging from 0.2 to 5.4/1000 live births, until the late 1990s, when maternal intrapartum chemoprophylaxis gained wide acceptance and incidence rates fell.[76,103,302] Invasive infection also occurs beyond the neonatal period in pregnant women, nonpregnant adults with underlying medical conditions, and elderly persons.[102,113,254]

MICROBIOLOGY

Isolation and Identification

GBS are facultative gram-positive diplococci that grow on a variety of bacteriologic media. Colonies are 3 to 4 mm in diameter, grayish white, flat, and somewhat mucoid. Colonies are surrounded by a narrow zone of β-hemolysis that for some strains is detectable only when the colony is lifted from the agar. Nonhemolytic strains account for 3 to 4 percent of isolates and can cause human disease.[13,293] Definitive identification of GBS relies on detection of the group B–specific antigen, a carbohydrate cell wall antigen common to all strains. The standard method, as described by Lancefield,[191] requires acid treatment of the bacteria to solubilize the carbohydrate group B antigen, followed by capillary precipitation with hyperimmune rabbit serum. Several newer methods using hyperimmune antisera have been developed, but latex agglutination is used widely because of the commercial availability of test kits, the ease of performing the assay, and the specificity of the results when organisms in pure culture are tested.[314] Other laboratory methods for presumptive identification include testing for resistance to bacitracin or trimethoprim-sulfamethoxazole, hydrolysis of sodium hippurate broth, failure to hydrolyze bile esculin, production of orange pigment when cultured under certain conditions, and CAMP (Christie, Atkinson, Munch, Peterson) testing. *CAMP* is an acronym of the names of the authors who first described the production of CAMP factor by GBS in the presence of the α-toxin of *Staphylococcus aureus* that results in synergistic hemolysis on sheep blood agar.[83] Chromogenic agars that undergo color change in the presence of β-hemolytic colonies can facilitate detection of β-hemolytic strains but will miss the majority of nonhemolytic strains (~4% of isolates).[326,341] In addition, recently developed rapid techniques for identifying group GBS from enrichment broth include DNA probes and nucleic acid amplification tests, such as polymerase chain reaction (PCR).[341]

Serologic Classification and Antigenic Structure

GBS possess two carbohydrate cell wall antigens—the group B–specific antigen and type-specific capsular polysaccharide. GBS are classified into serotypes based on type-specific capsular polysaccharides. Nine such polysaccharides are characterized: Ia, Ib, and II through VIII. The type-specific polysaccharides of GBS are repeating units of five to seven monosaccharides (glucose, galactose, glucosamine, and *N*-acetylneuraminic acid). All of the characterized polysaccharides include an *N*-acetylneuraminic acid (sialic acid) residue, which is important in the pathogenesis of type III human infection and perhaps other types.[29,309,352] Despite structural relatedness, antibody directed against the capsular polysaccharide of one type does not provide cross-protection against other capsular polysaccharide types.[192,343,354] A few strains isolated from patients with systemic infection contain type-specific capsular polysaccharide genes by genotypic methods but express low levels of capsule or have modified capsular structures that do not react with hyperimmune sera or monoclonal antibodies to the characterized capsular polysaccharides.[282] These strains are termed *nontypeable*.

Further differentiation of type Ia strains was based on the presence or absence of a protein antigen known as C, which led to the nomenclature of Ia and Ia/c serotypes. The C protein also is present in many other serotypes, except type III strains. Two components make up the C protein: α (trypsin resistant), found in 70 percent of non–type III capsular polysaccharide isolates, and β (trypsin

sensitive), found in approximately 20 percent of isolates.[120,172,221] GBS express additional surface proteins, including α-like repetitive proteins (Alp), also referred to as R proteins. R1 and R4 (shown to have an identical gene sequence to Rib) are the major R proteins found on clinical isolates.[122]

Pili, an essential virulence factor in many gram-negative pathogens, was discovered in GBS in 2005[197] and may play a role in attachment and invasion of host cells[218] and resistance to macrophage phagocytosis.[171] Ongoing analyses of the GBS genome sequenced from multiple pathogenic strains will provide further insights into its antigenic structure, genes contributing to its virulence, and targets for treatment and prevention.[134,235,328,329]

Extracellular Products

Several bacterial products are elaborated by GBS. Type-specific capsular polysaccharide is released from cells, and the amount elaborated has been correlated with virulence.[187,363] These soluble polysaccharides inhibit opsonophagocytic killing in vitro, thereby providing a mechanism for the documented increase in virulence.[200] Most strains possess C5a-ase, an enzyme of the serine esterase class that inactivates complement component C5a.[159] Because C5a is a potent chemoattractant for neutrophils, this enzyme helps the bacteria evade the host immune system by hindering the accumulation of neutrophils at the site of infection. The β-hemolysin elaborated by GBS was characterized in 1980; its role in virulence has been explored through the creation of nonhemolytic and hyperhemolytic mutants.[224,348] Expression of β-hemolysin correlated with tissue damage in an in vivo arthritis model[278]; activation of neutrophil signaling pathways in brain endothelium, leading to development of meningitis[97]; induced neuronal damage[287]; increased virulence in pulmonary infection in rats and rabbits[154,251]; and directly impaired cardiomyocyte viability and function.[153] Other bacterial products of GBS, including CAMP factor,[155,180] lipoteichoic acid,[152,248,249] pigment,[211,325] hippuricase,[121] hyaluronidase,[233] and nucleases,[123] have been described, but the contributions of these substances to pathogenesis are not clear.

Antimicrobial Susceptibility

To date, human isolates of GBS have remained uniformly susceptible to penicillin G. However, approximately 10-fold greater concentrations are required for inhibition and killing of GBS than for group A streptococci. GBS also are susceptible to other β-lactam agents, cephalosporins, vancomycin, and carbapenems. The prevalence of resistance to the macrolides (i.e., erythromycin, clindamycin, and clarithromycin) is increasing; from 1970 to 1990, resistance was reported in 3.4 to 7.4 percent of isolates, but more recent studies reported resistance to erythromycin in 17 to 40 percent of isolates and resistance to clindamycin in 7 to 30 percent.[56,91,118,291] Resistance is related to the presence of *ermTR*, *ermB*, or *mefA* genes.[91] Macrolide resistance is highest in type V strains.[205] Furthermore, inducible clindamycin resistance can occur in some strains that appear susceptible in broth susceptibility tests; therefore, D-zone testing is important to identify these strains.[291] Ninety-five percent of strains are resistant to tetracycline, and resistance to bacitracin, nalidixic acid, trimethoprim-sulfamethoxazole, and metronidazole is uniform.[268] Low-level gentamicin resistance is typical, but when gentamicin is combined with either penicillin G or ampicillin, synergistic killing of GBS occurs in vitro and in vivo.[298,299,322]

As many as 5 to 17 percent of GBS isolates have been reported to be tolerant to penicillin.[48,183] Expression of tolerance requires laboratory conditions that promote a greater than 16-fold discrepancy between minimal inhibitory concentrations and minimal bactericidal concentrations. Tolerant strains are characterized in vitro by delayed penicillin killing, similar rates of killing by penicillin whether growth is exponential or stationary, an additive rather than a synergistic response to the combination of penicillin and gentamicin, and deficient autolysis. The clinical significance, if any, of these laboratory-induced properties remains unknown.[184,185,312]

EPIDEMIOLOGY

Maternal Colonization

Asymptomatic colonization occurs at vaginal, rectal, urethral, and pharyngeal sites in up to one third of healthy young women.[53,238] Much effort has been exerted trying to define groups of women at enhanced risk for colonization with GBS. Factors found to be associated with vaginal acquisition include black ethnicity, multiple sex partners, frequent sexual intercourse, male-to-female oral sex, tampon use, and infrequent hand washing.[53,220,238] Women younger than 20 years of age have higher prevalence of colonization.[14,33] Several studies have found significantly higher colonization rates among blacks,[72,158,238] whereas others found higher rates in Hispanics.[285] Asians have the lowest colonization rates.[72,238]

Maternal vaginal or rectal colonization rates during pregnancy vary from 18 to 35 percent in reported studies.* These rates relate to the body sites sampled, microbiologic techniques employed, and the period of gestation in which the cultures are performed. Specimens from the distal portion of the vagina yield GBS more frequently than do specimens from the proximal vagina or cervix.[215] Concomitant sampling of the rectal site results in a 10 percent increase in detection over culturing the vaginal site alone.[21,94] Several investigators have suggested the gastrointestinal tract as the principal reservoir for this organism, and sometimes the rectal site is the only one that yields GBS.[21,94,162,269] The urinary tract is an important site of infection (asymptomatic bacteriuria) because it is a surrogate for a high-density or "heavy" (>10^5 colony-forming units/mL) genital inoculum and a marker of increased risk for development of early-onset sepsis in neonates.[246,359] Bacteriuria mandates therapy during pregnancy.[341]

The manner in which swab specimens from the vagina and rectum are processed also is important in accurately

*References 21, 24, 60, 94, 101, 232, 269, 285.

assessing colonization.[74,341] Anogenital culture swabs collected by patients themselves are as accurate as those performed by physicians.[17,277,331] Swabs can be placed in transport media at environmental temperatures for as long as 96 hours.[341] Specimens then should be placed in a selective antibiotic-containing enrichment broth medium, rather than on solid media, because solid media fail to detect as many as 50 percent of GBS carriers.[27,32] The addition of antibiotics to the broth limits the growth of competing flora, especially gram-negative enteric organisms, and enhances detection.[24,27,32,229] Todd-Hewitt broth with gentamicin and nalidixic acid (selective broth medium)[27] or with colistin and nalidixic acid (Lim broth)[204] is recommended for detection of GSB colonization.[341] After overnight incubation, the broth is subcultured onto a 5 percent sheep blood agar plate and is processed conventionally.

Of culture-positive women, 92 percent are identified if lower vaginal and rectal cultures are obtained at a single visit with correct laboratory detection methods.[100] The proximity to delivery affects the accuracy of predicting colonization at delivery. Cultures obtained at 35 and 37 weeks of gestation predict colonization at delivery with 87 percent sensitivity and 96 percent specificity.[60,269,362]

Infant Colonization

Colonization and infection in the neonate are associated significantly with the presence of GBS in the maternal genital tract at delivery. Vertical transmission from colonized mothers to their infants occurs in 41 to 72 percent of cases (mean, ~50%).* Only 1 to 12 percent (mean, ~5%) of colonized infants are born to noncolonized mothers.[15,24,99,232,365] Acquisition is presumed to occur either by the ascending route through ruptured membranes or from contact with the organism in the genital tract during parturition. Heavy maternal inocula in the genital tract ($>10^5$ colony-forming units/mL) greatly increase the rates of vertical transmission to neonates and rates of heavy infant colonization.[10,15,58,162,174] Other factors associated with increased colonization rates in infants include prolonged rupture of membranes (>12 to 18 hours) and vaginal delivery.[15,158] Maternal intrapartum antibiotic therapy substantially diminishes the vertical transmission of GBS.† The body sites in neonates most likely to yield GBS are the rectum and throat, findings reflecting replication of organisms at the respiratory or gastrointestinal tract sites after the ingestion of infected amniotic fluid or genital secretions.[158]

Horizontal transmission from nosocomial and community sources has been described.[1,100,129,253] Infant-to-infant or colonized staff member–to-infant spread may occur through hands of hospital personnel, but epidemics are rare.[100,253] The rate of community acquisition, presumably by an oral–fecal route, appears to be low, but this has not been investigated thoroughly.[129,222] Breast milk also has been proposed as a mode of transmission for late-onset infection; most reported cases involved a mother with postpartum mastitis.[50,189,288]

*References 7, 15, 24, 58, 100, 158, 232, 366.
†References 7, 58, 60, 158, 340, 366.

Incidence of Disease

Before the widespread use of maternal intrapartum chemoprophylaxis to prevent early-onset disease, reported attack rates for GBS disease in infants ranged from 1.8 to 4.0/1000 live births.[254,349,368] Early-onset disease (onset within the first 6 days of life) accounted for approximately 80 percent of cases.[302] Late-onset disease (onset from 7 through 89 days of life) rates ranged from 0.3 to 1.8/1000 live births.[95,262] With the 2002 guidelines for universal screening of pregnant women at 35 to 37 weeks of gestation and administration of intrapartum chemoprophylaxis to carriers, the incidence of early-onset disease decreased and stabilized at approximately 0.3 cases/1000 live births since 2004, a finding representing a decline of 81 percent from 1990.[302,341] However, the incidence of late-onset disease has remained unchanged.[175,341] Approximately 30 percent of cases of early-onset disease and up to 55 percent of those of late-onset and late, late-onset disease now occur in preterm infants.[207,301,305] Late, late-onset GBS disease occurs in infants older than 3 months of age and accounts for 7 to 13 percent of childhood GBS infections.[165,302,361] Affected infants typically were born before 34 weeks of gestation or have an underlying immunodeficiency or concomitant infection with human immunodeficiency virus (HIV).[92,165,361] Case-fatality rates for all infants with GBS disease have dropped from 46 percent in the 1970s to 4 to 6.5 percent in recent years.[170,271,341]

GBS disease also is a common finding in pregnant women, with clinical manifestations that include urinary tract infection (usually asymptomatic bacteriuria but also cystitis or pyelonephritis), intra-amniotic infection, postpartum endometritis (often with bacteremia), and puerperal sepsis.[261,306,367] Occasionally, meningitis, septic thrombophlebitis, and other serious complications occur.[367] Attack rates of 2/1000 deliveries have been reported, and 28 percent of maternal cases are associated with pregnancy loss or birth of an infant with early-onset GBS disease.[261,367] Nonpregnant women and men account for 68 percent of invasive GBS disease in adults.[113] These patients typically are older than 65 years of age or have underlying medical conditions, especially diabetes mellitus, but also malignancy (e.g., breast cancer or leukemia), HIV infection, liver disease, stroke and other neurologic disorders, decubitus ulcers, and neurogenic bladder.[102,113,168,254,335]

Risk Factors for Infant Disease

Vertical transmission is a prerequisite for the development of invasive, early-onset infection.[24,231,262] Evidence suggests that approximately 50 percent of late-onset infections also occur after vertical transmission or from spread within the household.[95] The degree of colonization or inoculum also increases the likelihood of infant disease; heavily colonized mothers are more likely to have infants with invasive infection, and heavily colonized infants are more likely to have either early- or late-onset disease.[61,95,174,203] Other maternal factors associated with the development of early-onset disease include delivery before 37 weeks of gestation, premature rupture of

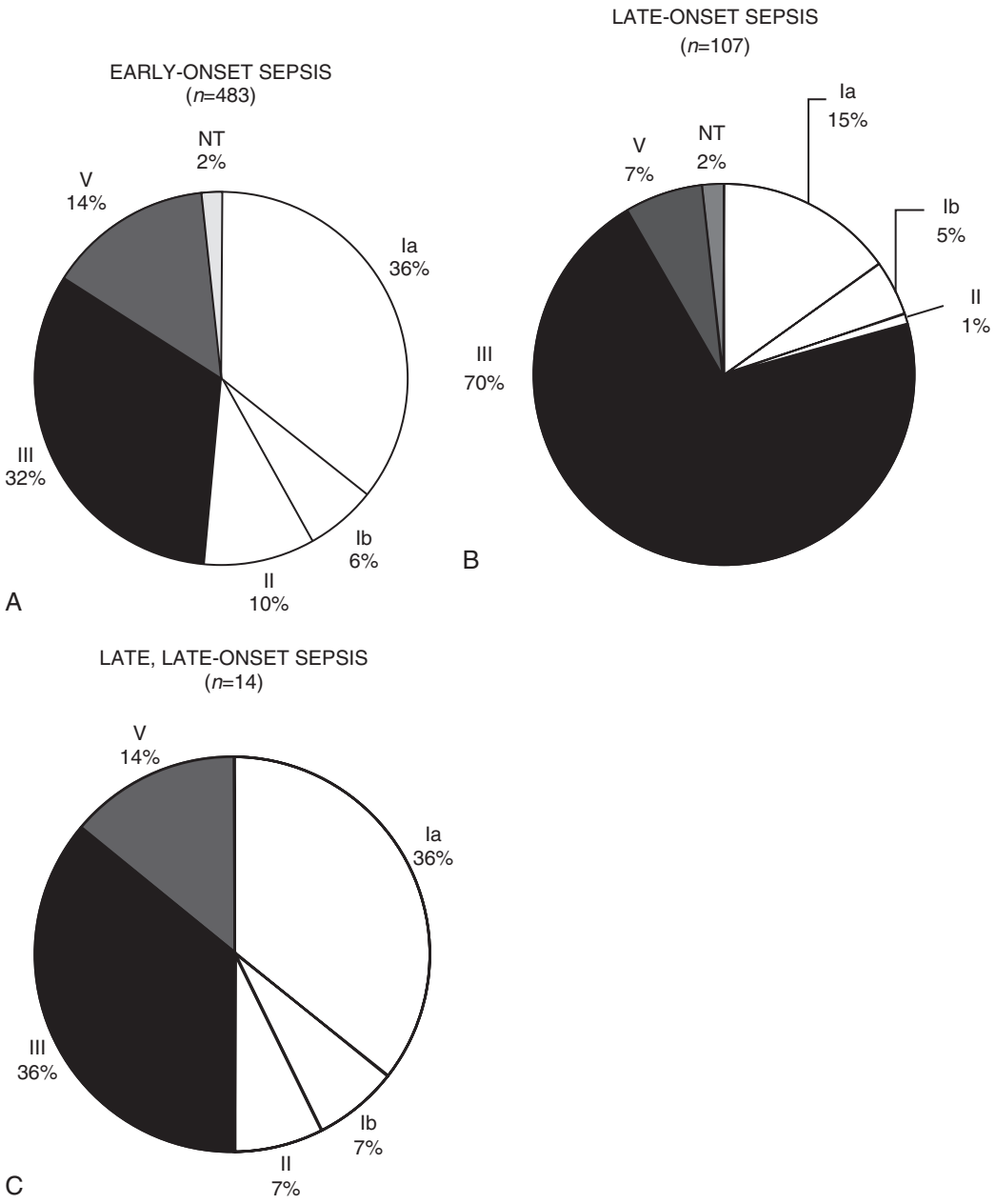

FIGURE 83-1 ■ Serotype distribution of invasive group B streptococcal strains causing early-onset, late-onset **(B),** and late, late-onset **(C)** disease in infants.[54,87,144,205,367] *NT,* Nontypeable.

membranes (i.e., rupture of membranes before onset of labor) or rupture of membranes more than 18 hours before delivery at any gestation, and intrapartum fever.* Women colonized with GBS also have a higher incidence of premature rupture of membranes and preterm labor, a finding suggesting a possible causal relationship between GBS and events leading to preterm birth.[6,230,234,285] Other maternal factors associated with an increased attack rate of early-onset infection are black ethnicity, age younger than 20 years, history of previous fetal loss, history of urinary tract infection with GBS during the current pregnancy, and primiparity.[305,306] Late-onset disease also is associated with young maternal age and black ethnicity.[306]

*References 13, 59, 60, 95, 125, 149, 262.

Siblings of infected infants who are the product of a multiple pregnancy (e.g., twins, triplets) have enhanced risk for development of early- and late-onset GBS disease.[106,260]

Capsular Polysaccharide Types Causing Disease

Reports from the 1970s indicated that the GBS capsular polysaccharide types colonizing pregnant women and neonates were divided fairly evenly among types I, II, and III.[28,354,355] The current distribution of capsule polysaccharide types in invasive disease from multiple North American studies is shown in Figure 83-1.[87,144,206,350,367] Serotypes Ia and III are the most common cause of

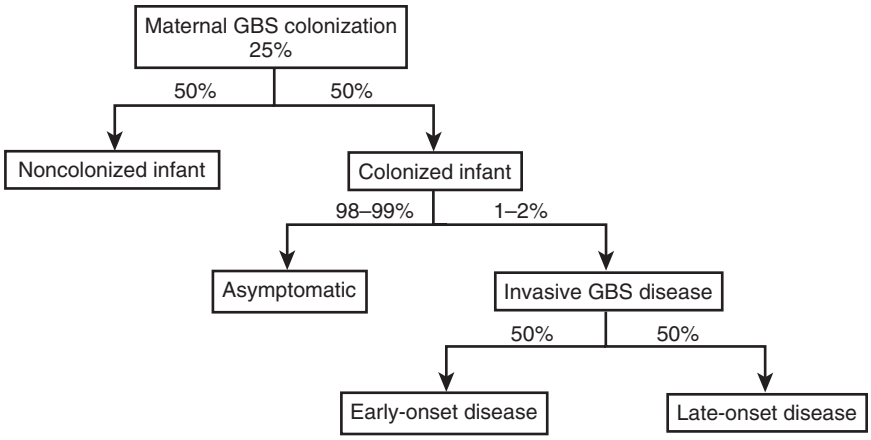

FIGURE 83-2 ■ Flow chart of vertical transmission and disease manifestation of group B streptococcal disease in the era of intrapartum chemoprophylaxis.

early-onset infection, whereas serotype III is the most common cause of late-onset infection. In Europe, type III predominates in both early- and late-onset disease.[124,332,350] Serotype III is implicated in 80 to 93 percent of meningitis cases, either as a focus of early-onset disease or as a manifestation of late-onset infection (80 to 93%).[25,31,355] Capsular polysaccharide type V emerged in the 1990s.[54,72,158,288] Type V causes both early- and late-onset infant disease and is the type most commonly isolated from adults with invasive disease.[54,289] In Japan, types VI and VIII are the most common isolates from pregnant women.[190] Recent studies from the Middle East and Africa suggest type IV may be an emerging pathogen.[93] The least common serotypes causing human disease are types Ib/c, VI, VII, and VIII.

PATHOGENESIS

For pediatricians, GBS disease predominantly afflicts neonates, a predilection that results from a unique combination of maternal, bacterial, and host factors. Figure 83-2 shows a simplified flow chart of GBS transmission. First, an organism carried by an asymptomatically colonized mother is transmitted vertically to her neonate. Evidence suggests that this transmission may occur in utero shortly before birth or during parturition. This continuum in time of acquisition is reflected in the time of onset of symptoms; infected neonates often are ill at or within 12 hours of birth (up to 90% of cases), whereas others may not have evidence of disease for a few days.[31,306] The organism successfully colonizes approximately 50 percent of all infants born to GBS carriers, yet disease develops in only 1 to 2 percent of these infants.[13,304] A breach in the delicate interplay of maternal, infant, and bacterial factors allows the organism to invade. Once invasion occurs, a combination of host defenses and therapeutic interventions may halt the progression of disease or the infant's defenses may fail and the disease may progress and result in tissue damage or death.

Maternal Factors

The bacterial inoculum in the maternal genital tract determines the likelihood that the organism will be transmitted vertically. Infants born to heavily colonized women are more likely to be colonized themselves and are more likely to have early-onset disease.* Infants delivered before 37 weeks of gestation have an increased risk for development of early-onset disease. Heavy maternal colonization may induce preterm delivery,[115,231,234,284,286] and disease is more likely to develop in the infant in this setting of high bacterial inoculum and immunologic immaturity. Invasive disease also has been reported in term neonates delivered by elective cesarean section with intact membranes, but this is a rare event.[13,90,109,181,261]

In addition to bacterial inoculum, another critical maternal factor is the concentration of serum antibody to the serotype-specific polysaccharide capsule of the colonizing strain of GBS at delivery. Antibody to the type-specific capsular polysaccharide is protective against the homologous serotype in the mouse model of lethal infection; antibody to the group B polysaccharide is not.[194] Baker and associates[28,34] determined that invasive disease with type III GBS occurred primarily in infants born to women with low serum concentrations of antibodies directed against the type III capsular polysaccharide. Several other investigators also reported a correlation between low concentrations of antibody to types Ia, Ib, II, and III capsular polysaccharide in maternal delivery sera and the occurrence of early- and late-onset GBS infant disease.[136,138] A correlation between antibody concentration and maternal age may explain the epidemiologic association of young maternal age and increased likelihood of neonatal disease.[13] Animal models have demonstrated that antibody directed against the α or β determinants of C protein is protective,[194,239] but definitive human studies are lacking. Invasive disease, but not colonization, elicits immunoglobulin (Ig)M and IgG antibodies to α and β C protein in humans.[255,256] Antibody to other protein determinants in some serotypes has been postulated to be partially protective. Titers of antibody to R protein were found to be higher in maternal serum from mothers colonized with R protein–bearing strains whose infants were healthy than from mothers whose infants were ill; such antibodies were protective in a mouse model against type II, but not type III strains.[208,209] Presence of maternal antibody to Rib also was associated

*References 10, 15, 60, 95, 162, 174.

with protection in neonates against Rib-expressing GBS strains.[196]

Bacterial Factors

To colonize the genital tract or cause disease effectively, organisms must be able to adhere to host tissue. GBS adhere well to many types of epithelium, including vaginal epithelium and chorioamniotic membranes.[128,225,315,324] Type III strains adhere more avidly to vaginal cells than do other serotypes in vitro.[57] Furthermore, invasive strains adhere better to epithelial tissue than do colonizing strains.[150] This capacity to adhere to and possibly invade chorioamniotic cells may explain the association of GBS colonization with preterm labor and premature rupture of membranes. If a breach occurs in the membranes, the bacteria can multiply in amniotic fluid.[151] After transmission and colonization occur, the capacity of the bacteria to adhere to neonatal epithelial cells may allow them to invade and disseminate. The lung, after aspiration of infected maternal fluids, is a frequent initial site of infection in a newborn with early-onset disease. GBS can adhere to and invade respiratory epithelial cell lines.[295] In addition, they can invade endothelial cells, which may be a mechanism for some of the pathologic features of disseminated disease.[132]

Several factors may confer adherence properties of GBS, including bacterial cell wall component lipoteichoic acid[249] or surface proteins.[68,324] Studies suggest that translocation across epithelial barriers is mediated by alpha C protein binding to host cell surface glycosaminoglycan.[42,78] Several proteins with potential roles in adherence and invasion were identified by detecting upregulation during cell invasion using proteomics and include an undefined surface antigen, penicillin-binding protein 2b, glyceraldehydes-3-phosphate dehydrogenase, and an iron-binding protein.[173] Mouse antisera to these five proteins inhibited binding of GBS to cervical epithelial cells. Translocation also may occur by a paracellular route.[270,318] Adherence to and invasion of the microvascular endothelial cells of the blood-brain barrier has been associated with proper anchoring of lipoteichoic acid,[98] β-hemolysin/cytolysin,[97] fibrinogen binding protein FbsA,[327] and pilus proteins.[218]

A well-defined virulence structure of GBS is the capsular polysaccharide. Mouse virulent strains are able to synthesize greater amounts of surface-bound type-specific polysaccharide than avirulent strains.[187,363] An unencapsulated mutant, created by inserting a transposon into the gene regulating capsule expression, has significantly less virulence in neonatal rats than does the parent type III strain.[296] As with other encapsulated organisms, the capsule is thought to confer virulence primarily by interfering with opsonophagocytosis.[200] In vitro, the capsule of type III GBS has been shown to prevent the deposition of C3,[227] and the presence of the terminal sialic acid residue on the repeating unit of this polysaccharide is crucial to this interference.[103] Removal of sialic acid residues from the polysaccharide leads to diminished virulence, and a desialylated mutant loses virulence in contrast to the parent strain.[353] Furthermore, the presence of this sialic acid moiety prevents activation of the alternative complement pathway, the predominant pathway used by the human host when minimal type-specific antibody is present.[103] In addition, when bacteria grow in the presence of human serum, the quantity of sialic acid increases, thereby potentiating its contribution to virulence.[273] Opsonization may be affected by cell surface components other than capsular sialic acid. C protein also lends relative resistance to opsonization to strains bearing the antigen.[172,264] β-Hemolysin/cytolysin contributes to cytolysis and apoptosis of phagocytes while protecting GBS by the linked carotenoid pigment against oxidative damage.[211]

Sialic acid residues on the capsule may interfere with another component of the immune system. In a serum-free system, the desialylated mutant of type III GBS elicited much larger quantities of leukotriene B_4 from macrophages than did the parent strain.[294] Leukotriene B_4 is a potent neutrophil chemoattractant, so this effect may result in diminished influx of effector cells. Similarly, C5a-ase may disable C5a, another host product capable of eliciting neutrophil influx. By enhancing phagocytosis and killing GBS, C5a also has direct stimulatory effects on neutrophils.[323] Bacterial elaboration of C5a-ase may affect both the accumulation of neutrophils and the efficiency of neutrophil function.

Once invasive infection is established, ongoing replication and digestion of bacteria can instigate host inflammatory responses that may be deleterious. Neonates recovering from GBS disease have circulating immune complexes for a prolonged period, and immune complexes can contribute to end-organ damage.[336] Additionally, immune complexes containing GBS components elicit inflammatory mediators such as leukotriene B_4 and interleukin (IL)-6. The cytokine response to gram-positive pathogens is not delineated as clearly as is that to gram-negative bacteria, partly because of the greater heterogeneity in structure of the latter.[55] Both group B and type III capsular polysaccharides induce the release of IL-6 from monocytes[318]; group B antigen also causes the release of tumor necrosis factor-α (TNF-α).[338] As with other gram-positive pathogens, the cell walls of GBS contain peptidoglycan and lipoteichoic acid. Peptidoglycans from other gram-positive organisms elicit a variety of proinflammatory cytokines such as TNF-α, IL-1, IL-6, and granulocyte colony-stimulating factor.[96,157,292] Lipoteichoic acid also induces the release of IL-1β, IL-6, and TNF-α.[49,182] GBS cell wall components likewise exert similar effects. Elaboration of these cytokines has been implicated in the clinical and hemodynamic effects of sepsis.[55,351] Specifically, blockade of IL-1 activity by administration of an IL-1 receptor antagonist in a piglet model of GBS sepsis ameliorated systemic hypotension and prolonged survival.[339]

Infant Host Factors

Neonates have several domains of immune dysfunction that affect their ability to mount a sufficient defense against GBS. Neutrophils are the primary effector cell in host defense against extracellular bacterial pathogens, and neutrophils from neonates have many functional abnormalities.[201] The generation of chemotactic activity

TABLE 83-1 **Features of Group B Streptococcal Disease in Infants**

Feature (References)	Early-Onset Disease	Late-Onset Disease	Late, Late-Onset Disease
Age at onset[59,65,306]	≤6 days; mean, 8 hr; median, 1 hr	7-89 days; mean, 36 days; median, 27 days	≥90 days
Infants affected[75,142,306,349]	Premature neonates, births after maternal obstetric complications	Term and preterm infants equal	Premature neonates <32 wk gestation, immunodeficiency
Clinical findings[103,199]	Acute respiratory distress, apnea, and hypotension common	Fever, irritability, nonspecific signs, occasionally fulminant	Fever, irritability, nonspecific signs
Manifestations[13,23,104,125,175,349,361]	Bacteremia (40%-55%), pneumonia (30%-45%), meningitis (6%-15%)	Bacteremia without a focus (55%-67%), meningitis (26%-35%), osteoarthritis (~5%), cellulitis/adenitis (~2%)	Bacteremia without a focus, focal infections as in late-onset disease
Case-fatality rate[76,175,302,306,349,361]	5%-15%	2%-6%	<5%

in neonatal serum in response to GBS is diminished, as is the release of chemotactic factors by neonatal monocytes.[11,12,20] Thus, the diminished ability of neutrophils to migrate is amplified by a diminished level of chemotactic stimulation. Phagocytosis and bacterial killing also are impaired when opsonic activity is poor, as is the usual pattern in neonates with sepsis.[201] The neutrophil storage pools of neonates rapidly become depleted during invasive infection, thereby leading to profound neutropenia,[82] an ominous prognostic indicator.[265] These defects in neonatal neutrophils are even more pronounced in infants born prematurely.[201]

Cells of the monocyte/macrophage lineage also may play a role in host defense against GBS streptococci, especially in the lungs, where the alveolar macrophage is the first effector cell to encounter pathogens. Defects in the functions of these cells have been described in neonates, especially preterm infants.[86] Cord blood monocytes have impaired phagocytosis and killing of GBS.[226] In animal models, oxidative metabolism, bacterial uptake, and migration to the site of infection by neonatal macrophages are diminished in response to GBS.[228,307,308]

Humoral immunity is compromised in neonates. Both the alternative and classical complement pathways are affected, and preterm neonates are impaired more severely than are term neonates.[89,105] Both pathways are important in the opsonization of GBS.[108] The concentration of type-specific antibody passively acquired from the mother is an important protective factor. Because passive transfer of IgG increases dramatically during the final 8 weeks of pregnancy, premature neonates are again at a disadvantage because they may not receive sufficient amounts of type-specific IgG if they are born before protective levels are transferred.[81]

The pattern of production of cytokines by neonatal immune cells frequently is altered in contrast to that of adults. For example, in response to GBS, the level of TNF-α and IL-6 released by neonatal monocytes is increased relative to adults,[337,338] but levels of IL-8 and leukotriene B_4 are decreased.[294,300] IL-12 and interferon-γ are associated with improved outcome in an animal model of GBS infection, but lower quantities of these mediators are released by neonatal mononuclear cells.[179,219] Impairment in the innate recognition systems, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors may lead to reduced cytokine production and pathogen clearance.[85,297] Monocytes from newborns show lower TLR-mediated cytokine production compared to adults.[86,297] Cytokine networks are important both in the manifestations of sepsis and in the stimulation of an appropriate immune response, so alterations in expression of cytokines may affect the neonate's response to this pathogen both clinically and immunologically.

CLINICAL MANIFESTATIONS

Early-Onset Disease

GBS remains the leading cause of neonatal sepsis since the 1970s and accounts for 40 to 50 percent of early-onset sepsis cases.[40,52,103,166] The bimodal distribution of GBS infections corresponding to age of neonates and young infants was described first in 1973 and now is divided into two types, termed *early-* and *late-onset disease*.[31,125] The syndromes of early- and late-onset disease differ in epidemiologic characteristics, pathogenesis, clinical findings, and prognosis. Their major clinical features are detailed in Table 83-1. Early-onset disease is defined as onset of infection in the first 6 days of life, but most neonates (61% to 95%) become ill within the first 24 hours (median, 1 hour).[59,65,149,306] Premature infants often are ill at birth or within 6 hours of birth and account for 17 to 32 percent of neonates with early-onset GBS disease.[22,279,302,306] In infants with very low birth-weight, GBS has dropped to the second most common cause of early-onset sepsis, after *Escherichia coli*, since the implementation of intrapartum antibiotic chemoprophylaxis.[320]

Early-onset disease often occurs in the setting of maternal complications, including labor before 37 weeks of gestation, prolonged rupture of membranes for more than 12 to 18 hours, intrapartum fever greater than 38° C (100.4° F), chorioamnionitis, and early postpartum febrile morbidity.* In one study, neonates born to a mother with one or more of these risk factors were 12.5 times more

*References 45, 59, 60, 95, 149, 349.

likely to develop early-onset GBS in contrast to those born to a mother with no risk factors.[59] Term infants can develop GBS disease without definable maternal risk factors, except vaginal or rectal colonization.[103] In other studies, 65 to 76 percent of mothers of neonates with early-onset disease had deliveries complicated by at least one of the previously mentioned identifiable risk factors or was known to be a GBS carrier.[149,279]

The three most frequent clinical manifestations are bacteremia without a focus, pneumonia, and meningitis.[13,349,361] The presentation for each may range from shock and respiratory failure at delivery to a healthy infant who has infection detected during evaluation because of maternal risk factors known to increase the risk for development of invasive infection.[279,349] Respiratory distress, including apnea, grunting respirations, tachypnea, and cyanosis, is the most common initial manifestation in all forms of early-onset GBS disease.[22,31,125,342,361] Other signs include lethargy, poor feeding, abdominal distention, pallor, jaundice, tachycardia, and hypotension. Fever usually is present in term neonates, but preterm infants often are normothermic or hypothermic.[349] Administration of intrapartum antibiotic prophylaxis to the mother does not alter the findings in infants in whom early-onset disease develops despite administration of intrapartum chemoprophylaxis; most still become clinically ill in the first 24 hours of life.[40,65]

Bacteremia without a focus is present in 27 to 87 percent of neonates with early-onset disease.[305,349,361] Signs of septicemia, such as respiratory distress and poor perfusion, often are present, especially in neonates born at less than 37 weeks of gestation.[349] Bacteremia in healthy-appearing term infants also may develop, and in one series it occurred in 22 percent of cases.[164,349,361] These infants probably remained healthy because of early evaluation and institution of empirical antimicrobial therapy.[349] Most often, bacteremic infants are mildly ill and have fever and nonspecific signs that should prompt bacteriologic evaluation. Bacteremia also frequently accompanies pneumonia and meningitis.

Radiographic findings of pulmonary involvement include infiltrates suggestive of congenital pneumonia, small pleural effusions, a pattern similar to that of hyaline membrane disease or respiratory distress syndrome, and increased vascular markings as seen in transient tachypnea of the newborn; radiographs may appear normal despite abnormal pulmonary signs.[202,212,342] In preterm neonates, the findings frequently are identical to those of respiratory distress syndrome; at autopsy, hyaline membranes containing bacteria and minimal inflammatory infiltrates have been described.[2,342]

Meningitis is documented in 6 to 15 percent of neonates with early-onset disease (Figure 83-3).[304,349,357] As a rule, no meningeal signs are present, thus underscoring the need to evaluate all neonates with presumed early-onset disease for the possibility of meningeal involvement.[349,357] Early in the course, the cerebrospinal fluid (CSF) white blood cell count may be normal despite isolation of GBS.[13] Again, respiratory distress is the most frequent clinical finding.[31,199,349] Seizures rarely are the initial feature but do occur in as many as 50 percent of affected neonates early in the course of therapy; often these seizures are focal and subtle.[31] Postmortem evaluation of infants who die of early-onset GBS meningitis reveals hemorrhage, prominent basilar involvement, and abundant bacteria with relatively sparse inflammation.[125]

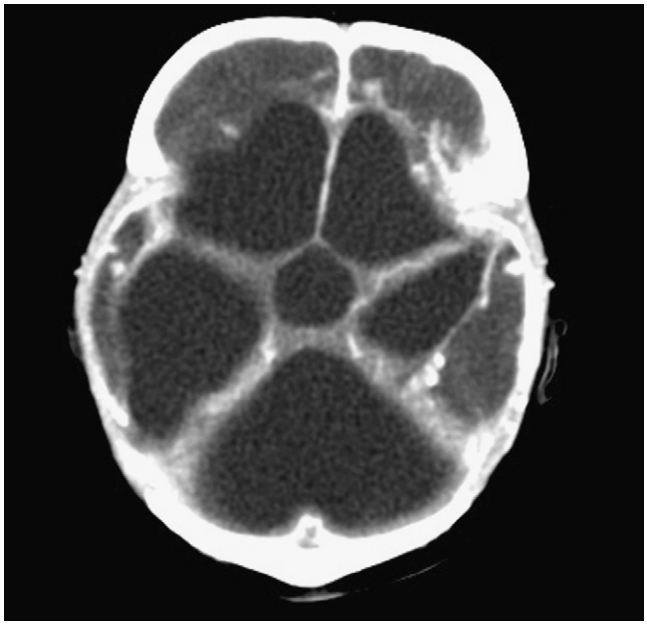

FIGURE 83-3 ■ Head computed tomography scan at 6 weeks of age from a term infant with early-onset group B streptococcal meningitis shows significant loss of normal brain matter.

Late-Onset Disease

Late-onset disease is defined as infection in infants 7 to 90 days of age, and it primarily affects term infants with an unremarkable maternal history and early neonatal course.[31,95,175] However, a recent case series suggested that late-onset infection develops in a larger proportion of infants born quite prematurely (<34 weeks of gestation).[207] Bacteremia without a focus and meningitis are the two most common manifestations of late-onset disease.[125,267,361] Osteoarticular infections and cellulitis are additional clinical foci.[23,104,361] Whereas early-onset disease most frequently occurs acutely with apnea and hypotension, late-onset disease often is manifested by fever, irritability, and other nonspecific signs.[103] More fulminant, rapidly progressive cases do occur, however.[31]

Meningitis is a frequent complication, occurring in 26 to 40 percent of late-onset disease cases.[31,175,349,361] GBS accounted for 86 percent of infants younger than 2 months of age with meningitis in a recent multistate epidemiologic study.[330] Late-onset meningitis most typically manifests with fever and lethargy, although respiratory distress, coma, and shock also may occur.[31] Classic signs of meningitis, such as a bulging fontanelle and nuchal rigidity, occur more commonly in neonates with late-onset than early-onset meningitis. Subdural effusions develop in as many as 20 percent of cases, but subdural empyema is a rare occurrence.[14,31,260] Infants

who die of late-onset meningitis have purulent leptomeningitis at postmortem examination.[125]

Sequelae occur in 20 to 30 percent of survivors of early- and late-onset meningitis. These sequelae include developmental delay and cognitive impairment, spastic quadriplegia, cortical blindness, deafness, uncontrolled seizures, hydrocephalus, and speech and language delay.[103,199,344] Signs at admission correlated with a high likelihood of a poor outcome include seizures, coma or semicoma, hypotension requiring initial pressor support, neutropenia, CSF protein levels greater than 300 mg/dL, CSF glucose lower than 20 mg/dL, and high concentrations of bacterial capsular polysaccharide antigen in CSF.[39,108,199,264]

Late, Late-Onset Disease

Contemporary studies show an increasing incidence of GBS infection in infants older than 3 months of age, defined as late, late-onset disease.[165,199,302,361] These infections tend to occur in infants who were born extremely prematurely and whose corrected postmenstrual age is less than 3 months.[165,302,361] Late, late-onset disease also occurs in children coinfected with HIV and those with immunodeficiencies,[92,165,361] a finding supporting the recommendation that immunodeficiency be considered in any child infected beyond the usual period of risk.[92] The clinical manifestations in these older infants are similar to those in patients with typical late-onset infection; bacteremia without a focus and meningitis are the most common clinical features.[92,165] Endocarditis and central venous catheter infection also have been noted.[165]

Septic Arthritis and Osteomyelitis

Osteoarthritis occurs in approximately 5 percent of infants with late-onset infection but appears to be diminishing since implementation of maternal intrapartum prophylaxis.[361] Among neonates and young infants with osteomyelitis, GBS have been identified as the causal agent in 7 to 38 percent of cases.[104,358] The osteoarticular disease usually is more indolent than the osteomyelitis caused by other etiologic agents in young infants. Diagnosis usually is established after a mean of 9 days of clinical signs at a mean age of 31 days.[103,104] Decreased motion of the involved extremity and pain with manipulation are common signs. Warmth and redness are uncommon findings but have been described.[104] Systemic signs and symptoms, including fever, are unusual manifestations.[104,234]

GBS has a predilection for the proximal humerus.[104] The femur is the second most commonly affected bone. Involvement of more than one bone is an unusual finding.[104] However, involvement of the adjacent joint is described.[236] Blood cultures infrequently are positive, unlike neonatal osteomyelitis caused by other etiologic agents. A lytic lesion often is seen on radiographs at first evaluation (Figure 83-4). This observation suggests that lytic lesions may be a late phenomenon that develops after seeding of the metaphysis during an episode of asymptomatic, early-onset bacteremia several weeks before presentation.[103]

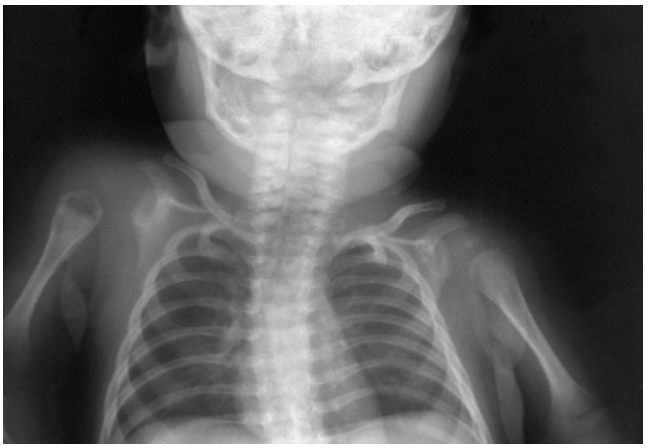

FIGURE 83-4 ■ Radiograph of the right arm of a 26-day-old infant with a 2-day history of diminished movement of that extremity. A well-defined, lytic lesion is present in the proximal end of the humerus. Necrotic material from this area was débrided surgically and grew group B streptococci on culture. (Courtesy Morven S. Edwards, MD.)

The mean age at diagnosis in infants who have septic arthritis without osteomyelitis is 20 days. The clinical picture typically is acute, with a mean of 2 days of abnormal findings before diagnosis is established. The lower extremities are involved most often, with the hip joint predominating. Infants typically present with local joint signs in the absence of systemic symptoms.[236]

Cellulitis and Adenitis

In one case series, 2 percent of late-onset GBS infections were associated with cellulitis and adenitis syndrome.[361] Diagnosis is made at a mean age of 5 weeks, with a preponderance of cases in male patients.[23] Typical presentation includes fever, irritability, poor feeding, and swelling of the affected soft tissue area. Enlarged adjacent lymph nodes become palpable within a few days. The most frequent sites of cellulitis are the face and neck,[23,146,263] with enlarged lymph nodes in the submandibular area. Ipsilateral otitis media was noted in four of five infants with facial or submandibular cellulitis in one case series.[23] Less commonly affected areas reported include the genital or inguinal region (Figure 83-5), the hand, and the prepatellar bursa.[23,62,356] Aspiration of the affected area of cellulitis often yields GBS, and concomitant bacteremia almost always is present.

Other Manifestations

Nearly every organ has been reported to be a site of GBS infection in young infants. Table 83-2 lists these reported unusual manifestations of early- and late-onset disease.

Recurrent Infections

Second (and rarely third) episodes of GBS infection occur in approximately 1 to 2 percent of cases after early- and late-onset infection.[139] In a case series from Houston, the mean age at recurrence was 44 days, and the mean

FIGURE 83-5 ■ This 3-month-old female infant who was born after 28 weeks of gestation had fever, lethargy, swelling of the external genitalia, and erythema that extended to the lower part of the abdomen and the thigh. Blood culture grew type Ib/c group B streptococci. (Courtesy Morven S. Edwards, MD.)

duration between the first course of therapy and recurrence was 19 days.[139] Molecular epidemiologic techniques have indicated that most, but not all, of these recurrent infections are caused by the strain implicated in the first episode; this finding suggests that persistent mucosal colonization after treatment of the first episode is followed by invasion of the bloodstream.[247] Contaminated breast milk also has been implicated as a source of recurrent infection.[189] No specific risk factors are evident in these infants, but most of them are born prematurely.[139]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Laboratory Studies

The diagnosis of GBS infection is confirmed by isolation of this pathogen from a normally sterile body site, including blood, CSF, pleural fluid, bone aspirate, joint fluid, or soft tissue. Cultures of the skin, umbilicus, or mucous membranes do not have clinical significance. Tracheal aspirate cultures that grow GBS indicate neonatal colonization but do not prove pulmonary invasion (pneumonia). Rather, isolation of the organism from blood (or in rare circumstances, lung tissue or the pleural space) is required.

Meningitis in early-onset disease is clinically indistinguishable from bacteremia without a focus, and 10 to 38 percent of neonates with meningitis have negative blood culture results,[357] so lumbar puncture is necessary to determine the presence or absence of meningeal involvement. Lumbar punctures also are indicated in patients with a focal site of GBS infection because each results from bacteremia. For example, GBS grew from CSF cultures in 24 percent of infants with cellulitis or adenitis who underwent lumbar puncture.[5] One study found that if selected criteria were used instead of routine lumbar punctures, the diagnosis of bacterial meningitis would have been missed or delayed in 17 percent of infants.[357]

CSF has had detectable group B antigen in 72 to 89 percent of neonates with meningitis; serum is much less likely to be positive. The only specimens recommended for antigen testing are blood and CSF; urine specimens should not be tested because they are unreliable. Antigen testing should not be used as a substitute for bacterial culture. Proper use of antigen testing should be limited to the setting of symptomatic infants in whom rapid diagnosis may alter therapy or allow proper tailoring of antibiotic choice; for example, a sick neonate with CSF pleocytosis and previous antibiotic administration. These tests are never useful in infants who appear healthy.

A limited study of real-time PCR to detect DNA in the blood of eight neonates with culture-proven GBS sepsis showed positive corresponding results.[177] However, larger, prospective studies are required to evaluate sensitivity and specificity.

The white blood cell count of a neonate with proven GBS sepsis may reflect leukopenia, neutropenia, or leukocytosis.[223,264,342] Manroe and associates[223] found the ratio of absolute immature neutrophils to absolute total neutrophils (I:T index) to be the most reliable index for distinguishing respiratory distress caused by GBS infection from that with a noninfectious origin. Most infected neonates had an elevation greater than 0.20 (91% vs. 4% of uninfected infants). Other markers of inflammation, such as C-reactive protein, may rise with acute infection and decrease in response to therapy.[272,276]

Differential Diagnosis

The signs and symptoms of early-onset GBS disease are clinically indistinguishable from neonatal sepsis caused by other bacterial pathogens such as *E. coli* and other enteric organisms. The timing is somewhat different, with GBS disease appearing in the first day and *E. coli* with onset on the second or third day of life.[170] The prominence of respiratory signs in early-onset disease may lead to confusion with noninfectious causes of respiratory distress such as transient tachypnea of the newborn, persistent fetal circulation, and respiratory distress syndrome.[2,22,264,342] Clinical features that suggest GBS infection include a history of prolonged rupture of membranes, apnea, shock in the first 24 hours of life, a 1-minute Apgar score of 5 or less, and rapid progression of pulmonary disease.[2,22,342]

TABLE 83-2 **Unusual Clinical Manifestations of Group B Streptococcal Disease in Infants and Children**

Site and Manifestation	References
Brain	
Abscess	312
Cerebritis	186
Chronic meningitis	317
Diabetes insipidus	216
Eosinophilic meningitis	242
Subdural empyema	117
Ventriculitis	243
Eye	
Conjunctivitis	16, 125, 274
Endophthalmitis	140, 198
Ear and Sinus	
Ethmoiditis	164
Otitis media/mastoiditis	23, 111, 310
Cardiovascular/Hematologic	
Asymptomatic bacteremia	164, 267, 283
Endocarditis	8, 43, 163, 253, 347
Myocarditis	44
Mycotic aneurysm	3
Pericarditis	143
Respiratory Tract	
Epiglottitis	364
Supraglottitis	210
Tracheitis	261
Pleural empyema	164, 316
Skin and Soft Tissue	
Breast abscess	250, 289
Bursitis	64
Cellulitis/adenitis	23, 146, 164, 195, 263, 356
Dactylitis	126
Fasciitis	135, 281
Impetigo neonatorum	46, 188, 213
Purpura fulminans	167, 214
Omphalitis	47, 169, 361
Rhabdomyolysis	334
Scalp abscess	114, 142
Abdomen	
Adrenal abscess	19, 73, 345
Delayed-onset diaphragmatic hernia	18, 41, 275, 321
Gallbladder distention	266
Peritonitis	71, 77
Urinary Tract	
Renal abscess	345, 360
Urinary tract infection	319, 361

Modified from Edwards MS, Nizet V. Group B streptococcal infections. In: Remington JS, Klein JO, Wilson CB, et al, editors. *Infectious diseases of the fetus and newborn infant.* 7th ed. Philadelphia: Saunders, 2011, pp. 419-469.

The differential diagnosis of late-onset disease depends on the focus of infection. Meningitis in infants of this age also is caused rarely by *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Listeria monocytogenes*, *Haemophilus influenzae* (type b and nontypeable), and, more commonly, viruses.[330] The findings of osteomyelitis may be subtle; refusal to move the arm may be ascribed to neuromuscular disease or Erb palsy.[104] Careful physical examination usually reveals tenderness over the involved area, and radiographs generally show a lytic defect in the metaphyses.[104] If the organism is isolated from a bone aspirate, the diagnosis is definitive. Finally, as seen in Table 83-2, many unusual manifestations of GBS infection have been described, so this organism should be included in the differential diagnosis of any focal infection occurring in the age group at risk.

TREATMENT

Empirical Treatment

The antimicrobial regimens recommended for the treatment of GBS infection in infants are summarized in Table 83-3. Penicillin G remains the drug of choice because susceptibility is uniform. In the usual circumstance, however, antimicrobial therapy for GBS infection is initiated before culture results are known. Initial empirical therapy for early-onset disease would include ampicillin and gentamicin for the treatment of neonatal pathogens in addition to GBS. Regardless of gestational age, neonates with suspected meningitis and those whose clinical condition will not permit lumbar puncture should receive high doses of ampicillin (300 mg/kg/day) plus gentamicin.[13] This combination is more effective than is either ampicillin or penicillin G alone in killing most GBS strains in vitro[298] and in vivo.[322] For suspected late-onset disease, the usual initial therapy includes intravenous ampicillin in combination with cefotaxime or ceftriaxone.[9] If an infant is receiving empirical vancomycin therapy and GBS meningitis has not been excluded, penicillin G or ampicillin should be added to the regimen because vancomycin is inhibitory rather than bactericidal in vitro, and CSF concentrations may not exceed the minimal inhibitory concentration if a high inoculum of GBS is present.[103]

Specific Treatment

Once GBS have been identified in cultures of blood, CSF, or other normally sterile body sites and blood and CSF cultures are shown to be sterile, penicillin G alone should be used to complete therapy. Recommendations concerning the optimal dose and duration have varied, but they

TABLE 83-3 **Treatment of Group B Streptococcal Infections in Infants**

Focus of Infection	Antibiotic	Daily Dose	Duration
Suspected meningitis (initial empirical therapy)	Ampicillin *plus* gentamicin	300-400 mg/kg 7.5 mg/kg	Until cerebrospinal fluid is sterile
Suspected sepsis* (initial empirical therapy)	Ampicillin *plus* gentamicin	150-200 mg/kg	Until bloodstream is sterile
Meningitis	Penicillin G	450,000-500,000 U/kg	14 days minimum†
Bacteremia	Penicillin G	200,000 U/kg	10 days
Arthritis	Penicillin G	200,000-300,000 U/kg	2-3 wk
Osteomyelitis	Penicillin G	200,000-300,000 U/kg	3-4 wk
Endocarditis	Penicillin G	400,000 U/kg	4 wk‡

*Assumes that lumbar puncture has been performed and the cerebrospinal fluid has no abnormalities.
†Should be extended to 21 days or longer if ventriculitis, cerebritis, subdural empyema, or other suppurative complications occur.
‡In combination with low-dose gentamicin for the first 14 days.

should be dictated by the focus and severity of the infection (see Table 83-3). The following issues should be considered when selecting the appropriate dose:

1. The usual minimal bactericidal concentration of penicillin for GBS ranges from 0.04 to 0.8 µg/mL.[185]
2. Only 10 percent of penicillin serum levels reach the CSF.
3. The inoculum of GBS in the CSF of infants with meningitis may reach 10^7 to 10^8 colony-forming units/mL.[116]
4. High doses of penicillin G and ampicillin are safe in neonates.[108]

To ensure rapid bacterial killing, especially in infants with meningitis, relatively high doses of penicillin are recommended for both early- and late-onset infections.

The infant with meningitis should undergo a second lumbar puncture 24 to 48 hours into therapy to document CSF sterility.[103] Infants with continued positive CSF cultures may have very high inoculum, ventriculitis with obstruction, severe infection with cerebritis and vasculitis, subdural empyema, or septic thrombophlebitis, or they may be receiving an insufficient dose of antibiotics; in this circumstance, appropriate studies should be initiated to determine which of these conditions is present. When CSF sterility is verified, penicillin G alone is given for a minimum of 14 days.[9] Longer treatment is indicated if the course is severe, the infant has ventriculitis, or CSF sterilization is delayed. Another lumbar puncture to evaluate CSF cell count and protein should be considered at the anticipated completion of therapy. Findings of polymorphonuclear cells greater than 30 percent or protein higher than 200 mg/dL may warrant further diagnostic evaluation and longer duration of therapy.[103] In addition, a contrast-enhanced neuroimaging study should be performed near completion of therapy in complicated cases, including infants who have prolonged fever (>5 days), cerebritis, abscess(es), subdural empyema, or venous thrombosis. Such complications often correlate with neurologic abnormalities that lead to a poor prognosis for complete central nervous system recovery.[108,344]

Infants with bacteremia without a focus should receive intravenous therapy for a total of 10 days.[9] A shorter duration has not been documented to be efficacious, and relapses, although rare, have been reported in these circumstances.[66,346] Patients with septic arthritis, osteomyelitis, or endocarditis should be treated for the durations summarized in Table 83-3. Oral therapy has no place in the management of infants with GBS disease.[9,107] Alternative agents such as the third-generation cephalosporins and vancomycin are active against GBS in vitro.[118,206] The clinical efficacy of these agents is unknown, however, and these drugs are not recommended in most circumstances.

Supportive Treatment

The importance of prompt, vigorous, and careful supportive therapy in the successful treatment of infant GBS infections cannot be overemphasized. Neonates with early-onset disease accompanied by pneumonia should be suspected of having early respiratory failure, and ventilatory support should be initiated before the onset of apnea, septic shock, or frank respiratory failure occurs. Persistent metabolic acidosis and delayed capillary refill should prompt treatment for shock. All patients with signs of impending respiratory or circulatory failure or meningitis should be treated in an intensive care unit. When present, hypoxemia, severe anemia, and acidosis should be corrected, and seizures should be controlled with anticonvulsants. In addition, fluid and electrolyte status should be monitored meticulously. Surfactant should be used as per nursery protocol; improved gas exchange is seen in infected premature neonates who receive surfactant, although the response is slower than in patients with respiratory distress syndrome.[156] Finally, if an infant has persistent pulmonary hypertension or if conventional ventilatory therapy has failed, extracorporeal membrane oxygenation may be considered, if available.

Adjunctive Treatment

Adjunctive treatment of life-threatening GBS disease is aimed at correcting poor host defenses. Such treatment previously studied includes intravenous human immunoglobulin, monoclonal antibodies to GBS polysaccharide

antigen, leukocyte transfusion, and growth factors such as granulocyte colony-stimulating factor and granulocyte-monocyte colony-stimulating factor for neutropenia.[70,80,133] The efficacy of these agents has not been established, and, although they may be used occasionally, they should be considered experimental.

Recurrent Infections

In the few infants who experience a recurrence, suppurative foci, HIV infection, or humoral immune deficiency should be excluded or treated. Ig levels should be determined to exclude humoral immune deficiency. Although it may be too early to document humoral immune deficiency unequivocally, total IgG usually is significantly lower than would be expected for age in weeks.[139] Tube dilution susceptibility of isolates from the first and recurrent episodes should be determined to ensure in vitro susceptibility to penicillin. If the reason for the recurrence remains unknown, persistent mucous membrane colonization with GBS probably is the source.[247] β-Lactam antibiotics, even when administered by the parenteral route, do not eradicate GBS colonization reliably.[259] Some studies have shown the benefit of rifampin 20 mg/kg/day to eradicate mucosal GBS colonization when given orally during the last 4 days of parenteral therapy.[139,240] However, a more recent study showed failure of rifampin to eradicate GBS colonization in infants.[119]

PROGNOSIS

The outcome of GBS disease is related to the severity and site of infection at initial evaluation. Improved outcomes after early-onset infection have resulted from greater awareness among pediatricians and improved obstetric management. Improvements in obstetrics include the use of intrapartum antibiotic prophylaxis in women at risk for delivering an infant with early-onset GBS infection. However, the mortality rate remains substantial at 2 to 8 percent, especially in neonates born at less than 37 weeks of gestation, in whom the mortality rate often exceeds 20 percent.[76,304]

Little information is available on the long-term prognosis of survivors of GBS sepsis without meningitis. In infants with septic shock, the development of periventricular leukomalacia has been reported and associated with neurodevelopmental sequelae.[112] However, the frequency of this association is not known.

For infants with early- or late-onset meningitis, 20 to 30 percent will have permanent neurologic sequelae, and 20 percent of these impaired survivors have global mental retardation, cortical blindness, hearing loss, spasticity, or paresis.[108,145,199,344] Despite advances in intensive care, a current assessment shows that rates of neurologic impairment remain unchanged.[199] This study evaluated term infants to avoid confounders of cognitive and behavioral deficits related to prematurity.

To date, the prognosis for infants with osteoarticular or soft tissue infections with GBS has been excellent.[23,104] However, omission of early surgical intervention for infections that involve either the hip or shoulder joints can result in epiphyseal injury.

PREVENTION

The continuing magnitude and severity of GBS disease and its attendant mortality and morbidity have led to investigations aimed at its prevention. Several general approaches have been proposed: maternal prophylaxis to decrease transmission, infant prophylaxis to decrease colonization, and immunoprophylaxis to increase protection against disease.

Intrapartum Antibiotic Prophylaxis

Maternal Prophylaxis

Epidemiologic studies during the 1980s showed that women with GBS colonization during pregnancy had more than 25 times likelihood of delivering infants with early-onset disease in contrast to women with negative prenatal cultures.[340,341] The idea to give antibiotic therapy to the mother to prevent vertical transmission to her fetus was suggested first by Franciosi and colleagues.[125] The first strategy to be evaluated was antenatal treatment of GBS maternal carriers with oral or intramuscular penicillin. This method temporarily suppresses colonization density but does not eradicate colonization or interrupt vertical transmission of GBS to neonates.[130,141,280]

The next strategy, maternal intrapartum chemoprophylaxis, has been demonstrated to be efficacious in the prevention of vertical transmission of GBS from colonized mothers to their neonates, the development of early-onset disease, and maternal febrile morbidity. Infants are less frequently colonized with GBS if they are born to mothers treated with ampicillin during labor than to mothers not given antibiotics.[60,61,365] Several controlled trials involving thousands of deliveries indicated that intrapartum penicillin G or ampicillin given intravenously to GBS carriers during labor prevents early-onset disease in neonates.[58,231,333] Early-onset infection occurred in 0 to 1.1 percent of infants whose mothers received intrapartum ampicillin in contrast to 5 to 9 percent of infants of mothers in the groups not receiving prophylaxis. A large trial involving more than 50,000 live births in Australia showed similar results.[131]

In 1996, guidelines supported by the Centers for Disease Control and Prevention (CDC), the American College of Obstetricians and Gynecologists, and the American Academy of Pediatrics recommended a risk-based or culture-based approach.[341] The risk-based approach was based on the presence of one or more of the following: premature labor at less than 37 weeks of gestation, prolonged rupture of membranes (>18 hours), GBS bacteriuria, intrapartum fever (38°C [≥100.4°F]) or delivery of a previous infant with GBS infection. The culture-based approach required intrapartum chemoprophylaxis for all women with positive vaginal and rectal cultures routinely obtained at 35 to 37 weeks of gestation regardless of risk factors. Use of these methods to define women who were candidates for intrapartum

chemoprophylaxis led to a 70 percent decline in the incidence of early-onset GBS disease.[302,341]

In 2002, a population-based study compared the two approaches and found the culture-based approach to be 50 percent more effective than the risk-based approach in preventing early-onset GBS disease.[303] Sixty-two percent of infants with early-onset disease were born to mothers who did not have any clinical risk factors. Therefore, revised guidelines published by the CDC in 2002 recommended universal vaginal and rectal screening cultures of all pregnant women between 35 and 37 weeks of gestation, with intrapartum chemoprophylaxis for all women identified as GBS carriers.[301] The risk-based approach was to be reserved for any women in labor whose colonization status is unknown. After release of these guidelines, active population surveillance showed that 85 percent of women were being actively screened, resulting in a 27 percent further decline in incidence of early-onset infection.[340] However, the retrospective cohort study also revealed missed opportunities for screening and chemoprophylaxis for women with unknown colonization status, especially those at risk for preterm delivery. Unpublished data from the CDC showed that antibiotic prophylaxis was 78 percent effective in preventing early-onset infection in preterm infants.[341] Thus, revised intrapartum chemoprophylaxis guidelines with specific algorithms for women with preterm labor or premature rupture of membranes were released in 2010.[341] If a woman with unknown colonization status is admitted with signs and symptoms of preterm labor or premature rupture of membranes, vaginal and rectal swabs should be obtained for culture and intrapartum chemoprophylaxis should be started and continued until delivery. If amniotic membranes are intact, prophylaxis should be discontinued if it is determined that the woman is not in true labor. Women with premature rupture of membranes who are not in labor should continue antibiotics per standard of care if receiving to prolong latency or continue antibiotics for 48 hours if receiving for GBS prophylaxis.

Colonization or bacteriuria during a previous pregnancy is not an indication for intrapartum chemoprophylaxis. Intrapartum chemoprophylaxis also is not indicated for cesarean section performed before onset of labor on a woman with intact amniotic membranes, regardless of GBS colonization status or gestational age. Women with GBS isolated from urine at a concentration of 10^4 colony-forming units/mL or greater during the current pregnancy should receive intrapartum chemoprophylaxis, because these women usually are heavily colonized. Women who previously gave birth to an infant with invasive GBS disease always should receive chemoprophylaxis. Table 83-4 shows the indications and nonindications for intrapartum chemoprophylaxis according to the current guidelines (released in 2010).[341]

Optimal culture techniques are discussed earlier in the chapter. Many rapid tests are being studied because they offer the advantage of detecting colonization status in women who present for preterm labor or who have not had prenatal care. These tests include latex agglutination, optical immunoassay, DNA hybridization, enzyme immunoassay, Islam starch medium tests, and PCR.[160] With the exception of PCR, all tests showed poor sensitivity or specificity in contrast to the standard culture method and are not sufficiently accurate to replace routine culture in decision making concerning intrapartum antibiotic prophylaxis. A multicenter study evaluated a real-time PCR assay that gave results in 40 minutes.[88] In contrast to intrapartum culture, the PCR assay had a sensitivity of 94 to 98 percent and a specificity of 96 to 99 percent.[88,110] Nucleic acid amplification tests, such as commercially available PCR assays, are an option for intrapartum testing for women who have unknown colonization status at labor onset.[341] However, these tests

TABLE 83-4 **Indications and Nonindications for Maternal Intrapartum Prophylaxis to Prevent Early-Onset Group B Streptococcal Infection**

Intrapartum GBS Prophylaxis Indicated	Intrapartum GBS Prophylaxis Not Indicated
Previous infant with invasive GBS disease	Colonization with GBS during a previous pregnancy
GBS bacteriuria during any trimester of current pregnancy*	GBS bacteriuria during previous pregnancy
Positive GBS vaginal-rectal screening culture within preceding 5 weeks*	Negative vaginal and rectal screening culture, regardless of intrapartum risk factors
Unknown GBS status at onset of labor and any of the following: Delivery at <37 weeks of gestation Prolonged rupture of membranes for ≥18 hr Intrapartum temperature ≥100.4°F (38°C)† Intrapartum NAAT positive for GBS‡	Cesarean delivery performed before onset of labor on a woman with intact amniotic membranes, regardless of GBS colonization status or gestational age

Modified from Centers for Disease Control and Prevention: Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1-32.

*Intrapartum chemoprophylaxis is not indicated in this circumstance if a cesarean delivery is performed before onset of labor on a woman with intact membranes.

†If amnionitis is suspected, broad-spectrum antibiotic therapy that includes an agent active against GBS should replace GBS prophylaxis.

‡GBS NAATs are optional and may not be available in all settings (see text). If intrapartum NAAT is negative for GBS but any other of these intrapartum risk factors is present, intrapartum GBS prophylaxis is indicated.

GBS, Group B streptococcus; *NAAT,* nucleic acid amplification test.

should not replace antenatal culture- or risk-based assessment of women with unknown colonization status. Nucleic acid amplification tests must be performed on samples placed in selective enrichment broths for adequate sensitivity of the assay, thereby lengthening the turnaround time and making it less useful in the real-world setting at this time.

Maternal chemoprophylaxis begins at hospital admission for delivery or at rupture of membranes and consists of intravenous penicillin G (initial dose, 5 million U; subsequent doses, 2.5 to 3 million U every 4 hours until delivery).[303,341] Although penicillin is preferred because of its narrow spectrum of activity, ampicillin (initial dose, 2 g; subsequent doses, 1 g every 4 hours) can be given as an alternative. For women who are allergic to penicillin, intrapartum chemoprophylaxis must take into account increasing resistance rates to clindamycin and erythromycin.[56,91] Women at low risk for developing anaphylaxis should be given cefazolin (initial dose, 2 g; subsequent doses, 1 g every 8 hours). Women at high risk for developing anaphylaxis should have susceptibility testing performed on their isolates obtained during screening at 35 to 37 weeks of gestation. Susceptibility testing should include checking for inducible clindamycin resistance. If the isolate is susceptible, clindamycin 900 mg every 8 hours can be used. Erythromycin is no longer an acceptable alternative.[341] If susceptibility testing is not available, the results are not known, or the isolate is resistant to clindamycin, vancomycin 1 g every 12 hours can be given as the alternative. The efficacy of vancomycin is not known.

Since the widespread adoption of chemoprophylaxis, the incidence of early-onset disease has plummeted.[302,340,341] Racial disparities in early-onset disease have declined significantly,[302] but the higher incidence of early-onset infection in black infants in contrast to nonblack infants remains a problem. Opportunities to prevent early-onset disease still are missed, however, because of laboratory practices,[74] hospital or procedural errors in communication of the screening results, failure to administer intrapartum chemoprophylaxis to a mother known to be colonized, or failure to recognize preterm delivery in mothers with unknown colonization status as an indication for prophylaxis.[279,340] The incidence of late-onset disease remains unaffected by intrapartum chemoprophylaxis.[175,341]

Management of an infant born to a mother given intrapartum prophylaxis depends on the clinical findings at birth, antibiotic regimen, and timing of doses administered to the mother (Figure 83-6). Amniotic fluid levels of penicillin that will kill GBS are not achieved until at least 3 hours after administration of the first dose.[63] If the infant of any gestational age appears healthy and has a mother who received at least one dose of intrapartum chemoprophylaxis with intravenous penicillin, ampicillin, or cefazolin at least 4 hours before delivery, neither diagnostic evaluation nor empirical antimicrobial therapy is required.[341] However, to ensure their ongoing stability, such infants should be observed in the hospital for at least 48 hours. Keeping the infant hospitalized through the second day of life has been challenging because the associated expense affects the cost-to-benefit ratio of intrapartum prophylaxis.[244] However, an analysis of all live births in the state of Florida for the years 1992 through 1994 demonstrated a 115 percent increase in the rate of readmission for GBS infection in infants discharged on the first day of life; this finding supports the recommendation to observe infants for 48 hours.[137]

Neonates with signs of sepsis are evaluated and treated empirically for sepsis. Healthy-appearing neonates born at 37 weeks of gestation or later to a mother with an indication for chemoprophylaxis but who had received inadequate intrapartum chemoprophylaxis should be observed at least 48 hours. An infant born at less than 37 weeks of gestation or after membrane rupture for 18 hours or longer to a mother with inadequate chemoprophylaxis should undergo limited laboratory evaluation (i.e., complete blood count and blood culture) and observation in the hospital for 48 hours without therapy. Adequate intrapartum chemoprophylaxis is defined as at least 4 hours of intravenous penicillin, ampicillin, or cefazolin. All other agents are considered inadequate.[341] If the subsequent clinical course or the laboratory results suggest infection, full diagnostic evaluation and therapy are initiated.

Concern that widespread use of intrapartum ampicillin use will result in a greater incidence of ampicillin-resistant neonatal infection has been supported by some case series.[51,178,237] Development of penicillin- or ampicillin-resistant GBS has not been seen.[79] In most studies, incidence rates of *E. coli* and other gram-negative pathogens have remained stable.[4,178,237,290] The rate of ampicillin-resistant *E. coli* infections is increasing in preterm infants. However, rates of ampicillin resistance in term neonates before and after widespread intrapartum chemoprophylaxis are the same.

Infant Chemoprophylaxis

The two controlled trials evaluating neonatal prophylaxis reached contradictory conclusions.[280,311] In the first trial, more than 16,000 newborns received either intramuscular penicillin G or topical tetracycline (prophylaxis for gonococcal ophthalmia neonatorum) within an hour of birth during alternating weeks.[311] In penicillin-treated newborns, the incidence of proved early-onset GBS disease was less common, but death rates were similar, and an increase in the number of penicillin-resistant infections in the penicillin group was noted. In the second controlled study, blood cultures were obtained before initiation of penicillin therapy, and rates of GBS bacteremia were similar in the treatment and control groups.[280] In this study of nearly 1200 neonates weighing 2000 g or less at birth, intramuscular penicillin (or no treatment) was given in the first hour of life and was continued for 3 days. Rates of GBS bacteremia were similar in the treatment and control groups. Penicillin prophylaxis probably was ineffective because almost 90 percent of neonates with early-onset GBS disease were bacteremic immediately after birth. The results of the second study are supported by numerous observations, thus indicating that early-onset disease often begins in

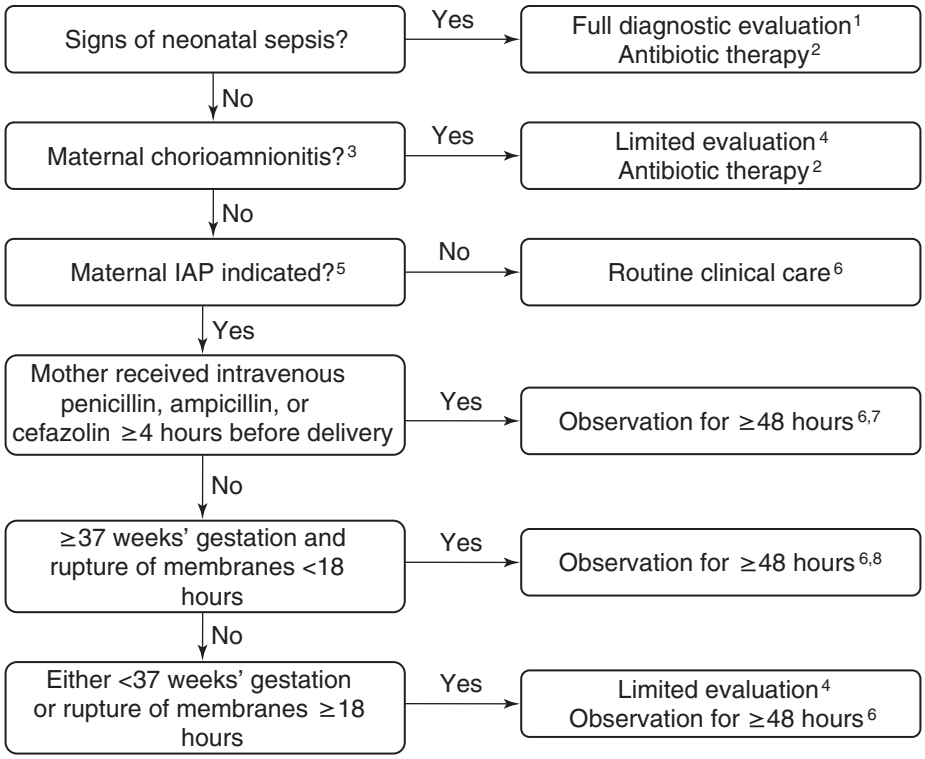

FIGURE 83-6 ■ Empirical management of a newborn for secondary prevention of early-onset group B streptococcal infection.
[1]Includes complete blood cell count (CBC) and differential, blood culture, and chest radiograph if respiratory abnormalities are present. When signs of sepsis are present, a lumbar puncture, if feasible, should be performed.
[2]Antibiotic therapy should be directed toward common causes of neonatal sepsis, including ampicillin for group B *Streptococcus* and coverage for *Escherichia coli* and other gram-negative pathogens, and should take into account local resistance patterns.
[3]Consultation with obstetric providers is important to determine the level of clinical suspicion for chorioamnionitis. Chorioamnionitis is diagnosed clinically, and some of the signs are nonspecific.
[4]Includes complete CBC and differential and blood culture.
[5]See Table 83-4 for indications for maternal intrapartum prophylaxis.
[6]If signs of sepsis develop, a full diagnostic evaluation should be conducted and antibiotic therapy initiated.
[7]A healthy-appearing infant who was ≥37 weeks of gestation at delivery and whose mother received ≥4 hours of appropriate intrapartum antibiotic prophylaxis (*IAP*) before delivery may be discharged home after 24 hours if other discharge criteria have been met, a person able to comply fully with instructions for home observation will be present, and access to medical care is readily available. If any one of these conditions is not met, the infant should be observed in the hospital for at least 48 hours and until criteria for discharge are achieved.
[8]Some experts recommend a CBC with differential at 6 to 12 hours. (Modified from the Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease—revised guidelines from CDC, 2010. *MMWR Morb Mortal Wkly Rep* 2010;59:1-32.)

utero.[61,262,305] Infant chemoprophylaxis currently is not recommended.[341]

Immunoprophylaxis

Although efforts to improve implementation of intrapartum chemoprophylaxis are ongoing, the most promising and potentially lasting method for prevention of both early- and late-onset infant infections is immunoprophylaxis. This approach remains investigational, and several reviews have summarized the rationale.[26,147,257] It is based on the observation that immunity to GBS correlates with antibody directed against the type-specific capsular polysaccharides of these organisms. These IgG class antibodies and complement promote opsonization, phagocytosis, and bacterial killing of GBS and protect animals against lethal challenge.[258,353] Thus, provision of protective levels of type-specific immunity to the infant could be achieved through active immunization of the mother. Baker and associates[37] immunized women at a mean gestation of 31 weeks with purified type III polysaccharide vaccine. Although the immune response was not optimal (63%), placental transport of maternal antibodies occurred in 90 percent in neonates born to women who did respond to vaccination, and most of these infants had protective levels of antibodies in their sera at 3 months of age. A more recent study immunizing women with a type III polysaccharide-tetanus toxoid conjugate reported a 95 percent immune response, with persistence of opsonophagocytic antibodies at 2 months of age in 100 percent of infants born to maternal responders.[27] Other studies immunizing nonpregnant adults with purified capsular polysaccharide–tetanus toxoid conjugate vaccines showed the safety and immunogenicity of these vaccines.[35,36,38]

Availability of the complete genome sequences for GBS has led to the discovery of many potential surface proteins and secreted proteins that can be expressed in

recombinant form and used for production of antibodies.[217,329] A set of proteins shared by the disease-causing serotypes theoretically can be used in combination to create a vaccine capable of providing protection against multiple serotypes. GBS pili with at least one of the pilus islands present in all sequenced GBS genomes may be included in a future vaccine.[67,171]

Because most pregnant women (estimated at 85% to 90%) have nonprotective levels of these antibodies in their sera at delivery, active immunization of childbearing-age women before or during pregnancy would optimize strategy to induce high maternal antibody levels. Human studies have demonstrated efficient antibody transfer to the neonate.[30,37] Transfer of high levels of maternal antibody would provide protection of the neonates from early- and late-onset GBS.[176] In addition, immunization also would offer protection for the mother against preterm deliveries and fetal loss caused by GBS infection. Finally, vaccination of elderly or nonpregnant adults with defined medical conditions could reduce the disease burden significantly. Because immunoprophylaxis should be the most cost-effective and beneficial prevention strategy for GBS disease,[148,245,313] it should be promoted by physicians, public health officials, parents, pharmaceutical manufacturers, and legislators.

NEW REFERENCES SINCE THE SIXTH EDITION

9. American Academy of Pediatrics. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 628–34.
17. Arya A, Cryan B, O'Sullivan K, et al. Self-collected versus health professional-collected genital swabs to identify the prevalence of group B streptococcus: a comparison of patient preference and efficacy. *Eur J Obstet Gynecol Reprod Biol* 2008;**139**:43–5.
20. Attipoe S, Park JY, Fenty N, et al. Oxidative stress levels are reduced in postmenopausal women with exercise training regardless of hormone replacement therapy status. *J Women Aging* 2008;**20**:31–45.
51. Bizzarro MJ, Dembry LM, Baltimore RS, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008;**121**: 689–96.
76. Centers for Disease Control and Prevention. Trends in perinatal group B streptococcal disease: United States, 2000-2006. *MMWR Morb Mortal Wkly Rep* 2009;**58**:109–12.
78. Chang YC, Wang Z, Flax LA, et al. Glycosaminoglycan binding facilitates entry of a bacterial pathogen into central nervous systems. *PLoS Pathog* 2011;**7**:e1002082.
85. Costa A, Gupta R, Signorino G, et al. Activation of the NLRP3 inflammasome by group B streptococci. *J Immunol* 2012;**188**: 1953–60.
86. Currie AJ, Curtis S, Strunk T, et al. Preterm infants have deficient monocyte and lymphocyte cytokine responses to group B streptococcus. *Infect Immun* 2011;**79**:1588–96.
93. Diedrick MJ, Flores AE, Hillier SL, et al. Clonal analysis of colonizing group B Streptococcus, serotype IV, an emerging pathogen in the United States. *J Clin Microbiol* 2010;**48**:3100–4.
103. Edwards MS, Nizet V. Group B streptococcal infections. In: Remington JS, Klein JO, Wilson CB, editors. *Infectious diseases of the fetus and newborn infant*. 7th ed. Philadelphia: Saunders; 2011. p. 419–69.
110. El Helali N, Nguyen JC, Ly A, et al. Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. *Clin Infect Dis* 2009;**49**:417–23.
115. Feikin DR, Thorsen P, Zywicki S, et al. Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women. *Am J Obstet Gynecol* 2001;**184**: 427–33.
124. Fluegge K, Siedler A, Heinrich B, et al. Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. *Pediatrics* 2006;**117**:e1139–45.
152. Henneke P, Dramsi S, Mancuso G, et al. Lipoproteins are critical TLR2 activating toxins in group B streptococcal sepsis. *J Immunol* 2008;**180**:6149–58.
153. Hensler ME, Miyamoto S, Nizet V. Group B streptococcal beta-hemolysin/cytolysin directly impairs cardiomyocyte viability and function. *PLoS One* 2008;**3**:e2446.
155. Hensler ME, Quach D, Hsieh CJ, et al. CAMP factor is not essential for systemic virulence of group B Streptococcus. *Microb Pathog* 2008;**44**:84–8.
171. Jiang S, Park SE, Yadav P, et al. Regulation and function of pilus island 1 in group B Streptococcus. *J Bacteriol* 2012;**194**:2479–90.
175. Jordan HT, Farley MM, Craig A, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease: a multistate, population-based analysis. *Pediatr Infect Dis J* 2008;**27**:1057–64.
176. Jordan HT, Prapasiri P, Areerat P, et al. A comparison of population-based pneumonia surveillance and health-seeking behavior in two provinces in rural Thailand. *Int J Infect Dis* 2009;**13**:355–61.
181. Katz V, Bowes WA. Perinatal group B streptococcal infections across intact amniotic membranes. *J Reprod Med* 1988;**33**:445–9.
199. Levent F, Baker CJ, Rench MA, et al. Early outcomes of group B streptococcal meningitis in the 21st century. *Pediatr Infect Dis J* 2010;**29**:1009–12.
220. Manning SD, Tallman P, Baker CJ, et al. Determinants of co-colonization with group B streptococcus among heterosexual college couples. *Epidemiology* 2002;**13**:533–9.
235. Meinke AL, Senn BM, Visram Z, et al. Immunological fingerprinting of group B streptococci: from circulating human antibodies to protective antigens. *Vaccine* 2010;**28**:6997–7008.
267. Peña BM, Harper MB, Fleisher GR. Occult bacteremia with group B streptococci in an outpatient setting. *Pediatrics* 1998;**102**: 67–72.
270. Pezzicoli A, Santi I, Lauer P, et al. Pilus backbone contributes to group B Streptococcus paracellular translocation through epithelial cells. *J Infect Dis* 2008;**198**:890–8.
271. Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA* 2008;**299**:2056–65.
277. Price D, Shaw E, Howard M, et al. Self-sampling for group B streptococcus in women 35 to 37 weeks pregnant is accurate and acceptable: a randomized cross-over trial. *J Obstet Gynaecol Can* 2006;**28**:1083–8.
287. Reiss A, Braun JS, Jäger K, et al. Bacterial pore-forming cytolysins induce neuronal damage in a rat model of neonatal meningitis. *J Infect Dis* 2011;**203**:393–400.
291. Richter SS, Howard WJ, Weinstein MP, et al. Multicenter evaluation of the BD Phoenix Automated Microbiology System for antimicrobial susceptibility testing of *Streptococcus* species. *J Clin Microbiol* 2007;**45**:2863–71.
297. Sadeghi K, Berger A, Langgartner M, et al. Immaturity of infection control in preterm and term newborns is associated with impaired toll-like receptor signaling. *J Infect Dis* 2007;**195**: 296–302.
326. Tazi A, Reglier-Poupet H, Dautezac F, et al. Comparative evaluation of Strepto B ID chromogenic medium and Granada media for the detection of group B streptococcus from vaginal samples of pregnant women. *J Microbiol Methods* 2008;**73**:263–5.
340. Van Dyke MK, Phares CR, Lynfield R, et al. Evaluation of universal antenatal screening for group B streptococcus. *N Engl J Med* 2009;**360**:2626–36.
341. Verani JR, McGee L, Schrag SJ, et al. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2010;**59**:1–36.
351. Wennekamp J, Henneke P. Induction and termination of inflammatory signaling in group B streptococcal sepsis. *Immunol Rev* 2008;**225**:114–27.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aber RC, Allen N, Howell JT, et al. Nosocomial transmission of group B streptococci. *Pediatrics* 1976;**58**:346–53.
2. Ablow RC, Driscoll SG, Effmann EL, et al. A comparison of early-onset group B steptococcal neonatal infection and the respiratory-distress syndrome of the newborn. *N Engl J Med* 1976;**294**:65–70.
3. Agarwala BN. Group B streptococcal endocarditis in a neonate. *Pediatr Cardiol* 1988;**9**:51–3.
4. Alarcon A, Peña P, Salas S, et al. Neonatal early onset *Escherichia coli* sepsis: trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. *Pediatr Infect Dis J* 2004;**23**:295–9.
5. Albanyan EA, Baker CJ. Is lumbar puncture necessary to exclude meningitis in neonates and young infants: lessons from the group B streptococcus cellulitis-adenitis syndrome. *Pediatrics* 1998;**102**:985–6.
6. Alger LS, Lovchik JC, Hebel JR, et al. The association of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and group B streptococci with preterm rupture of the membranes and pregnancy outcome. *Am J Obstet Gynecol* 1988;**159**:397–404.
7. Allardice JG, Baskett TF, Seshia MM, et al. Perinatal group B streptococcal colonization and infection. *Am J Obstet Gynecol* 1982;**142**:617–20.
8. Alsoub H, Najma F, Robida A. Group B streptococcal endocarditis in children beyond the neonatal period. *Pediatr Infect Dis J* 1997; **16**:418–20.
9. American Academy of Pediatrics. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 628–34.
10. Ancona RJ, Ferrieri P, Williams PP. Maternal factors that enhance the acquisition of group-B streptococci by newborn infants. *J Med Microbiol* 1980;**13**:273–80.
11. Anderson DC, Hughes BJ, Smith CW. Abnormal mobility of neonatal polymorphonuclear leukocytes: relationship to impaired redistribution of surface adhesion sites by chemotactic factor or colchicine. *J Clin Invest* 1981;**68**:863–74.
12. Anderson DC, Hughes BJ, Edwards MS, et al. Impaired chemotaxigenesis by type III group B streptococci in neonatal sera: relationship to diminished concentration of specific anticapsular antibody and abnormalities of serum complement. *Pediatr Res* 1983;**17**:496–502.
13. Anthony BF, Okada DM. The emergence of group B streptococci in infections of the newborn infant. *Annu Rev Med* 1977;**28**: 355–69.
14. Anthony BF, Okada DM, Hobel CJ. Epidemiology of group B Streptococcus: longitudinal observations during pregnancy. *J Infect Dis* 1978;**137**:524–30.
15. Anthony BF, Okada DM, Hobel CJ. Epidemiology of the group B streptococcus: maternal and nosocomial sources for infant acquisitions. *J Pediatr* 1979;**95**:431–6.
16. Armstrong JH, Zacarias F, Rein MF. Ophthalmia neonatorum: a chart review. *Pediatrics* 1976;**57**:884–92.
17. Arya A, Cryan B, O'Sullivan K, et al. Self-collected versus health professional-collected genital swabs to identify the prevalence of group B streptococcus: a comparison of patient preference and efficacy. *Eur J Obstet Gynecol Reprod Biol* 2008;**139**:43–5.
18. Ashcraft KW, Holder TM, Amoury RA, et al. Diagnosis and treatment of right Bochdalek hernia associated with group B streptococcal pneumonia and sepsis in the neonate. *J Pediatr Surg* 1983;**18**:480–5.
19. Atkinson G, Kodroff M, Gay B, et al. Adrenal abscess in the neonate. *Radiology* 1985;**155**:101–4.
20. Attipoe S, Park JY, Fenty N, et al. Oxidative stress levels are reduced in postmenopausal women with exercise training regardless of hormone replacement therapy status. *J Women Aging* 2008;**20**:31–45.
21. Badri MS, Zawaneh S, Cruz AC, et al. Rectal colonization with group B streptococcus: relation to vaginal colonization of pregnant women. *J Infect Dis* 1977;**135**:308–12.
22. Baker CJ. Early onset group B streptococcal disease. *J Pediatr* 1978;**93**:124–5.
23. Baker CJ. Group B streptococcal cellulitis-adenitis in infants. *Am J Dis Child* 1982;**136**:631–3.
24. Baker CJ, Barrett FF. Transmission of group B streptococci among parturient women and their neonates. *J Pediatr* 1973;**83**:919–25.
25. Baker CJ, Barrett FF. Group B streptococcal infections in infants. The importance of the various serotypes. *JAMA* 1974;**230**: 1158–60.
26. Baker CJ, Kasper DL. Group B streptococcal vaccines. *Rev Infect Dis* 1985;**7**:458–67.
27. Baker CJ, Clark DJ, Barrett FF. Selective broth medium for isolation of group B streptococci. *Appl Microbiol* 1973;**26**:884–5.
28. Baker CJ, Edwards MS, Kasper DL. Role of antibody to native type III polysaccharide of group B Streptococcus in infant infection. *Pediatrics* 1981;**68**:544–9.
29. Baker CJ, Kasper DL, Davis CE. Immunochemical characterization of the "native" type III polysaccharide of group B Streptococcus. *J Exp Med* 1976;**143**:258–70.
30. Baker CJ, Rench MA, McInnes P. Immunization of pregnant women with group B streptococcal type III capsular polysaccharide-tetanus toxoid conjugate vaccine. *Vaccine* 2003;**21**:3468–72.
31. Baker CJ, Barrett FF, Gordon RC, et al. Suppurative meningitis due to streptococci of Lancefield group B: a study of 33 infants. *J Pediatr* 1973;**82**:724–9.
32. Baker CJ, Goroff DK, Alpert SL, et al. Comparison of bacteriological methods for the isolation of group B Streptococcus from vaginal cultures. *J Clin Microbiol* 1976;**4**:46–8.
33. Baker CJ, Goroff DK, Alpert S, et al. Vaginal colonization with group B streptococcus: a study in college women. *J Infect Dis* 1977;**135**:392–7.
34. Baker CJ, Kasper DL, Tager IRAB, et al. Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B Streptococcus. *J Clin Invest* 1977;**59**: 810–18.
35. Baker CJ, Paoletti LC, Rench MA, et al. Immune response of healthy women to 2 different group B streptococcal type V capsular polysaccharide-protein conjugate vaccines. *J Infect Dis* 2004; **189**:1103–12.
36. Baker CJ, Paoletti LC, Wessels MR, et al. Safety and immunogenicity of capsular polysaccharide-tetanus toxoid conjugate vaccines for group B streptococcal types Ia and Ib. *J Infect Dis* 1999; **179**:142–50.
37. Baker CJ, Rench MA, Edwards MS, et al. Immunization of pregnant women with a polysaccharide vaccine of group B streptococcus. *N Engl J Med* 1988;**319**:1180–5.
38. Baker CJ, Rench MA, Fernandez M, et al. Safety and immunogenicity of a bivalent group B streptococcal conjugate vaccine for serotypes II and III. *J Infect Dis* 2003;**188**:66–73.
39. Baker CJ, Webb BJ, Jackson CV, et al. Countercurrent immunoelectrophoresis in the evaluation of infants with group B streptococcal disease. *Pediatrics* 1980;**65**:1110–14.
40. Baltimore RS, Huie SM, Meek JI, et al. Early-onset neonatal sepsis in the era of group B streptococcal prevention. *Pediatrics* 2001;**108**:1094–8.
41. Banagale RC, Watters JH. Delayed right-sided diaphragmatic hernia following group B streptococcal infection: a discussion of its pathogenesis, with a review of the literature. *Hum Pathol* 1983;**14**:67–9.
42. Baron MJ, Bolduc GR, Goldberg MB, et al. Alpha C protein of group B Streptococcus binds host cell surface glycosaminoglycan and enters cells by an actin-dependent mechanism. *J Biol Chem* 2004;**279**:24714–23.
43. Barton CW, Crowley DC, Uzark K, et al. A neonatal survivor of group B beta-hemolytic streptococcal endocarditis. *Am J Perinatol* 1984;**1**:214–15.
44. Bateman AC, Richards M, Pallett AP. Fatal myocarditis associated with a Lancefield group B streptococcus. *J Infect* 1998;**36**:354–5.
45. Becroft DM, Farmer K, Mason GH, et al. Perinatal infections by group B beta-haemolytic streptococci. *Br J Obstet Gynaecol* 1976;**83**:960–6.
46. Belgaumkar TK. Impetigo neonatorum congenita due to group B beta-hemolytic streptococcus infection (Letter). *J Pediatr* 1975; **86**:982–3.
47. Bergqvist G, Hurvell B, Malmborg AS, et al. Neonatal infections caused by group B streptococci. *Scand J Infect Dis* 1971;**3**: 157–62.
48. Betriu C, Gomez M, Sanchez A, et al. Antibiotic resistance and penicillin tolerance in clinical isolates of group B streptococci. *Antimicrob Agents Chemother* 1994;**38**:2183–6.

49. Bhakdi S, Klonisch T, Nuber P, et al. Stimulation of monokine production by lipoteichoic acids. *Infect Immun* 1991;**59**:4614–20.
50. Bingen E, Denamur E, Lambert-Zechovsky N, et al. Analysis of DNA restriction fragment length polymorphism extends the evidence for breast milk transmission in *Streptococcus agalactiae* late-onset neonatal infection. *J Infect Dis* 1992;**165**:569–73.
51. Bizzarro MJ, Dembry LM, Baltimore RS, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008;**121**: 689–96.
52. Bizzarro MJ, Raskind C, Baltimore RS, et al. Seventy-five years of neonatal sepsis at Yale: 1928-2003. *Pediatrics* 2005;**116**: 595–602.
53. Bliss SJ, Manning SD, Tallman P, et al. Group B Streptococcus colonization in male and nonpregnant female university students: a cross-sectional prevalence study. *Clin Infect Dis* 2002;**34**:184–90.
54. Blumberg HM, Stephens DS, Modansky M, et al. Invasive group B streptococcal disease: the emergence of serotype V. *J Infect Dis* 1996;**173**:365–73.
55. Bone RC. Gram-positive organisms and sepsis. *Arch Intern Med* 1994;**154**:26–34.
56. Borchardt SM, DeBusscher JH, Tallman PA, et al. Frequency of antimicrobial resistance among invasive and colonizing Group B streptococcal isolates. *BMC Infect Dis* 2006;**6**:57.
57. Botta GA. Hormonal and type-dependent adhesion of group B streptococci to human vaginal cells. *Infect Immun* 1979;**25**: 1084–6.
58. Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. *N Engl J Med* 1986;**314**:1665–9.
59. Boyer KM, Gadzala CA, Burd LI, et al. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. I. Epidemiologic rationale. *J Infect Dis* 1983;**148**:795–801.
60. Boyer KM, Gadzala CA, Kelly PD, et al. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. II. Predictive value of prenatal cultures. *J Infect Dis* 1983;**148**:802–9.
61. Boyer KM, Gadzala CA, Kelly PD, et al. Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease. III. Interruption of mother-to-infant transmission. *J Infect Dis* 1983;**148**:810–16.
62. Brady MT. Cellulitis of the penis and scrotum due to group B streptococcus. *J Urol* 1987;**137**:736–7.
63. Bray RE, Boe RW, Johnson WL. Transfer of ampicillin into fetus and amniotic fluid from maternal plasma in late pregnancy. *Am J Obstet Gynecol* 1966;**96**:938–42.
64. Brian MJ, O'Ryan M, Waagner D. Prepatellar bursitis in an infant caused by group B Streptococcus. *Pediatr Infect Dis J* 1992;**11**: 502–3.
65. Bromberger P, Lawrence JM, Braun D, et al. The influence of intrapartum antibiotics on the clinical spectrum of early-onset group B streptococcal infection in term infants. *Pediatrics* 2000; **106**:244–50.
66. Broughton DD, Mitchell WG, Grossman M, et al. Recurrence of group B streptococcal infection. *J Pediatr* 1976;**89**:182–5.
67. Buccato S, Maione D, Rinaudo CD, et al. Use of Lactococcus lactis expressing pili from group B Streptococcus as a broad-coverage vaccine against streptococcal disease. *J Infect Dis* 2006;**194**:331–40.
68. Bulgakova TN, Grabovskaya KB, Rýc M, et al. The adhesin structures involved in the adherence of group B streptococci to human vaginal cells. *Folia Microbiol (Praha)* 1986;**31**:394–401.
69. Butter MNW, DeMoor CE. Streptococcus agalactiae as a cause of meningitis in the newborn, and of bacteremia in adults. *Antonie Van Leeuwenhoek* 1967;**33**:439–50.
70. Cairo MS, Plunkett JM, Nguyen A, et al. Effect of stem cell factor with and without granulocyte colony-stimulating factor on neonatal hematopoiesis: in vivo induction of newborn myelopoiesis and reduction of mortality during experimental group B streptococcal sepsis. *Blood* 1992;**80**:96–101.
71. Callanan DL, Harris GG. Group B streptococcal infection in children with liver disease. *Clin Pediatr (Phila)* 1982;**21**:99–100.
72. Campbell JR, Hillier SL, Krohn MA, et al. Group B streptococcal colonization and serotype-specific immunity in pregnant women at delivery. *Obstet Gynecol* 2000;**96**:498–503.
73. Carty A, Stanley P. Bilateral adrenal abscesses in a neonate. *Pediatr Radiol* 1973;**1**:63–4.
74. Centers for Disease Control and Prevention. Laboratory practices for prenatal group B streptococcal screening: seven states, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:506–9.
75. Centers for Disease Control and Prevention. Early-onset and late-onset neonatal group B streptococcal disease: United States, 1996-2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1205–8.
76. Centers for Disease Control and Prevention. Trends in perinatal group B streptococcal disease: United States, 2000-2006. *MMWR Morb Mortal Wkly Rep* 2009;**58**:109–12.
77. Chadwick EG, Shulman ST, Yogev R. Peritonitis as a late manifestation of group B streptococcal disease in newborns. *Pediatr Infect Dis* 1983;**2**:142–3.
78. Chang YC, Wang Z, Flax LA, et al. Glycosaminoglycan binding facilitates entry of a bacterial pathogen into central nervous systems. *PLoS Pathog* 2011;**7**:e1002082.
79. Chen KT, Puopolo KM, Eichenwald EC, et al. No increase in rates of early-onset neonatal sepsis by antibiotic-resistant group B Streptococcus in the era of intrapartum antibiotic prophylaxis. *Am J Obstet Gynecol* 2005;**192**:1167–71.
80. Christensen KK, Christensen P. Intravenous gamma-globulin in the treatment of neonatal sepsis with special reference to group B streptococci and pharmacokinetics. *Pediatr Infect Dis* 1986;**5**: S189–192.
81. Christensen KK, Christensen P, Duc G, et al. Correlation between serum antibody-levels against group B streptococci and gestational age in newborns. *Eur J Pediatr* 1984;**142**:86–8.
82. Christensen RD, Rothstein G. Exhaustion of mature marrow neutrophils in neonates with sepsis. *J Pediatr* 1980;**96**:316–18.
83. Christie R, Atkins NE, Munch-Petersen, E. A note on a lytic phenomenon shown by group B streptococci. *Aust J Exp Biol Med Sci* 1944;**22**:197–200.
84. Congdon PM. Streptococcal infection in childbirth and septic abortion: source of infection and grouping of haemolytic strains. *Lancet* 1935;**226**:1287–8.
85. Costa A, Gupta R, Signorino G, et al. Activation of the NLRP3 inflammasome by group B streptococci. *J Immunol* 2012;**188**: 1953–60.
86. Currie AJ, Curtis S, Strunk T, et al. Preterm infants have deficient monocyte and lymphocyte cytokine responses to group B streptococcus. *Infect Immun* 2011;**79**:1588–96.
87. Davies HD, Raj S, Adair C, et al. Population-based active surveillance for neonatal group B streptococcal infections in Alberta, Canada: implications for vaccine formulation. *Pediatr Infect Dis J* 2001;**20**:879–84.
88. Davies HD, Miller MA, Faro S, et al. Multicenter study of a rapid molecular-based assay for the diagnosis of group B Streptococcus colonization in pregnant women. *Clin Infect Dis* 2004;**39**: 1129–35.
89. Davis CA, Vallota EH, Forristal J. Serum complement levels in infancy: age related changes. *Pediatr Res* 1979;**13**:1043–6.
90. Desa DJ, Trevenen CL. Intrauterine infections with group B beta-haemolytic streptococci. *Br J Obstet Gynaecol* 1984;**91**:237–9.
91. Desjardins M, Delgaty KL, Ramotar K, et al. Prevalence and mechanisms of erythromycin resistance in group A and group B Streptococcus: implications for reporting susceptibility results. *J Clin Microbiol* 2004;**42**:5620–3.
92. Di John D, Krasinski K, Lawrence R, et al. Very late onset of group B streptococcal disease in infants infected with the human immunodeficiency virus. *Pediatr Infect Dis J* 1990;**9**:925–8.
93. Diedrick MJ, Flores AE, Hillier SL, et al. Clonal analysis of colonizing group B Streptococcus, serotype IV, an emerging pathogen in the United States. *J Clin Microbiol* 2010;**48**:3100–4.
94. Dillon HC, Gray E, Pass MA, et al. Anorectal and vaginal carriage of group B streptococci during pregnancy. *J Infect Dis* 1982;**145**: 794–9.
95. Dillon HC, Khare S, Gray BM. Group B streptococcal carriage and disease: a 6-year prospective study. *J Pediatr* 1987;**110**: 31–6.
96. Dokter WH, Dijkstra AJ, Koopmans SB, et al. G(AnH)MTetra, a naturally occurring 1,6-anhydro muramyl dipeptide, induces granulocyte colony-stimulating factor expression in human monocytes: a molecular analysis. *Infect Immun* 1994;**62**:2953–7.
97. Doran KS, Liu GY, Nizet V. Group B streptococcal beta-hemolysin/cytolysin activates neutrophil signaling pathways in

brain endothelium and contributes to development of meningitis. *J Clin Invest* 2003;**112**:736–44.
98. Doran KS, Engelson EJ, Khosravi A, et al. Blood-brain barrier invasion by group B Streptococcus depends upon proper cell-surface anchoring of lipoteichoic acid. *J Clin Invest* 2005;**115**: 2499–507.
99. Easmon CS, Hastings MJ. GBS colonisation in mothers and babies. *Antibiot Chemother* 1985;**35**:28–39.
100. Easmon CS, Hastings MJ, Clare AJ, et al. Nosocomial transmission of group B streptococci. *Br Med J (Clin Res Ed)* 1981;**283**: 459–61.
101. Easmon CS, Hastings MJ, Neill J, et al. Is group B streptococcal screening during pregnancy justified? *Br J Obstet Gynaecol* 1985;**92**:197–201.
102. Edwards MS, Baker CJ. Group B streptococcal infections in elderly adults. *Clin Infect Dis* 2005;**41**:839–47.
103. Edwards MS, Nizet V. Group B streptococcal infections. In: Remington JS, Klein JO, Wilson CB, editors. *Infectious diseases of the fetus and newborn infant*. 7th ed. Philadelphia: Saunders; 2011. p. 419–69.
104. Edwards MS, Baker CJ, Wagner ML, et al. An etiologic shift in infantile osteomyelitis: the emergence of the group B streptococcus. *J Pediatr* 1978;**93**:578–83.
105. Edwards MS, Buffone GJ, Fuselier PA, et al. Deficient classical complement pathway activity in newborn sera. *Pediatr Res* 1983; **17**:685–8.
106. Edwards MS, Jackson CV, Baker CJ. Increased risk of group B streptococcal disease in twins. *JAMA* 1981;**245**:2044–6.
107. Edwards MS, Kasper DL, Jennings HJ, et al. Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci. *J Immunol* 1982;**128**:1278–83.
108. Edwards MS, Rench MA, Haffar AA, et al. Long-term sequelae of group B streptococcal meningitis in infants. *J Pediatr* 1985;**106**:717–22.
109. Eickhoff TC, Klein JO, Daly AK, et al. Neonatal sepsis and other infections due to group B beta-hemolytic streptococci. *N Engl J Med* 1964;**271**:1221–8.
110. El Helali N, Nguyen JC, Ly A, et al. Diagnostic accuracy of a rapid real-time polymerase chain reaction assay for universal intrapartum group B streptococcus screening. *Clin Infect Dis* 2009;**49**:417–23.
111. Ellis SS, Johnson A, Austin TL. Group B streptococcal meningitis associated with otitis media. *Am J Dis Child* 1976;**130**:1003–4.
112. Faix RG, Donn SM. Association of septic shock caused by early-onset group B streptococcal sepsis and periventricular leukomalacia in the preterm infant. *Pediatrics* 1985;**76**:415–19.
113. Farley MM, Harvey RC, Stull T, et al. A population-based assessment of invasive disease due to group B Streptococcus in nonpregnant adults. *N Engl J Med* 1993;**328**:1807–11.
114. Feder HM, MacLean WC, Moxon R. Scalp abscess secondary to fetal scalp electrode. *J Pediatr* 1976;**89**:808–9.
115. Feikin DR, Thorsen P, Zywicki S, et al. Association between colonization with group B streptococci during pregnancy and preterm delivery among Danish women. *Am J Obstet Gynecol* 2001;**184**: 427–33.
116. Feldman WE. Concentrations of bacteria in cerebrospinal fluid of patients with bacterial meningitis. *J Pediatr* 1976;**88**:549–52.
117. Ferguson L, Gotoff SP. Subdural empyema in an infant due to group B beta-hemolytic Streptococcus. *Am J Dis Child* 1977; **131**:97.
118. Fernandez M, Hickman ME, Baker CJ. Antimicrobial susceptibilities of group B streptococci isolated between 1992 and 1996 from patients with bacteremia or meningitis. *Antimicrob Agents Chemother* 1998;**42**:1517–19.
119. Fernandez M, Rench MA, Albanyan EA, et al. Failure of rifampin to eradicate group B streptococcal colonization in infants. *Pediatr Infect Dis J* 2001;**20**:371–6.
120. Ferrieri P, Flores AE. Surface protein expression in group B streptococcal invasive isolates. *Adv Exp Med Biol* 1997;**418**: 635–7.
121. Ferrieri P, Wannamaker LW, Nelson J. Localization and characterization of the hippuricase activity of group B streptococci. *Infect Immun* 1973;**7**:747–52.
122. Ferrieri P, Baker CJ, Hillier SL, et al. Diversity of surface protein expression in group B streptococcal colonizing & invasive isolates. *Indian J Med Res* 2004;**119**(suppl):191–6.
123. Ferrieri P, Gray ED, Wannamaker LW. Biochemical and immunological characterization of the extracellular nucleases of group B streptococci. *J Exp Med* 1980;**151**:56–68.
124. Fluegge K, Siedler A, Heinrich B, et al. Incidence and clinical presentation of invasive neonatal group B streptococcal infections in Germany. *Pediatrics* 2006;**117**:e1139–45.
125. Franciosi RA, Knostman JD, Zimmerman RA. Group B streptococcal neonatal and infant infections. *J Pediatr* 1973;**82**:707–18.
126. Frieden IJ. Blistering dactylitis caused by group B streptococci. *Pediatr Dermatol* 1989;**6**:300–2.
127. Fry RM. Fatal infections by haemolytic Streptococcus group B. *Lancet* 1938;**231**:199–201.
128. Galask RP, Varner MW, Petzold CR, et al. Bacterial attachment to the chorioamniotic membranes. *Am J Obstet Gynecol* 1984; **148**:915–28.
129. Gardner SE, Mason EO, Yow MD. Community acquisition of group B Streptococcus by infants of colonized mothers. *Pediatrics* 1980;**66**:873–5.
130. Gardner SE, Yow MD, Leeds LJ, et al. Failure of penicillin to eradicate group B streptococcal colonization in the pregnant woman: a couple study. *Am J Obstet Gynecol* 1979;**135**:1062–5.
131. Garland SM, Fliegner JR. Group B streptococcus (GBS) and neonatal infections: the case for intrapartum chemoprophylaxis. *Aust N Z J Obstet Gynaecol* 1991;**31**:119–22.
132. Gibson RL, Lee MK, Soderland C, et al. Group B streptococci invade endothelial cells: type III capsular polysaccharide attenuates invasion. *Infect Immun* 1993;**61**:478–85.
133. Givner LB. Human immunoglobulins for intravenous use: comparison of available preparations for group B streptococcal antibody levels, opsonic activity, and efficacy in animal models. *Pediatrics* 1990;**86**:955–62.
134. Glaser P, Rusniok C, Buchrieser C, et al. Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. *Mol Microbiol* 2002;**45**:1499–513.
135. Goldberg GN, Hansen RC, Lynch PJ. Necrotizing fasciitis in infancy: report of three cases and review of the literature. *Pediatr Dermatol* 1984;**2**:55–63.
136. Gotoff SP, Papierniak CK, Klegerman ME, et al. Quantitation of IgG antibody to the type-specific polysaccharide of group B streptococcus type 1b in pregnant women and infected infants. *J Pediatr* 1984;**105**:628–30.
137. Graven MA, Cuddeback JK, Wyble L. Readmission for group B streptococci or *Escherichia coli* infection among full-term, singleton, vaginally delivered neonates after early discharge from Florida hospitals for births from 1992 through 1994. *J Perinatol* 1999; **19**:19–25.
138. Gray BM, Pritchard DG, Dillon HC. Seroepidemiological studies of group B Streptococcus type II. *J Infect Dis* 1985;**151**:1073–80.
139. Green PA, Singh KV, Murray BE, et al. Recurrent group B streptococcal infections in infants: clinical and microbiologic aspects. *J Pediatr* 1994;**125**:931–8.
140. Greene GR, Carroll WL, Morozumi PA, et al. Endophthalmitis associated with group-B streptococcal meningitis in an infant. *Am J Dis Child* 1979;**133**:752–3.
141. Hall RT, Barnes W, Krishnan L, et al. Antibiotic treatment of parturient women colonized with group B streptococci. *Am J Obstet Gynecol* 1976;**124**:630–4.
142. Handrick W, Spencker FB, Künzel R. [Scalp infection caused by B-streptococci in a newborn infant following internal cardiotocography]. *Zentralbl Gynakol* 1984;**106**:1544–6.
143. Harper IA. The importance of group B streptococci as human pathogens in the British Isles. *J Clin Pathol* 1971;**24**:438–41.
144. Harrison LH, Elliott JA, Dwyer DM, et al. Serotype distribution of invasive group B streptococcal isolates in Maryland: implications for vaccine formulation. Maryland Emerging Infections Program. *J Infect Dis* 1998;**177**:998–1002.
145. Haslam RH, Allen JR, Dorsen MM, et al. The sequelae of group B beta-hemolytic streptococcal meningitis in early infancy. *Am J Dis Child* 1977;**131**:845–9.
146. Hauger SB. Facial cellulitis: an early indicator of group B streptococcal bacteremia. *Pediatrics* 1981;**67**:376–7.
147. Healy CM, Baker CJ. Prospects for prevention of childhood infections by maternal immunization. *Curr Opin Infect Dis* 2006;**19**: 271–6.
148. Heath PT. An update on vaccination against group B streptococcus. *Expert Rev Vaccines* 2011;**10**:685–94.

149. Heath PT, Balfour GF, Tighe H, et al. Group B streptococcal disease in infants: a case control study. *Arch Dis Child* 2009;**94**: 674–80.
150. Helmig R, Halaburt JT, Uldbjert N, et al. Increased cell adherence of group B streptococci from preterm infants with neonatal sepsis. *Obstet Gynecol* 1990;**76**:825–7.
151. Hemming VG, Nagarajan K, Hess LW, et al. Rapid in vitro replication of group B streptococcus in term human amniotic fluid. *Gynecol Obstet Invest* 1985;**19**:124–9.
152. Henneke P, Dramsi S, Mancuso G, et al. Lipoproteins are critical TLR2 activating toxins in group B streptococcal sepsis. *J Immunol* 2008;**180**:6149–58.
153. Hensler ME, Miyamoto S, Nizet V. Group B streptococcal beta-hemolysin/cytolysin directly impairs cardiomyocyte viability and function. *PLoS One* 2008;**3**:e2446.
154. Hensler ME, Liu GY, Sobczak S, et al. Virulence role of group B Streptococcus beta-hemolysin/cytolysin in a neonatal rabbit model of early-onset pulmonary infection. *J Infect Dis* 2005;**191**: 1287–91.
155. Hensler ME, Quach D, Hsieh CJ, et al. CAMP factor is not essential for systemic virulence of group B Streptococcus. *Microb Pathog* 2008;**44**:84–8.
156. Herting E, Gefeller O, Land M, et al. Surfactant treatment of neonates with respiratory failure and group B streptococcal infection. Members of the Collaborative European Multicenter Study Group. *Pediatrics* 2000;**106**:957–64; discussion 1135.
157. Heumann D, Barras C, Severin A, et al. Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes. *Infect Immun* 1994;**62**:2715–21.
158. Hickman ME, Rench MA, Ferrieri P, et al. Changing epidemiology of group B streptococcal colonization. *Pediatrics* 1999;**104**: 203–9.
159. Hill HR, Bohnsack JF, Morris EZ, et al. Group B streptococci inhibit the chemotactic activity of the fifth component of complement. *J Immunol* 1988;**141**:3551–6.
160. Honest H, Sharma S, Khan KS. Rapid tests for group B Streptococcus colonization in laboring women: a systematic review. *Pediatrics* 2006;**117**:1055–66.
161. Hood M, Janney A, Dameron G. Beta hemolytic streptococcus group B associated with problems of the perinatal period. *Am J Obstet Gynecol* 1961;**82**:809–18.
162. Hoogkamp-Korstanje JA, Gerards LJ, Cats BP. Maternal carriage and neonatal acquisition of group B streptococci. *J Infect Dis* 1982;**145**:800–3.
163. Horigome H, Okada Y, Hirano T, et al. Group B streptococcal endocarditis in infancy with a giant vegetation on the pulmonary valve. *Eur J Pediatr* 1994;**153**:140–1.
164. Howard JB, McCracken GH. The spectrum of group B streptococcal infections in infancy. *Am J Dis Child* 1974;**128**:815–18.
165. Hussain SM, Luedtke GS, Baker CJ, et al. Invasive group B streptococcal disease in children beyond early infancy. *Pediatr Infect Dis J* 1995;**14**:278–81.
166. Hyde TB, Hilger TM, Reingold A, et al. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. *Pediatrics* 2002;**110**: 690–5.
167. Issacman SH, Heroman WM, Lightsey AL. Purpura fulminans following late-onset group B beta-hemolytic streptococcal sepsis. *Am J Dis Child* 1984;**138**:915–16.
168. Jackson LA, Hilsdon R, Farley MM, et al. Risk factors for group B streptococcal disease in adults. *Ann Intern Med* 1995;**123**: 415–20.
169. Jacobs MR, Koornhof HJ, Stein H. Group B streptococcal infections in neonates and infants: report of 16 cases, with reference to the fatal outcome in late-onset leucopenic cases. *S Afr Med J* 1978;**54**:154–8.
170. Jeffery H, Mitchison R, Wigglesworth JS, et al. Early neonatal bacteraemia: comparison of group B streptococcal, other Gram-positive and Gram-negative infections. *Arch Dis Child* 1977;**52**: 683–6.
171. Jiang S, Park SE, Yadav P, et al. Regulation and function of pilus island 1 in group B Streptococcus. *J Bacteriol* 2012;**194**: 2479–90.
172. Johnson DR, Ferrieri P. Group B streptococcal Ibc protein antigen: distribution of two determinants in wild-type strains of common serotypes. *J Clin Microbiol* 1984;**19**:506–10.
173. Johri AK, Margarit I, Broenstrup M, et al. Transcriptional and proteomic profiles of group B Streptococcus type V reveal potential adherence proteins associated with high-level invasion. *Infect Immun* 2007;**75**:1473–83.
174. Jones DE, Kanarek KS, Lim DV. Group B streptococcal colonization patterns in mothers and their infants. *J Clin Microbiol* 1984;**20**:438–40.
175. Jordan HT, Farley MM, Craig A, et al. Revisiting the need for vaccine prevention of late-onset neonatal group B streptococcal disease: a multistate, population-based analysis. *Pediatr Infect Dis J* 2008;**27**:1057–64.
176. Jordan HT, Prapasiri P, Areerat P, et al. A comparison of population-based pneumonia surveillance and health-seeking behavior in two provinces in rural Thailand. *Int J Infect Dis* 2009;**13**:355–61.
177. Jordan JA, Durso MB. Real-time polymerase chain reaction for detecting bacterial DNA directly from blood of neonates being evaluated for sepsis. *J Mol Diagn* 2005;**7**:575–81.
178. Joseph TA, Pyati SP, Jacobs N. Neonatal early-onset *Escherichia coli* disease: the effect of intrapartum ampicillin. *Arch Pediatr Adolesc Med* 1998;**152**:35–40.
179. Joyner JL, Augustine NH, Taylor KA, et al. Effects of group B streptococci on cord and adult mononuclear cell interleukin-12 and interferon-gamma mRNA accumulation and protein secretion. *J Infect Dis* 2000;**182**:974–7.
180. Jürgens D, Sterzik B, Fehrenbach FJ. Unspecific binding of group B streptococcal cocytolysin (CAMP factor) to immunoglobulins and its possible role in pathogenicity. *J Exp Med* 1987;**165**: 720–32.
181. Katz V, Bowes WA. Perinatal group B streptococcal infections across intact amniotic membranes. *J Reprod Med* 1988;**33**: 445–9.
182. Keller R, Fischer W, Keist R, et al. Macrophage response to bacteria: induction of marked secretory and cellular activities by lipoteichoic acids. *Infect Immun* 1992;**60**:3664–72.
183. Kim KS. Antimicrobial susceptibility of GBS. *Antibiot Chemother* 1985;**35**:83–9.
184. Kim KS. Clinical perspectives on penicillin tolerance. *J Pediatr* 1988;**112**:509–14.
185. Kim KS, Anthony BF. Penicillin tolerance in group B streptococci isolated from infected neonates. *J Infect Dis* 1981;**144**:411–19.
186. Kim KS, Kaye KL, Itabashi HH, et al. Cerebritis due to group B streptococcus. *Scand J Infect Dis* 1982;**14**:305–8.
187. Klegerman ME, Boyer KM, Papierniak CK, et al. Type-specific capsular antigen is associated with virulence in late-onset group B Streptococcal type III disease. *Infect Immun* 1984;**44**:124–9.
188. Kline A, O'Donnell E. Group B streptococcus as a cause of neonatal bullous skin lesions. *Pediatr Infect Dis J* 1993;**12**:165–6.
189. Kotiw M, Zhang GW, Daggard G, et al. Late-onset and recurrent neonatal Group B streptococcal disease associated with breast-milk transmission. *Pediatr Dev Pathol* 2003;**6**:251–6.
190. Lachenauer CS, Kasper DL, Shimada J, et al. Serotypes VI and VIII predominate among group B streptococci isolated from pregnant Japanese women. *J Infect Dis* 1999;**179**:1030–3.
191. Lancefield RC. A serological differentiation of human and other groups of hemolytic streptococci. *J Exp Med* 1933;**57**:571–95.
192. Lancefield RC. Two serologic types of group B hemolytic streptococci with related, but not identical, type-specific substances. *J Exp Med* 1938;**67**:25–40.
193. Lancefield RC, Hare R. The serological differentiation of pathogenic and non-pathogenic strains of hemolytic streptococci from parturient women. *J Exp Med* 1935;**61**:335–49.
194. Lancefield RC, McCarty M, Everly WN. Multiple mouse-protective antibodies directed against group B streptococci: special reference to antibodies effective against protein antigens. *J Exp Med* 1975;**142**:165–79.
195. Lang ME, Vaudry W, Robinson JL. Case report and literature review of late-onset group B streptococcal disease manifesting as necrotizing fasciitis in preterm infants: is this a new syndrome? *Clin Infect Dis* 2003;**37**:e132–5.
196. Larsson C, Lindroth M, Nordin P, et al. Association between low concentrations of antibodies to protein alpha and Rib and invasive neonatal group B streptococcal infection. *Arch Dis Child Fetal Neonatal Ed* 2006;**91**:F403–8.
197. Lauer P, Rinaudo CD, Soriani M, et al. Genome analysis reveals pili in group B Streptococcus. *Science* 2005;**309**:105.

198. Lee SY, Chee SP. Group B Streptococcus endogenous endophthalmitis: case reports and review of the literature. *Ophthalmology* 2002;**109**:1879–86.
199. Levent F, Baker CJ, Rench MA, et al. Early outcomes of group B streptococcal meningitis in the 21st century. *Pediatr Infect Dis J* 2010;**29**:1009–12.
200. Levy NJ, Nicholson-Weller A, Baker CJ, et al. Potentiation of virulence by group B streptococcal polysaccharides. *J Infect Dis* 1984;**149**:851–60.
201. Lewis DB, Wilson CB. Developmental immunology and role of host defenses in neonatal susceptibility to infection. In: Remington JS, Klein JO, Wilson CB, Baker CJ, editors. *Infectious Diseases of the Fetus and Newborn Infant*, 6th ed. Philadelphia: WB Saunders; 2006. p. 87–210.
202. Lilien LD, Harris VJ, Pildes RS. Significance of radiographic findings in early-onset group B streptococcal infection. *Pediatrics* 1977;**60**:360–3.
203. Lim DV, Kanarek KS, Peterson ME. Magnitude of colonization and sepsis by group B streptococci in newborn infants. *Curr Microbiol* 1982;**7**:99–101.
204. Lim DV, Morales WJ, Walsh AF. Lim group B strep broth and coagglutination for rapid identification of group B streptococci in preterm pregnant women. *J Clin Microbiol* 1987;**25**:452–3.
205. Lin FY, Azimi PH, Weisman LE, et al. Antibiotic susceptibility profiles for group B streptococci isolated from neonates, 1995-1998. *Clin Infect Dis* 2000;**31**:76–9.
206. Lin FY, Clemens JD, Azimi PH, et al. Capsular polysaccharide types of group B streptococcal isolates from neonates with early-onset systemic infection. *J Infect Dis* 1998;**177**:790–2.
207. Lin FY, Weisman LE, Troendle J, et al. Prematurity is the major risk factor for late-onset group B streptococcus disease. *J Infect Dis* 2003;**188**:267–71.
208. Lindén V. Mouse-protective effect of rabbit anti-R-protein antibodies against group B streptococci type II carrying R-protein: lack of effect on type III carrying R-protein. *Acta Pathol Microbiol Immunol Scand B* 1983;**91**:145–51.
209. Lindén V, Christensen KK, Christensen P. Correlation between low levels of maternal IgG antibodies to R protein and neonatal septicemia with group B streptococci carrying R protein. *Int Arch Allergy Appl Immunol* 1983;**71**:168–72.
210. Lipson A, Kronick JB, Tewfik L, et al. Group B streptococcal supraglottitis in a 3-month-old infant. *Am J Dis Child* 1986;**140**:411–12.
211. Liu GY, Doran KS, Lawrence T, et al. Sword and shield: linked group B streptococcal beta-hemolysin/cytolysin and carotenoid pigment function to subvert host phagocyte defense. *Proc Natl Acad Sci U S A* 2004;**101**:14491–6.
212. Long WA, Lawson EE, Harned HS, et al. Pleural effusion in the first days of life: a prospective study. *Am J Perinatol* 1984;**1**:190–4.
213. Lopez JB, Gross P, Boggs TR. Skin lesions in association with beta-hemolytic Streptococcus group B. *Pediatrics* 1976;**58**:859–61.
214. Lynn NJ, Pauly TH, Desai NS. Purpura fulminans in three cases of early-onset neonatal group B streptococcal meningitis. *J Perinatol* 1991;**11**:144–6.
215. MacDonald SW, Manuel FR, Embil JA. Localization of group B beta-hemolytic streptococci in the female urogenital tract. *Am J Obstet Gynecol* 1979;**133**:57–9.
216. MacGilvray SS, Billow M. Diabetes insipidus as a complication of neonatal group B streptococcal meningitis. *Pediatr Infect Dis J* 1990;**9**:742–3.
217. Maione D, Margarit I, Rinaudo CD, et al. Identification of a universal group B streptococcus vaccine by multiple genome screen. *Science* 2005;**309**:148–50.
218. Maisey HC, Hensler M, Nizet V, et al. Group B streptococcal pilus proteins contribute to adherence to and invasion of brain microvascular endothelial cells. *J Bacteriol* 2007;**189**:1464–7.
219. Mancuso G, Cusumano V, Genovese F, et al. Role of interleukin 12 in experimental neonatal sepsis caused by group B streptococci. *Infect Immun* 1997;**65**:3731–5.
220. Manning SD, Tallman P, Baker CJ, et al. Determinants of co-colonization with group B streptococcus among heterosexual college couples. *Epidemiology* 2002;**13**:533–9.
221. Manning SD, Ki M, Marrs CF, et al. The frequency of genes encoding three putative group B streptococcal virulence factors among invasive and colonizing isolates. *BMC Infect Dis* 2006;**6**:116.
222. Manning SD, Neighbors K, Tallman PA, et al. Prevalence of group B streptococcus colonization and potential for transmission by casual contact in healthy young men and women. *Clin Infect Dis* 2004;**39**:380–8.
223. Manroe BL, Rosenfeld CR, Weinberg AG, et al. The differential leukocyte count in the assessment and outcome of early-onset neonatal group B streptococcal disease. *J Pediatr* 1977;**91**:632–7.
224. Marchlewicz BA, Duncan JL. Properties of a hemolysin produced by group B streptococci. *Infect Immun* 1980;**30**:805–13.
225. Mårdh PA, Westtöm L. Adherence of bacterial to vaginal epithelial cells. *Infect Immun* 1976;**13**:661–6.
226. Maródi L, Leijh PC, van Furth R. Characteristics and functional capacities of human cord blood granulocytes and monocytes. *Pediatr Res* 1984;**18**:1127–31.
227. Marques MB, Kasper DL, Pangburn MK, et al. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. *Infect Immun* 1992;**60**:3986–93.
228. Martin TR, Rubens CE, Wilson CB. Lung antibacterial defense mechanisms in infant and adult rats: implications for the pathogenesis of group B streptococcal infections in the neonatal lung. *J Infect Dis* 1988;**157**:91–100.
229. Mason EO, Wong P, Barrett FF. Evaluation of four methods for detection of group B streptococcal colonization. *J Clin Microbiol* 1976;**4**:429–31.
230. Matorras R, Garcia Perea A, Omeñaca F, et al. Group B streptococcus and premature rupture of membranes and preterm delivery. *Gynecol Obstet Invest* 1989;**27**:14–18.
231. Matorras R, García-Perea A, Omeñaca F, et al. Intrapartum chemoprophylaxis of early-onset group B streptococcal disease. *Eur J Obstet Gynecol Reprod Biol* 1991;**40**:57–62.
232. Matorras R, Garcia-Perea A, Usandizaga JA, et al. Natural transmission of group B Streptococcus during delivery. *Int J Gynaecol Obstet* 1989;**30**:99–103.
233. McClean D. The capsulation of streptococci and its relation to diffusion factor (hyaluronidase). *J Pathol Bacteriol* 1941;**53**:13–27.
234. McDonald H, Vigneswaran R, O'Loughlin JA. Group B streptococcal colonization and preterm labour. *Aust N Z J Obstet Gynaecol* 1989;**29**:291–3.
235. Meinke AL, Senn BM, Visram Z, et al. Immunological fingerprinting of group B streptococci: from circulating human antibodies to protective antigens. *Vaccine* 2010;**28**:6997–7008.
236. Memon IA, Jacobs NM, Yeh TF, et al. Group B streptococcal osteomyelitis and septic arthritis: its occurrence in infants less than 2 months old. *Am J Dis Child* 1979;**133**:921–3.
237. Mercer BM, Carr TL, Beazley DD, et al. Antibiotic use in pregnancy and drug-resistant infant sepsis. *Am J Obstet Gynecol* 1999;**181**:816–21.
238. Meyn LA, Moore DM, Hillier SL, et al. Association of sexual activity with colonization and vaginal acquisition of group B Streptococcus in nonpregnant women. *Am J Epidemiol* 2002;**155**:949–57.
239. Michel JL, Madoff LC, Kling DE, et al. Cloned alpha and beta C-protein antigens of group B streptococci elicit protective immunity. *Infect Immun* 1991;**59**:2023–8.
240. Millard DD, Bussey ME, Shulman ST, et al. Multiple group B streptococcal infections in a premature infant: eradication of nasal colonization with rifampin. *Am J Dis Child* 1985;**139**:964–5.
241. Minett FC, Stableforth AW, J ES. Studies on bovine mastitis. I. The bacteriology of mastitis. *J Comp Pathol* 1929;**42**:213–31.
242. Miron D, Snelling LK, Josephson SL, et al. Eosinophilic meningitis in a newborn with group B streptococcal infection. *Pediatr Infect Dis J* 1993;**12**:966–7.
243. Miyairi I, Causey KT, DeVincenzo JP, et al. Group B streptococcal ventriculitis: a report of three cases and literature review. *Pediatr Neurol* 2006;**34**:395–9.
244. Mohle-Boetani JC, Lieu TA, Ray GT, et al. Preventing neonatal group B streptococcal disease: cost-effectiveness in a health maintenance organization and the impact of delayed hospital discharge for newborns who received intrapartum antibiotics. *Pediatrics* 1999;**103**:703–10.
245. Mohle-Boetani JC, Schuchat A, Plikaytis BD, et al. Comparison of prevention strategies for neonatal group B streptococcal infection: a population-based economic analysis. *JAMA* 1993;**270**:1442–8.

246. Møller M, Thomsen AC, Borch K, et al. Rupture of fetal membranes and premature delivery associated with group B streptococci in urine of pregnant women. *Lancet* 1984;**2**:69–70.
247. Moylett EH, Fernandez M, Rench MA, et al. A 5-year review of recurrent group B streptococcal disease: lessons from twin infants. *Clin Infect Dis* 2000;**30**:282–7.
248. Nealon TJ, Mattingly SJ. Association of elevated levels of cellular lipoteichoic acids of group B streptococci with human neonatal disease. *Infect Immun* 1983;**39**:1243–51.
249. Nealon TJ, Mattingly SJ. Role of cellular lipoteichoic acids in mediating adherence of serotype III strains of group B streptococci to human embryonic, fetal, and adult epithelial cells. *Infect Immun* 1984;**43**:523–30.
250. Nelson JD. Bilateral breast abscess due to group B streptococcus (Letter). *Am J Dis Child* 1976;**130**:567.
251. Nizet V, Gibson RL, Rubens CE. The role of group B streptococci beta-hemolysin expression in newborn lung injury. *Adv Exp Med Biol* 1997;**418**:627–30.
252. Nocard M. Sur une mammite contagieuse des vaches laitières. *Ann Inst Pasteur* 1887;**1**:109–27.
253. Noya FJ, Rench MA, Metzger TG, et al. Unusual occurrence of an epidemic of type Ib/c group B streptococcal sepsis in a neonatal intensive care unit. *J Infect Dis* 1987;**155**:1135–44.
254. Opal SM, Cross A, Palmer M, et al. Group B streptococcal sepsis in adults and infants: contrasts and comparisons. *Arch Intern Med* 1988;**148**:641–5.
255. Pannaraj PS, Kelly JK, Madoff LC, et al. Group B Streptococcus bacteremia elicits beta C protein-specific Ig Mand IgG in humans. *J Infect Dis* 2007;**195**:353–6.
256. Pannaraj PS, Kelly JK, Rench MA, et al. Alpha C protein-specific immunity in humans with group B streptococcal colonization and invasive disease. *Vaccine* 2008;**26**:502–8.
257. Paoletti LC, Madoff LC. Vaccines to prevent neonatal GBS infection. *Semin Neonatol* 2002;**7**:315–23.
258. Paoletti LC, Wessels MR, Rodewald AK, et al. Neonatal mouse protection against infection with multiple group B streptococcal (GBS) serotypes by maternal immunization with a tetravalent GBS polysaccharide-tetanus toxoid conjugate vaccine. *Infect Immun* 1994;**62**:3236–43.
259. Paredes A, Wong P, Yow MD. Failure of penicillin to eradicate the carrier state of group B Streptococcus in infants. *J Pediatr* 1976;**89**:191–3.
260. Pass MA, Khare S, Dillon HC. Twin pregnancies: incidence of group B streptococcal colonization and disease. *J Pediatr* 1980;**97**:635–7.
261. Pass MA, Gray BM, Dillon HC. Puerperal and perinatal infections with group B streptococci. *Am J Obstet Gynecol* 1982; **143**:147–52.
262. Pass MA, Gray BM, Khare S, et al. Prospective studies of group B streptococcal infections in infants. *J Pediatr* 1979;**95**:437–43.
263. Patamasucon P, Siegel JD, McCracken GH. Streptococcal submandibular cellulitis in young infants. *Pediatrics* 1981;**67**: 378–80.
264. Payne NR, Ferrieri P. The relation of the Ibc protein antigen to the opsonization differences between strains of type II group B streptococci. *J Infect Dis* 1985;**151**:672–81.
265. Payne NR, Burke BA, Day DL, et al. Correlation of clinical and pathologic findings in early onset neonatal group B streptococcal infection with disease severity and prediction of outcome. *Pediatr Infect Dis J* 1988;**7**:836–47.
266. Peevy KJ, Wiseman HJ. Gallbladder distension in septic neonates. *Arch Dis Child* 1982;**57**:75–6.
267. Peña BM, Harper MB, Fleisher GR. Occult bacteremia with group B streptococci in an outpatient setting. *Pediatrics* 1998; **102**:67–72.
268. Persson KM, Forsgren A. Antimicrobial susceptibility of group B streptococci. *Eur J Clin Microbiol* 1986;**5**:165–7.
269. Persson K, Bjerre B, Elfström L, et al. Longitudinal study of group B streptococcal carriage during late pregnancy. *Scand J Infect Dis* 1987;**19**:325–9.
270. Pezzicoli A, Santi I, Lauer P, et al. Pilus backbone contributes to group B Streptococcus paracellular translocation through epithelial cells. *J Infect Dis* 2008;**198**:890–8.
271. Phares CR, Lynfield R, Farley MM, et al. Epidemiology of invasive group B streptococcal disease in the United States, 1999-2005. *JAMA* 2008;**299**:2056–65.
272. Philip AG. Response of C-reactive protein in neonatal Group B streptococcal infection. *Pediatr Infect Dis* 1985;**4**:145–8.
273. Platt MW, Correa N, Mold C. Growth of group B streptococci in human serum leads to increased cell surface sialic acid and decreased activation of the alternative complement pathway. *Can J Microbiol* 1994;**40**:99–105.
274. Pöschl JM, Hellstern G, Ruef P, et al. Ophthalmia neonatorum caused by group B Streptococcus. *Scand J Infect Dis* 2002;**34**: 921–2.
275. Potter B, Philipps AF, Bierny JP, et al. Neonatal radiology; acquired diaphragmatic hernia with group B streptococcal pneumonia. *J Perinatol* 1995;**15**:160–2.
276. Pourcyrous M, Bada HS, Korones SB, et al. Significance of serial C-reactive protein responses in neonatal infection and other disorders. *Pediatrics* 1993;**92**:431–5.
277. Price D, Shaw E, Howard M, et al. Self-sampling for group B streptococcus in women 35 to 37 weeks pregnant is accurate and acceptable: a randomized cross-over trial. *J Obstet Gynaecol Can* 2006;**28**:1083–8.
278. Puliti M, Nizet V, von Hunolstein C, et al. Severity of group B streptococcal arthritis is correlated with beta-hemolysin expression. *J Infect Dis* 2000;**182**:824–32.
279. Puopolo KM, Madoff LC, Eichenwald EC. Early-onset group B streptococcal disease in the era of maternal screening. *Pediatrics* 2005;**115**:1240–6.
280. Pyati SP, Pildes RS, Jacobs NM, et al. Penicillin in infants weighing two kilograms or less with early-onset group B streptococcal disease. *N Engl J Med* 1983;**308**:1383–9.
281. Ramamurthy RS, Srinivasan G, Jacobs NM. Necrotizing fasciitis and necrotizing cellulitis due to group B streptococcus. *Am J Dis Child* 1977;**131**:1169–70.
282. Ramaswamy SV, Ferrieri P, Flores AE, et al. Molecular characterization of nontypeable group B streptococcus. *J Clin Microbiol* 2006;**44**:2398–403.
283. Ramsey PG, Zwerdling R. Asymptomatic neonatal bacteremia. *N Engl J Med* 1976;**295**:225.
284. Regan JA, Chao S, James LS. Premature rupture of membranes, preterm delivery, and group B streptococcal colonization of mothers. *Am J Obstet Gynecol* 1981;**141**:184–6.
285. Regan JA, Klebanoff MA, Nugent RP. The epidemiology of group B streptococcal colonization in pregnancy: Vaginal Infections and Prematurity Study Group. *Obstet Gynecol* 1991;**77**:604–10.
286. Regan JA, Klebanoff MA, Nugent RP, et al. Colonization with group B streptococci in pregnancy and adverse outcome. VIP Study Group. *Am J Obstet Gynecol* 1996;**174**:1354–60.
287. Reiss A, Braun JS, Jäger K, et al. Bacterial pore-forming cytolysins induce neuronal damage in a rat model of neonatal meningitis. *J Infect Dis* 2011;**203**:393–400.
288. Rench MA, Baker CJ. Group B streptococcal breast abscess in a mother and mastitis in her infant. *Obstet Gynecol* 1989;**73**:875–7.
289. Rench MA, Baker CJ. Neonatal sepsis caused by a new group B streptococcal serotype. *J Pediatr* 1993;**122**:638–40.
290. Rentz AC, Samore MH, Stoddard GJ, et al. Risk factors associated with ampicillin-resistant infection in newborns in the era of group B streptococcal prophylaxis. *Arch Pediatr Adolesc Med* 2004;**158**: 556–60.
291. Richter SS, Howard WJ, Weinstein MP, et al. Multicenter evaluation of the BD Phoenix Automated Microbiology System for antimicrobial susceptibility testing of *Streptococcus* species. *J Clin Microbiol* 2007;**45**:2863–71.
292. Riesenfeld-Orn I, Wolpe S, Garcia-Bustos JF, et al. Production of interleukin-1 but not tumor necrosis factor by human monocytes stimulated with pneumococcal cell surface components. *Infect Immun* 1989;**57**:1890–3.
293. Roe MH, Todd JK, Favara BE. Nonhemolytic group B streptococcal infections. *J Pediatr* 1976;**89**:75–7.
294. Rowen JL, Smith CW, Edwards MS. Group B streptococci elicit leukotriene B4 and interleukin-8 from human monocytes: neonates exhibit a diminished response. *J Infect Dis* 1995;**172**:420–6.
295. Rubens CE, Smith S, Hulse M, et al. Respiratory epithelial cell invasion by group B streptococci. *Infect Immun* 1992;**60**:5157–63.
296. Rubens CE, Wessels MR, Heggen LM, et al. Transposon mutagenesis of type III group B Streptococcus: correlation of capsule expression with virulence. *Proc Natl Acad Sci U S A* 1987;**84**: 7208–12.

297. Sadeghi K, Berger A, Langgartner M, et al. Immaturity of infection control in preterm and term newborns is associated with impaired toll-like receptor signaling. *J Infect Dis* 2007;**195**: 296–302.
298. Schauf V, Deveikis A, Riff L, et al. Antibiotic-killing kinetics of group B streptococci. *J Pediatr* 1976;**89**:194–8.
299. Scheld WM, Alliegro GM, Field MR, et al. Synergy between ampicillin and gentamicin in experimental meningitis due to group B streptococci. *J Infect Dis* 1982;**146**:100.
300. Schibler KR, Trautman MS, Liechty KW, et al. Diminished transcription of interleukin-8 by monocytes from preterm neonates. *J Leukoc Biol* 1993;**53**:399–403.
301. Schrag S, Gorwitz R, Fultz-Butts K, et al. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC. *MMWR Recomm Rep* 2002;**51**:1–22.
302. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 2000;**342**:15–20.
303. Schrag SJ, Zell ER, Lynfield R, et al. A population-based comparison of strategies to prevent early-onset group B streptococcal disease in neonates. *N Engl J Med* 2002;**347**:233–9.
304. Schuchat A. Group B streptococcal disease in newborns: a global perspective on prevention. *Biomed Pharmacother* 1995;**49**:19–25.
305. Schuchat A, Deaver-Robinson K, Plikaytis BD, et al. Multistate case-control study of maternal risk factors for neonatal group B streptococcal disease. The Active Surveillance Study Group. *Pediatr Infect Dis J* 1994;**13**:623–9.
306. Schuchat A, Oxtoby M, Cochi S, et al. Population-based risk factors for neonatal group B streptococcal disease: results of a cohort study in metropolitan Atlanta. *J Infect Dis* 1990;**162**: 672–7.
307. Schuit KE, DeBiasio R. Kinetics of phagocyte response to group B streptococcal infections in newborn rats. *Infect Immun* 1980;**28**:319–24.
308. Sherman MP, Lehrer RI. Oxidative metabolism of neonatal and adult rabbit lung macrophages stimulated with opsonized group B streptococci. *Infect Immun* 1985;**47**:26–30.
309. Shigeoka AO, Rote NS, Santos JI, et al. Assessment of the virulence factors of group B streptococci: correlation with sialic acid content. *J Infect Dis* 1983;**147**:857–63.
310. Shurin PA, Howie VM, Pelton SI, et al. Bacterial etiology of otitis media during the first six weeks of life. *J Pediatr* 1978; **92**:893–6.
311. Siegel JD, McCracken GH, Threlkeld N, et al. Single-dose penicillin prophylaxis of neonatal group-B-streptococcal disease. *Lancet* 1982;**1**:1426–30.
312. Siegel JD, Shannon KM, DePasse BM. Recurrent infection associated with penicillin-tolerant group B streptococci: a report of two cases. *J Pediatr* 1981;**99**:920–4.
313. Sinha A, Lieu TA, Paoletti LC, et al. The projected health benefits of maternal group B streptococcal vaccination in the era of chemoprophylaxis. *Vaccine* 2005;**23**:3187–95.
314. Slifkin M, Pouchet-Melvin GR. Evaluation of three commercially available test products for serogrouping beta-hemolytic streptococci. *J Clin Microbiol* 1980;**11**:249–55.
315. Sobel JD, Myers P, Levison ME, et al. Comparison of bacterial and fungal adherence to vaginal exfoliated epithelial cells and human vaginal epithelial tissue culture cells. *Infect Immun* 1982; **35**:697–701.
316. Sokal MM, Nagaraj A, Fisher BJ, et al. Neonatal empyema caused by group B beta hemolytic streptococcus. *Chest* 1982;**81**:390–1.
317. Sokol DM, Demmler GJ, Baker CJ. Unusual presentation of group B streptococcal ventricultis. *Pediatr Infect Dis J* 1990;**9**: 525–7.
318. Soriani M, Santi I, Taddei A, et al. Group B Streptococcus crosses human epithelial cells by a paracellular route. *J Infect Dis* 2006; **193**:241–50.
319. St-Laurent-Gagnon T, Weber ML. Urinary tract streptococcus group B infection in a 6-week-old infant. *JAMA* 1978;**240**: 1269.
320. Stoll BJ, Hansen N, Fanaroff AA, et al. Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. *N Engl J Med* 2002;**347**:240–7.
321. Suresh BR, Rios A, Brion LP, et al. Delayed onset right-sided diaphragmatic hernia secondary to group B streptococcal infection. *Pediatr Infect Dis J* 1991;**10**:166–8.
322. Swingle HM, Bucciarelli RL, Ayoub EM. Synergy between penicillins and low concentrations of gentamicin in the killing of group B streptococci. *J Infect Dis* 1985;**152**:515–20.
323. Takahashi S, Nagano Y, Nagano N, et al. Role of C5a-ase in group B streptococcal resistance to opsonophagocytic killing. *Infect Immun* 1995;**63**:4764–9.
324. Tamura GS, Kuypers JM, Smith S, et al. Adherence of group B streptococci to cultured epithelial cells: roles of environmental factors and bacterial surface components. *Infect Immun* 1994;**62**: 2450–8.
325. Tapsall JW. Pigment production by Lancefield-group-B streptococci (*Streptococcus agalactiae*). *J Med Microbiol* 1986;**21**:75–81.
326. Tazi A, Reglier-Poupet H, Dautezac F, et al. Comparative evaluation of Strepto B ID chromogenic medium and Granada media for the detection of group B streptococcus from vaginal samples of pregnant women. *J Microbiol Methods* 2008;**73**:263–5.
327. Tenenbaum T, Bloier C, Adam R, et al. Adherence to and invasion of human brain microvascular endothelial cells are promoted by fibrinogen-binding protein FbsA of *Streptococcus agalactiae*. *Infect Immun* 2005;**73**:4404–9.
328. Tettelin H, Masignani V, Cieslewicz MJ, et al. Complete genome sequence and comparative genomic analysis of an emerging human pathogen, serotype V *Streptococcus agalactiae*. *Proc Natl Acad Sci U S A* 2002;**99**:12391–6.
329. Tettelin H, Masignani V, Cieslewicz MJ, et al. Genome analysis of multiple pathogenic isolates of *Streptococcus agalactiae:* implications for the microbial "pan-genome". *Proc Natl Acad Sci U S A* 2005;**102**:13950–5.
330. Thigpen MC, Whitney CG, Messonnier NE, et al. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011; **364**:2016–25.
331. Torok PG, Dunn JR. Self-collection of antepartum anogenital group B streptococcus cultures. *J Am Board Fam Pract* 2000; **13**:107–10.
332. Trijbels-Smeulders MA, Kimpen JL, Kollée LA, et al. Serotypes, genotypes, and antibiotic susceptibility profiles of group B streptococci causing neonatal sepsis and meningitis before and after introduction of antibiotic prophylaxis. *Pediatr Infect Dis J* 2006; **25**:945–8.
333. Tuppurainen N, Hallman M. Prevention of neonatal group B streptococcal disease: intrapartum detection and chemoprophylaxis of heavily colonized parturients. *Obstet Gynecol* 1989;**73**: 583–7.
334. Turner MC, Naumburg EG. Acute renal failure in the neonate: two fatal cases due to group B streptococci with rhabdomyolysis. *Clin Pediatr (Phila)* 1987;**26**:189–90.
335. Tyrrell GJ, Senzilet LD, Spika JS, et al. Invasive disease due to group B streptococcal infection in adults: results from a Canadian, population-based, active laboratory surveillance study—1996. Sentinel Health Unit Surveillance System Site Coordinators. *J Infect Dis* 2000;**182**:168–73.
336. Vallejo JG, Baker CJ, Edwards MS. Demonstration of circulating group B streptococcal immune complexes in neonates with meningitis. *J Clin Microbiol* 1994;**32**:2041–5.
337. Vallejo JG, Baker CJ, Edwards MS. Interleukin-6 production by human neonatal monocytes stimulated by type III group B streptococci. *J Infect Dis* 1996;**174**:332–7.
338. Vallejo JG, Baker CJ, Edwards MS. Roles of the bacterial cell wall and capsule in induction of tumor necrosis factor alpha by type III group B streptococci. *Infect Immun* 1996;**64**:5042–6.
339. Vallette JD, Goldberg RN, Suguihara C, et al. Effect of an interleukin-1 receptor antagonist on the hemodynamic manifestations of group B streptococcal sepsis. *Pediatr Res* 1995;**38**:704–8.
340. Van Dyke MK, Phares CR, Lynfield R, et al. Evaluation of universal antenatal screening for group B streptococcus. *N Engl J Med* 2009;**360**:2626–36.
341. Verani JR, McGee L, Schrag SJ, et al. Prevention of perinatal group B streptococcal disease: revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2010;**59**:1–36.
342. Vollman JH, Smith WL, Ballard ET, et al. Early onset group B streptococcal disease: clinical, roentgenographic, and pathologic features. *J Pediatr* 1976;**89**:199–203.
343. von Hunolstein C, D'Ascenzi S, Wagner B, et al. Immunochemistry of capsular type polysaccharide and virulence properties of type VI Streptococcus agalactiae (group B streptococci). *Infect Immun* 1993;**61**:1272–80.

344. Wald ER, Bergman I, Taylor HG, et al. Long-term outcome of group B streptococcal meningitis. *Pediatrics* 1986;**77**:217–21.
345. Walker KM, Coyer WF. Suprarenal abscess due to group B streptococcus. *J Pediatr* 1979;**94**:970–1.
346. Walker SH, Santos AQ, Quintero BA. Recurrence of group B III streptococcal meningitis. *J Pediatr* 1976;**89**:187–8.
347. Weinberg AG, Laird WP. Group B streptococcal endocarditis detected by echocardiography. *J Pediatr* 1978;**92**:335–6.
348. Weiser JN, Rubens CE. Transposon mutagenesis of group B streptococcus beta-hemolysin biosynthesis. *Infect Immun* 1987;**55**: 2314–16.
349. Weisman LE, Stoll BJ, Cruess DF, et al. Early-onset group B streptococcal sepsis: a current assessment. *J Pediatr* 1992;**121**: 428–33.
350. Weisner AM, Johnson AP, Lamagni TL, et al. Characterization of group B streptococci recovered from infants with invasive disease in England and Wales. *Clin Infect Dis* 2004;**38**:1203–8.
351. Wennekamp J, Henneke P. Induction and termination of inflammatory signaling in group B streptococcal sepsis. *Immunol Rev* 2008;**225**:114–27.
352. Wessels MR, Paoletti LC, Kasper DL, et al. Immunogenicity in animals of a polysaccharide-protein conjugate vaccine against type III group B Streptococcus. *J Clin Invest* 1990;**86**:1428–33.
353. Wessels MR, Rubens CE, Benedí VJ, et al. Definition of a bacterial virulence factor: sialylation of the group B streptococcal capsule. *Proc Natl Acad Sci U S A* 1989;**86**:8983–7.
354. Wilkinson HW. Analysis of group B streptococcal types associated with disease in human infants and adults. *J Clin Microbiol* 1978;**7**:176–9.
355. Wilkinson HW, Facklam RR, Wortham EC. Distribution by serological type of group B streptococci isolated from a variety of clinical material over a five-year period (with special reference to neonatal sepsis and meningitis). *Infect Immun* 1973;**8**:228–35.
356. Wirth J, Raymond J, Lapillonne A. Unusual report of diaper rash in a premature infant with group B streptococcal infection. *Pediatr Infect Dis J* 2006;**25**:750–1.
357. Wiswell TE, Baumgart S, Gannon CM, et al. No lumbar puncture in the evaluation for early neonatal sepsis: will meningitis be missed? *Pediatrics* 1995;**95**:803–6.
358. Wong M, Isaacs D, Howman-Giles R, et al. Clinical and diagnostic features of osteomyelitis occurring in the first three months of life. *Pediatr Infect Dis J* 1995;**14**:1047–53.
359. Wood EG, Dillon HC. A prospective study of group B streptococcal bacteriuria in pregnancy. *Am J Obstet Gynecol* 1981;**140**: 515–20.
360. Woods CR, Edwards MS. Renal abscess caused by group B Streptococcus. *Clin Infect Dis* 1994;**18**:662–3.
361. Yagupsky P, Menegus MA, Powell KR. The changing spectrum of group B streptococcal disease in infants: an eleven-year experience in a tertiary care hospital. *Pediatr Infect Dis J* 1991;**10**:801–8.
362. Yancey MK, Schuchat A, Brown LK, et al. The accuracy of late antenatal screening cultures in predicting genital group B streptococcal colonization at delivery. *Obstet Gynecol* 1996;**88**:811–15.
363. Yeung MK, Mattingly SJ. Biosynthetic capacity for type-specific antigen synthesis determines the virulence of serotype III strains of group B streptococci. *Infect Immun* 1984;**44**:217–21.
364. Young N, Finn A, Powell C. Group B Streptococcal epiglottitis. *Pediatr Infect Dis J* 1996;**15**:95–6.
365. Yow MD, Leeds LJ, Thompson PK, et al. The natural history of group B streptococcal colonization in the pregnant woman and her offspring. I. Colonization studies. *Am J Obstet Gynecol* 1980; **137**:34–8.
366. Yow MD, Mason EO, Leeds LJ, et al. Ampicillin prevents intrapartum transmission of group B streptococcus. *JAMA* 1979; **241**:1245–7.
367. Zaleznik DF, Rench MA, Hillier S, et al. Invasive disease due to group B Streptococcus in pregnant women and neonates from diverse population groups. *Clin Infect Dis* 2000;**30**:276–81.
368. Zangwill KM, Schuchat A, Wenger JD. Group B streptococcal disease in the United States, 1990: report from a multistate active surveillance system. *MMWR CDC Surveill Summ* 1992; **41**:25–32.

CHAPTER 84

Enterococcal and Viridans Streptococcal Infections

B. Keith English • Jerry L. Shenep

ENTEROCOCCAL INFECTIONS

The enterococci are gram-positive ovoid bacteria that are related closely to the streptococci but are phylogenetically distinct and make up the genus *Enterococcus*. These organisms are found in the normal bowel flora of humans and many animals and commonly are isolated from environmental sources. Enterococci generally are considered to be of low virulence but have been known to cause human infection since 1899 (reviewed by Murray[182]). These ubiquitous bacteria have become recognized increasingly as important causes of both nosocomial and community-acquired infection in adults* and children,† yet the role of *Enterococcus* spp. as a pathogen (or co-pathogen) in certain clinical settings, particularly intra-abdominal and pelvic infections, often remains unclear.

Enterococci are intrinsically resistant to many antimicrobial agents (including the cephalosporins, oxacillin, clindamycin, and the aminoglycosides). Since the emergence of high-level aminoglycoside resistance in *Enterococcus* spp. more than 40 years ago,[174] increasing percentages of these organisms have acquired clinically significant resistance to β-lactam antibiotics, vancomycin and other glycopeptides, and aminoglycosides. Vancomycin-resistant enterococcal (VRE) infections, especially those caused by vancomycin-resistant *Enterococcus faecium* (VREF), are of particular concern because VREF isolates frequently are resistant to other bactericidal antimicrobial agents.[4,184,186,233] The dramatic increase in nosocomial VRE infections[4,65,184,198] has resulted in failure of antimicrobial therapy[138] and has inspired comparisons with the preantibiotic period[5] and even speculation about a "postantimicrobial era."[46] Fortunately, new classes of antimicrobials active against most VRE isolates have become available recently, but pediatric experience with these agents is limited, and enterococci resistant to these agents already have been detected (see later discussion). Concern about the continued nosocomial spread of VRE isolates and the documented transfer of vancomycin resistance determinants to *Staphylococcus aureus*[29,30,264,274] led to the development of stringent hospital infection control guidelines designed to interrupt the spread of vancomycin-resistant organisms.[193] Although the impact of VRE infections in children has been less dramatic, many pediatric centers are reporting infections caused by these organisms.*

MICROBIOLOGY

The genus *Enterococcus* consists of gram-positive cocci that are catalase-negative and occur singly, in pairs, and in short chains. Morphologically, enterococci are indistinguishable from streptococci and traditionally were classified as members of the genus *Streptococcus*. Sherman's early classification scheme[243] divided the streptococci into four groups: pyogenic, viridans, lactic, and enterococcal. By the Lancefield criteria, enterococci were classified as group D streptococci, along with the "nonenterococcal" *Streptococcus bovis* group. However, genetic evidence has indicated that the enterococci are sufficiently different from the streptococci to merit establishment of a separate genus.[70]

Figure 84-2 is a scheme for the differentiation of enterococci from other gram-positive cocci. Catalase-negative gram-positive cocci that have been isolated from human sources include the streptococci, the enterococci, *Lactococcus* spp., *Leuconostoc* spp., *Pediococcus* spp., and *Gemella* spp. Most enterococci produce no (γ) or partial (α) hemolysis on blood agar; differentiation between enterococci and certain α-hemolytic or nonhemolytic streptococci and other nonstreptococcal gram-positive cocci may require a series of biochemical tests.[70,71] Clinical laboratories may identify an organism presumptively on a primary isolation plate as an enterococcus based on colony morphology, Gram stain, and the pyrrolidonyl arylamidase (PYR) test. Most enterococci produce PYR, as do *Streptococcus pyogenes* and nutritionally variant streptococci *(Abiotrophia)*, but not other streptococci. The PYR test is particularly useful for differentiating

*References 28, 65, 79, 96, 100, 101, 112, 152, 170, 171, 182, 184, 186, 197, 205, 215.

†References 8, 11, 12, 19-21, 32, 40, 62, 64, 75, 77, 98, 99, 113, 128, 135, 145, 149, 158, 168, 177, 189, 196, 198, 211, 213, 219, 221, 235, 236, 241, 242, 250, 257, 286.

*References 19, 32-40, 60-62, 75, 77, 78, 85, 98, 100, 103, 113, 135, 145, 149, 169, 177, 189, 196, 211, 212, 221, 257, 286.

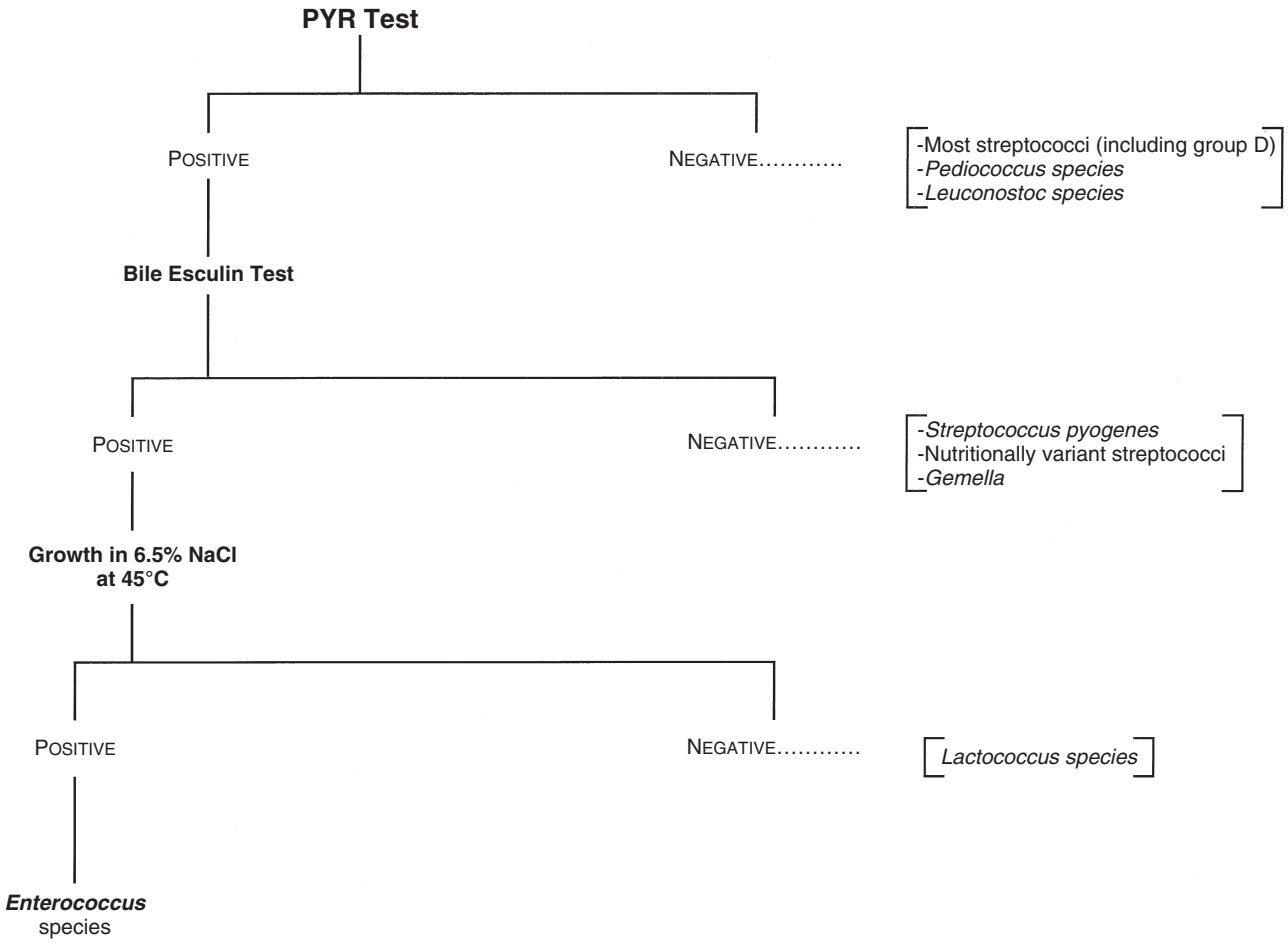

FIGURE 84-1 ■ Differentiation of enterococci from other gram-positive cocci. *PYR,* pyrrolidonyl.

enterococci from group D streptococci and *Leuconostoc* spp. (see Fig. 84-1). *S. pyogenes* and *Abiotrophia* spp. are distinguished easily from enterococci by colony morphology, hemolysis, and special growth requirements.

Enterococci are able to hydrolyze esculin in the presence of 40 percent bile salts; of the true streptococci, only group D streptococci (*S. bovis* group) and approximately 5 to 10 percent of viridans streptococci share this characteristic.[70] Enterococci are facultatively anaerobic and grow under harsh conditions that inhibit the growth of streptococci; growth in 6.5 percent sodium chloride at 45° C (113° F) is a useful confirmatory test. Enterococci produce leucine aminopeptidase, as do streptococci, lactococci, pediococci, and some *Gemella* strains. The presence of group D streptococcal antigen is of limited value because the *S. bovis* group, most pediococci, and half of the clinical *Leuconostoc* spp. isolates share this antigen.[70]

Occasional clinical isolates of *Leuconostoc* spp., *Pediococcus* spp., and *Lactococcus* spp. may be difficult to distinguish from the enterococci. Some strains of *Leuconostoc* spp. and *Pediococcus* spp. may grow in 6.5 percent NaCl at 45° C, but they are PYR-negative. Lactococci are PYR-positive, and some isolates will grow in 6.5 percent NaCl; however, most lactococci fail to grow (or grow very slowly) at 45° C. Consequently, definitive confirmation of an organism as an enterococcus may require complete identification to the species level. Molecular techniques allow rapid, reliable identification and speciation of enterococci,[52,59,63] but usually are available only in the research setting.

The genus *Enterococcus* now includes at least 14 "typical" species and 3 additional "atypical" species (the latter are PYR-negative and grow very slowly in the presence of 6.5% NaCl) (Table 84-1). However, most human clinical isolates are either *Enterococcus faecalis* (50% to 90%) or *E. faecium* (5% to 37% percent), although clusters of human infection caused by *Enterococcus raffinosus*,[35] *Enterococcus casseliflavus*,[191] *Enterococcus avium*,[203] and *Enterococcus durans*[237] and occasional human infections attributable to *Enterococcus gallinarum*, *Enterococcus mundtii*, and *Enterococcus flavescens* have been reported.[69] Even though *E. faecalis* and *E. faecium* continue to account for most clinical isolates, the percentage of *E. faecium* isolates has been increasing, and relatively more "other" (i.e., non-*faecalis* and non-*faecium*) enterococci are being identified by clinical laboratories.[121,123,153]

Speciation of enterococci has been useful primarily for epidemiologic purposes, but distinction between the more antibiotic-susceptible *E. faecalis* and the more antibiotic-resistant (see later discussion) *E. faecium* may be helpful in selecting optimal therapy for endocarditis

TABLE 84-1 ***Enterococcus* Species**

Typical Enterococci (PYR$^+$)
E. faecalis
E. faecium
E. avium
E. casseliflavus
E. durans
E. raffinosus
E. gallinarum
E. malodoratus
E. hirae
E. mundtii
E. pseudoavium
E. dispar
E. flavescens
E. sulfureus
Atypical Enterococci (PYR$^-$)
E. cecorum
E. columbae
E. saccharolyticus

PYR, Pyrrolidonyl arylamidase.

and other serious enterococcal infections. A panel of biologic tests can differentiate these two common enterococcal species readily.[70,129,182] Most *E. faecalis* isolates (unlike those of *E. faecium*) grow in the presence of 0.04 percent tellurite, reduce tetrazolium to formazan, and produce acid from sorbitol and glycerol.

A variety of molecular techniques are available to assist in the identification of enterococci and determination of the relatedness of enterococcal isolates.* These molecular techniques have been particularly valuable in investigations of possible nosocomial transmission of multiresistant enterococci.†

EPIDEMIOLOGY

Enterococci are normal flora of the gastrointestinal tract of most humans and have been found in as many as 97 percent of fecal samples from adults in Europe, Asia, and North America (reviewed by Murray[182]). Approximately half of newborn infants have become colonized with enterococci by the time they are 1 week of age.[194] Enterococci are isolated less commonly (<20% of specimens) from other sites, including the vagina, oral cavity, and skin. These organisms also are common inhabitants of the bowel flora of many animals and frequently are present in soil, water, and food. Enterococci are hardy organisms and may persist for long periods on environmental surfaces, thereby contributing to potential nosocomial spread of these bacteria.

Human infection caused by enterococci was reported before the beginning of the 20th century, but the initial reports included patients with infections that seldom are associated with enterococci today: enteritis, meningitis, and appendicitis (reviewed by Murray[182]). The ubiquitous presence of enterococci in fecal samples led to the mistaken impression that these organisms cause enteritis and food poisoning. Enterococci are isolated commonly as part of mixed flora in intra-abdominal and pelvic infections, but the contribution of these organisms to the pathogenesis of such infections remains unclear.[88,192] However, enterococci were identified as pathogens causing urinary tract infection and endocarditis as early as 1906,[4] with later confirmation by many studies. Subsequently, enterococci have been documented to cause invasive infection in neonates,* patients with malignancies,† recipients of bone marrow and solid organ transplants,‡ burn victims,[140] patients with indwelling catheters,§ and other immunosuppressed or debilitated patients.‖

In general, enterococcal infections occur less frequently in children (outside the neonatal period) than in adults, and enterococci are less common causes of pediatric (vs. adult) urinary tract infection[282] and endocarditis.[253] Much of the published experience with enterococcal infection in children focuses on the neonatal period,¶ and some evidence suggests that late-onset (but not early-onset) neonatal enterococcal infection may be increasing in frequency.[41,58] Relatively few series of pediatric enterococcal infection have been published.**

Enterococci are important nosocomial agents, and they rank as either the second or third most common hospital-acquired pathogens in this country.†† Evidence of nosocomial spread of enterococci is relatively recent. Initially, all enterococci isolated from hospitalized patients were thought to have originated from the patient's endogenous bowel flora. However, the emergence of multiantibiotic-resistant enterococci prompted the performance of careful epidemiologic studies that documented the nosocomial spread of these organisms.†† Many groups have reported that VRE and other enterococcal isolates may persist for long periods in the environment and may be spread either by direct patient-to-patient contact via the hands of colonized health care personnel[214] or from contaminated beds or other hospital material or contaminated patient equipment, including thermometers.[4,19,128,145,171]

The growing problem of nosocomial infection by VRE organisms is multifactorial; the diversity of isolates found in many hospitals indicates that resistant organisms may be introduced via multiple sources.‡‡ Consequently, outbreaks of VRE infection in institutions may be caused by single or multiple clones, although the first recognized outbreak of VRE infection or colonization in a hospital usually is associated with a single clone.[32,85]

*References 41, 42, 58, 63, 178, 278.
†References 41, 42, 59, 63, 70, 178, 201, 227, 278.

*References 8, 58, 84, 99, 133, 211.
†References 91, 93, 135, 177, 196, 242.
‡References 10, 75, 78, 127, 131, 169, 202, 231.
§References 10, 53, 171, 182, 185, 198.
‖References 31, 149, 154, 165, 169, 205, 217.
¶References 49, 58, 84, 99, 154, 214.
**References 15, 18, 23, 41, 53, 77, 91, 98, 158, 222.
††References 4, 28, 65, 124, 187, 220, 232, 284, 285.
‡‡References 40, 63, 84, 178, 185, 220, 243.

In many reports, distinguishing risk factors for VRE colonization from those for invasive disease caused by these organisms has not been possible, and certain factors probably increase the risk for both colonization and development of infection caused by VRE organisms. Major risk factors for VRE colonization or the development of infection, or for both, appear to be the severity of the underlying illness or immunosuppression; the proximity to patients colonized with VRE organisms (e.g., admission to an intensive care unit [ICU] or a transplant unit); receipt of a bone marrow or solid organ (especially liver) transplant; increasing length of hospital stay; recent cardiothoracic or abdominal surgery; the presence of indwelling central venous or urinary catheters; and previous treatment with vancomycin, broad-spectrum antibiotics (particularly those lacking appreciable antienterococcal activity, such as the third-generation cephalosporins), or agents with broad antianaerobic activity.* Patients infected with human immunodeficiency virus (HIV) also appear to be at higher risk for the development of VRE bacteremia.[10] Factors contributing to nosocomial spread of these organisms probably differ from those responsible for the gradual overall increase in resistant strains.[217]

Since the first report of a vancomycin-resistant isolate in 1988,[141] infection and colonization with VRE bacteria in the United States have been observed primarily in the ICUs of large teaching hospitals.[28,184,186] For example, data reported to the National Nosocomial Infection Surveillance (NNIS) system of the Centers for Disease Control and Prevention (CDC) from January 1989 through March 1993 revealed no vancomycin resistance in hospitals with fewer than 200 beds, a resistance rate of 1.8 percent in hospitals with 200 to 500 beds, and a rate of 3.6 percent in hospitals with more than 500 beds.[28] A 1995 report from the multicenter *Enterococcus* Study Group analyzed 1936 isolates collected from 97 laboratories in 47 states during the last quarter of 1992 and generally confirmed the findings of the NNIS survey.[121] However, VRE infections are not limited to ICUs. A 1999 report from the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) group's ongoing surveillance of nosocomial bloodstream infections in 49 U.S. hospitals revealed no overall differences in rates of vancomycin resistance in enterococci isolated from patients located in critical care versus hospital ward settings.[65] Fortunately, VRE infections have occurred much less frequently in children than in adults, and overall rates of vancomycin resistance in enterococci isolated from children's hospitals remain very low. However, many pediatric centers have reported VRE infections.†

In the United States, VRE isolates generally have not been detected in environmental sources or in patients who have not had exposure to hospitals,[182,184] with rare exceptions.[32] However, the ecology of VRE organisms in Europe differs: they have been detected in the feces of nonhospitalized patients and healthy volunteers in several European studies, with rates of VRE colonization as high as 28 percent in adults living in some parts of Belgium (reviewed by Murray[182,184]). The more widespread occurrence of VRE strains in Europe probably was related to the use of oral glycopeptides such as avoparcin in animal feed and the oral administration of bacterial preparations (possibly contaminated with resistant enterococci) to humans and animals for therapeutic purposes.[182-184]

Initial reports of VRE organisms in the United States were clustered in the northeast region, particularly New York, Maryland, and Pennsylvania,[28] and geographic differences in rates of VRE colonization and infection persist. However, subsequent reports indicate that VRE strains are found in most parts of the United States[120,153] and that rates of vancomycin resistance among enterococci isolated from patients in this country generally are higher than those found in isolates from Canada, Western Europe, or Latin America.[119,122,153] The reasons for these geographic differences remain unclear, but the high rates of resistance in the United States may be related to the increased use of vancomycin in this country during the past 3 decades.[132]

PATHOGENESIS AND VIRULENCE

Enterococci are organisms of low virulence, and their ubiquitous presence in the human gastrointestinal tract probably has contributed to both the spurious association of these organisms with some illnesses (e.g., enteritis) and the failure to recognize other situations in which enterococci are true pathogens (e.g., immunosuppressed and debilitated patients and neonates). When contrasted with organisms such as *S. pyogenes* and *S. aureus*, enterococci are much less virulent in animal models of infection,[115,170] but they are capable of causing disease at a higher inoculum.

Enterococci rarely cause primary cellulitis or abscesses, although these organisms are isolated frequently as components of polymicrobial wound infections and intra-abdominal and pelvic infections. The contribution of enterococci to the pathogenesis of these polymicrobial infections remains uncertain.[88,192] In animal models, synergy between enterococci and a variety of other organisms (particularly anaerobes) can be demonstrated, but enterococci injected alone have little propensity to cause either peritonitis or subcutaneous infection.[115,116,182,200] Many clinical trials have concluded that provision of antienterococcal therapy generally is not necessary to effect a cure of human intra-abdominal and pelvic infections, even though *Enterococcus* frequently (14% to 33% of cases) is isolated from primary peritoneal cultures.[88]

Enterococci are an important cause of urinary tract infections in adults (particularly in elderly men, patients with structural abnormalities of the urinary tract, and those with indwelling urinary catheters), but they are associated less frequently with urinary tract infections in children.[8,282] Enterococci also are important but less frequent (than in adults) causes of bacterial endocarditis in children.[166,254]

Adherence of bacteria to tissue is a necessary first step in the pathogenesis of both urinary tract infection and endocarditis, and evidence suggests that pathogenic

*References 11, 12, 42, 95, 214, 217, 220, 222, 250, 285.
†References 15, 18, 48, 58, 84, 133, 154, 163, 214, 247.

enterococci produce factors that mediate adherence to urinary epithelial cells and endocardial tissue (reviewed by Jett and colleagues[115] and Johnson[116]). Enterococcal adhesins include an enterococcal surface protein known as aggregation substance[38,136] and surface carbohydrates. Lipoteichoic acid, an important adhesin for *S. pyogenes*, does not appear to mediate adherence of enterococci,[249] but it may trigger the host inflammatory response to these organisms.[261] The innate immune response to enterococci is poorly understood, though one report has implicated the intracellular sensor nod2 in mediating the host inflammatory response to these organisms.[130] Recent studies have implicated an enterococcal surface protein, Esp, in biofilm formation by *E. faecalis*.[26,263] In addition, many virulent *E. faecalis* isolates express a cytolysin in the presence of target cells.[45,50]

Nosocomial enterococcal bacteremia in adults frequently is associated with urinary tract and wound infections, but catheter-related bacteremia is of increasing importance. Enterococcal bacteremia without a source occurs relatively more commonly in children,[18,217] although Bonadio[15] reported that many children with enterococcal bacteremia had an identifiable focus of infection. Enterococcal bacteremia occurs more frequently in patients with severe underlying disease and may be life-threatening. However, the precise contribution of enterococcal bacteremia to morbidity and mortality in severely ill patients remains unclear in individual patients. Adult ICU patients with enterococcal bacteremia have a very high mortality rate,[90] and some early studies suggested that isolation of *Enterococcus* from blood merely served to identify a very high-risk group of patients.[182] In several studies, clearance of VRE organisms from blood was not associated with a reduced mortality rate in this high-risk population. In other studies, spontaneous resolution of VRE bacteremia has been reported, thus suggesting that transient VRE bacteremia or pseudobacteremia (or both) may occur.[269] However, a series of subsequent studies clearly associated vancomycin resistance with an increased risk for mortality in certain patients and has demonstrated that effective anti-enterococcal therapy reduces the overall mortality rate in patients with VRE bacteremia.* Taken together, these findings indicate that at least a subset of high-risk patients with VRE bacteremia are at risk for the development of significant morbidity and mortality directly related to the infection.

CLINICAL MANIFESTATIONS

Enterococcal infections generally occur less frequently in children than in adults, but *Enterococcus* is a relatively frequent cause of neonatal infections.[58] The types of infections caused by enterococci in children are similar to those in adults,[15,18,53,98,217] although enterococcal bacteremia may be associated with fewer sequelae in children.[53] In children, as well as in adults, enterococci have become increasingly important nosocomial pathogens.† They are important causes of endocarditis, urinary tract infection, and bacteremia (particularly catheter-related bacteremia) in pediatric patients, and these organisms commonly are isolated as components of polymicrobial wound, intra-abdominal, and pelvic infections in children.[15,18,171,182] Meningitis[257] and septic arthritis[212] are rare manifestations of enterococcal infection. Enterococci are also important causes of peritonitis in children being treated with peritoneal dialysis.[257] Respiratory tract infections caused by enterococci are rarely recognized, though many neonates with enterococcal infection have pulmonary symptoms.[58]

Urinary Tract Infection

Urinary tract infections are the most common enterococcal infections in adults,[4,171] and enterococci are important urinary pathogens in children.[7,54,158] Most enterococcal urinary tract infections occur in elderly men after they have undergone urinary catheterization, instrumentation, or both.[73,180] Enterococci are infrequent (<5% of isolates) causes of cystitis and pyelonephritis in otherwise healthy children, infants, and neonates[7,282] and are infrequent causes of urinary tract infection in young women.[182] However, some centers are reporting an increasing incidence of both community-acquired and nosocomial urinary tract infection caused by enterococci.[73]

Most enterococcal urinary tract infections in children[7,54,147] and adults[73,81] are nosocomial. Risk factors for the development of urinary tract infection by *Enterococcus* include indwelling urinary catheters, instrumentation of the urinary tract, structural abnormalities of the urinary tract, and previous broad-spectrum antimicrobial therapy.[180,182] The increasing problem of nosocomial enterococcal urinary tract infection[73] is compounded by the growing problem of multiantibiotic-resistant enterococci.[28]

The genitourinary tract reportedly is the most common entry site leading to enterococcal bacteremia in adults,[90,137] but it is implicated much less frequently in the cause of enterococcal bacteremia in children.[15,18,53] Christie and colleagues[41] reported that urosepsis was the cause of 12 percent of episodes of nosocomial enterococcal bacteremia in one children's hospital, but Das and Gray[53] failed to detect any cases of urosepsis in 75 consecutive cases of enterococcal bacteremia in another pediatric hospital during a 3-year period. Other complications of enterococcal urinary tract infection in adults include prostatitis and perinephric abscess.[171]

Endocarditis

Enterococcal endocarditis was reported first in 1906,[3] and these organisms are important causes of native-valve and prosthetic-valve endocarditis in children and adults.[1,3,166,218] Either normal or previously damaged valves may be involved. Enterococci cause approximately 5 to 20 percent of cases of native-valve endocarditis in adults (excluding cases in intravenous drug users),[166] approximately 6 to 7 percent of prosthetic-valve endocarditis,[166,266] and approximately 5 to 10 percent of endocarditis in intravenous drug users.[182] Enterococcal

*References 55, 56, 64, 107, 184, 205.
†References 18, 28, 41, 62, 77, 78, 98, 135, 163, 170, 196, 220, 285.

endocarditis in intravenous drug users generally involves the aortic or mitral valves, unlike staphylococcal endocarditis in this setting, which usually involves the tricuspid valve.[182] As with other enterococcal infections, *E. faecalis* causes most cases of endocarditis.

Enterococcal endocarditis primarily is a disease of older men, and the genitourinary tract is the most commonly identified source of the initial bacteremia. Enterococci are relatively less frequent causes of endocarditis in children—less than 5 percent of cases in most series.* Typically, enterococcal endocarditis occurs after a subacute course that is clinically indistinguishable from that caused by streptococci.[166] Although the enterococcus is a common neonatal pathogen,[58] reports of neonatal enterococcal endocarditis are extremely rare.[262] The prognosis of enterococcal prosthetic-valve endocarditis is somewhat better than that of native-valve endocarditis caused by these organisms.[1,218] Aortic-valve involvement is associated with increased rates of morbidity and mortality.[166] Endocarditis rarely complicates nosocomial enterococcal bacteremia.[156,182,205] Consequently, VRE-associated endocarditis is a rare development, although cases have been reported.[255]

Bacteremia

Enterococcal bacteremia often represents a conundrum. Bacteremia in severely ill hospitalized patients, particularly that caused by VRE organisms, is associated with considerable morbidity and mortality. However, only a portion of that morbidity and mortality can be attributed directly to enterococcal bacteremia per se. Nosocomial enterococcal bacteremia frequently occurs as a component of polymicrobial bacteremia, with 21 to 45 percent of bloodstream isolates of *Enterococcus* being accompanied by one or more other pathogens.[53,156,205,245] As many as half of cases of catheter-related enterococcal bacteremia are polymicrobial.[163,205] Many,[97,156] but not all,[106,163] series of patients with enterococcal bacteremia have reported increased mortality rates in patients with polymicrobial bacteremia (including *Enterococcus*) versus isolated enterococcal bacteremia. Mortality in adults with nosocomial enterococcal bacteremia has ranged from 23 to 46 percent,[156,157,205] but patients at risk for the acquisition of nosocomial enterococcal bacteremia are severely ill and have a poor prognosis independent of the bacteremic event. However, hospital-acquired enterococcal infections can be life-threatening,[63,182,205,209] and specific therapy does appear to reduce the overall mortality rate.[64,107,205] Furthermore, a meta-analysis concluded that vancomycin resistance is independently correlated with increased mortality in patients with enterococcal bloodstream infections.[55,56] Nonetheless, many episodes of enterococcal bacteremia in high-risk patients apparently resolve in the absence of specific therapy.[107,269] The mortality rate associated with enterococcal bacteremia in children has been lower than that reported in adults in most studies but has ranged from 7.5 percent[53] to 12 percent[18] to 20 percent[15] to 26 percent,[41] depending on the population studied.

*References 117, 118, 252, 253, 262, 287.

In adults, many cases of enterococcal bacteremia are associated with a primary focus, most commonly a urinary tract infection.[57,91] In contrast, few children with enterococcal bacteremia have urosepsis, and most episodes of enterococcal bacteremia in children have not been associated with any identifiable focus.[18,217] However, Christie and coworkers[41] identified a primary focus in 21/57 children (37%) with nosocomial enterococcal bacteremia, including 7 patients with urosepsis and 6 with peritonitis. Many children with enterococcal bacteremia do have underlying disease involving the gastrointestinal or respiratory tract.[15,18] Central venous catheter–related enterococcal bacteremia is a significant problem in both children[15,18,41,53,58,205] and adults.[89,205] In earlier series, infections of vascular catheters were reported to account for only 2 to 14 percent of cases of enterococcal bacteremia (reviewed by Graninger and Ragette[90]), but subsequent reports have implicated intravascular devices in as many as 22 to 28 percent of these episodes in adults and children.[53,205]

Most episodes of enterococcal bacteremia do not lead to endocarditis,[156,182,205] and endocarditis is particularly uncommon in the setting of nosocomial enterococcal bacteremia. Maki and Agger[156] identified only one case of endocarditis in 118 episodes of hospital-acquired enterococcal bacteremia, whereas endocarditis was diagnosed in 12/35 patients with community-acquired enterococcal bacteremia.

Intra-abdominal Infections

Most of the published information about the role of *Enterococcus* in intra-abdominal and pelvic infections comes from series of adult patients. Enterococci commonly are isolated as components of polymicrobial infections involving the abdomen or pelvis,[88] and animal models suggest that these organisms can play a synergistic role in the pathogenesis of such infections.[115] However, evidence that the addition of specific antienterococcal therapy improves the outcome of human intra-abdominal and pelvic infections, even when enterococci are isolated from peritoneal cultures, is not compelling.[88]

Children with enterococcal bacteremia frequently have underlying conditions related to the gastrointestinal tract.[15,18,41] Bonadio[15] reported five cases of enterococcal bacteremia in previously healthy infants with gastroenteritis, six cases associated with bowel obstruction, and one case associated with acute appendicitis without perforation. Boulanger and coworkers[18] identified underlying conditions affecting the gastrointestinal system in 8/32 pediatric patients, but they were unable to specifically implicate any of these conditions as the source of the bacteremia. Das and Gray[53] detected underlying chronic gastrointestinal pathology (short-gut syndrome, congenital anomalies of the gastrointestinal tract, ulcerative colitis, chronic liver disease) in fully a third (25/75) of pediatric patients with enterococcal bacteremia.

Meningitis

Enterococci are rare causes of bacterial meningitis in adults and children. Stevenson and colleagues[256] found

only four cases of enterococcal meningitis among 493 episodes (0.8%) of bacterial meningitis in adults, and they identified an additional 90 cases in a literature search of the interval from 1966 to 1992. These authors reviewed 16 published cases of enterococcal meningitis in children: 11 of these 16 pediatric cases were complications of central nervous system (CNS) trauma or surgery, but four children (three of the four were neonates) had primary meningitis.[256] Enterococci are uncommon but well-recognized causes of infection involving cerebrospinal fluid (CSF) shunts and related devices.[113,134,188,235,286] Meningitis rarely complicates nosocomial bacteremia in adults,[156,205] but it has been reported more frequently in children (particularly neonates) with bacteremia. For example, meningitis developed in 7 percent (4/57) of episodes of nosocomial enterococcal bacteremia in children in Cincinnati[41] and in 15 percent (4/26) of premature neonates with late-onset enterococcal sepsis in Houston.[58]

Stevenson and associates[256] found that most adults with enterococcal meningitis were immunocompromised (most were receiving steroids) or had a history of CNS, surgery, or both. Enterococcal meningitis has been reported in an adult patient with HIV infection who had completed a course of steroids for presumed *Pneumocystis* pneumonia.[206] Although many children with enterococcal meningitis do have a history of CNS trauma or surgery or are premature infants, most do not have other identifiable predisposing conditions or a history of immunosuppressive therapy. As with the majority of enterococcal infections, most isolates from CSF are *E. faecalis*, but meningitis and ventriculoperitoneal shunt infections caused by *E. faecium* also have been reported in children and adults.[188,190,207]

Neonatal Infections

Enterococci are important neonatal pathogens,* and the published experience with neonatal enterococcal infections constitutes much of the pediatric experience with these organisms. Although several large series of neonatal sepsis included few cases of enterococcal infection (reviewed by Klein and Marcy[133]), many centers have reported that *Enterococcus* is a relatively frequent cause of neonatal bacteremia. Siegel and McCracken[247] found that enterococci were second only to group B streptococci as causes of neonatal sepsis at Parkland Hospital in Dallas from 1974 to 1977, with an incidence rate of approximately 1 case/1000 live births. Gladstone and colleagues[84] reported that *Enterococcus* caused 18/270 (6.7%) episodes of neonatal sepsis at Yale–New Haven Hospital during the period 1979 to 1988, which ranked fourth in incidence behind group B streptococci (64 cases, 23.7%), *Escherichia coli* (46 cases, 17.0%), and coagulase-negative staphylococci (36 cases, 13.3%). During the 1990s, reports from Houston,[58] Cincinnati,[41] and New York City[163] documented sharp increases in the rate of late-onset neonatal infection with enterococci in both hospitalized high-risk premature neonates and infants[58,163] and otherwise healthy term newborns with "community-acquired" infection.[58]

Enterococci cause both early-onset (<7 days of age) and late-onset (>7 days of age) neonatal sepsis. Early-onset disease is indistinguishable from that caused by other neonatal pathogens, but it tends to be less severe.[58] Dobson and Baker[58] identified 56 neonates with enterococcal sepsis during a 10-year period at Jefferson Davis Hospital in Houston; 18/56 (32%) had early-onset sepsis, 26/56 (46%) had late-onset sepsis, and 12/56 (21%) had sepsis associated with necrotizing enterocolitis (2 early onset, 10 late onset). In this study, 25/26 (96%) infants with late-onset enterococcal sepsis were premature infants. Christie and colleagues[41] identified 83 cases of enterococcal bacteremia between 1986 and 1992 at Children's Hospital of Cincinnati; 58 of the 83 episodes occurred in neonates. Most cases (57/83, 68.7%) were nosocomial, but many (26/83, 31.3%) were community acquired. Young infants (<3 months of age) accounted for almost all (24/26, 92.3%) of the community-acquired episodes and for many (34/57, 59.6%) of the nosocomial infections. Bonadio[15] and Boulanger and colleagues[18] also reported community-acquired enterococcal bacteremia in young infants.

McNeeley and associates[163] identified 138 episodes of enterococcal bacteremia in a New York City neonatal ICU during a 20-year period and reviewed 100 of the episodes in detail. These authors noted a sharp increase in the rate of enterococcal bacteremia during the second decade (1984 to 1994) of this study and found that most cases occurred in older infants; during this decade, the mean age at onset was 44.7 days (vs. 16.1 days during the first decade of the study), and 65 percent (51/78) of the episodes occurred after the infants reached 30 days of age. Most of the infections occurred in neonates with indwelling central venous catheters (77%), and more than half of the patients had evidence of gastrointestinal disease (necrotizing enterocolitis in 33% and abdominal distention in an additional 21%). The overall mortality rate in this study was 28 percent, although many of the deaths were not attributed to the enterococcal infection. Most (64%) of the episodes of enterococcal bacteremia in this series were polymicrobial.[163]

Nosocomial outbreaks of enterococcal infection, including VRE infection, have been reported in several neonatal units in the United States.[41,49,154,211,214] Indwelling central venous catheters, necrotizing enterocolitis, and intra-abdominal surgery are important predisposing factors for the development of nosocomial enterococcal bacteremia in neonates, whereas the genitourinary tract is implicated much less frequently as a source.[41,163]

Enterococcal bacteremia in neonates and young infants has been associated with diarrhea[15,58] and respiratory disease,[15,58] although a causative role for *Enterococcus* in the pathogenesis of gastroenteritis or pneumonia remains undefined. Enterococci rarely cause urinary tract infections in neonates,[15,41,58,163] but nosocomial and community-acquired cases have been reported. Enterococci have been noted to cause a variety of other

*References 6, 8, 31, 32, 34, 49, 64, 72, 84, 96, 99, 100, 128, 143, 154, 171, 179, 182, 183, 186, 193, 211, 216, 217, 222.

neonatal infections, including focal skin and soft tissue infections such as scalp abscess,[58] omphalitis,[58] and conjunctivitis.[273]

Most enterococcal infections in neonates are caused by *E. faecalis*, but outbreaks of infection with *E. faecium* have been reported.[49] In the series from Cincinnati reported by Christie and associates[41] and consisting largely of neonates, 82 percent of enterococcal isolates were *E. faecalis* and 14 percent were *E. faecium*. McNeeley and colleagues[163] reported the isolation of *E. faecalis* from 94/100 patients and *E. faecium* from 15/100 patients in their series (both organisms were isolated from nine of the patients). Six of the *E. faecium* isolates were resistant to vancomycin, and all six patients with VRE bacteremia died, although only one death appeared to be related directly to the VRE infection.

Septic Arthritis

Enterococci rarely have been reported to cause septic arthritis, but they can infect native or prosthetic joints. Raymond and colleagues[212] reported a case of enterococcal septic arthritis involving a prosthetic hip and reviewed an additional 18 cases from the literature. Of these 19 episodes, 11 involved prosthetic joints (2 hips, 9 knees), and 8 involved native joints; only 1 of the 8 individuals with native-joint arthritis had an underlying abnormality of the joint. In 7 of the 19 episodes, *Enterococcus* was isolated from synovial fluid, along with a second organism (3 with coagulase-negative staphylococci, 1 with group B streptococci, 1 with *Pseudomonas*, 1 with *Streptococcus*, and 1 with *Kingella kingae*). Only one pediatric case was identified: a 21-month-old girl with septic arthritis of the wrist whose joint aspirate grew *Enterococcus* spp. and *K. kingae*.[246]

DIAGNOSIS

Enterococcal infections usually are diagnosed by isolation of *Enterococcus* from a culture of blood or another normally sterile site. As discussed earlier, enterococci are ubiquitous inhabitants of the human gastrointestinal tract, and isolation of these organisms from stool or surface cultures is not evidence of invasive infection. Although enterococci are uncommon blood culture contaminants, transient bacteremia, pseudobacteremia, or both may be caused by these organisms.[107,269]

More problematic is the interpretation of a positive culture for enterococcus as a component of polymicrobial infections, particularly intra-abdominal and pelvic infections. In this setting, the role of the enterococcus in pathogenesis is unclear, and therapeutic regimens that do not include antienterococcal agents usually suffice to effect a clinical cure.[88] However, rare cases of breakthrough enterococcal bacteremia have been reported with these regimens, and one study found a decreased rate of abdominal surgical wound infections when antienterococcal coverage was provided in a prophylactic regimen.[276]

ANTIMICROBIAL SUSCEPTIBILITY AND RESISTANCE

The increasing importance of *Enterococcus* as a human pathogen, especially in the nosocomial setting,* is of particular concern because of the concomitant development of antimicrobial resistance by these organisms. Furthermore, antibiotic resistance probably has played a critical role in allowing the organism to persist and eventually cause disease in high-risk patients despite its relatively low virulence.

Some enterococci have acquired high-level resistance to all three classes of antimicrobial agents that traditionally have been used to treat life-threatening enterococcal infections—β-lactams, aminoglycosides, and glycopeptides. This acquired resistance has occurred in the context of intrinsic (usually lower level) resistance of enterococci to many antibiotics, and physicians have been confronted with the possibility that invasive disease caused by these organisms might not respond to any available antibiotics. Some enterococci also have acquired resistance to recently developed agents (e.g., linezolid, daptomycin) that are active against most antibiotic-resistant gram-positive cocci (see later discussion).

Intrinsic Resistance

β-Lactam Antibiotics

Relative resistance to β-lactam antibiotics is an intrinsic characteristic of enterococci that occurs even in human populations without previous exposure to antibiotics[172]; such resistance is due to the lower affinity of enterococcal (vs. streptococcal) penicillin-binding proteins, especially PBP-5.[171,279] In general, the minimal inhibitory concentration (MIC) of penicillin for most *E. faecalis* isolates (2 to 8 μg/mL) is at least 10 to 100 times higher than that of most streptococci, and *E. faecium* is even more resistant (MIC of 8 to 32 μg/mL or higher).[182] Ampicillin is the most active of the β-lactam antibiotics against enterococci, with average MIC values approximately twofold lower than those for penicillin.[171,172,182] Nafcillin generally is less active than penicillin, and methicillin is much less active (MIC >50 μg/mL for *E. faecalis*), as are carbenicillin and ticarcillin. Importantly, enterococci exposed to β-lactam antibiotics rapidly become tolerant of the killing effects of these agents.[171,182] Along with intrinsic resistance to these agents, tolerance limits the utility of β-lactam monotherapy for the treatment of endocarditis or other severe enterococcal infections.

Imipenem has some activity against *E. faecalis* but is much less active against *E. faecium*.[171,182] None of the cephalosporins currently approved for use in pediatrics have clinically useful activity against the enterococci, and frequent use of broad-spectrum cephalosporins and imipenem has been identified as a risk factor for the acquisition of nosocomial enterococcal infection.

*References 28, 65, 123, 163, 170, 182, 220, 284, 285.

Aminoglycosides

Enterococci are intrinsically resistant to all aminoglycosides because of diminished uptake of these drugs. For most *E. faecalis* isolates, the MIC for gentamicin or tobramycin ranges from 8 to 64 μg/mL and for streptomycin ranges from 12 to 250 μg/mL.[165,204,267] Moellering and Weinberg[173] and Zimmerman and colleagues[290] first demonstrated that the addition of a cell wall–active antibiotic results in dramatically increased aminoglycoside uptake by enterococci and that combinations of β-lactam and aminoglycoside antibiotics can lead to synergistic killing of these organisms. All *E. faecium* strains exhibit higher MICs (than does *E. faecalis*) to certain aminoglycosides, including tobramycin, netilmicin, kanamycin, and sisomicin, and these aminoglycosides do not exhibit synergy with β-lactam antibiotics against *E. faecium*.[36,182]

Other Antibiotics

Under carefully standardized laboratory conditions, enterococci are inhibited by the combination of trimethoprim and sulfamethoxazole (TMP-SMX). However, *Enterococcus* isolates should be considered resistant to TMP-SMX because these organisms are capable of using exogenous folinic acid to evade the antimicrobial action of TMP-SMX.[289] TMP-SMX fails to eradicate enterococci in animal models of infection,[33,95] and breakthrough enterococcal bacteremia has occurred in patients being treated with TMP-SMX for enterococcal urinary tract infection.[87] Enterococci also are intrinsically resistant to clindamycin, a drug with excellent activity against many other gram-positive cocci. Most enterococci have a clindamycin MIC of 12.5 to 100 μg/mL.[182] As with low-level β-lactam resistance, clindamycin resistance is found in enterococcal isolates from human populations with no previous antibiotic exposure.[172]

Acquired Resistance

Enterococci have acquired resistance to antibiotics by the acquisition of both narrow and broad host range plasmids and via the exchange of conjugative transposons (reviewed by Murray[182]). Resistance mediated by broad host range plasmids is of particular concern because glycopeptide resistance encoded by broad host range plasmids has been transferred to staphylococci in vitro[195] and in vivo.[29,30,265,275]

High-Level Resistance to Aminoglycosides

Intrinsic resistance of enterococci to aminoglycosides is caused by poor drug uptake by these organisms and can be overcome effectively both in vitro and in vivo by the addition of cell wall–active antibiotics. In contrast, high-level resistance to aminoglycosides is mediated either by the acquisition of plasmids encoding aminoglycoside-modifying enzymes (affecting all aminoglycosides via several different enzymes) or by ribosomal mutations (streptomycin only). High-level aminoglycoside resistance is of great clinical importance because it eliminates synergism between the affected aminoglycoside or aminoglycosides and β-lactam or glycopeptide antibiotics.[36,182,204] All *E. faecium* strains produce a chromosomally encoded aminoglycoside acetyltransferase that eliminates synergistic killing between cell wall–active antibiotics and certain aminoglycosides (including tobramycin, kanamycin, netilmicin, and sisomicin),[36] but it does not result in high-level resistance to these compounds. Consequently, these particular aminoglycosides should not be used to treat infections caused by *E. faecium*.

Enterococci with high-level resistance (MIC usually ≥2000 μg/mL) to streptomycin, kanamycin, and several other aminoglycosides (excluding gentamicin) were identified more than 40 years ago[174] and were widely prevalent in the United States by the mid-1970s.[24] High-level resistance to streptomycin occurs via two mechanisms, ribosomal mutation or enzymatic modification by 6′-adenylyltransferase. Initial reports of high-level resistance to kanamycin were associated with the production of 3′-phosphotransferase.[36,182]

Horodniceanu and colleagues[109] first reported high-level resistance to gentamicin in *E. faecalis* in 1979, and this resistance was shown later to be mediated by a fusion enzyme containing both 6′-acetyltransferase and 2″-phosphotransferase activity. Expression of this fusion enzyme conferred resistance to all clinically useful aminoglycosides except streptomycin.[142,143] Enterococci expressing this fusion protein usually have gentamicin MICs that are 2000 μg/mL or higher. Thus, enterococci expressing this fusion enzyme and the 6′-adenylyltransferase mediating streptomycin resistance (or chromosomally mediated streptomycin resistance) are highly resistant to all available aminoglycosides and generally fail to be killed synergistically by any combination of β-lactam antibiotics and aminoglycosides. Strains of *E. faecalis* resistant to both streptomycin and gentamicin (and thus to all aminoglycosides) were detected first in Houston, Bangkok, and Santiago in 1983.[165,182] Subsequently, strains of *E. faecalis*, *E. faecium*, and other enterococci resistant to gentamicin, streptomycin, or both (or all) aminoglycosides have become increasingly prevalent* (Table 84-2). Although high-level resistance to both streptomycin and gentamicin was described first in *E. faecalis*, it now is at least as common in *E. faecium*.[121]

Enterococci resistant to all aminoglycosides frequently have been isolated from clinical specimens for the past 2 decades. Jones and colleagues and the *Enterococcus* Study Group[120] found that fully 20 percent of 1936 enterococcal isolates from late 1992 (from 97 participating laboratories in 47 states) exhibited high-level resistance to both gentamicin and streptomycin. Low and associates and the SENTRY Antimicrobial Resistance Surveillance Program[153] reported similar rates of high-level resistance to both gentamicin and streptomycin from 1997 to 1999 (see Table 84-2). Almost a third (32.8%) of enterococci collected by the SENTRY group from 1997 to 2005 were highly resistant to gentamicin.[122] Many medical centers in the United States now report that most enterococcal isolates exhibit high-level resistance to all aminoglycosides.[186]

*References 89, 119, 120, 122, 153, 204.

TABLE 84-2 **Antimicrobial Resistance Patterns of Enterococci (United States)**

	Gordon et al.[89] (July 1988–April 1989)			Jones et al.[121] (Enterococcus Study Group) (October 1992–December 1992)			Edmond et al.[65] (April 1995–April 1998) (Bloodstream Isolates)			Low et al.[153] (SENTRY Antimicrobial Surveillance Program) (1997-1999)	Jones et al.[122] (SENTRY Antimicrobial Resistance Surveillance) (1997-2005)
Agents	*E. faecalis* (*N* = 632) (%)	*E. faecium* (*N* = 58) (%)	*Total* (*N* = 705) (%)	*E. faecalis* (*N* = 1428) (%)	*E. faecium* (*N* = 306) (%)	*Total* (*N* = 1936) (%)	*E. faecalis* (*N* = 598) (%)	*E. faecium* (*N* = 303) (%)	*Total* (*N* = 1354) (%)	*Total enterococcal isolates* (*N* = 2303) (%)	*E. faecium* (*N* = 1512) (%)
Ampicillin resistance (MIC ≥16 μg/mL)	0*	41†	4	0.6-0.7‡	58.7-59.3	12	2.7	81.1	NA	24	89
Streptomycin high-level resistance (MIC >2000 μg/mL)	14	33	16	31.5	55.7	36	NA	NA	NA	40	NA
Gentamicin high-level resistance (MIC >500 μg/mL)	11	2	10	26.0	30.8	27	NA	NA	NA	31	32.8
Vancomycin resistance (MIC >4 μg/mL)	0.3	0	0.3	2.0	21.9	5.6	3.2	50.5	17.7	17	69.4

*However, 11/632 (1.7%) *E. faecalis* isolates were β-lactamase producers.
†No β-lactamase–producing strains were identified.
‡Only two β-lactamase–producing isolates were identified.

Several new aminoglycoside-resistant genes were identified in enterococci during the late 1990s (reviewed by Chow[36]). Some enterococci produce three or more enzymes. Strains expressing some of the recently described resistant genes may fail to exhibit synergy between gentamicin and β-lactams despite MICs below those usually associated with high-level resistance. For example, the *aph (2″)-Ic* gene[39] found in clinical isolates of both *E. faecalis* and *E. faecium* results in gentamicin MICs of approximately 256 to 384 μg/mL, lower than the standard screening cutoff for high-level resistance to gentamicin (500 μg/mL). Nonetheless, these organisms are resistant to ampicillin-gentamicin synergism and would not be detected by standard screening methods.

High-Level Resistance to β-Lactams and Production of β-Lactamase

The mechanisms of resistance to penicillin, ampicillin, and other β-lactam antibiotics differ among enterococcal species. High-level β-lactam resistance (ampicillin MIC ≥16 μg/mL) of *E. faecium*[20,94,161] and some other non-*faecalis* enterococcal strains[20] is now common and is mediated by additional alterations in penicillin-binding proteins (PBPs), particularly PBP-5.[76,94,219] Thus, high-level resistance to ampicillin and other β-lactams in *E. faecium* (and other non-*faecalis* strains) represents an exaggerated form of intrinsic β-lactam resistance. *E. faecalis* isolates also are intrinsically resistant to β-lactams (though less so than *E. faecium*), but little change has occurred in the level of this resistance in recent years. However, some strains of *E. faecalis*[121,183,187] and *E. faecium*[48] have acquired clinically significant resistance to ampicillin and penicillin via the plasmid-mediated, constitutive production of a β-lactamase enzyme identical to that of *S. aureus*.[291] β-Lactamase–producing strains of *E. faecalis* and *E. faecium* will not be detected by routine susceptibility testing because of a pronounced inoculum effect. Therefore, enzymatic methods such as the nitrocefin test must be used to screen for β-lactamase–producing strains.[129,183] Fortunately, such strains remain very uncommon.[4,171]

Glycopeptide Resistance

Enterococci resistant to vancomycin were identified first in 1988[140,270] and rapidly have become a major nosocomial problem. Data collected by the CDC NNIS revealed a dramatic increase in the rate of vancomycin resistance in nosocomial isolates of *Enterococcus* during the interval 1989 to 1993.[28] The NNIS survey documented a 26-fold increase in vancomycin resistance among all nosocomial isolates (from 0.3% of enterococcal isolates in 1989 to 7.9% in 1993) and a 34-fold increase in vancomycin resistance among isolates obtained from adult patients in ICUs (from 0.4% of isolates in 1989 to 13.6% in 1993). Rates of vancomycin resistance in enterococci continued to increase during the next decade (see Table 84-2). The NNIS reported that fully 25 percent of enterococci associated with nosocomial infections in adult patients in ICUs in the United States were resistant to vancomycin in 1999 and 2000; by 2003 to 2004, approximately 30 percent of the enterococcal isolates from these patients were vancomycin-resistant.* Isolates of *E. faecium* are particularly likely to be vancomycin-resistant. Jones and colleagues[122] in the SENTRY Antimicrobial Resistance Surveillance Program reported that 69.4 percent of 1512 U.S. bloodstream isolates of *E. faecium* collected between 1997 and 2005 were resistant to vancomycin. Similarly, Wisplinghoff and colleagues[284] in the SCOPE surveillance study found that 60 percent of U.S. bloodstream isolates of *E. faecium* collected between 1995 and 2002 were resistant to vancomycin, whereas only 3 percent of *E. faecalis* bloodstream isolates were vancomycin-resistant.

Enterococci were the second most common organisms associated with pediatric bloodstream infections in both the 1992 to 1997 NNIS database[222] and the pediatric component of the 1995 to 2001 SCOPE survey.[284] However, VRE organisms were detected much less frequently in these pediatric patients; only 11 percent of *E. faecium* and 1 percent of *E. faecalis* bloodstream isolates (of 357 total enterococcal isolates) obtained from pediatric patients in the SCOPE study were vancomycin-resistant.[284] This finding is important because vancomycin-resistant isolates, particularly those of *E. faecium*, also may exhibit high-level resistance to both β-lactam antibiotics and aminoglycosides, thus rendering treatment of these infections extremely challenging.†

Vancomycin resistance among enterococci is phenotypically and genotypically heterogeneous[6,85,185,186] and may or may not be associated with resistance to other glycopeptides, including teicoplanin. Five major phenotypes of vancomycin resistance (VanA, VanB, VanC, VanD, and VanE) have been characterized. The VanA and VanB phenotypes are most common, and both are transferable. The VanA phenotype is characterized by high-level resistance to vancomycin and teicoplanin, whereas strains with the VanB phenotype exhibit variable levels of resistance to vancomycin but not teicoplanin.[6,85,185,186] The VanC phenotype is limited to *E. gallinarum* and *Enterococcus casseliflavus* and is associated with constitutive, low-level, chromosomally mediated (nontransferable) resistance to vancomycin but not teicoplanin.[85,185,186,217] VanD and VanE phenotypes occur uncommonly and are not transferable.

High-level resistance to both vancomycin and teicoplanin (the VanA phenotype) is found primarily in strains of *E. faecium*, whereas most vancomycin-resistant strains of *E. faecalis* express the VanB phenotype and remain susceptible to teicoplanin. Jones and associates and the *Enterococcus* Study Group found that 10/11 (91%) vancomycin-resistant strains of *E. faecalis* remained susceptible to teicoplanin (VanB phenotype), whereas 49/62 (79%) vancomycin-resistant strains of *E. faecium* were resistant to teicoplanin (VanA phenotype).[121]

The biochemical mechanisms responsible for the major vancomycin-resistant phenotypes are the subject of intense study (reviewed by Murray[184] and Gold[85]). Both

*See CDC website: http://www.cdc.gov/ncidod/dhqp/pdf/ar/ICU_RESTrend1995-2004.pdf.

†References 19, 81, 100, 124, 145, 177-179, 216, 217, 222, 241, 242.

VanA and VanB phenotypes are mediated by homologous enzymes that catalyze the formation of an altered, vancomycin-resistant depsipeptide that is incorporated into cell wall peptidoglycan.[6,186,217] The *vanA* and *vanB* gene clusters share functional similarities but are regulated quite differently.[85,186] VanA resistance is transferable by either a transposon or conjugative plasmids. VanB resistance often is encoded by chromosomal DNA but may be transferred by at least two different transposons.[85,186] Vancomycin induces the production of enzymes encoded by both the *vanA* and *vanB* gene clusters, whereas teicoplanin induces VanA but not VanB enzymes.[85]

Resistance to Other Antibiotics

Recently licensed antimicrobials active against most antibiotic-resistant, gram-positive cocci (including VRE organisms) include linezolid and daptomycin. The minocycline derivative tigecycline also is active against most enterococci but has not been well studied for the treatment of these infections. Finally, another agent, the streptogramin quinupristin-dalfopristin, is active against *E. faecium* but not *E. faecalis* isolates and because of this limitation and its considerable toxicity has little utility in pediatrics.

The oxazolidinones are a novel class of synthetic protein synthesis inhibitors that act to inhibit the formation of ribosomal initiation complexes in bacteria.[43] Linezolid, the first oxazolidinone antimicrobial approved for human use, is active against both *E. faecalis* and *E. faecium*, including most VRE isolates. Initial studies demonstrated virtually uniform activity of this agent against clinical isolates of enterococci,[17,67,198] with 100 percent of 180 strains (representing multiple resistance profiles) inhibited by linezolid concentrations of 1 to 4 μg/mL in one study.[67] Furthermore, initial in vitro studies suggested that the development of resistance would occur very rarely.[43,123,292] However, resistance to linezolid has been reported in several patients receiving prolonged courses of therapy with this drug[4,86,159,167,238] and in some patients who have not previously received the agent,[16] and it has been associated with clinical failures.[4,86] Resistance to linezolid is conferred by single nucleotide changes in varying numbers of copies of the bacterial genes encoding 23S ribosomal RNA.[167] Resistance increases in strains containing multiple copies of these mutations.[158,167] Although some centers have reported increasing rates of linezolid resistance in VRE isolates[167] and nosocomial spread of linezolid-resistant VRE strains,[105] overall rates of linezolid resistance in enterococci remain low. For example, only 0.9 percent of 1512 U.S. bloodstream isolates of *E. faecium* collected from 1997 to 2005 by the SENTRY group were resistant to linezolid[122] and only 0.75 percent of 934 enterococcal isolates studied in 2010 as part of the LEADER surveillance program were resistant to linezolid.[74]

Daptomycin is a novel cyclic lipopeptide antimicrobial that exhibits rapid, concentration-dependent bactericidal activity against gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and VRE strains.[27,254] Daptomycin has a novel method of action in which binding to bacterial membranes and triggering of rapid depolarization of membrane potential lead to inhibition of protein and nucleic acid synthesis.[81] Daptomycin was found to have potent activity against a panel of VRE isolates from the United States and Europe—all 75 VRE isolates tested (i.e., 55 *E. faecium* and 20 *E. faecalis*) were susceptible to this agent.[226] Daptomycin also was reported to be active against a collection of VRE strains resistant to linezolid or quinupristin-dalfopristin.[2] However, daptomycin resistance has developed during therapy with this agent,[148,181,224] although resistance rates remain very low. Sader and colleagues[225] studied 4312 *E. faecalis* isolates (195 VRE) and 2462 *E. faecium* isolates (1867 VRE) collected between 2005 and 2010; 99.98 percent of the *E. faecalis* isolates and 99.68 percent of the *E. faecium* isolates were susceptible to daptomycin. The mechanisms responsible for daptomycin resistance are poorly understood.

Streptogramins are protein synthesis inhibitor antibiotics and are natural combinations of two chemically unrelated molecules, streptogramin A and B.[151] Quinupristin-dalfopristin is a streptogramin agent that is active against *E. faecium* (including most strains of VREF) but lacks activity against *E. faecalis* at clinically achievable concentrations.[47] An early study found that more than 95 percent of 875 initial patient isolates of VREF were susceptible to quinupristin-dalfopristin,[68] but both emergence of resistance to this agent[175,226] and superinfection with *E. faecalis* and other organisms[37,283] have been reported during therapy with this drug. In the United States, overall rates of enterococcal resistance to quinupristin-dalfopristin remain low, in the range of 1 to 2 percent in most areas.[106]

Testing for Antimicrobial Resistance in Enterococci

All enterococci isolated from cultures of blood, CSF, or other normally sterile sites (with the possible exception of urine) should be tested for resistance to β-lactam antibiotics (i.e., ampicillin, penicillin, or both, including a test for β-lactamase production), vancomycin, and high levels of aminoglycosides (streptomycin and gentamicin)[70,212,217] by using the methodology and interpretative guidelines published by the Clinical and Laboratory Standards Institute (CLSI).[44] For multiantibiotic-resistant isolates, testing for susceptibility to alternative agents, including linezolid and daptomycin, should be performed.

Testing of enterococci for ampicillin (or penicillin) resistance must involve determination of the MIC of these agents and a test for β-lactamase production.[44] Jones and colleagues[120] and the *Enterococcus* Study Group compared three techniques that are used commonly to determine the ampicillin MIC of enterococcal isolates—disk diffusion, broth microdilution, and E-test strips—and found excellent agreement among the three methods. Enterococcal isolates with an ampicillin or penicillin MIC of 16 μg/mL or greater are considered resistant to these agents.[44] β-Lactamase–producing enterococci cannot be detected by these methods but are identified routinely by performance of the chromogenic nitrocefin assay.[258]

High-level resistance to gentamicin and streptomycin usually may be detected by either agar dilution (high-level resistance to gentamicin, MIC >500 μg/mL; high-level resistance to streptomycin, MIC >2000 μg/mL) or broth microdilution (high-level resistance to gentamicin, MIC >500 μg/mL; high-level resistance to streptomycin, MIC >1000 μg/mL).[44,258,259] These isolates also may be identified by disk diffusion with the use of high-content aminoglycoside disks (120-μg gentamicin disk, 300-μg streptomycin disks, ≥10-mm zone = susceptible)[259] or high-range E-test strips.[120,230] However, the recent identification of novel genes encoding gentamicin resistance in both *E. faecalis* and *E. faecium* has raised concern about continued use of the 500-μg/mL cutoff for detection of high-level resistance to gentamicin (reviewed by Chow[36]). These enterococcal isolates are not killed synergistically by combinations of ampicillin or penicillin and gentamicin, even though gentamicin MICs are 256 to 384 μg/mL. If these isolates become more widely prevalent, modification of the standard screening procedures for high-level aminoglycoside resistance may be required.

Commercially available automated antimicrobial susceptibility testing methods may fail to detect vancomycin resistance in enterococci, particularly the VanB phenotype of moderate resistance,[264] although newer versions of these systems appear to be much more reliable.[83,271] Routine disk diffusion testing is more dependable but requires an extended incubation time and the use of transmitted light for examination of zone size to be highly accurate.[260,264] Agar dilution screening using brain-heart infusion agar supplemented with 6 μg/mL vancomycin reliably identifies VRE strains,[44,258,277] as does the standard broth microdilution method.[121,264] E-test glycopeptide strips are a useful alternative to the more cumbersome broth dilution methodology.

THERAPY FOR ENTEROCOCCAL INFECTIONS

Before the emergence of VRE infection as a major nosocomial problem in high-risk patients, the optimal management of serious enterococcal infections (especially endocarditis) was well established. Successful treatment of enterococcal endocarditis required the administration of combination therapy consisting of a cell wall–active agent (usually ampicillin) and an aminoglycoside (gentamicin or streptomycin). Combination therapy also was recommended for other potentially life-threatening enterococcal infections (e.g., sepsis or meningitis) based on the extensive experience with endocarditis. Other enterococcal infections, including urinary tract infections, responded well to monotherapy with a variety of antimicrobials.

In contrast, the optimal therapy for many infections caused by VRE strains remains uncertain. Both linezolid and daptomycin have now been used extensively for the treatment of VRE infections in adults. However, only one of these antibiotics (linezolid) has been approved for use in children, and enterococci resistant to these new agents already have been detected.[167,181]

Treatment of Infections Caused by Antibiotic-Susceptible Enterococci

Any discussion of the treatment of serious enterococcal infection must begin with a review of the large experience in the treatment of enterococcal endocarditis. The difficulty of treating enterococcal endocarditis has been apparent since early reports showed that penicillin alone failed to cure as many as two thirds of patients with enterococcal endocarditis but was highly effective in the treatment of streptococcal endocarditis (reviewed by Murray[182]). The early failures of penicillin therapy stimulated studies of the in vitro and in vivo effects of β-lactam antibiotics on enterococci. These studies led to the discovery that enterococci were "tolerant" of the killing effects of cell wall–active agents and provided evidence that bactericidal therapy was required to cure bacterial endocarditis reliably.

For more than 65 years, standard therapy for enterococcal endocarditis has included an aminoglycoside plus a cell wall–active agent. Hunter[111] first reported clinical evidence of synergism between penicillin and streptomycin in the treatment of enterococcal endocarditis in 1947, and this synergism subsequently was confirmed for combinations of penicillin or ampicillin and streptomycin or gentamicin, both in vitro and in vivo (reviewed by Murray[182]). Moellering and Weinberg[173] first demonstrated that synergy between β-lactams and aminoglycosides was a consequence of increased aminoglycoside uptake by enterococci exposed to cell wall–active agents. Glycopeptides and aminoglycosides also exhibit in vitro and in vivo synergy against "susceptible" (not expressing high-level resistance) strains of enterococci.[108,171]

The preferred therapy for endocarditis caused by "susceptible" strains of enterococci in both adults* and children[52,253] consists of combination therapy with parenteral ampicillin (or penicillin G) plus parenteral gentamicin (or streptomycin) for a minimal duration of 4 to 6 weeks. Patients with severe penicillin allergy should be treated with vancomycin plus gentamicin or streptomycin. Selected adult patients with a short duration of symptoms and an uncomplicated course may be treated with 4-week regimens[280]; most other patients, including those with mitral valve involvement, a longer duration of symptoms (especially those with symptoms for >3 months), or prosthetic valve endocarditis, probably should receive 6-week courses of therapy.[166,280] Interestingly, several reports indicate that the prognosis of enterococcal prosthetic valve endocarditis is better than that of native valve disease, perhaps because of the generally shorter duration of symptoms before the diagnosis is made.[166,218] Many patients with prosthetic valve endocarditis caused by enterococci can be cured without surgery. Rice and colleagues[218] reported a 69 percent cure rate with medical therapy in patients with enterococcal endocarditis involving prosthetic valves.

The general consensus, based on experience with enterococcal endocarditis, is that other life-threatening enterococcal infections, including meningitis and septicemia,

*References 4, 14, 166, 171, 218, 280.

should be treated with bactericidal regimens.[170,182,256] The duration of therapy for uncommon enterococcal infections such as meningitis must be individualized, although 2- to 3-week courses of antibiotics have been reported to cure enterococcal meningitis.[256] The optimal treatment of enterococcal bacteremia, particularly that occurring in the nosocomial setting, remains controversial.

The prognosis for these patients varies widely and often is related more closely to the underlying disease than to the enterococcal infection. In adults, single-drug regimens generally are successful in the treatment of enterococcal bacteremia, thus indicating that bactericidal therapy is not required in all cases.[170,182]

Considerable clinical experience supports the routine use of single-drug therapy for uncomplicated enterococcal urinary tract infection and for soft tissue infection caused by enterococci. Urinary tract infections by susceptible strains of enterococci generally respond promptly to ampicillin, penicillin, nitrofurantoin, or vancomycin.[170,171,180,182]

Treatment of Infections Caused by Antibiotic-Resistant Enterococci (Including Vancomycin-Resistant Enterococci)

Unfortunately, the emergence of enterococci with clinically significant resistance to aminoglycosides, β-lactams, and glycopeptides has greatly complicated the management of endocarditis and other serious infections caused by these organisms. This discussion focuses on the management of endocarditis caused by drug-resistant enterococci because the need to provide bactericidal therapy has been well established in this setting. The general principles probably apply to the management of other life-threatening enterococcal infections (see earlier discussion).

Endocarditis caused by enterococci with high-level resistance to either streptomycin or gentamicin may be treated by substituting the other aminoglycoside in a combination regimen, but isolation of strains with high-level resistance to both aminoglycosides means that no available aminoglycoside will provide synergistic killing in concert with cell wall–active agents.[166,182,281] No reliably bactericidal regimen is available for the treatment of endocarditis caused by these strains, even if the isolates remain susceptible to other classes of antimicrobials. Based on animal studies, some experts recommend prolonged treatment (≥8 to 12 weeks) with high-dose intravenous ampicillin given by continuous infusion in this situation (if the isolate is susceptible).[66,171] Surgical excision of infected valves may be required in such patients.[218]

Endocarditis and other serious infections caused by enterococci resistant to β-lactam antibiotics also are increasing in frequency.[94] Endocarditis caused by enterococci (usually *E. faecium*) highly resistant to β-lactams may be treated with vancomycin plus an aminoglycoside (if the strain is susceptible to vancomycin). Endocarditis caused by β-lactamase–producing strains of *E. faecalis* (or, rarely, *E. faecium*) would be expected to respond to ampicillin-sulbactam because this combination is highly effective in animal models.[66] Higher-dose or continuous-infusion ampicillin may be effective in the treatment of endocarditis caused by some strains of enterococci that are "resistant" to ampicillin by current guidelines (MIC ≥16 μg/mL) because sustained plasma concentrations in excess of 100 μg/mL may be achieved with these regimens.[186] However, even high-dose ampicillin therapy probably will fail to cure endocarditis caused by enterococci with ampicillin MICs greater than 64 μg/mL.[22,85]

Enterococci resistant to vancomycin may remain susceptible to teicoplanin (VanB phenotype). Teicoplanin with or without the addition of an aminoglycoside has been used successfully in the treatment of serious enterococcal infections caused by susceptible isolates,[234] including some cases of endocarditis[209,234] and meningitis.[150] However, teicoplanin is not available in the United States, treatment of endocarditis with this agent has been associated with both treatment failure and relapse,[209,234] and high-level teicoplanin resistance may develop even without exposure to the drug.[102] Endocarditis caused by VanB strains of enterococci should be treated with high-dose ampicillin plus gentamicin or streptomycin if resistance to these agents is not present.

Similarly, endocarditis caused by enterococci highly resistant to both vancomycin and teicoplanin (VanA phenotype) but susceptible to β-lactams should be treated with ampicillin plus an aminoglycoside (if high-level resistance to aminoglycosides is not present). Unfortunately, the VanA phenotype is associated primarily with strains of *E. faecium*, which increasingly are resistant to β-lactams.[76,94,120] Endocarditis caused by enterococci highly resistant to both glycopeptides and β-lactams is especially difficult to treat. Combinations of ampicillin and vancomycin are not bactericidal against these isolates but may[244] or may not[31] provide additive or synergistic inhibition in vitro. If high-level resistance to aminoglycosides is not present, triple-combination therapy with a β-lactam, a glycopeptide, and an aminoglycoside may achieve bactericidal activity; such combinations are reported to be highly effective in animal models of endocarditis caused by ampicillin- and vancomycin-resistant *E. faecium*.[26] For endocarditis caused by enterococci exhibiting high-level resistance to both gentamicin and streptomycin along with resistance to β-lactams and glycopeptides, no proven effective therapies are available.

Newer Options for Treatment of Vancomycin-Resistant Enterococcal Infections

Four new antimicrobials with activity against most VRE strains recently have been licensed: linezolid, daptomycin, tigecycline, and quinupristin-dalfopristin. Of these drugs, only linezolid has been approved for use in children.

Linezolid, the first oxazolidinone antibiotic, was approved by the U.S. Food and Drug Administration (FDA) in April 2000 for the treatment of selected infections caused by antibiotic-resistant, gram-positive bacteria in adults, including those caused by VRE strains (*E. faecium* or *E. faecalis*). In December 2002, the FDA approved this agent for treatment of pediatric patients with pneumonia, skin and skin structure infections,

and VREF infections. Linezolid is available in both parenteral and oral formulations (with comparable bioavailability). Linezolid is active against many antibiotic-resistant, gram-positive pathogens, including MRSA, penicillin-resistant *S. pneumoniae*, and vancomycin-resistant isolates of both *E. faecium* and *E. faecalis*.[17,43,67,74,198] This agent is bacteriostatic against most susceptible organisms, including the enterococci, but it does exhibit bactericidal activity against some strains of pneumococci. Initial studies demonstrated virtually uniform activity of linezolid against clinical isolates of enterococci.[17,66,199]

Linezolid has been reported to effect both bacteriologic and clinical cure in patients with life-threatening VRE infections, including endocarditis,[9,13,35,254] bacteremia,[35,164,199] and meningitis,[240,286,288] although clinical experience with this agent in the treatment of endocarditis or meningitis remains limited. Linezolid therapy has been reported to cure several patients with VRE endocarditis,[9,13,34,255] including 10/22 patients in one study[13] and all of 3 patients who received at least 6 weeks of the drug in another report.[255] These results are encouraging, but enthusiasm for the use of linezolid to treat serious VRE infection must be tempered by its generally bacteriostatic activity and the potential for increasing resistance to this agent. Some experts suggest caution in using linezolid for treatment of VRE endocarditis because of reported therapeutic failures, toxicity, and potential for emergence of resistance.[4,221] Pediatric experience with linezolid is limited, but the drug was demonstrated to be well tolerated and as effective as vancomycin in the treatment of children with serious gram-positive infections in a large multicenter trial.[125]

Linezolid generally has been tolerated well in both adults and children.[42,80,229] Adverse effects most frequently reported in patients receiving linezolid have included nausea, diarrhea, and headache in adults and diarrhea and vomiting in children.[80] Reversible myelosuppression has been reported in adults.[80] Longer courses of linezolid therapy have been associated with more serious adverse effects, including optic neuropathy that usually is reversible and peripheral neuropathy that may be irreversible.[114,223] Enterococcal resistance to linezolid remains very uncommon,[74] but has been associated with clinical failures in both adults and children.

Daptomycin is a novel cyclic lipopeptide antimicrobial that exhibits rapid, concentration-dependent bactericidal activity against enterococci, including VRE strains.[27,254] It was approved by the FDA in September 2003 for the treatment of adult patients with complicated skin and soft tissue infections caused by gram-positive bacteria, including staphylococci, streptococci, and vancomycin-susceptible strains of *E. faecalis*. Daptomycin is available in an intravenous formulation. It is not approved for use in children, and pediatric experience with the drug is limited. Daptomycin should not be used for the treatment of pneumonia because it is inactivated by pulmonary surfactant.[248]

Considerable published experience exists now with daptomycin therapy (alone and in combination with other agents) in adult patients with VRE bloodstream infections, though pediatric data remain limited. Half (11/22) of the adult patients with VRE bacteremia or endocarditis treated with daptomycin summarized in three early reports were cured.[139,208,239] Subsequently, anecdotal reports have been published documenting successful treatment of VRE bacteremia or endocarditis with daptomycin alone or in combination with other agents.[4] Higher doses of daptomycin (e.g., 8 mg/kg/day in adults) may be more effective for the treatment of bacteremia caused by VRE (and other bacteria). Limited, retrospective comparisons have failed to identify significant differences in the outcomes of VRE bacteremia treated with daptomycin or linezolid.[51,268] The published pediatric experience is limited, and clinical trials in children are under way.

Daptomycin resistance remains uncommon among enterococci[225] but may develop during therapy. Patients being treated with daptomycin should be monitored for the development of muscle toxicity by serial measurement of serum creatine kinase concentrations and screening for muscle pain or weakness.

The streptogramin antibiotic quinupristin-dalfopristin[151] was approved by the FDA in September 1999 for the treatment of adults with infections caused by VREF. This agent is not approved for the treatment of *E. faecalis* infections, nor for pediatric use. Quinupristin-dalfopristin has been reported to effect clinical cures of several serious infections caused by multiresistant *E. faecium*, including ventriculoperitoneal shunt infections in two patients,[188,268] an infected aortic graft in one patient,[228] and VREF peritonitis in a series of adults.[155] Clinical response to quinupristin-dalfopristin has been reported in approximately 74 to 80 percent of patients with VREF bacteremia.* The lack of a consistent bactericidal effect may limit the utility of this agent in the treatment of endocarditis and other life-threatening infections. Quinupristin-dalfopristin therapy generally has been tolerated fairly well in both adults and children, although a high rate of venous phlebitis in recipients has led to a recommendation that the agent be administered through a central venous catheter. Myalgias and arthralgias are other commonly reported side effects of this agent. The potential utility of quinupristin-dalfopristin for the treatment of pediatric patients is limited by its venous toxicity, its lack of a consistent bactericidal effect against VREF, and its lack of clinically useful activity against *E. faecalis*.[47]

Tigecycline is a novel glycylcycline antibiotic derived from minocycline. It is FDA approved in adults for treatment of complicated skin and soft tissue infections and abdominal infections caused by susceptible bacteria, including vancomycin-susceptible *E. faecalis*. It is active in vitro against both vancomycin-susceptible and vancomycin-resistant *E. faecalis* and *E. faecium*, but the clinical experience with this agent for treatment of VRE infections is limited and mostly consists of case reports involving combination antimicrobial therapy.[4,25] Pediatric experience is even more limited.[113]

Several other promising new antibiotics with in vitro activity against VRE isolates are being studied in clinical trials, including the lipoglycopeptides oritavancin and

*References 82, 92, 144, 146, 160, 175, 272, 283.

telavancin,[168] as well as new members of the oxazolidinone and streptogramin classes of antibiotics.

PREVENTION OF ENTEROCOCCAL INFECTIONS

The explosive increase in nosocomial infections caused by vancomycin-resistant and multiantibiotic-resistant enterococci[28,64] has rendered prevention of enterococcal infections, particularly those caused by VRE strains, a public health priority in the United States.[32,85,186,213,241] In 1995, the Hospital Infection Control Practices Advisory Committee (HICPAC) recommended a series of overlapping strategies designed to prevent the development of serious VRE infections.[213] These strategies remain useful today and are designed to simultaneously reduce vancomycin resistance in enterococci and prevent nosocomial infections caused by these organisms. Implementation of such strategies has been reported to reduce or eliminate the transmission of VRE organisms in health care facilities.[201] However, overall rates of nosocomial infection by VRE continued to increase in the United States from 1995 to 2004,* perhaps because the HICPAC guidelines were followed inconsistently (reviewed by Cetinkaya and colleagues[32]).

Reversing the Trend Toward Vancomycin and Multiple-Antibiotic Resistance in Enterococci

The use of vancomycin[213,241] and treatment with broad-spectrum antibiotics, including the third-generation cephalosporins and carbapenems,† are risk factors for colonization and the development of infection with VRE strains. Consequently, the HICPAC and CDC recommend that all hospitals, even those at which VRE organisms have not been isolated, should (1) develop a comprehensive antimicrobial utilization plan, (2) oversee surgical prophylaxis, and (3) develop institution-specific guidelines for the proper use of vancomycin.[213] These strategies are echoed in the recommendations for development of antimicrobial stewardship programs that have been made jointly by the Society for Healthcare Epidemiology of America, the Infectious Diseases Society of America, and the Pediatric Infectious Diseases Society.[251]

Efforts to eliminate unnecessary use of vancomycin are of critical importance[213,241] but may be inadequate unless the use of other classes of antimicrobials is reduced as well (see later discussion). In 1995, HICPAC provided recommendations regarding the acceptable and appropriate use of vancomycin (Table 84-3), as well as recommendations regarding situations in which vancomycin use should be discouraged[213] (Table 84-4). These recommendations remain useful, but the recent emergence of community-acquired MRSA infections[104,110,126] has resulted in increased empirical and definitive use of vancomycin for an HICPAC-approved indication: "treatment of serious infections caused by β-lactam–resistant, gram-positive microorganisms."[213]

TABLE 84-3 Situations in Which Vancomycin Use Is Appropriate or Acceptable

Treatment
Treatment of serious infections caused by β-lactam–resistant, gram-positive microorganisms
Treatment of gram-positive infections in patients with serious allergies to β-lactams
Treatment of antibiotic-associated colitis only when it fails to respond to metronidazole or is severe and potentially life-threatening
Prophylaxis
Endocarditis prophylaxis after certain procedures in high-risk patients according to the American Heart Association guidelines
Prophylaxis for major surgical procedures involving implantation of prosthetic materials or devices (single-dose prophylaxis usually is adequate)

Modified from Recommendations for preventing the spread of vancomycin resistance: Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep 1995;**44**(RR-12):1-13.

TABLE 84-4 Situations in Which Vancomycin Use Should Be Discouraged

Treatment
Empiric therapy for febrile neutropenic patients (unless there is presumptive evidence of an infection caused by gram-positive organisms, such as a Hickman catheter exit-site infection, and the local prevalence of methicillin-resistant *Staphylococcus aureus* strains is substantial)
Treatment of an isolated, single blood culture positive for coagulase-negative *Staphylococcus*
Treatment (chosen for dosing convenience) of infections caused by β-lactam–susceptible, gram-positive microorganisms in patients with renal failure
Continued empirical use of presumed infection in patients whose cultures are negative for β-lactam–resistant gram-positive microorganisms
Primary treatment of antibiotic-associated diarrhea
Eradication of methicillin-resistant *S. aureus* colonization
Prophylaxis
Routine surgical prophylaxis except in patients with life-threatening allergy to β-lactams
Systemic or local prophylaxis for infection of indwelling intravascular catheters
Routine prophylaxis for infants with very low birth weight
Routine prophylaxis for dialysis patients
Use of vancomycin solution for topical application or irrigation
Selective decontamination of the gastrointestinal tract

Modified from Recommendations for preventing the spread of vancomycin resistance: Recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). MMWR Recomm Rep 1995;**44**(RR-12):1-13.

*See the CDC website: http://www.cdc.gov/ncidod/dhqp/pdf/ar/ICU_RESTrend1995-2004.pdf.

†References 64, 93, 162, 179, 184, 192, 216.

In addition, efforts should be undertaken to reduce the unnecessary use of broad-spectrum antibiotics and certain agents with potent antianaerobic activity, particularly in settings with high rates of nosocomial infection (such as ICUs). These efforts are necessary because many studies indicate that nonglycopeptide antibiotics also exert selective pressure for VRE strains and that limitation of the use of vancomycin alone has only a modest effect on reducing VRE colonization and infection (reviewed by Rice[216]). Exposure to agents with broad-spectrum activity (but lacking antienterococcal activity), such as the extended-spectrum cephalosporins, appears to predispose to colonization with VRE organisms,[61,216] whereas exposure to antimicrobials with potent antianaerobic activity (even if they are also active against enterococci) may promote high-density, prolonged VRE colonization (by eliminating gut anaerobes that may interfere with colonization by VRE strains).[60,210,216]

Preventing and Controlling the Spread of Nosocomial Infection by Vancomycin-Resistant Enterococci

The increasing prevalence of *Enterococcus* in nosocomial infections is related to the intrinsic and acquired antimicrobial resistance of these organisms, but the factors leading to increased antibiotic resistance in enterococci are not identical to those that predispose to enterococcal colonization, infection, or both. Enterococcal infections are concentrated increasingly in debilitated and immunocompromised patients, including those with malignancies, recipients of bone marrow and solid organ transplants, burn victims, premature neonates, and critically ill patients with indwelling intravascular catheters. Consequently, special attention should be paid to potential outbreaks of VRE infection in hospital wards caring for these high-risk patients.[32]

Efforts to prevent the spread of VRE colonization, infection, or both probably will be more successful if the VRE isolates are confined to a few patients in a single area of the hospital (Table 84-5). Widespread colonization with VRE strains may precede identification of infections by these organisms. Therefore, all hospitals should implement active VRE surveillance and formulate a multidisciplinary plan to prevent nosocomial spread of VRE strains if such organisms are identified. In hospitals that have not isolated VRE strains, periodic antimicrobial susceptibility testing should be performed on enterococcal isolates from all sources, particularly from high-risk patient populations such as those in intensive care or transplant units. If VRE organisms are identified, a comprehensive plan to prevent nosocomial spread of these bacteria should be instituted immediately. The HICPAC guidelines summarized the essential elements of such a plan.[213] Hospital infection control staff and clinical staff must be notified promptly when VRE strains are isolated from a clinical sample, and isolation precautions should be implemented immediately to prevent patient-to-patient transmission of VRE organisms. These precautions include gown and glove isolation, vigorous hand washing, dedicated use of noncritical patient items such as thermometers and stethoscopes, and prompt surveillance of any possibly exposed patients for VRE colonization. Additional measures may be necessary in hospitals with endemic or continued VRE transmission despite implementation of the aforementioned measures.[213] Ostrowsky and colleagues[201] reported that an active infection control program that included surveillance cultures and prompt isolation of infected patients successfully reduced the spread of VRE strains in health care facilities throughout the Sioux land region of Iowa, Nebraska, and South Dakota.

Once VRE organisms become endemic in a hospital unit, achieving complete eradication is very difficult. The duration of VRE colonization in individual patients may be weeks or months,[32,177] although spontaneous resolution of colonization occurs frequently.[32,176] Attempts to eradicate colonization in individual patients generally have been unsuccessful.[32,176] Consequently, the measures recommended by HICPAC and others are directed at preventing the initial establishment of VRE strains in hospitals. Implementation of these policies will require the involvement of hospital pharmacy and therapeutics committees, quality assurance programs, and medical staff. Ongoing monitoring of the efficacy of these programs is necessary.

NEW REFERENCES SINCE THE SIXTH EDITION

8. Ashkenazi-Hoffnung L, Livni G, Amir J, Bilavsky E. Serious bacterial infections in hospitalized febrile infants aged 90 days or younger: the traditional combination of ampicillin and gentamicin is still appropriate. *Scand J Infect Dis* 2011;**43**:489–94.
25. Cai Y, Wang R, Liang B, et al. Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease. *Antimicrob Agents Chemother* 2011;**55**: 1162–72.
44. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard*. 8th ed. Wayne, Pa: Clinical and Laboratory Standards Institute; 2009. http://ww.clsi.org/source/orders/free/m07-a8.pdf.
51. Crank CW, Scheetz MH, Brielmaier B, et al. Comparison of outcomes from daptomycin or linezolid treatment for vancomycin-resistant enterococcal bloodstream infection: a retrospective, multicenter, cohort study. *Clin Ther* 2010;**32**:1713–19.
62. Drews SJ, Richardson SE, Wray R, et al. An outbreak of vancomycin-resistant *Enterococcus faecium* in an acute care pediatric hospital: lessons from environmental screening and a case-control study. *Can J Infect Dis Med Microbiol* 2008;**19**:233–6.
74. Flamm RK, Farrell DJ, Mendes RE, et al. LEADER Surveillance program results for 2010: an activity and spectrum analysis of linezolid using 6801 clinical isolates from the United States (61 medical centers). *Diagn Microbiol Infect Dis* 2012;**74**:54–61.
75. Florescu DF, Qiu F, Langnas AN, et al. Bloodstream infections during the first year after pediatric small bowel transplantation. *Pediatr Infect Dis J* 2012;**31**:700–4.
77. Forgie S, Johnstone J, Durand J, et al. Outbreaks of vancomycin-resistant *Enterococcus faecium* in acute care pediatric hospitals. *Can J Infect Dis Med Microbiol* 2009;**20**:e13–14.
78. Fossati M, Cappelli B, Biral E, et al. Fatal vancomycin- and linezolid-resistant *Enterococcus faecium* sepsis in a child undergoing allogeneic haematopoietic stem cell transplantation for beta-thalassemia major. *J Med Microbiol* 2010;**59**:839–42.
98. Haas EJ, Zaoutis TE, Prasad P, et al. Risk factors and outcomes for vancomycin-resistant enterococcus bloodstream infection in children. *Infect Control Hosp Epidemiol* 2010;**31**:1038–42.
99. Hammoud MS, Al-Taiar A, Thalib L, et al. Incidence, aetiology, and resistance of late-onset neonatal sepsis: a five-year prospective study. *J Paediatr Child Health* 2012;**48**:604–9.

101. Hayakawa K, Marchaim D, Martin ET, et al. Comparison of the clinical characteristics and outcomes associated with vancomycin-resistant *Enterococcus faecalis* and vancomycin-resistant *E. faecium* bacteremia. *Antimicrob Agents Chemother* 2012;**56**:2452–8.
113. Jaspan HB, Brothers AW, Campbell AJ, et al. Multidrug-resistant *Enterococcus faecium* meningitis in a toddler: characterization of the organism and successful treatment with intraventricular daptomycin and intravenous tigecycline. *Pediatr Infect Dis J* 2010;**29**:379–81.
130. Kim YG, Shaw MH, Warner N, et al. Cutting edge: Crohn's disease-associated Nod2 mutation limits production of proinflammatory cytokines to protect the host from *Enterococcus faecalis*-induced lethality. *J Immunol* 2011;**187**:2849–52.
135. Kosack A, Riedel E, Kiehn TE, et al. Vancomycin-resistant enterococcus in pediatric oncology patients: an analysis of potential consequences of colonization and infection. *Pediatr Blood Cancer* 2009;**52**:300–2.
149. Lopez Marti MG, Jhaveri R. Bacteremia caused by an *Enterococcus faecalis* isolate with high-level linezolid resistance in a teenager with Crohn's disease. *Pediatr Infect Dis J* 2009;**28**:663–4.
158. Marcus N, Ashkenazi S, Samra Z, et al. Community-acquired enterococcal urinary tract infections in hospitalized children. *Pediatr Nephrol* 2012;**27**:109–14.
162. McKinnell JA, Knuz DF, Chamot E, et al. Association between vancomycin-resistant enterococci bacteremia and ceftriaxone usage. *Infect Control Hosp Epidemiol* 2012;**33**:718–24.
168. Mendes RE, Sader HS, Farrell DJ, Jones RN. Worldwide appraisal and update (2010) of telavancin activity tested against a collection of Gram-positive clinical pathogens from five continents. *Antimicrob Agents Chemother* 2012;**56**:2999–3004.
169. Milstone AM, Maragakis LL, Carroll KC, Perl TM. Targeted surveillance to identify children colonized with vancomycin-resistant enterococcus in the pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2010;**31**:95–8.
189. Nafedy-Hego H, Elgendy H, Moghazy WE, et al. Patterns of bacterial and fungal infections in the first 3 months after pediatric living donor liver transplantation: an 11-year single-center experience. *Liver Transpl* 2011;**17**:976–84.
196. Nolan SM, Gerber JS, Zaoutis T, et al. Outbreak of vancomycin-resistant enterococcus colonization among pediatric oncology patients. *Infect Control Hosp Epidemiol* 2009;**30**:338–45.
211. Pusch T, Kemp D, Trevino S, et al. Controlling outbreak of vancomycin-resistant *Enterococcus faecium* among infants caused by an endemic strain in adult inpatients. *Am J Infect Control* 2013;**41**:51–6.
221. Rivera AM, Boucher HW. Current concepts in antimicrobial therapy against select Gram-positive organisms: methicillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, and vancomycin-resistant enterococci. *Mayo Clin Proc* 2011;**86**:1230–42.
225. Sader HS, Moet GJ, Farrell DJ, Jones RN. Antimicrobial susceptibility of daptomycin and comparator agents tested against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: trend analysis of a 6-year period in U.S. medical centers (2005-2010). *Diag Microbiol Infect Dis* 2011;**70**:412–16.
251. Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). *Policy statement on antimicrobial stewardship*. http://www.jstor.org/stable/10.1086/665010.
257. Sutherland SM, Alexander SR, Feneberg R, et al. International Pediatric Peritonitis Registry: enterococcal peritonitis in children receiving chronic peritoneal dialysis. *Nephrol Dial Transplant* 2010;**25**:4048–54.
268. Twilla JD, Finch CK, Usery JB, et al. Vancomycin-resistant *Enterococcus* bacteremia: an evaluation of treatment with linezolid or daptomycin. *J Hosp Med* 2012;**7**:243–8.
286. Yilmaz A, Dalgic N, Musluman M, et al. Linezolid treatment of shunt-related cerebrospinal fluid infections in children. *J Neurosurg Pediatr* 2010;**5**:443–8.

The full reference list for this chapter is available at expertconsult.com.

VIRIDANS STREPTOCOCCAL INFECTIONS

The group of *Streptococcus* species characterized as viridans, alpha-hemolytic, or oral streptococci is ubiquitously present on the human oral mucosa. These organisms are important pathogens in children and adults alike; they cause infections ranging from caries and bacterial endocarditis in immunocompetent hosts to fatal sepsis in neutropenic persons.

None of the terms applied to this group is entirely satisfactory. Not all members of the alpha-hemolytic streptococci are alpha-hemolytic, some being gamma-hemolytic (nonhemolytic) or even beta-hemolytic. Further, *Streptococcus pneumoniae*, which is alpha-hemolytic, is not considered a member of the viridans group. The term "viridans streptococci" is likewise inadequate, because "viridans," derived from the Latin *viridis* (green), refers to the sheen caused by partial hemolysis around alpha-hemolytic colonies on sheep blood agar. The term "oral streptococci" circumvents the dilemma of outliers in the hemolytic classification schema; however, viridans streptococci are found in sites other than the oral cavity, and nonviridans streptococcal species are frequently present in the oral cavity. In accordance with the American Society for Microbiology's most recent guidance,[256] the term "viridans streptococci" is used here for this diverse group of bacteria, with the understanding that member organisms are typically but not invariably alpha-hemolytic. We further note that viridans refers not to a species of streptococci but rather to a group of species, notwithstanding erroneous references to *Streptococcus viridans*.[278]

Streptococci have been reclassified on the basis of molecular and genetic studies[62,256]; however, this reclassification may cause clinical confusion for the short term. For example, certain small-colony beta-hemolytic streptococci, including some in Lancefield group A, are now considered to be viridans streptococci. The hope is that classification based on molecular similarities will eventually lead to a clearer understanding of the infectious diseases associated with these organisms. One should recognize, however, that the current knowledge of viridans streptococcal infections is based predominantly on studies of the alpha-hemolytic members of the viridans group.

MICROBIOLOGY

Streptococci are gram-positive, catalase-negative bacteria that are spherical or ovoid and less than 2 μm in diameter. They are facultatively anaerobic and nonmotile and do

REFERENCES

Enterococcal Infections

1. Alminrante B, Tornos M, Gurgui M, et al. Prognosis of enterococcal endocarditis. *Rev Infect Dis* 1991;**13**:1248–9.
2. Anastasiou DM, Thorne GM, Luperchio SA, Alder JD. In vitro activity of daptomycin against clinical isolates with reduced susceptibilities to linezolid and quinupristin-dalfopristin. *Int J Antimicrob Agents* 2006;**28**:385–8.
3. Andrewes FW, Horder TJ. A study of the streptococci pathogenic for man. *Lancet* 1906;**2**:708–13.
4. Arias CA, Murray BE. *Enterococcus* species, *Streptococcus bovis* group and *Leuconostoc* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas and Bennett's principles and practices of infectious diseases*. 7th ed. Philadelphia: Elsevier; 2010. p. 2643–53.
5. Armstrong D, Neu H, Peterson LR, et al. The prospects of treatment failure in the chemotherapy of infectious diseases in the 1990s. *Microb Drug Resist* 1995;**1**:1–4.
6. Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* 1993;**37**: 1563–71.
7. Ashkenazi S, Even-Tov S, Samra Z, et al. Uropathogens of various childhood populations and their antibiotic susceptibility. *Pediatr Infect Dis J* 1991;**10**:742–6.
8. Ashkenazi-Hoffnung L, Livni G, Amir J, Bilavsky E. Serious bacterial infections in hospitalized febrile infants aged 90 days or younger: the traditional combination of ampicillin and gentamicin is still appropriate. *Scand J Infect Dis* 2011;**43**:489–94.
9. Babcock HM, Ritchie DJ, Christiansen E, et al. Successful treatment of vancomycin-resistant *Enterococcus* endocarditis with oral linezolid. *Clin Infect Dis* 2001;**32**:1373–5.
10. Bhavnani SM, Drake JA, Forrest A, et al. A nationwide, multicenter, case-control study comparing risk factors, treatment, and outcome for vancomycin-resistant and -susceptible enterococcal bacteremia. *Diagn Microbiol Infect Dis* 2000;**36**:145–58.
11. Bingen EH, Denamur E, Lambert-Zechovsky NY. Evidence for the genetic unrelatedness of nosocomial vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. *J Clin Microbiol* 1991;**29**:1888.
12. Bingen E, Lambert-Zechovsky N, Mariani-Kurkdjian P, et al. Bacteremia caused by a vancomycin-resistant enterococcus. *Pediatr Infect Dis J* 1989;**8**:475–6.
13. Birmingham MC, Rayner CR, Meagher AK, et al. Linezolid for the treatment of multidrug-resistant, Gram-positive infections: experience from a compassionate use program. *Clin Infect Dis* 2003;**36**:159–68.
14. Bisno AL, Dismukes WE, Durack DT, et al. Antimicrobial treatment of infective endocarditis due to viridans streptococci, enterococci, and staphylococci. *JAMA* 1989;**261**:1471–7.
15. Bonadio WA. Group D streptococcal bacteremia in children. *Clin Pediatr (Phila)* 1993;**32**:20–4.
16. Bonora MG, Ligozzi M, Luzzani A, et al. Emergence of linezolid resistance in *Enterococcus faecium* not dependent on linezolid treatment. *Eur J Clin Microbiol Infect Dis* 2006;**25**:197–8.
17. Bostic GD, Perri MB, Thal LA, et al. Comparative in vitro and bactericidal activity of oxazolidinone antibiotics against multidrug-resistant enterococci. *Diagn Microbiol Infect Dis* 1998; **30**:109–12.
18. Boulanger JM, Ford-Jones EL, Matlow AG. Enterococcal bacteremia in a pediatric institution: a four year review. *Rev Infect Dis* 1991;**13**:847.
19. Boyce JM, Opal SM, Chow JW. Outbreak of multi-drug resistant *Enterococcus faecium* with transferable vanB class vancomycin resistance. *J Clin Microbiol* 1994;**32**:1148–53.
20. Boyce JM, Opal SM, Potter-Bynoe G, et al. Emergence and nosocomial transmission of ampicillin-resistant enterococci. *Antimicrob Agents Chemother* 1992;**36**:1032–9.
21. Brown AE, de Lancastre H, Henning K, et al. *Epidemic nosocomial vancomycin-resistant* Enterococcus faecium *(VREF) on a pediatric oncology unit*. Presented at the 33rd Annual Meeting of the Infectious Diseases Society of America, San Francisco, September 16-18, 1995.
22. Bush L, Calman J, Cherney CL, et al. High-level penicillin resistance among isolates of enterococci: implications for treatment of enterococcal infections. *Ann Intern Med* 1989;**110**: 515–20.
23. Butler KM. Enterococcal infection in children. *Semin Pediatr Infect Dis* 2006;**17**:128–39.
24. Calderwood SA, Wennersten C, Moellering Jr RC. Resistance to six aminoglycosidic aminocyclitol antibiotics among enterococci: prevalence, evolution, and relationship to synergism with penicillin. *Antimicrob Agents Chemother* 1977;**12**:401–5.
25. Cai Y, Wang R, Liang B, et al. Systematic review and meta-analysis of the effectiveness and safety of tigecycline for treatment of infectious disease. *Antimicrob Agents Chemother* 2011;**55**: 1162–72.
26. Carniol K, Gilmore MS. Signal transduction, quorum-sensing, and extracellular protease activity in *Enterococcus faecalis* biofilm formation. *J Bacteriol* 2004;**186**:8161–3.
27. Carpenter CF, Chambers HF. Daptomycin: Another novel agent for treating infections due to drug-resistant gram-positive pathogens. *Clin Infect Dis* 2004;**38**:994–1000.
28. Centers for Disease Control and Prevention. Nosocomial enterococci resistant to vancomycin-United States, 1989-1993. *MMWR Morb Mortal Wkly Rep* 1993;**42**:597–9.
29. Centers for Disease Control and Prevention. *Staphylococcus aureus* resistant to vancomycin: United States, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:565–7.
30. Centers for Disease Control and Prevention. Brief report: vancomycin-resistant *Staphylococcus aureus:* New York, 2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:322–33.
31. Cercenado E, Eliopoulos GM, Wennersten CB, et al. Absence of synergistic activity between ampicillin and vancomycin against highly vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 1992;**36**:2201–3.
32. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. *Clin Microbiol Rev* 2000;**13**:686–707.
33. Chenoweth CE, Robinson KA, Schaberg DR. Efficacy of ampicillin versus trimethoprim-sulfamethoxazole in a mouse model of lethal enterococcal peritonitis. *Antimicrob Agents Chemother* 1990;**34**:1800–2.
34. Chien JW, Kucia ML, Salata RA. Use of linezolid, an oxazolidinone, in the treatment of multidrug-resistant gram-positive bacterial infections. *Clin Infect Dis* 2000;**30**:146–51.
35. Chirurgi VA, Oster SE, Goldberg AA, et al. Ampicillin-resistant *Enterococcus raffinosus* in an acute-care hospital: case-control study and antimicrobial susceptibilities. *J Clin Microbiol* 1991;**29**:2663–5.
36. Chow JW. Aminoglycoside resistance in enterococci. *Clin Infect Dis* 2000;**31**:586–9.
37. Chow JW, Davidson A, Sanford E 3rd et al. Superinfection with *Enterococcus faecalis* during quinupristin/dalfopristin therapy. *Clin Infect Dis* 1997;**24**:91–2.
38. Chow JW, Thal LA, Perri MB, et al. Plasmid-associated hemolysin and aggregation substance production contributes to virulence in experimental enterococcal endocarditis. *Antimicrob Agents Chemother* 1993;**37**:2474–7.
39. Chow JW, Zervos MJ, Lerner SA, et al. A novel gentamicin resistance gene in *Enterococcus*. *Antimicrob Agents Chemother* 1997;**41**: 511–14.
40. Christenson JC, Korgenski EK, Jenkins E, et al. Detection of vancomycin-resistant enterococci colonization in a children's hospital. *Am J Infect Control* 1998;**26**:569–71.
41. Christie C, Hammond J, Reising S, et al. Clinical and molecular epidemiology of enterococcal bacteremia in a pediatric teaching hospital. *J Pediatr* 1994;**125**:392–9.
42. Clark NC, Cooksey RC, Hill BC, et al. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *Antimicrob Agents Chemother* 1993;**37**:2311–17.
43. Clemett D, Markham A. Linezolid. *Drugs* 2000;**59**:815–27.
44. Clinical and Laboratory Standards Institute. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard*. 8th ed. Wayne, Pa: Clinical and Laboratory Standards Institute; 2009. http://ww.clsi.org/source/orders/free/m07-a8.pdf.
45. Coburn PS, Pillar CM, Jett BD, et al. *Enterococcus faecalis* senses target cells and in response expresses cytolysin. *Science* 2004;**306**: 2270–2.
46. Cohen M. Epidemiology of drug resistance: implications for a post-antibiotic era. *Science* 1992;**257**:1050–5.
47. Collins LA, Malanoski GJ, Eliopoulos GM, et al. In vitro activity of RP59500, an injectable streptogramin antibiotic, against vancomycin-resistant gram-positive organisms. *Antimicrob Agents Chemother* 1993;**37**:598–601.

48. Coudron PE, Markowitz SM, Wong ES. Isolation of a beta-lactamase–producing, aminoglycoside-resistant strain of *Enterococcus faecium*. *Antimicrob Agents Chemother* 1992;**36**:1125–6.
49. Coudron PE, Mayhall CG, Facklam RR. *Streptococcus faecium* outbreak in a neonatal intensive care unit. *J Clin Microbiol* 1984;**20**:1044.
50. Cox CR, Coburn PS, Gilmore MS. Enterococcal cytolysin: a novel two component peptide system that serves as a bacterial defense against eukaryotic and prokaryotic cells. *Curr Protein Pept Sci* 2005;**6**:77–84.
51. Crank CW, Scheetz MH, Brielmaier B, et al. Comparison of outcomes from daptomycin or linezolid treatment for vancomycin-resistant enterococcal bloodstream infection: a retrospective, multicenter, cohort study. *Clin Ther* 2010;**32**:1713–19.
52. Dajani AS. Infective endocarditis. In: Kaplan SL, editor. *Current therapy in pediatric infectious diseases*. 3rd ed. St. Louis: Mosby–Year Book; 1993. p. 129–33.
53. Das I, Gray J. Enterococcal bacteremia in children: a review of seventy-five episodes in a pediatric hospital. *Pediatr Infect Dis J* 1998;**17**:1154–8.
54. Davies H, Jones E, Sheng R, et al. Nosocomial urinary tract infections at a pediatric hospital. *Pediatr Infect Dis J* 1992;**11**:349–54.
55. DiazGranados CA, Jernigan JA. Impact of vancomycin resistance on mortality among patients with neutropenia and enterococcal bloodstream infection. *J Infect Dis* 2005;**191**:588–95.
56. DiazGranados CA, Zimmer SM, Klein M, Jernigan JA. Comparison of mortality associated with vancomycin-resistant and vancomycin-susceptible enterococcal bloodstream infections: a meta-analysis. *Clin Infect Dis* 2005;**41**:327–33.
57. Deshpande LM, Fritsche TR, Moet GJ, et al. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 2007;**58**:163–70.
58. Dobson SRM, Baker CJ. Enterococcal sepsis in neonates: features by age of onset and occurrence of focal infection. *Pediatrics* 1990;**85**:165.
59. Donabedian S, Chow JW, Shales DM, et al. DNA hybridization and contour-clamped homogeneous electric field electrophoresis for identification of enterococci to the species level. *J Clin Microbiol* 1995;**33**:141–5.
60. Donskey CJ, Chowdhry TK, Hecker MT, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *N Engl J Med* 2000;**343**:1925–32.
61. Donskey CJ, Hanrahan JA, Hutton RA, et al. Effect of parenteral antibiotic administration on the establishment of colonization with vancomycin-resistant *Enterococcus faecium* in the mouse gastrointestinal tract. *J Infect Dis* 2000;**181**:1830–3.
62. Drews SJ, Richardson SE, Wray R, et al. An outbreak of vancomycin-resistant *Enterococcus faecium* in an acute care pediatric hospital: lessons from environmental screening and a case-control study. *Can J Infect Dis Med Microbiol* 2008;**19**:233–6.
63. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. *J Clin Microbiol* 1995;**33**:24–7.
64. Edmond MB, Ober JF, Weinbaum DL, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. *Clin Infect Dis* 1995;**20**:1126–33.
65. Edmond MB, Wallace SE, McClish DK, et al. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis* 1999;**29**:239–44.
66. Eliopoulos GM, Thauvin-Eliopoulos C, Moellering Jr RC. Contribution of animal models in the search for effective therapy for endocarditis due to enterococci with high-level resistance to gentamicin. *Clin Infect Dis* 1992;**15**:58–62.
67. Eliopoulos GM, Wennersten CB, Gold HS, et al. In vitro activities in new oxazolidinone antimicrobial agents against enterococci. *Antimicrob Agents Chemother* 1996;**40**:1745–7.
68. Eliopoulos GM, Wennersten CB, Gold HS, et al. Characterization of vancomycin-resistant *Enterococcus faecium* isolates from the United States and their susceptibility in vitro to dalfopristin-quinupristin. *Antimicrob Agents Chemother* 1998;**42**:1088–92.
69. Facklam RR, Collins MD. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. *J Clin Microbiol* 1989;**27**:731–4.
70. Facklam RR, Sahm DR. *Enterococcus*. In: Murray PR, Baron EJ, Pfaller MA, et al, editors. *Manual of clinical microbiology*. 6th ed. Washington, DC: American Society for Microbiology; 1995. p. 308–14.
71. Facklam R, Pigott N, Franklin R, et al. Evaluation of three disk tests for identification of enterococci, leuconostocs, and pediococci. *J Clin Microbiol* 1995;**33**:885–7.
72. Fasola EL, Moody JA, Shanholtzer CJ, et al. Bactericidal action of gentamicin against enterococci that are sensitive, or exhibit low- or high-level resistance to gentamicin. *Diagn Microbiol Infect Dis* 1994;**19**:57–60.
73. Felmingham D, Wilson AP, Quintant AL, et al. *Enterococcus* species in urinary tract infection. *Clin Infect Dis* 1992;**15**:295–301.
74. Flamm RK, Farrell DJ, Mendes RE, et al. LEADER Surveillance program results for 2010: an activity and spectrum analysis of linezolid using 6801 clinical isolates from the United States (61 medical centers). *Diagn Microbiol Infect Dis* 2012;**74**:54–61.
75. Florescu DF, Qiu F, Langnas AN, et al. Bloodstream infections during the first year after pediatric small bowel transplantation. *Pediatr Infect Dis J* 2012;**31**:700–4.
76. Fontana R, Amalfitano G, Rossi L, et al. Mechanisms of resistance to growth inhibition and killing by β-lactam antibiotics in enterococci. *Clin Infect Dis* 1992;**15**:486–9.
77. Forgie S, Johnstone J, Durand J, et al. Outbreaks of vancomycin-resistant *Enterococcus faecium* in acute care pediatric hospitals. *Can J Infect Dis Med Microbiol* 2009;**20**:e13–14.
78. Fossati M, Cappelli B, Biral E, et al. Fatal vancomycin- and linezolid-resistant *Enterococcus faecium* sepsis in a child undergoing allogeneic haematopoietic stem cell transplantation for beta-thalassemia major. *J Med Microbiol* 2010;**59**:839–42.
79. Fraimow HS, Jungkind DL, Lander DW, et al. Urinary tract infection with an *Enterococcus faecalis* isolate that requires vancomycin for growth. *Ann Intern Med* 1994;**121**:22–6.
80. French G. Safety and tolerability of linezolid. *J Antimicrob Chemother* 2003;**51**(suppl. S2):ii45–53.
81. Fuchs PC, Barry AL, Brown SD. In vitro bactericidal activity of daptomycin against staphylococci. *J Antimicrob Chemother* 2002;**49**:467–70.
82. Furlong WB, Rakowski TA. Therapy with RP 59500 (quinupristin/dalfopristin) for prosthetic valve endocarditis due to enterococci with VanA/VanB resistance patterns. *Clin Infect Dis* 1997;**25**:163–4.
83. Garcia-Garrote F, Cercenado E, Bouza E. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. *J Clin Microbiol* 2000;**38**:2108–11.
84. Gladstone IJ, Ehrenkranz RA, Edberg SC, et al. A ten-year review of neonatal sepsis and comparison with the previous fifty-year experience. *Pediatr Infect Dis J* 1990;**9**:819–25.
85. Gold H. Vancomycin-resistant enterococci: mechanisms and clinical observations. *Clin Infect Dis* 2001;**33**:210–19.
86. Gonzales RD, Schreckenberger PC, Graham MB, et al. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. *Lancet* 2001;**357**:1179.
87. Goodhard GL. In vivo vs. in vitro susceptibility of *Enterococcus* to trimethoprim-sulfamethoxazole. *JAMA* 1984;**252**:2748–9.
88. Gorbach SL. Intraabdominal infections. *Clin Infect Dis* 1993;**17**:961–7.
89. Gordon S, Swenson JM, Hill BC, et al. Antimicrobial susceptibility patterns of common and unusual species of enterococci causing infections in the United States: Enterococcal Study Group. *J Clin Microbiol* 1992;**30**:2373–8.
90. Graninger W, Ragette R. Nosocomial bacteremia due to *Enterococcus faecalis* without endocarditis. *Clin Infect Dis* 1992;**15**:49–57.
91. Gray JW, George RH. Experience of vancomycin-resistant enterococci in a children's hospital. *J Hosp Infect* 2000;**45**:11–18.
92. Gray JW, Darbyshire PJ, Beath SV, et al. Experience with quinupristin/dalfopristin in treating infections with vancomycin-resistant *Enterococcus faecium* in children. *Pediatr Infect Dis J* 2000;**19**:234–8.
93. Gray JW, Pedler S, Kernahan J, et al. Enterococcal superinfection in paediatric oncology patients treated with imipenem. *Lancet* 1992;**13**:1487–8.
94. Grayson ML, Eliopoulos GM, Wennersten CB, et al. Increasing resistance to beta-lactam antibiotics among clinical isolates of

Enterococcus faecium: a 22-year review at one institution. *Antimicrob Agents Chemother* 1991;**35**:2180–4.
95. Grayson ML, Thauvin-Eliopoulos C, Eliopoulos GM, et al. Failure of trimethoprim-sulfamethoxazole therapy in experimental enterococcal endocarditis. *Antimicrob Agents Chemother* 1990; **34**:1792–4.
96. Green M, Shlaes JH, Barbadora K, et al. Bacteremia due to vancomycin-dependent *Enterococcus faecium*. *Clin Infect Dis* 1995; **20**:712–14.
97. Gullberg RM, Homann SR, Phair JP. Enterococcal bacteremia: analysis of 75 episodes. *Rev Infect Dis* 1989;**11**:74–85.
98. Haas EJ, Zaoutis TE, Prasad P, et al. Risk factors and outcomes for vancomycin-resistant enterococcus bloodstream infection in children. *Infect Control Hosp Epidemiol* 2010;**31**:1038–42.
99. Hammoud MS, Al-Taiar A, Thalib L, et al. Incidence, aetiology, and resistance of late-onset neonatal sepsis: a five-year prospective study. *J Paediatr Child Health* 2012;**48**:604–9.
100. Handwerger S, Raucher B, Altarac D, et al. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin and gentamicin. *Clin Infect Dis* 1993;**16**:750–5.
101. Hayakawa K, Marchaim D, Martin ET, et al. Comparison of the clinical characteristics and outcomes associated with vancomycin-resistant *Enterococcus faecalis* and vancomycin-resistant *E. faecium* bacteremia. *Antimicrob Agents Chemother* 2012;**56**:2452–8.
102. Hayden MK, Trenholme GM, Schultz JE, et al. In vivo development of teicoplanin resistance in a VanB *Enterococcus faecium* isolate. *J Infect Dis* 1993;**167**:1224–7.
103. Henning KJ, Delencastre H, Eagan J, et al. Vancomycin-resistant *Enterococcus faecium* on a pediatric oncology ward: duration of stool shedding and incidence of clinical infection. *Pediatr Infect Dis J* 1996;**15**:848–54.
104. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no predisposing factors. *JAMA* 1998;**279**:593–8.
105. Herrero IA, Issa NC, Patel R. Nosocomial spread of linezolid-resistant vancomycin-resistant *Enterococcus faecium*. *N Engl J Med* 2002;**346**:867–9.
106. Hershberger E, Donabedian S, Konstantinou K, Zervos MJ. Quinupristin-dalfopristin resistance in gram-positive bacteria: mechanism of resistance and epidemiology. *Clin Infect Dis* 2004; **38**:92–8.
107. Hoge CW, Adams J, Buchanan B, et al. Enterococcal bacteremia: to treat or not to treat, a reappraisal. *Rev Infect Dis* 1991;**13**: 600–5.
108. Hook EWI, Roberts RB, Sande MA. Antimicrobial therapy of experimental endocarditis. Antimicrob. *Agents Chemother* 1975;**8**: 564–70.
109. Horodniceanu T, Bougueleret T, El-Solh N, et al. High-level, plasmid-borne resistance to gentamicin in *Streptococcus faecalis* subsp. *zymogenes*. *Antimicrob Agents Chemother* 1979;**16**:686–9.
110. Hunt C, Dionne M, Delorme M, et al. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*: Minnesota and North Dakota, 1997-1999. *MMWR Morb Mortal Wkly Rep* 1999;**48**:707–10.
111. Hunter TH. Use of streptomycin in treatment of bacterial endocarditis. *Am J Med* 1947;**2**:436–42.
112. Jarvis WR, Martone WJ. Predominant pathogens in hospital infections. *J Antimicrob Chemother* 1992;**29**(suppl. A):19–24.
113. Jaspan HB, Brothers AW, Campbell AJ, et al. Multidrug-resistant *Enterococcus faecium* meningitis in a toddler: characterization of the organism and successful treatment with intraventricular daptomycin and intravenous tigecycline. *Pediatr Infect Dis J* 2010; **29**:379–81.
114. Javaheri M, Khurana RN, O'Hearn TM, et al. Linezolid-induced optic neuropathy: a mitochondrial disorder? *Br J Ophthalmol* 2007;**91**:111–15.
115. Jett BD, Huycke MM, Gilmore MS. Virulence of enterococci. *Clin Microbiol Rev* 1994;**7**:462–78.
116. Johnson AP. The pathogenicity of enterococci. *J Antimicrob Chemother* 1994;**33**:1083–9.
117. Johnson DH, Rosenthal A, Nadas AS. A forty-year review of bacterial endocarditis in infants and children. *Circulation* 1975; **51**:581–8.
118. Johnson DH, Rosenthal A, Nadas AS. Bacterial endocarditis in children under 2 years of age. *Am J Dis Child* 1975;**129**: 183–6.
119. Jones ME, Draghi DC, Thornsberry C, et al. Emerging resistance among bacterial pathogens in the intensive care unit: a European and North American surveillance study (2000-2002). *Ann Clin Microbiol Antimicrob* 2004;**3**:1–11.
120. Jones RN, Erwin ME, Anderson SC. Emerging multiply resistant enterococci among clinical isolates. II. Validation of the e-test to recognize glycopeptide-resistant strains. *Diagn Microbiol Infect Dis* 1995;**21**:95–100.
121. Jones RN, Sader HS, Erwin ME, Anderson SC. Emerging multiply resistant enterococci among clinical isolates. I. Prevalence data from 97 medical center surveillance study in the United States: *Enterococcus* Study Group. *Diagn Microbiol Infect Dis* 1995;**21**:85–93.
122. Jones RN, Turnidge J, Sader HS, et al. *Evolutionary trends in antimicrobial resistance in* E. faecium *bacteremia: Report from the SENTRY Antimicrobial Surveillance Program (1997-2005)*. Abstract C2-208. Presented at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, September 27-30, 2006.
123. Kaatz GW, Seo SM. In vitro activities of oxazolidinone compounds U100592 and U100766 against *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 1996;**40**: 799–801.
124. Kaplan AH, Gilligan PH, Facklam RR. Recovery of resistant enterococci during vancomycin prophylaxis. *J Clin Microbiol* 1988;**26**:1216–18.
125. Kaplan SL, Deville JG, Yogev R, et al. For the Linezolid Pediatric Study Group: Linezolid versus vancomycin for treatment of resistant gram-positive infections in children. *Pediatr Infect Dis J* 2003;**22**:677–85.
126. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
127. Kapur D, Dorsky D, Feingold JM, et al. Incidence and outcome of vancomycin-resistant enterococcal bacteremia following autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant* 2000;**25**:147–52.
128. Karanfil LV, Murphy M, Josephson A. A cluster of vancomycin-resistant *Enterococcus faecium* in an intensive care unit. *Infect Control Hosp Epidemiol* 1993;**13**:195–200.
129. Kaufhold A, Ferrieri P. The microbiologic aspects, including diagnosis, of beta-hemolytic streptococcal and enterococcal infections. *Infect Dis Clin North Am* 1993;**7**:235–56.
130. Kim YG, Shaw MH, Warner N, et al. Cutting edge: Crohn's disease-associated Nod2 mutation limits production of proinflammatory cytokines to protect the host from *Enterococcus faecalis*-induced lethality. *J Immunol* 2011;**187**:2849–52.
131. Kirkpatrick BD, Harrington SM, Smith D, et al. An outbreak of vancomycin-dependent *Enterococcus faecium* in a bone marrow transplant unit. *Clin Infect Dis* 1999;**29**:1268–73.
132. Kirst HA, Thompson DG, Nicas TI. Historical yearly usage of vancomycin. *Antimicrob Agents Chemother* 1998;**42**:1303–4.
133. Klein JO, Marcy SM. Bacterial sepsis and meningitis. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 4th ed. Philadelphia: W. B. Saunders; 1995. p. 835–90.
134. Koorevaar CT, Scherpenzeel PG, Neijens HJ, et al. Childhood meningitis caused by enterococci and viridans streptococci. *Infection* 1992;**20**:118–21.
135. Kosack A, Riedel E, Kiehn TE, et al. Vancomycin-resistant enterococcus in pediatric oncology patients: an analysis of potential consequences of colonization and infection. *Pediatr Blood Cancer* 2009;**52**:300–2.
136. Kreft B, Marre R, Schramm U, et al. Aggregation substance of *Enterococcus faecalis* mediates adhesion to cultured renal tubular cells. *Infect Immun* 1992;**60**:25–30.
137. Krieger JN, Kaiser DL, Wenzel RP. Urinary tract etiology of bloodstream infections in hospitalized patients. *J Infect Dis* 1983;**146**:719–23.
138. Kunin C. Resistance to antimicrobial drugs: a worldwide calamity. *Ann Intern Med* 1993;**118**:557–61.
139. Kvirikadze N, Suseno N, Vescio T, et al. Daptomycin for the treatment of vancomycin-resistant *Enterococcus faecium* bacteremia. *Scand J Infect Dis* 2006;**38**:290–2.
140. Law EJ, Blecher K, Still JM. Enterococcal infections as a cause of mortality and morbidity in patients with burns. *J Burn Care Rehabil* 1994;**15**:236–9.

141. LeClercq R, Derlot E, Duval J, et al. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N Engl J Med* 1988;**319**:157–61.
142. LeClercq R, Dutka-Malen S, Brisson-Noel A, et al. Resistance of enterococci to aminoglycosides and glycopeptides. *Clin Infect Dis* 1992;**15**:495–501.
143. LeClercq R, Dutka-Malen S, Brisson-Noel A, et al. Resistance of enterococci to aminoglycosides and glycopeptides [Letter]. *Clin Infect Dis* 1993;**16**:331.
144. Linden PK, Pasculle AW, McDevitt D, et al. Effect of quinupristin/dalfopristin on the outcome of vancomycin-resistant *Enterococcus faecium* bacteraemia: comparison with a control cohort. *J Antimicrob Chemother* 1997;**39**:145–51.
145. Livornese Jr LL, Dias S, Samel C, et al. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. *Ann Intern Med* 1992;**117**:112–16.
146. Loeffler AM, Drew RH, Perfect JR, et al. Safety and efficacy of quinupristin/dalfopristin for treatment of invasive gram-positive infections in pediatric patients. *Pediatr Infect Dis J* 2002;**21**:950–6.
147. Lohr JA, Donowitz LG, Sadler III JE. Hospital-acquired urinary tract infection. *Pediatrics* 1989;**83**:193–9.
148. Long JK, Choueiri TK, Hall GS, et al. Daptomycin-resistant *Enterococcus faecium* in a patient with acute myeloid leukemia. *Mayo Clin Proc* 2005;**80**:1215–16.
149. Lopez Marti MG, Jhaveri R. Bacteremia caused by an *Enterococcus faecalis* isolate with high-level linezolid resistance in a teenager with Crohn's disease. *Pediatr Infect Dis J* 2009;**28**:663–4.
150. Lososnky G, Wolf A, Schwalbe R, et al. Successful treatment of meningitis due to multiply resistant *Enterococcus faecium* with a combination of intrathecal teicoplanin and intravenous antimicrobial agents. *Clin Infect Dis* 1994;**19**:163–5.
151. Low DE. Quinupristin/dalfopristin: spectrum of activity, pharmacokinetics, and initial clinical experience. *Microb Drug Resist* 1995;**1**:223–34.
152. Low DE, Willey BM, McGeer AJ. Multidrug-resistant enterococci: a threat to the surgical patient. *Am J Surg* 1995;**169**(suppl.):8–12.
153. Low DE, Keller N, Barth A, et al. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 2001;**32**(suppl.):133–45.
154. Luginbuhl LM, Rotbart HA, Facklam RR, et al. Neonatal enterococcal sepsis: case-control study and description of an outbreak. *Pediatr Infect Dis J* 1987;**6**:1022–30.
155. Lynn WA, Clutterbuck E, Want S, et al. Treatment of CAPD-peritonitis due to glycopeptide-resistant *Enterococcus faecium* with quinupristin/dalfopristin. *Lancet* 1994;**344**:1025–6.
156. Maki DG, Agger WA. Enterococcal bacteremia: clinical features, the risk of endocarditis, and management. *Medicine (Baltimore)* 1988;**67**:248–69.
157. Malone DA, Wagner RA, Myers JP, et al. Enterococcal bacteremia in two large community teaching hospitals. *Am J Med* 1986;**81**:601–6.
158. Marcus N, Ashkenazi S, Samra Z, et al. Community-acquired enterococcal urinary tract infections in hospitalized children. *Pediatr Nephrol* 2012;**27**:109–14.
159. Marshall SH, Donskey CJ, Hutton-Thomas R, et al. Gene dosage and linezolid resistance in *Enterococcus faecium* and *Enterococcus faecalis*. *Antimicrob Agents Chemother* 2002;**46**:3334–6.
160. Matsumura S, Simor AE. Treatment of endocarditis due to vancomycin-resistant *Enterococcus faecium* with quinupristin/dalfopristin, doxycycline, and rifampin: a synergistic drug combination. *Clin Infect Dis* 1998;**27**:1554–6.
161. McCarthy A, Victor G, Ramotar K, et al. Risk factors for acquiring ampicillin-resistant enterococci and clinical outcomes at a Canadian tertiary-care hospital. *J Clin Microbiol* 1994;**32**:2671–6.
162. McKinnell JA, Knuz DF, Chamot E, et al. Association between vancomycin-resistant enterococci bacteremia and ceftriaxone usage. *Infect Control Hosp Epidemiol* 2012;**33**:718–24.
163. McNeeley DF, Saint-Louis F, Noel GJ. Neonatal enterococcal bacteremia: an increasingly frequent event with potentially untreatable pathogens. *Pediatr Infect Dis J* 1996;**15**:800–5.
164. McNeil SA, Clark NM, Chandrasekar PH, et al. Successful treatment of vancomycin-resistant *Enterococcus faecium* bacteremia with linezolid after failure of treatment with Synercid (quinupristin/dalfopristin). *Clin Infect Dis* 2000;**30**:403–4.
165. Mederski-Samoraj BD, Murray BE. High-level resistance to gentamicin in clinical isolates of enterococci. *J Infect Dis* 1983;**147**:751–7.
166. Megran DW. Enterococcal endocarditis. *Clin Infect Dis* 1992;**15**:63–71.
167. Meka VG, Gold HS. Antimicrobial resistance to linezolid. *Clin Infect Dis* 2004;**39**:1010–15.
168. Mendes RE, Sader HS, Farrell DJ, Jones RN. Worldwide appraisal and update (2010) of telavancin activity tested against a collection of Gram-positive clinical pathogens from five continents. *Antimicrob Agents Chemother* 2012;**56**:2999–3004.
169. Milstone AM, Maragakis LL, Carroll KC, Perl TM. Targeted surveillance to identify children colonized with vancomycin-resistant enterococcus in the pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2010;**31**:95–8.
170. Moellering Jr RC. Emergence of *Enterococcus* as a significant pathogen. *Clin Infect Dis* 1992;**14**:1173–6.
171. Moellering Jr RC. *Enterococcus* species, *Streptococcus bovis*, and *Leuconostoc* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 6th ed., Philadelphia: Churchill Livingstone; 2005.
172. Moellering Jr RC, Krogstad DJ. Antibiotic resistance in enterococci. In: Schlessinger D, editor. *Microbiology: 1979*. Washington, D.C.: American Society for Microbiology; 1979. p. 293–8.
173. Moellering Jr RC, Weinberg AN. Studies on antibiotic synergism against enterococci. II. Effect of various antibiotics on the uptake of C14-labeled streptomycin by enterococci. *J Clin Invest* 1971;**50**:2580–4.
174. Moellering Jr RC, Wennersten C, Medrek T. Prevalence of high-level resistance to aminoglycosides in clinical isolates of enterococci. *Antimicrob Agents Chemother* 1970;**1**:335–40.
175. Moellering Jr RC, Linden PK, Reinhardt J, et al. The efficacy and safety of quinupristin/dalfopristin for the treatment of infections caused by vancomycin-resistant *Enterococcus faecium*: Synercid Emergency-Use Study Group. *J Antimicrob Chemother* 1999;**44**:251–61.
176. Mondy KE, Shannon W, Mundy LM. Evaluation of zinc bacitracin capsules versus placebo for enteric eradication of vancomycin-resistant *Enterococcus faecium*. *Clin Infect Dis* 2001;**33**:473–6.
177. Montecalvo MA, Horowitz H, Gedris C. Outbreak of vancomycin-, ampicillin-, and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. *Antimicrob Agents Chemother* 1994;**38**:1363–7.
178. Moreno F, Grota P, Crisp C, et al. Clinical and molecular epidemiology of vancomycin-resistant *Enterococcus faecium* during its emergence in a city in southern Texas. *Clin Infect Dis* 1995;**21**:1234–7.
179. Morris Jr JG, Shay DK, Hebden JN, et al. Enterococci resistant to multiple antimicrobial agents, including vancomycin. *Ann Intern Med* 1995;**123**:250–9.
180. Morrison Jr AJ, Wenzel RP. Nosocomial urinary tract infections due to enterococcus: ten years' experience at a university hospital. *Arch Intern Med* 1986;**146**:1549–51.
181. Munoz-Price LS, Lolans K, Quinn JP. Emergence of resistance to daptomycin during treatment of vancomycin-resistant *Enterococcus faecalis* infection. *Clin Infect Dis* 2005;**41**:565–6.
182. Murray BE. The life and times of the enterococcus. *Clin Microbiol Rev* 1990;**3**:46–65.
183. Murray BE. Beta-lactamase–producing enterococci. *Antimicrob Agents Chemother* 1992;**36**:2355–9.
184. Murray BE. Editorial response: what can we do about vancomycin-resistant enterococci. *Clin Infect Dis* 1995;**20**:1134–6.
185. Murray BE. Diversity among multidrug-resistant enterococci. *Emerg Infect Dis* 1998;**4**:37–47.
186. Murray BE. Vancomycin-resistant enterococcal infections. *N Engl J Med* 2000;**342**:710–21.
187. Murray BE, Mederski-Samoraj B. Transferable beta-lactamase: a new mechanism for in vitro penicillin resistance in *Streptococcus faecalis*. *J Clin Invest* 1983;**72**:1168.
188. Nachman SA, Verma R, Egnor M. Vancomycin-resistant *Enterococcus faecium* shunt infection in an infant: an antibiotic cure. *Microb Drug Resist* 1995;**1**:95–6.
189. Nafedy-Hego H, Elgendy H, Moghazy WE, et al. Patterns of bacterial and fungal infections in the first 3 months after pediatric

living donor liver transplantation: an 11-year single-center experience. *Liver Transpl* 2011;**17**:976–84.
190. Nagai K, Yuge K, Ono E, et al. *Enterococcus faecium* meningitis in a child. *Pediatr Infect Dis J* 1994;**13**:1016–17.
191. Nauschuetz WF, Trevino SB, Harrison LS, et al. *Enterococcus casseliflavus* as an agent of nosocomial bloodstream infections. *Med Microbiol Lett* 1993;**2**:102–8.
192. Nichols RL, Muzik AC. Enterococcal infections in surgical patients: The mystery continues. *Clin Infect Dis* 1992;**15**:72–6.
193. Nicoletti G, Stefain S. Enterococci: Susceptibility patterns and therapeutic options. *Eur J Clin Microbiol Infect Dis* 1995;**14**(suppl.): 33–7.
194. Noble CJ. Carriage of group D streptococci in the human bowel. *J Clin Pathol* 1978;**31**:1182–6.
195. Noble WC, Virani Z, Cree R. Cotransfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992;**93**:195–8.
196. Nolan SM, Gerber JS, Zaoutis T, et al. Outbreak of vancomycin-resistant enterococcus colonization among pediatric oncology patients. *Infect Control Hosp Epidemiol* 2009;**30**:338–45.
197. Norris AH, Reilly JP, Edelstein PH, et al. Chloramphenicol for the treatment of vancomycin-resistant enterococcal infections. *Clin Infect Dis* 1995;**20**:1137–44.
198. Noskin GA, Siddiqui F, Stosor V, et al. In vitro activities of linezolid against important gram-positive bacterial pathogens including vancomycin-resistant enterococci. *Antimicrob Agents Chemother* 1999;**43**:2059–62.
199. Noskin GA, Siddiqui F, Stosor V, et al. Successful treatment of persistent vancomycin-resistant *Enterococcus faecium* bacteremia with linezolid and gentamicin. *Clin Infect Dis* 1999;**28**:689–90.
200. Onderdonk AB, Bartlett JG, Louie TJ, et al. Microbial synergy in experimental intra-abdominal abscess. *Infect Immun* 1976;**12**: 22–6.
201. Ostrowsky BE, Trick WE, Sohn AH, et al. Control of vancomycin-resistant enterococcus in health care facilities in a region. *N Engl J Med* 2001;**344**:1427–33.
202. Patel R, Allen SL, Manahan JM, et al. Natural history of vancomycin-resistant enterococcal colonization in liver and kidney transplant recipients. *Liver Transpl Surg* 2001;**7**:27–31.
203. Patel R, Keating MR, Cockerill FR, et al. Bacteremia due to *Enterococcus avium*. *Clin Infect Dis* 1993;**17**:1006–11.
204. Patterson JE, Zervos MJ. High-level gentamicin resistance in enterococcus: microbiology, genetic basis, and epidemiology. *Rev Infect Dis* 1990;**12**:644.
205. Patterson JE, Sweeney AH, Simms M, et al. An analysis of 110 serious enterococcal infections, epidemiology, antibiotic susceptibility, and outcome. *Medicine (Baltimore)* 1995;**74**:191–200.
206. Patton WN, Bienz N, Franklin IM, et al. Enterococcal meningitis in an HIV positive hemophilic patient. *J Clin Pathol* 1991;**44**: 608–9.
207. Perez Mato S, Robinson S, Begue RE. Vancomycin-resistant *Enterococcus faecium* meningitis successfully treated with chloramphenicol. *Pediatr Infect Dis J* 1999;**18**:483–4.
208. Poutsiaka DD, Skiffington S, Miller KB, et al. Daptomycin in the treatment of vancomycin-resistant *Enterococcus faecium* bacteremia in neutropenic patients. *J Infect* 2007;**54**:567–71.
209. Presterl E, Graninger W, Georgopoulos A. The efficacy of teicoplanin in the treatment of endocarditis caused by gram-positive bacteria. *J Antimicrob Chemother* 1993;**31**:755–66.
210. Pultz NJ, Stiefel U, Subramanyan S, et al. Mechanisms by which anaerobic microbiota inhibit the establishment in mice of intestinal colonization by vancomycin-resistant *Enterococcus*. *J Infect Dis* 2005;**191**:949–56.
211. Pusch T, Kemp D, Trevino S, et al. Controlling outbreak of vancomycin-resistant Enterococcus faecium among infants caused by an endemic strain in adult inpatients. *Am J Infect Control* 2013;**41**:51–6.
212. Raymond NJ, Henry J, Workowski KA. Enterococcal arthritis: case report and review. *Clin Infect Dis* 1995;**21**:516–22.
213. Recommendations for preventing the spread of vancomycin resistance: recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). *MMWR Recomm Rep* 1995;**44**(RR-12):1–13.
214. Rhinehart E, Smith NE, Wennersten C, et al. Rapid dissemination of beta-lactamase–producing, aminoglycoside-resistant *Enterococcus faecalis* among patients and staff on an infant-toddler surgical ward. *N Engl J Med* 1990;**323**:1814–18.
215. Ricaurte JC, Boucher HW, Turett GS, et al. Chloramphenicol treatment for vancomycin-resistant *Enterococcus faecium* bacteremia. *Clin Microbiol Infect* 2001;**7**:17–21.
216. Rice LB. Emergence of vancomycin-resistant enterococci. *Emerg Infect Dis* 2001;**7**:183–7.
217. Rice LB, Shlaes DM. Vancomycin resistance in the enterococcus. *Pediatr Clin North Am* 1995;**42**:601–18.
218. Rice LB, Calderwood SB, Eliopoulos GM, et al. Enterococcal endocarditis: a comparison of prosthetic and native valve disease. *Rev Infect Dis* 1991;**13**:1–7.
219. Rice LB, Carias LL, Hutton-Thomas R, et al. Penicillin-binding protein 5 and expression of ampicillin resistance in *Enterococcus faecium*. *Antimicrob Agents Chemother* 2001;**45**:1480–6.
220. Richards MJ, Edwards JR, Culver DH, et al. For the National Nosocomial Infections Surveillance System: nosocomial infections in pediatric intensive care units in the United States. *Pediatrics* 1999;**103**:e39.
221. Rivera AM, Boucher HW. Current concepts in antimicrobial therapy against select Gram-positive organisms: methicillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, and vancomycin-resistant enterococci. *Mayo Clin Proc* 2011;**86**: 1230–42.
222. Rubin LG, Tucci V, Cercenado E, et al. Vancomycin-resistant *Enterococcus faecium* in hospitalized children. *Infect Control Hosp Epidemiol* 1992;**13**:700–5.
223. Rucker JC, Hamilton SR, Bardenstein D, et al. Linezolid-induced toxic optic neuropathy. *Neurology* 2006;**66**:595–8.
224. Sabol K, Patterson JE, Lewis II JS, et al. Emergence of daptomycin resistance in *Enterococcus faecium* during daptomycin therapy. *Antimicrob Agents Chemother* 2005;**49**:1664–5.
225. Sader HS, Moet GJ, Farrell DJ, Jones RN. Antimicrobial susceptibility of daptomycin and comparator agents tested against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci: trend analysis of a 6-year period in U.S. medical centers (2005-2010). *Diag Microbiol Infect Dis* 2011;**70**:412–16.
226. Sader HS, Streit JM, Fritsche TR, Jones RN. Antimicrobial activity of daptomycin against multidrug-resistant gram-positive strains collected worldwide. *Diagn Microbiol Infect Dis* 2004;**50**: 201–4.
227. Sader HS, Pfaller MA, Tenover FC, et al. Evaluation and characterization of multiresistant *Enterococcus faecium* from 12 U.S. medical centers. *J Clin Microbiol* 1994;**31**:2840–2.
228. Sahgal VS, Urban C, Mariano N, et al. Quinupristin/dalfopristin (RP 59500) therapy for vancomycin-resistant *Enterococcus faecium* aortic graft infection: case report. *Microb Drug Resist* 1995;**1**: 245–7.
229. Saiman L, Goldfarb J, Kaplan SA, et al. Safety and tolerability of linezolid in children. *Pediatr Infect Dis J* 2003;**22**:S193–200.
230. Sanchez ML, Barrett MS, Jones RN. The E-test applied to susceptibility tests of gonococci, multiply resistant enterococci, and Enterobacteriaceae producing potent beta-lactamases. *Diagn Microbiol Infect Dis* 1992;**15**:459–62.
231. Sastry V, Brennan PJ, Levy MM, et al. Vancomycin-resistant enterococci: an emerging pathogen in immunosuppressed transplant recipients. *Transplant Proc* 1995;**27**:954–5.
232. Schaberg DR, Culver DH, Gaynes RP. Major trends in the microbial etiology of nosocomial infection. *Am J Med* 1991; **91**(suppl. 3B):72–5.
233. Schaberg DR, Dillon WI, Terpenning MS, et al. Increasing resistance of enterococci to ciprofloxacin. *Antimicrob Agents Chemother* 1992;**36**:2533–5.
234. Schmit J. Efficacy of teicoplanin for enterococcal infections: 63 cases and review. *Clin Infect Dis* 1992;**15**:302–6.
235. Schoenbaum SC, Gardner P, Shillito J. Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. *J Infect Dis* 1975;**131**:543–52.
236. Schuster F, Graubner UB, Schmid I, et al. Vancomycin-resistant-enterococci—colonization of 24 patients on a pediatric oncology unit. *Klin Padiatr* 1998;**210**:261–3.
237. Schwartz M, Slavoski L, Dash G, et al. *Nosocomial outbreak of multi-resistant* Enterococcus durans *(VRED): Description of the epidemiology and antimicrobial sensitivity testing. Abstract 68*. Presented at the 33rd Annual Meeting of the Infectious Diseases Society of America, San Francisco, September 16-18, 1995.

238. Seedat J, Zick G, Klare I, et al. Rapid emergence of resistance to linezolid during linezolid therapy of an *Enterococcus faecium* infection. *Antimicrob Agents Chemother* 2006;**50**:4217–19.
239. Segreti JA, Crank CW, Finney MS. Daptomycin for the treatment of gram-positive bacteremia and infective endocarditis: a retrospective case series of 31 patients. *Pharmacotherapy* 2006;**26**: 347–52.
240. Shaikh ZH, Peloquin CA, Ericsson CD. Successful treatment of vancomycin-resistant *Enterococcus faecium* meningitis with linezolid: case report and literature review. *Scand J Infect Dis* 2001; **33**:375–9.
241. Shay DK, Goldman DA, Jarvis WR. Reducing the spread of antimicrobial-resistant microorganisms: control of vancomycin-resistant enterococci. *Pediatr Clin North Am* 1995;**42**:703–16.
242. Shay DK, Maloney SA, Montecalvo M, et al. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. *J Infect Dis* 1995;**172**:993–1000.
243. Sherman JM. The streptococci. *Bacteriol Rev* 1937;**1**:3–97.
244. Shlaes DM, Etter L, Gutmann L. Synergistic killing of vancomycin-resistant enterococci of classes A, B, and C by combinations of vancomycin, penicillin and gentamicin. *Antimicrob Agents Chemother* 1991;**35**:776–9.
245. Shlaes DM, Levy J, Wolinsky E. Enterococcal bacteremia without endocarditis. *Arch Intern Med* 1981;**141**:578–81.
246. Shuler TE, Riddle Jr CD, Potts DW. Polymicrobic septic arthritis caused by *Kingella kingae* and *Enterococcus*. *Orthopedics* 1990;**13**: 254–6.
247. Siegel JS, McCracken Jr GH. Group D streptococcal infections. *J Pediatr* 1978;**93**:542–3.
248. Silverman JA, Mortin LI, Vanpraagh AD, et al. Inhibition of daptomycin by pulmonary surfactant: in vitro modeling and clinical impact. *J Infect Dis* 2005;**191**:2149–52.
249. Simpson WA, Courtney HS, Ofek I. Interactions of fibronectin with streptococci: the role of fibronectin as a receptor for *Streptococcus pyogenes*. *Rev Infect Dis* 1987;**9**(suppl.):351–9.
250. Singh-Naz N, Sleemi A, Pikis A, et al. Vancomycin-resistant *Enterococcus faecium* colonization in children. *J Clin Microbiol* 1999;**37**:413–16.
251. Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). *Policy Statement on Antimicrobial Stewardship*. Stable URL: http://www.jstor.org/stable/10.1086/665010.
252. Stanton BF, Baltimore RS, Clemens JD. Changing spectrum of infective endocarditis in children. *Am J Dis Child* 1984;**138**: 720–5.
253. Starke JR. Infective endocarditis. In: Feigin RD, Cherry JD, Demmler GJ, Kaplan SL, editors: *Textbook of pediatric infectious diseases*. 5th ed. Philadelphia: W. B. Saunders; 2004. p. 355–80.
254. Steenbergen JN, Alder J, Thorne GM, Tally FP. Daptomycin: a lipopeptide antibiotic for the treatment of serious gram-positive infections. *J Antimicrob Chemother* 2005;**55**:283–8.
255. Stevens MP, Edmond MB. Endocarditis due to vancomycin-resistant enterococci: case report and review of the literature. *Clin Infect Dis* 2005;**41**:1134–42.
256. Stevenson KB, Murray EW, Sarubbi FA. Enterococcal meningitis: report of four cases and review. *Clin Infect Dis* 1994;**18**:233–9.
257. Sutherland SM, Alexander SR, Feneberg R, et al. International Pediatric Peritonitis Registry: enterococcal peritonitis in children receiving chronic peritoneal dialysis. *Nephrol Dial Transplant* 2010;**25**:4048–54.
258. Swenson JM, Hindler JA, Peterson LR. Special tests for detecting antibacterial resistance. In: Murray PR, Baron EJ, Pfaller MA, et al, editors. *Manual of clinical microbiology*. 6th ed. Washington, DC: American Society for Microbiology; 1995. p. 1356–67.
259. Swenson JM, Clark NC, Ferraro MJ, et al. Development of a standardized screening method for detection of vancomycin-resistant enterococci. *J Clin Microbiol* 1994;**32**:1700–4.
260. Swenson JM, Ferraro MJ, Sahm DF, et al. New vancomycin disk diffusion breakpoints for enterococci. The National Committee for Clinical Laboratory Standards Working Group on enterococci. *J Clin Microbiol* 1992;**30**:2525–8.
261. Takada H, Kawabata Y, Arakaki R, et al. Molecular and structural requirements of a lipoteichoic acid from *Enterococcus hirae* ATCC 9790 for cytokine-inducing, antitumor, and antigenic activities. *Infect Immun* 1995;**63**:57–65.
262. Teixeira OH, Francis CK. Enterococcal endocarditis in early infancy. *Can Med Assoc J* 1982;**127**:612–13.
263. Tendolkar PM, Baghdayan AS, Gilmore MS, Shankar N. Enterococcal surface protein, Esp, enhances biofilm formation by *Enterococcus faecalis*. *Infect Immun* 2004;**72**:6032–9.
264. Tenover FC, Swenson JM, O'Hara CM, et al. Ability of commercial and reference antimicrobial susceptibility testing methods to detect vancomycin resistance in enterococci. *J Clin Microbiol* 1995;**33**:1524–7.
265. Tenover FC, Weigel LM, Appelbaum PC, et al. Vancomycin-resistant *Staphylococcus aureus* isolate from a patient in Pennsylvania. *Antimicrob Agents Chemother* 2004;**48**:275–80.
266. Threlkeld MG, Cobbs CG. Infectious disorders of prosthetic valves and intravascular devices. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practices of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 783–93.
267. Tofte RW, Solliday J, Crossley KB. Susceptibilities of enterococci to twelve antibiotics. *Antimicrob Agents Chemother* 1984;**25**: 532–3.
268. Twilla JD, Finch CK, Usery JB, et al. Vancomycin-resistant *Enterococcus* bacteremia: an evaluation of treatment with linezolid or daptomycin. *J Hosp Med* 2012;**7**:243–8.
269. Urdaneta M, Hollis F, Sperber SJ. *Vancomycin resistant enterococci in the blood: do we need to treat? Abstract 65*. Presented at the 33rd Annual Meeting of the Infectious Diseases Society of America, San Francisco, September 16-18, 1995.
270. Uttley AH, Collins CH, Naidoo J, et al. Vancomycin-resistant enterococci [Letter]. *Lancet* 1988;**1**:57–8.
271. van Den Braak N, Goessens W, van Belkum A, et al. Accuracy of the VITEK 2 system to detect glycopeptide resistance in enterococci. *J Clin Microbiol* 2001;**39**:351–3.
272. Verma A, Dhawan A, Philpott-Howard J, et al. Glycopeptide-resistant *Enterococcus faecium* infections in paediatric liver transplant recipients: safety and clinical efficacy of quinupristin/dalfopristin. *J Antimicrob Chemother* 2001;**47**:105–8.
273. Verma M, Chatwal J, Varughese P. Neonatal conjunctivitis: a profile. *Indian Pediatr* 1994;**31**:1357–61.
274. von Baum H, Schehl J, Geiss HK, et al. Prevalence of vancomycin-resistant enterococci among children with end-stage renal failure: Mid-European Pediatric Peritoneal Dialysis Study Group. *Clin Infect Dis* 1999;**29**:912–16.
275. Weigel LM, Donlan RM, Shin DH, et al. High-level vancomycin-resistant *Staphylococcus aureus* (VRSA) associated with a polymicrobial biofilm. *Antimicrob Agents Chemother* 2007;**51**: 231–8.
276. Weigelt JA, Easley SM, Thal ER, et al. Abdominal surgical wound infection is lowered with improved perioperative *Enterococcus* and *Bacteroides* therapy. *J Trauma* 1993;**34**:579–84.
277. Willey BM, Kreiswirth BN, Simor AE, et al. Detection of vancomycin resistance in enterococcus species. *J Clin Microbiol* 1992;**30**:1621–4.
278. Willey BM, McGeer AJ, Ostrowski MA, et al. The use of the molecular typing techniques in the epidemiologic investigation of resistant enterococci. *Infect Control Hosp Epidemiol* 1994;**15**: 548–56.
279. Williamson R, LeBouguenec C, Gutmann L, et al. One or two low affinity penicillin-binding proteins may be responsible for the range of susceptibility of *Enterococcus faecium* to benzylpenicillin. *J Gen Microbiol* 1985;**131**:1933–40.
280. Wilson WR, Karchmer AW, Dajani AS, et al. Antibiotic treatment of adults with infective endocarditis due to streptococci, enterococci, staphylococci, and HACEK microorganisms: American Heart Association. *JAMA* 1995;**274**:1706–13.
281. Wilson WR, Wilkowske CJ, Wright AJ, et al. Treatment of streptomycin-susceptible and streptomycin-resistant enterococcal endocarditis. *Ann Intern Med* 1984;**100**:816–23.
282. Winberg J, Anderson HJ, Bergstrom T. Epidemiology of symptomatic urinary tract infection in childhood. *Acta Paediatr Scand Suppl* 1974;**252**:1.
283. Winston DJ, Emmanouilides C, Kroeber A, et al. Quinupristin/dalfopristin therapy for infections due to vancomycin-resistant *Enterococcus faecium*. *Clin Infect Dis* 2000;**30**:790–7.
284. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in U.S. hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; **39**:309–17.

285. Wisplinghoff H, Seifert H, Tallent SM, et al. Nosocomial bloodstream infections in pediatric patients in United States hospitals: Epidemiology, clinical features and susceptibilities. *Pediatr Infect Dis J* 2003;**22**:686–91.
286. Yilmaz A, Dalgic N, Musluman M, et al. Linezolid treatment of shunt-related cerebrospinal fluid infections in children. *J Neurosurg Pediatr* 2010;**5**:443–8.
287. Zakrzewski T, Keith JD. Bacterial endocarditis in infants and children. *J Pediatr* 1965;**67**:1179–93.
288. Zeana C, Kubin CJ, Della-Latta P, et al. Vancomycin-resistant *Enterococcus faecium* meningitis successfully managed with linezolid: case report and review of the literature. *Clin Infect Dis* 2001;**33**:477–82.
289. Zervos MJ, Schaberg DS. Reversal of the in vitro susceptibility of enterococci to trimethoprim-sulfamethoxazole by folinic acid. *Antimicrob Agents Chemother* 1985;**28**:446–8.
290. Zimmerman RA, Moellering Jr RC, Weinberg AN. Mechanism of resistance to antibiotic synergism in enterococci. *J Bacteriol* 1971;**105**:873–9.
291. Zscheck KK, Murray BE. Genes involved in the regulation of beta-lactamase production in enterococci and staphylococci. *Antimicrob Agents Chemother* 1993;**37**:1966–70.
292. Zurenko GE, Yagi BH, Schaadt RD, et al. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. *Antimicrob Agents Chemother* 1996;**40**:839–45.

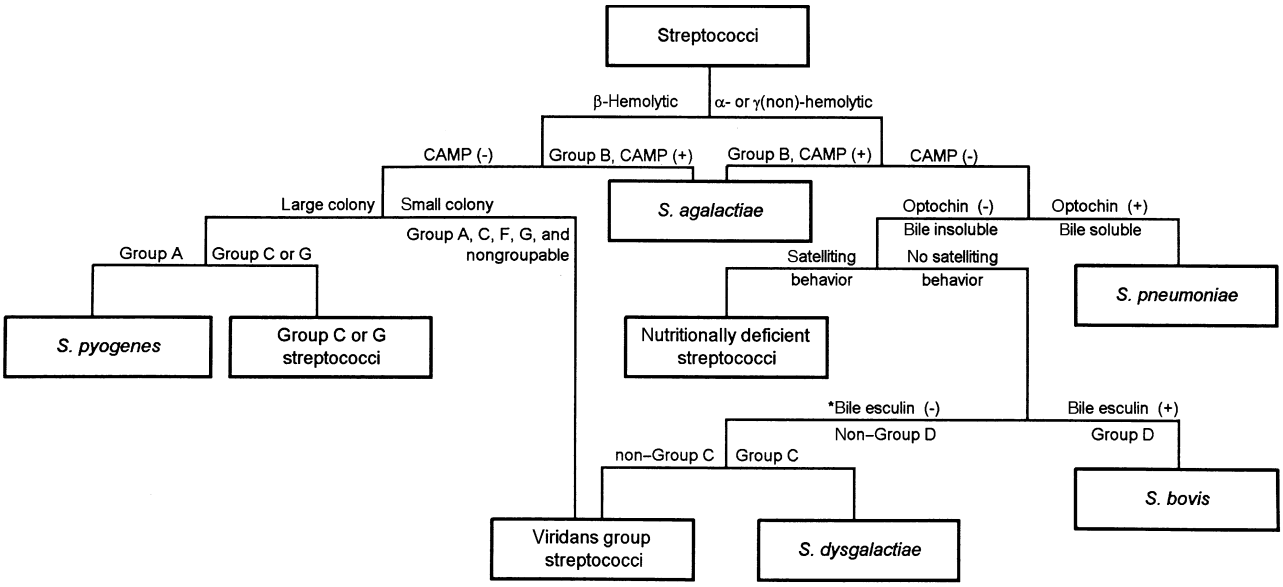

*Occasional viridans streptococcal strains are positive or weakly positive.

FIGURE 84-2 ■ Schema for the identification of clinically important streptococcal species. Viridans group species are, in general, those remaining after identification of other streptococcal species. *CAMP,* Christie, Atkins, and Munch-Peterson test.

not produce spores or gas. Some strains require an atmosphere enriched with carbon dioxide (5%). The enterococci (distinguished by their ability to grow in 6.5% sodium chloride) and lactococci (formerly Lancefield group N streptococci) were previously classified as streptococci but have now been assigned to separate genera.

Figure 84-2 is a classification schema for the clinically important streptococcal species. Hemolysis of blood agar remains a key tool for classifying streptococci. Strains that are beta-hemolytic are further characterized according to colony size and Lancefield group (a serologic classification system based on cell wall carbohydrate). Group B *Streptococcus agalactiae* strains can typically be identified by beta-hemolysis and a positive CAMP (Christie, Atkins, and Munch-Peterson) test result[256]; however, some strains of *S. agalactiae* that are alpha- or gamma-hemolytic also produce a positive CAMP test result.

Large-colony, beta-hemolytic, group A streptococci make up the species *Streptococcus pyogenes.* Most other large-colony, beta-hemolytic streptococci are group C or G streptococci, and they are occasionally pathogenic. Small-colony, beta-hemolytic streptococci, including groups A, C, G, and F and strains that cannot be grouped, are members of the *Streptococcus milleri* subgroup of viridans streptococci. Among the alpha- or gamma-hemolytic streptococci, *S. agalactiae* (group B streptococci) and *S. pneumoniae* are generally identified by positive CAMP and optochin test results, respectively. Bile solubility confirms an optochin-susceptible isolate as *S. pneumoniae*; however, some *Streptococcus mitis* isolates (as determined by genetic sequencing) yield similar results.[168]

Nutritionally deficient streptococci are identified by their requirement for the presence of a second bacterial species (*Staphylococcus aureus* is typically used in testing) to maintain growth on agar. These streptococci will generally grow in blood culture media in the absence of reduced nicotinamide adenine dinucleotide produced by a second bacterial species and may demonstrate some growth on agar in the absence of other bacteria. Nutritionally deficient streptococci were once classified as viridans streptococci, but more recent studies have assigned these organisms to a new genus: *Abiotrophia adjacens* and *Abiotrophia defectiva.*[153] With the exception of *Enterococcus* spp., streptococci that are alpha- or gamma-hemolytic, possess Lancefield group D antigen, and are bile esculin positive may be tentatively identified as *Streptococcus bovis.* This species was once considered a viridans streptococcus but is now classified separately.

Strains of one species of group C streptococci, *Streptococcus dysgalactiae*, are alpha- or gamma-hemolytic. These strains must also be distinguished from the viridans group. A species once included in the viridans group, *Streptococcus morbillorum*, was reclassified as *Gemella morbillorum.*[157] The remaining streptococcal organisms may tentatively be identified as viridans group streptococci. Thus, in practice, viridans streptococci continue to be characterized by the absence of features that distinguish other major streptococcal pathogens. Viridans streptococci have no characteristics that can be used to definitively confirm their identity in the standard microbiology laboratory.

Classification of species within the viridans streptococci group has also been challenging. Several schemata have been developed over the years, including those of Carlsson,[45] Coleman and Williams,[58] Facklam,[96] Ruoff and Kunz,[257,258] and Coykendall.[62] However, each of these schemata lacks reliable markers for member species, and clinical isolates are inconsistently classified as a result. Consequently, efforts to characterize the clinical features of infections according to individual viridans streptococcal species have been largely unsuccessful. Molecular and polymerase chain reaction–based taxonomies have been

TABLE 84-5 **Simplified Classification Schema for Viridans Streptococci**

Group	Species	Hemolysis	Sorbitol	Arginine	Voges-Proskauer	Mannitol	Esculin
S. milleri	*S. anginosus*	α, β, γ	–	+	+	±	±
	S. constellatus					–	
	S. intermedius					±	
S. mutans	*S. mutans*	β, γ, α	+	–	+	+	+
	S. sobrinus						
	S. rattus						
	S. cricetus						
S. salivarius	*S. salivarius*	γ, α	–	–	+	–	+
	S. vestibularis						
S. sanguis	*S. sanguis*	á	–	+	–	–	+
	S. gordonii						+
	S. parasanguis						±
	S. crista						–
S. mitis	*S. mitis**	á	–	–	–	–	–

Data from Coykendall AL. Classification and identification of the viridans streptococci. *Clin Microbiol Rev* 1989;**2**:315-328; and Ruoff KL. Streptococcus. In: Murray PR, Baron EJ, Pfaller MA, et al, editors. *Manual of clinical microbiology*. 6th ed. Washington, DC: American Society for Microbiology; 1995, p. 299-307.
*Previously included *S. mitis, S. sanguis II,* and *S. oralis.*

used to classify viridans streptococci,[5,62,217,255,338] but these techniques are generally unavailable in the clinical laboratory. Given the current flux in the taxonomy of these organisms, the American Society for Microbiology has advocated a simplified, practical approach to classifying viridans streptococci (Table 84-5).[256] The biochemical tests available in many clinical microbiology laboratories can be used to assign clinical isolates to one of the five groups to which the 14 clinically important viridans streptococcal species belong. Rapid molecular, nucleotide sequence analysis and polymerase chain reaction–based tests are available for tentative identification of individual viridans streptococcal species.[101,110,224,258,303] However, precise identification of species currently has clinical significance only as an epidemiologic tool.

EPIDEMIOLOGY

Viridans streptococci are the predominant oral microflora of humans; they are also commonly found in other areas of the upper respiratory tract, throughout the gastrointestinal tract, and in the female genital tract. Viridans streptococci are occasionally identified as skin flora.

Viridans streptococci begin colonizing neonates shortly after birth. By 1 month of age, virtually all infants are colonized with at least one species of viridans streptococci.[221] The mix of colonizing species varies with human ontogeny. For example, *Streptococcus mutans*, a species implicated in the development of caries, is rarely found in predentate children but is commonly present after the eruption of teeth.[47,84,301] The ecology of other viridans streptococcal species also appears to be affected by the eruption of teeth.[48,84,280,301] In addition to these temporal factors in the colonization of infants and children, individual viridans streptococcal species preferentially colonize certain anatomic areas of the oral cavity and pharynx. For example, *Streptococcus sanguis* is the predominant isolate of the buccal mucosa but is rarely found on the dorsum of the tongue, where *S. mitis* is the predominant species.[105] Diet may also affect viridans streptococcal ecology. For example, the low oral pH that can result from consumption of sugar-containing beverages and other carbohydrates favors colonization with caries-producing *S. mutans*.[118,298]

Little is known about the transmission of viridans streptococci in general, but studies have shown that *S. mutans* is commonly transmitted within families, especially from mother to child, and among young school children.[4,44,76,125,259] Toothbrushes may be an important vehicle of transmission of viridans streptococci in children.[181] A normal dishwasher cycle eliminates *S. mutans* from toothbrushes.[19] The hands of hospital personnel, especially those with skin disorders such as eczema, may also be a vehicle for transmission.[55] In addition, paper dental records have been suggested as a vehicle for the transmission of viridans streptococci.[64]

Viridans streptococci play an important role in the ecology of the oral flora by protecting against potentially more invasive pathogens via colonization resistance.[18,109] This mechanism was demonstrated by a double-blind study in which patients who had completed antibiotic therapy for group A streptococcal pharyngitis were treated with either a preparation containing four species of viridans streptococci or a placebo. None of the 17 patients in the treated group experienced recurrence of group A streptococcal pharyngitis, whereas pharyngitis recurred in 7 (37%) of the 19 patients in the placebo group.[248] A second double-blind, placebo-controlled multicenter trial by the same group confirmed these findings.[249] Viridans streptococci may also contribute to colonization resistance to pathogens such as methicillin-resistant *S. aureus* in the infant oral cavity[317]; nontypable *Haemophilus influenzae*, *S. pneumoniae*, and *Moraxella catarrhalis* at the eustachian tube orifice[300]; and *Neisseria gonorrhoeae* in the female genital tract.[191] In addition to

competing for mucosal adherence sites, viridans streptococci produce hydrogen peroxide and bacteriocins that inhibit certain competing bacteria; this mechanism may also contribute to colonization resistance.[65,67,318,323] Conversion of the hydrogen peroxide to ozone in the oral cavity may play a role in colonization resistance to other bacterial species and to *Candida* species.[316]

PATHOGENESIS

Viridans streptococci are typically low-virulence organisms; when they do cause infection, it is usually nonpyogenic with the exception of infections caused by the *Streptococcus milleri* subgroup. They are implicated most often in localized infections of the sinuses and oral cavity, including the teeth, after the tissues are directly invaded by colonizing organisms. Caries is a dental disease that develops during a period of years, often in association with *S. mutans* infection interacting with other oral flora.[161] Patients with cystic fibrosis may be particularly susceptible to caries because of a decreased buffering capacity.[46] Viridans streptococci are associated with a variety of gingival diseases, possibly including gingival hyperplasia accompanying chronic phenytoin administration. In an experimental animal model, phenytoin-induced gingival hyperplasia was more advanced in rats infected with *Streptococcus sobrinus* than in uninfected control animals.[206] On occasion, viridans streptococci, usually organisms of the *S. milleri* subgroup, cause pyogenic infections, including abscesses in the brain, lung, and abdomen. An intriguing preliminary study suggested that viridans streptococcal–related dental disease is associated with the subsequent development of coronary artery disease.[173] Viridans streptococci are among the microorganisms isolated from atherosclerotic plaque, although their role in the pathogenesis of atherosclerosis is unknown.[54] Typically, viridans streptococci cause life-threatening infection only when the oral mucosa is disrupted and the host's mechanisms of microbial clearance are compromised by conditions such as neutropenia or cardiac valve disease.

It is the preponderance of viridans streptococci in the oral flora, rather than their virulence, that accounts for their predominance in infections originating in the mouth. In a study of 36 children who underwent extraction of normal or abscessed teeth, 11 (30%) had postextraction positive blood cultures, and viridans streptococci were exclusively isolated in all 11.[287] Bacteremia occurred more commonly after removal of diseased teeth (37%), but it also occurred after removal of normal teeth (23%).

Another study of 58 children who underwent dental extraction included 26 who received penicillin, amoxicillin, or erythromycin prophylaxis because of a risk of endocarditis.[61] Bacteremia was detected in only 9 (35%) of the 26 children who received prophylaxis and in 20 (63%) of 32 children who did not ($P < .05$). In this study, viridans streptococci accounted for 37 percent of the blood isolates, strict anaerobes accounted for 26.5 percent, and a variety of organisms accounted for the remainder. The number of colony-forming units per milliliter of blood ranged from 2 to 12. Even within a single colony-forming unit, more than one bacterial species was sometimes identified after subculture.

In a study from Spain, bacteremia was detected in 96 percent, 64 percent, and 20 percent of patients 30 seconds, 15 minutes, and 1 hour after dental extractions.[310] This study indicates that bacteremia after dental procedures may not be as transient as was generally believed.

A study of 30 subjects with chronic periodontitis and 30 subjects with periodontal health showed that 40 percent and 41 percent had bacteremia during dental flossing; viridans streptococci were the most common isolate in both groups.[63] Roughly half of positive subjects remained positive 10 minutes after flossing.

Although these two studies differed in the diversity of organisms isolated, both indicated that viridans streptococci are the organisms most likely to invade the blood after trauma to the oral cavity. Viridans streptococcal bacteremia also occurs commonly during orthodontic banding[95] and esophageal stricture dilation, also reflecting the predominance of these organisms in the oral and salivary flora.[344]

The ability of viridans streptococci to bind to oral mucosa, dental surfaces, and dental plaque by interaction with specific microbial and host "receptors" accounts for the preferential colonization of the oral cavity by these organisms.[321] This interaction also helps explain the localization of the various viridans streptococcal species to distinct anatomic sites as well as the involvement of ontologic factors in colonization. The precise nature of bacterial adherence to the oral mucosa is not defined but appears to involve streptococcal lipoteichoic acid.[135] Through release of modulin protein I/II, viridans streptococci induce the expression of adhesion molecules such as E-selectin and intercellular (ICAM-1) and vascular cell (VCAM-1) adhesion molecules on endothelial cells, promote transendothelial migration of neutrophils in vitro, and induce cytokine release in epithelial (interleukin-6 [IL-6]) and endothelial (IL-6 and IL-8) cells.[325,326] Orthodontic appliances favor the proliferation of *S. mutans*.[312] The host's immune status also may be an important determinant of adherence and subsequent invasion. For example, immunocompetent persons produce secretory IgA to *S. mutans*,[12] and these antibodies are capable of preventing caries.[199] Viridans streptococci within the biofilm present in dental plaque are at least 500 times more resistant to antibiotic treatment than is predicted on the basis of their susceptibility in culture medium; this finding suggests that antibiotic treatment cannot eradicate viridans streptococci from dental plaque, even temporarily.[163]

At the University of Texas M. D. Anderson Cancer Center in Houston, the incidence of viridans streptococcal bacteremia increased extraordinarily between 1972 and 1989, from 1 to 47 cases per 10,000 admissions.[86] A risk factor analysis indicated that patients were significantly predisposed to the development of bacteremia by prophylactic administration of trimethoprim-sulfamethoxazole or a fluoroquinolone, profound neutropenia, or the administration of antacids or histamine type 2 receptor antagonists.[86] Presumably, administration of the implicated antibiotics and antacids favored the overgrowth of viridans streptococci and their proliferation

throughout the gastrointestinal tract, which in turn would favor viridans streptococcal bacteremia in an immunocompromised host, especially a neutropenic host whose mucosal barrier was disrupted by cytotoxic chemotherapy. Notably, although administration of cytosine arabinoside (cytarabine, Ara-C) was identified as a major risk factor for viridans streptococcal sepsis in many other studies,* it did not emerge as a risk factor in this study.

The ability to adhere to damaged cardiac valves and vegetations is a key factor in the pathogenesis of viridans streptococcal endocarditis. Strains of viridans streptococci carried by healthy children adhered less well to buccal and endocardial cells than did strains isolated from children with viridans streptococcal endocarditis.[268,269] Lipoteichoic acid is thought to help mediate adhesion of viridans streptococci to endocardium, and the effectiveness of penicillin prophylaxis may reflect its reduction of lipoteichoic acid on the bacterial cell surface.[175]

The ability of antibiotics to alter the surface properties of bacteria, even those resistant to the bactericidal action of the drug, may be an important determinant of the effectiveness of prophylactic antibiotics.[175,276] In the host, fibronectin is an important determinant of viridans streptococcal binding to damaged endothelium. Although viridans streptococci do not bind to soluble fibronectin, a reactive fibronectin binding domain becomes available when the fibronectin molecule is immobilized, as in endocarditis.[174] Mutant viridans streptococci that were unable to bind to fibronectin were shown to be significantly less virulent in an animal model of endocarditis.[174]

The ability of viridans streptococci to induce platelet aggregation also may be a factor in the pathogenesis of endocarditis.[102,182] In viridans streptococci–challenged, anticoagulated rabbits, only microscopic vegetations developed despite fulminant sepsis, indicating inability to limit the infection. In contrast, viridans streptococci–challenged, nonanticoagulated animals tended to have large vegetations and a subacute course.[138] These findings are concordant with those of a separate study showing that the density of bacteria within vegetations was greater in viridans streptococci–challenged thrombocytopenic rabbits than in nonthrombocytopenic control animals; these results suggest that platelets limit progression of the disease.[294] Surprisingly, neutropenia appears to have little effect on susceptibility to endocarditis in animal models, although it impedes containment of the infection.[194,195]

In neutropenic cancer patients and those who have undergone bone marrow transplantation, viridans streptococci cause septic shock and adult respiratory distress syndrome (ARDS). Viridans streptococci also induce nephritis in experimental models.[6,305] Little is known about the mechanisms involved in the pathogenesis of these conditions. Viridans streptococci produce no endotoxin, and no specific exotoxins have been identified. Nonetheless, products of these organisms can activate complement[204] and induce production of tumor necrosis factor (TNF)-α and TNF-β, IL-1, IL-2, IL-6, IL-8, interferon-γ, and nitric oxide.* Strains isolated from patients with sepsis were found to induce TNF-β and IL-8 production more potently than colonizing strains isolated from healthy subjects.[285] A study of two neutropenic patients with fatal viridans streptococcal sepsis showed high blood levels of IL-6, especially late in the course of infection, whereas levels of IL-1 and TNF-α were unremarkable.[89] Viridans streptococcal lipoteichoic acid induces production of cytokines and nitric oxide in vitro,[91,204] but the clinical significance of these observations is not known. Unlike blood-borne group A streptococci isolated from patients with septic shock, viridans streptococci isolated from the blood of pediatric cancer patients with septic shock do not express superantigens in vitro.[213] Thus, intravenous immune globulin, which has been suggested as an adjuvant therapy for group A streptococcal shock, may not be effective in cases of viridans streptococcal sepsis.

CLINICAL MANIFESTATIONS

The viridans streptococcal species are diverse, and consequently the infections they cause show a variety of clinical manifestations. Typically, the viridans streptococci cause nonpyogenic infections such as bacteremia and endocarditis, whereas the *S. milleri* group (also referred to as the *Streptococcus intermedius* group) tends to cause invasive pyogenic infections, including bone infections, brain abscesses, appendicitis, and pulmonary and abdominal abscesses.[207]

Sepsis in Immunocompromised Hosts

For obscure reasons, the relative incidence of gram-positive bacterial infections, especially viridans streptococcal infections, has increased in immunocompromised hosts during the past 2 or 3 decades. Viridans streptococci were first identified as an important cause of sepsis in neutropenic patients with cancer in 1978, when 29 episodes were reported in adults and children at the National Cancer Institute[228] and 6 episodes were reported in children at the M. D. Anderson Cancer Center.[134] The significance of blood isolates of viridans streptococci in the setting of cancer had previously not been generally appreciated. The fact that no deaths occurred in the original National Cancer Institute series or in a subsequent series from that institute[253] suggested that viridans streptococci produce a benign bacteremia similar to that caused by coagulase-negative staphylococci. In contrast, three of the six children at M. D. Anderson died. Several other centers in Europe and North America have reported fulminant, sometimes fatal viridans streptococcal sepsis in patients with cancer and in patients who have received transplants.† Overall, the reported death rate associated with viridans streptococcal sepsis has ranged from 0

*References 31, 32, 42, 78, 141, 186, 196, 242.

*References 20, 91, 127, 190, 215, 282, 284, 297.

†References 11, 14, 23, 31, 32, 42, 57, 59, 86, 117, 120, 129, 140, 164, 186, 189, 223, 240, 242, 275, 283, 290, 329.

percent to 50 percent; most centers report mortality rates of approximately 10 percent. In some centers, viridans streptococci are the most common cause of fatal and nonfatal sepsis.[23,31,117,124] The incidence of viridans streptococcal sepsis appears to be higher in children than in adults.[189,290]

The oral cavity is the most common portal of entry in immunocompromised hosts. Catheter-related viridans streptococcal bacteremia is unusual as determined by quantitative culture of simultaneous peripheral and catheter blood specimens. However, because administration of antacid is a risk factor,[86] the lower gastrointestinal tract is likely to be a portal of entry in some patients taking antacids and potentially in other circumstances. Several factors predispose patients to viridans streptococcal sepsis. Profound neutropenia is clearly a predisposing factor,[31,86] although viridans streptococcal bacteremia occasionally develops in cancer patients with absolute neutrophil counts greater than 1000/mm^3. Mucositis, especially oral mucositis, is a well-recognized risk factor.* Cytarabine appears to incur a risk even greater than that caused by its induction of clinically apparent mucositis,† although this greater risk has not been observed in at least one study.[111] Chemotherapy does markedly alter the oral flora, with 25 species identified only after chemotherapy in one study.[208] The use of prophylactic trimethoprim-sulfamethoxazole or quinolones is also an important risk factor.‡ The observation that administration of acyclovir may decrease the incidence of viridans streptococcal bacteremia in transplant patients suggests that herpes simplex virus infection may be a risk factor as well.[243] Allogeneic bone marrow transplantation has also been associated with increased risk of viridans streptococcal sepsis, which increases the mortality rate by a factor of 4.[186] Development of viridans streptococcal sepsis has also been observed to be associated with menstruation.[86]

The hallmark clinical feature of viridans streptococcal sepsis in an immunocompromised host is fever that typically is high, coincides with neutropenia and mucositis, and frequently lasts for several days after viable organisms are cleared from the blood. Most patients recover uneventfully. However, fulminant septic shock may occur. Shock may appear early, although it is often delayed for 2 or 3 days after the onset of sepsis, and it occurs despite prompt sterilization of blood by effective antibiotics.[290] ARDS frequently occurs in severe cases, usually 2 or 3 days after the initial bacteremia.[11,86,302] Focal complications, including pneumonia, encephalopathy,[265] and meningitis, are uncommon. Rash and palmar desquamation may be present but are not common.[86] For unexplained reasons, the incidence of aspergillosis is increased after viridans streptococcal sepsis in children with cancer.[214]

Several studies have implicated *S. mitis* as a more pathogenic species of viridans streptococci that has a greater likelihood of causing shock and ARDS in patients with cancer,* but other studies have found no clear relationship between clinical features and species.[86] *S. mitis* is the closest known relative of *S. pneumoniae*, and the two species share numerous pathogenicity genes.[71] *S. mitis* isolated from cancer patients with sepsis has also been found to be more antibiotic resistant than other species of viridans streptococci isolated from the same patient population.[126]

Neonatal Sepsis, Meningitis, and Other Infections

Viridans streptococci are normal inhabitants of the female genital tract and are a common cause of chorioamnionitis and subsequent abortion.[10] They are also a frequent cause of neonatal sepsis and meningitis.† Viridans streptococci do not ordinarily colonize the skin of newborns and should not be dismissed as contaminants when they are isolated from normally sterile sites.[2] At some newborn centers, the incidence of bacteremia and meningitis caused by viridans streptococci has approached or exceeded that caused by group B streptococci, although viridans streptococcal infections tend to be less severe.[37,205] *Streptococcus oralis (S. mitis)* caused more than half of the cases in one study.[332] The portal of entry of the organism is generally unknown in this setting, but a fetal scalp electrode was implicated in one case.[107] Unusual manifestations in newborns include pharyngitis and epiglottitis,[34] endocarditis,[193] and conjunctivitis.[160] Viridans streptococci also account for 6 to 12 percent of community acquired bacteremias, with a higher prevalence in children than in adults.[299]

Endocarditis

Viridans streptococci are a common cause of endocarditis at all ages because of the organism's ability to adhere to diseased endocardium and its frequent implication in bacteremia after dental procedures and routine mouth care. A study at a teaching hospital in Finland found that starting in 1995, *S. aureus* overtook viridans streptococci as a cause of endocarditis.[128] However, a study in Minnesota found no significant change in the etiology of endocarditis between 1970 and 2000; viridans streptococci remained the most common cause.[309] A study in Israel noted that *Candida albicans* was the most common cause of hospital-associated endocarditis in children, whereas viridans streptococcus was the most common cause of community acquired endocarditis in children.[183] Viridans streptococci tend to cause subacute endocarditis; blood cultures may be only intermittently positive. *S. sanguis* and *S. mitis* are the species most commonly identified.[79,238,244,295,334] Complications of viridans streptococcal endocarditis include septic pulmonary emboli, congestive heart failure, pericarditis, myocardial abscess, meningitis, osteomyelitis, and glomerulonephritis.[162,238] Shock and ARDS occur in immunocompromised patients but not in immunocompetent patients with endocarditis.

*References 31, 32, 86, 117, 186, 196.
†References 31, 32, 42, 78, 90, 141, 186, 196, 242.
‡References 31, 32, 56, 86, 117, 223.

*References 11, 32, 57, 82, 126, 192, 290, 302.
†References 2, 27, 37, 106, 171, 205, 332.

Pneumonia

Pulmonary infiltrates frequently complicate viridans streptococcal sepsis in neutropenic hosts. In most cases, these infiltrates represent ARDS and not primary pneumonia. However, several cases of primary viridans streptococcal pneumonia have been reported in previously healthy persons.* In some cases, the diagnosis was supported by multiple viridans streptococcus isolates from blood in the absence of endocarditis. The incidence of viridans streptococcal pneumonia may be significantly underestimated as tracheal isolates of viridans streptococci are usually discounted as contaminants, which they may not be in all cases.[250] In children with *S. milleri* group pleuropulmonary infections, computed tomography often shows complex pleural effusion and lung abscesses, which may require drainage for optimal treatment.[165] Viridans streptococci are increasingly recognized as contributing to pulmonary infections in children with cystic fibrosis, a population with a higher prevalence of antibiotic resistance among viridans streptococcal isolates.[119,218,279] Given the increasing incidence of viridans streptococcal infection and the escalating prevalence of antibiotic resistance among these organisms, clinicians should consider viridans streptococci as potential causes of pneumonia when they are isolated in the absence of other pathogens.

Osteomyelitis and Septic Arthritis

Viridans streptococci are unusual causes of osteomyelitis and septic arthritis. Extension of an oral infection into the mandible or maxilla is the most common cause of viridans streptococcal osteomyelitis,[220] but several cases of vertebral osteomyelitis caused by these organisms have been described.† Infection of the long bones[212,241] and septic arthritis[15] occur infrequently.

Caries

Although caries was recognized as an infectious disease in 1890 by workers trained in Robert Koch's laboratory, its infectious nature is still not accepted universally.[9,13,83] Evidence that *S. mutans* is the main cause of caries in children and adults alike is substantial,‡ but other viridans streptococci, *Lactobacillus* spp., and *Actinomyces* spp. also have cariogenic potential among the more than 700 species found in the indigenous microflora.[151,236,273,322] *S. mutans*, which colonizes the oral cavity only after the eruption of teeth, has a predilection for the dental surfaces, metabolizes sucrose, and produces a strong acid that weakens the mineral matrix of teeth, allowing the organisms to penetrate the dental structure.[49,273] Fluoridation of the water supply has been credited with strengthening tooth enamel, thereby fostering resistance to the harsh acids produced by *S. mutans*.[273] Fluoride exerts potent antibacterial activity against *S. mutans*, particularly at low pH.[49,324] Thus, fluoridation of water supplies may represent, albeit in a unique form, the most widespread and successful use of antibacterial prophylaxis. Topical treatments also provide protection against the cariogenicity of *S. mutans* and in some circumstances depend on the antibacterial action of fluoride.[133,308,320,342] Short courses of oral antibiotics can substantially reduce colonization with *S. mutans* and may be an important adjunct to the treatment of caries.[289] Specially designed culture systems are available to detect and measure the concentration of *S. mutans* in plaque to monitor the effects of therapy.[35,331] Because the development of cavities typically requires a few years of infection, there is ample opportunity to interrupt the pathogenesis of this disease.[9] A study in Germany showed that a special long-term program begun during pregnancy and continued through the teenage years increased caries-free dentitions from 57 percent in the control group to 90 percent in the treatment group.[197]

Abscesses and Other Infections

Suppurative infections produced by viridans streptococci are typically caused by the *S. milleri* group: *Streptococcus anginosus*, *Streptococcus constellatus*, and *S. intermedius*. Like *S. aureus*, members of this group resist killing by polymorphonuclear leukocytes and stimulate less chemotaxis than other viridans streptococci do.[328] *S. anginosus* is particularly prevalent in odontogenic abscesses, subdural empyemas, and epidural abscesses.[123,281] Because *S. milleri* inhabits the upper respiratory tract and the gastrointestinal tract and is relatively invasive, this organism causes sinusitis, otitis media, meningitis, and abdominal, splenic, and perianal abscesses, and it is often identified in brain abscesses, sometimes in association with tongue piercing.* *S. milleri* group organisms may infect the brain through a hematogenous route that originates in the oral cavity or intestinal tract or by direct invasion through the upper respiratory tract. Sepsis is uncommon in nonneutropenic patients, usually occurring in association with an intra-abdominal source.[178,261]

Other unusual infections caused by viridans streptococci include a large outbreak of *S. mitis* pharyngitis accompanied in 50 percent of cases by a toxic shock–like syndrome.[177] Meningitis resulting from both hematogenous and direct spread has been observed in healthy adults.[176] Iatrogenic meningitis due to contamination by oral viridans streptococci may complicate lumbar puncture or other central nervous system procedures.[93,267,339] Lung abscesses caused by viridans streptococci may result from the aspiration of saliva.[147,234] Empyema and mediastinitis are other reported thoracic infections associated with viridans streptococci.[21,147] Myositis complicated by rhabdomyolysis in children with leukemia has been reported.[262] Viridans streptococci also are found occasionally in liver abscesses,[17,103,112] peritonitis,[25] appendicitis,[229] and endophthalmitis.[52] In solid organ transplantation patients, viridans streptococci are a common cause of cholangitis.[292] Molecular analysis indicates that some cases of culture-negative intracranial abscess are caused by *S. anginosus*.[225]

*References 108, 116, 184, 209, 234, 264.
†References 3, 41, 70, 254, 319, 330.
‡References 9, 13, 35, 273, 304, 315, 322.

*References 26, 60, 88, 130, 167, 200, 203, 207, 261, 291.

DIAGNOSIS

Infections caused by viridans streptococci cannot be distinguished clinically from those caused by other gram-positive and gram-negative bacteria. Collection of adequate culture specimens is essential for diagnosis. Viridans streptococcal infections are typically diagnosed by culture of blood or other normally sterile tissues. The organisms may be present in low concentration. A volume of 30 mL of blood has been suggested as the optimal volume for culture in an adult-sized patient.[271] The addition of agents that neutralize the antibacterial effects of fresh blood, such as sodium polyanethol sulfonate, significantly improves the yield of blood cultures.[272] Chemotherapeutic agents may interfere with detection of viridans streptococci in blood.[222]

Viridans streptococci are normally not members of the skin flora and should not ordinarily be considered contaminants. Viridans streptococci have been reported to cross-react with *S. pneumoniae* omnisera, potentially causing false-positive results.[137]

ANTIBIOTIC SUSCEPTIBILITY

Viridans streptococci were once considered penicillin sensitive. Today, however, penicillin-resistant and penicillin-tolerant viridans streptococci are identified worldwide as causes of sepsis, endocarditis, meningitis, and other infections, including conjunctivitis of the newborn.* Penicillin resistance occurs commonly, particularly in patients receiving long-term penicillin therapy,[8,29,219,288,293] although even short courses of antibiotics may predispose patients to colonization with resistant viridans streptococci.[94,113,202] Monthly injections of penicillin do not appear to increase penicillin resistance in oral viridans streptococci.[28] Antibiotic resistance is found more commonly in viridans streptococci isolated from children compared with adults, perhaps reflecting greater use of antibiotics in children.[74] Infection with penicillin-resistant viridans streptococci is associated with a higher risk for mortality than is infection with penicillin-susceptible strains.[73,286] All species of viridans streptococci may develop antibiotic resistance, although *S. mutans* rarely exhibits resistance.[146]

Resistance to a variety of antibiotic classes has developed during the past 2 decades,[232] although resistance is still uncommon in community residents who have not had recent antibiotic treatment.[340] Resistance to cephalosporins is widespread; the general pattern of susceptibility is that cefotaxime and ceftriaxone are usually more active than cefepime and cefuroxime, which are both more active than ceftazidime and cephalexin.[75,185,226,335] Previous cephalosporin therapy is a risk factor for cephalosporin resistance, an observation particularly relevant to patients with cancer.[39,154] Resistance to fluoroquinolones, especially ciprofloxacin and ofloxacin, is common.† Levofloxacin has greater activity against viridans streptococci than the older fluoroquinolones do, and the newer agents garenoxacin, gatifloxacin, and moxifloxacin have enhanced activity against most gram-positive pathogens, including viridans streptococci.[245-247,281] However, resistance develops rapidly when patients receive prophylactic quinolones, including levofloxacin, gatifloxacin, and moxifloxacin.[233,306] At the Mayo Clinic in Rochester, Minnesota, 6 (16%) of 37 transplant patients receiving levofloxacin prophylaxis developed viridans streptococcal sepsis (3 experienced shock); blood isolates were resistant to levofloxacin and to the newer quinolones gatifloxacin and moxifloxacin.[239] Resistance was associated with mutations in the quinolone resistance–determining region of GyrA or ParC. Resistance of viridans streptococci to aminoglycosides,[152] tetracycline,[68,75,338] trimethoprim-sulfamethoxazole,[68,72,75,338] clindamycin,[72,313,338] erythromycin,[36,68,72,332,338] other macrolide antibiotics,[7] and vancomycin[277] has also been reported. Meropenem resistance of viridans streptococci appears to increase in children with cancer who receive multiple courses of empirical therapy.[1]

The penicillin resistance of viridans streptococci appears to involve chromosomally mediated alterations in the organisms' penicillin-binding proteins.[237,343] Initially, researchers suspected that genes conferring penicillin resistance had been acquired from *S. pneumoniae*.[81] Subsequent studies, however, indicated that penicillin resistance may have evolved first in *S. mitis* and that *S. pneumoniae* might have acquired genes mediating penicillin resistance from this and other closely related viridans streptococcal species,[50,80,231] although a recent study favors the other pathway.[142] Quinolone resistance determinants are also transmitted efficiently between viridans streptococci and *S. pneumoniae*,[145] and the *mef*(E) gene, which confers resistance to macrolides, is readily transmitted from viridans streptococci to *S. pyogenes*.[149]

The clinical impact of the development of penicillin resistance among viridans streptococci has been far-reaching. The emergence of penicillin-resistant viridans streptococci may in fact be encouraging the development of vancomycin-resistant enterococci because of the increased use of vancomycin to prevent and to treat viridans streptococcal infection in patients with cancer.

Overall, vancomycin is the antibiotic most reliably active against viridans streptococci. Teicoplanin also has been used successfully to treat viridans streptococcal endocarditis.[336] Daptomycin, dalbavancin, quinupristin/dalfopristin, tigecycline, and linezolid are active in vitro against most clinical isolates of viridans streptococci,[99,148,158,201,260] although breakthrough septic shock due to resistant *S. anginosus* has been reported during daptomycin therapy,[216] and 43 percent of viridans streptococci were resistant to quinupristin/dalfopristin in a study from Finland.[172]

TREATMENT

Empirical antibiotic therapy for viridans streptococci should be based on the pattern of antibiotic susceptibility among recent local clinical isolates. Antimicrobial susceptibility testing of viridans streptococcal isolates is

*References 33, 38, 85, 98, 115, 136, 160, 170, 230, 237.
†References 51, 68, 72, 155, 266, 338.

necessary. Limited data indicate that antibiotic susceptibility testing performed with the E-test is well correlated with agar dilution susceptibility testing.[251] Although vancomycin-resistant clinical isolates are rarely identified at present, inclusion of vancomycin in susceptibility testing is advisable. For infections other than endocarditis and meningitis, single-antibiotic therapy is usually preferred. However, in neutropenic patients with cancer, restricting antibiotic therapy to drugs active against gram-positive bacteria may increase the risk for gram-negative bacterial infections.[227] Some investigators have advocated reserving vancomycin for neutropenic patients with shock or ARDS,[313] whereas others have advocated inclusion of vancomycin in initial empirical therapy for patients with fever and neutropenia.[87,275] Viridans streptococcal sepsis may occur in neutropenic patients despite ongoing antibiotic therapy with β-lactam agents to which the organisms are susceptible in vitro[275]; however, this phenomenon has not been observed during vancomycin therapy, supporting the use of this antibiotic for viridans streptococcal sepsis in neutropenic patients. In addition, various methods to measure the susceptibility of viridans streptococcus to various antibiotics commonly yield discrepant results.[179]

Combination therapy is often advocated for treatment of viridans streptococcal endocarditis[30,104,188,327] and may be considered for treatment of meningitis, especially when the pathogen is penicillin tolerant.[92] A combination of either penicillins and aminoglycosides or of vancomycin and aminoglycosides is used most commonly. Penicillin and vancomycin are thought to increase the uptake of aminoglycosides, thereby providing synergistic bactericidal activity.[341] However, a meta-analysis of five clinical trials failed to identify any overall improvement in the outcome of patients with endocarditis who were treated with β-lactam and aminoglycoside combination therapy versus β-lactam monotherapy.[97] Vancomycin monotherapy has been successfully used to treat patients with penicillin-resistant viridans streptococcal endocarditis[139,159]; monotherapy with linezolid[211] or ceftriaxone[159,274] has also been used successfully.

Frequent dosing of antibiotics is generally recommended. However, because viridans streptococci exposed to combinations of penicillin or cephalosporin plus aminoglycoside appear to be susceptible to a postantibiotic effect,[43,156,166] longer dosing intervals may be satisfactory, although data are currently insufficient to support a recommendation. Viridans streptococcal endocarditis is usually treated for 4 to 6 weeks; the duration of therapy for infections at other sites has not been studied but can generally be guided by site-specific practice and individual clinical response.

In the treatment of endocarditis, penetration of antibiotics into the fibrin vegetation may be markedly impeded. Viridans streptococci produce an exopolysaccharide composed predominantly of dextran, which may limit penetration. Experimental studies indicate that the extent of exopolysaccharide production by viridans streptococcal strains affects the success rate of antimicrobial therapy.[235] Administration of dextranase to animals with experimental viridans streptococcal endocarditis enhances antibiotic efficacy, consistent with this observation.[198] In the future, such adjuvant therapies designed to reduce the size or density of valvular vegetations may offer promise for patients with endocarditis who are not helped by conventional antibiotic therapy.

Another setting in which adjuvant therapy may be considered is viridans streptococcal sepsis in neutropenic patients who have received cytarabine chemotherapy. One uncontrolled trial suggested that the early addition of high doses of corticosteroids to the antimicrobial therapeutic regimen may reduce the incidence of associated ARDS and death.[77] However, data are insufficient to recommend this approach routinely.

PREVENTION

Attempts to prevent viridans streptococcal infection have focused on three distinct settings: prevention of caries, prevention of endocarditis, and prevention of sepsis in neutropenic patients with cancer. Efforts have been successful in the first two settings. However, the emergence of penicillin-resistant viridans streptococci and concern about the future emergence of vancomycin resistance highlight the need for new approaches to prevent infection with these ubiquitous organisms. In developing such methods, investigators should keep in mind that the resistance to colonization provided by viridans streptococci can protect the host from more virulent pathogens (see Epidemiology).

The incidence of caries in the United States has been sharply reduced by fluoridation of water supplies, inclusion of fluoride in toothpaste, and modification of diet (e.g., use of sugar substitutes). Fluoride acts as an antibacterial agent that also strengthens the resistance of teeth to invasion by bacteria. The use of dental varnishes, gels, and rinses that contain fluoride or other antibacterial agents such as chlorhexidine or vancomycin may be beneficial in selected cases.[100,150,187,210] *Streptococcus salivarius*, a relatively nonpathogenic organism that produces potent bacteriocins, has been proposed for study as a probiotic.[263] There is evidence for and against the use of xylitol gum to reduce the levels of *S. mutans* in the oral cavity.[132,270]

The American Heart Association has led a successful effort to prevent endocarditis by the administration of systemic antibiotic prophylaxis to patients with known endocardial defects who are undergoing dental procedures.[66] These efforts are aimed especially at preventing viridans streptococcal endocarditis, and penicillin is the antibiotic most commonly used. The mechanism or mechanisms by which antibiotic prophylaxis prevents endocarditis are not completely understood. In animals, endocarditis can be prevented by administration of bacteriostatic antibiotics and by maintenance of serum levels of bactericidal antibiotics that are well below the minimum inhibitory concentration for the colonizing viridans streptococci.[114] Vancomycin has been observed to prevent development of vancomycin-tolerant *S. sanguis* endocarditis in experimentally challenged animals without reducing the incidence or level of bacteremia; thus, antibiotics may prevent endocarditis by reducing bacterial adherence to endocardium.[22] This hypothesis is

supported by a study in which 21 percent of children receiving antibiotic prophylaxis developed bacteremia, in some cases with antibiotic-resistant organisms, but endocarditis rarely occurred.[131] However, studies in animals have indicated that the probability of preventing endocarditis is related to the antibiotic susceptibility of the challenging streptococcal strains.[131] A study from Sydney found that 11 of 30 patients undergoing dental scaling developed viridans streptococcal bacteremia after mouth rinsing for 2 minutes with normal saline, whereas none of 30 patients developed viridans streptococcal bacteremia after rinsing with 7.5 percent povidone-iodine.[53] This provides a promising alternative, given the increasing resistance to antibiotics found in oral flora.

Prophylaxis should be carefully targeted[311] and administered immediately before dental procedures. An increase in the number of blood-borne antibiotic-resistant viridans streptococci can be detected within 6 hours of antibiotic treatment and persists for 9 days or longer.[169] Experimental studies of the prevention of endocarditis by antibiotic administration *after* challenge with bacterial inocula have yielded inconsistent results,[24,144,180] and the clinical utility of this approach is unknown. Topical treatment with vancomycin or chlorhexidine has been advocated as an adjuvant in preventing endocarditis, but its efficacy is unproven.[296,337]

In recent years, viridans streptococcal infections have presented a major problem in neutropenic patients with cancer and in patients who have received bone marrow transplants. Because penicillin-resistant viridans streptococci are widespread, some cancer centers include vancomycin in the initial empirical antibiotic regimen for febrile neutropenic patients.[275] In addition, despite concern about promoting vancomycin resistance, bone marrow transplant physicians are administering intravenous vancomycin prophylactically to high-risk patients in an effort to prevent viridans streptococcal sepsis; results of an observational cohort study support this practice.[143]

Uncontrolled trials of oral vancomycin paste[16] and vancomycin mouthwash[40] in children receiving cytotoxic chemotherapy suggested their efficacy in preventing viridans streptococcal infection. However, an increase in colonization and infection with vancomycin-resistant enterococci is a predictable consequence of increased vancomycin use. Therefore, the Centers for Disease Control and Prevention have recommended that empirical vancomycin therapy be avoided when it is feasible and encouraged investigators to explore alternatives to the empirical or prophylactic use of vancomycin.

In a comparative trial, penicillin prophylaxis was superior to trimethoprim-sulfamethoxazole prophylaxis in preventing viridans streptococcal infections in patients with cancer, despite extensive colonization with penicillin-resistant streptococci.[122] In other studies, oral prophylaxis with penicillin or roxithromycin (a macrolide antibiotic) also appeared to reduce the incidence of viridans streptococcal infection in patients with cancer in comparison to historical controls.[69,252,286] In contrast, prophylactic ampicillin did not reduce the incidence of viridans streptococcal sepsis in patients receiving autologous bone marrow transplants, and the incidence of penicillin resistance increased.[29] Other penicillin prophylaxis trials have also observed increased penicillin resistance.[29] The Centers for Disease Control and Prevention do not recommend the routine use of penicillin prophylaxis in patients receiving bone marrow transplants.[121]

Levofloxacin prophylaxis is currently commonly used to prevent bacterial infections in adults with cancer, but there is concern that viridans streptococci rapidly develop quinolone resistance.[307] Innovative prophylactic methods and carefully designed clinical trials are needed to identify effective prophylactic measures, especially for patients at high risk, such as children undergoing chemotherapy for acute myeloid leukemia.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abe M, Kamijo T, Matsuzawa S, et al. High incidence of meropenem resistance among alpha-hemolytic streptococci in children with cancer. *Pediatr Int* 2009;**51**:103–6.
19. Belanger-Giguere K, Giguere S, Belanger M. Disinfection of toothbrushes contaminated with *Streptococcus mutans*. *Am J Dent* 2011;**24**:155–8.
28. Bilavsky E, Eliahou R, Keller N, et al. Effect of benzathine penicillin treatment on antibiotic susceptibility of viridans streptococci in oral flora of patients receiving secondary prophylaxis after rheumatic fever. *J Infect* 2008;**56**:244–8.
44. Carletto-Korber FP, Gonzalez-Ittig RE, Jimenez MG, et al. Initial acquisition and genetic identity of *Streptococcus mutans* of mother-child pairs. *Pediatr Dent* 2010;**32**:205–11.
46. Catalan MA, Scott-Anne K, Klein MI, et al. Elevated incidence of dental caries in a mouse model of cystic fibrosis. *PLoS One* 2011;**6**:e16549.
52. Chen E, Lin MY, Cox J, et al. Endophthalmitis after intravitreal injection: the importance of viridans streptococci. *Retina* 2011; **31**:1525–33.
53. Cherry M, Daly CG, Mitchell D, et al. Effect of rinsing with povidone-iodine on bacteraemia due to scaling: a randomized-controlled trial. *J Clin Periodontol* 2007;**34**:148–55.
63. Crasta K, Daly CG, Mitchell D, et al. Bacteraemia due to dental flossing. *J Clin Periodontol* 2009;**36**:323–32.
71. Denapaite D, Bruckner R, Nuhn M, et al. The genome of *Streptococcus mitis* B6—what is a commensal? *PLoS One* 2010;**5**:e9426.
74. Doern CD, Burnham CA. It's not easy being green: the viridans group streptococci, with a focus on pediatric clinical manifestations. *J Clin Microbiol.* 2010;**48**:3829–35.
76. Domejean S, Zhan L, DenBesten PK, et al. Horizontal transmission of mutans streptococci in children. *J Dent Res* 2010;**89**: 51–5.
99. Farrell DJ, Mendes RE, Ross JE, et al. Linezolid surveillance program results for 2008 (LEADER Program for 2008). *Diagn Microbiol Infect Dis* 2009;**65**:392–403.
119. Grinwis ME, Sibley CD, Parkins MD, et al. Macrolide and clindamycin resistance in *Streptococcus milleri* group isolates from the airways of cystic fibrosis patients. *Antimicrob Agents Chemother* 2010;**54**:2823–9.
123. Gupta S, Vachhrajani S, Kulkarni AV, et al. Neurosurgical management of extraaxial central nervous system infections in children. *J Neurosurg Pediatr* 2011;**7**:441–51.
124. Hakim H, Flynn PM, Knapp KM, et al. Etiology and clinical course of febrile neutropenia in children with cancer. *J Pediatr Hematol Oncol* 2009;**31**:623–9.
130. Herskovitz MY, Goldsher D, Finkelstein R, et al. Multiple brain abscesses associated with tongue piercing. *Arch Neurol* 2009;**66**: 1292.
132. Hildebrandt G, Lee I, Hodges J. Oral mutans streptococci levels following use of a xylitol mouth rinse: a double-blind, randomized, controlled clinical trial. *Spec Care Dentist* 2010;**30**:53–8.
140. Huang WT, Chang LY, Hsueh PR, et al. Clinical features and complications of viridans streptococci bloodstream infection in pediatric hemato-oncology patients. *J Microbiol Immunol Infect* 2007;**40**:349–54.
142. Ip M, Chau SS, Chi F, et al. Fluoroquinolone resistance in atypical pneumococci and oral streptococci: evidence of horizontal

gene transfer of fluoroquinolone resistance determinants from *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2007;**51**: 2690–700.
148. Jones RN, Kohno S, Ono Y, et al. ZAAPS International Surveillance Program (2007) for linezolid resistance: results from 5591 gram-positive clinical isolates in 23 countries. *Diagn Microbiol Infect Dis* 2009;**64**:191–201.
151. Kanasi E, Johansson I, Lu SC, et al. Microbial risk markers for childhood caries in pediatricians' offices. *J Dent Res* 2010;**89**: 378–83.
159. Knoll B, Tleyjeh IM, Steckelberg JM, et al. Infective endocarditis due to penicillin-resistant viridans group streptococci. *Clin Infect Dis* 2007;**44**:1585–92.
161. Kuramitsu HK, Wang BY. The whole is greater than the sum of its parts: dental plaque bacterial interactions can affect the virulence properties of cariogenic *Streptococcus mutans*. *Am J Dent* 2011;**24**:153–4.
165. Lee EY, Khatwa U, McAdam AJ, et al. *Streptococcus milleri* group pleuropulmonary infection in children: computed tomographic findings and clinical features. *J Comput Assist Tomogr* 2010;**34**: 927–32.
167. Lee WS, Choi ST, Kim KK. Splenic abscess: a single institution study and review of the literature. *Yonsei Med J* 2011;**52**:288–92.
168. Leegaard TM, Bootsma HJ, Caugant DA, et al. Phenotypic and genomic characterization of pneumococcus-like streptococci isolated from HIV-seropositive patients. *Microbiology* 2010;**156**: 838–48.
172. Lindgren M, Jalava J, Rantakokko-Jalava K, et al. In vitro susceptibility of viridans group streptococci isolated from blood in southwest Finland in 1993-2004. *Scand J Infect Dis* 2007;**39**: 508–13.
179. Maeda Y, Goldsmith CE, Coulter WA, et al. Comparison of minimum inhibitory concentration by broth microdilution testing versus standard disc diffusion testing in the detection of penicillin, erythromycin and ciprofloxacin resistance in viridans group streptococci. *J Med Microbiol* 2011;**60**:1782–6.
183. Marom D, Levy I, Gutwein O, et al. Healthcare-associated versus community-associated infective endocarditis in children. *Pediatr Infect Dis J* 2011;**30**:585–8.
197. Meyer K, Geurtsen W, Gunay H. An early oral health care program starting during pregnancy: results of a prospective clinical long-term study. *Clin Oral Investig* 2010;**14**:257–64.
201. Moet GJ, Dowzicky MJ, Jones RN. Tigecycline (GAR-936) activity against *Streptococcus gallolyticus (bovis)* and viridans group streptococci. *Diagn Microbiol Infect Dis* 2007;**57**:333–6.
208. Napenas JJ, Brennan MT, Coleman S, et al. Molecular methodology to assess the impact of cancer chemotherapy on the oral bacterial flora: a pilot study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;**109**:554–60.
212. Nomura R, Nakano K, Makela K, et al. Isolation and characterization of *Streptococcus mitis* from blood of child with osteomyelitis. *Int J Paediatr Dent* 2011;**21**:192–9.
216. Palacio F, Lewis JS, Sadkowski L, et al. Breakthrough bacteremia and septic shock due to *Streptococcus anginosus* resistant to daptomycin in a patient receiving daptomycin therapy. *Antimicrob Agents Chemother* 2011;**55**:3639–40.
218. Parkins MD, Sibley CD, Surette MG, et al. The *Streptococcus milleri* group—an unrecognized cause of disease in cystic fibrosis: a case series and literature review. *Pediatr Pulmonol* 2008;**43**: 490–7.
225. Petti CA, Simmon KE, Bender J, et al. Culture-negative intracerebral abscesses in children and adolescents from *Streptococcus anginosus* group infection: a case series. *Clin Infect Dis* 2008;**46**: 1578–80.
236. Qi F, Kreth J. Characterization of anti-competitor activities produced by oral bacteria. *Methods Mol Biol* 2010;**666**:151–66.
263. Santagati M, Scillato M, Patane F, et al. Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. *FEMS Immunol Med Microbiol* 2012;**65**:23–31.
265. Sato Y, Okuya M, Hagisawa S, et al. Viridans streptococcal bacteremia-related encephalopathy in childhood with malignancy. *Pediatr Hematol Oncol* 2011;**28**:24–30.
270. Seki M, Karakama F, Kawato T, et al. Effect of xylitol gum on the level of oral mutans streptococci of preschoolers: block-randomised trial. *Int Dent J* 2011;**61**:274–80.
279. Sibley CD, Parkins MD, Rabin HR, et al. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients *Proc Natl Acad Sci U S A* 2008;**105**: 15070–5.
281. Sobottka I, Wegscheider K, Balzer L, et al. Microbiological analysis of a prospective, randomized, double-blind trial comparing moxifloxacin and clindamycin in the treatment of odontogenic infiltrates and abscesses. *Antimicrob Agents Chemother* 2012;**56**: 2565–9.
291. Stelzmueller I, Aigner F, Albright J, et al. Group milleri streptococci in perianal infections. *Colorectal Dis* 2010;**12**:e121–e127.
292. Stelzmueller I, Berger N, Wiesmayr S, et al. Group milleri streptococci: significant pathogens in solid organ recipients. *Transpl Int* 2007;**20**:51–6.
299. Tan LK, Lacey S, Mandalia S, et al. Hospital-based study of viridans streptococcal bacteraemia in children and adults. *J Infect* 2008;**56**:103–7.
303. Teles C, Smith A, Ramage G, et al. Identification of clinically relevant viridans group streptococci by phenotypic and genotypic analysis. *Eur J Clin Microbiol Infect Dis* 2011;**30**:243–50.
307. Timmers GJ, Simoons-Smit AM, Leidekker ME, et al. Levofloxacin vs. ciprofloxacin plus phenethicillin for the prevention of bacterial infections in patients with haematological malignancies. *Clin Microbiol Infect* 2007;**13**:497–503.
309. Tleyjeh IM, Steckelberg JM, Murad HS, et al. Temporal trends in infective endocarditis: a population-based study in Olmsted County, Minnesota. *JAMA* 2005;**293**:3022–8.
310. Tomas I, Alvarez M, Limeres J, et al. Prevalence, duration and aetiology of bacteraemia following dental extractions. *Oral Dis* 2007;**13**:56–62.
312. Topaloglu-Ak A, Ertugrul F, Eden E, et al. Effect of orthodontic appliances on oral microbiota—6 month follow-up. *J Clin Pediatr Dent* 2011;**35**:433–6.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

Viridans Streptococcal Infections

1. Abe M, Kamijo T, Matsuzawa S, et al. High incidence of meropenem resistance among alpha-hemolytic streptococci in children with cancer. *Pediatr Int* 2009;**51**:103–6.
2. Adams JT, Faix RG. *Streptococcus mitis* infection in newborns. *J Perinatol* 1994;**14**:473–8.
3. Adeotoye O, Kupfer R. *Streptococcus viridans* vertebral osteomyelitis. *J R Soc Med* 1999;**92**:306–7.
4. Alaluusua S. Transmission of mutans streptococci. *Proc Finn Dent Soc* 1991;**87**:443–7.
5. Alam S, Brailsford SR, Whiley RA, et al. PCR-based methods for genotyping viridans group streptococci. *J Clin Microbiol* 1999;**37**: 2772–6.
6. Albini B, Nisengard RJ, Glurich I, et al. *Streptococcus mutans*–induced nephritis in rabbits. *Am J Pathol* 1985;**118**:408–18.
7. Alcaide F, Carratala J, Linares J, et al. In vitro activities of eight macrolide antibiotics and RP-59500 (quinupristin-dalfopristin) against viridans group streptococci isolated from blood of neutropenic cancer patients. *Antimicrob Agents Chemother* 1996;**40**: 2117–20.
8. Alvarez M, Alvarez ME, Maiz L, et al. Antimicrobial susceptibility profiles of oropharyngeal viridans group streptococci isolates from cystic fibrosis and non–cystic fibrosis patients. *Microb Drug Resist* 1998;**4**:123–8.
9. Anderson MH, Molvar MP, Powell LV. Treating dental caries as an infectious disease. *Oper Dent* 1991;**16**:21–8.
10. Ariel I, Singer DB. *Streptococcus viridans* infections in midgestation. *Pediatr Pathol* 1991;**11**:75–83.
11. Arning M, Gehrt A, Aul C, et al. Septicemia due to *Streptococcus mitis* in neutropenic patients with acute leukemia. *Blut* 1990;**61**: 364–8.
12. Arnold RR, Mestecky J, McGhee JR. Naturally occurring secretory immunoglobulin A antibodies to *Streptococcus mutans* in human colostrum and saliva. *Infect Immun* 1976;**14**:355–62.
13. Asikainen S, Alaluusua S. Bacteriology of dental infections. *Eur Heart J* 1993;**14**(Suppl K):43–50.
14. Awada A, van der Auwera P, Meunier F, et al. Streptococcal and enterococcal bacteremia in patients with cancer. *Clin Infect Dis* 1992;**15**:33–48.
15. Barbadillo C, Trujillo A, Cuende E, et al. Septic arthritis due to streptococcus viridans. *Clin Exp Rheumatol* 1990;**8**:520–1.
16. Barker GJ, Call SK, Gamis AS. Oral care with vancomycin paste for reduction in incidence of alpha-hemolytic streptococcal sepsis. *J Pediatr Hematol Oncol* 1995;**17**:151–5.
17. Bateman NT, Eykyn SJ, Phillips I. Pyogenic liver abscess caused by *Streptococcus milleri*. *Lancet* 1975;**1**:657–9.
18. Beck A. Interference by an alpha-hemolytic streptococcus of beta-hemolytic pathogenic streptococci. *Inflammation* 1979;**3**:463–5.
19. Belanger-Giguere K, Giguere S, Belanger M. Disinfection of toothbrushes contaminated with *Streptococcus mutans*. *Am J Dent* 2011;**24**:155–8.
20. Benabdelmoumene S, Dumont S, Petit C, et al. Activation of human monocytes by *Streptococcus mutans* serotype f polysaccharide: immunoglobulin G Fc receptor expression and tumor necrosis factor and interleukin-1 production. *Infect Immun* 1991;**59**: 3261–6.
21. Berlot G, Tomasini A, Cioffi V, et al. Fatal *Streptococcus viridans* descending mediastinitis: case report and review of the literature. *Eur J Emerg Med* 1997;**4**:111–14.
22. Bernard JP, Francioli P, Glauser MP. Vancomycin prophylaxis of experimental *Streptococcus sanguis*. Inhibition of bacterial adherence rather than bacterial killing. *J Clin Invest* 1981;**68**:1113–16.
23. Berner R, Sauter S, Duffner U, et al. Bacteremic episodes in pediatric oncologic patients, especially caused by the *Streptococcus viridans* group. *Klin Padiatr* 1998;**210**:256–60.
24. Berney P, Francioli P. Successful prophylaxis of experimental streptococcal endocarditis with single-dose amoxicillin administered after bacterial challenge. *J Infect Dis* 1990;**161**:281–5.
25. Bert F, Noussair L, Lambert-Zechovsky N, et al. Viridans group streptococci: an underestimated cause of spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Eur J Gastroenterol Hepatol* 2005;**17**:929–33.
26. Bertrand B, Rombaux P, Eloy P, et al. Sinusitis of dental origin. *Acta Otorhinolaryngol Belg* 1997;**51**:315–22.
27. Bignardi GE, Isaacs D. Neonatal meningitis due to *Streptococcus mitis*. *Rev Infect Dis* 1989;**11**:86–8.
28. Bilavsky E, Eliahou R, Keller N, et al. Effect of benzathine penicillin treatment on antibiotic susceptibility of viridans streptococci in oral flora of patients receiving secondary prophylaxis after rheumatic fever. *J Infect* 2008;**56**:244–8.
29. Bilgrami S, Feingold JM, Dorsky D, et al. *Streptococcus viridans* bacteremia following autologous peripheral blood stem cell transplantation. *Bone Marrow Transplant* 1998;**21**:591–5.
30. Bisno AL, Dismukes WE, Durack DT, et al. Antimicrobial treatment of infective endocarditis due to viridans streptococci, enterococci, and staphylococci. *JAMA* 1989;**261**:1471–7.
31. Bochud PY, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. *Am J Med* 1994;**97**: 256–64.
32. Bochud PY, Eggiman P, Calandra T, et al. Bacteremia due to viridans streptococcus in neutropenic patients with cancer: clinical spectrum and risk factors. *Clin Infect Dis* 1994;**18**:25–31.
33. Boenning DA, Nelson LP, Campos JM. Relatively penicillin-resistant *Streptococcus sanguis* endocarditis in an adolescent. *Pediatr Infect Dis J* 1988;**7**:205–7.
34. Bos AP, Fetter WP, Baerts W, et al. Streptococcal pharyngitis and epiglottitis in a newborn infant. *Eur J Pediatr* 1992;**151**:874–5.
35. Bratthall D. Mutans streptococci—dental, oral and global aspects. *J Indian Soc Pedod Prev Dent* 1991;**9**:4–12.
36. Bromberg K, Orson JM, Triedman R, et al. Erythromycin-resistant *Streptococcus viridans* in oral flora with prolonged erythromycin therapy. *Ann Intern Med* 1980;**93**:931–2.
37. Broughton RA, Krafka R, Baker CJ. Non–group D alpha-hemolytic streptococci: new neonatal pathogens. *J Pediatr* 1981; **99**:450–4.
38. Bruckner L, Gigliotti F. Viridans group streptococcal infections among children with cancer and the importance of emerging antibiotic resistance. *Semin Pediatr Infect Dis* 2006;**17**:153–60.
39. Bruckner LB, Korones DN, Karnauchow T, et al. High incidence of penicillin resistance among alpha-hemolytic streptococci isolated from the blood of children with cancer. *J Pediatr* 2002;**140**: 20–6.
40. Brunet AS, Ploton C, Galambrun C, et al. Low incidence of sepsis due to viridans streptococci in a ten-year retrospective study of pediatric acute myeloid leukemia. *Pediatr Blood Cancer* 2006;**47**: 765–72.
41. Buchman AL. *Streptococcus viridans* osteomyelitis with endocarditis presenting as acute onset lower back pain. *J Emerg Med* 1990;**8**: 291–4.
42. Burden AD, Oppenheim BA, Crowther D, et al. Viridans streptococcal bacteraemia in patients with haematological and solid malignancies. *Eur J Cancer* 1991;**27**:409–11.
43. Buxbaum A, Georgopoulos A. Postantibiotic effect of ceftriaxone and gentamicin alone and in combination on *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Streptococcus viridans*. *Infection* 1996;**24**: 459–64.
44. Carletto-Korber FP, Gonzalez-Ittig RE, Jimenez MG, et al. Initial acquisition and genetic identity of *Streptococcus mutans* of mother-child pairs. *Pediatr Dent* 2010;**32**:205–11.
45. Carlsson J. A numerical taxonomic study of human oral streptococci. *Odontol Revy* 1968;**19**:137–60.
46. Catalan MA, Scott-Anne K, Klein MI, et al. Elevated incidence of dental caries in a mouse model of cystic fibrosis. *PLoS One* 2011;**6**:e16549.
47. Caufield PW, Cutter GR, Dasanayake AP. Initial acquisition of mutans streptococci by infants: evidence for a discrete window of infectivity. *J Dent Res* 1993;**72**:37–45.
48. Caufield PW, Dasanayake AP, Li Y, et al. Natural history of *Streptococcus sanguinis* in the oral cavity of infants: evidence for a discrete window of infectivity. *Infect Immun* 2000;**68**:4018–23.
49. Caufield PW, Wannemuehler YM. In vitro susceptibility of streptococcus mutans 6715 to iodine and sodium fluoride, singly and in combination, at various pH values. *Antimicrob Agents Chemother* 1982;**22**:115–9.
50. Chalkley L, Schuster C, Potgieter E, et al. Relatedness between *Streptococcus pneumoniae* and viridans streptococci: transfer of penicillin resistance determinants and immunological similarities of penicillin-binding proteins. *FEMS Microbiol Lett* 1991;**69**:35–42.
51. Chambers HF, Xiang Q, Liu, et al. Efficacy of levofloxacin for experimental aortic-valve endocarditis in rabbits infected with

viridans group streptococcus or *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1999;**43**:2742–6.
52. Chen E, Lin MY, Cox J, et al. Endophthalmitis after intravitreal injection: the importance of viridans streptococci. *Retina* 2011;**31**: 1525–33.
53. Cherry M, Daly CG, Mitchell D, et al. Effect of rinsing with povidone-iodine on bacteraemia due to scaling: a randomized-controlled trial. *J Clin Periodontol* 2007;**34**:148–55.
54. Chiu B. Multiple infections in carotid atherosclerotic plaques. *Am Heart J* 1999;**138**:S534–6.
55. Church DL, Bryant HE. Investigation of a *Streptococcus viridans* pseudobacteremia epidemic at a university teaching hospital. *Infect Control Hosp Epidemiol* 1989;**10**:416–21.
56. Classen DC, Burke JP, Ford CD, et al. *Streptococcus mitis* sepsis in bone marrow transplant patients receiving oral antimicrobial prophylaxis. *Am J Med* 1990;**89**:441–6.
57. Cohen J, Donnelly JP, Worsley AM, et al. Septicaemia caused by viridans streptococci in neutropenic patients with leukaemia. *Lancet* 1983;**2**:1452–4.
58. Coleman G, Williams REA. Taxonomy of some human viridans streptococci. In: Wannamaker LW, Matsen JM, editors. *Streptococci and streptococcal diseases: recognition, understanding, and management*. New York: Academic Press; 1972. p. 282–99.
59. Collin BA, Leather HL, Wingard JR, et al. Evolution, incidence, and susceptibility of bacterial bloodstream isolates from 519 bone marrow transplant patients. *Clin Infect Dis* 2001;**33**:947–53.
60. Corson MA, Postlethwaite KP, Seymour RA. Are dental infections a cause of brain abscess? Case report and review of the literature. *Oral Dis* 2001;**7**:61–5.
61. Coulter WA, Coffey A, Saunders ID, et al. Bacteremia in children following dental extraction. *J Dent Res* 1990;**69**:1691–5.
62. Coykendall AL. Classification and identification of the viridans streptococci. *Clin Microbiol Rev* 1989;**2**:315–28.
63. Crasta K, Daly CG, Mitchell D, et al. Bacteraemia due to dental flossing. *J Clin Periodontol* 2009;**36**:323–32.
64. Crompton N, Griffiths BM, Wilson M, et al. The transfer of bacteria to, and survival on, dental records. *Microbios* 1999;**99**: 181–7.
65. Dajani AS, Law DJ, Bollinger RO, et al. Ultrastructural and biochemical alterations effected by viridin B, a bacterocin of alpha-hemolytic streptococci. *Infect Immun* 1976;**14**:776–82.
66. Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis. Recommendations by the American Heart Association. *Circulation* 1997;**96**:358–66.
67. Dajani AS, Tom MC, Law DJ. Viridins, bacteriocins of alpha-hemolytic streptococci: isolation, characterization, and partial purification. *Antimicrob Agents Chemother* 1976;**9**:81–8.
68. de Azavedo JC, Trpeski L, Pong-Porter S, et al. In vitro activities of fluoroquinolones against antibiotic-resistant blood culture isolates of viridans group streptococci from across Canada. *Antimicrob Agents Chemother* 1999;**43**:2299–301.
69. Dekker AW, Rozenberg-Arska M, Verdonck LF. Prevention of bacteremias caused by alpha-hemolytic streptococci by roxithromycin in patients treated with intensive cytotoxic treatment. *Haematol Blood Transfus* 1990;**33**:551–4.
70. Demers C, Tremblay M, Lacourciere Y. Acute vertebral osteomyelitis complicating *Streptococcus sanguis* endocarditis. *Ann Rheum Dis* 1988;**47**:333–6.
71. Denapaite D, Bruckner R, Nuhn M, et al. The genome of *Streptococcus mitis* B6—what is a commensal? *PLoS One* 2010;**5**:e9426.
72. Diekema DJ, Beach ML, Pfaller MA, et al. Antimicrobial resistance in viridans group streptococci among patients with and without the diagnosis of cancer in the USA, Canada and Latin America. *Clin Microbiol Infect* 2001;**7**:152–7.
73. Docze A, Mraz M, Grey E, et al. Penicillin resistance in viridans streptococcal bacteremia is related with high mortality. *Scand J Infect Dis* 2003;**35**:916–7.
74. Doern CD, Burnham CA. It's not easy being green: the viridans group streptococci, with a focus on pediatric clinical manifestations. *J Clin Microbiol.* 2010;**48**:3829–35.
75. Doern GV, Ferraro MJ, Brueggemann AB, et al. Emergence of high rates of antimicrobial resistance among viridans group streptococci in the United States. *Antimicrob Agents Chemother* 1996;**40**:891–4.
76. Domejean S, Zhan L, DenBesten PK, et al. Horizontal transmission of mutans streptococci in children. *J Dent Res* 2010;**89**: 51–5.
77. Dompeling EC, Donnelly JP, Raemaekers JM, et al. Pre-emptive administration of corticosteroids prevents the development of ARDS associated with *Streptococcus mitis* bacteremia following chemotherapy with high-dose cytarabine. *Ann Hematol* 1994;**69**: 69–71.
78. Donnelly JP, Dompeling EC, Meis JF, et al. Bacteremia due to oral viridans streptococci in neutropenic patients with cancer: cytostatics are a more important risk factor than antibacterial prophylaxis. *Clin Infect Dis* 1995;**20**:469–70.
79. Douglas CW, Heath J, Hampton KK, et al. Identity of viridans streptococci isolated from cases of infective endocarditis. *J Med Microbiol* 1993;**39**:179–82.
80. Dowson CG, Coffey TJ, Kell C, et al. Evolution of penicillin resistance in *Streptococcus pneumoniae*; the role of *Streptococcus mitis* in the formation of a low affinity PBP2B in *S. pneumoniae*. *Mol Microbiol* 1993;**9**:635–43.
81. Dowson CG, Hutchison A, Woodford N, et al. Penicillin-resistant viridans streptococci have obtained altered penicillin-binding protein genes from penicillin-resistant strains of *Streptococcus pneumoniae*. *Proc Natl Acad Sci U S A* 1990;**87**:5858–62.
82. Dwyer R, Ringertz S. Viridans streptococci in blood cultures. Can we see any patterns of species related to patient category? Review of 229 cases of positive cultures with viridans streptococci. *APMIS* 1997;**105**:972–4.
83. Edelstein BL. The medical management of dental caries. *J Am Dent Assoc* 1994;**125**(Suppl):31S–9S.
84. Edwardsson S, Mejare B. *Streptococcus milleri* (Guthof) and *Streptococcus mutans* in the mouths of infants before and after tooth eruption. *Arch Oral Biol* 1978;**23**:811–14.
85. Elliott RH, Dunbar JM. Antibiotic sensitivity of oral alpha-haemolytic streptococcus from children with congenital or acquired cardiac disease. A prolonged survey. *Br Dent J* 1977;**142**: 283–5.
86. Elting LS, Bodey GP, Keefe BH. Septicemia and shock syndrome due to viridans streptococci: a case-control study of predisposing factors. *Clin Infect Dis* 1992;**14**:1201–7.
87. Elting LS, Rubenstein EB, Rolston KV, et al. Outcomes of bacteremia in patients with cancer and neutropenia: observations from two decades of epidemiological and clinical trials. *Clin Infect Dis* 1997;**25**:247–59.
88. Eng RH, Mangia AJ, Smith SM, et al. Meningitis following bacteremia with *Streptococcus sanguis*. *N Y State J Med* 1989;**89**: 625–6.
89. Engel A, Kern P, Kern WV. Levels of cytokines and cytokine inhibitors in the neutropenic patient with alpha-hemolytic streptococcus shock syndrome. *Clin Infect Dis* 1996;**23**:785–9.
90. Engelhard D, Elishoov H, Or R, et al. Cytosine arabinoside as a major risk factor for *Streptococcus viridans* septicemia following bone marrow transplantation: a 5-year prospective study. *Bone Marrow Transplant* 1995;**16**:565–70.
91. English BK, Patrick CC, Orlicek SL, et al. Lipoteichoic acid from viridans streptococci induces the production of tumor necrosis factor and nitric oxide by murine macrophages. *J Infect Dis* 1996;**174**:1348–51.
92. Entenza JM, Caldelari I, Glauser MP, et al. Importance of genotypic and phenotypic tolerance in the treatment of experimental endocarditis due to *Streptococcus gordonii*. *J Infect Dis* 1997;**175**: 70–6.
93. Enting RH, de Gans J, Blankevoort JP, et al. Meningitis due to viridans streptococci in adults. *J Neurol* 1997;**244**:435–8.
94. Erickson PR, Herzberg MC. Emergence of antibiotic resistant *Streptococcus sanguis* in dental plaque of children after frequent antibiotic therapy. *Pediatr Dent* 1999;**21**:181–5.
95. Erverdi N, Kadir T, Ozkan H, et al. Investigation of bacteremia after orthodontic banding. *Am J Orthod Dentofacial Orthop* 1999; **116**:687–90.
96. Facklam RR. Physiological differentiation of viridans streptococci. *J Clin Microbiol* 1977;**5**:184–201.
97. Falagas ME, Matthaiou DK, Bliziotis IA. The role of aminoglycosides in combination with a beta-lactam for the treatment of bacterial endocarditis: a meta-analysis of comparative trials. *J Antimicrob Chemother* 2006;**57**:639–47.

98. Farber BF, Eliopoulos GM, Ward JI, et al. Multiply resistant viridans streptococci: susceptibility to beta-lactam antibiotics and comparison of penicillin-binding protein patterns. *Antimicrob Agents Chemother* 1983;**24**:702–5.
99. Farrell DJ, Mendes RE, Ross JE, et al. Linezolid surveillance program results for 2008 (LEADER Program for 2008). *Diagn Microbiol Infect Dis* 2009;**65**:392–403.
100. Fine JB, Harper DS, Gordon JM, et al. Short-term microbiological and clinical effects of subgingival irrigation with an antimicrobial mouthrinse. *J Periodontol* 1994;**65**:30–6.
101. Flynn CE, Ruoff KL. Identification of "*Streptococcus milleri*" group isolates to the species level with a commercially available rapid test system. *J Clin Microbiol* 1995;**33**:2704–6.
102. Ford I, Douglas CW, Preston FE, et al. Mechanisms of platelet aggregation by *Streptococcus sanguis*, a causative organism in infective endocarditis. *Br J Haematol* 1993;**84**:95–100.
103. Ford JM, DuBois RE. Multiloculated hepatic abscess caused by alpha-hemolytic streptococcus. *South Med J* 1984;**77**:514–16.
104. Francioli PB, Glauser MP. Synergistic activity of ceftriaxone combined with netilmicin administered once daily for treatment of experimental streptococcal endocarditis. *Antimicrob Agents Chemother* 1993;**37**:207–12.
105. Frandsen EV, Pedrazzoli V, Kilian M. Ecology of viridans streptococci in the oral cavity and pharynx. *Oral Microbiol Immunol* 1991;**6**:129–33.
106. Fraser JJ Jr, Marks MI, Welch DF. Neonatal sepsis and meningitis due to alpha-hemolytic streptococcus. *South Med J* 1983;**76**: 401–2.
107. Freedman RM, Baltimore R. Fatal *Streptococcus viridans* septicemia and meningitis: relationship to fetal scalp electrode monitoring. *J Perinatol* 1990;**10**:272–4.
108. Freitas M, Castelo A, Petty G, et al. Viridans streptococci causing community acquired pneumonia. *Arch Dis Child* 2006;**91**:779–80.
109. Fujimori I, Kikushima K, Hisamatsu K, et al. Interaction between oral alpha-streptococci and group A streptococci in patients with tonsillitis. *Ann Otol Rhinol Laryngol* 1997;**106**:571–4.
110. Garnier F, Gerbaud G, Courvalin P, et al. Identification of clinically relevant viridans group streptococci to the species level by PCR. *J Clin Microbiol* 1997;**35**:2337–41.
111. Gassas A, Grant R, Richardson S, et al. Predictors of viridans streptococcal shock syndrome in bacteremic children with cancer and stem-cell transplant recipients. *J Clin Oncol* 2004;**22**:1222–7.
112. George S, Wadhera A, Mersich K, et al. Liver abscess due to *Streptococcus sanguis*. *Clin Infect Dis* 1996;**22**:191–2.
113. Ghaffar F, Friedland IR, Katz K, et al. Increased carriage of resistant non-pneumococcal alpha-hemolytic streptococci after antibiotic therapy. *J Pediatr* 1999;**135**:618–23.
114. Glauser MP, Francioli P. Successful prophylaxis against experimental streptococcal endocarditis with bacteriostatic antibiotics. *J Infect Dis* 1982;**146**:806–10.
115. Goldfarb J, Wormser GP, Glaser JH. Meningitis caused by multiply antibiotic-resistant viridans streptococci. *J Pediatr* 1984; **105**:891–5.
116. Goolam MA, Feldman C, Smith C, et al. Does primary *Streptococcus viridans* pneumonia exist? *S Afr Med J* 1992;**82**:432–4.
117. Graber CJ, de Almeida KN, Atkinson JC, et al. Dental health and viridans streptococcal bacteremia in allogeneic hematopoietic stem cell transplant recipients. *Bone Marrow Transplant* 2001;**27**: 537–42.
118. Grindefjord M, Dahllof G, Wikner S, et al. Prevalence of mutans streptococci in one-year-old children. *Oral Microbiol Immunol* 1991;**6**:280–3.
119. Grinwis ME, Sibley CD, Parkins MD, et al. Macrolide and clindamycin resistance in *Streptococcus milleri* group isolates from the airways of cystic fibrosis patients. *Antimicrob Agents Chemother* 2010;**54**:2823–9.
120. Groot-Loonen JJ, van der Noordaa J, de Kraker J, et al. Alpha-hemolytic streptococcal septicemia with severe complications during neutropenia in childhood cancer. *Pediatr Hematol Oncol* 1987;**4**:323–8.
121. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *MMWR Recomm Rep* 2000;**49**(RR10):1–7.
122. Guiot HF, van der Meer JW, van den Broek PJ, et al. Prevention of viridans-group streptococcal septicemia in oncohematologic patients: a controlled comparative study on the effect of penicillin G and cotrimoxazole. *Ann Hematol* 1992;**64**:260–5.
123. Gupta S, Vachhrajani S, Kulkarni AV, et al. Neurosurgical management of extraaxial central nervous system infections in children. *J Neurosurg Pediatr* 2011;**7**:441–51.
124. Hakim H, Flynn PM, Knapp KM, et al. Etiology and clinical course of febrile neutropenia in children with cancer. *J Pediatr Hematol Oncol* 2009;**31**:623–9.
125. Hamada S, Masuda N, Kotani S. Isolation and serotyping of *Streptococcus mutans* from teeth and feces of children. *J Clin Microbiol* 1980;**11**:314–8.
126. Han XY, Kamana M, Rolston KV. Viridans streptococci isolated by culture from blood of cancer patients: clinical and microbiologic analysis of 50 cases. *J Clin Microbiol* 2006;**44**:160–5.
127. Hanage WP, Cohen J. Stimulation of cytokine release and adhesion molecule expression by products of viridans streptococci. *J Infect Dis* 2002;**185**:357–67.
128. Heiro M, Helenius H, Makila S, et al. Infective endocarditis in a Finnish teaching hospital: a study on 326 episodes treated during 1980-2004. *Heart* 2006;**92**:1457–62.
129. Henslee J, Bostrom B, Weisdorf D, et al. Streptococcal sepsis in bone marrow transplant patients. *Lancet* 1984;**1**:393.
130. Herskovitz MY, Goldsher D, Finkelstein R, et al. Multiple brain abscesses associated with tongue piercing. *Arch Neurol* 2009;**66**: 1292.
131. Hess J, Dankert J, Durack D. Significance of penicillin tolerance in vivo: prevention of experimental *Streptococcus sanguis* endocarditis. *J Antimicrob Chemother* 1983;**11**:555–64.
132. Hildebrandt G, Lee I, Hodges J. Oral mutans streptococci levels following use of a xylitol mouth rinse: a double-blind, randomized, controlled clinical trial. *Spec Care Dentist* 2010;**30**:53–8.
133. Hirschfeld Z, Friedman M, Golomb G, et al. New sustained release dosage form of chlorhexidine for dental use: use for plaque control in partial denture wearers. *J Oral Rehabil* 1984;**11**:477–82.
134. Hoecker JL, Pickering LK, Groschel D. *Streptococcus salivarius* sepsis in children with malignancies. *J Pediatr* 1978;**92**:337–8.
135. Hogg SD, Manning JE. Inhibition of adhesion of viridans streptococci to fibronectin-coated hydroxyapatite beads by lipoteichoic acid. *J Appl Bacteriol* 1988;**65**:483–9.
136. Holbrook WP, Olafsdottir D, Magnusson HB, et al. Penicillin tolerance among oral streptococci. *J Med Microbiol* 1988;**27**: 17–22.
137. Holmberg H, Danielsson D, Hardie J, Krook A, Whiley R. Cross-reactions between alpha-streptococci and Omniserum, a polyvalent pneumococcal serum, demonstrated by direct immunofluorescence, immunoelectroosmophoresis, and latex agglutination. *J Clin Microbiol* 1985;**21**:745–8.
138. Hook EW, III, Sande MA. Role of the vegetation in experimental *Streptococcus viridans* endocarditis. *Infect Immun* 1974;**10**:1433–8.
139. Hsu RB, Lin FY. Effect of penicillin resistance on presentation and outcome of nonenterococcal streptococcal infective endocarditis. *Cardiology* 2006;**105**:234–9.
140. Huang WT, Chang LY, Hsueh PR, et al. Clinical features and complications of viridans streptococci bloodstream infection in pediatric hemato-oncology patients. *J Microbiol Immunol Infect* 2007;**40**:349–54.
141. Inoue S, Boyer D, Gordon R. Interstitial pneumonia and alpha-hemolytic streptococcus sepsis in a child with malignancy who recently received cytosine arabinoside. *Pediatr Infect Dis J* 1990;**9**: 598–600.
142. Ip M, Chau SS, Chi F, et al. Fluoroquinolone resistance in atypical pneumococci and oral streptococci: evidence of horizontal gene transfer of fluoroquinolone resistance determinants from *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2007;**51**: 2690–700.
143. Jaffe D, Jakubowski A, Sepkowitz K, et al. Prevention of peritransplantation viridans streptococcal bacteremia with early vancomycin administration: a single-center observational cohort study. *Clin Infect Dis* 2004;**39**:1625–32.
144. James J, MacFarlane TW, McGowan DA, et al. Failure of post-bacteraemia delayed antibiotic prophylaxis of experimental rabbit endocarditis. *J Antimicrob Chemother* 1987;**20**:883–5.
145. Janoir C, Podglajen I, Kitzis MD, et al. In vitro exchange of fluoroquinolone resistance determinants between *Streptococcus*

pneumoniae and viridans streptococci and genomic organization of the parE-parC region in *S. mitis*. *J Infect Dis* 1999;**180**:555–8.
146. Jarvinen H, Tenovuo J, Huovinen P. In vitro susceptibility of *Streptococcus mutans* to chlorhexidine and six other antimicrobial agents. *Antimicrob Agents Chemother* 1993;**37**:1158–9.
147. Jerng JS, Hsueh PR, Teng LJ, et al. Empyema thoracis and lung abscess caused by viridans streptococci. *Am J Respir Crit Care Med* 1997;**156**:1508–14.
148. Jones RN, Kohno S, Ono Y, et al. ZAAPS International Surveillance Program (2007) for linezolid resistance: results from 5591 gram-positive clinical isolates in 23 countries. *Diagn Microbiol Infect Dis* 2009;**64**:191–201.
149. Jonsson M, Swedberg G. Macrolide resistance can be transferred by conjugation from viridans streptococci to *Streptococcus pyogenes*. *Int J Antimicrob Agents* 2006;**28**:101–3.
150. Jordan HV, De Paola PF. Effect of a topically applied 3 percent vancomycin gel on *Streptococcus mutans* on different tooth surfaces. *J Dent Res* 1974;**53**:115–20.
151. Kanasi E, Johansson I, Lu SC, et al. Microbial risk markers for childhood caries in pediatricians' offices. *J Dent Res* 2010;**89**: 378–83.
152. Kaufhold A, Potgieter E. Chromosomally mediated high-level gentamicin resistance in *Streptococcus mitis*. *Antimicrob Agents Chemother* 1993;**37**:2740–2.
153. Kawamura Y, Hou XG, Sultana F, et al. Transfer of *Streptococcus adjacens* and *Streptococcus defectivus* to *Abiotrophia* gen. nov. as *Abiotrophia adiacens* comb. nov. and *Abiotrophia defectiva* comb. nov., respectively. *Int J Syst Bacteriol* 1995;**45**:798–803.
154. Kennedy HF, Gemmell CG, Bagg J, Gibson BE, Michie JR. Antimicrobial susceptibility of blood culture isolates of viridans streptococci: relationship to a change in empirical antibiotic therapy in febrile neutropenia. *J Antimicrob Chemother* 2001;**47**:693–6.
155. Armitage HT, McWhinney PH. Activity of quinolones against viridans group streptococci isolated from blood cultures of patients with haematological malignancy. *Support Care Cancer* 1999;**7**:28–30.
156. Kikuchi K, Enari T, Minami S, et al. Postantibiotic effects and postantibiotic sub-MIC effects of benzylpenicillin on viridans streptococci isolated from patients with infective endocarditis. *J Antimicrob Chemother* 1994;**34**:687–96.
157. Kilpper-Balz R, Schleifer K-H. Transfer of *Streptococcus morbillorum* to the genus *Gemella* as *Gemella haemolysans*. *Int J Syst Bacteriol* 1988;**38**:442–3.
158. Klastersky J. Role of quinupristin/dalfopristin in the treatment of gram-positive nosocomial infections in haematological or oncological patients. *Cancer Treat Rev* 2003;**29**:431–40.
159. Knoll B, Tleyjeh IM, Steckelberg JM, et al. Infective endocarditis due to penicillin-resistant viridans group streptococci. *Clin Infect Dis* 2007;**44**:1585–92.
160. Kontiainen S, Sivonen A. Multiply resistant *Streptococcus mitis* isolated from conjunctival exudate of newborns. *Eur J Clin Microbiol* 1987;**6**:53–5.
161. Kuramitsu HK, Wang BY. The whole is greater than the sum of its parts: dental plaque bacterial interactions can affect the virulence properties of cariogenic *Streptococcus mutans*. *Am J Dent* 2011;**24**:153–4.
162. Kurland S, Enghoff E, Landelius J, et al. A 10-year retrospective study of infective endocarditis at a university hospital with special regard to the timing of surgical evaluation in *S. viridans* endocarditis. *Scand J Infect Dis* 1999;**3**:87–91.
163. Larsen T, Fiehn NE. Resistance of *Streptococcus sanguis* biofilms to antimicrobial agents. *APMIS* 1996;**104**:280–4.
164. Leblanc T, Leverger G, Arlet G, et al. Frequency and severity of systemic infections caused by *Streptococcus mitis* and *sanguis* II in neutropenic children. *Pathol Biol (Paris)* 1989;**37**:459–64.
165. Lee EY, Khatwa U, McAdam AJ, et al. *Streptococcus milleri* group pleuropulmonary infection in children: computed tomographic findings and clinical features. *J Comput Assist Tomogr* 2010;**34**: 927–32.
166. Lee SY. Postantibiotic effects and postantibiotic sub-MIC effects of amoxicillin on *Streptococcus gordonii* and *Streptococcus sanguis*. *J Chemother* 2000;**12**:379–84.
167. Lee WS, Choi ST, Kim KK. Splenic abscess: a single institution study and review of the literature. *Yonsei Med J* 2011;**52**:288–92.
168. Leegaard TM, Bootsma HJ, Caugant DA, et al. Phenotypic and genomic characterization of pneumococcus-like streptococci isolated from HIV-seropositive patients. *Microbiology* 2010;**156**: 838–48.
169. Leviner E, Tzukert AA, Benoliel R, et al. Development of resistant oral viridans streptococci after administration of prophylactic antibiotics: time management in the dental treatment of patients susceptible to infective endocarditis. *Oral Surg Oral Med Oral Pathol* 1987;**64**:417–20.
170. Levy CS, Kogulan P, Gill VJ, et al. Endocarditis caused by penicillin-resistant viridans streptococci: 2 cases and controversies in therapy. *Clin Infect Dis* 2001;**33**:577–9.
171. Lilien LD, Wilks AK, Yeh TF. *Streptococcus sanguis* biotype II meningitis in a premature infant. *Clin Pediatr (Phila)* 1982; **21**:465.
172. Lindgren M, Jalava J, Rantakokko-Jalava K, et al. In vitro susceptibility of viridans group streptococci isolated from blood in southwest Finland in 1993-2004. *Scand J Infect Dis* 2007;**39**: 508–13.
173. Loesche WJ, Schork A, Terpenning MS, et al. Assessing the relationship between dental disease and coronary heart disease in elderly U.S. veterans. *J Am Dent Assoc* 1998;**129**:301–11.
174. Lowrance JH, Baddour LM, Simpson WA. The role of fibronectin binding in the rat model of experimental endocarditis caused by *Streptococcus sanguis*. *J Clin Invest* 1990;**86**:7–13.
175. Lowy FD, Chang DS, Neuhaus EG, et al. Effect of penicillin on the adherence of *Streptococcus sanguis* in vitro and in the rabbit model of endocarditis. *J Clin Invest* 1983;**71**:668–75.
176. Lu CH, Chang WN, Chang HW. Adults with meningitis caused by viridans streptococci. *Infection* 2001;**29**:305–9.
177. Lu HZ, Weng XH, Zhu B, et al. Major outbreak of toxic shock–like syndrome caused by *Streptococcus mitis*. *J Clin Microbiol* 2003; **41**:3051–5.
178. Macaluso A, Simmang C, Anthony T. *Streptococcus sanguis* bacteremia and colorectal cancer. *South Med J* 1998;**91**:206–7.
179. Maeda Y, Goldsmith CE, Coulter WA, et al. Comparison of minimum inhibitory concentration by broth microdilution testing versus standard disc diffusion testing in the detection of penicillin, erythromycin and ciprofloxacin resistance in viridans group streptococci. *J Med Microbiol* 2011;**60**:1782–6.
180. Malinverni R, Bille J, Glauser MP. Single-dose rifampin prophylaxis for experimental endocarditis induced by high bacterial inocula of viridans streptococci. *J Infect Dis* 1987;**156**:151–7.
181. Malmberg E, Birkhed D, Norvenius G, et al. Microorganisms on toothbrushes at day-care centers. *Acta Odontol Scand* 1994;**52**: 93–8.
182. Manning JE, Hume EB, Hunter N, et al. An appraisal of the virulence factors associated with streptococcal endocarditis. *J Med Microbiol* 1994;**40**:110–14.
183. Marom D, Levy I, Gutwein O, et al. Healthcare-associated versus community-associated infective endocarditis in children. *Pediatr Infect Dis J* 2011;**30**:585–8.
184. Marrie TJ. Bacteremic community-acquired pneumonia due to viridans group streptococci. *Clin Invest Med* 1993;**16**:38–44.
185. Marron A, Carratala J, Alcaide F, et al. High rates of resistance to cephalosporins among viridans-group streptococci causing bacteraemia in neutropenic cancer patients. *J Antimicrob Chemother* 2001;**47**:87–91.
186. Marron A, Carratala J, Gonzalez-Barca E, et al. Serious complications of bacteremia caused by viridans streptococci in neutropenic patients with cancer. *Clin Infect Dis* 2000;**31**:1126–30.
187. Marsh PD. Antimicrobial strategies in the prevention of dental caries. *Caries Res* 1993;**27**(Suppl. 1):72–6.
188. Martinez F, Martin-Luengo F, Garcia A, et al. Treatment with imipenem of experimental endocarditis caused by penicillin-resistant *Streptococcus sanguis*. *J Antimicrob Chemother* 1994;**33**: 1201–7.
189. Martino R, Subira M, Manteiga R, et al. Viridans streptococcal bacteremia and viridans streptococcal shock syndrome in neutropenic patients: comparison between children and adults receiving chemotherapy or undergoing bone marrow transplantation. *Clin Infect Dis* 1995;**20**:476–7.
190. Matsushita K, Fujimaki W, Kato H, et al. Immunopathological activities of extracellular products of *Streptococcus mitis*, particularly a superantigenic fraction. *Infect Immun* 1995;**63**:785–93.
191. McBride ME, Duncan WC, Knox JM. Bacterial interference of *Neisseria gonorrhoeae* by alpha-haemolytic streptococci. *Br J Vener Dis* 1980;**56**:235–8.

192. McWhinney PH, Gillespie SH, Kibbler CC, et al. *Streptococcus mitis* and ARDS in neutropenic patients. *Lancet* 1991;**337**:429.
193. Mecrow IK, Ladusans EJ. Infective endocarditis in newborn infants with structurally normal hearts. *Acta Paediatr* 1994;**83**: 35–9.
194. Meddens MJ, Thompson J, Eulderink F, et al. Role of granulocytes in experimental *Streptococcus sanguis* endocarditis. *Infect Immun* 1982;**36**:325–32.
195. Meddens MJ, Thompson J, Mattie H, et al. Role of granulocytes in the prevention and therapy of experimental *Streptococcus sanguis* endocarditis in rabbits. *Antimicrob Agents Chemother* 1984;**25**: 263–7.
196. Menichetti F, Del Favero A, Guerciolini R, et al. Viridans streptococci septicemia in cancer patients: a clinical study. *Eur J Epidemiol* 1987;**3**:316–8.
197. Meyer K, Geurtsen W, Gunay H. An early oral health care program starting during pregnancy: results of a prospective clinical long-term study. *Clin Oral Investig* 2010;**14**:257–64.
198. Mghir AS, Cremieux AC, Jambou R, et al. Dextranase enhances antibiotic efficacy in experimental viridans streptococcal endocarditis. *Antimicrob Agents Chemother* 1994;**38**:953–8.
199. Michalek SM, McGhee JR, Mestecky J, et al. Ingestion of *Streptococcus mutans* induces secretory immunoglobulin A and caries immunity. *Science* 1976;**192**:1238–40.
200. Michel RS, DeFlora E, Jefferies J, et al. Recurrent meningitis in a child with inner ear dysplasia. *Pediatr Infect Dis J* 1992;**11**: 336–8.
201. Moet GJ, Dowzicky MJ, Jones RN. Tigecycline (GAR-936) activity against *Streptococcus gallolyticus (bovis)* and viridans group streptococci. *Diagn Microbiol Infect Dis* 2007;**57**:333–6.
202. Mogi A, Nishi JI, Yoshinaga M, et al. Increased prevalence of penicillin-resistant viridans group streptococci in Japanese children with upper respiratory infection treated by beta-lactam agents and in those with oncohematologic diseases. *Pediatr Infect Dis* 1997;**16**:1140–4.
203. Molina JM, Leport C, Bure A, et al. Clinical and bacterial features of infections caused by *Streptococcus milleri*. *Scand J Infect Dis* 1991;**23**:659–66.
204. Monefeldt K, Helgeland K, Tollefsen T. In vitro activation of the classical pathway of complement by a streptococcal lipoteichoic acid. *Oral Microbiol Immunol* 1994;**9**:70–6.
205. Moomjian AS, Sokal MM, Vijayan S. Pathogenicity of alpha hemolytic streptococci in the neonate. *Am J Perinatol* 1984;**1**: 319–21.
206. Morisaki I, Mihara J, Kato K, et al. Phenytoin-induced gingival overgrowth in rats infected with *Streptococcus sobrinus* 6715. *Arch Oral Biol* 1990;**35**:753–8.
207. Murray HW, Gross KC, Masur H, et al. Serious infections caused by *Streptococcus milleri*. *Am J Med* 1978;**64**:759–64.
208. Napenas JJ, Brennan MT, Coleman S, et al. Molecular methodology to assess the impact of cancer chemotherapy on the oral bacterial flora: a pilot study. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010;**109**:554–60.
209. Nascimento-Carvalho CM, Brandileone MC, Guerra ML, et al. Do viridans streptococci cause pneumonia in children? *Pediatr Infect Dis J* 2001;**20**:726–8.
210. Newbrun E. Preventing dental caries: breaking the chain of transmission. *J Am Dent Assoc* 1992;**123**:55–9.
211. Ng KH, Lee S, Yip SF, et al. A case of *Streptococcus mitis* endocarditis successfully treated by linezolid. *Hong Kong Med J* 2005; **11**:411–3.
212. Nomura R, Nakano K, Makela K, et al. Isolation and characterization of *Streptococcus mitis* from blood of child with osteomyelitis. *Int J Paediatr Dent* 2011;**21**:192–9.
213. Ohuoba EF, Kansal RG, Hayden RT, et al. Failure of viridans group streptococci causing bacteremia in pediatric oncology patients to express superantigens. *J Pediatr Hematol Oncol* 2006; **28**:627–9.
214. Okamoto Y, Ribeiro RC, Srivastava DK, et al. Viridans streptococcal sepsis: clinical features and complications in childhood acute myeloid leukemia. *J Pediatr Hematol Oncol* 2003;**25**:696–703.
215. Orlicek SL, Branum KC, English BK, et al. Viridans streptococcal isolates from patients with septic shock induce tumor necrosis factor-alpha production by murine macrophages. *J Lab Clin Med* 1997;**130**:515–19.
216. Palacio F, Lewis JS, Sadkowski L, et al. Breakthrough bacteremia and septic shock due to *Streptococcus anginosus* resistant to daptomycin in a patient receiving daptomycin therapy. *Antimicrob Agents Chemother* 2011;**55**:3639–40.
217. Pan YP, Li Y, Caufield PW. Phenotypic and genotypic diversity of *Streptococcus sanguis* in infants. *Oral Microbiol Immunol* 2001; **16**:235–42.
218. Parkins MD, Sibley CD, Surette MG, et al. The *Streptococcus milleri* group—an unrecognized cause of disease in cystic fibrosis: a case series and literature review. *Pediatr Pulmonol* 2008;**43**: 490–7.
219. Parrillo JE, Borst GC, Mazur MH, et al. Endocarditis due to resistant viridans streptococci during oral penicillin chemoprophylaxis. *N Engl J Med* 1979;**300**:296–300.
220. Parrish LC, Kretzschmar DP, Swan RH. Osteomyelitis associated with chronic periodontitis: a report of three cases. *J Periodontol* 1989;**60**:716–22.
221. Pearce C, Bowden GH, Evans M, et al. Identification of pioneer viridans streptococci in the oral cavity of human neonates. *J Med Microbiol* 1995;**42**:67–72.
222. Peiris V, Oppenheim BA. Antimicrobial activity of cytotoxic drugs may influence isolation of bacteria and fungi from blood cultures. *J Clin Pathol* 1993;**46**:1124–5.
223. Persson L, Vikerfors T, Sjoberg L, et al. Increased incidence of bacteraemia due to viridans streptococci in an unselected population of patients with acute myeloid leukaemia. *Scand J Infect Dis* 2000;**32**:615–21.
224. Peterson EM, Shigei JT, Woolard A, et al. Identification of viridans streptococci by three commercial systems. *Am J Clin Pathol* 1988;**90**:87–91.
225. Petti CA, Simmon KE, Bender J, et al. Culture-negative intracerebral abscesses in children and adolescents from *Streptococcus anginosus* group infection: a case series. *Clin Infect Dis* 2008;**46**: 1578–80.
226. Pfaller MA, Jones RN. In vitro evaluation of contemporary beta-lactam drugs tested against viridans group and beta-haemolytic streptococci. *Diagn Microbiol Infect Dis* 1997;**27**:151–4.
227. Pizzo PA, Ladisch S, Ribichaud K. Treatment of gram-positive septicemia in cancer patients. *Cancer* 1980;**45**:206–7.
228. Pizzo PA, Ladisch S, Witebsky FG. Alpha-hemolytic, streptococci: clinical significance in the cancer patient. *Med Pediatr Oncol* 1978;**4**:367–70.
229. Poole PM, Wilson G. *Streptococcus milleri* in the appendix. *J Clin Pathol* 1977;**30**:937–42.
230. Potgieter E, Carmichael M, Koornhof HJ, et al. In vitro antimicrobial susceptibility of viridans streptococci isolated from blood cultures. *Eur J Clin Microbiol Infect Dis* 1992;**11**:543–6.
231. Potgieter E, Chalkley LJ. Reciprocal transfer of penicillin resistance genes between *Streptococcus pneumoniae*, *Streptococcus mitior* and *Streptococcus sanguis*. *J Antimicrob Chemother* 1991;**28**: 463–5.
232. Prabhu RM, Piper KE, Baddour LM, et al. Antimicrobial susceptibility patterns among viridans group streptococcal isolates from infective endocarditis patients from 1971 to 1986 and 1994 to 2002. *Antimicrob Agents Chemother* 2004;**48**:4463–5.
233. Prabhu RM, Piper KE, Litzow MR, et al. Emergence of quinolone resistance among viridans group streptococci isolated from the oropharynx of neutropenic peripheral blood stem cell transplant patients receiving quinolone antimicrobial prophylaxis. *Eur J Clin Microbiol Infect Dis* 2005;**24**:832–8.
234. Pratter MR, Irwin RS. Viridans streptococcal pulmonary parenchymal infections. *JAMA* 1980;**243**:2515–7.
235. Pulliam L, Dall L, Inokuchi S, et al. Effects of exopolysaccharide production by viridans streptococci on penicillin therapy of experimental endocarditis. *J Infect Dis* 1985;**151**:153–6.
236. Qi F, Kreth J. Characterization of anti-competitor activities produced by oral bacteria. *Methods Mol Biol* 2010;**666**:151–66.
237. Quinn JP, DiVincenzo CA, Lucks DA, et al. Serious infections due to penicillin-resistant strains of viridans streptococci with altered penicillin-binding proteins. *J Infect Dis* 1988;**157**:764–9.
238. Rapeport KB, Giron JA, Rosner F. *Streptococcus mitis* endocarditis. Report of 17 cases. *Arch Intern Med* 1986;**146**:2361–3.
239. Razonable RR, Litzow MR, Khaliq Y, et al. Bacteremia due to viridans group streptococci with diminished susceptibility to levofloxacin among neutropenic patients receiving levofloxacin prophylaxis. *Clin Infect Dis* 2002;**34**:1469–74.

240. Reed E, Arneson M, Vaughan W, et al. *Streptcoccus viridans:* a significant cause of neutropenic fever that caused death in some patients not empirically treated for gram-positive bacteria. *Proc Annu Meet Am Soc Clin Oncol* 1990;**9**:A1239.
241. Ribner BS, Freimer EH. Osteomyelitis caused by viridans streptococci. *Arch Intern Med* 1982;**142**:1739.
242. Richard P, Amador Del Valle G, Moreau P, et al. Viridans streptococcal bacteraemia in patients with neutropenia. *Lancet* 1995; **345**:1607–9.
243. Ringden O, Heimdahl A, Lonnqvist B, et al. Decreased incidence of viridans streptococcal septicaemia in allogeneic bone marrow transplant recipients after the introduction of acyclovir. *Lancet* 1984;**1**:744.
244. Roberts RB, Krieger AG, Schiller NL, et al. Viridans streptococcal endocarditis: the role of various species, including pyridoxal-dependent streptococci. *Rev Infect Dis* 1979;**1**:955–66.
245. Rolston KV, Frisbee-Hume S, LeBlanc B, et al. In vitro antimicrobial activity of moxifloxacin compared to other quinolones against recent clinical bacterial isolates from hospitalized and community-based cancer patients. *Diagn Microbiol Infect Dis* 2003; **47**:441–9.
246. Rolston KV, Vaziri I, Frisbee-Hume S, et al. In vitro antimicrobial activity of gatifloxacin compared with other quinolones against clinical isolates from cancer patients. *Chemotherapy* 2004;**50**: 214–20.
247. Rolston KV, Yadegarynia D, Kontoyiannis DP, et al. The spectrum of gram-positive bloodstream infections in patients with hematologic malignancies, and the in vitro activity of various quinolones against gram-positive bacteria isolated from cancer patients. *Int J Infect Dis* 2006;**10**:223–30.
248. Roos K, Holm SE, Grahn E, et al. Alpha-streptococci as supplementary treatment of recurrent streptococcal tonsillitis: a randomized placebo-controlled study. *Scand J Infect Dis* 1993;**25**:31–5.
249. Roos K, Holm SE, Grahn-Hakansson E, et al. Recolonization with selected alpha-streptococci for prophylaxis of recurrent streptococcal pharyngotonsillitis—a randomized placebo-controlled multicentre study. *Scand J Infect Dis* 1996;**28**:459–62.
250. Rose HD. Viridans streptococcal pneumonia. *JAMA* 1981;**245**:32.
251. Rosser SJ, Alfa MJ, Hoban S, et al. E test versus agar dilution for antimicrobial susceptibility testing of viridans group streptococci. *J Clin Microbiol* 1999;**37**:26–30.
252. Rozenberg-Arska M, Dekker A, Verdonck L, et al. Prevention of bacteremia caused by alpha-hemolytic streptococci by roxithromycin (RU-28965) in granulocytopenic patients receiving ciprofloxacin. *Infection* 1989;**17**:240–4.
253. Rubin M, Hathorn JW, Marshall D, et al. Gram-positive infections and the use of vancomycin in 550 episodes of fever and neutropenia. *Ann Intern Med* 1988;**108**:30–5.
254. Rubin MM, Sanfilippo RJ, Sadoff RS. Vertebral osteomyelitis secondary to an oral infection. *J Oral Maxillofac Surg* 1991;**49**: 897–900.
255. Rudney JD, Larson CJ. Identification of oral mitis group streptococci by arbitrarily primed polymerase chain reaction. *Oral Microbiol Immunol* 1999;**14**:33–42.
256. Ruoff KL. Streptococcus. In: Murray PR, Baron EJ, Pfaller MA, editors. *Manual of Clinical Microbiology, ed. 6*. Washington, DC: American Society for Microbiology; 1995. p. 299–307.
257. Ruoff KL, Kunz LJ. Identification of viridans streptococci isolated from clinical specimens. *J Clin Microbiol* 1982;**15**:920–5.
258. Ruoff KL, Kunz LJ. Use of the Rapid STREP system for identification of viridans streptococcal species. *J Clin Microbiol* 1983;**18**: 1138–40.
259. Saarela M, von Troil-Linden B, Torkko H, et al. Transmission of oral bacterial species between spouses. *Oral Microbiol Immunol* 1993;**8**:349–54.
260. Sader HS, Streit JM, Fritsche TR, et al. Antimicrobial susceptibility of gram-positive bacteria isolated from European medical centres: results of the Daptomycin Surveillance Programme (2002-2004). *Clin Microbiol Infect* 2006;**12**:844–52.
261. Salavert M, Gomez L, Rodriguez-Carballeira M, et al. Seven-year review of bacteremia caused by *Streptococcus milleri* and other viridans streptococci. *Eur J Clin Microbiol Infect Dis* 1996;**15**: 365–71.
262. Sandlund JT, Howard SC, Hijiya N, et al. Myositis complicating viridans streptococcal sepsis in childhood leukemia. *Pediatr Blood Cancer* 2005;**44**:277–9.
263. Santagati M, Scillato M, Patane F, et al. Bacteriocin-producing oral streptococci and inhibition of respiratory pathogens. *FEMS Immunol Med Microbiol* 2012;**65**:23–31.
264. Sarkar TK, Murarka RS, Gilardi GL. Primary *Streptococcus viridans* pneumonia. *Chest* 1989;**96**:831–4.
265. Sato Y, Okuya M, Hagisawa S, et al. Viridans streptococcal bacteremia-related encephalopathy in childhood with malignancy. *Pediatr Hematol Oncol* 2011;**28**:24–30.
266. Schmitz FJ, Fisher A, Boos M, et al. Quinolone-resistance mechanisms and in vitro susceptibility patterns among European isolates of *Streptococcus mitis*, *Streptococcus sanguis*, and *Streptococcus pneumoniae*. *Eur J Clin Microbiol Infect Dis* 2001;**20**:219–22.
267. Schneeberger PM, Janssen M, Voss A. Alpha-hemolytic streptococci: a major pathogen of iatrogenic meningitis following lumbar puncture. Case reports and a review of the literature. *Infection* 1996;**24**:29–33.
268. Schollin J. Adherence of alpha-hemolytic streptococci to human endocardial, endothelial and buccal cells. *Acta Paediatr Scand* 1988;**77**:705–10.
269. Schollin J, Danielsson D. Bacterial adherence to endothelial cells from rat heart, with special regard to alpha-hemolytic streptococci. *APMIS* 1988;**96**:428–32.
270. Seki M, Karakama F, Kawato T, et al. Effect of xylitol gum on the level of oral mutans streptococci of preschoolers: block-randomised trial. *Int Dent J* 2011;**61**:274–80.
271. Shanson DC, Thomas F, Wilson D. Effect of volume of blood cultured on detection of *Streptococcus viridans* bacteraemia. *J Clin Pathol* 1984;**37**:568–70.
272. Shanson DC, Thomas FD, Johnstone D. Improving detection of "viridans streptococcus" bacteraemia by adding sodium polyanethol sulphonate to blood cultures. *J Clin Pathol* 1985;**38**:1346–8.
273. Shaw JH. Causes and control of dental caries. *N Engl J Med* 1987;**317**:996–1004.
274. Shelburne SA III, Greenberg SB, Aslam S, et al. Successful ceftriaxone therapy of endocarditis due to penicillin non-susceptible viridans streptococci. *J Infect* 2007;**54**:e99–101.
275. Shenep JL, Hughes WT, Roberson PK, et al. Vancomycin, ticarcillin, and amikacin compared with ticarcillin-clavulanate and amikacin in the empirical treatment of febrile, neutropenic children with cancer. *N Engl J Med* 1988;**319**:1053–8.
276. Shibl AM. Effect of antibiotics on adherence of microorganisms to epithelial cell surfaces. *Rev Infect Dis* 1985;**7**:51–65.
277. Shlaes DM, Marino J, Jacobs MR. Infection caused by vancomycin-resistant *Streptococcus sanguis* II. *Antimicrob Agents Chemother* 1984;**25**:527–8.
278. Shulman ST. Streptococcus viridans—not! *Am J Dis Child* 1993; **147**:611.
279. Sibley CD, Parkins MD, Rabin HR, et al. A polymicrobial perspective of pulmonary infections exposes an enigmatic pathogen in cystic fibrosis patients *Proc Natl Acad Sci U S A* 2008;**105**: 15070–5.
280. Smith DJ, Anderson JM, King WF, et al. Oral streptococcal colonization of infants. *Oral Microbiol Immunol* 1993;**8**:1–4.
281. Sobottka I, Wegscheider K, Balzer L, et al. Microbiological analysis of a prospective, randomized, double-blind trial comparing moxifloxacin and clindamycin in the treatment of odontogenic infiltrates and abscesses. *Antimicrob Agents Chemother* 2012;**56**: 2565–9.
282. Soell M, Holveck F, Scholler M, et al. Binding of *Streptococcus mutans* SR protein to human monocytes: production of tumor necrosis factor, interleukin 1, and interleukin 6. *Infect Immun* 1994;**62**:1805–12.
283. Sotiropoulos SV, Jackson MA, Woods GM, et al. Alpha-streptococcal septicemia in leukemic children treated with continuous or large dosage intermittent cytosine arabinoside. *Pediatr Infect Dis J* 1989;**8**:755–8.
284. Soto A, Evans TJ, Cohen J. Proinflammatory cytokine production by human peripheral blood mononuclear cells stimulated with cell-free supernatants of viridans streptococci. *Cytokine* 1996;**8**: 300–4.
285. Soto A, McWhinney PH, Kibbler CC, et al. Cytokine release and mitogenic activity in the viridans streptococcal shock syndrome. *Cytokine* 1998;**10**:370–6.
286. Spanik S, Trupl J, Kunova A, et al. Viridans streptococcal bacteraemia due to penicillin-resistant and penicillin-sensitive streptococci: analysis of risk factors and outcome in 60 patients from a

single cancer centre before and after penicillin is used for prophylaxis. *Scand J Infect Dis* 1997;**29**:245–9.
287. Speck WT, Spear SS, Krongrad E, et al. Transient bacteremia in pediatric patients after dental extraction. *Am J Dis Child* 1976; **130**:406–7.
288. Sprunt K, Redman W, Leidy G. Penicillin resistant alpha streptococci in pharynx of patients given oral penicillin. *Pediatrics* 1968;**42**:957–68.
289. Staves E, Tinanoff N. Decline in salivary *S. mutans* levels in children who have received short-term antibiotic therapy. *Pediatr Dent* 1991;**13**:176–8.
290. Steiner M, Villablanca J, Kersey J, et al. Viridans streptococcal shock in bone marrow transplantation patients. *Am J Hematol* 1993;**42**:354–8.
291. Stelzmueller I, Aigner F, Albright J, et al. Group milleri streptococci in perianal infections. *Colorectal Dis* 2010;**12**:e121–e127.
292. Stelzmueller I, Berger N, Wiesmayr S, et al. Group milleri streptococci: significant pathogens in solid organ recipients. *Transpl Int* 2007;**20**:51–6.
293. Stimmel HM, Orchen JJ, Skaff DM, et al. Penicillin-resistant alpha-hemolytic streptococci in children with heart disease who take penicillin daily. *ASDC J Dent Child* 1981;**48**:29–32.
294. Sullam PM, Frank U, Yeaman MR, et al. Effect of thrombocytopenia on the early course of streptococcal endocarditis. *J Infect Dis* 1993;**168**:910–4.
295. Sussman JI, Baron EJ, Tenenbaum MJ, et al. Viridans streptococcal endocarditis: clinical, microbiological, and echocardiographic correlations. *J Infect Dis* 1986;**154**:597–603.
296. Svinhufvud LB, Heimdahl A, Nord CE. Effect of topical administration of vancomycin versus chlorhexidine on alpha-hemolytic streptococci in oral cavity. *Oral Med* 1988;**66**:304–9.
297. Takada H, Kawabata Y, Tamura M, et al. Cytokine induction by extracellular products of oral viridans group streptococci. *Infect Immun* 1993;**61**:5252–60.
298. Takahashi N, Horiuchi M, Yamada T. Effects of acidification on growth and glycolysis of *Streptococcus sanguis* and *Streptococcus mutans*. *Oral Microbiol Immunol* 1997;**12**:72–6.
299. Tan LK, Lacey S, Mandalia S, et al. Hospital-based study of viridans streptococcal bacteraemia in children and adults. *J Infect* 2008;**56**:103–7.
300. Tano K, Olofsson C, Grahn-Hakansson E, et al. In vitro inhibition of *S. pneumoniae*, nontypable *H. influenzae* and *M. catharralis* by alpha-hemolytic streptococci from healthy children. *Int J Pediatr Otorhinolaryngol* 1999;**47**:49–56.
301. Tappuni AR, Challacombe SJ. Distribution and isolation frequency of eight streptococcal species in saliva from predentate and dentate children and adults. *J Dent Res* 1993;**72**:31–6.
302. Tasaka T, Nagai M, Sasaki K, et al. *Streptococcus mitis* septicemia in leukemia patients; clinical features and outcome. *Intern Med* 1993;**32**:221–4.
303. Teles C, Smith A, Ramage G, et al. Identification of clinically relevant viridans group streptococci by phenotypic and genotypic analysis. *Eur J Clin Microbiol Infect Dis* 2011;**30**:243–50.
304. Thibodeau EA, O'Sullivan DM. Salivary mutans streptococci and incidence of caries in preschool children. *Caries Res* 1995;**29**: 148–53.
305. Thorig L, Daha MR, Eulderink F, et al. Experimental *Streptococcus sanguis* endocarditis: immune complexes and renal involvement. *Clin Exp Immunol* 1980;**40**:469–77.
306. Timmers GJ, Dijstelbloem Y, Simoons-Smit AM, et al. Pharmacokinetics and effects on bowel and throat microflora of oral levofloxacin as antibacterial prophylaxis in neutropenic patients with haematological malignancies. *Bone Marrow Transplant* 2004; **33**:847–53.
307. Timmers GJ, Simoons-Smit AM, Leidekker ME, et al. Levofloxacin vs. ciprofloxacin plus phenethicillin for the prevention of bacterial infections in patients with haematological malignancies. *Clin Microbiol Infect* 2007;**13**:497–503.
308. Tinanoff N. Review of the antimicrobial action of stannous fluoride. *J Clin Dent* 1990;**2**:22–7.
309. Tleyjeh IM, Steckelberg JM, Murad HS, et al. Temporal trends in infective endocarditis: a population-based study in Olmsted County, Minnesota. *JAMA* 2005;**293**:3022–8.
310. Tomas I, Alvarez M, Limeres J, et al. Prevalence, duration and aetiology of bacteraemia following dental extractions. *Oral Dis* 2007;**13**:56–62.
311. Tong DC, Rothwell BR. Antibiotic prophylaxis in dentistry: a review and practice recommendations. *J Am Dent Assoc* 2000;**131**: 366–74.
312. Topaloglu-Ak A, Ertugrul F, Eden E, et al. Effect of orthodontic appliances on oral microbiota—6 month follow-up. *J Clin Pediatr Dent* 2011;**35**:433–6.
313. Tunkel AR, Sepkowitz KA. Infections caused by viridans streptococci in patients with neutropenia. *Clin Infect Dis* 2002;**34**: 1524–9.
314. Tuohy M, Washington JA. Antimicrobial susceptibility of viridans group streptococci. *Diagn Microbiol Infect Dis* 1997;**29**:277–80.
315. Twetman S, Mattiasson A, Varela, et al. Mutans streptococci in saliva and dental caries in children living in a high and a low fluoride area. *Oral Microbiol Immunol* 1990;**5**:169–71.
316. Uehara Y, Agematsu K, Kikuchi K, et al. Secretory IgA, salivary peroxidase, and catalase-mediated microbicidal activity during hydrogen peroxide catabolism in viridans streptococci: pathogen coaggregation. *J Infect Dis* 2006;**194**:98–107.
317. Uehara Y, Kikuchi K, Nakamura T, et al. H_2O_2 produced by viridans group streptococci may contribute to inhibition of methicillin-resistant *Staphylococcus aureus* colonization of oral cavities in newborns. *Clin Infect Dis* 2001;**32**:1408–13.
318. Uehara Y, Kikuchi K, Nakamura T, et al. Inhibition of methicillin-resistant *Staphylococcus aureus* colonization of oral cavities in newborns by viridans group streptococci. *Clin Infect Dis* 2001;**32**: 1399–407.
319. Ullman RF, Strampfer MJ, Cunha BA. *Streptococcus mutans* vertebral osteomyelitis. *Heart Lung* 1988;**17**:319–21.
320. Ullsfoss BN, Ogaard B, Arends J, et al. Effect of a combined chlorhexidine and NaF mouthrinse: an in vivo human caries model study. *Scand J Dent Res* 1994;**102**:109–12.
321. van Houte J. Bacterial adherence in the mouth. *Rev Infect Dis* 1983;**5**(Suppl. 4):S659–69.
322. van Houte J. Role of micro-organisms in caries etiology. *J Dent Res* 1994;**73**:672–81.
323. van Loveren C, Buijs JF, ten Cate JM. Similarity of bacteriocin activity profiles of mutans streptococci within the family when the children acquire the strains after the age of 5. *Caries Res* 2000; **34**:481–5.
324. van Loveren C, Van de Plassche-Simons YM, De Soet JJ, et al. Acidogenesis in relation to fluoride resistance of *Streptococcus mutans*. *Oral Microbiol Immunol* 1991;**6**:288–91.
325. Vernier A, Diab M, Soell M, et al. Cytokine production by human epithelial and endothelial cells following exposure to oral viridans streptococci involves lectin interactions between bacteria and cell surface receptors. *Infect Immun* 1996;**64**:3016–22.
326. Vernier-Georgenthum A, Al-Okla S, Gourieux B, et al. Protein I/II of oral viridans streptococci increases expression of adhesion molecules on endothelial cells and promotes transendothelial migration of neutrophils in vitro. *Cell Immunol* 1998;**187**: 145–50.
327. Vicente MV, Olay T, Rodriguez A. Experimental endocarditis caused by *Streptococcus sanguis:* single and combined antibiotic therapy. *Antimicrob Agents Chemother* 1981;**20**:10–4.
328. Wanahita A, Goldsmith EA, Musher DM, et al. Interaction between human polymorphonuclear leukocytes and *Streptococcus milleri* group bacteria. *J Infect Dis* 2002;**185**:85–90.
329. Watanakunakorn C, Pantelakis J. Alpha-hemolytic streptococcal bacteremia: a review of 203 episodes during 1980-1991. *Scand J Infect Dis* 1993;**25**:403–8.
330. Weber M, Gubler J, Fahrer H, et al. Spondylodiscitis caused by viridans streptococci: three cases and a review of the literature. *Clin Rheumatol* 1999;**18**:417–21.
331. Weinberger SJ, Wright GZ. A comparison of *S. mutans* clinical assessment methods. *Pediatr Dent* 1990;**12**:375–9.
332. West PW, Al-Sawan R, Foster HA, et al. Speciation of presumptive viridans streptococci from early onset neonatal sepsis. *J Med Microbiol* 1998;**47**:923–8.
333. Westling K, Julander I, Ljungman P, et al. Viridans group streptococci in blood culture isolates in a Swedish university hospital: antibiotic susceptibility and identification of erythromycin resistance genes. *Int J Antimicrob Agents* 2006;**28**:292–6.
334. Westling K, Ljungman P, Thalme A, et al. *Streptococcus viridans* septicaemia: a comparison study in patients admitted to the departments of infectious diseases and haematology in a university hospital. *Scand J Infect Dis* 2002;**34**:316–9.

335. Wilcox MH, Winstanley TG, Douglas CW, et al. Susceptibility of alpha-haemolytic streptococci causing endocarditis to benzylpenicillin and ten cephalosporins. *J Antimicrob Chemother* 1993; **32**:63–9.
336. Wilson AP, Gaya H. Treatment of endocarditis with teicoplanin: a retrospective analysis of 104 cases. *J Antimicrob Chemother* 1996;**38**:507–21.
337. Wilson M, Patel H, Fletcher J. Susceptibility of biofilms of *Streptococcus sanguis* to chlorhexidine gluconate and cetylpyridinium chloride. *Oral Microbiol Immunol* 1996;**11**:188–92.
338. Wisplinghoff H, Reinert RR, Cornely O, et al. Molecular relationships and antimicrobial susceptibilities of viridans group streptococci isolated from blood of neutropenic cancer patients. *J Clin Microbiol* 1999;**37**:1876–80.
339. Yaniv LG, Potasman I. Iatrogenic meningitis: an increasing role for resistant viridans streptococci? Case report and review of the last 20 years. *Scand J Infect Dis* 2000;**32**:693–6.
340. Yap RL, Mermel LA, Maglio J. Antimicrobial resistance of community-acquired bloodstream isolates of viridans group streptococci. *Infection* 2006;**34**:339–41.
341. Yee Y, Farber B, Mates S. Mechanism of penicillin-streptomycin synergy for clinical isolates of viridans streptococci. *J Infect Dis* 1986;**154**:531–4.
342. Zickert I, Emilson CG, Ekblom K, et al. Prolonged oral reduction of *Streptococcus mutans* in humans after chlorhexidine disinfection followed by fluoride treatment. *Scand J Dent Res* 1987;**95**:315–9.
343. Zito ET, Daneo-Moore L. Transformation of *Streptococcus sanguis* to intrinsic penicillin resistance. *J Gen Microbiol* 1988;**134**: 1237–49.
344. Zuccaro G Jr, Richter JE, Rice TW, et al. Viridans streptococcal bacteremia after esophageal stricture dilation. *Gastrointest Endosc* 1998;**48**:568–73.

CHAPTER 85

Pneumococcal Infections

Stephen I. Pelton • Michael R Jacobs

The pneumococcus continues to be a leading cause of morbidity and mortality in persons of all ages, and its impact on child health is demonstrated by Elliott and Beeson's conclusion that it is one of the five pathogens that account for nearly half of infection-related deaths among children younger than 5 years of age (Fig. 85-1).[142] Pneumococcal infections include serious diseases such as meningitis, bacteremia, and bacteremic pneumonia, as well as milder but more common illnesses, such as community-acquired pneumonia, sinusitis, and otitis media. The causative agent, *Streptococcus pneumoniae*, is found in the nasopharynx of 30 percent of children in industrialized countries and as many as 80 percent of children in developing and emerging nations,* is transmitted mainly through respiratory droplets among children as well as to adult contacts, and was estimated by the World Health Organization as of 2008 to cause nearly 500,000 global deaths annually among children younger than 5 years of age.[586] Many children experience some form of pneumococcal infection (e.g., otitis media or pneumonia), but sepsis or meningitis occur in few. The continued incidence and severity of pneumococcal disease, coupled with significant case-fatality rates despite antimicrobial therapy, and the increasing prevalence of strains of pneumococci resistant to antimicrobial agents serve to underscore the necessity for ongoing surveillance and expanded research into treatment and prevention of pneumococcal infections.

HISTORY

Pasteur and Sternberg, working independently in 1880 and 1881, discovered the pneumococcus. Pasteur called the organism "microbe septicémique de la salive," and Sternberg called it "*Micrococcus pasteri.*" Each researcher recovered pneumococci from rabbits injected with human saliva. Friedlander demonstrated pneumococci in tissue from humans with pneumonia in 1882 and, in the following year, found them in most cases of acute pneumonia. Friedlander described both the characteristic capsule and colonial morphologic features of pneumococci and, in 1884, recovered pneumococci from the blood of patients with pneumonia for the first time. During the next few years, pneumococci were found in virtually all types of infection, including meningitis and otitis media. By 1890, researchers had established the pneumococcus as the most common cause of acute pneumonia, and, hence, the term *pneumococcus* emerged. In addition, the pneumococcus became recognized as a principal cause of meningitis and other serious infections. In 1926, the pneumococcus was called *Diplococcus pneumoniae* because it usually appears in pairs. In 1974, it was renamed *Streptococcus pneumoniae* because it forms long chains when grown in liquid medium.

Early researchers immunized animals with cell-free filtrates of pneumococci, demonstrated that serum from immune animals could protect against experimental pneumococcal infection, deduced the role of immunity in promoting phagocytosis, and noted agglutination of pneumococci by serum from immune animals. In 1897, Pane treated humans suffering from pneumonia with serum from such animals. By 1900, researchers had laid the foundation for immunotherapy for pneumococcal pneumonia, the only effective treatment until the advent of chemotherapy. Investigators noted that agglutination of pneumococci appeared to depend on the strain isolated. In 1910, Neufeld and Haendel classified pneumococci into several discrete serotypes on the basis of the appearance of capsular swelling (the quellung reaction). Only strains exposed to homologous serum showed capsular swelling. Their work made possible all subsequent epidemiologic investigations of pneumococcal infection, immunotherapy with type-specific serum, and the development of vaccines. The original classification of pneumococci was limited to types I, II, III, and IV (others). Currently, 93 serotypes have been identified, and certain serotypes have proved to be more virulent than others, with virulence depending, to some extent, on the species of animal infected.

The use of antisera for the treatment of pneumococcal pneumonia proved strikingly effective when type-specific sera were administered. As early as 1913, Cole and associates showed that treatment with antisera lowered fatality rates from 25 to 30 percent to 10.5 percent. In addition to allergic reactions, difficulties associated with this treatment included the necessity of identifying the causative serotype, the need for the earliest possible administration of antisera, and the availability of antisera to only types I, II, and III. White compared the efficacy of early antisera therapy and found that 403 of 1614 (25.0%) patients who did not receive any therapy died, 32 of 377 (8.5%) who received therapy within 3 days of onset died, and 24 of 127 (18.9%) who received therapy 4 or more days after onset died. Unfortunately, therapy with antisera had no beneficial effect on pneumococcal infections such as meningitis and endocarditis. Despite these drawbacks, the use of antisera soon became widespread. The advent

*References 5, 19, 100, 112, 115, 149, 156, 179, 203, 313, 351, 361, 598.

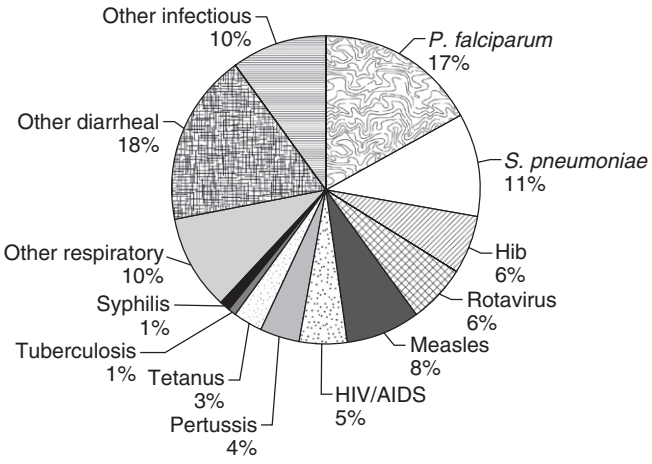

FIGURE 85-1 ■ Pathogen-specific infection-related mortality among children aged younger than 5 years. Among deaths from respiratory infection, 41% were attributed to *Streptococcus pneumoniae.* (From Elliott SR, Beeson JG. Estimating the burden of global mortality in children aged <5 years by pathogen-specific causes. *Clin Infect Dis* 2008;**46**:1794-5.)

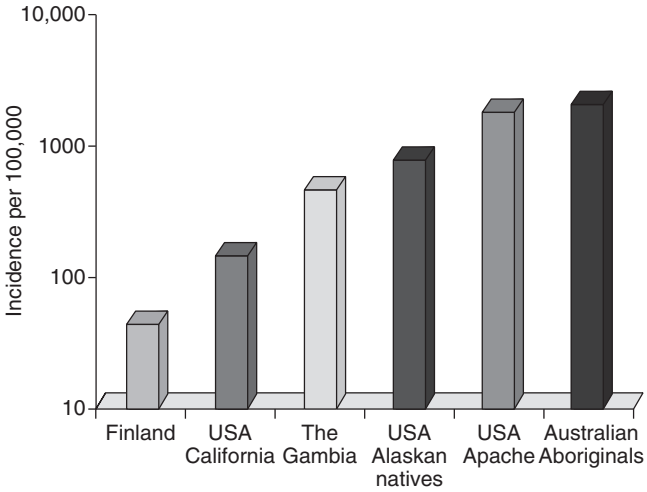

FIGURE 85-2 ■ Incidence of IPD in children age younger than 2 years in The Gambia and in various high- or low-risk populations. (From Greenwood B. The epidemiology of pneumococcal infection in children in the developing world. *Philos Trans R Soc Lond B Biol Sci* 1999;**354**:777-85.)

of chemotherapy—first with sulfa compounds, then penicillin—was followed by a precipitous decline in the use of antisera. Antimicrobial agents killed or inhibited pneumococci, regardless of serotype, and cured patients with previously incurable localized infections.[576] Coincident with research resulting in the use of antisera came research into the efficacy of pneumococcal vaccines. Proof of efficacy lagged, and indisputable evidence of protection induced by vaccination was not available until 1945. The ability of pneumococci to cause epidemic pneumococcal pneumonia in young men crowded into army camps or gold mines allowed large-scale trials to be performed. Highlights of the development of effective vaccines include the trial of Wright and associates[587] in South Africa beginning in 1911. With the use of a vaccine made with whole, killed pneumococci, this trial produced inconclusive results. Many trials followed, with some showing trends toward protection. In 1923, Heidelberger and Avery[223] published their classic article in which they stated that protective antibodies were reactive with surface capsular polysaccharides. In 1930, Francis and Tillett[166] showed capsular polysaccharides to be immunogenic for humans. Ekwurzel and colleagues[141] used a vaccine containing such polysaccharides during 1933 to 1937 and showed it to be effective. Smillie and associates used a preparation of serotype 1 polysaccharide to abort a hospital epidemic of pneumonia at a state hospital in Worcester, Massachusetts.[499]

Although many of these studies suggested that specific pneumococcal polysaccharide antigens could confer protection against severe pneumococcal infection, not until 1945, in a trial performed on U.S. Army and Air Force recruits, were they finally proved by MacLeod and associates to do so.[337] This trial showed vaccination to be strikingly effective in preventing pneumococcal pneumonia caused by serotypes contained in the vaccine but not in preventing disease caused by other serotypes, thus showing serotype-specific protection.

Regrettably, interest in vaccination waned rapidly with the general availability of penicillin, and manufacturers voluntarily withdrew their vaccines from the market. This unfortunate attitude persisted for the next 2 decades until the inability of chemotherapy to prevent many deaths from pneumococcal disease was recognized.[34] The rapid development and spread of antibiotic resistance among many clinically important strains further emphasized that prevention could be more effective than treatment for pneumococcal disease. Fortunately, a few farsighted individuals continued to maintain surveillance of the serotypes causing human disease, and their work allowed the reintroduction of pneumococcal vaccines. The current status of vaccines is discussed later in this chapter. Interested readers should consult both White's *The Biology of Pneumococcus* and Heffron's *Pneumonia, with Special Reference to Pneumococcus Lobar Pneumonia*, as well as a comprehensive review by Watson and colleagues, for a complete account of the long and fascinating history of this organism.[222,568,576]

EPIDEMIOLOGY

The incidence of pneumococcal disease is highest in children younger than 2 and adults older than 65 and varies substantially by country, by individual health status, and by season. The highest rates are observed in infants but decrease rapidly by 5 years of age. Greenwood reported that the incidence of invasive pneumococcal disease (IPD) in children younger than 2 years of age varies 10-fold, from less than 100 cases/100,000 in Finland to more than 1,000 cases/100,000 in Australian Aboriginal children (Fig. 85-2).[201] In part, the variability reflects the increased risk among children with sickle-cell disease or human immunodeficiency virus (HIV) infection, indigenous pediatric populations such as Native Americans, Inuits, or Aborigines, and malnourished children, but

because IPD impacts primarily healthy children the susceptibility of high-risk groups to infection does not explain the large geographic differences in incidence. Specific populations can have rates of IPD that are as much as 100-fold greater than those in age-matched healthy children residing within the same country. The incidence of IPD is also correlated with standard of living, with children in more industrialized countries having less disease than those living in less industrialized countries or in less privileged populations. The incidence of IPD in children younger than 5 to 6 years in western Europe (e.g., Finland, the United Kingdom, Germany, Switzerland, Denmark, Spain) was lower than 25 cases/100,000 population per year in various studies; in Chile, Australia, and New Zealand, it was 25 to 60 cases/100,000; and during the same period in the United States, the range was 65 to 75 cases/100,000.[218,245] The rates of IPD in U.S. children appear to be atypical, with higher rates reported than in comparable industrialized nations, although rates of pneumococcal meningitis appear more similar across countries.[218,338] The difference in U.S. incidence rates is most likely a reflection of the higher use of blood cultures in nontoxic febrile infants and toddlers in the U.S. than elsewhere.

Despite the high risk among children with specific comorbid illnesses, the majority of cases occur in healthy infants and toddlers. Such children represent an immunologically naïve population susceptible to bloodstream invasion or direct invasion of the lower respiratory tract. Differences in the proportion of disease by age also vary globally. In The Gambia and comparable countries, a larger proportion of disease occurs in children younger than 5 months of age compared with representative populations of U.S. children (Fig. 85-3). Similarly, in many countries, colonization is more prevalent and begins earlier, usually in the first months of life, compared with industrialized countries.[5]

Because virtually all children carry pneumococci belonging to several serotypes during their childhood, a focus on the epidemiology of carriage is warranted. In a study conducted in the United States, the prevalence of nasopharyngeal carriage in preschool children was 38 to 60 percent versus 29 to 35 percent and 9 to 25 percent in elementary school and junior high school students, respectively.[225] The prevalence in adults with no children at home was 6 percent. In studies in closed populations such as kibbutzim in Israel or a poor and crowded black community in southern Israel, the same differences between children and adults were noted.[66,111] Contact with young carriers increases the carriage rates in older children and adults. In the United States, although adults with no children had a carriage rate of 6 percent, the carriage rate increased to 29 percent when children were present at home. Similarly, primary school-aged children who have siblings younger than 2 years old carried *S. pneumoniae* more often than did those without young siblings.[334] In a study in Costa Rica, cultures in mother-child dyads showed an increased prevalence of carriage: from 6 percent at 1 month of age to 39 percent at 12 months of age. At the same time, carriage in mothers increased from 0 to 9.8 percent, thus supporting that pneumococci are transmitted from infants to adults, in this case mothers.[555] In a Swedish study, the observed average duration of carriage was longer in children who had family carriers of the same serotype and, therefore, was suggestive of rapid recirculation in the family.[140]

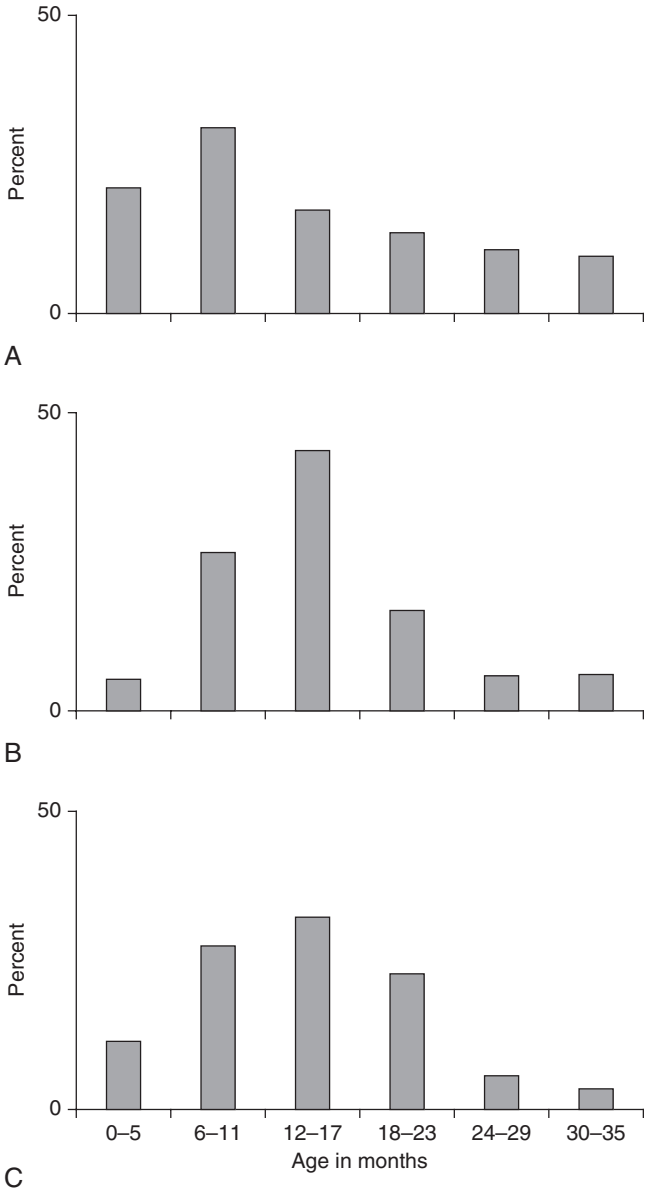

FIGURE 85-3 ■ Age distribution of IPD in young children in (**A**) The Gambia and high-risk (**B**) and low-risk (**C**) communities in the United States. (From Greenwood B. The epidemiology of pneumococcal infection in children in the developing world. *Philos Trans R Soc Lond B Biol Sci* 1999;**354**:777-85.)

Acquisition of *S. pneumoniae* may occur during the very first days of life. In infants aged 2 months, the prevalence of carriage ranges from less than 15 percent in most developed countries to greater than 60 percent in developing countries.[19] Colonization peaks toward the second to third year of life.[115,200,224] The relationship of age to carriage is not understood, but it depends, at least in part, on the development of specific anticapsular antibodies.[198,199] In toddlers vaccinated with a pneumococcal

conjugate vaccine, nasopharyngeal acquisition of new *S. pneumoniae* serotypes was inversely related to serum levels of specific antipolysaccharide IgG antibodies.[109] In another study, the presence of both circulating IgG and secretory IgA antibodies to the surface pneumococcal protein PsaA was associated with a lower prevalence of nasopharyngeal pneumococcal carriage.[438,493] A 2004 study showed that mucosal anticapsular IgA developed in response to colonization in preschool-aged children, regardless of vaccination status.[64] This phenomenon has been hypothesized to contribute to the falling carriage rates observed with increasing age.[600] Potentially, maturation of innate immunity may also contribute to the declining prevalence of colonization with increasing age; however, a specific role or mechanism has not been established.

Crowding is an important factor that facilitates the spread of *S. pneumoniae*. Therefore, one is not surprised that in the developed world the nasopharyngeal carriage rate and spread are highest in infants and toddlers attending daycare centers, with levels exceeding 90 percent in some studies, followed by those living with one or more siblings at home.[19,115,430,551] In addition, the viral infections that are very prevalent in infants and toddlers attending daycare centers enhance colonization of the nasopharynx with *S. pneumoniae*.[200,332,551] The combination of young age, poor hygienic behavior, and increased incidence of viral respiratory tract infections renders daycare centers an important site for transmission of *S. pneumoniae* from child to child and child to parent. Daycare centers, thus, are a nucleus of high carriage of *S. pneumoniae*, particularly antibiotic-resistant strains. This nucleus enables the dissemination of such pneumococci to other children, mainly young siblings of daycare center attendees.

The duration of carriage depends on age and serotype and may be related to additional factors such as antibiotic treatment, the immune status of the child, and other unknown factors. Carriage lasts longer in infants and young toddlers than in older children and adults.[110,140,198] In a study in Sweden,[140] infants were colonized for an average of 30 days and after 3 months 17 percent were carrying the same organism. In a study in the United States in adults, individual serotypes usually persisted for 2 to 4 weeks. The study showed that the pneumococcal serotype that initially colonizes infants (typically before they reach 6 months of age) can be detected for as long as 12 months (mean, 4 months).[198]

The relative risk for a specific serotype to colonize the nasopharynx of a child and its ability to cause respiratory tract or invasive infection appears to vary by serotype but not geographic location. Some serotypes, such as 6A, 6B, 9V, 14, 18C, 19A, 19F, and 23F, are among the most frequent colonizers in infants and young children in most parts of the world and thus often are considered "pediatric" serotypes.[68,111] These serotypes are acquired frequently by infants and young children and often are carried for prolonged periods. After children reach the age of 2 years, carriage of these "pediatric" serotypes declines rapidly. Although pneumococcal carriage decreases overall with age, the proportion of the "nonpediatric" serotypes increases with age. This phenomenon is demonstrated in Figure 85-4.

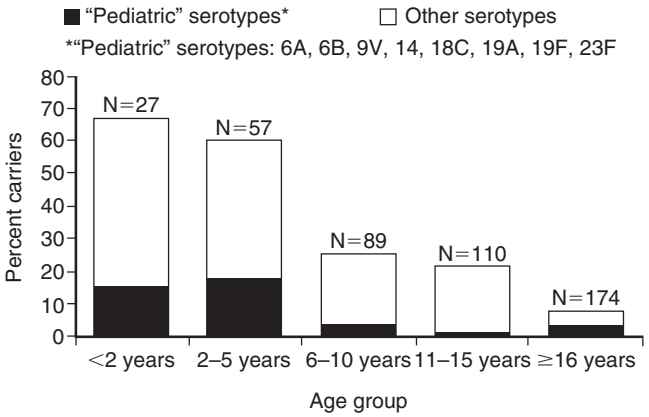

FIGURE 85-4 ■ Nasopharyngeal carriage of *Streptococcus pneumoniae* in closed community living in crowded conditions in Southern Israel. (Adapted from Dagan R, Gradstein S, Belmaker I, et al. An outbreak of *Streptococcus pneumoniae* serotype 1 in a closed community in southern Israel. *Clin Infect Dis* 2000; **30**:319-21.)

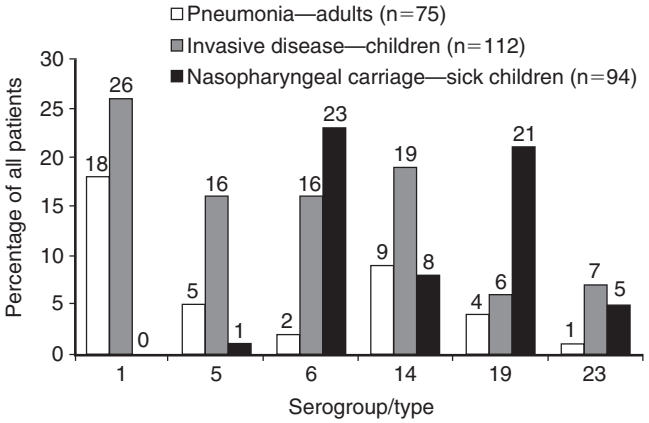

FIGURE 85-5 ■ Distribution of selected serogroups/serotypes in *Streptococcus pneumoniae* isolates from adults with pneumonia, children with IPD, and nasopharyngeal specimens from sick children in Kenya. (Adapted from Scott JAG, Hall AJ, Hannington A, et al. Serotype distribution and prevalence of resistance to benzylpenicillin in three representative populations of *Streptococcus pneumoniae* isolates from the coast of Kenya. *Clin Infect Dis* 1998;**27**:1442-50.)

In contrast to the "pediatric" serotypes, serotypes such as 1, 5, 7F, and 12 are carried infrequently and are eliminated from the nasopharynx rapidly. However, these serotypes are able to cause disease and even clusters or epidemics.[70,111] The different distribution of serotypes among colonized children, children with invasive infections, and adults with pneumonia is depicted in Figure 85-5.[480]

Although most colonization occurs without the development of disease, prospective, longitudinal studies have suggested that most systemic infections develop soon after colonization by a new pneumococcal serotype.[149,196,198] Some carriers are protected from invasive disease by the development of circulating antibodies. However, such antibodies do not always protect against

invasion of contiguous sites. Other investigators have shown that a substantial number of pneumococcal otitis media cases occur at any time during nasopharyngeal colonization by a specific serotype.[516]

One of the serious problems with pneumococcal strains clearly is the emergence of resistance to more than one antibiotic class.[251] The observed linkage between antimicrobial resistance to multiple antibiotic classes explains why the use of one antibiotic may result in carriage of *S. pneumoniae* resistant not only to the antibiotic to which the host was exposed but also to other, unrelated classes of antibiotics. In 1993, approximately a fifth of the pneumococcal strains in Iceland were penicillin nonsusceptible and 80 percent of them were multidrug resistant. When the risk for carriage of penicillin-resistant *S. pneumoniae* was investigated, a clear association was found not only with use of β-lactam drugs but also with the use of trimethoprim-sulfamethoxazole and erythromycin.[24] After treatment of otitis media, carriage of trimethoprim-sulfamethoxazole-resistant *S. pneumoniae* was found in 23 percent of patients by day 6 and in 33 percent by day 40. Additionally, non-penicillin-susceptible *S. pneumoniae* was carried at these times by 26 and 43 percent of children, respectively. This remarkable promotion of colonization with penicillin-resistant strains by trimethoprim-sulfamethoxazole treatment occurred because many strains were resistant to both penicillin and trimethoprim-sulfamethoxazole.

The dramatic change in nasopharyngeal flora after initiation of antibiotic therapy has two important consequences. First, previous treatment predisposes patients to new infections with more resistant organisms.[24] Antibiotic treatment not only can increase nasopharyngeal carriage of antibiotic-resistant *S. pneumoniae* but also can induce subsequent infection of the middle ear with a resistant strain within a few days.[112] Second, the increased prevalence of antibiotic-resistant *S. pneumoniae* in the nasopharynx may increase transmission in crowded conditions such as extended families and daycare centers.* The widespread use of antibiotics is likely to be responsible for the increase in antibiotic-resistant *S. pneumoniae*, especially in crowded populations, thus creating a vicious cycle that is difficult, if not impossible, to overcome (Fig. 85-6).[552]

Since the use of pneumococcal conjugate vaccine has become widespread in the United States, the serotypes found most commonly in nasopharyngeal colonization have been replaced by nonvaccine serotypes. A study evaluating nasopharyngeal colonization in 16 Massachusetts communities during 2001 to 2004 showed a decrease in serotypes found in the 7-valent pneumococcal conjugate vaccine (PCV7) from 36 percent to 14 percent along with an increase in non-PCV7 serotypes from 34 percent to 55 percent.[242] Another study showed that 25 percent of children colonized with pneumococci carried antibiotic-resistant, nonvaccine serotypes, including serotypes 19A and 35B, rarely detected before introduction of the pneumococcal conjugate vaccine. Further changes in the distribution of pneumococcal serotypes, specifically types 19A, 7F, and 6A, subsequent to the implementation of universal vaccination of children younger than 5 years of age with the 13-valent pneumococcal conjugate vaccine (PCV13) have been observed.[335] Both a reduction in overall carriage of the unique PCV13 serotypes as well as specific declines in serotypes 19A and 7F have been significant.

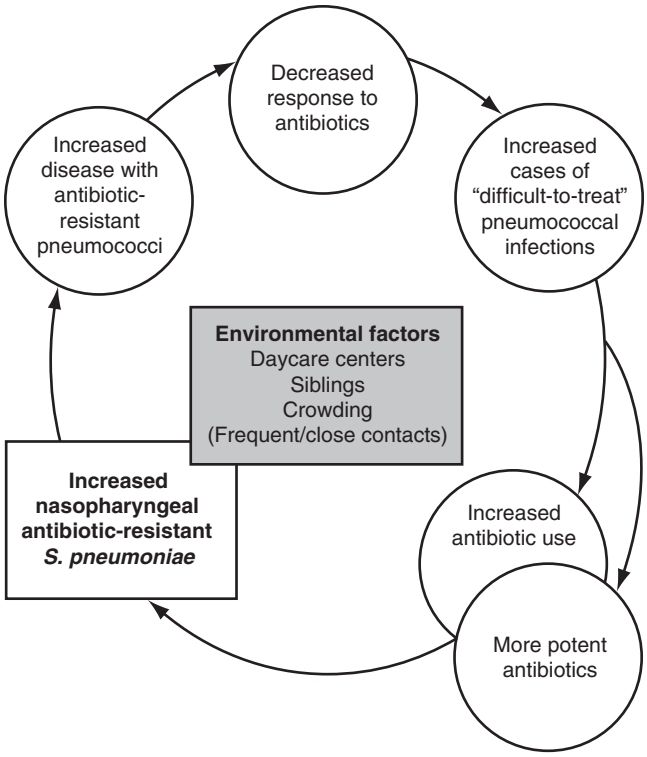

FIGURE 85-6 ■ Chain of events that create a viscous cycle in which treatment with antibiotics increases nasopharyngeal carriage of antibiotic-resistant pneumococci, which decreases responsiveness to antimicrobial treatment. The decreased responsiveness results in use of more potent antibiotics, which continues the cycle.

Risk Factors for Pneumococcal Infection

Demographic Features

Age. Young age is a major risk factor for pneumococcal infection because of immunologic immaturity and the absence of acquired immunity. The relationship between age and the development of protective immunity to pneumococcal infection was published in 1932 by Sutliff and Finland (Fig. 85-7).[515] Maternal antibody, passively transferred to the fetus, protects against invasive infection in the first few months of life. These antibodies decay over the first few months, and the incidence of IPD increases, peaking between 12 and 18 months of age, presumably as specific immunity is acquired after exposure. Following the introduction of pneumococcal conjugate vaccine (PCV), the age distribution has changed, with the peak incidence now between 6 and 12 months of age.[593] This represents the described naïveté of the infant immune system and the acquisition of protective

*References 115, 179, 224, 430, 456, 591.

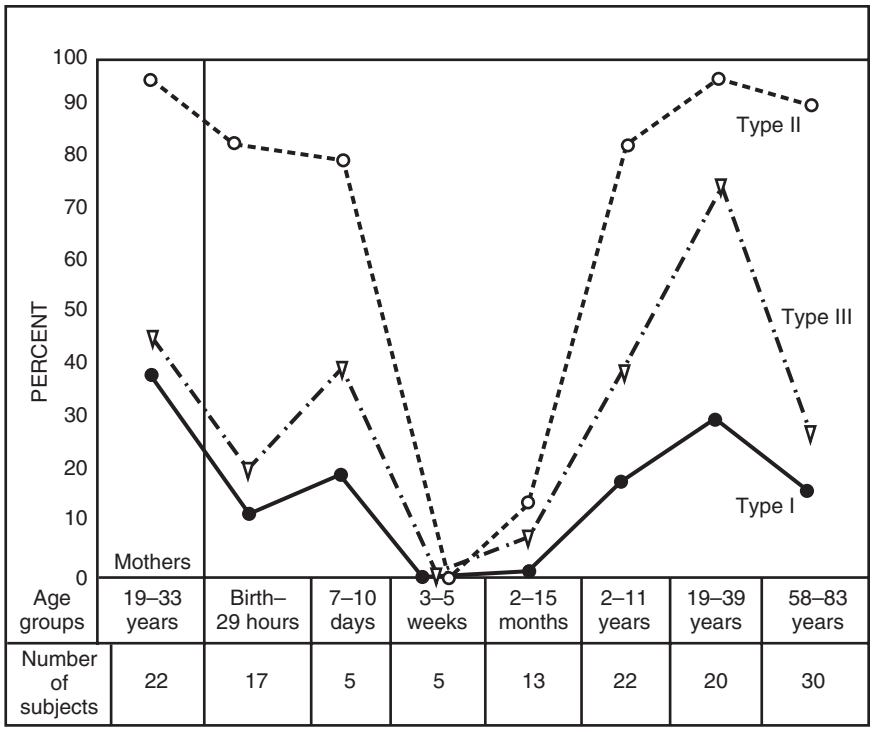

Age groups	19–33 years	Birth–29 hours	7–10 days	3–5 weeks	2–15 months	2–11 years	19–39 years	58–83 years
Number of subjects	22	17	5	5	13	22	20	30

FIGURE 85-7 ■ Percentage of subjects in different age groups whose blood is pneumocidal. (From Sutliff WD, Finland M. Antipneumococcic immunity reactions in individuals of different ages. *J Exp Med* 1932; **55**:837-52.)

antibody after immunization, with two doses of PCV administered in the first 4 months of providing protection.[343,362,578] As described earlier, IPD in developing countries occurs earlier in life. It is hypothesized that this is related to earlier and more intense exposure to pneumococci in such children.[5]

Gender. In most surveillance studies of pneumococcal disease there is a greater proportion of males than females. Yildirim and colleagues reported that the proportion of male cases was significantly higher (58.5% vs. 41.5%; $p = .01$), but gender-specific incidence rates were not significantly different.*

Race/Ethnicity. The incidence of pneumococcal bacteremia and meningitis in Alaskan Native children younger than 5 years ranges from 598 cases/100,000 population in those 6 to 11 months of age to 56 cases/100,000 population in those 36 to 47 months of age, which is approximately four times the incidence in similarly aged non-Alaskan Native/non-American Indian children.[122] The highest incidence for any ethnic group in the United States is found in Navajo and Apache populations living on reservations in the southwestern United States. The incidence in children aged 1 to 2 years in these populations ranges from 557 to 2,396/100,000.[102,398] Among children younger than 5 years, the incidence of IPD in African American children in the United States is two to three times higher than that in white children of the same age.[50,72,215,452] In a case-control study of risk factors for the development of invasive disease in young children, the association of race with disease risk was not statistically significant in an analysis that controlled for socioeconomic status.[322] However, other studies have reported persistence of increased risk even when controlling for income.[72,91,157,215,226] The reasons for the increased incidence in some populations in comparison to others in the United States are unclear.

In other geographic regions, differences among populations also can be observed, again being higher in populations that live in underprivileged conditions, which raises the question of the importance of genetic versus environmental risk factors. In southern Israel, the incidence of pneumococcal invasive infection during the first year of life in Bedouin infants (a population with a lifestyle, birth rate, and general disease incidence similar to that of the developing world) was fourfold higher than in Jewish infants (a population with standards of living comparable to the middle/low social class in the developed world).[168]

In New Zealand, when Maoris, Pacific Islanders, and others were compared, incidence rates of IPD in the first year of life per 100,000 population were 153, 276, and 52, respectively. These rates in children younger than 5 years were 67, 117, and 36 per 100,000, respectively.[558]

Seasonality

Pneumococcal infection occurs in a seasonal pattern, with increased cases of invasive infections, pneumonia, and otitis media between September/October and April/May in the Northern Hemisphere, with the opposite picture in the Southern Hemisphere. Peak incidence often occurs from December through February (Fig. 85-8).[134] The same seasonal variation in nasopharyngeal

*References 70, 135, 219, 358, 387, 407, 449, 488, 527, 593.

carriage of pneumococci is also observed, with lowest rates in the summer months.[200,332] This pattern is likely related to the seasonal variations in viral respiratory tract infections, which play an important role increasing pneumococcal carriage and subsequent infection, and to attendance at school and daycare centers. However, other factors are likely to be important. In one study, adults had a higher incidence of pneumococcal disease after school holidays and thus presumably from increased contact with children.[565] In Israel, the Jewish and Bedouin populations in southern Israel demonstrate distinct and different seasonal patterns. In the Jewish population the pattern appears similar to that of traditional Western populations with distinct seasonal variation. The Bedouin live in crowded and poor hygienic conditions with a high birth rate and a disease pattern similar to that in the developing world, without a clear pattern of seasonality.[323]

Lack of Breast-Feeding

Breast-feeding may be protective against pneumococcal infection. The reduction in colonization and decreased attachment to pharyngeal cells is thought to be mediated through several components of the immune system, including secretory IgA, lactoferrin, and lysozyme. Some studies have suggested that breast-feeding protects against otitis media,[20] but others have failed to show this effect.[47] Studies in the United States demonstrated a protective effect of breast-feeding against IPD in children in the general population[47] and in Alaskan natives.[178] However, in Finland no protective effect was observed.[520]

Crowding

Crowding contributes to many factors that increase the risk for acquiring pneumococcal infection, including viral infections, poor hygiene, and potentially increased person-to-person transmission of *S. pneumoniae*. Attendance at daycare centers was the most important risk factor for acquiring IPD in children and infants in several studies.* In a recent population-based, case-control study, adults aged 18 to 64 years who lived in households that included children attending daycare were at greater risk for acquiring IPD.[393] In developed countries, attendance at daycare centers is also the most important risk factor for acquiring acute otitis media (AOM), including pneumococcal otitis.[386,460]

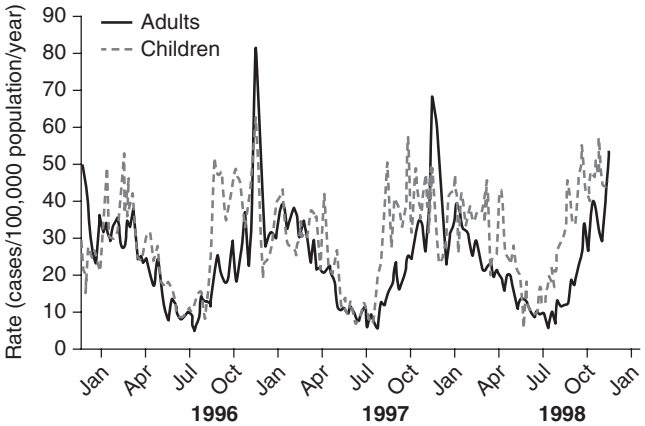

FIGURE 85-8 ■ Weekly rates of invasive pneumococcal disease in children (*dotted line:* ages birth to 17 years), and adults (*solid line:* age >18 years) in the United States, 1996 to 1998. (From Dowell SF, Whitney CG, Wright C, et al. Seasonal patterns of invasive pneumococcal disease. *Emerg Infect Dis* 2003;**9**:573-9.)

Immunocompromising Conditions

Congenital and Acquired Absence of Spleen or Splenic Function

The spleen is critical for the clearance of pneumococci from the bloodstream.[514,566] Children in whom the spleen is absent or dysfunctional are at risk for the development of overwhelming pneumococcal infection. In children with absence or dysfunction of the spleen, the mortality rate is multifold higher. Schutze and colleagues reported 27 percent mortality from prospective surveillance of eight U.S. children's hospitals.[478] Splenic dysfunction is also regarded as explaining the increased incidence and severity of pneumococcal bacteremia in sickle-cell disease and other sickle hemoglobinopathies (e.g., hemoglobin SC disease or S-β-thalassemia).[414,478,596] Children with hemoglobin SC disease, thalassemia, and other hemaglobinopathies demonstrate a higher incidence of IPD compared with healthy children, albeit lower than in persons with sickle-cell disease.[308,537] Other factors that contribute to the high incidence and poor outcome for pneumococcal infection in persons with sickle-cell disease include low levels of circulating antibodies and complement deficiency.[414,581] Penicillin prophylaxis is effective in reducing the risk for pneumococcal disease in children with absent or abnormal splenic function; however, the rate of disease remains increased compared with healthy children. PCV has been demonstrated to be immunogenic and effective in reducing disease due to vaccine serotypes in children with hemoglobinopathies (Fig. 85-9).[8,209,553] However, increased rates of disease due to serotypes not contained in the current vaccines are observed.†

Defective Antibody Formation

Reduced antibody formation is associated with increased risk for pneumococcal infection. Congenital agammaglobulinemia, acquired common variable hypogammaglobulinemia, and defective antibody production associated with diseases such as malignancies and human immunodeficiency virus (HIV) infection are associated with increased risk for pneumococcal disease.[106,545] In the era preceding the introduction of highly active antiretroviral treatment (HAART), HIV-infected children had a 100- to 1000-fold increase in incidence compared with healthy children.‡ In one study, the incidence of IPD was 6.1 cases/100 patient-years in HIV-infected children through the age of 7 years.[349] Despite the

*References 63, 188, 318, 389, 430, 434, 441, 456, 460, 517, 555.
†References 8, 159, 209, 411, 553, 583, 596.
‡References 17, 52, 151, 167, 341, 368.

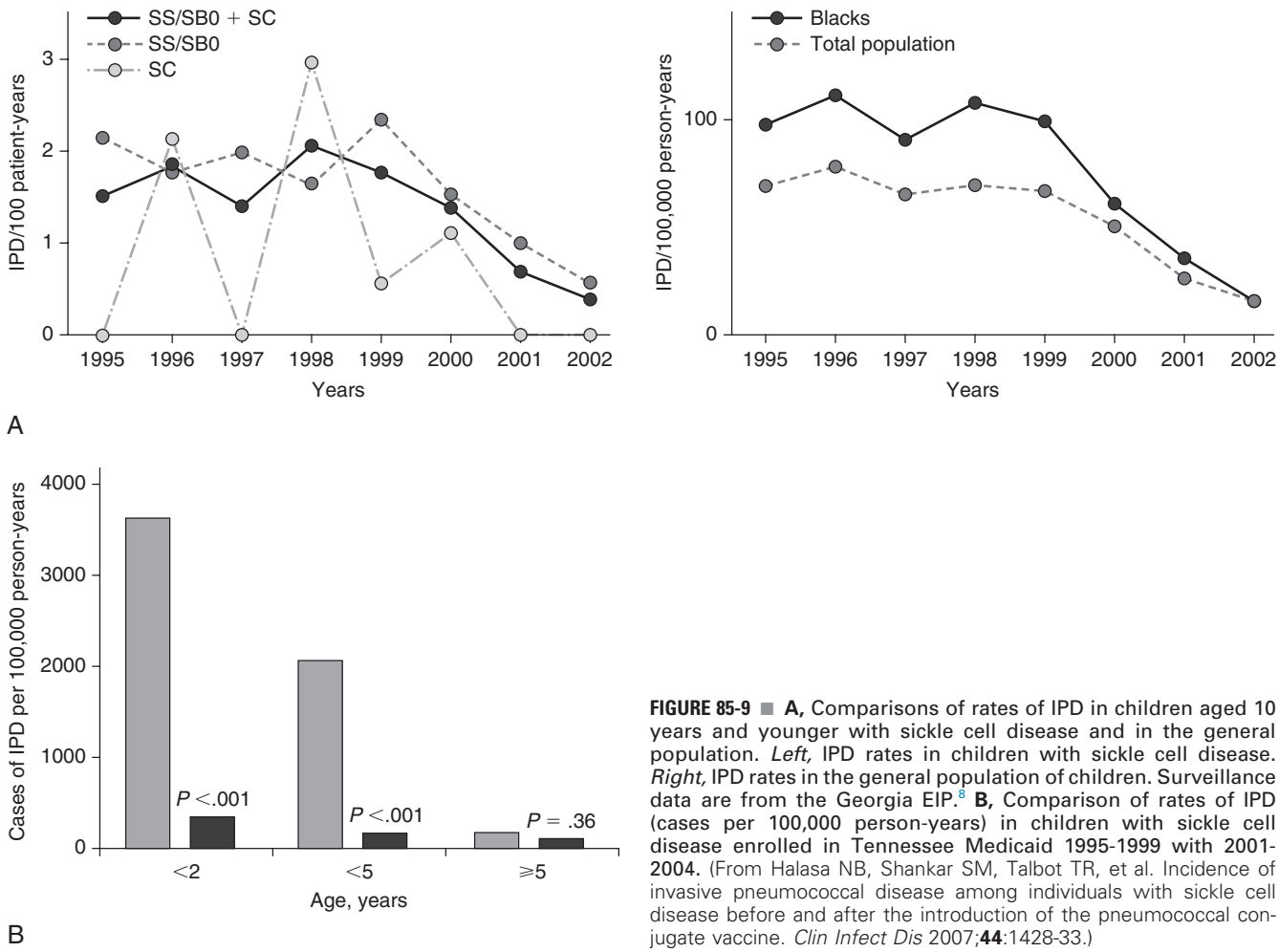

FIGURE 85-9 ■ **A,** Comparisons of rates of IPD in children aged 10 years and younger with sickle cell disease and in the general population. *Left,* IPD rates in children with sickle cell disease. *Right,* IPD rates in the general population of children. Surveillance data are from the Georgia EIP.[8] **B,** Comparison of rates of IPD (cases per 100,000 person-years) in children with sickle cell disease enrolled in Tennessee Medicaid 1995-1999 with 2001-2004. (From Halasa NB, Shankar SM, Talbot TR, et al. Incidence of invasive pneumococcal disease among individuals with sickle cell disease before and after the introduction of the pneumococcal conjugate vaccine. *Clin Infect Dis* 2007;**44**:1428-33.)

introduction of HAART, HIV-infected African children remain a high-risk group for IPD.[392]

Neutropenia and Neutrophil Dysfunction

The risk for pneumococcal disease is not uniform across all neutrophil abnormalities. Primary and secondary neutropenia, such as cyclic neutropenia, drug-induced neutropenia, and aplastic anemia, are associated with an increased incidence of severe pneumococcal infection. However, the incidence is not increased in other granulocyte dysfunction syndromes such as leukocyte adhesion deficiency syndromes.[16,171]

Genetic Variation in Mannose-Binding Lectin (MBL)

Approximately 5 percent of the population in Europe and North America and an even larger population in many developing countries are homozygous for MBL codon variants. These subjects have a greater than 2.5-fold increased risk for the acquisition of pneumococcal infection than do those who do not have this variant or are only heterozygous for this variant.

Immunocompetent Conditions

Studies of IPD since the introduction of PCV in 2000 have identified that an increasing proportion of cases occur in children with comorbid conditions. Children older than the age of 5 years with IPD were threefold more likely to have a comorbid condition compared with those younger than 5. Specific conditions identified by the Committee on Infectious Diseases of the American Academy of Pediatrics as indicating increased risk for pneumococcal disease include chronic lung and heart disease, diabetes mellitus, cerebrospinal fluid (CSF) leak, chronic renal failure and nephrotic syndrome (at least in part due to hypogammaglobulinemia), and cochlear implants (Table 85-1).[13] Recently presented data suggest that children with asthma and neurologic conditions including seizure disorders and muscular dystrophies are also at increased risk. The risk is further increased in children with two or more comorbidities.

Pneumococcal Serotype

A characteristic of most disease-causing strains of *S. pneumoniae* is the presence of a polysaccharide capsule. The capsular polysaccharides are composed of repeating units

TABLE 85-1 Underlying Medical Conditions That Are Indications for Pediatric Pneumococcal Immunization Listed by Risk Group

Risk Group	Condition
Immunocompetent children	Chronic heart disease*
	Chronic lung disease†
	Diabetes mellitus
	Cerebrospinal fluid leaks
	Cochlear implant
Children with functional or anatomic asplenia	Sickle-cell disease and other hemoglobinopathies
	Chronic or acquired asplenia or splenic dysfunction
Children with immunocompromising conditions	HIV infection
	Chronic renal failure and nephrotic syndrome
	Diseases associated with treatment with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, and Hodgkin disease or solid-organ transplantation
	Congenital immunodeficiency‡

*Particularly cyanotic congenital heart disease and cardiac failure.
†Including asthma if treated with prolonged high-dose oral corticosteroids.
‡Includes B- (humoral) or T-lymphocyte deficiency; complement deficiencies, particularly C_1, C_2, C_3, and C_4 deficiency; and phagocytic disorders (excluding chronic granulomatous disease).
HIV, human immunodeficiency virus.
Modified from American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 571-582.

TABLE 85-2 Capsular Serotypes of Streptococcus pneumoniae*

Serotypes (n = 25)	Serogroups (n = 21)
1	6A, 6B, 6C, 6D
2	7A, 7B, 7C, 7F
3	9A, 9L, 9N, 9V
4	10A, 10B, 10C, 10F
5	11A, 11B, 11C, 11D, 11E, 11F
8	12A, 12B, 12F
13	15A, 15B, 15C, 15F
14	16A, 16F
20	17A, 17F
21	18A, 18B, 18C, 18F
27	19A, 19B, 19C, 19F
29	22A, 22F
31	23A, 23B, 23F
34	24A, 24B, 24F
36	25A, 25F
37	28A, 28F
38	32A, 32F
39	33A, 33B, 33C, 33D, 33F
40	35A, 35B, 35C, 35F
42	41A, 41F
43	47A, 47F
44	
45	
46	
48	

*Includes 25 individual serotypes and 21 serogroups containing 68 serotypes.

of oligosaccharides, and for most of them the exact chemical structure is known. To date, 93 distinct capsular types have been described, several of them very recently (Table 85-2). Understanding serotype distribution and characteristics is important for several reasons. First, a selected number of serotypes are responsible for the majority of disease. However the serotypes vary somewhat by geography. For example, prior to the introduction of PCV, 7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) accounted for 80 percent of cases of IPD in North American children (Fig. 85-10A). Globally, a larger number of serotypes, including serotypes 1, 3, and 5 must be included to account for a similar 80 percent of IPD cases (see Fig. 85-10B).[266] A significant positive association between disease and serotype has been demonstrated in children younger than 36 months, with serotypes 1, 5, and 12F associated with IPD, serotypes 1, 3, 5, 12F, 19A, and 19F with AOM, and serotype 3 and nontypeable strains with conjunctivitis. A significant negative association with IPD was demonstrated for nontypeable *S. pneumoniae* and with AOM for serotypes 6A, 6B, 15A, and nontypeable strains. Understanding the distribution of serotypes causing serious pneumococcal disease is relevant to developing vaccine strategies for prevention and estimating the impact of a vaccine that provides coverage for a limited number of serotypes. Serotype distribution also varies over time, as recent experience with serotype 1 disease in Europe demonstrated.

The serotype distribution of nasopharyngeal carriage and invasive and other pneumococcal diseases varies because not all pneumococci have the same relative risk for progressing from carriage to disease. *Invasive capacity*, the term describing the relative risk for disease occurring after a colonizing event appears to be an intrinsic property of pneumococcal strains, predominantly determined by the capsular polysaccharide. The invasive capacity for common serotypes has been computed by several investigators. Large differences among serotypes (>10-fold) have been reported and, in general, estimates of relative risk have been similar across studies. Dagan and colleagues were able to demonstrate that the capacities to cause IPD and AOM were not comparable and that differences among serotypes were blunted for AOM compared with IPD (Fig. 85-11). After the introduction of PCV, investigators in many countries have reported increases in both carriage and disease due to serotype

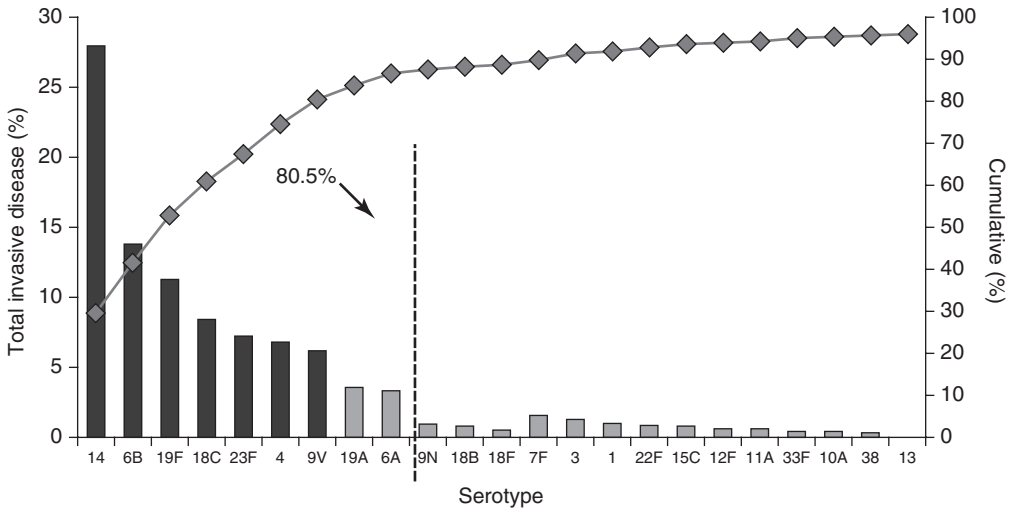

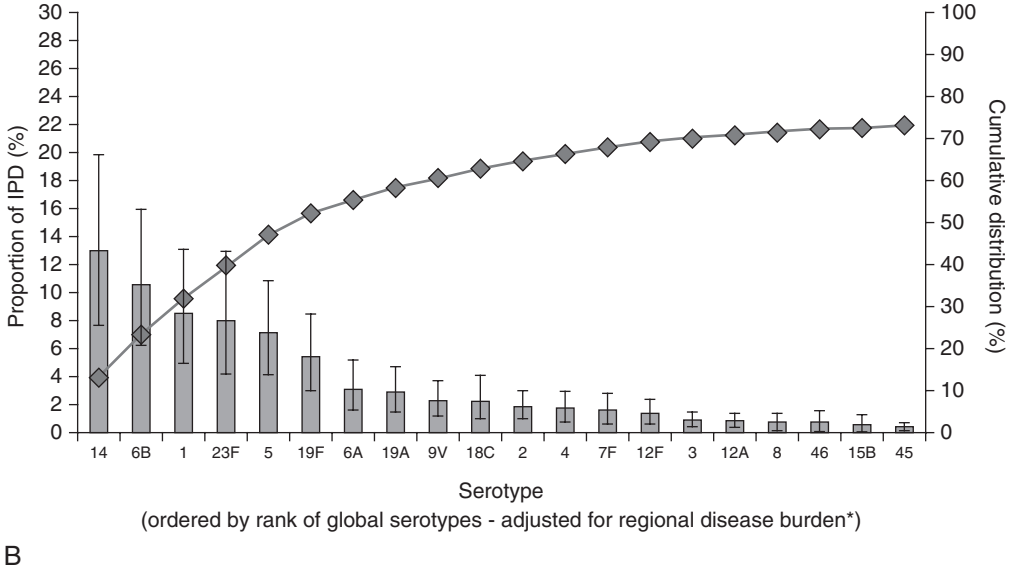

FIGURE 85-10 ■ **A,** Pneumococcal serotypes causing invasive disease in North American young children prior to PCV7. **B,** Proportion of IPD in young children due to the most common serotypes globally. Error bars indicate 95% confidence interval; cumulative line indicates proportion of IPD due to the 21 serotypes detailed. (**A,** from Hausdorff WP, Bryant J, Kloek C, et al. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. *Clin Infect Dis* 2000;**30**:122-40; **B,** from Johnson HL, Deloria-Knoll M, Levine OS, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS Med* 2010;**7**:e1000348.)

19A. In part, the increase in carriage resulted from intrinsic characteristics of specific clones of serotype 19A permitting them to "outcompete" other serotypes, from multidrug resistance, which allowed its persistence as a result of selective pressure from antimicrobial usage in the community, and from the lack of significant cross protection by antibodies generated in response to 19F capsular polysaccharide. It was initially hypothesized that including the capsular polysaccharide from serotype 19F in the vaccine would elicit cross-protective antibody against serotype 19A. The hypothesis was that antigenically related serotypes (those within serogroups; e.g., serotype 9A) would elicit cross-protective antibody against serotypes 9L, 9N, and 9V. However, although antibody to the 19A capsule increased after immunization with 19F as measured by enzyme-linked immunosorbent assay (ELISA), it was not sufficiently functional to prevent carriage or disease due to 19A. Proof of cross protection currently exists for antibody elicited by the 6B capsular polysaccharide, which demonstrates protection against disease and carriage due to serotype 6A. For serotype 23F, an increase in carriage of the cross-reacting serotypes 23A and 23B has been observed after immunization with PCV containing 23F capsular polysaccharide. Insufficient data are available to understand whether capsular polysaccharides elicit cross protection for other serotypes of the same serogroup. The extent of cross reactivity between types within the respective serogroups may

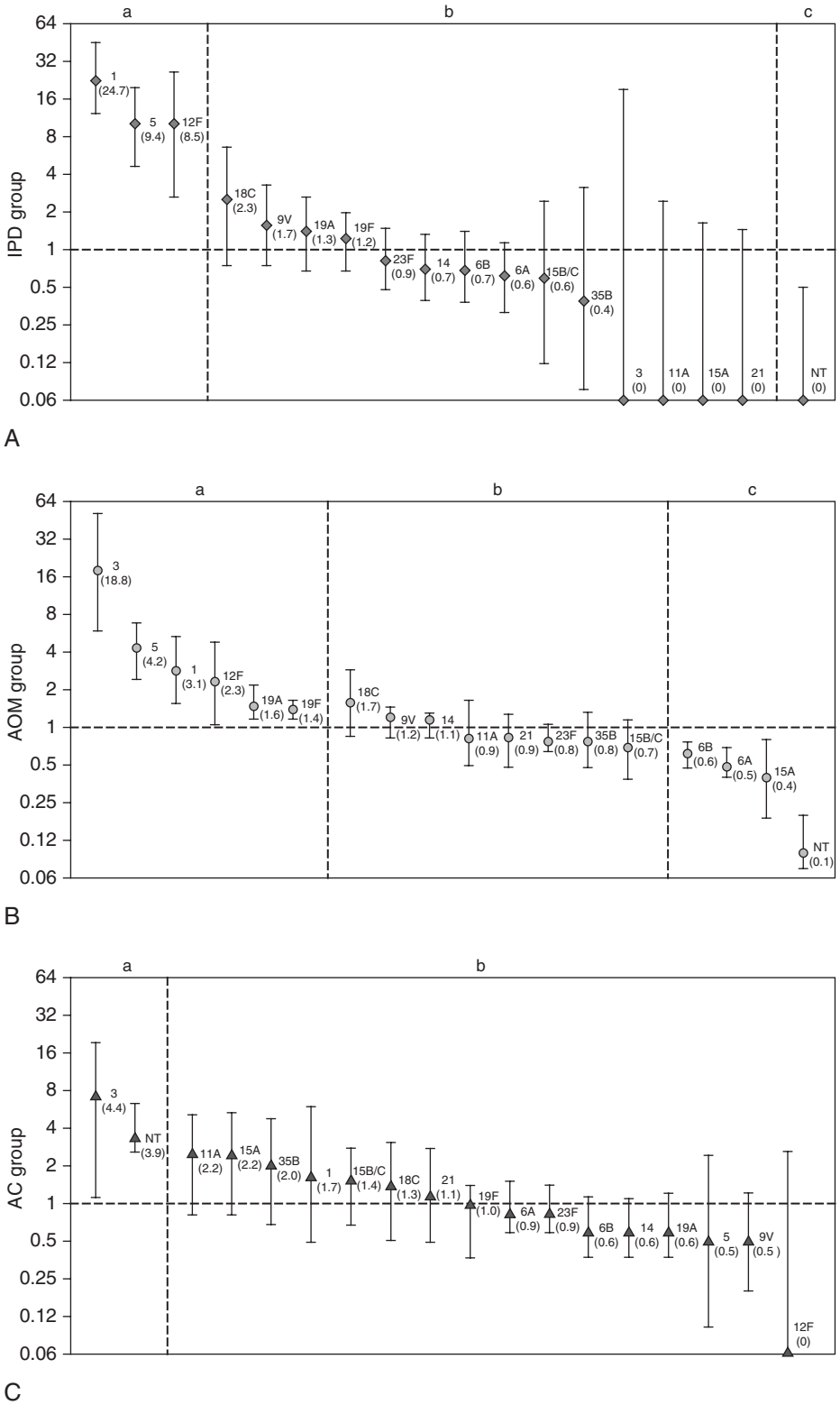

FIGURE 85-11 ■ Site-specific odds ratio for different *S. pneumoniae* serotypes for IPD, AOM, and acute conjunctivits. **A,** Serotypes with a significant positive associations. **B,** Serotypes with no significant positive or negative associations. **C,** Serotypes with a significant negative association. NT, nontypeable. Odds ratios are shown in parentheses. (From Shouval DS, Greenberg D, Givon-Lavi N, et al. Site-specific disease potential of individual *Streptococcus pneumoniae* serotypes in pediatric invasive disease, acute otitis media and acute conjunctivitis. *Pediatr Infect Dis J* 2006;**25**:602-7.)

differ; for example, serotypes 6A and 6B have identical chemical composition except for one of the bonds between two sugars and are extensively cross reactive whereas types 19F and 19A are clearly less cross reactive. Poolman suggested the process of protein conjugation of the 19F polysaccharide during vaccine production altered its structure such that antibody elicited was not functional against serotype 19A strains.

Another important reason for understanding serotype differences relates to clinical disease. Different serotypes may cause specific clinical syndromes. For example, serotype 3 is associated with necrotizing pneumonia and serotype 1 is associated with empyema as well as "epidemic disease" specifically in central Africa. Serotype 1 epidemiology also differs in that it causes disease across a broader age spectrum than the usual serotypes primarily found in infants.[41,177,550] High mortalities have been associated with specific serotypes as well.[427,548,571] Serotype is also linked to antimicrobial susceptibility. Before introduction of PCV, the majority of antibiotic-resistant pneumococci were associated with five serotypes: 6B, 9V, 14, 19F, and 23F.

Other attributes beyond capsular polysaccharide also impact on clinical syndromes. Recently, differences between strains of serotype 1 in Europe and Africa have been described. In Africa, disease due to serotype 1 is often meningitis and the strain is wild type with regard to the pneumolysin produced, whereas in Europe the clinical syndromes are bacteremia and pneumonia and the strain has a mutant pneumolysin that is unable to produce hemolysis.[364,571]

PATHOGENESIS

To cause disease, pneumococci, like other extracellular bacterial pathogens, must adhere to mammalian cells, replicate in situ, be carried to and replicate in parts of the body that normally are free of them, escape phagocytosis, and damage tissue by causing inflammation or producing substances that directly damage cells and, in some cases, invade the bloodstream.[379] The vast array of virulence mechanisms available to pneumococci are countered by numerous host defense mechanisms, although some host responses facilitate infection. Even though colonization with a pneumococcal strain can progress to disease, it usually does not occur, and the development of anticapsular type-specific antibodies occurs within 30 days in older children and adults.[197,198,381,382] If organisms find their way into the eustachian tubes, sinuses, or bronchi, clearance mechanisms, chiefly ciliary action, lead to their rapid removal. After the development of humoral immunity, colonization with a strain may persist for 1 to 12 months, during which time disease may occur in contiguous sites but the host is protected from invasive disease by circulating type-specific anticapsular IgG. Loss of colonization with a strain is followed, after a variable colonization-free interval, by colonization with a different serotype. Because 93 antigenically distinct serotypes exist, this cycle of colonization accompanied by the development of humoral immunity occurs many times.

Progression of colonization to disease usually requires the combination of two events: first, acquisition of a serotype to which the host is not immune, and, second, concurrent viral respiratory tract infection, chronic damage to respiratory epithelium (e.g., from smoking or occupational exposure), allergy, or other conditions that result in the development of disease rather than just colonization.[379] Many of these concurrent conditions initiate cytokine activation of the respiratory epithelium, which facilitates increased adhesion of pneumococci to respiratory epithelial cells, as well as invasion of these cells.[321] Mechanisms by which cytokine activation facilitates pneumococcal adhesion include expression of platelet-activating factor and polymeric immunoglobulin receptor (pIgR). This combination of factors leads to a higher density of colonizing organisms and enables pneumococci to cause infection, including pneumonia, acute exacerbations of chronic bronchitis, sinusitis, otitis media, and mastoiditis, in contiguous respiratory tract sites. Adherence of *S. pneumoniae* to host cells involves an array of surface adhesin molecules such as CbpA, PspA, PspC, Hyl, Ply, PsaA and both neuraminidases (see later section on virulence factors). These proteins are involved in interactions with the host complement system (PspA), degradation of hyaluronan of the extracellular matrix (Hyl), lysis of cholesterol-containing membranes (pneumolysin), and binding of metals (divalent cations) such as manganese or zinc (PsaA), followed by their transport inside the cytoplasm of pneumococci. Additionally, transepithelial and transendothelial transport of organisms into the bloodstream results in bacteremia and subsequent transport across other epithelial cells leads to infection of noncontiguous sites such as the leptomeninges, peritoneum, and joint spaces. Such infection occurs in nonimmune hosts by virtue of the fact that pneumococci are able to escape ingestion and killing by host phagocytic cells in the absence of type-specific antibody because the capsule inhibits phagocytosis. *S. pneumoniae* produces few toxins and largely causes disease by its capacity to replicate in host tissues and generate an intense inflammatory response. Cell wall teichoic acid (TA) and peptidoglycan stimulate the production of cytokines (interleukin [IL]-1, IL-6, IL-8, and tumor necrosis factor [TNF]) and activate complement by the alternative pathway. The polysaccharide capsule also activates the alternative complement pathway in vitro. Such activation is associated with the release of C5a, a potent attractant for polymorphonuclear neutrophils. The classic complement pathway also is activated by antibody to cell wall polysaccharides in the absence of anticapsular antibody, and an intense inflammatory response fueled by vigorous activation of both the alternative and classical complement pathways accompanies pneumococcal infection of an immunologically naïve host.[379] The disease process is largely a result of this inflammation, and its severity is in direct proportion to its intensity. Pneumolysin also is associated with the severity of disease, and injection of pneumolysin into rat lung causes all the histologic findings of pneumonia, whereas immunization of mice with pneumolysin before infection or challenge with pneumolysin knockout pneumococci is associated with a significant reduction in virulence.[40,155] Mitchell reports that

some nonmeningitis strains of serotype 1 have a mutant nonhemolytic pneumolysin.[364] As discussed later in the section on virulence factors, there are numerous other factors that contribute to the ability of pneumococcal strains to cause disease and regulate severity.

A 2006 study evaluated the association of serotype with pneumococcal disease among 189 isolates from blood or CSF, 3200 isolates from middle ear fluid, and 348 isolates from the conjunctiva of children aged younger than 36 months with pneumococcal infection. A positive association with IPD was demonstrated for serotypes 1, 5, and 12F; with AOM for serotypes 1, 3, 5, 12F, 19A, and 19F; and with acute conjunctivitis for serotype 3 and nontypeable *S. pneumoniae*.[488] Although pneumococci most commonly cause bacteremia, otitis media, pneumonia, and meningitis, they can produce disease in virtually any organ. Before the advent of immunotherapy and chemotherapy, such "unusual" infections were relatively common. Today, they are less so. Survival of a patient with a pneumococcal disease depends on numerous variables, including the site of infection, the underlying disease, and the patient's age. Before the advent of chemotherapy, pneumococcal meningitis was almost universally fatal, whereas pneumococcal pneumonia killed approximately 25 percent of hospitalized patients. Austrian and Gold, in 1964, dramatically illustrated the role of age and underlying disease when their survey of mortality associated with bacteremic pneumococcal pneumonia destroyed the complacency produced by the use of antimicrobial agents.[34] The aged and infirm were likely to die despite receiving immediate and appropriate therapy with penicillin. Today, normal children living in industrialized countries rarely die of pneumococcal disease; thus, the prognosis must be related to the likelihood of permanent sequelae occurring. Pneumococci do not usually cause necrosis in pulmonary tissue, and survivors rapidly regain normal pulmonary function.[261] Many children recovering from pneumococcal meningitis are found to have neurologic sequelae. Some investigators suggest that the first attack of pneumococcal otitis media in some way predisposes the individual to subsequent attacks of otitis media.[237] Before the availability of antimicrobial agents, recovery of a patient with pneumococcal pneumonia depended on the development of type-specific antibody. Although serum and white blood cells (WBCs) from nonimmune children kill pneumococci, probably by activation of the alternative complement pathway, they do so slowly. The importance of this pathway is illustrated best by the inability of children with sickle-cell disease to handle pneumococcal infection.[128,268,415] These children and others with asplenia may die rapidly despite the administration of prompt, vigorous therapy.[271,285,481]

MICROBIOLOGY

Structure

Pneumococcal cells are surrounded by a trilamellar, lipopolysaccharide, cytoplasmic membrane that has two electron-dense bands, each 25 to 30 Å wide. A cell wall surrounding the plasma membrane has two bands-an inner 30- to 40-Å-wide band and an outer 60- to 80-Å-wide band. Numerous bridges connect the cell wall and the plasma membrane. The polysaccharide capsule covers the cell wall in encapsulated strains and is seen as a wider, less-structured band.[534] A schematic representation of the major structural components and selected cell wall components is shown in Figure 85-12.

Cell Wall Structure

The predominant structural components of the pneumococcal cell wall are peptidoglycan, TA, lipoteichoic acid (LTA), and several choline-bound proteins. Choline is a lipid that is an essential growth factor for *S. pneumoniae*.

Peptidoglycan. Peptidoglycan, which accounts for approximately half of the cell wall mass, is a cell wall polymer linked by stem peptides to form a complex, three-dimensional structure.[484] Stem peptides are formed when transpeptidases (also known as *penicillin-binding proteins* [PBPs]) link pentapeptide chains into linear stem peptides in penicillin-susceptible strains. However, in non-penicillin-susceptible strains, branched and other variant stem peptides are produced.

Lipoteichoic Acid. LTA also is known as *pneumococcal Forssman (F) antigen*. The LTA of pneumococci possesses identical repeat and chain structures linked to a cell membrane glycolipid that anchors LTA to the cell.[161,162] Phosphocholine is attached to saccharide residues. PspA and other proteins (see later discussion) also are attached to choline residues on LTA.

Teichoic Acid. TA, also known as *pneumococcal C polysaccharide*, has a chain structure similar to that of LTA, except that the saccharide differs. TA chains are attached to the cell wall peptidoglycan. Some phosphocholine residues of both LTA and TA are expressed on the cell wall surface, where they are thought to serve three functions: (1) activation of the pneumococcal autolysin (LytA) enzyme, which is responsible for the autolysis of pneumococci; (2) binding of the choline-binding domain of LytA to choline on TA, which may regulate the activity of LytA; and (3) a function associated with transformability (choline-deficient cells lack transformability).[193,262]

Surface Proteins. Pneumococci have several surface proteins, with the five most important being *pneumococcal surface protein A* (PspA), *pneumococcal surface adhesin A* (PsaA), *choline-binding protein A* (CbpA), *hyaluronate lyase* (Hyl), and *pilin protein* (RrgB).

PspA is a cell wall protein with a molecular size of 67 to 99 kDa that is bound to TA and LTA by phosphocholine links.[262] This protein extends through the cell wall and capsule to the surface of the organism.[594] PspA exists in various antigenic forms, and epitopes within one PspA molecule can recombine into different types.[74] However, PspA variants usually are sufficiently cross reactive that immunization with one PspA serotype elicits immunity to other PspA serotypes.

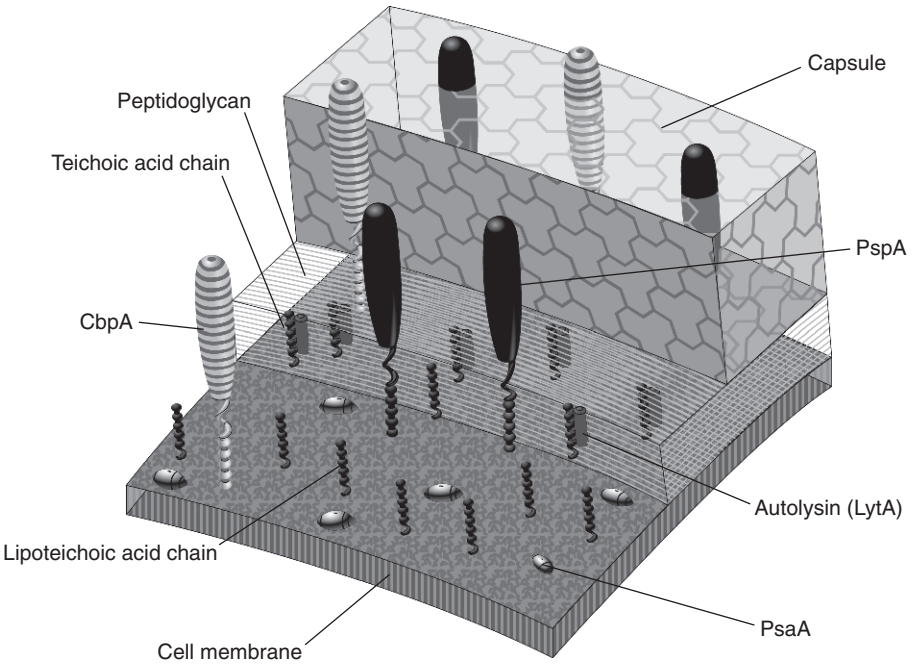

FIGURE 85-12 ■ Schematic three-dimensional representation of the major structural components of the cell membrane, cell wall, and capsule of *Streptococcus pneumoniae*. The locations of selected major virulence factors of the organism are shown as well. Chains of lipoteichoic acid are attached to cell membrane glycolipid, whereas surface proteins, such as PspA and CbpA, in turn are attached to the lipoteichoic acid chains via phosphocholine links. PsaA is found on the outer surface of the cell membrane. (Copyright Michael R. Jacobs, used with permission.)

PsaA is a 37-kDa surface protein thought to be anchored to the cell membrane and associated with magnesium and zinc transport.[129] It appears to be a lipoprotein and is common to virtually all *S. pneumoniae* isolates.[440] Considerable variation in the amino acid sequences of PsaA from different strains has been detected.[413] The relationship of this protein to other cell wall components has not been determined.[523]

CbpA is a protein similar to PspA and has a mass of 75 kDa. It is an adhesin involved in the adherence of pneumococci to cytokine-activated human cells.[458] Several other choline-binding proteins also have been identified.

Hyl is a hyaluronidase that results in breakdown of the hyaluronan and chondroitin sulfate present in the extracellular matrix of human tissues.[458]

Pilin protein (RrgB) is present on the surface of virulent pneumococci in the form of a pilus shaft, which is a long, thin proteinaceous filament extending from the cell wall that aids bacterial colonization in the nasopharynx through epithelial cell binding.[412] It has a large adhesive pilin at the tip, a polymeric shaft, and a smaller cell wall anchor and is composed of four immunoglobulin-like domains. Isopeptide bond formation in the N-terminal domains, which provides the covalent linkage to the next pilin subunit in the shaft, appears to take place only on assembly of the pili.

Capsule

The capsule of pneumococci consists of polysaccharides that vary in the make-up of monosaccharides, the sequence of monosaccharides in polysaccharides, the linkage of monosaccharides to each other, and the presence of nonsaccharide components.[156] Currently, 93 serotypes consisting of 25 individual serotypes and 68 serotypes grouped into 21 serogroups are known (Table 85-2). Each serotype has a specific capsular structure, and serotypes within a serogroup often have the same oligosaccharide sequences linked differently. The capsular structure of many serotypes has been determined.[351]

Several epidemic clones have been shown to have different serotypes, and extensive genetic changes involving the replacement of entire cassettes of genes related to capsule production are required for capsular switching to take place.[5]

Genome

The complete pneumococcal genome has been mapped. The genome has been estimated to be 2.0 to 2.1 megabases (Mb), approximately half that of *Escherichia coli*.[43,131] The locations of more than 100 genes, including 20 tRNA synthetase and 20 ribosomal protein genes, have been mapped. Genes involved in cell wall synthesis also have been identified. *S. pneumoniae* is a naturally transformable bacterium, which means that it is able to take up single-stranded DNA from its environment and incorporate this exogenous DNA into its genome. This process is known as *transformational recombination*.[373,505] Recombination is a powerful means of genome evolution and provides a great degree of genome flexibility to this organism. Transformation occurs only at high cell densities (10^5 to 10^8 colony-forming units/mL), and a peptide

pheromone quorum-sensing signal called *activator* or *competence factor* is required.[436] This factor also is termed *competence-stimulating peptide.*[508] The genes associated with capsule synthesis have been characterized for several serotypes as well. The complete nucleotide sequence of many of these genes of several *S. pneumoniae* serotypes has been determined.[27,172,370,569]

Virulence Factors

Animal models of pneumococcal infection have provided considerable insight into the pathogenesis of disease and the association of virulence factors with disease. However, the pneumococcus is primarily a human pathogen and the host defenses of animal models can vary significantly from those in humans. For example, pneumococci adhere to human but not to rabbit polymeric immunoglobulin receptor (pIgR).[49,601] Additionally, virulence in mice varies considerably with the strain of pneumococcus: pneumococci belonging to serogroups 6, 14, 19, and 23 rarely are virulent in mice, whereas serotypes 1, 2, and 3 usually are virulent.[73,601] Virulence also may vary according to the mouse strain.*

Some serotypes can be virulent to one species of animal but not to others. An example is serotype 19F, which rarely is virulent in mice[36] but is highly virulent in guinea pigs.[32] In addition, penicillin resistance appears to be linked to decreased virulence by virtue of the fact that virulence of isogenic mutants of a virulent, penicillin-susceptible strain was reduced significantly when transformed into a penicillin-resistant strain with an abnormal *pbp2x* gene.[445] Therefore, many animal models of pneumococcal virulence may not be representative of virulence in humans or representative of all pneumococcal serotypes or antimicrobial-resistant strains.

Although the polysaccharide capsule has been recognized as the major determinant of virulence, relatively little is known about the molecular basis of the pathogenesis of pneumococcal disease. A library of 1786 pneumococcal mutants created by insertion-duplication mutagenesis was analyzed for their ability to survive and replicate in murine models of pneumonia and bacteremia.[309] One hundred eighty-six mutant strains exhibited attenuated virulence; 56 of these strains were genetically characterized, and genomic DNA inserts were sequenced and subjected to database searches. Most of the insertions were in probable operons, but no pathogenicity islands were found. Forty-two novel virulence loci were identified. Five strains showed mutations in genes involved in gene regulation, cation transport, or stress tolerance; the virulence of these strains was shown to be highly attenuated in a murine respiratory tract infection model. Additional experiments also suggest that induction of competence for genetic transformation has a role in virulence.[309] This approach has revealed several previously unrecognized genes required for virulence.

A similar genomic approach was used to look for genes coding for surface-localized proteins that could be targets for protective humoral immunity. By exploiting the whole-genome sequence of *S. pneumoniae*, researchers found 130 open-reading frames encoding proteins with secretion motifs or similarity to predicted virulence factors.[413] Mice were immunized with 108 of these proteins, and 6 proteins conferred protection against disseminated pneumococcal infection. Each of the protective antigens showed broad strain distribution and immunogenicity in human infections. Some of these proteins have been identified as LytB, LytC, and a cell wall–anchored serine protease. Another genomic-based study used a genomic expression library of *S. pneumoniae* screened with convalescent-phase serum for immunoreactive proteins.[605] Six known and 17 unknown pneumococcal proteins were detected. Five of the known proteins, including PspA and SpsA (CbpA), were surface-located virulence factors, and 8 of the unknown proteins were putative membrane proteins. Use of these genomic approaches for the identification of novel microbial targets to elicit a protective immune response has been validated, and these new antigens may play roles in the development of improved vaccines against *S. pneumoniae.*

Capsule

The capsule is the major determinant of virulence in pneumococci. It prevents phagocytosis by polymorphonuclear leukocytes (PMNs) and macrophages, thereby allowing unrestricted extracellular multiplication of the organism. Because the pneumococcus has 93 antigenically distinct serotypes, production of anticapsular antibody in response to one serotype provides protection against only that serotype or serogroup, whereas nonencapsulated strains are considerably less virulent.[379] The importance of the capsule as a virulence factor is emphasized by the fact that protection from pneumococcal infection can be achieved by capsular-specific antibodies. Despite the large number of additional virulence factors, the capsule remains the single most important determinant of virulence in avoiding host defenses after the epithelial barriers have been breached. Other virulence factors are important in breaching host defenses such as epithelial barriers.

Neuraminidases

Neuraminidases are enzymes that cleave terminal sialic acid residues from glycolipids, glycoproteins, and oligosaccharides on eukaryotic cell surfaces; such cleavage may unmask cell surface receptors for pneumococcal adhesins.[413] The neuraminidase NanA has been implicated in the ability of *S. pneumoniae* to colonize and persist in the nasopharynx and middle ear.[535] A second neuraminidase, NanB, has much weaker activity than NanA does but exhibits optimal activity at pH 5, whereas NanA is most active at pH 7.[413]

Pneumolysin

Pneumolysin is a 53-kDa cytoplasmic protein produced by all pneumococci. It is essential for the initial binding to membrane cholesterol and the interaction leading to subsequent membrane damage.[37]

*References 1, 32, 36, 301, 445, 530.

Functions of pneumolysin include the following:

1. Pore formation in host epithelial cell membranes. Pneumolysin binds to cholesterol in host epithelial cell membranes, where oligomers of pneumolysin molecules assemble to form 35- to 45-nm pores in the cell membrane, which results in lysis of the targeted cell. Pneumolysin is, therefore, cytotoxic to epithelial cells, and it also slows ciliary beating of bronchial epithelial cells and disrupts the tight junctions between epithelial cells. In addition, pneumolysin disrupts alveolar epithelial cells and the alveolar-capillary boundary, thereby facilitating entry of pneumococci into the bloodstream and through the blood-brain barrier.[183,606]
2. Effects on phagocytic and immune cell function. Pneumolysin attracts neutrophils in the early phases of disease and lymphocytes at a later stage.
3. Direct activation of the complement system. Expression of pneumolysin by pneumococci reduces serum complement levels and serum opsonic activity.[10]
4. Promotion of nitric oxide (NO) production by macrophages. NO is produced by an inducible NO synthase (iNOS) during inflammation as an essential element of antimicrobial defense, but it also can contribute to host-induced tissue damage.[71]

Surface-Located Choline-Binding Proteins

The virulence of members of the choline-binding protein (Cbp) family and two recently described cell wall hydrolases, LytB and LytC, has been characterized.[193] Cbp-, LytB-, and LytC-deficient mutants show significantly reduced colonization of the nasopharynx. The following proteins of the Cbp family and their virulence mechanisms have been described:

1. PspA is a serologically variable protein that has undergone extensive recombination.[233] It is thought to exert its virulence function in systemic infection by interfering with deposition of the complement component C3b onto pneumococci or by blocking recruitment of the alternative pathway, thereby reducing the effectiveness of complement receptor-mediated pathways of clearance.[539] PspA has been shown to bind to lactoferrin, an iron-sequestering glycoprotein found in mucosal secretions, when the level of free extracellular iron is not sufficient for the growth of pneumococci. This binding is thought to overcome the iron limitation at mucosal surfaces and might represent a potential virulence mechanism for colonization of mucosal surfaces.[212]
2. CbpA, or SpsA, is a surface protein adhesin that acts as a bridging element between pneumococci and host-cell glycoconjugates on cytokine-activated host cells.[213] This process is thought to be associated with change from nasopharyngeal colonization to invasion of epithelial and endothelial cells.[458] One mechanism by which this process occurs, wherein CbpA binds to the pIgR of human epithelial cells, has been described.[599] The pneumococcus co-opts the IgA transcytosis machinery and gains entry into and across airway epithelial cells. This process is a novel example of a pathogen co-opting the transcytosis machinery to promote translocation across a mucosal barrier.[273]
3. PsaA is a surface protein that also is associated with virulence via adhesion to epithelial cells.
4. Pneumococcal histidine-containing protein A (PhpA) is a 20-kDa protein with putative proteolytic activity against the human complement component C3. PhpA is a potential candidate for use as a vaccine against systemic pneumococcal disease and otitis media.[601]
5. The pneumococcal histidine triad (Pht) proteins PhtA, PhtB, PhtD, and PhtE constitute a novel family of homologous surface proteins associated with virulence; they are also potential vaccine candidates.[9] Although antibodies targeting PhtA, PhtB, or PhtD are protective, the function of these proteins remains unknown. The number of histidine and tyrosine residues in these proteins suggests that they may be involved in metal or nucleoside binding.

Phase Variation

Phase variation in the colonial opacity of *S. pneumoniae* has been implicated as a factor in bacterial adherence, colonization, and invasion.[572] On clear media, colonies can appear as opaque or translucent when viewed under magnification with oblique, transmitted light. All strains of *S. pneumoniae* are thought to be capable of phase variation. Opaque colonies are less likely to autolyze, contain less TA but more PspA in their cell walls, and colonize the nasopharynx poorly in animal models but are more virulent when inoculated into sterile sites. Conversely, translucent colonies are more likely to autolyze, contain more TA but less PspA in their cell walls, and colonize the nasopharynx well but are less virulent when inoculated into sterile sites. Translucent colonies become umbilicated as a result of autolysis, whereas opaque colonies remain dome shaped.

Phosphorylcholine Esterase

This enzyme has activity that removes phosphorylcholine residues from cell wall TA and LTA.[557]

Inactivation of the gene encoding for the enzyme in pneumococcal strains caused a change in colony morphology from translucent (colonizing) to opaque (virulent) and a striking increase in virulence in the intraperitoneal mouse model. Phosphorylcholine esterase therefore appears to be a regulatory element involved in the interaction of *S. pneumoniae* with its host.

Pneumococcal Autolysin

Pneumococcal autolysin (LytA) is a 36-kDa cell wall protein attached to choline residues on TA and LTA. It is associated with unlinking of cell wall glycan from stem peptides during cell remodeling and division. Autolysin initially was considered to be a significant virulence factor, but more recent work has shown that it plays only a minor role and that immunization with autolysin does not provide protection.[413]

Cell Wall Stem Peptides

The peptidoglycan of gram-positive bacteria triggers the release of cytokines from peripheral blood mononuclear cells.[345] However, 100 to 1000 times more gram-positive peptidoglycan than gram-negative lipopolysaccharide endotoxin is required to release the same amount of cytokine. Simple stem peptides were 10-fold less active than was undigested peptidoglycan in stimulating TNF. In contrast, complex branched peptides such as tripeptides were at least 100-fold more potent than was the native material. These complex branched peptides represented 2 percent or less of the total material, but their activity in stimulating TNF was almost equal to that of endotoxin.

Iron Transport

The availability of iron is a major requirement for the growth and survival of many organisms, including *S. pneumoniae*. Two *S. pneumoniae* genetic loci, *pit1* and *pit2*, which encode homologs of ABC iron transporters, are required for uptake of iron by this organism.[78] Virulence in mouse models of pulmonary and systemic infection is attenuated moderately with a *pit2*-disrupted strain and attenuated strongly with a *pit1/pit2*-disrupted strain.

IgA Protease

IgA proteases belong to a family of proteins used by a diverse group of bacteria, including *S. pneumoniae*, for colonization and invasion. IgA1 protease allows bacteria to cleave human IgA1 in the hinge region. The exact role of these enzymes in bacterial pathogenesis is not understood completely, but they are important in bacterial colonization of mucosal membranes in the presence of secretory IgA antibodies by causing local IgA deficiency.[281] The IgA protease genes of *S. pneumoniae* and *Streptococcus mitis* show extensive polymorphism, which results in enzymes with considerable antigenic diversity.[429]

Phosphoglucomutase

Phosphoglucomutase is an enzyme that is necessary in one of the early steps in capsular polysaccharide synthesis, and *S. pneumoniae* mutants lacking this enzyme do not produce a capsule and are avirulent in immunocompetent but not in immunosuppressed mice.[214] Other metabolic pathways also are thought to be affected by this enzyme.

Free Oxygen Radicals

Release of free oxygen radicals has been implicated in the pathogenesis of otitis media by *S. pneumoniae*, and antibiotic killing of bacteria leads to the release of further free oxygen radicals, which results in tissue damage despite the administration of appropriate antibiotic therapy.[521] Reactive oxygen intermediates also mediate brain injury in bacterial meningitis.[30,333]

NADH Oxidase

Reduced nicotinamide adenine dinucleotide (NADH) oxidase has been shown to be a virulence factor necessary for *S. pneumoniae* infection. The basis of NADH oxidase as a virulence factor is the conversion of O_2 to H_2O. If O_2 is not reduced fully, it can form superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), both of which can be toxic to cells.[595]

Pyruvate Oxidase

Pyruvate oxidase decarboxylates pyruvate to acetyl phosphate plus H_2O_2 and CO_2 and appears to be associated with regulation of the multiple adhesive properties of pneumococci.[504] A pneumococcal mutant lacking the gene encoding pyruvate kinase showed a greater than 70 percent loss of the ability to attach to all cell types.

Plasminogen Binding and Penetration of the Basement Membrane

Binding of plasminogen plus penetration of the basement membrane is thought to be an essential step in the pathogenesis of bacterial meningitis.[137] Most strains adhere to reconstituted basement membrane, as well as to its purified laminin and collagen IV components, and to bound plasminogen. Penetration of the basement membrane was achieved within 3 to 4 hours in the presence of plasminogen, whereas without plasminogen no penetration occurred.

Hyaluronidase

Virtually all pneumococcal strains produce the enzyme hyaluronidase, a 107-kDa protein. Models used to simulate human meningitis generally involve the direct intracerebral route of infection. However, intranasal inoculation would provide a more realistic model, and this was achieved by intranasal administration of *S. pneumoniae* with hyaluronidase. This model induced meningitis in 50 percent of inoculated mice, whereas meningitis did not develop in any of the mice inoculated without hyaluronidase. Hyaluronidase was found to facilitate pneumococcal invasion of the bloodstream after colonization of the upper respiratory tract. This murine model mimics important features of human disease, which allows the model to be used to study issues related to the pathophysiology and treatment of pneumococcal meningitis.[604]

Peptidoglycan N-Acetylglucosamine Deacetylase A

The glucosamine and muramic acid residues of the pneumococcal cell wall traditionally are regarded as being *N*-acetylated. However, more than 80 percent of the glucosamine and 10 percent of the muramic acid residues have been shown to be deacetylated, thereby explaining the resistance of peptidoglycan to the hydrolytic action of lysozyme, a muramidase that cleaves the glycan backbone.[556] A gene that encodes for peptidoglycan *N*-acetylglucosamine deacetylase A has been identified. This gene may, therefore, contribute to pneumococcal virulence by providing protection against host lysozyme, which is known to accumulate in high concentration at sites of infection.

Phages

Whereas transformation is recognized as occurring in pneumococci, another mechanism of DNA transfer in pneumococci is transduction of DNA carried by bacteriophages (lysogeny). A high proportion (76% of 791 isolates) of clinical isolates of pneumococci were found to carry multiple copies of LytA, thus indicating the widespread occurrence of lysogeny in pneumococci.[437] The LytA hybridization pattern of a strain has been found to be stable during extensive serial culturing; it is specific for the clonal type of the strain and can be used as a molecular epidemiologic marker. In addition, phage DNA integrated into the pneumococcal genome acts as an integrase to facilitate the introduction of foreign genes into the pneumococcal chromosome.[184]

Tolerance

The ability of *S. pneumoniae* to escape lysis and killing by vancomycin and penicillin, a property termed *tolerance*, has been described.[228] Among 116 clinical isolates of pneumococci, 3 percent and 8 percent were tolerant to vancomycin and penicillin, respectively. Tolerance may contribute to treatment failure, particularly in meningitis, in which bactericidal activity is critical for eradication. A vancomycin- and cephalosporin-tolerant strain of *S. pneumoniae*, the Tupelo strain, has been isolated from the CSF of a patient in whom recrudescence of meningitis developed despite treatment with vancomycin and a third-generation cephalosporin.[355] The defect leading to tolerance in this strain involves the control pathway for triggering of autolysis via a mutated CiaH histidine kinase.[374] A frameshift mutation in the *lytA* gene encoding the main pneumococcal autolysin was associated with vancomycin tolerance in another strain.[374]

Viral Respiratory Tract Infections

The role of viral respiratory tract infection in predisposing the host to secondary bacterial infection, including pneumonia, empyema, and lung abscess, is well recognized.[321] The lungs of immunocompetent mice infected with influenza A virus on day 1 and *S. pneumoniae* on day 8 demonstrate greater *S. pneumoniae* colony counts, more extensive neutrophil infiltration, and higher lung levels of IL-1β and TNF-α after exposure to *S. pneumoniae* than do the lungs of control mice not preinfected with influenza virus.[321] Influenza A virus facilitates *S. pneumoniae* transmission and disease, with secondary bacterial pneumonia representing an important cause of excess mortality during both influenza epidemics and pandemics.[11,127] This lethal synergism between influenza virus and *S. pneumoniae* was first suggested by studies performed on samples collected during autopsy from victims of the 1918 influenza pandemic and recently confirmed by data collected during the 2009 A/H1N1v influenza pandemic.

Diagnostic Microbiology

S. pneumoniae is a gram-positive coccus that replicates in pairs and chains in liquid medium. The shape of the individual organism is a lanceolate coccus that usually occurs in pairs with the long axis forming a straight line. Elongated or pointed forms are commonly found.

Isolation

Pneumococci are cultured readily on blood and chocolate agar media, as well as in suitable liquid culture media for isolation from blood. Isolates are facultative anaerobes, and most strains require atmospheric enrichment with 5 to 10 percent CO_2 for primary isolation[33]; occasional strains are strict anaerobes. Strains can be adapted for growth without CO_2 supplementation by repeated subculture. Detection of nasopharyngeal carriage of pneumococci is a problem because of the presence of other flora; the use of antimicrobial-containing media, such as blood agar supplemented with gentamicin (5 μg/mL), has led to improvements in isolation of pneumococci, particularly resistant strains.[416,434] However, the sensitivity of current in vitro methods is poor in comparison to the sensitivity of mouse inoculation, although not all serotypes are virulent in mice.[232,293]

Identification

Pneumococci usually are identified readily by standard features such as colonial morphology, α-hemolysis, negative catalase reaction, optochin susceptibility, bile solubility, and specific reactions with antisera to capsular polysaccharides.[252] *S. pneumoniae* produces an autolytic intracellular enzyme, LytA, that causes the organism to autolyze rapidly when grown on artificial media. Bile salts and optochin accelerate this natural autolytic process by combining with the pneumococcal cell and activating its autolysin. Strains with atypical features, such as rounded rather than flat or concentrically ringed colonies, optochin resistance, or lack of capsules, do occur and can result in misidentification of such strains as viridans streptococci. Atypical strains are more likely to be encountered from normal flora sites and with

penicillin-resistant strains. Molecular methods for identification of pneumococci include polymerase chain reaction (PCR) detection of *ply* and *psaA* genes, multilocus sequence typing (MLST), 16S rRNA gene sequencing, and pyrosequencing. The presence of *psaA* is useful in distinguishing between atypical pneumococci and other streptococcal species.[252]

Strains with optochin zones greater than 14 mm can be identified presumptively as pneumococci, strains with 7- to 14-mm zones require confirmation by bile solubility, and strains with no zone usually are not *S. pneumoniae*. However, incubation in carbon dioxide has been recognized for a long time as decreasing the size of the zone around optochin disks, and incubation in room air generally results in an increase in zone size if the strain is a pneumococcus or a decrease if the strain is a member of the viridans group of streptococci.[435] Optochin-resistant variants of pneumococci can occur and usually are seen as a subpopulation within the zone of inhibition of an optochin disk, and optochin-resistant mutants can be selected by passage of strains in the presence of optochin.[378] Strains with equivocal optochin zones or atypical colonial morphology can be tested for bile solubility, either directly by placing a drop of bile salt solution (10% sodium deoxycholate) onto colonies and observing for lysis of the colonies or by suspension of organisms in a bile salt solution with a bile salt-free control. Care must be taken in the tube bile solubility test to avoid obtaining false-positive results, which can be caused by the organism suspension being too light or by organisms being suspended in broth rather than saline.[252]

Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) fingerprinting systems are increasingly being used for bacterial identification in the routine diagnostic microbiology laboratory but may not adequately differentiate pneumococci from species within the *Streptococcus mitis* group, which includes *S. pneumoniae*. A comparative analysis of MALDI-TOF spectra of several species from the *S. mitis* group suggests that it is possible to distinguish different species of the *S. mitis* group by close analysis of their mass peak profiles.[574]

Identification of the capsular polysaccharide serotype or serogroup also is useful in characterizing strains and confirming the identity of problem strains. Such identification is performed by the capsular swelling technique, in which equal volumes of an organism suspension, 0.3 percent methylene blue dye solution, and antiserum are mixed on a glass slide, covered with a coverslip, and read at 1000× magnification by phase-contrast microscopy. Alternatively, organism suspensions can be dried on slides and antiserum and methylene blue dye solution mixed on a coverslip, which then is placed on the slide. The polysaccharide capsule of the pneumococcal organism binds with type-specific antiserum, and organisms can be seen to clump or agglutinate; the resulting change in the refractive index of antibody-coated capsule causes the capsule to appear swollen. Antisera to each serotype or serogroup and factoring antisera to subtype serogroups are available from Statens Serum Institut, Copenhagen, Denmark.[227] The numbering system for pneumococcal serotypes is shown in Table 85-2.

Currently, 93 serotypes have been identified and are divided into 25 individual serotypes and 21 serogroups in the Danish classification, which now is used universally. Most serotypes have one antigenic determinant, whereas serogroups have one or more antigenic determinants common to the group and one or more determinants unique to each serotype. Serotypes within a serogroup usually are identified by the serogroup number followed by a letter indicating the serotype to which a strain belongs. Except for serogroups 6 and 9, the letters are F for the first subtype, followed by A, B, and so on. Each serogroup contains two to six related types, and the 21 serogroups currently include over 67 individual subtypes. Serotype numbers 26 and 30 are not in use. Serotype 2, an extremely uncommon pneumococcal serotype, has recently been detected as the leading pneumococcal serotype (20% of 221 cases) in childhood meningitis in Bangladesh.[465] These serotype 2 strains had three closely related pulsed-field gel electrophoresis types.

Omniserum containing antibodies to all serotypes is available and can be used to confirm the identity of isolates as pneumococci. Because many serotypes or serogroups are included in this reagent, reactions may not always be optimal and usually are stronger in pool or monovalent reagents. Nine antiserum pools classified from A to I, each containing four to seven serotypes or serogroups, also are available and can be used to identify strains in a group of serotypes before individual serotype or serogroup reagents are tested. Other methods of capsular typing, such as latex agglutination, coagglutination, and capillary precipitation, can be used, but these methods are not available commercially. The ability of a PCR method to identify the capsular serotype of pneumococci has been developed on the basis of polymorphisms in two genes common to the different capsule loci.[312] PCR analysis holds promise as a noncultural method for determining serotype.

Detection of Clonality

In addition to phenotypic features, such as serotype and antimicrobial resistance markers, various DNA fingerprint methods for epidemiologic typing of *S. pneumoniae* have been applied. The currently recognized reference method is multilocus sequence typing (MLST), in which 450 bp fragments of seven housekeeping loci are determined. The combination of alleles at these seven loci provided a sequence type (ST), and the relatedness between isolates is obtained by constructing a dendrogram from the matrix of pairwise differences between STs.[136,144,344] Other typing methods include ribotyping, BOX fingerprinting with the BOX repetitive sequence of *S. pneumoniae* used as a DNA probe, PCR fingerprinting with a primer homologous to the enterobacterial repetitive intergenic consensus sequence, pulsed-field gel electrophoresis of large DNA fragments digested by restriction enzymes, and detection of polymorphic DNA sequences for a multiple-locus variable-number tandem-repeat analysis (MLVA).[229,356]

Diagnosis of Pneumococcal Disease

Definitive diagnosis of pneumococcal infection is based on recovery of pneumococci from the site of infection or documentation of pneumococcal bacteremia, whereas presumptive diagnosis is based on detection of pneumococcal cellular components, such as capsular polysaccharide, and on species-specific DNA and RNA sequences from the site of infection or from remote sites such as urine.[252] Definitive diagnosis is confounded in many instances by the need for invasive procedures to obtain specimens (e.g., from the middle ear space) and by nasopharyngeal carriage of pneumococci when sputum is cultured. Pneumococci almost invariably are isolated from CSF in pneumococcal meningitis, even in patients receiving oral antibiotics.[459]

However, establishing the diagnosis of pneumococcal pneumonia is more challenging because sputum rarely is available from children and direct lung puncture is performed very infrequently. Detection of pneumococcal bacteremia to confirm the diagnosis of pneumococcal pneumonia or other localized infection is valuable, but it does not occur frequently and the actual prevalence of bacteremic pneumococcal pneumonia in children is not known,[529] although a study suggests that the prevalence is approximately 17 percent (11/64).[359] Therefore, pediatricians must use other methods to diagnose pneumococcal pneumonia. Signs and symptoms significantly associated with bacteremic pneumococcal pneumonia in children include high temperature (>38.9°C [>102°F]), leukocytosis (>15,000/mm^3), and lobar or segmental consolidation.[529] However, the frequency with which these findings are associated with nonbacteremic pneumococcal pneumonia is not known.

With the widespread deployment of pneumococcal vaccine, occult bacteremia caused by *S. pneumoniae* occurs less commonly. The likelihood that a positive blood culture will be a false-positive result is increased if the WBC count is less than 15,000/mm^3, the time for the blood culture to become positive exceeds 24 hours, or the Gram stain result is suggestive of a contaminant.[472] Overall, the frequency with which positive blood cultures are obtained from children with occult bacteremia has declined in comparison to the pre-pneumococcal vaccine era and has declined precipitously since the era before the deployment of the *H. influenzae* type b (Hib) vaccine.

Direct examination of Gram-stained smears of clinically appropriate material remains the fastest diagnostic method and can be augmented if necessary by direct demonstration of capsular swelling of organisms in the presence of anticapsular antisera. The availability of antigen-detection systems that can be used in urine, serum, CSF, and other specimens, such as capsular antigen detection by counterimmunoelectrophoresis and latex agglutination with polyvalent pneumococcal reagent, generally has not improved patient management because of the low sensitivity and specificity of these methods and the fact that they usually are positive only when a Gram stain also is positive. The concentration of pneumococcal capsular antigen in saliva was evaluated by latex agglutination in a study consisting of children with community-acquired pneumonia and healthy control subjects. None of the children with pneumonia in this study had a positive blood culture, and pneumococcal capsular antigen was detected in the saliva of 27 percent of children with pneumonia versus 17 percent of control subjects. More affected children (20%) than healthy control subjects (2%) had a pneumococcal capsular antigen titer of 10 or greater (p <0.01). Quantitative measurement of pneumococcal capsular antigen in saliva may be valuable in helping make an etiologic diagnosis in children with pneumonia, but its sensitivity is poor and it is confounded by false-positive results caused by pneumococcal carriage.[164]

A rapid (15-minute) immunochromatographic membrane test to detect pneumococcal polysaccharide capsular antigen in urine samples (Binax NOW) has been developed and was evaluated in the diagnosis of bacteremic and nonbacteremic pneumococcal pneumonia. Urine samples were studied in 51 patients with bacteremic and nonbacteremic pneumonia caused by *S. pneumoniae;* the pneumonia was diagnosed by blood culture, and pneumococcal polysaccharide capsular antigen was detected by counterimmunoelectrophoresis in urine samples. Pneumococcal antigen was detected in urine by the immunochromatographic membrane test in 41 of 51 patients with pneumococcal pneumonia (80.4%), including 23 of 28 bacteremic cases (82.1%) and 18 of 23 nonbacteremic cases (78.3%). Antigen also was detected in 7 of 16 patients with a diagnosis of presumptive pneumococcal pneumonia (43.7%) and in 1 of the 16 patients with pneumonia but in whom no pathogen was identified. The specificity of the immunochromatographic membrane test was 97.2 percent, but its sensitivity was only approximately 80 percent, thus limiting its value.[164] Furthermore, the usefulness of this test is questionable because the antigen was detected in the urine in 30 of 138 (22%) healthy children with nasopharyngeal carriage of *S. pneumoniae* versus only 3 of 71 (4%) noncarriers (p < .001).[211] Thus, the test was shown to be often positive in healthy pneumococcal carriers.

One recent study found that immunochromatographic testing for *S. pneumoniae* in CSF was both 100 percent sensitive and specific. The simplicity of the test and the longevity of the CSF antigen even after treatment suggest potential utility of this method in identifying *S. pneumoniae* meningitis in resource-poor countries with widespread prehospital antimicrobial use.[466] Another study showed immunochromatographic testing to be more sensitive than culture in detecting *S. pneumoniae* as the causative agent in thoracic empyema.[425]

Newer molecular-based methods for establishing the diagnosis of pneumococcal disease include the use of DNA probes to detect pneumolysin, autolysin, and PsaA protein, but considerable practical problems must be overcome before these methods will be applicable clinically.[117] Examples include a commercial method involving real-time PCR for simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *S. pneumoniae* in patients suspected of having meningitis and septicemia. This method is based on detection of the pneumolysin gene for *S. pneumoniae* and uses a single-tube, 5′-nuclease multiplex PCR assay on samples of CSF, plasma, serum, and whole blood. Amplified products

are monitored with sequence-specific, fluorescent dye-labeled probes. The sensitivity of using clinical samples (CSF, serum, plasma, and whole blood) from culture-confirmed cases of *S. pneumoniae* infection was 91.8 percent. The multiplex assay also was used to test a large number of culture-negative samples, which resulted in the detection of numerous cases of meningococcal, *H. influenzae*, and pneumococcal disease that had not been detected by culture.[518] However, whether these results are true positives or false positives is not known; and in another study, although the sensitivity of PCR amplification of the pneumolysin gene in the serum and CSF of infants and children with culture-proven pneumococcal bacteremia and meningitis was 100 percent, the specificity was poor, with 17 percent of healthy control subjects having positive results.[117] The prevalence of false-positive reactions was highest (33%) in 2-year-old children, the age group with the highest rate of nasopharyngeal carriage of pneumococci. Therefore, although PCR analysis of serum and CSF is a sensitive test for the detection of *S. pneumoniae* in these sites, its high rate of positivity in healthy control subjects as a result of nasopharyngeal carriage limits its utility in detecting systemic pneumococcal infection.[117]

Another rapid PCR method involving the use of a set of primers that amplify 273 base pairs of the autolysin gene has been developed to identify *S. pneumoniae*. In addition, three sets of primers were designed to amplify a 240-base pair fragment of the PBP2B gene *(pbp2b)* of penicillin-susceptible *S. pneumoniae* and two common *pbp2b* mutations present in penicillin-resistant *S. pneumoniae* so as to simultaneously identify the penicillin susceptibility of strains. The autolysin gene was identified in all 1062 clinical isolates of *S. pneumoniae* evaluated. In addition, 98.9 percent of 621 penicillin-susceptible isolates were shown to have DNA fragments amplified by the penicillin-susceptible primers, whereas 72.1 percent of 441 penicillin-resistant isolates were detected by the penicillin-resistant *S. pneumoniae* primers.[540] A gene probe for the gene encoding the PsaA protein also has been developed on the basis of PCR assay. PsaA was confirmed to be present in representative strains of all 90 known (at that time) serotypes of *S. pneumoniae*. The specificity of the assay was verified by the lack of signal from analysis of heterologous bacterial species (n = 30) and genera (n = 14), including viridans group streptococci. The potential of the assay for clinical application was shown by its ability to detect pneumococci in culture-positive nasopharyngeal specimens.[371]

Whereas a wide variety of molecular methods have been developed for the diagnosis of pneumococcal disease, use of these methods to rapidly detect *S. pneumoniae* remains problematic, with assays such as those targeting the pneumolysin gene cross reacting with other streptococcal species while assays that target the autolysin and *psaA* genes appear to be more specific.* The significance and clinical implications of *S. pneumoniae* DNA detected in clinical samples is therefore difficult to determine at this time.[58]

*References 4, 39, 85, 284, 350, 401, 475, 510, 590.

Susceptibility Testing

Susceptibility testing of pneumococci has been well standardized, and testing can be performed by determination of the minimal inhibitory concentration (MIC) and, for selected agents, by disk diffusion.[98] MICs can be performed by macrodilution or microdilution in cation-supplemented Mueller-Hinton broth enhanced with 5 percent whole defibrinated sheep or horse blood or 5 percent lysed and centrifuged horse blood.[98] If sulfonamides are tested, only the latter supplement should be used to avoid the presence of sulfonamide antagonists. MICs also can be determined by dilution in Mueller-Hinton agar supplemented as just described. Many systems based on frozen or dried microdilution trays are available commercially and used extensively for surveillance testing.[250] As with any system, commercial microdilution panels should be validated and used with appropriate quality controls. Another method for determination of MICs, the E-test, is much simpler to use than are the other methods for MIC determination. The E-test consists of a calibrated antibiotic-impregnated plastic strip that is applied to the surface of an inoculated agar plate. An antibiotic gradient is produced that results in an elliptical zone of inhibition after incubation. The MIC is read at the point where the ellipse of inhibition meets the strip. Evaluation of the E-test has shown that this method generally is reliable, although problems are encountered with some agents because acidification of the medium occurs during incubation of the plates in CO_2, which is required to ensure the growth of clinical isolates. Agents particularly affected are macrolides and some quinolones.

Disk diffusion also has been standardized well for testing pneumococci against selected agents. Distinction between susceptible and resistant strains is accomplished readily by using the current Clinical and Laboratory Standards Institute (CLSI) method involving macrolides, tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole, and clindamycin.[98,252] For testing penicillin and other β-lactams, disk diffusion is used best as a screening method with 1-μg oxacillin disks that have a susceptible cutoff zone of 20 mm or larger. Strains with zones of 20 mm or larger are fully susceptible to penicillin and other β-lactams. However, strains with zones that are less than 20 mm need to have MICs of penicillin and other appropriate β-lactams determined. Penicillin-susceptible strains with MICs of 0.06 μg/mL usually screen out with resistant strains.

Although interpretative categories for clarifying the significance of MIC values are available, many limitations to the currently available CLSI pneumococcal breakpoints exist, particularly for agents that can be administered in multiple-dosing regimens and by multiple routes of administration and that are used for infections in different body sites. Breakpoints for parenteral β-lactam agents generally are based on the use of agents in meningitis, whereas those of oral β-lactam agents are based on nonmeningeal infections such as otitis media.[98,252] Nonmeningeal breakpoints for some parenteral β-lactam agents were introduced in 2002, which to some extent will avoid the use of non-β-lactam agents for serious

nonmeningeal pneumococcal infections. These breakpoint changes classify strains previously interpreted as intermediate in sensitivity to penicillin G, cefotaxime, and ceftriaxone as susceptible if the agents are administered parenterally to treat pneumonia and other nonmeningeal infections. Pharmacokinetic and pharmacodynamic parameters recently have been shown to correlate with clinical outcome and offer a more rational approach to predicting antimicrobial efficacy and determining clinically relevant susceptibility breakpoints.[104,113,254,257]

The activity of antimicrobial agents against penicillin-susceptible, penicillin-intermediate, and penicillin-resistant pneumococci, based on the original penicillin breakpoints (susceptible, ≤0.06 μg/mL; intermediate, 0.12 to 1 μg/mL; resistant, ≥2 μg/mL), is shown in Table 85-3.[253] There is considerable variation in antimicrobial susceptibility between pneumococcal serotypes, and the in vitro activity of selected antimicrobial agents against a collection of 891 pneumococci collected in 2008 from 22 centers in the United States is shown in Table 85-4 for all isolates and for the 11 predominating serotypes.[255] All serotype 7F isolates were susceptible to all agents tested, as were more than 95% of serotype 3 isolates. In contrast, many isolates of serotypes 19A, 6C, and 19F were multidrug resistant.

Antibiotic Resistance

Resistance to β-Lactam Drugs

Widespread resistance to β-lactam and other drug classes has evolved in the most common pathogens, including *S. pneumoniae*. Although pneumococci are naturally transformable organisms, β-lactamase production never has been described in this organism. Instead, a much more complex resistance mechanism has evolved in *S. pneumoniae* that is mediated by sophisticated restructuring of the targets of the β-lactams, the PBPs, and by other newly described mechanisms.[208] The PBP targets in penicillin-resistant strains of *S. pneumoniae* are modified, low-binding affinity versions of the native PBPs. PBP targets may be modified by mutation or by transformation and homologous recombination with DNA from the PBP genes of viridans streptococci. The level of resistance is determined by how many and to what extent targets are modified.[89] Restructuring of PBPs is mediated by stepwise alterations in PBPs. The high-molecular-weight PBPs—types 1A, 2X, and 2B—that usually are detected in *S. pneumoniae* are involved in transpeptidase activity and play an important role in resistance.[208] Alterations in PBP-2B are associated with low-level resistance to penicillin, and alterations in PBP-2X mediate low-level resistance to cephalosporins. The additional alterations in PBP-1A raise penicillin MICs to 1 μg/mL or greater and cefotaxime MICs to 0.5 μg/mL or greater. Genomic comparison between *S. pneumoniae* and commensal *S. mitis* and *S. oralis* strains has documented the mosaic nature of PBPs among these species, with pneumococci acquiring their altered PBP genes from *S. mitis* and *S. oralis*.[207] Many other mosaic gene clusters not associated with penicillin resistance also have been found.[175] The capacity to produce branched cell wall stem peptides encoded by altered *murM* and *murN* genes, as well as altered PBPs, is required for expression of penicillin resistance in *S. pneumoniae*.[158] The *fibA* and *fibB* genes, which are homologous to the *Staphylococcus aureus femA/B* genes required for expression of methicillin resistance in this organism, encode proteins involved in the formation of interpeptide bridges and also are required for expression of PBP-mediated penicillin resistance.[569] Other mechanisms of β-lactam resistance have been described in laboratory mutants and in a clone of Hungarian pneumococcal strains with notably high levels of β-lactam resistance (penicillin MIC, 16 μg/mL; cefotaxime MIC, 4 μg/mL).[208,500]

Resistance to Non-β-Lactam Drugs

The molecular and genetic mechanisms of resistance to macrolides, chloramphenicol, tetracycline, fluoroquinolones, and trimethoprim-sulfamethoxazole in *S. pneumoniae* also have been determined. Resistance genes for several agents are carried on a transposon, Tn1545.[103] It confers resistance to three antimicrobial classes—kanamycin *(aphA-3)*, macrolide-lincosamide-streptogramin B-type antibiotics *(ermB)*, and tetracycline *(tetM)*. This transposon has been conjugated and transposed to the chromosome of *Enterococcus faecalis*, oral streptococci, and *Listeria monocytogenes*. The properties of this transposon account for the sudden emergence, rapid dissemination, and stabilization of resistance to multiple antibiotics in *S. pneumoniae* in the absence of plasmids.

Resistance mechanisms include the production of chloramphenicol acetyltransferase, an enzyme capable of catalyzing the conversion of chloramphenicol to nonfunctional derivatives. Chloramphenicol acetyltransferase is encoded by a chloramphenicol acetyltransferase *(cat)* gene identical to the *cat* gene from the *S. aureus* plasmid pC194. Tetracycline resistance occurs through ribosomal protection encoded by the genes *tetM* and *tetO*. The tetM and tetO proteins are thought to cause tetracycline to be released from the ribosome. Resistance to fluoroquinolones primarily involves mutations in the DNA gyrase gene *gyrA* and in the topoisomerase IV genes *parC* and *parE*, as well as an efflux mechanism that affects some fluoroquinolones. Resistance to trimethoprim is mediated through a single amino acid substitution in the chromosomal dihydrofolate reductase gene of *S. pneumoniae*, which is thought to disrupt the bond with trimethoprim without affecting the action of dihydrofolate reductase. Sulfonamide resistance appears to result from repetitions of one or two amino acids in the chromosomal dihydropteroate synthase.[579]

Two major mechanisms have been described for resistance to erythromycin. Coresistance to macrolides, clindamycin, and streptogramin B-type antibiotics is a result of modification of the ribosome through methylation of an adenine residue in domain V of the 23S rRNA. Methylation is encoded by a methylase gene, *ermB* (previously called *ermAM*). Resistance to 14- and 15-membered macrolides (erythromycin, azithromycin, and clarithromycin) but not to 16-membered macrolides (roxithromycin, josamycin, and spiramycin), ketolides, or clindamycin is a result of efflux of the antibiotic from the cell; such

TABLE 85-3 **Activity of Antimicrobial Agents Against Penicillin-Susceptible, Penicillin-Intermediate, and Penicillin-Resistant Pneumococci***

Agent Class	Antimicrobial Agent	Breakpoints (≤S/I/≥R) μg/mL	MIC_{90} (μg/mL)		
			Penicillin Susceptible	Penicillin Intermediate	Penicillin Resistant
Penicillins	Penicillin G (meningitis)	0.06/-/0.12	0.015-.06	0.5-1	2-4
	Penicillin G (nonmeningitis)	2/4/8	0.015-.06	0.5-1	2-4
	Amoxicillin	2/4/8	0.015-0.03	0.12-1	2
	Amoxicillin-clavulanate	2/4/8†	0.015-0.05	0.12-1	2
	Ampicillin	NA	0.015-0.06	0.5-2	2-4
	Ticarcillin	NA	1-2	64	64-128
	Piperacillin	NA	≤0.06	1-2	4-8
Oral cephalosporins	Cefdinir	0.5/1/2	0.06-0.125	1-4	8
	Cefpodoxime	0.5/1/2	0.06-0.25	1-4	4
	Cefuroxime axetil	1/2/4	0.03-0.125	1-4	4-8
	Cefaclor	1/2/4	0.5-2	8-16	16->32
	Cefixime	NA	0.25-1	8-32	>32
	Cefprozil	2/4/8	0.25-1	4-8	>32
Parenteral cephalosporins	Cefuroxime sodium	0.5/1/2	0.03-0.125	1-4	4-8
	Ceftriaxone (nonmeningitis)	1/2/4	0.01-0.06	0.5-1	1-2
	Ceftriaxone (meningitis)	0.5/1/2	0.01-0.06	0.5-1	1-2
	Cefotaxime (nonmeningitis)	1/2/4	0.01-0.12	0.25-0.5	1-4
	Cefotaxime (meningitis)	0.5/1/2	0.01-0.12	0.25-0.5	1-4
	Cefepime (nonmeningitis)	1/2/4	0.06-0.12	0.5-1	1-2
	Cefepime (meningitis)	0.5/1/2	0.06-0.12	0.5-1	1-2
	Ceftazidime	NA	0.25-0.5	16-32	>32
	Ceftaroline	0.25/–/–	0.015	0.06	0.25
Penems	Imipenem	0.12/0.25-0.5/1	≤0.008-0.015	0.12-0.25	0.25
	Meropenem	0.25/0.5/1	0.015-0.03	0.5	1
	Ertapenem	1/2/4	0.03	0.5	1
	Doripenem	NA	≤0.015	0.25	1
Macrolides, lincosamides, azalides, ketolides, streptogramins	Erythromycin	0.25/0.5/1	0.06-0.12	16->64	>64
	Azithromycin	0.5/1/2	0.12-0.25	16->64	>64
	Clarithromycin	0.25/0.5/1	0.03-0.06	8->64	>64
	Clindamycin	0.25/0.5/1	0.03	0.12	>64
	Telithromycin	1/2/4	0.03	0.25	0.25
	Quinupristin/ dalfopristin	1/2/4	0.5-1	0.5-1	0.5-1
Fluoroquinolones	Levofloxacin	2/4/8	2	2	2
	Moxifloxacin	1/2/4	0.25	0.25	0.25
Other agents	Tetracycline	2/4/8	≤2	>32	>32
	Doxycycline	0.25/-/0.5	0.12	8	8
	Vancomycin	1/–/–	0.12-0.5	0.12-0.5	0.12-0.5
	Rifampin	1/2/4	≤0.12	≤0.12	≤0.12
	Trimethoprim-sulfamethoxazole	0.5/-/1‡	>4	>4	>4

*Based on the original penicillin breakpoints (susceptible, ≤0.06 μg/mL; intermediate, 0.12-1 μg/mL; resistant, ≥2 μg/mL). Values are shown as minimal inhibitory concentration (MIC) values or ranges in μg/mL that inhibit 90% or more of isolates (MIC_{90}).
†Amoxicillin component.
‡Trimethoprim component.
Modified from Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae:* trends and management. *Expert Rev Anti Infect Ther* 2008;**6**:619–35.
NA, not available.

resistance is encoded by the gene *mefE* in *S. pneumoniae* and appears to be emerging rapidly as the predominant mechanism of resistance to erythromycin in many countries.[175] Other macrolide resistance mechanisms that have been described include mutations in position 2059 of the 23S rRNA and in genes encoding ribosomal protein L4.[519]

Vancomycin Tolerance

Although vancomycin resistance has not been described in pneumococci, antibiotic tolerance, or the ability of bacteria to survive but not grow in the presence of antibiotics, has been described. It has been shown to be caused by loss of function of the VncS histidine kinase

TABLE 85-4 **In Vitro Activity of Selected Antimicrobial Agents Against 891 Pneumococci Collected in 2008 from 22 Centers in the United States for All Isolates and for the 11 Predominating Serotypes**

Agent (CLSI Breakpoint for Susceptibility [μg/mL])	Percent Susceptibility of Isolates by Serotype (No. of Isolates)											
	All (891)	19A (189)	3 (82)	35B (59)	7F (52)	11A (49)	6C (43)	15A (38)	22F (35)	23A (33)	23B (33)	19F (29)
Penicillin G (≤2)	86.2	46.6	100	98.3	100	100	100	100	100	100	100	37.9
Ceftriaxone (≤1)	90.7	65.6	100	98.3	100	98.0	100	100	100	100	97.0	65.5
Imipenem (≤0.12)	76.2	40.2	100	15.3	100	95.9	90.7	100	100	100	100	20.7
Penicillin V (≤0.06)	58.6	13.8	98.8	10.2	100	95.9	37.2	10.5	100	36.4	84.8	20.7
Amoxicillin-clavulanate (≤2)	83.1	45.0	100	62.7	100	98.0	100	100	100	100	100	37.9
Cefuroxime (≤1)	70.1	36.1	100	9.6	100	95.2	47.5	91.4	100	96.4	96.8	20.7
Erythromycin (≤0.25)	61.6	26.5	95.1	64.4	100	75.5	41.9	5.3	88.6	78.8	75.8	17.2
Clindamycin (≤0.25)	79.2	43.4	96.3	100	100	95.9	97.7	10.5	100	81.8	100	31.0
Levofloxacin (≤2)	99.4	100	100	98.3	100	100	100	100	100	100	97.0	96.6
Moxifloxacin (≤1)	99.6	100	100	100	100	100	100	100	100	100	97.0	96.6
Vancomycin (≤1)	100	100	100	100	100	100	100	100	100	100	100	100
TMP-SMX (≤0.5)	66.2	16.9	97.6	100	100	87.8	34.9	55.3	97.1	90.9	84.8	17.2
Linezolid (≤2)	100	100	100	100	100	100	100	100	100	100	100	100

TMP-SMX, trimethoprim-sulfamethoxazole.
Modified from Jacobs MR, Good CE, Windau AR, et al. Activity of ceftaroline against recent emerging serotypes of *Streptococcus pneumoniae* in the United States. *Antimicrob Agents Chemother* 2010;**54**:2716–19.

of a two-component gene expression sensor-regulator system in *S. pneumoniae* that produces tolerance to vancomycin and other classes of antibiotics.[390]

Evolution of Antibiotic Resistance

Pneumococci initially were susceptible to many antimicrobial agents, but they became resistant with varying degrees of rapidity to many of these agents. The earliest example was the development of resistance to optochin (ethylhydrocupreine) when this agent was used experimentally in mice in the early part of the 20th century. With the introduction of sulfonamides in 1939, pneumococci similarly exhibited an ability to acquire resistance in experimental infections in mice, as well as in a human case of meningitis.[292] Sulfonamide resistance was identified sporadically thereafter, and a trimethoprim-sulfamethoxazole-resistant strain was recognized first in 1972. Trimethoprim-sulfamethoxazole resistance subsequently has become widespread in virtually all serotypes throughout the world, including developing countries, and resistance to this agent is greater than that to any other antimicrobial class worldwide.[231] Tetracycline resistance emerged in the 1960s and chloramphenicol resistance in 1970. However, little attention was paid to the development of resistance in this species until 1977, when isolates resistant to several antimicrobial classes, including penicillins, chloramphenicol, tetracyclines, macrolides, clindamycin, and trimethoprim-sulfamethoxazole, were detected in South Africa.[23,256]

Subsequently, multiresistant clones of pneumococci have spread throughout many regions of the world. Noteworthy is that multiresistant clones are confined mostly to serotype 14 and serogroups 6, 9, 19, and 23.[123] Whereas resistance to penicillins occurs in a stepwise fashion and in many cases can be overcome by using β-lactams with appropriate pharmacokinetics, resistance to other drug classes usually is absolute and distinct populations of strains are found to be susceptible and resistant to agents such as macrolides, clindamycin, tetracyclines, trimethoprim-sulfamethoxazole, and chloramphenicol. Unlike enterococci, resistance to vancomycin has not developed in pneumococci yet, although vancomycin-tolerant strains have been detected.[45,228]

Cross-resistance among *S. pneumoniae* to macrolides and other classes of antibiotics usually increases with increasing MICs to penicillin (Fig. 85-13).[254] Whereas only 6 percent of penicillin-susceptible pneumococci are resistant to macrolides and 14 percent to trimethoprim-sulfamethoxazole, approximately half of the penicillin-intermediate isolates were resistant to these agents. In the case of penicillin-resistant strains, three fourths were resistant to macrolides, 90 percent to trimethoprim-sulfamethoxazole, and 28 percent to clindamycin. However, this pattern is not the case in all countries and at least one multiresistant clone resistant to chloramphenicol, tetracycline, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole has remained susceptible to penicillin.[126,518]

Strains of *S. pneumoniae* were exquisitely susceptible to penicillin (MICs of 0.01 to 0.03 μg/mL) when this agent initially was used clinically in the 1940s and 1950s, and this MIC range is referred to as the baseline activity of penicillin against "wild-type" *S. pneumoniae*.[252] Evolution

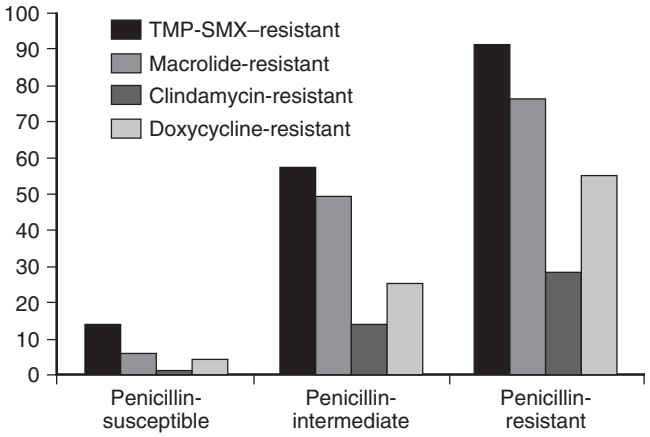

FIGURE 85-13 ■ Pneumococci often are resistant to several drug classes, and cross-resistance to macrolides and other classes of antibiotics increases as minimal inhibitory concentrations of penicillin increase. TMP-SMX, trimethoprim-sulfamethoxazole. (From Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae:* trends and management. *Expert Rev Anti Infect Ther* 2008;**6**:619–35.)

of resistance to this class of agents was noted first when a few strains of *S. pneumoniae* were isolated in the 1960s in Australia and New Guinea. These strains had decreased susceptibility to penicillin, with MICs of 0.1 to 0.25 μg/mL, approximately 10-fold higher than the MICs of baseline strains. Strains with penicillin MICs of 2 to 4 μg/mL, approximately 100-fold higher than baseline strains, were isolated in South Africa in 1977, and, subsequently, strains with even higher MICs (16 μg/mL, approximately 1000-fold higher than baseline strains) were described in Hungary.[500] Pneumococci conventionally are classified as penicillin-susceptible if the MICs are 0.06 μg/mL or less, intermediate if the MICs are 0.12 to 1.0 μg/mL, and resistant if the MICs are 2.0 μg/mL or greater. This classification is useful mainly in characterizing strains as fully susceptible to β-lactams if susceptible or as having decreased susceptibility if intermediate or resistant. Strains with such decreased susceptibility are better referred to as β-lactam drug challenged because the mechanism of resistance can be overcome if the pharmacokinetics of the β-lactam drug used in serum or at the site of infection exceeds the MIC for 40 to 50 percent of the dosing interval.[105] Similar variations in MIC ranges are seen with all β-lactams, although MIC ranges for many β-lactams are much higher than that for penicillin itself. Agents such as ampicillin, amoxicillin, cefotaxime, and ceftriaxone have MIC ranges similar to that of penicillin, whereas agents such as cefazolin, cefaclor, cefprozil, ceftazidime, and cefixime have much higher MIC ranges. For example, the baseline activity of cefaclor against *S. pneumoniae* is 0.5 to 1 μg/mL, which is a concentration 20- to 30-fold higher than that required for penicillin to inhibit the most susceptible strains. Changes in susceptibility that occur over the course of time were illustrated in a study of recent versus archived otitis media strains. In this study, the MIC_{90} for cefaclor against archived isolates was 1 μg/mL, whereas the MIC_{90} against recent isolates was greater than 64 μg/mL.[251] A few agents, such as imipenem and meropenem, have slightly lower MIC ranges than those of penicillin. The proportion of pneumococci that are penicillin susceptible, intermediate, and resistant vary considerably throughout the world.

Resistance to macrolides in strains of *S. pneumoniae* was noted first in 1964 and was detected sporadically in the United States until it became widespread in the latter half of the 1990s.[231,243,292] The baseline activity of macrolides (0.03 μg/mL) and MIC distributions (1000-fold concentration range, 0.03 to >32 μg/mL) against *S. pneumoniae* are somewhat similar to those of penicillin. The MIC distribution of macrolides is trimodal, with strains being exquisitely susceptible (erythromycin MIC, <0.03 μg/mL) or highly resistant (erythromycin MIC, >32 μg/mL) or demonstrating low level resistance (MICs of 1 to 16 μg/mL).[152] These distributions closely correlate with macrolide ribosomal methylase and efflux resistance mechanisms. The prevalence of macrolide resistance and reports of clinical failure resulting from strains with efflux and ribosomal methylase resistance mechanisms continue to increase. Some authors, however, have argued that isolates with efflux-mediated resistance could be susceptible to the high intracellular concentrations that these agents achieve in phagocytic cells and in epithelial lining fluid of the alveoli.[15] However, no clinical or animal data support these arguments for extracellular pathogens such as *S. pneumoniae*, whereas considerable clinical and animal data support the use of current breakpoints.[42,104,105,365] The rising incidence of macrolide-resistant pneumococci was directly proportional to the increasing use of macrolides in various communities and age groups.[173,243,423]

Strains with multiple antibiotic resistance have greater selective advantages than do strains resistant to just one antibiotic because the opportunity for positive selection is increased as the number of drug classes to which isolates are resistant increases.[291] Exposure to different classes of antibiotics allows more opportunity for selective advantage to a multiple antibiotic-resistant organism than to a monoresistant strain, which must wait to encounter the one antibiotic to which it is resistant and is likely to be killed by agents of other antibiotic classes. Thus, the increasing prevalence of antibiotic-resistant pneumococci is associated with the increasing prevalence of multidrug-resistant strains. Therefore, one is not surprised that the use of one class of antibiotics (mainly macrolides and trimethoprim-sulfamethoxazole) can be associated with an increase in resistance to other classes of antibiotics (mainly β-lactam drugs).[3,24,190,423] Many authorities now think that antibiotic agents such as the newer macrolides (e.g., clarithromycin and azithromycin) and trimethoprim-sulfamethoxazole are stronger promoters of antibiotic resistance among *S. pneumoniae* strains than are the β-lactam drugs.[24,190,423] Researchers also have suggested that among the β-lactam drugs, cephalosporins are stronger promoters of resistance in *S. pneumoniae* than are the aminopenicillins.[173,469] Although many strains are resistant to tetracyclines and macrolides, they are susceptible to the new tetracycline derivatives, the glycylcyclines, as well as to streptogramins, ketolides, glycopeptides, and rifampin. Many strains are resistant to

trimethoprim-sulfamethoxazole worldwide, with more than 40 percent being resistant in the United States. Fluoroquinolones with antipneumococcal activity (e.g., gatifloxacin, levofloxacin, moxifloxacin) are active against most strains of *S. pneumoniae;* however, in several countries where fluoroquinolones have been prescribed widely, clinically relevant levels of resistance have been described.[90,231] No doubt, antibiotic resistance will continue to evolve and challenge us.

HOST DEFENSES

Although anticapsular antibody is the most prominent protective mechanism against pneumococcal infection, many host responses to infection occur and many other factors are associated with protection against disease.[379] Pneumococcal infection and disease have been modeled in several animal species. Most are models of sepsis arising from intravenous or intraperitoneal inoculation of bacteria, and only a few were designed to study disease arising from intranasal infection. Chinchillas provide the only animal model of middle ear pneumococcal infection in which the disease can be produced by very small inocula injected into the middle ear or intranasally. This model, developed at the University of Minnesota in 1975, has been used to study pneumococcal pathogenesis at a mucosal site, the immunogenicity and efficacy of pneumococcal capsular polysaccharide vaccine antigens, and the kinetics and efficacy of antimicrobial drugs.[181]

Anticapsular Serum IgG Antibody

IgG to the capsular polysaccharide of *S. pneumoniae* is thought to provide the greatest degree of protection against systemic pneumococcal disease, as well as limited protection against colonization. The reference method for measurement of antibody is the opsonophagocytosis assay, which involves serial dilutions of serum, viable pneumococci, complement, and viable PMNs incubated together for 1 hour.[455] An infant mouse assay system for assessment of protective concentrations of human serum pneumococcal anticapsular antibodies correlated well with opsonophagocytic titer but not with naturally occurring IgG antibody concentrations or IgG produced in response to nonconjugated polysaccharide vaccines, as determined by ELISA.[267,384] However, the ELISA method of serotype-specific antibody assay with absorption of cross-reacting antibody to cell wall polysaccharides does correlate well with protection after vaccination with conjugated vaccine, and it is the method used most commonly to predict serotype-specific immunity.[22,546,547]

The development of a phagocytosis assay based on flow cytometry has not overcome the limitations of the ELISA method and is inferior to the opsonophagocytosis method.[260] Investigation of polymorphisms in the variable region of IgG that affect protective function has indicated that the capsular polysaccharide antibody repertoire in adults is derived from memory B-cell populations that have switched class and undergone extensive hypermutation.[336] Functionally disparate anticapsular polysaccharide antibodies can arise within individuals both by activation of independent clones and by intraclonal somatic mutation, which illustrates the complexity of assaying and interpreting serum capsular polysaccharide antibody levels.

Anticapsular IgA Antibody

The role of IgA in the control of invasive mucosal pathogens such as *S. pneumoniae* is understood poorly. Human pneumococcal capsular polysaccharide-specific IgA initiates dose-dependent killing of *S. pneumoniae* in the presence of complement and phagocytes. The majority of specific IgA in serum is of the polymeric form, and the efficiency of killing initiated by this polymeric form exceeds that of monomeric IgA-initiated killing. In the absence of complement, specific IgA induces minimal bacterial adherence, uptake, and killing. Killing of *S. pneumoniae* by resting phagocytes with immune IgA requires complement, predominantly via the C2-independent alternative pathway, which in turn requires factor B but not calcium. Pneumococcal capsule-specific IgA may have distinct roles in effecting the clearance of pneumococci in the presence or absence of inflammation, and the polymeric form may control pneumococcal infections locally and after the pathogen's entry into the bloodstream by several mechanisms.[258]

Phagocytosis and Leukocyte IgG Receptors

IgG-mediated phagocytosis by PMNs is the main defense against *S. pneumoniae*. Two leukocyte IgG receptors, FcγRIIa and FcγRIIIb, are expressed constitutively on PMNs. Blocking experiments have shown that FcγRIIa is crucial for opsonophagocytosis of serum-opsonized *S. pneumoniae*. In adults, serum-induced phagocytic activity depends mainly on antipneumococcal IgG2 antibodies.[454] However, in infants and young children, the main response to pneumococcal conjugate vaccines occurs in the IgG1 subclass.[22,554] Investigators have suggested that IgG1 subclass antibodies are at least as highly functional as is IgG2.[146] Recruitment and function of neutrophils also are important host defenses. In a pneumococcal infection model in immunocompetent and immunodeficient mice intranasally infected with *S. pneumoniae* type 2, immunocompetent BALB/c mice were resistant and immunodeficient CBA/Ca mice were susceptible to infection. BALB/c mice recruited significantly more neutrophils in the lungs, and inflammatory lesions were visible much earlier than in CBA/Ca mice.[185]

Antibodies to Surface Proteins and Pneumolysin

PspA, PsaA, and pneumolysin are common to virtually all pneumococcal isolates. The development of antibodies to PspA, PsaA, and pneumolysin as a result of pneumococcal infection and carriage in young children was determined by measurement of serum antibodies to these proteins by ELISA in children at ages 6, 12, 18, and 24 months and in their mothers. All age groups were shown to produce antibodies to the three proteins, which increased with age and were associated strongly with

pneumococcal exposure as a result of carriage or AOM.[439] IgA to PspA, PsaA, and pneumolysin has been detected by ELISA in the saliva of children aged 6 to 24 months.[493] This finding was associated with pneumococcal carriage and otitis media.

Serum antipneumolysin IgG at the time of hospital admission has been found to be higher in patients with nonbacteremic pneumococcal pneumonia than in those with bacteremic pneumococcal pneumonia or uninfected control subjects.[383] Serum antipneumolysin IgG levels also rose significantly during convalescence in patients with bacteremic pneumonia, and the levels attained were equal to those observed in nonbacteremic patients. Children aged 6 to 24 months were shown to produce antibodies to pneumolysin, and antibody concentrations increased with age and were associated strongly with pneumococcal exposure, whether by carriage or infection such as AOM.[439] Infants also have been shown to mount a specific antibody response to pneumolysin during AOM.[440]

Defense Mechanisms of the Spleen

The spleen is the principal organ that clears pneumococci from the bloodstream.[307,379] Opsonized particles are removed from the circulation by the liver; but with decreasing opsonization, the spleen increasingly assumes the role of clearance. The slow passage of blood through the spleen and the prolonged contact time with reticuloendothelial cells in the cords of Billroth and the splenic sinuses allow time for the removal of nonopsonized particles. Overwhelming pneumococcal infection occurs in children and adults whose spleens have been removed or do not function normally. Pneumococcal disease progresses so rapidly in such individuals that pneumonia is not detectable clinically or by chest radiographs, although it is seen at autopsy. The increase in the incidence of pneumococcal bacteremia and meningitis in children with sickle-cell disease is due largely to splenic dysfunction. Splenectomy also results in a marked, sustained elevation in risk for severe *S. pneumoniae* infection in HIV-infected patients.[428]

Vitamin A

The association of nasopharyngeal colonization with *S. pneumoniae* and vitamin A supplementation in infants in an area with endemic vitamin A deficiency in southern India showed that neonatal vitamin A supplementation delayed the age at which colonization occurs; therefore, it may play a role in lowering morbidity rates associated with pneumococcal disease.[101]

C-Reactive Protein

C-reactive protein (CRP) is a normal constituent of human serum that is synthesized by hepatocytes and induced by proinflammatory cytokines. The function of this acute-phase reactant includes activation of complement and enhancement of opsonophagocytosis. CRP binds to phosphorylcholine, a constituent of eukaryotic membranes that also is found on the cell surface of the major bacterial pathogens of the human respiratory tract, including *S. pneumoniae* and *H. influenzae*. CRP is present in inflamed (0.17 to 42 μg/mL) and uninflamed (<0.05 to 0.88 μg/mL) secretions from the human respiratory tract in sufficient quantities to have an antimicrobial effect. In addition, the *CRP* gene was expressed in human respiratory epithelial cell cultures. The complement-dependent bactericidal activity of normal nasal airway surface fluid and sputum was abolished when the secretions were pretreated to remove CRP. Human respiratory epithelial cells are capable of expressing CRP, and this protein may contribute to bacterial clearance in the human respiratory tract.[194]

Platelet-Activating Factor Receptors of Airway Epithelial Cells

Adherence of pneumococci to cultured human tracheal epithelial cells increased after exposure to acid and decreased after exposure to a specific inhibitor of the receptor for platelet-activating factor.[247] Exposure to acid thus may stimulate the adherence of *S. pneumoniae* to airway epithelial cells via increases in platelet-activating factor receptors. The clinical significance of these findings is not clear.

Cytokines

Polymorphonuclear granulocytes, which provide a major defense against *S. pneumoniae* infection, are attracted to and activated by various cytokines, including IL-1β, IL-6, TNF-α, IL-8, IL-10, IL-12, interferon-γ, and granulocyte-macrophage colony-stimulating factor.[28] The inflammatory response in bacterial meningitis also is mediated by TNF-α and IL-1, which are produced in the subarachnoid space by cells such as leukocytes, astrocytes, and microglia.[406] Inoculation of pneumococcal cell wall components directly into the CSF of rabbits also results in the induction of an inflammatory response with pleocytosis and increased levels of CSF TNF-α and IL-1.[195] Both TNF-α and IL-1β have been shown to increase mucosal adhesion of pneumococci to tracheal epithelium in a chinchilla trachea whole-organ perfusion model.[536]

L-Ascorbic Acid (Vitamin C)

Degradation of the connective tissue component hyaluronic acid by the hyaluronate lyase produced by *S. pneumoniae* is inhibited competitively by L-ascorbic acid (vitamin C).[324] One L-ascorbic acid molecule was found to bind to the active site of the enzyme. The high concentration of L-ascorbic acid in human tissues probably provides a low level of natural resistance to pneumococcal invasion by this mechanism.

Leukotrienes

Leukotrienes are produced by macrophages and are considered important for antibacterial defense in the lung. Leukotrienes comprise a group of highly potent lipid

mediators synthesized by the enzyme 5-lipoxygenase. Multidrug-resistance protein 1 (mrp1) is a transmembrane protein responsible for the cellular extrusion of leukotrienes from macrophages. In a mouse pneumonia model, mrp1-deficient mice display diminished growth of pneumococci in the lungs and low mortality by a mechanism that involves increased release of leukotriene B_4.[477] Pneumococci also induce the production of leukotrienes in the middle ear, which has been related to upregulation of two genes that govern the lipoxygenase pathway.[328]

Human Alveolar Macrophage Binding and Phagolysosomes

Human alveolar macrophages are the major resident phagocytic cells of the lung. After contact with macrophages, bacteria enter phagosomes, which gradually acquire the characteristics of terminal phagolysosomes, with incorporation of lysosome-associated membrane protein. Opsonization with serum containing immunoglobulin resulted in significantly greater binding of pneumococci to macrophages than did opsonization with immunoglobulin-depleted serum.[191] Binding, intracellular localization, and killing of pneumococci by macrophages all are increased significantly by opsonization with serum containing immunoglobulin, complement, or both.

Intracellular Killing

Once pneumococci undergo phagocytosis by "professional" phagocytes (leukocytes and macrophages), they are killed.[379] However, researchers have shown that pneumococci can enter and survive inside A549 cells, a human lung alveolar carcinoma (type II pneumocyte) cell line.[522] Not all clinical *S. pneumoniae* isolates were capable of penetrating these cells, and the presence of a polysaccharide capsule also reduced their capacity to penetrate A549 cells significantly. The intracellular activity of various antibiotics against pneumococci in A549 cells showed that in the presence of antibiotics for 18 hours, more than 98 percent of the A549 cells were viable and less than 3 percent of the pneumococci that initially were phagocytosed could be detected intracellularly after exposure to peak serum concentrations of penicillin G, azithromycin, moxifloxacin, trovafloxacin, rifampin, and telithromycin.[348]

In the absence of antibiotics, pneumococci were phagocytosed efficiently but then paradoxically went on to kill all the A549 cells within 18 hours. The clinical significance of these findings is unknown.

Complement

The second component of complement (C2) is an important factor associated with host defense against encapsulated organisms. Homozygous deficiency of C2 is the deficiency of complement most commonly inherited. Although C2 deficiency can be asymptomatic, patients usually have either autoimmune disease or recurrent pyogenic infection caused by encapsulated bacteria such as *S. pneumoniae*, *H. influenzae* type b, and *N. meningitidis*. An association between C2 deficiency and IgG subclass deficiency also has been described.[29] *S. pneumoniae* challenge of mice deficient in the third component of complement (C3) results in a 2000-fold increase in organism load in the bloodstream in comparison with control animals.[97] Binding of pneumococcal CbpA to epithelially produced C3 results in adhesion of pneumococci to type II pulmonary epithelial cells. CbpA-deficient pneumococcal mutants and lysates, therefore, fail to bind C3 and demonstrate a moderate decrease in adhesion to type II pulmonary epithelial cells, thus confirming the interaction of CbpA and C3 in adhesion.[501]

Polymeric Immunoglobulin Receptor

pIgR plays a crucial role in mucosal immunity against microbial infection by transporting polymeric immunoglobulins such as IgA across the mucosal epithelium. Polymeric IgA consists of two IgA molecules joined by a small polypeptide J chain. The J chain shows high affinity for the glycoprotein pIgRs of epithelial cells, which are responsible for externalization of polymeric IgA across cell membranes.[264] However, pIgR also can act as a "Trojan horse" and participate in the pathogenesis of IPD as pIgR binds to a major pneumococcal adhesin, CbpA.[599] Expression of pIgR in human nasopharyngeal cells greatly enhances pneumococcal adherence and invasion; this effect is abolished either by insertional knockout of CbpA in pneumococci or by antibodies against either pIgR or CbpA.

Basic Fibroblast Growth Factor

Basic fibroblast growth factor is a neurotrophic factor in the central nervous system that is expressed at high levels in response to seizure or stroke. It also occurs in pneumococcal meningitis, as shown in experimental bacterial meningitis in mice and in children with bacterial meningitis.[241] Patients with meningitis in whom major sequelae or death occurred had much higher levels of CSF basic fibroblast growth factor than did those who survived. In patients with bacterial meningitis who survived, basic fibroblast growth factor decreased significantly in CSF after 24 to 50 hours of administration of antibiotic therapy. However, its biologic role in the pathophysiology of bacterial meningitis is not known.

Granulocyte Colony-Stimulating Factor

In a limited study of 22 non-neutropenic adult patients with pneumococcal meningitis, granulocyte colony-stimulating factor (G-CSF), in addition to cefotaxime and dexamethasone, was administered subcutaneously for 6 days. All patients survived, and in only 1 patient did a complication develop (bilateral hearing deficit). Improvement of inflammation indices in CSF was rapid.[124] However, controlled clinical trials are needed. In a rabbit meningitis model, G-CSF increased the percentage of granulocytes in blood but not in CSF and increased CSF TNF-α and IL-1β concentrations.[474] However, G-CSF

did not reduce the density of apoptotic neurons in the dentate gyrus of the hippocampus. A second study in a rabbit meningitis model used longer pretreatment with G-CSF and showed more positive results.[405] G-CSF pretreatment attenuated meningeal inflammation and enhanced systemic killing of bacteria. Pretreatment with recombinant human G-CSF in a murine model of pneumococcal pneumonia resulted in improved survival with low, but not high, bacterial inocula. Therefore, the benefits of using G-CSF are limited because pneumococci already have recruited large numbers of neutrophils in the lungs by this time.[120]

Intracellular Signaling Pathways

Pneumococcal cell walls activate multiple intracellular signaling pathways in microglial brain cells, with induction of an outwardly rectifying K^+ channel, suppression of the constitutively expressed inwardly rectifying K^+ current, and release of TNF-α, IL-6, IL-12, and other inflammatory mediators.[431] The presence of serum strongly facilitated these effects. The mechanisms involved in microglial activation by pneumococcal cell walls were different from those activated by gram-negative lipopolysaccharide.

Lactoferrin

Human lactoferrin is an iron-binding glycoprotein that is particularly prominent in exocrine secretions and leukocytes and also is found in serum, especially during inflammation. It is able to sequester iron from microbes and has immunomodulatory functions, including inhibition of both activation of complement and production of cytokines. Binding of human lactoferrin to the surface of *S. pneumoniae* depends entirely on PspA.[206] Prevention of the binding of lactoferrin to pneumococcal PspA could be an important host defense mechanism.

Mannose-Binding Lectin

Mannose-binding lectin (MBL) is a key mediator of innate host immunity that activates the complement pathway and directly opsonizes some infectious pathogens. Mutations in three codons in the MBL gene have been identified, and individuals homozygous for a mutant genotype have very little or no serum MBL. In a study conducted in the United Kingdom of 229 patients in whom *S. pneumoniae* was isolated from sterile sites, 28 (12%) were homozygous for MBL codon variants versus only 18 of 353 (5%) control subjects (odds ratio, 2.59; 95% confidence interval [CI], 1.39 to 4.83).[461]

CLINICAL SYNDROMES

The pneumococcus causes disease over the entire age spectrum from early-onset neonatal sepsis to pneumonia, IPD, and meningitis in geriatric patients. It is found frequently in the nasopharynx of children younger than 5 years of age, yet only a small fraction of colonized children develop either invasive disease syndromes or respiratory tract infection. New data from both animal and human studies suggest that colonization is associated with a mild inflammatory response within the nasopharynx that, in general, resolves spontaneously.[272,588] In those children in whom the pneumococcus evades host defenses, the organism has the potential to cause local respiratory tract illnesses such as AOM, acute sinusitis, and nonbacteremic pneumonia or to enter the bloodstream and result in a spectrum of clinical syndromes, including bacteremia, sepsis, meningitis, or focal infections, such as septic arthritis, osteomyelitis, endocarditis, soft tissue infection, or peritonitis. Infection may also spread directly to the central nervous system from the nasopharynx in children with cochlear implants and in children with basal skull fracture and CSF leak. Peritonitis is also reported, most often in children with nephrotic syndrome. Colonization of the genital tract in women is also reported and may be the source for early-onset sepsis in vulnerable neonates or spread through the fallopian tubes to the peritoneal cavity.

The clinical signs and symptoms of patients with pneumococcal infection can be nonspecific, reflective of the bacteremia/sepsis syndrome, or specific to the organ system involved. In general, bacteremia presents as high fever and temperatures that can exceed 40° C (104° F).[38] Pneumococcal pneumonia most often presents as fever, cough, and tachypnea and possibly chest pain when consolidation is also present.

Bacteremia and "Occult" Bacteremia

Unsuspected or "occult" bacteremia in children 3 to 36 months of age who have fever without evidence of toxicity or focal infection has been reported in up to 4 percent of febrile infants and toddlers in the era before the introduction of pneumococcal conjugate vaccines. When clinical practice includes performance of blood cultures on febrile children with mild or moderate toxicity, bacteremia has been identified from emergency or walk-in clinics as well as office practice settings.[72] In the United States, *Streptococcus pneumoniae* represented approximately 60 percent of pathogens identified in children well enough to be discharged home whereas *H. influenzae* type b was identified in up to 25 percent of cases. This distribution was dramatically altered with the introduction of Hib conjugate vaccines, thus increasing the proportion of bacteremic cases from which *S. pneumoniae* was isolated from blood culture to 80 to 90 percent.[12,288,584] Age younger than 24 months and temperature greater than 39° C (102.2° F) were the demographic and clinical features initially described that characterized the majority of children with "occult" pneumococcal bacteremia in U.S. studies. Disease was less common in children younger than 6 months of age. Clinically, the risk for bacteremia increases with increasing temperature, being greatest with temperature greater than 40° C (104° F). Subtle clinical differences in alertness, color, quality of cry, hydration, and responsiveness have been described in bacteremic children compared with otherwise similar febrile children.[353,354,584] Laboratory studies reveal that elevated white blood cell counts above 15,000/mm^3 or absolute neutrophil counts above 5,000/mm^3 or elevated

erythrocyte sedimentation rate or procalcitonin level[18] all are associated with increased risk for pneumococcal bacteremia and all have poor predictive values. These tests are best used as negative predictors because a WBC count below 10,000/mm^3, normal absolute neutrophil count, or a normal erythrocyte sedimentation rate have negative predictive values that are near 99 percent.

The clinical significance of "occult" bacteremia in untreated children has been of considerable interest and debate because some have argued "occult" bacteremia is the prologue to serious pneumococcal disease and others believe it is primarily a self-resolving syndrome of less significance. Observational studies demonstrate spontaneous clearance in approximately one third of patients and persistent signs or symptoms of illness in as many as two thirds of patients.[288]

Persistent bacteremia, even in those with persistent clinical illness, is not common even in the absence of antimicrobial therapy. However, the development of focal, presumed pneumococcal infection is not uncommon (Table 85-5).[528] Temperature greater than 39°C (102.2°F) at follow-up best correlates the presence of persistent bacteremia or a new focus of infection. Persistent bacteremia and new focal infections were associated with higher temperature (mean of 38.8°C vs. 37.7°C [101.8°F vs. 99.9°F]), greater elevation in WBC count (18,900/mm^3 vs. 14,900/mm^3), age younger than 20 months, and no antibiotic treatment being prescribed during the initial visit.[38] Complications such as meningitis, pneumonia, cellulitis, periorbital cellulitis, and AOM are the most common focal infections reported at follow-up. Children in whom a lumbar puncture was performed at the initial presentation with fever without a focus, at the time of pneumococcal bacteremia as reflected by a positive culture of blood, have been reported to have a fivefold greater risk for having CSF pleocytosis and meningitis at follow-up.[584]

TABLE 85-5 **Outcome in Children with Pneumococcal Bacteremia Initially Managed at Home**

Outcome	No. of Children	Percent
Antimicrobial therapy at first visit	46	47
Improved at second visit	37	79
Not improved at second visit	9	20
Persistent bacteremia at second visit	2	4
Meningitis at second visit	2	4
No antimicrobial therapy at first visit	51	53
Improved at second visit	16	31
Not improved at second visit	35	69
Persistent bacteremia at second visit	13	25
Meningitis at second visit	2	4
Total	97	

From Teele DW, Marshall R, Klein JO. Unsuspected bacteremia in young children: a common and important problem. *Pediatr Clin North Am* 1979;**26**:773–84.

The role of antibiotics in initial management of the febrile child at risk for bacteremia for the prevention of serious focal complications is controversial. Bachur and Harper reported higher rates of persistent clinical illness, persistent bacteremia, and focal infection in those not treated at visit during which the blood culture was drawn compared with children receiving oral or parenteral therapy at initial visit.[38] He also identified that only one of eight patients with meningitis at follow-up returned prior to notification of a positive blood culture, suggesting the practice of blood culture at initial visit enhances the early identification of at-risk children prior to clinical progression of disease, at least in some children. Whether this syndrome is unique to the United States or industrialized countries or is universal including developing countries is unclear, largely because of differences in clinical practices in performing blood cultures in ambulant febrile children. The syndrome of "pneumococcal bacteremia without a focus" represents 61 to 70 percent of IPD in the United States.[596]

Comparable to reports from the United States, bacteremia without a focus, defined as a positive blood culture with no obvious focus after clinical and laboratory evaluation, was identified in 179 (70.5%) cases at a Canadian hospital in Alberta.[310] Forty percent of those admitted to the hospital and 89 percent of those not admitted to the hospital did not have a focus of infection. Twenty-nine percent of patients had focal infection; 8 percent had meningitis, almost 10 percent had pneumonia, 5.5 percent had periorbital cellulitis, and 6 percent had other clinical syndromes. Few cases were identified in the first 90 days of life, with the majority occurring in children between 3 and 36 months of age. Comorbid illness, primarily cardiac, respiratory, and neurologic conditions, was present in 21.6 percent of hospitalized children compared with 3.8 percent of those managed as outpatients. The overall mortality was low, 0.7 percent. In other industrialized countries, primarily Europe, a low proportion of IPD is characterized as bacteremia without a focus (18% to 20%). In developing countries the proportion is even lower (~1%).[311,400,485]

Reports from cities such as Bangkok suggest that the "benign" nature of bacteremia without a focus is not a universal observation. In a hospital-based study in Bangkok, IPD without a focus was associated with 23 percent mortality, which was especially high in children with comorbid conditions.[495] These reports suggest that the syndrome, IPD without a focus, is different in severity and associated complications in Thailand compared with that described in the United States and Canada, with a large proportion of patients developing pneumonia or meningitis and with high death rates during the initial few days of hospitalization. Earlier age at onset, nutritional status, comorbid conditions, or genetic factors may result in a different syndrome with more rapid progression of pneumococcal bacteremia and reduced likelihood of spontaneous clearance than the slower progression and relatively high rate of spontaneous resolution described in U.S. children. Yildirim and colleagues reported that during the years 2007 through 2009, bacteremia without

any focus was the most common clinical presentation among children younger than 24 months of age (46.9% of all cases), whereas the majority of the cases in children older than 24 months of age had bacteremic pneumonia (45.9%).[593]

Pneumococcal Sepsis with Purpura

Sepsis, characterized by hypotension and even purpura that mimics meningococcemia, is also reported with pneumococcemia (Fig. 85-14). It has been observed most often in children with sickle-cell anemia as well as in asplenic children, although it has also been reported in children with immunodeficiency as well as on rare occasion in otherwise healthy children. Unlike meningococci, pneumococci do not produce an endotoxin. However, pneumococcal autolysin has been proposed as a potential mechanism. This glycan-teichoic acid fragment released from the bacterial cell wall has produced purpura when injected to monkeys and mice.[93,220]

Acute hemorrhagic edema in association with pneumococcal sepsis has also been described, possibly suggesting a vasculitic mechanism.[372]

Pneumococcal Meningitis

Streptococcus pneumoniae has become the most common bacterial cause of bacterial meningitis in most countries after the introduction of Hib conjugate vaccines, as well as the falling incidence of meningococcal disease in North and South America and Europe, in part as a result of meningococcal C conjugate vaccine programs. Although meningitis is a less common presentation of IPD in children compared with bacteremia, sepsis, and pneumonia, the case-fatality rates are high (6% to 54%) and neurologic morbidity frequent in survivors (0% to 50%).[201,417] Traditional signs and symptoms of bacterial meningitis including lethargy/unconsciousness, neck stiffness, a bulging anterior fontanelle, and reduced feeding are each individually associated with increased risk for meningitis at presentation.[570] The duration of illness prior to presentation can be quite variable. A proportion of children, estimated at 20 percent, have abrupt onset of signs and symptoms.[65] In others, meningitis presents after several days of illness and precise determination of the onset of meningeal signs is challenging. Seizures develop in approximately a fourth of patients. Decreased consciousness and septic shock occur in 11 and 16 percent, respectively.[25]

FIGURE 85-14 ■ *Streptococcus pneumoniae* sepsis with purpura fulminans in a child who had undergone splenectomy for refractory idiopathic thrombocytopenic purpura. (See companion Expert Consult website for color version.) (From American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 571–82.)

Examination of CSF for the presence of bacteria by microscopy and culture yields a bacterial etiology in more than 70 percent of children who have not as yet received antimicrobial therapy.[201] Antigen-detection assays, such as latex agglutination and now PCR assays, enhance the identification of a bacterial pathogen and are of most value in those children who received antimicrobial treatment before performance of the lumbar puncture.[280] PCR assays can also effectively identify pneumococcal serotypes in CSF samples, especially when patients have already been started on antibiotic therapy.[88] The mean total CSF WBC count varies but in general is greater than 1000 WBC/mm^3.[473] The CSF protein level is generally increased, and the glucose value is often extremely low. Recent analysis suggests that patients are unlikely to have pneumococcal meningitis if all of the following criteria are absent: (1) positive CSF Gram stain, (2) CSF absolute neutrophil count of at least 1,000 cells/mm^3, (3) CSF protein of at least 80 mg/dL, (4) peripheral absolute neutrophil count of at least 10,000 cells/mm^3, and (5) a history of seizure before or at the time of presentation.[388]

Prolonged fever, lasting more than 10 days, or recurrence of fever after initial defervescence is reported in pneumococcal meningitis.[65] In such children a repeat lumbar puncture may often reveal minimal pleocytosis. Most often, an alternative diagnosis of a nosocomial infection is found in children with recurrent fever. Diagnoses such as viral infection, phlebitis, urinary tract infection (especially in children requiring catheterization), and drug fever are common. Occasionally, complications of pneumococcal infection such as subdural effusion, complicated pneumonia with effusion, endocarditis, or pericarditis may be the source.[121] When dexamethasone has been administered as part of the initial management, a fever spike after defervescence can occur after discontinuation.[421,432] Relapse of the meningitis has been reported rarely as the cause for prolonged or secondary fever.[121] There does not appear to be any increased morbidity or mortality in children with recurrent or prolonged fever.[148] The outcome and long-term complications

of pneumococcal meningitis include severe hearing loss, seizures, learning or mental difficulties, or paralysis.[25] Patients with pneumococcal meningitis have a higher mortality rate and are at greater risk for the development of neurologic sequelae than are patients with *N. meningitidis* or *H. influenzae* type b infection.[274,387] The mortality rate varies from 6.3 to 20 percent, with the highest mortality in the youngest children.[476] A peripheral WBC count of less than 5000/mm^3 at initial evaluation was recognized as a poor prognostic factor with a high mortality rate.[297] A trend toward a higher mortality rate also was noted if the CSF WBC count was less than 1000/mm^3. A similarly poor outcome can be predicted in patients with pneumococcal meningitis when low CSF glucose concentrations (<1.11 mmol/L)[165] or high CSF protein concentrations (>250 mg/dL)[297] are present. A multicenter U.S. study reported a 7.7 percent mortality rate, with 25 percent of children suffering neurologic morbidities and 32 percent with moderate or severe hearing loss.[25]

Children who have cochlear implants are more than 30 times more likely than healthy children to acquire pneumococcal meningitis, presumably from direct extension from the nasopharynx or middle ear. Implants with positioners were associated with a greater risk than those without. The risk period for development of pneumococcal meningitis appears to extend for 24 months after implantation.[53,443] Delayed sterilization of CSF cultures after antimicrobial treatment also has been associated with a poorer outcome.[314] In children with delayed sterilization, a statistically greater proportion had hearing loss at 1 year and more children had neurologic abnormalities, although these differences were not statistically significant.

Higher incidence rates, greater case-fatality rates, and more frequent neurologic sequelae have been observed in children from developing countries compared with those from industrialized countries. Case-fatality rates in excess of 50 percent have been reported from some African and Asian countries and included results from studies performed during the late 1990s and early 2000s.[83,400,409,453,467] The case-fatality rates from pneumococcal meningitis in developing countries and indigenous native populations in fact exceed the overall annual incidence rates of pneumococcal meningitis in industrialized countries by 15-fold to 100-fold.[506] The in-hospital case-fatality rates provide a minimal estimate as to the true mortality associated with pneumococcal meningitis, as indicated by studies with follow-up beyond the hospitalization period. A study in The Gambia showed that 23 percent of hospital survivors subsequently died in the community mainly related to neurologic sequelae resulting from the meningitis.[570] Late clinical presentation of cases to the hospital as well as delays in diagnosing meningitis (e.g., 22% initial erroneous misdiagnosis of pneumococcal meningitis as malaria in Malawi[367]) may contribute to this high mortality. The review by Peltola reported that mortality rates for pneumococcal meningitis (45%) were greater than for *H. influenzae* (29%) or *N. meningitidis* (8%).[417] A meta-analysis on mortality due to bacterial meningitis in developed countries also found higher mortality rates associated with pneumococcal meningitis (15.3% vs. 3.8% for *H. influenzae* type b and 7.5% for *N. meningitidis*).[46]

Pneumonia

Defining pneumococcal pneumonia in children remains a challenge because a specific pathogen cannot be documented in most cases unless bacteremia or empyema occurs. Vaccine probe studies have helped to redefine the spectrum of clinical pneumonia associated with *S. pneumoniae* and extended the clinical description beyond lobar pneumonia. Lobar pneumonia remains the classic pulmonary disease resulting from pneumococcal infections. High fever exceeding 40°C (104°F), general fatigue, a productive cough, and dyspnea are the prototypical presenting signs and symptoms. In older children, chest pain is often associated with a lobar infiltrate. Fever and cough are nearly universal, occurring in 90 and 70 percent, respectively. Severe abdominal pain is often a presenting symptom in left lower lobe disease, chest pain occurs in 10 to 50 percent of patients,[524] and meningeal signs may be present with apical infiltrates, especially in young children. Comparison of pneumococcal pneumonia with respiratory syncytial virus disease demonstrates significant overlap in presentation. Children with pneumococcal pneumonia less often have tachypnea but more often high WBC counts (mean of 20,800/mm^3 in pneumococcal pneumonia) and high CRP level (mean of 137 mg/L). Alveolar infiltrates are observed more frequently, but not exclusively, in pneumococcal pneumonia.[270] Children with bacteremic pneumococcal pneumonia were more ill appearing than children with nonbacteremic pneumococcal pneumonia. Lobar infiltrates on chest radiographs were present in the majority of bacteremic cases. Pleural effusion is common and occurs in as many as 40 percent of patients; however, only 10 percent have substantial fluid accumulation and 2 percent develop empyema.[326] Patients with pleural fluid secondary to pneumonia have the usual signs and symptoms, with chest pain, abdominal pain, egophony, and/or dullness on percussion. A pleural friction rub, abdominal pain, and dullness on percussion may also be present. Persistent fever, lasting as long as 3 weeks, can be associated with even relatively small effusions.[524] Several studies have reported *S. pneumoniae* as the most common cause of empyema in children.[82] It was most commonly reported in children between 3 and 5 years of age. Serotypes 1, 19A, and 3 were the most common pneumococcal serotypes identified in children with empyema.[81]

Establishing the diagnosis of a specific pathogen in children with pneumonia remains challenging. Blood culture is positive in no more than 10 percent of patients with pneumococcal pneumonia.[567] Children with bacteremic pneumococcal pneumonia usually have a high temperature and leukocytosis on admission, with peripheral WBC counts exceeding 15,000/mm^3, similar to those without bacteremia.[533] Based on vaccine probe studies, Madhi and colleagues reported that procalcitonin and CRP were equally useful in children infected and not infected with HIV to improve the specificity of the

pneumococcal pneumonia efficacy end point in the vaccine trial among children with radiologically confirmed pneumonia.[339]

No strict radiologic definition of pneumococcal pneumonia in children is accepted broadly. At one end is the typical appearance of severe lobar consolidation (Fig. 85-15), which is recognized as associated with bacterial pneumonia.[585] At the other end are the mild interstitial and perihilar changes that are often associated with viral infections or asthma and that may be part of the spectrum of normal appearances for children in developing countries. In studies performing lung aspiration in cases of radiologically proven lobar infiltrates, the proportion of patients in whom *S. pneumoniae* was detected was high.[150,559,563] *S. pneumoniae* was the predominant bacterial pathogen in most studies of children with lobar infiltrates (Table 85-6).[338] The role of the pneumococcus in children with lobar infiltrates is further supported by the observed 63 percent reduction in pneumonia with alveolar infiltrates in children vaccinated with a 7-valent pneumococcal conjugated vaccine compared with children receiving placebo.[57]

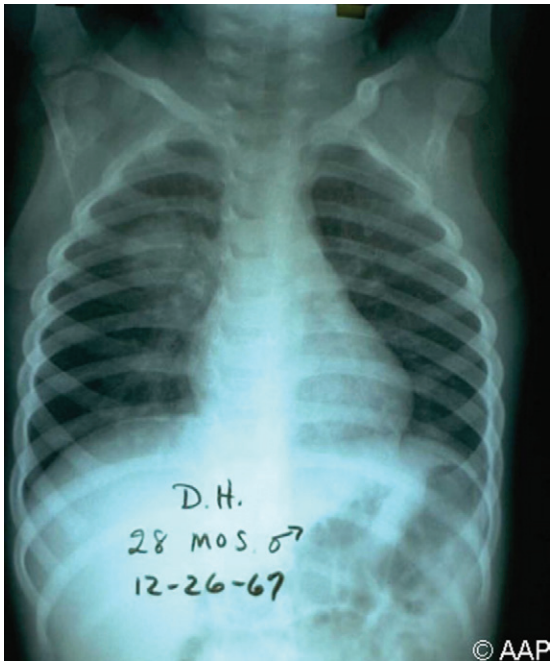

FIGURE 85-15 ■ Segmental (round) pneumococcal pneumonia in 28-month-old child. (See companion Expert Consult website for color version.) (From American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases.* Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 571–82.)

Although the role of the pneumococcus in children with lobar consolidation is known (see Table 85-6), its role in cases with other radiologic findings is not as well defined. Studies demonstrating a reduction in hospitalizations associated with "viral pneumonia" in children immunized with PCV suggest a role for the pneumococcus in disease manifesting as interstitial and perihilar infiltrates, at least in countries such as South Africa. A recent study found that the incidence of human metapneumovirus (hMPV) lower respiratory tract infection was reduced by 45 percent and the incidence of clinical pneumonia was reduced by 55 percent in non-HIV-infected children who had received three doses of conjugate pneumococcal vaccine. In fully vaccinated HIV-infected children, the incidence of hMPV-associated lower respiratory tract infection was reduced by 53 percent and that of clinical pneumonia by 65 percent.[340]

In the preantibiotic era, the natural course of pneumococcal pneumonia consisted of 7 to 9 days of a stationary phase of symptoms followed by worsening of the systemic signs ("pre-crisis") and high mortality. In those who recovered, defervescence, sweating, polyuria, and a brief febrile attack ("post-crisis") were observed.[31,404] Today, the mortality rate in industrialized countries is extremely low (<1%), even in cases of bacteremic pneumonia, and long-term complications are uncommon.[276] However, hemolytic-uremic syndrome in association with pneumococcal pneumonia and bacteremia is being reported with increasing frequency. Thirty-seven cases occurring between 1997 and 2009 in North America were recently reported from the Emerging Infection Network.[44] In general, the patients were severely ill and presented with pneumonia and bacteremia, and half of the patients required mechanical ventilation and chest tube placement or video-assisted thoracoscopic surgery. Ninety-two percent of cases were associated with

TABLE 85-6 **Etiology of Pneumonia Based on Lung Aspirate Studies**

Continent	Age	No. Patients	% Culture Positive	*Organism*				
				S. pneumoniae	*H. influenzae*	*S. aureus*	*E. coli*	Other
Europe	6 wk-11 yr	255	66	40 (32)	21 (17)	13 (5)	0.5 (0)	13 (10)
North America	2 mo-15 yr	240	34	77 (26)	5 (2)	12 (4)	2 (0.1)	15 (5)
South America	1 mo-14 yr	885	44	22 (10)	14 (6)	44 (19)	3 (1)	22 (9)
Africa	1 mo-12 yr	949	66	25 (17)	17 12)	29 (20)	4 (3)	44 (31)
Asia	11 days-14 yr	674	51	10 (2)	9 (5)	45 (24)	4 (2)	29 (15)
Oceania	1 mo-10 yr	101	58	57 (34)	71 (42)	1 (1)	0 (0)	39 (23)

Modified from Madhi S, Pelton SI. Epidemiology, diagnosis and treatment of serious pneumococcal infections in children from pneumococcal vaccines. In: Siber GR, Klugman KP, Makela PH, editors. *The impact of conjugate vaccine.* ASM Press; Washington, DC, 2008, p. 95–108.

PCV13 serotypes, with serotype 19A recovered from half of the children. Twenty cases have been reported from Taiwan.[315]

Otitis Media

Twenty-five to 60 percent of cases of AOM are attributable to the pneumococcus. The bacteriologic outcome of *S. pneumoniae* AOM is less favorable than is the outcome of AOM caused by other organisms when untreated or treated inappropriately.[289,380,402] The pathogenesis is thought to reflect recently acquired colonization in combination with viral respiratory tract infections in most cases.[527,543] Some studies have suggested that pneumococcal AOM is more severe than that caused by other pathogens; however, the overlap in signs and symptoms prevent clinical criteria alone from accurately predicting the etiologic pathogen. Observations from 1970 support that pneumococcal AOM is accompanied more frequently by great pain and higher fever than was AOM caused by other pathogens; it also is more purulent, as characterized by higher middle ear fluid white blood cell counts, and the tympanic membrane is more erythematous.[75,236] Subsequent studies have reported that when *S. pneumoniae* is recovered from the nasopharynx at the time acute otitis media is present there are greater abnormalities in the tympanic membrane, as reflected in higher severity score and greater likelihood of persistent symptoms on day 5, persistence of tympanic membrane abnormalities on day 28, and recurrence before day 28. Spontaneous remission of the infection occurs less commonly in patients with pneumococcal AOM than in those with other pathogens.[235] Tympanic membrane perforation is also more commonly observed with pneumococcal otitis.

Mastoiditis

Acute mastoiditis was the most common complication of otitis media and occurred in as many as 20 percent of untreated cases. Although now dramatically decreased since the routine use of antimicrobial therapy, it still remains the most common suppurative complication of AOM.[107] *S. pneumoniae* is the most common cause of acute mastoiditis in children and accounts for 25 to 46 percent of all culture-proven cases.[186,234] Increased cases of mastoiditis due to multidrug-resistant *S. pneumoniae* have been reported after the introduction of PCV7 in the United States.[403] Recent studies of the incidence of mastoiditis suggest a higher rate of disease in countries in which observation is the first-line approach to children with otitis media; however, the risk still remains remarkably small in such countries.[549]

Sinusitis

Studies suggest that 5 to 10 percent of all upper respiratory tract infections are complicated by bacterial sinusitis when defined as either acute, febrile illness with facial swelling (usually localized to the eye) or initial improvement in signs and symptoms and then recurrence of signs and symptoms or persistence of rhinorrhea, low-grade fever, and facial swelling without improvement beyond 10 days.[561] *S. pneumoniae* is the pathogen most commonly isolated from patients with sinusitis, and it causes 35 to 42 percent of all bacterial cases.[562] Clinical signs and symptoms are often age dependent and related to the maturation of the different sinuses affected. Ethmoidal and maxillary sinuses are most commonly involved, especially in children younger than 5 years old. Sinusitis is manifest initially as a viral infection, followed by a nasal discharge that frequently is purulent and accompanied by cough. The symptoms are often increased at night with accumulation of secretions from postnasal drip. Malodorous breath is frequent.[246] In cases of sinusitis, isolation of *S. pneumoniae* from the nasal discharge strongly suggests its involvement at the site of infection.

Conjunctivitis

Purulent bacterial conjunctivitis due to *S. pneumoniae* is well described, with nonencapsulated strains most often involved. Clinically, bilateral, purulent discharge is the most common clinical presentation and often distinguishes it from viral disease.[59,182,560,573] When conjunctivitis is present with acute otitis media, the so-called conjunctivitis-otitis syndrome, *H. influenzae* is the most common pathogen.[62]

In a study in Minnesota, children with conjunctivitis were evaluated in two cities.[80] Forty-nine percent of positive cultures showed *S. pneumoniae*. All isolates were nontypeable. Pulsed-field gel electrophoresis identified three clonal groups, with 84 percent of the isolates belonging to one clonal group. Multilocus sequence typing revealed that isolates had the same multilocus sequence type as that of isolates from a 2002 pneumococcal disease outbreak at a New England college. It has been suggested that certain pneumococcal strains have a predilection for causing conjunctivitis.[80] These reports suggest that pneumococcal conjunctivitis can be highly contagious in school settings.

Bone and Joint Infections

Septic arthritis caused by *S. pneumoniae* is a relatively rare condition that accounts for 2.2 to 9.7 percent of all cases.[205,463] Its main initial clinical features are single or multiple swollen, warm, and painful joints. The joints most commonly involved are the knee and the ankle.[248] In one study, the mean duration of symptoms before hospitalization was 2 days, and patients were hospitalized for an average of 11 days. The mean peripheral WBC count was 20,600/mm^3, the erythrocyte sedimentation rate usually was greater than 90 mm/hr, and the CRP concentration generally was higher than 100 mg/L. *S. pneumoniae* can be isolated from synovial fluid or blood in most patients.[463] The outcome is favorable in the majority of cases unless underlying diseases or sepsis occur concomitantly.[248]

Soft Tissue Infections

S. pneumoniae has become an uncommonly recognized etiologic agent of soft tissue infections but can cause

serious infections. Most commonly, facial cellulitis has been described in otherwise healthy children in conjunction with recovery of *S. pneumoniae* from either local or systemic cultures.[130] Periorbital infection can be a complication of sinus disease and can uncommonly spread to the orbit, resulting in orbital cellulitis or abscess manifested as proptosis and ophthalmoplegia.[486] Most patients with such complications are younger than 3 years of age and previously were healthy. Fever and peripheral WBC counts higher than 15,000/mm^3 can be observed in most patients, and blood cultures frequently are positive. Overall, response to therapy generally is good, and patients usually recover.[187] In patients with connective tissue diseases such as systemic lupus erythematosus, HIV infection, or corticosteroid treatment, pneumococcal cellulitis has been reported and may be severe.

Peritonitis

Primary peritonitis, where the pathogen travels from the blood or via the lymphatic system to the peritoneal cavity, is an uncommon condition that accounts for only 17 percent of all cases of peritonitis. *S. pneumoniae* is the major pathogen in primary peritonitis, responsible for 38 percent of all cases.[192] Primary pneumococcal peritonitis should be considered in the differential diagnosis of children with an acute abdominal syndrome,[169] particularly in children with nephrotic syndrome, immunocompromised status, or sickle-cell disease.[192] Colonization of the perineum and subsequent spread via the fallopian tube to the peritoneal cavity have been suggested as the pathogenesis of primary pneumococcal peritonitis and is consistent with the observation that 87 percent of cases occur in females.[483,496] Most of the patients are between 4 and 10 years of age.[483] Symptoms include diffuse abdominal pain, fever, vomiting, and diarrhea. Abdominal tenderness can be maximal in the right lower quadrant, which can lead to confusing it with acute appendicitis. In some cases, *S. pneumoniae* can be isolated as the only cause of peritonitis secondary to appendicitis. Cultures should include blood, peritoneal fluid, and a vaginal swab. Some patients need laparotomy or laparoscopy to exclude other pathologic processes such as appendicitis or a tuboovarian abscess.[496] Morbidity and mortality rates can be high when the period without treatment exceeds 1 week, but in most cases the outcome is favorable when treated adequately.[483]

Endocarditis

S. pneumoniae endocarditis, including that caused by nonpenicillin-susceptible strains, is most often reported to involve the aortic and mitral valves.[492] The syndrome of pneumococcal meningitis, pneumonia, and endocarditis was previously more common. It was initially described by Osler but is also known as Austrian syndrome. This triad is now uncommon, and *S. pneumoniae* accounts for less than 3 percent of cases of bacterial endocarditis currently.[249,376,525] This triad is rarely present in children. In children, mitral valve involvement has been described most often. A history of congenital heart disease is common. Medical therapy alone has a high mortality rate that was improved in a group of patients receiving combined medical and surgical interventions.[94]

Pericarditis

In the preantibiotic era, pneumococcal pericarditis was a relatively frequent complication of lobar pneumococcal pneumonia and the most common pathogen in purulent pericarditis in children. After the introduction of antibiotics, the pneumococcus declined as a cause of pericarditis, decreasing from 51 to 9 percent of cases and occurring mainly in adults. Currently, infections of the pericardium are rare in children and often are related to underlying medical conditions such as immunodeficiency.[419]

Infection in Immunocompromised Hosts

Patients with functional asplenia, including those with hemoglobinopathies, are at high risk for IPD. In 19 asplenic patients with IPD, the clinical findings included fever (86%), shock (27%), petechiae or purpura (27%), disseminated intravascular coagulopathy (18%), and respiratory distress (18%). Clinical illness included bacteremia alone (52%), meningitis (26%), bacteremia with otitis/sinusitis (13%), and bacteremia with pneumonia (9%). The mortality rate was 32 percent.[478]

HIV-infected children with pneumonia usually have the typical symptoms of fever, shortness of breath, and productive cough associated with pleuritic pain. The chest radiograph shows unilobar or multilobar infiltrates, and peripheral WBC counts often are elevated. A comparison between HIV-infected and non-HIV-infected children with community-acquired pneumonia showed no significant differences between groups with regard to the duration of hospitalization and mortality rates.[342] In contrast, when only patients infected with isolates belonging to the traditional "pediatric serotypes" were compared a higher rate of pneumonia with or without concurrent meningitis in the HIV-infected group was observed. Recurrence of pneumococcal infection within 6 months from the initial episode is also common in patients infected with HIV, particularly children.[176] Most HIV-infected patients recover without significant sequelae, and the clinical course of their systemic infection does not appear to be markedly different from that in healthy children.[259]

Patients with immunodeficiency disorders consisting of antibody and complement deficiencies are at high risk for acquisition of invasive and recurrent pneumococcal infection.[259] Children with nephrotic syndrome have a particularly increased risk for the development of pneumococcal peritonitis, mainly during relapse of their renal disease.[154] Children with sickle-cell disease are prone to the development of overwhelming *S. pneumoniae* bacteremia and sepsis; in children younger than 3 years of age, the incidence of bacteremia is 6.1 events per 100 patient-years, and the case-fatality rate is 24 percent for *S. pneumoniae* sepsis.[597]

S. pneumoniae is an important pathogen in patients undergoing bone marrow transplant (BMT) or periph-

eral blood stem cell transplant (PBSCT), with two patterns of disease recognized. Early onset is observed in both allogeneic and autologous BMT and PBSCT patients within the first 35 days. Later-onset disease occurs after 100 days and is more frequent in allogeneic compared with autologous BMT and is the most common bacterial infection in such patients. Functional hyposplenism secondary to irradiation, chronic graft-versus-host disease, and decreased IgG2 antibody contribute to the risk for IPD and the poor outcome. Case-fatality rates approximate 20 percent.[303] Chronic graft-versus-host disease is associated with long-term risk for pneumococcal infections in recipients of BMTs.[143,303]

Solid-organ transplant patients are at increased risk for pneumococcal disease. The risk varies with the nature of the transplant, but recipients have a lifelong increase in risk owing to the immunosuppressive therapy required for transplant survival, and recurrent disease is common. Of 42 children who had undergone bone marrow or solid organ transplantation and became infected with *S. pneumoniae*, 8 (19%) had recurrent pneumococcal infections.[479] Solid-organ recipients were more likely to have recurrent invasive disease than were recipients of BMTs. Death occurred in 2 of 42 recipients (5%). Cardiac transplant patients have an incidence of 39 cases of IPD per 1000 patients per year,[509] the highest risk for which occurs in African American heart transplant recipients who undergo transplantation because of idiopathic dilated cardiomyopathy.

TREATMENT

The pneumococcus is the causative bacterial agent in many respiratory and invasive infections, many of which are often treated empirically. Empirical and pathogen-directed treatment guidelines are available for the major disease syndromes associated with pneumococcal infection. In the absence of rapid methods for diagnosis of disease and identification of drug-resistant strains, empirical treatment will continue to be the rule in clinical practice.

Dosing regimens and susceptibility of β-lactam and non-β-lactam antimicrobial agents used to treat pneumococcal infections are shown in Tables 85-7 and 85-8, respectively.[253] Dosing regimens are shown as total recommended daily dose and number of doses per day and should be used in conjunction with local prescribing information. Susceptibility data reflect overall findings from U.S. and international studies, and local susceptibility data should be taken into consideration because susceptibility patterns may vary considerably.

Pneumonia

Guidelines for the management of infants and children older than 3 months of age with presumed bacterial community-acquired pneumonia have been published by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America.[69] These guidelines recommend no antibiotic treatment for a preschool-aged child with relatively mild disease because many of these infections are likely to be viral and the patient will not benefit from antibiotic therapy. Children with mild disease severity should be considered for outpatient treatment with antibiotics, with amoxicillin and amoxicillin-clavulanate recommended as first-line agents and cefpodoxime, cefuroxime, cefprozil, levofloxacin, and linezolid as alternatives. For children who do not have clinical, laboratory, or radiographic evidence that distinguishes bacterial from atypical pneumonia, a macrolide can be added to the β-lactam antibiotic. Hospitalization is required for any child requiring intravenous therapy, hydration, or respiratory support. For inpatient treatment of children fully immunized with conjugate pneumococcal and Hib vaccines, parenteral ampicillin, penicillin G, ceftriaxone, or cefotaxime is recommended, with the addition of clindamycin or vancomycin if community-acquired methicillin-resistant *S. aureus* is suspected. Except for ampicillin and penicillin G, the same agents are recommended for children who are not fully immunized. If atypical pneumonia cannot be excluded from the differential diagnosis, addition of azithromycin is recommended, with doxycycline and levofloxacin alternatives for older children. Other agents approved for treatment of pneumococcal pneumonia in adults include linezolid, tigecycline, and ceftaroline, a cephalosporin with anti-methicillin-resistant *S. aureus* activity.[263]

Sinusitis

Although fewer than 2 percent of cases of acute viral rhinitis are complicated by acute bacterial rhinosinusitis (ABRS), patients with symptoms lasting more than a week or increasing in severity after 5 to 7 days can have bacterial superinfection that requires treatment with antimicrobial agents. The most common cause of ABRS is *S. pneumoniae*.[95] Treatment guidelines suggest treating pediatric patients with mild disease and no antibiotic exposure in the previous 4 to 6 weeks with amoxicillin-clavulanate at 45/6.4 mg/kg/day.[95] Patients with type I β-lactam hypersensitivity can be treated with levofloxacin, whereas those with non–type I hypersensitivity can be treated with clindamycin plus either cefixime or cefpodoxime. Patients with more severe disease, recent antimicrobial use, or failure of initial therapy should be treated with amoxicillin-clavulanate at 90/6.4 mg/kg/day, clindamycin plus either cefixime or cefpodoxime, or levofloxacin. Ampicillin-sulbactam, ceftriaxone, cefotaxime, or levofloxacin is recommended for children with severe infection requiring hospitalization.

Acute Otitis Media

AOM is the most commonly treated bacterial infection in children. It most often occurs between age 6 months and 3 years and usually follows a viral respiratory tract infection. As in other respiratory infections, *S. pneumoniae* (25% to 50% of cases) and *H. influenzae* (23% to

TABLE 85-7 **Dosing Regimens and Susceptibility of β-Lactam Antimicrobial Agents Used to Treat Pneumococcal Infections***

Antimicrobial Agent	*Total Daily Dose (No. of Doses per Day)*		Susceptible Breakpoint (μg/mL)†	Percent Susceptibility
	Adolescents	Infants and Children		
Parenteral Agents—Meningitis				
Penicillin G	24 million units (6-8)	300,000-400,000 units/kg (4-6)	≤0.06	63.5
Ampicillin	12 g (6)	300 mg/kg (4)	Penicillin ≤0.06	68.3
Ceftriaxone	4 g (1-2)	100 mg/kg (1-2)	≤0.5	82.2-86.2
Cefotaxime	8-12 g (4-6)	225-300 mg/kg (4-6)	≤0.5	87.4
Cefepime	6 g (3)	150 mg/kg (3)	≤0.5	85.3
Meropenem	6 g (3)	120 mg/kg (3)	≤0.25	78.1-93.9
Parenteral Agents—Nonmeningeal Infections				
Penicillin G (regular dose)	12 million units (6)	250,000-400,000 units/kg (4-6)	≤2‡	92.6
Penicillin G (high dose)	18-24 million units (6)	400,000 units/kg (4-6)	≤4	99.7§
Ampicillin	4-8 g (4)	50-100 mg/kg (4)	penicillin ≤2	92.6
Ceftriaxone	1-2 g (1)	50-75 mg/kg (1-2)	≤1	95.2
Cefotaxime	3 g (3)	75-100 mg/kg (3-4)	≤1	95.1-96.5
Cefuroxime sodium	2.25 g (3)	50-100 mg/kg (3-4)	≤0.5	75.3
Cefepime	2 g (2)	100 mg/kg (2)	≤1	96.5
Ceftaroline	1.2 g (2)	NA	≤0.25	100
Meropenem	1.5-3 g (3)	60-120 mg/kg (3)	≤0.25	78.1-93.9
Imipenem	1-2 g (3-4)	60 mg/kg (4)	≤0.12	92.4-96.3
Ertapenem	1 g (1)	30 mg/kg (2)	≤1	93.2-93.3
Oral Agents				
Penicillin V	1-2 g (3-4)	25-50 mg/kg (3-4)	≤0.06‡	63.5
Amoxicillin (regular dose)	1.5 g (2-3)	45 mg/kg (2-3)	≤2	84-92.2
Amoxicillin-clavulanate (regular dose)	1.5 g/250 mg (2-3)	45/6.4 mg/kg (2-3)	≤2	84-93.6
Amoxicillin (high dose)	6 (3)	90 mg/kg (2-3)	≤4	86-92.2
Amoxicillin-clavulanate (high dose and extended release)	4 g/250 mg extended release (2)	90/6.4 mg/kg (2-3)	≤4	86-93.6
Cefaclor	750-1500 mg (3)	20-40 mg/kg (3)	≤0.5	60.2
Cefuroxime axetil	500-1000 mg (2)	20-30 mg/kg (2)	≤1	68-78.2
Cefixime	400 mg (1-2)	8 mg/kg (1-2)	≤1	68.3
Cefprozil	500-1000 mg (2)	15-30 mg/kg (2)	≤1	79.7
Cefdinir	600 mg (1-2)	14 mg/kg (1-2)	≤0.5	68-76.8

*Dosing regimens are shown as total recommended daily dose and number of doses per day (in parentheses) and should be used in conjunction with local prescribing information. Susceptibility data reflect overall findings from U.S. and international studies, and local susceptibility data should be taken into consideration because susceptibility patterns may vary considerably.
†Susceptibility breakpoint for agent shown unless otherwise specified.
‡Nonmeningeal isolates susceptible to penicillin G at this breakpoint can be considered susceptible to parenteral ampicillin, cefepime, cefotaxime, and ceftriaxone. Nonmeningeal isolates susceptible to ≤0.06 μg/mL of penicillin G also can be considered susceptible to these parenteral agents, as well as to parenteral ertapenem, imipenem, and meropenem and to oral ampicillin, amoxicillin, amoxicillin-clavulanate, cefaclor, cefdinir, cefditoren, cefprozil, cefuroxime, and cefpodoxime.
§Includes penicillin G susceptible (92.6%) and intermediate (7.1%) isolates based on new, nonmeningeal, parenteral penicillin G susceptibility breakpoints.
NA, not available.
Modified from Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae:* trends and management. *Expert Rev Anti Infect Ther* 2008;**6**:619–35.

67%) predominate as causes.[14] The incidence of AOM initially decreased after the introduction of PCV7 in 2000 but subsequently increased, associated with increases in antimicrobial resistant serotypes such as serotype 19A.[86] The introduction of PCV13 in 2010 is likely to reverse this trend.[513]

According to the practice guidelines published by the American Academy of Pediatrics and the American Academy of Family Physicians Subcommittee on Management of Acute Otitis Media, initial observation for 24 to 72 hours with necessary analgesia is acceptable to ascertain a diagnosis of probable bacterial AOM.[14] Once

TABLE 85-8 **Dosing Regimens and Susceptibility of Antimicrobial Agents Other Than β-Lactams Used to Treat Pneumococcal Infections***

Antimicrobial Agent	*Total Daily Dose (No. of Doses per Day)*		Susceptible Breakpoint (μg/mL)†	Percent Susceptibility
	Adolescents	Infants and Children		
Parenteral Agents—Meningitis				
Vancomycin	30-60 mg/kg (3-4)	60 mg/kg (4)	≤1	100
Rifampin	600 mg (1)	20 mg (1-2)	≤1	99.9
Chloramphenicol	4-6 g (4)	75-100 mg/kg (4)	≤4	88.1
Parenteral Agents—Nonmeningeal Infections				
Vancomycin	2 g (2-4)	40-45 mg/kg (3-4)	≤1	100
Oral Agents				
Doxycycline	200 mg day 1, then 100 mg (1)	NR	≤0.25	71.3
Co-trimoxazole	320/1600 mg (2)	8/40 mg/kg (2)	≤0.5/9.5	56-69.0
Oral and Parenteral Agents				
Levofloxacin	500-750 (1)	NR	≤2	99.2
Gemifloxacin	320 (1)	NR	≤0.25	99.9
Moxifloxacin	400 (1)	NR	≤1	99.6
Erythromycin	1-2 g (4)	30-50 mg/kg (4)	≤0.25	70.9
Clarithromycin	500-750 mg (1-2)	15 mg/kg (2)	≤0.25	71.1
Azithromycin	500 mg day 1, then 250-500 mg (1)	10 mg/kg day 1, then 5-10 mg/kg (1)	≤0.12	55-65.5
Clindamycin	600-1200 mg (4)	16-40 mg/kg (4)	≤0.25	66.7
Linezolid	1200 mg (2)	30 mg/kg (3)	≤2	100

*Dosing regimens are shown as total recommended daily dose and number of doses per day (in parentheses) and should be used in conjunction with local prescribing information. Susceptibility data reflect overall findings from U.S. and international studies, and local susceptibility data should be taken into consideration as susceptibility patterns may vary considerably.
†Susceptibility breakpoint for agent shown unless otherwise specified.
NR, not recommended for use in infants and children.
Modified from Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae:* trends and management. *Expert Rev Anti Infect Ther* 2008;**6**:619–35.

diagnosis is certain, initial treatment should be with either amoxicillin 80 to 90 mg/kg/day or, in case of high temperature (>39° C) or severe otalgia, amoxicillin-clavulanate 90/6.4 mg/kg/day. In a recent study of children 6 to 24 months old with no history of recurrent AOM and with bona fide AOM for whom clinical outcomes were assessed by validated otoscopists, 10 days of high-dose amoxicillin-clavulanate (80 mg/kg/day amoxicillin in two divided doses) or cefdinir (14 mg/kg/day in two divided doses) was significantly more effective than 5 days of cefdinir (86.5% vs. 71.0%; $p = .001$) as therapy for AOM.[87]

Bacteremia

S. pneumoniae is a common cause of bacteremia in children and is frequently associated with pneumonia. Treatment guidelines follow recommendations for treatment of pneumonia.[95,253]

Meningitis

After PCV7 introduction, an estimated 3330 pneumococcal meningitis hospitalizations and 394 deaths were prevented in persons of all ages during 2001 through 2004 in the United States, and pneumococcal meningitis is now more common in adults than in children.[538] An estimated 4100 cases and 500 deaths from bacterial meningitis occurred annually in the United States during 2003 through 2007, with 58 percent due to *S. pneumoniae.*[532] Practice guidelines for the management of bacterial meningitis recommend the following.[210] Patients suspected of having meningitis should have lumbar puncture and blood cultures performed as soon as possible, with empirical antimicrobial therapy begun immediately afterward. For infants and children 6 weeks of age and older, dexamethasone should be considered as adjunctive therapy. The issue remains controversial, but, if used, dexamethasone should be administered concurrently with the first dose of antibiotics. Initial empirical treatment for patients older than 1 month is vancomycin plus a third-generation cephalosporin such as ceftriaxone or cefotaxime. Treatment can be adjusted once culture and susceptibility results are obtained. Penicillin breakpoints specific for meningitis have recently been established: for susceptible disease, less than or equal to 0.06 μg/mL and for resistant disease, greater than or equal to 0.12 μg/mL.[98]

PREVENTION

Nonimmunologic Strategies

This category includes interventions that aim at (1) reducing risk factors predisposing to the development of pneumococcal infection, (2) providing chemical substances (e.g., antibiotics) to abort or prevent pneumococcal colonization or disease, and (3) modifying anatomic abnormalities that predispose to the development of pneumococcal infection.[290]

A factor that is important not only for pneumococcal infection but also for many other serious bacterial infections is the need to improve general health and nutrition worldwide, particularly in developing countries. Many risk factors for the acquisition of pneumococcal infection that can be alleviated include poor living conditions, overcrowding, poor hygiene, malnutrition, and a high prevalence of viral infections, particularly respiratory viruses, measles, and HIV infection. In the developed world, carriage of pneumococci, especially antibiotic-resistant strains that result in sporadic infections as well as outbreaks, is related to daycare attendance and is proportional to the number of children per group.* Therefore, reducing the number of children per group in daycare centers and developing alternative forms of childcare may reduce the rate of pneumococcal morbidity.

The importance of passive smoking (namely, being in close contact with smokers in the same household) has been highlighted as a risk factor for the development of pneumococcal disease.[393] Thus, efforts to prevent smoking in the home may have an important role in the prevention of pneumococcal infection. Breast-feeding may protect against some infections related to *S. pneumoniae*, such as otitis media.[19,77,135,457,543] Therefore, breast-feeding should be encouraged, especially in families in which otitis media occurs commonly, although the precise role of prolonged breast-feeding in protecting against pneumococcal infection has not been established.

Antimicrobial chemoprophylaxis is used predominantly for two indications: prevention of recurrent AOM and prevention of pneumococcal sepsis in children with anatomic or functional asplenia. Data now exist to support the common practice of prescribing regular doses of penicillin for children with asplenia or sickle-cell disease.[174] Chemoprophylaxis is used commonly for the prevention of middle ear infections. Controlled clinical trials have compared antimicrobial chemoprophylaxis with placebo, surgery, or historical controls.[60] Various antibiotics were tested (either as ongoing daily prophylaxis or as intermittent treatment during viral infections). Most of the studies, but not all, showed a benefit of chemoprophylaxis over controls, especially for amoxicillin, ampicillin, sulfonamides, and trimethoprim-sulfamethoxazole. However, those studies were performed before both the era of increased antibiotic resistance in *S. pneumoniae* and the use of pneumococcal conjugate vaccines. The dosing schedule recommended for prophylaxis usually is half the daily therapeutic dose given once a day, but the optimal dosing regimen has not been defined.

The potential benefits of otitis media chemoprophylaxis have been weighed against the ability of chemoprophylaxis to alter the nasopharyngeal flora, foster colonization with resistant organisms, and thereby compromise the long-term efficacy of the prophylactic drug and contribute to the propagation of resistant organisms throughout the community. As a result, the practice of otitis media chemoprophylaxis has been reduced greatly. In one study, prophylaxis with amoxicillin induced a dramatic increase in the carriage of penicillin-resistant *S. pneumoniae*, as well as increased carriage of other resistant pathogens.[76] The fear of increasing resistance led the Committee on Infectious Diseases of the American Academy of Pediatrics to issue a warning against the widespread use of otitis media prophylaxis and to state that "Antimicrobial prophylaxis should be reserved for control of recurrent acute otitis media, defined by 3 or more distinct and well-documented episodes during a period of 6 months or 4 or more episodes during a period of 12 months." Surgical otitis media prophylaxis by insertion of tympanostomy tubes or adenoidectomy (with or without tonsillectomy) has been recommended for otitis-prone children.[288] Some authorities now prefer this modality to the chemoprophylactic approach because it is not associated with altered flora. However, surgical risks also need to be considered.

A novel approach to prevention of pneumococcal disease is the potential for use of oligosaccharides to prevent attachment of organisms, including *S. pneumoniae*, to the respiratory mucosa. Because colonization of the respiratory mucosa can result in local and systemic disease, as well as spread to other individuals, prevention of colonization is a reasonable approach to prevent disease. Human milk oligosaccharides can prevent attachment of *S. pneumoniae* and other organisms to the mucosa of the respiratory tract[587,603] and have been shown to interfere with the establishment and progression of experimental pneumococcal pneumonia in infant rats.[244] Natural oligosaccharides act as decoys in mucosa (also in saliva, tears, urine, sweat, and breast milk) and bind the carbohydrate-binding proteins of the microbial pathogens and thereby prevent mucosal attachment.[290] Despite this promise, a large-scale study examining the efficacy of such an oligosaccharide (3-sialyllacto-*N*-neoteratose) given intranasally as prophylaxis for AOM and nasopharyngeal carriage of bacteria failed to show any beneficial effect on either outcome.[544]

Other experiments conducted in Finland showed that xylitol (a five-carbon sugar alcohol used extensively as a sweetener in toothpaste, chewing gum, and various foods) inhibited the growth of streptococci, including *S. pneumoniae*, in vitro.[295,296,541,542] Whether this approach eventually will develop into a practical strategy to prevent respiratory tract infections caused by *S. pneumoniae* and other organisms is not clear. The concept of reducing nasopharyngeal colonization and thereby the number of episodes of bacterial respiratory tract infection through competitive inhibition is novel, and additional agents, altered doses, and alternative methods of administration should be studied.[418]

*References 92, 99, 113, 115, 322, 441, 520.

Immunoprophylaxis

Unconjugated Capsular Polysaccharide Vaccines

Efforts to prevent pneumococcal infection by providing specific immunity started more than 100 years ago, as described at the beginning of this chapter. It is well established that both passive immunization by administration of specific antibodies and active immunization with polysaccharides can provide protection against many pneumococcal infections.

Passively administered serotype-specific antibodies can protect animals and humans against diseases caused by pneumococci, with administration of antisera associated with improved clinical outcome, including reduced mortality, in humans.[84] Human immunoglobulins can prevent experimental pneumococcal bacteremia in mice[96,462] and otitis media in chinchillas.[489] The finding of low cord blood IgG antibodies, mainly of the IgG1 subset, is predictive of early-onset AOM in infancy.[48,330,468] In several clinical studies, bacterial polysaccharide immune globulin (BPIG) obtained by immunizing healthy adults with a 14-valent pneumococcal vaccine (in addition to group C meningococcal and Hib polysaccharide vaccines) decreased the prevalence of AOM[491] and invasive infection[491] in children. High-dose BPIG administered to infant rats resulted in high serum concentrations of serotype-specific IgG against serotype 3 with protection against nasopharyngeal carriage of this serotype.[347]

Immunization with pneumococcal polysaccharide antigens, mainly in adults, has been studied for a long time. First, hexavalent polysaccharide vaccines were introduced in the late 1940s, followed by 14-valent vaccines in the late 1970s and 23-valent vaccines in the early 1980s. The last, produced by several manufacturers, contains 25 μg of purified, nonconjugated polysaccharide antigens per dose for serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F. These 23 serotypes accounted for approximately 90 percent of the serotypes responsible for IPD in all age groups in both developed and developing countries.[216,217,450,502] The 23-valent polysaccharide vaccines are tolerated well by healthy children for primary[316,482] or repeated immunization.[67] However, the presence of preexisting antibodies was associated with an increased incidence of adverse events at the site of infection.[61,299,396]

In general, these bacterial polysaccharide-based vaccines are poorly immunogenic in infants and toddlers for important disease-causing serotypes, which is to be expected because bacterial capsular polysaccharides induce antibody production primarily by T-cell-independent mechanisms that still are not fully developed in this age group.[446] Polysaccharide-specific IgG concentrations are relatively high in very young infants because they are acquired transplacentally and consist mainly of IgG1. Nasopharyngeal colonization with various serotypes of *S. pneumoniae* results in the natural production of serotype-specific antibodies.[79,221,531,582] The immune response to pneumococcal polysaccharides is serotype dependent, and some serotypes commonly associated with disease are especially poor immunogens until children reach the age of approximately 5 years.[133] Serotypes 6A, 6B, 12, 19A, 19F, and 23F are examples of this phenomenon.* The IgG subclasses that are produced after exposure to polysaccharide antigens of other bacterial species are mainly IgG2 and IgG4.[327] A second dose of polysaccharide vaccine does not provide a booster effect and even may result in a reduced immune response when compared with the response after the first dose, which may suggest an antigenic tolerance effect.[61,394,553] Children with many underlying conditions that predispose to the development of pneumococcal infection often respond more poorly to pneumococcal polysaccharide vaccines than do otherwise normal children. Published studies of children with recurrent respiratory tract infections all have demonstrated this phenomenon.†

In addition to a relatively poor systemic immune response, the polysaccharide vaccines have been shown to induce only minimal mucosal immune responses. Serotype-specific antibodies to pneumococcal serotypes 6A, 14, 18C, and 23F were measured in the sera and middle ear effusions of 14 children who had received a 14-valent pneumococcal capsular polysaccharide vaccine and in control subjects.[298] Serotype-specific antibody concentrations in middle ear effusions correlated with serum concentrations and generally were higher in pneumococcal vaccine recipients than in control vaccine recipients. IgM class antibodies frequently were seen only in the serum samples, thus suggesting that antibodies diffuse into the middle ear space rather than being synthesized in situ in response to the vaccine. This finding refutes previous theories that local production of antibodies occurs in the middle ear after vaccination.[300,497,498]

Limited data exist regarding the efficacy of polysaccharide pneumococcal vaccines in preventing disease in infants and children. A large study conducted in Papua New Guinea on more than 7000 children showed a reduction in mortality rates from acute lower respiratory tract disease.[317,447,448] In this study the effect was dramatic: a 59 percent reduction in mortality rates in all children vaccinated (5 months to 5 years of age) and a 50 percent reduction in children vaccinated before they reached 2 years of age. However, the vaccine did not protect against nonfatal disease. In the United States, researchers have suggested that the polysaccharide vaccines would be 62 percent effective in preventing IPD caused by vaccine serotypes in children aged 2 to 5 years.[159]

The effectiveness of polysaccharide pneumococcal vaccines in preventing otitis media is not clear. Several studies have shown some reduction in the incidence of otitis media in vaccinated children.‡ No effect of the use of polysaccharide pneumococcal vaccine on pneumococcal nasopharyngeal carriage could be demonstrated.[111,114] These studies on T-cell-independent polysaccharide pneumococcal vaccines clearly show that their benefit in children is at best marginal and limited to the minority of serotypes that are immunogenic in this age group.

*References 114, 133, 298, 316, 319, 503, 531, 582.
†References 2, 26, 54, 145, 170, 180, 230, 304, 420, 442, 444, 464, 470, 471, 505, 553.
‡References 132, 238, 277, 346, 526.

TABLE 85-9 **Pneumococcal Conjugate Vaccines Licensed in at Least One Country or Being Tested in Clinical Trials**

Vaccine	Valency	Pneumococcal Polysaccharides	Carrier Protein	Current Status	Manufacturer
PnOMPC7	7-valent	4, 6B, 9V, 14, 18C, 19F, 23F	Meningococcal outer-membrane protein complex	Research formulation only	Merck Research Laboratories
PnCRM7	7-valent	4, 6B, 9V, 14, 18C, 19F, 23F	CRM_{197} protein	Licensed	Pfizer
PnCRM9	9-valent	1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F	CRM_{197} protein	Research formulation only	Pfizer
PHiD-CV	10-valent	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F		Licensed	GSK bio
PncT/D	11-valent	1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	A mixture of tetanus and diphtheria toxoids	Not in commercial development	Aventis Pasteur
Pn-PD	11-valent	1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F	*Haemophilus influenzae*-protein D	Prototype for PHiD-CV; not in commercial development	GSK bio
PnCRM13	13-valent	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F	CRM_{197} protein	Licensed	Pfizer
PCV 15	15-valent	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, 33F	CRM_{197} protein	In clinical trials	Merck

Conjugated Capsular Polysaccharide Vaccines

The conjugation of pneumococcal polysaccharides to protein alters the immune response by recruiting T cells that both amplify the B-cell response and create memory B cells, in contrast to the T-cell-independent nature of the immune response that occurs after the administration of bacterial polysaccharides (Fig. 85-16).* These polysaccharide-protein antigens elicit protective antibody in infants and prime the immune system for future boosting with reexposure to either alone or conjugated. Antibodies elicited by polysaccharide-protein conjugates demonstrate improved functional capacity (determined by avidity and opsonization assays), persist, and result in a booster response with reexposure.[146] The concept of conjugation technology, demonstrated to be highly successful with capsular polysaccharide from *H. influenzae* type b, was modified to permit development of vaccines with multiple polysaccharides while maintaining immunogenicity for each individual polysaccharide. Currently, vaccines with 7 to 13 different conjugated pneumococcal polysaccharide antigens are licensed and next-generation vaccines with even greater numbers of polysaccharides are in clinical trials.[305]

Table 85-9 shows pneumococcal conjugate vaccines that are licensed in at least one country, are being tested in phase III efficacy studies, or are in advanced phase II (safety and immunology) stages. The selection of serotypes for inclusion in the various formulations is based on serotype epidemiology discussed earlier in this chapter. These conjugate vaccines were developed primarily targeting serotypes responsible for IPD, including meningitis, bacteremic pneumonia, and empyema.

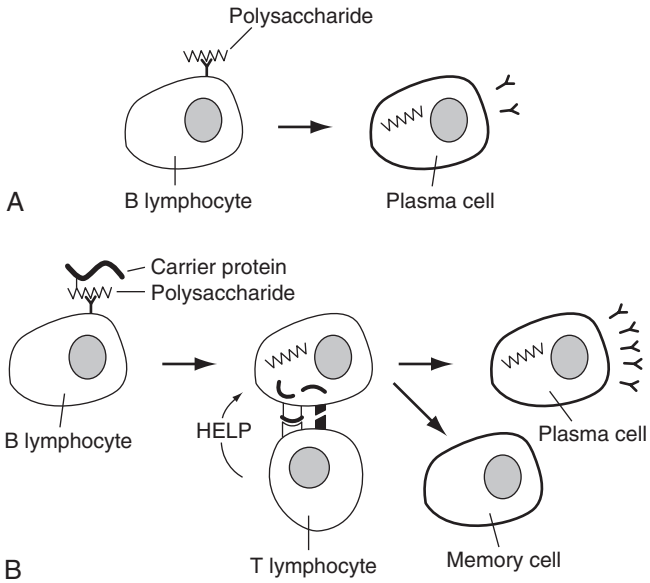

FIGURE 85-16 ■ T-cell-independent (**A**) and T-cell-dependent (**B**) antibody responses to polysaccharide or polysaccharide-protein conjugate antigens. (From Eskola J, Anttila M. Pneumococcal conjugate vaccines. *Pediatr Infect Dis J* 1999;**18**:543–51.)

The primary target population for polysaccharide conjugate vaccines is infants and toddlers because disease incidence is highest, global mortality substantial, and polysaccharide vaccines demonstrated to be poorly immunogenic and of limited value. Pneumococcal conjugate vaccines have been shown to be immunogenic, safe, and well tolerated, with vaccine reactions primarily being local, including pain, redness, and swelling at the injection site. The incidence of fever and irritability was

*References 35, 138, 279, 451, 490, 507.

somewhat higher than reported in studies with Hib conjugate vaccines or hepatitis B vaccines, perhaps because the pneumococcal vaccines represent 7 to 11 separate monovalent vaccines administered together as opposed to the monovalent Hib conjugate vaccine or hepatitis B vaccine. The 7-valent pneumococcal vaccine conjugated to CRM_{197} protein (PnCRM7) was found to be safe even when administered to low-birth-weight and preterm infants.[487]

PCV formulations were demonstrated to elicit high concentrations of IgG1 subclass antibodies, functional antibody in opsonophagocytic assays, and antibody with high avidity.[146,153] The immunogenicity of one formulation (PnCRM7) was demonstrated to be immunogenic in children with sickle-cell disease,[391,396,553] HIV infection,[6,286,287,385] solid-organ transplants,[329] allogeneic and autologous bone marrow transplants,[21,143] as well as in Alaska Natives and American Indians.[360] A 9-valent CRM (PnCRM9) pneumococcal vaccine also was immunogenic in children with sickle-cell disease.[189]

Clinical trials were used to evaluate the efficacy of the various pneumococcal conjugate vaccine formulations across a spectrum of end points and populations. The end points believed to be of most importance in evaluating these vaccines have been (1) prevention of IPD as defined by isolation of pneumococci from a normally sterile site (e.g., from blood, CSF, aspirates, and biopsy specimens); (2) prevention of mucosal infections such as otitis media, sinusitis, and nonbacteremic pneumonia; and (3) reduction of nasopharyngeal colonization, which in turn results in herd immunity due to reduced transmission of organisms.

Three efficacy studies of conjugate vaccines with the end point of a reduction in the number of invasive infections were completed by mid-2002, two with PnCRM7 and one with the PnCRM9 vaccine. The first study was a prospective double-blind study of 37,868 healthy infants in northern California to whom either the PnCRM7 vaccine or a control vaccine was administered at 2, 4, 6, and 12 to 15 months of age.[56] The vaccine was 97.4 percent efficacious (95% CI, 82.7% to 99.9%) against invasive disease caused by the seven serotypes included in the vaccine in fully vaccinated infants and 93.9 percent efficacious (95% CI, 79.6% to 98.5%) in partially vaccinated infants. No evidence of an increase in invasive disease caused by serotypes that were not included in the vaccine was detected, and thus the overall effect was a reduction in total IPD by 89.1 percent (95% CI, 73.7% to 95.8%). This trial led to U.S. licensure of the vaccine in February 2000, with subsequent licensure in many other countries. Additional analysis has shown that the vaccine was as effective in the subset of low-birth-weight and premature infants as it was in the full study cohort.[487] Secondary analyses demonstrated reductions in all-cause pneumonia and AOM.

A second large-scale efficacy study with the PnCRM7 vaccine was conducted among American Navajo and White Mountain Apache children in the United States using a cluster randomization strategy in which communities rather than individuals are randomized. This population has rates of IPD that are approximately five times those of the general U.S. population.[395] In this double-blind, community-randomized study, infants and young children aged 2 months to 2 years received the PnCRM7 vaccine or a control vaccine. A total of 8292 infants from 43 communities were enrolled; of these, 8091 lived in 38 communities that were randomized to the pneumococcal or the control vaccine. During the study period, two cases of IPD caused by the serotypes included in the vaccine occurred in the vaccine group versus eight in the control group. After controlling for community randomization, the primary efficacy of the vaccine was 76.8 percent (95% CI, 9.4% to 95.1%), and the intent-to-treat efficacy was 86.4 percent (95% CI, 11% to 96.1%).

A third efficacy study was conducted with the PnCRM9 vaccine among black infants in South Africa. In this double-blind, randomized, placebo-controlled study, PnCRM9 was administered to children at the ages of 6, 10, and 14 weeks according to the World Health Organization's Expanded Programme on Immunization. The infants were monitored until they reached 2 years of age, and the study was completed by the end of 2001. The intent-to-treat analysis showed a reduction of 82.5 percent (95% CI, 39.0% to 96.7%) in the number of invasive infections caused by the serotypes included in the vaccine for children not infected with HIV and a reduction of 65.4 percent (95% CI, 23.8% to 85.7%) in antiretroviral-naïve children infected with HIV.[294]

Once this vaccine was licensed in the United States (February 2000), universal immunization for children younger than 2 years of age and selected immunization for high-risk children was implemented. The Committee on Infectious Diseases of the American Academy of Pediatrics recommended routine administration as a four-dose series for infants at ages 2, 4, 6, and 12 to 15 months (known as the 3+1 schedule); catch-up immunization was recommended for all children up to 23 months of age. The PCV7 vaccine could be given along with other age-appropriate childhood immunizations in a separate syringe and at a separate injection site. Infants of very low birth-weight could be immunized when they reached a chronologic age of 6 to 8 weeks, regardless of their calculated gestational age. The PCV7 vaccine also was also recommended for all children younger than 60 months of age at high risk for acquiring IPD, including patients with sickle-cell disease, asplenia, HIV infection/acquired immunodeficiency syndrome, diabetes, cancer, liver disorders, lung diseases, and cardiac diseases. For some high-risk children, administration of PPV23 was recommended for supplemental protection. The only contraindication to receiving the vaccine was a serious allergic reaction to a previous dose of this vaccine or one of its components. Children with minor illnesses such as colds could be vaccinated.

The incidence of IPD decreased sharply in infants and toddlers after the widespread use of PCV7 in U.S. children.[55,239,577] Effectiveness studies demonstrated a rapid decline in IPD owing to vaccine serotypes in children younger than 5 years of age (Fig. 85-17) within 2 years of vaccine introduction, with a further gradual decline that persisted through 2010, when a second-generation pneumococcal conjugate vaccine was introduced (PCV13).[424,593] Rates of bacteremia without a focus,

TABLE 85-10 **Estimated PCV7 Reductions in Mortality During Hospitalizations for IPD, Pneumococcal Pneumonia, and All-Cause Pneumonia: 1999/2000 through 2004/2005**

Age Group	Estimated IPD Reduction	95% CI	Estimated Pneumococcal Pneumonia (IDC9 481) Reduction	95% CI	Estimated All-Cause Pneumonia Reduction	95% CI
<2	212	81-275	34	–66-67	548	425-646
2-4	46	12-62	26	–3-38	191	136-235
5-17	103	65-130	13	–14-29	350	252-431
18-39	810	674-922	1,088	888-1,245	2,495	2,220-2,754
40-64	1,045	709-1,349	2,304	1,990-2,596	8,513	7,674-9,312
≥65	4,007	3,246-4,693	10,895	9,772-11,934	71,556	61,644-81,013
Total*	6,222	4,788-7,432	14,360	12,568-15,908	83,653	72,351-94,391

*Number of deaths prevented.
CI, confidence interval.
Modified from Simonsen L,Taylor RJ, Young-Xu Y, et al. Impact of pneumococcal conjugate vaccination of infants on pneumonia and influenza hospitalization and mortality in all age groups in the United States. *MBio* 2011;**2**:e00309-10.

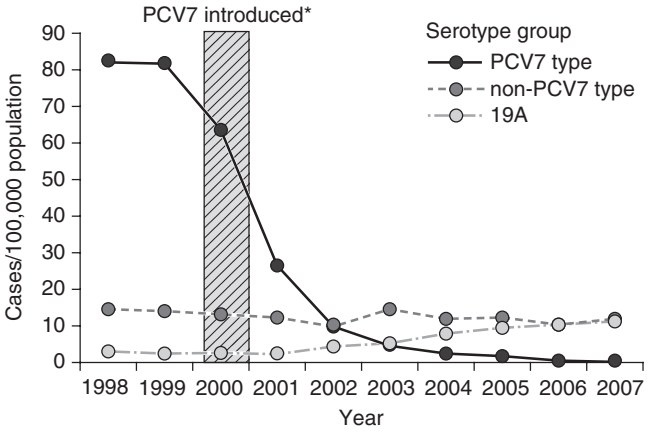

FIGURE 85-17 ■ Changes in IPD in U.S. children younger than 5 years of age by serotype groupings. PCV7 serotypes include 4, 6B, 9V, 14, 18C, 19F, and 23F. (From Pilishvili T, Lexau C, Farley MM, et al; Active Bacterial Core Surveillance/Emerging Infections Program. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.)

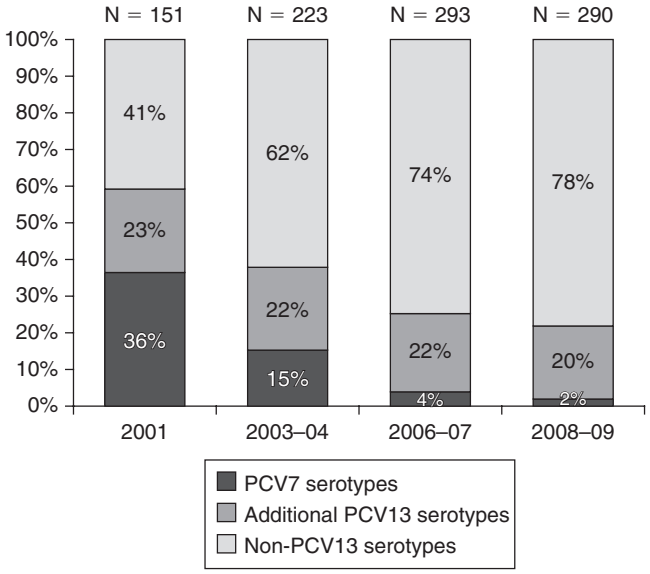

FIGURE 85-18 ■ Percent of pneumococcal isolates within each sampling period by vaccine serotype grouping. PCV7 serotypes are 4, 6B, 9V, 14, 19C, 19F, and 23F. Additional 13 valent serotypes are 1, 3, 5, 6A, 7F, and 19A. Non-PCV13 serotypes are all other serotypes. (From Wroe PC, Lee GM, Finkelstein JA, et al. Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. *Pediatr Infect Dis J* 2012; **31**:249–54.

meningitis, and bacteremic pneumonia all declined. By 2006 to 2007, PCV7 serotypes accounted for only 2 percent of IPD cases in children compared with 83 percent prior to 2000. By 2009, nasopharyngeal PCV7 serotypes comprised only 2 percent of pneumococcal isolates carried in the nasopharynx of children younger than 6 years of age (Fig. 85-18).[589] Despite the decline in carriage of PCV7 serotypes, overall pneumococcal carriage did not change as a result of increases in carriage of non-vaccine serotypes, particularly serotypes 19A, 15A, 15B, 15C, 6C, 23A, 23B, and 35B. These changes in serotype distribution resulted in reduced transmission of PCV7 serotypes and a decline in IPD due to vaccine serotypes in all age groups, including infants younger than 90 days of age and too young to be protected directly, as well as adults.[427] In fact, Simonsen noted that 90 percent of the reduction in pneumococcal disease mortality occurred though herd immunity among adults 18 years and older (Table 85-10).[494] Consistent with the observed differences in virulence between pneumococcal serotypes as discussed earlier, the current belief is the increase in "replacement" serotypes is primarily associated with serotypes with a lower capacity to produce disease after colonization. However, some of the serotypes, specifically 19A and 7F, are associated with modest increases in invasive disease both in children and adults. By 2007, the incidence of IPD due to serotype 19A had increased from 2.6 cases per 100,000 to 11.1 per 100,000 in children younger than 5 years of age and represented nearly 40 percent of all IPD in this age group (Fig. 85-19).[593]

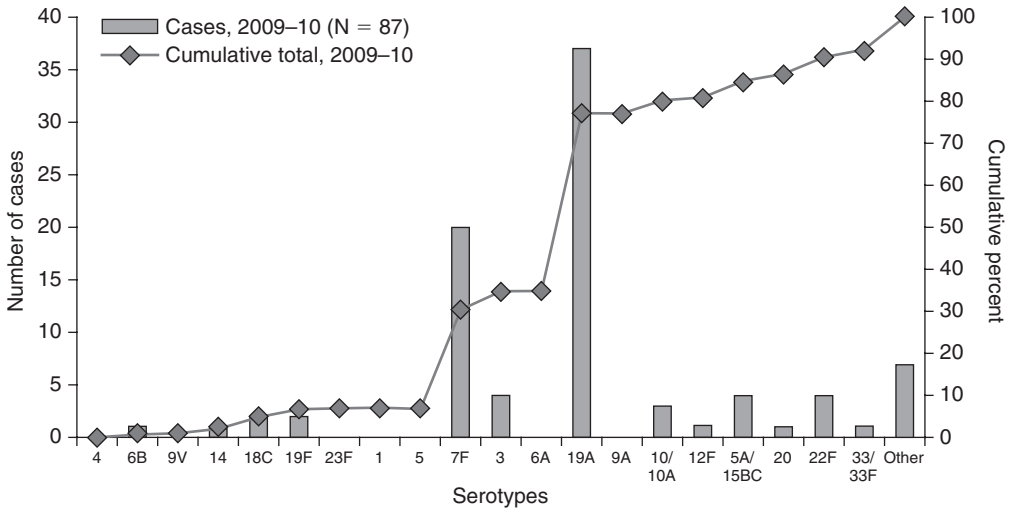

FIGURE 85-19 ■ Serotypes causing invasive pneumococcal disease in children younger than 18 years of age in Massachusetts between October 31, 2009, and September 1, 2010. (From Yildirim I, Stevenson A, Hsu KK, et al. Evolving picture of invasive pneumococcal disease in Massachusetts children: a comparison of disease in 2007-2009 with earlier periods. *Pediatr Infect Dis J* 2012;**31**:1016-21.)

PCV13 was introduced in 2010 in the United States for healthy children through age 5 years, children with chronic illness through age 6, and children 6 through 18 with sickle cell disease, HIV infection, and other high-risk conditions. Proportionally, PCV13 serotypes comprised nearly 75 percent of all IPD in children younger than 18 years of age, with 19A disease representing nearly 40 percent (see Fig. 85-19). The most recent reports from multiple U.S. surveillance programs[275,369,592] demonstrate further declines in IPD in children younger than 5 years of age and a decline in disease due to serotype 19A. PCV13 was introduced in the United Kingdom early in 2010, and a decline in IPD has been reported due to PCV13 unique serotypes.[362]

Global experience with PCV7 demonstrated similar observations with dramatic declines in IPD due to vaccine serotypes, initially in immunized children and then subsequently in unimmunized adults, despite the fact that alternative vaccine schedules (to 3+1) were employed in many countries. The United Kingdom and parts of Canada introduced a schedule of two primary doses with a booster dose at 12 months of age, whereas Australia introduced a schedule of three primary doses without a booster (a 3+0 schedule).[125,363,265] These schedules proved to be effective in both reducing IPD in children as well as creating indirect protection in unimmunized populations despite evidence that a primary two-dose series results in less serum antibody for serotypes 6B and 23F.[278,331]

PCV10 was introduced, adding serotypes 1, 5, and 7F to the seven serotypes in PCV7. Jokinen reported on the early effectiveness of PCV10 against IPD in Finnish children. A dramatic decline in vaccine serotype IPD among children 6 to 11 months of age was observed.[269]

Although invasive infections are the most dramatic part of the pneumococcal disease spectrum, respiratory tract infections such as otitis media are far more frequent.[325] Efficacy studies have shown protection against AOM due to vaccine serotypes in children immunized with pneumococcal conjugate vaccines. In one study, PnCRM7 vaccine reduced the number of clinically diagnosed otitis media episodes by 7 percent (95% CI, 4.1% to 7.9%).[160] The effectiveness of the PnCRM7 vaccine against frequent otitis media was 9.3 percent (95% CI, 3.0% to 15.1%) when three episodes in 6 months or four in 12 months were counted and 22.8 percent (95% CI, 6.7% to 36.2%) when a frequency of five episodes in 6 months or six in 12 months was considered. Children who received the vaccine were 20.1 percent (95% CI, 1.5% to 35.2%) less likely to require placement of a pressure-equalizing tube than control subjects. Otitis media was not a primary outcome measure in this study, and the efficacy of the vaccine against otitis media caused by vaccine serotypes could therefore only be determined on children with spontaneously draining ears; a 66 percent calculated efficacy against disease caused by vaccine serotypes was found in this group.

A second study, conducted in Finland, looked at the efficacy of PnCRM7 vaccine against pneumococcal AOM caused by vaccine serotypes as well as overall episodes.[147] The clinical diagnosis of AOM was based on predefined criteria, and bacteriologic diagnosis was based on culture of middle ear fluid obtained by myringotomy. The children were monitored through 24 months of age. The efficacy of the vaccine in reducing AOM caused by vaccine serotypes was 57 percent (95% CI, 46% to 67%). The point estimate for efficacy of the vaccine in reducing all culture-confirmed pneumococcal AOM was 34 percent. The vaccine was not effective against nonvaccine pneumococcal serotypes, except against serotype 6A. The vaccine also was ineffective against otitis media caused by *H. influenzae* and *Moraxella catarrhalis*, and the overall high protection provided against the serotypes included in the vaccine was offset partially by an increase in the number of episodes of otitis media caused by other serotypes of pneumococci and other pathogens. The overall reduction in the number of episodes of AOM of any cause in the pneumococcal vaccine group was 6 percent.

Although this difference did not reach statistical significance, it was similar to the 7 percent reduction in AOM observed in the California study.[56] In addition, similar to the California Study by the time children reached the age of 4 to 5 years, tympanostomy tube placement was reduced 39 percent compared with control subjects.[408]

A third study was conducted by the same Finnish group, in parallel with the study just described, with a different 7-valent vaccine (serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F conjugated to the meningococcal outer-membrane protein complex [OMPC]-PnOMPC7).[282] This vaccine was administered in four doses at 2, 4, 6, and 12 months of age. At 12 months of age, approximately 22 percent of the PnOMPC7 conjugate vaccine recipients received a nonconjugate 23-valent polysaccharide vaccine as a booster while the others received a fourth dose of PnOMPC7. The efficacy of the vaccine in reducing the number of cases of otitis media caused by the serotypes included in the vaccine was 56 percent, with 25 percent for reduction of any pneumococcal AOM. However, there was no overall reduction in the number of episodes of otitis media, with nonvaccine serotype pneumococci and other pathogens producing replacement disease.

Another study, using an 11-valent PCV (the prototype for PCV10), conjugated to outer membrane protein D of nontypeable *H. influenzae*, demonstrated a similar magnitude of protection against vaccine serotype AOM despite a markedly different study design.[433] Because the 11-valent PCV was conjugated to protein D from nontypeable *H. influenzae*, a 35 percent reduction in AOM episodes due to this pathogen was also reported, with an overall reduction in all episodes of AOM of approximately 33 percent. Further studies confirming the efficacy of PCV10, where some serotypes are conjugated to protein D, are pending completion.

Since licensure and universal immunization of children younger than age 2 with PCV7, several studies have shown that the reduction in AOM exceeds that reported in clinical trials.[202,426,564,602] Several hypotheses have been proposed to explain the observation. First, with widespread immunization and dramatic reduction in nasopharyngeal carriage of vaccine serotypes, effectiveness against vaccine serotypes will increase to nearly 100 percent. Second, prevention of early episodes of pneumococcal AOM will change the natural history of AOM in children, reducing subsequent episodes.[163] Pneumococcal AOM is now primarily caused by nonvaccine serotypes.[511,512]

Reports of AOM and mastoiditis unresponsive to antimicrobial therapy requiring drainage and systemic antimicrobial therapy due to multidrug-resistant serotype 19A have increased.[403,422] Because PCV13 includes the capsular polysaccharide from 19A, it is expected that such cases will diminish with decreasing prevalence of 19A nasopharyngeal colonization.

Because most cases of pneumococcal pneumonia are nonbacteremic, the role of *S. pneumoniae* can best be demonstrated indirectly in vaccine probe studies by determining the proportion of disease reduction after vaccine implementation. In the northern California PnCRM7 vaccine study, 3711 clinically diagnosed episodes of pneumonia were identified by the age of 3.5 years.[57] The efficacy of the vaccine in reducing the incidence of clinical pneumonia (intent-to-treat analysis) was 6.0 percent. For children in whom a chest radiograph was obtained, an 8.9 percent reduction was observed; for those with a positive chest radiograph (defined as parenchymal infiltrates, consolidation, or effusion), a 22.7 percent (95% CI, 8.7% to 34.5%) decline in disease occurred. Because the reduction in pneumonia was a secondary end point, the precision of the decline has some limitations. However, the findings suggest that more cases of clinically and radiologically proven pneumonia in children are caused by *S. pneumoniae* than previously suspected, with the reduction in disease extending beyond the "classic" lobar pneumonia pattern associated with *S. pneumoniae* to cases with non-lobar infiltrates. Comparable observations from South Africa documented a 22.1 percent reduction in children not infected with HIV immunized with PCV9.[294]

Additional supportive evidence that *S. pneumoniae* has an important role in a broader spectrum of respiratory tract infections can be derived from a study in southern Israel involving toddlers aged 12 to 35 months who attended daycare centers.[118] In this study a 15 percent reduction in non–otitis media upper respiratory tract infection was reported. Episodes of lower respiratory tract disease, including bronchiolitis, cough, and pneumonia were reduced. A reduction of 10 percent in the number of days that antibiotics were given for upper respiratory tract infections was achieved, as was a reduction of 47 percent for lower respiratory tract problems in the pneumococcal vaccine group.

Person-to-person spread is the major route of transmission for *S. pneumoniae*. The nonconjugate pneumococcal polysaccharide vaccines do not have a significant effect on carriage of *S. pneumoniae* in children and adults.[114,337] In contrast, the conjugate vaccines have a significant effect on carriage that expands its public health value. Table 85-11 shows selected studies conducted and published documenting the effect of pneumococcal vaccines on carriage of *S. pneumoniae*. Despite variations in the nature of the conjugate vaccines, populations, and the ages at which the vaccines were administered, a significant reduction in carriage of the serotypes included in the vaccine was observed in all studies. In most studies, serotype replacement was observed as the overall pneumococcal colonization remained relatively constant despite the decline in prevalence of vaccine serotypes.

A change in nasopharyngeal colonization is important because it reduces exposure of unimmunized children and adults to these potential pathogens. This reduction has been demonstrated in studies of toddlers attending daycare centers who were vaccinated with a PnCRM9 vaccine or a control vaccine.[110] In this study, the younger siblings of immunized and control children, who stayed home, were monitored. A marked reduction in the incidence of vaccine-type pneumococcal carriage was seen in the young siblings of those who were vaccinated with the PnCRM9 vaccine when compared with siblings of the control children. In a second study, Navajo and White Mountain Apache children younger than 2 years old were randomized according to their community of residence

TABLE 85-11 **Studies on the Effect of Conjugate Pneumococcal Vaccine on Carriage of *Streptococcus pneumoniae* and Antibiotic-Resistant *S. pneumoniae***

Author	Conjugate Vaccine (Valence)	Site	Age (mo) at Vaccination	Reduction in Serotypes Included in the Vaccine	Reduction in Resistant Pneumococci	Increase in Nonvaccine Serotypes
Dagan[115]	PnOMPC7	Israel	12-18	Yes	Yes	No
Dagan[116]	PnT, PnD (4-valent)	Israel	2, 4, 6	Yes	Yes	±
Obaro[399]	PnCRM5	The Gambia	2, 3, 4	Yes	ND	Yes
Kristinsson[302]	PnT, PnD (8-valent)	Iceland	3, 4, 6	Yes	ND	Yes
Mbelle[352]	PnCRM9	South Africa	1.5, 2.5, 3.5	Yes	Yes	Yes
Edwards[139]	PnCRM9	USA	2, 4, 6, 12	Yes	ND	Yes
Dagan[119]	PnT/D (11-valent)	Israel	2, 4, 6, 12	Yes	Yes	No
Dagan[108,110]	PnCRM9	Israel	12-35	Yes	Yes	Yes
O'Brien[397]	PnCRM7	USA (Native American)	2, 4, 6, 12-15	Yes	ND	Yes
Kilpi[283]	PnCRM7	Finland	2, 4, 6, 12	Yes	ND	ND

PnOMPC, 7-valent pneumococcal vaccine conjugated to the outer-membrane complex of *Neisseria meningitidis* B; PnT, pneumococcal vaccine conjugated to tetanus toxoid; PnD, pneumococcal vaccine conjugated to diphtheria toxoid; PnT/D, pneumococcal vaccine conjugated to a mixture of tetanus and diphtheria toxoid; PnCRM, pneumococcal vaccine conjugated to CRM_{197} protein (PnCRM5, 5-valent; PnCRM7, 7-valent; PnCRM9, 9-valent).

to receive either PnCRM7 or meningococcus C conjugate vaccine.[361]

A reduction in carriage of vaccine-associated serotypes was found both in infants who lived with a PnCRM7-vaccinated sibling and in those who did not have direct contact with a PnCRM7-vaccinated child but lived in a vaccine community, demonstrating the indirect protective effect of pneumococcal vaccination on those in the household. Because most antibiotic-resistant *S. pneumoniae* strains belong to only a few serotypes that are included in the conjugate pneumococcal vaccines or are related to these serotypes (see the sections on epidemiology and microbiology), conjugate vaccines are expected to reduce disease, carriage, and spread of antibiotic-resistant *S. pneumoniae* and may have an impact on the use of antibiotics. Indeed, in all vaccine studies that evaluated the effect of conjugate vaccines on nasopharyngeal carriage of antibiotic-resistant *S. pneumoniae*, a reduction in carriage of such strains was observed (see Table 85-11).[110,114,116,119,352]

Although invasive infections are the most dramatic part of the pneumococcal disease spectrum, respiratory tract infections such as otitis media and sinusitis occur far more frequently.[325] In a study investigating current estimates of pneumococcal disease burden, clinical outcome, and vaccine efficacy, researchers estimated that for each annual U.S. birth cohort, routine use of a 7-valent vaccine could prevent 12,000 cases of pneumococcal bacteremia and meningitis, 53,000 cases of pneumococcal pneumonia, and more than 1 million clinical cases of otitis media per year.[325] Pneumonia has a greater impact globally than does otitis media or even invasive infections because of its high mortality rate in children in the developing world.[51,204,320] Thus, the protection conferred by the conjugate pneumococcal vaccines with regard to reduction in the number of episodes of otitis media, pneumonia, and other mucosal infections could have an even greater public health impact on the burden of disease than on prevention of invasive infections.

Pneumococcal disease remains a burden on children throughout the globe. In young infants and children, the elderly, and those with serious comorbidities the disease can be deadly. Progress in prevention, both in the development of effective vaccines and in implementation of vaccine delivery programs in developing countries, has exceeded expectations, resulting in decreased incidence of and mortality from IPD, including meningitis and both bacteremic and nonbacteremic pneumonia.[375] The virtual elimination of nasopharyngeal colonization with vaccine serotypes enhances their public health value by reducing transmission of vaccine serotypes from children to adults, thereby reducing overall rates of pneumococcal disease. Vaccine failure is uncommon; Park and colleagues reported only 155 vaccine serotype infections identified by U.S. surveillance systems, with only 25 in fully vaccinated children, suggesting the combination of direct and indirect effects protects infants as well as children with chronic illness from vaccine serotype disease.[410] These findings demonstrate the major reduction in the burden of vaccine serotype pneumococcal disease in children associated with use of conjugate vaccines.

Nevertheless, potential challenges to the successful scenario observed to date are found in the distribution of disease owing to nonvaccine serotypes. The U.S. surveillance program reported a doubling of the proportion of children with comorbidities and IPD, which increased from 3 percent in 1998/1999 to 7 percent in 2007. In Massachusetts, a report noted that 16 percent of children with IPD have comorbidities that proportionally increased with age. Among the 155 cases of vaccine failure referred to previously, a disproportionate proportion were reported to occur in children with comorbidities (37%).[410] Ladhani and colleagues also reported that comorbidities were present in 17.7 percent of U.K. children with IPD

caused by PCV7 serotypes.[306] Case-fatality rates also reflected great vulnerability of children with comorbidities, with mortality from pneumococcal disease (4.4% overall) higher for meningitis (11.0%) and for children with comorbidities (9.1%).

The most commonly reported comorbidities associated with pneumococcal disease include malignancies and immunocompromised conditions.[240] Recent data demonstrate that children with asthma, diabetes, and neurologic disorders such as seizures and muscular dystrophies have increased relative risk for IPD compared with age-matched healthy control subjects.[575] Although the risk is modest with a single comorbidity, it escalates in children with two or more. Certainly the high incidence of both HIV infection and sickle-cell disease in developing countries in Africa is likely to result in these children being at high risk for nonvaccine serotype disease. Children with malnutrition or tuberculosis or exposed to second-hand smoke may also have higher risks for disease owing to nonvaccine serotypes to erode vaccine success that requires ongoing surveillance and analysis.

CONCLUSION

Pneumococcal disease, most notable invasive disease and pneumonia, has decreased considerably, specifically in children in industrialized countries over the past decade in association with the introduction of protein-conjugated polysaccharide vaccines as part of national childhood vaccination schedules. The decline in disease has been larger in unimmunized adults than in immunized children as a result of herd protection created by the reduction in carriage and transmission of vaccine serotypes. In addition, decreases in antimicrobial resistant vaccine serotypes has resulted.

However, in developing countries, acute respiratory tract infections in general and pneumonia in particular remain the leading causes of morbidity and mortality.[357] The mortality rate from respiratory tract infections in developing countries is estimated to be 10- to 30-fold higher than in developed countries.[377,580] Otitis media, although often not perceived as a severe problem in developing countries, may progress to chronic suppurative otitis media and acquired hearing loss in children.[7] Global Alliance for Vaccines and Immunization programs has been critical in reducing the time line for introduction of pneumococcal conjugate vaccine into developing countries.

These advances have been limited by replacement of nasopharyngeal colonization by vaccine serotypes with nonvaccine and frequently antimicrobial-resistant serotypes, resulting in some erosion of vaccine effectiveness as a result of disease with nonvaccine serotypes in IPD and pneumonia as well as other respiratory pathogens in otitis media. These limitations, in turn, have been countered by the introduction of "second generation" vaccines containing additional serotypes. However, because there are currently 93 recognized serotypes, with evolution of additional serotypes in this genetically promiscuous organism likely to continue, development of alternative, broadly conserved vaccine targets would be very beneficial in the long term.[366]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Abdeldaim GM, Stralin K, Korsgaard J, et al. Multiplex quantitative PCR for detection of lower respiratory tract infection and meningitis caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. *BMC Microbiol* 2010;**10**:310.
5. Abdullahi O, Karani A, Tigoi CC, et al. The prevalence and risk factors for pneumococcal colonization of the nasopharynx among children in Kilifi District, Kenya. *PLoS One* 2012;**7**:e30787.
8. Adamkiewicz TV, Silk BJ, Howgate J, et al. Effectiveness of the 7-valent pneumococcal conjugate vaccine in children with sickle cell disease in the first decade of life. *Pediatrics* 2008;**121**:562–9.
11. Alicino C, Iudici R, Alberti M, et al. The dangerous synergism between influenza and *Streptococcus pneumoniae* and innovative perspectives of vaccine prevention. *J Prev Med Hyg* 2011;**52**:102–6.
13. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 571–82.
14. American Academy of Pediatrics Subcommittee on Management of Acute Otitis. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.
39. Baggett HC, Rhodes J, Dejsirilert S, et al. Pneumococcal antigen testing of blood culture broth to enhance the detection of *Streptococcus pneumoniae* bacteremia. *Eur J Clin Microbiol Infect Dis* 2012;**31**:753–6.
69. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.
85. Casado Flores J, Nieto Moro M, Berron S, et al. Usefulness of pneumococcal antigen detection in pleural effusion for the rapid diagnosis of infection by *Streptococcus pneumoniae*. *Eur J Pediatr* 2010;**169**:581–4.
86. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2010;**29**:304–9.
87. Casey JR, Block SL, Hedrick J, et al. Comparison of amoxicillin/clavulanic acid high dose with cefdinir in the treatment of acute otitis media. *Drugs* 2012;**72**:1991–7.
88. Ceyhan M, Yildirim I, Sheppard CL, et al. Pneumococcal serotypes causing pediatric meningitis in Turkey: application of a new technology in the investigation of cases negative by conventional culture. *Eur J Clin Microbiol Infect Dis* 2010;**29**:289–93.
95. Chow AW, Benninger MS, Brook I, et al. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults. *Clin Infect Dis* 2012;**54**:e72–112.
98. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Publication M100-S22. Wayne, Pa: CLSI; 2012.
125. Deceuninck G, De Wals P, Boulianne N, et al. Effectiveness of pneumococcal conjugate vaccine using a 2+1 infant schedule in Quebec, Canada. *Pediatr Infect Dis J* 2010;**29**:546–9.
127. Diavatopoulos DA, Short KR, Price JT, et al. Influenza A virus facilitates *Streptococcus pneumoniae* transmission and disease. *FASEB J* 2010;**24**:1789–98.
136. Dunne EM, Ong EK, Moser RJ, et al. Multilocus sequence typing of *Streptococcus pneumoniae* by use of mass spectrometry. *J Clin Microbiol* 2011;**49**:3756–60.
142. Elliott SR, Beeson JG. Estimating the burden of global mortality in children aged <5 years by pathogen-specific causes. *Clin Infect Dis* 2008;**46**:1794–5.
156. Ferreira DM, Oliveira ML, Moreno AT, et al. Protection against nasal colonization with *Streptococcus pneumoniae* by parenteral immunization with a DNA vaccine encoding PspA (pneumococcal surface protein A). *Microb Pathog* 2010;**48**:205–13.
163. Fletcher MA, Fritzell B. Pneumococcal conjugate vaccines and otitis media: an appraisal of the clinical trials. *Int J Otolaryngol* 2012;**2012**:312935.

177. Gessner BD, Mueller JE, Yaro S. African meningitis belt pneumococcal disease epidemiology indicates a need for an effective serotype 1 containing vaccine, including for older children and adults. *BMC Infect Dis* 2010;**10**:22.
208. Hakenbeck R, Bruckner R, Denapaite D, et al. Molecular mechanisms of beta-lactam resistance in *Streptococcus pneumoniae*. *Future Microbiol* 2012;**7**:395–410.
210. Hameed N, Tunkel AR. Treatment of drug-resistant pneumococcal meningitis. *Curr Infect Dis Rep* 2010;**12**:274–81.
239. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009;**360**:244–56.
240. Hsu KK, Shea KM, Stevenson AE, et al; Members of the Massachusetts Department of Public Health. Underlying conditions in children with invasive pneumococcal disease in the conjugate vaccine era. *Pediatr Infect Dis J* 2011;**30**:251–3.
253. Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae:* trends and management. *Expert Rev Anti Infect Ther* 2008;**6**: 619–35.
255. Jacobs MR, Good CE, Windau AR, et al. Activity of ceftaroline against recent emerging serotypes of *Streptococcus pneumoniae* in the United States. *Antimicrob Agents Chemother* 2010;**54**: 2716–19.
263. Jinno S, Jacobs MR. Pneumonia due to drug-resistant *Streptococcus pneumoniae*. *Curr Infect Dis Rep* 2012;**14**:292–9.
265. Johnson DR, D'Onise K, Holland RA, et al. Pneumococcal disease in South Australia: vaccine success but no time for complacency. *Vaccine* 2012;**30**:2206–11.
266. Johnson HL, Deloria-Knoll M, Levine OS, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS Med* 2010;**7**(10).
269. Jokinen J, Nuorti P, Palmu A, et al. Monitoring the effectiveness of the 10-valent pneumococcal conjugate vaccine (PCV10) in the Finnish national vaccination programme. Presented at ISPPD 8. Iguacu, Brazil, March 11-15, 2012.
272. Kadioglu A, Weiser JN, Paton JC, et al. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. *Nat Rev Microbiol* 2008;**6**:288–301.
275. Kaplan SL, Barson WJ, Lin PL. Early trends for invasive pneumococcal infections in children after the introduction of the 13-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis* 2013;**32**:203–7.
284. Kim DW, Kilgore PE, Kim EJ, et al. The enhanced pneumococcal LAMP assay: a clinical tool for the diagnosis of meningitis due to *Streptococcus pneumoniae*. *PLoS One* 2012;**7**:e42954.
305. Musey L. Safety, tolerability, and immunogenicity of 15-valent pneumococcal conjugate vaccine (PCV15) in healthy adults. Poster 658. Presented at IDSA 2013, San Diego, Calif.
306. Ladhani SN, Slack MP, Andrews NJ, et al. Invasive pneumococcal disease after routine pneumococcal conjugate vaccination in children: England and Wales. *Emerg Infect Dis* 2013;**19**:61–8.
307. Lammers AJ, de Porto AP, Florquin S, et al. Enhanced vulnerability for *Streptococcus pneumoniae* sepsis during asplenia is determined by the bacterial capsule. *Immunobiology* 2011;**216**: 863–70.
315. Lee CS, Chen MJ, Chiou YH, et al. Invasive pneumococcal pneumonia is the major cause of paediatric haemolytic-uraemic syndrome in Taiwan. *Nephrology (Carlton)* 2012;**17**:48–52.
335. Loughlin AM, Hsu K, Silverio A, et al. *Impact of PCV13 vaccine on pneumococcal carriage in Massachusetts children.* Poster 176, ISPPD8, Iguazo Falls, Brazil, 2012.
338. Madhi S, Pelton SI. Epidemiology, diagnosis and treatment of serious pneumococcal infections in children from pneumococcal vaccines. In: Siber GR, Flugman KP, Makela PH, editors. *The impact of conjugate vaccine*. Washington, DC: ASM Press; 2008. p. 95–108.
350. Marchese A, Esposito S, Coppo E, et al. Detection of *Streptococcus pneumoniae* and identification of pneumococcal serotypes by real-time polymerase chain reaction using blood samples from Italian children </= 5 years of age with community-acquired pneumonia. *Microb Drug Resist* 2011;**17**:419–24.
351. Margolis E, Yates A, Levin BR. The ecology of nasal colonization of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus:* the role of competition and interactions with host's immune response. *BMC Microbiol* 2010;**10**:59.
361. Millar EV, Watt JP, Bronsdon MA, et al. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal colonization among unvaccinated household members. *Clin Infect Dis* 2008; **47**:989–96.
362. Miller E, Andrews NJ, Waight PA, et al. Effectiveness of the new serotypes in the 13-valent pneumococcal conjugate vaccine. *Vaccine* 2011;**29**:9127–31.
363. Miller E, Andrews NJ, Waight PA, et al. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. *Lancet Infect Dis* 2011;**11**:760–8.
364. Mitchell T. Streptococcus pneumoniae: *virulence factors and variation [Abstract 519]*. San Francisco: ICAAC; 2012.
366. Moffitt KL, Malley R. Next generation pneumococcal vaccines. *Curr Opin Immunol* 2011;**23**:407–13.
369. Moore M, Link-Gelles, Farley MM, et al. Impact of 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease, U.S., 2010-11. Abstract 1219. Presented at IDWEEK 2012, San Diego, Calif., 2012.
374. Moscoso M, Domenech M, Garcia E. Vancomycin tolerance in clinical and laboratory *Streptococcus pneumoniae* isolates depends on reduced enzyme activity of the major LytA autolysin or cooperation between CiaH histidine kinase and capsular polysaccharide. *Mol Microbiol* 2010. [Epub ahead of print].
375. Mueller JE. Conjugate vaccine introduction in the African meningitis belt: meeting surveillance objectives. *Trop Med Int Health* 2013;**18**:58–64.
392. Nunes MC, von Gottberg A, de Gouveia L, et al. The impact of antiretroviral treatment on the burden of invasive pneumococcal disease in South African children: a time series analysis. *AIDS* 2011;**25**:453–62.
401. Okitsu N, Yano H, Ohshima H, et al. Binax NOW® *Streptococcus pneumoniae* test of middle ear fluid for detecting causative pathogens in children with acute otitis media. *J Microbiol Methods* 2011;**84**:341–2.
403. Ongkasuwan J, Valdez TA, Hulten KG, et al. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics* 2008;**122**:34–9.
410. Park SY, Van Beneden CA, Pilishvili T, et al; Active Bacterial Core surveillance team. Invasive pneumococcal infections among vaccinated children in the United States. *J Pediatr* 2010;**156**:478–83 e2.
412. Paterson NG, Baker EN. Structure of the full-length major pilin from *Streptococcus pneumoniae:* implications for isopeptide bond formation in gram-positive bacterial pili. *PLoS One* 2011;**6**:e22095.
424. Pilishvili T, Lexau C, Farley MM, et al; Active Bacterial Core Surveillance/Emerging Infections Program. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.
428. Polizzotto MN, Jones P, Cameron PU, et al. The influence of splenectomy on the infectious complications and outcomes of people with HIV: marked, sustained elevation in risk for severe infection with bacteria including *Streptococcus pneumoniae*. *J Acquir Immune Defic Syndr* 2010;**55**:e24–6.
465. Saha SK, Al Emran HM, Hossain B, et al; Pneumococcal Study Group. *Streptococcus pneumoniae* serotype-2 childhood meningitis in Bangladesh: a newly recognized pneumococcal infection threat. *PLoS One* 2012;**7**:e32134.
475. Scholz CF, Poulsen K, Kilian M. Novel molecular method for identification of *Streptococcus pneumoniae* applicable to clinical microbiology and 16S rRNA sequence-based microbiome studies. *J Clin Microbiol* 2012;**50**:1968–73.
494. Simonsen L, Taylor RJ, Young-Xu Y, et al. Impact of pneumococcal conjugate vaccination of infants on pneumonia and influenza hospitalization and mortality in all age groups in the United States. *MBio* 2011;**2**:e00309–10.
508. Stevens KE, Chang D, Zwack EE, et al. Competence in *Streptococcus pneumoniae* is regulated by the rate of ribosomal decoding errors. *MBio* 2011;**2**:e00071–11.
510. Strachan RE, Cornelius A, Gilbert GL, et al; Australian Research Network. A bedside assay to detect *Streptococcus pneumoniae* in children with empyema. *Pediatr Pulmonol* 2011;**46**:179–83.
513. Strutton DR, Farkouh RA, Rubin JL, et al. Modeling the impact of the 13-valent pneumococcal conjugate vaccine serotype catch-up program using United States claims data. *BMC Infect Dis* 2012;**12**:175.

528. Teele DW, Marshall R, Klein JO. Unsuspected bacteremia in young children: a common and important problem. *Pediatr Clin North Am* 1979;**26**:773–84.
532. Thigpen MC, Whitney CG, Messonnier NE, et al; Emerging Infections Programs. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011;**364**:2016–25.
538. Tsai CJ, Griffin MR, Nuorti JP, et al. Changing epidemiology of pneumococcal meningitis after the introduction of pneumococcal conjugate vaccine in the United States. *Clin Infect Dis* 2008;**46**: 1664–72.
548. van Hoek AJ, Andrews N, Waight PA, et al. Effect of serotype on focus and mortality of invasive pneumococcal disease: coverage of different vaccines and insight into non-vaccine serotypes. *PLoS One* 2012;**7**:e39150.
550. Vanderkooi OG, Church DL, MacDonald J, et al. Community-based outbreaks in vulnerable populations of invasive infections caused by *Streptococcus pneumoniae* serotypes 5 and 8 in Calgary, Canada. *PLoS One* 2011;**6**:e28547.
552. Vergison A, Dagan R, Arguedas A, et al. Otitis media and its consequences: beyond the earache. *Lancet Infect Dis* 2010;**10**:195–203.
565. Walter ND, Taylor Jr TH, Dowell SF, et al; Active Bacterial Core Surveillance System. Holiday spikes in pneumococcal disease among older adults. *N Engl J Med* 2009;**361**:2584–5.
571. Weinberger DM, Harboe ZB, Sanders EA, et al. Association of serotype with risk for death due to pneumococcal pneumonia: a meta-analysis. *Clin Infect Dis* 2010;**51**:692–9.
574. Werno AM, Christner M, Anderson TP, et al. Differentiation of *Streptococcus pneumoniae* from nonpneumococcal streptococci of the *Streptococcus mitis* group by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2012;**50**:2863–7.
575. Weycker D, Farkouh R, Shea K, et al. Excess risk for invasive pneumococcal disease and pneumonia in persons with asthma or diabetes [Abstract 677]. Presented at IDSA Week, October 2012, San Diego, California.
586. World Health Organization. Pneumococcal vaccines. WHO position paper-2012 recommendations. *Vaccine* 2012;**30**:4717–18.
588. Wright AK, Ferreira DM, Gritzfeld JF, et al. Human nasal challenge with *Streptococcus pneumoniae* is immunising in the absence of carriage. *PLoS Pathog* 2012;**8**:e1002622.
589. Wroe PC, Lee GM, Finkelstein JA, et al. Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. *Pediatr Infect Dis J* 2012;**31**:249–54.
590. Xu Q, Kaur R, Casey JR, et al. Identification of *Streptococcus pneumoniae* and *Haemophilus influenzae* in culture-negative middle ear fluids from children with acute otitis media by combination of multiplex PCR and multi-locus sequencing typing. *Int J Pediatr Otorhinolaryngol* 2011;**75**:239–44.
592. Yildirim I, Stevenson A, Hsu K, et al. Invasive pneumococcal disease (IPD) surveillance in Massachusetts: evolving impact of PCV13 in children <2 years of age. Poster 186. Presented at ISPPD8, Iguazo Falls, Brazil, March 2012.
593. Yildirim I, Stevenson A, Hsu K, et al. Evolving picture of invasive pneumococcal disease in Massachusetts children: a comparison of disease in 2007-2009 with earlier periods. *Pediatr Infect Dis J* 2012;**31**:1016–21.
602. Zhou F, Shefer A, Kong Y, et al. Trends in acute otitis media-related health care utilization by privately insured young children in the United States, 1997-2004. *Pediatrics* 2008;**121**:253–60.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 86

Miscellaneous Gram-Positive Cocci

Randall G. Fisher

The focus of this chapter is on relatively uncommon gram-positive cocci that are of importance because of their unusual antimicrobial sensitivities and increasing recognition as pathogens in hospitalized patients. The reader is referred to a review of these organisms for more details.[19]

LEUCONOSTOC SPECIES

Bacteriology

Leuconostoc spp. are facultatively anaerobic, gram-positive cocci that usually appear in pairs or chains. They are catalase negative, Vogues-Proskauer positive, and leucine aminopeptidase positive. In addition, colonies often are α-hemolytic on blood agar and also may react with group D streptococcal antiserum. These properties are shared by viridans streptococci, with which *Leuconostoc* spp. often are confused.[21] They also may resemble enterococci, except that they are pyrrolidone carboxylyl peptidase negative. Differences include the production of gas from glucose and high-level vancomycin resistance. *Leuconostoc* spp. have been divided into three subclusters[17]; members of the first of these (the *L. mesenteroides* subcluster) are the only ones of human clinical importance. The most common pathogens are *L. mesenteroides*, *L. lactis*, *L. citreum*, *L. paramesenteroides*, and *L. pseudomesenteroides*.

Epidemiology

Leuconostoc spp. are found commonly on plants, especially sugar cane and leafy vegetables. They also are present in

REFERENCES

1. Aaberge IS, Eng J, Lermark G, Lovik M. Virulence of *Streptococcus pneumoniae* in mice: a standardized method for preparation and frozen storage of the experimental bacterial inoculum. *Microb Pathog* 1995;**18**:141–52.
2. Aaberge IS, Michaelsen TE, Heier HE. IgG subclass antibody responses to pneumococcal polysaccharide vaccine in splenectomized, otherwise normal, individuals. *Scand J Immunol* 1990;**31**: 711–16.
3. Abdel-Haq N, Abuhammour W, Asmar B, et al. Nasopharyngeal colonization with *Streptococcus pneumoniae* in children receiving trimethoprim-sulfamethoxazole prophylaxis. *Pediatr Infect Dis J* 1999;**18**:647–9.
4. Abdeldaim GM, Stralin K, Korsgaard J, et al. Multiplex quantitative PCR for detection of lower respiratory tract infection and meningitis caused by *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. *BMC Microbiol* 2010;**10**:310.
5. Abdullahi O, Karani A, Tigoi CC, et al. The prevalence and risk factors for pneumococcal colonization of the nasopharynx among children in Kilifi District, Kenya. *PLoS One* 2012;**7**:e30787.
6. Abzug MJ, Pelton SI, Song LY, et al. Immunogenicity, safety, and predictors of response after a pneumococcal conjugate and pneumococcal polysaccharide vaccine series in human immunodeficiency virus-infected children receiving highly active antiretroviral therapy. *Pediatr Infect Dis J* 2006;**25**:920–9.
7. Acuin J. Chronic suppurative otitis media. *Burden of illness and management options; child and adolescent health and development prevention of blindness and deafness*. Geneva: World Health Organization; 2004.
8. Adamkiewicz TV, Silk BJ, Howgate J, et al. Effectiveness of the 7-valent pneumococcal conjugate vaccine in children with sickle cell disease in the first decade of life. *Pediatrics* 2008;**121**:562–9.
9. Adamou JE, Heinrichs JH, Erwin AL, et al. Identification and characterization of a novel family of pneumococcal proteins that are protective against sepsis. *Infect Immun* 2001;**69**:949–58.
10. Alcantara RB, Preheim LC, Gentry-Nielsen MJ. Pneumolysin-induced complement depletion during experimental pneumococcal bacteremia. *Infect Immun* 2001;**69**:3569–75.
11. Alicino C, Iudici R, Alberti M, et al. The dangerous synergism between influenza and *Streptococcus pneumoniae* and innovative perspectives of vaccine prevention. *J Prev Med Hyg* 2011;**52**: 102–6.
12. Alpern ER, Alessandrini EA, Bell LM, et al. Occult bacteremia from a pediatric emergency department: current prevalence, time to detection, and outcome. *Pediatrics* 2000;**106**:505–11.
13. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 571–82.
14. American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.
15. Amsden GW. Pneumococcal macrolide resistance-myth or reality? *J Antimicrob Chemother* 1999;**44**:1–6.
16. Andersson B, Porras O, Hanson LA, et al. Inhibition of attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by human milk and receptor oligosaccharides. *J Infect Dis* 1986;**153**: 232–7.
17. Andiman WA, Mezger J, Shapiro E. Invasive bacterial infections in children born to women infected with human immunodeficiency virus type 1. *J Pediatr* 1994;**124**:846–52.
18. Andreola B, Bressan S, Callegaro S, et al. Procalcitonin and C-reactive protein as diagnostic markers of severe bacterial infections in febrile infants and children in the emergency department. *Pediatr Infect Dis J* 2007;**26**:672–7.
19. Aniansson G, Alm B, Andersson B, et al. Nasopharyngeal colonization during the first year of life. *J Infect Dis* 1992;**165**(Suppl. 1):S38–42.
20. Aniansson G, Alm B, Andersson B, et al. A prospective cohort study on breast-feeding and otitis media in Swedish infants. *Pediatr Infect Dis J* 1994;**13**:183–8.
21. Antin JH, Guinan EC, Avigan D, et al. Protective antibody responses to pneumococcal conjugate vaccine after autologous hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2005;**11**:213–22.
22. Anttila M, Voutilainen M, Jantti V, et al. Contribution of serotype-specific IgG concentration, IgG subclasses and relative antibody avidity to opsonophagocytic activity against *Streptococcus pneumoniae*. *Clin Exp Immunol* 1999;**118**:402–7.
23. Appelbaum PC, Bhamjee A, Scragg JN, et al. *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. *Lancet* 1977;**2**: 995–7.
24. Arason VA, Kristinsson KG, Sigurdsson JA, et al. Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. *BMJ* 1996;**313**: 387–91.
25. Arditi M, Mason Jr EO, Bradley JS, et al. Three-year multicenter surveillance of pneumococcal meningitis in children: clinical characteristics, and outcome related to penicillin susceptibility and dexamethasone use. *Pediatrics* 1998;**102**:1087–97.
26. Arpadi SM, Back S, O'Brien J, et al. Antibodies to pneumococcal capsular polysaccharides in children with human immunodeficiency virus infection given polyvalent pneumococcal vaccine. *J Pediatr* 1994;**125**:77–9.
27. Arrecubieta C, Lopez R, Garcia E. Molecular characterization of *cap3A*, a gene from the operon required for the synthesis of the capsule of *Streptococcus pneumoniae* type 3: sequencing of mutations responsible for the unencapsulated phenotype and localization of the capsular cluster on the pneumococcal chromosome. *J Bacteriol* 1994;**176**:6375–83.
28. Arva E, Andersson B. Induction of phagocyte-stimulating and Th1-promoting cytokines by in vitro stimulation of human peripheral blood mononuclear cells with *Streptococcus pneumoniae*. *Scand J Immunol* 1999;**49**:417–23.
29. Attwood JT, Williams Y, Feighery C. Impaired IgG responses in a child with homozygous C2 deficiency and recurrent pneumococcal septicaemia. *Acta Paediatr* 2001;**90**:99–101.
30. Auer M, Pfister LA, Leppert D, et al. Effects of clinically used antioxidants in experimental pneumococcal meningitis. *J Infect Dis* 2000;**182**:347–50.
31. Austrian R. Pneumonia in the later years. *J Am Geriatr Soc* 1981;**29**:481–9.
32. Austrian R. The enduring pneumococcus: unfinished business and opportunities for the future. In: Tomasz A, editor. Streptococcus pneumoniae: *molecular biology and mechanisms of disease*. Larchmont, NY: Mary Ann Liebert, Inc.; 2000. p. 3–7.
33. Austrian R, Collins P. Importance of carbon dioxide in the isolation of pneumococci. *J Bacteriol* 1966;**92**:1281–4.
34. Austrian R, Gold J. Pneumococcal bacteremia with especial reference to bacteremic pneumococcal pneumonia. *Ann Intern Med* 1964;**60**:759–76.
35. Avery OT, Goebel WF. Chemo-immunological studies on conjugated carbohydrate-proteins, part II. Immunological specificity of synthetic sugar-protein antigens. *J Exp Med* 1929;**50**:533–50.
36. Azoulay-Dupuis E, Rieux V, Muffat-Joly M, et al. Relationship between capsular type, penicillin susceptibility, and virulence of human *Streptococcus pneumoniae* isolates in mice. *Antimicrob Agents Chemother* 2000;**44**:1575–7.
37. Baba H, Kawamura I, Kohda C, et al. Essential role of domain 4 of pneumolysin from *Streptococcus pneumoniae* in cytolytic activity as determined by truncated proteins. *Biochem Biophys Res Commun* 2001;**281**:37–44.
38. Bachur R, Harper MB. Reevaluation of outpatients with *Streptococcus pneumoniae* bacteremia. *Pediatrics* 2000;**105**:502–9.
39. Baggett HC, Rhodes J, Dejsirilert S, et al. Pneumococcal antigen testing of blood culture broth to enhance the detection of *Streptococcus pneumoniae* bacteremia. *Eur J Clin Microbiol Infect Dis* 2012;**31**:753–6.
40. Balachandran P, Hollingshead SK, Paton JC, et al. The autolytic enzyme LytA of *Streptococcus pneumoniae* is not responsible for releasing pneumolysin. *J Bacteriol* 2001;**183**:3108–16.
41. Balicer RD, Zarka S, Levine H, et al. Control of *Streptococcus pneumoniae* serotype 5 epidemic of severe pneumonia among young army recruits by mass antibiotic treatment and vaccination. *Vaccine* 2010;**28**:5591–6.
42. Ball P. Therapy for pneumococcal infection at the millennium: doubts and certainties. *Am J Med* 1999;**107**:77S–85S.
43. Baltz RH, Norris FH, Matsushima P, et al. DNA sequence sampling of the *Streptococcus pneumoniae* genome to identify novel targets for antibiotic development. *Microb Drug Resist* 1998;**4**: 1–9.

44. Banerjee R, Hersh AL, Newland J, et al; Emerging Infections Network Hemolytic-Uremic Syndrome Study Group. *Streptococcus pneumoniae*-associated hemolytic-uremic syndrome among children in North America. *Pediatr Infect Dis J* 2011;**30**:736–9.
45. Baquero F, Loza E. Antibiotic resistance of microorganisms involved in ear, nose and throat infections. *Pediatr Infect Dis J* 1994;**13**:S9–14; discussion S20–2.
46. Baraff LJ, Lee SI, Schriger DL. Outcomes of bacterial meningitis in children: a meta-analysis. *Pediatr Infect Dis J* 1993;**12**:389–94.
47. Bauchner H, Leventhal JM, Shapiro ED. Studies of breast-feeding and infections: how good is the evidence? *JAMA* 1986;**256**: 887–92.
48. Becken ET, Daly KA, Lindgren BR, et al. Low cord blood pneumococcal antibody concentrations predict more episodes of otitis media. *Arch Otolaryngol Head Neck Surg* 2001;**127**:517–22.
49. Bedos JP, Rolin O, Bouanchaud DH, et al. [Relationship between virulence and resistance to antibiotics in pneumococci: contribution of experimental data obtained in an animal model]. *Pathol Biol (Paris)* 1991;**39**:984–90. in French.
50. Bennett NM, Buffington J, LaForce FM. Pneumococcal bacteremia in Monroe County, New York. *Am J Public Health* 1992;**82**: 1513–16.
51. Berman S, McIntosh K. Selective primary health care: strategies for control of disease in the developing world, part XXI. Acute respiratory infections. *Rev Infect Dis* 1985;**7**:674–91.
52. Bernstein LJ, Krieger BZ, Novick B, et al. Bacterial infection in the acquired immunodeficiency syndrome of children. *Pediatr Infect Dis* 1985;**4**:472–5.
53. Biernath KR, Reefhuis J, Whitney CG, et al. Bacterial meningitis among children with cochlear implants beyond 24 months after implantation. *Pediatrics* 2006;**117**:284–9.
54. Bjornson AB, Falletta JM, Verter JI, et al. Serotype-specific immunoglobulin G antibody responses to pneumococcal polysaccharide vaccine in children with sickle cell anemia: effects of continued penicillin prophylaxis. *J Pediatr* 1996;**129**:828–35.
55. Black S, France EK, Isaacman D, et al. Surveillance for invasive pneumococcal disease during 2000-2005 in a population of children who received 7-valent pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2007;**26**:771–7.
56. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* 2000;**19**:187–95.
57. Black SB, Shinefield HR, Ling S, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. *Pediatr Infect Dis J* 2002;**21**:810–15.
58. Blaschke AJ. Interpreting assays for the detection of *Streptococcus pneumoniae*. *Clin Infect Dis* 2011;**52**(Suppl. 4):S331–7.
59. Block SL, Hedrick J, Tyler R, et al. Increasing bacterial resistance in pediatric acute conjunctivitis (1997-1998). *Antimicrob Agents Chemother* 2000;**44**:1650–4.
60. Bluestone CD, Klein JO. *Otitis media in infants and children*. 2nd ed. Philadelphia: WB Saunders; 1995. p. 188–91.
61. Blum MD, Dagan R, Mendelman PM, et al. A comparison of multiple regimens of pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine and pneumococcal polysaccharide vaccine in toddlers. *Vaccine* 2000;**18**:2359–67.
62. Bodor FF. Conjunctivitis-otitis syndrome. *Pediatrics* 1982;**69**: 695–8.
63. Bogaert D, van Belkum A, Sluijter M, et al. Colonisation by *Streptococcus pneumoniae* and *Staphylococcus aureus* in healthy children. *Lancet* 2004;**363**:1871–2.
64. Bogaert D, Veenhoven RH, Ramdin R, et al. Pneumococcal conjugate vaccination does not induce a persisting mucosal IgA response in children with recurrent acute otitis media. *Vaccine* 2005;**23**:2607–13.
65. Bonsu BK, Harper MB. Fever interval before diagnosis, prior antibiotic treatment, and clinical outcome for young children with bacterial meningitis. *Clin Infect Dis* 2001;**32**:566–72.
66. Borer A, Meirson H, Peled N, et al. Antibiotic-resistant pneumococci carried by young children do not appear to disseminate to adult members of a closed community. *Clin Infect Dis* 2001;**33**: 436–44.
67. Borgono JM, McLean AA, Vella PP, et al. Vaccination and revaccination with polyvalent pneumococcal polysaccharide vaccines in adults and infants. *Proc Soc Exp Biol Med* 1978;**157**:148–54.
68. Box QT, Cleveland RT, Willard CY. Bacterial flora of the upper respiratory tract, part I. Comparative evaluation by anterior nasal, oropharyngeal, and nasopharyngeal swabs. *Arch Ped Adolesc Med* 1961;**102**:293–301.
69. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Diseases Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**53**:e25–76.
70. Bradley JS, Kaplan SL, Tan TQ, et al. Pediatric pneumococcal bone and joint infections. The Pediatric Multicenter Pneumococcal Surveillance Study Group (PMPSSG). *Pediatrics* 1998;**102**: 1376–82.
71. Braun JS, Novak R, Gao G, et al. Pneumolysin, a protein toxin of *Streptococcus pneumoniae*, induces nitric oxide production from macrophages. *Infect Immun* 1999;**67**:3750–6.
72. Breiman RF, Spika JS, Navarro VJ, et al. Pneumococcal bacteremia in Charleston County, South Carolina: a decade later. *Arch Intern Med* 1990;**150**:1401–5.
73. Briles DE, Crain MJ, Gray BM, et al. Strong association between capsular type and virulence for mice among human isolates of *Streptococcus pneumoniae*. *Infect Immun* 1992;**60**:111–16.
74. Briles DE, Tart RC, Swiatlo E, et al. Pneumococcal diversity: considerations for new vaccine strategies with emphasis on pneumococcal surface protein A (PspA). *Clin Microbiol Rev* 1998;**11**: 645–57.
75. Broides A, Leibovitz E, Dagan R, et al. Cytology of middle ear fluid during acute otitis media. *Pediatr Infect Dis J* 2002;**21**: 57–61.
76. Brook I, Gober AE. Prophylaxis with amoxicillin or sulfisoxazole for otitis media: effect on the recovery of penicillin-resistant bacteria from children. *Clin Infect Dis* 1996;**22**:143–5.
77. Brown CE, Magnuson B. On the physics of the infant feeding bottle and middle ear sequela: ear disease in infants can be associated with bottle feeding. *Int J Pediatr Otorhinolaryngol* 2000;**54**: 13–20.
78. Brown JS, Gilliland SM, Holden DW. A *Streptococcus pneumoniae* pathogenicity island encoding an ABC transporter involved in iron uptake and virulence. *Mol Microbiol* 2001;**40**:572–85.
79. Brussow H, Baensch M, Sidoti J. Seroprevalence of immunoglobulin M (IgM) and IgG antibodies to polysaccharides of *Streptococcus pneumoniae* in different age groups of Ecuadorian and German children. *J Clin Microbiol* 1992;**30**:2765–71.
80. Buck JM, Lexau C, Shapiro M, et al. A community outbreak of conjunctivitis caused by nontypeable *Streptococcus pneumoniae* in Minnesota. *Pediatr Infect Dis J* 2006;**25**:906–11.
81. Byington CL, Korgenski K, Daly J, et al. Impact of the pneumococcal conjugate vaccine on pneumococcal parapneumonic empyema. *Pediatr Infect Dis J* 2006;**25**:250–4.
82. Byington CL, Spencer LY, Johnson TA, et al. An epidemiological investigation of a sustained high rate of pediatric parapneumonic empyema: risk factors and microbiological associations. *Clin Infect Dis* 2002;**34**:434–40.
83. Campagne G, Schuchat A, Djibo S, et al. Epidemiology of bacterial meningitis in Niamey, Niger, 1981-96. *Bull World Health Organ* 1999;**77**:499–508.
84. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and development of passive antibody therapy. *Antimicrob Agents Chemother* 1994;**38**:1695–702.
85. Casado Flores J, Nieto Moro M, Berron S, et al. Usefulness of pneumococcal antigen detection in pleural effusion for the rapid diagnosis of infection by *Streptococcus pneumoniae*. *Eur J Pediatr* 2010;**169**:581–4.
86. Casey JR, Adlowitz DG, Pichichero ME. New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2010;**29**:304–9.
87. Casey JR, Block SL, Hedrick J, et al. Comparison of amoxicillin/clavulanic acid high dose with cefdinir in the treatment of acute otitis media. *Drugs* 2012;**72**:1991–7.
88. Ceyhan M, Yildirim I, Sheppard CL, et al. Pneumococcal serotypes causing pediatric meningitis in Turkey: application of a new

technology in the investigation of cases negative by conventional culture. *Eur J Clin Microbiol Infect Dis* 2010;**29**:289–93.
89. Chambers HF. Penicillin-binding protein-mediated resistance in pneumococci and staphylococci. *J Infect Dis* 1999;**179**(Suppl. 2): S353–9.
90. Chen DK, McGeer A, de Azavedo JC, et al. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. *N Engl J Med* 1999; **341**:233–9.
91. Chen FM, Breiman RF, Farley M, et al. Geocoding and linking data from population-based surveillance and the US census to evaluate the impact of median household income on the epidemiology of invasive *Streptococcus pneumoniae* infections. *Am J Epidemiol* 1998;**148**:1212–18.
92. Cherian T, Steinhoff MC, Harrison LH, et al. A cluster of invasive pneumococcal disease in young children in child care. *JAMA* 1994;**271**:695–7.
93. Chetty C, Kreger A. Characterization of pneumococcal purpura-producing principle. *Infect Immun* 1980;**29**:158–64.
94. Choi M, Mailman TL. Pneumococcal endocarditis in infants and children. *Pediatr Infect Dis J* 2004;**23**:166–71.
95. Chow AW, Benninger MS, Brook I, et al; Infectious Diseases Society of America. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults. *Clin Infect Dis* 2012;**54**:e72–112.
96. Chudwin DS. Prophylaxis and treatment of pneumococcal bacteremia by immune globulin intravenous in a mouse model. *Clin Immunol Immunopathol* 1989;**50**:62–71.
97. Circolo A, Garnier G, Fukuda W, et al. Genetic disruption of the murine complement C3 promoter region generates deficient mice with extrahepatic expression of C3 mRNA. *Immunopharmacology* 1999;**42**:135–49.
98. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. 22nd Informational Suppl. Publication M100-S22. Wayne, PA: CLSI; 2012.
99. Cohen R, Levy C, Boucherat M, et al. Characteristics and outcome of children with acute otitis media attending day care [abstract 776]. In: *Program and abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco)*. Washington, DC: American Society for Microbiology; 1999. p. 666.
100. Coles CL, Kanungo R, Rahmathullah L, et al. Pneumococcal nasopharyngeal colonization in young South Indian infants. *Pediatr Infect Dis J* 2001;**20**:289–95.
101. Coles CL, Rahmathullah L, Kanungo R, et al. Vitamin A supplementation at birth delays pneumococcal colonization in South Indian infants. *J Nutr* 2001;**131**:255–61.
102. Cortese MM, Wolff M, Almeido-Hill J, et al. High incidence rates of invasive pneumococcal disease in the White Mountain Apache population. *Arch Intern Med* 1992;**152**:2277–82.
103. Courvalin P, Carlier C. Transposable multiple antibiotic resistance in *Streptococcus pneumoniae*. *Mol Gen Genet* 1986;**205**:291–7.
104. Craig WA. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. *Clin Infect Dis* 1998;**26**:1–10; quiz 11–12.
105. Craig WA, Andes D. Pharmacokinetics and pharmacodynamics of antibiotics in otitis media. *Pediatr Infect Dis J* 1996;**15**:255–9.
106. Cunningham-Rundles C. Clinical and immunologic analyses of 103 patients with common variable immunodeficiency. *J Clin Immunol* 1989;**9**:22–33.
107. Dagan R, Engelhard D, Piccard E, et al. Epidemiology of invasive childhood pneumococcal infections in Israel. The Israeli Pediatric Bacteremia and Meningitis Group. *JAMA* 1992;**268**:3328–32.
108. Dagan R, Fraser D, Janco J, et al. Reduction of resistant pneumococcal nasopharyngeal colonization in toddlers attending day care centers after vaccination with a 9-valent CRM197 conjugate pneumococcal vaccine (PnCRM9) [Abstract 0-12]. Program and abstracts of the 18th annual meeting of the European Society for Paediatric Infectious Diseases (Noordwijk, The Netherlands). European Society for Paediatric Infectious Diseases; 2000. p. 19.
109. Dagan R, Givon-Lavi N, Fraser D, et al. Serum serotype-specific pneumococcal anticapsular immunoglobulin G concentrations after immunization with a 9-valent conjugate pneumococcal vaccine correlate with nasopharyngeal acquisition of pneumococcus. *J Infect Dis* 2005;**192**:367–76.
110. Dagan R, Givon-Lavi N, Zamir O, et al. Reduction of nasopharyngeal carriage of *Streptococcus pneumoniae* after administration of a 9-valent pneumococcal conjugate vaccine to toddlers attending day care centers. *J Infect Dis* 2002;**185**:927–36.
111. Dagan R, Gradstein S, Belmaker I, et al. An outbreak of *Streptococcus pneumoniae* serotype 1 in a closed community in southern Israel. *Clin Infect Dis* 2000;**30**:319–21.
112. Dagan R, Leibovitz E, Greenberg D, et al. Dynamics of pneumococcal nasopharyngeal colonization during the first days of antibiotic treatment in pediatric patients. *Pediatr Infect Dis J* 1998;**17**: 880–5.
113. Dagan R, Leibovitz E, Leiberman A, et al. Clinical significance of antibiotic resistance in acute otitis media and implication of antibiotic treatment on carriage and spread of resistant organisms. *Pediatr Infect Dis J* 2000;**19**:S57–65.
114. Dagan R, Melamed R, Muallem M, et al. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. *J Infect Dis* 1996; **174**:1271–8.
115. Dagan R, Melamed R, Muallem M, et al. Nasopharyngeal colonization in southern Israel with antibiotic-resistant pneumococci during the first 2 years of life: relation to serotypes likely to be included in pneumococcal conjugate vaccines. *J Infect Dis* 1996; **174**:1352–5.
116. Dagan R, Muallem M, Melamed R, et al. Reduction of pneumococcal nasopharyngeal carriage in early infancy after immunization with tetravalent pneumococcal vaccines conjugated to either tetanus toxoid or diphtheria toxoid. *Pediatr Infect Dis J* 1997; **16**:1060–4.
117. Dagan R, Shriker O, Hazan I, et al. Prospective study to determine clinical relevance of detection of pneumococcal DNA in sera of children by PCR. *J Clin Microbiol* 1998;**36**:669–73.
118. Dagan R, Sikuler-Cohen M, Zamir O, et al. Effect of a conjugate pneumococcal vaccine on the occurrence of respiratory infections and antibiotic use in day-care center attendees. *Pediatr Infect Dis J* 2001;**20**:951–8.
119. Dagan R, Zamir O, Tirosh N, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* in toddlers vaccinated during infancy with an 11-valent pneumococcal vaccine conjugated to diphtheria and tetanus toxoids [Abstract 47]. Presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, 2000, p. 236.
120. Dallaire F, Ouellet N, Simard M, et al. Efficacy of recombinant human granulocyte colony-stimulating factor in a murine model of pneumococcal pneumonia: effects of lung inflammation and timing of treatment. *J Infect Dis* 2001;**183**:70–7.
121. Daoud AS, Zaki M, al-Saleh QA. Prolonged and secondary fever in childhood bacterial meningitis. *Eur J Pediatr* 1989;**149**: 114–16.
122. Davidson M, Parkinson AJ, Bulkow LR, et al. The epidemiology of invasive pneumococcal disease in Alaska, 1986-1990-ethnic differences and opportunities for prevention. *J Infect Dis* 1994; **170**:368–76.
123. Davies T, Goering RV, Lovgren M, et al. Molecular epidemiological survey of penicillin-resistant *Streptococcus pneumoniae* from Asia, Europe, and North America. *Diagn Microbiol Infect Dis* 1999;**34**: 7–12.
124. de Lalla F, Nicolin R, Lazzarini L. Safety and efficacy of recombinant granulocyte colony-stimulating factor as an adjunctive therapy for *Streptococcus pneumoniae* meningitis in non-neutropenic adult patients: a pilot study. *J Antimicrob Chemother* 2000;**46**: 843–6.
125. Deceuninck G, De Wals P, Boulianne N, et al. Effectiveness of pneumococcal conjugate vaccine using a 2+1 infant schedule in Quebec, Canada. *Pediatr Infect Dis J* 2010;**29**:546–9.
126. del Castillo F, Ledesma F, Garcia-Perea A. Penicillin-susceptible and erythromycin-resistant *Streptococcus pneumoniae* in children with acute mastoiditis. *Eur J Clin Microbiol Infect Dis* 2001;**20**: 824–6.
127. Diavatopoulos DA, Short KR, Price JT, et al. Influenza A virus facilitates *Streptococcus pneumoniae* transmission and disease. *FASEB J* 2010;**24**:1789–98.
128. Dimitrov NV, Douwes FR, Bartolotta B, et al. Metabolic activity of polymorphonuclear leukocytes in sickle cell anemia. *Acta Haematol* 1972;**47**:283–91.

129. Dintilhac A, Alloing G, Granadel C, et al. Competence and virulence of *Streptococcus pneumoniae:* Adc and PsaA mutants exhibit a requirement for Zn and Mn resulting from inactivation of putative ABC metal permeases. *Mol Microbiol* 1997;**25**:727–39.
130. DiNubile MJ, Albornoz MA, Stumacher RJ, et al. Pneumococcal soft-tissue infections: possible association with connective tissue diseases. *J Infect Dis* 1991;**163**:897–900.
131. Dopazo J, Mendoza A, Herrero J, et al. Annotated draft genomic sequence from a *Streptococcus pneumoniae* type 19F clinical isolate. *Microb Drug Resist* 2001;**7**:99–125.
132. Douglas RM, Miles HB. Vaccination against *Streptococcus pneumoniae* in childhood: lack of demonstrable benefit in young Australian children. *J Infect Dis* 1984;**149**:861–9.
133. Douglas RM, Paton JC, Duncan SJ, et al. Antibody response to pneumococcal vaccination in children younger than five years of age. *J Infect Dis* 1983;**148**:131–7.
134. Dowell SF, Whitney CG, Wright C, et al. Seasonal patterns of invasive pneumococcal disease. *Emerg Infect Dis* 2003;**9**:573–9.
135. Duncan B, Ey J, Holberg CJ, et al. Exclusive breast-feeding for at least 4 months protects against otitis media. *Pediatrics* 1993;**91**: 867–72.
136. Dunne EM, Ong EK, Moser RJ, et al. Multilocus sequence typing of *Streptococcus pneumoniae* by use of mass spectrometry. *J Clin Microbiol* 2011;**49**:3756–60.
137. Eberhard T, Kronvall G, Ullberg M. Surface bound plasmin promotes migration of *Streptococcus pneumoniae* through reconstituted basement membranes. *Microb Pathog* 1999;**26**:175–81.
138. Eby R. Pneumococcal conjugate vaccines. *Pharm Biotechnol* 1995;**6**:695–718.
139. Edwards KM, Wandling G, Palmer P, et al. Carriage of pneumococci among infants immunized with a 9-valent pneumococcal conjugate vaccine at 2, 4, and 6 months of age [Abstract 34]. Presented at the 37th Annual Meeting of the Infectious Diseases Society of America (IDSA), Philadelphia, 1999, p. 28.
140. Ekdahl K, Ahlinder I, Hansson HB, et al. Duration of nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae:* experiences from the South Swedish Pneumococcal Intervention Project. *Clin Infect Dis* 1997;**25**:1113–17.
141. Ekwurzel GM, Simmons JS, Dublin LI, et al. Studies on immunizing substances in pneumococci, part VIII. Report on field tests to determine prophylactic value of a pneumococcus antigen. *Public Health Rep* 1938;**53**:1877–93.
142. Elliott SR, Beeson JG. Estimating the burden of global mortality in children aged <5 years by pathogen-specific causes. *Clin Infect Dis* 2008;**46**:1794–5.
143. Engelhard D, Cordonnier C, Shaw PJ, et al; Infectious Disease Working Party of the European Bone Marrow Transplantation (IDWP-EBMT). Early and late invasive pneumococcal infection following stem cell transplantation: a European Bone Marrow Transplantation survey. *Br J Haematol* 2002;**117**:444–50.
144. Enright MC, Spratt BG. A multilocus sequence typing scheme for *Streptococcus pneumoniae:* identification of clones associated with serious invasive disease. *Microbiology* 1998;**144** (Pt 11):3049–60.
145. Epstein MM, Gruskay F. Selective deficiency in pneumococcal antibody response in children with recurrent infections. *Ann Allergy Asthma Immunol* 1995;**75**:125–31.
146. Eskola J, Anttila M. Pneumococcal conjugate vaccines. *Pediatr Infect Dis J* 1999;**18**:543–51.
147. Eskola J, Kilpi T, Palmu A, et al. Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 2001;**344**:403–9.
148. Esterle TM, Edwards KM. Concerns of secondary fever in *Streptococcus pneumoniae* meningitis in an era of increasing antibiotic resistance. *Arch Pediatr Adolesc Med* 1996;**150**:552–4.
149. Faden H, Duffy L, Wasielewski R, et al. Relationship between nasopharyngeal colonization and the development of otitis media in children. Tonawanda/Williamsville Pediatrics. *J Infect Dis* 1997; **175**:1440–5.
150. Falade AG, Mulholland EK, Adegbola RA, et al. Bacterial isolates from blood and lung aspirate cultures in Gambian children with lobar pneumonia. *Ann Trop Paediatr* 1997;**17**:315–19.
151. Farley JJ, King Jr JC, Nair P, et al. Invasive pneumococcal disease among infected and uninfected children of mothers with human immunodeficiency virus infection. *J Pediatr* 1994;**124**:853–8.
152. Fasola EL, Bajaksouzian S, Appelbaum PC, et al. Variation in erythromycin and clindamycin susceptibilities of *Streptococcus pneumoniae* by four test methods. *Antimicrob Agents Chemother* 1997;**41**:129–34.
153. Fedson DS, Eskola J. Pneumococcal vaccine. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 3rd ed. Philadelphia: WB Saunders; 1999. p. 553–607.
154. Feldhoff C, Kleine L, Bachmann H. [Peritonitis and infection in children with idiopathic nephrotic syndrome]. *Klin Padiatr* 1988;**200**:40–4.
155. Feldman C, Munro NC, Jeffery PK, et al. Pneumolysin induces the salient histologic features of pneumococcal infection in the rat lung in vivo. *Am J Respir Cell Mol Biol* 1991;**5**:416–23.
156. Ferreira DM, Oliveira ML, Moreno AT, et al. Protection against nasal colonization with *Streptococcus pneumoniae* by parenteral immunization with a DNA vaccine encoding PspA (Pneumococcal surface protein A). *Microb Pathog* 2010;**48**:205–13.
157. Filice GA, Van Etta LL, Darby CP, et al. Bacteremia in Charleston County, South Carolina. *Am J Epidemiol* 1986;**123**:128–36.
158. Filipe SR, Tomasz A. Inhibition of the expression of penicillin resistance in *Streptococcus pneumoniae* by inactivation of cell wall muropeptide branching genes. *Proc Natl Acad Sci U S A* 2000;**97**: 4891–6.
159. Fiore AE, Levine OS, Elliott JA, et al. Effectiveness of pneumococcal polysaccharide vaccine for preschool-age children with chronic disease. *Emerg Infect Dis* 1999;**5**:828–31.
160. Fireman B, Black SB, Shinefield HR, et al. Impact of the pneumococcal conjugate vaccine on otitis media. *Pediatr Infect Dis J* 2003;**22**:10–16.
161. Fischer W. Pneumococcal lipoteichoic and teichoic acid. In: Tomasz A, editor. Streptococcus pneumoniae: *molecular biology and mechanisms of disease*. Larchmont, NY: Mary Ann Liebert, Inc.; 2000. p. 155–77.
162. Fischer W, Markwitz S, Labischinski H. Small-angle X-ray scattering analysis of pneumococcal lipoteichoic acid phase structure. *Eur J Biochem* 1997;**244**:913–17.
163. Fletcher MA, Fritzell B. Pneumococcal conjugate vaccines and otitis media: an appraisal of the clinical trials. *Int J Otolaryngol* 2012;**2012**:312935.
164. Foo RL, Graham SM, Suthisarnsuntorn U, et al. Detection of pneumococcal capsular antigen in saliva of children with pneumonia. *Ann Trop Paediatr* 2000;**20**:161–3.
165. Fortnum HM. Hearing impairment after bacterial meningitis: a review. *Arch Dis Child* 1992;**67**:1128–33.
166. Francis T, Tillett WS. Cutaneous reactions in pneumonia: the development of antibodies following the intradermal injection of type-specific polysaccharide. *J Exp Med* 1930;**52**:573–85.
167. Frankel RE, Virata M, Hardalo C, et al. Invasive pneumococcal disease: clinical features, serotypes, and antimicrobial resistance patterns in cases involving patients with and without human immunodeficiency virus infection. *Clin Infect Dis* 1996;**23**:577–84.
168. Fraser D, Givon-Lavi N, Bilenko N, et al. A decade (1989-1998) of pediatric invasive pneumococcal disease in 2 populations residing in 1 geographic location: implications for vaccine choice. *Clin Infect Dis* 2001;**33**:421–7.
169. Freij BJ, Votteler TP, McCracken Jr GH. Primary peritonitis in previously healthy children. *Am J Dis Child* 1984;**138**:1058–61.
170. Fuchshuber A, Kuhnemund O, Keuth B, et al. Pneumococcal vaccine in children and young adults with chronic renal disease. *Nephrol Dial Transplant* 1996;**11**:468–73.
171. Gallin JI, Buescher ES, Seligmann BE, et al. NIH conference. Recent advances in chronic granulomatous disease. *Ann Intern Med* 1983;**99**:657–74.
172. Garcia E, Llull D, Lopez R. Functional organization of the gene cluster involved in the synthesis of the pneumococcal capsule. *Int Microbiol* 1999;**2**:169–76.
173. Garcia-Rey C, Aguilar L, Baquero F, et al. Importance of local variations in antibiotic consumption and geographical differences of erythromycin and penicillin resistance in *Streptococcus pneumoniae*. *J Clin Microbiol* 2002;**40**:159–64.
174. Gaston MH, Verter JI, Woods G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia: a randomized trial. *N Engl J Med* 1986;**314**:1593–9.
175. Gay K, Stephens DS. Structure and dissemination of a chromosomal insertion element encoding macrolide efflux in *Streptococcus pneumoniae*. *J Infect Dis* 2001;**184**:56–65.

176. Gesner M, Desiderio D, Kim M, et al. *Streptococcus pneumoniae* in human immunodeficiency virus type 1-infected children. *Pediatr Infect Dis J* 1994;**13**:697–703.
177. Gessner BD, Mueller JE, Yaro S. African meningitis belt pneumococcal disease epidemiology indicates a need for an effective serotype 1 containing vaccine, including for older children and adults. *BMC Infect Dis* 2010;**10**:22.
178. Gessner BD, Ussery XT, Parkinson AJ, et al. Risk factors for invasive disease caused by *Streptococcus pneumoniae* among Alaska native children younger than two years of age. *Pediatr Infect Dis J* 1995;**14**:123–8.
179. Ghaffar F, Friedland IR, McCracken Jr GH. Dynamics of nasopharyngeal colonization by *Streptococcus pneumoniae*. *Pediatr Infect Dis J* 1999;**18**:638–46.
180. Gibb D, Spoulou V, Giacomelli A, et al. Antibody responses to *Haemophilus influenzae* type b and *Streptococcus pneumoniae* vaccines in children with human immunodeficiency virus infection. *Pediatr Infect Dis J* 1995;**14**:129–35.
181. Giebink GS. Otitis media: the chinchilla model. *Microb Drug Resist* 1999;**5**:57–72.
182. Gigliotti F, Williams WT, Hayden FG, et al. Etiology of acute conjunctivitis in children. *J Pediatr* 1981;**98**:531–6.
183. Gilbert RJ, Heenan RK, Timmins PA, et al. Studies on the structure and mechanism of a bacterial protein toxin by analytical ultracentrifugation and small-angle neutron scattering. *J Mol Biol* 1999;**293**:1145–60.
184. Gindreau E, Lopez R, Garcia P. MM1, a temperate bacteriophage of the type 23F Spanish/USA multiresistant epidemic clone of *Streptococcus pneumoniae:* structural analysis of the site-specific integration system. *J Virol* 2000;**74**:7803–13.
185. Gingles NA, Alexander JE, Kadioglu A, et al. Role of genetic resistance in invasive pneumococcal infection: identification and study of susceptibility and resistance in inbred mouse strains. *Infect Immun* 2001;**69**:426–34.
186. Ginsburg CM, Rudoy R, Nelson JD. Acute mastoiditis in infants and children. *Clin Pediatr (Phila)* 1980;**19**:549–53.
187. Givner LB, Mason Jr EO, Barson WJ, et al. Pneumococcal facial cellulitis in children. *Pediatrics* 2000;**106**:E61.
188. Givon-Lavi N, Fraser D, Porat N, et al. Spread of *Streptococcus pneumoniae* and antibiotic-resistant *S. pneumoniae* from day-care center attendees to their younger siblings. *J Infect Dis* 2002;**186**:1608–14.
189. Goldblatt D, Akoto AY, Ashton L, et al. Immunogenicity and the generation of immune memory following 9-valent pneumococcal conjugate vaccination in Ghanaian infants with sickle cell disease [Abstract 688]. Presented at the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, 2000, p. 245.
190. Goldstein FW. Penicillin-resistant *Streptococcus pneumoniae:* selection by both beta-lactam and non-beta-lactam antibiotics. *J Antimicrob Chemother* 1999;**44**:141–4.
191. Gordon SB, Irving GR, Lawson RA, et al. Intracellular trafficking and killing of *Streptococcus pneumoniae* by human alveolar macrophages are influenced by opsonins. *Infect Immun* 2000;**68**:2286–93.
192. Gorensek MJ, Lebel MH, Nelson JD. Peritonitis in children with nephrotic syndrome. *Pediatrics* 1988;**81**:849–56.
193. Gosink KK, Mann ER, Guglielmo C, et al. Role of novel choline binding proteins in virulence of *Streptococcus pneumoniae*. *Infect Immun* 2000;**68**:5690–5.
194. Gould JM, Weiser JN. Expression of C-reactive protein in the human respiratory tract. *Infect Immun* 2001;**69**:1747–54.
195. Granert C, Raud J, Waage A, et al. Effects of polysaccharide fucoidin on cerebrospinal fluid interleukin-1 and tumor necrosis factor alpha in pneumococcal meningitis in the rabbit. *Infect Immun* 1999;**67**:2071–4.
196. Gray BM, Converse 3rd GM, Dillon Jr HC. Epidemiologic studies of *Streptococcus pneumoniae* in infants: acquisition, carriage, and infection during the first 24 months of life. *J Infect Dis* 1980;**142**:923–33.
197. Gray BM, Converse 3rd GM, Huhta N, et al. Epidemiologic studies of *Streptococcus pneumoniae* in infants: antibody response to nasopharyngeal carriage of types 3, 19, and 23. *J Infect Dis* 1981;**144**:312–18.
198. Gray BM, Dillon Jr HC. Epidemiological studies of *Streptococcus pneumoniae* in infants: antibody to types 3, 6, 14, and 23 in the first two years of life. *J Infect Dis* 1988;**158**:948–55.
199. Gray BM, Dillon Jr HC. Natural history of pneumococcal infections. *Pediatr Infect Dis J* 1989;**8**:S23–5.
200. Gray BM, Turner ME, Dillon Jr HC. Epidemiologic studies of *Streptococcus pneumoniae* in infants: the effects of season and age on pneumococcal acquisition and carriage in the first 24 months of life. *Am J Epidemiol* 1982;**116**:692–703.
201. Greenwood B. The epidemiology of pneumococcal infection in children in the developing world. *Philos Trans R Soc Lond B Biol Sci* 1999;**354**:777–85.
202. Grijalva CG, Poehling KA, Nuorti JP, et al. National impact of universal childhood immunization with pneumococcal conjugate vaccine on outpatient medical care visits in the United States. *Pediatrics* 2006;**118**:865–73.
203. Guillemot D, Varon E, Bernede C, et al. Reduction of antibiotic use in the community reduces the rate of colonization with penicillin G-nonsusceptible *Streptococcus pneumoniae*. *Clin Infect Dis* 2005;**41**:930–8.
204. Gwatkin DR. How many die? A set of demographic estimates of the annual number of infant and child deaths in the world. *Am J Public Health* 1980;**70**:1286–9.
205. Hadari I, Dagan R, Gedalia A, et al. Pneumococcal osteomyelitis: an unusual cluster of cases. *Clin Pediatr (Phila)* 1985;**24**:143–5.
206. Hakansson A, Roche H, Mirza S, et al. Characterization of binding of human lactoferrin to pneumococcal surface protein A. *Infect Immun* 2001;**69**:3372–81.
207. Hakenbeck R, Balmelle N, Weber B, et al. Mosaic genes and mosaic chromosomes: intra- and interspecies genomic variation of *Streptococcus pneumoniae*. *Infect Immun* 2001;**69**:2477–86.
208. Hakenbeck R, Bruckner R, Denapaite D, et al. Molecular mechanisms of beta-lactam resistance in *Streptococcus pneumoniae*. *Future Microbiol* 2012;**7**:395–410.
209. Halasa NB, Shankar SM, Talbot TR, et al. Incidence of invasive pneumococcal disease among individuals with sickle cell disease before and after the introduction of the pneumococcal conjugate vaccine. *Clin Infect Dis* 2007;**44**:1428–33.
210. Hameed N, Tunkel AR. Treatment of drug-resistant pneumococcal meningitis. *Curr Infect Dis Rep* 2010;**12**:274–81.
211. Hamer DH, Egas J, Estrella B, et al. Assessment of the Binax NOW *Streptococcus pneumoniae* urinary antigen test in children with nasopharyngeal pneumococcal carriage. *Clin Infect Dis* 2002;**34**:1025–8.
212. Hammerschmidt S, Bethe G, Remane PH, et al. Identification of pneumococcal surface protein A as a lactoferrin-binding protein of *Streptococcus pneumoniae*. *Infect Immun* 1999;**67**:1683–7.
213. Hammerschmidt S, Talay SR, Brandtzaeg P, et al. SpsA, a novel pneumococcal surface protein with specific binding to secretory immunoglobulin A and secretory component. *Mol Microbiol* 1997;**25**:1113–24.
214. Hardy GG, Magee AD, Ventura CL, et al. Essential role for cellular phosphoglucomutase in virulence of type 3 *Streptococcus pneumoniae*. *Infect Immun* 2001;**69**:2309–17.
215. Harrison LH, Dwyer DM, Billmann L, et al. Invasive pneumococcal infection in Baltimore, Md: implications for immunization policy. *Arch Intern Med* 2000;**160**:89–94.
216. Hausdorff WP, Bryant J, Kloek C, et al. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. *Clin Infect Dis* 2000;**30**:122–40.
217. Hausdorff WP, Bryant J, Paradiso PR, et al. Which pneumococcal serogroups cause the most invasive disease: implications for conjugate vaccine formulation and use, part I. *Clin Infect Dis* 2000;**30**:100–21.
218. Hausdorff WP, Siber G, Paradiso PR. Geographical differences in invasive pneumococcal disease rates and serotype frequency in young children. *Lancet* 2001;**357**:950–2.
219. Hausdorff WP, Yothers G, Dagan R, et al. Multinational study of pneumococcal serotypes causing acute otitis media in children. *Pediatr Infect Dis J* 2002;**21**:1008–16.
220. Hawley HB, Yamada T, Mosher DF, et al. Disseminated intravascular coagulopathy during experimental pneumococcal sepsis: studies in normal and asplenic rhesus monkeys. *J Med Primatol* 1977;**6**:203–18.
221. Hazlewood M, Nusrat R, Kumararatne DS, et al. The acquisition of anti-pneumococcal capsular polysaccharide *Haemophilus influenzae* type b and tetanus toxoid antibodies, with age, in the UK. *Clin Exp Immunol* 1993;**93**:157–64.

222. Heffron R. *Pneumonia with special reference to pneumococcus lobar pneumonia*. A Commonwealth Fund Book. Cambridge, Mass.: Harvard University Press; 1939.
223. Heidelberger M, Avery OT. The soluble specific substance of pneumococcus. *J Exp Med* 1923;**38**:73–9.
224. Henderson FW, Gilligan PH, Wait K, et al. Nasopharyngeal carriage of antibiotic-resistant pneumococci by children in group day care. *J Infect Dis* 1988;**157**:256–63.
225. Hendley JO, Sande MA, Stewart PM, et al. Spread of *Streptococcus pneumoniae* in families, part I. Carriage rates and distribution of types. *J Infect Dis* 1975;**132**:55–61.
226. Henneberger PK, Galaid EI, Marr JS. The descriptive epidemiology of pneumococcal meningitis in New York City. *Am J Epidemiol* 1983;**117**:484–91.
227. Henrichsen J. Six newly recognized types of *Streptococcus pneumoniae*. *J Clin Microbiol* 1995;**33**:2759–62.
228. Henriques Normark B, Novak R, Ortqvist A, et al. Clinical isolates of *Streptococcus pneumoniae* that exhibit tolerance of vancomycin. *Clin Infect Dis* 2001;**32**:552–8.
229. Hermans PW, Sluijter M, Hoogenboezem T, et al. Comparative study of five different DNA fingerprint techniques for molecular typing of *Streptococcus pneumoniae* strains. *J Clin Microbiol* 1995; **33**:1606–12.
230. Hidalgo H, Moore C, Leiva LE, et al. Preimmunization and postimmunization pneumococcal antibody titers in children with recurrent infections. *Ann Allergy Asthma Immunol* 1996;**76**: 341–6.
231. Hoban DJ, Doern GV, Fluit AC, et al. Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 2001;**32**(Suppl. 2):S81–93.
232. Hodges RG, Mac LC, Bernhard WG. Epidemic pneumococcal pneumonia; pneumococcal carrier studies. *Am J Hyg* 1946;**44**: 207–30.
233. Hollingshead SK, Becker R, Briles DE. Diversity of PspA: mosaic genes and evidence for past recombination in *Streptococcus pneumoniae*. *Infect Immun* 2000;**68**:5889–900.
234. Hoppe JE, Koster S, Bootz F, et al. Acute mastoiditis-relevant once again. *Infection* 1994;**22**:178–82.
235. Howie VM, Ploussard JH. The "in vivo sensitivity test"-bacteriology of middle ear exudate, during antimicrobial therapy in otitis media. *Pediatrics* 1969;**44**:940–4.
236. Howie VM, Ploussard JH, Lester Jr RL. Otitis media: a clinical and bacteriological correlation. *Pediatrics* 1970;**45**:29–35.
237. Howie VM, Ploussard JH, Sloyer J. The "otitis-prone" condition. *Am J Dis Child* 1975;**129**:676–8.
238. Howie VM, Ploussard J, Sloyer JL, et al. Use of pneumococcal polysaccharide vaccine in preventing otitis media in infants: different results between racial groups. *Pediatrics* 1984;**73**:79–81.
239. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009;**360**:244–56.
240. Hsu KK, Shea KM, Stevenson AE, et al; Members of the Massachusetts Department of Public Health. Underlying conditions in children with invasive pneumococcal disease in the conjugate vaccine era. *Pediatr Infect Dis J* 2011;**30**:251–3.
241. Huang CC, Liu CC, Wang ST, et al. Basic fibroblast growth factor in experimental and clinical bacterial meningitis. *Pediatr Res* 1999;**45**:120–7.
242. Huang SS, Platt R, Rifas-Shiman SL, et al. Post-PCV7 changes in colonizing pneumococcal serotypes in 16 Massachusetts communities, 2001 and 2004. *Pediatrics* 2005;**116**:e408–13.
243. Hyde TB, Gay K, Stephens DS, et al; Active Bacterial Core Surveillance/Emerging Infections Program Network. Macrolide resistance among invasive *Streptococcus pneumoniae* isolates. *JAMA* 2001;**286**:1857–62.
244. Idanpaan-Heikkila I, Simon PM, Zopf D, et al. Oligosaccharides interfere with the establishment and progression of experimental pneumococcal pneumonia. *J Infect Dis* 1997;**176**:704–12.
245. Iglesias Sanchez L, Perez-Yarza EG, Garcia-Arenzana JM, et al. [Epidemiology of invasive pneumococcal disease in children in Gipuzkoa (Spain) from 1981 to 2001]. *An Esp Pediatr* 2002;**57**:401–7. In Spanish.
246. Isaacson G. Sinusitis in childhood. *Pediatr Clin North Am* 1996; **43**:1297–318.
247. Ishizuka S, Yamaya M, Suzuki T, et al. Acid exposure stimulates the adherence of *Streptococcus pneumoniae* to cultured human airway epithelial cells: effects on platelet-activating factor receptor expression. *Am J Respir Cell Mol Biol* 2001;**24**:459–68.
248. Ispahani P, Weston VC, Turner DP, et al. Septic arthritis due to *Streptococcus pneumoniae* in Nottingham, United Kingdom, 1985-1998. *Clin Infect Dis* 1999;**29**:1450–4.
249. Jackson MA, Rutledge J. Pneumococcal endocarditis in children. *Pediatr Infect Dis* 1982;**1**:120–2.
250. Jacobs MR. Emergence of antibiotic resistance in upper and lower respiratory tract infections. *Am J Manag Care* 1999;**5**:S651–61.
251. Jacobs MR. Increasing antibiotic resistance among otitis media pathogens and their susceptibility to oral agents based on pharmacodynamic parameters. *Pediatr Infect Dis J* 2000;**19**:S47–55; discussion S55–6.
252. Jacobs MR. Treatment and diagnosis of infections caused by drug-resistant *Streptococcus pneumoniae*. *Clin Infect Dis* 1992;**15**:119–27.
253. Jacobs MR. Antimicrobial-resistant *Streptococcus pneumoniae:* trends and management. *Expert Rev Anti Infect Ther* 2008;**6**: 619–35.
254. Jacobs MR, Bajaksouzian S, Zilles A, et al. Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 U.S. Surveillance study. *Antimicrob Agents Chemother* 1999; **43**:1901–8.
255. Jacobs MR, Good CE, Windau AR, et al. Activity of ceftaroline against recent emerging serotypes of *Streptococcus pneumoniae* in the United States. *Antimicrob Agents Chemother* 2010;**54**: 2716–19.
256. Jacobs MR, Koornhof HJ, Robins-Browne RM, et al. Emergence of multiply resistant pneumococci. *N Engl J Med* 1978;**299**: 735–40.
257. Jacobs MR, Weinberg W. Evidence-based guidelines for treatment of bacterial respiratory tract infections in the era of antibiotic resistance. *Manag Care Interface* 2001;**14**:68–80.
258. Janoff EN, Fasching C, Orenstein JM, et al. Killing of *Streptococcus pneumoniae* by capsular polysaccharide-specific polymeric IgA, complement, and phagocytes. *J Clin Invest* 1999;**104**:1139–47.
259. Janoff EN, Rubins JB. Invasive pneumococcal disease in the immunocompromised host. In: Tomasz, A, editor. *Streptococcus pneumoniae: molecular biology and mechanisms of disease*. Larchmont, NY: Mary Ann Liebert, Inc.; 2000. p. 321–41.
260. Jansen WT, Vakevainen-Anttila M, Kayhty H, et al. Comparison of a classical phagocytosis assay and a flow cytometry assay for assessment of the phagocytic capacity of sera from adults vaccinated with a pneumococcal conjugate vaccine. *Clin Diagn Lab Immunol* 2001;**8**:245–50.
261. Jay SJ, Johanson Jr WG, Pierce AK. The radiographic resolution of *Streptococcus pneumoniae* pneumonia. *N Engl J Med* 1975;**293**: 798–801.
262. Jedrzejas MJ. Pneumococcal virulence factors: structure and function. *Microbiol Mol Biol Rev* 2001;**65**:187–207; first page, table of contents.
263. Jinno S, Jacobs MR. Pneumonia due to drug-resistant *Streptococcus pneumoniae*. *Curr Infect Dis Rep* 2012;**14**:292–9.
264. Johansen FE, Braathen R, Brandtzaeg P. Role of J chain in secretory immunoglobulin formation. *Scand J Immunol* 2000;**52**: 240–8.
265. Johnson DR, D'Onise K, Holland RA, et al. Pneumococcal disease in South Australia: vaccine success but no time for complacency. *Vaccine* 2012;**30**:2206–11.
266. Johnson HL, Deloria-Knoll M, Levine OS, et al. Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: the pneumococcal global serotype project. *PLoS Med* 2010;**7**:e1000348.
267. Johnson SE, Rubin L, Romero-Steiner S, et al. Correlation of opsonophagocytosis and passive protection assays using human anticapsular antibodies in an infant mouse model of bacteremia for *Streptococcus pneumoniae*. *J Infect Dis* 1999;**180**:133–40.
268. Johnston Jr RB, Newman SL, Struth AG. An abnormality of the alternate pathway of complement activation in sickle-cell disease. *N Engl J Med* 1973;**288**:803–8.
269. Jokinen J, Nuorti P, Palmu A, et al. Monitoring the effectiveness of the 10-valent pneumococcal conjugate vaccine (PCV10) in the Finnish national vaccination programme. Presented at ISPPD 8. Iguacu, Brazil March 11-15. 2012.

270. Juven T, Mertsola J, Toikka P, et al. Clinical profile of serologically diagnosed pneumococcal pneumonia. *Pediatr Infect Dis J* 2001;**20**: 1028–33.
271. Kabins SA, Lerner C. Fulminant pneumococcemia and sickle cell anemia. *JAMA* 1970;**211**:467–71.
272. Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. *Nat Rev Microbiol* 2008;**6**:288–301.
273. Kaetzel CS. Polymeric Ig receptor: defender of the fort or Trojan horse? *Curr Biol* 2001;**11**:R35–8.
274. Kaplan SL. Clinical presentations, diagnosis, and prognostic factors of bacterial meningitis. *Infect Dis Clin North Am* 1999; **13**:579–94, vi–vii.
275. Kaplan SL, Barson WJ, Ling-Lin P. Early Trends for Invasive Pneumococcal Infections in Children After the Introduction of the 13-valent Pneumococcal Conjugate Vaccine. *Pediatr Infect Dis* 2013;**32**:203–7.
276. Kaplan SL, Mason Jr EO, Wald E, et al. Six year multicenter surveillance of invasive pneumococcal infections in children. *Pediatr Infect Dis J* 2002;**21**:141–7.
277. Karma P, Pukander J, Sipila M, et al. Prevention of otitis media in children by pneumococcal vaccination. *Am J Otolaryngol* 1985; **6**:173–84.
278. Kayhty H, Ahman H, Eriksson K, et al. Immunogenicity and tolerability of a heptavalent pneumococcal conjugate vaccine administered at 3, 5 and 12 months of age. *Pediatr Infect Dis J* 2005;**24**:108–14.
279. Kayhty H, Eskola J. New vaccines for the prevention of pneumococcal infections. *Emerg Infect Dis* 1996;**2**:289–98.
280. Kennedy WA, Chang SJ, Purdy K, et al. Incidence of bacterial meningitis in Asia using enhanced CSF testing: polymerase chain reaction, latex agglutination and culture. *Epidemiol Infect* 2007;**135**: 1217–26.
281. Kilian M, Reinholdt J, Lomholt H, et al. Biological significance of IgA1 proteases in bacterial colonization and pathogenesis: critical evaluation of experimental evidence. *APMIS* 1996;**104**: 321–38.
282. Kilpi T, Ahman H, Jokinen J, et al; Finnish Otitis Media Study Group. Protective efficacy of a second pneumococcal conjugate vaccine against pneumococcal acute otitis media in infants and children: randomized, controlled trial of a 7-valent pneumococcal polysaccharide-meningococcal outer membrane protein complex conjugate vaccine in 1666 children. *Clin Infect Dis* 2003;**37**: 1155–64.
283. Kilpi TM, Palmu A, Leinonen M, et al. Effect of a 7-valent pneumococcal conjugate vaccine on acute otitis media due to vaccine serotypes after boosting with conjugate or polysaccharide vaccines [Abstract G-2036]. Presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Chicago, 2001, p. 282.
284. Kim DW, Kilgore PE, Kim EJ, et al. The enhanced pneumococcal LAMP assay: a clinical tool for the diagnosis of meningitis due to *Streptococcus pneumoniae*. *PLoS One* 2012;**7**:e42954.
285. King H, Shumacker Jr HB. Splenic studies, part I. Susceptibility to infection after splenectomy performed in infancy. *Ann Surg* 1952;**136**:239–42.
286. King Jr JC, Vink PE, Farley JJ, et al. Comparison of the safety and immunogenicity of a pneumococcal conjugate with a licensed polysaccharide vaccine in human immunodeficiency virus and non-human immunodeficiency virus-infected children. *Pediatr Infect Dis J* 1996;**15**:192–6.
287. King Jr JC, Vink PE, Farley JJ, et al. Safety and immunogenicity of three doses of a five-valent pneumococcal conjugate vaccine in children younger than two years with and without human immunodeficiency virus infection. *Pediatrics* 1997;**99**:575–80.
288. Klein JO. The epidemiology of pneumococcal disease in infants and children. *Rev Infect Dis* 1981;**3**:246–53.
289. Klein JO. Otitis media. *Clin Infect Dis* 1994;**19**:823–33.
290. Klein JO. Nonimmune strategies for prevention of otitis media. *Pediatr Infect Dis J* 2000;**19**:S89–92.
291. Klugman KP. Antibiotic selection of multiply resistant pneumococci. *Clin Infect Dis* 2001;**33**:489–91.
292. Klugman KP. Pneumococcal resistance to antibiotics. *Clin Microbiol Rev* 1990;**3**:171–96.
293. Klugman KP, Koornhof HJ, Wasas A, et al. Carriage of penicillin resistant pneumococci. *Arch Dis Child* 1986;**61**:377–81.
294. Klugman KP, Madhi SA, Huebner RE, et al; Vaccine Trialists Group. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003;**349**:1341–8.
295. Kontiokari T, Svanberg M, Mattila P, et al. Quantitative analysis of the effect of xylitol on pneumococcal nasal colonisation in rats. *FEMS Microbiol Lett* 1999;**178**:313–17.
296. Kontiokari T, Uhari M, Koskela M. Effect of xylitol on growth of nasopharyngeal bacteria in vitro. *Antimicrob Agents Chemother* 1995;**39**:1820–3.
297. Kornelisse RF, Westerbeek CM, Spoor AB, et al. Pneumococcal meningitis in children: prognostic indicators and outcome. *Clin Infect Dis* 1995;**21**:1390–7.
298. Koskela M. Antibody response of young children to parenteral vaccination with pneumococcal capsular polysaccharides: a comparison between antibody levels in serum and middle ear effusion. *Pediatr Infect Dis* 1986;**5**:431–4.
299. Koskela M, Leinonen M, Haiva VM, et al. First and second dose antibody responses to pneumococcal polysaccharide vaccine in infants. *Pediatr Infect Dis* 1986;**5**:45–50.
300. Koskela M, Luotonen J. Recurrent pneumococcal otitis media: presence of pneumococcal antigens and antibody in middle ear effusion compared with antibody levels in serum. In: Lim DL, Bluestone CD, Klein JO, et al, editors. *Recent advances in otitis media with effusion*. New York: Marcel Decker; 1984. p. 251–5.
301. Kostyukova NN, Volkova MO, Ivanova VV, et al. A study of pathogenic factors of *Streptococcus pneumoniae* strains causing meningitis. *FEMS Immunol Med Microbiol* 1995;**10**:133–7.
302. Kristinsson KG, Sigurdardottir ST, Gudnason T, et al. Effect of vaccination with octavalent protein conjugated pneumococcal vaccines on pneumococcal carriage in infants [Abstract G-5]. Presented at the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Toronto, 1997, p. 193.
303. Kulkarni S, Powles R, Treleaven J, et al. Chronic graft versus host disease is associated with long-term risk for pneumococcal infections in recipients of bone marrow transplants. *Blood* 2000;**95**: 3683–6.
304. Kumar D, Welsh B, Siegal D, et al. Immunogenicity of pneumococcal vaccine in renal transplant recipients-three year follow-up of a randomized trial. *Am J Transplant* 2007;**7**:633–8.
305. Musey L. Safety, tolerability, and immunogenicity of 15-valent pneumococcal conjugate vaccine (PCV15) in healthy adults. Poster 658. Presented at IDSA 2012, San Diego, Calif.
306. Ladhani SN, Slack MP, Andrews NJ, et al. Invasive pneumococcal disease after routine pneumococcal conjugate vaccination in children, England and Wales. *Emerg Infect Dis* 2013;**19**:61–8.
307. Lammers AJ, de Porto AP, Florquin S, et al. Enhanced vulnerability for *Streptococcus pneumoniae* sepsis during asplenia is determined by the bacterial capsule. *Immunobiology* 2011;**216**:863–70.
308. Lane PA, Rogers ZR, Woods GM, et al. Fatal pneumococcal septicemia in hemoglobin SC disease. *J Pediatr* 1994;**124**:859–62.
309. Lau GW, Haataja S, Lonetto M, et al. A functional genomic analysis of type 3 *Streptococcus pneumoniae* virulence. *Mol Microbiol* 2001;**40**:555–71.
310. Laupland KB, Davies HD, Kellner JD, et al. Predictors and outcome of admission for invasive *Streptococcus pneumoniae* infections at a Canadian children's hospital. *Clin Infect Dis* 1998;**27**: 597–602.
311. Laurichesse H, Romaszko JP, Nguyen LT, et al. Clinical characteristics and outcome of patients with invasive pneumococcal disease, Puy-de-Dome, France, 1994-1998. *Eur J Clin Microbiol Infect Dis* 2001;**20**:299–308.
312. Lawrence ER, Arias CA, Duke B, et al. Evaluation of serotype prediction by cpsA-cpsB gene polymorphism in *Streptococcus pneumoniae*. *J Clin Microbiol* 2000;**38**:1319–23.
313. Leach AJ, Boswell JB, Asche V, et al. Bacterial colonization of the nasopharynx predicts very early onset and persistence of otitis media in Australian aboriginal infants. *Pediatr Infect Dis J* 1994;**13**:983–9.
314. Lebel MH, McCracken Jr GH. Delayed cerebrospinal fluid sterilization and adverse outcome of bacterial meningitis in infants and children. *Pediatrics* 1989;**83**:161–7.
315. Lee CS, Chen MJ, Chiou YH, et al. Invasive pneumococcal pneumonia is the major cause of paediatric haemolytic-uraemic syndrome in Taiwan. *Nephrology (Carlton)* 2012;**17**:48–52.

316. Lee HJ, Kang JH, Henrichsen J, et al. Immunogenicity and safety of a 23-valent pneumococcal polysaccharide vaccine in healthy children and in children at increased risk of pneumococcal infection. *Vaccine* 1995;**13**:1533–8.
317. Lehmann D, Marshall TF, Riley ID, et al. Effect of pneumococcal vaccine on morbidity from acute lower respiratory tract infections in Papua New Guinean children. *Ann Trop Paediatr* 1991;**11**: 247–57.
318. Leino T, Auranen K, Jokinen J, et al. Pneumococcal carriage in children during their first two years: important role of family exposure. *Pediatr Infect Dis J* 2001;**20**:1022–7.
319. Leinonen M, Sakkinen A, Kalliokoski R, et al. Antibody response to 14-valent pneumococcal capsular polysaccharide vaccine in preschool age children. *Pediatr Infect Dis* 1986;**5**:39–44.
320. Leowski J. Mortality from acute respiratory infections in children under 5 years of age: global estimates. *World Health Stat Q* 1986; **39**:138–44.
321. LeVine AM, Koeningsknecht V, Stark JM. Decreased pulmonary clearance of *S. pneumoniae* following influenza A infection in mice. *J Virol Methods* 2001;**94**:173–86.
322. Levine OS, Farley M, Harrison LH, et al. Risk factors for invasive pneumococcal disease in children: a population-based case-control study in North America. *Pediatrics* 1999;**103**:E28.
323. Levy A, Fraser D, Vardi H, et al. Hospitalizations for infectious diseases in Jewish and Bedouin children in southern Israel. *Eur J Epidemiol* 1998;**14**:179–86.
324. Li S, Taylor KB, Kelly SJ, et al. Vitamin C inhibits the enzymatic activity of *Streptococcus pneumoniae* hyaluronate lyase. *J Biol Chem* 2001;**276**:15125–30.
325. Lieu TA, Ray GT, Black SB, et al. Projected cost-effectiveness of pneumococcal conjugate vaccination of healthy infants and young children. *JAMA* 2000;**283**:1460–8.
326. Light RW, Girard WM, Jenkinson SG, et al. Parapneumonic effusions. *Am J Med* 1980;**69**:507–12.
327. Lim PL, Lau YL. Occurrence of IgG subclass antibodies to ovalbumin, avidin, and pneumococcal polysaccharide in children. *Int Arch Allergy Immunol* 1994;**104**:137–43.
328. Lin J, Vambutas A, Haruta A, et al. Pneumococcus activation of the 5-lipoxygenase pathway and production of glycoproteins in the middle ear of rats. *J Infect Dis* 1999;**179**:1145–51.
329. Lin PL, Michaels MG, Green M, et al. Safety and immunogenicity of the American Academy of Pediatrics-recommended sequential pneumococcal conjugate and polysaccharide vaccine schedule in pediatric solid organ transplant recipients. *Pediatrics* 2005;**116**: 160–7.
330. Lockhart NJ, Daly KA, Lindgren BR, et al. Low cord blood type 14 pneumococcal IgG1 but not IgG2 antibody predicts early infant otitis media. *J Infect Dis* 2000;**181**:1979–82.
331. Lockhart SP, Hackell JG, Fritzell B. Pneumococcal conjugate vaccines: emerging clinical information and its implications. *Expert Rev Vaccines* 2006;**5**:553–64.
332. Loda FA, Collier AM, Glezen WP, et al. Occurrence of *Diplococcus pneumoniae* in the upper respiratory tract of children. *J Pediatr* 1975;**87**:1087–93.
333. Loeffler JM, Ringer R, Hablutzel M, et al. The free radical scavenger alpha-phenyl-tert-butyl nitrone aggravates hippocampal apoptosis and learning deficits in experimental pneumococcal meningitis. *J Infect Dis* 2001;**183**:247–52.
334. Lopez B, Cima MD, Vazquez F, et al. Epidemiological study of *Streptococcus pneumoniae* carriers in healthy primary-school children. *Eur J Clin Microbiol Infect Dis* 1999;**18**:771–6.
335. Loughlin AM, Hsu K, Silverio A, et al. *Impact of PCV13 vaccine on pneumococcal carriage in Massachusetts children*. Poster 176, ISPPD 8, Iguazo Falls, Brazil. 2012.
336. Lucas AH, Moulton KD, Tang VR, et al. Combinatorial library cloning of human antibodies to *Streptococcus pneumoniae* capsular polysaccharides: variable region primary structures and evidence for somatic mutation of Fab fragments specific for capsular serotypes 6B, 14, and 23F. *Infect Immun* 2001;**69**:853–64.
337. Macleod CM, Hodges RG, Heidelberger M, Bernhard WG. Prevention of pneumococcal pneumonia by immunization with specific capsular polysaccharides. *J Exp Med* 1945;**82**:445–65.
338. Madhi S, Pelton SI. Epidemiology, diagnosis and treatment of serious pneumococcal infections in children from pneumococcal vaccines. In: Siber GR, Klugman KP, Makela PH, editors. *The impact of conjugate vaccine*. Washington, DC: ASM Press; 2008. p. 95–108.
339. Madhi SA, Heera JR, Kuwanda L, et al. Use of procalcitonin and C-reactive protein to evaluate vaccine efficacy against pneumonia. *PLoS Med* 2005;**2**:e38.
340. Madhi SA, Ludewick H, Kuwanda L, et al. Pneumococcal coinfection with human metapneumovirus. *J Infect Dis* 2006;**193**: 1236–43.
341. Madhi SA, Petersen K, Madhi A, et al. Increased disease burden and antibiotic resistance of bacteria causing severe community-acquired lower respiratory tract infections in human immunodeficiency virus type 1-infected children. *Clin Infect Dis* 2000;**31**: 170–6.
342. Madhi SA, Petersen K, Madhi A, et al. Impact of human immunodeficiency virus type 1 on the disease spectrum of *Streptococcus pneumoniae* in South African children. *Pediatr Infect Dis J* 2000; **19**:1141–7.
343. Mahon BE, Hsu K, Karumuri S, et al; U.S. Pediatric Multicenter Pneumococcal Surveillance Group; Massachusetts Department of Public Health Epidemiologists. Effectiveness of abbreviated and delayed 7-valent pneumococcal conjugate vaccine dosing regimens. *Vaccine* 2006;**24**:2514–20.
344. Maiden MC, Bygraves JA, Feil E, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc Natl Acad Sci U S A* 1998;**95**:3140–5.
345. Majcherczyk PA, Langen H, Heumann D, et al. Digestion of *Streptococcus pneumoniae* cell walls with its major peptidoglycan hydrolase releases branched stem peptides carrying proinflammatory activity. *J Biol Chem* 1999;**274**:12537–43.
346. Makela PH, Leinonen M, Pukander J, et al. A study of the pneumococcal vaccine in prevention of clinically acute attacks of recurrent otitis media. *Rev Infect Dis* 1981;**3**(Suppl.):S124–32.
347. Malley R, Stack AM, Ferretti ML, et al. Anticapsular polysaccharide antibodies and nasopharyngeal colonization with *Streptococcus pneumoniae* in infant rats. *J Infect Dis* 1998;**178**:878–82.
348. Mandell GL, Coleman EJ. Activities of antimicrobial agents against intracellular pneumococci. *Antimicrob Agents Chemother* 2000;**44**:2561–3.
349. Mao C, Harper M, McIntosh K, et al. Invasive pneumococcal infections in human immunodeficiency virus-infected children. *J Infect Dis* 1996;**173**:870–6.
350. Marchese A, Esposito S, Coppo E, et al. Detection of *Streptococcus pneumoniae* and identification of pneumococcal serotypes by real-time polymerase chain reaction using blood samples from Italian children </= 5 years of age with community-acquired pneumonia. *Microb Drug Resist* 2011;**17**:419–24.
351. Margolis E, Yates A, Levin BR. The ecology of nasal colonization of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Staphylococcus aureus:* the role of competition and interactions with host's immune response. *BMC Microbiol* 2010;**10**:59.
352. Mbelle N, Huebner RE, Wasas AD, et al. Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. *J Infect Dis* 1999;**180**:1171–6.
353. McCarthy PL, Grundy GW, Spiesel SZ, et al. Bacteremia in children: an outpatient clinical review. *Pediatrics* 1976;**57**:861–8.
354. McCarthy PL, Jekel JF, Stashwick CA, et al. Further definition of history and observation variables in assessing febrile children. *Pediatrics* 1981;**67**:687–93.
355. McCullers JA, English BK, Novak R. Isolation and characterization of vancomycin-tolerant *Streptococcus pneumoniae* from the cerebrospinal fluid of a patient who developed recrudescent meningitis. *J Infect Dis* 2000;**181**:369–73.
356. McGee L, McDougal L, Zhou J, et al. Nomenclature of major antimicrobial-resistant clones of *Streptococcus pneumoniae* defined by the pneumococcal molecular epidemiology network. *J Clin Microbiol* 2001;**39**:2565–71.
357. McIntosh K. Community-acquired pneumonia in children. *N Engl J Med* 2002;**346**:429–37.
358. Melander E, Molstad S, Persson K, et al. Previous antibiotic consumption and other risk factors for carriage of penicillin-resistant *Streptococcus pneumoniae* in children. *Eur J Clin Microbiol Infect Dis* 1998;**17**:834–8.
359. Michelow IC, Lozano J, Olsen K, et al. Diagnosis of *Streptococcus pneumoniae* lower respiratory infection in hospitalized children by

culture, polymerase chain reaction, serological testing, and urinary antigen detection. *Clin Infect Dis* 2002;**34**:E1–11.
360. Miernyk KM, Parkinson AJ, Rudolph KM, et al. Immunogenicity of a heptavalent pneumococcal conjugate vaccine in Apache and Navajo Indian, Alaska native, and non-native American children aged <2 years. *Clin Infect Dis* 2000;**31**:34–41.
361. Millar EV, Watt JP, Bronsdon MA, et al. Indirect effect of 7-valent pneumococcal conjugate vaccine on pneumococcal colonization among unvaccinated household members. *Clin Infect Dis* 2008; **47**:989–96.
362. Miller E, Andrews NJ, Waight PA, et al. Effectiveness of the new serotypes in the 13-valent pneumococcal conjugate vaccine. *Vaccine* 2011;**29**:9127–31.
363. Miller E, Andrews NJ, Waight PA, et al. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. *Lancet Infect Dis* 2011;**11**:760–8.
364. Mitchell T. *Streptococcus pneumoniae:* virulence factors and variation [Abstract 519]. ICAAC 2012, San Francisco, Calif. 2012.
365. Mitten MJ, Meulbroek J, Nukkala M, et al. Efficacies of ABT-773, a new ketolide, against experimental bacterial infections. *Antimicrob Agents Chemother* 2001;**45**:2585–93.
366. Moffitt KL, Malley R. Next generation pneumococcal vaccines. *Curr Opin Immunol* 2011;**23**:407–13.
367. Molyneux E, Walsh A, Phiri A, et al. Acute bacterial meningitis in children admitted to the Queen Elizabeth Central Hospital, Blantyre, Malawi in 1996-97. *Trop Med Int Health* 1998;**3**:610–18.
368. Moore D, Nelson M, Henderson D. Pneumococcal vaccination and HIV infection. *Int J STD AIDS* 1998;**9**:1–7.
369. Moore M, Link-Gelles, Farley MM, et al. *Impact of 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease, U.S, 2010-11* [Abstract 1219]. Presented at IDWEEK San Diego, California, 2012.
370. Morona JK, Miller DC, Coffey TJ, et al. Molecular and genetic characterization of the capsule biosynthesis locus of *Streptococcus pneumoniae* type 23F. *Microbiology* 1999;**145**(Pt 4):781–9.
371. Morrison KE, Lake D, Crook J, et al. Confirmation of psaA in all 90 serotypes of *Streptococcus pneumoniae* by PCR and potential of this assay for identification and diagnosis. *J Clin Microbiol* 2000;**38**:434–7.
372. Morrison RR, Saulsbury FT. Acute hemorrhagic edema of infancy associated with pneumococcal bacteremia. *Pediatr Infect Dis J* 1999;**18**:832–3.
373. Mortier-Barriere I, Humbert O, Martin B, et al. Control of recombination rate during transformation of *Streptococcus pneumoniae:* an overview. *Microb Drug Resist* 1997;**3**:233–42.
374. Moscoso M, Domenech M, Garcia E. Vancomycin tolerance in clinical and laboratory *Streptococcus pneumoniae* isolates depends on reduced enzyme activity of the major LytA autolysin or cooperation between CiaH histidine kinase and capsular polysaccharide. *Mol Microbiol* 2010. [Epub ahead of print].
375. Mueller JE. Conjugate vaccine introduction in the African meningitis belt: meeting surveillance objectives. *Trop Med Int Health* 2013;**18**:58–64.
376. Mufson MA, Oley G, Hughey D. Pneumococcal disease in a medium-sized community in the United States. *JAMA* 1982; **248**:1486–9.
377. Mulholland K. Global burden of acute respiratory infections in children: implications for interventions. *Pediatr Pulmonol* 2003;**36**: 469–74.
378. Munoz R, Fenoll A, Vicioso D, et al. Optochin-resistant variants of *Streptococcus pneumoniae*. *Diagn Microbiol Infect Dis* 1990;**13**: 63–6.
379. Musher DM. Streptococcus pneumoniae. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious disease*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 2128–147.
380. Musher D, Dagan R. Is the pneumococcus the one and only in acute otitis media? *Pediatr Infect Dis J* 2000;**19**:399–400.
381. Musher DM, Groover JE, Reichler MR, et al. Emergence of antibody to capsular polysaccharides of *Streptococcus pneumoniae* during outbreaks of pneumonia: association with nasopharyngeal colonization. *Clin Infect Dis* 1997;**24**:441–6.
382. Musher DM, Groover JE, Rowland JM, et al. Antibody to capsular polysaccharides of *Streptococcus pneumoniae:* prevalence, persistence, and response to revaccination. *Clin Infect Dis* 1993; **17**:66–73.
383. Musher DM, Phan HM, Baughn RE. Protection against bacteremic pneumococcal infection by antibody to pneumolysin. *J Infect Dis* 2001;**183**:827–30.
384. Musher DM, Phan HM, Watson DA, et al. Antibody to capsular polysaccharide of *Streptococcus pneumoniae* at the time of hospital admission for pneumococcal pneumonia. *J Infect Dis* 2000;**182**: 158–67.
385. Nachman S, Kim S, King J, et al; Pediatric AIDS Clinical Trials Group Study 292 Team. Safety and immunogenicity of a heptavalent pneumococcal conjugate vaccine in infants with human immunodeficiency virus type 1 infection. *Pediatrics* 2003;**112**: 66–73.
386. Nafstad P, Hagen JA, Oie L, et al. Day care centers and respiratory health. *Pediatrics* 1999;**103**:753–8.
387. Neuman HB, Wald ER. Bacterial meningitis in childhood at the Children's Hospital of Pittsburgh: 1988-1998. *Clin Pediatr (Phila)* 2001;**40**:595–600.
388. Nigrovic LE, Kuppermann N, Macias CG, et al; Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics. Clinical prediction rule for identifying children with cerebrospinal fluid pleocytosis at very low risk of bacterial meningitis. *JAMA* 2007;**297**:52–60.
389. Nilsson P, Laurell MH. Carriage of penicillin-resistant *Streptococcus pneumoniae* by children in day-care centers during an intervention program in Malmo, Sweden. *Pediatr Infect Dis J* 2001;**20**: 1144–9.
390. Novak R, Henriques B, Charpentier E, et al. Emergence of vancomycin tolerance in *Streptococcus pneumoniae*. *Nature* 1999;**399**: 590–3.
391. Nowak-Wegrzyn A, Winkelstein JA, Swift AJ, et al. Serum opsonic activity in infants with sickle-cell disease immunized with pneumococcal polysaccharide protein conjugate vaccine. The Pneumococcal Conjugate Vaccine Study Group. *Clin Diagn Lab Immunol* 2000;**7**:788–93.
392. Nunes MC, von Gottberg A, de Gouveia L, et al. The impact of antiretroviral treatment on the burden of invasive pneumococcal disease in South African children: a time series analysis. *AIDS* 2011;**25**:453–62.
393. Nuorti JP, Butler JC, Farley MM, et al. Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. *N Engl J Med* 2000;**342**:681–9.
394. O'Brien KL, Hochman M, Goldblatt D. Combined schedules of pneumococcal conjugate and polysaccharide vaccines: is hyporesponsiveness an issue? *Lancet Infect Dis* 2007;**7**:597–606.
395. O'Brien KL, Moulton LH, Reid R, et al. Efficacy and safety of seven-valent conjugate pneumococcal vaccine in American Indian children: group randomised trial. *Lancet* 2003;**362**:355–61.
396. O'Brien KL, Swift AJ, Winkelstein JA, et al. Safety and immunogenicity of heptavalent pneumococcal vaccine conjugated to CRM(197) among infants with sickle cell disease. Pneumococcal Conjugate Vaccine Study Group. *Pediatrics* 2000;**106**:965–72.
397. O'Brien KL, Bronsdon M, Carlone GM, et al. Effect of a seven valent pneumococcal conjugate vaccine on nasopharyngeal (NP) carriage among native American infants [Abstract 1463]. Pediatric Academic Societies Annual Meeting. *Pediatr Res* 2001; **49**:256.
398. O'Brien KL, Croll J, Parkinson AJ, et al. Active laboratory-based surveillance for invasive *Streptococcus pneumoniae* (pneumococcus) among Navajo people in the American southwest, 1989-1996 [Abstract 1187]. Presented at the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, 1999, p. 678.
399. Obaro SK, Adegbola RA, Banya WA, et al. Carriage of pneumococci after pneumococcal vaccination. *Lancet* 1996;**348**:271–2.
400. O'Dempsey TJ, McArdle TF, Lloyd-Evans N, et al. Pneumococcal disease among children in a rural area of west Africa. *Pediatr Infect Dis J* 1996;**15**:431–7.
401. Okitsu N, Yano H, Ohshima H, et al. Binax NOW® *Streptococcus pneumoniae* test of middle ear fluid for detecting causative pathogens in children with acute otitis media. *J Microbiol Methods* 2011;**84**:341–2.
402. Olson LC, Jackson MA. Only the pneumococcus. *Pediatr Infect Dis J* 1999;**18**:849–50.

403. Ongkasuwan J, Valdez TA, Hulten KG, et al. Pneumococcal mastoiditis in children and the emergence of multidrug-resistant serotype 19A isolates. *Pediatrics* 2008;**122**:34–9.
404. Ort S, Ryan JL, Barden G, et al. Pneumococcal pneumonia in hospitalized patients: clinical and radiological presentations. *JAMA* 1983;**249**:214–18.
405. Ostergaard C, Benfield T, Gesser B, et al. Pretreatment with granulocyte colony-stimulating factor attenuates the inflammatory response but not the bacterial load in cerebrospinal fluid during experimental pneumococcal meningitis in rabbits. *Infect Immun* 1999;**67**:3430–6.
406. Ostergaard C, Yieng-Kow RV, Benfield T, et al. Inhibition of leukocyte entry into the brain by the selectin blocker fucoidin decreases interleukin-1 (IL-1) levels but increases IL-8 levels in cerebrospinal fluid during experimental pneumococcal meningitis in rabbits. *Infect Immun* 2000;**68**:3153–7.
407. Paganini H, Guinazu JR, Hernandez C, et al. Comparative analysis of outcome and clinical features in children with pleural empyema caused by penicillin-nonsusceptible and penicillin-susceptible *Streptococcus pneumoniae*. *Int J Infect Dis* 2001;**5**:86–8.
408. Palmu AA, Verho J, Jokinen J, et al. The seven-valent pneumococcal conjugate vaccine reduces tympanostomy tube placement in children. *Pediatr Infect Dis J* 2004;**23**:732–8.
409. Parent du Chatelet I, Traore Y, Gessner BD, et al. Bacterial meningitis in Burkina Faso: surveillance using field-based polymerase chain reaction testing. *Clin Infect Dis* 2005;**40**:17–25.
410. Park SY, Van Beneden CA, Pilishvili T, et al; Active Bacterial Core Surveillance Team. Invasive pneumococcal infections among vaccinated children in the United States. *J Pediatr* 2010;**156**:478–83, e2.
411. Pastor P, Medley F, Murphy TV. Invasive pneumococcal disease in Dallas County, Texas: results from population-based surveillance in 1995. *Clin Infect Dis* 1998;**26**:590–5.
412. Paterson NG, Baker EN. Structure of the full-length major pilin from *Streptococcus pneumoniae:* implications for isopeptide bond formation in gram-positive bacterial pili. *PLoS One* 2011;**6**:e22095.
413. Paton JC, Berry AM, Lock RA. Molecular analysis of putative pneumococcal virulence proteins. *Microb Drug Resist* 1997;**3**: 1–10.
414. Pearson HA. Prevention of pneumococcal disease in sickle cell anemia. *J Pediatr* 1996;**129**:788–9.
415. Pearson HA, Cornelius EA, Schwartz AD, et al. Transfusion-reversible functional asplenia in young children with sickle-cell anemia. *N Engl J Med* 1970;**283**:334–7.
416. Peled N, Yagupsky P. Improved detection of *Streptococcus pneumoniae* in middle-ear fluid cultures by use of a gentamicin-containing medium. *J Clin Microbiol* 1999;**37**:3415–16.
417. Peltola H. Burden of meningitis and other severe bacterial infections of children in Africa: implications for prevention. *Clin Infect Dis* 2001;**32**:64–75.
418. Pelton S. Prevention of acute and recurrent otitis media. *Lancet* 2000;**356**:1370–1.
419. Perez Retortillo JA, Marco F, Richard C, et al. Pneumococcal pericarditis with cardiac tamponade in a patient with chronic graft-versus-host disease. *Bone Marrow Transplant* 1998;**21**:299–300.
420. Peters VB, Diamant EP, Hodes DS, et al. Impaired immunity to pneumococcal polysaccharide antigens in children with human immunodeficiency virus infection immunized with pneumococcal vaccine. *Pediatr Infect Dis J* 1994;**13**:933–4.
421. Pichard E, Gillis D, Aker M, et al. Rebound fever in bacterial meningitis: role of dexamethasone dosage. *Isr J Med Sci* 1994; **30**:408–11.
422. Pichichero ME, Casey JR. Emergence of a multiresistant serotype 19A pneumococcal strain not included in the 7-valent conjugate vaccine as an otopathogen in children. *JAMA* 2007;**298**:1772–8.
423. Pihlajamäki M, Kotilainen P, Kaurila T, et al; Finnish Study Group for Antimicrobial Resistance (FiRe-Network). Macrolide-resistant *Streptococcus pneumoniae* and use of antimicrobial agents. *Clin Infect Dis* 2001;**33**:483–8.
424. Pilishvili T, Lexau C, Farley MM, et al; Active Bacterial Core Surveillance/Emerging Infections Program Network. Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. *J Infect Dis* 2010;**201**:32–41.
425. Ploton C, Freydiere AM, Benito Y, et al. *Streptococcus pneumoniae* thoracic empyema in children: rapid diagnosis by using the Binax NOW immunochromatographic membrane test in pleural fluids. *Pathol Biol (Paris)* 2006;**54**:498–501.
426. Poehling KA, Szilagyi PG, Grijalva CG, et al. Reduction of frequent otitis media and pressure-equalizing tube insertions in children after introduction of pneumococcal conjugate vaccine. *Pediatrics* 2007;**119**:707–15.
427. Poehling KA, Talbot TR, Griffin MR, et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA* 2006;**295**:1668–74.
428. Polizzotto MN, Jones P, Cameron PU, et al. The influence of splenectomy on the infectious complications and outcomes of people with HIV: marked, sustained elevation in risk of severe infection with bacteria including *Streptococcus pneumoniae*. *J Acquir Immune Defic Syndr* 2010;**55**:e24–6.
429. Poulsen K, Reinholdt J, Jespersgaard C, et al. A comprehensive genetic study of streptococcal immunoglobulin A1 proteases: evidence for recombination within and between species. *Infect Immun* 1998;**66**:181–90.
430. Principi N, Marchisio P, Schito GC, et al. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Ascanius Project Collaborative Group. *Pediatr Infect Dis J* 1999; **18**:517–23.
431. Prinz M, Kann O, Draheim HJ, et al. Microglial activation by components of gram-positive and -negative bacteria: distinct and common routes to the induction of ion channels and cytokines. *J Neuropathol Exp Neurol* 1999;**58**:1078–89.
432. Prober CG. The role of steroids in the management of children with bacterial meningitis. *Pediatrics* 1995;**95**:29–31.
433. Prymula R, Peeters P, Chrobok V, et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and non-typable *Haemophilus influenzae:* a randomised double-blind efficacy study. *Lancet* 2006;**367**:740–8.
434. Radetsky MS, Istre GR, Johansen TL, et al. Multiply resistant pneumococcus causing meningitis: its epidemiology within a day-care centre. *Lancet* 1981;**2**:771–3.
435. Ragsdale AR, Sanford JP. Interfering effect of incubation in carbon dioxide on the identification of pneumococci by optochin discs. *Appl Microbiol* 1971;**22**:854–5.
436. Ramirez M, Morrison DA, Tomasz A. Ubiquitous distribution of the competence related genes comA and comC among isolates of *Streptococcus pneumoniae*. *Microb Drug Resist* 1997;**3**:39–52.
437. Ramirez M, Severina E, Tomasz A. A high incidence of prophage carriage among natural isolates of *Streptococcus pneumoniae*. *J Bacteriol* 1999;**181**:3618–25.
438. Rapola S, Jantti V, Eerola M, et al. Anti-PsaA and the risk of pneumococcal AOM and carriage. *Vaccine* 2003;**21**:3608–13.
439. Rapola S, Jantti V, Haikala R, et al. Natural development of antibodies to pneumococcal surface protein A, pneumococcal surface adhesin A, and pneumolysin in relation to pneumococcal carriage and acute otitis media. *J Infect Dis* 2000;**182**:1146–52.
440. Rapola S, Kilpi T, Lahdenkari M, et al. Antibody response to the pneumococcal proteins pneumococcal surface adhesin A and pneumolysin in children with acute otitis media. *Pediatr Infect Dis J* 2001;**20**:482–7.
441. Rauch AM, O'Ryan M, Van R, et al. Invasive disease due to multiply resistant *Streptococcus pneumoniae* in a Houston, Tex, day-care center. *Am J Dis Child* 1990;**144**:923–7.
442. Rautonen J, Siimes MA, Lundstrom U, et al. Vaccination of children during treatment for leukemia. *Acta Paediatr Scand* 1986;**75**:579–85.
443. Reefhuis J, Honein MA, Whitney CG, et al. Risk of bacterial meningitis in children with cochlear implants. *N Engl J Med* 2003;**349**:435–45.
444. Reinert RR, Kaufhold A, Kuhnemund O, et al. Serum antibody responses to vaccination with 23-valent pneumococcal vaccine in splenectomized patients. *Zentralbl Bakteriol* 1994;**281**:481–90.
445. Rieux V, Carbon C, Azoulay-Dupuis E. Complex relationship between acquisition of beta-lactam resistance and loss of virulence in *Streptococcus pneumoniae*. *J Infect Dis* 2001;**184**:66–72.
446. Rijkers GT, Sanders EAM, Breukels MA, et al. Responsiveness of infants to capsular polysaccharides: implications for vaccine development. *Rev Med Microbiol* 1996;**7**:3–12.
447. Riley ID, Lehmann D, Alpers MP. Pneumococcal vaccine trials in Papua New Guinea: relationships between epidemiology of

pneumococcal infection and efficacy of vaccine. *Rev Infect Dis* 1991;**13**(Suppl. 6):S535–41.
448. Riley ID, Lehmann D, Alpers MP, et al. Pneumococcal vaccine prevents death from acute lower-respiratory-tract infections in Papua New Guinean children. *Lancet* 1986;**2**:877–81.
449. Rios AM, de la Hoz F, Leal AL, et al. [The impact of antimicrobial resistance and *Streptococcus pneumoniae* serotype distribution on the mortality of children under 5 years of age with invasive disease]. *Rev Panam Salud Publica* 1999;**5**:69–76.
450. Robbins JB, Austrian R, Lee CJ, et al. Considerations for formulating the second-generation pneumococcal capsular polysaccharide vaccine with emphasis on the cross-reactive types within groups. *J Infect Dis* 1983;**148**:1136–59.
451. Robbins JB, Schneerson R. Polysaccharide-protein conjugates: a new generation of vaccines. *J Infect Dis* 1990;**161**:821–32.
452. Robinson KA, Baughman W, Rothrock G, et al; Active Bacterial Core Surveillance/Emerging Infections Program Network. Epidemiology of invasive *Streptococcus pneumoniae* infections in the United States, 1995-1998: Opportunities for prevention in the conjugate vaccine era. *JAMA* 2001;**285**:1729–35.
453. Roca A, Sigauque B, Quinto L, et al. Invasive pneumococcal disease in children <5 years of age in rural Mozambique. *Trop Med Int Health* 2006;**11**:1422–31.
454. Rodriguez ME, van der Pol WL, Sanders LA, et al. Crucial role of FcgammaRIIa (CD32) in assessment of functional anti-*Streptococcus pneumoniae* antibody activity in human sera. *J Infect Dis* 1999;**179**:423–33.
455. Romero-Steiner S, Libutti D, Pais LB, et al. Standardization of an opsonophagocytic assay for the measurement of functional antibody activity against *Streptococcus pneumoniae* using differentiated HL-60 cells. *Clin Diagn Lab Immunol* 1997;**4**:415–22.
456. Rosen C, Christensen P, Hovelius B, et al. A longitudinal study of the nasopharyngeal carriage of pneumococci as related to pneumococcal vaccination in children attending day-care centres. *Acta Otolaryngol* 1984;**98**:524–32.
457. Rosen IA, Hakansson A, Aniansson G, et al. Antibodies to pneumococcal polysaccharides in human milk: lack of relationship to colonization and acute otitis media. *Pediatr Infect Dis J* 1996;**15**: 498–507.
458. Rosenow C, Ryan P, Weiser JN, et al. Contribution of novel choline-binding proteins to adherence, colonization and immunogenicity of *Streptococcus pneumoniae*. *Mol Microbiol* 1997;**25**: 819–29.
459. Rothrock SG, Green SM, Wren J, et al. Pediatric bacterial meningitis: is prior antibiotic therapy associated with an altered clinical presentation? *Ann Emerg Med* 1992;**21**:146–52.
460. Rovers MM, Zielhuis GA, Ingels K, et al. Day-care and otitis media in young children: a critical overview. *Eur J Pediatr* 1999; **158**:1–6.
461. Roy S, Knox K, Segal S, et al; Oxford Pneumoccocal Surveillance Group. MBL genotype and risk of invasive pneumococcal disease: a case-control study. *Lancet* 2002;**359**:1569–73.
462. Rubin LG, Mardy GV, Pais L, et al. Human anti-capsular concentration required for protection against experimental pneumococcal bacteremia [Abstract G47]. Presented at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, 1995, p. 166.
463. Ryan MJ, Kavanagh R, Wall PG, et al. Bacterial joint infections in England and Wales: analysis of bacterial isolates over a four year period. *Br J Rheumatol* 1997;**36**:370–3.
464. Rytel MW, Dailey MP, Schiffman G, et al. Pneumococcal vaccine immunization of patients with renal impairment. *Proc Soc Exp Biol Med* 1986;**182**:468–73.
465. Saha SK, Al Emran HM, Hossain B, et al; Pneumococcal Study Group. *Streptococcus pneumoniae* serotype-2 childhood meningitis in Bangladesh: a newly recognized pneumococcal infection threat. *PLoS One* 2012;**7**:e32134.
466. Saha SK, Darmstadt GL, Yamanaka N, et al. Rapid diagnosis of pneumococcal meningitis: implications for treatment and measuring disease burden. *Pediatr Infect Dis J* 2005;**24**:1093–8.
467. Salaun-Saraux P, Saraux A, Lepage P, et al. [Septic meningitis in children in Rwanda from 1983 to 1990: retrospective study at the Kigali Hospital Center]. *Med Trop (Mars)* 1995;**55**:41–5. In French.
468. Salazar JC, Daly KA, Giebink GS, et al. Low cord blood pneumococcal immunoglobulin G (IgG) antibodies predict early onset acute otitis media in infancy. *Am J Epidemiol* 1997;**145**: 1048–56.
469. Samore MH, Magill MK, Alder SC, et al. High rates of multiple antibiotic resistance in *Streptococcus pneumoniae* from healthy children living in isolated rural communities: association with cephalosporin use and intrafamilial transmission. *Pediatrics* 2001; **108**:856–65.
470. Sanders LA, Rijkers GT, Kuis W, et al. Defective antipneumococcal polysaccharide antibody response in children with recurrent respiratory tract infections. *J Allergy Clin Immunol* 1993;**91**: 110–19.
471. Sanders LA, Rijkers GT, Tenbergen-Meekes AM, et al. Immunoglobulin isotype-specific antibody responses to pneumococcal polysaccharide vaccine in patients with recurrent bacterial respiratory tract infections. *Pediatr Res* 1995;**37**:812–19.
472. Sard B, Bailey MC, Vinci R. An analysis of pediatric blood cultures in the postpneumococcal conjugate vaccine era in a community hospital emergency department. *Pediatr Emerg Care* 2006;**22**: 295–300.
473. Schaad UB, Krucko J, Pfenninger J. An extended experience with cefuroxime therapy of childhood bacterial meningitis. *Pediatr Infect Dis* 1984;**3**:410–16.
474. Schmidt H, Stuertz K, Bruck W, et al. Intravenous granulocyte colony-stimulating factor increases the release of tumour necrosis factor and interleukin-1beta into the cerebrospinal fluid, but does not inhibit the growth of *Streptococcus pneumoniae* in experimental meningitis. *Scand J Immunol* 1999;**49**:481–6.
475. Scholz CF, Poulsen K, Kilian M. Novel molecular method for identification of *Streptococcus pneumoniae* applicable to clinical microbiology and 16S rRNA sequence-based microbiome studies. *J Clin Microbiol* 2012;**50**:1968–73.
476. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. Active Surveillance Team. *N Engl J Med* 1997;**337**:970–6.
477. Schultz MJ, Wijnholds J, Peppelenbosch MP, et al. Mice lacking the multidrug resistance protein 1 are resistant to *Streptococcus pneumoniae*-induced pneumonia. *J Immunol* 2001;**166**:4059–64.
478. Schutze GE, Mason Jr EO, Barson WJ, et al. Invasive pneumococcal infections in children with asplenia. *Pediatr Infect Dis J* 2002; **21**:278–82.
479. Schutze GE, Mason Jr EO, Wald ER, et al. Pneumococcal infections in children after transplantation. *Clin Infect Dis* 2001;**33**: 16–21.
480. Scott JA, Hall AJ, Muyodi C, et al. Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. *Lancet* 2000;**355**:1225–30.
481. Seeler RA, Metzger W, Mufson MA. *Diplococcus pneumoniae* infections in children with sickle cell anemia. *Am J Dis Child* 1972; **123**:8–10.
482. Sell SH, Wright PF, Vaughn WK. Clinical studies of pneumococcal vaccines in infants, part I. Reactogenicity and immunogenicity of two polyvalent polysaccharide vaccines. *Rev Infect Dis* 1981; **3**(Suppl.):97–107.
483. Sen S, Lalitha MK, Fenn AS, et al. Primary peritonitis in children. *Ann Trop Paediatr* 1983;**3**:53–6.
484. Severin A, Severina E, Tomasz A. Abnormal physiological properties and altered cell wall composition in *Streptococcus pneumoniae* grown in the presence of clavulanic acid. *Antimicrob Agents Chemother* 1997;**41**:504–10.
485. Shackley F, Knox K, Morris JB, et al. Outcome of invasive pneumococcal disease: a UK based study. Oxford Pneumococcal Surveillance Group. *Arch Dis Child* 2000;**83**:231–3.
486. Shapiro ED, Wald ER, Brozanski BA. Periorbital cellulitis and paranasal sinusitis: a reappraisal. *Pediatr Infect Dis* 1982;**1**:91–4.
487. Shinefield H, Black S, Ray P, et al. Efficacy, immunogenicity and safety of heptavalent pneumococcal conjugate vaccine in low birth weight and preterm infants. *Pediatr Infect Dis J* 2002;**21**:182–6.
488. Shouval DS, Greenberg D, Givon-Lavi N, et al. Site-specific disease potential of individual *Streptococcus pneumoniae* serotypes in pediatric invasive disease, acute otitis media and acute conjunctivitis. *Pediatr Infect Dis J* 2006;**25**:602–7.
489. Shurin PA, Giebink GS, Wegman DL, et al. Prevention of pneumococcal otitis media in chinchillas with human bacterial polysaccharide immune globulin. *J Clin Microbiol* 1988;**26**:755–9.
490. Siber GR. Pneumococcal disease: prospects for a new generation of vaccines. *Science* 1994;**265**:1385–7.

491. Siber GR, Thompson C, Reid GR, et al. Evaluation of bacterial polysaccharide immune globulin for the treatment or prevention of *Haemophilus influenzae* type b and pneumococcal disease. *J Infect Dis* 1992;**165**(Suppl. 1):S129–33.
492. Siegel M, Timpone J. Penicillin-resistant *Streptococcus pneumoniae* endocarditis: a case report and review. *Clin Infect Dis* 2001;**32**: 972–4.
493. Simell B, Korkeila M, Pursiainen H, et al. Pneumococcal carriage and otitis media induce salivary antibodies to pneumococcal surface adhesin A, pneumolysin, and pneumococcal surface protein A in children. *J Infect Dis* 2001;**183**:887–96.
494. Simonsen L, Taylor RJ, Young-Xu Y, et al. Impact of pneumococcal conjugate vaccination of infants on pneumonia and influenza hospitalization and mortality in all age groups in the United States. *MBio* 2011;**2**:e00309–10.
495. Sirinavin S, Vorachit M, Thakkinstian A, et al. Pediatric invasive pneumococcal disease in a teaching hospital in Bangkok. *Int J Infect Dis* 2003;**7**:183–9.
496. Sirotnak AP, Eppes SC, Klein JD. Tuboovarian abscess and peritonitis caused by *Streptococcus pneumoniae* serotype 1 in young girls. *Clin Infect Dis* 1996;**22**:993–6.
497. Sloyer Jr JL, Howie VM, Ploussard JH, et al. Immune response to acute otitis media in children, part I. Serotypes isolated and serum and middle ear fluid antibody in pneumococcal otitis media. *Infect Immun* 1974;**9**:1028–32.
498. Sloyer Jr JL, Ploussard JH, Howie VM. Efficacy of pneumococcal polysaccharide vaccine in preventing acute otitis media in infants in Huntsville, Alabama. *Rev Infect Dis* 1981;**3**(Suppl.):S119–23.
499. Smillie WG, Warnock GH, White HJ. A study of a type I pneumococcus epidemic at the State Hospital at Worcester, Mass. *Am J Public Health Nations Health* 1938;**28**:293–302.
500. Smith AM, Klugman KP. Non-penicillin-binding protein-mediated high-level penicillin and cephalosporin resistance in a Hungarian clone of *Streptococcus pneumoniae*. *Microb Drug Resist* 2000;**6**:105–10.
501. Smith BL, Hostetter MK. C3 as substrate for adhesion of *Streptococcus pneumoniae*. *J Infect Dis* 2000;**182**:497–508.
502. Sniadack DH, Schwartz B, Lipman H, et al. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children-implications for vaccine strategies. *Pediatr Infect Dis J* 1995;**14**:503–10.
503. Soininen A, Lahdenkari M, Kilpi T, et al. Antibody response to pneumococcal capsular polysaccharides in children with acute otitis media. *Pediatr Infect Dis J* 2002;**21**:186–92.
504. Spellerberg B, Cundell DR, Sandros J, et al. Pyruvate oxidase, as a determinant of virulence in *Streptococcus pneumoniae*. *Mol Microbiol* 1996;**19**:803–13.
505. Spika JS, Halsey NA, Le CT, et al. Decline of vaccine-induced antipneumococcal antibody in children with nephrotic syndrome. *Am J Kidney Dis* 1986;**7**:466–70.
506. Stanek RJ, Mufson MA. A 20-year epidemiological study of pneumococcal meningitis. *Clin Infect Dis* 1999;**28**:1265–72.
507. Stein KE. Thymus-independent and thymus-dependent responses to polysaccharide antigens. *J Infect Dis* 1992;**165**(Suppl. 1):S49–52.
508. Stevens KE, Chang D, Zwack EE, et al. Competence in *Streptococcus pneumoniae* is regulated by the rate of ribosomal decoding errors. *MBio* 2011;**2**:e00071–11.
509. Stovall SH, Ainley KA, Mason Jr EO, et al. Invasive pneumococcal infections in pediatric cardiac transplant patients. *Pediatr Infect Dis J* 2001;**20**:946–50.
510. Strachan RE, Cornelius A, Gilbert GL, et al. Australian Research Network in Empyema. A bedside assay to detect streptococcus pneumoniae in children with empyema. *Pediatr Pulmonol* 2011;**46**: 179–83.
511. Straetemans M, Sanders EA, Veenhoven RH, et al. Review of randomized controlled trials on pneumococcal vaccination for prevention of otitis media. *Pediatr Infect Dis J* 2003;**22**:515–24.
512. Straetemans M, Sanders EA, Veenhoven RH, et al. Pneumococcal vaccines for preventing otitis media. *Cochrane Database Syst Rev* 2004;**(2)**:CD001480.
513. Strutton DR, Farkouh RA, Rubin JL, et al. Modeling the impact of the 13-valent pneumococcal conjugate vaccine serotype catch-up program using United States claims data. *BMC Infect Dis* 2012;**12**:175.
514. Styrt B. Infection associated with asplenia: risks, mechanisms, and prevention. *Am J Med* 1990;**88**:33N–42N.
515. Sutliff WD, Finland M. Antipneumococcic immunity reactions in individuals of different ages. *J Exp Med* 1932;**55**:837–52.
516. Syrjanen RK, Auranen KJ, Leino TM, et al. Pneumococcal acute otitis media in relation to pneumococcal nasopharyngeal carriage. *Pediatr Infect Dis J* 2005;**24**:801–6.
517. Syrogiannopoulos GA, Grivea IN, Beratis NG, et al. Resistance patterns of *Streptococcus pneumoniae* from carriers attending day-care centers in southwestern Greece. *Clin Infect Dis* 1997;**25**: 188–94.
518. Syrogiannopoulos GA, Ronchetti F, Dagan R, et al. Mediterranean clone of penicillin-susceptible, multidrug-resistant serotype 6B *Streptococcus pneumoniae* in Greece, Italy and Israel. *Int J Antimicrob Agents* 2000;**16**:219–24.
519. Tait-Kamradt A, Davies T, Appelbaum PC, et al. Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. *Antimicrob Agents Chemother* 2000;**44**:3395–401.
520. Takala AK, Jero J, Kela E, et al. Risk factors for primary invasive pneumococcal disease among children in Finland. *JAMA* 1995; **273**:859–64.
521. Takoudes TG, Haddad Jr J. Free radical production by antibiotic-killed bacteria in the guinea pig middle ear. *Laryngoscope* 2001; **111**:283–9.
522. Talbot UM, Paton AW, Paton JC. Uptake of *Streptococcus pneumoniae* by respiratory epithelial cells. *Infect Immun* 1996;**64**: 3772–7.
523. Talkington DF, Brown BG, Tharpe JA, et al. Protection of mice against fatal pneumococcal challenge by immunization with pneumococcal surface adhesin A (PsaA). *Microb Pathog* 1996;**21**: 17–22.
524. Tan TQ, Mason Jr EO, Barson WJ, et al. Clinical characteristics and outcome of children with pneumonia attributable to penicillin-susceptible and penicillin-nonsusceptible *Streptococcus pneumoniae*. *Pediatrics* 1998;**102**:1369–75.
525. Taylor SN, Sanders CV. Unusual manifestations of invasive pneumococcal infection. *Am J Med* 1999;**107**:12S–27S.
526. Teele DW, Klein JO, Bratton L, et al. Use of pneumococcal vaccine for prevention of recurrent acute otitis media in infants in Boston. The Greater Boston Collaborative Otitis Media Study Group. *Rev Infect Dis* 1981;**3**(Suppl.):S113–18.
527. Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first seven years of life in children in greater Boston: a prospective, cohort study. *J Infect Dis* 1989;**160**:83–94.
528. Teele DW, Marshall R, Klein JO. Unsuspected bacteremia in young children: a common and important problem. *Pediatr Clin North Am* 1979;**26**:773–84.
529. Teele DW, Pelton SI, Grant MJ, et al. Bacteremia in febrile children under 2 years of age: results of cultures of blood of 600 consecutive febrile children seen in a "walk-in" clinic. *J Pediatr* 1975;**87**:227–30.
530. Teitelbaum R, Lifshitz S, Ling E, et al. Vulnerability to Streptococcus pneumoniae infection: Difference between microbial strains and genetically determined host response in adult mice [Abstract 983]. Presented at the 41st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 2001, p. 52.
531. Temple K, Greenwood B, Inskip H, et al. Antibody response to pneumococcal capsular polysaccharide vaccine in African children. *Pediatr Infect Dis J* 1991;**10**:386–90.
532. Thigpen MC, Whitney CG, Messonnier NE, et al; Emerging Infections Programs Network. Bacterial meningitis in the United States, 1998-2007. *N Engl J Med* 2011;**364**:2016–25.
533. Toikka P, Virkki R, Mertsola J, et al. Bacteremic pneumococcal pneumonia in children. *Clin Infect Dis* 1999;**29**:568–72.
534. Tomasz A. *Streptococcus pneumoniae:* functional anatomy. In: Tomasz A, editor. *Streptococcus pneumoniae: molecular biology and mechanisms of disease*. Larchmont, NY: Mary Ann Liebert, Inc.; 2000. p. 9–21.
535. Tong HH, Blue LE, James MA, et al. Evaluation of the virulence of a *Streptococcus pneumoniae* neuraminidase-deficient mutant in nasopharyngeal colonization and development of otitis media in the chinchilla model. *Infect Immun* 2000;**68**:921–4.
536. Tong HH, Fisher LM, Kosunick GM, et al. Effect of tumor necrosis factor alpha and interleukin 1-alpha on the adherence of

Streptococcus pneumoniae to chinchilla tracheal epithelium. *Acta Otolaryngol* 1999;**119**:78–82.
537. Topley JM, Cupidore L, Vaidya S, et al. Pneumococcal and other infections in children with sickle-cell hemoglobin C (SC) disease. *J Pediatr* 1982;**101**:176–9.
538. Tsai CJ, Griffin MR, Nuorti JP, et al. Changing epidemiology of pneumococcal meningitis after the introduction of pneumococcal conjugate vaccine in the United States. *Clin Infect Dis* 2008;**46**: 1664–72.
539. Tu AH, Fulgham RL, McCrory MA, et al. Pneumococcal surface protein A inhibits complement activation by *Streptococcus pneumoniae*. *Infect Immun* 1999;**67**:4720–4.
540. Ubukata K, Asahi Y, Yamane A, et al. Combinational detection of autolysin and penicillin-binding protein 2B genes of *Streptococcus pneumoniae* by PCR. *J Clin Microbiol* 1996;**34**:592–6.
541. Uhari M, Kontiokari T, Koskela M, et al. Xylitol chewing gum in prevention of acute otitis media: double blind randomised trial. *BMJ* 1996;**313**:1180–4.
542. Uhari M, Kontiokari T, Niemela M. A novel use of xylitol sugar in preventing acute otitis media. *Pediatrics* 1998;**102**:879–84.
543. Uhari M, Mantysaari K, Niemela M. A meta-analytic review of the risk factors for acute otitis media. *Clin Infect Dis* 1996;**22**: 1079–83.
544. Ukkonen P, Varis K, Jernfors M, et al. Treatment of acute otitis media with an antiadhesive oligosaccharide: a randomised, double-blind, placebo-controlled trial. *Lancet* 2000;**356**:1398–402.
545. Umetsu DT, Ambrosino DM, Quinti I, et al. Recurrent sinopulmonary infection and impaired antibody response to bacterial capsular polysaccharide antigen in children with selective IgG-subclass deficiency. *N Engl J Med* 1985;**313**:1247–51.
546. Vakevainen M, Eklund C, Eskola J, et al. Cross-reactivity of antibodies to type 6B and 6A polysaccharides of *Streptococcus pneumoniae*, evoked by pneumococcal conjugate vaccines, in infants. *J Infect Dis* 2001;**184**:789–93.
547. Vakevainen M, Jansen W, Saeland E, et al. Are the opsonophagocytic activities of antibodies in infant sera measured by different pneumococcal phagocytosis assays comparable? *Clin Diagn Lab Immunol* 2001;**8**:363–9.
548. van Hoek AJ, Andrews N, Waight PA, et al. Effect of serotype on focus and mortality of invasive pneumococcal disease: coverage of different vaccines and insight into non-vaccine serotypes. *PLoS One* 2012;**7**:e39150.
549. Van Zuijlen DA, Schilder AG, Van Balen FA, et al. National differences in incidence of acute mastoiditis: relationship to prescribing patterns of antibiotics for acute otitis media? *Pediatr Infect Dis J* 2001;**20**:140–4.
550. Vanderkooi OG, Church DL, MacDonald J, et al. Community-based outbreaks in vulnerable populations of invasive infections caused by *Streptococcus pneumoniae* serotypes 5 and 8 in Calgary, Canada. *PLoS One* 2011;**6**:e28547.
551. Varon E, Levy C, DeLa Rocque F, et al. Impact of antimicrobial therapy on nasopharyngeal carriage of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Branhamella catarrhalis* in children with respiratory tract infections. *Clin Infect Dis* 2000;**31**:477–81.
552. Vergison A, Dagan R, Arguedas A, et al. Otitis media and its consequences: beyond the earache. *Lancet Infect Dis* 2010;**10**: 195–203.
553. Vernacchio L, Neufeld EJ, MacDonald K, et al. Combined schedule of 7-valent pneumococcal conjugate vaccine followed by 23-valent pneumococcal vaccine in children and young adults with sickle cell disease. *J Pediatr* 1998;**133**:275–8.
554. Vidarsson G, Sigurdardottir ST, Gudnason T, et al. Isotypes and opsonophagocytosis of pneumococcus type 6B antibodies elicited in infants and adults by an experimental pneumococcus type 6B-tetanus toxoid vaccine. *Infect Immun* 1998;**66**:2866–70.
555. Vives M, Garcia ME, Saenz P, et al. Nasopharyngeal colonization in Costa Rican children during the first year of life. *Pediatr Infect Dis J* 1997;**16**:852–8.
556. Vollmer W, Tomasz A. The *pgdA* gene encodes for a peptidoglycan *N*-acetylglucosamine deacetylase in *Streptococcus pneumoniae*. *J Biol Chem* 2000;**275**:20496–501.
557. Vollmer W, Tomasz A. Identification of the teichoic acid phosphorylcholine esterase in *Streptococcus pneumoniae*. *Mol Microbiol* 2001;**39**:1610–22.
558. Voss L, Lennon D, Okesene-Gafa K, et al. Invasive pneumococcal disease in a pediatric population, Auckland, New Zealand. *Pediatr Infect Dis J* 1994;**13**:873–8.
559. Vuori-Holopainen E, Peltola H. Reappraisal of lung tap: review of an old method for better etiologic diagnosis of childhood pneumonia. *Clin Infect Dis* 2001;**32**:715–26.
560. Wald ER. Conjunctivitis in infants and children. *Pediatr Infect Dis J* 1997;**16**:S17–20.
561. Wald ER. Diagnosis and management of sinusitis in children. *Semin Pediatr Infect Dis* 1998;**9**:4–11.
562. Wald ER, Milmoe GJ, Bowen A, et al. Acute maxillary sinusitis in children. *N Engl J Med* 1981;**304**:749–54.
563. Wall RA, Corrah PT, Mabey DC, et al. The etiology of lobar pneumonia in the Gambia. *Bull World Health Organ* 1986;**64**: 553–8.
564. Wals PD, Carbon M, Sevin E, et al. Reduced physician claims for otitis media after implementation of pneumococcal conjugate vaccine program in the province of Quebec, Canada. *Pediatr Infect Dis J* 2009;**28**:e271–5.
565. Walter ND, Taylor Jr TH, Dowell SF, et al; Active Bacterial Core Surveillance System Team. Holiday spikes in pneumococcal disease among older adults. *N Engl J Med* 2009;**361**:2584–5.
566. Wara DW. Host defense against *Streptococcus pneumoniae:* the role of the spleen. *Rev Infect Dis* 1981;**3**:299–309.
567. Waterer GW, Jennings SG, Wunderink RG. The impact of blood cultures on antibiotic therapy in pneumococcal pneumonia. *Chest* 1999;**116**:1278–81.
568. Watson DA, Musher DM, Jacobson JW, et al. A brief history of the pneumococcus in biomedical research: a panoply of scientific discovery. *Clin Infect Dis* 1993;**17**:913–24.
569. Weber B, Ehlert K, Diehl A, et al. The fib locus in *Streptococcus pneumoniae* is required for peptidoglycan crosslinking and PBP-mediated beta-lactam resistance. *FEMS Microbiol Lett* 2000;**188**: 81–5.
570. Weber MW, Herman J, Jaffar S, et al. Clinical predictors of bacterial meningitis in infants and young children in The Gambia. *Trop Med Int Health* 2002;**7**:722–31.
571. Weinberger DM, Harboe ZB, Sanders EA, et al. Association of serotype with risk of death due to pneumococcal pneumonia: a meta-analysis. *Clin Infect Dis* 2010;**51**:692–9.
572. Weiser JN. Phase variation in colony opacity by *Streptococcus pneumoniae*. *Microb Drug Resist* 1998;**4**:129–35.
573. Weiss A, Brinser JH, Nazar-Stewart V. Acute conjunctivitis in childhood. *J Pediatr* 1993;**122**:10–14.
574. Werno AM, Christner M, Anderson TP, et al. Differentiation of *Streptococcus pneumoniae* from nonpneumococcal streptococci of the *Streptococcus mitis* group by matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2012;**50**:2863–7.
575. Weycker D, Farkouh R, Shea K, et al. Excess risk of invasive pneumococcal disease and pneumonia in persons with asthma or diabetes [Abstract 677]. Presented at IDSA Week, October 2012, San Diego, California.
576. White B. *The biology of pneumococcus*. Cambridge, Mass: Commonwealth Fund, Harvard University Press; 1938.
577. Whitney CG, Farley MM, Hadler J, et al; Active Bacterial Core Surveillance/Emerging Infections Program Network. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N Engl J Med* 2003;**348**: 1737–46.
578. Whitney CG, Pilishvili T, Farley MM, et al. Effectiveness of seven-valent pneumococcal conjugate vaccine against invasive pneumococcal disease: a matched case-control study. *Lancet* 2006; **368**:1495–502.
579. Widdowson CA, Klugman KP. Molecular mechanisms of resistance to commonly used non-betalactam drugs in *Streptococcus pneumoniae*. *Semin Respir Infect* 1999;**14**:255–68.
580. Williams BG, Gouws E, Boschi-Pinto C, et al. Estimates of world-wide distribution of child deaths from acute respiratory infections. *Lancet Infect Dis* 2002;**2**:25–32.
581. Winkelstein JA, Drachman RH. Deficiency of pneumococcal serum opsonizing activity in sickle-cell disease. *N Engl J Med* 1968;**279**:459–66.
582. Witt CS, Pomat W, Lehmann D, et al. Antibodies to pneumococcal polysaccharides in pneumonia and response to pneumococcal

vaccination in young children in Papua New Guinea. *Clin Exp Immunol* 1991;**83**:219–24.
583. Wong WY, Overturf GD, Powars DR. Infection caused by *Streptococcus pneumoniae* in children with sickle cell disease: epidemiology, immunologic mechanisms, prophylaxis, and vaccination. *Clin Infect Dis* 1992;**14**:1124–36.
584. Woods ER, Merola JL, Bithoney WG, et al. Bacteremia in an ambulatory setting: improved outcome in children treated with antibiotics. *Am J Dis Child* 1990;**144**:1195–9.
585. World Health Organization, Department of Vaccines and Biologicals. *Standardization of interpretation of chest radiographs for the diagnosis of pneumonia in children*. Geneva: World Health Organization, 2001.
586. World Health Organization. Pneumococcal vaccines. WHO position paper-2012 recommendations. *Vaccine* 2012;**30**:4717–18.
587. Wright AE, Morgan WP, Colebrook L, et al. Observations on prophylactic inoculation against pneumococcus infections, and on the results which have been achieved by it. *Lancet* 1914;**1**:87–95.
588. Wright AK, Ferreira DM, Gritzfeld JF, et al. Human nasal challenge with *Streptococcus pneumoniae* is immunising in the absence of carriage. *PLoS Pathog* 2012;**8**:e1002622.
589. Wroe PC, Lee GM, Finkelstein JA, et al. Pneumococcal carriage and antibiotic resistance in young children before 13-valent conjugate vaccine. *Pediatr Infect Dis J* 2012;**31**:249–54.
590. Xu Q, Kaur R, Casey JR, et al. Identification of *Streptococcus pneumoniae* and *Haemophilus influenzae* in culture-negative middle ear fluids from children with acute otitis media by combination of multiplex PCR and multi-locus sequencing typing. *Int J Pediatr Otorhinolaryngol* 2011;**75**:239–44.
591. Yagupsky P, Porat N, Fraser D, et al. Acquisition, carriage, and transmission of pneumococci with decreased antibiotic susceptibility in young children attending a day care facility in southern Israel. *J Infect Dis* 1998;**177**:1003–12.
592. Yildirim I, Stevenson A, Hsu K, et al. Invasive pneumococcal disease (IPD) surveillance in Massachusetts: evolving impact of PCV13 in children <2 years of age. Poster 186. Presented at ISPPD8, Iguazo Falls, Brazil, March 2012.
593. Yildirim I, Stevenson A, Hsu K, et al. Evolving picture of invasive pneumococcal disease in Massachusetts children: a comparison of disease in 2007-2009 with earlier periods. *Pediatr Infect Dis J* 2012;**31**:1016–21.
594. Yother J, Leopold K, White J, et al. Generation and properties of a *Streptococcus pneumoniae* mutant which does not require choline or analogs for growth. *J Bacteriol* 1998;**180**:2093–101.
595. Yu J, Bryant AP, Marra A, et al. Characterization of the *Streptococcus pneumoniae* NADH oxidase that is required for infection. *Microbiology* 2001;**147**:431–8.
596. Zangwill KM, Vadheim CM, Vannier AM, et al. Epidemiology of invasive pneumococcal disease in southern California: Implications for the design and conduct of a pneumococcal conjugate vaccine efficacy trial. *J Infect Dis* 1996;**174**:752–9.
597. Zarkowsky HS, Gallagher D, Gill FM, et al. Bacteremia in sickle hemoglobinopathies. *J Pediatr* 1986;**109**:579–85.
598. Zenni MK, Cheatham SH, Thompson JM, et al. *Streptococcus pneumoniae* colonization in the young child: association with otitis media and resistance to penicillin. *J Pediatr* 1995;**127**:533–7.
599. Zhang JR, Mostov KE, Lamm ME, et al. The polymeric immunoglobulin receptor translocates pneumococci across human nasopharyngeal epithelial cells. *Cell* 2000;**102**:827–37.
600. Zhang Q, Arnaoutakis K, Murdoch C, et al. Mucosal immune responses to capsular pneumococcal polysaccharides in immunized preschool children and controls with similar nasal pneumococcal colonization rates. *Pediatr Infect Dis J* 2004;**23**:307–13.
601. Zhang Y, Masi AW, Barniak V, et al. Recombinant PhpA protein, a unique histidine motif-containing protein from *Streptococcus pneumoniae*, protects mice against intranasal pneumococcal challenge. *Infect Immun* 2001;**69**:3827–36.
602. Zhou F, Shefer A, Kong Y, et al. Trends in acute otitis media-related health care utilization by privately insured young children in the United States, 1997-2004. *Pediatrics* 2008;**121**:253–60.
603. Zopf D, Roth S. Oligosaccharide anti-infective agents. *Lancet* 1996;**347**:1017–21.
604. Zwijnenburg PJ, van der Poll T, Florquin S, et al. Experimental pneumococcal meningitis in mice: a model of intranasal infection. *J Infect Dis* 2001;**183**:1143–6.
605. Zysk G, Bongaerts RJ, ten Thoren E, et al. Detection of 23 immunogenic pneumococcal proteins using convalescent-phase serum. *Infect Immun* 2000;**68**:3740–3.
606. Zysk G, Schneider-Wald BK, Hwang JH, et al. Pneumolysin is the main inducer of cytotoxicity to brain microvascular endothelial cells caused by *Streptococcus pneumoniae*. *Infect Immun* 2001;**69**:845–52.

dairy products and wine.[56] They are used in the food industry as starter cultures in food production.[37,69] Studies using culture methods have suggested that, although they occasionally are recovered from vaginal swabs in healthy individuals[60] and from mucosal surfaces in some hospitalized individuals,[30] *Leuconostoc* spp. are not part of the normal human flora.[3] Case reports of pediatric infection began to appear in the 1980s.[14,31,35,53,76]

Pathophysiology

Leuconostoc spp. rarely are pathogenic. Underlying disease states or compromised immune system, gastrointestinal tract disease (especially short-gut syndrome), previous or current antibiotic therapy, venous or gastrointestinal tract access devices, recent invasive procedures, and infancy are thought to be risk factors.[16,18,30,56] In patients with short-gut syndrome, the presence of central venous catheters and disrupted bowel mucosa are likely the most important risks.[22] Frequently, the organisms are isolated as part of a polymicrobial infection after a patient has been treated with vancomycin.[19] Of the first 21 cases reported in the English literature, 12 were in children, 10 of which occurred in patients younger than 1 year old. Documented portals of entry include central lines,[3,31] peritoneal dialysis and urinary catheters,[8] and gastrostomy tubes.[35] A case of nosocomial meningitis apparently caused by frequent lumbar puncture after intracranial hemorrhage has been reported.[32] Contaminated enteral formula also has been implicated.[9,35,51] One outbreak involving 42 patients in Spain was traced to contaminated total parenteral nutrition solution.[4] Occasional sporadic cases without a known risk factor have been described.[14,30]

Clinical Manifestations

Bacteremia, heralded by fever and usually leukocytosis, is by far the most common clinical manifestation of *Leuconostoc* infection. Gastrointestinal disturbances, especially diarrhea, are common manifestations.[3] Infants are prone to emesis. *Leuconostoc* bacteremia, in association with cough and chest radiographic findings consistent with pneumonia, was reported in a child with acquired immunodeficiency syndrome (AIDS).[56] *L. citreum* has been isolated from the lung tissue of a 33-year-old patient with AIDS who also had *Pneumocystis jiroveci* pneumonia.[25] Small necrotic cavities scattered through both lungs were associated with gram-positive cocci in tissue. Patients with associated dental infections,[76] peritonitis,[26] osteomyelitis,[47] and meningitis[14,23] also have been described. Meningitis has been reported in an otherwise healthy 16-year-old patient and in a neonate with fatal infection despite high cerebrospinal fluid (CSF) bactericidal antibiotic titers.[14,23] A nosocomial cluster of five cases of urinary tract infection caused by *L. pseudomesenteroides* (strictly a species of a new genus, *Weissella*)[13] has been reported,[8] as has a cluster of three cases of *Leuconostoc* septicemia in critically ill postsurgical patients.[63] In a series of 20 cases from Taiwan, 19 of the 20 had health care–associated bacteremia. Eleven of the 20 had an underlying malignancy, and the same number had been hospitalized for 30 days or more at the time the diagnosis was made.[42]

Diagnosis

Cultures usually are positive within 24 to 48 hours. Identification of vancomycin-resistant *Streptococcus* should raise the suspicion of *Leuconostoc* infection and prompt additional biochemical studies,[56] including gas production in MRS broth, failure to hydrolyze arginine, and delayed esculin hydrolysis.

Treatment

Treatment with relatively high doses of penicillin or ampicillin frequently is successful in eradicating infection. Many species produce biofilms[43]; when possible, access devices should be removed. Leuconostocs are most sensitive to the primitive β-lactam antibiotics, especially penicillin and ampicillin, although penicillin tolerance is a common finding.[35,72] They also frequently are sensitive to erythromycin, cephalothin, and the aminoglycosides. Leuconostocs are variably resistant to clindamycin and trimethoprim-sulfamethoxazole. Resistance increases with later generations of cephalosporins.[30,72] Leuconostocs are intrinsically vancomycin resistant because their pentapeptide cell wall precursors end in D-Ala-lactate rather than the usual D-Ala-alanine.[29] Vancomycin cannot bind the lactate. This mode of resistance is the same as that possessed by vancomycin-resistant enterococci. However, whereas resistance in enterococci is plasmid derived and transferable, in *Leuconostoc* spp. it is chromosomally mediated, constitutional, and not transferable.

PEDIOCOCCUS SPECIES

Bacteriology

Pediococci also are intrinsically vancomycin-resistant, facultatively anaerobic gram-positive cocci. They appear most characteristically in tetrads on Gram staining, although they may appear in pairs or clusters.[27] The genus name *Pediococcus* is derived from the Greek word *pedium*, which means "plane." This name, therefore, suggests that this is a genus of cocci that grow in a single plane. It is a misnomer, however, because pediococci are the only lactic acid bacteria that divide in two planes.[24] They are catalase and oxidase negative. They do not reduce nitrates, and no gas is produced in MRS broth.[67] They are pyrrolidone carboxylyl peptidase negative.[19] Most isolates react with Lancefield group D streptococcal antibodies.[67] They are leucine aminopeptidase positive, which distinguishes them from *Leuconostoc* spp.[20] They produce white, opaque, nonhemolytic colonies on sheep's blood agar. A new broth medium, a combination of 90 percent Iso-Sensitest broth and 10 percent MRS broth and called LAB susceptibility test medium, has been found to provide optimal support of growth and to yield the most accurate minimal inhibitory concentration values.[40]

Pediococci produce powerful bacteriocins, which are substances that kill other bacteria. The bacteriocins of pediococci are active against other gram-positive organisms in particular[12] but also are active against *Clostridium botulinum* spores,[52] some gram-negative organisms,[68] and *Listeria monocytogenes*. Reports have suggested that some species of *Pediococcus* have antifungal activity[45]; however, this activity in other studies has been found to be present only at low pH and has been attributed to acetic acid in the medium.[7]

Epidemiology

Like leuconostocs and other lactic acid bacteria, pediococci are found on plants, in dairy products, and in alcohol-containing beverages, where certain species are associated with spoilage.[73] They also are used in the formation of silage[37] and as starter cultures for some meat products. They are not thought to be part of the normal flora. They have been isolated from saliva and stool on rare occasion.[61] Although formerly thought to be nonpathogenic, they now are considered rare opportunistic pathogens with minimal virulence.

Pathophysiology

Pediococci rarely are pathogenic. Many cases of blood isolates are found in patients without symptoms of infection or in polymicrobial cultures in which the significance of the isolate could not be assessed adequately.[46] One patient with a clinical picture of septic shock in whom the only organism recovered was *P. pentosaceus* has been reported.[15] Risk factors for bacteremia, with or without symptoms, appear to be the extremes of age, recent abdominal surgery or tube feeding, broad-spectrum antimicrobial therapy, and the presence of severe underlying disease states.[46] However, because the overall number of reported cases is small, the relative risks of these factors are uncertain.

Clinical Manifestations and Diagnosis

Most patients either are asymptomatic or have fever as the only symptom. Six of the first 12 reported cases of *Pediococcus* bacteremia had concomitant pneumonia. Fifty-six percent of adult patients either were receiving tube feeding or had undergone abdominal surgery within 30 days of isolation of the organism.[46] The three reported pediatric cases occurred in infants, and all had underlying gastrointestinal tract anomalies. A 16-day-old infant with congenital jejunoileal atresia had undergone surgical repair just 8 days before being evaluated.[46] Her acute illness was characterized by emesis and a 200-g weight loss. She was afebrile when initially examined. The second patient was a 64-day-old infant with gastroschisis who had undergone two abdominal surgical procedures.[1] His fever reached 38.6°C (101.5°F), and his peripheral white blood cell count was 38,000/mm^3 with a significant left shift. The third patient was a 3-month-old female infant who had undergone surgical repair of gastroschisis on the first day of life.[2] Lethargy, direct hyperbilirubinemia, and a CSF pleocytosis were noted. Blood cultures grew *Pediococcus* spp., and the CSF (obtained after initiation of antibiotics) was sterile. The patient responded to a 21-day course of ampicillin and gentamicin. An adult case of infective endocarditis in a man who had a history of bowel transplantation for short-gut syndrome[36] and a case of persistent bacteremia in a patient with metastatic adenocarcinoma of the gallbladder have been reported[70]; both responded rapidly to therapy with daptomycin.

Pediococci are isolated frequently in localized infections, especially from abdominal sites, but they are virtually always part of a polymicrobial process. The relative importance of pediococci in these sites is difficult to assess. A case of *Pediococcus* bacteremic pneumonia was reported in a previously healthy pregnant woman.[62] Among 31 cases of *Pediococcus* infection submitted to the U.S. Centers for Disease Control and Prevention laboratory, 17 were from blood culture isolates (including two reported cases of endocarditis) and four were associated with urinary tract infections. Other sources included catheter tips, wounds, peritoneal fluid, CSF, lung, and bone.[19]

Diagnosis of infection with pediococci is established by identifying catalase-negative, vancomycin-resistant, gram-positive cocci in the characteristic tetrads. Many pediococci are misidentified initially as *Streptococcus equinus*, *S. constellatus*, or group D streptococcus, not *Enterococcus*. Reported cases of pediococcal infection may, therefore, represent only a fraction of the total number of infections.

Treatment

Pediococci generally are susceptible to penicillin, ampicillin, imipenem, clindamycin, and first- and second-generation cephalosporins. Although both imipenem and penicillin are highly active against pediococci, they do not appear to be bactericidal. Pediococci are moderately resistant to the quinolones, tetracycline,[73] and trimethoprim-sulfamethoxazole.[59] Pediococci, like leuconostocs, are intrinsically resistant to vancomycin. Resistance is not plasmid mediated, nor can it be transferred to other bacteria.[73] Sensitivity to ticarcillin and cefotaxime, when measured by agar dilution, is poor despite large zones of inhibition on disk susceptibility testing.[73] Occasionally, inducible resistance to erythromycin is found, although most isolates remain sensitive. Aminoglycoside sensitivity is variable. Daptomycin appears to be active.

AEROCOCCUS SPECIES

Bacteriology

The genus *Aerococcus* contains at least five species, but only two, *A. viridans* and *A. urinae*, have known clinical significance. In earlier papers, *A. urinae* was referred to as *Aerococcus*-like organism.[11] Aerococci are catalase-negative, nonmotile, gram-positive cocci that appear preferentially in tetrads but sometimes in pairs or clusters. These relatively slow-growing organisms produce

small, well-delineated, translucent, α-hemolytic colonies on blood agar.[10] They also are weakly bile esculin positive and pyrrolidone carboxylyl peptidase negative. They ferment mannose and mannitol.[57] Like enterococci, most aerococci will grow in 6.5 percent salt.[41]

Epidemiology

Aerococci are distributed throughout the world and are contaminants of air and dust.[33] They also have been found on meat, on raw vegetables, and in small numbers on human skin.[6] In hospitals, aerococci have been cultured from all areas, including operating suites and delivery rooms.[38] They also are found in salt water, where they cause a fatal disease in lobsters.[54]

Disease in humans is an uncommon event, and the organism usually is recovered from the bloodstream of patients with infective endocarditis. Most patients are elderly, but infection of infants and neonates also has been reported.[50,54] A rapidly fatal bacteremia has been described in patients with profound neutropenia.[39] Aerococci are agents of urinary tract infection in elderly and institutionalized persons[66]; these organisms can also cause pediatric urinary tract infections and even pyelonephritis, usually in the setting of obstructive uropathy.[44,48]

Pathophysiology

In most circumstances, aerococci are saprophytic. The exact conditions that favor the development of infection have not been elucidated clearly. Some cases of *A. urinae* infection have occurred after genitourinary tract surgery. One case of septic arthritis developed after an elective abortion.[74] Immunocompromised patients are at higher risk, but infection in otherwise well persons has been described.[57] One report of meningitis in neonates found adherence of aerococci to inflammatory cells and suggested a role of as-yet-undefined adhesion factors.[50] Work in laboratory animals also has shown that a protease isolated from *A. viridans* cleaves the hemagglutinin of influenza virus and potentiates both viral replication and disease in mice.[64] In vitro experiments show that the organism forms biofilms and causes platelet aggregation, features that explain why endocarditis is a common manifestation.[65]

Clinical Manifestations

Most cases of *A. viridans* bacteremia have been found in association with signs and symptoms of subacute infective endocarditis. Severe disease requiring valve replacement surgery has been described, but not in pediatric patients.[5] In a recent series of four cases of *A. viridans* infective endocarditis, two of the patients required surgical intervention.[58] *A. urinae* causes urinary tract infection with dysuria and frequency, usually in the absence of fever.[10] *Aerococcus* infection in childhood is an uncommon occurrence. One case of bacteremia in a 1-month-old infant has been reported.[54] The patient had a 2-day history of loose stools, irritability, and drowsiness and on physical examination was noted to have mottled skin, circumoral cyanosis, and agitation. CSF and urine studies were normal, but blood cultures exhibited pure growth of *A. viridans.* No predisposing factors were identified.

Nathavitharana and associates[50] reported three cases of meningitis caused by *A. viridans.* They occurred in a 7-month-old female infant with jerking movements of the extremities, irritability, and a bulging fontanelle; a 5-month-old female infant with fever, decreased appetite, and generalized convulsions; and a 24-month-old girl whose illness was manifested as fever, vomiting, and the neurologic signs of truncal ataxia, flaccidity, and hypoactive deep tendon reflexes. All three patients had elevated CSF white blood cell counts with 55 to 93 percent segmented forms. Remarkably, all these patients had a history of prolonged illness (1 week to 2 months) before being evaluated, in contrast to patients with other causes of bacterial meningitis. No risk factors for infection were identified in any of these patients, who were thought to have normal immune function.

Swanson and colleagues[71] reported a case of penicillin-resistant *A. viridans* bacteremia in an 11-month-old girl receiving prophylactic penicillin for sickle-cell disease. The patient responded clinically to a 10-day course of therapy with cephalosporins.

Bone and joint infections and wound or other localized infections are exceedingly rare occurrences and not distinguishable from similar syndromes caused by more common organisms. However, there are a number of case reports of spondylodiscitis in patients with *A. urinae* bacteremia or infective endocarditis.[49,75]

Diagnosis

Careful observation of both appearance on Gram stain and growth in culture is key to establishing the diagnosis of aerococcal infection. On Gram stain, aerococci resemble staphylococci. On blood agar, they resemble viridans group streptococci. One series revealed that 1 percent of 719 cultures called streptococci actually were aerococci[55]; another study reclassified 3 percent of 168 cultures.[6] In one study of 24 α-hemolytic, nonenterococcal bacterial isolates from urine, standard identification methods were compared with 16S rRNA sequencing and special biochemical profiling. Thirteen of the isolates were aerococci; all were identified correctly by sequencing, but typical procedures failed to differentiate them from nonaerococcal isolates. Ciprofloxacin and trimethoprim susceptibility and the ability to grow at 45°C improved the discrimination of standard testing.[28] In their propensity toward tetrad formation, they mimic pediococci. However, all aerococci are vancomycin sensitive. In their bile esculin hydrolysis and growth in 6.5 percent salt, they resemble enterococci; unlike enterococci, however, aerococci are pyrrolidone carboxylyl peptidase negative and do not form chains.

Treatment

Aerococci generally are sensitive to penicillin, ampicillin, the cephalosporins, chloramphenicol, and the macrolides. They usually are intermediately susceptible or resistant to sulfonamides and aminoglycosides.[6,33] Susceptibility testing for trimethoprim-sulfamethoxazole

appears to be affected by the medium used in the testing.[34] One report suggests that *A. viridans* and *A. urinae* have distinct antibiograms[41]; that is, *A. urinae* is more sensitive to penicillin and resistant to sulfonamides whereas *A. viridans* is more resistant to penicillin and sensitive to the sulfonamides.[6] Individual case reports do not confirm these in vitro observations, and no large clinical trials of antimicrobial susceptibility have been conducted.

Acknowledgments

The author would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Bou G, Luis Saleta J, Saez Nieto JA, et al. Nosocomial outbreaks caused by *Leuconostoc mesenteroides* subsp. *Mesenteroides*. *Emerg Infect Dis* 2008;**14**:968–71.
5. Bruegger D, Beiras-Fernandez A, Weis F, et al. Extracorporeal support in a patient with cardiogenic shock due to *Aerococcus urinae* endocarditis. *J Heart Valve Dis* 2009;**18**:418–20.
17. Endo A, Okada S. Reclassification of the genus *Leuconostoc* and proposals of *Fructobacillus fructosus* gen. nov., comb. nov., *Fructobacillus durionis* comb. nov., *Fructobacillus ficulneus* comb. nov. and *Fructobacillus pseudoficuleneus* comb. nov. *Int J Syst Evol Microbiol* 2008;**58**:2195–205.
22. Florescu D, Hill L, Sudan D, Iwen PC. *Leuconostoc* bacteremia in pediatric patients with short bowel syndrome: case series and review. *Pediatr Infect Dis J* 2008;**27**:1013–19.
32. Hatipoglu CA, Yildiz E, Koktekir E, et al. [A case of vancomycin resistant *Leuconostoc* meningitis.] *Mikrobiyol Bul* 2008;**42**:695–9.
34. Humphries RM, Lee C, Hindler JA. *Aerococcus urinae* and trimethoprim-sulfamethoxazole. *J Clin Microbiol* 2011;**49**:3934–5.
36. Iwen PC, Mindru C, Kalil AC, Florescu DF. *Pediococcus acidilactici* endocarditis successfully treated with daptomycin. *J Clin Microbiol* 2012;**50**:1106–8.
42. Lee MR, Huang YT, Lee PI, et al. Healthcare-associated bacteraemia caused by *Leuconostoc* species at a university hospital in Taiwan between 1995 and 2008. *J Hosp Infect* 2011;**78**:45–9.
43. Leathers TD, Bischoff KM. Biofilm formation by strains of *Leuconostoc citreum* and *L. mesenteroides*. *Biotechnol Lett* 2011;**33**:517–23.
44. Leite A, Vinhas-Da-Silva A, Felicio L, et al. *Aerococcus viridians* urinary tract infection in a pediatric patient with secondary pseudohypoaldosteronism. *Rev Argent Microbiol* 2010;**42**:269–70.
48. Murray TS, Muldrew KL, Finkelstein R, et al. Acute pyelonephritis caused by *Aerococcus urinae* in a 12-year-old boy. *Pediatr Infect Dis J* 2008;**27**:760–2.
49. Nasoodi A, Ali AG, Gray WJ, Hedderwick SA. Spondylodiscitis due to *Aerococcus viridians*. *J Med Microbiol* 2008;**57**:532–3.
65. Shannon O, Morgeilin M, Rasmussin M. Platelet activation and biofilm formation by *Aerococcus urinae*, an endocarditis-causing pathogen. *Infect Immun* 2010;**78**:4268–75.
66. Shelton-Dodge K, Vetter EA, Kohner PC, et al. Clinical significance and antimicrobial susceptibilities of *Aerococcus sanguinicola* and *Aerococcus urinae*. *Diagn Microbiol Infect Dis* 2011;**70**:448–51.
70. Suh B. Resolution of persistent *Pediococcus* bacteremia with daptomycin treatment: case report and review of the literature. *Diagn Microbiol Infect Dis* 2010;**66**:111–15.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 2 *GRAM NEGATIVE COCCI*

CHAPTER 87

Moraxella catarrhalis

Barbara W. Stechenberg

Once thought to be an unimportant commensal organism in the human respiratory tract, *Moraxella catarrhalis* now is recognized as an important pathogen in respiratory tract diseases, particularly otitis, sinusitis, and lower tract disease. The initial paper by Ghon and Pfeiffer[24] published in 1902 described gram-negative cocci in sputum referred to as *Micrococcus catarrhalis*. Since then, the organism has undergone several changes in nomenclature, first to *Neisseria catarrhalis* because of its resemblance to other *Neisseria* organisms. In 1970, in recognition of the contributions of Sarah Branham, the name was changed to *Branhamella catarrhalis*.[11] More recently, that

REFERENCES

1. Atkins JT, Tillman J, Tan TQ, et al. *Pediococcus pentosaceus* catheter-associated infection an infant with gastroschisis. *Pediatr Infect Dis J* 1994;**13**:75–6.
2. Barton LL, Rider ED, Coen RW. Bacteremic infection with *Pediococcus*: vancomycin-resistant opportunist. *Pediatrics* 2001;**107**: 775–6.
3. Bernaldo de Quiros JC, Munoz P, Cercenado E, et al. *Leuconostoc* species as a cause of bacteremia: two case reports and a literature review. *Eur J Clin Microbiol Infect Dis* 1991;**10**:505–9.
4. Bou G, Luis Saleta J, Saez Nieto JA, et al. Nosocomial outbreaks caused by *Leuconostoc mesenteroides* subsp. *Mesenteroides*. *Emerg Infect Dis* 2008;**14**:968–71.
5. Bruegger D, Beiras-Fernandez A, Weis F, et al. Extracorporeal support in a patient with cardiogenic shock due to *Aerococcus urinae* endocarditis. *J Heart Valve Dis* 2009;**18**:418–20.
6. Buu-Hoi A, Branger C, Acar JF. Vancomycin-resistant streptococci or *Leuconostoc* sp. *Antimicrob Agents Chemother* 1985;**28**:458–60.
7. Cabo ML, Braber AF, Koenraad PM. Apparent antifungal activity of several lactic acid bacteria against *Penicillium discolor* is due to acetic acid in the medium. *J Food Prot* 2002;**65**:1309–16.
8. Cappelli EA, Barros RR, Camello TC, et al. *Leuconostoc pseudomesenteroides* as a cause of nosocomial urinary tract infections. *J Clin Microbiol* 1999;**37**:4124–6.
9. Carapetis J, Bishop S, Davis J, et al. *Leuconostoc* sepsis in association with continuous enteral feeding: two case reports and a review. *Pediatr Infect Dis J* 1994;**13**:816–23.
10. Christensen JJ, Gutschik E, Friis-Moller A, et al. Urosepticemia and fatal endocarditis caused by aerococcus-like organisms. *Scand J Infect Dis* 1991;**23**:717–21.
11. Christensen JJ, Vibits H, Ursing J, et al. *Aerococcus*-like organism, a newly recognized potential urinary tract pathogen. *J Clin Microbiol* 1991;**29**:1049–53.
12. Cintas LM, Rodriguez JM, Fernandez MF, et al. Isolation and characterization of pediocin L50, a new bacteriocin from *Pediococcus acidilactici* with a broad inhibitory spectrum. *Appl Environ Microbiol* 1995;**61**:2643–8.
13. Collins MD, Samulis J, Metaxopoulos J, Wallbanks S. Taxonomic studies on some *Leuconostoc*-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. *J Appl Bacteriol* 1993;**75**:595–603.
14. Coovadia YM, Solwa Z, van den Ende J. Meningitis caused by vancomycin-resistant *Leuconostoc* sp. *J Clin Microbiol* 1987;**25**: 1784–5.
15. Corcoran GD, Gibbons N, Mulvihill TE. Septicaemia caused by *Pediococcus pentosaceus*: a new opportunistic pathogen. *J Infect* 1991; **23**:179–82.
16. Dhodapkar KM, Henry NK. *Leuconostoc* bacteremia in an infant with short-gut syndrome: case report and literature review. *Mayo Clin Proc* 1996;**71**:1171–4.
17. Endo A, Okada S. Reclassification of the genus *Leuconostoc* and proposals of *Fructobacillus fructosus* gen. nov., comb. nov., *Fructobacillus durionis* comb. nov., *Fructobacillus ficulneus* comb. nov. and *Fructobacillus pseudoficuleneus* comb. nov. *Int J Syst Evol Microbiol* 2008;**58**:2195–205.
18. Espinoza R, Kusne S, Pasculle AW, et al. *Leuconostoc* bacteremia after liver transplantation: another cause of vancomycin-resistant gram-positive infection. *Clin Transplant* 1997;**11**:322–4.
19. Facklam R, Elliott JA. Identification, classification, and clinical relevance of catalase-negative, gram-positive cocci, excluding the streptococci and enterococci. *Clin Microbiol Rev* 1995;**8**:479–95.
20. Facklam R, Hollis D, Collins MD. Identification of gram-positive coccal and coccobacillary vancomycin-resistant bacteria. *J Clin Microbiol* 1989;**27**:724–30.
21. Facklam R, Pigott N, Franklin R, et al. Evaluation of three disk tests for identification of enterococci, leuconostocs, and pediococci. *J Clin Microbiol* 1995;**33**:885–7.
22. Florescu D, Hill L, Sudan D, Iwen PC. *Leuconostoc* bacteremia in pediatric patients with short bowel syndrome: case series and review. *Pediatr Infect Dis J* 2008;**27**:1013–9.
23. Friedland IR, Snipelisky M, Khoosal M. Meningitis in a neonate caused by *Leuconostoc* sp. *J Clin Microbiol* 1990;**28**:2125–6.
24. Garvie EI. Genus *Pediococcus*. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG, editors. *Bergey's manual of systematic bacteriology*, vol. 2. Baltimore: Williams & Wilkins; 1984. p. 1075–9.
25. Giacometti A, Ranaldi R, Siquini FM, et al. *Leuconostoc citreum* isolated from lung in AIDS patient. *Lancet* 1993;**342**:622.
26. Gillespie RS, Symons JM, McDonald RA. Peritonitis due to *Leuconostoc* species in a child receiving peritoneal dialysis. *Pediatr Nephrol* 2002;**17**:966–8.
27. Green M, Barbadora K, Michaels M. Recovery of vancomycin-resistant gram-positive cocci from pediatric liver transplant recipients. *J Clin Microbiol* 1991;**29**:2503–6.
28. Grude N, Jenkins A, Tveten Y, Kristiansen BE. Identification of *Aerococcus urinae* in urine samples. *Clin Microbiol Infect* 2003;**9**: 976–9.
29. Handwerger S, Horowitz H, Coburn K, et al. Infection due to *Leuconostoc* species: six cases and review. *Rev Infect Dis* 1990;**12**: 602–10.
30. Handwerger S, Pucci MJ, Volk KJ, et al. Vancomycin-resistant *Leuconostoc mesenteroides* and *Lactobacillus casei* synthesize cytoplasmic peptidoglycan precursors that terminate in lactate. *J Bacteriol* 1994;**176**:260–4.
31. Hardy S, Ruoff KL, Catlin EA, et al. Catheter-associated infection with a vancomycin-resistant gram-positive coccus of the *Leuconostoc* sp. *Pediatr Infect Dis J* 1988;**7**:519–20.
32. Hatipoglu CA, Yildiz E, Koktekir E, et al. [A case of vancomycin resistant Leuconostoc meningitis.] *Mikrobiyol Bul* 2008;**42**:695–9.
33. Heilesen AM. Septicaemia due to *Aerococcus urinae*. *Scand J Infect Dis* 1994;**26**:759–60.
34. Humphries RM, Lee C, Hindler JA. *Aerococcus urinae* and trimethoprim-sulfamethoxazole. *J Clin Microbiol* 2011;**49**:3934–5.
35. Isenberg HD, Vellozzi EM, Shapiro J, et al. Clinical laboratory challenges in the recognition of *Leuconostoc* spp. *J Clin Microbiol* 1988;**26**:479–83.
36. Iwen PC, Mindru C, Kalil AC, Florescu DF. *Pediococcus acidilactici* endocarditis successfully treated with daptomycin. *J Clin Microbiol* 2012;**50**:1106–8.
37. Jeppesen VF, Huss HH. Characteristics and antagonistic activity of lactic acid bacteria isolated from chilled fish products. *Int J Food Microbiol* 1993;**18**:305–20.
38. Kerbaugh MA, Evans JB. *Aerococcus viridans* in the hospital environment. *Appl Microbiol* 1968;**16**:519–23.
39. Kern W, Vanek E. *Aerococcus* bacteremia associated with granulocytopenia. *Eur J Clin Microbiol* 1987;**6**:670–3.
40. Klare I, Konstabel C, Muller-Bertling S, et al. Evaluation of a new broth media for microdilution antibiotic susceptibility testing of lactobacilli, pediococci, lactococci, and bifidobacteria. *Appl Environ Microbiol* 2005;**71**:8982–6.
41. Kristensen B, Nielsen G. Endocarditis caused by *Aerococcus urinae*, a newly recognized pathogen. *Eur J Clin Microbiol Infect Dis* 1995; **14**:49–51.
42. Lee MR, Huang YT, Lee PI, et al. Healthcare-associated bacteraemia caused by *Leuconostoc* species at a university hospital in Taiwan between 1995 and 2008. *J Hosp Infect* 2011;**78**:45–9.
43. Leathers TD, Bischoff KM. Biofilm formation by strains of *Leuconostoc citreum* and *L. mesenteroides*. *Biotechnol Lett* 2011;**33**: 517–23.
44. Leite A, Vinhas-Da-Silva A, Felicio L, et al. *Aerococcus viridians* urinary tract infection in a pediatric patient with secondary pseudohypoaldosteronism. *Rev Argent Microbiol* 2010;**42**:269–70.
45. Magnusson J, Strom K, Roos S, et al. Broad and complex antifungal activity among environmental isolates of lactic acid bacteria. *FEMS Microbiol Lett* 2003;**14**:126–35.
46. Mastro TD, Spika JS, Lozano P, et al. Vancomycin-resistant *Pediococcus acidilactici*: nine cases of bacteremia. *J Infect Dis* 1990;**161**: 956–60.
47. Mulford JS, Mills J. Osteomyelitis caused by *Leuconostoc* species. *Aust N Z J Surg* 1999;**69**:541–2.
48. Murray TS, Muldrew KL, Finkelstein R, et al. Acute pyelonephritis caused by *Aerococcus urinae* in a 12-year-old boy. *Pediatr Infect Dis J* 2008;**27**:760–2.
49. Nasoodi A, Ali AG, Gray WJ, Hedderwick SA. Spondylodiscitis due to *Aerococcus viridians*. *J Med Microbiol* 2008;**57**:532–3.
50. Nathavitharana KA, Arseculeratne SN, Aponso HA, et al. Acute meningitis in early childhood caused by *Aerococcus viridans*. *BMJ (Clin Res Ed)* 1983;**286**:1248.
51. Noriega FR, Kotloff KL, Martin MA, et al. Nosocomial bacteremia caused by *Enterobacter sakazakii* and *Leuconostoc mesenteroides* resulting from extrinsic contamination of infant formula. *Pediatr Infect Dis J* 1990;**9**:447–9.

52. Okereke A, Montville TJ. Bacteriocin-mediated inhibition of *Clostridium botulinum* spores by lactic acid bacteria at refrigeration and abuse temperatures. *Appl Environ Microbiol* 1991;**57**:3423–8.
53. Park HK, Shim SS, Kim SY, et al. Molecular analysis of colonized bacteria in a human newborn infant gut. *J Microbiol* 2005;**43**:345–53.
54. Park JW, Grossman O. *Aerococcus viridans* infection: case report and review. *Clin Pediatr (Phila)* 1990;**29**:525–6.
55. Parker MT, Ball LC. Streptococci and aerococci associated with systemic infection in man. *J Med Microbiol* 1976;**9**:275–302.
56. Peters VB, Bottone EJ, Barzilai A, et al. *Leuconostoc* species bacteremia in a child with acquired immunodeficiency syndrome. *Clin Pediatr (Phila)* 1992;**31**:699–701.
57. Pien FD, Wilson WR, Kunz K, et al. *Aerococcus viridans* endocarditis. *Mayo Clin Proc* 1984;**59**:47–8.
58. Popescu GA, Benea E, Mitache E, et al. An unusual bacterium, *Aerococcus viridans*, and four cases of infective endocarditis. *J Heart Valve Dis* 2005;**14**:317–9.
59. Riebel WJ, Washington JA. Clinical and microbiologic characteristics of pediococci. *J Clin Microbiol* 1990;**28**:1348–55.
60. Rogosa M, Sharpe ME. Species differentiation of human vaginal lactobacilli. *J Gen Microbiol* 1960;**23**:197.
61. Ruoff KL, Kuritzkes DR, Wolfson JS, et al. Vancomycin-resistant gram-positive bacteria isolated from human sources. *J Clin Microbiol* 1988;**26**:2064–8.
62. Sarma PS, Mohanty S. *Pediococcus acidilactici* pneumonitis and bacteremia in a pregnant woman. *J Clin Microbiol* 1998;**36**:2392–3.
63. Scano F, Rossi L, Cattelan A, et al. *Leuconostoc* species: a case-cluster hospital infection. *Scand J Infect Dis* 1999;**31**:371–3.
64. Scheiblauer H, Reinacher M, Tashiro M, et al. Interactions between bacteria and influenza A virus in the development of influenza pneumonia. *J Infect Dis* 1992;**166**:783–91.
65. Shannon O, Morgeilin M, Rasmussin M. Platelet activation and biofilm formation by *Aerococcus urinae*, an endocarditis-causing pathogen. *Infect Immun* 2010;**78**:4268–75.
66. Shelton-Dodge K, Vetter EA, Kohner PC, et al. Clinical significance and antimicrobial susceptibilities of *Aerococcus sanguinicola* and *Aerococcus urinae*. *Diagn Microbiol Infect Dis* 2011;**70**: 448–51.
67. Sire JM, Donnio PY, Mesnard R, et al. Septicemia and hepatic abscess caused by *Pediococcus acidilactici*. *Eur J Clin Microbiol Infect Dis* 1992;**11**:623–5.
68. Skytta E, Haikara A, Mattila-Sandholm T. Production and characterization of antibacterial compounds produced by *Pediococcus damnosus* and *Pediococcus pentosaceus*. *J Appl Bacteriol* 1993;**74**: 134–42.
69. Stiles ME. Bacteriocins produced by *Leuconostoc* species. *J Dairy Sci* 1994;**77**:2718–24.
70. Suh B. Resolution of persistent *Pediococcus* bacteremia with daptomycin treatment: case report and review of the literature. *Diagn Microbiol Infect Dis* 2010;**66**:111–15.
71. Swanson H, Cutts E, Lepow M. Penicillin-resistant *Aerococcus viridans* bacteremia in a child receiving prophylaxis for sickle-cell disease. *Clin Infect Dis* 1996;**22**:387–8.
72. Swenson JM, Facklam RR, Thornsberry C. Antimicrobial susceptibility of vancomycin-resistant *Leuconostoc*, *Pediococcus*, and *Lactobacillus* species. *Antimicrob Agents Chemother* 1990;**34**:543–9.
73. Tankovic J, Leclercq R, Duval J. Antimicrobial susceptibility of *Pediococcus* spp. and genetic basis of macrolide resistance in *Pediococcus acidilactici* HM3020. *Antimicrob Agents Chemother* 1993;**37**: 789–92.
74. Taylor PW, Trueblood MC. Septic arthritis due to *Aerococcus viridans*. *J Rheumatol* 1985;**12**:1004–5.
75. Tekin A, Tekin G, Turunc T, et al. Infective endocarditis and spondylodiscitis in a patient due to *Aerococcus urinae:* first report. *Int J Cardiol* 2007;**115**:402–3.
76. Wenocur HS, Smith MA, Vellozzi EM, et al. Odontogenic infection secondary to *Leuconostoc* species. *J Clin Microbiol* 1988;**26**: 1893–4.

this organism should be a member of the genus *Moraxella* has become clear; thus, the name was changed to *M. catarrhalis*. With the transition in nomenclature, the last 2 decades have seen a parallel rebirth of this organism as a mucosal pathogen.

MICROBIOLOGY

M. catarrhalis is a gram-negative diplococcus that is morphologically indistinguishable from *Neisseria*. It has a tendency to resist decolorizing.[1] The organism is kidney shaped, with the flat sides abutting each other. The size varies, but it may be larger than meningococcus or gonococcus. However, the resemblance on sputum Gram stain or conjunctival smear to gonococcus can be striking and clinically confusing.

The organism grows well on blood or chocolate agar and forms small, opaque, grayish colonies that are circular and nonhemolytic. They have poor adhesion to the agar surface, on which they act like hockey pucks when pushed over the surface of the agar plate.[17] The use of selective media such as modified Thayer-Martin or TV broth (Müeller-Hinton broth supplemented with trimethoprim and vancomycin) increases the likelihood of recovering *M. catarrhalis* from the complex flora of the mucosal surfaces.

Isolates of *M. catarrhalis* cannot use maltose, glucose, lactose, or sucrose as carbohydrate sources. *M. catarrhalis* is oxidase-positive and produces deoxyribonuclease. Valuable differentiating tests are hydrolysis of DNA and tributyrin. Several rapid tests such as butyrate hydrolysis, Tween 80 hydrolysis, and selective DNase agar have been described.[51,56] Fatty-acid analysis also has been used to identify atypical strains.

The surface of *M. catarrhalis* is composed of outer-membrane proteins, lipo-oligosaccharide, and pili. The organism does not appear to have a capsule, and the role of the pili is not defined well. By using a technique involving the collection of outer membrane vesicles, which are released into the culture medium, and then examining by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), Bartos and Murphy[6] identified eight proteins designated outer membrane proteins A through H. The outer membrane protein patterns of strains from diverse geographic and clinical sources were strikingly homogeneous.

Preliminary studies of the lipo-oligosaccharide of *M. catarrhalis* show that it is more antigenically conserved than those of other gram-negative bacteria.[40] One study suggests that the lipo-oligosaccharide is not an adhesin, but its surface charge plays a critical role in the initial stage of attachment to human epithelial cells.[2] Several adhesins have been identified and may be potential candidates for a vaccine.[40]

Restriction endonuclease analysis has been used as an epidemiologic tool to distinguish strains of *M. catarrhalis*. Patterson and associates[43] used the technique to evaluate a nosocomial outbreak and demonstrate the lack of association of other strains. Dickinson and coworkers[16] used a similar technique to study isolates from children with otitis media.

PATHOGENESIS

The ability of *M. catarrhalis* to produce disease, particularly in sequestered areas such as the ear and lung, indicates that this organism possesses virulence mechanisms that allow it not only to grow in these anatomic sites but also to produce pathologic effects. However, a subset of strains of *M. catarrhalis* associated with selected virulence traits resulting in complement inactivation suggests that some strains are more virulent than others.[9,55]

A body of literature concerning the immune response to *M. catarrhalis* and the antigenic composition of this organism has accumulated.[12,13,35] Goldblatt and associates[25] demonstrated that children younger than 4 years old possess immunoglobulin (Ig)G1 and IgG2 that recognize an 82-kDa outer membrane protein exclusively but that older children mount an IgG3 response to a broad range of outer membrane proteins. Faden and colleagues[21] developed a technique to measure opsonic antibody with the use of outer membrane, antigen-coated beads. Convalescent sera opsonized homologous, antigen-coated sera significantly more often than did acute sera.

Unhanand and coworkers[53] used a murine model to study pulmonary clearance of *M. catarrhalis* from infected lungs. They investigated 10 strains and found marked variability in clearance rates and recruitment of phagocytic cells. With this model, Maciver and associates[37] actively immunized animals with the outer membrane vesicles of *M. catarrhalis* and passively immunized animals with rabbit antiserum raised against these vesicles. Both experiments resulted in enhanced pulmonary clearance of both homologous and heterologous strains. The same model has been used to evaluate the role of a large, antigenically conserved protein of *M. catarrhalis* in pulmonary clearance, as well as the role of outer membrane protein B in defense mechanisms of the lung.[31] The contribution of these proteins, as well as lipo-oligosaccharide, in host defense continues to unfold.

Initially thought to be an exclusively extracellular pathogen, *M. catarrhalis* invades multiple cell types. It is present intracellularly in human pharyngeal tissue, a potential reservoir for persistent colonization.[30] The organism forms biofilms, which may contribute to its role in chronic otitis with effusion.[28,29] Colonization of healthy children is characterized by genotypic heterogeneity, virulence gene diversity, and co-colonization with *Haemophilus influenzae*.[57]

EPIDEMIOLOGY

M. catarrhalis is a normal inhabitant of the upper respiratory tract and is recovered exclusively from humans. The rate of colonization is highest in the early years and then declines steadily to less than 5 percent in adult life.[54] Prevalence studies report that as many as 50 percent of children are colonized with *M. catarrhalis*.[33,38] Faden and associates[22] monitored a large cohort of children from birth to 2 years of age. Sixty-six percent became colonized by the time they reached 1 year of age, and 77.5 percent did so by 2 years of age. The frequency of

nasopharyngeal colonization was higher during visits for otitis media than during well-child visits. Otitis-prone children were colonized by 6 months of age. Restriction endonuclease analysis showed marked heterogeneity, with children acquiring and eliminating many different strains over time.[22,43]

Numerous studies have documented the increased prevalence of *M. catarrhalis* as a pathogen in otitis media, either alone or as a copathogen.[33,44] Shurin and colleagues[49] noted that isolation of *M. catarrhalis* from middle ear exudates increased from 6.4 percent between 1979 and 1980 to 26.5 percent between 1980 and 1982. A similar increase in *M. catarrhalis* as a pathogen in sinusitis has been reported.[58] In the pneumococcal conjugate vaccine (PCV7) era, a significantly higher proportion of PCV7-immunized children exhibit nasopharyngeal colonization with *M. catarrhalis*.[45]

Once colonization of the oropharynx occurs, colonization of the tracheobronchial tree may follow, but usually only if other risk factors are operative. In adults, such risk factors include previous cardiorespiratory disease, smoking, use of corticosteroids, use of immunosuppressive agents, malignancy, and intercurrent viral illnesses.[27,56] In children, many cases of pneumonia are associated with a preceding viral illness, prematurity, underlying lung disease, IgG deficiency, or other risk factors.

Nosocomial transmission has been well documented by DNA restriction endonuclease analysis.[14,43] In pediatric intensive care units, endotracheal intubation and frequent suctioning have been identified as risk factors for the development of pneumonia and bacterial tracheitis.[14,20]

Other interesting associations need to be investigated further. Seddon and associates[48] noted that colonization with *M. catarrhalis* occurred more commonly in asthmatic than in normal children. Whether the organism might be a trigger or pathogen in these children remains to be seen. Gottfarb and Brauner[26] found a high carriage rate of *M. catarrhalis* in children with severe persistent cough.

Infection with *M. catarrhalis* exhibits seasonality in both adults and children.[27,49] Infection is much more likely to occur in the winter and spring months.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

Otitis media caused by *M. catarrhalis* is indistinguishable clinically from otitis caused by other pathogens, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. *M. catarrhalis* may be isolated as a single agent or in combination with other organisms. There is some suggestion that otitis caused by this organism may be associated with less fever; a lower likelihood of a red, bulging membrane or perforation; and a lower C-reactive protein level.[40] Mastoiditis is not commonly seen with *M. catarrhalis*.

Because tympanocentesis usually is not performed in routine cases, the exact cause of an episode of acute otitis media generally is not known. One study found that *Moraxella* was an unusual organism as a single agent in children in whom acute otitis media had been treated recently.[29] Nonetheless, in a study of persistent otitis, Pichichero and Pichichero[44] demonstrated *Moraxella* in 7 percent of specimens. The spontaneous resolution rate of *M. catarrhalis* may be high,[4] and this fact should be considered when therapy is planned.

The clinical manifestations of acute sinusitis caused by *M. catarrhalis* are similar to those of other organisms; therefore, selection of antibiotics should be made with consideration of this organism as a potential pathogen.

Lower respiratory tract disease caused by *M. catarrhalis* may have a broad clinical spectrum; however, because sputum samples often are not available in children, many cases documented in the literature have been severe. Berg and Bartley[7] described five premature infants, all younger than 6 months of age, in whom precipitous clinical deterioration developed after 2 to 4 days of a prodrome consisting of cough, tachypnea, and intercostal retractions. They all required assisted ventilation, which is common in young infants with severe pneumonia.[7,14,19] Some cases of *M. catarrhalis* pneumonia have been associated with bacteremia.

Underlying conditions such as leukemia, acquired immunodeficiency syndrome, and trauma have been reported to predispose a patient to the development of infection with *M. catarrhalis*.[38,59] An association with Ig deficiency has been demonstrated in some patients with disease caused by this organism.[12] In adults, *M. catarrhalis* pneumonia occurs more commonly in patients with human immunodeficiency virus infection, malignancy, or chronic lung problems.[25,56] The role of this organism in less severe manifestations probably is limited.[34]

Bacterial tracheitis caused by *M. catarrhalis* has been reported in both immunocompromised hosts and those with normal immune systems.[20]

M. catarrhalis also has been responsible for a wide variety of other infections. Urethritis caused by this organism can be mistaken for infection with *Neisseria gonorrhoeae*.[50] Several cases of conjunctivitis have been reported; when present in the newborn period, *M. catarrhalis* can mimic the ophthalmia neonatorum of *N. gonorrhoeae*.[52] The relationship of neonatal infections to maternal vaginal carriage of *Moraxella* has not been established.[42]

More severe infections with *M. catarrhalis* include meningitis and bacteremia, so this organism should be considered in appropriate circumstances, especially in immunocompromised children.

Children with *M. catarrhalis* bacteremia may have many different manifestations. Some patients have petechial or purpuric rashes, thus rendering the clinical picture indistinguishable from that of meningococcemia. Others have been neutropenic and have required mechanical ventilation.[8] Baron and Shapiro[5] reported two cases of unsuspected bacteremia in children with nonspecific symptoms. Their manifestations were similar to those of children with occult pneumococcal bacteremia. An immunocompetent child with *M. catarrhalis* bacteremia and preseptal cellulitis has been reported.[47]

Meningitis can occur from hematogenous spread of *M. catarrhalis* from the nasopharynx or as a consequence of ventriculoperitoneal shunt placement or surgery.

Rarely, suppurative arthritis has been seen in children and adults.[32,39] Endocarditis is a rare occurrence, as is peritonitis.

TREATMENT

In the past, *M. catarrhalis* was susceptible to all β-lactam antibiotics. In the late 1970s, however, β-lactamase–producing strains of *M. catarrhalis* were isolated in Europe. In the United States, a parallel increase in the frequency of isolation of β-lactamase–producing *M. catarrhalis* has been noted, so it is not unusual for a laboratory to report that more than 90 percent of strains are β-lactamase producers.

The β-lactamases BRO-1, BRO-2, and BRO-3 are of chromosomal origin. BRO-1 is the most common β-lactamase in North American strains.[15] Because of the high prevalence of β-lactamase–producing strains, many laboratories choose not to do any such testing. The β-lactamase inhibitors clavulanic acid, sulbactam, and tazobactam are active against the enzymes produced by *Moraxella*. Most β-lactamase–positive isolates will respond to achievable concentrations of β-lactam/β-lactamase inhibitor antimicrobial combinations, cephalosporins, and cephamycins.[18,23]

Among the oral cephalosporins active against β-lactamase–positive strains of *Moraxella*, the minimal inhibitory concentration increases twofold to fourfold in the presence of the β-lactamase BRO-1 but not the β-lactamase BRO-2; cefixime is more active than are the older cephalosporins.[41] Resistance to tetracycline (by the nontransferable tetB determinant) and erythromycin has been reported, but such resistance is a rare finding.[10,46]

In vitro, *M. catarrhalis* usually is susceptible to ampicillin-sulbactam, amoxicillin-clavulanate, erythromycin, azithromycin, clarithromycin, trimethoprim-sulfamethoxazole, tetracyclines, chloramphenicol, fluoroquinolones (e.g., ciprofloxacin), aminoglycosides, and second- or third-generation cephalosporins, such as cefuroxime, cefprozil, cefpodoxime, cefixime, cefdinir, and loracarbef.[36] Levofloxacin also exhibits good activity against *M. catarrhalis*.[3] The *Moraxella* organism is resistant to clindamycin, vancomycin, and oxacillin.

PREVENTION

Prevention of nosocomial *M. catarrhalis* infection depends on sound practices of infection control, especially with regard to pulmonary toilet. Prevention of other infections with this organism has not been attempted. Development of a vaccine has been hampered by a lack of an animal model.

NEW REFERENCES SINCE THE SIXTH EDITION

28. Hall-Stoodley L, Hu FZ, Gieseke A, et al. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. *JAMA* 2006;**296**:202–11.
30. Heiniger N, Spaniol V, Troller R, et al. A reservoir of *Moraxella catarrhalis* in human pharyngeal lymphoid tissue. *J Infect Dis* 2007;**196**:1080–7.
40. Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. *Clin Infect Dis* 2009;**49**:124–31.
57. Verhaegh SJC, Snippe ML, Levy F, et al. Colonization of healthy children by *Moraxella catarrhalis* is characterized by genotype heterogeneity, virulence gene diversity and co-colonization with *Haemophilus influenzae*. *Microbiology* 2011;**157**:169–78.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 88

Meningococcal Disease

Marsha S. Anderson • Mary P. Glodé • Arnold L. Smith

Epidemic meningococcal meningitis was first described by Gaspard Vieusseux in Geneva in the spring of 1805. In a monograph entitled "The Disease Which Raged During the Spring of 1805," he wrote:

> *It commences suddenly with prostration of strength, often extreme: the face is distorted, the pulse feeble. There appears a violent pain in the head, especially over the forehead; then there comes pain of the heart or vomiting of greenish material, stiffness of the spine, and in infants, convulsions. In cases which were fatal, loss of consciousness occurred. The course of the disease is very rapid, termination by death or by cure. In most of the patients who died in 24 hours or a little after, the body is covered with purple spots at the moment of death or very little time afterward.*[217]

REFERENCES

1. Ainsworth SM, Nagy SB, Morgan LA, et al. Interpretation of gram-stained sputa containing *Moraxella* (*Branhamella*) *catarrhalis*. *Clin Microbiol* 1990;**28**:2559–60.
2. Akgul G, Erturk A, Turkoz M, et al. Role of lipooligosaccharide in the attachment of *Moraxella catarrhalis* to human pharyngeal epithelial cells. *Microbiol Immunol* 2005;**49**:931–5.
3. Arguedas A, Dagan R, Pichichero M, et al. An open-label, double tympanocentesis study of levofloxacin therapy in children with, or at high risk for, recurrent or persistent acute otitis media. *Pediatr Infect Dis J* 2006;**25**:1102–9.
4. Barnett ED, Klein JO. The problem of resistant bacteria for the management of acute otitis media. *Pediatr Clin North Am* 1995; **42**:509–18.
5. Baron J, Shapiro ED. Unsuspected bacteremia caused by *Branhamella catarrhalis*. *Pediatr Infect Dis J* 1985;**4**:100–1.
6. Bartos LC, Murphy TF. Comparison of the outer membrane proteins of 50 strains of *Branhamella catarrhalis*. *J Infect Dis* 1988; **158**:761–5.
7. Berg RA, Bartley DL. Pneumonia associated with *Branhamella catarrhalis* in infants. *Pediatr Infect Dis J* 1987;**6**:569–73.
8. Bonadio WA. *Branhamella catarrhalis* bacteremia in children. *Pediatr Infect Dis J* 1988;**7**:738–9.
9. Bootsma HJ, van der Heide HG, van de Pas S, et al. Analysis of *Moraxella catarrhalis* by DNA typing: evidence of a distinct subpopulation associated with virulence traits. *J Infect Dis* 2000;**181**: 1376–87.
10. Brown BA, Wallace RJ, Flanagan CW, et al. Tetracycline and erythromycin resistance among clinical isolates of *Branhamella catarrhalis*. *Antimicrob Agents Chemother* 1989;**33**:1631–3.
11. Catlin BW. Transfer of the organism named *Neisseria catarrhalis* to *Branhamella* gen. nov. *Int J Syst Bacteriol* 1970;**20**:155–9.
12. Catlin BW. *Branhamella catarrhalis:* an organism gaining respect as a pathogen. *Clin Microbiol Rev* 1990;**3**:293–320.
13. Chapman AJ, Musher DM, Jonsson S, et al. Development of bactericidal antibody during *Branhamella catarrhalis* infection. *J Infect Dis* 1985;**151**:878–82.
14. Cook PP, Heent DW, Syndman DR. Nosocomial *Branhamella catarrhalis* in a pediatric intensive care unit: risk factors for disease. *J Hosp Infect* 1989;**13**:299–307.
15. Deshpande LM, Sader HS, Fritsche TR, et al. Contemporary prevalence of BRO beta-lactamases in *Moraxella catarrhalis*: report from the SENTRY antimicrobial surveillance program (North America, 1997 to 2004). *J Clin Microbiol* 2006;**44**:3775–7.
16. Dickinson DP, Loos BG, Dryja DM, et al. Restriction fragment mapping of *Branhamella catarrhalis:* a new tool for studying the epidemiology of this middle ear pathogen. *J Infect Dis* 1988;**158**:208.
17. Doern GV. *Branhamella catarrhalis*: phenotypic characteristics. *Am J Med* 1990;**88**:335–55.
18. Doern GV, Jones RN. Antimicrobial susceptibility testing of *Haemophilus influenzae*, *Branhamella catarrhalis* and *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1988;**32**:1747–53.
19. Dyson C, Poonyth HO, Watkinson M, Rose SJ. Life threatening *Branhamella catarrhalis* pneumonia in young infants. *J Infect* 1990;**21**:305–7.
20. Ernst TN, Philp M. Bacterial tracheitis caused by *Branhamella catarrhalis*. *Pediatr Infect Dis J* 1987;**6**:574.
21. Faden H, Hong JJ, Pahade N. Immune response to *Moraxella catarrhalis* in children with otitis media: opsonophagocytosis with antigen-coated latex beads. *Ann Otol Rhinol Laryngol* 1994;**103**: 522–4.
22. Faden H, Harabuchi Y, Hong JJ, et al. Epidemiology of *Moraxella catarrhalis* in children during the first 2 years of life: relationship to otitis media. *J Infect Dis* 1994;**169**:1312–17.
23. Fung C-P, Yeo S-F, Livermore DM. Susceptibility of *Moraxella catarrhalis* isolates to β-lactam antibiotics in relation to β-lactamase pattern. *J Antimicrob Chemother* 1994;**33**:215–22.
24. Ghon A, Pfeiffer H. Der Mikvococcus catarrhalis (R. Pfeiffer) als Krankheitserreger. *Z Klin Med* 1902;**44**:263–81.
25. Goldblatt D, Turner MW, Levinsky RJ. *Branhamella catarrhalis*: Antigenic determinants and the development of the IgG subclass response in childhood. *J Infect Dis* 1990;**162**:1128–35.
26. Gottfarb P, Brauner A. Children with persistent cough: outcome with treatment and role of *Moraxella catarrhalis*? *Scand J Infect Dis* 1994;**26**:545–51.
27. Hager H, Verghese A, Alvarez S, et al. *Branhamella catarrhalis* respiratory infections. *Rev Infect Dis* 1987;**9**:1140–9.
28. Hall-Stoodley L, Hu FZ, Gieseke A, et al. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. *JAMA* 2006;**296**:202–11.
29. Harrison CJ, Marks JI, Welch DF. Microbiology of recently treated acute otitis media compared with previously untreated acute otitis media. *Pediatr Infect Dis J* 1985;**4**:641–6.
30. Heiniger N, Spaniol V, Troller R, et al. A reservoir of *Moraxella catarrhalis* in human pharyngeal lymphoid tissue. *J Infect Dis* 2007; **196**:1080–7.
31. Helminen ME, Maciver I, Paris M, et al. A mutation affecting expression of a major outer protein of *Moraxella catarrhalis* alters resistance and survival in vivo. *J Infect Dis* 1993;**168**:1194–201.
32. Izraeli S, Flasterstein B, Shamir R, et al. *Branhamella catarrhalis* as a cause of suppurative arthritis. *Pediatr Infect Dis J* 1989;**8**:256–7.
33. Klein JO. Otitis media. *Clin Infect Dis* 1994;**19**:823–33.
34. Korppi M, Katila ML, Jaaskelainen J, et al. Role of *Moraxella* (*Branhamella*) *catarrhalis* as a respiratory pathogen in children. *Acta Paediatr* 1992;**81**:993–6.
35. Leinonen M, Luotonen J, Herva E, et al. Preliminary serologic evidence for a pathogenic role of *Branhamella catarrhalis*. *J Infect Dis* 1981;**144**:570–4.
36. Lemmen SW, Anding K, Engels I, et al. Bactericidal activity of clarithromycin and cefaclor against *Streptococcus pneumoniae* and *Moraxella catarrhalis* in healthy volunteers. *J Antimicrob Chemother* 1994;**33**:673–4.
37. Maciver I, Unhanand M, McCracken GH, et al. Effect of immunization on pulmonary clearance of *Moraxella catarrhalis* in animal model. *J Infect Dis* 1993;**168**:469–72.
38. Marchant CD. Spectrum of disease due to *Branhamella catarrhalis* in children with particular reference to acute otitis media. *Am J Med* 1990;**88**(suppl. 5A):155–95.
39. Melendez PR, Johnson RH. Bacteremia and septic arthritis caused by *Moraxella catarrhalis*. *Rev Infect Dis* 1991;**13**:428–9.
40. Murphy TF, Parameswaran GI. *Moraxella catarrhalis*, a human respiratory tract pathogen. *Clin Infect Dis* 2009;**49**:124–31.
41. Nash DR, Flanagan C, Steele LC, et al. Comparison of the activity of cefixime and activities of other oral antibiotics against adult clinical isolates of *Moraxella* (*Branhamella*) *catarrhalis* containing BRO-1 and BRO-2 and *Haemophilus influenzae*. *Antimicrob Agents Chemother* 1991;**35**:192–4.
42. Ohlsson A, Bailey T. Neonatal pneumonia caused by *Branhamella catarrhalis*. *Scand J Infect Dis* 1985;**17**:225–8.
43. Patterson JE, Patterson TF, Farrel P, et al. Evaluation of restriction endonuclease analysis as an epidemiologic typing system for *Branhamella catarrhalis*. *J Clin Microbiol* 1989;**27**:944–6.
44. Pichichero ME, Pichichero CL. Persistent acute otitis media. I. Causative organisms. *Pediatr Infect Dis J* 1995;**14**:178–83.
45. Revai K, McCormick DP, Patel J, et al. Effect of pneumococcal conjugate vaccine in nasopharyngeal bacterial colonization during acute otitis media. *Pediatrics* 2006;**117**:1823–9.
46. Roberts MC, Pang Y, Spencer RC, et al. Tetracycline resistance in *Moraxella* (*Branhamella*) *catarrhalis*: demonstration of two clonal outbreaks by using pulsed-field gel electrophoresis. *Antimicrob Agents Chemother* 1991;**35**:2453–5.
47. Rotta AT, Asmar BI. *Moraxella catarrhalis* bacteremia and preseptal cellulitis. *South Med J* 1994;**87**:541–2.
48. Seddon PC, Sunderland D, O'Halloran SM, et al. *Branhamella catarrhalis* colonization in preschool asthmatics. *Pediatr Pulmonol* 1992;**13**:133–5.
49. Shurin PA, Marchant CD, Kim CH, et al. Emergence of beta-lactamase–producing strains of *Branhamella catarrhalis* as important agents of otitis media. *Pediatr Infect Dis J* 1983;**2**:34–8.
50. Smith G. *Branhamella catarrhalis* infection imitating gonorrhea in a man. *N Engl J Med* 1987;**326**:1277.
51. Speeleveld E, Fossepre JM, Gordts B, Van Landuyt HW. Comparison of three rapid methods, tributyrin, 4-methylumbelliferyl butyrate, and indoxyl acetate, for rapid identification of *Moraxella catarrhalis*. *J Clin Microbiol* 1994;**32**:1362–3.
52. Stull TL, Stanford EJ. Pseudogonococcal ophthalmia neonatorum caused by *Branhamella catarrhalis*. *Pediatr Infect Dis J* 1986;**5**: 104–5.
53. Unhanand M, Maciver I, Ramilo O, et al. Pulmonary clearance of *Moraxella catarrhalis* in an animal model. *J Infect Dis* 1992;**165**: 644–50.

54. Vaneechoutte M, Verschraegen G, Claeys G, et al. Respiratory tract carrier rates of *Moraxella Branhamella catarrhalis* in adults and children and interpretation of the isolation of *Moraxella catarrhalis* from sputum. *J Clin Microbiol* 1990;**28**:2674–80.
55. Verduin CM, Kools-Sijmons M, van der Plas J, et al. Complement-resistant *Moraxella catarrhalis* forms a genetically distinct lineage with the species. *FEMS Microbiol Lett* 2000;**184**:1–8.
56. Verghese A, Berk SL. *Moraxella* (*Branhamella*) *catarrhalis*. *Infect Dis Clin North Am* 1991;**5**:523–38.
57. Verhaegh SJC, Snippe ML, Levy F, et al. Colonization of healthy children by *Moraxella catarrhalis* is characterized by genotype heterogeneity, virulence gene diversity and co-colonization with *Haemophilus influenzae*. *Microbiol* 2011;**157**:169–78.
58. Wald ER, Reilly JS, Casselbrant M, et al. Treatment of acute maxillary sinusitis in childhood: a comparative study of amoxicillin and cefaclor. *J Pediatr* 1984;**104**:297–302.
59. Wong VK, Ross LA. *Branhamella catarrhalis* septicemia in an infant with AIDS. *Scand J Infect Dis* 1988;**20**:559–60.

Today, *Neisseria meningitidis* continues to be a cause of endemic and epidemic disease. In the United States, approximately 800 to 1500 cases of invasive disease occur each year and 95 to 97 percent of these cases are sporadic.[40,45] However, epidemics in developed and developing countries are still occurring and serve as a reminder of the potential virulence of this organism.

The reader is referred to the excellent reviews of meningococcal disease that have been published recently.[52,101]

MICROBIOLOGY

The *Neisseria* genus was named for Dr. Albert Neisser, who described the organism of gonorrhea in 1879. The genus *Neisseria* contains two species important in human disease, *N. gonorrhoeae* and *N. meningitidis*. In addition, several species are part of the normal flora of humans and animals. *N. sicca*, *N. lactamica*, *N. subflava*, *N. flavescens*, *N. mucosa*, *N. cinerea*, *N. polysaccharea*, and *N. elongata* are human commensals; they only occasionally cause human disease.[137]

N. meningitidis is a gram-negative coccus, usually less than 1 μm in diameter. It classically occurs in pairs, with adjacent sides flattened, resembling kidney beans. Occasionally, the organism divides in two planes at right angles to one another, which results in the formation of tetrads. The organisms are nonmotile, are aerobic (but facultatively anaerobic), produce catalase and oxidase, and may be encapsulated. *N. meningitidis* oxidizes glucose and maltose to acid. This distinguishes it from *N. gonorrhoeae*, which oxidizes only glucose. However, certain rare strains of *N. meningitidis* have been reported that fail to produce acid from either glucose or maltose. Fresh isolates have complex nutritional requirements, growing best on chocolate or blood agar. Incubation in a humidified 10 percent carbon dioxide environment is not essential, but enhances growth. Morphologically, the colonies are bluish-gray in appearance and will produce β-hemolysis after 48 to 72 hours of incubation on 5 percent horse blood agar.[28]

N. meningitidis can be transformed by DNA originating in other *Neisseria* spp. Thus, when a *Neisseria* spp. cohabiting the nasopharynx with *N. meningitidis* dies and releases its DNA, the coresident meningococcus can take up the DNA and incorporate it into its genome. Although this sequence of events is very rare, if the new DNA confers a selective advantage to the recipient meningococcus, the trait encoded by the incoming DNA may be retained in future progeny. This is the mechanism by which sulfonamide resistance and penicillin G resistance have arisen in *N. meningitidis*.[137] Many commensal *Neisseria* spp., which also inhabit the nasopharynx, are relatively resistant to penicillin G. DNA fragments from these commensal strains, encoding penicillin resistance, can be detected in penicillin-resistant *N. meningitidis*.[188] Transformation of new DNA into *N. meningitidis* also can introduce genes that modify the structure of the bacterium. Serotyping of groups A, B, and C meningococci has provided valuable macroepidemiologic information.[82] It is clear that as a population develops immunity to one capsular polysaccharide, the organism is capable of acquiring DNA encoding for an alternative capsule. Serogroup B to C and C to B switching has been reported.[197,204,218] Horizontal gene transfer appears to occur from commensal *Neisseria* to *N. meningitidis* of genes whose products aid the bacterium in surviving in the host, or are involved in housekeeping functions.[135] This phenomenon is probably responsible for the occasional report of commensal *Neisseria* causing meningitis.[10]

In addition to the transformation route for varying the antigenic phenotype, meningococci also may exhibit phase variation, which allows for variance of the number of surface antigens. It has been estimated that there are at least 65 genes in *N. meningitidis* subject to phase variation including *por*A.[77,211] Capsule synthesis may be switched on and off by this mechanism.

Like other gram-negative bacteria, *Neisseria's* outer and inner cell membranes are phospholipid bilayers. These membranes sandwich a layer of peptidoglycan. Lipo-oligosaccharide, which is similar to the lipopolysaccharide seen in gram-negative enteric bacteria, is associated with the outer leaflet of the outer cell membrane. Outer membrane proteins that function as porins are an integral part of the outer membrane. Some of these outer membrane proteins are opacity proteins and facilitate adherence and invasion.[151] Outside the outer membrane meningococci possess a polysaccharide capsule that protects the organism from phagocytosis.

Meningococci also have pili, which seem to be important in some phases of adherence to host cells, colonization, and invasion. The genes that code for pili can be turned on and off, so that the organism at times may not express pili. A recent study reported that posttranslational modification of the type IV pili by phosphoglycerol altered the pilin structure and destabilized the pili bundles, resulting in reduced bacterial aggregation on the mucosal surface, which allowed the organism to better cross epithelial monolayers to produce systemic invasion.[47] Antigenic variation of pili by a cassette mechanism allows the bacterium to escape the host's immune system.[93] In addition, both outer membrane proteins and lipo-oligopolysaccharide display antigenic variation by phase variation.

For epidemiologic purposes, meningococci are divided into serogroups, serotypes, and subtypes. Differences in capsular polysaccharides, outer membrane proteins, and lipo-oligosaccharides constitute the basis for these classifications. The capsular polysaccharides are antigenic and the basis for serogroup designation. Thirteen serogroups currently are recognized: A, B, C, D, H, I, K, L, X, Y, Z, W-135, and 29E.[226] Serogroups A, B, C, W-135, X, and Y are the most common causes of invasive disease worldwide.

Serotyping of organisms is based on antigenic differences in outer membrane proteins termed *porins*. Porins are protein channels in the outer membrane that permit nutrients and certain antibiotics to diffuse into the periplasmic space. A single locus encodes porin B, but two mutually exclusive alleles have antigenic differences. All meningococci have either antigenic class 2 or class 3 porin B. These differences permit serotyping. Subtypes are differentiated based on antigenic differences in porin

A. This highly variable antigen is called a class 1 outer membrane protein. Multiple serotypes and subtypes have been identified. Antigenic specificity is achieved by the generation of monoclonal antibodies to the major protein (primarily class 2 or 3), lipo-oligosaccharide, and class 1 protein. The designation B:2a:P1.1:L3,7 indicates that the serogroup B strain is an "a" subtype of class 2 proteins, is a "1" of class 1 protein, and possesses two lipo-oligosaccharide antigens: 3 and 7.[89]

Type 2 protein antigen is isolated from more than 50 percent of cases in the United States and Canada and is an antigenic determinant in serogroup B and C strains. These protein antigens not only are important as epidemiologic markers but also can induce protective bactericidal antibodies.[83]

EPIDEMIOLOGY

Epidemiology in the United States

Invasive infections caused by *N. meningitidis* are reportable through the National Notifiable Surveillance System in the United States. The number of U.S. cases reported to the Centers for Disease Control and Prevention (CDC) in 2008, 2009, 2010, and 2011 were 1172, 980, 845, and 759, respectively.[46] The recent numbers reflect the lowest number of cases ever reported in the United States.[46]

The incidence of meningococcal disease varies significantly by geographic location. Endemic disease, which occurs in developed countries such as the United States and Europe, has an incidence range of 0.15 to 3.0 cases/100,000 population per year.[40,101] The U.S. Active Bacterial Core Surveillance (ABC) system tracks invasive disease caused by encapsulated organisms in selected counties of 10 states geographically dispersed throughout the country. This reporting system covers a U.S. population of 42 million people. In 2010, the overall incidence of invasive meningococcal disease for the United States was estimated to be 0.15/100,000 population.[40] Of cases reported through the ABC system, 46 percent were meningitis, with a fatality rate of 12.8 percent, and 31 percent of cases were bacteremia, with a fatality of 1.1 percent. The rate of invasive disease was 0.27/100,000 population for African Americans and 0.2/100,000 for whites. Of cases in the 2010 ABC Surveillance Report, 20 percent were serogroup B, 30 percent were serogroup C, 48 percent were serogroup Y, and 2 percent were other serogroups. This serogroup analysis excluded Oregon because of an outbreak of serogroup B disease in Oregon at the time.[40]

Serogroups A, B, C, and Y account for more than 90 percent of meningococcal disease worldwide. The incidence of serogroup C and serogroup Y disease has increased in the United States over the last few years, associated with antigenic shifts involving outer membrane protein genes.[102] Disease caused by serogroup W-135 is infrequent, and like disease in serogroup Y has been associated with meningococcal pneumonia.[186,220]

In 2006, Brooks and colleagues[26] reported on an analysis of 69 outbreaks of meningococcal disease that occurred in the United States from 1992 to 2002. Outbreak-associated cases represented less than 2 percent of total cases of invasive disease. Additionally, outbreak-related cases were associated with a higher case fatality rate than sporadic cases (21% vs. 11%; odds ratio 3.3; 95% CI 2.0 to 5.5).[26]

Meningococcal disease, in the United States, peaks during the months November through March.[186] The highest attack rate is seen in February, and the lowest attack rate occurs in September.

The risk for meningococcal disease is related inversely to age, with the highest rates of disease occurring in children younger than 1 year of age.[34] Up to 49 percent of the cases occur in children younger than 2 years of age.[111] However, in epidemics an age shift occurs, with older children, adolescents, and young adults more often affected.[36] A progressive increase in the development of protective antibodies against meningococci occurs between the ages of 2 and 12 years. Neonates usually are protected, if the mother has antimeningococcal antibody, by passive in utero immunoglobulin (Ig)G transfer. In general, the development of bactericidal antibodies to meningococci increases in children at a rate of about 5 percent per year.[89]

International Epidemiology

In developing countries, the incidence rate of invasive meningococcal disease is about 10 to 20 times higher than in the United States (10 to 25/100,000 inhabitants/year).[94] The highest rate of meningococcal disease is in a multicountry belt across sub-Saharan Africa, which has been termed the *meningitis belt*. Serogroup A meningococcal disease is prevalent there and occurs in epidemics during the dry season.[174] During epidemics, the incidence of meningococcal disease is as high as 1000/100,000 inhabitants (1%). Spread of clonal strains of serogroup A meningococcus may be a factor in Africa's unusually high rate of epidemic disease. Clonal strains can migrate transcontinentally and cause epidemics, such as the clonal serogroup A meningococcus, electrophoretic type III-1 (ET III-1) that caused epidemics in China, Nepal, Saudi Arabia, Chad, and Kenya in the 1980s.[145,147,166]

Epidemics occur in the meningitis belt in sub-Saharan Africa each year, affecting thousands of individuals. In 2009, 14 African countries implemented enhanced surveillance during the epidemic season and reported 88,199 suspected cases, including 5352 deaths, the largest number since a 1996 epidemic. Although serogroup A is the predominant serogroup causing epidemic disease in Africa, serogroup X was responsible for more than half of the fatalities from meningococcal meningitis in Burkina Faso in 2010.[173,229] In Africa, epidemic disease occurs during the dry season and decreases once the rainy season starts.[166] Males and females are affected equally.

International outbreaks of *N. meningitidis* infections occurred in 1987 and 2000 and were associated with Muslims making the annual pilgrimage to Mecca. The disease spread to persons of all nationalities represented, some of whom returned home before becoming ill.[147,170] In some cases, close contacts of travelers were infected. Several cases in U.S. citizens were attributed to the outbreaks. The 1987 outbreak was linked to serogroup A,

and the 2000 outbreak was due to serogroup W-135.[35,147,170] Saudi Arabia now requires meningococcal vaccine for travelers entering the country to participate in the annual pilgrimage to Mecca.[123]

Colonization and Disease

The epidemiology of meningococcal disease was defined elegantly in a series of observations in New Jersey military recruits. An inverse correlation was noted between the age-related incidence of meningococcal disease in the United States and the prevalence of serum bactericidal activity against three pathogenic strains of *N. meningitidis*.[89,90] Investigators prospectively studied all incoming recruits to Fort Dix and demonstrated that recruits who lacked bactericidal activity to case strains had a 38 percent attack rate if they were exposed and acquired a pathogenic meningococcus strain.[89] Usually, nasopharyngeal carriage was an immunizing process. Carriage induced the formation of protective antibodies against homologous strains but also produced cross-reacting antibodies to heterologous strains of pathogenic meningococci. Antibody production typically was seen within 2 weeks of acquisition of carriage.[89,90]

In addition to nasopharyngeal carriage, other processes may contribute to meningococcal antibody formation. Certain strains of cross-reacting bacteria, such as *Escherichia coli* and *Bacillus*, produce capsular polysaccharides that immunologically are identical to the capsules of meningococci serogroups A, B, and C. This is a potential immunizing source against invasive meningococcal disease in the general population.[89,117,216]

Nasopharyngeal carriage of *N. meningitidis* is lowest in infants and children and highest in adolescents and young adults. One study determined that 2.4 percent of asymptomatic infants and children had a positive nasopharyngeal culture for *N. meningitidis* during a nonepidemic period.[140] A study of 1500 Norwegians found the carriage rate in persons 20 to 24 years of age was 32.7 percent and in individuals beyond 25 years of age was 10 percent.[33] Other investigators have shown that approximately 20 percent of children harbor meningococcal species in their nasopharynx, but the majority of these isolates are atypical, nontypable strains, which ferment lactose in addition to glucose and maltose.[89,90]

Nasopharyngeal meningococcal carriage in household contacts of persons with documented meningococcal disease is about 10 percent.[142,158,179] Several studies demonstrated that the carriage rate increases if infants or children are in the home.[118,158] In one outbreak of serogroup C meningococcal disease, the carriage rates were 37.8 percent in households with a case in an infant, 17.5 percent in households with a case in a child, and 6.9 percent in households with disease in an adult.[117]

Nasopharyngeal carriage is common after household contact with an index case, and colonization can persist for weeks to months. Without chemoprophylaxis, up to 35 percent of contacts become colonized by the eighth week. The median duration of carriage is 9 months.[140] However, in nearly 40 percent of individuals, it may exceed 16 months.[92] An association between smoking and increased rate of meningococcal carriage has been demonstrated.[196,202] In addition, passive smoke has been implicated in increasing the risk for meningococcal disease in children younger than 5 years of age by 7.5 times that of the general population.

The factors responsible for converting nasopharyngeal colonization to invasive disease have not been firmly established. Many people who subsequently develop invasive disease are colonized shortly before the illness begins. Likely, these individuals lack bactericidal antibody and for some reason do not develop protective antibody after acquisition of the pathogenic strain. Other risk factors associated with invasive disease include young age, crowding (e.g., new military recruits and college freshmen in dormitories),[5,27,37] lower socioeconomic class,[185] concurrent upper respiratory tract infections,[146,231] intimate kissing with multiple partners,[208] specific immune deficiencies (i.e., properdin or terminal complement),[78] functional or anatomic asplenia,[81] and active or passive smoking.[80,196]

Speculation as to the role of other upper respiratory tract pathogens in meningococcal disease prompted several observations. It was noted that the peak incidence of meningococcal infection mirrors the peaks of such agents as influenza and *Mycoplasma*. Several studies have shown an association between colonization or infection with respiratory tract viruses or *Mycoplasma* and the increased risk for development of meningococcal disease.[32,108,146,191] Simultaneous outbreaks of meningococcal and influenza or echovirus infections have been documented.[107,133,231] In addition, one study suggested that the incidence of meningococcal disease and the resultant morbidity and mortality increased in the 5 weeks after influenza-like syndromes.[146] It has been postulated that viral pathogens may temporarily affect the immune response and facilitate meningococcal disease. It is also possible that disruption of the normal respiratory epithelium occurs with viral infection, and this increases the likelihood that colonizing meningococci might become invasive.[146] Preceding viral respiratory tract disease is not a prerequisite for the establishment of meningococcal carriage or disease. However, its occurrence may increase the risk for invasive meningococcal disease.

A variety of host genetic factors also may influence susceptibility to invasive meningococcal disease. Individuals with late complement component deficiencies (C5, C6, C7, C8, or C9) and properdin pathway deficiencies are at increased risk for meningococcal disease.[78] This usually is an inherited defect, and a history of other members of the family with meningococcal disease or repeated meningococcal infections may exist. Complement factor H is a complement-related protein, and certain polymorphisms in the complement factor H gene have been associated with an increased susceptibility to meningococcal disease.[60,99] Acquired complement deficiencies associated with diseases such as systemic lupus erythematosus, nephrotic syndrome, and chronic liver disease also are associated with increased risk for meningococcal disease.[129] A Russian study found the incidence of complement deficiency in first episodes of meningococcal disease to be about 1 percent.[168] In Italy, the prevalence of complement deficiency in patients with meningococcal meningitis was 17 percent.[57] The decision

of whom to screen for complement deficiency should be made partly based on the prevalence of meningococcal disease in a given country. In countries where the incidence of meningococcal disease is high, complement deficiency is less likely to be found. In countries such as the United States, where the incidence is relatively low, complement screening should be considered because it has implications for family members who can be tested and then immunized and educated regarding their increased risk for meningococcal disease.

The chance of finding a complement or alternative pathway (properdin) deficiency substantially increases in patients with recurrent meningococcal disease or with uncommon serogroups. The prevalence of complement or properdin deficiency in a patient with infection by an unusual serogroup was found to range from 31 to 50 percent.[79,154] Therefore, screening all individuals with unusual serogroups is reasonable. CH_{50} is a generally available test and will screen for combined activity of C1 to C9. If complement deficiency is found, the individual should receive the meningococcal vaccine. In addition, family members should be screened for complement deficiency and all family members should be educated about the disease and immunized with quadrivalent polysaccharide or protein conjugate meningococcal vaccine. Most experts believe that the conjugate vaccine is preferred in the age groups for which the vaccine is licensed (9 months to 55 years).

Some investigators demonstrated that although persons with complement deficiency are more at risk for meningococcal disease, the disease they get often is mild. Meningococcal infection activates the complement system, and lipo-oligosaccharides present in the bacterium's outer membrane probably are responsible for the activation. In fatal cases, intense activation has continued until the time of death.[23] The case-fatality rate in complement-deficient individuals is about 3 percent.[187] It is thought that complement-deficient individuals are unable to maintain the high level complement pathway activation and therefore have less severe disease.

Properdin deficiency, which impairs activation of the alternative complement pathway, is X-linked and has been associated with fulminant meningococcal disease.[64,79] The case-fatality rate in one kindred was 75 percent for persons with meningococcal disease.[18] Screening of individuals with exceptionally fulminant disease or individuals who have a family history of meningococcal infections should be considered. AP_{50} will screen for properdin deficiency in the alternative complement pathway. If a deficiency is documented, those individuals and their family members should receive the quadrivalent meningococcal vaccine to prevent disease from serogroups of meningococcus in the vaccine (conjugate vaccine is preferred in the age groups for which the vaccine is licensed—9 months to 55 years).

Mannose binding lectin (MBL) is a pattern recognition receptor and a component of the innate immune system. Patients with meningococcal disease during childhood have a significantly increased incidence of structural mutations in the genes coding for MBL, leading to deficient MBL function in contrast to that in healthy age-matched controls.[75] Similarly, mutations in genes coding for Toll-like receptor (TLR)4 may also increase susceptibility to meningococcal disease. Human TLR4 mutations are associated with susceptibility to invasive meningococcal disease in infancy.[74]

PATHOLOGY AND PATHOGENESIS

The mechanisms by which meningococci invade humans are only partially understood. We do know that encapsulated, typable meningococci are virulent, whereas nonencapsulated strains are relatively nonpathogenic. Even among encapsulated strains, differences in virulence between case and carrier strains of *N. meningitidis* have been demonstrated in an animal model.[106] The presence of a polysaccharide capsule enhances invasiveness by resisting opsonization and subsequent phagocytosis.

The human nasopharynx is the only natural reservoir of *N. meningitidis*.[199] Once pathogenic meningococci have colonized the respiratory tract of susceptible persons, either the organisms become invasive or the individual develops antibody to the organism, conferring immunity. Meningococci adhere to nonciliated, columnar epithelial cells in the nasopharynx via pili. Binding induces endocytosis of the organism into the epithelial cell, and the bacteria may penetrate the epithelial barrier via phagocytotic vacuoles.[198] If antibody is insufficient and invasion occurs, the individual may become bacteremic. Occasionally, unsuspected meningococcal bacteremia has been detected on blood culture and spontaneously cleared on a follow-up culture without antibiotics.[202] However, in most cases, this does not occur and the individual becomes progressively sicker. Bacteria in the blood may seed the meninges and cause meningitis.

Meningococci are gram-negative organisms, and lipo-oligosaccharide is a major component of the bacterial cell membrane. Meningococci release blebs from their surfaces that contain an outer membrane laden with lipo-oligosaccharide. Lipo-oligosaccharide is a potent endotoxin and concentrations of it correlate with severity of disease.[23-25] Lipo-oligosaccharide induces release of a host of inflammatory and anti-inflammatory mediators whose concentrations are also correlated with severity of disease: tumor necrosis factor (TNF)-α, γ-interferon, interleukin (IL)-1, IL-6, IL-8, IL-10, and IL-1 receptor antagonist.[87,130,212,213,222] TNF-α downregulates thrombomodulin expression on endothelial cells, leading to decreased activity of proteins S and C.[53,152] Like TNF-α, many of the mediators directly or indirectly contribute to the formation of a procoagulant state, which results in the formation of microthrombi characteristically found in the skin, digits and extremities, and organs.

Cytokines play an important role in the development of shock frequently seen in meningococcemia. Cytokine release causes neutrophil activation and upregulation of adhesion molecules that may promote endothelial damage and capillary leak.[72] Vasodilation also may occur secondary to increased production of nitric oxide by the endothelial cells.[144] Compensatory vasoconstriction of splanchnic, skin, and renal vessels may subsequently not be enough to maintain adequate blood pressure. Endotoxin-related or cytokine-mediated cardiac

dysfunction also may contribute to development of heart failure and hypotension.[161] Profound capillary leak in the pulmonary bed may lead to the development of acute respiratory distress syndrome (ARDS).

The disseminated intravascular coagulation commonly seen in meningococcemia is believed to be a consequence of activation of the coagulation system by endotoxin.[182] Experimental animal work suggests a synergistic effect of meningococcal endotoxin and materials egested from leukocytes containing meningococci in the initiation of disseminated intravascular coagulation.[66]

The pathologic purpuric lesions seen in fulminant meningococcal disease are similar to those that the generalized Shwartzman reaction induces in rabbits by endotoxin. Pathologically, these lesions show evidence of microthrombi in the small dermal vessels, endothelial damage, and hemorrhage. Inflammatory vasculitis is mediated by upregulation of endothelial adhesion molecules, neutrophil activation, and effects of cytokines and bacterial endotoxin.[7,58,105] Circulating concentrations of endotoxin in patients with meningococcemia may be 50 to 100 times that of other gram-negative infections.[169] In addition, meningococcal endotoxin is more potent than endotoxin from enteric bacilli.[61]

A pathologic study of 200 fatal meningococcal infections illustrates the ability of the meningococcus to affect virtually any organ, either directly or indirectly.[100] Approximately 40 percent of the patients had both meningococcemia and meningitis. Except for adults with meningitis, the average survival time for all cases was 72 hours or less. The major organ systems involved at autopsy in these 200 cases were heart, central nervous system, skin, mucous and serous membranes, and adrenals. Myocarditis occurred in 78 percent of cases. Cutaneous hemorrhages occurred in 69 percent of the fatal infections and ranged from isolated petechiae to diffuse purpura. Acute meningitis was noted at autopsy in 68 percent, with brain abscesses noted in two patients. In almost half the cases with acute meningococcemia, *N. meningitidis* was isolated from otherwise normal cerebrospinal fluid (CSF). Adrenal hemorrhage and necrosis were found in 48 percent of autopsy cases. Diffuse adrenal hemorrhage occurred in approximately 50 percent of adult cases and in more than 80 percent of pediatric cases. Focal areas of inflammation and petechial hemorrhage were seen in many other tissues, including the synovium, skeletal muscle, and the tracheobronchial tree. An association was seen between the pathologic findings and the infecting serogroup of *N. meningitidis*. Serogroup A infections most frequently were associated with encephalitis; serogroups B and C infections were associated with necrotizing myocarditis.

CLINICAL MANIFESTATIONS AND DIFFERENTIAL DIAGNOSIS

The spectrum of disease caused by *N. meningitidis* ranges from asymptomatic transient bacteremia, which clears spontaneously, to fulminant sepsis resulting in death only a few hours after the first symptoms occur.[202] In 2006, Kaplan and colleagues (the U.S. Multicenter Meningococcal Surveillance Study Group)[116] reviewed 159 episodes of invasive meningococcal disease seen at 10 children's hospitals in the United States between January 2001 and March 2005. Meningitis was the most common manifestation of disease seen in 70 percent of cases. Of the children with meningitis, 27 percent had bacteremia alone and 4.4 percent had fulminant disease. Fifty-five percent of children had petechiae, and 38 percent had purpura. Hypotension was present in 10 percent and thrombocytopenia in 6 percent on admission. The overall mortality rate was 8 percent and was significantly greater in children older than 10 years than in children younger than 11 years ($p < .01$). Hearing loss occurred in 12.5 percent of children with meningitis. Skin necrosis occurred in 9.4 percent of children, and 2.4 percent required skin grafts. One percent of children underwent amputations; one child lost all 4 limbs. Only one penicillin intermediate isolate was identified in this study, and no penicillin-resistant isolates were found.

Meningococcemia and Meningitis

Serious or invasive disease usually presents in one of two ways: meningococcemia or meningitis (either with or without meningococcemia). It is important to consider other etiologies in the differential diagnosis of meningococcemia (Table 88-1).

The signs and symptoms of meningococcemia are variable. Early on, evidence of an upper respiratory tract infection may be seen, including coryza, pharyngitis, tonsillitis, and laryngitis. Patients generally are febrile, with complaints of headache, lethargy, and vomiting. Severe myalgias with muscle tenderness and joint pain also may be the presenting symptom.[109] The typical patient with meningococcemia has a short history of upper respiratory tract symptoms, fever, and a hemorrhagic rash. The patient often develops signs of severe circulatory collapse.

TABLE 88-1 **Differential Diagnosis Meningococcemia**

Infectious
Rocky Mountain spotted fever
Ehrlichia
Streptococcal pneumoniae sepsis
Haemophilus influenzae type B sepsis
Group A *Streptococcus* sepsis
Other bacterial sepsis with disseminated intravascular coagulation (i.e., enteric gram-negative organisms)
Infective endocarditis
Gonococcemia
Rat bite fever
Typhus
Secondary syphilis
Noninfectious
Henoch-Schönlein purpura
Idiopathic thrombocytopenic purpura
Collagen vascular disease
Neoplastic processes

FIGURE 88-1 ■ Petechial lesions are seen on the face and neck of a young child with meningococcemia.

FIGURE 88-2 ■ Extensive purpuric lesions occurring in a child with overwhelming meningococcemia and disseminated intravascular coagulation.

It is not unusual for purpura and shock to develop within hours of the first onset of symptoms.

The skin manifestations of meningococcemia range from diffuse mottling to extensive purpuric lesions (Figs. 88-1 and 88-2). Unfortunately, some variation exists in the type of rash seen. Petechiae or purpura are present in 50 to 60 percent of patients.[118,228] Maculopapular rash alone is reported in 10 to 13 percent of patients.[141,228] In children, 20 to 30 percent may have no rash at presentation.[228] This makes differentiation from a viral exanthem particularly difficult. A pink macular rash resembling early varicella is another variant sometimes seen in children with meningococcemia; these lesions often are tender. They may occur in crops, as do the petechiae, and are seen most frequently on the trunk and extremities.[68,104]

The finding of petechiae or purpura in a febrile child should increase the index of suspicion for meningococcemia or other serious disease (e.g., infectious, neoplastic, immunologic). Acral distribution of a rash particularly is worrisome for meningococcemia or another infectious vasculitic process. In two studies, involving more than 200 patients with fever and petechiae (not purpura), 8.0 to 20.2 percent had invasive bacterial disease.[215,228] Seven to ten percent of the total group had infections caused by *N. meningitidis*. Therefore, it seems reasonable to obtain blood cultures on most febrile patients with petechiae. Lumbar puncture should be performed if clinically indicated or if the blood culture ultimately is positive. Antibiotic therapy should include coverage for meningococcus.

Purpura is noted in 16 to 24 percent of patients.[171,228] When purpura is extensive and accompanied by shock it is referred to as *purpura fulminans*. Acquired protein S and C deficiencies have been described in some patients with purpura fulminans.[171,172] One study showed a mortality rate of 50 percent in patients who developed purpura. Progressive purpura was accompanied by declining protein C levels, and the level of protein C was related inversely to the clinical severity of the disease.[171]

Patients with meningitis often are febrile, with headache, vomiting, irritability, stiff neck, and sometimes seizures. A history of lethargy may be present, or the patient may be obtunded. In neonates, physical findings of meningismus, such as Kernig and Brudzinski signs, often are absent. The anterior fontanelle, if open, may be full and tense.

The most common neurologic complications of meningitis are hydrocephalus, cranial nerve palsies (especially hearing loss), subdural effusions or empyemas, cerebral edema, cortical vein thrombosis, and cerebral infarctions. Neurologic sequelae are much more common in patients with meningitis, but complications such as cerebral infarction also can be seen in children with meningococcemia and shock.[158]

Cerebral edema and cranial nerve palsies may be seen at presentation or develop shortly thereafter. Sixth nerve or third nerve palsies are suggestive of increased intracranial pressure and impending herniation, respectively. The development of either of these signs indicates an urgent intracranial process. Hearing loss occurs in 5 to 10 percent of patients with meningitis. Auditory testing should be performed on all patients with meningitis after recovery.

Subdural effusions or empyemas should be considered in patients with fever persisting after 8 days of therapy, vomiting, or development of signs of increased intracranial pressure after the initial few days of treatment. Drainage is recommended only if the effusions are infected (empyemas) or are large enough to produce either focal neurologic signs or increased intracranial pressure.[159]

Vascular thrombosis and cerebral infarction can be due to arterial or venous thrombosis. Venous thrombosis is more common and generally is not seen before the second week. Hemorrhagic infarction of the brain may occur then. Cerebral infarction also may be seen in patients without arterial or venous thrombosis who are in shock with prolonged hypotension and cerebral ischemia. Cerebral infarction in these individuals is an early event.

Hydrocephalus occurs most frequently in young children and those with delayed diagnosis or severe disease. It tends to present 3 to 4 weeks after the onset of illness. Inflammation, with collagen deposition and proliferation of fibroblasts in the meninges, produces an obstruction to the flow of CSF. Progressive increase in head circumference should alert the clinician to the possibility of hydrocephalus.[159] Imaging (i.e., computed tomography and magnetic resonance imaging) studies are diagnostic.

Neonatal meningococcemia and meningitis are uncommon but have been reported. In one report, a 2-week-old infant died after a brief febrile illness with both CSF and blood cultures positive for meningococcus. Maternal endocervical colonization was documented. In addition, the mother's pharyngeal culture grew *N. meningitidis*.[114] If endocervical colonization by *N. meningitidis* is found prenatally, treatment with antibiotics should be considered in an attempt to eradicate colonization before delivery or intrapartum antibiotics should be given. Ceftriaxone would seem to be a reasonable choice. Rifampin and ciprofloxacin are not advocated for use during pregnancy.

Chronic Meningococcemia

Chronic meningococcemia, first described in 1902, is defined as meningococcal septicemia without meningeal symptoms in which fever has persisted for at least a week before any antibiotic therapy.[70,157] Benoit[13] reviewed 148 cases of chronic meningococcemia in the United States in 1963 in patients ranging in age from 3 months to 62 years. The major symptoms included fever and chills (present in 100 percent of patients), skin rash (93.2%), arthralgias (70.3%), and headache (61.5%). The patients generally were not in a toxic state and were in good health before becoming infected. The mean duration of illness before diagnosis was 6 to 8 weeks (range, 1 to 40 weeks). Symptoms tended to be intermittent; the rash often appeared in association with fever and then disappeared over the next several days. Bacteremia also was intermittent in some patients. In the Benoit study, in the average patient, five blood cultures were obtained before meningococci were isolated. However, after a blood culture yielded the organism, most subsequent cultures were positive. In children, some investigators report that it is common to isolate the organism in the first blood culture.[131] The arthralgias also tended to be intermittent in nature.

In Benoit's series,[13] almost 40 percent of the patients with chronic meningococcemia developed localizing complications. The meninges were the most common site of localization; meningitis developed in 15.5 percent of patients. Other localized infection included carditis, nephritis, epididymitis, conjunctivitis, iritis, and retinitis. In only one instance was an organism recovered from the joint. The average duration of meningococcemia in those patients with complications was 10.2 weeks, in contrast to 4 to 8 weeks in patients without any localization.

The diagnosis is established by identifying the organism in blood cultures. Antibiotic therapy results in prompt defervescence and dramatic recovery.

The pathophysiologic mechanisms permitting chronic meningococcemia remain unclear. No evidence suggests that the organisms are less virulent than other meningococci; thus, a defect in host immunity has been suggested. A hypersensitivity basis for this disease has been postulated, and it is theorized that the skin changes and arthritis may be secondary to antigen-antibody complexes.[158] In contrast to acute meningococcemia, bacteria are almost never found by biopsy or culture of skin lesions. The histologic findings of the skin lesion are distinct from those seen in skin lesions of patients with acute meningococcemia.[13,100,158]

Meningococcal Pneumonia

Meningococcal pneumonia occurs in conjunction with meningococcemia or meningitis in 8 to 15 percent of cases.[122] However, the meningococcus also can play a role as a primary respiratory pathogen.

Primary meningococcal pneumonia, once considered a rare disease, now is recognized as the most common form of meningococcal disease in certain military recruit populations and has been reported to cause 4.5 percent of all bacterial pneumonias in a general hospital population.[122,134]

Patients with preceding viral pneumonias are at risk for meningococcal pneumonia; more than 100 cases of meningococcal pneumonia occurred during the influenza pandemic of 1918 and 1919. In addition to disease among military recruits, nosocomial acquisition of meningococcal pneumonia in hospitalized patients has been reported.[51]

The diagnosis of meningococcal pneumonia is difficult because isolation of the organism from the sputum does not distinguish a meningococcal carrier from an individual with meningococcal pneumonia. In addition, routine sputum cultures do not include media selective for the meningococcus. Blood cultures are positive in only 15 percent.[122]

Koppes and associates[122] reported on 68 cases of meningococcal pneumonia; diagnostic criteria included a compatible clinical syndrome and isolation of *N. meningitidis* from pleural fluid or blood.[122] All 68 meningococcal pneumonia cases were serogroup Y; during the same period, 10 cases of meningococcemia and 6 cases of

meningitis were caused by serogroup Y. The high ratio of pneumonia to meningitis in this study suggests that serogroup Y organisms may be more likely to cause pneumonia than other serogroups, which has been substantiated by other investigators.[175,185] Pneumonia caused by serogroup B or C meningococci has been reported, usually in association with meningococcemia or meningitis.[227]

Primary meningococcal pneumonia usually is associated with a gradual onset of symptoms and a history of an antecedent upper respiratory tract infection. Rales and fever are found in most patients, and 80 percent have pharyngitis. The radiograph often shows involvement of lower lobes with patchy alveolar infiltrates. More than one lobe is involved in 40 percent. Twenty-five percent have pleural effusions. Petechiae, purpura, or shock were not present in any of the patients with pneumonia reported by Koppes and colleagues.[122]

The pathogenesis of this disease is thought to be pulmonary infection via droplet inhalation. The epidemiologic importance of meningococcal pneumonia was emphasized in a report from the Centers for Disease Control and Prevention (CDC) discussing nosocomial transmission of serogroup Y *N. meningitidis* in oncology patients.[51] The index case had meningococcal pneumonia. One other patient in an adjacent room developed meningococcal bacteremia; in three additional patients, serogroup Y *N. meningitidis* was isolated from nasopharyngeal cultures. Airborne dissemination seemed to be the mode of transmission. Respiratory isolation for a patient with suspected meningococcal pneumonia is indicated.

Penicillin therapy of penicillin-sensitive meningococcal pneumonia results in a prompt clinical response. Of the patients reported by Koppes and colleagues,[122] 93 percent were afebrile after 3 days of therapy. Third-generation cephalosporins, such as ceftriaxone and cefotaxime, may be the current drugs of choice pending definitive identification.

Other Meningococcal Syndromes

Conjunctivitis

Primary meningococcal conjunctivitis is indistinguishable clinically from acute bacterial conjunctivitis caused by other organisms. Usually, it presents in children as the acute onset of unilateral purulent conjunctivitis.[11] It has been reported in individuals from 2 days of age to adulthood.[35] Gram stain of the purulent material typically shows gram-negative diplococci that may sometimes be confused with gonococcal conjunctivitis. Barquet and colleagues[12] showed that 44 percent of the isolates were serogroup B meningococci.

The complications of primary meningococcal conjunctivitis reported by Barquet and colleagues[12] included sepsis or meningitis in approximately 18 percent of patients. The symptoms of systemic meningococcal disease occurred 3 to 96 hours after the onset of the conjunctivitis (mean, 41 hours). Patients treated only with topical therapy at the time of conjunctivitis were 19 times more likely to develop systemic disease than were patients treated with systemic therapy ($p = .001$). Ocular complications occurred in 15.5 percent of patients and included corneal ulcers (10.7%), keratitis, hemorrhage, and iritis.

Pharyngitis

The diagnosis of meningococcal pharyngitis is difficult because, like group A β-hemolytic streptococci, the isolation of meningococcus from the pharynx does not establish that this organism is the etiologic agent. In fact, most individuals who harbor meningococci in their nasopharynges are asymptomatic carriers. However, Banks[9] noted overt nasopharyngitis in one third of patients with meningococcal sepsis or meningitis. Pizzi,[167] describing a severe epidemic of meningococcal meningitis, noted that individuals with sore throats often had a pure culture of meningococci.[169] Olcen and associates[158] cultured family members of 21 consecutive cases of meningococcal disease and found that 61 percent of family members with sore throat or other upper respiratory tract symptoms were meningococcal carriers, in contrast to 14 percent of asymptomatic family members.[158]

On occasion, culture samples are obtained in individuals in the community with symptomatic pharyngitis, and the laboratory reports growth of *N. meningitidis*. If the patient is febrile but otherwise appears well, we recommend a blood culture and intramuscular or intravenous ceftriaxone. If the blood culture result is negative, the patient should be treated with an antibiotic that will eradicate the meningococcus (see discussion of chemoprophylaxis). If the patient is afebrile and asymptomatic at the time the culture results are obtained, we recommend eradication of the organism using one of the antibiotics recommended for chemoprophylaxis.

Arthritis

Meningococcal arthritis occurs primarily in adults. The overall incidence, as a complication of bacteremia, is approximately 2 to 14 percent.[95,228] Meningococcal arthritis has two forms. The first is seen within the first few days of treatment and is characterized by severe arthralgias and few objective signs of joint inflammation. It is suggested that the pathogenesis of this arthritis is an inflammatory response to viable organisms that have seeded the synovium during the initial bacteremia. The second, more common, form appears to be an immune complex phenomenon. It usually is noted 3 to 7 days after the recognition of meningococcemia, often at a time when the patient appears to be improving from the meningitis or sepsis. The knee, wrist, elbow, and ankle joints are involved most commonly.[95]

In both forms, the arthritis usually is monoarticular or oligoarticular with an effusion, with minimal pain, erythema, and limitation of motion. It is very unusual to culture organisms from the effusion; joint fluid culture yields meningococci in fewer than 10 percent of cases. The exception to this is a child with suppurative arthritis on initial presentation. In one study, 8 percent of patients

with meningococcal infections had arthritis; 75 percent had positive culture results when cultures were obtained before receiving antibiotics.[227,228]

Synovial fluid leukocyte counts vary widely, but counts greater than 100,000/mm^3 have been reported.[165] The appearance of arthritis often is accompanied by a rise in temperature; in 7/47 patients with arthritis, a characteristic skin lesion appeared at the same time.[93] These lesions began as skin hyperpigmentation but progressed to vesiculation and ulceration; biopsies showed vasculitis. Additional evidence of concurrent vasculitis is suggested by reports of patients developing episcleritis and mild proteinuria simultaneously with arthritis.[93]

On histologic examination, the synovium is infiltrated with mononuclear cells that contain IgM, C3, and meningococcal antigen.[94] This strongly suggests an immune complex–mediated disease. No specific therapy is indicated, and the arthritis resolves spontaneously. Controversy exists regarding the role of intermittent closed drainage of the joint space.[19] Permanent joint deformity is uncommon in this disease, occurring in approximately 10 percent of cases.[190] Edwards and Baker[71] reported allergic complications of meningococcal disease in 10 percent of the 86 children followed prospectively. More than 83 percent were serogroup B, although late-onset arthritis and vasculitis also have been reported with serogroup A and C disease.

Permanent joint damage is unusual, occurring in about 1.5 percent of patients with arthritis. Potential sequelae include ankylosis, decreased range of motion, and bone necrosis.[129]

Pericarditis and Myocarditis

Pericarditis, as a complication of meningococcal disease, occurs in 3 to 5 percent of cases, although one series reported a 19 percent incidence in 32 patients with meningococcal meningitis.[69,148] It generally occurs in patients with meningococcemia but has been reported as an isolated event without septicemia or meningitis.[103]

Pericarditis is presumed to be a late complication of meningococcal disease because clinical symptoms such as fever, dyspnea, or substernal chest pain (or even cardiac tamponade) usually do not appear until the fourth to the seventh day of illness. However, several investigators have noted early evidence of pericarditis based on electrocardiographic or radiographic data of patients examined at the time of hospital admission.

Because most symptomatic pericardial effusions develop late in the course of the illness, are serous, and are sterile, the pathophysiologic mechanism is presumed to be an immune complex reaction. Uncontrolled studies report the successful use of steroids in the treatment of this complication.[104] However, one report documents pericarditis and the development of tamponade in a patient receiving steroids.[162]

The clinical course of meningococcal pericarditis usually is benign, but pericardial compression requiring pericardiocentesis occurs.[164] Early relapses also have been reported, but these were self-limited. The development of constrictive pericarditis requiring pericardectomy has been reported.[162,192,221]

Myocarditis was noted at autopsy in 78 percent of patients with fatal meningococcal disease.[100] Myocarditis was noted most often in adults but was more severe when it occurred in children. Rosenblatt and colleagues[183] noted myocarditis at autopsy in 10/12 children with fatal meningococcal infection. On pathologic examination, these cases showed collections of inflammatory cells in the myocardial interstitium and focal extravasation of erythrocytes with acute vasculitis. Abscesses and endocarditis were not seen. Inflammation occasionally may involve the atrioventricular node and has been reported as a cause of sudden death in a patient recovering from meningococcal meningitis.[181]

Miscellaneous Meningococcal Infections

Several unusual syndromes have been reported, including primary meningococcal pericarditis,[215] mesenteric adenitis,[126] peritonitis,[126] and genitourinary infections.[76]

LABORATORY FINDINGS AND DIAGNOSIS

Wong and colleagues[228] reviewed 100 cases of meningococcal infections in children seen at their institution between 1985 and 1988. Leukopenia (white blood cell count <5000/mm^3) was present in 21 percent, and thrombocytopenia was noted in 14 percent. Fifty-five percent of patients had meningitis. Eleven percent of those with culture-positive meningitis had no CSF abnormalities on chemistries or examination.

Hyponatremia is seen in some patients with meningitis. Inappropriate secretion of antidiuretic hormone is the mechanism. In a study of 43 children with meningococcal meningitis, 7 percent developed syndrome of inappropriate secretion of antidiuretic hormone.[73]

Other laboratory abnormalities seen in patients with sepsis or shock include abnormal coagulation panels (disseminated intravascular coagulation), acidosis, and abnormal liver function studies.

The gold standard for diagnosis is based on recovering the organism from blood, CSF, or petechiae. Blood culture alone is positive about 50 percent of the time in patients who have not received antibiotics.[228] Rapid diagnosis often can be made by Gram stain of the CSF in patients with meningitis. Characteristic gram-negative diplococci are seen. Caution should be exercised in relying solely on the Gram stain to determine the initial antibiotic regimen. Broad-spectrum antibiotic therapy should be initiated pending identification of the organism by culture. Overdecolorized gram-positive cocci of *Streptococcus pneumoniae*, on occasion, have been confused with meningococci on Gram stain.

In patients who have skin lesions, a rapid presumptive diagnosis of meningococcemia often can be made by needle aspiration and Gram stain of a skin lesion. Needle aspiration yields gram-negative diplococci on the Gram-stained specimen in about 50 percent of patients with acute meningococcal infections.[214] Culturing the obtained aspirate increases the yield further. Correlation of the Gram stain with the clinical presentation is important

because disseminated gonococcal infections also may present with skin lesions that yield gram-negative diplococci on Gram stain.

Latex agglutination has been used to detect circulating antigen in serum, CSF, and urine of patients with meningococcal disease.[224] Latex agglutinin tests using serum and urine are not recommended. Cross-reactions with certain *E. coli* K1 (cause of neonatal meningitis) or *Bacillus* strains may occur. Latex agglutinin tests using CSF may be helpful in patients pretreated with antibiotics and with a compatible clinical syndrome.[3] These tests are of particular benefit in cases of partially treated meningococcal meningitis in which culture and Gram stain may be negative.

Polymerase chain reaction (PCR), one of the newer tests for detection of *N. meningitides*, may be very helpful in diagnosis of patients who have partially treated meningococcemia or meningitis. Once antibiotics have been given, the chances that a blood culture result will be positive decrease to less than 5 percent.[31] One study showed the sensitivity and specificity of PCR for *N. meningitidis* to be 91 percent in cerebrospinal fluid specimens. Treatment with antibiotics before the test did not decrease the test's sensitivity or specificity.[153] Use of this test in confirming meningococcal infections in patients pretreated with antibiotics may be valuable in subsequent patient management, follow-up, and prompt institution of chemoprophylaxis to contacts. One report from the United Kingdom describes use of a multiplex PCR assay for the simultaneous detection of *N. meningitidis*, *H. influenzae*, and *S. pneumoniae* from clinical samples of CSF and whole blood. Corless and colleagues[55] found that the sensitivities for detection of the three organisms ranged from 88.4 percent to 91.8 percent, with 100 percent specificity. A 2012 article from China and Australia reported development of a multiplex PCR assay for detection of 12 meningococcal serogroups.[232] Commercially available assays such as this would greatly facilitate etiologic diagnosis in children with sepsis or meningitis. Currently, meningococcal PCR is in use in the United Kingdom but is only available in the United States as a research test. Investigators in the UK have utilized quantitative PCR of CSF in patients with culture-proved meningococcal disease. In their experience, the sensitivity of CSF PCR was 89 percent for detection of *N. meningitidis*. In addition, they noted that patients with serogroup B infection had extremely high quantities of bacteria within the CSF regardless of the peripheral blood bacterial quantitation.[59]

MORTALITY AND PROGNOSIS

The overall mortality of invasive meningococcal disease in the United States is 7 to 19 percent.[116,155,184,200] Various scoring systems have been devised in an attempt to predict prognosis in patients with meningococcal disease. These scoring systems were reviewed in an article by Kirsch and colleagues.[121]

The Glasgow Meningococcal Septicaemia Prognostic Score (GMSPS) is a validated scoring system developed to clinically assess patients and facilitate admission of the most severely ill children to intensive care units.[193,205] This scoring system, which evaluates seven key items (i.e., hypotension, difference in skin-core temperature, coma, acute deterioration, absence of meningismus, progressive purpura, and base deficit) has been used by many to define entry criteria into clinical trials.

In a review of 100 cases of meningococcal disease at the Los Angeles Children's Hospital, five features were identified that were correlated with poor prognosis: shock or seizures on presentation, hypothermia, total white blood cell count less than 5000/mm^3, platelet count less than 100,000/mm^3, and development of purpura fulminans. The overall mortality in this series was 10 percent.[228]

Most prognostic scoring systems and clinical reviews agree that purpura fulminans and shock uniformly are poor prognostic signs. Individuals with only meningitis have a lower case-fatality rate than those with bacteremia or an isolate from another source (2% vs. 12%).[184] Presumably, this is a function of virulence of the organism, ability of the immune system to contain the infection, or both. Case-fatality rates also differ by serogroup and are higher in W-135 disease (21%) than in serogroup C (14%), Y (9%), or B (6%).[184]

Evidence suggests a genetic component exists in host cytokine production that may be associated with severity of disease. Westendorp and associates[223] reported that families with low TNF production or high IL-10 production are at increased risk for fatal outcomes with meningococcal disease. Recently, investigators have been studying variants of proinflammatory host genes (TNF-α, IL-1) to see if polymorphisms in these might be linked to severity of disease. Read and colleagues found that homozygosis of certain alleles at the IL-1 locus increased risk for death in individuals with meningococcal disease, and suggested that IL-1 genotype may be associated with fatal outcomes.[178]

TREATMENT

Therapy for meningococcal disease has evolved over the last century. In the early 1900s, treatment consisted of administration of intravenous and intrathecal horse serum and CSF drainage. Mortality associated with this therapy was 26 percent.[104] The use of sulfonamides lowered the death rate to 5 or 10 percent.[96] With the emergence of sulfa-resistant strains, penicillin was added to the regimen and may still be used for susceptible meningococcal infections. In the late 1980s, reports began to appear of meningococci relatively resistant to penicillin. The mechanism of resistance is reduced binding affinity of penicillin-binding proteins. Relatively resistant strains have been reported in the United States, Canada, Europe, South Africa, Romania, and Croatia.[21,112,163,203,231] Isolates that are absolutely resistant to penicillin (minimum inhibitory concentration >1.0 μg/mL) have been documented from Spain and the United Kingdom.[163,203] These strains had acquired a gonococcal plasmid encoding for the production of a β-lactamase.[203]

In 2009, ciprofloxacin-resistant isolates were identified from four unrelated patients with invasive meningococcal

disease in three U.S. states. Three of the cases were infected with serogroup B meningococcus and one with serogroup Y meningococcus. Genetic studies suggested that the Y strain acquired resistance by a point mutation. However, the B strains may have acquired resistance through horizontal gene transfer from *N. lactamica*, a commensal organism carried in the nasopharynx.[230] Ceftriaxone-nonsusceptible strains have been reported from India.[139] Routine susceptibility testing of meningococcal strains is not generally available, but should be considered in special circumstances in which resistance is clinically or epidemiologically suspected.

Prompt institution of antibiotic therapy in suspected meningococcal infections may be life-saving. Children who present with fever and purpura should be considered to have meningococcemia until proved otherwise, while recognizing that other pathogens may present with fever and purpura. A purpura fulminans–like presentation also has rarely been described caused by *Staphylococcus aureus*,[124] group A *Streptococcus*,[67] group B *Streptococcus*, and overt sepsis with other organisms causing disseminated intravascular coagulation. Antibiotics should be administered as soon as possible. A systematic review of the effectiveness of antibiotics given before admission concluded that "the data are consistent with benefit when a substantial proportion of cases are treated."[97] If possible, blood cultures should be drawn before antibiotic administration, but specimen collection should not delay administration of antibiotics. A lumbar puncture can be performed in stable patients, but the procedure should not delay antibiotic administration. CSF obtained after antibiotic therapy may be sterile, but pleocytosis will be apparent. Patients who are unstable or have significant coagulopathy should have the lumbar puncture deferred. Shortly after administration of bactericidal antibiotics, some patients have marked clinical deterioration, including hypotension and sometimes death. Rapid liberation of endotoxin (and resultant stimulation of cytokine release) from lysing organisms may be the cause of this phenomenon.[142]

The clinical presentation of *N. meningitidis* meningitis may be similar to that of meningitis caused by *S. pneumoniae* or *H. influenzae*. Empirical antibiotic therapy should therefore take into consideration the most likely pathogens. In children older than 1 month of age with meningitis, vancomycin and cefotaxime (or vancomycin and ceftriaxone) is an appropriate regimen until a definitive diagnosis is made. Similarly, empirical therapy for children younger than 1 month of age includes ampicillin and cefotaxime, with consideration given to the addition of vancomycin.[186]

For penicillin-susceptible meningococcemia or meningitis, intravenous penicillin G 250,000 to 300,000 U/kg/day (maximum 12 million U/day) given in divided doses every 4 to 6 hours for 7 days, is effective. Third-generation cephalosporins, ceftriaxone 100 mg/kg/day intravenously in two divided doses or cefotaxime 200 mg/kg/day intravenously in four divided doses also are effective. Cefotaxime or ceftriaxone is recommended for travelers from Spain, Italy, and parts of Africa because of reports of penicillin resistance in these areas.[3]

In confirmed cases of meningococcal disease, comparisons of penicillin G with ceftriaxone have shown ceftriaxone to be as efficacious. Necrotic skin lesions were seen more commonly in the penicillin group, but otherwise complication and mortality rates were equivalent.[209] Meningococcal disease has been treated successfully with ceftriaxone intravenously in both the 80 to 100 mg/kg/day and 100 mg/kg/day in two divided doses regimens.[209] Chloramphenicol is an alternative for penicillin-allergic patients. Currently, routine susceptibility testing of meningococcal isolates is not recommended. However, in selected cases in which the patient is not responding as expected, susceptibility testing may be warranted. With all cases of meningococcal disease, eradicating colonization of the index case is important (see discussion of chemoprophylaxis).

Steroid administration in patients with septic shock and meningococcemia is controversial, but several studies have reported adrenal insufficiency (or partial adrenal insufficiency) in 10.3 to 16.9 percent of severely ill children with meningococcemia.[20,62,63,180] For patients with Waterhouse-Friderichsen syndrome, treatment with steroids is indicated. However, treatment of meningococcal meningitis with steroids has been debated in the literature for years. Steroid proponents point to the *Haemophilus influenzae* type b meningitis and *Streptococcus pneumoniae* studies, in which treatment with steroids decreases hearing loss and may reduce neurologic sequelae. They postulate that steroids, through anti-inflammatory effects, may decrease polymorphonuclear neutrophils, macrophages, and cytokines in the central nervous system and thus decrease central nervous system immune-mediated damage and hearing loss. Steroid opponents say that no conclusive studies show steroids to be of benefit in meningococcal meningitis and that risks include gastrointestinal ulceration, decreased penetration of antibiotics into the central nervous system (because of decreased meningeal inflammation), and steroid psychosis. If steroids are used, it seems prudent to use them early (preferably close to the time the first dose of antibiotic is administered). However, antibiotics should never be withheld waiting for steroids to be given.

Experimental and Adjunctive Therapies

Many adjunctive and experimental therapies have been tried or are being evaluated presently. Antiendotoxin therapies and protein C concentrate infusions are the most recent additions (Table 88-2). Two antiendotoxin therapies have been evaluated in clinical trials: HA-1A and recombinant bactericidal/permeability increasing protein (rBPI). HA-1A, a human monoclonal antibody to endotoxin, was evaluated in a randomized, double-blind, placebo-controlled trial in 269 children with severe meningococcemia. Although the 28-day mortality rate in the treatment group was 18 percent in contrast to 28 percent in the placebo group, this was not statistically significant ($p = .11$).[65] BPI is a naturally occurring protein in neutrophil azurophilic granules. BPI binds to and neutralizes the effects of endotoxin. Administration of rBPI was studied in 393 children with presumed

TABLE 88-2 **Recent Studies of Alternative Therapies for Severe Meningococcemia or Sepsis Syndrome**

Year	Study	First Author	Randomized/ Placebo Controlled	Eligible Patients	Patient Number (n)	Results
1997	Activated protein C	Smith[194]	No/No	3 mo–27 yr, severe meningococcemia with septic shock and purpura fulminans	12	0 deaths, 2 patients with amputations. Favorable results compared with historical mortality rate of 50% in patients with shock, and purpura fulminans, and Glasgow meningococcal septicemic prognostic score expected mortality of >80%.
1999	HA-1A	Derkx[65]	Yes/Yes	3 mo–18 yr, petechiae or purpura, hypotension, toxicity, or end organ dysfunction	269	No statistically significant reduction in 28-day mortality demonstrated. Mortality rate HA-1A 18%, placebo group 28% ($p = .11$).
2000*	rBPI	Levin[132]	Yes/Yes	2 wk–18 yr, petechiae or purpura, and severe disease	393	No statistically significant reduction in mortality (7.4% rBPI, 9.9% placebo). Treatment group had fewer amputations, trend toward improved outcomes.
2001†	Activated protein C	Bernard[14]	Yes/Yes	Severe sepsis (any organism, known or suspected) with organ dysfunction	1690	Significant reduction in mortality (30.8% placebo vs. 24.7% treatment group). Increased risk for bleeding in treatment group.
2005‡	Activated protein C	Nadel[150]	Yes/Yes	Pediatric patients with severe sepsis	477	Study stopped after review by Data Safety and Monitoring Committee. No difference in mortality or time to complete organ failure resolution.

*57 patients died before receiving drug or placebo (after randomization) and were not included in this analysis.
†This study enrolled adults presenting with severe clinical sepsis (meningococcemia not a criteria for entrance).
‡This study enrolled pediatric patients presenting with severe clinical sepsis of all types.

meningococcal disease. There was no difference in mortality rates (7.4% rBPI vs. 9.9% placebo). There was a trend in reduction of amputations in the treatment group that approached statistical significance ($p = .067$).[132]

Recombinant protein C concentrate infusions have been studied in adults in a large randomized, placebo-controlled trial of 1690 patients with severe sepsis. A reduction in mortality was demonstrated, but the treatment group had an increased risk for bleeding.[17] Smith and associates[194] compared 12 children with severe meningococcemia and purpura fulminans treated with protein C concentrate to historical controls. No deaths occurred in the treated patients, in contrast to 50 percent in historical controls. A large study, published in 2007, compared activated protein C to placebo in children with presumed meningococcal infection. The study was terminated by the Data and Safety Monitoring Board because of a low likelihood of improved outcome in the treatment group.

Additional supportive measures such as prophylactic low-dose heparin are used by some clinicians.[225] Many investigators have studied the effect of heparin on survival and disseminated intravascular coagulation in patients with meningococcemia, and no consistent beneficial effect on these parameters has been noted. Serious side effects directly attributed to heparin therapy have been reported rarely.[85] The difficulty in adjusting the dose in small infants may lead to heparin intoxication. A retrospective chart review of 24 patients with purpura fulminans showed less necrosis of the digits and extremities in patients treated with heparin, but the results were not statistically significant.[127]

Other experimental therapies have been attempted in patients with fulminant meningococcemia. Anecdotal reports or small case series describe use of tissue plasminogen activator,[2,149] antithrombin III infusion,[50] topical nitroglycerin,[110,143] plasmapheresis,[48] and extracorporeal membrane oxygenation (ECMO).[128] Continuous caudal

block has been used to restore lower extremity perfusion.[206] Insufficient evidence exists to say that any of these therapies have a significant impact on outcomes in meningococcal disease. Larger, multicenter studies are needed to evaluate efficacy.

CHEMOPROPHYLAXIS

The ability of *N. meningitidis* to spread from person to person and cause epidemic disease has been recognized since the 1800s. For this reason, droplet precautions should be continued for 24 hours after initiation of appropriate antibiotics in the hospitalized patient. The secondary attack rate in households with an index case is approximately 1000 times the attack rate in the general population.[4,196] Household crowding and young age are factors that increase the secondary attack rate. The mode of transmission is direct contact with respiratory droplets or secretions. For these reasons, chemoprophylaxis is recommended for household contacts of an index case and for young daycare center contacts. Persons who have had significant contact with the oral secretions of an index case also should be considered for prophylaxis. Several epidemiologic studies suggest that casual acquaintances (e.g., school-aged classmates) are not at increased risk, although some authors report secondary cases in this population.[113,115] Prophylaxis is indicated for health care workers who have had intimate exposure to nasopharyngeal secretions (e.g., mouth-to-mouth resuscitation and endotracheal intubation in the absence of droplet precautions).[86] The period of communicability of the index patient is not well established. Most public health authorities recommend that persons in contact with the patient up to 7 days before onset of illness be considered for prophylaxis. Prophylaxis should be instituted as soon as possible after identification of the index case. It is not necessary to wait for laboratory confirmation of a case if the clinical picture is most consistent with meningococcal infection. Nasopharyngeal cultures are not recommended, because many nonpathogenic *Neisseria* spp. may colonize the nasopharynx.

The index patient should receive chemoprophylaxis before discharge unless he or she has been treated with ceftriaxone (or cefotaxime). Abramson and Spika[1] reported that 4/14 (29%) of patients with meningococcal infection treated with intravenous penicillin had positive culture results from the respiratory tract 1 week after completion of therapy.

Secondary cases originally were defined as occurring more than 24 but less than 31 days after onset in the index case. Using this definition, approximately 50 percent of secondary cases will occur in the first 7 days after presentation of the index case. It is essential to initiate prophylaxis as soon as possible, based on clinical presentation of the index case (e.g., fulminant meningococcemia) or on laboratory culture data if the disease presents as bacterial meningitis, septic arthritis, and so forth.

The original drug used for chemoprophylaxis was sulfadiazine. However, a large percentage of strains now are resistant to sulfa. Table 88-3 lists four antibiotics that are highly effective in eradicating meningococcus from the nasopharynx. Rifampin is generally the drug of choice for chemoprophylaxis of children. It is generally well tolerated but has side effects, including orange urine and sweat, orange staining of contact lenses, and stimulation of liver microsomal enzymes leading to reduction in levels of other concurrent medications (e.g., oral contraceptives, anticoagulants, digoxin, and phenytoin). Development of resistant strains has been reported rarely.[54] Ciprofloxacin, ceftriaxone, and rifampin have been evaluated in a randomized, comparative study.[56] All three drugs were highly effective in carriage eradication. Ceftriaxone probably is the drug of choice for a pregnant contact and also may be indicated for children because a single intramuscular injection may result in greater compliance than four doses of rifampin. A single oral dose of ciprofloxacin is highly effective in eradicating carriage of meningococcus, but it is contraindicated in individuals younger than 18 years because of evidence of cartilage damage in juvenile beagles. In addition, a 2009 article reported four cases of invasive meningococcal disease in the United States with isolates resistant to ciprofloxacin.[230] Azithromycin was studied in a randomized, controlled trial comparing 500 mg of azithromycin orally once to rifampin 600 mg orally twice daily for 2 days. Carriage was eradicated in 56/60 (93%) colonized adults treated with azithromycin in contrast to eradication in 95 percent of the 59 colonized adults treated with rifampin.[88]

TABLE 88-3 **Chemoprophylaxis Regimens for Eradication of *Neisseria Meningitidis***

Drug	Age	Dose/Duration
Rifampin*	Children <1 mo	5 mg/kg/dose PO twice daily for 2 days (four doses)
	Children ≥1 mo	10 mg/kg/dose (maximum dose 600 mg) PO twice daily for 2 days (four doses)
	Adults	600 mg dose PO twice daily for 2 days (four doses)
Ceftriaxone	Children <15 yr	125 mg IM, single dose
	Children ≥15 yr	250 mg IM, single dose
	Adults	250 mg IM, single dose
Ciprofloxacin†	Children	Not recommended
	Adults	500 mg PO, single dose
Azithromycin‡	Children	10 mg/kg PO (maximum dose 500 mg), single dose
	Adults	500 mg PO, single dose

*Rifampin is not recommended for pregnant women (teratogenicity in laboratory animals). The reliability of oral contraceptives may be affected by rifampin therapy; therefore alternative contraceptive measures should be used during and for the month after rifampin administration.
†Ciprofloxacin is not recommended in children, pregnant women, or lactating women. Ciprofloxacin can be used in children if there is no acceptable alternative.
‡Azithromycin is not routinely recommended and is not listed in the CDC recommendations for prophylaxis.
IM, Intramuscularly; *PO,* orally.

As mentioned in the section on meningococcal vaccines, secondary disease also can be prevented by immunization of contacts. We recommend both immunization and chemoprophylaxis whenever possible (realizing that no protection against group B meningococci currently is provided by immunization).

The single most important element of chemoprophylaxis is education of contacts regarding the need for immediate medical attention if they develop signs or symptoms of a febrile illness. No prophylactic strategy is 100 percent effective, and ill contacts should be medically evaluated with a high suspicion for meningococcal disease.

MENINGOCOCCAL VACCINES

Primary prevention of meningococcal disease is essential for several reasons. The presentation may be fulminant, with no opportunity for antibiotics to influence the course of the disease. Antibiotic-resistant strains now are recognized, and chemoprophylaxis of contacts is a cumbersome and often ineffective public health measure. Mass immunization offers the opportunity to prevent both endemic and epidemic disease worldwide.

Two types of vaccines, meningococcal polysaccharide vaccine (MSPV4) and meningococcal conjugate vaccine (MCV), are currently licensed in many countries around the world for prevention of meningococcal disease. Although more costly, meningococcal conjugate vaccines are superior to polysaccharide vaccines in several ways. Children younger than 2 years of age do not produce high levels of antibody in response to pure polysaccharide antigens, polysaccharide antigens do not induce T-cell dependent immunity with memory cell production, and repeated boosting with MSPV4 has resulted in a blunting of antibody response, termed *hyporesponsiveness.*[22,136] In contrast, licensed conjugate vaccines have been shown to be immunogenic when used in an appropriate series with boosters, even in infants.[156] Conjugate vaccines produce stimulation of both T-cell and B-cell dependent immunity and have been shown to decrease nasopharyngeal carriage.[38] For these reasons, although meningococcal polysaccharide vaccines are licensed and available in many countries, meningococcal conjugate vaccines have significant advantages.

Meningococcal Vaccines in the United States

Meningococcal Polysaccharide Vaccine

Meningococcal polysaccharide vaccine (MPSV4, Menomune) is a capsular polysaccharide vaccine licensed for use in individuals older than 2 years of age who are at increased risk for meningococcal disease. This vaccine was licensed in 1981 and was the only meningococcal vaccine widely available in the United States until the licensing of meningococcal conjugate vaccines. Conjugate vaccines are generally preferred over meningococcal polysaccharide vaccines in people for whom conjugate vaccines are licensed.[38,43] However, meningococcal conjugate vaccines are not licensed for persons older than 55 years of age, so in this age group MSPV4 is the only U.S. licensed vaccine. MSPV4 is given subcutaneously as a single dose.

Meningococcal Conjugate Vaccines

Three meningococcal conjugate vaccines are currently licensed in the United States: MenACWY-D (Menactra), MenACWY-CRM (Menveo), and HibMenCY-TT (MenHibrix). MenACWY-D and MenACWY-CRM are quadrivalent vaccines that couple polysaccharide capsular antigens (i.e., serogroups A, C, Y, and W-135) to a protein carrier. In MenACWY-D, the protein carrier is diphtheria toxoid and in MenACWY-CRM, the protein carrier is CRM_{197} (mutant diphtheria toxin). MenHibrix is a combination *H. influenza* type B and bivalent meningococcal conjugate vaccine (i.e., serogroups C and Y) licensed in the United States for infants.

MenACWY-D, MenACWY-CRM, and HibMenCY-TT vaccines have been found to be safe and immunogenic in infants, children, adolescents, and adults.* Efficacy was inferred by the demonstration of development of serum bactericidal antibodies to each of the meningococcal antigens the vaccines contain at a level that was noninferior to the response generated by polysaccharide vaccines.[16,201]

Close monitoring for potential adverse events initially suggested a possible association between recent MenACWY-D (Menactra) and Guillain-Barré syndrome based on case reports through the Vaccine Adverse Event Reporting System (VAERS) shortly after the licensure of MenACWY-D.[6] Subsequently, the results of two large observational studies (collectively representing 2 million doses of vaccine) found no risk for Guillain-Barré associated with meningococcal conjugate vaccine.[1] Language about Guillain-Barré has been removed from the meningococcal vaccine information statement.

Meningococcal conjugate vaccines are administered intramuscularly (as opposed to MPSV4, which is administered subcutaneously). Soon after licensure of meningococcal conjugate vaccines, numerous cases of misadministration of MenACWY-D (Menactra) were reported. The CDC studied 100 such cases and found that misadministration of MenACWY-D by the subcutaneous route still resulted in a protective immune response. While this route of administration is not recommended, persons who received the vaccine by this route do not need to be revaccinated.[39]

The duration of protection of quadrivalent meningococcal vaccination was originally thought likely to persist for 10 years. This was part of the rationale to incorporate meningococcal conjugate vaccine into the U.S. preadolescent vaccine platform. It was presumed that by vaccinating 11- to 12-year-old children, antibody levels would be sufficient for protection through the early college years, when there is increased risk for meningococcal disease. Subsequent studies showed significant decline in meningococcal antibody (vaccine serogroups) in adolescents 3 to 5 years after vaccination. Additionally, a limited

*References 8, 16, 81, 98, 120, 156, 164, 210.

number of cases of meningococcal C or Y disease were reported to the CDC in previously vaccinated teenagers. The average time between vaccination and meningococcal disease was 3.25 years.[44,219] In 2010, as a result of concerns that adolescents may not be protected against invasive meningococcal disease for more than 5 years, the CDC's Advisory Committee on Immunization Practices (ACIP) recommended a booster dose of quadrivalent meningococcal vaccine be administered at age 16 years (Table 88-4).[44]

Meningococcal conjugate vaccines are currently licensed in the United States for persons as young as 9 months through 55 years. MenACWY-D is licensed in persons 9 months through 55 years; MenACWY-CRM is licensed in persons 2 through 55 years; HibMenCY-TT is licensed in infants and children 6 weeks through 18 months of age. As in adolescents, waning antibody has been detected 3 to 5 years after primary immunization and is the basis for the current recommendations for boosters if an ongoing risk factor exists for meningococcal disease (see Table 88-3).[44]

U.S. Recommendations for Use of Meningococcal Vaccines

Table 88-3 summarizes the ACIP recommendations for the use of vaccines to prevent meningococcal disease.[38,41,43,44,108] Meningococcal vaccine is recommended for all U.S. children (who have no contraindication) at the 11- to 12-year-old preadolescent visit, with a booster dose at age 16 years. Teenagers who receive their primary dose of meningococcal conjugate vaccine at 13 to 15 years should receive a booster at 16 to 18 years. If the primary dose of meningococcal conjugate vaccine is given on or after the 16th birthday, no booster is recommended. ACIP recommends use of quadrivalent meningococcal conjugate vaccines (MCV4; Menveo or Menactra) as the preferred products, but MPSV4 is an alternative.[16,38,44]

TABLE 88-4 **Recommendations for Meningococcal Vaccinations***

Targeted Group by Age and/or Risk Factor	Primary Series	Booster Recommendations
Persons ages 11-18 yr	Give one dose of MCV4,† preferably at age 11 or 12 yr[a]	Booster at age 16 yr if primary dose given at age 12 yr or younger Booster at ages 16-18 yr if primary dose given at ages 13-15 yr[b]
Persons ages 19-21 yr who are first-year college students and living in residence halls	Give one dose of MCV4[a] if person has never previously received MCV4 *or* if previous MCV4 given at age younger than 16 yr	No booster recommendations unless person has other risk factors for meningococcal disease (see specific categories in this table)
Travelers to or persons residing in countries where meningococcal disease is endemic or hyperendemic, persons in a defined risk group during an institutional or community outbreak,[c] and microbiologists routinely working with *Neisseria meningitidis*		
Ages 9-23 mo	Give two doses of MenACWY-D (Menactra), 3 mo apart[d]	If risk continues, give initial booster after 3 yr followed by boosters every 5 yr
Ages 2-55 yr	Give one dose of MCV4[a]	If risk continues, boost every 5 yr with MCV4[e,f]
Ages 56 years and older	Give one dose of MPSV	If risk continues, boost every 5 yr with MPSV[f]
Persons with persistent complement component deficiencies[g] or functional or anatomic asplenia		
Ages 9-23 mo with persistent complement component deficiencies only *(does not include children with functional or anatomic asplenia)*	Give two doses of MenACWY-D (Menactra), 3 mo apart	Initial booster 3 yr after completion of primary series, followed by boosters every 5 yr thereafter
Ages 9-23 mo with functional or anatomic asplenia (includes sickle cell disease)	Give two doses, 2 mo apart beginning at age 2 yr and ≥4 wk after completion of PCV13 vaccine[h]	Initial booster 3 yr after completing the primary series; thereafter boosters every 5 yr as long as child continues to be at risk
Ages 2-55 yr	2 doses of MCV4, 2 mo apart[h]	Boost every 5 yr with MCV4[e,i]
Ages 56 yr and older	Give one dose of MPSV	Boost every 5 yr with MPSV

Data from references 41, 43, 44, 108.
*Table adapted from Immunization Action Coalition. http://www.immunize.org/askexperts/experts_men.asp.
†MCV4; Menveo or Menactra
[a]If the person is HIV-positive, give 2 doses, 2 mo apart.
[b]The minimum interval between doses of MCV4 is 8 wk.
[c]Seek advice of local public health authorities to determine if vaccination is recommended.
[d]If child aged 9-23 mo will enter endemic area in <3 mo, give doses as soon as 2 mo apart.
[e]If primary dose(s) given when younger than age 7 yr, give initial booster after 3 yr, followed by boosters every 5 yr.
[f]Boosters are recommended if the person remains at increased risk.
[g]Persistent complement component deficiencies include C5-C9, properdin, factor H, and factor D.
[h]Children with functional or anatomic asplenia should complete a series of PCV13 vaccine before vaccination with MCV4; if MenACWY-D (Menactra) is to be given, begin meningococcal vaccination at least 4 wk after last dose of pneumococcal conjugate vaccine to avoid possible interference with immune response to the pneumococcal vaccine series.
[i]If the person received a one-dose primary series, give booster at the earliest opportunity, then boost every 5 yr.

Other persons at increased risk for meningococcal disease and recommendations for vaccine are listed in Table 88-3.

Meningococcal Vaccines Outside the United States

Meningococcal A Vaccine

The story of development and delivery of a conjugate serogroup A meningococcal vaccine to Africa is an inspirational public health achievement. Sub-Saharan Africa has long been afflicted with endemic and epidemic serogroup A disease. Approximately 430 million at-risk Africans reside in this "meningitis belt." Outbreaks of serogroup A meningococcal disease occur in the winter and are associated with dry Saharan winds and low humidity.[160] Every 7 to 12 years explosive outbreaks of disease occur, causing extensive morbidity and mortality. Control measures consisted largely of case identification and reactive mass immunization using a polysaccharide monovalent serogroup A meningococcal vaccine.

In 2001, a partnership was developed between the World Health Organization (WHO) and the Program for Appropriate Technology in Health (PATH) around the Meningitis Vaccine Project. This project was funded by the Bill and Melinda Gates Foundation, and its goal was to develop and introduce an affordable meningococcal conjugate serogroup A vaccine in Africa. The vaccine was developed with help from additional agencies at a cost of $0.40 per dose. Two studies (one in children 12 to 23 months of age and one in persons 2 to 29 years of age) showed the vaccine to be more immunogenic and with a similar safety profile to the polysaccharide serogroup A vaccine.[195] Burkina Faso became the first country to conduct a nationwide rollout of the meningococcal conjugate serogroup A vaccine MenAfriVac, nationwide. Over 11 million people ages 1 year to 29 years were immunized in a 10-day period in December 2010. During the subsequent 2011 winter dry season only 2665 suspected meningococcal cases were reported (80% reduction over prior years). From the suspect cases, 1437 specimens were cultured (or submitted for PCR), and only four isolates were identified to be serogroup A—all from unvaccinated individuals.[49] Carriage studies done before and after vaccine implementation in Burkina Faso show a decrease in oropharyngeal carriage. Before vaccine implementation, examination of 20,326 oropharyngeal cultures yielded a carriage rate of 0.4 percent. Fifteen thousand oropharyngeal samples obtained after the MenAfriVac implementation program revealed none of the samples were positive for serogroup A *N. meningitidis*.[42,125] More than 55 million Africans have now been vaccinated in at least 6 countries. Large-scale institution of this vaccine is estimated to dramatically decrease morbidity and mortality from serogroup A meningococcal disease in Africa in the next 10 years.

Meningococcal B Vaccines

Development of an immunogenic serogroup B meningococcal vaccine has been problematic because the polysaccharide capsule of meningococcus group B is poorly immunogenic in animals or humans. In 1983, Finnish investigators reported that horse antiserum to meningococcus group B reacted with glycoproteins isolated from human and rat brains.[17] Therefore, good immunogenic responses are not achievable with group B polysaccharide antigen vaccines because the body may see these antigens as "self" antigens (similar to neural tissue). Serogroup B outer membrane vesicle vaccines have been studied and used by some countries such as New Zealand to control outbreaks with moderate success.[84,119] However, outer membrane vesicle vaccines are largely directed against the PorA porin protein, which is antigenically variable among strains. Therefore, these vaccines are not broadly useful because strain variation occurs geographically.

A promising investigational multicomponent serogroup B vaccine contains antigens to factor H binding protein variant 1 (fHbpl), neisserial adhesion A (NadA) and *Neisseria* heparin binding antigen (NHBA) combined with outer membrane vesicles from a New Zealand strain (NZ98/254). This vaccine, referred to as 4CMenB, has been studied in clinical trials in several European countries. A large multicountry European study examined the immunogenicity and reactogenicity of 4CMenB in 1885 infants. The vaccine was highly immunogenic against two of the three meningococcal strains tested. For the third strain, 79 percent of infants developed sufficient antibody response that would be considered protective. Injection site pain and fever were relatively common after vaccination.[91] A study in more than 1600 Chilean adolescents showed that a two-dose series of 4CMenB performed better than a single dose in terms of good immunogenicity that lasted at least 6 months.[189]

The world awaits licensure and use of a highly immunogenic, safe vaccine with broad protection against multiple serogroup B meningococcal strains. Such a vaccine will have a substantial impact on control of serogroup B disease worldwide.

Meningococcal C Vaccines

Meningococcal C conjugate vaccination in the United Kingdom has demonstrated efficacy,[176,207] the ability to produce herd immunity (67% disease reduction in unvaccinated 1 to 17 year olds),[177] and a 66 percent reduction in adolescent nasopharyngeal carriage. A meningococcal serogroup C conjugate vaccine program was begun in the United Kingdom in 1999 with a 2-, 3-, 4-month primary series schedule for infants. Children 1 to 18 years of age received a single dose of meningococcal serogroup C conjugate vaccine (MCCV). In 2002, MCCV was offered to all individuals up to age 25 years. Later studies from the United Kingdom showed rapid waning of immunity in vaccinated infants (vaccinated at 2, 3, and 4 months). Despite high vaccine efficacy during the first year after immunization, vaccine efficacy fell significantly at 1 year or more after vaccination.[30] Subsequently, the infant vaccine schedule was changed to a two-dose primary series at 3 months and 4 months, with a booster dose given at 12 months of age.

The United Kingdom's meningococcal conjugate serogroup C program has been highly effective in producing a dramatic and sustained reduction in serogroup

C disease. The overall incidence rate of serogroup C disease has fallen by 98.7 percent in England and Wales, and no evidence exists for serogroup replacement.[29,138]

Several other countries also incorporated meningococcal C conjugate vaccines into their immunization programs because of circulating clonal serogroup C strains. Canada's staggered serogroup C vaccine implementation program allowed for a comparison of the meningococcal disease incidence in provinces with and without vaccine implementation; provinces with vaccine implementation showed substantial reduction in meningococcal C disease, and no serogroup replacement was seen.[15]

Future for Meningococcal Vaccines

A deeper understanding of the pathophysiologic mechanism of meningococcal disease and the bacterial structures critical for antigenicity and immunogenicity in humans is needed. A safe and effective vaccine that could induce protection against all encapsulated meningococci in all age groups is the ultimate goal.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Advisory Committee for Immunization Practices: Minutes of the ACIP Meeting, June 2010. 2012.
2. American Academy of Pediatrics. *Red Book: 2009 report on the Committee for Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2009.
3. Anonymous. Meningococcal disease among travelers returning from Saudi Arabia. *MMWR Morb Mortal Wkly Rep* 1987;**36**:559.
4. Anonymous. Analysis of endemic meningococcal disease by serogroup and evaluation of chemoprophylaxis. *J Infect Dis* 1976;**134**: 201–4.
5. Anonymous. Inadvertent misadministration of meningococcal conjugate vaccine: United States, June-August 2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1016–17.
8. Arguedas A, Soley C, Loaiza C, et al. Safety and immunogenicity of one dose of MenACWY-CRM, an investigational quadrivalent meningococcal glycoconjugate vaccine, when administered to adolescents concomitantly or sequentially with Tdap and HPV vaccine. *Vaccine* 2010;**28**:3171–9.
11. Barquet N, Domingo P, Cayla JA, et al. Prognostic factors in meningococcal disease: development of a bedside predictive model and scoring system. Barcelona Meningococcal Disease Surveillance Group. *JAMA* 1997;**278**:491–6.
15. Bettinger J, Scheifele D, Le Saux N, et al. The impact of childhood meningococcal serogroup C conjugate vaccine programs in Canada. *Pediatr Infect Dis J* 2009;**28**:220–4.
29. Campbell H, Andrews N, Borrow R, et al. Updated postlicensure surveillance of the meningococcal C conjugate vaccine in England and Wales: effectiveness, validation of serological correlates of protection, and modeling prediction of the duration of herd immunity. *Clin Vaccine Immunol* 2010;**17**:840–7.
30. Campbell H, Borrow R, Salisbury D, et al. Meningococcal C conjugate vaccine: the experience in England and Wales. *Vaccine* 2009;**27S**:B20–29.
33. Centers for Disease Control and Prevention. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2000;**49**:1–10.
34. Centers for Disease Control and Prevention. *Active bacterial core surveillance reports*. http://www.cdc.gov/abcs/reports-findings/surv-reports.html.
36. Centers for Disease Control and Prevention. Serogroup B meningococcal disease: Oregon, 1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**:121–4.
37. Centers for Disease Control and Prevention. Meningococcal disease and college students: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR—Morb Mortal Wkly Rep* 2000;**49**:13–20.
38. Centers for Disease Control and Prevention. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2005;**54**:1–21.
39. Centers for Disease Control and Prevention. Update: Guillain-Barré syndrome among recipients of Menactra® meningococcal conjugate vaccine: United States, June 2005–September 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1120–4.
40. Centers for Disease Control and Prevention. *Active bacterial core surveillance (ABCs) report: Emerging Infections Program Network* Neisseria meningitidis, 2010. http://www.cdc.gov/abcs/reports-findings/survreports/mening10.pdf
41. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391–2.
42. Centers for Disease Control and Prevention. *Division of Bacterial Diseases (CDC) winter news bulletin*. http://www.cdc.gov/ncird/div/DBD/newsletters/2011/winter/quarter3.html
43. Centers for Disease Control and Prevention. Licensure of a meningococcal conjugate vaccine in children 2 through 10 years of age. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1018–19.
44. Centers for Disease Control and Prevention. Updated recommendations for use of meningococcal conjugate vaccines: Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:72–6.
45. Centers for Disease Control and Prevention. Notifiable diseases and mortality. *MMWR* 2012;**61**:ND44–ND57.
46. Centers for Disease Control and Prevention. Notifiable diseases and mortality tables. *MMWR* 2012;**61**:ND283–ND296.
47. Chamot-Rooke J, Mikaty G, Malosse C, et al. Posttranslational modification of pili upon cell contact triggers *N. meningitidis* dissemination. *Science* 2011;**331**:778–82.
49. Clark T. *MenAfriVac™ (meningococcal A conjugate vaccine) introduction in Burkina Faso; presentation at the Advisory Committee of Immunization Practices*, June 2011. http://www.cdc.gov/vaccines/acip/meetings/downloads/min-archive/min-jun11.pdf
52. Cohn A, MacNeil J, Harrison L, et al. Changes in *Neisseria meningitidis* disease epidemiology in the United States, 1998–2007: implications for prevention of meningococcal disease. *Clin Infect Dis* 2010;**50**:184–91.
59. Darton T, Guiver M, Naylor S, et al. Bacterial genomic detection within cerebrospinal fluid of patients with meningococcal disease is influenced by microbial and host characteristics. *Clin Infect Dis* 2011;**53**:463–7.
60. Davila S, Wright V, Khor C. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat Genet* 2010;**42**:772.
84. Galloway Y, Stehr-Green P, McNicholas A, et al. Use of an observational cohort study to estimate the effectiveness of the New Zealand group B meningococcal vaccine in children aged under 5 years. *Int J Epidemiol* 2009;**38**:413–18.
98. Halperin S, Gupta A, Jeanfreau R, et al. Comparison of the safety and immunogenicity of an investigational and a licensed quadrivalent meningococcal conjugate vaccine in children 2-10 years of age. *Vaccine* 2010;**28**:7865–72.
99. Haralambous E, Dolly S, Hibberd M. Factor H, a regulator of complement activity, is a major determinant of meningococcal disease susceptibility in UK Caucasian patients. *Scand J Infect Dis* 2006;**38**:764.
101. Harrison L. Epidemiological profile of meningococcal disease in the United States. *Clin Infect Dis* 2010;**50**(suppl 2):S37–44.
108. Immunization Action Coalition. *Meningococcal disease: ask the experts*. http://www.immunize.org/askexperts/experts_men.asp.
119. Kelly C, Arnold R, Galloway Y, et al. A prospective study of the effectiveness of the New Zealand meningococcal B vaccine. *Am J Epidemiol* 2007;**166**:817–23.
125. Kristiansen P, Diomande F, Wei S, et al. Baseline meningococcal carriage in Burkina Faso before the introduction of a meningococcal serogroup A conjugate vaccine. *Clin Vaccine Immunol* 2011;**18**: 435–43.
138. Maiden MI-P, AB, Urwin R, Gray S, et al. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. *J Infect Dis* 2008;**197**:737–43.

156. Nolan T, Richmond P, Marshall H, et al. Immunogenicity and safety of an investigational combined *Haemophilus influenzae* type B-Neisseria meningitidis serogroups C and Y-tetanus toxoid conjugate vaccine. *Pediatr Infect Disease J* 2011;**30**:190–6.
159. Palmgren H. Meningococcal disease and climate. *Global Health Action* 2009.
173. ProMED. *Meningitis: meningococcal-Africa—Burkina Faso*, 2010. ProMED Digest, Archive No. 20100507.1484. http://www.promedmail.org/.
174. ProMed. *Meningococcal Disease Update 2007*. Accessed August 20, 2012, http://www.promedmail.org/pls/promed/f?p=2400:1202:103747487488488896256::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,36797.
189. Santolaya M, O'Ryan M, Valenzuela M, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. *Lancet* 2012;**379**:617–24.
195. Sow S, Okoko B, Diallo A, et al. Immunogenicity and safety of a meningococcal A conjugate vaccine in Africans. *N Engl J Med* 2011;**364**:2293–304.
197. Stefanelli P, CFazio C, Neri A, et al. First report of capsule replacement among electrophoretic type 37 *Neisseria meningitidis* strains in Italy. *J Clin Microbiol* 2003;**41**:5783–6.
229. World Health Organization. *Meningococcal meningitis: factsheet*. http://www.who.int/mediacentre/factsheets/fs141/en/.
230. Wu A, Harcourt B, Hatcher C, et al. Emergence of ciprofloxacin-resistant *Neisseria meningitidis* in North America. *N Engl J Med* 2009;**360**:886–92.
232. Zhu H, Wang Q, Wen L, et al. Development of a multiplex PCR assay for detection and genogrouping of *Neisseria meningitidis*. *J Clin Microbiol* 2012;**50**:46–51.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 89

Gonococcal Infections

Charles R. Woods, Jr.

Gonorrhea, the foremost manifestation of human disease caused by *Neisseria gonorrhoeae* (the gonococcus), is one of the oldest known human diseases. Hippocrates called the disease "strangury" in the 5th and 4th centuries BCE. Galen coined the name *gonorrhea*, meaning "flow of semen," in the 2nd century CE. Greco-Roman physicians recognized the association of the disease with sexual activity and prescribed sexual abstinence plus washing of the eyes of newborns for treatment. Gonorrhea has been known as *clap* since the late 1300s. This term may have derived from the Les Clapier district of Paris, where prostitutes were housed during the Middle Ages.[196,241]

After syphilis appeared in Europe in the late 15th century, the two diseases were thought to represent different manifestations of the same infection, and they often coexisted then as today. The description by Neisser of *N. gonorrhoeae* in stained smears of urethral and other exudates in 1879 and culture of the organism by Leistikow and Löffler in 1882 provided the foundation for modern understanding of the clinical spectrum of gonococcal diseases. The advent of safe and effective antimicrobial agents, first sulfonamides in 1936 and then penicillin in 1943, was the next major advance in combating gonorrhea.[196,241] Modern knowledge of the molecular pathogenesis of *N. gonorrhoeae* infection began in 1963 with the observation by Kellogg and colleagues[199] that gonococcal strains with differences in colony morphology also varied in virulence. Further understanding of the clinical aspects of the disease was facilitated by development of the Thayer-Martin medium in 1964.[338]

N. gonorrhoeae remains a major international public health problem because of reproductive sequelae of infection (e.g., infertility), the ever-growing resistance to antimicrobial agents, and disproportionate case burdens in public clinics.[59,198,224,225,289] Efforts to develop an effective vaccine are ongoing.[82,315,361] Additional information on gonococcal infections is provided in Chapter 42.

EPIDEMIOLOGY

Gonococcal infections remain second in incidence only to *Chlamydia* infections among reportable diseases in the United States.[61,67,70] Rates of gonococcal infection in the United States, Canada, and western and central Europe declined steadily in the 1980s and 1990s. Rates recently have increased in the United States and Canada. In 2010, 100.8 cases/100,000 population were reported, a slight increase from 98.1 in 2009, which was the lowest reported rate in U.S. history. In Canada 33.1 cases/100,000

REFERENCES

1. Advisory Committee for Immunization Practices: Minutes of the ACIP Meeting, June 2010. 2012.
2. Aiuto L, Barone S, Cohen P, et al. Recombinant tissue plasminogen activator restores perfusion in meningococcal purpura fulminans. *Crit Care Med* 1997;**25**:1079–82.
3. American Academy of Pediatrics. *Red book: 2009 report on the Committee for Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2009.
4. Anonymous. Analysis of endemic meningococcal disease by serogroup and evaluation of chemoprophylaxis. *J Infect Dis* 1976;**134**: 201–4.
5. Anonymous. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2000;**49**: 1–10.
6. Anonymous. Update: Guillain-Barré syndrome among recipients of Menactra® meningococcal conjugate vaccine: United States, June 2005–September 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1120–4.
7. Argenbright L, Barton R. Interactions of leukocyte integrins with intercellular adhesion molecule I in the production of inflammatory vascular injury in vivo: the Schwartzman reaction revisited. *J Clin Invest* 1992;**89**:259–72.
8. Arguedas A, Soley C, Loaiza C, et al. Safety and immunogenicity of one dose of MenACWY-CRM, an investigational quadrivalent meningococcal glycoconjugate vaccine, when administered to adolescents concomitantly or sequentially with Tdap and HPV vaccine. *Vaccine* 2010;**28**:3171–9.
9. Banks H. Meningococcosis: a protean disease. *Lancet* 1948;**2**: 635–40.
10. Baraldes M, Domingo P, Barrio J, et al. Meningitis due to *Neisseria subflava*: case report and review. *Clin Infect Dis* 2000;**30**:615–7.
11. Barquet N, Domingo P, Cayla JA, et al. Prognostic factors in meningococcal disease: development of a bedside predictive model and scoring system. Barcelona Meningococcal Disease Surveillance Group. *JAMA* 1997;**278**:491–6.
12. Barquet N, Gasser I, Domingo P, et al. Primary meningococcal conjunctivitis: report of 21 patients and review. *Rev Infect Dis* 1990; **12**:838–47.
13. Benoit F. Chronic meningococcemia. *Medicine* 1963;**35**:103.
14. Bernard G, Vincent J, Laterre P, et al. Efficacy and safety of recombinant human activated protein C for severe sepsis. *N Engl J Med* 2001;**344**:699–709.
15. Bettinger J, Scheifele D, Le Saux N, et al. The impact of childhood meningococcal serogroup C conjugate vaccine programs in Canada. *Pediatr Infect Dis J* 2009;**28**:220–4.
16. Bilukha OR, Rosenstein N. Prevention and control of meningococcal disease. *MMWR Recomm Rep* 2005;**54**:1–21.
17. Bjune G, Hoiby EA, Gronnesby JK, et al. Effect of outer membrane vesicle vaccine against group B meningococcal disease in Norway. *Lancet* 1991;**338**:1093–6.
18. Boger W. Fulminating meningococcemia. *N Engl J Med* 1944; **231**:385–7.
19. Boger W. Purulent meningococcal arthritis. *Am J Med Sci* 1944; **208**:708–17.
20. Bone M, Diver M, Selby A, et al. Assessment of adrenal function in the initial phase of meningococcal disease. *Pediatrics* 2002;**110**: 563–9.
21. Boras A, Bozinovic D, Tenover F, et al. First report of *Neisseria meningitidis* intermediately resistant to penicillin in Croatia. *J Clin Microbiol* 2001;**39**:823.
22. Borrow R, Joseph H, Andrews N, et al. Reduced antibody response to revaccination with meningococcal serogroup A polysaccharide vaccine in adults. *Vaccine* 2000;**19**:1129–32.
23. Brandtzaeg P, Mollnes TE, Kierulf P. Complement activation and endotoxin levels in systemic meningococcal disease. *J Infect Dis* 1989;**160**:58–65.
24. Brandtzaeg P, Halstensen A, Kierulf P, et al. Molecular mechanisms in the compartmentalized inflammatory response presenting as meningococcal meningitis or septic shock. *Microb Pathog* 1992;**13**:423–32.
25. Brandtzaeg P, Kierulf P, Gaustad P, et al. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. *J Infect Dis* 1989;**159**:195–294.
26. Brooks R, Woods CW, Benjamin Jr DK, et al. Increased case-fatality rate associated with outbreaks of *Neisseria meningitidis* infection, compared with sporadic meningococcal disease, in the United States, 1994-2002. *Clin Infect Dis* 2006;**43**:49–54.
27. Brundage J, Zollinger W. *Evolution of meningococcal disease epidemiology in the U.S. army*. Boca Raton, Fla: CRC Press; 1987.
28. Buchanan R, Gibbons N, editors. *Bergey's manual of determinative bacteriology*. Baltimore, Md: Williams & Wilkins; 1974.
29. Campbell H, Andrews N, Borrow R, et al. Updated postlicensure surveillance of the meningococcal C conjugate vaccine in England and Wales: effectiveness, validation of serological correlates of protection, and modeling prediction of the duration of herd immunity. *Clin Vaccine Immunol* 2010;**17**:840–7.
30. Campbell H, Borrow R, Salisbury D, et al. Meningococcal C conjugate vaccine: the experience in England and Wales. *Vaccine* 2009;**27S**:B20–29.
31. Cartwright K, Reilly S, White D, et al. Early treatment with parenteral penicillin in meningococcal disease. *BMJ* 1992;**305**: 143–7.
32. Cartwright KA, Jones DM, Smith AJ, et al. Influenza A and meningococcal disease. *Lancet* 1991;**338**:554–7.
33. Caugant DA, Hoiby EA, Magnus P, et al. Asymptomatic carriage of *Neisseria meningitidis* in a randomly sampled population. *J Clin Microbiol* 1994;**32**:323–30.
34. Centers for Disease Control and Prevention. *Active Bacterial Core Surveillance Reports*. http://www.cdc.gov/abcs/reports-findings/surv-reports.html
35. Anonymous. Meningococcal disease among travelers returning from Saudi Arabia. *MMWR Morb Mortal Wkly Rep* 1987;**36**:559.
36. Centers for Disease Control and Prevention. Serogroup B meningococcal disease: Oregon, 1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**:121–4.
37. Centers for Disease Control and Prevention. Meningococcal disease and college students: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR—Morb Mortal Wkly Rep* 2000;**49**:13–20.
38. Centers for Disease Control and Prevention. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2005;**54**:1–21.
39. Centers for Disease Control and Prevention. Inadvertent misadministration of meningococcal conjugate vaccine: United States, Jun-August 2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**: 1016–17.
40. Centers for Disease Control and Prevention. *Active Bacterial Core surveillance (ABCs) report: Emerging Infections Program Network* Neisseria meningitidis, 2010. http://www.cdc.gov/abcs/reports-findings/survreports/mening10.pdf.
41. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391–2.
42. Centers for Disease Control and Prevention. *Division of Bacterial Diseases (CDC) winter news bulletin*. http://www.cdc.gov/ncird/div/DBD/newsletters/2011/winter/quarter3.html.
43. Centers for Disease Control and Prevention. Licensure of a meningococcal conjugate vaccine in children 2 through 10 years of age. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1018–19.
44. Centers for Disease Control and Prevention. Updated recommendations for use of meningococcal conjugate vaccines: Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:72–6.
45. Centers for Disease Control and Prevention. Notifiable diseases and mortality. *MMWR* 2012;**61**:ND44–57.
46. Centers for Disease Control and Prevention. Notifiable diseases and mortality tables. *MMWR* 2012;**61**:ND283–96.
47. Chamot-Rooke J, Mikaty G, Malosse C, et al. Posttranslational modification of pili upon cell contact triggers *N. meningitidis* dissemination. *Science* 2011;**331**:778–82.
48. Churchwell K, McManus M, Kent P, et al. Intensive blood and plasma exchange for treatment of coagulopathy in meningococcemia. *J Clin Apheresis* 1995;**10**:171–7.
49. Clark T. *MenAfriVac™ (meningococcal A conjugate vaccine) introduction in Burkina Faso; presentation at the Advisory Committee of*

Immunization Practices, June 2011. http://www.cdc.gov/vaccines/acip/meetings/downloads/min-archive/min-jun11.pdf.
50. Cobroft R, Henderson A. Meningococcal purpura fulminans treated with antithrombin III concentrate: what is the optimal replacement therapy? *Aust N Z J Med* 1994;**24**:575–6.
51. Cohen MS, Steere AC, Baltimore R, et al. Possible nosocomial transmission of group Y *Neisseria meningitidis* among oncology patients. *Ann Intern Med* 1979;**91**:7–12.
52. Cohn A, MacNeil J, Harrison L, et al. Changes in *Neisseria meningitidis* disease epidemiology in the United States, 1998–2007: implications for prevention of meningococcal disease. *Clin Infect Dis* 2010;**50**:184–91.
53. Conway E, Rosenberg R. Tumor necrosis factor suppresses transcription of the thrombomodulin gene in endothelial cells. *Mol Cell Biol* 1988;**8**:5588–92.
54. Cooper E, Ellison R, Smith G, et al. Rifampin-resistant meningococcal disease in a contact patient given prophylactic rifampin. *J Pediatr* 1986;**108**:93–6.
55. Corless C, Guiver M, Borrow R, et al. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J Clin Microbiol* 2001;**39**:1553–8.
56. Cuevas LE, Kazembe P, Mughogho GK, et al. Eradication of nasopharyngeal carriage of *Neisseria meningitidis* in children and adults in rural Africa: a comparison of ciprofloxacin and rifampicin. *J Infect Dis* 1995;**171**:728–31.
57. D'Amelio R, Agostoni A, Biselli R, et al. Complement deficiency and antibody profile in survivors of meningococcal meningitis due to common serogroups in Italy. *Scand J Immunol* 1992;**35**:589–95.
58. Dahle J. Pathogenesis of hemorrhagic skin lesions in meningococcal disease. *NIPH Ann* 1983;**6**:49–53.
59. Darton T, Guiver M, Naylor S, et al. Bacterial genomic detection within cerebrospinal fluid of patients with meningococcal disease is influenced by microbial and host characteristics. *Clin Infect Dis* 2011;**53**:463–7.
60. Davila S, Wright V, Khor C. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. *Nat Genet* 2010;**42**:772.
61. Davis CE, Arnold K. Role of meningococcal endotoxin in meningococcal purpura. *J Exp Med* 1974;**140**:159–71.
62. De Kleign E, Joosten K, Van Rijn B, et al. Low serum cortisol in combination with high adrenocorticotrophic hormone concentrations are associated with poor outcome in children with severe meningococcal disease. *Pediatr Infect Dis J* 2002;**21**:330–6.
63. den Brinker M, Joosten K, Liem O, et al. Adrenal insufficiency in meningococcal sepsis: bioavailable cortisol levels and impact of interleukin-6 levels and intubation with etomidate on adrenal function and mortality. *J Clin Endocrinol Metab* 2005;**90**:5110–17.
64. Densen P, Weiler JM, Griffiss JM, et al. Familial properdin deficiency and fatal meningococcemia: correction of the bactericidal defect by vaccination. *N Engl J Med* 1987;**316**:922–6.
65. Derkx B, Wittes JRM. Randomized, placebo-controlled trial of HA-1A, a human monoclonal antibody to endotoxin in children with meningococcal septic shock. *Clin Infect Dis* 1999;**28**:770–7.
66. DeVoe IW, Gilka F. Disseminated intravascular coagulation in rabbits: synergistic activity of meningococcal endotoxin and materials egested from leucocytes containing meningococci. *J Med Microbiol* 1976;**9**:451–8.
67. Dhodapkar K, Corbacioglu S, Chang MKM, et al. Purpura fulminans caused by group A beta-hemolytic *Streptococcus* sepsis. *J Pediatr* 2000;**137**:562–7.
68. Dickson R, McKinnon N, Magner D, et al. Meningococcal infection. *Lancet* 1941;**2**:631–4.
69. Dixon LM, Sanford HS. Meningococcal pericarditis in the antibiotic era. *Mil Med* 1971;**136**:433–8.
70. Dock W. Intermittent fever of seven months duration due to meningococcemia. *JAMA* 1924;**83**:399–400.
71. Edwards MS, Baker CJ. Complications and sequelae of meningococcal infections in children. *J Pediatr* 1981;**99**:540–5.
72. Eley B, Levin M. Septic shock with special reference to meningococcal disease. *Curr Opin Infect Dis* 1994;**7**:345–50.
73. Ellsworth J, Marks MI, Vose A. Meningococcal meningitis in children. *CMAJ* 1979;**120**:155–8.
74. Faber J, Meyer C, Gemmer C, et al. Human toll-like receptor 4 mutations are associated with susceptibility to invasive meningococcal disease in infancy. *Pediatr Infect Dis J* 2006;**25**:80–1.
75. Faber J, Schuessler TFA, Murdoch C, et al. Age-dependent association of human mannose-binding lectin mutations with susceptibility to invasive meningococcal disease in childhood. *Pediatr Infect Dis J* 2007;**26**:243–6.
76. Faur Y, Weisburd M, Wilson M. Isolation of *Neisseria meningitidis* from the genito-urinary tract and anal canal. *J Clin Microbiol* 1975;**2**:178–82.
77. Feavers I. ABC of meningococcal diversity. *Nature* 2000;**404**:451–2.
78. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. *Clin Microbiol Rev* 1991;**4**:359–95.
79. Fijen C, Kuijper E, Hannema A, et al. Complement deficiencies in patients over ten years old with meningococcal disease due to uncommon serogroups. *Lancet* 1989;**2**:585–8.
80. Fisher M, Hedberg K, Cardosi P, et al. Tobacco smoke as a risk factor for meningococcal disease. *Pediatr Infect Dis J* 1997;**10**:979–83.
81. Francke E, Neu H. Postsplenectomy infection. *Surg Clin North Am* 1981;**61**:135–55.
82. Frasch C, Zollinger W, Poolman J. Serotype antigens of *Neisseria meningitidis* and a proposed scheme for design of serotypes. *Rev Infect Dis* 1985;**7**:504–10.
83. Frasch CE. Role of protein serotype antigens in protection against disease due to *Neisseria meningitidis*. *J Infect Dis* 1977;**136**(suppl):S84–90.
84. Galloway Y, Stehr-Green P, McNicholas A, et al. Use of an observational cohort study to estimate the effectiveness of the New Zealand group B meningococcal vaccine in children aged under 5 years. *Int J Epidemiol* 2009;**38**:413–18.
85. Gerard P, Moriau M, Bachy A, et al. Meningococcal purpura: report of 19 patients treated with heparin. *J Pediatr* 1973;**82**:780–6.
86. Gilmore A, Stuart J, Andrews N. Risk of secondary meningococcal disease in health-care workers. *Lancet* 2000;**356**:1654–5.
87. Girardin E, Grau GE, Dayer JM, et al. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med* 1988;**319**:397–400.
88. Girgis N, Sultan Y, Frenck Jr R, et al. Azithromycin compared with rifampin for eradication of nasopharyngeal colonization by *Neisseria meningitidis*. *Pediatr Infect Dis J* 1998;**17**:816–19.
89. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. I. The role of humoral antibodies. *J Exp Med* 1969;**129**:1307–26.
90. Goldschneider I, Gotschlich EC, Artenstein MS. Human immunity to the meningococcus. II. Development of natural immunity. *J Exp Med* 1969;**129**:1327–48.
91. Gossager N, Snape M, Yu L, et al. Immunogenicity and tolerability of recombinant serogroup b meningococcal vaccine administered with or without routine infant vaccinations according to different immunization schedules. *JAMA* 2012;**307**:573–82.
92. Greenfield S, Sheehe PR, Feldman HA. Meningococcal carriage in a population of "normal" families. *J Infect Dis* 1971;**123**:67–73.
93. Greenwood B, Whittle H. The pathogenesis of meningococcal arthritis. In: Dumonde D, Path M, editors. *Infection and immunology in rheumatic diseases*. Philadelphia: Lippincott; 1976. p. 119–27.
94. Greenwood BM, Bradley AK, Wall RA. Meningococcal disease and season in sub-Saharan Africa. *Lancet* 1985;**2**:829–30.
95. Greenwood BM, Hassan-King M, Whittle HC. Prevention of secondary cases of meningococcal disease in household contacts by vaccination. *Br Med J* 1978;**1**:1317–19.
96. Haggerty R, Zaia M. Acute bacterial meningitis. *Adv Pediatr* 1964;**13**:129–73.
97. Hahné S, Charlett A, Purcell B, et al. Effectiveness of antibiotics given before admission in reducing mortality from meningococcal disease: systematic review. *BMJ* 2006;**332**:1299–303.
98. Halperin S, Gupta A, Jeanfreau R, et al. Comparison of the safety and immunogenicity of an investigational and a licensed quadrivalent meningococcal conjugate vaccine in children 2-10 years of age. *Vaccine* 2010;**28**:7865–72.
99. Haralambous E, Dolly S, Hibberd M. Factor H, a regulator of complement activity, is a major determinant of meningococcal

disease susceptibility in UK Caucasian patients. *Scand J Infect Dis* 2006;**38**:764.
100. Hardman J. Fatal meningococcal infections: the changing pathologic picture in the '60s. *Mil Med* 1968;**133**:951–64.
101. Harrison L. Epidemiological profile of meningococcal disease in the United States. *Clin Infect Dis* 2010;**50**(suppl 2):S37–44.
102. Harrison L, Jolley K, Shutt K, et al. Antigenic shift and increased incidence of meningococcal disease. *J Infect Dis* 2006;**193**: 1266–74.
103. Herman R, Rubin H. Meningococcal pericarditis without meningitis presenting as tamponade. *N Engl J Med* 1974;**290**:143–4.
104. Herrick W. Extrameningeal meningococcus infections. *Arch Intern Med* 1919;**23**:409–18.
105. Hill W, Kinney T. The cutaneous lesions in acute meningococcemia: a clinical and pathologic study. *JAMA* 1947;**134**:513–18.
106. Holbein BE. Differences in virulence for mice between disease and carrier strains of *Neisseria meningitidis*. *Can J Microbiol* 1981; **27**:738–41.
107. Hubert B, Watier L, Garnerin P, et al. Meningococcal disease and influenza-like syndrome: a new approach to an old question. *J Infect Dis* 1992;**166**:542–5.
108. Immunization Action Coalition. *Meningococcal disease: ask the experts*. http://www.immunize.org/askexperts/experts_men.asp
109. Inkelis S, O'Leary D, Wang V, et al. Extremity pain and refusal to walk in children with invasive meningococcal disease. *Pediatrics* 2002;**110**:e3.
110. Irazuzta J, McManus ML. Use of topically applied nitroglycerin in the treatment of purpura fulminans. *J Pediatr* 1990;**117**: 993–5.
111. Jackson L, Wenger J. Laboratory-based surveillance for meningococcal disease in selected areas: United States, 1989-1991. *MMWR CDC Surveill Summ* 1993;**42**:21–30.
112. Jackson LA, Tenover FC, Baker C, et al. Prevalence of *Neisseria meningitidis* relatively resistant to penicillin in the United States, 1991. Meningococcal Disease Study Group. *J Infect Dis* 1994;**169**: 438–41.
113. Jacobson JA, Camargos PA, Ferreira JT, et al. The risk of meningitis among classroom contacts during an epidemic of meningococcal disease. *Am J Epidemiol* 1976;**104**:552–5.
114. Jones RN, Slepack J, Eades A. Fatal neonatal meningococcal meningitis: association with maternal cervical-vaginal colonization. *JAMA* 1976;**236**:2652–3.
115. Kaiser A, Hennekens C, Saslaw M, et al. Seroepidemiology and chemoprophylaxis of disease due to sulfonamide-resistant *Neisseria meningitidis* in a civilian population. *J Infect Dis* 1974;**130**: 217–24.
116. Kaplan S, Schutze G, Leake J, et al. Multicenter surveillance of invasive meningococcal infections in children. *Pediatrics* 2006; **118**:e979–e984.
117. Kaspar D, Winkelhake J, Zollinger W. Immunochemical similarity between polysaccharide antigens of *Escherichia coli* 07:K1(L):NM and group B *Neisseria meningitidis*. *J Immunol* 1973;**110**:262–8.
118. Kaufman B, Levy H, Zaleznak B, et al. Statistical analysis of 242 cases of meningococcus meningitis. *Pediatrics* 1951;**38**:705–16.
119. Kelly C, Arnold R, Galloway Y, et al. A prospective study of the effectiveness of the New Zealand meningococcal B vaccine. *Am J Epidemiol* 2007;**166**:817–23.
120. Keyserling H, Papa T, Koranyi K, et al. Safety, immunogenicity, and immune memory of a novel meningococcal (groups A, C, Y, and W-135) polysaccharide diphtheria toxoid conjugate vaccine (MCV-4) in healthy adolescents. *Arch Pediatr Adolesc Med* 2005;**159**:907–13.
121. Kirsch E, Barton P, Kitchen L, et al. Pathophysiology, treatment and outcome of meningococcemia: a review and recent experience. *Pediatr Infect Dis J* 1996;**15**:967–79.
122. Koppes G, Ellenbogen E, Gebhart R. Group Y meningococcal disease in United States Air Force recruits. *Am J Med* 1977; **62**:661–6.
123. Kozarsky P, Arguin P, Navin A. *Health information for international travel 2005-2006*. Atlanta, Ga: Department of Health and Human Services; 2006.
124. Kravitz G, Dries D, Peterson M, et al. Purpura fulminans due to *Staphylococcus aureus*. *Clin Infect Dis* 2005;**40**:941–7.
125. Kristiansen P, Diomande F, Wei S, et al. Baseline meningococcal carriage in Burkina Faso before the introduction of a meningococcal serogroup A conjugate vaccine. *Clin Vaccine Immunol* 2011;**18**:435–43.
126. Kunkel M, Brown LHB, et al. Meningococcal mesenteric adenitis and peritonitis in a child. *Pediatr Inf Dis J* 1984;**3**:327–8.
127. Kupperman N, Inkelis S, Saladino R. The role of heparin in the prevention of extremity and digit necrosis in meningococcal purpura fulminans. *Pediatr Infect Dis J* 1994;**13**:867–73.
128. Leclerc F, Martinot A, Cremer R, et al. ECMO for refractory cardiorespiratory failure due to meningococcal disease. *Lancet* 1997;**349**:1397–8.
129. Lehman TJ, Bernstein B, Hanson V, et al. Meningococcal infection complicating systemic lupus erythematosus. *J Pediatr* 1981; **99**:94–6.
130. Lehmann A, Halstensen A, Sornes S, et al. High levels of interleukin-10 during the initial phase of fulminant meningococcal septic shock. *J Infect Dis* 1995;**171**:229–32.
131. Leibel R, Fangman J, Ostrovsky M. Chronic meningococcemia in childhood. *Am J Dis Child* 1974;**127**:94–8.
132. Levin M, Quint P, Goldstein B, et al. Recombinant bactericidal/permeability-increasing protein (rBPI) as adjunctive treatment for children with severe meningococcal sepsis: a randomized trial. *Lancet* 2000;**356**:961–7.
133. Levitt L, Bond J, Hall Jr I, et al. Meningococcal and ECHO-9 meningitis: report of an outbreak. *Neurology* 1970;**20**:45–51.
134. Lewis J, Arnold C, Alexander J. Meningococcal pneumonia. *Am J Clin Pathol* 1973;**59**:388–90.
135. Linz B, Schenker M, Zhu P, et al. Frequent interspecific genetic exchange between commensal Neisseriae and *Neisseria meningitidis*. *Mol Microbiol* 2000;**36**:1049–58.
136. MacLennan J, Obaro S, Deeks J, et al. Immune response to revaccination with meningococcal A and C polysaccharides in Gambian children following repeated immunization during early childhood. *Vaccine* 1999;**17**:3086–93.
137. Maiden MC. Population genetics of a transformable bacterium: the influence of horizontal genetic exchange on the biology of *Neisseria meningitidis*. *FEMS Microbiol Lett* 1993;**112**:243–50.
138. Maiden MC, Ibarz-Pavón AB, Urwin R, et al. Impact of meningococcal serogroup C conjugate vaccines on carriage and herd immunity. *J Infect Dis* 2008;**197**:737–43.
139. Manchanda V, Bhalla P. Emergence of non-ceftriaxone-susceptible *Neisseria meningitidis* in India. *J Clin Microbiol* 2006; **44**:4290–1.
140. Marks MI, Frasch CE, Shapera RM. Meningococcal colonization and infection in children and their household contacts. *Am J Epidemiol* 1979;**109**:563–71.
141. Marzouk O, Thomson A, Sills J, et al. Features and outcome in meningococcal disease presenting with maculopapular rash. *Arch Dis Child* 1991;**66**:485–7.
142. Mellado M, Rodriguez-Contreras R, Mariscal A, et al. Effect of penicillin and chloramphenicol on the growth and endotoxin release by *N. meningitidis*. *Epidemiol Infect* 1991;**106**: 283–8.
143. Meyer M, Irazuzta J, Tozibikian H. Topical nitroglycerin and pain in purpura fulminans. *J Pediatr* 1999;**134**:639–41.
144. Moncada S, Higgs E. Endogenous nitric oxide: physiology, pathology and clinical relevance. *Eur J Clin Invest* 1991;**21**: 361–74.
145. Moore PS, Harrison LH, Telzak EE, et al. Group A meningococcal carriage in travelers returning from Saudi Arabia. *JAMA* 1988;**260**:2686–9.
146. Moore PS, Hierholzer J, DeWitt W, et al. Respiratory viruses and mycoplasma as cofactors for epidemic group A meningococcal meningitis. *JAMA* 1990;**264**:1271–5.
147. Moore PS, Reeves MW, Schwartz B, et al. Intercontinental spread of an epidemic group A *Neisseria meningitidis* strain. *Lancet* 1989; **2**:260–3.
148. Morse J, Oretsky M, Hudson J. Pericarditis as a complication of meningococcal meningitis. *Ann Intern Med* 1971;**74**:212–17.
149. Nadel S, De Munter C, Britto J, et al. Recombinant tissue plasminogen activator restores perfusion in meningococcal purpura fulminans. *Critical Care Medicine* 1998;**26**:971–2.
150. Nadel S, Goldstein B, Williams M, et al. Drotrecogin alfa (activated) in children with severe sepsis: a multicentre phase III randomized controlled trial. *Lancet* 2007;**369**:836–43.
151. Nassif X, So M. Interaction of pathogenic *Neisseriae* with nonphagocytic cells. *Clin Microbiol Rev* 1995;**8**:376–88.

152. Nawroth P, Stern D. Modulation of endothelial cell hemostatic properties by tumor necrosis factor. *J Exp Med* 1986;**320**: 1165–72.
153. Ni H, Knight A, Cartwright K, et al. Polymerase chain reaction for diagnosis of meningococcal meningitis. *Lancet* 1993;**340**: 1432–4.
154. Nielsen HE, Koch C, Magnussen P, et al. Complement deficiencies in selected groups of patients with meningococcal disease. *Scand J Infect Dis* 1989;**21**:389–96.
155. Niklasson P, Lundbergh P, Strandell T. Prognostic factors in meningococcal disease. *Scand J Infect Dis* 1971;**3**:17–25.
156. Nolan T, Richmond P, Marshall H, et al. Immunogenicity and safety of an investigational combined *Haemophilus influenzae* type B-*Neisseria meningitidis* serogroups C and Y-tetanus toxoid conjugate vaccine. *Pediatr Infect Dis J* 2011;**30**:190–6.
157. Ognibene A, Dito W. Chronic meningococcemia. *Arch Intern Med* 1964;**114**:29–32.
158. Olcen P, Kjellander J, Danielsson D, et al. Epidemiology of *Neisseria meningitidis*; prevalence and symptoms from the upper respiratory tract in family members to patients with meningococcal disease. *Scand J Infect Dis* 1981;**13**:105–9.
159. Oppenheimer E, Rosman N. Bacterial meningitis in childhood: neurologic complications and their management. *Pediatr Neurol* 1976;**287**:285–98.
160. Palmgren H. Meningococcal disease and climate. *Global Health Action* 2009;2:doi:10.3402.
161. Parillo J. Pathogenic mechanisms of septic shock. *N Engl J Med* 1993;**328**:307–43.
162. Penny J, Grace W, Kennedy R. Meningococcal pericarditis. *Am J Cardiol* 1966;**18**:281–5.
163. Perez-Trallero E, Aldamiz-Echeverria L, Perez-Yarza EG. Meningococci with increased resistance to penicillin. *Lancet* 1990;**335**: 1096.
164. Pichichero M, Casey J, Blatter M, et al. Comparative trial of the safety and immunogenicity of quadrivalent (A, C, Y, W-135) meningococcal polysaccharide-diphtheria conjugate vaccine versus quadrivalent polysaccharide vaccine in two- to ten-year-old children. *Pediatr Infect Dis J* 2005;**24**:57–62.
165. Pinals R, Ropes M. Meningococcal arthritis. *Arthritis Rheum* 1964;**7**:241–58.
166. Pinner RW, Onyango F, Perkins BA, et al. Epidemic meningococcal disease in Nairobi, Kenya, 1989. The Kenya/Centers for Disease Control (CDC) Meningitis Study Group. *J Infect Dis* 1992;**166**:359–64.
167. Pizzi M. A severe epidemic of meningococcus meningitis in Chile, 1941-2. *Am J Public Health* 1944;**34**:231–8.
168. Platonov A, Beloborodov V, Vershinina I. Meningococcal disease in patients with late complement deficiency: studies in the U.S.S.R. *Medicine* 1993;**72**:374–92.
169. Poolman J, van der Ley P, Tommassen J. *Surface structures and secreted products of meningococci*. Chichester, UK: Wiley; 1995.
170. Popovic T, Sacchi CT, Reeves MW, et al. *Neisseria meningitidis* serogroup W135 isolates associated with the ET-37 complex. *Emerg Infect Dis* 2000;**6**:428–9.
171. Powars D, Larsen R, Johnson J, et al. Epidemic meningococcemia and purpura fulminans with induced protein C deficiency. *Clin Infect Dis* 1993;**17**:254–61.
172. Powars D, Rogers Z, Patch M, et al. Purpura fulminans in meningococcemia: association with acquired deficiencies of proteins C and S. *N Engl J Med* 1987;**317**:571–2.
173. ProMED. *Meningitis, Meningococcal-Africa: Burkina Faso*, 2010. ProMED Digest, Archive No. 20100507.1484. http://www.promedmail.org/.
174. ProMed. *Meningococcal Disease Update* 2007. http://www.promedmail.org/pls/promed/f?p=2400:1202:10374748748848896256::NO::F2400_P1202_CHECK_DISPLAY,F2400_P1202_PUB_MAIL_ID:X,36797.
175. Racoosin J, Whitney C, Conover C, et al. Serogroup Y meningococcal disease in Chicago, 1991-1997. *JAMA* 1998;**280**:2094–8.
176. Ramsay M, Andrews N, Kaczmarski E, et al. Efficacy of meningococcal serogroup C conjugate vaccine in teenagers and toddlers in England. *Lancet* 2001;**357**:195–6.
177. Ramsay M, Andrews N, Trotter C, et al. Herd immunity from meningococcal serogroup C conjugate vaccination in England: database analysis. *BMJ* 2003;**326**:365–6.
178. Read R, Camp N, Di Giovine F, et al. An interleukin-1 genotype is associated with fatal outcome of meningococcal disease. *J Infect Dis* 2000;**182**:1557.
179. Riedo FX, Plikaytis BD, Broome CV. Epidemiology and prevention of meningococcal disease. *Pediatr Infect Dis J* 1995;**14**: 643–57.
180. Riordan F, Thomson A, Ratcliffe J, et al. Admission cortisol and adrenocorticotrophic hormone levels in children with meningococcal disease: evidence of adrenal insufficiency? *Crit Care Med* 1999;**27**:2257–61.
181. Robboy S. Atrioventricular node inflammation: mechanism of sudden death in protracted meningococcemia. *N Engl J Med* 1972;**286**:1091–3.
182. Rodriguez-Erdmann F. Intravascular activation of the clotting system with phospholipids. *Blood* 1965;**26**:541–53.
183. Rosenblatt J, Ray C, Enquist R. Meningococcal infections in children: observations on prognosis and therapy. *Personal communication* 1978.
184. Rosenstein NE, Perkins BA. Update on *Haemophilus influenzae* serotype b and meningococcal vaccines. *Pediatr Clin North Am* 2000;**47**:337–52, vi.
185. Rosenstein NE, Perkins BA, Stephens DS, et al. The changing epidemiology of meningococcal disease in the United States, 1992-1996. *J Infect Dis* 1999;**180**:1894–901.
186. Rosenstein NE, Perkins BA, Stephens DS, et al. Meningococcal disease. *N Engl J Med* 2001;**344**:1378–88.
187. Ross SC, Densen P. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. *Medicine (Baltimore)* 1984;**63**:243–73.
188. Saez-Nieto J, Lujan R, Berron S, et al. Epidemiology and molecular basis of penicillin-resistant *Neisseria meningitidis* in Spain: a 5-year history (1985-1989). *Clin Infect Dis Suppl* 1992;**1**:S12–20.
189. Santolaya M, O'Ryan M, Valenzuela M, et al. Immunogenicity and tolerability of a multicomponent meningococcal serogroup B (4CMenB) vaccine in healthy adolescents in Chile: a phase 2b/3 randomised, observer-blind, placebo-controlled study. *Lancet* 2012;**379**:617–24.
190. Scheim A. Articular manifestations of meningococcic infections. *Arch Intern Med* 1938;**62**:963–78.
191. Scholten RJ, Bijlmer HA, Tobi H, et al. Upper respiratory tract infection, heterologous immunisation and meningococcal disease. *J Med Microbiol* 1999;**48**:943–6.
192. Scott L, Knox D, Perry L, et al. Meningococcal pericarditis. *Am J Cardiol* 1972;**29**:104–8.
193. Sinclair J, Skeoch C, Hallworth D. Prognosis of meningococcal septicaemia. *Lancet* 1987;**3**:38.
194. Smith O, White B, Vaughn D, et al. Use of protein-C concentrate, heparin, and haemodiafiltration in meningococcus-induced purpura fulminans. *Lancet* 1997;**350**:1590–3.
195. Sow S, Okoko B, Diallo A, et al. Immunogenicity and safety of a meningococcal A conjugate in Africans. *N Engl J Med* 2011;**364**:2293–304.
196. Stanwell-Smith RE, Stuart JM, Hughes AO, et al. Smoking, the environment and meningococcal disease: a case control study. *Epidemiol Infect* 1994;**112**:315–28.
197. Stefanelli P, Fazio C, Neri A, et al. First report of capsule replacement among electrophoretic type 37 *Neisseria meningitidis* strains in Italy. *J Clin Microbiol* 2003;**41**:5783–6.
198. Stephens D, Farley M. Pathogenic events during infection of the human nasopharynx with *Neisseria meningitidis* and *Haemophilus influenzae*. *Rev Infect Dis* 1991;**12**:22–3.
199. Stephens D, Hoffman L, McGee Z. Interaction of *Neisseria meningitidis* with human nasopharyngeal mucosa: attachment and entry into columnar epithelial cells. *J Infect Dis* 1983;**148**: 369–76.
200. Stiehm E, Damrosch D. Factors in the prognosis of meningococcal infection. *J Pediatr* 1966;**68**:457–67.
201. Stuart JM, Cartwright KA, Robinson PM, et al. Effect of smoking on meningococcal carriage. *Lancet* 1989;**2**:723–5.
202. Sullivan T, La Scolea L. *Neisseria meningitidis* bacteremia in children: quantitation of bacteremia and spontaneous clinical recovery without antibiotic therapy. *Pediatrics* 1987;**80**:63–73.
203. Sutcliffe EM, Jones DM, El-Sheikh S, et al. Penicillin-insensitive meningococci in the UK. *Lancet* 1988;**1**:657–8.

204. Swartley J, Marfin A, Edupuganti S, et al. Capsule switching of *Neisseria meningitidis*. *Proc Acad Sci U S A* 1997;**94**:271–6.
205. Thompson A, Sills J, Hart C. Validation of the Glasgow meningococcal septicaemia prognostic score: a 10-year retrospective survey. *Crit Care Med* 1991;**19**:26–30.
206. Tobias J, Haun S, Helfaer M, et al. Use of continuous caudal block to relieve lower extremity ischemia caused by vasculitis in a child with meningococcemia. *J Pediatr* 1989;**115**:1019–21.
207. Trotter C, Andrews N, Kaczmarski E, et al. Effectiveness of meningococcal serogroup C conjugate vaccine 4 years after introduction. *Lancet* 2004;**364**:365–7.
208. Tully J, Viner R, Coen P. Risk and protective factors for meningococcal disease in adolescents: matched cohort study. *BMJ* 2006;**332**:445–50.
209. Tuncer A, Gar I, Ertem U, et al. Once daily ceftriaxone for meningococcemia and meningococcal meningitis. *Pediatr Infect Dis J* 1988;**7**:711–13.
210. U.S. Food and Drug Administration. *Product approval information-licensing action. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research*, 2005. http://www.fda.gov/cber/products/mpdtave011405.htm
211. van de Ende A, Hopman C, Dankert J. Multiple mechanism of phase variation of PorA in *Neisseria meningitidis*. *Infect Immun* 2000;**68**:6685–90.
212. van Deuren M, van der ven-Jongekrijg J, Demacker P, et al. Differential expression of proinflammatory cytokines and their inhibitors during the course of meningococcal infections. *J Infect Dis* 1994;**169**:157–61.
213. van Deuren M, van der ven-Jongekrijg J, Bartelink A, et al. Correlation between proinflammatory cytokines and anti-inflammatory mediators and the severity of disease in meningococcal infections. *J Infect Dis* 1995;**172**:433–9.
214. van Deuren M, van Dijke B, et al. Rapid diagnosis of acute meningococcal infections by needle aspiration or biopsy of skin lesions. *Br Med J* 1993;**307**:127.
215. Van Nguyen Z, Nguyen E, Weiner L. Incidence of invasive bacterial disease in children with fever and petechiae. *Pediatrics* 1984; **74**:77–80.
216. Vann W, Liu T, Robbins J. *Bacillus pumilus* polysaccharide cross-reactive with meningococcal group A polysaccharide. *Infect Immun* 1976;**13**:1654–62.
217. Vieusseux G. Memoire sur la maladie qui a regre a Geneve au printemps de 1805. *J Med Chir Pharm* 1806;**11**:163.
218. Vogel U, Claus H, Frosch M. Rapid serogroup switching in *Neisseria meningitidis*. *J Med* 2000;**342**:219–20.
219. Vu DM, Welsch JA, Zuno-Mitchell P, et al. Antibody persistence 3 years after immunization of adolescents with quadrivalent meningococcal conjugate vaccine. *J Infect Dis* 2006;**193**: 821–8.
220. Weightman NC, Johnstone DJ. Three cases of pneumonia due to *Neisseria meningitidis*, including serogroup W135. *Eur J Clin Microbiol Infect Dis* 1999;**18**:456–8.
221. Weis E, Silber E. Acute constrictive pericarditis. *J Pediatr* 1961;**58**:548–53.
222. Westendorp R, Langermans J, de Bel C, et al. Release of tumor necrosis factor: an innate host characteristic that may contribute to the outcome of meningococcal disease. *J Infect Dis* 1995; 1057–60.
223. Westendorp RG, Langermans JA, Huizinga T, et al. Genetic influence on cytokine production and fatal meningococcal disease. *Lancet* 1997;**349**:170–3.
224. Whittle H, Tugwell P, Egler L, et al. Rapid bacteriologic diagnosis of pyogenic meningitis by latex agglutination. *Lancet* 1974;**2**: 619–21.
225. Wilson F, Morse S. Therapy of acute meningococcal infections: early volume expansion and prophylactic low dose heparin. *Am J Med Sci* 1972;**264**:445–55.
226. Winn Jr W, Allen S, Janda W, et al. *Koneman's color atlas and textbook of diagnostic microbiology*. Baltimore, Md: Lippincott Williams & Wilkins; 2006.
227. Wolf R, Birbara C. Meningococcal infections at an army training center. *Am J Med* 1968;**44**:243–55.
228. Wong V, Hitchcock W, Mason W. Meningococcal infections in children: a review of 100 cases. *Pediatr Infect Dis J* 1989;**8**: 224–7.
229. World Health Organization. *Meningococcal meningitis: factsheet*. http://www.who.int/mediacentre/factsheets/fs141/en/.
230. Wu A, Harcourt B, Hatcher C, et al. Emergence of ciprofloxacin-resistant *Neisseria meningitidis* in North America. *N Engl J Med* 2009;**360**:886–92.
231. Young L, LaForce F, Head J, et al. A simultaneous outbreak of meningococcal and influenza infections. *N Engl J Med* 1972; **287**:5–9.
232. Zhu H, Wang Q, Wen L, et al. Development of a multiplex PCR assay for detection and genogrouping of *Neisseria meningitidis*. *J Clin Microbiol* 2012;**50**:46–51.

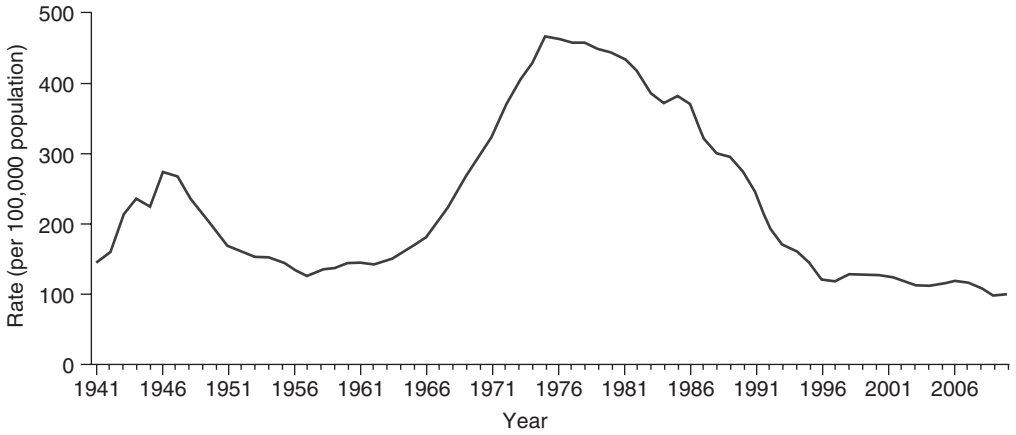

FIGURE 89-1 ■ Rates of gonorrhea in the United States, 1941 to 2010. After a 74 percent decline from 1975 through 1997, overall gonorrhea rates have plateaued in recent years. Gonorrhea is substantially underdiagnosed and underreported. (From Centers for Disease Control and Prevention: http://www.cdc.gov/std/stats10/gonorrhea-figs.htm.)

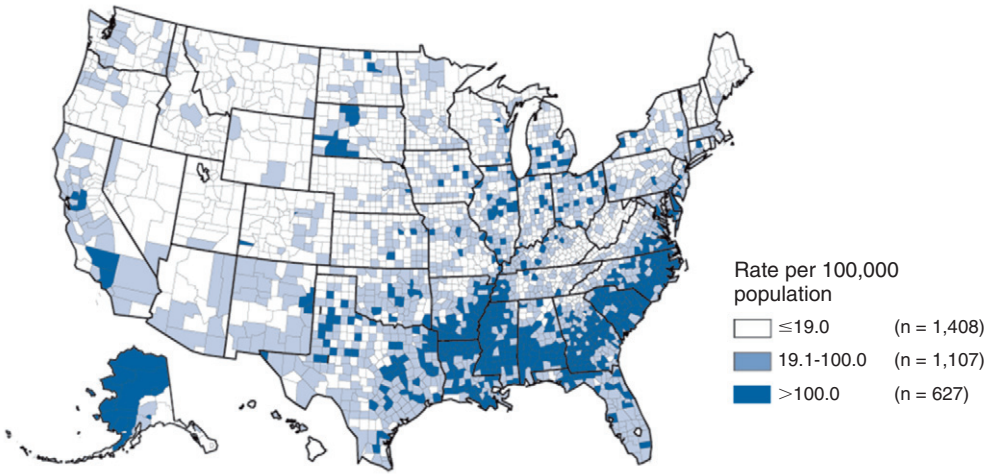

FIGURE 89-2 ■ Rates of gonorrhea by age and sex: United States, 2010. This pattern is the same as in previous decades. (See companion Expert Consult website for color version.) (From Centers for Disease Control and Prevention: http://www.cdc.gov/std/stats10/gonorrhea-figs.htm.)

population were reported in 2009, continuing an overall trend of increase from a nadir of 14.9 in 1996.[277] Regions with higher rates of adult disease have higher numbers of pediatric cases,[112,136] a finding that reflects the adult origin of virtually all pediatric cases.

An estimated 1 million new cases of gonococcal infection occur annually in the United States, only approximately a third of which are reported. At least half of the cases are thought to go undiagnosed or unreported. In 2010 in the United States, 309,341 cases were reported.[70] U.S. national trends in reported rates are shown in Figure 89-1. Rates of gonorrhea are based primarily on detection of symptomatic infection. Asymptomatic chronic infection in adults is well documented and may account for 5 percent of cases.[181] Such persons can transmit the infection. Some untreated cases will resolve over time. Data also indicate that asymptomatic infection occurs in children, including prepubertal children.[3,128,166,181] Gonococcal infections rarely are fatal (approximately four deaths per year in the United States), with death being caused by gonococcal sepsis.[61,225]

Gonorrhea rates remain highest among adolescents and young adults (Fig. 89-2). In 2010, the highest age group rates were 571/100,000 among adolescent females 15 to 19 years of age and 561 among males 20 to 24 years of age.[70] Rates among African Americans were 18.7 times higher than among whites (432 vs. 23). Low socioeconomic status, early onset of sexual activity, unmarried marital status, and past gonococcal infections are risk factors for acquiring gonococcal infection, as are prostitution and illicit drug use.[16,40] Limited access to quality health care also may contribute to an increased prevalence of disease in impoverished populations.[66] The prevalence of gonococcal infection in adolescents attending private clinics probably is lower than that in those seeking care in publicly funded settings.[22] For women, the use of hormonal contraceptives may increase the risk for acquiring infection, whereas the use of spermicides or diaphragms appears to be at least partially protective.[221,222]

Rates of gonococcal infection among men who have sex with men (MSM) are lower than in past years but remain higher than those among men who have sex only

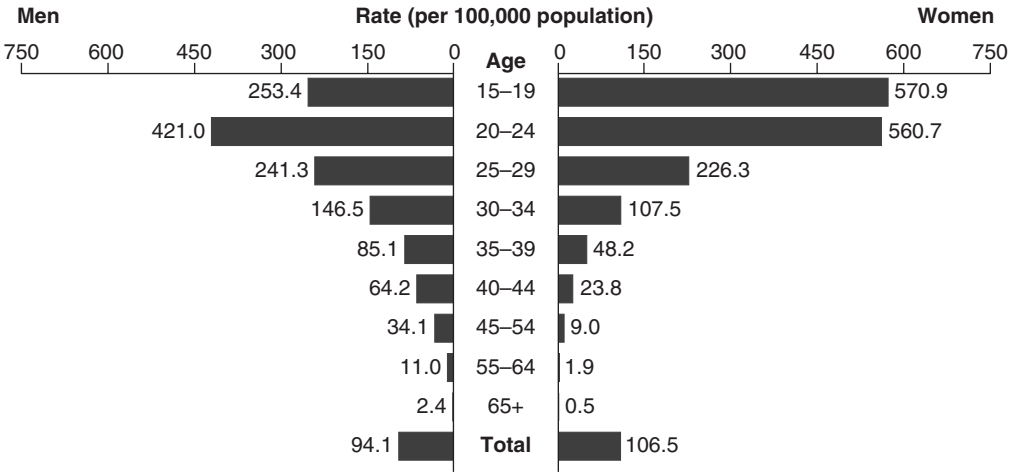

FIGURE 89-3 ■ Rates of gonorrhea by county in the United States and outlying areas, 2010. (From Centers for Disease Control and Prevention: http://www.cdc.gov/std/stats10/gonorrhea-figs.htm.)

with women (MSW).[70] The proportion of gonococcal infections among MSM versus MSW is higher in western states and New York City than in other regions of the United States. In a prospective study from 2001 to 2003, the prevalence of pharyngeal gonorrhea among human immunodeficiency virus (HIV)-negative MSM was 5.5 percent; 92 percent of cases were asymptomatic.[239]

The southeastern United States continues to have rates of gonorrhea higher than those of other regions of the country, but rates per 100,000 population in the Southeast fell from 175 to 134 between 2001 and 2010. Rates increased from 2009 to 2010 in the South, West, and Northeast but declined in the Midwest. About 50 percent of reported cases occurred in only 63 counties or independent cities in the United States in 2010 (Fig. 89-3). Most counties with more than 100 cases/100,000 population were located in the South.

Humans are the only reservoir of *N. gonorrhoeae*. Exudate and secretions from infected mucosal surfaces allow transmission to occur during the intimate contact of sexual acts, parturition, and, rarely, household exposure. The incubation period usually is 2 to 7 days. No evidence of airborne transmission of gonococci exists; contact with viable microbes is required for transmission. Gonococci survive on surfaces outside the human body for only short periods (probably minutes). Although organisms can be cultured from environmental sources (e.g., toilet seats) for up to 3 hours after artificial inoculation in large numbers, viable gonococci have not been recovered from random samplings in public restrooms.[139]

Nonsexual transmission of *N. gonorrhoeae* to the genitalia, rectum, or oropharynx of children rarely occurs and never should be presumed without extensive investigation of the social setting of the infected child.[252] Childhood infection potentially could be acquired when a child co-sleeps in the same bed with an infected family member,[139] and sharing of bath towels and other similar objects has been suspected as the cause of epidemics in prepubescent girls living together.[322] Although persistence of apparently perinatally acquired gonococcal infection for up to 1 year of age has been reported and fomite or maternal nonsexual transmission to young infants is plausible,[35] these possibilities should not be generalized beyond infancy. Gonococcal conjunctivitis can be acquired nonvenereally.[213]

The risk for males acquiring urethral infection after a single episode of vaginal intercourse with infected females is estimated to be 20 percent. With four exposures, the risk increases to 60 to 80 percent. The prevalence of infection in females named as sexual contacts of males with gonococcal urethritis has been reported to be 50 to 90 percent.[172,177]

The annual direct medical cost of treating sexually transmitted infections (STIs), including HIV, in the United States alone was estimated at $11 to $17 billion in 2003 U.S. dollars. Almost half of these costs accrue to persons aged 15 to 24 years. The reduction in gonorrhea and syphilis rates in the United States between 1990 and 2003 was estimated as being equivalent to $5 billion during this period.[79]

MICROBIOLOGY

Neisseria spp. are aerobic, gram-negative, nonmotile, non–spore-forming cocci that occur in pairs (diplococci), with adjacent sides flattened. They have the typical gram-negative microbial outer membrane overlying a thin peptidoglycan layer and cytoplasmic membrane. The species lacks a true polysaccharide capsule but produces a surface polyphosphate that provides a hydrophilic, negatively charged surface.[259] In Gram stains of clinical specimens, the microbes frequently are observed within phagocytes.[316]

Gonococci are able to use glucose, lactate, and pyruvate as carbon sources but cannot use other carbohydrates, which is the basis for the carbohydrate utilization tests for speciation of *Neisseria* (Table 89-1). Catalase and cytochrome oxidase are produced by the gonococcus, as in most *Neisseria* spp., but gonococci do not produce appreciable amounts of superoxide dismutase. Growth is optimal in a 5 percent carbon dioxide atmosphere at 35°

TABLE 89-1 **Biochemical Characteristics Differentiating Species of the Genus *Neisseria***

	N. Gonorrhoeae	*N. Meningitidis*	*N. Sicca*	*N. Subflava*	*N. Flavescens*	*N. Mucosa*	*N. Lactamica*
Acid from							
Glucose	+	+	+	+	–	+	+
Maltose	–	+	+	+	–	+	–
Sucrose	–	–	+	±	–	+	–
Lactose	–	–	–	–	–	–	+
Polysaccharide produced from 5% sucrose	0	0	+	±	+	+	0
Reduction of							
Nitrate	–	–	–	–	–	+	–
Nitrite	–	±	+	+	+	+	+
Pigment	–	–	±	+	+	+	–
Extra CO_2 for growth	+	+	–	–	–	–	–

to 37° C (95° to 98.6° F) and a pH of 6.5 to 7.5. Gonococci do not survive below pH 6.0, grow poorly below 30° C (86° F), and do not survive above 40° C (104° F).

Neisseria spp. require enriched media, including free iron, to support their growth. Multiple colony types are evident when a single isolate is grown on clear agar. Small convex glistening colonies are piliated, whereas larger, flatter colonies are nonpiliated. In vitro passage usually results in loss of pili. Under low-power microscopy, some colonies appear opaque or granular and others are transparent. The former represent colonies in which most cells express one or more opacity-associated proteins (Opa), whereas in the latter colonies, most cells do not express these proteins.*

Culture from Clinical Specimens

The organisms are cultured best on chocolate blood agar in an atmosphere enriched by carbon dioxide. If the clinical specimen has been obtained from a highly contaminated site (e.g., rectum or cervix), a selective medium containing nystatin, vancomycin, trimethoprim, and colistin (e.g., modified Thayer-Martin medium) to suppress contaminating flora allows the growth of most *Neisseria* spp. A few strains of gonococci are inhibited by vancomycin (see later).[38,237] Chocolate agar without antimicrobial agents is preferred if the culture is taken from a usually sterile area such as blood, cerebrospinal fluid (CSF), synovial fluid, or a skin lesion. Gonococcal colonies usually are evident on agar plates within 24 to 48 hours after inoculation. Frequent propagation is necessary to maintain isolates because viability is lost rapidly after 48 hours of growth.

Because gonococci cannot tolerate drying, clinical specimens must be plated as soon as possible onto appropriate media. Transport bottles that contain medium and a carbon dioxide–enriched atmosphere should be used if definitive processing of the specimen must be delayed. Transport bottles should be maintained upright to preserve the carbon dioxide atmosphere.

*References 38, 43, 199, 323, 334, 335, 349.

In addition to sugar fermentation patterns (see Table 89-1) and oxidase positivity, other biochemical reactions can be used to differentiate between *Neisseria* spp. or confirm that an isolate is *N. gonorrhoeae*.[71] Enzyme substrate tests that identify the presence or absence of 1-hydroxyprolylaminopeptidase, γ-glutamyl aminotransferase, and β-galactosidase are available as an adjunctive means of confirmation.[99] Gonococci possess only the former. These reactions alone will not distinguish *N. gonorrhoeae* from several commensal *Neisseria* spp. Antigen-detection–based tests, including fluorescent antibody staining, also have been used to confirm the identity of an isolate as *N. gonorrhoeae*.[200] Tests based on detection of gonococcal nucleic acid sequences, which are being used increasingly for diagnostic purposes, can be used to confirm the identity of gonococcal isolates from cultures (see later discussion of diagnostic testing and medicolegal issues).

Genetic Characteristics

Gonococci have a circular chromosome consisting of 2219 kilobases (kb), which is about half the size of the *Escherichia coli* genome.[110] The gonococcal genome has approximately 2250 predicted open-reading frames.[36] The entire genomes of *N. gonorrhoeae* strains FA1090, NCCP11945, and ST1407 have been sequenced. A macrorestriction map with the positions of many genetic markers has been available since 1991.[82,167,269] More than 1300 open-reading frame sequences have been validated.[36]

Piliated gonococcal cells (the natural in vivo state) are competent for genetic transformation by exogenous DNA at all stages of growth.[26] Only homologous DNA is taken into the cell.[117] Gonococci are highly autolytic and release DNA in a biologically active form. Thus, different strains are able to exchange genetic material readily. Such exchange can lead to further genetic and phenotypic diversity, which helps maintain the species in its human hosts and facilitates transfer of chromosomal antibiotic resistance genes.[304]

A 36-kb conjugal plasmid is present in many gonococci. It efficiently mobilizes its own transfer and other

non–self-mobilizable plasmids (e.g., the 4.5- and 7.5-kb penicillinase plasmids), but not chromosomal genes.[254,266,292] Extrachromosomal, nonplasmid DNA circles recently have been identified in wild-type gonococcal isolates. They may play a role in gene recombination, amplification of chromosomal genes, and transformation.[18] Gonococci possess multiple restriction endonucleases and their corresponding DNA methylases.[333] The species is relatively nonmutagenic and lacks photoreactivation and error-prone repair systems.[48]

Strain Typing

The ability to differentiate one strain from another allows researchers to investigate the epidemiology of transmission of gonorrhea and assessment of virulence factors. Numerous methods have been applied to gonococci.

Auxotyping classifies strains according to their ability to grow or not grow in the absence of 11 specific compounds, including amino acids (e.g., arginine and proline), purines, pyrimidines (e.g., hypoxanthine and uracil), and other nutrients (e.g., thiamine). Approximately 20 auxotype phenotypes are recognized. Genetic studies have shown that multiple mutations in the same biochemical pathway can lead to the same phenotype, such that a single auxotype can represent many genotypes.[311] Auxotypes are stable in vitro, however, and organisms cultured from sexual partners are of a similar auxotype.[51,55] The auxotype of greatest epidemiologic importance is designated arginine-, hypoxanthine-, and uracil-negative (AHU^-). Such strains are unable to grow in the absence of these compounds. AHU^- strains typically are more resistant to killing by normal human serum, are more likely to cause asymptomatic infections in males, and are found more frequently in patients with disseminated infection.[121,201] AHU^- strains often are susceptible to vancomycin.[237]

Numerous serotyping schemes have been attempted,[72] but the best and most widely available serologic technique is based on antigenic heterogeneity of the porin protein (formerly called protein I) contained in the outer membrane of the gonococcus.[10,189,202,301,302] Two immunochemically distinct serogroups, PorA and PorB, exist and can be subdivided into serovars by using sets of monoclonal antibodies. Serovars remain stable in vitro and are designated IA-x or IB-x, where *x* is the numeral of the serovar. At least 26 IA and 31 IB serotypes have been identified.[63] Serotyping can be combined with auxotyping to further discriminate among gonococcal strains.[176,274] Limitations of auxotype-serovar classification include restricted supplies of reagents and batch-to-batch variation in monoclonal antibodies. In addition, common serovars can have such significant genetic diversity that this typing method may not provide sufficient discrimination for some epidemiologic evaluations.

Genotyping methods have been applied to *N. gonorrhoeae* in the past 2 decades.[219] Simple restriction endonuclease methods with rare cutting enzymes[126,275] have been supplanted by polymerase chain reaction (PCR)-based methods and pulsed-field gel electrophoresis (PFGE). Opa typing, which uses PCR primers to generate DNA bands from each of the 11 *opa* genes, followed by restriction enzyme digestion, provides discrimination that is similar to that of PFGE.[213] Sequencing of the *porB* gene also appears to be highly discriminatory among strains.[87] PCR amplification of the *porB* gene with restriction enzyme digestion provides discrimination similar to that of combined auxotyping-serotyping.[114] Repetitive-element sequence-based PCR (rep-PCR) correlates well with PFGE for analysis of gonococcal strains, is rapid, and can be performed directly from colonies without DNA extraction.[274,359] *N. gonorrhoeae* multiantigen sequence typing is based on internal variation of two highly polymorphic loci and has been evaluated for cluster identification, tracing sexual contacts, and investigating treatment failures.[340] Multilocus, variable-number tandem repeat analysis also has been developed for *N. gonorrhoeae*.[168] These methods appear most useful for local microepidemiologic purposes.

Multilocus sequence typing is the primary typing method used now for macroepidemiologic analyses such as monitoring global spread of clones or clonal complexes with particular virulence traits or antimicrobial resistance patterns. At least two seven-loci–based schemes have been developed.[340]

PATHOGENESIS

A continuously expanding body of literature is devoted to the pathogenesis of *N. gonorrhoeae*.* *N. gonorrhoeae* is well adapted to its human host and is able to co-opt components of the innate immune system for its survival. Species specificity is demonstrated by greater adherence of gonococci to human cells than to nonhuman cells. Close interactions of *N. gonorrhoeae* with mucosal cells, erythrocytes, spermatozoa, and polymorphonuclear cells are well described.

Gonococci are able to survive in the urethra in the face of hydrodynamic forces that tend to wash other microbes away and persist there despite close proximity and even attachment to the hordes of neutrophils that respond to their presence. These observations indicate an ability to adhere to mucosal epithelia and evade the host acute innate immune response. Individuals also can have repeated infections by the same gonococcal strain, suggesting that the organism can thwart establishment of local adaptive immune responses. The tissue damage that occurs in the fallopian tubes during salpingitis implies that one or more directly toxic moieties or factors can trigger a deleterious host response.[129,322]

In the male human model of experimental infection, gonococci are recovered in urine at low levels for about 2 hours after urethral inoculation. The microbes then disappear from the urine for variable periods before becoming shed in increasing numbers. The bacteriuria persists until signs and symptoms of dysuria and urethritis appear after an incubation period of 1 to 6 days after inoculation. Severity of symptoms does not correlate with the shed microbial burden. Pyuria and detectable urinary concentrations of interleukins (IL)-6 and IL-8 usually appear within 24 hours after inoculation.

*References 93, 98, 119, 127, 170, 218, 280.

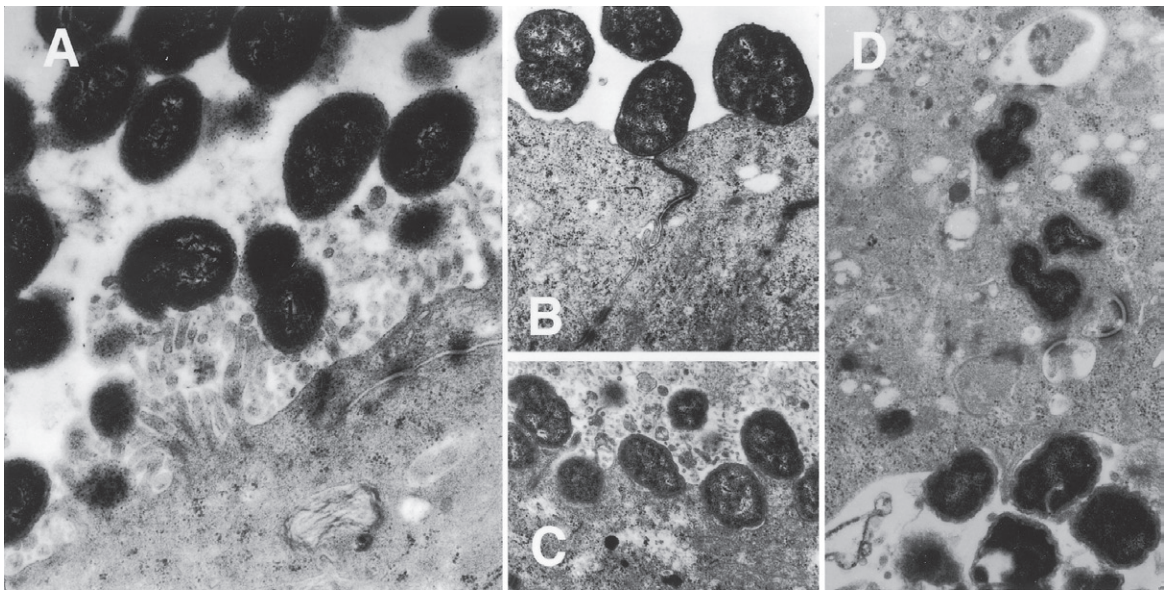

FIGURE 89-4 ■ Electron micrographs of *Neisseria gonorrhoeae* (strain MS11) interactions with polarized T84 human epithelial cell monolayers. At early stages of infection, the microbes adhere to the apical plasma membrane as microcolonies. Adherent bacteria are surrounded by a matrix of microvilli **(A)**. Bacteria subsequently disperse from the microcolony and adhere as a monolayer in which bacterial and host-cell membranes are tightly apposed **(B** and **C)**. The region of contact between the bacteria and the host-cell membrane enlarges **(C),** with subsequent internalization of the microbes **(D)**. Bacteria then traverse the host cell and exit via the basolateral membrane **(D).** (Photographs by Magdalene So, from the Annual Review of Cell and Developmental Biology, Volume 16. © 2000 by Annual Reviews. http://www.AnnualReviews.org.)

Phenotypic variation of gonococcal surface determinants is seen during the course of experimental infection.[170]

This human model and multiple in vitro molecular studies involving cell lines and fallopian tube organ cultures have provided many insights into the molecular pathogenesis of gonococcal infection.[84,165,227,228,285]

Resistance to Phagocytosis and Intracellular Killing

Gonococci are readily able to resist extracellular killing by neutrophils through mechanisms that are not completely understood. The microbes also resist phagocytosis via blocking deposition of opsonic antibodies and complement components on their own surfaces and by antigenic variation of their surface structures to evade any preexisting humoral immune responses. Antibody binding is inhibited by sialylation of surface lipo-oligosaccharide (LOS). Gonococci thwart the effects of complement via binding of the complement cascade inhibitor complement factor H to porin proteins and LOS.[98]

N. gonorrhoeae have multiple mechanisms by which they resist oxidative and nonoxidative killing after phagocytosis by neutrophils. The species produces catalase, cytochrome *c* peroxidase, superoxide dismutase, and a metalloproteinase that detoxify superoxide compounds produced in phagolysosomes. Gonococci also have repair systems for oxidatively damaged DNA. Nonoxidative antibacterial factors such as lysozyme, elastase, cathepsin G, defensins, and cathelicidins can be counteracted by gonococcal efflux pump systems, metalloproteinase, and other protein and regulatory systems.[98]

Invasion of Mucosal Epithelial Cells

In addition to the ability to resist killing by neutrophils, gonococci are able to persist in human hosts via their ability to invade epithelial cells. The initial steps of intracellular invasion involve attachment of microbes to epithelial cell surfaces through synergistic effects of pili, Opa proteins, and porin proteins.[19,98,151] Gonococci also exploit the production of complement factors, including C3, by cervical epithelial cells. In females, especially during the luteal phase and menses, C3 is secreted into the extracellular milieu, where it is activated to C3b. The gonococcal surface binds C3b and cleaves it to iC3b (inactive), which then serves as a ligand for the complement receptor 3 molecules on host cells that facilitates microbial attachment independently of pili and Opa proteins (see later).[119]

After adhering to host epithelial cells, some microbes are internalized by endocytosis triggered by interaction of gonococcal phospholipase D with host cell Akt (protein kinase B).[119] The vast majority of invading gonococci are ingested and killed by neutrophils. Gonococci are susceptible to the oxidative burst products of phagocytes, as well as to nonoxidative products such as cathepsin G.[52,308] Those that survive intracellularly may replicate inside the phagosomes and ultimately exit the basal (but not lateral) surface of the cell via exocytosis[228,232,250] (Fig. 89-4). Adjacent nonepithelial cells are sloughed, probably because of the toxicity of LOS and peptidoglycans.[149] Mucosal cell damage and submucosal invasion are followed by an influx of neutrophils along with the formation of submucosal microabscesses and exudation of purulent material into the lumen of the infected organ.[357]

Adherence of gonococci to mucosal epithelial cells causes activation of nuclear factor-κB (NFκB) and activator protein 1, which in turn leads to upregulation and release of multiple cytokines and chemokines, including macrophage colony-stimulating factor, tumor necrosis factor-α (TNF-α), tumor growth factor-β (TGF-β), monocyte chemoattractant protein 1, IL-1β, IL-6, and IL-8.[251,279]

N. gonorrhoeae can form biofilms during cervical infection. Membrane blebs that contain DNA elements and outer membrane structures are key components of these biofilms. A gonococcal thermonuclease is involved in remodeling of biofilm matrix. Cells in the biofilm activate gene sets involved in anaerobic respiration.[127]

Other specific microbial virulence factors are discussed in the following sections.

Virulence Factors

Specific *N. gonorrhoeae* virulence factors include (1) pili, (2) opacity proteins, (3) porin protein, (4) the ability to survive in low iron environments, (5) immunoglobulin (Ig)A protease, (6) LOS, (7) cell wall peptidoglycan, (8) reduction modifiable protein (Rmp), and (9) a type IV secretion system. Many other gonococcal gene products and molecular systems have been identified, and a more detailed and complex understanding of virulence at the molecular level is steadily forthcoming.

Pili

Gonococci are piliated predominantly in vivo and express type IV pili. Pili cover essentially the entire surface of the cell and are arranged in individual fibrils or fibrillar aggregates. Pili consist primarily of polymers of an 18-kd subunit now denoted as PilE. A single pilus is approximately 6 nm in diameter and up to several microns in length.[232] Each may contain thousands of PilE subunits.[44] The PilC protein is present in lower numbers in the pilus but appears to be the key pilus adhesin. PilC interacts with CD46 molecules, which are present on most human cells and serve as receptors for C3b, C4b, measles, and other viruses. PilC-CD46 attachment appears to be a key step in the initiation of microbial adherence to host cells.[46,195,204,296,298]

Binding of pili to a host-cell membrane induces at least two responses in the host cell: release of Ca^{2+} from intracellular stores[194] and cytoskeletal rearrangements with the formation of cortical plaques that represent an accumulation of actin, ezrin, and other phosphotyrosine-containing proteins.[233,283] The latter results in elongation of the microvilli that embrace the microbe. Formation of plaque appears to be induced by retraction of the attached pilus, and retraction depends on another pilus protein designated PilT.[234]

Gonococcal pili undergo both phase variation (switching from piliated to nonpiliated states) and high-frequency antigenic variation.[45] These variations occur during natural infection and in vitro passage.[306] Pili have a common N-terminal domain, semi-variable domains in the midportion, and two hypervariable regions in the carboxyl-terminal of the pilin protein subunit.[324] Relatively invariant regions occur between the variable domains. The pilin protein consists of 159 to 160 amino acids. A disulfide bridge is formed by cysteine residues at amino acids 121 and 151.

The genetic mechanism of pilus antigenic variation has been studied extensively. The chromosome contains either one or two complete pilin genes. The expressed gene contains an intact promoter region, a ribosome binding site, and a seven–amino acid signal sequence in addition to the pilin sequence. Scattered around the chromosome are six to eight loci that contain varying portions of the pilin sequence without the promoter region or the 5′ end of the structural gene. Some loci may contain many incomplete pilin sequences arranged head to tail that differ slightly from one another. Movement of these sequences into the active expression site through nonreciprocal combination events results in antigenic variation of the pilus protein. If recombination leads to a faulty pilin subunit product that cannot be processed into a mature pilus, the progeny of that organism become nonpiliated (phase variation). Subsequent recombination events can allow reversion back to the piliated phase.*

In addition to promoting adhesion to epithelial cells, pili also provide a twitching motility, are involved in DNA transformation, and may play a role in resistance to ingestion by phagocytic cells.[232] The presence of pili, perhaps mediated through their promotion of adhesion, affects the activation of $CD4^+$ T-lymphocytes and the production of IL-10. This property may help gonococci diminish the T-cell response to their presence.[271]

Opacity-Associated Proteins

Opa proteins also influence adherence of the gonococcus to host cells. The isolation of particular Opa protein phenotypes from different anatomic sites or at different times during the menstrual cycle has suggested that these proteins contribute to the ability of the organism to succeed in a given niche. Opa proteins (formerly designated protein II) are a set of as many as 12 related proteins that are variably present in gonococcal outer membranes. Opa proteins are 24 to 28 kDa in size and confer increased opacity to colonies of organisms by promoting adherence of the organisms to one another. A given strain has the capacity to make at least 10 different Opa proteins but appears to express no more than 5 at the same time. Some cells express no Opa proteins. Like pili, Opa proteins undergo both antigenic and phase variation. Both types of variations can occur within a single colony, so sector variations in opacity can be seen.[28,322,334,335]

Each *opa* gene is present as a complete gene with its own promoter and is transcribed at all times. Phase variation in expression occurs at the translational level. Each gene has a varying number of pentameric CTCTT repeat units adjacent to the ATG start codon. When the number of repeats is divisible by three, the transcribed mRNA is

*References 158, 235, 236, 256, 305, 324, 336.

translationally in frame and the Opa protein is expressed. The number of pentameric repeats is subject to high-frequency variation. Antigenic variation results from recombination between the two hypervariable regions in each of the *opa* genes.[88,325-337]

Two classes of host-cell receptors for Opa proteins have been identified. Heparan sulfate–proteoglycan (HSPG) receptors are present on epithelial cells and interact with one particular Opa protein variant (Opa_{50}). HSPG binding can stimulate the lipid hydrolysis enzymes phosphatidylcholine-specific phospholipase C and acid sphingomyelinase and thereby lead to clathrin-independent endocytosis. HSPG-Opa binding also can lead to interactions with serum factors such as vitronectin and fibronectin, which then mediate endocytosis via host-cell integrin receptors. The CD66 family is present on epithelial cells and neutrophils, recognizes many different Opa proteins, and mediates nonopsonic phagocytosis, a process distinct from antibody- and complement-mediated phagocytosis.*

Porin Protein

Formerly designated protein I, porin protein is the most common gonococcal outer-membrane protein. Porin is 34 to 36 kDa, is exposed on the surface, exists as a trimer in the membrane, and is physically proximate to LOS and Rmp.[29,169] The trimer forms an anion-specific pore in the bacterial membrane that allows small water-soluble molecules to enter the cell.[360] The *por* locus has two alleles that encode chemically and immunologically distinct classes of porin protein—PorA and PorB—that are similar to porin proteins in other gram-negative bacteria. A given strain expresses only PorA or PorB, and many antigenic variants of both exist (thereby forming the basis for serotyping).[50,202]

The porin trimer appears to translocate into the host-cell membrane and is able to disrupt neutrophil degranulation, the oxidative burst, and phagosome maturation,[159,220,242] in addition to induction of apoptosis in epithelial cells and neutrophils in vitro.[246] Exposure to gonococcal porin protein induces the typical structural and biochemical changes seen in apoptotic cells.[245] Porin translocation permits rapid influx of Ca^{2+} into the host cell from the external environment.[246] Porin proteins also may play a role in endocytosis mediated by the binding of HSPG and Opa protein[19] and in downregulation of complement by binding with C4b-binding protein.[278]

Iron Metabolism

Iron is an essential nutrient for *N. gonorrhoeae*, and, in host tissues, iron is sequestered in hemin compounds as ferritin or is bound to lactoferrin or transferrin. The species does not produce any siderophores but relies on an iron-repressible system that scavenges iron from transferrin, lactoferrin, and hemoglobin. This system comprises numerous proteins, some of which serve as receptors for the aforementioned iron-bearing ligands.[24,76,92,351] Other components include a TonB-dependent transporter and an associated lipoprotein that are expressed ubiquitously by gonococci, have limited antigenic variability, and are important for growth in vivo.[93] These proteins thus have potential as vaccine targets.

Immunoglobulin A Protease

All gonococci (and meningococci, but not nonpathogenic *Neisseria* spp.) make a protease that cleaves both serum and secretory IgA1 (but not IgA2) at the hinge region with the release of Fab and Fc fragments. This protease may help the organism evade host IgA at the mucosal surface, especially early in secondary infections, when preexisting antibodies may be present. However, mutant strains without the IgA protease have limited ability to grow inside epithelial cells. The protease appears to cleave a host-cell intracellular protein (LAMP1) involved in phagosome compartmentalization.[215] IgA1 protease is not required for gonococci to cause experimental urethritis,[188] thus suggesting that its intraphagosome function may be more important in pathogenesis.

Lipo-Oligosaccharide

Gonococci express LOS complexes of 3 to 7 kDa on their cell surface. LOS consists of a lipid A moiety and a core polysaccharide comprising ketodeoxyoctanoic acid, heptose, glucose, galactose, glucosamine, galactosamine, or any combination of these constituents.[153] The lack of a long polymeric sugar attached to the core distinguishes LOS from the lipopolysaccharides of other gram-negative bacteria. The core sugar antigens of LOS are subject to intrastrain and interstrain variation, and a single strain may express as many as six variants of LOS.[108,152] Numerous genes are involved in LOS synthesis; these genes undergo high-frequency phase variation similar to *opa* genes.[146] LOS terminal sugars mimic the structure of certain human glycosphingolipids.[153]

Gonococci with predominately short LOS molecules appear to be more sensitive to killing by human serum but also more able to invade eukaryotic cells. Strains with longer LOS are more serum-resistant but noninvasive.[347] Longer LOS moieties are sialylated readily with host neuraminic acid by a bacterial sialyltransferase that appears to shield both LOS and porin molecules from antibody binding, thus providing protection from complement-mediated killing in serum.[34,352]

LOS from serum-sensitive strains is able to activate the classic complement pathway and may do so in the absence of antibody.[309] LOS induces the production of cytokines (i.e., TNF-α, IL-1β, IL-6, and IL-8) by urethral epithelial cells[162] and can mediate host defensin-enhanced adherence to epithelial cells.[145]

Cell Wall Peptidoglycan and Lytic Transglycosylases

Gonococci shed membrane fragments with peptidoglycan into their environment during exponential growth. Peptidoglycan monomers have numerous biologic

*References 105, 106, 147, 207, 231, 232, 346.

properties, including activation of complement and modulation of mononuclear cell proliferation. These fragments also damage fallopian tube mucosa in organ culture, thus suggesting a pathogenic role for the compounds in invasive disease.[149,228] Production of peptidoglycan monomers is a by-product of lytic transglycosylases that act during cell growth and division. At least five such enzymes are encoded by *N. gonorrhoeae*. These also may facilitate gene transfers as part of type IV secretion.[74]

Reduction Modifiable Protein

Rmp (formerly designated protein III) is an antigenically conserved, 30- to 31-kDa protein that is present in all pathogenic *Neisseria*. Rmp is located proximate to LOS and porin in the outer membrane. Antibodies that bind to Rmp epitopes block the bactericidal effect of the complement-fixing IgM antibodies that recognize LOS. Women with preexisting anti-Rmp antibodies appear to be more susceptible to the development of infection than do those without such antibodies.[287,288,322]

Ribosomal Protein L12

Ribosomal protein L12 mimics the structure of human chorionic gonadotropin, the natural ligand for lutropin receptors. It is able to bind to lutropin receptors in the upper female genital tract, which may facilitate ascending infection in females.[118,326]

Type IV Secretion System

About 80 percent of *N. gonorrhoeae* strains encode a horizontally acquired 57-kb genetic island that encodes a type IV secretion system (T4SS). A T4SS is a multiprotein complex that functions as a conjugation system. In gonococci the T4SS secretes single-stranded chromosomal DNA into the extracellular environment. T4SS expression may enhance microbial survival inside human cells in addition to its role in genetic recombination and natural transformation among gonococcal strains.[280]

Characteristics of Strains Causing Disseminated Disease

The increased virulence of some strains is suggested by observations such as a microepidemic of gonorrhea involving one asymptomatic infected male and eight female contacts.[161] Seven of the women were infected symptomatically, and four experienced disseminated infection. Overall, many infections are asymptomatic and dissemination is relatively rare.

Strains of *N. gonorrhoeae* obtained from adult patients with disseminated disease usually are less susceptible to the bactericidal activity of sera[39,83,285,286,310] (see earlier discussion of LOS). These strains have an atypical growth pattern on agar[238] that reflects the absence of Opa protein expression, which suggests that the genetic variation capacity described earlier allows adaptation to different niches and probably helps organisms elude the host response.[23] Unlike most gonococcal strains, many invasive strains (designated AHU$^-$) require arginine, uracil, and hypoxanthine for growth.[199] A high degree of sensitivity to penicillin also has been characteristic of most strains from patients with disseminated disease.[356] Many also are susceptible to vancomycin, which may prevent detection in selective media. These strains should be detected in the usual media used for blood culture.

Host Response

The adult response to local and systemic infection with *N. gonorrhoeae* has been investigated extensively, but no such studies have been performed on infected children. Gonococcal infections usually are characterized by intense inflammation that involves neutrophilic and mononuclear granulocytes. The vast majority of gonococcal cells ingested by neutrophils are killed.[52] Gonococci are susceptible to the oxidative products produced by neutrophils but also can be killed efficiently by nonoxidative products such as cathepsin G.[308]

An intact complement system is essential for successful eradication of the organism. Properdin-mediated recruitment of C4b-binding protein facilitates antibody-mediated complement-dependent killing of gonococci that resist destruction via the classic complement pathway.[155] Persons with inherited or acquired complement deficiencies may be predisposed to contracting disseminated gonococcal infection, as is the case with meningococcal infection. Approximately 13 percent of patients with disseminated disease have complement deficiencies.[123,259]

Adherence of gonococci in vitro induces the activation of NFκB and activator protein 1, which leads to upregulation of messenger RNA (mRNA) and release of numerous cytokines and chemokines: macrophage colony-stimulating factor, TNF-α, TNF-β, monocyte chemoattractant protein 1, IL-1β, IL-6, and IL-8.[251] Intraurethral challenge leads to increased levels of IL-8, IL-6, and TNF-α in urine before the onset of symptoms and in plasma at the onset of symptoms.[279]

Multiple episodes of gonorrhea may occur in a single individual in a short period and even may be caused by the same strain. The antigenic diversity and ease of altering the antigenicity of both pili and opacity proteins undoubtedly contribute to the insufficiency of the host response in preventing reinfection. Noninvasive mucosal infections elicit production of low-affinity, broadly reactive IgM antibody by IgD$^+$, CD27$^+$ B cells in a T cell–independent manner that may partially explain the limited humoral response to typical infections.[321] In a mouse model of vaginal infection, gonococci induce Th17 inflammatory responses, which drive neutrophil influx. Th1- and Th2-adaptive specific antibody responses are suppressed by increased production of TGF-β plus enhanced development of T-regulatory cells. TGF-β production is mediated by interaction of Opa proteins with carcinoembryonic antigen related–cellular adhesion (CEACAM) molecules on human cells.[218]

Circulating humoral antibody to the infecting strain is measured by an assay of bactericidal antibody and is present in most persons who have prolonged mucosal colonization with *N. gonorrhoeae*.[182,197] In addition, women in whom pelvic inflammatory disease (PID) develops

usually produce bactericidal antibody during the infection. IgA- and IgG-blocking antibodies against some gonococcal antigens appear to block killing of the bacteria mediated by otherwise bactericidal IgG and IgM antibodies (see earlier discussion of Rmp).[285] Serum antibody responses are greater in patients with invasive disease (e.g., bacteremia or salpingitis).

Secretory IgA antibody is present in the urethral exudate of men with gonorrhea and in the genital secretions of women with gonorrhea.[260] The development of local IgA antibody occurs more rapidly and is more transient than development of the serum bactericidal response. Microbial IgA1 protease is not required for infection but may play a role in reinfection.

A cellular immune response to gonococcal antigens in patients with uncomplicated gonorrhea has been demonstrated in vitro.[210] The significance of this response in controlling or preventing infection is unknown.

PERINATAL GONOCOCCAL INFECTIONS

Since the 1970s, health clinics have encouraged the routine screening of sexually active women for gonorrhea. Such screening is especially important during pregnancy. Adolescent girls have a higher prevalence of gonorrhea than do older women of child-bearing age. This probably translates into higher rates of gonorrhea in pregnant adolescents. Rates of gonococcal infection in neonates reflect the frequency of infection in pregnant women. Recognition of gonorrhea early in pregnancy identifies a population at risk who should be monitored sequentially for reinfection throughout pregnancy.[190]

The spectrum of infection with *N. gonorrhoeae* appears to be similar in pregnant and nonpregnant women. Most are asymptomatic. Pharyngeal infection seems to occur more commonly during pregnancy, perhaps reflecting altered sexual practices. One study reported that 39 percent of patients with *N. gonorrhoeae* at any site had concurrent involvement of the pharynx and that 30 percent had pharyngeal infection as the sole manifestation.[91] In adolescent girls, pregnancy and menstruation are associated with disseminated disease.[171]

Gonorrheal infection puts both the mother and infant at risk for the development of other forms of gonococcal disease. Pregnant women have an increased risk for the development of gonococcal septic arthritis, and most such cases occur during the third trimester or in the immediate postpartum period.[41,171] Although the incidence of gonococcal arthritis is low, many cases have occurred in pregnant adolescents.

Gonococcal PID and acute salpingitis also may complicate the pregnancy of an infected woman.[137] These complications usually occur during the first trimester and have been associated with a high rate of fetal loss. An increased incidence of postpartum fever in women with untreated gonorrhea likewise has been noted. A new mother with complications of peripartum gonorrhea may have difficulty providing care for her child.[185]

Hazards to the fetus posed by maternal gonorrhea include septic abortion, perinatal death, prematurity, perinatal distress, and premature rupture of membranes. Prolonged rupture of membranes, premature delivery, chorioamnionitis, funisitis, and a clinical diagnosis of sepsis occur frequently in infants with *N. gonorrhoeae* detected in the gastric aspirate during delivery.[7,120,162,293] Table 89-2 tabulates the proportions in six studies of infants born to infected mothers who experienced these problems. A controlled study conducted in an area with a high prevalence of gonorrhea found that maternal infection with *N. gonorrhoeae* was associated significantly with preterm birth and that 14 percent of preterm births in this population were attributable to gonococcal infection.[122]

Gonococcal Ophthalmia Neonatorum

Epidemiology

The increased risk for ophthalmia neonatorum among infants born to women with vaginal discharge has been recognized for centuries. Recommendations for flushing the eyes of a newborn commonly were given before the 20th century.[230] In the late 1800s, Neisser helped establish the relationship between the gonococcus and neonatal ophthalmia.

TABLE 89-2 **Pregnancy Complications and Outcomes of Mothers Who Were Infected with *Neisseria Gonorrhoeae* at Delivery**

Pregnancy Complications and Outcomes	Charles et al[75] (*N* = 14)* (%)	Sarrel and Pruett[303] (*N* = 37) (%)	Israel et al[185] (*N* = 39) (%)	Amstey and Steadman[7] (*N* = 222)* (%)	Edwards et al[120] (*N* = 19)* (%)	Handsfield and Holmes[161] (*N* = 12)* (%)
Normal or term infant	—	13 (35)	30 (77)	142 (64)	7 (37)	—
Aborted	—	13 (35)	1 (2)	24 (11)	—	—
Perinatal death	—	3 (8)	1 (2)	15 (7)	2 (11)	—
Premature	—	6 (16)	5 (13)	49 (22)	8 (42)	8 (67)
Perinatal distress	—	—	2 (5)	—	2 (11)	—
Premature rupture of membranes	6 (43)	8 (22)	—	52 (23)	12 (63)	9 (75)

*Data were provided in which the outcomes of pregnancies of mothers not infected with *N. gonorrhoeae* were shown to be significantly more favorable.

By 1881, Dr. Carl Sigmund Franz Credé recognized that asymptomatic disease in the mother was a potential source of infection.[97] Gonorrhea was highly prevalent in Europe, and Credé described an increased incidence in patients from lower socioeconomic backgrounds. Mechanical cleansing of the birth canal failed to protect the infant from gonococcal ophthalmia neonatorum (GON), which was occurring in approximately 10 percent of newborns in major cities. The infection was the cause of a large proportion of admissions to schools for the blind.[17] Credé described the technique of instillation of 2 percent silver nitrate into the infant's conjunctival sac, which remains one method recommended for preventive procedures today.

By 1930, most states in the United States required that all newborns receive silver nitrate prophylaxis (i.e., the Credé procedure). At present, prophylaxis of some form is required by most states. GON decreased as a cause of admission to schools for the blind in the United States, from an average of 24 percent from 1906 to 1911 to 0.5 percent from 1951 to 1955.

The worldwide decline in adult gonorrhea in the 1950s probably contributed to the decreased recognition of disease in newborns during that period. The incidence of GON rose in the 1960s and 1970s along with the increased incidence of gonorrhea in the general population and was very high in some developing nations.[207] In Los Angeles, California, the rate rose from 9/100,000 live births in 1957 to 1958 to 56/100,000 live births in 1962 to 1963. In a New York hospital between 1970 and 1973, a rate of 145/100,000 births was reported; in a hospital in North Carolina during 1969 and 1970, a rate of 265/100,000 live births was proved by culture.[320] In the late 1970s and 1980s, GON again subsided in developed countries but has remained a problem in underdeveloped nations.

Factors associated with higher rates of GON include lower socioeconomic class of the mother and longer duration of rupture of membranes before delivery.[11] An increased incidence of GON in infants of unwed mothers and mothers who have not had prenatal care also may exist.[317] Previous treatment of gonorrhea during pregnancy also is associated with an increased incidence of GON.[190] The incidence of prematurity in infants with GON often is reported to be higher. Whether this is due to an adverse effect of gonorrhea on the pregnancy or because GON may be recognized more readily in premature infants has not been established. Premature rupture of membranes appears to increase the risk for GON.

Prevention

In the United States, prophylaxis for GON is recommended for all infants immediately after birth and is required by law in most states. This is considered a grade A recommendation by the U.S. Preventive Services Task Force.[343] Prophylaxis regimens using a 1 percent solution of silver nitrate, 1 percent tetracycline ointment, or 0.5 percent erythromycin ophthalmic ointment are equally effective. In the United States, erythromycin 0.5 percent ophthalmic ointment is the only one of these agents available for prophylaxis of GON. Single-dose units are preferred over multidose tubes. One unit should be instilled into the eyes of every neonate as soon as possible after delivery. Instillation may be delayed for as long as 1 hour to facilitate parent-infant bonding.

During a shortage of erythromycin ointment in 2009, azithromycin and gentamicin ophthalmic preparations were recommended. In one institution, 4/662 (0.6 percent) of newborns treated with gentamicin 0.3 percent ophthalmic ointment developed reactions with eye discharge, eyelid erythema, blistering, and edema within 48 hours of administration.[249] Povidone-iodine 2.5 percent solution may be effective for prophylaxis of GON but is not approved for this use in the United States at this time.[343] In randomized trial versus 1 percent tetracycline ointment, povidone-iodine 2.5 percent solution was less effective overall in preventing ophthalmia neonatorum of any type and more often was associated with chemical conjunctivitis.[101] Bacitracin is not effective for prevention of GON.[148]

The efficacies of tetracycline and erythromycin preparations in the prevention of tetracycline-resistant *N. gonorrhoeae* ophthalmia are unknown.[261] Both probably are effective because of the high concentration of drug in these preparations. Studies of these agents for prevention of *Chlamydia trachomatis* ophthalmia have been conflicting. Povidone-iodine may be more effective in preventing *C. trachomatis* ophthalmia than either topical silver nitrate or erythromycin. None of these agents prevents chlamydial colonization of the nasopharynx.

Silver Nitrate and Gonococcal Ophthalmia Neonatorum

Numerous studies have compared the efficacy of silver nitrate and no prophylaxis or prophylaxis with another agent in the prevention of GON.[77,240,294] Such studies, though not randomized, involved large numbers of infants and consistently showed lower rates of GON in treated versus untreated infants. Decreased frequency of blindness from GON in the population during the last half century, even during periods of increased rates of gonorrhea in women of child-bearing age, further supports the benefits of silver nitrate prophylaxis.

In a randomized trial of Kenyan infants born to mothers with gonorrhea reported in 1988, rates of GON were 42 percent in the control group, 7 percent in the group treated with 1 percent silver nitrate (83% reduction), and 3 percent in the group treated with 1 percent tetracycline (93% reduction). The two treatment arms were not statistically different.[209] In a randomized trial in the United States involving infants born to women without gonococcal infection, infants treated with 1 percent silver nitrate had a 39 percent lower rate (statistically significant) of conjunctivitis of any type in the first 2 months of life than did infants who received no prophylaxis. Infants treated with 0.5 percent erythromycin ointment in this trial had a 31 percent reduction in contrast to control infants, but this result was not statistically significant.[20]

The major advantages of the use of silver nitrate for prophylaxis are the lack of allergic potential, the absence of development of bacterial resistance to the compound, and very low cost. Two outbreaks of erythromycin-resistant staphylococcal conjunctivitis in newborn nurseries have been associated with the use of erythromycin eye ointment as ocular prophylaxis, but the outbreaks remitted when silver nitrate was substituted. Disadvantages include the occurrence of conjunctival irritation with associated development of exudate in many neonates. A solution of silver nitrate that becomes concentrated may cause ophthalmic injury, but this problem largely has been alleviated by dispensing doses in ampules, which prevents evaporation.

Failure of silver nitrate prophylaxis (as well as other agents for prevention of GON) does occur. Improper administration of silver nitrate such that the solution does not reach the conjunctival sac, irrigation performed too quickly after instillation, and inadvertent omission of prophylaxis can lead to apparent failure. When gonococcal infection of the eye has become established before birth, silver nitrate is not expected to prevent further progression. The increased risk for GON associated with premature rupture of membranes is due to exposure to and establishment of infection before actual delivery. In some cases of premature rupture of membranes, clinically apparent GON can be present at the time of birth.

Despite these problems, the use of 1 percent silver nitrate remains a widely accepted, carefully evaluated, and safe form of GON prophylaxis. The occasional failure of silver nitrate prophylaxis emphasizes that it is preferable to prevent GON through identification and treatment of pregnant women.

Clinical Features

The newborn eye is subject to colonization with numerous bacterial organisms that cause infections that usually are clinically mild, nonprogressive, and characterized by conjunctival discharge. The bacteria most commonly associated with conjunctival discharge are *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Neisseria cinerea*, *Klebsiella pneumoniae*, enterococci, and *C. trachomatis*.[113,206] Viral causes include herpes simplex virus and adenoviruses. In contrast, infection of the eye of a newborn with *N. gonorrhoeae* results in disease of a severity that, despite frequently being mild, can be rapidly destructive and lead to scarring and blindness. The chemical conjunctivitis that can result from silver nitrate prophylaxis typically starts within 6 to 24 hours after administration and disappears within 24 to 48 hours.

Colonization during delivery is followed by an incubation period of 2 to 5 days and usually less than 3 days, but cases occasionally may be recognized for 2 to 3 weeks after delivery.[135] A discharge typically develops that initially is watery but usually becomes thick and mucopurulent within a short time and may contain blood (Fig. 89-5). The disease generally is bilateral. Early findings include prominent edema of the conjunctivae and lids, followed by edema and later ulceration of the cornea or spread to wider areas in severe cases. Rapid arrest of the disease is essential because the degree of corneal involvement determines whether vision will be preserved. Some cases are self-limited and have benign outcomes, and occasional cases of asymptomatic GON have been discovered during routine screening.[273] Perforation of the globe and panophthalmitis can result from extensive local disease. The conjunctivae may serve as a portal of entry for gonococcal septicemia, arthritis, or other manifestations of invasive disease, but such events seldom occur.

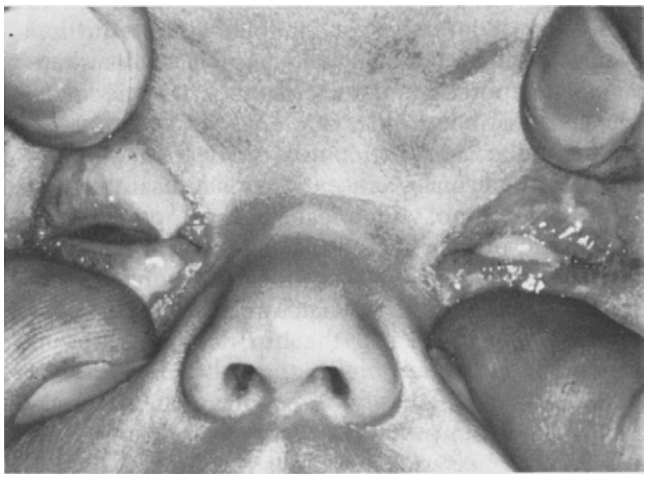

FIGURE 89-5 ■ Gonococcal ophthalmia neonatorum. The usual clinical finding is bilateral conjunctivitis that becomes progressively purulent if untreated. (From Gutman LT. Gonococcal infections. *Semin Pediatr Infect Dis* 2005;**16**:4.)

A presumptive diagnosis may be made by demonstrating typical gram-negative diplococci by Gram stain of conjunctival exudate. Because other organisms also may cause exudative infection of the conjunctivae, laboratory confirmation of the diagnosis of GON depends on culture results of *N. gonorrhoeae*.

Invasive disease rarely occurs in association with GON, but neonates with clinical evidence suggestive of GON should be evaluated for disseminated disease. The evaluation should include a thorough physical examination, especially of the joints. Exudate from the eyes or other sites of apparent local infection should be sent for Gram stain and culture on appropriate media.

Current recommendations in the United States are to obtain cultures of blood, eye discharge, and other potential sites of infection, such as CSF.[5] The absolute necessity of obtaining CSF for analysis and culture in otherwise well-appearing neonates is not certain, although it is reasonable to err on the side of caution. Neonates with GON generally should be hospitalized as part of initial management.

Gonococcal Scalp Abscess and Other Local Infections

Gonococcal infections of scalp wounds occur especially after fetal monitoring electrodes have been used on infants born to infected mothers.[102] The lesions may produce extensive local inflammatory disease and necrosis and can be a focus for disseminated infection. A scalp wound in a neonate should be cultured for gonococci, as

TABLE 89-3 **Incidence of Neonatal Gonococcal Disease in Exposed Infants**

Site of Neonatal Infection	Rate of Positive Cultures (%)	Population	Reference
Conjunctiva	0-10	Exposed infants who underwent silver nitrate ocular prophylaxis	Edwards et al, 1978[120] Allen and Barrere, 1949[4] Armstrong et al, 1976[11] Laga et al, 1989[207]
	2-48	Exposed infants who had no ocular prophylaxis	Rothenberg, 1979[294] Fransen et al, 1986[132] Laga et al, 1984[202]
Orogastric fluid	26-40	Infants of infected mothers	Handsfield et al, 1973[162] Edwards et al, 1978[120]
Oropharynx	35	Infants with gonococcal ophthalmia	Laga et al, 1989[207]
Disseminated disease as a proportion of all neonatal gonorrhea	0-1 (rare)	Reported series of neonatal gonococcal disease	Folland et al, 1977[130] Tomeh and Wilfert, 1973[339] Wald et al, 1980[348] Edwards et al, 1978[120] Fransen et al, 1986[132]

well as other likely pathogens, which include *Staphylococcus* spp., group B streptococci, *H. influenzae*, and gram-negative enteric flora. Herpes simplex also may be present in areas of injury to the scalp of newborns. Overall, approximately 1/200 births that are monitored with fetal scalp electrodes are complicated by infection at the monitoring site.[272]

Neonatal gonococcal vulvovaginitis, proctitis, rhinitis, funisitis, and urethritis have been described but are infrequent findings.[156] Gonococcal colonization of the oropharynx, gastric fluid, or both occurs relatively frequently in perinatally exposed infants, however. Pharyngeal colonization is present in as many as 35 percent of neonates who have GON.[132] A summary of selected studies on the incidence of neonatal gonococcal colonization and disease in exposed infants is presented in Table 89-3.

Infants with localized gonococcal infection other than GON should have cultures obtained of blood, CSF (although meningitis is rare), and the site of infection, if feasible. They should be hospitalized pending the availability of culture results (see the later discussion of treatment).

Systemic Disease in the Neonate

Disseminated disease occurs in 1 percent or fewer of infants who are perinatally exposed to gonococcal infection[13] (see Table 89-3). Septic arthritis is the most common form of disseminated gonococcal infection in neonates. Gonococcal arthritis of the newborn was described extensively between 1900 and 1930 by Coperman[89,90] and Wehrbein,[350] and recent case reports suggest that the disease remains similar. Clinical findings usually become evident when the infant is 1 to 4 weeks old. Although a few infants with gonococcal arthritis will have evidence of GON or other sites of mucosal or skin infection at the time of onset of arthritis, most do not. Concurrent gonococcal arthritis in both mother and newborn infant has been described.[150]

The signs and symptoms of gonococcal septic arthritis are similar to those of joint infection caused by other microbes in the neonatal period, including a predominance of polyarticular involvement. Infection most frequently involves the ankles, knees, wrists, and hands.[89] The hip may be infected with minimal signs other than pseudoparesis. Involvement of a joint may include suppurative arthritis, inflammatory disease of the periarticular structures, and tenosynovitis. Leukocytosis usually occurs, and most infants have a positive culture and compatible Gram stain results from the synovial fluid of the involved joint.[142]

Prompt drainage of septic hips, along with initiation of antimicrobial therapy, is necessary because of the risk for development of aseptic necrosis of the femoral head. Long-term dysfunction from gonococcal infection of other joints seldom occurs.[90,156,174,203]

Although gonococcal sepsis can develop in neonates with or without associated septic arthritis, the bacteremic phase of spread to the joints from the initial sites of infection generally is typically clinically silent. Premature infants seem to be more at risk for the development of sepsis with bacteremia than are term infants. Meningitis has been documented but is an exceedingly rare manifestation of neonatal gonococcal infection.[33,156,162]

GONOCOCCAL DISEASE BEYOND THE NEONATAL PERIOD

Lower Genital Tract Infection in Prepubertal Girls

Gonococcal vaginitis or vulvovaginitis is the most common form of gonorrhea in prepubertal girls beyond the neonatal period. In contrast to that of postpubertal females, the anestrogenic vaginal mucosa of prepubertal girls creates an alkaline environment that is colonized and infected more readily with *N. gonorrhoeae*. Infection of the endocervix, urethra, paraurethral and Bartholin's glands, and upper genital tract occurs only rarely.

Gonococcal vaginitis in prepubertal girls almost always is symptomatic, with vulvar erythema and a profuse vaginal discharge (Fig. 89-6). The girl may complain of dysuria, urinary frequency, vulvar discomfort, or pain while walking.[247,310,313] Asymptomatic cases are uncommon.

Signs and symptoms should resolve promptly within a few days after treatment is initiated, but acute manifestations may persist for several weeks if the child is not treated. The natural course of disease is for the inflammation to subside and the discharge to become scant and seropurulent. Infection may resolve spontaneously but occasionally may persist until the girl reaches puberty.

FIGURE 89-6 ■ Profuse, purulent vaginal discharge in an 18-month-old girl with gonococcal vulvovaginitis. Gonococcal infections in prepubertal children are almost always the result of sexual abuse. (See companion Expert Consult website for color version.) (From American Academy of Pediatrics 2012 Red Book Collection.)

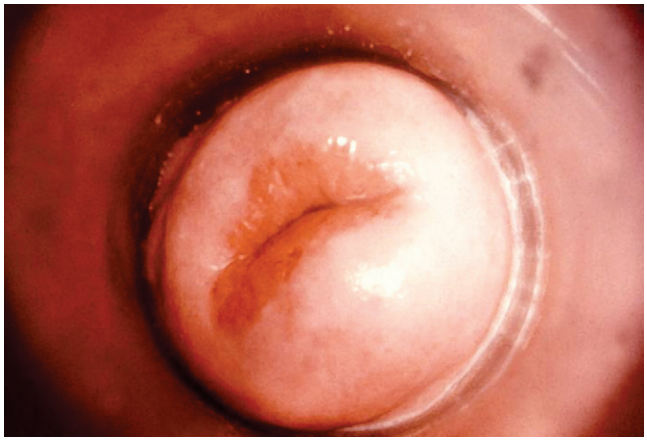

FIGURE 89-7 ■ Colposcopic view of cervix showing eroded ostium resulting from gonococcal infection. (See companion Expert Consult website for color version.) (From American Academy of Pediatrics 2012 Red Book Collection.)

Prepubertal vulvovaginitis can be caused by numerous irritative and infectious agents, including pinworms, foreign bodies, group A streptococci, *Neisseria meningitidis*, *Neisseria sicca*, and *Moraxella catarrhalis* (see also Chapter 42). Vulvovaginitis can mimic urinary tract infection, and pyuria can be seen on urinalysis with gonococcal and other causes of vulvovaginitis.[133,217]

Although ascending infection seldom occurs in this age group, it may result in salpingitis or peritonitis. One study found that 10 percent of young girls with gonorrhea had signs compatible with peritonitis, including fever, diffuse abdominal pain, leukocytosis, and decreased bowel sounds.[47] Salpingitis and periappendicitis may cause findings similar to those of appendicitis. Therefore, perineal examination for vaginal irritation or discharge should be done before performing abdominal surgery in young girls.[12]

In children with gonorrheal infection of the genitourinary tract, concomitant anorectal and tonsillopharyngeal colonization is a common finding[253] because sexual abuse usually is the means of infection.

Lower Genital Tract Infection in Postmenarcheal Females

Gonococcal infections in adolescent girls are similar to those in adults. The endocervix is the primary site of urogenital infection, although the external genitalia, urethra, vulvar mucosa, and vestibular glands also may be infected. Symptoms of acute infection usually begin 3 to 5 days after exposure with the development of a profuse, purulent vaginal discharge. The vulvar tissues are inflamed, with resultant pruritus and a burning sensation. Urethritis often is present initially and can lead to dysuria with urinary frequency and urgency. Urethral discharge can be seen but is much less prominent than in males. A purulent discharge from the vestibular and paraurethral glands also may be noted.

The endocervical mucosa is edematous, inflamed, and often friable (Fig. 89-7). A profuse yellow-green discharge is present and detaches easily from the surface. The zone of endocervical ectopy that normally is present in as many as 50 percent of adolescents may appear bright red, does not bleed easily when touched with swabs, and should not be mistaken for cervicitis. Ectopy that appears swollen and friable suggests cervicitis. Nabothian inclusion cysts (transparent and grayish) also are normal findings.

Infection of the Bartholin's (major vestibular) gland or paraurethral (Skene's) gland ducts may develop during acute gonorrhea. Bartholin's gland abscesses appear as large, circumscribed painful swellings of the dorsal aspect of the labium minus (Fig. 89-8). They occur in 5 percent of females with endocervical gonorrhea.[282] Paraurethral duct abscesses appear as small painful swellings in the urethrovaginal septum and may cause dysuria. Rupture into the urethra can create a urethral diverticulum. Bartholin's gland or paraurethral duct abscesses should be incised and drained, but small asymptomatic nodules do not require drainage.

If the gonococcal infection goes untreated, the acute symptoms generally subside in 8 to 10 weeks. In a cohort of 387 adolescents 14 to 17 years of age who were evaluated by weekly quantitative cultures, 16 developed acute infection. The number of gonococci shed remained stable during the first 6 weeks of infection, although shedding patterns varied widely among the individual patients. Microbial load did not correlate with genitourinary symptoms.[332]

Persisting acute symptoms are more likely to represent reinfection than chronic infection. Chronic urethritis, thickening of the vestibular glands and paraurethral ducts, and chronic cervicitis can occur. A secondary nonspecific vaginitis can develop as a result of irritation of the mucosa from persisting profuse endocervical discharge.

Mucopurulent cervicitis can be caused by *N. gonorrhoeae*, *C. trachomatis*, *Gardnerella vaginalis*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and group B streptococci.

FIGURE 89-8 ■ Acute Bartholin's duct abscess.

The overlap in clinical manifestations of infections caused by these microbes and the frequency of infection by two or more simultaneously preclude establishing the diagnosis by clinical findings alone.

Adolescents may be unaware of their infection, especially if they have a preexisting profuse discharge as a result of nonspecific vaginitis or trichomoniasis. Early recognition and treatment of lower genital tract gonococcal infection may prevent extension to and complications of upper genital tract infection. Therefore, sexually active adolescent females should be examined for gonococcal infection as part of their routine health care.[166,299]

A review of reported complications in 1232 cases of gonococcal vaginitis in the pre-antibiotic era revealed that 35 percent had urethritis, 19 percent had proctitis, and 6 percent had peritonitis.[21]

Urinalysis may provide clues to gonococcal infection in some cases. In a cross-sectional study of 296 sexually active females 14 to 22 years of age, gonorrhea or trichomoniasis was present in 65 percent who had sterile pyuria.[178]

Upper Genital Tract Infection in Postmenarcheal Females

PID will develop in 10 to 17 percent of females with endocervical gonococcal infection. Conditions encompassed by the term *pelvic inflammatory disease* include endometritis, parametritis, salpingitis, oophoritis, tubo-ovarian abscess, and pelvic peritonitis. PID can be caused by a variety of bacteria. Anaerobes, including *Bacteroides*, *Peptococcus*, and *Peptostreptococcus* spp., are the organisms usually recovered. *N. gonorrhoeae* is present in 25 to 50 percent of cases, similar to the proportion of cases from which *C. trachomatis* is recovered. Coliform organisms, *G. vaginalis*, *M. hominis*, *U. urealyticum*, and various streptococcal species also may be involved. PID develops when these organisms are able to ascend into the uterus, fallopian tubes, and beyond from the lower genital tract. Multiple species may be involved in a single episode. Gonococcal PID often occurs during or just after menses.[30,124,171] Identification of the specific microbial cause or causes of PID is complicated by the difficulty of obtaining fallopian tube specimens before initiating therapy.

Common findings in PID include an acute onset of lower abdominal pain with tenderness, fever, tenderness on lateral motion of the cervix, adnexal tenderness that generally is bilateral, and adnexal fullness. Vaginal discharge, urinary symptoms, and irregular vaginal bleeding also may be present. In moderate to severe cases, abdominal pain frequently is bilateral, exacerbated with movement, and continuous. Nausea, vomiting, marked abdominal tenderness, an abdomen that appears tense, and fever exceeding 39°C (102.2°F) may be present. Such patients often appear to be ill and may have tachycardia consistent with the height of fever.[307] Leukocytosis and elevated erythrocyte sedimentation rates are common findings. Alternatively, PID may be clinically silent or cause only mild pain without discernible tenderness, vaginal discharge, or leukocytosis. Such mild cases can go unrecognized by patients and physicians.

Establishing the diagnosis of PID may be difficult, and the differential diagnosis includes numerous other lower abdominal conditions, such as appendicitis, ectopic pregnancy, cholecystitis, mesenteric adenitis, pyelonephritis, and septic abortion. Sonography and pregnancy testing may be helpful in diagnostic decision making. No single symptom, sign, or laboratory finding is sensitive and specific for the diagnosis of PID. Combinations of findings can improve the sensitivity but do so at the expense of reduced specificity, and vice versa.

Initiation of empirical therapy for PID should be considered if the minimal criteria of lower abdominal tenderness, adnexal tenderness, and cervical motion tenderness all are present and no other cause for these findings is readily apparent. More elaborate evaluation may be needed in some cases because incorrect diagnosis and management might lead to unnecessary morbidity. Additional criteria that support the diagnosis of PID include an oral temperature higher than 38.3°C (101°F), an abnormal cervical or vaginal discharge, elevated erythrocyte sedimentation rate, increased C-reactive protein level, and laboratory documentation of cervical infection with *N. gonorrhoeae* or *C. trachomatis*. Some cases may require pursuit of definitive criteria for diagnosing PID, which include histopathologic evidence of endometritis on endometrial biopsy, transvaginal or abdominal ultrasonography or other imaging studies that show thickened fluid-filled fallopian tubes with or without free pelvic fluid or a tubo-ovarian abscess, and laparoscopic abnormalities consistent with PID.[60,186]

The outcome for fertility probably is improved with prompt and vigorous therapy. In the past, physicians recommended that all adolescents thought to have PID be hospitalized for therapy. More recently, inpatient observation and treatment have been recommended when a surgical emergency (e.g., ectopic pregnancy or appendicitis) cannot be excluded; when compliance with or tolerance of an outpatient treatment regimen and follow-up within 72 hours cannot be ensured; when the illness appears to be severe (e.g., pelvic or tubo-ovarian abscess, overt peritonitis); or when the patient is immunocompromised, is pregnant, or has failed to respond to outpatient therapy.[5] All courses of therapy should include treatment that is appropriate for *C. trachomatis* and other causes of PID, in addition to *N. gonorrhoeae*.

Complications of PID include tubo-ovarian abscess, perihepatitis (Fitz-Hugh-Curtis syndrome), future ectopic pregnancy, and infertility. Perihepatitis is characterized by right upper quadrant abdominal pain that may radiate to the shoulder. Nausea, fever, and other signs and symptoms of PID may be present as well. Leukocytosis occurs commonly, and liver enzymes are elevated in some cases. The differential diagnosis of perihepatitis includes pleuritis, cholelithiasis, subphrenic abscess, and perforated ulcers.[216]

An estimated 15 percent of women may become sterile after a single episode of PID and 50 percent after three infections. Because of scarring and fibrosis in the fallopian tube, patency is compromised and fertility is jeopardized.[53,124,125] PID in adolescents is particularly likely to result in infertility and ectopic pregnancy (Fig. 89-9), and PID is the single most common cause of infertility in young women.[44,243] Between 1970 and 1980, the rate of ectopic pregnancies per 1000 live births increased from 4.8 to 14.5, and between 1975 and 1981, the rate of hospitalization of females 15 to 19 years of age for salpingitis was 4/100,000.[348] These increases correlated with rising rates of gonococcal infection in the affected populations. Data from several studies of gonococcal disease in adolescents are presented in Table 89-4.

Risk factors for the development of PID include young age at the time of acquisition of gonococcal disease or other STIs, a history of previous PID, multiple sexual partners, and the use of an intrauterine device (IUD) for contraception. The immature cervix may be at particular risk for progression to upper tract disease.[315] Disseminated gonococcal infection may accompany asymptomatic infection of an IUD,[85] and the rate of acute PID in those who use IUDs may be increased.

Genital Tract Infection in Males

Gonococcal urethritis in prepubertal boys is a less frequent event than vaginitis in girls because of gender differences in rates of sexual abuse. In children with gonorrheal infection of the genitourinary tract, concomitant anorectal and tonsillopharyngeal colonization is a frequent finding.[253]

Urethritis is the primary manifestation of gonococcal infection in males of all ages beyond the neonatal period (Fig. 89-10). Even in young boys, the disease usually is symptomatic and resembles gonococcal urethritis in men.[96,136] Dysuria, purulent discharge, or both develop 2 to 7 days after exposure. Patients generally are afebrile. Purulence typically is greater than with nongonococcal urethritis, but symptoms often are mild enough that patients (or their parents) may delay seeking medical care for weeks. Associated inguinal adenopathy occurs rarely.

FIGURE 89-9 ■ Large hydrosalpinx resulting from gonococcal salpingitis in an adolescent female with repeated gonorrheal infections.

TABLE 89-4 **Prevalence of Gonorrhea and Other Sexually Transmitted Diseases in Clinics for Adolescents**

Reference	Location	Total Clinic Population Studied	Gonorrhea (%)	*Chlamydia* Infection (%)	Other
Shafer et al, 1984[309]	California	366	15	4	*Trichomonas* infection
Golden et al, 1984[143]	New York	186	10	10	Syphilis, *Trichomonas* infection
Demetriou et al, 1984[109]	Oklahoma	839	14	—	
Mulcahy and Lacey, 1987[244]	Leeds	210	14	16	*Trichomonas* infection
Jamson et al, 1995[187]	Colorado	632	7	—	Human papillomavirus infection
Giannini et al, 2010[138]	Ohio	16,039*	9	—	
		525†	16	—	

*Females 14-21 years of age seen at a children's hospital adolescent clinic, 2003-2007.
†Females 14-21 years of age seen at a health department sexually transmitted infection clinic, 2006-2007.

FIGURE 89-10 ■ Purulent male urethral discharge resulting from gonorrhea with an associated pyodermal lesion on the glans. (See companion Expert Consult website for color version.) (From American Academy of Pediatrics 2012 Red Book Collection; originally from Centers for Disease Control and Prevention.)

At least 5 percent of cases of gonococcal urethritis in males are asymptomatic, and asymptomatic infection can occur at all ages. Asymptomatic pyuria is a manifestation with which the pediatrician should be familiar.[103] It may be the only finding in some cases and should raise suspicion of gonococcal or chlamydial infection in boys who may have been sexually abused or in sexually active adolescent males.

Males with asymptomatic infection are a major reservoir for transmission to their sexual partners.[101] Untreated male urethral infection may persist for as long as 6 months.

Complications of gonococcal infection in males now occur much less frequently than in the pre-antibiotic era. Epididymitis is the most common complication. Unilateral swelling, pain, and erythema in the posterior aspect of the scrotum are the usual features. Fever may occur. If untreated, the infection may progress to involve the ipsilateral testis. Hydrocele may result from secretion of fluid into the potential space of the tunica vaginalis. Epididymitis can lead to testicular infarction, abscess, infertility, prostatitis, paraurethral abscesses, and penile lymphangitis, but these local complications are rare findings. Perihepatitis in males has been described but occurs extremely infrequently.[205]

Disseminated Disease

Dissemination requires invasion of the bloodstream from local infection of the mucous membranes of the genital tract, rectum, pharynx, or conjunctiva. Bacteremia then can lead to infection of other sites. The risk for dissemination in children after mucosal infection appears to be higher than the 1 to 3 percent rate in adults. Dissemination in adults seems to be more common with asymptomatic infections, many of which are caused by AHU^- gonococcal strains (see earlier discussion of pathogenesis).[200] Deficits in the complement system have been associated with disseminated disease in adults, but the frequency of such deficiencies in children is unknown.

As in neonates, gonococcal arthritis is the most common form of disseminated disease in older children, adolescents, and adults. The ankles, knees, wrists, and hands are involved most frequently. The clinical findings are not distinct from those of other microbial causes of septic arthritis. Polyarticular involvement occurs less frequently than in neonates.[94] Gram stain and synovial fluid cultures often are negative. Therefore, empirical coverage for *N. gonorrhoeae* must be considered when septic arthritis develops in a sexually active adolescent or a child who may have been sexually abused.

Typically, the patient has a single most severely affected joint, and associated myositis and tenosynovitis may be prominent findings. Gonococcal arthritis in older children and adolescents resembles that in adults and may be accompanied by cutaneous lesions (Fig. 89-11).[4] Osteomyelitis rarely occurs but has been reported in all age groups, usually in association with septic arthritis.[9]

Treatment of gonococcal arthritis depends on prompt recognition of the disease. Cultures of all mucous membranes (i.e., nasopharyngeal, rectal, vaginal or endocervical, and conjunctival), blood culture, and aspiration of the involved joint should be performed. The local signs of gonococcal arthritis may not respond to antibiotic therapy for several days. Serial needle aspiration rather than open drainage usually is sufficient for relief of pain and recovery without sequelae (the hip may be an exception).

Gonococcemia is clinically silent generally but can cause a syndrome of migratory polyarthralgia, fever, and rash that precedes the onset of arthritis by several days to a week. The symptomatic course normally is only mild to moderate in severity. Blood cultures often are negative by the time care is sought. Gonococcemia-related symptoms may resolve after several days, even without treatment. Rare cases can resemble meningococcemia with purpura and fulminant sepsis with disseminated intravascular coagulopathy. These cases sometimes can be fatal.[266]

The skin lesions associated with gonococcemia usually are pustules on an erythematous base (see Fig. 89-11), but petechiae, papules, and hemorrhagic bullae can develop. The development of skin lesions in conjunction with septic arthritis also has been referred to as the arthritis-dermatitis syndrome. The lesions usually arise on the extremities and are fewer than 20 in number. Low-grade fever is most common, but high fever with shaking chills may occur. Tenosynovitis is present in a fourth of these patients. Leukocytosis, pyuria, and elevated liver enzyme test results may be seen. The resultant septic joints generally become clinically apparent during the second week after the onset of disseminated infection.

Anorectal Gonorrhea

Gonococcal anorectal infection (proctitis) frequently is asymptomatic but can be associated with pruritus, tenesmus, purulent discharge, or rectal bleeding. Rectal infection can occur as a result of rectal intercourse or inoculation from vaginal secretions. Approximately 40 percent of females with genital gonococcal infection have positive anorectal cultures. Rectal infection is an unusual finding in males who have not engaged in rectal intercourse.[325]

FIGURE 89-11 ■ Skin lesion typical of the arthritis-dermatitis syndrome of disseminated gonococcal infection: necrotic pustule with an erythematous halo overlying proximal phalanx of index finger. (Courtesy Daniel P. Krowchuk, MD.)

Pharyngeal Gonorrhea

Pharyngeal gonococcal infection in all age groups beyond the neonatal period is acquired by orogenital contact. Pharyngeal gonorrhea may be asymptomatic, without evidence of inflammation, or may cause an exudative tonsillopharyngitis that can mimic group A streptococcal or viral infection. Cervical adenopathy may be present in some cases. In sexually abused children, the pharynx may be the only site of infection. It may be the sole culture-positive site in some cases of disseminated gonococcal infection, and pharyngeal infection possibly is a factor predisposing to dissemination. Pharyngeal infection usually resolves spontaneously within 10 to 12 weeks but should be treated when recognized.* In a recent study of 14- to 21-year-old females hospitalized in a children's hospital or seeking care in a public health clinic, pharyngeal gonorrhea prevalence among adolescents was 3.5 percent and 6.8 percent, respectively.[138]

Conjunctivitis Beyond Infancy

Gonococcal ophthalmia occasionally is seen in children and adults. Direct inoculation of the eye can occur as a result of transmission of fomites from infected persons. Clinical findings typically include a profuse purulent discharge, chemosis, eyelid edema, keratitis, and fever. The initial ocular discharge may be watery before turning purulent. The acute phase may mimic orbital cellulitis. Untreated infection, as with neonates, can lead to corneal opacification, ulceration, and rupture of the globe with resultant visual loss. Some cases may be minimally symptomatic, with a low-grade inflammatory response followed by spontaneous resolution.[213,217,273]

*References 1, 42, 73, 95, 154, 253, 263, 314, 353, 357.

Other Forms of Gonococcal Disease

Gonococcal meningitis is a rare condition that may occur with or without associated signs of gonococcemia or septic arthritis. Pyomyositis of the biceps and soft tissue abscesses remote from the genital area have been reported. A gonococcal abscess arising in an area of blunt trauma to a hand has been described in an adolescent with associated endocervical infection.[144] An abdominal wall abscess resulting from *N. gonorrhoeae* infection in a 22-month-old male child without other foci of infection has been described.[115] Ventriculoperitoneal shunt–associated infection, endocarditis, and myocarditis caused by *N. gonorrhoeae* have been reported in adults and can be expected to occur occasionally in children and adolescents.[171] These manifestations of gonococcal infection seldom are seen in children.

DIAGNOSTIC TESTING

Isolation of *N. gonorrhoeae* in culture remains the standard for diagnosing gonococcal infection, but nonculture, DNA-based tests have become widely used in recent years. Uses of these tests are listed in Table 89-5. Only culture should be used for rectal or pharyngeal specimens. Serologic tests based on complement fixation, latex agglutination, enzyme-linked immunosorbent assays, and other techniques have been developed, but the sensitivity of these methods is only about 70 percent, which limits their use primarily to studies of immune response and pathogenesis.[173,175,300]

The Centers for Disease Control and Prevention (CDC) also has defined three levels of diagnosis based on clinical and laboratory findings. They are more stringent than the case definitions used for public health surveillance. A *suggestive diagnosis* is defined by presence of

TABLE 89-5 **Laboratory Testing of Specimens for the Presence of *Neisseria Gonorrhoeae***

Clinical Indication for Testing	Testing Method and Specimen Type
Suspected female genitourinary tract infection	1. Culture performed on an endocervical swab specimen (adolescents or adults) or vaginal swab specimen from prepubescent females* 2. NAAT (transcription-mediated amplification tests preferred) performed on vaginal specimens or urine (may be acceptable for prepubescent children undergoing evaluation for sexual abuse; see text)
Suspected male urethral infection	1. Culture performed on an intraurethral swab specimen if collecting such a specimen is acceptable and transport and storage conditions are suitable for culture†,‡ (remains medicolegal forensic standard) 2. Gram stain of urethral discharge (positive if intracellular gram-negative diplococci are seen) 3. NAAT or NAHT performed on an intraurethral swab specimen if collecting such a specimen is acceptable 4. NAAT performed on urine
Suspected male or female rectal or pharyngeal infection	1. Culture performed on rectal or pharyngeal swab specimens* 2. NAAT preferred if off-label regulatory requirements have been met by an available clinical laboratory
Suspected gonococcal ophthalmia (neonatal or childhood) or disseminated infection	1. Culture performed on conjunctival exudate specimens, blood, synovial fluid, cerebrospinal fluid, or pustular skin lesions with the use of nonselective media (e.g., chocolate agar) 2. Gram stain on exudates or fluid samples (synovial, cerebrospinal fluid) can be useful as an initial point-of-care test that can help guide early treatment considerations pending culture results. Should be accompanied by culture 3. Some NAATs may be used for conjunctival specimens
Scenario for Confirmatory Testing	**Recommendations and Comments**
Presumptively positive culture (identification in the laboratory of typical gram-negative, oxidase-positive diplococci consistent with *N. gonorrhoeae*)	1. Preferred methods for confirmation of *N. gonorrhoeae* are (1) acid production from carbohydrates§ or (2) positive NAHT results (use of molecular method to confirm culture results) 2. Requiring both methods to be positive ensures high specificity 3. If an isolate cannot be conclusively identified as *N. gonorrhoeae* at a local laboratory, it should be sent to a reference laboratory for confirmation (especially in cases of alleged sexual abuse, sexual assault, or rape)
Positive nonculture test (e.g., NAAT, NAHT)	1. Culture with confirmation as above is the preferred additional test after a positive nonculture test if specimen transport and storage conditions are suitable. Confirmatory testing is not routinely required unless medicolegal issues exist or there is need for antibiotic susceptibility testing 2. Competitive probe format after a positive NAHT (probe) test, but this approach theoretically is less likely to detect a false-positive result 3. A different NAAT as an additional test after NAAT or NAHT has received limited evaluation; certain NAATs sometimes cross react with nongonococcal *Neisseria* spp. 4. Antibody tests that detect gonococcal antigens are not recommended for detection of *N. gonorrhoeae*

Compiled from Centers for Disease Control and Prevention: Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections: 2002. *MMWR Recomm Rep* 2002;**51**(RR-15):1-38; and Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):1-109.

*A selective medium (e.g., modified Thayer-Martin) should be used for culture of specimens from these sites.

†Transport cultures with an enriched CO_2 atmosphere can be used in some situations.

‡Selective or nonselective media may be used.

§*N. gonorrhoeae* produces acid reactions with glucose but not maltose, lactose, sucrose, or fructose. The species also does not reduce nitrates or hydrolyze tributyrin.

NAAT, Nucleic acid amplification test; *NAHT,* nucleic acid hybridization test.

mucopurulent endocervical or urethral exudate and sexual exposure to a person with gonococcal infection. A *presumptive diagnosis* requires two of three criteria: (1) typical gram-negative *intracellular* diplococci on Gram stain of urethral exudate from males or endocervical secretions; (2) growth of apparent *N. gonorrhoeae* from such specimens on culture medium, defined as typical colonial morphology, positive oxidase reaction, and typical gram-negative morphology; and (3) detection of *N. gonorrhoeae* by a nonculture laboratory test. A *definitive diagnosis* requires (1) isolation of *N. gonorrhoeae* from clinical specimens by culture, as in the second criterion for a presumptive diagnosis, and (2) confirmation of identity by biochemical, enzymatic, serologic, or nucleic acid testing (see Table 89-5).[71]

A diagnosis of *N. gonorrhoeae* infection at any site should prompt evaluation for the presence of other common STIs, if not already done (see also Chapter 42).[292]

Culture

In adults, culture of clinical specimens is 80 to 95 percent sensitive when promptly inoculated and incubated. Cultures of adult male urethral specimens, blood, and other normally sterile body sites tend to have sensitivities in the

higher range.[175] Because false-positive cultures (with appropriate laboratory confirmation; see later discussion) are not thought to occur, specificity and positive predictive values are 100 percent. Culture in adolescents and children probably has sensitivity similar to that of adults. Vaginal specimens are adequate for diagnosis in prepubertal girls, for whom obtaining endocervical specimens is unnecessary.

The use of selective media such as modified Thayer-Martin (which contains nystatin, vancomycin, trimethoprim, and colistin) is required for culture of endocervical, rectal, and pharyngeal specimens to suppress contaminating flora. Selective or nonselective media (chocolate agar) can be used with equal sensitivity for male urethral cultures. Plating of specimens on both types of media may improve the sensitivity, but the incremental yield is small and probably not cost-effective for routine practice.[31,100,283]

Gonococcal colonies become evident on agar plates within 24 to 48 hours after inoculation. Isolates are considered presumptively positive if Gram stain shows gram-negative diplococci and colonies are oxidase-positive. Further testing that demonstrates the gonococcal phenotypic pattern of acid production from selected carbohydrates or a positive result by a nucleic acid method, or both (see later discussion) is required for confirmation as *N. gonorrhoeae* (see Table 89-5).[64]

Gonococci do not tolerate drying, so clinical specimens must be plated onto appropriate media as soon as possible. Transport bottles containing medium and a carbon dioxide–enriched atmosphere should be used if definitive specimen processing will be delayed. Transport bottles should be maintained upright during inoculation to preserve the carbon dioxide atmosphere.

In sexually active females with endocervical gonococcal infection, the urethra, Bartholin's gland ducts, and Skene's gland ducts usually are infected also. Cultures of these sites may improve the overall yield or sensitivity, in part by avoiding the sampling errors that can occur with any single culture. However, the incremental yield is small enough to render obtaining such cultures unnecessary in routine clinical settings. Culture of the rectum and pharynx in females can be considered optional except when evaluating for sexual abuse.[31,192] However, in a recent study, addition of pharyngeal culture allowed detection of an additional 11 to 26 percent of cases versus cervical culture alone among 14- to 21-year-old females.[138]

In sexually active males, sites to be cultured depend on the sexual practices and the anatomic sites exposed. Among MSM, rectal and pharyngeal infections occur with frequencies similar to that of gonococcal urethritis.[37,175,229,239]

Gram-Stained Smears

In symptomatic males, a Gram stain of a urethral specimen that demonstrates polymorphonuclear leukocytes with intracellular gram-negative diplococci can be considered diagnostic of infection with *N. gonorrhoeae* (Fig. 89-12). Gram stain in this population has greater than 99 percent specificity and greater than 95 percent sensitivity.[65] Negative Gram stain results are not sufficient to exclude the diagnosis of gonorrhea. Although nonpathogenic *Neisseria* spp. and *N. meningitidis* are morphologically indistinguishable from gonococci, the former rarely are cell-associated.

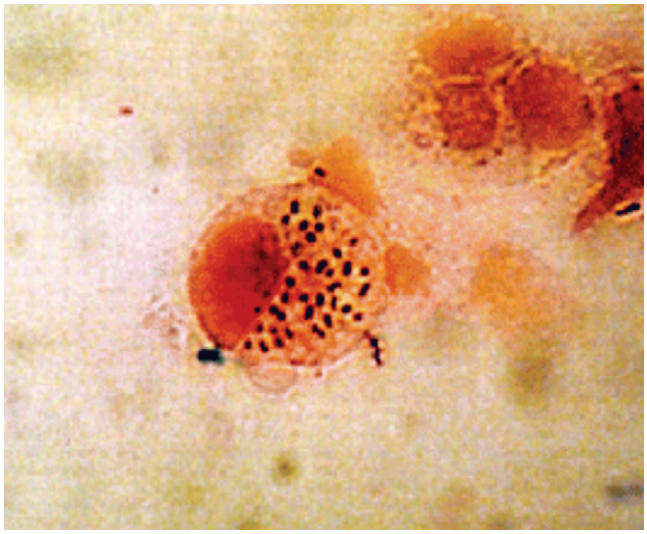

FIGURE 89-12 ■ Gram stain of gonorrheal exudate showing multiple intracellular diplococci. In male urethral specimens, this is considered diagnostic of gonococcal infection. In children and adolescent females, confirmation by culture or nucleic acid–based tests is needed (see text). (See companion Expert Consult website for color version.) (From American Academy of Pediatrics 2012 Red Book Collection.)

Gram stains of endocervical, pharyngeal, and rectal specimens are not recommended because of lower sensitivity in detection of infection at these sites. The specificity of a negative Gram stain appears to be at least 95 percent for specimens from the endocervix and rectum.[71,175,295]

Gram stains of eye exudates, joint fluid, and pustular fluid should be performed because the presence of gram-negative diplococci may help guide initial therapeutic interventions pending culture results.

Nonculture Diagnostics

Nonculture diagnostic tests have become widely used in the United States for establishing a rapid diagnosis of gonococcal (and chlamydial) infection. Many offer the advantage of using urine samples rather than the more invasive swabs and also allow *C. trachomatis* to be evaluated with the same specimen. Numerous gonococcal antigen-detection tests have been developed, but they have been supplanted largely by nucleic acid detection methods.

Nucleic acid hybridization tests (NAHTs) and nucleic acid amplification tests (NAATs) have been the nonculture tests used most commonly in the United States in recent years. NAHTs are based on a single-stranded DNA probe complementary to gonococcal ribosomal RNA (rRNA). Sensitivities are about 95 percent (less than culture), with a specificity of 99 percent.[160,175,214,265] Nucleic acid amplification tests (NAATs) use standard PCR-based assays, transcription-mediated amplification, or strand-displacement assays (SDA). These are more

sensitive than culture (see Table 89-5). False-positive reactions with PCR- or SDA-based NAATs can occur as a result of the carry-over contamination that occurs during processing or because of occasional cross reactions with commensal *Neisseria* spp. that may be present in some clinical specimens. Specificities are generally greater than 98 percent. False-negative results can occur as a result of target sequence differences (or absence) in some gonococcal clones. NAATs can be used to evaluate first-void urine specimens, which permits screening for gonococcal infection in males and females when genital examination is impractical.[80,319,344,345,354]

NAATs and DNA probe tests can detect the presence of nonviable gonococcal microbes, thereby possibly reducing the need for the stringent transport conditions often required for culture. Currently available NAATs are multiplex tests that also can detect *C. trachomatis*. NAATs are approved by the U.S. Food and Drug Administration for use with endocervical, vaginal, and urethral (male only) swab specimens and with urine specimens. They are not approved for rectal or pharyngeal specimens but are preferred for tests of these sites when clinical laboratories have met regulatory requirements for off-label procedures.[71] Some NAATs also can be used for conjunctival specimens.[5]

NAATs and DNA probe tests are adequate for rapid screening in clinical settings, and their results are adequate for making treatment decisions and public health case identifications. Culture remains the definitive test for medicolegal purposes (see the next section), although use of NAATs with confirmatory testing with a second NAAT based on an alternative DNA target is acceptable for forensic use on urine samples from girls but not boys.[27,71] In addition, because of ever-increasing resistance to the available antimicrobial agents, monitoring of gonococcal susceptibility by culture is necessary at the local level, at the very least in patients who do not respond to apparently adequate therapy.[64,354]

Medicolegal Issues Related to Diagnostic Tests

N. meningitidis and other members of the Neisseriaceae family are morphologically and often biochemically similar to *N. gonorrhoeae* and may be isolated from sites such as the vagina, blood, and nasopharynx.[111,116,355] Accurate identification of *Neisseria* organisms from any pediatric specimen is essential because misidentification of nongonococcal species as *N. gonorrhoeae* may lead to very serious social consequences for children and their families by precipitating concern regarding sexual abuse. A recent report of two cases of meningitis in young children initially misidentified as having *N. gonorrhoeae* but subsequently confirmed as having *N. meningitidis* illustrates that this is an ongoing issue.[327] Nonculture methods consistently identify more clinical specimens as positive than do standard cultures.[345] Determining whether such results indicate greater sensitivity, increased false positivity, or a combination of both has been difficult.

Because of this potential for obtaining false-positive results with nonculture NAAT or DNA probe tests, culture has been considered the medicolegal standard for children who require evaluation for suspected sexual victimization, including prepubertal girls with obvious vaginal discharge. Sequential testing using nonculture tests followed by culture if the former is positive has been proposed as a more sensitive approach than culture alone.[264] A recent multicenter study using NAATs on urine specimens, using a second NAAT based on an alternative DNA target or species-specific *N. gonorrhoeae* PCR to confirm positive results, found no false-positive results.[27] The NAAT-based approach increased the number of confirmed gonococcal infections among 485 girls who were suspected victims of sexual assault from 12, based on culture alone, to 16. This approach is now considered an alternative to culture on vaginal and urine specimens in girls. Culture remains preferred for urethral or urine specimens from boys and for pharyngeal and rectal specimens from all children.[71]

Proper Collection of Clinical Specimens

Urethral exudate from males may be obtained by passing small swabs or bacteriologic loops 2 to 4 cm into the urethra.[163] Endocervical specimens from postpubertal females are obtained by speculum examination with swabs inserted 1 to 2 cm into the external os after the cervix has been cleansed of external exudate and vaginal secretions.[291] The swab should be rotated one full revolution and withdrawn. Self-obtained vaginal swabs also provide adequate specimens for nonculture methods.[318] This technique has been studied in adolescents. Vaginal specimens collected with tampons also can be used with nonculture methods.[337]

In prepubertal girls, cultures can be obtained from the vaginal introitus by gently swabbing the hymenal opening. Deeper insertion is not necessary. Swabs of exudates emanating from the urethral meatus or vaginal orifice also are sufficient for culture.

In persons with symptomatic anorectal infection, rectal specimens should be obtained by anoscopic means, which increases the sensitivity. In asymptomatic persons, rectal specimens can be procured by blindly inserting a swab 2 to 3 cm into the anal canal and applying lateral pressure to avoid entering any fecal mass. Swabs that are grossly contaminated with fecal matter should be discarded.[104,358]

When urine specimens can be used for nonculture methods, the first 15 to 30 mL of voided urine should be collected.[223,248] Collection of urine should be delayed at least 1 hour after the most recent void to maximize sensitivity. The posterior pharynx, tonsillar areas, and faucial pillars should be swabbed to obtain adequate specimens for the diagnosis of pharyngeal infection.[175]

ANTIMICROBIAL RESISTANCE AMONG GONOCOCCI

Gonococcal strains have been able to acquire resistance to antibiotics since the first agents were introduced. In 1986, the CDC initiated the Gonococcal Isolate Surveillance Program (GISP) to monitor the antimicrobial sensitivities of *N. gonorrhoeae* in STI clinics in 21 cities in the

United States.[58] This system consisted of 29 sites in 2010.[70]

Sulfanilamide became available in 1936 and represented a major improvement over previous therapies that included local genital irrigation with solutions of silver nitrate or potassium permanganate.[268] Widespread resistance to sulfanilamide occurred by 1944. As successive agents have become available, multidrug-resistant strains are being seen with increasing frequency in many parts of the world. Ongoing analysis of gonococcal isolates for antibiotic susceptibility to the available antimicrobial agents remains essential for maintaining effective empirical therapeutic regimens.

In the 1940s, virtually all gonococcal isolates were highly susceptible to penicillin. Despite a gradual increase in the mean minimal inhibitory concentration (MIC) to penicillin from the mid-1950s through the mid-1970s, almost all strains had penicillin MICs of less than 0.5 μg/mL. This low-level resistance is mediated by alterations at a genetic locus called *penA* that result in modification of its product, penicillin-binding protein 2. Two other loci designated *mtr*, which encode an efflux pump that reduces concentrations of multiple antibiotics, and *penB*, which is an allele of *por*, the gene of the porin protein, also mediate low-level resistance to penicillins. *penB* encodes a porin with a mutation that decreases permeability to hydrophilic antibiotics.[140]

In 1976, strains of *N. gonorrhoeae* were discovered that had acquired plasmid-conferring resistance to penicillin through the production of penicillinase (a TEM-1 β-lactamase). These strains were found in many parts of the Far East and in London, and in the former they constituted approximately 30 percent of the isolates in some cities. The strains caused the expected spectrum of clinical disease, and treatment with penicillin was not effective. Penicillinase-producing gonococci contain one of two closely related 5.3- or 7.2-kb plasmids (Pc^r) that carry a Tn2 transposon system. This plasmid appears to have been acquired from *Haemophilus ducreyi*.[8,49,78,270] In 1983, an outbreak of chromosomally mediated, penicillin-resistant (MICs of 2 to 4 μg/mL), non–penicillinase-producing gonococci was reported from North Carolina.[129] Such strains were seen subsequently in other areas of the country.

Resistance to tetracycline antibiotics emerged in the 1980s and subsequently increased. Three chromosomal loci designated *mtr*, *penB*, and *tet* mediate low-level resistance. High-level resistance to tetracycline is conferred by *tetM*, which resides on a 38-kb plasmid (Tc^r) that is a derivative of the 36-kb conjugal plasmid. *tetM* produces a cytoplasmic protein that protects ribosomes from tetracycline. Tc^r gonococci can transfer this plasmid, as well as Pc^r, efficiently to other gonococcal strains.[246,329]

Spectinomycin resistance was described first in 1987 in U.S. military personnel in Korea. This agent had been introduced as the drug of choice there in 1981 because of high rates of penicillin resistance. Treatment failures actually began to occur in 1983. Resistance is chromosomally mediated and results in alteration of the ribosomal target site of the drug.[32,49] The widespread use of spectinomycin was associated with a decline in the rate of penicillin resistance.[32] Many gonococcal strains in some parts of the world also are resistant to streptomycin.[247] All gonococcal strains tested in the United States in recent years have been susceptible to streptomycin.[70]

Ciprofloxacin and other fluoroquinolone antibiotics became widely available for the treatment of gonococcal infection in the 1980s. Low-level resistance to fluoroquinolones is associated with mutations in the DNA gyrase gene *gyrA*, and high-level resistance has been linked to mutations in the topoisomerase gene *parC*.[184] Resistance to these agents has been noted since the early 1990s in areas of Southeast Asia.[131] In Hawaii, in 1999, 9.5 percent of isolates were resistant to ciprofloxacin, but resistance elsewhere in the United States was low, at 0.2 percent.[62,63] In 2000, fluoroquinolones no longer were recommended for initial treatment of gonococcal infection in Asia, the Pacific Islands, or Hawaii. In 2002, nonuse was extended to the state of California, and in 2004, to MSM throughout the United States. During the first 6 months of 2006, 13.3 percent of isolates collected through GISP were resistant to ciprofloxacin (and thus all other quinolone antibiotics). Among gonococcal isolates from MSM and heterosexual males from U.S. states other than California and Hawaii, ciprofloxacin-resistant rates were 30.7 and 5.1 percent, respectively. In April 2007, the CDC no longer recommended the use of fluoroquinolones for the treatment of gonococcal infections in the United States.[68] Quinolone resistance peaked at 14.8 percent of GISP isolates in 2007 and was 12.5 percent overall in 2010. Prevalence was 23.9 percent among isolates from MSM versus 7.9 percent among men who have sex only with women.[70]

In 1989, 13 percent of the *N. gonorrhoeae* isolates evaluated were resistant to penicillin, tetracycline, or both.[59] In 1999, such strains accounted for 28 percent of all isolates.[63] Rates of resistance to these two agents have fallen slightly in the United States since that time, with 9 percent being resistant to penicillin and 17 percent resistant to tetracycline in 2005.[67] Overall in 2010, 27.2 percent of isolates evaluated by GISP were resistant to penicillin, tetracycline, ciprofloxacin, or some combination of those antibiotics.[70] Tetracycline resistance was 21.6 percent in 2011.[72]

Strains resistant to azithromycin and erythromycin have been identified in the United States.[62] The distribution of azithromycin MICs has shifted upward in the last decade, although the proportion of GISP strains with MICs of 2.0 μg/mL or higher was 0.3 percent in 2011.[67,72] One isolate with azithromycin MIC greater than 256 μg/mL was found in Hawaii in 2011.[198] Rifampin resistance is also high in some areas of the world.[257]

During the past few years there has been upward creep in cefixime MICs in the United States and other countries, along with reports of treatment failures with the recommended 400 mg oral dose of this agent.[72,224,341] MICs of 0.5 μg/mL or higher are considered to have decreased susceptibility to cefixime. Nationally in the United States, in 2011, 1.5 percent of GISP isolates had cefixime MICs of 0.25 μg/mL or higher, a threshold used by GISP for monitoring emerging resistance to cephalosporins (Fig. 89-13). In the Western United States, 3.2

percent of isolates had MICs at or above 0.25 μg/mL. These observations prompted removal of cefixime from recommended options for empirical treatment of uncomplicated gonococcal infections in the United States in August 2012. Ceftriaxone MICs have increased minimally overall to date, but isolates with MICs of 1 to 2 μg/mL have been reported.[72,341]

These events raise concerns about emergence of multidrug resistant gonococcal strains. Ongoing monitoring of antimicrobial resistance in *N. gonorrhoeae* is warranted. New antimicrobial agents will be needed.

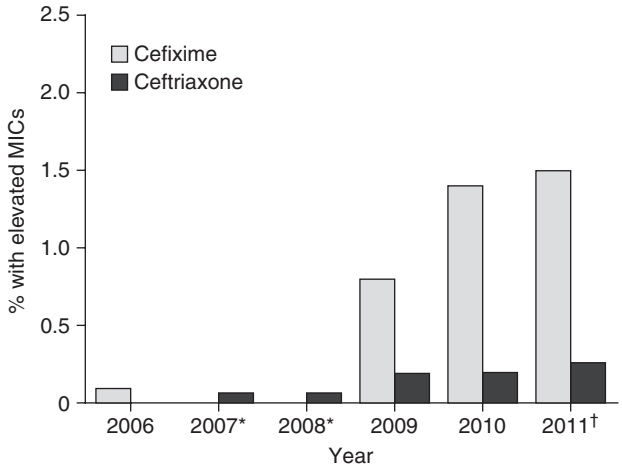

FIGURE 89-13 ■ Percentage of urethral *Neisseria gonorrhoeae* isolates (n = 32,794) with cefixime minimum inhibitory concentrations (MICs) ≥0.25 μg/mL and ceftriaxone MICs ≥0.125 μg/mL, from the Gonococcal Isolate Surveillance Project, United States, 2006 to August 2011. MICs have risen steadily during the past few years as a result of alterations in the microbial penicillin-binding proteins.
*Cefixime susceptibility was not tested during 2007 and 2008.
†Results from January through August, 2011.

TREATMENT

Because of ever-changing gonococcal susceptibility patterns and variations in susceptibility in different international regions, practitioners must remain alert for modifications of treatment guidelines for their respective geographic locations. Based on the current patterns of antimicrobial resistance in prevalent *N. gonorrhoeae* strains in the United States, the third-generation cephalosporin ceftriaxone is the only remaining antibiotic routinely recommended as initial therapy in children and adults as of August, 2012.[72]

The recommendations for treatment of childhood gonorrhea in the United States discussed in this section and listed in Tables 89-6 and 89-7 are based on 2010 guidelines from the CDC[65,68,69] and the 2012 Report of the Committee on Infectious Diseases of the American Academy of Pediatrics.[5] CDC guidelines are based on the stringent clinical efficacy criterion of an expected 95 percent efficacy with lower bound of 95 percent confidence interval of 90 percent.[254]

Treatment of Infants Born to Mothers with Gonococcal Infection

Infants born to mothers with untreated gonorrhea are at high risk for acquiring infection (e.g., GON and disseminated infection); consequently, even without overt signs of infection, such infants should be treated with a single injection of ceftriaxone (see Table 89-6). Ceftriaxone should be given cautiously to hyperbilirubinemic infants, especially premature ones. A single dose of cefotaxime 100 mg/kg intravenously or intramuscularly is an acceptable alternative. Topical prophylaxis for neonatal ophthalmia is not adequate therapy for documented infections of the eye or other sites.[71]

TABLE 89-6 **Treatment of Gonococcal Infections in the Neonatal Period**

Disease Category	Treatment Regimen	Comments
Infants born to mothers with gonococcal infection *or* Gonococcal ophthalmia neonatorum (GON) or other focal sites of infection (i.e., rectum, pharynx, vagina, urethra)	Ceftriaxone 25-50 mg/kg IV or IM, not to exceed 125 mg (single dose)*†‡	Infants with GON should receive eye irrigation with saline solution immediately on recognition and at frequent intervals subsequently until the discharge is eliminated
Disseminated gonococcal infection (septic arthritis, sepsis, meningitis) or scalp abscess	Ceftriaxone 25-50 mg/kg IV or IM once daily for 7 days (10-14 days for meningitis) *or* Cefotaxime 25 mg/kg IV or IM every 12 hours for 7 days (10-14 days for meningitis)	Cefotaxime is preferred for infants with hyperbilirubinemia who require more than a single dose of therapy If meningitis is present, higher or more frequent doses in the recommended ranges may be needed

Data compiled from Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):1-109; and American Academy of Pediatrics: Gonococcal infections. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012, p. 336-344.
*If ceftriaxone is unavailable or contraindicated, cefotaxime, 100 mg/kg IV or IM (single dose) is an alternative.
†Topical agents alone are inadequate and unnecessary when recommended systemic antibiotics are given.
‡For GON, some experts prefer to continue parenteral therapy with one of these agents until blood (with or without cerebrospinal fluid) cultures have been negative for 48 to 72 hours.
IM, Intramuscularly; *IV,* intravenously.

TABLE 89-7 **Treatment of Gonococcal Infections in Children Beyond the Neonatal Period**

	Prepubertal Children Who Weigh <100 lb (45 kg)[a]	Patients ≥8 Years Old and Who Weigh >100 lb (45 kg)[a]
Uncomplicated Gonococcal Infections		
Vulvovaginitis, endocervicitis, urethritis, proctitis or pharyngitis	Ceftriaxone 125 mg IM in a single dose[b]	Ceftriaxone 250 mg IM in a single dose[b]
Conjunctivitis[c]	Ceftriaxone 50 mg/kg (maximum, 1 g) IM in a single dose	Ceftriaxone 1 g IM in a single dose
Disseminated Gonococcal Infection		
Septic arthritis, sepsis, or arthritis-dermatitis syndrome[d]	Ceftriaxone 50 mg/kg/day (maximum, 1 g/day) IV or IM once daily for 7 days[e,f]	Ceftriaxone, 1 g IV or IM once daily for 7 days[e,f]
Meningitis or endocarditis[g]	Ceftriaxone 50 mg/kg/day (maximum, 2 g/day) IV or IM given twice daily; for meningitis, the duration is 10-14 days; for endocarditis, the duration is at least 28 days	Ceftriaxone 1-2 g IV twice daily; for meningitis, the duration is 10-14 days; for endocarditis, the duration is at least 28 days
Epididymitis Pelvic inflammatory disease		Ceftriaxone, 250 mg IM in a single dose
Treatment for presumed coinfection with *C. trachomatis*	Erythromycin base or ethylsuccinate 50 mg/kg/day (maximum 2 g/day) PO in 4 divided doses for 14 days *or* Azithromycin 20 mg/kg (maximum 1 g) PO in a single dose[i]	See text for description of options Azithromycin 1 g PO in a single dose *or* Doxycycline 100 mg PO twice daily for 7 days[h]

Note: See text for discussion of alternative treatment options, including those for patients with allergies to the recommended regimens above.

Data compiled from Centers for Disease Control and Prevention: Sexually transmitted diseases treatment guidelines 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):1-109; American Academy of Pediatrics: Gonococcal infections. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases.* 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012, p. 336-344; and Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases guidelines, 2010: oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:590-594.

[a]If ceftriaxone is not available, alternatives are (1) in children <45 kg weight: cefixime 8 mg/kg (maximum 400 mg) PO in a single dose, plus test of cure in 1 week; and (2) in children >8 years old and >45 kg weight: cefixime 400 mg in a single oral dose plus test of cure in 1 week.

[b]Therapy for potential (presumptive) coinfection with *Chlamydia trachomatis* also is recommended.

[c]Eyes should be lavaged with saline initially and at regular intervals until secretions no longer continue to accumulate.

[d]Hospitalization should be considered for children based on clinical severity, including those (1) who are unlikely to receive the prescribed treatment because of personal or parent/guardian failure to adhere to the regimen or (2) whose infection has not responded to outpatient therapy.

[e]Some experts advise a 10- to 14-day course of therapy for gonococcal sepsis or septic arthritis.

[f]For older children and adolescents, parenteral therapy can be discontinued 24 to 48 hours after improvement occurs and the 7-day course completed with an appropriate oral antimicrobial agent, based on antimicrobial susceptibility results.

[g]Children or adolescents with gonococcal meningitis or endocarditis should be hospitalized initially.

[h]Prevalence of tetracycline resistance is high among Gonococcal Isolate Surveillance Project isolates, particularly among those with elevated minimum inhibitory concentrations to cefixime. Azithromycin thus is the preferred second antimicrobial for treatment of presumed chlamydial coinfection.

[i]Data are limited regarding efficacy of azithromycin for treatment of chlamydia in young children, but the single dose and favorable side effect profile make this agent a reasonable choice.

IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

Treatment of Neonates with Gonococcal Infection

Neonates with suspected gonococcal infection of any site, including GON, should be admitted to the hospital for parenteral antibiotics and any needed supportive care. Many but not all experts recommend that CSF studies be performed even in afebrile, otherwise well-appearing infants whose only clinical manifestation is apparent GON.

The presence of typical gram-negative diplococci in Gram-stained specimens is sufficient justification to begin treatment of GON or gonococcal infection in other sites. The absence of gram-negative diplococci in Gram-stained specimens is not sufficient to abrogate presumptive treatment of GON in a neonate with conjunctival exudate.

Tests for concomitant *C. trachomatis* infection, HIV infection, and congenital syphilis should be performed in infants with gonococcal infection at any site. The mother and her partners should be evaluated for gonococcal infection (and other STIs) and treated according to the recommendations for gonococcal infection in adolescents and adults (see the next section).

Infants with GON can be treated with a single dose of ceftriaxone 25 to 50 mg/kg intravenously or intramuscularly, not to exceed 125 mg (see Table 89-6). This regimen also may be used for infants with other sites of nondisseminated gonococcal infection, including the rectum, pharynx, vagina, and urethra. A single dose of

cefotaxime 100 mg/kg given intravenously or intramuscularly is an alternative treatment of GON. Some experts prefer to continue parenteral therapy with one of these agents until blood cultures (with or without CSF cultures) have been negative for 48 to 72 hours. Infants with GON also should receive eye irrigation with saline solution immediately on recognition and at frequent intervals until the discharge is eliminated. Topical antimicrobial agents alone are inadequate and unnecessary when the recommended systemic antibiotics are given.[5,71,133,208]

Simultaneous infection with *C. trachomatis* should be considered a potential explanation for neonates who do not respond satisfactorily to the recommended treatment.

Disseminated Gonococcal Infection or Scalp Abscess

The recommended therapy for gonococcal arthritis, scalp abscess, and sepsis is ceftriaxone 25 to 50 mg/kg intravenously or intramuscularly given once per day for 7 days or cefotaxime 50 mg/kg/day given intravenously or intramuscularly in two divided doses for 7 days will be adequate for many infants (dose and interval adjustments may be needed depending on gestational and chronologic age and other clinical considerations). Cefotaxime is preferred for infants with hyperbilirubinemia. For gonococcal meningitis, treatment should be continued for 10 to 14 days, with consideration of the use of higher daily doses of these agents (see Table 89-6).[71]

Treatment of Gonococcal Infections Beyond the Neonatal Period

Treatment recommendations for children and adolescents with uncomplicated and complicated gonococcal infection, including PID, are outlined in Table 89-7. All treatment regimens include agents active against *C. trachomatis* in addition to *N. gonorrhoeae* (see later discussion).

Children who are postpubertal or who weigh more than 45.4 kg (100 lb) may be treated with dosage regimens as defined for adults. The recommended regimens generally have not been studied in populations of prepubertal children with either uncomplicated or complicated gonococcal infection, but these regimens are likely to be highly effective in most cases, as they are for older adolescents and adults. In adults with an uncomplicated gonococcal infection at any site, 99 percent of cases are cured by ceftriaxone. As a general rule, children treated with ceftriaxone do not require follow-up cultures, but if treatment regimens other than ceftriaxone are used, follow-up cultures may be indicated.[71]

The standard intramuscular dose for older children and adults was increased to 250 mg from 125 mg in 2010. This change was made due to increasingly widespread prevalence globally of strains with decreased susceptibility to cephalosporins in vitro, reports of treatment failures with the lower dose, and improved efficacy of the 250 mg dose in pharyngeal infections. Up-to-date information on treatment regimens for gonorrhea and other STIs can be obtained from the CDC website http://www.cdc.gov/std/treatment.

Alternatives to Ceftriaxone

Cefixime has an antimicrobial spectrum similar to that of ceftriaxone. Until recently, a single oral dose of cefixime was recommended for routine treatment of uncomplicated gonococcal infections. Increasing prevalence of gonococcal strains with decreased susceptibility to third-generation cephalosporins has led to the change in recommendations.[72] The 400-mg oral dose recommended until recently for adolescents and adults does not provide as high or as sustained a bactericidal concentration as that provided by a 125-mg parenteral doses of ceftriaxone. Cefixime remains effective for the majority of isolates that are fully susceptible to third-generation cephalosporins and can be used if susceptibility of a cultured isolate has been confirmed. The dosage in children weighing less than 45 kg is 8 mg/kg (maximum 400 mg) as a single oral dose. For children older than 8 years of age and weighing more than 45 kg, a single oral dose of 400 mg is used.

Other single-dose parenterally administered cephalosporins that are safe and likely effective against uncomplicated gonococcal infection in adolescents and adults include cefotaxime 500 mg intramuscularly, ceftizoxime 500 mg intramuscularly, and cefoxitin 2 g intramuscularly, with probenecid 1 g orally. None of these offer any advantage over recommended doses of ceftriaxone.[71]

Some evidence indicates that cefpodoxime 400 mg orally is effective for urogenital gonorrhea and cefuroxime axetil 1 g orally is effective for urogenital and rectal gonorrhea in older adolescents and adults. Neither agent is adequate for treatment of pharyngeal infection, at least as a single dose.[71] No data are available regarding use of these agents for gonococcal infections in children.

The fluoroquinolone antibiotics ciprofloxacin, ofloxacin, and levofloxacin have been used widely for treating gonococcal infection in adults. Cure rates for uncomplicated gonococcal infection with both agents exceed 98 percent in adults with susceptible isolates. These agents no longer are recommended for routine treatment of gonococcal infections in the United States and many other parts of the world because of substantial resistance rates.[68,69,72] They remain an option for adults, and potentially for children in selected circumstances, when the infecting gonococcal strain is known to be susceptible to them. These are contraindicated for women who are pregnant or nursing.

Spectinomycin would be an effective alternative in the United States, based on antimicrobial susceptibility surveillance, in most cases in which other agents cannot be used.[5] However, spectinomycin remains unavailable in the United States as of August 2012. Azithromycin (i.e., the higher, 2-g, dose in adolescents and adults) may be useful in circumstances in which patients are unable to take standard regimens because of allergies, pregnancy, or concerns of age-related toxicity (e.g., dental staining with tetracyclines) or when no other alternatives are available. Azithromycin 1 g orally as a single dose meets alternative regimen criteria, but treatment failures have been documented and there are ongoing concerns about

rapid emergence of macrolide resistance among *N. gonorrhoeae* should this regimen be used widely.[71]

Uncomplicated Pharyngeal Gonorrhea

Gonococcal infection of the pharynx is more difficult to eradicate than infection of urogenital and anorectal sites. Chlamydial infection of the pharynx is an unusual occurrence, but genital coinfection may be present. A single dose of ceftriaxone 250 mg intramuscularly is the preferred treatment of uncomplicated pharyngeal gonococcal infection. A single dose of an oral fluoroquinolone may be used if the isolate is known to be susceptible to these agents. Spectinomycin (when available) is unreliable against *N. gonorrhoeae* in the pharynx; it eradicates the organism in only approximately 50 percent of cases.[71]

Pelvic Inflammatory Disease

PID is presumed to be polymicrobial, including anaerobes, in addition to *N. gonorrhoeae*. Combination antimicrobial regimens are required. Hospitalization is warranted for adolescents who are pregnant; have potential surgical emergencies other than PID, including tubo-ovarian abscess; have not responded to an oral regimen already prescribed for the episode; are unable to follow or tolerate an oral regimen; or have severe illness, nausea and vomiting, or high fever.

Two parenteral regimens are recommended for those requiring hospitalization: (1) cefotetan 2 g intravenously every 12 hours *or* cefoxitin 2 g intravenously every 6 hours, combined with doxycycline 100 mg orally or intravenously every 12 hours; or (2) clindamycin 900 mg intravenously every 8 hours plus gentamicin, using a loading dose intravenously or intramuscularly of 2 mg/kg of body weight followed by maintenance doses of 1.5 mg/kg every 8 hours. Single daily dosing of gentamicin 3 to 5 mg/kg per dose can be used. Parenteral therapy can be discontinued 24 hours after clinical improvement. Oral doxycycline or clindamycin should then be continued to complete a total of 14 days of therapy. Clindamycin is preferred for course completion when tubo-ovarian abscess is present. An alternative regimen, based on limited data, is ampicillin-sulbactam 3 g intravenously every 6 hours plus doxycycline 100 mg orally or intravenously every 12 hours.[71]

Outpatient regimens recommended for adolescents who are not severely ill as discussed earlier and appear able to tolerate oral agents are (1) ceftriaxone 250 mg intramuscularly as a single dose plus doxycycline 100 mg orally twice daily for 14 days, with or without metronidazole 500 mg orally twice daily for 14 days; or (2) cefoxitin 2 g intramuscularly as a single dose, plus probenecid 1 g orally concurrently as a single dose, plus doxycycline 100 mg orally twice daily for 14 days, with or without metronidazole 500 mg orally twice daily for 14 days. Other parenteral third-generation cephalosporins (e.g., cefotaxime or ceftizoxime) may be substituted for ceftriaxone or cefoxitin in these regimens. Limited data suggest that amoxicillin-clavulanate plus doxycycline may be effective, although gastrointestinal symptoms may limit compliance. Ceftriaxone 250 mg intramuscularly as a single dose, plus azithromycin 1 g orally once per week for 2 weeks also may be effective. Addition of metronidazole to such alternative regimens should be considered.[71]

Quinolone-based oral regimens are no longer recommended for routine use because of prevalence of gonococcal resistance to these agents. However, if a cephalosporin-based regimen is not feasible, a fluoroquinolone plus metronidazole may be used (both for 14 days) if a culture for *N. gonorrhoeae* showing positive susceptibility to quinolones is documented. Levofloxacin 500 mg orally once daily or ofloxacin 400 mg orally once daily are the agents of choice.[71]

Substantial clinical improvement is expected within 72 hours after initiation of therapy. Those failing oral regimens should be hospitalized and switched to parenteral regimens. Diagnostic testing (i.e., imaging or laparoscopy) should be considered.

Severe Allergies to Cephalosporins

Selection of alternative antibiotic therapies for persons with a history of a reaction to a cephalosporin must be guided by the severity of the reaction and the availability of suitable alternative regimens.[64] Desensitization can be considered but often is impractical. Azithromycin is probably the best available choice at this time for persons with documented severe reactions to penicillins or cephalosporins.[71] If circumstances permit delay in treatment until antibiotic susceptibility test results can be obtained, other agents such as fluoroquinolones or doxycycline may be used if the isolate is shown to be susceptible.

Azithromycin 2 g orally is effective against uncomplicated gonococcal infection in adolescents and adults, but it is expensive and frequently associated with gastrointestinal problems. Because of these issues and concern about rapid emergence of resistance, it is not recommended for routine use.[65] A single dose of 20 mg/kg (maximum, 1 g) is recommended for the treatment of chlamydial infection in children, but no dose recommendations for azithromycin are available for the treatment of gonococcal infection in children.[5]

Patients should be observed for at least 30 minutes after the ingestion of a dose of 2 g of azithromycin to monitor tolerance of the medication.[56] An oral dose of 1 g of azithromycin cures approximately 93 percent of uncomplicated gonococcal infections in adults. Although better tolerated, this dose is not sufficiently effective for routine use.

Spectinomycin 40 mg/kg given intramuscularly, with a maximal dose of 2 g, remains effective for uncomplicated infections and, if available, would be a good choice for cephalosporin-allergic patients.

Infection with Human Immunodeficiency Virus

Children and adolescents infected with HIV who acquire a gonococcal infection should receive the same treatment as persons without HIV infection.

Concurrent Syphilis Infection

A single dose of ceftriaxone is not adequate for treating syphilis. Longer courses of therapy are required with penicillin-based regimens (see Chapter 143).

Pregnancy

Pregnant women should not be treated with quinolone or tetracycline agents. Those unable to take one of the recommended cephalosporin regimens can be treated with the 2 g dosage of azithromycin. Spectinomycin would be appropriate if available.

Presumptive Treatment of *Chlamydia Trachomatis* Coinfection

Persons with gonococcal infection, including children, are at high risk for acquiring concurrent chlamydial infection. Coinfections frequently occur and were present in 50/5877 students attending an urban U.S. high school in 1998 to 1999 who participated in a school-based screening program.[258] Among adolescents in 14 U.S. juvenile detention centers from 1997 through 2002, more than 50 percent of the teens with gonorrhea were coinfected with *C. trachomatis*.[193] In adolescents, treatment of gonococcal cervicitis with drug regimens that are effective against gonococci but not *Chlamydia* has been associated with a high incidence of residual salpingitis in females and urethritis in males because of the ongoing presence of *C. trachomatis*.[267,281,284,307]

Therefore, treatment recommendations for gonococcal infections beyond the neonatal period include agents active against both organisms (Table 89-7). Neonates with gonococcal infections also should be evaluated for *C. trachomatis* coinfection. Penicillin, amoxicillin, ceftriaxone, or spectinomycin alone will fail to eradicate *C. trachomatis*. Trimethoprim-sulfamethoxazole, tetracycline, doxycycline, azithromycin, and erythromycin are effective in vitro and in many clinical forms of chlamydial disease.

Follow-Up

The recommended ceftriaxone regimens have such high cure rates that routine testing for cure is not recommended.[57,60,65,71,81] Children or adolescents who receive treatment regimens other than ceftriaxone should have test of cure by culture or nonculture methods 1 week after treatment.[72] Those who remain in at-risk environments for reinfection or for whom adherence is uncertain also should have follow-up evaluations.[5]

Treatment failures can occur, and cultures should be repeated for antibiotic susceptibility testing when symptoms persist after treatment. This precaution may lead to recognition of an antibiotic-resistant strain that could have public health implications.

Apparent treatment failure usually is the result of reinfection. Reinfection may be associated with a higher risk for PID and other complications of gonococcal infection. Therefore, retesting in 3 months for reinfection (not test of cure) is advisable for sexually active adolescents and adults, regardless of whether patients think their sex partners have been treated.[5,65]

GONOCOCCAL INFECTION AND SEXUAL ABUSE OF CHILDREN

When gonococcal infection or any other STI is identified in a prepubertal child or in adolescents who are not sexually active within their peer groups, sexual abuse must be considered to have occurred unless proved otherwise. Nonsexual transmission never should be assumed without extensive investigation of the social setting of an infected child and even then rarely.[5,252] Isolation of *N. gonorrhoeae* in cultures from children should be reported to local public health authorities and also to child protective services to ensure that the child is not exposed to further abuse. Sexual abuse has been reported in approximately 10 percent of all self-reporting populations of girls during childhood in numerous studies, and rates in boys are about 3 percent.[14,297] Approximately 1 percent of children appear to experience serious forms of sexual abuse yearly.[6]

STIs occur in 3 to 20 percent of sexually abused children.* Gonococcal infections have been found in 5 to 7 percent of sexually abused children.[107] The absence of STDs does not rule out sexual abuse when other findings suggest that it has occurred. Table 89-8 summarizes the STD diagnoses from eight studies involving children who had been evaluated for suspected sexual abuse.

The prevalence and clinical manifestations of gonococcal infection in sexually abused children are determined by the prevalence of infection in the adults who constitute the population with whom the child resides, the age of the child, and the type and frequency of sexual contact.[264] Gonorrhea was the STI recognized most frequently in abused children when gonococcal infection was highly prevalent in the general population. As a result of improved control of gonorrhea in the 1980s, fewer pediatric cases have been recognized in the United States in recent years. Nonetheless, girls who are exposed to an infected male appear to have a high rate of acquired disease. In an outbreak in an orphanage, 53 of 95 abused girls were found to have contracted infection.[2]

Among prepubertal girls who have a vaginal discharge after being sexually abused, 9 to 24 percent have been found to have gonococcal infection.[141,181,310,313] The vast majority of cases of gonorrhea in prepubertal children are associated with vaginal discharge. A recent series documented gonococcal infection in vaginal specimens from two girls evaluated for suspected sexual abuse who did not have vaginal discharge.[141] The oropharynx also is a common site of gonococcal infection associated with sexual abuse of children. This may be the only site of infection in children forced to perform orogenital sexual acts with an infected abuser. Pharyngeal infection may be asymptomatic. Although few children who suffer sexual abuse subsequently contract an STI, the diagnosis of an STI is a very important indication that the child probably

*References 107, 141, 181, 182, 247, 290, 313, 348, 353.

TABLE 89-8 **Sexually Transmitted Diseases in Prepubertal Children Evaluated for Suspected Sexual Abuse**

Reference	No. of Children Evaluated	Number (%) Who Had A Diagnosis of				
		Gonorrhea (%)	Chlamydiosis (%)	Syphilis (%)	Trichomoniasis (%)	Condylomata Acuminata (%)
Wald et al, 1980[348]	189	28 (14.8)	ND	ND	ND	ND
Rimsza and Niggemann, 1982[290]	285	21 (7.4)	ND	0	ND	ND
White et al, 1983[353]	409	46 (10)	ND	6 (5.5)	4 (18)	3 (5.6)
Ingram et al, 1984[182]	50	10 (20)	3 (6)	ND	2 (4)	ND
DeJong, 1986[107]	532	25 (4.7)	ND	1 (0.2)	ND	3 (0.6)
Ingram et al, 1992[181]	1469	41 (2.8)	17 (1.2)	1 (0.1)	3 (2)	28 (2)
Siegel et al, 1995[313]	855*	12 (1.4)	11 (1.3)	0	4 (0.5)	ND
Muram et al, 1996[247]	865	12 (1.4)	ND	ND	ND	ND
Girardet et al, 2009[141]	485†	16 (3.3)	15 (3.1)	1 (0.2)	5 (5.9)‡	ND

*These children also were evaluated for human immunodeficiency virus infection, and all were found to be uninfected.
†These are the data for girls evaluated in the study. Fifty-one boys were included as well, and none had evidence of gonococcal or chlamydial infection.
‡Only 85 of the girls had wet mounts performed. The percentage positive reflects this subgroup.
ND, No data.

has been abused, and an STI may be the sole finding on physical examination.[179]

Children with suspected gonococcal infection (or suspected to have suffered sexual abuse) should undergo a thorough physical examination by someone familiar with the medicolegal and clinical aspects of sexually abused children. Genital, rectal, and pharyngeal cultures should be obtained. Vaginal cultures are satisfactory in prepubertal girls. Endocervical cultures should be obtained only after puberty. Blood culture should be performed if disseminated disease is a consideration. Other sites (e.g., conjunctivae and joint fluid) should be cultured if clinically indicated. Cultures should be handled in a manner that will ensure legal acceptance if needed (see later discussion).

Nonculture methods may be used in conjunction with routine cultures to increase sensitivity in detecting gonococcal infection. These test results, when rapidly available, may permit more rapid initiation of therapy than possible with standard culture results.[264] NAATs (see earlier discussion) may be used as an alternative to culture for forensic purposes for vaginal and urine specimens in girls, but not for these specimens from boys or extragenital specimens in boys or girls.[71]

Selective criteria based on the observations that most sexually abused children do not have gonococcal infection or other STIs and that the vast majority of prepubertal girls with gonococcal infection will have clinical signs of vulvovaginitis have been developed to determine which children undergoing evaluation for sexual abuse should be tested for the presence of gonococcal infection and other STIs.[180,247,310,312,313] These criteria are (1) the known presence of any STI in the child, a sibling, another household member, a close associate of the child, or the apparent perpetrator or (2) a history or physical findings suggesting that oral, genital, or rectal contact has occurred.[180] It is highly unlikely that a sexually abused child with gonococcal or chlamydial infection will be missed through the use of these criteria. Meanwhile, the number of potentially sexually abused children who must undergo culture is greatly decreased.[180,183] Because rectal and pharyngeal gonococcal infections in girls and boys and genital infections in boys often are asymptomatic, these selective criteria may be most relevant to vaginal cultures in girls.[59]

Children who are being evaluated for sexual abuse should be evaluated for other STIs, including chlamydial disease, syphilis, hepatitis B, and HIV infection. Girls also should be evaluated for evidence of *Trichomonas vaginalis* and bacterial vaginosis by wet mount of vaginal swabs.[5,71,157,299] (Children with confirmed gonococcal infections should be evaluated similarly.) Repeat physical examination and specimen collection may be warranted 2 weeks after initial evaluation, especially when the suspected abuse event was very recent. Repeat serologic testing for syphilis, hepatitis B, and HIV may be warranted at about 12 weeks after the most recent event. A single evaluation may be sufficient if suspected abuse occurred over an extended period and a substantial amount of time has elapsed since occurrence of the last suspected episode. This is determined on a case-by-case basis.[71]

The following are general issues for evaluation and management of children who may have been sexually abused:

1. Many institutions have a clinical service or clinician with training and skill in interviewing and examining children suspected of having experienced sexual abuse. Direct involvement by or consultation with persons with such expertise should be sought at the time of initial evaluation.[262]
2. If the child is symptomatic, all available and appropriate cultures and examinations should be completed before the child receives therapy. Because asymptomatic gonococcal infections of the oropharynx and rectum occur commonly, and vaginal infection in prepubertal girls can occur without discharge, strong consideration should be given to obtaining cultures, even in the absence of symptoms, especially for children and adolescents who meet the selective criteria described earlier.[30,128,166]
3. A chain-of-custody policy that ensures proper handling, labeling, and delivery of diagnostic samples to the appropriate laboratory is essential for test results to be used in any court procedures when issues of the child's safety are being considered.
4. If sexual abuse is confirmed or suspected by the social history, physical findings, or laboratory results, the child should not be allowed to leave the evaluation site until the child's safety has been ensured. This determination usually is made by the local department of social services and may require involvement of law enforcement agencies. Temporary hospitalization of the child may be needed to permit sufficient time for any additional investigations to be performed. Reporting of suspected and confirmed cases to the social service of the county in which the child resides is required by child abuse reporting laws.
5. Most children who have been victims of sexual abuse will not have specific physical findings. Orogenital contact is a common form of abuse, as are fondling and external genital contact. These forms of abuse may occur without physical signs of injury.
6. If the child is identified with an STI, other members of the family may be infected as well. Other female children are especially likely to be infected and should be examined.[128,136]
7. Internal pelvic examination of prepubertal children rarely is necessary unless there has been major trauma or there are concerns regarding foreign bodies. Internal examination of prepubertal girls should be performed by an experienced examiner with appropriate sedation or anesthesia.
8. Children often have compelling reasons to deny abuse and frequently do not disclose sexual abuse, even to skillful forensic interviewers.[212] Denial, therefore, is not compelling disproof of sexual abuse.
9. Sexual abuse of children can be a repeated event if the perpetrator has ongoing access to the child. If the child remains unprotected and the perpetrator has an STI, the child may be subject to recurrent episodes of that STI.[211]
10. Counseling with providers skilled in helping children who have been sexually abused should be part of long-term follow-up care. The likelihood of the child having serious emotional harm is greater when abuse has been more intrusive or violent, occurred repetitively over longer periods, or was committed by someone in a close relationship with the child.[86] Parents of victims also may need treatment and support.
11. Behavioral changes such as developmentally unusual sexual behavior or acting out can be a manifestation of child sexual abuse.[134] New onset of symptoms such as abdominal pain (which may lead to medical attention when it becomes persistent), enuresis, encopresis, sleep disturbances, and phobias can be manifestations of child sexual abuse. These issues also can arise from physical or emotional abuse or from non–abuse-related social stressors and do not indicate sexual abuse per se.[86]

PREVENTION AND CONTROL OF GONOCOCCAL INFECTIONS

All gonococcal infections must be reported to public health officials. Effort should be made to evaluate, counsel, and treat all sexual partners who were exposed to the index case within 60 days before the onset of symptoms or diagnosis in the index case. If a patient's last sexual intercourse occurred more than 60 days before the onset of symptoms, the most recent sexual partner of the patient should be evaluated and treated. Patients should be instructed to avoid engaging in sexual activity until therapy is completed and any symptoms have resolved.[69] Cases in prepubertal children must be investigated to identify the source of infection (and potential perpetrator of abuse).[5]

Condoms provide a high degree of protection against the acquisition and transmission of genital gonococcal infection.[191,342] Other barrier contraceptive measures and topical spermicidal and bactericidal agents likewise can reduce the likelihood of acquiring gonococcal (and chlamydial) infection.[15,54,226,331] Postexposure prophylactic antibiotics also reduce the risk for infection developing but are unlikely to be cost-effective.[164]

When patients, including infants with ophthalmia neonatorum,[5] with gonococcal infection are hospitalized, standard precautions are recommended.

Prevention of Neonatal Infection

Pregnant women should have an endocervical culture for *N. gonorrhoeae* as part of their initial prenatal care visit. A second culture late in the third trimester should be performed for those at high risk for exposure during pregnancy. Pregnant adolescents are at higher risk for contracting gonococcal infection than are older women. Treatment options are listed in Table 89-8. Fluoroquinolone and tetracycline antibiotics should not be used during pregnancy because of potential fetal toxicity.[5]

Infants born to mothers known to have active gonococcal infection are at risk for acquiring local and disseminated infection from colonization during birth. Such infants should be treated as listed in Table 89-7. Standard preventive measures for GON were described earlier.

Vaccine Development

Research into the development of vaccines for gonococcal infection is ongoing. Although natural infection induces antibody responses directed primarily against pili, Opa, porin protein, and LOS, these antibodies do not protect against reinfection even with the same strain. Serologic correlates of immunity remain elusive, making vaccine development more difficult. Two candidate vaccines have entered clinical trials. The first, a killed whole cell vaccine studied in the 1970s, induced antibody responses and was well tolerated but not protective. A purified pilin vaccine was studied in high-risk military personnel and also was not protective.[361]

Molecular analysis of gonococcal transferrin binding proteins may have potential as vaccine antigens because of their exposure on the microbial surface. These proteins are expressed in all strains and have highly conserved gene sequences. Natural infection, however, generates little systemic or local antibody response to transferrin binding proteins.[276] Viral replicon particles based on Venezuelan encephalitis virus that express antigenic components of transferrin binding proteins or porB have shown promise in a mouse model.[361] An oligosaccharide epitope (2C7 OS) of gonococcal LOS that is expressed on most strains and is widely conserved has been identified. A peptide that mimics the structure of 2C7 OS was able to induce antibodies in mice that possess dose-responsive, complement-dependent bactericidal activity against $2C7^{+}$ strains.[255]

As with meningococcal serogroup B strains, use of combinations of recombinant antigens, potentially delivered in different formats, may be necessary. The continued emergence of antimicrobial resistance in *N. gonorrhoeae* makes vaccine development more urgent.

NEW REFERENCES SINCE THE SIXTH EDITION

5. American Academy of Pediatrics. Red book: 2012 report of the Committee on Infectious Diseases. In: Pickering LK, editor. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
27. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
57. Centers for Disease Control and Prevention. *N. gonorrhoeae*. Available at *http://www.cdc.gov/std/Gonorrhea/lab/default.htm* (accessed 09/04/2012).
70. Centers for Disease Control and Prevention. *2010 sexually transmitted diseases surveillance, gonorrhea*. http://www.cdc.gov/std/stats10/gonorrhea.htm (accessed 09/02/2012).
71. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010; **59**(RR-12):1–109.
72. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases guidelines, 2010: oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:590–4.
73. Chacko MR, Phillips S, Jacobson MS. Screening for pharyngeal gonorrhea in the urban teenager. *Pediatrics* 1982;**70**:620–3.
74. Chan YA, Hackett KT, Dillard JP. The lytic transglycolases of *Neisseria gonorrhoeae*. *Microb Drug Resist* 2012;**18**:271–9.
82. Chung GT, Yoo JS, Oh HB, et al. Complete genome sequence of *Neisseria gonorrhoeae* NCCP11945. *J Bacteriol* 2008;**190**: 6035–6.
93. Cornelissen CN, Hollander A. TonB-dependent transporters expressed by *Neisseria gonorrhoeae*. *Front Microbiol* 2011. 2.117. doi: 10.3389/fmicb.2011.00117 (accessed 08/15/2012).
98. Criss AK, Seifert HS. A bacterial siren song: intimate interactions between *Neisseria* and neutrophils. *Nature Rev* 2012;**10**:178–90.
101. David M, Rumelt S, Weintraub Z. Efficacy comparison between povidone iodine 2.5% and tetracycline 1% in prevention of ophthalmia neonatorum. *Ophthalmology* 2011;**118**:1454–8.
115. Dickson SD, Alter SJ. Cutaneous gonococcal abscess of the abdomen in a child. *Pediatr Emerg Care* 2011;**27**:863–4.
119. Edwards JL, Bulter EK. The pathobiology of *Neisseria gonorrhoeae* lower female genital tract infection. *Front Microbiol* 2011. 2.102. doi: 10.3389/fmicb.2011.00102 (accessed 08/15/2012).
127. Falsetta ML, Steichen CT, McEwan AG, et al. The composition and metabolic phenotype of *Neisseria gonorrhoeae* biofilms. *Front Microbiol* 2011. 2.075 doi: 10.3389/fmicb.2011.00075 (accessed 08/15/2012).
138. Giannini CM, Kim HK, Mortensen J, et al. Culture of non-genital sites increases the detection of gonorrhea in women. *J Pediatr Adolesc Gynecol* 2010;**23**:246–52.
141. Girardet RG, Lahoti S, Howard LA, et al. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 2009;**124**:79–86.
155. Gulati S, Agarwal S, Vasudhev S, et al. Properdin is critical for antibody-dependent bactericidal activity against *Neisseria gonorrhoeae* that recruit C4b-binding protein. *J Immunol* 2012;**188**: 3416–25.
167. Hess D, Wu A, Golparian D, et al. Genome sequencing of *Neisseria gonorrhoeae* isolate of a successful international clone with decreased susceptibility/resistance to extended-spectrum cephalosporins. *Antimicrob Agents Chemother* 2012 epub PMID 22908152 (accessed 08/28/2012).
168. Heymans R, Golparian D, Bruisten SM, et al. Evaluation of *Neisseria gonorrhoeae* multiple-locus variable-number tandem-repeat analysis, *N. gonorrhoeae* multiantigen sequence typing, and full-length *porB* gene sequence analysis for molecular epidemiological typing. *J Clin Microbiol* 2012;**50**:180–3.
170. Hobbs MM, Sparling PF, Cohen MS, et al. Experimental gonococcal infection in male volunteers: cumulative experience with *Neisseria gonorrhoeae* strains FA1090 and MS11mkC. *Front Microbiol* 2011. 2.123 doi: 10.3389/fmicb.2011.00123 (accessed 08/15/2012).
198. Katz AR, Komeya AY, Soge OO, et al. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: case report of the first isolate identified in the United States. *Clin Infect Dis* 2012;**54**: 841–3.
218. Liu Y, Feinen G, Russell MW. New concepts in immunity to *Neisseria gonorrhoeae:* innate responses and suppression of adaptive immunity favor the pathogen, not the host. *Front Microbiol* 2011. 2.052. doi: 10.3389/fmicb.2011.00052 (accessed 08/15/2012).
224. Martin I, Sawatzky P, Allen V, et al. Emergence and characterization of *Neisseria gonorrhoeae* isolates with decreased susceptibilities to ceftriaxone and cefixime in Canada: 2001-2010. *Sex Transm Dis* 2012;**39**:316–23.
249. Nathawad R, Mendez H, Ahmad A, et al. Severe ocular reactions after neonatal ocular prophylaxis with gentamicin ophthalmic ointment. *Pediatr Infect Dis J* 2011;**30**:175–6.
277. Public Health Agency of Canada. *Reported cases and rates of gonorrhea by province/territory and sex, 1980 to 2009*. http://www.phac-aspc.gc.ca/std-mts/sti-its_tab/gonorrhea_pts-eng.php. (accessed 08/28/2012).
280. Ramsey ME, Woodhams KL, Dillard JP. The gonococcal genetic island and type IV secretion in pathogenic *Neisseria*. *Front Microbiol* 2011. 2.061. doi: 10.3389/fmicb.2011.00061. (accessed 08/15/2012).
321. So NS, Ostrowski MA, Gray-Owen SD. Vigorous response of human innate functioning IgM memory B cells upon infection by *Neisseria gonorrhoeae*. *J Immunol* 2012;**188**:4008–22.
327. Spivey MI, Paschall RT, Ferrett R, et al. *Neisseria:* avoiding the jump to conclusion. *J Child Sex Abuse* 2011;**20**:622–30.

332. Stupiansky NW, Van Der Pol B, Williams JA, et al. The natural history of incident gonococcal infection in adolescent women. *Sex Transm Dis* 2011;**38**:750–4.
340. Unemo M, Dillon J-AR. Review and international recommendation of methods for typing *Neisseria gonorrhoeae* isolates and their implications for improved knowledge of gonococcal epidemiology, treatment, and biology. *Clin Microbiol Rev* 2011;**24**:447–58.
341. Unemo M, Golparian D, Nicholas R, et al. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012;**56**:1273–80.
343. U.S. Preventive Services Task Force. Ocular prophylaxis for gonococcal ophthalmia neonatorum: reaffirmation recommendation statement. *Am Fam Physician* 2012;**85**:195–6.
361. Zhu W, Chen C-J, Thomas CE, et al. Vaccines for gonorrhea: can we rise to the challenge? *Front Microbiol* 2011. 2.124. doi: 10.3389/fmicb.2011.00124 (accessed 08/15/2012).

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 3 *GRAM-POSITIVE BACILLI*

CHAPTER 90

Diphtheria

Barbara W. Stechenberg

Diphtheria is an acute infectious disease caused by *Corynebacterium diphtheriae* or, less commonly, *Corynebacterium ulcerans*. *Corynebacterium pseudotuberculosis*, which primarily causes infections in sheep and goats, is not discussed here because it only rarely causes a diphtheria-like disease in humans. Infection by toxigenic strains of *C. diphtheriae* causes disease that is mediated by the production of an extracellular protein. Nontoxigenic strains also can cause disease, but it is usually less severe.

Before the discovery of antitoxin at the turn of the 20th century, the "strangling angel of children," as diphtheria once was called, was a significant cause of mortality in children and adults.[109] Apparent reference to diphtheria can be traced to the 5th century BC in the works of Hippocrates, *Epidemics III*, case 7.[79] In 1821, it was recognized as a specific entity by Brettoneau, who suggested that the disease was caused by a germ and that it could be transmitted from person to person. Brettoneau coined the origin of the modern term *diphtheria* from the Greek root *diphtheria*, which means "skin" or "hide."[50] In 1883, the causative agent was identified by Klebs in stained smears from diphtheritic membranes; in 1884, Löffler grew the organism on artificial media and showed that in guinea pigs it caused a fatal infection closely resembling human disease.

The toxin was purified in 1889 by Roux and Yersin, who found that toxin alone could cause the disease. Shortly thereafter, Behring and Kitasato discovered antitoxins when they immunized animals with toxins rather than bacteria. The use of antitoxin to treat children with diphtheria at the turn of the 20th century resulted in one of the largest decreases in mortality rates by a therapeutic intervention. In Germany alone, an estimated 45,000 lives were saved each year.[62]

ETIOLOGY

Corynebacteria (Klebs-Löffler bacilli) are irregularly staining, gram-positive, nonmotile, nonsporulating, pleomorphic bacilli.[54] The club-shaped appearance of the bacillus is not a true morphologic feature, but results from attempting to grow the bacillus on media that are nutritionally inadequate (Löffler media). The organism can be recovered most readily on media containing selective inhibitors that retard the growth of other microorganisms; a sheep blood agar–based medium containing fosfomycin (for selectivity) and Tindale medium (tellurite medium with cystine) are ideal.[43,44] *C. diphtheriae* and *C.*

REFERENCES

1. Abbott SL. Gonococcal tonsillitis-pharyngitis in a 5-year-old girl. *Pediatrics* 1973;**52**:287–9.
2. Ahmed HJ, Ilardi I, Antognoli A, et al. An epidemic of *Neisseria gonorrhoeae* in a Somali orphanage. *Int J STD AIDS* 1992;**3**: 52–3.
3. Alexander WJ, Griffith H, Housch JG, et al. Infections in sexual contacts and associates of children with gonorrhea. *Sex Transm Dis* 1984;**11**:156–8.
4. Allen JH, Barrere LE. Prophylaxis of gonorrhea ophthalmia of the newborn. *JAMA* 1949;**141**:522–5.
5. American Academy of Pediatrics. Red book: 2012 report of the Committee on Infectious Diseases. In: Pickering LK, editor. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
6. American Academy of Pediatrics Committee on Child Abuse and Neglect. Guidelines for the evaluation of sexual abuse of children. *Pediatrics* 1991;**87**:254–60.
7. Amstey MS, Steadman KT. Asymptomatic gonorrhea and pregnancy. *J Am Vener Dis Assoc* 1976;**3**:14–6.
8. Anderson B, Albritton WL, Biddle J, et al. Common β-lactamase–specifying plasmid in *Haemophilus ducreyi* and *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1984;**25**:296–7.
9. Angevine CD, Hall CB, Jacox RF. A case of gonococcal osteomyelitis: a complication of gonococcal arthritis. *Am J Dis Child* 1976;**130**:1013–14.
10. Apicella MA. Serogrouping of *Neisseria gonorrhoeae*: identification of four immunologically distinct acidic polysaccharides. *J Infect Dis* 1976;**134**:377–83.
11. Armstrong JH, Zacarias F, Rein MF. Ophthalmia neonatorum: a chart review. *Pediatrics* 1976;**57**:884–92.
12. Auman GL, Waldenberg LM. Gonococcal periappendicitis and salpingitis in a prepubertal girl. *Pediatrics* 1976;**58**:287–8.
13. Babl FE, Ram S, Barnett ED, et al. Neonatal gonococcal arthritis after negative prenatal screening and despite conjunctival prophylaxis. *Pediatr Infect Dis J* 2000;**19**:346–9.
14. Badgley RF, Allard HA, McCormick N, et al. *Sexual Offenses against Children*. Ottawa, Canada: Minister of Supply and Services; 1984.
15. Barlow D. The condom and gonorrhea. *Lancet* 1977;**2**:811–13.
16. Barnes RC, Holmes KK. Epidemiology of gonorrhea: current perspectives. *Epidemiol Rev* 1984;**6**:1–30.
17. Barsam PC. Specific prophylaxis of gonorrheal ophthalmia neonatorum: a review. *N Engl J Med* 1966;**274**:731–4.
18. Barten R, Meyer TF. DNA circle formation in *Neisseria gonorrhoeae*: a possible intermediate in diverse genomic recombination processes. *Mol Gen Genet* 2001;**264**:691–701.
19. Bauer FJ, Rudel T, Stein M, et al. Mutagenesis of the *Neisseria gonorrhoeae* porin reduces invasion in epithelial cells and enhances phagocyte responsiveness. *Mol Microbiol* 1999;**31**:903–13.
20. Bell TA, Grayston JT, Krohn MA, et al. Randomized trial of silver nitrate, erythromycin, and no eye prophylaxis for the prevention of conjunctivitis among newborns not at risk for gonococcal ophthalmitis. *Pediatrics* 1993;**92**:755–60.
21. Benson RA, Weinstock E. Gonorrheal vaginitis in children: a review of the literature. *Am J Dis Child* 1940;**59**:1083–96.
22. Best D, Ford CA, Miller WC. Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infection in pediatric private practice. *Pediatrics* 2001;**108**:e103.
23. Birji M, Everson JJ. Comparative virulence of opacity variance of *Neisseria gonorrhoeae* strain P9. *Infect Immun* 1981;**31**:965–70.
24. Biswas GD, Sparling PF. Characterization of *lbpA*, the structural gene for a lactoferrin receptor in *Neisseria gonorrhoeae*. *Infect Immun* 1995;**63**:2958–67.
25. Biswas GD, Blackman EY, Sparling PF. High-frequency conjugal transfer of a gonococcal penicillinase plasmid. *J Bacteriol* 1980;**143**: 1318–24.
26. Biswas GD, Sox T, Blackman E, et al. Factors affecting genetic transformation of *Neisseria gonorrhoeae*. *J Bacteriol* 1977;**129**: 983–92.
27. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
28. Black WJ, Schwalbe RS, Nachamkin I, et al. Characterization of *Neisseria gonorrhoeae* protein II phase variation by use of monoclonal antibodies. *Infect Immun* 1984;**45**:453–7.
29. Blake MS, Gotschlich EC. Purification and partial characterization of the major outer membrane protein of *Neisseria gonorrhoeae*. *Infect Immun* 1982;**36**:277–83.
30. Bogaerts J, Lepage P, DeClercq A, et al. Shigella and gonococcal vulvovaginitis in prepubertal Central African girls. *Pediatr Infect Dis J* 1992;**11**:890–2.
31. Bonin P, Tanino TT, Handsfield HH. Isolation of *Neisseria gonorrhoeae* on selective and nonselective media in a sexually transmitted disease clinic. *J Clin Microbiol* 1984;**92**:218–20.
32. Boslego JW, Tramont EC, Takafuji ET, et al. Effect of spectinomycin use on the prevalence of spectinomycin-resistant and of penicillinase-producing *Neisseria gonorrhoeae*. *N Engl J Med* 1987;**317**:272–8.
33. Bradford WL, Kelley HW. Gonococcic meningitis in a newborn infant. *Am J Dis Child* 1933;**46**:543–9.
34. Bramley J, Demarco de Hormaeche R, Constantinidou C, et al. A serum-sensitive, sialyltransferase-deficient mutant of *Neisseria gonorrhoeae* defective in conversion to serum resistance by CMP-NANA or blood cell extracts. *Microb Pathog* 1995;**18**:187–95.
35. Branch G, Paxton R. A study of gonococcal infections among infants and children. *Public Health Rep* 1965;**80**:347–52.
36. Brettin T, Altherr MR, Du Y, et al. Expression capable library for studies of *Neisseria gonorrhoeae*, version 1.0. *B M C Microbiol* 2005;**5**:50.
37. Bro-Jorgensen A, Jensen T. Gonococcal pharyngeal infections: report of 110 cases. *Br J Vener Dis* 1973;**49**:491.
38. Bronson JE, Holmberg I, Nygren B, et al. Vancomycin-sensitive strains of *Neisseria gonorrhoeae*: a problem for the diagnostic laboratory. *Pont J Vener Dis* 1973;**49**:452–3.
39. Brooks F, Israel KS, Petersen BH. Bactericidal and opsonic activity against *Neisseria gonorrhoeae* in sera from patients with disseminated gonococcal infection. *J Infect Dis* 1976;**134**:450–62.
40. Brooks GF, Darrow WW, Day JA. Repeated gonorrhea: an analysis of importance and risk factors. *J Infect Dis* 1978;**137**:161–9.
41. Brown D. Gonococcal arthritis in pregnancy. *South Med J* 1973;**66**:693–5.
42. Brown RT, Lossick JG, Mosure DJ, et al. Pharyngeal gonorrhea screening in adolescents: is it necessary? *Pediatrics* 1989;**84**: 623–5.
43. Brown WJ, Kraus ST. Gonococcal colony types. *JAMA* 1974;**228**:862–3.
44. Brunham RC, Binns B, Guijon F, et al. Etiology and outcome of acute pelvic inflammatory disease. *J Infect Dis* 1988;**158**:510–17.
45. Buchanan TM. Antigenic heterogeneity of gonococcal pili. *J Exp Med* 1975;**151**:1470.
46. Buchanan TM. Attachment role of gonococcal pili: optimum conditions and quantitation of adherence of isolated pili to human cells in vitro. *J Clin Invest* 1978;**61**:931.
47. Burry VF. Gonococcal vulvovaginitis and possible peritonitis in prepubertal girls. *Am J Dis Child* 1971;**121**:536–7.
48. Campbell LA, Yashin RE. Mutagenesis of *Neisseria gonorrhoeae*: absence or error-prone repair. *J Bacteriol* 1984;**106**:288–93.
49. Cannon JG, Sparling PF. The genetics of the gonococcus. *Annu Rev Microbiol* 1984;**38**:111–33.
50. Carbonetti NH, Simnad VI, Seifert HS, et al. Genetics of protein I of *Neisseria gonorrhoeae*: construction of hybrid porins. *Proc Natl Acad Sci U S A* 1988;**85**:6841–5.
51. Carifo K, Catlin BW. *Neisseria gonorrhoeae* autotyping: differentiation of clinical isolates based on growth responses on chemically defined media. *Appl Microbiol* 1973;**26**:223–30.
52. Casey SG, Shafer WM, Spitznagel JK. *Neisseria gonorrhoeae* survive intraleukocytic oxygen-independent antimicrobial capacities of anaerobic and aerobic granulocytes in the presence of pyocin lethal for extracellular gonococci. *Infect Immun* 1986;**52**: 384–9.
53. Cates W. Sexually transmitted organisms and infertility: the proof of the pudding. *Sex Transm Dis* 1984;**11**:113–16.
54. Cates Jr W, Weisner PJ, Curran JW. Sex and spermicides: preventing unintended pregnancy and infection. *JAMA* 1982;**248**: 1636–7.
55. Catlin BW. Nutritional profiles of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Neisseria lactamica* in chemically defined media

and the use of growth requirements for gonococcal typing. *J Infect Dis* 1973;**128**:178–94.
56. Centers for Disease Control and Prevention. *Alternatives to spectinomycin for the treatment of* Neisseria gonorrhoeae, n.d. Available at http://www.cdc.gov/std/specshortage.htm.
57. Centers for Disease Control and Prevention. *N. gonorrhoeae*, n.d. Available at http://www.cdc.gov/std/Gonorrhea/lab/default.htm (accessed 09/04/2012).
58. Centers for Disease Control and Prevention. Sentinel surveillance system for antimicrobial resistance in clinical isolates of *Neisseria gonorrhoeae*. *MMWR Morb Mortal Wkly Rep* 1987;**36**:585–6, 591–3.
59. Centers for Disease Control and Prevention. Plasmid-mediated antimicrobial resistance in *Neisseria gonorrhoeae*: United States, 1988 and 1989. *MMWR Morb Mortal Wkly Rep* 1990;**39**:284–93.
60. Centers for Disease Control and Prevention. 1998 Guidelines for treatment of sexually transmitted diseases. *MMWR Recomm Rep* 1998;**47**(RR-1):1–116.
61. Centers for Disease Control and Prevention. Summary of notifiable diseases, United States, 1999. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1–101.
62. Centers for Disease Control and Prevention. Fluoroquinolone-resistance in *Neisseria gonorrhoeae*, Hawaii, 1999, and decreased susceptibility to azithromycin in *N. gonorrhoeae*, Missouri, 1999. *MMWR Morb Mortal Wkly Rep* 2000;**49**:833–7.
63. Centers for Disease Control and Prevention. *Sexually Transmitted Diseases Surveillance 1999 Supplement: Gonococcal Isolate Surveillance Project (GISP) Annual Report—1999. Atlanta, U.S. Department of Health and Human Services, Public Health Service, October 2000*. Available at http://www.cdc.gov/nchstp/dstd/Stats_Trends/99GISP/gisp99.pdf.
64. Centers for Disease Control and Prevention. Screening tests to detect *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections—2002. *MMWR Recomm Rep* 2002;**51**(RR-15):1–38.
65. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines 2006. *MMWR Recomm Rep* 2006;**55**(RR-11):1–94.
66. Centers for Disease Control and Prevention. *Trends in reportable sexually transmitted diseases in the United States*, 2005. December 2006. Available at http://www.cdc.gov/std/stats05/trends2005.htm
67. Centers for Disease Control and Prevention. *Sexually Transmitted Disease Surveillance 2005 Supplement, Gonococcal Isolate Surveillance Project (GISP) annual report 2005*. Atlanta: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2007.
68. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases treatment guidelines, 2006: fluoroquinolones no longer recommended for treatment of gonococcal infections. *MMWR Morb Mortal Wkly Rep* 2007;**56**:332–6.
69. Centers for Disease Control and Prevention. *Updated recommended treatment regimens for gonococcal infections and associated conditions: United States*, April 2007. Available at http://www.cdc.gov/std/treatment/2006/updated-regimens.htm (accessed May 2007).
70. Centers for Disease Control and Prevention. *2010 sexually transmitted diseases surveillance, Gonorrhea*. http://www.cdc.gov/std/stats10/gonorrhea.htm (accessed 09/02/2012)
71. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):1–109.
72. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases guidelines, 2010: oral cephalosporins no longer a recommended treatment for gonococcal infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:590–4.
73. Chacko MR, Phillips S, Jacobson MS. Screening for pharyngeal gonorrhea in the urban teenager. *Pediatrics* 1982;**70**:620–3.
74. Chan YA, Hackett KT, Dillard JP. The lytic transglycolases of *Neisseria gonorrhoeae*. *Microb Drug Resist* 2012;**18**:271–9.
75. Charles AG, Cohen S, Kass MB, et al. Asymptomatic gonorrhea in prenatal patients. *Am J Obstet Gynecol* 1970;**108**:595–9.
76. Chen CJ, Sparling PF, Lewis LA, et al. Identification and purification of a hemoglobin-binding outer membrane protein from *Neisseria gonorrhoeae*. *Infect Immun* 1996;**64**:5008–14.
77. Chen J-Y. Prophylaxis of ophthalmia neonatorum: comparison of silver nitrate, tetracycline, erythromycin, and no prophylaxis. *Pediatr Infect Dis* 1992;**11**:1026–30.
78. Chen S-T, Clowes RC. Nucleotide sequence comparisons of plasmids pHD131, pJBl, pFA3, and pFA7 and p-lactamase expression in *Escherichia coli*, *Haemophilus influenzae*, and *Neisseria gonorrhoeae*. *J Bacteriol* 1987;**169**:3124–30.
79. Chesson HW, Gift TL, Pulver ALS. The economic value of reductions in gonorrhea and syphilis incidence in the United States, 1990-2003. *Prev Med* 2006;**43**:411–15.
80. Ching S, Lee H, Hook EW 3rd, et al. Ligase chain reaction for detection of *Neisseria gonorrhoeae* in urogenital swabs. *J Clin Microbiol* 1995;**33**:3111–14.
81. Christian CW, Pinto-Martin JA, McGowan KL. The management of prepubertal children with gonorrhea. *Clin Pediatr (Phila)* 1995;**34**:415–8.
82. Chung GT, Yoo JS, Oh HB, et al. Complete genome sequence of *Neisseria gonorrhoeae* NCCP11945. *J Bacteriol* 2008;**190**:6035–6.
83. Cohen MS, Sparling PF. Mucosal infection with *Neisseria gonorrhoeae*. *J Clin Invest* 1992;**89**:1699–707.
84. Cohen MS, Cannon JC, Jerse AE, et al. Human experimentation with *Neisseria gonorrhoeae*: rationale, methods, and implications for the biology of infection and vaccine development. *J Infect Dis* 1994;**169**:532–7.
85. Colin MJ, Weissmann G. Disseminated gonococcal infection and tenosynovitis from an asymptomatically infected intrauterine contraceptive device. *N Engl J Med* 1976;**294**:598–9.
86. Committee on Child Abuse and Neglect, American Academy of Pediatrics. Guidelines for evaluation of sexual abuse of children: subject review. *Pediatrics* 1999;**103**:186–91.
87. Connell TD, Black WJ, Kawula TH, et al. Recombination among protein II genes of *Neisseria gonorrhoeae* generates new coding sequences and increases structural variability in the protein II family. *Mol Microbiol* 1988;**2**:227–36.
88. Cooke SJ, de la Paz H, Poh CL, et al. Variation within serovars of *Neisseria gonorrhoeae* detected by structural analysis of outer-membrane protein PIB and by pulsed-field gel electrophoresis. *Microbiology* 1997;**143**:1415–22.
89. Cooperman MB. *Gonococcus* arthritis in infancy: a clinical study of forty-four cases. *Am J Dis Child* 1927;**33**:932–48.
90. Cooperman MB. End results of gonorrheal arthritis: a review of seventy cases. *Am J Surg* 1928;**5**:241–51.
91. Corman LC, Levison ME, Knight R, et al. The high frequency of pharyngeal gonococcal infection in a prenatal clinic population. *JAMA* 1974;**230**:568–70.
92. Cornelissen C, Sparling PF. Iron piracy: Acquisition of transferrin-bound iron by bacterial pathogens. *Mol Microbiol* 1994;**14**:843–50.
93. Cornelissen CN, Hollander A. TonB-dependent transporters expressed by *Neisseria gonorrhoeae*. *Front Microbiol* 2011. 2.117. doi: 10.3389/fmicb.2011.00117 (accessed 08/15/2012).
94. Coulter K. Migratory polyarthritis in a nine-year-old girl. *Pediatr Infect Dis J* 1990;**9**:856–7.
95. Cramolini GM, Litt IF. The pharynx as the only positive culture site in an adolescent with disseminated gonorrhea. *J Pediatr* 1982;**100**:644–6.
96. Crawford G, Knapp JS, Hale J, et al. Asymptomatic gonorrhea in men caused by gonococci with unique nutritional requirements. *Science* 1977;**196**:1352.
97. Credé CSF. Reports from the obstetrical clinic in Leipzig: prevention of eye inflammation in the newborn. *Am J Dis Child* 1971;**121**:3–4.
98. Criss AK, Seifert HS. A bacterial siren song: intimate interactions between *Neisseria* and neutrophils. *Nature Rev* 2012;**10**:178–90.
99. D'Amato RF, Eriquez LA, Tomfohrde KM, et al. Rapid identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by using enzymatic profiles. *J Clin Microbiol* 1978;**7**:77–81.
100. Danielsson D, Johannisson G. Culture diagnosis of gonorrhea: a comparison of the yield with selective and non-selective gonococcal culture media inoculated in the clinic and after treatment of specimens. *Acta Derm Venereol* 1973;**53**:75–80.
101. David M, Rumelt S, Weintraub Z. Efficacy comparison between povidone iodine 2.5% and tetracycline 1% in prevention of ophthalmia neonatorum. *Ophthalmology* 2011;**118**:1454–8.
102. D'Auria A, Tan L, Kreitzer M, et al. Gonococcal scalp-wound infection. *MMWR Morb Mortal Wkly Rep* 1975;**24**:115–16.
103. Dawar S, Hellerstein S. Gonorrhea as a cause of asymptomatic pyuria in adolescent boys. *J Pediatr* 1972;**81**:357–8.

104. Deheragoda P. Diagnosis of rectal gonorrhea by blind anorectal swabs compared with direct vision swabs taken via a proctoscope. *Br J Vener Dis* 1977;**53**:311.
105. Dehio C, Gray-Owen SD, Meyer TF. The role of neisserial opa proteins in interactions with host cells. *Trends Microbiol* 1998;**6**:489–95.
106. Dehio M, Gomez-Duarte OG, Dehio C, et al. Vitronectin-dependent invasion of epithelial cells by *Neisseria gonorrhoeae* involves alpha (V) integrin receptors. *F E B S Lett* 1998;**424**: 84–8.
107. DeJong AR. Sexually transmitted diseases in sexually abused children. *Sex Transm Dis* 1986;**13**:123–6.
108. Demarco de Hormaeche R, Jessop H, Senior K. Gonococcal variants selected by growth in vivo or in vitro have antigenically different LPS. *Microb Pathog* 1988;**4**:289–97.
109. Demetriou E, Sackett R, Welch DF, et al. Evaluation of an enzyme immunoassay for detection of *Neisseria gonorrhoea* in an adolescent population. *JAMA* 1984;**252**:247–50.
110. Dempsey JF, Litaker W, Madhure TL, et al. Physical map of the chromosome of *Neisseria gonorrhoeae* FA1090 with locations of genetic markers. *J Bacteriol* 1991;**173**:5476–86.
111. Denison MR, Perlman S, Anderson RD. Misidentification of *Neisseria* species in a neonate with conjunctivitis. *Pediatrics* 1988; **81**:877–88.
112. Desenclos J-CA, Garrity D, Wroten J. Pediatric gonococcal infection, Florida, 1984 to 1988. *Am J Public Health* 1992;**82**: 426–8.
113. Di Bartolomeo S, Mirta DH, Janer M, et al. Incidence of *Chlamydia trachomatis* and other potential pathogens in neonatal conjunctivitis. *Int J Infect Dis* 2001;**5**:139–43.
114. Dice LR. Measure of the amounts of ecological association between species. *Ecology* 1945;**26**:297–302.
115. Dickson SD, Alter SJ. Cutaneous gonococcal abscess of the abdomen in a child. *Pediatr Emergency Care* 2011;**27**:863–4.
116. Dossett JH, Appelbaum PC, Knapp JS, et al. Proctitis associated with *Neisseria cinerea* misidentified as *Neisseria gonorrhoeae* in a child. *J Clin Microbiol* 1985;**21**:575–7.
117. Dougherty TJ, Asmus A, Tomasz A. Specificity of DNA uptake in genetic transformation of gonococci. *Biochem Biophys Res Commun* 1979;**86**:97–104.
118. Edwards JL, Apicella MA. The molecular mechanisms used by *Neisseria gonorrhoeae* to initiate infection differ between men and women. *Clin Microbiol Rev* 2004;**17**:965–81.
119. Edwards JL, Bulter EK. The pathobiology of *Neisseria gonorrhoeae* lower female genital tract infection. *Front Microbiol* 2011. 2.102. doi: 10.3389/fmicb.2011.00102 (accessed 08/15/2012).
120. Edwards LE, Barrada MI, Hamann AA, et al. Gonorrhea in pregnancy. *Am J Obstet Gynecol* 1978;**132**:637–41.
121. Eisenstein BI, Lee TJ, Sparling PF. Penicillin sensitivity and serum resistance are independent attributes of strains of *Neisseria gonorrhoeae* causing disseminated gonococcal infection. *Infect Immun* 1977;**15**:834–41.
122. Elliott B, Brunham RC, Laga M, et al. Maternal gonococcal infection as a preventable risk factor for low birth weight. *J Infect Dis* 1990;**161**:531–6.
123. Ellison RT III, Curd JG, Kholer PF, et al. Underlying complement deficiency in patients with disseminated gonococcal infection. *Sex Transm Dis* 1987;**14**:201–4.
124. Eschenbach DA. Acute pelvic inflammatory disease: etiology, risk factors, and pathogenesis. *Clin Obstet Gynecol* 1976;**19**:147–69.
125. Eschenbach DA, Buchanan TM, Pollock HM, et al. Polymicrobial etiology of acute pelvic inflammatory disease. *N Engl J Med* 1975; **293**:166–71.
126. Falk ES, Danielsson D, Bjornvatn B, et al. Genomic fingerprinting in the epidemiology of gonorrhea. *Acta Derm Venereol* 1985;**65**: 2235–9.
127. Falsetta ML, Steichen CT, McEwan AG, et al. The composition and metabolic phenotype of *Neisseria gonorrhoeae* biofilms. *Front Microbiol* 2011. 2.075 doi: 10.3389/fmicb.2011.00075 (accessed 08/15/2012).
128. Farrell MK, Billimire ME, Shamroy JA, et al. Prepubertal gonorrhea: a multidisciplinary approach. *Pediatrics* 1981;**67**:151–3.
129. Faruki H, Kohmescher RN, McKinney WP, et al. A community-based outbreak of infection with penicillin-resistant *Neisseria gonorrhoeae* not producing penicillinase (chromosomally mediated resistance). *N Engl J Med* 1985;**313**:607–11.
130. Folland DS, Burke RE, Hinman AR, et al. Gonorrhea in preadolescent children: an inquiry into source of infection and mode of transmission. *Pediatrics* 1977;**60**:153–6.
131. Forsyth A, Moyes A, Young H. Increased ciprofloxacin resistance in gonococci isolated in Scotland. *Lancet* 2000;**356**:1984–5.
132. Fransen L, Nsanze H, Klaus V, et al. Ophthalmia neonatorum in Nairobi, Kenya: the roles of *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. *J Infect Dis* 1986;**153**:862–9.
133. Frewen TC, Bannatyne RM. Gonococcal vulvovaginitis in prepubertal girls. *Clin Pediatr (Phila)* 1979;**18**:491–3.
134. Friedrich WN, Grambsch P. Child sexual behavior inventory: normative and clinical comparisons. *Psychol Assess* 1992;**4**:303–11.
135. Friendly DS. Gonococcal conjunctivitis of the newborn. *Clin Pract Child Hosp* 1969;**25**:1–9.
136. Geidinghagen DH, Hoff GL, Biery RM. Gonorrhea in children: epidemiologic unit analysis. *Pediatr Infect Dis J* 1992;**11**:973–4.
137. Genadry RR, Thompson BH, Niebyl JR. Gonococcal salpingitis in pregnancy. *Am J Obstet Gynecol* 1976;**126**:512–13.
138. Giannini CM, Kim HK, Mortensen J, et al. Culture of non-genital sites increases the detection of gonorrhea in women. *J Pediatr Adolesc Gynecol* 2010;**23**:246–52.
139. Gilbaugh JH, Fuchs PC. The gonococcus and the toilet seat. *N Engl J Med* 1979;**301**:91–3.
140. Gill MJ, Simjee S, Al-Hattawi K, et al. Gonococcal resistance to β-lactams and tetracycline involves mutation in loop 3 of the porin encoded at the penB locus. *Antimicrob Agents Chemother* 1998;**42**:2799–803.
141. Girardet RG, Lahoti S, Howard LA, et al. Epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 2009;**124**:79–86.
142. Glaser S, Boxerbaum B, Kennell JH. Gonococcal arthritis in the newborn: report of a case and review of the literature. *Am J Dis Child* 1966;**112**:135–8.
143. Golden N, Hammerschlag M, Hewkoff S, et al. Prevalence of *Chlamydia trachomatis* cervical infections in female adolescents. *Am J Dis Child* 1984;**138**:562–4.
144. Gomperts BN, White LK. Gonococcal hand abscess. *Pediatr Infect Dis J* 2000;**19**:671–2.
145. Gorter AD, Oostrik J, van der Lay P, et al. Involvement of lipooligosaccharides of *Haemophilus influenzae* and *Neisseria meningitidis* in defensin-enhanced bacterial adherence to epithelial cells. *Microb Pathog* 2003;**34**:121–30.
146. Gotschlich EC. Genetic locus for the biosynthesis of the variable portion of *Neisseria gonorrhoeae* lipooligosaccharide. *J Exp Med* 1994;**180**:2181–2190.
147. Grassmé H, Gulbins E, Brenner B, et al. Acidic sphingomyelinase mediates entry of *N. gonorrhoeae* into nonphagocytic cells. *Cell* 1997;**91**:605–15.
148. Greenberg M, Vandow JE. Ophthalmia neonatorum: evaluation of different methods of prophylaxis in New York City. *Am J Public Health* 1961;**51**:836–45.
149. Gregg CR, Melly MA, Hellerqvist CG, et al. Toxic activity of purified lipopolysaccharide as *N. gonorrhoeae* for human fallopian tube mucosa. *J Infect Dis* 1983;**143**:432–9.
150. Gregory JE, Chisom JL, Meadows AT. Gonococcal arthritis in an infant. *Br J Vener Dis* 1972;**48**:306–7.
151. Griffiss JM, Lammel CJ, Wang J, et al. *Neisseria gonorrhoeae* coordinately uses Pili and Opa to activate HEC-1-B cell microvilli, which causes engulfment of the gonococci. *Infect Immun* 1999; **67**:3469–80.
152. Griffiss JM, O'Brien JP, Yamasaki R, et al. Physical heterogeneity of neisserial lipooligosaccharides reflects oligosaccharides that differ in apparent molecular weight, chemical composition, and antigenic expression. *Infect Immun* 1987;**55**:1792–800.
153. Griffiss JM, Schneider H, Mandrell RE, et al. Lipooligosaccharides: the principal glycolipids of the neisserial outer membrane. *Rev Infect Dis* 1988;**10**(suppl. 2):287–95.
154. Groothuis JR, Bischoff MC, Jauregui LE. Pharyngeal gonorrhea in young children. *Pediatr Infect Dis J* 1983;**2**:99–101.
155. Gulati S, Agarwal S, Vasudhev S, et al. Properdin is critical for antibody-dependent bactericidal activity against *Neisseria gonorrhoeae* that recruit C4b-binding protein. *J Immunol* 2012;**188**: 3416–25.
156. Gutman LT, Holmes KK. Gonococcal infections. In: Remington JS, Klein JO, editors. *Infectious Diseases of the Fetus and Newborn Infant*. 3rd ed. Philadelphia: W. B. Saunders; 1990.

157. Gutman LT, Herman-Giddens ME, McKinney Jr RE. Pediatric acquired immunodeficiency syndrome: barriers to recognizing the role of child sexual abuse. *Am J Dis Child* 1993;**147**:775–80.
158. Haas R, Schwartz H, Meyer TF. Release of soluble pilin antigen coupled with gene conversion in *Neisseria gonorrhoeae*. *Proc Natl Acad Sci U S A* 1987;**84**:9079–83.
159. Haines KA, Reibman J, Tang XY, et al. Effects of protein I of *Neisseria gonorrhoeae* on neutrophil activation: generation of diacylglycerol from phosphatidylcholine via a specific phospholipase C is associated with exocytosis. *J Cell Biol* 1991;**114**:433–42.
160. Hale YM, Melton ME, Lewis JS, et al. Evaluation of the PACE 2 *Neisseria gonorrhoeae* assay by three public health laboratories. *J Clin Microbiol* 1993;**31**:451–3.
161. Handsfield HH, Holmes KK. Microepidemic of virulent gonococcal infection. *J Am Vener Dis Assoc* 1974;**1**:20–2.
162. Handsfield HH, Hodson WA, Holmes KK. Neonatal gonococcal infection. 1. Orogastric contamination with *Neisseria gonorrhoeae*. *JAMA* 1973;**225**:697–701.
163. Handsfield HH, Lipman TO, Harnisch JP, et al. Asymptomatic gonorrhea in men: diagnosis, natural course, prevalence and significance. *N Engl J Med* 1974;**290**:117–23.
164. Harrison WO, Hooper RR, Weisner PJ, et al. A trial of minocycline given after exposure to prevent gonorrhea. *N Engl J Med* 1979;**300**:1074–8.
165. Harvey HA, Post DMB, Apicella MA. Immortalization of human urethral epithelial cells: a model for the study of the pathogenesis of and the inflammatory cytokine response to *Neisseria gonorrhoeae* infection. *Infect Immun* 2002;**70**:5808–15.
166. Hein K, Marks A, Cohen MI. Asymptomatic gonorrhea: prevalence in a population of urban adolescents. *J Pediatr* 1977;**90**:634–5.
167. Hess D, Wu A, Golparian D, et al. Genome sequencing of *Neisseria gonorrhoeae* isolate of a successful international clone with decreased susceptibility/resistance to extended-spectrum cephalosporins. *Antimicrob Agents Chemother* 2012 epub PMID 22908152 (accessed 08/28/2012).
168. Heymans R, Golparian D, Bruisten SM, et al. Evaluation of *Neisseria gonorrhoeae* multiple-locus variable-number tandem-repeat analysis, *N. gonorrhoeae* multiantigen sequence typing, and full-length porB gene sequence analysis for molecular epidemiological typing. *J Clin Microbiol* 2012;**50**:180–3.
169. Hitchcock PJ. Analyses of gonococcal lipopolysaccharide in whole cell lysates by sodium dodecyl sulfate–polyacrylamide gel electrophoresis: stable association of lipopolysaccharide with the major outer membrane protein (protein 1) of *Neisseria gonorrhoeae*. *Infect Immun* 1984;**46**:202–12.
170. Hobbs MM, Sparling PF, Cohen MS, et al. Experimental gonococcal infection in male volunteers: cumulative experience with *Neisseria gonorrhoeae* strains FA1090 and MS11mkC. *Front Microbiol* 2011. 2.123 doi: 10.3389/fmicb.2011.00123 (accessed 08/15/2012).
171. Holmes KK, Counts GW, Beaty HN. Disseminated gonococcal infection. *Ann Intern Med* 1971;**74**:979–93.
172. Holmes KK, Johnson DW, Trostle HJ. An estimate of the risk of men acquiring gonorrhea by sexual contact with infected females. *Am J Epidemiol* 1970;**91**:170–4.
173. Holmes KK, Buchanan TM, Adam JL, et al. Is serology useful in gonorrhea? A critical analysis of factors influencing serodiagnosis. In: Brook GF, Gotschlich EC, Sawyer WD, Young FE, editors. *Immunobiology of Neisseria gonorrhoeae*. Washington, D.C.: American Society for Microbiology; 1978. p. 370.
174. Holt LE. Gonococcus infections in children with especial reference to their prevalence in institutions and means of prevention. *N Y Med J* 1905;**81**:521–7.
175. Hook EW, Handsfield HH. Gonococcal infections in the adult. In: Holmes KK, Mårdh PA, Sparling PF, et al, editors. *Sexually Transmitted Diseases*. 3rd ed. New York: McGraw-Hill; 1999.
176. Hook EW 3rd, Judson FN, Handsfield HH, et al. Auxotype/serovar diversity and antimicrobial resistance of *Neisseria gonorrhoeae* in two mid-sized American cities. *Sex Transm Dis* 1987;**14**:141–6.
177. Hooper RR, Reynolds GH, Jones OG, et al. Cohort study of venereal disease. I. The risk of gonorrhea transmission from infected women to men. *Am J Epidemiol* 1978;**108**:136–44.
178. Huppert JS, Biro F, Lan D, et al. Urinary symptoms in adolescent females: STI or UTI? *J Adolesc Health* 2007;**40**:418–24.
179. Ingram DL. The gonococcus and the toilet seat revisited. *Pediatr Infect Dis* 1989;**8**:191.
180. Ingram DL, Everett VD, Flick LAR, et al. Vaginal gonococcal cultures in sexual abuse evaluations: evaluation of selective criteria for preteenaged girls. *Pediatrics* 1997;**99**:e8.
181. Ingram DL, Everett VD, Lyna PR, et al. Epidemiology of adult sexually transmitted disease agents in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 1992;**11**:945–50.
182. Ingram DL, Runyan DK, Collins AD, et al. Vaginal *Chlamydia trachomatis* infection in children with sexual contact. *Pediatr Infect Dis* 1984;**3**:97–9.
183. Ingram DM, Miller WC, Schoenbach VJ, et al. Risk assessment for gonococcal and chlamydial infections in young children undergoing evaluation for sexual abuse. *Pediatrics* 2001;**107**:e73.
184. Ison CA, Woodford PJ, Madders H, et al. Drift in susceptibility of *Neisseria gonorrhoeae* to ciprofloxacin and emergence of therapeutic failure. *Antimicrob Agents Chemother* 1998;**42**:2919–22.
185. Israel KS, Rissing KB, Brooks GF. Neonatal and childhood gonococcal infections. *Clin Obstet Gynecol* 1975;**18**:143–51.
186. Jacobson L, Westrom L. Objectivized diagnosis of acute pelvic inflammatory disease. *Am J Obstet Gynecol* 1969;**105**:1088–98.
187. Jamison JH, Kaplan DW, Hamman R, et al. Spectrum of genital human papillomavirus infection in a female adolescent population. *Sex Transm Dis* 1995;**22**:236.
188. Johannsen DB, Johnston DM, Koymen HO, et al. A *Neisseria gonorrhoeae* immunoglobulin A1 protease mutant is infectious in the human challenge model of urethral infection. *Infect Immun* 1999;**67**:3009–13.
189. Johnston KH, Holmes KK, Gotschlich EC. The serological classification of *Neisseria gonorrhoeae*. 1. Isolation of the outer membrane complex responsible for serotypic specificity. *J Exp Med* 1976;**143**:741–58.
190. Jones DED, Brame RG, Jones CP. Gonorrhea in obstetric patients. *J Am Vener Dis Assoc* 1976;**2**:30–2.
191. Judson FN, Maltz AB. A rational basis for the epidemiologic treatment of gonorrhea in a clinic for sexually transmitted diseases. *Sex Transm Dis* 1978;**5**:89.
192. Judson FN, Werness BA. Combining cervical and anal-canal specimens for gonorrhea on a single culture plate. *J Clin Microbiol* 1980;**12**:216.
193. Kahn RH, Mosure DJ, Blank S, et al. *Chlamydia trachomatis* and *Neisseria gonorrhoeae* prevalence and coinfection in adolescents entering selected US juvenile detention centers, 1997-2002. *Sex Transm Dis* 2005;**32**:255–9.
194. Kallstrom H, Islam MS, Berggren PO, et al. Cell signaling by the type IV pili of pathogenic *Neisseria*. *J Biol Chem* 1998;**273**:21777–82.
195. Kallstrom H, Liszewski MK, Atkinson JP, et al. Membrane cofactor protein (MCP or CD46) is a cellular pilus receptor for pathogenic *Neisseria*. *Mol Microbiol* 1997;**25**:639–47.
196. Kampmeier RH. Identification of the gonococcus by Albert Neisser. *Sex Transm Dis* 1978;**5**:71–2.
197. Kasper DL, Rice PA, McCormack WM. Bactericidal antibody in genital infections due to *Neisseria gonorrhoeae*. *J Infect Dis* 1977;**135**:243–51.
198. Katz AR, Komeya AY, Soge OO, et al. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: case report of the first isolate identified in the United States. *Clin Infect Dis* 2012;**54**:841–3.
199. Kellogg DS, Peacock Jr WL, Deacon WE, et al. *Neisseria gonorrhoeae*: 1. Virulence genetically linked to clonal variation. *J Bacteriol* 1963;**85**:1274–9.
200. Kellogg JA, Orwig LK. Comparison of Gonogen, Gono Gen II and Micro Trak Direct Fluorescent-Antibody Test with carbohydrate fermentation for confirmation of culture isolates of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1995;**33**:474–6.
201. Knapp JS, Holmes KK. Disseminated gonococcal infections caused by *Neisseria gonorrhoeae* with unique nutritional requirements. *J Infect Dis* 1975;**132**:204–8.
202. Knapp JS, Tam MR, Nowinski RC, et al. Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. *J Infect Dis* 1984;**150**:44–8.
203. Kohen DP. Neonatal gonococcal arthritis: three cases and review of the literature. *Pediatrics* 1974;**53**:436–40.

204. Koomey M. Implications of molecular contacts and signaling initiated by *Neisseria gonorrhoeae*. *Curr Opin Microbiol* 2001;**4**:53–7.
205. Krieger JN. Prostatitis, epididymitis, and orchitis. In: Mandell GL, Douglas RG, Bennett JE, editors. *Principles and Practice of Infectious Disease*. New York: Churchill Livingstone; 1985. p. 745–8.
206. Krohn MA, Hillier SL, Bell JA, et al. The bacterial etiology of conjunctivitis in early infancy. *Am J Epidemiol* 1993;**138**:326–32.
207. Laga M, Meheus A, Piot P. Epidemiology and control of gonococcal ophthalmia neonatorum. *Bull World Health Organ* 1989;**67**: 471–7.
208. Laga M, Naamara W, Brunham RC, et al. Single-dose therapy of gonococcal ophthalmia neonatorum with ceftriaxone. *N Engl J Med* 1986;**315**:1382–5.
209. Laga M, Plummer FA, Piot P, et al. Prophylaxis of gonococcal and chlamydial ophthalmia neonatorum. *N Engl J Med* 1988;**318**: 653–7.
210. Landolfo PJ, Marrie TJ, Nelson NA, et al. Cell-mediated immune response in gonococcal infection. *Can J Microbiol* 1981;**27**: 76–80.
211. Laras L, Craighill M, Woods ER, et al. Epidemiologic observations of adolescents with *Neisseria gonorrhoeae* genital infections treated at a children's hospital. *Adolesc Pediatr Gynecol* 1994;**7**: 9–12.
212. Lawson L, Chaffin M. False negatives in sexual abuse disclosure interviews. *J Interpersonal Violence* 1992;**7**:532–42.
213. Lewis LS, Glauser TA, Joffe MD. Gonococcal conjunctivitis in prepubertal children. *Am J Dis Child* 1990;**144**:546–8.
214. Limberger RJ, Biega R, Evancoe A, et al. Evaluation of culture and the Gen-Probe PACE 2 assay for detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in endocervical specimens transported to a state health laboratory. *J Clin Microbiol* 1992;**30**: 1162–6.
215. Lin L, Ayala P, Larson J, et al. The *Neisseria* type 2 IgA1 protease cleaves LAMP1 and promotes survival of bacteria within epithelial cells. *Mol Microbiol* 1997;**24**:1083–94.
216. Litt IF, Cohen MI. Perihepatitis associated with salpingitis in adolescents. *JAMA* 1978;**240**:1253.
217. Litt IF, Edberg SC, Finberg L. Gonorrhea in children and adolescents: a current review. *J Pediatr* 1974;**85**:595–607.
218. Liu Y, Feinen G, Russell MW. New concepts in immunity to *Neisseria gonorrhoeae:* innate responses and suppression of adaptive immunity favor the pathogen, not the host. *Front Microbiol* 2011. 2.052. doi: 10.3389/fmicb.2011.00052 (accessed 08/15/2012).
219. Looveren MV, Ison CA, Ieven M, et al. Evaluation of the discriminatory power of typing methods for *Neisseria gonorrhoeae*. *J Clin Microbiol* 1999;**37**:2183–8.
220. Lorenzen DR, Gunther D, Pandit J, et al. *Neisseria gonorrhoeae* porin modifies the oxidative burst of human professional phagocytes. *Infect Immun* 2000;**68**:6215–22.
221. Louv WC, Austin H, Alexander W, et al. A clinical trial of nonoxynol-9 for preventing gonococcal and chlamydial infections. *J Infect Dis* 1988;**158**:518–23.
222. Louv WC, Austin H, Perlman J, et al. Oral contraceptive use and the risk of chlamydial and gonococcal infection. *Am J Obstet Gynecol* 1989;**160**:396–402.
223. Luciano AA, Grubin L. Gonorrhea screening: comparison of three techniques. *JAMA* 1980;**243**:680–1.
224. Martin I, Sawatzky P, Allen V, et al. Emergence and characterization of *Neisseria gonorrhoeae* isolates with decreased susceptibilities to ceftriaxone and cefixime in Canada: 2001-2010. *Sex Transm Dis* 2012;**39**:316–23.
225. Mascola L, Albritton WL, Cates W, et al. Gonorrhea in American teenagers, 1960-1981. *Pediatr Infect Dis J* 1983;**2**:302–3.
226. McCormack WM, Reynolds GH. Effect of menstrual cycle and method of contraception on recovery of *Neisseria gonorrhoeae*. *JAMA* 1982;**247**:1292–4.
227. McGee ZA, Johnson AP, Taylor-Robinson D. Pathogenic mechanisms of *Neisseria gonorrhoeae*: observations on damage to human fallopian tubes in organ culture by gonococci of colony type I or type 4. *J Infect Dis* 1981;**143**:413–22.
228. McGee ZA, Stephens DS, Hoffman LH, et al. Mechanisms of mucosal invasion by pathogenic *Neisseria*. *Rev Infect Dis* 1983; **5**(suppl. 4):708.
229. McMillan A, Young H. Gonorrhea in the homosexual man: frequency of infection by culture site. *Sex Transm Dis* 1978;**5**:146.
230. Mellin GW. Ophthalmia neonatorum: yesterday, today, tomorrow. *Sight-Saver Rev* 1961;**31**:102–13.
231. Merz AJ, So M. Attachment of piliated, Opa^- and Opc^- gonococci and meningococci to epithelial cells elicits cortical actin rearrangements and clustering of tyrosine-phosphorylated proteins. *Infect Immun* 1997;**65**:4341–9.
232. Merz AJ, So M. Interactions of pathogenic *Neisseria* with epithelial cell membranes. *Annu Rev Cell Dev Biol* 2000;**16**:423–57.
233. Merz AJ, Enns CA, So M. Type IV pili of pathogenic *Neisseria* elicit cortical plaque formation in epithelial cells. *Mol Microbiol* 1999;**32**:1316–32.
234. Merz AJ, So M, Sheetz MP. Pilus retraction powers bacterial twitching motility. *Nature* 2000;**407**:98–102.
235. Meyer TF, Billyard E, Haas R, et al. Pilus genes of *Neisseria gonorrhoeae:* chromosomal organization and DNA sequence. *Proc Natl Acad Sci U S A* 1984;**81**:6110–14.
236. Meyer TF, Mlawer N, So M. Pilus expression in *Neisseria gonorrhoeae* involves chromosomal rearrangement. *Cell* 1982;**30**: 45–52.
237. Mirrett S, Reller LB, Knapp JS. *Neisseria gonorrhoeae* strains inhibited by vancomycin in selective media and correlation with auxotype. *J Clin Microbiol* 1981;**14**:94–9.
238. Morello JA, Lerner SA, Bohnhoff M. Characteristics of atypical *Neisseria gonorrhoeae* from disseminated and localized infections. *Infect Immun* 1976;**13**:1510–16.
239. Morris SR, Klausner JD, Buchbinder SP, et al. Prevalence and incidence of pharyngeal gonorrhea in a longitudinal sample of men who have sex with men: the EXPLORE study. *Clin Infect Dis* 2006;**43**:1284–9.
240. Morse SA, Johnson SR, Biddle JW, et al. High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal term determinant. *Antimicrob Agents Chemother* 1986;**30**: 664–70.
241. Gonorrhoea. In: Morton RS, editor. Major Problems in Dermatology, **vol. 9**. Philadelphia: W. B. Saunders; 1977.
242. Mosleh IM, Huber LA, Steinlein P, et al. *Neisseria gonorrhoeae* porin modulates phagosome maturation. *J Biol Chem* 1998;**273**: 35332–8.
243. Mueller BA, Luz-Jimenez M, Daling JR, et al. Risk factors for tubal infertility. *Sex Transm Dis* 1992;**19**:28–34.
244. Mulcahy FM, Lacey CJN. Sexually transmitted infections in adolescent girls. *Genitourin Med* 1987;**63**:119–21.
245. Muller A, Gunther D, Brinkmann V, et al. Targeting of the pro-apoptotic VDAC-like porin (PorB) of *Neisseria gonorrhoeae* to mitochondria of infected cells. *EMBO J* 2000;**19**:5332–43.
246. Muller A, Gunther D, Dux F, et al. Neisserial porin (PorB) causes rapid calcium influx in target cells and induces apoptosis by the activation of cysteine proteases. *EMBO J* 1999;**18**: 339–52.
247. Muram D, Speck PM, Dockter M. Child sexual abuse examination: is there a need for routine screening for *N. gonorrhoeae*? *J Pediatr Adolesc Gynecol* 1996;**9**:79–80.
248. Murray ES, Bentan MJ, Coppola SR, et al. New options for diagnosis and control of gonorrheal urethritis in males using uncentrifuged first voided urine (FVU) as a specimen for culture. *Am J Public Health* 1979;**69**:596–8.
249. Nathawad R, Mendez H, Ahmad A, et al. Severe ocular reactions after neonatal ocular prophylaxis with gentamicin ophthalmic ointment. *Pediatr Infect Dis J* 2011;**30**:175–6.
250. Naumann M, Rudel T, Meyer TF. Host cell interactions and signaling with *Neisseria gonorrhoeae*. *Curr Opin Microbiol* 1999;**2**: 62–70.
251. Naumann M, Wessler S, Bartsch C, et al. *Neisseria gonorrhoeae* epithelial cell interaction leads to the activation of the transcription factors nuclear factor κB and activator protein 1 and the induction of inflammatory cytokines. *J Exp Med* 1997;**186**: 247–58.
252. Neinstein LS, Goldenring J, Carpenter S. Nonsexual transmission of sexually transmitted diseases: an infrequent occurrence. *Pediatrics* 1984;**74**:67–76.
253. Nelson JD, Mohs E, Dajani AS, et al. Gonorrhea in preschool- and school-age children: report of the Prepubertal Gonorrhea Cooperative Study Group. *JAMA* 1976;**236**:1359–64.
254. Newman LM, Moran JS, Workowski KA. Update on the management of gonorrhea in adults in the United States. *Clin Infect Dis* 2007;**44**:S84–S101.

255. Ngampasutodol J, Rice PA, Walsh MT, et al. Characterization of a peptide vaccine candidate mimicking an oligosaccharide epitope of *Neisseria gonorrhoeae* and resultant immune responses and function. *Vaccine* 2006;**24**:157–70.
256. Nicolson IJ, Perry AC, Virji M, et al. Localization of antibody-binding sites by sequence analysis of cloned pilin genes from *Neisseria gonorrhoeae*. *J Gen Microbiol* 1987;**133**:825–33.
257. Noegel A, Gotschlich EC. Isolation of a high molecular weight polyphosphate from *Neisseria gonorrhoeae*. *J Exp Med* 1983;**157**: 2049–60.
258. Nsuami M, Cammarata CL, Brooks BN, et al. Chlamydia and gonorrhea co-occurrence in a high school population. *Sex Transm Dis* 2004;**31**:424–7.
259. O'Brien JP, Goldenberg DL, Rice PA. Disseminated gonococcal infection: a prospective analysis of 49 patients and a review of pathophysiology and immune mechanisms. *Medicine (Baltimore)* 1983;**2**:395–406.
260. O'Reilly RJ, Lee L, Welch BG. Secretory IgA antibody responses to *Neisseria gonorrhoeae* in the genital secretions of infected females. *J Infect Dis* 1976;**133**:113–25.
261. Oriel JD. Ophthalmia neonatorum: relative efficacy of current prophylactic practices and treatment. *J Antimicrob Chemother* 1984;**14**:209–20.
262. Orr DP, Preitto SV. Emergency management of sexually abused children: the role of the pediatric resident. *Am J Dis Child* 1979;**133**:628–31.
263. Osborne NG, Grubin L. Colonization of the pharynx with *Neisseria gonorrhoeae*. *Sex Transm Dis* 1979;**6**:253–6.
264. Palusci VJ, Reeves MJ. Testing for genital gonorrhea infections in prepubertal girls with suspected sexual abuse. *Pediatr Infect Dis J* 2003;**22**:618–23.
265. Panke ES, Yang LI, Leist PA, et al. Comparison of Gen-Probe DNA probe test and culture for the detection of *N. gonorrhoeae* in endocervical specimens. *J Clin Microbiol* 1991;**29**:883–8.
266. Pasquariello CA, Plotkin SA, Rice RJ, et al. Fatal gonococcal septicemia. *Pediatr Infect Dis* 1985;**4**:204–6.
267. Patamasucon P, Rettig PJ, Nelson JD. Cefuroxime therapy of gonorrhea and co-infection with *Chlamydia trachomatis* in children. *Pediatrics* 1981;**68**:534–8.
268. Pelouze PS. *Gonorrhea in the Male and Female*. Philadelphia: W. B. Saunders; 1941.
269. Perrin A, Nassif X, Tinsley C. Identification of regions of the chromosome of *Neisseria meningitidis* and *Neisseria gonorrhoeae* which are specific to the pathogenic *Neisseria* species. *Infect Immun* 1999;**67**:6119–29.
270. Phillips L. P-lactamase–producing penicillin-resistant gonococcus. *Lancet* 1976;**2**:656.
271. Plant LJ, Jonsson AB. Type IV pili of *Neisseria gonorrhoeae* influence the activation of human $CD4^+$ T cells. *Infect Immun* 2006; **74**:442–8.
272. Plavidal FJ, Werch A. Fetal scalp abscess secondary to intrauterine monitoring. *Am J Obstet Gynecol* 1976;**125**:65–8.
273. Podgore JK, Holmes KK. Ocular gonococcal infection with minimal or no inflammatory response. *JAMA* 1981;**246**:242–3.
274. Poh CL, Ramachandran V, Tapsall J. Genetic diversity of *Neisseria gonorrhoeae* IB-2 and IB-6 isolates revealed by whole-cell repetitive element sequence-based PCR. *J Clin Microbiol* 1996;**34**:292–5.
275. Poh CL, Khng HP, Lim CK, Loh GK. Molecular typing of *Neisseria gonorrhoeae* by restriction fragment length polymorphisms. *Genitourin Med* 1992;**68**:106–10.
276. Price GA, Hobbs MM, Cornelissen CN. Immunogenicity of gonococcal transferrin proteins during natural infections. *Infect Immun* 2004;**72**:277–83.
277. Public Health Agency of Canada. *Reported cases and rates of gonorrhea by province/territory and sex, 1980 to 2009*. http://www.phac-aspc.gc.ca/std-mts/sti-its_tab/gonorrhea_pts-eng.php. (accessed 08/28/2012).
278. Ram S, Gulati S, McQuillen DP, et al. Interactions between *Neisseria gonorrhoeae* and C4b-binding protein: a molecular basis for gonococcal serum resistance. *Abstract Mol Immunol* 1999;**36**:297.
279. Ramsey KH, Schneider H, Cross AS, et al. Inflammatory cytokines produced in response to experimental human gonorrhea. *J Infect Dis* 1995;**172**:186–91.
280. Ramsey ME, Woodhams KL, Dillard JP. The gonococcal genetic island and type IV secretion in pathogenic *Neisseria*. *Front Microbiol* 2011. 2.061. doi: 10.3389/fmicb.2011.00061. (accessed 08/15/2012).
281. Rawstrom SA, Hammerschlag MR, Gullans C, et al. Ceftriaxone treatment of penicillinase-producing *Neisseria gonorrhoeae* infections in children. *Pediatr Infect Dis* 1989;**8**:445–8.
282. Rees E. Gonococcal bartholinitis. *Br J Vener Dis* 1967;**43**:150–6.
283. Reichart CA, Rupkey LM, Brady WE, et al. Comparison of GC-Lect and modified Thayer-Martin media for isolation of *Neisseria gonorrhoeae*. *J Clin Microbiol* 1989;**27**:808–11.
284. Rettig PJ, Nelson JD. Genital tract infection with *Chlamydia trachomatis* in prepubertal children. *J Pediatr* 1981;**99**:206–10.
285. Rice PA, Kasper DL. Characterization of serum resistance of *Neisseria gonorrhoeae* that disseminate: roles of blocking antibody in gonococcal outer membrane protein. *J Clin Invest* 1982;**70**: 157–67.
286. Rice PA, McCormack WM, Kasper DL. Natural serum bactericidal activity against *N. gonorrhoeae* isolates from disseminated, locally invasive, and uncomplicated disease. *J Immunol* 1980;**124**: 2105–9.
287. Rice PA, Vayo HE, Tam MR, et al. Immunoglobulin G antibodies directed against protein III block killing of serum-resistant *Neisseria gonorrhoeae* by immune serum. *J Exp Med* 1986;**164**: 1735–48.
288. Rice PA, McQuillen DP, Gulati S, et al. Serum resistance of *Neisseria gonorrhoeae:* does it thwart the inflammatory response and facilitate the transmission of infection? *Ann N Y Acad Sci* 1994;**730**:7–14.
289. Rice RJ, Aral SO, Blount JH, et al. Gonorrhea in the United States 1975-1989: is the giant only sleeping? *Sex Transm Dis* 1987;**14**: 83–7.
290. Rimsza ME, Niggemann EH. Medical evaluation of sexually abused children: a review of 311 cases. *Pediatrics* 1982;**69**:8–14.
291. Ris HW, Dodge RW. Gonorrhea in adolescent girls in a closed population: prevalence, diagnosis and treatment. *Am J Dis Child* 1972;**123**:185.
292. Roberts M, Falkow S. Conjugal transfer of R plasmids in *Neisseria gonorrhoeae*. *Nature* 1977;**266**:630–1.
293. Rothbard MJ, Gregory T, Salerno LJ. Intrapartum gonococcal amnionitis. *Am J Obstet Gynecol* 1975;**121**:565–6.
294. Rothenberg R. Ophthalmia neonatorum due to *Neisseria gonorrhoeae*: prevention and treatment. *Sex Transm Dis* 1979;**6**(suppl. 2): 187–91.
295. Rothenberg RB, Simon R, Chipperfield E, et al. Efficacy of selected diagnostic tests for sexually transmitted diseases. *JAMA* 1976;**235**:49–51.
296. Rudel T, Scheurerpflug I, Meyer TF. *Neisseria* PilC protein identified as type-4 pilus tip–located adhesin. *Nature* 1995;**373**:357–9.
297. Russell DEH. The incidence and prevalence of intrafamilial and extrafamilial sexual abuse of female children. *Child Abuse Negl* 1983;**7**:133–42.
298. Rytkönen A, Johansson L, Asp V, et al. Soluble pilin of *Neisseria gonorrhoeae* interacts with human target cells and tissue. *Infect Immun* 2001;**69**:6419–26.
299. Sanders JM, Brookman RR, Brown RC, et al. Committee on Adolescence: role of the pediatrician in management of sexually transmitted diseases in children and adolescents. *Pediatrics* 1987; **79**:454–6.
300. Sandstrom E, Danielsson D. A survey of gonococcal serology. In: Danielsson D, Juhlin L, Mårdh P-A, editors. *Genital Infections and Their Complications*. Stockholm: Almqvist & Wiksell; 1975. p. 253.
301. Sandström EG, Danielsson D. Serology of *Neisseria gonorrhoeae*: classification by coagglutination. *Acta Pathol Microbiol Scand* 1980;**88**:27–38.
302. Sandström EG, Knapp JS, Buchanan TM. Serology of *Neisseria gonorrhoeae*: W-antigen serogrouping by coagglutination and protein I serotyping by enzyme-linked immunosorbent assay both detect protein I antigens. *Infect Immun* 1982;**35**:229–39.
303. Sarrel PM, Pruett KA. Symptomatic gonorrhea during pregnancy. *Obstet Gynecol* 1968;**32**:670–3.
304. Sarubbi FA, Jr Sparling PF. Transfer of antibiotic resistance in mixed cultures of *Neisseria gonorrhoeae*. *J Infect Dis* 1974;**130**: 660–3.
305. Segal E, Hagblom P, Seifert HS, et al. Antigenic variation of gonococcal pilus involves assembly of separated silent gene segments. *Proc Natl Acad Sci U S A* 1986;**83**:2177–81.

306. Seifert HS, Wright CJ, Jerse AE, et al. Multiple gonococcal pilin antigenic variants are produced during experimental human infections. *J Clin Invest* 1994;**93**:2744–9.
307. Shafer M-AB, Irwin CE, Sweet RL. Acute salpingitis in the adolescent female. *J Pediatr* 1982;**100**:339–50.
308. Shafer W, Onunka VC, Martin LE. Antigonococcal activity of human neutrophil cathepsin G. *Infect Immun* 1986;**54**:184–8.
309. Shafer WM, Joiner K, Guymon LF, et al. Serum sensitivity of *Neisseria gonorrhoeae*: the role of lipopolysaccharide. *J Infect Dis* 1984;**149**:175–83.
310. Shapiro RA, Schubert CJ, Siegel RM. *Neisseria gonorrhea* infections in girls younger than 12 years of age evaluated for vaginitis. *Pediatrics* 1999;**104**:e72.
311. Shinners EN, Catlin BW. Arginine and pyrimidine biosynthetic defects in *Neisseria gonorrhoeae* strains isolated from patients. *J Bacteriol* 1982;**151**:295–302.
312. Sicoli RA, Losek JD, Hudlett JM, et al. Indications for *Neisseria gonorrhoeae* cultures in children with suspected sexual abuse. *Arch Pediatr Adolesc Med* 1995;**149**:86–9.
313. Siegel RM, Schubert CJ, Myers PA, et al. The prevalence of sexually transmitted diseases in children and adolescents evaluated for sexual abuse in Cincinnati: rationale for limited STD testing in prepubertal girls. *Pediatrics* 1995;**96**:1090–4.
314. Silber TJ, Controni G. Clinical spectrum of pharyngeal gonorrhea in children and adolescents: a report of sixteen patients. *J Adolesc Health Care* 1983;**4**:51–4.
315. Singer A. The uterine cervix from adolescence to the menopause. *Br J Obstet Gynaecol* 1975;**82**:81–99.
316. Smith AL. *Principles of Microbiology*. 10th ed. St. Louis: Mirror/Mosby; 1985.
317. Smith JA. Ophthalmia neonatorum in Glasgow. *Scott Med J* 1969;**14**:272–9.
318. Smith K, Harrington K, Wingood G, et al. Self-obtained vaginal swabs for diagnosis of treatable sexually transmitted diseases in adolescent girls. *Arch Pediatr Adolesc Med* 2001;**155**:676–9.
319. Smith KR, Ching S, Lee H, et al. Evaluation of ligase chain reaction for use with urine for identification of *Neisseria gonorrhoeae* in females attending a sexually transmitted disease clinic. *J Clin Microbiol* 1995;**33**:455–7.
320. Snowe RJ, Wilfert CM. Epidemic reappearance of gonococcal ophthalmia neonatorum. *Pediatrics* 1973;**57**:110–14.
321. So NS, Ostrowski MA, Gray-Owen SD. Vigorous response of human innate functioning IgM memory B cells upon infection by *Neisseria gonorrhoeae*. *J Immunol* 2012;**188**:4008–22.
322. Sparling PF. Biology of *Neisseria gonorrhoeae*. In: Holmes KK, Mårdh P-A, Sparling PF, et al, editors. *Sexually Transmitted Diseases*. 3rd ed. New York: McGraw-Hill; 1999.
323. Sparling PF, Yobs AR. Colonial morphology of *Neisseria gonorrhoeae* isolated from males and females. *J Bacteriol* 1967; **93**:513.
324. Sparling PF, Cannon JG, So M. Phase and antigenic variation of pili and outer membrane protein II of *Neisseria gonorrhoeae*. *J Infect Dis* 1986;**153**:196–201.
325. Speck WT, Lawsky AR. Symptomatic anorectal gonorrhea in an adolescent female. *Am J Dis Child* 1971;**122**:438–9.
326. Spence JM, Tyler RE, Domaoal RA, et al. L12 enhances gonococcal transcytosis of polarized Hec1B cells via the lutropin receptor. *Microb Pathog* 2002;**32**:117–25.
327. Spivey MI, Paschall RT, Ferrett R, et al. *Neisseria:* avoiding the jump to conclusion. *J Child Sex Abuse* 2011;**20**:622–30.
328. Stern A, Meyer TF. Common mechanism controlling phase and antigenic variation in pathogenic *Neisseria*. *Mol Microbiol* 1987;**1**:5–12.
329. Stern A, Brown M, Nickel P, et al. Opacity genes in *Neisseria gonorrhoeae*: control of phase and antigenic variation. *Cell* 1986; **47**:61–71.
330. Stern A, Nickel P, Meyer TF, et al. Opacity determinants of *Neisseria gonorrhoeae*: gene expression and chromosomal linkage to the gonococcal pilus gene. *Cell* 1984;**37**:447–56.
331. Stone KM, Grimes DA, Magder LS. Personal protection against sexually transmitted diseases. *Am J Obstet Gynecol* 1986;**155**: 180–8.
332. Stupiansky NW, Van Der Pol B, Williams JA, et al. The natural history of incident gonococcal infection in adolescent women. *Sex Transm Dis* 2011;**38**:750–4.
333. Sullivan KM, MacDonald HJ, Saunders JR. Characterization of DNA restriction and modification activities in *Neisseria* species. *FEMS Microbiol Lett* 1987;**44**:389–93.
334. Swanson J. Studies on gonococcus infection: XIV—cell wall protein differences among color/opacity colony variants of *Neisseria gonorrhoeae*. *Infect Immun* 1978;**21**:292.
335. Swanson J. Colony opacity and protein II compositions of gonococci. *Infect Immun* 1982;**37**:359.
336. Swanson J, Robbins K, Barrera O, et al. Gene conversion variations generate structurally distinct pilin polypeptides in *Neisseria gonorrhoeae*. *J Exp Med* 1987;**165**:1016–25.
337. Tabrizi SN, Paterson BA, Fairley CK, et al. Comparison of tampon and urine as self-administered methods of specimen collection in the detection of *Chlamydia trachomatis*, *Neisseria gonorrhoeae* and *Trichomonas vaginalis* in women. *Int J S T D A I D S* 1998;**9**:347–9.
338. Thayer JD, Martin JE. Selective medium for the cultivation of *N. gonorrhoeae* and *N. meningitidis*. *Public Health Rep* 1964;**79**:49.
339. Tomeh MO, Wilfert CM. Venereal diseases of infants and children at Duke University Medical Center. *N C Med J* 1973;**34**:109–13.
340. Unemo M, Dillon J-AR. Review and international recommendation of methods for typing *Neisseria gonorrhoeae* isolates and their implications for improved knowledge of gonococcal epidemiology, treatment, and biology. *Clin Microbiol Rev* 2011;**24**:447–58.
341. Unemo M, Golparian D, Nicholas R, et al. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother* 2012;**56**:1273–80.
342. Upchurch DM, Brady WE, Reichart CA, et al. Behavioral contributions to acquisition and transmission of *Neisseria gonorrhoeae*. *J Infect Dis* 1990;**161**:938–41.
343. U.S. Preventive Services Task Force. Ocular prophylaxis for gonococcal ophthalmia neonatorum: reaffirmation recommendation statement. *Am Fam Physician* 2012;**85**:195–6.
344. Van Der Pol B, Ferrero D, Barrington LB, et al. Multicenter evaluation of the BDProbe Tec ET system for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in urine specimens, female endocervical swabs, and male urethral swabs. *J Clin Microbiol* 2001;**39**:1008–16.
345. Van Dyck E, Ieven M, Pattyn S, et al. Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* by enzyme immunoassay, culture, and three nucleic acid amplification tests. *J Clin Microbiol* 2001;**39**:1751–6.
346. Van Putten JP, Duensing TD, Cole RL. Entry of opaA$^+$ gonococci into Hep-2 cells requires concerted action of glycosaminoglycans, fibronectin and integrin receptors. *Mol Microbiol* 1998;**29**: 369–79.
347. Van Putten JPM, Robertson BD. Molecular mechanisms and implications for infection of lipopolysaccharide variation in *Neisseria*. *Mol Microbiol* 1995;**16**:847–53.
348. Wald ER, Woodward CL, Marston G, et al. Gonorrheal disease among children in a university hospital. *Sex Transm Dis* 1980;**7**: 41–3.
349. Walstad DL, Guymon LF, Sparling PF. Altered outer membrane protein in different colonial types of *Neisseria gonorrhoeae*. *J Bacteriol* 1977;**129**:1623–7.
350. Wehrbein HL. Gonococcus arthritis: a study of six hundred cases. *Surg Gynecol Obstet* 1929;**49**:105–13.
351. West SEH, Sparling PF. Response of *Neisseria gonorrhoeae* to iron limitation: alterations in expression of membrane proteins without apparent siderophore production. *Infect Immun* 1985; **47**:388–94.
352. Wetzler LM, Barry K, Blake MS, et al. Gonococcal lipooligosaccharide sialylation prevents complement-dependent killing by immune sera. *Infect Immun* 1992;**60**:39–43.
353. White ST, Loda FA, Ingram DL, et al. Sexually transmitted diseases in sexually abused children. *Pediatrics* 1983;**72**:16–21.
354. Whitley DM, Tapsall JW, Sloots TP. Nucleic acid amplification testing for *Neisseria gonorrhoeae*. *J Mol Diagn* 2006;**8**:3–15.
355. Whittington WL, Rice RJ, Biddle JW, et al. Incorrect identification of *Neisseria gonorrhoeae* from infants and children. *Pediatr Infect Dis* 1988;**7**:3–10.
356. Wiesner PJ, Handsfield HH, Holmes KK. Low antibiotic resistance of gonococci causing systemic infection. *N Engl J Med* 1973;**288**:1221–2.

357. Wiesner PJ, Tronca E, Bonin P, et al. Clinical spectrum of pharyngeal gonococcal infection. *N Engl J Med* 1973;**288**:181.
358. William DC, Felman YM, Riccardi NB. The utility of anoscopy in the rapid diagnosis of symptomatic anorectal gonorrhea in men. *Sex Trans Dis* 1981;**8**:16–17.
359. Woods CR, Versalovic J, Koeuth T, et al. Whole cell rep-PCR allows rapid assessment of clonal relationships of bacterial isolates. *J Clin Microbiol* 1993;**31**:1927–31.
360. Young JD-E, Blake M, Mauro A, et al. Properties of the major outer membrane protein from *Neisseria gonorrhoeae* incorporated into model lipid membranes. *Proc Natl Acad Sci U S A* 1983; **80**:3831–5.
361. Zhu W, Chen C-J, Thomas CE, et al. Vaccines for gonorrhea: can we rise to the challenge? *Front Microbiol* 2011. 2.124. doi: 10.3389/fmicb.2011.00124 (accessed 08/15/2012).

ulcerans grow on supplement-free blood agar, chocolate agar, and other standard media.[43]

Colonies of *C. diphtheriae* (with the exception of the lipophilic, gray *C. intermedius*) and *C. ulcerans* appear grayish white on Löffler medium. On tellurite medium, three diphtheria colony types can be distinguished: *mitis*, *gravis*, and *intermedius*. *Mitis* colonies are smooth, black, and convex; they do not ferment starch or glycogen and are hemolytic. *Gravis* colonies are gray, radially striate, and semirough; they ferment starch and glycogen and usually are not hemolytic. *Intermedius* colonies are small and smooth and have a black center; they do not ferment starch or glycogen and are not hemolytic. *C. ulcerans* colonies resemble *gravis* on Tindale medium but differ in that they are hemolytic. Similar to *gravis*, they ferment starch and glycogen. All diphtheria biotypes and *C. ulcerans* are characterized by cystinase activity and absence of pyrazinamidase activity. *C. ulcerans* may be distinguished from *C. diphtheriae* by its urease activity and ability to liquefy gelatin. Biotype *belfanti*, which does not occur in a toxigenic form, may be distinguished from the three potentially toxigenic diphtheria biotypes by its inability to reduce nitrate on Tindale medium and from *C. ulcerans* by its lack of production of urease.[43] Ribotyping and pulsed-field gel electrophoresis, both of which involve restriction digestion of genomic bacterial DNA, followed by gel electrophoresis and Southern blotting, permit more specific typing within each diphtheria biotype and aid in the epidemiologic study of outbreaks.[41,96]

C. diphtheriae biotypes *intermedius*, *gravis*, and *mitis* and *C. ulcerans* all have been observed in a toxigenic form. *Intermedius* was the biotype isolated most commonly in the United States between 1971 and 1981. Of the strains isolated, *intermedius* was found to be toxigenic more often than *mitis* or *gravis*.[36] In the United Kingdom between 1993 and 1998, and similarly in other parts of Europe, the biovar *gravis* has represented most nontoxigenic isolates, followed by *mitis* and *belfanti*.[45] Of the four nontoxigenic isolates obtained during a surveillance study of a U.S. Northern Plains Native American Community in 1996, two were of biotype *mitis* and two were *gravis*.[59] According to a United Kingdom diphtheria reference laboratory, between 1993 and 1998, the toxigenic isolates originating from Asia, Africa, and the Middle East were reported to be of the biotype *mitis* or *gravis*, with the exception of one *intermedius* isolate.[44] Golaz and associates[59] reported similar findings when they surveyed the different biotypes in South Dakota. A significant overall increase in the proportion of nontoxigenic isolates has been observed in Europe and Australia in recent years.[58] The reason for this increase is unclear, but one hypothesis is that increased immunity to toxigenic strains secondary to immunization has altered this epidemiology.

The complete genome sequence of *C. diphtheriae* biotype *gravis* has been elucidated.[33] The genome is approximately 2.48 Mb with a G+C content of 53 percent and has approximately 2320 predicted coding sequences. Metabolic analysis of the genome has revealed that *C. diphtheriae* has a complete set of enzymes for glycolysis, gluconeogenesis, pentose-phosphate pathways, anaerobic and aerobic respiration, amino acid biosynthesis, and purine nucleotide biosynthetic pathway. Most of the enzymes for the tricarboxylic acid cycle are present, with the exception of an enzyme succinyl coenzyme A (CoA) synthetase that catalyzes the conversion of succinate to succinyl CoA. An alternative enzyme present in *C. diphtheriae* may fulfill this conversion, however. The pyrimidine pathway in *C. diphtheriae* lacks an enzyme that interferes with the production of cytidine; the pathway to the biosynthesis of thymidine is complete. The genome has revealed 13 regions that are unique to *C. diphtheriae* and may serve as pathogenicity islands.[33]

No functional or significant differences have been detected in the exotoxins elaborated by the three strains of *C. diphtheriae* or by *C. ulcerans*. Only strains that are lysogenic for β-prophage or a closely related phage carrying the gene for toxin production produce diphtheria toxin. One or more *tox* gene sequences may exist in the bacterial genome, and the most highly toxigenic strains contain three or more copies.[82] Phage multiplication is not a prerequisite for the production of toxin. The capacity to synthesize toxin depends on genetic and nutritional factors. Toxin-producing cells apparently are cells in which spontaneous induction of the prophage to the phage occurs.[53] The most important factor controlling the yield of toxin is the concentration of inorganic iron in the culture medium.[39] Growth of *C. diphtheriae* in iron-deficient media prolongs the duration of induction lysis and is associated with a high yield of toxin. High concentrations of iron inhibit the production of toxin. Production of toxin also can be increased by the use of ultraviolet radiation. Conversion to a toxigenic strain occurs in nature, as has been shown by restriction enzyme studies of carriers of toxigenic and nontoxigenic strains in Manchester, England.

The ability of a strain of *C. diphtheriae* to elaborate diphtheria toxin can be shown by using several methods. In vivo studies involving necrosis of tissue in guinea pigs has been replaced by the widely used Elek[46] or modified Elek test.[49] Enzyme immunoassay[48] and polymerase chain reaction (PCR)[95] have been used to detect toxigenicity.

Diphtheria toxin is lethal to humans in an amount of approximately 130 μg/kg body weight. Cytoplasmic internalization of 1 molecule of toxin has been shown to cause cell death.[11] Toxigenic and nontoxigenic strains of *C. diphtheriae* can cause disease, but only strains that produce toxin cause disease with symptoms of myocarditis and neuritis.

EPIDEMIOLOGY

Asymptomatic human carriers serve as the reservoir for *C. diphtheriae*. Infection by *C. diphtheriae* is acquired by contact with either a carrier or an individual with active disease. The bacteria may be transmitted via droplets during coughing, sneezing, or talking. Rarely, transmission of *C. diphtheriae* occurs from skin lesions or fomites. Some reports suggest that skin carriers of *C. diphtheriae* are more infectious than nose or throat carriers and that skin carriers may serve as potential reservoirs for the initiation of epidemic spread.[12,68] In areas in which skin infections are endemic, levels of natural immunization

may be high.[21] This phenomenon is illustrated particularly well in a survey of tetanus and diphtheria immunity in a rural Kenyan community, where age was not found to be predictive of immunity and no correlation was found between levels of antibody for tetanus and diphtheria.[77]

Person-to-person transmission of *C. ulcerans* is not known to occur, although *C. ulcerans* was isolated from the siblings of two patients reported in the United Kingdom between 1995 and 1997.[18] Cases of respiratory diphtheria caused by *C. diphtheriae* and *C. ulcerans* have been documented in association with contaminated unpasteurized milk taken from cows with infected teats.[19,60,105] *C. diphtheriae* has been isolated from horses, dogs, and other domestic animals. *C. ulcerans* has been reported to infect ground squirrels in the United States, but transmission to humans has not been reported.[67,94] *C. ulcerans* is a commensal in animals and has been isolated in a wide range of wild and domestic animals.[106]

According to World Health Organization (WHO) reports, diphtheria is distributed worldwide and remains endemic in many developing areas of the world, including Asia, Africa, South America, and the Mediterranean regions.[20] Worldwide, diphtheria epidemics have occurred in a cyclic manner since the 16th century. The most recent epidemic began in 1990 in the Russian Federation, Ukraine, and the other newly independent states of the former Soviet Union.[24,107]

In the United States, the incidence and mortality rates from diphtheria in the 1920s were 140 to 150 cases/100,000, with 13,000 to 15,000 deaths each year. The number of cases gradually declined to 15 cases/100,000 population in 1945 with the extensive use of diphtheria toxoid vaccine. From 1970 through 1979, the average number of cases of diphtheria reported annually in the United States was 196.[8] From 1980 to 2004, only 57 cases of diphtheria were reported in the United States. Approximately 75 percent of the cases were in patients older than 15 years who were unimmunized or inadequately immunized.[8] From 1980 to 1995, only 4 fatal cases involving diphtheria and unimmunized children were reported.[14,87,97] In 1996, *C. diphtheriae* was isolated from a Native American population in South Dakota.[59] Only 5 cases of diphtheria have been reported since 2000. Maintenance of immunity in adults requires a booster vaccination every 10 years. The Centers for Disease Control and Prevention estimated in the mid-1990s that fewer than 50 percent of adults in the United States had received their 10-year boosters and that 40 to 50 percent of adults were susceptible to diphtheria.[52,97] In addition, the toxoid vaccine does not provide protection against nontoxigenic strains.

The incidence peaks during the cooler autumn, winter, and spring months. Several epidemics, primarily in the southern United States, have occurred in late summer and fall and corresponded to a high prevalence of *C. diphtheriae* skin infections. Between the years 1971 and 1981, the incidence of diphtheria was highest in the western United States. A 100-fold greater incidence of diphtheria occurs in Native Americans than in the general population.[36] This difference may be attributable to socioeconomic factors more than to race.

Evidence that diphtheria is diagnosed more frequently in chronic alcoholics and the indigent than in the general population is significant. In a 1993 to 1994 outbreak in St. Petersburg, Russia, 69 percent of a total of 42 deaths occurred in individuals classified as chronic alcoholics.[99] Between 1972 and 1982, three outbreaks occurred in the indigent alcoholic population living in Seattle's Skid Road.[66] Cutaneous infections accounted for 86 percent of the 1100 total cases. The first outbreak was caused by a single toxigenic *intermedius* biotype clone, whereas the other two involved nontoxigenic *mitis* and *gravis* strains. The incidence was highest in winter and spring.

Native Americans also represent a disproportionate number of diphtheria cases, the reasons for which are unclear, but apparently infection is endemic in some closely knit communities. During 1974 and 1975, 27 percent of Native Americans in the Skid Road population were affected, compared with 5 percent of the white population. In 1996, a surveillance study was conducted in a Northern Plains Native American community after *C. diphtheriae* was isolated from the skin of a resident of the community. The woman was a chronic alcoholic admitted to the hospital for detoxification and treatment of severe necrotizing leg ulcers, from which a toxigenic *mitis* biotype was isolated. In the following 4 months, 11 positive cultures were obtained from the community, including 4 positive throat swabs from asymptomatic household contacts of the index cases. Ribotyping indicated that the isolates were related closely to each other genetically and to strains obtained from past cases from the same area; they were different from organisms obtained from other parts of the United States and from the former Soviet Union, where an ongoing epidemic was occurring at the time.[26,59]

A similar study was conducted in a Koorie (Aborigine) community in Victoria, Australia, in 1994, after three cases of nontoxigenic *C. diphtheriae* endocarditis were diagnosed in the community. After screening 359 asymptomatic (with the exception of 4 people who had chronic skin ulcers swabbed) contacts of the index cases, 12 produced positive cultures for nontoxigenic *C. diphtheriae*. Of them, 5 were of the same biovar *gravis* clone as the three index cases.[69]

A major epidemic began in 1990 in the new independent states of the former Soviet Union and spread throughout the area. Between 1990 and 1997, approximately 150,000 cases of diphtheria were reported, with approximately 4000 fatalities.[65,96] The epidemic was attributed to decreasing immunization rates and immunity in adults and children and to movement of large numbers of people during the collapse of the former Soviet Union.[24,107] Apparently, multiple foci of infection existed across the continent. Most of the epidemic isolates from Ukraine and Russia were biotype *gravis*, but further molecular characterization of the *tox* genes from these areas revealed distinct epidemic strains in each location.[70] Superimposed over the steady increase in the number of cases from 1990 through 1994, a seasonal variation typical of the Northern Hemisphere also was observed, with a significant peak in the number of diagnoses occurring in October and November and a trough from April to July.[65]

A mass immunization program was initiated in Russia in 1993, with a resultant 10 percent decrease in the number of new cases reported between 1994 and 1995 (vs. twofold to threefold increases in the number of cases each year for the preceding 3 years).[57] The WHO also held training workshops and assembled laboratory kits to assist in establishing the proper diagnosis of diphtheria. As cases also began to appear in Europe with increasing frequency during this period, the European Working Group on Diphtheria (ELWGD) and reference laboratories were assembled to assist in an effort to increase routine screening for diphtheria. The ELWGD since has expanded and currently includes 20 participating countries, including representatives in Western and Eastern Europe, the United States, Australia, and Southeast Asia.[45] Surveillance from Europe from 2000 to 2009 has documented a decrease in incidence of more than 95 percent across the region, with the Russian Federation and Latvia accounting for 83 percent of the cases. In Western Europe, toxigenic *C. ulcerans* has increasingly been identified, often in association with contact with companion animals.[108]

PATHOGENESIS AND PATHOLOGY

Diphtheria is initiated by entry of *C. diphtheriae* into the nose or mouth, where the bacilli remain localized on the mucosal surfaces of the upper respiratory tract. Occasionally, the ocular or genital mucous membranes serve as the site of localization. The bacilli are unable to invade intact skin but may infect preexisting skin lesions. After a 2- to 4-day period of incubation, lysogenized strains may elaborate toxin.

Diphtheria toxin is secreted as a single polypeptide of 535 amino acids with a molecular weight of 58,342 D.[70] The toxin is composed of two subunits: a large B subunit that is involved in receptor binding and an A subunit that is the enzymatically active portion of the toxin. The toxin initially is absorbed onto the target cell membrane by binding a receptor on the cell surface and then undergoes receptor-mediated endocytosis. When it is in the endolysosomal complex, it undergoes a conformational change with subsequent release of the A subunit into the cytoplasm. The A subunit transfers an adenosine diphosphate (ADP)–ribosyl group from nicotinamide adenine dinucleotide (NAD) to elongation factor 2. This ADP-ribosylation inactivates elongation factor 2 and inhibits protein synthesis in the cell. Cholera and pertussis toxins also mediate target protein ribosylation by this mechanism. In addition to the inhibition of cellular protein synthesis, an independent mechanism of cytolysis has been described. In the presence of calcium and magnesium, diphtheria toxin has a nuclease-like activity that causes DNA fragmentation that results in cytolysis.[34,35,70,78]

Marked toxin-mediated tissue necrosis occurs in the vicinity of *C. diphtheriae* colonization and induces a robust local inflammatory response. The inflammatory response coupled with the necrotic tissue produces a patchy exudate that initially can be removed. As the infection progresses, the increased production of toxin causes a centrifugal widening of the area of infection, and eventually a fibrinous exudate develops. A tough adherent membrane results from coagulation of the exudate. The color of the pseudomembrane initially is white but over the course of time becomes dirty gray. Late in the course of the infection, green or black spots appear on the membrane, representing areas of necrosis. Histologic analysis of the pseudomembrane differs, based on the site of formation and maturation of the membrane. Analysis of the pharyngeal pseudomembrane shows fibrin; inflammatory cells, primarily composed of neutrophils, red blood cells, and colonies of organisms; and superficial epithelial cells.

With severe infections, significant vascular congestion, interstitial edema, fibrin exudate, and intense neutrophilic infiltration develop.[64] Profuse bleeding can occur when the membrane is torn off. The edematous tissue and the diphtheritic membrane may encroach on the airway. The membrane sloughs spontaneously during the recovery period, although sloughing can occur during the acute phase of the illness, leading to aspiration. Occasionally, secondary bacterial infection (classically caused by *Streptococcus pyogenes*) develops. Respiratory embarrassment or suffocation may occur, with involvement of the larynx or tracheobronchial tree. Bronchopneumonia may develop if the exudate enters the small airways and alveoli. Infection of these sites is an uncommon occurrence, however. Infections of the esophagus and stomach, with pseudomembranous lesions indistinguishable from lesions found in the respiratory tract, have been reported.[72]

Toxin produced at the site of infection is distributed throughout the body by the bloodstream and the lymphatics. This distribution occurs most readily when the pharynx and tonsils are covered by a diphtheritic membrane. Any organ or tissue can be damaged as a result of diphtheria toxin, but lesions of the heart, nervous system, and kidneys are particularly prominent. Clinical manifestations appear after a variable latent period of 10 to 14 days for myocarditis and 3 to 7 weeks for manifestations in the nervous system, such as peripheral neuritis. In a study of 102 patients who died of diphtheria caused by *C. diphtheriae*, the hearts appeared dilated, flabby, and pale, with a characteristic "streaky" appearance in the myocardium. The most prominent pathologic findings are necrosis and hyaline degeneration of the myocardium. The myocardium also appears edematous and is infiltrated with mononuclear cells with eosinophilic cytoplasm. In a significant proportion of cases, fatty accumulation in muscle fibers and the conducting system may be observed.[90] Burch and associates[22] showed mitochondrial damage with depletion of glycogen and accumulation of lipid droplets in the damaged myofibrils. Toxin may be observed within the myocardial cells with fluorescent antibody staining.[64] If the patient survives, muscle regeneration and interstitial fibrosis can be seen.

Peripheral neuropathy occurs secondary to *C. diphtheriae* infections. Histologic studies have shown that affected nerves have significant degeneration of myelin sheaths and axons. Toxic neuritis with fatty degeneration of paranodal myelin can be noted early in the disease course; segmental demyelination occurs later.[9] Axonal damage is secondary to the application of external

pressure from the swollen Schwann cell cytoplasm and myelin.[81]

C. diphtheriae infections also can lead to necrosis and hyaline degeneration of the liver, which can lead to hypoglycemia. Adrenal hemorrhage and acute tubular necrosis of the kidney have been known to occur secondary to *C. diphtheriae* infections.[64]

CLINICAL MANIFESTATIONS

The signs and symptoms of diphtheria depend on the site of infection, the immunization status of the host, and whether toxin has been distributed to the systemic circulation. The incubation period is 1 to 6 days (range, 1 to 10 days). Diphtheria can be classified clinically on the basis of the anatomic location of the initial infection and the diphtheritic membrane (nasal, pharyngeal/tonsillar, laryngeal or laryngotracheal, skin, and others) involved. More than one anatomic site may be involved simultaneously.

Nasal diphtheria initially resembles a common cold and is characterized by mild rhinorrhea and a paucity of systemic symptoms. Gradually, the nasal discharge becomes serosanguineous and then mucopurulent. A foul odor may be noticed, and careful inspection reveals a white membrane on the nasal septum. In severe cases, the infection may excoriate the nares and upper lip. Nasal diphtheria is a mild form of the disease because absorption of toxin usually is slow from this site. Frequently, delays in establishing an accurate diagnosis of nasal diphtheria occur because of the lack of systemic symptoms. The nasal form of the disease occurs most often in infants.

Pharyngeal and tonsillar diphtheria begin insidiously with anorexia, malaise, low-grade fever, and pharyngitis. Within 1 or 2 days, a membrane appears. The extent of membrane formation correlates with the immune status of the host; in some partially immune individuals, a membrane may not develop. The white or gray adherent membrane may cover the tonsils and pharyngeal walls and extend on to the uvula and soft palate or down on to the larynx and trachea. Attempts to remove the membrane are followed by bleeding. Cervical lymphadenitis varies. In some cases, it is associated with edema of the soft tissues of the neck and may be so severe that it gives the appearance of a "bull neck." In a 1970 epidemic, "erasure" edema of the neck was noted in patients with pharyngeal diphtheria.[85] Patients with erasure edema did not have a classic bull neck appearance, but the edema was characterized by obliteration of the sternocleidomastoid muscle border, the mandible, and the median border of the clavicle. The edema was brawny, pitting, warm to the touch, and tender to palpation. Erasure edema was noted in 29 percent of immunized patients and 30 percent of nonimmunized or inadequately immunized patients. It occurred most commonly in children older than 6 years and generally was associated with infection by the *gravis* or *intermedius* strain of *C. diphtheriae*.

The course of pharyngeal diphtheria depends on the degree of elaboration of the toxin and the extent of the membrane. In severe cases, respiratory and circulatory collapse may occur. The pulse rate is increased disproportionately to body temperature, which generally remains normal or slightly elevated. The palate may be paralyzed. This paralysis may be unilateral or bilateral and associated with difficulty swallowing and nasal regurgitation of swallowed fluids.[42] Stupor, coma, and death may occur within 7 to 10 days. In less severe cases, recovery may be slow and may be complicated by the development of myocarditis or neuritis. In mild cases, the membrane sloughs off in 7 to 10 days, and recovery is uneventful.

Laryngeal diphtheria generally reflects a downward extension of the membrane from the pharynx. Rarely, laryngeal diphtheria is primary and does not reflect an extension of disease from the pharynx. In these cases, toxicity and signs of toxemia generally are less prominent. Two cases of isolated diphtheritic tracheitis have been reported in the literature.[13,103] The clinical findings of laryngeal diphtheria are indistinguishable from those of other types of infectious croup. Noisy breathing, progressive stridor, hoarseness, and a dry, barking cough may be noted. Suprasternal, subcostal, and supraclavicular retractions reflect severe laryngeal obstruction, which may be fatal unless alleviated. Occasionally, in a mild case, an acute and fatal obstruction may occur because of a partially detached piece of membrane that occludes the airway. In severe cases of laryngeal diphtheria, the membrane may extend downward and invade the entire tracheobronchial tree.

Cutaneous disease, in contrast to pharyngeal disease, is more common in warmer climates and often is caused by nontoxigenic strains. In some countries with tropical and subtropical climates, such as Uganda, Tanzania, Sri Lanka, and Samoa, *C. diphtheriae* has been isolated from 60 percent of skin lesions in children.[74] Cutaneous diphtheria is more contagious than respiratory diphtheria. Cutaneous diphtheria may be an important source of person-to-person transmission of diphtheritic organisms and outbreaks in indigenous populations in which overcrowding and poor hygiene are important risk factors.[12,21,68] The skin lesions begin as vesicles or pustules that progress to typical ulcers with sharply defined borders, membranous bases, and surrounding erythema and edema. They may be covered with a dark pseudomembrane. The lesions occur most commonly on the legs, feet, and hands. For the first 1 to 2 weeks, the lesions are painful. Spontaneous healing generally takes 6 to 12 weeks, but lesions have been reported to persist for 1 year.[68]

Conjunctival, aural, and vulvovaginal diphtheria also may occur. Conjunctival lesions usually are limited to the palpebral conjunctiva, which appears red, edematous, and membranous. Rarely, conjunctival lesions have been associated with corneal erosion.[100] Diphtheria infections of the ear are characterized by the development of otitis externa with a persistent purulent and frequently foul-smelling discharge.

Clinical presentations other than typical diphtheria have been associated with isolation of the organism from patients with meningitis, endocarditis, osteomyelitis, and hepatitis. In most cases, these infections have occurred in patients with underlying problems, such as structural or valvular heart disease or intravenous drug use, or in individuals from poor socioeconomic backgrounds.[40,105]

Several cases of septic arthritis caused by nontoxigenic *C. diphtheriae* have been described.[1,63,105] Afghani and Stutman[1] reported the case of a 27-month-old child who had septic arthritis of the hip and skin lesions on the lower extremities. In this case, the skin lesions were presumed to be the portal of entry for nontoxigenic *C. diphtheriae*. Although the child had received four doses of diphtheria and tetanus toxoids and pertussis vaccines, immunization with toxoid does not provide protection against nontoxigenic strains of *C. diphtheriae*. In this case, the organism was sensitive to penicillin, cefuroxime, cephalothin, and clindamycin but was resistant to oxacillin, an antistaphylococcal antibiotic often used for the treatment of septic arthritis when the causative organism cannot be identified. A similar case was described in an immunocompetent, fully vaccinated 2-year-old child who had skin lesions from which *C. diphtheriae* was isolated. The skin lesions also were assumed to be the portal of entry for the organism because pan-sensitive *C. diphtheriae* were isolated from the skin and the articular aspirate.[64]

Within a 12-month period, in New South Wales, Australia, four cases of septic arthritis complicating endocarditis caused by the nontoxigenic *gravis* variety of *C. diphtheriae* were reported. In addition, the same strain caused three cases of endocarditis without the development of septic arthritis. Demographic distribution of these seven cases included a 12-year-old boy who died, five patients who were in their 20s, and a patient who was 49 years old. Three of the patients had underlying cardiac abnormalities, and one had a history of intravenous drug use.[105] This same clone of nontoxigenic *C. diphtheriae* was isolated from three patients in Koorie, an aborigine community in Victoria, Australia, who had endocarditis, and five asymptomatic contacts.[69] Two of the three patients with endocarditis were members of the same family, and one of them had a history of alcohol abuse. The third patient had a septic sternoclavicular joint, in addition to endocarditis, with isolation of the same organism. Nontoxigenic *C. diphtheriae* sepsis can lead to splenic and hepatic abscesses, as reported in a patient with chronic lymphocytic leukemia in British Columbia, Canada.[71]

Complications secondary to elaborated diphtheria toxin may affect any system, but myocarditis and involvement of the nervous system are most characteristic. Myocarditis may occur after mild and severe cases of diphtheria. Generally, it develops in patients in whom administration of antitoxin is delayed. Myocarditis most commonly appears in the second week of the disease, but it can appear as early as the first or as late as the sixth week of illness. Tachycardia, a muffled S_1, murmurs, and arrhythmias such as atrioventricular dissociation indicate myocardial involvement. Echocardiography may show left ventricular dysfunction.[3,61,80] Although some cases may result in cardiac failure, most myocardial complications are temporary.

Neurologic complications appear after a variable latent period. Approximately 75 percent of all patients with severe diphtheria develop neuropathies. The incidence of neurologic sequelae has been shown to correlate with the severity of respiratory symptoms; 20 percent of all patients with respiratory problems develop polyneuritis. Neurologic complications from diphtheria infections predominantly are bilateral, are motor rather than sensory, and usually resolve completely. Paralysis of the soft palate is the most common occurrence and generally appears in the third week. It is manifested by a nasal quality in the voice, nasal regurgitation, and difficulty swallowing. Ocular paralysis usually occurs around the fifth week of illness and is characterized by blurring of vision and difficulty with accommodation. Internal strabismus also may be noted. Paralysis of the diaphragm, peripheral neuropathy involving the limbs, and loss of deep tendon reflexes likewise are reported as complications of diphtheria. When they occur, along with an elevated cerebrospinal fluid protein, the syndrome is clinically indistinguishable from Guillain-Barré syndrome.

Rarely, 2 or 3 weeks after the onset of illness, involvement of the vasomotor centers results in hypotension and cardiac failure. Gastritis, hepatitis, nephritis, and hemolytic-uremic syndrome also have been reported as complications of diphtheria.[102]

Information on the effects, if any, of diphtheria on the fetus during pregnancy was unavailable until more recently. El Seed and associates[47] reported a case of pharyngeal diphtheria in a pregnant woman that occurred during the first trimester of pregnancy. Apart from vaginal bleeding, no complications of pregnancy were noted. Severe diphtheritic toxemia in the mother was characterized by quadriparesis, from which she fully recovered. A physically normal female infant was delivered at term. In this single case, severe diphtheritic toxemia during pregnancy was not associated with any teratogenic effect in the fetus and did not impair intrauterine fetal growth.

DIAGNOSIS

The diagnosis of diphtheria should be based on clinical findings because any delay in initiating therapy poses a serious risk to the patient. Isolation of the organism is used to confirm the clinical diagnosis. Material obtained from beneath the membrane, where organisms are concentrated most highly, or a portion of the membrane itself should be obtained for culture.[5,33]

C. diphtheriae is relatively resistant to drying. The use of a nonnutritive, moisture-reducing transport medium helps prevent the overgrowth of other microorganisms. The laboratory should be notified about the possibility of diphtheria so that appropriate culture media are inoculated. A Löffler slant, a tellurite plate, and a blood agar plate should be inoculated. Tellurite-containing media inhibit the growth of normal oral flora, allowing *C. diphtheriae* to grow into characteristic black colonies. Other corynebacteria, staphylococci, and yeast also can reduce tellurite and grow into black colonies.[14,38] Although examination of direct smears of colonies or diphtheritic lesions remains an important supplement to clinical examination, it often is inaccurate. Screening the colonies from the tellurite plate for catalase, urea, nitrate, pyrazinamidase, and cystinase is important. Most biotypes of *C. diphtheriae* are catalase-positive, urease-negative,

nitrate-positive (except biotype *belfanti*), pyrazinamidase-negative, and cystinase-positive.[43]

All diphtheria bacilli that are recovered should be tested for toxigenicity. In 1949, the Elek immunoprecipitation assay replaced in vivo testing for toxigenicity using guinea pigs or rabbits.[46] The Elek test is based on gel diffusion and immunoprecipitation of toxin, from organisms inoculated onto agar adjacent to an antitoxin-containing well. A strain that is positive for toxin is indicated by the formation of a precipitin band between the toxin and antitoxin.[44,46] The Elek test would take 48 hours to yield results on the toxigenic nature of a *C. diphtheriae* strain. A modified Elek test that consists of placement of an antitoxin-impregnated disk onto an agar plate surrounded by inoculates of clinical specimen and positive control has been described. In contrast to the conventional Elek test, the modified test has the advantage of using "spot" inoculations of numerous colonies directly from the primary plate. In addition, the modified Elek test has fewer false-positive and false-negative results and yields results more rapidly (16 to 24 hours).[43]

A rapid enzyme immunoassay is another test for detection of diphtheria toxin. This method uses equine polyclonal antibody to capture the diphtheria toxin and an alkaline phosphatase–labeled monoclonal antibody to detect fragment A of the toxin. It is a rapid test that takes 3 hours and has a limit of detection of 100 pg/mL.[48]

Rapid testing for diphtheria toxin by PCR specific for the "A" or "B" portion of the toxin gene, *tox*, is sensitive and has produced positive results in specimens stored for 12 months before performing the assay.* The absence of the *tox* gene by PCR excludes the diagnosis of diphtheria. PCR may give false-positive results, however, because it does not differentiate between partial or nonfunctional *tox* genes and functional *tox* gene products. An increasing number of cases of nontoxigenic diphtheria that are positive for the *tox* gene by PCR have been reported from Ukraine and Russia.[58,83]

The immune status of patients can be determined by toxin neutralization in Vero cells.[89] This method is used frequently, although it is difficult to standardize and relies heavily on individual interpretation of results. Enzyme-linked immunosorbent assay is a more rapid and quite sensitive method, but it detects some nonspecific antibodies; when antitoxin levels are in the low range, the assay may generate falsely elevated results.[86] Finally, a delayed fluorescence immune assay method was developed by Aggerbeck and colleagues[2] in 1996 and has been reported to have good sensitivity, specificity, and reproducibility.[17] Levels of diphtheria antitoxin of 0.01 IU/mL or greater generally are accepted as protective. A skin-testing method, the Schick test, also has been used to assess immunity.

The Schick test was used previously to determine the immune status of the patient. It is not helpful in establishing an early diagnosis because it cannot be read for several days, and currently it is not widely used. In the Schick test, a measured amount of purified diphtheria toxin (0.1 mL) is injected subcutaneously. A hypersensitivity reaction indicates an inadequate presence of antitoxin. A toxoid control also is injected, in the opposite arm, to help distinguish between a reaction to toxin and a reaction to other antigens in the toxin preparation.

Other laboratory studies are of little diagnostic value. The white blood cell count may be normal or elevated. Rarely, anemia develops as a result of rapid hemolysis of red blood cells. Examination of cerebrospinal fluid may reveal a minimal elevation of protein and, rarely, a mild pleocytosis in patients with diphtheritic neuritis. Hypoglycemia, glucosuria, or both may occur and reflect hepatic toxicity. An elevation in blood urea nitrogen may develop in patients with acute tubular necrosis. An electrocardiogram should be obtained and may reveal ST-segment and T-wave changes or arrhythmias indicative of myocarditis.

Differential Diagnosis

Mild forms of nasal diphtheria in a partially immunized host may resemble the common cold. When the nasal discharge is more serosanguineous or purulent, nasal diphtheria must be distinguished from a foreign body in the nose, sinusitis, adenoiditis, or the snuffles of congenital syphilis. Careful examination of the nose with a nasal speculum, sinus radiographs, and appropriate serologic tests for syphilis are helpful in excluding these disorders.

Tonsillar or pharyngeal diphtheria must be differentiated from streptococcal pharyngitis. Generally, streptococcal pharyngitis is associated with more severe pain on swallowing, higher temperature, and a nonadherent membrane limited to the tonsils. In some patients, pharyngeal diphtheria and streptococcal pharyngitis coexist.

Tonsillar and pharyngeal diphtheria also must be differentiated from infectious mononucleosis (lymphadenopathy and splenomegaly are common findings, atypical lymphocytes are generally present, and heterophile antibody may be present), nonbacterial membranous tonsillitis (white blood cell count generally is low, throat cultures reveal normal flora, and the course is unaffected by antibiotics), primary herpetic tonsillitis (presence of gingivitis, stomatitis, and discrete lesions of the tongue and palate may be helpful), Vincent angina (may be indistinguishable), and thrush (constitutional symptoms are absent, and lesions are present on the buccal mucosa and tongue). Tonsillar and pharyngeal diphtheria also must be differentiated from blood dyscrasias such as agranulocytosis and leukemia (complete blood count and bone marrow study are helpful); post-tonsillectomy faucial membranes (membranes are stationary and do not spread); and oropharyngeal involvement by toxoplasmosis, *Arcanobacterium*, cytomegalovirus, tularemia, and salmonellosis (associated signs and symptoms and appropriate cultures and serologic tests may be diagnostic).[55]

Laryngeal diphtheria must be differentiated from spasmodic or nonspasmodic croup; acute epiglottitis; laryngotracheobronchitis; aspirated foreign bodies; peripharyngeal and retropharyngeal abscesses; and laryngeal papillomas, hemangiomas, or lymphangiomas. A careful history, followed by careful visualization in the hospital under controlled conditions, aids in making a correct diagnosis.

*References 7, 74, 75, 92, 93, 95.

PREVENTION

Diphtheria is prevented on a community-wide basis most effectively by active immunization. Diphtheria toxoid is available in combination with tetanus toxoid as pediatric DT or adult Td and in combination with acellular pertussis as DTaP and Tdap. Combination regimens with DTaP and inactivated poliovirus and hepatitis B (Pediatrix) and inactivated poliovirus and *Haemophilus influenzae* type B (Pentacel) are also available. Pediatric formulations of diphtheria toxoid vaccines contain three to four times more diphtheria toxoid but the same tetanus toxoid in contrast to the adult formulations. Children younger than 7 years should be given the pediatric formulations of vaccine, whereas children older than 7 years should receive the adult Td. Two forms of Tdap are available: Boostrix, which is approved for children beginning at age 10 through 65 years or older, and Adacel, which is approved for individuals 11 to 64 years old.[23,27-30]

Primary immunization is carried out conveniently and effectively by giving diphtheria and tetanus toxoids and pertussis vaccine, DTaP, at 2, 4, and 6 months of age, with booster doses given at 15 to 18 months and again when the child is 4 to 6 years of age. Booster doses with the adult type of diphtheria and tetanus toxoids adsorbed (Td) should be given at 10-year intervals to all immunized individuals. Current recommendations are to give Tdap as the first booster dose in patients who are 11 to 12 years old and to anyone older who has not received a dose of Tdap.[31] Td and Tdap contain 2 to 2.5 flocculation units (Lf) diphtheria toxoid per dose in contrast to 7 to 25 Lf in the pediatric diphtheria, tetanus toxoid, and pertussis vaccine preparations (DTaP and DT). Primary immunization of children older than 7 years may be performed with Td. Two doses are given intramuscularly at least 4 weeks apart, with a booster dose provided 1 year later. Children and adults who are severely immunocompromised or undergoing long-term hemodialysis should use the standard immunization schedule, although response may be suboptimal.[51,56,76]

Most local and systemic reactions to the diphtheria and tetanus toxoids and whole cell pertussis vaccine (DTP), including fever, were related to the pertussis component.[10,37] Administration of tetanus and diphtheria toxoids is not followed by the high incidence of reactions associated with the use of pediatric DTP vaccines. At least one study showed that 7.5 Lf toxoid can be given safely to adults without a higher risk for reactions occurring.[15] Primary immunization against diphtheria for infants with progressive neurologic disorders and completion of the primary immunization series in patients who had experienced an untoward reaction to an earlier DTP vaccine injection may be performed with diphtheria and tetanus toxoids rather than diphtheria and tetanus toxoids and pertussis vaccine.[4]

A report of 97 preterm infants who received diphtheria and tetanus toxoids and acellular pertussis vaccine (DTaP) (94 of these infants also received *H. influenzae* type b vaccine) showed that most infants tolerated the vaccination without side effects, although a subgroup of infants with very low birth-weight (mean, 873 g) had either a recurrence or an increase in the number of apneic and bradycardic episodes in the 48 hours after receiving vaccination. The apneic and bradycardic episodes were present before immunization in every case.[101]

Booster doses of tetanus and diphtheria toxoids should be given at 10-year intervals to all immunized individuals. The Centers for Disease Control and Prevention Advisory Committee on Immunization Practices now recommends that all adults aged 19 years and older who have not received a dose of Tdap should receive a single dose.[32,84] As mentioned earlier, levels of diphtheria antitoxin of 0.01 IU/mL or greater generally are accepted as protective.

Diphtheria immunization is not always followed by complete protection.[88] Immunization is directed against the phage-mediated toxin, not against infection. Fully immunized individuals may be carriers or may have disease caused by nontoxigenic strains. An investigation conducted during an epidemic in Texas showed no statistical difference in the risk for diphtheria infection developing in individuals with full, lapsed, inadequate, or no previous diphtheria immunization; however, a 30-fold increased risk for development of symptomatic diphtheria in individuals with no immunization and an 11.5-fold increase in individuals with inadequate immunization were noted.[88]

The most important health problem in the United States today is inadequate immunization of the population. Immunization rates in adults are poorer than those in infants and children because of failure to maintain adequate immunity through appropriate booster immunization. A 70 to 80 percent immunization level is thought to be required to prevent epidemic spread.[36]

Prevention of diphtheria also depends on management of the contacts of known cases of diphtheria and carriers of the organism and on isolation of patients to minimize the spread of disease. Individuals at risk for contracting the disease from the index case include those who have had close respiratory or physical contact or prolonged close proximity with the infected individual, including members of the index case's household.[18] Specifically, family members who share body towels and cups or eating utensils, share a bed or a bedroom with more than two people, or take a bath less than once per week have a significantly greater risk for contracting the disease from the infected patient. A history of eczema in the contact also has been associated with a significantly increased risk for contracting diphtheria from the index case.[98] The patient is infectious until diphtheria bacilli no longer can be cultured from the site of infection. Two or three consecutive negative cultures at least 24 hours apart are required, and antibiotic therapy must be complete for 24 hours before the patient is released from isolation. If obtaining cultures is impossible, isolation may be ended after the completion of 14 days of appropriate antibiotic treatment.[6]

Cultures should be taken from the nose and throat of all close contacts, who should be kept under surveillance for 7 days (surveillance as an outpatient is acceptable).[5] Regardless of their immunization status, contacts should

be treated with a single intramuscular dose of benzathine penicillin G 600,000 U for individuals weighing less than 30 kg and 1.2 million U for individuals weighing more than 30 kg or a 7-day course of erythromycin 40 to 50 mg/kg/day (maximum 2 g/day) divided into four doses.[5] The immune status of each contact should be determined; individuals for whom immune status is inadequate, including individuals who have had the primary series but more than 5 years has elapsed since they received their last booster dose, should receive an injection of diphtheria toxoid. In addition, patients with diphtheria should be immunized during convalescence because infection may not confer immunity.[6]

Asymptomatic carriers who previously were not immunized against diphtheria should have cultures taken, receive diphtheria toxoid and penicillin or erythromycin (as described earlier), and be seen daily by a physician. Asymptomatic contacts who are found to carry a toxigenic strain should be subjected to the same isolation and treatment measures as the index case.[6] If daily surveillance is impossible, benzathine penicillin is preferred over erythromycin for treatment because failure to adhere to an oral drug regimen is a concern. If a contact is experiencing symptoms when seen, treatment of diphtheria is indicated. It is important to initiate prophylactic therapy in contacts who have not been immunized, before the results of culture are received. Management of carriers is described in the next section. Contacts whose occupations involve close contact with unimmunized children or food handling (especially milk) should refrain from working until cultures are confirmed to be negative.[6]

TREATMENT

Treatment of diphtheria is predicated on neutralization of free toxin and eradication of *C. diphtheriae* or *C. ulcerans* with antibiotics. Disease caused by *C. ulcerans* should be treated in the same manner as disease caused by *C. diphtheriae*.[3,25] The decision to administer equine antitoxin should be based on the site and size of the membrane, the degree of toxicity, and the duration of illness.[16]

Antitoxin can neutralize circulating toxin or toxin that is absorbed onto cells, but is ineffective when cells have been penetrated. Early treatment is essential to limit tissue damage. An adequate dose of antitoxin must be administered intravenously as early as possible to neutralize all free toxins. A single dose is used to avoid the risk for developing sensitization from repeated doses of horse serum. Tests for sensitivity to horse serum must be performed before antitoxin is administered. For this purpose, 0.02 mL of a 1:1000 dilution of antitoxin in saline can be given intracutaneously. Positive (histamine) and negative (isotonic saline) controls should be applied similarly. A positive reaction consists of a wheal at least 3 mm larger than the negative control, with surrounding erythema at the site of injection, developing within 15 to 20 minutes, and necessitates desensitization. The histamine control must be positive if test results are to be considered valid. Alternatively, the test may be done with a drop of serum diluted 1:100 and applied to the site of a superficial scratch, prick, or puncture on the volar aspect of the forearm. Controls should be applied and results interpreted as described for intracutaneous administration.[5]

If a patient has been shown to be sensitive to horse serum, the serum should be provided in a slowly increasing dosage given at 15-minute intervals. Several regimens have been used. One recommended regimen is as follows[5]:

- 0.1 mL of a 1:1000 dilution intravenously
- 0.3 mL of a 1:1000 dilution intravenously
- 0.6 mL of a 1:1000 dilution intravenously
- 0.1 mL of a 1:100 dilution intravenously
- 0.3 mL of a 1:100 dilution intravenously
- 0.6 mL of a 1:100 dilution intravenously
- 0.1 mL of a 1:10 dilution intravenously
- 0.3 mL of a 1:10 dilution intravenously
- 0.6 mL of a 1:10 dilution intravenously
- 0.1 mL undiluted intravenously
- 0.3 mL undiluted intravenously
- 0.6 mL undiluted intravenously
- 1 mL undiluted intravenously

The intravenous route for desensitization is considered safest because it offers good control, but protocols involving the intradermal, subcutaneous, and intramuscular routes also have been used frequently.[5]

If no reaction has occurred, the remaining material is given by slow intravenous infusion. Intravenous administration results in more rapid excretion of antitoxin into saliva, rendering it atoxic and preventing further absorption of toxin in the oropharynx, but it does not result in more rapid systemic elimination of antitoxin than intramuscular administration.[104] Reactions should be treated with aqueous epinephrine (1:1000) provided intravenously.

The antitoxin dosage is empirical. Pharyngeal or laryngeal disease of 48 hours' duration or less should be treated with 20,000 to 40,000 U, nasopharyngeal disease with 40,000 to 60,000 U, and severe pharyngeal or laryngeal diphtheria with 80,000 to 120,000 U of antitoxin. The last dose also should be given to patients with mixed clinical symptoms and to patients with brawny edema or disease of longer than 48 hours' duration. The value of antitoxin in the treatment of cutaneous disease is debated, but some experts recommend 20,000 to 40,000 U because toxic effects have been reported.[5]

Although antibiotics are not a substitute for treatment with antitoxin, they should be given when diphtheria is suspected clinically. Penicillin and erythromycin are still effective against most strains of *C. diphtheriae*. Penicillin and erythromycin also are effective in eradicating group A hemolytic streptococci, which may complicate 30 percent of cases of diphtheria. Treatment consists of a 14-day course of penicillin or erythromycin. Penicillin may be given as aqueous penicillin G 100,000 to 150,000 U/kg/day in four divided doses intravenously or as procaine penicillin 25,000 to 50,000 U/kg/day (maximum of 1.2 million U) in two divided doses intramuscularly. Patients who are sensitive to penicillin should be given erythromycin in a daily dosage of 40 to 50 mg/kg (maximum of 2 g/day) in four divided doses for 14 days. When the patient is able to tolerate oral

medications, erythromycin or penicillin V may be used in place of the intravenous antibiotics.[5]

Follow-up cultures should be obtained at least 2 weeks after antibiotic therapy is completed; if they are positive, erythromycin should be given for an additional 10 days.[5] Some resistance to erythromycin has been observed, but it is uncommon, and its epidemiologic significance is unknown.[5,6] Penicillin is recommended as first-line treatment in Vietnam, based on sensitivity data.[73] Amoxicillin, rifampin, and clindamycin provided in appropriate dosages also may be effective. Lincomycin and tetracycline have proved to be less effective, and cephalexin, oxacillin, and colistin have been shown to be ineffective against *C. diphtheriae*. The end point of therapy is two or three consecutive negative cultures at least 24 hours apart. In addition to receiving antibiotic therapy, patients with diphtheria should be immunized during convalescence because infection may not confer immunity.[5]

The carrier state has been treated effectively with a single intramuscular dose of benzathine penicillin G 600,000 U for children weighing less than 30 kg or 1.2 million U for individuals weighing 30 kg or greater or oral erythromycin 40 to 50 mg/kg/day for children and 1 g/day for adults for 7 to 10 days.[5] Carriers should have repeat pharyngeal cultures performed a minimum of 2 weeks after antibiotic therapy is complete; if the repeat cultures are positive, carriers should receive an additional course of antibiotics.

Treatment of deep infections caused by nontoxigenic *C. diphtheriae* can be accomplished with a wide variety of susceptible agents. Endocarditis with these organisms treated with either a β-lactam alone or in combination with an aminoglycoside has been associated with favorable outcomes.[91]

Supportive Treatment

Bed rest is extremely important and should be required for 2 to 3 weeks. Serial electrocardiograms should be obtained two or three times each week for 4 to 6 weeks to detect myocarditis as early as possible. Absolute bed rest must be enforced if myocarditis is detected, because sudden death has been precipitated by excessive activity. A patient with myocarditis may receive digitalization if congestive heart failure develops. Digitalization for arrhythmias caused by diphtheria may be contraindicated, however. In severe disease, prednisone 1 to 1.5 mg/kg/day for 2 weeks has been shown to lessen the incidence of myocarditis.

Hydration should be maintained, and a high-calorie liquid or soft diet should be provided. Secretions should be suctioned as needed to prevent aspiration. Palatal and pharyngeal paralysis increases the risk for aspiration occurring, so gavage via a nasogastric tube is indicated in these patients.

The quality of the voice and the gag reflex should be checked regularly for assessment of progression of the disease. Laryngeal diphtheria may require relief of obstruction with a tracheostomy. This procedure should be performed before the patient has become exhausted.

Adequate immunity does not develop in at least half of patients who recover from diphtheria, and they remain subject to reinfection. Immunization is indicated after the patient recovers.

PROGNOSIS

Many factors affect the prognosis in cases of diphtheria, the most important being the immunization status of the host. Morbidity and mortality rates are increased significantly in patients who are unimmunized or inadequately immunized. The rapidity with which medical care is sought and the diagnosis of diphtheria is suggested has a great impact on outcome. If specific treatment is provided on the first day of disease, the mortality rate may be reduced to less than 1 percent; delay in providing treatment until day 4 may be associated with a 20-fold increase in the mortality rate.

The virulence of the infecting organism and the location of infection are important prognostic factors. Infection with a nontoxigenic *C. diphtheriae* strain may cause disease but does not lead to myocarditis, neuritis, and other toxin-related phenomena. Toxigenic disease may vary from mild to severe. In cases of mild diphtheria, membrane sloughing and full recovery generally occur within 7 days. Disease caused by toxigenic *gravis* strains tends to be more severe and carries a poorer prognosis. Although diphtheria may affect the skin, nasopharynx, and other mucous membranes, involvement of the larynx heralds a more complicated course. Laryngeal diphtheria increases the risk for development of airway obstruction and promotes systemic absorption of the toxin. These patients require close monitoring of respiratory function and for involvement of other organ systems. Laryngeal diphtheria is more likely to be fatal in infants.

Few laboratory parameters indicate the severity of diphtheria. The development of amegakaryocytic thrombocytopenia and leukocytosis with counts of greater than 25,000 cells/mm^3 has been associated with a poor outcome.

The prognosis in a patient with diphtheria remains guarded until recovery is complete. At any time during the course of the illness, complications such as laryngeal obstruction, shock, and ventricular fibrillation may occur suddenly and unexpectedly. In patients with myocardial involvement, permanent damage to the heart, specifically fibrosis, may occur and lead to later complications. In addition, potentially severe neurologic manifestations, such as phrenic nerve paralysis, may appear late in the course of the disease.

Persistence of *C. diphtheriae* may be noted in the nasopharynx of 5 to 10 percent of convalescing patients. Recovery from diphtheria is followed by immunity that is demonstrable for at least 1 year after illness in 50 percent of patients. Second attacks are rare; nonetheless, immunization should be performed after the patient recovers.

Before the use of antitoxin and the availability of antibiotics, the mortality rate from diphtheria was 30 to 50 percent. Death was most common in children younger than 4 years old and was the result of suffocation. At present, the worldwide mortality rate is 5 to 10 percent, with no clear association with age.

NEW REFERENCES SINCE THE SIXTH EDITION

5. American Academy of Pediatrics. Diphtheria. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *2012 Red book: Report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2000. p. 63–6.
31. Centers for Disease Control and Prevention. Updated recommendations for the use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices (ACIP), 2012. *MMWR Morb Mortal Wkly Rep* 2011;**60**:13–15.
32. Centers for Disease Control and Prevention. Updated recommendations for the use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine in adults aged 65 years and older: Advisory Committee on Immunization Practices (ACIP), 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:468–70.
91. Muttaiyah S, Best EJ, Freeman JT, et al. *Corynebacterium diphtheriae* endocarditis: a case series and review of the treatment approach. *Intl J Infect Dis* 2011;**15**:e584–8.
106. Tiwari TSP, Golez A, Yu DT, et al. Investigation of 2 cases of diphtheria-like illness due to toxigenic *Corynebacterium ulcerans*. *Clin Infect Dis* 2008;**46**:3395–401.
108. Wagner KS, White JM, Lucenko I, et al. Diphtheria in the post-epidemic period, Europe, 2000-2009. *Emerg Infect Dis* 2012;**18**: 217–25.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 91

Anthrax

Morven S. Edwards

Anthrax is a toxigenic disease of herbivores for which humans are an incidental host. The term *anthrax*, derived from the Greek *anthrakos*, which means "coal," refers to the black eschar characteristic of cutaneous anthrax. The three clinical manifestations of anthrax are cutaneous, which accounts for 95 percent of infections in the United States; inhalational; and gastrointestinal. Each manifestation can occur in children,[29,42] and all can be complicated by meningitis.

HISTORICAL ASPECTS

Anthrax has been recognized since antiquity. The earliest reference to a disease thought to be anthrax is a description in Exodus of the plague that caused the death of all of the Egyptians' cattle. Hippocrates described carbuncles that are thought to represent the cutaneous form of anthrax. An account of anthrax in animals is detailed in the third of the Roman poet Virgil's four *Georgics*.[39] In the early 17th century, an anthrax pandemic referred to as the "Black Bane" caused 60,000 human deaths in Europe. By the 18th century, several excellent descriptions of the clinical disease in humans had been published.

Bacillus anthracis has a unique niche in infectious diseases. The organism, seen microscopically by Delafond in 1838, was isolated and cultivated in 1877 by Koch, who showed its proliferation in vivo, establishing a model for the causation of infectious disease. In 1881, both Pasteur and Greenfield[45] showed that a live-attenuated anthrax bacillus vaccine protected against subsequent challenge in animals, demonstrating the efficacy of immunization to prevent an infectious disease. In the late 1800s, anthrax was proved to be the cause of woolsorter's disease, or inhalation anthrax, contracted by factory workers handling anthrax-contaminated animal hides.

During World War I, *B. anthracis* was manufactured as an agent for biologic warfare. Because anthrax transmitted by inhalation is usually fatal and because *B. anthracis* spores are stable in the environment, anthrax continues to be a focus of biologic warfare research programs. The accidental release of anthrax spores from a military research facility in Sverdlovsk in the former Soviet Union in 1979 resulted in at least 68 deaths from inhalational anthrax.[32]

The first confirmed outbreak associated with intentional anthrax release in the United States occurred in October and November 2001.[22] Anthrax spores were disseminated by mail, resulting in 5 deaths from inhalation and 22 total cases of cutaneous and inhalational anthrax. Envelopes containing anthrax spores were sent through the U.S. Postal Service to offices of newspaper and broadcast media and U.S. senators. The spores were "weaponized," or finely milled, and treated with chemicals to prevent clumping so that they dispersed when the envelopes were opened and leaked from sealed envelopes as they passed through mail-sorting machines. A single anthrax strain was implicated. Outbreak-related expenditures exceeded billions of dollars, and the outbreak revealed the potential impact of a bioterrorism event on society.[6,10] It is estimated that the aerosolized release of

REFERENCES

1. Afghani B, Stutman HR. Bacterial arthritis caused by *Corynebacterium diphtheriae*. *Pediatr Infect Dis J* 1993;**12**:881–2.
2. Aggerbeck H, Norgaard-Pedersen B, Heron I. Simultaneous quantitation of diphtheria and tetanus antibodies by double antigen, time-resolved fluorescence immunoassay. *J Immunol Methods* 1996;**190**:171–83.
3. Ahmad N, Gainsborough N, Paul J. An unusual case of diphtheria and its complications. *Hosp Med* 2000;**61**:436–7.
4. American Academy of Pediatrics. *Report of the Committee on Infectious Diseases*. 22nd ed. Elk Grove Village, Ill: American Academy of Pediatrics; 1991. p. 195–9.
5. American Academy of Pediatrics. Diphtheria. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *2012 Red book: Report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2000. p. 63–6.
6. American Public Health Association: Diphtheria. In: Chin J, editor. *Control of communicable diseases manual*. Washington, DC: American Public Health Association; 2000. p. 165–70.
7. Aravina-Roman M, Bowman R, O'Neill G. Polymerase chain reaction for the detection of toxigenic *Corynebacterium diphtheriae*. *Pathology* 1995;**27**:71–3.
8. Atkinson W, Hamborsky J, McIntyre L, Wolfe S, editors. *Epidemiology and prevention of vaccine-preventable diseases*. 9th ed. Washington, DC: Public Health Foundation; 2006. p. 57–68.
9. Baba M, Gilliatt RW, Harding AE, Reiners K. Demyelination following diphtheria toxin in the presence of axonal atrophy. *J Neurol Sci* 1984;**64**:199–211.
10. Baraff LJ, Manclark CR, Cherry JD, et al. Analyses of adverse reactions to diphtheria and tetanus toxoids and pertussis vaccine by vaccine lot, endotoxin content, pertussis vaccine potency and percentage of mouse weight gain. *Pediatr Infect Dis J* 1989;**8**: 502–7.
11. Battistini A, Curatola AM, Gallinare P, et al. Inhibition of protein synthesis by diphtheria toxin induces a peculiar pattern of synthesized protein species. *Exp Cell Res* 1988;**176**:174–9.
12. Belsey MA, Sinclair M, Roder MR, et al. *Corynebacterium diphtheriae* skin infections in Alabama and Louisiana: a factor in the epidemiology of diphtheria. *N Engl J Med* 1969;**280**:135–41.
13. Berner R, Leititis JU, Furste HO, Brandis M. Bacterial tracheitis caused by *Corynebacterium diphtheriae*. *Eur J Pediatr* 1997;**156**: 207–8.
14. Bisgard KM, Hardy IRB, Popovic T, et al. Respiratory diphtheria in the United States, 1980 through 1995. *Am J Public Health* 1998; **88**:787–91.
15. Bjorkholm B, Granstrom M, Wahl M, et al. Adverse reactions and immunogenicity in adults to regular and increased dosage of diphtheria vaccine. *Eur J Clin Microbiol* 1987;**6**:637–40.
16. Bjorkholm B, Olling S, Larsson P, et al. An outbreak of diphtheria among Swedish alcoholics. *Infection* 1987;**15**:354–8.
17. Bonin E, Tiru M, Hallander H, Bredberg-Raden U. Evaluation of single- and dual antigen delayed fluorescence immunoassay in comparison to an ELISA and the in vivo toxin neutralisation test for detection of diphtheria toxin antibodies. *J Immunol Methods* 1999;**230**:131–40.
18. Bonnet JM, Begg NT. Control of diphtheria: guidance for consultants in communicable disease control. *Commun Dis Public Health* 1999;**2**:242–9.
19. Bostock AD, Gilbert FR, Lewis D, Smith DC. *Corynebacterium ulcerans* infection associated with untreated milk. *J Infect* 1984;**9**: 286–8.
20. Bowler CJ, Mandal BK, Schlecht B, et al. Diphtheria: the continuing hazard. *Arch Dis Child* 1988;**63**:194–5.
21. Bray JP, Burt EG, Potter EJ, et al. Epidemic diphtheria and skin infections in Trinidad. *J Infect Dis* 1972;**126**:34–40.
22. Burch GE, Sun SC, Sohal RS, et al. Diphtheritic myocarditis: a histochemical and electron microscopic study. *Am J Cardiol* 1968;**21**:261–8.
23. Centers for Disease Control and Prevention. Diphtheria, tetanus and pertussis: guidelines for vaccine prophylaxis and other preventive measures. *MMWR Morb Mortal Wkly Rep* 1981;**30**:392–407.
24. Centers for Disease Control and Prevention. Diphtheria epidemic: new independent states of the former Soviet Union, 1990-1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**:177–81.
25. Centers for Disease Control and Prevention. Respiratory diphtheria caused by *Corynebacterium ulcerans:* Terre Haute, Indiana, 1996. *MMWR Morb Mortal Wkly Rep* 1997;**46**:330–2.
26. Centers for Disease Control and Prevention. Toxigenic *Corynebacterium diphtheriae:* Northern Plains Indian Community, August-October 1996. *MMWR Morb Mortal Wkly Rep* 1997;**46**:506–10.
27. Centers for Disease Control and Prevention. Use of diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine as a five-dose series. *MMWR Morb Mortal Wkly Rep* 2000;**49**(RR13):1–8.
28. Centers for Disease Control and Prevention. Notice to readers: Food and Drug Administration approval of a fifth acellular pertussis vaccine for use among infants and young children—United States. *MMWR Morb Mortal Wkly Rep* 2002;**51**:574.
29. Centers for Disease Control and Prevention. Notice to readers: FDA licensure of diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis B (recombinant), and poliovirus vaccine combined (PEDIARIX) for use in infants. *MMWR Morb Mortal Wkly Rep* 2003;**52**:203–4.
30. Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, pertussis among adolescents: use of tetanus, reduced diphtheria toxoid, and acellular pertussis vaccine. *MMWR Morb Mortal Wkly Rep* 2006;**55**(RR03):1–34.
31. Centers for Disease Control and Prevention. Updated recommendations for the use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices (ACIP), 2012. *MMWR Morb Mortal Wkly Rep* 2011;**60**:13–15.
32. Centers for Disease Control and Prevention. Updated recommendations for the use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine in adults aged 65 years and older: Advisory Committee on Immunization Practices (ACIP), 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:468–70.
33. Cerdeno-Tarraga AM, Efstratiou A, Dover LG, et al. The complete genome sequence and analysis of *Corynebacterium diphtheriae* NCTC13129. *Nucl Acid Res* 2003;**31**:6516–23.
34. Chang MP, Baldwin RL, Bruce C, Wisnieski BJ. Second cytotoxic pathway of diphtheria toxin suggested by nuclease activity. *Science* 1989;**246**:1165.
35. Chang MP, Bramhall J, Graves S, et al. Internucleosomal DNA cleavage precedes diphtheria toxin-induced cytolysis: evidence that cell lysis is not a simple consequence of translation inhibition. *J Biol Chem* 1989;**264**:15261–7.
36. Chen RT, Broome CV, Weinstein RA, et al. Diphtheria in the United States, 1971-1981. *Am J Public Health* 1985;**75**:1393–7.
37. Cherry JD, Baraff LJ, Hewlett E. The past, present, and future of pertussis: the role of adults in epidemiology and future control. *West J Med* 1989;**150**:319–28.
38. Clarridge JE, Popovic T, Inzana TJ. Diphtheria and other corynebacterial and coryneform infections. In: Hausler WJ, Sussman M, editors. Topley and Wilson's microbiology and microbial infections, **vol. 3**. New York: Oxford University Press; 1998. p. 347–71.
39. Collier RJ, Kandel J. Structure and activity of diphtheria toxin. I. Thiol-dependent dissociation of a fraction of toxin into enzymatically active and inactive fragments. *J Biol Chem* 1971;**246**: 1496–503.
40. Davidson S, Rotem Y, Bogkowski B, Rubinstein E. *Corynebacterium diphtheriae* endocarditis. *Am J Med Sci* 1976;**271**:351–3.
41. DeZoysa A, Efstratiou A, George RC, et al. Molecular epidemiology of *Corynebacterium diphtheriae* from northwestern Russia and surrounding countries studied by using ribotyping and pulsed-field gel electrophoresis. *J Clin Microbiol* 1995;**33**:1080–3.
42. Dietze WE, Sudderth JF. Post-diphtheria polyneuritis: three case reports. *Laryngoscope* 1972;**82**:765–70.
43. Efstratiou A, Engler K, Mazurova IK, et al. Current approaches to the laboratory diagnosis of diphtheria. *J Infect Dis* 2000;**181** (suppl. 1):138–45.
44. Efstratiou A, George RC. Laboratory guidelines for the diagnosis of infections caused by *Corynebacterium diphtheriae* and *C. ulcerans*. *Commun Dis Public Health* 1999;**2**:250–7.
45. Efstratiou A, Roure C. The European Laboratory Working Group on Diphtheria: a global microbiologic network. *J Infect Dis* 2000;**181**(suppl. 1):146–51.
46. Elek SD. The plate virulence test for diphtheria. *J Clin Pathol* 1949;**2**:250–8.

47. El Seed AM, Dafalla AA, Abboud OI. Fetal immune response following maternal diphtheria during pregnancy. *Ann Trop Paediatr* 1981;**1**:217–19.
48. Engler KH, Efstratiou A. A rapid ELISA for confirmation of diphtheria caused by toxigenic *Corynebacterium* spp. (abstract 1522). In: Abstracts of the 8th European Congress of Clinical Microbiology and Infectious Diseases (Lausanne, Switzerland, May 1997). *Clin Microbiol Infect* 1997;**3**(suppl 2):14.
49. Engler KH, Glushkevich TG, Mazurova IK, et al. A modified Elek test for the detection of toxigenic corynebacteria. *J Clin Microbiol* 1997;**35**:495–8.
50. English PC. Diphtheria and theories of infectious disease: centennial appreciation of the critical role of diphtheria in the history of medicine. *Pediatrics* 1985;**76**:1–9.
51. Enke BU, Bokenkamp A, Offner G, et al. Response to diphtheria and tetanus booster vaccination in pediatric renal transplant recipients. *Transplantation* 1997;**64**:237–41.
52. Fontanarosa PB. Medical news and perspectives: diphtheria in Russia: a reminder of risk. *JAMA* 1995;**273**:1245.
53. Freeman VJ. Studies on virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. *J Bacteriol* 1951;**61**:675–88.
54. Funke G, Bernard K. Coryneform gram-positive rods. In: Murray PR, Baron EJ, Pfaller MA, et al, editors. *Manual of clinical microbiology*. 7th ed. Washington, DC: ASM Press; 1999. p. 319–40.
55. Gaston DA, Zurowski SM. *Arcanobacterium haemolyticum* pharyngitis and exanthem. *Arch Dermatol* 1996;**132**:61–4.
56. Ghio L, Pedrazzi C, Assael BM, et al. Immunity to diphtheria and tetanus in a young population on a dialysis regimen or with a renal transplant. *J Pediatr* 1997;**130**:987–9.
57. Gilbert L. Infections with *Corynebacterium diphtheriae:* changing epidemiology and clinical manifestations. *Commun Dis Intell* 1997;**21**:161–4.
58. Gluskevich TG, Zherebko G. Study of the role of non-toxigenic *C. diphtheriae* strains on the aetiology of diphtheria in the Ukraine (abstract). In: *Programme and abstracts of the Fifth International Meeting of the European Laboratory Working Group on Diphtheria (Halkidiki, Greece, June 1998)*. London: Public Health Laboratory Service; 1998. p. 32.
59. Golaz A, Lance-Parker S, Welty T, et al. Epidemiology of diphtheria in South Dakota. *S D J Med* 2000;**53**:281–5.
60. Goldie W, Maddock ECG. A milk-borne outbreak of diphtheria. *Lancet* 1943;**1**:285–6.
61. Groundstroem KWE, Molnar G, Lumio J. Echocardiographic follow-up of diphtheric myocarditis. *Cardiology* 1996;**87**:79–81.
62. Grundbacher FJ. Behring's discovery of diphtheria and tetanus antitoxins. *Immunol Today* 1992;**13**:188–90.
63. Guran P, Mollaret H, Chatelain R, et al. [Septic arthritis due to a nontoxigenic diphtheria bacillus.] [French.] *Arch Fr Pediatr* 1979;**36**:926–9.
64. Hadfield T, McEvoy P, Polotsky Y, et al. The pathology of diphtheria. *J Infect Dis* 2000;**181**(suppl. 1):116–20.
65. Hardy IRB, Dittmann S, Sutter RW. Current situation and control strategies for resurgence of diphtheria in newly independent states of the former Soviet Union. *Lancet* 1996;**347**:1739–44.
66. Harnisch JP, Tronca E, Nolan CM, et al. Diphtheria among alcoholic urban adults: a decade of experience in Seattle. *Ann Intern Med* 1989;**111**:71–82.
67. Hart RJC. *Corynebacterium ulcerans* in humans and cattle in North Devon. *J Hyg Camb* 1984;**92**:161–4.
68. Hofler W. Cutaneous diphtheria. *Int J Dermatol* 1991;**30**:845–7.
69. Hogg GG, Strachan JE, Huayi L, et al. Non-toxigenic *Corynebacterium diphtheriae* biovar *gravis:* Evidence for an invasive clone in a south-eastern Australian community. *Med J Aust* 1996;**164**:72–5.
70. Holmes R. Biology and molecular epidemiology of diphtheria toxin and the *tox* gene. *J Infect Dis* 2000;**181**(suppl. 1):156–67.
71. Isaac-Renton JL, Boyko WJ, Chan R, Crichton E. *Corynebacterium diphtheriae* septicemia. *Am J Clin Pathol* 1981;**75**:631–4.
72. Jennis F, Bale PM. Fatal respiratory and gastric diphtheria in an adult. *Med J Aust* 1966;**2**:760–2.
73. Kneen R, Pham NG, Solomon T, et al. Penicillin vs. erythromycin in the treatment of diphtheria. *Clin Infect Dis* 1998;**27**:845–50.
74. Kobaidze K, Popovic T, Nakao H, Quick L. Direct polymerase chain reaction for detection of toxigenic *Corynebacterium diphtheriae* strains from the Republic of Georgia after prolonged storage. *J Infect Dis* 2000;**181**(suppl. 1):152–5.
75. Komiya T, Shibata N, Ito M, et al. Retrospective diagnosis of diphtheria by detection of the *C. diphtheriae tox* gene in a formaldehyde-fixed throat swab using PCR and sequencing analysis. *J Clin Microbiol* 2000;**38**:2400–2.
76. Kreft B, Klouche M, Kreft R, et al. Low efficiency of active immunization against diphtheria in chronic hemodialysis patients. *Kidney Int* 1997;**52**:212–16.
77. Kurtzhals JAL, Kjeldsen K, Hey AS, et al. Immunity to tetanus and diphtheria in rural Africa. *Am J Trop Med Hyg* 1997;**56**:576–9.
78. Lessnick SL, Bruce C, Baldwin RL, et al. Does diphtheria toxin have nuclease activity? *Science* 1990;**250**:832–8.
79. Lloyd GER, editor. *Hippocratic writings*. New York: Penguin Books; 1983. p. 118.
80. Loukoushkina EF, Bobko PV, Kolbasova EV, et al. The clinical picture and diagnosis of diphtheritic carditis in children. *Eur J Pediatr* 1998;**157**:528–33.
81. Lozhnikova SM, Pirogov VN, Piradov MA, et al. Diphtheritic polyneuropath: clinico-morphologic study. *Arkh Patol* 1997;**59**:11–17.
82. Lubran MM. Bacterial toxins. *Ann Clin Lab Sci* 1988;**18**:58–71.
83. Mazurova IK. Characterization of non-toxigenic *tox* bearing *Corynebacterium diphtheriae* in the waning Russian epidemic (abstract). In: *Programme and abstracts of the Fifth International Meeting of the European Laboratory Working Group on Diphtheria (Halkidiki, Greece, June 1998)*. London: Public Health Laboratory Service; 1998. p. 33.
84. McCloskey RV. Diphtheria antitoxin titers in hospital workers after a single dose of adult-type diphtheria tetanus toxoid. *Am J Med Sci* 1969;**258**:209–13.
85. McCloskey RV, Eller JJ, Green M, et al. The 1970 epidemic of diphtheria in San Antonio. *Ann Intern Med* 1971;**75**:495–503.
86. Melville-Smith M, Balfour A. Estimation of *Corynebacterium diphtheriae* antitoxin in human sera: a comparison of an enzyme-linked immunosorbent assay with the toxin neutralisation test. *J Med Microbiol* 1988;**25**:279–83.
87. Miller LW, Older JJ, Drake J, et al. Diphtheria immunization: effect upon carriers and the control of outbreaks. *Am J Dis Child* 1972;**123**:197–9.
88. Mirchamsy H, Hamedi H, Fatch G, et al. Oral immunization against diphtheria and tetanus infections by fluid diphtheria and tetanus toxoids. *Vaccine* 1994;**12**:1167–72.
89. Miyamura K, Nishio S, Ito A, et al. Micro cell culture method for determination of diphtheria toxin and antitoxin titres using VERO cells, I. Studies on factors affecting the toxin and antitoxin titration. *J Biol Stand* 1974;**2**:189–201.
90. Morales AR, Vichitbandha P, Chandruang P, et al. Pathological features of cardiac conduction disturbances in diphtheric myocarditis. *Arch Pathol* 1971;**91**:1–7.
91. Muttaiyah S, Best EJ, Freeman JT, et al. *Corynebacterium diphtheriae* endocarditis: a case series and review of the treatment approach. *Intl J Infect Dis* 2011;**15**:e584–8.
92. Nakao H, Popovic T. Development of a direct PCR assay for detection of the *diphtheria* toxin gene. *J Clin Microbiol* 1997;**35**:1651–5.
93. Natu M, Borole D, Shaikh N, et al. Comparative assessment of laboratory procedures: diphtheria. *Indian J Pathol Microbiol* 1986;**29**:31–5.
94. Olson ME, Goemans I, Bolingbroke D, Lundberg S. Gangrenous dermatitis caused by *Corynebacterium ulcerans* in Richardson ground squirrels. *J Am Vet Med Assoc* 1988;**193**:367–8.
95. Pallen MJ, Hay AJ, Puckey LH, et al. Polymerase chain reaction for screening clinical isolates of corynebacteria for the production of diphtheria toxin. *J Clin Pathol* 1994;**47**:353–6.
96. Popovic T, Mazurova I, Efstratiou A, et al. Molecular epidemiology of diphtheria. *J Infect Dis* 2000;**181**(suppl. 1):168–77.
97. Popovic T, Wharton M, Wenger JD, et al. Are we ready for diphtheria? A report from the Diphtheria Diagnostic Workshop, Atlanta, 11 and 12 July 1994. *J Infect Dis* 1995;**171**:765–7.
98. Quick ML, Sutter RW, Kobaidze K, et al. Risk factors for diphtheria: a prospective case-control study in the Republic of Georgia, 1995-1996. *J Infect Dis* 2000;**181**(suppl. 1):121–9.
99. Rakhmanova AG, Lumio J, Groundstroem K, et al. Diphtheria outbreak in St. Petersburg: clinical characteristics of 1860 adult patients. *Scand J Infect Dis* 1996;**28**:37–40.

100. Rysselaere M, Vanneste L. Diphtheria of the eye. *Bull Soc Belge Ophtalmol* 1982;**201**:89–92.
101. Sanchez PJ, Laptook AR, Fisher L, et al. Apnea after immunization of preterm infants. *J Pediatr* 1997;**130**:746–52.
102. Sheth KJ, Sarff LD. Hemolytic uremic syndrome associated with *Corynebacterium diphtheria* infection. *Int J Pediatr Nephrol* 1986;**7**: 17–20.
103. Suresh GK, Dhawan A, Kohli V. Tracheal diphtheria mimicking bacterial tracheitis. *Pediatr Infect Dis J* 1992;**11**:502.
104. Tasman A, Minkenhof JE, Vink HH, et al. Importance of intravenous injection of diphtheria antiserum. *Lancet* 1958;**1**:1299–304.
105. Tiley SM, Kociuba KR, Heron LG, et al. Infective endocarditis due to nontoxigenic *Corynebacterium diphtheriae*: report of seven cases and review. *Clin Infect Dis* 1993;**16**:271–5.
106. Tiwari TSP, Golez A, Yu DT, et al. Investigation of 2 cases of diphtheria-like illness due to toxigenic *Corynebacterium ulcerans*. *Clin Infect Dis* 2008;**46**:3395–401.
107. Vitek CR, Wharton M. Diphtheria in the former Soviet Union: reemergence of a pandemic disease. *Emerg Infect Dis* 1998;**4**: 539–50.
108. Wagner KS, White JM, Lucenko I, et al. Diphtheria in the post-epidemic period, Europe, 2000-2009. *Emerg Infect Dis* 2012;**18**: 217–25.
109. Wilson GS. The necessity for a safe milk-supply. *Lancet* 1933; **2**:829–32.

ADDITIONAL READING

Barksdale L. *Corynebacterium diphtheriae* and its relatives. *Bacteriol Rev* 1970;**34**:378–422.

Pappenheimer Jr AM. Diphtheria toxin. In: Ajl SJ, Kadis S, Montie TC, editors. Microbial toxins, vol. 11B. New York: Academic Press; 1973.

Wood Jr WB. *From miasmas to molecules*. New York: Columbia University Press; 1961.

Zamiri I. Corynebacterium. In: Collee VG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie and McCartney practical medical microbiology*. 14th ed. New York: Churchill Livingstone; 1996. p. 299–307.

100 kg of anthrax spores upwind of Washington, D.C., could result in 130,000 to 3 million deaths.

BACTERIOLOGY

B. anthracis is an aerobic, nonmotile, spore-forming rod in the family Bacillaceae. Optimal growth occurs at 36° C in nonselective media. Colonies are gray-white, rough, and flat and can have comma-shaped projections caused by the outgrowth of chains of bacilli from the edges of the colony, giving it a "Medusa head" appearance. Individual colonies are 4 to 5 mm in diameter with a ground-glass appearance and exhibit tenacity or a "beaten egg white" appearance when lifted with an inoculating loop. The capsule, a γ-linked poly-D-glutamic acid, can be produced in blood or by culture in carbon dioxide. Gram stain of clinical material reveals large, square-ended, gram-positive rods singly or in short chains without a visible capsule. The equatorial or paracentral spores are invisible in smears fixed promptly after collection. After 24 to 48 hours of aerobic incubation, strands of rods are arranged in "boxcar" or "bamboo" fashion and sporulation occurs. Small numbers of bacteria are pathogenic for mice, guinea pigs, and rabbits, and death usually occurs 2 to 5 days after inoculation. Genotyping methods are available to determine molecular relatedness of clinical isolates and their geographic origin.[30]

EPIDEMIOLOGY AND TRANSMISSION

Domestic herbivores—cattle, sheep, horses, goats, and swine—are the most important agricultural sources of anthrax, but all domestic and many wild animals can serve as hosts. Animals are infected by ingestion of spores from infected pastures. Spores germinate in vivo, and death, associated with massive septicemia, usually occurs in 1 or 2 days. Anthrax is endemic in areas where an animal–soil–animal cycle is established because the spores can survive indefinitely in a dry environment. Uncultivated soil with a pH greater than 6.0 and ambient temperature greater than 15.5° C provides an environment favorable for persistence of spores.[47]

An estimated 20,000 to 100,000 human cases of anthrax occur yearly.[40] Regions of high endemicity include South and Central America, southern Europe, eastern Europe, Asia, Africa, the Caribbean, and the Middle East. The number of human cases probably correlates with the enzootic status of the disease in livestock in these countries. Familial clustering can occur in association with exposure to diseased animals.[35] Children who live in endemic areas often contract cutaneous anthrax from direct animal contact.[34] More than half of 448 patients from the Gambia with cutaneous anthrax were younger than 15 years of age, and 11 percent were younger than 2 years of age.[20] Epizootics of anthrax occur in the lower Mississippi valley and in parts of California, Texas, Missouri, Nebraska, and South Dakota[48]; sporadic cases have occurred in almost every state. Cases of illness were identified in four states and in Washington, D.C., during the 2001 bioterrorism-related outbreak.

The incidence of human anthrax in the United States has decreased markedly since the early 1900s, when more than 100 cases were reported annually. Cutaneous infections are reported sporadically. Cases often are associated with exposure to contaminated animal products in commercial preparations, but exposure to indigenous animal anthrax does occur.[43] Two members of a farmer's family ate hamburgers made from an anthrax-infected steer in Minnesota in 2000. The family members received antibiotic prophylaxis and anthrax vaccine and remained well.[11]

Direct contact with contaminated animal meat or carcasses or with contaminated animal products, such as hides, hair, wool, bone meal, and animal feed, are sources of transmission of anthrax spores to humans. Children of industrial workers have acquired infection, presumably from contact with their parents' contaminated clothing. Processed products, such as shaving brushes and saddle blankets, have been implicated as sources of infection. Use of contaminated goat hides from West Africa caused cutaneous anthrax in a drum maker in Connecticut and, through his work clothing or objects from his shed, led to cutaneous anthrax in his 8-year-old child.[13] Cutaneous anthrax is transmitted by deposition of spores or bacilli into abrasions or cuts in the skin. Blood-sucking insects, including mosquitoes and stable flies, can be vectors.[31,46,47]

The specific mode of acquisition may be difficult to determine in children; in one child with cutaneous anthrax, the only potential exposure was proximity to a bone meal factory that he walked past on his way to school.[26] Tying the umbilicus with a dirty thread was the presumed portal of entry for a neonate with *B. anthracis* sepsis.[34] Rubbing with contaminated fingers or deposition by an insect vector can lead to cutaneous involvement of the eyelids.[50] Discharge from cutaneous lesions theoretically is infectious, but person-to-person transmission has not been confirmed. Skin-to-skin contact through his mother's exposure at her workplace was proposed as the route of transmission to an infant in the bioterrorism-related outbreak in the United States in 2001.[19] Maternal anthrax has been associated with preterm delivery.[23]

Inhalational anthrax results from inhalation of spores. The estimated infectious dose in humans is 8,000 to 50,000 spores. Cases of inhalational anthrax in the United States have occurred in a weaver whose imported yarn was contaminated and in a drum maker whose imported hand-dried animal hide was contaminated with *B. anthracis*.[8,12] Eleven adults acquired inhalational anthrax during the 2001 bioterrorism outbreak of anthrax in the United States. Gastrointestinal anthrax usually is caused by ingestion of contaminated meat but also has occurred after exposure to a contaminated animal-hide drum.[15,24] A neonate was reported to have contracted anthrax meningitis from his mother, who was septicemic at the time of delivery.[20]

PATHOGENESIS AND PATHOLOGY

Anthrax toxicity results from the capsule and from the activity of three polypeptides—protective antigen, edema

factor, and lethal factor—that combine to form two exotoxins: lethal toxin and edema toxin. Protective antigen is integral to the action of both toxins. Toxin entry into cells is initiated when protective antigen binds to membrane receptors of susceptible cells. Two related proteins, tumor endothelial marker 8 and capillary morphogenesis protein 2, function as receptors. Cleaved protective antigen oligomerizes, and a heptamer of protective antigen provides binding sites for edema factor or lethal factor. The complex is endocytosed as edema toxin or lethal toxin.

Edema factor is a calmodulin-dependent adenylate cyclase that increases intracellular cyclic adenosine monophosphate levels and is responsible for the massive edema that occurs in cutaneous anthrax.[27] Lethal factor is a metalloproteinase that cleaves mitogen-activated protein kinase kinases so that they are unable to activate their downstream substrates, the mitogen-activated kinases.[18] Lethal factor stimulates production of tumor necrosis factor-α and interleukin-1β. The capsule of *B. anthracis* contributes to virulence by inhibiting phagocytosis.[22,49]

Cutaneous infection is initiated when anthrax spores are ingested at the site of entry by macrophages and germination occurs in skin tissues. Production of toxin results in local edema. Regional lymphangitis and lymphadenopathy can occur.[16] Interstitial edema, lymphatic dilation, and thrombosis and necrosis of blood vessels are characteristic microscopic features of cutaneous anthrax lesions. Erythrocytes extravasate freely into interstitial fluid. Few neutrophils or other inflammatory cells are present unless a lesion is infected secondarily. Hemorrhagic lymphadenitis involving regional lymph nodes occurs in all forms of anthrax.

In inhalation anthrax, spores entering the alveoli are ingested by alveolar macrophages and dendritic cells and carried to mediastinal lymph nodes, where they germinate. Inhalation anthrax is not pneumonia, although primary focal hemorrhagic necrotizing pneumonia can occur at the pulmonary portal of entry.[1] Massive hemorrhagic mediastinal lymphadenitis can block lymphatic drainage routes and can be causally related to the pulmonary edema, respiratory distress, and systemic disease observed clinically.

Gastrointestinal anthrax results from ingestion of spores or vegetative bacilli in contaminated meat. The primary sites of infection are the epithelium of the stomach or bowel wall.[7] Edema and small necrotic ulcers of the mucosa of the gastrointestinal tract, especially the ileum or cecum, are characteristic findings. Dissemination to the central nervous system can occur by hematogenous or lymphatic routes from any site of primary involvement. Hemorrhage involving the meninges and intense arteritis are uniform findings in patients who die of anthrax meningitis.

CLINICAL MANIFESTATIONS

Cutaneous Anthrax

The lesions of cutaneous anthrax occur mainly on exposed areas of the body.[2] In one report, the distribution in young children was 52 percent of lesions on the head and neck, 28 percent on the trunk, and 20 percent on the extremities, whereas in older children the distribution was 70 percent on the head and neck, 16 percent on the trunk, and 14 percent on the extremities.[21] Spores are introduced through abraded or injured skin. Children can have one or several lesions, and they are associated with regional adenitis.[28] After an incubation period of 2 to 5 days, a small, nontender, but frequently pruritic, papule develops at the site of inoculation. The lesion progresses to a serous or serosanguineous vesicle with surrounding nonpitting edema within 36 hours. Satellite vesicles, sometimes referred to as a "pearly wreath,"[50] can be seen occasionally.

The lesion undergoes central necrosis, and a 1- to 3-cm black eschar with well-defined margins forms 1 week to 10 days after onset of illness (Fig. 91-1).[38] The term *malignant edema* is used to describe severe lesions,

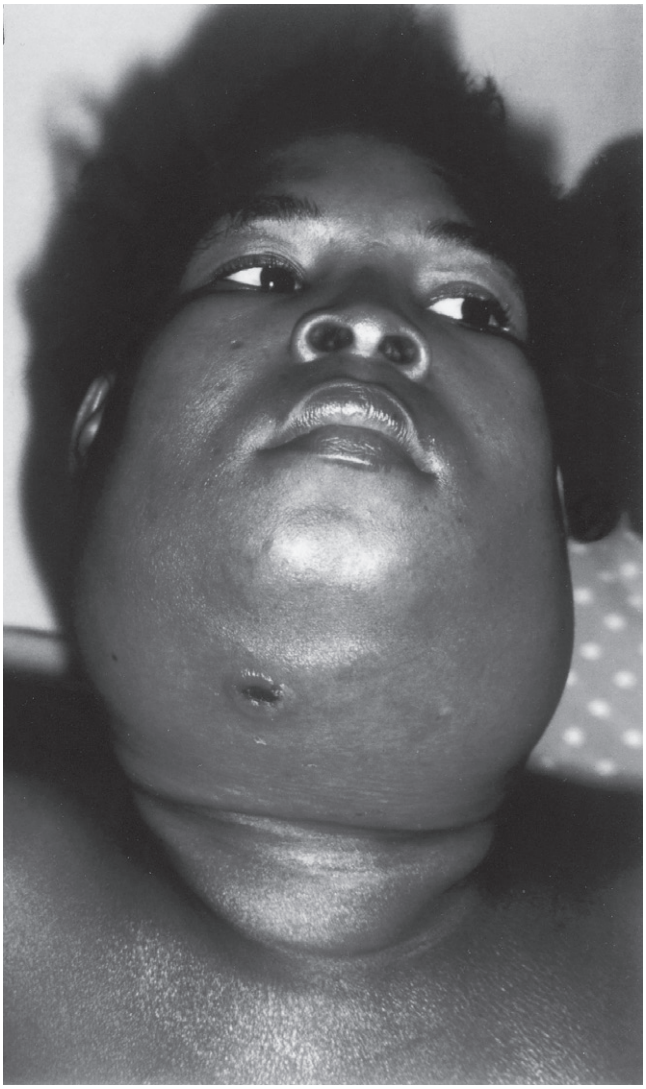

FIGURE 91-1 ■ Cutaneous anthrax with associated massive submental edema. The lesion is located at the site of a small, initially trivial laceration that served as the portal of entry for the anthrax endospores. The patient received ampicillin and recovered completely. (Courtesy M. Thomas Casey, MD.)

particularly lesions involving the head and neck, which can be associated with systemic toxicity and occlusion of the airway. Small children can appear acutely ill with a temperature of 39° to 40°C (102.2° to 104°F) and leukocytosis with counts of 20,000 to 30,000 cells/mm^3.[19,26] Associated bacteremia is uncommon. The edema usually resolves within 2 to 3 days, but separation of the eschar can take several weeks, and healing occurs with variable central scarring.

Inhalational Anthrax

Inhalational anthrax is a biphasic illness. Symptoms in the initial stage—malaise, low-grade fever, myalgia, and nonproductive cough—are nonspecific and resemble the symptoms of an upper respiratory tract illness. Adults who have inhalational anthrax are more likely to present with tachycardia, high hematocrit, and low albumin and sodium levels and are less likely to present with myalgias, headache, and nasal symptoms than are adults who have influenza-like illnesses.[25] After several days of illness, dyspnea and hypoxemia initiate onset of the second stage, which usually terminates fatally within 24 hours. Chest radiographs can show a widened mediastinum with smooth borders and evidence of hemorrhagic mediastinitis and pleural effusions. The pleural effusions often are large and usually contain bloody fluid. Pulmonary infiltrates usually are the result of superimposed bacterial infection. In the more recent cases from the United States, computed tomography of the chest showed the characteristic findings of hemorrhagic mediastinal and hilar lymph nodes and mediastinal edema and pleural effusions.[6]

Gastrointestinal Anthrax

Gastrointestinal anthrax can manifest as oropharyngeal or intestinal manifestations.[9] Each has an incubation period of 2 to 5 days after the ingestion of contaminated meat. The oropharyngeal form results in an oral or esophageal ulcer.[7,22,37] Presenting features include fever, severe sore throat, neck swelling caused by edema and enlargement of the cervical lymph nodes, dysphagia, and respiratory difficulty. Lesions on the tonsils or posterior oropharynx progress over 1 to 2 weeks from edema to a pseudomembrane-covered ulcer. The oropharyngeal form of anthrax, although uncommon, has a more favorable prognosis than does intestinal anthrax.

Initial features of intestinal anthrax are fever, nausea, anorexia, vomiting, and diffuse abdominal pain that progresses rapidly to severe abdominal pain with rebound tenderness. Vomiting of blood-tinged or coffee ground–like material and melena are common and are secondary to ulceration of the intestinal mucosa.[7,22,33] The pain decreases, and massive ascites develops within 24 to 48 hours of onset. Abdominal radiographs at this time show edematous loops of bowel and decreased air. If the abdomen is explored, findings include enlarged, erythematous mesenteric lymph nodes and straw-colored to purulent ascitic fluid in which organisms are readily visible.[3] Death usually occurs in association with significant blood loss, fluid and electrolyte imbalance, and shock. If the patient survives, the edema and melena subside in 10 to 14 days.

Meningitis

Most reports of anthrax meningitis are associated with cutaneous disease, likely owing to the higher frequency of cutaneous disease. Anthrax can disseminate to the meninges from any site of primary involvement. In the 2001 bioterrorism event, one adult with inhalation anthrax presented with meningitis and several others had features suggestive of central nervous system involvement.[36] In a report of 70 patients with anthrax meningitis who ranged from younger than 1 to 71 years of age, no primary focus was evident in 12 percent.

Anthrax meningitis is characterized by a fulminant, rapidly progressive course. Initial symptoms include intense headache, nausea and vomiting, myalgia, chills, dizziness, and, occasionally, a petechial rash. Meningismus is usually, but not invariably, present because of the acuity of the course. Progressive neurologic deterioration with delirium, convulsions, and coma can occur in hours or over 2 to 4 days. Overall survival is approximately 5 percent, but survival without sequelae has been reported in at least three children, two of whom had cutaneous lesions at diagnosis.[36,41,42,44]

Examination of cerebrospinal fluid reveals (1) gross or microscopic hemorrhage, (2) leukocytosis consisting predominantly of polymorphonuclear leukocytes, (3) elevated protein level, and (4) depressed glucose level. Gram-positive rods are easily visible on smears of cerebrospinal fluid. Peripheral leukocytosis is common, and the white blood cell count can be 60,000 to 80,000 cells/mm^3. Blood cultures yield the organism in 70 percent, and cerebrospinal fluid cultures are positive in virtually 100 percent of patients.[20] Neuroimaging findings are notable for multiple hemorrhages in the ventricles, subarachnoid space, and deep gray matter.[36]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

B. anthracis can be visualized by direct smear and cultured from tissue biopsy specimens or discharge from cutaneous lesions and from pleural fluid, blood, and cerebrospinal fluid in systemic infections. The commercially available QuickELISA Anthrax-PA Kit can be used as a screening test.[4] Definitive identification of suspected isolates can be obtained through the Laboratory Response Network in each state. Time-resolved fluorescent assay, real-time polymerase chain reaction, serologic tests to detect antibodies against protective antigen, and tissue immunochemistry are available through state health departments. The enzyme-linked immunosorbent assay used to detect IgG to *B. anthracis* protective antigen is

highly sensitive, has good specificity, and yields a positive result 10 days after the onset of symptoms.[6]

The lesion of cutaneous anthrax must be differentiated from ecthyma gangrenosum and from ulcerative skin lesions with regional lymphadenopathy, including rat-bite fever, ulceroglandular tularemia, plague, glanders, scrub typhus, rickettsialpox, cowpox, and orf. Staphylococcal lymphangitis can be distinguished by the discharge of purulent material and by the inflammatory response observed microscopically. Noninfectious causes of eschars include arachnoid bites and vasculitides.[22] The first stage of inhalational anthrax and the clinical features of the intestinal form are nonspecific, so a history of exposure is crucial to establishing the diagnosis. The mediastinal widening that occurs early in the course of inhalational anthrax can cause confusion with that seen in acute bacterial mediastinitis and in fibrous mediastinitis caused by *Histoplasma capsulatum*.[16] Gastrointestinal anthrax must be differentiated from other causes of acute abdominal illness and, if bleeding is present, from duodenal ulcer, typhoid, and intestinal tularemia. Anthrax meningitis must be differentiated from subarachnoid hemorrhage.[20]

TREATMENT

Most strains of naturally occurring *B. anthracis* are susceptible to penicillin and tetracycline, and treatment for 7 to 10 days is adequate. Treatment of bioterrorism-associated cutaneous disease should be initiated with ciprofloxacin 30 mg/kg per day orally divided every 12 hours, not to exceed 1000 mg every 24 hours, or doxycycline 100 mg orally two times per day for children 8 years of age or older or 4.4 mg/kg per day divided every 12 hours, not to exceed 200 mg per day for children younger than 8 years of age until susceptibility data are available. The antimicrobial regimen should continue for 60 days to provide postexposure prophylaxis and should be administered in conjunction with vaccine as indicated (see later).[4]

The initial regimen for treatment of inhalational anthrax, gastrointestinal anthrax, anthrax meningitis, or cutaneous anthrax with systemic signs should include intravenously administered ciprofloxacin 400 mg every 8 to 12 hours. One or two additional antimicrobial agents should be administered in conjunction with ciprofloxacin. Suggested agents with in vitro activity include penicillin, ampicillin, clindamycin, vancomycin, rifampin, meropenem, chloramphenicol, and clarithromycin.[17] Fluoroquinolones other than ciprofloxacin, including levofloxacin, also have excellent in vitro activity against *B. anthracis*. Therapy should be continued for at least 60 days.

For severe anthrax, Anthrax-Specific Hyperimmune Globulin 5 percent should be considered in consultation with the U.S. Centers for Disease Control and Prevention under an emergency investigative new drug (IND) proposal.[4] Supportive therapy includes attention to details of fluid and electrolyte balances, endotracheal intubation if indicated to maintain a patent airway, and local care for cutaneous lesions. Systemic corticosteroids can reduce the severity of infections in patients with massive edema or meningitis.[42]

PROGNOSIS

Before penicillin was introduced, cutaneous anthrax was fatal in approximately 20 percent of patients. With effective treatment, the mortality rate is less than 1 percent. Cutaneous anthrax of the eyelid may be complicated by ectropion of the upper lid and corneal scarring with blindness.[50] Immunity probably is lifelong in most patients. Although second attacks of cutaneous anthrax have been recorded, they have not been confirmed serologically and usually are mild.[21] Fatality rates are high for systemic anthrax and range from 50 to 100 percent for gastrointestinal anthrax to virtually 100 percent for inhalation anthrax, but children who have survived these infections have no apparent sequelae.

PREVENTION

The only human anthrax vaccine licensed in the United States is anthrax vaccine adsorbed (AVA), marketed as BioThrax, which is prepared from cell-free filtrates of microaerophilic cultures of a toxigenic, nonencapsulated strain of *B. anthracis* V770-NP1-R. Each 0.5-mL dose contains proteins including the protective antigen and contains no dead or live bacteria.[14] The anthrax vaccine is licensed for use in persons 18 to 65 years of age who are at high risk for exposure. Preexposure vaccination is recommended for persons who perform certain types of laboratory work, including those who work with high concentrations or pure cultures of *B. anthracis* spores or with environmental samples associated with anthrax investigations and those who work in spore-contaminated areas or settings with exposure to aerosolized *B. anthracis* spores. Preexposure vaccination is recommended for persons involved in environmental investigations or remediation efforts and, as recommended by the U.S. Department of Defense, for military personnel. The preexposure vaccination schedule consists of five intramuscular doses administered at day 0, 4 weeks and 6, 12 and 18 months, followed by an annual booster to maintain immunity. AVA is not licensed for use in children and, in a pre-event setting, vaccination of children is not recommended.

The use of AVA in children is not contraindicated in a postevent setting that poses a high risk for exposure to aerosolized *B. anthracis* spores. Recommendations for postexposure use of AVA in children from birth to 17 years of age are made by public health authorities on an event-by-event basis.[4,14] Under the existing IND protocol, postexposure vaccination should be administered as a three-dose subcutaneous series at 0, 2, and 4 weeks postexposure in conjunction with a 60-day course of an appropriate antimicrobial agent.[11]

The recommended postexposure prophylaxis after exposure to aerosolized *B. anthracis* spores for children younger than 18 years of age is one of the following for 60 days: (1) ciprofloxacin 30 mg/kg per day orally divided every 12 hours, not to exceed 1000 mg every 24 hours; or (2) doxycycline 100 mg orally two times per day for children older than 8 years of age and larger than 45 kg or 4.4 mg/kg per day divided every 12 hours, not to exceed 200 mg per day, for children 8 years of age and younger and for those older than 8 years of age but weighing less than or equal to 45 kg. If susceptibility testing demonstrates an amoxicillin minimal inhibitory concentration less than or equal to 0.125, oral amoxicillin 80 mg/kg/day orally divided into three doses given every 8 hours with a maximal dose of 500 mg should be used to complete therapy.

Worldwide, anthrax is controlled through livestock immunization programs. Procedures to prevent the spread of anthrax in animals include disposal of contaminated carcasses by burning and annual vaccination of livestock in known enzootic areas. Novel approaches to the treatment of anthrax are under investigation and include nonantimicrobial drugs with activity against anthrax toxin components and evaluation of agents that inhibit binding or assembly of toxins.[5] All suspected or proven cases of anthrax should be reported to public health officials. Hospitalized patients should be kept under isolation until the lesions are bacteriologically sterile. Contaminated dressings and clothing must be burned or sterilized, and the patient's room must be disinfected to destroy spores.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Akbayram S, Doğan M, Akgün C, et al. Clinical findings in children with cutaneous anthrax in Eastern Turkey. *Pediatr Dermatol* 2010; **27**:600–6.
4. American Academy of Pediatrics. Anthrax. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 Report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 228–32.
5. Artenstein AW, Opal SM. Novel approaches to the treatment of systemic anthrax. *Clin Infect Dis* 2012;**54**:1148–61.
9. Bravata DM, Holty J-EC, Wang E, et al. Inhalational, gastrointestinal, and cutaneous anthrax in children. *Arch Pediatr Adoles Med* 2007;**161**:896–905.
10. Bush LM, Perez MT. The anthrax attacks 10 years later. *Ann Intern Med* 2012;**156**:41–4.
13. Centers for Disease Control and Prevention. Cutaneous anthrax associated with drum making using goat hides from West Africa—Connecticut, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**: 628–31.
14. Centers for Disease Control and Prevention. Use of anthrax vaccine in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**(No. RR-6):1–30.
15. Centers for Disease Control and Prevention. Gastrointestinal anthrax after an animal hide drumming event—New Hampshire and Massachusetts, 2009. *MMWR Morb Mortal Wkly Rep* 2010; **59**:872–7.
24. Klempner MS, Talbot EA, Lee SI, et al. Case 25-2010: a 24-year-old woman with abdominal pain and shock. *N Engl J Med* 2010; **363**;766–77.
30. Marston CK, Allen CA, Beaudry J, et al. Molecular epidemiology of anthrax cases associated with recreational use of animal hides and yarn in the United States. *PLoS One* 2011;**6**:e28274.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 92

Bacillus cereus

Margot Anderson • Richard A. Oberhelman

Recognition of *Bacillus cereus* pathogenicity was delayed until clarification of the taxonomy of the genus *Bacillus* in the early 1950s. Multiple early reports of food poisoning and other infections, including gastroenteritis, bacteremia-septicemia, cellulitis, ear and eye infections, endocarditis, and urinary tract infections, that were attributed to *Bacillus subtilis* or to other *Bacillus* spp. probably were caused by *B. cereus.*[104] *B. cereus* can give rise to two distinct forms of foodborne diseases related to different toxins, the emetic and the diarrheal syndromes, and occasionally to localized and systemic disease.[43,66]

The diarrheal syndrome was recognized first by Hauge[51] in 1955 after four clinically similar outbreaks in Norway occurred. This common form of disease was related to a great variety of foods, such as meat and vegetable soup, poultry, pudding, sauce, pasta, cake, and milk. In 1974, Mortimer and McCann[82] described a vomiting syndrome associated with the consumption of fried

REFERENCES

1. Abramova FA, Grinberg LM, Yampolskaya OV, et al. Pathology of inhalational anthrax in 42 cases from the Sverdlovsk outbreak of 1979. *Proc Natl Acad Sci U S A* 1993;**90**:2291–4.
2. Akbayram S, Doğan M, Akgün C, et al. Clinical findings in children with cutaneous anthrax in Eastern Turkey. *Pediatr Dermatol* 2010; **27**:600–6.
3. Alizad A, Ayoub EM, Makki N. Intestinal anthrax in a two-year-old child. *Pediatr Infect Dis J* 1995;**14**:394–5.
4. American Academy of Pediatrics. Anthrax. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 228–32.
5. Artenstein AW, Opal SM. Novel approaches to the treatment of systemic anthrax. *Clin Infect Dis* 2012;**54**:1148–61.
6. Bartlett JG, Inglesby Jr TV, Borio L. Management of anthrax. *Clin Infect Dis* 2002;**35**:851–8.
7. Beatty ME, Ashford DA, Griffin PM, et al. Gastrointestinal anthrax: review of the literature. *Arch Intern Med* 2003;**163**:2527–31.
8. Brachman PS. Inhalation anthrax. *Ann NY Acad Sci* 1980;**353**: 83–93.
9. Bravata DM, Holty J-EC, Wang E, et al. Inhalational, gastrointestinal, and cutaneous anthrax in children. *Arch Pediatr Adoles Med* 2007;**161**:896–905.
10. Bush LM, Perez MT. The anthrax attacks 10 years later. *Ann Intern Med* 2012;**156**:41–4.
11. Centers for Disease Control and Prevention. Human ingestion of *Bacillus anthracis*—contaminated meat—Minnesota, August 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:813–32.
12. Centers for Disease Control and Prevention. Inhalation anthrax associated with dried animal hides—Pennsylvania and New York City, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:280–2.
13. Centers for Disease Control and Prevention. Cutaneous anthrax associated with drum making using goat hides from West Africa—Connecticut, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**: 628–31.
14. Centers for Disease Control and Prevention. Use of anthrax vaccine in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59** (No. RR-6):1–30.
15. Centers for Disease Control and Prevention. Gastrointestinal anthrax after an animal hide drumming event—New Hampshire and Massachusetts, 2009. *MMWR Morb Mortal Wkly Rep* 2010; **59**:872–7.
16. Dixon TC, Meselson M, Guillemin J, et al. Anthrax. *N Engl J Med* 1999;**341**:815–26.
17. Drago L, De Vecchi E, Lombardi A, et al. Bactericidal activity of levofloxacin, gatifloxacin, penicillin, meropenem and rokitamycin against *Bacillus anthracis* clinical isolates. *J Antimicrob Chemother* 2002;**50**:1059–63.
18. Duesbery NS, Webb CP, Leppla SH, et al. Proteolytic inactivation of MAP-kinase-kinase by anthrax lethal factor. *Science* 1998;**280**: 734–7.
19. Freedman A, Afonja O, Chang MW, et al. Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a 7-month-old child. *JAMA* 2002;**287**:869–74.
20. Gerhardt P. Cytology of *Bacillus anthracis*. *Fed Proc* 1967;**26**: 1504–17.
21. Heyworth B, Ropp ME, Voos UG, et al. Anthrax in the Gambia: an epidemiological study. *BMJ* 1975;**4**:79–82.
22. Inglesby TV, O'Toole T, Henderson DA, et al. Anthrax as a biological weapon, 2002: updated recommendations for management. *JAMA* 2002;**287**:2236–52.
23. Kadanali A, Tasyaran MA, Kadanali S. Anthrax during pregnancy: case reports and review. *Clin Infect Dis* 2003;**36**:1343–6.
24. Klempner MS, Talbot EA, Lee SI, et al. Case 25-2010: a 24-year-old woman with abdominal pain and shock. *N Engl J Med* 2010; **363**;766–77.
25. Kuehnert MJ, Doyle TJ, Hill HA, et al. Clinical features that discriminate inhalational anthrax from other acute respiratory illnesses. *Clin Infect Dis* 2003;**36**:328–36.
26. Lamb R. Anthrax. *BMJ* 1973;**1**:157–60.
27. Leppla SH. *Bacillus anthracis* calmodulin dependent adenylate cyclase: chemical and enzymatic properties and interactions with eucaryotic cells. *Adv Cyclic Nucleotide Protein Phosphorylation Res* 1984;**17**:189–98.
28. Maguiña C, Flores del Pozo J, Terashima A, et al. Cutaneous anthrax in Lima, Peru: retrospective analysis of 71 cases, including four with a meningoencephalitic complication. *Rev Inst Med Trop S Paulo* 2005;**47**:25–30.
29. Manios S, Kavaliotis I. Anthrax in children: a long forgotten, potentially fatal infection. *Scand J Infect Dis* 1979;**11**:203–6.
30. Marston CK, Allen CA, Beaudry J, et al. Molecular epidemiology of anthrax cases associated with recreational use of animal hides and yarn in the United States. *PLoS One* 2011;**6**:e28274.
31. McKendrick DRA. Anthrax and its transmission to humans. *Cent Afr J Med* 1980;**26**:126–9.
32. Meselson M, Guillemin J, Hugh-Jones M, et al. The Sverdlovsk anthrax outbreak of 1979. *Science* 1994;**266**:1202–8.
33. Nalin DR, Sultana B, Sahunja R, et al. Survival of a patient with intestinal anthrax. *Am J Med* 1977;**62**:130–2.
34. Özkaya E, Kirimi E, Berktas M, et al. *Bacillus anthracis* sepsis in a newborn. *Pediatr Infect Dis J* 2000;**19**:487–8.
35. Seboxa T, Goldhagen J. Anthrax in Ethiopia. *Trop Geogr Med* 1989;**41**:108–12.
36. Sejvar JJ, Tenover FC, Stephens DS. Management of anthrax meningitis. *Lancet Infect Dis* 2005;**5**:287–95.
37. Sirisanthana T, Navachareon N, Tharavichitkul P, et al. Outbreak of oral-pharyngeal anthrax: an unusual manifestation of human infection with *Bacillus anthracis*. *Am J Med Hyg* 1984;**33**:144–50.
38. Smego Jr RA, Gebrian B, Desmangels G. Cutaneous manifestations of anthrax in rural Haiti. *Clin Infect Dis* 1998;**26**:97–102.
39. Sternbach G. The history of anthrax. *J Emerg Med* 2003;**24**: 463–7.
40. Swartz MN. Recognition and management of anthrax—an update. *N Engl J Med* 2001;**345**:1621–6 (Erratum N Engl J Med *346*:634, 2002).
41. Tabatabaie P, Syadati A. *Bacillus anthracis* as a cause of bacterial meningitis. *Pediatr Infect Dis J* 1993;**12**:1035–7.
42. Tahernia AC, Hashemi G. Survival in anthrax meningitis. *Pediatrics* 1972;**50**:329–32.
43. Taylor JP, Dimmitt DC, Ezzell JW, et al. Indigenous human cutaneous anthrax in Texas. *South Med J* 1993;**86**:1–4.
44. Tengio FU. Anthrax meningitis: report of two cases. *East Afr Med J* 1973;**50**:337–9.
45. Tigertt WD. Anthrax. William Smith Greenfield, MD, FRCP, Professor Superintendent, The Brown Animal Sanatory Institution (1878-1881): concerning the priority due to him for the production of the first vaccine against anthrax. *J Hyg Camb* 1980;**85**:415–20.
46. Turell MJ, Knudson GB. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). *Infect Immun* 1987;**55**:1859–61.
47. Van Ness GB. Ecology of anthrax. *Science* 1971;**172**:1303–7.
48. Wolff AH, Heimann H. Industrial anthrax in the United States. *Am J Hyg* 1951;**53**:80–109.
49. Wright GG, Mandell GL. Anthrax toxin blocks priming of neutrophils by lipopolysaccharide and by muramyl dipeptide. *J Exp Med* 1986;**164**:1700–9.
50. Yorston D, Foster A. Cutaneous anthrax leading to corneal scarring from cicatricial ectropion. *Br J Ophthalmol* 1989;**73**:809–11.

rice in Chinese restaurants. Despite widespread recognition in Europe, *B. cereus* outbreaks have been reported infrequently in the United States. The first documented outbreak in the United States occurred in 1970.[79]

BACTERIOLOGY

Members of the genus *Bacillus* are aerobic or facultative anaerobic, gram-positive or gram-variable, spore-forming rods. They are distributed widely in the environment because of the high resistance of their endospores to extreme conditions, including heat, cold, desiccation, salinity, and radiation.[34,93] Based on the high variability in guanine and cytosine content (32% to 69%), the debate ensues regarding the classification of *Bacillus* spp.[107]

B. cereus belongs to the group of gram-positive rods that produce central or terminal ellipsoid or cylindrical spores that do not distend the sporangia.[107] Studies of DNA-DNA hybridization and 16S and 23S ribosomal RNA (rRNA) sequencing and enzyme electrophoretic patterns have shown a close relationship among *B. cereus*, *Bacillus anthracis*, *Bacillus mycoides*, *Bacillus pseudomycoides*, *Bacillus weihenstephanensis*, and *Bacillus thuringiensis;* these organisms are so closely related that they all may be considered variants of *B. cereus* and are labeled the *B. cereus* group. Differentiating, particularly between *B. cereus* and the insect pathogen *B. thuringiensis*, sometimes is difficult in the diagnostic laboratory, and overlap occurs among clinical syndromes reported.[50,59] Polymerase chain reaction (PCR) technology has been used for identifying species.[18]

B. cereus sensu stricto is a flagellated, motile, gram-positive rod, typically 1 to 1.2 μm in diameter by 3 to 5 μm in length. The organism sporulates freely on many media under well-aerated conditions, but vegetative cells also can grow anaerobically. It is able to metabolize glucose, fructose, and sucrose but not pentose and other sugar alcohols. It produces acid from glucose but not from arabinose, xylose, or mannitol. Starch hydrolysis and catalase production are similar to those of the other members of the genus. The presence of lipid globules or protoplasts is a characteristic that it shares with *Bacillus megaterium*.[67] Colonies on blood agar are large, flat, granular, and slightly green-tinged. *B. cereus* is differentiated from *B. anthracis* by motility, hemolysis, lack of lysis by γ phage, penicillin resistance, and absence of a capsule in *B. cereus*.[34] Identifying morphologic differentiation with nonmotile *B. cereus* strains and *B. anthracis* strains that are occasionally weakly hemolytic may be difficult.

Growth and multiplication of vegetative cells occur in a temperature range of 10° to 50°C (50° to 122°F), with an optimum of 28° to 35°C (82.4° to 95°F). Variations in toxin levels found in certain foods also can be related to pH levels, sugar content, the presence of other lactic acid bacteria, and aeration.[98,99] Some strains responsible for milk spoilage can grow at temperatures of 5°C (41°F),[67,93] but few strains are able to produce toxin at temperatures less than 7°C (44.6°F).[93] Ribosomal DNA characterization and genotyping based on the major cold shock protein homologue cspA have defined the species, *Bacillus weihenstephanensis*, which can grow at 4° to 7°C (39.2° to 44.6°F) but not at 43°C (109.4°F).[69] These strains form a subgroup of *B. cereus* organisms described as psychrotolerant or psychrotropic, meaning they are able to grow at temperatures of 7°C (44.6°F) and less.[68,89,100] Spore formation allows some *B. cereus* strains to survive pasteurization and heating.[68] A crystalline cell surface protein layer or "S-layer" has been described as a determinant of cell hydrophobicity. S-layer–producing cells are hydrophilic and able to bind to human matrix proteins, and presence of the S-layer in some *B. cereus* strains is associated with resistance to phagocytosis and increased radiation resistance against γ irradiation.[66]

Serologic differentiation of *Bacillus* spp. is hampered by cross-reactive antigens and autoagglutination of spores caused by hydrophobic surface properties. Serologic typing based on the flagellar (H) antigen can be used during an outbreak to distinguish among strains and to determine the similarity of isolates obtained from humans with the strains isolated from suspect foods.[34] The serotype scheme is based on H antisera raised against prototype strains. In addition to serology, biochemical typing, plasmid analysis, and phage typing have proved useful epidemiologically.[2,67,83,96,113] A *B. cereus*–specific, repetitive, extragenic, palindromic sequence-based PCR analysis (Bc-Rep-PCR) appears to be a promising newer technique.[87,91] Pulsed-field gel electrophoresis (PFGE) has been less successful in differentiating among *B. cereus* group species.[121]

Diagnosis of the less common extraintestinal infection is made by isolation from normally sterile sites (blood or tissue) after overnight incubation on nutrient or blood agar; clinical specimens from normally nonsterile sites (feces, vomitus) and food or environmental samples require selective techniques. Polymyxin B is used as a selective agent, and the lecithinase reaction of the organism on egg yolk and its inability to catabolize mannitol permit presumptive identification to be made with a variety of media: mannitol–egg yolk–polymyxin B, Kim and Goepfert medium, and polymyxin B–pyruvate–egg yolk–mannitol with bromothymol blue or bromocresol purple.[34]

EPIDEMIOLOGY

B. cereus in the spore and vegetative form is a ubiquitous organism found in soil, water, vegetation, and food products, especially cereals, dairy products, dried foods, spices, meat products, and vegetables.[67] The emetic syndrome typically is associated with cooked rice, usually fried, from Chinese restaurants.[64,82] Saving portions of boiled rice at room temperature overnight until required for frying previously was a common practice. Refrigerating boiled rice makes the grains stick together, which is less convenient for frying. The spores of *B. cereus* survive cooking and are capable of germination and outgrowth.[44,46] The optimal temperature for growth in boiled rice is 30° to 37°C (86° to 98.6°F), although growth does occur during storage at 15° to 43°C (59° to 109.4°F).[44] Most samples of uncooked rice contain multiple serotypes of *B. cereus*, and little difference occurs in

the growth rate of the various serotypes in boiled rice at 22°C (71.6°F).[85]

Starchy dried foods other than rice, such as cereals, frequently are contaminated.[16] Tortillas can be contaminated with the water used in preparation and from the hands of producers.[22] Specific foods incriminated in outbreaks in the United States include Chinese food (accounting for 50% of reported outbreaks between 1973 and 1987), followed by Mexican food, beef, fruits, and vegetables. Other foods implicated, particularly in the enterotoxin syndrome, include beef stew, turkey loaf, barbecued pork, macaroni and cheese, potatoes, spaghetti and other pastas, dairy and dried milk products, and seafoods.* In a study of 96 milk and milk product samples collected from retail shops in Nairobi, Kenya, 57 percent of samples were contaminated with *B. cereus* and 81 percent of the bacterial isolates (38/47) produced nonhemolytic enterotoxins, including 6 of 43 pasteurized milk products (43%).[84] Among the factors thought to contribute to outbreaks, the most frequent were inadequate cold-holding temperatures, preparing foods a half-day or more before serving, contaminated equipment, and storage in a contaminated environment.[8,23] Outbreaks of *B. cereus* have been recognized widely in Europe but less often in the United States. In the Netherlands, *B. cereus* reportedly was the cause of 22.4 percent of foodborne disease outbreaks of known bacterial cause. Similarly, in Finland, it accounted for 11.9 percent of outbreaks. In most places, *B. cereus* is incriminated in 0.9 to 7 percent of outbreaks of food-related disease and 0.7 to 3 percent of cases.[67] In the United States, between 1998 and 2002, *B. cereus* was identified as the cause of 37/6647 (0.6%) foodborne outbreaks reported to the Centers for Disease Control and Prevention (CDC), with a total of 571 cases and no deaths recorded.[75] The location of the outbreak is known for 24 of these outbreaks, and most of these (15/24 [62.5%]) occurred in commercial food outlets, such as restaurants, delicatessens, or workplace cafeterias. In outbreaks for which the implicated source was known, cereal products were the most common vehicle.[75] The susceptibility of children to this pathogen is evident from the report of an outbreak involving two daycare centers[64] and other reports that included neonates, children, and adolescents.† Only a small fraction of outbreaks are reported to the CDC; small outbreaks of mild, brief illness are less likely to be reported. It is thought that 10^5 to 10^8 organisms per gram need to be ingested to cause the emetic syndrome and 10^5 to 10^7 cells or spores (total dose) ingested to cause the enterotoxin syndrome.[47]

For infections not related to food, groups at risk include neonates,[37,86] immunocompromised hosts,‡ patients with recent eye surgery or trauma, intravenous drug users,[27,108] and patients with intravascular devices or artificial prostheses.§ *B. cereus* has been known to contaminate a variety of unusual environmental niches, including alcohol prep pads,[33] steam-dried towels,[32] and unopened boxes of nonsterile nitrile gloves.[62] Studies in three populations in South Africa and London, including school-aged children, found the organism in 18 to 43 percent of fecal samples.[106] The organism can be part of the normal intestinal flora. *B. cereus* does not persist in the intestine after ingestion and so is not spread by the fecal-oral route.[41]

PATHOGENESIS

B. cereus produces an enormous range of extracellular metabolites, including peptides with antibiotic properties (biocerin, cerein, thiocillins), β-lactamases, hydrolases, nuclease, urease, and proteases.[104] Two groups of toxins, known as diarrheal enterotoxins and emetic toxins, are responsible for the clinical syndromes of food poisoning.[77,111] Some strains may be able to produce both toxins based on the evidence that culture filtrates derived from strains isolated from emetic poisoning occasionally are able to produce a positive rabbit ileal loop assay.[93]

Biologic activities of diarrheal enterotoxins can be shown in multiple different assay systems. Three enterotoxins are produced. Purification plus isolation of multiple toxic fractions with some, but not all, of these activities has caused confusion.[34,103] Evaluation of the toxic activity of whole-cell suspension, cell-free culture filtrates, and the purified enterotoxin complex classically has included the rabbit ileal loop fluid accumulation assay, vascular permeability in rabbit skin, dermonecrosis and intestinonecrosis, mouse lethality, cytotoxicity, and hemolysis.* Hemolytic *B. cereus* enterotoxin has been characterized as a complex termed *hemolysin BL*, composed of a binding component B (35 kDa) and two lytic components, L1 and L2 (36 kDa and 45 kDa, respectively)[9]; all three are needed for fluid accumulation in the rabbit ileal loop.[13] Other *B. cereus* enterotoxins, a nonhemolytic enterotoxin and cytotoxin K, also have been described.[17,66,74]

The emetic toxin cereulide is a heat-stable (126°C [258.8°F] for 90 minutes), ring-shaped peptide with a molecular weight of 1.2 kDa[78]; it is stable between pH 2.0 and 11.0 and is protease-resistant. In contrast to the diarrheal toxins, it is preformed in foods, so the presence of living organisms at the time of ingestion is not necessary to cause symptoms.[43,104] Rice culture filtrates derived from emetic syndrome–associated strains cause cytoplasmic vacuolation and swollen mitochondria in HEp-2 cells, characteristics suggestive of uncoupling of oxidative phosphorylation.[90] Emetic activity occurs through the serotonin 5-HT_3 receptor and stimulation of the vagus afferent nerve.[1]

Among the multiple other substances with relevant activity, two groups are associated with local infection.[105,110] Cereolysin, or hemolysin I, is a thiol-activated cytolysin. Phospholipase C–like or lecithinase-like substances, including a sphingomyelinase and two hydrolases with preference for phosphatidylcholine and phosphatidylinositol, also exist. These enzymes induce the release of lysosomal enzymes from neutrophils that probably are

*References 31, 42, 55, 61, 66, 72.
†References 37, 53, 60, 86, 92, 108, 117.
‡References 4, 24, 36, 46, 53, 60, 96, 102.
§References 5, 7, 36, 37, 96, 97.

*References 11, 20, 71, 101, 109, 110.

involved in tissue damage, especially in wound and ocular infections.[34,119]

CLINICAL MANIFESTATIONS

Food Poisoning

Diarrheal Syndrome

The enterotoxins preformed in food or produced in vivo in the intestine after the ingestion of bacilli cause profuse, watery, nonbloody diarrhea accompanied by abdominal pain and cramps, nausea, and, occasionally, vomiting or low-grade fever.* The typical incubation period is 8 to 16 hours, and the clinical characteristics resemble the food poisoning of *Clostridium perfringens*. The interval between ingestion and the onset of symptoms reflects the time required for production of toxin in the gut. The symptoms resolve within approximately 12 to 24 hours but occasionally can last 2 days to 2 weeks.[42] The diarrhea (3 to 10 bowel movements per day) rarely leads to dehydration in healthy individuals. The most prevalent flagellar H serotypes are 1, 2, 6, 8, 10, 12, and 19. Serotyping is available at research laboratories.[16,31,72,106]

Emetic Syndrome

The usual illness is characterized by a rapid onset (within 1 to 5 hours after the ingestion of contaminated food) of nausea, vomiting, and malaise, occasionally followed by diarrhea hours later.[82] Infrequently, the diarrhea is reported to last several days.[114] The short incubation reflects the ingestion of preformed emetic toxin.

Extraintestinal Infections

Eye Infection

Keratitis, conjunctivitis, endophthalmitis, and panophthalmitis can be produced by *B. cereus*. Endophthalmitis that develops after the occurrence of penetrating wounds is caused by this organism in 27 to 46 percent of cases.[29,112] A history of soil contamination or the presence of a metal foreign body should raise clinical suspicion. Less frequently, corneal ulcers and surgical procedures are predisposing factors.[52,112] Exogenous endophthalmitis usually progresses rapidly, with deterioration of vision occurring in less than 48 hours. Severe pain is accompanied by chemosis, periorbital swelling, proptosis, and pus in the anterior chamber. The classic lesion is a corneal ring abscess, similar to that produced by *Pseudomonas* and *Proteus* spp. Endogenous cases may have subretinal exudation, retinal hemorrhage, and perivasculitis. Associated systemic symptoms are not unusual manifestations.[27] The outcome is poor, with almost half of patients left with visual acuity no better than simple light perception.[112] Many patients require enucleation. Endogenous ophthalmitis is associated with the use of illicit intravenous drugs or transfusion of contaminated blood products and subsequent bacteremic seeding of one eye.[27,96,102,112]

The pathogenesis of *B. cereus* on ocular tissue has been linked to the lecithinase activity of phospholipase C. A toxin fraction, hemolysin BL, which is formed by three separate components, produces similar destruction of retinal tissue in vitro and in animal experiments.[12] Its relationship with the diarrheal enterotoxin is unclear.[11]

Wound and Soft Tissue Infections

Wound infections of variable severity related to trauma, burns, or postsurgical complications occasionally are reported.[105,108,110,117] Because *Bacillus* is a common environmental contaminant, proof of the relevance of a *B. cereus* isolate is clearer if the organism is obtained from deep tissue in heavy pure growth. Severe infections in individuals involved in motor vehicle accidents can be complicated by necrotizing fasciitis and require extensive débridement.[117] Superficial, benignly infected wounds are common findings in the tropics.[35] Immunosuppressed patients may contract a severe gas gangrene–like infection with myonecrosis similar to disease caused by *Clostridium* spp.,[78] requiring amputation,[49] or a less severe primary cutaneous infection manifested by vesicles, pustules, or cellulitis.[53]

Skeletal Infections

Cases of chronic osteomyelitis occur rarely and result from accidental or surgical trauma. Radical débridement and antibiotic treatment are required.[96] Because *B. cereus* can be found as a copathogen with other, more frequent pathogenic bacteria, resolution of symptoms is delayed until eradication of the organism.[94] Acute osteomyelitis may occur in drug users.[96,102]

Bacteremia and Septicemia

Bacteremia occurs with indwelling catheters and other foreign bodies, contaminated intravenous drugs (e.g., heroin), and blood products, particularly platelets.[19,36,120] Immunosuppression and impaired neutrophil killing, such as occur in individuals with neutropenia secondary to malignancy or chemotherapy and in neonates with immaturity of the immune system, are major contributors to morbidity in systemic *B. cereus* infection.* Disseminated intravascular coagulation, multiorgan failure, and a fulminant course may occur in neonates and compromised hosts. Intestinal perforation with abdominal infection in neonates also has been described.[45] Outbreaks of sepsis in newborn nurseries have been linked to contaminated ventilation balloon devices.[115] Endocarditis is an infrequent complication of bacteremia that usually occurs in intravenous drug users or individuals with a long-term intravascular device.[96,102] Vegetations can form over mechanical prosthetic valves or pacemaker wires, requiring their replacement.[96,97] Morbidity and mortality rates are high in patients with valvular heart disease. Fatal serosanguineous pericarditis in a patient undergoing hemodialysis has been reported.[38]

*References 4, 31, 42, 61, 67, 73, 95.

*References 5, 20, 56, 57, 86, 88.

Pneumonia

Primary pulmonary disease rarely has been recorded. Underlying predisposing conditions include leukemia, alcohol abuse, chronic hepatitis, and steroid use.[14,36,56,96] Multiorgan disease in premature neonates with necrotizing pneumonia usually is fatal.[63] Outbreaks of *B. cereus* pneumonia in neonates have been linked to contaminated ventilators.[48] Blood culture is positive in 75 percent of cases, followed by pleural fluid and sputum culture in 40 to 60 percent.[14] The pleural space may become contaminated with *B. cereus* by mishandling of the thoracic drainage system in patients with other causes of pleuritis.[58]

A dramatic pulmonary syndrome similar to that of inhalational anthrax has been seen in adults (all metal workers) with *B. cereus* infection. The organisms in these cases possessed some *B. anthracis* toxin genes while being clearly phenotypically *B. cereus*.[3,54,55]

Infection of the Central Nervous System

Premature neonates are susceptible to meningeal seeding by dissemination of intravascular infection related to catheters.[86] As in patients with other serious infections, patients who are immunosuppressed are at higher risk.[56] The cerebrospinal fluid in *B. cereus* meningitis is purulent, with white blood cell counts of more than 1000/mm^3, a predominance of polymorphonuclear leukocytes, and moderate increments in protein content. Gram stain is positive in 70 percent of cases. Multiple brain abscesses may result from hematogenous spread in patients with leukemia.[56,60] Sequelae include hydrocephalus and brain damage. Often, these infections are fatal.

Intracranial shunts and penetrating surgical or traumatic cranial wounds expose the central nervous system to environmental *B. cereus*.[37,94] Spinal anesthesia[36] was associated with *Bacillus* infection in the past. Contamination in the operating room through the linen is a potential source.[6,7] These infections are typically more benign.

Liver Failure

Fulminant hepatitis and rhabdomyolysis have been associated with the emetic toxin. The toxin inhibits hepatic mitochondrial fatty acid oxidation. Liver changes include fatty infiltrates and midzonal necrosis.[76]

Pseudoinfections

Pseudoepidemics with *B. cereus* are a challenge for the clinical microbiologist.[80] Because these spore formers are so hardy, they are common laboratory contaminants. *Bacillus* spp. isolated from a single clinical specimen usually should be considered a contaminant, although their pathogenic potential is well known.* Pseudoinfections have been associated with contaminated blood culture media, syringes, blood culture analyzers, and fiberoptic bronchoscopes.[28] Colonization of umbilical cord stumps and eye surfaces by contaminated linens may cause pseudo-outbreaks in nurseries.[118]

*References 28, 36, 57, 88, 96, 102, 105, 110.

COMPLICATIONS

Death rarely ever results from the food-poisoning syndromes. Rapidly spreading wound infections may require amputation of extremities,[46] and ocular infections can lead to loss of vision with or without enucleation.[52,112,119] The risk for death in patients with septicemia, endocarditis, and meningitis is related to the underlying condition and severity of the disease.[5,53,81,86,97]

DIAGNOSIS

The laboratory finding of *B. cereus* in a foodstuff without quantitative cultures and without epidemiologic data is insufficient to establish its role in an outbreak.[114] In practice, appropriate specimens often either are unavailable or are submitted long after the incident, rendering their microbiologic significance questionable. During analysis of foods not involved in foodborne illness, the bacteria may be found in counts of 10^1 to 10^6 organisms per gram.[15,16,22,44,85,98] Diagnosis of the diarrheal form of *B. cereus* food poisoning is supported by the isolation of 10^5 or more organisms per gram from epidemiologically incriminated food.[81] The levels of *B. cereus* found in the implicated foods usually are in the range of 5×10^5 to 9.5×10^8 colony-forming units per gram. With the exception of milk, the products rarely appear to be spoiled, despite the bacteria's high density. Isolation of more than 10^5 organisms per gram in feces during an acute attack provides supportive evidence for the presumed diagnosis and confirms the association if the same serotype is isolated from incriminated food.

The organisms that produce emetic toxin are present in concentrations of 1×10^3 to 5×10^{10} colony-forming units per gram. Reheating may decrease or eliminate the organisms and leave the toxin intact, but render isolation of the organism difficult to accomplish.[34,114] Accurate PCR assays for the presence of the emetic toxin–producing *B. cereus* have been developed and can be performed on food samples.[39,65]

Commercial immunoassay kits (reversed passive latex agglutination test, enzyme-linked immunosorbent assay, and microslide immunodiffusion assay) are available for the detection of *B. cereus* diarrheal toxin. The kits may detect a variety of proteins, however.[10,12,30] Experimental techniques based on the detection of genes for phospholipase C and sphingomyelinase by PCR have been developed for the identification of *B. cereus* in food products.[78]

The relevance of clinical isolates in extraintestinal syndromes can be assessed by the degree of growth (heavy vs. scanty), the number of occasions that growth was obtained, the source of the material cultured, and predisposing or underlying conditions.[105] For suspected endophthalmitis, aqueous and vitreous samples should be obtained.[29]

TREATMENT

During a mild, self-limited attack of food poisoning, patients require only supportive therapy. Usually, oral fluid and electrolyte replacement is adequate (Table 92-1).[42]

TABLE 92-1 **Management and Complications Associated with *Bacillus Cereus* Infections**

Condition	Management	Complications
Food poisoning	Supportive measures; hydration	Usually none; liver failure rarely reported
Ocular	Systemic, topical, intravitreal antibiotics: vancomycin and/or ciprofloxacin; surgical—early vitrectomy[21]	Severe decreased visual acuity, blindness, enucleation
Septicemia	Removal of IV device, foreign body; IV antibiotics (according to sensitivity): vancomycin	Localization of infection: endophthalmitis, endocarditis, meningitis; death
Pneumonia/pleuritis	IV antibiotics; drainage of pleural space; resection of necrotic tissue	Death
Meningitis	IV antibiotics; removal of infected intracranial shunts or cerebrospinal fluid reservoirs	Hydrocephalus, brain damage, death
Wound/subcutaneous infection	IV antibiotics; surgical débridement	Amputation, death

IV, Intravenous.

No antibiotic therapy is required except in cases of nongastrointestinal infection. The production of three β-lactamases by most of the organisms renders penicillin derivatives, including third-generation cephalosporins, ineffective against *B. cereus*. Most strains are susceptible to chloramphenicol, vancomycin, clindamycin, aminoglycosides, erythromycin, tetracycline, imipenem, and ciprofloxacin.[5,25,105,110,116] Definitive antibiotic therapy should be based on the antibiogram susceptibility, but initial empirical therapy with clindamycin or vancomycin, with or without an aminoglycoside, is appropriate, pending susceptibility data.* Ciprofloxacin has also been reported to be useful in the treatment of recurrent pneumonia and bacteremia[40] and may have advantages in the penetration of respiratory and eye secretions.[70] Immediate empirical coverage for *B. cereus* is indicated for endophthalmitis in groups at risk. Vancomycin and ciprofloxacin administered intravitreally are the best options.[21] In cases of penetrating trauma, early vitrectomy should be considered.[29,112]

Surgical débridement of necrotic tissue, drainage of closed-space infections, and prompt removal of foreign bodies and indwelling catheters are important aspects of successful therapy.[14,26,92] Some bacteremic patients may be cured by removal of the intravenous catheter only.[96]

PREVENTION AND CONTROL

Low-level contamination in food products is difficult to avoid, but proper food handling should diminish the proliferation of bacilli.[93] Practical precautions for handling cereals and rice include not preparing large quantities at a single time and maintaining the food at a hot temperature (>63° C [96.8° F]) or cooling it quickly. The food must not be stored under warm conditions, especially in the range of 15° to 50° C (59° to 122° F).[16,44]

NEW REFERENCES SINCE THE SIXTH EDITION

3. Avashia SB, Riggins WS, Lindley C, et al. Fatal pneumonia among metalworkers due to inhalation exposure to *Bacillus cereus* containing *Bacillus anthracis* toxin genes. *Clin Infect Dis* 2007;**44**:414–16.
17. Bottone EJ. Bacillus cereus, a volatile human pathogen. *Clin Microbiol Rev* 2010;**23**:382–98.
21. Callegan MC, Cochran DC, Kane ST, et al. Virulence factor profiles and antimicrobial susceptibilities of ocular bacillus isolates. *Curr Eye Res* 2006;**31**:693–702.
23. Centers for Disease Control and Prevention. Surveillance for foodborne disease outbreaks: United States, 2006. *MMWR Morb Mortal Wkly Rep* 2009;**58**:609–15.
32. Dohmae S, Okubo T, Higuchi W, et al. *Bacillus cereus* nosocomial infection from reused towels in Japan. *J Hosp Infect* 2008;**69**: 361–7.
33. Dolan SA, Littlehorn C, Glode MP, et al. Association of *Bacillus cereus* infection with contaminated alcohol prep pads. *Infect Control Hosp Epidemiol* 2012;**33**:666–71.
39. Fricker M, Messelhausser U, Busch U, et al. Diagnostic real-time PCR assays for the detection of emetic *Bacillus cereus* strains in foods and recent food-borne outbreaks. *Appl Environ Microbiol* 2007;**73**:1892–8.
50. Guinebretiere MH, Velge P, Couvert O, et al. Ability of *Bacillus cereus* group strains to cause food poisoning varies according to phylogenetic affiliation (groups I to VII) rather than species affiliation. *J Clin Microbiol* 2010;**48**:3388–91.
54. Hoffmaster AR, Hill KK, Gee JE, et al. Characterization of *Bacillus cereus* isolates associated with fatal pneumonias: strains are closely related to *Bacillus anthracis* and harbor B. anthracis virulence genes. *J Clin Microbiol* 2006;**44**:3352–60.
57. Inoue D, Nagai Y, Mori M, et al. Fulminant sepsis caused by *Bacillus cereus* in patients with hematologic malignancies: analysis of its prognosis and risk factors. *Leuk Lymphoma* 2010;**51**: 860–9.
59. Jensen GB, Hansen BM, Eilenberg J, et al. The hidden lifestyles of *Bacillus cereus* and relatives. *Environ Microbiol* 2003;**5**:631–40.
62. Jeurissen A, Weyers L, Cossey V, et al. Dissemination of *Bacillus cereus* in the burn unit due to contaminated non-sterile gloves. *J Hosp Infect* 2010;**76**:92–3.
65. Kim JB, Kim JM, Kim SY, et al. Comparison of enterotoxin production and phenotypic characteristics between emetic and enterotoxic *Bacillus cereus*. *J Food Prot* 2010;**73**:1219–24.
83. Nishikawa Y, Kramer JM, Hanaoka M, et al. Evaluation of serotyping, biotyping, plasmid banding pattern analysis, and HEp-2 vacuolation factor assay in the epidemiological investigation of *Bacillus cereus* emetic-syndrome food poisoning. *Int J Food Microbiol* 1996;**31**:149–59.
87. Reyes-Ramirez A, Ibarra JE. Fingerprinting of *Bacillus thuringiensis* type strains and isolates by using *Bacillus cereus* group-specific repetitive extragenic palindromic sequence-based PCR analysis. *Appl Environ Microbiol* 2005;**71**:1346–55.
91. Sauka DH, Basile JI, Benintende G. Evidence of *Bacillus thuringiensis* intra-serovar diversity revealed by *Bacillus cereus* group-specific repetitive extragenic palindromic sequence-based PCR genomic fingerprinting. *J Mol Microbiol Biotechnol* 2011;**21**: 184–90.
121. Zhong W, Shou Y, Yoshida TM, et al. Differentiation of *Bacillus anthracis*, *B. cereus*, and *B. thuringiensis* by using pulsed-field gel electrophoresis. *Appl Environ Microbiol* 2007;**73**:3446–9.

*References 5, 14, 28, 60, 92, 117.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agata N, Ohta M, Mori M, Isobe M. A novel dodecadepsipeptide, cereulide, is an emetic toxin of *Bacillus cereus*. *FEMS Microbiol Lett* 1995;**129**:17–20.
2. Ahmed R, Sankar-Mistry P, Jackson S, et al. *Bacillus cereus* phage typing as an epidemiological tool in outbreaks of food poisoning. *J Clin Microbiol* 1995;**33**:636–40.
3. Avashia SB, Riggins WS, Lindley C, et al. Fatal pneumonia among metalworkers due to inhalation exposure to *Bacillus cereus* containing *Bacillus anthracis* toxin genes. *Clin Infect Dis* 2007;**44**:414–16.
4. Baddour LM, Gala SM, Griffin R, et al. A hospital cafeteria-related food-borne outbreak due to *Bacillus cereus*: unique features. *Infect Control* 1986;**7**:462–5.
5. Banerjee C, Bustamante CI, Wharton R, et al. *Bacillus* infections in patients with cancer. *Arch Intern Med* 1988;**148**:1769–74.
6. Barrie D, Hoffman PN, Wilson JA, et al. Contamination of hospital linen by *Bacillus cereus*. *Epidemiol Infect* 1994;**113**:297–306.
7. Barrie D, Wilson JA, Hoffman PN, et al. *Bacillus cereus* meningitis in two neurosurgical patients: an investigation into the source of the organism. *J Infect* 1992;**25**:291–7.
8. Bean NH, Griffin PM, Goulding JS, Ivey CB. Foodborne disease outbreaks, 5-year summary, 1983-1987. *MMWR C D C Surveill Summ* 1990;**39**:15–55.
9. Beecher DJ, Macmillan JD. Characterization of the components of hemolysin BL from *Bacillus cereus*. *Infect Immunol* 1991;**59**: 1778–84.
10. Beecher DJ, Wong AC. Identification and analysis of the antigens detected by two commercial *Bacillus cereus* diarrheal enterotoxin immunoassay kits. *Appl Environ Microbiol* 1994;**60**:4614–16.
11. Beecher DJ, Wong AC. Improved purification and characterization of hemolysin BL, a hemolytic dermonecrotic vascular permeability factor from *Bacillus cereus*. *Infect Immun* 1994;**62**:980–6.
12. Beecher DJ, Pulido JS, Barney NP, et al. Extracellular virulence factors in *Bacillus cereus* endophthalmitis: methods and implication of involvement of hemolysin BL. *Infect Immun* 1995;**63**:632–9.
13. Beecher DJ, Schoeni JL, Wong ACL. Enterotoxic activity of hemolysin BL from *Bacillus cereus*. *Infect Immunol* 1995;**63**: 4423–8.
14. Bekemeyer WB, Zimmerman GA. Life threatening complications associated with *Bacillus cereus* pneumonia. *Am Rev Respir Dis* 1985; **131**:466–9.
15. Beuchat LR, Ma-Lin CF, Carpenter JA. Growth of *Bacillus cereus* in media containing plant seed materials and ingredients used in Chinese cookery. *J Appl Bacteriol* 1980;**48**:397–407.
16. Blakey LJ, Priest FG. The occurrence of *Bacillus cereus* in some dried foods including pulses and cereals. *J Appl Bacteriol* 1980;**48**: 397–407.
17. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* 2010: **23**:382–98.
18. Brousseau R, Saint-Onge A, Prefontaine G, et al. Arbitrary primer polymerase chain reaction, a powerful method to identify *Bacillus thuringiensis* serovars and strains. *Appl Environ Microbiol* 1993;**59**: 114–9.
19. Bryce EA, Smith JA, Tweeddale M, et al. Dissemination of *Bacillus cereus* in an intensive care unit. *Infect Control Hosp Epidemiol* 1993;**14**:459–62.
20. Burdon AL, Davis JS, Wende RD. Experimental infection of mice with *Bacillus cereus*: studies of pathogenesis and pathologic changes. *J Infect Dis* 1967;**117**:307–16.
21. Callegan MC, Cochran DC, Kane ST, et al. Virulence factor profiles and antimicrobial susceptibilities of ocular bacillus isolates. *Curr Eye Res* 2006;**31**:693–702.
22. Capparelli E, Mata L. Microflora of maize prepared as tortillas. *Appl Microbiol* 1975;**29**:802–6.
23. Centers for Disease Control and Prevention. Surveillance for foodborne disease outbreaks: United States, 2006. *MMWR Morb Mortal Wkly Rep* 2009;**58**:609–15.
24. Christenson JC, Byington C, Korgenski EK, et al. *Bacillus cereus* infections among oncology patients at a children's hospital. *Am J Infect Control* 1999;**27**:543–6.
25. Conrod JD, Leadley PJ, Eickhoff TC. Antibiotic susceptibility of *Bacillus* species. *J Infect Dis* 1971;**123**:102–5.
26. Cotton DJ, Gill VJ, Marshal DJ, et al. Clinical features and therapeutic interventions in 17 cases of *Bacillus* bacteremia in an immunosuppressed patient population. *J Clin Microbiol* 1987;**25**:672–4.
27. Cowan Jr CL, Madden WM, Hatem GF, et al. Endogenous *Bacillus cereus* panophthalmitis. *Ann Ophthalmol* 1987;**19**:65–8.
28. Cunha BA. Pseudomeningitis: another nosocomial headache. *Infect Control Hosp Epidemiol* 1988;**9**:391–3.
29. David DB, Kirkby GR, Noble BA. *Bacillus cereus* endophthalmitis. *Br J Ophthalmol* 1994;**78**:577–80.
30. Day TL, Tatani SR, Notermans S, et al. A comparison of ELISA and RPLA for detection of *Bacillus cereus* diarrhoeal enterotoxin. *J Appl Bacteriol* 1994;**77**:9–13.
31. DeBuono BA, Brondum J, Kramer JM, et al. Plasmid, serotypic and enterotoxin analysis of *Bacillus cereus* in an outbreak setting. *J Clin Microbiol* 1988;**26**:1571–4.
32. Dohmae S, Okubo T, Higuchi W, et al. *Bacillus cereus* nosocomial infection from reused towels in Japan. *J Hosp Infect* 2008;**69**: 361–7.
33. Dolan SA, Littlehorn C, Glode MP, et al. Association of *Bacillus cereus* infection with contaminated alcohol prep pads. *Infect Control Hosp Epidemiol* 2012;**33**:666–71.
34. Drobniewski FA. *Bacillus cereus* and related species. *Clin Microbiol Rev* 1993;**6**:324–38.
35. Dryden MS, Kramer JM. Toxigenic *Bacillus cereus* as a cause of wound infections in the tropics. *J Infect* 1987;**15**:207–12.
36. Farrar Jr WE. Serious infections due to "non-pathogenic" organisms of the genus Bacillus. *Am J Med* 1963;**34**:134–41.
37. Feder HM, Garibaldi RA, Nurse BA, et al. *Bacillus* species isolates from cerebrospinal fluid in patients without shunts. *Pediatrics* 1988;**82**:909–13.
38. Fricchione LF, Sepkowitz DV, Gradon JD, et al. Pericarditis due to *Bacillus cereus* in an intravenous drug user. *Rev Infect Dis* 1991; **13**:774.
39. Fricker M, Messelhausser U, Busch U, et al. Diagnostic real-time PCR assays for the detection of emetic *Bacillus cereus* strains in foods and recent food-borne outbreaks. *Appl Environ Microbiol* 2007;**73**:1892–8.
40. Gascoigne AD, Richards J, Gould K, et al. Successful treatment of *Bacillus cereus* infection with ciprofloxacin. *Thorax* 1991;**46**:220–1.
41. Ghosh AC. Prevalence of *Bacillus cereus* in the faeces of healthy adults. *J Hyg* 1978;**80**:233–6.
42. Giannella RA, Brasile L. A hospital food-borne outbreak of diarrhea caused by *Bacillus cereus*: clinical, epidemiologic, and microbiologic studies. *J Infect Dis* 1979;**139**:366–70.
43. Gilbert RJ, Kramer JM. *Bacillus cereus* enterotoxins: present status. *Biochem Soc Trans* 1984;**12**:198–200.
44. Gilbert RJ, Stringer MF, Peace TC. The survival and growth of *Bacillus cereus* in boiled and fried rice in relation to outbreaks of food poisoning. *J Hyg* 1974;**73**:433–44.
45. Girisch M, Ries M, Zenker M, et al. Intestinal perforations in a premature infant caused by *Bacillus cereus*. *Infection* 2003;**31**: 192–3.
46. Gonzalez I, Lopez M, Mazas M, et al. The effect of recovery conditions on the apparent heat resistance of *Bacillus cereus* spores. *J Appl Bacteriol* 1995;**78**:548–54.
47. Granum PE, Lund T. *Bacillus cereus* and its food poisoning toxins. *FEMS Microbiol Lett* 1997;**157**:223–8.
48. Gray J, George RH, Durbin GM, et al. An outbreak of *Bacillus cereus* respiratory tract infections on a neonatal unit due to contaminated ventilator circuits. *J Hosp Infect* 1999;**41**:19–22.
49. Groschell D, Burgess MA, Bodey GP. Gas gangrene-like infection with *Bacillus cereus* in a lymphoma patient. *Cancer* 1976;**37**: 988–92.
50. Guinebretiere MH, Velge P, Couvert O, et al. Ability of *Bacillus cereus* group strains to cause food poisoning varies according to phylogenetic affiliation (groups I to VII) rather than species affiliation. *J Clin Microbiol* 2010:**48**:3388–91.
51. Hauge S. Food poisoning caused by aerobic spore-forming bacilli. *J Appl Bacteriol* 1955;**18**:591–5.
52. Hemadi R, Zaltas M, Paton B, et al. Bacillus-induced endophthalmitis: new series of 10 cases and review of the literature. *Br J Ophthalmol* 1990;**74**:26–9.
53. Henrickson KJ, Shenep JL, Flynn PM, et al. Primary cutaneous *Bacillus cereus* infection in neutropenic children. *Lancet* 1989;**1**: 601–3.
54. Hoffmaster AR, Hill KK, Gee JE, et al. Characterization of *Bacillus cereus* isolates associated with fatal pneumonias: strains are closely related to *Bacillus anthracis* and harbor B. anthracis virulence genes. *J Clin Microbiol* 2006;**44**:3352–60.

55. Holmes JR, Plunkett T, Pate P, et al. Emetic food poisoning caused by *Bacillus cereus*. *Arch Intern Med* 1981;**141**:766–7.
56. Ihde DC, Armstrong D. Clinical spectrum of infection due to *Bacillus* species. *Am J Med* 1973;**55**:839–46.
57. Inoue D, Nagai Y, Mori M, et al. Fulminant sepsis caused by *Bacillus cereus* in patients with hematologic malignancies: analysis of its prognosis and risk factors. *Leuk Lymphoma* 2010;**51**: 860–9.
58. Jacobs JA, Stobberingh EE. Infection due to a contaminated thoracic drainage system. *J Hosp Infect* 1993;**24**:23–8.
59. Jensen GB, Hansen BM, Eilenberg J, et al. The hidden lifestyles of *Bacillus cereus* and relatives. *Environ Microbiol* 2003;**5**:631–40.
60. Jenson HB, Levy SR, Duncan C, et al. Treatment of multiple brain abscesses caused by *Bacillus cereus*. *Pediatr Infect Dis J* 1989;**8**: 795–8.
61. Jephcott AE, Barton BW, Gilbert RJ, et al. An unusual outbreak of food poisoning associated with meals-on-wheels. *Lancet* 1977;**2**:129–30.
62. Jeurissen A, Weyers L, Cossey V, et al. Dissemination of *Bacillus cereus* in the burn unit due to contaminated non-sterile gloves. *J Hosp Infect* 2010;**76**:92–3.
63. Jevon GP, Dunne WM, Hicks MJ, et al. *Bacillus cereus* pneumonia in premature neonates: a report of two cases. *Pediatr Infect Dis J* 1993;**12**:251–3.
64. Khodr M, Hill S, Perkins L, et al. *Bacillus cereus* food poisoning associated with fried rice at two child day care centers: Virginia, 1993. *MMWR Morb Mortal Wkly Rep* 1994;**43**:177–8.
65. Kim JB, Kim JM, Kim SY, et al. Comparison of enterotoxin production and phenotypic characteristics between emetic and enterotoxic *Bacillus cereus*. *J Food Prot* 2010;**73**:1219–24.
66. Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of *Bacillus cereus* infections. *Microbes Infect* 2000;**2**: 189–98.
67. Kramer JM, Gilbert RJ. *Bacillus cereus* and other *Bacillus* species. In: Doyle MP, editor. *Foodborne bacterial pathogens*. New York: Marcel Dekker; 1989. p. 21–70.
68. Larsen HD, Jorgensen K. Growth of *Bacillus cereus* in pasteurized milk products. *Int J Food Microbiol* 1999;**46**:173–6.
69. Lechner S, Mayr R, Francis KP, et al. *Bacillus weihenstephanensis* sp. nov. is a new psychrotolerant species of *Bacillus cereus* group. *Int J Syst Microbiol* 1998;**48**:1373–82.
70. Lesk MR, Ammann H, Marcil G, et al. The penetration of oral ciprofloxacin into the aqueous humor, vitreous and subretinal fluid of humans. *Am J Ophthalmol* 1993;**115**:623–8.
71. Lettau LA, Benjamin D, Cantrell HF, et al. *Bacillus* species pseudomeningitis. *Infect Control Hosp Epidemiol* 1988;**9**:394–8.
72. Luby S, Jones J, Dowda H, et al. A large outbreak of gastroenteritis caused by diarrheal toxin-producing *Bacillus cereus*. *J Infect Dis* 1993;**167**:1452–5.
73. Lund BM. Foodborne disease due to *Bacillus* and *Clostridium* species. *Lancet* 1990;**336**:982–7.
74. Lund T, Granum PE. Characterisation of a non-haemolytic enterotoxin complex from *Bacillus cereus* after a foodborne outbreak. *FEMS Microbiol Lett* 1996;**141**:151–6.
75. Lynch M, Painter J, Woodruff R. Surveillance for foodbourne-disease outbreaks: United States, 1998-2002. *MMWR Morb Mortal Wkly Rep* 2006;**55**(SS-10):1–48.
76. Mahler H, Pasi A, Kramer JM, et al. Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*. *N Engl J Med* 1997;**336**:1142–8.
77. Melling J, Capel BJ, Turnbul PC, et al. Identification of a novel enterotoxigenic activity associated with *Bacillus cereus*. *J Clin Pathol* 1976;**29**:938–40.
78. Meredith FT, Fowler VG, Gautier M, et al. *Bacillus cereus* necrotizing cellulitis mimicking clostridial myonecrosis: case report and review of the literature. *Scand J Infect Dis* 1997;**29**: 528–9.
79. Midura T, Gerber M, Wood R, et al. Outbreak of food poisoning caused by *Bacillus cereus*. *Public Health Rep* 1970;**85**:45–8.
80. Morrell Jr RM, Wasilauskas BL. Tracking laboratory contamination by using a *Bacillus cereus* pseudoepidemic as an example. *J Clin Microbiol* 1992;**30**:1469–73.
81. Morris Jr JG. *Bacillus cereus* food poisoning. *Arch Intern Med* 1981;**141**:711.
82. Mortimer PR, McCann G. Food poisoning episodes associated with *Bacillus cereus* in fried rice. *Lancet* 1974;**1**:1043–5.
83. Nishikawa Y, Kramer JM, Hanaoka M, et al. Evaluation of serotyping, biotyping, plasmid banding pattern analysis, and HEp-2 vacuolation factor assay in the epidemiological investigation of *Bacillus cereus* emetic-syndrome food poisoning. *Int J Food Microbiol* 1996;**31**:149–59.
84. Ombui JN, Nduhiu JG. Prevalence of enterotoxigenic *Bacillus cereus* and its enterotoxins in milk and milk products in and around Nairobi. *East Afr Med J* 2005;**82**:280–4.
85. Parry JM, Gilbert RJ. Studies on the heat resistance of *Bacillus cereus* spores and growth of the organism in boiled rice. *J Hyg* 1980;**84**:77–82.
86. Patrick CC, Langston C, Baker CJ. *Bacillus* species infections in neonates. *Rev Infect Dis* 1989;**111**:612–15.
87. Reyes-Ramirez A, Ibarra JE. Fingerprinting of *Bacillus thuringiensis* type strains and isolates by using *Bacillus cereus* group-specific repetitive extragenic palindromic sequence-based PCR analysis. *Appl Environ Microbiol* 2005;**71**:1346–55.
88. Richard V, Van der Auwera P, Snoeck R, et al. Nosocomial bacteremia caused by *Bacillus* species. *Eur J Clin Microbiol Infect Dis* 1988;**7**:783–5.
89. Rowan NJ, Anderson GJ. Diarrheal enterotoxin production by psychrotropic *Bacillus cereus* present in reconstituted milk base infant formulae (MIF). *Lett Appl Microbiol* 1998;**26**:161–5.
90. Sakurai N, Koike KA, Irie Y, et al. The rice culture filtrate of *Bacillus cereus* isolated from emetic-type food poisoning causes mitochondrial swelling in a HEp-2 cell. *Microbiol Immunol* 1994; **38**:337–40.
91. Sauka DH, Basile JI, Benintende G. Evidence of *Bacillus thuringiensis* intra-serovar diversity revealed by *Bacillus cereus* group-specific repetitive extragenic palindromic sequence-based PCR genomic fingerprinting. *J Mol Microbiol Biotechnol* 2011;**21**: 184–90.
92. Schricker ME, Thompson GH, Schreiber JR. Osteomyelitis due to *Bacillus cereus* in an adolescent: case report and review. *Clin Infect Dis* 1994;**18**:863–7.
93. Schultz FJ, Smith JL. *Bacillus*: Recent advances in *Bacillus cereus* food poisoning research. In: Hui YH, Gorham JR, Murrel KD, editors. *Foodborne disease handbook*. New York: Marcel Dekker; 1994. p. 29–62.
94. Siegman-Igra Y, Lavochkin J, Schwartz D, et al. Meningitis and bacteremia due to *Bacillus cereus*: a case report and a review of *Bacillus* infections. *Isr J Med Sci* 1983;**19**:546–51.
95. Slaten DD, Oropeza RI, Werner SB. An outbreak of *Bacillus cereus* food poisoning: are caterers supervised sufficiently? *Public Health Rep* 1992;**107**:477–80.
96. Sliman R, Rehm S, Shlaes DM. Serious infections caused by *Bacillus* species. *Medicine (Baltimore)* 1987;**66**:218–23.
97. Steen MK, Bruno-Murtha LA, Chaux G, et al. *Bacillus cereus* endocarditis: report of a case and review. *Clin Infect Dis* 1992; **14**:945–6.
98. Sutherland AD. Toxin production by *Bacillus cereus* in dairy products. *J Dairy Res* 1993;**60**:569–74.
99. Sutherland AD, Limond AM. Influence of pH and sugars on the growth and production of diarrhoeagenic toxin by *Bacillus cereus*. *J Dairy Res* 1993;**60**:575–80.
100. TeGiffel MC, Beumer RR, Granum PE, et al. Isolation and characterization of *Bacillus cereus* from pasteurized milk in household refrigerators in the Netherlands. *Int J Food Microbiol* 1997;**34**: 307–18.
101. Thompson NE, Ketterhagen MJ, Bergdoll MS, et al. Isolation and some properties of an enterotoxin produced by *Bacillus cereus*. *Infect Immun* 1984;**43**:887–94.
102. Tuazon CU, Murray HW, Levy C, et al. Serious infections from *Bacillus* sp. *JAMA* 1979;**241**:1137–40.
103. Turnbull PC. Studies on the production of enterotoxins by *Bacillus cereus*. *J Clin Pathol* 1976;**29**:941–8.
104. Turnbull PC. *Bacillus cereus* toxins. *Pharmacol Ther* 1981;**13**: 453–505.
105. Turnbull PC, Kramer JM. Non-gastrointestinal *Bacillus cereus* infections: An analysis of exotoxin production by strains isolated over a two year period. *J Clin Pathol* 1983;**36**:1091–6.
106. Turnbull PC, Kramer JM. Intestinal carriage of *Bacillus cereus*: faecal isolation studies in three population groups. *J Hyg* 1985; **95**:629–38.
107. Turnbull PC, Kramer JM. Bacillus. In: Murray PR, Baron EJ, Pfaller MA, editors. *Manual of clinical microbiology*. 6th ed.

Washington, DC.: American Society for Microbiology; 1995. p. 349–56.
108. Turnbull PC, French TA, Dowsett EG. Severe systemic and pyogenic infections with *Bacillus cereus*. *BMJ* 1977;**1**:1628–9.
109. Turnbull PC, Nottingham JF, Ghosh AC. A severe necrotic enterotoxin produced by certain food, food poisoning and other clinical isolates of *Bacillus cereus*. *Br J Exp Pathol* 1977;**58**:273–80.
110. Turnbull PC, Jorgensen K, Kramer JM, et al. Severe clinical conditions associated with *Bacillus cereus* and the apparent involvement of exotoxins. *J Clin Pathol* 1979;**32**:289–93.
111. Turnbull PC, Kramer JM, Jorgensen K, et al. Properties and production characteristics of vomiting, diarrheal and necrotizing toxins of *Bacillus cereus*. *Am J Clin Nutr* 1979;**32**:219–28.
112. Vahey JB, Flynn HW, Jr. Results in the management of *Bacillus* endophthalmitis. *Ophthalmol Surg* 1991;**22**:681–6.
113. Vaisanen OM, Mwaisumo NJ, Salkinoja-Salonen MS. Differentiation of dairy strains of the *Bacillus cereus* group by phage typing, minimum growth temperature, and fatty acid analysis. *J Appl Bacteriol* 1991;**70**:315–24.
114. Vandeloski J, Gensheimer KF. *Bacillus cereus:* Maine. *MMWR Morb Mortal Wkly Rep* 1986;**35**:408–10.
115. Van Der Zwet WC, Parlevliet GA, Savelkoul PH, et al. Outbreak of *Bacillus cereus* infections in a neonatal intensive care unit traced to balloons used in manual ventilation. *J Clin Microbiol* 2000;**38**:4131–6.
116. Weber DJ, Saviteer SM, Rutala WA, et al. In vitro susceptibility of *Bacillus* spp to selected antimicrobial agents. *Antimicrob Agents Chemother* 1988;**32**:642–5.
117. Wong MT, Dolan MJ. Significant infections due to *Bacillus* species following abrasions associated with motor vehicle-related trauma. *Clin Infect Dis* 1992;**15**:855–7.
118. Young EJ, Wallace RJ, Ericsson CD, et al. Panophthalmitis due to *Bacillus cereus*. *Arch Intern Med* 1980;**140**:559–60.
119. Youngs ER, Roberts C, Kramer JM, et al. Dissemination of *Bacillus cereus* in a maternity unit. *J Infect* 1985;**10**:228–32.
120. Zaza S, Tokars JI, Yomtovian R, et al. Bacterial contamination of platelets at a university hospital: increased identification due to intensified surveillance. *Infect Control Hosp Epidemiol* 1994;**15**:82–7.
121. Zhong W, Shou Y, Yoshida TM, et al. Differentiation of *Bacillus anthracis*, *B. cereus*, and *B. thuringiensis* by using pulsed-field gel electrophoresis. *Appl Environ Microbiol* 2007;**73**:3446–9.

CHAPTER 93

Arcanobacterium haemolyticum

Natascha Ching

Arcanobacterium haemolyticum is a pleomorphic gram-positive coryneform rod that causes pharyngitis and exanthem in children and young adults.[18,40,44,50]

HISTORY

This diphtheroid was first noted by MacLean and colleagues[44] in association with exudative pharyngitis in American servicemen in the South Pacific during World War II. The organism originally was named *Corynebacterium haemolyticum* but was reclassified in 1986 as *A. haemolyticum* on the basis of phenetic, peptidoglycan, fatty acid, menaquinone, and DNA data.[16-18] The association between infection with *A. haemolyticum* and pharyngitis was observed repeatedly over the years, but a cause-and-effect relationship between the organism and illness has been established only relatively recently.[11,44,50]

THE ORGANISM

Microbiology[18,20,26,70,73]

A. haemolyticum is a gram-positive to gram-variable pleomorphic rod. Its laboratory characteristics are presented in Table 93-1. The organism grows best at 37° C (98.6° F) on a blood- or serum-enriched medium with the addition of 5 percent carbon dioxide. Alternatively, it grows well anaerobically. On rabbit or human blood agar, colonies are pinpoint (0.5 mm) at 24 hours; they increase to 1 to 1.5 mm after 48 hours. At this time, a unique black opaque dot is noted in the center of the colony, and this dot remains on the agar when the colony is scraped away.

At 24 hours, a 1-mm zone of hemolysis develops around colonies grown on human or rabbit blood agar. The hemolytic zone increases to 3 to 5 mm by 48 hours. Both growth and red cell hemolysis are minimal on horse and sheep blood agar. Because throat cultures usually are performed on sheep blood agar plates, the hemolytic activity of *A. haemolyticum* may be missed.

Colonies of *A. haemolyticum* can be of either the smooth or rough biotypes on horse blood agar.[10] These biotypes also differ in their hemolysis and biochemical properties. Smooth colonies predominate in wound infections and frequently use sucrose and trehalose, are β-hemolytic, and lack β-glucuronidase. Rough colonies are found almost exclusively in the respiratory tract and do not use sucrose and trehalose, are nonhemolytic, and are β-glucuronidase–positive.

A. haemolyticum resembles *Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*), a common cause of bovine mastitis and a rare cause of skin ulcers in children.[36] These organisms can be differentiated by several means. *A. pyogenes* is able to hydrolyze gelatin and ferment xylose. In addition, *A. haemolyticum* has a positive reverse Christie, Atkins, and Munch-Petersen (CAMP) test in that it inhibits the β-hemolysis of *Staphylococcus aureus*, whereas *A. pyogenes* shows slight enhancement of β-hemolysis.[25] The poor growth of *A. haemolyticum* on tellurite medium and lack of catalase help distinguish it from *Corynebacterium diphtheriae*, which also causes pharyngitis.[31,38,40]

Toxin Production

A. haemolyticum liberates three toxins: phospholipase D (PLD), a hemolysin, and neuraminidase.[18] PLD is a dermonecrotic toxin that after intradermal inoculation in rabbits and guinea pigs causes local hemorrhagic necrosis; injection of PLD also is lethal in rabbits.[58] The PLD gene of *A. haemolyticum* has a high degree of homology to that of *Corynebacterium pseudotuberculosis*.[19,47] PLD has been shown to be involved in the virulence of *C. pseudotuberculosis;* PLD mutants are less pathogenic in experimental infections in goats.[46] The PLD of *C. pseudotuberculosis* is similar biochemically and shares some biologic activity with the PLD that is found in brown recluse spider venom and that plays a role in the venom's toxicity.[6,40] *A. haemolyticum* carries a gene similar to the gene encoding the erythrogenic toxin of *Streptococcus pyogenes*.[18]

Antimicrobial Susceptibility

Almost all *A. haemolyticum* strains are highly susceptible to erythromycin (minimal inhibitory concentration <0.06 μg/mL). However, erythromycin is not bactericidal.[9,44,70] Carlson and colleagues[12] reported an *A. haemolyticum* isolate from a diabetic foot ulcer that was exceptionally resistant to macrolides, clindamycin, tetracycline, and ofloxacin. Waagner[70] noted that of 100 pharyngeal isolates, all were inhibited by concentrations of 0.25 μg/mL or less of penicillin G and by 1 μg/mL of penicillin V. However, tolerance to penicillin has been observed.[44,55] In one study, the minimal bactericidal concentration–minimal inhibitory concentration ratio varied from 1:1 to 1:8.[44] In addition to being susceptible to penicillin and erythromycin, *A. haemolyticum* also is susceptible to other β-lactams, clindamycin, chloramphenicol, azithromycin, vancomycin, ciprofloxacin,

TABLE 93-1 **Identification Characteristics of *Arcanobacterium Haemolyticum***

Test or Characteristic	Finding
Catalase	Negative
β-Hemolysis	Positive (a narrow zone of slight hemolysis after 48 hr on sheep blood)
Nitrate reduction	Negative
Pigment production	White or gray
Urease	Negative
Gelatin hydrolysis	Negative
Motility	Negative
Esculin hydrolysis	Negative
Carbohydrate usage	
Glucose	Positive
Maltose	Positive
Sucrose	Positive (requires rabbit serum for growth in peptone water)
Mannitol	Negative
Xylose	Negative

Data from Collins MD, Cummins CS. Genus *Corynebacterium*. In: Sneath PHA, Main NS, Sharpe ME, et al, editors. Bergey's manual of systemic bacteriology. Vol. 2. Baltimore, Md, Williams & Wilkins; 1986, p. 266-76.

tetracyclines, and rifampin; most strains are resistant to sulfonamides and trimethoprim-sulfamethoxazole.[9,12,70]

Carlson[8] compared the E test and agar dilution methods for susceptibility testing of 12 antimicrobial agents in 70 *A. haemolyticum* isolates. E test and agar dilution method results for benzylpenicillin, clindamycin, erythromycin, imipenem, levofloxacin, rifampin, and vancomycin were in 100 percent agreement. Tetracycline, ciprofloxacin and ofloxacin were in agreement between 97 and 99 percent of the time, but a lower percentage of agreement was found for cefotaxime and cefuroxime, at 93 and 84 percent, respectively. The author reinforces the concept that because there are no universal standards for the organism in regard to susceptible, intermediate, or resistant categories, results should be interpreted with caution for clinical care. Almuzara and associates[1] also evaluated the susceptibility of 19 strains of *A. haemolyticum* according to National Committee for Clinical and Laboratory Standards (now the Clinical and Laboratory Standards Institute [CLSI]) interpretative standards for aerobic organisms. The majority of isolates were susceptible to penicillin, cephalosporins, clindamycin, ciprofloxacin, vancomycin, and macrolides (erythromycin and azithromycin). However, only 68 percent of isolates were found to be sensitive to tetracycline.

Recommendations for antimicrobial susceptibility testing of *Arcanobacterium* are provided in CLSI document M45 entitled "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria." Table 5 is for *Corynebacterium* spp. and coryneforms and also applies to *Arcanobacterium* spp. The recommended MIC method for testing *Arcanobacterium* spp. uses broth microdilution with cation-adjusted Mueller-Hinton broth (CAMHB) supplemented with 2.5 to 5 percent lysed horse blood (CAMHB-LHB); there is currently no disk diffusion method recommended.[15] This method involves testing in cation-adjusted Mueller-Hinton broth supplemented with lysed horse blood (2.5% to 5% v/v). Some investigators have used agar dilution or E test satisfactorily.[8]

A vancomycin-resistant *A. haemolyticum* isolated from stool during an outbreak of vancomycin-resistant enterococci has been described. It contained the *vanA* gene primarily found in *Enterococcus faecium*.[59] Two other case reports have described organisms resistant to ciprofloxacin[68] and tetracycline.[39]

EPIDEMIOLOGY

Although similar organisms are common causes of infection in animals, humans appear to be the primary host of *A. haemolyticum*.[60,70] The organism is isolated primarily from throat specimens in patients with pharyngitis,[44,50,70] although it also may be a commensal of human skin.[43] It likewise may be a commensal in the throat but often is overlooked because laboratories frequently do not differentiate diphtheroids, which are considered "normal flora." In addition, because *A. haemolyticum* often is found in polymicrobial respiratory tract infections with classic respiratory pathogens, including *S. pyogenes*, it sometimes is missed when these more classic pathogens are identified.[40]

Although no definitive data are available, spread from person to person is assumed to be from the throat discharge of an infected person to the throat of a susceptible host. Transmission could occur directly or indirectly by fomites. Secondary cases in families indicate that spread is from person to person rather than from an environmental source.[23,50]

In an 8-year study, the organism was found to be isolated from throat specimens in each year, with isolation rates varying from 0.2 to 0.7 percent.[50] The peak age for contracting illness caused by *A. haemolyticum* is during the second decade of life; in contrast, the peak age for developing pharyngitis caused by *S. pyogenes* is during the first decade of life.[23,44,50] In two studies, illnesses occurred more commonly in females than in males.[23,50] In a review of *A. haemolyticum* systemic and deep-seated infections, a preponderance of males over females was found in adolescents with no risk factors for development of invasive disease.[64] No seasonal prevalence has been reported.

Screening throat cultures for pharyngotonsillitis in northern Israel was performed in 518 throat cultures in patients aged 1 to 90 years.[14] Only one culture was positive (0.2%) for *A. haemolyticum* in contrast to 26 percent recovery of group A *Streptococcus*. However, the one case was in a 9-year-old patient and only 58.7 percent of patients were 1 to 18 years old, the more common age of isolation for this organism.

PATHOGENESIS AND PATHOLOGY

Few data are available regarding pathogenesis and pathology. The dermonecrotic toxin probably plays a role in pharyngitis. Skin biopsy specimens were taken from the

exanthem in two patients, and both showed only a mild lymphohistiocytic perivascular infiltrate.[50] Cultures of both samples were negative, and no IgG, IgA, or IgM deposition was noted. These findings suggest that the rash may be toxin-mediated, like the rash in group A streptococcal infections.

A. haemolyticum has been found to persist intracellularly,[56] which might account for failure of penicillin treatment in some cases.

In one study, 5/42 patients were found to have apparent dual infections with Epstein-Barr virus and *A. haemolyticum*.[44] The authors of this study suggested that immune suppression by the virus contributes to a more marked effect of the bacterial infection in the throat.

With regard to the immune response to *A. haemolyticum*, paired acute and convalescent sera showed the development of a humoral response to four distinct cell wall–associated proteins on Western blot analysis in 7/8 patients with culture-confirmed infection.[54]

CLINICAL MANIFESTATIONS

Pharyngitis

Pharyngitis* is the most common finding in *A. haemolyticum* infection. Signs and symptoms associated with pharyngitis are presented in Table 93-2. The illness is indistinguishable from that caused by group A streptococci; frequently, it resembles Epstein-Barr virus infectious mononucleosis. Several patients with typical infectious mononucleosis had laboratory evidence of infection with both Epstein-Barr virus and *A. haemolyticum*.[28,29,44] Peritonsillar abscess caused by *A. haemolyticum* has been reported on several occasions.[4,38,41,49] The most common exanthem is scarlatiniform, which has an onset 1 to 4 days after the beginning of pharyngeal symptoms. The rash is most prominent on the extensor surfaces of the arms and legs (Fig. 93-1). Circumoral pallor, which is seen with group A streptococcal scarlet fever, does not seem to occur with *A. haemolyticum* infection. The rash may progress to involve the chest and back; it usually spares the palms, soles, and face, and it rarely involves the abdomen and buttocks.

The rash frequently is pruritic and may be urticarial. Erythema multiforme has been described.[3] Gaston and Zurowski[27] reported a 20-year-old man who had, in addition to pharyngitis, a rash involving mainly his hands and feet. His feet were swollen, and on the soles were erythematous macules, petechiae, and vesicles. His palms were tender, and 2- to 4-mm erythematous macular lesions that contained small central vesicles were present. Recently, Mehta[48] reported a 19-year-old woman who presented with pharyngitis and a pruritic rash on her arms and legs 4 days before evaluation. Physical examination revealed an erythematous, urticarial rash with large and small annular rings over the whole body with confluence on the upper thighs and smaller lesions groups on distal dorsal parts of the hands and feet.

Carlson and associates[13] also reported a 19-year-old man who presented with a 2-day history of a pruritic exanthem on the upper back and chest that spread centrifugally. The patient had pharyngitis that worsened to exudative tonsillitis, and a throat culture was positive for *A. haemolyticum*. He also had swollen fingers and worsening erythematous maculopapular exanthem over his trunk and proximal extremities. Interestingly, Pastia's lines were observed on the third day. Two weeks after antimicrobial treatment and discharge, a mild late desquamation was noted on the palms and around fingernails.

The duration of the exanthem has not been described adequately in the literature. In one study, exanthem was

*References 3, 11, 23, 27, 29, 31, 35, 43, 44, 50, 52, 53, 61-63, 69, 70.

TABLE 93-2 **Signs and Symptoms in Children, Adolescents, and Young Adults with *Arcanobacterium Haemolyticum* Infection**

Symptoms	Frequency (%)
Sore throat	100
Rash	40-70
Pruritus	50
Fever	40-75
Hoarseness	60
Cough (nonproductive)	40-60
Vomiting	30
Signs	
Pharyngitis or tonsillitis	100
Exudative	50-70
Palatal petechiae	30
Glossitis	25
Cervical lymphadenitis	40-75
Rash	40-70
Scarlatiniform	50
Urticarial	5
Maculopapular	25

Data from references 23, 32, 41, 46, 64.

FIGURE 93-1 ■ An erythematous maculopapular discrete and confluent rash on the thigh and arm of a 16-year-old girl with pharyngitis and *Arcanobacterium haemolyticum* isolated from her throat. (From Mackenzie A, Fuite LA, Chan FTH, et al. Incidence and pathogenicity of *Arcanobacterium haemolyticum* during a 2-year study in Ottawa. Clin Infect Dis 1995;**21**:177-81.)

noted to persist for longer than 2 days in 69 percent of patients.[46]

On occasion, *A. haemolyticum* infection has been manifested as a grayish-white pharyngeal pseudomembrane that has been confused with diphtheria.[3,31,34,38] *C. diphtheriae* and *A. haemolyticum* are diphtheroids that cannot be distinguished on Gram stain but can be differentiated by their biochemical properties.

Skin Infections

In the initial report of infections with *A. haemolyticum* in 1946, MacLean and associates[44] noted both pharyngitis in U.S. servicemen and skin infections in the native populations of the South Pacific Islands. Cutaneous infections have been observed mainly in tropical countries.[70] The most common manifestations are ulcerative lesions that resemble ecthyma. Cellulitis, wound infections, and paronychia all have been noted.* In wound infections, mixed infections with *A. haemolyticum* and other organisms are common findings.

Tan and colleagues[66] report five cases of *A. haemolyticum* bacteremia associated with soft tissue infections in adults. These patients had a history of soft tissue infections; two elderly bedridden patients had decubitus ulcers, and three diabetic patients had foot ulcers with extensive involvement that required surgical débridement. Four patients also had *A. haemolyticum* isolated from the suspected soft tissue focus of infection. A 3-year retrospective review of clinical samples over the same period as the five reported cases revealed 25 isolates of *A. haemolyticum*. Isolates from wound infections or cellulitis were found in 96 percent of cases; 20 percent of those patients also had bacteremia. Cultures from nonsterile sites were often polymicrobial, but the authors report *A. haemolyticum* was usually the predominant isolate.

Malini and colleagues[45] report three individuals with soft tissue infections with mixed infections with *A. haemolyticum* and β-hemolytic streptococci group G in a patient with cellulitis, β-hemolytic streptococci group A in postoperative wound infection, and *Proteus vulgaris* in a diabetic patient with chronic osteomyelitis. Each of these patients responded to erythromycin alone or in combination with penicillin with ciprofloxacin therapy. *A. haemolyticum* may have a synergistic role in tissue damage in these mixed soft tissue infections.

Other Manifestations

Isolated instances of septicemia, brain abscess, meningitis, meningoencephalitis, orbital cellulitis, endocarditis, osteomyelitis, septic arthritis, deep soft tissue infections, pleural empyema, cavitary pneumonia, pyothorax, Lemierre's disease, and sinusitis have been attributed to *A. haemolyticum* infection.† Most of these serious infections occurred in adults and frequently were associated with underlying conditions such as diabetes, a malignancy, or intravenous drug use. In a 1998 review of systemic and deep-seated infections caused by *A. haemolyticum*, Skov and associates[64] identified two groups of patients. The first group was composed of middle-aged to elderly adults who were immunocompromised or had other known risk factors for development of serious infectious disease. The second group consisted of preteens to young adults with no known risk factors except for one individual receiving steroid treatment for Epstein-Barr virus infection. Therriault and colleagues[67] report a case of sepsis with *A. haemolyticum* bacteremia, cavitary pneumonia, and pyomyositis that resolved with combination therapy of penicillin and azithromycin in an 18-year-old man with only a past history of mild asthma. Cultures were positive for *A. haemolyticum* from blood, bronchoalveolar fluid, and surface and surgical cultures from a lower extremity abscess.

A pyothorax with multiloculated hydropneumothorax has been reported in a 19-year-old man in India with no history of pharyngitis.[57] Pure growth of *A. haemolyticum* was obtained from the thoracocentesis. Treatment consisted of a therapeutic thoracocentesis and continued chest tube drainage for 1 month. Ceftriaxone and metronidazole were given for a month, and the patient had clinical and radiologic resolution.

A brain abscess was documented in an 18-year-old man in Venezuela with history of multiple periodontal problems, with periodontitis, dental caries, and multiple tooth extractions.[68] The patient had headache, vomiting, and neurologic deficits in the left extremities. Computed tomography (CT) of the brain revealed a left-sided hypodense frontoparietal lesion with cystic, contrast ring enhancement, edema, and midline mass effect. With worsening neurologic status, a craniotomy was performed and aspiration of purulent material from the encapsulated mass revealed positive cultures with pure *A. haemolyticum* growth. Therapy consisted of ceftriaxone and metronidazole for 1 week and was switched after susceptibility testing to penicillin for 3 weeks. Limjoco-Antonio and colleagues[39] report a 9-year-old girl with a swollen right eye after blunt trauma. She had ethmoid and maxillary sinusitis and orbital cellulitis, which required surgical drainage, débridement of subperiosteal abscess, and a second procedure with external ethmoidectomy and endoscopic sinus surgery. Cultures from the ethmoid sinus grew *A. haemolyticum*, and she was treated with ceftriaxone and clindamycin.

Infective endocarditis caused by *A. haemolyticum* has been reported in a 21-year-old woman with history of congenital heart disease repaired as a child; she was on lifelong warfarin anticoagulation therapy.[75] This patient had fever, lethargy, and a swollen, painful left calf. A transthoracic echocardiogram raised concerns of vegetations on the mitral valve and ventricular septal defect patch. As a result of the occurrence of a grand mal seizure, a large right frontal parenchymal hematoma with smaller frontal abscesses was found on CT of the head. She required a frontal craniotomy for evacuation of the hematoma. Blood culture revealed *A. haemolyticum* in 1/12 sets, which was confirmed by 16S rRNA gene sequencing. Antimicrobial therapy consisted of ceftriaxone with addition of gentamicin, for 6 weeks total therapy.

*References 7, 22, 23, 37, 49, 51, 70, 74.
†References 2, 5, 21, 25, 28, 29, 30, 32, 33, 39, 42, 57, 64, 66, 67, 69, 70-72, 75-77.

The initial therapy of vancomycin and metronidazole was given for only 2 weeks after identification of *A. haemolyticum*.

Fernandez-Suárez and colleagues[24] report *A. haemolyticum* has been associated with Lemierre's disease and septicemia in a 23-year-old male who presented with acute pharyngotonsillitis. The patient underwent cervical contrast-enhanced CT, which revealed complete thrombosis of the left internal jugular vein. Enoxaparin was initiated, with a transition to another anticoagulant (acenocoumarol) for 3 months as an outpatient. Antimicrobial therapy consisted initially of high-dose amoxicillin-clavulanate and metronidazole, but was changed to imipenem-cilastatin and metronidazole when right basal pneumonia was diagnosed along with bilateral pleural effusions. The patient completed 7 days of intravenous antibiotics and was transitioned to clindamycin as an outpatient for 10 days. All three blood cultures were positive for *A. haemolyticum*.

Lemierre's disease with coinfection of *A. haemolyticum* and *Fusobacterium necrophorum* bacteremia was reported by Younus and colleagues.[77] A 21-year-old man presented with fever, sore throat, tenderness along the border of his left sternocleidomastoid muscle, crackles, a blanching maculopapular rash, sepsis, and worsening pulmonary symptoms. The initial blood culture had *F. necrophorum*, and repeat blood cultures from day of admission had *A. haemolyticum* before antimicrobial therapy. CT of the chest revealed multiple nodular densities in the lungs, with evidence of central necrosis in one area. Therapy consisted of anticoagulation with heparin, initial antimicrobial therapy of gatifloxacin and metronidazole, which was switched to vancomycin and piperacillin-tazobactam for 2 weeks, followed by an oral course of amoxicillin-clavulanate for 3 months, but no surgical intervention was needed.

Differential Diagnosis

Pharyngitis caused by *A. haemolyticum* must be differentiated from all other causes of pharyngitis (see Chapter 9). Of particular importance is distinguishing *A. haemolyticum* pharyngitis from *S. pyogenes* pharyngitis. Such differentiation can be achieved with certainty only by specific culture. In *A. haemolyticum* infection, the rapid group A streptococcal antigen tests will be negative, as will the usual group A streptococcal culture. These negative tests in specimens from adolescents should suggest strongly the possibility of *A. haemolyticum* pharyngitis.

When exanthem occurs, the confusion with illness caused by *S. pyogenes* is more pronounced. In many cases, the rash in patients infected with *A. haemolyticum* is scarlatiniform. However, the lack of typical circumoral pallor and a tendency for more discrete lesions in *A. haemolyticum* infection occasionally may help in making the clinical diagnosis.

Other common causes of pharyngitis and exanthem in adolescents and young adults are *Mycoplasma pneumoniae* and Epstein-Barr virus infections. As noted by Mackenzie and colleagues,[43] *A. haemolyticum* and Epstein-Barr virus coinfections are not uncommon occurrences. Concurrent *M. pneumoniae* pneumonia and *A. haemolyticum* empyema and bacteremia have been reported in a previously healthy 20-year-old man.[65]

Cutaneous infections, including subacute ulcerations, wound infections, cellulitis, and paronychia caused by *A. haemolyticum*, must be differentiated from those caused by other organisms, such as staphylococci and streptococci.

SPECIFIC DIAGNOSIS

A specific diagnosis is made by culturing *A. haemolyticum* from the pharynx, a skin lesion, or a sterile body site in invasive infections. Culturing is done best with rabbit or human blood agar and the addition of 5 percent carbon dioxide.[18,20,70,73] An important note is that horse or sheep blood agar, which generally is used for culture of *S. pyogenes*, is not satisfactory for the growth and identification of *A. haemolyticum*. Using biochemical identification systems, such as the API (RAPID) Coryne strip or the Biolog system, can help differentiate *A. haemolyticum* from other coryneform bacteria. The Biolog system can make the identification in 4 hours.[26]

TREATMENT

A. haemolyticum is highly susceptible to numerous antibiotics.[9,10,44,53,70] Although no specific treatment studies have been performed, the experience in several large studies suggests that both penicillin and erythromycin are effective.[9,35,44,50] Clinical failure with penicillin has been noted.[3,52,70] Therefore, erythromycin has been suggested for first-line therapy for certain indications, such as *A. haemolyticum* tonsillitis.[12] Carlson and coworkers[12] suggest using either a broad-spectrum β-lactam antibiotic, clindamycin, or macrolides for serious systemic *A. haemolyticum* infection, although these authors do acknowledge that macrolides do not provide anaerobic coverage. An alternative approach would be to use high-dose penicillin plus an aminoglycoside.[64,70]

PROGNOSIS

The prognosis in cases of *A. haemolyticum* pharyngitis is good, even in untreated patients. However, invasive disease can be fatal, and peritonsillar abscess requires prompt surgical intervention and appropriate antimicrobial therapy.

NEW REFERENCES SINCE THE SIXTH EDITION

24. Fernandez-Suárez A, Benítez JM, Vidal AM, et al. Lemierre's syndrome and septicaemia caused solely by *Arcanobacterium haemolyticum* in a young immunocompetent patient. *J Med Microbiol* 2009; **58**:1645–8.
30. Goyal R, Singh NP, Mathur M. Septic arthritis due to *Arcanobacterium haemolyticum*. *Indian J Med Microbiol* 2005;**23**:63–5.
32. Hedman K, Brauner A. Septicaemia caused by *Arcanobacterium haemolyticum* smooth type in an immunocompetent patient. *J Med Microbiol* 2012;**61**:1328–9.
45. Malini A, Deepa EK, Manohar PV, et al. Soft tissue infections with *Arcanobacterium haemolyticum:* report of three cases. *Indian J Med Microbiol* 2008;**26**:192–5.

67. Therriault BL, Daniels LM, Carter YL, et al. Severe sepsis caused by *Arcanobacterium haemolyticum:* a case report and review of the literature. *Ann Pharmacother* 2008;**42**:1697–702.
69. Volante M, Corina L, Contucci AM, et al. *Arcanobacterium haemolyticum:* two case reports. *Acta Otorhinolaryngol Ital* 2008;**28**: 144–6.
75. Wong V, Turmezei T, Cartmeill, et al. Infective endocarditis caused by *Arcanobacterium haemolyticum:* a case report. *Ann Clin Microbiol Antimicrob* 2011;**10**:17.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 94

Erysipelothrix Rhusiopathiae

Randall G. Fisher

Erysipelothrix rhusiopathiae (insidiosa) was identified definitively first by Rosenbach[32] in 1884 as a cause of the cutaneous disease erysipeloid. Although most commonly associated with localized skin infection in humans, this organism has been associated with sepsis,[13,30,42] chronic skin eruption,[9,19] and endocarditis.*

BACTERIOLOGY

E. rhusiopathiae is a slender, pleomorphic, gram-positive, unencapsulated rod that produces 0.1-mm bluish colonies on blood agar. On Gram staining, the rods may appear singly, in short chains, or, rarely, in long, branching filaments. Although they are gram positive, they decolorize readily, sometimes producing a spotted appearance. Some strains produce α-hemolysis in 48 to 72 hours. Gelatin inoculated by stab inconsistently forms a "test-tube brush" or "pipe cleaner" appearance diagnostic for this organism.[13] *Erysipelothrix* is differentiated from morphologically similar *Listeria monocytogenes* and diphtheroids by the absence of motility and catalase production and by the presence of hydrogen sulfide production in triple-sugar iron medium.[13,42]

EPIDEMIOLOGY

First isolated from mice in 1880 by Koch, *Erysipelothrix* is a common commensal of wild and domestic mammals, birds, and fish.[13,42] The organism also may lead a saprophytic existence in soil. First identified by Loeffler in 1882 as the causative agent of swine erysipelas, it remains an important epidemic cause of disease in these animals, with losses greater than $25 million annually to this industry.[13] Sheep, rabbits, cattle, turkeys, and rats are subject to infection with this organism. *Erysipelothrix* has been recovered from wild moose and domestic emus.[5,14,23] *E. rhusiopathiae* survives salting and smoking procedures. Pieces of meat may contain the organism for 170 days after pickling, but exposure to moist heat for 15 minutes at 55° C kills most strains.[13,40] Fish handlers, meat processors, poultry workers, veterinarians, abattoir workers, and food handlers are at risk for exposure to *Erysipelothrix*.[26,28] Isolates of the same serotype may show genetic diversity, thus serotyping may not be completely reliable as an epidemiologic tool for tracking outbreaks.[6]

PATHOPHYSIOLOGY

Human infection is largely accidental and results from contamination of skin abrasions during handling of infected material. Males are infected more commonly than females, perhaps because of an increased risk for exposure. The presence of an antiphagocytic capsule may be a virulence factor for *E. rhusiopathiae*.[35] In vitro study shows that encapsulated strains are poorly phagocytized by macrophages, unless immune serum is provided; ingested bacteria are able to replicate within the macrophage. The ability to survive and replicate within macrophages probably is due to failure of the encapsulated strains to induce the oxidative burst.[34] The disease usually is self-limited, most often involving the hands. Biopsy of skin lesions shows a marked inflammatory response. Difficulty of establishing bacteriologic confirmation has been attributed to the organism's location in the deep part of the pars reticularis of the corium.[7]

CLINICAL MANIFESTATIONS

Human disease typically manifests as a mild, localized cutaneous eruption; a more severe, generalized cutaneous

*References 3, 13, 24, 25, 31, 42.

REFERENCES

1. Almuzara MN, de Mier C, Barberis CM, et al. *Arcanobacterium hemolyticum:* identification and susceptibility to nine antimicrobial agents. *Clin Microbiol Infect* 2002;**8**:828–9.
2. Altmann G, Bogokovsky B. Brain abscess due to *Corynebacterium haemolyticum*. *Lancet* 1973;**1**:338–9.
3. Banck G, Nyman M. Tonsillitis and rash associated with *Corynebacterium haemolyticum*. *J Infect Dis* 1986;**154**:1037–40.
4. Barnham M, Bradwell RA. Acute peritonsillar abscess caused by *Arcanobacterium haemolyticum*. *J Laryngol Otol* 1992;**106**:1000–1.
5. Ben-Yaacob D, Waron M, Boldur I, et al. Septicemia due to *Corynebacterium haemolyticum*. *Isr J Med Sci* 1984;**20**:431–3.
6. Bernheimer AW, Campbell BJ, Forrester LJ. Comparative toxinology of *Loxosceles reclusa* and *Corynebacterium pseudotuberculosis*. *Science* 1985;**228**:590–1.
7. Bowness P, Bower M, Montgomery J, et al. The bacteriology of skin sores in Goroka children. *Papua New Guinea Med J* 1984;**27**: 83–7.
8. Carlson P. Comparison of the E test and agar dilution methods for susceptibility testing of *Arcanobacterium haemolyticum*. *Eur J Clin Microbiol Infect Dis* 2000;**19**:891–3.
9. Carlson P, Kontiainen S, Renkonen OV. Antimicrobial susceptibility of *Arcanobacterium haemolyticum*. *Antimicrob Agents Chemother* 1994;**38**:142–3.
10. Carlson P, Lounatmaa K, Kontiainen S. Biotypes of *Arcanobacterium haemolyticum*. *J Clin Microbiol* 1994;**32**:1654–7.
11. Carlson P, Kontianinen S, Renkonen OV, et al. *Arcanobacterium haemolyticum* and streptococcal pharyngitis in army conscripts. *Scand J Infect Dis* 1995;**27**:17–8.
12. Carlson P, Korpela J, Walder M, et al. Antimicrobial susceptibilities and biotypes of *Arcanobacterium haemolyticum* blood isolates. *Eur J Clin Microbiol Infect Dis* 1999;**18**:915–7.
13. Carlson P, Seppanen M, Tarvainen K, et al. Pharyngitis and exanthema caused by *Arcanobacterium haemolyticum*. *Acta Derm Venereol* 2001;**81**:143–4.
14. Chen Y, Colodner R, Chazan B, Raz R. Pharyngotonsillitis due to *Arcanobacterium haemolyticum* in northern Israel. *Isr Med Assoc J* 2005;**7**:241–2.
15. Clinical and Laboratory Standards Institute. *Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria*. Approved guideline. CLSI document M45-A. Wayne, Pa: Clinical and Laboratory Standards Institute; 2006.
16. Collins MD, Cummins CS. Genus *Corynebacterium*. In: Sneath PHA, Mair NS, Sharpe ME, et al, editors. Bergey's manual of systemic bacteriology, vol. 2. Baltimore, Md: Williams & Wilkins; 1986. p. 266–76.
17. Collins MD, Jones D, Schofield GM. Reclassification of *Corynebacterium haemolyticum* (MacLean, Liebow and Rosenberg) in the genus *Arcanobacterium* gen. nov. as *Arcanobacterium haemolyticum* nom. rev., comb. nov. *J Gen Microbiol* 1982;**128**:1279–81.
18. Coyle MB, Lipsky BA. Coryneform bacteria in infectious diseases: clinical and laboratory aspects. *Clin Microbiol Rev* 1990;**3**:227–46.
19. Cuevas WA, Songer JG. *Arcanobacterium haemolyticum* phospholipase D is genetically and functionally similar to *Corynebacterium pseudotuberculosis* phospholipase D. *Infect Immun* 1993;**61**: 4310–6.
20. Cummings LA, Wu WK, Larson AM, et al. Effects of media, atmosphere, and incubation time on colonial morphology of *Arcanobacterium haemolyticum*. *J Clin Microbiol* 1993;**31**:3223–6.
21. Dobinsky S, Noesselt T, Rucker A, et al. Three cases of *Arcanobacterium haemolyticum* associated with abscess formation and cellulitis. *Eur J Clin Microbiol Infect Dis* 1999;**18**:804–6.
22. Esteban J, Zapardiel J, Soriano F. Two cases of soft-tissue infection caused by *Arcanobacterium haemolyticum*. *Clin Infect Dis* 1994;**18**: 835–6.
23. Fell HWK, Nagington J, Naylor GRE, et al. *Corynebacterium haemolyticum* infections in Cambridgeshire. *J Hyg Camb* 1977;**79**: 269–75.
24. Fernandez-Suárez A, Benítez JM, Vidal AM, et al. Lemierre's syndrome and septicaemia caused solely by *Arcanobacterium* haemolyticum in a young immunocompetent patient. *J Med Microbiol* 2009;**58**:1645–8.
25. Ford JG, Yeatts RP, Givner LB. Orbital cellulitis, subperiosteal abscess, sinusitis, and septicemia caused by *Arcanobacterium haemolyticum*. *Am J Ophthalmol* 1995;**120**:261–2.
26. Funke G, von Graevenitz A, Clarridge 3rd, JE, et al. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* 1997;**10**:125–59.
27. Gaston DA, Zurowski SM. *Arcanobacterium haemolyticum* pharyngitis and exanthem. *Arch Dermatol* 1996;**132**:61–4.
28. Givner LB, McGehee D, Taber LH, et al. Sinusitis, orbital cellulitis and polymicrobial bacteremia in a patient with primary Epstein-Barr virus infection. *Pediatr Infect Dis J* 1984;**3**:254–6.
29. Goudswaard J, van de Merwe DW, van der Sluys P, et al. *Corynebacterium haemolyticum* septicemia in a girl with mononucleosis infectiosa. *Scand J Infect Dis* 1988;**20**:339–40.
30. Goyal R, Singh NP, Mathur M. Septic arthritis due to Arcanobacterium haemolyticum. *Indian J Med Microbiol* 2005;**23**:63–5.
31. Green SL, LaPeter KS. Pseudodiphtheritic membranous pharyngitis caused by *Corynebacterium haemolyticum*. *J A M A* 1981;**245**: 2330–1.
32. Hedman K, Brauner A. Septicaemia caused by *Arcanobacterium haemolyticum* smooth type in an immunocompetent patient. *J Med Microbiol* 2012;**61**:1328–9.
33. Jobanputra RS, Swain CP. Septicaemia due to *Corynebacterum haemolyticum*. *J Clin Pathol* 1975;**28**:798–800.
34. Kain KC, Noble MA, Barteluk RL, et al. *Arcanobacterium hemolyticum* infection: confused with scarlet fever and diphtheria. *J Emerg Med* 1991;**9**:33–5.
35. Karpathios T, Drakonaki S, Zervoudaki A, et al. *Arcanobacterium haemolyticum* in children with presumed streptococcal pharyngotonsillitis or scarlet fever. *J Pediatr* 1992;**121**:735–7.
36. Kotrajaras RP, Buddhavudhikral S, Sukroongreung S, et al. Endemic leg ulcers caused by *Corynebacterium pyogenes* in Thailand. *Int J Dermatol* 1982;**21**:407–9.
37. Kotrajaras R, Tagami H. *Corynebacterium pyogenes:* its pathogenic mechanism in epidemic leg ulcers in Thailand. *Int J Dermatol* 1987;**26**:45–50.
38. Kovatch AL, Schuit KE, Michaels RH. *Corynebacterium haemolyticum* peritonsillar abscess mimicking diphtheria. *JAMA* 1983;**249**: 1757–8.
39. Limjoco-Antonio AD, Janda WM, Schreckenberger PC. *Arcanobacterium haemolyticum* sinusitis and orbital cellulitis. *Pediatr Infect Dis J* 2003;**22**:465–7.
40. Linder R. *Rhodococcus equi* and *Arcanobacterium haemolyticum*: two "coryneform" bacteria increasingly recognized as agents of human infection. *Emerg Infect Dis* 1997;**3**:145–53.
41. Lipsky BA, Goldberger AC, Tompkins LS, et al. Infections caused by nondiphtheria corynebacteria. *Rev Infect Dis* 1982;**4**:1220–35.
42. Locksley RM. The lowly diphtheroid: nondiphtheria corynebacterial infections in humans. *West J Med* 1982;**137**:45–52.
43. Mackenzie A, Fuite LA, Chan FTH, et al. Incidence and pathogenicity of *Arcanobacterium haemolyticum* during a 2-year study in Ottawa. *Clin Infect Dis* 1995;**21**:177–81.
44. MacLean PD, Liebow AA, Rosenberg AA. A hemolytic corynebacterium resembling *Corynebacterium ovis* and *Corynebacterium pyogenes* in man. *J Infect Dis* 1946;**79**:69–90.
45. Malini A, Deepa EK, Manohar PV, et al. Soft tissue infections with *Arcanobacterium haemolyticum*: report of three cases. *Indian J Med Microbiol* 2008;**26**:192–5.
46. McNamara PJ, Bradley GA, Songer JG. Targeted mutagenesis of the phospholipase D gene results in decreased virulence of *Corynebacterium pseudotuberculosis*. *Mol Microbiol* 1994;**12**:921–30.
47. McNamara PJ, Cuevas WA, Songer JG. Toxic phospholipases D of *Corynebacterium pseudotuberculosis, C. ulcerans* and *Arcanobacterium haemolyticum:* cloning and sequence homology. *Gene* 1995;**156**: 113–8.
48. Mehta CL. Arcanobacterium haemolyticum. *J Am Acad Dermatol* 2003;**48**:298–9.
49. Miller RA, Brancato F. Peritonsillar abscess associated with *Corynebacterium haemolyticum*. *West J Med* 1984;**140**:449–51.
50. Miller RA, Brancato F, Holmes KK. *Corynebacterium haemolyticum* as a cause of pharyngitis and scarlatiniform rash in young adults. *Ann Intern Med* 1986;**105**:867–72.
51. Montgomery J. The aerobic bacteriology of infected skin lesions in children of the Eastern Highlands Province. *Papua New Guinea Med J* 1985;**28**:93–103.
52. Moreno MM, Valle VA, Aguillar AL. Pharyngitis caused by *Arcanobacterium haemolyticum*. *An Esp Pediatr* 1989;**30**:209–10.
53. Nyman M, Banck G. The clinical picture in throat infections caused by *Corynebacterium haemolyticum*. *Hygiea Swedish Med Assoc* 1984;109–10.

54. Nyman M, Alugupalli KR, Stromberg S, et al. Antibody response to *Arcanobacterium haemolyticum* infection in humans. *J Infect Dis* 1997;**175**:1515–8.
55. Nyman M, Banck G, Thore M. Penicillin tolerance in *Arcanobacterium haemolyticum*. *J Infect Dis* 1990;**161**:261–5.
56. Osterlund A. Are penicillin treatment failures in *Arcanobacterium haemolyticum* pharyngotonsillitis caused by intracellularly residing bacteria? *Scand J Infect Dis* 1995;**27**:131–4.
57. Parija SC, Kaliaperumal V, Kumar SV, et al. *Arcanobacterium haemolyticum* associated with pyothorax: case report. *BMC Infect Dis* 2005;**5**:68.
58. Patocka F, Mara M, Soucek A, et al. Observations on the biological properties of atypical haemolytic corynebacteria isolated from man as compared with *Corynebacterium haemolyticum*, *Corynebacterium pyogenes bovis* and *Corynebacterium ovis*. *J Hyg Epidemiol Microbiol Imunol* 1962;**6**:1–12.
59. Power EG, Abdulla YH, Talsania HG, et al. *vanA* genes in vancomycin-resistant clinical isolates of *Oerskovia turbata* and *Arcanobacterium (Corynebacterium) haemolyticum*. *J Antimicrob Chemother* 1995;**36**:595–606.
60. Roberts RJ. Isolation of *Corynebacterium haemolyticum* from a case of ovine pneumonia. *Vet Rec* 1969;**84**:490.
61. Robinson BE, Murray DL. *Corynebacterium haemolyticum* and pharyngitis. *Ann Intern Med* 1987;**106**:778–9.
62. Ryan WJ. Throat infection and rash associated with an unusual corynebacterium. *Lancet* 1972;**2**:1345–7.
63. Selander B, Ljungh A. *Corynebacterium haemolyticum* as a cause of nonstreptococcal pharyngitis. *J Infect Dis* 1986;**154**:1041.
64. Skov RL, Sanden AK, Danchell VH, et al. Systemic and deep-seated infections caused by *Arcanobacterium haemolyticum*. *Eur J Clin Microbiol Infect Dis* 1998;**17**:578–82.
65. Stacey A, Bradlow A. *Arcanobacterium haemolyticum* and *Mycoplasma pneumoniae* co-infection. *J Infect* 1999;**38**:41–2.
66. Tan TY, Ng SY, Thomas H, et al. Arcanobacterium haemolyticum bacteraemia and soft-tissue infections: case report and review of the literature. *J Infect* 2006;**53**:e69–74.
67. Therriault BL, Daniels LM, Carter YL, et al. Severe sepsis caused by Arcanobacterium haemolyticum: a case report and review of the literature. *Ann Pharmacother* 2008;**42**:1697–702.
68. Vargas J, Hernandez M, Silvestri C, et al. Brain abscess due to *Arcanobacterium haemolyticum* after dental extraction. *Clin Infect Dis* 2006;**42**:1810–11.
69. Volante M, Corina L, Contucci AM, et al. *Arcanobacterium haemolyticum:* two case reports. *Acta Otorhinolaryngol Ital* 2008;**28**:144–6.
70. Waagner DC. *Arcanobacterium haemolyticum*: biology of the organism and diseases in man. *Pediatr Infect Dis J* 1991;**10**:933–9.
71. Waller KS, Wood BP. Cavitary pneumonia due to *Arcanobacterium haemolytic*. *Am J Dis Child* 1991;**145**:209–10.
72. Washington JA, Martin WJ, Spiekerman RE. Brain abscess with *Corynebacterium haemolyticum*: report of a case. *Am J Clin Pathol* 1971;**56**:212–5.
73. Wat LL, Fleming CA, Hodge DS, et al. Selective medium for isolation of *Arcanobacterium haemolyticum* and *Streptococcus pyogenes*. *Eur J Clin Microbiol Infect Dis* 1991;**10**:443–6.
74. Wickremesinghe RSB. *Corynebacterium haemolyticum* infections in Sri Lanka. *J Hyg Camb* 1981;**87**:271–7.
75. Wong V, Turmezei T, Cartmeill Soo S, et al. Infective endocarditis caused by *Arcanobacterium haemolyticum:* a case report. *Ann Clin Microbiol Antimicrob* 2011;**10**:17.
76. Worthington MG, Daly BDT, Smith FE. *Corynebacterium haemolyticum* endocarditis on a native valve. *South Med J* 1985;**78**:1261–2.
77. Younus F, Chua A, Tortora G, et al. Lemierre's disease caused by co-infection of *Arcanobacterium haemolyticum* and *Fusobacterium necrophorum:* a case report. *J Infect* 2002;**45**:114–17.

form; or a septicemia often associated with endocarditis. Localized cutaneous infection, the erysipeloid of Rosenbach,[32] is the most common manifestation of *Erysipelothrix* disease.[28] After a 1- to 4-day incubation period, an acute localized lesion appears at the site of an abrasion contaminated with *E. rhusiopathiae*–colonized material. Slowly progressive, purplish red, painful induration is typical. Absence of suppuration and involution without desquamation help to distinguish this lesion from streptococcal or staphylococcal infection. Occasionally, the skin may show sharply circumscribed bluish red lesions, which are similar to the cutaneous manifestations in swine.[13,42] Fever and other constitutional symptoms are uncommon manifestations, occurring in less than 10 percent of cases, unless bacteremia supervenes.[9,28] Untreated infection usually is self-limited, with an average duration of 3 weeks.

Lymphangitis and adenitis occur in 10 percent of cases; in 20 percent of cases, progression of disease extends from lesions on the hand to the wrist and forearm.[20] In one case, a patient with type 2 diabetes was discovered at the time of surgery to have necrotizing fasciitis; *E. rhusiopathiae* was the predominant but not only organism isolated from surgical specimens.[36] A 7-week-old infant with localized *E. rhusiopathiae* infection of the knee without a known source of exposure has been reported,[22] and a 6-year-old girl with *Erysipelothrix* pyopneumothorax has been described.[29] Septic arthritis has been described in a healthy 18-year-old man in whom disease developed after he underwent arthroscopic knee surgery,[2] in association with infective endocarditis,[33] and in a patient with systemic lupus erythematosus and chronic monarthritis without associated systemic symptoms.[41]

Cutaneous eruptions rarely may occur in areas distant from the site of inoculation,[21] appearing as violaceous lesions with advancing pink borders. Bullous vesiculation has been described.[9] In 1921, Prausnitz[30] reported the first case of apparent septicemia in childhood, isolating the organism from the blood of a 10-year-old boy.

E. rhusiopathiae rarely is associated with the bite of a domestic dog or cat.[1] In one prospective study of infected domesticated animal bite wounds, two patients who had been bitten by a cat infected with *E. rhusiopathiae* were identified.[37] Cultures were carefully processed and were performed in reference laboratories.

An uncommon but important complication of *Erysipelothrix* infection is endocarditis. Presumed or proven endocarditis accounts for 90 percent of serious *E. rhusiopathiae* infection.[12] Patients with congenital heart disease or heart valve damage secondary to acute rheumatic fever are at the greatest risk for development of endocarditis. However, previously normal heart valves can be infected.[13,24] Valvular and myocardial abscesses have been described.[27] In contrast to diphtheroid endocarditis, *E. rhusiopathiae* endocarditis usually does not involve prosthetic valves; and in contrast to *Bacillus* spp. endocarditis, it is not associated with intravenous drug abuse.[12] In a review of 1989 cases of endocarditis from 13 series,[3] *Erysipelothrix* was documented in two patients. *Erysipelothrix* endocarditis commonly involves the aortic or mitral valves or both. Overall mortality in reported cases is 38 percent, which is considerably higher than that associated with other pathogens of endocarditis.[11] Many patients with endocarditis are treated empirically with vancomycin, a drug to which all *Erysipelothrix* isolates are constitutively resistant.[16] No history nor physical evidence of cutaneous lesions is found in 50 percent of cases of endocarditis, and history of exposure to contaminated material often is lacking. Although immunocompromised individuals[25] may be at increased risk, serious infection also occurs in otherwise normal hosts,[4,13,24] particularly in association with occupational exposure.

Multiple case reports of septic arthritis, usually involving prosthetic joints, have been published.[15,38,39] Meningitis is rare and may be acute[17] or chronic.[18]

DIAGNOSIS

For localized disease, diagnosis depends largely on clinical appearance of the lesion in association with an appropriate history of exposure. Attempts to culture the organism from material collected by swab or aspirate of a local lesion almost always are unsuccessful, presumably because of the bacteria's location deep within the skin.[7,42] Biopsy specimens of affected skin cultured in broth generally yield the offending bacteria. Amplification and detection of *Erysipelothrix* DNA by polymerase chain reaction show promise in animal models of infection.[23] *Erysipelothrix* is isolated commonly from the blood of patients with septicemia or endocarditis and can be found in affected heart valves at autopsy or at the time of valve replacement.[9] Growth of the organism may be slow; cultures should be monitored for at least a week. A high index of suspicion is important for establishing the diagnosis of endocarditis. The organism has been misidentified as a viridans group streptococcus because of its pleomorphic coccoid appearance, α-hemolysis, and catalase-negative character. Abbreviated identification schema that do not include testing for hydrogen sulfide production sometimes lead to misidentification as *Lactobacillus* spp. or enterococci.[8]

TREATMENT

E. rhusiopathiae is exquisitely sensitive to penicillin. Most isolates also are sensitive to ceftriaxone.[10] Clindamycin or ciprofloxacin may be used for patients who are allergic to penicillin.[10] Localized disease usually can be treated with oral medication, but high parenteral doses occasionally are necessary, particularly for disseminated disease.[13] Treatment of endocarditis is similar to treatment of endocarditis caused by viridans streptococci. At least 12 million units of penicillin administered for 4 weeks has been curative in adult patients, but many cases have been treated for 6 weeks or longer. Concomitant administration of an aminoglycoside has been used in some cases. *Erysipelothrix* is resistant to vancomycin.[16] Prompt microbiologic differentiation of *E. rhusiopathiae* from other gram-positive organisms is important in guiding antimicrobial choice because vancomycin often is employed in empirical therapy for endocarditis. Risk for acquiring

disease is minimized by protecting individuals exposed to potentially contaminated materials.

Acknowledgments

The author would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

15. Hocqueloux L, Poisson DM, Sunder S, et al. Septic arthritis caused by *Erysipelothrix rhusiopathiae* in a prosthetic knee joint. *J Clin Microbiol* 2010;**48**:333–5.
17. Joo EJ, Kang CI, Kim WS, et al. Acute meningitis as an initial manifestation of *Erysipelothrix rhusiopathiae* endocarditis. *J Infect Chemother* 2011;**17**:703–5.
18. Kim SR, Kwon MJ, Lee JH, Lee NY. Chronic meningitis caused by *Erysipelothrix rhusiopathiae*. *J Med Microbiol* 2007;**56**:1405–6.
38. Traer EA, Williams MR, Keenan JN. *Erysipelothrix rhusiopathiae* infection of a total knee arthroplasty: an occupational hazard. *J Arthroplasty* 2008;**23**:609–11.
39. Troelsen A, Moller JK, Bolvig L, et al. Animal-associated bacteria, *Erysipelothrix rhusiopathiae*, as the cause of infection in a total hip arthroplasty. *J Arthroplasty* 2010;**25**:497.e21–3.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 95

Listeriosis

Tobias Kollmann • Robert Bortolussi • Timothy Mailman

Listeria monocytogenes is found widely in the environment in soil, water, and decaying vegetation.[3,42,108] Infection with *L. monocytogenes* was described first by Murray and associates in 1926 while they were investigating an epidemic of perinatal infection in laboratory rabbits.[91] Human disease caused by *L. monocytogenes* was identified 3 years later,[92] and neonatal infection was identified in 1936.[15] The first outbreak confirming transmission from animals to humans was reported in 1983.[109] *L. monocytogenes* since has been found to be a serious, lethal pathogen especially for neonates, pregnant women, the elderly, and immunocompromised individuals.[12,25,73] The recent upsurge in outbreaks has refocused attention on this organism.*

THE ORGANISM

Listeria comprise regular, short, facultatively anaerobic, non–spore-forming, gram-positive rods that are motile and form bluish gray colonies on nutrient agar. They are non–acid fast and catalase positive and do not form capsules. The genus is named in honor of *Lister*, the pioneer of antiseptics. Of the six *Listeria* spp. (*L. monocytogenes*, *Listeria innocua*, *Listeria grayi*, *Listeria welshimeri*, *Listeria seeligeri*, and *Listeria ivanovii*), *L. monocytogenes* is the major pathogen.[55,81] The few case reports regarding other species causing infection usually involve immunocompromised hosts.[105] The following features of *L. monocytogenes* separate it from nonpathogenic *Listeria* spp. as well as other gram-positive rods[114]:

1. All *Listeria* species exhibit tumbling motility at 25° C to 30° C, owing to peritrichous flagella, with reduced motility at 37° C (98.6° F).
2. *L. monocytogenes* grows with a narrow zone of β-hemolysis (nonhemolytic strains exist but are rare) and exhibits a rectangular area of increased hemolysis when streaked on blood agar in proximity to *Staphylococcus aureus* (Christie, Atkins, Munch-Peterson [CAMP] test).
3. *Listeria* are catalase positive.
4. *L. monocytogenes* ferments methyl-D-mannoside and L-rhamnose but not D-xylose.

Listeria tolerate low temperatures, high salt concentrations, and high pH, which allows replication in soil, water, sewage, manure, animal feed, and, significantly, refrigerated foods. Cold enrichment has been used to improve isolation from clinical samples.[9,41,55,113]

Paterson[96] and Seeliger and Finger[115] performed extensive serologic characterization of *L. monocytogenes*. At least 17 serotypes have been identified on the basis of somatic and flagellar antigens, with 3 (1/2a, 1/2b, 4b) accounting for most clinical and food isolates.[84,98]

*References 21, 22, 30, 73, 75, 110.

REFERENCES

1. Abedini S, Lester A. [*Erysipelothrix rhusiopathiae* bacteremia after dog bite.] *Ugeskr Laeger* 1997;**159**:4400–1. In Danish.
2. Allianatos PG, Tilentzoglou AC, Koutsoukou AD. Septic arthritis caused by *Erysipelothrix rhusiopathiae* infection after arthroscopically assisted anterior cruciate ligament reconstruction. *Arthroscopy* 2003;**19**:E26.
3. Ben-Chetrit E, Muiad N, Levo Y. Infective endocarditis caused by uncommon bacteria. *Scand J Infect Dis* 1983;**15**:179–83.
4. Callon RAJ, Brady PG. Toothpick perforation of the sigmoid colon: an unusual case associated with *Erysipelothrix rhusiopathiae* septicemia. *Gastrointest Endosc* 1990;**36**:141–3.
5. Campbell GD, Addison EM, Barker IK, et al. *Erysipelothrix rhusiopathiae*, serotype 17, septicemia in moose *(Alces alces)* from Algonquin Park, Ontario. *J Wild Dis* 1994;**30**:436–8.
6. Chooromoney KN, Hampson DJ, Eamens GJ, et al. Analysis of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by multilocus enzyme electrophoresis. *J Clin Microbiol* 1994;**32**: 371–6.
7. Dhttman G. Schweinrotlauf und erysipeloid. *Beitr Klin Chir* 1921;**123**:461–70.
8. Dunbar SA, Clarridge 3rd JE. Potential errors in recognition of *Erysipelothrix rhusiopathiae*. *J Clin Microbiol* 2000;**38**:1302–4.
9. Ehrlich JC. *Erysipelothrix rhusiopathiae* infection in man. *Arch Intern Med* 1944;**78**:565–77.
10. Fidalgo SG, Longbottom CJ, Riley TV. Susceptibility of *Erysipelothrix rhusiopathiae* to antimicrobial agents and home disinfectants. *Pathology* 2002;**34**:462–5.
11. Fidalgo SG, Riley TV. Detection of *Erysipelothrix rhusiopathiae* in clinical and environmental samples. *Methods Mol Biol* 2004;**268**: 199–205.
12. Gorby GL, Peacock JEJ. *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev Infect Dis* 1988;**10**:317–25.
13. Grieco MH, Sheldon C. Erysipelothrix rhusiopathiae. *Ann N Y Acad Sci* 1970;**174**:523–32.
14. Griffiths GL, Buller N. *Erysipelothrix rhusiopathiae* infection in semi-intensively farmed emus. *Austr Vet J* 1991;**68**:121–2.
15. Hocqueloux L, Poisson DM, Sunder S, et al. Septic arthritis caused by *Erysipelothrix rhusiopathiae* in a prosthetic knee joint. *J Clin Microbiol* 2010;**48**:333–5.
16. Johnson AP, Uttley AH, Woodford N, et al. Resistance to vancomycin and teicoplanin: an emerging clinical problem. *Clin Microbiol Rev* 1990;**3**:280–91.
17. Joo EJ, Kang CI, Kim WS, et al. Acute meningitis as an initial manifestation of *Erysipelothrix rhusiopathiae* endocarditis. *J Infect Chemother* 2011;**17**:703–5.
18. Kim SR, Kwon MJ, Lee JH, Lee NY. Chronic meningitis caused by *Erysipelothrix rhusiopathiae*. *J Med Microbiol* 2007;**56**: 1405–6.
19. Klauder JV. Erysipeloid as an occupational disease. *JAMA* 1938;**111**:1345–8.
20. Klauder JV. *Erysipelothrix rhusiopathiae* infection in swine and in human beings. *Arch Dermatol Syph* 1944;**50**:151–9.
21. Kramer MR, Gombert ME, Corrado ML, et al. *Erysipelothrix rhusiopathiae* endocarditis. *South Med J* 1982;**75**:892.
22. Lacroix J, Delage G, Mitchell G. Erysipeloid in an infant. *J Pediatr* 1981;**99**:745–6.
23. Makino S, Okada Y, Maruyama T, et al. Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J Clin Microbiol* 1994;**32**:1526–31.
24. Morris CA, Schwabacher H, Lynch PG, et al. Two fatal cases of septicaemia due to *Erysipelothrix insidiosa*. *J Clin Pathol* 1965;**18**: 614–17.
25. Muirhead N, Reid TMS. *Erysipelothrix rhusiopathiae* endocarditis. *J Infect* 1980;**2**:83–5.
26. Mutalib A, Keirs R, Austin F. Erysipelas in quail and suspected erysipeloid in processing plant employees. *Avian Dis* 1995;**39**: 191–3.
27. Nandish S, Khardori N. Valvular and myocardial abscesses due to *Erysipelothrix rhusiopathiae*. *Clin Infect Dis* 1999;**29**:1351–2.
28. Nelson E. Five hundred cases of erysipeloid. *Rocky Mountain Med J* 1955;**52**:40–2.
29. Panhotra BR, Agarwal KC, Kumar L, et al. *Erysipelothrix rhusiopathiae* infection in a child: a case report with review of literature. *Indian Pediatr* 1979;**16**:547–9.
30. Prausnitz C. Bakteriologische untersuchung über schweinrotlauf beim menschen. *Zentralbl Bakteriol Mikrobiol Hyg* 1921;**85**:362.
31. Reboli AC, Farrar WE. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clin Microbiol Rev* 1989;**2**:354–9.
32. Rosenbach FJ. Experimentelle morphologische und klinische Studie ber die (Krankheitserregenden) Mikroorganismen des Schweinrotlaufs, des Erysipeloids und der Muse sepsis. *Z Hyg Infektionskrankheit* 1909;**63**:343–71.
33. Ruiz ME, Richards JS, Kerr GS, Kan VL. *Erysipelothrix rhusiopathiae* septic arthritis. *Arthritis Rheum* 2003;**48**:1156–7.
34. Shimoji Y, Yokomizo Y, Mori Y. Intracellular survival and replication of *Erysipelothrix rhusiopathiae* within murine macrophages: failure of induction of the oxidative burst of macrophages. *Infect Immun* 1996;**64**:1789–93.
35. Shimoji Y, Yokomizo Y, Sekizaki T, et al. Presence of a capsule in *Erysipelothrix rhusiopathiae* and its relationship to virulence for mice. *Infect Immun* 1994;**62**:2806–10.
36. Simionescu R, Grover S, Shekar R, West BC. Necrotizing fasciitis caused by *Erysipelothrix rhusiopathiae*. *South Med J* 2003;**96**:937–9.
37. Talan DA, Citron DM, Abrahamian FM, et al. Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group. *N Engl J Med* 1999;**340**:85–92.
38. Traer EA, Williams MR, Keenan JN. *Erysipelothrix rhusiopathiae* infection of a total knee arthroplasty: an occupational hazard. *J Arthroplasty* 2008;**23**:609–11.
39. Troelsen A, Moller JK, Bolvig L, et al. Animal-associated bacteria, *Erysipelothrix rhusiopathiae*, as the cause of infection in a total hip arthroplasty. *J Arthroplasty* 2010;**25**:497.e21–3.
40. Watarai M, Sawada T, Nakagomi M, et al. Comparison of etiological and immunological characteristics of two attenuated *Erysipelothrix rhusiopathiae* strains of serotypes 1a and 2. *J Vet Med Sci* 1993;**55**:595–600.
41. Wong RC, Kong KO, Lin RV, Barkham T. Chronic monoarthritis of the knee in systemic lupus erythematosus. *Lupus* 2003;**12**: 324–6.
42. Woodbine M. *Erysipelothrix rhusiopathiae*: bacteriology and chemotherapy. *Bacteriol Rev* 1947;**14**:161–78.

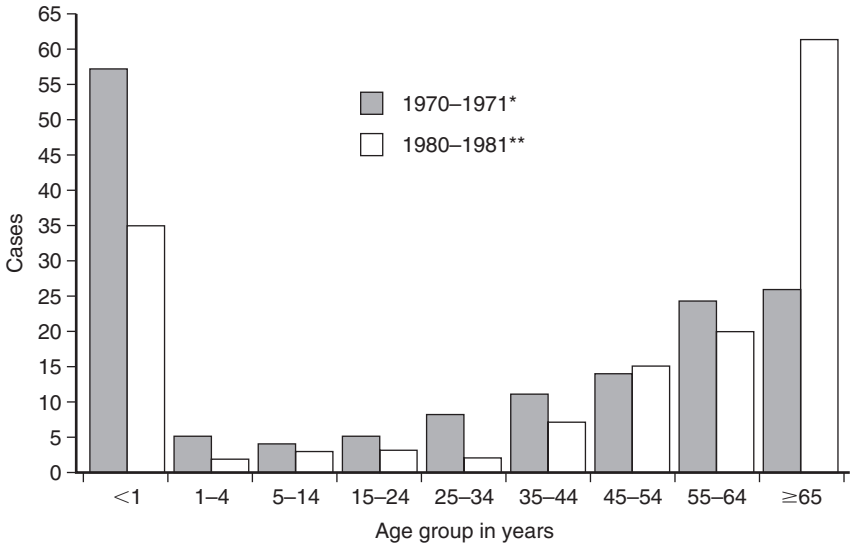

* Age unknown for 63 cases
** Age unknown for 3 cases

FIGURE 95-1 ■ Human listeriosis cases by age group for the United States, 1970-1971 and 1980-1981. (From Albritton WL, Cochi SL, Feeley JC. Overview of neonatal listeriosis. Clin Invest Med 1984;**7**:311-14.)

TRANSMISSION

The ability of *L. monocytogenes* to tolerate high salt concentrations and an alkaline environment allows it to survive for months in soil, water, animal feed, and refrigerated foods.[23] Contaminated food is a common source of severe human *Listeria* infection.[17,51,71] In spite of earlier successes in controlling foodborne *L. monocytogenes* infection, the incidence remains high.[17] *L. monocytogenes* still is the most common cause of foodborne illness related hospitalization and death in the United States.[17] Yet most infections are sporadic and have no epidemiologic explanation.[130] Furthermore, asymptomatic fecal carriage in healthy individuals is common with a prevalence reported at 2 to 10 percent[108] or higher.[61,73] Dissemination of *L. monocytogenes* from colonized humans may play a role in transmission.[30] Although maternal colonization does not invariably lead to invasive illness, pregnancy-associated infection represents approximately 17 percent of all invasive cases.[58] About 20 percent of pregnancy-associated infection results in fetal loss or neonatal death.[58] Transmission of *L. monocytogenes* from mother to fetus can occur in utero by inhalation or ingestion of infected amniotic fluid or transplacentally from the maternal circulation.[73] However, the factors contributing to most cases of late-onset infection of the newborn (up to 2 months of age) are unknown.[10,63,100] Nosocomial transmission has also been described.[35,49,74,112]

EPIDEMIOLOGY

Despite its ubiquitous presence, and regular outbreaks, *L. monocytogenes* is a relatively uncommon cause of infection in the general population (ranging from 0.1 to 11.3/1 million population).[12,25,40,104,121] Listeriosis has a nonuniform age distribution, with most cases occurring in newborns or elderly individuals (Fig. 95-1). Furthermore, symptomatic listeriosis has a disproportionately high mortality rate in the unborn, the newborn, and the elderly, ranging from 20 to over 60 percent.[104] Invasive *L. monocytogenes* infection is 20- to 100-fold more common in pregnancy than in the general population.[47,58,73] Perinatal attack rates during outbreaks are extremely high and can reach 1 to 2 percent of all deliveries.[10,100] Although preventative measures, monitoring, and voluntary recall of contaminated food products have resulted in reduced perinatal listeriosis in recent decades,[73] risk for foodborne infection remains high and incidence in older adults has, in fact, increased.[10,86,104]

PATHOGENESIS AND PATHOLOGY

Infection with *L. monocytogenes* begins with the ingestion of the bacteria. In adults, the infectious dose is estimated to be 10^4 to 10^6 organisms per gram of ingested food but may be as low as 10 organisms in immunocompromised hosts and patients who have diminished gastric acidity or injured intestinal lining.[10,107,108,110] *L. monocytogenes* infects intestinal M cells through phagocytosis and intestinal epithelial cells through actions of listerial surface proteins called internalin A (InlA).[39,78] InlA binds to host E-cadherin that is transiently exposed to the luminal surface of the intestinal villi during remodeling of epithelial junctions.[97] InlA is possibly also involved in the crossing of the placental barrier because E-cadherin is expressed on syncytiotrophoblasts.[8,79] After infection of the intestinal epithelium, *L. monocytogenes* translocates and reaches the liver, spleen, mesenteric lymph nodes, and other organs via the blood and lymph.[131] Binding of listerial internalin B (InlB) to the host hepatocyte growth factor receptor (also known as the tyrosine kinase receptor Met) and complement component C1q[6] permits efficient hepatocyte entry via endocytosis.* InlB may also

*References 13, 14, 29, 50, 66, 95.

have a role facilitating the transfer of *L. monocytogenes* across the blood-brain barrier.[6,50]

After cell entry, *L. monocytogenes* eventually escapes from endocytic vacuole and replicates in the cytosol of the host cell; from there it spreads to neighboring cells, avoiding destruction by antibodies or neutrophils.[72] This vacuolar escape is assisted by two virulence-associated bacterial molecules: listeriolysin O (LLO) and phospholipase C.[23] LLO is a pore-forming cholesterol-dependent cytolysin and one of the major virulence determinants of *L. monocytogenes*.[5,64,111,132] Although LLO is absolutely required for vacuolar escape in murine cells, it appears dispensable in human cells, where phospholipases are more centrally involved instead.[99] Once in the cytoplasm, *L. monocytogenes* induces polymerization of host actin filaments; this mobility allows it to avoid intracellular degradation (autophagy) and to spread from cell to cell.[88,124-126] Although ActA is one of the major virulence determinants of *L. monocytogenes*,[27] strains lacking ActA retain the ability to infect the placenta, although at a lower rate than wild-type strains.[77]

From the moment *L. monocytogenes* adheres to a target host cell, a response is set into motion that aims to restrict bacterial invasion and survival.[23,129] This has been best studied in the mouse macrophage, where *L. monocytogenes* activates at least three pathways[137]: (1) a MyD88-dependent pathway emanating from the cell surface and phagosome leading to expression of cytokines and reactive oxygen species; (2) a STING/IRF3-dependent pathway emanating from the cytosol leading to the expression of interferon-γ (IFN-γ) and co-regulated genes; and (3) an AIM2/caspase-1–dependent inflammasome pathway resulting in proteolytic activation and secretion of interleukin (IL)-1 and IL-18 and pyroptotic cell death. *L. monocytogenes*, on the other hand, has evolved elaborate mechanisms trying to counterbalance these host defense strategies.[26] The outcome of this molecular tug of war depends on the bacterium, the host cell, and the host. Cytokines and chemokines released by the infected cells recruit neutrophils, which in turn destroy extracellular bacteria and digest dying cells.[68] Infected murine macrophages for example produce IL-12p70, which induces the synthesis of IFN-γ by natural killer cells and CD4 T helper-1 cells; IFN-γ in turn activates infected macrophages to become listericidal through the production of free oxygen and nitrogen radicals.[94,132] This process also leads to formation of granulomas at sites of infection, thereby "walling off the infection."[116] Although multiple granulomas are characteristic of disseminated disease, the basic pathologic change of suppurative inflammation is more common.[67] These innate immune effector cascades limit the spread of *L. monocytogenes* during the early phase of infection. In the immune-competent host, adaptive T-cell immune responses peak about 1 week after primary infection, leading to complete clearance of infected cells; this process also leads to acquired resistance to subsequent infection (immune memory).[95] Acquired immunity is not dependent on clinical symptomatic infection, because T cells specific for *L. monocytogenes* are frequently present in healthy individuals without a history of listeriosis.*

*References 24, 43, 70, 89, 100, 101.

There are many differences in both innate and adaptive immunity between the pregnant, the neonate, the young adult, and the older adult[22,69,80]; and the host response of the human to *Listeria* has not been studied in sufficient detail to know what specific aspect of immune-mediated protection fails to protect those most susceptible from severe listeriosis. In newborn animals, susceptibility to *L. monocytogenes* is associated with delayed production of innate cytokines, resulting in slower activation of T cells and natural killer cells.[10] In older children and young adults, *L. monocytogenes* infection is rare; however, underlying diseases or medications that interfere with cell-mediated immunity are known to increase susceptibility.* On the other hand, neutropenia and disorders of the humoral immune system (complement or immunoglobulin) are not associated with excessive prevalence of listeriosis.[73] This suggests a central role of at least the innate and adaptive cell–mediated immune system in optimal defense against *L. monocytogenes* in the human host.

CLINICAL MANIFESTATIONS

Most infections with *L. monocytogenes* in the healthy host remain entirely asymptomatic.[10] Clinical symptomatic infection with *L. monocytogenes* is called "listeriosis"[107] and exists in two forms depending on the immunologic status of the host—noninvasive gastrointestinal listeriosis and invasive listeriosis.[10,104,132] In immunocompetent children and adults, noninvasive listeriosis develops as a febrile gastroenteritis.[107,110] However, in vulnerable groups, namely, the pregnant female and unborn fetus, neonates and infants up to approximately 2 months of age,[136] the elderly (>60 years of age), and some immune-compromised children and adults,[10,1104] *L. monocytogenes* can spread from the gastrointestinal tract into the systemic circulation and from there to other parts of the body.[10] The main target organs are the placenta, liver, and spleen, as well as the central nervous system.[10,30,100,109] This clinical pattern of invasive listeriosis derives from the unique capacity of *L. monocytogenes* to cross the intestinal, blood-brain, and placental barriers.[78]

In pediatric listeriosis, most severe infections occur in the first 2 months of life. The initial clinical features can be divided into early and late-onset diseases, similar to those of group B streptococcal infection (Table 95-1). In 50 to 74 percent of early-onset cases the mother reports a history of an influenza-like illness, with 65 percent reporting fever, 21 percent backache (which may be mistaken for a urinary tract infection), 10 percent headache, 7 percent vomiting and diarrhea, and 4 percent muscle pains or sore throat; however, approximately one third of infected pregnant women may be entirely asymptomatic.[73] Spontaneous abortion occurs in 10 to 20 percent of clinically symptomatic listeriosis cases during pregnancy, with another approximately 50 percent delivering preterm and 11 percent ending in intrauterine fetal demise. Thirty-four percent of infected pregnancies demonstrate fetal distress, and 75 percent display meconium-stained amniotic fluid; microabscesses are frequently histologically evident in infected placentas.[73]

*References 10, 20, 28, 57, 100, 117, 129, 137.

TABLE 95-1 **Clinical and Laboratory Findings of Early-Onset and Late-Onset Neonatal Listeriosis**

Feature	Early Onset[1,2,34,60,73]	Late Onset[2,36,65,73,114]
Median age in days (range)	1 (birth to 6)	14 (7-35)
Male (%)	60	67
Preterm (%)	65	20
Mortality (%)	38 (22-63)	3 (0-10)
Respiratory involvement (%)	38-50	10
Meningitis (%)	24	67-93
Blood isolate (%)	81-88	17-95
Maternal perinatal illness (%)	50-74	0

Early-onset neonatal listeriosis has a high mortality rate (20 to 60 percent); even in those who survive, a significant number (12.7%) develop long-term neurologic sequelae.[10,73] At birth, respiratory distress, apnea, lethargy, and fever are common manifestations; diarrhea, conjunctivitis, and myocarditis also have been described.[10] The respiratory symptoms may mimic symptoms of respiratory distress syndrome. Patchy bronchopneumonic infiltrates, probably caused by aspiration of infected amniotic fluid, may be seen on chest radiographs.[10] Occasionally, discrete roseolar or pustular lesions on the skin and pharynx occur that on histology appear as microabscesses and macroabscesses and contain culturable *L. monocytogenes;* this rash has been termed *granulomatosis infantisepticum* (Fig. 95-2).

The late-onset form of neonatal listeriosis is less common than the early-onset form and usually affects term infants, who appear healthy until the onset of meningitis or, less commonly, septicemia and colitis 1 to 8 weeks after birth.[36,56,65,74] Clinical manifestations of late-onset meningitis are often subtle and include fever, irritability, lethargy, and poor feeding.[65,113] Cerebrospinal fluid findings vary; although pleocytosis usually is significant, not all infections have a polymorphonuclear cell predominance. The maternal history in these cases usually is negative, suggesting postnatal, possibly even nosocomial, exposure in affected infants.[112]

Although the incidence of listeriosis in immunocompromised children is higher, the clinical features are similar to those of nonimmunosuppressed patients with *L. monocytogenes* infection and include most commonly meningitis or septicemia, but rhombencephalitis, brain abscess, arthritis, osteomyelitis, endocarditis, endophthalmitis, liver abscess, and peritonitis may occur as well.[82,112,113,117]

FIGURE 95-2 ■ Typical pustular rash on the abdomen of a stillborn infant with listeriosis. Note the small pale granuloma measuring 1 to 3 mm and the dark erythema surrounding these lesions.

DIAGNOSIS

Appropriate specimens for staining and culture vary with the clinical syndrome, but the organism can be readily cultured from blood, cerebrospinal fluid, amniotic fluid, and genital secretions. Examination of Gram-stained material (ear, meconium, and placenta) from neonates is recommended in suspected, early-onset sepsis.[77] Pathologic specimens (e.g., biopsy material, placental or fetal tissue) may reveal the characteristic Gram-stain morphology and pathologic features, such as microabscesses and granulomas. Selective culture media, such as PALCAM (polymyxin B–acriflavine–lithium chloride–ceftazidime–esculin–mannitol agar) or modified Oxford agar, may be helpful for isolation from nonsterile sites such as stool or vaginal secretions.[83,98] Laboratories undertaking primary isolation must be aware of the similarities between *L. monocytogenes* and frequently discarded "commensals."[84]

After 48 hours of incubation at 37°C on 5 percent sheep blood agar, typical colonies are gray with a narrow zone of β-hemolysis. The appearance of short *Listeria* rods on Gram stain has been confused with lancet-shaped pneumococci. Traditionally, identification is based on morphology; tumbling motility; β-hemolysis; positive catalase, esculin, and CAMP tests; and carbohydrate use pattern. Commercial kits are available for the rapid identification of *Listeria* from culture. These kits include DNA probes, latex agglutination, and enzyme immunoassay methods. Despite extensive attempts to develop serologic techniques for the diagnosis of listeriosis,

none has proved satisfactory and few centers attempt serodiagnosis.

Histologic diagnosis from pathologic material should be attempted if the organism is not cultured. Specific fluorescent antibody staining,[18] nucleic acid hybridization,[120] and polymerase chain reaction[4,59] have all been used for detection of *Listeria*. The application of molecular detection to food safety programs has been growing rapidly, with clinical applications of molecular *Listeria* detection lagging somewhat behind with regard to the commercial options available. Still, several centers have reported the development of sensitive molecular assays for detection of *L. monocytogenes* from clinical samples, in particular cerebrospinal fluid. For cases of suspected meningitis with prior antibiotic treatment, broad-range 16S polymerase chain reaction is becoming widely used in North America and can be key in establishing the diagnosis.[11,76]

TREATMENT

Intravenous ampicillin and an aminoglycoside, typically gentamicin, remain the treatment of choice for invasive *L. monocytogenes* infections. Ampicillin may be used alone in immunocompetent patients with less severe infections or once clinical response has occurred. Antibiotic resistance in clinical *Listeria* isolates remains low, although surveillance of food and dairy products has shown an increase in resistance to multiple antibiotics including ampicillin.[52,118,135] Although human data for dual treatment are lacking, ampicillin plus gentamicin has demonstrable synergy on most *Listeria* strains both in vitro and in animal models[31,87,127] *L. monocytogenes* shows tolerance to some antibiotics in vitro.[32,85] Antibiotics commonly recommended for treatment—including ampicillin—are bacteriostatic at concentrations usually achieved in blood.[32,44,45,128,133]

For penicillin-allergic patients, trimethoprim-sulfamethoxazole has been used successfully.[51,119] This is supported by in vivo data with and without the addition of rifampin.[37,53,54,87,106] In an in vivo model of *L. monocytogenes* encephalitis, the combinations ampicillin/gentamicin and trimethoprim-sulfamethoxazole/rifampin were highly active against intracerebral bacteria.[7] Trimethoprim-sulfamethoxazole cannot be recommended for use in perinatal infections because of the concern of bilirubin toxicity developing with sulfonamides.[37]

For patients with both penicillin and sulfa allergies, vancomycin has been used successfully, albeit with much less accumulated clinical experience than ampicillin-based regimens.[102] One group has reported a bacteremia with vancomycin-resistant *L. grayi*, the first documented invasive infection with this species.[105]

More recently, cases of invasive *Listeria* infections treated successfully with linezolid have been published.[87,90] This oxazolidinone antimicrobial exhibits bactericidal activity against *Listeria*.

Notably, *L. monocytogenes* is intrinsically resistant to cephalosporin antibiotics.[32] Similarly, resistance to clindamycin, tetracyclines, quinolones, and macrolides is common and clinical experience with these agents is very limited. Antagonism also seems to occur between certain antibiotic combinations (erythromycin and penicillins, erythromycin and aminoglycosides, penicillin and chloramphenicol, and penicillin and tetracycline).[32,44]

Duration of therapy depends on the clinical syndrome, the presence of underlying disease, and the response to treatment. For most invasive infections without meningitis, 10 to 14 days of therapy is usually sufficient. When meningitis is present, many experts recommend 2 to 3 weeks of therapy. Longer courses may be necessary for patients with endocarditis, rhombencephalitis, and severe, complicated infections. When treating central nervous system infections, diagnostic imaging of the brain near the end of anticipated therapy may be used to evaluate the possibility of parenchymal involvement and determine the need for an extended duration of therapy.

Listeria gastroenteritis, in otherwise healthy individuals, is generally a self-limited illness and no data exist with regard to the efficacy of antimicrobial therapy. In high-risk individuals such as pregnant women and those with immunocompromise, who have either symptomatic illness or who are known to have ingested a contaminated food, it may be prudent to administer treatment or prophylaxis with a course of oral amoxicillin or trimethoprim-sulfamethoxazole.[93]

PROGNOSIS

Maternal listeriosis may result in abortion, stillbirth, or early neonatal death.[73] Although the relative risk for fetal death is difficult to estimate, spontaneous abortion rates of 10 to 20 percent and stillborn rates of 11 percent have been noted.[73] Fetal distress and/or meconium staining of amnionic fluid is estimated to occur in 75 percent of perinatal cases.[73] Convincing evidence that *L. monocytogenes* is associated with repeated abortions is lacking.[48]

Among cases of early-onset sepsis, the mortality rate in North America is approximately 40 percent (see Table 95-1).[1,34] Most survivors appear normal at short-term follow up.[33,34,123] Sequelae are related to the associated complications of prematurity, pneumonia, and sepsis; hydrocephalus and cerebral palsy also have been reported.[33,62] Early treatment of maternal disease affects fetal and neonatal outcome favorably.[34,62]

Late-onset *Listeria* meningitis has a mortality rate of less than 10 percent. The outcome after *Listeria* meningitis may be more favorable than the outcome associated with other types of bacterial meningitis.[65,134] Major sequelae are hydrocephalus and mental retardation. Beyond the neonatal period, the outcome of listeriosis depends on the nature of any underlying disease and the availability of intensive medical care. Among adults with no underlying malignancy, the long-term outcome of *Listeria* meningitis is excellent.[103]

PREVENTION

The sporadic nature of the disease in North America emphasized the need for collaborative investigation and reporting of infections by physicians and veterinarians to

public health authorities.[9,41] In the early 1990s, listeriosis became a notifiable disease in the United States and Canada.[98,122] Since then, the important role of food in the transmission of sporadic cases has been firmly established.[113,122] Inspection of food production facilities and dissemination of educational materials may have contributed to a general reduction of outbreaks of listeriosis since then as well.[4,38,46,122] Strict adherence to regulations for pasteurization of raw milk is important to inactivate the organism and prevent listeriosis.[16] Contamination of food can occur during the preparation and processing of pasteurized milk products and ready-to-eat meat or poultry products. The source of an outbreak is sometimes found by tracking strains of *L. monocytogenes* isolated from a patient's food to the retail sources. Recommendations for individuals at high risk, such as pregnant women and immunocompromised patients, include avoiding soft cheeses and delicatessen meats and avoiding reheating leftover foods or ready-to-eat foods (e.g., hot dogs).[98] In spite of this, many hospitals have failed to implement appropriate food preparation policies.[19] During an outbreak of *Listeria*, prompt investigation and treatment of pregnant women with a febrile "influenza-like" illness have been advocated.[9,62] Although treatment of symptomatic mothers may prevent perinatal listeriosis, there is no evidence to show that treatment of colonized infants can either eradicate carriage or prevent infection. Careful attention given to the handling of infected infants in a neonatal unit in an attempt to prevent transmission is important for preventing nosocomial infection.[35,112]

NEW REFERENCES SINCE THE SIXTH EDITION

3. Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect* 2010;**16**(1):16–23.
5. Beauregard KE, Lee KD, Collier RJ, et al. pH-dependent perforation of macrophage phagosomes by listeriolysin O from Listeria monocytogenes. *J Exp Med* 1997;**186**:1159–63.
6. Bierne H, Cossart P. InlB, a surface protein of Listeria monocytogenes that behaves as an invasin and a growth factor. *J Cell Sci* 2002;**115**:3357–67.
8. Bonazzi M, Veiga E, Pizarro-Cerdá J, Cossart P. Successive post-translational modifications of E-cadherin are required for InlA-mediated internalization of *Listeria monocytogenes*. *Cell Microbiol* 2008;**10**(11):2208–22.
10. Bortolussi R. Listeriosis: a primer. *CMAJ* 2008;**179**(8):795–7.
11. Bøving MK, Pedersen LN, Møller JK. Eight-plex PCR and liquid-array detection of bacterial and viral pathogens in cerebrospinal fluid from patients with suspected meningitis. *J Clin Microbiol* 2009;**47**(4):908–13.
13. Braun L, Ghebrehiwet B, Cossart P. gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of Listeria monocytogenes. *EMBO J* 2000;**19**:1458–66.
14. Braun L, Ohayon H, Cossart P. The InIB protein of Listeria monocytogenes is sufficient to promote entry into mammalian cells. *Mol Microbiol* 1998;**27**:1077–87.
17. Centers for Disease Control and Prevention. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 1996-2010. *MMWR Morb Mortal Wkly Rep* 2011; **60**(22):749–55.
19. Cokes C, France AM, Reddy V, et al. Serving high-risk foods in a high-risk setting: survey of hospital food service practices after an outbreak of listeriosis in a hospital. *Infect Control Hosp Epidemiol* 2011;**32**(4):380–6.
20. Condotta SA, Richer MJ, Badovinac VP, Harty JT. Probing CD8 T cell responses with *Listeria monocytogenes* infection. *Adv Immunol* 2012;**113**:51–80.
21. Conly J, Johnston B. *Listeria:* a persistent food-borne pathogen. *Can J Infect Dis Med Microbiol* 2008;**19**(5):327–8.
22. Corbett NP, Blimkie D, Ho KC, et al. Ontogeny of Toll-like receptor mediated cytokine responses of human blood mononuclear cells. *PLoS One* 2010;**5**(11):e15041.
23. Cosgrove S. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August-September 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**(39):1357–8.
24. Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium *Listeria monocytogenes*. *Proc Natl Acad Sci U S A* 2011;**108**(49):19484–91.
25. Davies JW, Ewan EP, Varughese P, et al. Listeria monocytogenes infections in Canada. *Clinical and Investigative Medicine. Medecine clinique et experimentale* 1984;**7**:315–20.
26. Diacovich L, Gorvel JP. Bacterial manipulation of innate immunity to promote infection. *Nat Rev Microbiol* 2010;**8**:117–28.
27. Domann E, Wehland J, Rohde M, et al. A novel bacterial virulence gene in Listeria monocytogenes required for host cell microfilament interaction with homology to the proline-rich region of vinculin. *Embo J* 1992;**11**:1981–90.
29. Dramsi S, Biswas I, Maguin E, et al. Entry of Listeria monocytogenes into hepatocytes requires expression of inIB, a surface protein of the internalin multigene family. *Mol Microbiol* 1995; **16**:251–61.
30. Dussurget O. New insights into determinants of Listeria monocytogenes virulence. *Int Rev Cell Mol Biol* 2008;**270**:1–38.
38. Furyk JS, Swann O, Molyneux E. Systematic review: neonatal meningitis in the developing world. *Trop Med Int Health* 2011; **16**(6):672–9.
39. Gaillard JL, Berche P, Frehel C, et al. Entry of L. monocytogenes into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* 1991;**65**:1127–41.
40. Gaulin C, Ramsay D, Bekal S. Widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008. *J Food Prot* 2012;**75**:71–8.
42. Gianfranceschi MV, D'Ottavio MC, Gattuso A, et al. Distribution of serotypes and pulsotypes of *Listeria monocytogenes* from human, food and environmental isolates (Italy 2002-2005). *Food Microbiol* 2009;**26**:520–6.
43. Goossens PL, Milon G, Cossart P, et al. Attenuated Listeria monocytogenes as a live vector for induction of CD8+ T cells in vivo: a study with the nucleoprotein of the lymphocytic choriomeningitis virus. *Int Immunol* 1995;**7**:797–805.
47. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis* 2012;**54**:652–60.
50. Greiffenberg L, Goebel W, Kim KS, et al. Interaction of Listeria monocytogenes with human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. *Infect Immun* 1998; **66**:5260–7.
58. Jackson KA, Iwamoto M, Swerdlow D. Pregnancy-associated listeriosis. *Epidemiol Infect* 2010;**138**:1503–9.
60. Jiao Y, Zhang W, Ma J, et al. Early onset of neonatal listeriosis. *Pediatr Int* 2011;**53**:1034–7.
61. Kampelmacher EH, Huysinga WT, van Noorle Jansen LM. The presence of Listeria monocytogenes in feces of pregnant women and neonates. *Zentralbl Bakteriol Orig A* 1972;**222**:258–62.
63. Kaur S, Malik SV, Vaidya VM, et al. Listeria monocytogenes in spontaneous abortions in humans and its detection by multiplex PCR. *J Appl Microbiol* 2007;**103**:1889–96.
64. Kayal S, Charbit A. Listeriolysin O: a key protein of Listeria monocytogenes with multiple functions. *FEMS Microbiol Rev* 2006;**30**:514–29.
66. Khelef N, Lecuit M, Bierne H, et al. Species specificity of the Listeria monocytogenes InlB protein. *Cell Microbiol* 2006;**8**:457–70.
68. Kobayashi SD, Braughton KR, Whitney AR, et al. Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. *Proc Natl Acad Sci U S A* 2003;**100**:10948–53.
69. Kollmann TR, Crabtree J, Rein-Weston A, et al. Neonatal innate TLR-mediated responses are distinct from those of adults. *J Immunol* 2009;**183**:7150–60.
70. Kollmann TR, Reikie B, Blimkie D, et al. Induction of protective immunity to Listeria monocytogenes in neonates. *J Immunol* 2007;**178**:3695–701.

71. Kovacevic J, Mesak LR, Allen KJ. Occurrence and characterization of *Listeria* spp. in ready-to-eat retail foods from Vancouver, British Columbia. *Food Microbiol* 2012;**30**:372–8.
72. Lam GY, Czuczman MA, Higgins DE, et al. Interactions of *Listeria monocytogenes* with the autophagy system of host cells. *Adv Immunol* 2012;**113**:7–18.
73. Lamont RF, Sobel J, Mazaki-Tovi S, et al. Listeriosis in human pregnancy: a systematic review. *J Perinat Med* 2011;**39**:227–36.
75. Lavi O, Louzoun Y, Klement E. Listeriosis: a model for the fine balance between immunity and morbidity. *Epidemiology* 2008;**19**: 581–7.
76. Le Monnier A, Abachin E, Beretti JL, et al. Diagnosis of *Listeria monocytogenes* meningoencephalitis by real-time PCR for the *hly* gene. *J Clin Microbiol* 2011;**49**:3917–23.
79. Lecuit M, Nelson DM, Smith SD, et al. Targeting and crossing of the human maternofetal barrier by Listeria monocytogenes: role of internalin interaction with trophoblast E-cadherin. *Proc Natl Acad Sci U S A* 2004;**101**:6152–7.
80. Leng J, Goldstein DR. Impact of aging on viral infections. *Microbes Infect* 2010;**12**:1120–4.
86. Mook P, O'Brien SJ, Gillespie IA. Concurrent conditions and human listeriosis, England, 1999-2009. *Emerg Infect Dis* 2011;**17**: 38–43.
87. Morosi S, Francisci D, Baldelli F. A case of rhombencephalitis caused by Listeria monocytogenes successfully treated with linezolid. *J Infect* 2006;**52**:e73–5.
89. Munk ME, Kaufmann SH. Listeria monocytogenes reactive T lymphocytes in healthy individuals. *Microb Pathog* 1988;**5**:49–54.
90. Munoz P, Ferreira L, Marin M, et al. First report of Listeria monocytogenes endocarditis treated with linezolid. *Int J Antimicrob Agents* 2006;**28**:480–1.
94. Pamer EG. Immune responses to Listeria monocytogenes. *Nat Rev Immunol* 2004;**4**:812–23.
95. Parida SK, Domann E, Rohde M, et al. Internalin B is essential for adhesion and mediates the invasion of Listeria monocytogenes into human endothelial cells. *Mol Microbiol* 1998;**28**:81–93.
97. Pentecost M, Otto G, Theriot JA, et al. Listeria monocytogenes invades the epithelial junctions at sites of cell extrusion. *PLoS Pathog* 2006;**2**:e3.
100. Posfay-Barbe KM, Wald ER. Listeriosis. *Semin Fetal Neonatal Med* 2009;**14**:228–33.
101. Reikie BA, Smolen KK, Fortuno 3rd ES, et al. A single immunization near birth elicits immediate and lifelong protective immunity. *Vaccine* 2010;**29**:83–90.
103. Roed C, Engsig FN, Omland LH, et al. Long-term mortality in patients diagnosed with *Listeria monocytogenes* meningitis: a Danish nationwide cohort study. *J Infect* 2012;**64**:34–40.
104. Ruzante JM, Majowicz SE, Fazil A, et al. Hospitalization and deaths for select enteric illnesses and associated sequelae in Canada, 2001-2004. *Epidemiol Infect* 2011;**139**:937–45.
105. Salimnia H, Patel D, Lephart PR, et al. *Listeria grayi:* vancomycin-resistant, gram-positive rod causing bacteremia in a stem cell transplant recipient. *Transpl Infect Dis* 2010;**12**:526–8.
107. Schlech 3rd WF. Listeria gastroenteritis—old syndrome, new pathogen. *N Engl J Med* 1997;**336**:130–2.
110. Schlech 3rd WF, Schlech WF, Haldane H, et al. Does sporadic *Listeria* gastroenteritis exist? A 2-year population-based survey in Nova Scotia, Canada. *Clin Infect Dis* 2005;**41**:778–84.
111. Schnupf P, Portnoy DA. Listeriolysin O: a phagosome-specific lysin. *Microbes Infect* 2007;**9**:1176–87.
116. Serbina NV, Shi C, Pamer EG. Monocyte-mediated immune defense against murine *Listeria monocytogenes* infection. *Adv Immunol* 2012;**113**:119–34.
118. Srinivasan V, Nam HM, Nguyen LT, et al. Prevalence of antimicrobial resistance genes in Listeria monocytogenes isolated from dairy farms. *Foodborne Pathog Dis* 2005;**2**:201–11.
121. Taillefer C, Boucher M, Laferriere C, et al. Perinatal listeriosis: Canada's 2008 outbreaks. *J Obstet Gynaecol Can* 2010;**32**:45–8.
124. Theriot JA, Mitchison TJ, Tilney LG, et al. The rate of actin-based motility of intracellular Listeria monocytogenes equals the rate of actin polymerization. *Nature* 1992;**357**:257–60.
125. Tilney LG, Connelly PS, Portnoy DA. Actin filament nucleation by the bacterial pathogen, Listeria monocytogenes. *J Cell Biol* 1990;**111**:2979–88.
126. Tilney LG, Portnoy DA. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, Listeria monocytogenes. *J Cell Biol* 1989;**109**:1597–608.
129. Unanue ER, Carrero JA. Studies with *Listeria monocytogenes* lead the way. *Adv Immunol* 2012;**113**:1–5.
131. Vazquez-Boland JA, Dominguez-Bernal G, Gonzalez-Zorn B, et al. Pathogenicity islands and virulence evolution in Listeria. *Microbes Infect* 2001;**3**:571–84.
132. Vazquez-Boland JA, Kuhn M, Berche P, et al. Listeria pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001;**14**:584–640.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 96

TUBERCULOSIS

Andrea T. Cruz • Jeffrey R. Starke

Tuberculosis ranks as one of the three most important infectious diseases in the world in terms of morbidity and mortality. The disease is recognizable in skeletons from the Stone Age and in mummified corpses from the Egyptian Old Kingdom. For centuries, it was believed that the touch of royalty could cure scrofula. Edward the Confessor (mid-11th century) was one of the first to wash the diseased flesh of his subjects.[228] Although this tradition lapsed with King William III in the late 17th century, it may have contributed to the consumptive deaths of

REFERENCES

1. Ahlfors CE, Goetzman BW, Halsted CC, et al. Neonatal listeriosis. *Am J Dis Child* 1977;**131**:405–8.
2. Albritton WL, Wiggins GL, Feeley JC. Neonatal listeriosis: distribution of serotypes in relation to age at onset of disease. *J Pediatr* 1976;**88**:481–3.
3. Allerberger F, Wagner M. Listeriosis: a resurgent foodborne infection. *Clin Microbiol Infect* 2010;**16**:16–23.
4. Allmann M, Hofelein C, Koppel E, et al. Polymerase chain reaction (PCR) for detection of pathogenic microorganisms in bacteriological monitoring of dairy products. *Res Microbiol* 1995;**146**: 85–97.
5. Beauregard KE, Lee KD, Collier RJ, et al. pH-dependent perforation of macrophage phagosomes by listeriolysin O from *Listeria monocytogenes*. *J Exp Med* 1997;**186**:1159–63.
6. Bierne H, Cossart P. InlB, a surface protein of *Listeria monocytogenes* that behaves as an invasin and a growth factor. *J Cell Sci* 2002;**115**:3357–67.
7. Blanot S, Boumaila C, Berche P. Intracerebral activity of antibiotics against *Listeria monocytogenes* during experimental rhombencephalitis. *J Antimicrob Chemother* 1999;**44**:565–8.
8. Bonazzi M, Veiga E, Pizarro-Cerdá J, Cossart P. Successive post-translational modifications of E-cadherin are required for InlA-mediated internalization of *Listeria monocytogenes*. *Cell Microbiol* 2008;**10**(11):2208–22.
9. Bortolussi R. An ongoing problem: perinatal infection due to *Listeria monocytogenes*, an old pathogen reborn. *Clin Invest Med* 1984;**7**:213–15.
10. Bortolussi R. Listeriosis: a primer. *CMAJ* 2008;**179**:795–7.
11. Bøving MK, Pedersen LN, Møller JK. Eight-plex PCR and liquid-array detection of bacterial and viral pathogens in cerebrospinal fluid from patients with suspected meningitis. *J Clin Microbiol* 2009;**47**(4):908–13.
12. Bowmer EJ, McKiel JA, Cockcroft WH, et al. *Listeria monocytogenes* infections in Canada. *CMAJ* 1973;**109**:125–9 passim.
13. Braun L, Ghebrehiwet B, Cossart P. gC1q-R/p32, a C1q-binding protein, is a receptor for the InlB invasion protein of *Listeria monocytogenes*. *EMBO J* 2000;**19**:1458–66.
14. Braun L, Ohayon H, Cossart P. The InIB protein of *Listeria monocytogenes* is sufficient to promote entry into mammalian cells. *Mol Microbiol* 1998;**27**:1077–87.
15. Burn CG. Clinical and pathological features of an infection reused by new pathogen of the genus. *Listere Am J Pathol* 1936;**12**:341.
16. Centers for Disease Control and Prevention. Update—listeriosis and pasteurized milk. *MMWR Morb Mortal Wkly Rep* 1988;**37**: 764–6.
17. Centers for Disease Control and Prevention. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food—Foodborne Diseases Active Surveillance Network, 10 U.S. sites, 1996-2010. *MMWR Morb Mortal Wkly Rep* 2011; **60**(22):749–55.
18. Cherry WB, Moody MD. Fluorescent-antibody techniques in diagnostic bacteriology. *Bacteriol Rev* 1965;**29**:222–50.
19. Cokes C, France AM, Reddy V, et al. Serving high-risk foods in a high-risk setting: survey of hospital food service practices after an outbreak of listeriosis in a hospital. *Infect Control Hosp Epidemiol* 2011;**32**(4):380–6.
20. Condotta SA, Richer MJ, Badovinac VP, Harty JT. Probing CD8 T cell responses with *Listeria monocytogenes* infection. *Adv Immunol* 2012;**113**:51–80.
21. Conly J, Johnston B. *Listeria:* a persistent food-borne pathogen. *Can J Infect Dis Med Microbiol* 2008;**19**(5):327–8.
22. Corbett NP, Blimkie D, Ho KC, et al. Ontogeny of Toll-like receptor mediated cytokine responses of human blood mononuclear cells. *PLoS One* 2010;**5**(11):e15041.
23. Cosgrove S. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe—United States, August-September 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**(39):1357–8.
24. Cossart P. Illuminating the landscape of host-pathogen interactions with the bacterium *Listeria monocytogenes*. *Proc Natl Acad Sci U S A* 2011;**108**(49):19484–91.
25. Davies JW, Ewan EP, Varughese P, et al. *Listeria monocytogenes* infections in Canada. *Clin Invest Med* 1984;**7**:315–20.
26. Diacovich L, Gorvel JP. Bacterial manipulation of innate immunity to promote infection. *Nat Rev Microbiol* 2010;**8**:117–28.
27. Domann E, Wehland J, Rohde M, et al. A novel bacterial virulence gene in *Listeria monocytogenes* required for host cell microfilament interaction with homology to the proline-rich region of vinculin. *Embo J* 1992;**11**:1981–90.
28. Doorduyn Y, de Jager CM, van der Zwaluw WK, et al. Invasive *Listeria monocytogenes* infections in the Netherlands, 1995-2003. *Eur J Clin Microbiol Infect Dis* 2006;**25**:433–42.
29. Dramsi S, Biswas I, Maguin E, et al. Entry of *Listeria monocytogenes* into hepatocytes requires expression of inIB, a surface protein of the internalin multigene family. *Mol Microbiol* 1995;**16**:251–61.
30. Dussurget O. New insights into determinants of *Listeria monocytogenes* virulence. *Int Rev Cell Mol Biol* 2008;**270**:1–38.
31. Edmiston Jr CE, Gordon RC. Evaluation of gentamicin and penicillin as a synergistic combination in experimental murine listeriosis. *Antimicrob Agents Chemother* 1979;**16**:862–3.
32. Espaze EP, Reynaud AE. Antibiotic susceptibilities of *Listeria:* in vitro studies. *Infection* 1988;**16**(Suppl 2):S160–4.
33. Evans JR, Allen AC, Bortolussi R, et al. Follow-up study of survivors of fetal and early onset neonatal listeriosis. *Clin Invest Med* 1984;**7**:329–34.
34. Evans JR, Allen AC, Stinson DA, et al. Perinatal listeriosis: report of an outbreak. *Pediatr Infect Dis* 1985;**4**:237–41.
35. Facinelli B, Varaldo PE, Casolari C, et al. Cross-infection with *Listeria monocytogenes* confirmed by DNA fingerprinting. *Lancet* 1988;**2**:1247–8.
36. Filice GA, Cantrell HF, Smith AB, et al. *Listeria monocytogenes* infection in neonates: investigation of an epidemic. *J Infect Dis* 1978;**138**:17–23.
37. Forna F, McConnell M, Kitabire FN, et al. Systematic review of the safety of trimethoprim-sulfamethoxazole for prophylaxis in HIV-infected pregnant women: implications for resource-limited settings. *AIDS Rev* 2006;**8**:24–36.
38. Furyk JS, Swann O, Molyneux E. Systematic review: neonatal meningitis in the developing world. *Trop Med Int Health* 2011; **16**(6):672–9.
39. Gaillard JL, Berche P, Frehel C, et al. Entry of *L. monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. *Cell* 1991;**65**: 1127–41.
40. Gaulin C, Ramsay D, Bekal S. Widespread listeriosis outbreak attributable to pasteurized cheese, which led to extensive cross-contamination affecting cheese retailers, Quebec, Canada, 2008. *J Food Prot* 2012;**75**:71–8.
41. Gellin BG, Broome CV. Listeriosis. *JAMA* 1989;**261**:1313–20.
42. Gianfranceschi MV, D'Ottavio MC, Gattuso A, et al. Distribution of serotypes and pulsotypes of *Listeria monocytogenes* from human, food and environmental isolates (Italy 2002-2005). *Food Microbiol* 2009;**26**:520–6.
43. Goossens PL, Milon G, Cossart P, et al. Attenuated *Listeria monocytogenes* as a live vector for induction of $CD8^+$ T cells in vivo: a study with the nucleoprotein of the lymphocytic choriomeningitis virus. *Int Immunol* 1995;**7**:797–805.
44. Gordon RC, Barrett FF, Clark DJ. Influence of several antibiotics, singly and in combination, on the growth of *Listeria monocytogenes*. *J Pediatr* 1972;**80**:667–70.
45. Gordon RC, Barrett FF, Yow MD. Ampicillin treatment of listeriosis. *J Pediatr* 1970;**77**:1067–70.
46. Goulet V, de Valk H, Pierre O, et al. Effect of prevention measures on incidence of human listeriosis, France, 1987-1997. *Emerg Infect Dis* 2001;**7**:983–9.
47. Goulet V, Hebert M, Hedberg C, et al. Incidence of listeriosis and related mortality among groups at risk of acquiring listeriosis. *Clin Infect Dis* 2012;**54**:652–60.
48. Gray ML, Seeliger HPR, Patel J. Perinatal infections due to *Listeria monocytogenes:* do these affect subsequent pregnancies? *Clin Pediatr (Phila)* 1963;614–23.
49. Green HT, Macaulay MB. Hospital outbreak of *Listeria monocytogenes* septicaemia: a problem of cross infection? *Lancet* 1978;**2**: 1039–40.
50. Greiffenberg L, Goebel W, Kim KS, et al. Interaction of *Listeria monocytogenes* with human brain microvascular endothelial cells: InlB-dependent invasion, long-term intracellular growth, and spread from macrophages to endothelial cells. *Infect Immun* 1998; **66**:5260–7.
51. Grif K, Patscheider G, Dierich MP, et al. Incidence of fecal carriage of *Listeria monocytogenes* in three healthy volunteers: a

one-year prospective stool survey. *Eur J Clin Microbiol Infect Dis* 2003;**22**:16–20.
52. Hansen JM, Gerner-Smidt P, Bruun B. Antibiotic susceptibility of *Listeria monocytogenes* in Denmark 1958-2001. *APMIS* 2005;**113**: 31–6.
53. Hawkins AE, Bortolussi R, Issekutz AC: In vitro and in vivo activity of various antibiotics against *Listeria monocytogenes* type 4b: clinical and investigative medicine. *Med Clin Exp* 1984;**7**:335–41.
54. Hof H, Waldenmeier G. Therapy of experimental listeriosis—an evaluation of different antibiotics. *Infection* 1988;**16**(Suppl 2): S171–4.
55. Holt JG, Krieg NR, Sneath PHA, et al., editors. *Bergey's manual of determinative bacteriology*. Baltimore: Williams & Wilkins; 1994. p. 566–7.
56. Hood M. Listeriosis as an infection of pregnancy manifested in the newborn. *Pediatrics* 1961;**27**:390–6.
57. Hugin AW, Cerny A, Wrann M, et al. Effect of cyclosporin A on immunity to *Listeria monocytogenes*. *Infect Immun* 1986;**52**:12–17.
58. Jackson KA, Iwamoto M, Swerdlow D. Pregnancy-associated listeriosis. *Epidemiol Infect* 2010;**138**:1503–9.
59. Jaton K, Sahli R, Bille J. Development of polymerase chain reaction assays for detection of *Listeria monocytogenes* in clinical cerebrospinal fluid samples. *J Clin Microbiol* 1992;**30**:1931–6.
60. Jiao Y, Zhang W, Ma J, et al. Early onset of neonatal listeriosis. *Pediatr Int* 2011;**53**:1034–7.
61. Kampelmacher EH, Huysinga WT, van Noorle Jansen LM. The presence of *Listeria monocytogenes* in feces of pregnant women and neonates. *Zentralbl Bakteriol Orig A* 1972;**222**:258–62.
62. Katz VL, Weinstein L. Antepartum treatment of *Listeria monocytogenes* septicemia. *South Med J* 1982;**75**:1353–4.
63. Kaur S, Malik SV, Vaidya VM, et al. *Listeria monocytogenes* in spontaneous abortions in humans and its detection by multiplex PCR. *J Appl Microbiol* 2007;**103**:1889–96.
64. Kayal S, Charbit A. Listeriolysin O: a key protein of *Listeria monocytogenes* with multiple functions. *FEMS Microbiol Rev* 2006; **30**:514–29.
65. Kessler SL, Dajani AS. *Listeria meningitis* in infants and children. *Pediatr Infect Dis J* 1990;**9**:61–3.
66. Khelef N, Lecuit M, Bierne H, et al. Species specificity of the *Listeria monocytogenes* InlB protein. *Cell Microbiol* 2006;**8**:457–70.
67. Klatt EC, Pavlova Z, Teberg AJ, et al. Epidemic perinatal listeriosis at autopsy. *Hum Pathol* 1986;**17**:1278–81.
68. Kobayashi SD, Braughton KR, Whitney AR, et al. Bacterial pathogens modulate an apoptosis differentiation program in human neutrophils. *Proc Natl Acad Sci U S A* 2003;**100**: 10948–53.
69. Kollmann TR, Crabtree J, Rein-Weston A, et al. Neonatal innate TLR-mediated responses are distinct from those of adults. *J Immunol* 2009;**183**:7150–60.
70. Kollmann TR, Reikie B, Blimkie D, et al. Induction of protective immunity to *Listeria monocytogenes* in neonates. *J Immunol* 2007; **178**:3695–701.
71. Kovacevic J, Mesak LR, Allen KJ. Occurrence and characterization of *Listeria* spp. in ready-to-eat retail foods from Vancouver, British Columbia. *Food Microbiol* 2012;**30**:372–8.
72. Lam GY, Czuczman MA, Higgins DE, et al. Interactions of *Listeria monocytogenes* with the autophagy system of host cells. *Adv Immunol* 2012;**113**:7–18.
73. Lamont RF, Sobel J, Mazaki-Tovi S, et al. Listeriosis in human pregnancy: a systematic review. *J Perinat Med* 2011;**39**:227–36.
74. Larsson S, Cederberg A, Ivarsson S, et al. *Listeria monocytogenes* causing hospital-acquired enterocolitis and meningitis in newborn infants. *BMJ* 1978;**2**:473–4.
75. Lavi O, Louzoun Y, Klement E. Listeriosis: a model for the fine balance between immunity and morbidity. *Epidemiology* 2008;**19**: 581–7.
76. Le Monnier A, Abachin E, Beretti JL, et al. Diagnosis of *Listeria monocytogenes* meningoencephalitis by real-time PCR for the *hly* gene. *J Clin Microbiol* 2011;**49**:3917–23.
77. Le Monnier A, Autret N, Join-Lambert OF, et al. ActA is required for crossing of the fetoplacental barrier by *Listeria monocytogenes*. *Infect Immun* 2007;**75**:950–7.
78. Lecuit M. Understanding how *Listeria monocytogene*s targets and crosses host barriers. *Clin Microbiol Infect* 2005;**11**:430–6.
79. Lecuit M, Nelson DM, Smith SD, et al. Targeting and crossing of the human maternofetal barrier by *Listeria monocytogenes:* role of internalin interaction with trophoblast E-cadherin. *Proc Natl Acad Sci U S A* 2004;**101**:6152–7.
80. Leng J, Goldstein DR. Impact of aging on viral infections. *Microbes Infect* 2010;**12**:1120–4.
81. Lessing MP, Curtis GD, Bowler IC. *Listeria ivanovii* infection. *J Infect* 1994;**29**:230–1.
82. Massarotti EM, Dinerman H. Septic arthritis due to *Listeria monocytogenes:* report and review of the literature. *J Rheumatol* 1990;**17**:111–13.
83. McBride ME, Girard KF. Procedure for the selective isolation of *Listeria monocytogenes*. *J Lab Clin Med* 1960;**55**:153–7.
84. McLaughlin J. Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. *Eur J Clin Microbiol Infect Dis* 1990;**9**:210–13.
85. Miguel-Yanes JM, Gonzalez-Ramallo VJ, Pastor L. Outcome of *Listeria monocytogenes* prosthetic valve endocarditis: as bad as it looks? *Scand J Infect Dis* 2004;**36**:709–11.
86. Mook P, O'Brien SJ, Gillespie IA. Concurrent conditions and human listeriosis, England, 1999-2009. *Emerg Infect Dis* 2011;**17**: 38–43.
87. Morosi S, Francisci D, Baldelli F. A case of rhombencephalitis caused by *Listeria monocytogenes* successfully treated with linezolid. *J Infect* 2006;**52**:e73–5.
88. Mounier J, Ryter A, Coquis-Rondon M, et al. Intracellular and cell-to-cell spread of *Listeria monocytogenes* involves interaction with F-actin in the enterocytelike cell line Caco-2. *Infect Immun* 1990;**58**:1048–58.
89. Munk ME, Kaufmann SH. *Listeria monocytogenes* reactive T lymphocytes in healthy individuals. *Microb Pathog* 1988;**5**:49–54.
90. Munoz P, Ferreira L, Marin M, et al. First report of *Listeria monocytogenes* endocarditis treated with linezolid. *Int J Antimicrob Agents* 2006;**28**:480–1.
91. Murray MGD, Webb RA, Swann MBR. A disease of rabbits characterized by large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes*. *J Pathol Bacteriol* 1926;**29**:407–39.
92. Nyfeldt A. Etiologie do la mononucleose infectieuse. *Compt Rend Soc Biol* 1929;**101**:590–1.
93. Ooi ST, Lorber B. Gastroenteritis due to *Listeria monocytogenes*. *Clin Infect Dis* 2005;**40**:1327–32.
94. Pamer EG. Immune responses to *Listeria monocytogenes*. *Nat Rev Immunol* 2004;**4**:812–23.
95. Parida SK, Domann E, Rohde M, et al. Internalin B is essential for adhesion and mediates the invasion of *Listeria monocytogenes* into human endothelial cells. *Mol Microbiol* 1998;**28**:81–93.
96. Paterson JS. The antigenic structure of organisms of genus *Listerella*. *J Pathol Bacteriol* 1940;427–36.
97. Pentecost M, Otto G, Theriot JA, et al. *Listeria monocytogenes* invades the epithelial junctions at sites of cell extrusion. *PLoS Pathog* 2006;**2**:e3.
98. Pinner RW, Schuchat A, Swaminathan B, et al. Role of foods in sporadic listeriosis, part II: microbiologic and epidemiologic investigation. The Listeria Study Group. *JAMA* 1992;**267**: 2046–50.
99. Portnoy DA, Jacks PS, Hinrichs DJ. Role of hemolysin for the intracellular growth of *Listeria monocytogenes*. *J Exp Med* 1988; **167**:1459–71.
100. Posfay-Barbe KM, Wald ER. Listeriosis. *Semin Fetal Neonatal Med* 2009;**14**:228–33.
101. Reikie BA, Smolen KK, Fortuno 3rd ES, et al. A single immunization near birth elicits immediate and lifelong protective immunity. *Vaccine* 2010;**29**:83–90.
102. Renoult T, Chabot F, Aymard B, et al. Treatment of *Listeria* bacteremia with vancomycin. *Rev Infect Dis* 1991;**13**:181–4.
103. Roed C, Engsig FN, Omland LH, et al. Long-term mortality in patients diagnosed with *Listeria monocytogenes* meningitis: a Danish nationwide cohort study. *J Infect* 2012;**64**:34–40.
104. Ruzante JM, Majowicz SE, Fazil A, et al. Hospitalization and deaths for select enteric illnesses and associated sequelae in Canada, 2001-2004. *Epidemiol Infect* 2011;**139**:937–45.
105. Salimnia H, Patel D, Lephart PR, et al. *Listeria grayi:* vancomycin-resistant, gram-positive rod causing bacteremia in a stem cell transplant recipient. *Transpl Infect Dis* 2010;**12**:526–8.
106. Scheld WM. Evaluation of rifampin and other antibiotics against *Listeria monocytogenes* in vitro and in vivo. *Rev Infect Dis* 1983; **5**(Suppl 3):S593–9.

107. Schlech 3rd WF. *Listeria* gastroenteritis—old syndrome, new pathogen. *N Engl J Med* 1997;**336**:130–2.
108. Schlech 3rd WF. Foodborne listeriosis. *Clin Infect Dis* 2000;**31**: 770–5.
109. Schlech 3rd WF, Lavigne PM, Bortolussi RA, et al. Epidemic listeriosis—evidence for transmission by food. *N Engl J Med* 1983;**308**:203–6.
110. Schlech 3rd WF, Schlech WF, Haldane H, et al. Does sporadic *Listeria* gastroenteritis exist? A 2-year population-based survey in Nova Scotia, Canada. *Clin Infect Dis* 2005;**41**:778–84.
111. Schnupf P, Portnoy DA. Listeriolysin O: a phagosome-specific lysin. *Microbes Infect* 2007;**9**:1176–87.
112. Schuchat A, Lizano C, Broome CV, et al. Outbreak of neonatal listeriosis associated with mineral oil. *Pediatr Infect Dis J* 1991; **10**:183–9.
113. Schuchat A, Swaminathan B, Broome CV. Epidemiology of human listeriosis. *Clin Microbiol Rev* 1991;**4**:169–83.
114. Seeliger HPR. *Listeriosis*. New York: S. Karger; 1961.
115. Seeliger HPR, Finger H. Analytical serology of *Listeria*. In: Kwapinski JBG, editor. *Analytical serology of microorganisms*. New York: John Wiley and Sons; 1969. p. 549–609.
116. Serbina NV, Shi C, Pamer EG. Monocyte-mediated immune defense against murine *Listeria monocytogenes* infection. *Adv Immunol* 2012;**113**:119–34.
117. Skogberg K, Syrjanen J, Jahkola M, et al. Clinical presentation and outcome of listeriosis in patients with and without immunosuppressive therapy. *Clin Infect Dis* 1992;**14**:815–21.
118. Srinivasan V, Nam HM, Nguyen LT, et al. Prevalence of antimicrobial resistance genes in *Listeria monocytogenes* isolated from dairy farms. *Foodborne Pathog Dis* 2005;**2**:201–11.
119. Stamm AM, Dismukes WE, Simmons BP, et al. Listeriosis in renal transplant recipients: report of an outbreak and review of 102 cases. *Rev Infect Dis* 1982;**4**:665–82.
120. Steinman CR. Specific detection and semiquantitation of microorganisms in tissue by nucleic acid hybridization, part I: characterization of the method and application to model systems. *J Lab Clin Med* 1975;**86**:164–74.
121. Taillefer C, Boucher M, Laferriere C, et al. Perinatal listeriosis: Canada's 2008 outbreaks. *J Obstet Gynaecol Can* 2010;**32**:45–8.
122. Tappero JW, Schuchat A, Deaver KA, et al. Reduction in the incidence of human listeriosis in the United States: effectiveness of prevention efforts? The Listeriosis Study Group. *JAMA* 1995; **273**:1118–22.
123. Teberg AJ, Yonekura ML, Salminen C, et al. Clinical manifestations of epidemic neonatal listeriosis. *Pediatr Infect Dis J* 1987;**6**:817–20.
124. Theriot JA, Mitchison TJ, Tilney LG, et al. The rate of actin-based motility of intracellular *Listeria monocytogenes* equals the rate of actin polymerization. *Nature* 1992;**357**:257–60.
125. Tilney LG, Connelly PS, Portnoy DA. Actin filament nucleation by the bacterial pathogen, *Listeria monocytogenes*. *J Cell Biol* 1990;**111**:2979–88.
126. Tilney LG, Portnoy DA. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, *Listeria monocytogenes*. *J Cell Biol* 1989;**109**:1597–608.
127. Traub WH. Perinatal listeriosis. *Chemotherapy* 1981;423–31.
128. Tsai YH, Hirth RS, Leitner F. A murine model for listerial meningitis and meningoencephalomyelitis: therapeutic evaluation of drugs in mice. *Chemotherapy* 1980;**26**:196–206.
129. Unanue ER, Carrero JA. Studies with *Listeria monocytogenes* lead the way. *Adv Immunol* 2012;**113**:1–5.
130. Varma JK, Samuel MC, Marcus R, et al. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. *Clin Infect Dis* 2007;**44**:521–8.
131. Vazquez-Boland JA, Dominguez-Bernal G, Gonzalez-Zorn B, et al. Pathogenicity islands and virulence evolution in *Listeria*. *Microbes Infect* 2001;**3**:571–84.
132. Vazquez-Boland JA, Kuhn M, Berche P, et al. *Listeria* pathogenesis and molecular virulence determinants. *Clin Microbiol Rev* 2001;**14**: 584–640.
133. Vischer WA, Rominger C. Rifampicin against experimental listeriosis in the mouse. *Chemotherapy* 1978;**24**:104–11.
134. Visintine AM, Oleske JM, Nahmias AJ. Infection in infants and children. *Am J Dis Child* 1977;**131**:393–7.
135. Walsh D, Duffy G, Sheridan JJ, et al. Antibiotic resistance among *Listeria*, including *Listeria monocytogenes*, in retail foods. *J Appl Microbiol* 2001;**90**:517–22.
136. Wilson CB. Immunologic basis for increased susceptibility of the neonate to infection. *J Pediatr* 1986;**108**:1–12.
137. Witte CE, Archer KA, Rae CS, et al. Innate immune pathways triggered by *Listeria monocytogenes* and their role in the induction of cell-mediated immunity. *Adv Immunol* 2012;**113**:135–56.

many members of European royal families. Tuberculosis became more widespread in western Europe after the plague years of the Middle Ages and the epidemic during the era of urbanization and industrialization in the 18th and 19th centuries.[177] At that time, scrofula affected more than half the young inhabitants of workhouses and orphanages.

During the 18th and 19th centuries in the eastern cities of the United States (Boston, New York, and Philadelphia), the mortality rate from tuberculosis was about 400 per 100,000 population. With improving socioeconomic conditions, the mortality rate fell to 200 per 100,000 around 1900 and to 26 per 100,000 by 1950 even before the advent of effective chemotherapy. Stress in all its forms—famine, war, rationing, long working hours, child labor, population displacement, crowded living and working conditions—favors the spread of tuberculosis in humans, whereas years of peace and plenty favor its rapid decline.[176,515] The decrease in the Western world in the incidence of tuberculosis was accentuated by the discovery, development, and widespread use of antituberculosis drugs beginning in the late 1940s.

Another important factor leading to the decline of tuberculosis in Western countries was the recognition in the 1920s of the importance of bovine tuberculosis and its successful eradication as a public health problem in the United States by gradual slaughter of infected cattle and almost universal pasteurization of milk.[220] Although *Mycobacterium bovis* disease now accounts for a small fraction (1% to 2%) of all tuberculosis in the United States,[253] it has been implicated in up to 10 percent of cases in children along border regions of the United States.[144]

STAGES: EXPOSURE, INFECTION, AND DISEASE

The pathophysiologic process of tuberculosis is complicated, and the delay between acquisition of infection and manifestation of disease renders certain pathophysiologic events less distinct.[12] There are three major stages of tuberculosis: exposure, infection, and disease.[616]

Exposure means that the child has had significant contact with an adult or adolescent with infectious pulmonary tuberculosis. The contact investigation—examination of individuals close to a person suspected of having tuberculosis by performing a tuberculin skin test, chest radiograph, and physical examination—is the most important activity in a community to prevent cases of tuberculosis in children.[14,58,265] The most frequent setting for exposure of a child is the household, but it can occur in a school, daycare center, or other closed setting.[153,257] In this stage, the tuberculin skin test result is negative, the chest radiograph is normal, and the child lacks signs or symptoms of disease. Some exposed children may have inhaled droplet nuclei infected with *M. tuberculosis* and have early infection, but the clinician cannot know it because delayed hypersensitivity to tuberculin—a positive skin test response—takes up to 3 months to develop. Children younger than 5 years old or immunocompromised children of any age who are in the exposure stage should be treated to prevent the rapid development of disseminated or meningeal tuberculosis, which can occur before the skin test becomes reactive.[184,371,462,493,631]

Infection occurs when the individual inhales droplet nuclei containing *M. tuberculosis*, which becomes established intracellularly within the lung and associated lymphoid tissue. The hallmark of latent tuberculosis infection is a reactive tuberculin skin test or a positive interferon gamma release assay (IGRA). In this stage, the child has no signs or symptoms and the chest radiograph is either normal or reveals only granuloma or calcifications in the lung parenchyma or regional lymph nodes or in both tissues. In industrialized countries, virtually all children with tuberculosis infection should receive treatment, usually with isoniazid, to prevent the development of disease in the near or distant future.

Disease occurs when signs or symptoms or radiographic manifestations caused by *M. tuberculosis* become apparent. The word *tuberculosis* refers to disease. Not all infected individuals have the same risk for contracting disease. An immunocompetent adult with untreated tuberculosis infection has a 5 to 10 percent lifetime risk for development of disease; half the risk exists in the first 2 to 3 years after infection occurs. Adults with tuberculosis infection who then become infected with human immunodeficiency virus (HIV) have a 5 to 10 percent annual risk for development of tuberculosis disease.[564] Historical studies have shown that often serious, life-threatening forms of the disease will develop within 1 to 2 years in as many as 40 percent of immunocompetent infants with untreated tuberculosis infection.[395]

EPIDEMIOLOGY

Incidence and Prevalence

Between 20 and 45 percent of the world's population (approximately 2 billion people) are infected with *M. tuberculosis*, and more than 90 percent of new cases occur in the developing world. The World Health Organization (WHO) estimates that 8.8 million incident cases, 12 million prevalent cases, and 1.45 million deaths from tuberculosis occur annually worldwide.[713] The annual rates per 100,000 persons are 128 for incidence, 178 for prevalence, and 15 for mortality, respectively.[713]

Steps toward tuberculosis elimination have been thwarted by the HIV pandemic. Each disease makes the other more difficult to treat, and diagnosing tuberculosis in HIV-infected patients is challenging. It has been estimated that 13 percent of persons with tuberculosis are coinfected with HIV,[713] although there is wide variation. The highest rates of HIV coinfection are seen in sub-Saharan Africa, where up to 40 percent of newly diagnosed tuberculosis patients have HIV infection.[713] Approximately one fourth of deaths from tuberculosis occur in HIV-infected persons,[713] and tuberculosis is the most common infectious cause of death in HIV-infected persons, particularly in patients with high CD4 cell counts.[23] True rates of HIV and tuberculosis coinfection are unclear, because many nations do not screen all newly diagnosed tuberculosis patients for HIV.

According to the WHO, in 1997, only 32 percent of the world's population lived in areas where effective tuberculosis control programs were fully operational.[457] Poor tuberculosis control selects for drug-resistant isolates. Multidrug-resistant tuberculosis (MDR-TB), where the isolate is resistant to at least isoniazid and rifampin, and extensively drug-resistant tuberculosis (XDR-TB),[572] with resistance to isoniazid, rifampin, non-streptomycin injectable drugs, and fluoroquinolones, have become important problems in many areas of the world.[183,713,726] MDR-TB was not described until 1990[91] but has spread widely. In adults, drug resistance in *M. tuberculosis* often is secondary, the resistance emerging during therapy because treatment is inadequate or interrupted.[206] In children, drug resistance usually is primary in that the child is infected with a strain that already has become resistant.[460,546,548,645] Rates of drug resistance in children tend to mirror those in adults in the same population[622,623] and may be higher in developing countries because of difficulty in completing therapy, inadequate supply of medications, and use of over-the-counter cough medications that often contain isoniazid or rifampin. Although 4 percent of tuberculosis cases globally (650,000 estimated MDR-TB cases in 2010)[713] are MDR-TB, rates as high as 23 to 26 percent have been reported in countries formerly part of the Soviet Union.[713,715] The true rates of drug-resistant tuberculosis are unclear. Limitations to accurate estimates include variation in laboratory capacity and reporting and selection of particular patients for drug susceptibility testing (e.g., patients who are failing standard therapy). Additionally, not all patients have culture-confirmed tuberculosis. Finally, the manner in which rates are reported is important. Although incident cases are a better marker of spread of strains in the community, prevalence rates provide a better reflection of the chronicity of MDR-TB and XDR-TB.

In developing countries where the burden of disease is greatest, the only available diagnostic test often is an acid-fast smear of sputum, the result of which rarely is positive from infants and children with pulmonary tuberculosis. This leads to underestimation of the pediatric tuberculosis disease burden. Up to 11 percent of deaths from adolescence to early adulthood are attributable to tuberculosis and HIV infection/AIDS in high-prevalence areas.[489] In areas with both accurate reporting and high rates of tuberculosis, it is thought that children younger than 15 years of age comprise 15 to 20 percent of the disease burden.[399] This figure varies widely among countries: although only 6 percent of all cases in the United States occur in children,[103] children are reported to account for 1.4 percent (Nigeria) to 17 percent (Swaziland) of cases in sub-Saharan African nations.[714] Approximately 81 percent of childhood cases occur in the 22 countries with the highest burden. There also are indirect impacts of tuberculosis on child health. It has been estimated that 9.7 million children were orphaned in 2009 after at least one of their parents died of tuberculosis.[713] The inability to control tuberculosis despite the availability of effective, relatively inexpensive therapy is one of the greatest medical failures of our time.[239,615]

The situation in the United States is markedly different. Between 1953, when reporting began, and 1985, the number of annually reported tuberculosis cases in the United States fell by 74 percent from 84,304 to 22,201.[96] In 1985, the overall case rate was approximately 10 per 100,000 population. Between 1985 and 1992 in the United States, the total tuberculosis case numbers rose 20 percent.[96] During that same time, the number of pediatric tuberculosis cases rose 40 percent[294,669] (Fig. 96-1). Most experts cite four probable causes for the increases: (1) the co-epidemic of HIV infection; (2) the increasing rates of tuberculosis in foreign-born individuals in the United States[78]; (3) the increased transmission among adults in congregate settings, including jails and prisons, nursing homes, homeless shelters, HIV treatment facilities, hospitals, and, rarely, schools[40,43,60,418]; and (4) a decline in the public health infrastructure in many areas of the country.[73,123,516,617] After several years of intense and expensive effort were expended, the number of tuberculosis cases in the United States declined again.[447]

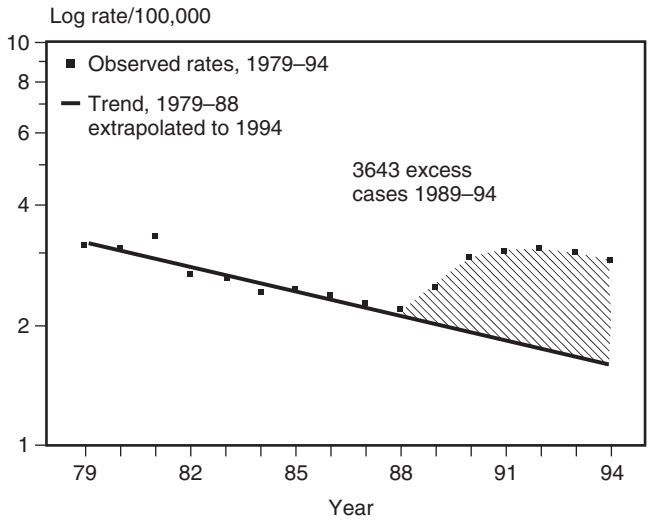

FIGURE 96-1 ■ Observed and expected tuberculosis cases in children younger than 15 years old in the United States, 1979 to 1994.

In 2011, a total of 10,521 incident tuberculosis cases (3.4 cases per 100,000 population) were reported in the United States, the lowest reported rate since 1953.[103] Since treatment became available in the late 1940s, tuberculosis has been concentrated in certain high-risk groups in the United States[97] (Table 96-1). Tuberculosis occurs most commonly in areas with ethnically diverse populations, including large urban areas, coastal states, and states bordering Mexico[90,98] (Fig. 96-2); California, Florida, New York, and Texas accounted for almost one half of all U.S. tuberculosis cases in 2011.[103] Tuberculosis disproportionately affects ethnic and minority populations in the United States. In 2011, when compared with non-Hispanic whites, tuberculosis rates among Hispanics, non-Hispanic blacks, and Asians were 7, 8, and 25 times greater, respectively.[103] In 2011, the Centers for Disease Control and Prevention (CDC) reported the highest overall number of cases (3147) and case rates (21.4/100,000) in Asians.[103]

The majority (62.5%) of cases of tuberculosis in the United States occur in foreign-born persons, who have rates of tuberculosis 12 times higher than persons born

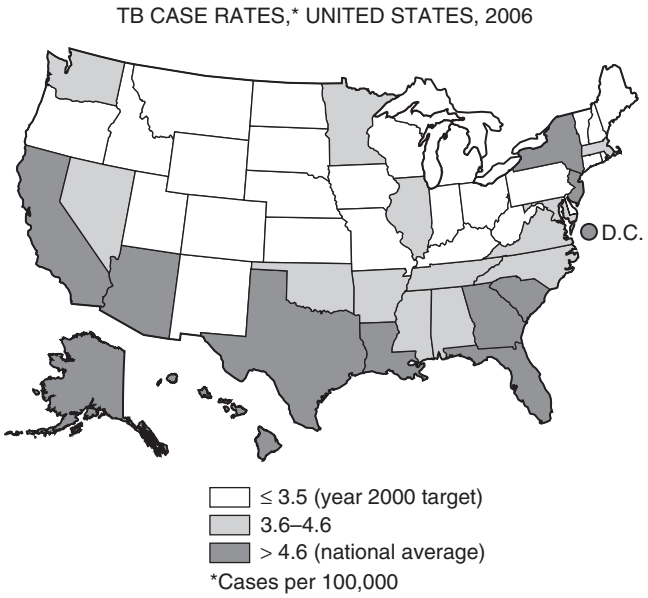

FIGURE 96-2 ■ Tuberculosis case rates (cases per 100,000) by state, 2006. (From Centers for Disease Control and Prevention. *Reported tuberculosis in the United States, 2006*. Atlanta, Ga.: U.S. Department of Health and Human Services, CDC; October 2007.)

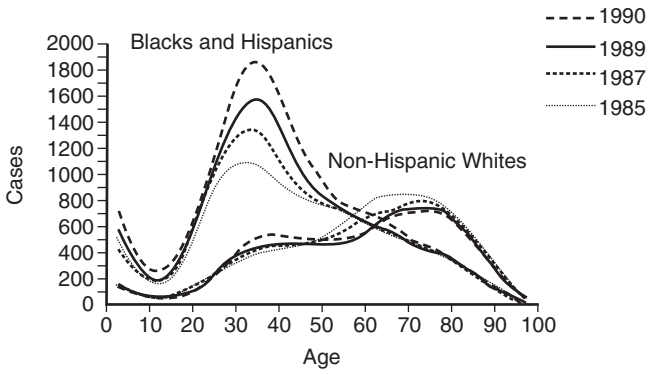

FIGURE 96-3 ■ Tuberculosis cases in Hispanics and blacks versus non-Hispanic whites in the United States, 1985 to 1990.

TABLE 96-1 High-Risk Groups for Tuberculosis Infection and Disease

Groups at High Risk for Exposure or Infection
Close contacts of person with tuberculosis
Foreign-born persons from high-risk countries (Asia, Africa, Latin America, Russia, Eastern Europe)
Residents and employees of high-risk congregate settings (correctional institutions, nursing homes, homeless shelters, hospitals serving high-risk populations, drug treatment centers)
Medically underserved, low-income populations
High-risk racial or ethnic minority populations
Injection drug users
Children exposed to adults in high-risk categories
Groups at Higher Risk for Disease Once Infected
Immunosuppressed patients, including those HIV infected
Recent tuberculosis infection (within past 2 years)
Persons with certain medical conditions (diabetes mellitus, silicosis, cancer, end-stage renal disease, gastrectomy, body weight ≤90% of ideal)
Injection drug users
History of inadequately treated tuberculosis
Children age ≤4 years, especially infants

in the United States.[103] Tuberculosis is endemic in most developing countries, and between 30 and 50 percent of recent immigrants to the United States have latent tuberculosis infection on entry into the country.[119,642] During the immigration process, adults are screened for tuberculosis disease with a chest radiograph, but skin testing to detect tuberculosis infection is not required. Among children in the United States, being born in a country with a high rate of tuberculosis is the most important risk factor for having tuberculosis infection. Foreign-born adopted children also are at risk for having tuberculosis infection and disease.[205,346,531] People who enter the United States illegally may be unable or afraid to seek medical treatment, even when they are ill. Studies have shown that in most immigrants in whom tuberculosis develops, it does so within 5 years of immigration, thus indicating that many cases could be prevented if appropriate screening and treatment programs were conducted.

Other important risk factors for tuberculosis in adults include lower socioeconomic status, migrant work, HIV infection, drug use, homelessness, travel to high-prevalence countries, history of incarceration, and occupations with exposure to high-risk populations (see Table 96-1). Children from high-risk population groups or children who have contact with adults in these groups may be at increased risk for development of tuberculosis infection.[369] Age has an important influence on tuberculosis case rates.[451] During 2010, 6 percent of cases in the United States occurred in children younger than 15 years, 11 percent in persons 15 to 24 years old, 33 percent in persons 25 to 44 years old, 31 percent in persons 45 to 64 years old, and 20 percent in persons older than 64 years.[103] The highest case rates among children are in those younger than 5 years. Children aged 5 to 14 years, the so-called favored age, have a consistently lower case rate than that of any other segment of the population.[393,451] In early childhood, the incidence is not significantly different in girls and boys, although higher rates of disease are generally experienced by adolescent girls than adolescent boys.[69]

The age at which tuberculosis disease initially develops currently varies among ethnic groups in the United States.[95] White persons develop tuberculosis more commonly as older adults, whereas African Americans and Hispanics have a higher incidence of disease as young adults or children (Fig. 96-3).[632] Many of the risk factors for development of tuberculosis, such as HIV infection, drug use, history of incarceration, and recent immigration, occur more commonly in young adults in their 20s, 30s, and 40s, a time when contact with children is more likely. Other factors, including recent immigration, socioeconomic status, high-risk behavior, and possibly genetic susceptibility, may influence the peak age distribution of tuberculosis among different groups.[384]

Inherited susceptibility to tuberculosis may contribute to differences among various ethnic groups.[384] Laboratory animals and humans have been shown to differ in genetic susceptibility to tuberculosis.[376,567] In the United States, highly urbanized immigrants, such as Jews from European ghettos, fared much better than did their rural Irish and African counterparts, presumably because generations of exposure to tuberculosis in previous European epidemics had selected in favor of more resistant individuals.[503] As the disease spread to other continents, previously unexposed populations may have been more susceptible to tuberculosis and experienced higher rates of disease.

Cross-sectional prevalence of tuberculosis infection is difficult to ascertain, because this primarily has been described in high-risk groups. One 2011 review found tuberculosis infection in up to 20 percent of foreign-born persons, 13 to 32 percent of homeless individuals, 28 percent of injection drug users, and 2 to 17 percent of prisoners.[262] An estimated 4 to 6 percent of the U.S. population, or approximately 11 million people, are infected with *M. tuberculosis.* The percentage of infected Americans varies widely by racial and ethnic groups and socioeconomic status. Foreign-born persons residing in the United States had a prevalence of infection of 19 percent; African-Americans, 7 percent; Mexican-Americans, 9 percent; and persons living in poverty, 6 percent.[45] This group of infected persons represents a large reservoir from which cases of tuberculosis disease will emerge in the future if these individuals are not treated.[6]

In some U.S. locales that have had recent increases in tuberculosis, the demographic groups with the greatest tuberculosis morbidity also have large numbers of HIV-infected persons.[114,256,448] Sixty to 70 percent of persons with tuberculosis are screened for HIV, and HIV seroprevalence in tuberculosis patients is 6 to 9 percent in the United States.[103] Data on HIV coinfection are incomplete, because HIV screening is not mandatory for tuberculosis patients and is not reported to the CDC by all states. The HIV epidemic has had a profound effect on the epidemiology of tuberculosis in children by two mechanisms[541]: (1) most important in the United States, HIV-infected adults with tuberculosis may transmit *M. tuberculosis* to children in their environment and tuberculosis disease will develop in some of them[326]; and (2) important in many developing countries, children with HIV infection are at increased risk for progressing from asymptomatic tuberculosis infection to disease.[275,375] Several studies have demonstrated increased rates of childhood tuberculosis associated with increased rates of disease among HIV-infected adults in the community.[238,299] Tuberculosis probably is underdiagnosed in HIV-infected children, especially in the developing world, because of the similarity of its clinical manifestations with other opportunistic pulmonary diseases and the difficulty of confirming the diagnosis with the skin test or culture. All children with suspected tuberculosis disease should have HIV serotesting because the two infections are linked epidemiologically and many experts prolong treatment in HIV-infected children with tuberculosis.[231,549,702]

In addition to the association with HIV, the resurgence of tuberculosis in the United States between 1985 and 1992 was associated with the emergence of MDR-TB.[726] MDR-TB mortality rates may be as high as 50 percent in complicated cases.[217,283] Since 1993, the incidence of resistance to isoniazid has remained stable and the incidence of MDR-TB has decreased. In 2010, 8 percent of U.S. isolates were resistant to at least isoniazid and 1.2 percent were MDR-TB. Over 80 percent of cases of MDR-TB occurred in foreign-born persons. XDR-TB has been uncommon in the United States, with five cases reported in 2008, none in 2009, and one in 2010.[103]

Children comprise a small fraction of tuberculosis cases in the United States, in part because of low rates of coinfection with HIV and because the United States has a different population structure than many developing nations. The site of manifestation of tuberculosis disease differs between adults and children. Although pulmonary disease is most common for all ages, extrapulmonary tuberculosis occurs more often in children.[451] Because HIV coinfection increases the risk for development of extrapulmonary disease, rates have increased in adults during the past 15 years. In general, 25 percent of pediatric cases are extrapulmonary, with 75 percent being pulmonary. In 1985, before widespread HIV infection occurred, 85 percent of adults had pulmonary disease and 15 percent had extrapulmonary disease. With the spread of HIV infection, these numbers have shifted; and in recent years, the CDC reported that 78 percent of all new cases, including those in adults and children, were pulmonary and 22 percent were extrapulmonary.[103] Comparison of the site of extrapulmonary disease in children and adults in 1985, before the AIDS epidemic, shows some important differences. First, approximately 70 percent of extrapulmonary tuberculosis in children involved the lymph nodes, as opposed to 25 percent in adults (Fig. 96-4). Second, tuberculous meningitis accounted for 13 percent of extrapulmonary disease in children versus 4 percent in adults. Genitourinary involvement occurred in 16 percent of adults, but it was a rare finding in children. Although the proportion of extrapulmonary cases in adults has increased as a result of the HIV-AIDS epidemic, extrapulmonary disease still occurs more commonly in children.

Transmission

Transmission of tuberculosis is from one human to another, usually through infected droplets of mucus that become airborne when an individual coughs, sneezes, or laughs.[113] The droplets dry and become droplet nuclei, which may remain suspended in air for hours. Only particles less than 10 μm in diameter are small enough to reach the alveoli.[542] Transmission sometimes occurs by direct contact with infected discharges (sputum, saliva, urine, or drainage from an open sinus or abscess); it occasionally occurs by means of heavily contaminated fomites, such as shoes, gastric lavage tubes, bronchoscopes, or syringes prepared by someone with positive sputum.[252] Rare cases of tuberculosis transmitted by a lung or kidney transplant have been reported.[101,446] Dogs may be a source of infection for children because dogs

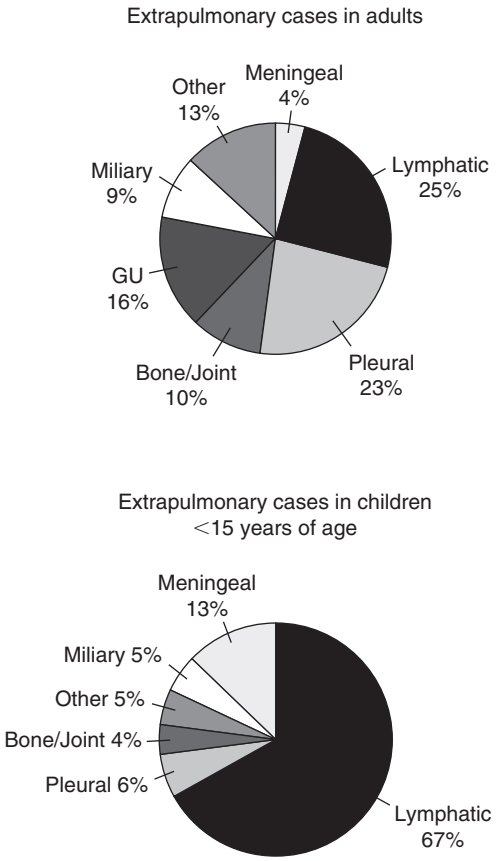

FIGURE 96-4 ■ Extrapulmonary disease by site in adults and children in the United States. (From the Centers for Disease Control and Prevention.)

are susceptible to the human type of tubercle bacillus.[521] One necropsy study showed that the prevalence of tuberculosis disease and infection in dogs in a high-prevalence setting (South Africa) was 1 percent and 50 percent, respectively.[487]

The collective experience of many clinicians is that children usually are infected by an adult or adolescent in the immediate household, most often a parent, grandparent, older sibling, boarder, or household employee.[42] Casual extrafamilial contact is the source of infection much less often, but physicians, babysitters, schoolteachers, music teachers, school bus drivers, parishioners, nurses, gardeners, and candy store keepers have been implicated in individual cases and in hundreds of mini-epidemics.* Attention has been drawn to the prevalence of active tuberculosis among residents of nursing homes for the elderly.[286] Children visiting their grandparents have contracted tuberculosis in this setting. Within the household of an infectious adult, infants and toddlers most often become infected. Adults with pulmonary disease who are receiving regular, appropriate chemotherapy probably rarely infect children; much more dangerous are those with chronic tuberculous disease that is unrecognized, inadequately treated, or in relapse because of the development of resistance.

*References 26, 67, 179, 351, 359, 696.

Wallgren[689] was the first to point out that children with tuberculosis rarely infect other children. Many children with the disease have tuberculin-negative siblings and parents. Children with tuberculosis often have been cared for by their families or in hospitals and institutions without infecting their contacts.[131,445] When transmission of *M. tuberculosis* has been documented in children's hospitals, it almost invariably has come from an adult with undiagnosed pulmonary tuberculosis.[27,305,306,434,698] Adults accompanying a child with suspected tuberculosis disease should be screened as soon as possible for pulmonary tuberculosis.* In tuberculous children, tubercle bacilli in endobronchial secretions are relatively sparse and cough is not characteristic of endothoracic tuberculosis or miliary disease. When young children cough, they lack the tussive force of adults. Specimens collected by bronchoalveolar lavage or early-morning gastric aspiration from children with clinically suspected tuberculosis seldom have acid-fast bacilli seen on smears. Only approximately 40 percent eventually will grow *M. tuberculosis* by culture.[602] Young infants with congenital tuberculosis or advanced, postnatally acquired pulmonary disease are more likely to have smear- or culture-positive specimens (80%) than older children.[545,670] Therefore, specimens and secretions from infants, children with cavitary lesions, and intubated patients should be handled as potentially infectious.[100,406] Most preadolescent children with tuberculosis, however, are not contagious and do not require isolation. Tuberculosis in adolescents may be more typical of adult-type reactivation disease, including the presence of cavitary lesions with smear- and culture-positive sputum. Children or adolescents who have symptomatic pulmonary tuberculosis with features of adult-type tuberculosis should be treated as potentially contagious until mycobacterial smears and cultures are negative.[81,136]

Children, nonetheless, play an extremely important role in the transmission of tuberculosis, not so much because they are likely to contaminate their immediate environment but rather because they may harbor a partially healed infection that lies dormant, only to be reactivated as infectious pulmonary tuberculosis many years later under the social, emotional, and physiologic stresses arising during adolescence, pregnancy, or old age. Thus, children infected with *M. tuberculosis* constitute a long-lasting reservoir of tuberculosis in the population. From an epidemiologic perspective, the diagnosis of tuberculosis disease in a child is a marker of recent transmission in the community, owing to the short incubation periods seen for childhood tuberculosis.

The risk for infection developing in child contacts of adults receiving antituberculosis chemotherapy often is a matter of practical concern. Several studies reveal that most contacts are infected by the index case before the diagnosis is made and treatment is initiated. Although it is not possible to carry out a definitive clinical study, evidence indicates that patients receiving effective chemotherapy rarely transmit *M. tuberculosis*. Nonetheless, it seems prudent to avoid exposure of children to adults with positive sputum smears or positive cultures and to

*References 70, 100, 118, 131, 307, 308, 445, 614.

assume that adults positive by smear or culture remain infectious for at least several weeks after the start of therapy.

MYCOBACTERIOLOGY

The genus *Mycobacterium*, closely related by its cell wall antigens to the genera *Corynebacterium* and *Nocardia*, is classified in the order Actinomycetales and the family Mycobacteriaceae. Several species are termed *M. tuberculosis* complex (MTBC): *M. tuberculosis*, *M. africanum*, *M. bovis*, *M. canetti*, and *M. microti*. Phylogenetic analysis indicates an origin for MTBC approximately 40,000 years ago, coinciding with expansion of humans out of eastern Africa.[706]

Mycobacteria are nonmotile, non-spore-forming, pleomorphic, weakly gram-positive rods measuring 1 to 5 μm long and typically slender and slightly "bent." Some appear beaded, and some are clumped. In general, species pathogenic for humans are more acid fast, have more exacting nutritional requirements, grow more slowly, form less pigment, and are more sensitive to chemotherapeutic agents than are the saprophytic species. The cell wall constituents of mycobacteria determine their most striking biologic properties. The cell walls contain 20 to 60 percent lipids by dry weight, largely bound to proteins and carbohydrates. These organisms are more resistant than are most others to light, alkali, acid, and the bactericidal action of antibodies. Their growth is slow, with a generation time of 14 to 24 hours, perhaps because of the slow metabolic exchange through the waxy "capsule." Their hydrophobic properties render them difficult to study.

Acid-fastness, that is, the capacity to form stable mycolate complexes with certain aryl methane dyes (specifically, carbolfuchsin, crystal violet, auramine, and rhodamine, which then are not removed readily even by rinsing with 95 percent ethanol plus hydrochloric acid), is the hallmark of mycobacteria. The cells appear red when stained with fuchsin (as with the Ziehl-Neelsen or Kinyoun stains), appear purple with crystal violet, or exhibit yellow-green fluorescence under ultraviolet light (when stained with auramine and rhodamine, as in Truant stain). Truant stain, in experienced hands, is considered the best stain for specimens expected to contain small numbers of organisms. Fluorescent staining can decrease staining time by 50 percent and increase sensitivity capacity in high-volume laboratories in developing countries when compared with Ziehl-Neelsen staining.[352] One study of LED fluorescence microscopy in adults showed that fluorescence microscopy was more sensitive but less specific than Ziehl-Neelsen staining, in part because in the fluorescence microscopy artifacts can fluoresce, leading to false-positive results.[134]

Identification of mycobacteria depends on their staining properties and on their biochemical and metabolic characteristics. Mycobacteria are obligate aerobes. On the whole, their growth requirements are simple. *M. tuberculosis* can grow in "classic" media, whose essential ingredients are egg yolk and glycerin (Löwenstein-Jensen, Petragnani, Dorset); often, a dye such as malachite green to inhibit contaminants; and, sometimes, potatoes and charcoal, which probably neutralize growth inhibitors. They also can grow in simple synthetic media, frequently with an admixture of asparagine, glutamate, or amino acid mixtures (Middlebrook 7H9, Tween-albumin). Once grown, they can be replated on media also containing antituberculosis drugs to determine drug susceptibility patterns. Isolation on solid media often takes 3 to 6 weeks, followed by another 2 to 4 weeks for drug susceptibility results. Improvements in laboratory methods have permitted more rapid culture, identification, and drug susceptibility testing of mycobacteria, such as by an automatic radiometric method known as the BACTEC method, in which a decontaminated, concentrated specimen is inoculated into a bottle of medium containing carbon 14–labeled palmitic acid as the substrate.[581] As mycobacteria metabolize the carbon 14–labeled palmitic acid, carbon dioxide 14 accumulates in the head space of the bottle, where radioactivity can be measured. Unfortunately, cross-contamination of bottles has been reported and has resulted in false-positive culture results.[178] The addition of appropriate dilutions of antituberculosis drugs permits an evaluation of drug susceptibility to be made. The time for identification and drug susceptibility testing can be reduced to 1 to 3 weeks, depending on the size of the inoculum.

Restriction fragment length polymorphism (RFLP) analysis and whole-genome analysis of mycobacterial DNA have become powerful tools for determining strain relatedness in both outbreaks and routine epidemiology of tuberculosis in a community, allowing for social network analysis.[9,211,586,710] Bacteriophage typing is of comparably limited utility compared with RFLP and other sequencing modalities.

RESISTANCE AND IMMUNITY

Natural resistance to tuberculosis infection varies greatly among animal species; humans, guinea pigs, and rabbits are highly susceptible. However, Lurie[376] experimentally bred resistant rabbits and showed that although the tubercle bacilli disseminated just as well in the resistant rabbits, multiplication within tissues was inhibited. Thus, the differences between resistant and susceptible rabbits appeared to lie in the ability of the resistant rabbits to produce an effective immune response, and this ability seemed to be controlled genetically. Multiple single nucleotide polymorphisms (SNPs) have been associated with disease susceptibility in a number of racial and ethnic groups: CCL2-2518G in Asians and Latinos,[201] *IRGM*[323] and interleukin-12B in African-Americans, and P2X7 and Toll-like receptor 2 and 8 mutations in Turkish children.[138,139,646] However, few mutations, apart from *SLC11A1*,[356,384] have been identified that appear to increase susceptibility across racial and ethnic groups. Many of these mutations are in genes influencing macrophage-mediated destruction of the bacillus.[323,646]

Additional evidence that genetic factors influence susceptibility comes from data involving twins. Kallmann and Reisner[300] noted that when one homozygous twin suffered from tuberculosis, the other twin had a higher

chance of being affected than was the case with heterozygous twins. Gender affects resistance, and females appear to be especially susceptible during adolescence. The negative nitrogen and calcium balance that can arise during adolescence may account in part for susceptibility at this age.[298]

Likewise, young age appears to predispose one to tuberculosis.[451,544,670] However, one cannot be sure that the apparent susceptibility is not due to a larger dose of bacteria because of more intimate contact between very young children and their infectors. Diabetes mellitus is a risk factor for progression from infection to disease, with escalating risk associated with worsening glycemic control.[697] Many viral infections depress tuberculin reactivity, but only measles and perhaps influenza have been incriminated in lowering resistance to tuberculosis.[44,246,729] Unfortunately, natural resistance is ill defined and poorly understood.

Cell-mediated immunity is regarded as most important in host defense against *M. tuberculosis*.[169,355] The T cell–mediated immune response involves a variety of cell subsets that are involved in numerous functions, including protection, delayed hypersensitivity, cytolysis, and establishment of memory immunity.[476] The functions also involve an array of cytokines, several of which direct cells of the monocyte-macrophage axis to contain and destroy the invading bacilli.[15,637] The exact role of individual cytokines is not yet clear, but an emerging concept is that much of the clinical response to the presence of *M. tuberculosis* is determined by the balance of the cellular-cytokine response,[169] which to some degree is under genetic influence.[476] In general, T-helper 1 (Th1) responses are more beneficial to the host than are Th2 responses, in part owing to the former's role in activating macrophages in granulomas.[169] Unfortunately, infants and young children have increased propensity to develop Th2 responses to mycobacterial immunogens.[355] Although different strains of *M. tuberculosis* can evoke different host immune responses, there is no apparent association between the genotype and the phenotype expressed in children with disease.[401]

More details about the immune response to *M. tuberculosis* are emerging as new immunotherapies are developed. During the past decade, several monoclonal antibodies directed against tumor necrosis factor–α have been used to manage inflammatory bowel disease and rheumatologic disorders. Unfortunately, use of these agents, particularly infliximab, has been associated with up to a 25-fold increased risk for tuberculosis disease.[601] All immunocompromised patients, including those who will undergo any form of immunotherapy, should be evaluated carefully for tuberculosis infection or disease.

PATHOGENESIS

Portal of Entry

The tubercle bacillus usually is inhaled. The observations of Riley[524] suggest that a single tubercle bacillus can initiate infection. Ghon, Kuedlich, and their associates (Table 96-2) reported that the primary focus found in 2114 autopsies on children was the lung in 95.93 percent of cases. Especially significant is that their study was done at a time when bovine tuberculosis, which might have produced many primary gastrointestinal foci, was much more common than it is today. Ingestion probably accounts for a small percentage of primary pulmonary foci and for some gastrointestinal foci, particularly in infants who have consumed milk containing bovine tubercle bacilli. *M. tuberculosis* has also been transmitted by organ transplantation.[101] Contamination of marijuana bongs has been associated both with clusters of pulmonary tuberculosis[444] and uvulitis.[229] Contamination of a superficial skin or mucous membrane lesion, such as an abrasion of the sole of the foot or the elbow, insect bite, ritual circumcision, or infection of the vulva, may lead to infection. Infection by inoculation with a sputum-contaminated syringe has been reported in more recent years.[252] True congenital infection, although rare, may be a result of either lymphohematogenous spread in the mother during pregnancy or smoldering endometritis.[672]

TABLE 96-2 **Portal of Entry of Tubercle Bacilli**

Respiratory (%)		Nonrespiratory (%)	
Lung	95.93*	Bowel	1.14
Tonsils	0.09	Skin	0.14
Nose	0.09	Eye	0.05
Middle ear	0.09	Parotid	0.05
Total†	*96.20*	*Total*	*1.38*

*Of 2114 autopsies on children.
†Undetermined, 2.4 percent.
Data from Ghon A, Kuedlich H. Die Eintrittspforten der Infektion. In: Engel S, Pirquet C, editors. *Handbuch der Kindertuberkulose*. Stuttgart: Georg Thieme; 1930.

Incubation Period

The incubation period from the time that the tubercle bacillus enters the body until cutaneous sensitivity develops has been found to be 3 weeks to 3 months.[395] With both bacille Calmette-Guérin (BCG) and experimental infections, the incubation period is shorter when the inoculum is large, and clinical experience suggests that the same is true in humans. Debré, for example, noted long ago that tuberculosis acquired by an infant from its mother was likely to be much more severe than was an infection acquired from a visitor to the home.[148] Animal experiments support this concept. The end of the incubation period coincides with the onset of tuberculin hypersensitivity and may be accompanied by a period lasting 1 to 3 weeks that Wallgren called fever of onset or fever of invasion. At this time, the tissue reaction intensifies throughout the primary complex and may permit the complex to be visible on radiographs.

The "Timetable" of Tuberculosis

Wallgren's tremendous experience with tuberculous children in institutions permitted him to recognize and to describe the usual early course and timing of the initial infection and each of its best-known complications.[692]

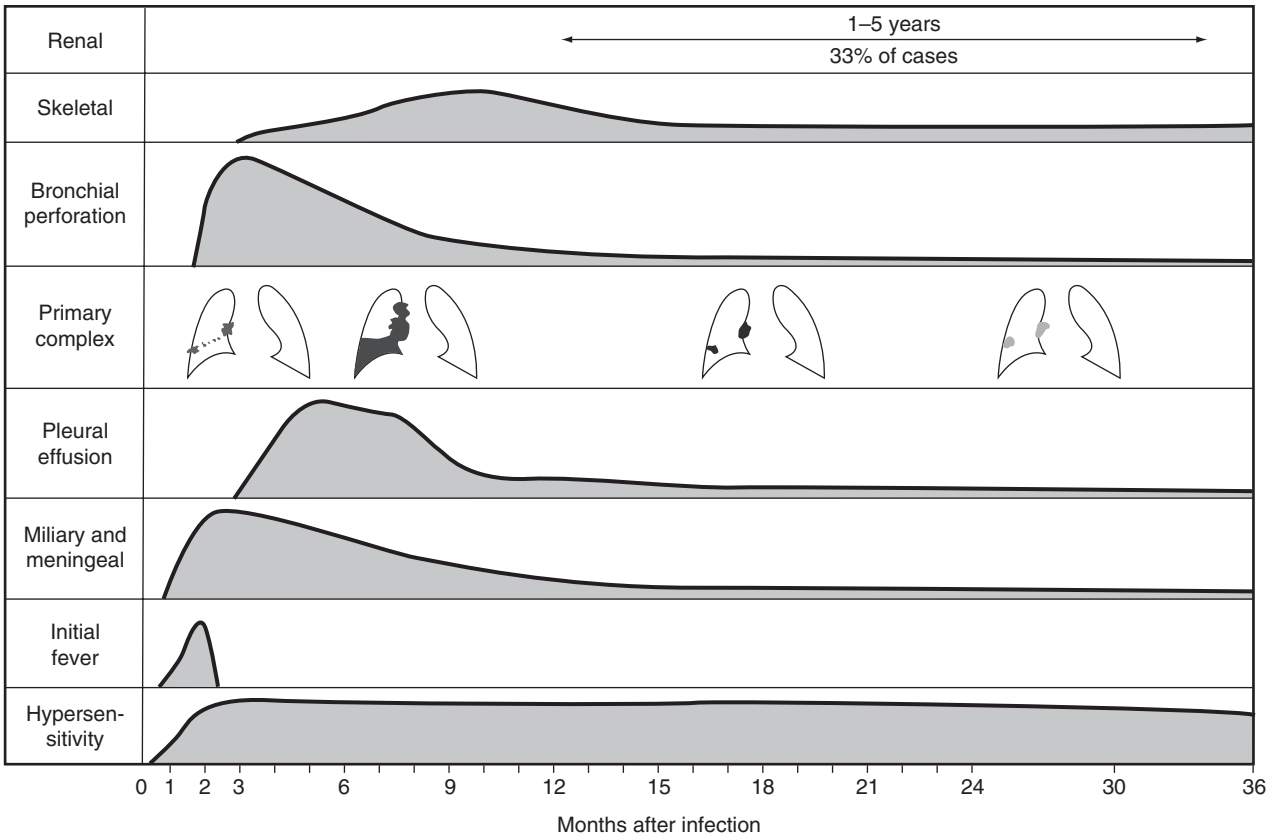

FIGURE 96-5 ■ The timetable of tuberculosis.

His timetable concept is an extremely useful one for clinicians because it permits a realistic prognosis, an understanding of what complications to look for and when, and a more productive approach to finding the infectious source case (Fig. 96-5).[395] Of young adults who progressed from infection to disease, 45 percent developed symptoms within 1 year, 62 percent within 2 years, and 83 percent within 5 years of contact with a source case.[64] One Peruvian study followed contacts of patients with MDR and XDR tuberculosis and showed that 2.6 percent had disease at the time of the contact investigation and that 6 percent developed disease during the 4-year follow-up. The frequency of disease was almost twice as high in contacts of XDR-TB versus MDR-TB patients.[39]

Symptomatic, massive lymphohematogenous spread (i.e., miliary or acute meningeal tuberculosis) is seen in only 0.5 to 3 percent of infected children. When it does occur, the usual onset is 2 to 6 months after initial infection. Endobronchial tuberculosis, possibly with segmental pulmonary lesions, develops slightly later on average. The metastatic lesions of bones and joints, which can be expected in 5 percent of untreated infected children, usually do not appear until approximately 1 year after infection occurs, at the earliest. Renal lesions come later still, 5 to 25 years after initial infection. The relationship between the anatomic site of tuberculosis and the median age at onset in children is shown in Table 96-3. The interval between the acquisition of initial infection and the appearance of chronic pulmonary tuberculosis is extremely variable but can be months to decades, depending mainly on the age of the child at the time of infection. The interval is likely to be short in adolescents but much longer in infants.

In summary, the first 5 years after initial acquisition of tuberculosis infection in childhood, especially the first year, is when complications usually occur. Later in life, during times of stress, a previously silent or arrested lesion may reactivate and become dangerous to the patient as well as highly infectious to others.

TABLE 96-3 **Median Age of Children* with Tuberculosis by Predominant Site of Involvement, United States, 1988**

Site	No. of Cases (%)	Median Age (yr)
Pulmonary	1213 (77.5)	6
Lymphatic	209 (13.3)	5
Pleural	49 (3.1)	16
Meningeal	29 (1.9)	2
Bone or joint	19 (1.2)	8
Other	15 (1.0)	12
Miliary	14 (0.9)	1
Genitourinary	13 (0.8)	16
Peritoneal	4 (0.3)	13
Not stated	1 (0.1)	—
Total	*1566 (100.0)*	*6*

*Younger than 20 years.

CLINICAL FORMS OF TUBERCULOSIS IN CHILDREN

Endothoracic

Asymptomatic Tuberculosis Infection

Asymptomatic (or latent) infection can be defined as infection associated with tuberculin hypersensitivity and a positive tuberculin test result but with no striking clinical or radiographic manifestations. Computed tomography (CT) may reveal enlarged lymph nodes in the chest, even though the plain radiograph is normal.[18,150] On occasion, low-grade fever is found at the onset, usually by chance. If the child has been in recent contact with a person who has contagious tuberculosis and the tuberculin test result is positive, disease should be ruled out immediately with a chest radiograph and a thorough physical examination. Asymptomatic tuberculosis infection occurs more frequently in children of elementary school age than in adolescents or infants. Forty to 50 percent of infections in infants younger than 1 year and 80 to 95 percent of infections in older children can be expected to cause no specific recognizable symptom or radiographic findings.[491] Gastric washings in these patients, even when performed with great care, yield a very low percentage of positive results.

A clinician making a diagnosis of tuberculosis infection in a child must assume, however, that the patient might be in the earliest stage of infection and at risk for the development of symptomatic disease in the near future.[395] A careful history and investigation of contacts should be undertaken immediately for determination, if possible, of the date of exposure. Chemotherapy must be started, and the patient closely monitored not only to detect any toxic effect of chemotherapy and to monitor adherence with treatment but also to be sure that disease does not develop.

The Endothoracic Primary Complex and Its Complications

The primary complex, described by Ghon,[213] includes three elements: the primary focus, lymphangitis, and regional lymphadenitis. This complex holds true for every primary infection, regardless of the portal of entry. Ghon noted that at least 70 percent of primary pulmonary foci are subpleural. Thus, pleurisy is almost a regular feature of the primary complex.

Evolution of the primary pulmonary focus begins with an acute inflammatory reaction around tubercle bacilli inhaled into an alveolus, with the localized alveolar consolidation varying from the size of a pea to the size of a walnut. Macrophages appear within hours in the inflammatory exudate and change into clusters of epithelioid cells to form tubercles. In turn, these tubercles may resolve and disappear or central caseation consisting of incomplete cell autolysis may develop. The caseous lesion contains large numbers of multiplying tubercle bacilli that spread rapidly from the primary focus through the regional lymphatic vessels to the regional lymph nodes, with areas of inflammation being set up along the way that later may caseate and calcify.[502]

TABLE 96-4 **Location of the Primary Pulmonary Focus**

Location	No. of Patients	%
Right upper lobe	138	27
Right middle lobe	40	7
Right lower lobe	107	20
Left upper lobe	122	24
Left lower lobe	104	20
Total	*511*	*98*

Data from Ghon A, Kuedlich H. Die Eintrittspforten der Infektion. In: Engel S, Pirquet C, editors. *Handbuch der Kindertuberkulose.* Stuttgart: Georg Thieme; 1930.

The primary pulmonary focus has been studied carefully by numerous investigators (Table 96-4). Plotting their locations on a normal chest radiograph creates a pattern resembling the scatter of birdshot on a paper target. Thus, all parts of the lung apparently are at equal risk for being seeded. The thought that the primary focus has a predilection for the lower fields of the lung probably arises from the fact that the lung is pyramid shaped, with more basilar than apical lung tissue.

Many investigators concur that 70 to 85 percent of primary infections are initiated by one focus.[123,639] In a study of 170 cases, Ghon[213] found two foci in 15 percent, three in 7 percent, four in 3 percent, and five in 2 percent. Multiple lung foci can result, although rarely, from the ingestion and inhalation of tubercle bacilli. This pattern of disease was shown clearly in the results of pathologic studies of the 71 infants who died in the Lübeck disaster of the 1930s, an incident in which 251 neonates mistakenly had been given live tubercle bacilli by mouth instead of BCG vaccine. Fifteen of the neonates were found to have primary lung lesions at autopsy, and all 251 had primary intestinal lesions.

Although the lymphadenitis cannot be detected clinically and rarely is apparent even on radiographs, the hallmark of initial tuberculosis infection is the relatively large size and importance of the adenitis as opposed to the relatively insignificant size of the initial focus in the lung, skin, or elsewhere. The development of tuberculin hypersensitivity within 3 to 12 weeks after initial acquisition of infection enhances the cellular reaction throughout the primary complex, but with a particularly prominent effect on the primary focus and the regional lymph nodes. At this time, the infection may spread along nearby lymphatic chains to involve more distant nodes. The lymphatic drainage of the lungs is outlined in Table 96-5; it occurs predominantly from left to right. One is not surprised, therefore, that the nodes in the right upper paratracheal area appear to be the ones most often affected. Many primary lesions are subpleural, and the lymphatic drainage of the apical pleura is to the cervical nodes. Moreover, the paratracheal chains have communications with both the deep cervical nodes and the abdominal nodes, as shown in Figure 96-6.

TABLE 96-5 Lymphatic Drainage of the Lung

Right upper lobe	→	Right paratracheal chain
Right middle lobe	→	Right and left paratracheal nodes
Right lower lobe	→	Subcarinal nodes
Left upper lobe	→	Left paratracheal nodes
Left lower lobe	→	Left paratracheal nodes
Lingula	→	Subcarinal nodes
Subcarinal nodes	→	Right paratracheal nodes

Based on data of Rouviere, cited in Courtice FC, Simmonds WJ. Physiological significance of lymph drainage of the serous cavities and lungs. *Physiol Rev* 1954;**34**:419–42.

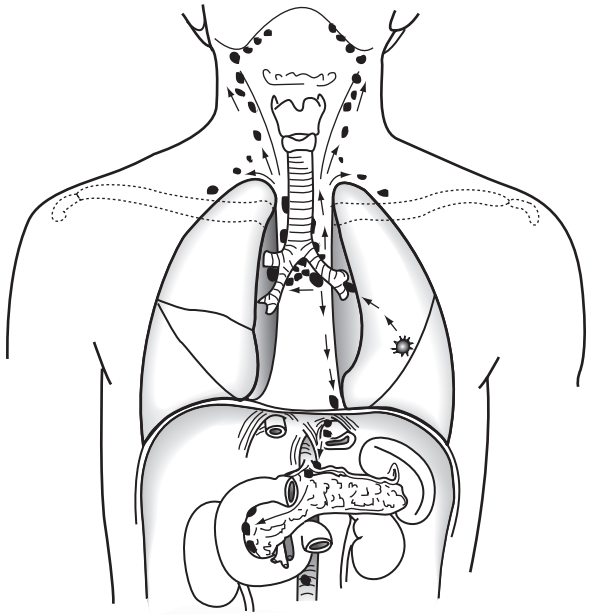

FIGURE 96-6 ■ Schematic composite drawing illustrating wide lymphogenic spread of the tuberculosis infection from a primary pulmonary focus in the base of the left upper lobe. The infection extended cephalad to the submandibular nodes in the head and caudad as far as the pancreatic nodes in the abdomen. (From Caffey J. *Pediatric x-ray diagnosis*. 7th ed. Chicago: Year Book; 1978.)

Bronchial obstruction as a result of enlargement of the peribronchial lymph nodes was reported by Ghon[188] and others. Not until the 1920s did Wallgren[690] and others elucidate the role of these large nodes in producing the radiographic shadows variously called epituberculosis, collapse-consolidation, and segmental lesions that so often are seen in cases of childhood tuberculosis.[362,394,690] At the end of the incubation period, as tuberculin sensitivity develops, the hilar lymph nodes enlarge greatly and, in many cases, caseous foci appear within them. Acid-fast studies of smears and sections have confirmed that this caseum has few tubercle bacilli. As the nodes enlarge, they frequently impinge on the neighboring regional bronchus and compress it and cause diffuse inflammation of its wall, even to the point of obstructing the lumen.[362,557,608] Daly and colleagues,[141] in their study of endobronchial tuberculosis in children at Bellevue, found this mechanism to be the cause of obstruction in half their patients. Other mechanisms of obstruction include damage to the bronchial cartilage leading to gradual (or, rarely, abrupt) perforation of the bronchus and the formation of plugs of semiliquid toothpaste-like caseum that partially or completely occludes the bronchus. In some cases, endobronchial granulomatous tissue forms around the stoma of the fistula and obstructs the lumen.

Three immediate results of bronchial obstruction are possible. The first is sudden death by asphyxia, fortunately an extremely rare event.[343]

The second possible result is obstructive hyperaeration (also called *obstructive emphysema*) of a lobar segment, a lobe, or even an entire lung. This unusual result usually affects children younger than 2 years old, and it may be accompanied by wheezing. Physical examination generally is of little help; radiographs, best taken on expiration, show hyperaeration, which usually is not accompanied by mediastinal displacement, probably because of fixation by the tuberculous mediastinal nodes (Fig. 96-7). Aspiration of a foreign body always must be considered in the differential diagnosis. The obstruction ultimately resolves by itself; however, corticosteroids may be added to the chemotherapeutic regimen to hasten recovery.[452,453,660] Surgical removal of the obstructing nodes has been successful but rarely is performed.[147]

The third possible result of bronchial obstruction is the appearance of a segmental lesion that is fan shaped on a radiograph, representing mainly atelectasis and almost always involving the very segment occupied by the primary pulmonary focus[339,394,437] (Fig. 96-8). Actually, the radiographic opacity results from a combination of several elements: the primary pulmonary focus, the caseous material from an eroded bronchus, the inflammatory response elicited by the caseum, and atelectasis. In some instances, acute secondary infection plays a role. Children with secondary bacterial pneumonia often initially have high fever, cough, and rales; the signs and symptoms respond to conventional antibiotics, but the chest radiographic findings usually do not clear because of the underlying tuberculosis. The relative roles of these various elements often cannot be assessed; sometimes atelectasis is conspicuous, and at other times consolidation is the salient pathologic process, with the volume of the segment undiminished or even increased (hence the descriptive term *collapse-consolidation lesion*, preferred by some clinicians to the less accurate term *atelectasis*).

The percentage of infected children in whom segmental lesions develop has been estimated by several investigators. All agree that the younger the child, the more frequently collapse-consolidation lesions occur (Table 96-6). The segmental lesion is likely to form during the first 3 to 6 months after acquisition of infection (50 of 65 in Payne's series[491]). Multiple segmental lesions can occur simultaneously (18 of 160 children in Payne's experience). When multiple lesions develop, the segmental lesions usually form in one lung, but occasionally the lobes or segments of both lungs are affected. Sometimes, segmental lesions and obstructive hyperaeration occur simultaneously. The physical signs and symptoms of segmental lesions—cough, rales, localized wheezing,

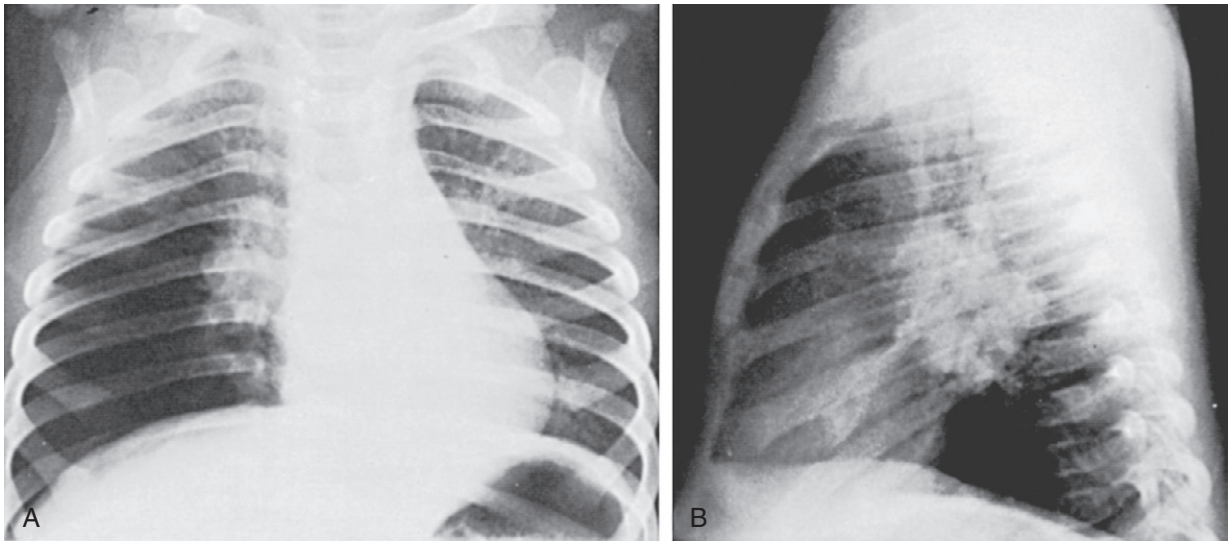

FIGURE 96-7 ■ Radiograph of an 8-month-old girl with obstructive hyperaeration of the right lower lobe as a result of tuberculosis. **A,** Note the hyperlucent right lower lobe and shift of the heart and mediastinum away from the ball-valve obstruction to the left. **B,** Note the large hilar lymph nodes that are compressing the right lower lobe bronchi. Tuberculosis should be considered in patients with hyperaeration of unknown cause.

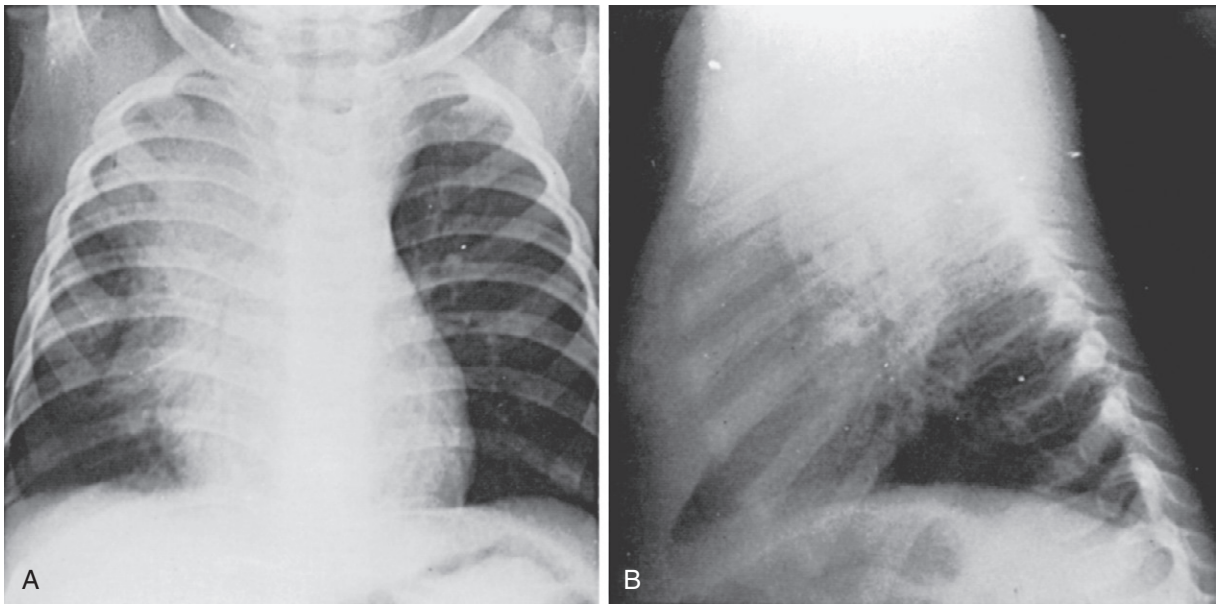

FIGURE 96-8 ■ **A** and **B,** Radiographs of an 8-month-old boy with primary tuberculosis. The posteroanterior film shows collapse-consolidation of the right upper lobe, hilar and paratracheal adenopathy, and pleural reaction. Note the narrowed right bronchi.

egophony—are surprisingly meager but are seen more frequently in infants because of the smaller size of their airways.

Although segmental lesions and hyperaeration are the most common findings produced by enlarging thoracic lymph nodes, others occur. Enlarged peritracheal nodes may cause stridor and respiratory distress.[391] Subcarinal nodes may impinge on the esophagus and cause difficulty swallowing, followed occasionally by the formation of an esophageal diverticulum, or the nodes may rupture directly into the esophagus and produce a bronchoesophageal fistula. Enlarged lymph nodes may compress the subclavian vein and produce edema of the hand and arm, or they may erode major blood vessels, including the aorta. They also may rupture into the mediastinum and point in the left or more often the right supraclavicular fossa. Compression of the left recurrent laryngeal nerve has been reported. Compression of the left phrenic nerve leads to paralysis of the left leaf of the diaphragm in an estimated 0.1 to 0.3 percent of tuberculous children. Rupture into the pericardial sac is described later.

The late results of bronchial obstruction include the following possibilities: complete reexpansion of the lung and resolution of the radiographic findings; disappearance of the segmental lesion, with residual calcification of the primary focus or the regional lymph nodes; or

TABLE 96-6 **Percentage of Tuberculin Converters and Age at Which Segmental Lesions Develop**

Age at Infection (yr)	Converters with Segmental Lesions		
	NUMBER	PERCENT	TOTAL CONVERTERS (NO.)
Birth-1	77	43	180
1-5	35	24	147
6-10	31	25	121
11-15	16	16	97
Total	*159*	*29*	*545*

After Payne M, cited in Miller FJW, Seale RME, Taylor MD. *Tuberculosis in children.* Boston: Little, Brown; 1963.

scarring and progressive contraction of the lobe or segment, usually associated with bronchiectasis.[362,395] Permanent anatomic sequelae result from segmental lesions in approximately 60 percent of all cases, even though the abnormality usually is not apparent on plain radiographs. Cylindrical (rarely saccular) bronchiectasis, sometimes stenoses, and elongation or shortening can be demonstrated on bronchography. Fortunately, most of these abnormalities are asymptomatic in the upper lobes. However, secondary infection may occur in the middle and lower lobes and cause the middle lobe syndrome.[72] On occasion, the chronic vascularity that accompanies bronchiectasis leads to poor oxygen saturation during exercise and to restricted body growth. In addition, bronchogenic carcinoma may arise years later in the old scarred lesions remaining in the bronchus.

Calcification of the primary complex, when it appears, always results from caseation. In calcified caseum, as in bone, the predominant calcium salt is tribasic calcium phosphate. Calcification of caseous lesions occurs much more readily in children than in adults, probably because children's calcium and phosphorus plasma levels are higher. In the infants who were victims of the Lübeck disaster, autopsy showed calcification to be present as early as 58 days after the onset of massive infection. Payne,[491] in a study of calcification in 299 children from Newcastle-on-Tyne, reported calcification visible on the chest radiographs of one child within 6 months of infection and in 38 within 12 months, 104 within 18 months, 165 within 24 months, 252 within 3 years, and all 299 within 4 years.

Calcium usually is deposited as fine particles and creates a stippled effect (Fig. 96-9), but it may be deposited in large, even enormous masses. Calcification may persist without much change, or it may start resorbing within 5 years and eventually disappear completely. It occasionally progresses to ossification with the formation of true bone and functional bone marrow. Calcification, if it is visible at all on radiographs, most often involves the regional lymph nodes.[68] Sometimes, however, the primary pulmonary focus or the entire primary complex, including the lymphangitis, calcifies (Fig. 96-10).

Calcification took place in 75 to 80 percent of the 525 children with pulmonary tuberculosis monitored by Payne.[491] Currently, extensive calcification occurs uncommonly in the Western world, probably because tuberculous lesions treated early with isoniazid rarely caseate and caseation is a prerequisite for calcification.

Pleural Effusion

Pleural effusion can be localized or generalized and unilateral or bilateral.[360] The majority of children will have concomitant pulmonary parenchymal disease and/or intrathoracic lymphadenopathy.[132] All tuberculous serous effusions probably originate in the discharge of bacilli into the cavity from an adjacent lesion—in the case of the pleura, from a subpleural pulmonary focus or from subpleural caseous lymph nodes. The breakthrough may be small and the pleuritis localized and asymptomatic, or it may occur in the form of a generalized effusion, usually 3 to 6 months after infection occurs (Fig. 96-11). In 75 percent of Wallgren's[690] cases, later calcification of the pulmonary focus or regional lymph nodes proved the effusion to be on the same side as the original primary focus. Seemingly without logical explanation are the following clinical observations: tuberculous pleural effusion is a rare occurrence in children younger than 2 years and an uncommon one in children younger than 5 years (perhaps because sensitivity to tuberculin is lower in the very young), occurs more frequently in boys than in girls, almost never is associated with a segmental lesion, and rarely is associated with miliary tuberculosis.

The onset of pleurisy usually is abrupt and resembles bacterial pneumonia, with fever, chest pain, shortness of breath, and, on physical examination, dullness to percussion and diminished breath sounds. Fever may be high and, in untreated cases, last for several weeks. On occasion, differentiation of an effusion from an extensive pneumonic lesion is difficult; lateral decubitus radiographic views are helpful in confirming the presence of pleural fluid.

Thoracentesis is, of course, the essential diagnostic procedure. The puncture should be made in the area shown on the radiograph to have the greatest accumulation of fluid. No more than 30 mL of pleural fluid should be withdrawn; otherwise, the protein loss from this usually protein-rich fluid may be considerable. The fluid generally is greenish yellow, occasionally tinged with blood, with a specific gravity of 1.012 to 1.022, a high protein content, and often a low glucose level (<30 mg/dL), and it has several hundred white cells per cubic millimeter with a predominance of neutrophils or lymphocytes, depending on the age of the effusion. Polymerase chain reaction (PCR), adenosine deaminase levels of 38 IU/L, and absolute lymphocyte count of greater than or equal to 275 cells/mm^3 in pleural fluid had a sensitivity and specificity of 74 percent and 88 percent, 81 percent and 75 percent, and 90 percent and 83 percent, respectively, in one study of children with tuberculous effusions.[426] The cells in tuberculous pleural effusion are predominantly T lymphocytes, which are present in a higher proportion than in blood.[193,577] Tubercle bacilli generally are present in such small numbers that results from direct smears and cultures are likely to be disappointing; smears almost always are negative, and pleural fluid cultures are positive in less than 30 percent of cases.

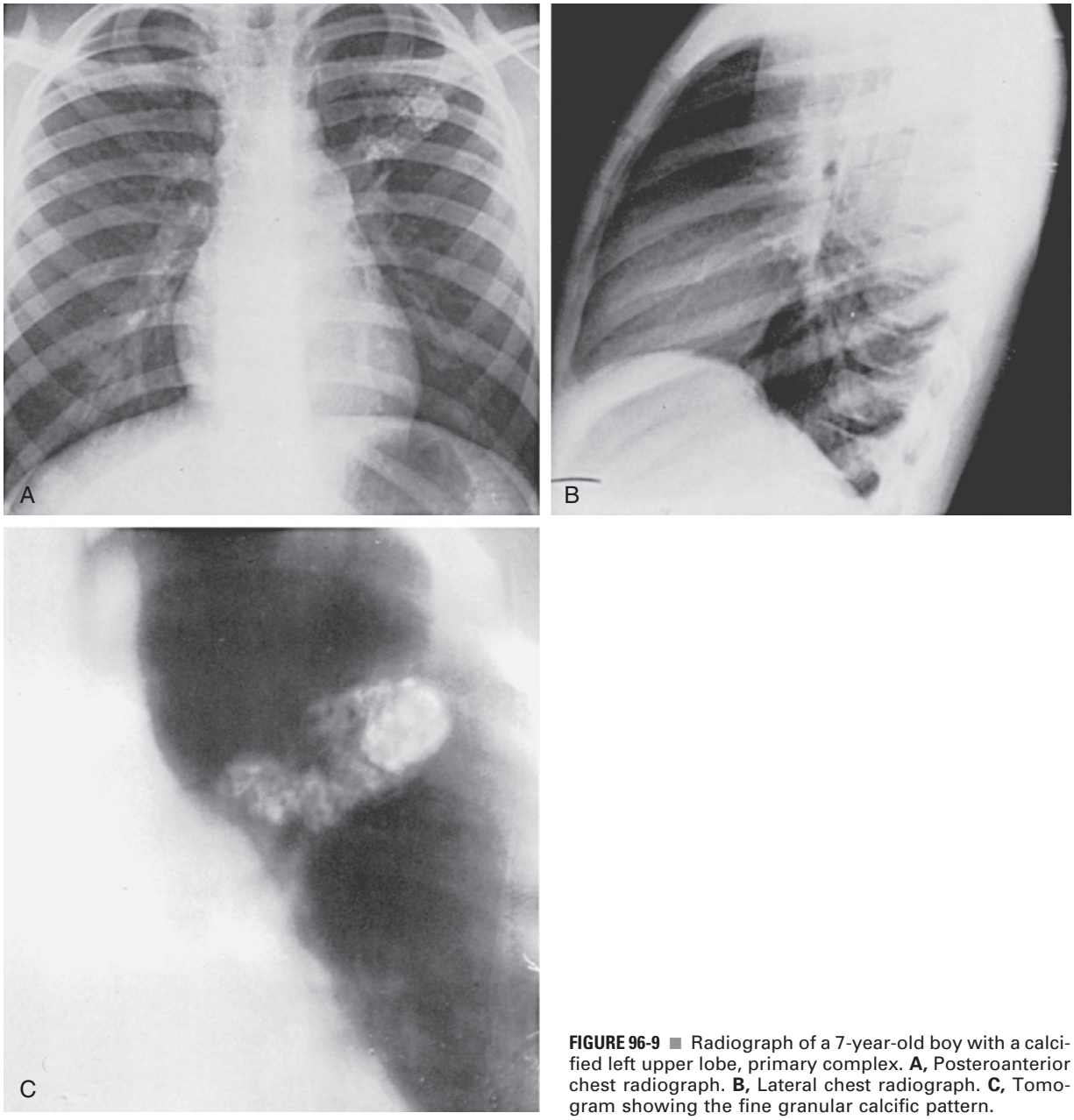

FIGURE 96-9 ■ Radiograph of a 7-year-old boy with a calcified left upper lobe, primary complex. **A,** Posteroanterior chest radiograph. **B,** Lateral chest radiograph. **C,** Tomogram showing the fine granular calcific pattern.

Pleural biopsy is a useful diagnostic procedure because both the finding of typical tubercles on histologic study and culture of the tissue are much more likely to establish the diagnosis than is culture of pleural fluid.[354]

The prognosis for children with tuberculous effusion always has been relatively good compared with that for other overt forms of tuberculosis, even in the days before chemotherapy.[364] Clinical improvement occurs well before radiographic resolution of disease, and the majority of children have radiographic abnormalities at the completion of therapy.[132] Permanent impairment of pulmonary function is a surprisingly uncommon event after pleural effusion.[204] The development of scoliosis is a remote possibility and should be guarded against while the patient recovers.

Progressive Pulmonary Tuberculosis

In this serious complication of the primary complex, the primary pulmonary focus, instead of resolving or calcifying, enlarges steadily and develops a large caseous center. This center then liquefies and empties into an adjacent bronchus to create a "primary cavity"[644,677] (Fig. 96-12); the liquefaction is associated with particularly large numbers of tubercle bacilli. The tubercle bacilli further disseminate to other parts of the lobe and to the entire lung, where other foci of infection form. On rare occasion, an enlarging primary focus ruptures into the pleural cavity and creates a pneumothorax, bronchopleural fistula, or caseous pyopneumothorax or ruptures into the pericardial sac or the mediastinum.

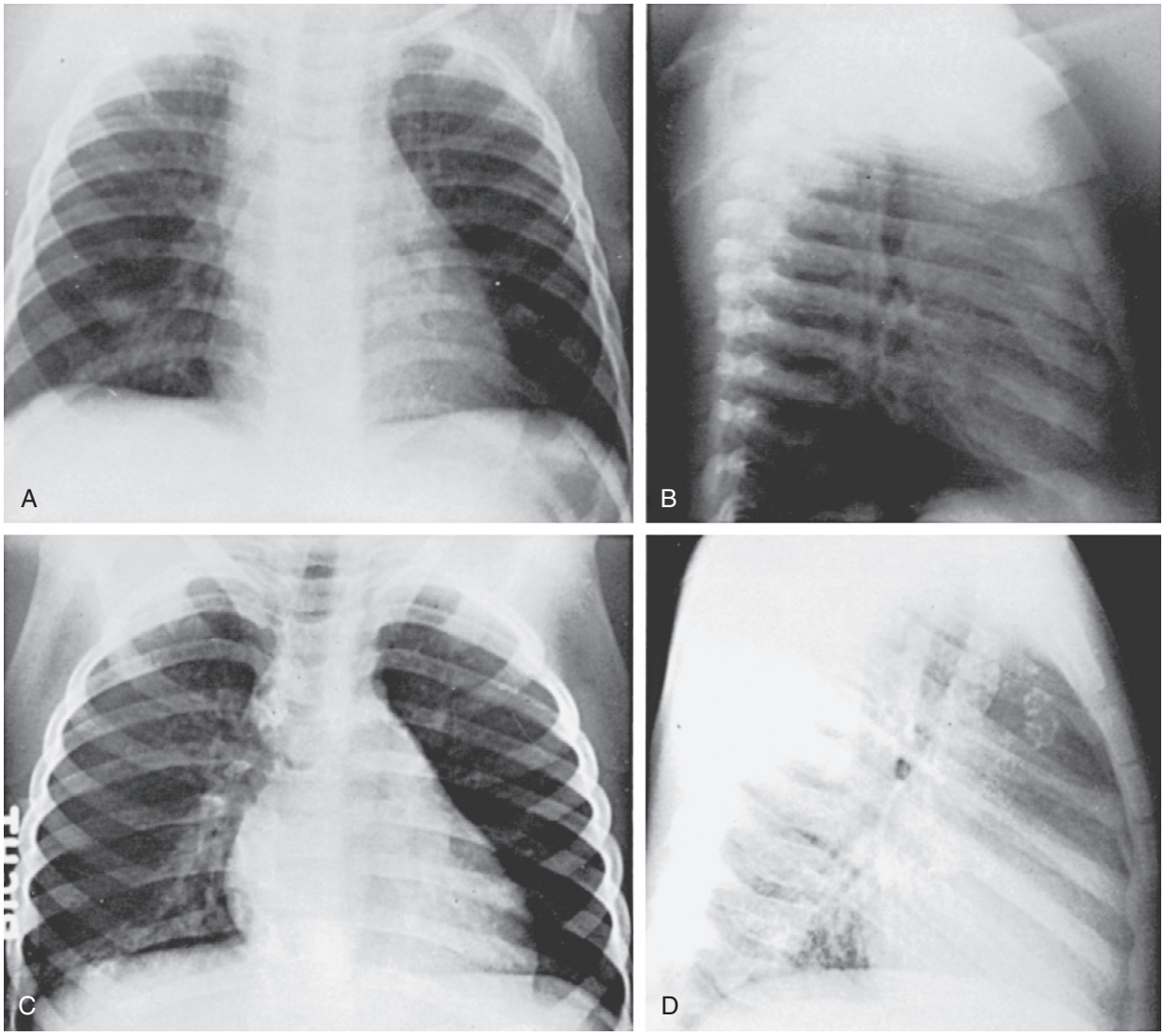

FIGURE 96-10 ■ **A** and **B,** Radiographs of a 7-month-old girl with mild fullness of the right upper mediastinum and a hazy infiltrate in the right lower lobe. The result of a skin test for tuberculosis was positive. She was treated for tuberculosis and clinically improved. **C,** The same patient 3.5 years later has a calcified primary complex in the right upper and lower lobes over the diaphragm. **D,** The anterior location of the upper lobe complex is seen clearly on the lateral chest radiograph.

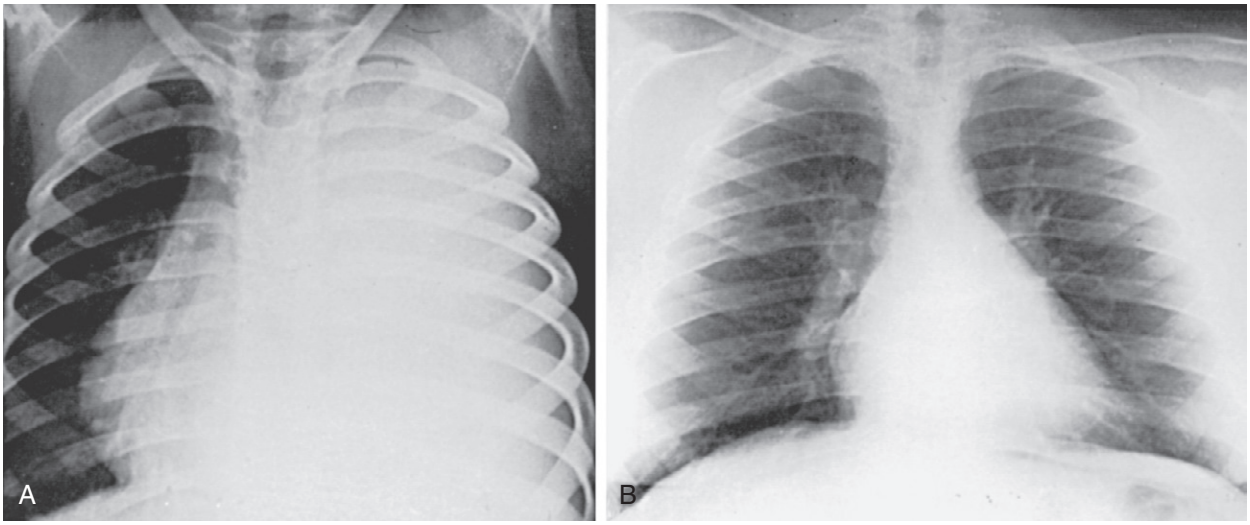

FIGURE 96-11 ■ **A,** Posteroanterior chest radiograph of a 5-year-old boy with massive left pleural effusion caused by tuberculosis. **B,** Normal radiograph 6 years later from the same patient who had no physical complaint.

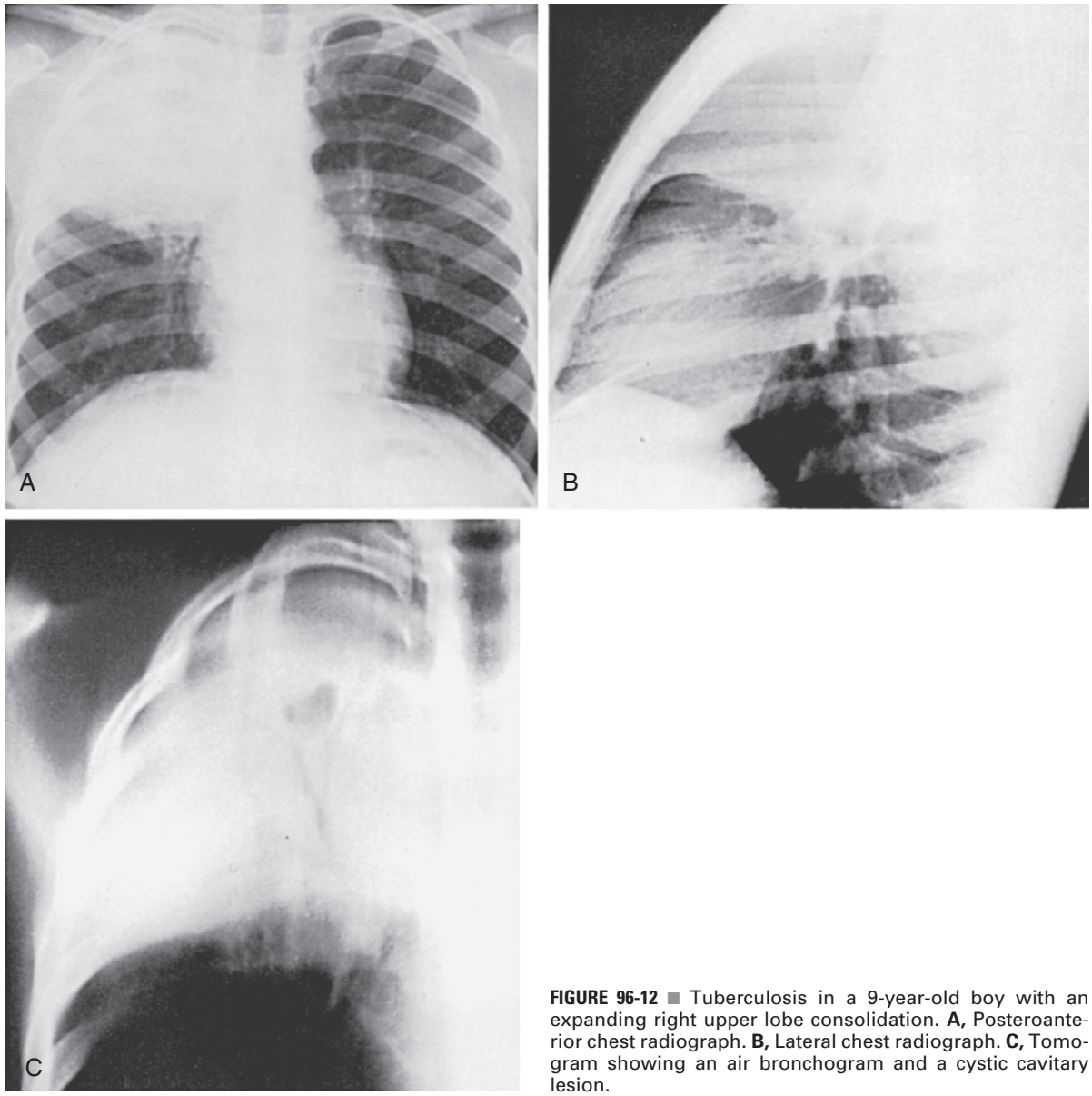

FIGURE 96-12 ■ Tuberculosis in a 9-year-old boy with an expanding right upper lobe consolidation. **A,** Posteroanterior chest radiograph. **B,** Lateral chest radiograph. **C,** Tomogram showing an air bronchogram and a cystic cavitary lesion.

Although clinical symptoms often are minimal when the primary focus is uncomplicated, a progressive lesion often is accompanied by more severe fever, cough, malaise, and weight loss as well as classic signs of cavitation, such as egophony.[227,545] Before chemotherapy was available, the inability to contain the primary focus was associated with a grave outlook; 25 to 65 percent of patients thus affected died. Now, with appropriate treatment, the prognosis is good.

Distinguishing between progressive pulmonary tuberculosis and a simple tuberculous focus with a superimposed acute bacterial pneumonia caused by *Staphylococcus*, *Klebsiella*, or anaerobes may be difficult. Antimicrobial agents effective against these pathogens may be indicated, in addition to appropriate antituberculosis drugs. Sometimes, especially during convalescence from pulmonary parenchymal lesions, bullous lesions appear and persist for several months. They seem to be associated, in children as in adults, either with "tears" in damaged alveolar walls or with the emptying of caseum out of cavities.[407]

Chronic Pulmonary Tuberculosis

Chronic pulmonary tuberculosis, sometimes referred to as adult or reactivation tuberculosis, is the type of disease seen in pulmonary tissue sensitized and immunized by an earlier tuberculosis infection. For many years, ongoing debate centered on whether the lesions of chronic pulmonary tuberculosis, more localized than those of the initial tuberculous lesion and less likely to spread to lymph nodes and the bloodstream, were due to "endogenous reinfection." Evidence that has accumulated during subsequent decades indicates that endogenous reinfection is the usual event.[619] However, reinfection with a different strain of *M. tuberculosis* has been documented and may be more common in areas where tuberculosis is prevalent.[587]

Careful long-term studies of children have revealed a continuum of involvement in many cases: first the primary focus, followed within a few years in some patients by infraclavicular small round foci in the lung apices that often were calcified (Assmann foci, Simon foci) and thought to result from hematogenous spread at the time of initial infection. Later, these foci disappear spontaneously or remain visible as tiny calcifications or as larger "round foci," which may, if untreated, progress to the typical lesions of chronic pulmonary tuberculosis.

Even before antituberculosis drugs were discovered, chronic pulmonary tuberculosis was a rare occurrence in children (e.g., 6% to 7% in the series of Lincoln and colleagues[361] of closely monitored patients at Bellevue Hospital). It appears more frequently in children in the lower socioeconomic strata of society and more frequently in girls than in boys. Chronic pulmonary tuberculosis rarely develops in children who survive with a healed, untreated tuberculosis infection acquired before they have reached 2 years of age, but it is much more frequent in children who acquire their initial infection after reaching 7 years of age and particularly if they become infected close to the onset of puberty. In that case, the "adult" type of lesion often develops in the very lobe where the primary lesion occurred. In this situation, progressive pulmonary tuberculosis cannot be differentiated from chronic pulmonary tuberculosis. Now, with effective treatment, such differentiation is unimportant.[240]

Cough, fever of unknown origin, chest pain, hemoptysis, and supraclavicular adenitis are the most common clinical manifestations. Essential diagnostic procedures are the tuberculin skin test and appropriate chest radiographs, often including such special procedures as tomograms and lordotic views. An intense search for tubercle bacilli must be made in sputum, gastric washings, and, if necessary, secretions obtained by bronchoscopy.

Myocardial and Pericardial Tuberculosis

Tubercles often are found in the heart in miliary tuberculosis. Although it is exceedingly rare, myocardial caseation has been described that is usually secondary to direct spread from mediastinal glands and accompanied by paroxysmal tachycardia or arrhythmias.[691]

Tuberculous pericarditis, although more common than symptomatic tuberculous myocarditis, occurred in only 0.4 percent of 2500 children monitored by Lincoln and Sewell[364] at Bellevue Hospital and in 4 percent of 200 children in Boyd's series.[66] It occurs more commonly in males than in females. In most cases, tuberculous pericarditis probably arises by direct invasion or by lymphatic drainage from caseous lymph nodes in the subcarinal area or from nodes close to the ductus arteriosus, with resulting exudation of hemorrhagic fluid and the development of granulation tissue on both the parietal and visceral surfaces of the pericardium. Pericardial fluid may be serofibrinous or hemorrhagic; tubercle bacilli rarely are found on smears.[273] Sometimes, extensive fibrosis leads to obliteration of the pericardial sac, with the development, usually years later, of constrictive pericarditis.

The initial symptoms generally are nonspecific: low-grade fever, poor appetite, failure to gain weight, and, rarely, chest pain. On examination, a pericardial friction rub may be heard, or if a large effusion already is present, distant heart sounds, tachycardia, and a narrow pulse pressure may suggest the diagnosis. The diagnosis then is confirmed by radiography, echocardiography, electrocardiography, tuberculin skin test, and aspiration of fluid for culture. Before the advent of chemotherapy, approximately half the patients succumbed. Now, with appropriate drugs and possibly corticosteroids to diminish the size of the effusion[633] and also occasional partial pericardiectomy, the outlook is excellent.

Lymphohematogenous Spread

Tubercle bacilli from the lymphadenitis of the primary complex are disseminated during the incubation period in all cases of tuberculosis infection. The results of liver biopsy of young asymptomatic tuberculin converters (indicating recent infection) show that the liver always is involved.[117] Tubercle bacilli can reach deep, distant organs through the bloodstream or lymphatic channels. Autopsies on individuals who have died soon after development of initial infection show that bacilli often are deposited in the liver, spleen, skin, and apical pulmonary tissue.

The clinical picture produced by lymphohematogenous spread probably is determined by host susceptibility at the time of spread and by the quantity of tubercle bacilli released.[574,679] Three clinical forms can be recognized:

1. The lymphohematogenous spread may be occult, in which case it usually remains so, or it may be occult initially with metastatic, extrapulmonary lesions appearing months or years later (e.g., renal tuberculosis).[521]
2. So-called protracted hematogenous tuberculosis, rarely seen today, is characterized by high, spiking fever, marked leukocytosis, hepatomegaly and splenomegaly, and general glandular enlargement, sometimes with repeated evidence of metastatic seeding in the choroid, kidneys, and skin. Calcifications may appear subsequently, often in large numbers, in the pulmonary apices (Simon foci) and in the spleen, thus attesting to the earlier dissemination of tubercle bacilli through blood. The tuberculin skin test result usually is strongly positive. Bone marrow biopsy may confirm the clinical impression, but treatment often must be started on a presumptive basis.[527] Although this type of tuberculosis in past years often ended tragically in tuberculous meningitis, today it is completely treatable if it is diagnosed in time.
3. The third form of lymphohematogenous spread, analogous to sepsis with pyogenic bacteria, is miliary tuberculosis.[275,574] It usually arises from discharge of a caseous focus, often a lymph node, into a blood vessel such as a pulmonary vein; it may be self-propagating, with repeated discharge arising at various sites. Most common during the first 2 to 6 months after infection in infancy, it can arise even in adults who have apparently well-healed, calcified lesions.[321,556]

Miliary disease provides a striking illustration of the difference in susceptibility of tissue to tubercle bacilli; tubercles tend to be larger and more numerous in the lung, spleen, liver, and bone marrow than in the heart, pancreas, and brain. The number of fixed intravascular phagocytes, as well as the relative tortuosity of the smaller blood vessels themselves, must play an important role in determining tissue susceptibility. In acute caseating miliary tuberculosis, the lesions are likely to be numerous and sometimes almost coalescent.

The clinical picture of miliary tuberculosis varies greatly, probably depending on the number of bacilli in the bloodstream. Sometimes, the patient is afebrile and appears to be well, and the condition is diagnosed by chance during contact investigation of another individual with infectious tuberculosis. The onset can be insidious, often occurring after the patient has had another precipitating infection. In rare cases, the onset is abrupt. Drowsiness, loss of weight and appetite, persistent fever, weakness, rapid breathing with a rustling sound on auscultation of the lungs, occasionally cyanosis, and almost always a palpable spleen are the clinical manifestations that lead the clinician to obtain a chest radiograph.

Usually within no more than 3 weeks after the onset of symptoms, tubercles, sometimes tiny and at times large, can be seen evenly distributed throughout both lung fields[473]; in the early stages, they often are detected best on a lateral view of the retrocardiac space (Fig. 96-13). The incidence of choroidal tubercles varies greatly; it has been reported variously as 13 and 87 percent. Recurrent pneumothorax, subcutaneous emphysema, pneumomediastinum, and pleural effusion are less serious but well-recognized complications of miliary tuberculosis. Second attacks have been reported.[716] Cutaneous lesions, including painful nodules, papulonecrotic tuberculids, and purpuric lesions, may appear in crops.[314]

The diagnosis usually is established by means of the clinical picture and a chest radiograph. Sometimes it is confirmed by a liver or skin biopsy; by culture of *M. tuberculosis* from the gastric aspirate, urine, or bone marrow[117,245]; or by fiberoptic bronchoscopy and transbronchial biopsy.[74] Treatment usually is successful.

Extrathoracic Spread

Central Nervous System Tuberculosis

Tubercle bacilli are distributed by the bloodstream into all parts of the central nervous system (CNS) during lymphohematogenous spread.[363,613] Surprisingly, they do not multiply as well in nervous tissue as in other areas such as the lung. Thus, CNS tuberculosis, although an early manifestation of infection, usually does not appear simultaneously with miliary spread but days later. The tubercle bacilli can affect the CNS in various ways[668,686] and produce tuberculous meningitis,[278,636] serous meningitis,[358,668] tuberculoma,[661,668] or tuberculous brain abscess, or it can affect mainly the spinal cord and cause spinal tuberculous leptomeningitis.[465]

Tuberculous meningitis arises from caseous foci, often very small ones, situated in the brain or meninges.[522] These lesions arise from hematogenous spread early in the infection.[165,290] In time, the caseous foci discharge tubercle bacilli directly into the subarachnoid space. The thick, gelatinous exudate lies in the meshes of the pia-arachnoid in the brain, where it infiltrates the walls of meningeal arteries and veins and produces inflammation, caseation, and obstruction; the exudate can extend along

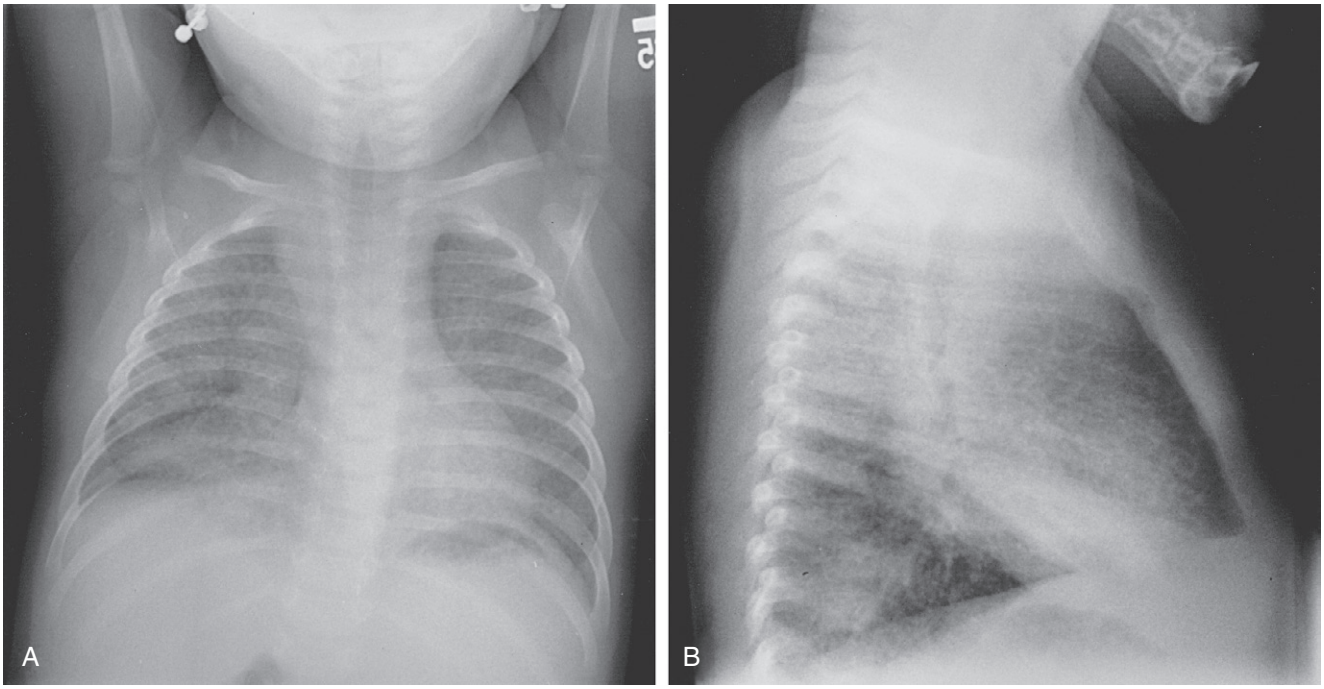

FIGURE 96-13 ■ Miliary tuberculosis in an infant. The numerous tubercles can be seen on both the posteroanterior **(A)** and lateral **(B)** views.

small vessels into the cortex, where it causes occlusion and produces infarcts. This same exudate interferes with normal flow of cerebrospinal fluid (CSF) in and out of the ventricular system and with absorption of the fluid by the pacchionian bodies. The predilection of the exudate for the base of the brain accounts for the frequent involvement of the third, sixth, and seventh nerves and the optic chiasm. The combination of vascular lesions producing infarcts,[353] interference with CSF flow resulting in hydrocephalus, and direct cranial nerve involvement, especially of the eye, causes the devastating damage that all too often results from tuberculous meningitis.[22]

Tuberculous meningitis has been estimated to develop in 1 of every 300 untreated infections.[289] Virtually never seen in infants younger than 4 months, it occurs most frequently in children younger than 6 years, usually appears within 2 to 6 months after initial infection, and accompanies miliary tuberculosis in approximately 50 percent of cases. Tuberculosis should be suspected as the cause of meningitis that is accompanied by cranial nerve involvement, hydrocephalus, or evidence of inflammation at the base of the brain.[673]

The onset of tuberculous meningitis usually is gradual and occurs during a period of approximately 3 weeks; in some cases, it seems to be precipitated by a viral infection, a fall, or a blow on the head. On occasion, the onset is abrupt and marked by a convulsion. A convenient approach is for the clinician to divide the course into three stages. The first stage is characterized by personality change, irritability, anorexia, listlessness, and some fever. After 1 or 2 weeks, the disease passes into the second stage, when signs of increased intracranial pressure and cerebral damage appear: drowsiness, stiff neck, cranial nerve palsies, inequality of the pupils, vomiting, tache cérébrale, absence of the abdominal reflexes, and convulsions that may be tonic or clonic and focal or generalized. The third stage is characterized by coma, irregular pulse and respirations, and rising fever. Papilledema occasionally is noted.

Aids in establishing the diagnosis are a history of contact with an adult who has tuberculosis (however, the family history of tuberculosis often is "negative" because the incubation period of meningitis is short and the contagious adult has not been discovered yet)[159]; a tuberculin skin test (positive result in only 50% of cases); positive tuberculin skin test results in siblings; a chest radiograph, which often reveals pulmonary disease; and changes in, and the characteristic findings from, CSF. However, the ventricular CSF may be relatively normal because it is obtained proximal to the site of inflammation. The lumbar CSF usually is clear and under substantially increased pressure. It will contain 50 to 500 white blood cells/mm^3, with polymorphonuclear leukocytes predominant early and lymphocytes predominant later. The CSF glucose level may be at the lower limits of normal if the patient is examined early in the second stage, and it falls by 5 mg or so each day; by the third stage, it usually is very low. The protein content may be normal at the time of the first spinal tap, but it rises steadily to very high concentrations, at which time a pellicle will develop on the fluid on standing. Tubercle bacilli may be found in the pellicle but are scarce at best. Only approximately 50 percent of cases of tuberculous meningitis in children can be confirmed by culture of CSF. Gastric washings should be cultured, not only to confirm the diagnosis in retrospect but also to permit drug susceptibility testing of the organisms.[723]

CT or magnetic resonance imaging (MRI)[19,22,230,469,707] is recommended for the evaluation of all patients with tuberculous meningitis. Both permit recognition and follow-up of tuberculomas,[667,704] infarction or vasculitis, and hydrocephalus that might require shunting.[22,135,295,627] Involvement of the basal ganglia often is a diagnostic clue for the presence of tuberculosis.[19] Profound abnormalities in the electrolyte pattern of intracellular and extracellular fluid and CSF have been reported in patients with tuberculous meningitis. These abnormalities consist mainly of low sodium and chloride levels, with low or normal plasma potassium levels, accompanied by high sodium levels in red blood cells and skeletal muscle.[241] High levels of antidiuretic hormone (the syndrome of "inappropriate" antidiuretic hormone secretion) may cause hypotonic expansion of the extracellular fluid. Because intense vomiting and dehydration usually accompany tuberculous meningitis, the electrolyte disturbances usually include severe hypochloremia. Salt wasting in the urine occurs in rare cases. Determination of the CSF chloride concentration is useless because it merely reflects the plasma level.

A good prognosis depends on immediate initiation of treatment without awaiting epidemiologic and bacteriologic confirmation of the diagnosis.[274,533] Before chemotherapy was used, every case of true tuberculous meningitis was fatal within 3 to 4 weeks, whereas today appropriate treatment during the first stage allows survival in nearly every case, although the patient's intelligence may never return to its previous level. When it is initiated during the second stage, treatment results in survival in 75 percent or more of patients. If patients are in coma when treatment is begun, they rarely recover unscathed. Very young age and the occurrence of convulsions generally are poor prognostic factors.[533,554] Hydrocephalus, usually of the communicating type, occurs frequently (38% in one series) and is largely responsible for poor outcomes. Relieving the hydrocephalus surgically appears to improve the sensorium, vision, and neurologic deficits. Studies support the early use of ventriculoperitoneal shunting in patients severely ill at the time of admission.[340,482] Infarcts caused by vasculitis also can leave catastrophic residua. The presence of drug-resistant tuberculous meningitis greatly worsens the prognosis,[479] as does coinfection with HIV.[676] The role of corticosteroids in the treatment of intracranial tuberculosis is discussed later in this chapter.

The long-term sequelae of tuberculous meningitis are numerous and include blindness, deafness, intracranial calcification, diabetes insipidus, syndrome of inappropriate antidiuretic hormone secretion, obesity, paraplegia, and mental retardation.[554]

Serous tuberculous meningitis is an uncommon occurrence (13% of 500 cases in the experience of Udani and associates[668]). Apparently, it develops when a tuberculous

focus close to the subarachnoid space causes a lymphocytic reaction in the subarachnoid space without the actual presence of tubercle bacilli.[727] In the days before the advent of chemotherapy, "serous" meningitis was differentiated readily from "true" tuberculous meningitis because the serous type was the only nonfatal form of tuberculous meningitis. Today, such differentiation no longer is possible because treatment is begun immediately, so true tuberculous meningitis does not develop.

Isolated tuberculoma is manifested clinically as a brain tumor.[29,146,160] As many as 30 percent of brain tumors are tuberculomas, depending on the incidence of tuberculosis in the particular population under study (in India, for instance). Tuberculomas occur most often in children younger than 10 years old and often are located at the base of the brain around the cerebellum.[688] In contrast, tuberculomas in adults more often are supratentorial. Headache, convulsions, fever, and other symptoms and signs usually associated with brain abscess or tumor also characterize tuberculoma. Only careful evaluation, including inquiry about exposure to tuberculosis, a tuberculin skin test, chest radiography, and CT, will permit these cases to be recognized in time to begin appropriate chemotherapy before neurosurgical intervention is needed. The widespread use of neuroimaging has revealed that small, multiple tuberculomas are a frequent feature of tuberculous meningitis in infants and young children.[19,22] A phenomenon recognized fairly recently is a symptomatic intracranial tuberculoma appearing and enlarging during treatment of meningeal, miliary, and even pulmonary tuberculosis.[5,350,514,575,650] This phenomenon appears to be mediated immunologically; it usually responds to corticosteroids and does not necessitate a change in antituberculosis chemotherapy. In addition, some small children with severe pulmonary or disseminated tuberculosis have one or several tuberculomas but normal findings on CSF evaluation.[159,291] In one series, HIV-infected children were more likely to have cerebral atrophy and less likely to have intense basal meningeal enhancement than HIV-uninfected children.[149] CT or MRI of the head should be performed whenever neurologic signs or symptoms accompany tuberculosis.

Tuberculous brain abscess is a rarely reported form of CNS tuberculosis that tends to occur at an older age than tuberculoma does.[335,703] On pathologic examination, the lesion lacks the giant cell and granulomatous reaction associated with tuberculoma. Focal neurologic signs occur commonly. CT and MRI, if used routinely in cases of tuberculous meningitis, may permit this type of abscess, which requires surgical intervention as well as chemotherapy, to be recognized more frequently. Intramedullary tuberculoma of the spinal cord is exceedingly rare.[296,378] It can be manifested as recurrent abdominal pain.[51,465]

Spinal tuberculous leptomeningitis occurs more often in older children and adults than in infants[373]; in the series of 500 cases studied by Udani and coworkers,[668] only 2 percent fell into this last age group. Protein levels in the CSF are elevated substantially, and sometimes partial or total block may be noted on myelography. Exudate may completely surround the spinal cord.

Cutaneous Tuberculosis

The manifestations of cutaneous tuberculosis can be classified in children according to a modification of the earlier classifications designed for adults[333]: (1) lesions produced by inoculation from an exogenous source[498] in a previously uninfected child, in a previously infected child, or after inoculation with BCG vaccine; (2) lesions resulting from hematogenous dissemination[314]; (3) lesions arising from an endogenous source; and (4) erythema nodosum. A newer classification has been proposed and perhaps is more useful for adults than for children.[50]

Skin lesions associated with the primary complex may be caused by direct inoculation of tubercle bacilli into a traumatized area, such as a lesion on the sole of a child's bare foot, a mosquito bite on the face, an abraded elbow, or the foreskin at the time of ritual circumcision.[484,563] The initial skin focus usually is a small, painless nodule, sometimes with tiny satellite lesions that soon turn into indolent ulcers without surrounding inflammation. The most striking feature is regional lymphadenitis, which often is what convinces the patient to see a physician. Fever and systemic reactions generally are minimal; low-grade pyogenic infection and cat-scratch fever (*Bartonella* infection) always must be considered in the differential diagnosis. A strongly positive tuberculin skin test result usually is obtained. Needle aspiration and culture may differentiate disease caused by *M. tuberculosis* from that caused by environmental mycobacteria.

Scrofuloderma indicates tuberculosis of the skin overlying a caseous lymph node (most often in the cervical area) that has ruptured to the outside and left either a shallow ulcer or a deep sinus, sometimes surrounded by a cluster of nodules. In the past, scrofuloderma was a frequent manifestation of tuberculosis in children and usually left extensive scars. Today, it is a rare occurrence because the diagnosis generally is made before the node ruptures. Administration of chemotherapy often forestalls the need for surgical excision.

Manifestations that result from hematogenous dissemination are papulonecrotic tuberculids and tuberculosis verrucosa cutis. Papulonecrotic tuberculids[584] are miliary tubercles in the skin that usually appear as tiny papules with "apple jelly" centers, most often on the trunk, thighs, and face. They frequently are similar to papular urticaria or early varicella lesions, or they may be confused with the skin lesions of Letterer-Siwe disease. Skin biopsy provides a reliable diagnosis. Lupus vulgaris is a rare form of chronic, indolent tuberculosis, usually on the face, that often seems to evolve from tuberculids, very rarely at the site of BCG inoculation.

Tuberculosis verrucosa cutis is a condition characterized by large (several centimeters in diameter) papulonecrotic tuberculids. The lesions usually appear on the arms, legs, or buttocks, suggesting that trauma may play some part in their causation. Fungal infection is the main consideration in the differential diagnosis.

Erythema nodosum formerly was a common manifestation of hypersensitivity to tuberculin. It occurs mostly in young teenage girls. Usually beginning with fever and systemic toxicity manifesting soon after initial infection develops, erythema nodosum is characterized by large,

deep, painful, indurated nodules on the shins and sometimes on the thighs, elbows, and forearms. The nodules gradually change from light pink to a bruise-like color. Erythema nodosum is not specific for tuberculosis but also occurs with streptococcal and meningococcal infections, histoplasmosis, coccidioidomycosis, sarcoidosis, drug sensitivity reactions, and perhaps cat-scratch disease. Tuberculin hypersensitivity is pronounced in children with tuberculosis underlying erythema nodosum, and tuberculin skin testing should be performed with caution. These patients may or may not have associated tuberculous lesions, but some clinicians hold that these patients have a greater chance of suffering complications. Skin biopsy reveals only nonspecific changes regardless of cause and, therefore, is useless in establishing the diagnosis. Both fever and nodules clear within 2 or 3 weeks.

Skeletal Tuberculosis

Bone and joint tuberculosis can be expected in 1 to 5 percent of children whose initial infection with tubercle bacilli is untreated. Usually, the tubercle bacilli are disseminated to skeletal structures during lymphohematogenous spread of the initial infection.[35] The disease becomes symptomatic during the first 1 to 3 years after infection occurs. Each of the most frequently involved bones and joints has a characteristic "incubation period" (e.g., 1 month for dactylitis but about 30 months for tuberculosis of the hip). In very young children, blood flow through growing bone is intense; consequently, they suffer from skeletal tuberculosis more often than older children do. The lesion usually starts as an area of endarteritis in the metaphysis of the long bone, where the blood supply is particularly abundant; lesions can be single or multiple.[511,573] Bone infection can be initiated by two other mechanisms, particularly in the vertebrae: (1) direct extension through the lymphatics from a caseous paravertebral lymph node[75] and (2) direct local hematogenous or lymphatic extension from a neighboring bone. As bone is destroyed progressively by pressure necrosis and formation of a cold abscess, a nearby joint may become involved.[512,671,687]

The bones most often affected are the vertebrae.[472] In one study of 1074 patients with the disease, 440 had involvement of the vertebrae; 89, the knee; 81, the hip; and 51, the elbow.[665] The upper extremities and non–weight-bearing bones, such as the skull, clavicle, and mandible, rarely are involved.[244,247] Often a history of trauma is present and may play some part in activation of an underlying lesion or may serve simply to draw attention to the process.

Tuberculous spondylitis, or tuberculosis of the vertebrae, frequently affects the thoracic vertebrae, particularly the 12th one.[255] In one series of 64 cases of spinal tuberculosis in children, the lesions were in the thoracic area in 24 children, in the lumbar area in 19, and in both areas in 13. Cervical involvement is a rare finding.[269] Usually, two vertebrae are involved, but sometimes 3, 4, or even as many as 11 (usually contiguous, but at times with "skips") are affected. The body of the vertebra is affected far more often than the spinous processes or the arch.

The progression of tuberculous spondylitis, as seen on radiographs, is from slight narrowing of the disk space with only minimal disk involvement and slight collapse and "wedging" of the vertebral body; to marked narrowing of the disk space with collapse, wedging of the bodies, and resulting angulation of the spine (gibbus); and to extensive destruction of the bodies with severe kyphosis (Pott disease).

Paravertebral abscess (Pott abscess), retropharyngeal abscess, psoas abscess, and neurologic lesions are serious complications to be expected in 10 to 30 percent of cases of spondylitis. Neurologic complications most often arise from cervical and lumbar vertebral lesions and consist of various degrees of neuroplegia, paraplegia, or even quadriplegia. The complications are caused by inflammation of the spinal cord secondary to a neighboring cold abscess, by caseum or granuloma in the extradural space, or by spinal vessel thrombosis.

The signs and symptoms of spondylitis include "night cries" and restless sleep, a low-grade daily fever, and a peculiar position (e.g., torticollis with cervical lesions) or gait. Findings on physical examination may include marked "guarding" because of dorsal muscle spasm, pain when the back is "pounded," a deformity (e.g., gibbus), or reflex changes (including clonus).[408] On occasion, the presence of referred chest pain leads to discovery of a paravertebral abscess on the chest radiograph. With chemotherapy and, when necessary, surgery, the outlook for both eventual clinical healing and neural recovery is in the range of 80 to 90 percent, although patients may not gain their full expected height.

Tuberculosis of the knee can be divided into several clinical types[254]: effusion into the joint without bone erosion and with little restriction of motion; thickening and fibrosis of the synovial membrane without bone erosion but often with considerable restriction of motion; synovial disease and a bone focus but an intact joint space; and synovial disease with diminished range of motion.[629] Some degree of pain, stiffness, and limping, usually intermittent in milder cases, first calls attention to the problem.

Tuberculosis of the hip, in some series of patients more common than tuberculosis of the knee, should be considered in the differential diagnosis when a child refuses to walk or a limp develops. Today, when the disease still is limited at the time of discovery to the acetabulum or to the head of the femur and no intra-articular disease is present, good mobility of the joint usually can be preserved.

Tuberculous dactylitis (spina ventosa)[237] is the most common form of skeletal tuberculosis in infants. Endarteritis is followed by swelling (often painless), and cystic bone lesions are seen on radiographs. A cold abscess may form and drain spontaneously. The prognosis for recovery without deformity is surprisingly good and was so even before chemotherapy was available.

Tuberculous arthritis is a rare finding in children. Usually monarticular, it involves primarily the weight-bearing joints and only exceptionally the joints of the upper extremities. Bacterial cultures and histologic examination of the synovium establish the diagnosis.[687] Poncet disease is a rare form of rheumatic joint disease associated with tuberculosis; it is an exceedingly rare finding in

children.[140] The diagnosis of skeletal tuberculosis should be considered immediately in any child who is known to have been infected with tubercle bacilli and in whom a bone or joint lesion develops and in any child with a persistent, not otherwise explained bone or joint lesion.[684] The differential diagnosis must include low-grade infections caused by *Staphylococcus*, *Haemophilus influenzae*, *Salmonella*, and *Brucella;* fungal infections; rheumatoid arthritis; malignant disease of bone; eosinophilic granuloma, particularly with skull or pelvic lesions; and osteochondrosis resulting from aseptic necrosis of bone, particularly Legg-Calvé-Perthes disease and tuberculosis of the hip.

Children with bone and joint tuberculosis usually react strongly to tuberculin. Although the number of tubercle bacilli in an active bone lesion is much lower than in a lung lesion, the organisms almost invariably can be recovered on culture, and a great effort to do so should be made by means of aspiration or open biopsy.[226]

Treatment of skeletal tuberculosis includes both chemotherapeutic and orthopedic interventions, with the chemotherapeutic interventions by far the more important.[509] Orthopedic procedures can be used for several purposes: establishment of the diagnosis, evacuation of caseum and necrotic bone, immobilization of a joint, and reconstruction or strengthening of damaged bone. Since the advent of chemotherapy, any indicated surgical procedure can be performed without fear of subsequent development of sinus formation. However, the trend has been toward reliance on drug therapy with increasingly conservative surgical management. Controlled trials of treatment undertaken in different parts of the world (Southeast Asia) by the Medical Research Council Working Party on Tuberculosis of the Spine and others have demonstrated the remarkable effectiveness of ambulatory treatment with different regimens of chemotherapy in children and adults,[280,412-414] including short-course chemotherapy.[235]

Tuberculosis of the Superficial Lymph Nodes (Scrofula)

Striking enlargement of the superficial regional lymph nodes is an integral component of the primary tuberculous complex. The tonsillar and submandibular nodes are involved most often and probably represent extension from the paratracheal lymph nodes (and not from a primary lesion in the tonsil, as once was thought); occasionally, these nodes are involved when the primary lesion occurs in the mucous membranes of the mouth, a rare event. Enlarged supraclavicular nodes may accompany a primary pulmonary lesion in the upper lung fields.[558] Enlarged axillary and epitrochlear nodes can result from a primary skin lesion of the elbow or hand, often a small, insignificant-looking area that has, however, been present for some time. Preauricular adenitis suggests a focus on the scalp or forehead or in the lacrimal sac, sometimes attributed to an insect bite. With inguinal adenitis, careful examination of the sole of the foot may reveal a small ulcer, often at the base of the toes.

When the superficial lymph nodes are involved early in the course of infection—the normal event—the enlargement usually is painless and the node or nodes are rubbery and discrete.[400] Low-grade fever generally is present, sometimes unnoticed by the parents. On occasion, acute respiratory tract infections seem to precipitate or to aggravate superficial tuberculous lymphadenitis and the patient has high fever, some local pain, and perilymphadenitis. Rarely, the patient is seen first with a fluctuant mass and the overlying skin is shiny and erythematous.[143,605]

The diagnosis usually is not obvious, so it is wise in all cases of superficial cervical and supraclavicular lymphadenitis to perform a throat culture, radiography of the chest, and a Mantoux tuberculin test. If the node is fluctuant, it should be aspirated and cultured for mycobacteria as well as for pyogenic bacteria.[344] In preauricular, axillary, and inguinal adenitis, a possible primary skin lesion should be sought diligently. If a lesion is found, a biopsy should be performed, in addition to a tuberculin skin test, chest radiography, and a careful history.

Currently, in addition to pyogenic infection, the following need to be considered as possible causes: cat-scratch disease, tularemia, malignant tumors, sarcoid, and infection caused by mycobacteria other than *M. tuberculosis*.[34,338] If the adenitis is caused by infection with *M. tuberculosis*, the induration from a Mantoux tuberculin skin test usually is greater than 15 mm, whereas mycobacteria other than *M. tuberculosis* produce a less intense reaction. The key to distinguishing tuberculosis from infection by another mycobacterium generally is epidemiologic—has the child been exposed to tuberculosis?

Because infection with pyogenic bacteria often enhances mycobacterial adenitis, frequently a wise approach is to institute therapy with a conventional antibacterial agent while awaiting the results of the skin test, chest radiography, and cultures. If the adenitis persists and evidence of mycobacterial infection is elicited, antituberculosis drug therapy should be initiated and surgical excision seriously considered.[338,557] Surgical excision currently is the treatment of choice for adenitis caused by mycobacteria other than *M. tuberculosis*. In the case of lymphadenitis caused by *M. tuberculosis*, the response to antituberculosis drugs is likely to be good. On the other hand, the adenitis is more likely to extend down into the mediastinum and to be difficult to remove. Under these circumstances, surgical excision probably is unwise, whereas a few weeks of corticosteroid and antituberculosis therapy may be effective.[293,338]

Superficial tuberculous lymphadenitis sometimes occurs early in the course of lymphohematogenous spread, in which case it often is manifested as "general glandular enlargement" accompanied by swinging fever, malaise, and weight loss. The tuberculin test result almost always is positive in immunocompetent patients, and the tuberculin test should be included in the investigation of every patient with general glandular enlargement.

Finally, tuberculous adenitis, either localized or generalized, can occur in adolescents or young adults who were infected months or even years earlier, whose infection has been quiescent, and in whom the appearance of lymphadenitis heralds reactivation of the tuberculosis infection.[337]

Ocular Tuberculosis

Ocular tuberculosis is an uncommon finding in children. When it does occur, the conjunctiva and the cornea are the areas most often involved.[158]

The conjunctiva can serve as the initial portal of entry for tubercle bacilli, especially after trauma. Unilateral lacrimation and reddening may lead to the discovery of yellowish gray nodules, usually on the palpebral conjunctiva. Preauricular adenitis appears early; the submandibular and cervical nodes also may enlarge. A tuberculin skin test and biopsy with culture can be performed to confirm the diagnosis.

Phlyctenular conjunctivitis probably is one of the hypersensitivity phenomena of childhood tuberculosis. Tubercle bacilli have, on rare occasion, been isolated from the small, grayish, jelly-like nodules usually clustered on the limbus and surrounded by dilated conjunctival vessels. Pain and photophobia are intense, and the lesions may recur in crops for weeks and affect one or both eyes. In the differential diagnosis, foreign body, herpes simplex virus conjunctivitis, vernal conjunctivitis, and trachoma should be considered. Tuberculin sensitivity is likely to be pronounced, and 1 tuberculin unit of purified protein derivative (PPD) should be used in the initial tuberculin skin test. Fortunately, hydrocortisone drops are effective in controlling both a strong local reaction to the diagnostic tuberculin test and the discomfort from the underlying disease. The prognosis for complete recovery is excellent, provided the phlyctenules do not ulcerate and leave corneal scars. Systemic chemotherapy should be started immediately after the diagnostic procedures are completed.

Although tuberculosis of the ciliary body and iris has been reported rarely in children, tubercles of the choroid often have been found in patients with miliary tuberculosis (up to 70% of patients in some series) and occasionally in children with a seemingly uncomplicated infection.[438,470] Frequently multiple, the tubercles heal slowly with deposition of retinal pigment; residual scarring apparently can be prevented with corticosteroid therapy. Tuberculous uveitis and tuberculosis manifested as an orbital mass are rare clinical entities.[576]

Tuberculosis of the Middle Ear

Tuberculosis of the middle ear is a relatively rare manifestation of the disease.[194,374,377,443,538] It occurs as a primary focus in the area of the eustachian tube (because of reflux up the tube) in neonates who have aspirated infected amniotic fluid or in older infants who have ingested tuberculous material. It can occur as a metastatic lesion in older children who have a primary focus elsewhere. If it is a primary focus, regional lymphadenitis involves the preauricular lymph node or the anterior cervical chain and facial paralysis occurs frequently. The primary focus always is unilateral. Otorrhea is a common occurrence and painless but may become foul smelling because of bacterial contamination with enteric organisms. Older patients may complain of tinnitus and "funny noises." The eardrum often is damaged extensively. A large central perforation or several perforations are characteristic findings. A tuberculin skin test, biopsy, and careful cultures for tubercle bacilli are essential. Once greatly feared for the almost inevitable loss of hearing and the frequent occurrence of tuberculous meningitis, tuberculous otitis media now heals well with appropriate chemotherapy.[443]

Gastrointestinal and Abdominal Tuberculosis

Tuberculosis of the mouth occurred more commonly in the days of bovine tuberculosis than it does today; at that time, scrofula often represented a primary complex in the mouth or tonsils, with associated submandibular or cervical lymphadenitis.[423] A primary focus in the mouth generally consists of a painless ulcer or mass of granulation tissue around a tooth socket or in the gingivolabial sulcus, with enlarged submental or submandibular nodes. Primary tuberculosis of the tonsils begins as a painless swelling of one tonsil, sometimes with an ulcer or yellowish node and, of course, enlargement of the regional lymph nodes. If tubercles found on histologic examination of a tonsil removed at tonsillectomy are unaccompanied by lymphadenitis, the lesion is considered a metastatic rather than a primary lesion. Tuberculosis of the esophagus occurs rarely if ever in children; sometimes, dysphagia is produced by a mass of mediastinal nodes, which may rupture into the lumen and later heal, possibly leaving an esophageal diverticulum.

Abdominal tuberculosis may occur after the ingestion of tubercle bacilli or as a part of generalized lymphohematogenous spread, but tuberculous enteritis always has been uncommon.[21,52,530,640,643] When tubercle bacilli penetrate the gut wall, they usually do so through the Peyer patches or the appendix, where they give rise to local ulcers followed by mesenteric lymphadenitis and sometimes peritonitis.[20] On occasion, especially in older children, tuberculous enteritis accompanies extensive pulmonary cavitation. Symptoms and signs include vague abdominal pain, intussusception, blood in the stool, and sinus formation after a seemingly routine appendectomy.

The spleen is seeded during the initial lymphohematogenous spread. Only rarely are the tubercles numerous and large enough to undergo caseation and to calcify.[327] The reticuloendothelial system of the liver is involved also. Symptoms rarely are manifested except in miliary tuberculosis, in which the liver may be enlarged markedly, or in congenital tuberculosis, in which both the liver and spleen usually are enlarged.[382]

Mesenteric lymphadenitis can arise as part of an intra-abdominal primary complex or by extension from tuberculous thoracic or pelvic lymph nodes; often asymptomatic, it may be discovered later when calcified. It can cause ascites and dilation of the superficial abdominal veins, but the symptom most frequently attributed to mesenteric lymphadenitis is colicky abdominal pain after exercise, probably because adhesions are stretched.

Tuberculous peritonitis can be a result of direct extension from a primary intestinal focus, adjacent mesenteric lymph nodes, or tuberculous salpingitis.[109,157,212] It may be "plastic" or accompanied by a serous effusion. On palpation, a mass of lymph nodes often can be felt and the abdomen may have a characteristic "doughy" feeling. Paracentesis should be performed with care because the

intestine may be immobilized by adhesions. Even with a large effusion, absorption usually occurs within a month. Malignant disease must, of course, be considered strongly in the differential diagnosis, and it may be necessary to obtain a biopsy specimen even if the tuberculin skin test result is positive. Laparoscopy frequently is useful, as is fine-needle aspiration[367] and ultrasonography.[304] CT is more sensitive for intra-abdominal lymphadenopathy, but less helpful for tuberculous ascites, because it does not detect septations well.[674] The ascitic fluid–blood glucose ratio of the aspirated peritoneal fluid can be helpful in differentiating tuberculous peritonitis from ascites of other causes.[704]

Renal Tuberculosis

Renal tuberculosis is a late and uncommon complication of pulmonary disease; it rarely occurs less than 4 or 5 years after primary infection and is therefore likely to be diagnosed during adolescence.[187,588] However, tubercle bacilli can be recovered from the urine in many cases of miliary tuberculosis and some cases of pulmonary tuberculosis in young children. Hematogenous dissemination can give rise to tubercles in the glomeruli, with resultant caseating and sloughing but tiny lesions, which then discharge tubercle bacilli into the tubules. An encapsulated caseous mass occasionally develops in the zone between the renal pyramid and cortex; it may calcify in situ or discharge into the pelvis of the kidney and form a cavity analogous to a pulmonary cavity. Infection can be unilateral or bilateral and can spread downward to involve the bladder. Frequently, dysuria, hematuria, and "sterile" pyuria are the initial findings in the urine; they may occur grossly but often not until late in the course of a disease that causes strikingly few symptoms. Appropriate examination and culture of single early-morning urine specimens rarely fail to reveal tubercle bacilli. Intensive chemotherapy renders surgical intervention a rare necessity.[588] Urine from patients with renal tuberculosis is highly infectious, and such children should be isolated until their urine is sterile.

Dialysis- and Renal Transplant–Associated Tuberculosis

Infections are a common cause of morbidity and mortality in patients with end-stage renal disease. Depressed cellular immunity, as manifested by cutaneous anergy, delayed homograft rejection, and depressed lymphocyte count, has been demonstrated in uremic patients, whereas cell-mediated immunity appears to recover in stable patients treated by long-term hemodialysis. Thus, it is not surprising that tuberculosis occurs more frequently in patients maintained on dialysis than in the general population and that when it does become active, it does so before or early in the course of dialysis. Extrapulmonary involvement (mediastinal, meningeal, pleural, osseous, and renal) and miliary tuberculosis appear relatively frequently in this group. In these patients, fever of unknown origin should lead to suspicion of active tuberculosis.[16] Tuberculosis also can originate in the transplanted organ.[446,683]

Genital Tuberculosis

Tuberculosis of the genital tract is an uncommon finding in both sexes before puberty.[588] It usually arises as a metastatic lesion during lymphohematogenous spread and occasionally by direct extension from an adjacent lesion of bone, gut, or the urinary tract. Genital tuberculosis is a particular hazard for adolescent girls with tuberculosis infection. Frequently, other forms of tuberculosis associated with initial infection, such as a pleural effusion, also are present. The fallopian tubes are involved in 90 to 100 percent of cases, the endometrium in 50 percent, the ovaries in 20 to 30 percent, and the cervix in 2 to 4 percent.[551] With tubal involvement, peritoneal tuberculosis occurs frequently.

Lower abdominal pain and amenorrhea usually prompt the patient to seek medical care. A lower abdominal mass, free peritoneal fluid, and constitutional symptoms may or may not be present. Chemotherapy is effective, but infertility remains a potential sequela of the disease.

Tuberculosis of the external genitalia has been seen as a manifestation of child abuse.

Genital tuberculosis can occur in males as primary tuberculosis of the penis after ritual circumcision; many such instances were reported in the past.[258,484] Massive inguinal lymphadenopathy in a circumcised infant should arouse suspicion of a possible tuberculous etiology. Epididymitis or epididymo-orchitis can occur in early childhood.[221] These disorders are characterized by nodular, painless swelling of the scrotum and a dragging pain in the groin and have a gradual onset rather than the acute onset that results after trauma, torsion of the testis, mumps orchitis, or epididymitis associated with bacterial infection.

Inoculation Tuberculosis

More than 200 cases of syringe-transmitted tuberculosis have been described in the world literature.[252] It has become more common since the widespread use of injectable penicillin and routine childhood immunization and usually has been a result of contamination of the syringe or the solution by an individual with infectious sputum. If the recipient has not been infected previously, the lesion in the muscle or subcutaneous tissue will be the primary focus and the regional lymph nodes will enlarge, caseate, and, under favorable circumstances, calcify. However, at least 10 infants thus infected have died as a result of generalized tuberculosis and in others bone tuberculosis has developed. If the recipient already is tuberculin positive, a tuberculous abscess will form without regional lymphadenitis. In this case, an injection abscess must be differentiated from a deep tuberculous abscess arising in the stage of hematogenous dissemination.

Perinatal Tuberculosis (Congenital and Postnatal)

Transmission of *M. tuberculosis* from mother to infant through the placenta or amniotic fluid has been reported in approximately 300 patients.[80] Infection of the placenta

has been demonstrated, and tubercle bacilli have been grown from the tissues of stillborn infants and from living infants within a few days of birth. Perinatal tuberculosis can be acquired by the infant through one of several routes:

1. By transplacental spread through the umbilical vein from a mother with primary hematogenous tuberculosis occurring during pregnancy (i.e., true congenital tuberculosis). The liver is enlarged, enlarged lymph nodes may be present at the porta hepatis, and evidence of widespread miliary disease may be seen in the infant (i.e., the infant may have a primary liver complex or primary lung complexes).
2. By in utero aspiration of amniotic fluid infected from endometritis in the mother or from the placenta. This route of infection also constitutes true congenital tuberculosis. In both these situations, clinical onset of signs and symptoms is likely to be rapid (by 2 to 3 weeks of age) and to include failure to thrive, fever, respiratory distress, and hepatosplenomegaly.
3. By ingestion of infected amniotic fluid or secretions during delivery. This mechanism would seem to be less well documented than the first two routes, but it certainly is a possibility.
4. By inhalation of tubercle bacilli at or soon after birth from the mother, other relatives, or attendants with infectious pulmonary tuberculosis. This route is the most common mode of transmission to neonates.
5. By ingestion of infected breast milk or cow's milk.

One cannot always be sure of the type of infection in a particular neonate; only clear-cut evidence of a primary complex in the liver establishes a definite diagnosis of congenital tuberculosis. However, the presence of early forms of tuberculosis (e.g., pleural, miliary, or meningeal) in the mother during pregnancy or the puerperium also is strong evidence of true congenital tuberculosis in the infant.

The last two listed routes are really means of "postnatally" acquired tuberculosis and need, for epidemiologic purposes, to be distinguished from the first three routes. Neonates with these types of tuberculosis usually lack striking clinical features until they are 1 or 2 months of age.

The diagnosis of perinatal tuberculosis is likely to be difficult to establish and often delayed.[349] Disease in the mother often is overlooked; the mother may have pleural effusion, fever of unknown origin, cough, endometritis, and other symptoms without the tuberculous etiology being recognized. The early symptoms and signs in the neonate likewise are overlooked frequently and may be similar to those caused by other congenital infections. Once the diagnosis is suspected, treatment should be started immediately, and diagnostic procedures should be carried out rapidly and aggressively.

The clinical manifestations vary, probably depending on the size of the infecting dose of bacilli and the site and size of the caseous lesions.[409,454] In many of the reported cases, tuberculosis disease was discovered only at autopsy.[272] Symptoms usually appeared during the second week of life and included loss of appetite and failure to gain weight, fever, nasal or ear discharge, cough, bronchopneumonia, jaundice, hepatomegaly, and splenomegaly occurring later.[80] Wasting has been noted frequently and, in one case, clearly was shown to be caused by hypoadrenocorticism.[435]

Because the result of the tuberculin skin test very rarely is positive in infants, demonstration of tubercle bacilli in gastric washings, middle ear fluid, lymph node biopsy specimens, skin biopsy specimens, bone marrow aspirate, endotracheal aspirate, or lung biopsy specimens is essential.[232] Examination of the placenta for organisms or characteristic histopathologic changes can be extremely helpful and should be done when tuberculosis is diagnosed in the mother around the time of delivery. Successful treatment of congenital tuberculosis has been reported by several investigators, although clinical response may be very slow, and extensive calcification of the lungs, liver, spleen, and muscles may result.[309,313,454,594,607]

Tuberculosis in Adolescents

Tuberculosis in adolescents has become relatively more important as the incidence of infection in childhood has lessened.[151,390,593] Logically, it should be considered in two ways: first, tuberculosis acquired as an initial infection during the adolescent years and, second, tuberculosis infection acquired in early life and reactivated during adolescence.[393,456] In actual practice, separating the two often is difficult or impossible, and most clinical reports and studies do not do so.

Tuberculosis in adolescents may occur exactly as it does in young children, or a classic primary complex may progress rapidly to chronic pulmonary tuberculosis while the hilar lymph node involvement characteristic of childhood tuberculosis remains present. The Medical Research Council in 1963 published a report of 504 cases of tuberculosis in adolescents, including 316 cases of pulmonary tuberculosis, 44 cases of pleural effusion, 44 with hilar lymph node enlargement, 6 cases of miliary and 5 of meningeal tuberculosis, 13 with bone and joint disease, and 8 with genitourinary tuberculosis. In a series of cases of primary tuberculosis in adults, Stead[619] included 11 adolescents, 5 with simple primary tuberculosis, 5 with pleurisy and effusion, and 1 with progressive pulmonary tuberculosis.

From the extensive clinical experience of Lincoln and Sewell[364] and others, several observations emerge. Tuberculosis infection in early infancy rarely leads to pulmonary tuberculosis in adolescence, perhaps because it has several years in which to heal, whereas tuberculosis infection acquired after 7 years of age and, in particular, acquired after 10 years of age is likely to progress. When *M. tuberculosis* is acquired during adolescence, chronic pulmonary tuberculosis may develop within 1 to 3 years. Moreover, the risk for acquiring pulmonary tuberculosis is two to six times greater for adolescent girls than for adolescent boys. In both sexes, the adolescent growth spurt is the time of greatest risk. The work of Johnston[298] suggests that the depressant effect of puberty on calcium and nitrogen retention, at least in girls, may be correlated with the failure of tuberculosis infection to heal.

Tuberculosis and Pregnancy

In the era before chemotherapy, whether pregnancy and tuberculosis affected each other adversely was an ongoing controversy. Since the advent of chemotherapy, however, the prognosis has improved greatly.[203] The main problems now are serious unrecognized tuberculosis in a pregnant woman, sometimes with a fatal outcome, and serious unrecognized disease in her infant. Another problem is whether pregnancy influences the risk for progression of tuberculosis infection to disease; the data are conflicting in this regard. Tuberculin skin testing probably is valid during pregnancy; chest radiographs (with shielding) should be obtained for all tuberculin-positive pregnant patients. Therapy, when indicated, can safely include isoniazid (which crosses the placenta but apparently without ill effects), rifampin, and ethambutol. The safety of pyrazinamide in pregnancy has not been established, but a growing number of experts recommend it because anecdotal data have not shown it to be harmful to the mother or fetus. Streptomycin, because of fetal ototoxicity, is not recommended.[599] However, one study of seven pregnant women with multidrug-resistant tuberculosis showed no adverse effects on their neonates when mothers were treated with second-line drugs, including aminoglycosides.[174,578] Should a mother receiving antituberculosis therapy breast-feed? It is probably safe for her to do so because the drugs, although found in milk, are present only in small amounts.[600]

Tuberculosis and Human Immunodeficiency Virus Infection

In adults infected with both HIV and *M. tuberculosis*, the rate of progression from asymptomatic infection to disease is increased greatly.[542] The clinical manifestations of tuberculosis in HIV-infected adults are typical when the $CD4^+$ cell count is higher than 500/mm^3. As the $CD4^+$ cell count falls, manifestations become "atypical." Extrapulmonary foci occur in as many as 60 percent of profoundly immunocompromised patients.[31] Pulmonary cavities are rare findings; lower lobe infiltrates or nodules often accompanied by thoracic adenopathy are common occurrences, especially if the patient's tuberculosis infection is recent. Of course, many patients have a nonreactive tuberculin skin test. Sputum is less likely to be produced or to contain visible acid-fast organisms on stained smears; more invasive procedures, such as bronchoscopy, often are required to isolate *M. tuberculosis* and to rule out other causes of opportunistic lung disease.[397] Malabsorption of antituberculosis drugs in HIV-infected patients can lead to prolonged symptoms and disease.[496]

When tuberculosis develops in HIV-infected children, the clinical features tend to be fairly typical of childhood tuberculosis in immunocompetent children, although the disease often progresses more rapidly and the clinical manifestations are more severe.* Many children have sizeable pulmonary radiographic findings with minimal respiratory signs or symptoms. An increased tendency for extrapulmonary disease may be noted. Nonspecific signs and symptoms,[693] overlap in symptomatology with other opportunistic infections, and higher rates of culture-positive patients with negative radiographs make the diagnosis challenging. Consequently, studies evaluating the utility of symptom-based scoring systems have shown discrepant results.[185,392,494] Establishing the diagnosis can be very difficult; a diligent search for an infectious adult in the child's environment may yield the best clues to the correct diagnosis.[325] Recurrent disease and relapse occur more frequently in HIV-infected children.[549] The prognosis generally is good if tuberculosis disease is not far advanced when the patient is diagnosed and appropriate antituberculosis drugs are available.[251]

*References 61, 105, 115, 185, 189, 263, 320, 439, 481, 654.

Tuberculosis and Immune Reconstitution Inflammatory Syndrome

The introduction of highly active antiretroviral therapy (HAART) into regions where tuberculosis is prevalent has had a profound effect on the epidemiology and clinical expression of both diseases. One manifestation may be immune reconstitution inflammatory syndrome (IRIS). IRIS manifestations can include either worsening of tuberculosis symptoms while on effective therapy (paradoxical IRIS) or new-onset tuberculosis symptoms after initiation of HAART (unmasking IRIS).[417,610] Sometimes it is difficult to tell if a deterioration in the clinical course of a patient with an opportunistic infection who is started on HAART is due to IRIS, poor compliance with treatment for the opportunistic infection, or drug resistance.[345-347] Consensus case definitions were created in 2008 to help differentiate among these diagnostic possibilities. Paradoxical IRIS is defined for patients diagnosed with tuberculosis before starting HAART and who showed an initial response to antituberculous medication. Major criteria include new or enlarging lymph nodes or focal tissue involvement, central nervous system findings, or serositis or new or worsening radiographic findings. Minor criteria include new or worsening constitutional or respiratory symptoms or new or worsening abdominal pain accompanied by peritonitis, organomegaly, or intra-abdominal lymphadenopathy. At least one major or two minor criteria are needed for diagnosis. Unmasking IRIS is defined in patients not receiving tuberculosis treatment when HAART is initiated who then develop tuberculosis with evidence of substantial inflammation (e.g., tuberculous lymphadenitis, pulmonary tuberculosis accompanied by respiratory failure).[417]

Factors suggesting IRIS are temporal association (within 3 months of starting HAART), unusual clinical manifestations, unexpected clinical course, exclusion of alternative explanations, evidence of preceding immune restoration (rise in CD4 lymphocyte count), and fall in HIV viral load. In adults, the risk for developing IRIS in patients with tuberculosis who are started on HAART is greatest if they have extrapulmonary tuberculosis, high initial HIV viral loads and low CD4 lymphocyte counts, and rapid response to HAART.[63,442] One meta-analysis showed that paradoxical IRIS developed in 16 percent of adults with tuberculosis.[442] Although the risk for IRIS is higher when HAART is initiated shortly after antituberculosis medications are begun, most reactions are mild

and the survival benefit in patients receiving HAART outweighs the risk for IRIS.[57,302]

Published reports of IRIS in children have been rare,* although anecdotal evidence from pediatric acquired immune deficiency clinics in Africa suggest it is becoming common as HAART is introduced to more children with HIV infection. One study indicated that IRIS was seen in almost 40 percent of HIV-infected children and was more common in boys than girls and more common in children 5 to 12 years of age and that unmasking IRIS comprised more than three fourths of cases.[475] Other studies have shown lower incidence of IRIS,[506,694] but the finding that IRIS is more common in children with disseminated disease and malnutrition has been documented in multiple studies.[475,694] IRIS in children has been associated with nontuberculous mycobacteria,[506] disseminated viral[506,694] or fungal infections,[475] bacterial pneumonia,[475] and previous BCG vaccination.[331,507,580] The finding that IRIS occurs in children with tuberculosis and HIV infection who receive HAART is not surprising. For decades, so-called paradoxical reactions have been described in HIV-uninfected children with tuberculosis after antituberculosis therapy is started. The most common manifestations are fever, cough, new skin lesions, enlarging lymph nodes in the thorax or neck, and appearance or enlargement of tuberculomas in the brain, with or without accompanying meningitis.[417,475] The risk for unmasking IRIS appears to be highest in the first 3 months after initiation of HAART.[30]

IRIS also can be seen in HIV-uninfected children. One study in HIV-uninfected children showed that unexpected deterioration while on therapy was seen in almost 15 percent of children, most commonly manifest as enlarging intrathoracic lymphadenopathy. In this cohort, IRIS was more common in children with multiple sites of disease and in children whose weight was in the lowest quartile.[652]

The best management of IRIS is unknown. As with paradoxical reactions that cause tissue damage or obstruction, corticosteroids may be beneficial to "quiet" the local immune response, although no randomized clinical trials have been reported. Less severe reactions may respond to nonsteroidal anti-inflammatory drugs. The high frequency of IRIS associated with tuberculosis has led some experts to recommend delay of HAART until appropriate (for drug susceptibility pattern) antituberculosis chemotherapy has been given for at least 2 months.[357] No consensus on this approach has been reached, however, and data from children may indicate that they are more likely to die of complications of HIV than of tuberculosis when they are dually infected.[251]

DIAGNOSIS

In the developing world, the only way that children with tuberculosis disease are discovered is passively, when they have a profound illness that is consistent with a manifestation of tuberculosis. Having an ill adult contact is an obvious clue to establishment of the correct diagnosis. The only available laboratory test usually is an acid-fast smear of sputum, which the child rarely produces. In many regions, chest radiography is not available. To aid in establishing the diagnosis, a variety of scoring systems based on available tests, clinical signs and symptoms, and known exposures have been devised.[422,700] However, no single clinical scoring system has been validated, the sensitivity and specificity of these systems can be very low and lead to both overdiagnosis and underdiagnosis of tuberculosis, differing prevalences of tuberculosis can skew predictive values, and scoring systems perform poorly in HIV-infected children.[317,400,422]

In industrial countries, children with tuberculosis usually are discovered in one of two ways.[172,322,544] Obviously, one way is to consider tuberculosis as the cause of a symptomatic illness.[534] Discovering an adult contact with infectious tuberculosis is an invaluable aid to establishing the diagnosis; the "yield" from a contact investigation usually is higher than that from cultures from the child.[41] Culture from the infectious adult case may yield the only drug susceptibility results for the child because cultures from children with tuberculosis frequently are negative. Children often are the first in the family to be diagnosed with tuberculosis, potentially owing to different thresholds for seeking medical attention for children and adults and more rapid onset of severe manifestations in young children. A negative exposure history does not rule out tuberculosis in a child. The yield of contact investigations is higher for children than adults, owing to the former's more limited social network. Yield of contact investigations may be lower in foreign-born children, whose source cases may still reside in their country of origin.[571] The second way is to discover a child with pulmonary tuberculosis during the contact investigation of an adult with tuberculosis.[402,431,603,631,634] Typically, the affected child has few or no symptoms, but investigation reveals a positive tuberculin skin test result and an abnormal chest radiograph. In some areas of the United States, as many as 50 percent of children with pulmonary tuberculosis are discovered in this manner, before significant symptoms have begun.

The recent use of molecular epidemiology techniques has shown, however, occasional discordance between the isolate from the child and that from the presumed source, meaning that the real source of transmission to the child is undiscovered.[710,719] It is less common to find tuberculosis disease in a U.S.-born child as the result of a community- or school-based tuberculin skin testing program, although foreign-born children with tuberculosis may be found in this manner.[173,493]

Tuberculin Sensitivity and the Skin Test

Sensitization to tuberculin is induced by infection with living tubercle bacilli. Specific tuberculin sensitivity to either *M. tuberculosis* or other mycobacteria can be transferred in humans by injection of lymphocytes from sensitized donors and also by injection of certain purified mycobacterial protein antigens. Sensitization to tuberculin as a result of infection with *M. tuberculosis* tends to persist undiminished for life.[186,236,266]

*References 301, 450, 475, 506, 694, 720.

The time of appearance of sensitivity in animals after infection with *M. tuberculosis* has occurred depends on the number of tubercle bacilli in the infective dose and on the virulence (i.e., the rate of multiplication of the organisms), and such is also likely to be the case in humans.[32] For all practical purposes in humans, tuberculin reactivity seems to appear in 3 to 6 weeks, and occasionally as long as 3 months after initial infection. At first, an inflammatory reaction appears at the site of injection, with predominance of segmented neutrophils, followed by immigration of macrophages and T lymphocytes, until the entire area of induration consists of mononuclear cells. The size of the induration depends on the amount of tuberculoprotein injected and the availability of sensitized T lymphocytes in sufficient number. The size of the induration seems to depend on at least two additional factors: the local behavior of the skin (the disappearance time for wheals of normal saline solution, the so-called Aldrich-McClure test, is accelerated during fever, pregnancy, cachexia, and extreme malnutrition) and the number of actively multiplying tubercle bacilli in the body, which can be demonstrated in animals and probably in humans as well. That still other factors must be involved is clear because 10 to 20 percent of immunocompetent patients with proven tuberculosis are tuberculin negative during initial disease, with tuberculin sensitivity often regained during treatment.[501] Decreased T-lymphocyte blastogenesis has been demonstrated in some cases, and inhibition by B lymphocytes may play a role.

Falsely negative tuberculin skin tests can be seen in a number of circumstances. Temporary desensitization to tuberculin occurs most strikingly during measles and has been studied carefully. Full reactivity diminished during the incubation period and returned within approximately 1 month after appearance of the exanthem.[246] Influenza and administration of influenza and measles vaccines tend to depress sensitivity but rarely suppress it entirely.[330,729] Whether other viral diseases and vaccines regularly are less active in this regard and what the important factors may be are not known.[44] If a temporary desensitizing effect occurs in bacterial infections such as scarlet fever, it probably depends on factors such as hyperthermia and dehydration. Corticosteroids,[65,379,552] HIV infection,[388,513] adrenocorticotropic hormone,[537] nitrogen mustard,[695] and irradiation[142] diminish tuberculin reactivity, perhaps simply by inducing lymphopenia.[616]

False-positive results of tuberculin skin tests also are seen. That degrees of sensitivity to *M. tuberculosis* are induced by nontuberculous mycobacteria also is clear.[233] Previous receipt of BCG vaccine can cause increased reactivity to tuberculin, but the association is weaker than many clinicians suspect.[10,84,372,440,566] Less than 50 percent of infants given BCG vaccination have a reactive tuberculin skin test at 9 to 12 months of age, and the great majority will have a nonreactive skin test by the time they are 5 years of age. Older children and adults who receive BCG vaccination have a reactive skin test and keep it longer, but by 10 to 15 years after vaccination most individuals have lost tuberculin skin test reactivity.[125,420,540] Repeated administration of BCG vaccine can maintain tuberculin reactivity.[279] Repeated tuberculin skin tests in a person sensitized previously by BCG vaccination, infection with *M. tuberculosis*, or probably infection by an environmental mycobacterium may increase the reaction to subsequent tuberculin skin tests (called the booster phenomenon).[48,208,565,657]

The antigens currently used in tuberculin testing are crude extracts consisting of a mixture of many antigens, some species specific, some shared among many species. Standardized, isolated, purified mycobacterial antigens are needed in clinical practice. The major antigen used in tuberculin testing is PPD, obtained from filtrates of heat-killed tubercle bacilli. Batch 49608, prepared by Dr. Florence Seibert in 1939, was designated by the WHO as the international standard tuberculin, the only one to be called PPD-S. All other PPD preparations are referred to simply as PPD or as PPD-T. Each batch must be stabilized with Tween 80 at 5 ppm to minimize adherence to glass and plastic and must be identified by manufacturer and lot number. However, the potency of PPD doses varies from batch to batch. The tuberculin vial should be refrigerated (2° to 8° C) and protected from light.

The Mantoux test is the reference test. A graduated syringe and a 26- or 27-gauge needle are used to inject 0.1 mL of PPD into the most superficial layer of the epidermis of the forearm, which raises an immediate wheal (Fig. 96-14). Under optimal circumstances, the needle should not be withdrawn for a few seconds to minimize leakage. If there is leakage or if the injection was too deep and no wheal is noted, the skin test should be repeated at least 5 cm from the original site. The reference test uses a dose of 5 tuberculin units of PPD-S or 2 units of RT-23. The reading should be made at 48 to 72 hours, with the forearm slightly flexed. Any induration (not erythema) should be measured, preferably with calipers, and the diameter at right angles to the axis of administration recorded in millimeters. Any blistering or ulceration also should be noted. Use of the words "negative" and "positive" should be avoided because interpretation can change as more epidemiologic information becomes known. No study has demonstrated that parents

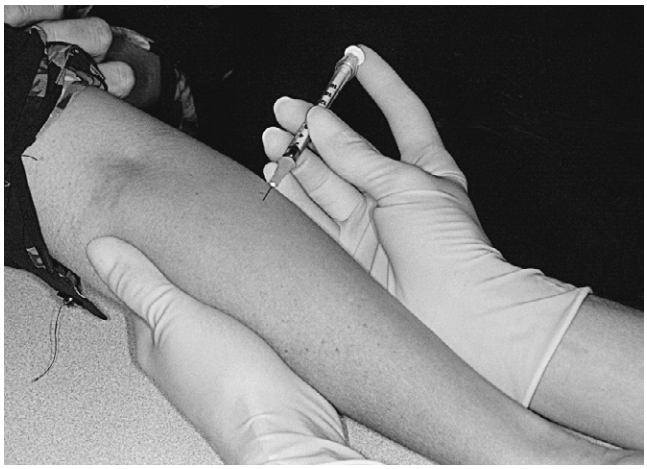

FIGURE 96-14 ■ A useful technique for performing a Mantoux tuberculin skin test on a child. The needle is applied perpendicular to the long axis of the arm to attain better control.

or patients can read positive skin test results accurately, but that they may not correctly interpret or report positive results has been well documented.[111,112,224,264,477] Two studies have demonstrated that pediatric care providers tend to underread Mantoux tuberculin skin tests.[77,285]

Several studies have shown great variability in readings of Mantoux tests.[37,83,312,477] Such variability can be minimized only by ongoing training of both testers and readers and by concentrating the responsibility of testing and reading to a small number of trained individuals.

The importance of the tuberculin test cannot be overemphasized as the main criterion for diagnosis of tuberculosis infection in an individual child. Only rarely is the tuberculin test response negative in an infected child, usually as a result of anergy from overwhelming infection, from viral infection, from HIV infection, from the use of immunosuppressive drugs, or because of factors not yet understood.[625] Anergy in tuberculosis can be selective for tuberculin; results of "control" skin tests may be positive but the Mantoux test result negative in a child with tuberculosis disease.[618] The utility of candidal or tetanus controls is limited and they are not recommended. In lieu of controls, clinicians need to be cognizant of the decreased sensitivity of the test in young children and immunocompromised patients.

The Mantoux test has a sensitivity and specificity of only approximately 95 percent at 10 mm of induration.[270] When a test with these characteristics is applied to a population with a 90 percent prevalence of tuberculosis infection, the positive predictive value of the skin test is 99 percent, an excellent result. However, if the same test is applied to a population with only a 1 percent prevalence of infection, the positive predictive value drops to 15 percent; 85 percent of the "positive" results are false-positive findings created by biologic variability, nonspecific reactions, and infection with environmental mycobacteria. These false-positive results lead to unnecessary treatment, cost, and anxiety for the patient, family, and clinician. In short, the low sensitivity and specificity of the tuberculin skin test render the test undesirable for use in persons from low-prevalence groups. The trend in the United States is to reduce or to eliminate routine testing of low-risk children but to target children with specific risk factors for one-time or periodic tuberculosis skin testing.[11,94,432]

In lieu of mass screening using tuberculin skin tests, children should be screened by history. Several recently published studies have shown that a questionnaire can be used in the United States to identify children with significant risk factors for tuberculosis infection, who then can receive a tuberculin skin test.[209,369,478,532] Factors that consistently correlate with having a positive tuberculin skin test result include contact with a case of tuberculosis, other family members with a positive tuberculin skin test result, and foreign birth or extensive travel to a high-prevalence country.[555] The predictive value of these questions may be diminished in high-prevalence areas.

The CDC and the American Academy of Pediatrics (AAP) have recommended varying the size of induration considered positive in various groups, according to their risk factors (Table 96-7). This is an attempt to increase sensitivity in children most likely to have rapid progression of asymptomatic tuberculosis infection to disease and to increase specificity in persons with no known risk factors for tuberculosis. In general in the United States, previous receipt of BCG vaccine should not influence interpretation of the initial tuberculin skin test of a child.[297]

TABLE 96-7 Amount of Induration in Reaction to a Mantoux Tuberculin Skin Test Considered Positive (Indicating Probable Infection with *Mycobacterium tuberculosis*)

Reaction Size	Risk Factors
≥5 mm	Contact with infectious cases
	Abnormal chest radiograph
	HIV infection or other immunocompromise
≥10 mm	Birth or previous residence in a high-prevalence country
	Residence in long-term care or corrections facility
	Certain medical risk factors: diabetes mellitus, silicosis, renal disease
	Occupation in health care field, exposure to patients with tuberculosis
	Member of a local high-risk group
	Close contact with a high-risk adult (except health care workers)
	Age <4 yr
≥15 mm	No risk factors

Interferon-γ Release Assays

Advances in molecular biology and genomics have led to use of alternatives to the tuberculin skin test.[480,609] Two new in vitro formats have been developed that measure interferon-γ release by sensitized lymphocytes after stimulation by *M. tuberculosis* antigens. Both formats use proteins—early secreted antigen target 6 and culture filtrate protein (CFP)-10—that are found on *M. tuberculosis* and only a few species of nontuberculous mycobacteria (*M. kansasii*, *M. marinum*, and *M. szulgai*) but not on the bacilli BCG or the *M. avium* complex. The first test licensed in the United States was QuantiFERON-TB and includes an extra antigen (TB7.7). The test measures whole-blood interferon production. The second format licensed in the United States is the enzyme-linked immunospot (T-SPOT.TB). This technique measures the number of mononuclear cells that produce interferon.

Both formats of interferon gamma release assays (IGRAs) have been studied primarily in adults.[480] Sensitivity in HIV-uninfected adults with disease has ranged from 73 to 96 percent,[421] and reported specificities range from 86 to 99 percent.[155,568] In HIV-infected adults with disease, sensitivity and specificity have ranged from 30 to 92 percent and 52 to 76 percent, respectively.[110,421] One meta-analysis of IGRA performance in HIV-uninfected adults with infection demonstrated specificities ranging from 98 to 100 percent.[154] There is some evidence that

the T-SPOT.TB is less affected by immunosuppression in HIV-infected adults than the QuantiFERON.[87]

In many clinical situations, these tests have a higher specificity than that of the tuberculin skin test, better correlation with surrogate measures of recent exposure to *M. tuberculosis* in low-incidence settings, and less cross-reactivity than does the tuberculin skin test because of previous BCG vaccination. Like the tuberculin skin test, interferon release assays cannot differentiate between tuberculosis infection and disease. Two clear advantages of the interferon release assays are the need for only one patient encounter (vs. two with the tuberculin skin test) and the lack of possible boosting of the result, because the patient is not exposed to any biologic material. It is unclear to what degree an IGRA can be boosted by a preceding tuberculin skin test. One meta-analysis reported substantial within-subject variation in IGRAs before and after tuberculin skin testing, but the frequency varied dramatically (16% to 80%).[675] Two more recent studies did not demonstrated boosting.[525,543] Although the potential risk for boosting can be avoided by obtaining the IGRA before or at the same time as placing the tuberculin skin test, prior receipt of a tuberculin skin test is not a contraindication to IGRA use.

There are fewer pediatric studies of IGRAs. Sensitivity in children with microbiologically confirmed or all children diagnosed with tuberculosis disease (clinically diagnosed disease and microbiologically confirmed) has ranged from 53 to 100 percent and from 53 to 94 percent, respectively.[380] There is no reference standard for the diagnosis of latent tuberculosis infection. Concordance between the tuberculin skin test and IGRAs varies widely. Although high concordance (> 90%) has been reported from low-prevalence settings where BCG is not used, concordance decreases in BCG-immunized children.[46,130,380] When tuberculin skin testing and IGRAs offer discordant results, the clinician must make the determination of which test to rely on. One reasonable approach is to use the more specific IGRA in an older (≥ 5 years old) immunocompetent child lacking specific TB risk factors, aside from birth in a high-prevalence nation. For younger children, children identified via contact investigations or with contact with persons suspected of having tuberculosis disease, immunocompromised children of any age, or children in whom tuberculosis disease is suspected, then any test being positive would be considered positive.

The performance of IGRAs is limited in certain groups. Immunocompromised children, including those with cancer[620] and HIV infection,[128,303,387] are more likely to have indeterminate test results,[243] as are children younger than 2 years of age.[303] Whereas some studies have shown no association between indeterminate results (usually due to failure of the positive control) and age,[490] others have reported high rates of indeterminate test results in children younger than 4 or 5 years of age.[46,130] Helminthic infections have also been associated with indeterminate results,[655] a particular concern when screening international adoptees.

Reversions and conversions of IGRAs have been described in HIV-infected children being treated for tuberculosis disease.[128] Determining performance for children with tuberculosis infection is more challenging, because there is no reference standard. One proxy is degree of exposure to a documented source case. Sensitivity and specificity for infection have ranged from 90 to 92 percent and 91 to 95 percent, respectively.[380] IGRAs appear to correlate more closely than tuberculin skin tests for BCG-vaccinated children identified in contact investigations.[199] However, data are conflicting and other studies have shown similar performance for the tuberculin skin test and IGRAs for the diagnosis of pediatric tuberculosis infection.[4] The sensitivity of IGRAs and the tuberculin skin test declines with decreasing age and in HIV-infected children.[387]

The CDC has suggested that IGRAs be used with caution in children younger than 5 years of age given the relative paucity of data for this age group.[102] One optimal use for these assays may be in the BCG-immunized older child (≥5 years of age) who lacks tuberculosis risk factors aside from birth in a high-prevalence nation. Another use may be for a child whose family is reluctant to treat infection based on a tuberculin skin test result alone. In children in whom one suspects disease, obtaining IGRAs may provide indirect evidence for tuberculosis in instances where the child is anergic to the tuberculin skin test. Because most studies have not shown a consistent, significant difference between the 2 IGRAs, the CDC recommends that the assays may be used interchangeably.[102]

Given the brief period of time during which IGRAs have been used, many questions remain unanswered. Some relate to the dynamics of IGRAs—when they become positive after exposure to a person with tuberculosis disease, how long they remain positive, and the degree to which conversions and reversions occur on therapy. These questions may be best answered with longitudinal studies.

Diagnostic Mycobacteriology in Children

The demonstration of acid-fast bacilli in stained smears of sputum is presumptive evidence of pulmonary tuberculosis in most patients.[536] However, in children, tubercle bacilli usually are relatively few. Gastric washings, which often are used in lieu of sputum, can be contaminated with acid-fast organisms from the mouth. Tubercle bacilli in CSF, pleural fluid, lymph node aspirate, and urine are sparse; thus, only rarely are direct-stained smears for tubercle bacilli of any use in pediatric practice.[628] Cultures for tubercle bacilli are of great importance, not only to confirm the diagnosis but increasingly to permit testing for drug susceptibility. If culture and drug susceptibility data are available from the associated adult case and the child has classic features of tuberculosis (positive skin test result, consistent abnormal chest radiograph), obtaining cultures from the child adds little to management.

Painstaking collection of specimens is essential for diagnosis of children because fewer organisms usually are present than in adults.[579] Gastric lavage should be performed in the very early morning, when the patient has had nothing to eat or drink for 8 hours and before the patient has a chance to wake up and start swallowing

saliva, which could dilute the bronchial secretions that were brought up during the night and made their way into the stomach. The stomach contents should be aspirated first. No more than 50 to 75 mL of sterile distilled water (not saline) should be injected through the stomach tube and the aspirate added to the first collection. The gastric acidity (poorly tolerated by tubercle bacilli) should be neutralized immediately. Concentration and culture should be performed as soon as possible after collection. However, even with optimal, in-hospital collection of three early-morning gastric aspirate samples, *M. tuberculosis* can be isolated from only 30 to 40 percent of children and 70 percent of infants with pulmonary tuberculosis.[76,618,670]

Sputum induction is an alternative to gastric aspiration. Sputum induction is an outpatient procedure that can be performed in clinic settings[436] and requires the child to be fasting for only 3 hours. A nebulized treatment with a β agonist is given, followed by a nebulized treatment with hypertonic saline. The β agonist decreases the risk for bronchospasm occasionally seen with hypertonic saline. Saline increases the tussive force in children (even those who had no cough initially). As children begin to cough up secretions, the child's oropharynx is suctioned. One study from a high-prevalence nation showed that one specimen obtained via sputum induction had the same smear and culture yield as three gastric aspirates.[282,722] Sputum induction in HIV-infected and HIV-uninfected adults had the same diagnostic yield as specimens obtained via bronchoscopy.[127] In most studies, the yield of *M. tuberculosis* from bronchoscopy specimens has been lower than from properly obtained gastric aspirates.[1,104]

CSF, pleural fluid, and synovial fluid (as much fluid as possible should be collected) usually are centrifuged, and the sediment is used for stained smear and culture. An overnight urine specimen should be obtained in the early morning and taken immediately to the laboratory for processing because the organisms tolerate the low pH of urine poorly. Lymph node aspirates and biopsy tissue can be inoculated directly into a fluid medium such as Middlebrook 7H9.

Staining and examination of smears as well as inoculation of special media, incubation in a carbon dioxide environment, strain differentiation based on many cultural characteristics, and drug susceptibility testing require equipment, skills, and experience beyond those available in the usual clinic or hospital laboratory.[250] Thus, most laboratories depend on regional or reference laboratories for procedures beyond their scope.

Traditional drug susceptibility testing ordinarily is performed for isoniazid, rifampin, ethambutol, and streptomycin. Pyrazinamide susceptibilities are not routinely performed owing to testing complexities. Drug susceptibilities are performed using one of two systems: agar proportions and growth in liquid media. For agar proportions, the organism is inoculated onto two plates: one with a specified concentration of an antibiotic and one that is drug free. The proportion of colonies on each plate is compared, and if there is more growth on the antibiotic plate, the isolate is considered resistant to that drug. For automated liquid media systems (e.g., BACTEC), the isolate is inoculated into vials with and without antibiotic. If the drug-containing vial grows faster than or at the same rate as the drug-free vial, then the isolate is considered resistant to that drug. Susceptibilities to other medications can be requested if the isolate is resistant to first-line medications.

Pyrosequencing, detecting pyrophosphate release during DNA synthesis, has been used to detect mutations associated with resistance to several first- and second-line medications. One advantage is the ability of pyrosequencing to detect mutations in multiple loci in several isolates in real time.[197]

Nucleic Acid Amplification

The main form of nucleic acid amplification studied in children with tuberculosis is PCR, which uses specific DNA sequences as markers for microorganisms.[188,389,433] Various PCR techniques, most using the mycobacterial insertion element IS6110 as the DNA marker for *M. tuberculosis* complex organisms,[11,88] have a sensitivity and specificity of more than 90 percent in comparison to sputum culture for detecting pulmonary tuberculosis in adults. Other advantages include the ability to detect even low organism burdens, a particular consideration in children with paucibacillary disease; rapidity of results and decreased time to initiation of therapy and patients lost to follow-up; ability to differentiate between *M. tuberculosis* and nontuberculous mycobacterial species; and potentially diminished biosafety requirements.[458] Disadvantages include diminished sensitivity in patients with smear-negative disease; expense of equipment; variation in test performance even among reference laboratories[463,705]; and concern for cross-contamination.

Evaluation of PCR in childhood tuberculosis has been limited. Compared with a clinical diagnosis of pulmonary tuberculosis in children, the sensitivity of PCR has varied from 25 to 83 percent and specificity has varied from 80 to 100 percent.[433,466,488,553,590] PCR of stool had a sensitivity of 30 to 40 percent and a specificity of 100 percent in children with pulmonary tuberculosis,[709] offering a noninvasive diagnostic modality. PCR of gastric aspirates may be positive in a recently infected child even when the chest radiograph is normal, demonstrating the occasional arbitrariness of the distinction between tuberculosis infection and disease in children. PCR may have a useful but limited role in evaluating children for tuberculosis. A negative PCR analysis never eliminates tuberculosis as a diagnostic possibility, and a positive result does not confirm it. PCR may be particularly helpful in evaluating immunocompromised children with pulmonary disease, especially those with HIV infection, although published reports of its performance in such children are lacking. PCR also may aid in confirming the diagnosis of extrapulmonary tuberculosis, but only a few reports have been published.[218,386,425]

Xpert TB/RIF is a real-time PCR assay for *M. tuberculosis* that simultaneously detects rifampin resistance. Rifampin resistance is used as a proxy for MDR-TB, because rifampin monoresistance is uncommon.[171] Rifampin mutations, in contrast to isoniazid mutations, cluster on a small part of the mycobacterial genome and

synonymous mutations are rare, facilitating molecular detection of drug resistance. This assay uses a self-contained cartridge system and is not as operator dependent as traditional PCR. Sensitivity and specificity were 72 to 77 percent and 99 percent in smear-negative adults and 98 to 99 percent and 99 to 100 percent in smear-positive adults, respectively and performed equivalently in HIV-infected and HIV-uninfected adults.[62] The two pediatric studies to date comparing Xpert TB/RIF to culture showed a sensitivity of 100 percent in smear-positive children and 61 to 67 percent in smear-negative children, with a specificity of 99 to 100 percent.[459,508] Smear microscopy, in contrast, had a sensitivity of 39 percent and a specificity of 100 percent.[459] Although cartridges for the Xpert system are expensive, the self-contained system offers advantages in rapid detection of MDR-TB and the prospect of increasing available diagnostics in settings that lack laboratory infrastructure. However, Xpert should not replace the need for obtaining mycobacterial cultures. The need to identify additional drugs to which the isolate is susceptible is crucial for advancing patient care.

TREATMENT

Management of Tuberculous Children

Treatment of cavitary tuberculosis in adults is one of the most scientifically accurate areas in all of medicine and one of the finest examples of international professional cooperation in all of history. The ability to quantify the extent of disease and the large numbers of cases of tuberculosis have allowed researchers to address specific questions about optimal drug regimens and doses, duration of therapy, and nonmedical management. Historically, treatment recommendations for children have been extrapolated from data in adults. However, during the past 3 decades, a large number of treatment trials for children have been reported, which has led to dramatic changes in the therapeutic approach to childhood tuberculosis.

As recently as the early 1980s, the recommended treatment duration for children with tuberculosis disease was 12 to 18 months. Although these regimens are effective when they are used properly, actual failure rates are high because of poor adherence during the long period of treatment. Newer regimens often are called short-course chemotherapy because treatment durations as short as 6 months are routinely successful. However, the key to the new approach is not the short duration but the intensive initial therapy with three or more antituberculosis drugs.

Antituberculosis Drugs (Tables 96-8 and 96-9)

Isoniazid is the mainstay of therapy for tuberculosis in children. Inexpensive, bactericidal, of low molecular weight and therefore readily diffusible to all tissues in the body, [163] and relatively nontoxic to children, isoniazid is one of the most nearly perfect drugs in the pediatrician's armamentarium. It can be administered orally, intramuscularly, or intravenously. When the drug is taken orally, high plasma, sputum, and CSF levels are reached within a few hours and persist for at least 6 to 8 hours.[342,471] Because of the slow multiplication of *M. tuberculosis*, the total daily dose can be given at one time. The usual level necessary to inhibit multiplication of tubercle bacilli is 0.02 to 0.05 μg/mL.

Human variation in the acetylation rate of isoniazid to an inactive compound is known to be determined genetically.[405] Rapid acetylation occurs more frequently in blacks and Asians than in whites. Although a simple method, specifically the use of a urine sample, now is available for classifying patients as slow or rapid inactivators of isoniazid, the normal way of coping with the problem in children has been to give a sufficiently large dose of isoniazid to ensure an adequate level even in rapid inactivators.[403,681]

The principal toxic effects of isoniazid are peripheral neuritis and hepatitis. Peripheral neuritis resulting from competitive inhibition of pyridoxine utilization is a rare event in North American children because both milk and meat are the main dietary sources of pyridoxine.[54] In some well-nourished children, serum pyridoxine concentrations are depressed by isoniazid but clinical signs are not apparent.[495] In the case of most children, therefore, the use of supplementary pyridoxine is not necessary. However, in teenagers whose diets may be inadequate, in children from ethnic groups with low milk and meat intake, in HIV-infected children, and in breast-fed infants, pyridoxine supplementation (25 to 50 mg/day) is important. Peripheral neuritis, when it does occur, usually is manifested by "pins and needles" sensations in the hands and feet.

The risk for development of hepatotoxicity from isoniazid, which is rare in children, increases in frequency with age.* Its cause is unclear.[332] Rapid acetylators are no more susceptible than are slow acetylators.[190,403] Simultaneous use of alcohol, phenytoin, piperazine, and especially rifampin seems to increase the likelihood of hepatotoxicity.[86,336] Monitoring of aspartate aminotransferase and alanine aminotransferase activity sometimes reveals transient increases during treatment with isoniazid, but the levels usually return spontaneously to normal without interruption of treatment.[483] Liver enzyme abnormalities in adolescents receiving isoniazid are common occurrences and usually disappear spontaneously, but severe hepatitis can occur.[370,595] The estimated incidence of hepatic failure in children on isoniazid monotherapy was 3.2/100,000,[717] jaundice was rare (0.06%), and transient elevations in hepatic transaminase levels were seen in 8 percent.[162] In children receiving isoniazid as part of multidrug therapy, rates of jaundice and elevated hepatic transaminase levels are higher, but still extremely low.[162]

The possible occurrence of hepatitis raises the question of routine monitoring of liver enzyme levels once a month in all children receiving isoniazid. The advantage of doing so has to be weighed not only against the expense but particularly against the difficulty of ensuring regular

*References 38, 86, 329, 366, 449, 468, 510, 606, 621.

TABLE 96-8 Commonly Used Drugs for the Treatment of Tuberculosis in Children

Drug	Dosage Forms	Daily Dose (mg/kg/day)	Twice-Weekly Dose (mg/kg/dose)	Thrice-Weekly Dose (mg/kg/dose)	Maximal Daily Dose
Ethambutol	Tablets 100 mg 400 mg	20-25	50	Not recommended for young children 1.5 g if 40-55 kg 2.5 g if 56-75 kg 3 g if >75 kg	2.5 g
Isoniazid*†	Scored tablets: 100 mg 300 mg Syrup:‡ 10 mg/mL	10-15†	20-30	15	Daily: 300 mg Twice weekly: 900 mg Thrice weekly: 900 mg
Pyrazinamide	Scored tablets: 500 mg	20-40	50 (max: 2 g) if <56 kg 3 g if 56-75 kg 4 g if >75 kg	Not recommended for young children 1.5 g if 40-55 kg 3 g if 56-75 kg 4 g if >75 kg	2 g
Rifampin*	Capsules: 150 mg 300 mg Syrup: Formulated in syrup from capsules	10-20	10-20	Not recommended for young children Adolescents: 10	Daily: 600 mg Twice weekly: 900 mg
Streptomycin	Vials: 1 g 4 g	20-40	20-40		1 g

*Rifamate is a capsule containing 150 mg of isoniazid and 300 mg of rifampin. Two capsules provide the usual adult (>50 kg body weight) daily doses of each drug.
†When isoniazid is used in combination with rifampin, the incidence of hepatotoxicity increases if the isoniazid dose exceeds 10 mg/kg/day.
‡Most experts advise against the use of isoniazid syrup because of instability and a high rate of gastrointestinal adverse reaction (diarrhea, cramps).

TABLE 96-9 Drugs for Treatment of Drug-Resistant Tuberculosis in Children*

Drugs	Dosage Forms	Daily Dosage (mg/kg/day)	Maximum Daily Dose
Amikacin	Vials: 500 mg, 1 g	15-30 (IM, IV)	1 g
Capreomycin	Vials: 1 g	15-30 (IM, IV)	1 g
Clofazimine	Capsules: 50 mg 100 mg	50-100 mg/day	200 mg
Cycloserine	Capsules: 250 mg	10-20	1 g
Ethionamide	Tablets: 250 mg	15-20 given in two or three divided doses	1 g
Kanamycin	Vials: 75 mg/2 mL 500 mg/2 mL 1 g/3 mL	15-30 (IM, IV)	1 g
Levofloxacin	Tablets: 250 mg 500 mg 750 mg	Adults: 500-750 mg total/day Pediatric: 7.5-10 mg	1000 mg
Linezolid	Vials: 2 mg/mL Tablets: 600 mg Suspension: 20 mg/mL	10 (PO, IV)	600 mg
p-Aminosalicylic acid	Packets: 4 g	200-300 given in two to four divided doses	12 g

IM, intramuscularly; *IV,* intravenously; *PO,* orally.
*There are no data to support intermittent dosing for second-line medications except for injectable agents.

monthly visits if the patient and parents know that every clinic visit entails a venipuncture. Most experts prefer to substitute routine questions about appetite and well-being, determination of weight, and a check of the appearance of the sclera and the size of the liver.[77,493] Patients should be counseled to stop taking isoniazid and to contact the clinician immediately if significant nausea, vomiting, abdominal pain, or jaundice occurs during the use of isoniazid.

Allergic manifestations of isoniazid hypersensitivity are extraordinarily rare. Convulsions have been reported after doses of 100 mg/kg or more, as in suicide attempts.[410,461,485,570] The treatment for isoniazid overdose is intravenous pyridoxine.

The usual dosage in children is 10 to 15 mg/kg/day, to a maximum of 300 mg/day. Isoniazid is available in tablets of 100 and 300 mg. A syrup of isoniazid in sorbitol (10 mg/mL) also is available; however, it is unstable at 37° C and should be kept cool. Significant gastrointestinal intolerance (nausea, diarrhea) develops in many children while they are taking the isoniazid suspension. If tablets are used, they are crushed easily in a dessert spoon, to which then is added in the same spoon a vehicle such as applesauce, mashed banana, or another palatable medium. The crushed tablets must never be added to the nursing bottle or offered in milk or water because they will be ingested only partially. If isoniazid is given concurrently with rifampin, the dose should not exceed 10 mg/kg/day.[468] If the intramuscular form is used, for example, in a child with meningitis who is vomiting, the daily dose is the same as the daily oral dose but usually is divided and given every 8 to 12 hours. Isoniazid can interact with several other drugs, particularly theophylline, and the dosage of each may need to be modified in a patient taking several drugs.[28] Isoniazid also can increase serum phenytoin levels by blocking its metabolism in the liver, thereby leading to toxicity.[424]

RIF is a semisynthetic drug derived from *Streptomyces mediterranei*. Active against a wide variety of both intracellular and extracellular organisms, it is bactericidal and more effective against mycobacteria than is any other drug except isoniazid. Most clinical isolates are susceptible to 5 μg/mL or less. The drug is absorbed readily from the gastrointestinal tract in the fasting state; peak serum levels of 6 to 10 μg/mL are achieved within 2 hours, and the drug is distributed widely in body fluids and tissues, including CSF. Excretion is mainly through the biliary tract; however, effective levels are achieved in the kidneys and urine. In many patients receiving treatment with rifampin, tears, saliva, urine, and stool turn orange as the result of a harmless metabolite, but patients and parents always must be warned in advance. Drawbacks include the relatively high cost; the rare occurrence of explosive hypersensitivity reactions with hemolytic anemia (associated with intermittent rifampin therapy[214]); and the occasional occurrence of leukopenia or thrombocytopenia (associated with daily rifampin[225]). Rifampin has a number of drug interactions, including inactivation of oral contraceptives. It decreases levels of protease inhibitors, thus limiting its use in HIV-coinfected children on antiretroviral therapy. Rifabutin is preferred in this circumstance. Finally, there is a "therapeutic orphan" clause in the United States for children younger than 5 years, which also means that no formulation is commercially available for young children. However, rifampin easily can be made into a suspension for use in children.

Rifampin should be used alone only in treating tuberculosis infection with an isoniazid-resistant organism or for children who do not tolerate isoniazid. If isoniazid 20 mg/kg and RIF 15 to 20 mg/kg are used, the incidence of hepatotoxicity is appreciable. Therefore, when the two are used together, one would be wise to approximate the doses to isoniazid 10 mg/kg and RIF 15 to 20 mg/kg. Rifamate is a capsule containing both isoniazid (150 mg) and rifampin (300 mg). Two capsules supply the usual adult (more than 50 kg) daily dose of each drug. Rifamate may be appropriate for older children and adolescents.[3] Rifater contains isoniazid, rifampin, and pyrazinamide together in one pill in varying concentrations. Rifapentine is a new rifamycin with a very long half-life, allowing for weekly administration. It is now recommended in combination therapy with isoniazid for the treatment of tuberculosis infection for persons 12 years of age or above.[626] This regimen currently is recommended only under directly observed preventive therapy. Few pharmacokinetic data for rifapentine are available for young children. It appears that children require larger weight-normalized doses than adults.[56] Long-term efficacy data for children and adolescents are scant.

Pyrazinamide contributes to the killing of *M. tuberculosis*, particularly at a low pH, such as within macrophages.[535] The exact mechanism of action of this agent is a subject of controversy. Pyrazinamide has no effect on extracellular tubercle bacilli in vitro but clearly contributes to the killing of intracellular bacilli. Primary resistance is very rare, except that *Mycobacterium bovis* is intrinsically resistant. The drug diffuses readily into all areas, including CSF.[161,191] The usual adult daily dose is 30 to 40 mg/kg. The adult dose is tolerated well by children, results in high CSF concentrations, and clearly is effective in therapy trials for tuberculosis in children.[167,528,539,611] However, serum concentrations are lower in children than in adults taking a weight-equivalent dose.[222] Pyrazinamide appears to exert its maximal effect during the first 2 months of therapy. Hepatotoxicity can occur at high doses but rarely does at the usual dose. Pyrazinamide routinely causes an increase in serum uric acid concentration by inhibiting its excretion through the kidneys. Toxic reactions in adults include flushing, cutaneous hypersensitivity, arthralgia, and overt gout; however, the considerable experience with this drug in children in Latin American countries, Hong Kong, and the United States has revealed few problems. It plays a major role in intensive, short-course treatment regimens.[215,611] Use of pyrazinamide for the first 2 months allows therapy to be shortened from 9 to 6 months for most nonskeletal, nonmeningeal forms of tuberculosis.

Ethambutol has been used for many years as a companion drug for isoniazid in adults. The usual oral dose is 20 mg/kg/day.[164] At this dose, the drug primarily is bacteriostatic, its major role being to prevent the emergence of resistance to other drugs. However, at doses of 25 mg/kg/day or 50 mg/kg given twice a week,

ethambutol has some bactericidal action.[129,210] Unfortunately, at these higher doses, optic neuritis or red-green color blindness has occurred in some adults. Regular visual field and color chart testing should detect these reversible effects early. The incidence of ophthalmologic toxicity in children is extremely low (0.05%), if it occurs at all, in part because children metabolize the drug faster than adults.[164] It is used frequently and safely in children, including in preverbal children in whom visual acuity evaluation is not possible, with life-threatening forms of tuberculosis or who are at risk for drug-resistant tuberculosis. However, it diffuses poorly into CSF.[161] In the United States, it is used with isoniazid, rifampin, and pyrazinamide as first-line therapy for children with suspected tuberculosis when drug-susceptibility results are not yet known.

Ethionamide is an effective and well-tolerated drug in children at a dose of 15 to 20 mg/kg/day divided into two or three doses given after meals. Children rarely complain about its sulfurous taste, which is repulsive to adults. Related to isoniazid, it likewise diffuses readily into CSF.[166,168,271] Ethionamide is used in cases of drug-resistant tuberculosis and tuberculous meningitis, where it can replace ethambutol in the regimen. Unfortunately, no convenient pediatric dosage form is available. Use of ethionamide may be limited by its most common side effect—vomiting. Ethionamide resistance is common in children with isoniazid resistance.[550]

Second-line medications are used when patients have drug-resistant strains of *M. tuberculosis* or when they are intolerant of first-line mediations. Second-line medications are notable for more frequent or severe adverse effects, fewer pediatric pharmacokinetic data, and fewer available child-friendly formulations than first-line drugs. Drug susceptibility testing for some second-line agents (cycloserine, linezolid, clofazimine) is less well-validated than for first-line medications, and correlation between in vitro susceptibility and clinical response is less evident.

Streptomycin is used in conjunction with isoniazid and rifampin in life-threatening forms of tuberculosis. It is bactericidal and tolerated well in children in the usual dose of 20 to 40 mg/kg/day intramuscularly up to 1 g. Usually, streptomycin can be discontinued within 1 to 3 months if clinical improvement is definite, whereas the other two or three drugs are continued by mouth. Use of streptomycin and other injectable drugs (amikacin, capreomycin, kanamycin) is limited by the dose-dependent incidence of ototoxicity and nephrotoxicity. An isolate may be resistant to some injectable agents and remain susceptible to others.

p-Aminosalicylic acid formerly was part of the standard treatment of tuberculosis. However, it is a purely bacteriostatic drug that has been superseded by more powerful drugs (e.g., rifampin, pyrazinamide). It is used only for the treatment of drug-resistant tuberculosis. Cycloserine, linezolid, and clofazimine are other drugs sometimes used in patients with MDR-TB.[561] Cycloserine diffuses well into tissues and CSF,[161] but its use may be limited by neuropsychiatric side effects,[656] which are more common in adults. Linezolid demonstrates modest early bactericidal activity but little extended early bactericidal activity[156]; its use may be limited by optic neuritis, peripheral neuropathy, and bone marrow suppression. Clofazimine has anti-inflammatory properties, and there is little documented resistance; its use may be limited by skin pigment changes.[116]

Several of the fluoroquinolones, especially levofloxacin and moxifloxacin, have significant antituberculosis activity,[315,430] but they cannot be used routinely in children with drug-susceptible tuberculosis because of the possible destruction of growing cartilage seen in animal models. However, the dire consequences of drug-resistant tuberculosis lead many experts to use them successfully and with little apparent toxicity in children with MDR-TB disease.[562] These drugs are bactericidal and diffuse into tissues and CSF well.[161] If an isolate is resistant to one fluoroquinolone, resistance to other fluoroquinolones is common.

Microbiologic Basis for Treatment

Laboratory observations of *M. tuberculosis* and the results of clinical therapy trials have led to a hypothesis concerning the actions of various drugs and drug combinations.[181,225,427,428] The tubercle bacillus can be killed only during replication, which occurs in organisms that are active metabolically. In one model, bacilli in a host exist in different populations (Table 96-10). They are active metabolically and replicate freely where oxygen tension is high and the pH is neutral or alkaline. Environmental conditions for growth are best within cavities, and such conditions can lead to a large bacterial population. Adults with reactivation-type pulmonary tuberculosis usually have all three populations of tubercle bacilli. Children with pulmonary tuberculosis and patients of all ages with only extrapulmonary tuberculosis are infected with a much smaller number of tubercle bacilli because the cavitary population is not present.

Naturally occurring drug-resistant mutant organisms occur within large populations of tubercle bacilli even before chemotherapy is started. All known genetic loci for drug resistance in *M. tuberculosis* are located on the chromosome; no plasmid-mediated resistance is known. The rate of resistance within populations of organisms is

TABLE 96-10 **In Vivo Location of *Mycobacterium tuberculosis*: A Model**

Location	Population Size	Metabolism and Replication	pH	Most Effective Drugs
Cavity	10^7-10^9	Active and rapid	Neutral or alkaline	INH, RIF, STM
Closed caseous lesions	10^5-10^7	Slow and intermittent	Neutral	RIF, INH
Within macrophages	10^4-10^6	Very slow	Acid	PZA, RIF, INH

INH, isoniazid; *PZA,* pyrazinamide; *RIF,* rifampin; *STM,* streptomycin.

related to the rate of mutations at these loci.[416,641,647,724,725] Although a large population of bacilli as a whole may be considered drug susceptible, a subpopulation of drug-resistant organisms occurs at a fairly predictable rate. The mean frequency of these drug-resistant mutants is about 10^{-6} but varies among drugs: streptomycin, 10^{-5}; isoniazid, 10^{-6}; and rifampin, 10^{-7}.[143] A cavity containing 10^{9} tubercle bacilli has thousands of single drug–resistant mutant organisms, whereas a closed caseous lesion contains few if any resistant mutants.

The two microbiologic properties of population size and drug resistance mutation explain why single antituberculosis drugs cannot cure cavitary tuberculosis. In the mid 1940s, streptomycin alone was given to adults with cavitary pulmonary tuberculosis.[411] Within 3 months, 80 percent of patients had significant numbers of streptomycin-resistant organisms. This phenomenon has been observed for every antituberculosis drug developed subsequently. However, the natural occurrence of resistance to one drug is independent of resistance to any other drug because the resistance loci are not linked. The chance of having even one organism "naturally" resistant to two drugs is on the order of 10^{-11} to 10^{-13}. Populations of this size in patients are extremely rare, and mutants naturally resistant to two drugs are nonexistent.

The population size of tubercle bacilli within a patient determines the appropriate therapy. For patients with large bacterial populations (adults with cavities or extensive infiltrates), many single drug–resistant mutants are present and at least two antituberculosis drugs must be used.[145] Conversely, for patients with tuberculosis infection but no disease, the bacterial population is small (10^{3} to 10^{4} organisms), drug-resistant mutants are rare findings, and a single drug can be used. Children with pulmonary tuberculosis and patients of all ages with extrapulmonary tuberculosis have medium-sized populations in which drug-resistant mutants may or may not be present. In general, these patients should be treated with at least two drugs.

Some antituberculosis drugs, such as isoniazid, rifampin, streptomycin, and fluoroquinolones are bactericidal against *M. tuberculosis*. Other drugs, including ethionamide, *p*-aminosalicylic acid, and low-dose ethambutol, are bacteriostatic. The earliest treatment regimens for tuberculosis combined the killing action of a bactericidal drug with a bacteriostatic drug that would suppress replication of drug-resistant mutant organisms. A small number of organisms survived despite administration of chemotherapy, and 18 to 24 months of treatment was necessary to permit host defenses to eliminate persisting organisms. Despite the prolonged treatment period, relapse rates were 5 to 15 percent, mostly a result of poor adherence to treatment.

The availability of rifampin and the rediscovery of pyrazinamide in the early 1970s effected radical change in antituberculosis chemotherapy. These two drugs have the most potent sterilizing action, the ability to kill tubercle bacilli within lesions as quickly as possible. The addition of rifampin to isoniazid for the treatment of pulmonary tuberculosis leads to cure rates approaching 100 percent with only 9 months of treatment. The further addition of pyrazinamide shortens the necessary treatment duration to only 6 months.

Treatment of the Stages of Tuberculosis

Exposure

Children exposed to potentially infectious adults with pulmonary tuberculosis should begin treatment, usually with isoniazid only, if the child is younger than age 5 years or has other risk factors for the rapid development of tuberculosis disease, such as immunocompromise.[631,711] Failure to do so may result in the development of severe tuberculosis disease even before the tuberculin skin test becomes reactive; the "incubation period" of disease may be shorter than that for the skin test. The child is treated for a minimum of 2 months after contact with the infectious case is broken (by physical separation or by effective treatment of the case). After 2 months, the tuberculin skin test is repeated. If the second test result is positive, infection is documented and isoniazid should be continued for a total duration of 9 months; if the second skin test result is negative, treatment can be discontinued. If the exposure was to a person with an isoniazid-resistant but rifampin-susceptible isolate, then rifampin is the recommended treatment.

Two special circumstances of exposure deserve attention. A difficult situation arises when exposed children are anergic because of HIV infection. These children are particularly vulnerable to rapid progression of tuberculosis, and it will not be possible to determine whether infection has occurred. In general, these children should be treated as though they have tuberculosis infection.

The second situation is potential exposure of a neonate to a mother (or other adult) with a positive tuberculin skin test result or, rarely, a nursery worker with contagious tuberculosis.[357,624] Management is based on further evaluation of the mother:

1. *The mother has a normal chest radiograph:* no separation of the infant and mother is required. Although the mother should receive treatment for tuberculosis infection and other household members should be evaluated for tuberculosis infection or disease, the infant needs no further workup and no treatment is needed unless a case of disease is found.
2. *The mother has an abnormal chest radiograph:* the mother and child should be separated until the mother has been evaluated thoroughly. If the radiograph, history, physical examination, and analysis of sputum reveal no evidence of pulmonary tuberculosis in the mother, a reasonable assumption is that the infant is at low risk for acquiring infection. The radiographic abnormality is due to another cause or a quiescent focus of previous tuberculosis infection. However, if the mother remains untreated, contagious tuberculosis may develop later and the infant will be exposed. Both the mother and infant should receive appropriate follow-up care, but the infant does not need treatment. If the radiograph and clinical history are suggestive of pulmonary tuberculosis, the child and mother should remain

separated until both have begun appropriate chemotherapy. The infant should be evaluated for congenital tuberculosis. The placenta should be examined. If the mother has no risk factors for drug-resistant tuberculosis, the infant should receive isoniazid and close follow-up care. The infant should have a tuberculin skin test at 3 or 4 months after the mother is judged to be contagious no longer; evaluation of the infant at this time follows the guidelines for other exposure of children. If no infection is documented at this time, repeating the tuberculin skin test in 6 to 12 months would be prudent. If the mother has tuberculosis caused by a multidrug-resistant isolate of *M. tuberculosis* or she has poor adherence to therapy, the child should remain separated from her until she is no longer contagious or the infant can be given BCG vaccine and be kept separated until the vaccine "takes" (marked by a reactive tuberculin skin test).

Infection

The recommendation for treatment of asymptomatic tuberculin-positive individuals is based on data from several well-controlled studies; it applies particularly to children and adolescents who are at high risk for the development of overt disease but at very low risk for development of the main toxic manifestation of isoniazid therapy, which is hepatitis.[170,202,455,468,597] The large, carefully controlled U.S. Public Health Study of 1955, followed by others both in this country and abroad, demonstrated the favorable effect of 12 months of isoniazid on the incidence of complications as a result of both lymphohematogenous and pulmonary spread.[268] The younger the tuberculin reactor, the greater the benefit.[126]

The American Thoracic Society and the CDC[13] recommend that isoniazid for treatment of tuberculosis infection be given to the following groups:

- Household members and other close associates of potentially infectious tuberculosis cases. All contacts of any age with a Mantoux tuberculin skin test reading of 5 mm or greater and without a documented history of reaction in the past or with a positive interferon gamma release assay (IGRA) should be considered recently infected and receive therapy if they have not been previously treated.
- Newly infected people, regardless of age, who have had a tuberculin skin test or IGRA conversion within the past 2 years.
- People with HIV infection who have a reaction of 5 mm or greater to a Mantoux test.
- People of any age with tuberculosis infection in the past who received inadequate treatment.
- People of any age with a positive tuberculin skin test or positive IGRA and an abnormal but stable chest radiograph.
- People with significant tuberculin reactions or positive IGRAs who have special clinical situations, including silicosis, diabetes mellitus, prolonged corticosteroid therapy, immunosuppressive therapy, hematologic malignant disease, and end-stage renal disease.
- All children and adolescents with a "positive" tuberculin skin test reaction or interferon release assay result.

The question arises regarding how long the protective effect can be expected to last. Comstock and associates,[124] in their final report on isoniazid prophylaxis in Alaska, demonstrated the protective effect of 1 year of chemoprophylaxis to be at least 19 years. Hsu[267] described 2494 patients monitored for up to 30 years and showed that adequate drug prophylaxis prevented reactivation of tuberculosis infection during adolescence and into young adulthood. It seems reasonable to hope that the decreased risk for active tuberculosis after isoniazid prophylaxis may, in fact, be lifelong in individuals infected with isoniazid-susceptible tubercle bacilli. Failure of isoniazid after exposure to isoniazid-resistant *M. tuberculosis* has been documented. No controlled study of an alternative regimen has been reported. Rifampin alone is recommended and widely used, although failures have been reported.[368]

The duration of isoniazid treatment initially was set arbitrarily at 12 months.[281] A large trial comparing regimens of daily isoniazid for 12, 24, and 52 weeks with placebo for their ability to prevent tuberculosis disease was conducted in eastern Europe with adults who had old fibrotic lesions caused by tuberculosis. Therapy for 1 year was most effective, especially if the patients were adherent to it. However, therapy for 24 weeks afforded a fairly high level of protection. A subsequent analysis concluded that the 24-week duration of preventive therapy was more cost effective for adults than was the 52-week duration.[596] Subsequently, many health departments have accepted 6 months of isoniazid preventive therapy as their standard regimen for adults. However, the cost-effectiveness analysis does not apply to children. A recent review of all published studies concluded that the effectiveness of isoniazid therapy increased up to 9 months' duration, but no additional benefit was achieved with a longer duration.[13] AAP and CDC recommended regimens[13] for tuberculosis infection include the following:

- 9 months of therapy with isoniazid—administered daily by family or twice weekly under direct observation
- 6 months of therapy with rifampin—for patients whose source case has an isoniazid-resistant, rifampin-susceptible *M. tuberculosis* strain or in patients who cannot tolerate isoniazid
- 12 doses of isoniazid and rifapentine administered weekly via direct observation for children 12 years of age and older[626]
- For children whose source case has MDR-TB, combination therapy with two drugs to which the isolate is susceptible may be considered; expert consultation should be sought

A shorter duration of effective treatment for tuberculosis infection is highly desirable to improve compliance and effectiveness.[396] In England, a 3- to 4-month course of isoniazid plus rifampin is used frequently. There

have been published case-control series, and a recent meta-analysis of available data suggests that especially when compliance is considered, a 3- to 4-month course of isoniazid plus rifampin may be as effective as is a 9-month course of isoniazid.[195] One small, randomized study also yielded favorable results with this regimen.[604] However, this regimen is not yet recommended by the CDC, AAP, or WHO.

Isoniazid preventive therapy in HIV-infected patients has been best studied in adults.[404] The theoretical advantages of this therapy in settings with high tuberculosis prevalence are that persons may be exposed without knowledge of a specific source case; HIV-infected patients may be anergic to the tuberculin skin test (and have high rates of indeterminate IGRA results); and HIV-infected persons with tuberculosis infection are at high risk for rapidly progressing to tuberculosis disease. The potential disadvantages are the potential of selecting for isoniazid-resistant isolates, inadvertently treating patients for infection when they have disease, drug toxicity in a patient who may not have infection, and cost. Randomized trials have shown IPT to be effective in preventing tuberculosis disease-free survival in adults, irrespective of the use of HAART. Isoniazid preventive therapy in HIV-infected children is controversial. Two large randomized studies have evaluated its impact on survival and tuberculosis disease-free survival in HIV-infected African children. Whereas one study showed decreased all-cause mortality and decreased cases of tuberculosis disease,[721] a second study found no benefit.[381] Additional data are needed to determine what role isoniazid preventive therapy should have in national tuberculosis programs in settings with high prevalence of both HIV and tuberculosis.

Disease in Adults

In adults, a shorter duration of antituberculosis chemotherapy is desirable for several reasons. It may be significantly less expensive than traditional therapy; the patient is exposed to potentially toxic drugs for shorter periods of time; and more time and resources can be allotted to ensure adherence with treatment. Finally, if a patient absconds from treatment, a greater likelihood will exist that bacteriologic cure already has been achieved as a result of the early and rapid sterilizing activity of the newer regimens.

A 9-month regimen of isoniazid and rifampin cures more than 98 percent of cases of drug-susceptible pulmonary tuberculosis in adults.[585] Both drugs are given daily for the first 2 weeks to 2 months. There is no difference in outcome for patients who receive medication 7 days per week versus 5 days per week. Consequently, 5 day-per-week regimens are utilized to facilitate directly observed therapy. After this, medications can be given daily, twice weekly, or thrice weekly under directly observed therapy for the remaining 7 to 8 months with equivalent results and rates of adverse reactions. When it is given twice weekly, the rifampin dose is the same as the daily dose, but the isoniazid dose is increased to 900 mg in adults. Twice-weekly and thrice-weekly administration is supported by pharmacologic and animal model data determining the area-under-the-curve characteristics for these antituberculosis drugs. Unfortunately, durations of therapy with only isoniazid and rifampin for less than 9 months are unacceptable because failure and relapse rates exceed 10 percent.

When three or more antituberculosis drugs are used initially, treatment durations of 6 months are routinely successful.[120] Regimens using isoniazid, rifampin, pyrazinamide, and streptomycin during the initial phase (2 months), followed by isoniazid and rifampin in the continuation phase (4 months), routinely yield cure rates greater than 98 percent and relapse rates below 4 percent.[25,582] If pyrazinamide is excluded from the initial phase, the rate of bacteriologic failure rises to 7 to 10 percent.[259] However, exclusion of streptomycin does not affect cure or relapse rates appreciably.[259,598] Use of pyrazinamide beyond the first 2 months of therapy does not add any benefit for patients with drug-susceptible isolates.[260] Regimens of 4 months' total duration have unacceptably high relapse rates of 10 percent or greater.[292] On the basis of all reported studies, the American Thoracic Society (ATS) and CDC currently recommend for the treatment of pulmonary tuberculosis in adults a 6-month regimen using isoniazid, rifampin, pyrazinamide, and ethambutol for 2 months, followed by 4 months of daily or twice-weekly doses of isoniazid and rifampin.[59,92]

Duration of therapy may need to be extended in HIV-infected patients. One meta-analysis found that relapse and failure rates were higher in thrice-weekly compared with daily therapy and there was a trend toward higher relapse rates in patients who were treated for 6 months when compared with those treated for at least 8 months.[318]

Chemotherapy for Children

Clinical trials of antituberculosis drugs in children are difficult to perform, mostly because of the difficulty in obtaining positive cultures at diagnosis or relapse and the need for long-term follow-up.[611,612] Historically, recommendations for treating children with tuberculosis have been extrapolated from clinical trials of adults with pulmonary tuberculosis.[592] However, during the past 2 decades, a large number of clinical trials involving only children have been reported. In 1983, Abernathy and colleagues[2] reported successful treatment of 50 children with tuberculosis in Arkansas using isoniazid and rifampin daily for 1 month, then twice weekly for 8 months. The success rate virtually was 100 percent. Most pulmonary infiltrates cleared by the end of therapy, but hilar adenopathy usually was still present radiographically and then gradually cleared during a period of 2 to 3 years. Patients with only hilar adenopathy can be treated successfully with isoniazid and rifampin for 6 months.[287,518] In children with cavitary lung disease or children with persistently positive sputum cultures after 2 months of effective therapy, therapy may be extended to 9 months to decrease relapse rates.[99]

Several major studies of 6-month therapy in children with at least three drugs in the initial phase have been reported.* The regimen used most commonly was 6

*References 8, 53, 277, 334, 638, 651, 663.

months of isoniazid and rifampin supplemented during the first 2 months with pyrazinamide. The overall success rate has been greater than 98 percent and the incidence of clinically significant adverse reactions less than 2 percent. Regimens not using streptomycin were as successful as those that included it. Use of twice-weekly medications (under directly observed therapy) during the continuation phase was as effective and safe as was daily administration. Three studies used twice-weekly therapy throughout the treatment regimen with excellent success.[334,651,680] The 6-month, three-drug regimen is successful, tolerated well, and less expensive.[629,664] It also effects a cure faster, so the likelihood of successful treatment is greater if the child becomes nonadherent later in therapy. Current CDC and ATS recommendations are to include ethambutol with isoniazid, rifampin, and pyrazinamide during the initial phase unless the child's source case is known to have an *M. tuberculosis* isolate susceptible to first-line medications.

The optimal duration of therapy and dosing interval for antituberculous medications in children coinfected with HIV is unclear. Relapse or failure in up to 12 percent of HIV-infected children treated for 6 months has been reported.[549] The optimal timing for initiation of HAART in children with tuberculosis disease is unclear, but extrapolation from adult data suggests a survival benefit from initiation within 2 to 8 weeks.[302,571] Selection of a HAART regimen may be complicated by the known interaction of rifampin with protease inhibitors. Although these interactions are decreased by using rifabutin, some nations have deferred protease inhibitors to second-line HAART because of these interactions. Further complicating care of the coinfected child is the risk for IRIS and that children on both HAART and antituberculous medications tend to tolerate both classes of medication more poorly than children receiving one or the other.[682]

Extrapulmonary Tuberculosis

Controlled treatment trials for various forms of extrapulmonary tuberculosis are rare. In most reports, extrapulmonary cases have been combined with pulmonary cases and often are not analyzed separately. Several of the 6-month, three-drug trials in children included extrapulmonary cases.[53,334] Most non–life-threatening forms of extrapulmonary tuberculosis respond well to a 9-month course of isoniazid and rifampin[180,182] or to a 6-month regimen including isoniazid, rifampin, and pyrazinamide.[284] One exception may be bone and joint tuberculosis, which may have a high failure rate when 6-month chemotherapy is used, especially if surgical intervention has not occurred.[180] Tuberculous meningitis usually is not included in trials of extrapulmonary tuberculosis therapy because of its serious nature and low incidence. Treatment with isoniazid and rifampin for 12 months generally is effective.[685] In the 1950s, Lorber[373] treated children with tuberculous meningitis for only 6 months with good results. A more recent study from Thailand showed that a 6-month regimen that included pyrazinamide for serious tuberculous meningitis led to fewer deaths and better outcomes than did longer regimens that did not contain pyrazinamide.[288] Most children are treated initially with four drugs (isoniazid, rifampin, pyrazinamide, and ethionamide or streptomycin). Ethambutol crosses the blood-brain barrier very poorly, [161] making it a suboptimal choice for treating children with tuberculous meningitis. Treatment with pyrazinamide and the fourth drug is stopped after 2 months, and isoniazid and rifampin are continued for a total of 9 to 12 months.[166]

Drug-Resistant Tuberculosis in Children

The incidence of drug-resistant tuberculosis is increasing because of poor adherence by the patient, the availability of some antituberculosis drugs in noncontrolled over-the-counter formulations, and poor management by physicians.[55,206,207,383,464] In the United States, approximately 13 percent of *M. tuberculosis* isolates are resistant to at least one drug and 1.2 percent are MDR-TB[103]; rates of drug resistance are more than twice as high in foreign-born cases than in U.S.-born persons with disease. Initial drug resistance rates of up to 80 percent have been noted in adults with pulmonary tuberculosis in some countries,[385] and rates of 20 to 30 percent are common findings. Resistance is most common to streptomycin and isoniazid and still is relatively rare to rifampin.[148,261,429,728] Rifampin monoresistance is unusual[171]; in the vast majority of cases, rifampin resistance is seen in conjunction with isoniazid resistance. Certain epidemiologic factors—disease in an Asian or Hispanic immigrant to the United States, homelessness in some communities, and history of previous antituberculosis therapy—correlate with drug resistance in adult patients.[17,103] Patterns of drug resistance in children tend to mirror those found in adult patients in the population.[89,547,569,623] Outbreaks of drug-resistant tuberculosis in children occurring at schools have been reported.[89,523] Individual cases also have been recognized. The key to determining drug resistance in childhood tuberculosis usually comes from the drug susceptibility results of the infectious adult contact case's isolate.

Therapy for drug-resistant tuberculosis is successful only when at least two bactericidal drugs are given to which the infecting strain of *M. tuberculosis* is susceptible.[79,217,486,546,648] If only one effective drug is given, secondary resistance will develop. Exact treatment regimens must be tailored to the specific pattern of drug resistance. The duration of therapy usually is extended to at least 9 to 12 months if either isoniazid or rifampin can be used and to at least 18 to 24 months if resistance to both drugs is present.[283] Children tend to tolerate second-line antituberculosis drugs well, and community-based treatment usually is successful.[175,441] This is reflected in the lower mortality rates seen in children than in adults with MDR-TB. One pediatric series of children with MDR-TB in South Africa demonstrated 25-fold and almost 40-fold increases in mortality for children with MDR-TB and HIV coinfection and extrapulmonary tuberculosis, respectively.[562] On occasion, surgical resection of a diseased lung or lobe is required.[285,718] One meta-analysis showed that estimated success rates for pulmonary resection in adults with MDR-TB were over 80 percent,[717] a substantial improvement over the 50 to 65 percent survival seen with medical therapy alone.[474]

Treatment of a child with MDR-TB or XDR-TB exposure or infection is controversial. The benefit of preventing progression to disease, especially in a child with ongoing exposure, must be balanced against the scant pharmacokinetic, safety, and efficacy data available for many second-line medications. If resources permit, these children should be treated with two drugs to which the organism is susceptible either to complete a course of therapy for tuberculosis infection or until sequential tuberculin skin testing has been performed.[7] An expert in tuberculosis always should be involved in the management of children with drug-resistant tuberculosis infection or disease.

Adherence and Directly Observed Therapy

Nonadherence with drug treatment by patients is a major problem in control of tuberculosis because of the long-term nature of its treatment.[419,635,678] As treatment regimens become shorter in duration, adherence assumes an even greater importance. Suspected cases of tuberculosis must be reported to the local health department so that it can perform the necessary contact investigations and assist both patients and health care providers in overcoming barriers to adherence. To comply, the patient and family must know what is expected of them through verbal and written instructions in the patient's preferred language. An assessment of potential nonadherence should be made at the beginning of therapy.[36] Missed appointments should be brought quickly to the attention of the responsible public health officials, who may be able to use incentives or enablers, behavior modification, or, rarely, confinement to ensure adherence. The success of twice-weekly therapy, especially after a period of daily administration of medications, allows directly observed therapy to be given by a health care professional in cases of proven or suspected nonadherence.[107,108,284,653] Most experts hold that twice-weekly medication should be administered only under the direct observation of a health care worker.[328,699] Direct observation means that a health care worker or other nonrelated third party (e.g., teacher, school nurse, social worker) is physically present while the patient ingests the medication. A substantial number of children and their families are nonadherent with therapy. One study of isoniazid preventive therapy after tuberculosis exposure showed that 33 percent took medications for at least 4 months and only 12 percent completed a 6-month course.[211a] Adherence is inaccurately predicted by physicians. There are no documented differences in completion rates by child's age or duration of time the family has been in the United States.[504] In one study, the only factor associated with completion of tuberculosis infection therapy was health department–enabled medication administration.[133] In most communities in the United States and in an increasing number of other nations, directly observed therapy is the standard of care for all patients with tuberculosis disease.[94,196] However, when directly observed therapy cannot be used, structured behavior interactions can increase compliance with treatment.[85,651]

Summary of Treatment Recommendations

1. A regimen of isoniazid and rifampin for 6 months, supplemented with pyrazinamide during the first 2 months and ethambutol for 2 months or until the source case is known to be susceptible to all first-line agents, is standard therapy for children with suspected intrathoracic tuberculosis in the United States and Canada.
2. An alternative regimen is isoniazid and rifampin for 9 months. The disadvantages of this regimen include a longer duration, the potential for increased drug resistance during therapy, and less effectiveness if the patient absconds from treatment. This regimen should be used only if pyrazinamide cannot be tolerated.
3. After an initial 2 weeks to 2 months of daily drug administration, drugs can be given twice weekly under directly observed therapy with excellent effectiveness.[500] With patients for whom social or other restraints prevent reliable daily self-administration even during the initial phase of therapy, drugs can be given two or three times per week from the beginning under directly observed therapy.
4. In most cases, extrapulmonary tuberculosis can be treated with the same regimens as used for pulmonary tuberculosis, although data for tuberculous meningitis and bone or joint disease are relatively lacking. The duration of therapy is extended for tuberculous meningitis, osteoarticular tuberculosis, and children with substantial pulmonary disease after 6 months of therapy.[99,712]
5. Optimal therapy for tuberculosis in children with HIV infection has not been established.[630] Most HIV-infected adults with tuberculosis respond well to antituberculosis drugs but may require longer durations of treatment; higher failure and relapse rates were noted if rifamycins were used for only 6 months.[318] Immunosuppressed children with tuberculosis, including those with HIV infection, should be treated with at least three drugs initially, and treatment should be continued for a minimum of 9 months. Intermittent therapy is not recommended for HIV-infected children.[712] HIV testing is recommended for all infants and children with tuberculosis disease.
6. Tuberculosis disease occurring during pregnancy should be treated with a 9-month regimen of isoniazid and rifampin, supplemented during the initial phase with ethambutol (streptomycin should not be used). The use of pyrazinamide in pregnant patients is controversial although probably safe.

Corticosteroids

Corticosteroids have a place in the treatment of patients with tuberculosis. They should never be used except under cover of effective antituberculosis drugs. Corticosteroids would be expected to be beneficial in situations

in which the host inflammatory reaction is contributing to tissue damage or is impairing function.

Corticosteroids often are a useful addition to antituberculosis drugs if suppression of inflammatory reaction is desired, such as in the following situations[591]:

1. In patients with tuberculous meningitis in whom increased intracranial pressure is present.[658] The major actions are to reduce vasculitis, inflammation, and, ultimately, intracranial pressure. Not only is reduction of pressure desirable, but lowering of the pressure also probably favors the circulation of chemotherapeutic drugs through the brain and meninges.[200] One meta-analysis demonstrated lower rates of mortality and long-term neurologic sequelae in patients with tuberculous meningitis treated with corticosteroids than in children and adults not treated with corticosteroids.[505]
2. In patients with acute pericardial effusion in whom tamponade is occurring. Relief of symptoms takes place within hours. Use of corticosteroids reduced symptoms and mortality but not echocardiographic resolution of the effusion in both HIV-infected[234] and HIV-uninfected[633] children and adults. There appears to be a reluctance to use corticosteroids in HIV-infected patients with tuberculous pericarditis,[708] but increased mortality has not occurred when corticosteroids are utilized in conjunction with antituberculosis chemotherapy.
3. In patients with pleural effusion, a shift of the mediastinum, and acute respiratory embarrassment.[348] The long-term course probably is the same with or without corticosteroids, but symptomatic improvement usually is dramatic. One randomized trial of corticosteroid use for pleural tuberculosis in HIV-infected adults showed more rapid disease resolution, but increased rates of Kaposi sarcoma and no change in mortality, in corticosteroid recipients[192]; although both groups had similar CD4 cell counts and viral loads, no antiretroviral agents were provided, making it difficult to extrapolate the findings. Similar data are unavailable for children.
4. In patients with miliary tuberculosis if the inflammatory reaction is so severe that it produces alveolocapillary block with cyanosis.
5. In patients with enlarged mediastinal lymph nodes that are causing respiratory difficulty or a severe collapse-consolidation lesion, particularly in the middle or lower lobes, when bronchiectasis is likely to be a troublesome sequela.[453] Under either of these circumstances, a course of corticosteroids is warranted, with the realization that it will be more successful in an early infection because inflammation characterizes the first stages of tuberculosis. If caseation already is advanced, corticosteroids will be of little benefit.
6. In patients with IRIS whose reactions are either severe (e.g., airway compromise, brain stem herniation) or whose symptoms are refractory to therapy with nonsteroidal anti-inflammatory medications.[417]

The dosage of corticosteroids should be in the anti-inflammatory range, that is, prednisone 1 to 2 mg/kg/day for 4 to 6 weeks with gradual withdrawal. Some experts prefer dexamethasone, but no comparative trials have been published.

Activity

Activity need not be restricted in children with tuberculosis, except when a particular complication is inevitable (shortness of breath in pleural effusion, immobilization for a vertebral lesion).

Isolation

Isolation should be maintained for children with cavitary lesions, productive cough with acid-fast stain–positive sputum, draining sinuses, or renal tuberculosis until their secretions are negative on smear and preferably on culture. Young children are virtually noninfectious because they rarely cough and because their bronchial secretions contain few bacilli compared with those of adults with tuberculosis. Guidelines issued by the CDC state that most children with typical tuberculosis do not require isolation in the hospital.[100] Children with possible pulmonary tuberculosis should be treated as potentially infectious if they have a cavity or extensive upper lobe infiltrate, if they have a productive cough (especially if the sputum is acid-fast smear positive), or during high-risk procedures such as bronchoscopy. Although most children are not contagious with tuberculosis, the same cannot be said of their adult caregivers. Two studies in a relatively high-prevalence area of the United States showed that up to 17 percent of children admitted to the hospital for tuberculosis were accompanied by an adult with as yet undiagnosed tuberculosis.[131,445] Consideration should be given to screening caregivers of these children symptomatically or radiographically.

Follow-Up

Follow-up of children treated with antituberculosis drugs has become somewhat more streamlined in recent years. While receiving chemotherapy, the patient should be seen monthly, both to encourage regular intake of the prescribed drugs and to check, by a few simple questions (concerning appetite, well-being) and a few observations (weight gain; appearance of the skin and sclerae; palpation of the liver, spleen, and lymph nodes), that the disease is not spreading and that toxic effects of the drugs are not appearing.[701] Repeated chest radiographs should be obtained 1 to 2 months after the onset of chemotherapy to ascertain the maximal extent of disease before chemotherapy takes effect; thereafter, radiographs rarely are necessary.[324] Radiographic worsening at this time may be an indication of IRIS, the presence of drug-resistant tuberculosis, and/or adherence difficulties. Therefore, it is important to obtain this radiograph before stopping four-drug therapy and starting isoniazid and rifampin. If the radiograph at 1 to 2 months has normalized, subsequent imaging is unnecessary unless the child's disease worsens clinically. If the radiograph at 1 to 2 months is abnormal, repeating imaging at the conclusion of therapy

is helpful to reestablish a new radiographic baseline for the child. Chemotherapy has been so successful that follow-up beyond its termination is not necessary, except for children with serious disease, such as tuberculous meningitis, or those with extensive residual chest radiographic findings at the end of chemotherapy.

Case Reporting

Every case of definite or suspected tuberculosis, by United States law,[93] must be reported immediately by telephone to the health authority[216] to ensure prompt contact investigation.[265] Free antituberculosis drugs are available for diagnosed cases and for intimate contacts in almost every state of the United States and in many countries.

Prevention

Prevention of tuberculosis can be subdivided logically to consider the following circumstances:

1. Protection against exposure to the disease
2. Use of antituberculosis drugs in tuberculin-negative individuals at high risk for development of infection[559]
3. Immunization of tuberculin-negative individuals

Protection against exposure to disease is the ideal form of prevention.[398,415] It presupposes thorough preemployment and ongoing case-finding programs among all who come in contact with children, including daycare center and school personnel, Sunday school personnel, teachers, health care workers, babysitters, household servants, food handlers, beauticians, and barbers. Numerous epidemics, mini-epidemics, and mass exposures in neonatal nurseries have been traced to such infected individuals.

Immunization

Immunization against tuberculosis theoretically would be a tremendous boon to humanity, but in practice it has been fraught with very great difficulty. Various strains of mycobacteria and diverse nonliving immunogenic fractions have been studied. The impossibility of standardizing vaccines in the early days, the lack of any clinically useful test reflecting the immune status of the individual, and the relatively slow course of the disease have handicapped epidemiologic studies. A number of new vaccines are in human clinical trials. These studies use surrogate markers of interferon, interleukin, and other cytokine responses as measures of vaccine take, because finding correlates of protection remain elusive.[242,560]

BCG was developed at the Institut Pasteur in Paris by Calmette and Guérin, who, starting in 1908, made 231 passages of a strain of *M. bovis* on a beef bile medium, thereby producing marked attenuation. Injected into laboratory animals, this strain was shown to increase resistance to challenge with virulent *M. tuberculosis*. In 1921, it first was administered orally to neonates and since then has been given to more than 4 billion people.

BCG vaccine attempts to replace the potentially dangerous primary infection with *M. tuberculosis* with an innocuous primary infection with the bacillus of Calmette and Guérin, thus activating host cell-mediated immunity with minimal chance of causing progressive disease so that an infection with *M. tuberculosis* will be of the "reinfection" type.[365]

Variations in strains and lack of standardization are basic problems in evaluating the results of immunization.[49,223,649] BCG was maintained for many years by serial passage at the Institut Pasteur and distributed to hundreds of laboratories all over the world. Not until 1966 did the WHO Expert Committee on Biological Standardization adopt formal requirements for the maintenance of frozen "seed lots" to minimize the inevitable mutations that have produced BCG vaccines with widely varying characteristics. Routine quality control measures carried out by the production laboratory include an identity test, a test for contamination, a safety test in guinea pigs, an estimate of the total bacillary mass, viability, and a test of heat stability. Periodic assessment of the allergenic capacity in humans is part of quality control testing. The WHO, through its International Reference Preparation for BCG vaccine and through quality control testing, has helped decrease the gross variations in BCG vaccine found until recent years.

Vaccination techniques and dosages are variable. Intradermal injection is the most precise technique. Multiple-puncture techniques are popular because they are easy, but reported results consistently are inferior to those obtained with intradermal injection. Oral vaccination, the original method of administration, largely has been abandoned because of poor results. The actual dose of BCG at present usually is approximately 10^6 culturable particles. Because in animals large doses produce better resistance to challenge than small ones do, the largest convenient dose is used. However, in neonates, who have a higher incidence of untoward reactions, the customary approach is to halve the dose generally used in older infants and children to prevent local complications.

The usual local reaction to intradermal BCG vaccine is the development of a papule at the site of vaccination, and this papule reaches its maximal diameter (10 to 20 mm) in the sixth week. A small crust that may form on the papule detaches at about this time, with only a small ulcer remaining that may discharge a surprising amount of pus. Most ulcers are healed by the 10th week. A small scar is visible in over 80 percent of BCG-vaccinated individuals.[319,540] Enlargement of the regional lymph nodes occurs regularly and is painless, sometimes ending in calcification. Formation of an abscess with breakdown is a rare event, but it occurs more often in infants.

Untoward reactions to BCG rarely have been a problem.[666] Fatalities caused by progressive disease have been reported in no more than 60 vaccine recipients (of an estimated 3+ billion), usually (but not always) children with well-documented immunodeficiency.* No return of the attenuated strain to virulence has ever been noted. In countries where BCG is used routinely for immunization of neonates, osteomyelitis has been diagnosed in some 5

*References 82, 152, 198, 219, 492, 497.

TABLE 96-11 **Summary of Eight Large Controlled Trials of Bacillus Calmette-Guérin (BCG) Immunization Against Tuberculosis**

Subjects	Investigators	Intake Period	Vaccine Laboratory	Duration of Observation (yr)	% Protection from BCG
North America: American Indians	Stein and Aronson, 1953	1935-1938	Phipps	9-11	80
Chicago: infants	Rosenthal et al., 1961	1937-1948	Tice	12-23	75
Britain: schoolchildren	Medical Research Council, 1971	1950-1952	Copenhagen	15	78
South India: rural population	Frimodt-Moller et al., 1964	1950-1955	Madras	2.5-7	60/31*
Puerto Rico: children	Palmer et al., 1958	1949-1951	New York State	5.5-7.5	31
Georgia, Alabama: population	Comstock and Palmer, 1966	1950	Tice	14	14
Georgia: schoolchildren	Comstock and Webster, 1969	1947	Tice	20	0
Brazil: schoolchildren†	Pereira et al., 2011	1996-1998	Moreaux	9	25

*The initial estimate of efficacy was 60 percent. Subsequently, when follow-up was extended to 9 to 14 years, the efficacy figure declined to 31 percent.
†BCG vaccine given for the first time to children 7 to 14 years of age.
Modified from Sutherland, quoted by Eickhoff TC. The current status of BCG immunization against tuberculosis. *Annu Rev Med* 1977;**28**:411–23.

per 100,000 neonates. It usually becomes manifested when the child is between 5 and 33 months of age, when a tender swelling is noted near a joint; bone destruction is well localized and responds to conservative treatment.[106] On the whole, BCG is one of the safest vaccines in use.

In many countries where BCG is given routinely, the incidence of HIV infection in adults and children is high.[71,529] Reports of local and systemic complications from BCG vaccine in HIV-infected people are increasing, but the true magnitude of the interaction is not yet known.* However, in more recent studies, the rate of adverse BCG reactions in HIV-infected children appears to be higher than thought previously.[248,249] In most cases, BCG complications occur shortly after vaccination, but in one man with AIDS, adenitis as a result of BCG occurred 30 years after inoculation.[520] Routine treatment of patients with previous BCG vaccination who subsequently become immunocompromised is not recommended, but the clinician should be aware of previous BCG vaccination if signs or symptoms of mycobacterial infection occur.

The efficacy of BCG vaccines in humans has been evaluated in several large, well-controlled studies (Table 96-11). Three of these trials showed excellent protection, two showed mediocre protection, and two showed little or no effect of BCG. Another study, not tabulated because its numbers are relatively small, is the "experiment of nature" reported by Hyge[276] in 1957. Hyge[276] observed an epidemic of tuberculosis in a school for girls, in which 105 girls initially were tuberculin negative, 130 were tuberculin positive, and 133 were BCG immunized. In this group, the total incidence of tuberculosis was 23 times as high in the tuberculin-negative girls as in the BCG-immunized girls. Explanations for these differences in outcomes among trials must be sought in the quality and characteristics of the BCG vaccine used in the particular trial, the possible immunizing effect (in Georgia and Alabama) of infections with other mycobacteria,[589] the possibly greater effectiveness of BCG vaccine in areas of high tuberculosis prevalence, and methodological variations among the trials.

The most recent large study of BCG effectiveness is the Chingleput study, started in 1968 in Chingleput District near Madras, South India, an area where sensitization with nontuberculous mycobacteria is prevalent. People of all ages were vaccinated with one of two BCG vaccines or a placebo; only the incidence of adult-type pulmonary tuberculosis in the three groups was compared (i.e., not the forms usually found in children). During the ensuing years, no difference in incidence was noted among the three groups.[24] This disturbing result has been the subject of several WHO investigations because BCG is one of the vaccines recommended for all children in the Expanded Program of Immunization sponsored by the WHO itself.[519] Another study of neonatal vaccination with BCG in England reported very favorable results with BCG.[137] Most countries administer BCG to infants. In some nations, multiple doses of BCG are used. The few data that are available on revaccination show a modest efficacy (9%).[33,526] Revaccination with BCG is not recommended by the World Health Organization. Higher effectiveness (up to 25%) is seen when BCG is given to school-aged children who never received BCG as infants.[499]

A group at the Harvard School of Public Health reviewed all published studies of BCG efficacy in a meta-analysis.[122] Most published trials were not analyzed because of serious flaws in their experimental design or reporting. Among all trials and case-control studies included, the average protection against tuberculosis disease by various BCG preparations was 50 percent. The

*References 47, 248, 249, 467, 517.

protective levels were higher for disease in children, particularly for meningitis and tuberculosis-associated death.[121,662] However, ascertainment bias and lack of standardized case definitions render the results of these analyses very difficult to interpret. The BCG vaccines prevent many cases of tuberculosis in children, but the effect is quite variable. Variation in effectiveness of BCG in pulmonary tuberculosis has been seen across a number of trials. It has been hypothesized that these differences may be due to differences in BCG strains, overattenuation of BCG strains, interference in immunologic response caused by nontuberculous mycobacteria, and polymorphisms seen in human populations.[316] That BCG vaccines are not an instrument of tuberculosis control also has become apparent because they do not prevent infection with *M. tuberculosis*, their protective effect is short lived, and vaccination of infants does little to prevent future cases of contagious tuberculosis among adults in a community.

The role of BCG vaccine in the United States today is very limited. The Advisory Committee on Immunization Practices of the U.S. Public Health Service and the Advisory Council for the Elimination of Tuberculosis recommend BCG only for tuberculin-negative infants and children in the United States who (1) are at high risk for having intimate and prolonged exposure to persistently untreated or ineffectively treated adults with infectious pulmonary tuberculosis, cannot be removed from the source of infection, and cannot be prescribed long-term preventive therapy or (2) continuously are exposed to people with tuberculosis resistant to isoniazid and rifampin.[95] A few experts, however, are more inclined toward the use of BCG in neonates who are at any risk for exposure to tuberculosis whatsoever.[310,311,583,659]

Contraindications to the use of BCG for prevention of tuberculosis include congenital immunodeficiency, known HIV infection (in the United States), leukemia, lymphoma, and generalized malignant disease as well as treatment with corticosteroids, alkylating agents, antimetabolites, and radiation. The WHO recommends giving BCG to asymptomatic HIV-infected infants who reside in areas with high tuberculosis rates.

SELECTED READINGS

American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America. Controlling tuberculosis in the United States. *Am J Respir Crit Care Med* 2005; **172**:1169–227.

Brailey ME. *Tuberculosis in white and Negro children, part II: the epidemiologic aspects of the Harriet Lane study*. Cambridge, Mass.: Harvard University Press; 1958.

Dubos R, Dubos J. *The white plague: tuberculosis, man and society*. Boston: Little, Brown; 1952. Reissued by Rutgers University Press, 1987.

Hardy JB. *Tuberculosis in white and Negro children, part I: the roentgenologic aspects of the Harriet Lane study*. Cambridge, MA: Harvard University Press; 1958.

Lincoln EM, Sewell EM. *Tuberculosis in children*. New York: McGraw-Hill; 1963.

Raviglione MC, editor. *Reichman and Hershfields' tuberculosis: a comprehensive international approach*. 3rd ed. New York: Informa Healthcare; 2006.

Reichman LB, Hershfield ES, editors. *Tuberculosis: a comprehensive international approach*. New York: Marcel Dekker; 1999.

Seminars in Pediatric Infectious Disease, vol. 10, no. 4, 1993. (Entire volume devoted to tuberculosis in children.)

NEW REFERENCES SINCE THE SIXTH EDITION

4. Adetifa IMO, Ota MOC, Jeffries DJ, et al. Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent *Mycobacterium tuberculosis* infection in childhood contacts in the Gambia. *Pediatr Infect Dis J* 2010;**29**: 439–43.
7. Al-Dabbagh M, Lapphra K, McGloin R, et al. Drug-resistant tuberculosis: pediatric guidelines. *Pediatr Infect Dis J* 2011;**30**: 501–5.
17. Andrews JR, Shah NS, Weissman D, et al. Predictors of multidrug- and extensively drug-resistant tuberculosis in a high HIV prevalence community. *PLoS One* 2010;**5**:e15735.
23. Anglaret X, Minga A, Gabillard D, et al. AIDS and non-AIDS morbidity and mortality across the spectrum of CD4 cell counts in HIV-infected adults before starting antiretroviral therapy in Côte d'Ivoire. *Clin Infect Dis* 2012;**54**:714–23.
30. Bakeera-Kitaka S, Conesa-Botella A, Dhabangi A, et al. Tuberculosis in human immunodeficiency virus infected Ugandan children starting on antiretroviral therapy. *Int J Tuberc Lung Dis* 2011;**15**: 1082–6.
33. Barreto ML, Pereira SM, Pilger D, et al. Evidence of an effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: second report of the BCG-REVAC cluster-randomised trial. *Vaccine* 2011;**29**:4875–7.
39. Becerra MC, Appleton SC, Franke MF, et al. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: a retrospective cohort study. *Lancet* 2011;**377**:147–52.
45. Bennett DE, Courval JM, Onorato I, et al. Prevalence of tuberculosis infection in the United States population: the National Health and Nutrition Examination Survey, 1999-2000. *Am J Respir Crit Care Med* 2008;**177**:348–55.
46. Bergamini BM, Losi M, Vaienti F, et al. Performance of commercial blood tests for the diagnosis of latent tuberculosis infection in children and adolescents. *Pediatrics* 2009;**123**:e419–24.
57. Blanc F-X, Sok T, Laureillard D, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *N Engl J Med* 2011;**365**:1471–81.
62. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011;**377**: 1495–505.
64. Borgdorff MW, Sebek M, Geskus RB, et al. The incubation period distribution of tuberculosis estimated with a molecular epidemiological approach. *Int J Epidemiol* 2011;**40**:964–70.
87. Cattamanchi A, Smith R, Steingart KR, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. *J Acquir Immune Defic Syndr* 2011;**56**:230–8.
101. Centers for Disease Control and Prevention. Transplantation-transmitted tuberculosis—Oklahoma and Texas, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:333–6.
102. Centers for Disease Control and Prevention. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1–13.
103. Centers for Disease Control and Prevention. Trends in tuberculosis – United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012; **61**:181–5.
106. Chang CK, Chang WC, Lee HS, et al. Subcutaneous abscess in the lower leg caused by BCG-related osteomyelitis. *South Med J* 2010;**103**:686–9.
110. Chen J, Zhang R, Wang J, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis in HIV-infected patients: a systematic review and meta-analysis. *PloS One* 2011;**6**: e26827.
116. Cholo MC, Steel HC, Fourie PB, et al. Clofazimine: current status and future prospects. *J Antimicrob Chemother* 2012;**67**: 290–8.

128. Connell TG, Davies MA, Johannisen C, et al. Reversion and conversion of *Mycobacterium tuberculosis* IFN-gamma ELISpot results during anti-tuberculous treatment of HIV-infected children. *BMC Infect Dis* 2010;**10**:138.
130. Cruz AT, Geltemeyer AM, Starke JR, et al. Comparing the tuberculin skin test and T-SPOT.TB blood test in children. *Pediatrics* 2011;**127**:e31–8.
131. Cruz AT, Medina D, Whaley EM, et al. Tuberculosis among families of children with suspected tuberculosis and employees at a children's hospital. *Infect Control Hosp Epidemiol* 2011;**32**: 188–90.
132. Cruz AT, Ong LT, Starke JR. Childhood pleural tuberculosis: a review of 45 cases. *Pediatr Infect Dis J* 2009;**28**:981–4.
133. Cruz AT, Starke JR. Increasing adherence for latent tuberculosis infection therapy with health department-administered therapy. *Pediatr Infect Dis J* 2012;**31**:193–5.
134. Cuevas LE, Al-Sonboli N, Lawson L, et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multicenter cross-sectional evaluation. *PloS Med* 2011;**8**:e1001057.
138. Dalgic N, Tekin D, Kayaalti Z, et al. Arg753Gln polymorphism of the human Toll-like receptor 2 gene from infection to disease in pediatric tuberculosis. *Hum Immunol* 2011;**72**:440–5.
139. Dalgic N, Tekin D, Kayaalti Z, et al. Relationship between Toll-like receptor 8 gene polymorphisms and pediatric pulmonary tuberculosis. *Dis Markers* 2011;**31**:33–8.
149. Dekker G, Andronikou S, van Toorn R, et al. MRI findings in children with tuberculous meningitis: a comparison of HIV-infected and non-infected children. *Childs Nerv Syst* 2011;**27**: 1943–9.
154. Diel R, Goletti D, Ferrara G, et al. Interferon-gamma release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis. *Eur Respir J* 2011; **37**:88–99.
155. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a meta-analysis. *Chest* 2010;**137**:952–68.
156. Dietze R, Hadad DJ, McGee B, et al. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2008;**178**:1180–5.
161. Donald PR. Cerebrospinal fluid concentrations of antituberculosis agents in children and adults. *Tuberculosis (Edinb)* 2010;**90**:279–92.
162. Donald PR. Antituberculosis drug-induced hepatotoxicity in children. *Pediatr Rep* 2011;**3**:e16.
169. Dorhoi A, Reece ST, Kaufmann SH. For better or for worse: the immune response against *Mycobacterium tuberculosis* balances pathology and protection. *Immunol Rev* 2011;**240**:235–51.
197. Engström A, Morcillo N, Imperiale B, et al. Detection of first- and second-line drug resistance in *Mycobacterium tuberculosis* clinical isolates using pyrosequencing. *J Clin Microbiol* 2012;**50**:2026–33.
201. Feng WX, Flores-Villanueva PO, Mokrousov I, et al. CCL2-2518 (A/G) polymorphisms and tuberculosis susceptibility: a meta-analysis. *Int J Tuberc Lung Dis* 2012;**16**:150–6.
211. Gardy JL, Johnston JC, Ho Sui SJ, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. *N Engl J Med* 2011;**364**:730–9.
211a. Garie KT, Yassin MA, Cuevas LE. Lack of adherence to isoniazid chemoprophylaxis in children in contact with adults with tuberculosis in southern Ethiopia. *PloS One* 2011;**6**:e26452.
220. Good M, Duignan A. Perspectives on the history of bovine TB and the role of tuberculin bovine TB eradication. *Vet Med Int* 2011;**2011**:410470.
242. Hatherill M. Prospects for elimination of childhood tuberculosis: the role of new vaccines. *Arch Dis Child* 2011;**96**:851–6.
243. Haustein T, Ridout DA, Hartley JC, et al. The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis J* 2009;**28**:669–73.
253. Hlavsa MC, Moonan PK, Cowan LS, et al. Human tuberculosis due to *Mycobacterium bovis* in the United States, 1995-2005. *Clin Infect Dis* 2008;**47**:168–75.
262. Horsburgh CR Jr, Rubin EJ. Latent tuberculosis infection in the United States. *N Engl J Med* 2011;**364**:1441–8.
302. Karim SS, Naidoo K, Grobler A, et al. Integration of antiretroviral therapy with tuberculosis treatment. *N Engl J Med* 2011;**365**: 1492–501.
303. Kasambira TS, Shah M, Adrian PV, et al. QuantiFERON-TB Gold In-Tube for the detection of *Mycobacterium tuberculosis* infection in children with household tuberculosis contact. *Int J Tuberc Lung Dis* 2011;**15**:628–34.
316. Kernodle DS. Decrease in the effectiveness of bacille Calmette-Guérin vaccine against pulmonary tuberculosis: a consequence of increased immune suppression by microbial antioxidants, not overattenuation. *Clin Infect Dis* 2010;**51**:177–84.
318. Khan FA, Minion J, Pai M, et al. Treatment of active tuberculosis in HIV-coinfected patients: a systematic review and meta-analysis. *Clin Infect Dis* 2010;**50**:1288–99.
319. Kheir AE, Alhaj AA, Ibrahim SA. The sensitivity of BCG scar as an indicator of previous vaccination among Sudanese infants. *Vaccine* 2011;**29**:8189–91.
323. King KY, Lew JD, Ha NP, et al. Polymorphic allele of human IRGM1 is associated with susceptibility to tuberculosis in African-Americans. *PloS One* 2011;**6**:e16371.
352. Lehman LG, Ngapmen Yamadji AL, Ngo Sack F, et al. The CyScope fluorescence microscope, a reliable tool for tuberculosis diagnosis in resource-limited settings. *Am J Trop Med Hyg* 2010; **83**:906–8.
356. Li X, Yang Y, Zhou F, et al. SLC11A1 (NRAMP1) polymorphisms and tuberculosis susceptibility: updated systematic review and meta-analysis. *PloS One* 2011;**6**:e15831.
370. Lobato MN, Jereb JA, Starke JR. Unintended consequences: mandatory tuberculin skin testing and severe isoniazid hepatotoxicity. *Pediatrics* 2008;**121**:e1732–3.
380. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children. *Pediatr Infect Dis J* 2011;**30**:694–700.
381. Madhi SA, Nachman S, Violari A, et al. Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011;**365**:21–31.
387. Mandalakas AM, Detjen AK, Hesseling AC, et al. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011;**15**:1018–32.
399. Marais BJ, Schaaf HS. Childhood tuberculosis: an emerging and previously neglected problem. *Infect Dis Clin North Am* 2010;**24**: 727–49.
404. Martinson NA, Barnes GL, Moulton LH, et al. New regimens to prevent tuberculosis in adults with HIV infection. *N Engl J Med* 2011;**365**:11–20.
408. Mawachaka PM, Ranketi SS, Nchafatso OG, et al. Spinal tuberculosis among human immunodeficiency virus-negative patients in a Kenyan tertiary hospital: a 5-year synopsis. *Spine J* 2011;**11**: 265–9.
417. Meintjes G, Lawn SD, Scano F, et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect Dis* 2008;**8**: 516–23.
421. Metcalfe JZ, Everett CK, Steingart KR, et al. Interferon-gamma release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries: systematic review and meta-analysis. *J Infect Dis* 2011;**204**(Suppl. 4):S1120–9.
436. Moore HA, Apolles P, de Villiers PJ, et al. Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting. *Int J Tuberc Lung Dis* 2011;**15**: 1185–90.
442. Müller M, Wandel S, Colebunders R, et al. Immune reconstitution syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:251–61.
458. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. *Paediatr Respir Rev* 2011;**12**:16–21.
459. Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. *Lancet Infect Dis* 2011;**11**:819–24.
466. Oberhelman RA, Soto-Castellares G, Gilman RH, et al. Diagnostic approaches for paediatric tuberculosis by use of different

specimen types, culture methods, and PCR: a prospective case-control study. *Lancet* 2010;**10**:612–20.
474. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis; systematic review and meta-analysis. *Lancet Infect Dis* 2009;**9**:153–61.
475. Orikiiriza J, Bakeera-Kitaka S, Musiime V, et al. The clinical pattern, prevalence, and factors associated with immune reconstitution inflammatory syndrome in Ugandan children. *AIDS* 2009; **24**:2009–17.
484. Papaevangelou V, Vintila A, Papaparaskevas J, et al. Infant penile tuberculosis following circumcision. *J Infect* 2009;**58**:83–5.
487. Parsons SD, Warren RM, Ottenhoff TH, et al. Detection of *Mycobacterium tuberculosis* infection in dogs in a high-risk setting. *Res Vet Sci* 2012;**92**:414–19.
489. Patton GC, Coffey C, Sawyer SM, et al. Global patterns of mortality in young people: a systematic analysis of population health data. *Lancet* 2009;**374**:881–92.
490. Pavic I, Topic RZ, Raos M, et al. Interferon-gamma release assay for the diagnosis of latent tuberculosis infection in children younger than 5 years of age. *Pediatr Infect Dis J* 2011;**30**:866–70.
494. Pedrozo C, Sant'Anna C, de Fatima March M, et al. Clinical scoring system for paediatric tuberculosis in HIV-infected and non-infected children in Rio de Janeiro. *Int J Tuberc Lung Dis* 2009;**13**:413–15.
499. Pereira SM, Barreto ML, Pilger D, et al. Effectiveness and cost-effectiveness of first BCG vaccination against tuberculosis in school-age children without previous tuberculin test (BCG-REVAC Trial): a cluster-randomised trial. *Lancet Infect Dis* 2012; **12**:300–6.
504. Powell DA, Perkins L, Wang S-H, et al. Completion of therapy for latent tuberculosis in children of different nationalities. *Pediatr Infect Dis J* 2008;**28**:272–4.
505. Prasad K, Singh MB. Corticosteroids for managing tuberculous meningitis. *Cochrane Database Syst Rev* 2008;23:CD002244.
508. Rachow A, Clowes P, Saathoff E, et al. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. *Clin Infect Dis* 2012;**54**:1388–96.
525. Ritz N, Yau C, Connell TG, et al. Absence of interferon-gamma release assay conversion following tuberculin skin testing. *Int J Tuberc Lung Dis* 2011;**15**:767–9.
527. Rose PC, Schaaf HS, Marais BJ, et al. Value of bone marrow biopsy in children with suspected disseminated mycobacterial disease. *Int J Tuberc Lung Dis* 2011;**15**:200–4.
543. Sauzullo I, Massetti AP, Mengoni F, et al. Influence of previous tuberculin skin test on serial IFN-gamma release assays. *Tuberculosis (Edinb)* 2011;**91**:322–6.
550. Schaaf HS, Victor TC, Venter A, et al. Ethionamide cross- and co-resistance in children with isoniazid-resistant tuberculosis. *Int J Tuberc Lung Dis* 2009;**13**:1355–9.
560. Scriba TJ, Tameris M, Mansoor N, et al. Dose-finding study of the novel tuberculosis vaccine, MVA85A, in healthy BCG-vaccinated infants. *J Infect Dis* 2011;**203**:1832–43.
561. Seddon JA, Hesseling AC, Marais BJ, et al. Paediatric use of second-line anti-tuberculosis agents: a review. *Tuberculosis (Edinb)* 2012;**92**:9–17.
562. Seddon JA, Hesseling AC, Willemse M, et al. Culture-confirmed multidrug-resistant tuberculosis in children: clinical features, treatment, and outcome. *Clin Infect Dis* 2012;**54**:157–66.
568. Sester M, Sotgiu G, Lange C, et al. Interferon gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2011;**37**:100–11.
601. Solovic I, Sester M, Gomez-Reino JJ, et al. The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. *Eur Respir J* 2010;**36**:1185–206.
620. Stefan DC, Dippenaar A, Detjen AK, et al. Interferon-gamma release assays for the detection of *Mycobacterium tuberculosis* infection in children with cancer. *Int J Tuberc Lung Dis* 2010;**14**: 689–94.
626. Sterling TR, Villarino MA, Borisov AS, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. *N Engl J Med* 2011;**365**:2155–66.
633. Strang JI, Nunn AJ, Johnson DA, et al. Management of tuberculous constrictive pericarditis and tuberculous pericardial effusion in Transkei: results at 10 years follow-up. *QJM* 2004;**97**: 525–35.
646. Tekin D, Kayaalti Z, Dalgic N, et al. Polymorphism in the *P2X7* gene increases susceptibility to extrapulmonary tuberculosis in Turkish children. *Pediatr Infect Dis J* 2010;**29**:779–81.
652. Thampi N, Stephens D, Rea E, et al. Unexplained deterioration during antituberculous therapy in children and adolescents: clinical presentation and risk factors. *Pediatr Infect Dis J* 2012;**31**: 129–33.
655. Thomas TA, Mondal D, Noor Z, et al. Malnutrition and helminth infection affect performance of an interferon gamma release assay. *Pediatrics* 2010;**126**:e1522–9.
656. Thomas TA, Shenoi SV, Heysell SK, et al. Extensively drug-resistant tuberculosis in children with human immunodeficiency virus in rural South Africa. *Int J Tuberc Lung Dis* 2010;**14**: 1244–51.
675. Van Zyl-Smit RN, Zwerling A, Dheda K, et al. Within-subject variability of the interferon-gamma assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. *PloS One* 2009;**4**:e8517.
682. Verhagen LM, Warris A, van Soolingen D, et al. Human immunodeficiency virus and tuberculosis coinfection in children: challenges in diagnosis and treatment. *Pediatr Infect Dis J* 2010;**29**: e63–70.
693. Walters E, Cotton MF, Rabie H, et al. Clinical presentation and outcome of tuberculosis in human immunodeficiency virus infected children on antiretroviral therapy. *BMC Pediatrics* 2008; **8**:1.
694. Wang ME, Castillo ME, Montano SM, et al. Immune reconstitution inflammatory syndrome in human immunodeficiency virus-infected children in Peru. *Pediatr Infect Dis J* 2009;**28**:900–3.
697. Webb EA, Hesseling AC, Schaaf HS, et al. High prevalence of *Mycobacterium tuberculosis* infection and disease in children and adolescents with type 1 diabetes mellitus. *Int J Tuberc Lung Dis* 2009;**13**:868–74.
705. Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clin Infect Dis* 2011;**52**:1350–5.
706. Wirth T, Hildebrand F, Allix-Bequec C, et al. Origin, spread, and demography of the *Mycobacterium tuberculosis* complex. *PloS Pathog* 2008;**4**:e1000160.
708. Wiysonge CS, Ntsekhe M, Gumedze F, et al. Contemporary use of adjunctive corticosteroids in tuberculous pericarditis. *Int J Cardiol* 2008;**124**:388–90.
709. Wolf H, Mendez M, Gilman RH, et al. Diagnosis of pediatric pulmonary tuberculosis by stool PCR. *Am J Trop Med Hyg* 2008;**79**:893–8.
712. World Health Organization. *Rapid Advice 2010: Treatment of tuberculosis in children*. Geneva: World Health Organization; 2010.
713. World Health Organization. *Global tuberculosis control 2011*. Geneva: World Health Organization; 2011.
714. World Health Organization. *Tuberculosis country profiles*. Available online at http://www.who.int/tb/country/data/profiles/en/index.html. Accessed December 12, 2011.
715. Wright A, Zignol M, Van Deun A, et al. Epidemiology of antituberculosis drug resistance 2002-2007: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. *Lancet* 2009;**373**:1861–73.
718. Xu HB, Jiang RH, Li L. Pulmonary resection for patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *J Antimicrob Chemother* 2011;**66**:1687–95.
721. Zar HJ, Cotton MF, Strauss S, et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007;**334**:136.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abadco D, Steiner P. Gastric lavage is better than bronchoalveolar lavage for isolation of *Mycobacterium tuberculosis* in childhood pulmonary tuberculosis. *Pediatr Infect Dis J* 1992;**11**:735–8.
2. Abernathy RS, Dutt AK, Stead WW, et al. Short-course chemotherapy for tuberculosis in children. *Pediatrics* 1983;**72**:801–6.
3. Acocella G. The use of fixed dose combinations in antituberculous chemotherapy: rationale for their application in daily, intermittent and pediatric regimes. *Bull Int Union Tuberc Lung Dis* 1990;**65**: 77–83.
4. Adetifa IMO, Ota MOC, Jeffries DJ, et al. Commercial interferon gamma release assays compared to the tuberculin skin test for diagnosis of latent *Mycobacterium tuberculosis* infection in childhood contacts in the Gambia. *Pediatr Infect Dis J* 2010;**29**: 439–43.
5. Afghani B, Lieberman JM. Paradoxical enlargement or development of intracranial tuberculomas during therapy: case report and review. *Clin Infect Dis* 1994;**19**:1092–9.
6. Albisua I, Artigao FB, Del Castillo F, et al. Twenty years of pulmonary tuberculosis in children: what has changed? *Pediatr Infect Dis J* 2002;**21**:91–7.
7. Al-Dabbagh M, Lapphra K, McGloin R, et al. Drug-resistant tuberculosis: pediatric guidelines. *Pediatr Infect Dis J* 2011;**30**: 501–5.
8. Al-Dossary FS, Ong LT, Correa AG, et al. Treatment of childhood tuberculosis with a six month directly observed regimen of only two weeks of daily therapy. *Pediatr Infect Dis J* 2002;**21**:91–7.
9. Alland D, Kolkut GE, Moss A, et al. Transmission of tuberculosis in New York City: an analysis by DNA fingerprinting and conventional epidemiologic methods. *N Engl J Med* 1994;**330**: 1710–16.
10. Almeida LMD, Barbieri MA, Da Paixao AC, et al. Use of purified protein derivative to assess the risk of infection in children in close contact with adults with tuberculosis in a population with high Calmette-Guérin bacillus coverage. *Pediatr Infect Dis J* 2001;**20**: 1061–5.
11. American Thoracic Society. Rapid diagnostic tests for tuberculosis: what is the appropriate use? *Am J Respir Crit Care Med* 1997; **15**:1804–14.
12. American Thoracic Society. Diagnostic standards and classification of tuberculosis in adults and children. *Am J Respir Crit Care Med* 2000;**161**:1376–95.
13. American Thoracic Society and Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. *Am J Respir Crit Care Med* 2000; **161**(Suppl.):221–47.
14. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America. Controlling tuberculosis in the United States. *Am J Respir Crit Care Med* 2005;**172**:1169–227.
15. Anderson P, Munk ME, Pollock JM, et al. Specific immune-based diagnosis of tuberculosis. *Lancet* 2000;**356**:1099–104.
16. Andrew OT, Schoenfeld RY, Hopewell PC, et al. Tuberculosis in patients with end-stage renal disease. *Am J Med* 1980;**68**:59–65.
17. Andrews JR, Shah NS, Weissman D, et al. Predictors of multidrug- and extensively drug-resistant tuberculosis in a high HIV prevalence community. *PLoS One* 2010;***5***:e15735.
18. Andronikou S, Joseph E, Lucas S, et al. CT scanning for the detection of tuberculous mediastinal and hilar adenopathy in children. *Pediatr Radiol* 2004;**34**:232–6.
19. Andronikou S, Smith B, Hatherill M, et al. Definitive neuroradiological diagnostic features of tuberculous meningitis in children. *Pediatr Radiol* 2004;**34**:876–85.
20. Andronikou S, Welman CJ, Kader E. The CT features of abdominal tuberculosis in children. *Pediatr Radiol* 2002;**32**:75–81.
21. Andronikou S, Wieselthaler N. Modern imaging of tuberculosis in children: Thoracic central nervous system and abdominal tuberculosis. *Pediatr Radiol* 2004;**34**:861–75.
22. Andronikou S, Wieselthaler N, Smith B, et al. Value of early followup CT in pediatric tuberculous meningitis. *Pediatr Radiol* 2005;**35**:1092–9.
23. Anglaret X, Minga A, Gabillard D, et al. AIDS and non-AIDS morbidity and mortality across the spectrum of CD4 cell counts in HIV-infected adults before starting antiretroviral therapy in Côte d'Ivoire. *Clin Infect Dis* 2012;***54***:714–23.
24. Anonymous. Fifteen year follow up of trial of BCG vaccines in south India for tuberculosis prevention. Tuberculosis Research Centre (ICMR), Chennai. *Indian J Med Res* 1999;**110**:56–69.
25. Aquinas SM. Short-course therapy for tuberculosis. *Drugs* 1982; **24**:118–32.
26. Askew GL, Finelli L, Hutton M, et al. *Mycobacterium tuberculosis* transmission from a pediatrician to patient. *Pediatrics* 1997;**100**: 19–23.
27. Aznar J, Safi H, Romero J, et al. Nosocomial transmission of tuberculosis infection in pediatrics wards. *Pediatr Infect Dis J* 1995;**14**:44–8.
28. Baciewicz AM, Self TH. Isoniazid interactions. *South Med J* 1985;**78**:714–18.
29. Bagga A, Kalra V, Ghai OP. Intracranial tuberculoma evaluation and treatment. *Clin Pediatr (Phila)* 1988;**27**:487–90.
30. Bakeera-Kitaka S, Conesa-Botella A, Dhabangi A, et al. Tuberculosis in human immunodeficiency virus infected Ugandan children starting on antiretroviral therapy. *Int J Tuberc Lung Dis* 2011;***15***: 1082–6.
31. Barber TW, Craven DE, McCabe WR. Bacteremia due to *Mycobacterium tuberculosis* in patients with human immunodeficiency virus infection: a report of 9 cases and a review of the literature. *Medicine (Baltimore)* 1990;**69**:375–83.
32. Barclay WR. Does a positive tuberculin test indicate the presence of live tubercle bacilli? *JAMA* 1975;**232**:755.
33. Barreto ML, Pereira SM, Pilger D, et al. Evidence of an effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: second report of the BCG-REVAC cluster-randomised trial. *Vaccine* 2011;**29**:4875–7.
34. Barton LL, Feigin RD. Childhood cervical lymphadenitis: a reappraisal. *J Pediatr* 1974;**84**:846–52.
35. Bavadekan AV. Osteoarticular tuberculosis in children. *Prog Pediatr Surg* 1982;**15**:131–51.
36. Bayer R, Wilkinson D. Directly observed therapy for tuberculosis: history of an idea. *Lancet* 1995;**345**:1545–8.
37. Bearman JE, Kleinman H, Glyer VV, et al. Study of variability in tuberculin skin reading. *Am Rev Respir Dis* 1964;**90**:913–19.
38. Beaudry PH, Brickman HF, Wise MB. Liver enzyme disturbances during isoniazid chemoprophylaxis in children. *Am Rev Respir Dis* 1974;**110**:581–4.
39. Becerra MC, Appleton SC, Franke MF, et al. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: a retrospective cohort study. *Lancet* 2011;***377***:147–52.
40. Beck-Sague C, Dooley SW, Hutton MD, et al. Hospital outbreak of multidrug-resistant *Mycobacterium tuberculosis* infections: factors in transmission to staff and HIV-infected patients. *JAMA* 1992; **268**:1280–6.
41. Behr MA, Hopewell PC, Paz EA, et al. Predictive value of contact investigation for identifying recent transmission of *Mycobacterium tuberculosis*. *Am J Respir Crit Care Med* 1998;**158**:465–9.
42. Behr MA, Warren SA, Salamon H, et al. Transmission of *Mycobacterium tuberculosis* from patients smear negative for acid-fast bacilli. *Lancet* 1999;**353**:444–9.
43. Bellin EY, Fletcher DD, Safyer SM. Association of tuberculosis infection with increased time in or admission to the New York City jail system. *JAMA* 1993;**269**:2228–31.
44. Belsey MA. Tuberculosis and varicella infections in children. *Am J Dis Child* 1967;**113**:444–8.
45. Bennett DE, Courval JM, Onorato I, et al. Prevalence of tuberculosis infection in the United States population: the National Health and Nutrition Examination Survey, 1999-2000. *Am J Respir Crit Care Med* 2008;***177***:348–55.
46. Bergamini BM, Losi M, Vaienti F, et al. Performance of commercial blood tests for the diagnosis of latent tuberculosis infection in children and adolescents. *Pediatrics* 2009;***123***:e419–24.
47. Besnard M, Sauvion S, Offredo C, et al. Bacillus Calmette-Guérin infection after vaccination of human immunodeficiency virus–infected children. *Pediatr Infect Dis J* 1993;**12**:993–7.
48. Besser RE, Pakiz B, Schulte J, et al. Risk factors for positive Mantoux tuberculin skin tests in children in San Diego, California: evidence for boosting and possible food borne transmission. *Pediatrics* 2001;**108**:305–10.
49. Beyazova U, Rota S, Ceuheroglu C, et al. Humoral immune response in infants after BCG vaccination. *Tubercle Lung Dis* 1995;**76**:248–53.

50. Beyt BE Jr, Ortbals DW, Santa Cruz DJ, et al. Cutaneous mycobacteriosis: analysis of 34 cases with a new classification of the disease. *Medicine (Baltimore)* 1980;**60**:96–109.
51. Bhagwati SN. Spinal intramedullary tuberculoma in children. *Childs Brain* 1979;**5**:568.
52. Bhansali SK. Abdominal tuberculosis: experience with 300 cases. *Am J Gastroenterol* 1977;**67**:324–37.
53. Biddulph J. Short-course chemotherapy for childhood tuberculosis. *Pediatr Infect Dis J* 1990;**9**:794–801.
54. Biehl JP, Vilter RW. Effects of isoniazid on pyridoxine metabolism. *JAMA* 1954;**156**:1549–52.
55. Bifani PJ, Plikaytis BB, Kapur V, et al. Origin and interstate spread of a New York City multidrug-resistant *Mycobacterium tuberculosis* clone family. *JAMA* 1996;**275**:452–7.
56. Blake MJ, Abdel-Rahman SM, Jacobs RF, et al. Pharmacokinetics of rifapentine in children. *Pediatr Infect Dis J* 2006;***25***: 405–9.
57. Blanc F-X, Sok T, Laureillard D, et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *N Engl J Med* 2011;***365***:1471–81.
58. Bloch AB, Snider DE Jr. How much tuberculosis in children must we accept? *Am J Public Health* 1986;**76**:14–15.
59. Blumberg HM, Burman WJ, Chaisson RE, et al; American Thoracic Society/Centers for Disease Control and Prevention/ Infectious Diseases Society of America. Treatment of tuberculosis. *Am J Respir Crit Care Med* 2003;**167**:603–62.
60. Blumberg HM, Watkins DL, Berschling JD, et al. Preventing the nosocomial transmission of tuberculosis. *Ann Intern Med* 1995; **122**:658–63.
61. Blusse van Oud-Alblas HJ, van Vliet ME, Kimpen JL, et al. Human immunodeficiency virus infection in children hospitalized with tuberculosis. *Ann Trop Paediatr* 2002;**22**:115–23.
62. Boehme CC, Nicol MP, Nabeta P, et al. Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet* 2011;***377***: 1495–505.
63. Bonnet MB, Pinoges LP, Varaine FV, et al. Tuberculosis after HAART initiation in HIV-positive patients from five countries with a high tuberculosis burden. *AIDS* 2006;**20**:1275–9.
64. Borgdorff MW, Sebek M, Geskus RB, et al. The incubation period distribution of tuberculosis estimated with a molecular epidemiological approach. *Int J Epidemiol* 2011;***40***:964–70.
65. Bovornkitti S, Kangsdal P, Sathirapat P, et al. Reversion and reconversion rate of tuberculin skin test reactions in correlation with the use of prednisone. *Dis Chest* 1960;**38**:51–5.
66. Boyd GL. Tuberculosis pericarditis in children. *Am J Dis Child* 1953;**86**:293–300.
67. Braden CR. Infectiousness of a university student with laryngeal and cavitary tuberculosis. Investigative team. *Clin Infect Dis* 1995;**21**:565–70.
68. Brailey ME. Observations on the development of intrathoracic calcification in tuberculin-positive infants. *Bull Johns Hopkins Hosp* 1937;**61**:258–71.
69. Brailey ME. *Tuberculosis in white and Negro children, part II: the epidemiologic aspects of the Harriet Lane study*. Cambridge, MA: Harvard University Press; 1958.
70. Bratcher DF, Stover BH, Lane NE, et al. Compliance with national recommendations for tuberculosis screening and immunization of health care workers in a children's hospital. *Infect Control Hosp Epidemiol* 2000;**21**:338–40.
71. Braun MM, Byers RH, Heyward WL, et al. Acquired immunodeficiency syndrome and extrapulmonary tuberculosis in the United States. *Arch Intern Med* 1990;**150**:1913–16.
72. Brock RC. Post-tuberculous bronchostenosis and bronchiectasis of the middle lobe. *Thorax* 1950;**5**:5–39.
73. Brudney K, Dobkin J. Resurgent tuberculosis in New York City: human immunodeficiency virus, homelessness and the decline of tuberculosis control programs. *Am Rev Respir Dis* 1991;**144**: 745–9.
74. Burk JR, Viroslav J, Bynum LJ. Miliary tuberculosis diagnosed by fiberoptic bronchoscopy and transbronchial biopsy. *Tubercle* 1978;**59**:107–9.
75. Burke HE. Pathogenesis of Pott's disease. *Trans Am Clin Climatol Assoc* 1948;**59**:122–37.
76. Burroughs M, Beitel A, Kawamura A, et al. Clinical presentation of tuberculosis in culture-positive children. *Pediatr Infect Dis J* 1999;**18**:440–6.
77. Byrd RB, Horn BR, Solomon DA, et al. Toxic effects of isoniazid in tuberculous chemoprophylaxis: role of biochemical monitoring in 1,000 patients. *JAMA* 1979;**241**:1239–41.
78. Cain KP, Haley CA, Armstrong LR, et al. Tuberculosis among foreign-born persons in the United States. *Am J Respir Crit Care Med* 2007;**175**:75–9.
79. Caminero JA. Treatment of multidrug-resistant tuberculosis: evidence and controversies. *Int J Tuberc Lung Dis* 2006;**10**: 829–37.
80. Cantwell M, Shehab Z, Costello A, et al. Brief report: congenital tuberculosis. *N Engl J Med* 1994;**330**:1051–4.
81. Cardona M, Bek MD, Mills K, et al. Transmission of tuberculosis from a seven-year-old child in a Sydney school. *J Paediatr Child Health* 1999;**35**:375–8.
82. Carlgren LE, Hansson CG, Henricsson L, et al. Fatal BCG infection in an infant with congenital lymphocytopenic agammaglobulinemia. *Acta Paediatr Scand* 1966;**55**:636–44.
83. Carter ER, Lee CM. Interpretation of the tuberculin skin test reaction by pediatric providers. *Pediatr Infect Dis J* 2002;**21**: 200–3.
84. Carvalho AC, Kritski L, De Reimer K. Tuberculin skin testing among BCG-vaccinated children who are household contacts. *Int J Tuberc Lung Dis* 2001;**5**:297.
85. Cass AD, Talavera GA, Gresham LS, et al. Structural behavioral intervention to increase children's adherence to treatment for latent tuberculosis infection. *Int J Tuberc Lung Dis* 2005;**9**: 415–20.
86. Casteels-Van Daele M, Igodt-Ameye L, Corbeel L, et al. Hepatotoxicity of rifampin and isoniazid in children. *J Pediatr* 1975; **86**:739–41.
87. Cattamanchi A, Smith R, Steingart KR, et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. *J Acquir Immune Defic Syndr* 2011;***56***:230–8.
88. Cave M, Eisenach K, McDermott P, et al. IS6110: Conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. *Mol Cell Probes* 1991;**5**:73–80.
89. Centers for Disease Control and Prevention. Interstate outbreak of drug-resistant tuberculosis involving children—California, Montana, Nevada, Utah. *MMWR Morb Mortal Wkly Rep* 1983; **32**:516–18.
90. Centers for Disease Control and Prevention. A strategic plan for the elimination of tuberculosis from the United States. *MMWR Morb Mortal Wkly Rep* 1989;**38**:269–72.
91. Centers for Disease Control and Prevention. Outbreak of multidrug-resistant tuberculosis—Texas, California, and Pennsylvania. *MMWR Morb Mortal Wkly Rep* 1990;***39***:369.
92. Centers for Disease Control and Prevention. Initial therapy for tuberculosis in the era of multidrug resistance. *MMWR Recomm Rep* 1993;**42**(RR-7):1–8.
93. Centers for Disease Control and Prevention. Tuberculosis control laws—United States, 1993. *MMWR Recomm Rep* 1993;**42**(RR-15): 1–28.
94. Centers for Disease Control and Prevention. Screening for tuberculosis and tuberculosis infection in high-risk populations. Recommendations of the Advisory Council for the Elimination of Tuberculosis. *MMWR Recomm Rep* 1995;**44**(RR-11):19–34.
95. Centers for Disease Control and Prevention. The role of BCG vaccine in the prevention and control of tuberculosis in the United States: a joint statement by the Advisory Council for the Elimination of Tuberculosis and the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 1996;**45**(RR-4):1–18.
96. Centers for Disease Control and Prevention. Tuberculosis elimination revisited: obstacles, opportunities, and a renewed commitment. Advisory Council for the Elimination of Tuberculosis (ACET). *MMWR Recomm Rep* 1999;**48**(RR-9):1–13.
97. Centers for Disease Control and Prevention. *Core curriculum on tuberculosis: what a clinician should know*. 4th ed. Atlanta: U. S. Department of Health and Human Services; 2000.
98. Centers for Disease Control and Prevention. Preventing and controlling tuberculosis along the U.S.-Mexican border. *MMWR Recomm Rep* 2001;**50**(RR-1):1–27.

99. Centers for Disease Control and Prevention. Treatment of tuberculosis. *MMWR Morb Mortal Wkly Rep* 2003;**52**(RR-11):1–88.
100. Centers for Disease Control and Prevention. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in healthcare settings. *MMWR Recomm Rep* 2005;**54**(RR-17):1–141.
101. Centers for Disease Control and Prevention. Transplantation-transmitted tuberculosis—Oklahoma and Texas, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:333–6.
102. Centers for Disease Control and Prevention. Updated guidelines for using interferon gamma release assays to detect *Mycobacterium tuberculosis* infection—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1–13.
103. Centers for Disease Control and Prevention. Trends in tuberculosis—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:181–5.
104. Chan S, Abadco D, Steiner P. Role of flexible fiberoptic bronchoscopy in the diagnosis of childhood endobronchial tuberculosis. *Pediatr Infect Dis J* 1994;**13**:506–9.
105. Chan SP, Birnbaum J, Rao M. Clinical manifestation and outcome of tuberculosis in children with acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 1996;**15**:443–7.
106. Chang CK, Chang WC, Lee HS, et al. Subcutaneous abscess in the lower leg caused by BCG-related osteomyelitis. *South Med J* 2010;**103**:686–9.
107. Chaulk CP, Kazandijian VA. Directly observed therapy for treatment completion of pulmonary tuberculosis. Consensus statement of the Public Health Tuberculosis Guidelines Panel. *JAMA* 1998; **279**:943–8.
108. Chaulk CP, Moore-Rice K, Rizzo R, et al. Eleven years of community-based directly observed therapy for tuberculosis. *JAMA* 1995;**274**:945–51.
109. Chavalittamvong B, Talalak P. Tuberculous peritonitis in children. *Prog Pediatr Surg* 1982;**15**:161–7.
110. Chen J, Zhang R, Wang J, et al. Interferon-gamma release assays for the diagnosis of active tuberculosis in HIV-infected patients: a systematic review and meta-analysis. *PloS One* 2011;**6**:e26827.
111. Cheng TL, Miller EB, Ottolini M, et al. Tuberculosis testing: physician attitudes and practices. *Arch Pediatr Adolesc Med* 1996; **150**:682–5.
112. Cheng TL, Ottolin M, Getson P, et al. Poor validity of parent reading of skin test induration in a high risk population. *Pediatr Infect Dis J* 1996;**15**:90–1.
113. Chin DP, Crane CM, Diul MY, et al. Spread of *Mycobacterium tuberculosis* in a community implementing recommended elements of tuberculosis control. *JAMA* 2000;**283**:2968–74.
114. Chintu C, Bhat G, Luo C, et al. Seroprevalence of human immunodeficiency virus type 1 infection in Zambian children with tuberculosis. *Pediatr Infect Dis J* 1993;**12**:499–504.
115. Chintu C, Mwaba P. Tuberculosis in children with human immunodeficiency virus infection. *Int J Tuberc Lung Dis* 2005;**9**:477–84.
116. Cholo MC, Steel HC, Fourie PB, et al. Clofazimine: current status and future prospects. *J Antimicrob Chemother* 2012;**67**: 290–8.
117. Choremis C, Vlachos J, Vlachou CA, et al. Needle biopsy of the liver in various forms of childhood tuberculosis. *J Pediatr* 1963; **62**:203–7.
118. Christie CDC, Contaniou P, Marx ML, et al. Low risk for tuberculosis in a regional pediatric hospital: nine-year study of community rates and the mandatory employee tuberculin skin test program. *Infect Control Hosp Epidemiol* 1998;**19**:168–74.
119. Cohen T, Murray M. Incident tuberculosis among recent U.S. immigrants and exogenous reinfection. *Emerg Infect Dis* 2005;**11**: 725–8.
120. Cohn DL, Catlin BJ, Peterson KC, et al. A 62-dose, 6-month therapy for pulmonary and extrapulmonary tuberculosis. *Ann Intern Med* 1990;**112**:407–15.
121. Colditz G, Berkey CS, Mosteller F, et al. The efficacy of bacillus Calmette-Guérin vaccination of newborns and infants in the prevention of tuberculosis: meta-analysis of the published literature. *Pediatrics* 1995;**96**:29–35.
122. Colditz G, Brewer T, Berkey C, et al. Efficacy of BCG vaccine in the prevention of tuberculosis: meta-analysis of the published literature. *JAMA* 1994;**271**:698–702.
123. Comstock GW. Variability of tuberculosis trends in a time of resurgence. *Clin Infect Dis* 1994;**19**:1015–22.
124. Comstock GW, Baum C, Snider DE Jr. Isoniazid prophylaxis among Alaskan Eskimos: final report of the Bethel isoniazid studies. *Am Rev Respir Dis* 1979;**119**:827–30.
125. Comstock GW, Edwards LB, Nabangxang H. Tuberculin sensitivity eight to fifteen years after BCG vaccination. *Am Rev Respir Dis* 1971;**103**:572–5.
126. Comstock GW, Livesay VT, Woolpert SF. Prognosis of a positive tuberculin reaction in childhood and adolescence. *Am J Epidemiol* 1974;**99**:131–8.
127. Conde MB, Soares SL, Mello FC, et al. Comparison of sputum induction with fiberoptic bronchoscopy in the diagnosis of tuberculosis: experience at an acquired immune deficiency syndrome reference center in Rio de Janeiro, Brazil. *Am J Respir Crit Care Med* 2000;**162**:2238–40.
128. Connell TG, Davies MA, Johannisen C, et al. Reversion and conversion of *Mycobacterium tuberculosis* IFN-gamma ELISpot results during anti-tuberculous treatment of HIV-infected children. *BMC Infect Dis* 2010;**10**:138.
129. Crowle AJ, Sbarbaro JA, Judson FN, et al. The effect of ethambutol on tubercle bacilli within cultured human macrophages. *Am Rev Respir Dis* 1985;**132**:742–5.
130. Cruz AT, Geltemeyer AM, Starke JR, et al. Comparing the tuberculin skin test and T-SPOT.TB blood test in children. *Pediatrics* 2011;**127**:e31–8.
131. Cruz AT, Medina D, Whaley EM, et al. Tuberculosis among families of children with suspected tuberculosis and employees at a children's hospital. *Infect Control Hosp Epidemiol* 2011;**32**: 188–90.
132. Cruz AT, Ong LT, Starke JR. Childhood pleural tuberculosis: a review of 45 cases. *Pediatr Infect Dis J* 2009;**28**:981–4.
133. Cruz AT, Starke JR. Increasing adherence for latent tuberculosis infection therapy with health department–administered therapy. *Pediatr Infect Dis J* 2012;**31**:193–5.
134. Cuevas LE, Al-Sonboli N, Lawson L, et al. LED fluorescence microscopy for the diagnosis of pulmonary tuberculosis: a multicenter cross-sectional evaluation. *PloS Med* 2011;**8**:e1001057.
135. Curless RG, Mitchell CD. Central nervous system tuberculosis in children. *Pediatr Neurol* 1991;**7**:270–4.
136. Curtis A, Ridzon R, Vogel R, et al. Extensive transmission of *Mycobacterium tuberculosis* from a child. *N Engl J Med* 1999;**341**: 1491–5.
137. Curtis HM, Bamford FN, Leck I. Incidence of childhood tuberculosis after neonatal BCG vaccination. *Lancet* 1984;**1**:145–8.
138. Dalgic N, Tekin D, Kayaalti Z, et al. Arg753Gln polymorphism of the human Toll-like receptor 2 gene from infection to disease in pediatric tuberculosis. *Hum Immunol* 2011;**72**:440–5.
139. Dalgic N, Tekin D, Kayaalti Z, et al. Relationship between Toll-like receptor 8 gene polymorphisms and pediatric pulmonary tuberculosis. *Dis Markers* 2011;**31**:33–8.
140. Dall L, Long L, Stanford J. Poncet's disease: tuberculosis rheumatism. *Rev Infect Dis* 1989;**11**:105–7.
141. Daly JF, Brown DS, Lincoln EM, et al. Endobronchial tuberculosis in children. *Dis Chest* 1952;**22**:380–98.
142. Damian DL, Halliday GM. Measurement of ultraviolet radiation–induced suppression of recall contact and delayed-type hypersensitivity in humans. *Methods* 2002;**28**:34–45.
143. Dandapat MC, Mishra BM, Dash SP, et al. Peripheral lymph node tuberculosis: review of 80 cases. *Br J Surg* 1990;**77**:911–12.
144. Danker WM, Davis CE. *Mycobacterium bovis* as a significant cause of tuberculosis in children residing along the United States–Mexico border in the Baja California region. *Pediatrics* 2000; **105**:e79.
145. David HL. Probability distribution of the drug-resistant mutants in unselected populations of *Mycobacterium tuberculosis*. *Appl Microbiol* 1970;**20**:810–14.
146. De Angelis LM. Intracranial tuberculoma: case report and review of the literature. *Neurology* 1981;**31**:1133–6.
147. de Blic J, Azevedo I, Burren C, et al. The value of flexible bronchoscopy in childhood pulmonary tuberculosis. *Chest* 1991;**100**: 188–92.
148. Debré R, Noufflard H, Brissaud HE, et al. Infection of children by strains of tubercle bacilli initially resistant to streptomycin or to isoniazid. *Am Rev Respir Dis* 1959;**80**:326–31.
149. Dekker G, Andronikou S, van Toorn R, et al. MRI findings in children with tuberculous meningitis: a comparison of

HIV-infected and non-infected children. *Childs Nerv Syst* 2011; **27**:1943–9.
150. Delacourt C, Mani TM, Bonnerot V, et al. Computed tomography with normal chest radiograph in tuberculous infection. *Arch Dis Child* 1993;**69**:430–2.
151. de Pontual L, Balu L, Ovetchkine P, et al. Tuberculosis in adolescents: a French retrospective study of 52 cases. *Pediatr Infect Di J* 2006;**25**:930–2.
152. Deeks SL, Clark M, Scheifele P, et al. Serious adverse events associated with bacilli Calmette-Guérin vaccine in Canada. *Pediatr Infect Dis J* 2005;**24**:538–41.
153. Dewan PK, Banouvong H, Abernathy N, et al. A tuberculosis outbreak in a private-home family child care center in San Francisco, 2002 to 2004. *Pediatrics* 2006;**117**:863–9.
154. Diel R, Goletti D, Ferrara G, et al. Interferon-gamma release assays for the diagnosis of latent *Mycobacterium tuberculosis* infection: a systematic review and meta-analysis. *Eur Respir J* 2011; **37**:88–99.
155. Diel R, Loddenkemper R, Nienhaus A. Evidence-based comparison of commercial interferon-gamma release assays for detecting active TB: a meta-analysis. *Chest* 2010;**137**:952–68.
156. Dietze R, Hadad DJ, McGee B, et al. Early and extended early bactericidal activity of linezolid in pulmonary tuberculosis. *Am J Respir Crit Care Med* 2008;**178**:1180–5.
157. Dineen P, Homan WP, Grafe WR. Tuberculous peritonitis: 43 years' experience in diagnosis and treatment. *Ann Surg* 1976;**184**: 717–22.
158. Dinning WJ, Mauston S. Cutaneous and ocular tuberculosis: a review. *J R Soc Med* 1985;**78**:576–81.
159. Doerr CA, Starke JR, Ong LT. Clinical and public health aspects of tuberculous meningitis in children. *J Pediatr* 1995;**127**:27–33.
160. Domingo Z, Peter JC. Intracranial tuberculomas: an assessment of a therapeutic 4-drug trial in 35 children. *Pediatr Neurosci* 1989; **15**:161–7.
161. Donald PR. Cerebrospinal fluid concentrations of antituberculosis agents in children and adults. *Tuberculosis (Edinb)* 2010;**90**: 279–92.
162. Donald PR. Antituberculosis drug-induced hepatotoxicity in children. *Pediatr Rep* 2011;**3**:e16.
163. Donald PR, Gent WL, Seifart H, et al. Cerebrospinal fluid isoniazid concentrations in children with tuberculous meningitis: the influence of dosage and acetylation status. *Pediatrics* 1992;**89**: 247–50.
164. Donald PR, Mahan D, Maritz JS, et al. Ethambutol dosage for the treatment of children: literature review and recommendations. *Int J Tuberc Lung Dis* 2006;**10**:1318–30.
165. Donald PR, Schaaf HS, Schoeman JF. Tuberculous meningitis and miliary tuberculosis: the Rich focus revisited. *J Infect* 2005;**50**: 193–5.
166. Donald PR, Schoeman JF, VanZyl LE, et al. Intensive short-course chemotherapy in the management of tuberculous meningitis. *Int J Tuberc Lung Dis* 1998;**2**:704–11.
167. Donald PR, Seifart H. Cerebrospinal fluid pyrazinamide concentrations in children with tuberculous meningitis. *Pediatr Infect Dis J* 1988;**7**:469–71.
168. Donald PR, Seifart HI. Cerebrospinal fluid concentrations of ethionamide in children with tuberculous meningitis. *J Pediatr* 1989;**115**:483–6.
169. Dorhoi A, Reece ST, Kaufmann SH. For better or for worse: the immune response against Mycobacterium tuberculosis balances pathology and protection. *Immunol Rev* 2011;**240**:235–51.
170. Dormer BA, Harrison I, Swart JA, et al. Prophylactic isoniazid protection of infants in a tuberculosis hospital. *Lancet* 1959;**2**: 902–3.
171. Driver CR, Frieden TR, Bloch AB, et al. Drug resistance among tuberculosis patients, New York City, 1991 and 1992. *Public Health Rep* 1994;**109**:632–6.
172. Driver C, Luallen J, Good W, et al. Tuberculosis in children younger than five years old: New York City. *Pediatr Infect Dis J* 1995;**14**:117–21.
173. Driver CR, Valway SE, Cantwell ME, et al. Tuberculin skin test screening of school children in the United States. *Pediatrics* 1996;**98**:97–102.
174. Drobac PC, del Castillo H, Sweetland A, et al. Treatment of a multidrug-resistant tuberculous during pregnancy: long-term follow-up of 6 children with intrauterine exposure to second-line agents. *Clin Infect Dis* 2005;**40**:1689–92.
175. Drobac PC, Mukherjee JS, Joseph JK, et al. Community-based therapy for children with multidrug-resistant tuberculosis. *Pediatrics* 2006;**117**:2022–8.
176. Drucker E, Alcabes P, Bosworth W, et al. Childhood tuberculosis in the Bronx, New York. *Lancet* 1994;**343**:1482–5.
177. Dubos R, Dubos J. *The white plague: tuberculosis, man and society*. New Brunswick, NJ: Rutgers University Press; 1987.
178. Dunlap NE, Harris RH, Benjamin WH Jr, et al. Laboratory contamination of *Mycobacterium tuberculosis* cultures. *Am J Respir Crit Care Med* 1995;**152**:1702–4.
179. Dutt AK, Mehta JB, Whitaker BJ, et al. Outbreak of tuberculosis in a church. *Chest* 1995;**107**:447–52.
180. Dutt AK, Moers D, Stead WW. Short-course chemotherapy for extrapulmonary tuberculosis. *Ann Intern Med* 1986;**107**:7–12.
181. Dutt AK, Stead WW. Present chemotherapy for tuberculosis. *J Infect Dis* 1982;**146**:698–704.
182. Dutt AK, Stead WW. Tuberculous pleural effusion: 6-month therapy with isoniazid and rifampin. *Am Rev Respir Dis* 1992; **145**:1429–32.
183. Dye C, Espinal MA, Watt CJ, et al. Worldwide incidence of multidrug resistant tuberculosis. *J Infect Dis* 2002;**185**:1197–202.
184. Eamranond P, Jaramillo E. Tuberculosis in children: reassessing the need for improved diagnosis in global control strategies. *Int J Tuberc Lung Dis* 2001;**5**:594–603.
185. Edwards DJ, Kitetele F, Van Rie A. Agreement between clinical scoring systems used for the diagnosis of pediatric tuberculosis in the HIV era. *Int J Tuberc Lung Dis* 2007;**11**:263–9.
186. Edwards LB, Hardy JB. Relation of the degree of sensitivity to tuberculin to the persistence of sensitivity and to prognosis in young children. *Bull Johns Hopkins Hosp* 1946;**78**:13–20.
187. Ehrlich RM, Lattimer J. Urogenital tuberculosis in children. *J Urol* 1971;**105**:461–5.
188. Eisenach KD, Sifford MD, Cave MD, et al. Detection of *Mycobacterium tuberculosis* in sputum samples using a polymerase chain reaction. *Am Rev Respir Dis* 1991;**144**:1160–3.
189. Elenga N, Kouakoussui KA, Bonard D, et al. Diagnosed tuberculosis during the follow-up of a cohort of human immunodeficiency virus–infected children in Abidjon, Coté d'Ivoire. ANRS 1278 study. *Pediatr Infect Dis J* 2005;**24**:1077–82.
190. Ellard GA. Hepatic toxicity of isoniazid among rapid and slow acetylators of the drug. *Am Rev Respir Dis* 1978;**118**:628–9.
191. Ellard GA, Humphries MJ, Gabriel M, et al. Penetration of pyrazinamide into the cerebrospinal fluid in tuberculous meningitis. *BMJ* 1987;**294**:284–5.
192. Elliott AM, Luzze H, Quigley MA, et al. A randomized, double-blind, placebo-controlled trial of the use of prednisolone as an adjunct to treatment in HIV-1–associated pleural tuberculosis. *J Infect Dis* 2004;**190**:869–78.
193. Ellner JJ. Pleural fluid and peripheral blood lymphocyte function in tuberculosis. *Ann Intern Med* 1978;**89**:932–3.
194. Emmett JR, Fischer ND, Biggers WP. Tuberculous mastoiditis. *Laryngoscope* 1977;**87**:1157–63.
195. Ena J, Valls V. Short-course therapy with rifampin plus isoniazid, compared with standard therapy with isoniazid for latent tuberculosis infection: a meta-analysis. *Clin Infect Dis* 2005;**40**:670–6.
196. Enarson DA. The International Union Against Tuberculosis and Lung Disease model National Tuberculosis Programmes. *Tubercle Lung Dis* 1995;**76**:95–9.
197. Engström A, Morcillo N, Imperiale B, et al. Detection of first- and second-line drug resistance in *Mycobacterium tuberculosis* clinical isolates using pyrosequencing. *J Clin Microbiol* 2012;**50**:2026–33.
198. Esterly JR, Sturner WQ, Esterly NB, et al. Disseminated BCG in twin boys with presumed chronic granulomatous disease of childhood. *Pediatrics* 1977;**48**:141–4.
199. Ewer K, Decks J, Alvarez L, et al. Comparison of T-cell–based assay with tuberculin skin test for diagnosis of *Mycobacterium tuberculosis* infection in a school tuberculosis outbreak. *Lancet* 2003;**361**:1168–73.
200. Excobar JA, Belsey MA, Dueñas A, et al. Mortality from tuberculous meningitis reduced by steroid therapy. *Pediatrics* 1975;**56**: 1050–5.
201. Feng WX, Flores-Villanueva PO, Mokrousov I, et al. CCL2-2518 (A/G) polymorphisms and tuberculosis susceptibility: a meta-analysis. *Int J Tuberc Lung Dis* 2012;**16**:150–6.

202. Ferrebee SH. Controlled chemoprophylaxis trials in tuberculosis: a general review. *Adv Tuberc Res* 1969;**17**:28–106.
203. Figueroa-Damian R, Arredondo-Garcia JL. Neonatal outcome of children born to women with tuberculosis. *Arch Med Res* 2001;**32**:66–9.
204. Filler J, Porter M. Physiologic studies of the sequelae of tuberculous pleural effusion in children treated with antimicrobial drugs and prednisone. *Am Rev Respir Dis* 1963;**88**:181–8.
205. Francis J, Reed A, Yohannes F, et al. Screening for tuberculosis among orphans in a developing country. *Am J Prev Med* 2002;**22**: 117–19.
206. Frieden TR, Fujiwara PI, Washko RM, et al. Tuberculosis in New York City: turning the tide. *N Engl J Med* 1995;**333**:229–33.
207. Frieden TR, Sterling T, Pablos-Mendez A, et al. The emergence of drug-resistant tuberculosis in New York City. *N Engl J Med* 1993;**328**:521–6.
208. Friedland IR. The booster effect with repeat tuberculin testing in children and its relationship to BCG vaccination. *S Afr Med* 1990;**77**:387–9.
209. Froehlich H, Ackerson LM, Morozumi PA. Targeted testing of children for tuberculosis: validation of a risk assessment questionnaire. *Pediatrics* 2001;**107**:e54.
210. Gangadharam PRJ, Pratt PF, Perumal UK, et al. The effects of exposure time, drug concentration, and temperature on the activity of ethambutol versus *Mycobacterium tuberculosis*. *Am Rev Respir Dis* 1990;**141**:1478–82.
211. Gardy JL, Johnston JC, Ho Sui SJ, et al. Whole-genome sequencing and social-network analysis of a tuberculosis outbreak. *N Engl J Med* 2011;**364**:730–9.
211a. Garie KT, Yassin MA, Cuevas LE. Lack of adherence to isoniazid chemoprophylaxis in children in contact with adults with tuberculosis in southern Ethiopia. *PloS One* 2011;**6**:e26452.
212. Gaur S, Kesarwala H, Frenkel LD. Tuberculous peritonitis in an adolescent female. *Pediatr Infect Dis J* 1987;**18**:859–62.
213. Ghon A. *The primary lung focus of tuberculosis in children*. London: JA Churchill; 1916.
214. Girling DJ. Adverse reactions to rifampicin in antituberculosis regimens. *J Antimicrob Chemother* 1977;**3**:115.
215. Girling DJ. Role of pyrazinamide in primary chemotherapy for pulmonary tuberculosis. *Tubercle* 1984;**65**:1–4.
216. Glassroth J, Bailey WC, Hopewell PC, et al. Why tuberculosis is not prevented. *Am Rev Respir Dis* 1990;**141**:1236–40.
217. Goble M, Iseman MD, Madsen LA, et al. Treatment of 171 patients with pulmonary tuberculosis resistant to isoniazid and rifampin. *N Engl J Med* 1993;**328**:527–32.
218. Gomez LP, Morris SL, Panduro A. Rapid and efficient detection of extra-pulmonary *Mycobacterium tuberculosis* by PCR analysis. *Int J Tuberc Lung Dis* 2000;**4**:361–70.
219. Gonzalez B, Moreno S, Burdach R, et al. Clinical presentation of bacillus Calmette-Guérin infections in patients with immunodeficiency syndromes. *Pediatr Infect Dis J* 1989;**8**:201–6.
220. Good M, Duignan A. Perspectives on the history of bovine TB and the role of tuberculin bovine TB eradication. *Vet Med Int* 2011;**2011**:410470.
221. Gorse GJ, Belshe RB. Male genital tuberculosis: a review of the literature with instructive case reports. *Rev Infect Dis* 1985;**7**: 511–24.
222. Graham SM, Bell DJ, Nyirongo S, et al. Low levels of pyrazinamide and ethambutol in children with tuberculosis and impact of age, nutritional status, and human immunodeficiency virus infection. *Antimicrob Agents Chemother* 2006;**50**:407–13.
223. Grange JM, Gibson J, Osborn TW, et al. What is BCG? *Tubercle* 1983;**64**:129–39.
224. Graziani AL, MacGregor RR. Self-reading of tuberculin testing vs. physician reading. *Infect Dis Clin Pract* 1995;**4**:72–4.
225. Grosset J. Bacteriologic basis for short-course chemotherapy for tuberculosis. *Clin Chest Med* 1980;**1**:231–41.
226. Grosskopf I, David A, Charach G, et al. Bone and joint tuberculosis: a 10-year review. *Isr J Med Sci* 1994;**30**:278–83.
227. Groussard P, Gie RP, Kling S, et al. Expansile pneumonia in children caused by *Mycobacterium tuberculosis*: clinical, radiological and bronchoscopic appearances. *Pediatr Pulmonol* 2004;**38**:451–5.
228. Grzybowski S, Allen EA. History and importance of scrofula. *Lancet* 1995;**346**:1472–4.
229. Guarisco JL, Cheney ML, LeJeune FE Jr, et al. Isolated uvulitis secondary to marijuana use. *Laryngoscope* 1988;**98**:1309–12.
230. Gupta R, Gupta S, Dingh D, et al. MR imaging and angiography in tuberculous meningitis. *Neuroradiology* 1994;**36**:87–92.
231. Gutman L, Moye J, Zimmer B, et al. Tuberculosis in human immunodeficiency virus–exposed or –infected United States children. *Pediatr Infect Dis J* 1994;**13**:963–8.
232. Hageman J, Shulman S, Schreiber M, et al. Congenital tuberculosis: critical reappraisal of clinical findings and diagnostic procedures. *Pediatrics* 1980;**66**:980–4.
233. Haimi-Cohen Y, Zeharia A, Mimouni M, et al. Skin indurations in response to tuberculin testing in patients with nontuberculous mycobacterial lymphadenitis. *Clin Infect Dis* 2001;**33**:1786–8.
234. Hakim JG, Ternouth I, Mushangi E, et al. Double blind randomised placebo controlled trial of adjunctive prednisolone in the treatment of effusive tuberculous pericarditis in HIV seropositive patients. *Heart* 2000;**84**:183–8.
235. Hannachi J, Martin M, Boulabal F, et al. Comparison of three daily short-course regimens in osteoarticular tuberculosis in Algiers. *Bull Int Union Tuberc* 1982;**57**:46–7.
236. Hardy JB. Persistence of hypersensitivity to old tuberculin following primary tuberculosis in childhood: a long-term study. *Am J Public Health* 1946;**36**:1417–26.
237. Hardy JB, Hartmann JR. Tuberculous dactylitis in childhood. *J Pediatr* 1947;**30**:146–56.
238. Harries AD. Tuberculosis and human immunodeficiency virus infection in developing countries. *Lancet* 1990;**335**:387–90.
239. Harries AD, Hargreaves NJ, Graham S, et al. Childhood tuberculosis in Malawi: nationwide case-finding and treatment outcomes. *Int J Tuberc Lung Dis* 2002;**6**:424–31.
240. Harris VJ, Dida F, Lander SS, et al. Cavitary tuberculosis in children. *J Pediatr* 1977;**90**:660–1.
241. Harrison HE, Finberg L, Fleischman E. Disturbances of ionic equilibrium of intracellular and extracellular electrolytes in patients with tuberculous meningitis. *J Clin Invest* 1952;**31**: 300–8.
242. Hatherill M. Prospects for elimination of childhood tuberculosis: the role of new vaccines. *Arch Dis Child* 2011;**96**:851–6.
243. Haustein T, Ridout DA, Hartley JC, et al. The likelihood of an indeterminate test result from a whole-blood interferon-gamma release assay for the diagnosis of *Mycobacterium tuberculosis* infection in children correlates with age and immune status. *Pediatr Infect Dis J* 2009;**28**:669–73.
244. Haygood T, Williamson S. Radiographic findings of extremity tuberculosis in childhood: back to the future? *Radio Graphics* 1994;**14**:561–70.
245. Heinle EW Jr, Jensen NN, Westerman MP. Diagnostic usefulness of marrow biopsy in disseminated tuberculosis. *Am Rev Respir Dis* 1965;**91**:701–5.
246. Helms S, Helms P. Tuberculin sensitivity during measles. *Acta Tuberc Scand* 1956;**35**:166–71.
247. Heney C, Baise T, Cohen MA. Tuberculosis of the mandible: a case report. *Pediatr Infect Dis J* 1988;**7**:74–6.
248. Hesseling AC, Marais BJ, Gie RP, et al. The risk of disseminated bacille Calmette-Guérin (BCG) disease in HIV-infected children. *Vaccine* 2007;**25**:14–18.
249. Hesseling AC, Rabie H, Marais BJ, et al. Bacille Calmette-Guérin vaccine–induced disease in HIV-infected and HIV-uninfected children. *Clin Infect Dis* 2006;**42**:548–58.
250. Hesseling AC, Schaaf HS, Gie RP, et al. A critical review of diagnostic approaches used in the diagnosis of childhood tuberculosis. *Int J Tuberc Lung Dis* 2002;**6**:1039–45.
251. Hesseling AC, Westra AE, Werschkull H, et al. Outcome of HIV-infected children with culture-confirmed tuberculosis. *Arch Dis Child* 2005;**90**:1171–4.
252. Heycock JB, Noble TC. Four cases of syringe-transmitted tuberculosis. *Tubercle* 1961;**42**:25–7.
253. Hlavsa MC, Moonan PK, Cowan LS, et al. Human tuberculosis due to *Mycobacterium bovis* in the United States, 1995-2005. *Clin Infect Dis* 2008;**47**:168–75.
254. Hoffman EB, Allin J, Campbell JA, et al. Tuberculosis of the knee. *Clin Orthop Relat Res* 2002;**398**:100–6.
255. Hoffman EB, Crosier JH, Cremin BJ. Imaging in children with spinal tuberculosis. *J Bone Joint Surg Br* 1993;**75**:233–9.
256. Hoffman ND, Kelly C, Futterman D. Tuberculosis infection in human immunodeficiency virus–positive adolescents and young adults: a New York City cohort. *Pediatrics* 1996;**97**: 198–203.

257. Hoge C, Fisher L, Donnell D, et al. Risk factors for transmission of *Mycobacterium tuberculosis* in a primary school outbreak: lack of racial difference in susceptibility to infection. *Am J Epidemiol* 1994;**139**:520–30.
258. Holt LE. Tuberculosis acquired through ritual circumcision. *JAMA* 1913;**61**:99–102.
259. Hong Kong Chest Service/British Medical Research Council Fifth Collaborative Study. Controlled clinical trial of five 6-month regimens of chemotherapy for pulmonary tuberculosis. *Am Rev Respir Dis* 1987;**136**:1339–42.
260. Hong Kong Chest Service/British Medical Research Council. Controlled trial of 2, 4 and 6 months of pyrazinamide in 6-month, three times weekly regimens for smear positive pulmonary tuberculosis, including an assessment of a combined preparation of isoniazid, rifampin and pyrazinamide: results at 30 months. *Am Rev Respir Dis* 1991;**143**:700–6.
261. Honore N, Cole S. Streptomycin resistance in mycobacteria. *Antimicrob Agents Chemother* 1994;**38**:238–41.
262. Horsburgh CR Jr, Rubin EJ. Latent tuberculosis infection in the United States. *N Engl J Med* 2011;**364**:1441–8.
263. Houde C, Dery P. *Mycobacterium bovis* sepsis in an infant with human immunodeficiency virus infection. *Pediatr Infect Dis J* 1988;**7**:810.
264. Howard TP, Soloman DA. Reading the tuberculin skin test: who, when and how? *Arch Intern Med* 1988;**148**:2457–9.
265. Hsu KHK. Contact investigation: a practical approach to tuberculosis eradication. *Am J Public Health* 1963;**53**:1761–9.
266. Hsu KHK. Tuberculin reaction in children treated with isoniazid. *Am J Dis Child* 1983;**137**:1090–2.
267. Hsu KHK. Thirty years after isoniazid: Its impact on tuberculosis in children and adolescents. *JAMA* 1984;**251**:1283–5.
268. Hsu KHK, Starke JR. Diagnosis and treatment of tuberculous infection. *Semin Pediatr Infect Dis* 1993;**4**:283–90.
269. Hsu LCS, Leong JCY. Tuberculosis of the lower cervical spine (C2 to C7): a report on 40 cases. *J Bone Joint Surg Br* 1984; **66**:1–5.
270. Huebner RE, Schein MF, Bass JB. The tuberculin skin test. *Clin Infect Dis* 1993;**17**:968–75.
271. Hughes IE, Smith H, Kane PO. Ethionamide: its passage into the cerebrospinal fluid in man. *Lancet* 1962;**1**:616–17.
272. Hughesdon MR. Congenital tuberculosis. *Arch Dis Child* 1946; **21**:121–38.
273. Hugo-Hamman CT, Scher H, DeMoor MMA. Tuberculous pericarditis in children: a review of 44 cases. *Pediatr Infect Dis J* 1994;**13**:13–18.
274. Humphries MJ, Teoh R, Lau J, et al. Factors of prognostic significance in Chinese children with tuberculous meningitis. *Tubercle* 1990;**71**:161–8.
275. Hussey G, Chisholm T, Kibel M. Miliary tuberculosis in children: a review of 94 cases. *Pediatr Infect Dis J* 1991;**10**:832–6.
276. Hyge TV. The efficacy of BCG-vaccination: tuberculosis epidemic in a state school with an observation period of 12 years. *Dan Med Bull* 1957;**4**:13–15.
277. Ibanez S, Ross G. Quimioterapia abreviada de 6 meses en tuberculosis pulmonar infantil. *Rev Chil Pediatr* 1980;**51**:249–52.
278. Idriss ZH, Sinno A, Kronfol NM. Tuberculous meningitis in childhood: forty-three cases. *Am J Dis Child* 1976;**130**: 364–7.
279. Ildirim I, Hacimustafaoglu M, Ediz B. Correlation of tuberculin induration with the number of bacillus Calmette-Guérin vaccines. *Pediatr Infect Dis J* 1995;**14**:1060–3.
280. Ingalhalikar VT, Deostale DA, Abhyankar VK. Nonimmobilization of surgically treated tuberculosis of spine in children. *Prog Pediatr Surg* 1982;**15**:153–7.
281. International Union Against Tuberculosis Committee on Prophylaxis. Efficacy of various durations of isoniazid preventive therapy for tuberculosis: five years of follow-up in the IUAT trial. *Bull World Health Organ* 1982;**60**:555–64.
282. Iriso R, Mudido PM, Karamagi C, et al. The diagnosis of childhood tuberculosis in an HIV-endemic setting and the use of induced sputum. *Int J Tuberc Lung Dis* 2005;**9**:716–26.
283. Iseman MD. Treatment of multidrug-resistant tuberculosis. *N Engl J Med* 1993;**329**:784–91.
284. Iseman MD, Cohn DL, Sbarbaro JA. Directly observed treatment of tuberculosis: We can't afford not to try it. *N Engl J Med* 1993; **328**:576–8.
285. Iseman MD, Madsen L, Goble M, et al. Surgical intervention in the treatment of pulmonary disease caused by drug-resistant *Mycobacterium tuberculosis*. *Am Rev Respir Dis* 1990;**141**:623–5.
286. Ijaz K, Dillaha JA, Yang Z, et al. Unrecognized tuberculosis in a nursing home causing death with spread of tuberculosis to the community. *J Am Geriatr Soc* 2002;**50**:1213–18.
287. Jacobs RF, Abernathy RS. The treatment of tuberculosis in children. *Pediatr Infect Dis* 1985;**4**:513–17.
288. Jacobs RF, Sunakorn P, Chotpitayasunonah T, et al. Intensive short course chemotherapy for tuberculous meningitis. *Pediatr Infect Dis J* 1992;**11**:194–8.
289. Jaffe IP. Tuberculous meningitis in childhood. *Lancet* 1982;**1**:738.
290. Jain SK, Paul-Satyaseela M, Lamichhane G, et al. *Mycobacterium tuberculosis* invasion and traversal across an in vitro human blood-brain barrier as a pathogenic mechanism for central nervous system tuberculosis. *J Infect Dis* 2006;**193**:1287–95.
291. Janner D, Kirk S, McLeary M. Cerebral tuberculosis without neurologic signs and with normal cerebrospinal fluid. *Pediatr Infect Dis J* 2000;**19**:763–4.
292. Jasmer RM, Seaman CB, Gonzalez LC, et al. Tuberculosis treatment outcomes. *Am J Respir Crit Care Med* 2004;**170**:561–6.
293. Jawahar MS, Sivasubramanian S, Vijayan VK, et al. Short course chemotherapy for tuberculous lymphadenitis in children. *BMJ* 1990;**301**:359–62.
294. Jereb J, Kelly G, Porterfield D. The epidemiology of tuberculosis in children. *Semin Pediatr Infect Dis* 1993;**4**:220–31.
295. Jinkins JR. Computed tomography of intracranial tuberculosis. *Neuroradiology* 1991;**33**:126–35.
296. John JF, Douglas RG. Tuberculous arachnoiditis. *J Pediatr* 1975; **86**:235–7.
297. Johnson H, Lee B, Doherty E, et al. Tuberculin sensitivity and the BCG scar in tuberculosis contacts. *Tubercle Lung Dis* 1995; **76**:122–5.
298. Johnston JA. *Nutritional studies in adolescent girls and their relation to tuberculosis*. Springfield, Ill.: Charles C Thomas; 1953.
299. Jones D, Malecki J, Bigler W, et al. Pediatric tuberculosis and human immunodeficiency virus infection in Palm Beach County, Florida. *Am J Dis Child* 1992;**146**:1166–70.
300. Kallmann IJ, Reisner D. Twin studies on the genetic factors in tuberculosis. *Am Rev Tuberc* 1943;**47**:549.
301. Kampann B, Tena-Coki GN, Nicol MP, et al. Reconstruction of antimycobacterial immune responses in HIV-infected children receiving HAART. *AIDS* 2006;**20**:1011–18.
302. Karim SS, Naidoo K, Grobler A, et al. Integration of antiretroviral therapy with tuberculosis treatment. *N Engl J Med* 2011;**365**: 1492–501.
303. Kasambira TS, Shah M, Adrian PV, et al. QuantiFERON-TB Gold In-Tube for the detection of *Mycobacterium tuberculosis* infection in children with household tuberculosis contact. *Int J Tuberc Lung Dis* 2011;**15**:628–34.
304. Kedar RP, Shah PP, Shivde RS, et al. Sonographic findings in gastrointestinal and peritoneal tuberculosis. *Clin Radiol* 1994;**49**: 24–9.
305. Kellerman SE, Saiman L, San Gabriel P, et al. Observational study of the use of infection control interventions for *Mycobacterium tuberculosis* in pediatric facilities. *Pediatr Infect Dis J* 2001;**20**: 566–70.
306. Kellerman SE, Saiman L, Soto-Irizarry M, et al. Costs associated with tuberculosis control programs at hospitals caring for children. *Pediatr Infect Dis J* 1999;**18**:604–8.
307. Kellerman SE, Simonds D, Banerjee S, et al. APIC and CDC survey of *Mycobacterium tuberculosis* isolation and control practices in hospitals caring for children, part I: patient and family isolation policies and procedures. *Am J Infect Control* 1998;**26**:478–82.
308. Kellerman SE, Simonds D, Banerjee S, et al. APIC and CDC survey of *Mycobacterium tuberculosis* isolation and control practices in hospitals caring for children, part II: environmental and administrative controls. *Am J Infect Control* 1998;**26**:483–7.
309. Kendig EL Jr. Tuberculosis in the very young: report of three cases of infants less than one month of age. *Am Rev Tuberc* 1954; **70**:161–5.
310. Kendig EL Jr. Prognosis of infants born of tuberculous mothers. *Pediatrics* 1960;**26**:97–100.
311. Kendig EL Jr. The place of BCG vaccine in the management of infants born of tuberculous mothers. *N Engl J Med* 1969; **281**:520–3.

312. Kendig EL Jr, Kirkpatrick BV, Carter WH, et al. Underreading of the tuberculin skin test reaction. *Chest* 1998;**113**:1175–7.
313. Kendig EL Jr, Rodgers WL. Tuberculosis in the neonatal period. *Am Rev Tuberc Pulm Dis* 1958;**77**:418–22.
314. Kennedy C, Knowles GK. Miliary tuberculosis presenting with skin lesions. *BMJ* 1975;**3**:356.
315. Kennedy N, Fox R, Kisyombe GM, et al. Early bactericidal and sterilizing activities of ciprofloxacin in pulmonary tuberculosis. *Am Rev Respir Dis* 1993;**148**:1547–51.
316. Kernodle DS. Decrease in the effectiveness of bacille Calmette-Guérin vaccine against pulmonary tuberculosis: a consequence of increased immune suppression by microbial antioxidants, not overattenuation. *Clin Infect Dis* 2010;**51**:177–84.
317. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for better methods. *Emerg Infect Dis* 1995;**1**: 115–23.
318. Khan FA, Minion J, Pai M, et al. Treatment of active tuberculosis in HIV-coinfected patients: a systematic review and meta-analysis. *Clin Infect Dis* 2010;**50**:1288–99.
319. Kheir AE, Alhaj AA, Ibrahim SA. The sensitivity of BCG scar as an indicator of previous vaccination among Sudanese infants. *Vaccine* 2011;**29**:8189–91.
320. Khouri Y, Mastrucci M, Hutto C, et al. *Mycobacterium tuberculosis* in children with human immunodeficiency virus type 1 infection. *Pediatr Infect Dis J* 1992;**11**:950–5.
321. Kim JH, Langston AA, Gallis HA. Miliary tuberculosis: epidemiology, clinical manifestations, diagnosis and outcome. *Rev Infect Dis* 1990;**12**:583–90.
322. Kimerling ME, Vaughn ES, Dunlap NE. Childhood tuberculosis in Alabama: epidemiology of disease and indicators of program effectiveness, 1983 to 1993. *Pediatr Infect Dis J* 1995; **14**:678–84.
323. King KY, Lew JD, Ha NP, et al. Polymorphic allele of human IRGM1 is associated with susceptibility to tuberculosis in African-Americans. *PloS One* 2011;**6**:e16371.
324. Kisembo HN, Kawooya MG, Zirembozi G, et al. Serial chest radiographs in the management of children with a clinical suspicion of pulmonary tuberculosis. *J Trop Pediatr* 2001;**47**:276–83.
325. Kiwanuka J, Graham SM, Coulter JB, et al. Diagnosis of pulmonary tuberculosis in children in an HIV-endemic area, Malawi. *Ann Trop Paediatr* 2001;**21**:5–14.
326. Klausner JD, Ryder RW, Baende E, et al. *Mycobacterium tuberculosis* in household contacts of human immunodeficiency virus type 1–seropositive patients with active pulmonary tuberculosis in Kinshasa, Zaire. *J Infect Dis* 1993;**168**:106–11.
327. Kohli V, Kumar L, Kataria S. Multiple hepatosplenic tuberculous abscesses in an eight-year-old boy. *Pediatr Infect Dis J* 1996;**15**: 178–9.
328. Kohn MR, Arden MR, Vasilakis J, et al. Directly observed preventive therapy: turning the tide against tuberculosis. *Arch Pediatr Adolesc Med* 1996;**150**:727–9.
329. Kopanoff DE, Snider DE, Caras GJ. Isoniazid-related hepatitis: a United States Public Health Service Cooperative Surveillance Study. *Am Rev Respir Dis* 1978;**117**:991–1001.
330. Kröger L. Influence of MPR revaccination on tuberculin and sensitin skin reactions in children. *Acta Paediatr* 1994;**83**:232–4.
331. Kroidl A, Huck K, Weinspach S, et al. Immune reconstitution inflammatory syndrome (IRIS) due to bacille Calmette-Guérin (BCG) in an HIV-positive child. *Scand J Infect Dis* 2006;**38**: 716–18.
332. Kumar A, Misra PK, Mehotra R, et al. Hepatotoxicity of rifampin and isoniazid: is it all drug-induced hepatitis? *Am Rev Respir Dis* 1991;**143**:1350–2.
333. Kumar B, Rai R, Kaur I, et al. Childhood cutaneous tuberculosis: a study over 25 years from northern India. *Int J Dermatol* 2001; **40**:26–32.
334. Kumar L, Dhand R, Singhi PD, et al. A randomized trial of fully intermittent vs. daily followed by intermittent short-course chemotherapy for childhood tuberculosis. *Pediatr Infect Dis J* 1990; **9**:802–6.
335. Kumar R, Pandery CK, Bose N, et al. Tuberculosis brain abscess: clinical presentation, pathophysiology and treatment (in children). *Childs Nerv Syst* 2002;**18**:118–23.
336. Kutt H, Brennan R, Dehejia H, et al. Diphenylhydantoin intoxication: a complication of isoniazid therapy. *Am Rev Respir Dis* 1970; **101**:377–84.
337. Lai KK, Stottmeier KD, Sherman IH, et al. Mycobacterial cervical lymphadenopathy: relation of etiologic agents to age. *JAMA* 1984;**251**:1286–8.
338. Lake AM, Oski FA. Peripheral lymphadenopathy in childhood: ten-year experience with excisional biopsy. *Am J Dis Child* 1978; **132**:357–9.
339. Lamont A, Cremin B, Pettenet B. Radiologic patterns of pulmonary tuberculosis in the pediatric age group. *Pediatr Radiol* 1986; **16**:2–7.
340. Lamprecht D, Schoeman J, Donald P, et al. Ventriculoperitoneal shunting in childhood tuberculosis meningitis. *Br J Neurosurg* 2001;**15**:119–25.
341. Lange WR, Warnock-Eckhart E, Bean ME. *Mycobacterium tuberculosis* infection in foreign born adoptees. *Pediatr Infect Dis J* 1989;**8**:625–9.
342. Lanier VS, Russell WF Jr, Heaton A, et al. Concentrations of active isoniazid in serum and cerebrospinal fluid of patients with tuberculosis treated with isoniazid. *Pediatrics* 1958;**21**: 910–15.
343. Larmola E. Two cases of sudden death in infants recovering from primary tuberculosis. *Acta Tuberc Scand* 1949;**21**(Suppl.):67.
344. Lau SK, Kwan S, Lee J, et al. Source of tubercle bacilli in cervical lymph nodes: a prospective study. *J Laryngol Otol* 1991;**105**: 558–61.
345. Lawn SD, Bekker LG, Miller RF. Immune reconstitution disease associated with mycobacterial infections in HIV-infected individuals receiving antiretrovirals. *Lancet Infect Dis* 2005;**5**:361–73.
346. Lawn SD, Myer L, Bekker LG, et al. Tuberculosis-associated immune reconstitution disease: incidence, risk factors and impact in an antiretroviral treatment service in South Africa. *AIDS* 2007;**21**:335–41.
347. Lawn SD, Wood R. Incidence of tuberculosis during highly active antiretroviral therapy in high-income and low-income countries. *Clin Infect Dis* 2005;**41**:1783–6.
348. Lee C, Wang W, Lan R, et al. Corticosteroids in the treatment of tuberculous pleurisy: a double-blind, placebo-controlled randomized study. *Chest* 1988;**94**:1256–9.
349. Lee LH, Le Vea CM, Graman PS. Congenital tuberculosis in a neonatal intensive care unit: case report, epidemiologic investigation and management of exposures. *Clin Infect Dis* 1998;**27**: 474–7.
350. Lees A, Macleod A, Marshall J. Cerebral tuberculoma developing during treatment of tuberculous meningitis. *Lancet* 1980;**1**: 1208–11.
351. Leggiadro RJ, Collery B, Dowdy S. Outbreak of tuberculosis in a family day care home. *Pediatr Infect Dis J* 1989;**8**:52–4.
352. Lehman LG, Ngapmen Yamadji AL, et al. The CyScope fluorescence microscope, a reliable tool for tuberculosis diagnosis in resource-limited settings. *Am J Trop Med Hyg* 2010;**83**:906–8.
353. Leiguarda R, Berthier M, Starkstein S, et al. Ischemic infarction in 25 children with tuberculous meningitis. *Stroke* 1988;**19**: 200–4.
354. Levine H, Metzger W, Lacera S, et al. Diagnosis of tuberculous pleurisy by culture of pleural biopsy specimen. *Arch Intern Med* 1970;**126**:269–71.
355. Lewinsohn DA, Gennaro ML, Scholuinck L, et al. Tuberculosis immunology in children: diagnostic and therapeutic challenges and opportunities. *Int J Tuberc Lung Dis* 2004;**8**:658–74.
356. Li X, Yang Y, Zhou F, et al. *SLC11A1 (NRAMP1)* polymorphisms and tuberculosis susceptibility: updated systematic review and meta-analysis. *PloS One* 2011;**6**:e15831.
357. Light IJ, Saidleman M, Sutherland JM. Management of newborns after nursery exposure to tuberculosis. *Am Rev Respir Dis* 1974; *10***9**:415–19.
358. Lincoln EM. Tuberculous meningitis in children: with special reference to serous meningitis. *Am Rev Tuberc* 1947;**56**:75–94, 95-109.
359. Lincoln EM. Epidemics of tuberculosis. *Adv Tuberc Res* 1965; **14**:159–97.
360. Lincoln EM, Davies PA, Bovornkitti S. Tuberculous pleurisy with effusion in children. *Am Rev Tuberc* 1958;**77**:271–89.
361. Lincoln EM, Gilbert L, Morales SM. Chronic pulmonary tuberculosis in individuals with known previous primary tuberculosis. *Dis Chest* 1960;**38**:473–82.
362. Lincoln EM, Harris LC, Bovornkitti S, et al. Endobronchial tuberculosis in children. *Am Rev Tuberc Pulm Dis* 1958;**77**:39–61.

363. Lincoln EM, Sabato VR, Davies PA. Tuberculous meningitis in children. *J Pediatr* 1960;**57**:807–23.
364. Lincoln EM, Sewell EM. *Tuberculosis in children*. New York: McGraw-Hill; 1963.
365. Lindgren I. Pathology of tuberculous infection in BCG-vaccinated humans. *Adv Tuberc Res* 1965;**14**:203–31.
366. Linna O, Uhari M. Hepatotoxicity of rifampicin and isoniazid in children treated for tuberculosis. *Eur J Pediatr* 1980;**134**: 227–9.
367. Liu KW, Chan YL, Tseng R, et al. Childhood abdominal tuberculosis: the role of echo-guided fine-needle aspiration in its management. *Surg Endosc* 1994;**8**:326–8.
368. Livengood JR, Sigler TG, Foster LR, et al. Isoniazid-resistant tuberculosis: a community outbreak and report of a rifampin prophylaxis failure. *JAMA* 1985;**253**:2847–9.
369. Lobato M, Hopewell PC. *Mycobacterium tuberculosis* infection after travel to or contact with visitors from countries with high prevalence of tuberculosis. *Am J Respir Crit Care Med* 1998; **158**:1871–5.
370. Lobato MN, Jereb JA, Starke JR. Unintended consequences: mandatory tuberculin skin testing and severe isoniazid hepatotoxicity. *Pediatrics* 2008;**121**:e1732–3.
371. Lobato MN, Mohle-Boetani JC, Royce SE. Missed opportunities for preventing tuberculosis among children younger than five years of age. *Pediatrics* 2000;**106**:e75.
372. Lockman S, Tappero JW, Kenyon TA, et al. Tuberculin reactivity in a pediatric population with high BCG vaccination coverage. *Int J Tuberc Lung Dis* 1999;**3**:23–30.
373. Lorber J. Isoniazid and streptomycin in tuberculous meningitis. *Lancet* 1964;**1**:1140–2.
374. Lucente FE, Tobias GW, Parisier SC, et al. Tuberculous otitis media. *Laryngoscope* 1978;**88**:1107–16.
375. Luo C, Chintu C, Bhat G, et al. Human immunodeficiency virus type-1 infection in Zambian children with tuberculosis: changing seroprevalence and evaluation of a thiacetazone-free regimen. *Tubercle Lung Dis* 1994;**75**:110–15.
376. Lurie MB. *Resistance to tuberculosis: experimental studies in native and acquired defense mechanisms*. Cambridge, MA: Harvard University Press; 1964.
377. MacAdam AM, Rubio T. Tuberculous otomastoiditis in children. *Am J Dis Child* 1977;**131**:152–6.
378. MacDonnell AH, Baird RW, Bronze MS. Intramedullary tuberculomas of the spinal cord: case report and review. *Rev Infect Dis* 1990;**12**:432–9.
379. MacGregor RR, Sheagren JN, Lipsett MB, et al. Alternate-day prednisone therapy: evaluation of delayed hypersensitivity response, control of disease and steroid side effects. *N Engl J Med* 1969;**280**:1427–31.
380. Machingaidze S, Wiysonge CS, Gonzalez-Angulo Y, et al. The utility of an interferon gamma release assay for diagnosis of latent tuberculosis infection and disease in children. *Pediatr Infect Dis J* 2011;**30**:694–700.
381. Madhi SA, Nachman S, Violari A, et al. Primary isoniazid prophylaxis against tuberculosis in HIV-exposed children. *N Engl J Med* 2011;**365**:21–31.
382. Maharaj B, Leary WP, Pudifin DJ. A prospective study of hepatic tuberculosis in 41 black patients. *Q J Med* 1987;**242**: 517–22.
383. Mahmoudi A, Iseman M. Pitfalls in the care of patients with tuberculosis: common errors and their association with the acquisition of drug resistance. *JAMA* 1993;**270**:65–8.
384. Malik S, Abel L, Tooker H, et al. Alleles of *NRAMP-1* gene are risk factors for pediatric tuberculosis disease. *Proc Natl Acad Sci U S A* 2005;**102**:12183–8.
385. Manalo F, Tan F, Sbarbaro JA, et al. Community-based short-course treatment of pulmonary tuberculosis in a developing nation: initial report of an eight-month, largely intermittent, regimen in a population with a high prevalence of drug resistance. *Am Rev Respir Dis* 1990;**142**:1301–5.
386. Mancao MY, Nolte FS, Nahmias AJ, et al. Use of polymerase chain reaction for diagnosis of tuberculous meningitis. *Pediatr Infect Dis J* 1994;**13**:154–5.
387. Mandalakas AM, Detjen AK, Hesseling AC, et al. Interferon-gamma release assays and childhood tuberculosis: systematic review and meta-analysis. *Int J Tuberc Lung Dis* 2011;**15**: 1018–32.
388. Mandalakas AM, Guay L, Musoke P, et al. Human immunodeficiency virus status and delayed-type hypersensitivity skin testing in Ugandan children. *Pediatrics* 1999;**103**:e21.
389. Manjunath N, Shankar P, Rajan L, et al. Evaluation of a polymerase chain reaction for the diagnosis of tuberculosis. *Tubercle* 1991;**72**:21–7.
390. Marais BJ, Gie RP, Hesseling AC, et al. Adult-type pulmonary tuberculosis in children aged 10-14 years. *Pediatr Infect Dis J* 2005;**24**:743–4.
391. Marais BJ, Gie RP, Hesseling AC, et al. Radiographic signs and symptoms in children treated for tuberculosis. Possible implications for symptom-based screening in resource-limited settings. *Pediatr Infect Dis J* 2006;**25**:237–40.
392. Marais BJ, Gie GP, Hesseling AC, et al. A refined symptom-based approach to diagnose pulmonary tuberculosis in children. *Pediatrics* 2006;**118**:e1350–9.
393. Marais BJ, Gie RP, Schaaf HS, et al. The clinical epidemiology of childhood pulmonary tuberculosis: a critical review of literature from the pre-chemotherapy era. *Int J Tuberc Lung Dis* 2004;**8**: 278–85.
394. Marais BJ, Gie RP, Schaaf HS, et al. A proposed radiographic classification of childhood intra-thoracic tuberculosis. *Pediatr Radiol* 2004;**33**:886–94.
395. Marais BJ, Gie RP, Schaaf HS, et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of the pre-chemotherapy literature. *Int J Tuberc Lung Dis* 2004;**8**:392–402.
396. Marais BJ, Gie RP, Schaaf HS, et al. Childhood pulmonary tuberculosis. Old wisdom and new challenges. *Am J Respir Crit Care Med* 2006;**173**:1078–90.
397. Marais BJ, Graham SM, Cotton MF, et al. Diagnostic and management challenges for childhood tuberculosis in the era of HIV. *J Infect Dis* 2007;**196**(Suppl. 1):576–85.
398. Marais BJ, Obihara CC, Warren RM, et al. The burden of childhood tuberculosis: a public health perspective. *Int J Tuberc Lung Dis* 2005;**9**:1305–13.
399. Marais BJ, Schaaf HS. Childhood tuberculosis: an emerging and previously neglected problem. *Infect Dis Clin North Am* 2010; **24**:727–49.
400. Marais BJ, Wright CA, Schaaf HS, et al. Tuberculous lymphadenitis as a cause of persistent cervical lymphadenopathy in children from a tuberculous endemic area. *Pediatr Infect Dis J* 2006;**25**: 142–6.
401. Maree F, Hesseling AC, Schaaf HS, et al. Absence of an association between *Mycobacterium tuberculosis* genotype and clinical features in children with tuberculous meningitis. *Pediatr Infect Dis J* 2007;**26**:13–18.
402. Marks SM, Taylor Z, Qualls NL, et al. Outcomes of contact investigations of infectious tuberculosis patients. *Am J Respir Crit Care Med* 2000;**162**:2033–8.
403. Martinez-Roig A, Cami J, Llorens-Terol J, et al. Acetylation phenotype and hepatotoxicity in the treatment of tuberculosis in children. *Pediatrics* 1986;**77**:912–15.
404. Martinson NA, Barnes GL, Moulton LH, et al. New regimens to prevent tuberculosis in adults with HIV infection. *N Engl J Med* 2011;**365**:11–20.
405. Mason E, Russell DW. Isoniazid acetylation rates (phenotypes) of patients being treated for tuberculosis. *Bull World Health Organ* 1971;**45**:617–24.
406. Matlow AG, Harrison A, Monteath A, et al. Nosocomial transmission of tuberculosis (TB) associated with a case of an infant with peritoneal TB. *Infect Control Hosp Epidemiol* 2000;**21**:222–3.
407. Matsaniotis N, Kattanis C, Economou-Mavrou C, et al. Bullous emphysema in childhood tuberculosis. *J Pediatr* 1967;**71**:703–8.
408. Mawachaka PM, Ranketi SS, Nchafatso OG, et al. Spinal tuberculosis among human immunodeficiency virus–negative patients in a Kenyan tertiary hospital: a 5-year synopsis. *Spine J* 2011;**11**: 265–9.
409. McCray MK, Esterly MB. Cutaneous eruptions in congenital tuberculosis. *Arch Dermatol* 1981;**117**:460–4.
410. McKenzie SA, McNab AJ, Katz G. Neonatal pyridoxine responsive convulsions due to isoniazid therapy. *Arch Dis Child* 1976; **51**:567–8.
411. Medical Research Council Investigation. Streptomycin treatment of pulmonary tuberculosis. *BMJ* 1948;**2**:769–82.
412. Medical Research Council Working Party on Tuberculosis of the Spine. Five-year assessment of controlled trials of inpatient and

outpatient treatment and of plaster-of-paris jackets for tuberculosis of the spine in children on standard chemotherapy: studies in Masan and Pusan, Korea. *J Bone Joint Surg Br* 1976;**58**:399–411.
413. Medical Research Council Working Party on Tuberculosis of the Spine. Five-year assessment of controlled trials of ambulatory treatment, débridement and anterior spinal fusion in the management of tuberculosis of the spine: studies in Bulawayo (Rhodesia) and in Hong Kong. *J Bone Joint Surg Br* 1978;**60**:163–77.
414. Medical Research Council Working Party on Tuberculosis of the Spine. Twelfth report: controlled trial of short-course regimens of chemotherapy in the ambulatory treatment of spinal tuberculosis. *J Bone Joint Surg Br* 1993;**75**:240–8.
415. Mehta JB, Bentley S. Prevention of tuberculosis in children: missed opportunities. *Am J Prev Med* 1992;**8**:283–6.
416. Meier A, Kirschner P, Bange F, et al. Genetic alterations in streptomycin-resistant *Mycobacterium tuberculosis*: mapping of mutations conferring resistance. *Antimicrob Agents Chemother* 1994;**38**:228–33.
417. Meintjes G, Lawn SD, Scano F, et al. Tuberculosis-associated immune reconstitution inflammatory syndrome: case definitions for use in resource-limited settings. *Lancet Infect Dis* 2008;**8**: 516–23.
418. Menzies R, Fanning A, Yuan L, et al. Tuberculosis among health-care workers. *N Engl J Med* 1995;**332**:92–8.
419. Menzies R, Rocher I, Vissandjee B. Factors associated with compliance in treatment of tuberculosis. *Tubercle Lung Dis* 1993;**74**: 32–7.
420. Menzies R, Vissandjee B. Effect of bacille Calmette-Guérin vaccination on tuberculin reactivity. *Am Rev Respir Dis* 1992;**141**: 621–5.
421. Metcalfe JZ, Everett CK, Steingart KR, et al. Interferon-gamma release assays for active pulmonary tuberculosis diagnosis in adults in low- and middle-income countries: systematic review and meta-analysis. *J Infect Dis* 2011;**204**(Suppl. 4):S1120–9.
422. Migliori GB, Borghesi A, Rossanigo P, et al. Proposal of an improved score method for the diagnosis of pulmonary tuberculosis in childhood in developing countries. *Tubercle Lung Dis* 1992;**73**:145–9.
423. Miller FJW, Seale RME, Taylor MD. *Tuberculosis in children*. Boston: Little, Brown; 1963, p. 214.
424. Miller RR, Porter J, Greenblatt DJ. Clinical importance of the interaction of phenytoin and isoniazid: a report from the Boston Collaborative Drug Surveillance Program. *Chest* 1979;**75**:356–8.
425. Miorner H, Sjobring U, Nayak P, et al. Diagnosis of tuberculous meningitis: a comparative analysis of 3 immunoassays, an immune complex assay and the polymerase chain reaction. *Tubercle Lung Dis* 1995;**76**:381–6.
426. Mishra OP, Kumar R, Ali Z, et al. Evaluation of polymerase chain reaction and adenosine deaminase assay for the diagnosis of tuberculous effusions in children. *Arch Dis Child* 2006;**91**:985–9.
427. Mitchison DA. Basic mechanisms of chemotherapy. *Chest* 1979; **76**(Suppl.):771–81.
428. Mitchison DA. The action of anti-tuberculous drugs in short-course chemotherapy. *Tubercle* 1985;**66**:219–25.
429. Mitchison DA, Nunn AJ. Influence of initial drug resistance on the response to short-course chemotherapy of pulmonary tuberculosis. *Am Rev Respir Dis* 1986;**133**:423–8.
430. Mohanty KC, Dhamgaye TM. Controlled trial of ciprofloxacin in short-term chemotherapy for pulmonary tuberculosis. *Chest* 1993; **104**:1194–8.
431. Mohle-Boetani JC, Flood J. Contact investigations and the continued commitment to control tuberculosis. *JAMA* 2002;**287**: 1040–1.
432. Mohle-Boetani JC, Miller B, Halpern M, et al. School-based screening for tuberculous infection: a cost benefit analysis. *JAMA* 1995;**274**:613–19.
433. Montenegro S, Gilman R, Sheen P, et al. Improved detection of *Mycobacterium tuberculosis* in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. *Clin Infect Dis* 2003;**3**:16–23.
434. Moore M, Schulte J, Valway S, et al. Evaluation of transmission of *Mycobacterium tuberculosis* in a pediatric setting. *J Pediatr* 1998;**133**:108–12.
435. Morens DM, Baublis JV, Heidelberger KP. Congenital tuberculosis and associated hypoadrenocorticism. *South Med J* 1979;**72**:160–1, 165.
436. Moore HA, Apolles P, de Villiers PJ, et al. Sputum induction for microbiological diagnosis of childhood pulmonary tuberculosis in a community setting. *Int J Tuberc Lung Dis* 2011;**15**: 1185–90.
437. Morrison JB. Natural history of segmental lesions in primary pulmonary tuberculosis. *Arch Dis Child* 1973;**48**:90–8.
438. Morse ML, Karr DJ, Menddman PM. Ocular tuberculosis in a five-month-old. *Pediatr Infect Dis J* 1988;**7**:514–16.
439. Moss WJ, Dedyo T, Suarez M, et al. Tuberculosis in children infected with human immunodeficiency virus: a report of five cases. *Pediatr Infect Dis J* 1992;**11**:114–20.
440. Mudido PM, Guwratudde D, Nakakeeto MK, et al. The effect of bacilli Calmette-Guérin vaccination at birth on tuberculin skin test reactivity in Ugandan children. *Int J Tuberc Lung Dis* 1999;**3**: 891–5.
441. Mukherjee JA, Joseph KS, Rich ML, et al. Clinical and programmatic considerations in treatment of MDR-TB in children: a series of 16 patients in Lima, Peru. *Int J Tuberc Lung Dis* 2003;**7**: 637–44.
442. Müller M, Wandel S, Colebunders R, et al. Immune reconstitution syndrome in patients starting antiretroviral therapy for HIV infection: a systematic review and meta-analysis. *Lancet Infect Dis* 2010;**10**:251–61.
443. Mumtaz MA, Schwartz RH, Grundfast KM, et al. Tuberculosis of the middle ear and mastoid. *Pediatr Infect Dis J* 1983;**2**:234–6.
444. Munckhof WJ, Konstantinos A, Wamsley M, et al. A cluster of tuberculosis associated with use of a marijuana water pipe. *Int J Tuberc Lung Dis* 2003;**7**:860–5.
445. Munoz FM, Ong LT, Seavy D, et al. Tuberculosis among adult visitors of children with suspected tuberculosis and employees at a children's hospital. *Infect Control Hosp Epidemiol* 2002;**23**: 568–72.
446. Munoz P, Rodriguez C, Bouza E. *Mycobacterium tuberculosis* infection in recipients of solid organ transplants. *Clin Infect Dis* 2005;**40**:581–7.
447. Munsiff SS, Li J, Cook SV, et al. Trends in drug-resistant *Mycobacterium tuberculosis* in New York City, 1991-2003. *Clin Infect Dis* 2006;**42**:1702–10.
448. Murray JF. Cursed duet: HIV infection and tuberculosis. *Respiration* 1990;**57**:210–20.
449. Nakajo MM, Rao M, Steiner P. Incidence of hepatotoxicity in children receiving isoniazid chemoprophylaxis. *Pediatr Infect Dis J* 1989;**8**:649–50.
450. Narendran G, Swaminathan S, Sathish S, et al. Immune reconstitution syndrome in a child with TB and HIV. *Indian J Pediatr* 2006;**73**:627–9.
451. Nelson LJ, Schneider E, Wells CD, et al. Epidemiology of childhood tuberculosis in the United States, 1993-2001: the need for continued vigilance. *Pediatrics* 2004;**114**:333–41.
452. Nemir RL, Cardona J, Lacoius A, et al. Prednisone therapy as an adjunct in the treatment of lymph node–bronchial tuberculosis in childhood: a double-blind study. *Am Rev Respir Dis* 1963;**88**: 189–98.
453. Nemir RL, Cardona J, Vaziri F, et al. Prednisone as an adjunct in the chemotherapy of lymph node–bronchial tuberculosis in childhood: a double-blind study, part II: further term observation. *Am Rev Respir Dis* 1967;**95**:402–10.
454. Nemir RL, O'Hare D. Congenital tuberculosis. *Am J Dis Child* 1985;**139**:284–7.
455. Nemir RL, O'Hare D. Tuberculosis in children 10 years of age and younger: three decades of experience during the chemotherapeutic era. *Pediatrics* 1991;**88**:236–41.
456. Nemir RL, Teichner A. Management of tuberculin reactors in children and adolescents previously vaccinated with BCG. *Pediatr Infect Dis J* 1983;**2**:446–51.
457. Netto EM, Dye C, Raviglione MC. Progress in global tuberculosis control 1995-1996, with emphasis on 22 high-incidence countries. Global Monitoring and Surveillance Project. *Int J Tuberc Lung Dis* 1999;**3**:310–20.
458. Nicol MP, Zar HJ. New specimens and laboratory diagnostics for childhood pulmonary TB: progress and prospects. *Paediatr Respir Rev* 2011;**12**:16–21.
459. Nicol MP, Workman L, Isaacs W, et al. Accuracy of the Xpert MTB/RIF test for the diagnosis of pulmonary tuberculosis in children admitted to hospital in Cape Town, South Africa: a descriptive study. *Lancet Infect Dis* 2011;**11**:819–24.

460. Nivin B, Nicholas P, Gayer M, et al. A continuing outbreak of multi-drug-resistant tuberculosis, with transmission in a hospital nursery. *Clin Infect Dis* 1998;**26**:303–7.
461. Nolan CM, Elarth AM, Barr HW. Intentional isoniazid overdose in young Southeast Asian refugee women. *Chest* 1988;**93**:803–6.
462. Nolan R Jr. Childhood tuberculosis in North Carolina: A study of the opportunities for intervention in the transmission of tuberculosis in children. *Am J Public Health* 1986;**76**:26–30.
463. Noordhoek G, Kolk A, Bjune G, et al. Sensitivity and specificity of PCR for detection of *Mycobacterium tuberculosis*: a blind comparison study among seven laboratories. *J Clin Microbiol* 1994;**32**: 277–84.
464. Nunn P, Felten M. Surveillance of resistance to antituberculosis drugs in developing countries. *Tubercle Lung Dis* 1994;**75**:163–7.
465. Obaegbulam SC. Spinal extraosseous extradural tuberculoma. *Tubercle* 1977;**58**:97.
466. Oberhelman RA, Soto-Castellares G, Gilman RH, et al. Diagnostic approaches for paediatric tuberculosis by use of different specimen types, culture methods, and PCR: a prospective case-control study. *Lancet* 2010;**10**:612–20.
467. O'Brien K, Ruff A, Louis M, et al. Bacillus Calmette-Guérin complications in children born to HIV-1–infected women with a review of the literature. *Pediatrics* 1995;**95**:414–18.
468. O'Brien RJ, Long MW, Cross FS, et al. Hepatotoxicity from isoniazid and rifampin among children treated for tuberculosis. *Pediatrics* 1983;**72**:491–9.
469. Offenbacher H, Fazekas F, Schmidt R, et al. MRI in tuberculous meningoencephalitis: report of four cases and review of the neuroimaging literature. *J Neurol* 1991;**238**:340–4.
470. Olazabal F. Choroidal tubercles: a neglected sign. *JAMA* 1967; **200**:104–7.
471. Olson WA, Pruitt AW, Dayton PG. Plasma concentration of isoniazid in children with tuberculous infections. *Pediatrics* 1981;**67**: 876–8.
472. Omari B, Robertson JM, Nelson RJ, et al. Pott's disease: a resurgent challenge to the thoracic surgeon. *Chest* 1989;**95**:145–50. (See also follow-up letters, Chest 96:955-956, 1989.)
473. Optican RJ, Ost A, Ravin CE. High-resolution computed tomography in the diagnosis of miliary tuberculosis. *Chest* 1992;**102**: 941–3.
474. Orenstein EW, Basu S, Shah NS, et al. Treatment outcomes among patients with multidrug-resistant tuberculosis; systematic review and meta-analysis. *Lancet Infect Dis* 2009;**9**:153–61.
475. Orikiiriza J, Bakeera-Kitaka S, Musiime V, et al. The clinical pattern, prevalence, and factors associated with immune reconstitution inflammatory syndrome in Ugandan children. *AIDS* 2009; **24**:2009–17.
476. Orme IM, Andersen P, Boom WH. T cell response to *Mycobacterium tuberculosis*. *J Infect Dis* 1993;**167**:1481–97.
477. Ozuah PO, Burton W, Lerro KA, et al. Assessing the validity of tuberculin skin test readings by trained professionals and patients. *Chest* 1999;**116**:104–6.
478. Ozuah PO, Ozuah TP, Stein REK, et al. Evaluation of a risk assessment questionnaire used to target tuberculin skin testing in children. *JAMA* 2001;**285**:451–3.
479. Padoyatchi N, Bambar S, Dawood H, et al. Multidrug-resistant tuberculosis meningitis in children in Durban, South Africa. *Pediatr Infect Dis J* 2006;**25**:147–50.
480. Pai M, Kalantri S, Dheda K. New tools and emerging technologies for the diagnosis of tuberculosis, part I: latent tuberculosis. *Expert Rev Mol Diagn* 2006;**6**:413–22.
481. Palme IB, Gudetta B, Bruchfield J, et al. Impact of human immunodeficiency virus 1 infection on clinical presentation, treatment outcome and survival in a cohort of Ethiopian children with tuberculosis. *Pediatr Infect Dis J* 2002;**21**:1053–61.
482. Palur R, Rajohekhar V, Chandy MJ, et al. Shunt surgery for hydrocephalus in tuberculous meningitis: a long-term follow-up study. *J Neurosurg* 1991;**74**:64–9.
483. Paluschi VJ, O'Hare D, Lawrence RM. Hepatotoxicity and transaminase measurement during isoniazid chemoprophylaxis in children. *Pediatr Infect Dis J* 1995;**14**:144–8.
484. Papaevangelou V, Vintila A, Papaparaskevas J, et al. Infant penile tuberculosis following circumcision. *J Infect* 2009;**58**:83–5.
485. Parish RE, Brownstein D. Emergency department management of children with acute isoniazid poisoning. *Pediatr Emerg Care* 1986;**2**:88–90.
486. Park MM, Davis AL, Schluger NW, et al. Outcome of MDR-TB patients, 1983-1993: prolonged survival with appropriate therapy. *Am J Respir Crit Care Med* 1996;**153**:317–24.
487. Parsons SD, Warren RM, Ottenhoff TH, et al. Detection of *Mycobacterium tuberculosis* infection in dogs in a high-risk setting. *Res Vet Sci* 2012;**92**:414–19.
488. Pastrana DG, Torronteras R, Caro P, et al. Comparison of Amplicor, in-house polymerase chain reactions and conventional culture for the diagnosis of tuberculosis in children. *Clin Infect Dis* 2001;**32**:17–22.
489. Patton GC, Coffey C, Sawyer SM, et al. Global patterns of mortality in young people: a systematic analysis of population health data. *Lancet* 2009;**374**:881–92.
490. Pavic I, Topic RZ, Raos M, et al. Interferon-gamma release assay for the diagnosis of latent tuberculosis infection in children younger than 5 years of age. *Pediatr Infect Dis J* 2011;**30**:866–70.
491. Payne M, quoted in Miller FJW, Seale RME, Taylor MD. *Tuberculosis in children*. Boston: Little, Brown; 1963.
492. Pederson FK, Schiotz PO, Valerius NH, et al. Fatal BCG infection in an immunocompetent girl. *Acta Paediatr Scand* 1978;**67**: 19–23.
493. Pediatric Tuberculosis Collaborative Group. Targeted tuberculin skin testing and treatment of latent tuberculosis infection in children and adolescents. *Pediatrics* 2004;**114**:1175–207.
494. Pedrozo C, Sant'Anna C, de Fatima March M, et al. Clinical scoring system for paediatric tuberculosis in HIV-infected and non-infected children in Rio de Janeiro. *Int J Tuberc Lung Dis* 2009;**13**:413–15.
495. Pellock JM, Howell J, Kendig EL Jr, et al. Pyridoxine deficiency in children treated with isoniazid. *Chest* 1985;**87**:658–61.
496. Peloquin CA, MacPhee AA, Berning SE. Malabsorption of antimycobacterial medications. *N Engl J Med* 1993;**329**:1122–3.
497. Peltola H, Salmi I, Vahvanen V, et al. BCG vaccination as a cause of osteomyelitis and subcutaneous abscess. *Arch Dis Child* 1984; **59**:157–61.
498. Pereira CA, Webber B, Orson JM. Primary tuberculous complex of the skin. *JAMA* 1976;**235**:942.
499. Pereira SM, Barreto ML, Pilger D, et al. Effectiveness and cost-effectiveness of first BCG vaccination against tuberculosis in school-age children without previous tuberculin test (BCG-REVAC Trial): a cluster-randomised trial. *Lancet Infect Dis* 2012; **12**:300–6.
500. Perry S, Starke JR. Adherence to prescribed treatment and public health aspects of tuberculosis in children. *Semin Pediatr Infect Dis* 1993;**4**:291–8.
501. Pesanti E. The negative tuberculin skin test: tuberculin, HIV and anergy panels. *Am J Respir Crit Care Med* 1994;**149**:1699–709.
502. Pineda P, Leung A, Muller N, et al. Intrathoracic pediatric tuberculosis: a report of 202 cases. *Tubercle Lung Dis* 1993;**74**: 261–6.
503. Pospelov LE, Matrakshin AG, Chernousova LN, et al. Association of various genetic markers with tuberculosis and other lung disease in Tuvinian children. *Tubercle Lung Dis* 1996;**77**:77–80.
504. Powell DA, Perkins L, Wang S-H, et al. Completion of therapy for latent tuberculosis in children of different nationalities. *Pediatr Infect Dis J* 2008;**28**:272–4.
505 Prasad K, Singh MB. Corticosteroids for managing tuberculous meningitis. *Cochrane Database Syst Rev* 2008;23:CD002244.
506. Puthanaki T, Oberdorfen P, Akarathum N, et al. Immune reconstitution syndrome after highly active antiretroviral therapy in human immunodeficiency virus–infected Thai children. *Pediatr Infect Dis J* 2006;**25**:53–8.
507. Puthanakit T, Oberdorfen P, Punjaisee S, et al. Immune reconstitution syndrome due to bacillus Calmette-Guérin after initiation of antiretroviral therapy in children with HIV infection. *Clin Infect Dis* 2005;**41**:1049–52.
508. Rachow A, Clowes P, Saathoff E, et al. Increased and expedited case detection by Xpert MTB/RIF assay in childhood tuberculosis: a prospective cohort study. *Clin Infect Dis* 2012;**54**:1388–96.
509. Ramachondran S, Clifton IJ, Collyns TA, et al. The treatment of spinal tuberculosis: a retrospective study. *Int J Tuberc Lung Dis* 2005;**9**:541–4.
510. Rapp RS, Campbell RW, Howell JC, et al. Isoniazid hepatotoxicity in children. *Am Rev Respir Dis* 1978;**118**:794–6.
511. Rasool M. Osseous manifestations of tuberculosis in children. *J Pediatr Orthop* 2001;**21**:749–55.

512. Rasool M, Govender S, Naidoo K. Cystic tuberculosis of bone in children. *J Bone Joint Surg Br* 1994;**76**:113–17.
513. Raszka WV, Moriarty RA, Ottolini MG, et al. Delayed-type hypersensitivity skin testing in human immunodeficiency virus-infected pediatric patients. *J Pediatr* 1996;**129**:245–50.
514. Ravenscroft A, Schoeman JF, Donald PR. Tuberculous granulomas in childhood tuberculous meningitis: radiologic features and course. *J Trop Pediatr* 2001;**47**:5–12.
515. Raviglione MC, Rieder HL, Styblo K, et al. Tuberculosis trends in Eastern Europe and the former USSR. *Tubercle Lung Dis* 1994;**75**:400–16.
516. Reichler MR, Reves R, Bur S, et al. Evaluation of investigations conducted to detect and prevent transmission of tuberculosis. *JAMA* 2002;**287**:991–5.
517. Reichman LB. Why hasn't BCG proved dangerous in HIV-infected patients? *JAMA* 1989;**261**:3246.
518. Reis FJ, Bedran MB, Mowra JA, et al. Six-month isoniazid-rifampin treatment for pulmonary tuberculosis in children. *Am Rev Respir Dis* 1990;**142**:996–9.
519. Report of a WHO Study Group. BCG vaccination policies. WHO Technical Report Series No. 652, 1980-1981.
520. Reynes J, Perez C, Lamaury I, et al. Bacille Calmette-Guérin adenitis 30 years after immunization in a patient with AIDS. *J Infect Dis* 1989;**160**:727.
521. Rich AR. *The pathogenesis of tuberculosis*. 2nd ed. Springfield, Ill.: Charles C Thomas; 1951.
522. Rich AR, McCordock HA. The pathogenesis of tuberculous meningitis. *Bull Johns Hopkins Hosp* 1933;**52**:5–35.
523. Ridzon R, Kent JH, Valway S, et al. Outbreak of drug-resistant tuberculosis with secondary-generation transmission in a high school in California. *J Pediatr* 1997;**131**:863–8.
524. Riley RL. Airborne transmission. In: Johnson JE, editor. *Rational therapy and control of tuberculosis*. Gainesville: University of Florida Press; 1970.
525. Ritz N, Yau C, Connell TG, et al. Absence of interferon-gamma release assay conversion following tuberculin skin testing. *Int J Tuberc Lung Dis* 2011;**15**:767–9.
526. Rodriguez LC, Pereira SM, Cunha SS, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. *Lancet* 2005; **366**:1290–5.
527. Rose PC, Schaaf HS, Marais BJ, et al. Value of bone marrow biopsy in children with suspected disseminated mycobacterial disease. *Int J Tuberc Lung Dis* 2011;**15**:200–4.
528. Roy V, Tekur U, Chopra K. Pharmacokinetics of pyrazinamide in children suffering from pulmonary tuberculosis. *Int J Tuberc Lung Dis* 1999;**3**:133–7.
529. Ryder RW, Oxtoby MJ, Mvula M, et al. Safety and immunogenicity of bacille Calmette-Guérin, diphtheria-tetanus-pertussis, and oral polio vaccines in newborn children in Zaire infected with human immunodeficiency virus type 1. *J Pediatr* 1993;**122**: 697–702.
530. Saczek KB, Schaaf HS, Vors M, et al. Diagnostic dilemmas in abdominal tuberculosis in children. *Pediatr Surg Int* 2001;**17**: 111–15.
531. Saiman L, Aronson J, Zhou J, et al. Prevalence of infectious diseases among internationally adopted children. *Pediatrics* 2001; **108**:608–12.
532. Saiman L, San Gabriel P, Schultz J, et al. Risk factors for latent tuberculosis infection among children in New York City. *Pediatrics* 2001;**107**:999–1003.
533. Saitah A, Pong A, Waecker NJ Jr, et al. Prediction of neurologic sequelae in childhood tuberculous meningitis: a review of 20 cases and proposal of a novel scoring system. *Pediatr Infect Dis J* 2005;**24**: 207–12.
534. Salazar GE, Schmitz TL, Cama R, et al. Pulmonary tuberculosis in a developing country. *Pediatrics* 2001;**108**:448–53.
535. Salfinger M, Crowle AJ, Reller LB. Pyrazinamide and pyrazinoic acid activity against tubercle bacilli in cultured human macrophages and in the BACTEC system. *J Infect Dis* 1990;**162**: 201–7.
536. Salfinger M, Pfyfler GE. The new diagnostic mycobacteriology laboratory. *Eur J Clin Microbiol* 1994;**13**:961–79.
537. Salomon H, Angel JH. Corticotropin-induced changes in the tuberculin skin test: controlled study in advanced tuberculosis. *Am Rev Respir Dis* 1961;**83**:235–42.
538. Saltzman SJ, Feigin RD. Tuberculous otitis media and mastoiditis. *J Pediatr* 1971;**79**:1004–6.
539. Sanchez-Albisua I, Vidal ML, Joya-Verde G, et al. Tolerance of pyrazinamide in short course chemotherapy for pulmonary tuberculosis in children. *Pediatr Infect Dis J* 1997;**16**:760–3.
540. Santiago EM, Lawson E, Gillenwater K, et al. A prospective study of bacillus Calmette-Guérin scar formation and tuberculin skin test reactivity in infants in Lima, Peru. *Pediatrics* 2003;**112**: e298.
541. Sassan-Morokro M, DeCock KM, Ackah A, et al. Tuberculosis and HIV infection in children in Abidjon, Côté d'Ivoire. *Trans R Soc Trop Med Hyg* 1994;**88**:178–81.
542. Sathe SS, Reichman LB. Mycobacterial disease in patients infected with human immunodeficiency virus. *Clin Chest Med* 1989;**10**: 445–63.
543. Sauzullo I, Massetti AP, Mengoni F, et al. Influence of previous tuberculin skin test on serial IFN-gamma release assays. *Tuberculosis (Edinb)* 2011;**91**:322–6.
544. Schaaf HS, Beyers N, Gie RP, et al. Respiratory tuberculosis in childhood: the diagnostic value of clinical features and special investigations. *Pediatr Infect Dis J* 1995;**14**:189–94.
545. Schaaf HS, Gie RP, Beyers N, et al. Tuberculosis in infants less than 3 months of age. *Arch Dis Child* 1993;**69**:371–4.
546. Schaaf HS, Gie RP, Beyers N, et al. Primary drug-resistant tuberculosis in children. *Int J Tuberc Lung Dis* 2000;**4**:1–7.
547. Schaaf HS, Gie RP, Beyers N, et al. Primary drug-resistant tuberculosis. *Pediatr Infect Dis J* 2000;**19**:695–9.
548. Schaaf HS, Gie RP, Kennedy M, et al. Evaluation of young children in contact with adult multi-drug resistant pulmonary tuberculosis: a 30-month follow-up. *Pediatrics* 2002;**109**:765–71.
549. Schaaf HS, Krook S, Hollermans DW, et al. Recurrent culture-confirmed tuberculosis in human immunodeficiency virus–infected children. *Pediatr Infect Dis J* 2005;**24**:685–91.
550. Schaaf HS, Victor TC, Venter A, et al. Ethionamide cross- and co-resistance in children with isoniazid-resistant tuberculosis. *Int J Tuberc Lung Dis* 2009;**13**:1355–9.
551. Schaefer G. *Tuberculosis in obstetrics and gynecology*. Boston: Little, Brown; 1956.
552. Schick B, Dolgin J. The influence of prednisone on the Mantoux reaction in children. *Pediatrics* 1963;**31**:856–9.
553. Schluger N, Kinney D, Harkin T, et al. Clinical utility of the polymerase chain reaction in the diagnosis of infections due to *Mycobacterium tuberculosis*. *Chest* 1994;**105**:1116–21.
554. Schoeman J, Wait J, Burger M, et al. Long-term followup of childhood tuberculous meningitis. *Dev Med Child Neurol* 2002; **44**:522–6.
555. Scholten JW, Fujiwara PI, Frieden TR. Prevalence and factors associated with tuberculosis infection among new school entrants, New York City, 1991-1993. *Int J Tuberc Lung Dis* 1999;**3**:31–41.
556. Schuit KE. Miliary tuberculosis in children. *Am J Dis Child* 1979;**133**:583–5.
557. Schuit KE, Powell DA. Mycobacterial lymphadenitis in childhood. *Am J Dis Child* 1978;**132**:675–7.
558. Schwartz P. Lymph node tuberculosis: a decisive factor in pulmonary pathology. *Arch Pediatr* 1957;**74**:159–77, 201–18.
559. Schwartzman K, Menzies D. Tuberculosis screening of immigrants to low-prevalence countries. *Am J Respir Crit Care Med* 2000;**161**:780–9.
560. Scriba TJ, Tameris M, Mansoor N, et al. Dose-finding study of the novel tuberculosis vaccine, MVA85A, in healthy BCG-vaccinated infants. *J Infect Dis* 2011;**203**:1832–43.
561. Seddon JA, Hesseling AC, Marais BJ, et al. Paediatric use of second-line anti-tuberculosis agents: a review. *Tuberculosis (Edinb)* 2012;**92**:9–17.
562. Seddon JA, Hesseling AC, Willemse M, et al. Culture-confirmed multidrug-resistant tuberculosis in children: clinical features, treatment, and outcome. *Clin Infect Dis* 2012;**54**:157–66.
563. Sehgal VN, Wagh SA. Cutaneous tuberculosis. *Int J Dermatol* 1990;**29**:237–52.
564. Selwyn P, Hartel D, Lewis V, et al. A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection. *N Engl J Med* 1989;**320**:545–50.
565. Sepulveda RL, Burr C, Ferrer X, et al. Booster effect of tuberculosis testing in healthy 6-year-old school children vaccinated with bacille Calmette-Guérin at birth in Santiago, Chile. *Pediatr Infect Dis J* 1988;**7**:578–82.

566. Sepulveda RL, Heiba IM, King A, et al. Evaluation of tuberculin reactivity in BCG-immunized siblings. *Am J Respir Crit Care Med* 1994;**149**:620–4.
567. Sepulveda RL, Heiba IM, Navarrete C, et al. Tuberculin reactivity after newborn BCG immunization in mono- and dizygotic twins. *Tubercle Lung Dis* 1994;**75**:138–43.
568. Sester M, Sotgiu G, Lange C, et al. Interferon gamma release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis. *Eur Respir J* 2011;**37**:100–11.
569. Shafer R, Small P, Larkin C, et al. Temporal trends and transmission patterns during the emergence of multidrug-resistant tuberculosis in New York City: a molecular epidemiologic assessment. *J Infect Dis* 1995;**171**:170–6.
570. Shah BR, Santucci K, Sinert R, et al. Acute isoniazid neurotoxicity in an urban hospital. *Pediatrics* 1995;**95**:700–4.
571. Shah NS, Harrington T, Huber M, et al. Increased reported cases of tuberculosis among children younger than 5 years of age, Maricopa County, Arizona, 2002-2003. *Pediatr Infect Dis J* 2006;**25**: 151–5.
572. Shah NS, Wright A, Bai GH, et al. Worldwide emergency of extensively drug-resistant tuberculosis. *Emerg Infect Dis* 2007;**13**: 380–7.
573. Shannon FB, Moore M, Houkom JA, et al. Multifocal cystic tuberculosis of bone. *J Bone Joint Surg Am* 1990;**72**:1089–92.
574. Sharma SK, Mohan A, Sharma A, et al. Miliary tuberculosis: new insights into an old disease. *Lancet Infect Dis* 2005;**5**:415–530.
575. Shepard WE, Field ML, James DH, et al. Transient appearance of intracranial tuberculomas during treatment of tuberculous meningitis. *Pediatr Infect Dis J* 1986;**5**:599–601.
576. Sheridan PH, Edman JB, Starr SE. Tuberculosis presenting as an orbital mass. *Pediatrics* 1981;**67**:874–5.
577. Shimokata K, Kawachi H, Kishumoto H, et al. Local cellular immunity in tuberculous pleurisy. *Am Rev Respir Dis* 1982;**126**: 822–4.
578. Shin S, Guerra D, Rich M, et al. Treatment of multidrug-resistant tuberculosis during pregnancy: A report of 7 cases. *Clin Infect Dis* 2003;**15**:996–1003.
579. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. *Lancet Infect Dis* 2003;**3**:624–32.
580. Siberry GK, Tessema S. Immune reconstitution syndrome precipitated by bacille Calmette-Guérin after initiation of antiretroviral therapy. *Pediatr Infect Dis J* 2006;**25**:648–9.
581. Siddiqi SH, Hwangbo CC, Silcox V, et al. Rapid radiometric methods to detect and differentiate *Mycobacterium tuberculosis/M. bovis* from other mycobacterial species. *Am Rev Respir Dis* 1984; **130**:634–40.
582. Singapore Tuberculosis Service/British Medical Research Council. Five-year follow-up of a clinical trial of three 6-month regimens of chemotherapy given intermittently in the continuation phase in the treatment of pulmonary tuberculosis. *Am Rev Respir Dis* 1988;**137**:1147–50.
583. Sirinavin S, Chotpitayasunondh T, Suwanjutha S, et al. Efficacy of neonatal bacillus Calmette-Guérin vaccination against tuberculosis. *Pediatr Infect Dis J* 1991;**10**:359–65.
584. Sloan JB. Papulonecrotic tuberculid in a 9-year-old American girl: case report and review of the literature. *Pediatr Dermatol* 1990;**7**: 191–5.
585. Slutkin G, Schecter GF, Hopewell PC. The results of 9-month isoniazid-rifampin therapy for pulmonary tuberculosis under program conditions in San Francisco. *Am Rev Respir Dis* 1988; **138**:1622–4.
586. Small P, Hopewell P, Singh S, et al. The epidemiology of tuberculosis in San Francisco: a population-based study using conventional and molecular methods. *N Engl J Med* 1994;**330**: 1703–9.
587. Small PM, Shafer RW, Hopewell PC, et al. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in patients with advanced HIV infection. *N Engl J Med* 1993;**328**:1137–44.
588. Smith AM, Lattimer JK. Genitourinary tract involvement in children with tuberculosis. *NY State J Med* 1973;**73**:2325–8.
589. Smith D, Reeser P, Musa S. Does infection with environmental mycobacteria suppress the protective response to subsequent vaccination with BCG? *Tubercle* 1985;**66**:17–23.
590. Smith KC, Starke JR, Eisenach K, et al. Detection of *Mycobacterium tuberculosis* in clinical specimens from children using a polymerase chain reaction. *Pediatrics* 1996;**97**:155–60.
591. Smith MHD. The role of adrenal steroids in the treatment of tuberculosis. *Pediatrics* 1958;**22**:774–6.
592. Smith MHD. What about short course and intermittent chemotherapy for tuberculosis in children? *Pediatr Infect Dis J* 1982;**1**: 298–303.
593. Smith MHD. Tuberculosis in children and adolescents. *Clin Chest Med* 1989;**10**:381–95.
594. Snider DE Jr, Block AB. Congenital tuberculosis. *Tubercle* 1984;**65**:81–2.
595. Snider DE Jr, Caras GJ. Isoniazid-associated hepatitis deaths: a review of available information. *Am Rev Respir Dis* 1992;**145**: 494–7.
596. Snider DE Jr, Caras GJ, Kaplan JP. Preventive therapy with isoniazid: cost-effectiveness of different durations of therapy. *JAMA* 1986;**255**:1579–83.
597. Snider DE Jr, Farer LS. Preventive therapy for tuberculous infection: an intervention in need of improvement. *Am Rev Respir Dis* 1984;**130**:355–6.
598. Snider DE, Graczyk J, Bek E, et al. Supervised six-month treatment of newly diagnosed pulmonary tuberculosis using isoniazid, rifampin, and pyrazinamide with and without streptomycin. *Am Rev Respir Dis* 1984;**130**:1091–4.
599. Snider DE Jr, Layde PM, Johnson MW, et al. Treatment of tuberculosis during pregnancy. *Am Rev Respir Dis* 1980;**122**:65–79.
600. Snider DE Jr, Powell KE. Should women taking antituberculosis drugs breastfeed? *Arch Intern Med* 1984;**144**:589–90.
601. Solovic I, Sester M, Gomez-Reino JJ, et al. The risk of tuberculosis related to tumour necrosis factor antagonist therapies: a TBNET consensus statement. *Eur Respir J* 2010;**36**:1185–206.
602. Somu N, Swaminathan S, Paramisivan CN, et al. Value of bronchoalveolar lavage and gastric lavage in the diagnosis of pulmonary tuberculosis in children. *Tuberc Lung Dis* 1995;**76**:295–9.
603. Soren K, Saiman L, Irigoyen M, et al. Evaluation of household contacts of children with positive tuberculin skin tests. *Pediatr Infect Dis J* 1999;**18**:949–55.
604. Spyridis NP, Spyridis PG, Gelesme A, et al. The effectiveness of a 9-month regimen of isoniazid alone versus 3- and 4- month regimens of isoniazid plus rifampin for treatment of latent tuberculosis infection in children: results of an 11 year randomized study. *Clin Infect Dis* 2007;**45**:715–22.
605. Spyridis P, Maltezou HC, Hantzakos A, et al. Mycobacterial cervical adenitis in children: clinical and laboratory factors of importance for differential diagnosis. *Scand J Infect Dis* 2001;**33**:362–6.
606. Spyridis P, Sinaniotis C, Papadea I, et al. Isoniazid liver injury during chemoprophylaxis in children. *Arch Dis Child* 1979;**54**: 65–7.
607. Stallworth JR, Brasfield DM, Tiller RE. Congenital miliary tuberculosis proved by open lung biopsy specimen and successfully treated. *Am J Dis Child* 1980;**134**:320–1.
608. Stansberr SD. Tuberculosis in infants and children. *J Thorac Imaging* 1990;**5**:17–27.
609. Starke JR. Interferon-gamma release assays for diagnosis of tuberculosis infection in children. *Pediatr Infect Dis J* 2006;**25**:941–2.
610. Starke JR. New concepts in childhood tuberculosis. *Curr Opin Pediatr* 2007;**19**:306–13.
611. Starke JR. Multidrug therapy for tuberculosis in children. *Pediatr Infect Dis J* 1990;**9**:785–93.
612. Starke JR. Current chemotherapy for tuberculosis in children. *Infect Dis Clin North Am* 1992;**6**:215–38.
613. Starke JR. Tuberculosis of the central nervous system in children. *Semin Pediatr Neurol* 1999;**6**:318–31.
614. Starke JR. Transmission of *Mycobacterium tuberculosis* to and from children and adolescents. *Semin Pediatr Infect Dis* 2001;**12**: 115–23.
615. Starke JR. Childhood tuberculosis: ending the neglect. *Int J Tuberc Lung Dis* 2002;**6**:373–4.
616. Starke JR, Correa AG. Management of mycobacterial infection and disease in children. *Pediatr Infect Dis J* 1995;**14**:455–70.
617. Starke JR, Jacobs R, Jereb J. Resurgence of tuberculosis in children. *J Pediatr* 1992;**120**:839–55.
618. Starke JR, Taylor-Watts KT. Tuberculosis in the pediatric population of Houston, Texas. *Pediatrics* 1989;**84**:28–35.
619. Stead WW. Pathogenesis of a first episode of chronic pulmonary tuberculosis in man: recrudescence of residuals of the primary infection or exogenous reinfection? *Am Rev Respir Dis* 1967;**95**: 729–45.

620. Stefan DC, Dippenaar A, Detjen AK, et al. Interferon-gamma release assays for the detection of *Mycobacterium tuberculosis* infection in children with cancer. *Int J Tuberc Lung Dis* 2010;**14**: 689–94.
621. Stein MT, Liang D. Clinical hepatotoxicity of isoniazid in children. *Pediatrics* 1979;**64**:499–505.
622. Steiner P, Rao M. Drug-resistant tuberculosis in children. *Semin Pediat Infect Dis* 1993;**4**:275–82.
623. Steiner P, Rao M, Mitchell M. Primary drug-resistant tuberculosis in children: correlation of drug-susceptibility patterns of matched patient and source-case strains of *Mycobacterium tuberculosis*. *Am J Dis Child* 1985;**139**:780–2.
624. Steiner P, Rao M, Victoria MS, et al. Miliary tuberculosis in two infants after nursery exposure: epidemiologic, clinical, and laboratory findings. *Am Rev Respir Dis* 1976;**113**:267–71.
625. Steiner P, Rao M, Victoria MS, et al. Persistently negative tuberculin reactions: their presence among children culture positive for *Mycobacterium tuberculosis*. *Am J Dis Child* 1980;**134**:747–50.
626. Sterling TR, Villarino MA, Borisov AS, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. *N Engl J Med* 2011;**365**:2155–66.
627. Stevens DL, Everett ED. Sequential computerized axial tomography in tuberculous meningitis. *JAMA* 1978;**239**:642.
628. Stop TB Partnership Childhood TB Subgroup. Chapter 1: Introduction and diagnosis of tuberculosis in children. *Int J Tuberc Lung Dis* 2006;**10**:1091–7.
629. Stop TB Partnership Childhood TB Subgroup. Chapter 2: Antituberculosis treatment in children. *Int J Tuberc Lung Dis* 2006; **10**:1205–11.
630. Stop TB Partnership Childhood TB Subgroup. Chapter 3: Management of TB in the HIV-infected child. *Int J Tuberc Lung Dis* 2006;**10**:1331–6.
631. Stop TB Partnership Childhood TB Subgroup. Chapter 4: Childhood contact screening and management. *Int J Tuberc Lung Dis* 2007;**11**:12–15.
632. Stout JE, Saharia KK, Nageswaron S, et al. Racial and ethnic disparities in pediatric tuberculosis in North Carolina. *Arch Pediatr Adolesc Med* 2006;**160**:631–7.
633. Strang JI, Nunn AJ, Johnson DA, et al. Management of tuberculous constrictive pericarditis and tuberculous pericardial effusion in Transkei: results at 10 years follow-up. *QJM* 2004;**97**:525–35.
634. Sullama PM, Slutkin G, Hopewell PC. The benefits of evaluating close associates of child tuberculin reactors from a high prevalence group. *Am J Public Health* 1986;**76**:1109–11.
635. Sumartojo E. When tuberculosis treatment fails: a social behavior account of patient adherence. *Am Rev Respir Dis* 1993;**147**: 1311–20.
636. Sumaya CV, Simek M, Smith MHD. Tuberculous meningitis in children during the isoniazid era. *J Pediatr* 1975;**87**:43–9.
637. Swaminathan S, Gong J, Zhang M, et al. Cytokine production in children with tuberculous infection and disease. *Clin Infect Dis* 1999;**28**:1290–3.
638. Swaminathan S, Raghavan A, Duraipandian M, et al. Short-course chemotherapy for paediatric respiratory tuberculosis: 5-year report. *Int J Tuberc Lung Dis* 2005;**9**:693–6.
639. Sweany HC. Studies on the pathogenesis of primary tuberculous infection. *Am Rev Tuberc* 1933;**27**:559–88.
640. Tabrisky J, Lindstrom RR, Peters R, et al. Tuberculous enteritis. *Am J Gastroenterol* 1975;**63**:49–57.
641. Takiff H, Salazar L, Guerrero C, et al. Cloning and nucleotide sequence of *Mycobacterium tuberculosis* gyrA and gyrB genes and detection of quinoline resistance mutations. *Antimicrob Agents Chemother* 1994;**38**:773–80.
642. Talbot EA, Moore M, McCray E, et al. Tuberculosis among foreign-born persons in the United States, 1993-1998. *JAMA* 2000;**284**:2894–900.
643. Talwar BS, Talwar R, Chowdhary B, et al. Abdominal tuberculosis in children: an Indian experience. *J Trop Pediatr* 2000;**46**: 368–70.
644. Teeratkulpisarn J, Lumbigagnon P, Pairojkul S, et al. Cavitary tuberculosis in a young infant. *Pediatr Infect Dis J* 1994;**13**: 545–6.
645. Teixeira L, Perkins MD, Johnson JL, et al. Infection and disease among household contacts of patients with multi-drug resistant tuberculosis. *Int J Tuberc Lung Dis* 2001;**5**:321–8.
646. Tekin D, Kayaalti Z, Dalgic N, et al. Polymorphism in the *P2X7* gene increases susceptibility to extrapulmonary tuberculosis in Turkish children. *Pediatr Infect Dis J* 2010;**29**:779–81.
647. Telenti A, Imboden P, Marchesi F, et al. Detection of rifampin-resistance mutations in *Mycobacterium tuberculosis*. *Lancet* 1993; **341**:647–50.
648. Telzak EE, Sepkowitz K, Alpert P, et al. Multidrug-resistant tuberculosis in patients without HIV infection. *N Engl J Med* 1995;**333**:907–11.
649. tenDam HG. Research on BCG vaccination. *Adv Tuberc Res* 1984; **21**:79–106.
650. Teoh R, Humphries MJ, O'Mahony G. Symptomatic intracranial tuberculoma developing during treatment of tuberculosis: report of 10 patients and review of the literature. *Q J Med* 1987;**63**: 449–60.
651. Te Water Naude JM, Donald PR, Hussey GD, et al. Twice weekly vs. daily chemotherapy for childhood tuberculosis. *Pediatr Infect Dis J* 2000;**19**:405–10.
652. Thampi N, Stephens D, Rea E, et al. Unexplained deterioration during antituberculous therapy in children and adolescents: clinical presentation and risk factors. *Pediatr Infect Dis J* 2012;**31**:129–33.
653. Thiam S, LeFeure A, Hane F, et al. Effectiveness of a strategy to improve adherence to tuberculosis treatment in a resource-poor setting. *JAMA* 2007;**297**:380–6.
654. Thomas P, Bornschlegel K, Singh TP, et al. Tuberculosis in human immunodeficiency virus–infected and human immunodeficiency virus–exposed children in New York City. *Pediatr Infect Dis J* 2000; **19**:700–6.
655. Thomas TA, Mondal D, Noor Z, et al. Malnutrition and helminth infection affect performance of an interferon gamma release assay. *Pediatrics* 2010;**126**:e1522–9.
656. Thomas TA, Shenoi SV, Heysell SK, et al. Extensively drug-resistant tuberculosis in children with human immunodeficiency virus in rural South Africa. *Int J Tuberc Lung Dis* 2010;**14**: 1244–51.
657. Thompson WJ, Glassroth JL, Snider DE Jr, et al. The booster phenomenon in serial tuberculin testing. *Am Rev Respir Dis* 1979; **119**:587–97.
658. Thwaites GE, Nguyen DB, Nguyen HD, et al. Dexamethasone for the treatment of tuberculous meningitis in adolescents and adults. *N Engl J Med* 2004;**351**:1741–51.
659. Tidjani O, Amedome A, tenDam HG. Protective effect of BCG vaccination of the newborn against childhood tuberculosis in an African community. *Tubercle* 1986;**67**:269–81.
660. Toppet M, Malfroot A, Derde MP, et al. Corticosteroids in primary tuberculosis with bronchial obstruction. *Arch Dis Child* 1990;**65**:1222–6.
661. Traub M, Colchester AC, Kingsley DP, et al. Tuberculosis of the central nervous system. *Q J Med* 1984;**53**:81–100.
662. Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* 2006; **367**:1173–80.
663. Tsakalidis D, Pratsidou P, Hitoglou-Makedou A, et al. Intensive short course chemotherapy for treatment of Greek children with tuberculosis. *Pediatr Infect Dis J* 1992;**11**:1036–42.
664. Tuberculosis in Children. Guidelines for diagnosis, prevention and treatment (Statement of the Scientific Committee of the International Union Against Tuberculosis and Lung Diseases). *Bull Int Union Tuberc Lung Dis* 1991;**66**:61–7.
665. Tuli SM. *Tuberculosis of the spine*. New Delhi: Amerind Publishing; 1975.
666. Turnbull FM, McIntyre PB, Achat HM, et al. National study of adverse reactions after vaccination with bacille Calmette-Guérin. *Clin Infect Dis* 2002;**34**:447–53.
667. Tyler B, Bennett H, Kim J. Intracranial tuberculomas in a child: computed tomographic scan diagnosis and nonsurgical management. *Pediatrics* 1983;**71**:952–4.
668. Udani PM, Parekh UC, Dastur DK. Neurological and related syndromes in CNS tuberculosis: clinical features and pathogenesis. *J Neurol Sci* 1971;**14**:341–57.
669. Ussery XT, Valway SE, McKenna M, et al. Epidemiology of tuberculosis among children in the United States. *Pediatr Infect Dis J* 1996;**15**:697–704.
670. Vallejo J, Ong L, Starke J. Clinical features, diagnosis and treatment of tuberculosis in infants. *Pediatrics* 1994;**94**:1–7.

671. Vallejo J, Ong LT, Starke JR. Tuberculous osteomyelitis of the long bones in children. *Pediatr Infect Dis J* 1995;**14**:542–6.
672. Vallejo JG, Starke JR. Tuberculosis and pregnancy. *Clin Chest Med* 1992;**13**:693–707.
673. Van den Bosch A, Terken M, Ypma L, et al. Tuberculous meningitis and miliary tuberculosis in young children. *Trop Med Int Health* 2004;**9**:309–13.
674. Vanhoenacker FM, De Backer AI, Op de Beek B, et al. Imaging of gastrointestinal and abdominal tuberculosis. *Eur Radiol* 2004; **14**:e103–15.
675. Van Zyl-Smit RN, Zwerling A, Dheda K, et al. Within-subject variability of the interferon-gamma assay results for tuberculosis and boosting effect of tuberculin skin testing: a systematic review. *PloS One* 2009;**4**:e8517.
676. vander Weert EM, Hertgers NM, Schaaf HS, et al. Comparison of diagnostic criteria of tuberculous meningitis in human immunodeficiency virus–infected and uninfected children. *Pediatr Infect Dis J* 2006;**25**:65–9.
677. Van Hest R, De Vries G, Morbano G, et al. Cavitating tuberculosis in an infant. *Pediatr Infect Dis J* 2004;**23**:667–70.
678. Van Zyl S, Marais BJ, Hesseling AC, et al. Adherence to antituberculosis chemoprophylaxis and treatment in children. *Int J Tuberc Lung Dis* 2006;**10**:13–18.
679. Van Zwanenberg D. Influence of the number of bacilli on the development of tuberculous disease in children. *Am Rev Respir Dis* 1960;**82**:31–44.
680. Varudkar BL. Short-course chemotherapy for tuberculosis in children. *Indian J Pediatr* 1985;**52**:593–7.
681. Venkataraman P, Menon NK, Nair NGB, et al. Classification of subjects as slow or rapid inactivators of isoniazid based on the ratio of urinary excretion of acetylisoniazid to isoniazid. *Tubercle* 1972;**53**:84–91.
682. Verhagen LM, Warris A, van Soolingen D, et al. Human immunodeficiency virus and tuberculosis coinfection in children: challenges in diagnosis and treatment. *Pediatr Infect Dis J* 2010;**29**: e63–70.
683. Verma A, Dhawan A, Wade JJ, et al. *Mycobacterium tuberculosis* infection in pediatric liver transplant recipients. *Pediatr Infect Dis J* 2000;**19**:625–30.
684. Versfeld GA, Soloman A. A diagnostic approach to tuberculosis of bones and joints. *J Bone Joint Surg Br* 1982;**64**:446–9.
685. Visudhiphan P, Chiemchanya S. Tuberculous meningitis in children: treatment with isoniazid and rifampin for twelve months. *J Pediatr* 1989;**114**:875–9.
686. Waeker NJ Jr, Connor JD. Central nervous system tuberculosis in children: a review of 30 cases. *Pediatr Infect Dis J* 1990;**9**: 539–43.
687. Wallace K, Cohen AS. Tuberculous arthritis: report of two cases with review of biopsy and synovial fluid findings. *Am J Med* 1976;**61**:277–82.
688. Wallace RC, Burton EM, Barrett FF, et al. Intracranial tuberculosis in children: CT appearance and clinical outcome. *Pediatr Radiol* 1991;**21**:241–6.
689. Wallgren A. On contagiousness of childhood tuberculosis. *Acta Paediatr* 1937;**22**:229–34.
690. Wallgren A. *Pulmonary tuberculosis in adults and children*. New York: Thomas Nelson & Sons; 1939.
691. Wallgren A. Tuberculous heart disease. *Acta Med Scand* 1947; **196**(Suppl.):132–44.
692. Wallgren A. The time-table of tuberculosis. *Tubercle* 1948;**29**: 245–51.
693. Walters E, Cotton MF, Rabie H, et al. Clinical presentation and outcome of tuberculosis in human immunodeficiency virus infected children on antiretroviral therapy. *BMC Pediatrics* 2008; **8**:1.
694. Wang ME, Castillo ME, Montano SM, et al. Immune reconstitution inflammatory syndrome in human immunodeficiency virus–infected children in Peru. *Pediatr Infect Dis J* 2009;**28**:900–3.
695. Ward JR, Cloud RS, Krawitt EL, et al. Studies on adjuvant-induced polyarthritis in rates: the effect of 'immunosuppressive agents' on arthritis and tuberculin hypersensitivity. *Arthritis Rheum* 1964;**7**:654–61.
696. Washko R, Robinson E, Fehrs LJ, et al. Tuberculosis transmission in a high school choir. *J Sch Health* 1998;**68**:256–9.
697. Webb EA, Hesseling AC, Schaaf HS, et al. High prevalence of *Mycobacterium tuberculosis* infection and disease in children and adolescents with type 1 diabetes mellitus. *Int J Tuberc Lung Dis* 2009;**13**:868–74.
698. Weinstein J, Barrett C, Baltimore R, et al. Nosocomial transmission of tuberculosis from a hospital visitor on a pediatrics ward. *Pediatr Infect Dis J* 1995;**14**:232–4.
699. Weis S, Slocum P, Blais F, et al. The effects of directly observed therapy on the rates of drug resistance and relapse in tuberculosis. *N Engl J Med* 1994;**330**:1179–84.
700. Weismuller MM, Graham SM, Claessens NJM, et al. Diagnosis of childhood tuberculosis in Malawi: an audit of hospital practice. *Int J Tuberc Lung Dis* 2002;**6**:432–8.
701. Werhane MJ, Snukst-Torbeck G, Schraufnagel DE. The tuberculosis clinic. *Chest* 1989;**96**:815–18.
702. Whalen C, Horsburgh C, Hom D, et al. Accelerated course of human immunodeficiency virus infection after tuberculosis. *Am J Respir Crit Care Med* 1995;**151**:129–35.
703. Whitener DR. Tuberculous brain abscess. *Arch Neurol* 1978;**35**: 148–55.
704. Wilkins EGL. Tuberculous peritonitis: Diagnostic value of the ascitic/blood glucose value. *Tubercle* 1984;**65**:47–52.
705. Wilson ML. Recent advances in the laboratory detection of *Mycobacterium tuberculosis* complex and drug resistance. *Clin Infect Dis* 2011;**52**:1350–5.
706. Wirth T, Hildebrand F, Allix-Bequec C, et al. Origin, spread, and demography of the *Mycobacterium tuberculosis* complex. *PloS Pathog* 2008;**4**:e1000160.
707. Witrak BJ, Ellis GT. Intracranial tuberculosis: Manifestations on computerized tomography. *South Med J* 1985;**78**:386–92.
708. Wiysonge CS, Ntsekhe M, Gumedze F, et al. Contemporary use of adjunctive corticosteroids in tuberculous pericarditis. *Int J Cardiol* 2008;**124**:388–90.
709. Wolf H, Mendez M, Gilman RH, et al. Diagnosis of pediatric pulmonary tuberculosis by stool PCR. *Am J Trop Med Hyg* 2008; **79**:893–8.
710. Wootten SH, Gonzalez BE, Pawlak B, et al. Epidemiology of pediatric tuberculosis using traditional and molecular techniques: Houston, Texas. *Pediatrics* 2005;**116**:1141–7.
711. World Health Organization. *Guidance for national tuberculosis programmes on the management of tuberculosis in children*. Geneva: World Health Organization; 2006.
712. World Health Organization. *Rapid advice 2010: treatment of tuberculosis in children*. Geneva: World Health Organization; 2010.
713. World Health Organization. *Global tuberculosis control 2011*. Geneva: World Health Organization; 2011.
714. World Health Organization. *Tuberculosis country profiles*. Available online at http://www.who.int/tb/country/data/profiles/en/index.html. Accessed December 12, 2011.
715. Wright A, Zignol M, Van Deun A, et al. Epidemiology of antituberculosis drug resistance 2002-2007: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. *Lancet* 2009;**373**:1861–73.
716. Wright FW, Hamilton WS. Miliary tuberculosis twice. *Br J Dis Chest* 1974;**68**:210–12.
717. Wu SS, Chao CS, Vargas JH, et al. Isoniazid-related hepatic failure in children: a survey of liver transplantation centers. *Transplantation* 2007;**84**:173–9.
718. Xu HB, Jiang RH, Li L. Pulmonary resection for patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *J Antimicrob Chemother* 2011;**66**:1687–95.
719. Yeo IKT, Tannenbaum T, Scott AN, et al. Contact investigation and genotyping to identify tuberculosis transmission to children. *Pediatr Infect Dis J* 2006;**25**:1037–43.
720. Zampoli M, Kilborn T, Eley B. Tuberculosis during early antiretroviral induced immune reconstitution in HIV-infected children. *Int J Tuberc Lung Dis* 2007;**11**:417–23.
721. Zar HJ, Cotton MF, Strauss S, et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ* 2007;**334**:136.
722. Zar HJ, Hanslo D, Apollos P, et al. Induced sputum versus gastric lavage for microbiological confirmation of pulmonary tuberculosis in infants and young children: A prospective study. *Lancet* 2005; **365**:130–4.
723. Zarabi M, Sane S, Girdany BR. Chest roentgenogram in the early diagnosis of tuberculous meningitis in children. *Am J Dis Child* 1971;**121**:389–92.

724. Zhang Y. Genetic basis of isoniazid resistance of *Mycobacterium tuberculosis*. *Rev Microbiol* 1993;**144**:143–50.
725. Zhang Y, Heym B, Allen B, et al. Catalase-peroxidase gene and isoniazid resistance of *Mycobacterium tuberculosis*. *Nature* 1992; **358**:591–3.
726. Zignol M, Hosseini MS, Wright A, et al. Global incidence of multidrug-resistant tuberculosis. *J Infect Dis* 2006;**194**:479–85.
727. Zinneman HH, Hall WH. Transient tuberculous meningitis. *Am Rev Respir Dis* 1976;**114**:1185–8.
728. Zitrin CM, Lincoln EM. Initial tuberculous infection due to drug-resistant organisms: with a review of the world literature on initial infection due to isoniazid-resistant tubercle bacilli. *J Pediatr* 1961;**58**:219–23.
729. Zweiman B, Pappano JE Jr, Hildrath EA. Effect of influenza vaccine administration on tuberculin skin sensitivity. *Dis Chest* 1967;**52**:46–9.

CHAPTER 97

Other Mycobacteria

W. Matthew Linam • Richard F. Jacobs

The definition of mycobacteria other than tubercle bacilli is quite confusing. Runyon[156] in his address to the International Conference on Atypical Mycobacteria probably defined them best: "Tubercle bacilli include *Mycobacterium tuberculosis*, *Mycobacterium bovis*, and *Mycobacterium africanum*. Together with *Mycobacterium microti* (not pathogenic for humans), these organisms constitute the tubercle bacillus complex." Any mycobacteria not listed in this group are considered to be in the "other" grouping. Mycobacteria other than those causing tuberculosis and leprosy were not recognized as causes of disease in humans until the 1950s.[189] The incidence of disease caused by these organisms remained stable until the acquired immunodeficiency syndrome (AIDS) epidemic began in the 1980s. The most common forms of the disease are chronic pulmonary disease resembling tuberculosis (occurring mainly in adults), cervical adenopathy in children, skin and soft tissue infection, and disseminated disease in immunocompromised individuals.[135] In the mid-1980s, the incidence of infections with nontuberculous mycobacteria (NTM) increased markedly, probably because of the increased number of immunocompromised patients (e.g., because of AIDS and organ transplantation) and the significant improvement in microbiologic methods for cultivating these organisms.[216,221]

EPIDEMIOLOGY

NTM are ubiquitous in nature. They are found in soil, water, animals, milk,[50] and food. Of importance in some hospital-acquired infections or infections in immunocompromised hosts is the presence of the organisms in common tap water.[18,70,121,182] Exposure to environments, especially soil, colonized by these organisms seems to be important for acquisition of disease in children. Organisms commonly found in soil include *Mycobacterium scrofulaceum*, *Mycobacterium flavescens*, *Mycobacterium avium* complex (MAC), *Mycobacterium gastri*, *Mycobacterium terrae*, *Mycobacterium fortuitum*, and *Mycobacterium chelonae*. Water is an important source for all of the previously named organisms and for *Mycobacterium kansasii*, *Mycobacterium marinum*, *Mycobacterium gordonae*, and *Mycobacterium xenopi*.

In contrast, humans are the only known reservoirs for *M. tuberculosis*. In a survey from the Centers for Disease Control and Prevention (CDC), 35 percent of mycobacteria isolated in laboratories were nontuberculous.[63] MAC (*Mycobacterium avium* and *Mycobacterium intracellulare*) accounted for 66 percent of the nontuberculous isolates, followed by *M. fortuitum* (19%), *M. kansasii* (9%), and *M. scrofulaceum* (6%). These data were collected in 1980, before the present AIDS epidemic and before the rapid increase in the number and types of immunocompromised patients; therefore, current rates probably are much higher. Two studies from 2009 showed a similar distribution of nontuberculous isolates. The distribution in the Australian study was MAC (48.5%), *Mycobacterium abscessus* or *M. chelonae* (13.2%), *M. fortuitum* (11.8%), and *Mycobacterium ulcerans* (5.9%).[15] The distribution in a California study was MAC (47%), *M. abscessus* or *M. chelonae* (22%), and *M. fortuitum* (5%).[150] Some of the variation seen may be due to geographic differences.

Geography apparently has some bearing on the prevalence of these infections. The southeastern part of the United States has much higher rates of infection in children than does the Northeast or the Northwest. MAC was seen most commonly along the coastal borders of the United States and in the states bordering Canada. The highest rates were seen in Hawaii (10.9/100,000 population), Connecticut (8.9 cases per 100,000 population), and Florida (8.4 cases/100,000 population). High rates also were seen in Kansas and the desert Southwest, however, showing the widespread nature of this organism in causing disease. In contrast, *M. kansasii* was seen most commonly in the Midwest of the United States and almost never in the Southeast.[53] States with rates of *M. kansasii* greater than 0.75/100,000 included Missouri, Illinois, Kentucky, Indiana, Kansas, Nebraska, Louisiana, Texas, Arizona, and Florida. North Carolina, a state with one of the highest rates of MAC (>4.8 cases/100,000 population), had few cases of *M. kansasii* (0 to 0.25/100,000 population). *M. marinum* frequently was isolated from coastal areas, whereas *M. xenopi* was scattered across the United States, with 50 percent of cases found in just three states (Connecticut, Wisconsin, and California). Internationally, incidence rates of NTM infections in children range from 0.84/100,000 (Australia) to 3.1/100,000 (Germany).[15,149] For all of the nontuberculous species, males and rural residents had a much higher incidence of infection.[162] Extrapolation of these rates to determine disease patterns is not without problems, however. The number of mycobacterial isolates could be skewed easily by the presence of multiple isolates from a single patient, or it may just represent colonization. Nonetheless, these rates can help predict which species of NTM are most likely to be encountered in a particular geographic region while the physician awaits final identification and sensitivity testing on an isolated nontuberculous organism.

The site of isolation from the human source can be helpful in determining the type of mycobacteria that may be involved in the disease process. MAC is responsible for lymphadenitis, particularly in children. MAC also is responsible for pulmonary disease and disseminated disease to bones and occasionally to the meninges. *M. kansasii* is associated most commonly with infections of pulmonary origin and disseminated lesions in adults; rarely, it is associated with adenitis and skin granuloma in children. *M. scrofulaceum* also causes lymphadenitis in children, and *M. marinum* is responsible for skin granuloma and ulcers after exposure to certain salt water beaches, swimming pools, and tropical fish tanks. The rapidly growing mycobacteria, such as *M. fortuitum* and *M. chelonae*, often cause skin and soft tissue infection and, rarely, pulmonary and disseminated disease in adults and children. A notable exception is the rapidly growing mycobacterium *M. abscessus*, which is the third most common NTM species recovered from respiratory specimens.[68] Health care–associated NTM infections have been reported in patients with long-term vascular catheters, peritoneal dialysis catheters, and various surgical procedures. Rapidly growing mycobacteria are frequently implicated. The source is often contaminated tap water.

A familial immune defect predisposing to disseminated NTM infection in childhood has been reported.[109] Interleukin (IL)-12 and interferon (IFN)-γ play an important role in the control of mycobacterial infections, primarily through upregulation of tumor necrosis factor (TNF)-α. Defects in either the synthesis or signaling pathways of these cytokines have resulted in disseminated NTM infections.[48] In addition, patients receiving anti–TNF-α therapy may be at increased risk for NTM disease, especially if they are receiving additional immune suppressive medications.[214]

MICROBIOLOGY

Runyon and Timpe[154,155,189] in their monumental work on NTM suggested a useful classification system based on three characteristics of the organisms: production of pigment, rate of growth, and colonial characteristics. The four groups are as follows: group I—photochromogens, which produce bright yellow to red pigment in the presence of light; group II—scotochromogens, which produce yellow to orange pigment in the dark; group III—nonphotochromogens, which are non–pigment producers; and group IV—rapid growers, which generally grow in less than 1 week. Kubica[100] published an updated version of Runyon's classification in 1978, and it was useful for many years. Subsequently, a more "simplified" classification based on the growth rates of the organisms alone was proposed.[216] More recently, reliable DNA sequencing, using the highly conserved *16S rRNA* gene, has allowed for rapid recognition of new species. A sequence difference of greater than 1 percent usually indicates a new species. Currently, 154 NTM species have been identified.[55,125,191]

The NTM still are differentiated by most clinical laboratories based on various morphologic, physiologic, and biochemical characteristics (Tables 97-1 and 97-2). The difficulty with identifying many of these organisms is their slow growth rates with standard techniques. Because antibiotic susceptibility varies for different nosocomial tracheobronchitis (NTB), it is important to identify organisms to the species level to ensure the appropriate therapy is chosen.[69] Beginning sensitivity testing as soon as an organism is cultivated and thought to be the responsible pathogen is useful because several months may be required for proper identification and susceptibility testing. Serologic tests can be helpful in identifying some

TABLE 97-1 **Characteristics of Slow-Growing Pathogenic Mycobacteria**

Organism	Growth Present (°C)			Growth Rate (Days)	Niacin	Nitrate Reduction	Pigment Dark	Pigment Light	Growth in 5% NaCl
	25	37	45						
M. tuberculosis	–	+	–	12-28	+	+	–	–	–
M. bovis	–	+	–	21-40	–	–	–	–	–
Photochromogens, Runyon group I									
M. kansasii	+	+	–	10-21	–	+	–	+	–
M. marinum	+	±	–	7-14	–	–	–	+	–
Scotochromogens, Runyon group II									
M. scrofulaceum	+	+	–	10-28	–	–	+	+	–
M. szulgai	+	+	–	12-28	–	+	+	+	–
M. gordonae	+	+	–	10-28	–	–	+	+	–
Nonchromogens, Runyon group III									
M. avium	+	+	+	10-21	–	–	–	–	–
M. intracellulare	+	+	–	10-21	–	–	–	–	–
M. ulcerans	–	–	–	28-60	–	–	–	–	?
M. xenopi	–	+	–	14-28	–	–	–	–	–

TABLE 97-2 **Characteristics of Rapid Mycobacterial Growers**

Runyon Group IV	Optimal Temperature (°C)	Growth Rate (Days)	Niacin	Nitrate Reduction	Growth in 5% NaCl
M. fortuitum	37	3-7	–	+	+
M. abscessus	37	3-7	–	–	+
M. chelonae	37	3-7	–	–	–

of the organisms.* In clinical medicine, however, they rarely are valuable for establishing a diagnosis in an individual patient. A study using humoral immunoglobulins against mycobacterial antigens failed to diagnose tuberculosis or nontuberculous adenitis in children.[196] Blood cultures for mycobacteria are best performed with the Isolator lysis-centrifugation system or the radiometric BACTEC 13A blood culture bottle.[2,92,93] Blood collected in ethylenediaminetetraacetic acid or coagulated blood is unacceptable. Body fluids such as cerebrospinal fluid, pleural fluid, and peritoneal fluid can be inoculated directly into BACTEC or Septi-Chek broth, particularly if only small volumes are available. To aid in detection and growth, cultures should include both solid and broth media, whenever possible. Gene probes became commercially available in the late 1980s. They involve DNA probes complementary to species-specific sequences of rRNA for the identification of *M. tuberculosis*, MAC, *M. gordonae*, and *M. kansasii*.[65]

Polymerase chain reaction (PCR) assay has been evaluated for the routine detection of *M. tuberculosis* in the clinical laboratory and compared with fluorochrome smear and culture. Multiplex, real-time PCRs also have been developed to aid in the rapid identification of NTM. In a study by Kim and colleagues,[97] a multiplex, real-time PCR was developed capable of identifying 23 mycobacterial species and was tested using 77 reference strains and 369 clinical isolates. The test correctly identified all 189 (100%) isolates of the *M. tuberculosis* and 169 (93.9%) of the NTM isolates. Another multiplex, real-time PCR test using 314 clinical isolates had a 95 to 99 percent sensitivity and 99 to 100 percent specificity for distinguishing *M. tuberculosis* complex, MAC, the *M. chelonae–M. abscessus* group, the *M. fortuitum* group, and *Mycobacterium mucogenicum*.[150] The PCR testing offered by commercial laboratories at present is not approved by the U.S. Food and Drug Administration for in vitro diagnosis.

MANIFESTATIONS OF NONTUBERCULOUS MYCOBACTERIAL INFECTION IN CHILDREN

Lymphadenitis

Lymphadenitis is the most common manifestation of NTM infection in children. It also can occur rarely in adults.[44,105,113] All nodes in the cervical chain can be affected, but the nodes of the submandibular region seem to be most commonly involved.[46,119] The parotid gland also can be affected.[39] The differential diagnosis frequently centers on deciding whether a malignant process or a nonmalignant process is present. Nonmalignant processes to consider include mononucleosis, bacterial adenitis, cat-scratch disease, toxoplasmosis, tularemia, and *M. tuberculosis* infection.

Most mycobacterial cases of lymphadenitis are caused by the nontuberculous organisms. The clinician also must consider the possibility of *M. tuberculosis*, however.[35] No clinical features help the clinician discern between NTM infection and tuberculosis. The use of histopathology has been postulated to help differentiate between atypical and tuberculous infections.[140] In a retrospective study, the findings of ill-defined (nonpalisading) granulomas, irregular or serpiginous granulomas, a predominantly nonspecific granulomatous response, predominantly sarcoidlike granulomas, or lack of significant caseation were seen more commonly with nontuberculous lymphadenitis. Additionally, nontuberculous infections had neutrophils predominantly in the center of the necrosis, whereas in tuberculous infections, neutrophils were scattered throughout the specimen.[140] A prospective study investigating these findings has not been published to date, and other authors have not noted similar patterns.[12]

In a Canadian study, the rate of NTM as a cause of lymphadenitis was 1.21 cases/100,000 children, whereas the rate for tuberculosis was 0.3 case/100,000 children.[171] Investigators from San Diego reported a marked increase in the number of infections in children.[141] Other authors around the world have reported a markedly increased incidence of infection with these organisms in immunocompetent children.[61] In one study, the incidence increased from 1 case between 1987 and 1990 to 85 cases between 1991 and 1993.[104] In a large study compiled by Lincoln and Gilbert[112] involving 243 children, more than 50 percent were younger than 3 years of age and 80 percent were younger than 5 years. In contrast, one study showed that the mean age had increased to 5.2 years.[210] More recently, a Canadian study compared the incidence of NTM cervicofacial adenitis in children from 1994 to 1998 (25.1/100,000 hospitalizations) and 1999 to 2004 (107.4/100,000 hospitalizations) and showed a statistically significant increase. Contact with NTM sources such as soil and water may account for the frequency of cervical lymphadenitis in young children.[193]

Most patients have no systemic symptoms and exhibit normal chest radiographs; other laboratory studies generally are not helpful. The mean duration of swelling is approximately 6 weeks. Affected lymph notes are often unilateral and nontender. Over time, NTM infections involving superficial lymph nodes may develop a violaceous discoloration of the overlying skin. Nodes may spontaneously rupture or develop draining sinus tracts.

*References 42, 101, 154, 195, 203, 216, 218, 221.

Infections are caused mainly by MAC and *M. scrofulaceum*. A large prospective study spanning 32 years from 1958 to 1990 showed that MAC has become the predominant etiologic agent and has surpassed *M. scrofulaceum* from earlier in the study.[219] Current data estimate that 72 percent of NTM lymphadenitis cases are caused by *M. avium*.[139] A series of 190 patients from India showed, however, that 60 percent of the nontuberculous adenitis cases were caused by *M. scrofulaceum*, followed by 40 percent by *M. avium-intracellulare*.[88] This difference may be due in part to differences in water chlorination. Case reports involving *M. fortuitum* also have been published.[151] Lymphadenitis caused by *Mycobacterium haemophilum* is being diagnosed in immunocompetent children in increasing numbers.[8,159] Samra and colleagues[159] showed that the BACTEC radiometric system or MB Redox broth is superior to Löwenstein-Jensen media for isolation of *M. haemophilum*. This organism is thought to have been underdiagnosed previously because of the widespread use of Löwenstein-Jensen media. *M. malmoense* also has been described as an etiologic agent of cervical lymphadenitis in children.[192] Haas and associates[73] described *Mycobacterium heidelbergense*, a new agent of mycobacterial lymphadenitis in children.

Currently, no NTM skin test is commercially available in the United States. All patients with suspected NTM lymphadenitis should have a tuberculin skin test placed. Children with NTM lymphadenitis can have a variety of reactions to tuberculin skin tests, ranging from 0 mm to greater than 10 mm of induration. Needle aspiration of an affected node can be a valuable diagnostic tool. Cultures for bacterial and mycobacterial etiologies should be performed, with recovery rates in children with cervical lymphadenitis ranging from 60 percent to 88 percent.[10,16,81,91,224] The aspirate should be inoculated onto aerobic and anaerobic media and Sabouraud agar and mycobacterial media (Löwenstein-Jensen slants or Middlebrook media). Use of the BACTEC system can be helpful, especially in isolation of more fastidious species. Mycobacteria can be isolated 12 to 17 days after inoculation.[176] More recently, preoperative diagnosis of *M. avium* lymphadenitis was accomplished with the use of PCR of gastric aspirates in two children.[72] PCR is being used with increasing frequency in the diagnosis in NTM lymphadenitis.[31,139]

The best treatment of lymphadenitis caused by NTM is complete excision of the involved lymph node.[120,200] Incision and drainage without excision results in a high rate of secondary drainage, and subsequent excision of the remaining tissue is required for cure.[5,158,165] Complete excision may not be possible when affected nodes are adjacent to large vessels or the facial nerve. In those situations, adjunctive antibiotic therapy may be necessary. The majority (59%) of clinicians manage NTM lymphadenitis by surgical excision followed by adjunctive antibiotics.[139] A randomized controlled trial involving 100 children with NTM cervicofacial lymphadenitis compared surgical excision to antibiotic therapy with clarithromycin and rifabutin.[114] Esthetic outcome was significantly better in the surgical group. Another small study showed successful nonsurgical management of deep cervical NTM lymphadenitis.[75]

Acquired Immunodeficiency Syndrome and Nontuberculous Mycobacteria

Before the availability of highly active antiretroviral therapy (HAART), NTM infections, most commonly MAC, were the second most common opportunistic infections in children in the United States with human immunodeficiency virus (HIV) infection. The incidence rate in the pre-HAART era was 1.3 to 1.8 episodes per 100 person-years.[62,133] Approximately 6 percent of adults and 4 percent of children with AIDS reported to the CDC had disseminated MAC infection as the AIDS-defining disease.[22] Autopsy studies show that MAC infection is present in 20 percent to 50 percent of adult HIV-infected patients.[147,208,212] In a retrospective study from 1990 to 1996 in New York City, 26 percent of children had evidence of MAC infection at the time of their deaths.[89] MAC infection was thought to be the cause of death in 13 percent of the 54 children with HIV infection, and the mean age at death was 7.8 years. In two autopsy studies of opportunistic infection in HIV-infected children in Latin America and Argentina, 2.7 percent and 3.4 percent, respectively, had evidence of disseminated disease.[49,144] In these studies, 62 percent (Argentina) and 72 percent (Latin America) of the children studied were younger than 1 year of age. These data indicate that MAC infection is seen less commonly in younger children with HIV. Since the availability of HAART, incidence of MAC has decreased to 0.14 to 0.2 episodes per 100 person-years.[62,133]

In the CDC study, MAC was the NTM most commonly isolated. *M. kansasii* and *M. scrofulaceum* were found in one case each. In adult studies, the incidence of infection with *M. kansasii* and *M. scrofulaceum* also is low.[45,84] In patients with HIV, *M. fortuitum* complex is the most frequently isolated rapidly growing mycobacteria and typically causes lymphadenitis and pulmonary disease.[145] More than 70 percent of children with MAC and AIDS had evidence of disseminated disease. Almost all had $CD4^+$ counts less than 100 cells/mm^3. Clinical findings included failure to maintain growth curves, anorexia, persistent or recurrent fever, fatigue, and abdominal pain. MAC has been shown to infect the esophagus, stomach, and intestine of pediatric patients with AIDS.[90] Patients with extensive MAC infection of the small and large intestines have severe, persistent diarrhea. Respiratory symptoms are uncommon. Clinical findings may include hepatomegaly, splenomegaly, or lymphadenopathy. Bone marrow suppression also may occur. The median age at diagnosis was 46 months, with a median of 9 months elapsing between the onset of symptoms and positive cultures. When NTM infection was diagnosed in these patients, they survived less than 10 months. Blood cultures have 90 percent to 95 percent sensitivity in detecting disseminated MAC infection in adult patients with AIDS.[76] Infection is also confirmed by isolation of MAC from a normally sterile body site such as lymph node or bone marrow.

Mycobacterium genavense has been shown to cause infection in children with HIV infection.[132] The children were febrile and had abdominal cramps and diarrhea. $CD4^+$ lymphocyte counts were less than 400/mm^3. The

organism was found in numerous stool samples and lymph node specimens. Multiple-drug regimens that include amikacin, ethambutol, rifampin, and clarithromycin may be useful in treating this infection.

For treatment of MAC infection in HIV-infected children, combination therapy is recommended.[127] Clarithromycin 15 to 30 mg/kg/day divided twice daily (maximum 500 mg) or azithromycin 10 to 12 mg/kg once daily and ethambutol 15 to 25 mg/kg/day in a single dose (maximum of 2.5 g) should be included. Additionally, the use of rifabutin 10 to 20 mg/kg/day once daily (maximum 300 mg) or ciprofloxacin 20 to 30 mg/kg/day divided twice daily intravenous or orally (maximum 1.5 g) can be considered. The use of granulocyte colony-stimulating factor as an adjunct to antimicrobial therapy for disseminated MAC infection has been reported,[136] but additional data are needed to determine the usefulness of this agent. Immune reconstitution inflammatory syndrome has been reported in adults and children with MAC infection receiving HAART.[37,167]

Secondary prophylaxis is recommended for all children with a history of disseminated MAC infection. Clarithromycin 7.5 mg/kg orally in two doses (maximum 500 mg) is recommended in combination with ethambutol 15 to 25 mg/kg/day once daily (maximum 2.5 g), with or without rifabutin 5 mg/kg/day once daily (maximum 300 mg). In most situations, secondary prophylaxis is lifelong. Guidelines are available for discontinuing prophylaxis in children who have completed appropriate therapy, are without evidence of ongoing infection, are stable on HAART, and have sustained recovery of their $CD4^+$ cells above the threshold for initiating primary prophylaxis.[127]

The use of clarithromycin 7.5 mg/kg orally twice daily (maximum 500 mg) or azithromycin 20 mg/kg once weekly (maximum 1.2 g) for prophylaxis is recommended for adults and children with advanced immunosuppression. The U.S. Public Health Service and the Infectious Disease Society of America have published guidelines for the prevention of opportunistic infections, including disseminated MAC.[127] These guidelines incorporate age-specific $CD4^+$ counts at which prophylaxis should be used: children 6 years of age or older, fewer than 50 cells/mm^3; children 2 to 6 years old, fewer than 75 cells/mm^3; children 1 to 2 years old, fewer than 500 cells/mm^3; and children younger than 1 year, fewer than 750 cells/mm^3. Before initiating prophylaxis, patients should be evaluated for disseminated MAC infection by obtaining a blood culture for MAC. In HIV-infected children older than 2 years of age, primary prophylaxis may be discontinued once they are stable on HAART (>6 months) with sustained immunologic response for over 3 months (children 6 years of age or older, >100 cells/mm^3; children 2 to 6 years old, >200 cells/mm^3). A phase I/II study of prophylactic rifabutin for prevention of disseminated MAC infection in children showed a side effect of bilateral, stellate corneal deposits without associated uveitis in 6 of 25 children.[173]

The emergence of resistance is a concern with the MAC prophylactic regimens in patients with AIDS. In some trials, 9 percent of adults receiving azithromycin prophylaxis and 5 percent of adults receiving clarithromycin prophylaxis had breakthrough MAC bacteremia. Of these cases, 11 percent and 58 percent of the azithromycin and clarithromycin, respectively, breakthrough isolates were macrolide-resistant.[6,77]

Pulmonary Infections

Reviews from the late 1970s showed that pulmonary disease caused by NTM usually was caused by *M. kansasii* and MAC.[32,64,152] MAC infection most commonly occurred during the sixth decade, whereas *M. kansasii* infection occurred in individuals a decade younger. Men were affected most commonly, in ratios as high as 4:1. Chronic obstructive pulmonary disease (COPD) as noted by radiographic findings was found in 50 to 60 percent of patients with NTM pulmonary infections. Bullous lung disease was seen in 24 to 39 percent. The lobar distribution and severity of disease were similar for NTM agents and *M. tuberculosis*, with one exception—*M. kansasii* was much more prone to producing unilateral disease, which occurred nearly 60 percent of the time in contrast to 35 percent of the time in MAC or *M. tuberculosis* infection. Typically, disease begins in the posterior portions of the upper lobes. Progression to cavitary disease occurred in 87 percent of patients with *M. tuberculosis* and MAC infections and in 96 percent of patients with *M. kansasii* infection. Rare case reports of mediastinal mass lesions in children caused by NTM have been published.[56]

Two recent prevalence studies have shown that the epidemiology and clinical features have changed. An Oregon study estimated the 2-year prevalence of NTM pulmonary disease to be 8.6 cases/100,000 persons (20.4/100,000 for persons older than 50 years).[215] In a large multisite study, the age-adjusted annual prevalence was 5.4 cases/100,000 persons.[142] Both studies show an increased prevalence of NTM pulmonary disease. This increase is most evident in women, especially those older than 60 years of age.[142] MAC was the most common isolate (80% to 87.5%), followed by *M. abscessus* or *chelonae* (6% to 12%).[142,215] Other species causing NTM pulmonary disease include *Mycobacterium simiae*, *M. kansasii*, *M. fortuitum*, *M. mucogenicum*, and *M. xenopi*.[142] Underlying comorbidities such as COPD, bronchiectasis, and lung cancer were common, with bronchiectasis more common in women.[142,215] Most common imaging findings were nodules (59%), bronchiectasis (39.7%), and cavitary lesions (8.5%).[142] NTM pulmonary disease is uncommon in children and most frequently seen in children with cystic fibrosis.[38]

Patients with cystic fibrosis have been noted to have an increased incidence of infection with NTM. Prevalence rates of 2 to 20 percent have been reported.[4,79,80,95] A large multicenter prospective prevalence study of NTM in patients with cystic fibrosis from France included 1582 patients.[153] The prevalence of patients with at least one positive NTM culture was 6.6 percent. The prevalence of patients meeting criteria for NTM infection was 3.6 percent. *M. abscessus* complex and MAC were the most common species responsible for both colonization and disease. Mycobacterial infections are seen more commonly in older patients with cystic fibrosis. The peak

prevalence for *M. abscessus* was 11 to 15 years and 25 years for MAC.[153] Frequent intravenous antibiotic use is a possible risk factor for colonization with NTM.[190] *M. abscessus* has emerged as a clinically persistent pathogen in patients with cystic fibrosis, especially patients on steroid therapy for allergic bronchopulmonary aspergillosis.[54,131,190] Persistent *M. abscessus* infection has been associated with lung function decline.[53] Other organisms cultured from patients with cystic fibrosis include *Mycobacterium peregrinum*, *M. kansasii*, *M. fortuitum*, *M. chelonae*, and *M. xenopi*. Bacterial contamination, particularly with *Pseudomonas aeruginosa*, of the acid-fast bacilli cultures from patients with cystic fibrosis has been a major problem that has rendered isolation of mycobacteria more difficult.[174] Another confounding problem is the difficulty in differentiating infection from colonization in these patients.[95]

The American Thoracic Society recommends radiographic changes (i.e., nodules, cavitary opacities, or multifocal bronchiectasis), isolation of multiple colonies of the same species, and the absence of other potential pathogens as criteria for the diagnosis of pathogenic infection with NTM.[3] Discernment is even more difficult in the setting of the chronic lung disease seen in patients with cystic fibrosis. Kilby and associates[95] suggest that repeated isolation of NTM associated with pulmonary cavities or infiltrates that do not improve with aggressive standard antibacterial treatment could indicate active mycobacterial disease in patients with cystic fibrosis.

Treatment of NTM pulmonary disease recommended by the American Thoracic Society requires prolonged courses using multidrug regimens. Drugs are administered for 18 to 24 months, with a minimum of 12 months of culture negativity required while receiving therapy. Treatment of MAC pulmonary infections involves clarithromycin or azithromycin, ethambutol, and rifampin. In fibrocavitary or other severe disease, streptomycin or amikacin may be added.[69] Treatment success is variable. The median efficacy of macrolide-based regimens reported in a recent review of 12 clinical trials was 56 percent (range, 26% to 71%).[57]

M. xenopi has been described in adults as a cause of infection of the pulmonary tract.[172] The mean age at infection was 62 years, and it occurred in a mainly Canadian population. Eighty-six percent of the patients had underlying pulmonary pathology, including COPD, previous pulmonary tuberculosis, carcinoma of the lung, sarcoidosis, and cystic fibrosis. Additionally, a case was reported of a 7-year-old boy with leukemia in whom pneumonia with *M. xenopi* developed and was treated successfully with 2 years of therapy that included ethambutol and clarithromycin.[108]

M. simiae has been isolated in adults with underlying pulmonary abnormalities.[11,99] *M. simiae* is the most drug-resistant of all the NTM. Some isolates are resistant to all drugs tested.

Mycobacterium szulgai has been described in several case series as a cause of lung disease.[118,206] Disease occurred in elderly white men and resembled chronic tuberculosis. Therapeutic regimens effective against *M. avium* seemed to provide good clinical outcomes.

Skin Infections

Mycobacterium marinum

M. marinum is photochromogenic and was identified as a pathogen in fish in 1926 by Aronson.[112] Skin lesions usually result from light trauma (abrasions) in swimming pools or other bodies of water when the surfaces of the pool are colonized by *M. marinum*.[41,128] Fish tanks also have been implicated and generally involve the upper limb or a finger.[9,186] Most cases occur in children aged 10 to 16 years old. The most common sites are the elbows, knees, and ankles. Cooler superficial portions of the body are affected most frequently. Other exposed body areas can be involved, depending on what part of the body has made contact with the water containing the mycobacterium (e.g., the nose in divers).[129] Lesions are usually solitary but may have a sporotrichoid pattern. Regional spread of lesions has been reported.[179]

The incubation period from exposure to formation of a small, indurated papule that ulcerates generally is 3 weeks. The lesion then crusts and forms a granuloma with a small crater. The lesions usually are painless and resolve in several months; occasionally, they can last longer. In contrast to other mycobacterial diseases, regional nodes are usually not involved.

Infection with this organism usually is benign. A main consequence, however, is that patients with *M. marinum* infection frequently have conversion of their purified protein derivative stabilized with Tween (PPD-T) test to positive.[112] The natural reservoir for *M. marinum*, which requires a cool incubator (32° C [89.6° F]), is in fish and other cold-blooded animals. Generally, only a few organisms may be isolated in some granulomas.

M. marinum is usually susceptible to rifampin, ethambutol, clarithromycin, and trimethoprim-sulfamethoxazole and intermediately susceptible to doxycycline. Treatment regimens usually include two active agents. Treatment of *M. marinum* has been successful with rifampin and ethambutol,[198,220] and one report showed good results with rifampin alone.[47] An accompanying editorial cautioned against the use of rifampin alone, however, and recommended using rifampin with ethambutol.[19] Other studies have shown success with a combination of clarithromycin and rifampin.[9] The duration of therapy, according to the literature, varies from several weeks to 18 months. Generally, response to therapy is rapid, and treatment should be continued for 4 to 6 weeks after clinical resolution.[47]

Mycobacterium ulcerans

In 1948, MacCullum[117] reported the first cases of disease caused by *Mycobacterium ulcerans*. Most cases since then have occurred in remote, tropical, or subtropical areas of the world, including parts of Africa and Australia.[116,148] In a 1-year period, 23 cases of Buruli ulcer caused by *M. ulcerans* occurred in Lambarene (Gabon).[21] Cases also have been reported in Mexico.[112] The natural reservoir for *M. ulcerans* is unknown. One report suggests the spines of a tall prickly grass known as *Echinochloa pyramidalis* as a reservoir.[180] An Australian study found evidence that mosquitoes may play a role in the transmission of *M.*

ulcerans.[106] The lesions caused by *M. ulcerans* occur mainly on the cooler superficial portions of the body. Patients harboring this organism are in otherwise good health without underlying immunodeficiency.[33] Scraping of the skin by thorns or pieces of wood has been implicated as the route of inoculation in many of the cases. The organism is fastidious, with growth requiring 6 to 12 weeks and seen only between 25° C and 33° C (77° to 91.4° F).

The incubation period for this painless infection is approximately 3 weeks. Regional lymphadenitis rarely occurs with this organism. The infection has three distinct stages, and knowledge of them can be helpful in making the diagnosis and providing treatment. The disease begins as a hard, mobile nodule. It frequently is associated with pruritus, and in Zaire is termed *mputa matadi* (the itching stone). This stage is known as the pre-ulcerative stage. In some patients, the infection resolves spontaneously, but in others it progresses to the ulcerative stage. In contrast to *M. marinum*, in which the lesions are short-lived and do not progress past the ulcerative stage, the lesions of *M. ulcerans* usually last 6 to 9 months and frequently progress and lead to deformities of limbs that may require amputation.[112] The organisms can be isolated in large numbers from the periphery of ulcers adjacent to normal tissue, which again contrasts with infection by *M. marinum*, in which few organisms are found in the lesions. The infection generally involves subcutaneous adipose tissue and leads to areas of fat necrosis, which then proceeds to overlying necrosis of the adjacent skin. The lesions can become enormous, sometimes involving a complete limb. Although numerous organisms are seen in the progressing edge of the infection, little evidence of a cellular immune response is apparent. Additionally, anergy to skin reagents prepared from *M. ulcerans* (burulin) frequently is noted at this stage.[181] The next stage, called the *reactive phase*, is then reached. Cellular infiltrates with granuloma formation occur in the lesion. The number of organisms in the lesion decreases dramatically, and a positive skin test reaction develops to the burulin. Infection with *M. ulcerans* also can cause conversion of a patient's PPD response to positive; however, conversion is seen in approximately 50 percent of cases.[33] Finally, healing may occur, but with fibrosis that often results in limb deformity.

Treatment of *M. ulcerans* infection is anecdotal at best. Treatment choices are based on the stage of infection. Lesions in the pre-ulcerative stage are best treated with excision and primary closure.[66] Successful therapy in the anergic progressive stage is considered the most difficult and involves appropriate antimycobacterial therapy for the infection. Success has been reported with isonicotinic acid hydrazide and streptomycin or diaminodiphenylsulfone and oxytetracycline combinations[33] and with sulfamethoxazole, rifampin, minocycline,[177] and clofazimine.[116] A combination of clarithromycin and rifampin has also been recommended.[67] In the final stage, healing should be promoted with as little deformity or loss of function as possible, using skin grafting and splinting and excision of fibrous tissue.[66,148] Disseminated infection in an immunocompetent child along with the development of multifocal osteomyelitis has been described.[81] (See also Chapter 98.)

Other Mycobacteria in Skin Disease

M. haemophilum has produced painful subcutaneous nodules in immunocompromised patients, particularly patients with renal transplants.[43,218] In addition, *M. haemophilum* has produced disseminated disease, including bacteremia, osteomyelitis, and pulmonary disease, in immunocompromised patients. A study of archived skin biopsy specimens using PCR for NTM identified additional NTM skin infections.[197] *M. haemophilum* was the most common organism identified. *M. chelonae* has been found to be a cause of disseminated cutaneous infection.[202] Steroid use is the predisposing factor for infection with this organism. *M. fortuitum* has been implicated in cutaneous lesions in a child involved in a motor scooter accident, with the subsequent development of lesions at the site of knee lacerations; regional adenopathy of the inguinal nodes also developed.[170] *M. fortuitum-chelonae* complex has been responsible for superficial skin abscesses in children.[14] *M. fortuitum* in adult studies also has been implicated in severe infections in immunocompromised hosts; these infections usually are rapidly disseminating, with high mortality.[217] *M. avium* has been isolated from an eyelid abscess with drainage.[164] Contaminated pedicure footbaths have been implicated in outbreaks of NTM skin infections, including a recent outbreak from *Mycobacterium bolletii* and *Mycobacterium massiliense*.[209] NTM skin infections also have occurred at insulin injection sites, acupuncture sites, and filler injection sites, with many of these caused by rapidly growing mycobacteria.[107,178] Treatment of cutaneous disease caused by these organisms is difficult at best. Four- or five-drug therapy has been tried, but with poor results.

ORGANISMS SEEN IN CHILDREN

Specific organisms and treatment guidelines are discussed in the following sections. Table 97-3 provides a quick guide to some of the more commonly used antimycobacterial agents.

Mycobacterium avium-intracellulare Complex

MAC consists of *M. avium* and *M. intracellulare*. These organisms are slow-growing, obligate aerobes that require 2 to 6 weeks for colony formation on solid media. Colonies usually are smooth but may be rough and can be transparent or opaque. These organisms grow on routine bacterial media, but growth is achieved best on selective mycobacterial media, such as Löwenstein-Jensen medium or Middlebrook 7K10 and 7K11 agar. Nucleic acid hybridization probes using target sequences of ribosomal RNA are available commercially for rapid identification of clinical isolates.[111,130] MAC infection is diagnosed most commonly by culture of blood or bone marrow.

In a study of 56 isolates from pediatric patients involving sequence analysis of the ribosomal internal transcribed spacer, Hazra and colleagues[78] showed that the closely related Mav-B and Mav-A sequevars caused most disease. Patients from geographically diverse areas of the

TABLE 97-3 **Antimycobacterial Agents***

Drug	Dosage	Form
Amikacin (A)	15-30 mg/kg/day divided three times daily	IV or IM
Azithromycin (Z)	500 mg bid (adults/adolescents); 10-12 mg/kg/day (children)	PO
Cefoxitin (X)	80-160 mg/kg/day divided four to six times daily	IV or IM
Ciprofloxacin (C)	20-30 mg/kg/day divided twice daily (adults only in United States)	PO or IV
Clarithromycin (CL)	15-30 mg/kg/day divided twice daily	PO
Clofazimine (CLO)	1-2 mg/kg/day	PO
Doxycycline (D)	2-4 mg/kg/day divided twice daily (>8 yr old)	PO, IV
Ethambutol (ETB)	15 mg/kg/day	PO
Ethionamide (ETH)	15-20 mg/kg/day divided twice daily	PO
Imipenem (IMP)	60-100 mg/kg/day divided four times daily	IV
Isoniazid (I)	5 mg/kg/day	PO
Pyrazinamide (PZA)	15-30 mg/kg/day	PO
Rifabutin (RIB)	5-10 mg/kg/day; maximum 300 mg/day	PO
Rifampin (RIF)	10-20 mg/kg/day once or divided twice daily	PO or IV
Streptomycin (S)	20-30 mg/kg/day	IM

Infections

Disseminated MAC: HIV infected: CL (or Z) + ETB (± RIB)

MAC lung disease: CL (or Z) + ETB + RIF ± S or A (severe or cavitary disease)

Mycobacterium abscessus: CL or Z + 1 or more of A, X, or IMP

Mycobacterium chelonae: CL alone (skin disease), 2 or more of the following: CL, linezolid, IMP, or tobramycin (invasive disease)

Mycobacterium fortuitum: 2 or more of the following: CL, C, A, X, IMP, or TMP-SMX

Mycobacterium kansasii: RIF + ETB + I

Mycobacterium marinum: ETB + RIF, or CL + RIF

Data from references 69, 70, 84, 108-110, 156, 196, 204, 206, 216, 221.
HIV, Human immunodeficiency virus; *MAC, M. avium* complex; *TMP-SMX,* trimethoprim-sulfamethoxazole.

United States (i.e., Boston, Miami, and Los Angeles) had isolates with closely related patterns. The finding of related strains causing disease in epidemiologically unrelated patients is most consistent with two hypotheses: a similar subset of *M. avium* strains is more virulent and more likely to cause disease in humans, and pathogenic strains are more prevalent in the environment.

M. avium was recognized in 1890 as the causative agent of disease in chickens.[218] *M. intracellulare* was designated in 1967 and at the time was difficult to distinguish routinely from *M. avium*, thus the name *M. avium-intracellulare*. Today, with the use of DNA probes, most seroagglutination types have been discerned between the two groups. *M. avium* is the most common NTM causing disease in humans, but isolates from environmental sources are more likely to be *M. intracellulare*. Both organisms can be found in birds, soil, dust, and fresh or salt water. Infections caused by MAC strains isolated from adult patients with AIDS could be identified as either serotype 4 or 8, in contrast to patients without AIDS, in whom no predominant serotype has been identified.[94,223] Nearly all isolates of MAC from patients with AIDS have been identified as *M. avium*; in patients without AIDS, the rate of *M. avium* is approximately 55 percent, and the rate of *M. intracellulare* 32 to 40 percent.[71,223]

Lung disease has been the major manifestation of MAC infection in nonimmunocompromised adults. Most investigators thought that MAC infection occurred mainly in patients with deficient immunity or underlying lung disease. Later reports seemed to indicate, however, that normal adult hosts are at risk for development of infection with MAC and that rates are increasing.[87,143] The incidence of NTM lung disease in postmenopausal women has increased over the last 20 years, especially in slender women with scoliosis or pectus excavatum.[23] Case reports involving children are lacking in detail because they usually appear within discussions of adult patients.[146]

Pediatric case reports of disseminated disease caused by MAC have appeared in the literature. Children have had ulcerative lesions of the colon[40]; mesenteric disease with abscess formation[164]; hematogenous spread to the liver, spleen, kidneys, and adrenal cortex; lesions of the epididymis[175]; bone lesions[201]; and skin lesions. Disseminated osteomyelitis rarely is caused by NTM, but if it occurs, *M. intracellulare* most commonly is isolated.[30,98] Septic arthritis also has been reported in association with osteomyelitis.[60] Immunocompromised patients with disseminated MAC infection historically require multiple-drug therapy, including a combination of azithromycin or clarithromycin and ethambutol. Rifabutin is frequently added as a third agent.[69] Some reports indicate, however, that disseminated disease in HIV-infected patients may respond to only two agents, as mentioned earlier in the discussion of AIDS. The addition of other agents may be necessary because of the high incidence of resistant

organisms. A preliminary report showed that IFN-γ might be effective when combined with conventional therapy in some patients who are refractory to standard chemotherapy alone.[83]

Bacterial peritonitis is a common occurrence in patients regularly undergoing ambulatory peritoneal dialysis for chronic renal failure. Reports of NTM causing peritonitis have been noted.[74,137,211] In cases involving NTM with foreign bodies, such as Tenckhoff catheters, the development of infected sinus tracts is frequent. Additionally, antituberculous drug regimens in these cases generally are unsuccessful. Although the mycobacteria were sensitive to the agents used, the patients continued to have sinus tract drainage without improvement, even after removal of the foreign body.

Mycobacterium scrofulaceum

M. scrofulaceum has many characteristics similar to those of *M. avium* and *M. intracellulare* and can be found in soil, water, and dairy products. This organism is associated most commonly with lymphadenitis in children 1 to 5 years old and rarely causes other manifestations in humans. Skin and bone lesions have been reported in two children chronically infected with *M. scrofulaceum* for 10 years.[51,207] Disseminated disease caused by *M. scrofulaceum* is rare, but was recently reported in a child with IFN-γ receptor 1 deficiency.[122] Few data are available on chemotherapeutic agents for treatment of infection with this organism. Based on susceptibility patterns, three or more drugs may be necessary for treatment of serious disease. Lymphadenitis can be cured with complete excision of the lymph node.

Mycobacterium kansasii

Of the photochromogens, *M. kansasii* is the one most commonly isolated in humans. It is also the second most commonly identified NTM causing disease in the United States. In contrast to *M. avium-intracellulare* and *M. scrofulaceum*, *M. kansasii* rarely is isolated in soil but has been cultured from water and milk.[26,27] Chronic pulmonary infection is the most common manifestation of this disease and is seen mainly in adults, particularly adults with AIDS. A survey from Israel of 56 adults with *M. kansasii* pulmonary infection showed that 64 percent occurred in men, 59 percent had underlying lung disease, and none was infected with HIV.[168] Pulmonary disease occurs infrequently in children. Some children have a course similar to that of adults, with underlying pulmonary disease caused by previous tuberculosis or chronic pulmonary disease.[13,126] The clinical presentation of *M. kansasii* lung disease is similar to that of *M. tuberculosis*, including cavitary infiltrates and more frequent involvement of the upper lobes, although bilateral disease pleural effusions and lymphadenopathy are less common.[169] In contrast, other children have acute symptoms of classic bacterial pneumonia with an abrupt onset of fever and sputum production and lung consolidation on physical examination and radiographs.[13,25]

In contrast to some other nontuberculous organisms, *M. kansasii* is sensitive to most of the antituberculous drugs, particularly rifampin. Most authorities recommend the use of three drugs, including rifampin, isoniazid, and ethambutol. The treatment course usually requires a minimum of 12 months of negative sputum cultures, with therapy for 24 months needed in some patients. A study of 18 patients showed that thrice-weekly clarithromycin, ethambutol, and rifampin was effective, with a mean follow-up of 46 months.[67] Patients with AIDS and *M. kansasii* infection have responded to this three-drug regimen, but the total duration of therapy is unknown at this time. In children with AIDS, the diagnosis of *M. kansasii* infection is rare, and the clinical response to therapy has been poor.[85] Cases have been reported in other immunocompromised children, including a 7-month-old boy with disseminated *M. kansasii* infection and numerous organisms found in his spleen at autopsy.[124] *M. kansasii* also has been reported to cause meningitis; the patients died despite the use of antimycobacterial therapy.[86,161] In another report, an 18-month-old previously healthy boy developed chronic septic arthritis of the ankle with adjacent osteomyelitis of the distal tibia[213] resulting in significant bone destruction.

Mycobacterium malmoense

Buchholz and coworkers[20] reviewed infections with *M. malmoense* in the United States from 1993 to 1995. Only 1 of 73 patients was younger than 10 years. This patient had cervical lymphadenitis and was cured with surgical excision alone. This organism, which frequently is overlooked on standard Löwenstein-Jensen egg medium, grows at 25°C to 37°C (77° to 98.6°F). The organism is slow-growing, and it may require 8 to 12 weeks for colonies to become visible on solid media. The BACTEC system was shown to be superior in isolating *M. malmoense* in one study of children with lymphadenitis.[82] Bone marrow involvement also was described in a patient with chronic granulocytic leukemia.[52] PCR may improve the identification of this organism. The study by Buchholz and colleagues[20] showed that prolonged combination therapy with isoniazid, rifampin, ethambutol, and pyrazinamide after surgical excision was effective in some cases.

Rapidly Growing Mycobacteria

NTM that form colonies on subculture within 7 or fewer days are referred to as *rapidly growing mycobacteria*. The *M. chelonae-abscessus* group consists of *M. chelonae*, *M. abscessus*, *M. bolletii*, *and M. massiliense*. The *M. fortuitum* complex consists of *M. fortuitum*, *M. peregrinum*, and biovariant 3.[103,222] Multilocus DNA sequencing may be needed to differentiate rapidly growing mycobacteria to the species level.[1,227] Patients typically have an underlying condition such as immune suppression or cystic fibrosis and may have a catheter or other implanted device. The most frequent manifestations of rapidly growing mycobacteria include pulmonary infections, bloodstream infections, skin and soft tissue infections, osteomyelitis, device-associated infections, and disseminated disease.[145,222]

M. abscessus, as stated earlier, is related closely to *M. chelonae* but should be designated as a separate species.[103]

Manifestations of *M. abscessus* infection usually involve pulmonary, bloodstream, cutaneous, or occasionally disseminated disease.[68,204,222] *M. abscessus* is the most common rapidly growing mycobacteria isolate causing pulmonary disease.[68] Underlying conditions such as bronchiectasis, gastroesophageal disorders with chronic vomiting, and cystic fibrosis are frequently present. Multilobar nodular bronchiectatic disease is the most common presentation. Cutaneous disease is often related to surgery or trauma and manifests as purple nodules. Maxson and associates[123] reported a case of osteomyelitis caused by *M. abscessus*.

Wallace and associates[202] provided the largest series of patients with skin, soft tissue, and bone involvement with *M. chelonae*, which is the primary clinical manifestation. Steroid use seemed to be the factor associated most commonly with the development of disease. Adult renal transplant patients also have been described with skin and subcutaneous tissue involvement caused by *M. chelonae*.[36] *M. chelonae*, likewise, has been described as an etiologic agent for otitis media, probably from contamination of ear, nose, and throat instruments with colonized water sources.[115] Cases of keratitis caused by *M. chelonae* have been reported after laser in situ keratomileusis (LASIK).[59,225] Pulmonary disease is less common.[68] With the improved ability to distinguish *M. abscessus* and *M. chelonae*, many isolates that were once identified as *M. chelonae* may now be identified as *M. abscessus*.[222]

M. fortuitum complex is one of the most frequently isolated rapidly growing mycobacteria and causes a range of diseases, including pulmonary, wound, lymphadenitis, and catheter infections.[145,222] *M. fortuitum* and *M. chelonae* have been implicated in sternal wound infections and endocarditis and have occurred in outbreak-type settings.[102,157,187] Patients responded to surgical débridement and amikacin with cefoxitin. A case series from Hong Kong reported successful treatment of *M. fortuitum* sternotomy infections with the use of a single daily dose ofloxacin as monotherapy in three patients.[226] Pulmonary disease with findings similar to those in *M. abscessus* occur in patients with *M. fortuitum*, but primarily in patients with gastroesophageal disorders with chronic vomiting.[68]

Other rapidly growing mycobacteria include *M. mucogenicum*, which is commonly isolated in cancer patients. In this population, *M. mucogenicum* is the rapidly growing mycobacteria most frequently associated with bloodstream and catheter-associated infections.[222] An outbreak of *M. mucogenicum* catheter-associated bloodstream infections occurred in eight pediatric hematology and oncology patients.[166] Six of the patients were successfully treated with catheter removal alone. *Mycobacterium neoaurum* also has been associated with catheter-associated bloodstream infections in immune-suppressed patients, including children.[205] *Mycobacterium smegmatis*, which resembles *M. fortuitum*, is a rapidly growing mycobacterium responsible for skin and soft tissue infections.[203] A case of disseminated infection has been reported in a child with inherited IFN-γ receptor deficiency.[138] *M. massiliense*, which is a rapidly growing mycobacterium closely related to *M. abscessus*, was associated with an outbreak of intramuscular injection–associated soft tissue infections in adults and children.[96] *Mycobacterium septicum* is a recently described rapidly growing mycobacterial species associated with catheter-related bacteremia.[163] *Mycobacterium wolinskyi* is an uncommon cause of posttraumatic and postsurgical wound infections and osteomyelitis.[7] As our ability to detect and distinguish different mycobacterial species improves, the number of mycobacterial species implicated in human infection will likely continue to increase.

Treatment of rapidly growing mycobacteria usually requires combination therapy involving at least two agents. Because susceptibility differs across species, these bacteria should be identified to the species level and treatment based on susceptibility testing. In general, skin and soft tissue disease should be treated for at least 4 months and bone disease for at least 6 months. Surgery may be required for extensive disease. For infections involving implants, the foreign body should be removed. Pulmonary disease often is difficult to treat, but a goal of at least 12 months of negative sputum is reasonable.[69]

OTHER SITES OF INFECTION

Carpal tunnel syndrome in adults has been reported as being caused by *M. szulgai*, an uncommon scotochromogenic mycobacterium.[184] In a review of 21 cases of *M. szulgai*, pulmonary disease was most common, with disseminated disease more common in immune-suppressed patients.[199] Effective treatment included débridement, ethambutol, and rifampin. Other infections in humans include choroiditis,[34] panniculitis,[160] parotitis,[29] genitourinary tract infection,[17,188] and synovitis.[185] Ear infections with NTM also have been reported, as has mastoiditis.[58,134,183] More recently, infection of Broviac catheters in pediatric patients with leukemia and hemodialysis and peritoneal dialysis catheters has been described.[24,28,108,166,194] In all cases, removal of the catheter was required for resolution of the infection.

With the continued proliferation of immunocompromised patients because of AIDS and new treatment modalities that induce an immunocompromised state (e.g., organ transplantation, new immunosuppressive drugs), NTM probably will continue to remain important pathogens. With newer isolation techniques and new technology such as DNA probes, the ability to diagnose these infections and understanding of the pathogenesis of the infections that these organisms produce should improve, as should the ability to treat these infections.

NEW REFERENCES SINCE THE SIXTH EDITION

7. Ariza-Heredia EJ, Dababneh AS, Wilhelm MP, et al. *Mycobacterium wolinskyi*: a case series and review of the literature. *Diagn Microbiol Infect Dis* 2011;**71**:421–7.
15. Blyth CC, Best EJ, Jones CA, et al. Nontuberculous mycobacterial infection in children: a prospective national study. *Pediatr Infect Dis J* 2009;**28**:801–5.
23. Chan ED, Iseman MD. Slender, older women appear to be more susceptible to nontuberculous mycobacterial lung disease. *Gend Med* 2010;**7**:5–18.
24. Chang CY, Tsay RW, Lin LC, et al. Venous catheter-associated bacteremia caused by rapidly growing mycobacteria at a medical center in central Taiwan. *J Microbiol Immunol Infect* 2009;**42**:343–50.

29. Chen CC, Chen SY, Chen YS, et al. *Mycobacterium fortuitum*-induced persistent parotitis: successful therapy with clarithromycin and ciprofloxacin. *Head Neck* 2007;**29**:1061–4.
31. Choi P, Qin X, Chen EY, et al. Polymerase chain reaction for pathogen identification in persistent pediatric cervical lymphadenitis. *Arch Otolaryngol Head Neck Surg* 2009;**135**:243–8.
37. Cotugno N, Douagi I, Rossi P, et al. Suboptimal immune reconstitution in vertically HIV infected children: a view on how HIV replication and timing of HAART initiation can impact on T and B-cell compartment. *Clin Dev Immunol* 2012;**2012**:805151.
38. Cruz A, Ong LT, Starke JR. Mycobacterial infections in Texas children: a 5-year case series. *Pediatr Infect Dis* 2010;**29**:772–4.
48. Dorman SE, Holland SM. Interferon-gamma and interleukin-12 pathway defects and human disease. *Cytokine Growth Factor Rev* 2000;**11**:321–33.
53. Esther CR Jr, Esserman DA, Gilligan P, et al. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. *J Cyst Fibrosis* 2010;**9**:117–23.
55. Euzeby JP. *List of prokaryotic names with standing in nomenclature: genus* Mycobacterium. Available from: http://www.bacterio.cict.fr/m/mycobacterium.html.
59. Freitas D, Alvarenga L, Sampaio J, et al. An outbreak of *Mycobacterium chelonae* infection after LASIK. *Ophthalmology* 2003;**110**:276–85.
62. Gona P, Van Dyke RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *JAMA* 2006;**296**:292–300.
68. Griffith DE, Girard WM, Wallace RJ Jr. Clinical features of pulmonary disease caused by rapidly growing mycobacteria: an analysis of 154 patients. *Am Rev Respir Dis* 1993;**147**:1271–8.
69. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
75. Harris RL, Modayil P, Adam J, et al. Cervicofacial nontuberculous *Mycobacterium* lymphadenitis in children: is surgery always necessary? *Int J Pediatr Otorhinolaryngol* 2009;**73**:1297–301.
96. Kim HY, Yun YJ, Park CG, et al. Outbreak of *Mycobacterium massiliense* infection associated with intramuscular injections. *J Clin Microbiol* 2007;**45**:3127–30.
97. Kim JU, Cha CH, An HK. Multiplex real-time PCR assay and melting curve analysis for identifying *Mycobacterium* tuberculosis complex and nontuberculous mycobacteria. *J Clin Microbiol* 2012;**50**:483–7.
106. Lavender CJ, Fyfe JA, Azuolas J, et al. Risk of Buruli ulcer and detection of *Mycobacterium ulcerans* in mosquitoes in southeastern Australia. *PLoS Negl Trop Dis* 2011;**5**:e1305.
107. Lee WJ, Kang SM, Sung H, et al. Non-tuberculous mycobacterial infections of the skin: a retrospective study of 29 cases. *J Dermatol* 2010;**37**:965–72.
114. Lindeboom JA, Lindeboom R, Bruijnesteijn van Coppenraet ES, et al. Esthetic outcome of surgical excision versus antibiotic therapy for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Pediatr Infect Dis J* 2009;**28**:1028–30.
122. Marazzi MG, Chapgier A, Defilippi AC, et al. Disseminated *Mycobacterium scrofulaceum* infection in a child with interferon-gamma receptor 1 deficiency. *Int J Infect Dis* 2010;**14**:e167–70.
125. McNabb A, Eisler D, Adie K, et al. Assessment of partial sequencing of the 65-kilodalton heat shock protein gene *(hsp65)* for routine identification of *Mycobacterium* species isolated from clinical sources. *J Clin Microbiol* 2004;**42**:3000–11.
127. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1–166.
133. Nesheim SR, Kapogiannis BG, Soe MM, et al. Trends in opportunistic infections in the pre- and post-highly active antiretroviral therapy eras among HIV-infected children in the Perinatal AIDS Collaborative Transmission Study, 1986-2004. *Pediatrics* 2007;**120**:100–9.
139. Pilkington EF, MacArthur CJ, Beekmann SE, et al. Treatment patterns of pediatric nontuberculous mycobacterial (NTM) cervical lymphadenitis as reported by nationwide surveys of pediatric otolaryngology and infectious disease societies. *Int J Pediatr Otorhinolaryngol* 2010;**74**:343–6.
142. Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med* 2010;**182**:970–6.
145. Redelman-Sidi G, Sepkowitz KA. Rapidly growing mycobacteria infection in patients with cancer. *Clin Infect Dis* 2010;**51**:422–34.
149. Reuss AM, Wiese-Posselt M, Weissmann B, et al. Incidence rate of nontuberculous mycobacterial disease in immunocompetent children: a prospective nationwide surveillance study in Germany. *Pediatr Infec Dis J* 2009;**28**:642–4.
150. Richardson ET, Samson D, Banaei N. Rapid identification of *Mycobacterium tuberculosis* and nontuberculous mycobacteria by multiplex, real-time PCR. *J Clin Microbiol* 2009;**47**:1497–502.
153. Roux AL, Catherinot E, Ripoll F, et al. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. *J Clin Microbiol* 2009;**47**:4124–8.
166. Shachor-Meyouhas Y, Sprecher H, Eluk O, et al. An outbreak of *Mycobacterium mucogenicum* bacteremia in pediatric hematology-oncology patients. *Pediatr Infect Dis J* 2011;**30**:30–2.
167. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy. *AIDS* 2005;**19**:399–406.
169. Shitrit D, Priess R, Peled N, et al. Differentiation of *Mycobacterium kansasii* infection from *Mycobacterium tuberculosis* infection: comparison of clinical features, radiological appearance, and outcome. *Eur J Clin Microbiol Infect Dis* 2007;**26**:679–84.
178. Sousa TS, Matherne RJ, Wilkerson MG. Case report: cutaneous nontuberculous mycobacterial abscesses associated with insulin injections. *J Drugs Dermatol* 2010;**9**:1439–42.
191. Tortoli E. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. *Clin Microbiol Rev* 2003;**16**:319–54.
193. Tremblay V, Ayad T, Lapointe A, et al. Nontuberculous mycobacterial cervicofacial adenitis in children: epidemiologic study. *J Otolaryngol Head Neck Surg* 2008;**37**:616–22.
194. Tse KC, Lui SL, Cheng VC, et al. A cluster of rapidly growing mycobacterial peritoneal dialysis catheter exit-site infections. *Am J Kidney Dis* 2007;**50**:e1–5.
197. van Coppenraet LS, Smit VT, Templeton KE, et al. Application of real-time PCR to recognize atypical mycobacteria in archival skin biopsies: high prevalence of *Mycobacterium haemophilum*. *Diag Mol Pathol* 2007;**16**:81–6.
199. van Ingen J, Boeree MJ, de Lange WC, et al. Clinical relevance of *Mycobacterium szulgai* in The Netherlands. *Clin Infect Dis* 2008;**46**:1200–5.
205. Washer LL, Riddell JT, Rider J, et al. *Mycobacterium neoaurum* bloodstream infection: report of 4 cases and review of the literature. *Clin Infect Dis* 2007;**45**:e10–13.
209. Wertman R, Miller M, Groben P, et al. *Mycobacterium bolletii/Mycobacterium massiliense* furunculosis associated with pedicure footbaths: a report of 3 cases. *Arch Dermatol* 2011;**147**:454–8.
213. Williams B, Neth O, Shingadia D, et al. *Mycobacterium kansasii* causing septic arthritis and osteomyelitis in a child. *Pediatr Infect Dis J* 2010;**9**:88–9.
214. Winthrop KL, Chang E, Yamashita S, et al. Nontuberculous mycobacteria infections and anti-tumor necrosis factor-alpha therapy. *Emerg Infect Dis* 2009;**15**:1556–61.
215. Winthrop KL, McNelley E, Kendall B, et al. Pulmonary nontuberculous mycobacterial disease prevalence and clinical features: an emerging public health disease. *Am J Respir Crit Care Med* 2010;**182**:977–82.
222. Xiang YH, De I, Jacobson KL. Rapidly growing mycobacteria. *Am J Clin Pathol* 2007;**128**:612–21.
225. Yamaguchi T, Bissen-Miyajima H, Hori-Komai Y, et al. Infectious keratitis outbreak after laser in situ keratomileusis at a single laser center in Japan. *J Cataract Refract Surg* 2011;**37**:894–900.
227. Zelazny AM, Root JM, Shea YR, et al. Cohort study of molecular identification and typing of *Mycobacterium abscessus*, *Mycobacterium massiliense*, and *Mycobacterium bolletii*. *J Clin Microbiol* 2009;**47**:1985–95.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adekambi T, Berger P, Raoult D, Drancourt M. *rpoB* gene sequence-based characterization of emerging non-tuberculous mycobacteria with descriptions of *Mycobacterium bolletii* sp. nov., *Mycobacterium phocaicum* sp. nov. and *Mycobacterium aubagnense* sp. nov. *Int J Syst Evol Microbiol* 2006;**56**:133–43.
2. Agy MB, Wallis CK, Plorde JJ, et al. Evaluation of four mycobacterial blood culture media: BACTEC 13A, Isolator/BACTEC 12B, Isolator/Middlebrook agar, and a biphasic medium. *Diagn Microbiol Infect Dis* 1989;**12**:303–8.
3. Ahn CH, McLarty JW, Ahn SS, et al. Diagnostic criteria for pulmonary disease caused by *Mycobacterium kansasii* and *Mycobacterium intracellulare*. *Am Rev Respir Dis* 1982;**125**:388–91.
4. Aitken ML, Burke W, McDonald G, et al. Nontuberculous mycobacterial disease in adult cystic fibrosis patients. *Chest* 1993;**103**: 1096–9.
5. Altman RP, Margileth AM. Cervical lymphadenopathy from atypical mycobacteria: diagnosis and surgical treatment. *J Pediatr Surg* 1975;**10**:419–22.
6. Alvarez-Elcoro S, Enzler MJ. The macrolides: erythromycin, clarithromycin, and azithromycin. *Mayo Clin Proc* 1999;**74**: 613–34.
7. Ariza-Heredia EJ, Dababneh AS, Wilhelm MP, et al. *Mycobacterium wolinskyi*: a case series and review of the literature. *Diagn Microbiol Infect Dis* 2011;**71**:421–7.
8. Armstrong KL, James RW, Dawson DJ, et al. *Mycobacterium haemophilum* causing perihilar or cervical lymphadenitis in healthy children. *J Pediatr* 1992;**121**:202–5.
9. Aubry A, Chosidow O, Caumes E, et al. Sixty-three cases of *Mycobacterium marinum* infection: clinical features, treatment, and antibiotic susceptibility of causative isolates. *Arch Intern Med* 2002;**162**:1746–52.
10. Barton LL, Feigin RD. Childhood cervical lymphadenitis: a reappraisal. *J Pediatr* 1974;**84**:846–52.
11. Bell RC, Higuchi JH, Donovan WN, et al. *Mycobacterium simiae:* clinical features and follow-up of twenty-four patients. *Am Rev Respir Dis* 1983;**127**:35–8.
12. Benjamin DR. Granulomatous lymphadenitis in children. *Arch Pathol Lab Med* 1987;**111**:750–3.
13. Bialkin G, Pollak A, Weil AJ. Pulmonary infection with *Mycobacterium kansasii*. *Am J Dis Child* 1961;**101**:739–48.
14. Blacklock ZM, Dawson DJ. Atypical mycobacteria causing nonpulmonary disease in Queensland. *Pathology* 1979;**11**:283–7.
15. Blyth CC, Best EJ, Jones CA, et al. Nontuberculous mycobacterial infection in children: a prospective national study. *Pediatr Infect Dis J* 2009;**28**:801–5.
16. Brook I. Aerobic and anaerobic bacteriology of cervical adenitis in children. *Clin Pediatr* 1980;**19**:693–6.
17. Brooker WJ, Aufderheide AC. Genitourinary tract infections due to atypical mycobacteria. *J Urol* 1980;**124**:242–4.
18. Brooks RW, Parker BC, Gruft H, et al. Epidemiology of infection by nontuberculous mycobacteria. V. Numbers in eastern United States soils and correlation with soil characteristics. *Am Rev Respir Dis* 1984;**130**:630–3.
19. Brown JW 3rd, Sanders CV. *Mycobacterium marinum* infections: a problem of recognition, not therapy? *Arch Intern Med* 1987;**147**: 817–18.
20. Buchholz UT, McNeil MM, Keyes LE, et al. *Mycobacterium malmoense* infections in the United States, January 1993 through June 1995. *Clin Infect Dis* 1998;**27**:551–8.
21. Burchard GD, Bierther M. Buruli ulcer: clinical pathological study of 23 patients in Lambarene, Gabon. *Trop Med Parasitol* 1986; **37**:1–8.
22. Centers for Disease Control and Prevention. *HIV/AIDS surveillance year-end edition: U. S. AIDS cases reported through December 1992*. 1993.
23. Chan ED, Iseman MD. Slender, older women appear to be more susceptible to nontuberculous mycobacterial lung disease. *Gend Med* 2010;**7**:5–18.
24. Chang CY, Tsay RW, Lin LC, et al. Venous catheter-associated bacteremia caused by rapidly growing mycobacteria at a medical center in central Taiwan. *J Microbiol Immunol Infect* 2009;**42**: 343–50.
25. Chapman JS. Varieties of tuberculosis in children. *Minn Med* 1959;**42**:1773–8.
26. Chapman JS, Speight M. Isolation of atypical mycobacteria from pasteurized milk. *Am Rev Respir Dis* 1968;**98**:1052–4.
27. Chapman JS, Bernard JS, Speight M. Isolation of mycobacteria from raw milk. *Am Rev Respir Dis* 1965;**91**:351–5.
28. Chawla PG, Nevins TE. Management of hemodialysis catheter-related bacteremia: a 10-year experience. *Pediatr Nephrol* 2000;**14**: 198–202.
29. Chen CC, Chen SY, Chen YS, et al. *Mycobacterium fortuitum*-induced persistent parotitis: successful therapy with clarithromycin and ciprofloxacin. *Head Neck* 2007;**29**:1061–4.
30. Chicoine L, Lapointe N, Simoneau R, et al. "Anonymous" mycobacterial infection causing disseminated osteomyelitis and skin lesions. *Can Med Assoc J* 1968;**98**:1059–62.
31. Choi P, Qin X, Chen EY, et al. Polymerase chain reaction for pathogen identification in persistent pediatric cervical lymphadenitis. *Arch Otolaryngol Head Neck Surg* 2009;**135**:243–8.
32. Christensen EE, Dietz GW, Ahn CH, et al. Initial roentgenographic manifestations of pulmonary *Mycobacterium tuberculosis*, *M kansasii*, and *M intracellularis* infections. *Chest* 1981;**80**:132–6.
33. Clancey JK, Dodge OG, Lunn HF, et al. Mycobacterial skin ulcers in Uganda. *Lancet* 1961;**2**:951–4.
34. Clever VG. Choroidal involvement with *Mycobacterium intracellulare*. *Ann Ophthalmol* 1980;**12**:1409–11.
35. Colville A. Retrospective review of culture-positive mycobacterial lymphadenitis cases in children in Nottingham, 1979-1990. *Eur J Clin Microbiol* 1993;**12**:192–5.
36. Cooper JF, Lichtenstein MJ, Graham BS, et al. *Mycobacterium chelonae*: a cause of nodular skin lesions with a proclivity for renal transplant recipients. *Am J Med* 1989;**86**:173–7.
37. Cotugno N, Douagi I, Rossi P, et al. Suboptimal immune reconstitution in vertically HIV infected children: a view on how HIV replication and timing of HAART initiation can impact on T and B-cell compartment. *Clin Dev Immunol* 2012;**2012**: 805151.
38. Cruz A, Ong LT, Starke JR. Mycobacterial infections in Texas children: a 5-year case series. *Pediatr Infect Dis* 2010;**29**:772–4.
39. Currarino G, Votteler T, Weinberg A. Atypical mycobacterial infection of intraparotid lymph nodes. Clinical and sialographic observations. *Pediatr Radiol* 1977;**6**:10–12.
40. Cuttino JT. Infectious keratitis outbreak after laser in situ keratomileusis at a single laser center in Japan. *Am J Pathol* 1949; **25**:1–47.
41. Dailloux M, Morlot M, Sirbat C. [Study of factors affecting presence of atypical mycobacteria in water of a swimming pool (author's transl)]. *Rev Epidemiol Sante Publique* 1980;**28**:299–306.
42. Davidson PT. International conference on atypical mycobacteria: introduction. *Rev Infect Dis* 1981;**3**:816–18.
43. Davis BR, Brumbach J, Sanders WJ, et al. Skin lesions caused by *Mycobacterium haemophilum*. *Ann Intern Med* 1982;**97**:723–4.
44. Deepe GS Jr, Capparell R, Coonrod JD. Atypical mycobacterial lymphadenitis in an adult. *Chest* 1980;**78**:882–3.
45. Delabie J, De Wolf-Peeters C, Bobbaers H, et al. Immunophenotypic analysis of histiocytes involved in AIDS-associated *Mycobacterium scrofulaceum* infection: similarities with lepromatous lepra. *Clin Exp Immunol* 1991;**85**:214–18.
46. Dhooge I, Dhooge C, De Baets F, et al. Diagnostic and therapeutic management of atypical mycobacterial infections in children. *Eur Arch Otorhinolaryngol* 1993;**250**:387–91.
47. Donta ST, Smith PW, Levitz RE, et al. Therapy of *Mycobacterium marinum* infections: use of tetracyclines vs rifampin. *Arch Intern Med* 1986;**146**:902–4.
48. Dorman SE, Holland SM. Interferon-gamma and interleukin-12 pathway defects and human disease. *Cytokine Growth Factor Rev* 2000;**11**:321–33.
49. Drut R, Anderson V, Greco MA, et al. Opportunistic infections in pediatric HIV infection: a study of 74 autopsy cases from Latin America. The Latin American AIDS Pathology Study Group. *Pediatr Pathol Lab Med* 1997;**17**:569–76.
50. Dunn BL, Hodgson DJ. "Atypical" mycobacteria in milk. *J Appl Bacteriol* 1982;**52**:373–6.
51. Dustin P, Demol P, Derks-Jacobovitz D, et al. Generalized fatal chronic infection by *Mycobacterium scrofulaceum* with severe amyloidosis in a child. *Pathol Res Pract* 1980;**168**:237–48.
52. Engervall P, Bjorkholm M, Petrini B, et al. Disseminated *Mycobacterium malmoense* infection in a patient with chronic granulocytic leukaemia. *J Intern Med* 1993;**234**:231–3.

53. Esther CR Jr, Esserman DA, Gilligan P, et al. Chronic *Mycobacterium abscessus* infection and lung function decline in cystic fibrosis. *J Cyst Fibrosis* 2010;**9**:117–23.
54. Esther CR Jr, Henry MM, Molina PL, et al. Nontuberculous mycobacterial infection in young children with cystic fibrosis. *Pediatr Pulmonol* 2005;**40**:39–44.
55. Euzeby JP. *List of prokaryotic names with standing in nomenclature: genus* Mycobacterium. Available from: http://www.bacterio.cict.fr/m/mycobacterium.html.
56. Fergie JE, Milligan TW, Henderson BM, et al. Intrathoracic *Mycobacterium avium* complex infection in immunocompetent children: case report and review. *Clin Infect Dis* 1997;**24**:250–3.
57. Field SK, Fisher D, Cowie RL. *Mycobacterium avium* complex pulmonary disease in patients without HIV infection. *Chest* 2004; **126**:566–81.
58. Flint D, Mahadevan M, Gunn R, et al. Nontuberculous mycobacterial otomastoiditis in children: four cases and a literature review. *Int J Pediatr Otorhinolaryngol* 1999;**51**:121–7.
59. Freitas D, Alvarenga L, Sampaio J, et al. An outbreak of *Mycobacterium chelonae* infection after LASIK. *Ophthalmology* 2003;**110**: 276–85.
60. Frosch M, Roth J, Ullrich K, et al. Successful treatment of *Mycobacterium avium* osteomyelitis and arthritis in a non-immunocompromised child. *Scand J Infect Dis* 2000;**32**:328–9.
61. Gill MJ, Fanning EA, Chomyc S. Childhood lymphadenitis in a harsh northern climate due to atypical mycobacteria. *Scand J Infect Dis* 1987;**19**:77–83.
62. Gona P, Van Dyke RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *JAMA* 2006;**296**:292–300.
63. Good RC, Snider DE Jr. Isolation of nontuberculous mycobacteria in the United States. 1980. *J Infect Dis* 1982;**146**:829–33.
64. Gorse GJ, Fairshter RD, Friedly G, et al. Nontuberculous mycobacterial disease: experience in a southern California hospital. *Arch Intern Med* 1983;**143**:225–8.
65. Goto M, Oka S, Okuzumi K, et al. Evaluation of acridinium-ester-labeled DNA probes for identification of *Mycobacterium tuberculosis* and *Mycobacterium avium-Mycobacterium intracellulare* complex in culture. *J Clin Microbiol* 1991;**29**:2473–6.
66. Grange JM. Mycobacteria and the skin. *Int J Dermatol* 1982;**21**: 497–503.
67. Griffith DE, Brown-Elliott BA, Wallace RJ Jr. Thrice-weekly clarithromycin-containing regimen for treatment of *Mycobacterium kansasii* lung disease: results of a preliminary study. *Clin Infect Dis* 2003;**37**:1178–82.
68. Griffith DE, Girard WM, Wallace RJ Jr. Clinical features of pulmonary disease caused by rapidly growing mycobacteria: an analysis of 154 patients. *Am Rev Respir Dis* 1993;**147**: 1271–8.
69. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
70. Gruft H, Falkinham JO 3rd, Parker BC. Recent experience in the epidemiology of disease caused by atypical mycobacteria. *Rev Infect Dis* 1981;**3**:990–6.
71. Guthertz LS, Damsker B, Bottone EJ, et al. *Mycobacterium avium* and *Mycobacterium intracellulare* infections in patients with and without AIDS. *J Infect Dis* 1989;**160**:1037–41.
72. Haas WH, Amthor B, Engelmann G, et al. Preoperative diagnosis of *Mycobacterium avium* lymphadenitis in two immunocompetent children by polymerase chain reaction of gastric aspirates. *Pediatr Infect Dis J* 1998;**17**:1016–20.
73. Haas WH, Butler WR, Kirschner P, et al. A new agent of mycobacterial lymphadenitis in children: *Mycobacterium heidelbergense* sp. nov. *J Clin Microbiol* 1997;**35**:3203–9.
74. Hakim A, Hisam N, Reuman PD. Environmental mycobacterial peritonitis complicating peritoneal dialysis: three cases and review. *Clin Infect Dis* 1993;**16**:426–31.
75. Harris RL, Modayil P, Adam J, et al. Cervicofacial nontuberculous *Mycobacterium* lymphadenitis in children: is surgery always necessary? *Int J Pediatr Otorhinolaryngol* 2009;**73**:1297–301.
76. Havlir D, Kemper CA, Deresinski SC. Reproducibility of lysis-centrifugation cultures for quantification of *Mycobacterium avium* complex bacteremia. *J Clin Microbiol* 1993;**31**:1794–8.
77. Havlir DV, Dube MP, Sattler FR, et al. Prophylaxis against disseminated *Mycobacterium avium* complex with weekly azithromycin, daily rifabutin, or both. California Collaborative Treatment Group. *New Engl J Med* 1996;**335**:392–8.
78. Hazra R, Lee SH, Maslow JN, et al. Related strains of *Mycobacterium avium* cause disease in children with AIDS and in children with lymphadenitis. *J Infect Dis* 2000;**181**:1298–303.
79. Hjelt K, Hojlyng N, Howitz P, et al. The role of Mycobacteria Other Than Tuberculosis (MOTT) in patients with cystic fibrosis. *Scand J Infect Dis* 1994;**26**:569–76.
80. Hjelte L, Petrini B, Kallenius G, et al. Prospective study of mycobacterial infections in patients with cystic fibrosis. *Thorax* 1990;**45**: 397–400.
81. Hofer M, Hirschel B, Kirschner P, et al. Brief report: disseminated osteomyelitis from *Mycobacterium ulcerans* after a snakebite. *New Engl J Med* 1993;**328**:1007–9.
82. Hoffner SE, Henriques B, Petrini B, et al. *Mycobacterium malmoense*: an easily missed pathogen. *J Clin Microbiol* 1991;**29**: 2673–4.
83. Holland SM, Eisenstein EM, Kuhns DB, et al. Treatment of refractory disseminated nontuberculous mycobacterial infection with interferon gamma: a preliminary report. *New Engl J Med* 1994;**330**:1348–55.
84. Horsburgh CR Jr, Selik RM. The epidemiology of disseminated nontuberculous mycobacterial infection in the acquired immunodeficiency syndrome (AIDS). *Am Rev Respir Dis* 1989;**139**:4–7.
85. Hoyt L, Oleske J, Holland B, et al. Nontuberculous mycobacteria in children with acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 1992;**11**:354–60.
86. Huempener HR, Kingsolver WR, Deuschle KW. Tuberculous meningitis caused by both *Mycobacterium tuberculosis* and atypical mycobacteria. *Am Rev Respir Dis* 1966;**94**:612–14.
87. Iseman MD. *Mycobacterium* avium complex and the normal host: the other side of the coin. *New Engl J Med* 1989;**321**:896–8.
88. Jindal N, Devi B, Aggarwal A. Mycobacterial cervical lymphadenitis in childhood. *Indian J Med Sci* 2003;**57**:12–5.
89. Johann-Liang R, Cervia JS, Noel GJ. Characteristics of human immunodeficiency virus-infected children at the time of death: an experience in the 1990s. *Pediatr Infect Dis J* 1997;**16**:1145–50.
90. Kahn E. Gastrointestinal manifestations in pediatric AIDS. *Pediatr Pathol Lab Med* 1997;**17**:171–208.
91. Kent DC. Tuberculous lymphadenitis: not a localized disease process. *Am J Med Sci* 1967;**254**:866–74.
92. Kiehn TE, Cammarata R. Laboratory diagnosis of mycobacterial infections in patients with acquired immunodeficiency syndrome. *J Clin Microbiol* 1986;**24**:708–11.
93. Kiehn TE, Cammarata R. Comparative recoveries of *Mycobacterium avium-M. intracellulare* from isolator lysis-centrifugation and BACTEC 13A blood culture systems. *J Clin Microbiol* 1988;**26**: 760–1.
94. Kiehn TE, Edwards FF, Brannon P, et al. Infections caused by *Mycobacterium avium* complex in immunocompromised patients: diagnosis by blood culture and fecal examination, antimicrobial susceptibility tests, and morphological and seroagglutination characteristics. *J Clin Microbiol* 1985;**21**:168–73.
95. Kilby JM, Gilligan PH, Yankaskas JR, et al. Nontuberculous mycobacteria in adult patients with cystic fibrosis. *Chest* 1992;**102**:70–5.
96. Kim HY, Yun YJ, Park CG, et al. Outbreak of *Mycobacterium massiliense* infection associated with intramuscular injections. *J Clin Microbiol* 2007;**45**:3127–30.
97. Kim JU, Cha CH, An HK. Multiplex real-time PCR assay and melting curve analysis for identifying *Mycobacterium* tuberculosis complex and nontuberculous mycobacteria. *J Clin Microbiol* 2012;**50**:483–7.
98. Koenig MG, Collins RD, Heyssel RM. Disseminated mycobacteriosis caused by Battey type mycobacteria. *Ann Intern Med* 1966; **64**:145–54.
99. Krasnow I, Gross W. *Mycobacterium simiae* infection in the United States: a case report and discussion of the organism. *Am Rev Respir Dis* 1975;**111**:357–60.
100. Kubica GP. [Committee of Bacteriology and Immunology. Current nomenclature of mycobacteria: 1978]. *Bull Int Union Tuberc* 1978;**53**:211–17.
101. Kubica GP, Wayne LG, editors. *The mycobacteria: A sourcebook*. New York: Marcel Dekker; 1984.

102. Kuritsky JN, Bullen MG, Broome CV, et al. Sternal wound infections and endocarditis due to organisms of the *Mycobacterium fortuitum* complex. *Ann Intern Med* 1983;**98**:938–9.
103. Kusunoki S, Ezaki T. Proposal of *Mycobacterium peregrinum* sp. nov., nom. rev., and elevation of *Mycobacterium chelonae* subsp. *abscessus* (Kubica et al.) to species status: *Mycobacterium abscessus* comb. nov. *Int J Syst Bacteriol* 1992;**42**:240–5.
104. Kuth G, Lamprecht J, Haase G. Cervical lymphadenitis due to mycobacteria other than tuberculosis: an emerging problem in children? *J Otorhinolaryngol* 1995;**57**:36–8.
105. Lai KK, Stottmeier KD, Sherman IH, et al. Mycobacterial cervical lymphadenopathy: relation of etiologic agents to age. *JAMA* 1984;**251**:1286–8.
106. Lavender CJ, Fyfe JA, Azuolas J, et al. Risk of Buruli ulcer and detection of *Mycobacterium ulcerans* in mosquitoes in southeastern Australia. *PLoS Negl Trop Dis* 2011;**5**:e1305.
107. Lee WJ, Kang SM, Sung H, et al. Non-tuberculous mycobacterial infections of the skin: a retrospective study of 29 cases. *J Dermatol* 2010;**37**:965–72.
108. Levendoglu-Tugal O, Munoz J, Brudnicki A, et al. Infections due to nontuberculous mycobacteria in children with leukemia. *Clin Infect Dis* 1998;**27**:1227–30.
109. Levin M, Newport MJ, D'Souza S, et al. Familial disseminated atypical mycobacterial infection in childhood: a human mycobacterial susceptibility gene? *Lancet* 1995;**345**:79–83.
110. Lewis LL. Nontuberculous mycobacterial infections. In: Pizzo PA, Wilfert CM, editors. *Pediatric AIDS: The challenge of HIV infection in infants, children, and adolescents*. 2nd ed. Baltimore: Williams & Wilkins; 1994. p. 308–20.
111. Lim SD, Todd J, Lopez J, et al. Genotypic identification of pathogenic *Mycobacterium* species by using a nonradioactive oligonucleotide probe. *J Clin Microbiol* 1991;**29**:1276–8.
112. Lincoln EM, Gilbert LA. Disease in children due to mycobacteria other than *Mycobacterium* tuberculosis. *Am Rev Respir Dis* 1972; **105**:683–714.
113. Lindberg MC, Thomas GG. Lymphadenitis due to atypical mycobacteria. *Ala Med* 1989;**59**:19–21.
114. Lindeboom JA, Lindeboom R, Bruijnesteijn van Coppenraet ES, et al. Esthetic outcome of surgical excision versus antibiotic therapy for nontuberculous mycobacterial cervicofacial lymphadenitis in children. *Pediatr Infect Dis J* 2009;**28**:1028–30.
115. Lowry PW, Jarvis WR, Oberle AD, et al. *Mycobacterium chelonae* causing otitis media in an ear-nose-and-throat practice. *New Engl J Med* 1988;**319**:978–82.
116. Lunn HF, Ree RJ. Treatment of mycobacterial skin ulcers in Uganda with a riminophenazine derivative (B.663). *Lancet* 1964; **1**:247–9.
117. MacCullum P. A new mycobacterial infection in man. I. Clinical aspects. *J Pathol Bacteriol* 1948;**60**:93–102.
118. Maloney JM, Gregg CR, Stephens DS, et al. Infections caused by *Mycobacterium szulgai* in humans. *Rev Infect Dis* 1987;**9**:1120–6.
119. Maltezou HC, Spyridis P, Kafetzis DA. Nontuberculous mycobacterial lymphadenitis in children. *Pediatr Infect Dis* 1999;**18**: 968–70.
120. Mandell DL, Wald ER, Michaels MG, et al. Management of nontuberculous mycobacterial cervical lymphadenitis. *Arch Otolaryngol Head Neck Surg* 2003;**129**:341–4.
121. Mankiewicz E, Majdaniw O. Atypical mycobacteria in tapwater. *Can J Public Health* 1982;**73**:358–60.
122. Marazzi MG, Chapgier A, Defilippi AC, et al. Disseminated *Mycobacterium scrofulaceum* infection in a child with interferon-gamma receptor 1 deficiency. *Int J Infect Dis* 2010;**14**:e167–70.
123. Maxson S, Schutze GE, Jacobs RF. *Mycobacterium* abscessus osteomyelitis: treatment with clarithromycin. *Infect Dis Clin Pract* 1994;**3**:203–6.
124. McCracken GH Jr, Reynolds RC. Primary lymphopenic immunologic deficiency: disseminated *Mycobacterium kansasii* infection. *Am J Dis Child* 1970;**120**:143–7.
125. McNabb A, Eisler D, Adie K, et al. Assessment of partial sequencing of the 65-kilodalton heat shock protein gene (*hsp65*) for routine identification of *Mycobacterium* species isolated from clinical sources. *J Clin Microbiol* 2004;**42**:3000–11.
126. Merckx JJ, Soule EH, Karlson AG. The histopathology of lesions caused by infection with unclassified acid-fast bacteria in man: report of 25 cases. *Am J Clin Pathol* 1964;**41**:244–55.
127. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1–166.
128. Mollohan CS, Romer MS. Public health significance of swimming pool granuloma. *Am J Public Health Nations Health* 1961;**51**: 883–91.
129. Morgan JK, Blowers R. Swimming-pool granuloma in Britain. *Lancet* 1964;**1**:1034–6.
130. Musial CE, Tice LS, Stockman L, et al. Identification of mycobacteria from culture by using the Gen-Probe Rapid Diagnostic System for *Mycobacterium avium* complex and *Mycobacterium tuberculosis* complex. *J Clin Microbiol* 1988;**26**:2120–3.
131. Mussaffi H, Rivlin J, Shalit I, et al. Nontuberculous mycobacteria in cystic fibrosis associated with allergic bronchopulmonary aspergillosis and steroid therapy. *Eur Respir J* 2005;**25**:324–8.
132. Nadal D, Caduff R, Kraft R, et al. Invasive infection with *Mycobacterium genavense* in three children with the acquired immunodeficiency syndrome. *Eur J Clin Microbiol Infect Dis* 1993;**12**: 37–43.
133. Nesheim SR, Kapogiannis BG, Soe MM, et al. Trends in opportunistic infections in the pre- and post-highly active antiretroviral therapy eras among HIV-infected children in the Perinatal AIDS Collaborative Transmission Study, 1986-2004. *Pediatrics* 2007; **120**:100–9.
134. Nylen O, Alestig K, Fasth A, et al. Infections of the ear with nontuberculous mycobacteria in three children. *Pediatr Infect Dis J* 1994;**13**:653–6.
135. O'Brien RJ. The epidemiology of nontuberculous mycobacterial disease. *Clin Chest Med* 1989;**10**:407–18.
136. Peacock KH, Lewis L, Lavoie S. Erosive mediastinal lymphadenitis associated with *Mycobacterium avium* infection in a pediatric acquired immunodeficiency syndrome patient. *Pediatr Infect Dis J* 2000;**19**:576–8.
137. Perlino CA. *Mycobacterium avium* complex: an unusual cause of peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. *Clin Infect Dis* 1993;**17**:1083–4.
138. Pierre-Audigier C, Jouanguy E, Lamhamedi S, et al. Fatal disseminated *Mycobacterium smegmatis* infection in a child with inherited interferon gamma receptor deficiency. *Clin Infect Dis* 1997;**24**:982–4.
139. Pilkington EF, MacArthur CJ, Beekmann SE, et al. Treatment patterns of pediatric nontuberculous mycobacterial (NTM) cervical lymphadenitis as reported by nationwide surveys of pediatric otolaryngology and infectious disease societies. *Int J Pediatr Otorhinolaryngol* 2010;**74**:343–6.
140. Pinder SE, Colville A. Mycobacterial cervical lymphadenitis in children: Can histological assessment help differentiate infections caused by non-tuberculous mycobacteria from *Mycobacterium tuberculosis*? *Histopathology* 1993;**22**:59–64.
141. Pransky SM, Reisman BK, Kearns DB, et al. Cervicofacial mycobacterial adenitis in children: endemic to San Diego? *Laryngoscope* 1990;**100**:920–5.
142. Prevots DR, Shaw PA, Strickland D, et al. Nontuberculous mycobacterial lung disease prevalence at four integrated health care delivery systems. *Am J Respir Crit Care Med* 2010;**182**: 970–6.
143. Prince DS, Peterson DD, Steiner RM, et al. Infection with *Mycobacterium avium* complex in patients without predisposing conditions. *New Engl J Med* 1989;**321**:863–8.
144. Quijano G, Siminovich M, Drut R. Histopathologic findings in the lymphoid and reticuloendothelial system in pediatric HIV infection: a postmortem study. *Pediatr Pathol Lab Med* 1997;**17**: 845–56.
145. Redelman-Sidi G, Sepkowitz KA. Rapidly growing mycobacteria infection in patients with cancer. *Clin Infect Dis* 2010;**51**:422–34.
146. Reich JM, Johnson RE. *Mycobacterium avium* complex pulmonary disease: incidence, presentation, and response to therapy in a community setting. *Am Rev Respir Dis* 1991;**143**:1381–5.
147. Reichert CM, O'Leary TJ, Levens DL, et al. Autopsy pathology in the acquired immune deficiency syndrome. *Am J Pathol* 1983;**112**:357–82.

148. Reid IS. *Mycobacterium ulcerans* infection: a report of 13 cases at the Port Moresby General Hospital, Papua. *Med J Aust* 1967; **1**:427–31.
149. Reuss AM, Wiese-Posselt M, Weissmann B, et al. Incidence rate of nontuberculous mycobacterial disease in immunocompetent children: a prospective nationwide surveillance study in Germany. *Pediatr Infec Dis J* 2009;**28**:642–4.
150. Richardson ET, Samson D, Banaei N. Rapid identification of *Mycobacterium tuberculosis* and nontuberculous mycobacteria by multiplex, real-time PCR. *J Clin Microbiol* 2009;**47**:1497–502.
151. Rivron MJ, Hughes EA, Sibert JR, et al. Cervical lymphadenitis in childhood due to mycobacteria of the Fortuitum group. *Arch Dis Child* 1979;**54**:312–13.
152. Rosenzweig DY. Pulmonary mycobacterial infections due to *Mycobacterium intracellulare-avium* complex: clinical features and course in 100 consecutive cases. *Chest* 1979;**75**:115–19.
153. Roux AL, Catherinot E, Ripoll F, et al. Multicenter study of prevalence of nontuberculous mycobacteria in patients with cystic fibrosis in France. *J Clin Microbiol* 2009;**47**:4124–8.
154. Runyon EH. Anonymous mycobacteria in pulmonary disease. *Med Clin North Am* 1959;**43**:273–90.
155. Runyon EH. Pathogenic mycobacteria. *Bibl Tuberc* 1965;**21**: 235–87.
156. Runyon EH. Mycobacteria: an overview. *Rev Infect Dis* 1981;**3**: 819–21.
157. Safranek TJ, Jarvis WR, Carson LA, et al. *Mycobacterium chelonae* wound infections after plastic surgery employing contaminated gentian violet skin-marking solution. *New Engl J Med* 1987;**317**: 197–201.
158. Salyer KE, Votteler TP, Dorman GW. Surgical management of cervical adenitis due to atypical mycobacteria in children. *JAMA* 1968;**204**:1037–40.
159. Samra Z, Kaufmann L, Zeharia A, et al. Optimal detection and identification of *Mycobacterium haemophilum* in specimens from pediatric patients with cervical lymphadenopathy. *J Clin Microbiol* 1999;**37**:832–4.
160. Sanderson TL, Moskowitz L, Hensley GT, et al. Disseminated *Mycobacterium avium*-intracellulare infection appearing as a panniculitis. *Arch Pathol Lab Med* 1982;**106**:112–14.
161. Sanford JP, Barnett JA. Atypical mycobacterial organisms as encountered in a general hospital and its clinics. In: Chapman JS, editor. *The anonymous mycobacteria in human disease*. Springfield, Ill: Charles C. Thomas; 1960. p. 98.
162. Schaefer WB. Incidence of the serotypes of *Mycobacterium avium* and atypical mycobacteria in human and animal diseases. *Am Rev Respir Dis* 1968;**97**:18–23.
163. Schinsky MF, McNeil MM, Whitney AM, et al. *Mycobacterium septicum* sp. nov., a new rapidly growing species associated with catheter-related bacteraemia. *Int J Syst Evol Microbiol* 2000;**50**: 575–81.
164. Schonell ME, Crofton JW, Stuart AE, et al. Disseminated infection with *Mycobacterium avium*. I. Clinical features, treatment and pathology. *Tubercle* 1968;**49**:12–30.
165. Schuit KE, Powell DA. Mycobacterial lymphadenitis in childhood. *Am J Dis Child* 1978;**132**:675–7.
166. Shachor-Meyouhas Y, Sprecher H, Eluk O, et al. An outbreak of *Mycobacterium mucogenicum* bacteremia in pediatric hematology-oncology patients. *Pediatr Infect Dis J* 2011;**30**:30–2.
167. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy. *AIDS* 2005;**19**: 399–406.
168. Shitrit D, Baum GL, Priess R, et al. Pulmonary *Mycobacterium kansasii* infection in Israel, 1999-2004: clinical features, drug susceptibility, and outcome. *Chest* 2006;**129**:771–6.
169. Shitrit D, Priess R, Peled N, et al. Differentiation of *Mycobacterium kansasii* infection from *Mycobacterium tuberculosis* infection: comparison of clinical features, radiological appearance, and outcome. *Eur J Clin Microbiol Infect Dis* 2007;**26**:679–84.
170. Shocket E, Drosd RE, Tate CF Jr. Granuloma of the skin due to *Mycobacterium fortuitum*. *South Med J* 1964;**57**:1352–6.
171. Sigalet D, Lees G, Fanning A. Atypical tuberculosis in the pediatric patient: Implications for the pediatric surgeon. *J Pediatr Surg* 1992;**27**:1381–4.
172. Simor AE, Salit IE, Vellend H. The role of *Mycobacterium xenopi* in human disease. *Am Rev Respir Dis* 1984;**129**:435–8.
173. Smith JA, Mueller BU, Nussenblatt RB, et al. Corneal endothelial deposits in children positive for human immunodeficiency virus receiving rifabutin prophylaxis for *Mycobacterium avium* complex bacteremia. *Am J Ophthalmol* 1999;**127**:164–9.
174. Smith MJ, Efthimiou J, Hodson ME, et al. Mycobacterial isolations in young adults with cystic fibrosis. *Thorax* 1984;**39**:369–75.
175. Snijder J. Morphological aspect of atypical mycobacterioses. In: *Selected papers*, vol. 10. The Hague: Royal Netherlands Tuberculosis Association; 1967.
176. Sommers HM, Good RC. *Mycobacterium*. In: Lennette EH, Balows A, Hausler WJ Jr, Shadomy HJ, editors. *Manual of clinical microbiology*. Washington, D. C.: American Society for Microbiology; 1985. p. 216–40.
177. Song M, Vincke G, Vanachter H, et al. Treatment of cutaneous infection due to *Mycobacterium ulcerans*. *Dermatologica* 1985;**171**: 197–9.
178. Sousa TS, Matherne RJ, Wilkerson MG. Cutaneous nontuberculous mycobacterial abscesses associated with insulin injections [case report]. *J Drugs Dermatol* 2010;**9**:1439–42.
179. Speight EL, Williams HC. Fish tank granuloma in a 14-month-old girl. *Pediatr Dermatol* 1997;**14**:209–12.
180. Stanford JL, Paul RC. A preliminary report on some studies of environmental mycobacteria. *Ann Soc Belg Med Trop* 1973;**53**: 389–93.
181. Stanford JL, Revill WD, Gunthorpe WJ, Grange JM. The production and preliminary investigation of Burulin, a new skin test reagent for *Mycobacterium ulcerans* infection. *J Hyg (London)* 1975;**74**:7–16.
182. Steadham JE. High-catalase strains of *Mycobacterium kansasii* isolated from water in Texas. *J Clin Microbiol* 1980;**11**:496–8.
183. Stewart MG, Troendle-Atkins J, Starke JR, et al. Nontuberculous mycobacterial mastoiditis. *Arch Otolaryngol Head Neck Surg* 1995; **121**:225–8.
184. Stratton CW, Phelps DB, Reller LB. Tuberculoid tenosynovitis and carpal tunnel syndrome caused by *Mycobacterium szulgai*. *Am J Med* 1978;**65**:349–51.
185. Sutker WL, Lankford LL, Tompsett R. Granulomatous synovitis: the role of atypical mycobacteria. *Rev Infect Dis* 1979;**1**:729–35.
186. Swift S, Cohen H. Granulomas of the skin due to *Mycobacterium balnei* after abrasions from a fish tank. *New Engl J Med* 1962; **267**:1244–6.
187. Syed AU, Hussain R, Bhat AN, et al. Mediastinitis due to *Mycobacterium fortuitum* infection following Fontan operation in a child. *Scan Cardiovasc J* 1997;**31**:311–13.
188. Thomas E, Hillman BJ, Stanisic T. Urinary tract infection with atypical mycobacteria. *J Urol* 1980;**24**:748–50.
189. Timpe A, Runyon EH. The relationship of atypical acid-fast bacteria to human disease: a preliminary report. *J Lab Clin Med* 1954;**44**:202–9.
190. Torrens JK, Dawkins P, Conway SP, et al. Non-tuberculous mycobacteria in cystic fibrosis. *Thorax* 1998;**53**:182–5.
191. Tortoli E. Impact of genotypic studies on mycobacterial taxonomy: the new mycobacteria of the 1990s. *Clin Microbiol Rev* 2003;**16**:319–54.
192. Tortoli E, Piersimoni C, Bartoloni A, et al. *Mycobacterium malmoense* in Italy: the modern Norman invasion? *Eur J Epidemiol* 1997;**13**:341–6.
193. Tremblay V, Ayad T, Lapointe A, et al. Nontuberculous mycobacterial cervicofacial adenitis in children: epidemiologic study. *J Otolaryngol Head Neck Surg* 2008;**37**:616–22.
194. Tse KC, Lui SL, Cheng VC, et al. A cluster of rapidly growing mycobacterial peritoneal dialysis catheter exit-site infections. *Am J Kidney Dis* 2007;**50**:e1–5.
195. Tsukamura M. A review of the methods of identification and differentiation of mycobacteria. *Rev Infect Dis* 1981;**3**:841–61.
196. Turneer M, Van Nerom E, Nyabenda J, et al. Determination of humoral immunoglobulins M and G directed against mycobacterial antigen 60 failed to diagnose primary tuberculosis and mycobacterial adenitis in children. *Am J Respir Crit Care Med* 1994;**150**:1508–12.
197. van Coppenraet LS, Smit VT, Templeton KE, et al. Application of real-time PCR to recognize atypical mycobacteria in archival skin biopsies: high prevalence of *Mycobacterium haemophilum*. *Diag Mol Pathol* 2007;**16**:81–6.
198. Van Dyke JJ, Lake KB. Chemotherapy for aquarium granuloma. *JAMA* 1975;**233**:1380–1.

199. van Ingen J, Boeree MJ, de Lange WC, et al. Clinical relevance of *Mycobacterium szulgai* in The Netherlands. *Clin Infect Dis* 2008;**46**:1200–5.
200. Venkatesh V, Everson NW, Johnstone JM. Atypical mycobacterial lymphadenopathy in children: is it underdiagnosed? *J R Coll Surg Edinb* 1994;**39**:301–3.
201. Volini F, Colton R, Lester W. Disseminated infection caused by battey type mycobacteria. *Am J Clin Pathol* 1965;**43**:39–46.
202. Wallace RJ Jr, Brown BA, Onyi GO. Skin, soft tissue, and bone infections due to *Mycobacterium chelonae chelonae*: importance of prior corticosteroid therapy, frequency of disseminated infections, and resistance to oral antimicrobials other than clarithromycin. *J Infect Dis* 1992;**166**:405–12.
203. Wallace RJ Jr, Nash DR, Tsukamura M, et al. Human disease due to *Mycobacterium smegmatis*. *J Infect Dis* 1988;**158**:52–9.
204. Wallace RJ Jr, Swenson JM, Silcox VA, et al. Spectrum of disease due to rapidly growing mycobacteria. *Rev Infect Dis* 1983;**5**: 657–79.
205. Washer LL, Riddell JT, Rider J, et al. *Mycobacterium neoaurum* bloodstream infection: report of 4 cases and review of the literature. *Clin Infect Dis* 2007;**45**:e10–13.
206. Wayne LG, Sramek HA. Agents of newly recognized or infrequently encountered mycobacterial diseases. *Clin Microbiol Rev* 1992;**5**:1–25.
207. Weed LA, Karlson AG, Ivins JC, et al. Recurring migratory chronic osteomyelitis associated with saprophytic acid-fast bacilli: report of a case of 10 years' duration apparently cured by surgery. *Proc Staff Meet Mayo Clin* 1956;**31**:238–46.
208. Welch K, Finkbeiner W, Alpers CE, et al. Autopsy findings in the acquired immune deficiency syndrome. *JAMA* 1984;**252**:1152–9.
209. Wertman R, Miller M, Groben P, et al. *Mycobacterium bolletii/ Mycobacterium massiliense furunculosis* associated with pedicure footbaths: a report of 3 cases. *Arch Dermatol* 2011;**147**:454–8.
210. White MP, Bangash H, Goel KM, et al. Non-tuberculous mycobacterial lymphadenitis. *Arch Dis Child* 1986;**61**:368–71.
211. White R, Abreo K, Flanagan R, et al. Nontuberculous mycobacterial infections in continuous ambulatory peritoneal dialysis patients. *Am J Kidney Dis* 1993;**22**:581–7.
212. Wilkes MS, Fortin AH, Felix JC, et al. Value of necropsy in acquired immunodeficiency syndrome. *Lancet* 1988;**2**:85–8.
213. Williams B, Neth O, Shingadia D, et al. *Mycobacterium kansasii* causing septic arthritis and osteomyelitis in a child. *Pediatr Infect Dis J* 2010;**9**:88–9.
214. Winthrop KL, Chang E, Yamashita S, et al. Nontuberculous mycobacteria infections and anti-tumor necrosis factor-alpha therapy. *Emerg Infect Dis* 2009;**15**:1556–61.
215. Winthrop KL, McNelley E, Kendall B, et al. Pulmonary nontuberculous mycobacterial disease prevalence and clinical features: an emerging public health disease. *Am J Respir Crit Care Med* 2010;**182**:977–82.
216. Wolinsky E. Nontuberculous mycobacteria and associated diseases. *Am Rev Respir Dis* 1979;**119**:107–59.
217. Wolinsky E. Mycobacteria: Significance of speciation and sensitivity tests. In: Lorian V, editor. *Significance of medical microbiology in the care of patients*. 2nd ed. Baltimore: Williams & Wilkins; 1982. p. 103–10.
218. Wolinsky E. Mycobacterial diseases other than tuberculosis. *Clin Infect Dis* 1992;**15**:1–10.
219. Wolinsky E. Mycobacterial lymphadenitis in children: a prospective study of 105 nontuberculous cases with long-term follow-up. *Clin Infect Dis* 1995;**20**:954–63.
220. Wolinsky E, Gomez F, Zimpfer F. Sporotrichoid *Mycobacterium marinum* infection treated with rifampin-ethambutol. *Am Rev Respir Dis* 1972;**105**:964–7.
221. Woods GL, Washington JA 2nd. Mycobacteria other than *Mycobacterium tuberculosis*: review of microbiologic and clinical aspects. *Rev Infect Dis* 1987;**9**:275–94.
222. Xiang YH, De I, Jacobson KL. Rapidly growing mycobacteria. *Am J Clin Pathol* 2007;**128**:612–21.
223. Yakrus MA, Good RC. Geographic distribution, frequency, and specimen source of *Mycobacterium avium* complex serotypes isolated from patients with acquired immunodeficiency syndrome. *J Clin Microbiol* 1990;**28**:926–9.
224. Yamauchi T, Ferrieri P, Anthony BF. The aetiology of acute cervical adenitis in children: serological and bacteriological studies. *J Med Microbiol* 1980;**13**:37–43.
225. Yamaguchi T, Bissen-Miyajima H, Hori-Komai Y, et al. Infectious keratitis outbreak after laser in situ keratomileusis at a single laser center in Japan. *J Cataract Refract Surg* 2011;**37**:894–900.
226. Yew WW, Kwan SY, Ma WK, et al. Single daily-dose ofloxacin monotherapy for *Mycobacterium fortuitum* sternotomy infection. *Chest* 1989;**96**:1150–2.
227. Zelazny AM, Root JM, Shea YR, et al. Cohort study of molecular identification and typing of *Mycobacterium abscessus, Mycobacterium massiliense*, and *Mycobacterium bolletii*. *J Clin Microbiol* 2009;**47**: 1985–95.

CHAPTER 98

Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses

Andrea T. Cruz

Mycobacterial infections in humans date to at least the 10th millennium BCE, with evidence of tuberculosis in Neolithic skeletons from Heidelberg, Germany,[14] Egyptian mummies,[299] and from pre-Columbian Mesoamerica.[79] Evidence of lepromatous leprosy has been found in burials in India dating back to 2000 BCE, correlating with Vedic scriptural references to leprosy.[287] Although the origins of mycobacterial infections in humans are unknown, most authorities speculate that domestication of animals in the Neolithic era promoted the transmission of mutants of *Mycobacterium tuberculosis* from livestock to humans. The origins of leprosy and Buruli ulcer and their respective etiologic agents are understood less well but may involve interplay among environmental mycobacteria, animals, and humans.[317] After tuberculosis, leprosy and Buruli ulcer are the second and third most common mycobacterial infections in humans. Leprosy and Buruli ulcer are the two most common cutaneous mycobacterioses.

Tuberculosis and other cutaneous mycobacterial infections are discussed in Chapters 96 and 97. This chapter is devoted to the two cutaneous mycobacterioses that are of greatest medical importance: leprosy and Buruli ulcer.

LEPROSY

Leprosy is a chronic infectious disease caused by *Mycobacterium leprae* that principally affects the cooler parts of the body, especially the skin, upper respiratory tract, testes, eyes, and superficial segments of peripheral nerves. Although leprosy has affected nearly every part of the world at some time, its geographic origin is only now being unraveled.[239]

The World Health Organization (WHO) reported that in 1999, approximately 800,000 patients were being treated for active leprosy and that, also in 1999, 738,000 new patients were reported.[407] By 2010, these numbers had decreased to 192,000 and 228,000, respectively.[412] Many authorities consider that the total global prevalence of patients with active leprosy is much higher (1.5 to 2 million),[11] however, and are underreported because of the stigma associated with leprosy.[143] Several million more patients experience serious sequelae.[193]

In the Middle Ages, leprosy was common in Europe, reported in up to one quarter of skeletons in one ordinary (nonleper colony) Danish village cemetery.[26] Single nucleotide polymorphisms (SNPs) have indicated relatively low levels of genetic diversity, but phylogenetic analysis indicates that the most diverse strains are found in central Asia or eastern Africa.[239] European emigration and the West African slave trade, rather than crossing from Asia via the Bering Strait, likely introduced leprosy to the New World. The Silk Road may have then spread leprosy from Europe to China.[240] At least two foci in the United States were established in the 19th century by specific immigrations: Asians brought leprosy to the Hawaiian Islands and started an epidemic in the highly susceptible Hawaiians,[243] and Scandinavians introduced leprosy into the northern Midwest region of the United States.[203]

Patients with leprosy frequently experience severe stigmas, and, in Western cultures, this is attributable at least partially to a misunderstanding of what is called leprosy in the Old Testament.[30] The Hebrew word *tsara'ath* was rendered *lepra* when the Old Testament was translated into Greek in the 3rd and 2nd centuries BCE. In preparing the Latin Vulgate version in AD 405, Jerome used the word *lepra* directly from the Greek. In the first English translation from the Vulgate in 1384, Wycliffe translated *lepra* as *leprosy*, perhaps because leprosy, then common in Europe and Great Britain, seemed to portray an image of an unholy and loathsome human condition. In the original text, *tsara'ath* was not a specific disease but probably a group of diseases, the identities of which are obscure, and the word more generally referred to ceremonial uncleanness. More contemporary biblical editions include this description: "the priest shall examine the diseased spot on the skin of his body; and if the hair in the diseased spot has turned white and the disease appears to be deeper than the skin of his body, it is a leprous disease; when the priest has examined him he shall pronounce him unclean" (Leviticus 13 : 4).

Other cultures not influenced by Judaic laws and traditions have similar or more severe attitudes toward patients with leprosy.[143] Chinese literature indicates that in the 8th century BCE, patients with symptoms now recognized as symptoms of leprosy were stigmatized.[339] Similarly, the *Laws of Manu* (India, circa 1500 BCE) punish persons marrying into families with leprosy.[34] Although suicide was considered a sin under Hinduism, leprosy was an exception.[143] The stigma persists to the modern era. In 2009, the chairman of India's human rights commission recommended prosecuting leprosy patients if they had children.[38] Continuing efforts must be made to minimize the stigma peculiarly associated with leprosy. To help achieve this goal, the Fifth International Leprosy Congress in 1948 adopted a resolution to abandon the word *leper* for leprosy patient.[190] Some physicians prefer Hansen disease as a synonym for leprosy.

ORGANISM

M. leprae is a species in the order Actinomycetales and the family Mycobacteriaceae. This bacillus was seen first by Hansen in 1873 in Bergen, Norway, in lepromas from Norwegian patients, and this organism was the first reported bacterium causing chronic disease in humans.

M. leprae is an acid-fast bacillus 0.3 to 0.5 μm wide by 4 to 7 μm long. The acid-fastness of *M. leprae* is weaker than that of other mycobacteria. Viable *M. leprae* organisms stain solidly, but degenerating bacilli first stain irregularly, then become granular and eventually lose acid-fastness completely. The persistence of bacillary carcasses can be verified by silver staining techniques.[382] Staining quality provides a rapid method for determining the effectiveness of therapy. In vitro cultivation of *M. leprae* frequently is claimed, but not substantiated.[170,290] Because *M. leprae* still cannot be cultivated, identification depends on criteria other than those used routinely for cultivable mycobacteria. Current criteria for *M. leprae* are the following: (1) It does not grow on routine laboratory media, (2) it infects the footpads of mice in a characteristic manner,[192,329] (3) acid-fastness is extractable with pyridine,[52] (4) the organism invades nerves of the host, (5) suspensions of dead bacilli produce a characteristic pattern of reactions when injected into the skin of patients (lepromin reaction) with the various clinical forms of leprosy, (6) it produces the species-specific antigen phenolic glycolipid-1 (PGL-1),[109] and (7) it exhibits species-specific DNA sequences.[401]

Electron micrographs of *M. leprae* reveal a cell wall 15 to 20 nm thick around a cytoplasmic membrane that gives rise to mesosomes extending into the cytoplasm. Its cell walls contain arabinogalactan, mycolates, peptidoglycan, and protein.[211]

The genome of *M. leprae* is small (3.3 million base pairs) in contrast to that of *M. tuberculosis* (~4.4 million base pairs).[240] Only half the genome contains protein-encoding genes; the remainder comprises pseudogenes and noncoding elements. Gene deletion and decay have markedly limited the metabolic activities of *M. leprae* and may contribute significantly to failure to cultivate the organism and to its long generation time in the mouse footpad (14 days).[28,88] Localization of infections to the cooler parts of the body,[25] selective growth in the footpads of immunologically intact mice and in the ears of hamsters, and the high susceptibility of the armadillo (central body temperature of 32° to 35° C [89.6° to 95° F]) to disseminated infections all suggest that the optimal temperature for growth of *M. leprae* is less than 37° C (98.6° F).[217]

TRANSMISSION

The modes of transmission of *M. leprae* in nature have not been fully established. The frequency in children of a single early lesion in skin that usually is covered by clothing argues against the development of such lesions at the site of contact with *M. leprae*.[17] For many years, skin-to-skin contact between the patient and healthy subjects was considered the most important means of transmission, and this concept cannot be abandoned readily.[189] Intact skin of heavily infected patients discharges a few *M. leprae*, but ulcers in the skin may be a source of numerous bacilli. In some studies, bacillary load was the only factor associated with leprosy spread to index case contacts.[303] Skin-to-skin contact and fomites containing *M. leprae* could be sources of infection, but this mode has been minimized in recent years in favor of the nasorespiratory route.[153]

That the nasal mucosa of lepromatous patients harbors massive numbers of *M. leprae* has been known since Hansen's original discovery, and studies suggest that the respiratory passages could be an important source of infecting bacilli.[75] In one study, 80 percent of patients with multibacillary leprosy had *M. leprae* DNA in skin washings and 60 percent had nasal secretions testing positive for *M. leprae* by polymerase chain reaction (PCR). Household contacts were PCR-positive from skin and nasal secretion samples in 17 percent and 4 percent of instances, respectively.[153] *M. leprae* may bind to nasal mucosal cells by first binding fibronectin and attaching to fibronectin receptors on mucosal cells.[40] *M. leprae* organisms ejected in nose blowing remain viable under ambient conditions for 1 week,[66] and disseminated leprosy develops in immunosuppressed mice after the inhalation of aerosol that contains *M. leprae*.[283] Breast tissue and milk from lepromatous patients contain *M. leprae*, and infants may acquire infection from this source.[266]

Placental transmission of leprosy has been a subject of conjecture for some time, but evidence is growing for a significant influence of leprosy on fetal development and for intrauterine infection of the fetus. In a study of 156 pregnant leprosy patients in Ethiopia, the placentas were small and infants had lower birth-weight, higher risk for intrauterine growth restriction, and higher infant mortality rates than infants of nonlepromatous mothers.[83] Immunoglobulin A (IgA) and IgM antibodies for *M. leprae* are present in the cord blood of 30 to 50 percent of infants delivered by mothers with lepromatous leprosy.[213] Evidence is strong for synthesis of fetal antibodies to *M. leprae*. Occasionally, *M. leprae* has been shown in placentas and cord blood.[141,368] *M. leprae*–specific IgA and IgM levels increased in infants of lepromatous

mothers during the 3- to 24-month period after birth,[212] and two such infants had clinical leprosy at 9 and 17 months of age.[82]

Leprosy in young infants may be a common occurrence in areas of high endemicity.[116] In a report combining cases from the literature and observations by experienced leprologists, a total of at least 49 patients younger than 1 year of age with leprosy were identified.[32] In only half of these infants did the mother have leprosy or a history of leprosy. The youngest infant was 2.5 months old at the time of diagnosis. The fact that many of the mothers never had clinical leprosy suggests that they had an evanescent *M. leprae* bacteremia during gestation. A substantial bacteremia is a common finding in multibacillary disease[80,181] and is detectable in 15 percent of paucibacillary patients.[171]

The discoveries of a naturally acquired leprosy-like disease in recently captured wild armadillos in Louisiana,[363,391] chimpanzees,[78,120] a mangabey monkey from West Africa,[227] and a macaque from the Philippines provide reason to consider that leprosy is a zoonosis.[216,369,392] Reports of naturally acquired leprosy in armadillos range from 3 to 85 percent in the southern United States.[152,360-363] In all of these species, the histopathologic features resemble those in leprosy in humans, and the bacilli that cause the infection cannot be distinguished from *M. leprae*.[24,220,226] Leprosy has been transmitted successfully from the mangabey monkey to other mangabey, rhesus, and African green monkeys.[155,403] Some authorities suggest that insects, including *Aedes aegypti* mosquitoes, may ingest *M. leprae* during a blood meal from lepromatous patients and harbor viable bacteria.[12] The natural transmission of leprosy by insects remains unproven, however.

EPIDEMIOLOGY

The highest incidence rates of leprosy are found in Southeast Asia (including India), which accounted for 68 percent of new leprosy cases in 2010. The Americas accounted for 17 percent, followed by Africa (11%), Western Pacific nations (2.2%), and Eastern Mediterranean countries (1.7%).[412] While prevalence has fallen substantially in recent years, this decrease is partially due to decreased treatment durations.[289] Based on limited whole-population surveys in endemic areas, the total number of active patients may exceed the number reported by the WHO by a significant margin. The stigma of the disease and inefficiency in health care delivery systems contribute to this disparity in statistics.[143]

It is estimated that approximately 6500 persons in the United States have leprosy, of whom 3300 actively are receiving therapy; 150 new patients were reported in 2008.[251] This is a decline from a peak of over 360 new diagnoses annually seen in the 1980s. This surge was attributed in part to an influx of refugees from Southeast Asia.[191] Since 2000, an increased number of cases in immigrants from the Marshall Islands and Micronesia has been reported.[405] Most patients in the United States are immigrants, but a few indigenous patients regularly come from Hawaii, Louisiana, Texas, and other southeastern states.[182] No instances of secondary transmission from imported cases within the United States have been reported; immigrants with leprosy present no known public health risk to the population of the United States. The same situation probably is true for other nonendemic countries that receive many immigrants from endemic areas.

Hansen's discovery of the leprosy bacillus developed from his conviction that leprosy was a specific contagious disease, based on clinical, anatomic, and epidemiologic observations. In 1871 and 1872, he studied 69 families in western Norway in which several members had leprosy. The prevailing concept of that era was that leprosy was hereditary, but from data gathered on these families, Hansen showed that patients always had contact with another leprosy patient. Members of the same families with no such contacts were free of leprosy.[126] Hansen reasoned that the spread of leprosy depended on dissemination of this etiologic agent in a susceptible population. The leprosy epidemic in Nauru in the central Pacific area shows how rapidly leprosy can spread in a leprosy-naïve population.[121] Leprosy was introduced into this small island in 1912, and by 1924, one third of the 2500 inhabitants had leprosy.

The prevailing concept has been that an individual becomes infected only after experiencing repeated exposure. This concept now is doubted, and a single exposure may be sufficient in optimal conditions. One report describes leprosy transmission occurring after a single exposure from a patient to a surgeon who practiced in a leprosy nonendemic area.[2] Other reports describe inoculation leprosy after injury with a broken glass bangle[115] and after tattooing.[337] In any patient-contact situation, the number of viable *M. leprae* being shed by the patient and the degree of susceptibility of the contact both may vary. Long periods of association may be necessary before optimal conditions for infection exist.

Lymphocyte transformation studies show that occupational contacts of leprosy patients in Ethiopia have the highest rate of sensitization (58%) to *M. leprae*, followed closely by household contacts (47%). Noncontacts living in endemic areas have a lower rate of sensitization, but approximately 29 percent of the population still is sensitized.[117]

Geographic, ethnic, and socioeconomic factors may contribute to the spread of leprosy by affecting the number of untreated or ineffectively treated bacillary-positive patients and the opportunities for exposure. The percentage of patients who harbor large numbers of bacilli—generally, patients with lepromatous leprosy—is related to ethnic background. In some Asian populations, 50 percent or more of patients with leprosy have lepromatous leprosy; in Africans, this figure is 5 to 10 percent. One case-control study in Brazil noted that low educational level, prior history of food shortages, bathing weekly in open water bodies, and low frequency of bed linen changes were associated with leprosy transmission.[173] Another study, in Bangladesh, found that recent food shortage, rather than poverty per se, was the only socioeconomic factor associated with leprosy disease.[96] The association of nutritional depletion on leprosy has been described historically; the Nauru leprosy epidemic,

indolent from 1912 through 1920, became rampant after a devastating epidemic of influenza (30% mortality) left a debilitated population with marked dietary deficiencies. During the next 4 years, the incidence of leprosy increased from 4 to 346 patients.[121] Wartime malnutrition in Malaya[300] and China[340] was associated with increased morbidity and mortality with leprosy. Nonetheless, convincing evidence that the prevalence of leprosy is unusually high in chronically malnourished populations is lacking, in part because of the colinearity between malnutrition and other markers of socioeconomic deprivation.

Improvements in housing and other living conditions may play a role in the declining prevalence of leprosy. No other factor satisfactorily explains the virtual disappearance of leprosy from northern Europe after the Middle Ages and from Scandinavia in the 20th century, long before any effective chemotherapy was available. If the disease is predominantly airborne, the construction of dwellings that provide less confined sleeping quarters in this era could have contributed in a major way to the disappearance of leprosy in northern Europe and Scandinavia. Consistent with this concept is the inadequate housing that prevails in all geographic areas in which leprosy is common today. Another explanation for declining leprosy cases in Europe after the 15th century was that the rudimentary infection control and isolation of lepromatous patients may have had an impact.[327]

The presumed increased susceptibility of children is difficult to establish and may depend more on exposure to contagious patients and genetic predisposition than on other factors. The proportion of children among all detected patients is 20 to 30 percent.[110,259] Of 615 patients who were diagnosed in Louisiana between 1855 and 1970, 5 percent had disease onset at 0 to 9 years of age, and 19 percent were in the 10- to 19-year-old age group.[97] Lara,[184] in a study of 2000 children who lived in a leprosarium in the Philippines in an era when effective chemotherapy was unavailable, noted that leprosy developed in 470 (23%). Of these 470 patients, 254 were monitored closely, and in approximately 75 percent, the lesions healed spontaneously. Active, persistent disease developed in approximately 6 percent of children heavily exposed to leprosy. In most populations studied, only 5 to 10 percent of individuals are susceptible to leprosy. In adults, leprosy occurs more commonly in men than in women (2 : 1 to 3 : 1). In children, the sex ratio is approximately 1 : 1.

Genetic factors likely influence the susceptibility of some individuals to leprosy and the form of disease that develops.[98,102,103,331] Earlier studies found that if one twin has leprosy, the chance of leprosy developing in a monozygotic twin is 60 to 85 percent versus a 15 to 25 percent risk for dizygotic twins.[126] More recently, genome screening in Indian patients found an association with leprosy susceptibility on chromosome 10p13, near the gene for mannose receptor C, a phagocytic receptor on macrophages, and on chromosome 6, within the major histocompatibility complex (MHC).[334] Within the MHC, associations exist between leprosy and MHC class II genes in Indian patients, and with the tumor necrosis factor (TNF) gene in Brazilian patients.[328] Polymorphisms in the promoter regions of the interleukin (IL)-10 and TNF genes are associated with leprosy,[306] and the TNF promoter especially for the development of lepromatous or multibacillary leprosy.[297]

Certain human leukocyte antigens (HLA-DR) seem to be associated with specific forms of leprosy. *HLA-DR2* and *HLA-DR3* alleles are associated with tuberculoid disease, and *HLA-DQ1* is associated with lepromatous disease.[57] In Suriname, *HLA-DR3* is found frequently in mixed populations with tuberculoid leprosy and rarely in lepromatous patients; however, in Indians with tuberculoid leprosy, *HLA-DR2* predominates.[372] HLA-DR antigens may influence the presentation of antigens of *M. leprae* to T cells and may affect the immune response to leprosy.[262]

Growing genetic evidence supports interpopulation heterogeneity in leprosy susceptibility, and population-based studies have allowed for discrimination between household spread resulting from increased contact with secretions versus genetic predisposition. However, studies have varied widely in genes evaluated, suspected penetrance, and associated risk factors. One study in a former leprosy colony in Brazil estimated that penetrance for homozygous individuals ranged from 56 to 85 percent.[188] On chromosome 6, within the leprosy susceptibility locus region q25-q26,[229] further investigation revealed a significant association between leprosy and 17 markers near the Parkinson disease gene *PARK2* and the coregulated gene *PACRG* in Vietnamese and Brazilian families.[228] Having two of the alleles was associated with increased susceptibility to leprosy. In Malawi, a large case-control candidate gene study of leprosy susceptibility found that homozygotes for a silent T-to-C change in codon 352 of the vitamin D receptor gene were at higher risk, whereas homozygotes for the McCoy b blood group defining variant K1590E of the complement receptor 1 gene seemed to be protective.[103] One report from Iran showed that interferon (IFN)-γ receptor-1 gene promoter (IFNGR1) polymorphisms increased susceptibility to leprosy in a family.[377] A genomewide association study from eastern China indicated that SNPs in the nucleotide-binding oligomerization domain-containing protein 2 (NOD2)-mediated signaling pathway, which helps mediate innate immune responses, were associated with increased leprosy susceptibility.[419]

Toll-like receptors (TLRs), molecules present on the surface of innate or native immune cells, mediate cytokine production on encountering a foreign invader, including mycobacteria, and ultimately may influence the pattern of specific immunity, which is especially relevant in leprosy.[205,294] Studies in Korea found that lepromatous leprosy occurred more commonly in patients with a mutation in the TLR-2, associated with altered production of IL-10 and IL-12, underscoring the role early cytokine responses against *M. leprae* may play.[159-161] Another SNP, this one in the TLR-1 transmembrane domain, was associated with diminished nuclear factor-κB (NFκ-B) activity and found to have reduced cytokine responses to *M. leprae*.[230] Some TLR-1 mutations are protective and are more commonly seen in European populations than in Africa.[404] In African patients, polymorphisms in the *NRAMP1* gene, a gene also associated

with cellular immunity to *M. tuberculosis*, are associated with lepromatous leprosy,[210] as shown in the Mitsuda reaction.[7,279]

In Texas and Louisiana, the ratios of autochthonous to imported leprosy patients are the highest in the continental United States. Indigenous leprosy is highly prevalent in armadillos in only those two states,[25,343,390,391] but also has been reported in armadillos found in Alabama, Arkansas, Mississippi, and northern Mexico.[182,194,363,398] No cases of transmission of leprosy to humans from naturally infected mangabey monkeys or chimpanzees have been reported, but this potential exists.[120,219]

PATHOGENESIS AND PATHOLOGY

M. leprae causes disease by its ability to survive and multiply in macrophages (Fig. 98-1).[317] If macrophages of the host digest the bacilli early, disease is undetectable or the patient has only minimal lesions. If the macrophages are totally incapable of destroying the organisms, widely disseminated lepromatous leprosy follows. Survival of *M. leprae* in macrophages depends on the immune response of the patient; knowledge of immunity to *M. leprae* is necessary for understanding the mechanism of pathologic changes in leprosy.

Immunity

The immunopathogenesis of leprosy can be understood by examining the polar nature of the condition, whereby tuberculoid disease is characterized by one or several well-demarcated lesions, borderline disease manifests with a modest number of medium-sized lesions, and lepromatous disease manifests with widespread poorly demarcated lesions (Table 98-1). Each type is associated with a different immunologic profile, especially within the lesions.

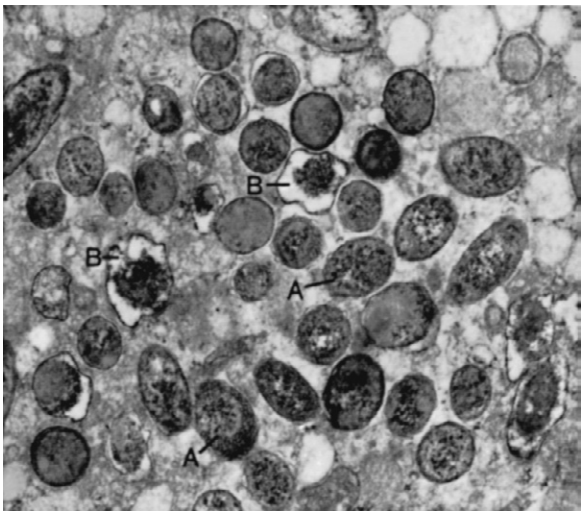

FIGURE 98-1 ■ Electron micrograph of a portion of a globus of *M. leprae* within a histiocyte in a leproma in the skin. Cross sections of well-preserved (**A**) and degenerated (**B**) bacilli are presented (×45,000). (Courtesy Dr. S. C. Chang.)

The ability of an individual to resist *M. leprae* is assessed readily by the induration provoked by an intradermal injection of a suspension of killed *M. leprae* prepared from lepromatous tissue. Lepromatous nodules of patients were the traditional source, but now infected tissue from armadillos commonly is used.[221] The reagent is known as *lepromin*, and the response is known as the *lepromin reaction*.[232] This reaction has two components: an early response at 48 hours (Fernandez reaction) and a late response at 3 to 4 weeks (Mitsuda reaction). The Mitsuda reaction is the most consistent and is used by clinicians as an aid in classifying the clinical forms of leprosy.

Mitsuda reactions are strongly positive (>5 mm in diameter) in tuberculoid patients, weak or negative (0 to 2 mm) in lepromatous patients, and intermediate (3 to 5 mm) in patients with borderline disease. The reactions are a direct measure of delayed hypersensitivity or cell-mediated immunity to *M. leprae* antigens; lepromatous patients are anergic to *M. leprae*, akin to anergy to the tuberculin skin test (TST) seen in children with tuberculous meningitis or miliary tuberculosis. Also similar to the TST, the lepromin reaction has no value in establishing the diagnosis because a high percentage of any population is Mitsuda-positive. One study evaluated the Mitsuda response in Indian children residing in leprosy-endemic regions. Percent positivity was higher in low-endemic regions than high-endemic areas (93% and 88%, respectively), indicating that the Mitsuda response is independent of *M. leprae* exposure.[324] Modifications of the lepromin reaction with the use of concentrated lepromin show that macrophages at the test site in lepromatous patients cannot clear *M. leprae* from the skin, whereas in tuberculoid patients, the bacilli are destroyed efficiently.[54]

Nonspecific factors participate in host defense against *M. leprae*. Complement promotes phagocytosis of leprosy bacilli.[311] After phagocytosis occurs, phagolysosomal fusion and intracellular killing ensue, perhaps by oxygen-independent mechanisms. Although the precise mechanisms of specific immunity remain elusive, abundant experimental evidence indicates that in a lepromatous patient, cell-mediated immunity to *M. leprae* is markedly suppressed. Delayed hypersensitivity skin test reactions to many antigens often are depressed, but they are depressed most consistently and most severely to *M. leprae*.[36] The degree of suppression is gradually less pronounced in clinical forms of disease that are progressively nearer tuberculoid leprosy.[392]

A continuous decrease occurs in the sensitivity of peripheral blood lymphocytes to *M. leprae* that proceeds from tuberculoid to lepromatous patients.[247] Many investigators consider that the defect in cell-mediated immunity to *M. leprae* is in T-lymphocyte function or in the interaction of T lymphocytes with macrophages. Total numbers of circulating T lymphocytes are decreased in lepromatous patients,[86] but no consistent alteration occurs in the percent distribution of circulating T-cell subsets, particularly in the helper-suppressor (T_H1/T_H2) cell ratio.[281]

Within tuberculoid and lepromatous lesions, immunophenotypic and functional profiles of the infiltrating T

TABLE 98-1 **Criteria for Classification of Leprosy**

Group	Clinical Features	Histologic Features	Lepromin Reaction (Mitsuda)	Bacillary Density
Tuberculoid	A single or few anesthetic macules or plaques Borders well defined Peripheral nerve involvement common	Epithelioid-lymphocyte granulomas, with or without giant cells, in skin and nerves No subepidermal clear zone Bacilli in nerves, but rare	Strongly positive	Rare
Borderline-tuberculoid	Lesions similar to those of tuberculoid, but more numerous Borders of lesions less distinct Satellite lesions sometimes present around larger lesions Peripheral nerve involvement common	Granulomas similar to those in tuberculoid Nerves are infiltrated Bacilli frequently found in nerves	Positive	Scanty
Borderline	More lesions than in borderline-tuberculoid Borders more vague Satellite lesions often seen Peripheral nerve involvement common	Epithelioid cells and histiocytic infiltrations focalized by lymphocytes Nerves show increased cellularity Bacilli readily found in nerves	Negative or weakly positive	Moderate
Borderline-lepromatous	Lesions are numerous and similar to those of borderline Some nerve damage	Histiocytic infiltrations show a tendency to evolve toward epithelioid cells and foamy cells Lymphocytes present Nerves have less cellular infiltration Bacilli plentiful in nerves	Negative	Heavy
Lepromatous	Multiple, nonanesthetic, macular or papular, symmetrically distributed lesions No neural lesions until late Late complications of madarosis, leonine facies, testicular damage	Foamy histiocytes containing large numbers of bacilli Few or no lymphocytes Subepidermal clear zone Numerous bacilli in nerves and perineurium without significant intraneural cellular infiltration	Negative	Very heavy
Indeterminate	Vaguely defined hypopigmented or erythematous macule	Often indistinguishable from mild nonspecific dermatitis Lymphocytes and histiocytes around skin appendages and nerves	Weakly positive or negative	Negative or scanty

cells differ markedly and are important factors in understanding the immunopathogenesis of leprosy.[236,237,278] In tuberculoid lesions, the CD4/CD8 T cell ratio is approximately 2:1, and the lesions are characterized by a T_H1- or T_H1-like profile, containing abundant mRNA transcripts for numerous proinflammatory cytokines that confer strong cell-mediated immunity, including IL-2, IFN-γ, and IL-12.[417] Notably, $CD4^+$ T cells in tuberculoid lesions produce IFN-γ.[304] In lepromatous lesions, the CD4/CD8 ratio is approximately 1:2, and the lesions are characterized by a T_H2- or T_H2-like profile, containing mRNA transcripts for anti-inflammatory cytokines that include IL-4 and IL-10, factors associated with weak cell-mediated immunity but strong humoral responses.[417] The $CD8^+$ T cells in lepromatous disease produce IL-4.[304] In contrast to Buruli ulcer (see later discussion of Buruli ulcer), induction of apoptosis in the lesions does not seem to play a major role in pathogenesis.[386] This immunologic dichotomy between tuberculoid and lepromatous lesions is consistent with the paradigm that robust cell-mediated immunity limits disease progression and that humoral immunity has little or no effect. In addition to genetic predisposition (see discussion of epidemiology), an important immunologic factor may relate to host innate responses, when *M. leprae* is first encountered. TLRs on innate immune cells may recognize mycobacterial lipoproteins, generating cytokines that mediate specific or adoptive responses toward a T_H1 or T_H2 direction.[29,233]

In lepromatous lesions, T_H1 and T_H2 cells are admixed among the macrophages. Suppressor activity is generated by lepromin in vitro in peripheral leukocytes from lepromatous patients but not in cells from tuberculoid patients.[209] This suppressor activity also may be induced by PGL-1 of *M. leprae*,[136,208] but it is unrelated to the type of leprosy.[273] PGL-1 abounds in the tissues of

lepromatous patients. Secondary immunosuppression in advanced lepromatous leprosy may result from blockade of thymus-dependent areas of lymph nodes by *M. leprae*–laden macrophages.[365] Specific suppressor T-cell activity in immunosuppression in leprosy remains controversial.[164]

Macrophages of lepromatous patients are thought to have the capacity to kill, digest, and clear *M. leprae* if they are activated.[133] Patients with lepromatous leprosy fail to produce IL-2, but IL-2 restores the proliferation of lymphocytes in response to specific antigens.[128] Defective IFN-γ is produced by lymphocytes from lepromatous patients on stimulation by *M. leprae* antigens.[257] IL-2–bearing lymphocytes are reduced markedly in lepromatous infiltrations in tissues.[235] Suppressor T cells may influence IL-2 production in situ and reduce the proliferation of specifically sensitized T cells to release IFN-γ, with the result that macrophages are not activated. The injection of IFN-γ or recombinant IL-2 into the skin of lepromatous patients causes a local influx of $CD4^+$ T cells along with the formation of epithelioid and giant cells and a reduction in bacillary load.[163,379] Therapy with IL-2 or IFN-γ induces the secretion of TNF-α; however, the activity of this toxic molecule may be inhibited by thalidomide or pentoxifylline.[162,357] Whether the increasing use of TNF-α antagonists would be associated with the development of leprosy from latent infections or perhaps be useful in the management of leprosy reactions remains unclear.[91,315]

Immunoglobulin production (i.e., IgG, IgA, and IgM) usually is elevated only slightly in tuberculoid patients but is elevated markedly in lepromatous patients, consistent with the predominant T_H1 versus T_H2 responses.[37] The total number of B lymphocytes is increased in the blood of lepromatous patients, as is the circulating antibody to mycobacterial antigens.[107,253]

The role of immunologic processes in damage to nerves in leprosy is poorly understood. Some observations suggest that antineural antibodies in the sera of many patients, especially patients with lepromatous disease, are related to such damage.[264] TNF-α is associated with macrophage infiltration of peripheral nerves in reversal reactions.[174] Infected Schwann cells present antigens to T cells, rendering them targets for immune attack.[317]

Histopathology

Biopsy specimens from well-defined lesions of leprosy should be taken from the active border and fixed in buffered 10 percent formalin or other suitable fixative. The Fite-Faraco staining method conventionally is used because the Ziehl-Neelsen stain does not show *M. leprae* optimally in tissue sections. However, Fite-Faraco staining is time-consuming and can result in observer fatigue, particularly in paucibacillary cases. One study showed that rhodamine-auramine fluorescent staining demonstrated organisms in 70 percent of cases, in contrast to 45 percent for Fite-Faraco staining.[254] A histopathologic diagnosis of leprosy must not be made unless the evidence is convincing. In nonendemic regions, providing additional history to the pathologist can allow for clinicopathologic correlation.[292]

FIGURE 98-2 ■ Hypopigmented macule of indeterminate leprosy on the anterior surface of the leg of an Indian girl. (See companion Expert Consult website for color version.)

Indeterminate Leprosy

In indeterminate leprosy (Fig. 98-2), the immune potential of the patient is not portrayed clearly in the cellular reaction. Only a mild chronic inflammation is found, with small infiltrations of lymphocytes or histiocytes along neurovascular channels and sometimes around appendages (Fig. 98-3). If leprosy is suspected, all nerves in the dermis and subcutaneous tissue in numerous sections of the biopsy specimen must be searched for acid-fast bacilli, even if no inflammatory changes within the nerves are present. Sometimes acid-fast bacilli appear only in the arrector pili muscles or subepidermal zone,[285] but are rarely seen in normal-appearing skin.[33] Angiogenesis is not common in indeterminate leprosy skin lesions, in contrast to other forms.[20] A histopathologic diagnosis of leprosy cannot be made unreservedly in indeterminate leprosy without showing acid-fast bacilli. Molecular biologic studies rarely are helpful in diagnosing indeterminate leprosy.

Tuberculoid Leprosy

Patients with tuberculoid leprosy (Fig. 98-4) have a high level of cell-mediated immunity to *M. leprae*, which is reflected in the cellular reaction. Granulomata composed of epithelioid cells, Langhans giant cells, and lymphocytes are present in the dermis or subcutaneous tissue (Fig. 98-5A). Frequently, upper dermal granulomata invade the lower layers of the epidermis (see Fig. 98-5A). Damage to nerves is a distinctive feature; in old, advanced lesions, all cutaneous nerves may be damaged beyond recognition (see Fig. 98-5B). Occasionally, S-100

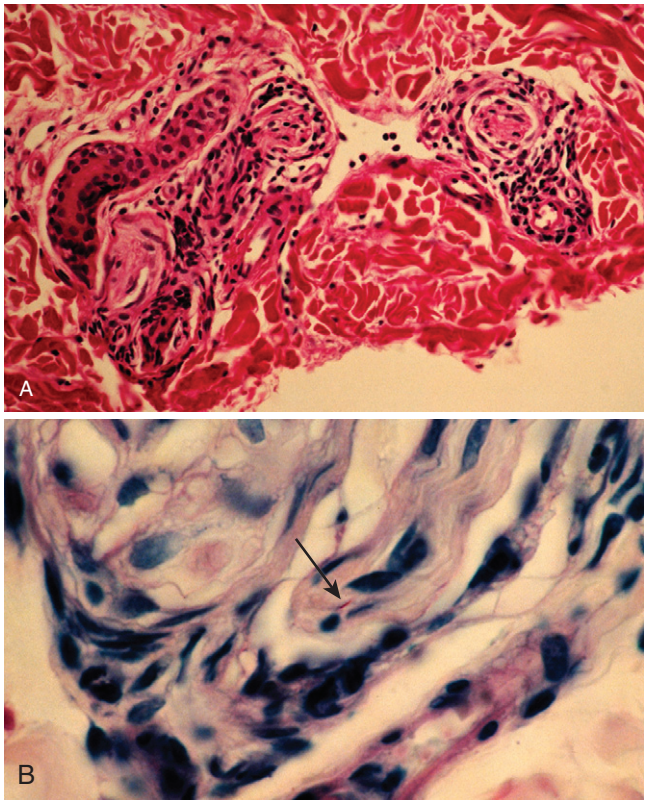

FIGURE 98-3 ■ **A,** Indeterminate leprosy showing only mild infiltrations along neurovascular channels in the deep dermis (hematoxylin and eosin stain). **B,** Only a single acid-fast bacillus was found in one deep dermal nerve (*arrow*) after a search of multiple sections (Fite-Faraco stain). (See companion Expert Consult website for color version.)

FIGURE 98-4 ■ Tuberculoid leprosy in a 12-year-old Congolese boy. This was the only lesion, and it has a well-defined papulated border with central healing. (See companion Expert Consult website for color version.)

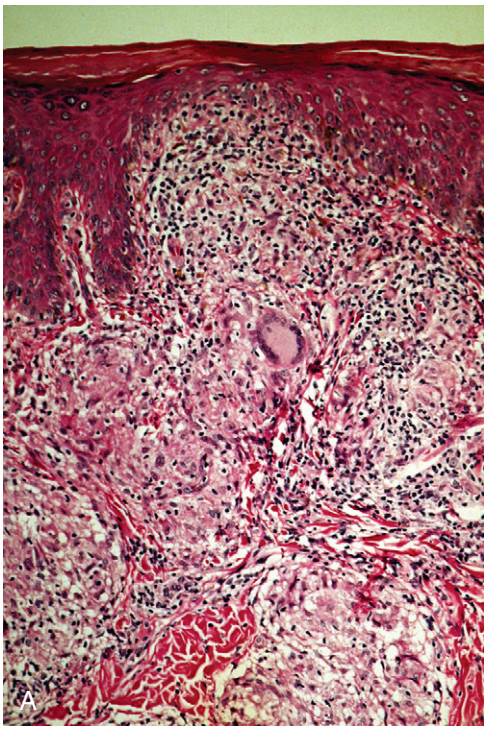

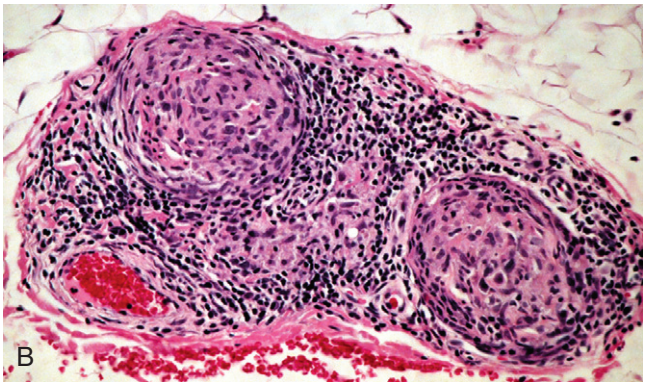

FIGURE 98-5 ■ **A,** Tuberculoid leprosy with a dense granulomatous infiltration that invades the epidermis. Granulomata contain epithelioid cells, Langhans giant cells, and lymphocytes (hematoxylin and eosin stain). **B,** High magnification of a nerve bundle in the subcutaneous tissue of section in **A.** Granulomata have nearly completely destroyed the nerves. Remnants of nerves contained rare acid-fast bacilli (hematoxylin and eosin stain). (See companion Expert Consult website for color version.)

immunostaining helps reveal a neural pattern of Schwann cells if a question exists as to whether nerves are damaged. Schwann cells are increased in number in early lesions, and the nerves are invaded by mononuclear cells. Bacilli rarely are found, and often many sections must be searched to locate a single bacillus. The bacilli usually are within remnants of dermal nerves but sometimes are located just beneath the epidermis. When major nerve trunks are involved, they contain typical tuberculoid infiltrates that eventually may replace the entire nerve. Occasionally, caseous "abscesses" are present in large nerves, but they are rare in children.

Borderline Leprosy

Borderline leprosy represents a broad spectrum of clinical (Fig. 98-6) and histopathologic variations (see Table 98-1). In the borderline-tuberculoid variety, strong cell-mediated immunity still is evident by the large numbers of epithelioid cells and lymphocytes. The nerves usually are damaged less and are identifiable more readily than

FIGURE 98-6 ■ Borderline lepromatous leprosy in a Filipino. There are many plaques, some with well-defined borders and others vaguely defined. The erythematous plaques indicate that the patient is undergoing an upgrading reversal reaction (type 1). (See companion Expert Consult website for color version.)

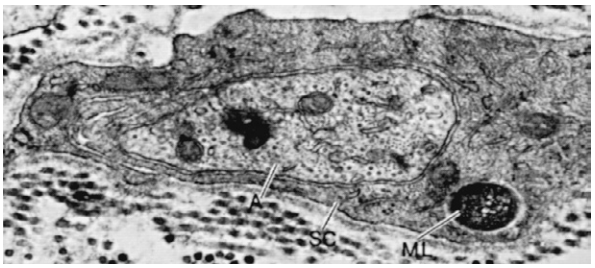

FIGURE 98-7 ■ Electron micrograph of a portion of a damaged dermal nerve in borderline leprosy. A nonmyelinated axon (*A*) is surrounded by a Schwann cell (*SC*) that contains a single *M. leprae* bacillus (*ML*) (×60,000). (Courtesy Dr. S. C. Chang.)

in tuberculoid leprosy. *M. leprae* often remain rare but are seen more readily than in tuberculoid leprosy in nerves (Fig. 98-7) or in the subdermal zone. Acid-fast bacilli can be seen in arrector pili muscle in normal-appearing skin in 17 to 25 percent of cases of borderline leprosy.[33] In the central portion of the borderline area of leprosy, the epithelioid cells are not surrounded by large numbers of lymphocytes and Langhans giant cells are uncommon. The subepidermal area is free of infiltrating cells and nerves are not damaged severely, but the perineurium often is thickened by epithelioid cells. Some histopathologists do not recognize the borderline area of leprosy as an entity because there is usually a suggestion that the lesion is on either the borderline-tuberculoid or the borderline-lepromatous side of the spectrum of the disease.[151] Borderline-lepromatous lesions reveal a low level of cell-mediated immunity. The

FIGURE 98-8 ■ Advanced lepromatous leprosy in an adolescent Filipino. Skin of most of the face, especially the alae nasi, is diffusely thickened. Eyebrows are thinned. (See companion Expert Consult website for color version.)

granulomata comprise mostly macrophages, but contain many irregularly distributed lymphocytes. A few nests of epithelioid cells may be present. The perineurium of the nerves is infiltrated with cellular exudates. Nerves are easily identified and contain many bacilli.

Lepromatous Leprosy

Prelepromatous lesions show only a mild proliferation of macrophages around vessels, nerves, and appendages. Acid-fast bacilli are few and often difficult to show. Anergy to *M. leprae* becomes apparent early in the lepromatous lesion (Fig. 98-8), with the bacilli-laden macrophage (Virchow cell or lepra cell) being the predominant inflammatory cell. In early lesions, they tend to accumulate around vessels, nerves, and appendages, but they eventually may replace the entire dermis (Fig. 98-9A). The infected macrophages are supported by a delicate stroma and supplied by a rich network of capillaries. As the macrophages age, they become vacuolated (foamy), largely from their lipid content. In developing lesions, the intracellular bacilli are arranged in small bundles (see Fig. 98-9B); in advanced lesions, dense masses of bacilli termed *globi* may replace nearly the entire cytoplasm of the macrophage. Infiltrating cells do not invade the epidermis but leave a narrow subepidermal clear zone. Many bacilli are found in the dermal nerves and frequently in endothelial cells, walls of blood vessels, arrector pili muscles, and epithelial cells of hair follicles.[245] In one case series, acid-fast bacilli were found in arrector pili muscle in normal-appearing skin in more than 50 percent of patients with lepromatous leprosy,[33] with implications for both disease extent and recurrence risk. Plasma cells vary in number and probably reflect B-lymphocyte hyperactivity.[107] Few lymphocytes are in a lepromatous lesion. Large nerve trunks may show typical lepromatous infiltrations.

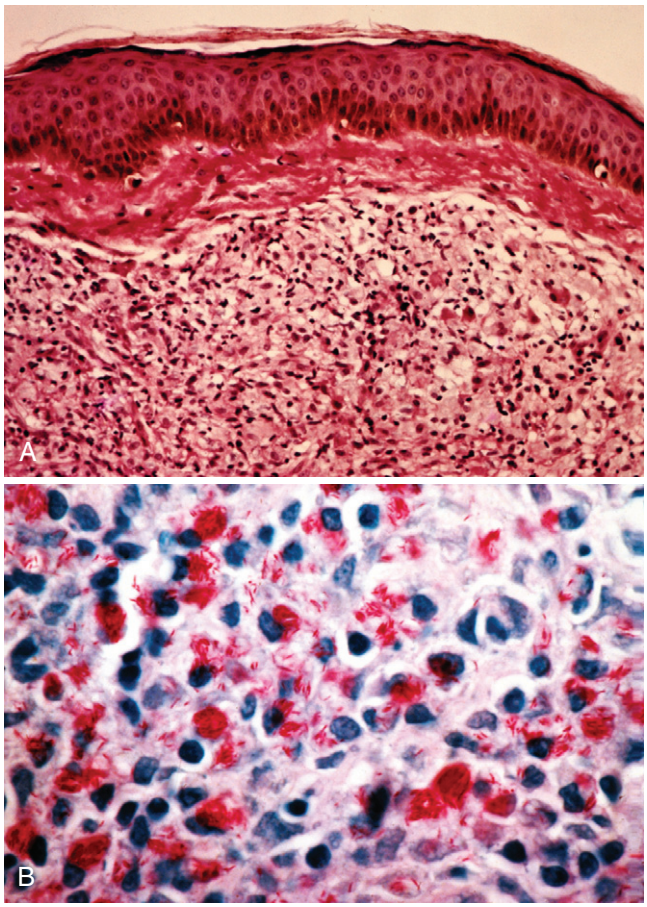

FIGURE 98-9 ■ A, Advanced lepromatous leprosy showing replacement of dermis by foamy histiocytes and very few lymphocytes. Note thin subepidermal clear zone (hematoxylin and eosin stain). **B,** Higher magnification of same biopsy specimen showing clumps of *M. leprae* (globi) in histiocytes in a leproma in the skin (Fite-Faraco stain). (See companion Expert Consult website for color version.)

Occasionally, in patients with lepromatous leprosy, elevated firm nodules form in the skin, especially in relapsing disease. Because of the characteristic histologic pattern of these lesions, in which the spindle-shaped histiocytes resemble fibrocytes, this form is called *histoid leprosy.*[318,383] Lepromatous leprosy is disseminated widely, and lepromatous infiltrations frequently are found in the upper respiratory tract, as far down as the larynx, and in the eyes and testes. In adults, the testes sometimes are densely infiltrated, with subsequent development of sterility and gynecomastia, but these complications are rare findings in children. Lymph nodes often are infiltrated by bacilli-laden macrophages, especially in the medulla and paracortical areas.

CLINICAL MANIFESTATIONS

The incubation period varies (usually 2 to 5 years), and no prodromal manifestations are well established. Some experienced clinicians working in areas of high prevalence recognize early signs of nerve involvement (i.e., localized paresthesia, itching, or numbness) before any visible lesions develop. Paucibacillary leprosy is defined as having one to five skin lesions, and includes all smear-negative cases. Multibacillary leprosy includes more than five skin lesions and includes all smear-positive cases. The polar forms of leprosy include tuberculoid (paucibacillary disease) and lepromatous (multibacillary) leprosy, with other forms falling between these extremes. Differentiation of paucibacillary from multibacillary disease is important for selection of the number of empirical medications started, duration of therapy, and risk for relapse and sequelae.[412]

After the incubation period, various lesions appear. The nature of the lesions depends on the immune response of the patient to *M. leprae*. So far, no strain variations of the bacillus except in drug sensitivity have been detected.[399,400] Most clinicians today follow the classification scheme outlined by Ridley and Jopling (see Table 98-1).[286] Classification is important because it aids in establishing the prognosis and treatment program for the patient. The distribution of leprosy types varies geographically. One study from the National Leprosy Control Program in Mexico reviewed more than 6000 skin biopsies obtained between 1978 and 2002. Lepromatous leprosy was the most common form, seen in 60 percent of cases; twice as many cases were multibacillary as paucibacillary.[375] In contrast, trends over 50 years in one region of India showed that in recent decades, borderline leprosy was more common and multibacillary rates had declined.[258]

Virtually all patients with leprosy have peripheral neuropathy if cutaneous sensory changes are included, and approximately 25 percent have significant deformity. In experimental studies, the pathogenesis of peripheral neuritis in leprosy involves bacillation of the endothelial cells of epineural and perineural blood vessels and lymphatics.[314,316,317] Surface proteins of *M. leprae* may bind the bacillus to Schwann cells via laminin.[276,332]

Ocular complications in leprosy are well known, especially in lepromatous leprosy. These lesions can include corneal scarring; conjunctivitis; and eyelash abnormalities, including ectropion (eversion of lid margin from globe), lagophthalmos (inability to close eyelids completely), madarosis (loss of eyelashes), and trichiasis (eyelashes growing back toward globe).[58,63,64,87,131] All patients with leprosy should be evaluated by an ophthalmologist at diagnosis and periodically thereafter, especially during any reactional episodes. Reactional episodes can involve ocular hypotension, punctate keratitis, and uveitis.[65]

Indeterminate Leprosy

An indeterminate lesion is the first manifestation of leprosy in most patients and may heal spontaneously, remain unchanged for months or years, or gradually progress toward tuberculoid or lepromatous disease. Patients with indeterminate leprosy have a single or a few macules in the skin (see Fig. 98-2). The macule is defined poorly and is mildly hypopigmented in deeper pigmented skin and slightly erythematous in lighter skin. Skin texture, sensation, and sweating within early macules are normal or only slightly altered. Peripheral nerves are

normal, and skin smears from lesions rarely contain bacilli. The definitive diagnosis can be made only by finding acid-fast bacilli in histopathologic sections.

Tuberculoid Leprosy

Tuberculoid leprosy is the paucibacillary extreme of leprosy. Patients with tuberculoid leprosy have a single or several asymmetrically distributed hypopigmented skin lesions (see Fig. 98-4). Tuberculoid lesions arise de novo or evolve from indeterminate macules. The lesion may be macular or infiltrated, but the borders always are sharply demarcated from the surrounding normal skin and frequently are finely papulated. Lesions range in size from less than 1 cm to large enough to cover entire regions, such as the thigh or buttock. Many tuberculoid lesions heal spontaneously. In large, active lesions, the centers often are healed and repigmented, although somewhat atrophic. The lesions often are hairless, hypohidrotic, and anesthetic. On the face, because of its rich innervation, the detection of hypoesthesia in early lesions requires discriminating tests. Conversely, clinicians may mistakenly diagnose leprosy in areas of the body that normally are hypoesthetic (e.g., over the elbows or knees). Despite hypoesthesia, deep pain on percussion of lesions over bone in tuberculoid leprosy, termed the *tap sign*, has been described, with a sensitivity of 67 percent and specificity of 100 percent.[179]

Involvement of peripheral nerves commonly occurs in tuberculoid leprosy (Fig. 98-10), and cutaneous nerves often can be palpated adjacent to or within lesions. The regional nerve trunks most commonly enlarged are the ulnar from the olecranon groove to midarm, lateral popliteal just distal to the head of the fibula, and posterior tibial in the medial aspect of the ankle. Enlarged or tender nerves anywhere should alert the clinician to the possibility of leprosy. Any readily palpable cutaneous nerve probably is enlarged, but evaluating the size of nerve trunks requires experience because of the wide range in normal size.

FIGURE 98-10 ■ Enlargement of the great auricular nerve in an adolescent Congolese boy. A large macule of tuberculoid leprosy over the angle of the mandible is now nearly inactive and barely visible. (See companion Expert Consult website for color version.)

Borderline Leprosy

Borderline leprosy, sometimes called *dimorphous* or *intermediate leprosy*, has features of the lepromatous and tuberculoid forms and represents a continuous spectrum of disease ranging from near-tuberculoid to near-lepromatous. It is an unstable form of leprosy and may evolve gradually toward tuberculous leprosy by undergoing reversal reactions or be downgraded toward lepromatous leprosy. Table 98-1 describes the three major subgroups of borderline leprosy: borderline-tuberculoid, borderline, and borderline-lepromatous.

In borderline-tuberculoid leprosy, the number of lesions usually is greater than in tuberculoid leprosy, and the borders of each lesion, macule, or plaque are defined less sharply than in tuberculoid leprosy. Small satellite lesions may develop around larger macules or plaques. Borderline-lepromatous leprosy often manifests with widespread nodular infiltrations or plaques of varying size (see Fig. 98-6).

Damage to nerves and the resulting deformity develop early and often are widespread. Neuropathic pain or neurotropic changes (e.g., sensory changes that lead to damaged hands or feet or a muscular weakness such as footdrop) frequently bring the patient to the physician. Severe damage to nerves occurs infrequently in young children, but can be disastrous. Prevention of this complication is an important goal of leprosy detection programs and of treatment in every patient with leprosy.

Lepromatous Leprosy

In contrast to tuberculoid leprosy, lepromatous leprosy is the classic multibacillary disease extreme. Here, the bacilli multiply freely, peripheral blood smears can be positive for acid-fast bacilli (up to 80% in one study),[80] and the disease disseminates widely, often before striking cutaneous manifestations develop. Lepromatous leprosy may evolve from an indeterminate or borderline area leprosy or may be the first recognizable form. In its earliest form, lepromatous leprosy manifests as "juvenile leprosy," a clinical entity delineated from observations of large numbers of children in homes for children of patients with leprosy in India.[244] This form, also called *prelepromatous leprosy*, is difficult to detect and frequently goes unrecognized until a more advanced stage develops. Skin texture may be altered slightly, but the vague macules with indistinct borders are detected only under appropriate lighting, preferably daylight. No changes in sensation or sweating occur in the macules, and frequently acid-fast bacilli are not detectable in skin smears. Histopathologic sections may reveal a few bacilli to confirm the diagnosis; however, if leprosy is suspected, the patient should be monitored until an explanation for the mild skin changes is found. If leprosy is present and not detected and treated, advanced forms of lepromatous leprosy develop in many of these patients (Fig. 98-11).

FIGURE 98-11 ■ Progression of lepromatous leprosy in a Hawaiian boy. The photograph on the *left* was taken in 1931, when the patient was 13 years old, and the photograph on the *right* was taken 2 years later. No effective chemotherapy was available in that era.

The hypopigmented or slightly erythematous macules of early lepromatous leprosy, similar to those of juvenile leprosy, are missed easily because they are vague and have slight, if any, sensory changes. These macules usually are small but gradually may coalesce and cover large areas of skin, even nearly the entire body. Clinical diagnosis often is missed, and over the course of a few years, advanced lepromatous leprosy develops. If skin smears or biopsy specimens are taken in the macular stage, diagnosis is virtually ensured. If the disease is not diagnosed and treated in the macular stage, infiltration of the skin increases gradually and nodules may develop. The skin is infiltrated most heavily in the cooler portions of the body, notably the ears (pinnae) and face. By this time, nerves usually are enlarged, with early signs of sensory loss in the hands and feet. Eyebrows are thinned and eventually lost, beginning at the lateral edges. These advanced changes of lepromatous leprosy are not common findings in young children but are well known.

Histoid leprosy, one variant of multibacillary leprosy, often is classified with lepromatous leprosy. Here, firm, dome-shaped nodular or papular lesions show constriction around the base. They are more commonly found in the axial skeleton.[318]

Patients of Latin American ancestry, especially patients from Mexico and Costa Rica, may contract the highly anergic diffuse form of lepromatous leprosy called *Lucio leprosy*. The disease may be so diffuse that it is not recognized until sensory changes appear in the hands and feet, and the eyebrows and other body hair begin to disappear. In advanced forms of Lucio leprosy, a marked obstructive vasculitis in the skin is present, with the production of dermal infarcts and irregular ulcers (Lucio phenomenon).[185,280] Lucio leprosy has been reported in children 7 years old.[310]

Neuritic Leprosy

Neurotic leprosy, a relatively rare form of the disease, manifests as motor or sensory impairment in one or multiple nerves in the absence of cutaneous changes. Symptoms include anesthesia, paresis, and wasting of muscles in the affected area. Nerve trunks frequently are painful, enlarged, and tender. Leprosy must be suspected in patients with any peripheral neuritis that has these features. Chronic neuropathy and nerve enlargement often persist for years after the patient has completed chemotherapy for leprosy.[130] Large leprotic nerve abscesses are rare occurrences but are exquisitely painful and may require surgical intervention for drainage.[321] Most clinicians prefer to treat such lesions with corticosteroids.

Leprosy and Acquired Immunodeficiency Syndrome

Given the history of acquired immunodeficiency syndrome (AIDS) altering the epidemiology and undermining control efforts for the other mycobacterial species with human-to-human transmission, *M. tuberculosis*, concern exists that a similar phenomenon might be seen with *M. leprae*.[367] In one study in Zambia, antibodies to human immunodeficiency virus (HIV) were found in 33 percent of patients with newly diagnosed leprosy versus 7 percent of controls.[206] Perhaps the positivity of some of these patients with leprosy can be explained by cross-reactivity between antibodies to HIV-1 and the lipoarabinomannan of *M. leprae*.[168] In other populations in Africa, HIV infection constituted an overall risk factor of 2.2 for leprosy, with 4 to 23 percent of multibacillary leprosy cases attributable to HIV coinfection.[27]

However, most studies have shown that HIV seroprevalence among leprosy patients is no higher than the general population and that clinical manifestations are similar in coinfected patients and patients with leprosy alone.* Other prospective studies show that HIV infection may not be a risk factor for acquisition of leprosy in some populations.[172,320] These observations are supported by a study of the parameters of cell-mediated immunity in coinfected patients in Brazil.[305] In these patients with borderline leprosy, the quality of the granulomata in the infiltrations of leprosy was not altered significantly despite low $CD4^+$ T-cell counts. The incidence of reversal reactions (type 1, described later) and neuritis is increased in multibacillary patients coinfected with HIV.[39] These reactions, similar to immune reconstitution inflammatory syndrome (IRIS) have been described for both forms of IRIS.[367] Paradoxical IRIS, in which worsening of leprosy lesions is seen while on both appropriate leprosy and antiretroviral therapy, has been described with borderline leprosy; reactions often are local, not systemic.[15] Unmasking IRIS, in which leprosy symptoms develop anew in a person on antiretroviral therapy, has been described in a patient with borderline tuberculoid leprosy.[187]

REACTIONS

The course of leprosy, treated or nontreated, often is interrupted by acute episodes known as reactions, which fall into two general categories: reversal reactions (or type 1) and erythema nodosum leprosum (or type 2).[74]

*References 147, 172, 180, 241, 320, 367.

Reversal Reactions

Reversal reactions complicate borderline leprosy and represent delayed hypersensitivity reactions, with an upgrading of cell-mediated immunity toward tuberculoid leprosy. Lesions become erythematous and edematous, and neuritis is a common manifestation (Fig. 98-12). Risk factors for reversal reactions include being lepromin-positive and having PGL-1 IgM antibodies.[288] Proliferation of sensitized T lymphocytes initiates reversal reactions and amplifies the inflammatory response by releasing lymphokines and activating macrophages.[293] Effective chemotherapy for paucibacillary and multibacillary patients may activate cell-mediated immunity and provoke clinical or subclinical reversal reactions. Expression of HLA-DR is increased, which may enhance production of IFN-γ by lymphocytes in granulomata.[61] Histopathologically, edema is accompanied by increased lymphocytes, epithelioid cells, and giant cells. In severe reactions, necrosis may occur, almost always in nerves. Increased levels of TNF-α may partially explain this necrosis.[22] Cyclooxygenase-2 levels are elevated in nerves and blood vessels during reversal reactions, a finding that explains why nonsteroidal anti-inflammatory drugs, including selective cyclooxygenase-2 inhibitors, often improve the condition.[267] Patients experiencing such reactions must be observed closely so that sensory loss and deformities are minimized.

FIGURE 98-12 ■ Reversal reaction (type 1) in an 8-year-old Congolese boy with borderline-tuberculoid leprosy. The left side of his face is swollen and displays mild palsy from facial nerve damage. The patient responded rapidly to oral steroid therapy. (See companion Expert Consult website for color version.)

Differentiating reversal reactions from relapsing lesions frequently is difficult and requires careful correlation of clinical and histopathologic findings. This correlation is becoming increasingly important in endemic areas, where shorter term chemotherapeutic regimens of fixed duration are used.[104,215] The following criteria for differentiating relapses and reversal reactions are suggested: a relapse involves an increased number of lesions, positive skin smears for acid-fast bacilli (for patients with a borderline area of leprosy and borderline-lepromatous leprosy), tissue reaction inconsistent with a reversal reaction, and a favorable response to chemotherapy; a reversal reaction involves an exacerbation of existing lesions, skin smears negative for acid-fast bacilli, tissue reaction consistent with a reversal reaction, and a rapid response to anti-inflammatory drugs.

Erythema Nodosum Leprosum

Formerly, erythema nodosum leprosum developed in approximately 50 percent of lepromatous leprosy patients after they had undergone a few months of chemotherapy; however, with the addition of clofazimine to the standard therapeutic regimen, the frequency is much reduced.[93] Tender erythematous subcutaneous nodules develop rapidly (Fig. 98-13); the nodules often are accompanied by fever, synovitis, and iridocyclitis.[269] Erythema nodosum leprosum resembles the Arthus reaction and is thought to result from immune complex formation.[111] Erythema nodosum leprosum is associated with serum TNF-α elevation[22,309] and intralesional increases in helper T lymphocytes.[234]

In the nodule, neutrophils and sometimes an intense vasculitis are present. Ulceration of the skin frequently accompanies severe erythema nodosum leprosum. Glomerulonephritis sometimes complicates this condition; secondary amyloidosis is a late sequela of repeated reactions and may be a consequence of the neutrophilic leukocytosis.[202] Although neutrophilic infiltration is considered by many physicians to be the hallmark of the tissue reaction, tissue from a rare patient with clinically typical erythema nodosum leprosum may not show neutrophils. In such patients, demonstration of serum amyloid A and C-reactive protein may aid in establishing

FIGURE 98-13 ■ Erythema nodosum leprosum in a Filipino adolescent girl with lepromatous leprosy. (See companion Expert Consult website for color version.)

the diagnosis.[137] Occasional patients may have mixed reversal (upgrading) reactions and erythema nodosum leprosum. These patients usually have leprosy in the borderline-lepromatous area of the spectrum.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The cardinal signs of leprosy are hypoesthetic lesions of the skin, enlarged peripheral nerve or nerves, and acid-fast bacilli in skin smears. In the absence of another clear explanation, any one of these signs strongly suggests leprosy.

An experienced observer can establish a clinical diagnosis in most patients, except those with early leprosy, with a high degree of accuracy. Because leprosy is considered one of the "great imitators,"[388] however, histopathologic evaluation is strongly recommended to supplement and confirm the clinical diagnosis. Patients with leprosy may be found in almost any geographic area, an awareness of which would minimize missed and delayed diagnoses, especially in areas of low prevalence. In the United States, the usual delay in establishing the diagnosis after the patient's first visit to a physician for symptoms related to leprosy is approximately 1.5 to 2 years. This delay often significantly worsens the prognosis.

History is important. Contact with patients with leprosy or residence in an endemic area raises suspicion of leprosy in a patient with a chronic lesion of the skin. Sensory loss or unexplained damage to hands or feet suggests damage to nerve trunks. Sometimes, a footdrop or clawhand brings the patient to a physician. Occasionally, patients with lepromatous leprosy consult an otolaryngologist first because of a chronic stuffy nose.

Clinicians must evaluate sensory changes in a lesion using the precautions already mentioned. The modalities usually tested are light touch with the use of a few fibers of cotton or calibrated nylon threads and heat and cold discrimination with the use of warm and cold water in test tubes. Much patience and repeated testing often are necessary in evaluating young children. Spontaneous sweating can be observed directly, or induced sweating can be evaluated. Hair may be completely preserved in early lesions but lost in advanced lesions. The main nerve trunks must be palpated for tenderness and enlargement. Skin in the area of discrete lesions also must be palpated gently to detect enlargement of cutaneous nerves. In the world population, leprosy is the most common cause of peripheral neuropathy and must be considered in any patient with peripheral neuropathy.[301]

Obtaining and examining smears for acid-fast bacilli is an important diagnostic procedure and should be controlled carefully by experienced laboratories. Briefly, smears are made from the edge of discrete macules or plaques, nodules, ear lobes, and nasal mucosa. Fine needle aspirate has a sensitivity over 90 percent.[207] Skin smears are made by squeezing and holding a fold of skin between the thumb and forefinger to avoid getting blood in the smear and by making a short, shallow slit in the skin with a razor blade or scalpel. The instrument is turned at a right angle to the slit, and the edges of the incision are scraped. The cells and fluid thus obtained are spread on a slide, heat-fixed, and stained by the Ziehl-Neelsen method.[378] Evaluation of smears should not be done by researchers unfamiliar with their interpretation. An occasional acid-fast bacillus may be a harmless contaminant in the staining reagents. DNA probes specific for *M. leprae* are available and are useful in identifying leprosy bacilli in tissue or nasal secretions.[75,401]

The lepromin reaction is useless for the diagnosis of leprosy.[35] Currently available skin tests with soluble *M. leprae* antigens are unreliable.[125] Enzyme-linked immunosorbent assays and gelatin particle agglutination tests for antibodies to the PGL-1 of *M. leprae* are available.[45,119,142] Although specificity for *M. leprae* is high, these tests detect antibodies to PGL-1 in only 50 percent of patients with paucibacillary leprosy. Other serologic tests for antibodies to *M. leprae*–specific epitopes on protein moieties of the bacillus are being evaluated.[265] PGL-1 antigen is detectable in the serum and urine of most multibacillary patients.[260,418] PGL-1 antibodies also have been used for early leprosy diagnosis in household contacts.[16]

Reliance on DNA probes and PCR technology may prove useful in establishing the diagnosis of leprosy in tissue sections, skin smears, and nasal smears.[75,356,399,401] Because these methods can detect a single leprosy bacillus, interpreting results, particularly in highly endemic areas, is difficult. Careful clinicopathologic correlation is essential when basing a diagnosis on DNA findings.[370] Multiplex PCR has also been piloted to detect early cases of leprosy in community-based contact tracing, and was more sensitive than acid-fast bacillus smear examination.[13] One pediatric study showed that whereas slit-skin acid-fast bacillus smears were positive in only 20 percent of cases, PCR was positive in almost 90 percent of children with borderline and lepromatous leprosy and 72 percent of leprosy cases overall.[158] Specimens for PCR amplification should be preserved in 70 percent ethyl alcohol.[44,99,122]

Historically, mouse footpad (MFP) assays have been used to screen for *M. leprae* drug resistance; however, the MFP assay is time-consuming (often requiring more than 6 months before result availability). Molecular targets for drug resistance have been identified. Resistance to rifampin (similar to *M. tuberculosis*) is associated with the *rpoB* gene, fluoroquinolone resistance with the *gyrA* gene, and dapsone resistance is associated with the *folP1* gene.[250] In one study, sensitivity was higher for PCR than for MFP.[319]

The differential diagnosis of leprosy in children is an extensive subject and can be discussed only briefly here. Superficial mycoses and postinflammatory changes commonly are confused with early leprosy. Changes in pigmentation may be caused by scars, birthmarks, and actinic dermatitis. In areas where dermal filariasis is endemic, vague macules in a black-skinned patient may appear identical to the early macules of leprosy.[31,204,218] Among the many infiltrated lesions of the skin that can resemble leprosy are leishmaniasis, lymphoma, granuloma annulare, granuloma multiforme (Mkar disease), lupus erythematosus, psoriasis, pityriasis rosea, sarcoidosis, and

neurofibromatosis. Histoid leprosy especially mimics other diseases—von Recklinghausen lesions, molluscum, erythema nodosum, subcutaneous rheumatoid nodules, and nodular syphilis.[318] Peripheral neuropathies that may simulate leprosy are those seen in Morvan syndrome, syringomyelia, lead intoxication, diabetes mellitus, primary amyloidosis of nerves, and familial hypertrophic neuropathy. Pure neuritic leprosy is in the differential diagnosis of hypertrophic neuropathies, such as those caused by amyloidosis and chronic relapsing polyneuritis, and progressive hypertrophic interstitial lepromatous leprosy frequently gives false-positive reactions for syphilis.

PROGNOSIS

Without chemotherapy, the prognosis in all patients except those with limited and self-healing disease potentially is poor. Patients with borderline or advanced tuberculoid leprosy frequently become mutilated because of neuropathy. Borderline patients can decline toward lepromatous leprosy. In patients with lepromatous leprosy, the disease is progressive and can cause death from laryngeal obstruction. Secondary amyloidosis is a frequent late sequela. Blindness may result from repeated episodes of iridocyclitis or ocular desiccation and/or keratitis from eyelash anomalies. Nerve damage as identified by electrophysiologic tests may be more widespread than realized on clinical examination. In one series of adults with multibacillary leprosy, before treatment, 92 percent of patients had abnormal nerve conduction studies, with sensory nerves being more involved than motor nerves (52% vs. 37%), and almost two thirds of patients had involvement of five or more sensory or motor nerves or both.[41] General debility and deformity eventually prevent gainful employment in many patients. Additionally, the stigma associated with the disease may have pervasive effects on the lives of affected children and adults.

If therapy is started early, the prognosis usually is excellent, and deformity and mutilation are prevented. Even after receiving successful chemotherapy, however, some patients continue to experience significant neuritis and loss of peripheral nerve function. Sometimes this "silent neuropathy" goes unnoticed by the patient and the physician.[67,395] Appropriate early attention to anesthetic hands and feet and restoration of function by reconstructive surgery can prevent most mutilation.

The lepromin test is a valuable prognostic tool because it measures the cell-mediated immunity potential of the host to acquisition of infection by *M. leprae*.[150] Patients with early macular lesions who are lepromin-negative have a poorer prognosis than patients who are lepromin-positive if treatment cannot be administered. Histopathologic evaluation made before instituting chemotherapy is important for determining the prognosis and, if available, should not be neglected.

TREATMENT

When a diagnosis of leprosy is established, chemotherapy must be initiated and appropriate measures must be instituted for preventing or correcting deformity in patients with neurotropic changes.[47,105,183] The three chemotherapeutic agents most commonly used are dapsone, clofazimine, and rifampin.[242] Although the WHO recommends only clinical findings for assessment of the therapeutic response in field programs, clinicians may wish to use additional evaluations. The effectiveness of chemotherapy is assessed readily in patients with lepromatous or near-lepromatous leprosy by the staining quality of *M. leprae* in skin smears. The response to chemotherapy in patients with tuberculoid leprosy and most borderline patients is determined by the clinical response and by histopathologic evaluation. Viability of *M. leprae* in tissues is assessed in unusual cases in the mouse footpad.

Combined chemotherapy is being accepted increasingly for all forms of active leprosy. Although experimental data are limited,[330] multidrug therapy has replaced monotherapy (Table 98-2).[76] Monotherapy with any chemotherapeutic agent is not advised for concern for selection of resistant isolates. As previously discussed, conventional methodologies for leprosy drug susceptibility are cumbersome, leading to increased emphasis on detection of resistance using molecular modalities. This has also resulted in increased surveillance capabilities. During 2010, sentinel surveillance data from nine nations (i.e., Brazil, China, Colombia, India, Myanmar, Pakistan, the Philippines, Vietnam, and Yemen) evaluated drug resistance in patients with relapsed multibacillary leprosy. Of 88 samples tested for drug resistance, 9 were dapsone-resistant, 1 was rifampin-resistant, and none were fluoroquinolone-resistant. No isolate was found to be multidrug-resistant.[413]

Dapsone

Dapsone, a bacteriostatic sulfone antimetabolite for *M. leprae*, was the first chemotherapeutic agent found to be effective; it was first used in 1941 in Carville, Louisiana.[92] The drug usually is given orally at 1 to 2 mg/kg/day as a single daily dose. The adult dose is 100 mg daily, and in the United States, dapsone is available as 25- and 100-mg tablets. The WHO guidelines recommend dapsone as part of multidrug therapy for all forms of leprosy.[411] The effect of dapsone on the bacilli is slow; 3 to 6 months of treatment is necessary to render bacilli from patients with lepromatous leprosy noninfectious for the mouse footpad. Sulfone-resistant strains of *M. leprae* are being detected with increasing frequency[395,396,413]; monotherapy with dapsone is not recommended. Dapsone usually is well tolerated but may provoke one or more of the following signs or symptoms: fever, exfoliative dermatitis, generalized lymphadenopathy, hepatosplenomegaly, or psychosis. Laboratory findings may include leukocytosis, hemolytic anemia, eosinophilia, or elevated bilirubin and serum transaminases.[5] Consideration should be given to screening children for glucose-6-phosphate dehydrogenase (G6PD) deficiency before initiation of therapy.

Rifampin

Rifampin is a bactericidal antibiotic inhibiting bacterial DNA-dependent RNA polymerase. A single large dose

TABLE 98-2 **Suggested Dosing Regimens for Leprosy from the World Health Organization[410,411] and National Hansen's Disease Program[252,*]**

Leprosy Classification	Drug	Dose†			Duration
		Age <10 Yr	Age 10-14 Yr	Age ≥ 15 Yr	
Paucibacillary[1]	Dapsone	1-2 mg/kg/day	1-2 mg/kg/day	100 mg daily	WHO: 6 months NHDP: 12 months
	Rifampin	10-20 mg/kg/day	10-20 mg/kg/day	600 mg daily (NHDP) 600 mg monthly (WHO)	
Multibacillary[2]	Dapsone	1-2 mg/kg/day	1-2 mg/kg/day	100 mg daily	WHO: 12 months NHDP: 24 months
	Rifampin	10 mg/kg/day	10 mg/kg/day	600 mg monthly (WHO) 600 mg daily (NHDP)	
	Clofazimine[3]	50 mg biweekly + 100 mg monthly (WHO) 1 mg/kg/day (NHDP)	50 mg every other day + 150 mg monthly (WHO) 1 mg/kg/day (NHDP)	50 mg daily (NHDP, WHO) ± 300 mg load monthly (WHO)	
	Minocycline (second line for dapsone or clofazimine)	25 mg monthly if <15 kg; 50 mg monthly if 15-30 kg (WHO) ND (NHDP)	50 mg monthly (WHO) ND (NHDP)	100 mg monthly (WHO) 100 mg daily (NHDP)	Extend multidrug therapy to 2 years[380]
	Ethionamide (second line for clofazimine)	50-75 mg daily if <15 kg, 150-175 mg daily if 15-30 kg (WHO) ND (NHDP)	275-375 mg daily (WHO) ND (NHDP)	275-375 mg daily	
	Ofloxacin (second line for clofazimine or rifampin)	100 mg monthly if <15 kg, 200 mg monthly if 15-30 kg (WHO) Not recommended (NHDP)	200 mg monthly (WHO) Not recommended (NHDP)	400 mg monthly (WHO) 400 mg daily (NHDP)	

[1]Tuberculoid leprosy, borderline-tuberculoid, and indeterminate.
[2]Lepromatous, borderline, and borderline-lepromatous.
[3]Higher doses may be used for patients with erythema nodosum leprosum.
*Second-line medications are italicized.
†Pediatric dosing should never exceed adult maximum. Approximate pediatric dosing,[156] as a percentage of adult dosing, can be estimated as 25% for children <15 kg, 50% if 15-30 kg, 75% if 30-45 kg, and 100% if >45 kg.
Because the National Hansen's Disease Program may change its recommendation for patient management, clinicians in the United States are advised to seek consultation prior to treating any patient with leprosy (telephone: 1-800-642-2477 or 1-225-756-3709, or e-mail, Mtemplet@hrsa.gov; it also has a website available at www.bphc.hrsa.gov/nhdp/).
ND, No dosing information available; *NHDP,* National Hansen's Disease Program; *WHO,* World Health Organization.

can render a highly positive lepromatous patient noninfectious within 1 week. Available formulations include capsules (150 and 300 mg) and a parenteral formulation. The WHO recommends rifampin as part of multidrug therapy for all forms of leprosy. The dose for persons 15 years of age and older is 600 mg, with pediatric doses of approximately 10 mg/kg given as a single daily dose. Optimal doses for children with leprosy have not been reported. Adult doses range from 300 to 600 mg daily.[415] Many side effects and drug–drug interactions with rifampin have been described, and the relevant literature must be consulted.[36] Rifampin-resistant leprosy has been reported,[144,402] so monotherapy with rifampin is not recommended. Rifampin is not felt to interact with dapsone or clofazimine.

Clofazimine

Clofazimine (Lamprene) is a bacteriostatic riminophenazine dye that has anti–*M. leprae* and anti-inflammatory activities, of particular utility for patients with erythema nodosum leprosum reactions, and is part of multidrug therapy for multibacillary leprosy (in conjunction with dapsone and rifampin).[93] The mechanism of action may involve enhancement of oxygen-dependent killing of *M. leprae* and binding to bacterial DNA. Clofazimine is available in 50- and 100-mg capsules, but is not commercially available in the United States (the National Hansen's Disease Program [NHDP] can facilitate U.S. practitioners obtaining the medication). The WHO recommends clofazimine for patients with multibacillary leprosy.[411] The lower dosages are used for maintenance therapy when a good clinical response has been achieved, and the higher dosages (up to 100 to 300 mg daily in two or three divided doses) may be needed to control erythema nodosum leprosum; these regimens have not been well-studied in children. The major side reactions to clofazimine are hyperpigmentation of skin and enteritis. Enteritis is experienced only at the higher dosages generally used for erythema nodosum leprosum. The pink to

black hyperpigmentation is most prominent on exposed body parts, usually begins within 1 to 4 weeks of therapy initiation, subsides with the clinical improvement in leprosy, and resolves after drug withdrawal. Pigmentary staining of the cornea and lacrimal fluid have been reported, but infrequently affects visual acuity. Although two instances of clofazimine-resistant *M. leprae* infection have been reported, the validity of these observations is uncertain.[165,393] Cross-resistance between clofazimine and dapsone or rifampin has not been reported. For some time, clofazimine has been considered safe in pregnancy, but one report described three neonatal deaths in 15 observed pregnancies.[94] Whether the drug was associated with the deaths was not established. Monotherapy with clofazimine is not recommended.

Multidrug Therapy

Because of the existence of drug-resistant *M. leprae*, combined drug regimens are mandatory for the treatment of all forms of leprosy.[89,330,394,395] The first large-scale multidrug therapy for leprosy was initiated in Malta in 1972. Rifampin, dapsone, prothionamide, and isoniazid were used. Evaluation of the patients approximately 20 years later revealed a single relapse; however, presumed "persisting" *M. leprae* organisms were detected.[108,157]

Combined drug therapy minimizes development of drug-resistant strains of *M. leprae* and may eliminate some "persisting" organisms. Persisting *M. leprae* are viable bacilli that can be isolated in small numbers from patients who are clinically responding well to therapy. These persisting *M. leprae* bacilli are sensitive to the drug in question when tested in the mouse footpad and may account for relapses when treatment is discontinued. Persisting *M. leprae* organisms have been detected after 5 years of therapy with rifampin, 6 years of clofazimine therapy, and 22 years of dapsone therapy.

In 1982, a WHO study group recommended the multidrug therapy regimens that are described hereafter.[406] The multidrug therapy regimens were designed primarily for field programs, and they use pulsed supervised monthly rather than daily rifampin.[19] Multidrug therapy is well tolerated, and compliance in large-scale control programs has been satisfactory. The efficacy of multidrug therapy has been promising.[42,395] In two surveys involving approximately 112,000 patients with multibacillary disease monitored for 9 years after receiving therapy, the cumulative risk for relapse was 0.77 percent. Anecdotal descriptions of certain groups of highly bacilliferous patients with relapse rates of 20 percent and recurrences developing 5 years or more after therapy have been reported.[146] These and other results suggest that therapeutic regimens for multibacillary patients should be given for longer than 2 years. In most reports, relapse rates in paucibacillary patients exceed those in multibacillary patients. The potential for relapse after receiving multidrug therapy regimens must await long-term, large-scale follow-up results.[145,342] One study indicated that IgG antibodies against several recombinant leprosy proteins (including PGL-1) decline during therapy and may serve as a marker for disease recurrence.[85] Peripheral neuropathy sometimes persists after completion of these therapeutic regimens.[4,130]

The trend since 2001 has been to reduce the duration of treatment and change the therapeutic regimens—even to the extreme of a single-dose regimen composed of rifampin, ofloxacin, and minocycline (ROM) for single-lesion therapy. One meta-analysis concluded that single-dose ROM was less effective than multidrug therapy for paucibacillary leprosy.[322] Many of these innovations are interwoven into the WHO Elimination of Leprosy Program and have provoked critical concern by some authorities.[100,193,248,344] The NHDP recommends daily therapy,[252] as opposed to the intermittent dosing recommendations for rifampin recommended by the WHO for resource-limited settings. Both WHO- and NHDP-recommended regimens are listed in Table 98-2. Laboratory monitoring should include baseline complete blood count, transaminases, bilirubin, blood urea nitrogen, creatinine, and calcium and then repeated every 3 months.

Other Drugs Under Investigation

Recent years have seen escalating use of fluoroquinolones, macrolides, tetracyclines, and ethionamide for patients intolerant of standard regimens.[112,113,123,148] Knowledge of the *M. leprae* genome will potentially lead to targeted drug development after computational genomic analysis. For example, one study identified several genes mapping to peptidoglycan cell wall biosynthesis; these *Mur* genes have no homolog to human genes, decreasing the risk for cross-reactivity of agents.[325]

Treatment of Reactions

Patients experiencing a reaction should be observed daily in the early stages and hospitalized if the symptoms are severe. Formerly, specific therapy was stopped or the dosage reduced during reactions, but these measures are no longer recommended.[406] Damage to eyes and neurotropic changes may ensue rapidly without immediate attention. Nerve tenderness and function must be assessed frequently during reactions. Acute inflammation of isolated lesions without damage to nerves is likely to be of little consequence except for cosmetic considerations, but the patient should be monitored closely.

Reversal (Type 1) Reaction

Patients with painful, tender nerves must receive immediate care, usually in the hospital. Analgesics are given, and the affected area is rested. Large daily doses of corticosteroids are started and tapered to a minimal effective dose until the reaction subsides. Conversion to alternate-day steroid regimens may be attempted when long-term treatment is necessary. One randomized, double-blind trial compared methylprednisolone with placebo in conjunction with oral corticosteroids for adults with type 1 reactions; persons treated with methylprednisolone were less likely to have nerve damage and recurrent type 1 reactions.[384] However, one meta-analysis indicated that although corticosteroids may be useful for treating acute

nerve damage, long-term benefit may not be seen.[41,374] A 12-week course of topical tacrolimus was used in one case of steroid-refractory type 1 reaction in a child with borderline lepromatous leprosy, with resolution of all skin lesions.[302] Some clinicians use clofazimine for chronic reversal reactions, but it is not recommended for the initial treatment of reactions with acute neuritis. For reactions, clofazimine probably is consistently efficacious only for erythema nodosum leprosum.[140]

Erythema Nodosum Leprosum (Type 2) Reaction

Mild erythema nodosum leprosum is treated with analgesics; more severe disease is treated with thalidomide or corticosteroids.[381] Pediatric doses of thalidomide in erythema nodosum leprosum have not been established, but the initial adult dose is 100 mg four times daily followed by a minimal effective dose, usually 100 mg daily. In the United States, thalidomide is available for erythema nodosum leprosum under the Calgene S.T.E.P.S. Program.* The teratogenic action of thalidomide demands that appropriate measures be taken in the treatment of fertile women. For the rare patient who does not respond to thalidomide or in fertile women, corticosteroids or clofazimine is used. Corticosteroids, if used, are administered in the usual dosage schedules (1 to 2 mg/kg daily), beginning with large doses and tapering to a minimal effective level. One systemic review indicated that clofazimine was superior to thalidomide or corticosteroids, but analysis was hindered by variation in the quality of studies in the pooled data.[373] Clofazimine is effective in most patients with erythema nodosum leprosum and does not have the disadvantages of thalidomide or corticosteroids. The anti-inflammatory action of clofazimine is not manifested until after 4 to 6 weeks of continuous use. The dosage must be adjusted to the minimal effective level, and high doses are recommended by the WHO for erythema nodosum leprosum reactions.[411] A few studies suggest that pentoxifylline or pentoxifylline plus clofazimine is effective.[255,357,397] One case report described the successful treatment of a chronic erythema nodosum leprosum reaction with a 6-week course and slow taper of the TNF inhibitor etanercept.[277]

Iridocyclitis requires emergency measures. Local corticosteroids must be added to systemic anti-inflammatory regimens. Ophthalmologic consultation should be obtained.

PREVENTION

Precise recommendations for the prevention of leprosy in individuals have not been formulated. Control programs today are based on the general principles that (1) the number of contagious patients is reduced by chemotherapy and (2) the surveillance of contacts detects early leprosy. To accomplish these goals, appropriate education of the public and medical personnel and population surveys in areas of higher prevalence must be implemented. In endemic areas, improved housing probably is a highly important preventive measure by reducing close contact of patients with healthy individuals. In health care settings, infection control precautions include hand hygiene for all patients with lepromatous leprosy. Standard precautions are indicated for the hospitalized patient. Leprosy is a reportable disease in the United States.

Chemoprophylaxis

Chemoprophylaxis for close contacts was first attempted with dapsone, showing efficacy rates ranging from 34 to 99 percent,[342] but was not recommended for large populations[67] given concerns that long-term use would select for dapsone-resistant isolates.[415] More recently, rifampin has been evaluated for chemoprophylaxis. One study on leprosy hyperendemic Indonesian islands used rifampin every 3.5 months, showing protective efficacy of 75 to 90 percent.[10] A cluster randomized trial in Bangladesh showed that single-dose rifampin had protective efficacy of 57 percent within 2 years of administration.[238] Single-dose rifampin chemoprophylaxis has been shown to be both culturally acceptable[95] and cost effective, particularly in social contacts and neighbors of cases.[139] However, concern exists that single-dose rifampin chemoprophylaxis may select for rifampin-resistant *M. leprae* isolates.

Vaccination, Immunoprophylaxis, and Immunotherapy

M. leprae antigens for vaccines originally were obtained from experimentally infected armadillos. Vaccines composed of heat-killed whole *M. leprae* have been found to be safe and to induce delayed-type hypersensitivity to *M. leprae* in a high percentage of lepromin-negative individuals. Several other vaccines based on cultivable mycobacteria (*Mycobacterium vaccae*, *Mycobacterium "w,"* and the Indian Cancer Research Centre bacillus) induce similar responses.[21,166,347] Field trials of these vaccines for the immunoprophylaxis of leprosy have been conducted; however, because of the chronicity and low prevalence of the disease, meaningful evaluation of their efficacy would require extended follow-up observations.[23] Because infection-induced immunity is not observed regularly in leprosy, a reasonable doubt exists that vaccines containing only *M. leprae* would be protective. Consequently, combined vaccines of killed *M. leprae* and live bacillus Calmette-Guérin (BCG) have been studied. Such vaccines convert lepromin-negative contacts of patients with leprosy to positive reactors and upgrade patients with lepromatous leprosy toward the tuberculoid region of the disease spectrum.[53,222]

BCG immunization alone had a protective effect of 26 percent in one meta-analysis; this analysis also found that age at immunization did not affect efficacy and that receipt of a second dose of BCG vaccine was more protective for leprosy than receipt of a single BCG dose.[323] Heterogeneity among trials and overestimation of

*Calgene S.T.E.P.S. Program: 1-888-771-0141.

protective effect (61%) was seen in observational studies in contrast to experimental studies.[323] Large-scale studies in Malawi and Venezuela suggested that the addition of killed *M. leprae* to BCG did not protect against leprosy.[55,167] BCG vaccination was combined with single-dose rifampin for close contacts of leprosy patients in Bangladesh, showing a protective effect of 80 percent.[313] The WHO does not recommend BCG vaccination for the prevention of leprosy.[18] This decision was based on the highly variable results of extensive studies in Burma, Papua New Guinea, and Uganda.[406]

Leprosy vaccines as adjuvant to multidrug therapy also have been attempted in multibacillary leprosy patients. *Mycobacterium w* vaccine given in 3-month intervals (up to 8 doses) was associated with improvement in clinical scores and decline in bacteriologic indices for up to 2 to 3 years, but no impact was seen on clinical relapse or incidence of neuropathy after this interval. Rates of type 1 reactions were more common in the vaccine group in contrast to a placebo group receiving multidrug therapy alone.[326]

ELIMINATION

In 1991, the World Health Assembly defined leprosy elimination as a prevalence of fewer than 1 case/10,000 persons. By this definition, only three nations with populations over 1 million have yet to attain this goal: Brazil, Nepal, and Timor-Leste (East Timor).[38] The prospect of truly eliminating leprosy is poor due to several factors. First, a zoonotic focus[219,365] can lead to continued introduction into human populations. Second, vaccination development efforts have been hindered by poor correlates of immunity. Third, surveillance for new cases can be hindered by the stigma attached to the disease. This can lead to either failure to seek medical attention or seeking medical attention in alternative medical settings, where reporting of cases may not occur.[143] One study modeled the impact of various interventions on leprosy incidence rates. Variables included passive versus active case detection, multidrug therapy, contact tracing, BCG vaccination of infants, early diagnosis of subclinical infection, and chemoprophylaxis. A multifaceted approach including BCG vaccine coverage, contact tracing, early diagnosis, and chemoprophylaxis of household contacts seemed to most substantially reduce leprosy incidence rates.[101] However, these strategies would require an infrastructure and investment that fall outside the health budgets of many developing nations. Given the lack of tools to diagnose subclinical infection, long treatment duration, and continued uncertainty regarding aspects of patterns of exposure, modes of transmission, persistence of leprosy in treated cases, and early host response, many experts believe that control, rather than eradication, should be the goal.[341]

BURULI ULCER

Mycobacterium ulcerans causes indolent, necrotizing cutaneous ulcers that are known classically as *Buruli ulcers*.[195,371] They are named after the county in Uganda, near the Nile, where the first large epidemic was described in 1897.[46,56,77] Other names for these lesions include Bairnsdale or Searles ulcer in Australia and Kumusi ulcer in Papua New Guinea. Today, researchers recognize that many infections with *M. ulcerans* are not manifested as ulcers and *M. ulcerans* infection is a technically more appropriate name for the disease. A few infections have been acquired by travelers returning from endemic areas,[50] as well as patients from endemic areas referred to medical centers in industrialized countries.[274]

The WHO has recognized Buruli ulcer as a reemerging infectious disease in western Africa with an important public health impact. The WHO Global Buruli Ulcer Initiative has held annual meetings to evaluate all aspects of the disease since 1998, and in 2004 the World Health Assembly adopted a resolution for all member states to intensify research to develop tools for diagnosing, treating, and preventing Buruli ulcer.[408]

ORGANISM

MacCullum and associates[196] in 1948 in Australia were the first to isolate the etiologic agent in culture. *M. ulcerans* is strongly acid-fast, with an optimal growth temperature of 30° to 33°C (86° to 91.4°F) on routine mycobacteriologic media such as Löwenstein-Jensen medium. Unlike *M. marinum*, *M. ulcerans* is slow-growing, nonphotochromogenic, microaerophilic, and heat sensitive.[223,263]

Comparative genomic analysis indicates that *M. ulcerans* arose from *M. marinum* via horizontal transfer of a virulence plasmid.[73] The two species have 98 percent genomic homology, but *M. ulcerans* has lost 1.1 Mb of DNA from the 6.6 Mb *M. marinum* genome.[354] However, differences in clinical manifestations between the two species are attributed in large part to one addition to the genome. *M. ulcerans* is the only mycobacterial pathogen of humans known to elaborate a necrotizing, immunosuppressive exotoxin, mycolactone.[62,282,336] The genomic sequences of the plasmid-encoded synthases that produce mycolactone have been described more recently.[352] The mycolactone mechanism of action is unclear, but evidence exists for the toxin triggering apoptosis[127,388] and decreasing satellite cell proliferation.[134]

Specific insertion sequences for *M. ulcerans* have been characterized and are available for identification of the organism by PCR.[1,335,353] Variations in the 3′ end of the 16S rRNA gene sequence are related to geographic origin and are used to divide the organism into African, American, Asian, and Australian strains.[272]

TRANSMISSION AND EPIDEMIOLOGY

Endemic foci of *M. ulcerans* infections usually appear in rural settings near permanent wetlands in warm countries, especially in terrain subject to seasonal flooding. Reports of patients with Buruli ulcers have come from at least 30 countries, principally in the tropics.[409] A few patients live in nontropical climates, such as China,[90] Japan,[364] and southern Australia.[376] Today, the largest numbers of reported patients live in West Africa (i.e., Benin, Côte d'Ivoire, and Ghana).[371] In these countries, the disease is reemerging rapidly, with an estimated total annual incidence exceeding 4000 patients. Other countries in which the disease is known to be endemic include Angola, Cameroon, the Democratic Republic of the Congo, Equatorial Guinea, French Guiana, Gabon, Indonesia, Malaysia, Papua New Guinea, Peru, Suriname, and Uganda.[69,132,215,268] In the Western Hemisphere, cases have been reported primarily in French Guyana, but also in Brazil, Mexico, Peru, and Suriname.[409]

Observers attribute this reemergence to environmental factors such as deforestation, artificial topographic alterations (e.g., dams and irrigation systems),[68,197] increasing populations engaged in basic manual agriculture in wetlands, and possibly climactic changes.[225] These man-made environmental modifications may lead to changes in water temperature or turbidity, potentially protecting *M. ulcerans* biofilms.[214] In North America, two cases of Buruli ulcer were reported in 2005 in central Mexico, the nearest location to the United States to date.[48] Individuals of all ages are affected, but the highest frequencies are in children 15 years of age or younger—approximately 50 to 75 percent of all cases.[201] A second peak occurs in the seventh to eighth decade of life, suggesting that latent infection can reactivate with age.[70] The sexes are affected equally, and a racial predilection is unknown.[70] Anecdotal observations of children in families of multiple parentage have suggested a possible genetic predisposition, now supported by molecular studies.[348] Seasonal variations in incidence occur in some foci.[129,284] Approximately 80 percent of the lesions are on the limbs, with the highest frequencies involving the lower extremities.

Although the ultimate source of *M. ulcerans* remains obscure, the organism has been discovered in aquatic insects, such as water bugs, firefly larvae, and beetles, and obtained from stagnant water in endemic areas of West Africa, as well as newly dammed areas in Cameroon.[197,200,271] In some cases, the organisms were found only in the salivary glands of aquatic insects, supporting the notion that insect bites may play a role in transmitting *M. ulcerans*.[198,199] Adult mosquitoes trapped outside Victoria, Australia also had detectable *M. ulcerans* DNA. *Aedes* spp. were most likely to have DNA identified, followed by *Anopheles* spp.; lowest detection was seen in *Culex* spp.[186] This may explain the observation that having daily contact with a natural water source was a risk factor for Buruli ulcer disease according to one study in Benin,[345] and that lower rates of Buruli ulcer were seen in higher altitude villages in Benin.[346] Wading into rivers was a risk factor for Buruli ulcer in one study, which also found that the most common lesions occurred on uncovered extremities[275]; both also suggest transmission from biting aquatic insects. A group from Melbourne, Australia, evaluated both environmental sources and nonhuman animal feces for *M. ulcerans* DNA. Real-time PCR detected DNA in soil, water residue, and aquatic and terrestrial vegetation in one endemic area. The group also found that 41 percent of opossum feces tested positive.[106] Koalas and small rodents also have been found to harbor *M. ulcerans*.[84,231] *M. ulcerans* DNA was not detected in fecal samples from Buruli ulcer patients, suggesting the human gastrointestinal tract is not likely to be a significant reservoir.[307] After the development of molecular biologic techniques for the identification of *M. ulcerans*, the organism was detected in the environment in Australia by IS2404 PCR.[295,296,350] However, these molecular assays do not detect only viable organisms, so some instances of detection from environmental sources may represent dead organisms sticking to environmental substrates.

Buruli ulcer rarely, if ever, is contagious. The distribution of patients, even in highly endemic foci, is random, suggesting that each patient is exposed to environmental sources, such as swamps where villagers work their gardens and obtain water for domestic use and especially where children play. The mode or modes of transmission to humans have not yet been delineated completely; however, the most plausible route is by trauma at sites of skin recently contaminated by *M. ulcerans*.[81,224] Many patients give a history of specific antecedent penetrating trauma at the site of their initial lesion, which may include wounds from a gunshot or land mine, thrown stones, hypodermic injection, or even a human bite (Fig. 98-14).[72] The organism may be spread by aerosol from the surface

FIGURE 98-14 ■ A major Buruli ulcer in the deltoid area of a 12-year-old Angolan boy. This pristine ulcer developed 3 months after a documented hypodermic anticholera vaccine injection at this site. Typical Buruli ulcer features are represented—undermining of borders, necrotic base, and induration of the adjacent skin. (See companion Expert Consult website for color version.)

of ponds or be carried by fomites or insects to skin surfaces. Insects may introduce *M. ulcerans* into the skin, but this means of transmission has not been proved. Although proposed by some authorities, transmission by nasorespiratory passage with a subsequent bacteremia seems to be unlikely.

PATHOGENESIS AND PATHOLOGY

Three properties determine the pathologic features of early *M. ulcerans* infection: optimal growth at 30° to 33° C (86° to 91.4° F), slow growth rate, and elaboration of the toxin mycolactone.[336] The temperature requirement tends to favor the development of lesions in the skin (versus deeper tissues). The slow growth rate results in slowly progressive lesions. Mycolactone destroys tissues and suppresses immune responses. The spectrum of clinical and histopathologic forms of infection suggests that some patients have innate resistance, or resistance develops soon after infection is acquired, whereas others acquire resistance later or, occasionally, never.

Many of the differences in pathogenicity between *M. ulcerans* and *M. marinum* may be explained by the presence of mycolactone in the former species. Mycolactone has broad-spectrum cytotoxic activity for a variety of cell types. Necrosis is due in part to mycolactone triggering massive apoptosis, vasculitis, and thrombosis of subcutaneous and dermal vessels. However, most studies have evaluated mycolactone levels in peripheral blood, and it is unclear to what degree this reflects concentrations in ulcerative lesions.[336] Mycolactone is also thought to inhibit TNF-γ and IL-2 production and augment IL-10 production.[3,60,177] Other pathogenic mechanisms may include *M. ulcerans* cell wall phospholipases, which have homology to *M. tuberculosis*.[118] These activities partly explain the immunologic unresponsiveness and reduced inflammatory reaction at the site of the lesion. One study in Benin found that the rate of hemoglobinopathies was almost seven times higher in Buruli ulcer osteomyelitis patients than the general population, suggesting these disorders facilitate the growth of the organism in bony matrices.[249]

Based on the current understanding of the natural history of *M. ulcerans* infection, pathogenesis may proceed as follows: after inoculation of the etiologic agent deep into the skin or subcutaneous tissue, a latent phase occurs during which the mycobacterium proliferates slightly, probably initially intracellularly,[261] and begins to elaborate small amounts of toxin that causes necrosis, especially of fatty tissue. This necrosis provides a microaerophilic environment and perhaps nutrients favorable for the accelerated growth of *M. ulcerans* and elaboration of increased amounts of toxin. During this necrotic phase, no significant cellular response occurs.[49,50,77]

In some patients, at this stage, a subcutaneous nodule begins to develop, with clusters of *M. ulcerans* in the center surrounded by a zone of necrosis. In highly resistant patients, this lesion may self-heal, perhaps without ulceration, or form a small, sharply delineated ulcer. In others, the skin is undermined by the necrosis and eventually breaks down into larger ulcers with widely undermined skin. In the least resistant individuals, a nodule never develops and the necrosis spreads rapidly and widely to cover large body surface areas, but ulceration, if it occurs at all, is a late event.

Eventually, the necrotic stage ceases in most patients, either because the toxin is neutralized or because production is interrupted. At this time, a granulomatous stage begins to develop, followed by healing and scarring. Some of the variability in clinical presentation and progression of the disease may be related to heterogeneity in the *M. ulcerans* genome and in the plasmid that encodes the production of mycolactone.[1,246,351,355]

Microscopically, the active ulcer shows extensive coagulation necrosis of the subcutaneous tissue down to and often including the fascia (Fig. 98-15).[124] The deep layers of necrosis reveal masses of acid-fast bacilli, often with mineralization and extensive vasculitis. Marked edema is present, and fat cells enlarge and die, with only their cellular ghost outlines remaining (Fig. 98-16). The dermis and surrounding tissue seldom contain acid-fast bacilli. Lesions sometimes provoke a reactive (contiguous)

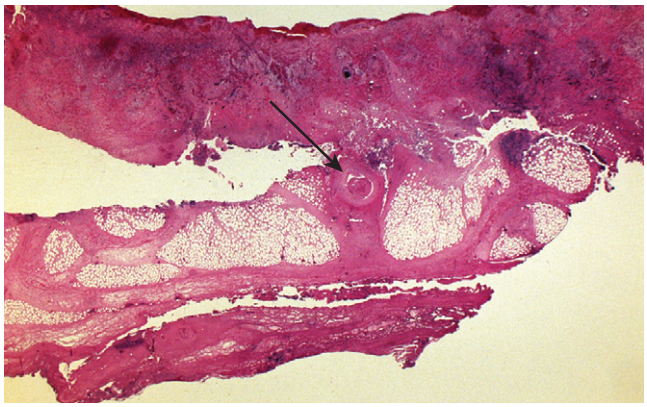

FIGURE 98-15 ■ Histopathologic section of the undermined edge of a major Buruli ulcer. Note the coagulation necrosis of the entire panniculus and fascia, and vasculitis with thrombosis of a blood vessel *(arrow)* (hematoxylin and eosin stain). (See companion Expert Consult website for color version.)

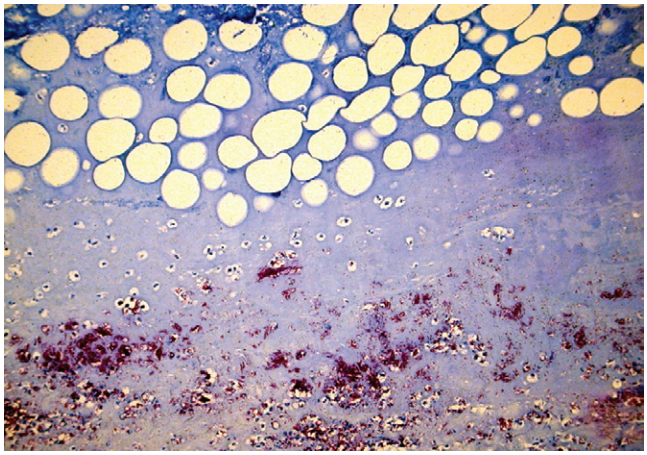

FIGURE 98-16 ■ High magnification of the necrotic panniculus of a Buruli ulcer showing the dead fat cell "ghosts," coagulation necrosis, and masses of acid-fast bacilli (Ziehl-Neelsen stain). (See companion Expert Consult website for color version.)

osteitis that leads to necrosis of cortical bone and osteomyelitis. Metastatic lesions may develop in the skin and bone from bacteremia and produce skin lesions distant from the original lesion and frequently focal or multifocal osteomyelitis. Regional lymph nodes may show massive necrosis and contain large numbers of acid-fast bacilli, especially in the capsule. Visceral organs are not known to be involved, but no necropsies of patients who died of disseminated disease have been reported.[359]

An understanding of the pathogenesis and developing improved therapies for Buruli ulcer has been slowed by the lack of an experimental animal model that replicates the spectrum of features found in human disease. Laboratory rats and mice develop skin lesions after intradermal inoculation with *M. ulcerans*, but they lack the extensive ulceration found in human disease. Multimammate rats *(Mastomys natalensis)* rapidly develop systemic infections and die.[338] In the mouse footpad, *M. ulcerans* multiplies, but necrosis without ulceration destroys the limb and causes death.[282] Guinea pigs develop inflammatory lesions at the inoculation sites that may resolve without formation of ulcers.[178,282] The nine-banded armadillo is susceptible to *M. ulcerans* and develops cutaneous lesions after experimental intradermal infection, approximating those of the human disease, but is phylogenetically distant.[387] The cynomolgus monkey (crab-eating macaque) is moderately susceptible to experimental *M. ulcerans* infection and develops some of the clinical and histologic features of Buruli ulcer.[382,385]

CLINICAL MANIFESTATIONS

Incubation and Forms of Lesions

In one study of specific trauma related to lesions, the incubation period ranged from 2 weeks to 3 years, with a mean of 3 months.[224] The following are the various forms of lesions of *M. ulcerans* infection according to WHO designations.[413] Figure 98-17 presents a proposed classification of the clinical forms of *M. ulcerans* disease and their possible interrelationships. The WHO also designates three categories of lesions. Category I consists of single lesions less than 5 cm in diameter; these may heal completely with medical therapy. Category II consists of a single lesion 5 to 15 cm in diameter; only some heal completely with medical therapy. Category III lesions encompass large (>15 cm diameter) lesions, lesions in multiple locations, osteomyelitis, or lesions in critical sites (e.g., ocular, genitalia, or breast). These lesions usually require combined medical and surgical therapy.[409,410]

Papule

The papular stage has been described only in patients from Australia. These papules are painless, elevated, and measure up to 1 cm in diameter.

Nodule

Nodules are primarily subcutaneous and firm, measure approximately 2 cm in diameter, and are painless, although they are often pruritic. The overlying skin may be discolored. Although usually mobile, they can become attached both to the skin and to deeper tissues.[366] This stage is the initial one in most Africans, and in the Kikongo language, the disease is called *mputa matadi* because the nodule is a "rock-hard lesion." Unlike ulcerative forms, acid-fast bacilli are aggregated in the center of nodules.[366] Nodular lesions have fewer functional limitations than other forms of Buruli ulcer disease.[312]

Plaque

Plaques are firm, elevated, painless, well-defined lesions more than 2 cm in largest dimension. Their borders are irregular. This stage often is the one in which physicians initially see the patient, but the lesion may or may not arise from a nodule. The skin over the lesion is reddened or discolored. These lesions may ulcerate in the late stages. These lesions are more likely to relapse after surgical therapy than ulcerative forms.[176]

Edematous Form

Most edematous lesions do not begin in the nodular stage but spread directly from the initial nidus of infection.

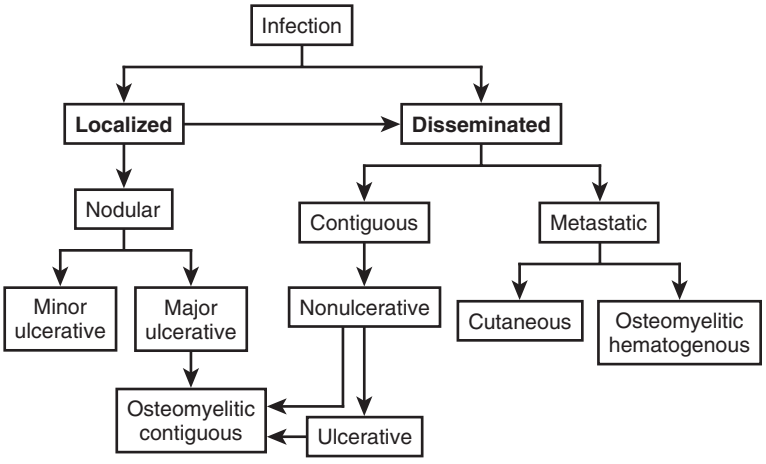

FIGURE 98-17 ■ Proposed classification of clinical forms of *M. ulcerans* infections, indicating possible progression of lesions after the initial cutaneous infection. Without treatment, lesions may self-heal at any stage, or advance according to the schema shown at left.

FIGURE 98-18 ■ Edematous form of *M. ulcerans* infection in a 6-year-old Ghanaian girl after surgery to remove all necrotic tissue. There was never any ulceration. Note ablation of breast tissue. (See companion Expert Consult website for color version.)

Spread often is rapid and wide and covers entire limbs or major portions of the trunk (Fig. 98-18). This type of lesion is characterized by diffuse nonpitting swelling and vague margins. Lesions are firm and frequently painful, with changes in color and scaling on the skin surface. These lesions, along with plaques, are more likely to relapse after surgical therapy than ulcerative forms.[176]

Ulcerative Forms

When fully developed, pristine ulcers have undermined edges surrounded by a zone of induration and often desquamation of the epidermis (see Fig. 98-14). In the base of the ulcerated area, a whitish necrotic slough and sometimes eschar develop. An oily exudate frequently oozes from the dependent area. Old ulcers tend to begin healing in the uppermost part while activity continues in the dependent portion. Collections of fluid in this area probably continue to support the growth of *M. ulcerans*, which sustains progression of the lesion.

The ulcerative forms are divided into major and minor ulcers. Both forms tend to self-heal. A major ulcer is large and chronic and can form a contiguous osteomyelitis. Minor ulcers are small (1 to 2 cm in diameter), are sharply delineated, and heal early. Both ulcerative forms begin as subcutaneous nodules.

Bone Involvement

Contiguous Osteomyelitis

Reactive osteitis occasionally develops beneath destroyed overlying skin and soft tissue, particularly with major ulcers. Bone becomes devitalized and necrotic, with the development of sequestra (Fig. 98-19).

Metastatic Osteomyelitis

M. ulcerans–specific osteomyelitis develops in approximately 10 percent of all patients, underscoring that Buruli ulcer should always be considered a potentially systemic disease.[274] Most likely, metastatic osteomyelitis is a result of lymphohematogenous spread of *M. ulcerans* from a distant, earlier cutaneous lesion (Fig. 98-20). The overlying skin ordinarily is intact, but swelling and inflammation occur over the site of bone involvement. If the

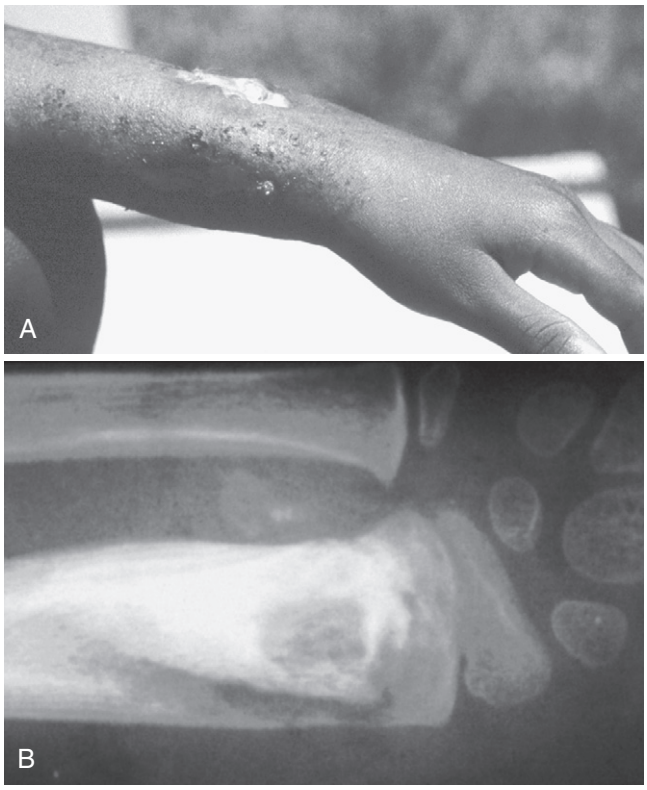

FIGURE 98-19 ■ **A,** *M. ulcerans* infection in a young Congolese boy. Note swelling and ulcer on dorsum of forearm. (See companion Expert Consult website for color version.) **B,** Radiograph of forearm shows reactive osteitis, necrosis of cortex of distal end of the radius, and early sequestration with osteomyelitis.

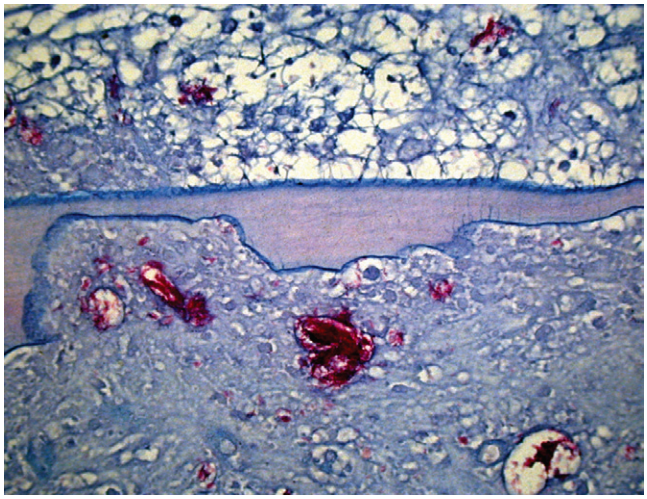

FIGURE 98-20 ■ Ziehl-Neelsen–stained section shows metastatic osteomyelitis of the tibia in a 6-year-old boy in Benin. This lesion developed after *M. ulcerans* infection of the contralateral leg. Note necrosis of marrow, erosion of a trabecula, and many clusters of acid-fast bacilli. (Ziehl-Neelsen stain). (See companion Expert Consult website for color version.)

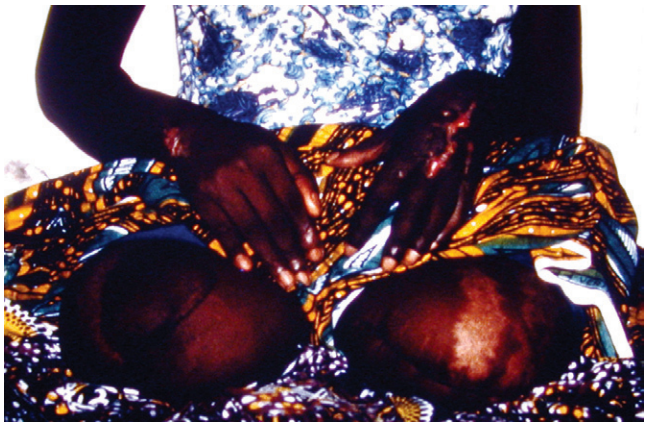

FIGURE 98-21 ■ This 13-year-old girl in Benin initially had a cutaneous lesion of Buruli ulcer. Subsequently, metastatic spread to bones of the lower extremities occurred, resulting in bilateral high-thigh amputations. Note also the spread to bones of both wrists and left hand. (See companion Expert Consult website for color version.)

FIGURE 98-22 ■ Ghanaian adolescent girl with healed *M. ulcerans* infection that extended from the wrist to the shoulder. The scarring resulted in contraction deformities of the wrist and elbow and disuse atrophy of the entire extremity. Appropriate physiotherapy would have minimized these sequelae. (See companion Expert Consult website for color version.)

condition goes untreated, a draining fistula usually develops. Osteomyelitis often requires amputation (Fig. 98-21).

Complications

Infection may traverse the deep fascia and damage tendons, nerves, joints, genitalia, and periorbital tissues, requiring subsequent enucleation of the eye. Healing leads to fibrosis and scarring, causing functional limitations.[349] The scar may form keloids and often causes major contraction deformities, especially in lesions that cross joints (Fig. 98-22). Squamous cell carcinoma may develop in healed lesions, especially nonpigmented lesions.[169] Skin grafting and physiotherapy prevent most of these complications. Most disease-related deaths result from septicemia, gas gangrene, or tetanus.

HUMAN IMMUNODEFICIENCY VIRUS AND BURULI ULCER

Because Buruli ulcer primarily is a disease of children in rural areas, very few patients have been reported with Buruli ulcer and HIV infection.[8,175] One study in Benin suggested that HIV infection increased the odds of Buruli ulcer eightfold.[154] Relevant reports indicate, however, that HIV infection may render Buruli ulcer disease highly aggressive; rapidly spreading *M. ulcerans* osteomyelitis has developed in several patients with AIDS.[175,303] Chronic diarrhea in an HIV-infected adult has also been attributed to *M. ulcerans*.[135]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

To an experienced observer, an accurate clinical diagnosis often can be made with ease.[414,416] In the ulcerated forms, a Ziehl-Neelsen stain of exudate from the ulcer edge obtained with a cotton swab reveals clusters of extracellular acid-fast bacilli. Punch biopsies and fine needle aspiration also have been used to obtain samples from nonulcerative lesions.[410] Smears have an estimated sensitivity of 40 percent.[409] The same material, obtained by swab after decontamination, may be used for culture on Löwenstein-Jensen or other suitable mycobacterial media. The incubation temperature must be 30° to 33° C (86° to 91.4° F). If culture cannot be performed locally, transport media may be inoculated with material from the cotton swab and maintained at 4° C (39.2° F) while in transport to a specialized laboratory. The transport medium is composed of Middlebrook 7H9 broth supplemented with polymyxin B, amphotericin B, nalidixic acid, trimethoprim, azlocillin, and 0.5 percent agar. Cultures often require at least 6 to 8 weeks to grow and have a sensitivity of 20 to 60 percent, in part because of the fastidious temperature requirements of the organism.[409] PCR is available and increasing in popularity and convenience.[1,335,350] PCR sensitivity is high (~98%).[409] Tissue for histopathologic analysis should be obtained from the edge of the ulcer and must include all levels, including the fascia. Fixation in 4 percent buffered formalin is adequate. Histopathology has a sensitivity of 90 percent in experienced hands.[409] Mycolactone can be found in peripheral blood and ulcer exudates (including exudates obtained at the conclusion of therapy) by high-performance liquid chromatography. This modality may have potential for diagnostic testing and stratifying risk for disease recurrence in the future.[308]

Some of the differential diagnostic possibilities are as follows:

- *Papules:* Insect bites, verruca vulgaris, pityriasis, granuloma annulare
- *Nodules:* Lipoma, sebaceous cyst, onchocerciasis, furuncle
- *Plaques:* Leprosy, mycosis, necrobiosis, psoriasis
- *Edema:* Bacterial cellulitis, actinomycosis, elephantiasis, pyomyositis
- *Ulcers:* Tropical phagedenic ulcer, noma, stasis ulcer, leishmaniasis

PROGNOSIS

Without treatment, Buruli ulcer often leads to deforming depressed scars, contraction deformities, or amputation. One study found that almost 60 percent of patients had functional limitations a median of 4 years after completing therapy. Functional limitations and risk factors for stopping work or school were collinear and included lesion over a joint or on hands or feet, female sex, older age, and presence of a persistent wound.[349] The stigma of the deformities and the socioeconomic burden of the disease often are marked.[9] With early appropriate treatment, including excision and grafting, the prognosis usually is excellent. Metastatic lesions and local recurrences occur frequently enough, however, to warrant vigilant follow-up.[6] Paradoxical worsening of lesions while on appropriate antibiotic therapy has been noted, similar to immune reconstitution inflammatory syndromes seen for other mycobacterial infections. These are thought to be due to mycobacterial antigens and inflammatory cytokines released from clinically unrecognized bacterial foci.[298]

TREATMENT

Treatment options for Buruli ulcer include antibiotics and surgical intervention, the latter commonly used for category III lesions. In the preantibiotic era, wide local excision was commonly used.[366] The WHO now recommends surgery as an adjunct to medical therapy, particularly to remove necrotic tissue, cover skin defects, and correct deformities resulting in functional limitations.[410]

The WHO recommends an 8-week course of rifampin 10 mg/kg (maximum 600 mg) daily and either streptomycin or amikacin 15 mg/kg (maximum 1 g) daily as first-line therapy for all forms of active disease.[410,411] Intramuscular administration of aminoglycosides in children may be limited by the small muscle mass in many children. Monitoring for side effects is essential. For streptomycin, treatment should be stopped if hearing impairment or vertigo with nystagmus develops. For rifampin, treatment should be stopped if hepatitis, jaundice, or acute renal failure occurs, side effects that generally are associated with intermittent therapy of more than 10 mg/kg. One randomized trial in Ghana for patients with ulcers less than 10 cm in diameter compared the WHO-recommended regimen to streptomycin and rifampin for 4 weeks, followed by rifampin and clarithromycin for 4 weeks; they found no difference in response to therapy acutely or at 1-year follow-up.[256] An entirely oral regimen of 8 weeks of rifampin and clarithromycin was evaluated in a pilot study of 30 Beninese patients with lesions less than 10 cm in diameter. The treatment was well tolerated, and all patients had healed by 12 months without disease recurrence.[43] The extent to which these studies would apply to patients with more invasive or multifocal disease is unclear. Mouse models have evaluated the activity of other agents against *M. ulcerans*, showing that 8-week regimens of rifampin with either moxifloxacin or linezolid displayed bactericidal effects and clinical response comparable to those of rifampin with an aminoglycoside.[149] Although this study is promising, linezolid may be cost-prohibitive in many resource-limited nations, and the use of fluoroquinolones in countries with high rates of tuberculosis also is of concern.

If surgical intervention is chosen, to minimize the danger of development of *M. ulcerans* bacteremia many surgeons prescribe clarithromycin and rifampin 1 to 2 weeks before performing surgery and have the patients continue antibiotic therapy for several weeks after surgery. Although reasonable, this antibiotic complement to surgery is not of proved efficacy. Papules and preulcerative nodules seldom are diagnosed, even in endemic areas; however, wide excision and primary closure usually are curative.[366]

Plaques and edematous forms are excised widely down to the fascia, or through the fascia if it is necrotic. Muscle usually is not damaged, but if so, the excision is extended into muscle. The lateral extent of excision often is difficult to determine. By careful palpation, the physician can establish an approximate limit of the disease. Exploratory incisions and blunt dissection may help determine the limit of induration and necrosis. Use of real-time PCR for determining the extent of disease is being evaluated.[291]

Very small ulcers can be excised and closed primarily, as for nodules. Large ulcers are excised widely. The required extent of surgery may be determined by exploratory lateral excision and blunt dissection. Split-skin autografting of surgical defects usually is performed after a bed of granulation tissue has formed. Postoperative care, including physiotherapy, should be designed to prevent contractures.

Bone lesions should be referred to specialists. Heat therapy without surgical excision has been successful for appropriate lesions, but must be applied assiduously, with all necessary controls.[223] Recurrences after surgical treatment are frequent, but rates are not well established.[71]

PREVENTION

In an endemic tropical rural setting where children usually are scantily attired, prevention of contamination of the skin from environmental sources is virtually impossible. Wearing long trousers seems to prevent development of infection.[201] Protected water supplies in villages would reduce exposure; however, such protective measures usually are futile in rural areas of developing countries. Standard precautions are indicated for the hospitalized patient. Buruli ulcer disease is not a reportable disease in the United States.

Vaccination with BCG has a moderate (40% to 50%) protective effect against *M. ulcerans* infections for 6 to 12 months,[138] although effect wanes with time and has been variable in both human and animal studies.[51] One form of Buruli ulcer disease for which BCG appears to have a sustained protective effect for children and adults is for the disseminated osteomyelitic form.[270] Studies also are evaluating whether repeated BCG vaccination may render the population more immune to *M. ulcerans* infection, although this was not seen in animal models,[270,358]

in contrast to what was found for leprosy.[167,323] One *M. ulcerans*–specific vaccine target would be mycolactone, but this polyketide is poorly immunogenic, and antimycolactone antibodies are not detected in either cases or controls.[51] Mycobacterial heat shock protein (HSP) 45 is very immunogenic and protective in mouse models, but homology to a human HSP increases risk for autoimmune reactions.[59] In the absence of an effective vaccine, control strategies have focused on early detection and treatment, combined with physiotherapy to reduce scarring and functional limitations.[409]

NEW REFERENCES SINCE THE SIXTH EDITION

13. Banerjee S, Sarkar K, Gupta S, et al. Multiplex PCR technique could be an alternative approach for early detection of leprosy among close contacts: a pilot study from India. *BMC Infect Dis* 2010;**10**:252.
16. Bazan-Furini R, Motta AC, Simao JC, et al. Early detection of leprosy by examination of household contacts, determination of serum anti-PGL-1 antibodies, and consanguinity. *Mem Inst Oswaldo Cruz* 2011;**16**:536–40.
33. Budhiraja V, Rastogi R, Khare S, et al. Histopathological changes in the arrector pili muscle of normal appearing skin in leprosy patients. *Int J Infect Dis* 2010;**145**:e70–72.
38. Burki T. Old problems still mar fight against ancient disease. *Lancet* 2009;**373**:287–9.
41. Capadia GD, Shetty VP, Khambati FA, et al. Effect of corticosteroid usage combined with multidrug therapy on nerve damage assessed using nerve conduction studies: a prospective cohort of 365 untreated multibacillary leprosy patients. *J Clin Neurophysiol* 2010;**27**:38–47.
43. Chauty A, Ardant M-F, Marsollier L, et al. Oral treatment for *Mycobacterium ulcerans* infection: results from a pilot study in Benin. *Clin Infect Dis* 2011;**52**:94–6.
51. Converse PJ, Almeida DV, Nuermberger EL, et al. BCG-mediated protection against *Mycobacterium ulcerans* infection in the mouse. *PLoS Negl Trop Dis* 2011;**15**:e985.
65. Daniel E, Rao PS, Ffytche TJ, et al. Ocular hypotension and hypotony in multibacillary leprosy patients; at diagnosis, during, and after completion of multidrug therapy. *Indian J Lepr* 2010;**82**:181–8.
73. Demangel C, Stinear TP, Cole ST. Buruli ulcer: reductive evolution enhances pathogenicity of *Mycobacterium ulcerans*. *Nat Rev Microbiol* 2009;**7**:50–60.
84. Durnez L, Suykerbuyk P, Nicholas V, et al. Terrestrial small mammals as reservoirs of *Mycobacterium ulcerans* in Benin. *Appl Environ Microbiol* 2010;**76**:4574–7.
85. Duthie MS, Hay MN, Rada EM, et al. Specific IgG antibody responses may be used to monitor leprosy treatment efficacy and as recurrence prognostic markers. *Eur J Clin Microbiol Infect Dis* 2011;**30**:1257–65.
87. Ebeigbe JA, Kio F. Ocular leprosy in institutionalized Nigerian patients. *Ghana Med J* 2011;**45**:50–3.
95. Feenstra SG, Nahar Q, Pahan D, et al. Acceptability of chemoprophylaxis for household contacts of leprosy patients in Bangladesh: a qualitative study. *Lep Rev* 2011;**82**:178–87.
96. Feenstra SG, Nahar Q, Pahan D, et al. Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study. *PLoS Negl Trop Dis* 2011;**5**:e1029.
101. Fischer EA, de Vlas SJ, Habbema JD, et al. The long term effect of current and new interventions on the new case detection of leprosy: a modeling study. *PLoS Negl Trop Dis* 2011;**5**:e1330.
106. Fyfe JA, Lavender CJ, Handasyde KA, et al. A major role for mammals in the ecology of *Mycobacterium ulcerans*. *PLoS Negl Trop Dis* 2010;**4**:e791.
115. Ghorpade A. Post-traumatic inoculation tuberculoid leprosy after injury with a glass bangle. *Lepr Rev* 2009;**80**:215–18.
134. Houngbedji GM, Bouchard P, Frenette J. *Mycobacterium ulcerans* infections cause progressive muscle atrophy and dysfunction, and mycolactone impairs satellite cell proliferation. *Am J Physiol Regul Integr Comp Physiol* 2011;**300**:R724–R732.
138. Huygen K, Adjei O, Affolabi D, et al. Buruli ulcer disease: prospects for a vaccine. *Med Microbiol Immunol* 2009;**198**:69–77.
139. Idema WJ, Majer IM, Pahan D, et al. Cost-effectiveness of a chemoprophylactic intervention with single dose rifampicin in contacts of new leprosy patients. *PLoS Negl Trop Dis* 2010;**4**:e874.
158. Kamal R, Natrajan M, Katoch K, et al. Evaluation of the diagnostic role of in situ PCR on slit-skin smears in pediatric leprosy. *Indian J Lepr* 2010;**82**:195–200.
169. Kassi K, Kouame K, Allen W, et al. Squamous cell carcinoma secondary to Buruli ulcer: a clinical case report in a young girl. *Bacteriol Virusol Parazitol Epidemol* 2010;**55**:25–8.
175. Kibadi K, Colebunders R, Muyembe-Tamfum JJ, et al. Buruli ulcer lesions in HIV-positive patient. *Emerg Infect Dis* 2010;**16**:738–9.
176. Kibadi K, Mputu-Yamba JB, Mokassa B, et al. Relapse after surgical treatment of *Mycobacterium ulcerans* infection: study of risk factors in 84 patients in the Democratic Republic of the Congo. *Med Trop (Mars)* 2009;**69**:471–4.
186. Lavender CJ, Fyfe JA, Azuolas J, et al. Risk of Buruli ulcer and detection of *Mycobacterium ulcerans* in mosquitoes in southeastern Australia. *PLoS Negl Trop Dis* 2011;**5**:e1305.
188. Lazaro FP, Werneck RI, Mackert CCO, et al. A major gene controls leprosy susceptibility in a hyperendemic isolated population north of Brazil. *J Infect Dis* 2010;**201**:1598–605.
191. Levis WR, Paraskevas L-R, Jacobson M, et al. Endemic leprosy in New York City. *Arch Dermatol* 2011;**147**:624–5.
197. Marion E, Landier J, Boisier P, et al. Geographic expansion of Buruli ulcer disease, Cameroon. *Emerg Infect Dis* 2011;**17**:551–3.
207. Mehdi G, Maheshwari V, Ansari HA, et al. Modified fine-needle aspiration technique for diagnosis of granulomatous skin lesions with special reference to leprosy and cutaneous tuberculosis. *Diagn Cytopathol* 2010;**38**:391–6.
214. Merritt RW, Walker ED, Small PLC, et al. Ecology and transmission of Buruli ulcer disease: a systematic review. *PLoS Negl Trop Dis* 2010;**4**:e911.
240. Monot M, Honore N, Garnier T, et al. Comparative genomic and phylogenetic analysis of *Mycobacterium leprae*. *Nat Genet* 2009;**41**:1282–9.
250. Nakata N, Kai M, Makino M. Mutational analysis of the *Mycobacterium leprae* folP1 gene and dapsone resistance. *Antimicrob Agent Chemotherap* 2011;**55**:762–6.
251. National Hansen's Disease Program. *Epidemiology*. Available online at: http://www.hrsa.gov/hansensdisease. Accessed October 5, 2011.
252. National Hansen's Disease Program. *Recommended leprosy treatment regimens*. Available online at: http://www.hrsa.gov/hansensdisease/diagnosis/recommendedtreatment.html. Accessed September 27, 2011.
256. Nienhuis WA, Stienstra Y, Thompson WA, et al. Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial. *Lancet* 2010;**375**:664–72.
277. Ramien ML, Wong A, Keystone JS. Severe refractory erythema nodosum leprosum successfully treated with the tumor necrosis factor etanercept. *Clin Infect Dis* 2011;**52**:e133–e135.
287. Robbins G, Tripathy VM, Misra VN, et al. Ancient skeletal evidence for leprosy in India (2000 B.C.). *PLoS One* 2009;**4**:e5669.
289. Rodrigues LC, Lockwood DNJ. Leprosy now: epidemiology, progress, challenges, and research gaps. *Lancet Infect Dis* 2011;**11**:464–70.
292. Rongioletti F, Gallo R, Cozzani E, et al. Leprosy: a diagnostic trap for dermatopathologists in nonendemic areas. *Am J Dermatopathol* 2009;**31**:607–10.
298. Ruf MT, Chauty A, Adeye A, et al. Secondary Buruli ulcer skin lesions emerging several months after completion of chemotherapy: paradoxical reaction or evidence for immune protection? *PLoS Negl Trop Dis* 2011;**5**:e1252.
302. Safa G, Darrieux L, Coic A, et al. Type 1 leprosy reversal reaction treated with topical tacrolimus along with systemic corticosteroids. *Indian J Med Sci* 2009;**63**:359–62.
303. Sales AM, Ponce de Leon A, Duppre NC, et al. Leprosy among patient contacts: a multilevel study of risk factors. *PLoS Negl Trop Dis* 2011;**5**:e1013.
307. Sarfo FS, Lavender CJ, Fyfe JA, et al. *Mycobacterium ulcerans* DNA not detected in faecal samples from Buruli ulcer patients: results of a pilot study. *PLoS One* 2011;**6**:e19611.
308. Sarfo FS, Le Chevalier F, Aka N, et al. Mycolactone diffuses into the peripheral blood of Buruli ulcer patients: implications for diagnosis and disease monitoring. *PLoS Negl Trop Dis* 2011;**5**:e1237.

312. Schunk M, Thompson W, Klutse E, et al. Outcome of patients with Buruli ulcer after surgical treatment with or without antimycobacterial treatment in Ghana. *Am J Trop Med Hyg* 2009;**81**:75–81.
313. Schuring RP, Richardus JH, Pahan D, et al. Protective effect of the combination of BCG vaccination and rifampin prophylaxis in leprosy prevention. *Vaccine* 2009;**27**:7125–8.
318. Sehgal VN, Srivastava G, Singh N. Histoid leprosy: histopathological connotations' relevance in contemporary context. *Am J Dermatopathol* 2009;**31**:268–71.
319. Sekar B, Arunagiri K, Kumar BN, et al. Detection of mutations in folP1, rpoB, and gyrA genes of *M. leprae* by PCR-direct sequencing: a rapid tool for screening drug resistance in leprosy. *Lepr Rev* 2011;**82**:36–45.
322. Setia MS, Shinde SS, Jerajani HR, et al. Is there a role for rifampicin, ofloxacin, and minocycline (ROM) therapy in the treatment of leprosy? Systematic review and meta-analysis. *Trop Med Int Health* 2011;**16**:1541–51.
325. Shanmugam A, Natarajan J. Computational genome analysis of metabolic enzymes in *Mycobacterium leprae* for drug target identification. *Bioinformation* 2010;**4**:392–5.
333. Siddaraju N, Sistla SC, Singh N, et al. Pure neuritic leprosy with nerve abscess presenting as a cystic soft tissue mass. *Diagn Cytopathol* 2009;**37**:355–8.
336. Silva MT, Portaels F, Pedrosa J. Pathogenetic mechanisms of the intracellular parasite *Mycobacterium ulcerans* leading to Buruli ulcer. *Lancet Infect Dis* 2009;**9**:699–710.
345. Sopoh GE, Barogui YT, Johnson RC, et al. Family relationship, water contact, and occurrence of Buruli ulcer in Benin. *PLoS Negl Trop Dis* 2010;**4**:e746.
346. Sopoh GE, Johnson RC, Anagonou SY, et al. Buruli ulcer prevalence and altitude, Benin. *Emerg Infect Dis* 2011;**17**:153–4.
354. Stinear TP, Seemann T, Harrison PF, et al. Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. *Genome Res* 2008;**18**:729–41.
363. Truman RW, Singh P, Sharma R, et al. Probable zoonotic leprosy in the southern United States. *N Engl J Med* 2011;**364**:1626–33.
373. Van Veen NH, Lockwood DN, Van Brakel WH, et al. Interventions for erythema nodosum leprosum: a Cochrane review. *Lepr Rev* 2009;**80**:355–72.
377. Velayati AA, Farnia P, Khalizadeh S, et al. Interferon-gamma receptor-1 gene promoter polymorphisms and susceptibility to leprosy in children of a single family. *Am J Trop Med Hyg* 2011;**84**:627–9.
384. Walker SL, Nicholls PG, Dhakal S, et al. A phase two randomized controlled double blind trial of high dose intravenous methylprednisolone and oral prednisolone versus intravenous normal saline and oral prednisolone in individuals with leprosy type 1 reactions and/or nerve function impairment. *PloS Negl Trop Dis* 2011;**5**:e1041.
404. Wong SH, Gochhait S, Malhotra D, et al. Leprosy and the adaptation of human toll-like receptor 1. *PLoS Pathol* 2010;**6**:e1000979.
405. Woodall P, Scollard D, Rajan L. Hansen Disease among Micronesian and Marshallese persons living in the United States. *Emerg Infect Dis* 2011;**17**:1202–8.
406. World Health Organization. Surveillance of drug resistance in leprosy: 2010. *Wkly Epidemiol Rep* 2011;**23**:237–40.
410. World Health Organization. *Buruli ulcer: treatment recommendations*. Available online at: http://www.who.int/buruli/en. Accessed October 5, 2011.
411. World Health Organization. *Leprosy elimination, WHO-recommended multidrug therapy (MDT) regimens*. Available online at: http://www.who.int/lep/mdt/regimens/en/index.html. Accessed September 27, 2011.
412. World Health Organization. Leprosy update, 2011. *Wkly Epidemiol Rep* 2011;**3**:389–400.
419. Zhang F-R, Huang W, Chen S-M, et al. Genomewide association study of leprosy. *N Engl J Med* 2009;**361**:2609–18.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 99

Nocardia

Nada Harik • Richard F. Jacobs

Nocardia spp. are obligate aerobic bacilli that exist throughout the world as soil and dust saprotrophs. These organisms are non–spore-forming, thin, branching, gram-positive, partially acid-fast, filamentous bacteria. Humans become infected with *Nocardia* by two primary routes: inhalation of contaminated airborne dust particles or traumatic implantation of the bacterium into subcutaneous tissue. Pulmonary disease is the form of nocardiosis recognized most commonly in the United States.[2] The pulmonary event may be subclinical or transient, or it may provoke an acute or chronic process mimicking staphylococcal or fungal pneumonia, tuberculosis, or carcinoma. Hematogenous dissemination may occur, especially in immunocompromised hosts. The central nervous system is the most common site of dissemination, with involvement manifested most often as a brain abscess. Cutaneous nocardiosis may be acute, subacute, or chronic. This form of disease is seen predominantly in immunocompetent hosts, with *Nocardia brasiliensis* being the agent identified most frequently.

In 1888, Nocard, a veterinarian, noted an aerobic actinomycete in bovine farcy, a chronic wasting disease in cattle characterized by pulmonary abscesses and draining cutaneous sinus tracts. Eppinger first described human

REFERENCES

1. Ablordey A., Kotlowski R, Swings J, et al. PCR amplification with primers based on IS2404 and GC-rich repeated sequence reveals polymorphism in *Mycobacterium ulcerans*. *J Clin Microbiol* 2005;**43**: 448–51.
2. Achilles EG, Hagel C, Vierbuchen M, et al. Leprosy accidentally transmitted from a patient to a surgeon in a nonendemic area. *Ann Intern Med* 2004;**141**:W51.
3. Adusumilli S, Mve-Obiang A, Sparer T, et al. *Mycobacterium ulcerans* toxic macrolide, mycolactone modulates the host immune response and cellular location of *M. ulcerans* in vitro and in vivo. *Cell Microbiol* 2005;**7**:1295–304.
4. Agrawal A, Pandit L, Dalal M, et al. Neurological manifestations of Hansen's disease and their management. *Clin Neurol Neurosurg* 2005;**107**:445–54.
5. Agrawal S, Agarwalla A. Dapsone hypersensitivity syndrome: a clinico-epidemiological review. *J Dermatol* 2005;**32**:883–9.
6. Aguiar J, Steunou C. Les ulcères de Buruli en zone rurale au Bénin pris en charge de 635 cas [Buruli ulcers in rural areas of Benin: management of 635 cases]. *Med Trop (Mars)* 1997;**57**:83–90.
7. Alcais A, Sanchez FO, Thuc NV, et al. Granulomatous reaction to intradermal injection of lepromin (Mitsuda reaction) is linked to the human NRAMP1 gene in Vietnamese leprosy sibships. *J Infect Dis* 2000;**181**:302–8.
8. Allen S. Buruli ulcer and HIV infection. *Int J Dermatol* 1992; **31**:744–5.
9. Asiedu K, Etuaful S. Socioeconomic implications of Buruli ulcer in Ghana: a three-year review. *Am J Trop Med Hyg* 1998;**59**: 1015–22.
10. Bakker MI, Hatta M, Kwenang A, et al. Prevention of leprosy using rifampicin as chemoprophylaxis. *Am J Trop Med Hyg* 2005; **72**:443–8.
11. Balagon MV, Cellona RV, Fajardo TT, et al. Detection of new leprosy cases at a walk-in skin clinic in Cebu City, Philippines, highlights surveillance. *Int J Dermatol* 1999;**38**:796–7.
12. Banerjee R, Banerjee BD, Chaudhury S, et al. Transmission of viable *Mycobacterium leprae* by *Aedes aegypti* from lepromatous leprosy patients to the skin of mice through interrupted feeding. *Lepr Rev* 1991;**62**:21–6.
13. Banerjee S, Sarkar K, Gupta S, et al. Multiplex PCR technique could be an alternative approach for early detection of leprosy among close contacts: a pilot study from India. *BMC Infect Dis* 2010;**10**:252.
14. Bartels P. Tuberculose in der Jüngeren Steinzeit. *Arch Anthropol* 1907;**6**:243–55.
15. Batista MD, Porro AM, Maeda SM, et al. Leprosy reversal reaction as immune reconstitution inflammatory syndrome in patients with AIDS. *Clin Infect Dis* 2008;**46**:e56–60.
16. Bazan-Furini R, Motta AC, Simao JC, et al. Early detection of leprosy by examination of household contacts, determination of serum anti-PGL-1 antibodies, and consanguinity. *Mem Inst Oswaldo Cruz* 2011;**16**:536–40.
17. Bechelli LM, Garbajosa PG, Gyi MM, et al. Site of early skin lesions in children with leprosy. *Bull World Health Organ* 1973; **48**:107–11.
18. Bechelli LM, Garbajosa PG, Gyi MM, et al. BCG vaccination of children against leprosy: seven-year findings of the controlled WHO trial in Burma. *Bull World Health Organ* 1973;**48**:323–34.
19. Becx-Bleuminck M. Operational aspects of multidrug therapy. *Int J Lepr Other Mycobact Dis* 1989;**57**:540–51.
20. Bhandarkar SS, Cohen C, Kuruvila M, et al. Angiogenesis in cutaneous lesions of leprosy: implications for treatment. *Arch Dermatol* 2007;**143**:1527–9.
21. Bhatki WS, Chullawala RG, Chaturvedi RM, et al. Lepromin conversion induced by a "sub-unit" vaccine from ICRC bacilli. *Indian J Med Res* 1988;**87**:545–54.
22. Bhattacharya SN, Chattopadhaya D, Saha K. Tumor necrosis factor: status in reactions in leprosy before and after treatment. *Int J Dermatol* 1993;**32**:436–9.
23. Bhutani LK, Nath I, Mehra NK. Grand round: leprosy. *Lancet* 1995;**345**:697–703.
24. Binford CH, Meyers WM, Walsh GP. Leprosy: state of the art. *JAMA* 1982;**247**:2283–92.
25. Binford CH, Meyers WM, Walsh GP, et al. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): histopathologic and microbiologic studies of tissues. *J Reticuloendothel Soc* 1977;**22**:377–88.
26. Boldsen JL. Leprosy and mortality in the medieval Danish village of Tirup. *Am J Phys Anthropol* 2005;**126**:159–68.
27. Borgdorff MW, van den Broek J, Chum HJ, et al. HIV-1 infection as a risk factor for leprosy: a case-control study in Tanzania. *Int J Lepr Other Mycobact Dis* 1993;**61**:556–62.
28. Brennan PJ, Vissa VD. Genomic evidence for the retention of the essential mycobacterial cell wall in the otherwise defective *Mycobacterium leprae*. *Lepr Rev* 2001;**72**:415–28.
29. Brightbill HD, Libraty DH, Krutzik SR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 1999;**285**:732–6.
30. Brody SN. *The Disease of the Soul: Leprosy in Medieval Literature*. Ithaca, N.Y.: Cornell University Press; 1974.
31. Browne SG. Onchocercal depigmentation. *Trans R Soc Trop Med Hyg* 1960;**54**:325–34.
32. Brubaker ML, Meyers WM, Bourland J. Leprosy in children one year of age and under. *Int J Lepr Other Mycobact Dis* 1985;**53**: 517–23.
33. Budhiraja V, Rastogi R, Khare S, et al. Histopathological changes in the arrector pili muscle of normal appearing skin in leprosy patients. *Int J Infect Dis* 2010;**145**:e70–72.
34. Buhler G. The Laws of Manu. In: The Sacred Books of the East, **vol. 25**. Oxford: Clarenden Press. 1886.
35. Bullock WE. Studies of immune mechanisms in leprosy. I. Depression of delayed allergic response to skin test antigens. *N Engl J Med* 1968;**278**:298–304.
36. Bullock WE. Rifampin in the treatment of leprosy. *Rev Infect Dis* 1983;**5**(Suppl. 3):S606–13.
37. Bullock WE, Ho MF, Chen MJ. Studies of immune mechanisms in leprosy. II. Quantitative relationships of IgG, IgA, and IgM immunoglobulins. *J Lab Clin Med* 1970;**75**:863–70.
38. Burki T. Old problems still mar fight against ancient disease. *Lancet* 2009;**373**:287–9.
39. Bwire R, Kawuma HJS. Type 1 reactions in leprosy, neuritis and steroid therapy: the impact of the human immunodeficiency virus. *Trans R Soc Trop Med Hyg* 1994;**88**:315–16.
40. Byrd SR, Gelber R, Bermudez LE. Roles of soluble fibronectin and β_1 integrin receptors in the binding of *Mycobacterium leprae* to nasal epithelial cells. *Clin Immunol Immunopathol* 1993;**69**: 266–71.
41. Capadia GD, Shetty VP, Khambati FA, et al. Effect of corticosteroid usage combined with multidrug therapy on nerve damage assessed using nerve conduction studies: a prospective cohort of 365 untreated multibacillary leprosy patients. *J Clin Neurophysiol* 2010;**27**:38–47.
42. Cellona RV, Balagon MF, dela Cruz EC, et al. Long-term efficacy of 2 year WHO multiple drug therapy (MDT) in multibacillary (MB) leprosy patients. *Int J Lepr Other Mycobact Dis* 2003;**71**: 308–19.
43. Chauty A, Ardant M-F, Marsollier L, et al. Oral treatment for *Mycobacterium ulcerans* infection: results from a pilot study in Benin. *Clin Infect Dis* 2011;**52**:94–6.
44. Chedore P, Broukhanski G, Shainhouse Z, et al. False-positive amplified *Mycobacterium tuberculosis* direct test results for samples containing *Mycobacterium leprae*. *J Clin Microbiol* 2006;**44**: 612–13.
45. Cho SN, Chatterjee D, Brennan PJ. A simplified serological test for leprosy based on a 3,6-di-*O*-methylglucose-containing synthetic antigen. *Am J Trop Med Hyg* 1986;**35**:167–72.
46. Clancey JK, Dodge OG, Lunn HF, et al. Mycobacterial skin ulcers in Uganda. *Lancet* 1961;**2**:951–4.
47. Cochrane RG, Davey TF. *Leprosy in Theory and Practice*. 2nd ed. Bristol, England: John Wright & Sons; 1964.
48. Coloma JN, Navarrete-Franco G, Iribe P, et al. Ulcerative cutaneous mycobacteriosis due to *Mycobacterium ulcerans*: report of two Mexican cases. *Int J Lepr Other Mycobact Dis* 2005;**73**:5–12.
49. Connor DH, Lunn HF. Buruli ulceration: a clinicopathologic study of 38 Ugandans with *Mycobacterium ulcerans* infection. *Arch Pathol* 1966;**81**:183–9.
50. Connor DH, Meyers WM, Krieg RE. Infection by *Mycobacterium ulcerans*. In: Connor DH, Binford CH, editors. *Pathology of Tropical and Extraordinary Diseases: An Atlas*, vol. 1. Washington, DC: Armed Forces Institute of Pathology; 1976. p. 226.

51. Converse PJ, Almeida DV, Nuermberger EL, et al. BCG-mediated protection against *Mycobacterium ulcerans* infection in the mouse. *PLoS Negl Trop Dis* 2011;**15**:e985.
52. Convit J, Pinardi ME. A simple method for the differentiation of *Mycobacterium leprae* from other mycobacteria through routine staining technics. *Int J Lepr Other Mycobact Dis* 1972;**40**:130–2.
53. Convit J, Aranzazu N, Ulrich M, et al. Immunotherapy with a mixture of *Mycobacterium leprae* and BCG in different forms of leprosy and in Mitsuda-negative contacts. *Int J Lepr Other Mycobact Dis* 1982;**50**:415–24.
54. Convit J, Avila JL, Goihman M, et al. A test for the determination of competency in clearing bacilli in leprosy patients. *Bull World Health Organ* 1972;**46**:821–6.
55. Convit J, Sampson C, Zuniga M, et al. Immunoprophylactic trial with combined *Mycobacterium leprae*/BCG vaccine against leprosy: preliminary results. *Lancet* 1992;**339**:446–50.
56. Cook A. Mengo Hospital Notes. Kampala, Uganda, Makerere Medical School Library, 1897 (cited in BMJ 2:37-9, 1970).
57. Cooke GS, Hill AV. Genetics of susceptibility to human infectious disease. *Nat Rev Genet* 2001;**2**:967–77.
58. Courtright P, Daniel E, Sundarrao M, et al. Eye disease in multibacillary leprosy patients at the time of their leprosy diagnosis: findings from the Longitudinal Study of Ocular Leprosy (LOSOL) in India, the Philippines and Ethiopia. *Lepr Rev* 2002;**73**: 225–38.
59. Coutanceau E, Legras P, Marsollier L, et al. Immunogenicity of *Mycobacterium ulcerans* Hsp65 and protective efficacy of a *Mycobacterium leprae* Hsp65-based DNA vaccine against Buruli ulcer. *Microbes Infect* 2006;**8**:2075–81.
60. Coutanceau E, Marsollier L, Brosch R, et al. Modulation of the host immune response by a transient intracellular stage of *Mycobacterium ulcerans*: the contribution of endogenous mycolactone toxin. *Cell Microbiol* 2005;**7**:1187–96.
61. Cree IA, Coghill G, Subedi AM, et al. Effects of treatment on the histopathology of leprosy. *J Clin Pathol* 1995;**48**:304–7.
62. Daniel AK, Lee RE, Portaels F, et al. Analysis of *Mycobacterium* species for the presence of a macrolide toxin, mycolactone. *Infect Immun* 2004;**72**:123–32.
63. Daniel E, Ffytche TJ, Kempen JH, et al. Incidence of ocular complications in patients with multibacillary leprosy after completion of a 2 year course of multidrug therapy. *Br J Ophthalmol* 2006;**90**:949–54.
64. Daniel E, Ffytche TJ, Sundar Rao PS, et al. Incidence of ocular morbidity among multibacillary leprosy patients during a 2 year course of multidrug therapy. *Br J Ophthalmol* 2006;**90**:568–73.
65. Daniel E, Rao PS, Ffytche TJ, et al. Ocular hypotension and hypotony in multibacillary leprosy patients; at diagnosis, during, and after completion of multidrug therapy. *Indian J Lepr* 2010; **82**:181–8.
66. Davey TF, Rees RJW. The nasal discharge in leprosy: clinical and bacteriological aspects. *Lepr Rev* 1974;**45**:121–34.
67. Dayal R, Bharadwaj VP. Prevention and early detection of leprosy in children. *J Trop Pediatr* 1995;**41**:132–8.
68. Debacker M, Portaels F, Aguiar J, et al. Risk factors for Buruli ulcer, Benin. *Emerg Infect Dis* 2006;**12**:1325–31.
69. Debacker M, Aguiar J, Steunou C, et al. *Mycobacterium ulcerans* disease (Buruli ulcer) in rural hospital, Southern Benin, 1997-2001. *Emerg Infect Dis* 2004;**10**:1391–8.
70. Debacker M, Aguiar J, Steunou C, et al. *Mycobacterium ulcerans* disease: role of age and gender in incidence and morbidity. *Trop Med Int Health* 2004;**9**:1297–304.
71. Debacker M, Aguiar J, Steunou C, et al. Buruli ulcer recurrence, Benin. *Emerg Infect Dis* 2005;**11**:584–9.
72. Debacker M, Zinsou C, Aguiar J, et al. First case of *Mycobacterium ulcerans* disease (Buruli ulcer) following a human bite. *Clin Infect Dis* 2003;**36**:e67–e68.
73. Demangel C, Stinear TP, Cole ST. Buruli ulcer: reductive evolution enhances pathogenicity of *Mycobacterium ulcerans*. *Nat Rev Microbiol* 2009;**7**:50–60.
74. de Rijk AJ, Gabre S, Byass P, et al. Field evaluation of WHO-MDT of fixed duration at ALERT, Ethiopia: the AMFES project, II: Reaction and neuritis during and after MDT in PB and MB leprosy patients. *Lepr Rev* 1994;**65**:320–32.
75. de Wit MY, Douglas JT, McFadden J, et al. Polymerase chain reaction for detection of *Mycobacterium leprae* in nasal swab specimens. *J Clin Microbiol* 1993;**31**:502–6.
76. Dietrich M, Gaus W, Kern P, et al. An international randomized study with long-term follow-up of single versus combination chemotherapy of multibacillary leprosy. *Antimicrob Agents Chemother* 1994;**38**:2249–57.
77. Dodge OG. Mycobacterial skin ulcers in Uganda: histopathological and experimental aspects. *J Pathol Bacteriol* 1964;**88**:167–74.
78. Donham KJ, Leininger JR. Spontaneous leprosy-like disease in a chimpanzee. *J Infect Dis* 1977;**136**:132–6.
79. Donoghue HD, Spigelman M, Greenblatt CL, et al. Tuberculosis: from prehistory to Robert Koch, as revealed by ancient DNA. *Lancet Infect Dis* 2004;**4**:584–92.
80. Drutz DJ, Chen TS, Lu WH. The continuous bacteremia of lepromatous leprosy. *N Engl J Med* 1972;**287**:159–64.
81. Duker AA, Portaels F, Hale M. Pathways of *Mycobacterium ulcerans* infection: a review. *Environ Int* 2006;**32**:567–73.
82. Duncan ME, Melsom R, Pearson JM, et al. A clinical and immunological study of four babies of mothers with lepromatous leprosy, two of whom developed leprosy in infancy. *Int J Lepr Other Mycobact Dis* 1983;**51**:7–17.
83. Duncan ME, Miko T, Howe R, et al. Growth and development of children of mothers with leprosy and healthy controls. *Ethiop Med J* 2007;**45**(Suppl. 1):9–23.
84. Durnez L, Suykerbuyk P, Nicholas V, et al. Terrestrial small mammals as reservoirs of *Mycobacterium ulcerans* in Benin. *Appl Environ Microbiol* 2010;**76**:4574–7.
85. Duthie MS, Hay MN, Rada EM, et al. Specific IgG antibody responses may be used to monitor leprosy treatment efficacy and as recurrence prognostic markers. *Eur J Clin Microbiol Infect Dis* 2011;**30**:1257–65.
86. Dwyer JM, Bullock WE, Fields JP. Disturbances of the blood: T:B lymphocyte ratio in lepromatous leprosy. *N Engl J Med* 1973; **288**:1036–9.
87. Ebeigbe JA, Kio F. Ocular leprosy in institutionalized Nigerian patients. *Ghana Med J* 2011;**45**:50–3.
88. Eiglmeier K, Parkhill J, Honore N, et al. The decaying genome of *Mycobacterium leprae*. *Lepr Rev* 2001;**72**:387–98.
89. Ellard GA. Rationale of the multidrug regimens recommended by a World Health Organization study group on chemotherapy of leprosy for control programs. *Int J Lepr Other Mycobact Dis* 1984; **52**:395–401.
90. Faber WR, Jensema AJ, Goldschmidt WF. Treatment of recurrent erythema nodosum leprosum with infliximab. *N Engl J Med* 2006;**355**:739.
91. Faber WR, Arias-Bouda LM, Zeegelaar JE, et al. First reported case of *Mycobacterium ulcerans* infection in a patient from China. *Trans R Soc Trop Med Hyg* 2000;**94**:277–9.
92. Faget GH, Pogge RC, Johansen FA, et al. The Promin treatment of leprosy: a progress report. *Public Health Rep* 1943;**58**: 1729–41.
93. Fajardo TT, Abalos RM, dela Cruz EC, et al. Clofazimine therapy for lepromatous leprosy: a historical perspective. *Int J Dermatol* 1999;**38**:70–4.
94. Farb H, West DP, Pedvis-Leftick A. Clofazimine in pregnancy complicated by leprosy. *Obstet Gynecol* 1982;**59**:122–3.
95. Feenstra SG, Nahar Q, Pahan D, et al. Acceptability of chemoprophylaxis for household contacts of leprosy patients in Bangladesh: a qualitative study. *Lep Rev* 2011;**82**:178–87.
96. Feenstra SG, Nahar Q, Pahan D, et al. Recent food shortage is associated with leprosy disease in Bangladesh: a case-control study. *PLoS Negl Trop Dis* 2011;**5**:e1029.
97. Feldman RA, Sturdivant M. Leprosy in Louisiana 1855-1970: an epidemiologic study of long-term trends. *Am J Epidemiol* 1975; **102**:303–10.
98. Fernando SL, Britton WJ. Genetic susceptibility to mycobacterial disease in humans. *Immunol Cell Biol* 2006;**84**:125–37.
99. Fiallo P, Williams DL, Chan GP, et al. Effects of fixation on polymerase chain reaction detection of *Mycobacterium leprae*. *J Clin Microbiol* 1992;**30**:3095–8.
100. Fine PE, Warndorff DK. Leprosy by the year 2000: what is being eliminated? [editorial]. *Lepr Rev* 1997;**68**:201–2.
101. Fischer EA, de Vlas SJ, Habbema JD, et al. The long term effect of current and new interventions on the new case detection of leprosy: a modeling study. *PLoS Negl Trop Dis* 2011;**5**: e1330.
102. Fitness J, Tosh K, Hill AV. Genetics of susceptibility to leprosy. *Genes Immun* 2002;**3**:441–53.

103. Fitness J, Floyd S, Warndorff DK, et al. Large-scale candidate gene study of leprosy susceptibility in the Karonga district of northern Malawi. *Am J Trop Med Hyg* 2004;**71**:330–40.
104. Flageul B, Wallach D, Vignon-Pennamen MD, et al. Late onset of a reversal reaction in borderline leprosy. *J Am Acad Dermatol* 1989;**20**:857–60.
105. Fritschi EP. *Reconstructive Surgery in Leprosy*. Bristol, England: John Wright & Sons. 1971.
106. Fyfe JA, Lavender CJ, Handasyde KA, et al. A major role for mammals in the ecology of *Mycobacterium ulcerans*. *PLoS Negl Trop Dis* 2010;**4**:e791.
107. Gajl-Peczalska KJ, Lim SD, Jacobson RR, et al. B lymphocytes in lepromatous leprosy. *N Engl J Med* 1973;**288**:1033–5.
108. Gatt P. The Malta experience: 1972-1992—20 years after starting the eradication project in Malta. *Int J Lepr* 1993;**61**(Suppl.):5A.
109. Gaylord H, Brennan PJ. Leprosy and the leprosy bacillus: recent developments in characterization of antigens and immunology of the disease. *Ann Rev Microbiol* 1987;**41**:645–75.
110. Gehr E. Leprosy in childhood. *Doc Med Geog Trop* 1957;**9**: 101–24.
111. Gelber RH, Drutz DJ, Epstein WV, et al. Clinical correlates of C1Q-precipitating substances in the sera of patients with leprosy. *Am J Trop Med Hyg* 1974;**23**:471–5.
112. Gelber RH, Fukuda K, Byrd S, et al. A clinical trial of minocycline in lepromatous leprosy. *BMJ* 1992;**304**:91–2.
113. Gelber RH, Murray LP, Siu P, et al. Efficacy of minocycline in single dose and at 100 mg twice daily for lepromatous leprosy. *Int J Lepr Other Mycobact Dis* 1994;**62**:568–73.
114. George KM, Pascopella L, Welty DM, et al. A *Mycobacterium ulcerans* toxin, mycolactone, causes apoptosis in guinea pig ulcers and tissue culture cells. *Infect Immun* 2000;**68**:877–83.
115. Ghorpade A. Post-traumatic inoculation tuberculoid leprosy after injury with a glass bangle. *Lepr Rev* 2009;**80**:215–18.
116. Girdhar BK, Girdhar A, Ramu G, et al. Borderline leprosy (BL) in an infant: report of a case and a brief review. *Lepr India* 1983; **55**:333–7.
117. Godal T. Growing points in leprosy research: (3) Immunological detection of sub-clinical infection in leprosy. *Lepr Rev* 1974;**45**: 22–30.
118. Gomez A, Mve-Obiang A, Vray B, et al. Biochemical and genetic evidence for phospholipase C activity in *M. ulcerans*. *Infect Immunol* 2000;**68**:2995–7.
119. Gonzalez-Abreu E, Mora N, Perez M, et al. Serodiagnosis of leprosy in patients' contacts by enzyme-linked immunosorbent assay. *Lepr Rev* 1990;**61**:145–50.
120. Gormus BJ, Xu KY, Alford PL, et al. A serologic study of naturally acquired leprosy in chimpanzees. *Int J Lepr Other Mycobact Dis* 1991;**59**:450–7.
121. Grant AMB. Leprosy at Nauru since 1928. *Int J Lepr* 1934;**2**: 305–10.
122. Groathouse NA, Brown SE, Knudson DL, et al. Isothermal amplification and molecular typing of the obligate intracellular pathogen *Mycobacterium leprae* isolated from tissues of unknown origins. *J Clin Microbiol* 2006;**44**:1502–8.
123. Grosset JH. Progress in the chemotherapy of leprosy. *Int J Lepr Other Mycobact Dis* 1994;**62**:268–77.
124. Guarner J, Bartlett J, Whitney EA, et al. Histopathologic features of *Mycobacterium ulcerans* infection. *Emerg Infect Dis* 2003;**9**: 651–6.
125. Gupte MD, Anantharaman DS, Nagaraju B, et al. Experiences with *Mycobacterium leprae* soluble antigens in a leprosy endemic population. *Lepr Rev* 1990;**61**:132–44.
126. Harboe M. The work and concepts of Armauer Hansen: how do they stand today? *Ethiop Med J* 1983;**21**:123–6.
127. Harboe M. The immunology of leprosy. In: Hastings RC, editor. *Leprosy (Medicine in the Tropics Series)*. Edinburgh: Churchill Livingstone; 1985. p. 53.
128. Haregewoin A, Godal T, Mustafa AS, et al. T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. *Nature* 1983;**303**:342–4.
129. Hayman J. Clinical features of *Mycobacterium ulcerans* infection. *Australas J Dermatol* 1985;**26**:67–73.
130. Hietaharju A, Croft R, Alam R, et al. Chronic neuropathic pain in treated leprosy. *Lancet* 2000;**356**:1080–1.
131. Hogeweg M, Keunen JE. Prevention of blindness in leprosy and the role of the Vision 2020 Programme. *Eye* 2005;**19**:1099–105.
132. Horsburgh CR, Meyers WM. Buruli ulcer. In: Horsburgh CR Jr, Nelson AM, editors. *Pathology of Emerging Infections*. Washington, DC: American Society for Microbiology; 1997. p. 119.
133. Horwitz MA, Levis WR, Cohn ZA. Defective production of monocyte-activating cytokines in lepromatous leprosy. *J Exp Med* 1984;**159**:666–78.
134. Houngbedji GM, Bouchard P, Frenette J. *Mycobacterium ulcerans* infections cause progressive muscle atrophy and dysfunction, and mycolactone impairs satellite cell proliferation. *Am J Physiol Regul Integr Comp Physiol* 2011;**300**:R724–R732.
135. Huh JG, Kim YS, Lee JS, et al. *Mycobacterium ulcerans* infection as a cause of chronic diarrhea in an AIDS patient: a case report. *World J Gastroenterol* 2008;**14**:808–11.
136. Hunter SW, Fujiwara T, Brennan PJ. Structure and antigenicity of the major specific glycolipid antigen of *Mycobacterium leprae*. *J Biol Chem* 1982;**257**:15072–8.
137. Hussain R, Lucas SB, Kifayet A, et al. Clinical and histological discrepancies in diagnosis of ENL reactions classified by assessment of acute phase proteins SAA and CRP. *Int J Lepr Other Mycobact Dis* 1995;**63**:222–30.
138. Huygen K, Adjei O, Affolabi D, et al. Buruli ulcer disease: prospects for a vaccine. *Med Microbiol Immunol* 2009;**198**:69–77.
139. Idema WJ, Majer IM, Pahan D, et al. Cost-effectiveness of a chemoprophylactic intervention with single dose rifampicin in contacts of new leprosy patients. *PLoS Negl Trop Dis* 2010;**4**:e874.
140. Imkamp FMJH. Clofazimine (lamprene or B663) in lepra reactions. *Lepr Rev* 1981;**52**:135–40.
141. Inaba T. Ueber die Histopathologischen und Bakteriologischen Untersuchungen der Plazenta bei Leprosen. *La Lepro* 1938;**9**(Suppl. III).
142. Izumi S, Fujiwara T, Ikeda M, et al. Novel gelatin particle agglutination test for serodiagnosis of leprosy in the field. *J Clin Microbiol* 1990;**28**:525–9.
143. Jacob JT, Franco-Paredes C. The stigmatization of leprosy in India and its impact on future approaches to elimination and control. *PLoS Negl Trop Dis* 2008;**2**:e113.
144. Jacobson RR, Hastings RC. Rifampin-resistant leprosy. *Lancet* 1976;**2**:1304–5.
145. Jakeman P. Risk of relapse in multibacillary leprosy. *Lancet* 1995;**345**:4–5.
146. Jamet P, Ji B. Marchoux Chemotherapy Study Group. Relapse after long-term follow up of multibacillary patients treated by WHO multidrug regimen. *Int J Lepr Other Mycobact Dis* 1995; **63**:195–201.
147. Janssen F, Wallach D, Khuong MA, et al. Association de maladie de Hansen et d'infection par le virus de l'immunodéficience humaine: Deux observations [Association of Hansen's disease and human deficiency virus infection: 2 cases]. *Presse Med* 1988;**17**: 1652–3.
148. Ji B, Lefrancois S, Robert J, et al. In vitro and in vivo activities of rifampin, streptomycin, amikacin, moxifloxacin, R207910, linezolid, and PA-824 against *Mycobacterium ulcerans*. *Antimicrob Agents Chemotherap* 2006;**50**:1921–6.
149. Ji B, Jamet P, Perani EG, et al. Powerful bactericidal activities of clarithromycin and minocycline against *Mycobacterium leprae* in lepromatous leprosy. *J Infect Dis* 1993;**168**:188–90.
150. Job CK. The Kellersberger Memorial Lecture 1983. The lepromin test and its role in the management of leprosy. *Ethiop Med J* 1983;**21**:233–42.
151. Job CK, Chacko CJG. A simplified 6 group classification of leprosy. *Lepr India* 1982;**54**:26–32.
152. Job CK, Harris EB, Allen JL, et al. A random survey of leprosy in wild nine-banded armadillos in Louisiana. *Int J Lepr Other Mycobact Dis* 1986;**54**:453–7.
153. Job CK, Jayakumar J, Kearney M, et al. Transmission of leprosy: a study of skin and nasal secretions of household contacts of leprosy patients using PCR. *Am J Trop Med Hyg* 2008;**78**: 518–21.
154. Johnson RC, Nackers F, Glynn JR, et al. Association of HIV infection and *Mycobacterium ulcerans* disease in Benin. *AIDS* 2008;**22**: 901–3.
155. Johnstone PAS, Meyers WM, Binford CH. Recent advances in the development of nonhuman primates as animal models for leprosy. *Scand J Lab Anim Sci* 1989;**16**(Suppl. 1):102–5.
156. Jopling WH. *Handbook of Leprosy*. London: William Heinemann; 1984.

157. Jopling WH, Ridley MJ, Bonnici E, et al. A follow-up investigation of the Malta Project. *Lepr Rev* 1984;**55**:247–53.
158. Kamal R, Natrajan M, Katoch K, et al. Evaluation of the diagnostic role of in situ PCR on slit-skin smears in pediatric leprosy. *Indian J Lepr* 2010;**82**:195–200.
159. Kang TJ, Chae GT. Detection of Toll-like receptor 2 (TLR2) mutation in the lepromatous leprosy patients. *FEMS Immunol Med Microbiol* 2001;**31**:53–8.
160. Kang TJ, Lee SB, Chae GT. A polymorphism in the Toll-like receptor 2 is associated with IL-12 production from monocyte in lepromatous leprosy. *Cytokine* 2002;**20**:56–62.
161. Kang TJ, Yeum CE, Kim BC, et al. Differential production of interleukin-10 and interleukin-12 in mononuclear cells from leprosy patients with a Toll-like receptor 2 mutation. *Immunology* 2004;**112**:674–80.
162. Kaplan G. Recent advances in cytokine therapy in leprosy. *J Infect Dis* 1993;**167**(Suppl. 1):S18–22.
163. Kaplan G, Mathur NK, Job CK, et al. Effect of multiple interferon gamma injections on the disposal of *Mycobacterium leprae*. *Proc Natl Acad Sci USA* 1989;**86**:8073–7.
164. Kaplan G, Sampaio EP, Walsh GP, et al. Influence of *Mycobacterium leprae* and its soluble products on the cutaneous responsiveness of leprosy patients to antigen and recombinant interleukin 2. *Proc Natl Acad Sci USA* 1989;**86**:6269–73.
165. Kar HK, Bhatia VN, Harikrishnan S. Combined clofazimine- and dapsone-resistant leprosy: a case report. *Int J Lepr* 1986;**54**: 389–91.
166. Kar HK, Sharma AK, Misra RS, et al. Reversal reaction in multibacillary leprosy patients following MDT with and without immunotherapy with a candidate for an antileprosy vaccine, *Mycobacterium w*. *Lepr Rev* 1993;**64**:219–26.
167. Karonga Prevention Trial Group. Randomised controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. *Lancet* 1996;**348**:17–24.
168. Kashala O, Marlink R, Ilunga M, et al. Infection with human immunodeficiency virus type 1 (HIV-1) and human T cell lymphotropic viruses among leprosy patients and contacts: Correlation between HIV-1 cross-reactivity and antibodies to lipoarabinomannan. *J Infect Dis* 1994;**169**:296–304.
169. Kassi K, Kouame K, Allen W, et al. Squamous cell carcinoma secondary to Buruli ulcer: a clinical case report in a young girl. *Bacteriol Virusol Parazitol Epidemol* 2010;**55**:25–8.
170. Kato L. Leprosy associated mycobacteria: implications. *Acta Leprol* 1989;**7**:1–6.
171. Kaur I, Kaur S, Sharma VK, et al. Bacillaemia and *Mycobacterium leprae* cell wall antigen in paucibacillary leprosy. *Indian J Lepr* 1993;**65**:283–8.
172. Kawuma HJS, Bwire R, Adatu-Engwau F. Leprosy and infection with the human immunodeficiency virus in Uganda: a case-control study. *Int J Lepr* 1994;**62**:521–6.
173. Kerr-Pontes LR, Barreto ML, Evangelista CM, et al. Socioeconomic, environmental, and behavioural risk factors for leprosy in Northeast Brazil: results of a case-control study. *Int J Epidemiol* 2006;**35**:994–1000.
174. Khanolkar-Young S, Rayment N, Brickell PM, et al. Tumour necrosis factor-alpha (TNF-alpha) synthesis is associated with the skin and peripheral nerve pathology of leprosy reversal reactions. *Clin Exp Immunol* 1995;**99**:196–202.
175. Kibadi K, Colebunders R, Muyembe-Tamfum JJ, et al. Buruli ulcer lesions in HIV-positive patient. *Emerg Infect Dis* 2010;**16**: 738–9.
176. Kibadi K, Mputu-Yamba JB, Mokassa B, et al. Relapse after surgical treatment of *Mycobacterium ulcerans* infection: study of risk factors in 84 patients in the Democratic Republic of the Congo. *Med Trop (Mars)* 2009;**69**:471–4.
177. Kiszewski AE, Becerril E, Aguilar LD, et al. The local immune response in ulcerative lesions of Buruli disease. *Clin Exp Immunol* 2006;**143**:445–51.
178. Krieg RE, Hockmeyer WT, Connor DH. Toxin of *Mycobacterium ulcerans*: production and effects in guinea pig skin. *Arch Dermatol* 1974;**110**:783–8.
179. Kumarasinghe SP, Kumarasinghe MP, Amarasinghe UT. "Tap sign" in tuberculoid and borderline tuberculoid leprosy. *Int J Lepr Other Mycobact Dis* 2004;**72**:291–5.
180. Lamfers EJ, Bastiaans AH, Mravunac M, et al. Leprosy in the acquired immunodeficiency syndrome. *Ann Intern Med* 1987;**107**: 111–12.
181. Lane JE, Balagon MV, Dela Cruz EC, et al. *Mycobacterium leprae* in untreated lepromatous leprosy: more than skin deep. *Clin Exp Dermatol* 2006;**31**:469–70.
182. Lane JE, Walsh DS, Meyers WM, et al. Borderline tuberculoid leprosy in a woman from the state of Georgia with armadillo exposure. *J Am Acad Dermato* 2006;**55**:714–16.
183. Languillon J, Carayon A. *Précis de Léprologie*. Paris: Masson; 1969.
184. Lara CB. Leprosy in children: general considerations—initial and early changes. *Philipp J Lepr* 1966;**1**:22–57.
185. Latapi F, Zamora AC. The "spotted" leprosy of Lucio: an introduction to its clinical and histological study. *Int J Lepr Other Mycobact Dis* 1948;**16**:421–9.
186. Lavender CJ, Fyfe JA, Azuolas J, et al. Risk of Buruli ulcer and detection of *Mycobacterium ulcerans* in mosquitoes in southeastern Australia. *PLoS Negl Trop Dis* 2011;**5**:e1305.
187. Lawn SD, Wood C, Lockwood DN. Borderline tuberculoid leprosy: an immune reconstitution phenomenon in a human immunodeficiency virus-infected patient. *Clin Infect Dis* 2003; **36**:e5–6.
188. Lazaro FP, Werneck RI, Mackert CCO, et al. A major gene controls leprosy susceptibility in a hyperendemic isolated population north of Brazil. *J Infect Dis* 2010;**201**:1598–605.
189. Leiker DL. On the mode of transmission of *Mycobacterium leprae*. *Lepr Rev* 1977;**48**:9–16.
190. Leprosy News and Notes. The words "leper" and "leprosy." Fifth International Leprosy Congress, 1948. *Int J Lepr Other Mycobact Dis* 1948;**16**:243.
191. Levis WR, Paraskevas L-R, Jacobson M, et al. Endemic leprosy in New York City. *Arch Dermatol* 2011;**147**:624–5.
192. Levy L, Ji B. The mouse foot-pad technique for cultivation of *Mycobacterium leprae*. *Lepr Rev* 2006;**77**:5–24.
193. Lockwood DN, Suneetha S. Leprosy: Too complex a disease for a simple elimination paradigm. *Bull World Health Organ* 2005;**83**: 230–5.
194. Lumpkin LR, Cox GF, Wolf JE. Leprosy in five armadillo handlers. *J Am Acad Dermatol* 1983;**9**:899–903.
195. Lupi O, Madkan V, Tyring SK. Tropical dermatology: bacterial tropical diseases. *J Am Acad Dermatol* 2006;**54**:559–78; quiz 578-580.
196. MacCallum P, Tolhurst JC, Buckle G, et al. A new mycobacterial infection in man. *J Pathol Bacteriol* 1948;**60**:93–122.
197. Marion E, Landier J, Boisier P, et al. Geographic expansion of Buruli ulcer disease, Cameroon. *Emerg Infect Dis* 2011;**17**:551–3.
198. Marsollier L, Aubry J, Coutanceau E, et al. Colonization of the salivary glands of *Naucoris cimicoides* by *Mycobacterium ulcerans* requires host plasmatocytes and a macrolide toxin, mycolactone. *Cell Microbiol* 2005;**7**:935–43.
199. Marsollier L, Aubry J, Saint-André JP, et al. Ecologie et transmission de *Mycobacterium ulcerans* [Ecology and transmission of *Mycobacterium ulcerans*]. *Pathol Biol (Paris)* 2003;**51**:490–5.
200. Marsollier L, Robert R, Aubry J, et al. Aquatic insects as a vector for *Mycobacterium ulcerans*. *Appl Environ Microbiol* 2002;**68**: 4623–8.
201. Marston BJ, Diallo MO, Horsburgh CR, et al. Emergence of Buruli ulcer disease in the Daloa region of Côte d'Ivoire. *Am J Trop Med Hyg* 1995;**52**:219–24.
202. McAdam KP, Anders RF, Smith SR, et al. Association of amyloidosis with erythema nodosum leprosum reactions and recurrent neutrophil leucocytosis in leprosy. *Lancet* 1975;**2**:572–3.
203. McCoy W. History of leprosy in the United States. *Am J Trop Med* 1938;**18**:19–34.
204. McDougall AC, Waudby H. Dermal microfiliariasis and leprosy. *Lepr Rev* 1977;**48**:161–8.
205. McInturff JE, Modlin RL, Kim J. The role of toll-like receptors in the pathogenesis and treatment of dermatological disease. *J Invest Dermatol* 2005;**125**:1–8.
206. Meeran K. Prevalence of HIV infection among patients with leprosy and tuberculosis in rural Zambia. *BMJ* 1989;**298**:364–5.
207. Mehdi G, Maheshwari V, Ansari HA, et al. Modified fine-needle aspiration technique for diagnosis of granulomatous skin lesions with special reference to leprosy and cutaneous tuberculosis. *Diagn Cytopathol* 2010;**38**:391–6.

208. Mehra V, Brennan PJ, Rada E, et al. Lymphocyte suppression in leprosy induced by unique *M. leprae* glycolipid. *Nature* 1984;**308**:194–6.
209. Mehra V, Mason LH, Rothman W, et al. Delineation of a human T cell subset responsible for lepromin-induced suppression in leprosy patients. *J Immunol* 1980;**125**:1183–8.
210. Meisner SJ, Mucklow S, Warner G, et al. Association of NRAMP1 polymorphism with leprosy type but not susceptibility to leprosy per se in west Africans. *Am J Trop Med Hyg* 2001;**65**:733–5.
211. Melancon-Kaplan J, Hunter SW, McNeil M, et al. Immunological significance of *Mycobacterium leprae* cell walls. *Proc Natl Acad Sci USA* 1988;**85**:1917–21.
212. Melsom R, Harboe M, Duncan ME, et al. IgA and IgM antibodies against *Mycobacterium leprae* in cord sera and in patients with leprosy: an indicator of intrauterine infection in leprosy. *Scand J Immunol* 1981;**14**:343–52.
213. Melsom R, Harboe M, Duncan ME. IgA, IgM and IgG anti-*M. leprae* antibodies in babies of leprosy mothers during the first two years of life. *Clin Exp Immunol* 1982;**49**:532–42.
214. Merritt RW, Walker ED, Small PLC, et al. Ecology and transmission of Buruli ulcer disease: a systematic review. *PLoS Negl Trop Dis* 2010;**4**:e911.
215. Meyers WM. Mycobacterial infections of the skin (including leprosy, tuberculosis of the skin, Buruli ulcer, and less common mycobacterial infections). In: Doerr W, Seifert G, editors. *Tropical Pathology*, vol. 8. 2nd ed. Berlin: Springer-Verlag; 1995. p. 291.
216. Meyers WM, Gormus BJ, Walsh GP. Nonhuman sources of leprosy. *Int J Lepr Other Mycobact Dis* 1992;**60**:477–80.
217. Meyers WM, Binford CH, Walsh GP, et al. Animal models of leprosy. In: Leive L, Schlessinger D, editors. *Microbiology: 1984*. Washington, DC: American Society for Microbiology; 1984. p. 307.
218. Meyers WM, Connor DH, Harman LE, et al. Human streptocerciasis: a clinico-pathologic study of 40 Africans (Zairians) including identification of the adult filaria. *Am J Trop Med Hyg* 1972;**21**:528–45.
219. Meyers WM, Gormus BJ, Walsh GP, et al. Naturally acquired and experimental leprosy in nonhuman primates. *Am J Trop Med Hyg* 1991;**44**:24–7.
220. Meyers WM, Gormus BJ, Walsh GP. Experimental leprosy. In: Hastings RC, editor. *Leprosy (Medicine in the Tropics Series)*. 2nd ed. Edinburgh: Churchill Livingstone; 1994. p. 385.
221. Meyers WM, Kvernes S, Binford CH. Comparison of reactions to human and armadillo lepromins in leprosy. *Int J Lepr Other Mycobact Dis* 1975;**43**:218–25.
222. Meyers WM, McDougall AC, Fleury RN, et al. Histologic responses in sixty multibacillary leprosy patients inoculated with autoclaved *Mycobacterium leprae* and live BCG. *Int J Lepr Other Mycobact Dis* 1988;**56**:302–9.
223. Meyers WM, Shelly WM, Connor DH. Heat treatment of *Mycobacterium ulcerans* infections without surgical excision. *Am J Trop Med Hyg* 1974;**23**:924–9.
224. Meyers WM, Shelly WM, Connor DH, et al. Human *Mycobacterium ulcerans* infections developing at sites of trauma to skin. *Am J Trop Med Hyg* 1974;**23**:919–23.
225. Meyers WM, Tignokpa N, Priuli GB, et al. *Mycobacterium ulcerans* infection (Buruli ulcer): first reported patients in Togo. *Br J Dermatol* 1996;**134**:1116–21.
226. Meyers WM, Walsh GP, Brown HL, et al. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): reactions in leprosy patients to lepromins prepared from naturally infected armadillos. *J Reticuloendothel Soc* 1977;**22**:369–75.
227. Meyers WM, Walsh GP, Brown HL, et al. Leprosy in a mangabey monkey: naturally acquired infection. *Int J Lepr Other Mycobact Dis* 1985;**53**:1–14.
228. Mira MT, Alcais A, Nguyen VT, et al. Susceptibility to leprosy is associated with PARK2 and PACRG. *Nature* 2004;**427**:636–40.
229. Mira MT, Alcais A, Van Thuc N, et al. Chromosome 6q25 is linked to susceptibility to leprosy in a Vietnamese population. *Nat Genet* 2003;**33**:412–15.
230. Misch EA, Macdonald M, Ranjit C, et al. Human TLR1 deficiency is associated with impaired mycobacterial signaling and protection from leprosy reversal reaction. *PLoS Negl Trop Dis* 2008;**2**:e231.
231. Mitchell PJ. Jerrett IV, Slee KJ. Skin ulcers caused by *Mycobacterium ulcerans* in koalas near Bairnsdale, Australia. *Pathology* 1984;**16**:256–60.
232. Mitsuda K. On the value of a skin reaction to a suspension of leprous nodules. *Reprinted in English in Int J Lepr Other Mycobact Dis* 1953;**21**:347–58. (First published in Japanese: Hifuka Hinyoka Zasshi [Jpn. J. Dermatol. Urol.] *19*:697-708, 1919.)
233. Modlin RL, Brightbill HD, Godowski PJ. The toll of innate immunity on microbial pathogens. *N Engl J Med* 1999;**340**:1834–5.
234. Modlin RL, Gebhard JF, Taylor CR, et al. *In situ* characterization of T lymphocyte subsets in the reactional states of leprosy. *Clin Exp Immunol* 1983;**53**:17–24.
235. Modlin RL, Hofman FM, Horwitz DA, et al. *In situ* identification of cells in human leprosy granulomas with monoclonal antibodies to interleukin 2 and its receptor. *J Immunol* 1984;**132**:3085–90.
236. Modlin RL, Hofman FM, Taylor CR, et al. T lymphocyte subsets in the skin lesions of patients with leprosy. *J Am Acad Dermatol* 1983;**8**:182–9.
237. Modlin RL, Melancon-Kaplan J, Young SM, et al. Learning from lesions: patterns of tissue inflammation in leprosy. *Proc Natl Acad Sci USA* 1988;**85**:1213–17.
238. Moet FJ, Pahan D, Oskam L, et al. Effectiveness of single dose rifampicin in preventing leprosy in close contacts of patients with newly diagnosed leprosy: cluster randomized controlled trial. *BMJ* 2008;**336**:761–4.
239. Monot M, Honore N, Garnier T, et al. On the origin of leprosy. *Science* 2005;**308**:1040–2.
240. Monot M, Honore N, Garnier T, et al. Comparative genomic and phylogenetic analysis of *Mycobacterium leprae*. *Nat Genet* 2009;**41**:1282–9.
241. Moran CA, Nelson AM, Tuur SM, et al. Leprosy in five human immunodeficiency virus-infected patients. *Mod Pathol* 1995;**8**:662–4.
242. Moschella SL. An update on the diagnosis and treatment of leprosy. *J Am Acad Dermatol* 2004;**51**:417–26.
243. Mourit AAM. *The path of the destroyer: a history of leprosy in the Hawaiian Islands*. Honolulu: Honolulu Star-Bulletin; 1916.
244. Muir E. Juvenile leprosy. *Int J Lepr Other Mycobact Dis* 1936;**4**:45–8.
245. Mukherjee A, Meyers WM. Endothelial cell bacillation in lepromatous leprosy: a case report. *Lepr Rev* 1987;**58**:419–24.
246. Mve-Obiang A, Lee RE, Portaels F, et al. Heterogeneity of mycolactones produced by clinical isolates of *Mycobacterium ulcerans*: implications for virulence. *Infect Immun* 2003;**71**:774–83.
247. Myrvang B, Godal T, Ridley DS, et al. Immune responsiveness to *Mycobacterium leprae* and other mycobacterial antigens throughout the clinical and histopathological spectrum of leprosy. *Clin Exp Immunol* 1973;**14**:541–53.
248. Naaf B. Treatment of leprosy: science or politics? *Trop Med Int Health* 2006;**11**:268–78.
249. Nackers F, Tonglet R, Slachmuylder V, et al. Association between haemoglobin variants S and C and *Mycobacterium ulcerans* disease (Buruli ulcer): a case-control study in Benin. *Trop Med Int Health* 2007;**12**:511–18.
250. Nakata N, Kai M, Makino M. Mutational analysis of the *Mycobacterium leprae folP1* gene and dapsone resistance. *Antimicrob Agent Chemotherap* 2011;**55**:762–6.
251. National Hansen's Disease Program. *Epidemiology*. Available online at: http://www.hrsa.gov/hansensdisease. Accessed October 5, 2011.
252. National Hansen's Disease Program. *Recommended leprosy treatment regimens*. Available online at: http://www.hrsa.gov/hansensdisease/diagnosis/recommendedtreatment.html. Accessed September 27, 2011.
253. Navalkar RG, Norlin M, Ouchterlony O. Characterization of leprosy sera with various mycobacterial antigens using double diffusion-in-gel analysis. *Int Arch Allergy* 1965;**28**:250–60.
254. Nayak SV, Shivarudrappa AS, Mukkanil AS. Role of fluorescent microscopy in detecting *Mycobacterium leprae* in tissue sections. *Ann Diagn Pathol* 2003;**7**:78–81.
255. Nery JA, Perisse AR, Sales AM, et al. The use of pentoxifylline in the treatment of type 2 reactional episodes in leprosy. *Indian J Lepr* 2000;**72**:457–67.

256. Nienhuis WA, Stienstra Y, Thompson WA, et al. Antimicrobial treatment for early, limited *Mycobacterium ulcerans* infection: a randomised controlled trial. *Lancet* 2010;**375**:664–72.
257. Nogueira N, Kaplan G, Levy E, et al. Defective gamma interferon production in leprosy: reversal with antigen and interleukin 2. *J Exp Med* 1983;**158**:2165–70.
258. Norman G, Raja SB, Samuel P. Trends in leprosy over fifty years in Gudiyatham Talu, Vellore, Tamil Nadu. *Indian J Lepr* 2006;**78**: 167–85.
259. Noussitou FM. *Leprosy in Children*. Geneva: World Health Organization; 1976.
260. Olcen P, Harboe M, Warndorff T, et al. Antigens of *Mycobacterium leprae* and anti-*M. leprae* antibodies in the urine of leprosy patients. *Lepr Rev* 1983;**54**:203–16.
261. Oliveira MS, Fraga AG, Torrado E, et al. Infection with *Mycobacterium ulcerans* induces persistent inflammatory responses in mice. *Infect Immun* 2005;**73**:6299–310.
262. Ottenhoff THM. State of the Art Lectures. Immunology of leprosy: lessons from and for leprosy. *Int J Lepr Other Mycobact Dis* 1994;**62**:108–21.
263. Palomino JC, Obiang AM, Realini L, et al. Effect of oxygen on growth of *Mycobacterium ulcerans* growth in the BACTEC system. *J Clin Microbiol* 1998;**36**:3420–2.
264. Park JY, Cho SN, Youn JK, et al. Detection of antibodies to human nerve antigens in sera from leprosy patients by ELISA. *Clin Exp Immunol* 1992;**87**:368–72.
265. Parkash O, Chaturvedi V, Girdhar BK, et al. A study on performance of two serological assays for diagnosis of leprosy patients. *Lepr Rev* 1995;**66**:26–30.
266. Pedley JC. The presence of *M. leprae* in human milk. *Lepr Rev* 1967;**38**:239–42.
267. Pesce C, Grattarola M, Menini S, et al. Cyclooxygenase 2 expression in vessels and nerves in reversal reaction leprosy. *Am J Trop Med Hyg* 2006;**74**:1076–7.
268. Phanzu DM, Bafende EA, Dunda BK, et al. *Mycobacterium ulcerans* disease (Buruli ulcer) in a rural hospital in Bas-Congo, Democratic Republic of Congo, 2002-2004. *Am J Trop Med Hyg* 2006;**75**: 311–14.
269. Pocaterra L, Jain S, Reddy R, et al. Clinical course of erythema nodosum leprosum: an 11-year cohort study in Hyderabad, India. *Am J Trop Med Hyg* 2006;**74**:868–79.
270. Portaels F, Aguiar J, Debacker M, et al. *Mycobacterium bovis* BCG vaccination as prophylaxis against *Mycobacterium ulcerans* osteomyelitis in Buruli ulcer disease. *Infect Immun* 2004;**72**:62–5.
271. Portaels F, Elsen P, Guimares-Peres A, et al. Insects in the transmission of *Mycobacterium ulcerans*. *Lancet* 1999;**353**:986.
272. Portaels F, Fonteyene PA, de Beenhouwer H, et al. Variability in 3′ end of 16S rRNA sequence of *Mycobacterium ulcerans* is related to geographic origin of isolates. *J Clin Microbiol* 1996;**34**:962–5.
273. Prasad HK, Mishra RS, Nath I. Phenolic glycolipid-I of *Mycobacterium leprae* induces general suppression of in vitro concanavalin A responses unrelated to leprosy type. *J Exp Med* 1987;**165**: 239–44.
274. Pszolla N, Sarkar MR, Strecker W, et al. Buruli ulcer: a systemic disease. *Clin Infect Dis* 2003;**37**:e78–e82.
275. Raghunathan PL, Whitney EAS, Asamoa K, et al. Risk factors for Buruli ulcer disease (*Mycobacterium ulcerans* infection): results from a case-control study in Ghana. *Clin Infect Dis* 2005;**40**: 1445–53.
276. Rambukkana A, Yamada H, Zanazzi G, et al. Role of alpha-dystroglycan as a Schwann cell receptor for *Mycobacterium leprae*. *Science* 1998;**282**:2076–9.
277. Ramien ML, Wong A, Keystone JS. Severe refractory erythema nodosum leprosum successfully treated with the tumor necrosis factor etanercept. *Clin Infect Dis* 2011;**52**:e133–e135.
278. Rangdaeng S, Scollard DM, Suriyanon V, et al. Studies of human leprosy lesions in situ using suction-induced blisters. I. Cellular components of new, uncomplicated lesions. *Int J Lepr Other Mycobact Dis* 1989;**57**:492–8.
279. Ranque B, Alcais A, Thuc NV, et al. A recessive major gene controls the Mitsuda reaction in a region endemic for leprosy. *J Infect Dis* 2005;**192**:1475–82.
280. Rea TH, Jerskey RS. Clinical and histologic variations among thirty patients with Lucio's phenomenon and pure and primitive diffuse lepromatosis (Latapi's lepromatosis). *Int J Lepr Other Mycobact Dis* 2005;**73**:169–88.
281. Rea TH, Bakke AC, Parker JW, et al. Peripheral blood T lymphocyte subsets in leprosy. *Int J Lepr Other Mycobact Dis* 1984; **52**:311–17.
282. Read JK, Heggie CM, Meyers WM, et al. Cytotoxic activity of *Mycobacterium ulcerans*. *Infect Immun* 1974;**9**:1114–22.
283. Rees RJW, McDougall AC. Airborne infection with *Mycobacterium leprae* in mice. *J Med Microbiol* 1977;**10**:63–8.
284. Revill WDL, Barker DJP. Seasonal distribution of mycobacterial skin ulcers. *Br J Prev Soc Med* 1972;**26**:23–7.
285. Ridley DS. *Skin Biopsy in Leprosy*. 2nd ed. Basel: Geigy; 1985.
286. Ridley DS, Jopling WH. Classification of leprosy according to immunity: a five-group system. *Int J Lepr Other Mycobact Dis* 1966;**34**:255–73.
287. Robbins G, Tripathy VM, Misra VN, et al. Ancient skeletal evidence for leprosy in India (2000 B.C.). *PLoS One* 2009;**4**:e5669.
288. Roch PW, Theuvenet WL, Britton WJ. Risk factors for type-1 reactions in borderline leprosy patients. *Lancet* 1991;**338**:654–7.
289. Rodrigues LC, Lockwood DNJ. Leprosy now: epidemiology, progress, challenges, and research gaps. *Lancet Infect Dis* 2011;**11**: 464–70.
290. Rojas-Espinosa O, Løvik M. *Mycobacterium leprae* and *Mycobacterium lepraemurium* in domestic and wild animals. *Rev Sci Tech Off Int Epiz* 2001;**20**:219–51.
291. Rondini S, Mensah-Quainoo E, Troll H, et al. Development and application of real-time PCR assay for quantification of *Mycobacterium ulcerans* DNA. *J Clin Microbiol* 2003;**41**:4231–7.
292. Rongioletti F, Gallo R, Cozzani E, et al. Leprosy: a diagnostic trap for dermatopathologists in nonendemic areas. *Am J Dermatopathol* 2009;**31**:607–10.
293. Rook GA. The immunology of leprosy. *Tubercle* 1983;**64**: 297–312.
294. Rook GA, Martinelli R, Brunet LR. Innate immune responses to mycobacteria and the downregulation of atopic responses. *Curr Opin Allergy Clin Immunol* 2003;**3**:337–42.
295. Ross BC, Johnson PD, Oppedisano F, et al. Detection of *Mycobacterium ulcerans* in environmental samples during an outbreak of ulcerative disease. *Appl Environ Microbiol* 1997;**63**:4135–8.
296. Ross BC, Marino L, Oppedisano F, et al. Development of a PCR assay for rapid diagnosis of *Mycobacterium ulcerans* infection. *J Clin Microbiol* 1997;**35**:1696–700.
297. Roy S, McGuire W, Mascie-Taylor CG, et al. Tumor necrosis factor promoter polymorphism and susceptibility to lepromatous leprosy. *J Infect Dis* 1997;**176**:530–2.
298. Ruf MT, Chauty A, Adeye A, et al. Secondary Buruli ulcer skin lesions emerging several months after completion of chemotherapy: paradoxical reaction or evidence for immune protection? *PLoS Negl Trop Dis* 2011;**5**:e1252.
299. Ruffer MA. Pott'sche Krankeit an Einer Ägyptischen Mumie aus der Zeit der 21 Dynastie (um 1000 v. Chr.). In: *Zur Historischen Biol d Krankheitserreger*, vol. 3. Giessen: Topelmann; 1910.
300. Ryrie GA. Some impressions of Sungei Buloh Leper Hospital under Japanese occupation. *Lepr Rev* 1947;**18**:10–17.
301. Sabin TD, Swift TR, Jacobson RR. *Peripheral Neuropathy*. Philadelphia: W. B. Saunders; 1993.
302. Safa G, Darrieux L, Coic A, et al. Type 1 leprosy reversal reaction treated with topical tacrolimus along with systemic corticosteroids. *Indian J Med Sci* 2009;**63**:359–62.
303. Sales AM, Ponce de Leon A, Duppre NC, et al. Leprosy among patient contacts: a multilevel study of risk factors. *PLoS Negl Trop Dis* 2011;**5**:e1013.
304. Salgame P, Abrams JS, Clayberger C, et al. Differing lymphokine profiles of functional subsets of human CD4 and CD8 T cell clones. *Science* 1991;**254**:279–82.
305. Sampaio EP, Caneshi JR, Nery JA, et al. Cellular immune response to *Mycobacterium leprae* infection in human immunodeficiency virus-infected individuals. *Infect Immun* 1995;**63**:1848–54.
306. Santo AR, Suffys PN, Vanderborght PR, et al. Role of tumor necrosis factor-alpha and interleukin-10 promoter gene polymorphisms in leprosy. *J Infect Dis* 2002;**186**:1687–91.
307. Sarfo FS, Lavender CJ, Fyfe JA, et al. *Mycobacterium ulcerans* DNA not detected in faecal samples from Buruli ulcer patients: results of a pilot study. *PLoS One* 2011;**6**:e19611.
308. Sarfo FS, Le Chevalier F, Aka N, et al. Mycolactone diffuses into the peripheral blood of Buruli ulcer patients: implications for diagnosis and disease monitoring. *PLoS Negl Trop Dis* 2011;**5**: e1237.

309. Sarno EN, Grau GE, Vieira LM, et al. Serum levels of tumour necrosis factor-alpha and interleukin-1 beta during leprosy reactional states. *Clin Exp Immunol* 1991;**84**:103–8.
310. Saul A, Novales J. La lèpre de Lucio-Latapi et le phénomène de Lucio. *Acta Leprol* 1983;**92**:115–32.
311. Schlesinger LS, Horwitz MA. Complement receptors and complement component C3 mediate phagocytosis of *Mycobacterium tuberculosis* and *Mycobacterium leprae*. *Int J Lepr Other Mycobact Dis* 1990;**58**:200–1.
312. Schunk M, Thompson W, Klutse E, et al. Outcome of patients with Buruli ulcer after surgical treatment with or without antimycobacterial treatment in Ghana. *Am J Trop Med Hyg* 2009; **81**:75–81.
313. Schuring RP, Richardus JH, Pahan D, et al. Protective effect of the combination of BCG vaccination and rifampin prophylaxis in leprosy prevention. *Vaccine* 2009;**27**:7125–8.
314. Scollard DM. Endothelial cells and the pathogenesis of lepromatous neuritis: insights from the armadillo model. *Microbes Infect* 2000;**2**:1835–43.
315. Scollard DM, Joyce MP, Gillis TP. Development of leprosy and type 1 leprosy reactions after treatment with infliximab: a report of 2 cases. *Clin Infect Dis* 2006;**43**:e19–e22.
316. Scollard DM, McCormick G, Allen JL. Localization of *Mycobacterium leprae* to endothelial cells of epineurial and perineurial blood vessels and lymphatics. *Am J Pathol* 1999;**154**:1611–20.
317. Scollard DM, Adams LB, Gillis TP, et al. The continuing challenges of leprosy. *Clin Microbiol Rev* 2006;**19**:338–81.
318. Sehgal VN, Srivastava G, Singh N. Histoid leprosy: histopathological connotations' relevance in contemporary context. *Am J Dermatopathol* 2009;**31**:268–71.
319. Sekar B, Arunagiri K, Kumar BN, et al. Detection of mutations in folP1, rpoB, and gyrA genes of *M. leprae* by PCR-direct sequencing: a rapid tool for screening drug resistance in leprosy. *Lepr Rev* 2011;**82**:36–45.
320. Sekar B, Jayasheela M, Chattopadhya D, et al. Prevalence of HIV infection and high-risk characteristics among leprosy patients of south India: a case-control study. *Int J Lepr Other Mycobact Dis* 1994;**62**:527–31.
321. Sethi SK, Solanki RS, Mendiratta V. Leprotic nerve abscess. *Appl Radiol* 2006;**35**:44–7.
322. Setia MS, Shinde SS, Jerajani HR, et al. Is there a role for rifampicin, ofloxacin, and minocycline (ROM) therapy in the treatment of leprosy? Systematic review and meta-analysis. *Trop Med Int Health* 2011;**16**:1541–51.
323. Setia MS, Steinmaus C, Ho CS, et al. The role of BCG in prevention of leprosy: a meta-analysis. *Lancet Infect Dis* 2006;**6**:162–70.
324. Shah DH, Vartak RR, Naik SS, et al. Epidemiological studies in children of a low-endemic region, a high-endemic region, and dwellers of a leprosy colony: evaluation of anti-ND-BSA antibodies and lepromin response. *Int J Lepr Other Mycobact Dis* 1994; **62**:539–46.
325. Shanmugam A, Natarajan J. Computational genome analysis of metabolic enzymes in *Mycobacterium leprae* for drug target identification. *Bioinformation* 2010;**4**:392–5.
326. Sharma P, Misra RS, Kar HK, et al. Mycobacterium w vaccine, a useful adjuvant to multidrug therapy in multibacillary leprosy: a report on hospital-based immunotherapeutic clinical trials with a follow-up of 1-7 years after treatment. *Lepr Rev* 2000;**71**:179–92.
327. Sharp D. Leprosy lessons from old bones. *Lancet* 2007;**369**: 808–9.
328. Shaw MA, Donaldson IJ, Collins A, et al. Association and linkage of leprosy phenotypes with HLA class II and tumour necrosis factor genes. *Genes Immun* 2001;**2**:196–204.
329. Shepard CC. The experimental disease that follows the injection of human leprosy into footpads of mice. *J Exp Med* 1960;**112**: 445–54.
330. Shepard CC. Combinations involving dapsone, rifampicin, clofazimine and ethionamide in the treatment of *M. leprae* infections in mice. *Int J Lepr Other Mycobact Dis* 1976;**44**:135–9.
331. Shields ED, Russell DA, Pericak-Vance MA. Genetic epidemiology of the susceptibility to leprosy. *J Clin Invest* 1987;**79**: 1139–43.
332. Shimoji Y, Ng V, Matsumura K, et al. A 21-kDa surface protein of *Mycobacterium leprae* binds peripheral nerve laminin-2 and mediates Schwann cell invasion. *Proc Natl Acad Sci USA* 1999;**96**: 9857–62.
333. Siddaraju N, Sistla SC, Singh N, et al. Pure neuritic leprosy with nerve abscess presenting as a cystic soft tissue mass. *Diagn Cytopathol* 2009;**37**:355–8.
334. Siddiqui MR, Meisner S, Tosh K, et al. A major susceptibility locus for leprosy in India maps to chromosome 10p13. *Nat Genet* 2001; **27**:439–41.
335. Siegmund V, Adjei O, Racz P, et al. Dry-reagent-based PCR as a novel tool for laboratory confirmation of clinically diagnosed *Mycobacterium ulcerans*-associated disease in areas in the tropics where *M. ulcerans* is endemic. *J Clin Microbiol* 2005;**43**:271–6.
336. Silva MT, Portaels F, Pedrosa J. Pathogenetic mechanisms of the intracellular parasite *Mycobacterium ulcerans* leading to Buruli ulcer. *Lancet Infect Dis* 2009;**9**:699–710.
337. Singh A, Sharma S, Sarvjot V. Inoculation leprosy developing after tattooing. *Indian J Pathol Microbiol* 2007;**50**:841–2.
338. Singh NB, Srivastava A, Verma VK, et al. *Mastomys natalensis*: a new animal model for *Mycobacterium ulcerans* research. *Indian J Exp Biol* 1984;**22**:393–4.
339. Skinsnes OK. Leprosy in society. II. The pattern of concept and reaction to leprosy in Oriental antiquity. *Lepr Rev* 1964;**35**: 106–22.
340. Skinsnes OK, Higa LH. The role of protein malnutrition in the pathogenesis of ulcerative "lazarine" leprosy. *Int J Lepr Other Mycobact Dis* 1976;**44**:346–58.
341. Smith C, Richardus JH. Leprosy strategy is about control, not eradication. *Lancet* 2008;**371**:969–70.
342. Smith CM, Smith WCS. Chemoprophylaxis is effective in the prevention of leprosy in endemic countries: a systematic review and meta-analysis. *J Infect* 2000;**41**:137–42.
343. Smith JH, Folse DS, Long EG, et al. Leprosy in wild armadillos (*Dasypus novemcinctus*) of the Texas Gulf Coast: epidemiology and mycobacteriology. *J Reticuloendothel Soc* 1983;**34**:75–88.
344. Smith WCS. We need to know what is happening to the incidence of leprosy [editorial]. *Lepr Rev* 1997;**68**:195–200.
345. Sopoh GE, Barogui YT, Johnson RC, et al. Family relationship, water contact, and occurrence of Buruli ulcer in Benin. *PLoS Negl Trop Dis* 2010;**4**:e746.
346. Sopoh GE, Johnson RC, Anagonou SY, et al. Buruli ulcer prevalence and altitude, Benin. *Emerg Infect Dis* 2011;**17**:153–4.
347. Stanford JL, Rook GA, Bahr GM, et al. *Mycobacterium vaccae* in immunoprophylaxis and immunotherapy of leprosy and tuberculosis. *Vaccine* 1990;**8**:525–30.
348. Stienstra Y, van der Werf TS, Oosterom E, et al. Susceptibility to Buruli ulcer is associated with the SLC11A1 (NRAMP1) D543N polymorphism. *Genes Immun* 2006;**7**:185–9.
349. Stienstra Y, van Roest MH, van Wezel MJ, et al. Factor associated with functional limitations and subsequent employment or schooling in Buruli ulcer patients. *Trop Med Int Health* 2005;**10**: 1251–7.
350. Stinear T, Davies JK, Jenkin GA, et al. Identification of *Mycobacterium ulcerans* in the environment from regions in Southeast Australia in which it is endemic with sequence capture-PCR. *Appl Environ Microbiol* 2000;**66**:3206–13.
351. Stinear TP, Hong H, Frigui W, et al. Common evolutionary origin for the unstable virulence plasmid pMUM found in geographically diverse strains of *Mycobacterium ulcerans*. *J Bacteriol* 2005;**187**:1668–76.
352. Stinear TP, Mve-Obiang A, Small PL, et al. Giant plasmid-encoded polyketide synthases produce the macrolide toxin of *Mycobacterium ulcerans*. *Proc Natl Acad Sci USA* 2004;**101**: 1345–9.
353. Stinear T, Ross BC, Davies JK, et al. Identification and characterization of IS*2404* and IS*2606*: two distinct repeated sequences for detection of *Mycobacterium ulcerans* by PCR. *J Clin Microbiol* 1999;**37**:1018–23.
354. Stinear TP, Seemann T, Harrison PF, et al. Insights from the complete genome sequence of *Mycobacterium marinum* on the evolution of *Mycobacterium tuberculosis*. *Genome Res* 2008;**18**: 729–41.
355. Stragier P, Ablordey A, Bayonne LM, et al. Heterogeneity among *Mycobacterium ulcerans* isolates from Africa. *Emerg Infect Dis* 2006; **12**:844–7.
356. Sung KJ, Kim SB, Choi JH, et al. Detection of *Mycobacterium leprae* DNA in formalin-fixed, paraffin-embedded samples from multibacillary and paucibacillary leprosy patients by polymerase chain reaction. *Int J Dermatol* 1993;**32**:710–13.

357. Talhari S, Orsi AT, Talhari AC, et al. Pentoxifylline may be useful in the treatment of type 2 leprosy reaction. *Lepr Rev* 1995;**66**: 261–3.
358. Tanghe A, Adnet PY, Gartner T, et al. A booster vaccination with *Mycobacterium bovis* BCG does not increase the protective effect of the vaccine against experimental *Mycobacterium ulcerans* infection in mice. *Infect Immune* 2007;**75**:2642–4.
359. Toll A, Gallardo F, Ferran M, et al. Aggressive multifocal Buruli ulcer with associated osteomyelitis in an HIV-positive patient. *Clin Exp Dermatol* 2005;**30**:649–51.
360. Truman R. Leprosy in wild armadillos. *Lepr Rev* 2005;**76**: 198–208.
361. Truman RW, Job CK, Hastings RC. Antibodies to the phenolic glycolipid-1 antigen for epidemiologic investigations of enzootic leprosy in armadillos (*Dasypus novemcinctus*). *Lepr Rev* 1990;**61**: 19–24.
362. Truman RW, Kumaresan JA, McDonough CM, et al. Seasonal and spatial trends in the detectability of leprosy in wild armadillos. *Epidemiol Infect* 1991;**106**:549–60.
363. Truman RW, Singh P, Sharma R, et al. Probable zoonotic leprosy in the southern United States. *N Engl J Med* 2011;**364**: 1626–33.
364. Tsukamura M, Kaneda K, Imaeda T, et al. [A taxonomic study on a mycobacterium which caused a skin ulcer in a Japanese girl and resembled *Mycobacterium ulcerans*]. *Kekkaku* 1989;**64**:691–7.
365. Turk JL. Cell-mediated immunological processes in leprosy. *Lepr Rev* 1970;**41**:207–22.
366. Uganda Buruli Group. Clinical features and treatment of pre-ulcerative Buruli lesions (*Mycobacterium ulcerans* infection). *BMJ* 1970;**2**:390–3.
367. Ustianowski AP, Lawn SD, Lockwood DN. Interactions between HIV infection and leprosy: a paradox. *Lancet Infect Dis* 2006;**6**: 350–60.
368. Valla MC. *Lèpre et Grossesse*. Lyon, France: Thèse de Médicine; 1976.
369. Valverde CR, Canfield D, Tarara R, et al. Spontaneous leprosy in a wild-caught cynomolgus macaque. *Int J Lepr Other Mycobact Dis* 1998;**66**:140–8.
370. van Beers SM, Izumi S, Madjid B, et al. An epidemiological study of leprosy infection by serology and polymerase chain reaction. *Int J Lepr Other Mycobact Dis* 1994;**62**:1–9.
371. van der Werf TS, Stienstra Y, Johnson RC, et al. *Mycobacterium ulcerans* disease. *Bull World Health Organ* 2005;**83**:785–91.
372. van Eden W, deVries RR, D'Amaro J, et al. HLA-DR-associated genetic control of the type of leprosy in a population from Surinam. *Hum Immunol* 1982;**4**:343–50.
373. Van Veen NH, Lockwood DN, Van Brakel WH, et al. Interventions for erythema nodosum leprosum: a Cochrane review. *Lepr Rev* 2009;**80**:355–72.
374. Van Veen NH, Nicholls PG, Smith WC, et al. Corticosteroids for treating nerve damage in leprosy. *Cochrane Database Syst Rev* 2007;**18**:CD005491.
375. Vargas-Ocampo F. Analysis of 6000 skin biopsies of the national leprosy control program in Mexico. *Int J Lepr Other Mycobact Dis* 2004;**72**:427–36.
376. Veitch MG, Johnson PD, Flood PE, et al. A large localized outbreak of *Mycobacterium ulcerans* infection on a temperate southern Australian island. *Epidemiol Infect* 1997;**119**:313–18.
377. Velayati AA, Farnia P, Khalizadeh S, et al. Interferon-gamma receptor-1 gene promoter polymorphisms and susceptibility to leprosy in children of a single family. *Am J Trop Med Hyg* 2011;**84**: 627–9.
378. Vettom L, Pritze S. Reliability of skin smear results: experiences with quality control of skin smears in different routine services in leprosy control programmes. *Lepr Rev* 1989;**60**:187–96.
379. Villahermosa LG, Abalos RM, Walsh DS, et al. Recombinant interleukin-2 in lepromatous leprosy lesions: Immunological and microbiological consequences. *Clin Exp Dermatol* 1997;**22**: 134–40.
380. Villahermosa LG, Fajardo TT, Abalos RM, et al. Parallel assessment of 24 monthly doses of rifampin, ofloxacin, and minocycline versus two years of World Health Organization multi-drug therapy for multi-bacillary leprosy. *Am J Trop Med Hyg* 2004;**70**: 197–200.
381. Villahermosa LG, Fajardo TT, Abalos RM, et al. A randomized, double-blind, double-dummy, controlled dose comparison of thalidomide for treatment of erythema nodosum leprosum. *Am J Trop Med Hyg* 2005;**72**:518–26.
382. Wabitsch KR, Meyers WM. Histopathologic observations on the persistence of *Mycobacterium leprae* in the skin of multibacillary leprosy patients under chemotherapy. *Lepr Rev* 1988;**59**:341–6.
383. Wade HW. The histoid variety of lepromatous leprosy. *Int J Lepr Other Mycobact Dis* 1963;**31**:129–42.
384. Walker SL, Nicholls PG, Dhakal S, et al. A phase two randomized controlled double blind trial of high dose intravenous methylprednisolone and oral prednisolone versus intravenous normal saline and oral prednisolone in individuals with leprosy type 1 reactions and/or nerve function impairment. *PloS Negl Trop Dis* 2011;**5**: e1041.
385. Walsh DS, dela Cruz EC, Abalos RM, et al. Clinical and histological features of skin lesions in a cynomolgus monkey experimentally infected with *Mycobacterium ulcerans* (Buruli ulcer) by intradermal inoculation. *Am J Trop Med Hyg* 2007;**76**:132–4.
386. Walsh DS, Lane JE, Abalos RM, et al. TUNEL and limited immunophenotypic analyses of apoptosis in paucibacillary and multibacillary leprosy lesions. F. E. M. S. *Immunol Med Microbiol* 2004;**41**:265–9.
387. Walsh DS, Meyers WM, Krieg RE, et al. Transmission of *Mycobacterium ulcerans* to the nine-banded armadillo. *Am J Trop Med Hyg* 1999;**61**:694–7.
388. Walsh DS, Meyers WM, Portaels F, et al. High rates of apoptosis in human *Mycobacterium ulcerans* culture-positive Buruli ulcer skin lesions. *Am J Trop Med Hyg* 2005;**73**:410–15.
389. Walsh DS, Prieto-Go D, Abalos RM, et al. Malignant T-cell lymphoma mimicking lepromatous leprosy. *Clin Exp Dermatol* 2001;**26**:173–5.
390. Walsh GP, Meyers WM, Binford CH, et al. Leprosy as a zoonosis: an update. *Acta Leprol* 1988;**6**:51–60.
391. Walsh GP, Storrs EE, Meyers WM, et al. Naturally acquired leprosy-like disease in the nine-banded armadillo (*Dasypus novemcinctus*): recent epizootiologic findings. *J Reticuloendothel Soc* 1977;**22**:363–7.
392. Walsh DS, Villahermosa LG, Balagon MV, et al. Cutaneous delayed-type hypersensitivity responsiveness in lepromatous and borderline lepromatous leprosy patients as determined by MULTITEST CMI. *Southeast Asian J Trop Med Public Health* 1999;**30**: 518–26.
393. Warndorff Van Diepen T. Clofazimine-resistant leprosy, a case report. *Int J Lepr Other Mycobact Dis* 1982;**50**:139–42.
394. Waters MFR. The diagnosis and management of dapsone-resistant leprosy. *Lepr Rev* 1977;**48**:95–105.
395. Waters MFR. The treatment of leprosy. *Tubercle* 1983;**64**: 221–32.
396. Waters MFR. Relapse following various types of multidrug therapy in multibacillary leprosy [editorial]. *Lepr Rev* 1995;**66**: 1–9.
397. Welsh O, Gomez M, Mancias C, et al. A new therapeutic approach to type II leprosy reaction. *Int J Dermatol* 1999;**38**:931–3.
398. West BC, Todd JR, Lary CH, et al. Leprosy in six isolated residents of northern Louisiana: time-clustered cases in an essentially nonendemic area. *Arch Intern Med* 1988;**148**:1987–92.
399. Williams DL, Gillis TP. A study of the relatedness of *Mycobacterium leprae* isolates using restriction fragment length polymorphism analysis. *Acta Leprol* 1989;**7**(Suppl. 1):226–30.
400. Williams DL, Gillis TP. Molecular detection of drug resistance in *Mycobacterium leprae*. *Lepr Rev* 2004;**75**:118–30.
401. Williams DL, Gillis TP, Booth RJ, et al. The use of a specific DNA probe and polymerase chain reaction for the detection of *Mycobacterium leprae*. *J Infect Dis* 1990;**162**:193–200.
402. Williams DL, Waguespack C, Eisenach K, et al. Characterization of rifampin-resistance in pathogenic mycobacteria. *Antimicrob Agents Chemother* 1994;**38**:2380–6.
403. Wolf RH, Gormus BJ, Martin LN, et al. Experimental leprosy in three species of monkeys. *Science* 1985;**227**:529–31.
404. Wong SH, Gochhait S, Malhotra D, et al. Leprosy and the adaptation of human toll-like receptor 1. *PLoS Pathog* 2010;**6**:e1000979.
405. Woodall P, Scollard D, Rajan L. Hansen Disease among Micronesian and Marshallese persons living in the United States. *Emerg Infect Dis* 2011;**17**:1202–8.
406. World Health Organization. *Chemotherapy of leprosy for control programmes. World Health Organ Tech Rep Ser No. 675*. Geneva: WHO; 1982.

407. World Health Organization. *Leprosy: global situation. Wkly Epidemiol Rec No. 28 (July 14)*. Geneva: WHO; 2000.
408. World Health Organization. *World Health Assembly, Seventh Plenary Meeting (A57/VR/7)*. Geneva: WHO; 2004.
409. World Health Organization. Buruli ulcer: progress report, 2004-2008. *Wkly Epidemiol Rec* 2008;**83**:145–56.
410. World Health Organization. *Buruli ulcer: treatment recommendations*. Available online at: http://www.who.int/buruli/en. Accessed October 5, 2011.
411. World Health Organization. *Leprosy elimination: WHO-recommended multidrug therapy (MDT) regimens*. Available online at: http://www.who.int/lep/mdt/regimens/en/index.html. Accessed September 27, 2011.
412. World Health Organization. Leprosy update, 2011. *Wkly Epidemiol Rec* 2011;**3**:389–400.
413. World Health Organization. Surveillance of drug resistance in leprosy: 2010. *Wkly Epidemiol Rept* 2011;**23**:237–40.
414. World Health Organization; Asiedu K, Scherpbier R, Raviglione M, editors. *Buruli Ulcer: Mycobacterium ulcerans Infection (WHO/CDS/CPE/GBUI/2000.1)*. Geneva: WHO; 2000.
415. World Health Organization. *Expert Committee on Leprosy. World Health Organ Tech Rep Ser No. 607*. Geneva: WHO; 1977.
416. World Health Organization; Portaels F, Johnson PDR, Meyers WM, editors. *Diagnosis of Mycobacterium ulcerans Disease (Buruli Ulcer) (WHO/CDS/CPE/GBUI/2001.3)*. Geneva: WHO; 2001.
417. Yamamura M, Uyemura K, Deans RJ, et al. Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science* 1991;**254**:277–9.
418. Young DB, Harnisch JP, Knight J, et al. Detection of phenolic glycolipid I in sera from patients with lepromatous leprosy. *J Infect Dis* 1985;**152**:1078–81.
419. Zhang F-R, Huang W, Chen S-M, et al. Genomewide association study of leprosy. *N Engl J Med* 2009;**361**:2609–18.

disease in 1890. The earliest pediatric case was documented in 1895 in a boy with pulmonary and subcutaneous infection.[5]

ORGANISM

Nocardia spp. are included among the aerobic actinomycetes. They are gram-positive bacteria that are filamentous and branched and grow more slowly than other aerobic and facultatively anaerobic bacteria. They commonly produce a fungus-like mycelium that fragments or breaks up into rod-shaped and short coccoid forms. *Nocardia* spp. grow well aerobically on a variety of simple media (e.g., blood agar and brain-heart infusion agar); adding carbon dioxide (10%) promotes more rapid growth. The organisms are inhibited by antibiotics and antifungal agents, so media containing such agents do not support the growth of *Nocardia*. Because of their growth on commonly used fungal media (e.g., Sabouraud dextrose agar) and on some mycobacterial media (e.g., Löwenstein-Jensen medium), many *Nocardia* samples may be misdirected to the mycology or mycobacteriology section of clinical laboratories for identification.

In the past, microscopic and colonial morphology, biochemical tests, chemotaxonomic methods, or antimicrobial susceptibility patterns were used to speciate *Nocardia*. 16S rRNA gene sequencing and polymerase chain reaction (PCR) restriction fragment length polymorphism (RFLP) analysis are the current methods most frequently used for speciation; use of these molecular techniques has resulted in changes in the nomenclature of *Nocardia* spp.[11]

More than 30 species of *Nocardia* of human clinical significance have been identified.[11] Formerly the term *Nocardia asteroides* complex was used to account for the heterogeneity in antimicrobial resistance patterns seen among *N. asteroides*. Detailed molecular analyses and susceptibility testing have established clearly that *N. asteroides* complex represented many different *Nocardia* spp.[11] The most medically important species of *Nocardia* include: *N. asteroides*, *Nocardia abscessus*, *Nocardia brasiliensis*, *Nocardia farcinica*, *Nocardia brevicatena/paucivorans* complex, *Nocardia nova* complex, *Nocardia otitidiscaviarum* complex, *Nocardia pseudobrasiliensis*, and *Nocardia transvalensis* complex.

Nocardia spp. grow best at temperatures of 25°C to 45°C (77° to 113°F); growth at higher temperatures may be used for differentiation. In pure culture, small, chalky white, heaped, wrinkled, or verrucous colonies appear in 3 to 5 days. Mature colonies usually are light orange and have a velvety appearance caused by the production of rudimentary aerial mycelia. They have the odor of a musty basement or freshly turned soil. Detection of colonies from clinical specimens, such as respiratory secretions, may require 2 to 4 weeks. In mixed culture, rapidly growing bacteria may obscure small *Nocardia* colonies. Use of modified Thayer-Martin medium may enhance recovery.[46]

Gram stain of a portion of a colony shows delicate, branching filaments no more than 1 μm in diameter. The delicate filaments may fragment and produce bacillary or coccoid forms. Many *Nocardia* spp. are partially acid-fast (i.e., in contrast to *Mycobacterium* spp., they retain fuchsin less tenaciously). A modified Ziehl-Neelsen or Kinyoun stain that decolorizes with 1 percent sulfuric acid instead of the more active acid alcohol is best for showing acid-fast *Nocardia* in clinical specimens. Acid-fastness is characteristic of *Nocardia* in tissue or primary colonial isolates but is lost quickly on subculture; not all pathogenic strains of *Nocardia* are acid-fast.[6] *N. asteroides* often survives the *N*-acetylcysteine digestion procedure (without sodium hydroxide) performed on sputum or bronchial washings; however, some positive sputum specimens may be rendered falsely negative.[44] Cultures of sputum and bronchial washings for isolation of *Nocardia* should be performed before and after the digestion procedure.

EPIDEMIOLOGY, TRANSMISSION, AND PATHOGENESIS

Nocardia spp. occasionally can be skin contaminants or upper respiratory tract saprotrophs.[23,51,60] Bronchial obstruction or decreased bronchociliary clearance predisposes to colonization.[51] The presence of signs and symptoms that correlate with nocardiosis can help ascertain the clinical significance of a positive *Nocardia* culture.

Most infections occur in the lungs, via inhalation, with dissemination to the central nervous system occurring in one third of affected patients. Although nocardiosis occurs in immunocompetent individuals, 70 percent of patients are immunosuppressed by medications or underlying disease.[2] The typical patient has compromised cellular immunity (secondary to steroids, organ transplantation, cytotoxic chemotherapy, chronic granulomatous disease, chronic alcoholism, diabetes mellitus, or human immunodeficiency virus [HIV] infection). Nocardiosis also is seen more commonly in patients with chronic pulmonary disease or a history of surgery or trauma.[41] Person-to-person communicability has not been a problem. Historically the incidence of nocardiosis in the United States has been estimated to be approximately 500 to 1000 new cases per year.[4] However, this number most likely is an underrepresentation given the marked increase in the number of immunocompromised individuals. In addition, nocardial infections can be difficult to recognize, resulting in underestimation of the true incidence of nocardiosis. Presumed nosocomial nocardiosis has been reported.

Cutaneous nocardiosis usually is an infection of immunocompetent hosts. *N. brasiliensis* is isolated from approximately 80 percent of cases of primary cutaneous or subcutaneous nocardiosis.[53] Traumatic implantation of *Nocardia* into deep subcutaneous tissue may result in an indolent condition termed *actinomycotic mycetoma* (to distinguish it from the eumycotic mycetomas caused by true fungi). Mycetoma is a chronic, progressive infection that can extend to underlying bone. Actinomycotic mycetomas usually involve the lower extremities or hands and typically are caused by *Actinomadura* spp., non–acid-fast aerobic actinomycetes, or *Streptomyces* spp. Actinomycotic mycetomas caused by *Nocardia* frequently involve the chest and back. Mycetomas, the cause of which may be *N. brasiliensis*, have been described in Mexican and

South American field workers. This *Nocardia* spp. also has been documented as an opportunistic pathogen prevalent in Florida, with a predilection for diabetics.[56]

In addition to the classic mycetoma, traumatic introduction of *Nocardia* spp. from soil may result in wound infections that occur after a more acute or subacute course. The organism can be introduced into the skin by tick[37] and other insect bites[20,47] or by a cat scratch[21,52] and result in cellulitis, pustules, or pyoderma; these conditions occasionally disseminate in immunocompromised individuals.[30,31] Posttraumatic endophthalmitis[22] and poststernotomy mediastinitis[61,69] have been described.

Nocardiosis previously was considered a rare disease in humans; however, it is being recognized more frequently.[44] It has been diagnosed in individuals of all ages (4 weeks to 82 years of age). Except in cases of cutaneous nocardiosis, almost all patients have one or more severe underlying diseases (e.g., lupus erythematosus, asthma, glomerulonephritis, ulcerative colitis, bronchiectasis, tuberculosis, rheumatoid arthritis, diabetes mellitus, or sarcoidosis) or are immunocompromised (e.g., steroids, organ transplantation, chemotherapy, chronic granulomatous disease, or HIV infection). Patients with lymphoreticular neoplasms and transplant recipients seem to be particularly at risk. In addition, the risk for acquiring infection is increased in patients with chronic pulmonary disease and in any patient who is receiving long-term corticosteroid treatment. Infliximab treatment also has been associated with the development of nocardial infection.[67] This catalase-positive organism is one of the five main pathogens seen in children with chronic granulomatous disease and can cause severe infection.[27] Although *Nocardia* is not a surveillance organism for acquired immunodeficiency syndrome (AIDS), patients with AIDS may contract nocardiosis.[28] In a review of all cases (n = 25) of nocardiosis at an urban adult teaching hospital from 1999 to 2004, 76 percent of patients with nocardiosis also were infected with HIV; all had CD4 counts less than 100 cells/mm^3.[13]

In a retrospective review of cases of pediatric nocardiosis admitted to Arkansas Children's Hospital, five children with nocardiosis were identified from more than 100,000 admissions in a 10-year period. Four of the five patients had received a transplant within the previous year. Three of these patients had pulmonary disease caused by *N. asteroides*, and one transplant recipient had skin involvement with *N. brasiliensis* at a central venous line site. One immunocompetent 5-year-old boy contracted central nervous system infection caused by *N. asteroides* 2 months after incurring a penetrating brain injury.[25]

N. farcinica is a medically important pathogen.[55,66] In a series of 200 cases of *Nocardia* infection reported by Wallace and associates,[66] the isolates designated *N. farcinica* were from patients with severe illness, 56 percent of whom had disseminated infections. *N. farcinica* isolates were characterized by increased resistance to antimicrobials, specifically third-generation cephalosporins. Evidence from mouse models indicates that *N. farcinica* seems to be more virulent than are other *Nocardia* spp.[18] Because the various species of *Nocardia* seem to have different pathogenicities, determination of the infecting species of *Nocardia* is important.

The immune response to *Nocardia* is multifaceted.[2] Neutrophils are mobilized to the site of infection and are the predominant cell type found in lesions. Neutrophils only inhibit the organisms, however, and limit the spread of infection until an adequate cell-mediated immune response develops or effective antimicrobial agents are administered. Immune T cells are vital in clearing *Nocardia* from the lung and preventing dissemination; many predisposing conditions for nocardiosis involve inadequate cell-mediated immunity. Activated macrophages induce cytotoxic T cells effective against *N. asteroides*. *Nocardia* may survive inside neutrophils and macrophages by inhibiting phagosome–lysosome fusion and production of catalase and superoxide dismutase, which inactivate the myeloperoxidase system. *Nocardia* organisms exhibit a differential ability to evade phagosome–lysosome fusion that depends on their state of growth, possibly related to specific cell wall mycolic acids detected only in log-phase cells.[3] Differential cell wall characteristics also may influence the ability of *Nocardia* organisms to exhibit specific organ tropism (e.g., the brain).[7] Antibody may play a role in host defense through enhancement of macrophage activities. Although antecedent conditions of nocardiosis frequently involve dysfunctional cellular immunity, other preconditions include neutrophil disorders (chronic granulomatous disease) and immunoglobulin disorders.

PATHOLOGY

The lesions of nocardiosis are suppurative, whether in the lung or in subcutaneous tissue, and they involve primarily proliferation of neutrophils rather than formation of granulomas. Pulmonary nocardiosis in immunocompetent patients often resembles pulmonary actinomycosis in that it results in a chronic localized pneumonia that often abuts the pleura.[26] Indolent progressive fibrosis resembling fibronodular tuberculosis may occur. Nocardiosis is more aggressive in immunocompromised patients and is manifested as multifocal necrotizing pneumonia with confluent abscess formation. *Nocardia* spp. tend to invade the pleura and chest wall, with tissue planes disregarded in the process. Little evidence of encapsulation is characteristic of all organs invaded and probably accounts for the ready dissemination of organisms from the initial pulmonary focus.

Nocardia spp. appear as delicate, beaded, branching filaments in tissue stained with Gram stain or modified acid-fast stain (Fig. 99-1). *Nocardia* spp. are invisible in hematoxylin and eosin preparations or in sections stained with periodic acid–Schiff for fungi. Methenamine silver preparations sometimes detect tissue organisms; overstaining with silver enhances visualization.

CLINICAL MANIFESTATIONS AND DIAGNOSIS

The most common manifestation of nocardiosis (>40% of all cases) in the United States is pulmonary disease in a patient with underlying immunosuppression.[16,49,51] Infection may remain localized to the lung or may

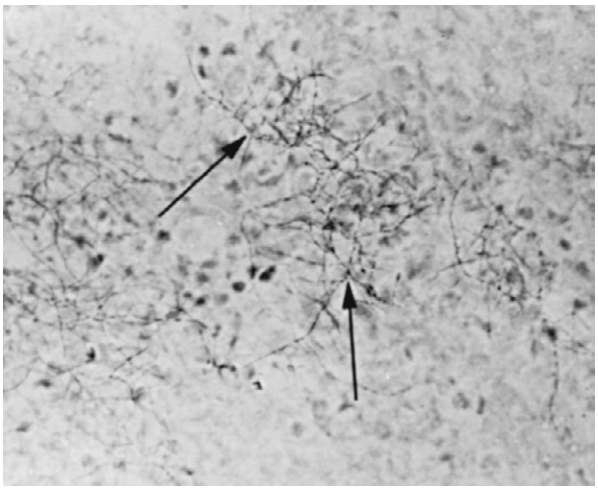

FIGURE 99-1 ■ Appearance of *Nocardia asteroides* and *Nocardia brasiliensis (arrows)* in a properly decolorized acid-fast smear. Organisms appear as fragmented bacilli with stain concentrated in a beaded fashion along portions of the filaments (×160).

disseminate hematogenously to the central nervous system and skin and, more rarely, to almost any organ in the body. In high-risk patients, the diagnosis should be suspected when central nervous system manifestations, particularly signs of a brain tumor, abscess, or soft tissue swelling, develop in conjunction with a current or recent subacute or chronic pulmonary infection. Although sulfonamides are the most effective drugs for treatment of nocardiosis, invasive infections have been described in immunocompromised individuals receiving oral trimethoprim-sulfamethoxazole (TMP-SMX) as prophylaxis for *Pneumocystis* pneumonia[14,63] and in patients with chronic granulomatous disease receiving TMP-SMX prophylaxis.[57]

Clinical manifestations of pulmonary nocardiosis are not specific and include anorexia, weight loss, productive cough, pleural pain, dyspnea, and, occasionally, hemoptysis.[16] Untreated pulmonary nocardiosis usually runs a chronic course, similar to that of tuberculosis, but it also may clear spontaneously and obscure the source of subsequent metastatic infection. The diverse clinical and radiographic manifestations, including acute bronchopneumonia, lobar pneumonia or necrotizing pneumonia with single or multiple abscesses, and pleural empyema, may mimic more common pulmonary infections, such as mycobacterial, staphylococcal, and fungal pneumonia. For example, common computed tomography (CT) findings of pulmonary nocardiosis include solitary or more often multiple lung nodules and lung consolidation with or without cavitation that are nonspecific.[32] Pleural involvement (i.e., thickening or effusion) and chest wall extension are seen less commonly on CT.[32] Normal hosts or patients with only slightly impaired host defenses may have only mild respiratory tract symptoms of several months' duration.[19]

The central nervous system is the most common secondary site of infection, and such infection occurs in one third of patients.[2] Most experts recommend performing routine cranial CT for patients with pulmonary nocardiosis even when they are asymptomatic because of the frequency of involvement of the central nervous system. Brain abscesses are the most common manifestation; meningitis is reported less frequently.[9,14]

Traumatic inoculation may result in localized disease manifested as cellulitis, subcutaneous abscess, or a lymphocutaneous syndrome in which one or more cutaneous nodules are associated with regional adenopathy or suppurative lymphadenitis.[36] Over a 5-year period, 31 cases of localized cutaneous nocardiosis with *N. brasiliensis* were identified in immunocompetent children in South Texas.[21] *Nocardia* spp. may cause cervicofacial disease and cervical adenitis in children.[35] Cutaneous nocardiosis can be mistaken for sporotrichosis, tularemia, mycobacterial infection, or superficial cellulitis.

Other reported clinical manifestations include tracheitis, peritonitis, iliopsoas abscess, hematogenous endophthalmitis, keratitis, endocarditis, mediastinitis, septic arthritis, ventriculitis and choroid plexitis, and osteomyelitis.[16,34,42]

Bacteremic nocardiosis is reported rarely and usually is associated with endovascular foreign bodies in patients receiving long-term steroid therapy.[33] Catheter-associated *Nocardia* bacteremia also has been reported in a child with acute lymphoblastic leukemia (ALL) on maintenance chemotherapy.[68] In a report of six cases of central venous catheter–related *Nocardia* bacteremia, four cases were in children 18 years or younger.[20] All six patients received antibiotic therapy; the catheter was retained in three of the six. Relapse occurred in one of the three patients in whom the catheter was not removed.[20]

The diagnosis of nocardiosis is established in one third of cases by sputum analysis and culture. Humoral methods used to diagnose nocardiosis generally lack specificity because of the high degree of serologic cross reactivity that occurs among *Nocardia* spp. and between *Mycobacterium* and *Streptomyces* spp.[8] The probable decreased sensitivity for detection of an antibody response in immunocompromised individuals also likely is a limitation of serologic testing. It is important to notify the laboratory when nocardial infection is suspected so that steps can be taken to optimize its recognition and recovery. Although *Nocardia* colonies usually are evident by 3 to 5 days after culture, prolonged incubation may be necessary.

Although *Nocardia* spp. sometimes can be respiratory saprotrophs, withholding therapy from immunocompromised patients when cultures repeatedly are positive is difficult. Bronchoalveolar lavage or lung biopsy may be required to establish the diagnosis. Although these procedures are invasive, given the overlap in symptoms with other infectious and neoplastic processes and potential for rapid progression of disease, bronchoalveolar lavage or lung biopsy should be considered early in immunodeficient patients to establish a definitive diagnosis. The demonstration of tissue invasion confirms active infection.

Tentative species identification of most *Nocardia* can be made by biochemical methods, chemotaxonomic methods, or antimicrobial susceptibility patterns. However, only *N. brasiliensis*, *N. farcinica*, and *N. pseudobrasiliensis* can be identified reliably by these methods.[11] Molecular confirmation (e.g., DNA probes, PCR and PCR-RFLP molecular analyses, DNA sequencing, pyrosequencing, and ribotyping) of species identification is

the method of choice. Molecular testing by hsp 65 PCR and 16S restriction enzyme analysis identifies more than 90 percent of currently recognized clinical species.[11] PCR assays for identifying *Nocardia* spp. are not yet commercially available.

TREATMENT AND PROGNOSIS

Sulfonamides have been the best-studied drugs for the treatment of nocardiosis.[54,57,65] In the United States, TMP-SMX, the only intravenous sulfonamide formulation available, has been the agent of choice for treatment of nocardiosis. TMP-SMX has been used successfully at doses of 15 mg/kg/day of TMP and 75 mg/kg/day of SMX, either parenterally or orally.[57,62,64] Few cases have been reported of failure of TMP-SMX therapy for the treatment of nocardiosis.[1,24,48,58] TMP-SMX resistance among *Nocardia* spp. has been documented. However, a study of sulfonamide resistance in isolates of *Nocardia* spp. from six major laboratories across the United States from 2005 and 2011 showed only 2 percent resistance to TMP-SMX or sulfamethoxazole.[10] The 2011 Clinical and Laboratory Standards Institute document on susceptibility testing of mycobacteria and other aerobic actinomycetes recommends that all clinically significant isolates of *Nocardia* spp. be tested for susceptibility to multiple antimicrobial agents.[15] No published prospective clinical trials have correlated in vitro susceptibilities with clinical outcome for nocardiosis. Susceptibility testing is the best available guide for selecting appropriate combination therapy and alternative therapies if sulfonamides fail or cannot be given because of patient intolerance or allergy.

No prospective randomized trials have established the optimal antimicrobial therapy for nocardiosis. Recommended antimicrobial therapy regimens are based on efficacy in animal models of nocardiosis and in vitro susceptibility and synergy testing.[11]

TMP-SMX as a single agent usually is sufficient for patients with cutaneous nocardiosis. Many infectious disease specialists administer empirical combination drug therapy, consisting of TMP-SMX, amikacin, and a carbapenem or third-generation cephalosporin, for patients with severe disease, central nervous system nocardiosis, or disseminated disease, and for immunosuppressed patients because of documented treatment failure and mortality associated with sulfonamide monotherapy in those settings.[39,44,58] Currently, no *Nocardia* spp. are resistant to amikacin and a β-lactam in vitro; the use of both (plus TMP-SMX) ensures that all clinical isolates would be susceptible to at least one other drug.[11] Antimicrobial therapy can be narrowed to two agents when results of in vitro susceptibility testing become available. Because antimicrobial resistance patterns vary by species, clinical isolates identified initially as *Nocardia* should be speciated further using molecular techniques. Children allergic to sulfonamides can be treated with amikacin and a carbapenem or third-generation cephalosporin.

The oxazolidinone linezolid is the first antimicrobial shown to be active in vitro against all clinically important *Nocardia* spp.[12] Linezolid, which is available for oral administration, has been used successfully to treat 11 patients with nocardiosis.[28,43] In one study, linezolid-associated hematologic toxicity was seen in three of six patients.[43] Peripheral neuropathy has also been observed in individuals with nocardiosis treated with linezolid.[29] Myelosuppression occurs more commonly in individuals receiving linezolid for longer than 2 weeks. Therefore the use of linezolid for nocardial infection is limited by the prolonged treatment required for nocardiosis.

Clinical experience with minocycline, the tetracycline with best in vitro activity against *Nocardia*, has been encouraging. Amoxicillin-clavulanic acid has been effective in treating infections with *N. brasiliensis*, commonly a β-lactamase producer.[64] However, a case of acquired resistance to β-lactamase inhibitor antibiotics has been reported.[59] Other species with susceptibility to amoxicillin-clavulanic acid include *N. abscessus* and *N. farcinica*, whereas isolates of the *N. nova* complex are ampicillin susceptible or intermediate but resistant to amoxicillin-clavulanic acid.[11] Susceptibility of some *Nocardia* spp. to fluoroquinolones has been reported. In an in vitro study gatifloxacin and moxifloxacin generally were more active than was ciprofloxacin against isolates of *Nocardia*, especially against isolates of *N. farcinica* and *N. brasiliensis*.[11] A patient with disseminated *N. farcinica* nocardiosis initially treated with aspiration, TMP-SMX, and imipenem had recurrence of brain lesions after a change to monotherapy with moxifloxacin despite in vitro susceptibility to moxifloxacin.[17]

Clinical improvement typically is seen within 7 to 10 days after initiation of appropriate therapy.[41] Patients with unchanged or worsening symptoms after receiving 2 weeks of therapy should be reassessed thoroughly. Intravenous antibiotic therapy may be changed to an oral regimen after at least 3 to 6 weeks if clinical improvement is noted. The variable and chronic course of nocardiosis precludes determining precise therapeutic end points. Metastatic lesions can appear during or after an otherwise effective course of sulfonamide therapy with maintenance of the recommended 100- to 150-μg/mL level in serum or plasma. Because the tendency for relapse or the late appearance of metastatic disease is a concern, therapy often is continued for many months. Patients with mycetomas may require 6 to 12 months of therapy; localized cutaneous nocardiosis usually is treated for 3 to 6 months. Therapy for 6 to 12 months is suggested for patients with serious infection. Therapy for 12 months is indicated for patients with central nervous system nocardiosis and for immunocompromised patients.[38] Patients with central nervous system disease should be monitored with serial neuroimaging studies. Patients with AIDS probably should be treated with suppressive therapy indefinitely.

Surgical drainage of abscesses is important because metastatic abscesses can appear in the face of adequate therapy until surgical drainage is achieved.[23] Select brain abscesses may respond to antimicrobial treatment without surgery.[40]

Despite specific therapy, the overall mortality rate is 25 to 40 percent.[50,51] Most reported cases involving dissemination to the central nervous system have been fatal.[50] Disseminated nocardiosis has a poor prognosis, with a mortality rate of greater than 85 percent in

immunocompromised hosts.[4] Factors associated with increased mortality rates in one reported patient series were treatment with corticosteroids or antineoplastic agents, underlying Cushing disease, disseminated disease involving two or more noncontiguous organs or the central nervous system, and the presence of symptoms for less than 3 weeks before initial evaluation.[50]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Ameen M, Arenas R, Vásquez del Mercado E, et al. Efficacy of imipenem therapy for Nocardia actinomycetomas refractory to sulfonamides. *J Am Acad Dermatol* 2010;**62**:239–46.
10. Brown-Elliott BA, Biehle J, Conville PS, et al. Sulfonamide resistance in isolates of Nocardia spp. from a US multicenter survey. *J Clin Microbiol* 2012;**50**:670.
14. Chow E, Moore T, Deville J, Nielsen K. Nocardia asteroides brain abscesses and meningitis in an immunocompromised 10-year-old child. *Scand J Infect Dis* 2005;**37**:511–13.
24. Geiseler PJ, Check F, Lamothe F, et al. Failure of trimethoprim/sulfamethoxazole in invasive Nocardia asteroides infection. *Arch Intern Med* 1979;**139**:355–6.
29. Jodlowski TZ, Melnychuk I, Conry J. Linezolid for the treatment of Nocardia spp. infections. *Ann Pharmacother* 2007;**41**:1694–8.
32. Kanne JP, Yandow DR, Mohammed T-LH, et al. CT findings of pulmonary nocardiosis. *AJR Am J Roentgenol* 2011;**197**:W266–W272.
34. Lalitha P. Nocardia keratitis. *Curr Opin Opthalmol* 2009;**20**:318–23.
42. Mongkolrattanothai K, Ramakrishnan S, Zagardo M, Gray B. Ventriculitis and choroid plexitis caused by multidrug-resistant Nocardia pseudobrasiliensis. *Pediatr Infect Dis J* 2008;**27**:666–8.
48. Overkamp D, Waldmann B, Lins T, et al. Successful treatment of brain abscess caused by Nocardia in an immunocompromised patient after failure of co-trimoxazole. *Infection* 1992;**20**:365–6.
68. Watson ME, Estabrook MM, Burnham C-AD. Catheter-associated Nocardia higoensis bacteremia in a child with acute lymphocytic leukemia. *J Clin Microbiol* 2011;**49**:469–71.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 100

Corynebacterium and Rhodococcus

Randall G. Fisher

The gram-positive rods encompass a vast number of species. They are widespread in the environment and are part of the normal flora of animals and humans. The focus of this chapter is on gram-positive bacilli seldom encountered as pathogens in healthy individuals. Many of these organisms show increasing prominence as causes of disease in immunocompromised patients.[13] Bacteria from the genera *Corynebacterium* (other than *Corynebacterium diphtheriae*) and *Rhodococcus* are discussed.

CORYNEBACTERIUM

The genus *Corynebacterium* comprises a wide number of organisms possessing little pathogenic potential, with some notable exceptions. The most infamous member of this genus, *C. diphtheriae* (see Chapter 90), is a cause of a potentially lethal pharyngeal infection with systemic manifestations. *Corynebacterium jeikeium* (formerly *Corynebacterium* group JK) can be a major nosocomial agent of bacteremia and endocarditis; other species commonly are associated with infections in animals and rarely cause invasive infection in humans.[83,119] For an exhaustive consideration of coryneform bacteria, the reader is referred to the review by Funke and colleagues.[50]

BACTERIOLOGY

Corynebacterium spp. derive their name from their club-like shape. Because of their resemblance to *C. diphtheriae*, they are included in the heterogeneous group of diphtheroids. Snapping division produces the angular and palisade arrangement of cells responsible for their characteristic gram-positive, "Chinese letters" microscopic appearance.[27] Organisms are facultatively anaerobic or aerobic and do not produce spores. They are nonmotile and catalase positive and contain mycolic acid in their cell walls. Clinically relevant species include *C. diphtheriae*,

REFERENCES

1. Ameen M, Arenas R, Vásquez del Mercado E, et al. Efficacy of imipenem therapy for *Nocardia actinomycetomas* refractory to sulfonamides. *J Am Acad Dermatol* 2010;**62**:239–46.
2. Beaman BL, Beaman L. *Nocardia* species: host-parasite relationships. *Clin Microbiol Rev* 1994;**7**:213–64.
3. Beaman BL, Moring SE. Relationship among cell wall composition, state of growth, and virulence of *Nocardia asteroides* GUH-2. *Infect Immun* 1988;**56**:557–63.
4. Beaman BL, Burnside J, Edwards B, Causey W. Nocardial infections in the United States, 1972-1974. *J Infect Dis* 1976;**134**: 286–9.
5. Beckmeyer WJ. Nocardiosis: report of a successfully treated case of cutaneous granuloma. *Pediatrics* 1959;**23**:33–9.
6. Berd D. Laboratory identification of clinically important aerobic actinomycetes. *Appl Microbiol* 1973;**25**:665–81.
7. Black CM, Paliescheskey M, Beaman BL, et al. Acidification of phagosome in murine macrophages: blockage by *Nocardia asteroides*. *J Infect Dis* 1986;**154**:952–8.
8. Boiron P, Stynen D. Immunodiagnosis of nocardiosis. *Gene* 1992;**115**:219–22.
9. Bross JE, Gordon G. Nocardial meningitis case reports and review. *Rev Infect Dis* 1991;**3**:160–5.
10. Brown-Elliott BA, Biehle J, Conville PS, et al. Sulfonamide resistance in isolates of *Nocardia* spp. from a US multicenter survey. *J Clin Microbiol* 2012;**50**:670.
11. Brown-Elliott BA, Brown JM, Conville PS, Wallace RJ Jr. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. *Clin Microbiol Rev* 2006;**19**:259–82.
12. Brown-Elliott BA, Ward SC, Crist CJ, et al. In vitro activities of linezolid against multiple *Nocardia* species. *Antimicrob Agents Chemother* 2001;**45**:1295–7.
13. Castro JG, Espinoza L. *Nocardia* species infections in a large county hospital in Miami: 6 years experience. *J Infect* 2006;**54**: 358–61.
14. Chow E, Moore T, Deville J, Nielsen K. *Nocardia asteroides* brain abscesses and meningitis in an immunocompromised 10-year-old child. *Scand J Infect Dis* 2005;**37**:511–13.
15. Clinical and Laboratory Standards Institute. *Susceptibility Testing of Mycobacteria, Nocardiae, and Other Aerobic Actinomycetes*. Approved Standard: 2nd ed. CLSI document M24-A2. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.
16. Curry WA. Human nocardiosis: a clinical review with selected case reports. *Arch Intern Med* 1980;**140**:818–26.
17. Dahan K, El Kabbaj D, Venditto M, et al. Intracranial *Nocardia* recurrence during fluorinated quinolones therapy. *Transpl Infect Dis* 2006;**8**:161–5.
18. Desmond EP, Flores M. Mouse pathogenicity studies of *Nocardia asteroides* complex species and clinical correlation with human isolates. *FEMS Microbiol Lett* 1993;**110**:281–4.
19. Feigin DS. Nocardiosis of the lung: chest radiographic findings in 21 cases. *Radiology* 1986;**159**:9–14.
20. Feng Y-H, Huang W-T, Tsao C-J. Venous access port-related *Nocardia* bacteremia with successful short-term antibiotics treatment. *J Chin Med Assoc* 2004;**67**:416–8.
21. Fergie JE, Purcell K. Nocardiosis in South Texas children. *Pediatr Infect Dis J* 2001;**20**:711–14.
22. Ferry AP, Font RL, Weinberg RS, et al. Nocardial endophthalmitis: report of two cases studied histopathologically. *Br J Ophthalmol* 1988;**72**:55–61.
23. Frazier AR, Rosenow EC III, Roberts GD. Nocardiosis: a review of 25 cases occurring during 24 months. *Mayo Clin Proc* 1975;**50**: 657–663.
24. Geiseler PJ, Check F, Lamothe F, et al. Failure of trimethoprim/sulfamethoxazole in invasive *Nocardia asteroides* infection. *Arch Intern Med* 1979;**139**:355–6.
25. Harik NK, Jacobs RF, Darville T. Nocardiosis in children: five cases. *J Invest Med* 2001;**49**:108.
26. Heffner JE. Pleuropulmonary manifestations of actinomycosis and nocardiosis. *Semin Respir Infect* 1988;**3**:352–61.
27. Idriss ZH, Cunningham RJ, Wilfert CM. Nocardiosis in children. *Pediatrics* 1975;**55**:479–84.
28. Javalry K, Horowitz HW, Wormser GP. Nocardiosis in patients with human immunodeficiency virus infection: report of 2 cases and review of the literature. *Medicine (Baltimore)* 1980;**71**:128–38.
29. Jodlowski TZ, Melnychuk I, Conry J. Linezolid for the treatment of *Nocardia* spp. infections. *Ann Pharmacother* 2007;**41**:1694–8.
30. Kahn FW, Gornick CC, Tofte RW. Primary cutaneous *Nocardia asteroides* infection with dissemination. *Am J Med* 1981;**70**: 859–63.
31. Kalb RE, Kaplan MH, Grosman ME. Cutaneous nocardiosis. *J Am Acad Dermatol* 1985;**13**:125–33.
32. Kanne JP, Yandow DR, Mohammed T-LH, et al. CT findings of pulmonary nocardiosis. *AJR Am J Roentgenol* 2011;**197**: W266–W272.
33. Kontoyiannis DP, Ruoff K, Hooper DC. *Nocardia* bacteremia: report of 4 cases and review of the literature. *Medicine (Baltimore)* 1998;**77**:255–67.
34. Lalitha P. Nocardia keratitis. *Curr Opin Opthalmol* 2009;**20**:318–23.
35. Lampe RM, Baker CJ, Septimus EJ, et al. Cervicofacial nocardiosis in children. *J Pediatr* 1981;**99**:593–5.
36. Law BL, Marks MI. Pediatric nocardiosis. *Pediatrics* 1982;**70**: 560–4.
37. Leggiadro RJ, Miller RB. Cutaneous nocardiosis presenting as a tick-borne infection. *Pediatr Infect Dis J* 1987;**6**:421–2.
38. Lerner PI. Nocardiosis. *Clin Infect Dis* 1996;**22**:891–903.
39. Long PF. A retrospective study of *Nocardia* infections associated with the acquired immune deficiency syndrome (AIDS). *Infection* 1994;**22**:362–4.
40. Mamelak AN, Obana WG, Flaherty JF, et al. Nocardial brain abscess: treatment strategies and factors influencing outcome. *Neurosurgery* 1994;**35**:622–31.
41. McNeil MM, Brown JM. The medically important aerobic actinomycetes: epidemiology and microbiology. *Clin Microbiol Rev* 1994;**7**:357–417.
42. Mongkolrattanothai K, Ramakrishnan S, Zagardo M, Gray B. Ventriculitis and choroid plexitis caused by multidrug-resistant *Nocardia pseudobrasiliensis*. *Pediatr Infect Dis J* 2008;**27**:666–8.
43. Moylett EH, Pacheco SE, Brown-Elliott BA, et al. Clinical experience with linezolid for the treatment of *Nocardia* infection. *Clin Infect Dis* 2003;**36**:313–18.
44. Murray JF, Finegold SM, Froman S, et al. The changing spectrum of nocardiosis. *Am Rev Respir Dis* 1961;**83**:315–30.
45. Murray PR, Neeren RL, Niles AC. Effect of decontamination procedures on recovery of *Nocardia* spp. *J Clin Microbiol* 1987;**25**: 2010–11.
46. Reference deleted in proofs.
47. O'Conner PT, Dire DJ. Cutaneous nocardiosis associated with insect bites. *Cutis* 1992;**50**:301–2.
48. Overkamp D, Waldmann B, Lins T, et al. Successful treatment of brain abscess caused by *Nocardia* in an immunocompromised patient after failure of co-trimoxazole. *Infection* 1992;**20**:365–6.
49. Palmer DL, Harvey RL, Wheeler JK. Diagnostic and therapeutic considerations in *Nocardia asteroides* infection. *Medicine (Baltimore)* 1974;**53**:391–401.
50. Present CA, Wiernik PH, Serpick AA. Factors affecting survival in nocardiosis. *Am Rev Respir Dis* 1973;**108**:1444–51.
51. Rosett W, Hodges GR. Recent experiences with nocardial infections. *Am J Med Sci* 1978;**276**:279–85.
52. Sachs MK. Lymphocutaneous *Nocardia brasiliensis* infection acquired from a cat scratch: case report and review. *Clin Infect Dis* 1992;**15**:710–11.
53. Satterwhite TK, Wallace RJ Jr. Primary cutaneous nocardiosis. *JAMA* 1979;**242**:333–6.
54. Schiff TA, McNeil MM, Brown JM. Cutaneous *Nocardia farcinica* infection in a nonimmunocompromised patient: case report and review. *Clin Infect Dis* 1993;**16**:756–60.
55. Shetty AK, Arvin AM, Gutierrez KM. *Nocardia farcinica* pneumonia in chronic granulomatous disease. *Pediatrics* 1999;**104**:961–4.
56. Smego RA Jr, Gallis HA. The clinical spectrum of *Nocardia brasiliensis* infection in the United States. *Rev Infect Dis* 1984;**6**:164–80.
57. Smego RA Jr, Moeller MG, Gallis HA. Trimethoprim-sulfamethoxazole therapy for *Nocardia* infections. *Arch Intern Med* 1983;**143**:711–18.
58. Stamm AM, McFall DW, Dismukes WE. Failure of sulfonamides and trimethoprim in the treatment of nocardiosis. *Arch Intern Med* 1983;**143**:383–5.
59. Steingrube VA, Wallace RJ Jr, Brown BA, et al. Acquired resistance of *Nocardia brasiliensis* to clavulanic acid related to a change in

β-lactamase following therapy with amoxicillin-clavulanic acid. *Antimicrob Agents Chemother* 1991;**35**:524–8.
60. Stropnik Z. Isolation of *Nocardia asteroides* from human skin. *Sabouraudia* 1965;**4**:41–4.
61. Thaler F, Gotainer B, Teodori G, et al. Mediastinitis due to *Nocardia asteroides* after cardiac transplantation. *Intensive Care Med* 1992;**18**:127–8.
62. van Burik J-A, Hackman RC, Nadeem SQ, et al. Nocardiosis after bone marrow transplantation: a retrospective study. *Clin Infect Dis* 1997;**22**:1154–60.
63. Wallace RJ Jr, Steele LC. Susceptibility testing on *Nocardia asteroides* for the clinical laboratory. *Diagn Microbiol Infect Dis* 1988;**9**: 155–66.
64. Wallace RJ Jr, Nash DR, Johnson WK, et al. β-Lactam resistance in *Nocardia brasiliensis* is mediated by β-lactamase and reversed in the presence of clavulanic acid. *J Infect Dis* 1987;**156**:959–66.
65. Wallace RJ, Septimus EJ Jr, Williams TW, et al. Use of trimethoprim-sulfamethoxazole for treatment of infections due to *Nocardia*. *Rev Infect Dis* 1982;**4**:315–25.
66. Wallace RJ Jr, Tsukamura M, Brown BA, et al. Cefotaxime-resistant *Nocardia asteroides* strains are isolates of the controversial species *Nocardia farcinica*. *J Clin Microbiol* 1990;**28**:2726–32.
67. Wallis RS, Broder MS, Wong JY, et al. Granulomatous infectious diseases associated with tumor necrosis factor antagonists. *Clin Infect Dis* 2004;**38**:1261–5.
68. Watson ME, Estabrook MM, Burnham CA. Catheter-associated *Nocardia higoensis* bacteremia in a child with acute lymphocytic leukemia. *J Clin Microbiol* 2011;**49**:469–71.
69. Yew WW, Wong PC, Kwan SYL, et al. Two cases of *Nocardia asteroides* sternotomy infection treated with ofloxacin and a review of other active antimicrobial agents. *J Infect Dis* 1991;**23**: 297–302.

C. jeikeium, *C. pseudotuberculosis*, *C. xerosis*, *C. amycolatum*, *C. pseudodiphtheriticum*, *C. minutissimum*, *C. striatum*, *C. ulcerans*, and *C. urealyticum*. Species can be differentiated according to biochemical tests and fermentation of sugars.[27,50,115]

EPIDEMIOLOGY

Corynebacterium spp. can be part of the normal skin and upper respiratory tract flora and commonly colonize hospitalized patients. Nosocomial acquisition of *C. jeikeium* has been characterized most completely. Thirty-five percent of hospitalized patients may be colonized with *C. jeikeium*, and the organism may be isolated at the time of admission.[52] The skin, groin, and rectum are common sites of recovery of organisms. Wounds and suppurative sites quickly become colonized.[52] Longer duration of hospitalization, receipt of broad-spectrum antimicrobial agents, and impaired skin integrity are risk factors for invasive infection. Hospital personnel caring for oncology patients have higher rates of hand colonization with pathogenic *Corynebacterium* spp. than do nurses caring for dermatology patients.[66] Transmission from patient to patient in the hospital environment can occur, and selective antibiotic pressure has been shown to augment colonization with *Corynebacterium* spp.[81,95,154] Outbreaks of bacteremic infection have been reported in hematology wards,[112,132] and DNA restriction fragment analysis and hybridization techniques have been used to document spread.[81,109]

PATHOPHYSIOLOGY

C. jeikeium and *C. urealyticum* are lipophilic, which may account for their ability to proliferate on skin that has a higher lipid content; organisms have been isolated from sebum-filled eccrine gland biopsy specimens in an adolescent with malignancy.[71] Breach of the integrity of the skin is an important risk factor for acquisition of local infection and bacteremia with *Corynebacterium*. Plastic intravascular catheters increase the risk for infection.[113] Certain isolates of *C. pseudotuberculosis* and *C. ulcerans* can express diphtheria-like toxins,[152] which may confer virulence.

Some *Corynebacterium* spp., notably *C. jeikeium* and *C. urealyticum*, commonly show resistance to penicillins, cephalosporins, aminoglycosides, erythromycin, and tetracycline.* Strains with a significant DNA relationship to the *C. jeikeium*–type strain show penicillin resistance.[114] Resistant organisms have been noted to have significantly thickened cell walls compared with susceptible strains, but the functional importance of this feature is unknown.[16]

CLINICAL MANIFESTATIONS

Systemic infections with *Corynebacterium* spp. generally are not clinically distinguishable from serious infections caused by other pathogens. Immunocompromised patients are at the highest risk for developing disease caused by *Corynebacterium*,[62,118] but infections also have been described in neonates and immunocompromised older children.[14,38,74] Risk factors include male gender, neutropenia, broad-spectrum antibiotic exposure, and prolonged hospital stay.[112,132] Catalase production may be responsible for the rare infection with non-JK *Corynebacterium* spp. in children with chronic granulomatous disease.[74] *Corynebacterium* spp. are responsible for approximately 9 percent of early-onset and 4 percent of late-onset prosthetic valve endocarditis.[151]

C. jeikeium is a pathogen of particular concern. In 1976, this agent first was described as a cause of serious infection in four patients, including an 11-year-old boy with a ventriculoatrial shunt.[62] The other three patients had hematologic malignancies and were neutropenic at the time of infection. Immunocompromised patients, particularly patients with leukemia, are vulnerable to bacteremia. These high-risk subjects may show a local inflammatory lesion at the site of infection or a disseminated, hemorrhagic, or necrotic papular exanthem.[105] Subcutaneous nodules also may be seen. *C. jeikeium* has been recovered from disseminated lesions in a 14-year-old boy with leukemia.[71] A literature review of 83 neutropenic patients (nearly all adults) with *C. jeikeium* sepsis found the following risk factors for development of infection: presence of a central venous catheter, being male, profound and prolonged neutropenia, and exposure to multiple antibiotics. Skin lesions were reported in 48 percent of patients and pulmonary infiltrates were found in 36 percent of patients. The overall case-fatality rate was 34 percent, but it was reduced to 5 percent in patients with recovery from neutropenia.[144]

Infections with *C. jeikeium* also have been reported after trauma, ventriculoperitoneal shunting procedures,[3,76] orthopedic procedures,[26] bone marrow aspiration,[38] and central venous catheter placement.[38] *C. jeikeium* now is recognized to be one of the most common causes of prosthetic valve endocarditis in adults.[102] Cutaneous findings of bacteremia so commonly observed in patients with cancer generally are absent in patients with endocarditis.[33] In a series of 38 patients with *C. jeikeium* endocarditis, 74 percent of patients had prosthetic valves. The mortality rate was 33 percent and was not altered by valve replacement surgery.[100] According to a newer study, cases of endocarditis caused by *C. jeikeium* are more likely to require valve replacement than are those caused by other *Corynebacterium* spp.[12] Exit-site infections in patients on continuous ambulatory peritoneal dialysis also may be caused by *Corynebacterium* spp.; in the largest series published to date, *C. striatum* caused almost twice as many of these infections as did *C. jeikeium*. Most infections are manageable without removal of the dialysis catheter.[123]

Although primarily a pathogen in farm animals, *C. pseudotuberculosis* can cause localized suppurative granulomatous lymphadenitis in humans[83,99]; almost all cases are associated with animal contact, particularly sheep and goats.[53] Presentation and histology may mimic the much more common cat-scratch disease, and Gram stain and culture are required to differentiate the two entities.[83]

*References 38, 52, 83, 95, 102, 115, 130.

Most cases have been reported from Australia.[107] The complete genome of an isolate taken from a 12-year-old girl with suppurative lymphadenitis has been sequenced.[142]

C. xerosis has been reported as a rare cause of endocarditis, arthritis, and ventriculoperitoneal shunt infection.[7,17] Most individuals with endocarditis have a prosthetic heart valve.[42,86] In one case an implantable cardioversion device became infected with the organism.[90] *C. xerosis* also can be a cause of eye infections in patients with superficial corneal foreign bodies; in one study of 101 patients, *C. xerosis* caused 3 of 15 culture-positive cases.[87] It also is an extremely rare cause of post-LASIK (laser-assisted in-situ keratomileusis) infectious keratitis.[37] Using chemical testing and molecular genetic investigation, Funke and colleagues[49] showed that many isolates tentatively identified as *C. xerosis* are actually *C. amycolatum*. *C. amycolatum* is much more likely to be multidrug-resistant than *C. xerosis*.[50] *C. amycolatum* is a comparatively common cause of bacteremia in children with cancer; of 12 cases of corynebacterial bacteremia reported from St. Jude Children's Research Hospital, 3 were caused by this species. In contrast to reports in adults, corynebacterial bacteremia in children usually started in the outpatient setting, happened in patients who were non-neutropenic, occurred relatively late in the course of cancer therapy, and had a relatively benign outcome.[1] In adults with cancer, *C. amycolatum* was often recovered from the upper urinary or lower respiratory tracts or found in surgical wounds or as an infection of ulcerating tumors.[93] A case of fatal sepsis in a premature neonate caused by *C. amycolatum* has been reported.[14]

C. pseudodiphtheriticum, a commensal of the oropharynx, can cause pneumonia, bronchitis, or tracheitis.[2,28,31] Lung disease usually occurs in the context of underlying cardiopulmonary pathology or immunocompromise.[60] Onset typically is acute, but fever may be noticeably absent.[88] One cystic fibrosis center in France reported that 10 children had exacerbations that appeared to be related to respiratory tract infection with *C. pseudodiphtheriticum;* matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry was used to identify the isolates.[15] At least 18 cases of *C. pseudodiphtheriticum* endocarditis, including three infections in children with congenital heart disease, have been reported.[101] Infection of allograft material is a common finding; native heart valves may be involved but usually in the context of preexisting lesions or intravenous drug abuse. In adults, endocarditis due to *C. pseudodiphtheriticum* has a predilection for men who have had a previous prosthetic valve replacement; infection with toxigenic strains appears to have a higher mortality rate.[12] Three cases of exudative pharyngitis with a pseudomembrane mimicking diphtheria, including one in a 4-year-old girl from Arkansas, have been reported to be caused by *C. pseudodiphtheriticum*.[70] In one case, a surface swab from a draining wound after arthroscopy was reported to harbor "normal skin flora" but joint fluid grew the same organism, which was finally identified as *C. pseudodiphtheriticum*. Treatment resulted in resolution of symptoms of septic arthritis.[75] Patients who are immunocompromised and have corneal epithelial defects can get severe eye infections with this organism.[82] Rarely, ulcerative skin lesions have been described due to *C. pseudodiphtheriticum*.[22]

C. minutissimum traditionally has been regarded as the cause of the mild cutaneous disease erythrasma, which is characterized by scaly, pruritic red-brown patches, usually occurring in the axilla or groin. Although *C. minutissimum* may play a role, erythrasma probably is a polymicrobial process.[50] *C. minutissimum* is recognized as a rare nosocomial infectious complication of malignancy and dialysis and has been implicated in a case of pyelonephritis in an 8-month-old infant with posterior urethral valves.[30] An adult who developed native-valve endocarditis caused by *C. minutissimum* has been reported.[6] A newer case report describes bacteremia and meningitis due to this organism in a patient with sickle-cell disease with an intravenously implanted right subclavian port.[32]

C. striatum has been recovered from purulent sputum of hospitalized patients and from infected central venous catheter sites.[125,148] It also is a cause of exit-site infections in patients on long-term ambulatory peritoneal dialysis.[123] It has been reported as a cause of fatal pulmonary infection and endocarditis.[81,92,119] *C. striatum* was responsible for a nosocomial outbreak among 14 patients over a 12-month period in a surgical intensive care unit. Endotracheal intubation for longer than 24 hours was the only risk factor found to predispose to infection.[19] A second intensive care unit outbreak was reported from Italy; in this series, one of the patients was a 16-year-old admitted for severe trauma that resulted in having a sepsis-like picture and three blood cultures positive for *C. striatum*. Despite therapy with teicoplanin and meropenem, the patient died.[68] *C. striatum* also has been reported as a cause of meningitis and ventriculoperitoneal shunt infections.[67,149] One case of septic arthritis that developed 20 days after a therapeutic knee aspiration has been reported.[124]

C. ulcerans derives its name from its association with ulcerative pharyngitis. Although it is more commonly a pathogen of nonhuman primates,[106] infection can occur in humans after contact with an animal or consumption of contaminated raw milk.[11,34] Toxigenic strains of *C. ulcerans* can produce a syndrome indistinguishable from that caused by toxigenic *C. diphtheriae*.[23,140] In the United Kingdom, domestic *C. ulcerans* is now a more common cause of diphtheria than *C. diphtheriae*.[147] Cases in the United States are decidedly rare; the case of an 80-year-old man in Idaho reported in 2010 was the first case in 5 years.[24] Skin lesions that exactly mimic cutaneous diphtheria, caused by diphtheria toxin–secreting strains of *C. ulcerans*, also have been reported.[146]

C. urealyticum (formerly *Corynebacterium* group D2) is a cause of alkaline-encrusted cystitis and pyelitis, primarily in the elderly.[44,130] A case series of four children with encrusted cystitis and pyelitis has been published. The mean age was 9 years (range, 4 to 13 years). Treatment was with prolonged antibiotics and endoscopic debulking. Cure was effected in three of the four patients; the other patient was a renal transplant recipient who lost the graft.[98] A prospective study of renal transplant recipients suggested that it is likely an underdiagnosed cause of obstructive uropathy in this group of patients; risk factors for infection were receipt of antibiotics in the previous

month, nephrostomy, and skin colonization with the organism.[85] Routine urine culture may miss the organism; it may grow with prolonged incubation of sheep's blood agar.[127] It is associated less commonly with infection at other sites and with bacteremia.[129] An 8-year-old boy with chemotherapy-induced neutropenia and a necrotic soft tissue infection of the scrotum caused by *C. urealyticum* has been reported.[120] Cure of bacteremia in a patient with obstruction due to prostate cancer was not effected until his percutaneous nephrostomy tube was removed.[25]

DIAGNOSIS

Diagnosis of *Corynebacterium* infection is based on isolation of the organism from clinical material. This organism commonly is accompanied by other pathogens. Similar to *Mycobacterium* and *Nocardia* spp., *Corynebacterium* organisms have mycolic acids in their cell wall. The chains are shorter, however, and the organisms are not acid fast. *Corynebacterium* spp. may be difficult to distinguish from some *Rhodococcus* spp.[27] Colonies of *C. jeikeium* may show a metallic sheen when grown on agar.[62] Growth of *C. jeikeium* is enhanced by the addition of lipids to the medium. Most *Corynebacterium* spp. can be differentiated quickly from each other by sugar fermentation, hydrolysis of urea, and reduction of nitrate.[139] Selective media containing kanamycin or trimethoprim-sulfamethoxazole have been useful in the recovery of multidrug-resistant strains of *Corynebacterium*.[61] The API Coryne system version 2.0 correctly identified 162 of 178 (91%) isolates in one study.[5] Polymerase chain reaction (PCR) testing and MALDI-TOF spectrometry are quite accurate for specific identification at the species level, but these tests are not yet widely available. Erythrasma usually is diagnosed by the typical coral red fluorescence under Wood lamp examination.

TREATMENT

Empirical therapy for infection must account for the frequency of infection with *C. jeikeium*, which often is multiresistant to antibiotics but is susceptible to vancomycin.[50] In some series of immunocompromised patients, this organism is the most common *Corynebacterium* spp. encountered.[131,150] Vancomycin is recommended for empirical therapy for suspected *Corynebacterium* infection until susceptibilities are known. Treatment can be changed to a penicillin or cephalosporin, if appropriate. In vitro, *C. jeikeium* is sensitive to linezolid,[55] and most strains also are sensitive to quinupristin-dalfopristin[121] and daptomycin.[54] A majority of strains is also sensitive to tigecycline, but experience with the antibiotic in childhood is sparse.[45] Linezolid is bacteriostatic against all species of *Corynebacterium*, and in vitro its activity is slower against *C. jeikeium* than it is against *C. striatum* and *C. amycolatum*.[55]

Two-drug therapy generally is recommended for treatment of *Corynebacterium* endocarditis; for gentamicin-susceptible strains, penicillin-gentamicin combinations have been shown to be synergistic, regardless of whether the strains are susceptible to penicillin.[102] Rarely, resistance to vancomycin is encountered. A woman with prosthetic valve endocarditis caused by a vancomycin-resistant *Corynebacterium* spp. was treated successfully with imipenem and ciprofloxacin.[10] Removal of infectious sources, such as central nervous system shunts and central venous catheters, may be required for cure. Scrupulous attention given to skin hygiene may reduce colonization of hospital personnel and the incidence of patient-to-patient transmission of pathogenic strains.[40,112] Management of toxigenic *C. ulcerans* infection is identical to that for infection caused by toxigenic *C. diphtheriae*, including the use of antitoxin.[140] Erythrasma usually responds to treatment with a macrolide.

RHODOCOCCUS

The genus *Rhodococcus* contains at least 15 species, of which *Rhodococcus equi* is the most clinically relevant to humans. Infections with other species are rare. Suppurative keratitis caused by *Rhodococcus ruber* after penetrating trauma has been described.[78] This organism derives its name from its role as a cause of pyogranulomatous pneumonia in young horses.[110] It has assumed a prominent role as a cause of human pulmonary disease in immunocompromised patients, particularly in patients with acquired immunodeficiency syndrome (AIDS).[43,63,125] For a comprehensive discussion of this organism, the reader is referred to the review by Cornish and Washington.[29]

BACTERIOLOGY

R. equi is a catalase-positive, urease-positive, oxidase-negative, gram-positive rod. The organism assumes a more coccoid morphology in solid media and a more bacillary form in liquid media. Its cell wall contains mycolic acid, rendering it acid fast when grown on Lowenstein-Jensen media and stained with Kinyoun stain.[56]

EPIDEMIOLOGY

R. equi is a soil organism, and its growth is enriched by the manure of herbivores. Despite the common occurrence of this pathogen as a cause of veterinary infections, exposure to animals apparently is not necessary for human infection to occur[110]; most reported human patients have not had farm or animal exposure.[63] Hospital outbreaks of infection associated with patient-to-patient transmission have not been reported. In a retrospective analysis of 24 cases of *R. equi* infection, however, six patients had shared

a hospital room with a patient with *R. equi* pneumonia, raising the possibility of nosocomial transmission.[8] Most patients diagnosed with *Rhodococcus* infection are immunocompromised; AIDS is the most common underlying diagnosis, but infection also has been reported in transplant patients[9] and rarely in patients believed to be immunocompetent.[143] In one case, infective endocarditis with severe complications including subarachnoid hemorrhage and subdural hematoma was reported in a patient with a normal immune workup.[94] On the other hand, a chronic, nonhealing ulcerative lesion caused by *R. equi* in a man believed to be immunocompetent led to a diagnostic workup that proved the cause to be a CD8 deficit.[36]

Chemotherapeutic agents such as fludarabine, which causes prolonged CD4 lymphocytopenia, can predispose to *R. equi* infection.[51] Receipt of high-dose corticosteroids[116] and alemtuzumab (an anti-CD52 monoclonal antibody) have also been reported as antecedents of infection.[97]

PATHOPHYSIOLOGY

The prominence of pulmonary infection suggests that the respiratory tract is a common portal of entry. After gaining access to the lower respiratory tract, organisms are taken up by alveolar macrophages; Mac-1 macrophage receptors and complement are required for binding.[64] The appearance of pyogranulomatous lesions is consistent with the role of *R. equi* as an intracellular parasite containing mycolic acid, a possible virulence factor in the cell wall.[57,111] Surface 15- and 17-kDa antigens expressed by an 85-kb plasmid seem to confer virulence in mice and foals,[134] and virulent strains seem to have an increased capacity for intracellular survival in macrophages.[65]

Most *R. equi* isolates from patients with AIDS express either the 15- to 17-kDa antigens or a 20-kDa antigen that seems to confer intermediate virulence. Most isolates from patients without AIDS express none of these antigens, however.[135] Other factors may play a role in promoting *Rhodococcus* disease in humans.[136] Death of parasitized macrophages may release enzymes, which contribute further to tissue damage. $CD4^+$ lymphocytes are essential for pulmonary clearance of *R. equi* in a mouse model,[73] which may help to explain the high risk for infection associated with cellular immunodeficiency.

CLINICAL MANIFESTATIONS

Infection typically manifests as a subacute pneumonia developing over several weeks. Symptoms such as cough and fever are common, but progression of disease may be silent. Although most infections currently occur in patients with AIDS, malignancy and transplantation also pose risks. Pulmonary infection in children with leukemia has been described.[4,96] Infection may be accompanied by other pathogens, particularly in patients with AIDS.

Pulmonary infection often is pleura based and associated with cavitation.[63] Empyema may occur as a complication. Computed tomography most frequently reveals pneumonia with cavitation, but other patterns, including "ground-glass" opacities, peribronchial nodules, and centrilobular nodules, may be seen.[89] Lung tissue showing malakoplakia, an unusual-appearing granulomatous inflammation with aggregates of histiocytes that contain concentrically layered basophilic inclusions, should raise suspicion of the presence of *R. equi* infection.[21,58,122]

Extrapulmonary disease is seen at diagnosis in 7 percent of patients with pneumonia.[145] Manifestations of infection include otitis/mastoiditis,[4,69,84] abscesses,[46] osteomyelitis,[18,47,104] meningitis,[35,126] pericarditis,[80] lymphadenitis,[80] and endophthalmitis.[41] The organism has been grown from a biopsy of a granulomatous skin lesion in an immunocompetent 7-year-old girl.[91] *R. equi* also has been reported as a cause of peritonitis in patients receiving long-term peritoneal dialysis.[20,137] A case report of ventriculitis in a premature infant with a ventriculoperitoneal shunt has been reported.[133]

DIAGNOSIS

Diagnosis relies on isolation of *Rhodococcus* from clinical material. Although sputum specimens may be positive, bronchoalveolar lavage or lung biopsy may be required. Blood cultures may be positive in half or more of patients with AIDS and with focal pneumonic disease.[141] The physician should be alert to the possible coexistence of *R. equi* with other pathogens. In the laboratory, confusing this organism with *Corynebacterium*, acid-fast organisms, and other gram-positive coccobacilli has been shown to delay establishment of the diagnosis.[39] Positive findings on Gram stain and Kinyoun stain should be interpreted in the context of clinical information.[125] Organisms appear salmon-pink when grown on blood agar and orange on Lowenstein-Jensen medium.[13] Differentiating from acid-fast bacteria on smear sometimes can be difficult. Combined use of a siderophore detection medium, ethylene glycol degradation, and β-galactosidase activity may help differentiate *Rhodococcus* from *Nocardia* and rapid-growing mycobacteria.[48] DNA restriction fragment analysis and ribotyping show promise in aiding the identification and tracking of *Rhodococcus* spp.[79] An *R. equi*–specific PCR assay has been used to confirm infection in some cases in which identification was difficult.[1]

Differentiation of *R. equi* from other species of the genus *Rhodococcus* can be difficult and may frequently be incorrect; 16S rRNA genome sequencing analysis sometimes identifies a different species of *Rhodococcus* from specimens originally signed out as *R. equi*.[59] Also, organisms of the new genus *Dietzia* are often misidentified as *R. equi*.[77] In one study, only 7 of 15 clinical specimens originally called *R. equi* by the microbiology laboratory were actually *Rhodococcus* isolates; the 8 others were all members of the genus *Dietzia*.[108]

TREATMENT

Clinical isolates commonly are resistant to penicillins and cephalosporins. Even if susceptible in vitro, β-lactam

antibiotics should be avoided because of rapid development of resistance.[72] Erythromycin, clarithromycin, azithromycin, clindamycin, rifampin, aminoglycosides, vancomycin, fluoroquinolones, and imipenem are active against *R. equi*.[103] Moxifloxacin is more active in vitro than is either ciprofloxacin or levofloxacin.[117] Synergy has been shown with various combinations of these agents. Including rifampin or erythromycin in a two-drug combination has been recommended because of penetrance of macrophages.[13,128] Combinations of antibiotics that included vancomycin were found to be most effective in clearing infection in a mouse model.[103] Cure rates in adults with lung infection are approximately 60 percent when antibiotic therapy alone is employed but may reach 75 percent when combined with surgical resection of infected pulmonary tissue.[63] Surgery has not been shown to increase survival rates, however.[8]

Pediatric patients generally have fared better than adults, but most reported cases in children have been in non-AIDS patients.[13] Relapse is a common occurrence, but the optimal duration of therapy to prevent relapse is unknown. For patients with AIDS, some authors recommend a minimum of 2 months of therapy followed by long-term suppressive therapy.[72] Relapse has been reported to occur at extrapulmonary sites in 13 percent of immunocompromised patients,[145] often without reappearance of pulmonary disease. Treatment of *R. equi* peritonitis in patients receiving peritoneal dialysis has been reported to be successful with intraperitoneal imipenem or vancomycin for 14 days.[20,138] Removal of the peritoneal dialysis catheter may be required for cure.

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Adderson EE, Boudreaux JW, Hayden RT. Infections caused by coryneform bacteria in pediatric oncology patients. *Pediatr Infect Dis J* 2008;**27**:136–41.
5. Almuzara MN, DeMier C, Rodriguez CR, et al. Evaluation of API Coryne System, version 2.0, for diphtheroid gram-positive rod identification with clinical relevance. *Rev Argent Microbiol* 2006;**38**:197–201.
12. Belmares J, Detterline S, Pak JB, Parada JP. *Corynebacterium* endocarditis species-specific risk factors and outcomes. *BMC Infect Dis* 2007;**7**:4.
15. Bittar F, Cassagne C, Bosdure E, et al. Outbreak of *Corynebacterium pseudodiphtheriticum* infection in cystic fibrosis patients, France. *Emerg Infect Dis* 2010;**16**:1231–6.
22. Cantarelli VV, Brodt TC, Secchi C, et al. Cutaneous infection caused by *Corynebacterium pseudodiphtheriticum:* a microbiological report. *Rev Inst Med Trop Sao Paulo* 2008;**50**:51–2.
24. Centers from Disease Control and Prevention. Notes from the field: respiratory diphtheria-like illness caused by toxigenic *Corynebacterium ulcerans*–Idaho, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:77.
25. Chung CS, Liao CH, Cheng SL, et al. Percutaneous nephrostomy tube–associated bacteremia caused by *Corynebacterium urealyticum*. *J Microbiol Immunol Infect* 2008;**41**:525–7.
32. Dalal A, Likhi R. *Corynebacterium minutissimum* bacteremia and meningitis: a case report and review of the literature. *J Infect* 2008;**56**:77–9.
36. Denes E, Peignon-Orsoni D, Terrade FX. Nonhealing wound due to *Rhodococcus equi* in an apparently immunocompetent patient, revealing CD8+ T-lymphocyte deficiency. *J Clin Microbiol* 2010; **48**:4658–60.
45. Fernandez-Roblas R, Adames H, Martin-de-Hijas NZ, et al. In vitro activity of tigecycline and 10 other antimicrobials against clinical isolates of the genus *Corynebacterium*. *Int J Antimicrob Agents* 2009;**33**:453–5.
51. Gelfand MS, Cleveland KO, Brewer SC. *Rhodococcus equi* pneumonia in a patient with fludarabine-treated chronic lymphocytic leukemia and CD4-lymphopenia. *Am J Med Sci* 2010;**340**:80–1.
59. Gurtler V, Mayall BC, Seviour R. Can whole genome analysis refine the taxonomy of the genus *Rhodococcus? REMS Microbiol Rev* 2004;**28**:377–403.
68. Iaria C, Stass G, Costa GB, et al. Outbreak of multi-resistant *Corynebacterium striatum* infection in an Italian general intensive care unit [Letter]. *J Hosp Infect* 2007;**67**:102–4.
77. Koerner RJ, Goodfellow MI, Jones AL. The genus *Dietzia:* a new home for some known and emerging opportunistic pathogens. *FEMS Immunol Med Microbiol* 2009;**55**:296–305.
85. Lopez-Medrano F, Garcia-Bravo M, Morales JM, et al. Urinary tract infection due to *Corynebacterium urealyticum* in kidney transplant recipients: an underdiagnosed etiology for obstructive uropathy and graft dysfunction—results of a prospective cohort study. *Clin Infect Dis* 2008;**46**:825–30.
90. Marti J, Anton E, Idolate A. Implantable cardioverter-defibrillator infection due to *Corynebacterium xerosis*. *Int J Cardiol* 2008;**128**: e1–2.
93. Martins C, Faria L, Souza M, et al. Microbiological and host features associated with corynebacteriosis in cancer patients: a five-year study. *Mem Inst Oswaldo Cruz* 2009;**104**:905–13.
94. Matsushita H, Hanayama N, Hobo K, et al. Infectious endocarditis caused by *Rhodococcus equi*. *Ann Thorac Surg* 2010;**89**:957–9.
97. Meeuse JJ, Sprenger HG, van Assen S, et al. *Rhodococcus equi* infection after alemtuzumab therapy for T-cell prolymphocytic leukemia. *Emerg Infect Dis* 2007;**13**:1942–3.
105. Olson JM, Nguyen VQ, Yoo J, Keuchle MK. Cutaneous manifestations of *Corynebacterium jeikeium* sepsis. *Int J Dermatol* 2009; **48**:886–8.
108. Pilares L, Aguero J, Vazquez-Boland JA, et al. Identification of atypical *Rhodococcus*-like clinical isolates as *Dietzia* spp. by 16S rRNA sequencing. *J Clin Microbiol* 2010;**48**:1904–7.
116. Roda RH, Young M, Timpone J, et al. *Rhodococcus equi* pulmonary–central nervous system syndrome: brain abscess in a patient on high-dose steroids—a case report and review of the literature. *Diagn Microbiol Infect Dis* 2009;**63**:96–9.
124. Scholle D. A spontaneous joint infection with *Corynebacterium striatum*. *J Clin Microbiol* 2007;**45**:656–8.
133. Strunk T, Gardiner K, Simmer K, et al. *Rhodococcus equi* meningitis after ventriculoperitoneal shunt insertion in a preterm infant. *Pediatr Infect Dis J* 2007;**26**:1076–7.
142. Trost E, Ott L, Schneider J, et al. The complete genome sequence of *Corynebacterium pseudotuberculosis* FRC41 isolated from a 12-year-old girl with necrotizing lymphadenitis reveals insights into gene-regulatory networks contributing to virulence. *BMC Genomics* 2010;**11**:728.
147. Wagner KS, White JM, Crowcroft NS, et al. Diphtheria in the United Kingdom, 1986-2008; the increasing role of *Corynebacterium ulcerans*. *Epidemiol Infect* 2010;**138**:1519–30.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adderson EE, Boudreaux JW, Hayden RT. Infections caused by coryneform bacteria in pediatric oncology patients. *Pediatr Infect Dis J* 2008;**27**:136–41.
2. Ahmed K, Kawakami K, Watanabe K, et al. *Corynebacterium pseudodiphtheriticum*: a respiratory tract pathogen. *Clin Infect Dis* 1995; **20**:41–6.
3. Allen KD, Green HT. Infections due to a "Group JK" *Corynebacterium*. *J Infect* 1986;**13**:41–4.
4. Allen VD, Niec A, Kerem E, et al. *Rhodococcus equi* pneumonia in a child with leukemia. *Pediatr Infect Dis J* 1989;**8**:656–8.
5. Almuzara MN, DeMier C, Rodriguez CR, et al. Evaluation of API Coryne System, version 2.0, for diphtheroid gram-positive rod identification with clinical relevance. *Rev Argent Microbiol* 2006; **38**:197–201.
6. Aperis G, Moyssakis I. *Corynebacterium minutissimum* endocarditis: a case report and review. *J Infect* 2007;**54**:e79–81.
7. Arisoy ES, Demmler GJ, Dunne WM Jr. *Corynebacterium xerosis* ventriculoperitoneal shunt infection in an infant: report of a case and review of the literature. *Pediatr Infect Dis J* 1993;**12**:536–8.
8. Arlotti M, Zoboli G, Moscatelli GL, et al. *Rhodococcus equi* infection in HIV-positive subjects: a retrospective analysis of 24 cases. *Scand J Infect Dis* 1996;**28**:463–7.
9. Ayra B, Hussian S, Hariharan S. *Rhodococcus equi* pneumonia in a renal transplant patient: a case report and review of literature. *Clin Transplant* 2004;**18**:748–52.
10. Barnass S, Holland K, Tabaqchali S. Vancomycin-resistant *Corynebacterium* species causing prosthetic valve endocarditis successfully treated with imipenem and ciprofloxacin. *J Infect* 1991;**22**: 161–9.
11. Barrett NJ. Communicable disease associated with milk and dairy products in England and Wales: 1983-1984. *J Infect* 1986;**12**: 265–72.
12. Belmares J, Detterline S, Pak JB, Parada JP. *Corynebacterium* endocarditis species-specific risk factors and outcomes. *BMC Infect Dis* 2007;**7**:4.
13. Berkowitz FE. The gram-positive bacilli: a review of the microbiology, clinical aspects, and antimicrobial susceptibilities of a heterogeneous group of bacteria. *Pediatr Infect Dis J* 1994;**13**: 1126–38.
14. Berner R, Pelz K, Wilhelm C, et al. Fatal sepsis caused by *Corynebacterium amycolatum* in a premature infant. *J Clin Microbiol* 1997;**35**:1011–12.
15. Bittar F, Cassagne C, Bosdure E, et al. Outbreak of *Corynebacterium pseudodiphtheriticum* infection in cystic fibrosis patients, France. *Emerg Infect Dis* 2010;**16**:1231–6.
16. Blom J, Heltberg O. The ultrastructure of antibiotic-susceptible and multi-resistant strains of group JK diphtheroid rods isolated from clinical specimens. *Acta Pathol Microbiol Immunol Scand B* 1986;**94**:301–8.
17. Booth LV, Richards RH, Chandran DR. Septic arthritis caused by *Corynebacterium xerosis* following vascular surgery. *Rev Infect Dis* 1991;**13**:548–9.
18. Bouchou K, Cathebras P, Dumollard JM, et al. Chronic osteitis due to *Rhodococcus equi* in an immunocompetent patient [Letter]. *Clin Infect Dis* 1995;**20**:718–20.
19. Brandenburg AH, van Belkum A, van Pelt C, et al. Patient-to-patient spread of a single strain of *Corynebacterium striatum* causing infections in a surgical intensive care unit. *J Clin Microbiol* 1996; **34**:2089–94.
20. Brown E, Hendler E. *Rhodococcus* peritonitis in a patient treated with peritoneal dialysis. *Am J Kidney Dis* 1989;**14**:417–18.
21. Byard RW, Thorner PS, Edwards V, et al. Pulmonary malacoplakia in a child. *Pediatr Pathol* 1990;**10**:417–24.
22. Cantarelli VV, Brodt TC, Secchi C, et al. Cutaneous infection caused by *Corynebacterium pseudodiphtheriticum:* a microbiological report. *Rev Inst Med Trop Sao Paulo* 2008;**50**:51–2.
23. Centers for Disease Control and Prevention. Respiratory diphtheria caused by *Corynebacterium ulcerans*—Terre Haute, Indiana, 1996. *MMWR Morb Mortal Wkly Rep* 1997;**46**:330–2.
24. Centers for Disease Control and Prevention. Notes from the field: respiratory diphtheria-like illness caused by toxigenic *Corynebacterium ulcerans*—Idaho, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:77.
25. Chung CS, Liao CH, Cheng SL, et al. Percutaneous nephrostomy tube–associated bacteremia caused by *Corynebacterium urealyticum*. *J Microbiol Immunol Infect* 2008;**41**:525–7.
26. Claeys G, Vershchraegen G, DeSmet L, et al. *Corynebacterium* JK (Johnson-Kay strain) infection of a Kuntscher-nailed tibial fracture. *Clin Orthop Rel Res* 1986;**202**:227–9.
27. Collins MD, Cummins CS. Corynebacterium. In: Garrity GM, editor. *Bergey's manual of systematic bacteriology*. New York: Springer; 1986. p. 1266–76.
28. Colt HG, Morris JF, Marston BJ, et al. Necrotizing tracheitis caused by *Corynebacterium pseudodiphtheriticum:* unique case and review. *Rev Infect Dis* 1991;**13**:73–6.
29. Cornish N, Washington JA. *Rhodococcus equi* infections: clinical features and laboratory diagnosis. *Curr Clin Top Infect Dis* 1999;**19**: 198–215.
30. Craig J, Grigor W, Doyle B, et al. Pyelonephritis caused by *Corynebacterium minutissimum*. *Pediatr Infect Dis J* 1994;**13**: 1151–2.
31. Craig TJ, Maguire FE, Wallace MR. Tracheobronchitis due to *Corynebacterium pseudodiphtheriticum*. *South Med J* 1991;**84**: 504–6.
32. Dalal A, Likhi R. *Corynebacterium minutissimum* bacteremia and meningitis: a case report and review of the literature. *J Infect* 2008;**56**:77–9.
33. Dan M, Somer I, Knobel B, et al. Cutaneous manifestations of infection with *Corynebacterium* group JK. *Rev Infect Dis* 1988;**10**: 1204–7.
34. de Carpentier JP, Flanagan PM, Singh IP, et al. Nasopharyngeal *Corynebacterium ulcerans*: a different diphtheria. *J Laryngol Otol* 1992;**106**:824–6.
35. DeMarais PL, Kocka FE. *Rhodococcus* meningitis in an immunocompetent host. *Clin Infect Dis* 1995;**20**:167–9.
36. Denes E, Peignon-Orsoni D, Terrade FX. Nonhealing wound due to *Rhodococcus equi* in an apparently immunocompetent patient, revealing CD8+ T-lymphocyte deficiency. *J Clin Microbiol* 2010; **48**:4658–60.
37. deOliveira GC, Solari HP, Ciola FB, et al. Corneal infiltrates after excimer laser photorefractive keratectomy and LASIK. *J Refract Surg* 2006;**22**:159–65.
38. Dietrich MC, Watson DC, Kumar ML. *Corynebacterium* group JK infections in children. *Pediatr Infect Dis J* 1989;**8**:233–6.
39. Doig C, Gill MJ, Church DL. *Rhodococcus equi*—an easily missed opportunistic pathogen. *Scand J Infect Dis* 1991;**23**:1–6.
40. Eagan JA, Blevins A, Armstrong D. Prevention of skin colonization and subsequent bacteremia with CDC-JK organisms in patients with cancer. *Cancer Pract* 1993;**1**:325–8.
41. Ebersole LL, Paturzo JL. Endophthalmitis caused by *Rhodococcus equi* Prescott serotype 4. *J Clin Microbiol* 1988;**26**:1221–2.
42. Eliakim R, Silkoff P, Lugassy G, et al. *Corynebacterium xerosis* endocarditis. *Arch Intern Med* 1983;**143**:1995.
43. Emmons W, Reichwein B, Winslow DL. *Rhodococcus equi* infection in the patient with AIDS: literature review and report of an unusual case. *Rev Infect Dis* 1991;**13**:91–6.
44. Estorc JJ, de La Coussaye JE, Viel EJ, et al. Teicoplanin treatment of alkaline encrusted cystitis due to *Corynebacterium* group D2. *Eur J Med* 1992;**1**:183–4.
45. Fernandez-Roblas R, Adames H, Martin-de-Hijas NZ, et al. In vitro activity of tigecycline and 10 other antimicrobials against clinical isolates of the genus *Corynebacterium*. *Int J Antimicrob Agents* 2009;**33**:453–5.
46. Fierer J, Wolf P, Seed L, et al. Non-pulmonary *Rhodococcus equi* infections in patients with acquired immune deficiency syndrome (AIDS). *J Clin Pathol* 1987;**40**:556–8.
47. Fischer L, Sterneck M, Albrecht H, et al. Vertebral osteomyelitis due to *Rhodococcus equi* in a liver transplant recipient. *Clin Infect Dis* 1998;**26**:749–52.
48. Fiss E, Brooks GF. Use of a siderophore detection medium, ethylene glycol degradation, and beta-galactosidase activity in the early presumptive differentiation of *Nocardia*, *Rhodococcus*, *Streptomyces*, and rapidly growing *Mycobacterium* species. *J Clin Microbiol* 1991;**29**:1533–5.
49. Funke G, Lawson PA, Bernard KA, et al. Most *Corynebacterium xerosis* strains identified in the routine clinical laboratory correspond to *Corynebacterium amycolatum*. *J Clin Microbiol* 1996;**34**: 1124–8.

50. Funke G, von Graevenitz A, Clarridge JE 3rd, et al. Clinical microbiology of coryneform bacteria. *Clin Microbiol Rev* 1997;**10**: 125–59.
51. Gelfand MS, Cleveland KO, Brewer SC. *Rhodococcus equi* pneumonia in a patient with fludarabine-treated chronic lymphocytic leukemia and CD4-lymphopenia. *Am J Med Sci* 2010;**340**: 80–1.
52. Gill VJ, Manning C, Lamson M, et al. Antibiotic-resistant group JK bacteria in hospitals. *J Clin Microbiol* 1981;**13**:472–7.
53. Goldberger AC, Lipsky BA, Plorde JJ. Suppurative granulomatous lymphadenitis caused by *Corynebacterium ovis* (pseudotuberculosis). *Am J Clin Pathol* 1981;**76**:486–90.
54. Goldstein EJ, Citron DM, Merriam CV, et al. In vitro activities of daptomycin, vancomycin, quinupristin-dalfopristin, linezolid, and five other antimicrobials against 307 gram-positive anaerobic and 31 *Corynebacterium* clinical isolates. *Antimicrob Agents Chemother* 2003;**47**:1486.
55. Gomez-Garces JL, Alos JI, Tamayo J. In vitro activity of linezolid and 12 other antimicrobials against coryneform bacteria. *Int J Antimicrob Agents* 2007;**29**:688–92.
56. Goodfellow M. *Rhodococcus*. In: Garrity GM, editor. *Bergey's manual of systematic bacteriology*. New York: Springer; 1986. p. 1266–76.
57. Gotoh K, Mitsuyama M, Imaizumi S, et al. Mycolic acid–containing glycolipid as a possible virulence factor of *Rhodococcus equi* for mice. *Microbiol Immunol* 1991;**35**:175–85.
58. Guerrero MF, Ramos JM, Renedo G, et al. Pulmonary malacoplakia associated with *Rhodococcus equi* infection in patients with AIDS: case report and review. *Clin Infect Dis* 1999;**28**:1334–6.
59. Gurtler V, Mayall BC, Seviour R. Can whole genome analysis refine the taxonomy of the genus Rhodococcus? *REMS Microbiol Rev* 2004;**28**:377–403.
60. Gutierrez-Rodero F, Ortiz de la Tabla V, Martinez C, et al. *Corynebacterium pseudodiphtheriticum*: an easily missed respiratory pathogen in HIV-infected patients. *Diagn Microbiol Infect Dis* 1999;**33**:209–16.
61. Hamilton DJ, Ulness BK, Baugher LK, et al. Comparison of a novel trimethoprim-sulfamethoxazole-containing medium (XT80) with kanamycin agar for isolation of antibiotic-resistant organisms from stool and rectal cultures of marrow transplant patients. *J Clin Microbiol* 1987;**25**:1886–90.
62. Hande KR, Witebsky FG, Brown MS, et al. Sepsis with a new species of *Corynebacterium*. *Ann Intern Med* 1976;**85**:423–6.
63. Harvey RL, Sunstrum JC. *Rhodococcus equi* infection in patients with and without human immunodeficiency virus infection. *Rev Infect Dis* 1991;**13**:139–45.
64. Hondalus MK, Diamond MS, Rosenthal LA, et al. The intracellular bacterium *Rhodococcus equi* requires Mac-1 to bind to mammalian cells. *Infect Immun* 1993;**61**:2919–29.
65. Hondalus MK, Mosser DM. Survival and replication of *Rhodococcus equi* in macrophages. *Infect Immun* 1994;**62**:4167–75.
66. Horn WA, Larson EL, McGinley KJ, et al. Microbial flora on the hands of health care personnel: differences in composition and antibacterial resistance. *Infect Control Hosp Epidemiol* 1988;**9**: 189–93.
67. Hoy CM, Kerr K, Livingston JH. Cerebrospinal fluid-shunt infection due to *Corynebacterium* striatum [Letter; comment]. *Clin Infect Dis* 1997;**25**:1486–7.
68. Iaria C, Stass G, Costa GB, et al. Outbreak of multi-resistant *Corynebacterium striatum* infection in an Italian general intensive care unit (Letter). *J Hosp Infect* 2007;**67**:102–4.
69. Ibarra R, Jinkins JR. Severe otitis and mastoiditis due to *Rhodococcus equi* in a patient with AIDS: case report. *Neuroradiology* 1999;**41**:699–701.
70. Izurieta HS, Strebel PM, Youngblood T, et al. Exudative pharyngitis possibly due to *Corynebacterium pseudodiphtheriticum*, a new challenge in the differential diagnosis of diphtheria [see comments]. *Emerg Infect Dis* 1997;**3**:65–8.
71. Jerdan MS, Shapiro RS, Smith NB, et al. Cutaneous manifestations of *Corynebacterium* group JK sepsis. *J Am Acad Dermatol* 1987;**16**:444–7.
72. Johnson DH, Cunha BA. *Rhodococcus equi* pneumonia. *Semin Respir Infect* 1997;**12**:57–60.
73. Kanaly ST, Hines SA, Palmer GH. Failure of pulmonary clearance of *Rhodococcus equi* infection in CD4+ T-lymphocyte-deficient transgenic mice. *Infect Immun* 1993;**61**:4929–32.
74. Kaplan A, Israel F. *Corynebacterium aquaticum* infection in a patient with chronic granulomatous disease. *Am J Med Sci* 1988;**296**: 57–8.
75. Kemp M, Holtz K, Andersen K, Christensen JJ. Demonstration by PCR and DNA sequencing of *Corynebacterium pseudodiphtheriticum* as a cause of joint infection and isolation of the same organism from a surface swab from the patient. *J Med Microbiol* 2005;**54**:689–91.
76. Keren G, Geva T, Bogokovsky B, et al. *Corynebacterium* group JK pathogen in cerebrospinal fluid shunt infection: report of two cases. *J Neurosurg* 1988;**68**:648–50.
77. Koerner RJ, Goodfellow MI, Jones AL. The genus *Dietzia:* a new home for some known and emerging opportunistic pathogens. *FEMS Immunol Med Microbiol* 2009;**55**:296–305.
78. Lalitha P, Srinivasan M, Prajna V. *Rhodococcus ruber* as a cause of keratitis. *Cornea* 2006;**25**:238–9.
79. Lasker BA, Brown JM, McNeil MM. Identification and epidemiological typing of clinical and environmental isolates of the genus *Rhodococcus* with use of a digoxigenin-labeled rDNA gene probe. *Clin Infect Dis* 1992;**15**:223–33.
80. Lee-Chiong T, Sadigh M, Simms M, et al. Case reports: pericarditis and lymphadenitis due to *Rhodococcus equi*. *Am J Med Sci* 1995;**310**:31–3.
81. Leonard RB, Nowowiejski DJ, Warren JJ, et al. Molecular evidence of person-to-person transmission of a pigmented strain of *Corynebacterium* striatum in intensive care units. *J Clin Microbiol* 1994;**32**:164–9.
82. Li A, Lal S. *Corynebacterium pseudodiphtheriticum* keratitis and conjunctivitis: a case report. *Clin Exp Ophthalmol* 2000;**28**:60–1.
83. Lipsky BA, Goldberger AC, Tompkins LS, et al. Infections caused by nondiphtheria corynebacteria. *Rev Infect Dis* 1982;**4**: 1220–35.
84. Lopes Cardoso FL, Machado ES, Souza MJ, et al. *Rhodococcus equi* mastoiditis in a patient with AIDS. *Clin Infect Dis* 1996;**22**:713.
85. Lopez-Medrano F, Garcia-Bravo M, Morales JM, et al. Urinary tract infection due to *Corynebacterium urealyticum* in kidney transplant recipients: an underdiagnosed etiology for obstructive uropathy and graft dysfunction—results of a prospective cohort study. *Clin Infect Dis* 2008;**46**:825–30.
86. Lortholary O, Buu-Hoi A, Fagon JY, et al. Mediastinitis due to multiple resistant *Corynebacterium xerosis*. *Clin Infect Dis* 1993; **16**:172.
87. Macedo Filho ET, Lago A, Duarte K, et al. Superficial corneal foreign body: laboratory and epidemiologic aspects. *Arq Bras Oftalmol* 2005;**68**:821–32.
88. Manzella JP, Kellogg JA, Parsey KS. *Corynebacterium pseudodiphtheriticum*: a respiratory tract pathogen in adults. *Clin Infect Dis* 1995;**20**:37–40.
89. Marchiori E, de Mendonca RG, Capone D, et al. *Rhodococcus equi* infection in acquired immunodeficiency syndrome: computed tomography aspects. *J Bras Pneumonol* 2006;**32**:405–9.
90. Marti J, Anton E, Idolate A. Implantable cardioverter-defibrillator infection due to *Corynebacterium xerosis*. *Int J Cardiol* 2008;**128**: e1–2.
91. Martin T, Hogan DJ, Murphy F, et al. *Rhodococcus* infection of the skin with lymphadenitis in a nonimmunocompromised girl. *J Am Acad Dermatol* 1991;**24**:328–32.
92. Martinez-Martinez L, Suarez AI, Ortega MC, et al. Fatal pulmonary infection caused by *Corynebacterium striatum*. *Clin Infect Dis* 1994;**19**:806–7.
93. Martins C, Faria L, Souza M, et al. Microbiological and host features associated with corynebacteriosis in cancer patients: a five-year study. *Mem Inst Oswaldo Cruz* 2009;**104**:905–13.
94. Matsushita H, Hanayama N, Hobo K, et al. Infectious endocarditis caused by *Rhodococcus equi*. *Ann Thorac Surg* 2010;**89**:957–9.
95. McGowan JE Jr. JK coryneforms: a continuing problem for hospital infection control. *J Hosp Infect* 1988;**11**(Suppl. A): 358–66.
96. McGowan KL, Mangano MF. Infections with *Rhodococcus equi* in children. *Diagn Microbiol Infect Dis* 1991;**14**:347–52.
97. Meeuse JJ, Sprenger HG, van Assen S, et al. *Rhodococcus equi* infection after alemtuzumab therapy for T-cell prolymphocytic leukemia. *Emerg Infect Dis* 2007;**13**:1942–3.
98. Meria P, Margaryan M, Haddad E, et al. Encrusted cystitis and pyelitis in children: an unusual condition with potentially severe consequences. *Urology* 2004;**64**:569–73.

99. Mills AE, Mitchell RD, Lim EK. *Corynebacterium pseudotuberculosis* is a cause of human necrotising granulomatous lymphadenitis. *Pathology* 1997;**29**:231–3.
100. Mookadam F, Cikes M, Baddour LM, et al. *Corynebacterium jeikeium* endocarditis: a systematic overview spanning four decades. *Eur J Clin Microbiol Infect Dis* 2006;**25**:349–53.
101. Morris A, Guild I. Endocarditis due to *Corynebacterium pseudodiphtheriticum*: five case reports, review, and antibiotic susceptibilities of nine strains. *Rev Infect Dis* 1991;**13**:887–92.
102. Murray BE, Karchmer AW, Moellering RC Jr. Diphtheroid prosthetic valve endocarditis: a study of clinical features and infecting organisms. *Am J Med* 1980;**69**:838–48.
103. Nordmann P, Kerestedjian JJ, Ronco E. Therapy of *Rhodococcus equi* disseminated infections in nude mice. *Antimicrob Agents Chemother* 1992;**36**:1244–8.
104. Novak RM, Polisky EL, Janda WM, et al. Osteomyelitis caused by *Rhodococcus equi* in a renal transplant recipient. *Infection* 1988;**16**:186–8.
105. Olson JM, Nguyen VQ, Yoo J, et al. Cutaneous manifestations of *Corynebacterium jeikeium* sepsis. *Int J Dermatol* 2009;**48**:886–8.
106. Panaitescu M, Maximescu P, Michel J, et al. Respiratory pathogens in non-human primates with special reference to *Corynebacterium ulcerans*. *Lab Anim* 1977;**11**:155–7.
107. Peel MM, Palmer GG, Stacpoole AM, et al. Human lymphadenitis due to *Corynebacterium pseudotuberculosis:* report of ten cases from Australia and review. *Clin Infect Dis* 1997;**24**:185–91.
108. Pilares L, Aguero J, Vazquez-Boland JA, et al. Identification of atypical *Rhodococcus*-like clinical isolates as *Dietzia* spp. By 16S rRNA sequencing. *J Clin Microbiol* 2010;**48**:1904–7.
109. Pitcher D, Johnson A, Allerberger F, et al. An investigation of nosocomial infection with *Corynebacterium jeikeium* in surgical patients using a ribosomal RNA gene probe. *Eur J Clin Microbiol Infect Dis* 1990;**9**:643–8.
110. Prescott JF. *Rhodococcus equi*: an animal and human pathogen. *Clin Microbiol Rev* 1991;**4**:20–34.
111. Prescott JF, Johnson JA, Markham RJ. Experimental studies on the pathogenesis of *Corynebacterium equi* infection in foals. *Can J Comp Med* 1980;**44**:280–8.
112. Quinn JP, Arnow PM, Weil D, et al. Outbreak of JK diphtheroid infections associated with environmental contamination. *J Clin Microbiol* 1984;**19**:668–71.
113. Riebel W, Frantz N, Adelstein D, et al. *Corynebacterium* JK: a cause of nosocomial device-related infection. *Rev Infect Dis* 1986;**8**: 42–9.
114. Riegel P, de Briel D, Prevost G, et al. Genomic diversity among *Corynebacterium jeikeium* strains and comparison with biochemical characteristics and antimicrobial susceptibilities. *J Clin Microbiol* 1994;**32**:1860–5.
115. Riley PS, Hollis DG, Utter GB, et al. Characterization and identification of 95 diphtheroid (group JK) cultures isolated from clinical specimens. *J Clin Microbiol* 1979;**9**:418–24.
116. Roda RH, Young M, Timpone J, et al. *Rhodococcus equi* pulmonary–central nervous system syndrome: brain abscess in a patient on high-dose steroids—a case report and review of the literature. *Diagn Microbiol Infect Dis* 2009;**63**:96–9.
117. Rolston KV, Frisbee-Hume S, Le Blanc B, et al. In vitro antimicrobial activity of moxifloxacin compared to other quinolones, against recent clinical bacterial isolates from hospitalized and community-based cancer patients. *Diagn Microbiol Infect Dis* 2003; **47**:441–9.
118. Rozdzinski E, Kern W, Schmeiser T, et al. *Corynebacterium jeikeium* bacteremia at a tertiary care center. *Infection* 1991;**19**: 201–4.
119. Rufael DW, Cohn SE. Native valve endocarditis due to *Corynebacterium* striatum: case report and review. *Clin Infect Dis* 1994; **19**:1054–61.
120. Saavedra J, Rodriguez JN, Fernandez-Jurado A, et al. A necrotic soft-tissue lesion due to *Corynebacterium urealyticum* in a neutropenic child. *Clin Infect Dis* 1996;**22**:851–2.
121. Sanchez Hernandez J, Mora Peris B, Yague Guirae G, et al. In vitro activity of new antibiotics against *Corynebacterium jeikeium*, *Corynebacterium amycolatum* and *Corynebacterium urealyticum*. *Int J Antimicrob Agents* 2003;**22**:492–6.
122. Scannell KA, Portoni EJ, Finkle HI, et al. Pulmonary malacoplakia and *Rhodococcus equi* infection in a patient with AIDS. *Chest* 1990;**97**:1000–1.
123. Schiffl H, Mucke C, Lang SM. Exit-site infections by non-diphtheria corynebacteria in CAPD. *Perit Dial Int* 2004;**24**:454–9.
124. Scholle D. A spontaneous joint infection with *Corynebacterium striatum*. *J Clin Microbiol* 2007;**45**:656–8.
125. Scott MA, Graham BS, Verrall R, et al. *Rhodococcus equi*—an increasingly recognized opportunistic pathogen: report of 12 cases and review of 65 cases in the literature. *Am J Clin Pathol* 1995; **103**:649–55.
126. Scotton PG, Tonon E, Giobbia M, et al. *Rhodococcus equi* nosocomial meningitis cured by levofloxacin and shunt removal. *Clin Infect Dis* 2000;**30**:223–4.
127. Simoons-Smit AM, Savelkoul PH, Newling DW, et al. Chronic cystitis caused by *Corynebacterium urealyticum* detected by polymerase chain reaction. *Eur J Clin Microbiol Infect Dis* 2000;**19**: 949–52.
128. Sirera G, Romeu J, Clotet B, et al. Relapsing systemic infection due to *Rhodococcus equi* in a drug abuser seropositive for human immunodeficiency virus. *Rev Infect Dis* 1991;**13**:509–10.
129. Soriano F, Ponte C, Ruiz P, et al. Non-urinary tract infections caused by multiply antibiotic-resistant *Corynebacterium urealyticum*. *Clin Infect Dis* 1993;**17**:890–1.
130. Soriano F, Ponte C, Santamaria M, et al. *Corynebacterium* group D2 as a cause of alkaline-encrusted cystitis: report of four cases and characterization of the organisms. *J Clin Microbiol* 1985;**21**: 788–92.
131. Soriano F, Zapardiel J, Nieto E. Antimicrobial susceptibilities of *Corynebacterium* species and other non–spore-forming gram-positive bacilli to 18 antimicrobial agents. *Antimicrob Agents Chemother* 1995;**39**:208–14.
132. Stamm WE, Tompkins LS, Wagner KF, et al. Infection due to *Corynebacterium* species in marrow transplant patients. *Ann Intern Med* 1979;**91**:167–73.
133. Strunk T, Gardiner K, Simmer K, et al. *Rhodococcus equi* meningitis after ventriculoperitoneal shunt insertion in a preterm infant. *Pediatr Infect Dis J* 2007;**26**:1076–7.
134. Takai S, Iie M, Watanabe Y, et al. Virulence-associated 15- to 17-kilodalton antigens in *Rhodococcus equi*: temperature-dependent expression and location of the antigens. *Infect Immun* 1992;**60**: 2995–7.
135. Takai S, Imai Y, Fukunaga N, et al. Identification of virulence-associated antigens and plasmids in *Rhodococcus equi* from patients with AIDS. *J Infect Dis* 1995;**172**:1306–11.
136. Tan C, Prescott JF, Patterson MC, et al. Molecular characterization of a lipid-modified virulence-associated protein of *Rhodococcus equi* and its potential in protective immunity. *Can J Vet Res* 1995;**59**:51–9.
137. Tang S, Lo CY, Lo WK, et al. *Rhodococcus peritonitis* in continuous ambulatory peritoneal dialysis. *Nephrol Dial Transplant* 1996;**11**: 201–2.
138. Tang S, Lo CY, Lo WK, et al. Optimal treatment regimen for CAPD peritonitis caused by *Rhodococcus* species [Letter; comment]. *Nephrol Dial Transplant* 1997;**12**:1080–1.
139. Thompson JS, Gates-Davis DR, Yong DC. Rapid microbiochemical identification of *Corynebacterium diphtheriae* and other medically important corynebacteria. *J Clin Microbiol* 1983;**18**:926–9.
140. Three cases of toxigenic *Corynebacterium ulcerans* infection. *Commun Dis Rep CDR Wkly* 2000;**10**:49, 52.
141. Torres-Tortosa M, Arrizabalaga J, Villanueva JL, et al. Prognosis and clinical evaluation of infection caused by *Rhodococcus equi* in HIV-infected patients: a multicenter study of 67 cases. *Chest* 2003;**123**:1970–6.
142. Trost E, Ott L, Schneider J, et al. The complete genome sequence of *Corynebacterium pseudotuberculosis* FRC41 isolated from a 12-year-old girl with necrotizing lymphadenitis reveals insights into gene-regulatory networks contributing to virulence. *BMC Genomics* 2010;**11**:728.
143. Ulivieri S, Oliveri G. Cerebellar abscess due to *Rhodococcus equi* in an immunocompetent patient: case report and literature review. *J Neurosurg Sci* 2006;**50**:127–9.
144. van der Lelie H, Leverstein-Van Hall M, Mertens M, et al. *Corynebacterium* CDC group JK *(Corynebacterium jeikeium)* sepsis in haematological patients: a report of three cases and a systematic literature review. *Scand J Infect Dis* 1995;**27**:581–4.
145. Verville TD, Huycke MM, Greenfield RA, et al. *Rhodococcus equi* infections of humans: 12 cases and a review of the literature. *Medicine (Baltimore)* 1994;**73**:119–32.

146. Wagner J, Ignatius R, Voss S, et al. Infection of the skin caused by *Corynebacterium ulcerans* and mimicking classical cutaneous diphtheria. *Clin Infect Dis* 2001;**33**:1598–600.
147. Wagner KS, White JM, Crowcroft NS, et al. Diphtheria in the United Kingdom, 1986-2008; the increasing role of *Corynebacterium ulcerans*. *Epidemiol Infect* 2010;**138**:1519–30.
148. Watkins DA, Chahine A, Creger RJ, et al. *Corynebacterium striatum*: a diphtheroid with pathogenic potential. *Clin Infect Dis* 1993;**17**:21–5.
149. Weiss K, Labbe AC, Laverdiere M. *Corynebacterium striatum* meningitis: case report and review of an increasingly important *Corynebacterium* species [see comments]. *Clin Infect Dis* 1996;**23**: 1246–8.
150. Williams DY, Selepak ST, Gill VJ. Identification of clinical isolates of nondiphtherial *Corynebacterium* species and their antibiotic susceptibility patterns. *Diagn Microbiol Infect Dis* 1993;**17**:23–8.
151. Wilson WR, Danielson GK, Giuliani ER, et al. Prosthetic valve endocarditis. *Mayo Clin Proc* 1982;**57**:155–61.
152. Wong TP, Groman N. Production of diphtheria toxin by selected isolates of *Corynebacterium ulcerans* and *Corynebacterium pseudotuberculosis*. *Infect Immun* 1984;**43**:1114–16.
153. Yoo SJ, Sung H, Chae JD, et al. *Rhodococcus equi* pneumonia in a heart transplant recipient in Korea, with emphasis on microbial diagnosis. *Clin Microbiol Infect* 2003;**9**:230–3.
154. Young VM, Meyers WF, Moody MR, et al. The emergence of coryneform bacteria as a cause of nosocomial infections in compromised hosts. *Am J Med* 1981;**70**:646–50.

SUBSECTION 4 *GRAM-NEGATIVE BACILLI*

CHAPTER 101

CITROBACTER

Randall G. Fisher

Citrobacter, a genus of enteric gram-negative rods closely related to *Salmonella*, has been associated increasingly with human disease. Although *Citrobacter* strains usually are not considered normal inhabitants of the intestinal tract of humans and animals,[56,92] a study using 16S rDNA polymerase chain reaction (PCR) showed that normal newborns are colonized with these organisms by day 6 of life.[66] They have been associated with urinary tract infections,* osteomyelitis,[52] diarrhea,[18,30,95] and invasive disease in the immunocompromised host.[37,41,52,56] As detailed in a review by Doran,[16] *Citrobacter* commonly is associated with sepsis, meningitis, and brain abscess in neonates.

BACTERIOLOGY

In 1931, Werkman and Gillen[92] proposed the generic term *Citrobacter* for citrate-positive coli-aerogenes intermediates isolated from stool. This genus now includes 11 species, of which the most commonly identified are *Citrobacter freundii*, *Citrobacter koseri* (formerly *Citrobacter diversus*), and *Citrobacter farmeri* (formerly *Citrobacter amalonaticus*).[7] *Citrobacter* are straight, facultatively anaerobic bacilli possessing peritrichous flagellae that confer motility. In addition to using citrate, these organisms hydrolyze urea and ferment glucose, with production of gas.[79] They grow on ordinary media as gray, opaque, round colonies that produce a strong, fetid odor. In contrast to *Salmonella*, *Citrobacter* grows in the presence of potassium cyanide. Indole-negative strains that produce hydrogen sulfide are classified as *C. freundii*. Indole-positive, hydrogen sulfide–negative strains are differentiated by their ability to ferment malonate; *C. koseri* ferments malonate, whereas *C. farmeri* does not.[2] Antigenic schemata have been developed to classify the O somatic antigens of *Citrobacter*[25,29,95]; these antigens show cross reactivity with O antigens of other Enterobacteriaceae.

EPIDEMIOLOGY

Meningitis caused by *Citrobacter* was reported first in 1960, with two cases of *C. freundii*.[34] In the decade from 1970 to 1979, 69 cases of *Citrobacter* meningitis were reported,[26] and 4 percent of neonatal meningitis cases reported in the First Neonatal Meningitis Cooperative Study Group were caused by *Citrobacter*.[61] *C. koseri* is the species usually isolated with meningitis; central nervous system infection caused by *C. freundii* occurs less commonly.[16,26] Most cases in the United States are reported from southern states; biotype d or serotypes O2 and O1[26] are the most common isolates of *C. koseri* encountered.

Most cases of neonatal meningitis caused by this organism have been sporadic. The source of sporadic cases usually is unknown, but nine cases clearly have been documented to be vertically transmitted from mother to infant.[16] In addition, several well-documented nosocomial outbreaks of infection have been reported.* The source usually is the gastrointestinal tract or hands of nursery staff. One cluster was associated with contaminated formula.[86] When *Citrobacter* is introduced into the neonatal nursery, colonization may exceed 79 percent.[25] Parry and associates[67] described a nursery outbreak in which 11 of 128 infants were colonized with *C. koseri* over an observation period of 3 months; 2 of the colonized infants developed meningitis. Additional colonization of neonates seemed to be eliminated by removal of a nurse with persistent hand carriage of the organism.

In another outbreak,[95] introduction of *C. koseri* into the nursery was linked to an infant admitted with meningitis. Thirty-one percent of infants in the nursery subsequent to the index case were found to be colonized with *C. koseri* of the same serotype and biotype. A second infant from this study developed meningitis with the organism during the observation period. Umbilical colonization of infants in this cluster was more common than was rectal colonization, but rectal colonization was more persistent, lasting 4 months. Two nurses were found to

*References 6, 23, 41, 49, 52, 56.

*References 19, 23, 25, 51, 67, 74, 95.

have hand colonization with the organism, and the reintroduction of these bacteria into the nursery was linked to a pregnant nurse who had perineal cultures at delivery yielding the epidemic strain. She was implicated in the colonization of her own infant and three other neonates. Corresponding culture data from a reference hospital revealed an overall neonatal colonization rate with *Citrobacter* of 1 to 10 percent over a 5-year period, with no invasive disease.

Although *Citrobacter* has been isolated increasingly from debilitated adult patients,[93] particularly as a urinary tract,[41,52,56] soft tissue,[17,52,56] and bone[56] pathogen, *Citrobacter* infection in children is unusual after the first 2 months of life. One case of meningitis was reported in a teenager who lacked apparent risk factors or immunodeficiency.[73] In older children, *Citrobacter* spp. are more likely to cause opportunistic infection in an immunocompromised host or, occasionally, urinary tract infection. One study showed *Citrobacter* spp. to be responsible for 37 (1.4%) urinary tract infections in children over a 3-year period.[21] One quarter of infections were nosocomially acquired, and one third occurred in children with urinary tract abnormalities. A more recent study from Kosovo of 299 children with urinary tract infections found that *Citrobacter* spp. caused 5.4 percent of cases, making it the fourth most common cause.[81]

PATHOPHYSIOLOGY

Citrobacter infrequently colonizes the intestinal tract and perineum of humans.[41] Vertical transmission of strains shown to be identical by DNA typing shows that the newborn can acquire colonization at the time of passage through the birth canal of a colonized mother.[35,65] Onset of disease beyond the first week of life is related commonly to colonization of the infant in the nursery. As with other types of gram-negative neonatal meningitis, central nervous system infection results from bacteremia in a colonized infant, leading to seeding of the meninges.

The basis for the particular invasiveness of *Citrobacter* in the neonate and its propensity to cause multiple brain abscesses are largely unexplained. *Citrobacter* spp. possess the ability to invade, transcytose, and multiply within human brain microvascular endothelial cells in vitro.[3] Additionally, in the neonatal rat model, after being taken up into macrophages, *C. koseri* is able to survive phagolysosomal fusion and replicate therein.[87] Many strains of *C. koseri* seem to be able to produce brain pathologic processes in the mouse, but the degree of damage seems to be related to virulence of the strain and the age of the mouse.[47,80] Differences in strains, related to the presence of an outer-membrane protein with a molecular weight of 32,000, have been associated with differences in brain histopathology in one infant rat model of *C. koseri* meningitis.[46] Strains isolated from cerebrospinal fluid of infants with meningitis more commonly possess this outer-membrane protein than do strains isolated from other body sites.[47] In the mouse model, early proinflammatory mediator release was dependent on MyD88 (a signaling adapter of Toll-like receptor 4 [TL4]). MyDD88 knockout mice had enhanced mortality and elevated bacterial burdens versus wild-type mice.[53] In vitro, microglial cells respond with inflammation via TL4 and MyD88.[54]

In an immunocompromised patient, broad use of antimicrobial agents may produce selective pressure, leading to increased colonization with *Citrobacter*. Increased bacterial density combined with a blunted immune response may result in invasive disease. Some strains of *C. freundii* have been shown to produce Shiga toxins (verotoxins) nearly identical to those produced by enterohemorrhagic strains of *Escherichia coli*.[77] At least one outbreak of gastroenteritis and the hemolytic-uremic syndrome caused by Shiga-toxin–producing *C. freundii* has been reported.[89]

Citrobacter spp. contain type 3 fimbriae, which are vital to the production of biofilms.[64] The formation of biofilms in *Citrobacter* isolates is important in the pathogenesis of catheter-associated urinary tract infections.[64]

CLINICAL MANIFESTATIONS

Citrobacter, similar to other neonatal pathogens, can cause early-onset and late-onset infection. In a review of 74 cases of neonatal meningitis caused by these bacteria, the mean age of onset reported for early disease was 7 days; 85 percent of patients were included in this group.[26] Fifteen percent of cases occurred after 3 weeks of age. Twenty-three (31%) of 74 patients were younger than 36 weeks of gestational age at birth, suggesting that preterm infants are at increased risk for acquisition of *Citrobacter* infection. Prematurity is even more common (71%) in cases that are proved to be vertically acquired.[16]

Clinical signs and symptoms are typical of neonatal sepsis. Fever, lethargy, poor feeding, vomiting, irritability, bulging fontanelle, seizures, and jaundice are common presenting features. Umbilical infection and surgical manipulation of colonized umbilical stumps occasionally have preceded development of bacteremia and meningitis.[67] The white blood cell count may show leukocytosis or leukopenia. Cerebrospinal fluid findings are consistent with those of most types of neonatal bacterial meningitis and usually show polymorphonuclear cell elevation, elevated protein, and depressed glucose; gram-negative rods may be seen on smear. Although growing the organism in culture normally is not difficult, in one reported case standard cultures were negative, but the organism was recovered by direct inoculation of cerebrospinal fluid into a BACTEC blood culture bottle.[14] Of *Citrobacter* meningitis cases in which the results of blood cultures are reported, 80 percent document concurrent bacteremia.[16]

Citrobacter is a particularly devastating cause of neonatal meningitis. The most common *Citrobacter* spp. causing neonatal meningitis is *C. koseri*, which accounts for more than 80 percent of the cases.[16] Central nervous system infection with this organism produces multiple brain abscesses with unusually high frequency.[26,32,42,47,50] In the extensive reviews by Graham and Band[26] and by Doran,[16] three quarters of *Citrobacter* meningitis cases resulted in intracerebral abscesses. By comparison, the incidence of abscess formation in non-*Citrobacter*

gram-negative meningitis is reported to be as low as 10 percent.[26] The case-fatality rate for *Citrobacter* meningitis is approximately 30 percent, and at least three quarters of surviving infants have neurologic sequelae, such as mental retardation, hemiparesis, seizures, and developmental delay.[16]

The presence of brain abscess seems to contribute significantly to morbidity and mortality.[15,25,26] For unknown reasons, neonates with vertically acquired *Citrobacter* meningitis seem to be less likely to develop intracerebral abscesses.[16] At least three cases in which diffuse pneumocephalus developed in association with *Citrobacter* meningitis have been reported; in one, gas was noted to accumulate within the brain and in the anterior chamber of the eye, a condition known as *pneumatosis oculi*.[1,72] Rarely, *Citrobacter* infection in the neonatal period may lead to focal infection not involving the central nervous system. A case of septic arthritis and osteomyelitis of the shoulder in a 3-week-old infant has been reported.[36]

In adults, *Citrobacter* is isolated most commonly from the urinary tract.[41,52,56] In earlier studies, 5 to 12 percent of bacterial isolates from urinary tract infections in adult patients were *Citrobacter*.[18,94] More recently, in a health maintenance organization, *Citrobacter* spp. accounted for only 0.8 percent of 4342 isolates from women with acute uncomplicated cystitis.[31] *Citrobacter* spp. are a similarly uncommon cause of urinary tract infection in children.[21] Sputum is the second most common clinical specimen to yield *Citrobacter* in adults[41]; lung abscess,[22] pneumonia,[52,56] bronchitis,[37] and septic arthritis[52] have been reported. *Citrobacter* is an occasional cause of bacteremia in hospitalized patients, accounting for approximately 0.5 percent of blood culture isolates.[70,78] In one series, all 45 patients had at least one underlying disease, with malignancies (particularly intra-abdominal tumors) and hepatobiliary stones being the most frequent coexisting conditions.[78] Polymicrobial bacteremia occurred in one third of patients. The case-fatality rate was 18 percent. In a series of 6 bacteremic patients who had *Citrobacter* species other than *freundii* or *koseri*, 5 of the 6 had associated intra-abdominal infection; cancer and cirrhosis were the most common underlying diseases.[48]

Gastrointestinal disease occasionally has been attributed to *Citrobacter*, but frequent isolation of this agent from normal stools often renders this diagnosis equivocal. This genus was implicated first in an outbreak of mild gastroenteritis by Barnes and Cherry[5] in 1946, and an outbreak of watery diarrhea in a Virginia infant care unit included two infants in whom isolates of enterotoxin-liberating *Citrobacter* were obtained from the stool.[30] Some studies have found higher incidences of *Citrobacter* isolation from the stool of patients with enterocolitis syndrome than from stools of control patients.[95] Shiga-toxin (verotoxin)-producing *C. freundii* isolated from organically grown parsley was associated with an outbreak of diarrhea and hemolytic-uremic syndrome in a daycare setting.[89] *C. freundii* has been found as a cause of appendicitis in a healthy adult,[52] peritonitis in adults with liver disease or pancreatitis,[52] neutropenic colitis after chemotherapy for breast cancer,[13] and meningitis in adults as a complication of neurosurgery.[85] A case of Meleney gangrene occurring after cesarean section delivery has been reported.[84]

A patient with diabetes developed necrotizing fasciitis caused by *C. freundii* associated with injury from a fish fin.[12] Bone and soft tissue infections occur[8,27,82]; 3 percent of *Citrobacter* pathogens were isolated from joint or bone in one adult series.[52] One 53-year-old man developed infection 3 weeks after total hip arthroplasty, a condition normally expected to be caused by *S. aureus*.[43] Eye infections, including keratitis,[24] traumatic endophthalmitis,[9] and endogenous endophthalmitis[11] have been reported. A case of *C. freundii* pharyngitis complicated by retropharyngeal abscess and mediastinitis was cured by minimally invasive surgery and intravenous antibiotics.[88]

DIAGNOSIS

Biochemical characteristics of *C. koseri* include lack of hydrogen sulfide production on triple-sugar iron agar, negative Voges-Proskauer reaction, use of citrate, motility, production of indole, decarboxylation of ornithine but not lysine, and production of acid from adonitol.[2] Identification of this organism as a pathogen in a nursery setting should heighten suspicion of its possible role in subsequent neonatal infections. *C. freundii* is indole-negative and hydrogen sulfide–positive, which differentiates it from *C. koseri*. *C. farmeri* differs from *C. koseri* because of the former's inability to ferment malonate. *C. freundii* and *C. farmeri* account for a significant portion of disease caused by *Citrobacter* in immunocompromised individuals and should be suspected particularly in this group of patients.[37] In some cases, *C. freundii* can be misidentified as *Cronobacter* spp. by commercial systems.[20]

Infants with invasive *Citrobacter* disease present in a similar fashion to infants with sepsis and meningitis of other causes. Such infants should undergo a thorough evaluation, including blood and urine culture and cerebrospinal fluid studies. Brain imaging studies should be done when the diagnosis is established. Computed tomography is the most common test used, although ultrasound often is more feasible for an unstable neonate and may be nearly as sensitive in detecting abscesses.[50,62,96] Serial imaging studies should be done because abscesses may develop during the first few weeks of illness. In cases in which the cerebrospinal fluid cultures are negative because of prior antimicrobial therapy, surgical aspiration of abscesses sometimes enables identification of the organism.

TREATMENT

Most *C. koseri* organisms are resistant to ampicillin (97% in one series)[52] and sensitive to aminoglycosides and third-generation cephalosporins.[16] A 4-year experience with neonatal septicemia caused by *C. koseri* has been described, however, in which all of 13 isolates were resistant to gentamicin but susceptible to third-generation cephalosporins.[19] The resistance patterns of *C. freundii* were reported in a national surveillance study of nosocomial bloodstream infections.[70] Of the 23 *C. freundii*

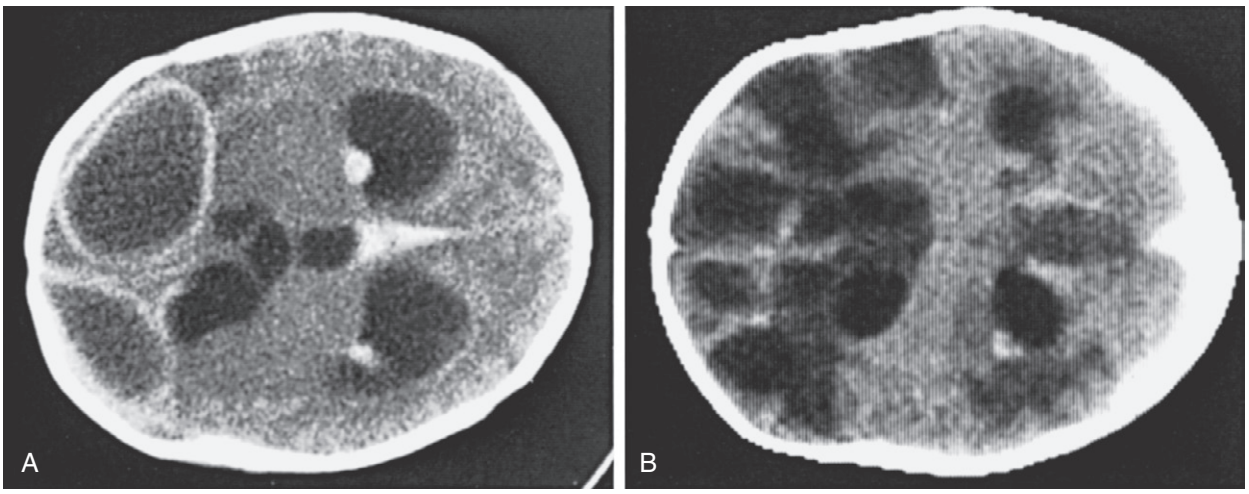

FIGURE 101-1 ■ Computed tomography scans showing progressive abscess formation and encephalomalacia in an infant at 3 weeks of age **(A)** and 6 weeks of age **(B),** despite bacteriologic "cure" of *Citrobacter* meningitis.

isolates tested, resistance to piperacillin, piperacillin/tazobactam, ceftriaxone, and ceftazidime was a common (39% to 48%) finding. Isolates generally were susceptible to the aminoglycosides and ciprofloxacin (91% to 96%), and all *C. freundii* tested were susceptible to cefepime and imipenem.

Some *Citrobacter* isolates contain chromosomally mediated group I β-lactamases. These bacteria possess a gene that, when triggered by exposure to cephalosporins or by spontaneous mutation, produces a cephalosporinase capable of inactivating cephalosporins.[38] Clinically, it manifests as treatment failure and emergence of drug resistance to various cephalosporins despite initial susceptibility.[57] In one study, the presence of group I β-lactamases was much more common with *C. freundii* (9/22 isolates) than with *C. koseri* (0/7 isolates).[38] Resistance was associated with previous receipt of an extended-spectrum, β-lactam antibiotic. Reliable estimates of the percentage of *Citrobacter* strains that contain the chromosomal resistance gene are difficult to obtain because most studies lump *Citrobacter*, *Enterobacter*, and *Serratia* isolates together. In one Korean study, of 152 *Enterobacter/Citrobacter/Serratia* isolates, 45 (30%) were derepressed AmpC mutants.[66] In an in vitro study, AmpC production was inducible in 8/9 clinical isolates of *C. freundii* and in 1/3 isolates of *C. koseri*. AmpC synthesis was stably derepressed in 1/9 *C. freundii* isolates.[17] Cefepime seems to be less likely to induce production of these β-lactamases and more resistant to hydrolysis by them,[76] although a highly cefepime-resistant strain has been described.[4] Of 3030 ceftazidime-resistant Enterobacteriaceae in a U.S. study, 99 percent retained susceptibility to imipenem and 96.7 percent of ceftazidime-resistant *Citrobacter* isolates were susceptible to cefepime.[71] Many different types of β-lactamases, including a novel TEM-type (TEM-134),[68] the class A β-lactamase CKO,[69] and a VIM-1 metallo-β-lactamase, have been described in various isolates of *Citrobacter*.[91] More recently, carbapenem resistance resulting from *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) has been described.[10,58] Quinolone resistance via *qnr* genes has also been found in *C. koseri*[40]; in one study from Korea, 29 percent of *C. freundii* isolates were quinolone resistant. Most of these had *qnrB* genes.[39] Extensively resistant isolates are more likely to be sensitive to colistin[55] and polymyxin B[97] than to tigecycline.

Treatment of *Citrobacter* meningitis often requires a multidisciplinary effort involving the neurosurgeon and the pediatrician. Although cerebral abscesses usually are aspirated or drained surgically, some patients are treated with antibiotics alone and neither approach is clearly shown to be superior. When abscesses are inaccessible or small and not progressive, conservative management may be considered.[16] Ventriculostomy and craniectomy with open drainage of abscesses have been required in some children to effect bacteriologic cure, and placement of a shunt for hydrocephalus often is required.

Generally, antibiotic therapy for gram-negative neonatal meningitis has proved disappointing (see Chapter 66).[59,60] No evidence supports one combination of antibiotics over another in the treatment of *Citrobacter* meningitis. Usually a third-generation or fourth-generation cephalosporin or a carbapenem (usually meropenem) is used in combination with an aminoglycoside initially.[16] Chloramphenicol,[15,25,33] imipenem-cilastatin,[18,33] and trimethoprim-sulfamethoxazole[28] also have been used successfully.

Poor meningeal penetration of aminoglycosides in addition to the presence of intracranial abscesses render antibiotic therapy for *Citrobacter* meningitis especially difficult. The ability of this organism to persist in the brain is shown by its recovery 4 years after neonatal infection.[18] Cranial computed tomography usually is used for evaluation of complications such as hydrocephalus and multicystic encephalomalacia (Fig. 101-1). Administration of antibiotics intrathecally or directly into abscess cavities has been tried but has not been shown convincingly to be beneficial.* In a randomized controlled trial, intraventricular administration of gentamicin in the treatment of neonates with gram-negative meningitis was associated with a poorer outcome.[61]

*References 28, 29, 32, 45, 51, 63, 75, 83, 90.

Neonates with gram-negative meningitis should undergo repeat lumbar puncture approximately 72 hours after beginning therapy to document sterilization of cerebrospinal fluid. Duration of therapy with intravenous antibiotics generally is a minimum of 21 days for gram-negative neonatal meningitis. For cases complicated by intracranial abscesses, prolonged therapy (usually 4 to 6 weeks after sterilization of cerebrospinal fluid) is indicated.[16,44]

Scrupulous attention given to preventive infection control practices has been recommended to stem nursery outbreaks.[25,67,95] These prophylactic measures include care of skin and the umbilical cord, elimination of crowding with isolation of infected infants and carriers, and good hand washing practices. Exclusion of colonized personnel and temporary closing of the nursery have been followed by a reduction in neonatal *C. koseri* colonization. Although cohorting of colonized infants is a reasonable practice, multiple sources of introduction of *Citrobacter* may limit the efficacy of this approach in some outbreaks.[23]

Treatment of *Citrobacter* infection beyond the neonatal period requires choice of an appropriate antibiotic, with drainage of abscesses and appropriate débridement of wounds. Therapy should be guided by antimicrobial susceptibility testing. Outcome depends largely on the preceding debility of the host and location of the infection. Significant mortality is associated with immunocompromised patients with septicemia or pulmonary disease.[37,41,52,56,78]

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Chen KJ. Diffuse retinal vasculitis in *Citrobacter koseri* traumatic endophthalmitis with intraocular foreign body. *J Trauma* 2010; **68**:504.
10. Chen S, Hu F, Liu Y, et al. Detection and spread of carbapenem-resistant *Citrobacter freundii* in a teaching hospital in China. *Am J Infect Control* 2011;**39**:e55–60.
11. Chiu CH, Peng MY, Wang YC, Chang FY. Endogenous endophthalmitis caused by *Citrobacter koseri*. *Am J Med Sci* 2009;**338**: 509–10.
20. Giammanco GM, Aleo A, Guida I, Mammina C. Molecular epidemiological survey of *Citrobacter freundii* misidentified as *Cronobacter* spp (*Enterobacter sakazakii*) and *Enterobacter hormaechei* isolated from powdered infant milk formula. *Foodborne Pathog Dis* 2011;**8**: 517–25.
24. Goold LA, Warrier SK, Wittles NK, Nathan F. Microbial keratitis secondary to infection with *Citrobacter koseri*. *Cornea* 2010;**29**:479.
39. Jeong HS, Bae IK, Shin JH, et al. Fecal colonization of Enterobacteriaceae carrying plasmid-mediated quinolone resistance determinants in Korea. *Microb Drug Resist* 2011;**17**:507–12.
40. Jeong HS, Bae IK, Shin JH, et al. Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and AmpC beta-lactamase in Enterobacteriaceae. *Korean J Lab Med* 2011;**31**:257–64.
43. Kaufmann AM, Watters TS, Henderson RA, Wellman SS, Bolognesi MP. *Citrobacter koseri* as a cause of early periprosthetic infection after primary total hip arthroplasty. *J Arthroplasty* 2011; **978**:e13–16.
48. Lai CC, Tan CK, Lin SH, et al. Bacteremia caused by non-freundii, non-*koseri Citrobacter* species in Taiwan. *J Hosp Infect* 2010;**76**: 332–5.
64. Ong CL, Beatson SA, Totsika M, et al. Molecular analysis of type 3 fimbrial genes from *Escherichia coli*, *Klebsiella* and *Citrobacter* species. *BMC Microbiol* 2010;**24**:183.
81. Spahiu L, Hasbahta V. Most frequent causes of urinary tract infections in children. *Med Arh* 2010;**64**:88–90.
97. Zhang R, Cai JC, Zhou HW, et al. Genotypic characterization and in vitro activities of tigecycline and polymyxin B for members of the Enterobacteriaceae with decreased susceptibility to carbapenems. *J Med Microbiol* 2011;**60**:1813–19.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 102

ENTEROBACTER

Laura A. Sass • Randall G. Fisher

Enterobacter is a genus of Enterobacteriaceae that is an increasingly frequent cause of health care–associated pediatric infection. *Enterobacter* can cause infection of postsurgical wounds, meningitis, and infection of the gastrointestinal, urinary, and respiratory tracts. Development of resistance to antibiotics commonly used for treatment of infection is an increasingly common challenge. For a more detailed overview of *Enterobacter* spp., the reader is referred to the review by Sanders and Sanders.[170]

REFERENCES

1. Altmann G, Sechter I, Cahan D, et al. *Citrobacter diversus* isolated from clinical material. *J Clin Microbiol* 1976;**3**:390–2.
2. Alviedo JN, Sood BG, Aranda JV, Becker C. Diffuse pneumocephalus in neonatal *Citrobacter* meningitis. *Pediatrics* 2006;**118**: e1576–9.
3. Badger JL, Stins MF, Kim KS. *Citrobacter freundii* invades and replicates in human brain microvascular endothelial cells. *Infect Immun* 1999;**67**:4208–15.
4. Barnaud G, Labia R, Raskine L, et al. Extension of resistance to cefepime and cefpirome associated to a six amino acid deletion in the H-10 helix of the cephalosporinase of an *Enterobacter cloacae* clinical isolate. *FEMS Microbiol Lett* 2001;**195**:185–90.
5. Barnes LA, Cherry CB. A group of paracolon organisms having apparent pathogenicity. *Am J Public Health* 1946;**36**:481–3.
6. Barton LL, Walentik C. *Citrobacter diversus* urinary tract infection. *Am J Dis Child* 1982;**136**:467–8.
7. Brenner DJ, O'Hara CM, Grimont PA, et al. Biochemical identification of *Citrobacter* species defined by DNA hybridization and description of *Citrobacter gillenii* sp. nov. (formerly *Citrobacter* genomospecies 10) and *Citrobacter murliniae* sp. nov. (formerly *Citrobacter genomospecies* 11). *J Clin Microbiol* 1999;**37**:2619–24.
8. Bruehl CL, Listernick R. *Citrobacter freundii* septic arthritis. *J Paediatr Child Health* 1992;**28**:402–3.
9. Chen KJ. Diffuse retinal vasculitis in *Citrobacter koseri* traumatic endophthalmitis with intraocular foreign body. *J Trauma* 2010;**68**:504.
10. Chen S, Hu F, Liu Y, Zhu D, Wang H, Zhang Y. Detection and spread of carbapenem-resistant *Citrobacter freundii* in a teaching hospital in China. *Am J Infect Control* 2011;**39**:e55–60.
11. Chiu CH, Peng MY, Wang YC, Chang FY. Endogenous endophthalmitis caused by Citrobacter koseri. *Am J Med Sci* 2009;**338**: 509–10.
12. Chuang YM, Tseng SP, Teng LJ, et al. Emergence of cefotaxime resistance in *Citrobacter freundii* causing necrotizing fasciitis and osteomyelitis. *J Infect* 2006;**53**:e161–3.
13. Clemons MJ, Valle JW, Harris M, et al. *Citrobacter freundii* and fatal neutropenic enterocolitis following adjuvant chemotherapy for breast cancer. *Clin Oncol (R Coll Radiol)* 1997;**9**:172–5.
14. Cohen-Wolkowicz M, Laufer M. Enhanced culture detection of *Citrobacter koseri* from cerebrospinal fluid in BacTec. *Pediatr Infect Dis J* 2005;**24**:750.
15. Curless RG. Neonatal intracranial abscess: two cases caused by *Citrobacter* and a literature review. *Ann Neurol* 1980;**8**:269–72.
16. Doran TI. The role of *Citrobacter* in clinical disease of children: Review. *Clin Infect Dis* 1999;**28**:384–94.
17. Dunne WM, Hardin DJ. Use of several inducer and substrate antibiotic combinations in a disk approximation assay format to screen for AmpC induction in patient isolates of *Pseudomonas aeruginosa*, *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp. *J Clin Microbiol* 2005;**43**:5945–9.
18. Eppes SC, Woods CR, Mayer AS, et al. Recurring ventriculitis due to *Citrobacter diversus*: clinical and bacteriologic analysis. *Clin Infect Dis* 1993;**17**:437–40.
19. Giacoia GP, West K. Sepsis with *Citrobacter diversus* in sick newborns. *Am J Perinatol* 1989;**6**:49–54.
20. Giammanco GM, Aleo A, Guida I, Mammina C. Molecular epidemiological survey of *Citrobacter freundii* misidentified as *Cronobacter* spp (*Enterobacter sakazakii*) and *Enterobacter hormaechei* isolated from powdered infant milk formula. *Foodborne Pathog Dis* 2011;**8**:517–25.
21. Gill MA, Schutze GE. *Citrobacter* urinary tract infections in children. *Pediatr Infect Dis J* 1999;**18**:889–92.
22. Gilman RM, Irwin RS, Garrity FL. Community-acquired *Citrobacter diversus* infections. *Respir Care* 1980;**25**:66–71.
23. Goering RV, Ehrenkranz NJ, Sanders CC, et al. Long term epidemiological analysis of *Citrobacter diversus* in a neonatal intensive care unit. *Pediatr Infect Dis J* 1992;**11**:99–104.
24. Goold LA, Warrier SK, Wittles NK, Nathan F. Microbial keratitis secondary to infection with *Citrobacter koseri*. *Cornea* 2010;**29**:479.
25. Graham DR, Anderson RL, Ariel FE, et al. Epidemic nosocomial meningitis due to *Citrobacter diversus* in neonates. *J Infect Dis* 1981; **144**:203–9.
26. Graham DR, Band JD. *Citrobacter diversus* brain abscess and meningitis in neonates. *JAMA* 1981;**245**:1923–5.
27. Grant MD, Horowitz HI, Lorian V. Gangrenous ulcer and septicemia due to *Citrobacter*. *N Engl J Med* 1969;**280**:1286–7.
28. Greene GR, Heitlinger L, Madden JD. *Citrobacter ventriculitis* in a neonate responsive to trimethoprim-sulfamethoxazole. *Clin Pediatr (Phila)* 1983;**22**:515–17.
29. Gross RJ, Rowe B, Easton JA. Neonatal meningitis caused by *Citrobacter koseri*. *J Clin Pathol* 1973;**26**:138–9.
30. Guerrant RL, Dickens MD, Wenzel RP, et al. Toxigenic bacterial diarrhea: nursery outbreak involving multiple bacterial strains. *J Pediatr* 1976;**89**:885–91.
31. Gupta K, Scholes D, Stamm WE. Increasing prevalence of antimicrobial resistance among uropathogens causing acute uncomplicated cystitis in women [see comments]. *JAMA* 1999;**281**: 736–8.
32. Gwynn CM, George RH. Neonatal *Citrobacter* meningitis. *Arch Dis Child* 1973;**48**:455–8.
33. Haimi-Cohen Y, Amir J, Weinstock A, et al. The use of imipenem-cilastatin in neonatal meningitis caused by *Citrobacter diversus*. *Acta Paediatr* 1993;**82**:530–2.
34. Harris D, Cone TE Jr. *Escherichia freundii* meningitis: report of two cases. *J Pediatr* 1960;**56**:774–7.
35. Harvey BS, Koeuth T, Versalovic J, et al. Vertical transmission of *Citrobacter diversus* documented by DNA fingerprinting. *Infect Control Hosp Epidemiol* 1995;**16**:564–9.
36. Hayani KC. *Citrobacter koseri* osteomyelitis in an infant. *Acta Paediatr Jpn* 1997;**39**:390–1.
37. Hodges GR, Degener CE, Barnes WG. Clinical significance of *Citrobacter* isolates. *Am J Clin Pathol* 1978;**70**:37–40.
38. Jacobson KL, Cohen SH, Inciardi JF, et al. The relationship between antecedent antibiotic use and resistance to extended-spectrum cephalosporins in group I beta-lactamase-producing organisms. *Clin Infect Dis* 1995;**21**:1107–13.
39. Jeong HS, Bae IK, Shin JH, et al. Fecal colonization of Enterobacteriaceae carrying plasmid-mediated quinolone resistance determinants in Korea. *Microb Drug Resist* 2011;**17**:507–12.
40. Jeong HS, Bae IK, Shin JH, et al. Prevalence of plasmid-mediated quinolone resistance and its association with extended-spectrum beta-lactamase and AmpC beta-lactamase in Enterobacteriaceae. *Korean J Lab Med* 2011;**31**:257–64.
41. Jones SR, Ragsdale AR, Kutscher E, et al. Clinical and bacteriologic observations on a recently recognized species of Enterobacteriaceae, *Citrobacter diversus*. *J Infect Dis* 1973;**128**:563–5.
42. Kaplan AM, Itabashi HH, Yoshimori R, et al. Cerebral abscesses complicating neonatal *Citrobacter freundii* meningitis. *West J Med* 1977;**127**:418–22.
43. Kaufmann AM, Watters TS, Henderson RA, et al. *Citrobacter koseri* as a cause of early periprosthetic infection after primary total hip arthroplasty. *J Arthroplasty* 2011;**978**:e13–16.
44. Kline MW. *Citrobacter* meningitis and brain abscess in infancy: epidemiology, pathogenesis, and treatment. *J Pediatr* 1988;**113**: 430–4.
45. Kline MW, Kaplan SL. *Citrobacter diversus* and neonatal brain abscess. *Pediatr Neurol* 1987;**3**:178–80.
46. Kline MW, Kaplan SL, Hawkins EP, et al. Pathogenesis of brain abscess formation in an infant rat model of *Citrobacter diversus* bacteremia and meningitis. *J Infect Dis* 1988;**157**:106–12.
47. Kline MW, Mason EO Jr, Kaplan SL. Characterization of *Citrobacter diversus* strains causing neonatal meningitis. *J Infect Dis* 1988;**157**:101–5.
48. Lai CC, Tan CK, Lin SH, et al. Bacteremia caused by non-*freundii*, non-*koseri Citrobacter* species in Taiwan. *J Hosp Infect* 2010;**76**: 332–5.
49. Lesseva MI, Hadjiski OG. Analysis of bacteriuria in patients with burns. *Burns* 1995;**21**:3–6.
50. Levine RS, Rosenberg HK, Zimmerman RA, et al. Complications of *Citrobacter* neonatal meningitis: assessment by real-time cranial sonography correlated with CT. *AJNR Am J Neuroradiol* 1983;**4**: 668–71.
51. Lin FC, Devoe WF, Morrison C, et al. Outbreak of neonatal *Citrobacter diversus* meningitis in a suburban hospital. *Pediatr Infect Dis J* 1987;**6**:50–5.
52. Lipsky BA, Hook EW 3rd, Smith AA, et al. *Citrobacter* infections in humans: experience at the Seattle Veterans Administration Medical Center and a review of the literature. *Rev Infect Dis* 1980;**2**:746–60.

53. Liu S, Kielian T. Microglial activation by *Citrobacter koseri* is mediated by TLR4- and MyDD88-dependent pathways. *J Immunol* 2009;**183**:5537–47.
54. Liu S, Kielian T. MyD88 is pivotal for immune recognition of *Citrobacter koseri* and astrocyte activation during CNS infection. *J Neuroinflammation* 2011;**8**:35.
55. Livermore DM, Warner M, Mushtag S, et al. What remains against carbapenem-resistant Enterobacteriaceae? Evaluation of chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline. *Int J Antimicrob Agents* 2011;**37**:415–19.
56. Madrazo A, Geiger J, Lauter CB. *Citrobacter diversus* at Grace Hospital, Detroit, Michigan. *Am J Med Sci* 1975;**270**:497–501.
57. Marshall WF, Blair JE. The cephalosporins. *Mayo Clin Proc* 1999; **74**:187–95.
58. Mavroidi A, Neonakis K, Liakopoulos A, et al. Detection of *Citrobacter koseri* carrying beta-lactamase KPC-2 in a hospitalized patient, Greece, July 2011. *Euro Surveill* 2011;**16**:pii.19990.
59. McCracken GH Jr. New developments in the management of children with bacterial meningitis. *Pediatr Infect Dis* 1984;**3**:S32–4.
60. McCracken GH Jr, Mize SG. A controlled study of intrathecal antibiotic therapy in gram-negative enteric meningitis of infancy: report of the neonatal meningitis cooperative study group. *J Pediatr* 1976;**89**:66–72.
61. McCracken GH Jr, Mize SG, Threlkeld N. Intraventricular gentamicin therapy in gram-negative bacillary meningitis of infancy: report of the Second Neonatal Meningitis Cooperative Study Group. *Lancet* 1980;**1**:787–91.
62. Meier A, Chusid MJ, Sty JR. Neonatal *Citrobacter* meningitis: neurosonographic observations. *J Ultrasound Med* 1998;**17**: 399–401.
63. Morgan MG, Stuart C, Leanord AT, et al. *Citrobacter diversus* brain abscess: case reports and molecular epidemiology. *J Med Microbiol* 1992;**36**:273–8.
64. Ong CL, Beatson SA, Totsika M, et al. Molecular analysis of type 3 fimbrial genes from *Escherichia coli*, *Klebsiella* and *Citrobacter* species. *BMC Microbiol* 2010;**24**:183.
65. Papasian CJ, Kinney J, Coffman S, et al. Transmission of *Citrobacter koseri* from mother to infant documented by ribotyping and pulsed-field gel electrophoresis. *Diagn Microbiol Infect Dis* 1996;**26**:63–7.
66. Park HK, Shim SS, Kim SY, et al. Molecular analysis of colonized bacteria in a human newborn infant gut. *J Microbiol* 2005;**43**: 345–53.
67. Parry MF, Hutchinson JH, Brown NA, et al. Gram-negative sepsis in neonates: a nursery outbreak due to hand carriage of *Citrobacter diversus*. *Pediatrics* 1980;**65**:1105–9.
68. Perilli M, Mugnaioli C, Luzzaro F, et al. Novel TEM-type extended-spectrum beta-lactamase, TEM-134, in a *Citrobacter koseri* clinical isolate. *Antimicrob Agents Chemother* 2005;**49**: 1564–6.
69. Petrella S, Renard M, Ziental-Gelus N, et al. Characterization of the chromosomal class A beta-lactamase CKO from *Citrobacter koseri*. *F E M S Micribiol Lett* 2006;**254**:285–92.
70. Pfaller MA, Jones RN, Marshall SA, et al. Inducible amp C beta-lactamase producing gram-negative bacilli from blood stream infections: frequency, antimicrobial susceptibility, and molecular epidemiology in a national surveillance program (SCOPE). *Diagn Microbiol Infect Dis* 1997;**28**:211–19.
71. Pfaller MA, Sader HS, Fritsche TR, Jones RN. Antimicrobial activity of cefepime tested against ceftazidime-resistant Gram-negative clinical strains from North American Hospitals: report from the SENTRY Antimicrobial Surveillance Program (1998-2004). *Diagn Microbiol Infect Dis* 2006;**56**:63–8.
72. Pooboni SK, Mathur SK, Dux A, et al. Pneumocephalus in neonatal meningitis: diffuse, necrotizing meningoencephalitis in *Citrobacter* meningitis presenting with pneumatosis oculi and pneumocephalus. *Pediatr Crit Care Med* 2004;**5**:393–5.
73. Prais D, Nussinovitch M, Harel L, Amir I. *Citrobacter koseri* (*diversus*) meningitis in an otherwise healthy adolescent. *Scand J Infect Dis* 2003;**35**:202–4.
74. Ribeiro CD, Davis P, Jones DM. *Citrobacter koseri* meningitis in a special care baby unit. *J Clin Pathol* 1976;**29**:1094–6.
75. Rose SJ. Neonatal meningitis due to *Citrobacter koseri*. *J Perinat Med* 1979;**7**:273–5.
76. Sanders WE Jr, Tenney JH, Kessler RE. Efficacy of cefepime in the treatment of infections due to multiply resistant *Enterobacter* species. *Clin Infect Dis* 1996;**23**:454–61.
77. Schmidt H, Montag M, Bockemuhl J, et al. Shiga-like toxin II-related cytotoxins in *Citrobacter freundii* strains from humans and beef samples. *Infect Immun* 1993;**61**:534–43.
78. Shih CC, Chen YC, Chang SC, et al. Bacteremia due to *Citrobacter* species: significance of primary intraabdominal infection. *Clin Infect Dis* 1996;**23**:543–9.
79. Smith RF, Dayton SL, Chipps DD. Recognition of *Citrobacter diversus* in the clinical laboratory. *Appl Microbiol* 1973;**25**:157–8.
80. Soriano AL, Russell RG, Johnson D, et al. Pathophysiology of *Citrobacter diversus* neonatal meningitis: comparative studies in an infant mouse model. *Infect Immun* 1991;**59**:1352–8.
81. Spahiu L, Hasbahta V. Most frequent causes of urinary tract infections in children. *Med Arh* 2010;**64**:88–90.
82. Stricker T, Frohlich S, Nadal D. Osteomyelitis and septic arthritis due to *Citrobacter freundii* and *Haemophilus influenzae* type b. *J Paediatr Child Health* 1998;**34**:90–1.
83. Tamborlane WVF, Soto EV. Experience and reason: briefly recorded—*Citrobacter diversus* meningitis. A case report. *Pediatrics* 1975;**55**:739–41.
84. Tandon A, Sharma D, Dawar P, et al. Conservative debridement of postoperative Meleney's gangrene following a caesarean section. *J Wound Care* 2006;**15**:445–6.
85. Tang LM, Chen ST, Lui TN. *Citrobacter* meningitis in adults. *Clin Neurol Neurosurg* 1994;**96**:52–7.
86. Thurm V, Gericke B. Identification of infant food as a vehicle in a nosocomial outbreak of *Citrobacter freundii*: epidemiological subtyping by allozyme, whole-cell protein and antibiotic resistance. *J Appl Bacteriol* 1994;**76**:553–8.
87. Townsend SM, Gonzalez-Gomez I, Badger JL. fliP influences *Citrobacter koseri* macrophage uptake, cytokine expression and brain abscess formation in the neonatal rat. *J Med Microbiol* 2006;**55**: 1631–40.
88. Trinidade A, Sekhawat V, Andreou Z, et al. *Citrobacter freundii* causing pharyngitis and secondary retropharyngeal abscess with intrathoracic extension to the diaphragm: minimally invasive management of a rare case. *J Laryngol Otol* 2010;**124**:e4.
89. Tschape H, Prager R, Streckel W, et al. Verotoxinogenic *Citrobacter freundii* associated with severe gastroenteritis and cases of haemolytic uraemic syndrome in a nursery school: green butter as the infection source. *Epidemiol Infect* 1995;**114**:441–50.
90. Vogel LC, Ferguson L, Gotoff SP. *Citrobacter* infections of the central nervous system in early infancy. *J Pediatr* 1978;**93**:86–8.
91. Weile J, Gfroer S, Schroeppel K, et al. First detection of a VIM-1 metallo-beta-lactamase in a carbapenem-resistant *Citrobacter freundii* clinical isolate in an acute hospital in Germany. *Scand J Infect Dis* 2007;**39**:264–6.
92. Werkman CH, Gillen GF. Bacteria producing trimethylene glycol. *J Bacteriol* 1932;**23**:167–82.
93. Werthamer S, Weiner M. Subacute bacterial endocarditis due to *Flavobacterium meningosepticum*. *Am J Clin Pathol* 1972;**57**: 410–12.
94. Wientzen RL, McCracken GH Jr, Petruska ML, et al. Localization and therapy of urinary tract infections of childhood. *Pediatrics* 1979;**63**:467–74.
95. Williams WW, Mariano J, Spurrier M, et al. Nosocomial meningitis due to *Citrobacter diversus* in neonates: new aspects of the epidemiology. *J Infect Dis* 1984;**150**:229–35.
96. Wilson DA, Nguyen DL, Marshall K. Sonography of brain abscesses complicating *Citrobacter* neonatal meningitis. *Am J Perinatol* 1988;**5**:37–9.
97. Zhang R, Cai JC, Zhou HW, et al. Genotypic characterization and in vitro activities of tigecycline and polymyxin B for members of the Enterobacteriaceae with decreased susceptibility to carbapenems. *J Med Microbiol* 2011;**60**:1813–19.

BACTERIOLOGY

Enterobacter spp. are named for their enteric recovery as gram-negative bacteria.[165] They commonly are found in soil, water, and sewage. *Enterobacter* spp. also cause botanical disease. These organisms are facultatively anaerobic and motile by peritrichous flagella, with the exception of *Enterobacter asburiae*. Most species yield positive results on malonate, citrate, sucrose fermentation, and Voges-Proskauer tests. *Enterobacter sakazakii* is distinguishable from the other species by its yellow-pigmented colonies; it has recently been renamed *Cronobacter sakazakii*.[86] Taxonomic studies have led to several classifications of species contained in this genus. With the reclassification of *Enterobacter agglomerans* as *Pantoea agglomerans* (see Chapter 114), *Enterobacter cloacae* and *Enterobacter aerogenes* are the most common species recovered from clinical material.*

Additional species in the *Enterobacter* genus rarely recovered from human infections include *Enterobacter amnigenus*, *Enterobacter asburiae*, *Enterobacter gergoviae*, *Enterobacter cancerogenus* (formerly *Enterobacter taylorae*), *Enterobacter kobei* (formerly National Institutes of Health group 21),[102] *E. cloacae* subsp. dissolvens (formerly *Enterobacter dissolvens*), *Enterobacter nimipressuralis*, and *Enterobacter hormaechei*.† NIH group 42 strains now have become *Enterobacter cowanii*.[85] *Enterobacter intermedius* now has been reclassified to *Kluyvera intermedia* (formerly *Kluyvera cochleae*).[148] *Enterobacter ludwigii* is another new species isolated from clinical specimens that is closely related to the *E. cloacae* complex.[83]

EPIDEMIOLOGY

Enterobacter is encountered most commonly as a hospital-acquired pathogen in patients with chronic illness.‡ A surveillance study at 50 American medical centers showed *Enterobacter* as the eighth most common cause of health care–associated bloodstream infections, accounting for 230/4725 (5%) isolates.[155] Of these, 71 percent were *E. cloacae*, 23 percent were *E. aerogenes*, and 6 percent were other species of *Enterobacter*. *Enterobacter* infections are particularly common in intensive care units (ICUs).[129] A report from the Centers for Disease Control and Prevention (CDC) describing the epidemiology of health care–associated infections in 61 pediatric ICUs in the United States revealed that *Enterobacter* spp. were isolated with increasing frequency from patients with pneumonia and were the most common gram-negative isolates from bloodstream infections.[88] The frequency of *Enterobacter* spp. increased from 7 to 12 percent of reported pathogens over a 6-year period. *Enterobacter* spp. also were found to be second only to *Pseudomonas aeruginosa* in prevalence in health care–associated sinusitis.[166]

In other CDC surveys focusing on data from the National Healthcare Safety Network (NHSN), *Enterobacter* was one of the five pathogens most commonly encountered in ICUs and accounted for 5.0 to 8.6 percent of reported health care–associated infections.[79,88] In one large academic hospital, the relative proportion of *Enterobacter* spp. in health care–associated infections had decreased as a result of an increase in gram-positive and anaerobic infections.[93] During a 10-year period in six Brazilian neonatal ICUs, gram-negative pathogens accounted for more than half of all bloodstream infections, with *Enterobacter* spp. representing 2 percent.[41] In Canada, similar results were found in a nationwide surveillance program, with *E. cloacae* found in 2.2% of all health care–associated infections, including blood, wound, and urine sources.[216] Multiple outbreaks caused by *Enterobacter* have been described in neonatal ICUs.[66,80,169] *E. cloacae* has been found in some neonatal ICUs as the predominant pathogen, representing almost 40 percent of bloodstream isolates,[119] in addition to being responsible for outbreaks of sepsis.[45] In some series, one third of *Enterobacter* bacteremias were polymicrobial, which represents a greater prevalence than that of bacteremia caused by other gram-negative organisms.[21,61]

The most frequently cited risk factor for *Enterobacter* infection is the recent receipt of antibiotics, particularly third-generation cephalosporins.* Other risk factors include prolonged hospital stay, especially in an ICU, presence of serious underlying illness (e.g., burns, malignancy, and diabetes); prematurity in a neonate; immunosuppression; organ transplantation; and the presence of a foreign device.† Vertical spread of *Enterobacter* from mother to infant may occur at the time of birth,[60] but infant colonization is usually from an environmental source. A surveillance program in a neonatal ICU that screens for *E. cloacae* colonization determined that only 8.8 percent of colonized infants were the result of mother-to-child transmission.[66] Environmental sources implicated in outbreaks of infection have included intravenous fluids,‡ chronic hand dermatitis of a care provider,[12] contaminated infant formula,[10,15,30,38,137] blood gas machines,[2,106] contaminated transesophageal echocardiography probes,[91] cardioplegia ice,[24] therapeutic bed mattresses and mattress covers,[190] and rectal thermometers.[189]

Ribotyping, pulsed-field electrophoresis, and restriction-fragment polymorphism analysis of DNA from clinical isolates have been useful in discriminating possible sources of contamination and patient-to-patient transfer of individual strains.§ Integrons, discrete mobile units of DNA that can confer antibiotic resistance, have been reported in *Enterobacter* spp. and are more common findings in infections acquired in a health care setting.[44] Molecular diagnostic testing has proved useful in outbreak situations, especially in tracking antibiotic-resistant strains caused by plasmid acquisition of extended-spectrum β-lactamases.[64] Most cases are not outbreak-associated, and endogenous origin of infection is a more common means of acquisition than is the patient's environment.[155] This factor explains why recent antimicrobial

*References 8, 18, 34, 37, 52, 61, 88, 200, 202.
†References 1, 39, 65, 82, 96, 126, 127, 138, 165, 205.
‡References 8, 18, 37, 52, 88, 200, 202, 209.

*References 4, 6, 8, 17, 26, 61, 89, 197.
†References 3, 44, 57, 80, 117, 169, 170, 194, 217.
‡References 9, 28, 31, 120, 123, 185, 195, 199, 201.
§References 16, 24, 30, 37, 38, 71, 75, 76, 108, 154, 170, 174, 191.

use by the patient is such a strong predictor of infection.

PATHOPHYSIOLOGY

Newborns often are colonized by *Enterobacter* spp. in the gastrointestinal tract soon after birth,[14,145] and acquisition of hospital strains in immunocompromised newborns is common.[6,14,55,59,192] *Enterobacter* spp. may contaminate the compromised respiratory tract. The oropharynx commonly is colonized by *Enterobacter* by the time the infant is 1 month of age; colonization rates generally are lower in breast-fed infants.[11] Newborns with gastrointestinal abnormalities requiring prolonged parenteral nutrition have higher rates of colonization with *Enterobacter* and a higher incidence of sepsis.[156,157] Other risk factors for increased *Enterobacter* colonization in neonates include prematurity and prior antibiotic use.[150] Enteric organisms are recovered less frequently from the oropharynx of healthy older children and adults, but increased colonization, including increased risk for third-generation cephalosporin–resistant strains, especially with prior cephalosporin treatment of at least 3 days, is noted during illness.[204]

The ability of *Enterobacter* spp. to develop inducible resistance to penicillins and cephalosporins increases their pathogenic potential. All *Enterobacter* spp. possess an inducible chromosomally encoded class C (Bush group I) β-lactamase (*ampC*), which usually is produced in small amounts.[170] An inducible plasmid-encoded *ampC* (gene $bla_{\text{ACT-1}}$) also has been described and can be present in *Enterobacter* spp. and other Enterobacteriaceae.[168] Both enzymes have a high affinity for third-generation cephalosporins but a low maximum hydrolysis rate.[126] Consequently, β-lactamase mediates resistance to these antibiotics only when it is produced in large quantities or large numbers of plasmids are present.[113,164]

Resistance emerges after a mutation occurs in the *ampD* gene, which normally prevents high-level expression of β-lactamase.[87,90] Such mutants are considered "stably derepressed" and may be induced by exposure to β-lactam antibiotics, especially ampicillin and cephalosporins. Mutations in the *ampR* gene produce similar effects.[105] The addition of a β-lactamase inhibitor (e.g., clavulanic acid) does not increase the activity of β-lactam antibiotics against *Enterobacter* spp.; rather, it may decrease the activity against the organism by inducing the class C β-lactamase, as can the presence of *ampR* mutants.[90,105,193]

Emergence of resistance can occur during therapy for an isolate that initially is shown to be susceptible.[78,170] Resistance can be detected 24 hours after initiation of therapy or may be delayed 2 to 3 weeks.[36] In addition, more reports of resistant strains of *Enterobacter* that produce plasmid-mediated, extended-spectrum β-lactamases (ESBL) have been published.[84,121,158] Various ESBL genes, including genes from the TEM, SHV, and CTX-M families, have been found.[29,64,146,180,215] This variation can complicate choosing antimicrobial agents because discerning the source of the β-lactam resistance in the clinical microbiology laboratory often is difficult without performing specific testing, such as the double-disk diffusion method. A survey of 11 clinical laboratories in Germany to determine ESBL prevalence found that 40 percent of *E. cloacae* complex organisms were resistant to extended-spectrum cephalosporins, and only 6 percent carried ESBL genes.[84] This finding is in contrast to other reports with prevalences of 36 percent in some Asia-Pacific regions.[13]

The global spread of carbapenemase-producing Enterobacteriaceae has affected *Enterobacter* species, as well as other members of the class. Infections with these multidrug resistant organisms have been reported worldwide. Class A carbapenemases are plasmid mediated, effectively hydrolyze carbapenems, and are partially inhibited by clavulanic acid, with one of the most common being *Klebsiella pneumoniae* carbapenemases (KPC).[136] KPC-mediated resistance in *Enterobacter* spp. has been reported.[115,173] Class B metallo-β-lactamases (MBLs) are mostly Verona integron–encoded metallo-β-lactamase (VIM) and, more recently, New Delhi metallo-β-lactamase–1 (NDM-1). VIM producers can be endemic to a certain region, but have been reported globally in outbreaks and sporadic infections.[73,77,141] These enzymes hydrolyze all β-lactam antibiotics except aztreonam. NDM-1 producers have also been reported worldwide and are likely endemic in India. These genes are not associated with a single clone and have varied resistance to carbapenems. Of concern is the likelihood of other antibiotic resistance genes to be included on the $bla_{\text{NDM-1}}$ gene, including ESBL, aminoglycoside, macrolide, and rifampin resistance.[27,136] The CDC had three reports of NDM-1 isolates in 2010, one of which was *E. cloacae*. All patients had recent medical care in India as their risk factor.[33]

In one study, gram-negative fecal aerobic flora were eradicated completely after 24 hours of ceftriaxone therapy, only to be replaced within 10 days (mean, 6.7 days) of therapy by *P. aeruginosa*, *Enterobacter*, and *Citrobacter* resistant to all β-lactam antibiotics.[72] Although uncommon, the combination of reduced outer-membrane permeability and high-level β-lactamase production renders some clinical isolates of *Enterobacter* resistant to carbapenems (imipenem, meropenem, and ertapenem) and fourth-generation cephalosporins (cefepime).*

Other than the presence of endotoxin, factors responsible for the virulence of *Enterobacter* spp. are not well defined but may include cytotoxins, type III secretion systems, altered pili, and efflux pumps.† In vitro, it is possible to transfer and express an *Escherichia coli* enterotoxigenic plasmid in *Enterobacter*, implying the potential for transfer of virulence factors to *Enterobacter* spp. from other species.[214] Shiga toxin–producing *E. cloacae* has been isolated from the stool of a 5-month-old girl with hemolytic-uremic syndrome.[147] Shiga-toxin producing *E. coli* OR:H9 also was isolated from the child's stool, rendering the cause of the child's symptoms unclear. Similar to other organisms associated with central venous catheter infections, *Enterobacter* spp. may adhere irreversibly to catheter material, promoting colonization and infection via biofilm formation.[97,99,151] Certain ribotypes have

*References 19, 46, 49, 110, 112, 126, 152, 181, 187.
†References 95, 104, 109, 142, 144, 153.

been encountered more commonly as community or bloodstream isolates, indicating the presence of as yet undefined factors affecting virulence.[203] The propensity of *C. sakazakii* to produce neonatal meningitis complicated by abscesses and cerebral infarction is unexplained.[15,38,144,208] Proposed mechanisms have included brain capillary and intestinal endothelial cell invasion, persistence in human macrophages, biofilm formation, and a possible role for outer membrane protein A (OmpA).*

CLINICAL MANIFESTATIONS

Infection caused by *Enterobacter* commonly is indistinguishable from illness caused by other enteric pathogens. Sources of infection include central venous catheters and the urinary and biliary tracts.† *Enterobacter* commonly colonizes the respiratory tract secretions of intubated patients, and it is implicated increasingly as a cause of ventilator-associated pneumonia.[88,166,172] It can also be a cause of severe community-acquired pneumonia, generally in patients with serious comorbidities. This type of pneumonia strongly resembles health care–associated pneumonia, and patients may appear septic. Empirical antibiotic therapy may be very difficult because of unpredictable resistance patterns.[23] It also is one of the most common causes of pneumonia after lung transplantation[118] and can be found as part of the oral flora in patients with strokes.[70] Neonatal infection warrants special mention because of the prominence of *C. sakazakii* as a cause of devastating meningitis.

Similar to *Citrobacter koseri* (see Chapter 101), *C. sakazakii* causes neonatal meningitis complicated by cerebral abscesses or infarctions.‡ Poor feeding, irritability, jaundice, a full anterior fontanelle, and fever or hypothermia are presenting features shared with other gram-negative causes of bacterial meningitis. The mortality rate has been reported to range from 33 to 80 percent, and almost all survivors experience severe neurologic complications, including quadriplegia, developmental impedance, and impaired sight and hearing.[50,208] This severe morbidity is consistent with the development of multiple cystic lesions of the brain in 50 percent of surviving neonates. Serial computed tomography (CT) scans commonly reveal evolution of lesions most consistent with initial cerebral infarction, rather than primary abscess formation.[62,107,208] Subsequent cystic lesions may be purulent abscesses, from which the organism can be cultured; alternatively, they sometimes represent sterile fluid collections.[27] Meningitis tended to develop in infants of greater gestational age and birth-weight than in infants with only bacteremia, but they tended to be younger in chronologic age.[22]

Enterobacter also may be associated with necrotizing enterocolitis in the newborn, which became more apparent during the outbreaks of *C. sakazakii* associated with contaminated powdered infant formula.[107,188] *Enterobacter* spp. were the third most common isolate recovered from peritoneal fluid in a series of patients with necrotizing enterocolitis.[107,134]

In older immunocompromised children, bacteremia complicated by sepsis is a significant risk. Central venous catheterization and gastrointestinal tract pathologic processes seem to pose greater risks for development of bacteremia than infection of the urinary tract.[8,37,61,116] Bacteremia is accompanied by shock in almost one third of patients, but disseminated intravascular coagulation occurs in fewer than 5 percent.[17,21,61] Seeding of metastatic foci is uncommon. Overall case-fatality rates with bacteremia vary but usually are approximately 30 percent.[170] Predictors of poor prognosis include age younger than 18 months, inadequacy of antimicrobial chemotherapy, infection with extended-spectrum cephalosporin-resistant isolates, septic shock, type of underlying disease, presence of pulmonary infection, thrombocytopenia, unknown primary site of infection, and requirement for intensive care.* Absence of fever during the course of infection may be a particularly ominous sign; four of five afebrile subjects died in one series.[17] One study found that the use of an aminoglycoside as part of the empirical antibiotic management was a factor contributing to survival of *Enterobacter* or *Citrobacter* bacteremia.[47]

Some *Enterobacter* spp. recovered from children with diarrhea have been reported to produce enterotoxin,[35] although a causative role in enteritis has not been established. Other presentations of *Enterobacter* infection include endophthalmitis,[101,128,130] endometritis,[68] wound infections,[61] diskitis,[159,178] endocarditis,[186] and osteomyelitis.[40,51,100,179] *Enterobacter* also has caused syndromes classically associated with other agents, such as gas gangrene,[56] childhood purpura fulminans,[74] ecthyma gangrenosum,[160] and necrotizing fasciitis.[103]

DIAGNOSIS

Diagnosis of infection caused by *Enterobacter* relies primarily on isolation of the organism in culture from clinical material. Motility, production of ornithine decarboxylase, and the absence of deoxyribonuclease help distinguish the genus from *Klebsiella* and *Serratia*. The presence of yellow-pigmented colonies can help identify *C. sakazakii*. Patterns of sugar fermentation and production of decarboxylase also distinguish the species. Molecular techniques that are becoming available for rapid identification of bacteria include polymerase chain reaction (PCR)–single-strand conformation polymorphism analysis using 16S ribosomal DNA. This technique allows the potential identification of pathogens to be made from a wide range of clinical specimens with low numbers of bacteria.[206,212] Newer technology, including matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF) and real-time PCR for rapid nonculture detection of *Enterobacter* and other clinical isolates, are still under investigation.[124] These applications may have promise for clinical

*References 42, 97, 131, 133, 183, 184.
†References 8, 10, 61, 81, 88, 116.
‡References 15, 38, 50, 94, 208, 210.

*References 17, 21, 34, 47, 61, 92, 122.

applications.[149,167] In light of the increase of *C. sakazakii* invasive disease after ingestion of contaminated powdered infant formula, several techniques have been introduced for the rapid identification of this pathogen clinically and in foodstuffs. The use of a specific PCR amplification of the outer membrane protein A gene *(ompA)* is being investigated,[132] as is reverse-transcriptase[114] and the use of selective media.[5,48,98] DNA microarray technology is also being investigated for use.[198] Ribotyping is a highly discriminatory and reproducible method for the typing of *E. cloacae*, the most common cause of infection.[63] Other useful molecular techniques include restriction endonuclease analysis of chromosomal DNA, pulsed-field gel electrophoresis, random amplification of polymorphic DNA, and amplification of short interspersed repetitive sequences.[170] Most often, molecular typing methods are used in concert with biotyping, serotyping, or bacteriocin typing.

TREATMENT

Treatment of *Enterobacte*r infection is made problematic by inducible resistance to cephalosporins and intrinsic resistance to aminopenicillins, cefazolin, and cefoxitin.[20] The recognition that *Enterobacter* spp. may carry ESBL plasmids in addition to expressing chromosomal *ampC* β-lactamases can be problematic using automated ESBL detection systems. The use of the double-disk synergy method for ESBL detection can indicate the presence of plasmid-mediated resistance but is challenging because high production of *ampC* can mask the inhibition of ESBL by clavulanic acid.[207] New selective media for the identification of ESBL-producing isolates are available, but still may have problems with high *ampC* producers.[161,163] Identification of carbapenemase-producing isolates is more difficult. The Clinical and Laboratory Standards Institute (CLSI) lowered carbapenem breakpoints in the United States in 2010 to allow for better identification, but this has not occurred in Europe. The modified Hodge test has potential, but like the double-disk synergy method for ESBL detection, it is manual and can be time consuming, while still lacking in specificity and sensitivity (i.e., allows detection of high level *ampC* producers, but with weak detection of NDM-1 producers). Molecular techniques, mostly PCR, are now the standard for detection.[136] It is important to know the local antibiotic resistance patterns to determine the risk for multidrug resistance. Therapeutic options in this setting can include the use of older medications, including fosfomycin and colistin, in addition to newer antibiotics such as tigecycline and doripenem.[67]

Antibiotic resistance may be present at the time of initial isolation or may develop during therapy.[37,78] This problem is compounded by the common observation of resistance to extended-spectrum penicillins and, to a lesser extent, aminoglycosides.[7] The risks for development of resistance to cephalosporins, piperacillin, and aminoglycosides have been reported to be higher at a tertiary care center than at a primary care hospital.[54] Previous administration of third-generation cephalosporins increases the risk for having multiresistant *Enterobacter* isolates in an initial positive blood culture.[8,36,135] Hospitalized newborns quickly may acquire multiresistant strains, even though they themselves have not been treated with cephalosporins.[14] Isolation of multiresistant *Enterobacter* spp. in blood culture is associated with a higher case-fatality rate in contrast to mortality after isolation of a more sensitive *Enterobacter*.[36,175] Although less common, resistance to imipenem has been reported.[53,110,182] Cefepime, a fourth-generation cephalosporin, generally maintains activity against *Enterobacter* spp. that possess the class C β-lactamase,[171] although minimum inhibitory concentrations may be higher.[126]

Emergence of cefepime resistance during therapy has been described in a liver transplant recipient with a hepatic abscess caused by *E. aerogenes*.[112] The accompanying editorial warns about the risk for failure using cefepime in patients who have high-density infections (e.g., poorly drained liver abscess) caused by ceftazidime-resistant strains of *Enterobacter*.[126] The presence of an SHV-type ESBL has been associated with cefepime resistance, highlighting the need to identify those strains.[178] Vigilant antibiotic stewardship may aid in decreasing resistance. One institution restricted the use of cefepime and compared the 2 years before with the 2 years after the policy, demonstrating an improvement in ciprofloxacin susceptibility in their *Enterobacter* clinical isolates.[140]

In a nationwide survey of nosocomial bloodstream infections, rates of *Enterobacter* resistance to third-generation cephalosporins (ceftazidime, ceftriaxone) and broad-spectrum semisynthetic penicillins (piperacillin) with or without a β-lactamase inhibitor (tazobactam) was high, ranging from 35 to 50 percent.[155] Cefepime and imipenem inhibited 97 to 100 percent of isolates. Susceptibility to aminoglycosides and fluoroquinolones ranged from 92 to 98 percent, and 85 to 96 percent of isolates were susceptible to trimethoprim-sulfamethoxazole. Fluoroquinolone resistance is now being reported and likely is plasmid-mediated.[143,213] Plasmid-mediated quinolone resistance can be more common in ESBL-producing isolates as well, so caution should be used.[177] Because resistance patterns may vary based on geographic location, local susceptibility data should be used to guide initial therapy.

Some investigators recommend initial combination therapy that includes an aminoglycoside plus either cefepime or a carbapenem (imipenem or meropenem), until results of susceptibility testing are available. Third-generation cephalosporins should be used with caution, even when initial susceptibility results seem favorable. When strains are resistant to gentamicin and tobramycin, amikacin may be a suitable alternative. Good responses to therapy and return of gentamicin susceptibility of hospital *Enterobacter* strains have occurred after routine substitution of amikacin for gentamicin.[162,176] Trimethoprim-sulfamethoxazole alone (or combined with an aminoglycoside) and quinolones seem to be good alternatives for the treatment of *Enterobacter* infections, including meningitis.[8,43,69,127,210] Fluoroquinolones have been reported to be effective in bone and joint infections, in combination with cefepime.[111] As newer antimicrobial agents become available, more testing will be required to determine their effectiveness. Tigecycline has promise,

especially in the treatment of multidrug resistant strains.[196,211] Colistin has also been used, but it is hampered by its side effect profile.

Enterobacter meningitis creates special concerns. As is common with other forms of neonatal enteric meningitis, even susceptible organisms often persist in cerebrospinal fluid for 5 days or longer.[94] Monotherapy with a cell wall–active agent seems to be less effective than combination therapy with an aminoglycoside. Trimethoprim-sulfamethoxazole also has been used successfully.[58,210] Intrathecal administration of antibiotics does not seem to be beneficial in infants,[125] but it has been used with some success in adults.[139] The physician should anticipate the potential development of cerebral abscesses, infarctions, and cysts in newborns with *C. sakazakii* infection. Serial CT scans should be considered, and a neurosurgeon should be sought for drainage of abscesses and management of fluid accumulation.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Akhter K, Timpone J, Matsumoto C, et al. Six-month incidence of bloodstream infections in intestinal transplant patients. *Transpl Infect Dis* 2013;**14**:242–7.
5. Al-Holy MA, Shin JH, Osaili TM, Rasco BA. Evaluation of a new enrichment broth for detection of *Cronobacter* spp. in powdered infant formula. *J Food Prot* 2011;**74**:387–93.
6. Anderson B, Nicholas S, Sprague B, et al. Molecular and descriptive epidemiology of multidrug-resistant Enterobacteriaceae in hospitalized infants. *Infect Control Hosp Epidemiol* 2008;**29**:250–5.
25. Boyer A, Amadeo B, Vargas F, et al. Severe community-acquired *Enterobacter* pneumonia: a plea for greater awareness of the concept of health-care-associated pneumonia. *BMC Infect Dis* 2011;**11**:120.
27. Brink AJ, Coetzee J, Clay CG, et al. Emergence of New Delhi metallo-beta-lactamase (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC-2) in South Africa. *J Clin Microbiol* 2012;**50**: 525–7.
33. Centers for Disease Control and Prevention (CDC). Detection of Enterobacteriaceae isolates carrying metallo-beta-lactamase: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:750.
34. Chang EP, Chiang DH, Chen TL, et al. Clinical characteristics and predictors of mortality in patients with *Enterobacter aerogenes* bacteremia. *J Microbiol Immunol Infect* 2009;**42**:329–35.
39. Corti G, Mondanelli N, Losco M, et al. Post-traumatic infection of the lower limb caused by rare Enterobacteriaceae and *Mucorales* in a young healthy male. *Int J Infect Dis* 2009;**13**:e57–60.
42. Cruz A, Xicohtencatl-Cortes J, Gonzalez-Pedrajo B, et al. Virulence traits in *Cronobacter* species isolated from different sources. *Can J Microbiol* 2011;**57**:735–44.
45. Dalben M, Varkulja G, Basso M, et al. Investigation of an outbreak of *Enterobacter cloacae* in a neonatal unit and review of the literature. *J Hosp Infect* 2008;**70**:7–14.
49. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. *J Antimicrob Chemother* 2009;**63**:659–67.
64. Garza-Gonzalez E, Ibarra SIM, Llaca-Diaz JM, Gonzalez GM. Molecular characterization and antimicrobial susceptibility of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolates at a tertiary-care centre in Monterrey, Mexico. *J Med Microbiol* 2011;**60**:84–90.
66. Gbaguidi-Haore H, Talon D, Thouverez M, et al. Molecular epidemiology of *Enterobacter cloacae* in a neonatal department: a 2 year surveillance study. *Eur J Clin Microbiol Infect Dis* 2008;**27**: 643–8.
67. Giamarellou H, Poulakou G. Multidrug-resistant Gram-negative infections: what are the treatment options? *Drugs* 2009;**69**: 1879–901.
73. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011;**53**:60–7.
77. Heller I, Grif K, Orth D. Emergence of VIM-1-carbapenemase-producing *Enterobacter cloacae* in Tyrol, Austria. *J Med Microbiol* 2012;**61**:567–71.
79. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol* 2008;**29**:996–1011.
80. Ho TS, Wang SM, Wu YH, et al. Long-term characteristics of healthcare-associated infections in a neonatal intensive care unit. *J Microbiol Immunol Infect* 2010;**43**:407–15.
86. Iversen C, Mullane N, McCardell B, et al. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov., and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *Int J Syst Evol Microbiol* 2008;**58**(Pt 6):1442–7.
93. Kang J, Sickbert-Bennett EE, Brown VM, et al. Relative frequency of health care-associated pathogens by infection site at a university hospital from 1980 to 2008. *Am J Infect Control* 2012;**40**:416–20.
96. Kim DM, Jang SJ, Neupane GP, et al. *Enterobacter nimipressuralis* as a cause of pseudobacteremia. *BMC Infect Dis* 2010;**10**:315.
98. Kim SA, Rhee MS. A new cost-effective, selective and differential medium for the isolation of *Cronobacter* spp. *J Microbiol Methods* 2011;**85**:149–54.
99. Kim SM, Lee HW, Choi YW, et al. Involvement of curli fimbriae in the biofilm formation of *Enterobacter cloacae*. *J Microbiol* 2012;**50**:175–8.
104. Kryzminska S, Mokrachka J, Koczura R, Kaznowski A. Cytotoxic activity of *Enterobacter cloacae* human isolates. *FEMS Immunol Med Microbiol* 2009;**56**:248–52.
109. Lavigne JP, Sotto A, Nicolas-Chanoine MH, et al. Membrane permeability, a pivotal function involved in antibiotic resistance and virulence in Enterobacter aerogenes clinical isolates. *Clin Microbiol Infect* 2012;**18**:539–45.
115. Lo A, Verrall R, Williams J, et al. Carbapenem resistance via the *blaKPC-2* gene in *Enterobacter cloacae* blood culture isolate. *South Med J* 2010;**103**:453–4.
122. Marcos M, Inurrieta A, Soriano A, et al. Effect of antimicrobial therapy on mortality in 377 episodes of *Enterobacter* spp. bacteraemia. *J Antimicrob Chemother* 2008;**62**:397–403.
131. Mittal R, Wang Y, Hunter CJ, et al. Brain damage in newborn rat model of meningitis by *Enterobacter sakazakii:* a role for outer membrane protein A. *Lab Invest* 2009;**89**:263–77.
136. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011;**17**:1791–8.
140. Oliveira PR, Paula AP, Dal-Paz K, et al. Impact of restriction of cefepime use on the antimicrobial susceptibility of gram-negative bacilli related to healthcare-associated infections in an orthopedic hospital. *Infect Drug Resist* 2011;**4**:149–51.
141. Oteo J, Hernandez-Almaraz JL, Gil-Anton J, et al. Outbreak of vim-1-carbapenemase-producing *Enterobacter cloacae* in a pediatric intensive care unit. *Pediatr Infect Dis J* 2010;**29**:1144–6.
142. Paauw A, Caspers MP, Leverstein-van Hall MA, et al. Identification of resistance and virulence factors in an epidemic *Enterobacter hormaechei* outbreak strain. *Microbiology* 2009;**155**: 1478–88.
149. Pavlovic M, Konrad R, Iwobi AN, et al. A dual approach employing MALDI-TOF MS and real-time PCR for fast species identification within the Enterobacter cloacae complex. *FEMS Microbiol Lett* 2012;**328**:46–53.
153. Perez A, Poza M, Fernandez A, et al. Involvement of the AcrAB-TolC efflux pump in resistance, fitness, and virulence of *Enterobacter cloacae*. *Antimicrob Agents Chemother* 2012;**56**:2084–90.
156. Pichler J, Horn V, Macdonald S, Hill S. Sepsis and its etiology among hospitalized children less than 1 year of age with intestinal failure on parenteral nutrition. *Transplant Proc* 2010;**42**:24–5.
161. Randall LP, Kirchner M, Teale CJ, et al. Evaluation of CHROMagar CTX, a novel medium for isolating CTX-M-ESBL-positive Enterobacteriaceae while inhibiting AmpC-producing strains. *J Antimicrob Chemother* 2009;**63**:302–8.

163. Reglier-Poupet H, Naas T, Carrer A, et al. Performance of chromID ESBL, a chromogenic medium for detection of Enterobacteriaceae producing extended-spectrum beta-lactamases. *J Med Microbiol* 2008;**57**:310–5.
167. Risch M, Radjenovic D, Han JN, et al. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. *Swiss Med Wkly* 2010;**140**:w13095.
169. Samanta S, Farrer K, Breathnach A, Heath PT. Risk factors for late onset gram-negative infections: a case-control study. *Arch Dis Child Fetal Neonatal Ed* 2011;**96**:F15–8.
172. Sandiumenge A, Rello J. Ventilator-associated pneumonia caused by ESKAPE organisms: cause, clinical features, and management. *Curr Opin Pulm Med* 2012;**18**:187–93.
173. Sheng JF, Li JJ, Tu S, et al. bla(KPC) and rmtB on a single plasmid in *Enterobacter amnigenus* and *Klebsiella pneumoniae* isolates from the same patient. *Eur J Clin Microbiol Infect Dis* 2012;**31**:1585–91.
177. Silva-Sanchez J, Barrios H, Reyna-Flores F, et al. Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum beta-lactamase-producing Enterobacteriaceae isolates in Mexico. *Microb Drug Resist* 2011;**17**:497–505.
183. Townsend SM, Hurrell E, Caubilia-Barron J, et al. Characterization of an extended-spectrum beta-lactamase *Enterobacter hormaechei* nosocomial outbreak, and other *Enterobacter hormaechei* misidentified as *Cronobacter* (*Enterobacter*) *sakazakii*. *Microbiology* 2008;**154**:3659–67.
198. Wang M, Cao B, Gao Q, et al. Detection of *Enterobacter sakazakii* and other pathogens associated with infant formula powder by use of a DNA microarray. *J Clin Microbiol* 2009;**47**:3178–84.
216. Zhanel GG, DeCorby M, Adam H, et al. Prevalence of antimicrobial-resistant pathogens in Canadian hospitals: results of the Canadian Ward Surveillance Study (CANWARD 2008). *Antimicrob Agents Chemother* 2008;**52**:1430–7.
217. Zhong L, Men TY, Li H, et al. Multidrug-resistant gram-negative bacterial infections after liver transplantation: spectrum and risk factors. *J Infect* 2012;**64**:299–310.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 103

EXTRAINTESTINAL PATHOGENIC *ESCHERICHIA COLI*

Patrick C. Seed

Escherichia coli are common among terrestrial mammals, including humans, occupying approximately 0.1 percent of the microbes in the human enteric tract.[25] Although *E. coli* has normal homeostatic functions within intestinal-associated microbial communities, some subtypes have developed the capacity to invade other niches and produce infectious diseases.[59,69] Genetic mutations and the loss and gain of genetic material through genetic rearrangements, transposons, phages, and plasmids have produced *E. coli* lineages with a wide capacity to occupy different host niches and yield different clinical syndromes.[69] Animal and human subtypes are closely related and in many cases are the same, differentiated based only on the hosts from which they were isolated. The pathotypes of *E. coli* have specific associated virulence factors and genetic islands.

E. coli is the common cause of a wide range of bacterial infections within and outside of the intestinal tract, including traveler's diarrhea, dysentery, cholecystitis, cholangitis, urinary tract infection, bacteremia, neonatal meningitis, and pneumonia (Table 103-1). The pathogenic *E. coli* may be divided into two major categories: extraintestinal pathogenic *E. coli* (ExPEC) and diarrheogenic *E. coli*. ExPEC will be the focus of this chapter.

Escherichia coli are facultative anaerobic gram-negative bacilli in the Enterobacteriaceae family.[40] Common physiologic and biochemical features include fermentation of lactose (~90% of isolates), indole production, and lysine decarboxylase activity.[77] Many isolates are motile, with peritrichous flagella constituting 1/56 flagellar antigen (H) serotypes.[75] In addition, there are 171 somatic antigens (O). The most common O subtypes have been reported as O1, O2, O4, O6, O7, O8, O18, O25, O68, and O75.[14,36,107,132]

EPIDEMIOLOGY

E. coli produces three major types of extraintestinal invasive diseases in children: (1) urinary tract infections (UTIs), (2) bacteremia and sepsis, and (3) meningitis. Lower incidence infections produced by ExPEC include pneumonia, osteomyelitis, and endocarditis. Antibiotic resistance in ExPEC has undergone a shift in the past

REFERENCES

1. Abbott SL, Janda JM. *Enterobacter cancerogenus* ("*Enterobacter taylorae*") infections associated with severe trauma or crush injuries. *Am J Clin Pathol* 1997;**107**:359–61.
2. Acolet D, Ahmet Z, Houang E, et al. *Enterobacter cloacae* in a neonatal intensive care unit: Account of an outbreak and its relationship to use of third generation cephalosporins. *J Hosp Infect* 1994;**28**:273–86.
3. Akhter K, Timpone J, Matsumoto C, et al. Six-month incidence of bloodstream infections in intestinal transplant patients. *Transpl Infect Dis* 2012;**14**:242–7.
4. al Ansari N, McNamara EB, Cunney RJ, et al. Experience with *Enterobacter* bacteraemia in a Dublin teaching hospital. *J Hosp Infect* 1994;**27**:69–72.
5. Al-Holy MA, Shin JH, Osaili TM, Rasco BA. Evaluation of a new enrichment broth for detection of *Cronobacter* spp. in powdered infant formula. *J Food Prot* 2011;**74**:387–93.
6. Anderson B, Nicholas S, Sprague B, et al. Molecular and descriptive epidemiology of multidrug-resistant Enterobacteriaceae in hospitalized infants. *Infect Control Hosp Epidemiol* 2008;**29**:250–5.
7. Anderson EL, Hieber JP. An outbreak of gentamicin-resistant *Enterobacter cloacae* infections in a pediatric intensive care unit. *Infect Control* 1983;**4**:148–52.
8. Andresen J, Asmar BI, Dajani AS. Increasing *Enterobacter bacteremia* in pediatric patients. *Pediatr Infect Dis J* 1994;**13**:787–92.
9. Archibald LK, Ramos M, Arduino MJ, et al. *Enterobacter cloacae* and *Pseudomonas aeruginosa* polymicrobial bloodstream infections traced to extrinsic contamination of a dextrose multidose vial. *J Pediatr* 1998;**133**:640–4.
10. Ashkenazi S, Even-Tov S, Samra Z, et al. Uropathogens of various childhood populations and their antibiotic susceptibility. *Pediatr Infect Dis J* 1991;**10**:742–6.
11. Baltimore RS, Duncan RL, Shapiro ED, et al. Epidemiology of pharyngeal colonization of infants with aerobic gram-negative rod bacteria. *J Clin Microbiol* 1989;**27**:91–5.
12. Beck-Sague CM, Chong WH, Roy C, et al. Outbreak of surgical wound infections associated with total hip arthroplasty. *Infect Control Hosp Epidemiol* 1992;**13**:526–34.
13. Bell JM, Turnidge JD, Jones RN, et al. Prevalence of extended-spectrum beta-lactamase-producing *Enterobacter cloacae* in the Asia-Pacific region: results from the SENTRY antimicrobial surveillance program, 1998 to 2001. *Antimicrob Agents Chemother* 2003;**47**:3989–93.
14. Berkowitz FE, Metchock B. Third generation cephalosporin-resistant gram-negative bacilli in the feces of hospitalized children. *Pediatr Infect Dis J* 1995;**14**:97–100.
15. Biering G, Karlsson S, Clark NC, et al. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. *J Clin Microbiol* 1989;**27**:2054–6.
16. Bingen E, Denamur E, Lambert-Zechovsky N, et al. Rapid genotyping shows the absence of cross-contamination in *Enterobacter cloacae* nosocomial infections. *J Hosp Infect* 1992;**21**:95–101.
17. Bodey GP, Elting LS, Rodriguez S. Bacteremia caused by *Enterobacter*: 15 years of experience in a cancer hospital. *Rev Infect Dis* 1991;**13**:550–8.
18. Bonadio WA, Margolis D, Tovar M. *Enterobacter cloacae* bacteremia in children: a review of 30 cases in 12 years. *Clin Pediatr (Phila)* 1991;**30**:310–13.
19. Bornet C, Davin-Regli A, Bosi C, et al. Imipenem resistance of *Enterobacter aerogenes* mediated by outer membrane permeability. *J Clin Microbiol* 2000;**38**:1048–52.
20. Bouza E, Cercendado E. *Klebsiella* and *Enterobacter*: antibiotic resistance and treatment implications. *Semin Respir Infect* 2002; **17**:215–30.
21. Bouza E, Garcia de la Torre M, Erice A, et al. *Enterobacter* bacteremia: an analysis of 50 episodes. *Arch Intern Med* 1985;**145**:1024–7.
22. Bowen AB, Braden CR. Invasive *Enterobacter sakazakii* disease in infants. *Emerg Infect Dis* 2006;**12**:1185–9.
23. Boyer A, Amadeo B, Vargas F, et al. Severe community-acquired *Enterobacter* pneumonia: a plea for greater awareness of the concept of health-care-associated pneumonia. *BMC Infect Dis* 2011;**11**:120.
24. Breathnach AS, Riley PA, Shad S, et al. An outbreak of wound infection in cardiac surgery patients caused by *Enterobacter cloacae* arising from cardioplegia ice. *J Hosp Infect* 2006;**64**:124–8.
25. Brink AJ, Coetzee J, Clay CG, et al. Emergence of New Delhi metallo-beta-lactamase (NDM-1) and *Klebsiella pneumoniae* carbapenemase (KPC-2) in South Africa. *J Clin Microbiol* 2012;**50**: 525–7.
26. Burchard KW, Barrall DT, Reed M, et al. *Enterobacter* bacteremia in surgical patients. *Surgery* 1986;**100**:857–62.
27. Burdette JH, Santos C. *Enterobacter sakazakii* brain abscess in the neonate: the importance of neuroradiologic imaging. *Pediatr Radiol* 2000;**30**:33–4.
28. Campos LC, Lobianco LF, Seki LM, et al. Outbreak of *Enterobacter hormaechei* septicemia in newborns caused by contaminated parenteral nutrition in Brazil. *J Hosp Infect* 2007;**66**:95–7.
29. Cantón R, Oliver A, Coque TM, et al. Epidemiology of extended-spectrum beta-lactamase-producing *Enterobacter* isolates in a Spanish hospital during a 12 year period. *J Clin Microbiol* 2002;**40**: 1237–47.
30. Caubilla-Barron J, Hurrell E, Townsend S, et al. Genotypic and phenotypic analysis of *Enterobacter sakazakii* strains from a fatal outbreak in a neonatal intensive care unit in France. *J Clin Microbiol* 2007;**45**:3979–85.
31. Centers for Disease Control and Prevention. *Enterobacter cloacae* bloodstream infections associated with contaminated prefilled saline syringes: California, November 1998. *MMWR Morb Mortal Wkly Rep* 1998;**47**:959–60.
32. Centers for Disease Control and Prevention. *Enterobacter sakazakii* infections associated with the use of powdered infant formula: Tennessee, 2001. *MMWR Morb Mortal Wkly Rep* 2002;**51**: 297–300.
33. Centers for Disease Control and Prevention (CDC). Detection of Enterobacteriaceae isolates carrying metallo-beta-lactamase United States, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**(24): 750.
34. Chang EP, Chiang DH, Chen TL, et al. Clinical characteristics and predictors of mortality in patients with Enterobacter aerogenes bacteremia. *J Microbiol Immunol Infect* 2009;**42**(4):329–35.
35. Chatterjee BD, Thawani G, Sanyal SN. Etiology of acute childhood diarrhoea in Calcutta. *Trop Gastroenterol* 1989;**10**: 158–66.
36. Chow JW, Fine MJ, Shlaes DM, et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* 1991;**115**:585–90.
37. Chow JW, Yu VL, Shlaes DM. Epidemiologic perspectives on *Enterobacter* for the infection control professional. *Am J Infect Control* 1994;**22**:195–201.
38. Clark NC, Hill BC, O'Hara CM, et al. Epidemiologic typing of *Enterobacter sakazakii* in two neonatal nosocomial outbreaks. *Diagn Microbiol Infect Dis* 1990;**13**:467–72.
39. Corti G, Mondanelli N, Losco M, et al. Post-traumatic infection of the lower limb caused by rare Enterobacteriaceae and *Mucorales* in a young healthy male. *Int J Infect Dis* 2009;**13**:e57–60.
40. Corti G, Panunzi I, Losco M, et al. Postsurgical osteomyelitis caused by *Enterobacter sakazakii* in a healthy young man. *J Chemother* 2007;**19**:94–6.
41. Couto RC, Carvalho EAA, Pedrosa TMG, et al. A 10 year prospective surveillance of nosocomial infections in neonatal intensive care units. *Am J Infect Control* 2007;**35**:183–9.
42. Cruz A, Xicohtencatl-Cortes J, Gonzalez-Pedrajo B, et al. Virulence traits in *Cronobacter* species isolated from different sources. *Can J Microbiol* 2011;**57**:735–44.
43. D'Antuono VS, Brown I. Successful treatment of enterobacter meningitis with ciprofloxacin. *Clin Infect Dis* 1998;**26**:206–7.
44. Daikos GL, Kosmidis C, Tassios PT, et al. Enterobacteriaceae bloodstream infections: presence of integrons, risk factors and outcome. *Antimicrob Agents Chemother* 2007;**51**:2366–72.
45. Dalben M, Varkulja G, Basso M, et al. Investigation of an outbreak of *Enterobacter cloacae* in a neonatal unit and review of the literature. *J Hosp Infect* 2008;**70**:7–14.
46. De Gheldre Y, Maes N, Rost F, et al. Molecular epidemiology of an outbreak of multidrug-resistant *Enterobacter aerogenes* infections and in vivo emergence of imipenem resistance. *J Clin Microbiol* 1997;**35**:152–60.
47. Deal EN, Micek ST, Ritchie DJ, et al. Predictors of in-hospital mortality for bloodstream infections caused by *Enterobacter* species or *Citrobacter freundii*. *Pharmacotherapy* 2007;**27**:191–9.
48. Derzelle S, Dilasser F, Maladen V, et al. Comparison of three chromogenic media and evaluation of two molecular based

identification systems for the detection of *Enterobacter sakazakii* from environmental samples from infant formulae factories. *J Food Prot* 2007;**70**:1678–84.
49. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. *J Antimicrob Chemother* 2009;**63**:659–67.
50. Drudy D, Mullane NR, Quinn T, et al. *Enterobacter sakazakii*: an emerging pathogen in powdered infant formula. *Clin Infect Dis* 2006;**42**:996–1002.
51. Dubey L, Krasinski K, Hernanz-Schulman M. Osteomyelitis secondary to trauma or infected contiguous soft tissue. *Pediatr Infect Dis J* 1988;**7**:26–34.
52. Ehni WF, Reller LB, Ellison RT 3rd. Bacteremia in granulocytopenic patients in a tertiary-care general hospital. *Rev Infect Dis* 1991;**13**:613–19.
53. Ehrhardt AF, Sanders CC, Thomson KS, et al. Emergence of resistance to imipenem in *Enterobacter* isolates masquerading as *Klebsiella pneumoniae* during therapy with imipenem/cilastatin. *Clin Infect Dis* 1993;**17**:120–2.
54. Ellner PD, Fink DJ, Neu HC, et al. Epidemiologic factors affecting antimicrobial resistance of common bacterial isolates. *J Clin Microbiol* 1987;**25**:1668–74.
55. Fanaro S, Chierici R, Guerrini P, et al. Intestinal microflora in early infancy: composition and development. *Acta Paediatr Suppl* 2003;**91**:48–55.
56. Fata F, Chittivelu S, Tessler S, et al. Gas gangrene of the arm due to *Enterobacter cloacae* in a neutropenic patient. *South Med J* 1996;**89**:1095–6.
57. Fok TF, Lee CH, Wong EM, et al. Risk factors for *Enterobacter* septicemia in a neonatal unit: case-control study. *Clin Infect Dis* 1998;**27**:1204–9.
58. Foster D, Rhoney D. *Enterobacter* meningitis: Organism susceptibilities, antimicrobial therapy and related outcomes. *Surg Neurol* 2005;**63**:533–7.
59. Fryklund B, Tullus K, Burman LG. Epidemiology of enteric bacteria in neonatal units: influence of procedures and patient variables. *J Hosp Infect* 1991;**18**:15–21.
60. Fryklund B, Tullus K, Berglund B, et al. Importance of the environment and the faecal flora of infants, nursing staff and parents as sources of gram-negative bacteria colonizing newborns in three neonatal wards. *Infection* 1992;**20**:253–7.
61. Gallagher PG. *Enterobacter* bacteremia in pediatric patients. *Rev Infect Dis* 1990;**12**:808–12.
62. Gallagher PG, Ball WS. Cerebral infarctions due to CNS infection with *Enterobacter sakazakii*. *Pediatr Radiol* 1991;**21**:135–6.
63. Garaizar J, Kaufmann ME, Pitt TL. Comparison of ribotyping with conventional methods for the type identification of *Enterobacter cloacae*. *J Clin Microbiol* 1991;**29**:1303–7.
64. Garza-Gonzalez E, Ibarra SIM, Llaca-Diaz JM, Gonzalez GM. Molecular characterization and antimicrobial susceptibility of extended-spectrum beta-lactamase-producing Enterobacteriaceae isolates at a tertiary-care centre in Monterrey, Mexico. *J Med Microbiol* 2011;**60**:84–90.
65. Gaston MA. Enterobacter: An emerging nosocomial pathogen. *J Hosp Infect* 1988;**11**:197–208.
66. Gbaguidi-Haore H, Talon D, Thouverez M, et al. Molecular epidemiology of *Enterobacter cloacae* in a neonatal department: a 2 year surveillance study. *Eur J Clin Microbiol Infect Dis* 2008;**27**: 643–8.
67. Giamarellou H, Poulakou G. Multidrug-resistant gram-negative infections: what are the treatment options? *Drugs* 2009;**69**: 1879–901.
68. Gibbs RS, Blanco JD, Bernstein S. Role of aerobic gram-negative bacilli in endometritis after cesarean section. *Rev Infect Dis* 1985; **7**(suppl. 4):S690–5.
69. Goepp JG, Lee CK, Anderson T, et al. Use of ciprofloxacin in an infant with ventriculitis. *J Pediatr* 1992;**121**:303–5.
70. Gosney MA, Martin MV, Wright AE, et al. *Enterobacter sakazakii* in the mouths of stroke patients and its association with aspiration pneumonia. *Eur J Intern Med* 2006;**17**:185–8.
71. Grattard F, Pozzetto B, Berthelot P, et al. Arbitrarily primed PCR, ribotyping, and plasmid pattern analysis applied to investigation of a nosocomial outbreak due to *Enterobacter cloacae* in a neonatal intensive care unit. *J Clin Microbiol* 1994;**32**:596–602.
72. Guggenbichler JP, Kofler J, Allerberger F. The influence of third-generation cephalosporins on the aerobic intestinal flora. *Infection* 1985;**13**:S137–9.
73. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbaepenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011;**53**:60–7.
74. Gurses N, Ozkan A. Neonatal and childhood purpura fulminans: review of seven cases. *Cutis* 1988;**41**:361–3.
75. Haertl R, Bandlow G. Molecular typing of *Enterobacter cloacae* by pulsed-field gel electrophoresis of genomic restriction fragments. *J Hosp Infect* 1993;**25**:109–16.
76. Harbarth S, Sudre P, Dharan S, et al. Outbreak of *Enterobacter cloacae* related to understaffing, overcrowding, and poor hygiene practices. *Infect Control Hosp Epidemiol* 1999;**20**:598–603.
77. Heller I, Grif K, Orth D. Emergence of VIM-1-carbapenemase-producing *Enterobacter cloacae* in Tyrol, Austria. *J Med Microbiol* 2012;**61**:567–71.
78. Heusser MF, Patterson JE, Kuritza AP, et al. Emergence of resistance to multiple beta-lactams in *Enterobacter cloacae* during treatment for neonatal meningitis with cefotaxime. *Pediatr Infect Dis J* 1990;**9**:509–12.
79. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol* 2008;**29**: 996–1011.
80. Ho TS, Wang SM, Wu YH, et al. Long-term characteristics of healthcare-associated infections in a neonatal intensive care unit. *J Microbiol Immunol Infect* 2010;**43**:407–15.
81. Hoffmann H, Schmoldt S, Trülzsch K, et al. Nosocomial urosepsis caused by *Enterobacter kobei* with aberrant phenotype. *Diagn Microbiol Infect Dis* 2005;**53**:143–7.
82. Hoffmann H, Stindl S, Ludwig W, et al. Reassignment of *Enterobacter dissolvens* to *Enterobacter cloacae* as *E. cloacae* subspecies dissolvens comb. nov. and emended description of *Enterobacter asburiae* and *Enterobacter kobei*. *Syst Appl Microbiol* 2005;**28**: 196–205.
83. Hoffmann H, Stindl S, Stumpf A, et al. Description of *Enterobacter ludwigii* sp. nov., a novel *Enterobacter* species of clinical relevance. *Syst Appl Microbiol* 2005;**28**:206–12.
84. Hoffmann H, Stürenburg E, Heesemann J, et al. Prevalence of extended-spectrum beta-lactamases in isolates of the *Enterobacter cloacae* complex from German hospitals. *Clin Microbiol Infect* 2006; **12**:322–30.
85. Inouse K, Sugiyama K, Kosako Y, et al. *Enterobacter cowanii* sp. nov., a new species of the family Enterobacteriaceae. *Curr Microbiol* 2000;**41**:417–20.
86. Iversen C, Mullane N, McCardell B, et al. *Cronobacter* gen. nov., a new genus to accommodate the biogroups of *Enterobacter sakazakii* gen. nov., comb. nov., *Cronobacter malonaticus* sp. nov., *Cronobacter turicensis* sp. nov., *Cronobacter muytjensii* sp. nov., *Cronobacter dublinensis* sp. nov., *Cronobacter* genomospecies 1, and of three subspecies, *Cronobacter dublinensis* subsp. *dublinensis* subsp. nov., *Cronobacter dublinensis* subsp. *lausannensis* subsp. nov., and *Cronobacter dublinensis* subsp. *lactaridi* subsp. nov. *Int J Syst Evol Microbiol* 2008;**58**(Pt 6):1442–7.
87. Jacobs C, Joris B, Jamin M, et al. AmpD, essential for both β-lactamase regulation and cell wall recycling, is a novel cytosolic n-acetylmuramyl-L-alanine amidase. *Mol Microbiol* 1995;**15**:553–9.
88. Jarvis WR, Martone WJ. Predominant pathogens in hospital infections. *J Antimicrob Chemother* 1992;**29**(suppl. A):19–24.
89. Johnson MP, Ramphal R. Beta-lactam-resistant *Enterobacter* bacteremia in febrile neutropenic patients receiving monotherapy. *J Infect Dis* 1990;**162**:981–3.
90. Kaneko K, Okamoto R, Nakano R, et al. Gene mutations responsible for overexpression of AmpC beta-lactamase in some clinical isolates of *Enterobacter cloacae*. *J Clin Microbiol* 2005;**43**:2955–8.
91. Kanemitsu K, Endo S, Oda K, et al. An increased incidence of *Enterobacter cloacae* in a cardiovascular ward. *J Hosp Infect* 2007; **66**:130–4.
92. Kang CI, Kim SH, Park WB, et al. Bloodstream infections caused by *Enterobacter* species: predictors of 30 day mortality rate and impact of broad-spectrum cephalosporin resistance on outcome. *Clin Infect Dis* 2004;**39**:812–18.

93. Kang J, Sickbert-Bennett EE, Brown VM, et al. Relative frequency of health care-associated pathogens by infection site at a university hospital from 1980 to 2008. *Am J Infect Control* 2012; **40**:416–20.
94. Kaplan SL, Patrick CC. Cefotaxime and aminoglycoside treatment of meningitis caused by gram-negative enteric organisms. *Pediatr Infect Dis J* 1990;**9**:810–14.
95. Keller R, Pedroso MZ, Ritchmann R, et al. Occurrence of virulence-associated properties in *Enterobacter cloacae*. *Infect Immun* 1998;**66**:645–9.
96. Kim DM, Jang SJ, Neupane GP, et al. *Enterobacter nimipressuralis* as a cause of pseudobacteriemia. *BMC Infect Dis* 2010;**10**:315.
97. Kim H, Ryu JH, Beuchat LR. Attachment of and biofilm formation by *Enterobacter sakazakii* on stainless steel and enteral feeding tubes. *Appl Environ Microbiol* 2006;**72**:5846–56.
98. Kim SA, Rhee MS. A new cost-effective, selective and differential medium for the isolation of *Cronobacter* spp. *J Microbiol Methods* 2011;**85**:149–54.
99. Kim SM, Lee HW, Choi YW, et al. Involvement of curli fimbriae in the biofilm formation of *Enterobacter cloacae*. *J Microbiol* 2012;**50**:175–8.
100. Klein JD, Leach KA. Pediatric pelvic osteomyelitis. *Clin Pediatr (Phila)* 2007;**46**:787–90.
101. Korah S, Braganza A, Jacob P, et al. An "epidemic" of post cataract surgery endophthalmitis by a new organism. *Indian J Ophthalmol* 2007;**55**:464–6.
102. Kosako Y, Tamura K, Sakazaki R, et al. *Enterobacter kobei* sp. nov., a new species of Enterobacteriaceae resembling *Enterobacter cloacae*. *Curr Microbiol* 1996;**33**:261–5.
103. Kronish JW, McLeish WM. Eyelid necrosis and periorbital necrotizing fasciitis: report of a case and review of the literature. *Ophthalmology* 1991;**98**:92–8.
104. Kryzminska S, Mokrachka J, Koczura R, Kaznowski A. Cytotoxic activity of *Enterobacter cloacae* human isolates. *FEMS Immunol Med Microbiol* 2009;**56**:248–52.
105. Kuga A, Okamoto R, Inoue M. ampR gene mutations that greatly increase class C beta-lactamase activity in *Enterobacter cloacae*. *Antimicrob Agents Chemother* 2000;**44**:561–7.
106. Lacey SL, Want SV. An outbreak of *Enterobacter cloacae* associated with contamination of a blood gas machine. *J Infect* 1995;**30**: 223–6.
107. Lai KK. *Enterobacter sakazakii* infections among neonates, infants, children and adults. *Medicine* 2001;**80**:113–22.
108. Lambert-Zechovsky N, Bingen E, Denamur E, et al. Molecular analysis provides evidence for the endogenous origin of bacteremia and meningitis due to *Enterobacter cloacae* in an infant. *Clin Infect Dis* 1992;**15**:30–2.
109. Lavigne JP, Sotto A, Nicolas-Chanoine MH, et al. Membrane permeability, a pivotal function involved in antibiotic resistance and virulence in *Enterobacter aerogenes* clinical isolates. *Clin Microbiol Infect* 2012;**18**:539–45.
110. Lee EH, Nicolas MH, Kitzis MD, et al. Association of two resistance mechanisms in a clinical isolate of *Enterobacter cloacae* with high-level resistance to imipenem. *Antimicrob Agents Chemother* 1991;**35**:1093–8.
111. Legout L, Senneville E, Stern R, et al. Treatment of bone and joint infections caused by gram-negative bacilli with a cefepime-fluoroquinolone combination. *Clin Microbiol Infect* 2006;**12**: 1030–3.
112. Limaye AP, Gautom RK, Black D, et al. Rapid emergence of resistance to cefepime during treatment. *Clin Infect Dis* 1997;**25**: 339–40.
113. Lindberg F, Normark S. Contribution of chromosomal beta-lactamases to beta-lactam resistance in enterobacteria. *Rev Infect Dis* 1986;**8**(Suppl. 3):S292–304.
114. Liu Y, Cai X, Zhang X, et al. Real time PCR using TaqMan and SYBR Green for detection of *Enterobacter sakazakii* in infant formula. *J Microbiol Methods* 2006;**65**:21–31.
115. Lo A, Verrall R, Williams J, et al. Carbapenem resistance via the *blaKPC-2* gene in *Enterobacter cloacae* blood culture isolate. *South Med J* 2010;**103**:453–4.
116. Lohr JA, Donowitz LG, Sadler JE 3rd. Hospital-acquired urinary tract infection. *Pediatrics* 1989;**83**:193–9.
117. Loiwal V, Kumar A, Gupta P, et al. *Enterobacter aerogenes* outbreak in a neonatal intensive care unit. *Pediatr Int* 1999;**41**:157–61.
118. Low DE, Kaiser LR, Haydock DA, et al. The donor lung: infectious and pathologic factors affecting outcome in lung transplantation. *J Thorac Cardiovasc Surg* 1993;**106**:614–21.
119. Mahapatra MA, Ghosh SK, Mishra S, et al. *Enterobacter cloacae*: a predominant pathogen in neonatal septicemia. *Indian J Med Microbiol* 2002;**20**:110–12.
120. Maki DG, Rhame FS, Mackel DC, et al. Nationwide epidemic of septicemia caused by contaminated intravenous products. I. Epidemiologic and clinical features. *Am J Med* 1976;**60**: 471–85.
121. Manzur A, Tubau F, Pujol M, et al. Nosocomial outbreak due to extended-spectrum beta-lactamase-producing *Enterobacter cloacae* in a cardiothoracic intensive care unit. *J Clin Microbiol* 2007;**45**: 2365–9.
122. Marcos M, Inurrieta A, Soriano A, et al. Effect of antimicrobial therapy on mortality in 377 episodes of *Enterobacter* spp. bacteraemia. *J Antimicrob Chemother* 2008;**62**:397–403.
123. Matsaniotis NS, Syriopoulou VP, Theodoridou MC, et al. *Enterobacter* sepsis in infants and children due to contaminated intravenous fluids. *Infect Control* 1984;**5**:471–7.
124. Matsuhisa A, Saito Y, Sakamoto Y, et al. Detection of bacteria in phagocyte-smears from septicemia-suspected blood by in situ hybridization using biotinylated probes. *Microbiol Immunol* 1994; **38**:511–17.
125. McCracken GH Jr, Mize SG. A controlled study of intrathecal antibiotic therapy in gram-negative enteric meningitis of infancy: report of the neonatal meningitis cooperative study group. *J Pediatr* 1976;**89**:66–72.
126. Medeiros AA. Relapsing infection due to *Enterobacter* species: lessons of heterogeneity. *Clin Infect Dis* 1997;**25**:341–2.
127. Meis JF, Groot-Loonen J, Hoogkamp-Korstanje JA. A brain abscess due to multiply-resistant *Enterobacter cloacae* successfully treated with meropenem. *Clin Infect Dis* 1995;**20**:1567.
128. Milewski SA, Klevjer-Anderson P. Endophthalmitis caused by *Enterobacter cloacae*. *Ann Ophthalmol* 1993;**25**:309–11.
129. Mireya UA, Marti PO, Xavier KV, et al. Nosocomial infections in paediatric and neonatal intensive care units. *J Infect* 2007;**54**: 212–20.
130. Mirza GE, Karakucuk S, Doganay M, et al. Postoperative endophthalmitis caused by an *Enterobacter* species. *J Hosp Infect* 1994; **26**:167–72.
131. Mittal R, Wang Y, Hunter CJ, et al. Brain damage in newborn rat model of meningitis by *Enterobacter sakazakii:* a role for outer membrane protein A. *Lab Invest* 2009;**89**:263–77.
132. Mohan Nair MK, Venkitanarayanan K. Role of bacterial OmpA and host cytoskeleton in the invasion of human intestinal epithelial cells by *Enterobacter sakazakii*. *Pediatr Res* 2007;**62**:664–9.
133. Mohan Nair MK, Venkitanarayanan KS. Cloning and sequencing of the *ompA* gene of *Enterobacter sakazakii* and development of an *ompA*-targeted PCR for rapid detection of *Enterobacter sakazakii* in infant formula. *Appl Environ Microbiol* 2006;**72**:2539–46.
134. Mollitt DL, Tepas JJ 3rd, Talbert JL. The microbiology of neonatal peritonitis. *Arch Surg* 1988;**123**:176–9.
135. Muller A, Lopez-Lozano JM, Bertrand X, et al. Relationship between ceftriaxone use and resistance to third-generation cephalosporins among clinical strains of *Enterobacter cloacae*. *J Antimicrob Chemother* 2004;**54**:173–7.
136. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. *Emerg Infect Dis* 2011;**17**:1791–8.
137. Noriega FR, Kotloff KL, Martin MA, et al. Nosocomial bacteremia caused by *Enterobacter sakazakii* and *Leuconostoc mesenteroides* resulting from extrinsic contamination of infant formula. *Pediatr Infect Dis J* 1990;**9**:447–9.
138. O'Hara CM, Steigerwalt AG, Hill BC, et al. *Enterobacter hormaechei*, a new species of the family Enterobacteriaceae formerly known as enteric group 75. *J Clin Microbiol* 1989;**27**:2046–9.
139. O'Neill E, Humphreys H, Phillips J, et al. Third-generation cephalosporin resistance among gram-negative bacilli causing meningitis in neurosurgical patients: significant challenges in ensuring effective antibiotic therapy. *J Antimicrob Chemother* 2006;**57**:356–9.
140. Oliveira PR, Paula AP, Dal-Paz K, et al. Impact of restriction of cefepime use on the antimicrobial susceptibility of gram-negative bacilli related to healthcare-associated infections in an orthopedic hospital. *Infect Drug Resist* 2011;**4**:149–51.

141. Oteo J, Hernandez-Almaraz JL, Gil-Anton J, et al. Outbreak of vim-1-carbapenemase-producing *Enterobacter cloacae* in a pediatric intensive care unit. *Pediatr Infect Dis J* 2010;**29**:1144–6.
142. Paauw A, Caspers MP, Leverstein-van Hall MA, et al. Identification of resistance and virulence factors in an epidemic *Enterobacter hormaechei* outbreak strain. *Microbiology* 2009;**155**:1478–88.
143. Paauw A, Fluit AC, Verhoef J, et al. *Enterobacter cloacae* outbreak and emergence of quinolone resistance gene in a Dutch hospital. *Emerg Infect Dis* 2006;**12**:807–12.
144. Pagotto FJ, Nazarowec-White M, Bidawid S, et al. *Enterobacter sakazakii*: infectivity and enterotoxin production in vitro and in vivo. *J Food Prot* 2003;**66**:370–5.
145. Park HK, Shim SS, Kim SY, et al. Molecular analysis of colonized bacteria in a human newborn infant gut. *J Microbiol* 2005;**43**: 345–53.
146. Park YJ, Park SY, Oh EJ, et al. Occurrence of extended-spectrum beta-lactamases among chromosomal AmpC-producing *Enterobacter cloacae*, *Citrobacter freundii* and *Serratia marcescens* in Korea and investigation of screening criteria. *Diagn Microbiol Infect Dis* 2005;**51**:265–9.
147. Paton AW, Paton JC. *Enterobacter cloacae* producing a *Shiga*-like toxin II-related cytotoxin associated with a case of hemolytic-uremic syndrome. *J Clin Microbiol* 1996;**34**:463–5.
148. Pavan ME, Franco RJ, Rodriguez JM, et al. Phylogenetic relationships of the genus *Kluyvera*: Transfer of *Enterobacter intermedius* Izard et al. 1980 to the genus *Kluyvera* as *Kluyvera intermedia* comb. nov. and reclassification of *Kluyvera cochleae* as a later synonym of *K. intermedia*. *Int J Syst Evol Microbiol* 2005;**55**: 437–42.
149. Pavlovic M, Konrad R, Iwobi AN, et al. A dual approach employing MALDI-TOF MS and real-time PCR for fast species identification within the *Enterobacter cloacae* complex. *FEMS Microbiol Lett* 2012;**328**:46–53.
150. Penders J, Thijs C, Vink C. Factors influencing the composition of the intestinal microbiota in early infancy. *Pediatrics* 2006;**118**: 511–21.
151. Penner J, Allerberger F, Dierich MP, et al. In vitro experiments on catheter-related infections due to gram-negative rods. *Chemotherapy* 1993;**39**:336–54.
152. Pérez A, Canle D, Latasa C, et al. Cloning, nucleotide sequencing, and analysis of the AcrAB-TolC efflux pump of *Enterobacter cloacae* and determination of its involvement in antibiotic resistance in a clinical isolate. *Antimicrob Agents Chemother* 2007;**51**: 3247–53.
153. Perez A, Poza M, Fernandez A, et al. Involvement of the AcrAB-TolC efflux pump in resistance, fitness, and virulence of *Enterobacter cloacae*. *Antimicrob Agents Chemother* 2012;**56**:2084–90.
154. Peters SM, Bryan J, Cole MF. Enterobacterial repetitive intergenic consensus polymerase chain reaction typing of isolates of *Enterobacter cloacae* from an outbreak of infection in a neonatal intensive care unit. *Am J Infect Control* 2000;**28**:123–9.
155. Pfaller MA, Jones RN, Marshall SA, et al. Inducible amp C beta-lactamase producing gram-negative bacilli from blood stream infections: frequency, antimicrobial susceptibility, and molecular epidemiology in a national surveillance program (SCOPE). *Diagn Microbiol Infect Dis* 1997;**28**:211–19.
156. Pichler J, Horn V, Macdonald S, Hill S. Sepsis and its etiology among hospitalized children less than 1 year of age with intestinal failure on parenteral nutrition. *Transplant Proc* 2010;**42**:24–5.
157. Pierro A, van Saene HK, Jones MO, et al. Clinical impact of abnormal gut flora in infants receiving parenteral nutrition. *Ann Surg* 1998;**227**:547–52.
158. Pitout JD, Thomson KS, Hanson ND, et al. Plasmid-mediated resistance to expanded-spectrum cephalosporins among *Enterobacter aerogenes* strains. *Antimicrob Agents Chemother* 1998;**42**: 596–600.
159. Porter P, Wray CC. *Enterobacter agglomerans* spondylodiscitis: a possible, unrecognized complication of tetracycline therapy. *Spine* 2000;**25**:1287–9.
160. Rajan RK. Spontaneous bacterial peritonitis with ecthyma gangrenosum due to *Escherichia coli*. *J Clin Gastroenterol* 1982;**4**: 145–8.
161. Randall LP, Kirchner M, Teale CJ, et al. Evaluation of CHROMagar CTX, a novel medium for isolating CTX-M-ESBL-positive Enterobacteriaceae while inhibiting AmpC-producing strains. *J Antimicrob Chemother* 2009;**63**:302–8.
162. Raz R, Sharir R, Shmilowitz L, et al. The elimination of gentamicin-resistant gram-negative bacteria in a newborn intensive care unit. *Infection* 1987;**15**:32–4.
163. Reglier-Poupet H, Naas T, Carrer A, et al. Performance of chromID ESBL, a chromogenic medium for detection of Enterobacteriaceae producing extended-spectrum beta-lactamases. *J Med Microbiol* 2008;**57**:310–15.
164. Reisbig MD, Hossain A, Hanson ND. Factors influencing gene expression and resistance for gram-negative organisms expressing plasmid-encoded *ampC* genes of *Enterobacter* origin. *J Antimicrob Chemother* 2003;**51**:1141–51.
165. Richard C. *Enterobacter*. In: Garrity GM, editor. *Bergey's manual of systematic bacteriology*. New York: Springer; 1984. p. 465–7.
166. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in pediatric intensive care units in the United States. *Pediatrics* 1999;**103**:e39–45.
167. Risch M, Radjenovic D, Han JN, et al. Comparison of MALDI TOF with conventional identification of clinically relevant bacteria. *Swiss Med Wkly* 2010;**140**:w13095.
168. Rottman M, Benzerara Y, Hanau-Berçot B, et al. Chromosomal ampC genes in *Enterobacter* species other than *Enterobacter cloacae*, and ancestral association of the ACT-1 plasmid-encoded cephalosporinase to *Enterobacter asburiae*. *FEMS Microbiol Lett* 2002;**210**: 87–92.
169. Samanta S, Farrer K, Breathnach A, Heath PT. Risk factors for late onset gram-negative infections: a case-control study. *Arch Dis Child Fetal Neonatal Ed* 2011;**96**:F15–F18.
170. Sanders WE Jr, Sanders CC. *Enterobacter* spp. Pathogens poised to flourish at the turn of the century. *Clin Microbiol Rev* 1997;**10**: 220–41.
171. Sanders WE Jr, Tenney JH, Kessler RE. Efficacy of cefepime in the treatment of infections due to multiply resistant *Enterobacter* species. *Clin Infect Dis* 1996;**23**:454–61.
172. Sandiumenge A, Rello J. Ventilator-associated pneumonia caused by ESKAPE organisms: cause, clinical features, and management. *Curr Opin Pulm Med* 2012;**18**:187–93.
173. Sheng JF, Li JJ, Tu S, et al. bla(KPC) and rmtB on a single plasmid in *Enterobacter amnigenus* and *Klebsiella pneumoniae* isolates from the same patient. *Eur J Clin Microbiol Infect Dis* 2012;**31**: 1585–91.
174. Shi ZY, Liu PY, Lau YJ, et al. Epidemiological typing of isolates from an outbreak of infection with multidrug-resistant *Enterobacter cloacae* by repetitive extragenic palindromic unit b1-primed PCR and pulsed-field gel electrophoresis. *J Clin Microbiol* 1996; **34**:2784–90.
175. Shlaes DM. The clinical relevance of *Enterobacter* infections. *Clin Ther* 1993;**15**:21–8.
176. Shulman ST, Yogev R. Treatment of pediatric infections with amikacin as first-line aminoglycoside. *Am J Med* 1985;**79**:43–50.
177. Silva-Sanchez J, Barrios H, Reyna-Flores F, et al. Prevalence and characterization of plasmid-mediated quinolone resistance genes in extended-spectrum beta-lactamase-producing Enterobacteriaceae isolates in Mexico. *Microb Drug Resist* 2011;**17**:497–505.
178. Solans R, Simeon P, Cuenca R, et al. Infectious discitis caused by *Enterobacter cloacae*. *Ann Rheum Dis* 1992;**51**:906–7.
179. Syrogiannopoulos GA, McCracken GH Jr, Nelson JD. Osteoarticular infections in children with sickle cell disease. *Pediatrics* 1986;**78**:1090–6.
180. Szabó D, Bonomo RA, Silveira F, et al. SHV-type extended-spectrum beta-lactamase production is associated with reduced cefepime susceptibility in *Enterobacter cloacae*. *J Clin Microbiol* 2005;**43**:5058–64.
181. Szabó D, Silveira F, Hujer AM, et al. Outer membrane changes and efflux pump expression together may confer resistance to ertapenem in *Enterobacter cloacae*. *Antimicrob Agents Chemother* 2006;**50**:2833–5.
182. Thomson KS, Sanders CC, Chmel H. Imipenem resistance in *Enterobacter*. *Eur J Clin Microbiol Infect Dis* 1993;**12**:610–13.
183. Townsend SM, Hurrell E, Caubilia-Barron J, et al. Characterization of an extended-spectrum beta-lactamase *Enterobacter hormaechei* nosocomial outbreak, and other *Enterobacter hormaechei* misidentified as *Cronobacter* (*Enterobacter*) *sakazakii*. *Microbiology* 2008;**154**:3659–67.
184. Townsend SM, Hurrell E, Gonzalez-Gomez I, et al. *Enterobacter sakazakii* invades brain capillary endothelial cells, persists in human macrophages influencing cytokine secretion and induces

severe brain pathology in the neonatal rat. *Microbiology* 2007;**153**:3538–47.
185. Tresoldi AT, Padoveze MC, Trabasso P, et al. *Enterobacter cloacae* sepsis outbreak in a newborn unit caused by contaminated total parenteral nutrition solution. *Am J Infect Control* 2000;**28**: 258–61.
186. Tunkel AR, Fisch MJ, Schlein A, et al. *Enterobacter* endocarditis. *Scand J Infect Dis* 1992;**24**:233–40.
187. Tzouvelekis LS, Tzelepi E, Kaufmann ME, et al. Consecutive mutations leading to the emergence in vivo of imipenem resistance in a clinical strain of *Enterobacter aerogenes*. *J Med Microbiol* 1994;**40**:403–7.
188. vanAcker J, deSmet F, Muyldermans G, et al. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *J Clin Microbiol* 2001;**39**:293–7.
189. van den Berg RW, Claahsen HL, Niessen M, et al. *Enterobacter cloacae* outbreak in the NICU related to disinfected thermometers. *J Hosp Infect* 2000;**45**:29–34.
190. van der Mee-Marquet N, Girard S, Lagarrigue F, et al. Multiresistant *Enterobacter cloacae* outbreak in an intensive care unit associated with therapeutic beds. *Crit Care* 2006;**10**:405.
191. van Nierop WH, Duse AG, Stewart RG, et al. Molecular epidemiology of an outbreak of *Enterobacter cloacae* in the neonatal intensive care unit of a provincial hospital in Gauteng, South Africa. *J Clin Microbiol* 1998;**36**:3085–7.
192. van Rossem MC, de Waal WJ, van Hannen EJ, et al. *Enterobacter* colonization in newborn infants: predictors, follow-up and implications for infection control. *J Hosp Infect* 2007;**67**:142–8.
193. Varaldo PE, Biavasco F, Mannelli S, et al. Distribution and antibiotic susceptibility of extraintestinal clinical isolates of *Klebsiella*, *Enterobacter* and *Serratia* species. *Eur J Clin Microbiol Infect Dis* 1988;**7**:495–500.
194. Verhamme KM, DeCoster W, DeRoo L, et al. Pathogens in early-onset and late-onset intensive care unit-acquired pneumonia. *Infect Control Hosp Epidemiol* 2007;**28**:389–97.
195. Vonberg RP, Gastmeier P. Hospital-acquired infections related to contaminated substances. *J Hosp Infect* 2007;**65**:15–23.
196. Waites KB, Duffy LB, Dowzicky MJ. Antimicrobial susceptibility among pathogens collected from hospitalized patients in the United States and in vitro activity of tigecycline, a new glycylcycline antimicrobial. *Antimicrob Agents Chemother* 2006;**50**:3479–84.
197. Walder M, Haeggman S, Tullus K, et al. A hospital outbreak of high-level beta-lactam-resistant *Enterobacter* spp. association more with ampicillin and cephalosporin therapy than with nosocomial transmission. *Scand J Infect Dis* 1996;**28**:293–6.
198. Wang M, Cao B, Gao Q, et al. Detection of *Enterobacter sakazakii* and other pathogens associated with infant formula powder by use of a DNA microarray. *J Clin Microbiol* 2009;**47**:3178–84.
199. Wang SA, Tokars JI, Bianchine PJ, et al. *Enterobacter cloacae* bloodstream infections traced to contaminated human albumin. *Clin Infect Dis* 2000;**30**:35–40, 156.
200. Watanakunakorn C, Weber J. *Enterobacter* bacteremia: a review of 58 episodes. *Scand J Infect Dis* 1989;**21**:1–8.
201. Watson JJ, Jones RC, Siston AM, et al. Outbreak of catheter-associated *Klebsiella oxytoca* and *Enterobacter cloacae* bloodstream infections in an oncology chemotherapy center. *Arch Intern Med* 2005;**165**:2639–43.
202. Weischer M, Kolmos HJ. Retrospective 6-year study of enterobacter bacteraemia in a Danish university hospital. *J Hosp Infect* 1992;**20**:15–24.
203. Weischer M, Kolmos HJ. Ribotyping of selected isolates of *Enterobacter cloacae* and clinical data related to biotype, phage type, O-serotype, and ribotype. *A P M I S* 1993;**101**:879–86.
204. Wendt C, Lin D, von Baum H. Risk factors for colonization with third-generation cephalosporin-resistant enterobacteriaceae. *Infection* 2005;**33**:327–32.
205. Wenger PN, Tokars JI, Brennan P, et al. An outbreak of *Enterobacter hormaechei* infection and colonization in an intensive care nursery. *Clin Infect Dis* 1997;**24**:1243–4.
206. Widjojoatmodjo MN, Fluit AC, Verhoef J. Molecular identification of bacteria by fluorescence-based PCR-single-strand conformation polymorphism analysis of the 16S rRNA gene. *J Clin Microbiol* 1995;**33**:2601–6.
207. Wiegand I, Geiss HK, Mack D, et al. Detection of extended-spectrum beta-lactamases among Enterobacteriaceae by use of semiautomated microbiology systems and manual detection procedures. *J Clin Microbiol* 2007;**45**:1167–74.
208. Willis J, Robinson JE. *Enterobacter sakazakii* meningitis in neonates. *Pediatr Infect Dis J* 1988;**7**:196–9.
209. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; **39**:309–17.
210. Wolff MA, Young CL, Ramphal R. Antibiotic therapy for enterobacter meningitis: a retrospective review of 13 episodes and review of the literature. *Clin Infect Dis* 1993;**16**:772–7.
211. Woodford N, Hill RLR, Livermore DM. In vitro activity of tigecycline against carbapenem-susceptible and -resistant isolates of *Klebsiella* spp. and *Enterobacter* spp. *J Antimicrobiol Chemother* 2007;**59**:582–3.
212. Xu J, Moore JE, Millar BC, et al. Improved laboratory diagnosis of bacterial and fungal infections in patients with hematological malignancies using PCR and ribosomal RNA sequence analysis. *Leuk Lymphoma* 2004;**45**:1637–41.
213. Xu X, Wu S, Ye X, et al. Prevalence and expression of the plasmid-mediated quinolone resistance determinant qnrA1. *Antimicrob Agents Chemother* 2007;**51**:4105–10.
214. Yamamoto T, Honda T, Miwatani T, et al. A virulence plasmid in *Escherichia coli* enterotoxigenic for humans: Intergenetic transfer and expression. *J Infect Dis* 1984;**150**:688–98.
215. Yu WL, Cheng KC, Chi JC, et al. Characteristics and molecular epidemiology of extended-spectrum beta-lactamase-producing *Enterobacter cloacae* isolated from a district teaching hospital in Taiwan. *Clin Microbiol Infect* 2006;**12**:579–82.
216. Zhanel GG, DeCorby M, Adam H, et al. Prevalence of antimicrobial-resistant pathogens in Canadian hospitals: results of the Canadian Ward Surveillance Study (CANWARD 2008). *Antimicrob Agents Chemother* 2008;**52**:1430–7.
217. Zhong L, Men TY, Li H, et al. Multidrug-resistant gram-negative bacterial infections after liver transplantation: spectrum and risk factors. *J Infect* 2012;**64**:299–310.

TABLE 103-1 **Major *Escherichia coli* Classifications**

Main Group	Subgroup	Associated Diseases
Intestinal pathogenic *E. coli* (IPEC)	Enterohemorrhagic *E. coli* (EHEC)	Afebrile, bloody colitis
	Enteropathogenic *E. coli* (EPEC)	Watery diarrhea
	Enterotoxigenic *E. coli* (ETEC)	Watery diarrhea
	Enteroaggregative *E. coli* (EAEC)	Chronic, persistent diarrhea
	Enteroinvasive *E. coli* (EIEC)	Watery diarrhea or dysentery
Extraintestinal pathogenic *E. coli* (ExPEC)	Uropathogenic *E. coli* (UPEC)	Urinary tract infection, urosepsis
	Neonatal meningitis *E. coli* (NMEC)	Meningitis
	Sepsis-causing *E. coli* (SEPEC)	Bacteremia, sepsis
Animal-associated pathogenic *E. coli* (AAPEC) reservoir groups	Avian pathogenic *E. coli* (APEC)	Bird sepsis, chronic respiratory disease, swollen head syndrome
	Mammary pathogenic *E. coli* (MPEC)	Bovine mastitis
	Endometrial pathogenic *E. coli* (EnPEC)	Bovine endometriosis

TABLE 103-2 **Risk Factors for Extraintestinal Pathogenic *Escherichia Coli* Urinary Tract Infection in Children**

Factor	Type	Mechanism	References
Female gender	Physical-anatomic	Shorter urethra and closer proximity to the rectum increases access of uropathogens to the urinary tract	139
Vesicoureteral reflux	Physical-anatomic	Reflux of urine through the ureteral valves moves contaminated urine from the bladder to the kidneys	7, 11, 117
Bladder dyssynergia	Physical-anatomic	Incoordination of voiding; failure to clear microbes	120
Urinary tract anomalies	Physical-anatomic	Urinary stasis; failure to clear microbes	7
Catheterization	Physical-anatomic	Introduction of organisms into urinary tract; persistence of less virulent organisms	73, 112, 126
Sexual intercourse	Behavioral	Introduction of periurethral organisms into urinary tract	26, 91
Spermicide use	Behavioral	Inhibition of vaginal lactobacilli with outgrowth of *E. coli*	45
Diaphragm use	Behavioral	Spring-rim–mediated minor bladder trauma; co-use of spermicidal gels	45
Diabetes mellitus	Immune	Glucosuria; neutrophil functional impairment	32, 93, 98

decade and has important implications in the treatment of ExPEC-related infections.

Urinary Tract Infection

E. coli is the leading cause of culture-proved UTI in children, producing more than 80 percent of community-acquired UTI, regardless of the presence of vesicoureteral reflux.[44] Other etiologies in pediatric community-acquired UTI include *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus* spp., and, primarily in female adolescents, *Staphylococcus saprophyticus*. *E. coli* is also the leading cause of hospital-acquired UTI among children. Unlike in adult patients, in whom *E. coli* may account for more than 50 percent of nosocomial UTIs, *E. coli* produces approximately 30 percent of nosocomial UTIs in children.[72,79] Organisms such as *Candida* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa* assume a greater proportion of pediatric nosocomial UTIs.

In general, the prevalence of UTI is 7 percent among infants with febrile illness, of which, as noted earlier, *E. coli* is by far the most likely cause.[116] Among female infants, 7.5 percent with febrile illnesses have a UTI. Of male infants, the prevalence differs greatly based on whether the infant was circumcised. Among uncircumcised male infants, the prevalence of UTI is 20.1 percent, in contrast to a prevalence of 2.4 percent among those who are circumcised. Circumcision status does not alter the high proportion of UTIs associated with *E. coli*. Black infants have a lower prevalence of UTI, at approximately 4.7 percent, in contrast to white children, at 8 percent. The prevalence of UTI drops significantly after the age of 1 year but increases during adolescence such that the pooled prevalence among children younger than 19 years of age is 7.8 percent.[116] Factors increasing the risk for urinary tract infection include vesicoureteral reflux, bladder dyssynergia, other urinary tract anomalies, sexual intercourse, spermicide use, pregnancy, and diabetes mellitus (Table 103-2).

Fever without localizing signs is a major cause of presentation for medical care among infants younger than 90 days of age. Recent studies indicate that in the era of vaccinations for *Haemophilus influenza* type b and *Streptococcus pneumoniae*, *E. coli* UTI is the leading cause of

fever without localizing signs.[133,134] UTI was responsible for 76 percent of all episodes, and bacteremia and meningitis were 19 percent and 3 percent, respectively. Of the UTIs, *E. coli* was the causative agent in 96 percent of the cases.

Some female children experience colonization of the bladder without urinary symptoms, termed asymptomatic bacteriuria (ASB). *E. coli* is also the most common cause of ASB. In U.S. female school children at 5 to 6 years of age, the incidence of ASB was observed to be 1.9 to 2.2 percent, whereas only 0.2 percent of age-matched boys had ASB.[20,113] The cumulative prevalence of ASB between the ages of 6 and 12 years is reported to be 4.6 percent. No ethnic or socioeconomic risk factors have been associated with ASB. In addition to sex, risk factors include diabetes and sickle cell disease.[17,106]

Bacteremia and Sepsis

E. coli may produce bacteremia or sepsis. Pediatric *E. coli* bacteremia and sepsis occur mostly in young infants and special groups of children such as patients with chemotherapy-induced immunodeficiency, central venous catheters, urinary tract anomalies, and human immunodeficiency virus.[16,108] Patients in the neonatal and pediatric intensive care units have a heightened risk for bloodstream *E. coli* infections, as do patients with obstructive urinary tract disease and UTI, leading to urosepsis.

Among infants, *E. coli* bloodstream infections are most common in the first month of life, with decreasing numbers of cases in the second to third months of life. Among neonates, *E. coli* has been associated with approximately 18 percent of early-onset sepsis and a similar percentage of late-onset sepsis, placing it as the second or third most common etiology in this age group.[130] In preterm infants, the infection rate for *E. coli* was over 10 percent, with a contemporary study demonstrating a rate of 29 percent.[9,123] Prematurity, low birth-weight, prolonged labor, prolonged rupture of membranes, intrapartum maternal fever, and maternal antibiotics may increase the risk for complicated, high morbidity or mortality infections.[128] Neonatal early-onset group B streptococcal disease has decreased significantly with the use of intrapartum ampicillin for women at risk because of known colonization, prolonged rupture of membranes, or maternal fever. However, more than 50 percent of *E. coli* organisms are resistant to ampicillin such that prophylaxis has not had a positive impact on early-onset *E. coli* infection. Retrospective data suggest that ampicillin-resistant *E. coli* may be responsible for more fulminant and higher mortality disease among premature infants,[1,9,55] although this remains controversial and in need of further study.[115]

Meningitis

In the pediatric population, *E. coli* meningitis occurs almost exclusively among young infants and highly immunocompromised children. It is the leading cause of meningitis among preterm infants and equal to group B streptococcus.[19,122] *E. coli* meningitis frequently results in intensive care, severe neurologic impairment, and high mortality, at 34 percent, 21 percent, and 14 percent, respectively.[48,49] Twenty-eight percent of infants have intercurrent UTI and are older than 6 days of life.[48] Concurrent bacteremia is present in 81 percent.

Peritonitis

Peritonitis in children is most typically a complication of appendicitis. Intestinal perforation is an infrequent cause in children. Overall, it is a rare entity in children in contrast to adults. *E. coli* accounts for more than 50 percent of bacterial peritonitis in children, with 60 percent of the infections being polymicrobial.[23]

Pneumonia, Endocarditis, and Osteomyelitis

E. coli is an infrequent cause of infections of the lungs, heart, and bones. Pneumonia may arise from oropharyngeal or hematogenous sources and endocarditis through hematogenous spread after translocation of bacteria from the intestinal tract or following urosepsis. Osteomyelitis is most frequently hematogenous after intestinal translocation or urosepsis but also may occur after traumatic injuries and direct inoculation into areas proximal to bone.

PATHOGENESIS

ExPEC produces a wide variety of invasive infections outside of the gastrointestinal tract, including those of the urinary tract, bloodstream, and central nervous system (CNS). ExPEC is a normal commensal organism in the enteric and genital tracts, and colonization alone does not have any measurable consequence to individuals harboring these strains. ExPEC colonizes through oral-fecal transmission where food may be contaminated, through cross-transmission between humans, or, as more recently described, through transmission between domesticated animals such as cats and dogs.[53] Sexual transmission of ExPEC also has been shown to occur, with male partners appearing to transmit ExPEC bearing virulence gene characteristics typical of those producing UTIs to their female partners.[28] The relative frequency of acquisition of ExPEC by oral-fecal and sexual routes is not clear at the present time.

ExPEC infections occur after bacteria invade sterile sites such as the urinary tract or the bloodstream from commensal reservoirs such as the enteric tract and vagina. When the immune system is unable to limit the spread of bacteria, invasive infections such as UTI, bacteremia, meningitis, and, more rarely, pneumonia or osteomyelitis may occur.

Urinary Tract Infection

ExPEC is the leading cause of UTIs,[27] and UTI may precede more invasive infections of the bloodstream, bones, heart, and CNS. ExPEC colonizing the

periurethral area may be introduced into the urethra and ascend through the urinary tract. The infection may involve only the urethra (urethritis), but most commonly involves the bladder, resulting in cystitis. More advanced infections may ascend through the ureters to the kidneys, resulting in pyelitis, pyelonephritis, or pyelonephrosis. Anatomic variation or defects, such as voiding dysfunction and vesicoureteral reflux, increase the risk for lower tract and upper tract UTI.[30]

Infrequently, ExPEC UTI may occur through hematogenous spread in which bloodstream organisms infect the kidneys and are subsequently isolated from the urine in a descending infection. This may occur during bacteremia after organisms have translocated from the enteric tract. Perinephric abscesses may be the sole consequence of hematogenous infection of the urinary tract.

The specific host and bacterial factors that favor transition from the external genital region into the urethra remain poorly understood in the prepubescent, nonsexually active child. After the initiation of sexual activity, UTI rates increase.[27] Spermicides and intercourse are both associated with an increased risk for UTI in women, primarily through promotion of ExPEC in the vagina and mechanical introduction of bacteria into the female urethra, respectively. The size of the inoculum necessary to start an infection in humans is not well understood and is likely overestimated in animal models.

Many of the molecular events during ExPEC cystitis have been recently elucidated through animal and human studies (Fig. 103-1), and ExPEC produces virulence factors to persist in the urinary tract (Table 103-3). Once in the urinary bladder, ExPEC adheres to the bladder epithelium to initiate an infection. Attachment is essential because micturition is a potent host defense to flush the bacteria out of the organ.[135] Type 1 pili, fibrous extensions from the bacteria with adhesive tips, have been established as a major *E. coli* factor promoting adherence to the bladder epithelium. On attachment of ExPEC to the apical surface of the bladder epithelium via type 1 pili, ExPEC can invade into bladder epithelial cells through a variety of routes, including lipid rafts and caveolin, clathrin-coated pits, and fusiform vesicles.[8,24,61] Within the epithelium, ExPEC may persist long-term in late endosomes[86,87] or escape into the cytosol of the epithelial cell and form intracellular bacterial communities (IBCs), biofilm-like structures with bacteria enmeshed in type 1 pili, aggregative antigens, and polysaccharide capsules.[3,4] From the IBC state, bacteria may amass and escape as long filaments, only to bind to more bladder epithelial cells and reinvade naïve epithelium.[56,57]

ExPEC isolates from children with pyelonephritis are more likely to produce P pili, hemolysin, and cytotoxic necrotizing factor (CNF-1) and be serum resistant than cystitis strains or commensal isolates.[21,49] In the kidneys,

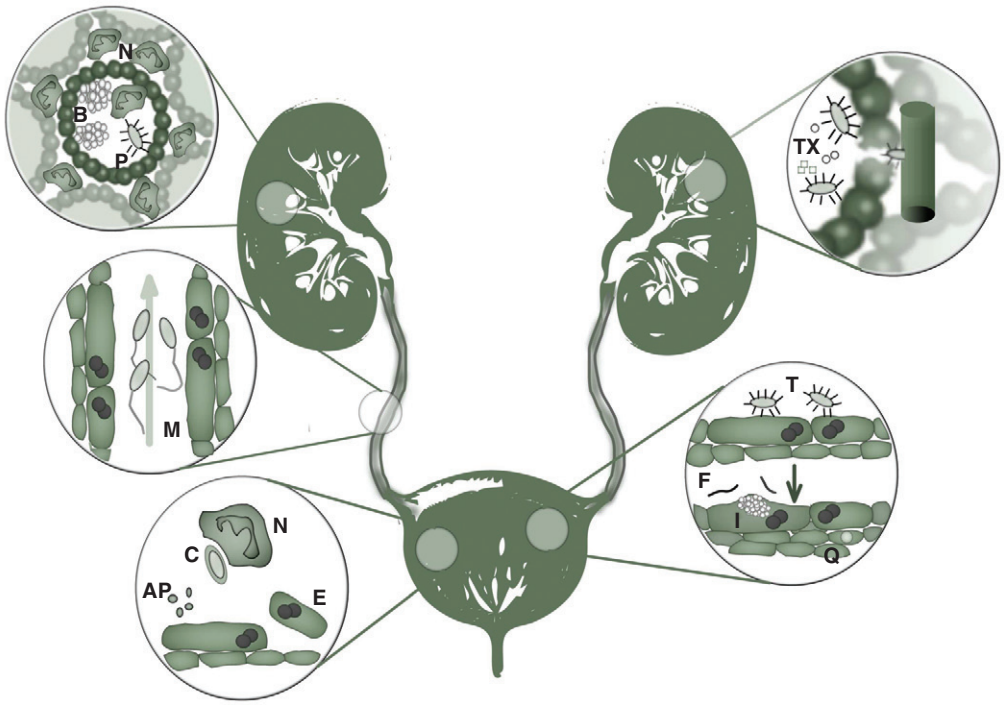

FIGURE 103-1 ■ Pathogenesis of extraintestinal pathogenic *E. coli* (ExPEC) urinary tract infection. ExPEC produces lower and upper tract infection. In the bladder, *E. coli* adheres to the bladder epithelium by using adherence factors such as type 1 pili *(T, lower right circle).* ExPEC can invade the epithelium, replicate, and reemerge as filamentous bacteria *(F).* Infection of the bladder results in a strong inflammatory response *(lower left circle)* involving production of antimicrobial peptides *(P),* neutrophil infiltration *(N),* and exfoliation, or sloughing, of the epithelium *(E).* ExPEC produces a capsule *(C)* to protect against the innate immune response. ExPEC can migrate up the ureters *(middle left circle)* against urinary flow using flagella *(M).* In the kidneys, ExPEC produces P pili *(P)* and other adhesive factors to bind to the renal epithelium, where biofilms *(B)* may be formed. An inflammatory response includes neutrophils *(N)* and other factors such as complement and antimicrobial peptides. From the kidney tubules, ExPEC may also produce toxins *(TX, upper right circle),* damaging the local epithelial and endothelial borders to invade capillaries and disseminate in the bloodstream, producing bacteremia. (See companion website on Expert Consult for a color version of this figure.)

TABLE 103-3 **Representative Extraintestinal *Escherichia Coli* Virulence Factors**

Functional Category	Virulence Factor	Role	References
Adhesin	Type 1 pili *(fim)*	Adherence to urinary tract epithelium, particularly in bladder; lectin-recognizing mannosylated glycoproteins	50, 51, 62
	P pili *(pap/prf)*	Adherence to urinary tract epithelium, particularly in bladder; lectin-recognizing glycolipids	70, 78, 105, 129
	S pili *(sfa/foc)*		135
	Auf pili		15
	Dr adhesins	Examples include Dr and Afa I-IV; binding to decay-activating factor	33, 95
	TosA	Repeats-In-Toxin (RTX) protein; adherence to renal tubular cells	131
	UpaB	Autotransporter-type adhesion; binding to extracellular matrix proteins	2
	Tsh	Serine protease-type autotransporters; associated with pyelonephritis strains	42
Invasin	IbeA	Invasion of the brain endothelium	54, 78
	OmpA	Outer membrane protein A	92
Toxins	HlyA	α-Hemolysin	83
	Cyclomodulins	Examples include cytolethal distending toxin (CDT-1) and cytotoxic necrotizing factor 1 (CNF-1)	22
	Sat	Serine autotransporter	37
Protectins	Groups II and III polysaccharide capsules	K1, K2, and K5 capsules, among others	3, 5
	OmpT	Outer membrane protease T	124
	Curli	Amyloid-like extracellular fibers	58
Iron acquisition	IreA	Siderophore receptor	31
	Iro	Salmochelin	31
	Iuc	Aerobactin	31
	SitA	Periplasmic iron binding protein	31
	Ybt	Yersiniabactin	31
Immune regulators	TcpC	Immunomodulator	78
	SisA/B	Inflammation suppressors	76
Other	DsdA	D-Serine deaminase	39
	Flagella	Motility	71, 136
	Cellulose	Biofilm formation	94

ExPEC uses Ppili and type 1 pili to adhere to the renal tubule epithelium and persist in the kidney.[70,81] Local toxin production, including hemolysin, CNF-1, and Sat, damage the epithelium and may permit bacterial penetration and translocation across the border into the adjacent capillary system.[80,119] The toxins also may inhibit infiltrating granulocytes and limit their capacity to contain the infection.[18]

In response to acute infection of the bladder, the lipopolysaccharide-recognition receptor Toll-like receptor 4 (TLR4) is triggered by ExPEC, initiating a signal cascade that results in proinflammatory events, including the expression of cytokines interleukin (IL)-6, IL-8, and granulocyte-macrophage colony-forming unit (GM-CSF).[41,121] Neutrophils and macrophages are recruited into the bladder to assist in clearing the infection.[68,137] The superficial bladder epithelium is also shed in the process of exfoliation, resulting in elimination of attached and invasive intracellular ExPEC. Microscopy of urine from young women with acute cystitis revealed leukocytes and exfoliated bladder epithelial cells, including filamentous ExPEC and cells harboring IBC.[110] In animal studies, the clearance of ExPEC is incomplete, even with the application of antibiotics, resulting in chronic latent intravesical infection, predicted to be a reservoir for some recurrent infections.[87,114]

The determinants that balance acute inflammatory infections and ASB are not fully known. ASB and cystitis isolates have reduced virulence gene numbers relative to strains causing pyelonephritis.[10,125] Despite similar known virulence gene content in cystitis strains of ExPEC, a prototypic ASB isolate of *E. coli* has been previously shown to have reduced virulence factor gene expression in contrast to cystitis isolates and ASB isolates may outcompete cystitis strains for growth in urine.[109] The host response to bacteria within the urinary tract is likely to be important to the development of ASB. Children with reduced TLR4 expression have been shown to have an increased risk for ASB.[104]

Bacteremia

ExPEC are frequent causes of bacteremia secondary to translocation from the intestinal tract into the

bloodstream. This frequently occurs in patients with loss of intestinal epithelial barrier integrity or compromised immunity. ExPEC produces protective factors that favor survival in serum, including polysaccharide capsules known to inhibit complement deposition, opsonization, and phagocytosis by granulocytes.[12,47,111] Toxins such as hemolysin A and CNF-1 have roles in inhibiting phagocyte function,[18,103] thus limiting ExPEC clearance. ExPEC also responds to the bloodstream environment by downregulating factors that may stimulate immunity or promote phagocytosis such as the adhesive factor type 1 pili.

Critical metabolic pathways are necessary for successful dissemination of ExPEC in bloodstream infections. Sialic acid usage and iron acquisition have been previously shown to be essential for ExPEC survival during disseminated infection.[118]

Meningitis

ExPEC disseminates from mucosal surfaces through the bloodstream to the CNS. Neonatal animal models have suggested that ExPEC disseminates to the CNS within macrophages and that dissemination depends on nitric oxide production.[82] The severity of bacteremia correlates with the likelihood of developing meningitis in both infant and adult animal models.[67] At the blood-brain barrier, ExPEC transverses the brain microvascular endothelial cells by first binding and invading the cells through actin rearrangement and transcellular traversal of the barrier.[66] Type 1 pili and OmpA (Table 103-4) are key adhesive factors mediating the initial interaction between *E. coli* via CD48 and gp96, respectively.[64,102] The *E. coli* factor IbeA has been shown to enhance brain microvascular endothelial cell invasion.[29,101] K1 polysaccharide encapsulation, common among ExPEC causing neonatal meningitis prevents late endosome–lysosomal fusion and thus killing of intracellular *E. coli*, whereas lack of encapsulation results in bacteria proceeding through early to late endosomes and lysosomal fusion with eventual degradation of the bacteria-containing vacuoles.[65] The consequence of brain endothelial cell invasion by *E. coli* is blood-brain barrier dysfunction resulting from cytokine release such as the proinflammatory factor IL-8, brain endothelial cell apoptosis, and increased permeability, ultimately leading to encephalopathy.[66]

TABLE 103-4 **Susceptibility to Antibiotic-Class Representative Agents Commonly Used in the Treatment of Urinary Tract Infection and Invasive *Escherichia Coli* Infections**

Antibiotic*	Susceptibility (%)	Reference
Ampicillin/amoxicillin	61.8	[138]
Amoxicillin-sulbactam	59	Unpublished†
Trimethoprim-sulfamethoxazole	77.4	[138]
Nitrofurantoin	97.3	[138]
Ciprofloxacin‡	94.4	[138]
Levofloxacin‡	94.5	[138]
Ceftriaxone	97	Unpublished†
Gentamicin	92	Unpublished†
Tobramycin	92	Unpublished†
Amikacin	100	Unpublished†
Meropenem	100	Unpublished†

*Oral agents are listed at the top of the table.
†Data are representative of the Research Triangle Region in North Carolina.
‡U.S. Food and Drug Administration–approved for complicated *E. coli* urinary tract infections.
Note: Children <15 yr of age were ≤6.4% of the subjects from whom isolates were obtained.[138]

CLINICAL PRESENTATION

Urinary Tract Infection

Acute UTI with *E. coli* is associated with dysuria, urinary frequency, urgency, and often cloudy or odorous urine. Cystitis typically has low-grade fever or no elevation in temperature and may include anterior abdominal or pelvic pain or pressure. In contrast, *E. coli* pyelonephritis includes flank or lower back pain, and high fever, often higher than 38.9°C (102°F). Pyelonephritis may also include chills, sweats, rigors, nausea, vomiting, and headache. In patients with perinephric abscesses or intrarenal necrosis, the patient may have pleuritic chest pain from irritation of the diaphragm, and a flank mass may be present on physical examination. Bacteremia and urosepsis may develop secondary to pyelonephritis, especially among patients with diabetes, urinary tract abnormalities, urinary obstruction, or pregnancy. Pyelonephritis with and without urosepsis must be differentiated from intra-abdominal processes, including appendicitis, psoas abscess, ruptured ovarian cyst, and ectopic pregnancy.

E. coli UTIs are not easily distinguished symptomatically from the syndromes caused by other organisms, including *S. saprophyticus* in the female adolescent, *K. pneumoniae*, *P. mirabilis*, or group B streptococcus.

Bacteremia and Sepsis

Bacteremia by *E. coli* is not readily distinguished in signs and symptoms from bloodstream infections produced by other gram-negative bacteria. Children may have fever, fatigue, lethargy, or irritability. Nausea and vomiting may accompany bloodstream infections. Some children may have a diffuse erythematous rash and, in the case of sepsis, generalized edema. Tachycardia may occur in the presence and absence of fever. Urine output may be diminished accompanying renal failure in cases of severe infection.

Meningitis

Neonates with *E. coli* meningitis present with general nonspecific features, including fever, vomiting, irritability, and failure to thrive. In advanced circumstances,

neurologic signs, including apnea, seizures, decreased muscular tone, and ocular or limb palsies, may be present. Jaundice may accompany the presentation. In the infant older than 3 months, nuchal rigidity and a tense fontanelle may be part of the presentation. Acute *E. coli* meningitis in the older child is accompanied by headache, nausea, vomiting, lethargy, confusion, and fever. Neurologic features such as seizure or focal symptoms may accompany *E. coli* meningitis. The clinical presentation of *E. coli* meningitis overlaps with other etiologies of meningitis.

E. coli meningitis may be a rare complication of neurosurgical procedures. The clinical presentation may not be different from that of acute meningitis in the nonsurgical patient. However, infectious meningitis must be differentiated from other infectious and noninfectious complications of surgery.

Acute *E. coli* meningitis must be differentiated from other processes, including seizure disorder, brain abscess, aneurysm, sepsis, and, in the case of the neonate, tetanus.

Intra-abdominal Infection

E. coli produces cholecystitis and cholangitis associated with right upper quadrant pain, fever, and jaundice. In advanced cases, bacteremia and sepsis may complicate the infections. Both infections are associated with high fever, typically above 38.9°C (102°F). Cholangitis is accompanied by chills and sometimes hepatic abscess. Children with *E. coli* cholecystitis or cholangitis develop right upper quadrant pain, fever, and jaundice. In severe cases, hypotension and confusion also develop. Cholecystitis manifests with fever (>38.9°C [102°F]). Cholangitis manifests with fever (>38.9°C [102°F]), shaking chills, and right upper quadrant pain and can be complicated by hepatic abscess. Other infectious etiologies may bear resemblance to *E. coli* intra-abdominal infection, including *Klebsiella* spp., enterococcus, and, with appropriate exposure histories, amoebic abscess or *Echinococcosis* spp.

E. coli also may cause intra-abdominal abscesses with and without peritonitis. Abscess alone may be associated with low-grade fever and nonspecific abdominal pain, as well as occasional malaise and anorexia. Peritonitis is associated with higher fever and rebound pain. In severe cases, septic shock may accompany peritonitis. Patients with *E. coli* intra-abdominal abscesses may have low-grade fever, but the spectrum of clinical presentations ranges from nonspecific abdominal examination findings to frank septic shock. Peritonitis manifests as localized pain with rebound and fever. Abdominal abscess with E. coli are often polymicrobial with anaerobes, gram-negative bacilli, and enterococci.

Pneumonia

Children with *E. coli* pneumonia typically present with a febrile illness, shortness of breath, tachypnea, increased respiratory secretions, and rales and crackles with auscultation. *E. coli* pneumonia may be ventilator-associated and bacteremia-associated. *E. coli* is a rare cause of community-acquired pneumonia in a child with otherwise normal immune defenses. *E. coli* pneumonia is difficult to distinguish clinically from other bacterial pneumonia.

DIAGNOSIS

General

The definitive diagnosis of ExPEC infection is isolation of the organism from a sterile site such as urine, blood, or cerebrospinal fluid (CSF). Isolation from sputum or wounds is also indicated in some circumstances, such as pneumonia and abdominal infections, respectively. In these circumstances, recovery of the bacterium must be regarded in the context of the patient's clinical presentation to determine if the isolation of the organism is compatible with infection or represents colonization.

E. coli is readily isolated from a wide variety of clinical samples at 37°C (98.6°F) and aerobic conditions.[40] Nonselective and selective media, including MacConkey agar, will isolate *E. coli*. Approximately 90 percent of isolates are lactose fermenting, and approximately 99 percent produce indole, making this an optimal biochemical test for the identification of *E. coli*.[89] Many isolates are β-hemolytic, although a significant percentage from sites such as the urinary tract may not display β hemolysis on blood agar. *E. coli* does not typically produce pigments.

Urinary Tract Infection

For suspicion of UTI, a urine dipstick test may be performed to rapidly determine if the patient has pyuria or bacteriuria based on the detection of leukocyte esterase and nitrites, respectively. Definitive diagnosis is based on urine culture results. The specimen should be collected from a midstream clean void in children old enough to provide such a sample or by straight sterile catheterization in younger children. A contaminated specimen and asymptomatic colonization must be differentiated from infection based on clinical symptoms and signs, as well as the urinalysis consistent with inflammation. In cases of infection, pyuria usually is present. In urinalysis, leukocyte esterase and nitrite each have a sensitivity of 0.88 and a false positive rate of 0.05. Bacteria identified by Gram stain on noncentrifuged samples have a sensitivity of 0.93 and false positive rate of 0.05. White blood cells alone have poor sensitivity and increased false positive rate.[34]

Routine screening of the urine for cure after antibiotic therapy and screening for asymptomatic colonization are not recommended because screening produces a large number of false positive results and does not alter rates of renal scarring.[60,63]

Imaging studies may be indicated in some patients to assist in making a diagnosis of *E. coli* infection, determining the necessity for an interventional procedure, or assessing the prognosis. For UTI, renal ultrasonography, computed tomography, or dimethylsuccinic acid scans

may be indicated to evaluate the kidneys and look for any other source of abscess, stones, or obstruction. Vesicoureterogram may be indicated for the diagnosis of vesicoureteral reflux, which may predispose a patient to pyelonephritis.

Other Infections

Recovery of the organism in contaminated sites, such as sputum and wounds, must be analyzed in the context of the patient's clinical state to determine if it represents colonization or infection. Recovery from sterile sites, such as the CSF, should be considered diagnostic of infection.

Lumbar puncture and a CSF culture for *E. coli* will establish the diagnosis of acute *E. coli* meningitis. Before culture data are available, CSF parameters alone will not be specific for *E. coli* infection. Elevated protein, depressed glucose, and the presence of leukocytes with a neutrophilic predominance may be present. Before culture information is available, Gram stain may reveal gram-negative bacilli without being solely indicative of *E. coli*. Justification for this lumbar puncture and analysis must be made based on indications for possible CNS infection, including a positive blood culture, altered behavior, and abnormal neurologic signs, such as seizure.

Patients with pneumonia should undergo blood cultures and sputum Gram stain and culture. The results of a Gram stain of the sputum help differentiate a good specimen (i.e., many polymorphonuclear [PMN] leukocytes and few squamous epithelial cells) from a bad specimen (i.e., few PMN leukocytes and many squamous epithelial cells). In addition, obtain the sputum culture before antibiotic therapy is initiated.

Additional studies may be indicated for non-UTI ExPEC infections. In pneumonia, chest radiography or CT is indicated. In cholecystitis and cholangitis, ultrasonography or CT of the right upper quadrant is indicated. For suspicion of intra-abdominal abscess, abdominal and pelvic ultrasound or CT may be indicated.

TREATMENT

General

Therapy for ExPEC infections depends on the site and severity of infection. The choice of antibiotics must be determined on the basis of local region antibiotic susceptibilities, site to which the antibiotic is to be delivered, and appropriateness and tolerance of oral or parenteral agents.

Urinary Tract Infection

Determination of therapy for UTI is first contingent on the appropriateness of oral therapy or intravenous antibiotics. Most community-acquired UTIs may be treated with oral therapy, assuming antibiotic resistance that would preclude therapy with an available oral agent is not suspected, the child is able to tolerate oral therapy (e.g., not vomiting), and the child does not have underlying urinary tract anomalies or other comorbidities such as diabetes mellitus, renal transplantation, systemic lupus erythematosus, or other conditions. Infants with UTI in the first month of life should be given parenteral antibiotics at the start of their treatment because of the challenge of clinically differentiating lower tract and upper tract infections, the high prevalence of concomitant bacteremia (~10%), and concurrent urinary tract pathology, including posterior urethral valves, obstructed duplex systems, and high-grade vesicoureteric reflux with serious metabolic disturbance, such as hyperkalemia and hypernatremia. Furthermore, past trials have excluded young infants, leaving no specific data to further guide management of UTI in this group.

The duration of therapy for cystitis should be 5 to 7 days, although pediatric-specific data regarding sufficient minimum duration of therapy are not available. Caution must be taken because it may be difficult in the young child to differentiate lower and upper urinary tract involvement, especially given that fever is a typical presenting symptom suggesting upper tract involvement. Typical therapy entails oral trimethoprim-sulfamethoxazole (TMP-SMX), trimethoprim alone, cephalexin, amoxicillin-clavulanate, or nitrofurantoin. The fluoroquinolone ciprofloxacin is not approved for use in children except for complicated *E. coli* UTI.

Prior research has indicated that oral therapy for pyelonephritis using the cephalosporins cefixime and ceftibuten or amoxicillin-clavulanate is effective in treating acute infections and does not result in a significant increase in renal scarring in contrast to initial parenteral therapy.[43,85,90] Pyelonephritis should be treated for a longer duration of 10 to 14 days. In children who are not candidates for initial oral therapy, 2 to 3 days of parenteral therapy should be provided until the child is able to tolerate an oral agent. However, no difference in renal scarring was identified in children receiving initial parenteral therapy before oral therapy in contrast to children receiving oral therapy for the entire course.[90] Longer initial intravenous therapy (8 days vs. 3 days) also was not shown to decrease renal scarring.[13] The combined duration of parenteral and oral therapy should then be the same as for children receiving only oral therapy.

E. coli UTI complicated by perinephric or intrarenal abscess may require surgical drainage and longer therapy. Ultrasound evaluation may be indicated before discontinuation of therapy.

The role of prophylactic antibiotics for the prevention of *E. coli* UTI has changed in recent years as more studies indicate they do not provide significant protection from recurrent infections[31,84,96,99] or in studies demonstrating protection from recurrent *E. coli* infection, prophylactic antibiotics, particularly cephalosporins, may result in subsequent infections with resistant organisms.[35,88] Exceptions may be for children with high-grade vesicoureteral reflux grades IV and V, in which data are currently insufficient to fully determine whether the benefits of daily antibiotic prophylaxis to prevent recurrent UTI and renal scarring outweigh the risks that include cost and the emergence of multidrug-resistant organisms. Trimethoprim-sulfamethoxazole or nitrofurantoin may be considered in these circumstances.

Other Infections

The treatment of invasive infections for *E. coli* should entail parenteral antibiotics of the appropriate spectrum, accounting for local resistance patterns and, in the case of empirical coverage, for other possible etiologic agents. Two antimicrobials with independent mechanisms of action such as a β-lactam and an aminoglycoside should be considered in patients at risk for multidrug-resistant *E. coli* infections. Past hospitalization and antibiotic exposure are among the risk factors for multidrug resistance.

The treatment of *E. coli* bloodstream infections is generally 10 to 14 days. Serious consideration should be given to the removal of central venous catheters in the presence of a high degree of suspicion that the catheter may be involved or responsible for the infection, although mixed data exist for the necessity of catheter removal.[6,16,38,97] *E. coli* meningitis should be treated for a minimum of 21 days or 14 days after obtaining a negative CSF culture, whichever is longer.[100] Parenteral therapy is indicated for both bloodstream infection and meningitis. Treatment of *E. coli* meningitis should include an agent with known penetration of the blood-brain barrier, for example, an advanced cephalosporin such as cefotaxime or a carbapenem such as meropenem, as well as an aminoglycoside, until full antibiotic susceptibilities are available.

Antibiotic Susceptibility

Specific antibiotic susceptibility data for pediatric *E. coli* isolates in North America is severely lacking. However, the antibiotic resistance of *E. coli* causing invasive infections among the population as a whole, particularly UTI, has undergone major shifts over the past decade. Previously *E. coli* was widely susceptible to a range of antibiotics, but in the past decade, resistance has increased in prevalence (see Table 103-4). Susceptibilities of *E. coli* to cephalexin and cefixime, often employed in treating pediatric *E. coli* infections in the outpatient setting, are notably unavailable. Antibiotic resistance to ampicillin, amoxicillin, and TMP-SMX is now broadly distributed. Neonates delivered to mothers treated with ampicillin for the prevention of group B streptococcus infection have an increased risk for ampicillin-resistant *E. coli* early-onset sepsis.[9] Furthermore, multidrug resistance is common. Of the *E. coli* resistant to TMP-SMX, they are 2 times more likely to be resistant to ampicillin and 33 times more likely to be resistant to the fluoroquinolones and nitrofurantoin.[138]

Risk factors associated with infection with extended-spectrum β-lactamase (ESBL)-producing *E. coli* include underlying illness and hospitalization in the previous 3 months.[127]

OUTCOMES

General

Outcomes of *E. coli* infections depend on the degree of invasiveness and status of the host immunologic defenses.

Urinary Tract Infection

Cystitis has the lowest morbidity, mostly incurred in terms of socioeconomic costs and emergence of antibiotic resistance. In contrast, pyelonephritis and other renal infections require more extended antibiotic therapy, incurring more cost and lost time from school and work. Furthermore, renal infections may result in progressive renal scarring with the concern for loss of renal function and early renal insufficiency. In some circumstances, particularly in young children, children with anatomic variations of the urinary tract and those with diabetes, UTI may precede bloodstream infections.

Other Infections

The mortality rate for early-onset and late-onset sepsis in neonates has been previously shown to be approximately 15 percent and 9 percent, respectively.[52] In infants with very low birth-weight (<1500 g) the odds ratio of death with early-onset and late-onset sepsis was 1.45 and 1.30, respectively, with *E. coli* contributing to this degree of risk.[46] Among older children with central venous catheter–related *E. coli* bloodstream infections, the mortality rate is approximately 7 percent despite appropriate antibiotic therapy, with parenteral nutrition and mechanical ventilation being predictors for poor outcome.[16]

In a 25-year retrospective study of neonates with meningitis in a tertiary medical center, of which *E. coli* was one of the top three pathogens, approximately 14 percent died and 40 percent had significant morbidity, including seizures and hearing loss.[74]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Alarcon A, Peña P, Salas S, et al. Neonatal early onset *Escherichia coli* sepsis: trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. *The Pediatr Infect Dis J* 2004;**23**:295–9.
2. Allsopp LP, Beloin C, Ulett GC, et al. Molecular characterization of UpaB and UpaC: two new autotransporter proteins of uropathogenic *Escherichia coli* CFT073. *Infect Immun* 2012;**80**: 321–32.
3. Anderson GG, Goller CC, Justice S, et al. Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. *Infect Immun* 2010;**78**: 963–75.
4. Anderson GG, Martin SM, Hultgren SJ. Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes Infect* 2004;**6**:1094–101.
5. Bahrani-Mougeot FK, Buckles EL, Lockatell CV, et al. Type 1 fimbriae and extracellular polysaccharides are preeminent uropathogenic *Escherichia coli* virulence determinants in the murine urinary tract. *Mol Microbiol* 2002;**45**:1079–93.
6. Benjamin DKJ, Miller W, Garges H, et al. Bacteremia, central catheters, and neonates: when to pull the line. *Pediatrics* 2001; **107**:1272–6.
7. Bhat RG, Katy TA, Place FC. Pediatric urinary tract infections. *Emerg Med Clin North Am* 2011;**29**:637–53.
8. Bishop BL, Duncan MJ, Song J, et al. Cyclic AMP-regulated exocytosis of *Escherichia coli* from infected bladder epithelial cells. *Nat Med* 2007;**13**:625–30.
9. Bizzarro MJ, Dembry LM, Baltimore RS, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008;**121**:689–96.
10. Blanco M, Blanco JE, Alonso MP, et al. Virulence factors and O groups of *Escherichia coli* isolates from patients with acute

pyelonephritis, cystitis and asymptomatic bacteriuria. *Eur J Epidemiol* 1996;**12**:191–8.
11. Blumenthal I. Vesicoureteric reflux and urinary tract infection in children. *Postgrad Med J* 2006;**82**:31–5.
12. Bortolussi R, Ferrieri P, Quie PG. Influence of growth temperature of *Escherichia coli* on K1 capsular antigen production and resistance to opsonization. *Infect Immun* 1983;**39**:1136–41.
13. Bouissou F, Munzer C, Decramer S, et al. Prospective, randomized trial comparing short and long intravenous antibiotic treatment of acute pyelonephritis in children: dimercaptosuccinic acid scintigraphic evaluation at 9 months. *Pediatrics* 2008;**121**:e553–60.
14. Brooks HJ, Benseman BA, Peck J, et al. Correlation between uropathogenic properties of *Escherichia coli* from urinary tract infections and the antibody-coated bacteria test and comparison with faecal strains. *J Hyg* 1981;**87**:53–61.
15. Buckles EL, Bahrani-Mougeot FK, Molina A, et al. Identification and characterization of a novel uropathogenic *Escherichia coli*-associated fimbrial gene cluster. *Infect Immun* 2004;**72**:3890–901.
16. Buckley J, Coffin SE, Lautenbach E, et al. Outcome of *Escherichia coli* and/or *Klebsiella* bloodstream infection in children with central venous catheters. *Infect Arch Pédiatr Hosp Epidemiol* 2007;**28**:1308–10.
17. Cumming V, Ali S, Forrester T, et al. Asymptomatic bacteriuria in sickle cell disease: a cross-sectional study. *BMC Infect Dis* 2006;**6**:46.
18. Davis JM, Rasmussen SB, O'Brien AD. Cytotoxic necrotizing factor type 1 production by uropathogenic *Escherichia coli* modulates polymorphonuclear leukocyte function. *Infect Immun* 2005;**73**:5301–10.
19. Dawson KG, Emerson JC, Burns JL. Fifteen years of experience with bacterial meningitis. *Pediatr Infect Dis J* 1999;**18**:816–22.
20. Dodge WF, West EF, Travis LB. Bacteriuria in school children: observations on outcome following detection in 110 girls. *Arch Pediatr Adolesc Med* 1974;**127**:364–70.
21. Draghijeva E. Virulence factors in *Escherichia coli* from children with pyelonephritis. *Clin Microbiol Infect* 2000;**6**:328–30.
22. Dubois D, Delmas J, Cady A, et al. Cyclomodulins in urosepsis strains of *Escherichia coli*. *J Clin Microbiol* 2010;**48**:2122–9.
23. Dumont R, Cinotti R, Lejus C, et al. The microbiology of community-acquired peritonitis in children. *Pediatr Infect Dis J* 2011;**30**:131–5.
24. Duncan MJ, Li G, Shin JS, et al. Bacterial penetration of bladder epithelium through lipid rafts. *J Biol Chem* 2004;**279**:18944–51.
25. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science (New York)* 2005;**308**:1635–8.
26. Finer G, Landau D. Pathogenesis of urinary tract infections with normal female anatomy. *Lancet Infect Dis* 2004;**4**:631–5.
27. Foxman B. The epidemiology of urinary tract infection. *Nat Rev Urol* 2010;**7**:653–60.
28. Foxman B. Uropathogenic *Escherichia coli* are more likely than commensal *E. coli* to be shared between heterosexual sex partners. *Am J Epidemiol* 2002;**156**:1133–40.
29. Gabizon A, Catane R, Uziely B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res* 1994;**54**:987–92.
30. Garin EH, Olavarria F, Garcia Nieto V, et al. Clinical significance of primary vesicoureteral reflux and urinary antibiotic prophylaxis after acute pyelonephritis: a multicenter, randomized, controlled study. *Pediatrics* 2006;**117**:626–32.
31. Garénaux A, Caza M, Dozois CM. The ins and outs of siderophore mediated iron uptake by extra-intestinal pathogenic *Escherichia coli*. *Vet Microbiol* 2011;**153**:89–98.
32. Geerlings SE. Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. *Int J Antimicrob Agents* 2008;**31**(Suppl. 1):S54–7.
33. Goluszko P, Moseley SL, Truong LD, et al. Development of experimental model of chronic pyelonephritis with *Escherichia coli* O75:K5:H-bearing Dr fimbriae: mutation in the Dra region prevented tubulointerstitial nephritis. *J Clin Invest* 1997;**99**:1662–72.
34. Gorelick MH, Shaw KN. Screening tests for urinary tract infection in children: a meta-analysis. *Pediatrics* 1999;**104**:e54.
35. Gryllos I, Tran-Winkler HJ, Cheng MF, et al. Induction of group A streptococcus virulence by a human antimicrobial peptide. *Proc Natl Acad Sci U S A* 2008;**105**:16755–60.
36. Grüneberg RN. Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection. *Lancet* 1969;**2**:766–8.
37. Guyer DM, Radulovic S, Jones FE, et al. Sat, the secreted autotransporter toxin of uropathogenic *Escherichia coli*, is a vacuolating cytotoxin for bladder and kidney epithelial cells. *Infect Immun* 2002;**70**:4539–46.
38. Hanna H, Afif C, Alakech B, et al. Central venous catheter-related bacteremia due to gram-negative bacilli: significance of catheter removal in preventing relapse. *Infect Control Hosp Epidemiol* 2004;**25**:646–9.
39. Haugen BJ, Pellett S, Redford P, et al. In vivo gene expression analysis identifies genes required for enhanced colonization of the mouse urinary tract by uropathogenic *Escherichia coli* strain CFT073 dsdA. *Infect Immun* 2007;**75**:278–89.
40. Hawkey PM. Identification of Enterobacteriaceae. In: *Principles and practice of clinical bacteriology*. John Wiley & Sons, Ltd; 2006. p.341–5.
41. Hedges S, Anderson P, Lidin-Janson G, et al. Interleukin-6 response to deliberate colonization of the human urinary tract with gram-negative bacteria. *Infect Immun* 1991;**59**:421–7.
42. Heimer SR, Rasko DA, Lockatell CV, et al. Autotransporter genes *Pic* and *Tsh* are associated with *Escherichia coli* strains that cause acute pyelonephritis and are expressed during urinary tract infection. *Infect Immun* 2004;**72**:593–7.
43. Hoberman A, Wald ER, Hickey RW, et al. Oral versus initial intravenous therapy for urinary tract infections in young febrile children. *Pediatrics* 1999;**104**(1 Pt 1):79–86.
44. Honkinen O, Lehtonen OP, Ruuskanen O, et al. Cohort study of bacterial species causing urinary tract infection and urinary tract abnormalities in children. *BMJ* 1999;**318**:770–1.
45. Hooton TM, Scholes D, Stapleton AE, et al. A prospective study of asymptomatic bacteriuria in sexually active young women. *N Engl J Med* 2000;**343**:992–7.
46. Hornik CP, Fort P, Clark RH, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. *Early Hum Dev* 2012;**88**(Suppl. 2):S69–74.
47. Horwitz MA, Silverstein SC. Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. *J Clin Invest* 1980;**65**:82–94.
48. Houdouin V, Bonacorsi S, Bidet P, et al. Clinical outcome and bacterial characteristics of 99 *Escherichia coli* meningitis in young infants. *Arch De Pédiatr* 2008;**15**(Suppl. 3):S138–47.
49. Houdouin V, Bonacorsi S, Mahjoub-Messai F, et al. Phylogenetic groups and virulence factors of *Escherichia coli* strains causing pyelonephritis in children with and without urinary tract abnormalities. *Clin Microbiol Infect* 2007;**13**:740–2.
50. Hultgren SJ, Porter TN, Schaeffer AJ, et al. Role of type 1 pili and effects of phase variation on lower urinary tract infections produced by *Escherichia coli*. *Infect Immun* 1985;**50**:370–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alarcon A, Peña P, Salas S, et al. Neonatal early onset *Escherichia coli* sepsis: trends in incidence and antimicrobial resistance in the era of intrapartum antimicrobial prophylaxis. *The Pediatr Infect Dis J* 2004;**23**:295–9.
2. Allsopp LP, Beloin C, Ulett GC, et al. Molecular characterization of UpaB and UpaC: two new autotransporter proteins of uropathogenic *Escherichia coli* CFT073. *Infect Immun* 2012;**80**; 321–32.
3. Anderson GG, Goller CC, Justice S, et al. Polysaccharide capsule and sialic acid-mediated regulation promote biofilm-like intracellular bacterial communities during cystitis. *Infect Immun* 2010;**78**: 963–75.
4. Anderson GG, Martin SM, Hultgren SJ. Host subversion by formation of intracellular bacterial communities in the urinary tract. *Microbes Infect* 2004;**6**:1094–101.
5. Bahrani-Mougeot FK, Buckles EL, Lockatell CV, et al. Type 1 fimbriae and extracellular polysaccharides are preeminent uropathogenic *Escherichia coli* virulence determinants in the murine urinary tract. *Mol Microbiol* 2002;**45**:1079–93.
6. Benjamin DKJ, Miller W, Garges H, et al. Bacteremia, central catheters, and neonates: when to pull the line. *Pediatrics* 2001;**107**: 1272–6.
7. Bhat RG, Katy TA, Place FC. Pediatric urinary tract infections. *Emerg Med Clin North Am* 2011;**29**:637–53.
8. Bishop BL, Duncan MJ, Song J, et al. Cyclic AMP-regulated exocytosis of *Escherichia coli* from infected bladder epithelial cells. *Nat Med* 2007;**13**:625–30.
9. Bizzarro MJ, Dembry LM, Baltimore RS, et al. Changing patterns in neonatal *Escherichia coli* sepsis and ampicillin resistance in the era of intrapartum antibiotic prophylaxis. *Pediatrics* 2008;**121**: 689–96.
10. Blanco M, Blanco JE, Alonso MP, et al. Virulence factors and O groups of *Escherichia coli* isolates from patients with acute pyelonephritis, cystitis and asymptomatic bacteriuria. *Eur J Epidemiol* 1996;**12**:191–8.
11. Blumenthal I. Vesicoureteric reflux and urinary tract infection in children. *Postgrad Med J* 2006;**82**:31–5.
12. Bortolussi R, Ferrieri P, Quie PG. Influence of growth temperature of *Escherichia coli* on K1 capsular antigen production and resistance to opsonization. *Infect Immun* 1983;**39**:1136–41.
13. Bouissou F, Munzer C, Decramer S, et al. Prospective, randomized trial comparing short and long intravenous antibiotic treatment of acute pyelonephritis in children: dimercaptosuccinic acid scintigraphic evaluation at 9 months. *Pediatrics* 2008;**121**: e553–60.
14. Brooks HJ, Benseman BA, Peck J, et al. Correlation between uropathogenic properties of *Escherichia coli* from urinary tract infections and the antibody-coated bacteria test and comparison with faecal strains. *J Hyg* 1981;**87**:53–61.
15. Buckles EL, Bahrani-Mougeot FK, Molina A, et al. Identification and characterization of a novel uropathogenic *Escherichia coli*-associated fimbrial gene cluster. *Infect Immun* 2004;**72**:3890–901.
16. Buckley J, Coffin SE, Lautenbach E, et al. Outcome of *Escherichia coli* and/or *Klebsiella* bloodstream infection in children with central venous catheters. *Infect Control Hosp Epidemiol* 2007;**28**:1308–10.
17. Cumming V, Ali S, Forrester T, et al. Asymptomatic bacteriuria in sickle cell disease: a cross-sectional study. *BMC Infect Dis* 2006; **6**:46.
18. Davis JM, Rasmussen SB, O'Brien AD. Cytotoxic necrotizing factor type 1 production by uropathogenic *Escherichia coli* modulates polymorphonuclear leukocyte function. *Infect Immun* 2005; **73**:5301–10.
19. Dawson KG, Emerson JC, Burns JL. Fifteen years of experience with bacterial meningitis. *Pediatr Infect Dis J* 1999;**18**:816–22.
20. Dodge WF, West EF, Travis LB. Bacteriuria in school children: observations on outcome following detection in 110 girls. *Arch Pediatr Adolesc Med* 1974;**127**:364–70.
21. Draghijeva E. Virulence factors in *Escherichia coli* from children with pyelonephritis. *Clin Microbiol Infect* 2000;**6**:328–30.
22. Dubois D, Delmas J, Cady A, et al. Cyclomodulins in urosepsis strains of *Escherichia coli*. *J Clin Microbiol* 2010;**48**:2122–9.
23. Dumont R, Cinotti R, Lejus C, et al. The microbiology of community-acquired peritonitis in children. *Pediatr Infect Dis J* 2011;**30**:131–5.
24. Duncan MJ, Li G, Shin JS, et al. Bacterial penetration of bladder epithelium through lipid rafts. *J Biol Chem* 2004;**279**: 18944–51.
25. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. *Science (New York)* 2005;**308**:1635–8.
26. Finer G, Landau D. Pathogenesis of urinary tract infections with normal female anatomy. *Lancet Infect Dis* 2004;**4**:631–5.
27. Foxman B. The epidemiology of urinary tract infection. *Nat Rev Urol* 2010;**7**:653–60.
28. Foxman B. Uropathogenic *Escherichia coli* are more likely than commensal *E. coli* to be shared between heterosexual sex partners. *Am J Epidemiol* 2002;**156**:1133–40.
29. Gabizon A, Catane R, Uziely B, et al. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res* 1994;**54**:987–92.
30. Garin EH, Olavarria F, Garcia Nieto V, et al. Clinical significance of primary vesicoureteral reflux and urinary antibiotic prophylaxis after acute pyelonephritis: a multicenter, randomized, controlled study. *Pediatrics* 2006;**117**:626–32.
31. Garénaux A, Caza M, Dozois CM. The ins and outs of siderophore mediated iron uptake by extra-intestinal pathogenic *Escherichia coli*. *Vet Microbiol* 2011;**153**:89–98.
32. Geerlings SE. Urinary tract infections in patients with diabetes mellitus: epidemiology, pathogenesis and treatment. *Int J Antimicrob Agents* 2008;**31**(Suppl. 1):S54–7.
33. Goluszko P, Moseley SL, Truong LD, et al. Development of experimental model of chronic pyelonephritis with *Escherichia coli* O75:K5:H-bearing Dr fimbriae: mutation in the Dra region prevented tubulointerstitial nephritis. *J Clin Invest* 1997;**99**: 1662–72.
34. Gorelick MH, Shaw KN. Screening tests for urinary tract infection in children: a meta-analysis. *Pediatrics* 1999;**104**:e54.
35. Gryllos I, Tran-Winkler HJ, Cheng MF, et al. Induction of group A streptococcus virulence by a human antimicrobial peptide. *Proc Natl Acad Sci U S A* 2008;**105**:16755–60.
36. Grüneberg RN. Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection. *Lancet* 1969;**2**:766–8.
37. Guyer DM, Radulovic S, Jones FE, et al. Sat, the secreted autotransporter toxin of uropathogenic *Escherichia coli*, is a vacuolating cytotoxin for bladder and kidney epithelial cells. *Infect Immun* 2002;**70**:4539–46.
38. Hanna H, Afif C, Alakech B, et al. Central venous catheter-related bacteremia due to gram-negative bacilli: significance of catheter removal in preventing relapse. *Infect Control Hosp Epidemiol* 2004; **25**:646–9.
39. Haugen BJ, Pellett S, Redford P, et al. In vivo gene expression analysis identifies genes required for enhanced colonization of the mouse urinary tract by uropathogenic *Escherichia coli* strain CFT073 dsdA. *Infect Immun* 2007;**75**:278–89.
40. Hawkey PM. Identification of Enterobacteriaceae. In: *Principles and practice of clinical bacteriology*. John Wiley & Sons, Ltd; 2006. p.341–5.
41. Hedges S, Anderson P, Lidin-Janson G, et al. Interleukin-6 response to deliberate colonization of the human urinary tract with gram-negative bacteria. *Infect Immun* 1991;**59**:421–7.
42. Heimer SR, Rasko DA, Lockatell CV, et al. Autotransporter genes *Pic* and *Tsh* are associated with *Escherichia coli* strains that cause acute pyelonephritis and are expressed during urinary tract infection. *Infect Immun* 2004;**72**:593–7.
43. Hoberman A, Wald ER, Hickey RW, et al. Oral versus initial intravenous therapy for urinary tract infections in young febrile children. *Pediatrics* 1999;**104**(1 Pt 1):79–86.
44. Honkinen O, Lehtonen OP, Ruuskanen O, et al. Cohort study of bacterial species causing urinary tract infection and urinary tract abnormalities in children. *BMJ* 1999;**318**:770–1.
45. Hooton TM, Scholes D, Stapleton AE, et al. A prospective study of asymptomatic bacteriuria in sexually active young women. *N Engl J Med* 2000;**343**:992–7.
46. Hornik CP, Fort P, Clark RH, et al. Early and late onset sepsis in very-low-birth-weight infants from a large group of neonatal intensive care units. *Early Hum Dev* 2012;**88**(Suppl. 2):S69–74.
47. Horwitz MA, Silverstein SC. Influence of the *Escherichia coli* capsule on complement fixation and on phagocytosis and killing by human phagocytes. *J Clin Invest* 1980;**65**:82–94.

48. Houdouin V, Bonacorsi S, Bidet P, et al. [Clinical outcome and bacterial characteristics of 99 *Escherichia coli* meningitis in young infants]. *Arch De Pédiatr* 2008;**15**(Suppl. 3):S138–47.
49. Houdouin V, Bonacorsi S, Mahjoub-Messai F, et al. Phylogenetic groups and virulence factors of *Escherichia coli* strains causing pyelonephritis in children with and without urinary tract abnormalities. *Clin Microbiol Infect* 2007;**13**:740–2.
50. Hultgren SJ, Porter TN, Schaeffer AJ, et al. Role of type 1 pili and effects of phase variation on lower urinary tract infections produced by *Escherichia coli*. *Infect Immun* 1985;**50**:370–7.
51. Hultgren SJ, Schwan WR, Schaeffer AJ, et al. Regulation of production of type 1 pili among urinary tract isolates of *Escherichia coli*. *Infect Immun* 1986;**54**:613–20.
52. Isaacs D, Barfield CP, Grimwood K, et al. Systemic bacterial and fungal infections in infants in Australian neonatal units. Australian Study Group for Neonatal Infections. *Med J Australia* 1995;**162**: 198–201.
53. Johnson JR, Clabots C, Kuskowski MA. Multiple-host sharing, long-term persistence, and virulence of *Escherichia coli* clones from human and animal household members. *J Clin Microbiol* 2008;**46**: 4078–82.
54. Johnson JR, Kuskowski MA, Gajewski A, et al. Extended virulence genotypes and phylogenetic background of *Escherichia coli* isolates from patients with cystitis, pyelonephritis, or prostatitis. *J Infect Dis* 2005;**191**:46–50.
55. Joseph TA, Pyati SP, Jacobs N. Neonatal early-onset *Escherichia coli* disease. the effect of intrapartum ampicillin. *Arch Pediatr Adolesc Med* 1998;**152**:35–40.
56. Justice SS, Hunstad DA, Seed PC, et al. Filamentation by *Escherichia coli* subverts innate defenses during urinary tract infection. *Proc Natl Acad Sci U S A* 2006;**103**:19884–9.
57. Justice SS, Lauer SR, Hultgren SJ, et al. Maturation of intracellular *Escherichia coli* communities requires SurA. *Infect Immun* 2006;**74**:4793–800.
58. Kai-Larsen Y, Lüthje P, Chromek M, et al. Uropathogenic *Escherichia coli* modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. *PLoS Pathogens* 2010;**6**:e1001010.
59. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2004;**2**:123–40.
60. Kaplan RE, Springate JE, Feld LG. Screening dipstick urinalysis: a time to change. *Pediatrics* 1997;**100**:919–21.
61. Kau AL, Hunstad DA, Hultgren SJ. Interaction of uropathogenic *Escherichia coli* with host uroepithelium. *Curr Opin Microbiol* 2005; **8**:54–9.
62. Keith BR, Maurer L, Spears PA, et al. Receptor-binding function of Type 1 pili effects bladder colonization by a clinical isolate of *Escherichia coli*. *Infect Immun* 1986;**53**:693–6.
63. Kemper KJ, Avner ED. The case against screening urinalyses for asymptomatic bacteriuria in children. *Am J Dis Child (1960)* 1992;**146**:343–6.
64. Khan NA, Kim Y, Shin S, et al. FimH-mediated *Escherichia coli* K1 invasion of human brain microvascular endothelial cells. *Cell Microbiol* 2007;**9**:169–78.
65. Kim KJ, Elliott SJ, Di Cello F, et al. The K1 capsule modulates trafficking of *E. coli*-containing vacuoles and enhances intracellular bacterial survival in human brain microvascular endothelial cells. *Cell Microbiol* 2003;**5**:245–52.
66. Kim KS. Mechanisms of microbial traversal of the blood-brain barrier. *Nat Rev Microbiol* 2008;**6**:625–34.
67. Kim KS, Itabashi H, Gemski P, et al. The K1 capsule is the critical determinant in the development of *Escherichia coli* meningitis in the rat. *J Clin Invest* 1992;**90**:897–905.
68. Kjeldsen L, Bainton DF, Sengelov H, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel matrix protein of specific granules in human neutrophils. *Blood* 1994;**83**:799–807.
69. Köhler C-D, Dobrindt U. What defines extraintestinal pathogenic *Escherichia coli*? *Int J Med Microbiol* 2011;**301**:642–7.
70. Lane MC, Mobley HLT. Role of P-fimbrial-mediated adherence in pyelonephritis and persistence of uropathogenic *Escherichia coli* (UPEC) in the mammalian kidney. *Kidney Int* 2007;**72**:19–25.
71. Lane MC, Alteri CJ, Smith SN, et al. Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract. *Proc Natl Acad Sci U S A* 2007;**104**: 16669–74.
72. Langley JM, Hanakowski M, Leblanc JC. Unique epidemiology of nosocomial urinary tract infection in children. *Am J Infect Control* 2001;**29**:94–8.
73. Leuck A-M, Wright D, Ellingson L, et al. Complications of Foley catheters: is infection the greatest risk? *J Urol* 2012;**187**:1662–6.
74. Lin M-C, Chi H, Chiu N-C, et al. Factors for poor prognosis of neonatal bacterial meningitis in a medical center in northern Taiwan. *J Microbiol Immunol Infect* 2012;**45**:442–7.
75. Lior H. *Escherichia Coli in domestic animals and humans*. Wallingford, Oxon, UK: CAB International; 1994.
76. Lloyd AL, Smith SN, Eaton KA, et al. Uropathogenic *Escherichia coli* suppresses the host inflammatory response via pathogenicity island genes sisA and sisB. *Infect Immun* 2009;**77**:5322–33.
77. Madigan M. *Brock biology of microorganisms*. Upper Saddle River, NJ: Pearson Prentice Hall; 2006.
78. Mahjoub-Messai F, Bidet P, Caro V, et al. *Escherichia coli* isolates causing bacteremia via gut translocation and urinary tract infection in young infants exhibit different virulence genotypes. *J Infect Dis* 2011;**203**:1844–9.
79. Matlow AG, Wray RD, Cox PN. Nosocomial urinary tract infections in children in a pediatric intensive care unit: a follow-up after 10 years. *Pediatr Crit Care Med* 2003;**4**:74–7.
80. Melican K, Boekel J, Mansson LE, et al. Bacterial infection-mediated mucosal signalling induces local renal ischaemia as a defence against sepsis. *Cell Microbiol* 2008;**10**:1987–98.
81. Melican K, Sandoval RM, Kader A, et al. Uropathogenic *Escherichia coli* P and type 1 fimbriae act in synergy in a living host to facilitate renal colonization leading to nephron obstruction. *PLoS Pathog* 2011;**7**:e1001298.
82. Mittal R, Prasadarao NV. Nitric oxide/cGMP signalling induces *Escherichia coli* K1 receptor expression and modulates the permeability in human brain endothelial cell monolayers during invasion. *Cell Microbiol* 2010;**12**:67–83.
83. Mobley HL, Chippendale GR. Hemagglutinin, urease, and hemolysin production by *Proteus mirabilis* from clinical sources. *J Infect Dis* 1990;**161**:525–30.
84. Montini G, Rigon L, Zucchetta P, et al. Prophylaxis after first febrile urinary tract infection in children? A multicenter, randomized, controlled, noninferiority trial. *Pediatrics* 2008;**122**:1064–71.
85. Montini G, Toffolo A, Zucchetta P, et al. Antibiotic treatment for pyelonephritis in children: multicentre randomised controlled non-inferiority trial. *BMJ (Clin Res Ed)* 2007;**335**:386.
86. Mulvey MA, Schilling JD, Hultgren SJ. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infect Immun* 2001;**69**:4572–9.
87. Mysorekar IU, Hultgren SJ. Mechanisms of uropathogenic *Escherichia coli* persistence and eradication from the urinary tract. *Proc Natl Acad Sci U S A* 2006;**103**:14170–5.
88. Nagler EV, Williams G, Hodson EM, et al. Interventions for primary vesicoureteric reflux. *Cochrane Database Syst Rev* 2011;(6):CD001532.
89. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998;**11**:142–201.
90. Neuhaus TJ, Berger C, Buechner K, et al. Randomised trial of oral versus sequential intravenous/oral cephalosporins in children with pyelonephritis. *Eur J Pediatr* 2008;**167**:1037–47.
91. Nguyen H, Weir M. Urinary tract infection as a possible marker for teenage sex. *South Med J* 2002;**95**:867–9.
92. Nicholson TF, Watts KM, Hunstad DA. OmpA of uropathogenic *Escherichia coli* promotes postinvasion pathogenesis of cystitis. *Infect Immun* 2009;**77**:5245–51.
93. Nicolle LE. Urinary tract infection in diabetes. *Curr Opin Infect Dis* 2005;**18**:49–53.
94. Norinder BS, Lüthje P, Yadav M, et al. Cellulose and PapG are important for *Escherichia coli* causing recurrent urinary tract infection in women. *Infection* 2011;**39**(6):571–4.
95. Nowicki B, Selvarangan R, Nowicki S. Family of *Escherichia coli* Dr adhesins: decay-accelerating factor receptor recognition and invasiveness. *J Infect Dis* 2001;**183**(Suppl.):S24–7.
96. Oualha M, Larakeb SA, Dunand O, et al. Stop of antibiotic prophylaxis in mild/moderate grade vesico-ureteral reflux. *Arch De Pédiatr* 2010;**17**:3–9.
97. Page S, Abel G, Stringer MD, et al. Management of septicaemic infants during long-term parenteral nutrition. *Int J Clin Pract* 2000;**54**:147–50.

98. Patterson JE, Andriole VT. Bacterial urinary tract infections in diabetes. *Infect Dis Clin North Am* 1997;**11**:735–50.
99. Pennesi M, Travan L, Peratoner L, et al. Is antibiotic prophylaxis in children with vesicoureteral reflux effective in preventing pyelonephritis and renal scars? A randomized, controlled trial. *Pediatrics* 2008;**121**:e1489–94.
100. Pickering LK, Baker CJ, Kimberlin DW, et al. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009.
101. Prasadarao NV, Wass CA, Huang SH, et al. Identification and characterization of a novel Ibe10 binding protein that contributes to *Escherichia coli* invasion of brain microvascular endothelial cells. *Infect Immun* 1999;**67**:1131–8.
102. Prasadarao NV, Srivastava PK, Rudrabhatla RS, et al. Cloning and expression of the *Escherichia coli* K1 outer membrane protein A receptor, a Gp96 homologue. *Infect Immun* 2003;**71**:1680–8.
103. Pruett TL, Chenoweth DE, Fiegel VD, et al. *Escherichia coli* and human neutrophils. Effect of bacterial supernatant with hemolysin activity upon chemotaxin receptors. *Arch Surg (Chicago, 1960)* 1985;**120**:212–16.
104. Ragnarsdóttir B, Samuelsson M, Gustafsson MCU, et al. Reduced Toll-like receptor 4 expression in children with asymptomatic bacteriuria. *J Infect Dis* 2007;**196**:475–84.
105. Rene P, Silverblatt FJ. Serological response to *Escherichia coli* pili in pyelonephritis. *Infect Immun* 1982;**37**:749–54.
106. Renko M, Tapanainen P, Tossavainen P, et al. Meta-analysis of the significance of asymptomatic bacteriuria in diabetes. *Diabetes Care* 2011;**34**:230–5.
107. Roberts AP, Linton JD, Waterman AM, et al. Urinary and faecal *Escherichia coli* O-sero-groups in symptomatic urinary-tract infection and asymptomatic bacteriuria. *J Med Microbiol* 1975;**8**:311–18.
108. Rongkavilit C, Rodriguez ZM, Gómez-Marín O, et al. Gram-negative bacillary bacteremia in human immunodeficiency virus type 1-infected children. *Pediatr Infect Dis J* 2000;**19**:122–8.
109. Roos V, Ulett GC, Schembri MA, et al. The asymptomatic bacteriuria *Escherichia coli* strain 83972 outcompetes uropathogenic *E. coli* strains in human urine. *Infect Immun* 2006;**74**:615–24.
110. Rosen DA, Hooton TM, Stamm WE, et al. Detection of intracellular bacterial communities in human urinary tract infection. *PLoS Med* 2007;**4**:e329.
111. Russo TA, Davidson BA, Carlino-MacDonald UB, et al. The effects of *Escherichia coli* capsule, O-antigen, host neutrophils, and complement in a rat model of gram-negative pneumonia. *FEMS Microbiol Lett* 2003;**226**:355–61.
112. Saint S. Clinical and economic consequences of nosocomial catheter-related bacteriuria. *Am J Infect Control* 2000;**28**:68–75.
113. Savage DCL, Wilson MI, McHardy M, et al. Covert bacteriuria of childhood: a clinical and epidemiological study. *Arch Dis Child* 1973;**48**:8–20.
114. Schilling JD, Lorenz RG, Hultgren SJ. Effect of trimethoprim-sulfamethoxazole on recurrent bacteriuria and bacterial persistence in mice infected with uropathogenic *Escherichia coli*. *Infect Immun* 2002;**70**:7042–9.
115. Schrag SJ, Stoll BJ. Early-onset neonatal sepsis in the era of widespread intrapartum chemoprophylaxis. *Pediatr Infect Dis J* 2006;**25**:939–40.
116. Shaikh N, Morone NE, Bost JE, et al. Prevalence of urinary tract infection in childhood: a meta-analysis. *Pediatr Infect Dis J* 2008;**27**:302–8.
117. Smellie JM, Prescod NP, Shaw PJ, et al. Childhood reflux and urinary infection: a follow-up of 10-41 years in 226 adults. *Pediatr Nephrol (Berlin, Germany)* 1998;**12**:727–36.
118. Smith SN, Hagan EC, Lane MC, et al. Dissemination and systemic colonization of uropathogenic *Escherichia coli* in a murine model of bacteremia. *MBio 1* 2010, doi: 10.1128/mBio.00262-10.
119. Smith YC, Rasmussen SB, Grande KK, et al. Hemolysin of uropathogenic *Escherichia coli* evokes extensive shedding of the uroepithelium and hemorrhage in bladder tissue within the first 24 hours after intraurethral inoculation of mice. *Infect Immun* 2008;**76**:2978–90.
120. Snodgrass W. Relationship of voiding dysfunction to urinary tract infection and vesicoureteral reflux in children. *Urology* 1991;**38**:341–4.
121. Song J, Duncan MJ, Li G, et al. A novel TLR4-mediated signaling pathway leading to IL-6 responses in human bladder epithelial cells. *PLoS Pathog* 2007;**3**:e60.
122. Stoll BJ. The global impact of neonatal infection. *Clin Perinatol* 1997;**24**:1–21.
123. Stoll BJ, Hansen NI, Sanchez PJ, et al. Early onset neonatal sepsis: the burden of group b streptococcal and *E. coli* disease continues. *Pediatrics* 2011;**127**:817–26.
124. Stumpe S, Schmid R, Stephens DL, et al. Identification of OmpT as the protease that hydrolyzes the antimicrobial peptide protamine before it enters growing cells of *Escherichia coli*. *Bacteriol* 1998;**180**:4002–6.
125. Takahashi A, Kanamaru S, Kurazono H, et al. *Escherichia coli* isolates associated with uncomplicated and complicated cystitis and asymptomatic bacteriuria possess similar phylogenies, virulence genes, and O-serogroup profiles. *J Clin Microbiol* 2006;**44**:4589–92.
126. Tambyah PA, Maki DG. Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1,497 catheterized patients. *Archives of Internal Medicine* 2000;**160**(5):678–82.
127. Topaloglu R, Er I, Dogan BG, et al. Risk factors in community-acquired urinary tract infections caused by ESBL-producing bacteria in children. *Pediatr Nephrol (Berlin, Germany)* 2010;**25**:919–25.
128. Tsai C-H, Chen Y-Y, Wang K-G, et al. Characteristics of early-onset neonatal sepsis caused by *Escherichia coli*. *Taiwan J Obstet Gynecol* 2012;**51**:26–30.
129. Vejborg RM, Hancock V, Schembri MA, et al. Comparative genomics of *Escherichia coli* strains causing urinary tract infections. *Appl Environ Microbiol* 2011;**77**:3268–78.
130. Vergnano S, Menson E, Kennea N, et al. Neonatal infections in England: the NeonIN Surveillance Network. *Arch Dis Child Fetal Neonatal Ed* 2011;**96**:F9–F14.
131. Vigil PD, Wiles TJ, Engstrom MD, et al. The Repeat-In-Toxin (RTX) family member TosA mediates adherence of uropathogenic *Escherichia coli* and survival during bacteremia. *Infect Immun* 2011;**80**:493–505.
132. Vosti KL, Monto AS, Rantz LA. Host-parasite interaction in patients with infections due to *Escherichia coli*. II. Serologic response of the host. *J Lab Clin Med* 1965;**66**:613–26.
133. Waddle E, Jhaveri R. Outcomes of febrile children without localising signs after pneumococcal conjugate vaccine. *Arch Dis Child* 2009;**94**:144–7.
134. Watt K, Waddle E, Jhaveri R. Changing epidemiology of serious bacterial infections in febrile infants without localizing signs. *PLoS One* 2010;**5**:e12448.
135. Wright KJ, Hultgren SJ. Sticky fibers and uropathogenesis: bacterial adhesins in the urinary tract. *Future Microbiol* 2006;**1**:75–87.
136. Wright KJ, Seed PC, Hultgren SJ. Development of intracellular bacterial communities of uropathogenic *Escherichia coli* depends on type 1 pili. *Cell Microbiol* 2007;**9**:2230–41.
137. Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *Pediatr Nephrol (Berlin, Germany)* 2009;**24**:2387–92.
138. Zhanel GG, Hisanaga TL, Laing NM, et al. Antibiotic resistance in outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int J Antimicrob Agents* 2005;**26**:380–8.
139. Zhang L, Foxman B. Molecular epidemiology of *Escherichia coli* mediated urinary tract infections. *Front Biosci* 2003;**8**:e235–44.

CHAPTER 104

Diarrhea-Causing and Dysentery-Causing *Escherichia coli*

Jorge J. Velarde • Andrea A. Berry • James P. Nataro

Escherichia coli has long been recognized as the most common facultative anaerobe in the human gastrointestinal tract. Virtually all *Homo sapiens* (and most other mammals) harbor this bacterium. Yet within the *E. coli* biomass lurk highly evolved pathogenic subtypes that have adapted themselves to a new niche—human pathogenicity. These pathotypes may prefer the gastrointestinal tract, the genitourinary tract, or disseminated sites (including the meninges)—the last almost exclusively in neonates. This chapter focuses on *E. coli* associated with enteric infections.

The first implication of *E. coli* with diarrhea occurred more than 75 years ago, when Adam[3] postulated the existence of a group of "dyspepsia" *E. coli* responsible for neonatal and infantile diarrhea. In the 1940s, Bray reported that many, but not all, *E. coli* from severe infant diarrhea cases in summer agglutinated with antiserum prepared from a diarrheal isolate, whereas *E. coli* organisms from other sources did not.[11] The isolate used by Bray to develop the antiserum was later serotyped by the Kauffman scheme as O111:B4, one of the now classic serogroups of enteropathogenic *E. coli* (EPEC) (Table 104-1). Although this initial description was made possible by serologic fingerprinting, the advent of molecular biology rapidly produced a plethora of reports describing the several discrete *E. coli* pathotypes.

CAUSATIVE ORGANISMS

Six distinct diarrheogenic pathotypes are recognized currently on the basis of clinical, biochemical, and molecular or genetic criteria (Table 104-2), as follows:

1. Enterotoxigenic *E. coli* (ETEC), a major cause of traveler's diarrhea and infant diarrhea in developing countries, elaborates the heat-stable enterotoxin, the heat-labile enterotoxin, or both and causes infection of the small intestine.
2. EPEC, the original pathotype defined by Bray,[11] causes infant diarrhea in less-developed countries. The bacteria induce a characteristic attaching and effacing lesion in the small intestine. Classic EPEC requires the chromosomally encoded locus of enterocyte effacement and a high-molecular-weight virulence plasmid termed EPEC adherence factor plasmid.
3. Enterohemorrhagic *E. coli* (EHEC) causes outbreaks of hemorrhagic colitis and hemolytic-uremic syndrome in temperate climates. The essential virulence factor is the Shiga toxin, which circulates through the bloodstream and acts by inhibition of protein synthesis in the target cell.
4. Enteroinvasive *E. coli* (EIEC) is an unusual cause of diarrhea and dysentery. It is similar to *Shigella* epidemiologically and pathogenetically and shares similar virulence genes.
5. Enteroaggregative *E. coli* (EAEC) causes diarrhea in individuals of all ages in industrialized countries and in less-developed areas and in travelers. The organism adheres to the intestinal mucosa and secretes several enterotoxins and cytotoxins.[123]
6. Diffusely adherent *E. coli* (DAEC) is the sixth category. As a pathotype it is very heterogeneous, and its association with diarrhea is controversial. The epidemiologic scenario for this organism has not been well elucidated.

TRANSMISSION AND EPIDEMIOLOGY

Diarrheogenic *E. coli* strains are worldwide in distribution. The route of infection is fecal-oral, predominantly via contaminated food and water, although person-to-person transmission may occur with EHEC and possibly EPEC in infants.[150] As discussed subsequently, environmental contamination by EHEC is an emerging problem in industrialized countries, whereas ETEC continues to be ubiquitous worldwide. Table 104-3 lists the age-specific predilections of the various *E. coli* pathogens.

Enterotoxigenic *Escherichia coli*

The infectious dose for ETEC is approximately 10^8 bacteria.[59] The incubation period is 14 to 50 hours.[148] Transmission typically occurs via contamination of food and water,[203] and person-to-person transmission is thought to be uncommon because of its high infectious dose. In developing countries, contamination of food and water sources occurs commonly.[181] ETEC has additionally been detected in the United States in numerous food samples, including cheese, hamburger, sausage, and seafood.[101,137,203] A domestic U.S. outbreak of diarrhea at Crater Lake National Park in Oregon was traced to ETEC organisms in the water supply.[196] Because large numbers of organisms are required for experimental infection, transmission probably would require multiplication of the

TABLE 104-1 Serotypes Characteristic of the Diarrheogenic Pathotypes of *Escherichia coli*

Enteropathogenic	Enterotoxigenic	Enteroinvasive	Enterohemorrhagic	Enteroaggregative
O44:H34	O6:H-, 12, 16, 40	O28:H-	O26:H11	Nontypeable
O55:H6, 7, 32	O8:H-, 42	O29:H-	O77:H18	Rough
O86:H2, 34	O25:H-, 42	O32:H-	O103:H2	O3:H2
O111:H2, 7, 12	O27:H7	O42:H-	O104:H21	O6:H1
O114:H2	O29:H21	O89:H-	O111:H8	O11:H16
O119:H6	O63:H-, 12	O112:H-	O113:H21	O15:H21
O124:H?	O78:H11, 12	O121:H-	O128:H2	O44:H18
O125:H21	O117:H4	O124:H-	O145:H-	O92:H23
O127:H4, 6, 21	O125:H30	O136:H-	O157:H-, 7	O111:H21
O128:H2, H21	O126:H10, 27	O144:H-	O178:H19	O126:H2, 27, H-
O142:H6, 34	O127:H2	O152:H-		O104:H4
O158:H23	O128:H8, 35			
	O143:H-			
	O146:H39			
	O148:H28			
	O153:H45			
	O159:H4, 21			

TABLE 104-2 Relationship of *Escherichia coli* Virulence Genes to Clinical Patterns of Diarrhea

	Virulence Genes										
Pathogen Group	***EAF***	***A/E***	***LA***	***AA***	***LT/ST***	***CFA***	***Afa/Dr/F1845***	**Invasion Factors**	***Stx***	***ShET-2***	**Clinical Disease**
ETEC	–	–	–	–	+	+	–	–	–	–	Watery
EPEC	+	+	+	–	–	–	–	–	–	–	Watery
EHEC	–	+	–	–	–	–	–	–	+	–	Bloody (hemorrhagic colitis)
EIEC	–	–	–	–	–	–	–	+	–	+	Bloody (dysentery)
EAEC	–	–	–	+	+*	–	–	–	–	–	Watery (persistent)
DAEC	–	–	–	–	–	–	+	+	–	–	Watery

*Enteroaggregative stable toxin (EAST) is a member of the ST family.

DAEC, Diffusely adherent *E. coli; ETEC,* enterotoxigenic *E. coli; EPEC,* enteropathogenic *E. coli; EHEC,* enterohemorrhagic *E. coli; EIEC,* enteroinvasive *E. coli; EAEC,* enteroaggregative *E. coli.*

AA, Autoaggregative attaching pattern; *A/E,* attaching and effacing changes; *Afa/Dr/F1845,* DAEC adherence factors; *CFA,* colonization factor antigens; *EAF,* EPEC adherence factor; *LA,* localized adherence pattern; *LT/ST,* heat-labile and heat-stable toxins; invasion factors, chromosomal and plasmid factors mediating cell invasion; *ShET-2,* plasmid-encoded *Shigella* enterotoxin-2, highly homologous to the plasmid-encoded EIEC toxin; *Stx,* Shiga family cytotoxins.

TABLE 104-3 Age-Related Patterns of *Escherichia coli* Diarrhea

Pathogen Classification	Age at Highest Risk	*Characteristics of Diarrhea*		
		Bloody	***Watery***	***Inflammatory***
EAEC	<6 mo	–	+++	–
EPEC	<1 yr	–	+++	–
ETEC	<2 yr	–	+++	–
EIEC	>2 yr	++	+	+++
EHEC	2-10 yr	+++	+	–
DAEC	>1 yr	–	+++	–

DAEC, Diffusely adherent *E. coli; EAEC,* enteroaggregative *E. coli; EHEC,* enterohemorrhagic *E. coli; EIEC,* enteroinvasive *E. coli; EPEC,* enteropathogenic *E. coli; ETEC,* enterotoxigenic *E. coli.*

inoculum within the vehicle, which probably contributes to the well-documented propensity of ETEC infection to occur in warm months.[181]

ETEC is a major cause of diarrhea in infants and children in the developing world.[78,181] The incidence typically increases during the first 6 months of life and decreases after 2 years. ETEC has been estimated to cause approximately 20 percent of diarrheal episodes in the developing world,[181] although this percentage varies substantially by location. A study in rural Egypt found that ETEC accounted for 66 percent of all first episodes of diarrhea, with an incidence of 1.7 episodes per child-year during the first 6 months of life and 2.3 during the second 6 months.[184] In Tehran, researchers found that ETEC could be isolated from 15.5 percent of patients younger than 5 years old with diarrhea. [206] The

prevalence was 18 percent in Bangladesh,[180] 33 to 37.5 percent in Mexico,[47,65] and 20.5 percent among children with diarrhea in Nicaragua.[254] A study in Hanoi found, however, that only 2.2 percent of diarrheal episodes could be attributed to ETEC.[158] Prevalence was 9.2 percent in a study in the Brazilian northeast,[160] and the frequency in Mongolia[209] was similarly low.

ETEC disease is primarily a childhood problem, with the incidence decreasing after the child reaches 5 years of age.[180,181] After infection with a particular ETEC strain, the host is resistant to disease but still may excrete the organism asymptomatically, a characteristic that contributes to the environmental burden.[150,233] Adults still may be susceptible to ETEC diarrhea.[78,181] In a study of elderly individuals in Dhaka, Bangladesh, researchers found that 13 percent of adults older than 60 years with diarrhea were noted to have ETEC.[66] Although the incidence of ETEC diarrhea decreases after age 5 years, the incidence seems to increase again in patients older than 15 years, and 25 percent of ETEC infections are seen in adults.[180,181]

ETEC is the most common cause of traveler's diarrhea, typically accounting for approximately 30 to 40 percent of cases, except in southeast Asia, where it has been found to account for approximately 7 percent of cases.[2,104,212,217] Illness is acquired by ingestion of contaminated food or water. The diarrhea is acute and watery and typically resolves spontaneously without severe limitation of activities.[2]

ETEC organisms are extremely diverse, and children in endemic areas experience multiple infections. The basis of immunity, which increases with repeated exposure, is unknown.[233] Strains producing heat-stable enterotoxin cause more severe disease than strains producing only heat-labile enterotoxin, and the former is not immunogenic. Colonization of the small intestinal mucosa is mediated by surface fimbriae termed *colonization factor antigens*, which are immunogenic and may elicit some element of protective immunity.[185] Despite the existence of more than 25 known colonization factor antigens, a few are overrepresented among clinical isolates[159,201,218,254]; in a study from Bangladesh for instance, seven colonization factor antigens constituted more than 75 percent of the colonization factor antigens isolated.[180] In an analysis of multiple studies, Wolf [260] suggested that most ETEC express colonization factor antigens I, II (CS1, CS2, CS3), or IV (CS4, CS5, CS6). In some epidemiologic studies, however, more than 50 percent of strains do not express known colonization factor antigens.[218,219] This may be secondary to the presence of colonization factor antigens that are as yet unrecognized.[147] ETEC is predominantly an infection of warm weather months in endemic areas, coincident with conditions promoting bacterial replication in the environment. Some sites report that a second peak occurs after the rainy season.[180,181]

Enteropathogenic *Escherichia coli*

Neter and associates[157] first coined the term *EPEC* to denote a group of *E. coli* serotypes associated with dramatic and highly lethal nursery outbreaks of diarrhea in the United States and the United Kingdom (see Table 104-1). We now recognize that these serotype antigens serve as surrogate markers for a package of chromosomal and plasmid-borne virulence genes, which together orchestrate enteric pathogenesis. The epidemiology of EPEC has changed since its original description was provided. Gone are the lethal outbreaks formerly reported in industrialized countries, to be replaced by sporadic endemic disease in resource-poor countries. The infection retains its dramatic predilection for infants and children younger than 24 months, is rarely seen in older children and adults, and is not a cause of traveler's diarrhea.[46,47,79,247] The basis of this age-dependent incidence is unknown.

Globally, EPEC is among the most common causes of infant diarrhea.* EPEC has a propensity to cause persistent infection and wasting, suggesting that its burden may comprise more than simple dehydration. Breast-feeding offers some protection against EPEC because of the presence of immune factors and oligosaccharides that inhibit adherence of EPEC to epithelial cells.[49] In industrialized countries such as the United States, EPEC generally is no longer considered to be a cause of endemic diarrhea, although EPEC may be present in infants immigrating from or visiting resource-poor settings. Rare indigenous cases may occur.[82] In an outpatient setting in the United States, 2 of 147 cases of nondysenteric acute diarrhea were caused by EPEC.[33] In 166 Swiss children admitted to hospitals with diarrhea, 13 (7.8%) had EPEC isolated from stool cultures.[64]

As noted previously, EPEC strains of the classic serotypes (also known as typical EPEC) require the chromosomal locus and the EPEC adherence factor plasmid to cause diarrhea. Controversy exists over whether so-called atypical EPEC (which are positive for locus of enterocyte effacement and negative for EPEC adherence factor) are a cause of diarrhea.[149] The description of an outbreak of diarrhea in Japan caused by an atypical EPEC strain suggests that at least some of these strains are pathogenic.[263] Recent molecular epidemiologic studies have suggested that atypical EPEC may require a chromosomally encoded adhesion factor termed *Efa1*,[4,163] which may substitute for the EPEC adherence factor plasmid-encoded bundle-forming pilus.

Enterohemorrhagic *Escherichia coli*

Since its discovery in 1977, EHEC has emerged as an important and increasingly common human pathogen. Although most occurrences of disease are sporadic and not all occurrences are related to ground beef,[243] EHEC is notably carried asymptomatically in cattle and other ruminants, from whence it can be transmitted widely. The organisms may be excreted by cattle herds, and large outbreaks of human infection have been associated with the ingestion of undercooked hamburger and other foods contaminated directly and indirectly by cattle manure. Careful prospective epidemiologic surveillance in Minnesota has revealed an increase in the incidence of EHEC from 0.5 to 2/100,000 children younger than 18 years of age during the period 1979 to 1988,[133] similar to the incidence of EHEC determined in the state of

*References 46, 47, 79, 163, 164, 191.

Washington in the late 1980s: 2.1/100,000. In a multicenter study of mild acute diarrhea in outpatients in the United States, 5 (3.4%) of 147 cases were caused by EHEC,[33] a frequency similar to that in Swiss children hospitalized for community-acquired diarrhea.[64] Very young children are not commonly infected; rather, infection occurs most commonly and severely in children 2 to 10 years old or in the elderly.

Ground beef is identified as the most common source of EHEC infection. Several outbreaks have been associated with fast-food consumption.[183] In meat-processing plants where bulk ground beef is prepared for the fast-food industry, meat from one contaminated carcass can contaminate and distribute the organisms to a huge number of beef patties. Several other food sources of transmission that have been documented include other beef; produce, including spinach and lettuce; milk; water; and processed foods, including unpasteurized apple cider, mayonnaise, and dry fermented sausage. Person-to-person, cattle-to-person, and waterborne outbreaks have been described. Person-to-person spread as sequelae of point-source epidemics is common.[216] Many unusual modes of transmission linked with cattle have occurred and include poorly washed apples dropped in cow pastures and used to make cider, surface water supplies in proximity to areas where cattle graze, spread of aerosolized particles at agricultural fairs, and transmission by contact at petting zoos. Contaminated swimming holes have been implicated in several outbreaks.

A multistate outbreak of O157:H7 EHEC infections associated with the consumption of fresh bagged spinach occurred in the United States in 2006, affecting 26 states and resulting in 205 confirmed illnesses, 103 hospitalizations, 31 cases of hemolytic-uremic syndrome, and 3 deaths.[13,34] This outbreak exemplifies the widespread effect of a foodborne outbreak caused by the mass production and distribution of produce.

Hemolytic-uremic syndrome is a not-infrequent sequelae of EHEC-associated infection. A review of O157:H7 outbreaks (defined as two or more cases) reported to the Centers for Disease Control and Prevention (CDC) in the United States from 1982 to 2002 found 350 outbreaks, representing 8598 cases, 1493 (17%) hospitalizations, 354 (4%) cases of hemolytic-uremic syndrome, and 40 (0.5%) deaths.[183] During a large outbreak of *E. coli* O157:H7 infection associated with fast-food restaurant hamburgers on the West Coast of the United States in 1993, of the 732 affected individuals identified, 195 were admitted to the hospital, hemolytic-uremic syndrome developed in 55 (7.5%), and 4 died.[82] Investigation of this outbreak showed that small numbers of bacteria (in the range of a few hundred) constituted an infectious dose. Among 93 cases of O157:H7 infection reported in Washington State in 1987, hemolytic-uremic syndrome or thrombotic thrombocytopenic purpura developed in 11 (12%), for an approximate incidence of 0.23 per 100,000.[171]

The highest incidence of hemolytic-uremic syndrome occurs in children younger than 5 years, in whom the age-specific incidence ranged from 2.6 to 5.8/100,000 in Minnesota,[133] Washington,[171] and Oregon.[194] More recently, improvements in animal processing and public health interventions, including detection and investigation of outbreaks, has led to a U.S. national decrease in the incidence of hemolytic-uremic syndrome to fewer than 1 case/100,000.[38] The incidence of hemolytic-uremic syndrome in Argentina, 21.7/100,000, is four to eight times higher and is the highest reported incidence of hemolytic-uremic syndrome in the world.[129]

In the United Kingdom and the United States, hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura are associated predominantly with Shiga toxin 2–producing *E. coli* O157:H7[133,172]; however, this finding may represent more facile detection of this serotype. In addition, the incidence of non-O157:H7 infections in the United States is increasing (serotypes associated with EHEC infection are presented in Table 104-1).[31,106,259] In Argentina, non-O157:H7, Shiga toxin 2–producing strains are isolated most commonly.[129] Similarly, in Australia, other serotypes, especially O111, have been associated with major outbreaks and a significant number of hemolytic-uremic syndrome cases.[36] Many of these serotypes have been implicated in sporadic and outbreak disease in the United States[30] (e.g., an outbreak caused by O104:H21 acquired from contaminated milk).[37]

Non-O157:H7 EHEC infections have increased in prevalence in the United States and are now known to account for 20 to 50 percent of all EHEC infections.[106] Although some non-O157 strains are not pathogenic, others have been shown to cause disease as severe as that caused by O157:H7 strains, suggesting that a clinical suspicion for EHEC should be maintained even when testing for O157:H7 is negative.[106] Non-O157, Shiga toxin 2–producing *E. coli* infection was made a reportable disease in the United States in 2000.[31]

Hemolytic-uremic syndrome occurs seasonally in the United States and Canada (most common during the summer months), although no specific seasonal risk factor or factors for infection with Shiga toxin–producing *E. coli* are known. Other epidemiologic data suggest that poor children are at lower risk for the development of hemolytic-uremic syndrome, perhaps because they already have immunity to EHEC or Shiga toxins through early contact with the organisms in their environment.[42]

Enteroinvasive *Escherichia coli*

EIEC is closely related to *Shigella* microbiologically and pathogenetically. The pathotype typically is found in areas with a high burden of *Shigella*. The infectious dose may be higher than that of *Shigella*, and person-to-person transmission seldom is reported. Food-borne outbreaks occur sporadically.[80,132] The first description of EIEC as a pathotype occurred in 1971, when bloody diarrhea was traced to the consumption of French Camembert cheese contaminated with *E. coli* O124.[132] Small outbreaks or sporadic cases involving a limited number of serotypes have been reported from numerous countries, including the United States, France, Japan, and Brazil.[132,165,227,248,251] The prevalence varies by region. In a rural community in Ecuador up to 20.5 percent of diarrheal cases were due to EIEC[200]; in Tunisia 11.3 percent of children with diarrhea had EIEC.[8] Prospective studies in Thailand using DNA probes for EIEC pathogenicity genes indicated that 6 percent of cases of dysentery may be caused by

EIEC strains.[60] Similarly, studies in Nicaragua, South India, Vietnam, Gabon, Tanzania, Denmark, and multiple sites in Africa, including Kenya, Ghana, and Nigeria, demonstrated low to nondetectable rates of EIEC.* The epidemiology of the cases is complicated by the fact that in contrast to the initial descriptions of EIEC-associated disease, most EIEC infections probably are neither dysenteric nor characterized by bloody diarrhea but instead manifest as watery diarrhea with low-grade fever, similar to that caused by viral agents and ETEC.

Infection with 10^8 bacteria has been shown to be necessary for experimental dysentery in human volunteers,[59] a dose substantially higher than that for *Shigella* spp. ($\leq 10^4$ organisms). In a food-borne outbreak caused by a nontypeable EIEC, secondary person-to-person transmission was not reported.[227]

Enteroaggregative *Escherichia coli*

EAEC was implicated first as a cause of diarrheal disease in studies of children in Chile, Mexico, and India.[23,24,48,153] The pathogenicity of at least some EAEC strains has been established by several observations. First, volunteer studies by Nataro and associates[152] revealed that some EAEC could elicit diarrhea in healthy subjects, whereas other strains may not be pathogenic. Second, EAEC strains have been implicated in outbreaks of diarrhea, the largest of which involved nearly 2700 schoolchildren in Japan.[88,100,264] Finally, but most emphatically, a meta-analysis performed by Huang and colleagues[97] of more than 20 years of studies revealed convincingly that EAEC is a cause of diarrhea in infants; adults, especially those with human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) in developing countries; children in industrialized countries; and adult travelers to less-developed areas. In addition, a study from the United States by Nataro and colleagues[154] suggested that EAEC was the most frequent bacterial cause of diarrhea among all ages in Baltimore, Maryland, and New Haven, Connecticut.

A persisting controversy surrounding EAEC epidemiology and pathogenesis is the definition of the pathotype. The meta-analysis described earlier implicated EAEC, as defined by the characteristic stacked-brick adherence pattern to epithelial cells in culture.[97] A study of infant diarrhea in Cincinnati suggested, however, that only strains hybridizing with an EAEC gene probe (see later discussion) were truly associated with clinical diarrhea.[44] A full understanding of what constitutes a truly pathogenic EAEC strain is unavailable.

Diffusely Adherent *Escherichia coli*

The association between DAEC and diarrhea remains controversial. When DAEC was given to adult volunteers, it failed to convincingly elicit symptoms.[239] Additionally, this strain has been found in both healthy individuals and patients with diarrhea.[166,215] An age-related association may exist in children older than 12 to 18 months.[150,168,211,232] Of note, the commonly used *daaC* probe has been found to cross react with some strains of EAEC, which may have led to overestimation of DAEC in some studies.[226] Finally, DuPont and colleagues[140] suggest that DAEC may also play a role in traveler's diarrhea previously identified as pathogen negative.

*References 94, 146, 166, 167, 182, 188, 207.

CLINICAL MANIFESTATIONS

Diarrhea-causing *E. coli* may be responsible for a variety of clinical syndromes because virtually all known mechanisms of diarrhea, including secretory toxins, cytotoxic toxins, invasion, and pathogenic adherence, are manifested by the various *E. coli* pathotypes. The clinical manifestations are largely a function of the complement of virulence genes (see Table 104-2), and identification of the essential pathogenicity genes provides the definitive diagnostic maneuver. As with other pathogens, invasive *E. coli* strains (i.e., EIEC) produce inflammatory diarrhea with fever, abdominal pain, nausea, vomiting, and leukocytes and blood in the stool. Noninvasive, cytotoxin-producing EHEC strains cause a frankly bloody diarrhea associated with leukocytosis but without fecal leukocytes or fever. Producers of heat-stable or heat-labile enterotoxins (i.e., ETEC) elicit brisk, watery diarrhea with the potential for significant dehydration.

Enterotoxigenic *Escherichia coli*

ETEC causes watery, nonmucoid, nonbloody diarrhea in infants, older children, and adults, and it is a common cause of traveler's diarrhea.[181] The onset of diarrhea is abrupt, with an incubation period of 14 to 50 hours.[148] The frequency of stools varies from a few to more than 10 per day, and a striking absence of leukocytes is noted when the diarrheal stool is examined by light microscopy. Young affected children may vomit and commonly are febrile (38° to 40° C [100.4° to 104° F]), although adults typically are afebrile. ETEC disease cannot be distinguished clinically from most other causes of acute nonspecific watery diarrhea. The illness usually is self-limited to 3 to 5 days but occasionally lasts more than 1 week. Severely dehydrating illness that resembles clinical cholera can occur but is uncommon.

Variability in the clinical picture may be due to age differences and preexisting immunity, but it also may be attributed to differences in the infecting inoculum. DuPont and colleagues[58] produced mild diarrhea (three watery stools per day for 2 to 3 days) when 10^8 bacteria were fed to adult volunteers, whereas 10^{10} organisms caused more pronounced diarrhea (more than five stools per day for 4 to 5 days), with mucus but no blood observed in some subjects. Many subjects also had abdominal cramping, but no tenesmus was present, and all remained afebrile. This clinical picture is typical of traveler's diarrhea in adults.[142] ETEC diarrhea can result in severe dehydration necessitating aggressive fluid therapy.

Enteropathogenic *Escherichia coli*

EPEC diarrhea typically is copious and watery, without blood or fecal leukocytes.[124] Patients may have low-grade fever. In a Swiss study conducted with modern diagnostic methods, the mean age of 13 hospitalized children with

EPEC diarrhea was 1.4 years (range, 0.5 to 3 years).[64] Fever was low-grade, and vomiting occurred in 69 percent. Volume depletion was considered moderate in five children and severe in two. Although nursery outbreaks no longer occur commonly, severe illness still can develop in infants infected with EPEC,[103] with high mortality rates ranging from 25 to 70 percent.[53] The cause of death of patients in nursery outbreaks is not well documented. Investigations using an experimental EPEC infection model in adult volunteers (who are not naturally susceptible) revealed that watery diarrhea occurred 3 to 16 hours after inoculation and generally lasted less than 2 days. Diarrhea occasionally was copious and in some cases was associated with abdominal cramps, nausea, vomiting, malaise, and fever.[56] This clinical picture is consistent with disease caused by outbreaks of EPEC in adults.

EPEC also has been implicated in chronic diarrhea in the United States and elsewhere, with serious nutritional consequences that may require total parenteral nutrition and hospital stays of 120 days.[197] In prospective studies of EPEC diarrhea in young infants in Brazil[79] and Ethiopia,[246] fever, vomiting, and dehydration all were observed commonly. The clinical significance of chronic diarrhea may be increased by the underlying malnutrition of infants in resource-poor countries. In many settings, especially where effective oral rehydration programs are in place, chronic diarrhea and associated malnutrition are now more important causes of diarrheal deaths than are acute diarrhea and dehydration.[28]

In the course of the classic, severe nursery outbreaks caused by typical EPEC strains, the first signs and symptoms were mild and nonspecific, but they commonly were followed by vomiting and diarrhea of increasing severity. Stools contained neither mucus nor blood, and the volume fluctuated over a period of weeks. Plain abdominal radiographs revealed only nonspecific dilation of small bowel loops, although severe ileus not attributable to hypokalemia developed in many patients. Infected infants lost as much as 15 percent of body weight, leading to profound electrolyte disturbances and severe dehydration, along with central nervous system manifestations, such as irritability, hypertonicity, seizures, and coma. Circulatory collapse occurred despite adequate replacement of fluids and achievement of electrolyte balance. In an epidemic in Virginia, Belnap and O'Donnell[19] described fatal infections caused by *E. coli* O111:B4 occurring after 3 weeks of illness and associated with renal failure, coma, and signs of disseminated intravascular coagulation, but blood cultures typically remained negative. The fatality rate was age-dependent; although the overall mortality was 16 percent, the rate in neonates was 40 percent.

Enterohemorrhagic *Escherichia coli*

In 1971, a distinctive clinical diarrheal syndrome was recognized that was characterized by afebrile bloody diarrhea in addition to cramping and colonic inflammatory changes, usually right-sided.[189] In 1983, this illness was associated with an otherwise rare *E. coli* serotype, O157:H7, and a characteristic syndrome, hemorrhagic colitis, was defined.[190] O157:H7 subsequently was recognized as the most virulent prototype of a Shiga toxin–producing pathotype designated EHEC. EHEC now has been shown to cause a wide spectrum of diseases that may be confined to the gastrointestinal tract or, in a sizable proportion (typically 5% to 10%), can become systemic.[18,243] After an incubation period of 3 to 4 days (range, 1 to 8 days), the local gastrointestinal illness usually begins with nonbloody diarrhea that can progress to bloody diarrhea after 1 or 2 days. Hemorrhagic colitis occurs in 50 to 90 percent of O157:H7 infections.[171,243]

Further progression may occur over the course of the next day or two and result in the passage of frank blood, the pathognomonic clinical feature of hemorrhagic colitis. Associated manifestations include vomiting in approximately 50 percent of patients and abdominal pain. Fever may occur but typically is low-grade. This clinical picture may be confused with other conditions, such as appendicitis, intussusception, inflammatory bowel disease, ischemic colitis, and diverticulitis. The difficulty in establishing the diagnosis can lead to inappropriate drug therapy or even surgery.[82,98]

A serious complication of hemorrhagic colitis is the development of hemolytic-uremic syndrome.[82] Hemolytic-uremic syndrome is characterized by the triad of acute renal failure, thrombocytopenia, and hemolytic anemia and develops in approximately 10 to 20 percent of infected children younger than 10 years.[150] It is the most common cause of acquired renal failure in the United States.[249,250] The acute mortality rate is approximately 5 percent, and renal failure is expected to develop eventually in many more patients during the next several decades. After renal failure, cerebrovascular accident and colonic perforation are the most common serious sequelae. Thrombotic thrombocytopenic purpura and hemolytic-uremic syndrome have some overlapping clinical and pathologic features, but thrombotic thrombocytopenic purpura is increasingly regarded as a separate clinical entity that is not associated with an antecedent EHEC infection[242]; the separation is supported by its separate pathophysiology (see later discussion).

A prospective study in Canada identified *E. coli* O157:H7 in 15 percent of 125 patients with grossly bloody diarrhea over a 6-month period.[173] The age range was 15 months to 73 years; however, almost half of the patients were younger than 10 years of age. The illness was similar to that described earlier, with a mean duration of 7.8 days, but it was significantly longer in children (9.1 ± 2 days) than in adults (6.6 ± 1.1 days). Sigmoidoscopy findings were abnormal in 7/8 adults examined, with hyperemic mucosa in 6 and superficial ulcerations in 1. Biopsy samples showed mild mucosal inflammation in 4/5 patients. In another study, the findings on colonoscopy in 10 patients with hemorrhagic colitis caused by *E. coli* O157:H7 included severe inflammation (predominantly right-sided), marked edema, easy hemorrhage, and the frequent appearance of longitudinal ulcerlike lesions.[221]

Because local and systemic manifestations in EHEC infection are caused, at least partially, by Shiga toxins, the presence of these same toxins in the intestinal lumen in patients with non-O157:H7 infection puts them at risk for development of hemorrhagic colitis and hemolytic-uremic syndrome. Available longitudinal data suggest

that O157:H7 infection results in more bloody diarrhea, hospitalization, and hemolytic-uremic syndrome than non-O157:H7 EHEC infection.[87,91]

Enteroinvasive *Escherichia coli*

Naturally acquired EIEC infection causes a mild to moderately severe dysentery syndrome consisting of fever, malaise, diarrhea, tenesmus, and abdominal cramping.[59,251] Watery diarrhea usually occurs at the onset of the illness and progresses to mucoid diarrhea with streaks of blood or microscopic hematochezia but rarely to the classic small-volume, grossly bloody, dysenteric stool seen with *Shigella* infection. Of 229 cases reported in one study, grossly bloody stool occurred in only 4.[132] In two of these patients, sigmoidoscopy revealed superficial ulcerations in one and hyperemia alone in the second patient. As in *Shigella* infection, the stool reveals abundant polymorphonuclear cells, compatible with the inflammatory, invasive nature of the organism. Vomiting and dehydration can occur; the latter generally is mild. Fever of 38° to 39.5°C (1040.4° to 103.1°F) is a typical symptom that occurs early in association with malaise, myalgia, and headache and lasts for 2 to 3 days. In most instances, the diarrhea ceases in 1 week or less, but in some patients, it may continue for 2 weeks or more.

In adult volunteers with experimentally induced EIEC disease, febrile illness developed approximately 11 hours (range, 8 to 24 hours) after the inoculum was ingested.[59] Chills, myalgia, headache, and profuse diarrhea or abdominal cramps and tenesmus rapidly followed. In 2/13 subjects, this picture was associated with systemic toxicity and transient hypotension consistent with bacteremia, even though blood cultures were negative for all patients. Clinical dysentery with bloody stools occurred in several subjects, and reddened, friable mucosa with multiple bleeding points was seen by sigmoidoscopy. Clinical illness was controlled quickly with parenteral ampicillin therapy.

Enteroaggregative *Escherichia coli*

Descriptions of EAEC clinical features are derived from outbreak investigations, volunteer studies, and studies of traveler's diarrhea.[97] Most EAEC infections are accompanied by watery diarrhea, which may progress to persistent diarrhea that lasts 14 days or longer.[24,150,153] Bloody diarrhea is observed in a subset of patients.[48] An EAEC strain was implicated in a school lunch outbreak in Tajimi City, Japan, in 1993.[100] Of 6636 children who ate the school lunch at 16 schools, 2697 (rate of attack 40.6%) reported gastrointestinal symptoms. Major symptoms were abdominal pain (73.6%), nausea (51.1%), and diarrhea (39.9%). The incubation period for the onset of gastrointestinal illness was 40 to 50 hours on average. Duration of symptoms was not reported. A nursery outbreak in Serbia involved 19 normal infants.[43] All experienced watery diarrhea, which persisted beyond 2 weeks in 3 patients. A report of 17 pediatric patients with illness caused by EAEC of serotype O126:H27 in Israel suggested that patients typically manifested diarrhea (in all), vomiting (in 8), and fever (in 12, ≤40°C [104°F]).[220] In this report, one 6-week-old infant developed diarrhea persisting for 40 days. Studies in travelers suggest that EAEC disease may be mildly inflammatory, although fever and fecal leukocytes are present in a few patients.[97]

The particularly severe epidemic linked to an O104:H4 Shiga toxin–producing EAEC strain[187] differed from O157:H7-induced disease in several ways. Sequelae were more severe in adults than children, and overall the frequency of hemolytic-uremic syndrome and death was unusually high. Diarrhea in patients infected with O104:H4 was similar to that described in patients infected with O157:H7, suggesting that presentation of Shiga toxin at the site of infection does not require a set of virulence factors unique to EHEC strains.

Diffusely Adherent *Escherichia coli*

The association between DAEC and diarrhea remains controversial. Typically in cases in which a relationship is thought to exist, it causes a watery diarrhea that can be prolonged.[150]

PATHOGENESIS

Enterotoxigenic *Escherichia coli*

ETEC was identified first as a porcine pathogen,[86,224] which elicited fluid secretion when injected into ligated ileal loops.[86] Two secretogenic activities were characterized; one could be inactivated by heating and the other could not, and the activities could be found both in the same strain or independently. These activities now have been assigned to two well-characterized enterotoxins, heat-stable and heat labile. Heat-labile enterotoxin is genetically, structurally, and mechanistically similar to the cholera toxin produced by *Vibrio cholerae* O1 strains.[76,150] Heat-stable enterotoxin is actually two distinct toxins: STa (including closely related STI peptides STI-a and STI-b) is associated with human disease, whereas STb causes disease only in animals.[70,150]

The essential pathogenic strategy for ETEC involves colonization of the proximal small intestine, followed by release of one or both of the enterotoxins. Colonization of the intestinal epithelium (Fig. 104-1) is likely initially mediated by the ETEC flagella aided by the tip adhesin, EtpA, that has been recently described.[198,199] It then becomes dependent on proteinaceous, hairlike bacterial surface appendages termed *fimbriae* (or *pili*), of which more than 25 antigenic types are known to exist.[147,260] These adherence factors are termed *colonization factor antigens* or *coli surface antigens* (Fig. 104-2).[74]

Human STa is a small, nonimmunogenic molecule that is translated as a 72–amino acid polypeptide.[228] The first 19 amino acids serve as a signal sequence for secretion into the periplasmic space; a further cleavage event occurs after secretion, which results in the final 18 (ST-Ia) to 19 (ST-Ib) amino acid active peptide.[70,150,186] The sequence of the mature heat-stabile enterotoxin has six cysteine residues that form stabilizing disulfide bonds, contributing to the high stability of the toxin to heat and proteases.[265] The heat-stable enterotoxin receptor

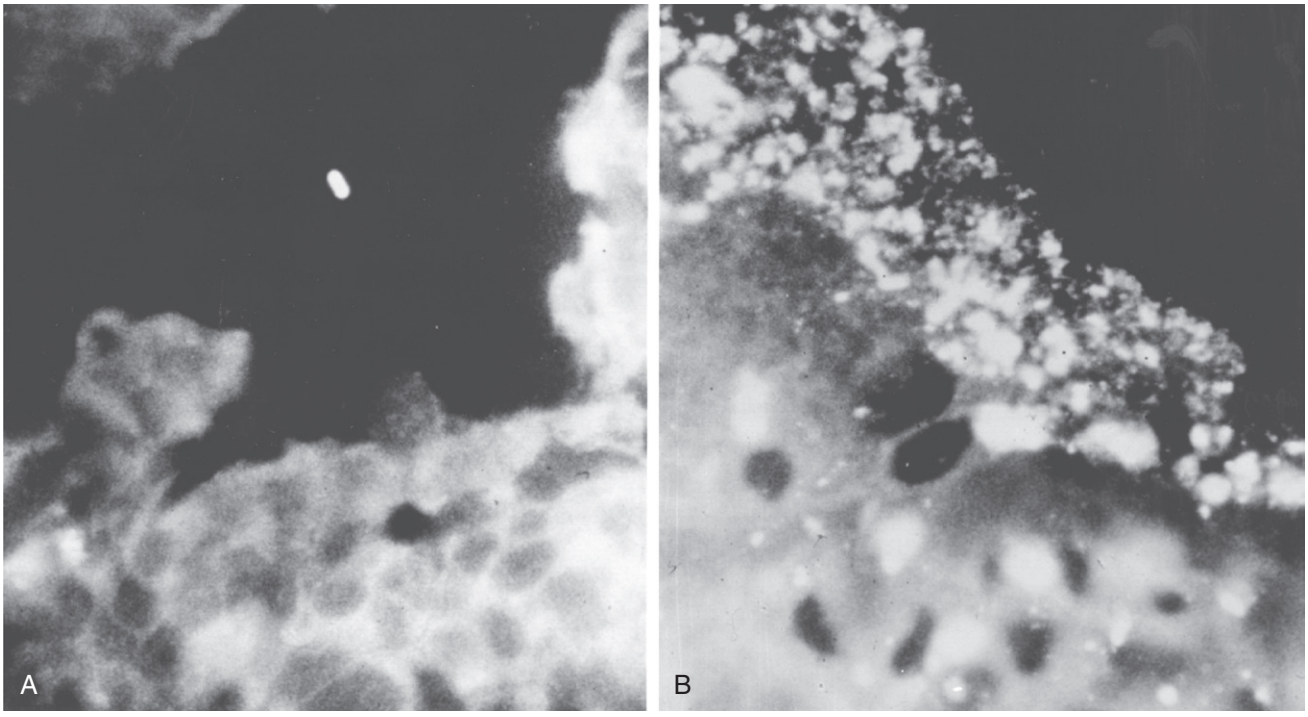

FIGURE 104-1 ■ A, Laboratory-passaged avirulent *Escherichia coli* H10407 in infant rabbit intestine. No adherent, colonizing bacteria are seen. **B,** Fresh, virulent H10407 organisms at the same time interval in infant rabbit intestine are closely adherent to the brush border (indirect immunofluorescent stained section, ×1000). (Courtesy Drs. Dolores and Doyle Evans, Department of Microbiology, Baylor College of Medicine.)

FIGURE 104-2 ■ A, Electron micrograph of negatively stained cells of avirulent plasmid-cured *Escherichia coli* H10407. Note the bald appearance of the outer surface of the organism. **B,** Similar view of virulent, fresh *E. coli* H10407. Note the hairy surface of the organism as a result of colonization factor antigen/I (×20,000). (Courtesy Drs. Dolores and Doyle Evans, Department of Microbiology, Baylor College of Medicine.)

on the apical surface of the intestinal epithelial cells is guanylyl cyclase C. This receptor is a large transmembrane protein with an extracellular ligand-binding domain and intracellular catalytic domain; binding to guanylyl cyclase C results in its activation, which is responsible for an increase in cellular cyclic guanosine monophosphate (cGMP).[213] cGMP is an intracellular signaling molecule that induces activation of apical chloride channels and inhibition of absorptive mechanisms, contributing to a secretory diarrhea.[69,83,143] Heat-stabile enterotoxin exploits an endogenous signaling pathway, the natural agonist of which is the peptide guanylin, a natural controller of electrolyte homeostasis.[50]

The heat-labile family of toxins includes LT-I, which has significant homology in structure and function with cholera toxin, and LT-II, which does not seem to cause human or animal disease.[150] LT-I shares 80 percent identity with cholera toxin and similarly has an A_1-B_5 stoichiometry.[77,141,150,223,235] The 28-kDa A subunit is the catalytically active moiety, whereas the 11-kDa B subunit pentamer mediates cell binding and entry. Heat-labile enterotoxin interacts with G_{M1} gangliosides on the surface of the epithelial cells.[70,122,150] The A subunit is composed of two domains, A1 and A2, which are bound via a disulfide bond. A1 has an adenosine diphosphate–ribosyl transferase, which targets the cellular guanosine triphosphate binding protein $G_{s\alpha}$, a regulator of cellular cyclic adenosine monophosphate (cAMP) levels. Ribosylation of $G_{s\alpha}$ leads to constitutive activation of adenylate cyclase, which greatly increases cAMP levels.[70,214] Increased cAMP leads to increased phosphorylation of chloride channels, especially cystic fibrosis transmembrane conductance regulator (CFTR), by protein kinase A, and increased chloride secretion, which also may contribute to secretory diarrhea.[70,214] Heat-labile enterotoxin additionally seems to promote *E. coli* adherence to epithelial cells, a function dependent on ADP-ribosylation, although the mechanism remains unclear.[105]

Enteropathogenic *Escherichia coli*

EPEC pathogenesis is complex and only partly understood. In contrast to ETEC, it possesses no potent secretogenic toxin but rather induces changes to the enterocyte signal transduction environment, which transforms the enterocyte from an absorptive cell to a secretory cell. EPEC pathogenesis has been described as a multistep process. The first step comprises attachment to the small bowel mucosa, an event mediated by the EPEC adherence factor plasmid-encoded bundle-forming pilus. The bundle-forming pilus is a complex surface fimbrial structure that may undergo contraction, not only tethering the bacteria to the cell but also pulling itself close to the cell's apical membrane.[117] Approach of the bacteria is followed by induction of a characteristic lesion, termed *attaching and effacing*. Under electron microscopy, the normal microvillus architecture is dissolved, and the bacterium adheres tightly (<20 nm distance) to the now undulating membrane. The bacteria may be observed sitting atop a cup or pedestal of protruding cell membrane (Fig. 104-3). This deceptively simple phenotype is mediated and accompanied by a dizzying array of invisible changes to the cell's internal environment.[53]

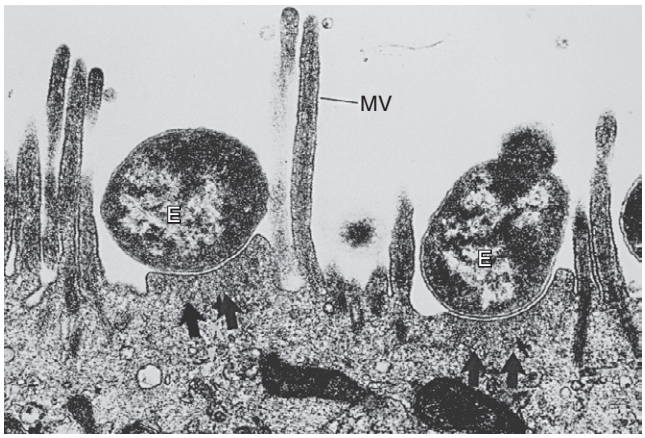

FIGURE 104-3 ■ High-power electron micrograph of intestinal epithelium infected by typical enteropathogenic *Escherichia coli* strains *(E),* with occasional intact microvilli *(MV)* and pedestal formation indicated by *double arrows.* This figure shows not only the structural alterations and loss of the brush border but also the close apposition of the organisms to the epithelial cells. (Courtesy Ralph A. Gianella, Department of Medicine, University of Cincinnati School of Medicine.)

Cellular changes are induced largely by a platoon of protein toxins that the bacterium injects directly into the cytoplasm of the target host cell. This injection process is mediated by a complex bacterial organelle termed an *injectisome*, also known as a *type III secretion system*. The first toxin injected is referred to as the *translocated intimin receptor* (Tir). Tir inserts itself into the apical plasma membrane, where it serves as the receptor for the bacterial outer membrane adhesion protein, *intimin*. As its name suggests, intimin mediates very close attachment of the bacterium to the cell and contributes to the resulting cytoskeletal damage.[39]

The net effect of Tir, intimin, and other injected toxins is dissolution of the microfilament and microtubule networks, phosphorylation of membrane ion transporters, and disruption of tight junctions at the cell's periphery. Diarrhea apparently is caused by a combination of ion transport and paracellular leakage.[258] Release of proinflammatory cytokines may complete the pathogenic cascade. The relative contributions of each of these events to EPEC disease are unknown.[39]

EPEC adhere to HEp-2 epithelial cells in a characteristic localized pattern, which has been suggested as a diagnostic assay (Fig. 104-4).[53] Also, EPEC causes actin polymerization of target cells at sites of attachment, a virulence property readily detected in tissue culture (e.g., HEp-2 cells) by a fluorescent actin-staining test (Fig. 104-5).[116] As discussed subsequently, these phenotypic assays, although useful for pathogenesis studies, have largely been replaced by molecular diagnostic tests.

Enterohemorrhagic *Escherichia coli*

The cardinal pathogenic feature of the EHEC pathotype is the production of Shiga toxin, accompanied by a cohort of accessory virulence factors. As with EPEC, these

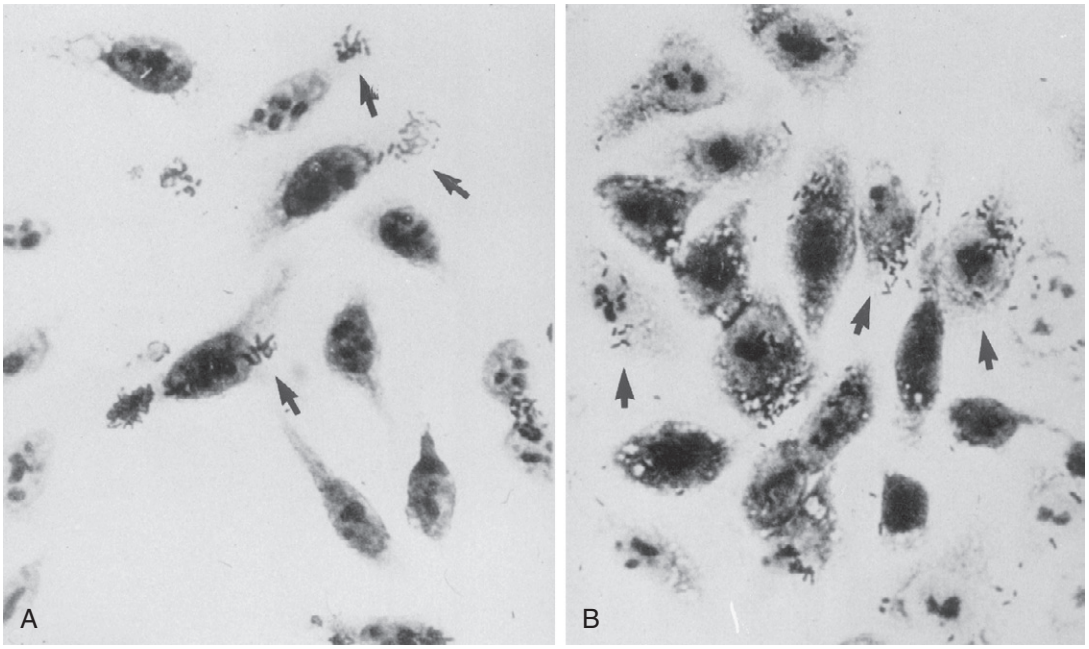

FIGURE 104-4 ■ Patterns of adherence of *Escherichia coli* to HEp-2 cells in tissue culture. Typical enteropathogenic *E. coli* associates with HEp-2 cells in the pattern shown in **A,** termed localized adherence (LA). *Arrows* point to cells where growing microcolonies of bacteria are attached to focal areas of the cell membrane. Other *E. coli* organisms isolated from patients with diarrhea adhere over the entire HEp-2 cell membrane, as shown in the cells identified with *arrows* in **B.** This pattern of interaction is termed diffuse adherence (DA). Although the LA phenotype has been associated definitively with the capacity to induce diarrhea in experimental models, evidence of virulence in DA strains remains controversial.

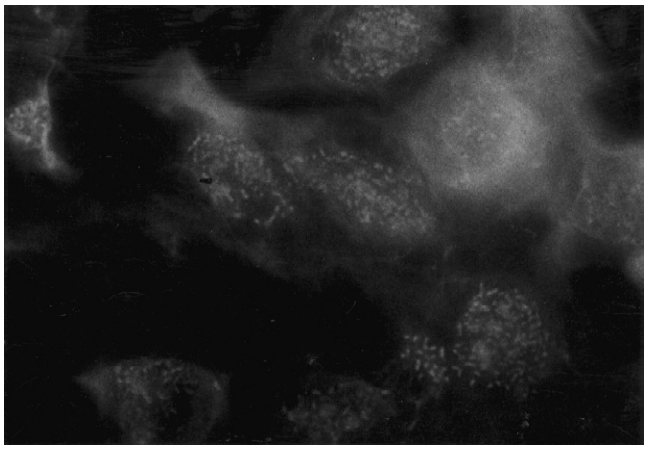

FIGURE 104-5 ■ Some *Escherichia coli* strains have the capacity to attach to the eukaryotic cell membrane and induce polymerization of actin beneath the membrane. This property may be shown by the fluorescent actin staining test, in which polymerized F-actin is detected by a fluorescein-tagged mushroom toxin specific for this form of actin. In this figure, the bacteria are attached to HEp-2 cells, and the induced actin polymerization revealed by the fluorescent reagent highlights the organisms, which are seen as bright rods on the infected cells.

factors are encoded by the bacterial chromosome and a high-molecular-weight virulence plasmid. Shiga toxin is encoded on a lysogenic phage, which is capable of infecting some non-EHEC *E. coli* strains. The full package of virulence genes has been well characterized in serotype O157:H7; studies that characterize locally obtained EHEC strains suggest that non-O157 pathogens are a heterogeneous group and tend to share some, but not all, of the virulence traits, accounting for their reduced virulence and frequency.[17,109,131] Shiga toxins were discovered by Konowalchuk and associates,[118] who reported finding Vero cell toxic activity in culture filtrates of certain strains of *E. coli* isolated from patients with diarrhea (Shiga toxin occasionally has been referred to by the archaic synonym *Vero toxin*).

Shiga toxins constitute a family of protein toxins that share identical enzymatic action and target cell binding specificity. Shiga toxins are subdivided into two families, Stx1 and Stx2, each consisting of the major Stx type and variants (i.e., Stx1c, Stx1d, Stx2c, Stx2c2, $Stx2d_{EH250}$, $Stx2d_{activatable}$, Stx2e, and Stx2f).[26] Stx2 and Stx2c have been associated with severe disease, including hemorrhagic colitis and hemolytic-uremic syndrome.[73,172] $Stx2d_{activatable}$ can be activated by mouse and human intestinal mucus and is associated with a severe clinical phenotype.[26] The greater pathogenicity of Shiga toxin 2 may be due to greater accessibility of the active site on the Shiga toxin 2 structure.[71] The *stx1* gene differs from the *Shigella stx* gene by three nucleotide changes, which result in a single conservative amino acid substitution in the A subunit: threonine to serine.[95]

Shiga toxins have the same structure and mechanism of action. They have an A_1-B_5 stoichiometry, comprising a single enzymatically active A subunit and a pentamer of B subunits responsible for toxin binding. The B subunits bind to globotrioacyl ceramide (Gb_3) and related glycolipids on host cells, including epithelial enterocytes, vascular endothelial cells, smooth muscle cells, renal endothelial cells, and erythrocytes.[54,120] The catalytic A subunit cleaves the *N*-glycosidic bond in a specific adenosine of the 28S rRNA in the 60S ribosomal subunit.[61] This single cleavage event results in irreversible cessation of protein synthesis and ultimately leads to cell death via

multiple signal transduction pathways.[45] Intestinal villus cells are susceptible to the action of Shiga toxin, which includes reduced absorption of sodium. Data suggest that Shiga toxin induces local inflammatory cytokine production, with potential effects on epithelial cell and mucosal integrity. This host response may induce a vicious cycle because both hydrogen peroxide and neutrophils augment production of Shiga toxins.[257]

Epidemiologic evidence strongly links Shiga toxin–producing strains to hemorrhagic colitis and to the associated systemic complications of hemolytic-uremic syndrome.[81,111,189] The mechanisms underlying hemolytic-uremic syndrome are unclear but seem to be due to the effects of toxin on vascular endothelial cells, possibly in concert with lipopolysaccharide and a variety of cytokines, which initiate events resulting in endothelial cell injury and platelet thrombi and, subsequently, the characteristic thrombotic microangiopathy.[42,112,162] Shiga toxin also activates complement and binds factor H, a complement regulator, leading to excess complement activation and deposition.[169] Other possible initiation factors include abnormal levels of von Willebrand factor, but whether this abnormality is a cause or a consequence of disease is unknown.[144] Shiga toxin 1 has been shown to decrease production of prostacyclins; however, the role of this event in the pathogenesis of hemolytic-uremic syndrome is unclear.[112] By contrast, thrombotic thrombocytopenic purpura results from autoantibodies against or mutations in the von Willebrand factor metalloprotease ADAMTS13.[242]

Similar to EPEC, EHEC express the intimin outer membrane protein and a type III secretion apparatus.[150] EHEC are capable of inducing complex cytoskeletal alterations similar to those caused by EPEC infection, although certain subtle differences are observed. EHEC infects the human colon, whereas EPEC is an infection of the small bowel. The genes encoding intimin, Tir, the type III secretion system, and secreted proteins reside on a 35-kb pathogenicity island called the locus of enterocyte effacement, which may be more crucial for EPEC than for EHEC for virulence.[54] A 60-Md plasmid, pO157, which contains genes encoding an enterohemolysin, is commonly found in O157:H7 strains, but its role in production of disease is unclear.[150] Subtilase cytotoxin (SubAB), a novel cytotoxin with an A_1-B_5 stoichiometry, is associated with hemolytic-uremic syndrome in diverse EHEC strains in Australia and the United States.[114,176] SubAB is heat-stable, more toxic to Vero cells than Shiga toxin, and lethal when injected in mice.[114,176] Cytotoxicity is the result of direct cleavage of an endoplasmic reticulum chaperone protein, BiP.[175]

Enteroinvasive *Escherichia coli*

In 1967, Trabulsi and coworkers[248] in Brazil and Sakazaki and associates[205] in Japan described the isolation of certain *E. coli* serotypes from patients with a disease resembling bacillary dysentery, but with cultures negative for *Shigella*. These isolates possessed a critical virulence hallmark associated with *Shigella*—the ability to invade intestinal and other epithelial cells (Fig. 104-6).[162] They have been referred to as EIEC, and a limited number of serotypes, distinct from the EPEC O groups, but often cross reactive with *Shigella* O antigens, have been found to possess this property (see Table 104-1). The genetic and molecular basis of invasion by *Shigella* was well defined in the 1990s, and the same plasmid and chromosomal genes encoding invasion properties and mechanisms seem to be present in EIEC as well.[96,125,174,208]

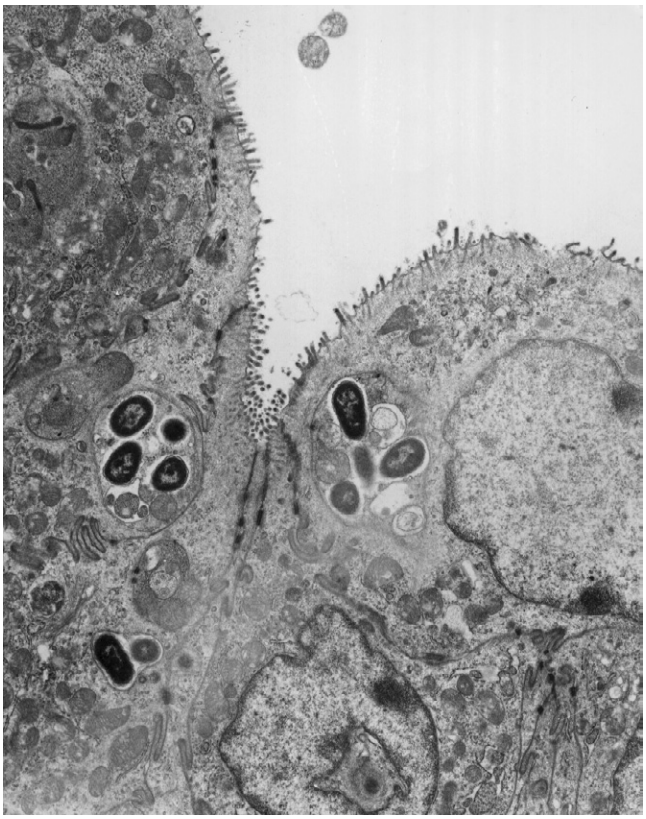

FIGURE 104-6 ■ Electron micrograph of enteroinvasive *Escherichia coli* infection of intestinal mucosa. The intracellular location of the bacteria clearly is seen within membrane-bound vesicles in two adjacent infected cells. The pathogenesis of enteroinvasive *E. coli* infection involves the invasion of intestinal cells and local cell-to-cell spread, as shown here, by a mechanism identical to that of *Shigella* spp. (Courtesy Saul Tzipori, D.V.M., Department of Comparative Medicine, Tufts University School of Veterinary Medicine.)

The invasive process for EIEC is thought to be the same as that for *Shigella* spp. It involves four main steps: (1) initial entry into cells, (2) intracellular multiplication, (3) intracellular and intercellular spread, and (4) host-cell killing. The process is complex and involves multiple genes on the invasion plasmid and the chromosome.[1,10,253] Of interest, intracellular *Shigella* and likely EIEC are able to escape autophagy.[10] They additionally take advantage of the immune response to promote an invasive phenotype.[253] EIEC produces toxins reported to be structurally distinct from the Shiga toxin of *Shigella dysenteriae* type 1 and the Shiga toxins 1 and 2 of EHEC.[67] Nonetheless, studies suggest that EIEC toxins possess many properties in common with the Shiga family of toxins, including the ability to cause fluid secretion in animal models.[67] EIEC may contain a plasmid-borne gene that encodes a 63-kDa protein named *ShET2* (*Shigella* enterotoxin 2); a mutation in this gene substantially decreases the enterotoxic activity of the parent strain.[155] The virulence of EIEC is

notably less severe than that of *Shigella*. Several groups have noted that the expression of virulence genes by EIEC using in vitro models of infection is diminished in contrast to *Shigella*.[229,231] However, the differences leading to less severe disease in vivo are still not clear.

Enteroaggregative *Escherichia coli*

The pathogenesis of EAEC includes adherence to the intestinal epithelium as a thick biofilm, accompanied by mucus hypersecretion, intestinal damage, and induction of an inflammatory response. Most of these phenotypes seem to be mediated by a set of bacterial cytotoxins and enterotoxins.[89]

Adherence and colonization of the intestinal epithelium are mediated by pili called *aggregative adherence fimbriae*, of which several allelic variants exist. EAEC may colonize the large and small intestines.[93] All known aggregative adherence fimbriae variants are encoded on high-molecular-weight virulence plasmids collectively termed *pAA*. Also encoded on pAA is a transcriptional activator of the fimbrial genes, AggR. AggR is emerging as a global regulator of virulence functions in EAEC[57]; also under AggR control is a gene encoding a surface protein nicknamed *dispersin*. In the absence of dispersin, the aggregative adherence fimbriae adhesins collapse onto the surface of the bacterium, rendering it unable to colonize the intestine (J. Nataro, unpublished data, 2004). Several cryptic genes, including a large chromosomal locus, also are under AggR control.

Damage to the intestinal mucosa has been linked to the presence of a secreted enterotoxin named plasmid-encoded toxin (Pet).[63] Pet is a protease that is internalized by target epithelial cells, where it cleaves cytoskeletal proteins, including spectrin. The net effect of these cleavage events is induction of fluid, secretion of electrolytes, and, ultimately, exfoliation of cells from the mucosal surface.[92]

EAEC strains also harbor a homolog of the ETEC heat-stable toxin, enteroaggregative *E. coli* heat-stable enterotoxin[210] and a chromosomally encoded toxin termed *Shigella* enterotoxin 1.[68] Inflammation by EAEC has been linked to the expression of the bacterial flagellum, although studies in polarized epithelial monolayers suggest that additional factors under control of AggR also are required.[89]

Diffusely Adherent *Escherichia coli*

As their name implies, DAEC are categorized by their adherence pattern on Hep-2 cells. Their pathogenesis is not well delineated. They characteristically contain nonfimbrial adhesins referred to as Afa/Dr adhesins (Afa/Dr DAEC). Along with a fimbrial adhesin, F1845, found in prototypical strain C1845 and a majority of DAEC strains, these adhesins bind to human decay-accelerating factor and, depending on the subtype of DAEC, carcinoembryonic antigen–related molecules.[108,121,215] Binding then leads to invasion by a zipperlike mechanism[107] and a resulting inflammatory response.[15,20,21,35,215] Few virulence factors have been elucidated for DAEC. However, Sat, a member of the autotransporter family of toxins, has been found in DAEC strains thought to be diarrheagenic, causes lesions and triggers autophagy in vitro in epithelial cells, and causes intestinal damage in an animal model.[85,126,241]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Diarrheogenic *E. coli* can be identified best by detecting their defining virulence genes. Although many molecular tests are described for this purpose, few laboratories in the United States use them. For most pathotypes, consultation with an expert, such as the senior author or the *E. coli* reference laboratories at the CDC, is recommended. Detection of *E. coli* generally is indicated for severe and persistent diarrhea lacking any other diagnosis (particularly in returning travelers) or in the setting of diarrheal outbreaks of unknown cause. With the exception of EHEC, most infections caused by diarrheogenic pathotypes are self-limiting.

Because of the potential of EHEC to cause serious sequelae, establishing a specific diagnosis is of clinical and epidemiologic importance. The earlier in the course of illness that a stool specimen is obtained, the more likely that *E. coli* O157:H7 will be recovered[244]; recovery more than 7 days after onset of diarrhea is unusual. The laboratory can use sorbitol-MacConkey agar to screen colonies for lack of sorbitol fermentation or substrates such as 4-methylumbelliferyl-β-D-glucuronide (MUG) for the production of β-glucuronidase; *E. coli* O157:H7 is almost uniquely sorbitol-negative and glucuronidase-negative. These techniques have limited sensitivity, with the sensitivity of sorbitol-MacConkey agar reported as 50 to 60 percent.[113] Some sorbitol-fermenting O157:H7 isolates and many other sorbitol-fermenting, non-O157 EHEC capable of causing severe illness and hemolytic-uremic syndrome would be missed by sorbitol-MacConkey agar.

Detecting Shiga toxin in stool specimens, either directly or after overnight broth enrichment, currently is the most sensitive nonmolecular method for identifying EHEC.[113] These tests are more sensitive than culture techniques and are not dependent on serotype. Commercial enzyme immunoassays for Shiga toxin have been approved by the U.S. Food and Drug Administration for either confirmation of isolates as toxin producers or rapid direct diagnosis based on the detection of Shiga toxin 1 or 2 in stool.[22,151] Despite the detection of Shiga toxin by these rapid assays, isolation of the organism, usually performed at reference laboratories, remains crucial for serotyping and other epidemiologic purposes. Serotype-specific commercial antigen immunoassays also are available.[151]

Diagnosis of ETEC traditionally would require identifying the heat-stable or heat-labile enterotoxin genes in an isolate or, after isolation and growth in vitro, by DNA probes or by detecting their products using enzyme-linked immunosorbent assay (ELISA). Multiplex PCR assays for detection of toxins and colonization factor antigens have been developed and are rapid, sensitive, and being used with increasing frequency.[51,193] PCR techniques are additionally available that allow virulence

factors to be detected directly from stool or environmental sources without the need for performing culture.[32,130] A retrospective diagnosis can be made by detecting an increase in antitoxin antibody, especially for heat-labile enterotoxin. ELISA for colonization factor antigens of ETEC also could be useful.

EPEC can be detected reliably by DNA probes for the 60-Md EPEC adherence factor plasmid or by adherence to cells in culture. The ability of *E. coli* to adhere to HEp-2 cells in culture in distinct morphologies led to the description of EPEC, EAEC, and DAEC as different pathotypes (see Fig. 104-3). After a 3-hour incubation of bacteria with cells, EPEC adheres to the cell in tight microcolonies, referred to as the *localized adherence* pattern. Although it remains sensitive and specific for EPEC, this method has been replaced by molecular detection methods to detect the locus of enterocyte effacement island (via the intimin-encoding gene *eae*) and the EPEC adherence factor plasmid (often via detection of the bundle-forming pilus–encoding genes).[55]

EAEC can be detected by the presence of the aggregative adherence pattern in the Hep-2 adherence assay (Fig. 104-7). DNA probes and PCR are available to detect the AggR regulon, and these tests are likely to provide more specific detection of EAEC pathogens.[44]

EIEC is detected best using molecular techniques that are specific for detection of pathogenesis-related genes. Several multiplex PCR assays that allow the user to screen for multiple *E. coli* pathotypes simultaneously have been described.[14,127,178] Additionally, it is possible to differentiate between *Shigella* spp. and EIEC using these techniques,[177] which can be introduced readily into any molecular biology laboratory and produce excellent results.

DAEC can be detected by a diffuse adherence pattern on Hep-2 cells.[166,215] A probe, commonly used in epidemiologic studies, which targets *daaC*, part of the F1845 operon, has recently been shown to cross react with some strains of EAEC.[215,226] PCR methods are available for detection from stool.[140,215]

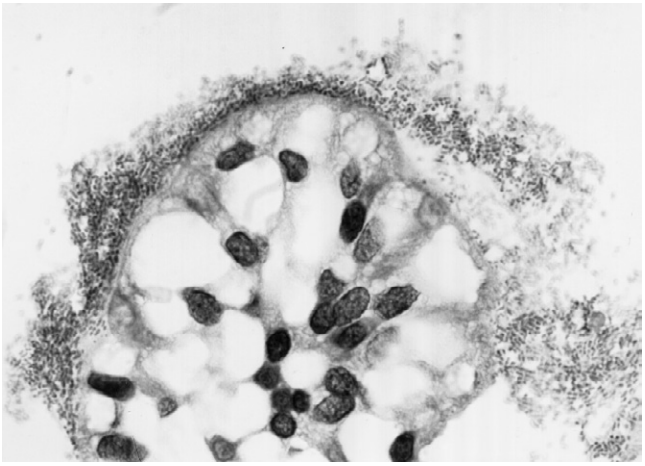

FIGURE 104-7 ■ Enteroaggregative *Escherichia coli* (EAEC) infection of the gnotobiotic pig intestine. This photomicrograph shows the characteristic "stacked-brick" appearance of the aggregative organisms over the surface of the intestinal epithelium in vivo in the same manner described for EAEC adherence to HEp-2 cells in tissue culture. (Courtesy Saul Tzipori, D.V.M., Department of Comparative Medicine, Tufts University School of Veterinary Medicine.)

PROGNOSIS

EPEC, EAEC, and ETEC do not cause systemic infections or complications except those resulting from dehydration or the consequences of nutritional depletion. The prognosis is related directly to the availability and adequacy of fluid therapy. When this need is dealt with correctly in an otherwise healthy and well-nourished patient, the principal complication is the rare instance of monosaccharide intolerance. In infants or young children in developing countries with protein-energy malnutrition, however, chronic diarrhea and progressive worsening of nutritional status frequently are observed, sometimes resulting in mortality. When food is withheld from either well-nourished or poorly nourished children, hypoglycemia may occur and produce seizures, coma, or death. Rarely, loss of water in excess of salt causes hypertonic dehydration, with serum sodium concentrations greater than 160 mEq/L, a situation that may cause seizures, coma, and death.

As noted earlier, EAEC has been associated with persistent diarrhea lasting longer than 14 days in young infants and children. Persistent diarrhea leads to nutritional deterioration, may be difficult to control, and may culminate in death from sepsis or other infections. Inflammatory diarrhea caused by EIEC also results in nutritional deterioration, with significant protein losses occurring via the gut.

EHEC infections can be followed by hemolytic-uremic syndrome and neurologic and intestinal complications. Ten percent of patients may die early in the course of the systemic phase of hemolytic-uremic syndrome. Although the renal failure of hemolytic-uremic syndrome may be reversible with good management of fluid and electrolytes and the use of dialysis as needed, permanent damage to the kidneys is, contrary to earlier more optimistic assessments, likely to occur in 25 percent or more of patients over the course of 1 or 2 decades, and many of them require permanent dialysis or transplantation in the future.[75] Symptoms of chronic renal disease may appear more than 1 year after the initial presentation of hemolytic-uremic syndrome, suggesting that follow-up of these patients should be continued for at least 5 years, even for patients who appear to be recovered.[195]

TREATMENT

In all age groups, the principal treatment of the intestinal manifestations of *E. coli* enteric infection is replacement of fluids and electrolytes; with maintenance of fluid balance, the disease is self-limited to 1 week or less in most patients and lasts no more than 2 weeks in nearly all patients. The earlier that fluid replacement therapy is begun, the better the prognosis, particularly considering that clinical signs of dehydration do not develop until a 5 percent loss of body weight occurs and that sustained

loss of more than 10 percent of body weight is incompatible with survival.

When shock is present (usually with altered consciousness and an absent or thready pulse) or oral rehydration is unsuccessful because of persistent vomiting, patients must be rehydrated by intravenous infusion of an isotonic electrolyte solution, such as lactated Ringer solution or normal saline. Alternatively, several prerequisites must be ensured for oral therapy: (1) the patient must not be in shock, (2) the patient must be fully conscious, (3) the patient must be able to drink (vomiting, particularly common in children, is not an absolute contraindication because frequent small oral feedings usually are largely retained; when the metabolic abnormality begins to reverse toward normal, vomiting ceases), (4) bowel sounds must be present, and (5) renal function must be normal. Current recommendations for oral rehydration in the United States and Europe suggest the use of hypotonic fluid containing 60 to 75 mEq of sodium per liter (along with potassium and carbohydrates). In the developing world, where cholera is a common occurrence, the World Health Organization recommends that a solution containing 75 mEq of sodium be used for all cases of diarrhea. Monitoring for electrolyte imbalances may be required. Rice-based solutions for oral rehydration currently are favored in many diarrheal centers because of their reduced osmotic load.[115] Additionally, zinc supplementation may be of benefit in reducing the duration of acute diarrhea and preventing further episodes.[25,115,234]

A prospective cohort study comparing *E. coli* O157:H7 hemolytic-uremic syndrome patients who developed oligoanuric renal failure requiring dialysis with patients with nonoligoanuric renal failure found that the patients who required dialysis had received less intravascular volume expansion and sodium than the patients who did not require dialysis, suggesting that intravascular volume expansion with isotonic fluid was protective for oligoanuric renal failure when given early in the course of O157 infection.[7] The authors advocate hospitalizing all children with suspected EHEC infection for volume expansion with isotonic fluids and close monitoring.

Although rehydration therapy is the cornerstone of management, antibiotic therapy should be considered in some cases. In most *E. coli*–associated diarrheal illnesses, the disease is mild and of short duration and no specific antimicrobial therapy is required. Studies to address this issue have found antibiotics to be beneficial in some circumstances. In traveler's diarrhea secondary to ETEC, antibiotic therapy can shorten the duration of illness and decrease its severity.[12,62] Prophylactic antibiotic therapy probably carries more risk than benefit, however, and generally is not recommended unless a significant reason exists for the traveler to require prophylaxis. Because of increasing rates of resistance to trimethoprim-sulfamethoxazole (TMP-SMX) it is no longer recommended for treatment or prophylaxis.[58] For moderate to severe traveler's diarrhea (both ETEC and EAEC), rifaximin, ciprofloxacin, and azithromycin for 1 to 3 days have been shown to have some efficacy. Rifaximin is not absorbed from the gastrointestinal tract, which provides a more favorable side effect profile. However, if symptoms occur, such as high fever (39.4°C [102.9°F]) or grossly bloody stools, suggesting a more invasive pathogen, azithromycin is recommended instead (10 mg/kg/day for 3 days in children older than 6 months; children younger than 1 year should be watched closely by a physician).[51,58] Fluoroquinolones (ciprofloxacin) are considered to have a potential undesirable side effect profile in children, although 1 to 3 days, as recommended for traveler's diarrhea, would likely be safe. Of note, resistance to fluoroquinolones does continue to increase, which warrants continued monitoring and judicious use.[139] Children in developing countries with ETEC diarrhea also may benefit from receiving antibiotic therapy.[145,161]

EIEC infection theoretically would benefit even more from antibiotic therapy, given its pathogenic similarity to shigellosis and the known benefits of the early use of antibiotics. No controlled studies have validated this benefit for EIEC, however. In experimentally induced disease, DuPont and colleagues[59] reported that parenteral ampicillin produced a bacteriologic cure and rapid clinical response with defervescence and improvement of diarrhea in adults. They have also used ampicillin in the pediatric population for severe dysentery caused by EIEC.[230] TMP-SMX and ampicillin are the current drugs of choice, although resistance has been reported.[6]

Immunocompromised hosts, especially children with AIDS, may require prolonged antibiotic therapy for protracted or recrudescent diarrhea, even when it is caused by bacteria that normally produce only self-limited disease. Malnourished children and children with other serious underlying illness also fit this category. In these patients, systemic invasion may develop along with associated complications, including shock and renal failure. In addition, prolonged carrier states are common findings, with frequent relapses requiring long-term antibiotic therapy for suppression of relapse.

Epidemic EPEC infection, especially in newborns, seems to be affected favorably by antibiotic therapy.[156] The potential for this pathogen to cause prolonged disease and a history of high rates of mortality in neonates suggest the need for antibiotic trials in this age group. Based on limited data, either TMP-SMX or oral nonabsorbable antibiotics, such as gentamicin or colistin, usually are recommended, although antibiotic resistance is common and may alter the choice of antibiotics based on local susceptibility patterns.[16,255]

Antibiotic treatment of EHEC is not recommended. Antibiotics in clinical use do not improve the course or prevent sequelae, and they may exacerbate the illness by promoting production and release of Shiga toxin.[9,27,40] In vitro data suggest that subinhibitory concentrations of TMP-SMX and other antibiotics may increase expression of Shiga toxin.[16,110,134] The most rigorous prospective study to address this question found that the cohort who received antibiotics had a higher incidence of hemolytic-uremic syndrome (of 25 patients who received antibiotics, 9 developed hemolytic-uremic syndrome) than the cohort who did not (of 234 patients who did not receive antibiotics, 27 developed hemolytic-uremic syndrome; $p < .001$).[261,262] Other studies have found no association.[171,204]

Notably, no studies have used a randomized placebo-controlled design, so the association may reflect selection bias because both the use of antibiotics and systemic

complications are associated with more severe disease. A meta-analysis[204] did not show a higher risk for hemolytic-uremic syndrome associated with antibiotics; however, this meta-analysis has been criticized because it included a study in which all of the patients received antibiotics[99] and because comparisons were made among different antibiotics, which may not induce similar sequelae.

Eculizumab, a monoclonal C5 antibody that targets terminal complement complex formation, has been shown in a small case series (n = 3) to be effective in improving refractory severe hemolytic-uremic syndrome.[119] Further follow-up of hundreds of patients in Germany who had Shiga toxin–positive EAEC during a 2011 continental outbreak and received eculizumab is ongoing.[170]

Antimotility agents such as loperamide generally are not needed and should be used cautiously if prescribed, with great attention paid to dosage, especially in very young patients.[245] Dysentery is a contraindication to the use of antimotility agents, which may be a risk factor for the development of ileus and abdominal distention.[3] The prolonged use of antimotility agents is reported to be associated with more serious systemic complications of EHEC infection and is not recommended.[40,41]

In most instances of *E. coli* diarrhea, the clinician is left to make therapeutic decisions without knowing the etiologic agent responsible. For reasons already outlined, routine diagnostic microbiology laboratories cannot distinguish pathogenic from nonpathogenic *E. coli*, with the exception of classic EPEC serotypes and Shiga toxin–producing *E. coli*, including O157:H7 EHEC. Clinical decisions regarding the administration of antibiotic therapy are made on purely clinical grounds with criteria such as the history, duration, and severity of the illness; the age and immunologic competence of the patient; and the nature of the diarrheal stool (e.g., watery, inflammatory, bloody, or dysenteric). Empirical antimicrobial therapy is more justifiable for immunocompromised hosts, for patients with prolonged or severe illness or a history of relevant risk factors (e.g., specific food ingestion, travel, or exposure to known contacts), and for cases of inflammatory or dysenteric illness.

Research on alternative treatments of EHEC and prevention of hemolytic-uremic syndrome is ongoing. A synthetic verotoxin receptor, SYNSORB Pk, was not shown to prevent death, dialysis, or serious extrarenal events in a trial of 145 children with EHEC-induced HUS.[252] Although antibodies against *E. coli* O157 intimin were protective for colonization and disease in a pig model,[52] plasmapheresis and intravenous immunoglobulin have not been shown to ameliorate hemolytic-uremic syndrome in humans.[192,256] Investigation of monoclonal antibodies directed against Shiga toxin are under way.[252] The complete genome sequence of *E. coli* O157:H7 is now known and may lead to greater understanding of the pathogenesis of hemorrhagic colitis and hemolytic-uremic syndrome and perhaps innovative approaches to treatment and prevention.[90]

PREVENTION

Epidemic nursery outbreaks of diarrhea in newborns can be controlled by the application of classic principles of preventive medicine. Prompt diagnosis and treatment and scrupulous attention given to details of hand washing and environmental sanitation to eliminate person-to-person transmission are still effective, whereas prophylactic antimicrobials have no role. Outbreaks in neonatal nurseries can be contained by epidemiologic control measures such as cohorting; by screening staff for carriage; and, if necessary, by closing the unit until it is decontaminated. In contrast, preventing sporadic cases of *E. coli* diarrhea is difficult. In communities with obvious deficits in water supply and feces disposal, correction of these problems would lead to a diminished incidence of diarrheal diseases in general.

For traveler's diarrhea, avoidance of contaminated sources such as tap water, unpeeled fresh fruits, and large quantities of ice can be effective in prevention. Several studies additionally indicate that prophylactic antibiotics can protect adult travelers, at least for a limited time.[84] The risk for selection of resistant organisms and drug side effects limits the use of antibiotic prophylaxis to short-term travelers with business or diplomatic missions that would be hindered significantly by an episode of diarrhea. Fluoroquinolones, azithromycin, and rifaximin have been evaluated for prophylaxis and presumptive treatment, and all are efficacious.[222] Some evidence has been presented for the efficacy of bismuth compounds, such as bismuth subsalicylate, for the prevention of ETEC diarrhea in adults. The concern for bismuth toxicity with prolonged use in young children would render it a problematic solution for pediatric *E. coli* diarrhea and its prevention.

ETEC vaccine development has been propelled by the observation that ETEC infection confers some protection from reinfection with the same strain,[233] also evident in the decreasing rates of infection in children older than 5 years.[181] The challenge is that more than 25 colonization factor antigens have been identified, and strain serotypes vary tremendously across the world.[78] As noted previously, however, it also is known that certain serotypes and colonization factor antigens are most common (i.e., colonization factor antigens I, II, and IV) and tend to co-segregate.[74,78,260] This factor would limit the antigenic variability needed in an effective vaccine. Many isolates of ETEC expressing only heat-labile enterotoxin do not express known colonization factors and would be unaffected by a colonization factor antigen–based vaccine. Although no ETEC vaccine currently is licensed and available, several approaches have been attempted. Dukoral, a vaccine licensed in Europe and Canada for prevention of cholera, has been demonstrated to have some short-term efficacy against ETEC-associated traveler's diarrhea and may be useful for travelers to high-risk areas.[102] Among the most successful ETEC-specific vaccines to date is one composed of recombinant cholera toxin B (as a surrogate for the LTB toxin subunit) with strains of formalin-killed ETEC expressing the most prevalent colonization factor antigens in developing countries (rCTB-CF ETEC).[236] This vaccine was found to prevent 77 percent of nonmild ETEC diarrhea in travelers to developing countries.[181,202,236] In a study of Egyptian children, however, it was not efficacious in preventing disease.[29,181] Studies are now ongoing to increase the expression of colonization

factor antigens in a vaccine construct and improve the immune response to these antigens.[236]

Other avenues of vaccine development that have been pursued include live, attenuated ETEC vaccines[136] and subunit vaccines. Heat-stable enterotoxin does not create a significant immune response; however, heat-labile enterotoxin is a known adjuvant similar to cholera toxin and has been conjugated to heat-stabile enterotoxin.[29,78,128,266] Concerns for human use of this construct come, however, from the homology of heat-stabile enterotoxin to guanylin, which is produced endogenously.[29,50] Live, attenuated *Shigella* vaccine vectors also are being studied in an attempt to elicit a mucosal immune response. Researchers have studied new methods of delivering antigens to the mucosa, including encapsulation in microspheres, delivery in recombinant foods,[237,238,240] and a transcutaneous heat-labile enterotoxin patch vaccine.[72,135] Finally, new vaccine candidates are being identified, such as the EtpA adhesin that has been shown to elicit an immune response and offer some protection in mice.[198,199] Although no vaccine is yet licensed in the United States, work continues to advance in this field in an attempt to decrease the incidence and severity of morbidity from dysentery in the developing world and from traveler's diarrhea.

Vaccine development for other diarrheogenic *E. coli* strains lags behind that for ETEC. Because EHEC-induced hemolytic-uremic syndrome is a rare disease, vaccine development for humans is limited. However, one group is pursuing vaccination as part of the primary infant series because young age is a risk factor for the development of hemolytic-uremic syndrome. An O157:H7 O–specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* (O157-rEPA) vaccine was shown to be safe and immunogenic in children in a phase 2 study.[5,138] Vaccine development for cattle, the major reservoir, is ongoing[225] and has now yielded two available cattle vaccines.[138] A vaccine composed of type III secreted proteins resulted in decreased shedding of *E. coli* O157:H7 in contrast to controls, although prevalence of shedding increased over the study period. Repeat vaccination of cattle may serve as a mode to decrease human exposure to EHEC.[179]

NEW REFERENCES SINCE THE SIXTH EDITION

10. Andrade JA, Freymuller E, Fagundes-Neto U. Adherence of enteroaggregative *Escherichia coli* to the ileal and colonic mucosa: an in vitro study utilizing the scanning electron microscopy. *Arq Gastroenterol* 2011;**48**:199–204.
15. Arikawa K, Nishikawa Y. Interleukin-8 induction due to diffusely adherent *Escherichia coli* possessing *Afa/Dr* genes depends on flagella and epithelial Toll-like receptor 5. *Microbiol Immunol* 2010; **54**:491–501.
17. Aslani MM, Bouzari S. Characterization of virulence genes of non-O157 Shiga toxin-producing *Escherichia coli* isolates from two provinces of Iran. *Jpn J Infect Dis* 2009;**62**:16–19.
35. Cane G, Ginouves A, Marchetti S, et al. HIF-1alpha mediates the induction of IL-8 and VEGF expression on infection with Afa/Dr diffusely adhering *E. coli* and promotes EMT-like behaviour. *Cell Microbiol* 2010;**12**:640–53.
38. Centers for Disease Control and Prevention. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food—foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 749–55.
51. de la Cabada Bauche J, Dupont HL. New developments in traveler's diarrhea. *Gastroenterol Hepatol (N Y)* 2011;**7**:88–95.
58. DuPont HL, Ericsson CD, Farthing MJ, et al. Expert review of the evidence base for self-therapy of travelers' diarrhea. *J Travel Med* 2009;**16**:161–71.
65. Estrada-Garcia T, Lopez-Saucedo C, Thompson-Bonilla R, et al. Association of diarrheagenic *Escherichia coli* pathotypes with infection and diarrhea among Mexican children and association of atypical enteropathogenic *E. coli* with acute diarrhea. *J Clin Microbiol* 2009;**47**:93–8.
70. Fleckenstein JM, Hardwidge PR, Munson GP, et al. Molecular mechanisms of enterotoxigenic *Escherichia coli* infection. *Microbes Infect* 2010;**12**:89–98.
87. Hadler JL, Clogher P, Hurd S, et al. Ten-year trends and risk factors for non-O157 Shiga toxin-producing *Escherichia coli* found through Shiga toxin testing, Connecticut, 2000-2009. *Clin Infect Dis* 2011;**53**:269–76.
91. Hedican EB, Medus C, Besser JM, et al. Characteristics of O157 versus non-O157 Shiga toxin-producing *Escherichia coli* infections in Minnesota, 2000-2006. *Clin Infect Dis* 2009;**49**:358–64.
105. Johnson AM, Kaushik RS, Francis DH, et al. Heat-labile enterotoxin promotes *Escherichia coli* adherence to intestinal epithelial cells. *J Bacteriol* 2009;**191**:178–86.
109. Kappeli U, Hachler H, Giezendanner N, et al. Human infections with non-O157 Shiga toxin-producing *Escherichia coli*, Switzerland, 2000-2009. *Emerg Infect Dis* 2011;**17**:180–5.
119. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 2011;**364**: 2561–3.
126. Lievin-Le Moal V, Comenge Y, Ruby V, et al. Secreted autotransporter toxin (Sat) triggers autophagy in epithelial cells that relies on cell detachment. *Cell Microbiol* 2011;**13**:992–1013.
127. Liu J, Gratz J, Maro A, et al. Simultaneous detection of six diarrhea-causing bacterial pathogens with an in-house PCR-Luminex assay. *J Clin Microbiol* 2011;**50**:98–103.
128. Liu M, Ruan X, Zhang C, et al. Heat-labile- and heat-stable-toxoid fusions (LTRG-STaPF) of human enterotoxigenic *Escherichia coli* elicit neutralizing antitoxin antibodies. *Infect Immun* 2011;**79**:4002–9.
131. Mag T, Nogrady N, Herpay M, et al. Characterisation of verotoxin-producing *Escherichia coli* strains isolated from human patients in Hungary over a 7-year period. *Eur J Clin Microbiol Infect Dis* 2010;**29**:249–52.
134. McGannon CM, Fuller CA, Weiss AA. Different classes of antibiotics differentially influence shiga toxin production. *Antimicrob Agents Chemother* 2010;**54**:3790–8.
138. Melton-Celsa A, Mohawk K, Teel L, et al. Pathogenesis of Shiga-toxin producing *Escherichia coli*. *Curr Top Microbiol Immunol* 2012;**357**:67–103.
139. Mendez Arancibia E, Pitart C, Ruiz J, et al. Evolution of antimicrobial resistance in enteroaggregative *Escherichia coli* and enterotoxigenic *Escherichia coli* causing traveller's diarrhoea. *J Antimicrob Chemother* 2009;**64**:343–7.
145. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012;**54**: 185–92.
147. Nada RA, Shaheen HI, Khalil SB, et al. Discovery and phylogenetic analysis of novel members of class b enterotoxigenic *Escherichia coli* adhesive fimbriae. *J Clin Microbiol* 2011;**49**:1403–10.
159. Nicklasson M, Klena J, Rodas C, et al. Enterotoxigenic *Escherichia coli* multilocus sequence types in Guatemala and Mexico. *Emerg Infect Dis* 2010;**16**:143–6.
160. Nunes MR, Penna FJ, Franco RT, et al. Enterotoxigenic *Escherichia coli* in children with acute diarrhoea and controls in Teresina/PI, Brazil: distribution of enterotoxin and colonization factor genes. *J Appl Microbiol* 2011;**111**:224–32.
163. Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. *Curr Opin Infect Dis* 2011;**24**:478–83.
164. Ochoa TJ, Barletta F, Contreras C, et al. New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Trans R Soc Trop Med Hyg* 2008;**102**:852–6.
166. Okeke IN. Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *J Infect Dev Ctries* 2009;**3**: 817–42.

168. Opintan JA, Bishar RA, Newman MJ, et al. Carriage of diarrhoeagenic *Escherichia coli* by older children and adults in Accra, Ghana. *Trans R Soc Trop Med Hyg* 2010;**104**:504–6.
169. Orth D, Khan AB, Naim A, et al. Shiga toxin activates complement and binds factor H: evidence for an active role of complement in hemolytic uremic syndrome. *J Immunol* 2009;**182**:6394–400.
170. Orth-Holler D, Riedl M, Wurzner R. Inhibition of terminal complement activation in severe Shiga toxin-associated HUS: perfect example for a fast track from bench to bedside. *EMBO Mol Med* 2011;**3**:617–19.
177. Pavlovic M, Luze A, Konrad R, et al. Development of a duplex real-time PCR for differentiation between *E. coli* and *Shigella* spp. *J Appl Microbiol* 2011;**110**:1245–51.
182. Rajendran P, Ajjampur SS, Chidambaram D, et al. Pathotypes of diarrheagenic *Escherichia coli* in children attending a tertiary care hospital in South India. *Diagn Microbiol Infect Dis* 2010;**68**:117–22.
187. Rasko DA, Webster DR, Sahl JW, et al. Origins of the *E. coli* strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med* 2011;**365**:709–17.
188. Reyes D, Vilchez S, Paniagua M, et al. Diversity of intestinal *Escherichia coli* populations in Nicaraguan children with and without diarrhoea. *J Med Microbiol* 2009;**58**:1593–600.
193. Rodas C, Iniguez V, Qadri F, et al. Development of multiplex PCR assays for detection of enterotoxigenic *Escherichia coli* colonization factors and toxins. *J Clin Microbiol* 2009;**47**:1218–20.
195. Rosales A, Hofer J, Zimmerhackl LB, et al. Need for long-term follow-up in enterohemorrhagic *Escherichia coli*-associated hemolytic uremic syndrome due to late-emerging sequelae. *Clin Infect Dis* 2012;**54**:1413–21.
199. Roy K, Hilliard GM, Hamilton DJ, et al. Enterotoxigenic *Escherichia coli* EtpA mediates adhesion between flagella and host cells. *Nature* 2009;**457**:594–8.
201. Sabui S, Ghosal A, Dutta S, et al. Allelic variation in colonization factor CS6 of enterotoxigenic *Escherichia coli* isolated from patients with acute diarrhoea and controls. *J Med Microbiol* 2010;**59**:770–9.
217. Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. *Am J Trop Med Hyg* 2009;**80**:609–14.
218. Shaheen HI, Abdel Messih IA, Klena JD, et al. Phenotypic and genotypic analysis of enterotoxigenic *Escherichia coli* in samples obtained from Egyptian children presenting to referral hospitals. *J Clin Microbiol* 2009;**47**:189–97.
222. Singh E, Redfield D. Prophylaxis for travelers' diarrhea. *Curr Gastroenterol Rep* 2009;**11**:297–300.
225. Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the faeces of domestic ruminants. *Zoonoses Public Health* 2012;**59**:126–38.
226. Snelling AM, Macfarlane-Smith LR, Fletcher JN, et al. The commonly-used DNA probe for diffusely-adherent *Escherichia coli* cross-reacts with a subset of enteroaggregative *E. coli*. *BMC Microbiol* 2009;**9**:269.
232. Spano LC, Sadovsky AD, Segui PN, et al. Age-specific prevalence of diffusely adherent Escherichia coli in Brazilian children with acute diarrhoea. *J Med Microbiol* 2008;**57**:359–63.
240. Tacket CO. Plant-based oral vaccines: results of human trials. *Curr Top Microbiol Immunol* 2009;**332**:103–17.
242. Tarr PI. Shiga toxin-associated hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: distinct mechanisms of pathogenesis. *Kidney Int Suppl* 2009;**112**:S29–32.
253. Usein CR, Tatu-Chitoiu D, Ciontea S, et al. *Escherichia coli* pathotypes associated with diarrhea in Romanian children younger than 5 years of age. *Jpn J Infect Dis* 2009;**62**:289–93.
254. Vidal RM, Valenzuela P, Baker K, et al. Characterization of the most prevalent colonization factor antigens present in Chilean clinical enterotoxigenic *Escherichia coli* strains using a new multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2009;**65**:217–23.
258. Weflen AW, Alto NM, Hecht GA. Tight junctions and enteropathogenic *E. coli*. *Ann N Y Acad Sci* 2009;**1165**:169–74.
262. Wong CS, Mooney JC, Brandt JR, et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. *Clin Infect Dis* 2012;**55**:33–41.
266. You J, Xu Y, He M, et al. Protection of mice against enterotoxigenic *E. coli* by immunization with a polyvalent enterotoxin comprising a combination of LTB, STa, and STb. *Appl Microbiol Biotechnol* 2011;**89**:1885–93.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 105

KLEBSIELLA

Randall G. Fisher

Klebsiella is a genus of Enterobacteriaceae, a frequent cause of nosocomial pediatric infection. Classically described by Friedländer[42] as a cause of pneumonia, *Klebsiella* can cause infections of the urinary tract, lung, and central venous catheters in high-risk newborns and immunocompromised older children.[20]

BACTERIOLOGY

Klebsiella organisms were named for Edwin Klebs, the noted German bacteriologist.[99] Distinguishing features of *Klebsiella* spp. include the absence of motility and the presence of a polysaccharide capsule that gives rise to

REFERENCES

1. Acheson D, Keusch G. *Shigella* and enteroinvasive *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JI, et al., editors. *Infections of the Gastrointestinal Tract*. New York: Raven Press, Ltd.; 1995. p. 763–84.
2. Adachi JA, Ericsson CD, Jiang ZD, et al. Natural history of enteroaggregative and enterotoxigenic *Escherichia coli* infection among US travelers to Guadalajara, Mexico. *J Infect Dis* 2002;**185**: 1681–3.
3. Adam A. Biology of colon bacillus in dyspepsia, and its relation to pathogenesis and to intoxication. *J Kinderheilk* 1923;**101**: 295–314.
4. Afset JE, Bruant G, Brousseau R, et al. Identification of virulence genes linked with diarrhea due to atypical enteropathogenic *Escherichia coli* by DNA microarray analysis and PCR. *J Clin Microbiol* 2006;**44**:3703–11.
5. Ahmed A, Li J, Shiloach Y, et al. Safety and immunogenicity of *Escherichia coli* O157 O-specific polysaccharide conjugate vaccine in 2-5-year-old children. *J Infect Dis* 2006;**193**:515–21.
6. Ahmed AM. Molecular characterization of a multidrug-resistant strain of enteroinvasive *Escherichia coli* O164 isolated in Japan. *J Med Microbiol* 2005;**54**:273–8.
7. Ake JA, Jelacic S, Ciol MA, et al. Relative nephroprotection during *Escherichia coli* O157:H7 infections: association with intravenous volume expansion. *Pediatrics* 2005;**115**:e673–680.
8. Al-Gallas N, Bahri O, Bouratbeen A, et al. Etiology of acute diarrhea in children and adults in Tunis, Tunisia, with emphasis on diarrheagenic *Escherichia coli:* prevalence, phenotyping, and molecular epidemiology. *Am J Trop Med Hyg* 2007;**77**:571–82.
9. Al-Qarawi S, Fontaine RE, Al-Qahtani MS. An outbreak of hemolytic uremic syndrome associated with antibiotic treatment of hospital inpatients for dysentery. *Emerg Infect Dis* 1995;**1**: 138–40.
10. Andrade JA, Freymuller E, Fagundes-Neto U. Adherence of enteroaggregative *Escherichia coli* to the ileal and colonic mucosa: an in vitro study utilizing the scanning electron microscopy. *Arq Gastroenterol* 2011;**48**:199–204.
11. Anonymous. Bray's discovery of pathogenic *Esch. coli* as a cause of infantile gastroenteritis. *Arch Dis Child* 1973;**48**:923–6.
12. Anonymous. Travelers' diarrhea: National Institutes of Health Consensus Development Conference. Bethesda, Maryland, January 28-30, 1985. *Rev Infect Dis* 1986;**8**(Suppl. 2):S109–233.
13. Anonymous. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach: United States, September 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1045–6.
14. Aranda KR, Fabbricotti SH, Fagundes-Neto U, et al. Single multiplex assay to identify simultaneously enteropathogenic, enteroaggregative, enterotoxigenic, enteroinvasive and Shiga toxin-producing *Escherichia coli* strains in Brazilian children. *FEMS Microbiol Lett* 2007;**267**:145–50.
15. Arikawa K, Nishikawa Y. Interleukin-8 induction due to diffusely adherent *Escherichia coli* possessing *Afa/Dr* genes depends on flagella and epithelial Toll-like receptor 5. *Microbiol Immunol* 2010;**54**:491–501.
16. Ashkenazi S, Cleary TG. Antibiotic treatment of bacterial gastroenteritis. *Pediatr Infect Dis J* 1991;**10**:140–8.
17. Aslani MM, Bouzari S. Characterization of virulence genes of non-O157 Shiga toxin-producing *Escherichia coli* isolates from two provinces of Iran. *Jpn J Infect Dis* 2009;**62**:16–19.
18. Bell BP, Griffin PM, Lozano P, et al. Predictors of hemolytic uremic syndrome in children during a large outbreak of *Escherichia coli* O157:H7 infections. *Pediatrics* 1997;**100**:E12.
19. Belnap WD, O'Donnell JJ. Epidemic gastroenteritis due to *Escherichia coli* 0-111; a review of the literature, with the epidemiology, bacteriology, and clinical findings of a large outbreak. *J Pediatr* 1955;**47**:178–93.
20. Betis F, Brest P, Hofman V, et al. Afa/Dr diffusely adhering *Escherichia coli* infection in T84 cell monolayers induces increased neutrophil transepithelial migration, which in turn promotes cytokine-dependent upregulation of decay-accelerating factor (CD55), the receptor for Afa/Dr adhesins. *Infect Immun* 2003;**71**: 1774–83.
21. Betis F, Brest P, Hofman V, et al. The Afa/Dr adhesins of diffusely adhering *Escherichia coli* stimulate interleukin-8 secretion, activate mitogen-activated protein kinases, and promote polymorphonuclear transepithelial migration in T84 polarized epithelial cells. *Infect Immun* 2003;**71**:1068–74.
22. Bettelheim KA, Beutin L. Rapid laboratory identification and characterization of verocytotoxigenic (Shiga toxin producing) *Escherichia coli* (VTEC/STEC). *J Appl Microbiol* 2003;**95**:205–17.
23. Bhan MK, Khoshoo V, Sommerfelt H, et al. Enteroaggregative *Escherichia coli* and *Salmonella* associated with nondysenteric persistent diarrhea. *Pediatr Infect Dis J* 1989;**8**:499–502.
24. Bhan MK, Raj P, Levine MM, et al. Enteroaggregative *Escherichia coli* associated with persistent diarrhea in a cohort of rural children in India. *J Infect Dis* 1989;**159**:1061–4.
25. Bhandari N, Bahl R, Taneja S, et al. Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young north Indian children. *Pediatrics* 2002;**109**:e86.
26. Bielaszewska M, Friedrich AW, Aldick T, et al. Shiga toxin activatable by intestinal mucus in *Escherichia coli* isolated from humans: predictor for a severe clinical outcome. *Clin Infect Dis* 2006;**43**: 1160–7.
27. Bin Saeed AA, El Bushra HE, Al-Hamdan NA. Does treatment of bloody diarrhea due to *Shigella dysenteriae* type 1 with ampicillin precipitate hemolytic uremic syndrome? *Emerg Infect Dis* 1995;**1**: 134–7.
28. Black RE. Persistent diarrhea in children of developing countries. *Pediatr Infect Dis J* 1993;**12**:751–61.
29. Boedeker EC. Vaccines for enterotoxigenic *Escherichia coli:* current status. *Curr Opin Gastroenterol* 2005;**21**:15–19.
30. Bokete TN, O'Callahan CM, Clausen CR, et al. Shiga-like toxin-producing *Escherichia coli* in Seattle children: a prospective study. *Gastroenterology* 1993;**105**:1724–31.
31. Brooks JT, Sowers EG, Wells JG, et al. Non-O157 Shiga toxin-producing *Escherichia coli* infections in the United States, 1983-2002. *J Infect Dis* 2005;**192**:1422–9.
32. Caeiro JP, Estrada-Garcia MT, Jiang ZD, et al. Improved detection of enterotoxigenic *Escherichia coli* among patients with travelers' diarrhea, by use of the polymerase chain reaction technique. *J Infect Dis* 1999;**180**:2053–5.
33. Caeiro JP, Mathewson JJ, Smith MA, et al. Etiology of outpatient pediatric nondysenteric diarrhea: a multicenter study in the United States. *Pediatr Infect Dis J* 1999;**18**:94–7.
34. California Food Emergency Response Team. *Investigation of an* Escherichia coli *O157:H7 outbreak associated with Dole Pre-Packaged Spinach*. Sacramento: California Department of Health Services; 2007.
35. Cane G, Ginouves A, Marchetti S, et al. HIF-1alpha mediates the induction of IL-8 and VEGF expression on infection with Afa/Dr diffusely adhering E. coli and promotes EMT-like behaviour. *Cell Microbiol* 2010;**12**:640–53.
36. Centers for Disease Control and Prevention. Community outbreak of hemolytic uremic syndrome attributable to *Escherichia coli* O111:NM: South Australia 1995. *MMWR Morb Mortal Wkly Rep* 1995;**44**:550–1.557–8.
37. Centers for Disease Control and Prevention. Outbreak of acute gastroenteritis attributable to *Escherichia coli* serotype O104:H21: Helena, Montana, 1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**: 501–3.
38. Centers for Disease Control and Prevention. Vital signs: incidence and trends of infection with pathogens transmitted commonly through food: foodborne diseases active surveillance network, 10 U.S. sites, 1996-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 749–55.
39. Chen HD, Frankel G. Enteropathogenic *Escherichia coli:* unravelling pathogenesis. *FEMS Microbiol Rev* 2005;**29**:83–98.
40. Cimolai N, Carter JE, Morrison BJ, et al. Risk factors for the progression of *Escherichia coli* O157:H7 enteritis to hemolytic-uremic syndrome. *J Pediatr* 1990;**116**:589–92.
41. Cimolai N, Morrison BJ, Carter JE. Risk factors for the central nervous system manifestations of gastroenteritis-associated hemolytic-uremic syndrome. *Pediatrics* 1992;**90**:616–21.
42. Cleary TG, Lopez EL. The shiga-like toxin-producing *Escherichia coli* and hemolytic uremic syndrome. *Pediatr Infect Dis J* 1989;**8**: 720–4.
43. Cobeljic M, Miljkovic-Selimovic B, Paunovic-Todosijevic D, et al. Enteroaggregative *Escherichia coli* associated with an outbreak of diarrhoea in a neonatal nursery ward. *Epidemiol Infect* 1996;**117**: 11–16.

44. Cohen MB, Nataro JP, Bernstein DI, et al. Prevalence of diarrheagenic *Escherichia coli* in acute childhood enteritis: a prospective controlled study. *J Pediatr* 2005;**146**:54–61.
45. Colpoys WE, Cochran BH, Carducci TM, et al. Shiga toxins activate translational regulation pathways in intestinal epithelial cells. *Cell Signal* 2005;**17**:891–9.
46. Cravioto A, Reyes RE, Ortega R, et al. Prospective study of diarrhoeal disease in a cohort of rural Mexican children: incidence and isolated pathogens during the first two years of life. *Epidemiol Infect* 1988;**101**:123–34.
47. Cravioto A, Reyes RE, Trujillo F, et al. Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. *Am J Epidemiol* 1990;**131**: 886–904.
48. Cravioto A, Tello A, Navarro A, et al. Association of Escherichia coli HEp-2 adherence patterns with type and duration of diarrhoea. *Lancet* 1991;**337**:262–4.
49. Cravioto A, Tello A, Villaf NH, et al. Inhibition of localized adhesion of enteropathogenic *Escherichia coli* to HEp-2 cells by immunoglobulin and oligosaccharide fractions of human colostrum and breast milk. *J Infect Dis* 1991;**163**:1247–55.
50. Currie MG, Fok KF, Kato J, et al. Guanylin: an endogenous activator of intestinal guanylate cyclase. *Proc Natl Acad Sci U S A* 1992;**89**:947–51.
51. de la Cabada Bauche J, Dupont HL. New developments in traveler's diarrhea. *Gastroenterol Hepatol (N Y)* 2011;**7**:88–95.
52. Dean-Nystrom EA, Gansheroff LJ, Mills M, et al. Vaccination of pregnant dams with intimin(O157) protects suckling piglets from *Escherichia coli* O157:H7 infection. *Infect Immun* 2002;**70**: 2414–18.
53. Donnenberg MS. Enteropathogenic *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JI, et al., editors. *Infections of the Gastrointestinal Tract*. New York: Raven Press; 1995. p. 709–26.
54. Donnenberg MS, Whittam TS. Pathogenesis and evolution of virulence in enteropathogenic and enterohemorrhagic *Escherichia coli*. *J Clin Invest* 2001;**107**:539–48.
55. Donnenberg MS, Giron JA, Nataro JP, et al. A plasmid-encoded type IV fimbrial gene of enteropathogenic *Escherichia coli* associated with localized adherence. *Mol Microbiol* 1992;**6**:3427–37.
56. Donnenberg MS, Tacket CO, James SP, et al. Role of the *eaeA* gene in experimental enteropathogenic *Escherichia coli* infection. *J Clin Invest* 1993;**92**:1412–17.
57. Dudley EG, Thomson NR, Parkhill J, et al. Proteomic and microarray characterization of the AggR regulon identifies a pheU pathogenicity island in enteroaggregative *Escherichia coli*. *Mol Microbiol* 2006;**61**:1267–82.
58. DuPont HL, Ericsson CD, Farthing MJ, et al. Expert review of the evidence base for self-therapy of travelers' diarrhea. *J Travel Med* 2009;**16**:161–71.
59. DuPont HL, Formal SB, Hornick RB, et al. Pathogenesis of *Escherichia coli* diarrhea. *N Engl J Med* 1971;**285**:1–9.
60. Echeverria P, Sethabutr O, Serichantalergs O, et al. *Shigella* and enteroinvasive *Escherichia coli* infections in households of children with dysentery in Bangkok. *J Infect Dis* 1992;**165**: 144–7.
61. Endo Y, Tsurugi K, Yutsudo T, et al. Site of action of a Vero toxin (VT2) from *Escherichia coli* O157:H7 and of Shiga toxin on eukaryotic ribosomes. RNA N-glycosidase activity of the toxins. *Eur J Biochem* 1988;**171**:45–50.
62. Ericsson CD, DuPont HL, Mathewson JJ, et al. Treatment of traveler's diarrhea with sulfamethoxazole and trimethoprim and loperamide. *JAMA* 1990;**263**:257–61.
63. Eslava C, Navarro-Garcia F, Czeczulin JR, et al. Pet, an autotransporter enterotoxin from enteroaggregative *Escherichia coli*. *Infect Immun* 1998;**66**:3155–63.
64. Essers B, Burnens AP, Lanfranchini FM, et al. Acute community-acquired diarrhea requiring hospital admission in Swiss children. *Clin Infect Dis* 2000;**31**:192–6.
65. Estrada-Garcia T, Lopez-Saucedo C, Thompson-Bonilla R, et al. Association of diarrheagenic *Escherichia coli* pathotypes with infection and diarrhea among Mexican children and association of atypical enteropathogenic *E. coli* with acute diarrhea. *J Clin Microbiol* 2009;**47**:93–8.
66. Faruque AS, Malek MA, Khan AI, et al. Diarrhoea in elderly people: aetiology, and clinical characteristics. *Scand J Infect Dis* 2004;**36**:204–8.
67. Fasano A, Kay BA, Russell RG, et al. Enterotoxin and cytotoxin production by enteroinvasive *Escherichia coli*. *Infect Immun* 1990; **58**:3717–23.
68. Fasano A, Noriega FR, Liao FM, et al. Effect of *Shigella* enterotoxin 1 (ShET1) on rabbit intestine in vitro and in vivo. *Gut* 1997;**40**:505–11.
69. Field M, Graf LH Jr, Laird WJ, et al. Heat-stable enterotoxin of *Escherichia coli:* in vitro effects on guanylate cyclase activity, cyclic GMP concentration, and ion transport in small intestine. *Proc Natl Acad Sci U S A* 1978;**75**:2800–4.
70. Fleckenstein JM, Hardwidge PR, Munson GP, et al. Molecular mechanisms of enterotoxigenic *Escherichia coli* infection. *Microbes Infect* 2010;**12**:89–98.
71. Fraser ME, Fujinaga M, Cherney MM, et al. Structure of shiga toxin type 2 (Stx2) from *Escherichia coli* O157:H7. *J Biol Chem* 2004;**279**:27511–17.
72. Frech SA, Dupont HL, Bourgeois AL, et al. Use of a patch containing heat-labile toxin from *Escherichia coli* against travellers' diarrhoea: a phase II, randomised, double-blind, placebo-controlled field trial. *Lancet* 2008;**371**:2019–25.
73. Friedrich AW, Bielaszewska M, Zhang WL, et al. *Escherichia coli* harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J Infect Dis* 2002;**185**:74–84.
74. Gaastra W, Vennerholm AM. Colonization factors of human enterotoxigenic *Escherichia coli* (ETEC). *Trends Microbiol* 1996;**4**: 444–52.
75. Garg AX, Suri RS, Barrowman N, et al. Long-term renal prognosis of diarrhea-associated hemolytic uremic syndrome: a systematic review, meta-analysis, and meta-regression. *JAMA* 2003; **290**:1360–70.
76. Gill DM, Richardson SH. Adenosine diphosphate-ribosylation of adenylate cyclase catalyzed by heat-labile enterotoxin of *Escherichia coli:* comparison with cholera toxin. *J Infect Dis* 1980;**141**: 64–70.
77. Gill DM, Clements JD, Robertson DC, et al. Subunit number and arrangement in *Escherichia coli* heat-labile enterotoxin. *Infect Immun* 1981;**33**:677–82.
78. Girard MP, Steele D, Chaignat CL, et al. A review of vaccine research and development: human enteric infections. *Vaccine* 2006;**24**:2732–50.
79. Gomes TA, Rassi V, MacDonald KL, et al. Enteropathogens associated with acute diarrheal disease in urban infants in Sao Paulo, Brazil. *J Infect Dis* 1991;**164**:331–7.
80. Gordillo ME, Reeve GR, Pappas J, et al. Molecular characterization of strains of enteroinvasive *Escherichia coli* O143, including isolates from a large outbreak in Houston, Texas. *J Clin Microbiol* 1992;**30**:889–93.
81. Griffin PM. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*. In: Blaser MJ, Smith PD, Ravdin JI, et al., editors. *Infections of the Gastrointestinal Tract*. New York: Raven Press; 1995. p. 739–61.
82. Griffin PM, Tauxe RV. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol Rev* 1991;**13**: 60–98.
83. Guandalini S, Rao MC, Smith PL, et al. cGMP modulation of ileal ion transport: in vitro effects of *Escherichia coli* heat-stable enterotoxin. *Am J Physiol* 1982;**243**:G36–41.
84. Guerrant RL, Van Gilder T, Steiner TS, et al. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis* 2001; **32**:331–51.
85. Guignot J, Chaplais C, Coconnier-Polter MH, et al. The secreted autotransporter toxin, Sat, functions as a virulence factor in Afa/Dr diffusely adhering *Escherichia coli* by promoting lesions in tight junction of polarized epithelial cells. *Cell Microbiol* 2007; **9**:204–21.
86. Gyles CL, Barnum DA. A heat-labile enterotoxin from strains of *Escherichia coli* enteropathogenic for pigs. *J Infect Dis* 1969;**120**: 419–26.
87. Hadler JL, Clogher P, Hurd S, et al. Ten-year trends and risk factors for non-O157 Shiga toxin-producing *Escherichia coli* found through Shiga toxin testing, Connecticut, 2000-2009. *Clin Infect Dis* 2011;**53**:269–76.
88. Harada T, Hiroi M, Kawamori F, et al. A food poisoning diarrhea outbreak caused by enteroaggregative *Escherichia coli* serogroup O126:H27 in Shizuoka, Japan. *Jpn J Infect Dis* 2007;**60**:154–5.

89. Harrington SM, Dudley EG, Nataro JP. Pathogenesis of enteroaggregative *Escherichia coli* infection. *FEMS Microbiol Lett* 2006; **254**:12–18.
90. Hayashi T, Makino K, Ohnishi M, et al. Complete genome sequence of enterohemorrhagic *Escherichia coli* O157:H7 and genomic comparison with a laboratory strain K-12. *DNA Res* 2001;**8**:11–22.
91. Hedican EB, Medus C, Besser JM, et al. Characteristics of O157 versus non-O157 Shiga toxin-producing *Escherichia coli* infections in Minnesota, 2000-2006. *Clin Infect Dis* 2009;**49**:358–64.
92. Henderson IR, Hicks S, Navarro-Garcia F, et al. Involvement of the enteroaggregative *Escherichia coli* plasmid-encoded toxin in causing human intestinal damage. *Infect Immun* 1999;**67**:5338–44.
93. Hicks S, Candy DC, Phillips AD. Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa in vitro. *Infect Immun* 1996;**64**:4751–60.
94. Hien BT, Scheutz F, Cam PD, et al. Diarrheagenic *Escherichia coli* and Shigella strains isolated from children in a hospital case-control study in Hanoi, Vietnam. *J Clin Microbiol* 2008;**46**:996–1004.
95. Hofmann SL. Southwestern Internal Medicine Conference: Shiga-like toxins in hemolytic-uremic syndrome and thrombotic thrombocytopenic purpura. *Am J Med Sci* 1993;**306**:398–406.
96. Hromockyj AE, Maurelli AT. Identification of an *Escherichia coli* gene homologous to virR, a regulator of *Shigella* virulence. *J Bacteriol* 1989;**171**:2879–81.
97. Huang DB, Nataro JP, DuPont HL, et al. Enteroaggregative *Escherichia coli* is a cause of acute diarrheal illness: a meta-analysis. *Clin Infect Dis* 2006;**43**:556–63.
98. Hunt CM, Harvey JA, Youngs ER, et al. Clinical and pathological variability of infection by enterohaemorrhagic (Vero cytotoxin producing) *Escherichia coli*. *J Clin Pathol* 1989;**42**:847–52.
99. Ikeda K, Ida O, Kimoto K, et al. Effect of early fosfomycin treatment on prevention of hemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. *Clin Nephrol* 1999;**52**:357–62.
100. Itoh Y, Nagano I, Kunishima M, et al. Laboratory investigation of enteroaggregative *Escherichia coli* O untypeable:H10 associated with a massive outbreak of gastrointestinal illness. *J Clin Microbiol* 1997;**35**:2546–50.
101. Jain S, Chen L, Dechet A, et al. An outbreak of enterotoxigenic *Escherichia coli* associated with sushi restaurants in Nevada, 2004. *Clin Infect Dis* 2008;**47**:1–7.
102. Jelinek T, Kollaritsch H. Vaccination with Dukoral against travelers' diarrhea (ETEC) and cholera. *Expert Rev Vaccines* 2008;**7**: 561–7.
103. Jerse AE, Martin WC, Galen JE, et al. Oligonucleotide probe for detection of the enteropathogenic *Escherichia coli* (EPEC) adherence factor of localized adherent EPEC. *J Clin Microbiol* 1990; **28**:2842–4.
104. Jiang ZD, Lowe B, Verenkar MP, et al. Prevalence of enteric pathogens among international travelers with diarrhea acquired in Kenya (Mombasa), India (Goa), or Jamaica (Montego Bay). *J Infect Dis* 2002;**185**:497–502.
105. Johnson AM, Kaushik RS, Francis DH, et al. Heat-labile enterotoxin promotes *Escherichia coli* adherence to intestinal epithelial cells. *J Bacteriol* 2009;**191**:178–86.
106. Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. *Clin Infect Dis* 2006;**43**:1587–95.
107. Kansau I, Berger C, Hospital M, et al. Zipper-like internalization of Dr-positive *Escherichia coli* by epithelial cells is preceded by an adhesin-induced mobilization of raft-associated molecules in the initial step of adhesion. *Infect Immun* 2004;**72**:3733–42.
108. Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2004;**2**:123–40.
109. Kappeli U, Hachler H, Giezendanner N, et al. Human infections with non-O157 Shiga toxin-producing *Escherichia coli*, Switzerland, 2000-2009. *Emerg Infect Dis* 2011;**17**:180–5.
110. Karch H, Strockbine N, O'Brien AD. Growth of *Escherichia coli* in the presence of trimethoprim-sulfamethoxazole facilitates detection of Shiga-like toxin producing strains by colony blot assay. *FEMS Microbiology Letters* 1986;**35**:141–5.
111. Karmali MA, Petric M, Lim C, et al. The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J Infect Dis* 1985;**151**:775–82.
112. Kavi J, Wise R. Causes of the haemolytic uraemic syndrome. *BMJ* 1989;**298**:65–6.
113. Kehl SC. Role of the laboratory in the diagnosis of enterohemorrhagic *Escherichia coli* infections. *J Clin Microbiol* 2002;**40**: 2711–15.
114. Khaitan A, Jandhyala DM, Thorpe CM, et al. The operon encoding SubAB, a novel cytotoxin, is present in shiga toxin-producing *Escherichia coli* isolates from the United States. *J Clin Microbiol* 2007;**45**:1374–5.
115. King CK, Glass R, Bresee JS, et al. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* 2003;**52**:1–16.
116. Knutton S, Baldwin T, Williams PH, et al. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. *Infect Immun* 1989;**57**:1290–8.
117. Knutton S, Shaw RK, Anantha RP, et al. The type IV bundle-forming pilus of enteropathogenic *Escherichia coli* undergoes dramatic alterations in structure associated with bacterial adherence, aggregation and dispersal. *Mol Microbiol* 1999;**33**:499–509.
118. Konowalchuk J, Speirs JI, Stavric S. Vero response to a cytotoxin of *Escherichia coli*. *Infect Immun* 1977;**18**:775–9.
119. Lapeyraque AL, Malina M, Fremeaux-Bacchi V, et al. Eculizumab in severe Shiga-toxin-associated HUS. *N Engl J Med* 2011;**364**: 2561–3.
120. LeBlanc JJ. Implication of virulence factors in *Escherichia coli* O157:H7 pathogenesis. *Crit Rev Microbiol* 2003;**29**:277–96.
121. Le Bouguenec C, Servin AL. Diffusely adherent *Escherichia coli* strains expressing Afa/Dr adhesins (Afa/Dr DAEC): hitherto unrecognized pathogens. *FEMS Microbiol Lett* 2006;**256**:185–94.
122. Lencer WI, Hirst TR, Holmes RK. Membrane traffic and the cellular uptake of cholera toxin. *Biochim Biophys Acta* 1999;**1450**: 177–90.
123. Levine MM. *Escherichia coli* infections. *N Engl J Med* 1985;**313**: 445–7.
124. Levine MM, Edelman R. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. *Epidemiol Rev* 1984;**6**:31–51.
125. Levine MM, Xu JG, Kaper JB, et al. A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *J Infect Dis* 1987;**156**:175–82.
126. Lievin-Le Moal V, Comenge Y, Ruby V, et al. Secreted autotransporter toxin (Sat) triggers autophagy in epithelial cells that relies on cell detachment. *Cell Microbiol* 2011;**13**:992–1013.
127. Liu J, Gratz J, Maro A, et al. Simultaneous detection of six diarrhea-causing bacterial pathogens with an in-house PCR-Luminex assay. *J Clin Microbiol* 2011;**50**:98–103.
128. Liu M, Ruan X, Zhang C, et al. Heat-labile- and heat-stable-toxoid fusions (LTRG-STaPF) of human enterotoxigenic *Escherichia coli* elicit neutralizing antitoxin antibodies. *Infect Immun* 2011;**79**:4002–9.
129. Lopez EL, Diaz M, Grinstein S, et al. Hemolytic uremic syndrome and diarrhea in Argentine children: the role of Shiga-like toxins. *J Infect Dis* 1989;**160**:469–75.
130. Lothigius A, Janzon A, Begum Y, et al. Enterotoxigenic *Escherichia coli* is detectable in water samples from an endemic area by real-time PCR. *J Appl Microbiol* 2008;**104**:1128–36.
131. Mag T, Nogrady N, Herpay M, et al. Characterisation of verotoxin-producing *Escherichia coli* strains isolated from human patients in Hungary over a 7-year period. *Eur J Clin Microbiol Infect Dis* 2010;**29**:249–52.
132. Marier R, Wells J, Swanson RC, et al. An outbreak of enteropathogenic *Escherichia coli* foodborne disease traced to imported French cheese. *Lancet* 1973;**302**:1376–8.
133. Martin DL, MacDonald KL, White KE, et al. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. *N Engl J Med* 1990;**323**:1161–7.
134. McGannon CM, Fuller CA, Weiss AA. Different classes of antibiotics differentially influence shiga toxin production. *Antimicrob Agents Chemother* 2010;**54**:3790–8.
135. McKenzie R, Bourgeois AL, Frech SA, et al. Transcutaneous immunization with the heat-labile toxin (LT) of enterotoxigenic *Escherichia coli* (ETEC): protective efficacy in a double-blind, placebo-controlled challenge study. *Vaccine* 2007;**25**:3684–91.

136. McKenzie R, Darsley M, Thomas N, et al. A double-blind, placebo-controlled trial to evaluate the efficacy of PTL-003, an attenuated enterotoxigenic *E. coli* (ETEC) vaccine strain, in protecting against challenge with virulent ETEC. *Vaccine* 2008;**26**: 4731–9.
137. Mehlman IJ, Fishbein M, Gorbach SL, et al. Pathogenicity of *Escherichia coli* recovered from food. *J Assoc Off Anal Chem* 1976; **59**:67–80.
138. Melton-Celsa A, Mohawk K, Teel L, et al. Pathogenesis of Shiga-toxin producing *Escherichia coli*. *Curr Top Microbiol Immunol* 2012;**357**:67–103.
139. Mendez Arancibia E, Pitart C, Ruiz J, et al. Evolution of antimicrobial resistance in enteroaggregative *Escherichia coli* and enterotoxigenic *Escherichia coli* causing traveller's diarrhoea. *J Antimicrob Chemother* 2009;**64**:343–7.
140. Meraz IM, Jiang ZD, Ericsson CD, et al. Enterotoxigenic *Escherichia coli* and diffusely adherent *E. coli* as likely causes of a proportion of pathogen-negative travelers' diarrhea: a PCR-based study. *J Travel Med* 2008;**15**:412–18.
141. Merritt EA, Pronk SE, Sixma TK, et al. Structure of partially-activated heat-labile enterotoxin (LT) at 2.6 A resolution. *FEBS Lett* 1994;**337**:88–92.
142. Merson MH, Morris GK, Sack DA, et al. Travelers' diarrhea in Mexico: a prospective study of physicians and family members attending a congress. *N Engl J Med* 1976;**294**:1299–305.
143. Mezoff AG, Giannella RA, Eade MN, et al. *Escherichia coli* enterotoxin (STà) binds to receptors, stimulates guanyl cyclase, and impairs absorption in rat colon. *Gastroenterology* 1992;**102**:816–22.
144. Moake JL. Haemolytic-uraemic syndrome: basic science. *Lancet* 1994;**343**:393–7.
145. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012;**54**: 185–92.
146. Moyo SJ, Maselle SY, Matee MI, et al. Identification of diarrheagenic *Escherichia coli* isolated from infants and children in Dar es Salaam, Tanzania. *BMC Infectious Diseases* 2007;**7**:92.
147. Nada RA, Shaheen HI, Khalil SB, et al. Discovery and phylogenetic analysis of novel members of class b enterotoxigenic *Escherichia coli* adhesive fimbriae. *J Clin Microbiol* 2011;**49**:1403–10.
148. Nalin DR, McLaughlin JC, Rahaman M, et al. Enteropathogenic *Escherichia coli* and idiopathic diarrhoea in Bangladesh. *Lancet* 1975;**2**:1116–19.
149. Nataro JP. Atypical enteropathogenic *Escherichia coli:* typical pathogens? *Emerg Infect Dis* 2006;**12**:696.
150. Nataro JP, Kaper JB. Diarrheagenic *Escherichia coli*. *Clin Microbiol Rev* 1998;**11**:142–201.
151. Nataro JP, Bopp CA, Fields PI, et al. *Escherichia*, *Shigella*, and *Salmonella*. In: Murray PR, Baron EJ, Jorgenson JH, et al., editors. *Manual of Clinical Microbiology*. 9th ed. Washington, DC: American Society for Microbiology; 2007. p. 670–87.
152. Nataro JP, Deng Y, Cookson S, et al. Heterogeneity of enteroaggregative *Escherichia coli* virulence demonstrated in volunteers. *J Infect Dis* 1995;**171**:465–8.
153. Nataro JP, Kaper JB, Robins-Browne R, et al. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. *Pediatr Infect Dis J* 1987;**6**:829–31.
154. Nataro JP, Mai V, Johnson J, et al. Diarrheagenic *Escherichia coli* infection in Baltimore, Maryland, and New Haven, Connecticut. *Clin Infect Dis* 2006;**43**:402–7.
155. Nataro JP, Seriwatana J, Fasano A, et al. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. *Infect Immun* 1995;**63**: 4721–8.
156. Nelson JD. Duration of neomycin for enteropathogenic *Escherichia coli* diarrheal disease: a comparative study of 113 cases. *Pediatrics* 1971;**48**:248–58.
157. Neter E, Korns RF, Trussel RE. Association of *Escherichia coli* serogroup 0111 with two hospital outbreaks of epidemic diarrhea of the newborn infant in New York State during 1947. *Pediatrics* 1953;**12**:377–83.
158. Nguyen TV, Le Van P, Le Huy C, et al. Detection and characterization of diarrheagenic *Escherichia coli* from young children in Hanoi, Vietnam. *J Clin Microbiol* 2005;**43**:755–60.
159. Nicklasson M, Klena J, Rodas C, et al. Enterotoxigenic *Escherichia coli* multilocus sequence types in Guatemala and Mexico. *Emerg Infect Dis* 2010;**16**:143–6.
160. Nunes MR, Penna FJ, Franco RT, et al. Enterotoxigenic *Escherichia coli* in children with acute diarrhoea and controls in Teresina/PI, Brazil: distribution of enterotoxin and colonization factor genes. *J Appl Microbiol* 2011;**111**:224–32.
161. Oberhelman RA, Laborde D, Mera R, et al. Colonization with enteroadherent, enterotoxigenic and enterohemorrhagic *Escherichia coli* among day-care center attendees in New Orleans, Louisiana. *Pediatr Infect Dis J* 1998;**17**:1159–62.
162. Obrig TG, Del Vecchio PJ, Brown JE, et al. Direct cytotoxic action of Shiga toxin on human vascular endothelial cells. *Infect Immun* 1988;**56**:2373–8.
163. Ochoa TJ, Contreras CA. Enteropathogenic *Escherichia coli* infection in children. *Curr Opin Infect Dis* 2011;**24**:478–83.
164. Ochoa TJ, Barletta F, Contreras C, et al. New insights into the epidemiology of enteropathogenic *Escherichia coli* infection. *Trans R Soc Trop Med Hyg* 2008;**102**:852–6.
165. Ogawa H, Nakamura A, Sakazaki R. Pathogenic properties of "enteropathogenic" *Escherichia coli* from diarrheal children and adults. *Jpn J Med Sci Biol* 1968;**21**:333–49.
166. Okeke IN. Diarrheagenic *Escherichia coli* in sub-Saharan Africa: status, uncertainties and necessities. *J Infect Dev Ctries* 2009;**3**: 817–42.
167. Olesen B, Neimann J, Bottiger B, et al. Etiology of diarrhea in young children in Denmark: a case-control study. *J Clin Microbiol* 2005;**43**:3636–41.
168. Opintan JA, Bishar RA, Newman MJ, et al. Carriage of diarrhoeagenic *Escherichia coli* by older children and adults in Accra, Ghana. *Trans R Soc Trop Med Hyg* 2010;**104**:504–6.
169. Orth D, Khan AB, Naim A, et al. Shiga toxin activates complement and binds factor H: evidence for an active role of complement in hemolytic uremic syndrome. *J Immunol* 2009;**182**:6394–400.
170. Orth-Holler D, Riedl M, Wurzner R. Inhibition of terminal complement activation in severe Shiga toxin-associated HUS: perfect example for a fast track from bench to bedside. *EMBO Mol Med* 2011;**3**:617–19.
171. Ostroff SM, Kobayashi JM, Lewis JH. Infections with Escherichia coli O157:H7 in Washington State: the first year of statewide disease surveillance. *JAMA* 1989;**262**:355–9.
172. Ostroff SM, Tarr PI, Neill MA, et al. Toxin genotypes and plasmid profiles as determinants of systemic sequelae in *Escherichia coli* O157:H7 infections. *J Infect Dis* 1989;**160**:994–8.
173. Pai CH, Gordon R, Sims HV, et al. Sporadic cases of hemorrhagic colitis associated with *Escherichia coli* O157:H7: clinical, epidemiologic, and bacteriologic features. *Ann Intern Med* 1984;**101**: 738–42.
174. Pal T, Formal SB, Hale TL. Characterization of virulence marker antigen of *Shigella* spp. and enteroinvasive *Escherichia coli*. *J Clin Microbiol* 1989;**27**:561–3.
175. Paton AW, Beddoe T, Thorpe CM, et al. AB5 subtilase cytotoxin inactivates the endoplasmic reticulum chaperone BiP. *Nature* 2006;**443**:548–52.
176. Paton AW, Srimanote P, Talbot UM, et al. A new family of potent AB(5) cytotoxins produced by Shiga toxigenic *Escherichia coli*. *J Exp Med* 2004;**200**:35–46.
177. Pavlovic M, Luze A, Konrad R, et al. Development of a duplex real-time PCR for differentiation between *E. coli* and *Shigella* spp. *J Appl Microbiol* 2011;**110**:1245–51.
178. Persson S, Olsen KE, Scheutz F, et al. A method for fast and simple detection of major diarrhoeagenic *Escherichia coli* in the routine diagnostic laboratory. *Clin Microbiol Infect* 2007;**13**: 516–24.
179. Potter AA, Klashinsky S, Li Y, et al. Decreased shedding of *Escherichia coli* O157:H7 by cattle following vaccination with type III secreted proteins. *Vaccine* 2004;**22**:362–9.
180. Qadri F, Das SK, Faruque AS, et al. Prevalence of toxin types and colonization factors in enterotoxigenic *Escherichia coli* isolated during a 2-year period from diarrheal patients in Bangladesh. *J Clin Microbiol* 2000;**38**:27–31.
181. Qadri F, Svennerholm AM, Faruque AS, et al. Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. *Clin Microbiol Rev* 2005;**18**:465–83.

182. Rajendran P, Ajjampur SS, Chidambaram D, et al. Pathotypes of diarrheagenic *Escherichia coli* in children attending a tertiary care hospital in South India. *Diagn Microbiol Infect Dis* 2010;**68**: 117–22.
183. Rangel JM, Sparling PH, Crowe C, et al. Epidemiology of *Escherichia coli* O157 : H7 outbreaks, United States, 1982-2002. *Emerg Infect Dis* 2005;**11**:603–9.
184. Rao MR, Abu-Elyazeed R, Savarino SJ, et al. High disease burden of diarrhea due to enterotoxigenic *Escherichia coli* among rural Egyptian infants and young children. *J Clin Microbiol* 2003;**41**: 4862–4.
185. Rao MR, Wierzba TF, Savarino SJ, et al. Serologic correlates of protection against enterotoxigenic *Escherichia coli* diarrhea. *J Infect Dis* 2005;**191**:562–70.
186. Rasheed JK, Guzman-Verduzco LM, Kupersztoch YM. Two precursors of the heat-stable enterotoxin of *Escherichia coli:* evidence of extracellular processing. *Mol Microbiol* 1990;**4**:265–73.
187. Rasko DA, Webster DR, Sahl JW, et al. Origins of the *E. coli* strain causing an outbreak of hemolytic-uremic syndrome in Germany. *N Engl J Med* 2011;**365**:709–17.
188. Reyes D, Vilchez S, Paniagua M, et al. Diversity of intestinal *Escherichia coli* populations in Nicaraguan children with and without diarrhoea. *J Med Microbiol* 2009;**58**:1593–600.
189. Riley LW. The epidemiologic, clinical, and microbiologic features of hemorrhagic colitis. *Annu Rev Microbiol* 1987;**41**:383–407.
190. Riley LW, Remis RS, Helgerson SD, et al. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N Engl J Med* 1983;**308**:681–5.
191. Robins-Browne RM, Still CS, Miliotis MD, et al. Summer diarrhoea in African infants and children. *Arch Dis Child* 1980;**55**: 923–8.
192. Robson WL, Fick GH, Jadavji T, et al. The use of intravenous gammaglobulin in the treatment of typical hemolytic uremic syndrome. *Pediatr Nephrol* 1991;**5**:289–92.
193. Rodas C, Iniguez V, Qadri F, et al. Development of multiplex PCR assays for detection of enterotoxigenic *Escherichia coli* colonization factors and toxins. *J Clin Microbiol* 2009;**47**:1218–20.
194. Rogers MF, Rutherford GW, Alexander SR, et al. A population-based study of hemolytic-uremic syndrome in Oregon, 1979-1982. *Am J Epidemiol* 1986;**123**:137–42.
195. Rosales A, Hofer J, Zimmerhackl LB, et al. Need for long-term follow-up in enterohemorrhagic *Escherichia coli*-associated hemolytic uremic syndrome due to late-emerging sequelae. *Clin Infect Dis* 2012;**54**:1413–21.
196. Rosenberg ML, Koplan JP, Wachsmuth IK, et al. Epidemic diarrhea at Crater Lake from enterotoxigenic *Escherichia coli:* a large waterborne outbreak. *Ann Intern Med* 1977;**86**:714–18.
197. Rothbaum R, McAdams AJ, Giannella R, et al. A clinicopathological study of enterocyte-adherent *Escherichia coli:* a cause of protracted diarrhea in infants. *Gastroenterol* 1982;**83**:441–54.
198. Roy K, Hamilton D, Allen KP, et al. The EtpA exoprotein of enterotoxigenic *Escherichia coli* promotes intestinal colonization and is a protective antigen in an experimental model of murine infection. *Infect Immun* 2008;**76**:2106–12.
199. Roy K, Hilliard GM, Hamilton DJ, et al. Enterotoxigenic *Escherichia coli* EtpA mediates adhesion between flagella and host cells. *Nature* 2009;**457**:594–8.
200. Ruttler ME, Yanzon CS, Cuitino MJ, et al. Evaluation of a multiplex PCR method to detect enteroaggregative *Escherichia coli*. *Biocell* 2006;**30**:301–8.
201. Sabui S, Ghosal A, Dutta S, et al. Allelic variation in colonization factor CS6 of enterotoxigenic *Escherichia coli* isolated from patients with acute diarrhoea and controls. *J Med Microbiol* 2010;**59**: 770–9.
202. Sack DA, Shimko J, Torres O, et al. Randomised, double-blind, safety and efficacy of a killed oral vaccine for enterotoxigenic *E. coli* diarrhoea of travellers to Guatemala and Mexico. *Vaccine* 2007;**25**:4392–400.
203. Sack RB, Sack DA, Mehlman IJ, et al. Enterotoxigenic *Escherichia coli* isolated from food. *J Infect Dis* 1977;**135**:313–17.
204. Safdar N, Said A, Gangnon RE, et al. Risk of hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 enteritis: a meta-analysis. *JAMA* 2002;**288**:996–1001.
205. Sakazaki R, Tamura K, Saito M. Enteropathogenic *Escherichia coli* associated with diarrhea in children and adults. *Jpn J Med Sci Biol* 1967;**20**:387–99.
206. Salmanzadeh-Ahrabi S, Habibi E, Jaafari F, et al. Molecular epidemiology of *Escherichia coli* diarrhoea in children in Tehran. *Ann Trop Paediatr* 2005;**25**:35–9.
207. Samie A, Obi CL, Dillingham R, et al. Enteroaggregative *Escherichia coli* in Venda, South Africa: distribution of virulence-related genes by multiplex polymerase chain reaction in stool samples of human immunodeficiency virus (HIV)-positive and HIV-negative individuals and primary school children. *Am J Trop Med Hyg* 2007;**77**:142–50.
208. Sansonetti PJ. Genetic and molecular basis of epithelial cell invasion by *Shigella* species. *Rev Infect Dis* 1991;**13**(Suppl. 4):S285–292.
209. Sarantuya J, Nishi J, Wakimoto N, et al. Typical enteroaggregative *Escherichia coli* is the most prevalent pathotype among *E. coli* strains causing diarrhea in Mongolian children. *J Clin Microbiol* 2004;**42**:133–9.
210. Savarino SJ, Fasano A, Robertson DC, et al. Enteroaggregative *Escherichia coli* elaborate a heat-stable enterotoxin demonstrable in an in vitro rabbit intestinal model. *J Clin Invest* 1991;**87**:1450–5.
211. Scaletsky IC, Fabbricotti SH, Carvalho RL, et al. Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: a case-control study. *J Clin Microbiol* 2002; **40**:645–8.
212. Schultsz C, Ende JVD, Cobelens F, et al. Diarrheagenic Escherichia coli and acute and persistent diarrhea in returned travelers. *J Clin Microbiol* 2000;**38**:3550–4.
213. Schulz S, Green CK, Yuen PS, et al. Guanylyl cyclase is a heat-stable enterotoxin receptor. *Cell* 1990;**63**:941–8.
214. Sears CL, Kaper JB. Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. *Microbiological Reviews* 1996;**60**:167–215.
215. Servin AL. Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clin Microbiol Rev* 2005;**18**:264–92.
216. Seto EY, Soller JA, Colford JM Jr. Strategies to reduce person-to-person transmission during widespread *Escherichia coli* O157:H7 outbreak. *Emerg Infect Dis* 2007;**13**:860–6.
217. Shah N, DuPont HL, Ramsey DJ. Global etiology of travelers' diarrhea: systematic review from 1973 to the present. *Am J Trop Med Hyg* 2009;**80**:609–14.
218. Shaheen HI, Abdel Messih IA, Klena JD, et al. Phenotypic and genotypic analysis of enterotoxigenic *Escherichia coli* in samples obtained from Egyptian children presenting to referral hospitals. *J Clin Microbiol* 2009;**47**:189–97.
219. Shaheen HI, Khalil SB, Rao MR, et al. Phenotypic profiles of enterotoxigenic *Escherichia coli* associated with early childhood diarrhea in rural Egypt. *J Clin Microbiol* 2004;**42**:5588–95.
220. Shazberg G, Wolk M, Schmidt H, et al. Enteroaggregative *Escherichia coli* serotype O126:H27, Israel. *Emerg Infect Dis* 2003; **9**:1170–3.
221. Shigeno T, Akamatsu T, Fujimori K, et al. The clinical significance of colonoscopy in hemorrhagic colitis due to enterohemorrhagic *Escherichia coli* O157:H7 infection. *Endoscopy* 2002;**34**:311–14.
222. Singh E, Redfield D. Prophylaxis for travelers' diarrhea. *Curr Gastroenterol Rep* 2009;**11**:297–300.
223. Sixma TK, Pronk SE, Kalk KH, et al. Crystal structure of a cholera toxin-related heat-labile enterotoxin from *E. coli*. *Nature* 1991;**351**:371–7.
224. Smith HW, Linggood MA. Observations on the pathogenic properties of the K88, Hly and Ent plasmids of *Escherichia coli* with particular reference to porcine diarrhoea. *J Med Microbiol* 1971; **4**:467–85.
225. Snedeker KG, Campbell M, Sargeant JM. A systematic review of vaccinations to reduce the shedding of *Escherichia coli* O157 in the faeces of domestic ruminants. *Zoonoses Public Health* 2012; **59**:126–38.
226. Snelling AM, Macfarlane-Smith LR, Fletcher JN, et al. The commonly-used DNA probe for diffusely-adherent *Escherichia coli* cross-reacts with a subset of enteroaggregative *E. coli*. *BMC Microbiol* 2009;**9**:269.
227. Snyder JD, Wells JG, Yashuk J, et al. Outbreak of invasive *Escherichia coli* gastroenteritis on a cruise ship. *Am J Trop Med Hyg* 1984;**33**:281–4.
228. So M, McCarthy BJ. Nucleotide sequence of the bacterial transposon Tn1681 encoding a heat-stable (ST) toxin and its identification in enterotoxigenic *Escherichia coli* strains. *Proc Natl Acad Sci U S A* 1980;**77**:4011–15.

229. Sobieszczanska BM, Osek J. Enteroaggregative and cell-detaching *Escherichia coli* strains among Polish children with and without diarrhea. *Folia Microbiol (Praha)* 2003;**48**:823–8.
230. Sobieszczanska BM, Osek J, Gryko R, et al. Association between cell-bound haemolysin and cell-detaching activity of *Escherichia coli* isolated from children. *J Med Microbiol* 2006;**55**:325–30.
231. Sobieszczanska BM, Osek J, Wasko-Czopnik D, et al. Association of enteroaggregative *Escherichia coli* with irritable bowel syndrome. *Clin Microbiol Infect* 2007;**13**:404–7.
232. Spano LC, Sadovsky AD, Segui PN, et al. Age-specific prevalence of diffusely adherent *Escherichia coli* in Brazilian children with acute diarrhoea. *J Med Microbiol* 2008;**57**:359–63.
233. Steinsland H, Valentiner-Branth P, Gjessing HK, et al. Protection from natural infections with enterotoxigenic *Escherichia coli:* longitudinal study. *Lancet* 2003;**362**:286–91.
234. Strand TA, Chandyo RK, Bahl R, et al. Effectiveness and efficacy of zinc for the treatment of acute diarrhea in young children. *Pediatrics* 2002;**109**:898–903.
235. Streatfield SJ, Sandkvist M, Sixma TK, et al. Intermolecular interactions between the A and B subunits of heat-labile enterotoxin from *Escherichia coli* promote holotoxin assembly and stability in vivo. *Proc Natl Acad Sci U S A* 1992;**89**:12140–4.
236. Svennerholm AM, Tobias J. Vaccines against enterotoxigenic *Escherichia coli*. *Expert Rev Vaccines* 2008;**7**:795–804.
237. Tacket CO. Plant-based vaccines against diarrheal diseases. *Trans Am Clin Climatol Assoc* 2007;**118**:79–87.
238. Tacket CO. Plant-based oral vaccines: results of human trials. *Curr Top Microbiol Immunol* 2009;**332**:103–17.
239. Tacket CO, Moseley SL, Kay B, et al. Challenge studies in volunteers using *Escherichia coli* strains with diffuse adherence to HEp-2 cells. *J Infect Dis* 1990;**162**:550–2.
240. Tacket CO, Reid RH, Boedeker EC, et al. Enteral immunization and challenge of volunteers given enterotoxigenic *E. coli* CFA/II encapsulated in biodegradable microspheres. *Vaccine* 1994;**12**:1270–4.
241. Taddei CR, Fasano A, Ferreira AJ, et al. Secreted autotransporter toxin produced by a diffusely adhering *Escherichia coli* strain causes intestinal damage in animal model assays. *FEMS Microbiol Lett* 2005;**250**:263–9.
242. Tarr PI. Shiga toxin-associated hemolytic uremic syndrome and thrombotic thrombocytopenic purpura: distinct mechanisms of pathogenesis. *Kidney Int Suppl* 2009;**112**:S29–32.
243. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 2005;**365**:1073–86.
244. Tarr PI, Neill MA, Clausen CR, et al. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. *J Infect Dis* 1990;**162**:553–6.
245. Taylor DN, Sanchez JL, Candler W, et al. Treatment of travelers' diarrhea: ciprofloxacin plus loperamide compared with ciprofloxacin alone: a placebo-controlled, randomized trial. *Ann Intern Med* 1991;**114**:731–4.
246. Thoren A, Stintzing G, Tufvesson B, et al. Aetiology and clinical features of severe infantile diarrhoea in Addis Ababa, Ethiopia. *J Trop Pediatr* 1982;**28**:127–31.
247. Toledo MR, Alvariza Mdo C, Murahovschi J, et al. Enteropathogenic *Escherichia coli* serotypes and endemic diarrhea in infants. *Infect Immun* 1983;**39**:586–9.
248. Trabulsi LR, Fernandes MR, Zuliani ME. Novas bacterias patogenicas para o intestino do homen. *Rev Inst Med Trop Sao Paulo* 1967;**9**:31–9.
249. Trachtman H, Christen E. Pathogenesis, treatment, and therapeutic trials in hemolytic uremic syndrome. *Curr Opin Pediatr* 1999;**11**:162–8.
250. Trachtman H, Cnaan A, Christen E, et al. Effect of an oral Shiga toxin-binding agent on diarrhea-associated hemolytic uremic syndrome in children: a randomized controlled trial. *JAMA* 2003;**290**:1337–44.
251. Tulloch EF Jr, Ryan KJ, Formal SB, et al. Invasive enteropathic *Escherichia coli* dysentery: an outbreak in 28 adults. *Ann Intern Med* 1973;**79**:13–17.
252. Tzipori S, Sheoran A, Akiyoshi D, et al. Antibody therapy in the management of shiga toxin-induced hemolytic uremic syndrome. *Clin Microbiol Rev* 2004;**17**:926–41.
253. Usein CR, Tatu-Chitoiu D, Ciontea S, et al. Escherichia coli pathotypes associated with diarrhea in Romanian children younger than 5 years of age. *Jpn J Infect Dis* 2009;**62**:289–93.
254. Vidal RM, Valenzuela P, Baker K, et al. Characterization of the most prevalent colonization factor antigens present in Chilean clinical enterotoxigenic *Escherichia coli* strains using a new multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2009;**65**:217–23.
255. Vila J, Vargas M, Casals C, et al. Antimicrobial resistance of diarrheagenic *Escherichia coli* isolated from children under the age of 5 years from Ifakara, Tanzania. *Antimicrobial Agents & Chemotherapy* 1999;**43**:3022–4.
256. von Baeyer H. Plasmapheresis in thrombotic microangiopathy-associated syndromes: review of outcome data derived from clinical trials and open studies. *Ther Apher* 2002;**6**:320–8.
257. Wagner PL, Acheson DW, Waldor MK. Human neutrophils and their products induce Shiga toxin production by enterohemorrhagic *Escherichia coli*. *Infect Immun* 2001;**69**:1934–7.
258. Weflen AW, Alto NM, Hecht GA. Tight junctions and enteropathogenic *E. coli*. *Ann N Y Acad Sci* 2009;**1165**:169–74.
259. Wickham ME, Lupp C, Mascarenhas M, et al. Bacterial genetic determinants of non-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. *J Infect Dis* 2006;**194**:819–27.
260. Wolf MK. Occurrence, distribution, and associations of O and H serogroups, colonization factor antigens, and toxins of enterotoxigenic *Escherichia coli*. *Clin Microbiol Rev* 1997;**10**:569–84.
261. Wong CS, Jelacic S, Habeeb RL, et al. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000;**342**:1930–6.
262. Wong CS, Mooney JC, Brandt JR, et al. Risk factors for the hemolytic uremic syndrome in children infected with *Escherichia coli* O157:H7: a multivariable analysis. *Clin Infect Dis* 2012;**55**:33–41.
263. Yatsuyanagi J, Saito S, Miyajima Y, et al. Characterization of atypical enteropathogenic *Escherichia coli* strains harboring the *astA* gene that were associated with a waterborne outbreak of diarrhea in Japan. *J Clin Microbiol* 2003;**41**:2033–9.
264. Yatsuyanagi J, Saito S, Sato H, et al. Characterization of enteropathogenic and enteroaggregative *Escherichia coli* isolated from diarrheal outbreaks. *J Clin Microbiol* 2002;**40**:294–7.
265. Yoshimura S, Ikemura H, Watanabe H, et al. Essential structure for full enterotoxigenic activity of heat-stable enterotoxin produced by enterotoxigenic *Escherichia coli*. *FEBS Lett* 1985;**181**:138–42.
266. You J, Xu Y, He M, et al. Protection of mice against enterotoxigenic *E. coli* by immunization with a polyvalent enterotoxin comprising a combination of LTB, STa, and STb. *Appl Microbiol Biotechnol* 2011;**89**:1885–93.

large mucoid colonies on solid media. The organisms are oxidase-negative and citrate-positive; they ferment inositol and hydrolyze urea but do not produce ornithine decarboxylase or hydrogen sulfide. Acetoin and 2,3-butanediol predominate over acidic end products during sugar fermentation (positive result on the Voges-Proskauer test). Four species of *Klebsiella* commonly are agreed on by microbiologists: *Klebsiella pneumoniae* (the most common human pathogen), *Klebsiella oxytoca* (a less common human pathogen), *Klebsiella terrigena*, and *Raoultella planticola*. Previously, *R. planticola* was recovered almost exclusively from soil and aquatic environments; reports now suggest that this organism may be a relatively common neonatal pathogen in some parts of the world.[106,130] *R. planticola* may express virulence factors similar to those of *K. pneumoniae*.[107] Organisms are defined serologically by their capsular polysaccharide (K antigens) and lipopolysaccharide (O antigens). Significant cross-reactivity exists between the capsule of some pneumococci (e.g., 19F) and *Klebsiella*.[79] The reader is referred to a review by Podschun and Ullman[105] for a detailed description of *Klebsiella* spp.

EPIDEMIOLOGY

Friedländer[42] proposed that *K. pneumoniae* was the most common cause of community-acquired pneumonia, an observation that was refuted by Fraenkel's[40] observations on pneumococcal pneumonia. *K. pneumoniae* accounts for fewer than 10 percent of hospitalized cases of pneumonia in adults.[22] *Klebsiella* spp. now are in greatest evidence as opportunistic nosocomial pathogens of the urinary tract, respiratory tract, biliary tract, and bloodstream. In one survey of the Centers for Disease Control and Prevention, the infection rate of nosocomial *K. pneumoniae* was 16.7 infections/10,000 patients discharged.[61] Hand carriage generally is regarded as the common mode of transmission.[46] Environmental sources of *Klebsiella* spp. include contaminated blood pressure monitoring equipment,[112] ventilator traps,[46] dialysate,[75] ultrasound coupling gel,[44] dextrose solution,[77] and hand disinfectant.[115] The emergence of plasmid-mediated, β-lactamase resistance can be responsible for the rapid spread of resistant organisms to susceptible patients in intensive care units (ICUs).[12,14] Outbreaks may be complex; patient-to-patient transmission of epidemic strains containing different plasmids may be interspersed with sporadic, nonepidemic *Klebsiella* infections.[14] *Klebsiella* spp. are second only to *Escherichia coli* as causes of sepsis,[45] with the highest rates of infection being reported from larger hospitals affiliated with medical schools.

Klebsiella spp. commonly are highlighted as pathogens of debilitated adults and alcoholics,[68] but by 1985, nearly 50 percent of reported outbreaks of *Klebsiella* were in neonatal ICUs.[61] Outbreaks in newborns continue to occur frequently worldwide.* Most outbreaks in newborns have been associated with *K. pneumoniae* infection, but scattered outbreaks of *K. oxytoca* infection in nurseries also have been reported.[8,129] A high percentage of infants in ICUs may become colonized with hospital strains of *Klebsiella*.[51] In one longitudinal study in which weekly rectal swabs were cultured, 80/368 (22%) neonates in an ICU harbored extended-spectrum β-lactamase (ESBL)–producing *Klebsiella* spp.[17] Infecting organisms have been isolated from care providers and from mothers of colonized infants.[26] One report described an outbreak among newborns associated with infestation of a neonatal unit by cockroaches colonized with infecting *Klebsiella* strains.[27] *Klebsiella* may spread from newborn units to adult units; interhospital and international spread of resistant strains has been described.[25,33,121] In a newer report, 31 neonates became colonized, 10 were infected, and 5 died from a neonatal ICU (NICU) outbreak in Maryland. Understaffing of an overcrowded NICU was thought to be important in the genesis of the outbreak.[128] In another NICU outbreak, the use of proton pump inhibitors and prior receipt of third-generation cephalosporins were identified as the principal risk factors for colonization and infection with an ESBL-producing strain.[49] Outbreak control usually depends on cohorting, careful hand washing, and intensive cleaning. However, *K. pneumoniae* isolates that are extremely antibiotic resistant also may be more resistant to chlorhexidine; cleaning with this agent in such cases may provide a selective advantage to the resistant strain.[95]

Ribotyping, pulsed-field gel electrophoresis, and DNA amplification techniques have proved valuable in characterizing *Klebsiella* strains associated with outbreaks.[81,123,124] Experimental techniques include multiplex real-time polymerase chain reaction, which can identify carbapenem-resistant *K. pneumoniae* strains in 2 hours.[127] The modified Hodge test is used in resource-limited settings and works quite well at identifying organisms that produce carbapenemases.[4] Different ribotypes that share plasmids conferring antibiotic resistance can be responsible for pediatric infections in a particular institution.[14] Strains expressing ESBL may become endemic and may present a complex and diverse pattern of production of enzymes with resistance to β-lactamase inhibitors.[34,39] Although broad-spectrum resistance to β-lactams and carbapenems[83] has been described, some longitudinal studies have shown that the frequency of ESBLs in *K. pneumoniae* isolates is decreasing.[118] Active surveillance, carrier cohorting, and increased barrier protection resulted in a decrease in the frequency of KPC-producing *K. pneumoniae* in one Greek hospital.[109]

PATHOPHYSIOLOGY

Pneumonias caused by *Klebsiella* most commonly arise from colonization of the upper respiratory tract, followed by aspiration of organisms to the lower respiratory tract. Some degree of gram-negative oropharyngeal colonization is a normal finding in newborns. The oropharynx of nearly one third of healthy newborns is colonized by gram-negative rods, including *Klebsiella*, by the time infants reach 1 month of age; colonization rates generally are lower in breast-fed infants.[11] Antibiotic pressure in high-risk newborns and older children has been observed to promote overgrowth of *Klebsiella*.[13,120] Enteric organisms are recovered less frequently from the oropharynx of healthy older children and adults; oral

*References 2, 9, 26, 33, 54, 103, 114.

colonization with gram-negative rods is increased during illness,[64] after postoperative viral infections,[65,111] and in debilitated adults.[84] Increased adherence of gram-negative rods to oropharyngeal cells contributes to increased colonization.[65] Elastase made by polymorphonuclear cells contributes to such colonization by reducing the fibronectin coating of sugar receptors.[30] The capsule plays an initial role in interactions of epithelial cells but is not required for an adhesin interaction with the cell surface.[37] Adherence properties may be affected by plasmid content[32] and may be transferred between *E. coli* and *K. pneumoniae*.[60]

In animal models of sepsis, capsular polysaccharide (K antigens) is a virulence factor; monoclonal antibodies to the K antigens reduce the severity of illness in mice.[78] In a mouse model of urinary tract infection, the K antigens seemed to be more important in infection than the lipopolysaccharide (O antigens), and clinical strains deficient in lipopolysaccharide retained virulence by resistance of capsule to complement.[3,21] In one series of adult patients, capsular type K2 frequently was associated with asymptomatic bacteriuria and cystitis, but not pyelonephritis; the presence of type 1 fimbriae bore a closer relationship to upper urinary tract infection.[108] In a retrospective study, patients bacteremic with hyperviscous strains were more likely to develop invasive localized disease, such as liver abscess, meningitis, pleural empyema, or endophthalmitis.[80] Patients bacteremic with the hypermucoviscous phenotype are more likely to come to the hospital from the community and less likely to have underlying disease states, suggesting increased virulence.[69] Liver abscess is almost always caused by hypermucoviscous organisms[38]; surprisingly, disruption of the genes that produce this phenotype did not affect serum sensitivity in vitro and did not alter abscess formation in a mouse model.[74] In another study, the lipopolysaccharide O1 was found to be important; O1 serotypes had higher serum resistance and mutation of the O1 gene greatly reduced the virulence of the organism in a mouse model.[58] Neutrophils play an important role in clearance of *Klebsiella*, and phagocytosis is augmented by leukotrienes.[85]

CLINICAL MANIFESTATIONS

Klebsiella infection shows little clinical distinction from diseases produced by other enteric pathogens. The organism generally is less common than group B streptococcus or *E. coli* as a cause of early-onset or late-onset infection in newborns.[47,88] Investigators from Spain,[54] however, reported one 7-year interval in which *K. pneumoniae* was the most common cause of bacteremia in newborns. In Yemen, gram-negative rods accounted for almost all of 90 cases of neonatal sepsis; *E. coli* outnumbered *K. pneumoniae* by only one case.[2a] Risk factors for neonatal *Klebsiella* infection include prematurity, presence of indwelling catheters, previous antibiotic treatment, and parenteral nutrition.[114] Infection in newborns is characterized by typical features of pneumonia, sepsis, and meningitis.[88] In one unusual case, *K. pneumoniae* bacteremia manifested on day 4 of life as a morbilliform maculopapular exanthem, more severe on the face. The infant was afebrile, but the mother had had fever in the peripartum period, and the amniotic fluid had been meconium-stained.[23]

Although hepatic abscesses have been associated principally with adults with poorly controlled diabetes mellitus, one case report described a 32-week-gestation neonate with *K. pneumoniae* sepsis who developed a liver abscess large enough to be noted on physical examination as an abdominal "lump."[125] In adults, hepatic abscess occasionally is complicated by endophthalmitis or central nervous system infection, with devastating outcomes. A study from Taiwan found that infection with genotype K1 was the only significant risk factor for these complications.[36] This series also revealed that 8/19 (42%) of the complicated K1 strain infections occurred in patients without identifiable underlying medical disease.[36] Other investigators have emphasized that serotype K2 also can be virulent in patients with liver abscess.[43] Careful study of the pathophysiology and epidemiology of complicated *K. pneumoniae* liver abscesses is ongoing, and the picture is likely to continue to be clarified.

Klebsiella spp. have been isolated commonly from blood and peritoneal fluid in outbreaks of necrotizing enterocolitis.[48,92] Less common manifestations in infants include toxic epidermal necrolysis,[52,102] conjunctivitis,[76] parotitis,[24] retropharyngeal abscess,[28] subdural hematoma,[97] psoas abscess,[5] and renal abscess.[126]

Klebsiella is an unusual cause of infection in an otherwise healthy older child. The classic Friedländer pneumonia that occurs in debilitated adults[42] is rare in children. The identification of pulmonary infection should suggest the possibility of underlying immunodeficiency or significant malnutrition, if not suspected previously.[59,66] If pneumonia caused by *Klebsiella* does occur, progression to lung abscesses should be anticipated.

Lung abscesses may develop within days to weeks after *Klebsiella* infection. Formation of abscesses occurs more frequently during *Klebsiella* pulmonary infection than during any other community-acquired infection.[22] A rare but devastating outcome is massive pulmonary gangrene—the rapid total destruction of part of the lung presumed to be due to vascular compromise. This complication is heralded by radiographs that show small cavities that later coalesce into a large cavity with an intracavitary mass of necrotic lung.[94,98] Some researchers speculate that *Klebsiella* lung infection is accompanied by coincident anaerobic infection that contributes to or is primarily responsible for the pathologic process.[22]

Catheterization of the urinary tract can be associated with urinary tract *Klebsiella* infection, but bacteremia is an uncommon complication in an immunocompetent child.[31,82] Approximately 10 percent of nosocomial urinary tract infections observed in infants after surgery are caused by *Klebsiella*.[31] Focal renal infection progressing to renal abscess has been described.[73] *K. pneumoniae* bacteremia has been associated with lesions of the gastrointestinal tract, presence of an indwelling central venous catheter, and neutropenia. Patients with short-bowel syndrome seem to be at greater risk than are patients with inflammatory bowel disease or malignancy for development of catheter-associated *Klebsiella* or *Enterobacter* bacteremia. *K. pneumoniae* was a constituent of polymicrobial bacteremia in 15 such patients (26%).[16] Mortality rates have ranged from 5 to 20 percent, with

higher death rates occurring in children infected with an aminoglycoside-resistant strain.[15,61] Pneumonia, shock, and disseminated intravascular coagulation are poor prognostic factors in children with underlying malignancy. Rare clinical presentations include multifocal osteomyelitis[72] and endophthalmitis.[86] Pediatric recipients of solid organ transplants may have high rates of acquisition of drug-resistant *Klebsiella*.[113] *Klebsiella* spp. have been described as a frequent pathogen in children and adults with sickle cell disease in West Africa.[1]

Of four patients who received organ transplants from people who had been infected with KPC-producing *K. pneumoniae*, one developed invasive infection despite tigecycline prophylaxis.[7] *K. pneumoniae* was the most common cause of bloodstream infection in children undergoing liver transplantation in Korea, accounting for 22 percent of all infections.[116] In New York City, *K. pneumoniae* was the second most common etiology of infection among adult liver transplant recipients. The mortality rate was 71 percent among those who contracted KPC-positive strains; the odds ratio for death was 4.9 for those with the KPC-producing isolates.[70]

Of six patients with clinical and colonoscopic findings suggestive of antibiotic-associated hemorrhagic colitis (pseudomembranous enterocolitis) but negative evaluation for *Clostridium difficile*, five had stool cultures positive for *K. oxytoca*.[57] All five patients had been receiving penicillin therapy. *K. oxytoca* was cultured from only 1.6 percent of 385 healthy controls. Additionally, the disease could be reproduced in a rat model when rats were inoculated with *K. oxytoca* and administered amoxicillin-clavulanate, but disease was not seen if rats were not given the injection and the antibiotic treatment.[57]

DIAGNOSIS

Klebsiella spp. characteristically grow as large mucoid colonies on MacConkey agar. Citrate-containing media can be used to facilitate isolation of *Klebsiella* strains because these organisms can use citrate as a sole carbon source.[99] Serotyping with specific antisera usually is determined by countercurrent immunoelectrophoresis or a Quellung test.[10] In situ hybridization techniques have been used to identify *Klebsiella* in phagocytes from blood specimens,[87] and restriction-enzyme analysis and ribotyping of clinical isolates have been used to characterize nosocomial spread of antibiotic-resistant strains.[14,50] Conventional, commonly used microbiologic methods may misidentify some *Klebsiella* spp., particularly *R. planticola* and *K. terrigena*.[93] Rarely, blood cultures have required longer than 72 hours of incubation for radiometric detection of *Klebsiella*.[89]

TREATMENT

Empirical antimicrobial therapy should be guided by an understanding of antimicrobial susceptibilities of *Klebsiella* in the hospital. Therapy with a cephalosporin plus an aminoglycoside (rather than a cephalosporin alone) has been associated with a more favorable outcome in patients with cancer who are infected with susceptible strains.[15] Antimicrobial therapy of *Klebsiella* spp. is made problematic, however, by the resistance to penicillins and cephalosporins conferred by ESBLs.[12,14,18,53] *Klebsiella* with ESBL are widely distributed, and regional differences in susceptibility occur.[67] Outer membrane protein changes and porin deficiencies of some strains can augment resistance to third-generation cephalosporins.[6,117] Some investigators have reported significant correlation between production of ESBL and resistance to ciprofloxacin.[101,122] Plasmid-mediated resistance to aminoglycosides also is common.[8,26,45,96] Endocarditis isolates that developed resistance to piperacillin-tazobactam and to ciprofloxacin during therapy have been reported.[131]

Effective treatment of multidrug-resistant isolates sometimes requires creativity or use of antibiotics no longer commonly employed. In one case report, a 15-year-old girl developed bacteremia with a meropenem-intermediate strain; bacteremia persisted despite thrice-daily administration of meropenem combined with gentamicin, to which the isolate was sensitive. Clearance was achieved eventually with meropenem administered as a continuous infusion.[35] In another case, bacteremia and peritonitis developed in a patient on continuous ambulatory peritoneal dialysis; infection persisted despite removal of the catheter and treatment with meropenem and amikacin (to which the isolate was intermediately resistant). Cure required a switch to intravenous polymyxin B.[100] Treatment of carbapenem-resistant strains is even more difficult. In vitro, the combination of doripenem and colistin produced bactericidal activity in 75 percent and synergy against 50 percent of colistin-resistant KPC-positive strains.[62] In an animal model, isolates that were carbapenem-resistant because of OXA-48 were more sensitive to ceftazidime than to piperacillin-tazobactam, imipenem, ertapenem, or cefotaxime.[91] New approaches to the treatment of so-called pan-resistant strains include the novel β-lactamase inhibitor MK-7655[56] and an antibacterial "designer peptide" called Api88, which binds a chaperone protein and rescues mice from lethal challenge.[29]

Antibiotic pressure is important in increasing the risk for development of resistant isolates.[110] In some nursery outbreaks, switching from gentamicin to amikacin has been associated with return of the susceptibility of *Klebsiella* isolates to gentamicin.[8,55] Imipenem or the combination of piperacillin and tazobactam may show good antimicrobial activity against multiply-resistant organisms.[63,104,110] The combination of β-lactamase and porin deficiency has been associated with resistance to imipenem,[19] and hyperproduction of some β-lactamases can limit the effectiveness of β-lactam–β-lactamase inhibitor combinations.[41] Experience with use of ciprofloxacin in young children is limited because of observations of irreversible cartilage injury in laboratory animals after administration of this quinolone. Successful treatment of a multidrug-resistant *K. pneumoniae* has been reported in a preterm infant, however, without observable short-term adverse effects.[71] Strict adherence to infection control policies that promote restricted antibiotic use, cohorting, and hand washing may help to prevent the spread of resistant *Klebsiella* strains.[8,26,51,90,119]

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

2a. Al-Shamahy HA, Sabrah AA, Al-Rosabi AB, et al. Type of bacteria associated with neonatal sepsis in Al-Thawra University Hospital, Sama'a, Yemen, and their antimicrobial profile. *Sultan Qaboos Univ Med J* 2012;**12**:48–54.
4. Amjad A, Mirza IA, Abbasi S, et al. Modified Hodge test: a simple and effective test for detection of carbapenemase production. *Iran J Microbiol* 2011;**3**:189–93.
7. Ariza-Heredia EJ, Patel R, Blumberg EA, et al. Outcomes of transplantation using organs from a donor infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing K. pneumoniae. *Transpl Infect Dis* 2012;**14**:229–36.
29. Czihal P, Knappe D, Fritsche S, et al. Api88 is a novel antibacterial designer peptide to treat systemic infections with multidrug-resistant gram-negative pathogens. *ACS Chem Biol* 2012;**7**:1281–91.
38. Fierer J. Biofilm formation and *Klebsiella pneumoniae* liver abscess: true, tru, and unrelated? *Virulence* 2012;**3**:241–2.
49. Guyot K, Biran V, Doit C, et al. Raman spectroscopic analysis of the clonal and horizontal spread of CTX-M-15-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *Eur J Clin Microbiol Infect Dis* 2012; Epub ahead of print.
56. Hirsch EB, Ledesma KR, Chang KT, et al. In vitro activity of MK-7655, a novel beta-lactamase inhibitor, in combination with imipenem against carbapenem resistant gram-negative bacteria. *Antimicrob Agents Chemother* 2012;**56**:2237–40.
58. Hsieh PF, Lin TL, Yang FL, et al. Lipopolysaccharide O1 antigen contributes to the virulence in *Klebsiella pneumoniae* causing pyogenic liver abscess. *PLoS One* 2012;**7**:e33155.
62. Jernigan MG, Press EG, Nguyen MH, et al. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2012;**56**:3395–8.
69. Jung SW, Chae HJ, Park YJ, et al. Microbiological and clinical characteristics of bacteremia caused by the hypermucoviscosity phenotype of *Klebsiella pneumoniae* in Korea. *Epidemiol Infect* 2012;**14**:1–7.
70. Kalpoe JS, Sonnenberg E, Factor SH, et al. Mortality associated with carbapenem-resistant *Klebsiella pneumoniae* infections in liver transplant recipients. *Liver Transpl* 2012;**18**:468–74.
74. Kong Q, Beanan J, Olson R, et al. Biofilm formed by a hypervirulent (hypermucoviscous) variant of *Klebsiella pneumoniae* does not enhance serum resistance or survival in an in vivo abscess model. *Virulence* 2012;**3**:309–18.
91. Mimoz O, Gregoire N, Poirel L, et al. Broad-spectrum beta-lactam antibiotics for treating experimental peritonitis in mice due to *Klebsiella pneumoniae* producing the carbapenemase OXA-48. *Antimicrob Agents Chemother* 2012;**56**:2759–60.
95. Naparstek L, Carmeli Y, Chmelnitsky I, et al. Reduced susceptibility to chlorhexidine among extremely-drug-resistant strains of *Klebsiella pneumoniae*. *J Hosp Infect* 2012;**81**:15–19.
109. Poulou A, Voulgari E, Vrioni G, et al. Imported KPC-producing *Klebsiella pneumoniae* clones in a Greek hospital: impact of infection control measures to restrain their dissemination. *J Clin Microbiol* 2012;**50**:2618–23.
116. Rhee KW, Oh SH, Kim KM, et al. Early bloodstream infection after pediatric living donor transplantation. *Transplant Proc* 2012;**44**:794–6.
127. Spanu T, Fiori B, D'Inzeo T, et al. Evaluation of the new NucliSENS EasyQ test for rapid detection of *Klebsiella pneumoniae* carbapenemase gene (blaKPC). *J Clin Microbiol* 2012;**50**:2783–5.
128. Tamma PD, Savard P, Pal T, et al. An outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2012;**33**:631–4.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 106

Morganella morganii

Randall G. Fisher

Similar to *Proteus* and *Providencia* spp., *Morganella morganii* has emerged as an important nosocomial pathogen, most often associated with urinary tract or wound infections. Although most descriptions of infection are in adults, infections in children do occur.

BACTERIOLOGY

M. morganii (formerly *Proteus morganii*) is a motile gram-negative bacillus commonly found in the feces of humans, other mammals, and reptiles.[33] The organism was elevated to genus rank because of genetic differences from *Proteus*, with which it is otherwise biologically similar.[7] Most strains do not ferment lactose. *M. morganii*, *Proteus* spp., and *Providencia* spp. are distinguished from other Enterobacteriaceae by their ability to deaminate phenylalanine and lysine. Similar to *Proteus* and *Providencia* spp., *M. morganii* produces urease, but this enzyme is unrelated genetically and serologically to urease produced by the other two genera.[21,33] In contrast to *Proteus* spp., neither *Providencia* spp. nor *M. morganii* shows swarming activity

REFERENCES

1. Aken'Ova YA, Bakare RA, Okunade MA, Olaniyi J. Bacterial causes of acute osteomyelitis in sickle cell anaemia: changing infection profile. *W Afr J Med* 1995;**14**:255–8.
2. Akindele JA, Gbadegesin RA. Outbreak of neonatal *Klebsiella* septicaemia at the University College Hospital, Ibadan, Nigeria: appraisal of predisposing factors and preventive measures. *Trop Geogr Med* 1994;**46**:151–3.

2a. Al-Shamahy HA, Sabrah AA, Al-Rosabi AB, et al. Type of bacteria associated with neonatal sepsis in Al-Thawra University Hospital, Sama'a, Yemen, and their antimicrobial profile. *Sultan Qaboos Univ Med J* 2012;**12**:48–54.

3. Alvarez D, Merino S, Tomas JM, et al. Capsular polysaccharide is a major complement resistance factor in lipopolysaccharide O side chain-deficient *Klebsiella pneumoniae* clinical isolates. *Infect Immun* 2000;**68**:953–5.
4. Amjad A, Mirza IA, Abbasi S, et al. Modified Hodge test: a simple and effective test for detection of carbapenemase production. *Iran J Microbiol* 2011;**3**:189–93.
5. Andreou A, Karasavvidou A, Papadopoulou F, Koukoulidis A. Iliopsoas abscess in a neonate. *Am J Perinatol* 1997;**14**:519–21.
6. Ardanuy C, Linares J, Dominguez MA, et al. Outer membrane profiles of clonally related *Klebsiella pneumoniae* isolates from clinical samples and activities of cephalosporins and carbapenems. *Antimicrob Agents Chemother* 1998;**42**:1636–40.
7. Ariza-Heredia EJ, Patel R, Blumberg EA, et al. Outcomes of transplantation using organs from a donor infected with *Klebsiella pneumoniae* carbapenemase (KPC)-producing K. pneumoniae. *Transpl Infect Dis* 2012;**14**:229–36.
8. Aronsson B, Eriksson M, Herin P, et al. Gentamicin-resistant *Klebsiella* spp. and *Escherichia coli* in a neonatal intensive care unit. *Scand J Infect Dis* 1991;**23**:195–9.
9. Arredondo-Garcia JL, Diaz-Ramos R, Solorzano-Santos F, et al. Neonatal septicaemia due to *K. pneumoniae:* septicaemia due to *Klebsiella pneumoniae* in newborn infants—nosocomial outbreak in an intensive care unit. *Rev Latinoam Microbiol* 1992;**34**:11–16.
10. Ayling-Smith B, Pitt TL. State of the art in typing: *Klebsiella* spp. *J Hosp Infect* 1990;**16**:287–95.
11. Baltimore RS, Duncan RL, Shapiro ED, et al. Epidemiology of pharyngeal colonization of infants with aerobic gram-negative rod bacteria. *J Clin Microbiol* 1989;**27**:91–5.
12. Bauernfeind A, Rosenthal E, Eberlein E, et al. Spread *of Klebsiella pneumoniae* producing SHV-5 beta-lactamase among hospitalized patients. *Infection* 1993;**21**:18–22.
13. Bennet R, Eriksson M, Nord CE, et al. Fecal bacterial microflora of newborn infants during intensive care management and treatment with five antibiotic regimens. *Pediatr Infect Dis* 1986;**5**: 533–9.
14. Bingen EH, Desjardins P, Arlet G, et al. Molecular epidemiology of plasmid spread among extended broad-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates in a pediatric hospital. *J Clin Microbiol* 1993;**31**:179–84.
15. Bodey GP, Elting LS, Rodriquez S, et al. *Klebsiella* bacteremia: a 10-year review in a cancer institution. *Cancer* 1989;**64**:2368–76.
16. Bonadio WA. *Klebsiella pneumoniae* bacteremia in children: fifty-seven cases in 10 years. *Am J Dis Child* 1989;**143**:1061–3.
17. Boo NY, Ng SF, Lim VK. A case-control study of risk factors associated with rectal colonization of extended-spectrum beta-lactamase producing *Klebsiella* sp. in newborn infants. *J Hosp Infect* 2005;**61**:68–74.
18. Bradford PA, Cherubin CE, Idemyor V, et al. Multiply resistant *Klebsiella pneumoniae* strains from two Chicago hospitals: identification of the extended-spectrum TEM-12 and TEM-10 ceftazidime-hydrolyzing beta-lactamases in a single isolate. *Antimicrob Agents Chemother* 1994;**38**:761–6.
19. Bradford PA, Urban C, Mariano N, et al. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC beta-lactamase, and the loss of an outer membrane protein. *Antimicrob Agents Chemother* 1997;**41**:563–9.
20. Brown RB, Cipriani D, Schulte M, et al. Community-acquired bacteremias from tunneled central intravenous lines: results from studies of a single vendor. *Am J Infect Control* 1994;**22**:149–51.
21. Camprubi S, Merino S, Benedi VJ, et al. The role of the O-antigen lipopolysaccharide and capsule on an experimental *Klebsiella pneumoniae* infection of the rat urinary tract. *FEMS Microbiol Lett* 1993;**111**:9–13.
22. Carpenter JL. *Klebsiella* pulmonary infections: occurrence at one medical center and review. *Rev Infect Dis* 1990;**12**:672–82.
23. Casacci M, Carpentier O, Truffert P, et al. Neonatal maculopapular exanthema revealing septicemia due to *Klebsiella pneumoniae* via maternofetal infection. *Ann Dermatol Venereol* 2006;**133**:31–3.
24. Coban A, Ince Z, Ucsel R, et al. Neonatal suppurative parotitis: a vanishing disease? *Eur J Pediatr* 1993;**152**:1004–5.
25. Cookson B, Johnson AP, Azadian B, et al. International inter- and intrahospital patient spread of a multiple antibiotic-resistant strain of *Klebsiella pneumoniae*. *J Infect Dis* 1995;**171**:511–13.
26. Coovadia YM, Johnson AP, Bhana RH, et al. Multiresistant *Klebsiella pneumoniae* in a neonatal nursery: the importance of maintenance of infection control policies and procedures in the prevention of outbreaks. *J Hosp Infect* 1992;**22**:197–205.
27. Cotton MF, Wasserman E, Pieper CH, et al. Invasive disease due to extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal unit: the possible role of cockroaches. *J Hosp Infect* 2000;**44**:13–17.
28. Coulthard M, Isaacs D. Retropharyngeal abscess. *Arch Dis Child* 1991;**66**:1227–30.
29. Czihal P, Knappe D, Fritsche S, et al. Api88 is a novel antibacterial designer peptide to treat systemic infections with multidrug-resistant gram-negative pathogens. *ACS Chem Biol* 2012;**7**: 1281–91.
30. Dal Nogare AR, Toews GB, Pierce AK. Increased salivary elastase precedes gram-negative bacillary colonization in postoperative patients. *Am Rev Respir Dis* 1987;**135**:671–5.
31. Davies HD, Jones EL, Sheng RY, et al. Nosocomial urinary tract infections at a pediatric hospital. *Pediatr Infect Dis J* 1992;**11**: 349–54.
32. Denoya CD, Trevisan AR, Zorzopulos J. Adherence of multiresistant strains of *Klebsiella pneumoniae* to cerebrospinal fluid shunts: correlation with plasmid content. *J Med Microbiol* 1986;**21**: 225–31.
33. Eisen D, Russell EG, Tymms M, et al. Random amplified polymorphic DNA and plasmid analyses used in investigation of an outbreak of multiresistant *Klebsiella pneumoniae*. *J Clin Microbiol* 1995;**33**:713–17.
34. Essack SY, Hall LM, Pillay DG, et al. Complexity and diversity of *Klebsiella pneumoniae* strains with extended-spectrum beta-lactamases isolated in 1994 and 1996 at a teaching hospital in Durban, South Africa. *Antimicrob Agents Chemother* 2001;**45**: 88–95.
35. Falagas ME, Siempos II, Tsakoumis I. Cure of persistent, post-appendectomy *Klebsiella pneumoniae* septicemia with continuous intravenous administration of meropenem. *Scand J Infect Dis* 2006; **38**:807–10.
36. Fang CT, Lai SY, Yi WC, et al. *Klebsiella pneumoniae* genotype K1: An emerging pathogen that causes septic ocular or central nervous system complications from pyogenic liver abscess. *Clin Infect Dis* 2007;**45**:284–93.
37. Favre-Bonte S, Joy B, Forestier C. Consequences of reduction of *Klebsiella pneumoniae* capsule expression on interactions of this bacterium with epithelial cells. *Infect Immun* 1999;**67**:554–61.
38. Fierer J. Biofilm formation and *Klebsiella pneumoniae* liver abscess: true, tru, and unrelated? *Virulence* 2012;**3**:241–2.
39. Fiett J, Palucha A, Miaczynska B, et al. A novel complex mutant beta-lactamase, TEM-68, identified in a *Klebsiella pneumoniae* isolate from an outbreak of extended-spectrum beta-lactamase-producing klebsiellae. *Antimicrob Agents Chemother* 2000;**44**: 1499–505.
40. Fraenkel A. Bakteriologische mitteilungen. *Leitsch F Klin Med* 1886;**10**:401–11.
41. French GL, Shannon KP, Simmons N. Hospital outbreak of *Klebsiella pneumoniae* resistant to broad-spectrum cephalosporins and beta-lactam-beta-lactamase inhibitor combinations by hyperproduction of SHV-5 beta-lactamase. *J Clin Microbiol* 1996;**34**: 358–63.
42. Friedländer C. Über die schizomyceten bei der acuten fibrosen pneumonie. *Arch Pathol Anat Physiol Klin Med* 1882;**87**:319–24.
43. Fung CP, Siu LK. Virulence of *Klebsiella pneumoniae* serotype K2 should not be underestimated in *K. pneumoniae* liver abscess. *Clin Infect Dis* 2007;**45**:1530–1.

44. Gaillot O, Maruejouls C, Abachin E, et al. Nosocomial outbreak of *Klebsiella pneumoniae* producing SHV-5 extended-spectrum beta-lactamase, originating from a contaminated ultrasonography coupling gel. *J Clin Microbiol* 1998;**36**:1357–60.
45. Garcia de la Torre M, Romero-Vivas J, Martinez-Beltran J, et al. *Klebsiella* bacteremia: an analysis of 100 episodes. *Rev Infect Dis* 1985;**7**:143–50.
46. Gorman LJ, Sanai L, Notman AW, et al. Cross infection in an intensive care unit by *Klebsiella pneumoniae* from ventilator condensate. *J Hosp Infect* 1993;**23**:27–34.
47. Grauel EL, Halle E, Bollmann R, et al. Neonatal septicaemia: Incidence, etiology and outcome: a 6-year analysis. *Acta Paediatr Scand* 1989;**360**(Suppl.):113–19.
48. Gregersen N, Van Nierop W, Von Gottberg A, et al. *Klebsiella pneumoniae* with extended spectrum beta-lactamase activity associated with a necrotizing enterocolitis outbreak. *Pediatr Infect Dis J* 1999;**18**:963–7.
49. Guyot K, Biran V, Doit C, et al. Raman spectroscopic analysis of the clonal and horizontal spread of CTX-M-15-producing Klebsiella pneumoniae in a neonatal intensive care unit. *Eur J Clin Microbiol Infect Dis* 2012;**31**:2827–34.
50. Haertl R, Bandlow G. Use of small fragment restriction endonuclease analysis (SF-REA) for epidemiological fingerprinting of *Klebsiella oxytoca*. *Int J Med Microbiol Virol Parasitol Infect Dis* 1994;**280**:312–18.
51. Hambraeus A, Lagerqvist-Widh A, Zettersten U, et al. Spread of *Klebsiella* in a neonatal ward. *Scand J Infect Dis* 1991;**23**:189–94.
52. Hawk RJ, Storer JS, Daum RS. Toxic epidermal necrolysis in a 6-week-old infant. *Pediatr Dermatol* 1985;**2**:197–200.
53. Heritage J, Hawkey PM, Todd N, et al. Transposition of the gene encoding a TEM-12 extended-spectrum beta-lactamase. *Antimicrob Agents Chemother* 1992;**36**:1981–6.
54. Hervas JA, Alomar A, Salva F, et al. Neonatal sepsis and meningitis in Mallorca, Spain, 1977-1991. *Clin Infect Dis* 1993;**16**:719–24.
55. Hesseling PB, Mouton WL, Henning PA, et al. A prospective study of long-term use of amikacin in a paediatrics department: indications, administration, side-effects, bacterial isolates and resistance. *S Afr Med J* 1990;**78**:192–5.
56. Hirsch EB, Ledesma KR, Chang KT, et al. In vitro activity of MK-7655, a novel beta-lactamase inhibitor, in combination with imipenem against carbapenem resistant gram-negative bacteria. *Antimicrob Agents Chemother* 2012;**56**:2237–40.
57. Hogenauer C, Langner C, Beubler E, et al. *Klebsiella oxytoca* as a causative organism of antibiotic-associated hemorrhagic colitis. *N Engl J Med* 2006;**355**:2418–26.
58. Hsieh PF, Lin TL, Yang FL, et al. Lipopolysaccharide O1 antigen contributes to the virulence in *Klebsiella pneumoniae* causing pyogenic liver abscess. *PLoS One* 2012;**7**:e33155.
59. Hughes WT. Pneumonia in the immunocompromised child. *Semin Resp Infect* 1987;**2**:177–83.
60. Jallat C, Darfeuille-Michaud A, Girardeau JP, et al. Self-transmissible R plasmids encoding CS31A among human *Escherichia coli* strains isolated from diarrheal stools. *Infect Immun* 1994;**62**:2865–73.
61. Jarvis WR, Munn VP, Highsmith AK, et al. The epidemiology of nosocomial infections caused by *Klebsiella pneumoniae*. *Infect Control* 1985;**6**:68–74.
62. Jernigan MG, Press EG, Nguyen MH, et al. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2012;**56**:3395–8.
63. Jett BD, Ritchie DJ, Reichley R, et al. In vitro activities of various beta-lactam antimicrobial agents against clinical isolates of *Escherichia coli* and *Klebsiella* spp. resistant to oxyimino cephalosporins. *Antimicrob Agents Chemother* 1995;**39**:1187–90.
64. Johanson WG, Pierce AK, Sanford JP. Changing bacterial flora of hospitalized patients. *N Engl J Med* 1969;**281**:1137–40.
65. Johanson WG, Higuchi JH, Chaudhuri TR, et al. Bacterial adherence to epithelial cells in bacillary colonization of the respiratory tract. *Am Rev Respir Dis* 1980;**121**:55–63.
66. Johnson AW, Osinusi K, Aderele WI, et al. Bacterial aetiology of acute lower respiratory infections in pre-school Nigerian children and comparative predictive features of bacteraemic and non-bacteraemic illnesses. *J Trop Pediatr* 1993;**39**:97–106.
67. Jones RN, Jenkins SG, Hoban DJ, et al. In vitro efficacy of six cephalosporins tested against Enterobacteriaceae isolated at 38 North American medical centers participating in the SENTRY Antimicrobial Surveillance Program, 1997-1998. *Int J Antimicrob Agents* 2000;**15**:111–18.
68. Jong GM, Hsiue TR, Chen CR, et al. Rapidly fatal outcome of bacteremic *Klebsiella pneumoniae* pneumonia in alcoholics. *Chest* 1995;**107**:214–17.
69. Jung SW, Chae HJ, Park YJ, et al. Microbiological and clinical characteristics of bacteremia caused by the hypermucoviscosity phenotype of *Klebsiella pneumoniae* in Korea. *Epidemiol Infect* 2012;**14**:1–7.
70. Kalpoe JS, Sonnenberg E, Factor SH, et al. Mortality associated with carbapenem-resistant Klebsiella pneumoniae infections in liver transplant recipients. *Liver Transpl* 2012;**18**:468–74.
71. Khaneja M, Naprawa J, Kumar A, Piecuch S. Successful treatment of late-onset infection due to resistant *Klebsiella pneumoniae* in an extremely low birth weight infant using ciprofloxacin. *J Perinatol* 1999;**19**:311–14.
72. Kishan J, Mir NA, Elzouki AY, et al. Radiological case of the month: *Klebsiella* multifocal osteomyelitis. *Am J Dis Child* 1988; **142**:687–8.
73. Klar A, Hurvitz H, Berkun Y, et al. Focal bacterial nephritis (lobar nephronia) in children. *J Pediatr* 1996;**128**:850–3.
74. Kong Q, Beanan J, Olson R, et al. Biofilm formed by a hypervirulent (hypermucoviscous) variant of *Klebsiella pneumoniae* does not enhance serum resistance or survival in an in vivo abscess model. *Virulence* 2012;**3**:309–18.
75. Kolmos HJ. *Klebsiella pneumoniae* in a nephrological department. *J Hosp Infect* 1984;**5**:253–9.
76. Krohn MA, Hillier SL, Bell TA, et al. The bacterial etiology of conjunctivitis in early infancy: Eye Prophylaxis Study Group. *Am J Epidemiol* 1993;**138**:326–32.
77. Lalitha MK, Kenneth J, Jana AK, et al. Identification of an IV-dextrose solution as the source of an outbreak of *Klebsiella pneumoniae* sepsis in a newborn nursery. *J Hosp Infect* 1999;**43**: 70–3.
78. Lang AB, Bruderer U, Senyk G, et al. Human monoclonal antibodies specific for capsular polysaccharides of *Klebsiella* recognize clusters of multiple serotypes. *J Immunol* 1991;**146**:3160–4.
79. Lee CJ. Bacterial capsular polysaccharides: biochemistry, immunity and vaccine. *Mol Immunol* 1987;**24**:1005–19.
80. Lee HC, Chuang YC, Yu WL, et al. Clinical implications of hypermucoviscosity phenotype in *Klebsiella pneumoniae* isolates: association with invasive syndrome in patients with community-acquired bacteremia. *J Intern Med* 2006;**259**:606–14.
81. Lhopital S, Bonacorsi S, Meis D, et al. Molecular markers for differentiation of multiresistant *Klebsiella pneumoniae* isolates in a pediatric hospital. *Infect Control Hosp Epidemiol* 1997;**18**: 743–8.
82. Lohr JA, Donowitz LG, Sadler JE. Hospital-acquired urinary tract infection. *Pediatrics* 1989;**83**:193–9.
83. Lomaestro BM, Tobin EH, Shang W, Gootz T. The spread of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* to upstate New York. *Clin Infect Dis* 2006;**43**:e26–8.
84. Mackowiak PA, Martin RM, Jones SR, et al. Pharyngeal colonization by gram-negative bacilli in aspiration-prone persons. *Arch Intern Med* 1978;**138**:1224–7.
85. Mancuso P, Nana-Sinkam P, Peters-Golden M. Leukotriene B4 augments neutrophil phagocytosis of *Klebsiella pneumoniae*. *Infect Immun* 2001;**69**:2011–16.
86. Margo CE, Mames RN, Guy JR. Endogenous *Klebsiella* endophthalmitis: report of two cases and review of the literature. *Ophthalmology* 1994;**101**:1298–301.
87. Matsuhisa A, Saito Y, Sakamoto Y, et al. Detection of bacteria in phagocyte-smears from septicemia-suspected blood by in situ hybridization using biotinylated probes. *Microbiol Immunol* 1994; **38**:511–17.
88. McCracken GHJ, Mize SG, Threlkeld N. Intraventricular gentamicin therapy in gram-negative bacillary meningitis of infancy: report of the Second National Meningitis Cooperative Study Group. *Lancet* 1980;**1**:787–91.
89. Meadow WL, Schwartz IK. Time course of radiometric detection of positive blood cultures in childhood. *Pediatr Infect Dis* 1986; **5**:333–6.
90. Meyer KS, Urban C, Eagan JA, et al. Nosocomial outbreak of *Klebsiella* infection resistant to late-generation cephalosporins. *Ann Intern Med* 1993;**119**:353–8.

91. Mimoz O, Gregoire N, Poirel L, et al. Broad-spectrum beta-lactam antibiotics for treating experimental peritonitis in mice due to *Klebsiella pneumoniae* producing the carbapenemase OXA-48. *Antimicrob Agents Chemother* 2012;**56**:2759–60.
92. Mollitt DL, Tepas JJ, Talbert JL. The microbiology of neonatal peritonitis. *Arch Surg* 1988;**123**:176–9.
93. Monnet D, Freney J, Brun Y, et al. Difficulties in identifying *Klebsiella* strains of clinical origin. *Int J Med Microbiol* 1991;**274**: 456–64.
94. Moon WK, Im JG, Yeon KM, et al. Complications of *Klebsiella* pneumonia: CT evaluation. *J Comput Assist Tomogr* 1995;**19**: 176–81.
95. Naparstek L, Carmeli Y, Chmelnitsky I, et al. Reduced susceptibility to chlorhexidine among extremely-drug-resistant strains of *Klebsiella pneumoniae*. *J Hosp Infect* 2012;**81**:15–19.
96. Nathoo KJ, Mason PR, Gwanzura L, et al. Severe *Klebsiella* infection as a cause of mortality in neonates in Harare, Zimbabwe: evidence from postmortem blood cultures. *Pediatr Infect Dis J* 1993;**12**:840–4.
97. Ng PC, Fok TF, Lee CH, et al. Massive subdural haematoma: an unusual complication of septicaemia in preterm very low birthweight infants. *J Pediatr Child Health* 1998;**34**:296–8.
98. O'Reilly GV, Dee PM, Otteni GV. Gangrene of the lung: successful medical management of three patients. *Diagn Radiol* 1978;**126**: 575–9.
99. Orskov I. *Klebsiella*. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*, vol. 1. Baltimore: Williams & Wilkins; 1984. p. 461–5.
100. Parchuri S, Mohan S, Cunha BA. Extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* chronic ambulatory peritoneal dialysis peritonitis treated successfully with polymyxin B. *Heart Lung* 2005;**34**:360–3.
101. Paterson DL, Mulazimoglu L, Casellas JM, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000;**30**:473–8.
102. Picard E, Gillis D, Klapholz L, et al. Toxic epidermal necrolysis associated with *Klebsiella pneumoniae* sepsis. *Pediatr Dermatol* 1994;**11**:331–4.
103. Pierce JR, Merenstein GB, Stocker JT. Immediate postmortem cultures in an intensive care nursery. *Pediatr Infect Dis J* 1984;**3**: 510–13.
104. Pillay T, Pillay DG, Adhikari M, Sturm AW. Piperacillin/tazobactam in the treatment of *Klebsiella pneumoniae* infections in neonates. *Am J Perinatol* 1998;**15**:47–51.
105. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: Epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 1998;**11**:589–603.
106. Podschun R, Acktun H, Okpara J, et al. Isolation of *Klebsiella planticola* from newborns in a neonatal ward. *J Clin Microbiol* 1998;**36**:2331–2.
107. Podschun R, Fischer A, Ullman U. Expression of putative virulence factors by clinical isolates of *Klebsiella planticola*. *J Med Microbiol* 2000;**49**:115–19.
108. Podschun R, Sievers D, Fischer A, et al. Serotypes, hemagglutinins, siderophore synthesis, and serum resistance of *Klebsiella* isolates causing human urinary tract infections. *J Infect Dis* 1993; **168**:1415–21.
109. Poulou A, Voulgari E, Vrioni G, et al. Imported KPC-producing *Klebsiella pneumoniae* clones in a Greek hospital: impact of infection control measures to restrain their dissemination. *J Clin Microbiol* 2012;**50**:2618–23.
110. Quinn JP. Clinical significance of extended-spectrum beta-lactamases. *Eur J Clin Microbiol Infect Dis* 1994;**13**(Suppl. 1): S39–42.
111. Ramirez-Ronda CH, Fuxench-Lopez Z, Nevarez M. Increased pharyngeal bacterial colonization during viral illness. *Arch Intern Med* 1981;**141**:1599–603.
112. Ransjo U, Good Z, Jalakas K, et al. An outbreak of *Klebsiella oxytoca* septicemias associated with the use of invasive blood pressure monitoring equipment. *Acta Anaesthesiol Scand* 1992; **36**:289–91.
113. Rebuck JA, Olsen KM, Fey PD, et al. Characterization of an outbreak due to extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a pediatric intensive care unit transplant population. *Clin Infect Dis* 2000;**31**:1368–72.
114. Reish O, Ashkenazi S, Naor N, et al. An outbreak of multiresistant *Klebsiella* in a neonatal intensive care unit. *J Hosp Infect* 1993;**25**: 287–91.
115. Reiss I, Borkhardt A, Fussle R, et al. Disinfectant contaminated with *Klebsiella oxytoca* as a source of sepsis in babies. *Lancet* 2000; **356**:310.
116. Rhee KW, Oh SH, Kim KM, et al. Early bloodstream infection after pediatric living donor transplantation. *Transplant Proc* 2012; **44**:794–6.
117. Rice LB, Carias LL, Hujer AM, et al. High-level expression of chromosomally encoded SHV-1 beta-lactamase and an outer membrane protein change confer resistance to ceftazidime and piperacillin-tazobactam in a clinical isolate of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000;**44**:362–7.
118. Romero L, Lopez L, Rodriguez-Bano J, et al. Long-term study of the frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing extended-spectrum beta-lactamases. *Clin Microbiol Infect* 2005;**11**:625–31.
119. Royle J, Halasz S, Eagles G, et al. Outbreak of extended spectrum beta lactamase producing *Klebsiella pneumoniae* in a neonatal unit. *Arch Dis Child Fetal Neonatal Ed* 1999;**80**:F64–8.
120. Sakata H, Fujita K, Yoshioka H. The effect of antimicrobial agents on fecal flora of children. *Antimicrob Agents Chemother* 1986;**29**: 225–9.
121. Saurina G, Quale JM, Manikal VM, et al. Antimicrobial resistance in Enterobacteriaceae in Brooklyn, NY: epidemiology and relation to antibiotic usage patterns. *J Antimicrob Chemother* 2000;**45**: 895–8.
122. Schumacher H, Scheibel J, Moller JK. Cross-resistance patterns among clinical isolates of *Klebsiella pneumoniae* with decreased susceptibility to cefuroxime. *J Antimicrob Chemother* 2000;**46**: 215–21.
123. Sechi LA, Spanu T, Sanguinetti M, et al. Molecular analysis of *Klebsiella pneumoniae* strains isolated in pediatric wards by ribotyping, pulsed field gel electrophoresis and antimicrobial susceptibilities. *New Microbiol* 2001;**24**:35–45.
124. Shannon K, Fung K, Stapleton P, et al. A hospital outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* investigated by RAPD typing and analysis of the genetics and mechanisms of resistance. *J Hosp Infect* 1998;**39**:291–300.
125. Sharma S, Mohta A, Sharma P. Hepatic abscess in a preterm neonate. *Indian Pediatr* 2007;**44**:226–8.
126. Sood SK, Mulvihill D, Daum RS. Intrarenal abscess caused by *Klebsiella pneumoniae* in a neonate: modern management and diagnosis. *Am J Perinatol* 1989;**6**:367–70.
127. Spanu T, Fiori B, D'Inzeo T, et al. Evaluation of the new NucliSENS EasyQ test for rapid detection of *Klebsiella pneumoniae* carbapenemase gene (blaKPC). *J Clin Microbiol* 2012;**50**: 2783–5.
128. Tamma PD, Savard P, Pal T, et al. An outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2012;**33**: 631–4.
129. Tullus K, Ayling-Smith B, Kuhn I, et al. Nationwide spread of *Klebsiella oxytoca* K55 in Swedish neonatal special care wards. *A P M I S* 1992;**100**:1008–14.
130. Westbrook GL, O'Hara CM, Roman SB, Miller JM. Incidence and identification of *Klebsiella planticola* in clinical isolates with emphasis on newborns. *J Clin Microbiol* 2000;**38**:1495–7.
131. Zimhony O, Chmelnitsky I, Bardenstein R, et al. Endocarditis caused by extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*: emergence of resistance to ciprofloxacin and piperacillin-tazobactam during treatment despite initial susceptibility. *Antimicrob Agents Chemother* 2006;**50**:3179–82.

on 1.5 percent agar[33]; *M. morganii* is ornithine positive, whereas *Providencia* spp. are ornithine negative. *M. morganii* organisms do not hydrolyze gelatin and do not produce hydrogen sulfide. For a detailed discussion of taxonomy and characterization of *M. morganii*, the reader is referred to the review by O'Hara and colleagues.[31]

EPIDEMIOLOGY

Similar to *Proteus* and *Providencia* spp., *M. morganii* organisms commonly are found in soil, sewage, and manure. Similar to *Proteus mirabilis*, *M. morganii* often invades the instrument-fitted urinary tract and surgical wounds; adults in inpatient surgical units have the greatest risk for colonization and infection.[1] Institutionalized elderly patients also are infected frequently with these organisms. Urinary tract colonization with *M. morganii* accompanying groin skin colonization in elderly individuals may account for the greater frequency of urinary tract infections in this population.[17] *M. morganii* accounted for nearly 10 percent of 145 consecutive complicated, multidrug-resistant urinary tract infections.[13] *Escherichia coli* and *P. mirabilis* account for most urinary tract infections in children, but *M. morganii* has been implicated in some cases of cystitis and pyelonephritis. In contrast to well-described nursery epidemics of *P. mirabilis* infection,[3,9] no *M. morganii* neonatal outbreaks have been described, and infections of the central nervous system are rare in newborns.[15,43] A fatal case of early-onset sepsis in which both the mother's lochia and the neonate's blood were culture positive has been reported. The baby died at 17 days of age despite appropriate antimicrobial therapy.[32] Ribotyping is a sensitive method for molecular characterization of isolates that may aid in analyzing outbreaks.[35]

PATHOPHYSIOLOGY

Factors that predispose the urinary tract to invasion by *P. mirabilis* and *Providencia* spp. also may favor *M. morganii* colonization and infection. These organisms all split urea, forming ammonium hydroxide and increasing local pH, which results in toxicity to renal cells and potentiation of urolithiasis.[6,29] The ability of *P. mirabilis* to regenerate more rapidly in urine with faster generation of alkaline pH compared with *M. morganii* may provide a selective advantage for the former organism in establishing itself as a urinary tract pathogen.[40]

CLINICAL MANIFESTATIONS

Urinary tract infection with *M. morganii* often is associated with an elevated urinary pH. Urolithiasis can occur, although perhaps less frequently than during *P. mirabilis* infection.[4] *M. morganii* has been recovered from less than 10 percent of adult bacteremic episodes, but mortality rates have exceeded 20 percent.[1] Approximately three fourths of all cases of adult *M. morganii* bacteremia are hospital associated.[24] In a series of 132 patients with bacteremia from Proteeae, preexisting biliary or hepatic disease (especially the presence of biliary drainage catheters) was associated with *Morganella* bacteremia.[24] In a series of 73 patients with *Morganella* bacteremia in Taiwan, 92 percent had at least one underlying disease, the most common of which was solid tumor. The only independent risk factor for mortality was inappropriate antibiotic treatment.[26] Other reported complications identified in immunocompromised or instrument-fitted patients include meningitis,[19,27] arthritis,[23,39] empyema,[20] peritonitis,[20] otitis media,[18] and skin infection.[2] Many cases of postoperative *M. morganii* endophthalmitis and panophthalmitis have been reported, as have rare cases of endogenous endophthalmitis, all in adults.[10,42,44,45] *M. morganii* has been recovered alone or in combination with other organisms from surgical wounds[13] and soft tissue abscesses in children.[8] A 12-year-old with meningoencephalitis, whose immune system was apparently normal, has been reported.[30] Another rare manifestation of *M. morganii* infection was pericarditis, seen in a child with X-linked agammaglobulinemia.[11] A 3-year-old boy developed hemorrhagic bullae and necrotizing fasciitis after being scratched by a chicken; cultures grew both *M. morganii* and enterococci.[25] Perinatal bacterial sepsis and brain abscess have been described.[36-38,43] In three more recently reported cases of early-onset neonatal sepsis, premature rupture of membranes and maternal receipt of antepartum amoxicillin were documented.[5,16]

DIAGNOSIS

M. morganii produces a reddish brown pigment when cultured on nutrient media supplemented with 5 percent tryptophan.[33] Production of urease and deamination of tryptophan help to distinguish this bacterium from other organisms. In contrast to the closely related *Proteus* and *Providencia* spp., *M. morganii* generally ferments only glucose and mannose and does not produce a red color on lysine iron agar.[33] *M. morganii* may be missed or mistaken for other organisms in the common circumstance of polymicrobial infection in a catheterized patient. In one series, *M. morganii* was among the most common bacteriuric species in patients on long-term catheterization but commonly was missed by reference laboratories.[14] The clinical laboratory should be directed to look for this organism, particularly in circumstances of nosocomial urinary tract infection and sepsis.

TREATMENT

Effective treatment of local infections or septicemia relies on appropriate choice of an antibiotic, often including an aminoglycoside, combined with surgical débridement and drainage of abscesses, as necessary. Antimicrobial susceptibility can vary widely among *M. morganii* and the related *Proteus* and *Providencia* strains, emphasizing the importance of species identification and susceptibility testing.[22,34] Complex combinations of aminoglycoside resistance that differ by hospital and geographic region can occur.[28] Most isolates are intrinsically resistant to

ampicillin and first- and second-generation cephalosporins. Some carry a chromosomal broad-spectrum AmpC β-lactamase that can be stably derepressed on treatment with third-generation cephalosporins, similar to *Enterobacter* spp., although it appears that this problem arises much less frequently among *Morganella* isolates.[12] Most urinary tract infections respond to treatment with third-generation cephalosporins. Failure to clear bacteriuria should alert the physician to the possibility of urolithiasis or structural abnormality[4]; stone removal or surgical correction of anatomic defects often is required for cure. The approach to the treatment of sepsis or serious infection of sites other than the urinary tract should be considered carefully in light of local susceptibility patterns, keeping in mind that isolates with AmpC β-lactamases may seem to be sensitive to cephalosporins on initial testing but develop resistance during therapy. Treatment failure, with prolonged culture-positive ventriculitis, has been described in an infant with *M. morganii* meningitis after initial therapy that included cefotaxime led to stable derepression of an AmpC β-lactamase, showing the potentially devastating clinical relevance of this phenomenon.[41] Aztreonam has shown effectiveness in therapy for 98 percent of multidrug-resistant strains.[13]

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

11. Cho YK, Kook H, Woo YJ, et al. *Morganella morganii* pericarditis in a child with X-linked agammaglobulinemia. *Pediatr Int* 2010;**52**:489–91.
12. Choi SH, Lee JE, Park SJ, et al. Emergence of antibiotic resistance during therapy for infections caused by Enterobacteriaceae producing AmpC beta-lactamase: implications for antibiotic use. *Antimicrob Agents Chemother* 2008;**52**:995–1000.
25. Lee CY, Lee HF, Huang FL, Chen PY. Haemorrhagic bullae associated with a chicken scratch. *Ann Trop Paediatr* 2009;**29**:309–11.
30. Ndiaye M, Sène MS, Sow AD, et al. [Meningoencephalitis due to *Morganella morganii:* a case report]. *Bull Soc Pathol Exot* 2010;**103**:230–2. In French.
32. Ovalle A, Martinez MA, Kakarieka E, et al. [Fatal neonatal sepsis caused by vertical transmission of *Morganella morganii:* report of one case]. *Rev Med Chil* 2009;**137**:1201–4. In Spanish.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 107

Proteus

Randall G. Fisher

Proteus spp. are pathogens that are associated increasingly with pediatric illness. Neonatal meningitis and pediatric urinary tract infections are the most common childhood conditions in which these organisms are isolated, but infections of other organ systems have been described.

BACTERIOLOGY

Proteus spp. are motile, gram-negative bacilli that do not ferment lactose and are distinguished from other Enterobacteriaceae by their ability to deaminate phenylalanine and lysine. Rapid and abundant production of urease further differentiates *Proteus* spp. from *Providencia* spp.[37] *Proteus vulgaris* and *Proteus mirabilis* tend to form a thin, spreading growth (swarm) on the surface of moist agar media, often overgrowing other bacterial isolates. They also produce hydrogen sulfide and liquefy gelatin. *P. mirabilis* is distinguished from other *Proteus* spp. (e.g., *P. vulgaris*) by its inability to produce indole from tryptophan. Most indole-negative clinical *Proteus* isolates are classified by laboratories as *P. mirabilis;* however, in one study, 8 of 61 were found to be *P. penneri* when extensive biochemical testing was performed.[35] Disparate DNA content and anomalous biochemical and serologic reactions have caused *Proteus morganii* to be renamed *Morganella morganii* (see Chapter 106),[37] and both terms for this organism appear in the clinical literature. For detailed discussion of taxonomy and characterization of *Proteus* spp., the reader is referred to the review by O'Hara and colleagues.[49]

REFERENCES

1. Adler JL, Burke JP, Martin DF, et al. *Proteus* infection in a general hospital, part II: some clinical and epidemiological characteristics. *Ann Intern Med* 1971;**75**:531–6.
2. Bagel J, Grossman ME. Hemorrhagic bullae associated with *Morganella morganii* septicemia. *J Am Acad Dermatol* 1985;**12**:575–6.
3. Becker AH. Infection due to *Proteus mirabilis* in a newborn nursery. *Am J Dis Child* 1962;**104**:355–9.
4. Bensman A, Roubach L, Allouch G, et al. Urolithiasis in children: presenting signs, etiology, bacteriology and localisation. *Acta Paediatr Scand* 1983;**72**:879–83.
5. Boussemart T, Piet-Duroux S, Manouana M, et al. *Morganella morganii* and early-onset neonatal infection. *Arch Pediatr* 2004;**11**:37–9.
6. Braude AI, Siemienski J. Role of bacterial urease in experimental pyelonephritis. *J Bacteriol* 1960;**80**:171–9.
7. Brenner DJ, Farmer JJ III, Fanning GR, et al. Deoxyribonucleic acid relatedness of *Proteus* and *Providencia* species. *Int J Syst Bacteriol* 1978;**28**:269–82.
8. Brook I, Martin WJ. Aerobic and anaerobic bacteriology of perirectal abscess in children. *Pediatrics* 1980;**66**:282–4.
9. Burke JP, Ingall D, Klein JO, et al. *Proteus mirabilis* infections in a hospital nursery traced to a human carrier. *N Engl J Med* 1971; **284**:115–21.
10. Christensen SR, Hansen AB, LaCour M, Fledelius HC. Bilateral endogenous bacterial endophthalmitis: a report of four cases. *Acta Ophthalmol Scand* 2004;**82**:306–10.
11. Cho YK, Kook H, Woo YJ, et al. *Morganella morganii* pericarditis in a child with X-linked agammaglobulinemia. *Pediatr Int* 2010;**52**: 489–91.
12. Choi SH, Lee JE, Park SJ, et al. Emergence of antibiotic resistance during therapy for infections caused by Enterobacteriaceae producing AmpC beta-lactamase: implications for antibiotic use. *Antimicrob Agents Chemother* 2008;**52**:995–1000.
13. Cox CE. Aztreonam therapy for complicated urinary tract infections caused by multidrug-resistant bacteria. *Rev Infect Dis* 1985; **7**(Suppl. 4):S767–71.
14. Damron DJ, Warren JW, Chippendale GR, et al. Do clinical microbiology laboratories report complete bacteriology in urine from patients with long-term urinary catheters? *J Clin Microbiol* 1986; **24**:400–4.
15. Darby CP, Hill O. *Proteus morganii* meningitis treated with trimethoprim-sulfamethoxazole (co-trimoxazole). *Clin Pediatr* 1975;**14**:669–72.
16. Dutta S, Narang A. Early onset neonatal sepsis due to *Morganella morganii*. *Indian Pediatr* 2004;**41**:1155–7.
17. Ehrenkranz NJ, Alfonso BC, Eckert DG, et al. *Proteeae* species bacteriuria accompanying *Proteeae* species groin skin carriage in geriatric outpatients. *J Clin Microbiol* 1989;**27**:1988–91.
18. Haddad JJ, Inglesby TVJ, Addonizio L. Head and neck infections in pediatric cardiac transplant patients. *Ear Nose Throat J* 1995;**74**: 422–5.
19. Isaacs RD, Ellis-Pegler RB. Successful treatment of *Morganella morganii* meningitis with pefloxacin mesylate. *J Antimicrob Chemother* 1987;**20**:769–70.
20. Isobe H, Motomura K, Kotou K, et al. Spontaneous bacterial empyema and peritonitis caused by *Morganella morganii*. *J Clin Gastroenterol* 1994;**18**:87–8.
21. Jones BD, Mobley HL. Genetic and biochemical diversity of ureases of *Proteus*, *Providencia*, and *Morganella* species isolated from urinary tract infection. *Infect Immun* 1987;**55**:2198–203.
22. Kaslow RA, Lindsey JO, Bison AL, et al. Nosocomial infection with highly resistant *Proteus rettgeri*: report of an epidemic. *Am J Epidemiol* 1976;**104**:278–86.
23. Katz LM, Lewis RJ, Borenstein DG. Successful joint arthroplasty following *Proteus morganii (Morganella morganii)* septic arthritis: a four-year study. *Arthritis Rheum* 1987;**30**:583–5.
24. Kim BN, Kim NJ, Kim MN, et al. Bacteraemia due to tribe Proteeae: a review of 132 cases during a decade (1991-2000). *Scand J Infect Dis* 2003;**35**:98–103.
25. Lee CY, Lee HF, Huang FL, Chen PY. Haemorrhagic bullae associated with a chicken scratch. *Ann Trop Paediatr* 2009;**29**: 309–11.
26. Lee IK, Liu JW. Clinical characteristics and risk factors for mortality in *Morganella morganii* bacteremia. *J Microbiol Immunol Infect* 2006;**39**:328–34.
27. Mastroianni A, Coronado O, Chiodo F. *Morganella morganii* meningitis in a patient with AIDS. *J Infect* 1994;**29**:356–7.
28. Miller GH, Sabatelli FJ, Hare RS, et al. The most frequent aminoglycoside resistance mechanisms—changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. *Clin Infect Dis* 1997;**24**: S46–62.
29. Musher DM, Griffith DP, Yawn D, et al. Role of urease in pyelonephritis resulting from urinary tract infection wit*h Proteus*. *J Infect Dis* 1975;**131**:177–81.
30. Ndiaye M, Sène MS, Sow AD, et al. [Meningoencephalitis due to *Morganella morganii*: a case report]. *Bull Soc Pathol Exot* 2010;**103**:230–2. In French.
31. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin Microbiol Rev* 2000;**13**:534–46.
32. Ovalle A, Martinez MA, Kakarieka E, et al. [Fatal neonatal sepsis caused by vertical transmission of *Morganella morganii:* report of one case]. *Rev Med Chil* 2009;**137**:1201–4. In Spanish.
33. Penner JL. *Morganella*. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*, vol. 1. Baltimore: Williams & Wilkins; 1984. p. 497–8.
34. Piccolomini R, Cellini L, Allocati N, et al. Comparative in vitro activities of 13 antimicrobial agents against *Morganella-Proteus-Providencia* group bacteria from urinary tract infections. *Antimicrob Agents Chemother* 1987;**31**:1644–7.
35. Pignato S, Giammanco GM, Grimont F, et al. Molecular characterization of the genera *Proteus*, *Morganella*, and *Providencia* by ribotyping. *J Clin Microbiol* 1999;**37**:2840–7.
36. Ranu SS, Valencia GB, Piecuch S. Fatal early onset infection in an extremely low birth weight infant due to *Morganella morganii*. *J Perinatol* 1999;**19**:533–5.
37. Rowen JL, Lopez SM. *Morganella morganii* early onset sepsis. *Pediatr Infect Dis J* 1998;**17**:1176–7.
38. Salen PN, Eppes S. *Morganella morganii*: a newly reported rare cause of neonatal sepsis. *Acad Emerg Med* 1997;**4**:711–14.
39. Schonwetter RS, Orson FM. Chronic *Morganella morganii* arthritis in an elderly patient. *J Clin Microbiol* 1988;**26**:1414–15.
40. Senior BW. *Proteus morganii* is less frequently associated with urinary tract infections than *Proteus mirabilis*: an explanation. *J Med Microbiol* 1983;**16**:317–22.
41. Sinha AK, Kempley ST, Price E, et al. Early onset *Morganella morganii* sepsis in a newborn infant with emergence of cephalosporin resistance caused by derepression of AmpC beta-lactamase production. *Pediatr Infect Dis J* 2006;**25**:376–7.
42. Tsanaktsidis G, Agarwal SA, Maloof AJ, et al. Postoperative *Morganella morganii* endophthalmitis association with subclinical urinary tract infection. *J Cataract Refract Surg* 2003;**29**:1011–13.
43. Verboon-Maciolek M, Vandertop WP, Peters AC, et al. Neonatal brain abscess caused by *Morganella morganii*. *Clin Infect Dis* 1995;**20**: 471.
44. Wang TJ, Huang JS, Hsueh PR. Acute postoperative *Morganella morganii* panophthalmitis. *Eye* 2005;**19**:713–15.
45. Zaninetti M, Baglivo E, Safran AB. *Morganella morganii* endophthalmitis after vitrectomy: case report and review of the literature. *Klin Monatsbl Augenheilkd* 2003;**220**:207–9.

EPIDEMIOLOGY

Proteus spp. commonly are found in soil, sewage, and manure. Although they are normal inhabitants of the colon and perineum, their numbers can be increased in individuals receiving antibiotic therapy.[33]

First reported by Buisine and Henninot in 1949,[12] neonatal meningitis caused by *P. mirabilis* accounts for approximately 4 percent of all neonatal meningitis cases.[45] Outbreaks in nurseries have been attributed to contaminated equipment and human carriers. Vertical transmission from mother to infant has been confirmed by DNA fingerprinting and ribotyping methods.[9] In Becker's series[6] of *P. mirabilis* neonatal meningitis, all affected infants came from the same nursery and all were exposed to mist from an apparatus that yielded *P. mirabilis*. The importance of hand carriage was well documented by Burke and associates[13]; neonatal umbilical colonization and invasive disease were linked to a single nurse from whom *P. mirabilis* was cultured from her hands, rectum, and vagina. Ribotyping is a sensitive method for molecular characterization of isolates and may aid in analyzing outbreaks.[53]

Proteus infection after the first few months of life most commonly involves the urinary tract. Although *Escherichia coli* accounts for most urinary tract infections in children, *Proteus* spp. commonly are implicated in reported series of cystitis and pyelonephritis[8,23,34,50,70]; in a large pediatric series, they were the third most common cause of urinary tract infection, after *E. coli* and *Klebsiella pneumoniae*.[10] *P. mirabilis* is the most common species of *Proteus* isolated: it has been cultured more frequently from the urethra of uncircumcised than of circumcised male infants and has replaced *E. coli* as the most prevalent pathogen in one consecutive series of male patients presenting with initial urinary tract infection.[34,71]

Urinary tract infection with *Proteus* spp. is one of the most common presenting signs of urolithiasis in children,[19] and *P. mirabilis* supplants *E. coli* as the major urinary tract pathogen in children prone to renal stone formation.[8] Diagnosis of greater than 50 percent of pediatric urolithiasis cases is based on preceding urinary tract infection, and *Proteus* is responsible for 65 percent of these infections.[8] Isolation of this organism as a pathogen on urine culture should alert the physician to the possible presence of a urinary tract stone.

PATHOPHYSIOLOGY

Most cases of central nervous system infection caused by *Proteus* spp. occur in neonates and are thought to arise by bacteremic spread of the organism to the brain or meninges. Contiguous spread to the brain from localized infections is reported occasionally.[44,61] As is the case with *Citrobacter* meningitis, a propensity for formation of abscesses of the central nervous system remains unexplained. Rabbit models of *P. mirabilis* meningitis have shown that in vivo concentrations of gentamicin necessary to produce bacterial killing are 10 to 30 times higher than the concentrations predicted from in vitro susceptibility testing.[68] Reduced aminoglycoside effect may be secondary to depressed cerebrospinal fluid pH associated with *P. mirabilis* infection.[68] Whatever the mechanism, lack of effective antimicrobial activity in the ventricles accounts partly for the persistence of organisms at these sites.

Crystallization of urinary catheters and stents leads to obstruction of outflow of urine. Urease produced by *Proteus* infection causes calcium phosphate and magnesium phosphate to precipitate and accumulate in the catheter lumen.[67] This process is less likely to occur when the urine is dilute and acidic.[67] Mannose-resistant/*Proteus*-like fimbriae (often shortened to MR/P fimbriae) are important in the formation of biofilms on catheter material.[57] *Proteus* isolates obtained from catheter-associated urinary tract infection have increased swarming motility and form biofilms more readily than *Proteus* isolates from stool of healthy volunteers.[28] Methods of preventing crystallization, including the use of low-energy acoustic waves generated by electrically activated piezo elements, which interferes with docking and attachment, are being studied.[27] More conventional approaches include coating the catheters with agarose[66] and impregnating catheters with chlorhexidine and triclosan.[22]

Numerous factors may predispose the urinary tract to invasion by *Proteus* spp. *Proteus* spp. split urea, forming ammonium hydroxide and increasing local pH, which results in toxicity to renal cells and potentiation of urolithiasis.[11,47] The ability of *P. mirabilis* to regenerate more rapidly in urine with faster generation of alkaline pH when compared with *M. morganii* may provide a selective advantage for the former organism in establishing itself as a urinary tract pathogen.[60] Biochemically complex struvite ($MgNH_4PO_4$) stones provide a refuge for *Proteus* organisms and form a barrier to effective antimicrobial therapy.[8] Formation of struvite stones is a major cause of urinary bacterial persistence in women without azotemia,[65] and a similar case probably can be made for pediatric patients with urolithiasis. *P. mirabilis* ureases showed lower affinities for substrate but hydrolyzed urea 6 to 25 times faster than enzymes from other species, which may explain the frequent association of this species with formation of stones.[30]

Organisms have been shown to be taken up by human renal epithelium, by an actin-independent mechanism.[15] Pili may enhance the virulence of *Proteus* in pyelonephritis by increasing adherence of organisms to the renal pelvis.[57,62] Flagella have been implicated in the spread of this organism in the urinary tract[40]; the ability to invade host uroepithelial cells is coupled closely with the ability of *P. mirabilis* to differentiate into hyperflagellated, filamentous swarm cells capable of rapid spread on the surface of moist agar media.[3] The role of fimbriae as a factor predisposing to ascending infection is less clear.[5,43] Swarming behavior might inherently assist ascending colonization of the urinary tract, as shown in a mouse model of infection.[4] A putative gene regulator for swarming behavior (RsbA) may act to identify environmental conditions that favor swarming.[7] The reader is referred to the review by Rozalski and colleagues[58] for a more in-depth review of *Proteus* virulence factors.

A growing body of literature suggests that adult rheumatoid arthritis may be triggered by infection of the urinary tract with *Proteus* spp. In one study, increased levels of total and class-specific IgG antibodies directed at three *Proteus* peptides were found in patients with rheumatoid arthritis but not in control subjects.[56] Patients with active disease had measurable IgM, and a positive correlation was found between antibody indices and inflammatory markers.[56] This association is still speculative and is being investigated; at this time, no suggestion has been made that juvenile rheumatoid arthritis is related in any way to *Proteus* infection.

CLINICAL MANIFESTATIONS

P. mirabilis can produce a broad spectrum of symptoms associated with neonatal infection. Most patients have typical symptoms of early-onset neonatal sepsis, including nonspecific lethargy, fever, and poor feeding; manifestations of sepsis may include septic arthritis and osteomyelitis. In a few patients the disease presents after the first week of life. Meningitis may occur with either early-onset or late-onset disease. Brain abscesses associated with subtle clinical abnormalities rarely develop for weeks to months before presentation.[13] *Proteus* brain abscesses are associated with a high degree of mortality, frequent complications, and increased risks of neurologic deficits in survivors.[17,31,39,63] Hydrocephalus is a particularly frequent complication and should be anticipated. Destruction of the brain may progress to porencephaly or compartmentalization of ventricles and often requires surgical intervention.[31] Computed tomography is useful, especially in diagnosing and following progression of cerebral complications.[63]

Urinary tract infection with these bacteria involves predominantly younger patients and often is associated with elevated urinary pH; clinical findings and urine abnormalities often are less striking than in patients with *E. coli* urinary tract infections.[34] Thirty percent of patients may show recurrent infection during the 12 months after receiving initial treatment.[34] Indwelling urinary catheters increase the risk for *Proteus* colonization and infection. Long-term indwelling urinary catheters or stents may become blocked by encrustations of aggregated struvite crystals; prolonged colonization with urease-producing *P. mirabilis* is associated with this complication.[38] *P. mirabilis* is the most common pathogen associated with xanthogranulomatous pyelonephritis,[2] which can mimic Wilms tumor and usually is not seen in childhood except in malnourished patients.

Proteus spp. often are implicated as agents of septicemia in adult patients and account for approximately 8 percent of gram-negative bacteremias in this group.[20,36] In 60 percent of *Proteus* bacteremic episodes in adults, the urinary tract has been determined to be the source[20]; no anatomic source is identified in 20 percent of cases of *Proteus* bacteremia. *P. mirabilis* and *P. vulgaris* are the species responsible for most cases of *Proteus* bacteremia. The overall incidence of gram-negative enteric bacteremia in pediatric patients is lower than that of adults; 5 percent of such cases are caused by *Proteus* spp.[20] As in adults, the genitourinary tract is the source identified most frequently.[20] Predictors of bacteremia in adults with *Proteus* urinary tract infection are community acquisition, hydronephrosis, bandemia greater than 10 percent, a C-reactive protein value greater than 10 mg/dL, and hypothermia or hyperthermia at diagnosis.[14] Mortality rates average less than 40 percent and strongly depend on the severity of underlying disease in the host.[20,36]

Osteomyelitis,[32,52,68] pneumonia,[20,36,54] mastoiditis,[44] and wound infections[36] also occur. Rarely, native-valve endocarditis has been caused by *P. mirabilis*.[16] Severe multifocal osteomyelitis requiring bilateral above-knee amputation for cure has been reported in a patient with human immunodeficiency virus infection despite a reasonably good $CD4^+$ cell count.[51] *Proteus* spp. have been isolated commonly from chronic suppurative otitis media on several different continents.[24,69] Reports that *Proteus* spp. are major pathogens of otogenic brain abscess in pediatric and adult patients support this association.[25,55] Pediatric osteomyelitis secondary to contiguous infection of traumatized soft tissue often is polymicrobial, and *Proteus* spp. have been implicated as co-pathogens in at least 10 percent of such cases.[52] Sickle cell anemia is a risk factor.[1,32]

DIAGNOSIS

Proteus is suspected readily because of its ability to swarm on the surface of moist agar. A selective medium developed for the isolation of *Proteus* organisms relies on the ability of all members to produce a dark brown pigment in medium containing DL-tryptophan.[26] Production of urease, lack of indole production from tryptophan, and a positive result with ornithine decarboxylase testing distinguish *P. mirabilis* from *Providencia* spp. and other *Proteus* spp.[37]

TREATMENT

Treatment of meningitis caused by *Proteus* spp. should conform to standard regimens recommended for gram-negative meningitis. *P. mirabilis* usually is sensitive to ampicillin, however, and this drug alone or combined with an aminoglycoside often is suitable therapy when the identity and susceptibilities of the infecting organism are known.[59] A third-generation cephalosporin often is an alternative, but resistant extended-spectrum β-lactamase–producing strains and β-lactamase inhibitor–resistant strains have been described in pediatric patients.[18,40] Consecutive lumbar punctures should be performed for *Proteus* meningitis until cerebrospinal fluid cultures are sterile. A minimum of 2 weeks of antibiotic therapy is recommended after bacteriologic cure has been achieved. Ventricular aspiration or drainage of abscesses may be required to direct therapy, based on persistence of organisms at these sites. Open drainage of abscesses often is necessary, but resolution of abscess formation with the use of antibiotic therapy alone has been reported.[64] Intraventricular antibiotics are not of proven benefit in terms of mortality or morbidity but

have been used to clear ventricular colonization. A 15-year-old boy with *Proteus* mastoiditis and meningitis has been treated successfully with intravenous trimethoprim-sulfamethoxazole.[44]

Effective treatment of local infections or septicemia relies on appropriate choice of antibiotics, often including an aminoglycoside, combined with surgical débridement and drainage of abscesses as necessary. Many experts recommended "double coverage"—a cell wall–active agent plus an aminoglycoside—especially when the infection is severe or is caused by an indole-positive strain. In a large, three-continent longitudinal survey, approximately 5 percent of *P. mirabilis* strains exhibited phenotypic extended-spectrum, β-lactamase resistance patterns.[21] In France, 59 percent of 1008 urinary *P. mirabilis* isolates were resistant to amoxicillin, 48 percent were resistant to piperacillin, 34 percent were resistant to amoxicillin-clavulanate, and 2.8 percent were resistant to piperacillin-tazobactam.[41] One third of isolates were resistant to trimethoprim-sulfamethoxazole.[41] In Japan, extended-spectrum β-lactamase production in *P. mirabilis* went up from 7 percent in 2000 to 12.9 percent in 2009.[48] A report from China details the combination of carbapenem resistance and quinolone resistance in 19 isolates; all of them had the *Klebsiella pneumoniae* carbapenemase-2 gene.[29] Complex combinations of aminoglycoside resistance that differ by hospital and geographic region can occur.[46] In one study, amikacin retained better activity than did other aminoglycosides.[41] Most *P. mirabilis* urinary tract infections respond to ampicillin, but some organisms have been shown to acquire a plasmid-mediated β-lactamase.[42] Failure to clear bacteria should alert the physician to the possibility of urolithiasis or structural abnormality; removal of stones or surgical correction of anatomic defects often is required for cure.

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

14. Chen CY, Chen YH, Lu PL, et al. *Proteus mirabilis* urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. *J Microbiol Immunol Infect* 2012;**45**:228–36.
28. Hola V, Peroutkova T, Ruzicka F. Virulence factors in *Proteus* bacteria from biofilm communities of catheter-associated urinary tract infections. *FEMS Immunol Med Microbiol* 2012;**65**(2):343–9.
29. Hu YY, Cai JC, Zhang R, et al. Emergence of *Proteus mirabilis* harboring blaKPC-2 and qnrD in a Chinese hospital. *Antimicrob Agents Chemother* 2012;**56**:2278–82.
35. Kishore J. Isolation, identification, and characterization of *Proteus penneri*—a missed rare pathogen. *Indian J Med Res* 2012;**135**:341–5.
48. Nakamura T, Komatsu M, Yamasaki K, et al. Epidemiology of *Escherichia coli*, *Klebsiella* species, and *Proteus mirabilis* strains producing extended-spectrum beta-lactamases from clinical samples in the Kinki Region of Japan. *Am J Clin Pathol* 2012;**137**:620–6.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 108

Providencia

Randall G. Fisher

The genus *Providencia* comprises pathogens most commonly associated with urinary tract infection. *Providencia* spp. are encountered most often as pathogens in hospitals or long-term care facilities and can be responsible for outbreaks of multidrug-resistant infection.[40]

BACTERIOLOGY

Providencia spp. (named after the city of Providence, Rhode Island) are motile gram-negative bacilli that do not ferment lactose and are distinguished from other Enterobacteriaceae by their ability to deaminate phenylalanine and lysine.[8,31] The genus distinguishes "urease-negative" organisms, *P. rettgeri*, *P. stuartii*, *P. alcalifaciens*, *P. rustigianii*, and *P. heimbachae*, from the otherwise biochemically similar "urease-positive" *Proteus* spp.[15,31] Urease is produced by most strains of *P. rettgeri* and by 15 percent or less of *P. stuartii* strains.[27] *Providencia* spp. also differ from other Proteeae in their ability to produce acid from inositol. Strains are differentiated further by reactivity with straight-chain hydroxy alcohols.[31] For a detailed discussion of taxonomy and characterization of *Providencia* spp., the reader is referred to the review by O'Hara and colleagues.[28]

REFERENCES

1. Aken'Ova YA, Bakare RA, Okunade MA, Olaniyi J. Bacterial causes of acute osteomyelitis in sickle cell anaemia: changing infection profile. *W Afr J Med* 1995;**14**:255–8.
2. Al-Ghazo MA, Ghalayini IF, Matalka II, et al. Xanthogranulomatous pyelonephritis: analysis of 18 cases. *Asian J Surg* 2006;**29**: 257–61.
3. Allison C, Coleman N, Jones PL, et al. Ability of *Proteus mirabilis* to invade human urothelial cells is coupled to motility and swarming differentiation. *Infect Immun* 1992;**60**:4740–6.
4. Allison C, Emody L, Coleman N, et al. The role of swarm cell differentiation and multicellular migration in the uropathogenicity of *Proteus mirabilis*. *J Infect Dis* 1994;**169**:1155–8.
5. Bahrani FK, Massad G, Lockatell CV, et al. Construction of an MR/P fimbrial mutant of *Proteus mirabilis*: role in virulence in a mouse model of ascending urinary tract infection. *Infect Immun* 1994;**62**:3363–71.
6. Becker AH. Infection due to *Proteus mirabilis* in a newborn nursery. *Am J Dis Child* 1962;**104**:355–9.
7. Belas R, Schneider R, Melch M. Characterization of *Proteus mirabilis* precocious swarming mutants: identification of rsbA, encoding a regulator of swarming behavior. *J Bacteriol* 1998;**180**:6126–39.
8. Bensman A, Roubach L, Allouch G, et al. Urolithiasis in children: presenting signs, etiology, bacteriology and localisation. *Acta Paediatr Scand* 1983;**72**:879–83.
9. Bingen E, Boissinot C, Desjardins P, et al. Arbitrarily primed polymerase chain reaction provides rapid differentiation of *Proteus mirabilis* isolates from a pediatric hospital. *J Clin Microbiol* 1993;**31**: 1055–9.
10. Bonsu BK, Shuler L, Sawicki L, et al. Susceptibility of recent bacterial isolates to cefdinir and selected antibiotics among children with urinary tract infections. *Acad Emerg Med* 2006;**13**:76–81.
11. Braude AI, Siemienski J. Role of bacterial urease in experimental pyelonephritis. *J Bacteriol* 1960;**80**:171–9.
12. Buisine A, Henninot E. Les meningites à *Proteus* chez l'enfant. *Ann Biol Clin* 1949;**7**:448.
13. Burke JP, Ingall D, Klein JO, et al. *Proteus mirabilis* infections in a hospital nursery traced to a human carrier. *N Engl J Med* 1971; **284**:115–21.
14. Chen CY, Chen YH, Lu PL, et al. *Proteus mirabilis* urinary tract infection and bacteremia: risk factors, clinical presentation, and outcomes. *J Microbiol Immunol Infect* 2012;**45**:228–36.
15. Chippendale GR, Warren JW, Trifillis AL, Mobley HL. Internalization of *Proteus mirabilis* by human renal epithelial cells. *Infect Immun* 1994;**62**:3115–21.
16. Claassen DO, Batsis JA, Orenstein R. *Proteus mirabilis:* a rare cause of infective endocarditis. *Scand J Infect Dis* 2007;**39**:373–5.
17. Darby CP, Conner E, Kyong CU. *Proteus mirabilis* brain abscess in a neonate. *Dev Med Child Neurol* 1978;**20**:366–75.
18. de Champs C, Bonnet R, Sirot D, et al. Clinical relevance of *Proteus mirabilis* in hospital patients: a two year survey. *J Antimicrob Chemother* 2000;**45**:537–9.
19. Diamond DA. Clinical patterns of paediatric urolithiasis. *Br J Urol* 1991;**68**:195–8.
20. duPont HL, Spink WH. Infections due to gram-negative organisms: an analysis of 860 patients with bacteremia at the University of Minnesota Medical Center, 1958-1966. *Medicine* 1969;**48**: 307–29.
21. Fedler KA, Biedenbach DJ, Jones RN. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY antimicrobial surveillance program on three continents. *Diagn Microbiol Infect Dis* 2006;**56**:427–36.
22. Gaonkar TA, Caraos L, Modak S. Efficacy of a silicone urinary catheter impregnated with chlorhexidine and triclosan against colonization with *Proteus mirabilis* and other uropathogens. *Infect Control Hosp Epidemiol* 2007;**28**:596–8.
23. Ginsburg CM, McCracken GH Jr. Urinary tract infections in young infants. *Pediatrics* 1982;**69**:409–12.
24. Gul HC, Kurnaz A, Turhan V, et al. [Microorganisms isolated from middle ear cultures and their antibacterial susceptibility in patients with chronic suppurative otitis media]. *Kulak Burun Bogaz Ihtis Derg* 2006;**16**:164–8. In Polish.
25. Hafidh MA, Keogh I, Walsh RM, et al. Otogenic intracranial complications: a 7-year retrospective review. *Am J Otolaryngol* 2006;**27**: 390–5.
26. Hawkey PM, McCormick A, Simpson RA. Selective and differential medium for the primary isolation of members of the Proteeae. *J Clin Microbiol* 1986;**23**:600–3.
27. Hazan Z, Zumeris J, Jacob H, et al. Effective prevention microbial biofilm formation on medical devices by low-energy surface acoustic waves. *Antimicrob Agents Chemother* 2006;**50**:4144–52.
28. Hola V, Peroutkova T, Ruzicka F. Virulence factors in *Proteus* bacteria from biofilm communities of catheter-associated urinary tract infections. *FEMS Immunol Med Microbiol* 2012;**65**(2):343–9.
29. Hu YY, Cai JC, Zhang R, et al. Emergence of *Proteus mirabilis* harboring blaKPC-2 and qnrD in a Chinese hospital. *Antimicob Agents Chemother* 2012;**56**:2278–82.
30. Jones BD, Mobley HL. Genetic and biochemical diversity of ureases of *Proteus*, *Providencia*, and *Morganella* species isolated from urinary tract infection. *Infect Immun* 1987;**55**:2198–203.
31. Kalsbeck JE, DeSousa AL, Kleiman MB, et al. Compartmentalization of the cerebral ventricles as a sequela of neonatal meningitis. *J Neurosurg* 1980;**52**:547–52.
32. Kanfaoui A, Graide D, Petein M, et al. *Proteus mirabilis* osteomyelitis of the ribs in a girl with sickle cell anaemia. *Eur J Pediatr* 1999;**158**:767.
33. Kaslow RA, Lindsey JO, Bison AL, et al. Nosocomial infection with highly resistant *Proteus rettgeri*: report of an epidemic. *Am J Epidemiol* 1976;**104**:278–86.
34. Khan AJ, Ubriani RS, Bombach E, et al. Initial urinary tract infection caused by *Proteus mirabilis* in infancy and childhood. *J Pediatr* 1978;**93**:791–3.
35. Kishore J. Isolation, identification, and characterization of *Proteus penneri*—a missed rare pathogen. *Indian J Med Res* 2012;**135**: 341–5.
36. Kreger BE, Craven DE, Carling PC, et al. Gram-negative bacteremia, part III: reassessment of etiology, epidemiology and ecology in 612 patients. *Am J Med* 1980;**68**:332–43.
37. Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*. Baltimore: Williams & Wilkins; 1984. p. 491–4.
38. Kunin CM. Blockage of urinary catheters: role of microorganisms and constituents of the urine on formation of encrustations. *J Clin Epidemiol* 1989;**42**:835–42.
39. Levy HL, Ingall D. Meningitis in neonates due to *Proteus mirabilis*. *Am J Dis Child* 1967;**114**:320–4.
40. Luzzaro F, Perilli M, Amicosante G, et al. Properties of multidrug-resistant, ESBL-producing *Proteus mirabilis* isolates and possible role of beta-lactam/beta-lactamase inhibitor combinations. *Int J Antimicrob Agents* 2001;**17**:131–5.
41. Mahamat A, Lavigne JP, Bouziges N, et al. Antimicrobial susceptibility of *Proteus mirabilis* urinary tract isolates from 1999 to 2005 at Nimes University Hospital. *Pathol Biol (Paris)* 2006;**54**:456–61. In French.
42. Mariotte S, Nordmann P, Nicolas MH. Extended-spectrum beta-lactamase in *Proteus mirabilis*. *J Antimicrob Chemother* 1994;**33**: 925–35.
43. Massad G, Lockatell CV, Johnson DE, et al. *Proteus mirabilis* fimbriae: construction of an isogenic pmfA mutant and analysis of virulence in a CBA mouse model of ascending urinary tract infection. *Infect Immun* 1994;**62**:536–42.
44. McConville JH, Manzella JP. Parenteral trimethoprim/sulfamethoxazole for gram-negative bacillary meningitis. *Am J Med Sci* 1984;**287**:43–5.
45. McCracken GH Jr. New developments in the management of children with bacterial meningitis. *Pediatr Infect Dis* 1984;**3**: S32–4.
46. Miller GH, Sabatelli FJ, Hare RS, et al. The most frequent aminoglycoside resistance mechanisms—changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. *Clin Infect Dis* 1997;**24**: S46–62.
47. Musher DM, Griffith DP, Yawn D, et al. Role of urease in pyelonephritis resulting from urinary tract infection with *Proteus*. *J Infect Dis* 1975;**131**:177–81.
48. Nakamura T, Komatsu M, Yamasaki K, et al. Epidemiology of *Escherichia coli*, *Klebsiella* species, and *Proteus mirabilis* strains producing extended-spectrum beta-lactamases from clinical samples in the Kinki Region of Japan. *Am J Clin Pathol* 2012;**137**:620–6.
49. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin Microbiol Rev* 2000;**13**:534–46.

50. Pazin GJ, Braude AI. Immobilizing antibodies in urine, part 2: prevention of ascending spread of *Proteus mirabilis*. *Invest Virol* 1974;**122**:129–33.
51. Petsatodis G, Symeonidis PD, Karatagalis D, Pournaras J. Multifocal *Proteus mirabilis* osteomyelitis requiring bilateral amputation in an HIV-positive patient. *J Bone Joint Surg Br* 2007;**89**:249–51.
52. Pichichero ME, Friesen HA. Polymicrobial osteomyelitis: report of three cases and review of the literature. *Rev Infect Dis* 1982;**4**: 86–96.
53. Pignato S, Giammanco GM, Grimont F, et al. Molecular characterization of the genera *Proteus*, *Morganella*, and *Providencia* by ribotyping. *J Clin Microbiol* 1999;**37**:2840–7.
54. Pine JR, Hollman JL. Elevated pleural fluid pH in *Proteus mirabilis* empyema. *Chest* 1983;**84**:109–11.
55. Rashid T, Ebringer A, Wilson C, et al. The potential use of antibacterial peptide antibody indices in the diagnosis of rheumatoid arthritis and ankylosing spondylitis. *J Clin Rheumatol* 2006;**12**:1–2.
56. Rashid T, Jayakumar KS, Binder A, et al. Rheumatoid arthritis patients have elevated antibodies to cross-reactive and non-cross reactive antigens from *Proteus* microbes. *Clin Exp Rheumatol* 2007; **25**:259–67.
57. Rocha SP, Pelayo JS, Elias WP. Fimbriae of uropathogenic *Proteus mirabilis*. *F E M S Immunol Med Microbiol* 2007;**51**:1–7.
58. Rozalski A, Sidorczyk Z, Kotelko K. Potential virulence factors of *Proteus* bacilli. *Microbiol Mol Biol Rev* 1997;**61**:65–89.
59. Scherzer AL, Kaye D, Shinefield HR. *Proteus mirabilis* meningitis: report of two cases treated with ampicillin. *J Pediatr* 1966;**68**: 731–40.
60. Senior BW. *Proteus morganii* is less frequently associated with urinary tract infections than *Proteus mirabilis*: an explanation. *J Med Microbiol* 1983;**16**:317–22.
61. Sennaroglu L, Sozeri B. Otogenic brain abscess: review of 41 cases. *Otolaryngol Head Neck Surg* 2000;**123**:751–5.
62. Silverblatt FJ. Host-parasite interaction in the rat renal pelvis: a possible role for pili in the pathogenesis of pyelonephritis. *J Exp Med* 1974;**140**:1696–711.
63. Smith ML, Mellor D. *Proteus mirabilis* meningitis and cerebral abscess in the newborn period. *Arch Dis Child* 1980;**55**:308–10.
64. Spirer Z, Jurgenson U, Lazewnick R, et al. Complete recovery from an apparent brain abscess treated without neurosurgery: the importance of early CT scanning. *Clin Pediatr* 1982;**21**:106–9.
65. Stamey TA. *Pathogenesis and treatment of urinary tract infections*. Baltimore: Williams & Wilkins; 1980.
66. Stickler DJ, Lear JC, Morris NS, et al. Observations on the adherence of *Proteus mirabilis* onto polymer surfaces. *J Appl Microbiol* 2006;**100**:1028–33.
67. Stickler DJ, Morgan SD. Modulation of crystalline *Proteus mirabilis* biofilm development on urinary catheters. *J Med Microbiol* 2006; **55**:489–94.
68. Strausbaugh LJ, Sande MA. Factors influencing the therapy of experimental *Proteus mirabilis* meningitis in rabbits. *J Infect Dis* 1978;**137**:251–60.
69. Wariso BA, Ibe SN. Bacteriology of chronic discharging ears in Port Harcourt, Nigeria. *West Afr J Med* 2006;**25**:219–22.
70. Wientzen RL, McCracken GH Jr, Petruska ML, et al. Localization and therapy of urinary tract infections of childhood. *Pediatrics* 1979;**63**:467–73.
71. Wiswell TE, Miller GM, Gelston HM, et al. Effect of circumcision status on periurethral bacterial flora during the first year of life. *J Pediatr* 1988;**113**:442–6.

EPIDEMIOLOGY

Providencia organisms are recovered uncommonly from stool in healthy humans, but they frequently colonize indwelling or condom urinary catheters, particularly in patients receiving antibiotic therapy.[5,10,16,40,41] In a Japanese study of patients with traveler's diarrhea, *Providencia* spp. were recovered, using a new selective medium, from 23 of 130 specimens tested.[42] *Providencia* spp. have been recognized as pathogens for more than 50 years[7]; *P. rettgeri* and *P. stuartii* are the most common species implicated in urinary tract infection.[10,12,13,24,41] Multiple biotypes of *P. stuartii* have been identified in hospital outbreaks, indicating the probability of multiple sources of colonization.[2] Ribotyping is a sensitive method for molecular characterization of isolates that may aid in analysis of outbreaks.[33]

PATHOPHYSIOLOGY

P. stuartii does not seem to have greater access to the urinary tract compared with other bacteria; in patients with long-term catheters, the incidence of bacteriuria caused by this organism is equivalent to that caused by other uropathogens.[40] Rather, *P. stuartii* manifests an extraordinary ability to persist within the catheterized urinary tract.[41] Bacteriuria may take weeks to months to clear. A mannose-resistant, *Klebsiella*-like hemagglutinin may play an important role in the persistence and adherence of *P. stuartii* to urinary tract catheters.[26]

Despite the similarities between *P. mirabilis* (the major pathogen responsible for urolithiasis in children)[4] and urease-producing *Providencia* spp., the latter organisms rarely are associated with formation of stones. *P. stuartii* occasionally produces urease with a higher affinity for substrate, but *P. mirabilis* ureases hydrolyze urea 6 to 25 times faster.[14] Restriction-enzyme analysis of genes coding for the respective enzymes shows significant divergence.[14] These differences may explain the more frequent association of *P. mirabilis* with formation of stones.

Some strains of *P. alcalifaciens* have been isolated more commonly in children with diarrhea, and enteropathogenicity has been shown in HEp-2 cells and a rabbit model.[2,3] In vitro, *P. alcalifaciens* prefers basolateral to apical entry into Caco-2 cells; they are taken up by endocytosis and appear to require an invasion.[23] Ex vivo *P. alcalifaciens* strains show the ability to translocate and to resist complement-mediated lysis.[39] In children with diarrhea, *P. alcalifaciens* often is associated with other enteric pathogens, so its role in pathogenesis remains unclear.[1]

CLINICAL MANIFESTATIONS

Although *Escherichia coli* and *Proteus* spp. account for most urinary tract infections in children, *Providencia* spp. have been reported as a cause of infection in children with spinal injury and long-term urinary tract catheterization.[17,24] Most infections have been described, however, in elderly patients or adults with spinal injury who require long-term urinary tract catheterization.[40] Clinical findings are typical of those associated with urinary tract infection. Bacteremia is uncommon but devastating.[18] In a study of 132 cases of bacteremia caused by members of the tribe Proteeae, only 8 were caused by *Providencia* spp., but four of those patients died of the infection.[18] A single case of endocarditis and a case of relapsing pericarditis both caused by *P. stuartii* have been reported.[19,35]

Eye infections caused by *P. rettgeri*, including conjunctivitis, dacryocystitis, keratitis, and endophthalmitis, have been reported. These infections generally occurred in patients with compromise in ocular surface or in immunity.[29]

Providencia spp. have been implicated in the so-called purple urine bag syndrome, in which an enzyme from the organism causes 3-indoxyl sulfate to be formed, which discolors the urine blue or blue-violet. This syndrome usually occurs in patients with indwelling cystotomy or nephrostomy tubes.[20,38] Xanthogranulomatous pyelonephritis with a nephrocutaneous fistula has also been reported.[21]

Although central nervous system infection with *Providencia* spp. is extremely rare, meningitis occurred after a neurosurgical procedure in a patient in Turkey[36] and a left frontal lobe cystic mass was reported that was originally thought to be a tumor but proved to be a brain abscess caused by *P. stuartii*.[27]

DIAGNOSIS

Infection with *Providencia* spp. should be suspected when indole-positive, urease-negative, gram-negative rods, which oxidatively deaminate tryptophan, are isolated in culture. Because patients with long-term catheterization may be colonized with multiple organisms, *Providencia* spp. frequently are overlooked or misidentified.[7] Clinical laboratories should be encouraged to identify all bacterial colonies in patients with long-term catheterization in whom infection is suspected. Identification is particularly important because of the marked differences in susceptibility of uropathogens.[32]

TREATMENT

Empirical therapy should be guided by antimicrobial susceptibility testing of the patient's isolate and knowledge of susceptibilities of previously identified *Providencia* within the care facility. Removal of urinary tract catheters speeds eradication of these pathogens. Strains of *P. stuartii* and *P. rettgeri* commonly are resistant to many antibiotics. In a longitudinal Italian study of 223 *P. stuartii* isolates, 116 (52%) were extended-spectrum β-lactamase positive.[37] The rate of extended-spectrum β-lactamase–positive isolates increased from 31 percent in 1999 to 62 percent in 2002.[31] Since the 1970s, multidrug resistance has emerged[30,34]; many strains are resistant to sulfonamides, trimethoprim, nitrofurantoin, nalidixic acid, penicillins, cephalosporins, and aminoglycosides; some singular strains are resistant to most antibiotics in common use.[32] Imipenem-resistant strains have now been

described.[34] One case report describes an isolate that carried *blaPER-1*, *blaVIM-2*, and *armA* (a 16S methylase gene that confers resistance to aminoglycosides).[22] Much of the observed resistance seems to be plasmid based[11]; quinolones and aztreonam have shown some promise in the treatment of such cases.[6,9] Complex combinations of aminoglycoside resistance that differ by hospital and geographic region can occur.[25] Organisms that are resistant to gentamicin and tobramycin may remain susceptible to amikacin.

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

21. Lee G, Hong JH. Xanthogranulomatous pyelonephritis with nephrocutaneous fistula due to *Providencia rettgeri* infection. *J Med Microbiol* 2011;**60**:1050–2.
23. Maszewska A, Torzewska A, Staczek P, Tozalski A. Enterocyte-like Caco-2 cells as a model for in vitro studies of diarrhoeagenic *Providencia alcalifaciens* invasion. *Microb Pathog* 2010;**49**:285–93.
27. Muccio CF, Leonini S, Esposito G, Cerase A. Pyogenic abscess from *Providencia stuartii* mimicking necrotic tumor at perfusion-weighted imaging. *Neurol Sci* 2011;**32**:919–23.
35. Simon C, Dieli M, Brucato A, et al. Images in cardiovascular medicine. Bacterial pericarditis due to *Providencia stuartii:* an atypical case of relapsing pericarditis. *Circulation* 2010;**122**:e401–3.
36. Sipahi OR, Bardak-Ozcem S, Ozgiray E, et al. Meningitis due to *Providencia stuartii*. *J Clin Microbiol* 2010;**48**:4667–8.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 109

Shigella

Theresa J. Ochoa • Margaret Kosek

HISTORICAL BACKGROUND

The term *dysentery* classically has been used to describe the frequent, painful passage of stools containing blood and mucus. The syndrome has been recognized since the time of Hippocrates. The differentiation of dysentery into bacillary and amebic forms followed the recognition by Shiga in 1898 that one form of dysentery was associated with a bacterium in the stools of affected individuals; their sera also were found to agglutinate the bacillus. In recognition of Shiga's achievement, the genus was eventually named after him.

The most important subsequent advance has been the recognition of the molecular basis of *Shigella* virulence. In the 1960s, researchers showed that shigellae invade the corneal epithelium of guinea pigs and cause keratoconjunctivitis (Sereny test). Subsequently, Formal and colleagues[85] showed that *Shigella flexneri* invades the intestinal epithelium. Since 1980, Sansonetti and colleagues[231,233] and other investigators[235] have identified multiple plasmid and chromosomal virulence genes.

BACTERIOLOGY

Shigellae are small, nonencapsulated gram-negative rods that are members of the Enterobacteriaceae. Technically, they are *Escherichia coli*, but for reasons of tradition and clinical usefulness, the designation as a separate genus has been preserved. Shigellae do not ferment lactose or do so slowly and are nonmotile (they lack the H [flagellar] antigen). They lack urease and do not produce hydrogen sulfide on triple sugar iron media or gas during metabolism of carbohydrate.[78] The somatic antigen (or O antigen) side chains that determine serotype and serogroup are attached as multiple repeating units to the lipid A core and core oligosaccharides shared with other members of Enterobacteriaceae. Envelope or K antigens that are heat labile also have been described, although their clinical relevance is uncertain.

Serogroup Classification

Four serogroups, or species, of *Shigella* are defined on the basis of serologic similarities and biochemical reactions. Group A *(Shigella dysenteriae)*, mannitol nonfermenters, includes 13 serotypes having O antigens that do not cross react immunologically. Two additional provisional serovars, types 14 and 15, also are pathogenic.[11] Serogroup D *(Shigella sonnei)*, ornithine decarboxylase–positive, slow lactose fermenters, share the same lipopolysaccharide. *Shigella* strains that ferment mannitol (in contrast to *S. dysenteriae*) but do not decarboxylate ornithine or ferment lactose (in contrast to *S. sonnei*) are

REFERENCES

1. Albert MJ, Faruque AS, Mahalanabis D. Association of *Providencia alcalifaciens* with diarrhea in children. *J Clin Microbiol* 1998;**36**: 1433–5.
2. Albert MJ, Alam K, Ansaruzzaman M, et al. Pathogenesis of *Providencia alcalifaciens*–induced diarrhea. *Infect Immun* 1992;**60**: 5017–24.
3. Albert MJ, Ansaruzzaman M, Bhuiyan NA, et al. Characteristics of invasion of HEp-2 cells by *Providencia alcalifaciens*. *J Med Microbiol* 1995;**42**:186–90.
4. Bensman A, Roubach L, Allouch G, et al. Urolithiasis in children: presenting signs, etiology, bacteriology and localisation. *Acta Paediatr Scand* 1983;**72**:879–83.
5. Breitenbucher RB. Bacterial changes in the urine samples of patients with long-term indwelling catheters. *Arch Intern Med* 1984; **144**:1585–8.
6. Cox CE. Aztreonam therapy for complicated urinary tract infections caused by multidrug-resistant bacteria. *Rev Infect Dis* 1985; **7**(Suppl. 4):S767–71.
7. Damron DJ, Warren JW, Chippendale GR, et al. Do clinical microbiology laboratories report complete bacteriology in urine from patients with long-term urinary catheters? *J Clin Microbiol* 1986; **24**:400–4.
8. Ewing WH, Tanner KE, Dennard DA. The Providence group: an intermediate group of enteric bacteria. *J Infect Dis* 1954;**94**: 134–40.
9. Fang GD, Brennen C, Wagener M, et al. Use of ciprofloxacin versus use of aminoglycosides for therapy of complicated urinary tract infection: prospective, randomized clinical and pharmacokinetic study. *Antimicrob Agents Chemother* 1991;**35**:1849–55.
10. Fierer J, Ekstrom M. An outbreak of *Providencia stuartii* urinary tract infections: patients with condom catheters are a reservoir of the bacteria. *JAMA* 1981;**245**:1553–5.
11. Hawkey PM. *Providencia stuartii*: a review of a multiply antibiotic-resistant bacterium. *J Antimicrob Chemother* 1984;**13**:209–26.
12. Hawkey PM, Penner JL, Potten MR, et al. Prospective survey of fecal, urinary tract, and environmental colonization by *Providencia stuartii* in two geriatric wards. *J Clin Microbiol* 1982;**16**: 422–6.
13. Hollick GE, Nolte FS, Calnan BJ, et al. Characterization of endemic *Providencia stuartii* isolates from patients with urinary devices. *Eur J Clin Microbiol* 1984;**3**:521–5.
14. Jones BD, Mobley HL. Genetic and biochemical diversity of ureases of *Proteus*, *Providencia*, and *Morganella* species isolated from urinary tract infection. *Infect Immun* 1987;**55**:2198–203.
15. Jones BD, Mobley HL. *Proteus mirabilis* urease: genetic organization, regulation, and expression of structural genes. *J Bacteriol* 1988;**170**:3342–9.
16. Kaslow RA, Lindsey JO, Bison AL, et al. Nosocomial infection with highly resistant *Proteus rettgeri*: report of an epidemic. *Am J Epidemiol* 1976;**104**:278–86.
17. Keren G, Tyrrel DL. Gram-negative septicemia caused by *Providencia stuartii*. *Int J Pediatr Nephrol* 1987;**8**:91–4.
18. Kim BM, Kim NJ, Kim MN, et al. Bacteremia due to tribe Proteeae: a review of 132 cases during a decade (1991-2000). *Scand J Infect Dis* 2003;**35**:98–103.
19. Krake PR, Tandon N. Infective endocarditis due to *Providencia stuartii*. *South Med J* 2004;**97**:1022–3.
20. Lazimy Y, Delotte J, Machiavella JC, et al. Purple urine bag syndrome: a case report. *Prog Urol* 2007;**17**:864–5.
21. Lee G, Hong JH. Xanthogranulomatous pyelonephritis with nephrocutaneous fistula due to *Providencia rettgeri* infection. *J Med Microbiol* 2011;**60**:1050–2.
22. Lee HW, Kang HY, Shin KS, Kim J. Multidrug-resistant *Providencia* isolates carrying *blaPER-1*, *blaVIM-2*, and *armA*. *J Microbiol* 2007;**45**:272–4.
23. Maszewska A, Torzewska A, Staczek P, Tozalski A. Enterocyte-like Caco-2 cells as a model for in vitro studies of diarrhoeagenic *Providencia alcalifaciens* invasion. *Microb Pathog* 2010;**49**:285–93.
24. McHale PJ, Walker F, Scully B, et al. *Providencia stuartii* infections: a review of 117 cases over an eight-year period. *J Hosp Infect* 1981;**2**:155–65.
25. Miller GH, Sabatelli FJ, Hare RS, et al. The most frequent aminoglycoside resistance mechanisms—changes with time and geographic area: a reflection of aminoglycoside usage patterns? Aminoglycoside Resistance Study Groups. *Clin Infect Dis* 1997;**24**: S46–62.
26. Mobley HL, Chippendale GR, Tenney JH, et al. MR/K hemagglutination of *Providencia stuartii* correlates with adherence to catheters and with persistence in catheter-associated bacteriuria. *J Infect Dis* 1988;**157**:264–71.
27. Muccio CF, Leonini S, Esposito G, Cerase A. Pyogenic abscess from *Providencia stuartii* mimicking necrotic tumor at perfusion-weighted imaging. *Neurol Sci* 2011;**32**:919–23.
28. O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin Microbiol Rev* 2000;**13**:534–46.
29. Oreishi AF, Schechter BA, Karp CL. Ocular infections caused by *Providencia rettgeri*. *Ophthalmology* 2006;**113**:1463–6.
30. Overturf GD, Wilkins J, Ressler R. Emergence of resistant *P. stuartii* to multiple antibiotics: specification and biochemical characterization of *Providencia*. *J Infect Dis* 1974;**129**:353–7.
31. Penner JL. *Providencia*. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*, vol. 1. Baltimore: Williams & Wilkins; 1984. p. 494–6.
32. Piccolomini R, Cellini L, Allocati N, et al. Comparative in vitro activities of 13 antimicrobial agents against *Morganella-Proteus-Providencia* group bacteria from urinary tract infections. *Antimicrob Agents Chemother* 1987;**31**:1644–7.
33. Pignato S, Giammanco GM, Grimont F, et al. Molecular characterization of the genera *Proteus*, *Morganella*, and *Providencia* by ribotyping. *J Clin Microbiol* 1999;**37**:2840–7.
34. Shiroto K, Ishii Y, Kimura S, et al. Metallo-beta-lactamase IMP-1 in *Providencia rettgeri* from two different hospitals in Japan. *J Med Microbiol* 2005;**54**:1065–70.
35. Simon C, Dieli M, Brucato A, et al. Images in cardiovascular medicine: bacterial pericarditis due to *Providencia stuartii:* an atypical case of relapsing pericarditis. *Circulation* 2010;**122**:e401–3.
36. Sipahi OR, Bardak-Ozcem S, Ozgiray E, et al. Meningitis due to *Providencia stuartii*. *J Clin Microbiol* 2010;**48**:4667–8.
37. Tumbarello M, Citton R, Spanu T, et al. ESBL-producing multidrug-resistant *Providencia stuartii* infections in a university hospital. *J Antimicrob Chemother* 2004;**53**:277–82.
38. Vaidyanathan S, Soni BM. Bluish discolouration of urine drainage tube and bag in a female patient with spina bifida, paraplegia, and suprapubic cystostomy. *Sci World J* 2007;**3**:1070–2.
39. Vieira AB, Koh IH, Guth BE. *Providencia alcalifaciens* strains translocate from the gastrointestinal tract and are resistant to the lytic activity of serum complement. *J Med Microbiol* 2003;**52**:633–6.
40. Warren JW. *Providencia stuartii*: a common cause of antibiotic-resistant bacteriuria in patients with long-term indwelling catheters. *Rev Infect Dis* 1986;**8**:61–7.
41. Warren JW, Tenney JH, Hoopes JM, et al. A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. *J Infect Dis* 1982;**146**:719–23.
42. Yoh M, Matsuyama J, Ohnishi M, et al. Importance of *Providencia* species as a major cause of travellers' diarrhea. *J Med Microbiol* 2005;**54**:1077–82.

classified as serogroups B and C. Of these, the strains that express lipopolysaccharides that are related to each other immunologically are group B *(Shigella flexneri)*, whereas the strains whose O antigens are unrelated to each other or to other shigellae are group C *(Shigella boydii)*. Multiple serotypes of *S. flexneri* (15 serotypes and subtypes) and of *S. boydii* (18 serotypes) exist.

EPIDEMIOLOGY

Based on the findings of the Child Health Epidemiology Reference Group (CHERG) of the World Health Organization (WHO), it is estimated that globally 89 million community cases and 37 million outpatient cases of shigellosis occur annually in children younger than age 5 years.[140] Most of these cases occur in developing countries. In 1999, a review of the literature for 1966 through 1997 suggested that around 1.1 million persons die annually of shigellosis, including around 880,000 in Asia.[134] A recent review of the literature for 1990 through 2009 indicates that around 125 million shigellosis cases occur annually in Asia, of which 14,000 are fatal.[28] This estimate for illnesses is similar to the earlier estimate, but the number of deaths is 98 percent lower. The lower estimate of deaths is associated with markedly reduced case-fatality rates rather than fewer cases.

Generally, *Shigella* spp. are associated with 5 to 10 percent of all diarrhea cases and 30 percent of dysentery cases.[183] Although epidemic *Shigella* dysentery is the most dramatic manifestation of *Shigella* infections in developing countries, most infections are caused by endemic shigellosis. Despite the fact that severe dehydration usually is not seen in shigellosis, shigellosis is disproportionately represented in severe and fatal cases of diarrhea, particularly in children younger than 5 years old. The incidence of *Shigella* is increased by undernutrition,[131] and *Shigella* is one of the enteric infections that has shown to be most strongly correlated with linear growth deficits after infection.[41] *Shigella* is a common cause of prolonged and persistent episodes of diarrhea, and the dominant cause of the clinical dysentery syndrome in populations living in poverty or exposed to conditions where hygiene and water quality are suboptimal. Therefore, the empirical management of dysentery should always include an antibiotic that has been demonstrated to have low local levels of resistance.

According to surveillance reports of the Centers for Disease Control and Prevention, 10,000 to 15,000 cases of shigellosis have been documented each year during the past 30 years in the United States,[57] and it is currently estimated that nearly 500,000 cases occur annually in the United States, one fourth of which are domestically acquired.[236] *Shigella* is the third most important Foodborne Diseases Active Surveillance Network pathogen in 10 sentinel states in the United States, with a population-based incidence of 3.99 per 100,000 population. However, incidence rates have decreased significantly in 2009 when compared with rates documented in 1996 and 1998 (55% decrease).[58]

Because shigellae are spread through a fecal-oral route, they are especially prevalent where hygiene is poor. The organisms can be cultured from around toilets in homes where shigellae have caused disease. Toilet paper does not prevent contamination of fingers. Shigellae are transmitted easily from person to person because the inoculum size required to cause disease is only 10 organisms in the case of *S. dysenteriae* serotype 1[74] and a few hundred organisms in the cases of *S. sonnei* and *S. flexneri*.[72] Hand washing and wearing gloves are mandatory procedures for individuals caring for patients with bacillary dysentery. Patients who lack the acid barrier provided by a normally functioning stomach because of prior gastrectomy or use of antacids are at increased risk for acquiring infection.

Epidemics usually are associated with exposure to contaminated water or food, although as might be predicted from the inoculum size, outbreaks related to swimming also occur.[156] Houseflies can be mechanical vectors of shigellosis, particularly where the fly population is large. *Shigella* infection shows seasonal variation. In North America, few cases occur in the winter, whereas in tropical regions, the peak is during the rainy season. Shigellae are worldwide and thrive where susceptible individuals are grouped together (including institutions for retarded or mentally ill individuals, prisoner-of-war camps, Indian reservations, the military, daycare centers, and the developing world).[125,206,249] The devastating impact of *S. dysenteriae* type 1 epidemics in vulnerable concentrated populations is still notable as can be seen in refugee camps in Goma and Tanzania in the 1990s where attack rates ranged from 6.3 to 39.1 percent and case-fatality rates were estimated to be as high as 9 percent at some camps.[92,124]

The species of *Shigella* causing most infections varies according to regions. In developed countries, *S. sonnei* is the most common species, followed by *S. flexneri* (approximately 70% *S. sonnei* and 25% *S. flexneri*); however, in the developing world, this pattern is reversed, with *S. flexneri* being more common than *S. sonnei*. *S. dysenteriae* serotype 1 occurs primarily in Africa, India, and Bangladesh. *S. boydii* is found primarily on the Indian subcontinent.[185] However, *Shigella* has shown temporal procession in serogroup dominance. Recently, epidemiologic transition has favored the emergence of *S. sonnei* as the dominant serogroup in some countries (i.e., Israel, Argentina, Vietnam), although the reason for this is not clear.[130] A large molecular epidemiology investigation in China has provided convincing evidence for numerous serotype switching events, as well as changing antibiotic resistance, of an emergent sequence type that has replaced *S. flexneri* 2a as the dominant serotype.[265]

Humans and other primates can be infected with shigellae, and an age-related risk for acquiring symptomatic shigellosis exists. In contrast to *Salmonella* spp., which causes disease most frequently in the first few months of life, shigellae infrequently cause illness in the first 6 months of life. The peak incidence occurs in children between 1 and 4 years of age, with fewer cases occurring in children aged 5 to 9 years old. Adults are at lower risk.

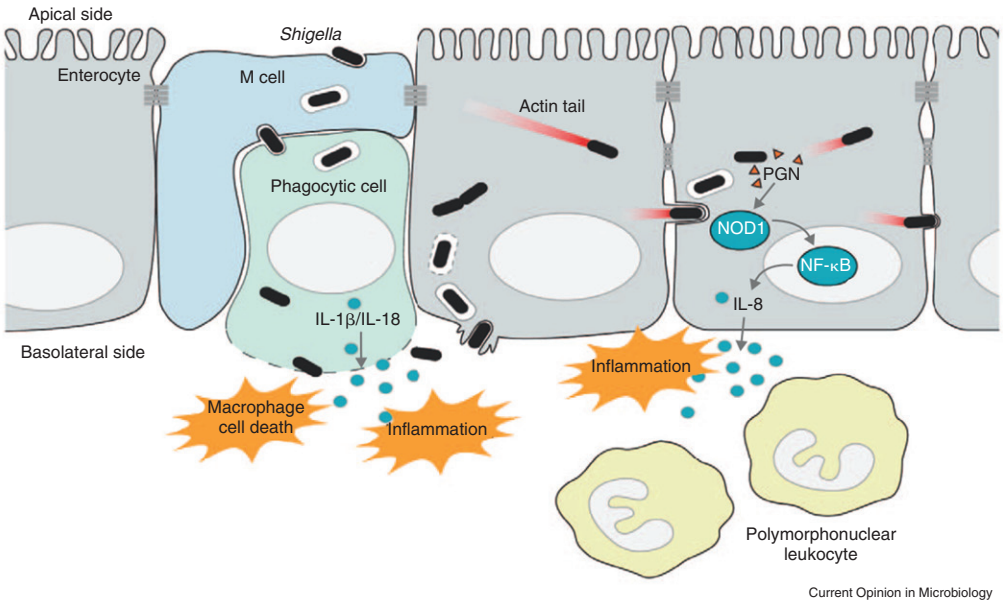

FIGURE 109-1 ■ Cellular pathogenesis of *Shigella* spp. *Shigella* passes the epithelial cells barrier by transcytosis through M cells and encounters resident macrophages. The bacteria evade degradation in macrophages by inducing an apoptosis-like cell death, which is accompanied by proinflammatory signaling. Free bacteria invade the epithelial cells from the basolateral side, move into the cytoplasm by vectorial actin polymerization, and spread to adjacent cells. Proinflammatory signaling by macrophages and epithelial cells further activates the innate immune response and attracts polymorphonuclear leukocytes. (See companion website on Expert Consult for a color version of this figure.) (From Ashida H, Ogawa M, Kim M, et al. *Shigella* deploy multiple countermeasures against host innate immune responses. *Curr Opin Microbiol* 2011;**14**:16-23.)

PATHOGENESIS

Invasiveness and Toxin Production

The ability to invade mammalian cells is the most important virulence trait of *Shigella* spp.* Uptake by M cells overlying Peyer patches with ingestion by macrophages under the M cells induces production of cytokines and recruitment of polymorphonuclear leukocytes (Fig. 109-1). Apoptosis is induced in macrophages after ingestion of shigellae; these events are accompanied by release of interleukin (IL)-1, IL-1β, and IL-18, which triggers other inflammatory events.[233,272,274] Polymorphonuclear leukocytes enter the gut lumen by moving between epithelial cells. The gaps between epithelial cells may be the portal of entry of bacteria into the epithelium. After penetration of intestinal epithelial cells, shigellae are located in vacuoles derived from the cytoplasmic membrane of the mucosal cells. The bacteria lyse these vacuoles, move intracellularly, multiply, kill the epithelial cells, and infect adjacent cells. Cell death is followed by formation of ulcerations and microabscesses in the colon. In contrast to *Salmonella* infection, *Shigella* infection rarely spreads beyond the lamina propria, so bacteremia and metastatic infections are uncommon.

The genetic basis of virulence has been studied extensively.[192,196] Invasiveness is the result primarily of genes on a large virulence plasmid (200 kb), containing a mosaic of around 100 genes and insertion sequences.† These genes encode a molecular apparatus called a type III secretion system (TTSS) that is capable of injecting bacterial proteins through bacterial and host membranes into host cells (translocation) or the extracellular milieu (secretion) to influence host biochemistry and cell physiology directly.[196] More than 30 effector proteins of the TTSS have been identified and their virulence function and phenotype described.[191,237] Recent structural and functional studies suggest that two different sets of effectors are involved in inducing actin cytoskeleton reorganization to promote entry of bacteria into epithelial cells and in modulating cell signaling pathways to dampen innate immune responses induced on infection, respectively. Effectors involved in entry are produced independently of the TTSS activity, whereas effectors involved in controlling the cell responses are produced on activation of the TTSS.[197] The invasive plasmid antigen (ipa) region includes genes for four polypeptides needed for invasion: *ipaA*, *ipaB*, *ipaC*, and *ipaD*. The proteins produced by these loci are recognized by the humoral immune system. The product of the *ipaB* locus is essential for induction of apoptosis in macrophages.[273] The IpaH protein family has E3 ubiquitin ligase activity.[219] The mxi-spa region of the virulence plasmid is necessary for orientation of the ipa-encoded proteins into the outer membrane of the bacteria. The *virG* gene encodes a protein that causes intracellular and intercellular spread of shigellae after invasion of epithelial cells. The *virF* gene regulates a locus *(virB)* that is responsible for positive regulation of the ipa genes.[223]

Chromosomal loci regulate expression of the virulence plasmid.[195] The keratoconjunctivitis provocation *(kcpA)*

*References 90, 139, 147, 180, 190, 237.
†References 98, 99, 104, 230-232, 235, 237.

gene is a positive regulator of the *virG* virulence plasmid gene that determines ability to spread within and between cells. Lipopolysaccharides play a role in resistance to nonspecific host defense mechanisms that are encountered during invasion of tissue. The *Shigella* lipopolysaccharides induce the trafficking of Toll-like receptor 4, the dominant mediator of the innate immune response.[52] Smooth colonies express the complete complex of lipopolysaccharide O side chains required for full virulence—the ability to invade epithelial cells, to multiply within them, and to resist phagocytosis.[94,193] Rough colonial variants that lack complete lipopolysaccharides do not penetrate epithelial cells efficiently and are avirulent.

Some strains of *Shigella* spp. produce toxins that injure mammalian cells. A chromosomal locus in *S. dysenteriae* serotype 1 encodes a protein synthesis–inhibiting exotoxin (Shiga toxin) that is a major virulence factor in this serotype (and in the enterohemorrhagic *E. coli* serotypes that have the same or closely related genes).[31,242] This toxin is composed of a single copy of an A subunit (32,000 daltons) that is linked to five copies of B subunits (7,790 daltons).[187] The B subunits bind to a glycolipid cell receptor, globotriaosylceramide, followed by internalization. The A subunit cleaves an adenine residue from the eukaryotic 28S ribosomal subunit. The resulting block in elongation factor 1–dependent binding of aminoacyl tRNA to the ribosome causes cell death through inhibition of protein synthesis.[187] Shiga toxin previously was considered to be a neurotoxin because its administration to mice or rabbits caused paralysis and death[46,56,107]; it now is thought to target primarily the vascular endothelium. It causes fluid accumulation in rabbit ileal loops[80,126] that probably is related to reduced fluid uptake by damaged villus cells.

The severity of *S. dysenteriae* serotype 1 infection relative to other *Shigella* serotypes is thought to be caused by production of Shiga toxin. Enterohemorrhagic *E. coli* serotypes, such as *E. coli* O157:H7, produce an identical (Shiga toxin 1) or similar (Shiga toxin 2) toxin and, similar to *S. dysenteriae* serotype 1, cause bloody diarrhea and hemolytic-uremic syndrome. Enteroinvasive *E. coli* serotypes do not produce these toxins.[64]

Shigella spp. also produce two enterotoxins, ShET-1 and ShET-2.[257] All *S. flexneri* 2a strains produce ShET-1, whereas only 3.3 percent of other *Shigella* serotypes and no enteroinvasive *E. coli* serotypes possess the gene for this toxin.[184] ShET-2 is produced by all *Shigella* spp.[257] Genes of an additional toxin, designated *Shigella* enterotoxin, or Sen, have been found in 75 percent of enteroinvasive *E. coli* serotypes and 83 percent of *Shigella* strains.[177] These enterotoxins may contribute to the high-volume, watery diarrhea often seen in the initial stages of the disease.[23] Vaccine studies using a mutant with deletions of these toxin genes have shown that one or both enterotoxins play a role in human disease.[133]

Immune Response

Serum IgG, IgM, and IgA responses occur to the lipopolysaccharide and invasion plasmid antigens of shigellae.[189] Secretory IgA specific to both of these sets of antigens occurs in human milk and feces.[65] Protection is thought to be serotype specific. The level of IgG antibodies to lipopolysaccharides present in an individual before infection occurs determines whether symptomatic shigellosis develops.[69] No clear proof currently exists about the role of antibodies to the invasion plasmid virulence proteins or to the toxins. Cell-mediated immunity against shigellae also may play a role in resolution of infection. Production of αβ T cells and natural killer cells mediates interferon-γ production that may be essential to defense from *Shigella*.[142,262] Antibody-dependent cellular cytotoxicity against shigellae has been shown.

During infection, shigellae use several effectors to direct various cellular signaling pathways and to modify the innate immune activation of the host.[192,202] Several bacterial components such as lipopolysaccharides, peptidoglycan, and effector proteins delivered via the TTSS (i.e., rod protein)[168] are recognized as pathogen-associated molecular patterns (PAMPs) by Nod-like receptors (NLRs), including NOD1, NOD2, NLRP3 (NALP3), and NLRC4 (Ipaf), which stimulate an array of host innate immune systems that leads to the production of proinflammatory cytokines, chemokines, and antimicrobial peptides.[14,15] In addition, the remnants of the host cell membrane that result from the disruption of the phagocytic vacuolar membrane may also stimulate inflammatory responses.[75] All this causes activation of the nuclear factor κB and other terminal kinase pathways, with IL-8 appearing as a major chemokine mediating the inflammatory burst that is dominated by massive infiltration of the mucosa by polymorphonuclear leukocytes.[202] The levels of cytokines tumor necrosis factor-α, IL-1β, IL-1RA, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor in stool correlate with the severity of shigellosis. In contrast to other cytokines, interferon-γ and its receptor[214] are depressed early and increase during recovery. Fecal concentrations of tumor necrosis factor-α, IL-1β, IL-1RA, IL-6, IL-8, and granulocyte-macrophage colony-stimulating factor are significantly higher in patients with *S. dysenteriae* serotype 1 than in patients with *S. flexneri* infection.[216] Elevated tumor necrosis factor-α and IL-6 levels in stool and serum have been associated with complications during infection with *S. dysenteriae* serotype 1.[240] Infiltration of polymorphonuclear leukocytes and lymphocytes into the intestine is controlled by these cytokines. Epithelial cell production of IL-8 and a low ratio of IL-1RA to IL-1 are responsible for the severe inflammation.[13,229] T lymphocytes with suppressor-cytotoxic and helper-inducer phenotypes are recruited into the epithelium and lamina propria during infection with *Shigella*, related partly to the induction of HLA-DR expression in the rectal mucosa.[215] Peripheral blood lymphocytes of infected individuals respond to *Shigella* antigens in vitro with production of interferon-γ and IL-10.[192,228] The delivery of as yet uncharacterized TTSS-secreted proteins enables *Shigella* to suppress the transcription of genes that encode antimicrobial peptides, which promotes the advance of bacteria deeper into the crypts in the intestine.[245]

Shigella spp. elicit an inflammatory response during multiplication within macrophages and epithelial cells.

Shigella multiplication in macrophage cytoplasm induces cell death, which is achieved by two distinctive pathways: activation of caspase-1 by IpaB secreted by the TTSS and translocation of lipid A from the cytosol, which occurs independent of caspase-1 and Toll-like receptor 4 activities. Recent studies have characterized *Shigella*-induced macrophage cell death as pyroptosis or pyronecrosis, which is accompanied by severe inflammation.[14]

PATHOLOGY

The main morphologic changes of shigellosis (superficial ulcerations, focal hemorrhages, mucosal edema, erythema, and friability) occur in the colon where the organisms invade.[111,243] The rectosigmoid and distal segments of the colon typically are involved more severely than is the proximal colon. The epithelial cell damage may cause development of a pseudomembrane composed of thick, fibropurulent exudate tightly adherent to the necrotic ulcerated colonic mucosa. Pseudopolyposis also has been reported.[48] On microscopic examination, damage to epithelial cells, ulcerations, goblet cell depletion, and intense polymorphonuclear and mononuclear infiltration with crypt abscesses are seen. Rectal mucosa shows increased numbers of $CD8^+$ and $\gamma\delta^+$ T cells.[110] Vessels in the lamina propria are congested or thrombosed. Perforation of the colon usually does not occur as part of the colitis.[27] Evidence of inflammation and proinflammatory cytokines persists for at least 1 month after clinical resolution.[213] Histologic changes generally are more severe and persistent[96] with *S. dysenteriae* than with *S. flexneri*.[10]

CLINICAL MANIFESTATIONS

The incubation period ranges from 12 hours to a few days (if a low number of organisms is ingested). Onset of high fever, toxicity, and crampy abdominal pain is sudden. During the first 48 hours, high-volume watery diarrhea may occur (small bowel phase of disease); subsequently, low-volume, bloody, and mucous diarrhea develops in association with urgency and tenesmus (large bowel or "dysenteric" disease). In some cases, the watery diarrhea persists for several days without subsequent development of dysentery. Other children may have bloody or mucous diarrhea.

Physical examination shows fever, signs of toxicity, tenderness over the lower abdominal quadrants, and hyperactive bowel sounds. Signs of dehydration may be present. Rectal examination reveals severe tenderness. Rectal prolapse may be present, particularly when diarrhea is associated with malnutrition.

The course without therapy typically lasts 7 to 10 days. Although it is true that many enteric infections exacerbate undernutrition, initiating a "viscous cycle" of diarrhea and undernutrition, *Shigella* infections are particularly likely to do so. *Shigella* incidence is increased in underweight children.[131] *Shigella* causes a severe protein-losing enteropathy[42] (which is lessened by early appropriate antibiotic therapy)[38] and wasting of large amounts of vitamin A and zinc.[55,170] Shigellosis is associated with prolonged and persistent illness and linear growth shortfalls in children.[41]

TABLE 109-1 **Complications of Shigellosis**

Abdominal
Persistent diarrhea
Postdysenteric irritable bowel syndrome
Ileus, toxic megacolon, intestinal perforation
Protein-losing enteropathy, malnutrition
Surgical complications: intestinal perforation and obstruction, appendicitis, intra-abdominal abscesses
Neurologic
Seizures
Headache, lethargy, disorientation, hallucinations
Coma
Severe toxin encephalopathy or ekiri syndrome
Bacteremia
In malnourished children, young infants, and AIDS patients
Hemolytic-Uremic Syndrome
Only with *Shigella dysenteriae* serotype 1
Urogenital
Vulvovaginitis, urinary tract infections
Other
Conjunctivitis, keratitis, corneal ulcers
Reactive arthritis
Reiter syndrome
Hepatitis
Myocarditis

The need for surgical intervention in shigellosis is rare but is associated with significant morbidity and mortality. In a series of 57 children with complications of shigellosis requiring surgical intervention,[169] intestinal obstruction (53%), appendicitis (28%), and colonic perforation (17%) were the most common occurrences (Table 109-1).

Although the organism usually is excreted for only a few days or weeks (range, 1 to 30 days), carriage of the organism for many months sometimes occurs.[67,148] The illness usually is acute; a chronic carrier state can occur in malnourished individuals. Asymptomatic infection of toddlers living in an endemic area occurs commonly and is usually transient, lasting no more than a few days.

Extraintestinal Manifestations and Complications

Multiple extraintestinal complications have been described with *Shigella* infection (see Table 109-1). Seizures have been reported in 10 to 45 percent of hospitalized children with culture-proven shigellosis.[21,25,30,71,83] In outpatient settings, the frequency of seizures is very low. Children who develop neurologic complaints may have lethargy, severe headache, disorientation, hallucinations, or self-limited convulsions lasting less than 15 minutes.[19,22,25,174] Seizures are most likely to occur in very young patients, patients with a high peak body temperature, and patients with a family history of convulsive disorders.[21,135] Seizures can be focal, although usually they are generalized.

When symptoms related to the nervous system occur, they are likely to appear early in the illness, even preceding development of diarrhea. Death rarely has been described[106]; most children recover completely with no residual neurologic deficits.[22] In contrast, in Bangladesh, where seizures in shigellosis typically are associated with factors known to alter consciousness (e.g., hypoglycemia, hyponatremia, fever), mortality rates are high.[129] Children with *Shigella* encephalopathy have significantly higher case-fatality rates than children with shigellosis without encephalopathy.[60] The pathogenesis of neurologic signs and symptoms during episodes of shigellosis is unclear. Hypoglycemia and electrolyte abnormalities are found in a few patients.[22] Direct invasion of the central nervous system during *Shigella* bacteremia is very rare.[263] Simple febrile seizures might explain convulsions in a few children with dysentery, but some children who have seizures during episodes of shigellosis do not experience seizures during other febrile infections and are outside the age range usually associated with febrile seizures.[22] Shiga toxin formerly was thought to cause the neurologic symptoms because it was considered to be a neurotoxin; however, data now clearly show that Shiga toxin is not responsible.[20] In an animal model of *Shigella*-related seizures, corticotropin-releasing hormone, a stress neurohormone, was associated with increased susceptibility to seizures after exposure to *S. dysenteriae*.[268] Work from the same group has demonstrated that proinflammatory mediators are involved in *Shigella*-related seizures[267]; however, these researchers found that prostaglandins, which are host mediators induced during the inflammatory process, are not involved in the enhanced seizure response after exposure to *Shigella*.[269]

Severe toxic encephalopathy has been described. This syndrome (ekiri), as originally described in Japan, was characterized by dysentery with hyperpyrexia, convulsions, sensory disturbances, and rapid progression to death.[224] The children died of cerebral edema early in the course of disease (6 to 48 hours after onset). Mild hyponatremia has been a common finding.[93] Children with ekiri did not have sepsis, disseminated intravascular coagulation, hemolytic-uremic syndrome, or severe dehydration. This toxic encephalopathy is rare. Whether this syndrome is part of a continuum of central nervous system dysfunctions with seizures and other encephalopathic symptoms or has a completely different pathogenesis is unclear.

Hemolytic-uremic syndrome (microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure) or isolated hemolysis has been reported, mainly after infections with *S. dysenteriae* serotype 1 and rarely after infection with *S. flexneri*.[211] Vascular endothelial cell damage by Shiga toxin is considered to be the initial event, although endotoxin absorbed from the gut also may play a role.[132]

Ileus that progresses to toxic megacolon with distended loops and eventual intestinal perforation occurs.[26] It is seen mainly with *S. dysenteriae* serotype 1 infection.

Septicemia in shigellosis is a rare occurrence except in human immunodeficiency virus (HIV)-infected patients, malnourished children, young infants, and children with *S. dysenteriae* serotype 1 infections.[100,123,164,238] The mortality rate is at least twice as high in dysentery-associated sepsis as in shigellosis uncomplicated by bacteremia. The serogroup-related mortality risk in cases of *Shigella* bacteremia has been reported to be 85 percent for infection with *S. dysenteriae*, 43 percent for infection with *S. flexneri*, and 25 percent for infection with *S. sonnei;* bacteremia rarely is reported with *S. boydii* infection.[161] In Bangladesh, *Shigella* bacteremia was found in 4 percent of patients.[250] When bacteremia occurs with dysentery, it is as likely to be caused by other enteric bacteria as by the *Shigella* itself. The occurrence of *Klebsiella*, *E. coli*, and other enteric pathogens in blood cultures of children with shigellosis presumably reflects loss of the barrier function during severe colitis.[179] *Shigella* bacteremia may be complicated by disseminated intravascular coagulation and multiorgan failure. Bronchopneumonia may develop in septicemic children,[5] but its pathogenesis is unclear. Children who die of shigellosis often have pneumonia at autopsy.[48] HIV-infected patients with systemic shigellosis are more likely to die than patients not infected with HIV.[123]

Other extraintestinal infections rarely are caused by *Shigella* spp. Vaginitis with a bloody discharge (sometimes lasting for months in the absence of specific therapy) may occur, usually without concurrent or recent diarrhea.[70,176] *Shigella* cystitis, not always associated with diarrhea, has been described, usually in girls.[30] Conjunctivitis, keratitis, corneal ulcers, and iritis are other uncommon manifestations of shigellosis that usually are assumed to occur after autoinoculation.[30,253] Hepatitis with mildly abnormal liver function test results has been described.[248] Myocarditis manifested clinically by hypotension despite fluid replacement, arrhythmia, heart block, or low voltage on electrocardiography and pathologically by interstitial lymphocytic infiltrates and focal necrosis has been described.[221]

Postinfectious complications of shigellosis include reactive arthritis and postinfectious irritable bowel syndrome. Reactive arthritis may develop after infections with enteric pathogens such as *Shigella*, *Campylobacter*, *Salmonella*, or *Yersinia*.[53] However, the lack of a clear case definition precludes assessing the real disease burden of postinfectious arthritis. In general, reactive arthritis after shigellosis occurs especially in adults who are HLA-B27 positive; is uncommon in children[50]; and is unlikely to be associated with *Shigella* vaccines in developing countries.[88] Postdysenteric irritable bowel syndrome occurs in some patients after recovery.[261] In a recent case-control study of children in Italy, 43 percent of children with prior *Shigella* infections were found to have symptoms of postinfectious irritable bowel syndrome versus 11 percent of controls.[234] However, in a study conducted in the Netherlands the prevalence of postinfectious irritable bowel syndrome after *Campylobacter*, *Salmonella*, or *Shigella* infections was 9 percent.[97]

Shigellosis in the Neonatal Period

Bacillary dysentery is rare in neonates.[31,76,81,101,141] More than half of the reported neonatal cases occurred during the first 3 days of life, which is consistent with fecal-oral

transmission during delivery,[95,181] usually from a symptomatic mother. Although a neonate with shigellosis usually has only low-grade fever with diarrhea of variable severity,[6,137,172] septicemia[145,222] and chronic diarrhea are more common than in older children. Intestinal perforation has been reported in neonatal shigellosis.[247] Diarrhea more often is nonbloody in infants; fever also occurs less commonly than in older children.[109] Data on age-related mortality caused by shigellosis in developing countries suggest that the mortality rate of the neonate is more than twice that of older children.

Shigellosis in Acquired Immunodeficiency Syndrome

In patients with acquired immunodeficiency syndrome (AIDS), shigellosis not only is more common and more severe but also is associated more often with bacteremia.[32,108,123] In contrast to the usual short, self-limited course of shigellosis, *Shigella* infection in patients with AIDS may be chronic and relapsing, despite appropriate antibiotic treatment.[138,241]

LABORATORY FINDINGS

The fecal leukocyte examination is helpful in evaluating a patient with fever and watery diarrhea. Direct microscopic examination of fecal mucus stained with methylene blue shows many polymorphonuclear leukocytes in patients with colitis, including most patients with shigellosis.[103] The blood leukocyte count in patients with shigellosis often is normal, although leukopenia or leukocytosis may occur.[18] The differential of the blood leukocyte count typically shows an increased percentage of band forms. Approximately one third of children with shigellosis have more bands than segmented neutrophils in their peripheral blood smear. A leukemoid reaction, with a peripheral leukocyte count greater than 50,000/mm^3, has been reported, mainly with infections caused by *S. dysenteriae* serotype 1.[49] A leukemoid reaction has been reported in 10 percent of patients infected with the Shiga bacillus. When examination of cerebrospinal fluid is performed in children with neurologic symptoms, normal results usually are obtained, although some patients have a mild lymphocytic pleocytosis. Likewise, when electroencephalography is performed, the results usually are normal.[22]

DIAGNOSIS

Bacillary dysentery usually is suspected in children who present with bloody diarrhea, high fever, tenesmus, and generalized toxicity. Approximately half of children do not develop bloody diarrhea during the course of their disease. This fact is especially relevant in developed countries, where most infections are caused by *S. sonnei*. The presence of only watery diarrhea does not exclude the possibility of shigellosis in an ill patient with high fever.

Isolation Techniques

Proof of the diagnosis of suspected bacillary dysentery often is problematic. Definite diagnosis of *Shigella* infection depends on isolation of the organism from stool specimens or rectal swabs. The bacteria may not survive in fecal specimens during transit, however, and special selective media are necessary for isolation. Recovery of shigellae is easier early in the course of the disease than later because the number of viable organisms in stools decreases significantly during late stages of the disease. Even in adult volunteer studies, when appropriate stool cultures were obtained daily, cultures failed to isolate shigellae in approximately 20 percent of volunteers who had ingested the organism and developed diarrhea.

Several measures increase the likelihood of isolating *Shigella*. Specimens should be processed without delay. Pre-enrichment broth does not significantly impact isolation rates, and the main determinant of success in isolating *Shigella* is rapid processing time (time between voiding and storage in an appropriate transport medium) and minimal time to primary plating. If a specimen cannot be processed immediately, a transport medium, such as buffered glycerol saline or Cary-Blair, should be used. More than one stool culture or rectal swab should be obtained and inoculated promptly onto at least two different culture media. Specimens should be plated lightly onto MacConkey, xylose-lysine-deoxycholate, or Hektoen enteric agar, whereas a heavier plating is necessary for the more inhibitory *Shigella-Salmonella* medium.[173,252] After overnight incubation at 37° C, lactose-negative colonies are transferred to triple sugar iron and lysine iron agar slants and incubated again overnight. Slants showing characteristic reactions of alkaline red slants, acid butt, and production of gas are tested biochemically for presumptive identification and then serologically for definitive identification.

Other Diagnostic Methods

Because presumptive identification of *Shigella* takes at least 48 hours and definite identification takes approximately 72 hours, attempts to develop rapid diagnostic methods are being made, especially because institution of early treatment is important in shigellosis. Identification of shigellae by specific DNA probes with use of either restriction fragments or synthetic oligonucleotides[182] based on detecting virulence genes located on the 120- to 140-MDa plasmid has been described. Because the invasion plasmids of shigellae, similar to other plasmids, may be lost spontaneously, a DNA probe derived from *S. flexneri ipaH* gene, a multicopy element that is found on the chromosome and on the invasion plasmid of shigellae, has been developed and found to be more sensitive than the probes that were used previously.[258] Use of polymerase chain reaction (PCR) in vitro amplification of nucleic acids, to detect shigellae directly from stool specimens, has been reported.[86] PCR can detect as few as 10 colony-forming units of *S. flexneri* in stool specimens, whereas the sensitivities of DNA probe hybridization (with no amplification) and standard

biochemical methods are 10^3 and 10^6 colony-forming units, respectively. PCR for *ipaH* genes seems to be more sensitive than culture; 28 percent of patients with dysentery with a positive PCR assay result are culture negative.[89,113] Another new method for the identification of *Shigella* is microarray analysis using the *gyrB* genes.[118] This novel approach also renders it possible to identify simultaneously other enteric pathogens, such as *Salmonella* spp. and diarrheogenic *E. coli*.[118]

Serologic studies are not helpful in establishing the diagnosis of shigellosis in individual patients; humoral antibodies develop after clinical recovery. Serologic studies may be helpful, however, in epidemiologic studies to define the level of endemicity of shigellosis.

Sigmoidoscopy and barium enema study are unnecessary, unless they are indicated to rule out other conditions. When these procedures are performed in patients with a possibility of having shigellosis, caution is necessary because of the diffuse acute colitis and risk for perforation.

DIFFERENTIAL DIAGNOSIS

Colitis of any etiology manifesting as acute-onset bloody diarrhea with fever and abdominal cramps can mimic shigellosis. Etiologic agents to be considered include *Campylobacter* spp., *Salmonella* spp., *Clostridium difficile*, *Yersinia enterocolitica*, *Shigella* spp., *Vibrio parahaemolyticus*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli* (e.g., serotype O157:H7), *Balantidium coli*, and *Entamoeba histolytica*. The initial presentation of inflammatory bowel disease can mimic shigellosis.

An etiologic diagnosis of acute colitis syndrome on the basis of clinical presentation is difficult to make, although some data suggest a specific causative agent. In developed countries, *Campylobacter* is the most common cause of acute infectious colitis. Shigellosis should be suspected when evidence exists of person-to-person spread and when convulsions or other neurologic symptoms develop. A history of previous antibiotic treatment suggests diarrhea related to *C. difficile*, and previous consumption of seafood suggests *V. parahaemolyticus*. *Yersinia* infections are found mainly in the cooler regions of Europe and North America; the disease may mimic acute appendicitis because of the right lower quadrant pain associated with mesenteric lymphadenitis.

Enterohemorrhagic *E. coli* infection often causes bloody diarrhea with little or no fever, in contrast to shigellosis, in which high fever is typical. Negative stool cultures for the mentioned bacterial pathogens may suggest infection by enteroinvasive *E. coli*. Amebiasis causes a colitis similar to the colitis caused by *Shigella*,[244] although it is of slower onset, with a lower degree of fever; the findings on fecal leukocyte examination are negative. The involvement of the colon with amebiasis is less diffuse than in shigellosis; areas of normal mucosa are found between ulcerations.

A prolonged course with negative cultures should raise concern about the possible presence of either ulcerative colitis or Crohn disease. When watery diarrhea is present, the list of possible etiologic agents is even longer, although many agents that cause watery diarrhea are associated with little or no fever and are not often confused with shigellosis. The diagnosis usually cannot be made by clinical presentation alone and requires laboratory confirmation.

TREATMENT

Fluid Administration

Dehydration is less a problem with shigellosis than with rotavirus or toxigenic *E. coli* infection. Some children with shigellosis, particularly young infants, have dehydration during the course of the disease. The high-volume watery diarrhea seen early in the course of the disease may cause excess losses of fluids and electrolytes; likewise, in patients with severe colitis, systemic toxicity and vomiting may cause anorexia that interferes with fluid intake.

Assessment of the hydration status of the patient on admission is mandatory, with early institution of appropriate fluid and electrolyte therapy needed. Low osmolarity oral rehydration (total osmolarity 245 mOsm/L, 75 mEq sodium, 20 mEq potassium, 10 mmol/L citrate, 75 mmol/L glucose), an improved choice especially in undernourished children, should be administered when available. Oral rehydration with commercially available solutions (e.g., Pedialyte) also is acceptable, and continued feeding is to be encouraged whenever possible. Administration of intravenous fluid therapy is necessary in children who are comatose, have an ileus, or are in shock. Early (12 to 24 hours after oral fluids are begun) breast-feeding and age-appropriate feeding in these children is a priority, especially in undernourished patients. Children with severe disease, seizures, and/or obtundation should have glucose and serum sodium levels monitored because hyponatremia and hypoglycemia are treatable complications seen in severe disease and possibilities to consider in severe cases or in patients who decompensate while receiving appropriate antibiotic therapy.

Antibiotic Therapy

Children who have dysentery always should be treated empirically with antimicrobial agents. WHO guidelines suggest that presumptive shigellosis should be treated with antibiotics, the choice being decided by the antimicrobial susceptibility pattern of locally circulating *Shigella* strains (WHO/CDR/95.3). If after 2 days of therapy, the patient's condition improves, a full course of therapy for 5 days should be given. If the patient does not improve, the antibiotic should be changed. If after the second antibiotic the patient does not show signs of improvement, the diagnosis must be reviewed, and stool microscopy, culture, and susceptibility testing should be performed where available.[183]

Appropriate antimicrobial therapy for shigellosis shortens the duration of fever and diarrhea and reduces

TABLE 109-2 **Antibiotic Treatment of Shigellosis in Children**

Antibiotic	Dosage	Comments
Azithromycin	12 mg/kg/day once daily PO on day 1, followed by 6 mg/kg/day once daily on days 2-5 (maximum 500 mg/day)	Although published data in children are limited, its use is recommended by many experts.
Nalidixic acid	55 mg/kg/day PO divided every 6 hr (maximum 2 g/day)	Not available in the United States; contraindicated in infants <3 mo old. Rates of resistance are rapidly increasing in Southeast Asia.
Ciprofloxacin	15-30 mg/kg/day PO divided every 12 hr (maximum 1.5 g/day)	Rates of resistance are increasing in Southeast Asia.
Ceftriaxone	50 mg/kg/day IM or IV (maximum 2 g/day)	Current drug of choice for empirical therapy for severe dysentery in children
Pivmecillinam	80 mg/kg/day divided four times a day	
Meropenem	120 mg/kg/day IV divided three times a day	Option for severe disease in patients with drug-resistant or suspected drug-resistant strains (from Southeast Asia)
Cefixime	8 mg/kg/day PO divided every 12-24 hr (maximum 400 mg/day)	Not recommended as therapy in adults

IV, intravenously; PO, orally.

the risk for developing complications.[16] Shedding of the organisms in stools stops within 1 to 2 days, so that intrafamilial spread may be decreased. Therapy should be stopped or changed on the basis of culture results (e.g., another pathogen or a resistant *Shigella* strain is isolated) and clinical response.

The choice of antimicrobial agent is complicated by the increasing frequency of antibiotic resistance.[79,82,130,227] Organisms resistant to ampicillin, trimethoprim-sulfamethoxazole, tetracycline, and chloramphenicol have been reported from the Middle East,* Africa,† South America,[151,177,185,209] Europe,‡ Eurasia,[27,194,270] Asia,§ and the South Pacific. Currently, ampicillin and trimethoprim-sulfamethoxazole are inappropriate for empirical therapy; they should be used only if the organism has been shown to be susceptible.[16]

Multiresistant strains also are found commonly in some parts of the United States. Among 344 *Shigella* isolates, from 1999 to 2001, 95 percent were resistant to one or more antimicrobial agents and 70 percent were resistant to two or more agents.[204] Among 1118 *Shigella* strains isolated from the Foodborne Diseases Active Surveillance Network and National Antimicrobial Resistance Monitoring System during 2000 through 2010, 74 percent of strains were resistant to ampicillin, 36 percent to trimethoprim-sulfamethoxazole, 28 percent to tetracycline, 2 percent to nalidixic acid, and 0.5 percent to ciprofloxacin. However, antimicrobial resistance differed by race, ethnicity, age, travel, and species.[239]

Multiresistant *Shigella* spp. are particularly likely to emerge in individuals who are exposed to multiple antibiotics[251] (e.g., patients with AIDS) and individuals who recently have traveled to areas with known resistance. Likewise, children in daycare centers are at risk for acquiring resistant organisms because the frequent use of antibiotics for otitis media may favor selection and emergence of resistant enteric organisms and because crowded living conditions and poor hygiene facilitate transmission.[45]

Generally, shigellae are susceptible in vitro to azithromycin, ceftriaxone, cefotaxime, cefixime, nalidixic acid, and quinolones. However, resistance to these antibiotics is being reported in several regions. Nalidixic acid–resistant *Shigella* has rapidly developed principally in Asia and Africa.[62,96] Resistance to ciprofloxacin is increasingly common in India and in travelers returning from India.[167,246] These fluoroquinolone-resistant strains have mutations in *gyrA* and *parC* and have developed efflux pumps.[200] Resistance to ceftriaxone[158,260] and azithromycin has also been reported in some countries.[44]

In vitro susceptibility does not always predict clinical efficacy or superiority of one drug over another, however. The first-generation and second-generation cephalosporins are active in vitro but have been ineffective in clinical trials. Clinical studies have shown some drugs to be clearly superior to others when susceptibility data have suggested that either might work well.

Prevalent serotypes and resistance patterns vary from year to year in a given locale. Typically, rates of resistance are related to the severity of disease caused by a given serotype. The more likely an organism is to cause severe disease, the more likely that resistant strains will emerge. *S. dysenteriae* serotype 1 is more likely to be multiply resistant than *S. flexneri; S. flexneri* is more likely to be resistant than *S. sonnei*. In Africa[43,121] and Asia,[34,36,63,157,175] *Shigella dysenteriae* resistant to nalidixic acid and ciprofloxacin have been reported. Nalidixic acid–resistant *S. flexneri* also have been recognized occasionally.[62] Local resistance patterns, history of travel to an area of frequent resistance,[251] and severity of illness should determine treatment.

Given the frequent occurrence of resistant organisms, optimal empirical therapy in children with dysentery should include azithromycin, a third-generation cephalosporin, nalidixic acid, or ciprofloxacin (Table 109-2).*

*References 2, 7, 12, 23, 80, 102, 118, 266.
†References 2, 43, 54, 121, 163, 178.
‡References 31, 59, 115, 119, 136, 159, 259.
§References 3, 8, 40, 127, 150, 152, 154, 157.

*References 16, 33, 77, 114, 143, 183, 204.

Although less well studied, ampicillin-sulbactam and pivmecillinam also have been shown to be effective in children. In children, oral cefixime seems to be superior to ampicillin-sulbactam.[105] Adults do not respond well to usual doses of cefixime.[226]

Local resistance patterns, history of travel to an area of frequent resistance,[251] and severity of illness should determine treatment. In the absence of local susceptibility data, the WHO recommendations should be followed.[254,264] First-line therapy is ciprofloxacin (in all age groups, including children), and second-line therapy is pivmecillinam (where available), ceftriaxone, or azithromycin. Use of quinolones in children has been controversial. An oral fluoroquinolone (ciprofloxacin, norfloxacin) seems to be optimal for adults.* A considerable body of evidence from the long-term use of these drugs in children with cystic fibrosis has been accumulated as has evidence from short-term use to treat typhoid fever and dysentery in children[143]; no evidence of bone or joint toxicity or growth impairment has been found, except for transient musculoskeletal events (arthralgia) in some children.[1,220] Nalidixic acid does not cause arthropathy or limit growth when it is used for a short time.[186]

Data for children suggest that norfloxacin at a dose of 10 to 15 mg/kg/day or ciprofloxacin at a dose of 10 mg/kg every 12 hours (maximum, 500 mg/dose) for 5 days is effective therapy.[93,144,155,225] In adults, short-course therapy (1 or 2 days) with ciprofloxacin has been effective for treatment of infections caused by *Shigella* spp. other than *S. dysenteriae*, for which a 10-dose, 5-day regimen is superior.[37] Pediatric data suggest that even for *S. dysenteriae* serotype 1 a short course of ciprofloxacin 15 mg/kg/dose every 12 hours for 3 days is as effective as a 5-day course.[271]

Because the patterns of antibiotic resistance of shigellae change, susceptibility testing should be performed on all clinical isolates if facilities are available, and the treatment should be changed accordingly. The recommended duration of antibiotic therapy for shigellosis usually is 5 days. Studies in adults and children have shown, however, that short-course treatment is nearly as effective as multiple doses in terms of symptomatic improvement,[91,160] although eradication of the organism from stools is less likely to occur with a single-dose regimen.[116] Because a major goal of antibiotic therapy is to reduce person-to-person transmission, multiple doses are preferred.

After initiation of therapy, a resistant organism can be suspected in the event of persistence of fever, grossly bloody stools, or unchanged frequency of stools by day 3 of therapy.[112] Persistent presence of numerous fecal leukocytes (>50 per high-power field) and erythrocytes (>5 per high-power field) at day 5 also suggests resistance. These findings are important because morbidity and mortality rates are higher when the organism is not susceptible to the initial drug of choice.[117] Protein-losing enteropathy is a more likely occurrence with resistant *Shigella* spp. if an inadequate agent has been used.[38]

*References 17, 33, 39, 105, 144, 160, 208.

Adjunctive Therapy

As with other forms of infectious colitis, antimotility agents should be avoided. Antimotility drugs, such as diphenoxylate (Lomotil), prolong the duration of fever, diarrhea, and excretion of the organism.[73] It has been speculated that intestinal motility and the constant fluid flow are important host defense factors for clearing of the organism and recovery from the infection.

Nutritional therapy is a key in undernourished children living in poverty, where the burden of global *Shigella* infections is focused. A high-protein, high-calorie diet during convalescence is important, and children should receive at least one additional meal a day until pre-illness weight is achieved. Nutritional therapy based on green bananas, a food rich in amylase-resistant starches, has been shown to be more efficacious than diet therapy with rice- or yogurt-based therapies in different trials in Bangladesh and Venezuela.[9,210] Because this is an affordable staple in many regions where shigellosis is endemic, its adjunctive use should be considered. Zinc should be given to all children at 20 mg/day (dosed as elemental zinc with sulfate and gluconate preparations being equally efficacious) for 10 to 14 days in children older than the age of 6 months; younger children should receive 10 mg/day for the same duration.

PROGNOSIS

Most patients recover eventually with or without specific antimicrobial therapy, although illness may be prolonged and severe if it is not treated with appropriate antibiotics.[47] The mortality rate in developed countries is less than 1 percent, and life-threatening complications are rare events. With appropriate antibiotic therapy, defervescence usually occurs within 24 hours and the diarrhea decreases dramatically in 1 to 2 days. If left untreated, the disease usually lasts 1 week or more. In developing countries, childhood shigellosis is associated with significant morbidity and mortality (10% to 30%),[35] particularly if it is caused by *S. dysenteriae* serotype 1. Children with malnutrition are particularly likely to have a complicated course.[61] Shigellosis in malnourished children often causes a vicious cycle of further impaired nutrition, and repeated infections may be associated with impaired growth. Young infants and severely undernourished children are more likely to have bacteremia, which markedly increases mortality rates.[188]

PREVENTION

In developed countries where person-to-person transmission of shigellae is the major mode of infection, personal hygiene measures are most important.[128] Special attention to hygiene should be given in daycare centers, which sometimes play a central role in community-wide outbreaks of shigellosis.[171] The close contact among children too young to control their excretions renders this setting ideal for fecal-oral spread of the organism. Children attending daycare centers frequently transmit

infection to their families. Washing hands after defecating and before eating or preparing meals is important and helpful in preventing spread.[128] Daycare personnel who prepare food should avoid performing diaper-changing duties. Sick children should be excluded from the daycare center or cohorted, and mothers should be educated regarding the possibility of being infected by their children and the use of the necessary precautions. Proper cooking of potentially infected food, appropriate refrigeration, and exclusion of individuals with diarrhea from handling food are important precautions. Education of staff members in proper hygiene is essential to infection control.[205]

Patients with diarrhea in institutional and hospital settings should be isolated for prevention of outbreaks. Aggressive investigation and early initiation of appropriate antibiotic therapy in cases of bacillary dysentery are important measures in reducing excretion of virulent shigellae and stopping spread of the disease. Use of antibiotics for prophylaxis is not recommended.

In developing countries, a safe water supply and appropriate sanitation systems are important measures for reducing the risk for shigellosis. Chlorination of drinking water is important. Water stored in vessels that permit hand dipping has been defined as a risk factor.[256] Food prepared by street vendors also has been recognized as a risk factor. Prolonged breast-feeding is the best practical strategy for prevention of shigellosis (and most other enteric infections) in infants in most of the developing world.[4,66,165] Educational efforts to promote breast-feeding in these areas are key to children's survival. Human milk contains specific secretory IgA antibodies against *Shigella* lipopolysaccharides and virulence plasmid-coded antigens.[65] Lactoferrin and other nonspecific (nonantibody) factors in human milk, the effect of human milk on the type of intestinal flora, and the supply of an uncontaminated food source all may contribute to the protective effect of breast-feeding against diarrheal disease.

Natural clinical *Shigella* infection confers approximately 75 percent protection against illness on subsequent exposure to the homologous *Shigella* serotype and in some instances against heterologous serotypes. Antibodies (serum or mucosal) directed against the lipopolysaccharide O-antigen appear to play a major role in protection. The first line of defense, however, occurs at the mucosa.[198] Serotype-specific (lipopolysaccharide-based) vaccines have been produced.[84,85] Although early studies showed that immunization by the parenteral route with killed vaccines was ineffective, interest in this approach continues.[24,68,146] Several oral, live organism–based *Shigella* vaccines have been studied. Avirulent mutants of *S. flexneri* that lack the ability to invade the intestinal mucosa are safe and effective in monkeys. Multiple doses of large numbers of organisms were required to protect humans, however. Attenuated vaccines prepared from streptomycin-dependent mutant strains were effective but unstable.[166] Genetically attenuated *S. flexneri* strains conferred protection but caused diarrhea when fed to some volunteers. Prototype-attenuated *S. flexneri* strains CVD 1208[133] and WRSS[122] have provided encouraging results in early clinical trials. Two nonliving vaccine approaches include the oral administration of proteosomes[87] and of inactivated *Shigella* bacteria.

A recent trial of a new set of O-specific polysaccharide conjugate vaccines in Israeli children has demonstrated immunogenicity; however, there was no significant level of protection for the *S. sonnei* conjugate in children 1 to 2 years old, the age when *Shigella* infections peak in most endemic regions. Additionally, the incidence rates at the study site did not allow for estimates of the efficacy of the *S. flexneri* conjugate.[199] New studies are being conducted using improved techniques for enhancing immunogenicity of the polysaccharide unit and using synthetic conjugates.[203,218] Other new candidate vaccines are being evaluated, including the use of the major outer membrane proteins (OmpA) of *S. flexneri* 2a[207]; outer membrane vesicles (OMVs) from *S. flexneri*[51]; invasin-complex-based vaccines (Invaplex) from *S. flexneri* 2a containing lipopolysaccharides[217,255]; type III secretion system (TTSS) IpaB and IpaD proteins, which are more likely to be cross protective[162]; and second-generation *virG (iscA) S. sonnei* live attenuated candidate vaccines.[29,212]

No effective, licensed vaccine against shigellosis is available.* Whatever prototype *Shigella* vaccines prove to be well tolerated, immunogenic, and protective, the final formulation will have to confer protection against multiple epidemiologically important serotypes, including *S. dysenteriae* 1 (the cause of epidemic disease from Shiga toxin), all *S. flexneri* serotypes and subtypes (the most important agents of endemic disease in developing countries), and *S. sonnei* (responsible for 5% to 15% of shigellosis in developing countries and an important cause of traveler's shigellosis and of shigellosis in diarrhea in daycare centers in industrialized countries).[146]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Adefurin A, Sammons H, Jacqz-Aigrain E, et al. Ciprofloxacin safety in paediatrics: a systematic review. *Arch Dis Child* 2011;**96**:874–80.
9. Alvarez-Acosta T, León C, Acosta-González S, et al. Beneficial role of green plantain *(Musa paradisiaca)* in the management of persistent diarrhea: a prospective randomized trial. *J Am Coll Nutr* 2009;**28**:169–76.
14. Ashida H, Ogawa M, Kim M, et al. *Shigella* deploy multiple countermeasures against host innate immune responses. *Curr Opin Microbiol* 2011;**14**:16–23.
15. Ashida H, Ogawa M, Mimuro H, et al. *Shigella* are versatile mucosal pathogens that circumvent the host innate immune system. *Curr Opin Immunol* 2011;**23**:448–55.
28. Bardhan P, Faruque ASG, Naheed A, et al. Decrease in shigellosis-related deaths without *Shigella* spp.–specific interventions, Asia. *Emerg Infect Dis* 2010;**16**:1718–23.
29. Barnoy S, Baqar S, Kaminski RW, et al. *Shigella sonnei* vaccine candidates WRSs2 and WRSs3 are as immunogenic as WRSS1, a clinically tested vaccine candidate, in a primate model of infection. *Vaccine* 2011;**29**:6371–8.
44. Boumghar-Bourtchai L, Mariani-Kurkdjian P, Bingen E, et al. Macrolide-resistant *Shigella sonnei*. *Emerging Infect Dis* 2008;**14**:1297–9.
51. Camacho AI, de Souza J, Sánchez-Gómez S, et al. Mucosal immunization with *Shigella flexneri* outer membrane vesicles induced protection in mice. *Vaccine* 2011;**29**:8222–9.
53. Carter JD, Hudson AP. Reactive arthritis: clinical aspects and medical management. *Rheum Dis Clin North Am* 2009;**35**:21–44.

*References 120, 130, 146, 149, 153, 198, 201.

58. Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:418–22.
60. Chisti MJ, Faruque ASG, Khan WA, et al. Characteristics of children with *Shigella* encephalopathy: experience from a large urban diarrhea treatment center in Bangladesh. *Pediatr Infect Dis J* 2010;**29**:444–7.
75. Dupont N, Lacas-Gervais S, Bertout J, et al. *Shigella* phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. *Cell Host Microbe* 2009;**6**:137–49.
77. Erdman SM, Buckner EE, Hindler JF. Options for treating resistant *Shigella* species infections in children. *J Pediatr Pharmacol Ther* 2008;**13**:29–43.
88. Gaston JSH, Inman RD, Ryan ET, et al. Vaccination of children in low-resource countries against *Shigella* is unlikely to present an undue risk of reactive arthritis. *Vaccine* 2009;**27**:5432–4.
96. Gu B, Cao Y, Pan S, et al. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of *Shigella* between Europe-America and Asia-Africa from 1998 to 2009. *Int J Antimicrob Agents* 2012;**40**:9–17.
97. Haagsma JA, Siersema PD, De Wit NJ, et al. Disease burden of post-infectious irritable bowel syndrome in The Netherlands. *Epidemiol Infect* 2010;**138**:1650–6.
120. Kaminski RW, Oaks EV. Inactivated and subunit vaccines to prevent shigellosis. *Expert Rev Vaccines* 2009;**8**:1693–704.
123. Keddy KH, Sooka A, Crowther-Gibson P, et al. Systemic shigellosis in South Africa. *Clin Infect Dis* 2012;**54**:1448–54.
124. Kernéis S, Guerin PJ, von Seidlein L, et al. A look back at an ongoing problem: *Shigella dysenteriae* type 1 epidemics in refugee settings in Central Africa (1993-5). *PLoS One* 2009;**4**:e4494.
130. Kosek M, Yori PP, Olortegui MP. Shigellosis update: advancing antibiotic resistance, investment empowered vaccine development, and green bananas. *Curr Opin Infect Dis* 2010;**23**:475–80.
131. Kosek M, Yori PP, Pan WK, et al. Epidemiology of highly endemic multiply antibiotic-resistant shigellosis in children in the Peruvian Amazon. *Pediatrics* 2008;**122**:e541–549.
158. Mandal J, Mondal N, Mahadevan S, et al. Emergence of resistance to third-generation cephalosporin in *Shigella*—a case report. *J Trop Pediatr* 2010;**56**:278–9.
162. Martinez-Becerra FJ, Kissmann JM, Diaz-McNair J, et al. Broadly protective *Shigella* vaccine based on type III secretion apparatus proteins. *Infect Immun* 2012;**80**:1222–31.
167. Mensa L, Marco F, Vila J, et al. Quinolone resistance among *Shigella* spp. isolated from travellers returning from India. *Clin Microbiol Infect* 2008;**14**:279–81.
168. Miao EA, Mao DP, Yudkovsky N, et al. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci U S A* 2010;**107**:3076–80.
191. Ogawa M, Handa Y, Ashida H, et al. The versatility of *Shigella* effectors. *Nat Rev Microbiol* 2008;**6**:11–16.
197. Parsot C. *Shigella* type III secretion effectors: how, where, when, for what purposes? *Curr Opin Microbiol* 2009;**12**:110–16.
198. Pasetti MF, Simon JK, Sztein MB, et al. Immunology of gut mucosal vaccines. *Immunol Rev* 2011;**239**:125–48.
199. Passwell JH, Ashkenzi S, Banet-Levi Y, et al. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year-old Israeli children. *Vaccine* 2010;**28**:2231–5.
200. Pazhani GP, Niyogi SK, Singh AK, et al. Molecular characterization of multidrug-resistant *Shigella* species isolated from epidemic and endemic cases of shigellosis in India. *J Med Microbiol* 2008;**57**:856–63.
201. Phalipon A, Mulard LA, Sansonetti PJ. Vaccination against shigellosis: is it the path that is difficult or is it the difficult that is the path? *Microbes Infect* 2008;**10**:1057–62.
203. Phalipon A, Tanguy M, Grandjean C, et al. A synthetic carbohydrate-protein conjugate vaccine candidate against *Shigella flexneri* 2a infection. *J Immunol* 2009;**182**:2241–7.
207. Pore D, Mahata N, Pal A, et al. Outer membrane protein A (OmpA) of *Shigella flexneri* 2a, induces protective immune response in a mouse model. *PLoS One* 2011;**6**:e22663.
210. Rabbani GH, Ahmed S, Hossain I, et al. Green banana reduces clinical severity of childhood shigellosis: a double-blind, randomized, controlled clinical trial. *Pediatr Infect Dis J* 2009;**28**:420–5.
212. Ranallo RT, Fonseka S, Boren TL, et al. Two live attenuated *Shigella flexneri* 2a strains WRSf2G12 and WRSf2G15: a new combination of gene deletions for 2nd generation live attenuated vaccine candidates. *Vaccine* 2012;**30**:5159–71.
217. Riddle MS, Kaminski RW, Williams C, et al. Safety and immunogenicity of an intranasal *Shigella flexneri* 2a Invaplex 50 vaccine. *Vaccine* 2011;**29**:7009–19.
218. Robbins JB, Kubler-Kielb J, Vinogradov E, et al. Synthesis, characterization, and immunogenicity in mice of *Shigella sonnei* O-specific oligosaccharide-core-protein conjugates. *Proc Natl Acad Sci U S A* 2009;**106**:7974–8.
220. Rosanova MT, Lede R, Capurro H, et al. [Assessing fluoroquinolones as risk factor for musculoskeletal disorders in children: a systematic review and meta-analysis.] *Arch Argent Pediatr* 2010;**108**:524–31.
234. Saps M, Pensabene L, Di Martino L, et al. Post-infectious functional gastrointestinal disorders in children. *J Pediatr* 2008;**152**:812–16, 816.e1.
236. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.
237. Schroeder GN, Hilbi H. Molecular pathogenesis of *Shigella* spp.: controlling host cell signaling, invasion, and death by type III secretion. *Clin Microbiol Rev* 2008;**21**:134–56.
239. Shiferaw B, Solghan S, Palmer A, et al. Antimicrobial susceptibility patterns of *Shigella* isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000-2010. *Clin Infect Dis* 2012;**54**(Suppl. 5):S458–63.
245. Sperandio B, Regnault B, Guo J, et al. Virulent *Shigella flexneri* subverts the host innate immune response through manipulation of antimicrobial peptide gene expression. *J Exp Med* 2008;**205**:1121–32.
246. Srinivasa H, Baijayanti M, Raksha Y. Magnitude of drug-resistant shigellosis: a report from Bangalore. *Indian J Med Microbiol* 2009;**27**:358–60.
254. Traa BS, Walker CLF, Munos M, et al. Antibiotics for the treatment of dysentery in children. *Int J Epidemiol* 2010;**39**(Suppl. 1):i70–4.
255. Turbyfill KR, Kaminski RW, Oaks EV. Immunogenicity and efficacy of highly purified invasin complex vaccine from *Shigella flexneri* 2a. *Vaccine* 2008;**26**:1353–64.
260. Vinh H, Baker S, Campbell J, et al. Rapid emergence of third generation cephalosporin resistant *Shigella* spp. in Southern Vietnam. *J Med Microbiol* 2009;**58**:281–3.
265. Ye C, Lan R, Xia S, et al. Emergence of a new multidrug-resistant serotype X variant in an epidemic clone of *Shigella flexneri*. *J Clin Microbiol* 2010;**48**:419–26.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adefurin A, Sammons H, Jacqz-Aigrain E, et al. Ciprofloxacin safety in paediatrics: a systematic review. *Arch Dis Child* 2011;**96**: 874–80.
2. Adeleye IA. Conjugal transferability of multiple antibiotic resistance in three genera of Enterobacteriaceae in Nigeria. *J Diarrhoeal Dis Res* 1992;**10**:93–6.
3. Agarwal SK, Tewari M, Banerjee G. A study on transferable R-plasmids among *Shigella* species at Lucknow. *J Commun Dis* 1997;**29**:351–4.
4. Ahmed F, Clemens JD, Rao MR, et al. Community-based evaluation of the effect of breast-feeding on the risk of microbiologically confirmed or clinically presumptive shigellosis in Bangladeshi children. *Pediatrics* 1992;**90**:406–11.
5. Alam AN, Chowdhury AA, Kabir IA, et al. Association of pneumonia with undernutrition and shigellosis. *Indian Pediatr* 1984;**21**: 609–13.
6. Aldrich JA, Flowers RP 3rd, Hall FK. *Shigella* sonnei septicemia in a neonate: a case report. *J Am Osteopath Assoc* 1979;**79**:93–8.
7. al-Eissa Y, al-Zamil F, al-Kharashi M, et al. The relative importance of *Shigella* in the aetiology of childhood gastroenteritis in Saudi Arabia. *Scand J Infect Dis* 1992;**24**:347–51.
8. Aleksić S, Katz A, Aleksić V, et al. Antibiotic resistance of *Shigella* strains isolated in the Federal Republic of Germany 1989-1990. *Zentralbl Bakteriol* 1993;**279**:484–93.
9. Alvarez-Acosta T, León C, Acosta-González S, et al. Beneficial role of green plantain *(Musa paradisiaca)* in the management of persistent diarrhea: a prospective randomized trial. *J Am Coll Nutr* 2009;**28**:169–76.
10. Anand BS, Malhotra V, Bhattacharya SK, et al. Rectal histology in acute bacillary dysentery. *Gastroenterology* 1986;**90**:654–60.
11. Ansaruzzaman M, Kibriya AK, Rahman A, et al. Detection of provisional serovars of *Shigella dysenteriae* and designation as *S. dysenteriae* serotypes 14 and 15. *J Clin Microbiol* 1995;**33**: 1423–5.
12. Araj GF, Uwaydah MM, Alami SY. Antimicrobial susceptibility patterns of bacterial isolates at the American University Medical Center in Lebanon. *Diagn Microbiol Infect Dis* 1994;**20**:151–8.
13. Arondel J, Singer M, Matsukawa A, et al. Increased interleukin-1 (IL-1) and imbalance between IL-1 and IL-1 receptor antagonist during acute inflammation in experimental shigellosis. *Infect Immun* 1999;**67**:6056–66.
14. Ashida H, Ogawa M, Kim M, et al. *Shigella* deploy multiple countermeasures against host innate immune responses. *Curr Opin Microbiol* 2011;**14**:16–23.
15. Ashida H, Ogawa M, Mimuro H, et al. *Shigella* are versatile mucosal pathogens that circumvent the host innate immune system. *Curr Opin Immunol* 2011;**23**:448–55.
16. Ashkenazi S. *Shigella* infections in children: new insights. *Semin Pediatr Infect Dis* 2004;**15**:246–52.
17. Ashkenazi S, Amir J, Waisman Y, et al. A randomized, double-blind study comparing cefixime and trimethoprim-sulfamethoxazole in the treatment of childhood shigellosis. *J Pediatr* 1993;**123**: 817–21.
18. Ashkenazi S, Amir Y, Dinari G, et al. Differential leukocyte count in acute gastroenteritis. An aid to early diagnosis. *Clin Pediatr (Phila)* 1983;**22**:356–8.
19. Ashkenazi S, Bellah G, Cleary TG. Hallucinations as an initial manifestation of childhood shigellosis. *J Pediatr* 1989;**114**:95–6.
20. Ashkenazi S, Cleary KR, Pickering LK, et al. The association of Shiga toxin and other cytotoxins with the neurologic manifestations of shigellosis. *J Infect Dis* 1990;**161**:961–5.
21. Ashkenazi S, Dinari G, Weitz R, et al. Convulsions in shigellosis: evaluation of possible risk factors. *Am J Dis Child* 1983;**137**: 985–7.
22. Ashkenazi S, Dinari G, Zevulunov A, et al. Convulsions in childhood shigellosis: clinical and laboratory features in 153 children. *Am J Dis Child* 1987;**141**:208–10.
23. Ashkenazi S, May-Zahav M, Sulkes J, et al. Increasing antimicrobial resistance of *Shigella* isolates in Israel during the period 1984 to 1992. *Antimicrob Agents Chemother* 1995;**39**:819–23.
24. Ashkenazi S, Passwell JH, Harlev E, et al. Safety and immunogenicity of *Shigella sonnei* and *Shigella flexneri* 2a O-specific polysaccharide conjugates in children. *J Infect Dis* 1999;**179**: 1565–8.
25. Avital A, Maayan C, Goitein KJ. Incidence of convulsions and encephalopathy in childhood *Shigella* infections: survey of 117 hospitalized patients. *Clin Pediatr (Phila)* 1982;**21**:645–8.
26. Aysev AD, Guriz H. Drug resistance of *Shigella* strains isolated in Ankara, Turkey, 1993-1996. *Scand J Infect Dis* 1998;**30**:351–3.
27. Azad MA, Islam M, Butler T. Colonic perforation in *Shigella dysenteriae* 1 infection. *Pediatr Infect Dis* 1986;**5**:103–4.
28. Bardhan P, Faruque ASG, Naheed A, et al. Decrease in shigellosis-related deaths without *Shigella* spp.–specific interventions, Asia. *Emerg Infect Dis* 2010;**16**:1718–23.
29. Barnoy S, Baqar S, Kaminski RW, et al. *Shigella sonnei* vaccine candidates WRSs2 and WRSs3 are as immunogenic as WRSS1, a clinically tested vaccine candidate, in a primate model of infection. *Vaccine* 2011;**29**:6371–8.
30. Barrett-Connor E, Connor JD. Extraintestinal manifestations of shigellosis. *Am J Gastroenterol* 1970;**53**:234–45.
31. Barton LL, Pickering LK. Shigellosis in the first week of life. *Pediatrics* 1973;**52**:437–8.
32. Baskin DH, Lax JD, Barenberg D. *Shigella* bacteremia in patients with the acquired immune deficiency syndrome. *Am J Gastroenterol* 1987;**82**:338–41.
33. Basualdo W, Arbo A. Randomized comparison of azithromycin versus cefixime for treatment of shigellosis in children. *Pediatr Infect Dis J* 2003;**22**:374–7.
34. Bennish M, Eusof A, Kay B, et al. Multiresistant *Shigella* infections in Bangladesh. *Lancet* 1985;**2**:441.
35. Bennish ML, Harris JR, Wojtyniak BJ, et al. Death in shigellosis: incidence and risk factors in hospitalized patients. *J Infect Dis* 1990;**161**:500–6.
36. Bennish ML, Salam MA, Hossain MA, et al. Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983-1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. *Clin Infect Dis* 1992;**14**: 1055–60.
37. Bennish ML, Salam MA, Khan WA, et al. Treatment of shigellosis, part III: comparison of one- or two-dose ciprofloxacin with standard 5-day therapy: a randomized, blinded trial. *Ann Intern Med* 1992;**117**:727–34.
38. Bennish ML, Salam MA, Wahed MA. Enteric protein loss during shigellosis. *Am J Gastroenterol* 1993;**88**:53–7.
39. Bhattacharya K, Bhattacharya MK, Dutta D, et al. Double-blind, randomized clinical trial for safety and efficacy of norfloxacin for shigellosis in children. *Acta Paediatr* 1997;**86**:319–20.
40. Bhattacharya MK, Bhattacharya SK, Paul M, et al. Shigellosis in Calcutta during 1990-1992: antibiotic susceptibility pattern and clinical features. *J Diarrhoeal Dis Res* 1994;**12**:121–4.
41. Black RE, Brown KH, Becker S, et al. Longitudinal studies of infectious diseases and physical growth of children in rural Bangladesh, part II: incidence of diarrhea and association with known pathogens. *Am J Epidemiol* 1982;**115**:315–24.
42. Black RE, Levine M. Intestinal protein loss in shigellosis. *Nutr Res* 1991;**11**:1215–20.
43. Bogaerts J, Verhaegen J, Munyabikali JP, et al. Antimicrobial resistance and serotypes of *Shigella* isolates in Kigali, Rwanda (1983 to 1993): increasing frequency of multiple resistance. *Diagn Microbiol Infect Dis* 1997;**28**:165–71.
44. Boumghar-Bourtchai L, Mariani-Kurkdjian P, Bingen E, et al. Macrolide-resistant *Shigella* sonnei. *Emerg Infect Dis* 2008;**14**: 1297–9.
45. Brian MJ, Van R, Townsend I, et al. Evaluation of the molecular epidemiology of an outbreak of multiply resistant *Shigella* sonnei in a day-care center by using pulsed-field gel electrophoresis and plasmid DNA analysis. *J Clin Microbiol* 1993;**31**:2152–6.
46. Bridgwater FA, Morgan RS, Rowson KE, et al. The neurotoxin of *Shigella shigae:* morphological and functional lesions produced in the central nervous system of rabbits. *Br J Exp Pathol* 1955; **36**:447–53.
47. Burry VF, Thurn AN, Co TG. Shigellosis: an analysis of 239 cases in a pediatric population. *Mo Med* 1968;**65**:671–4.
48. Butler T, Dunn D, Dahms B, et al. Causes of death and the histopathologic findings in fatal shigellosis. *Pediatr Infect Dis J* 1989; **8**:767–72.
49. Butler T, Islam M, Bardhan P. The leukemoid reaction in shigellosis. In: *Shigellosis: a continuing global problem*. Bangladesh: International Centre for Diarrhoeal Disease Research; 1983. p. 154.

50. Calin A, Fries JF. An "experimental" epidemic of Reiter's syndrome revisited: follow-up evidence on genetic and environmental factors. *Ann Intern Med* 1976;**84**:564–6.
51. Camacho AI, de Souza J, Sánchez-Gómez S, et al. Mucosal immunization with *Shigella flexneri* outer membrane vesicles induced protection in mice. *Vaccine* 2011;**29**:8222–9.
52. Cario E. Toll-like receptors and gastrointestinal diseases: from bench to bedside? *Curr Opin Gastroenterol* 2002;**18**:696–704.
53. Carter JD, Hudson AP. Reactive arthritis: clinical aspects and medical management. *Rheum Dis Clin North Am* 2009;**35**:21–44.
54. Casalino M, Nicoletti M, Salvia A, et al. Characterization of endemic *Shigella flexneri* strains in Somalia: antimicrobial resistance, plasmid profiles, and serotype correlation. *J Clin Microbiol* 1994;**32**:1179–83.
55. Castillo-Duran C, Vial P, Uauy R. Trace mineral balance during acute diarrhea in infants. *J Pediatr* 1988;**113**:452–7.
56. Cavanagh JB, Howard JG, Whitby JL. The neurotoxin of *Shigella shigae*; a comparative study of the effects produced in various laboratory animals. *Br J Exp Pathol* 1956;**37**:272–8.
57. Centers for Disease Control and Prevention (CDC). Summary of notifiable diseases—United States, 2001. *MMWR Morb Mortal Wkly Rep* 2003;**50**:i–xxiv, 1–108.
58. Centers for Disease Control and Prevention (CDC). Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food—10 states, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:418–22.
59. Cheasty T, Skinner JA, Rowe B, et al. Increasing incidence of antibiotic resistance in *Shigella* from humans in England and Wales: recommendations for therapy. *Microb Drug Resist* 1998;**4**: 57–60.
60. Chisti MJ, Faruque ASG, Khan WA, et al. Characteristics of children with *Shigella* encephalopathy: experience from a large urban diarrhea treatment center in Bangladesh. *Pediatr Infect Dis J* 2010;**29**:444–7.
61. Chopra M, Wilkinson D, Stirling S. Epidemic *Shigella* dysentery in children in northern KwaZulu-Natal. *S Afr Med J* 1997;**87**: 48–51.
62. Chu YW, Houang ET, Lyon DJ, et al. Antimicrobial resistance in *Shigella flexneri* and *Shigella sonnei* in Hong Kong, 1986 to 1995. *Antimicrob Agents Chemother* 1998;**42**:440–3.
63. Chunder N, Bhattacharya SK, Biswas D, et al. Isolation of a fluoroquinolone-resistant *Shigella dysenteriae* 1 strain from Calcutta. *Indian J Med Res* 1997;**106**:494–6.
64. Cleary TG, Murray BE. Lack of Shiga-like cytotoxin production by enteroinvasive *Escherichia coli*. *J Clin Microbiol* 1988;**26**: 2177–9.
65. Cleary TG, Winsor DK, Reich D, et al. Human milk immunoglobulin A antibodies to *Shigella* virulence determinants. *Infect Immun* 1989;**57**:1675–9.
66. Clemens JD, Stanton B, Stoll B, et al. Breast feeding as a determinant of severity in shigellosis: evidence for protection throughout the first three years of life in Bangladeshi children. *Am J Epidemiol* 1986;**123**:710–20.
67. Clements D, Ellis CJ, Allan RN. Persistent shigellosis. *Gut* 1988;**29**:1277–8.
68. Cohen D, Ashkenazi S, Green MS, et al. Double-blind vaccine-controlled randomised efficacy trial of an investigational *Shigella sonnei* conjugate vaccine in young adults. *Lancet* 1997;**349**: 155–9.
69. Cohen D, Green MS, Block C, et al. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. *J Infect Dis* 1988;**157**:1068–71.
70. Davis TC. Chronic vulvovaginitis in children due to *Shigella flexneri*. *Pediatrics* 1975;**56**:41–4.
71. Donald WD, Winkler CH Jr, Bargeron LM Jr. The occurrence of convulsions in children with *Shigella* gastroenteritis. *J Pediatr* 1956;**48**:323–7.
72. DuPont HL, Hornick RB, Dawkins AT, et al. The response of man to virulent *Shigella flexneri* 2a. *J Infect Dis* 1969;**119**:296–9.
73. DuPont HL, Hornick RB. Adverse effect of Lomotil therapy in shigellosis. *JAMA* 1973;**226**:1525–8.
74. DuPont HL, Levine MM, Hornick RB, et al. Inoculum size in shigellosis and implications for expected mode of transmission. *J Infect Dis* 1989;**159**:1126–8.
75. Dupont N, Lacas-Gervais S, Bertout J, et al. *Shigella* phagocytic vacuolar membrane remnants participate in the cellular response to pathogen invasion and are regulated by autophagy. *Cell Host Microbe* 2009;**6**:137–49.
76. Emanuel B, Sherman JO. Shigellosis in a neonate: successful treatment with an oral elemental diet. *Clin Pediatr (Phila)* 1975;**14**:725–6.
77. Erdman SM, Buckner EE, Hindler JF. Options for treating resistant *Shigella* species infections in children. *J Pediatr Pharmacol Ther* 2008;**13**:29–43.
78. Ewing WH. *Edwards and Ewing's identification of enterobacteriaceae*. 4th ed. New York: Elsevier Science; 1986.
79. Farrar WE Jr, Eidson M, Guerry P, et al. Interbacterial transfer of R factor in the human intestine: in-vivo acquisition of R-factor–mediated kanamycin resistance by a multiresistant strain of *Shigella sonnei*. *J Infect Dis* 1972;**126**:27–33.
80. Fernandez A, Sninsky CA, O'Brien AD, et al. Purified *Shigella* enterotoxin does not alter intestinal motility. *Infect Immun* 1984; **43**:477–81.
81. Floyd TM, Higgins AR, Kader MA. Studies in shigellosis, part V: the relationship of age to the incidence of *Shigella* infections in Egyptian children, with special reference to shigellosis in the newborn and in infants in the first six months of life. *Am J Trop Med Hyg* 1956;**5**:119–30.
82. Fontaine O. Antibiotics in the management of shigellosis in children: what role for the quinolones? *Rev Infect Dis* 1989; **11**(Suppl. 5):S1145–50.
83. Forbes GB. Neurologic complications of systemic disease. *Postgrad Med* 1954;**15**:157–62.
84. Formal SB, Hale TL, Kapfer C. *Shigella* vaccines. *Rev Infect Dis* 1989;**11**(Suppl. 3):S547–51.
85. Formal SB, Kent TH, Austin S, et al. Fluorescent-antibody and histological study of vaccinated and control monkeys challenged with *Shigella flexneri*. *J Bacteriol* 1966;**91**:2368–76.
86. Frankel G, Riley L, Giron JA, et al. Detection of *Shigella* in feces using DNA amplification. *J Infect Dis* 1990;**161**:1252–6.
87. Fries LF, Montemarano AD, Mallett CP, et al. Safety and immunogenicity of a proteosome-*Shigella flexneri* 2a lipopolysaccharide vaccine administered intranasally to healthy adults. *Infect Immun* 2001;**69**:4545–53.
88. Gaston JSH, Inman RD, Ryan ET, et al. Vaccination of children in low-resource countries against *Shigella* is unlikely to present an undue risk of reactive arthritis. *Vaccine* 2009;**27**:5432–4.
89. Gaudio PA, Sethabutr O, Echeverria P, et al. Utility of a polymerase chain reaction diagnostic system in a study of the epidemiology of shigellosis among dysentery patients, family contacts, and well controls living in a shigellosis-endemic area. *J Infect Dis* 1997;**176**:1013–18.
90. Gemski P Jr, Takeuchi A, Washington O, et al. Shigellosis due to *Shigella* dysenteriae, part 1: relative importance of mucosal invasion versus toxin production in pathogenesis. *J Infect Dis* 1972; **126**:523–30.
91. Gilman RH, Spira W, Rabbani H, et al. Single-dose ampicillin therapy for severe shigellosis in Bangladesh. *J Infect Dis* 1981; **143**:164–9.
92. Goma Epidemiology Group. Public health impact of Rwandan refugee crisis: what happened in Goma, Zaire, in July, 1994? *Lancet* 1995;**345**:339–44.
93. Goren A, Freier S, Passwell JH. Lethal toxic encephalopathy due to childhood shigellosis in a developed country. *Pediatrics* 1992; **89**:1189–93.
94. Gots RE, Formal SB, Giannella RA. Indomethacin inhibition of *Salmonella typhimurium*, *Shigella flexneri*, and cholera-mediated rabbit ileal secretion. *J Infect Dis* 1974;**130**:280–4.
95. Greenberg M, Frant S, Shapiro R. Bacillary dysentery acquired at birth. *J Pediatr* 1940;**17**:363–6.
96. Gu B, Cao Y, Pan S, et al. Comparison of the prevalence and changing resistance to nalidixic acid and ciprofloxacin of *Shigella* between Europe-America and Asia-Africa from 1998 to 2009. *Int J Antimicrob Agents* 2012;**40**:9–17.
97. Haagsma JA, Siersema PD, De Wit NJ, et al. Disease burden of post-infectious irritable bowel syndrome in The Netherlands. *Epidemiol Infect* 2010;**138**:1650–6.
98. Hale TL, Oaks EV, Formal SB. Identification and antigenic characterization of virulence-associated, plasmid-coded proteins of *Shigella* spp. and enteroinvasive *Escherichia coli*. *Infect Immun* 1985;**50**:620–9.
99. Hale TL, Sansonetti PJ, Schad PA, et al. Characterization of virulence plasmids and plasmid-associated outer membrane proteins

in *Shigella flexneri*, *Shigella sonnei*, and *Escherichia coli*. *Infect Immun* 1983;**40**:340–50.
100. Haltalin KC, Nelson JD. Coliform septicemia complicating shigellosis in children. *JAMA* 1965;**192**:441–3.
101. Haltalin KC. Neonatal shigellosis: report of 16 cases and review of the literature. *Am J Dis Child* 1967;**114**:603–11.
102. Harnett N. High level resistance to trimethoprim, cotrimoxazole and other antimicrobial agents among clinical isolates of *Shigella* species in Ontario, Canada—an update. *Epidemiol Infect* 1992;**109**: 463–72.
103. Harris JC, Dupont HL, Hornick RB. Fecal leukocytes in diarrheal illness. *Ann Intern Med* 1972;**76**:697–703.
104. Harris JR, Wachsmuth IK, Davis BR, et al. High-molecular-weight plasmid correlates with *Escherichia coli* enteroinvasiveness. *Infect Immun* 1982;**37**:1295–8.
105. Helvaci M, Bektaşlar D, Ozkaya B, et al. Comparative efficacy of cefixime and ampicillin-sulbactam in shigellosis in children. *Acta Paediatr Jpn* 1998;**40**:131–4.
106. Hoefnagel D. Fulminating, rapidly fatal shigellosis in children. *N Engl J Med* 1958;**258**:1256–7.
107. Howard JG. Observations on the intoxication produced in mice and rabbits by the neurotoxin of *Shigella shigae*. *Br J Exp Pathol* 1955;**36**:439–46.
108. Huebner J, Czerwenka W, Gruner E, et al. Shigellemia in AIDS patients: case report and review of the literature. *Infection* 1993; **21**:122–4.
109. Huskins WC, Griffiths JK, Faruque AS, et al. Shigellosis in neonates and young infants. *J Pediatr* 1994;**125**:14–22.
110. Islam D, Christensson B. Disease-dependent changes in T-cell populations in patients with shigellosis. *APMIS* 2000;**108**: 251–60.
111. Islam MM, Azad AK, Bardhan PK, et al. Pathology of shigellosis and its complications. *Histopathology* 1994;**24**:65–71.
112. Islam MR, Alam AN, Hussain MS, et al. Effect of antimicrobial (nalidixic acid) therapy in shigellosis and predictive values of outcome variables in patients susceptible or resistant to it. *J Trop Med Hyg* 1995;**98**:121–5.
113. Islam MS, Hossain MS, Hasan MK, et al. Detection of shigellae from stools of dysentery patients by culture and polymerase chain reaction techniques. *J Diarrhoeal Dis Res* 1998;**16**:248–51.
114. Jain SK, Gupta A, Glanz B, et al. Antimicrobial-resistant *Shigella sonnei:* limited antimicrobial treatment options for children and challenges of interpreting in vitro azithromycin susceptibility. *Pediatr Infect Dis J* 2005;**24**:494–7.
115. Jensen G, Wandall DA, Gaarslev K, et al. Antibiotic resistance in *Shigella* and *Salmonella* in a region of Lithuania. *Eur J Clin Microbiol Infect Dis* 1996;**15**:872–6.
116. Kabir I, Butler T, Khanam A. Comparative efficacies of single intravenous doses of ceftriaxone and ampicillin for shigellosis in a placebo-controlled trial. *Antimicrob Agents Chemother* 1986;**29**: 645–8.
117. Kagalwalla AF, Khan SN, Kagalwalla YA, et al. Childhood shigellosis in Saudi Arabia. *Pediatr Infect Dis J* 1992;**11**:215–19.
118. Kakinuma K, Fukushima M, Kawaguchi R. Detection and identification of *Escherichia coli*, *Shigella*, and *Salmonella* by microarrays using the gyrB gene. *Biotechnol Bioeng* 2003;**83**:721–8.
119. Kaminski N, Bogomolski V, Stalnikowicz R. Acute bacterial diarrhoea in the emergency room: therapeutic implications of stool culture results. *J Accid Emerg Med* 1994;**11**:168–71.
120. Kaminski RW, Oaks EV. Inactivated and subunit vaccines to prevent shigellosis. *Expert Rev Vaccines* 2009;**8**:1693–704.
121. Kariuki S, Muthotho N, Kimari J, et al. Molecular typing of multi-drug resistant *Shigella dysenteriae* type 1 by plasmid analysis and pulsed-field gel electrophoresis. *Trans R Soc Trop Med Hyg* 1996;**90**:712–14.
122. Katz DE, Coster TS, Wolf MK, et al. Two studies evaluating the safety and immunogenicity of a live, attenuated *Shigella flexneri* 2a vaccine (SC602) and excretion of vaccine organisms in North American volunteers. *Infect Immun* 2004;**72**:923–30.
123. Keddy KH, Sooka A, Crowther-Gibson P, et al. Systemic shigellosis in South Africa. *Clin Infect Dis* 2012;**54**:1448–54.
124. Kernéis S, Guerin PJ, von Seidlein L, et al. A look back at an ongoing problem: *Shigella dysenteriae* type 1 epidemics in refugee settings in Central Africa (1993-5). *PLoS One* 2009;**4**:e4494.
125. Keusch GT, Bennish ML. Shigellosis: recent progress, persisting problems and research issues. *Pediatr Infect Dis J* 1989;**8**:713–19.
126. Keusch GT, Jacewicz M. The pathogenesis of *Shigella* diarrhea, part V: relationship of Shiga enterotoxin, neurotoxin, and cytotoxin. *J Infect Dis* 1975;**131**(Suppl.):S33–9.
127. Khalil K, Khan SR, Mazhar K, et al. Occurrence and susceptibility to antibiotics of *Shigella* species in stools of hospitalized children with bloody diarrhea in Pakistan. *Am J Trop Med Hyg* 1998;**58**: 800–3.
128. Khan MU. Interruption of shigellosis by hand washing. *Trans R Soc Trop Med Hyg* 1982;**76**:164–8.
129. Khan WA, Dhar U, Salam MA, et al. Central nervous system manifestations of childhood shigellosis: prevalence, risk factors, and outcome. *Pediatrics* 1999;**103**:E18.
130. Kosek M, Yori PP, Olortegui MP. Shigellosis update: advancing antibiotic resistance, investment empowered vaccine development, and green bananas. *Curr Opin Infect Dis* 2010;**23**:475–80.
131. Kosek M, Yori PP, Pan WK, et al. Epidemiology of highly endemic multiply antibiotic-resistant shigellosis in children in the Peruvian Amazon. *Pediatrics* 2008;**122**:e541–9.
132. Koster F, Levin J, Walker L, et al. Hemolytic-uremic syndrome after shigellosis: relation to endotoxemia and circulating immune complexes. *N Engl J Med* 1978;**298**:927–33.
133. Kotloff KL, Pasetti MF, Barry EM, et al. Deletion in the *Shigella* enterotoxin genes further attenuates *Shigella flexneri* 2a bearing guanine auxotrophy in a phase 1 trial of CVD 1204 and CVD 1208. *J Infect Dis* 2004;**190**:1745–54.
134. Kotloff KL, Winickoff JP, Ivanoff B, et al. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull World Health Organ* 1999;**77**: 651–66.
135. Kowlessar M, Forbes GB. The febrile convulsion in shigellosis. *N Engl J Med* 1958;**258**:520–6.
136. Kozlova NS. [Plasmids of antibiotic-resistant strains of *Shigella* isolated in Leningrad and Leningrad region.] *Antibiot Khimioter* 1993;**38**:9–14.
137. Kraybill EN, Controni G. Septicemia and enterocolitis due to *Shigella* sonnei in a newborn infant. *Pediatrics* 1968;**42**:529–31.
138. Kristjánsson M, Viner B, Maslow JN. Polymicrobial and recurrent bacteremia with *Shigella* in a patient with AIDS. *Scand J Infect Dis* 1994;**26**:411–16.
139. Labrec EH, Schneider H, Magnani TJ, et al. Epithelial cell penetration as an essential step in the pathogenesis of bacillary dysentery. *J Bacteriol* 1964;**88**:1503–18.
140. Lanata C, Mendoza W. Improving diarrhoea estimates. *WHO Report* 2002.
141. Landsberger M. Bacillary dysentery in a newborn infant. *Arch Pediatr* 1942;**59**:330–2.
142. Le-Barillec K, Magalhaes JG, Corcuff E, et al. Roles for T and NK cells in the innate immune response to *Shigella flexneri*. *J Immunol* 2005;**175**:1735–40.
143. Leibovitz E. The use of fluoroquinolones in children. *Curr Opin Pediatr* 2006;**18**:64–70.
144. Leibovitz E, Janco J, Piglansky L, et al. Oral ciprofloxacin vs. intramuscular ceftriaxone as empiric treatment of acute invasive diarrhea in children. *Pediatr Infect Dis J* 2000;**19**:1060–7.
145. Levin SE. *Shigella* septicemia in the newborn infant. *J Pediatr* 1967;**71**:917–18.
146. Levine MM. Enteric infections and the vaccines to counter them: future directions. *Vaccine* 2006;**24**:3865–73.
147. Levine MM, DuPont HL, Formal SB, et al. Pathogenesis of *Shigella* dysenteriae 1 (Shiga) dysentery. *J Infect Dis* 1973;**127**: 261–70.
148. Levine MM, DuPont HL, Khodabandelou M, et al. Long-term *Shigella*-carrier state. *N Engl J Med* 1973;**288**:1169–71.
149. Levine MM, Kotloff KL, Barry EM, et al. Clinical trials of *Shigella* vaccines: two steps forward and one step back on a long, hard road. *Nat Rev Microbiol* 2007;**5**:540–53.
150. Lim YS, Tay L. Serotype distribution and antimicrobial resistance of *Shigella* isolates in Singapore. *J Diarrhoeal Dis Res* 1991;**9**: 328–31.
151. Lima AA, Lima NL, Pinho MC, et al. High frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, streptomycin, chloramphenicol, and tetracycline isolated from patients with shigellosis in northeastern Brazil during the period 1988 to 1993. *Antimicrob Agents Chemother* 1995;**39**:256–9.
152. Lin SR, Chang SF. Drug resistance and plasmid profile of *Shigellae* in Taiwan. *Epidemiol Infect* 1992;**108**:87–97.

153. Lindberg AA, Pál T. Strategies for development of potential candidate *Shigella* vaccines. *Vaccine* 1993;**11**:168–79.
154. Ling JM, Shaw PC, Kam KM, et al. Molecular studies of plasmids of multiply-resistant *Shigella* spp. in Hong Kong. *Epidemiol Infect* 1993;**110**:437–46.
155. Lolekha S, Vibulbandhitkit S, Poonyarit P. Response to antimicrobial therapy for shigellosis in Thailand. *Rev Infect Dis* 1991; **13**(Suppl. 4):S342–6.
156. Makintubee S, Mallonee J, Istre GR. Shigellosis outbreak associated with swimming. *Am J Public Health* 1987;**77**:166–8.
157. Mamun KZ, Tabassum S, Hussain MA, et al. Antimicrobial susceptibility of *Shigella* from a rural community in Bangladesh. *Ann Trop Med Parasitol* 1997;**91**:643–7.
158. Mandal J, Mondal N, Mahadevan S, et al. Emergence of resistance to third-generation cephalosporin in *Shigella*—a case report. *J Trop Pediatr* 2010;**56**:278–9.
159. Maraki S, Georgiladakis A, Christidou A, et al. Antimicrobial susceptibilities and beta-lactamase production of *Shigella* isolates in Crete, Greece, during the period 1991-1995. *APMIS* 1998;**106**: 879–83.
160. Martin JM, Pitetti R, Maffei F, et al. Treatment of shigellosis with cefixime: two days vs. five days. *Pediatr Infect Dis J* 2000;**19**: 522–6.
161. Martin T, Habbick BF, Nyssen J. Shigellosis with bacteremia: a report of two cases and a review of the literature. *Pediatr Infect Dis* 1983;**2**:21–6.
162. Martinez-Becerra FJ, Kissmann JM, Diaz-McNair J, et al. Broadly protective *Shigella* vaccine based on type III secretion apparatus proteins. *Infect Immun* 2012;**80**:1222–31.
163. Mason PR, Nathoo KJ, Wellington M, et al. Antimicrobial susceptibilities of *Shigella* dysenteriae type 1 isolated in Zimbabwe—implications for the management of dysentery. *Cent Afr J Med* 1995;**41**:132–7.
164. Mata L. *The Children of Santa Maria Cauque: a prospective field study of health and growth*. Cambridge: MIT Press; 1978.
165. Mata LJ, Urrutia JJ, García B, et al. *Shigella* infection in breast-fed Guatemalan Indian neonates. *Am J Dis Child* 1969;**117**:142–6.
166. Mel DM, Terzin AL, Vuksić L. Studies on vaccination against bacillary dysentery, part 3: effective oral immunization against *Shigella flexneri* 2a in a field trial. *Bull World Health Organ* 1965; **32**:647–55.
167. Mensa L, Marco F, Vila J, et al. Quinolone resistance among *Shigella* spp. isolated from travellers returning from India. *Clin Microbiol Infect* 2008;**14**:279–81.
168. Miao EA, Mao DP, Yudkovsky N, et al. Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome. *Proc Natl Acad Sci USA* 2010;**107**:3076–80.
169. Miron D, Sochotnick I, Yardeni D, et al. Surgical complications of shigellosis in children. *Pediatr Infect Dis J* 2000;**19**:898–900.
170. Mitra AK, Alvarez JO, Guay-Woodford L, et al. Urinary retinol excretion and kidney function in children with shigellosis. *Am J Clin Nutr* 1998;**68**:1095–103.
171. Mohle-Boetani JC, Stapleton M, Finger R, et al. Communitywide shigellosis: control of an outbreak and risk factors in child day-care centers. *Am J Public Health* 1995;**85**:812–16.
172. Moore EE. *Shigella sonnei* septicaemia in a neonate. *BMJ* 1974; **1**:22.
173. Morris GK, Koehler JA, Gangarosa EJ, et al. Comparison of media for direct isolation and transport of shigellae from fecal specimens. *Appl Microbiol* 1970;**19**:434–7.
174. Mulligan K, Nelson S, Friedman HS, et al. Shigellosis-associated encephalopathy. *Pediatr Infect Dis J* 1992;**11**:889–90.
175. Munshi MH, Sack DA, Haider K, et al. Plasmid-mediated resistance to nalidixic acid in *Shigella dysenteriae* type 1. *Lancet* 1987;**2**: 419–21.
176. Murphy TV, Nelson JD. *Shigella* vaginitis: report of 38 patients and review of the literature. *Pediatrics* 1979;**63**:511–16.
177. Nataro JP, Seriwatana J, Fasano A, et al. Identification and cloning of a novel plasmid-encoded enterotoxin of enteroinvasive *Escherichia coli* and *Shigella* strains. *Infect Immun* 1995;**63**:4721–8.
178. Navia MM, Capitano L, Ruiz J, et al. Typing and characterization of mechanisms of resistance of *Shigella* spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. *J Clin Microbiol* 1999;**37**:3113–17.
179. Neglia TG, Marr TJ, Davis AT. *Shigella* dysentery with secondary *Klebsiella* sepsis. *J Pediatr* 1976;**89**:253–4.
180. Neill RJ, Gemski P, Formal SB, et al. Deletion of the Shiga toxin gene in a chlorate-resistant derivative of *Shigella* dysenteriae type 1 that retains virulence. *J Infect Dis* 1988;**158**:737–41.
181. Neter E. *Shigella sonnei* infection at term and its transfer to the newborn. *Obstet Gynecol* 1961;**17**:517–19.
182. Newland JW, Neill RJ. DNA probes for Shiga-like toxins I and II and for toxin-converting bacteriophages. *J Clin Microbiol* 1988; **26**:1292–7.
183. Niyogi SK. Shigellosis. *J Microbiol* 2005;**43**:133–43.
184. Noriega FR, Liao FM, Formal SB, et al. Prevalence of *Shigella* enterotoxin 1 among *Shigella* clinical isolates of diverse serotypes. *J Infect Dis* 1995;**172**:1408–10.
185. Notario R, Morales E, Carmelengo E, et al. [Enteropathogenic microorganisms in children with acute diarrhea in 2 hospitals of Rosario, Argentina.] *Medicina (B Aires)* 1993;**53**:289–99.
186. Nuutinen M, Turtinen J, Uhari M. Growth and joint symptoms in children treated with nalidixic acid. *Pediatr Infect Dis J* 1994; **13**:798–800.
187. O'Brien AD, Holmes RK. Shiga and Shiga-like toxins. *Microbiol Rev* 1987;**51**:206–20.
188. O'Connor HJ, O'Callaghan U. Fatal *Shigella sonnei* septicaemia in an adult complicated by marrow aplasia and intestinal perforation. *J Infect* 1981;**3**:277–9.
189. Oaks EV, Hale TL, Formal SB. Serum immune response to *Shigella* protein antigens in rhesus monkeys and humans infected with *Shigella* spp. *Infect Immun* 1986;**53**:57–63.
190. Ogawa H. Experimental approach in studies on pathogenesis of bacillary dysentery–with special references to the invasion of bacilli into intestinal mucosa. *Acta Pathol Jpn* 1970;**20**:261–77.
191. Ogawa M, Handa Y, Ashida H, et al. The versatility of *Shigella* effectors. *Nat Rev Microbiol* 2008;**6**:11–16.
192. Ogawa M, Sasakawa C. Intracellular survival of *Shigella*. *Cell Microbiol* 2006;**8**:177–84.
193. Okamura N, Nagai T, Nakaya R, et al. HeLa cell invasiveness and O antigen of *Shigella flexneri* as separate and prerequisite attributes of virulence to evoke keratoconjunctivitis in guinea pigs. *Infect Immun* 1983;**39**:505–13.
194. Oztürk MK, Caksen H, Sümerkan B. Convulsions in childhood shigellosis and antimicrobial resistance patterns of *Shigella* isolates. *Turk J Pediatr* 1996;**38**:183–8.
195. Pál T, Newland JW, Tall BD, et al. Intracellular spread of *Shigella flexneri* associated with the kcpA locus and a 140-kilodalton protein. *Infect Immun* 1989;**57**:477–86.
196. Parsot C. *Shigella* spp. and enteroinvasive *Escherichia coli* pathogenicity factors. *FEMS Microbiol Lett* 2005;**252**:11–18.
197. Parsot C. *Shigella* type III secretion effectors: how, where, when, for what purposes? *Curr Opin Microbiol* 2009;**12**:110–16.
198. Pasetti MF, Simon JK, Sztein MB, et al. Immunology of gut mucosal vaccines. *Immunol Rev* 2011;**239**:125–48.
199. Passwell JH, Ashkenzi S, Banet-Levi Y, et al. Age-related efficacy of *Shigella* O-specific polysaccharide conjugates in 1-4-year-old Israeli children. *Vaccine* 2010;**28**:2231–5.
200. Pazhani GP, Niyogi SK, Singh AK, et al. Molecular characterization of multidrug-resistant *Shigella* species isolated from epidemic and endemic cases of shigellosis in India. *J Med Microbiol* 2008; **57**:856–63.
201. Phalipon A, Mulard LA, Sansonetti PJ. Vaccination against shigellosis: is it the path that is difficult or is it the difficult that is the path? *Microbes Infect* 2008;**10**:1057–62.
202. Phalipon A, Sansonetti PJ. *Shigella's* ways of manipulating the host intestinal innate and adaptive immune system: a tool box for survival? *Immunol Cell Biol* 2007;**85**:119–29.
203. Phalipon A, Tanguy M, Grandjean C, et al. A synthetic carbohydrate-protein conjugate vaccine candidate against *Shigella flexneri* 2a infection. *J Immunol* 2009;**182**:2241–7.
204. Pickering LK. Antimicrobial resistance among enteric pathogens. *Semin Pediatr Infect Dis* 2004;**15**:71–7.
205. Pickering LK, Bartlett AV, Woodward WE. Acute infectious diarrhea among children in day care: epidemiology and control. *Rev Infect Dis* 1986;**8**:539–47.
206. Pickering LK, Woodward WE. Diarrhea in day care centers. *Pediatr Infect Dis* 1982;**1**:47–52.
207. Pore D, Mahata N, Pal A, et al. Outer membrane protein A (OmpA) of *Shigella flexneri* 2a, induces protective immune response in a mouse model. *PLoS One* 2011;**6**:e22663.

208. Prado D, López E, Liu H, et al. Ceftibuten and trimethoprim-sulfamethoxazole for treatment of *Shigella* and enteroinvasive *Escherichia coli* disease. *Pediatr Infect Dis J* 1992;**11**:644–7.
209. Prado V, Pidal P, Arellano C, et al. [Antimicrobial multiresistance of *Shigella* sp strains in a semi-rural community of northern Santiago.] *Rev Med Chil* 1998;**126**:1464–71.
210. Rabbani GH, Ahmed S, Hossain I, et al. Green banana reduces clinical severity of childhood shigellosis: a double-blind, randomized, controlled clinical trial. *Pediatr Infect Dis J* 2009;**28**:420–5.
211. Rahaman MM, Jamiul Alam AK, Islam MR, et al. Shiga bacillus dysentery associated with marked leukocytosis and erythrocyte fragmentation. *Johns Hopkins Med J* 1975;**136**:65–70.
212. Ranallo RT, Fonseka S, Boren TL, et al. Two live attenuated *Shigella flexneri* 2a strains WRSf2G12 and WRSf2G15: a new combination of gene deletions for 2nd generation live attenuated vaccine candidates. *Vaccine* 2012;**30**:5159–71.
213. Raqib R, Lindberg AA, Wretlind B, et al. Persistence of local cytokine production in shigellosis in acute and convalescent stages. *Infect Immun* 1995;**63**:289–96.
214. Raqib R, Ljungdahl A, Lindberg AA, et al. Local entrapment of interferon gamma in the recovery from *Shigella* dysenteriae type 1 infection. *Gut* 1996;**38**:328–36.
215. Raqib R, Reinholt FP, Bardhan PK, et al. Immunopathological patterns in the rectal mucosa of patients with shigellosis: expression of HLA-DR antigens and T-lymphocyte subsets. *APMIS* 1994;**102**:371–80.
216. Raqib R, Wretlind B, Andersson J, et al. Cytokine secretion in acute shigellosis is correlated to disease activity and directed more to stool than to plasma. *J Infect Dis* 1995;**171**:376–84.
217. Riddle MS, Kaminski RW, Williams C, et al. Safety and immunogenicity of an intranasal *Shigella flexneri* 2a Invaplex 50 vaccine. *Vaccine* 2011;**29**:7009–19.
218. Robbins JB, Kubler-Kielb J, Vinogradov E, et al. Synthesis, characterization, and immunogenicity in mice of *Shigella sonnei* O-specific oligosaccharide-core-protein conjugates. *Proc Natl Acad Sci USA* 2009;**106**:7974–8.
219. Rohde JR, Breitkreutz A, Chenal A, et al. Type III secretion effectors of the IpaH family are E3 ubiquitin ligases. *Cell Host Microbe* 2007;**1**:77–83.
220. Rosanova MT, Lede R, Capurro H, et al. [Assessing fluoroquinolones as risk factor for musculoskeletal disorders in children: a systematic review and meta-analysis.] *Arch Argent Pediatr* 2010;**108**:524–31.
221. Rubenstein JS, Noah ZL, Zales VR, et al. Acute myocarditis associated with *Shigella* sonnei gastroenteritis. *J Pediatr* 1993;**122**:82–4.
222. Ruderman JW, Stoller KP, Pomerance JJ. Bloodstream invasion with *Shigella* sonnei in an asymptomatic newborn infant. *Pediatr Infect Dis* 1986;**5**:379–80.
223. Sakai T, Sasakawa C, Makino S, et al. DNA sequence and product analysis of the virF locus responsible for Congo red binding and cell invasion in *Shigella flexneri* 2a. *Infect Immun* 1986;**54**:395–402.
224. Sakamoto A, Kamo S. Clinical, statistical observations on ekiri and bacillary dysentery; a study of 785 cases. *Ann Paediatr* 1956;**186**:1–18.
225. Salam MA, Dhar U, Khan WA, et al. Randomised comparison of ciprofloxacin suspension and pivmecillinam for childhood shigellosis. *Lancet* 1998;**352**:522–7.
226. Salam MA, Seas C, Khan WA, et al. Treatment of shigellosis, part IV: cefixime is ineffective in shigellosis in adults. *Ann Intern Med* 1995;**123**:505–8.
227. Salzman TC, Scher CD, Moss R. Shigellae with transferable drug resistance: outbreak in a nursery for premature infants. *J Pediatr* 1967;**71**:21–6.
228. Samandari T, Kotloff KL, Losonsky GA, et al. Production of IFN-gamma and IL-10 to *Shigella* invasins by mononuclear cells from volunteers orally inoculated with a Shiga toxin-deleted *Shigella dysenteriae* type 1 strain. *J Immunol* 2000;**164**:2221–32.
229. Sansonetti PJ, Arondel J, Huerre M, et al. Interleukin-8 controls bacterial transepithelial translocation at the cost of epithelial destruction in experimental shigellosis. *Infect Immun* 1999;**67**:1471–80.
230. Sansonetti PJ, Hale TL, Dammin GJ, et al. Alterations in the pathogenicity of *Escherichia coli* K-12 after transfer of plasmid and chromosomal genes from *Shigella flexneri*. *Infect Immun* 1983;**39**:1392–402.
231. Sansonetti PJ, Kopecko DJ, Formal SB. *Shigella* sonnei plasmids: evidence that a large plasmid is necessary for virulence. *Infect Immun* 1981;**34**:75–83.
232. Sansonetti PJ, Kopecko DJ, Formal SB. Involvement of a plasmid in the invasive ability of *Shigella flexneri*. *Infect Immun* 1982;**35**:852–60.
233. Sansonetti PJ, Phalipon A, Arondel J, et al. Caspase-1 activation of IL-1beta and IL-18 are essential for *Shigella flexneri*–induced inflammation. *Immunity* 2000;**12**:581–90.
234. Saps M, Pensabene L, Di Martino L, et al. Post-infectious functional gastrointestinal disorders in children. *J Pediatr* 2008;**152**:812–16, 816.e1.
235. Sasakawa C, Kamata K, Sakai T, et al. Molecular alteration of the 140-megadalton plasmid associated with loss of virulence and Congo red binding activity in *Shigella flexneri*. *Infect Immun* 1986;**51**:470–5.
236. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.
237. Schroeder GN, Hilbi H. Molecular pathogenesis of *Shigella* spp.: controlling host cell signaling, invasion, and death by type III secretion. *Clin Microbiol Rev* 2008;**21**:134–56.
238. Scragg JN, Rubidge CJ, Appelbaum PC. *Shigella* infection in African and Indian children with special reference to *Shigella* septicemia. *J Pediatr* 1978;**93**:796–7.
239. Shiferaw B, Solghan S, Palmer A, et al. Antimicrobial susceptibility patterns of *Shigella* isolates in Foodborne Diseases Active Surveillance Network (FoodNet) sites, 2000-2010. *Clin Infect Dis* 2012;**54**(Suppl. 5):S458–63.
240. de Silva DG, Mendis LN, Sheron N, et al. Concentrations of interleukin 6 and tumour necrosis factor in serum and stools of children with *Shigella dysenteriae* 1 infection. *Gut* 1993;**34**:194–8.
241. Simor AE, Poon R, Borczyk A. Chronic *Shigella flexneri* infection preceding development of acquired immunodeficiency syndrome. *J Clin Microbiol* 1989;**27**:353–5.
242. Smith H, Scotland H, Rowe B. Vero cytotoxin production and presence of VT genes in strains of *Escherichia coli* and *Shigella*. *FEMS Microbiol Lett* 1987;**42**:173.
243. Speelman P, Kabir I, Islam M. Distribution and spread of colonic lesions in shigellosis: a colonoscopic study. *J Infect Dis* 1984;**150**:899–903.
244. Speelman P, McGlaughlin R, Kabir I, et al. Differential clinical features and stool findings in shigellosis and amoebic dysentery. *Trans R Soc Trop Med Hyg* 1987;**81**:549–51.
245. Sperandio B, Regnault B, Guo J, et al. Virulent *Shigella flexneri* subverts the host innate immune response through manipulation of antimicrobial peptide gene expression. *J Exp Med* 2008;**205**:1121–32.
246. Srinivasa H, Baijayanti M, Raksha Y. Magnitude of drug-resistant shigellosis: a report from Bangalore. *Indian J Med Microbiol* 2009;**27**:358–60.
247. Starke JR, Baker CJ. Neonatal shigellosis with bowel perforation. *Pediatr Infect Dis* 1985;**4**:405–7.
248. Stern MS, Gitnick GL. *Shigella* hepatitis. *JAMA* 1976;**235**:2628.
249. Stoll BJ, Glass RI, Huq MI, et al. Surveillance of patients attending a diarrhoeal disease hospital in Bangladesh. *BMJ (Clin Res Ed)* 1982;**285**:1185–8.
250. Struelens MJ, Patte D, Kabir I, et al. *Shigella* septicemia: prevalence, presentation, risk factors, and outcome. *J Infect Dis* 1985;**152**:784–90.
251. Tauxe RV, Puhr ND, Wells JG, et al. Antimicrobial resistance of *Shigella* isolates in the USA: the importance of international travelers. *J Infect Dis* 1990;**162**:1107–11.
252. Taylor WI, Harris B. Isolation of shigellae, part II: comparison of plating media and enrichment broths. *Am J Clin Pathol* 1965;**44**:476–9.
253. Tobias JD, Starke JR, Tosi MF. *Shigella* keratitis: a report of two cases and a review of the literature. *Pediatr Infect Dis J* 1987;**6**:79–81.
254. Traa BS, Walker CLF, Munos M, et al. Antibiotics for the treatment of dysentery in children. *Int J Epidemiol* 2010;**39**(Suppl. 1):i70–4.
255. Turbyfill KR, Kaminski RW, Oaks EV. Immunogenicity and efficacy of highly purified invasin complex vaccine from *Shigella flexneri* 2a. *Vaccine* 2008;**26**:1353–64.

256. Tuttle J, Ries AA, Chimba RM, et al. Antimicrobial-resistant epidemic *Shigella* dysenteriae type 1 in Zambia: modes of transmission. *J Infect Dis* 1995;**171**:371–5.
257. Vargas M, Gascon J, Jimenez De Anta MT, et al. Prevalence of *Shigella* enterotoxins 1 and 2 among *Shigella* strains isolated from patients with traveler's diarrhea. *J Clin Microbiol* 1999;**37**:3608–11.
258. Venkatesan MM, Buysse JM, Kopecko DJ. Use of *Shigella flexneri ipa*C and *ipa*H gene sequences for the general identification of *Shigella* spp. and enteroinvasive *Escherichia coli*. *J Clin Microbiol* 1989;**27**:2687–91.
259. Vila J, Gascon J, Abdalla S, et al. Antimicrobial resistance of *Shigella* isolates causing traveler's diarrhea. *Antimicrob Agents Chemother* 1994;**38**:2668–70.
260. Vinh H, Baker S, Campbell J, et al. Rapid emergence of third generation cephalosporin resistant *Shigella* spp. in Southern Vietnam. *J Med Microbiol* 2009;**58**:281–3.
261. Wang L-H, Fang X-C, Pan G-Z. Bacillary dysentery as a causative factor of irritable bowel syndrome and its pathogenesis. *Gut* 2004;**53**:1096–101.
262. Way SS, Borczuk AC, Dominitz R, et al. An essential role for gamma interferon in innate resistance to *Shigella flexneri* infection. *Infect Immun* 1998;**66**:1342–8.
263. Whitfield C, Humphries JM. Meningitis and septicemia due to *Shigellae* in a newborn infant. *J Pediatr* 1967;**70**:805–6.
264. World Health Organization. *Guidelines for the control of shigellosis, including epidemics due to* Shigella *dysenteriae type 1*. Geneva: WHO Press; 2005.
265. Ye C, Lan R, Xia S, et al. Emergence of a new multidrug-resistant serotype X variant in an epidemic clone of *Shigella flexneri*. *J Clin Microbiol* 2010;**48**:419–26.
266. Yolken RH, Ojeh C, Khatri IA, et al. Intestinal mucins inhibit rotavirus replication in an oligosaccharide-dependent manner. *J Infect Dis* 1994;**169**:1002–6.
267. Yuhas Y, Weizman A, Ashkenazi S. Bidirectional concentration-dependent effects of tumor necrosis factor alpha in *Shigella dysenteriae*-related seizures. *Infect Immun* 2003;**71**:2288–91.
268. Yuhas Y, Weizman A, Chrousos GP, et al. Involvement of the neuropeptide corticotropin-releasing hormone in an animal model of *Shigella*-related seizures. *J Neuroimmunol* 2004;**153**:36–9.
269. Yuhas Y, Weizman A, Vanichkin A, et al. Involvement of prostaglandins in an animal model of *Shigella*-related seizures. *J Neuroimmunol* 2005;**168**:34–9.
270. Yurdakök K, Sahin N, Ozmert E, et al. *Shigella* gastroenteritis: clinical and epidemiological aspects, and antibiotic susceptibility. *Acta Paediatr Jpn* 1997;**39**:681–4.
271. Zimbabwe, Bangladesh, South Africa (Zimbasa) Dysentery Study Group. Multicenter, randomized, double blind clinical trial of short course versus standard course oral ciprofloxacin for *Shigella dysenteriae* type 1 dysentery in children. *Pediatr Infect Dis J* 2002;**21**:1136–41.
272. Zychlinsky A, Fitting C, Cavaillon JM, et al. Interleukin 1 is released by murine macrophages during apoptosis induced by *Shigella flexneri*. *J Clin Invest* 1994;**94**:1328–32.
273. Zychlinsky A, Kenny B, Ménard R, et al. *Ipa*B mediates macrophage apoptosis induced by *Shigella flexneri*. *Mol Microbiol* 1994;**11**:619–27.
274. Zychlinsky A, Perdomo JJ, Sansonetti PJ. Molecular and cellular mechanisms of tissue invasion by *Shigella flexneri*. *Ann NY Acad Sci* 1994;**730**:197–208.

CHAPTER 110

Serratia

Randall G. Fisher

Similar to other members of the Enterobacteriaceae, the genus *Serratia* contains species increasingly associated with opportunistic infection in the compromised host. One of the oldest bacterial organisms to be named,[116] *Serratia marcescens* is the chief species associated with disease in humans and has been associated with infection of the urinary tract, the respiratory tract, local wounds, and central venous catheters. Illness may be complicated by bacteremia and meningitis. Treatment of infection may be made exceptionally difficult because these organisms frequently are resistant to penicillins, cephalosporins, and aminoglycosides.

BACTERIOLOGY

S. marcescens can produce a red pigment resembling blood on contaminated foodstuffs. In the 6th century, the "miraculous" appearance of blood on food provoked superstition and scientific investigation. Troops were goaded into battle, and religious beliefs gained support because of the fortuitous growth of the saprophyte in bread.[116] In vitro, the red pigment produced by these strains (called "prodigiosin") is toxic to cancer cells but not to nonmalignant cell lines.[33] In addition, prodigiosin has activity against the mitochondria of *Trypanosoma cruzi*[44] and is also larvicidal against *Aedes aegypti*.[91]

In 1819, *S. marcescens* was named by Bizio, who correctly interpreted the discoloration of cornmeal to be due to a living organism.[76] The genus name honors the Italian physicist Serrafino Serrati, who Bizio thought had been slighted in favor of Robert Fulton as inventor of the steamboat; the species name *marcescens* was drawn from the Latin word meaning "to decay."

We now recognize the genus *Serratia* as comprising organisms that are straight, motile, catalase-positive, gram-negative rods. On solid agar, colonies are opaque; iridescent; and white, pink, or red. Organisms appear positive with the Voges-Proskauer test.[53] The genus may be distinguished from other Enterobacteriaceae by its use of caprylate or L-fucose as a sole carbon source and its hydrolysis of gelatin.[53,111] Clinically relevant strains include *S. marcescens*, *Serratia liquefaciens*, *Serratia odorifera*, *Serratia ficara*, and *Serratia plymuthica*.[19,41] For a detailed review of the properties of *S. marcescens*, the reader is referred to the review by Hejazi and Falkiner.[54]

EPIDEMIOLOGY

S. marcescens was thought to be nonpathogenic in earlier times and was used as a biologic marker of transmission in 1906. In that year, N. H. Gordon, commissioned to study the atmospheric hygiene of the British House of Commons, gargled a liquid culture of *S. marcescens* and then quoted Shakespeare to an audience of agar plates in the otherwise empty House of Commons chamber.[48,116] The organism subsequently was recovered from the plates, documenting the possibility of aerosol transmission of bacteria (Gordon reported no ill effects). The importance of *S. marcescens* as a biologic marker for hand-to-hand bacterial transmission, ascension of bacteria in the urinary tract in catheterized patients, and bacteremia after dental extraction is reviewed in detail by Yu.[116] Most remarkably, in investigations in 1950 and 1952 to judge the threat of biologic warfare to the United States, the U.S. Navy released *S. marcescens* into the Pacific, where it became aerosolized and drifted 80 miles inland.[13] Although an epidemic of *S. marcescens* infection in a San Francisco hospital coincided with this event, subsequent serotype and biotype analyses cast doubt on any relationship to the U.S. Navy experiments.[38] Rather, the early San Francisco hospital experience heralded the increased frequency of nosocomial infections that would be observed in subsequent years.[116]

In some studies, *S. marcescens* is the organism most frequently isolated from contact lens solutions and contact lens cases.[92,118] Ex vivo mouse cornea studies show that the organism adheres to injured but not to intact corneas.[92] All commercial multipurpose disinfectant solutions are active against the type strain but vary in their activity against actual clinical isolates. Polyquaternium-1–based solutions fared the worst in their activity against circulating strains.[57]

Sporadic nosocomial outbreaks of infection were reported first in the 1950s and 1960s.[72,93,114] Early outbreaks in a pediatric ward and neonatal nursery were attributed to contaminated intravenous solution and caps of bottles containing saline used to moisten umbilical cords.[72] As reviewed by Yu,[116] environmental sources before 1979 included disinfectants, water from ultrasonic nebulizers, respirators, arterial pressure monitors, and fiberoptic bronchoscopes. Environmental sources since have included suction traps,[81] intra-aortic pressure transducers,[10,112] contaminated handwashing brushes,[5] illicit intravenous drug paraphernalia,[29] contaminated urologic instruments,[36] colonized disinfectants or soaps,[37,69,74] contaminated infant parenteral nutrition fluid,[42] contaminated whole blood or blood products,[47,94] and inadequately sterilized breast milk pumps.[50] In one report, a woman elected to discontinue breastfeeding when the breast pump tubing turned bright pink and was culture positive for *S. marcescens*.[39]

Hand-to-hand transmission seems to be the primary mechanism of nosocomial spread. In one dramatic outbreak, spread of an organism with the same serotype, phage type, and antimicrobial sensitivity pattern was documented among four geographically separated teaching hospitals in the same region[99]; spread likely was due to passive carriage of *S. marcescens* on the hands of rotating personnel. By 1979, it was apparent that nosocomial increase in *S. marcescens* infection was becoming a worldwide concern.[19]

Outbreaks in neonatal units and pediatric wards have been widespread, persistent, and associated with high morbidity and mortality rates.[5,16,81,107] At the peak of one epidemic of invasive *S. marcescens* disease in a neonatal nursery, more than 90 percent of infants were colonized with the epidemic strain.[35] Increased rates of colonization have been associated with nearly 10-fold increases in rates of *S. marcescens* bacteremia and meningitis.[119] In a case-control study, neonates with *S. marcescens* bloodstream infection were at least three times more likely to have associated meningitis than were neonates whose blood cultures grew *Escherichia coli*.[14] Outbreaks of multidrug-resistant strains have been especially troublesome in surgical subspecialty wards, and an outbreak has been reported in a bone marrow transplant unit.[20,62] In the United States a multistate outbreak of *Serratia* infections was caused by contaminated prefilled saline and heparin syringes.[24]

Biotyping may be successful in characterizing isolates, which can be traced in the hospital environment.[52,106] Ribotyping or identification of a unique biochemical characteristic has proved useful for showing cross-contamination across hematology, gastroenterology, and neonatology units in a pediatric hospital.[12,43] Use of typing methods may be particularly important because drug-resistant and drug-susceptible isolates of *Serratia* organisms may co-circulate.[27] Banding differences from pulsed-field gel electrophoresis of DNA digests are restricted in number from outbreak strains; increases in banding pattern may reflect genetic drift over time.[7] Such DNA techniques have aided decisions regarding cohorting and closure of neonatal units and have been used to show cross-contamination of wards.[56,79] DNA amplification techniques offer promise for characterizing isolates in future outbreaks and defining antimicrobial susceptibility.[55,69,102]

PATHOPHYSIOLOGY

Pathologic findings of sepsis are similar to the findings of other gram-negative enteric bacilli. Postmortem examination of the lungs of patients with radiologic findings of *S. marcescens* pneumonia reveals a focal necrotizing pneumonia in most cases and hemorrhagic manifestations in some.[9]

Several properties may enhance virulence of *Serratia* organisms in human infection. The 56-kDa protease of *S. marcescens* seems to possess properties of a virulence factor. It enhances vascular permeability through activation of the Hageman factor–kallikrein–kinin pathway in vivo.[71] The protease also has the capacity to degrade host proteins important in humoral immune response, such as immunoglobulins and fibronectin,[80] and inactivates the chemotactic effect of C5a.[86] *Serratia* hemolysin indirectly may increase vascular permeability, local edema, and granulocyte accumulation.[63] Clinical strains seem to possess increased adherence properties compared with environmental isolates.[8] Compared with some enteric organisms, *Serratia* organisms adhere better to epithelial cells of the bladder, which may facilitate development of urinary tract infection.[31] Outer membrane vesicles (OMVs) are formed by *S. marcescens* in response to stress; OMVs likely play a role in virulence because it appears they function in toxin delivery.[73]

Cell-mediated immunity and humoral immunity may be important in protection from *Serratia* infection and illness. In a murine model of immunization against *S. marcescens*, only the transfer of antiserum and spleen cells from vaccinated mice increased bacterial clearance from the liver and survival after infection developed.[66] In nonphagocytic cells in vitro, *S. marcescens* can survive and even proliferate within autophage-like vacuoles by delaying or preventing fusion with lysosomes.[40]

Resistance of *S. marcescens* to aminoglycosides generally is plasmid mediated. Resistance to aminoglycosides may be conferred by one of several genes producing acetylating, phosphorylating, or adenylating enzymes.[1,2,20,58,77] Risk for acquiring infection with aminoglycoside resistance increases with exposure to these agents.[49,117] In some patients, repeated hospitalizations have shown greater importance, however, than aminoglycoside use as a risk factor for developing infection with resistant strains.[6] High levels of resistance to penicillins and cephalosporins are mediated by one or more plasmids. Cephalosporin resistance also may be derived chromosomally (see also Chapter 102).[27,45,75,89] Chromosomally mediated β-lactam resistance may be inducible in the presence of high levels of penicillin or cephalosporins, particularly when plasmid-derived β-lactamase is blocked by clavulanic acid.[21]

A group of researchers found and cloned a multidrug efflux pump of the major facilitator superfamily from *S. marcescens*.[104] Transposable plasmid elements may seem partly responsible for the rapid spread of multidrug resistance.[95,96,101] In one survey, 2.6 percent of *S. marcescens* isolates were deemed "multidrug resistant," defined as resistant to three or more classes of antimicrobial agents.[34] Plasmids conferring multidrug resistance are transferable from *S. marcescens* to *Klebsiella* spp. and may be responsible for sequential nosocomial outbreaks of different genera sharing common drug-resistance patterns.[110] A report from Spain suggested probable in vivo transmission between *E. coli* and *S. marcescens* of a plasmid containing both β-lactamase and quinolone resistance genes.[70]

CLINICAL MANIFESTATIONS

First described in a patient with bronchiectasis as a cause of "blood-tinged" sputum colored by the organism,[115] *S. marcescens* commonly is associated with urinary tract, respiratory tract, central venous catheter, and bacteremic infections.[2] Other species, including *S. liquefaciens*,

S. ficara, *S. odorifera*, and *S. plymuthica*, are less common causes of disease.[14,25,32,41,103]

Chromogenic *S. marcescens* was responsible for the historically interesting and reportedly benign "red diaper syndrome," which persisted for 7 months in the infant of a genetics professor.[113] In at least one series, the organism has been identified as one of the top five causes of neonatal sepsis[51] and now is recognized as a major pathogen of compromised newborns. Disease in neonatal intensive care units is associated commonly with high rates of underlying respiratory illness.[83] Other preexisting risk factors include necrotizing enterocolitis, surgical procedures, intravenous catheters, prolonged intubation, and cardiac disease.[14] Clinical illness shares features in common with other neonatal enteric pathogens; apnea, hypotension, and respiratory distress are seen frequently. Pneumonia with empyema has been reported.[61] Meningitis occurs as a complication in 24 percent of cases of neonatal bacteremia,[14] and antibiotic resistance may emerge during therapy for bacteremia or localized infection.[22] Significant brain injury caused by ventriculitis, brain abscesses, or porencephalic cysts is observed in most infants with meningitis.[22,67]

In older children and adults, *Serratia* spp. are isolated most frequently from the urinary tract.[2,99] Instrumentation, catheterization, and clustering of susceptible individuals are important risk factors.[6,23,58,97,100] By the 1970s, increased frequencies of serious infection, such as endocarditis, were noted in intravenous drug abusers.[29] In some series of respiratory and urinary tract infections, *S. marcescens* was observed to be associated more commonly with the complication of bacteremia than was any other enteric pathogen.[60,65] As is true of other causes of gram-negative sepsis, *Serratia* sepsis characterized by shock, pneumonia, or hemorrhage confers a substantially poorer prognosis.[15,17,98] The risk for these complications in cancer patients was observed by Saito and associates[98] to be lower, however, than their previous experience with other pathogens, such as *E. coli* and *Pseudomonas aeruginosa*. When predictive factors of mortality were sought in 385 subjects with nosocomial bacteremia, *S. marcescens* was not an independent predictor of death.[78] Other infections caused by *Serratia* include soft tissue infections, abscesses, endophthalmitis (including a case occurring after septicemia in an infant),[3,4] osteomyelitis and arthritis,[109] spinal epidural abscess, and peritonitis in dialysis patients.[28] A severe case of epiglottitis including fasciitis, myositis, and bacteremia was seen in a 58-year-old woman; prior cases were all in immune-compromised patients.[82] In one report, sternal osteomyelitis recurred 15 years after apparent successful treatment.[90]

DIAGNOSIS

The diagnosis of *Serratia* infection relies primarily on isolation of organisms from clinical material. Nonspecific laboratory tests occasionally may be misleading. *Serratia* meningitis in a neonate may be accompanied by a normal cerebrospinal fluid white blood cell count or only a modest cerebrospinal fluid pleocytosis.[22] Although *S. marcescens* is famous historically because of its chromogenic potential, most strains are nonpigmented. Hydrolysis of gelatin distinguishes *S. marcescens* from *Klebsiella* and *Enterobacter* in the clinical microbiology laboratory. The presence of ornithine decarboxylase and fermentation of sorbitol but not arabinose helps to differentiate further *S. marcescens* from other *Serratia* spp.[53] Biotyping,[52] DNA and RNA detection techniques,[69] and antimicrobial susceptibilities[106] can be used to characterize strains.

TREATMENT

Empirical decisions about antibiotic treatment of *Serratia* infection should rely on knowledge of hospital flora. Therapy should be tailored when susceptibilities are known. In newborns, meningitis and its complications should be suspected and interventions should be guided by imaging of the central nervous system. Imaging techniques may be useful in guiding needle aspiration of abscesses.[85] Recommended antibiotic therapy for neonatal meningitis is a cephalosporin and an aminoglycoside for susceptible strains. Mortality rates remain high (>45%) even with appropriate antibiotic management.[22]

In older children and adults, reported response rates for bacteremic infection have been 75 percent for patients who received appropriate antibiotics, 22 percent for patients who received inappropriate antibiotics, and 29 percent for patients who received no antibiotics.[98] Patients who continue to have positive blood culture results while receiving appropriate antibiotic therapy have a poor prognosis. In the review by Saito and associates[98] of 118 patients with *Serratia* bacteremia, patients who received only an aminoglycoside had the poorest response rate among those who received appropriate therapy; patients who received a cephalosporin, alone or in combination, fared better. *Serratia* organisms generally have proven susceptible to third-generation cephalosporins in surveys of hospitals from North America.[59] The physician needs to be wary, however, of potential resistance to cephalosporins and penicillins owing to the production of extended-spectrum β-lactamases. An extended-spectrum, metallo-β-lactamase–mediating resistance to imipenem has been described,[88] but it is uncommon. Physicians should consider strongly empirical use of a carbapenem in combination with an aminoglycoside in critically ill patients.[84] A worldwide surveillance study from 2004 to 2009 suggested that over 90 percent of *Serratia* strains were susceptible to meropenem, imipenem, and tigecycline.[11] However, outbreaks due to meropenem-resistant clones have also been described.[108]

Amikacin historically has been effective in the treatment of gentamicin-resistant strains.[30,68] In 1985, amikacin was recommended as a first-line antibiotic for treatment of pediatric nosocomial infection when *Serratia* spp. or other enteric agents with resistance potential were suspected.[105] Since the 1980s, however, outbreaks of *S. marcescens* infections caused by amikacin-resistant strains have been reported.[87] Quinolones have been used successfully for treatment of organisms resistant to other

agents,[46] but resistance to these drugs also has been identified.[64]

Cohorting and attempts to remove environmental sources of infection have been successful in ending epidemics but typically require several months.[26] Rarely, neonatal intensive care units have been closed to admissions to halt epidemics.[18,81]

Acknowledgments

I would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

11. Bertrand X, Dowzicky MJ. Antimicrobial susceptibility among gram-negative isolates collected from intensive care units in North America, Europe, the Asia-Pacific rim, Latin America, the Middle East, and Africa between 2004 and 2009 as part of the tigecycline evaluation and surveillance trial. *Clin Ther* 2012;**34**:124–37.
24. Chemaly RF, Rathod DB, Sikka MK, et al. *Serratia marcescens* bacteremia because of contaminated prefilled heparin and saline syringes: a multi-state report. *Am J Infect Control* 2011;**39**:521–4.
39. Faro J, Katz A, Berens P, Ross PJ. Premature termination of nursing secondary to *Serratia marcescens* breast pump contamination. *Obstet Gynecol* 2011;**117**:485–6.
40. Fedrigo GV, Campoy EM, De Venanzio G, et al. *Serratia marcescens* is able to survive and proliferate in autophagic-like vacuoles inside non-phagocytic cells. *PLoS One* 2011;**6**:324054.
44. Genes C, Baquero E, Echieverri F, et al. Mitochondrial dysfunction in *Trypanosoma cruzi:* the role of *Serratia marcescens* prodigiosin in the alternative treatment of Chagas disease. *Parasit Vectors* 2011;**6**:66.
70. Mata C, Miro E, Mirelis B, et al. In vivo transmission of a plasmid coharbouring *bla* and *znrB* genes between *Escherichia coli* and *Serratia marcescens*. *FEMS Microbiol Lett* 2010;**308**:24–8.
73. McMahon KJ, Castelli ME, Garcia Vescovi E, Feldman MF. Biogenesis of outer membrane vesicles in *Serratia marcescens* is thermoregulated and can be induced by activation of the Rcs phosphorelay system. *J Bacteriol* 2012;**194**(12):3241–9.
82. Musham CK, Jarathi A, Agarwal A. Acute epiglottitis due to *Serratia marcescens* in an immunocompetent adult. *Am J Med Sci* 2012;**344**(2):153–4.
90. Paquin A, Lepelletier D, Leprince C, et al. Relapse of *Serratia marcescens* sternal osteitis 15 years after the first episode. *J Clin Microbiol* 2012;**50**:184–6.
91. Patil CD, Patil SV, Salunke BK, Salunkhe RB. Prodigiosin produced by *Serratia marcescens* NMCC46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi*. *Parasitol Res* 2011;**109**:1179–87.
108. Suh B, Bae IK, Kim J, et al. Outbreak of meropenem-resistant *Serratia marcescens* comediated by chromosomal AmpC beta-lactamase overproduction and outer membrane protein loss. *Antimicrob Agents Chemother* 2010;**54**:5057–61.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 111

SALMONELLA

Theresa J. Ochoa • Javier Santisteban-Ponce

MICROBIOLOGY

Salmonellae are motile (owing to peritrichous flagella), nonencapsulated, gram-negative bacilli and facultative anaerobes of the Enterobacteriaceae family. The classification of *Salmonella* is confusing because multiple nomenclature systems are used (Table 111-1). In this chapter, we use the current designation of the Centers for Disease Control and Prevention (CDC), rather than either the complete name or the traditional clinical shorthand that referred to each of the more than 2500 serovars of *Salmonella* as though they were separate species. In hospital laboratories, *S.* ser. Choleraesuis and *S.* ser. Typhi are distinguished biochemically from other *Salmonella* spp. serogroup, based on O (somatic) antigen, also usually is determined on initial isolation, and organisms that are not *S.* ser. Typhi or *S.* ser. Choleraesuis are reported as *Salmonella* serogroup A, B, C1, D1, and so on. Common *Salmonella* spp. and their serogroups are shown in Table 111-2.

Salmonella serotype is defined by the serogroup antigens, the flagellar (H) antigens, and the virulence (Vi) antigen. H antigens can be either phase 1 (nonspecific) or phase 2 (specific). The Vi antigen, a heat-labile polysaccharide found on *S.* ser. Typhi, *S.* ser. Dublin, and *S.* ser. Paratyphi C, may block agglutination caused by antibodies to O antigen. Serotyping is an important epidemiologic tool for defining outbreaks, although it is most useful when an unusual type is associated with disease. When a common serotype is associated with an outbreak

REFERENCES

1. Acar JF. *Serratia marcescens* infections. *Infect Control* 1986;**7**: 273–8.
2. Acar JF, Witchitz JL, Goldstein F, et al. Susceptibility of aminoglycoside-resistant gram-negative bacilli to amikacin: delineation of individual resistance patterns. *J Infect Dis* 1976; **134**(Suppl.):S280–5.
3. al Hazzaa SA, Tabbara KF, Gammon JA. Pink hypopyon: a sign of *Serratia marcescens* endophthalmitis. *Br J Ophthalmol* 1992;**76**: 764–5.
4. Alvarez R, Adan A, Martinez JA, et al. Haematogenous *Serratia marcescens* endophthalmitis in an HIV-infected intravenous drug addict. *Infection* 1990;**18**:29–30.
5. Anagnostakis D, Fitsialos J, Koutsia C, et al. A nursery outbreak of *Serratia marcescens* infection: evidence of a single source of contamination. *Am J Dis Child* 1981;**135**:413–14.
6. Arroyo JC, Milligan WL, Postic B, et al. Clinical, epidemiologic and microbiologic features of a persistent outbreak of amikacin-resistant *Serratia marcescens*. *Infect Control* 1981;**2**:367–72.
7. Aucken HM, Boquete T, Kaufmann ME, Pitt TL. Interpretation of band differences to distinguish strains of *Serratia marcescens* by pulsed-field gel electrophoresis of XbaI DNA digests. *Epidemiol Infect* 2000;**125**:63–70.
8. Aucken HM, Pitt TL. Antibiotic resistance and putative virulence factors of *Serratia marcescens* with respect to O and K serotypes. *J Med Microbiol* 1998;**47**:1105–13.
9. Balikian JP, Herman PG, Godleski JJ. *Serratia* pneumonia. *Radiology* 1980;**137**:309–11.
10. Beck-Sague CM, Jarvis WR. Epidemic bloodstream infections associated with pressure transducers: a persistent problem. *Infect Control Hosp Epidemiol* 1989;**10**:54–9.
11. Bertrand X, Dowzicky MJ. Antimicrobial susceptibility among gram-negative isolates collected from intensive care units in North America, Europe, the Asia-Pacific rim, Latin America, the Middle East, and Africa between 2004 and 2009 as part of the tigecycline evaluation and surveillance trial. *Clin Ther* 2012;**34**: 124–37.
12. Bingen EH, Mariani-Kurkdjian P, Lambert-Zechovsky NY, et al. Ribotyping provides efficient differentiation of nosocomial *Serratia marcescens* isolates in a pediatric hospital. *J Clin Microbiol* 1992;**30**:2088–91.
13. *Biologic testing involving human subjects by the Department of Defense, 1977: Hearings before the Subcommittee on Health and Science Research of the United States Senate*. Washington, D.C.: Government Printing Office; 1977.
14. Bizzarro MJ, Dembry LM, Baltimore RS, Gallagher PG. Case-control analysis of endemic *Serratia marcescens* bacteremia in a neonatal intensive care unit. *Arch Dis Child Fetal Neonatal Ed* 2007;**92**:F120–6.
15. Bollet C, Grimont P, Gainnier M, et al. Fatal pneumonia due to *Serratia proteamaculans* subsp. *quinovora*. *J Clin Microbiol* 1993;**31**: 444–5.
16. Bollmann R, Halle E, Sokolowska-Kohler W, et al. Nosocomial infections due to *Serratia marcescens*: clinical findings, antibiotic susceptibility patterns and fine typing. *Infection* 1989;**17**:294–300.
17. Bouza E, Garcia de la Torre M, Erice A, et al. *Serratia* bacteremia. *Diagn Microbiol Infect Dis* 1987;**7**:237–47.
18. Braver DJ, Hauser GJ, Berns L, et al. Control of a *Serratia marcescens* outbreak in a maternity hospital. *J Hosp Infection* 1987;**10**: 129–37.
19. Brooks HJ, Chambers TJ, Tabaqchali S. The increasing isolation of *Serratia* species from clinical specimens. *J Hyg* 1979;**82**: 31–40.
20. Bullock DW, Bidwell JL, Reeves DS, et al. Outbreaks of hospital infection in southwest England caused by gentamicin-resistant *Serratia marcescens*. *J Hosp Infect* 1982;**3**:263–73.
21. Bush K, Flamm RK, Ohringer S, et al. Effect of clavulanic acid on activity of beta-lactam antibiotics in *Serratia marcescens* isolates producing both a TEM beta-lactamase and a chromosomal cephalosporinase. *Antimicrob Agents Chemother* 1991;**35**:2203–8.
22. Campbell JR, Diacovo T, Baker CJ. *Serratia marcescens* meningitis in neonates. *Pediatr Infect Dis J* 1992;**11**:881–6.
23. Cann KJ, Johnstone D, Skene AI. An outbreak of *Serratia marcescens* infection following urodynamic studies. *J Hosp Infect* 1987; **9**:291–3.
24. Chemaly RF, Rathod DB, Sikka MK, et al. *Serratia marcescens* bacteremia because of contaminated prefilled heparin and saline syringes: a multi-state report. *Am J Infect Control* 2011;**39**: 521–4.
25. Chmel H. *Serratia odorifera* biogroup 1 causing an invasive human infection. *J Clin Microbiol* 1988;**26**:1244–5.
26. Christensen GD, Korones SB, Reed L, et al. Epidemic *Serratia marcescens* in a neonatal intensive care unit: importance of the gastrointestinal tract as a reservoir. *Infect Control* 1982;**3**:127–33.
27. Coleman D, Falkiner FR, Carr ME, et al. Simultaneous outbreaks of infection due to *Serratia marcescens* in a general hospital. *J Hosp Infect* 1984;**5**:270–82.
28. Connacher AA, Old DC, Phillips G, et al. Recurrent peritonitis caused by *Serratia marcescens* in a diabetic patient receiving continuous ambulatory peritoneal dialysis. *J Hosp Infect* 1988;**11**: 155–60.
29. Cooper R, Mills J. *Serratia* endocarditis: a follow-up report. *Arch Intern Med* 1980;**140**:199–202.
30. Craven PC, Jorgensen JH, Kaspar RL, et al. Amikacin therapy of patients with multiply antibiotic-resistant *Serratia marcescens* infections: development of increasing resistance during therapy. *Am J Med* 1977;**62**:902–10.
31. Daifuku R, Stamm WE. Bacterial adherence to bladder uroepithelial cells in catheter-associated urinary tract infection. *N Engl J Med* 1986;**314**:1208–13.
32. Darbas H, Jean-Pierre H, Paillisson J. Case report and review of septicemia due to *Serratia ficaria*. *J Clin Microbiol* 1994;**32**: 2285–8.
33. Deorukhkar AA, Chander R, Ghosh SB, Sainis KB. Identification of a red-pigmented bacterium producing a potent anti-tumor *N*-alkylated prodigiosin as *Serratia marcescens*. *Res Microbiol* 2007; **158**:399–404.
34. DiPersio JR, Dowzicky MJ. Regional variations in multidrug resistance among Enterobacteriaceae in the USA and comparative activity of tigecycline, a new glycylcycline antimicrobial. *Int J Antimicrob Agents* 2007;**29**:518–27.
35. Duggan TG, Leng RA, Hancock BM, et al. *Serratia marcescens* in a newborn unit: microbiological features. *Pathology* 1984;**16**: 189–91.
36. Echols RM, Palmer DL, King RM, et al. Multidrug-resistant *Serratia marcescens* bacteriuria related to urologic instrumentation. *South Med J* 1984;**77**:173–7.
37. Ehrenkranz NJ, Bolyard EA, Wiener M, et al. Antibiotic-sensitive *Serratia marcescens* infections complicating cardiopulmonary operations: contaminated disinfectant as a reservoir. *Lancet* 1980;**2**: 1289–92.
38. Farmer JI, Davis BR, Grimont PAD. Source of American *Serratia*. *Lancet* 1977;**2**:459–60.
39. Faro J, Katz A, Berens P, Ross PJ. Premature termination of nursing secondary to *Serratia marcescens* breast pump contamination. *Obstet Gynecol* 2011;**117**:485–6.
40. Fedrigo GV, Campoy EM, De Venanzio G, et al. *Serratia marcescens* is able to survive and proliferate in autophagic-like vacuoles inside non-phagocytic cells. *PLoS One* 2011;**6**:324054.
41. Fitzgerald P, Drew JH, Kruszelnicki I. *Serratia*: a problem in a neonatal nursery. *Aust Paediatr J* 1984;**20**:205–7.
42. Frean JA, Arntzen L, Rosekilly I, et al. Investigation of contaminated parenteral nutrition fluids associated with an outbreak of *Serratia odorifera* septicaemia. *J Hosp Infect* 1994;**27**:263–73.
43. Geiseler PJ, Harris B, Andersen BR. Nosocomial outbreak of nitrate-negative *Serratia marcescens* infections. *J Clin Microbiol* 1982;**15**:728–30.
44. Genes C, Baquero E, Echieverri F, et al. Mitochondrial dysfunction in *Trypanosoma cruzi:* the role of *Serratia marcescens* prodigiosin in the alternative treatment of Chagas disease. *Parasit Vectors* 2011;**6**:66.
45. Gianneli D, Tzelepi E, Tzouvelekis LS, et al. Dissemination of cephalosporin-resistant *Serratia marcescens* strains producing a plasmidic SHV type beta-lactamase in Greek hospitals. *Eur J Clin Microbiol Infect Dis* 1994;**13**:764–7.
46. Goldstein EJ, Alpert ML, Najem A, et al. Norfloxacin in the treatment of complicated and uncomplicated urinary tract infections: a comparative multicenter trial. *Am J Med* 1987;**82**:65–9.
47. Gong J, Hogman CF, Hambraeus A, et al. Transfusion-associated *Serratia marcescens* infection: studies of the mechanism of action. *Transfusion* 1993;**33**:802–8.

48. Gordon WH. *Report on an investigation of the ventilation of the debating chamber of the House of Commons*. Parliamentary Command Paper; 1906. p. 3035.
49. Graham DR, Clegg HWD, Anderson RL, et al. Gentamicin treatment associated with later nosocomial gentamicin-resistant *Serratia marcescens* infections. *Infect Control* 1981;**2**:31–7.
50. Gransden WR, Webster M, French GL, et al. An outbreak of *Serratia marcescens* transmitted by contaminated breast pumps in a special care baby unit. *J Hosp Infect* 1986;**7**:149–54.
51. Grauel EL, Halle E, Bollmann R, et al. Neonatal septicaemia: incidence, etiology and outcome: a 6-year analysis. *Acta Paediatr Scand* 1989;**360**(Suppl.):113–19.
52. Grimont PA, Grimont F. Biotyping of *Serratia marcescens* and its use in epidemiological studies. *J Clin Microbiol* 1978;**8**:73–83.
53. Grimont PA, Grimont F. Serratia. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*, vol. 1. Baltimore: Williams & Wilkins; 1984. p. 476–84.
54. Hejazi A, Falkiner FR. Serratia marcescens. *J Med Microbiol* 1997;**46**:903–12.
55. Hejazi A, Keane CT, Falkiner FR. The use of RAPD-PCR as a typing method for *Serratia marcescens*. *J Med Microbiol* 1997;**46**: 913–19.
56. Hoyen C, Rice L, Conte S, et al. Use of real time pulsed field gel electrophoresis to guide interventions during a nursery outbreak of *Serratia marcescens* infection. *Pediatr Infect Dis J* 1999;**18**: 357–60.
57. Hume EB, Zhu H, Cole N, et al. Efficacy of contact lens multipurpose solutions against *Serratia marcescens*. *Optom Vis Sci* 2007; **84**:316–20.
58. John JFJ, McNeill WF. Characteristics of *Serratia marcescens* containing a plasmid coding for gentamicin resistance in nosocomial infections. *J Infect Dis* 1981;**143**:810–17.
59. Jones RN, Jenkins SG, Hoban DJ, et al. In vitro efficacy of six cephalosporins tested against Enterobacteriaceae isolated at 38 North American medical centres participating in the SENTRY Antimicrobial Surveillance Program, 1997-1998. *Int J Antimicrob Agents* 2000;**15**:111–18.
60. Karnad A, Alvarez S, Berk SL. Pneumonia caused by gram-negative bacilli. *Am J Med* 1985;**79**:61–7.
61. Khan EA, Wafelman LS, Garcia-Prats JA, Taber LH. *Serratia marcescens* pneumonia, empyema and pneumatocele in a preterm neonate. *Pediatr Infect Dis J* 1997;**16**:1003–5.
62. Knowles S, Herra C, Devitt E, et al. An outbreak of multiply resistant *Serratia marcescens*: The importance of persistent carriage. *Bone Marrow Transplant* 2000;**25**:873–7.
63. Konig W, Faltin Y, Scheffer J, et al. Role of cell-bound hemolysin as a pathogenicity factor for *Serratia* infections. *Infect Immun* 1987;**55**:2554–61.
64. Korner RJ, Nicol A, Reeves DS, et al. Ciprofloxacin-resistant *Serratia marcescens* endocarditis as a complication of non-Hodgkin's lymphoma. *J Infect* 1994;**29**:73–6.
65. Krieger JN, Kaiser DL, Wenzel RP. Urinary tract etiology of bloodstream infections in hospitalized patients. *J Infect Dis* 1983;**148**:57–62.
66. Kumagai Y, Okada K, Sawae Y. The effect of humoral and cell-mediated immunity in resistance to systemic *Serratia* infection. *J Med Microbiol* 1992;**36**:245–9.
67. Lam AH, Berry A, deSilva M, et al. Intracranial *Serratia* infection in preterm newborn infants. *A J N R Am J Neuroradiol* 1984;**5**: 447–51.
68. Leonard JM, McGee ZA, Alford RH. Gentamicin-resistant bacillary infection: clinical features and amikacin therapy. *Arch Intern Med* 1978;**138**:201–5.
69. Liu PY, Lau YJ, Hu BS, et al. Use of PCR to study epidemiology of *Serratia marcescens* isolates in nosocomial infection. *J Clin Microbiol* 1994;**32**:1935–8.
70. Mata C, Miro E, Mirelis B, et al. In vivo transmission of a plasmid coharbouring *bla* and *znrB* genes between *Escherichia coli* and *Serratia marcescens*. *FEMS Microbiol Lett* 2010;**308**:24–8.
71. Matsumoto K, Yamamoto T, Kamata R, et al. Pathogenesis of serratial infection: activation of the Hageman factor–prekallikrein cascade by serratial protease. *J Biochem* 1984;**96**:739–49.
72. McCormack RC, Kunin CM. Control of a single source nursery epidemic due to *Serratia marcescens*. *Pediatrics* 1966;**37**:750–5.
73. McMahon KJ, Castelli ME, Garcia Vescovi E, Feldman MF. Biogenesis of outer membrane vesicles in *Serratia marcescens* is thermoregulated and can be induced by activation of the Rcs phosphorelay system. *J Bacteriol* 2012;**194**(12):3241–9.
74. McNaughton M, Mazinke N, Thomas E. Newborn conjunctivitis associated with triclosan 0.5 percent antiseptic intrinsically contaminated with *Serratia marcescens*. *Can J Infect Control* 1995;**10**: 7–8.
75. Medeiros AA, O'Brien TF. Contributions of R factors to the antibiotic resistance of hospital isolates of *Serratia*. *Antimicrob Agents Chemother* 1968;**8**:30–5.
76. Merlino CP. Bartolomeo Bizio's letter to the most eminent priest, Angelo Bellani, concerning the phenomenon of the red-colored polenta. *J Bacteriol* 1924;**9**:527–43.
77. Meyer RD. Patterns and mechanisms of emergence of resistance to amikacin. *J Infect Dis* 1977;**136**:449–52.
78. Miller PJ, Wenzel RP. Etiologic organisms as independent predictors of death and morbidity associated with bloodstream infections. *J Infect Dis* 1987;**156**:471–7.
79. Miranda G, Kelly C, Solorzano F, et al. Use of pulsed-field gel electrophoresis typing to study an outbreak of infection due to *Serratia marcescens* in a neonatal intensive care unit. *J Clin Microbiol* 1996;**34**:3138–41.
80. Molla A, Matsumoto K, Oyamada I, et al. Degradation of protease inhibitors, immunoglobulins, and other serum proteins by *Serratia* protease and its toxicity to fibroblast in culture. *Infect Immun* 1986;**53**:522–9.
81. Montanaro D, Grasso GM, Annino I, et al. Epidemiological and bacteriological investigation of *Serratia marcescens* epidemic in a nursery and in a neonatal intensive care unit. *J Hyg* 1984;**93**: 67–78.
82. Musham CK, Jarathi A, Agarwal A. Acute epiglottitis due to *Serratia marcescens* in an immunocompetent adult. *Am J Med Sci* 2012;**344**(2):153–4.
83. Newport MT, John JF, Michel YM, et al. Endemic *Serratia marcescens* infection in a neonatal intensive care nursery associated with gastrointestinal colonization. *Pediatr Infect Dis* 1985;**4**:160–7.
84. Nicasio AM, Quintiliani R Jr., Deryke CA, et al. Treatment of *Serratia marcescens* meningitis with prolonged infusion of meropenem. *Ann Pharmacother* 2007;**41**:1077–81.
85. Obana WG, Cogen PH, Callen PW, et al. Ultrasound-guided aspiration of a neonatal brain abscess. *Child Nerv Syst* 1991;**7**:272–3; discussion 274.
86. Oda T, Kojima Y, Akaike T, et al. Inactivation of chemotactic activity of C5a by the serratial 56-kilodalton protease. *Infect Immun* 1990;**58**:1269–72.
87. Okuda T, Endo N, Osada Y, et al. Outbreak of nosocomial urinary tract infections caused by *Serratia marcescens*. *J Clin Microbiol* 1984;**20**:691–5.
88. Osano E, Arakawa Y, Wacharotayankun R, et al. Molecular characterization of an enterobacterial metallo-beta-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. *Antimicrob Agents Chemother* 1994;**38**:71–8.
89. Pagani L, Luzzaro F, Ronza P, et al. Outbreak of extended-spectrum beta-lactamase-producing *Serratia marcescens* in an intensive care unit. *FEMS Immunol Med Microbiol* 1994;**10**:39–46.
90. Paquin A, Lepelletier D, Leprince C, et al. Relapse of *Serratia marcescens* sternal osteitis 15 years after the first episode. *J Clin Microbiol* 2012;**50**:184–6.
91. Patil CD, Patil SV, Salunke BK, Salunkhe RB. Prodigiosin produced by *Serratia marcescens* NMCC46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi*. *Parasitol Res* 2011;**109**:1179–87.
92. Prabha V, Singh N, Chopra P. Bacteriological evaluation of conjunctiva, contact lens storage cases and solutions during contact lens wear. *Indian J Pathol Microbiol* 2007;**50**:101–3.
93. Rabinowitz K, Schiffrin R. A ward contamination by *Serratia marcescens*. *Acta Med Orient* 1952;**11**:181–4.
94. Roth VR, Arduino MJ, Nobiletti J, et al. Transfusion-related sepsis due to *Serratia liquefaciens* in the United States. *Transfusion* 2000;**40**:931–5.
95. Rubens CE, Farrar WEJ, McGee ZA, et al. Evolution of a plasmid mediating resistance to multiple antimicrobial agents during a prolonged epidemic of nosocomial infections. *J Infect Dis* 1981; **143**:170–81.
96. Rubens CE, McNeill WF, Farrar WEJ. Evolution of multiple-antibiotic-resistance plasmids mediated by transposable plasmid deoxyribonucleic acid sequences. *J Bacteriol* 1979;**140**:713–19.

97. Rutala WA, Kennedy VA, Loflin HB, et al. *Serratia marcescens* nosocomial infections of the urinary tract associated with urine measuring containers and urinometers. *Am J Med* 1981;**70**: 659–63.
98. Saito H, Elting L, Bodey GP, et al. *Serratia* bacteremia: review of 118 cases. *Rev Infect Dis* 1989;**11**:912–20.
99. Schaberg DR, Alford RH, Anderson R, et al. An outbreak of nosocomial infection due to multiply resistant *Serratia marcescens*: evidence of interhospital spread. *J Infect Dis* 1976;**134**:181–8.
100. Schaberg DR, Haley RW, Highsmith AK, et al. Nosocomial bacteriuria: a prospective study of case clustering and antimicrobial resistance. *Ann Intern Med* 1980;**93**:420–4.
101. Schaberg DR, Rubens CE, Alford RH, et al. Evolution of antimicrobial resistance and nosocomial infection: lessons from the Vanderbilt experience. *Am J Med* 1981;**70**:445–8.
102. Senda K, Arakawa Y, Ichiyama S, et al. PCR detection of metallo-beta-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams. *J Clin Microbiol* 1996;**34**:2909–13.
103. Serruys-Schoutens E, Rost F, Depre G. A nosocomial epidemic of *Serratia liquefaciens* urinary tract infection after cystometry. *Eur J Clin Microbiol* 1984;**3**:316–17.
104. Shahcheraghi F, Minato Y, Chen J, et al. Molecular cloning and characterization of a multidrug efflux pump, SmfY, from *Serratia marcescens*. *Biol Pharm Bull* 2007;**30**:798–800.
105. Shulman ST, Yogev R. Treatment of pediatric infections with amikacin as first-line aminoglycoside. *Am J Med* 1985;**79**:43–50.
106. Sifuentes-Osornio J, Ruiz-Palacios GM, Groschel DH. Analysis of epidemiologic markers of nosocomial *Serratia marcescens* isolates with special reference to the Grimont biotyping system. *J Clin Microbiol* 1986;**23**:230–4.
107. Stamm WE, Kolff CA, Dones EM, et al. A nursery outbreak caused by *Serratia marcescens*: scalp-vein needles as a portal of entry. *J Pediatr* 1976;**89**:96–9.
108. Suh B, Bae IK, Kim J, et al. Outbreak of meropenem-resistant *Serratia marcescens* comediated by chromosomal AmpC beta-lactamase overproduction and outer membrane protein loss. *Antimicrob Agents Chemother* 2010;**54**:5057–61.
109. Svensson O, Parment PA, Blomgren G. Orthopaedic infections by *Serratia marcescens*: a report of seven cases. *Scand J Infect Dis* 1987;**19**:69–75.
110. Thomas FE, Jackson RT, Melly A, et al. Sequential hospitalwide outbreaks of resistant *Serratia* and *Klebsiella* infections. *Arch Intern Med* 1977;**137**:581–4.
111. Verrall R. Serratia marcescens. *Infect Control* 1983;**4**:469–71.
112. Villarino ME, Jarvis WR, O'Hara C, et al. Epidemic of *Serratia marcescens* bacteremia in a cardiac intensive care unit. *J Clin Microbiol* 1989;**27**:2433–6.
113. Waisman HA, Stone WH. The presence of *Serratia marcescens* as the predominating organism in the intestinal tract of the newborn: the occurrence of the "red diaper syndrome." *Pediatrics* 1958;**21**: 8–12.
114. Wheat RP, Zuckerman A, Rantz LA. Infection due to chromobacteria: report of eleven cases. *Arch Intern Med* 1951;**88**:461–6.
115. Woodward HMM, Clarke KB. A case of infection in man by the *Bacterium prodigiosum*. *Lancet* 1913;**1**:314–15.
116. Yu VL. *Serratia marcescens*: historical perspective and clinical review. *N Engl J Med* 1979;**300**:887–93.
117. Yu VL, Oakes CA, Axnick KJ, et al. Patient factors contributing to the emergence of gentamicin-resistant *Serratia marcescens*. *Am J Med* 1979;**66**:468–72.
118. Yung MS, Boost M, Cho P, Yap M. Microbial contamination of contact lenses and lens care accessories of soft contact lens wearers (university students) in Hong Kong. *Ophthalmic Physiol Opt* 2007; **27**:11–21.
119. Zaidi M, Sifuentes J, Bobadilla M, et al. Epidemic of *Serratia marcescens* bacteremia and meningitis in a neonatal unit in Mexico City. *Infect Control Hosp Epidemiol* 1989;**10**:14–20.

TABLE 111-1 **Examples of Current *Salmonella* Nomenclature**

CDC Designation	Complete Name	Previous Designation
S. ser. Typhi	*S. enterica** subsp. *enterica* ser. Typhi	*S. typhi*
S. ser. Enteritidis	*S. enterica* subsp. *enterica* ser. Enteritidis	*S. enteritidis*
S. IIIa 18:z_4,z_{23}: -	*S. enterica* subsp. *arizonae* ser. 18:z_4,z_{23}: -	*Arizona hinshawii* ser. 7a, 7b:1,2,5:
S. ser. Marina	*S. enterica* subsp. *houtenae* ser. Marina	*S. marina*

**S. choleraesuis* and *S. enteritidis* also are designations commonly used for the species.
CDC, Centers for Disease Control and Prevention.

TABLE 111-2 ***Salmonella* Species Included in Major Serogroups**

Serogroup*	Representative Serotypes
A	*S.* ser. Paratyphi A
B	*S.* ser. Paratyphi B
	S. ser. Saint-Paul
	S. ser. Agona
	S. ser. Derby
	S. ser. Typhimurium
	S. ser. Heidelberg
C1	*S.* ser. Paratyphi C
	S. ser. Choleraesuis
	S. ser. Montevideo
	S. ser. Infantis
C2	*S.* ser. *Newport*
C3	*S.* ser. Santiago
D1	*S.* ser. Typhi
	S. ser. Enteritidis
	S. ser. Dublin
D2	*S.* ser. Strasbourg
E1	*S.* ser. Anatum
E2	*S.* ser. Newington
E3	*S.* ser. Illinois

*Human infections with organisms in serogroups E4, F, G1, G2, H, and I and the O antigens not given serogroup designation (O17 through O67) are uncommon.

(e.g., *S.* ser. Typhimurium), biochemical phenotype, antibiogram, plasmid characterization, bacteriophage typing, outer-membrane protein analysis, pulsed-field gel electrophoresis, and randomly amplified polymorphic DNA may help determine whether a single-strain, common-source outbreak is in progress.

Six subgroups of *Salmonella* have been proposed on the basis of DNA relatedness. Most serotypes, including almost all of the serotypes important in human and animal disease, belong to subgroup I. The former genus *Arizona* is reclassified as *Salmonella*.

Based in host adaptability, Salmonellae are divided in three groups. Group 1 englobes *Salmonella* highly adapted to humans and primates, group 2 identifies highly specific animal-adapted *Salmonella*, and group 3 includes those serovars not host adapted but capable of infecting both humans and a wide range of animals and consisting mainly of *Salmonella* ser. Typhimurium and *S.* ser. Enteritidis.[6] Blood agar or chocolate agar supports their growth when they are present as the sole organisms in blood, cerebrospinal fluid (CSF), or joint fluid. For specimens containing mixed flora (e.g., stool), selective media such as *Salmonella-Shigella* (SS) agar or bismuth sulfate agar must be used.

EPIDEMIOLOGY

Nontyphoidal *Salmonella*

Public Health Issues

In most of the world, the prevalence of *Salmonella* varies according to the water supply, waste disposal, food preparation practices, and climate. The incidence of nontyphoidal salmonellosis is 93.8 million gastroenteritis cases worldwide each year, causing 155,000 deaths globally.[196] In the United States the incidence of the disease has increased steadily, despite good public health interventions, while nontyphoidal *Salmonella*-related mortality declined over years.[76] During 1996 to 2009, incidence of foodborne infections in the United States has increased 44 percent,[50] and a more than sixfold increase in reported nontyphoidal *Salmonella* infection in the United States has occurred during the last 40 years, with an estimate of 168,000 physician office visits, 15,000 hospitalizations, and 400 deaths annually during 1996 to 1999.[322] This increase reflects industrial-scale food production and distribution,[49] misuse of antimicrobial agents (in humans and animals) that alter the competing gastrointestinal flora and induce multidrug-resistant strains of *Salmonella*,[317] and probably an increasing number of immunocompromised individuals in the population. Targeted interventions, including on-farm control measures, refrigeration, and education contributed to an important decrease in the incidence of *S.* ser. Enteritidis infections in the United States,[200] highlighting the importance of these efforts to further reduce the rate of *Salmonella* infection. The most recent 44 percent increase in food-related incidence of *S.* ser. Enteritidis infections is linked to eating chicken in domestically acquired episodes.[48] In some developing countries, mortality rates of nontyphoidal bacteremic *Salmonella* infections in infants remain at 20 percent or higher over years.[123]

Significance of Animal Reservoirs and Other Food Sources

In contrast to *Shigella* spp., which infect only primates, nontyphoidal *Salmonella* spp. infect a variety of animals (including poultry, livestock, and pet reptiles and rodents).[298] Salmonella has been isolated from flies, mites, and other arthropods taken from animal farm environments; it is able to multiply inside some insects and is

transmitted within insects and to birds, rodents, and livestock.[328] Those arthropods, birds, and rodents could contribute to *Salmonella* transmission to the wildlife animals.[140] Animals and animal products (including meat and dairy products), water, and infected humans can be the source of infection. *Salmonella* is the leading cause of foodborne illness in the United States, and the second most common cause of death from foodborne pathogens.[190] Spread of resistant organisms from food animals to humans has been shown.[216] *Salmonella* spp. have been isolated from 50 percent of poultry,[36] 16 percent of pork, 5 percent of beef, and 40 percent of frozen egg products in retail stores. Slicing food with contaminated tools and surfaces transfer *Salmonella* in progressively decreasing amounts per slice, resulting in higher bacterial contamination for the firstly sliced portions.[48] Undercooked eggs (e.g., in Caesar salad, egg-dipped bread, and homemade eggnog) may be contaminated by organisms on the shell surface or transovarially directly through the egg yolk. Grade A shell eggs have been implicated in more than 40 percent of more recent outbreaks.[190] Even in the absence of recognized outbreaks, undercooked eggs probably are important vehicles of infection.[139]

The impact of *Salmonella* outbreaks was shown when milk contaminated with *S.* ser. Typhimurium was distributed in Chicago, Illinois. Reports estimated that more than 150,000 people became ill, with more than 16,000 culture-confirmed cases, 2777 individuals hospitalized, and 14 fatalities.[27] Ice cream, cream cakes, and mayonnaise commonly have been incriminated as the source of infections. Fruits and vegetables are vehicles reported less frequently in outbreaks but better identified and reported in recent years as a result of molecular methods and country-wide database reporting.[28,107,186,197]

Of the 10 most frequent *Salmonella* serovars causing disease in humans, 8 are included within the 10 serovars most frequently identified in at least one of the major food-animal species.[109] Some serotypes are associated with particular reservoirs. *S.* ser. Dublin is associated with dairy cattle and is frequently found in individuals who drink raw milk.[305] *S.* ser. Choleraesuis is associated with pigs.[59] *S.* ser. Typhimurium is associated with contact with pet rodents.[298] Infection with *S.* ser. Marina is associated with contact with pet iguanas. *Salmonella* group F, *S.* ser. Typhimurium, *S.* ser. Muenchen, and *S.* ser. Java infections have been traced to pet turtles. Reptiles, including rattlesnakes, are important *S.* IIIa 18:z_4,z_{23}: *(Arizona hinshawii)* reservoirs.

Humans as a Reservoir

After infection occurs, nontyphoidal *Salmonella* spp. are excreted in feces for a median of 5 weeks. Children younger than 5 years of age may excrete the organisms for 20 weeks after having an illness, but older children and adults usually excrete *Salmonella* for less than 8 weeks. Up to 5 percent of children younger than 5 years of age have been found to excrete nontyphoidal *Salmonella* for 1 year after disease.[42] *S.* ser. Typhi may be excreted chronically, particularly in the presence of gallbladder disease and sustained by biofilms.[72] Food handlers who are excreting *Salmonella* spp. represent an important risk group. Nontyphoidal *Salmonella* infection index cases have been mapped to isolation of the same clones in 65 percent from children and adults living in the same household versus less than 2 percent of home environment samples in Africa.[164] Nontyphoidal *Salmonella* spp. may be excreted for longer periods in feces of children with primary immunodeficiency.[236]

Bacterial Characteristics Favoring Survival

Salmonella spp. survive refrigeration and sometimes heating; they may remain viable at ambient or reduced temperatures for weeks and even in low-moisture foods.[250] Salmonellae may remain viable in foods after cooking for less than 12 minutes at less than 65°C (140°F). *Salmonella* spp. are killed by heating to 54.4°C (130°F) for 1 hour or 60°C (140°F) for 15 minutes. Food industry ingredients with low water activity and high fat content provide a marked thermal resistance to *Salmonella*, making necessary use of higher heating temperatures and longer time.[250] Salmonellae survive for hours on the hands of slaughterhouse workers.[243] The organisms have been found to survive in flour for nearly 1 year. *S.* ser. Tennessee has been reported to remain viable for 2 to 8 days on glass, stainless steel, enameled surfaces, a rubber mattress, linen, and a rubber tabletop.[330] *Salmonella* small inoculates (<100 cfu/g) are able to survive 30 to 40 days in vacuum dust.[138] Nosocomial infections have been related to contaminated medical equipment (e.g., endoscopes) and diagnostic or pharmacologic preparations, particularly those of animal origin (e.g., bile salts, pancreatic extracts, pepsin, and vitamins). *Salmonella* capability to form complex surface structures called biofilms in both host and nonhost environments are an important mechanism for survival and persistence.[47,173] Biofilm formation increases resistance of *Salmonella* to disinfectants and even to antibiotics, resulting in 2000-fold resistance to ciprofloxacin in vitro.[293]

Relationship of Age to Risk for Disease

The highest incidence rates occur in children younger than 5 years of age, especially infants younger than 1 year, and in individuals older than 70 years. Among infants in the United States, the incidence of invasive salmonellosis (7.8 cases/100,000 inhabitants) is the highest within age groups.[323] Nursery outbreaks often can be traced to an infected mother,[20,176] with subsequent spread occurring through health care personnel.[280,331] The mother of the index case can be symptomatic[108,157,270] or asymptomatic,[331] recovering from recent infection,[228,284] or a chronic carrier.[277] Low-birth-weight infants seem to be at higher risk than full-term infants for acquiring *Salmonella* infection.[24,280,331] The source of infection occasionally is contaminated food, but more often it is fomites (e.g., delivery room resuscitators,[269] rectal thermometers,[153,205] suction devices,[167] water baths for heating formula,[261] soap dispensers,[213] scales,[5,25,331] tables,[331] air-conditioning filters,[331] and plumbing[210]). Outbreaks in nurseries often are extraordinarily difficult to stop. They have been reported to last months[176,233,331] to years.[108,210,301] Contamination sometimes can become so widespread that other areas

of the hospital also experience cases.[202,283] These outbreaks occur far more commonly with *Salmonella* than with other bacterial enteropathogens. Such outbreaks sometimes are caused by multiresistant *Salmonella*.[176] Multidrug-resistant *Salmonella* has been isolated from cockroaches found in a neonatal intensive care unit in Africa.[310]

Seasonality

Salmonella infection occurs throughout the year, with predominance for warm months.[177]

Inoculum Size Required to Cause Disease

The estimated inoculum size required to cause symptomatic disease in healthy adult volunteers is 10^5 to 10^{10} organisms,[34] but the number of organisms required to cause symptoms in infants and children probably is much lower. In contrast, large inocula are not required for *Shigella* infection, which occurs in adult volunteers exposed to only 10 organisms. In some outbreaks, very small inocula of *Salmonella* seem to have caused disease. Pharmacologic suppression of gastric acid secretion increase the risk for disease caused by *Salmonella*.[26] Large inocula (e.g., 10^9 organisms) may cause severe symptoms, even in healthy children.[305] The incubation period usually is 6 to 72 hours but depends on inoculum size, bacterial virulence, and host immunocompetence. Communicability parallels the duration of fecal excretion. The probability of salmonellosis occurring is increased when a member of the household is infected. Infants especially may be susceptible to acquiring *Salmonella* infection directly or indirectly from ill family members. Infants also may acquire infection via exposure to raw meat while riding in a grocery cart or while having contact with infected pets.[158] In a retrospective review of 187 infants younger than 1 year old with *Salmonella* gastroenteritis, 39 percent had at least one family contact with diarrhea, and 71 percent of the contacts had stool cultures positive for *Salmonella*.[332] *Salmonella* spp. rarely have been isolated during studies of gastroenteritis in daycare centers, suggesting that larger inocula are required to cause illness in toddlers and older children.[60,184,246]

Antibiotic Selection Pressure

Since the mid-1960s, *Salmonella* spp. have become increasingly resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX). Multiresistant strains have included *S.* ser. Typhimurium, which is the most common serotype in Europe and the United States. Antibiotic resistance usually is transferable between organisms through plasmids that carry genes encoding resistance factors. Some serotype-specific virulence plasmids form hybrid plasmids through recombination with resistance plasmids or acquire gene cassettes consisting of multiple resistance genes. Such evolutionary events provide a virulent strain with the advantage of survival in an unfavorable drug environment.[297] Patients who are infected with antibiotic-resistant strains are more likely to be hospitalized, to be very young, to be black, and to have been exposed recently to antibiotic agents.[178] Previous use of antimicrobial agents for treatment of other illnesses is a significant risk factor for acquiring multiresistant *Salmonella* infection.[193,262]

In the United States, 30.5 percent of nontyphoidal *Salmonella* isolates from blood from 1996 to 2007 were found resistant to at least one antimicrobial tested.[75]

Perhaps the most important factor is the overuse and misuse of antibiotics in animals raised for food.[67,144,145,183,292] Subtherapeutic concentrations of antibiotics used to enhance growth and prevent infection in animals promote intestinal colonization by antibiotic-resistant bacteria, including *Salmonella;* these organisms may be found in feces and may contaminate instruments and meat at the time of slaughter.[47,107] Plasmid analysis and antibiotic susceptibility patterns have linked *Salmonella* outbreaks to specific farms and slaughterhouses.[144,145,216]

Salmonella Ser. Typhi

The CDC estimates that 22 million typhoid cases occur each year in the world, with an annual incidence varying from less than 10 to more than 1000 cases/100,000 population.[276] The global mortality estimates from typhoid also have been revised downward from 600,000 to 200,000, on the basis of regional extrapolations.[74] *S.* ser. Typhi is the most common *Salmonella* isolate in many developing countries. Although the overall ratio of disease caused by *S.* ser. Typhi to disease caused by *S.* ser. Paratyphi is about 10:1, the proportion of *S. paratyphi* infections is increasing in some parts of the world,[30] sometimes paralleling *S.* ser. Typhi isolations.[73] The human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) epidemic in Africa has been associated with a concomitant increase in community-acquired bacteremia caused by nontyphoidal *Salmonella*.[29,125] In the United States, approximately 1700 total cases were reported (1 case/100,000) in 1955. In 1988, approximately 400 total cases were reported (0.018 case/100,000). Approximately 28 percent of infections occurred in individuals 19 years old or younger. Frequently, the highest incidence is stated as occurring in individuals 5 to 12 years of age. More recent population-based studies from South Asia and India suggest, however, that the incidence is highest in children younger than 5 years of age, with higher rates of complications and hospitalization.[30,287]

In the United States, individuals traveling to developing countries are a high-risk group; 62 to 81 percent of infections are related to foreign travel, especially to Mexico, India, the Philippines, Pakistan, El Salvador, and Haiti. Of these areas, the Indian subcontinent has the highest incidence of typhoid among travelers.[3,48,211,274]

Reservoir

Humans are the reservoir for *S.* ser. Typhi; infection implies direct or indirect contact with an infected person. Animal products transmit *S.* ser. Typhi if they are contaminated by infected humans during processing. The most common mode of transmission is food or water contaminated by human feces. Waterborne typhoid fever

epidemics are especially important. Congenital transmission can occur from a bacteremic mother to her fetus transplacentally[215,281] or at the time of delivery.[215]

Relevance of Inoculum Size to Disease

As with nontyphoidal *Salmonella*, more than 10^5 organisms are required to cause clinical illness in adults.[147,148]

Antibiotic Resistance

The worldwide frequency of antibiotic-resistant *S.* ser. Typhi has been increasing since the 1960s[289] but remains much lower than that of nontyphoidal *Salmonella*. Extensive protracted outbreaks have been reported throughout Asia, the Middle East, and Central and South America. These outbreaks may have been related to widespread and inappropriate use of antimicrobial agents in these areas. After sporadic outbreaks of chloramphenicol-resistant typhoid between 1970 and 1985, many strains of *S.* ser. Typhi developed plasmid-mediated, multidrug resistance to the three primary antimicrobials used (i.e., ampicillin, chloramphenicol, and TMP-SMX).[267] This pattern of simultaneous resistance of *S.* ser. Typhi to formerly recommended first-line antimicrobials (i.e., ampicillin, chloramphenicol, and TMP-SMX) is defined as multidrug-resistant typhoid fever.[344] Chromosomally acquired quinolone resistance in *S.* ser. Typhi and *S.* ser. Paratyphi has been described more frequently in some parts of Asia and the rest of the world, possibly related to the widespread and indiscriminate use of quinolones.[258,286] With the advent of quinolone resistance, third-generation cephalosporins were used for treatment, followed by occasional reports of resistance.[344]

PATHOPHYSIOLOGY

The severity and outcome of *Salmonella* infections depend on the combination of the virulence of the infecting bacterial strain, the infectious dose, the route of infection, and the genetic makeup and immunologic status of the host.

Salmonella can (1) adhere to, invade, and multiply in intestinal epithelium; (2) produce cholera toxin–like enterotoxin that increases cyclic adenosine monophosphate levels within intestinal crypt cells, causing a net efflux of electrolytes and water into the intestinal lumen; (3) be taken up by M cells overlying Peyer's patches of the distal ileum and proximal colon; (4) survive in macrophages of Peyer's patches, mesenteric lymph nodes, and the extraintestinal reticuloendothelial system; and (5) survive in the bloodstream.[106,108,116] Specific genes encode virulence factors necessary for each step in these processes and for specific disease manifestations (Table 111-3).

Pathologic findings in *S.* ser. Typhi infection include hypertrophy and hyperplasia of the intestinal and mesenteric lymphoid tissues, liver, and spleen. In contrast, *S.* ser. Typhimurium and other nontyphoidal serotypes cause diffuse colitis, mucosal edema, and crypt abscesses as the major pathologic abnormalities.[36,80]

Some virulence genes are shared by all *Salmonella* strains, whereas others are serotype-specific. Differences in invasiveness of various serotypes exist. *S.* ser. Typhi, *S.* ser. Choleraesuis, *S.* ser. Heidelberg,[152,207] and *S.* ser. Dublin[305] are more likely to enter the blood and to seed distant sites. Virulence plasmids have been identified in *S.* ser. Typhi, *S.* ser. Typhimurium, and *S.* ser. Dublin.[21] The presence of virulence plasmids seems to be more common in blood isolates of *S.* ser. Typhimurium than in fecal isolates (76% vs. 42%).[103]

Nursery outbreaks of *Salmonella* have shown dramatically the variability in severity of illness related to strain or serotype. In nursery outbreaks of *S.* ser. Oranienburg[301] and *S.* ser. Newport,[176] grossly bloody stools were found in 76 to 90 percent of infected infants, with 10 to 11 percent febrile and only 9 to 11 percent asymptomatic. Watery, green, nonbloody diarrhea has been common with *S.* ser. Typhimurium,[1] *S.* ser. Virchow,[266] and *S.* ser. Nienstedten.[283] A high frequency of asymptomatic infections has been seen during nursery outbreaks with *S.* ser. Heidelberg (38% asymptomatic),[24] *S.* ser. Virchow (42% asymptomatic),[266] and *S.* ser. Tennessee (100% asymptomatic).[331]

Which genes are required for disease in humans versus animals remains unclear. *S.* ser. Typhimurium has genes

TABLE 111-3 **Putative Pathophysiologic Basis of Selected Clinical Features of Salmonellosis**

Disease Manifestation	Mechanisms and Bacterial Genes
Bloody diarrhea	*sip* A-D–mediated invasion and interleukin-8–mediated inflammation
Watery diarrhea	*stn* enterotoxin (cholera-like toxin)
	SopB-mediated intestinal inflammation and fluid secretion
	Serotypes that induce transepithelial polymorphonuclear leukocyte migration (e.g., *S.* ser. Typhimurium) are more likely to cause diarrhea than are serotypes that do not (e.g., *S.* ser. Typhi)
Bacteremia	*viaB* (Vi synthesis) capsular antigen interferes with C3 binding (*S.* ser. Typhi, *S.* ser. Dublin, *S.* ser. Paratyphi C)
	rck resistance to serum complement (virulence plasmid encoded)
	rfb encodes lipopolysaccharide synthesis; lipopolysaccharide contributes to persistence of bacteremia
Relapses, prolonged fever, failure of certain antibiotics	Survival in macrophages (*sseABC, spiC, mgtCB,* cytotoxin and virulence plasmid genes *spvRABCD*)

that allow it to cause a nondiarrheal typhoidal illness in mice; in humans, it typically causes symptoms related primarily to intestinal involvement. An estimated more than 200 genes determine *S.* ser. Typhimurium virulence in mice. The clinical variability in host range and disease manifestations is due to the fact that *Salmonella* strains vary in their possession and expression of virulence genes.[200]

Salmonella has developed two complex virulence functions to actively interact with the host and modify host cell functions. These interactions are hallmarks of *Salmonella* pathogenesis: (1) *Salmonella* infects cells of the gastrointestinal epithelium, and are thus able to invade nonphagocytic cells; and (2) *Salmonella* may survive and replicate inside the phagocytes. Of critical importance for these interactions are the *Salmonella* pathogenicity islands (SPI), in particular SPI-1 (invasion and penetration of the gastrointestinal epithelium) and SPI-2 (systemic spread and colonization of host organs) (Fig. 111-1).[135] Both SPIs encode a molecular apparatus termed type III secretion system (TTSS). This apparatus is structurally and functionally related to the flagella assembly systems and typically contains more than 20 different protein subunits located in the inner and outer membrane and in the periplasm and cytoplasm of the bacterial cells.[135] TTSSs are capable of injecting bacterial proteins through bacterial and host membranes into host cells (translocation) or the extracellular milieu (secretion) to influence host biochemistry and cell physiology directly.[65]

Genes relevant to the intestinal phase of illness are encoded primarily in SPI-1. The *invA-H* chromosomal genes are necessary for adherence to and invasion of intestinal mucosal cells[105]; most of the genes described so far seem to be involved in secretion or transport of virulence proteins.[128,316] Genes related to the *Shigella* invasion plasmid antigens *(ipaA-D)* have been described in *Salmonella* spp.[162,163]; these *Salmonella* genes *(sipA-D)* encode the proteins that interact with host cells to cause bacterial uptake and intracellular movement. The major TTSS effector proteins used to invade epithelial cells and induce inflammation in the *Salmonella typhimurium* mouse model are SipA, SopB, SopE, and SopE2.[160] Although initially characterized as an invasiveness island, SPI-1 has additional functions related to the activation of the innate immune pathway, including (1) induction of polymorphonuclear recruitment across the intestinal epithelium by the SPI-1 secreted effect SipA, (2) activation of nuclear factor-κB signaling by the concerted activity of SPI-1 translocated effectors, and (3) activation of caspase-1–mediated interleukin (IL)-1β/IL-18 activation

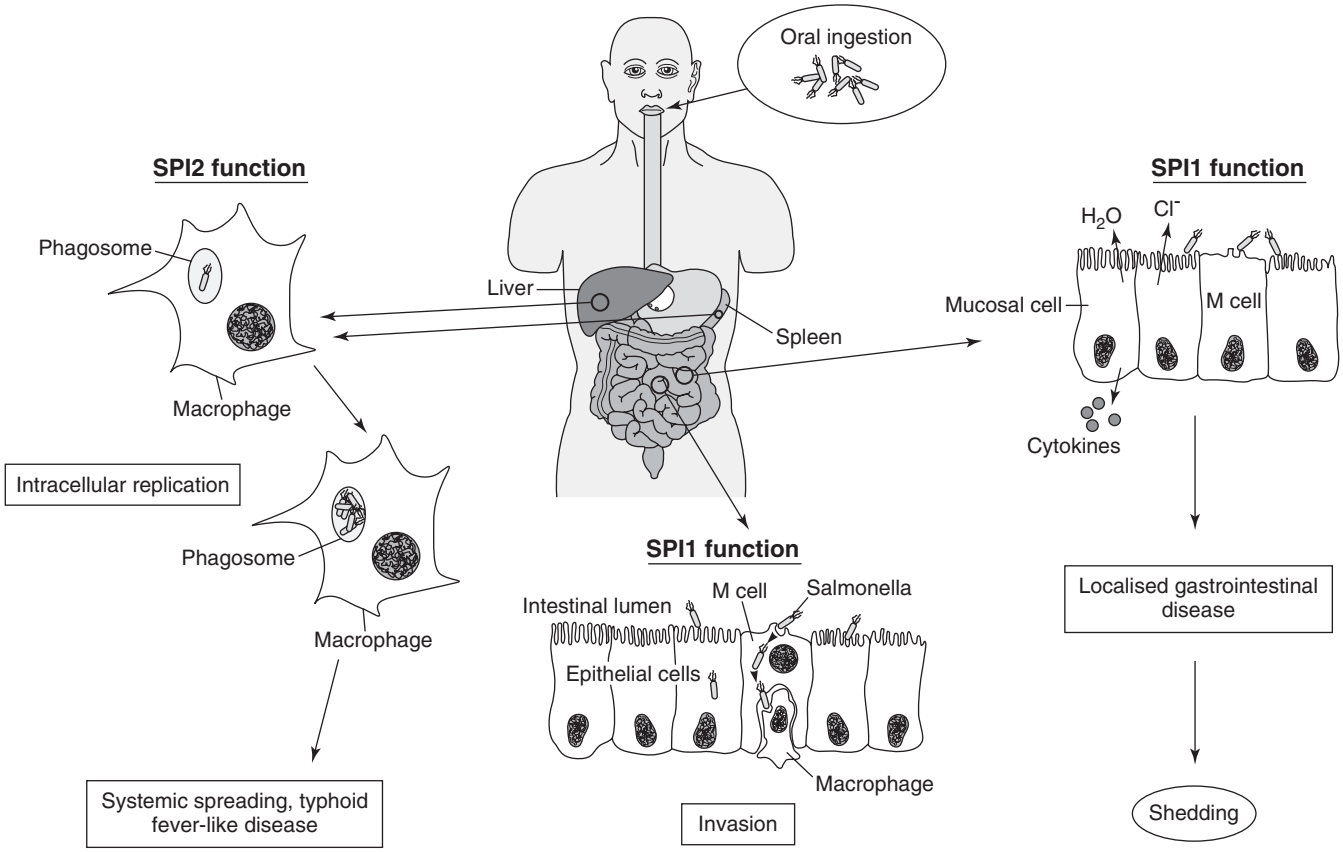

FIGURE 111-1 ■ Host-pathogen interactions during pathogenesis of *Salmonella* infection. SPI1 function is required for the initial stages of salmonellosis, that is, the entry of *Salmonella* into nonphagocytic cells by triggering invasion and the penetration of the gastrointestinal epithelium. Furthermore, SPI1 function is required for the onset of diarrheal symptoms during localized gastrointestinal infections. The function of SPI2 is required for later stages of the infection, that is, systemic spread and the colonization of host organs. The role of SPI2 for survival and replication in host phagocytes appears to be essential for this phase of pathogenesis. (From Hansen-Wester I, Hensel M. *Salmonella* pathogenicity islands encoding type III secretion systems. *Microbes Infect* 2001;**3**:549–59)

and proinflammatory cell death by the SPI-1 translocator effector SipB.[65]

The role of host cells in the invasion process is complex. After *S.* ser. Typhimurium comes in contact with epithelial cells, activation of epidermal growth factor receptor occurs, which activates a kinase that turns on phospholipase A_2 so that arachidonic acid is generated. Arachidonic acid is converted to leukotriene D_4, which opens calcium channels and causes membrane ruffling, cytoskeletal changes, and uptake of bacteria.[105] Nonphagocytic cells, including epithelial cells, are adapted poorly for killing of internalized bacteria. Not only do *Salmonella* spp. survive in vacuoles within epithelial cells but they also can replicate actively.[106] *S.* ser. Typhi survives better in human than in mouse macrophages, whereas *S.* ser. Typhimurium survives better in mouse macrophages.[41,81,282] *Salmonella* is capable of infecting a wide variety of cells, including dendritic cells, macrophages, hepatocytes, neutrophils, colonocytes, and other epithelial cells. In vitro, within minutes of contact with cells, *Salmonella* organisms are internalized and take up residence in a unique membrane-bound compartment distinct from a phagosome or lysosome, the *Salmonella*-containing vacuole.[124] Live neutrophils recruited during acute *Salmonella* colitis engulf pathogens in the gut lumen and may thus actively engage in shaping the environment of pathogens and commensals in the inflamed gut.[187]

SPI-2 is essential for intracellular parasitism and systemic virulence in murine typhoid and for evasion of phagocyte oxidase machinery of the host.[320] Intracellular replication occurs in a specialized membrane compartment, the *Salmonella*-containing vacuole, and depends on translocation of approximately 30 effector proteins via the SPI-2 TTSS into the host endomembrane system and cytoplasm.[104] SPI-2 has additional roles in inflammatory disease, such as induction of cyclooxygenase and modulation of host cytokine expression and signaling. In addition, SPI-2 function affects antimicrobial defense mechanisms of the host, intracellular transport processes, integrity and function of the cytoskeleton, and host cell death.[174] SPI-2 is crucial for early and complete induction of enterocolitis and systemic disease.[65]

Several pathogen-associated molecular patterns of pathophysiologic importance are presented by *Salmonella* during infection, principally lipopolysaccharides and flagellin. The activation of Toll-like receptor (TLR)4 in response to *Salmonella* lipopolysaccharides is essential for inducing host responses.[319] *Salmonella* flagellin is a potent inducer of host inflammation in polarized epithelial monolayers when delivered to the basolateral surface of the epithelium. When delivered there, *Salmonella* flagellin induces IL-8 secretion by stimulating basolateral TLR5.[345] Flagellin stimulation of innate immune responses is crucial for intestinal inflammation but not for murine typhoid.[65]

The development of diarrhea depends on host and pathogen factors. An influx of polymorphonuclear leukocytes into the mucosa must occur for diarrhea to develop.[329] Neutropenic animals fail to develop fluid secretion when they are infected with *Salmonella*[329]; infiltration of leukocytes is thought to trigger production of prostaglandin because fluid secretion can be blocked by indomethacin.[117] A cholera toxin–like enterotoxin is made by approximately two thirds of *Salmonella* strains, including *S.* ser. Typhimurium and *S.* ser. Typhi.[155]

For most nontyphoidal *Salmonella* strains, infection does not extend beyond the lamina propria and the local lymphatics. In contrast, *S.* ser. Typhi, *S.* ser. Dublin, and *S.* ser. Choleraesuis rapidly invade the bloodstream, with little intestinal involvement. Some virulence genes confer a survival advantage to the organisms if they get into the extraintestinal milieu. Vi capsular antigen present in *S.* ser. Typhi, *S.* ser. Dublin, and *S.* ser. Paratyphi C interferes with C3 binding. Mutations in lipopolysaccharide genes decrease invasiveness of *S.* ser. Typhi and *S.* ser. Choleraesuis, but not of *S.* ser. Typhimurium.[106,222] *S.* ser. Dublin, *S.* ser. Typhimurium, and *S.* ser. Enteritidis have virulence genes that confer resistance to complement by preventing the formation and insertion of the C5b-9 membrane attack complex.

The mechanisms governing homeostasis and immune defense are of great importance for *Salmonella* infection. *Salmonella* uses multiple strategies to evade and modulate host innate and adaptive immune responses, to persist in the presence of a robust immune response.[219] Chronically infected hosts are often asymptomatic and transmit disease to naïve hosts via fecal shedding of bacteria, thereby serving as a critical reservoir for disease. The ability of *Salmonella* to persist depends on a balance between immune responses that lead to the clearance of the pathogen and avoidance of damage to host tissues.[271] In addition, the intestinal microbiota plays a critical role in controlling *Salmonella* infection, disease, and transmissibility.[219] However, the molecular nature of the homeostatic interactions, the pathogen's ability to overcome colonization resistance, and the triggering of native and adaptive mucosal immune responses are still poorly understood.[160,161]

Host Factors

Multiple host defense strategies have evolved to deal with the bacteria virulence factors; host susceptibility often can be related directly to defects in these defense mechanisms (Table 111–4). The host tries to kill ingested organisms in the stomach, to inhibit their growth in the gut, to limit their spread beyond the intestine, and to clear them by immune mechanisms. At a pH of 2.0, most *Salmonella* spp. are killed rapidly.[115] When gastric pH is increased by oral administration of antacid, susceptibility increases.[114,147,148,262] A *Salmonella* inoculum ingested in water passes through the stomach more rapidly than when the same inoculum is ingested in food. Rapid transit through the small bowel decreases the contact time of organisms with the mucosa. Patients with decreased intestinal motility caused by medication or anatomic factors have increased severity and complications and may have a prolonged carrier state. Prior antimicrobial exposure increases the risk for incurring infection with antimicrobial-susceptible and antimicrobial-resistant strains of *Salmonella*.[242] The normal flora may compete for substrates, lower the local pH by production of short-chain fatty acids, and produce antibacterial substances such as colicins. Some patients with gastroenteritis have

TABLE 111-4 **Susceptibility to Salmonella Species Infection**

Patient Group at Risk	Mechanism
Newborn	Achlorhydria, rapid gastric emptying
	Poorly developed cell-mediated immunity
	Complement deficiency
	Immunoglobulin deficiency in premature infants
Sickle-cell anemia	Reticuloendothelial system overload owing to hemolysis
	Functional asplenia
	Tissue infarcts
	Defective opsonization
Neutropenia (congenital or acquired)	Polymorphonuclear neutrophils needed for killing
Chronic granulomatous disease	Defective killing by polymorphonuclear neutrophils
Defects of immune system IL-12/interferon-γ axis	Defective signaling resulting in failure to activate macrophages and recurrent/persistent infection by nontyphoid *Salmonella*
Acquired immunodeficiency syndrome	Low CD4
	Effects of malnutrition on cell-mediated immunity
	Survival of organisms in macrophages (owing to *Salmonella* genes *PhoP/PhoQ, spvA-D, R*)
Organ transplantation, immunosuppression	Defective cell-mediated immunity
Gastrectomy	Loss of stomach acid barrier
Malaria	Reticuloendothelial overload during hemolysis
	Abnormal complement levels
	Abnormal macrophage function
Bartonellosis (verruga peruana)	Reticuloendothelial overload during hemolysis
Schistosomiasis	*Salmonella* sequestered in schistosomes protected from host defenses and antibiotics

progression or exacerbation of symptoms when antibiotics are given.[265]

Children with compromised immune systems are at increased risk for serious focal infections. *Salmonella* organisms are able to survive in macrophages but not in polymorphonuclear leukocytes. Patients with neutropenia (e.g., congenital or related to chemotherapy) or neutrophil dysfunction (e.g., chronic granulomatous disease) are at high risk for development of disseminated infection. Patients who have been bacteremic with a nontyphoidal *Salmonella* strain are at increased risk for having a relapse if leukopenia is present.[112]

An increased risk for acquiring disease exists in settings in which reticuloendothelial function or cell-mediated immunity is impaired[226,291] or immature.[225] Hemolytic anemias are thought to cause reticuloendothelial overload. Children with sickle-cell anemia are at risk for developing bacteremia and osteomyelitis.[4,199,299,338] Children with sickle-cell and S-Thal also sometimes develop osteomyelitis.[66] Patients with sickle-cell anemia have complement defects and defects in opsonization of *S.* ser. Typhimurium.[134] Newborns also have complement deficiencies that may explain the high frequency of *Salmonella* infection in newborns and the susceptibility of newborns to bacteremic complications seldom seen in normal hosts.

Malaria predisposes to salmonellosis by multiple mechanisms.[192] Schistosomiasis predisposes to development of *Salmonella* infections and prolonged bacteremia[262]; reticuloendothelial cell killing of *Salmonella* is impaired, and *Salmonella* colonizes the schistosomes. Pili on *Salmonella* adhere to the surface of *Schistosoma mansoni* and *Schistosoma haematobium*.[188] In Gabonese children with bacteremic nontyphoidal *Salmonella* infection, rectal biopsy specimens showed the eggs of *Schistosoma intercalatum* in 90 percent of cases.[113]

Immune Response

The innate immune system provides the first line of defense against invading microorganisms by inducing a variety of inflammatory and antimicrobial responses. A distinctive feature of *Salmonella* is that it has not only adapted to survive in a strong inflammatory environment, but it also uses this adaptation as a strategy to gain a growth advantage over the intestinal microbiota. The gastric innate immune response to *Salmonella* is complex. First, following the invasion of the mucosa, the presence of *Salmonella* is detected by pattern recognition receptors. Extracellular *Salmonella* are detected by TLRs inducing a transcriptional response leading to the expression of proinflammatory cytokines such as IL-23. Intracellular *Salmonella* activate NOD-like receptors that can induce IL-23 expression, as well as the assembly of NLRC4/NLRP3 inflammasomes that activate caspase-1, promoting the secretion of mature IL-1b and IL-18. SPI-1 mediated activation of caspase-1 in epithelial cells might contribute to IL-18 secretion. Furthermore, IL-18 and IL-23 amplify the inflammatory response by paracrine signaling. IL-18 induces the release of IFNc from T cells, and IL-23 induces the release of IL-22 and IL-17. These cytokines induce the increased production of mucins and antimicrobial peptides, and promote the release of CXC chemokines, leading to an influx of neutrophils into the mucosa.[39]

Cell-mediated immunity generally is thought to be more important than humoral immunity in clearance of *Salmonella* organisms. T-cell activation of macrophages is necessary to kill intracellular *Salmonella* organisms.[195] Oral immunization with an attenuated typhoid vaccine primes lymphocytes to produce cytokines typical of a T_H1 response (high interferon [IFN]-γ/low IL-4) to the flagellar antigen.[302] Healthy individuals vaccinated with either oral or parenteral typhoid vaccines develop

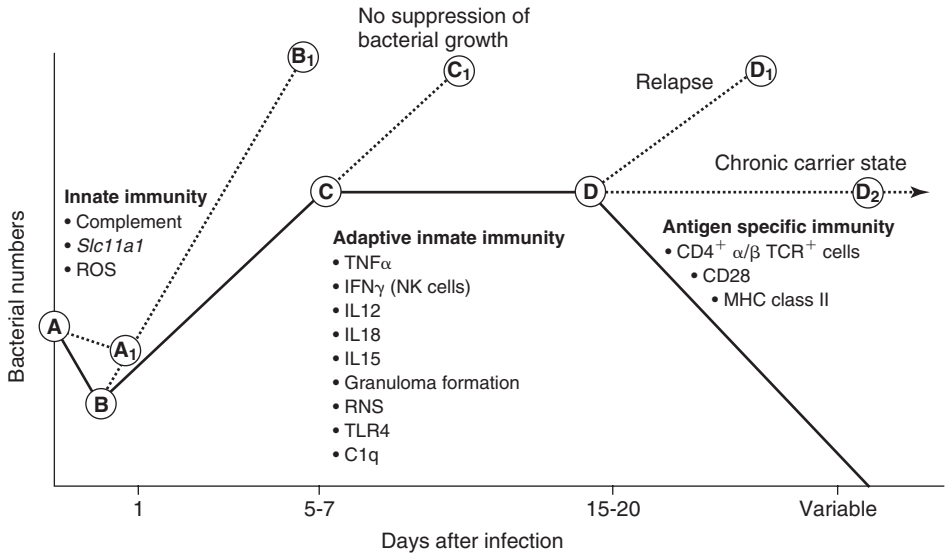

FIGURE 111-2 ■ Immunologic mechanisms that control the progression of a sublethal *S. enterica* infection in the mouse model. The *solid line* in the graph shows the course of a sublethal infection in wild-type immunocompetent mice. The *dotted lines* show the course of the infection when immunologic mechanisms required at points *A→D* are absent. Lack of complement and/or reactive oxygen species (*ROS*) affects the early blood clearance and killing of the bacteria with a shift of the growth curve from *A→B* to *A→A1*. The *Slc11a1* gene and ROS influence the net growth rate and their absence determines a shift of the curve from *B→C* to *B→B1*. Point *C* coincides with the onset of the adaptive innate immunity response. The lack of any of the immunologic factors that form part of adaptive innate immunity determines failure to suppress bacterial growth in the tissues *(C→D)* and the unrestrained progression of the infection process *(C→C1)*. Point *D* coincides with the intervention of antigen-specific immunity that is required to clear the infection and prevent relapse *(D→D1)* of the establishment of a chronic carrier state *(D→D2)*. (From Mastroeni P, Maskell D, editors. *Salmonella infections*. Cambridge, Mass: Cambridge University Press; 2006, p. 209).

antibody-dependent cellular cytotoxicity mediated by IgA, IgG, or both.[77] Studies of serum and secretory antibodies to O and H antigens have not shown protection, however; relapses of typhoid fever have occurred despite high antibody titers. Immunity may be short-lived. In a study of 14 individuals (17 to 28 years old) with acute typhoid fever, cell-mediated immunity persisted for 16 weeks; intestinal secretory IgA persisted for 48 weeks; and IgG, IgM, and anti-O and anti-H agglutinins persisted for 2 years, 16 weeks, 16 weeks, and 36 weeks, respectively.[279]

Cytokines play a crucial role in initiating and regulating the innate and adaptive immune response against *Salmonella*. These bacteria can trigger synthesis of cytokines and chemokines in epithelial cells, macrophages, and dendritic cells. The consequences of cytokine activation vary. Although IFN-γ, IL-12, tumor necrosis factor-α, IL-18, transforming growth factor-β, and CCL2 have protective functions during *Salmonella* infection, IL-4 and IL-10 interfere with host defenses.[94] The main immunologic mechanisms that control the course of *Salmonella* infection in mice are described in Figure 111-2.[203]

Impaired cell-mediated immunity probably explains the high frequency of bacteremia with nontyphoidal *Salmonella* strains in children with HIV infection[273] and with malnutrition.[288] Defective cell-mediated immunity can be congenital or acquired (from tumors,[132] collagen vascular disease, organ transplantation,[96] chemotherapy, glucocorticosteroids).[256] Inherited deficiency in the IL-12/IL-23/IFN-γ pathway results in susceptibility to recurrent *Salmonella* and *Mycobacterium* infections.[86] Defects have been described in which patients have mutations in IL-12, IL-12 receptor, IL-23, IL-23 receptor, IFN-γ receptor, and signal transducer and activator of transcription (STAT). Complete and partial deficiency syndromes have been described.[10,15,159] Isolated persistent or recurrent nontyphoidal *Salmonella* infection without history of infectious episodes by other microorganisms has been reported in 15 percent of children with deficiency of β-1 receptor for IL-12.[81]

Humoral immunity is less important. Preterm neonates who are infected with *S.* ser. Typhimurium may have a lower risk for developing complications (e.g., intestinal perforation, meningitis, endophthalmitis, sepsis, and pyelitis), however, if they are given intravenous immunoglobulin plus cefoperazone than if given cefoperazone alone (16% vs. 82%); the mortality rate also is decreased (12% vs. 41%).[122]

CLINICAL MANIFESTATIONS

Salmonella spp. may cause acute or chronic asymptomatic infection. Symptomatic infections include acute gastroenteritis, bacteremia with or without local suppuration, and enteric fever.

Acute Asymptomatic Infection

Asymptomatic infections usually are identified by stool cultures obtained during prospective research studies or

epidemiologic investigations. A study of Mexican infants showed that 74 percent of nontyphoidal *Salmonella* infections were asymptomatic.[71]

Acute Gastroenteritis

The most common clinical illness caused by *Salmonella* infection is gastroenteritis. Nausea, vomiting, and crampy abdominal pain begin 6 to 72 hours (median, 24 hours) after ingestion of contaminated food or water. The abdominal pain may be severe enough to suggest appendicitis. Diarrhea usually is moderate in volume and, depending on the serotype, may contain blood. Dysentery is a more common presentation in children from developed countries in contrast to those from developing regions.[245] Headaches, malaise, myalgias, and fevers are common. These symptoms usually resolve in approximately 1 week without antibiotic therapy; symptoms may persist in very young patients and patients with underlying diseases. In neonates, loose, green, mucous stools or, less often, bloody diarrhea is seen; fever is common with *Salmonella* gastroenteritis during the first months of life.[152] Reactive arthritis develops rarely in children after having *Salmonella* gastroenteritis.

Bacteremia with or Without Metastatic Focal Infection

Some *Salmonella* serotypes (e.g., *S.* ser. Typhi; *S.* ser. Choleraesuis; *S.* ser. Paratyphi A, B, and C; *S.* ser. Heidelberg; *S.* ser. Typhimurium; *S.* ser. Enteritidis; *S.* ser. Saint-Paul; *S.* ser. Newport; *S.* ser. Panama; and *S.* ser. Dublin) have a propensity to invade the bloodstream; others (e.g., *S.* ser. Tennessee and *S.* ser. Weltevreden)[334] rarely seem to cause bacteremia. *Salmonella* has been found as one cause of occult bacteremia in infants and children,[8,170] and when not treated with appropriate parenteral antibiotics, 45 to 68 percent of infected children developed persistent *Salmonella* bacteremia.[175] *Salmonella* is also found within cases of clinically suspected community-acquired invasive bacterial disease in children from tropical regions.[13] Fever, chills, diaphoresis, myalgias, anorexia, and weight loss may last for days or weeks. Stool cultures may be negative. A child sometimes can have afebrile diarrhea and yet be bacteremic for several days.[165] Warm climate, previous gastrointestinal disease, and recent exposure to antibiotics are some reported risk factors for bacteremia in children.[127] Severe malnutrition, HIV infection, prolonged illness, and recent hospital admission are risk factors for nontyphoidal *Salmonella* bacteremia in developing countries.[37] The true frequency of bacteremia is unclear. Depending on the patient's age, geographic location, and nature of the study (prospective vs. retrospective), 2 to 45 percent of infections are bacteremic.* Bacteremia probably occurs more commonly in newborns (in some studies 30% to 50%) than in older children,[152] although this is debatable.[207] The true risk for bacteremia developing in the first year of life is likely to be 2 to 6 percent.[78,311]

*References 78, 152, 207, 225, 288, 311, 333, 334, 341.

TABLE 111-5 **Focal Infections in Nontyphoidal Salmonellosis**

Organ or System	Focal Infection
Brain	Meningitis
	Brain abscess and empyema
Osteoarticular	Arthritis
	Osteomyelitis
	Pyomyositis and psoas abscess
	Prosthetic osteoarthritis
	Subcutaneous abscess
Cardiovascular	Endocarditis
	Pericarditis
	Mycotic aneurysm
	Myocardial abscess
	Septic thrombophlebitis
Respiratory tract	Retropharyngeal and pharyngeal abscess
	Pneumonia and empyema
Other	Urinary tract infection
	Epididymitis and orchitis
	Reiter syndrome
	Endophthalmitis
	Suppurative lymphadenitis

Hemolytic anemia, especially sickle-cell anemia, is associated with a high risk for development of *Salmonella* bacteremia.[221] Persistent or recurrent bacteremia occurs in patients with AIDS, schistosomiasis, and intravascular focal infection.[221] Immunocompromised adults who become bacteremic with *Salmonella* are more likely to do so without having had preceding gastroenteritis and to have a high mortality rate.[136] Children more typically are relatively immunocompetent; most often, they develop bacteremia associated with diarrhea and have a much better prognosis.[179,207] In a large study in African children, nontyphoidal *Salmonella* spp. were the second most common cause of community-acquired bacteremia in all children and in HIV-positive patients.[29] The risk for development of focal infections during bacteremia is higher (36%) in children with underlying conditions than in previously healthy children (2.5%).[342]

Focal suppurative infections may occur almost anywhere (Table 111-5); the most common sites are bones (particularly in sickle-cell anemia)[214,260] and the central nervous system.* Meningitis has a high morbidity, with acute hydrocephalus, seizures, ventriculitis, abscesses, subdural empyema, and cerebral infarction.[339] Long-term neurologic sequelae include developmental delay, which could be severe, motor disabilities (i.e., cranial nerve palsy, hemiparesis, cuadriparesis), chronic hydrocephalus, epilepsy, visual and auditory impairment, and athetosis.[67,235,339] Neurologic sequelae are particularly common in infants with delayed presentation, prolonged fever (>10 days) or seizures during hospitalization, and marked alteration of consciousness on admission.[150,235,339] Mortality rates from meningitis were 40 to 60 percent before

*References 40, 90, 169, 189, 264, 342.

the introduction of third-generation cephalosporins, fluoroquinolones, and carbapenems. With the use of these antibiotics the mortality rate has decreased to almost 10 percent but still with a high rate of sequelae.[235,339] Relapses even after prolonged therapy occur commonly (reflecting the intracellular localization of *Salmonella* and the difficulty of achieving adequate intracellular levels of antibiotics). Of nontyphoidal *Salmonella* meningitis, 50 to 75 percent occur in the first 4 months of life.[66,235] The serotypes causing meningitis, including *S.* ser. Typhimurium, *S.* ser. Heidelberg, *S.* ser. Enteritidis, *S.* ser. Saint-Paul, *S.* ser. Havana, *S.* ser. Oranienburg, *S.* ser. Newport, and *S.* ser. Panama,[66,332] are commonly associated with bacteremia. In infants, complications include pneumonia,[24] osteomyelitis,[84,172,338] septic arthritis,[24,280] pericarditis,[133,206] pyelitis,[301] peritonitis,[1] otitis media,[1] mastitis,[224] cholecystitis,[131] endophthalmitis,[70] cutaneous abscesses,[253] and infected cephalohematoma.[84] In adults and occasionally in older children, femoral and distal aorta (mycotic aneurysms),[66] heart valves,[66] scrotum,[314] testicles,[66] prostate,[278] ovaries,[66] and fallopian tubes[278] also may be infected.

Hemolytic-uremic syndrome associated with *S.* ser. Typhimurium[91,204] and *S.* ser. Typhi[22] has been reported. Because the cytotoxins produced by various *Salmonella* strains are distinct immunologically from Shiga toxin produced by *Shigella dysenteriae* 1 and the Shiga toxins produced by enterohemorrhagic *Escherichia coli*,[16] the association of hemolytic-uremic syndrome with salmonellosis may represent undiagnosed dual infection with toxin-producing organisms.

Enteric Fever

Enteric fever usually is caused by *S.* ser. Typhi and, less often, other invasive *Salmonella*, including *S.* ser. Paratyphi and *S.* ser. Choleraesuis. The onset of symptoms in enteric fever is usually insidious.[147,148] After an incubation period of 10 to 14 days (range, 6 to 21 days), which generally is related to the inoculum size, fever, malaise, anorexia, and abdominal pain develop over 2 to 3 days. The incubation period tends to be shorter with paratyphoid fever. The temperature rises in small increments, usually reaching 40° to 40.5°C (104° to 104.9°F) by the end of the first week of illness. The temperature does not return to normal but rather rises to higher peaks each afternoon, with higher nadirs occurring each subsequent morning during the first week. Eventually, the fever is unremitting; spikes in temperature occur without return to normal. In younger children, onset of fever is more abrupt on onset and then could turn intermittent or remain sustained.[97]

Constipation occurs in older children and tends to appear later on in the course,[168] whereas diarrhea is more frequent in younger children and occurs in almost one third of patients.[208,308] When diarrhea develops, it usually does so after the patient has been febrile for several days[168] and tends to be more frequent in children from nonendemic settings, where it can even lead to dehydration.[295] It is small in volume and of loose consistency, resembling a greenish pea soup; it contains erythrocytes but usually is not grossly bloody.[92,208,294] Fecal leukocytes are present in nearly all patients with diarrhea.[92] Diarrhea occurs more commonly with paratyphoid than with typhoid fever.[308] Vomiting is mild and not sustained.

A dull, continuous frontal headache begins during the first 2 days of fever and is easier to explore in adolescents.[168] Children commonly complain of headache and often are drowsy, irritable, or delirious.[83]

A relative bradycardia for the degree of fever can be found over the first week, but it is not a constant or specific finding.[79,303] Patients have a toxic facies; coated tongue; a musty, "damp hay–like" odor; and a tender, doughy abdomen with slight guarding. Occasionally, a child may have a low-level cough. The skin is dry with little sweating. Meningismus may occur early in the illness.

During the second week, rose-colored spots measuring 2 to 4 mm may appear on the abdomen or chest and rarely on surrounding locations. They are blanching, erythematous, slightly raised lesions that last approximately 3 days. Rose spots occur in a few patients and are difficult to recognize in dark-skinned individuals. Infrequently, new crops of rose spots continue for 1 to 2 weeks.

A soft, tender spleen becomes palpable by the second week of illness. Respiratory symptoms may progress, and epistaxis occasionally may occur. If left untreated, enteric fever has a prolonged course, with continuous high temperature of 39.5° to 40.5°C (103.1° to 104.9°F) for up to 4 weeks, followed by a gradual return to normal beginning during the third or fourth week. A rapid decrease in temperature late in illness suggests intestinal hemorrhage or perforation[33]; such a decline in temperature typically is followed by an increase a few hours later as peritonitis develops. Intestinal hemorrhage and intestinal perforation[33,44,118] may occur in the second to fourth week in 3 percent of patients with typhoid fever.[44]

Complications occur in 10 to 15 percent of patients; most of these develop during the second or third week of illness. Many complications, of which gastrointestinal bleeding, intestinal perforation, and typhoid encephalopathy are the most important, have been described (Table 111-6).[181,234,239,255,333] Gastrointestinal bleeding is the most common, occurring in 10 percent of patients.[239] It results from erosion of a necrotic Peyer's patch through the wall of an enteric vessel. Suppurative lymphadenitis,[223] tonsillitis,[156,278] endocarditis,[88] and pancreatitis[14,201,340] rarely occur. Patients who have thalassemia or glucose-6-phosphate dehydrogenase deficiency may have hemolysis during typhoid fever.[308]

The relapse rate is 5 to 10 percent, even when appropriate therapy has been given.[7] Relapses typically occur 2 to 3 weeks after the resolution of fever and are milder than the initial illness. The *Salmonella* isolate from a patient in relapse usually has the same antibiotic susceptibility pattern as the isolate obtained from the patient during the original episode. Reinfection also may occur and can be distinguished from relapse by molecular typing.[325]

In some geographic areas, such as Indonesia, where an exceptionally virulent *S.* ser. Typhi is endemic, toxemia, delirium, obtundation, coma, and shock sometimes occur.[147,148,181] Some serotypes in Indonesia (e.g., H1-j) seem to be less virulent than others, suggesting that

TABLE 111-6 **Complications of Typhoid Fever**

Organ/system	Complication
Abdominal	Gastrointestinal hemorrhage
	Intestinal perforation
	Hepatitis
	Cholecystitis
	Pancreatitis
Neuropsychiatric	Encephalopathy
	Delirium
	Psychosis
	Meningitis
	Impairment of coordination
Cardiovascular	Asymptomatic electrocardiographic changes
	Myocarditis
	Infected prosthetic valve
	Shock
Respiratory	Bronchitis
	Pneumonia
Hematologic	Anemia
	Disseminated intravascular coagulation
Other	Focal abscess
	Suppurative lymphadenitis
	Pharyngitis
	Tonsillitis
	Osteomyelitis
	Arthritis
	Parotiditis
	Orchitis
	Pyelonephritis
	Miscarriage
	Relapse
	Chronic carriage

Modified from Parry CM, Hien TT, Dougan G, et al. Typhoid fever. *N Engl J Med* 2002;**347**:1770-82.

properties of the flagellar antigen may be important to virulence.[129]

Typhoid fever varies in its clinical course. Variations on the classic theme include a completely afebrile course occurring in debilitated patients, a high spiking fever from the first day (particularly in children), a focal presentation (e.g., pneumonia and nephritis), and a severe course during relapses. Infants are said to be at higher risk for development of massive hepatomegaly, thrombocytopenia, and other complications.[254] The mortality rate is high in the neonatal period.[257] Children younger than 5 years of age may have a nonspecific illness that is not recognized clinically as typhoid[287]; infants and toddlers often have a febrile illness misinterpreted as a "viral syndrome." In children younger than 2 years, the fever may last for only 1 to 5 days, despite the presence of *S.* ser. Typhi or *S.* ser. Paratyphi in the blood; low-grade fever (temperature of 38.3° to 38.8°C [100.9° to 101.8°F]) and cough may be the only findings in such children.[102] The case-fatality rates are highest among children younger than 1 year and among elderly patients. The most important contributor to a poor outcome is probably a delay in instituting effective antibiotic treatment.[239]

Enteric fever from infection with multidrug resistant *Salmonella* ser. Typhi (MDRST) in children is more frequently associated with the presence of fever, toxic appearance, hepatomegaly, splenomegaly, and abdominal tenderness and distention on admission in contrast non-MDRST.[344]

Typhoid and nontyphoidal *Salmonella* infections during pregnancy increase the risk for spontaneous abortion.[213,296] Spontaneous abortion or premature labor usually can be prevented by early treatment.[285] Transmission of *S.* ser. Typhi rarely occurs in utero.[55] Typically, premature delivery occurs during the second to fourth week of untreated maternal typhoid fever.[126] In the preantibiotic era, 40 percent of women with typhoid delivered prematurely; the remainder carried to term, although only 17 percent of infants survived.[82] If infection occurs late in gestation and is treated appropriately, the infant may survive.

Asymptomatic Chronic Carrier State

Chronic carriers excrete *Salmonella* organisms in stools for longer than 1 year after having gastroenteritis-enterocolitis or enteric fever. Approximately 1 to 4 percent of patients who recover from enteric fever caused by *S.* ser. Typhi chronically excrete the organism[212]; fewer than 1 percent of patients with nontyphoidal *Salmonella* excrete for a prolonged period.[42,51] Twenty-five percent of prolonged carriers have no history of typhoid.[239] Nontyphoidal infection is associated with excretion for a mean of 5 weeks, although children younger than 5 years of age,[42] female patients, elderly patients, and patients with biliary tract disease are more likely to become carriers. The biliary tract is infected in almost all chronic carriers of *S.* ser. Typhi. As many as 10^6 organisms per 1 g of feces may be excreted.[212]

Chronic excretion in such patients serve as the source of infection to their contacts. Chronic carriers represent an epidemiologically important reservoir of *S.* ser. Typhi; they often are the source of outbreaks of typhoid fever. In the United States, although typhoid fever generally is imported, 30 percent of infections result from exposure to previously diagnosed or newly diagnosed chronic carriers.[275]

Patients who have a history of *Salmonella haematobium* or tuberculous infections of the urinary tract may develop chronic urinary carriage after a bout of typhoid fever.[99,263]

DIAGNOSIS

The symptoms in *Salmonella* gastroenteritis overlap sufficiently with symptoms seen in other diarrheal illnesses that laboratory studies generally are required to prove the diagnosis. Proctoscopy is rarely done; typical findings include mucosal edema, hyperemia, friability, and hemorrhages.[80] The fecal leukocyte examination is positive for polymorphonuclear leukocytes in 36 to 82 percent of nontyphoidal cases,[137] but this finding is nonspecific. Stool culture is preferable to swab culture, particularly for evaluation of long-term carriers. Overnight enrichment in selenite broth increases the yield from stool

cultures. The optimal agar for isolation of the organism (SS, Hektoen enteric, MacConkey, xylose-lysine-deoxycholate, xylose-lysine-Tergitol 4, brilliant green, or modified semisolid Rappaport-Vassiliadis medium) is debated. Modified semisolid Rappaport-Vassiliadis medium has a high yield but cannot be used for isolating *S.* ser. Typhi or nonmotile strains and has lower specificity than SS agar.[272] Increasing the volume of stool sample improves the yield of *Salmonella* in cultures.[324]

Salmonella organisms usually can be isolated readily from blood by use of conventional media if the patient is bacteremic. In patients with extraintestinal focal nontyphoidal infection, specimens from the affected areas may have positive Gram stains and grow the organism.

Enteric fever should be suspected on the basis of the setting and clinical course. Laboratory abnormalities are common but nonspecific findings. Normocytic, normochromic anemia and leukopenia or neutropenia, perhaps caused by hemophagocytosis in the bone marrow, often are present.[198] Clotting abnormalities consistent with disseminated intravascular coagulation (e.g., thrombocytopenia, hypofibrinogenemia) may occur[43] but usually are transient and not associated with clinically significant bleeding. In enteric fever, electrolyte values usually are normal, but increases in alkaline phosphatase, serum lactate dehydrogenase, serum aspartate aminotransferase, and serum cholesterol occur frequently. Transient proteinuria sometimes occurs during the first week of enteric fever.

Cultures from multiple sites should be submitted for suspected enteric fever; culture of bone marrow has the highest yield,[141,143,315] particularly if the patient has had antibiotic pretreatment. During the first week of typhoid fever, approximately 90 percent of patients have positive blood and bone marrow cultures, but negative stool and urine cultures. During subsequent weeks, the yield of blood and bone marrow cultures decreases as the yield of stool and urine cultures increases. Culture of duodenal fluid obtained by string capsules can be as sensitive as culture of bone marrow aspirates.[19,143,315] Over the third week of enteric fever, bone marrow culture could remain positive despite negative blood cultures.[327] Ratio of nonviable to viable *Salmonella* bacteria in blood ranges from 1000:1 to 47,000:1.[23] The overall frequencies of positive cultures during the course of typhoid are found in blood (40% to 54%), urine (7% to 10%), stool (approximately 35%), bone marrow (80% to 90%), rose spots (~65%), and duodenal string test culture (58% to 85%).[119,143]

Blood volume is the most important factor for *Salmonella* isolation in blood cultures, and it is recommended 10 to 15 mL be drawn per sample from infants, as in adults.[335] Because bacterial counts in blood are higher in children[324] and the volume of samples are sensible both for personnel and caregivers, recommended blood volume for toddlers and infants is 2 to 4 mL.[335] The Widal test measures antibodies against the O and H antigens of *S.* ser. Typhi. Although many patients with enteric fever may have a fourfold increase in the titer of paired sera during the second week of illness, many false-negative and false-positive test results occur. Widal test titers could rise before clinical onset of disease, preventing the expected fourfold rise in titers of paired sera.[241] False-positive results of the Widal test can be caused by infection with other Enterobacteriaceae, malaria, dengue, nontyphoidal *Salmonella*, and other diseases and previous exposure to *Salmonella* antigens (including typhoid vaccines). Variability and poor standardization of commercial test antigens are identified causes for both false-positive and false-negative results.[232] Widal test titers may be more useful in children with typhoid who are living in a nonendemic area. Interpretation of Widal test results is aided by information about seropositivity in the population to which the patient belongs.[62,313] Some centers have found the Widal test helpful when it is used with locally determined cutoff points.[64,238] Only under unfavorable circumstances when no confirmatory microbiologic test result is available, the Widal test could be used to diagnose typhoid fever, but underscoring this approach is not to be encouraged.[232] Various diagnostic kits, including serologic tests such as passive hemagglutination, passive bacterial agglutination, latex particle agglutination slide tests, counterimmunoelectrophoresis, radioimmunoassay, and enzyme-linked immunosorbent assay with use of monoclonal antibodies, have been developed.[154] These commercial assays (i.e., Typhidot, Typhidot-M, and Tubex) have not proved to be sufficiently robust in large-scale evaluations in community settings.[30] Molecular techniques used primarily in epidemiologic studies include DNA hybridization studies, phage typing, chromosome analysis, and plasmid analysis. A salivary anti–*Salmonella typhi* lipopolysaccharide IgA enzyme-linked immunosorbent assay (ELISA) test for diagnosis of typhoid fever in children has shown promising results, but more research is needed.[343]

DIFFERENTIAL DIAGNOSIS

Salmonella gastroenteritis cannot be distinguished clinically from other infectious causes of acute diarrhea reliably, although history and epidemiology sometimes may suggest an etiologic agent. Bloody diarrhea with mucus can be caused by *Salmonella*, *Shigella*, enteroinvasive *E. coli*, enterohemorrhagic *E. coli*, *Campylobacter* spp., *Yersinia enterocolitica*, *Clostridium difficile*, *Trichuris trichiura*, and *Entamoeba histolytica*. Watery diarrhea may be caused by rotavirus or other viral enteropathogens or enterotoxin-producing bacterial pathogens. When abdominal pain and tenderness are severe, the differential diagnosis includes appendicitis, perforated viscus, and mesenteric adenitis.

Enteric fever can mimic other infections of the reticuloendothelial system,[31] including Epstein-Barr virus infection, disseminated histoplasmosis, tuberculosis, ehrlichiosis, brucellosis, leptospirosis, tularemia, plague, malaria, systemic *Bartonella henselae* infection, and typhus. Noninfectious illnesses with prolonged fever that sometimes can be confused with typhoid include juvenile rheumatoid arthritis and other collagen vascular diseases, Kawasaki syndrome, and lymphomas. An early diagnosis often is difficult to establish because the findings are nonspecific. Findings that are particularly helpful in discriminating typhoid fever from other prolonged febrile illnesses include severe cough and chest pain (more

TABLE 111-7 **Antibiotics Commonly Used in the Treatment of *Salmonella* Infections**

Drug	Dose	Comments
Ciprofloxacin	10-20 mg/kg/day in two doses PO or IV	First-line therapy (*)
Ceftriaxone	50-100 mg/kg/day in one or two doses IM or IV	First-line therapy
Cefotaxime	100-200 mg/kg/day in three doses IM or IV	First-line therapy
Cefixime	10-20 mg/kg/day in one or two doses PO	Alternative therapy
Azithromycin	8-12 mg/kg/day in one dose PO	Alternative therapy
Chloramphenicol	50-75 mg/kg/day in four doses PO	High frequency of resistance; use only for susceptible strains
Ampicillin	200 mg/kg/day in four doses PO, IM, or IV	High frequency of resistance; use only for susceptible strains
TMP-SMX	10 mg/kg/day TMP, 50 mg/kg/day SMX in two doses PO or IV	High frequency of resistance; use only for susceptible strains

*Non-FDA approved in children younger than 18 years old, while current expert opinion agrees in recommending this agent as an effective therapy for regions with susceptible *Salmonella* strains and especially for severe infections.
TMP-SMX, Trimethoprim-sulfamethoxazole.

typical of lobar pneumonia), diarrhea with grossly obvious blood (more typical of dysentery), acute onset of chills (more typical of malaria), and marked lower abdominal pain early in the febrile illness (more typical of bacillary dysentery, *Y. enterocolitica* infection, and salpingitis). For patients in countries where typhoid is not endemic, a history of travel is crucial. In endemic developing countries, the widely variable false-positive and false-negative results of the Widal test complicate the differential diagnosis workup, favoring the misuse of antimicrobials.[241] *Salmonella* infections could be a cause of fever of unknown origin, especially in developing countries.[61]

TREATMENT

For children with salmonellosis for whom antibiotic treatment is appropriate, the interpretation of antimicrobial susceptibility studies is important. Drugs such as aminoglycosides, polymyxins, tetracyclines, and first-generation and second-generation cephalosporins (e.g., cephalothin, cefazolin, cefuroxime, cefamandole) have a poor clinical track record, despite apparent in vitro susceptibility. Table 111-7 shows the drugs that typically are useful in treatment of children with *Salmonella* infections. The emergence of *S.* ser. Typhimurium DT104 in the United States has led to a dramatic increase in multidrug-resistant (i.e., ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) organisms.[121]

Gastroenteritis

As with all forms of gastroenteritis, fluid and electrolyte replacement and maintenance are the first objective of management. For most patients, oral rehydration is all that is necessary to treat *Salmonella* gastroenteritis. Generally, *Salmonella* gastroenteritis should not be treated with antibiotics because these agents do not shorten the course of illness* but prolong excretion of *Salmonella*.†

*References 15, 17, 57, 58, 166, 194, 227, 244, 275.
†References 15, 17, 85, 166, 227, 244.

Salmonella serotypes typically have been grouped together for these treatment studies, however, as though they were all the same organism. Given the variability in expression of virulence genes, whether treatment may be useful for some serotypes that possess particular virulence traits remains an open question.

Exceptions to the generalization that *Salmonella* gastroenteritis should not be treated include children at high risk for developing complications, including children with underlying diseases or receiving therapies that impair host defenses. Examples of children who probably ought to be given antibiotics are infants 6 months or younger; children with AIDS or malignant diseases; those splenectomized; children with primary immunodeficiencies; children with chronic hepatopathy; and children with hemolytic anemias, particularly sickle-cell anemia. Treatment of these patients is debatable; the data from neonates suggest that antibiotics make little difference in the course.[1,95,166,260,300] Because the risk for development of bacteremia is high, however, antibiotics likely will continue to be used in such settings. Bacteremia occurs in a small fraction of infections; therefore, proving the concept is impossible without conducting a massive placebo-controlled clinical trial. When treatment is given, probably 5 days or fewer of antibiotics is indicated, barring complications. Ciprofloxacin or a third-generation cephalosporin (i.e., ceftriaxone or cefotaxime) are recommended in preference to former suggestions in favor of ampicillin or amoxicillin.[38] Parenteral therapy with a third-generation cephalosporin or fluoroquinolone has been related to reductions in duration of fever and hospitalization in one retrospective study of children with more severe gastroenteritis.[312]

Extraintestinal Infections

Any child who appears to be sufficiently toxic that bacteremia is suspected also should be started on antibiotic treatment until blood cultures exclude the diagnosis. For children with bacteremic nontyphoidal *Salmonella* and focal extraintestinal complications, a third-generation cephalosporin (e.g., ceftriaxone, cefotaxime) or fluoroquinolone is an appropriate choice. If the patient seems

TABLE 111-8 **Recommended Antibiotic Treatment of Typhoid Fever**

Susceptibility	Antibiotic	Daily Dose (mg/kg)	Days
Full susceptible	Chloramphenicol *or*	50-75	14-21
	Amoxicillin *or*	75-100	14
	Trimethoprim-sulfamethoxazole *or*	8/40	14
	Third-generation cephalosporin (e.g., cefixime) *or*	15-20	7-10
	Fluoroquinolone (e.g., ofloxacin or ciprofloxacin)	30	7-10
Multidrug-resistant	Azithromycin	20	5-7
	Third-generation cephalosporin (e.g., cefixime) *or*	15-20	7-10
	Fluoroquinolone (e.g., ofloxacin or ciprofloxacin)	30	7-10
Quinolone-resistant	Azithromycin *or*	20	5-7
	Third-generation cephalosporin (e.g., cefixime) *or*	20	7-10
	Parenteral third-generation cephalosporin (e.g., ceftriaxone) *or*	80	7-10
	Fluoroquinolone (e.g., ofloxacin or ciprofloxacin)	30	7-10

to have a life-threatening infection, ampicillin and chloramphenicol should be used only if evidence exists that the pathogen is not resistant to ampicillin or chloramphenicol and that no meningitis or related involvement is suspected. Children at high risk for having recurrence of bacteremia (children with congenital or acquired immunodeficiencies, such as AIDS) may require a third-generation cephalosporin or a fluoroquinolone to achieve cure.[68,146] Frequent recurrences of life-threatening infection sometimes necessitate use of prolonged or life-long maintenance therapy with an oral agent, after controlling the episode with prolonged combined parenteral therapy guided by antibiogram results.[146]

Apparent recurrent episodes of nontyphoidal salmonellosis in the same child are actually a persistent infection by the same clone in nearly 80 percent of cases[231] and merit evaluation in search of primary or acquired immune deficiencies.

Meningitis should be treated with a third-generation cephalosporin because these agents have good penetration into the CSF; ampicillin and chloramphenicol use is associated with higher relapse rates and lower cure rates.[169,235] Meningitis must be treated for at least 4 weeks; approximately three fourths of patients who have relapses have been treated for 3 weeks or less.[66] *Salmonella* may persist for more than 3 weeks inside CSF mononuclear leukocytes despite negative CSF cultures.[58] Some authors suggest combined antimicrobial therapy with cefotaxime or ceftriaxone plus ciprofloxacin for meningitis.[53,130,235,251] Dexamethasone before or at the time of initiation of antimicrobials aimed to reduce the severity of sequelae of bacterial meningitis in children is recommended by extension to *Salmonella* meningitis by some authors[12,149,339] but available data are very limited to lead to definitive conclusions.[217,218,318] Timely initiation of appropriate active bactericidal antimicrobials and severity of disease on arrival are important determinants of outcome, especially in developing countries.[89] Carbapenems such as imipenem and meropenem have been used with good results in a few cases of *Salmonella* meningitis,[52,235] but risk for seizures with imipenem therapy must be considered.[251] Regarding ceftriaxone effectiveness in contrast to ceftazidime and cefotaxime, data from case series suggest a better response to ceftriaxone, especially compared to ceftazidime.[180]

For other extraintestinal infections in children, the duration of antibiotic treatment usually is 10 to 14 days for bacteremia and 4 to 6 weeks for acute osteomyelitis. Collections of pus should be drained, and for intracranial collections, the total duration of antimicrobial therapy should be extended to 4 to 6 weeks after drainage if this is feasible.[52] Nonaccessible collections in the central nervous system would need more prolonged therapy and complicate outcomes. Schistosomiasis, when present, must be treated to achieve resolution of the coincident *Salmonella* infection.[263]

Typhoid Fever

The response to antibiotics is slow. Fever may persist for many days, even after bacteremia has resolved.[344] The emergence and spread of MDRST since 1989, defined as combined resistance to chloramphenicol, TMP-SMX, and ampicillin,[344] caused a shift in empirical therapy from once first-line drugs to a fluoroquinolone; a third-generation cephalosporin, such as ceftriaxone; or azithromycin in children (Table 111-8). MDRST is particularly common in the Indian subcontinent, Southeast Asia, and Africa, and resistance to ciprofloxacin is increasing.[63,267]

In the United States, MDRST has been less problematic than it is elsewhere, but resistance to ampicillin, chloramphenicol, and TMP-SMX has continuously increased since the 1990s.[3,191,274] Currently, most *S.* ser. Typhi isolates are still sensitive to ceftriaxone and ciprofloxacin, although nalidixic acid–resistant isolates are common in travelers returning from the Indian subcontinent.[3,191] Such strains respond poorly to fluoroquinolones, with a 30-fold chance of requiring retreatment.[326] When the patient has a history of recent travel to an area with MDRST or of contact with an individual returning from such an area, the choice of empirical treatment should take this information into account. New minimum inhibitory concentration (MIC) breakpoints have been established for determination of *Salmonella* susceptibility, intermediate sensitivity, or resistance against ciprofloxacin.[151] As a result, MICs of 1 μg/mL or

less but 0.06 μg/mL or greater are associated with delayed antimicrobial responses to ciprofloxacin or even to clinical failure.[151] Third-generation cephalosporins are effective against MDRST* and appropriate for children in such circumstances. Although older small trials in adults suggested shorter courses could be comparable to longer courses of treatment, caution is granted for decisions on treatment duration for children given to low quality of evidence and ever-changing susceptibility patterns over time.[336] For MDRST in children, ceftriaxone given at 65 mg/kg/day in children was associated with relapses with short-course therapy (7 days) in contrast to 14 days of therapy.[32]

Current evidence supports ceftriaxone, cefotaxime, azithromycin, and fluoroquinolones as the most effective drugs for the treatment of typhoid fever.[11,38,336] Fluoroquinolone drugs have proved safe in all age groups, are rapidly effective, and are associated with lower rates of stool carriage than traditional first-line drugs. The three main issues regarding the use of fluoroquinolones are (1) the potential for toxic effects in children, (2) the cost, and (3) the potential emergence of resistance.[240] In preclinical testing, the fluoroquinolones damaged the articular cartilage of young beagles. A considerable body of evidence exists for supporting safety of fluoroquinolones from the long-term use of these drugs in children with cystic fibrosis and from short-term use to treat typhoid fever and dysentery in children. Availability of generic fluoroquinolones in many developing countries favored the emergence of fluoroquinolone resistance resulting from its misuse, which turn as the greatest limitation on their use.[239]

In the developed world, described concerns about toxicity of fluoroquinolones in children have limited their use to situations of MDRST infections with confirmed resistance to all of the usual antibiotics but sensitive to a fluoroquinolone. If a fluoroquinolone is used, ciprofloxacin or ofloxacin is preferred because of better oral bioavailability. Ciprofloxacin 15 mg/kg/dose twice daily for 7 to 10 days in children causes defervescence in an average of 4.2 days with infrequent relapses, even with MDRST.[9] Children with MDRST who have been treated with ciprofloxacin 10 mg/kg/day became afebrile in 3.3 days, and 94 percent achieved clinical cure, with no relapses or carriers detected on follow-up.[93] Ofloxacin is associated with more rapid defervescence and better cure rates than cefixime.[46] Very short courses of ofloxacin (2 or 3 days) may be followed by relapse.[229,321]

Fluoroquinolone treatment for typhoid fever produced lower relapses (relative risk [RR], 0.04 to 0.52) and length of hospital stay (−3.53 to −1.62 days) in contrast to chloramphenicol.[307] Clinical failure (RR, 0.01 to 0.54) and microbiologic failure (RR, 0.03 to 0.64) were reduced for fluoroquinolones in contrast to β-lactam antibiotics treatment.[307] Fluoroquinolones performed better than third-generation cephalosporins leading to lower clinical failures and duration of hospital stay.[307]

Other agents have been described that occasionally may be useful. Furazolidone 7.5 mg/kg/day is nearly as effective as is chloramphenicol in strains susceptible to both drugs (86% vs. 90% cure).[66] Despite low serum levels, azithromycin seems to be equivalent to chloramphenicol or ciprofloxacin; the high intracellular concentration in macrophages (>100 times serum levels) of azithromycin presumably accounts for its efficacy.[45,120] When MDRST is resistant to nalidixic acid, azithromycin is more effective than ofloxacin, resulting in faster resolution of fever and very effective control of fecal carriage in contrast to ofloxacin and even to combined therapy of azithromycin with ofloxacin.[54,240]

A 5-day course of oral azithromycin 20 mg/kg/day (maximum 1000 mg/day) is as effective as intravenous ceftriaxone 75 mg/kg/day (maximum 2.5 g/day for 5 days).[111] Various nonantimicrobial measures should be considered as part of the management of *S.* ser. Typhi infections.

No good level of evidence exists to establish superiority of fluoroquinolones over first-line antibiotics for typhoid fever specifically in children, but fluoroquinolones are suggested at this time as first-line therapy. Because of the high level of resistance in some regions of the world, the World Health Organization recommendations for second-line therapy options include third-generation cephalosporins and azithromycin.[336]

Dexamethasone, although potentially increasing the relapse rate,[69] is indicated for patients with severe typhoid fever with delirium, stupor, shock, or coma; the dose is 3 mg/kg initially and then eight doses of 1 mg/kg every 6 hours for 48 hours. This therapy reduces mortality rates from 35 to 55 percent to 10 percent.[142,252] Parenteral fluoroquinolones are probably the antibiotic of choice for severe infections, but no randomized trials of such treatment have been performed.[93] Antipyretics were thought at one time to be dangerous in typhoid fever[87]; whether this concern is correct is doubtful on the basis of more recent experience.[230]

Intestinal hemorrhage or perforation during enteric fever generally is considered to be an indication for surgical intervention.[33,44,118,185] Resection of 10 cm of intestine proximal and distal to the perforation seems to improve outcome in contrast to other surgical approaches.[18] Antibiotic coverage should be broadened to include anaerobes and gram-negative enterics when perforation occurs.[33]

Chronic Carriers

Generally, patients who are not food handlers probably should not be cultured or given special treatment after having a bout of gastroenteritis caused by a nontyphoidal *Salmonella* strain. Carriers of *S.* ser. typhi should be decolonized to decrease the risk to close contacts. Carriers who have a normal gallbladder can be treated with oral ciprofloxacin or norfloxacin for 4 weeks; these drugs concentrate highly in bile.[11] If oral fluoroquinolone therapy is not tolerated, then high-dose intravenous ampicillin for 4 weeks could be indicated.[11] Other options are oral ampicillin or amoxicillin combined with probenecid for 6 weeks. Chronic carriers who cannot be decolonized are treated with cholecystectomy if cholelithiasis or cholecystitis is present; these patients should receive

*References 98, 154, 169, 209, 220, 237, 290, 309.

ampicillin intravenously for 7 to 10 days before and 30 days after cholecystectomy.

PREVENTION

Public Health Measures

Recognition of an increased frequency of human infections with an unusual serotype should be followed by an epidemiologic investigation aimed at detecting the source and vehicle. Intervention to stop such outbreaks then can be attempted. Judicious use of antibiotics in dairy and livestock animals,[144] careful food processing and storage, and proper preparation of foods are helpful in decreasing transmission of infection. Appropriate sewage disposal, assurance of a safe water supply, prevention of sale of pet turtles, inspection of cosmetics for contamination, and adequate cleaning of medical equipment are important public health strategies. Families with small children should be informed of the risks associated with pet reptiles and encouraged to avoid such unnecessary risks.

Personal Hygienic Measures

Person-to-person spread can be decreased by hand washing after defecating or changing diapers, frequent hand washing during preparation of foods that might be contaminated (e.g., meat), and excluding of infected individuals from food-handling tasks.

Infection Control

Hospitalized children with *Salmonella* gastroenteritis should be isolated (enteric precautions) until stool cultures are negative. Children with extraintestinal infections should be isolated until stool studies exclude intestinal infection or colonization.

Nursery Outbreaks

Neonatal *Salmonella* infection outbreaks should be investigated to determine the source. Cultures of fomites sometimes reveal a removable focus. Neonates and staff members caring for them should be cohorted during outbreaks, with use of enteric precautions in dealing with infants who are excreting the organism. Surveillance cultures should be done on feces of not only sick infants but also well infants to cohort more appropriately. With current early postpartum discharge policies, reporting *Salmonella* infections in infants is important in detecting outbreaks. Isolation and cohorting can be effective in controlling such outbreaks.[259]

Breast-Feeding

In the developing world, breast-feeding is key in prevention because human milk contains secretory IgA and other factors that protect infants from *Salmonella* spp.[35,101,110,247,248] In a case-control study conducted in the United States, breast-feeding was found also to have a strong protective effect against sporadic *Salmonella* infections.[268] Pediatric health care providers and community education programs should encourage mothers to breast-feed.

Vaccination

Several vaccines have been developed for typhoid fever. Vaccination of children is indicated when the risk for development of typhoid fever is high (e.g., living with a chronic carrier or in an endemic area) but probably is underused.[306] Two vaccines are available: (1) an oral live attenuated Ty21a vaccine (Vivotif Berna) and (2) a parenteral purified Vi capsular polysaccharide vaccine (Typhim Vi).[171,249]

The Ty21a vaccine has been evaluated in liquid and capsule forms. Ty21a oral vaccine is well tolerated; abdominal pain, nausea, vomiting, and rashes occur rarely. The form licensed in the United States is an enteric-coated capsule preparation meant to be given in four separate doses on alternate days taken 1 hour before meals. It is not recommended for children younger than 6 years of age. The four doses should be completed 1 week before potential exposure.[11] Revaccination with the entire four-dose series is recommended every 5 to 7 years in high-risk settings. Because the Ty21a oral vaccine is a live-attenuated *Salmonella*, it should not be used in immunocompromised hosts or in individuals taking antibiotics at the time of receiving vaccination.[48] The antimalarial mefloquine inhibits growth of the attenuated organism, and vaccination should be delayed for 24 hours after its use. Malaria prophylaxis with atovaquone and proguanil does not interfere with immune response to Ty21a.[100]

Several large field trials suggest that the Vi capsular vaccine as a single 25-μg dose (0.5 mL) has an efficacy of 55 percent and 75 percent in adults and children older than 5 years.[2,249] Although fever, malaise, local pain, and tenderness occur with this vaccine, it has two major advantages over the Ty21a oral vaccines: It does not require refrigeration, and only a single dose is required for protection and then repeated every 3 years.[337] It may be used in children 2 years of age. Vaccination must occur at least 2 weeks before exposure.[11]

A parenteral Vi vaccine conjugated with tetanus toxoid is licensed and produced in India (Peda Typh). The first dose is advised for subjects older than 2 years of age, followed by a second dose at 4- to 8-week intervals, and then by a booster every 10 years.[182] Several vaccine producers from Asia, Russia, and Cuba manufacture local-use parenteral polysaccharide Vi vaccines.[182] Typhoid vaccination is recommended additionally for curtailing typhoid fever outbreaks.[337]

PROGNOSIS

Salmonella gastroenteritis usually is a self-limited disease in the normal host, although chronic diarrhea sometimes develops after an acute episode. Extraintestinal focal infections with nontyphoidal *Salmonella* strains are difficult to cure, particularly if they involve the meninges or occur in compromised hosts. *Salmonella* spp. meningitis may relapse if the course of treatment is too short.

Likewise, bacteremia and focal infection recur after treatment in severely compromised hosts, particularly patients with AIDS. Relapse after typhoid fever has long been recognized as a risk.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Agbaje M, Begum RH, Oyekunle MA, et al. Evolution of *Salmonella* nomenclature: a critical note. *Folia Microbiol (Praha)* 2011; **56**:497–503.
7. Ahmad KA, Khan LH, Roshan B, et al. Factors associated with typhoid relapse in the era of multiple drug resistant strains. *J Infect Dev Countries* 2011;**5**:727–31.
11. American Academy of Pediatrics. *Salmonella* infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 635–40.
12. Ananth Ramakrishnan K, Levin M, Faust SN. Bacterial meningitis and brain abscess. *Medicine* 2009;**37**:567–73.
13. Andrade AL, Arguedas A, Benavides J, et al. Bacteriology of community-acquired invasive disease found in a multicountry prospective, population-based, epidemiological surveillance for pneumococcus in children in Latin America. *Pediatr Infect Dis J* 2012;**31**:1312–14.
23. Baker S, Favorov M, Dougan G. Searching for the elusive typhoid diagnostic. *BMC Infect Dis* 2010;**10**:45.
26. Bavishi C, DuPont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther* 2011;**34**:1269–81.
28. Berger CN, Sodha SV, Shaw RK, et al. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ Microbiol* 2010;**12**:2385–97.
38. British Medical Association, Royal Pharmaceutical Society, and Royal College of Paediatrics and Child Health, et al. *British National Formulary (BNF) for Children 2011-2012: The essential resource for clinical use of medicines in children*. London: BMJ Group; Royal Pharmaceutical Society; RCPCH; 2011.
39. Broz P, Ohlson MB, Monack DM. Innate immune response to *Salmonella typhimurium*, a model enteric pathogen. *Gut Microbes* 2012;**3**:1–9.
47. Carrasco E, Morales-Rueda A, García-Gimeno RM. Cross-contamination and recontamination by *Salmonella* in foods: a review. *Food Res Int* 2012;**45**:545–56.
50. Chai SJ, White PL, Lathrop SL, et al. *Salmonella enterica* Serotype enteritidis: increasing incidence of domestically acquired infections. *Clin Infect Dis* 2012;**54**(Suppl. 5):S488–97.
52. Chen KM, Lee HF, Chi CS, et al. Obscure manifestations of *Salmonella* subdural empyema in children: case report and literature review. *Childs Nerv Sys* 2011;**27**:591–5.
53. Chimalizeni Y, Kawaza K, Molyneux E. The epidemiology and management of non typhoidal salmonella infections. *Adv Exp Med Biol* 2010;**659**:33–46.
61. Chow A, Robinson JL. Fever of unknown origin in children: a systematic review. *World J Pediatr* 2011;**7**:5–10.
63. Chuang C-H, Su L-H, Perera J, et al. Surveillance of antimicrobial resistance of *Salmonella enterica* serotype Typhi in seven Asian countries. *Epidemiol Infect* 2009;**137**:266–9.
72. Crawford RW, Rosales-Reyes R, de La Ramírez-Aguilar ML, et al. Gallstones play a significant role in *Salmonella* spp. gallbladder colonization and carriage. *Proc Natl Acad Sci USA* 2010;**107**: 4353–8.
73. Crump JA, Mintz ED. Global trends in typhoid and paratyphoid Fever. *Clin Infect Dis* 2010;**50**:241–6.
75. Crump JA, Medalla FM, Joyce KW, et al. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. *Antimicrob Agents Chemother* 2011;**55**: 1148–54.
76. Cummings PL, Sorvillo F, Kuo T. Salmonellosis-related mortality in the United States, 1990-2006. *Foodborne Pathog Dis* 2010;**7**: 1393–9.
81. de Beaucoudrey L, Samarina A, Bustamante J, et al. Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)* 2010;**89**:381–402.
104. Figueira R, Holden DW. Functions of the *Salmonella* pathogenicity island 2 (SPI-2) type III secretion system effectors. *Microbiology (Reading, UK)* 2012;**158**(Pt 5):1147–61.
107. Finstad S, O'Bryan CA, Marcy JA, et al. *Salmonella* and broiler processing in the United States: relationship to foodborne salmonellosis. *Food Res Int* 2012;**45**:789–94.
140. Hilbert F, Smulders FJM, Chopra-Dewasthaly R, et al. *Salmonella* in the wildlife-human interface. *Food Res Int* 2012;**45**:603–8.
151. Humphries RM, Fang FC, Aarestrup FM, et al. In vitro susceptibility testing of fluoroquinolone activity against *Salmonella*: recent changes to CLSI standards. *Clin Infect Dis* 2012;**55**:1107–13.
160. Kaiser P, Hardt W-D. *Salmonella typhimurium* diarrhea: switching the mucosal epithelium from homeostasis to defense. *Curr Opin Immunol* 2011;**23**:456–63.
161. Kaiser P, Diard M, Stecher B, et al. The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response. *Immunol Rev* 2012;**245**:56–83.
177. Laupland KB, Schønheyder HC, Kennedy KJ, et al. *Salmonella enterica* bacteraemia: a multi-national population-based cohort study. *BMC Infect Dis* 2010;**10**:95.
182. Levine MM. Typhoid fever vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 6th ed. Edinburgh: Saunders; 2012. p. 812–36.
186. Loharikar A, Newton A, Rowley P, et al. Typhoid fever outbreak associated with frozen mamey pulp imported from Guatemala to the Western United States, 2010. *Clin Infect Dis* 2012;**55**:61–6.
187. Loetscher Y, Wieser A, Lengefeld J, et al. *Salmonella* transiently reside in luminal neutrophils in the inflamed gut. *PLoS One* 2012; **7**:e34812.
196. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 2010;**50**: 882–9.
214. Millet A, Hullo E, Armari Alla C, et al. Drépanocytose et salmonelloses invasives ostéo-articulaires. *Arch Pédiatr* 2012;**19**: 267–70.
217. Molyneux EM, Mankhambo LA, Phiri A, et al. The outcome of non-typhoidal salmonella meningitis in Malawian children, 1997-2006. *Ann Trop Paediatr* 2009;**29**:13–22.
219. Monack DM. *Salmonella* persistence and transmission strategies. *Curr Opin Microbiol* 2012;**15**:100–7.
241. Parry CM, Wijedoru L, Arjyal A, et al. The utility of diagnostic tests for enteric fever in endemic locations. *Expert Rev Anti Infect Ther* 2011;**9**:711–25.
245. Pfeiffer ML, DuPont HL, Ochoa TJ. The patient presenting with acute dysentery: a systematic review. *J Infect* 2012;**64**: 374–86.
250. Podolak R, Enache E, Stone W, et al. Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods. *J Food Prot* 2010;**73**:1919–36.
260. Richards LH, Howard J, Klein JL. Community-acquired *Salmonella* bacteraemia in patients with sickle-cell disease 1969-2008: a single centre study. *Scand J Infect Dis* 2011;**43**:89–94.
271. Ruby T, McLaughlin L, Gopinath S, et al. *Salmonella's* long-term relationship with its host. *FEMS Microbiol Rev* 2012;**36**: 600–15.
293. Steenackers H, Hermans K, Vanderleyden J, et al. *Salmonella* biofilms: an overview on occurrence, structure, regulation and eradication. *Food Res Int* 2012;**45**:502–31.
310. Tilahun B, Worku B, Tachbele E, et al. High load of multi-drug resistant nosocomial neonatal pathogens carried by cockroaches in a neonatal intensive care unit at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Antimicrob Resist Infect Control* 2012;**1**:12.
312. Tsai MH, Huang YC, Lin TY, et al. Reappraisal of parenteral antimicrobial therapy for nontyphoidal *Salmonella* enteric infection in children. *Clin Microbiol Infect* 2011;**17**:300–5.
317. Van TT, Nguyen HN, Smooker PM, et al. The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* isolated from food-producing animals, retail meat and humans in South East Asia. *Int J Food Microbiol* 2012;**154**:98–106.
328. Wales AD, Carrique-Mas JJ, Rankin M, et al. Review of the carriage of zoonotic bacteria by arthropods, with special reference to *Salmonella* in mites, flies and litter beetles. *Zoonoses Public Health* 2010;**57**:299–314.

336. World Health Organization. Recommendations for management of common childhood conditions: newborn conditions, dysentery, pneumonia, oxygen use and delivery, common causes of fever, severe acute malnutrition and supportive care. Geneva, Switzerland: World Health Organization; 2012.
337. World Health Organization. Report of the ad-hoc consultation on typhoid vaccine introduction and typhoid surveillance. WHO/IVB/12.02: 18-20 April 2011, Plaza Athenee Hotel, Bangkok. Geneva, Switzerland: World Health Organization; 2012.
339. Wu HM, Huang WY, Lee ML, et al. Clinical features, acute complications, and outcome of *Salmonella* meningitis in children under one year of age in Taiwan. *BMC Infect Dis* 2011; **11**:30.
343. Zaka-ur-Rab Z, Abqari S, Shahab T, et al. Evaluation of salivary anti-*Salmonella typhi* lipopolysaccharide IgA ELISA for serodiagnosis of typhoid fever in children. *Arch Dis Child* 2012;**97**:236–8.
344. Zaki SA, Karande S. Multidrug-resistant typhoid fever: a review. *J Infect Dev Countries* 2011;**5**:324–37.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 112

PLAGUE (*YERSINIA PESTIS*)

Gary D. Overturf

HISTORY AND EPIDEMIOLOGY

Yersinia pestis has been responsible for the most devastating epidemics in human history. Plague was first mentioned in 1320 BCE in the Old Testament in I Samuel, chapters 5 and 6. The first of three pandemics was heralded by the Justinian plague in northern Africa and the Mediterranean (541-700).[48] The second pandemic occurred in Sicily in 1346; also in that year, plague appeared during the siege of the city of Kaffa in the Crimea, thereafter spreading throughout most of Europe, where it became known as the "Black Death," having been introduced by accompanying rodent populations from China along Asian-European trade routes. One third of the population of Europe died in its aftermath. Between the 14th and 20th centuries, plague occurred throughout Europe and Russia, with resultant frequent outbreaks.[43]

In 1894, Yersin and Kitasato, working independently, first described the plague bacillus while investigating plague that spread from mainland China to Hong Kong,[47] and the role played by rats and fleas in the spread of the disease was defined.[61] In 1900, plague was introduced into San Francisco by rats aboard ships docking there. The disease spread to ground squirrels and then to other wild animals of the American Southwest.[46] In 1943, effective antibiotics against *Y. pestis* became available. Although antibiotics have been effective in the treatment and prophylaxis of plague, plasmid-borne antibiotic resistance is increasingly being noted.[19] The potential for aerosolized *Y. pestis* as a biologic weapon has led to renewed interest in the understanding and study of this organism.[28]

Plague is now a worldwide disease. The World Health Organization reported 38,359 human cases of plague from 1989 to 2003 that resulted in 2,845 deaths. Approximately 80 percent of the cases occurred in Africa with endemic sites in east Africa and Madagascar; 15 percent were reported from Asia, including China, southeast Asia, and India; and 5 percent occurred in the Americas.[63] Plague remains endemic in the American Southwest, with more than 80 percent of cases occurring in New Mexico, Arizona, and Colorado and approximately 10 percent in California[11,27]; plague, however, occurs in rodent populations of 17 contiguous states west of the 100th meridian.[19,20]

BACTERIOLOGY

Y. pestis is in the family Enterobacteriaceae and is one of 14 *Yersinia* spp. It is a small, pleomorphic, nonmotile, gram-negative bacillus and is closely related to one of the three pathogenic representatives of the genus, *Yersinia pseudotuberculosis*.[49,57,60] With Wayson, Giemsa, and Gram stains, the bacillus takes on bipolar or "safety pin" morphologic features.[17] *Y. pestis* grows on blood, chocolate, and MacConkey agars at temperatures ranging from 0°C to 40°C (32°F to 104°F); the optimal temperature is 28°C (82.4°F). On first isolation at 35°C (95°F) on 5 percent blood agar, the colonies are pinpoint in size, growing to 1 to 2 mm after 2 days. They are nonhemolytic on 5 percent sheep blood agar.[60]

Depending on the clinical nature of the disease, blood cultures, sputum samples, or aspirates of enlarged nodes

REFERENCES

1. Abroms IF, Cochran WD, Holmes LB, et al. A *Salmonella newport* outbreak in a premature nursery with a one-year follow-up: effect of ampicillin following bacteriologic failure of response to kanamycin. *Pediatrics* 1966;**37**:616–23.
2. Acharya IL, Lowe CU, Thapa R, et al. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi:* a preliminary report. *N Engl J Med* 1987;**317**:1101–4.
3. Ackers ML, Puhr ND, Tauxe RV, et al. Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. *JAMA* 2000;**283**:2668–73.
4. Adeyokunnu AA, Hendrickse RG. *Salmonella* osteomyelitis in childhood: a report of 63 cases seen in Nigerian children of whom 57 had sickle cell anaemia. *Arch Dis Child* 1980;**55**:175–84.
5. Adler JL, Anderson RL, Boring JR, et al. A protracted hospital-associated outbreak of salmonellosis due to a multiple-antibiotic-resistant strain of *Salmonella indiana*. *J Pediatr* 1970;**77**:970–5.
6. Agbaje M, Begum RH, Oyekunle MA, et al. Evolution of *Salmonella* nomenclature: a critical note. *Folia Microbiol (Praha)* 2011; **56**:497–503.
7. Ahmad KA, Khan LH, Roshan B, et al. Factors associated with typhoid relapse in the era of multiple drug resistant strains. *J Infect Dev Countries* 2011;**5**:727–31.
8. Akintemi OB, Roberts KB. Evaluation and management of the febrile child in the conjugated vaccine era. *Advances in Pediatrics* 2006;**53**(1):255–78.
9. Alam MN, Haq SA, Das KK, et al. Efficacy of ciprofloxacin in enteric fever: comparison of treatment duration in sensitive and multidrug-resistant *Salmonella*. *Am J Trop Med Hyg* 1995;**53**: 306–11.
10. Alvarez-Elcoro S, Soto-Ramirez L, Mateos-Mora M. Salmonella bacteremia in patients with prosthetic heart valves. *Am J Med* 1984;**77**:61–3.
11. American Academy of Pediatrics. *Salmonella* infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 635–40.
12. Ananth Ramakrishnan K, Levin M, Faust SN. Bacterial meningitis and brain abscess. *Medicine* 2009;**37**:567–73.
13. Andrade AL, Arguedas A, Benavides J, et al. Bacteriology of community-acquired invasive disease found in a multicountry prospective, population-based, epidemiological surveillance for pneumococcus in children in Latin America. *Pediatr Infect Dis J* 2012;**31**:1312–14.
14. Asano T, Kuwabara K, Takagi A, et al. Acute pancreatitis complicating typhoid fever in a 4-year-old girl. *Pediatr Int* 2007;**49**:1004–6.
15. Aserkoff B, Bennett JV. Effect of antibiotic therapy in acute salmonellosis on the fecal excretion of salmonellae. *N Engl J Med* 1969;**281**:636–40.
16. Ashkenazi S, Cleary TG, Murray BE, et al. Quantitative analysis and partial characterization of cytotoxin production by *Salmonella* strains. *Infect Immun* 1988;**56**:3089–94.
17. Association for the Study of Infectious Disease. Effect of neomycin in non-invasive salmonella infections of the gastrointestinal tract. Joint Project by Members of the Association for the Study of Infectious Disease. *Lancet* 1970;**2**:1159–61.
18. Athié CG, Guizar CB, Alcantara AV. Twenty-five years of experience in the surgical treatment of perforation of the ileum caused by *Salmonella typhi* at the General Hospital of Mexico City, Mexico. *Surgery* 1998;**123**:632–6.
19. Avendano A, Herrera P, Horwitz I, et al. Duodenal string cultures: practicality and sensitivity for diagnosing enteric fever in children. *J Infect Dis* 1986;**153**:359–62.
20. Baine WB, Gangarosa EJ, Bennett JV, et al. Institutional salmonellosis. *J Infect Dis* 1973;**128**:357–60.
21. Baird GD, Manning EJ, Jones PW. Evidence for related virulence sequences in plasmids of *Salmonella dublin* and *Salmonella typhimurium*. *J Gen Microbiol* 1985;**131**:1815–23.
22. Baker NM, Mills AE, Rachman I, et al. Haemolytic-uraemic syndrome in typhoid fever. *BMJ* 1974;**2**:84–7.
23. Baker S, Favorov M, Dougan G. Searching for the elusive typhoid diagnostic. *BMC Infect Dis* 2010;**10**:45.
24. Bannerman CH. *Salmonella Heidelberg* enteritis: an outbreak in the neonatal unit Harare Central Hospital. *Cent Afr J Med* 1985;**31**:1–4.
25. Bate JG, James U. *Salmonella typhimurium* infection dust-borne in a children's ward. *Lancet* 1958;**2**:713–15.
26. Bavishi C, DuPont HL. Systematic review: the use of proton pump inhibitors and increased susceptibility to enteric infection. *Aliment Pharmacol Ther* 2011;**34**:1269–81.
27. Bean NH, Griffin PM, Goulding JS, et al. Foodborne disease outbreaks, 5-year summary, 1983-1987. *MMWR CDC Surveill Summ* 1990;**39**:15–57.
28. Berger CN, Sodha SV, Shaw RK, et al. Fresh fruit and vegetables as vehicles for the transmission of human pathogens. *Environ Microbiol* 2010;**12**:2385–97.
29. Berkley J, Lowe B, Mwangi I, et al. Bacteremia among children admitted to a rural hospital in Kenya. *N Engl J Med* 2005;**352**: 39–47.
30. Bhutta ZA. Current concepts in the diagnosis and treatment of typhoid fever. *BMJ* 2006;**333**:78–82.
31. Bhutta ZA. Typhoid fever: current concepts. *Infect Dis Clin Pract* 2006;**14**:266–72.
32. Bhutta ZA, Khan IA, Shadmani M. Failure of short-course ceftriaxone chemotherapy for multidrug-resistant typhoid fever in children: a randomized controlled trial in Pakistan. *Antimicrob Agents Chemother* 2000;**44**:450–2.
33. Bitar R, Tarpley J. Intestinal perforation in typhoid fever: a historical and state-of-the-art review. *Rev Infect Dis* 1985;**7**:257–71.
34. Blaser MJ, Newman LS. A review of human salmonellosis. I. Infective dose. *Rev Infect Dis* 1982;**4**:1096–106.
35. Borgnolo G, Barbone F, Scornavacca G, et al. A case-control study of *Salmonella* gastrointestinal infection in Italian children. *Acta Paediatr* 1996;**85**:804–8.
36. Boyd JF. Pathology of the alimentary tract in *Salmonella typhimurium* food poisoning. *Gut* 1985;**26**:935–44.
37. Brent AJ, Oundo JO, Mwangi I, et al. *Salmonella* bacteremia in Kenyan children. *Pediatr Infect Dis J* 2006;**25**:230–6.
38. British Medical Association, Royal Pharmaceutical Society, and Royal College of Paediatrics and Child Health, et al. *British National Formulary (BNF) for Children 2011-2012: The essential resource for clinical use of medicines in children*. London: BMJ Group; Royal Pharmaceutical Society; RCPCH; 2011.
39. Broz P, Ohlson MB, Monack DM. Innate immune response to *Salmonella typhimurium*, a model enteric pathogen. *Gut Microbes* 2012;**3**:1–9.
40. Bryan JP, Rocha H, Scheld WM. Problems in salmonellosis: rationale for clinical trials with newer beta-lactam agents and quinolones. *Rev Infect Dis* 1986;**8**:189–207.
41. Buchmeier NA, Heffron F. Intracellular survival of wild-type *Salmonella typhimurium* and macrophage-sensitive mutants in diverse populations of macrophages. *Infect Immun* 1989;**57**:1–7.
42. Buchwald DS, Blaser MJ. A review of human salmonellosis. II. Duration of excretion following infection with nontyphi *Salmonella*. *Rev Infect Dis* 1984;**6**:345–56.
43. Butler T, Bell WR, Levin J, et al. Typhoid fever: studies of blood coagulation, bacteremia, and endotoxemia. *Arch Intern Med* 1978; **138**:407–10.
44. Butler T, Knight J, Nath SK, et al. Typhoid fever complicated by intestinal perforation: a persisting fatal disease requiring surgical management. *Rev Infect Dis* 1985;**7**:244–56.
45. Butler T, Sridhar CB, Daga MK, et al. Treatment of typhoid fever with azithromycin versus chloramphenicol in a randomized multicenter trial in India. *J Antimicrob Chemother* 1999;**44**: 243–50.
46. Cao XT, Kneen R, Nguyen TA, et al. A comparative study of ofloxacin and cefixime for treatment of typhoid fever in children. The Dong Nai Pediatric Center Typhoid Study Group. *Pediatr Infect Dis J* 1999;**18**:245–8.
47. Carrasco E, Morales-Rueda A, García-Gimeno RM. Cross-contamination and recontamination by *Salmonella* in foods: a review. *Food Res Int* 2012;**45**:545–56.
48. Centers for Disease Control and Prevention. Typhoid immunization: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1990;**39**(RR-10):1–5.
49. Centers for Disease Control and Prevention. Multistate outbreak of *Salmonella poona* infections: United States and Canada, 1991. *MMWR Morb Mortal Wkly Rep* 1991;**40**:549–52.
50. Chai SJ, White PL, Lathrop SL, et al. *Salmonella enterica* Serotype Enteritidis: increasing incidence of domestically acquired infections. *Clin Infect Dis* 2012;**54**(Suppl. 5):S488–97.

51. Challapalli M, Cherubin C, Cunningham DG. Lack of chronic carriage of *Salmonella typhimurium*. *Pediatr Infect Dis J* 1989;**8**: 539–40.
52. Chen KM, Lee HF, Chi CS, et al. Obscure manifestations of *Salmonella* subdural empyema in children: case report and literature review. *Childs Nerv Sys* 2011;**27**:591–5.
53. Chimalizeni Y, Kawaza K, Molyneux E. The epidemiology and management of non typhoidal salmonella infections. *Adv Exp Med Biol* 2010;**659**:33–46.
54. Chinh NT, Parry CM, Ly NT, et al. A randomized controlled comparison of azithromycin and ofloxacin for treatment of multidrug-resistant or nalidixic acid-resistant enteric fever. *Antimicrob Agents Chemother* 2000;**44**:1855–9.
55. Chin KC, Simmonds EJ, Tarlow MJ. Neonatal typhoid fever. *Arch Dis Child* 1986;**61**:1228–30.
56. Chiu CH, Lin TY, Ou JT. A pilot study of seven days of ceftriaxone therapy for children with *Salmonella* enterocolitis. *Changgeng Yi Xue Za Zhi* 1997;**20**:115–21.
57. Chiu CH, Lin TY, Ou JT. A clinical trial comparing oral azithromycin, cefixime and no antibiotics in the treatment of acute uncomplicated *Salmonella* enteritis in children. *J Paediatr Child Health* 1999;**35**:372–4.
58. Chiu CH, Ou JT. Persistence of *Salmonella* species in cerebrospinal fluid of patients with meningitis following ceftriaxone therapy. *Clin Infect Dis* 1999;**28**:1174–5.
59. Chiu C-H, Chuang C-H, Chiu S, et al. *Salmonella enterica* serotype Choleraesuis infections in pediatric patients. *Pediatrics* 2006; **117**:e1193–6.
60. Chorba TL, Meriwether RA, Jenkins BR, et al. Control of a nonfoodborne outbreak of salmonellosis: day care in isolation. *Am J Public Health* 1987;**77**:979–81.
61. Chow A, Robinson JL. Fever of unknown origin in children: a systematic review. *World J Pediatr* 2011;**7**:5–10.
62. Chow CB, Wang PS, Cheung MW, et al. Diagnostic value of the Widal test in childhood typhoid fever. *Pediatr Infect Dis J* 1987;**6**:914–17.
63. Chuang C-H, Su L-H, Perera J, et al. Surveillance of antimicrobial resistance of *Salmonella enterica* serotype Typhi in seven Asian countries. *Epidemiol Infect* 2009;**137**:266–9.
64. Clegg A, Passey M, Omena M, et al. Re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea. *Acta Trop* 1994;**57**: 255–63.
65. Coburn B, Grassl G, Finlay B. *Salmonella:* the host and disease—a brief review. *Immunol Cell Biol* 2007;**85**:112–18.
66. Cohen JI, Bartlett JA, Corey GR. Extra-intestinal manifestations of salmonella infections. *Medicine (Baltimore)* 1987;**66**: 349–88.
67. Cohen ML, Tauxe RV. Drug-resistant *Salmonella* in the United States: an epidemiologic perspective. *Science* 1986;**234**:964–9.
68. Connolly MJ, Snow MH, Ingham HR. Ciprofloxacin treatment of recurrent *Salmonella typhimurium* septicaemia in a patient with acquired immune deficiency syndrome. *J Antimicrob Chemother* 1986;**18**:647–8.
69. Cooles P. Adjuvant steroids and relapse of typhoid fever. *J Trop Med Hyg* 1986;**89**:229–31.
70. Corman LI, Poirier RH, Littlefield CA, et al. Endophthalmitis due to *Salmonella enteritidis*. *J Pediatr* 1979;**95**:1001–2.
71. Cravioto A, Reyes RE, Trujillo F, et al. Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. *Am J Epidemiol* 1990;**131**: 886–904.
72. Crawford RW, Rosales-Reyes R, de La Ramírez-Aguilar ML, et al. Gallstones play a significant role in *Salmonella* spp. gallbladder colonization and carriage. *Proc Natl Acad Sci USA* 2010; **107**:4353–8.
73. Crump JA, Mintz ED. Global trends in typhoid and paratyphoid Fever. *Clin Infect Dis* 2010;**50**:241–6.
74. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bull World Health Organ* 2004;**82**:346–53.
75. Crump JA, Medalla FM, Joyce KW, et al. Antimicrobial resistance among invasive nontyphoidal *Salmonella enterica* isolates in the United States: National Antimicrobial Resistance Monitoring System, 1996 to 2007. *Antimicrob Agents Chemother* 2011;**55**: 1148–54.
76. Cummings PL, Sorvillo F, Kuo T. Salmonellosis-related mortality in the United States, 1990-2006. *Foodborne Pathog Dis* 2010;**7**: 1393–9.
77. D'Amelio R, Tagliabue A, Nencioni L, et al. Comparative analysis of immunological responses to oral (Ty21a) and parenteral (TAB) typhoid vaccines. *Infect Immun* 1988;**56**:2731–5.
78. Davis RC. *Salmonella* sepsis in infancy. *Am J Dis Child* 1981; **135**:1096–9.
79. Davis TM, Makepeace AE, Dallimore EA, et al. Relative bradycardia is not a feature of enteric fever in children. *Clin Infect Dis* 1999;**28**:582–6.
80. Day DW, Mandal BK, Morson BC. The rectal biopsy appearances in *Salmonella* colitis. *Histopathology* 1978;**2**:117–31.
81. de Beaucoudrey L, Samarina A, Bustamante J, et al. Revisiting human IL-12Rβ1 deficiency: a survey of 141 patients from 30 countries. *Medicine (Baltimore)* 2010;**89**(6):381–402.
82. Diddle AW, Stephens RL. Typhoid fever in pregnancy. *Am J Obstet Gynecol* 1939;**38**:300–5.
83. Dietrich HF. Typhoid fever in children. *J Pediatr* 1937;**10**: 191–201.
84. Diwan N, Sharma KB. Isolation of *Salmonella typhimurium* from cephalhematoma and osteomyelitis. *Indian J Med Res* 1978;**67**: 27–9.
85. Dixon JM. Effect of antibiotic treatment on duration of excretion of *Salmonella typhimurium* by children. *Br Med J* 1965;**2**: 1343–5.
86. Doffinger R, Patel S, Kumararatne DS. Human immunodeficiencies that predispose to intracellular bacterial infections. *Curr Opin Rheumatol* 2005;**17**:440–6.
87. Dowle E. The reaction of patients with typhoid fever to the administration of aspirin. *S Afr Med J* 1956;**30**:474–7.
88. Du Plessis JP, Govendrageloo K, Levin SE. Right-sided endocarditis due to *Salmonella typhi*. *Pediatr Cardiol* 1997;**18**:443–4.
89. Duke T, Curtis N, Fuller DG. The management of bacterial meningitis in children. *Expert Opin Pharmacother* 2003;**4**:1227–40.
90. Dunn DW, McAllister J, Craft JC. Brain abscess and empyema caused by *Salmonella*. *Pediatr Infect Dis* 1984;**3**:54–7.
91. Dutta P, Bhattacharya SK, Dutta D, et al. Hemolytic uraemic syndrome following *Salmonella typhimurium* enteritis. *Indian J Pediatr* 1989;**56**:409–10.
92. Dutta P, Mitra U, Rasaily R, et al. Multi-drug resistant typhoid fever with diarrhea. *Indian Pediatr* 1997;**34**:891–9.
93. Dutta P, Rasaily R, Saha MR, et al. Ciprofloxacin for treatment of severe typhoid fever in children. *Antimicrob Agents Chemother* 1993;**37**:1197–9.
94. Eckmann L, Kagnoff MF. Cytokines in host defense against *Salmonella*. *Microbes Infect* 2001;**3**:1191–200.
95. Edgar WM, Lacey BW. Infection with *S. heidelberg:* an outbreak presumably not foodborne. *Lancet* 1963;**1**:161–3.
96. Ejlertsen T, Aunsholt NA. *Salmonella* bacteremia in renal transplant recipients. *Scand J Infect Dis* 1989;**21**:241–4.
97. El-Radhi AS, Carroll J, Klein N. *Clinical manual of fever in children*. Berlin: Springer; 2009.
98. Farid Z, Girgis N, Abu el Ella A. Successful treatment of typhoid fever in children with parenteral ceftriaxone. *Scand J Infect Dis* 1987;**19**:467–8.
99. Farid Z, Bassily S, Kent DC, et al. Chronic urinary salmonella carriers with intermittent bacteraemia. *J Trop Med Hyg* 1970; **73**(6):153–6.
100. Faucher JF, Binder R, Missinou MA, et al. Efficacy of atovaquone/proguanil for malaria prophylaxis in children and its effect on the immunogenicity of live oral typhoid and cholera vaccines. *Clin Infect Dis* 2002;**35**:1147–54.
101. Feachem RG, Koblinsky MA. Interventions for the control of diarrhoeal diseases among young children: promotion of breastfeeding. *Bull World Health Organ* 1984;**62**:271–91.
102. Ferreccio C, Levine MM, Manterola A, et al. Benign bacteremia caused by *Salmonella typhi* and paratyphi in children younger than 2 years. *J Pediatr* 1984;**104**:899–901.
103. Fierer J, Krause M, Tauxe R, et al. *Salmonella typhimurium* bacteremia: association with the virulence plasmid. *J Infect Dis* 1992; **166**:639–42.
104. Figueira R, Holden DW. Functions of the *Salmonella* pathogenicity island 2 (SPI-2) type III secretion system effectors. *Microbiology (Reading, UK)* 2012;**158**(Pt 5):1147–61.

105. Finlay BB. Molecular and cellular mechanisms of *Salmonella* pathogenesis. *Curr Top Microbiol Immunol* 1994;**192**:163–85.
106. Finlay BB, Falkow S. Comparison of the invasion strategies used by *Salmonella cholerae-suis*, *Shigella flexneri* and *Yersinia enterocolitica* to enter cultured animal cells: endosome acidification is not required for bacterial invasion or intracellular replication. *Biochimie* 1988;**70**:1089–99.
107. Finstad S, O'Bryan CA, Marcy JA, et al. *Salmonella* and broiler processing in the United States: relationship to foodborne salmonellosis. *Food Res Int* 2012;**45**:789–94.
108. Foley AR. An outbreak of paratyphoid B fever in the nursery of a small hospital. *Can J Public Health* 1947;**38**:73–5.
109. Foley SL, Lynne AM. Food animal-associated *Salmonella* challenges: pathogenicity and antimicrobial resistance. *J Anim Sci* 2008;**86**(14 suppl):E173–87.
110. France GL, Marmer DJ, Steele RW. Breast-feeding and *Salmonella* infection. *Am J Dis Child* 1980;**134**:147–52.
111. Frenck RW Jr, Mansour A, Nakhla I, et al. Short-course azithromycin for the treatment of uncomplicated typhoid fever in children and adolescents. *Clin Infect Dis* 2004;**38**:951–7.
112. Galofré J, Moreno A, Mensa J, et al. Analysis of factors influencing the outcome and development of septic metastasis or relapse in *Salmonella* bacteremia. *Clin Infect Dis* 1994;**18**:873–8.
113. Gendrel D, Kombila M, Beaudoin-Leblevec G, et al. Nontyphoidal salmonellal septicemia in Gabonese children infected with *Schistosoma intercalatum*. *Clin Infect Dis* 1994;**18**:103–5.
114. Giannella RA, Broitman SA, Zamcheck N. *Salmonella enteritis*. I. Role of reduced gastric secretion in pathogenesis. *Am J Dig Dis* 1971;**16**:1000–6.
115. Giannella RA, Broitman SA, Zamcheck N. Influence of gastric acidity on bacterial and parasitic enteric infections: a perspective. *Ann Intern Med* 1973;**78**:271–6.
116. Giannella RA, Formal SB, Dammin GJ, et al. Pathogenesis of salmonellosis: studies of fluid secretion, mucosal invasion, and morphologic reaction in the rabbit ileum. *J Clin Invest* 1973;**52**: 441–53.
117. Giannella RA, Gots RE, Charney AN, et al. Pathogenesis of *Salmonella*-mediated intestinal fluid secretion: activation of adenylate cyclase and inhibition by indomethacin. *Gastroenterology* 1975;**69**:1238–45.
118. Gibney EJ. Typhoid perforation. *Br J Surg* 1989;**76**:887–9.
119. Gilman RH, Terminel M, Levine MM, et al. Relative efficacy of blood, urine, rectal swab, bone-marrow, and rose-spot cultures for recovery of *Salmonella typhi* in typhoid fever. *Lancet* 1975;**1**: 1211–13.
120. Girgis NI, Butler T, Frenck RW. Azithromycin versus ciprofloxacin for treatment of uncomplicated typhoid in a randomized trial in Egypt that included patients with multidrug resistance. *Antimicrob Agents Chemother* 1999;**43**:1441–4.
121. Glynn MK, Bopp C, Dewitt W, et al. Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. *N Engl J Med* 1998;**338**:1333–8.
122. Gökalp AS, Toksoy HB, Turkay S, et al. Intravenous immunoglobulin in the treatment of *Salmonella typhimurium* infections in preterm neonates. *Clin Pediatr (Phila)* 1994;**33**:349–52.
123. Gordon MA, Graham SM, Walsh AL, et al. Epidemics of invasive *Salmonella enterica* serovar enteritidis and *S. enterica* serovar typhimurium infection associated with multidrug resistance among adults and children in Malawi. *Clin Infect Dis* 2008;**46**: 963–9.
124. Gorvel JP, Méresse S. Maturation steps of the *Salmonella*-containing vacuole. *Microbes Infect* 2001;**3**:1299–303.
125. Graham SM. Salmonellosis in children in developing and developed countries and populations. *Curr Opin Infect Dis* 2002;**15**: 507–12.
126. Griffith JPC, Ostheimer M. Typhoid fever in children. *Am J Med Sci* 1902;**124**:868–88.
127. Grisaru-Soen G. Risk factors for development of nontyphoid *Salmonella* bacteremia. *Clin Pediatr (Phila)* 2004;**43**:825–9.
128. Groisman EA, Ochman H. Cognate gene clusters govern invasion of host epithelial cells by *Salmonella typhimurium* and *Shigella flexneri*. *EMBO J* 1993;**12**:3779–87.
129. Grossman DA, Witham ND, Burr DH, et al. Flagellar serotypes of *Salmonella typhi* in Indonesia: relationships among motility, invasiveness, and clinical illness. *J Infect Dis* 1995;**171**:212–16.
130. Guillaumat C, Dang-Duy TL, Levy C, et al. Méningites á salmonelles du nouveau-né et du nourrisson. Intérêt des fluoroquinolones. *Arch Pediatr* 2008;**15**(Suppl. 3):S161–6.
131. Guthrie KJ, Montgomery GI. Infections with *Bacterium enteritidis* in infancy with the triad of enteritis, cholecystitis and meningitis. *J Pathol Bacteriol* 1939;**49**:393–409.
132. Hadfield TL, Monson MH, Wachsmuth IK. An outbreak of antibiotic-resistant *Salmonella enteritidis* in Liberia, West Africa. *J Infect Dis* 1985;**151**:790–5.
133. Haggman DL, Rehm SJ, Moodie DS, et al. Nontyphoidal *Salmonella* pericarditis: a case report and review of the literature. *Pediatr Infect Dis* 1986;**5**:259–64.
134. Hand WL, King NL. Serum opsonization of salmonella in sickle cell anemia. *Am J Med* 1978;**64**:388–95.
135. Hansen-Wester I, Hensel M. *Salmonella* pathogenicity islands encoding type III secretion systems. *Microbes Infect* 2001;**3**: 549–59.
136. Han T, Sokal JE, Neter E. Salmonellosis in disseminated malignant diseases: a seven-year review (1959-1965). *N Engl J Med* 1967;**276**:1045–52.
137. Harris JC, DuPont HL, Hornick RB. Fecal leukocytes in diarrheal illness. *Ann Intern Med* 1972;**76**:697–703.
138. Haysom IW, Sharp K. The survival and recovery of bacteria in vacuum cleaner dust. *J R Soc Promot Health* 2003;**123**:39–45.
139. Hedberg CW, David MJ, White KE, et al. Role of egg consumption in sporadic *Salmonella enteritidis* and *Salmonella typhimurium* infections in Minnesota. *J Infect Dis* 1993;**167**:107–11.
140. Hilbert F, Smulders FJM, Chopra-Dewasthaly R, et al. *Salmonella* in the wildlife-human interface. *Food Res Int* 2012;**45**:603–8.
141. Hoffman SL, Edman DC, Punjabi NH, et al. Bone marrow aspirate culture superior to streptokinase clot culture and 8 ml 1:10 blood-to-broth ratio blood culture for diagnosis of typhoid fever. *Am J Trop Med Hyg* 1986;**35**:836–9.
142. Hoffman SL, Punjabi NH, Kumala S, et al. Reduction of mortality in chloramphenicol-treated severe typhoid fever by high-dose dexamethasone. *N Engl J Med* 1984;**310**:82–8.
143. Hoffman SL, Punjabi NH, Rockhill RC, et al. Duodenal string-capsule culture compared with bone-marrow, blood, and rectal-swab cultures for diagnosing typhoid and paratyphoid fever. *J Infect Dis* 1984;**149**:157–61.
144. Holmberg SD. Drug-resistant *Salmonella* species from animals fed antimicrobics. *Infect Dis Newsl* 1986;**5**:25.
145. Holmberg SD, Osterholm MT, Senger KA, et al. Drug-resistant *Salmonella* from animals fed antimicrobials. *N Engl J Med* 1984; **311**:617–22.
146. Hoppe JE, Dopfer R, Huber S, et al. Eradication of *Salmonella dublin* in an immunodeficient child by combined use of ceftriaxone and ciprofloxacin after failure of either agent alone. *Infection* 1989;**17**:399–400.
147. Hornick RB, Greisman SE, Woodward TE, et al. Typhoid fever: pathogenesis and immunologic control. *N Engl J Med* 1970; **283**:686–91.
148. Hornick RB, Greisman SE, Woodward TE, et al. Typhoid fever: pathogenesis and immunologic control. 2. *N Engl J Med* 1970;**283**:739–46.
149. Huang DB, DuPont HL. Problem pathogens: extra-intestinal complications of *Salmonella enterica* serotype Typhi infection. *Lancet Infect Dis* 2005;**5**:341–8.
150. Huang LT, Ko SF, Lui CC. *Salmonella* meningitis: clinical experience of third-generation cephalosporins. *Acta Paediatr* 1997;**86**: 1056–8.
151. Humphries RM, Fang FC, Aarestrup FM, et al. In vitro susceptibility testing of fluoroquinolone activity against *Salmonella:* recent changes to CLSI standards. *Clin Infect Dis* 2012;**55**:1107–13.
152. Hyams JS, Durbin WA, Grand RJ, et al. *Salmonella* bacteremia in the first year of life. *J Pediatr* 1980;**96**:57–9.
153. Im SW, Chow K, Chau PY. Rectal thermometer mediated cross-infection with *Salmonella wandsworth* in a paediatric ward. *J Hosp Infect* 1981;**2**:171–4.
154. Isomäki O, Vuento R, Granfors K. Serological diagnosis of salmonella infections by enzyme immunoassay. *Lancet* 1989;**1**:1411–14.
155. Jiwa SF. Probing for enterotoxigenicity among the salmonellae: an evaluation of biological assays. *J Clin Microbiol* 1981;**14**:463–72.
156. Johnson PC. Typhoid tonsillitis. *JAMA* 1980;**244**:362.

157. Jones DMM, Pantin CG. Neonatal diarrhoea due to *Salmonella paratyphi* B. *J Clin Pathol* 1956;**9**:128–30.
158. Jones TF, Ingram LA, Fullerton K, et al. A case-control study of the epidemiology of sporadic *Salmonella* infection in infants. *Pediatrics* 2006;**118**:2380–7.
159. Jouanguy E, Doffinger R, Dupuis S, et al. IL-12 and IFN-g in host defense against mycobacteria and *Salmonella* in mice and men. *Curr Opin Immunol* 1999;**11**:346–51.
160. Kaiser P, Hardt W-D. *Salmonella typhimurium* diarrhea: switching the mucosal epithelium from homeostasis to defense. *Curr Opin Immunol* 2011;**23**:456–63.
161. Kaiser P, Diard M, Stecher B, et al. The streptomycin mouse model for *Salmonella* diarrhea: functional analysis of the microbiota, the pathogen's virulence factors, and the host's mucosal immune response. *Immunol Rev* 2012;**245**:56–83.
162. Kaniga K, Trollinger D, Galán JE. Identification of two targets of the type III protein secretion system encoded by the inv and spa loci of *Salmonella typhimurium* that have homology to the *Shigella* IpaD and IpaA proteins. *J Bacteriol* 1995;**177**:7078–85.
163. Kaniga K, Tucker S, Trollinger D, et al. Homologs of the *Shigella* IpaB and IpaC invasins are required for *Salmonella typhimurium* entry into cultured epithelial cells. *J Bacteriol* 1995;**177**:3965–71.
164. Kariuki S, Revathi G, Kariuki N, et al. Invasive multidrug-resistant non-typhoidal *Salmonella* infections in Africa: zoonotic or anthroponotic transmission? *J Med Microbiol* 2006;**55**(Pt 5):585–91.
165. Katz BZ, Shapiro ED. Predictors of persistently positive blood cultures in children with "occult" *Salmonella* bacteremia. *Pediatr Infect Dis* 1986;**5**:713–14.
166. Kazemi M, Gumpert TG, Marks MI. A controlled trial comparing sulfamethoxazole-trimethoprim, ampicillin, and no therapy in the treatment of salmonella gastroenteritis in children. *J Pediatr* 1973;**83**:646–50.
167. Khan MA, Abdur-Rab M, Israr N, et al. Transmission of *Salmonella worthington* by oropharyngeal suction in hospital neonatal unit. *Pediatr Infect Dis J* 1991;**10**:668–72.
168. Khosla SN. *Typhoid fever: its cause*, *transmission and prevention*. New Delhi, Atlantic, 2008.
169. Kinsella TR, Yogev R, Shulman ST, et al. Treatment of *Salmonella* meningitis and brain abscess with the new cephalosporins: two case reports and a review of the literature. *Pediatr Infect Dis J* 1987;**6**:476–80.
170. Klein JO. Management of the febrile child without a focus of infection in the era of universal pneumococcal immunization. *Pediatr Infect Dis J* 2002;**21**:584–8; discussion 613–14.
171. Klugman KP, Gilbertson IT, Koornhof HJ, et al. Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet* 1987;**2**:1165–9.
172. Konzert W. Uber eine Salmonella-Osteomyelitis im Rahmen einer *Salmonella-typhimurium*-Epidemie auf einer Neugeborenen-Station. *Wien Klin Wochenschr* 1969;**81**:713–16.
173. Kroupitski Y, Pinto R, Brandl MT, et al. Interactions of *Salmonella enterica* with lettuce leaves. *J Appl Microbiol* 2009;**106**:1876–85.
174. Kuhle V, Hensel M. Cellular microbiology of intracellular *Salmonella enterica:* functions of the type III secretion system encoded by *Salmonella* pathogenicity island 2. *Cell Mol Life Sci* 2004;**61**: 2812–26.
175. Kuppermann N. Occult bacteremia in young febrile children. *Pediatr Clin North Am* 1999;**46**:1073–109.
176. Lamb VA, Mayhall CG, Spadora AC, et al. Outbreak of *Salmonella typhimurium* gastroenteritis due to an imported strain resistant to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole in a nursery. *J Clin Microbiol* 1984;**20**:1076–9.
177. Laupland KB, Schønheyder HC, Kennedy KJ, et al. *Salmonella enterica* bacteraemia: a multi-national population-based cohort study. *BMC Infect Dis* 2010;**10**:95.
178. Lee LA, Puhr ND, Maloney EK, et al. Increase in antimicrobial-resistant *Salmonella* infections in the United States, 1989-1990. *J Infect Dis* 1994;**170**:128–34.
179. Lee SC, Yang PH, Shieh WB, et al. Bacteremia due to non-typhi *Salmonella:* analysis of 64 cases and review. *Clin Infect Dis* 1994;**19**: 693–6.
180. Lee WS, Puthucheary SD, Omar A. *Salmonella* meningitis and its complications in infants. *J Paediatr Child Health* 1999;**35**:379–82.
181. Levine MM. Typhoid fever and enteric fever. In: Kass E, Platt R, editors. *Current therapy in infectious diseases*. Toronto: B.C., Decker; 1986.
182. Levine MM. Typhoid fever vaccines. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 6th ed. Edinburgh: Saunders; 2012. p. 812–36.
183. Levy SB. Man, animals and antibiotic resistance. *Pediatr Infect Dis* 1985;**4**:3–5.
184. Lieb S, Gunn RA, Taylor DN. Salmonellosis in a day-care center. *J Pediatr* 1982;**100**:1004–5.
185. Lizarralde E. Typhoid perforation of the ileum in children. *J Pediatr Surg* 1981;**16**:1012–16.
186. Loharikar A, Newton A, Rowley P, et al. Typhoid fever outbreak associated with frozen mamey pulp imported from Guatemala to the Western United States, 2010. *Clin Infect Dis* 2012;**55**:61–6.
187. Loetscher Y, Wieser A, Lengefeld J, et al. *Salmonella* transiently reside in luminal neutrophils in the inflamed gut. *PLoS One* 2012;**7**:e34812.
188. LoVerde PT, Amento C, Higashi GI. Parasite-parasite interaction of *Salmonella typhimurium* and *Schistosoma*. *J Infect Dis* 1980;**141**: 177–85.
189. Low LC, Lam BC, Wong WT, et al. *Salmonella* meningitis in infancy. *Aust Paediatr J* 1984;**20**:225–8.
190. Lynch M, Painter J, Woodruff R, et al. Surveillance for foodborne-disease outbreaks: United States, 1998-2002. *MMWR Surveill Summ* 2006;**55**:1–42.
191. Lynch MF, Blanton EM, Bulens S, et al. Typhoid fever in the United States, 1999-2006. *JAMA* 2009;**302**:859–65.
192. Mabey DC, Brown A, Greenwood BM. *Plasmodium falciparum* malaria and *Salmonella* infections in Gambian children. *J Infect Dis* 1987;**155**:1319–21.
193. MacDonald KL, Cohen ML, Hargrett-Bean NT, et al. Changes in antimicrobial resistance of *Salmonella* isolated from humans in the United States. *JAMA* 1987;**258**:1496–9.
194. Macdonald WB, Friday F, McEacharn M. The effect of chloramphenicol in *Salmonella* enteritis of infancy. *Arch Dis Child* 1954; **29**:238–41.
195. Mackaness GB, Blanden RV, Collins FM. Host-parasite relations in mouse typhoid. *J Exp Med* 1966;**124**:573–83.
196. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal *Salmonella* gastroenteritis. *Clin Infect Dis* 2010;**50**: 882–9.
197. Maki DG. Coming to grips with foodborne infection: peanut butter, peppers, and nationwide salmonella outbreaks. *N Engl J Med* 2009;**360**:949–53.
198. Mallouh AA, Sa'di AR. White blood cells and bone marrow in typhoid fever. *Pediatr Infect Dis J* 1987;**6**:527–9.
199. Mallouh AA, Salamah MM. Pattern of bacterial infections in homozygous sickle cell disease: a report from Saudi Arabia. *Am J Dis Child* 1985;**139**:820–2.
200. Marcus R, Rabatsky-Ehr T, Mohle-Boetani JC, et al. Dramatic decrease in the incidence of *Salmonella* serotype Enteritidis infections in 5 FoodNet sites: 1996-1999. *Clin Infect Dis* 2004;**38**(Suppl. 3):S135–41.
201. Martinez-Roig A, Bonet-Alcaina M, Casellas-Montagut M, et al. Pancreatitis in typhoid fever relapse. *Pediatr Infect Dis J* 2009;**28**:74.
202. Marzetti G, Laurenti F, Caro M de, et al. *Salmonella München* infections in newborns and small infants: an analysis of the clinical expressions and manner of spread, as studied in two outbreaks. *Clin Pediatr (Phila)* 1973;**12**:93–7.
203. Mastroeni P. Mechanisms of immunity to *Salmonella* infection. In: Mastroeni P, Maskell D, editors. *Salmonella infections: clinical, immunological and molecular aspects*. Cambridge: Cambridge University Press; 2006. p. 207–54.
204. Maudgil A, Bhan MK, Khoshoo V. Hemolytic uremic syndrome associated with *Salmonella typhimurium*. *Indian Pediatr* 1987;**24**: 608–9.
205. McAllister TA, Roud JA, Marshall A, et al. Outbreak of *Salmonella eimsbuettel* in newborn infants spread by rectal thermometers. *Lancet* 1986;**1**:1262–4.
206. McKinlay B. Infectious diarrhea of the newborn caused by an unclassified species of *Salmonella* 54:1252, 1937. *Am J Dis Child* 1937;**54**:1252–6.
207. Meadow WL, Schneider H, Beem MO. *Salmonella enteritidis* bacteremia in childhood. *J Infect Dis* 1985;**152**:185–9.
208. Mehta PN. Clinical features and differential diagnosis of typhoid fever. In: Ganguly N, Kundu R, Ghosh TK, editors. *Common MDR infections in children: typhoid*, *tuberculosis*, *malaria*. New Delhi: CBS Publishers; 2005. p. 10–13.

209. Meloni T, Marinaro AM, Desole MG, et al. Ceftriaxone treatment of *Salmonella* enteric fever. *Pediatr Infect Dis J* 1988;**7**:734–5.
210. Mendis NM, de La Motte PU, Gunatillaka PD, et al. Protracted infection with *Salmonella bareilly* in a maternity hospital. *J Trop Med Hyg* 1976;**79**:142–50.
211. Mermin JH, Townes JM, Gerber M, et al. Typhoid fever in the United States, 1985-1994: changing risks of international travel and increasing antimicrobial resistance. *Arch Intern Med* 1998; **158**:633–8.
212. Merselis JG, Kaye D, Connolly CS, et al. Quantitative bacteriology of the typhoid carrier state. *Am J Trop Med Hyg* 1964;**13**: 425–9.
213. Michel J, Malpuech G, Godenèche P, et al. Etude clinique et bactériologique d'une épidémie de salmonellose en milieu hospitalier (Salmonella Oranienburg). *Pediatrie* 1970;**25**:13–19.
214. Millet A, Hullo E, Armari Alla C, et al. Drépanocytose et salmonelloses invasives ostéo-articulaires. *Arch Pédiatrie* 2012;**19**: 267–70.
215. Mohanty S, Gaind R, Sehgal R, et al. Neonatal sepsis due to *Salmonella* Typhi and Paratyphi A. *J Infect Dev Countries* 2009;**3**: 633–8.
216. Mølbak K, Baggesen DL, Aarestrup FM, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium DT104. *N Engl J Med* 1999;**34**:1420–5.
217. Molyneux EM, Mankhambo LA, Phiri A, et al. The outcome of non-typhoidal salmonella meningitis in Malawian children, 1997-2006. *Ann Trop Paediatr* 2009;**29**:13–22.
218. Molyneux EM, Walsh AL, Forsyth H, et al. Dexamethasone treatment in childhood bacterial meningitis in Malawi: a randomised controlled trial. *Lancet* 2002;**360**:211–18.
219. Monack DM. *Salmonella* persistence and transmission strategies. *Curr Opin Microbiol* 2012;**15**:100–7.
220. Moosa A, Rubidge CJ. Once daily ceftriaxone vs. chloramphenicol for treatment of typhoid fever in children. *Pediatr Infect Dis J* 1989;**8**:696–9.
221. Morpeth SC, Ramadhani HO, Crump JA. Invasive non-Typhi Salmonella disease in Africa. *Clin Infect Dis* 2009;**49**(4):606–11.
222. Mroczenski-Wildey MJ, Di Fabio JL, Cabello FC. Invasion and lysis of HeLa cell monolayers by *Salmonella typhi:* the role of lipopolysaccharide. *Microb Pathog* 1989;**6**:143–52.
223. Naqvi SH, Thobani S, Moazam F, et al. Generalized suppurative lymphadenitis with typhoidal salmonellosis. *Pediatr Infect Dis J* 1988;**7**:882–3.
224. Nelson JD. Suppurative mastitis in infants. *Am J Dis Child* 1973;**125**:458–9.
225. Nelson SJ, Granoff D. *Salmonella* gastroenteritis in the first three months of life: a review of management and complications. *Clin Pediatr (Phila)* 1982;**21**:709–12.
226. Nelson JD, McCracken GH. What next? *Pediatr Infect Dis J* 1990; **16**:10–16.
227. Nelson JD, Kusmiesz H, Jackson LH, et al. Treatment of *Salmonella* gastroenteritis with ampicillin, amoxicillin, or placebo. *Pediatrics* 1980;**65**:1125–30.
228. Neter E. Observations on the transmission of salmonellosis in man. *Am J Public Health Nations Health* 1950;**40**:929–33.
229. Nguyen TC, Solomon T, Mai XT, et al. Short courses of ofloxacin for the treatment of enteric fever. *Trans R Soc Trop Med Hyg* 1997; **91**:347–9.
230. Noyola DE, Fernandez M, Kaplan SL. Reevaluation of antipyretics in children with enteric fever. *Pediatr Infect Dis J* 1998;**17**: 691–5.
231. Okoro CL, Kingsley RA, Quail MA, et al. High-resolution single nucleotide polymorphism analysis distinguishes recrudescence and reinfection in recurrent invasive nontyphoidal salmonella typhimurium disease. *Clin Infect Dis* 2012;**54**:955–63.
232. Olopoenia LA, King AL. Widal agglutination test: 100 years later—still plagued by controversy. *Postgrad Med J* 2000;**76**: 80–4.
233. Omland T, Gardborg O. *Salmonella enteritidis* infections in infancy with special reference to a small nosocomial epidemic. *Acta Paediatr* 1960;**49**:583–90.
234. Ortiz-Neu C, Marr JS, Cherubin CE, et al. Bone and joint infections due to *Salmonella*. *J Infect Dis* 1978;**138**:820–8.
235. Owusu-Ofori A, Scheld WM. Treatment of *Salmonella* meningitis: two case reports and a review of the literature. *Int J Infect Dis* 2003;**7**:53–60.
236. Ozen M. Recurrent *Salmonella* bacteremia in interleukin-12 receptor 1 deficiency. *J Trop Pediatr* 2005;**52**:296–8.
237. Pape JW, Gerdes H, Oriol L, et al. Typhoid fever: successful therapy with cefoperazone. *J Infect Dis* 1986;**153**:272–6.
238. Parry CM, Hoa NT, Diep TS, et al. Value of a single-tube Widal test in diagnosis of typhoid fever in Vietnam. *J Clin Microbiol* 1999;**37**:2882–6.
239. Parry CM, Hien TT, Dougan G, et al. Typhoid fever. *N Engl J Med* 2002;**347**:1770–82.
240. Parry CM, Ho VA, Le Phuong T, et al. Randomized controlled comparison of ofloxacin, azithromycin, and an ofloxacin-azithromycin combination for treatment of multidrug-resistant and nalidixic acid-resistant typhoid fever. *Antimicrob Agents Chemother* 2007;**51**:819–25.
241. Parry CM, Wijedoru L, Arjyal A, et al. The utility of diagnostic tests for enteric fever in endemic locations. *Expert Rev Anti Infect Ther* 2011;**9**:711–25.
242. Pavia AT, Shipman LD, Wells JG. Epidemiologic evidence that prior antimicrobial exposure decreases resistance to infection by antimicrobial-sensitive *Salmonella*. *J Infect Dis* 1990;**161**: 255–60.
243. Pether JV, Gilbert RJ. The survival of salmonellas on finger-tips and transfer of the organisms to foods. *J Hyg (Lond)* 1971;**69**: 673–81.
244. Pettersson T, Klemola E, Wager O. Treatment of acute cases of *Salmonella* infection and *Salmonella* carriers with ampicillin and neomycin. *Acta Med Scand* 1964;**175**:185–90.
245. Pfeiffer ML, DuPont HL, Ochoa TJ. The patient presenting with acute dysentery: a systematic review. *J Infect* 2012;**64**:374–86.
246. Pickering LK. Bacterial and parasitic enteropathogens in day care. *Semin Pediatr Infect Dis* 1990;**1**:263–9.
247. Pickering LK, Ruiz-Palacios G. Antibodies in milk directed against specific enteropathogens. In: Hamosh A, Goldman AS, editors: *Human lactation 2*. New York: Plenum; 1986. p. 499.
248. Pickering LK, Kohl S, Cleary TG. Humoral factors in breast milk that protect against diarrhea. In: Jensen RG, Neville MC, editors. *Human lactation*. New York: Plenum; 1985. p. 63.
249. Plotkin SA, Bouveret-Le Cam N. A new typhoid vaccine composed of the Vi capsular polysaccharide. *Arch Intern Med* 1995;**155**: 2293–9.
250. Podolak R, Enache E, Stone W, et al. Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods. *J Food Prot* 2010;**73**: 1919–36.
251. Price EH, de Louvois J, Workman MR. Antibiotics for *Salmonella* meningitis in children. *J Antimicrob Chemother* 2000;**46**:653–5.
252. Punjabi NH, Hoffman SL, Edman DC, et al. Treatment of severe typhoid fever in children with high dose dexamethasone. *Pediatr Infect Dis J* 1988;**7**:598–600.
253. Puri V, Thirupuram S, Khalil A, et al. Nosocomial *Salmonella typhimurium* epidemic in a neonatal special care unit. *Indian Pediatr* 1980;**17**:233–9.
254. Rajajee S, Anandi TB, Subha S, et al. Patterns of resistant *Salmonella typhi* infection in infants. *J Trop Pediatr* 1995;**41**:52–4.
255. Ramachandran S, Godfrey JJ, Perera MV. Typhoid hepatitis. *JAMA* 1974;**230**:236–40.
256. Ramos JM, García-Corbeira P, Aguado JM, et al. Clinical significance of primary vs. secondary bacteremia due to nontyphoid *Salmonella* in patients without AIDS. *Clin Infect Dis* 1994;**19**: 777–80.
257. Reed RP, Klugman KP. Neonatal typhoid fever. *Pediatr Infect Dis J* 1994;**13**:774–7.
258. Renuka K, Sood S, Das BK, et al. High-level ciprofloxacin resistance in *Salmonella enterica* serotype Typhi in India. *J Med Microbiol* 2005;**54**(Pt 10):999–1000.
259. Rice PA, Craven C, Wells JG. *Salmonella heidelberg* enteritis and bacteremia: an epidemic on two pediatric wards. *Am J Med* 1976;**60**:509–16.
260. Richards LH, Howard J, Klein JL. Community-acquired *Salmonella* bacteraemia in patients with sickle-cell disease 1969-2008: a single centre study. *Scand J Infect Dis* 2011;**43**:89–94.
261. Riley LW, Cohen ML. Plasmid profiles and salmonella epidemiology. *Lancet* 1982;**1**:573.
262. Riley LW, Cohen ML, Seals JE, et al. Importance of host factors in human salmonellosis caused by multiresistant strains of *Salmonella*. *J Infect Dis* 1984;**149**:878–83.

263. Rocha H, Kirk JW, Hearey CD. Prolonged *Salmonella* bacteremia in patients with *Schistosoma mansoni* infection. *Arch Intern Med* 1971;**128**:254–7.
264. Rodriguez RE, Valero V, Watanakunakorn C. *Salmonella* focal intracranial infections: review of the world literature (1884-1984) and report of an unusual case. *Rev Infect Dis* 1986;**8**:31–41.
265. Rosenthal SL. Exacerbation of salmonella enteritis due to ampicillin. *N Engl J Med* 1969;**280**:147–8.
266. Rowe B, Giles C, Brown GL. Outbreak of gastroenteritis due to *Salmonella virchow* in a maternity hospital. *Br Med J* 1969;**3**: 561–4.
267. Rowe B, Ward LR, Threlfall EJ. Multidrug-resistant *Salmonella typhi:* a worldwide epidemic. *Clin Infect Dis* 1997;**24**(Suppl. 1): S106–9.
268. Rowe SY, Rocourt JR, Shiferaw B, et al. Breast-feeding decreases the risk of sporadic salmonellosis among infants in FoodNet sites. *Clin Infect Dis* 2004;**38**(Suppl. 3):S262–70.
269. Rubinstein AD, Fowler RN. Salmonellosis of the newborn with transmission by delivery room resuscitators. *Am J Public Health Nations Health* 1955;**45**:1109–14.
270. Rubinstein AD, Feemster RF, Smith HM. Salmonellosis as a public health problem in wartime. *Am J Public Health* 1944; **34**:841.
271. Ruby T, McLaughlin L, Gopinath S, et al. *Salmonella's* long-term relationship with its host. *FEMS Microbiol Rev* 2012;**36**: 600–15.
272. Ruiz Gomez J, Lorente Salinas I, Perez Salmerón J, et al. Evaluation of methods for isolation of *Salmonella* species using modified semisolid Rappaport-Vassiliadis medium and Salmonella-Shigella agar. *Eur J Clin Microbiol Infect Dis* 1998;**17**:791–3.
273. Ruiz-Contreras J, Ramos JT, Hernández-Sampelayo T, et al. Sepsis in children with human immunodeficiency virus infection. The Madrid HIV Pediatric Infection Collaborative Study Group. *Pediatr Infect Dis J* 1995;**14**:522–6.
274. Ryan CA, Hargrett-Bean NT, Blake PA. *Salmonella typhi* infections in the United States, 1975-1984: increasing role of foreign travel. *Rev Infect Dis* 1989;**11**:1–8.
275. Sánchez C, García-Restoy E, Garau J, et al. Ciprofloxacin and trimethoprim-sulfamethoxazole versus placebo in acute uncomplicated *Salmonella* enteritis: a double-blind trial. *J Infect Dis* 1993; **168**:1304–7.
276. Sanchez-Vargas FM, Abu-El-Haija MA, Gomez-Duarte OG. Salmonella infections: an update on epidemiology, management, and prevention. *Travel Med Infect Dis* 2011;**9**:263–77.
277. Sanders DY, Sinal SH, Morrison L. Chronic salmonellosis in infancy. *Clin Pediatr (Phila)* 1974;**13**:640–3.
278. Saphra I, Winter JW. Clinical manifestations of salmonellosis in man: an evaluation of 7779 human infections identified at the New York Salmonella Center. *N Engl J Med* 1957;**256**(24):1128–34.
279. Sarasombath S, Banchuin N, Sukosol T, et al. Systemic and intestinal immunities after natural typhoid infection. *J Clin Microbiol* 1987;**25**:1088–93.
280. Sasidharan CK, Rajagopal KC, Panicker CK. *Salmonella typhimurium* epidemic in newborn nursery. *Indian J Pediatr* 1983; **50**:599–605.
281. Schloesser RL, Schaefer V, Groll AH. Fatal transplacental infection with non-typhoidal *Salmonella*. *Scand J Infect Dis* 2004;**36**: 773–4.
282. Schwan WR, Huang X, Hu L, et al. Differential bacterial survival, replication and apoptosis-inducing ability of *Salmonella* serovars within human and murine macrophages. *Infect Immun* 2000;**68**:1005–13.
283. Seals JE, Parrott PL, McGowan JE, et al. Nursery salmonellosis: delayed recognition due to unusually long incubation period. *Infect Control* 1983;**4**:205–8.
284. Seligman E. Mass invasion of *Salmonella* in a babies ward. *Ann Paediatr* 1949;**172**:406.
285. Seoud M, Saade G, Uwaydah M, et al. Typhoid fever in pregnancy. *Obstet Gynecol* 1988;**71**:711–14.
286. Shirakawa T, Acharya B, Kinoshita S, et al. Decreased susceptibility to fluoroquinolones and *gyrA* gene mutation in the *Salmonella enterica* serovar Typhi and Paratyphi A isolated in Katmandu, Nepal, in 2003. *Diagn Microbiol Infect Dis* 2006;**54**:299–303.
287. Sinha A, Sazawal S, Kumar R, et al. Typhoid fever in children aged less than 5 years. *Lancet* 1999;**354**:734–7.
288. Sirinavin S, Jayanetra P, Lolekha S, et al. Predictors for extraintestinal infection in Salmonella enteritis in Thailand. *Pediatr Infect Dis J* 1988;**7**:44–8.
289. Smith SM, Palumbo PE, Edelson PJ. *Salmonella* strains resistant to multiple antibiotics: therapeutic implications. *Pediatr Infect Dis* 1984;**3**:455–60.
290. Soe GB, Overturf GD. Treatment of typhoid fever and other systemic salmonelloses with cefotaxime, ceftriaxone, cefoperazone, and other newer cephalosporins. *Rev Infect Dis* 1987;**9**:719–36.
291. Sperber SJ, Schleupner CJ. Salmonellosis during infection with human immunodeficiency virus. *Rev Infect Dis* 1987;**9**:925–34.
292. Spika JS, Waterman SH, Hoo GW, et al. Chloramphenicol-resistant *Salmonella newport* traced through hamburger to dairy farms: a major persisting source of human salmonellosis in California. *N Engl J Med* 1987;**316**:565–70.
293. Steenackers H, Hermans K, Vanderleyden J, et al. *Salmonella* biofilms: an overview on occurrence, structure, regulation and eradication. *Food Res Int* 2012;**45**:502–31.
294. Stephens I, Levine MM. Management of typhoid fever in children. *Pediatr Infect Dis J* 2002;**21**:157–8.
295. Stormon MO, McIntyre PB, Morris J, et al. Typhoid fever in children: diagnostic and therapeutic difficulties. *Pediatr Infect Dis J* 1997;**16**:713–14.
296. Stuart BM, Pullen RL. Typhoid; clinical analysis of 360 cases. *Arch Intern Med* 1946;**78**:629–61.
297. Su LH, Chiu CH, Chu C, et al. Antimicrobial resistance in nontyphoid Salmonella serotypes: a global challenge. *Clin Infect Dis* 2004;**39**:546–51.
298. Swanson SJ, Snider C, Braden CR. Multidrug-resistant *Salmonella enterica* serotype Typhimurium associated with pet rodents. *N Engl J Med* 2007;**356**:21–8.
299. Syrogiannopoulos GA, McCracken GH, Nelson JD. Osteoarticular infections in children with sickle cell disease. *Pediatrics* 1986; **78**:1090–6.
300. Szanton VL. Epidemic salmonellosis; a 30-month study of 80 cases of *Salmonella* Oranienburg infection. *Pediatrics* 1957; **20**(5, Part 1):794–808.
301. Szmuness W, Sikorska J, Szymanek E, et al. The microbiological and epidemiological properties of infections caused by *Salmonella enteritidis*. *J Hyg (Lond)* 1966;**64**:9–21.
302. Sztein MB, Wasserman SS, Tacket CO, et al. Cytokine production patterns and lymphoproliferative responses in volunteers orally immunized with attenuated vaccine strains of *Salmonella typhi*. *J Infect Dis* 1994;**170**:1508–17.
303. Taneja PN, Ghai OP. Enteric fever in children. *Indian J Pediatr* 1957;**24**:237–45.
304. Taylor DN, Bied JM, Munro JS, et al. *Salmonella dublin* infections in the United States, 1979-1980. *J Infect Dis* 1982;**146**:322–7.
305. Taylor DN, Bopp C, Birkness K, et al. An outbreak of salmonellosis associated with a fatality in a healthy child: a large dose and severe illness. *Am J Epidemiol* 1984;**119**:907–12.
306. Taylor DN, Levine MM, Kuppens L, et al. Why are typhoid vaccines not recommended for epidemic typhoid fever? *J Infect Dis* 1999;**180**:2089–90.
307. Thaver D, Zaidi AK, Critchley J, et al. A comparison of fluoroquinolones versus other antibiotics for treating enteric fever: meta-analysis. *BMJ* 2009;**338**:b1865.
308. Thisyakorn U, Mansuwan P, Taylor DN. Typhoid and paratyphoid fever in 192 hospitalized children in Thailand. *Am J Dis Child* 1987;**141**:862–5.
309. Ti TY, Monteiro EH, Lam S, et al. Ceftriaxone therapy in bacteremic typhoid fever. *Antimicrob Agents Chemother* 1985;**28**:540–3.
310. Tilahun B, Worku B, Tachbele E, et al. High load of multi-drug resistant nosocomial neonatal pathogens carried by cockroaches in a neonatal intensive care unit at Tikur Anbessa specialized hospital, Addis Ababa, Ethiopia. *Antimicrob Resist Infect Control* 2012;**1**:12.
311. Torrey S, Fleisher G, Jaffe D. Incidence of *Salmonella* bacteremia in infants with *Salmonella* gastroenteritis. *J Pediatr* 1986; **108**(5 Pt 1):718–21.
312. Tsai MH, Huang YC, Lin TY, et al. Reappraisal of parenteral antimicrobial therapy for nontyphoidal *Salmonella* enteric infection in children. *Clin Microbiol Infect* 2011;**17**:300–5.
313. Tsang RS, Chau PY, Lam SK, et al. Antibody response to the lipopolysaccharide and protein antigens of *Salmonella typhi* during

typhoid infection. I. Measurement of serum antibodies by radioimmunoassay. *Clin Exp Immunol* 1981;**46**:508–14.
314. Uwyyed K, Korman SH, Bar-Oz B, et al. Scrotal abscess with bacteremia caused by *Salmonella* group D after ritual circumcision. *Pediatr Infect Dis J* 1990;**9**:65–6.
315. Vallenas C, Hernandez H, Kay B, et al. Efficacy of bone marrow, blood, stool and duodenal contents cultures for bacteriologic confirmation of typhoid fever in children. *Pediatr Infect Dis* 1985; **4**:496–8.
316. van Gijsegem F, Genin S, Boucher C. Conservation of secretion pathways for pathogenicity determinants of plant and animal bacteria. *Trends Microbiol* 1993;**1**:175–80.
317. Van TT, Nguyen HN, Smooker PM, et al. The antibiotic resistance characteristics of non-typhoidal *Salmonella enterica* isolated from food-producing animals, retail meat and humans in South East Asia. *Int J Food Microbiol* 2012;**154**:98–106.
318. Vaudry W, Core LAJ, Hoog M de. Two commentaries on 'corticosteroids for acute bacterial meningitis'. *Evid Based Child Health* 2008;**3**:453–7.
319. Vazquez-Torres A, Vallance BA, Bergman MA, et al. Toll-like receptor 4 dependence of innate and adaptive immunity to *Salmonella:* importance of the Kupffer cell network. *J Immunol* 2004; **172**:6202–8.
320. Vazquez-Torres A, Xu Y, Jones-Carson J, et al. *Salmonella* pathogenicity island 2-dependent evasion of the phagocyte NADPH oxidase. *Science* 2000;**287**:1655–8.
321. Vinh H, Duong NM, Le Phuong T, et al. Comparative trial of short-course ofloxacin for uncomplicated typhoid fever in Vietnamese children. *Ann Trop Paediatr* 2005;**25**:17–22.
322. Voetsch AC, Angulo FJ, Rabatsky-Ehr T, et al. Laboratory practices for stool-specimen culture for bacterial pathogens, including *Escherichia coli* O157:H7, in the FoodNet sites, 1995-2000. *Clin Infect Dis* 2004;**38**(Suppl. 3):S190–7.
323. Vugia DJ, Samuel M, Farley MM, et al. Invasive *Salmonella* infections in the United States, FoodNet, 1996-1999: incidence, serotype distribution, and outcome. *Clin Infect Dis* 2004;**38**(Suppl. 3): S149–56.
324. Wain J, Diep TS, Bay PV, et al. Specimens and culture media for the laboratory diagnosis of typhoid fever. *J Infect Dev Countries* 2008;**2**:469–74.
325. Wain J, Hien TT, Connerton P, et al. Molecular typing of multiple-antibiotic-resistant *Salmonella enterica* serovar Typhi from Vietnam: application to acute and relapse cases of typhoid fever. *J Clin Microbiol* 1999;**37**:2466–72.
326. Wain J, Hoa NT, Chinh NT, et al. Quinolone-resistant *Salmonella typhi* in Viet Nam: molecular basis of resistance and clinical response to treatment. *Clin Infect Dis* 1997;**25**:1404–10.
327. Wain J, Pham VB, Ha V, et al. Quantitation of bacteria in bone marrow from patients with typhoid fever: relationship between counts and clinical features. *J Clin Microbiol* 2001;**39**:1571–6.
328. Wales AD, Carrique-Mas JJ, Rankin M, et al. Review of the carriage of zoonotic bacteria by arthropods, with special reference to *Salmonella* in mites, flies and litter beetles. *Zoonoses Public Health* 2010;**57**:299–314.
329. Wallis TS, Starkey WG, Stephen J, et al. The nature and role of mucosal damage in relation to *Salmonella typhimurium*-induced fluid secretion in the rabbit ileum. *J Med Microbiol* 1986;**22**:39–49.
330. Watt J, Carlton E. Studies of the acute diarrheal diseases, XVI: an outbreak of *S. typhimurium* infection among newborn premature infants. *Public Health Rep* 1945;**60**(Pt I):734–810.
331. Watt J, Wegman ME, Brown OW, et al. Salmonellosis in a premature nursery unaccompanied by diarrheal disease. *Pediatrics* 1958;**22**(4 Pt 1):689–705.
332. Wilson R, Feldman RA, Davis J, et al. Salmonellosis in infants: the importance of intrafamilial transmission. *Pediatrics* 1982;**69**:436–8.
333. Winkler AP, Gleich S. Acute acalculous cholecystitis caused by *Salmonella typhi* in an 11-year-old. *Pediatr Infect Dis J* 1988;**7**: 125–8.
334. Wittler RR, Bass JW. Nontyphoidal *Salmonella* enteric infections and bacteremia. *Pediatr Infect Dis J* 1989;**8**:364–7.
335. World Health Organization. Background document: the diagnosis, treatment and prevention of typhoid fever: WHO/V&B/03.07. Geneva: World Health Organization; 2003.
336. World Health Organization. Recommendations for management of common childhood conditions: newborn conditions, dysentery, pneumonia, oxygen use and delivery, common causes of fever, severe acute malnutrition and supportive care. Geneva, Switzerland: World Health Organization; 2012.
337. World Health Organization. Report of the ad-hoc consultation on typhoid vaccine introduction and typhoid surveillance. WHO/IVB/12.02: April 18-20, 2011, Plaza Athenee Hotel, Bangkok. Geneva: World Health Organization; 2012.
338. Wright J, Thomas P, Serjeant GR. Septicemia caused by *Salmonella* infection: an overlooked complication of sickle cell disease. *J Pediatr* 1997;**130**:394–9.
339. Wu HM, Huang WY, Lee ML, et al. Clinical features, acute complications, and outcome of *Salmonella* meningitis in children under one year of age in Taiwan. *BMC Infect Dis* 2011;**11**:30.
340. Yacaman-Handal R, Flores-Nava G, Escobedo-Chávez E, et al. Pancreatitis aguda secundaria a fiebre tifoidea en un preescolar. *Rev Gastroenterol Mex* 2000;**65**:30–3.
341. Yamamoto LG, Ashton MJ. Salmonella infections in infants in Hawaii. *Pediatr Infect Dis J* 1988;**7**:48–52.
342. Zaidi E, Bachur R, Harper M. Non-typhi *Salmonella* bacteremia in children. *Pediatr Infect Dis J* 1999;**18**:1073–7.
343. Zaka-ur-Rab Z, Abqari S, Shahab T, et al. Evaluation of salivary anti-*Salmonella typhi* lipopolysaccharide IgA ELISA for serodiagnosis of typhoid fever in children. *Arch Dis Child* 2012;**97**:236–8.
344. Zaki SA, Karande S. Multidrug-resistant typhoid fever: a review. *J Infect Dev Countries* 2011;**5**:324–37.
345. Zeng H, Wu H, Sloane V, et al. Flagellin/TLR5 responses in epithelia reveal intertwined activation of inflammatory and apoptotic pathways. *Am J Physiol Gastrointest Liver Physiol* 2006;**290**: G96–8.

should be examined for typical bacilli. The isolated bacilli can be identified by the following criteria. *Y. pestis*, the sole *Yersinia* spp. that is nonmotile at 37° C and 22° C (98.6° F and 71.6° F), also is the sole *Yersinia* spp. that is urease negative, but it may be positive in freshly isolated strains. The organism is positive for esculin, β-galactosidase, catalase, and methyl red. Oxidase, indole, and Voges-Proskauer reactions are negative. It ferments glucose, maltose, salicin, xylose, arabinose, dextrin, trehalose, and mannitol. It does not produce acid from lactose, sucrose, rhamnose, melibiose, adonitol, cellobiose, sorbose, or dulcitol. It does not use citrate, and it does not grow in potassium cyanide. *Y. pestis* is negative for lysine, ornithine decarboxylase, and arginine dihydrolase.[44,53]

Positive cultures show pinpoint colonies within 24 to 48 hours after inoculation. Many laboratories use fully automated or semiautomated identification systems (e.g., Vitek) that may not detect *Y. pestis*, often confusing *Y. pestis* and *Y. pseudotuberculosis* or *Yersinia enterocolitica* with plague bacillus. Nonautomated laboratories may require 6 days to identify the organism. Because of lack of standardized susceptibility testing procedures, suspected isolates must be referred to public health or other reference laboratories to comply with the select agent requirements of the Title II Enhanced Controls for Dangerous Biological Agents and Toxins Act.[28]

Several chromosomal-mediated virulence factors are responsible for the virulence of *Y. pestis*, including (1) an antiphagocytic capsular material known as fraction 1, (2) the endogenous purine synthesis that allows the organism to grow within macrophages, and (3) the ability to absorb iron from the medium.[5] Several plasmids have been implicated in the development of other virulence factors.[24,57] A plasmid of 9-kb pairs contains the determinant of secretory protein that kills other bacterial strains. A plasmid of 72-kb pairs, which all pathogenic *Y. pestis* strains contain, confers the requirement for environmental calcium to be present for the organism to grow at 37° C (98.6° F). When grown under this condition, *Y. pestis* produces V and W antigens that are necessary for virulence.[21] An exotoxin and an endotoxin have been found to contribute to the lethal effects of plague.[53,62]

TRANSMISSION

Host

More than 200 mammalian species have been reported to be naturally infected with *Y. pestis*. Epidemics of plague usually are transmitted by the fleas of infected domestic rats, however. This form of spread is more likely to occur in urban, rat-infested, and crowded dwellings and may result in epidemics. In the United States, plague is transmitted sporadically to humans after contact with an enzootic sylvatic focus.[31] Infected wild rodents perpetuate the plague bacillus in a given ecosystem by virtue of their ability to withstand an inoculum of *Y. pestis* many times greater than that necessary to cause disease in humans or domestic animals. After inoculation, wild rodents may become bacteremic and infect fleas that feed on them; these fleas transmit the plague bacillus to another rodent. Hibernating animals are especially resistant to clinical infection. Animals inoculated before going into hibernation may survive through the winter and not die until after they come out of their burrows, reintroducing the bacillus in the new season.[25] Carnivores are relatively resistant to infection but contribute to the spread of the organism by transporting infected fleas from one area to another.[31,61]

The role of domestic animals in bridging the gap between sylvatic plague and human infection has been studied extensively.[29,41,47] Cats and dogs are susceptible to natural and experimental plague.[56] Epizootics in cats have been observed in conjunction with plague epidemics in humans.[47] Experimentally infected cats develop severe systemic illness, with bacteremia and abscess formation at the site of the inoculum. Between 1977 and 1998, 23 cases of feline-associated human plague infection were reported.[22] Five of these cases were fatal; two of the patients had primary pneumonic plague, and one had septicemic plague. Many of these cases were misdiagnosed at presentation, leading to delays in treatment and, in some cases, fatalities. This diagnosis should be considered especially in the western states of Arizona, California, Colorado, and New Mexico. No seasonal variation in the occurrence of cat-associated illness was noted.[20,22]

Ten other cases reported in the literature of feline transmission of plague to humans involved four veterinarians.[18] Dogs also are susceptible, but the disease is milder.[29] Swine are resistant to plague, the only evidence of subclinical infection being the presence of antibodies to the fraction 1 antigen of *Y. pestis*. Domestic animals, by virtue of their intimate contact with wildlife and humans, may be responsible for some cases of human plague. This danger is accentuated by a dearth of symptoms in some animals.[41]

Vector

Plague is transmitted to humans by the bite of an infected flea, the skinning and evisceration of infected animals,[32] or the inhalation of infected droplets from a case of pneumonic plague.[35] Infrequent portals of entry include the conjunctiva[41] and the pharynx.[40,49]

The efficiency of the flea as a vector for human disease depends on the likelihood that the infected flea will feed on a person and that the flea will regurgitate the bacillus into the victim's bloodstream in the process of feeding.[31] Flea species vary in both of these attributes. Wild rodent fleas are reluctant to feed on humans and do it only under duress (e.g., when the natural host dies). Fleas of domestic animals are more likely to bite humans. The Oriental rat flea, *Xenopsylla cheopis*, is the most efficient transmitter of plague because of its willingness to bite people and its propensity for regurgitating large numbers of bacilli in the process.[31] When the Oriental rat flea ingests infected blood, the actions of a coagulase produced by *Y. pestis* and a trypsin-like enzyme present in the flea's stomach result in the formation of an infected clot that blocks the flea's proventriculus. In obstructing the flea's intestinal tract, the clot allows further replication of bacteria. When the flea tries to feed again, it regurgitates large numbers of plague bacilli. The formation and dissolution of the fibrin clot are temperature dependent. At temperatures greater

than 27°C (>80.6°F), a fibrinolytic enzyme is activated that dissolves the clot and allows the flea to dispose of the bacillus. One postulation is that this temperature-dependent phenomenon is responsible for the observed cyclic nature of urban plague epidemics, which tend to subside with the advent of hot weather.[7]

In contrast, fleas in which the intestinal tracts do not become blocked contribute to the endemicity of plague by harboring the organism and transmitting it in sublethal doses.[9] Sylvatic plague depends on the rodent flea as the vector. This flea, although not as efficient as the rat flea in transmitting the bacillus, may itself become a reservoir of *Y. pestis* by surviving for 12 to 15 months after the original host dies. In the new season, it reintroduces the plague bacillus into the new rodent population.[47] The observation that *Y. pestis* can survive in the soil during interepizootics suggests another possible mechanism of transmission of plague.[52]

EPIDEMIOLOGY

More recent studies have suggested that plague is an ancient disease of mammals; the plague bacterium emerged 1,500 to 20,000 years ago as a clone that evolved from *Y. pseudotuberculosis* that first caused outbreaks in Africa.[1] The plague bacillus exists in enzootic cycles involving wild animals or domestic rats. In urban plague, the course of events usually is initiated by the introduction of the plague bacillus from an enzootic focus into a susceptible rat population. With humans and rats living in proximity, an epizootic in rats may be followed by an epidemic in humans.[47] The epidemic may subside with the advent of hot, humid weather[7] or the obliteration of the rat population.[47] Such epidemics rarely occur today but have been described in the former South Vietnam[4,33] and more recently in India.[13] Between 1985 and 1999, 23 countries reported a total of 29,020 cases of plague to the World Health Organization, with an average mortality rate of 11 percent; major epidemics and outbreaks occurred in Tanzania (1991), Zaire (1992, 1993), Peru (1993, 1994), India (1994), and Madagascar (1995), with fatality rates ranging from 4.6 to 22.3 percent.[55]

In the United States today, humans become infected most frequently by direct contact with a sylvatic reservoir of infection. Sporadic human cases usually result from working or hunting in a plague-infested area[31] and increasingly from living near foci of infection as suburban spread encroaches on the natural habitats of rodents.[14] In recent years, domestic animals, especially cats, have been responsible for a significant proportion of human cases. During 1990 to 2005, 107 cases of plague were reported in the United States, a median of 7 cases a year. By September 2006, 13 plague cases, with two deaths (case-fatality rate, 15%), had been reported from four states (New Mexico, Colorado, California, and Texas). Such resurgences often occur after ecologic changes resulting in population increases in ground rodents such as prairie dogs, ground squirrels, mice, and rats in the American Southwest.[15]

Sylvatic plague epizootics occur in the summer. Most cases of rural plague occur between April and September. The rare occurrence of human plague in the winter usually is associated with hunting and direct exposure to infected tissues.[12,32,37]

The continental United States has a large enzootic focus that includes 130 counties in 15 western states. Surveillance for plague in rodents during the 1990s has identified infected animals farther east than ever before reported. The plague bacillus now has been isolated in wild rodents in eastern Montana, western Nebraska, western North Dakota, and eastern Texas.[14] Between 1925 and 1965, the number of reported cases in the United States averaged between two and three per year.[8,10] During the 1970s, 105 cases were reported.[32] The number of cases reported in 1980 through 1982 showed a similar increasing trend.[10] Between 1970 and 1979, 53 percent of cases were in females, in contrast to the period 1926 to 1969, when only 27 percent were in females. Approximately 60 percent of cases occur in individuals younger than 20 years.[32] Of 10 confirmed cases reported in the United States in 1993, however, the age distribution was 22 to 96 years. Five of the patients were older than 65 years.[14] Native Americans living on reservations in Arizona, New Mexico, and Utah are at increased risk. In the period 1970 to 1979, 35 percent of cases in these three states occurred in Native Americans.[10,32] Many of the patients were infected within 1 mile of their residence and almost all within their state of residence.[26] Seven of the 10 patients described in 1993 were exposed in their home sites, and one, a veterinarian, was exposed at work.[14]

Occasionally, plague has been acquired by a traveler in an endemic area who then traveled during the incubation period to a plague-free region of the country. This set of circumstances shows why all physicians need to be aware of the presenting symptoms and signs of plague and to obtain an accurate travel history.[34]

PATHOGENESIS AND PATHOLOGY

The portal of entry of the plague bacillus determines, to some extent, the form of disease. The most common portal of entry is the skin when it is bitten by an infected flea. Broken skin may provide access for direct inoculation while infected animals are being handled. After overcoming the skin barrier, the organisms move through the lymphatics to the regional lymph nodes, where they elicit an inflammatory response. The infection may be localized at this site, with subsequent formation of antibody and recovery. This clinical form is known as *pestis minor*. The bacillus commonly is disseminated through the bloodstream. Distant organ involvement may include the liver, spleen, kidneys, lungs, and meninges. Disseminated intravascular coagulation is common in fatal cases. Coagulation defects, including thrombocytopenia and elevated fibrin split products[4] and fibrin deposits in the glomeruli,[21,47] may be present. Bacteremia is not synonymous with severe disease and occurs commonly in mild cases.[47]

The major determinant of severity seems to be the presence of high levels of endotoxin. The toxin of *Y. pestis* has the biologic properties of typical endotoxin. When injected into experimental animals, it can cause the clinical symptoms and signs and pathologic changes characteristic of endotoxic shock and death. The quantity of

endotoxin necessary to kill is estimated to be comparable to that present in a lethal dose of live bacteria.[2,54] The murine toxin of *Y. pestis* has a direct inhibitory effect in vitro on the respiration of heart mitochondria of rats and mice, whereas it has little or no effect on the mitochondria of rabbits, chimpanzees, dogs, and monkeys. The differing sensitivities in vitro correlate with the susceptibilities in vivo of these species to *Y. pestis* infection.[50]

Achieving high levels of toxin depends on the ability of the bacillus to replicate in the infected host. Resistance to phagocytosis had been assumed to be related to virulence. More recent experimental evidence has shown that virulent *Y. pestis* organisms are phagocytosed, but, in contrast to avirulent ones, are not killed. They continue to replicate freely in macrophages, allowing the accumulation of endotoxin.[30,50]

When the lung is the portal of entry, the disease usually is more fulminant. After being inhaled, bacilli replicate freely in the alveolar spaces. Severe pneumonia, endotoxemia, and septicemia ensue and, if untreated, cause death. In fatal cases, the thoracic lymph nodes show infarction, necrosis, and liquefaction, with pus formation. Edema and inflammation of the surrounding tissue are common.[47] The mucosa of trachea and bronchi is covered by bloody, frothy exudate. Submucosal hemorrhages and areas of necrosis may surround the trachea. The pleural surfaces contain hemorrhagic lesions and fibrinous adhesions. The lung parenchyma may be consolidated or show signs of acute edema.[47] The predominant histologic feature is an alveolar exudate consisting of histiocytes and polymorphonuclear leukocytes.[21]

Other organs also are involved. The kidneys may appear grossly hemorrhagic and contain areas of necrosis. Microscopic examination reveals leukocytic infiltrates of congested veins and capillaries. Glomeruli with fibrin thrombi frequently are found in patients with disseminated intravascular coagulation.[21] Biopsy of purpuric skin lesions reveals subepithelial hemorrhages and fibrin deposit in the capillaries. These changes are indistinguishable from the changes seen in a generalized Shwartzman reaction.[4]

CLINICAL MANIFESTATIONS

The incubation period of *Y. pestis* generally is 3 to 4 days but ranges from a few hours to 10 days. The onset of illness usually is abrupt, beginning with fever, malaise, weakness, and headache.[47,48] Fever is high, frequently accompanied by shaking chills.[41] The appearance of a visible and palpable bubo may be preceded by pain and tenderness at that site.[48]

On physical examination, the patient is "toxic," apprehensive, and tachycardic. The inoculation site in the skin may not be evident, or it may be marked by a carbuncle. In bubonic plague, typical large, fixed, edematous, and exquisitely tender nodes are present at one anatomic site.[33] In decreasing order of frequency, the areas of nodal involvement are the groin (including femoral and inguinal nodes), axilla, and neck.[47] Any lymph node may suppurate, sometimes presenting an atypical picture (e.g., if intra-abdominal nodes are involved, an acute abdominal emergency may be suspected).[48] Septicemia as an initial presentation of *Y. pestis* infection is common.[26] Twenty-five percent of the patients presented without adenopathy in the 71 confirmed cases of plague in New Mexico from 1980 to 1984. All patients with septicemic presentation had fever and chills, and most had tachycardia, tachypnea, and relative hypotension. Seventy-two percent had gastrointestinal symptoms. Plague pneumonia was twice as likely to occur among septicemic patients as among patients with bubonic plague. Septicemic patients were significantly older and more likely to die than were patients with a bubonic presentation. Although septicemic plague occurred more often in older patients, patients younger than 30 years with septicemic presentation were more likely to die.[26]

As a result of its nonspecific presentation, septicemic plague is difficult to diagnose early. Of 27 patients with plague admitted to Indian Medical Center in Gallup, New Mexico, between 1965 and 1989, 5 had a nonspecific febrile syndrome with upper respiratory tract symptoms. They were prescribed penicillin. Three of the 5 patients died. Another 5 patients had a nonspecific febrile syndrome associated with chills, myalgias, and anorexia. These patients were not treated initially with antibiotics, and 3 died.[18] The index of suspicion must be high because early diagnosis is imperative for avoiding a high risk of mortality. Individuals who appear to have what seems to be community-acquired, gram-negative sepsis and who reside in or have a history of recent travel to endemic areas of plague must be evaluated for and treated with antibiotics effective against *Y. pestis*.[39]

Gastrointestinal symptoms occur in patients with plague, especially patients with septicemic plague.[25] Between 1980 and 1984, more than half of the 71 patients with plague in New Mexico had gastrointestinal symptoms that sometimes preceded the appearance of the buboes in the bubonic cases. Common symptoms are abdominal pain, nausea, vomiting, and diarrhea. These symptoms are believed to be a general response of the body to gram-negative septicemia. Occasionally, hepatosplenomegaly and mesenteric or retroperitoneal lymphadenopathy have masqueraded as an acute abdomen.[27]

Neurologic manifestations caused by the effects of toxin on the brain are common. A patient with plague may have insomnia, delirium, stupor, weakness, staggering gait, vertigo, disorders of speech, and loss of memory.[38] *Y. pestis* meningitis is a rare occurrence, but it does occur. Children younger than 15 years old seem to be more susceptible, and septicemic patients are four times more likely to develop meningitis than are patients with bubonic plague.[42] It often manifests while the patient is well into a course of antibiotic therapy for bubonic or septicemic plague.[3] When intravascular coagulation supervenes, renal involvement may manifest as acute cortical or tubular necrosis. Hepatic involvement may be evidenced by mildly elevated liver enzymes.[47] Hantavirus pulmonary syndrome may mimic septicemic or pneumonic plague; both share a similar geographic distribution, and patients for whom this diagnosis is contemplated should be treated with antibiotics to cover the possibility of plague.[45]

Primary pneumonic plague has identical constitutional symptoms but follows a fulminant course with a more

pronounced pulmonary component. Within 20 to 24 hours after the onset of the illness, tachypnea, dyspnea, and cough productive of bloody mucopurulent sputum supervene. If early and effective treatment is not instituted, the patient usually dies.[47]

DIFFERENTIAL DIAGNOSIS

Because of the rare incidence of plague today, the diagnosis often is delayed or missed. Bubonic plague may be confused with other diseases affecting the skin and lymph nodes. The diagnosis of staphylococcal or streptococcal adenitis can be established easily by culture. Lymphogranuloma venereum is more indolent, has milder systemic symptoms, and is associated with anogenital ulcer. Syphilitic adenitis usually is nontender. With cat-scratch disease and *Pasteurella multocida* infections, the constitutional symptoms are few and the patient typically has a history of animal exposure. Tularemia has a more gradual onset.[47] In their later stages, the ulcerated skin lesions of plague may resemble anthrax.[33]

DIAGNOSIS

The most important factor in promptly establishing the diagnosis of plague is having a high index of suspicion. Suspicion should trigger immediate notification to the local or state health department. The state reference laboratory can arrange for rapid diagnostic tests.

Bacterial staining of lymph node material by Giemsa, Wayson, or Wright stain often shows the typical bipolar plague organisms. In the septicemic form of the disease, similar bacterial staining of venous blood frequently permits visualization of the plague bacillus.[36] Fluorescent antibody staining of direct smears and tissues may provide a rapid, presumptive diagnosis of plague.[28] More recent rapid tests, such as enzyme-linked immunosorbent assay for F1 antigen[16] or the polymerase chain reaction for F1 antigen genes (*pla* and *caf1*), have shown promise in the laboratory or in outbreaks, but they are not widely available.[12]

TREATMENT

Therapeutic decisions cannot await culture results. All patients suspected of having plague should receive prompt antimicrobial therapy after appropriate blood and tissue samples have been obtained for cultures, fluorescent antibody staining, and serologic testing.

The sulfonamides and streptomycin proved effective when they were introduced first in the 1940s. Resistant strains to one or the other of these antibiotics soon appeared.[6,49] Despite the paucity of published trials in humans on antibiotic effectiveness, other than streptomycin and tetracycline, the Working Group on Civilian Biodefense Consensus Statement[28] recommends gentamicin as an effective alternative to streptomycin for patients needing parenteral antibiotics. Gentamicin has been used frequently in recent years and has shown comparable outcomes to streptomycin in one case series.[3,5] In vitro and in vivo studies in mice corroborate its effectiveness against *Y. pestis* infections.[53,59]

For acutely ill patients thought to have plague infection, streptomycin and gentamicin are the drugs of choice. If available, streptomycin is given intramuscularly, 20 to 30 mg/kg/day in two divided doses up to a maximum of 1 g every 12 hours.[38] Gentamicin is administered intravenously or intramuscularly, 7.5 mg/kg/day to children and 3 to 5 mg/kg/day to adults in three divided doses. Although single daily doses of gentamicin are commonly recommended today and may be effective in infections due to *Yersinia pestis*, they have not been studied to date. Antibiotic susceptibility testing should be done because *Y. pestis* plasmid-mediated, multiple-antibiotic resistance has been described.[23]

When plague meningitis develops, chloramphenicol, 50 to 100 mg/kg/day (after administration of an initial dose of 25 mg/kg) intravenously in four divided doses (the maximal dose for chloramphenicol is 4.0 g/day), is the treatment of choice. Duration of therapy is determined by the length and severity of the illness. Treatment is continued for at least 7 days in patients with uncomplicated disease.[13]

Patients older than 8 years of age who do not require hospitalization receive tetracycline at a dose of 25 to 50 mg/kg/day every 4 to 6 hours up to a total daily dose of 1 g in children and 2 g in adults. When outpatient treatment is given, the patient should be observed closely for the first 3 days to ensure resolution of the disease.[13] Sulfonamides may be used for prophylaxis in pediatric patients as an alternative to the tetracycline class of antibiotics.[28] Doxycycline at a dose of 4 mg/kg/day (up to a maximum of 200 mg) divided into two doses may be substituted for tetracycline.

PROGNOSIS

In outbreaks of untreated plague, the mortality rate has ranged from 40 to 70 percent. Pneumonic plague almost invariably is fatal without treatment. With prompt specific antimicrobial therapy, the overall mortality rate for plague has decreased to 5 percent.[35] Complications during convalescence include polyarthritis, small lung abscesses, delayed suppuration of buboes,[33] and meningitis. *Staphylococcus aureus* and *Pseudomonas* spp. may superinfect involved lymph nodes.[48] Immunity usually ensues after clinical or asymptomatic infection occurs, but natural reinfection rarely has been observed.[48]

PREVENTION AND CONTROL

Institution of hygienic measures and eradication of rats from areas of human habitations have all but eliminated epidemics of urban plague. When epizootics occur in wild rodents, control measures must be directed against rodents and fleas. Vector control can be achieved by the use of insecticides in fields and housing areas. In plague-endemic areas, the public must be instructed to avoid burrows, not to handle sick or dead rodents, to de-flea household pets, and to eliminate trash near living areas.[10]

The immune status of domestic animals can be used as a surveillance tool to ascertain the presence of *Y. pestis* in the community. Dogs, cats, and swine develop antibodies to the fraction 1 antigen of *Y. pestis*.[29,41]

Patients with plague should be isolated with respiratory precautions until they are bacteriologically sterile. Contacts of patients with pneumonic plague should receive chemoprophylaxis with tetracycline at 25 to 50 mg/kg/day up to 2 g in adults and up to 1 g in children 8 years and older. Younger children receive trimethoprim-sulfamethoxazole at 40 mg/kg/day (sulfamethoxazole) in two equal doses orally.[29] The 6-day quarantine period for international travel for contacts of patients with plague does not guarantee the clearance of the bacillus from asymptomatic pharyngeal carriers.[6] Public and professional education in endemic zones is paramount for ensuring prompt reporting of human and animal cases.[52]

Plague vaccines had been used since the late 19th century for individuals at high risk for occupational exposure. They are no longer being manufactured in the United States. Research in this area is continuing.[28,58]

NEW REFERENCES SINCE THE SIXTH EDITION

5. Boulanger LL, Ettestad P, Fogarty DT, et al. Gentamicin and tetracyclines for the treatment of human plague: review of 75 cases in New Mexico 1985-1999. *Clin Infect Dis* 2004;**38**:663–9.
19. Cot S, Bertherat E. Bubonic plague: the old scourge is alive and well. Inter-regional Conference on Prevention and Control of Bubonic Plague, April 7-11, Antananarivo, Madagascar. *Med Trop (Mars)* 2007;**67**:117–18. In French.
20. Craven BM, Maupin GO, Beard TJ, et al. Reported cases of human plague infections in the United States 1970-1991. *J Med Entomol* 1993;**30**:758–61.
49. Perry RD, Fetherson JD. *Yersinia pestis*—etiologic agent of plague. *Clin Microbiol Rev* 1997;**10**:35–66.
52. Prevention of plague: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1996;**45**(RR-14):1–5.
57. Schriefer MR, Petersen JM. *Yersinia*. In: Versalovic J, Carrol KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press; 2011. p. 627–38.
58. Smiley ST. Current challenges in the development of vaccines for pneumonic plague. *Expert Rev Vaccines* 2008;**7**:209–21.
61. Stenseth NC, Atahabar BB, Begon M, et al. Plague: past, present and future. *PLoS Med* 2008;**5**:e3.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 113

Other *Yersinia* Species

Charles R. Woods, Jr.

Yersinia spp. are gram-negative, coccobacillary organisms that are primarily zoonotic. The genus is a member of the family Enterobacteriaceae and consists of 11 species, 3 of which clearly are human pathogens[74]: *Yersinia pestis*, *Yersinia pseudotuberculosis* (both formerly included in the genus *Pasteurella*), and *Yersinia enterocolitica*. *Y. pestis*, the causative agent of plague, is found in rodents and insect vectors and is discussed in Chapter 112.

Y. enterocolitica and *Y. pseudotuberculosis* are responsible for a variety of syndromes, some of which originally were called *pseudotuberculosis*. Infections caused by these primarily enteropathogenic *Yersinia* spp. now are collectively referred to as *yersiniosis*, which is the focus of this chapter. Molecular phylogenetic analysis suggests that *Y. pestis* evolved from *Y. pseudotuberculosis* 1500 to 20,000 years ago.[4] The genomes of representative strains of the three pathogenic *Yersinia* spp. have been sequenced.[213]

During the past 3 decades, *Y. enterocolitica* has been recognized as an important human pathogen worldwide and is a common cause of gastroenteritis in some pediatric populations of the industrialized world.[1,50,61,127] *Y. enterocolitica* also has drawn attention because of its immunologic or postinfectious manifestations, which include reactive arthritis and erythema nodosum.[50] *Y. pseudotuberculosis*, although widespread, is a much less common cause of human disease.[74] In Japan, it has been associated with clinical illness that sometimes has resembled Kawasaki disease.

HISTORICAL ASPECTS

In 1883, Malassez and Vignal[140] described a bacterium that produced a disease they named *pseudotuberculosis*. When injected into guinea pigs, the organism produced tuberculosis-like lesions. It grew at 4° C (39.2° F) and multiplied better at 22° C (71.6° F) than at 37° C (98.6° F). This observation was confirmed in 1910 by Albrecht,[11]

REFERENCES

1. Achtmann M, Zurth K, Morelli G, et al. *Yersinia pestis*, the cause of plague, is a recent emerged clone of *Yersinia pseudotuberculosis*. *Proc Natl Acad Sci U S A* 1999;**96**:14043–8.
2. Albizo JM, Surgalla MJ. Isolation and characterization of *Pasteurella pestis* endotoxin. *Infect Immun* 1970;**2**:229–36.
3. Becker TM, Poland JD, Quan TJ, et al. Plague meningitis: a retrospective analysis of cases reported in the United States, 1970-1979. *West J Med* 1987;**147**:554–7.
4. Bonacorsi SP, Scavizzi A, Guiyoule JH, et al. Assessment of a fluoroquinolone, three beta-lactams, two aminoglycosides, and cycline in the treatment of murine *Yersinia pestis* infection. *Antimicrob Agents Chemother* 1994;**38**:481–6.
5. Boulanger LL, Ettestad P, Fogarty DT, et al. Gentamicin and tetracyclines for the treatment of human plague: review of 75 cases in New Mexico 1985-1999. *Clin Infect Dis* 2004;**38**:663–9.
6. Butler T. A clinical study of bubonic plague. *Am J Med* 1972;**53**: 268–76.
7. Cantey JR. Plague in Vietnam. *Arch Intern Med* 1974;**133**:280–3.
8. Cavanaugh DC. Specific effect of temperature upon transmission of the plague bacillus by the Oriental rat flea, *Xenopsylla cheopis*. *Am J Trop Med Hyg* 1971;**20**:264–73.
9. Centers for Disease Control and Prevention. Plague: United States, 1976. *MMWR Morb Mortal Wkly Rep* 1977;**28**:159.
10. Centers for Disease Control and Prevention. Plague vaccine. *MMWR Morb Mortal Wkly Rep* 1982;**31**:301–3.
11. Centers for Disease Control and Prevention. Plague in the United States, 1982. *MMWR Morb Mortal Wkly Rep* 1983;**32**:19SS–24SS.
12. Centers for Disease Control and Prevention. Plague: human plague in the United States, 1983. *MMWR Morb Mortal Wkly Rep* 1983;**32**: 329–30.
13. Centers for Disease Control and Prevention. Plague: winter plague—Colorado, Washington, Texas, 1983-1984. *MMWR Morb Mortal Wkly Rep* 1984;**33**:145–8.
14. Centers for Disease Control and Prevention. *Plague treatment guidelines*. Fort Collins, CO: Bacteriologic Zoonoses Branch, Division of Vector-Borne Infectious Diseases. Available at www.cdc.gov/plague/healthcare/clinicians.html, 2013.
15. Centers for Disease Control and Prevention. Human plague: United States, 1993-1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**:242–6.
16. Centers for Disease Control and Prevention. Human plague—four states 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:940–3.
17. Chanteau S, Rahalison L, Ratsitorahina M, et al. Early diagnosis of bubonic plague using F1 antigen capture ELISA assay and rapid immunogold dipstick. *Int J Med Microbiol* 2000;**290**:279–83.
18. Chen TH, Elberg SS. *Yersinia*, *Pasteurella*, and *Francisella*. In: Braude AI, Davis CE, Fierer J, editors. *Medical microbiology and infectious diseases*. Philadelphia: Saunders; 1981. p. 393–9.
19. Cot S, Bertherat E. Bubonic plague: the old scourge is alive and well. Inter-regional Conference on Prevention and Control of Bubonic Plague, April 7-11, Antananarivo, Madagascar. *Med Trop (Mars)* 2007;**67**:117–18. In French.
20. Craven BM, Maupin GO, Beard TJ, et al. Reported cases of human plague infections in the United States 1970-1991. *J Med Entomol* 1993;**30**:758–61.
21. Crook D, Tempest B. Plague: a review of 27 cases. *Arch Intern Med* 1992;**152**:1253–6.
22. Dennis DT, Hughes JM. Multidrug resistance in plague. *N Engl J Med* 1997;**337**:702–4.
23. Eidson M, Tierney L, Rollag OJ, et al. Feline plague in New Mexico: risk factors and transmission to humans. *Am J Public Health* 1988;**78**:1333–5.
24. Finegold MJ. Pathogenesis of plague. *Am J Med* 1968;**45**:549–55.
25. Gage KL, Dennis DT, Orloski KA, et al. Cases of cat-associated human plague in the western US, 1977-1998. *Clin Infect Dis* 2000; **30**:893–900.
26. Galimand M, Guiyoule A, Berbaud G, et al. Multidrug resistance in *Yersinia pestis* mediated by transferable plasmid. *N Engl J Med* 1997;**337**:677–80.
27. Ganem DE. Plasmids and pestilence: biological and clinical aspects of bubonic plague. *West J Med* 1986;**144**:447–51.
28. Goldenberg MI, Kartman L. Role of soil in the ecology of *Pasteurella pestis*. *Bacteriol Proc* 1966;**66**:54–7.
29. Historical notes—plague. *Can Med Assoc J* 1978;**119**:10.
30. Hull HF, Montes JM, Mann JM. Plague masquerading as gastrointestinal illness. *West J Med* 1986;**145**:485–7.
31. Hull HF, Montes JM, Mann JM. Septicemic plague in New Mexico. *J Infect Dis* 1987;**155**:113–18.
32. Humphrey M, McGiuney R, Perkins C, et al. *Yersinia pestis*: a case of mistaken identity. *Pediatr Infect Dis J* 1988;**7**:365–6.
33. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon. *JAMA* 2000;**283**:2281–90.
34. Janssen WA, Surgalla MJ. Plague bacillus: survival within host phagocytes. *Science* 1969;**163**:950–2.
35. Kartman L, Goldenberg MI, Hubbert WT. Recent observations on the epidemiology of plague in the United States. *Am J Public Health* 1966;**56**:1554–69.
36. Kaufmann AF, Boyce JM, Martone WJ. Trends in human plague in the United States. *J Infect Dis* 1980;**141**:522–4.
37. Legters LJ, Cottingham AJ, Hunter DH. Clinical and epidemiologic notes on a defined outbreak of plague in Vietnam. *Am J Trop Med Hyg* 1970;**19**:639–52.
38. Leopold JC. Septicemic plague in a 14-month old. *Pediatr Infect Dis J* 1986;**5**:108–10.
39. Maegraith BG. Plague. In: Adams AR, Maegraith BG, editors. *Clinical tropical diseases*. Oxford: Blackwell Scientific; 1970. p. 325–36.
40. Mann JM, Hull HF, Schmid GP, et al. Plague and the peripheral smear. *JAMA* 1984;**251**:953.
41. Mann JM, Martone WJ, Boyce JM, et al. Endemic human plague in New Mexico: risk factors associated with infection. *J Infect Dis* 1979;**140**:397–401.
42. Mann JM, Schaudler L, Cushing A. Pediatric plague. *Pediatrics* 1982;**69**:762–7.
43. Mann JM, Schmid GP, Stoesz PA, et al. Peripatetic plague. *JAMA* 1982;**247**:47–8.
44. Marshall JD, Guy DV, Gibson FL. Asymptomatic pharyngeal plague infection in Vietnam. *Am J Trop Med Hyg* 1967;**16**:175–7.
45. Marshall JD, Harrison DN, Murr JA, et al. The role of domestic animals in the epidemiology of plague, part III: Experimental infection in swine. *J Infect Dis* 1972;**125**:556–9.
46. Martin AR, Hurtado FP, Plessak RA, et al. Plague meningitis. *Pediatrics* 1967;**40**:610–16.
47. McNeill WH, editor. *Plagues and people*. Garden City, NY: Anchor Press of Doubleday; 1976.
48. Mollaret HH, Thal E. *Yersinia*. In: Buchanan RE, Gibbons NE, editors. *Bergey's manual of determinative bacteriology*. 8th ed. Baltimore: Williams & Wilkins; 1974. p. 330–2.
49. Perry RD, Fetherson JD. *Yersinia pestis*—etiologic agent of plague. *Clin Microbiol Rev* 1997;**10**:35–66.
50. Peters CJ, Simpson GL, Levy H. Spectrum of hantavirus infection. *Annu Rev Med* 1999;**50**:531–45.
51. Pollitzer R. *Plague*. WHO Monogr. No. 22. Geneva: World Health Organization; 1954.
52. Prevention of plague: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1996; **45**(RR-14):1–5.
53. Reed WP, Palmer DL, Williams RC, et al. Bubonic plague in the southwestern United States. *Medicine (Baltimore)* 1970;**49**:465–86.
54. Reiley CG, Kates ED. The clinical spectrum of plague in Vietnam. *Arch Intern Med* 1970;**126**:990–4.
55. Rust JH, Jr., Cavanaugh DC, Kadis S, et al. Plague toxin: its effect in vitro and in vivo. *Science* 1963;**142**:408–9.
56. Rust JH, Cavanaugh DC, O'Shita R, et al. The role of domestic animals in the epidemiology of plague, part I: Experimental infection of dogs and cats. *J Infect Dis* 1971;**124**:522–6.
57. Schriefer MR, Petersen JM. *Yersinia*. In: Versalovic J, Carrol KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press; 2011. p. 627–38.
58. Smiley ST. Current challenges in the development of vaccines for pneumonic plague. *Expert Rev Vaccines* 2008;**7**:209–21.
59. Smith MD, Vinh DX, Nguyen TT, et al. In vitro antimicrobial susceptibilities of strains of *Yersinia pestis*. *Antimicrob Agents Chemother* 1995;**39**:2153–4.
60. Sonnenwirth AC. *Yersinia*. In: Lennette EH, Spaulding EH, Trauant JP, editors. *Manual of clinical microbiology*. 2nd ed. Washington DC: American Society for Microbiology; 1974. p. 222–9.
61. Stenseth NC, Atahabar BB, Begon M, et al. Plague: past, present and future. *PLoS Med* 2008;**5**:e3.
62. Walker RV, Bomes MG, Thiggins ED. Composition of and physiopathology produced by plague endotoxins. *Nature* 1966;**209**:1246.
63. World Health Organization. Human plague in 1998 and 1999. *Wkly Epidemiol Rec* 2000;**75**:338–44.

who labeled the disease *enteritis follicularis suppurativa*. The first case of mesenteric adenitis, the most common syndrome produced by *Y. pseudotuberculosis*, was reported in 1913 by Saisawa.[187] A second instance of disease that indicated its capacity to produce death (i.e., bacteremia and multiple hepatic abscesses) was recorded in 1949 by Hassig and colleagues.[86] Masshoff[142] was the first to recover the organism from a culture of mesenteric lymph nodes of a patient with the clinical picture of acute appendicitis. Masshoff and Dolle[143] subsequently described the histologic picture produced by *Y. pseudotuberculosis*. In 1954, Knapp and Masshoff[115] first reported the clinical features of infection produced by this organism. Only 14 cases of this infection had been described up to that time.[60]

The existence of a species of *Yersinia* other than that causing pseudotuberculosis was suggested in 1933 by Gilbert,[67] who reported an unusual infection in animals. Schleifstein and Coleman[188,189] examined numerous organisms isolated between 1933 and 1957 from stool cultures of human cases of diarrhea from which *Salmonella* and *Shigella* organisms could not be recovered and that resembled the infections in animals reported by Gilbert. These investigators identified an organism that had not been described previously and named it *Bacterium enterocoliticum*. In 1964, it was named *Y. enterocolitica* by Frederiksen.[64]

The genus is named for A. J. Yersin, the French bacteriologist who first isolated the plague bacillus.[203] During the past 40 years, an extensive literature detailing the microbiology, pathology, epidemiology, molecular pathogenesis, and clinical features of disease caused by *Y. pseudotuberculosis* and *Y. enterocolitica* has accumulated.*

MICROBIOLOGY

Yersinia organisms are large (0.5 to 1 μm × 1 to 2 μm or larger), gram-negative, and ovoid or rod-shaped. *Y. enterocolitica* and *Y. pseudotuberculosis* are motile at 22° to 25°C (71.6° to 77°F) but not at 37°C (98.6°F). Similar to other members of Enterobacteriaceae, *Yersinia* organisms are facultative anaerobes and grow well on ordinary media. On Gram staining, *Y. pseudotuberculosis* appears as a large coccobacillus. Staining with methylene blue and carbol fuchsin discloses a bipolar (safety pin) morphology of most, but not all, strains. *Y. enterocolitica* is smaller and shows little, if any, bipolarity.[200,203]

Yersinia organisms may be confused with coliforms, such as *Morganella*, *Proteus*, *Shigella*, *Salmonella*, and *Providencia* spp., or *Escherichia coli* or with *Y. pestis* or *Brucella*, and *Achromobacter* organisms, unless careful biochemical and physiologic studies are conducted. *Yersinia* organisms reduce nitrates and are oxidase-negative, catalase-positive, urease-positive, and citrate-negative. They ferment glucose, maltose, mannitol, glycerol, xylose, and fructose, producing acid but no gas with each sugar. *Yersinia* organisms usually do not ferment lactose but produce α-galactosidase. They do not ferment dulcitol, inositol, raffinose, or rhamnose. On lysine-iron agar slants, *Yersinia* organisms produce an alkaline slant with an acid butt. They do not produce hydrogen sulfide. The Voges-Proskauer reaction is negative at 37°C (98.6°F) but may be positive at 25°C (77°F) for some strains of *Y. enterocolitica*. All strains of *Y. pseudotuberculosis* and most strains of *Y. enterocolitica* isolated in Europe are indole-negative. Most strains of *Y. enterocolitica* found in the United States have been indole-positive.*

Although these two species of *Yersinia* share many properties, they are distinguishable on the basis of several biochemical activities, antigenic structure, and sensitivity to various *Yersinia* phages.[184] *Y. enterocolitica* produces an acid slant and acid butt on triple sugar iron agar caused by fermentation of sucrose, whereas *Y. pseudotuberculosis* produces an alkaline slant and an acid butt. *Y. enterocolitica* elaborates ornithine decarboxylase and ferments sucrose and amygdalin. *Y. pseudotuberculosis* does none of these, but it ferments adonitol, which *Y. enterocolitica* does not.[95,203]

Commercially available tests used to identify Enterobacteriaceae in clinical laboratories may not contain the biochemical reactions needed to identify specific *Yersinia* spp. Traditional macroscale biochemical testing may be required to distinguish *Y. enterocolitica* and *Y. pseudotuberculosis* from nonpathogenic *Yersinia* spp.[80,131] On solid culture media, colonies of the pathogenic species typically are small, smooth, opaque colonies. The colonies of nonpathogenic species are larger and more translucent.[109] The emerging bacterial identification method, matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry, appears reliable for distinguishing *Yersinia* isolates at the species level.[205]

Genomes and Typing of *Yersinia* Strains

Biotyping and serotyping have been the predominant methods used to characterize strains of *Y. enterocolitica*. At least 54 to 60 serotypes of *Y. enterocolitica* exist on the basis of variability of somatic O antigens, but only 11 typically are associated with human disease.[24,26,61,74,216] Six biotypes of *Y. enterocolitica*, designated 1A, 1B, and 2 to 5, have been defined on the basis of differences in the following: lipase activity; salicin acid production; esculin hydrolysis; indole production; ornithine decarboxylase; Voges-Proskauer test; pyrazinamide activity; nitrate reduction; and fermentations of xylose, trehalose, sorbose, and inositol.[26,36,215]

Biotypes 1A, 2, 3, 4, and 5, now designated as *Y. enterocolitica* subsp. *palearctica*, are considered generally low pathogenic.[194] Biotype 1B, now designated as *Y. enterocolitica* subsp. *enterocolitica*, is considered highly pathogenic. Further subspecies distinctions may be forthcoming.[195] The genomes of biotypes 1A, 1B, and 2 to 5 are about 4.9, 4.6, and 4.5 Mb, respectively.[177] Genomic size differences are primarily related to number and distribution of insertion sequences.

Only a few serotype:biotype combinations are regarded as human pathogens: O:8, O:4, O:13a,13b,

*References 14, 24, 26, 28, 48, 50, 58, 60, 88, 89, 94, 100, 113-116, 127, 135, 147, 183, 200, 202, 207, 219, 232.

*References 53, 71, 95, 157, 164, 200, 203.

O:18, O:20, and O:21 (biotype 1B); O:9 and O:5,27 (biotype 2); O:1,2,3 and O:5,27 (biotype 3); O:3 (biotype 4); and O:2,3 (biotype 5).[221] These pathogenic strains, which carry *Yersinia* virulence plasmids (pYV), generally have negative test results for pyrazinamidase activity, esculin hydrolysis, and salicin fermentation. Nonpathogenic strains, generally of biotype 1A, have positive test results for each of them.[44,51,107] Data suggest that some biotype 1A strains that lack pYV and other *Yersinia* virulence factors also may cause gastroenteritis.[73,194,196]

Strains of *Y. enterocolitica* can be typed genetically by multilocus sequence typing (MLST),[195] repetitive element-based (interrepeat) polymerase chain reaction (PCR), pulsed-field gel electrophoresis,[152] and ribotyping.[132] These methods allow distinction among strains within biotypes and serotypes and may be useful for outbreak and other epidemiologic investigations.

The complete genomes of the "Old World" biotype 3/O:9 strain 105.5R(r), prototypical of isolates common in Europe and Japan, and "New World" biotype 1B/O:8 strain 8081, prototypical of isolates in China, have been compared. The type III secretion systems differ, and strain 8081 has lost ancestral clusters of genes that contribute to enteric survival and pathogenesis.[226] Other genomic studies have shown that low pathogenic *Y. enterocolitica* subsp. *palearctica* biotype 4/O:3, which causes sporadic human disease and commonly colonizes pigs worldwide, also possess a type III secretion system and other alternative virulence determinants in contrast to the high pathogenic *Y. enterocolitica* subsp. *enterocolitica* (biotype 2) strains associated with outbreaks of human disease in Northern Europe.[17]

At least 11 antigenic groups of *Y. pseudotuberculosis* exist on the basis of variation of somatic O antigens. They have been labeled 1a, 1b, 2a, 2b, 2c, 3, 4a, 4b, 5a, 5b, and 6.[77,99,139] Type 2 is related antigenically to *Salmonella* group B, and type 4 is related to *Salmonella* groups D and H.[71] Two clusters of *Y. pseudotuberculosis* have been described using MLST. Strains of one cluster are distributed worldwide and usually harbor pYV. The second cluster lacks pYV and is currently restricted to the Far East.[43]

EPIDEMIOLOGY

Although most of the early reports of yersiniosis caused by *Y. pseudotuberculosis* and *Y. enterocolitica* came from northern Europe, these microbes have been identified with increasing frequency in all parts of the world,[13] with the possible exception of South America. *Y. enterocolitica* infections are more common in the United States than *Y. pseudotuberculosis*. Between 1996 and 2007, 1355 cases of foodborne *Y. enterocolitica* infections were identified by FoodNet in contrast to only 18 cases of foodborne *Y. pseudotuberculosis*.[134] The median ages of infected persons were 6 years (0 to 94 years) and 47 years (16 to 86 years), respectively.

During 1996 to 2009, FoodNet data show the rate of foodborne disease caused by *Y. enterocolitica* in the United States was stable at 0.5/100,000 population.[160] Rates have been highest in blacks but declined from 3.9 to 0.4/100,000 during this time period. The rate in Black children less than five years old declined from 41.5 to 3.9 per 100,000.

Yersinia enterocolitica

Y. enterocolitica is distributed worldwide but is isolated most frequently in cooler climates.[148] Whether such geographic differences reflect differences in reservoirs or culinary practices that may enhance the risk for acquisition of this organism, or rather represent differences in surveillance for the disease and use of more sensitive culturing techniques in these areas, is unclear.[50] Increased frequency of infections during fall and winter has been reported from Europe[192] and the United States,[1] but no seasonality is evident among outbreaks of disease in which more than three cases of *Y. enterocolitica* disease have been identified.[50]

Geographic differences in serotype distribution and frequency also exist. Sporadic infections caused by biotype 4/serotype O:3 and biotype 3/serotype O:9 strains are common in Europe,[9,93] but outbreaks rarely have occurred.[50] In North America, multiple serotypes have been responsible for sporadic disease,* but more recently serotype O:3 has become predominant.[36,54,127] Five outbreaks in the United States have been caused by serotype O:8, and two outbreaks in Canada have been caused by serotypes O:5 and O:5,27.[50] Disease caused by serotype O:8 has been reported in Europe.[93] Biotype 2/serotype O:9 is predominant in China.[226]

The true incidence and prevalence of *Y. enterocolitica* infection are unknown.[50] The reported proportional frequency of isolation of *Y. enterocolitica* from stool cultures from patients with diarrhea has ranged from 0 to 3.2 percent in series from Europe, the United States, and New Zealand (Table 113-1).† Symptomatic infection occurs more commonly in children. Most series show a slight male predominance of approximately 1.3 : 1.[54,61,141,215] Surveillance at five FoodNet sites in the United States during 1996 to 1999 found that African-American infants had far higher rates than white infants, with a peak occurrence from November to February (Fig. 113-1).[174]

Animals and water sources are the primary environmental reservoirs for *Y. enterocolitica*, but the biotypes and serotypes of the strains found in them usually differ from those causing human disease.[36,50,192] Blood transfusions also may be a source of *Y. enterocolitica* infection.[37]

Animal Reservoirs

Y. enterocolitica strains have been isolated from a wide variety of mammals (i.e., dogs, pigs, sheep, rabbits, guinea pigs, cows, horses, chinchillas, and monkeys), frogs, fish, flies, fleas, snails, crabs, and oysters. Birds do not seem to be a major reservoir for *Y. enterocolitica*, although avian isolates have been reported.[50,95,135] The proportion of enteric illness attributable to animal contact in the United States in recent years is 1 percent.[79]

*References 19, 25, 36, 70, 192, 198.
†References 52, 61, 93, 127, 141, 146, 192.

TABLE 113-1 **Percentage of Stool Cultures Yielding *Yersinia Enterocolitica***

Country	Years	Population	Total Cultures	Percentage *Y. Enterocolitica*
Canada[141]	1977-1978	Symptomatic children	6364	2.8
The Netherlands[92]	1982-1984	Enteritis patients <40 yr	827	2.9
Italy[146]	1981-1985	Children with diarrhea	2500	1.4*
New Zealand[61]	1988-1993	Patients with gastroenteritis	231,128	0.6
U.S. (Detroit, MI)[52]	May-November 1977	Children with diarrhea	1262	0
U.S. (New York State)[192]	1976-1980	Survey of cultures from a state laboratory, six hospitals, and several daycare centers	2487	0.9†
U.S.‡ (7 cities)[127]	November 1989-January 1990	All stool cultures submitted to seven hospitals	4841	0.8
U.S. (St. Louis, MO)[112]	1998-2001	Children presenting to an emergency department with diarrhea	1626	0.1

*Yearly percentages during the 5 years ranged from 0 to 4.4 percent.
†This increased to 4 percent of 3035 isolates when cultures from an outbreak and other screenings were included.
‡Includes Detroit, MI.

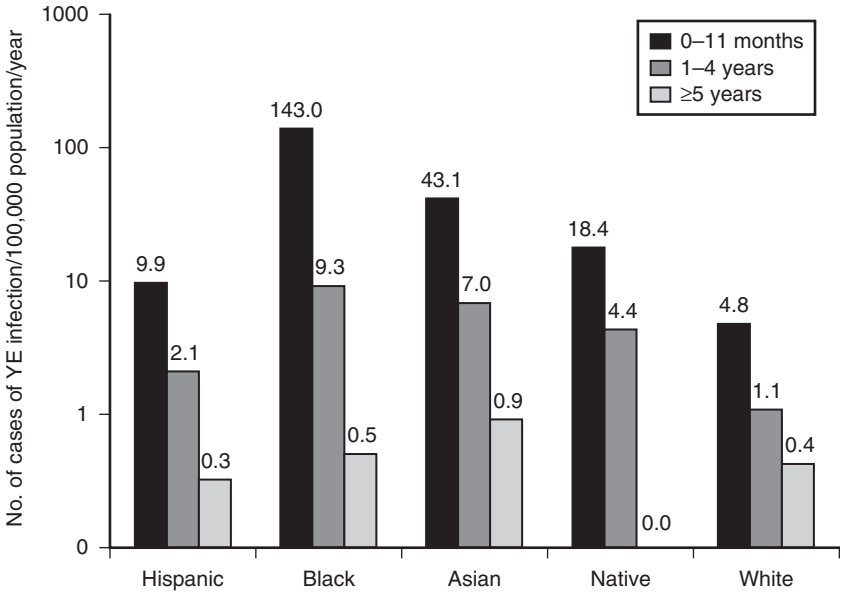

FIGURE 113-1 ■ Adjusted incidence of *Yersinia enterocolitica (YE)* infection, by race and age. Average incidences are given over the 4-year surveillance period (1996 to 1999) by age group and adjusted race/ethnicity. (From Ray SM, Ahuja SD, Blake PA, et al. Population-based surveillance for *Yersinia enterocolitica* infections in FoodNet sites, 1996-1999: higher risk for disease in infants and minority populations. *Clin Infect Dis* 2004;**38**:S181-9.)

Pigs seem to be an important reservoir for the human pathogenic serotypes O:3 and O:9 in Europe and Japan and serotype O:3 in North America and South Africa.[50,127,170] The biochemical and phage typing profiles of isolates from pigs are similar to those of strains commonly responsible for human infections.[219] *Y. enterocolitica* has been isolated from the tongue, tonsils, and cecal contents of swine and from pork, ham, and butcher shop cutting boards.[50,71] Pig farmers in Finland were 3 times and 2.4 times as likely to have seropositivity to serotypes O:3 and O:9 than were berry farmers.[191]

Wild rodents captured in areas of Japan where human infections caused by *Y. enterocolitica* serotype O:8 had occurred were shown to harbor isolates of the same serotype.[46] Two distinct serotype O:8 strains, defined by restriction enzyme analysis of the virulence plasmids, were isolated from humans and rodents. This finding suggests that rodents are a potential source of sporadic human infection in Japan.

Apparent transmission to humans from dogs and cats also has been reported. A fecal-oral or oral-oral route has been postulated but not confirmed. Little evidence supports airborne or insect vector–borne transmission.[50]

Foods and Water

In countries with high numbers of cases of yersiniosis, ingestion of raw pork has been common. Infection with serotypes O:3 and O:9 was highly associated with ingestion of raw pork during the 2 weeks preceding the illness in a case-control study.[211] In Belgium, laboratory surveillance that began in 1967 showed yearly increases in cases through 1986. After a media campaign was launched to dissuade people from eating raw or undercooked pork or pork products and to educate consumers regarding good hygiene practices during food preparation, the number of isolations of *Y. enterocolitica* decreased from a high of 1469 in 1986 to 707 in 1996.[221] Still, eating raw minced

pork or other foods prepared from raw pork products remains an ongoing risk factor for *Y. enterocolitica* infection for young children in several European countries.[23,97,182] Changes in techniques for slaughtering may have contributed to this decline. Preparation of chitterlings in the household was a risk factor for *Y. enterocolitica* infection among children in Michigan[1] and Illinois.[38]

Ingestion of water contaminated with serotype O:8 has led to sporadic cases and outbreaks.[50] Water and malnutrition have been associated with clinical illness in Gaza.[57] Bean sprouts that had been immersed in contaminated water were the source of an outbreak in Pennsylvania in 1982. Ingestion of tofu (bean curd) packed in untreated spring water that subsequently was found to be contaminated with *Y. enterocolitica* caused 44 cases of symptomatic infection in Washington state in 1981 and 1982.[209] Serotypes commonly found in water samples rarely are isolated from humans with symptomatic disease, however.[36]

Contaminated milk has been implicated as the source of several large outbreaks of *Y. enterocolitica* infection.[20,50,216] Whipped cream and ice cream may harbor the organism. Contamination of milk products after pasteurization has been documented. *Y. enterocolitica* has been found in raw milk samples from cows and goats. Samples of beef, lamb, poultry, oysters, and a variety of vegetables also have been found to be contaminated with *Y. enterocolitica*.[33,50,71]

Serotypes O:3, O:4,33, O:5,27, O:7,8, O:8, O:10, O:13, and O:16 cause most human disease in North America but rarely are isolated in surveillance of water or food samples. Serotype O:8 strains have been cultured from cattle, milk, and water samples[157]; serotype O:4,33 strains have been isolated from pigs and cattle; and serotype O:4,32 strains have been found in cheese, ham, sausage, raw beef, and one pancake specimen.[36,192,216]

Incubation, Carriage, and Transmission in Humans

The incubation period of *Y. enterocolitica* enterocolitis ranges from 1 to 14 days, with a median of approximately 4 days.[36,50,127] The minimal infective dose of *Y. enterocolitica* is unknown. Ingestion of 3.5×10^9 organisms by a volunteer resulted in diarrhea in less than 1 day, but such large inocula are unlikely to be encountered clinically. The duration of excretion of the organism after development of infection in children ranges from 14 to 97 days (mean 42 days).[141] The impact, if any, of antibiotic treatment on the duration of carriage is unknown.

Transmission to household members occurs uncommonly, even among young children, who are at higher risk for development of symptomatic disease.[20,141,209] Six percent of household contacts developed disease in one outbreak,[209] but several large outbreaks with no secondary household cases have been reported.[50]

Yersinia enterocolitica *and Blood Transfusion–Related Sepsis*

In this era of screening for viral infections in blood donations, bacterial sepsis has become the most frequent infectious complication of blood transfusions in developed countries. Sporadic cases of *Y. enterocolitica* sepsis related to contamination of transfusions of red blood cells (RBCs) have been recognized since 1987 and have occurred in the United States, Europe, and Australia.[37,75] *Y. enterocolitica* is the most common cause of transfusion-related sepsis.[105] Rapid-onset septic shock, fever, chills, and atypical signs such as explosive diarrhea are common. Case fatality is about 50 percent.[45,75]

In many cases of transfusion-related sepsis, the contaminated RBC units had been stored for 25 days or more.[87] After experimental inoculation of small numbers of *Y. enterocolitica* into packed cells kept at 4°C (39.2°F), the organisms continue to replicate, reaching concentrations of 100 colony-forming units (CFU)/mL in 7 days and 10^6 CFU/mL in 21 days. This lag of 1 to 3 weeks in rapid growth is consistent with the increasing iron stores released from hemolyzing RBCs during storage. *Y. enterocolitica* multiplication is supported by glucose and adenine, which are part of RBC anticoagulant and additive solutions. Although growth is calcium-dependent during human infection at body temperatures, *Y. enterocolitica* can replicate in calcium-free media (e.g., RBCs anticoagulated via citrate chelation) at temperatures less than 30°C (86°F).[75] High levels of endotoxin can result from bacterial replication in RBC products and have been documented in samples from RBC units that led to transfusion-related sepsis.[45] Timely administration of effective antimicrobial agents appears to improve outcomes, suggesting a significant contribution from viable microbes.[75]

Human Outbreaks

Outbreaks of *Y. enterocolitica* disease have involved communities, families (with interfamily spread), hospitals, and schools. The sources of the organism have been various foods and animals, especially dogs. A review of these outbreaks suggests that infection may occur more commonly than has been recognized. Yersiniosis also resembles disease caused by *Salmonella* and *Shigella* organisms in many respects, including the environmental sources of the organisms, the clinical syndromes, and the occurrence of asymptomatic infection.

Outbreaks in Schools and Communities. A community outbreak caused by a serotype O:8 strain occurred in New York State in 1976.[19] At least 222 children and employees had a yersiniosis-like illness during a 10-week period. Illness was associated with drinking chocolate milk that was contaminated after pasteurization during hand mixing with chocolate syrup. Transmission of infection from ill children to household contacts was not observed. School-related outbreaks also have occurred in Japan.[15,234]

In the summer of 1982, thousands of individuals in several southern states who consumed milk from a single dairy developed yersiniosis. A total of 172 culture-positive infections were confirmed, many as a result of hospitalization for illness. Seventeen patients underwent appendectomy; 24 others had extraintestinal spread of infection. The strain involved was designated serotype O:13a,13b.[216] An outbreak associated with consumption of pasteurized

milk in Vermont and New Hampshire in 1995 probably resulted from contamination after pasteurization with a serotype O:8 strain, possibly from rinsing bottles with untreated well water.[5]

During November to December 2002, nine infants in the Chicago area developed gastroenteritis caused by *Y. enterocolitica*. Eight were African-American and were exposed to caretakers who had prepared chitterlings for holiday meals. All recovered, although six were hospitalized for a median of 5 days.[38]

Family Epidemics. In North Carolina, 21 individuals from four families were involved in an outbreak of yersiniosis that had an unusually high attack rate.[76] Of the 21 affected individuals, 18 were children 3 to 13 years of age; 16 of the 21 had diarrhea and fever, and 5 were asymptomatic. *Y. enterocolitica* was recovered from the spleen of the youngest child at autopsy. The diagnosis was established serologically in the others. A dog that had given birth to puppies that died of a diarrheal illness a week before the families became ill seemed to be the source of the infection.

Nosocomial Outbreaks. A young child who was hospitalized in Finland with acute gastroenteritis was the source case of infection in a housekeeping worker and four nurses who cared for her.[214] The infecting strain was serotype O:9. Another hospital outbreak involved nine patients in Canada.[173] It was caused by a serotype O:5 strain. Person-to-person contact was considered the likely mode of transmission.

Prevention of Disease

During outbreaks, efforts should be made to identify environmental sources and vehicles of transmission.[50] A single environmental source can harbor multiple serotypes of *Y. enterocolitica*, such that resulting outbreaks may be polyclonal.[209] Enteric precautions should be used for hospitalized patients with diarrhea caused by *Y. enterocolitica* (as with other causes of gastroenteritis).[50] At the population level, decreased consumption of raw or undercooked pork products potentially can reduce the incidence of infection.[211,221]

Yersinia pseudotuberculosis

Y. pseudotuberculosis may infect individuals of all ages, but at least 75 percent of patients with clinically apparent disease are children younger than 15 years of age.[99,184] Infection in young infants has been reported.[99,228] Of 130 cases diagnosed in Great Britain from 1959 to 1970, boys were involved three times more frequently than girls.[135] Infections occur more commonly during the cold months of the year.[99,185] The seasonal winter peak of human infection produced by *Y. pseudotuberculosis* is similar to that seen in wild and domesticated animals.[113,114,184]

The attack rates for children living in rural and urban areas seem to be the same. *Y. pseudotuberculosis* occasionally has been recovered from healthy individuals. Exposure to the organism seems to be uncommon; antibody to *Y. pseudotuberculosis* was detected in only 1/2000 sera samples from individuals with no history of yersiniosis.[64,184]

Y. pseudotuberculosis is distributed worldwide in a large variety of animals and birds, but infection seldom occurs.[68] Guinea pigs, rodents, and rabbits are infected most often[135] and may experience a plaguelike illness.[68,95] Lesions in guinea pigs may be confused easily with lesions caused by *Y. pestis*. Rats and other rodents also may have plaguelike disease caused by *Y. pseudotuberculosis*. The microbe can be cultured from tongue, tonsils, intestines, and large organs of infected animals.[156]

Infection has been reported in various domestic animals (i.e., cattle, sheep, goats, cats, dogs, hamsters), commercially raised fur bearers (chinchillas, mink, coypu), and other wild or captive animals (rabbits, raccoons, foxes, deer, beavers, monkeys, puma, kangaroos). *Y. pseudotuberculosis* has been found in more than 50 species of birds,[82,108,135] and epizootics have occurred among turkeys, ducks, pigeons, and doves and in aviaries of canaries and finches. Strains obtained from animals and birds in the United States are predominantly of serotypes 1a, 1b, and 3.[135,184]

The incubation period for human disease ranges from 41 hours to 20 days.[99] In a food-associated outbreak in Finland, the median incubation period was 8 days (Fig. 113-2).[102] The organism can survive in fresh tap water for 46 days at room temperature and for 8 months at 4° C (39.2° F). It can survive at 4° C in meat for 145 days and in milk and bread for 2 to 3 weeks.[135]

A family outbreak of mesenteric adenitis caused by *Y. pseudotuberculosis* that involved four siblings aged 7 to 14 years has been reported.[172] A pet dog was shown to have increasing antibody titers at the time the children were ill.

Periodic outbreaks have been reported in Japan, northern Europe, and areas of the former Soviet Union. Sandwiches prepared by a single bakery were the primary risk factor for 67 cases that occurred in a 3-week period in Japan.[99] Drinking unchlorinated well water or mountain stream water has been the source of other outbreaks. Children were much more likely to have clinical disease than adults. More recent outbreaks in Finland have been associated with contaminated lettuce and grated carrots.[102]

PATHOLOGY

The diseases produced by *Y. enterocolitica* and *Y. pseudotuberculosis* are similar and share the histopathologic theme of involvement of the lymphoid tissues of the intestinal mucosa and mesentery.

Yersinia enterocolitica

Y. enterocolitica infection predominantly affects the gastrointestinal tract. The most severe clinical symptoms correlate with an acute terminal ileitis. The mucosal surface of the ileum and other involved sites may be inflamed diffusely. Ulcerations may occur throughout the gastrointestinal tract and may be small and superficial or extend to the muscularis propria. Mucosal and submucosal

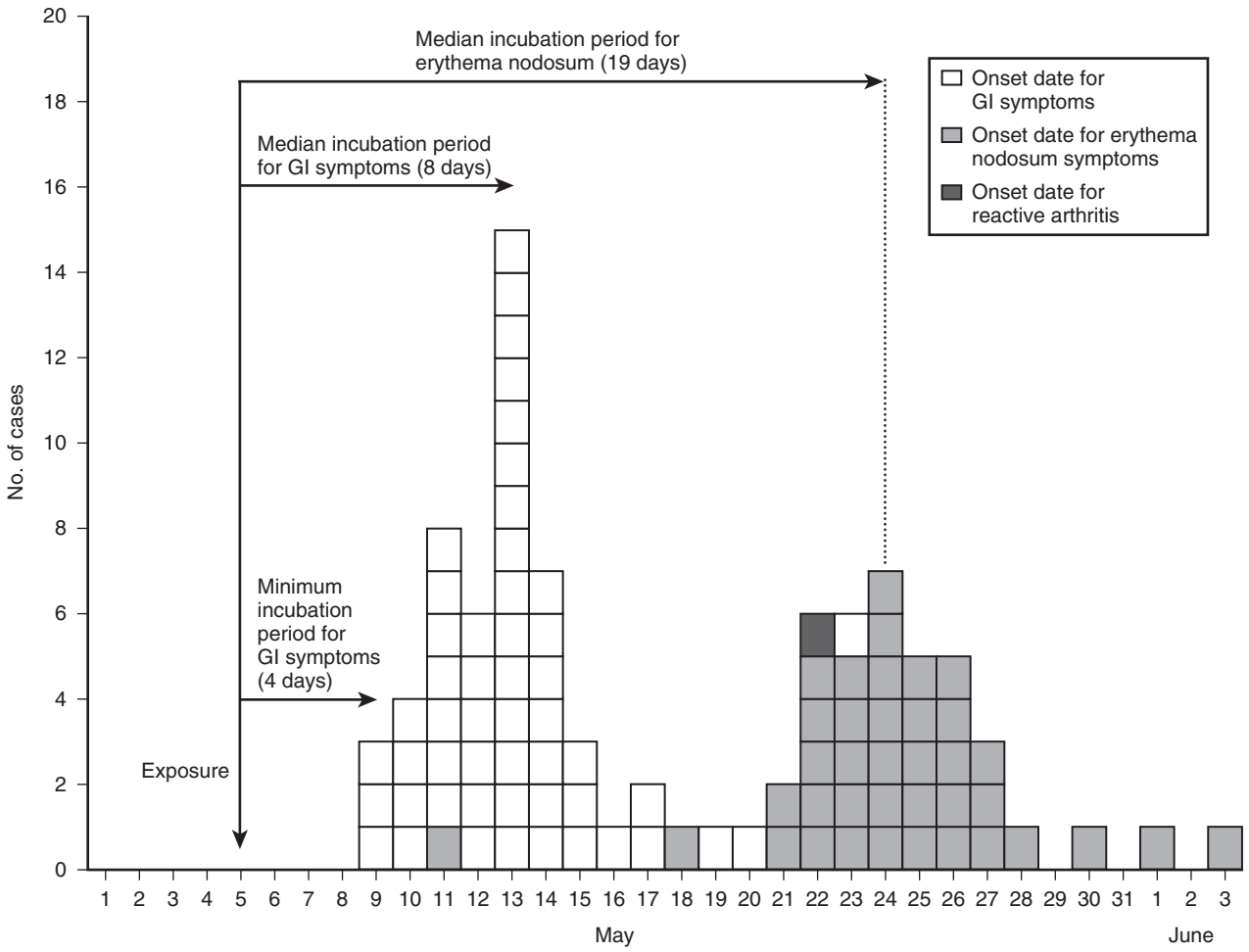

FIGURE 113-2 ■ Distribution of incubation periods for *Yersinia pseudotuberculosis* infection to onset of gastrointestinal (GI) symptoms and erythema nodosum after point source exposure to contaminated carrots. Of 72 case patients with a known date of onset, 51 had GI symptoms and 38 had erythema nodosum only; 28 had GI symptoms and erythema nodosum and appear twice in the graph. (From Jalava K, Hakkinen M, Valkonen M, et al. An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with *Yersinia pseudotuberculosis*. *J Infect Dis* 2006;**194**:1209-1216.)

hyperplasia of Peyer's patches occurs with scattered microabscess formation.

Ulcers occur primarily over the sites of lymphoid tissue within the mucosa, which accounts for their more longitudinal appearance in the small intestine and an oval or punctate appearance in the stomach and colon. Ulcerations are characterized by necrosis of the epithelial layer. In the colon, the necrosis also may extend through the superficial third of the crypts. Large colonic ulcerations covered by pseudo-membranes or mucoid debris are seen occasionally. Ulcerations may progress to perforation, with subsequent development of peritonitis or gastrointestinal hemorrhage in severe cases.[30,69,219,220]

The inflammatory response in the mucosa consists mainly of neutrophils and mononuclear cells. Lymphocytes and plasma cells also may be seen. Giant cells are not seen, although a granulomatous appearance can be imparted by the presence of plump epithelioid histiocytes. Numerous colonies of gram-negative bacteria often can be seen beneath the mucosal ulcerations and within the microabscesses that occur in the lymphoid tissues.[69,219,220]

The appendix usually appears normal on gross inspection, but small focal ulcerations frequently are present.[7] Large areas of necrosis are found occasionally, and acute, suppurative appendicitis has been reported.[30,104,141] Periappendicular inflammation may result from a true appendicitis or an adjacent terminal ileitis.[219]

Mesenteric adenitis, the hallmark of infection caused by *Y. pseudotuberculosis*, also is a common feature of enterocolitis caused by *Y. enterocolitica*. The lymph nodes usually show numerous large pyroninophilic cells and mitotic figures in the cortical area and marginal sinuses. Small collections of leukocytes in the germinal centers are seen in some cases and suggest formation of microabscesses. In severe cases, extensive areas of necrosis circumscribed by a neutrophilic infiltrate may be seen. The sinusoids can become filled with neutrophils and mononuclear cells. The germinal centers often appear reactive.

The histopathologic appearance of the mesenteric adenitis of infection caused by *Y. enterocolitica* can resemble the adenitides caused by cat scratch disease *(Bartonella henselae)*, toxoplasmosis, infectious mononucleosis, and *Y. pseudotuberculosis*.[7,219,231] The necrotizing epithelioid

granulomata that may be present in mesenteric adenitis caused by *Y. pseudotuberculosis* have not been described in infection caused by *Y. enterocolitica*. A more recent study of six cases suggests that biotype 1B strains may be more likely to cause suppurative inflammation, whereas biotype 2 through 5 strains may be more likely to cause granulomatous infection.[124]

Yersinia pseudotuberculosis

Numerous reports collectively have described the pathology of infection caused by *Y. pseudotuberculosis*.* Grossly enlarged, soft, and inflamed mesenteric lymph nodes are the predominant finding on laparotomy. They frequently are located at the ileocecal angle. Punctate hemorrhages and small, yellow microabscesses may be present on the surfaces of the nodes at the height of infection. The appendix usually appears normal, but the terminal ileum and cecum occasionally appear inflamed. A necrotic purulent mass sometimes is seen in the mesentery.

Histopathologic findings in the mesenteric lymph nodes include enlarged follicles and small abscesses; hyperplasia of reticulum cells; necrosis of the nodes with infiltrates of neutrophilic leukocytes and plasma cells; and, in some instances, punctate hemorrhages. Scattered clusters of neutrophils may be seen within the sinusoids and germinal centers without formation of abscess or necrosis. Atypical mononuclear cells, some of which may be mitotic, may be present in the sinusoids. When necrosis is absent, large numbers of eosinophilic leukocytes occasionally are seen surrounding reticulogranulocytic infiltrates in the nodes. Giant cells can occur and may cause the histologic picture to be confused with that of tuberculosis.

The mesenteric adenitis produced by *Y. pseudotuberculosis* seems to progress through histopathologic stages akin to those of other pyogenic infections that can lead to formation of abscesses.[53,77] Reticulogranulocytic infiltration is followed by formation of an abscess, with subsequent organization and clearing of the abscess. The inflammatory process of *Y. pseudotuberculosis* seems to remain confined to the lymph nodes without rupture through the nodal capsule.

Focal mucosal ulcerations may be seen in the ileum, and are more likely to be found at the site of Peyer's patches. Aggregates of neutrophilic leukocytes similar to those in mesenteric nodes may be seen in germinal centers within mucosal lymphoid tissue. Fibrinoid material may be a prominent feature at these sites. Small areas of necrosis surrounded by reticulum cells and leukocytes also may be present in the submucosal follicles.[62,114,116,227] Ulcerated lymphoid follicles in the intestinal wall are connected to the regional lymph nodes by a lymphangitis. This anatomic situation is analogous to the primary complex of tuberculosis.

Despite a clinical picture of acute appendicitis as the presenting feature of infection by *Y. pseudotuberculosis*, the appendix typically is grossly and microscopically normal. Inflammatory changes, when present, usually are in the form of a periappendicitis. Phlegmonous appendicitis can result from infection caused by *Y. pseudotuberculosis*, but is rare.[53,91]

*References 53, 60, 62, 94, 114, 136, 142, 143, 147, 172, 227.

PATHOGENESIS

The pathogenesis of infection by the two enteropathogenic *Yersinia* spp. has been studied extensively. After ingestion and successful transit to the small intestine have occurred, these *Yersinia* spp. are able to penetrate into the lamina propria primarily by passing through the cytoplasm of M cells that reside on the mucosal surface of Peyer's patches. The bacteria are internalized in membrane-bound vacuoles in which they survive but do not replicate. After reaching the lamina propria, the microbes multiply as extracellular microcolonies in lymph follicles and Peyer's patches, where they may reach densities of 10^9 CFU/g of tissue.[56]

Neutrophils and macrophages infiltrate these sites in response to the infection, ultimately creating microabscesses, but *Y. enterocolitica* is able to resist phagocytosis and intracellular killing by neutrophils and macrophages. *Yersinia* spp. also inhibit production of tumor necrosis factor (TNF)-α and interferon (IFN)-γ and induce apoptosis in host macrophages. The microbes disseminate to the mesenteric lymph nodes, apparently through lymphatic vessels. Infection usually is contained at this point, although abscesses often develop within the nodes. Systemic spread occurs occasionally, more commonly with infection by serotype O:8 strains.

Control of infection caused by *Y. enterocolitica* is the result of T-cell responses that lead to restriction of bacterial growth in infected organs through stimulation of production of *Yersinia*-specific antibody by B cells and cytokine-mediated activation of macrophages. These responses ultimately overcome the antiphagocyte strategies of *Yersinia*. Production of TNF-α and IFN-γ seems to be an essential part of the host response to *Yersinia* infections. *Yersinia* microbes generally do not survive within granulocytes after they have been successfully phagocytosed.[46,48,49,161]

Only a few of the many serotypes of *Yersinia* spp. are capable of infecting humans. Numerous virulence factors have been identified among these "pathogenic" strains (Table 113-2). Most of them are encoded on the *Yersinia* virulence plasmid pYV. At least eight chromosomal loci that encode novel *Yersinia* virulence factors have been found by tagged mutagenesis studies.[67] Much of this knowledge has been gained through mouse and rabbit models of human infection and observations of interactions of *Yersinia* organisms with in vitro cell culture models.[29,46,90,137,168]

The distinctions in pathogenesis between the two enteropathogenic *Yersinia* spp. and *Y. pestis* seem to be caused partly by the presence in *Y. pestis* of (1) two additional plasmids that encode a plasmin activator and a mouse exotoxin and (2) a hemin storage locus on its chromosome. These additional factors may enable *Y. pestis* to survive in and transmit between fleas and rodents. Such transmission does not occur with the enteropathogenic *Yersinia* spp.[180]

TABLE 113-2 **Known and Putative *Yersinia* Virulence Factors***

Stage of Pathogenesis	Determinant	Genomic Origin†	Function	Conditions of Expression
Gastric transit	Urease	Chromosome	Aids survival of gastric acidity.	
Mucosal invasion	Invasin (inv)	Chromosome	Attachment, invasion via β_1 integrins; broad host/cell range.	28°C
	Attachment invasion locus (AiL)	Chromosome	Attachment, lesser invasion factor; more host-specific than invasin; serum resistance.	37°C
	Yersinia adhesin A (YadA)	Plasmid	Attachment, invasion; reduces opsonization by C3b by binding complement factor H; ? role in resistance to antibacterial polypeptides (e.g., bactericidal permeability-increasing protein).	37°C and *any* calcium concentration
Disruption of phagocyte function	Effector proteins (act on host cells)	Plasmid		37°C and low calcium concentration
	YopH		Tyrosine phosphorylase that prevents phagocytosis by macrophages by preventing assembly of focal adhesion structures of phagocytes.	
	YopE		Phagocytosis resistance via induction of host cell actin filament rearrangements.	
	YopJ/P		Cysteine protease/acetyltransferase that inhibits mitogen-activated protein kinase and nuclear factor κB signaling via interruption of posttranslational covalent additions of ubiquitin-like molecules to these enzymes. This prevents production of cytokines (tumor necrosis factor-α), inhibits the host immune response, and triggers apoptosis of macrophages. Inhibits kinase phosphorylation via an acetyltransferase function.	
	YpkA/YopO		Serine kinase, causes rounding up of cells, specific target unknown.	
	YopT		Disruption of actin filaments.	
	YopM		Role unclear. Inhibits thrombin-induced platelet activation in vitro but is delivered intracellularly during infection. Leads to depletion of natural killer cells and alters expression of IL-15 and IL-15 receptors.	
	Membrane attachment, pore insertion into host cell	Plasmid		37°C and low calcium concentration
	YopB and YopD		Pore formation in host cell membrane to allow translocation of effector proteins into the host cell. Suppression of IL-8 secretion by epithelial cells	
	YopQ (YopK)		Controls translocation via modulating the size of the YopB, YopD pore in the host cell membrane	
	YopN		Involved in control of translocation, perhaps stabilizing contact between the bacterium and host cell membranes	

TABLE 113-2 **Known and Putative Yersinia Virulence Factors*** (Continued)

Stage of Pathogenesis	Determinant	Genomic Origin†	Function	Conditions of Expression
	LcrV[e]		Assists extrusion of YopB and YopD to the host cell membrane. Homologue of the *Y. pestis* V antigen. Antibodies against V antigen seem to be protective at the serotype level.	
	Yop secretion apparatus (Ysc) (28 proteins in total; part of microbe)	Plasmid		37°C and low calcium concentration
	YscD, YscR, YscU, and LcrD/YscV		Form a complex that spans the inner bacterial membrane (gated pore/channel).	
	YscC secretin		Forms a pore in the outer bacterial membrane that may connect with the inner membrane pore.	
	YscN		ATPase that energizes Yop transfer across the membranes.	
	LcrQ (in *Y. pseudotuberculosis*), YscM1 and YscM2 (in *Y. enterocolitica*)		Negative *yop* gene regulators that, when secreted, decrease in concentration in the bacterium, allowing *yop* expression.	
	Cytosolic chaperones (Syc) (6 proteins in total; within microbe)	Plasmid	Secretion/translocation pilots or antidegradation roles for effector proteins.	37°C and low calcium concentration
Colonization of Peyer's patches and lymph nodes	*Yersinia* phospholipase A	Plasmid	Serum resistance, inhibition of phagocytosis.	37°C and low calcium concentration
Iron metabolism	Yersiniabactin	Chromosome (high pathogenicity island)	Siderophore.	37°C, iron starvation
Diarrhea	*Yersinia* heat-stable enterotoxin (Yst)	Chromosome	Fluid secretion in intestine. Precise role in pathogenesis of disease in humans is unclear, but found in clinical isolates from children with diarrhea.	28°C (in vitro)
Systemic invasion	O antigen	Chromosome	Component of lipopolysaccharide. Complement/serum resistance.	

Data from references 26, 47, 48, 72, 76, 111, 120, 128, 150, 151, 161, 180, 190, 196, 197, 235, 236.
*The complete pathogenesis/virulence roles of several gene products encoded on the *Yersinia* virulence plasmid pVY and chromosomal sites have yet to be determined.
†Plasmid refers to the *Yersinia* virulence plasmid pYV.
IL, Interleukin; *Lcr,* low calcium response; *Yop, Yersinia* outer membrane protein (although not all such designated proteins reside in the outer membrane).

Bacterial Determinants of Mucosal Invasion

Two outer-membrane proteins encoded by chromosomal genes permit entry into various mammalian cell types in vitro and are likely to be responsible for the ability of *Yersinia* to invade into and through the intestinal mucosal epithelium. They have been named *invasin* and the *attachment invasin locus protein* (Ail). All isolates of *Y. enterocolitica* that are virulent in humans contain DNA sequences that encode for invasin and Ail. Nonpathogenic strains do not contain the genetic code for Ail but most contain invasin genes that cannot be expressed because of chromosomal rearrangements. These proteins apparently are unique to the genus *Yersinia*.[100,145,165]

Invasin attaches to receptors in the β_1 integrin family and induces an actin-mediated endocytosis of the microbe. It binds to β_1-chain integrin receptors with approximately 100-fold higher affinity than do natural ligands, such as fibronectin. Such high-affinity binding triggers endocytosis-like internalization that involves clathrin.[49] Invasin is expressed maximally at ambient temperatures when the organism is in stationary phase. *Y. enterocolitica* microbes living in the environment probably exist in a stationary phase–like state and may be primed maximally for invasion of the host after ingestion. Invasin expression also can remain elevated at 37°C (98.6°F) when the pH is 5.5, and such conditions are encountered during passage through the intestinal tract. Ail is expressed maximally at 37°C, functions as an adhesin and an invasion

factor, and plays a role in the resistance of *Y. enterocolitica* to the bactericidal activity of human serum.[100,101,165]

High levels of β_1-chain integrin receptors are found on the luminal surface of M cells, which are antigen-sampling epithelial cells found in highest numbers overlying Peyer's patches in the intestinal tract.[83] Flagellum-dependent motility seems to be required for invasin-mediated invasion of cells by *Y. enterocolitica*.[233]

The following determinants are primarily relevant to *Y. enterocolitica*, although *Y. pseudotuberculosis* shares many of these features.

Yersinia Adhesin A

Yersinia adhesin A (YadA) is a multifunctional virulence factor of *Y. enterocolitica*. YadA is approximately 50 kDa and probably forms tetrameric fibrillae on the microbial surface. Its gene resides on the pYV plasmid and is transcribed at 37°C independently of the calcium concentration. YadA binds to extracellular matrix proteins, such as collagen, and mediates adhesion to cells. It may play a role in translocation of Yop effector proteins into eukaryotic phagocytic cells. YadA also inhibits the terminal complement attack complex, reduces opsonization by C3b through binding of complement factor H, and contributes to the ability of *Y. enterocolitica* to resist killing by antimicrobial polypeptides of human granulocytes. YadA knockout mutants are far less virulent in mice than is the parent wild-type strain.[48,89,224]

Virulence Plasmid and Type III Secretion System

Pathogenic strains of *Y. enterocolitica* harbor a plasmid that consists of approximately 70 kb (denoted pYV) and encodes the low calcium response system of *Yersinia* spp., which involves a complex response to environmental conditions of 37°C and calcium concentrations of less than 2.5 mM, both of which describe the intracellular compartment of mammalian cells.[48,197] Plasmid-encoded factors are required for survival and extracellular multiplication after reaching Peyer's patches. Plasmid-cured derivatives are ingested rapidly and killed by neutrophils in Peyer's patches, whereas wild-type strains are able to proliferate and spread through the lamina propria to adjacent villi. Plasmid-encoded factors are not required for *Y. enterocolitica* to penetrate the intestinal mucosa.[89]

In DNA cross-hybridization studies, the pYVs of *Y. enterocolitica* serogroups O:9, O:3, and O:5 show 90 percent nucleotide identity with one another, 75 percent identity with the pYV of serogroup O:8, and 55 percent identity with the pYV of *Y. pestis* and *Y. pseudotuberculosis*. Despite the nucleotide divergence among the pYVs of the three *Yersinia* spp., the overall structures and most of the genes are highly similar among them. The *Yersinia* low calcium response plasmids are nonconjugative.[48,197]

The *Y. enterocolitica* pYV contains approximately 70 genes, of which around 53 have protein products with known or putative functions (many of these are listed among the virulence factors in Table 113-2). pYV encodes *Yersinia* adhesin A and a system of six effector proteins that are delivered into host cells (primarily phagocytic granulocytes) through a complex mechanism of regulatory proteins, secretion chaperones, and proteins that act as secretion channels or pores in the bacterial and host cell membranes. This system, called a type III secretion system, denotes secretion of the bacterial proteins only in response to contact with a mammalian cell. The effector proteins and several others involving pore formation are designated Yops (*Yersinia* outer-membrane proteins), although we now know that most of them do not reside in the outer membrane. Similar type III secretion systems are found in *Salmonella* and *Shigella* spp.[48,197]

The secretion system is composed partially of syringe-like organelles—called *Ysc injectisomes*—that develop when *Yersinia* organisms are incubated at mammalian body temperatures. These injectisomes are protein pumps that span the peptidoglycan layer and the two bacterial cell membranes, topped by a needle-like structure protruding outside the bacterium. Twenty-seven proteins are involved in the structure; 10 of them are located internally and appear to be similar to the basal body of a flagellum. The structure also includes an ATPase that functions as a proton pump. A homomultimeric ring-shaped structure extends through the outer membrane, with a central pore diameter of about 50 Å.[34]

As the injectisomes assemble, stocks of intracellular Yops are synthesized. Active transcription of *Yop* genes is limited to bacteria in close contact with eukaryotic cells. The secretion channel of the injectisome remains closed, with negative feedback mechanisms to prevent overproduction of Yops. On close contact with a phagocyte, the injectisome channels open, and secretion of effector Yops into the target cell begins.[34] This is accomplished through insertion of YopB and YopD into the host cell membrane, where these form a multimeric complex that serves as a pore for effector injection.[150]

After secretion into a phagocytic cell occurs, YopH, YopE, YopT, and YopO/YpkA disrupt its cytoskeletal dynamics, blocking phagocytosis (see Table 113-2). YopT is a cysteine protease that cleaves and inactivates Rho proteins, which are small membrane-associated GTPases that control the host cell actin cytoskeleton.[190] Other Yops inhibit production of TNF-α, IL-8, and other inflammatory chemokines and induce apoptosis of macrophages.[34]

A similar type III secretion system is present in *Y. pseudotuberculosis*, which is considered to be primarily an extracellular pathogen.[236] *Y. pseudotuberculosis* can survive within macrophages, and mouse studies suggest a small minority of invading microbes may reside intracellularly in multiple tissue sites. This system is able to transport Yops from phagosomes in macrophages into the cytosol and to inject Yops into mouse dendritic cells. YopJ inhibits dendritic cell–mediated T-cell responses via induction of apoptosis and reduction of surface molecule expression.[210]

Summary *of Yersinia* Pathogenesis

Pathogenic strains of *Y. enterocolitica* require calcium concentrations equivalent to those of serum and extracellular fluids in humans for growth at body temperature. Plasmid proteins are synthesized maximally, however, at 37°C

under conditions of low calcium concentrations, such as those found intracellularly. These regulatory effects of environmental calcium concentrations permit free growth of *Yersinia* organisms during extracellular life and production of factors that inhibit phagocytosis by macrophages and neutrophils when *Yersinia* organisms pass through the intracellular compartment during mucosal invasion or come into contact with these granulocytes.[46,48,89]

A model of how pathogenic *Yersinia* organisms living in cold (≤28° C [≤82.4° F) environmental reservoirs are able to establish infection in the mammalian host can be described at the molecular level (see Table 113-2). *Yersinia* microbes living in the environment express invasin, which renders them ready to attach and invade the intestinal mucosal M cells after ingestion. Expression of invasin continues at body temperature during gastric passage to the intestines. Survival of gastric acidity is assisted by a urease-producing system.

During intracellular passage through M cells in the small intestine, *pYV* genes are likely to be activated (conditions of low calcium, 37° C). YadA can be produced at body temperature and may assist in mucosal invasion. Transcytosis from the intestinal lumen to the lamina propria seems to occur by a clathrin-mediated process that probably is a normal function of the host cell, after internalization is triggered. Ail also is synthesized after ingestion occurs and probably promotes attachment to migrating cells in the lamina propria that may facilitate extracellular spread of the microbes to regional lymph nodes and perhaps the liver and spleen.

To survive this journey, *Yersinia* organisms must evade phagocytosis by macrophages and neutrophils. YadA can inhibit complement opsonization, reducing the likelihood that phagocytosis will occur. When contact between the microbe and a host granulocyte occurs, the previously activated type III secretion system (described earlier) encoded by the pYV plasmid comes into play. A contiguous pore is inserted into the granulocyte cell membrane (involving YopB, YopD, YopQ, YopN, and LcrV). Effector proteins (YopE, YopH, YopJ/P, YopO/A, YopM, and YopT) then are translocated into the granulocyte cytoplasm, disrupting its abilities to ingest the bacterium and produce cytokines and triggering apoptotic cell death (see Table 113-2). Although this system of virulence factors is robust enough to facilitate short-lived infection that often causes mild to moderate clinical symptoms, the microbes that do not succumb to the innate immune response ultimately are contained and then eliminated by the adaptive host response.

Y. pseudotuberculosis, which does not express Ail or invasin, is able to penetrate the intestinal epithelial barrier through a cascade of steps initiated via binding to Toll-like receptor 2 on monocytes. This involves caspase 1 and IL-1β production that ultimately leads to myosin light chain kinase activation in intestinal epithelial cells that disrupts tight junctions between them.[106]

Iron Metabolism and Virulence

Iron is an essential growth factor for most bacteria, many of which release siderophores (high-affinity chelators) that bind ferric iron and then are taken up again through receptors by the microbe. *Y. enterocolitica* serotype O:8 and other biotype 1B strains synthesize a chromosomally encoded siderophore, designated *yersiniabactin*, which sits on the outer membrane of the bacterium. The presence of this siderophore decreases the concentration of environmental iron required for optimal growth and probably accounts for the increased virulence observed for serotype O:8 strains.[50,89] The gene encoding yersiniabactin and the genes required for its biosynthesis, transport, and regulation compose the core of what is termed a *high-pathogenicity island* because of the high lethality for mice that its presence confers.[171]

Serotypes O:8, O:4, O:13, O:18, O:20, and O:21 (all biotype 1B and historically considered "American" strains) are highly lethal to mice after intraperitoneal injection and are able to evoke a keratoconjunctivitis after inoculation into the conjunctival sac of guinea pigs (the positive Sereny test result). Oral infection in mice predominantly produces the features of mesenteric adenitis and systemic infection, rather than simple gastroenteritis, which is characteristic of human infection by American strains. The "European" serotypes, O:3, O:9, and O:5,27, yield a negative Sereny test result and cause mild diarrhea but not death in mice. The European serotypes of *Y. enterocolitica* do not produce yersiniabactin, but are able to use siderophores synthesized by other organisms.[46,89,145]

Deferoxamine, a *Streptomyces*-derived siderophore used clinically to treat iron overload states, can be used by *Y. enterocolitica* strains as a source of iron. The increased availability of ferric iron that exists in iron overload states—for example, hemochromatosis and diseases such as thalassemia, which require frequent red blood cell transfusions—also facilitates survival and growth of *Y. enterocolitica*. Iron overloading and deferoxamine are independent risk factors for development of systemic disease after intestinal infection with *Y. enterocolitica*.[50]

Enterotoxin Production

All enteropathogenic strains of *Y. enterocolitica* produce a heat-stable enterotoxin that closely resembles the heat-stable toxin of *E. coli*. Both enterotoxins induce increases in levels of cyclic guanosine monophosphate levels in intestinal epithelial cells. The *Y. enterocolitica* enterotoxin is not plasmid-encoded, and its presence does not correlate with the expression of other virulence phenotypes.[68] Because the enterotoxin is not produced in vitro at temperatures exceeding 30° C (86° F), production in the gastrointestinal tract and a causative role in diarrhea were thought to be unlikely.[50] Observations in the young rabbit oral infection model have shown, however, that enterotoxin-negative mutants did not induce diarrhea and the wild-type strain did. Clinical biotype 1A isolates from children with diarrhea in India produce *Y. enterocolitica* stable toxin-b. These findings suggest that *Yersinia* enterotoxins may play a role in causing the diarrhea frequently associated with *Y. enterocolitica* infection in children.[89,164,196]

TABLE 113-3 **Clinical Features of *Yersinia Enterocolitica* Infection in Children**

	Sweden,[18] 1967-1973	Finland,[138] 1974-1978	Canada, 1972[54]	Canada,[141] 1977-1978	U.S.,[127] 1989-1990	U.S.,[153] 1988-1991	Combined Totals
No.	31	35	40	57	37*	48	248
Age ≤5 yr	28	26	19	NS†	≥28	NS	≥101/142 (≥71%)
Fever (>38°C)	15	6	36	39	35	44	175/248 (71%)
Diarrhea	31	26	32	56	37	45	227/248 (92%)
Grossly bloody diarrhea	2	NS	7	NS	14	22	45/156 (29%)
Abdominal pain	4	6	20	31/48	NS	NS	61/154 (40%)
Vomiting	NS	12	12	22	18	23	87/217 (40%)
Rash‡	NS	2	2	NS	NS	NS	4/75 (5%)
Appendectomy	0	0	4	1	0	0	5/200 (2%)
Serotype O:3	31	NS	34	57	34	NS	156/165 (94%)

*All were black children; 7 different cities, 3-month period.
†More than half of these children were <2 years old.
‡Maculopapular rash or urticaria.
NS, Not specified.

Gastric Acidity as a Protective Host Factor

Although *Y. enterocolitica* is able to grow under conditions at pH 5.0 to 9.0, optimal growth occurs at pH 7.0 to 8.0. Gastric acidity may play a protective role against some *Yersinia* inocula, although pathogenic *Yersinia* organisms produce a urease enzyme that facilitates survival. Therapeutic agents or clinical conditions that result in reduced gastric acidity may predispose patients to development of infection. *Y. enterocolitica* bacteremia has been reported after gastrectomy.[50,120]

CLINICAL MANIFESTATIONS

Clinical disease caused by *Y. enterocolitica* occurs far more frequently than that caused by *Y. pseudotuberculosis*.[61,129,130] Historically, diarrheal illness has been considered the hallmark of *Y. enterocolitica* and the pseudo-appendicular syndrome of mesenteric adenitis indicative of *Y. pseudotuberculosis*. Each species can cause enterocolitis and mesenteric adenitis, however. Various other clinical infections and postinfection syndromes also are caused by these microbes. In a series of patients presenting with acute abdominal pain suggestive of appendicitis, the incidence of serologic evidence of *Yersinia* infection has ranged from 7 to 31 percent.[16]

Yersinia enterocolitica

The clinical features of the disease caused by *Y. enterocolitica*, primarily an acute enteritis, have been described by many investigators.* The clinical manifestations depend partly on the age and physiologic condition of the host.[50,141,172] Enterocolitis is the most common presentation and occurs most often in young children. The pseudo-appendicular syndrome, which results primarily from mesenteric adenitis and mimics acute appendicitis, occurs more commonly in older children and young adults.[20,50,92,104,158,179] Asymptomatic infection can occur, but the relative frequency in contrast to symptomatic disease is unknown. The predominant clinical features of *Y. enterocolitica* infection in children are summarized in Table 113-3. Disease manifestations may be strain-related in some cases. The so-called pathogenic strains of biotype 1B are more likely to cause fever, whereas those of so-called nonpathogenic strains of biotype 1A may cause more vomiting.[97]

Enterocolitis

Y. enterocolitica enterocolitis is characterized by diarrhea and abdominal pain. The diarrhea usually persists for 7 to 14 days.[127,141,219] A range of 1 to 46 days of diarrhea has been reported.[109] Ten percent of cases may persist for 30 days or more,[141] and chronic diarrhea persisting for several months has been described.[54]

During the first week of symptoms, patients commonly have 3 to 10 stools per day, with a gradual decrease in frequency thereafter. Stools typically are greenish, exhibit variable consistency (usually watery or mucoid), and are not remarkably malodorous. Gross blood is noted in approximately 25 to 50 percent of patients. Vomiting occurs in 40 percent of cases. Nausea is a common symptom. The abdominal pain can be colicky, diffuse, or localized to the right lower quadrant or epigastrium. Fever occurs commonly and usually is of low grade, but may exceed 40°C (104°F); it usually resolves within 1 week.[1,138]

Most cases are self-limited, but some children require hospitalization. Among 60 children hospitalized in Michigan between 1990 and 1997, the mean number of hospital days required was 4 (range, 1 to 17 days).[1]

Fecal leukocytes are present commonly but not universally. The peripheral white blood cell count may range from 5600/mm^3 to more than 30,000/mm^3. Most counts are greater than 15,000/mm^3. Band forms often exceed 15 percent of the total. Infants frequently exhibit an immature-to-total neutrophil ratio greater than 0.5.[1,127,138] An absolute monocytosis may be seen in two thirds of

*References 18, 40, 50, 54, 81, 119, 126, 127, 137, 138, 141, 173, 179, 215, 232.

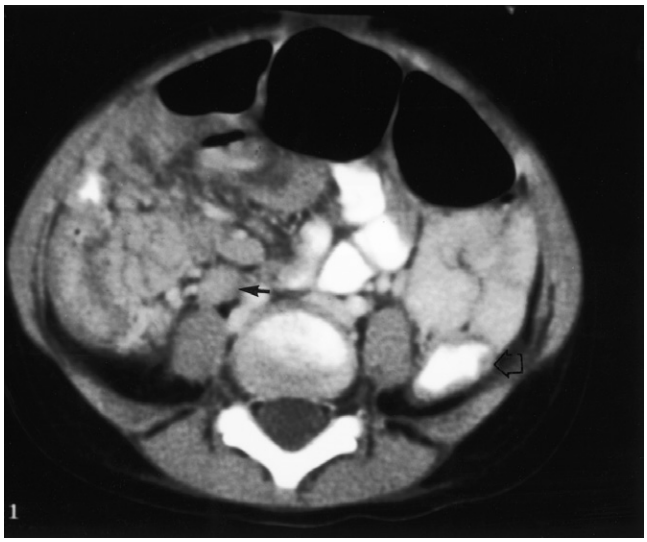

FIGURE 113-3 ■ Mesenteric lymphadenopathy *(solid arrow)* and thickened bowel wall *(open arrow)* seen on computed tomography scan of a toddler with a 1-week history of severe abdominal pain, fever, vomiting, and nonbloody diarrhea. Stool culture on cefsulodin-irgasan-novobiocin agar yielded growth of *Yersinia enterocolitica,* serotype O:8. (From Tuohy AM, O'Gorman M, Byington C, et al. *Yersinia enterocolitis* mimicking Crohn's disease in a toddler. *Pediatrics* 1999;**104**:e36.)

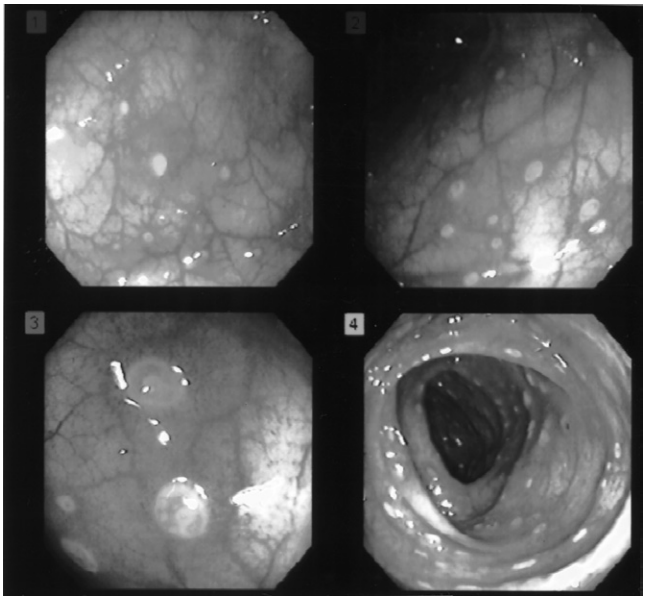

FIGURE 113-4 ■ Multiple mucosal aphthoid ulcerations, 2 to 3 mm in size, as seen during colonoscopy in the child described in Figure 113-3. (From Tuohy AM, O'Gorman M, Byington C, et al. *Yersinia enterocolitis* mimicking Crohn's disease in a toddler. *Pediatrics* 1999;**104**:e36.)

patients.[122] Culture-negative cerebrospinal fluid pleocytosis can occur.[1]

Radiologic examination by upper gastrointestinal barium studies in 24 adult patients with severe diarrhea caused by *Y. enterocolitica* showed abnormalities of the terminal ileum in 21 cases.[220] Diffuse thickening of the mucosal folds was seen in 16 cases, and nodular filling defects were seen in 11. The radiographic appearance suggested the presence of one or more ulcerations of the terminal ileum in 11 patients. Dilation of the terminal ileum was noted in 12 patients, and extrinsic compression, presumably from enlarged lymph nodes, was present in 4. In some instances, the findings suggested the terminal ileitis of Crohn disease. Follow-up studies performed 2 months after acute illness showed decreased but persistent thickening of mucosal folds in eight patients. Barium enema studies were done in 15 patients and showed no striking abnormalities other than mucosal ulcerations, which were seen best on air-contrast studies.[219]

Mesenteric lymphadenopathy can be seen on computed tomography scans (Fig. 113-3).[218] Among 13 adults who had colonoscopy or sigmoidoscopy for severe diarrhea caused by *Y. enterocolitica*, abnormalities were seen in 8; the mucosa appeared diffusely swollen, erythematous, and friable in 6 individuals, and 2 had only small, 1- to 2-mm aphthoid ulcerations. Serial procedures showed macroscopic and microscopic healing of ulcers within 4 to 5 weeks.[220] Aphthoid ulcerations may be seen throughout the colon (Fig. 113-4).[218]

Pseudoappendicitis-Mesenteric Adenitis

The syndrome of pseudoappendicitis, characterized in most cases by a normal appendix and an intense suppurative mesenteric adenitis, has attracted considerable attention since it was reported first in 1953.[142,143] The first recognized cases were caused by *Y. pseudotuberculosis*, but most cases reported in recent years have been caused by *Y. enterocolitica*.[31,103,126]

Fever, abdominal pain, right lower quadrant tenderness, and leukocytosis are the primary features of *Y. enterocolitica*–induced pseudo-appendicular syndrome.[50,104] Some patients also have features of enterocolitis (i.e., nausea, vomiting, and diarrhea). The clinical presentation often is highly suggestive of acute appendicitis, such that laparotomy is required. Among a series of 581 patients in Scandinavia who underwent laparotomy for suspected appendicitis, 3.8 percent of cultures of stool or operative specimens yielded *Y. enterocolitica*.[158] Another 284 patients with similar symptoms were observed, and 5.6 percent of stool cultures from these cases yielded *Y. enterocolitica*. In a similar Scandinavian series of 205 patients who underwent appendectomy, 22 subsequently were diagnosed by serology as having *Y. enterocolitica* infection.[104] The findings on laparotomy usually are mesenteric lymphadenitis, terminal ileitis, and a normal or slightly inflamed appendix.[20,50,104,158]

A study of 40 cases of granulomatous appendicitis found evidence suggesting infection caused by *Y. enterocolitica* in four specimens, *Y. pseudotuberculosis* in four specimens, and both in two specimens by PCR analysis.[125] Two patients in the series subsequently were diagnosed as having Crohn disease, but causation cannot be inferred.

Asymptomatic Infection

In an unknown number of cases, infection by *Y. enterocolitica* is entirely asymptomatic. In a study of the

distribution of antibodies to *Y. enterocolitica* in sera collected for various purposes in Ontario, Canada, from 4209 individuals who had no evidence of infection by this organism, specific antibody was present in 199 (serotype O:3 in 158 and serotype O:9 in 41).[215]

Other Presentations of Acute Infection

Bacteremia can occur[35,41,50,149] and may result in spread of infection to virtually any body site. Such events occur uncommonly and are seen more often in adults than in children. In children, bacteremia occurs more commonly in infants than in older children.[1] The risk for development of bacteremia during gastroenteritis in infants younger than 3 months may be 30 percent.[153] Bacteremias may be transient and asymptomatic or lead to septic shock and death. Septic cases tend to occur among patients with underlying illnesses and are associated with mortality rates of 34 to 50 percent.[50]

Y. enterocolitica may cause focal infections in many extraintestinal sites, even in the absence of detectable bacteremia. Pharyngitis has been reported and occurs primarily in adults. Cervical adenopathy may be associated with *Y. enterocolitica* pharyngitis, and gastrointestinal symptoms may be absent.[50] One adult with pharyngitis died of associated septic shock.[181] Conjunctivitis and panophthalmitis caused by *Y. enterocolitica* have been described. Parinaud oculoglandular syndrome,[42] inguinal adenopathy,[228] and suppurative lymphadenitis[217] have been reported, including a neonate with inguinal adenitis.[159]

Cellulitis, soft tissue abscesses, and wound infections also have been reported. Cellulitis may have associated vesiculobullous lesions. An erysipelas-like rash, maculopapular rash, and urticaria also have been described in association with infection caused by *Y. enterocolitica*.*

Pancreatitis, cholecystitis, diverticulitis, and intestinal perforation have been described.[129,169] Peritonitis also can occur but is extremely rare,[175] especially considering the frequency of mesenteric adenitis. Pneumatosis intestinalis has been reported in an infant.[1] Pneumonia, pleural empyema, lung abscess, hepatic and splenic abscesses, urinary tract infection, and renal abscess also have been reported.[50] Glomerulonephritis that usually is transient has been reported.[51,65] Cases of meningitis, osteomyelitis, septic arthritis, pyomyositis (including psoas muscle abscess), endocarditis, mycotic aneurysm, and intravenous catheter–related infection caused by *Y. enterocolitica* have been described.† Thrombocytopenia[70] and hemolytic anemia[117] have occurred in association with infection caused by *Y. enterocolitica*.

Underlying Conditions That Predispose to Bacteremia

Y. enterocolitica bacteremias that occur in patients beyond early infancy most often occur in patients with chronic illnesses or iron overload states. Thalassemias are such conditions that occur most commonly in children.[40,63,110] Among 144 Italian children with thalassemia who were receiving deferoxamine therapy and frequent blood transfusions, 14 developed infection caused by *Y. enterocolitica* during a 12-month period.[40] Septicemia occurred in 5 of the 14 and was preceded by enterocolitis or mesenteric adenitis in each case. All 14 recovered after receiving 2 weeks of therapy with intravenous trimethoprim-sulfamethoxazole. A similar proportion of children with thalassemias observed in two centers in Canada between 1979 and 1994 also developed invasive disease caused by *Y. enterocolitica*.[6] Bacteremia can be associated temporally with blood transfusions in such patients, suggesting that transfusions may be the source of infection or predispose to development of infection in some cases.[129]

Hemochromatosis, cirrhosis, and other liver diseases may facilitate development of *Y. enterocolitica* bacteremia, also on the basis of excess availability of serum iron. Deferoxamine therapy itself is a risk factor for development of sepsis caused by *Y. enterocolitica* because of the ability of the microbe to extract iron from this compound. Immunosuppressive therapies, diabetes mellitus, and malnutrition also may predispose to development of *Y. enterocolitica* bacteremia.[50]

Postinfectious Syndromes

A reactive arthritis may occur 1 to 14 (usually 4 to 10) days after the cessation of acute illness.* Most such events occur in adults, with a slight female predominance, but 8/74 cases in one series from Sweden were in children aged 11 to 20 years old (5 boys, 3 girls).[230] In a series from the Netherlands, 10 percent of children with yersiniosis, most of whom were 7 years of age or older, developed arthritis.[93] The knees, ankles, and wrists are affected most commonly, and in approximately 50 percent of cases, only one or two joints are involved. Hands, fingers, toes, shoulders, hips, and elbows also may be involved. Pain usually is severe, and the arthritis is additive and usually not migratory. The inflammatory process is self-limited and may persist for 2 months or longer in two thirds of cases, with one third persisting for 4 months or longer.

The erythrocyte sedimentation rate exceeds 60 mm/hr in approximately 50 percent of cases. Joint effusions usually are inflammatory, but cell counts and differentials vary and occasionally mimic septic arthritis. Immune complexes have been found in joint fluid. Nonsteroidal anti-inflammatory drugs and corticosteroids, intra-articular and systemically administered, have been used for symptomatic relief for this process.[178]

Erythema nodosum also occurs as a postinfectious manifestation, more often in adults than in children. It can occur alone or in association with arthritis.[86] Tendinitis, myositis, myocarditis, urethritis, uveitis, and conjunctivitis also can occur in association with arthritis. Many but not all patients who develop these postinfectious reactions are human leukocyte antigen (HLA)-B27 positive.[1] Some patients manifest full Reiter syndrome.[93,118,185,199] Acute glomerulonephritis has been

*References 18, 31, 50, 78, 192, 209, 229.
†References 12, 32, 50, 90, 201, 203.

*References 7, 8, 10, 13, 90, 93, 179, 212, 220, 230.

linked to infection with serotype O:3 strains in one series of adults.[65]

Yersinia antigens, but not intact bacteria, have been found in synovial tissue obtained several weeks to months after onset of reactive arthritis. *Y. enterocolitica* is able to survive within in vitro cultures of human synovial cells for 6 weeks, with resultant deposition of residual antigen aggregates within the cells.[98] Synovial fluid–derived T cells from patients with *Y. enterocolitica*–induced reactive arthritis have been shown to respond to several *Y. enterocolitica* antigens: heat shock protein 60, urease β subunit, ribosomal L2 protein, and a region of the plasmid-encoded tyrosine phosphatase YopH that is highly homologous to the catalytic domain of eukaryotic protein tyrosine phosphatases.[123,144,167]

Epitopes on *Yersinia*-produced proteins likely trigger cross-reactive immunologic recognition of host proteins that leads to chronic inflammation in susceptible individuals.[95] High levels of antibodies that react with 60-kDa recombinant *Yersinia* heat shock protein have been found in HLA-B27–positive individuals with acute anterior uveitis or pars planitis.[34]

Antibodies against *Y. enterocolitica* have been detected in patients with disorders of the thyroid, including Graves disease, thyroid adenoma, and Hashimoto thyroiditis.[193] A Danish twin study found no increased risk for thyroid antibodies after *Y. enterocolitica* infection, however.[84] These observations may reflect autoantibodies that cross react with *Yersinia* epitopes, rather than a causal link between yersiniosis and thyroid disease.[50,176]

Brachial plexus neuropathy and transverse myelitis, each occurring after the resolution of gastrointestinal symptoms caused by *Y. enterocolitica*, have been described in one patient.[204] Various chronic ailments have been described among a group of 160 Scandinavian patients observed for 4 to 14 years after having acute yersiniosis.[186] Ailments included persistent joint complaints, ankylosing spondylitis (in HLA-B27–positive patients), iridocyclitis, chronic hepatitis, chronic abdominal pain, rheumatoid arthritis, chronic nephritis, thyroid disease, and neurologic ailments. Observed deaths among these patients exceeded the expected number. These findings require confirmation before causal links can be considered.

Yersinia pseudotuberculosis

The pseudo-appendicular syndrome that results from mesenteric adenitis is the primary disease produced by *Y. pseudotuberculosis*.* The chief complaint is abdominal pain, either diffuse or localized to the right lower quadrant. Fever (38° to 40° C) (100.4° to 104° F) almost always is present. Tenderness over the McBurney point usually is present. All of these symptoms are highly suggestive of acute appendicitis. Diarrhea may occur but often is absent. Mild leukocytosis occurs, but white blood cell counts usually are less than 20,000/mm^3. The clinical course almost always is benign, with recovery typically beginning approximately the fifth day of illness. On laparotomy, the appendix is normal in most cases but occasionally appears inflamed or suppurative. The mesenteric lymph nodes are enlarged and may appear necrotic.

Efforts have been made to distinguish the pseudoappendicular syndrome caused by *Y. pseudotuberculosis* from that caused by *Y. enterocolitica*.[28,71] *Y. pseudotuberculosis* adenitis is less likely to have associated enterocolitis and may have a shorter febrile course, but no clear distinction can be made on clinical grounds alone.

A fulminant typhoidal or septicemic form of infection caused by *Y. pseudotuberculosis* can occur but seems to affect primarily older adults with debilitating conditions, such as diabetes or liver disease. This syndrome often is fatal.[114,185] Isolated cervical adenitis, liver abscess, and terminal ileitis have been described.[86,227] Subacute and recurrent disease can occur.[136] Erythema nodosum[185] and nonsuppurative arthritis[39] also have been reported in association with infection caused by *Y. pseudotuberculosis*. In an outbreak related to eating contaminated grated carrots in Finland in 2003, erythema nodosum was seen in numerous patients with gastrointestinal illness.[102] Median time from exposure to development of gastrointestinal symptoms was 8 days. Median time from exposure to development of erythema nodosum was 19 days (see Fig. 113-2). One patient developed reactive arthritis in a similar time frame.

Yersinia pseudotuberculosis *and Kawasaki Disease–Like Illness*

In Korea and Japan, *Y. pseudotuberculosis* strains have been responsible for a clinical syndrome that can mimic Kawasaki disease.[39] This manifestation of disease occurs primarily in outbreaks and has been described as scarlet fever–like.[162] A transient (2 to 3 days) erythematous maculopapular rash, strawberry tongue, conjunctivitis, and desquamation can occur, generally in association with gastrointestinal symptoms and fever. Erythema nodosum, lymphadenopathy, uveitis, and coronary aneurysms have occurred in some of these cases, as has acute interstitial nephritis, which can lead to transient renal failure.[3,121,162] Among a series of 33 patients (median age, 5 years) with such presentations, 20 had elevated antibody titers against *Y. pseudotuberculosis*–derived mitogen, which can function as a superantigen.[3] As in Kawasaki disease, Vβ3 T lymphocytes were increased in many of these patients in contrast to healthy control subjects.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of *Yersinia* enterocolitis includes viral and other bacterial causes of acute gastroenteritis. When the symptoms of mesenteric adenitis are predominant and severe, appendicitis and other causes of an acute abdomen must be considered. The acute terminal ileitis caused by *Yersinia* infections also can be similar to the gastrointestinal manifestations of Crohn disease, ulcerative colitis, cat scratch disease, anisakiasis, amebiasis, actinomycosis, typhoid fever, and lymphoma.[69,93,162,219,227] Cases of infection caused by *Y. enterocolitica* with concomitant recovery of *Salmonella* and *Campylobacter* spp.

*References 21, 53, 60, 62, 71, 91, 96, 113, 114, 136, 162, 183, 185, 222, 227.

and rotavirus antigen in stool specimens have been observed.[1]

DIAGNOSIS

The most effective approach to the diagnosis of yersiniosis is isolation of the organism from the stool of patients with enteritis caused by *Y. enterocolitica* or from the infected mesenteric lymph nodes of patients infected by *Y. pseudotuberculosis*. *Y. enterocolitica* occasionally can be recovered from involved mesenteric lymph nodes or the distal ileum.[50] Cultures of feces from individuals with acute suppurative mesenteric adenitis usually fail to grow either organism.

Isolating *Yersinia* organisms from extraintestinal specimens such as lymph nodes and blood is not difficult because they grow on ordinary media (e.g., blood agar) and on several selective and differential media employed for enteric bacteria. Isolating from fecal specimens is more difficult, however, because *Yersinia* multiply more slowly than other enteric bacteria at 37° C (98.6° F) and have no characteristic colony morphology. Selective media have been developed, but many clinical laboratories culture stool specimens for *Yersinia* spp. only on request because of the costs of these media and the relatively low frequency of occurrences of these pathogens in the community.

Yersinia organisms grow well on MacConkey agar but are much smaller than are other enteric bacteria after standard incubation at 37° C. Cefsulodin-irgasan-novobiocin agar plates have been designed specifically for the isolation of *Yersinia* spp. from stool specimens. After being incubated for 48 hours, *Yersinia* colonies appear dark pink with translucent borders and occasionally are surrounded by a zone of precipitated bile. Cefsulodin-irgasan-novobiocin agar inhibits the growth of most other bacteria except for *Citrobacter* spp. (positive citrate reactions of which allow their distinction). If a dedicated medium for *Yersinia* isolation is not used, MacConkey agar can be examined after 24 hours at 35° to 37° C (95° to 98.6° F) for small colorless colonies that become much larger after an additional 24 hours of incubation at room temperature. Most *Y. enterocolitica* strains are lactose-negative.[74] *Yersinia* spp. can be differentiated readily from *Salmonella* spp. because the latter are motile at 37° C, urease-negative, citrate-positive, and lysine-positive. Most *Salmonella* strains also produce gas during fermentation and produce hydrogen sulfide. *Shigella* are urease-negative and lack motility at room temperature.[203]

Yersinia spp. grow faster at 37° C than at room temperature. Growth occurs readily at 22° to 28° C (71.6° to 82.4° F), however, and these lower temperatures are recommended for primary isolation.[74] Because of the ability of *Yersinia* spp. to grow at even colder temperatures, specimens can be inoculated into phosphate-buffered saline, refrigerated at 4° to 6° C (39.2° to 42.8° F), and subcultured periodically (up to 4 weeks) if the routine plates that were inoculated with the specimen remain negative. Such "cold enrichment" greatly enhances the isolation rate of *Yersinia* spp. and may be the most reliable method for isolating these organisms from fecal specimens. Many of the *Yersinia* isolates recovered by cold enrichment, however, represent either *Y. enterocolitica* serotypes, which usually are not associated with human disease, or other *Yersinia* spp., which have roles that remain unclear.

Pathologic or virulent *Yersinia* strains can be distinguished in most instances from nonpathogenic strains by three biochemical tests that are associated with absence of the virulence plasmid. The virulent strains lack pyrazinamidase activity, do not ferment salicin, and do not hydrolyze esculin. On Congo red–magnesium oxalate agar during incubation at 36° C (96.8° F), fresh pathologic isolates (but not those that have been subcultured serially) grow as small red colonies, showing the virulence plasmid-determined properties of Congo red dye uptake and calcium-dependent growth.[59]

Serology

Serology can be performed with microtiter techniques and is most reliable for serotypes O:3 and O:9 of *Y. enterocolitica*. Antibody to the infecting serotype usually is absent at the onset of disease. Peak titers usually are reached 3 to 4 weeks after onset of clinical illness and decrease during the next 3 to 5 months. Low postconvalescent titers may persist for months. Microhemagglutination, complement fixation, and enzyme immunoassays are available in a few commercial laboratories.[27,74,219]

Agglutinin titers of 1 : 128 or higher for *Y. enterocolitica* in previously normal healthy individuals suggest infection. Titers of 1 : 200 or greater were present within 3 weeks of onset of illness in 62/65 Canadian children who had infection with serotype O:3. Fourfold increases in titer rarely were seen. Negative or minimal titers (≤1 : 32) do not rule out yersiniosis in infants or immunosuppressed patients. Serologic responses occur more commonly and are of higher titer among patients with extraintestinal systemic infection. Prozone reactions may occur at dilutions of 1 : 32 or lower. Marked cross agglutination occurs between *Y. enterocolitica* serotype O:9 and *Brucella abortus*, *Morganella morganii*, and *Salmonella*.[27,141] Antibodies to *Y. pseudotuberculosis* often are detectable at the onset of clinical signs of infection and may be highest during the acute phase of illness.[91]

Molecular Techniques

PCR assays have been developed for *Y. enterocolitica* and *Y. pseudotuberculosis* with use of primers that allow assessment of 16S rRNA sequences and of specific plasmid-encoded and chromosome-encoded genes.* PCR techniques have been used to identify *Yersinia* organisms in blood, tissue, stool, water, and food samples.[85,124]

TREATMENT

Most patients with yersiniosis do not require treatment because the disease usually is self-limited. Seriously ill

*References 39, 125, 154, 155, 223, 225.

patients generally have responded to treatment with chloramphenicol, gentamicin, or tetracyclines, but clinical success has not been uniform. Of these agents, tetracyclines have been the traditional agent of choice.[71,219] *Y. enterocolitica* isolates resistant to the tetracyclines have been reported in recent years, however. Two percent of a sample of *Y. enterocolitica* isolates from Canada in 1992 were resistant to tetracycline,[166] and 10 percent of a sample of *Y. enterocolitica* isolates from the Netherlands from 1982 to 1991 were resistant to doxycycline.[206]

More than 99 percent of 1060 isolates of *Y. enterocolitica* collected in Canada in the years 1972 to 1976, 1980, 1985, and 1990 were susceptible in vitro to piperacillin, cefotaxime, aztreonam, gentamicin, tobramycin, amikacin, trimethoprim-sulfamethoxazole, chloramphenicol, and ciprofloxacin. No evidence of decreasing susceptibility to any of these agents was found across the periods sampled. These results were mirrored by a study of 335 isolates obtained in the Netherlands from 1982 to 1991. All of these isolates were susceptible to ceftazidime, cefepime, imipenem, trimethoprim-sulfamethoxazole, ciprofloxacin, and ofloxacin. Aminoglycosides were effective against more than 99 percent, chloramphenicol was effective against 94 percent, and cefuroxime was effective against 90 percent. Seven multidrug-resistant isolates were present among the isolates from Canada, but none was found among the isolates from the Netherlands.[166,206]

Most *Y. enterocolitica* isolates, regardless of serotype, are resistant to ampicillin, ticarcillin, and first-generation cephalosporins. Most of them also are resistant to amoxicillin-clavulanic acid. This is likely due to expression of β-lactamase *BlaA* and/or *BlaB* genes, because most *Y. enterocolitica* strains harbor both.[22] Azithromycin was active in vitro against 50 percent of the 335 isolates in the Netherlands, but almost all isolates were resistant to erythromycin and clarithromycin.[166,206] Antimicrobial resistance of *Y. enterocolitica* strains in Europe does not appear to have increased in recent years.[22]

The decreasing effectiveness of tetracyclines in vitro raises the question of whether these agents should be the first choice for treatment of infection caused by *Y. enterocolitica*. A retrospective review of 43 cases (with patient ages ranging from 3 to 89 years) treated for *Y. enterocolitica* septicemia in France between 1985 and 1991 showed that third-generation cephalosporins were effective in 85 percent of cases in which they were used, although aminoglycosides or fluoroquinolones usually were administered concurrently.[66] Fluoroquinolones alone or in combination with other agents cured all 15 patients. Seven children with bacteremia and diarrhea in Michigan responded well to cefotaxime.[1]

In a double-blind, placebo-controlled trial of trimethoprim-sulfamethoxazole for treatment of children with gastroenteritis caused by *Y. enterocolitica*, the clinical course of illness was not shortened.[163] The children had been ill for a mean of 12 days before treatment was begun, however.

Systemic infections, extraintestinal focal infections, and enterocolitis in compromised hosts should be treated with antibiotics.[50] The in vitro susceptibilities and limited clinical data suggest that children with such infections should be treated with a third-generation cephalosporin, an aminoglycoside, or both. Trimethoprim-sulfamethoxazole also may be used.[40,93] Fluoroquinolones also probably would be effective. Cefotaxime and ceftriaxone were effective in treating bacteremia in a series of 12 children.[2]

Even fewer clinical and in vitro susceptibility data are available for *Y. pseudotuberculosis*. These infections probably can be managed identically to *Y. enterocolitica* infections. A recent analysis of 12 strains in Germany found no resistance to agents tested, including ampicillin.[22] All isolates of *Y. enterocolitica* and *Y. pseudotuberculosis* should be examined for susceptibility to a variety of antibacterial drugs.

VACCINES

There are no vaccines for *Yersinia* species at this time, although work to develop a new and improved vaccine against *Y. pestis* is ongoing. An attenuated *Y. pseudotuberculosis* strain (IP32953) transformed to express the *Y. pestis* F1 capsule conferred 100 percent protection in mice against a high challenge dose of *Y. pestis* after a single oral dose of the vaccine strain.[55]

OTHER *YERSINIA* SPECIES

Eleven other "non-pestis" *Yersinia* spp. (*Y. frederiksenii*, *Y. intermedia*, *Y. kristensenii*, *Y. aldovae*, *Y. bercovieri*, *Y. mollaretii*, *Y. rohdei*, *Y. alecksiciae*, *Y. similiensis*, *Y. massiliensis*, and *Y. ruckeri*) occasionally have been isolated from clinical specimens, but their roles as human pathogens remain unclear.[74,192,194,207] Each except *Y. aldovae* has been isolated from humans worldwide, including individuals with gastrointestinal disorders.[207] These microbes also can be found in fresh-water sources, sewage, dogs, pigs, cattle, wild mammals, birds, reptiles, fish, and some foods, especially milk and meat products.

These organisms are similar biochemically to one another and have been termed atypical *Y. enterocolitica*.[50] None contains the *Yersinia* virulence plasmid pYV.[145] Some contain other large plasmids distinct from pYV, carriage of which may be associated with the ability to cause diarrhea in humans.[164,207] A heat-stable enterotoxin has been found in isolates of *Y. bercovieri*.[208] These *Yersinia* spp. can grow at 4° C (39.2° F) and on cefsulodin-irgasan-novobiocin agar and can multiply in refrigerated foods.

These species occasionally may cause diarrhea and other gastrointestinal symptoms, especially in immunocompromised hosts or individuals with gastric acid suppression.[133] Use of cold enrichment techniques may enhance recovery of these organisms. Their isolation from clinical specimens should not be disregarded or deemed causative of clinical disease without careful epidemiologic considerations.[74] Isolates of these species sometimes can be misidentified as *Y. enterocolitica* by commercial identification systems used in many clinical laboratories. Differences in colony morphology, pyrazinamide reactions (negative for *Y. enterocolitica*), and other biochemical tests can be used to distinguish them from *Y. enterocolitica*.[80,109]

NEW REFERENCES SINCE THE SIXTH EDITION

17. Batzilla J, Antonenka U, Höpper D, et al. *Yersinia enterocolitica palearctica* serobiotype O:3/4: a successful group of emerging zoonotic pathogens. *BMC Genomics* 2011;**12**:348. http://www.biomedcentral.com/1471-2164/12/348.
22. Bonke R, Wacheck S, Stüber E, et al. Antimicrobial susceptibility and distribution of β-lactamase A (*blaA)* and β-lactamase B *(blaB)* genes in enteropathogenic *Yersinia* species. *Microbial Drug Resist* 2011;**17**:575–81.
23. Boqvist S, Pettersson H, Svensson A, et al. Sources of sporadic *Yersinia enterocolitica* infection in children in Sweden, 2004: a case-control study. *Epidemiol Infect* 2008;**137**:897–905.
43. Ch'ng SL, Octavia S, Xia Q, et al. Population structure and evolution of pathogenicity of *Yersinia enterocolitica*. *Applied Environ Microbiol* 2011;**77**:768–75.
55. Derbise A, Marin AC, Ave P, et al. An encapsulated *Yersinia pseudotuberculosis* is a highly efficient vaccine against pneumonic plague. *PLOS Negl Trop Dis* 2012;**6**:e1528.
57. El Qouqa IA, El Jarou MA, Abu Samaha AS, et al. *Yersinia enterocolitica* infection among children aged less than 12 years: a case-control study. *Int J Infect Dis* 2011;**15**:e48–53.
75. Guinet F, Carniel E, Leclercq A. Transfusion-transmitted *Yersinia enterocolitica* sepsis. *Clin Infect Dis* 2011;**53**:583–91.
79. Hale CR, Scallan E, Cronquist AB, et al. Estimates of enteric illness attributable to contact with animals and their environments in the United States. *Clin Infect Dis* 2012;**54**:S472–9.
97. Huovinen E, Sihvonen LM, Virtanen MJ, et al. Symptoms and sources of *Yersinia enterocolitica*-infection: a case-control study. *BMC Infect Dis* 2010;**10**:122. http://www.biomedcentral.com/1471-2334/10/122.
106. Jung, C, Meinzer U, Montcuquet N, et al. *Yersinia pseudotuberculosis* disrupts intestinal barrier integrity through hematopoietic TLR-2 signaling. *J Clin Invest* 2012;**122**:2239–51.
134. Long C, Jones TF, Vugia DJ, et al. *Yersinia pseudotuberculosis* and *Yersinia entercolitica* infections, FoodNet, 1996-2007. *Emerg Infect Dis* 2010;**16**:566–7.
150. Montagner C, Arquint C, Cornelis GR. Translocators YopB and YopD from *Yersinia enterocolitica* form a multimeric integral membrane complex in eukaryotic cell membranes. *J Bacteriol* 2011; **193**:6923–8.
159. Nunes Rosado FG, Stratton CW, Mosse CA. Clinicopathologic correlation of epidemiologic and histopathologic features of pediatric bacterial lymphadenitis. *Arch Pathol Lab Med* 2011;**135**:1490–3.
160. Ong KL, Gould H, Chen DL, et al. Changing epidemiology of *Yersinia enterocolitica* infections: markedly decreased rates in young Black children, Foodborne Diseases Active Surveillance Network (FoodNet) 1996-2009. *Clin Infect Dis* 2012;**54**:S385–90.
177. Reuter S, Thomson NR, McNally A. Evolutionary dynamics of the *Yersinia enterocolitica* complex. *Adv Experimental Med Biol* 2012;**954**:15–22.
182. Rosner BM, Stark K, Höhle M, et al. Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009-2010. *Epidemiol Infect* 2012;**140**:1738–47.
190. Schmidt G. *Yersinia enterocolitica* outer protein T (YopT). *Eur J Cell Biol* 2011;**90**:955–8.
194. Sihvonen LM, Haukka K, Kuusi M, et al. *Yersinia enterocolitica* and *Y. enterocolitica*-like species in clinical stool specimens of humans: identification and prevalence of bio/serotypes in Finland. *Eur J Clin Microbiol Infect Dis* 2009;**28**:757–65.
195. Sihvonen LM, Jalkanen K, Huovinen E, et al. Clinical isolates of *Yersinia enterocolitica* biotype 1A represent two phylogenetic lineages with differing pathogenicity-related properties. *BMC Microbiol* 2012;**12**:208.
205. Stephan R, Cernela N, Ziegler D, et al. Rapid species specific identification and subtyping of *Yersinia enterocolitica* by MALDI-TOF mass spectrometry. *J Microbiol Methods* 2011;**87**:150–3.
210. Tansini A, Verdan da Silva FF, Rodrigues dos Santos R, et al. Yop effector proteins from *Yersinia pseudotuberculosis* impair dendritic cell activation. *Adv Experimental Med Biol* 2012;**954**:203–8.
226. Wang X, Yang L, Jing H, et al. Complete genome sequence of a *Yersinia enterocolitica* "Old World" (3/O:9) strain and comparison with the "New World" (1B/O:8) strain. *J Clin Microbiol* 2011;**49**: 1251–9.
236. Zhang Y, Romanov G, Bliska JB. Type III secretion system-dependent translocation of ectopically expressed Yop effectors into macrophages by intracellular *Yersinia pseudotuberculosis*. *Infect Immun* 2011;**79**:4322–31.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 114

Miscellaneous Enterobacteriaceae

Randall G. Fisher

Three less commonly isolated organisms of the family Enterobacteriaceae are *Edwardsiella tarda*, *Hafnia alvei*, and *Pantoea agglomerans.* Each of these organisms, although uncommon, can cause significant disease in certain clinical circumstances.[65]

EDWARDSIELLA TARDA

BACTERIOLOGY

E. tarda is a non–lactose-fermenting, gram-negative bacillus that is indole positive and produces hydrogen sulfide. It ferments only glucose and maltose. The species name *tarda* reflects its biochemical inactivity. It usually is lysine positive and ornithine decarboxylase positive.[27] The organism resembles *Salmonella* biochemically and

REFERENCES

1. Abdel-Haq NM, Asmar BI, Abuhammour WM, et al. *Yersinia enterocolitica* infection in children. *Pediatr Infect Dis J* 2000;**19**: 954–8.
2. Abdel-Haq NM, Papadopol R, Asmar BI, et al. Antibiotic susceptibilities of *Yersinia enterocolitica* recovered from children over a 12-year period. *Int J Antimicrob Agents* 2006;**27**:449–52.
3. Abe J, Onimaru M, Matsumoto S, et al. Clinical role for a superantigen in *Yersinia pseudotuberculosis* infection. *J Clin Invest* 1997; **99**:1823–30.
4. Achtman M, Zurth K, Morelli G, et al. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proc Natl Acad Sci U S A* 1999;**96**:14043–8.
5. Ackers ML, Schoenfeld S, Markman J, et al. An outbreak of *Yersinia enterocolitica* O:8 infections associated with pasteurized milk. *J Infect Dis* 2000;**181**:1834–7.
6. Adamkiewicz TV, Berkovitch M, Krishman C, et al. Infection due to *Yersinia enterocolitica* in a series of patients with β-thalassemia: incidence and predisposing factors. *Clin Infect Dis* 1998;**27**: 1362–6.
7. Ahlqvist J, Ahvonen P, Räsänen JA, et al. Enteric infection with *Yersinia enterocolitica*: large pyroninophilic cell reaction in mesenteric lymph nodes associated with early production of specific antibodies. *Acta Pathol Microbiol Scand* 1971;**79A**:109–22.
8. Aho K, Ahvonen P, Lassus A, et al. HL-A 27 in reactive arthritis: a study of *Yersinia* arthritis and Reiter's disease. *Arthritis Rheum* 1974;**17**:521–6.
9. Ahvonen P. Human yersiniosis in Finland. I. Bacteriology and serology. *Ann Clin Res* 1972;**4**:30–8.
10. Ahvonen P, Sievers K, Aho K. Arthritis associated with *Yersinia enterocolitica* infection. *Acta Rheumatol Scand* 1969;**15**:232–53.
11. Albrecht H. Zur Aetiologie der *Enteritis follicularis* suppurativa. *Wien Klin Wochenschr* 1910;**23**:991.
12. alMohsen I, Luedtke G, English BK. Invasive infections caused by *Yersinia enterocolitica* in infants. *Pediatr Infect Dis J* 1997;**16**: 253–5.
13. Anonymous. Worldwide spread of infection with *Yersinia enterocolitica*. *WHO Chron* 1976;**30**:494–6.
14. Arvastson B, Damgaard K, Winblad S. Clinical symptoms of infection with *Yersinia enterocolitica*. *Scand J Infect Dis* 1971;**3**:37–40.
15. Asakawa Y, Akahane S, Kheata N, et al. Two community outbreaks of human infection with *Yersinia enterocolitica*. *J Hyg (Camb)* 1973; **71**:715–23.
16. Attwood SE, Healy K, Caffarkey MT, et al. *Yersinia* infection and abdominal pain. *Lancet* 1987;**1**:529–33.
17. Batzilla J, Antonenka U, Höpper D, et al. *Yersinia enterocolitica palearctica* serobiotype O:3/4—a successful group of emerging zoonotic pathogens. *BMC Genomics* 2011;**12**:348. http://www.biomedcentral.com/1471-2164/12/348.
18. Bergstrand CG, Winblad S. Clinical manifestations of infection with *Yersinia enterocolitica* in children. *Acta Paediatr Scand* 1974; **63**:875–7.
19. Bissett ML. *Yersinia enterocolitica* isolates from humans in California, 1968-1975. *J Clin Microbiol* 1976;**4**:137–44.
20. Black RE, Jackson RJ, Tsai T, et al. Epidemic *Yersinia enterocolitica* infection due to contaminated chocolate milk. *N Engl J Med* 1978; **298**:76–9.
21. Blattner RJ. Acute mesenteric lymphadenitis. *J Pediatr* 1969; **74**:479–81.
22. Bonke R, Wacheck S, Stüber E, et al. Antimicrobial susceptibility and distribution of β-lactamase A (*blaA)* and β-lactamase B *(blaB)* genes in enteropathogenic *Yersinia* species. *Microbial Drug Resis* 2011;**17**:575–81.
23. Boqvist S, Pettersson H, Svensson A, et al. Sources of sporadic *Yersinia enterocolitica* infection in children in Sweden, 2004: a case-control study. *Epidemiol Infect* 2008;**137**:897–905.
24. Bottone EJ. *Yersinia enterocolitica*: a panoramic view of a charismatic microorganism. *Crit Rev Microbiol* 1977;**5**:211–41.
25. Bottone EJ. Current trends of *Yersinia enterocolitica* isolates in the New York City area. *J Clin Microbiol* 1983;**17**:63–7.
26. Bottone EJ. *Yersinia enterocolitica:* overview and epidemiologic correlates. *Microbes Infect* 1999;**1**:323–33.
27. Bottone EJ, Sheehan DJ. *Yersinia enterocolitica*: guidelines for serologic diagnosis of human infections. *Rev Infect Dis* 1983;**5**: 898–906.
28. Bottone EJ, Chester B, Malowany MS, et al. Unusual *Yersinia enterocolitica* isolates not associated with mesenteric lymphadenitis. *Appl Microbiol* 1974;**27**:858–61.
29. Bovallius A, Nilsson G. Ingestion and survival of *Y. pseudotuberculosis* in HeLa cells. *Can J Microbiol* 1975;**21**:1997–2007.
30. Bradford W, Noce P, Gutman L. Pathologic features of enteric infection with *Yersinia enterocolitica*. *Arch Pathol* 1974;**98**:17–22.
31. Braunstein H, Tucker EB, Gibson BC. Mesenteric lymphadenitis due to *Yersinia enterocolitica:* report of a case. *Am J Clin Pathol* 1971;**55**:506–10.
32. Brennessel DJ, Robbins H, Hindman S. Pyomyositis caused by *Yersinia enterocolitica*. *J Clin Microbiol* 1984;**20**:293–4.
33. Brocklehurst TE, Zaman-Wong CH, Lund BM. A note on the microbiology of retail packs of prepared salad vegetables. *J Appl Bacteriol* 1987;**63**:409–15.
34. Cancino-Diaz JC, Vargas-Rodriguez L, Grinberg-Zylberbaum N, et al. High levels of IgG class antibodies to recombinant HSP60 kDA of *Yersinia enterocolitica* in sera of patients with uveitis. *Br J Ophthalmol* 2004;**88**:247–50.
35. Caplan LM. *Yersinia enterocolitica* septicemia. *Am J Clin Pathol* 1978;**69**:189.
36. Caprioli T, Drapeau AJ, Kasatiya S. *Yersinia enterocolitica:* serotypes and biotypes isolated from humans and the environment in Quebec, *Canada J Clin Microbiol* 1978;**8**:7–11.
37. Centers for Disease Control. *Yersinia enterocolitica* bacteremia and endotoxin shock associated with red blood cell transfusions: United States, 1991. *MMWR Morb Mortal Wkly Rep* 1991;**40**: 176–8.
38. Centers for Disease Control and Prevention. *Yersinia enterocolitica* gastroenteritis among infants exposed to chitterlings: Chicago, Illinois, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:956–8.
39. Cheong HI, Park HW, Koo JW, et al. Diagnosis of *Yersinia pseudotuberculosis* infection by polymerase chain reaction. *Pediatr Infect Dis J* 1996;**15**:596–9.
40. Cherchi GB, Pacifico L, Cossellu S, et al. Prospective study of *Yersinia enterocolitica* infection in thalassemic patients. *Pediatr Infect Dis J* 1995;**14**:579–84.
41. Chessum B, Frengley JD, Fleck DG, et al. Case of septicemia due to *Yersinia enterocolitica*. *B M J* 1971;**3**:466.
42. Chin GN, Noble RC. Ocular involvement in *Yersinia enterocolitica* infections presenting as Parinaud's oculoglandular syndrome. *Am J Ophthalmol* 1977;**83**:19–23.
43. Ch'ng SL, Octavia S, Xia Q, et al. Population structure and evolution of pathogenicity of *Yersinia enterocolitica*. *Applied Environ Microbiol* 2011;**77**:768–75.
44. Cimolai N, Trombley C, Blair G. Implications of *Yersinia enterocolitica* biotyping. *Arch Dis Child* 1994;**70**:19–21.
45. Cookson ST, Arduino MJ, Aguero SM, et al. *Yersinia enterocolitica*–contaminated red blood cells (RBCs): an emerging threat to blood safety. Abstract J99. Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La, American Society for Microbiology, September 15-18, 1996, p. 237.
46. Cornelis G, Laroche Y, Balligand G, et al. *Yersinia enterocolitica*, a primary model for bacterial invasiveness. *Rev Infect Dis* 1987;**9**: 64–86.
47. Cornelis GR. *Yersinia* type III secretion: send in the effectors. *J Cell Biol* 2002;**158**:401–8.
48. Cornelis GR, Boland A, Boyd AP, et al. The virulence plasmid of *Yersinia*, an antihost genome. *Microbiol Mol Biol Rev* 1998;**62**: 1315–52.
49. Cossart P. Subversion of the mammalian cell cytoskeleton by invasive bacteria. *J Clin Invest* 1997;**100**(Suppl.):S33–7.
50. Cover TL, Aber RC. *Yersinia enterocolitica*. *N Engl J Med* 1989; **321**:16–24.
51. Cusack D, Martin P, Schinittger T, et al. Nephropathy in association with *Yersinia enterocolitica*. *Ir J Med Sci* 1983;**152**:311–12.
52. Dajani A, Maurer M. Is *Yersinia enterocolitica* gastroenteritis a Canadian disease? *J Pediatr* 1980;**97**:165–6.
53. Daniels JJH. Enteric infections with *Pasteurella pseudotuberculosis:* an acute abdominal syndrome. *J Int Coll Surg* 1962;**38**:397–411.
54. Delorme J, Laverdiere M, Martineau B, et al. Yersiniosis in children. *Can Med Assoc J* 1974;**110**:281–4.
55. Derbise A, Marin AC, Ave P, et al. An encapsulated *Yersinia pseudotuberculosis* is a highly efficient vaccine against pneumonic

plague. *PLOS Negl Trop Dis* 2012;**6**:e1528. Doi:10.1371/journal.pntd.0001528.
56. Dube PH, Handley SA, Lewis J, et al. Protective role of interleukin 6 during *Yersinia enterocolitica* infection is mediated through the modulation of inflammatory cytokines. *Infect Immun* 2004;**72**:3561–70.
57. El Qouqa IA, El Jarou MA, Abu Samaha AS, et al. *Yersinia enterocolitica* infection among children aged less than 12 years: a case-control study. *Int J Infect Dis* 2011;**15**:e48–53.
58. Fällman M, Persson C, Wolf-Watz H. *Yersinia* proteins that target host cell signaling pathways. *J Clin Invest* 1997;**100**(Suppl.):S15–18.
59. Farmer JJ, III, Carter GP, Miller VL, et al. Pyrazinamidase, CR-MOX agar, salicin fermentation-esculin hydrolysis, and d-xylose fermentation for identifying pathogenic serotypes of *Yersinia enterocolitica*. *J Clin Microbiol* 1992;**30**:2589–94.
60. Feldman WH, Karlson AG. Pseudotuberculosis. In: Hull T, editor. *Diseases Transmitted from Animals to Man*. 5th ed. Springfield, IL: Charles C Thomas; 1963.
61. Fenwick SG, McCarthy MD. *Yersinia enterocolitica* is a common cause of gastroenteritis in Auckland. *N Z Med J* 1995;**108**:269–71.
62. Finlayson NB, Fagundes B. *Pasteurella pseudotuberculosis* infection: three cases in the United States. *Am J Clin Pathol* 1971;**55**:24–9.
63. Fontani C, Valeri M, Pifferesi M. *Yersinia enterocolitica* septicemia in a girl with thalassemia major. *Pediatr Med Clin* 1988;**10**:657–8.
64. Frederiksen W. Human pseudotuberculosis in Denmark. *Symp Series Immunobiol Stand* 1968;**9**:137.
65. Friedberg M, Denneberg T, Brun C, et al. Glomerulonephritis in infections with *Yersinia enterocolitica* O-serotype 3. *Acta Med Scand* 1981;**209**:103–10.
66. Gayraud M, Scavizzi MR, Mollaret HH, et al. Antibiotic treatment of *Yersinia enterocolitica* septicemia: a retrospective review of 43 cases. *Clin Infect Dis* 1993;**17**:405–10.
67. Gilbert R. *Interesting cases and unusual specimens*. Annual Report of the Division of Laboratory Research. Albany, NY: New York State Department of Health; 1933. p. 57.
68. Gillespie JH, Timoney JF. *Hagan and Bruner's Infectious Diseases of Domestic Animals*. 7th ed. Ithaca, NY: Comstock; 1981. p. 96–7.
69. Gleason TH, Patterson SD. The pathology of *Yersinia enterocolitica* ileocolitis. *Am J Surg Pathol* 1982;**6**:347–55.
70. Glud TK, Laursen B. *Yersinia enterocolitica* infection complicated by severe thrombocytopenia resistant to high-dose intravenous immunoglobulin. *Acta Med Scand* 1985;**217**:233–4.
71. Goodwin CS. *Yersinia* infections including mesenteric adenitis, and gastrointestinal tuberculosis. In: Goodwin CS, editor. *Microbes and Infection of the Gut*. Melbourne: Blackwell Scientific; 1984. p. 241–51.
72. Gort AS, Miller VL. Identification and characterization of *Yersinia enterocolitica* genes induced during systemic infection. *Infect Immun* 2000;**68**:6635–42.
73. Grant T, Bennett-Wood V, Robins-Browne RM. Characterization of the interaction between *Yersinia enterocolitica* biotype 1A and phagocytes and epithelial cells in vitro. *Infect Immun* 1999;**67**:4307–75.
74. Gray LD. *Escherichia*, *Salmonella*, *Shigella*, and *Yersinia*. In: Murray PR, editor. *Manual of Clinical Microbiology*. 6th ed. Washington D.C.: ASM Press; 1995. p. 450–6.
75. Guinet F, Carniel E, Leclercq A. Transfusion-transmitted *Yersinia enterocolitica* sepsis. *Clin Infect Dis* 2011;**53**:583–91.
76. Gutman LT, Ottesen EA, Quan TJ, et al. An inter-familial outbreak of *Yersinia enterocolitica* enteritis. *N Engl J Med* 1973;**288**:1372–7.
77. Haenselt V. Zur Kenntnis der abscedierenden reticulocytaren Lymphadenitis (Masshoff). *Arzt Wochenschr* 1957;**12**:509.
78. Hagan AG, Lassen J, Berge LN. Erysipelas-like disease caused by *Yersinia enterocolitica*. *Scand J Infect Dis* 1974;**6**:101–2.
79. Hale CR, Scallan E, Cronquist AB, et al. Estimates of enteric illness attributable to contact with animals and their environments in the United States. *Clin Infect Dis* 2012;**54**:S472–9.
80. Hallanvuo S, Peltola J, Heiskanen T, Siitonen A. Simplified phenotypic scheme evaluated by 16S rRNA sequencing for differentiation between *Yersinia enterocolitica* and *Y. enterocolitica*-like species. *J Clin Microbiol* 2006;**44**:1077–80.
81. Hallstrom K, Sairanen E, Ohela K. A pilot clinical study of yersinioses in South-Eastern Finland. *Acta Med Scand* 1972;**191**:485–91.
82. Hamasaki S, Hayashidani H, Keneko K, et al. A survey for *Yersinia pseudotuberculosis* in migratory birds in coastal Japan. *J Wildl Dis* 1989;**25**:401–3.
83. Handley SA, Newberry RD, Miller VL. *Yersinia enterocolitica* invasion-dependent and invasion-independent mechanisms of systemic dissemination. *Infect Immun* 2005;**73**:8453–5.
84. Hansen PS, Wenzel BE, Brix TH, et al. *Yersinia enterocolitica* infection does not confer an increased risk of thyroid antibodies: evidence from a Danish twin study. *Clin Exp Immunol* 2006;**146**:32–8.
85. HaoXuan Z, JiDe W, MingJun Z, et al. *Yersinia enterocolitica* identification in stool samples using real time PCR. *Diagn Microbiol Infect Dis*. doi:10.1016/j.diagmicrobio. 2006.07.016.
86. Hassig A, Karrer J, Pusterla F. Über Pseudotuberculoses beim Menschen. *Schweiz Med Wochenschr* 1949;**79**:791.
87. Hastings JGM, Batta K, Gourevitch D, et al. Fatal transfusion reaction due to *Yersinia enterocolitica*. *J Hosp Infect* 1993;**27**:75–9.
88. Hayashidani H, Ohtomo Y, Toyokawa Y, et al. Potential sources of sporadic human infection with *Yersinia enterocolitica* serovar O:8 in Aomori Prefecture, Japan. *J Clin Microbiol* 1995;**33**:1253–7.
89. Heesemann J, Gaede K, Autenrieth IB. Experimental *Yersinia enterocolitica* infection in rodents: a model for human yersiniosis. *A P M I S* 1993;**101**:417–29.
90. Hewstone AS, Davidson GP. *Yersinia enterocolitica* septicaemia with arthritis in a thalassaemic child. *Med J Aust* 1972;**1**:1035–8.
91. Hnatko SI, Rodin AE. *Pasteurella pseudotuberculosis* infection in man. *Can Med Assoc J* 1963;**88**:1108–12.
92. Hoogkamp-Korstanje JAA, Stolk-Engelaar VMM. *Yersinia enterocolitica* infection in children. *Pediatr Infect Dis J* 1995;**14**:771–5.
93. Hoogkamp-Korstanje JAA, De Koning J, Samsom JP. Incidence of human infection with *Yersinia enterocolitica* serotypes O3, O8, and O9 and the use of indirect immunofluorescence in diagnosis. *J Infect Dis* 1986;**153**:138–41.
94. Horstebrock R. Zur Frage der "abscedierenden, retikulocytaren Lymphadenitis (Masshoff)." *Zentralbl Allg Pathol* 1954;**91**:221.
95. Hubbert WT. Yersiniosis in mammals and birds in the United States: case reports and review. *Am J Trop Med Hyg* 1972;**21**:458–63.
96. Hubbert WT, Petenyi CW, Glasgow LA, et al. *Yersinia tuberculosis* infection in the United States: septicemia, appendicitis, and mesenteric lymphadenitis. *Am J Trop Med Hyg* 1971;**20**:679–84.
97. Huovinen E, Sihvonen LM, Virtanen MJ, et al. Symptoms and sources of *Yersinia enterocolitica*-infection: a case-control study. *BMC Infect Dis* 2010;**10**:122. http://www.biomedcentral.com/1471-2334/10/122.
98. Huppertz H, Heesemann J. Experimental *Yersinia* infection of human synovial cells: persistence of live bacteria and generation of bacterial antigen deposits including "ghosts," nucleic acid-free bacterial rods. *Infect Immun* 1996;**64**:1484–7.
99. Inoue H, Nakashima H, Ishida T, et al. Three outbreaks of *Yersinia pseudotuberculosis* infections. *Zentralbl Bakteriol Mikrobiol Hyg* 1988;**186**:504–11.
100. Isberg RR. Mammalian cell adhesion functions and cellular penetration of enteropathogenic *Yersinia* species. *Mol Microbiol* 1989;**3**:1449–53.
101. Isberg RR, Voorhis DL, Falkow S. Identification of invasin: a protein that allows enteric bacteria to penetrate cultured mammalian cells. *Cell* 1987;**50**:769–78.
102. Jalava K, Hakkinen M, Valkonen M, et al. An outbreak of gastrointestinal illness and erythema nodosum from grated carrots contaminated with *Yersinia pseudotuberculosis*. *J Infect Dis* 2006;**194**:1209–16.
103. Jansson E, Wallgren GR, Ahvonen P. *Yersinia enterocolitica* as a cause of acute mesenteric lymphadenitis. *Acta Paediatr Scand* 1968;**57**:448–50.
104. Jepsen OB, Korner B, Lauritsen KB, et al. *Yersinia enterocolitica* infection in patients with acute surgical abdominal disease: a prospective study. *Scand J Infect Dis* 1976;**8**:189–94.
105. Jones BL, Hanson MF. Prevention of transfusion of *Yersinia enterocolitica*. *J Hosp Infect* 1994;**28**:236–8.
106. Jung, C, Meinzer U, Montcuquet N, et al. *Yersinia pseudotuberculosis* disrupts intestinal barrier integrity through hematopoietic TLR-2 signaling. *J Clin Invest* 2012;**122**:2239–51.

107. Kandolo K, Wauters G. Pyrazinamidase activity in *Yersinia enterocolitica* and related organisms. *J Clin Microbiol* 1985;**21**: 980–2.
108. Kato Y, Ito K, Kubo Y, et al. Occurrence of *Yersinia enterocolitica* in wild-living birds. *Appl Environ Microbiol* 1985;**49**:198–200.
109. Kay BA, Wachsmuth K, Gemski P, et al. Virulence and phenotypic characterization of *Yersinia enterocolitica* isolated from humans in the United States. *J Clin Microbiol* 1983;**17**:128–38.
110. Kelly DA, Price E, Jani B, et al. *Yersinia* enterocolitis vs. iron overload. *J Pediatr Gastroenterol Nutr* 1987;**6**:643–5.
111. Kerschen EJ, Cohen DA, Kaplan AM, et al. The plague virulence protein YopM targets the innate immune response by causing a global depletion of NK cells. *Infect Immun* 2004;**72**:4589–602.
112. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. *Clin Infect Dis* 2006;**43**:807–13.
113. Knapp W. *Pasteurella pseudotuberculosis* als erreger einer mesenterialen Lymphadenitis beim Menschen. *Zentralbl Bakteriol* 1954; **161**:422–4.
114. Knapp W. Mesenteric adenitis due to *Pasteurella pseudotuberculosis* in young people. *N Engl J Med* 1958;**259**:776–8.
115. Knapp W, Masshoff W. Zur Ätiologie der abszedierenden retikulozytaren Lymphadenitis: einer praktisch wichtigen, vielfach unter dem Bilde einer akuten Appendizitis verlaufenden Erkrankung. *Dtsch Med Wochenschr* 1954;**79**:1266–71.
116. Knapp W, Steuter W. Untersuchungen über den Nachweis komplementbindender und agglutinierender Antikorper gegen *Pasteurella pseudotuberculosis* in Sera infizierter und immunisierter Menschen and Tiere. *Z Immunitatsforsch Exp Ther* 1956;**113**: 370–4.
117. Knorring von J, Petterson T. Haemolytic anaemia complicating *Yersinia enterocolitica* infection: report of a case. *Scand J Haematol* 1972;**9**:149.
118. Kobayashi S, Ogasawara M, Maeda K, et al. Antibodies against *Yersinia enterocolitica* in patients with Reiter's syndrome. *J Lab Clin Med* 1985;**105**:380–9.
119. Kohl S, Jacobson JA, Nahmias A. *Yersinia enterocolitica* infections in children. *J Pediatr* 1976;**89**:77–9.
120. Koning-Ward TF, Robins-Browne RM. Analysis of the urease gene complex of members of the genus *Yersinia*. *Gene* 1996; **182**:225–8.
121. Koo JW, Park SN, Choi SM, et al. Acute renal failure associated with *Yersinia pseudotuberculosis* infection in children. *Pediatr Nephrol* 1996;**10**:582–6.
122. Krogstad P, Mendelman PM, Miller VL, et al. Clinical and microbiologic characteristics of cutaneous infection with *Yersinia enterocolitica*. *J Infect Dis* 1992;**165**:740–3.
123. Lahesmaa R, Soderberg C, Bliska J, et al. Pathogen antigen- and superantigen-reactive synovial fluid T cells in reactive arthritis. *J Infect Dis* 1995;**172**:1290–7.
124. Lamps LW, Havens JM, Gilbrech LJ, et al. Molecular biogrouping of pathogenic *Yersinia enterocolitica*: development of a diagnostic PCR assay with histologic correlation. *Am J Clin Pathol* 2006; **125**:658–64.
125. Lamps LW, Madhusudhan KT, Greenson JK, et al. The role of *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* in granulomatous appendicitis: a histologic and molecular study. *Am J Surg Pathol* 2001;**25**:508–15.
126. Larsen JH. Human yersiniose. *Ugeskr Laeger* 1972;**134**:431.
127. Lee LA, Taylor J, Carter GP, et al. *Yersinia enterocolitica* O:3: An emerging cause of pediatric gastroenteritis in the United States. *J Infect Dis* 1991;**163**:660–3.
128. Lee VT, Schneewind O. Type III secretion machines and the pathogenesis of enteric infections caused by *Yersinia* and *Salmonella* spp. *Immunol Rev* 1999;**168**:241–55.
129. Leino R, Kalliomaki JL. Yersiniosis as an internal disease. *Ann Intern Med* 1974;**81**:458–61.
130. Leino R, Granfars K, Havia T, et al. Yersiniosis as a gastrointestinal disease. *Scand J Infect Dis* 1987;**19**:63–8.
131. Linde H-J, Neubauer H, Meyer H, et al. Identification of *Yersinia* species by the Vitek GNI Card. *J Clin Microbiol* 1999; **37**:211–14.
132. Lobato MJ, Landeras E, González-Hevia MA, et al. Genetic heterogeneity of clinical strains of *Yersinia enterocolitica* traced by ribotyping and relationships between ribotypes, serotypes, and biotypes. *J Clin Microbiol* 1998;**36**:3297–302.
133. Loftus CG, Harewood GC, Cockerill FR, III, et al. Clinical features of patients with novel *Yersinia* species. *Dig Dis Sci* 2002;**47**: 2805–10.
134. Long C, Jones TF, Vugia DJ, et al. *Yersinia pseudotuberculosis* and *Yersinia entercolitica* infections, FoodNet, 1996-2007. *Emerg Infect Dis* 2010;**16**:566–7.
135. Mair NS. Yersiniosis (infections due to *Yersinia pseudotuberculosis* and *Yersinia enterocolitica*). In: Hubbert WT, McCollough WF, Schnurrenberger PR, editors. *Diseases Transmitted from Animals to Man*. 6th ed. Springfield, IL: Charles C Thomas; 1975. p. 174–85.
136. Mair NS, Mair HJ, Stirk EM, et al. Three cases of acute mesenteric lymphadenitis due to *Pasteurella pseudotuberculosis*. *J Clin Pathol* 1960;**13**:432–9.
137. Maki M, Gronroos P, Vesikari T. In vitro invasiveness of *Yersinia enterocolitica* isolated from children with diarrhea. *J Infect Dis* 1978;**138**:677–80.
138. Maki M, Vesikari T, Rantala I, et al. Yersiniosis in children. *Arch Dis Child* 1980;**55**:861–5.
139. Makino S, Okada Y, Maruyama T, et al. PCR-based random amplified polymorphic DNA fingerprinting of *Yersinia pseudotuberculosis* and its practical applications. *J Clin Microbiol* 1994;**32**: 65–9.
140. Malassez L, Vignal W. Tuberculos zoologique (forme ou aspect ou tuberculose sans bacillus). *Arch Physiol Norm Pathol* 1883; **53**:2.
141. Marks MI, Pai CH, Lafleur L, et al. *Yersinia enterocolitica* gastroenteritis: a prospective study of clinical, bacteriologic, and epidemiologic features. *J Pediatr* 1980;**96**:26–31.
142. Masshoff W. Eine neuartige Form der mesenterialen Lymphadenitis. *Dtsch Med Wochenschr* 1953;**78**:532–5.
143. Masshoff W, Dolle W. Über eine besondere Form der sog. mesenterialen Lymphadenopathie: "Die abscedierende reticulocytare Lymphadenitis." *Virchows Arch Pathol Anat* 1953;**323**: 664–84.
144. Mertz AKH, Ugrinovic S, Lauster R, et al. Characterization of the synovial T cell response to various recombinant *Yersinia* antigens in *Yersinia enterocolitica*–triggered reactive arthritis. *Arthritis Rheum* 1998;**41**:315–26.
145. Miller VL, Farmer JJ III, Hill WE, et al. The Ail locus is found uniquely in *Yersinia enterocolitica* serotypes commonly associated with disease. *Infect Immun* 1989;**57**:121–31.
146. Mingrone MG, Fantasia M, Figura N, et al. Characteristics of *Yersinia enterocolitica* isolated from children with diarrhea in Italy. *J Clin Microbiol* 1987;**25**:1301–4.
147. Mollaret H-H. Un domaine pathologique nouveau. *Ann Biol Clin* 1972;**30**:1–5.
148. Mollaret HH, Bercovier H, Alonso JM. Summary of the data received at the WHO Reference Center for *Yersinia enterocolitica*. *Contrib Microbiol Immunol* 1979;**5**:174–84.
149. Mollaret H-H, Omland T, Henriksen SD, et al. Les septicemies humaines: "*Yersinia enterocolitica*": propos de dix-sept cas recents. *Presse Med* 1971;**19**:345.
150. Montagner C, Arquint C, Cornelis GR. Translocators YopB and YopD from *Yersinia enterocolitica* form a multimeric integral membrane complex in eukaryotic cell membranes. *J Bacteriol* 2011;**193**:6923–8.
151. Mukherjee S, Keitany G, Li Y, et al. *Yersinia* YopJ acetylates and inhibits kinase activation by blocking phosphorylation. *Science* 2006;**312**:1211–14.
152. Najdenski H, Iteman I, Carniel E. Efficient subtyping of pathogenic *Yersinia enterocolitica* strains by pulsed-field gel electrophoresis. *J Clin Microbiol* 1994;**32**:2913–20.
153. Naqvi SH, Swierkosz EM, Gerard J, et al. Presentation of *Yersinia enterocolitica* enteritis in children. *Pediatr Infect Dis J* 1993;**12**: 386–9.
154. Neubauer H, Hensel A, Aleksic S, et al. Evaluation of a *Yersinia* adhesion gene (yadA) specific PCR for the identification of enteropathogenic *Yersinia enterocolitica*. *Int J Food Microbiol* 2000; **57**:225–7.
155. Neubauer H, Hensel A, Aleksic S, et al. Identification of *Yersinia enterocolitica* within the genus *Yersinia*. *Syst Appl Microbiol* 2000;**23**:58–62.
156. Nikolova S, Tzvetkov Y, Najdenski H, et al. Isolation of pathogenic *Yersinia* from animals in Bulgaria. *J Vet Med B Infect Dis Vet Public Health* 2001;**48**:203–9.

157. Nilehn B. Studies on *Yersinia enterocolitica*: with special reference to bacterial diagnosis and occurrence in human enteric disease. *Acta Pathol Microbiol Scand* 1969;**206**(Suppl.):1–48.
158. Nilehn B, Sjostrom B. Studies on *Yersinia enterocolitica*: occurrence in various groups of acute abdominal disease. *Acta Pathol Microbiol Scand* 1967;**71**:612–28.
159. Nunes Rosado FG, Stratton CW, Mosse CA. Clinicopathologic correlation of epidemiologic and histopathologic features of pediatric bacterial lymphadenitis. *Arch Pathol Lab Med* 2011; **135**:1490–3.
160. Ong KL, Gould H, Chen DL, et al. Changing epidemiology of *Yersinia enterocolitica* infections: markedly decreased rates in young Black children, Foodborne Diseases Active Surveillance Network (FoodNet) 1996-2009. *Clin Infect Dis* 2012;**54**:S385–90.
161. Orth K, Xu Z, Mudgett MB, et al. Disruption of signaling by *Yersinia* effector YopJ, a ubiquitin-like protein protease. *Science* 2000;**290**:1594–7.
162. Paff JR, Triplett DA, Saari TN. Clinical and laboratory aspects of *Yersinia pseudotuberculosis* infection with a report of two cases. *Am J Clin Pathol* 1976;**66**:101–10.
163. Pai CH, Mors V. Production of enterotoxin by *Yersinia enterocolitica*. *Infect Immun* 1978;**19**:908–11.
164. Pai CH, Gillis F, Tuomanen E, et al. Placebo-controlled double-blind evaluation of trimethoprim-sulfamethoxazole treatment of *Yersinia enterocolitica* gastroenteritis. *J Pediatr* 1984;**104**:308–11.
165. Pepe JC, Badger JL, Miller VL. Growth phase and low pH affect the thermal regulation of the *Yersinia enterocolitica* inv gene. *Mol Microbiol* 1994;**11**:123–5.
166. Preston MA, Brown S, Borczyk AA, et al. Antimicrobial susceptibility of pathogenic *Yersinia enterocolitica* isolated in Canada from 1972-1990. *Antimicrob Agents Chemother* 1994;**38**:2121–4.
167. Probst P, Hermann E, Hermann-Meyer zum Buschenfelde K, et al. Identification of the *Yersinia enterocolitica* urease B subunit as a target antigen for human synovial T lymphocytes in reactive arthritis. *Infect Immun* 1993;**61**:4507–9.
168. Quan TJ, Meek JL, Tsuchiya KR, et al. Experimental pathogenicity of recent North American isolates of *Yersinia enterocolitica*. *J Infect Dis* 1974;**129**:341–4.
169. Rabinovitz M, Stremple TH, Granforo K, et al. *Yersinia enterocolitica* infections complicated by intestinal perforation. *Arch Intern Med* 1987;**147**:1062–3.
170. Rabson AR, Koornhof HJ. *Yersinia enterocolitica* infections in South Africa. *S Afr Med J* 1972;**46**:798–803.
171. Rakin A, Noelting S, Schubert S, Heesemann J. Common and specific characteristics of the high-pathogenicity island of *Yersinia enterocolitica*. *Infect Immun* 1999;**67**:5265–74.
172. Randall KJ, Mair NS. Family outbreak of *Pasteurella pseudotuberculosis* infection. *Lancet* 1962;**1**:1042–3.
173. Ratman S, Mercer E, Picco B, et al. A nosocomial outbreak due to *Yersinia enterocolitica* serotype O:5, biotype 1. *J Infect Dis* 1982;**145**:242–7.
174. Ray SM, Ahuja SD, Blake PA, et al. Population-based surveillance for *Yersinia enterocolitica* infections in FoodNet sites, 1996-1999: higher risk for disease in infants and minority populations. *Clin Infect Dis* 2004;**38**:S181–9.
175. Reed RP, Robins-Browne RM, Williams ML. *Yersinia enterocolitica* peritonitis. *Clin Infect Dis* 1997;**25**:1468–9.
176. Resetkova E, Notenboom R, Arreaza G, et al. Seroreactivity to bacterial antigens is not a unique phenomenon in patients with autoimmune thyroid diseases in Canada. *Thyroid* 1994;**4**:269–74.
177. Reuter S, Thomson NR, McNally A. Evolutionary dynamics of the *Yersinia enterocolitica* complex. *Adv Experimental Med Biol* 2012;**954**:15–22.
178. Rodnan GP, Schumacher HR, Zvaifler NJ. *Primer on the Rheumatic Diseases*. 8th ed. Atlanta, GA: Arthritis Foundation; 1983. p. 93–4.
179. Rodriguez WJ, Controni G, Cohen GJ, et al. *Yersinia enterocolitica* in children. *JAMA* 1979;**242**:1978–80.
180. Roggenkamp A, Geiger AM, Leitritz L, et al. Passive immunity to infection with *Yersinia* spp. mediated by anti-recombinant V antigen is dependent on polymorphism of V antigen. *Infect Immun* 1997;**65**:446–51.
181. Rose EB, Camp CJ, Antes EJ. Family outbreak of fatal *Yersinia enterocolitica* pharyngitis. *Am J Med* 1987;**82**:636–7.
182. Rosner BM, Stark K, Höhle M, et al. Risk factors for sporadic *Yersinia enterocolitica* infections, Germany 2009-2010. *Epidemiol Infect* 2012;**140**:1738–47.
183. Ryser RJ, Hornick RB. A review of "new" bacterial strains causing diarrhea. In: Remington JS, Swartz MN, editors. *Clinical Topics in Infectious Diseases*. New York: McGraw-Hill; 1981. p. 184–210.
184. Saari TN, Tripplet DA. *Yersinia pseudotuberculosis* mesenteric adenitis. *J Pediatr* 1974;**85**:656–9.
185. Saari M, Make M, Paivonsal T, et al. Acute anterior uveitis and conjunctivitis following *Yersinia* infection in children. *Int Ophthalmol Med* 1986;**9**:237–41.
186. Saebo A, Lassen J. *Yersinia enterocolitica:* an inducer of chronic inflammation. *Int J Tissue React* 1994;**16**:51–7.
187. Saisawa K. Über die Pseudotuberkulose beim Menschen. *Zeitschr Hyg* 1913;**73**:353.
188. Schleifstein J, Coleman MB. An identified microorganism resembling *B. ligneri* and *Past. pseudotuberculosis* and pathogenic for man. *N Y State J Med* 1939;**39**:1749.
189. Schleifstein J, Coleman MB. *Bacterium enterocoliticum*. Annual Report, Division of Laboratories and Research. Albany, NY: New York State Department of Health; 1943. p. 56.
190. Schmidt G. *Yersinia enterocolitica* outer protein T (YopT). *Eur J Cell Biol* 2011;**90**:955–8.
191. Seuri M, Granfors K. Antibodies against *Yersinia* among farmers and slaughterhouse workers. *Scand J Work Environ Health* 1992; **18**:128–32.
192. Shayegani M, DeForge L, McGlynn DM, et al. Characteristics of *Yersinia enterocolitica* and related species isolated from human and environmental sources. *J Clin Microbiol* 1981;**14**:304–12.
193. Shenkman L, Bottone EJ. Antibodies to *Yersinia enterocolitica* in thyroid disease. *Ann Intern Med* 1976;**85**:735–9.
194. Sihvonen LM, Haukka K, Kuusi M, et al. *Yersinia enterocolitica* and *Y. enterocolitica*-like species in clinical stool specimens of humans: identification and prevalence of bio/serotypes in Finland. *Eur J Clin Microbiol Infect Dis* 2009;**28**:757–65.
195. Sihvonen LM, Jalkanen K, Huovinen E, et al. Clinical isolates of *Yersinia enterocolitica* biotype 1A represent two phylogenetic lineages with differing pathogenicity-related properties. *BMC Microbiol* 2012;**12**:208.
196. Singh I, Virdi JS. Production of *Yersinia* stable toxin (YST) and distribution of *yst* genes in biotype 1A strains of *Yersinia enterocolitica*. *J Med Microbiol* 2004;**53**:1065–8.
197. Snellings NJ, Popek M, Lindler LE. Complete DNA sequence of *Yersinia enterocolitica* serotype O:8 low-calcium-response plasmid reveals a new virulence plasmid-associated replicon. *Infect Immun* 2001;**69**:4627–38.
198. Snyder JD, Christenson E, Feldman RA. Human *Yersinia enterocolitica* infections in Wisconsin: clinical, laboratory and epidemiologic features. *Am J Med* 1982;**72**:768–74.
199. Solem J, Lassen J. Reiter's disease following *Yersinia enterocolitica* infection. *Scand J Infect Dis* 1971;**3**:83.
200. Sonnenwirth AC. *Yersinia*. In: Lennett EH, Spaulding EH, Truant JP, editors. *Manual of Clinical Microbiology*. 2nd ed. Washington D.C.: American Society of Microbiology; 1954. p. 222–9.
201. Sonnenwirth AC. *Yersinia enterocolitica* as an etiologic agent in meningitis. *Bact Proc Abstr M* 1969;**128**:87.
202. Sonnenwirth AC. *Yersinia enterocolitica*. *N Engl J Med* 1970; **283**:1468.
203. Sonnenwirth AC. Isolation and characterization of *Yersinia enterocolitica*. *Mt Sinai J Med* 1976;**43**:736–45.
204. Sotaniemi KA. Neurologic complications associated with yersiniosis. *Neurology* 1983;**33**:95–7.
205. Stephan R, Cernela N, Ziegler D, et al. Rapid species specific identification and subtyping of *Yersinia enterocolitica* by MALDI-TOF mass spectrometry. *J Microbiol Methods* 2011;**87**:150–3.
206. Stolk-Engelaar VMM, Meis JFGM, Mulder JA, et al. In-vitro antimicrobial susceptibility of *Yersinia enterocolitica* isolates from stools of patients in The Netherlands from 1982-1991. *J Antimicrob Chemother* 1995;**36**:839–43.
207. Sulakvelidze A. *Yersinia* other than *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis*: the ignored species. *Microbes Infect* 2000;**2**: 497–513.
208. Sulakvelidze A, Kreger A, Joseph A, et al. Production of enterotoxin by *Yersinia* bercovieri, a recently identified *Yersinia enterocolitica*–like species. *Infect Immun* 1999;**67**:968–71.
209. Tackett CO, Ballard J, Harris N, et al. An outbreak of *Yersinia enterocolitica* infections caused by contaminated tofu (soybean curd). *Am J Epidemiol* 1985;**121**:705–11.

210. Tansini A, Verdan da Silva FF, Rodrigues dos Santos R, et al. Yop effector proteins from *Yersinia pseudotuberculosis* impair dendritic cell activation. *Adv Experimental Med Biol* 2012;**954**:203–8.
211. Tauxe RV, Vandepitta J, Mautero G, et al. *Yersinia enterocolitica* infections and pork: the missing link. *Lancet* 1987;**1**:1129–33.
212. Thomas AF, Solomon L, Rabson A. Polyarthritis associated with *Yersinia enterocolitica* infection. *S Afr Med J* 1975;**49**:18–20.
213. Thomson NR, Howard S, Wren BW, et al. The complete genome sequence and comparative genome analysis of the high pathogenicity *Yersinia enterocolitica* strain 8081. *PLoS Genet* 2006;**2**: 2039–51.
214. Toivanen P, Toivanen A, Olkkonen L, et al. Hospital outbreak of *Yersinia enterocolitica* infection. *Lancet* 1973;**1**:801–3.
215. Toma S. Survey on the incidence of *Yersinia enterocolitica* in the province of Ontario. *Can J Public Health* 1973;**64**:477–87.
216. Toma S, Wauters G, McClure HM, et al. O:13a, 13b, a new pathogenic serotype of *Yersinia enterocolitica*. *J Clin Microbiol* 1984;**20**:843–5.
217. Toshniwal R, Kocka FE, Kallick CA. Suppurative lymphadenitis with *Yersinia enterocolitica*. *Eur J Clin Microbiol* 1985;**4**:587–8.
218. Tuohy AM, O'Gorman M, Byington C, et al. *Yersinia* enterocolitis mimicking Crohn's disease in a toddler. *Pediatrics* 1999; **104**:e36.
219. Vantrappen G, Geboes K, Ponette E. *Yersinia* enteritis. *Med Clin North Am* 1982;**66**:639–53.
220. Vantrappen G, Ponette E, Geboes K, et al. *Yersinia* enteritis and enterocolitis: gastroenterological aspects. *Gastroenterology* 1977; **72**:220–7.
221. Verhaegen J, Charlier J, Lemmens P, et al. Surveillance of human *Yersinia enterocolitica* infections in Belgium: 1967-1996. *Clin Infect Dis* 1998;**27**:59–64.
222. Vilinskas J, Tilton RC, Kriz JJ. A new clinical entity: human infection with *Yersinia* presenting as an acute abdomen. *Am J Surg* 1971;**7**:568.
223. Vishnubhatla A, Fung DY, Oberst RD, et al. Rapid 5′ nuclease (TaqMan) assay for detection of virulent strains of *Yersinia enterocolitica*. *Appl Environ Microbiol* 2000;**66**:4131–5.
224. Visser LG, Hiemstra PS, Van Den Barselaar MT, et al. Role of YadA in resistance to killing of *Yersinia enterocolitica* by antimicrobial polypeptides of human granulocytes. *Infect Immun* 1996;**64**:1653–8.
225. Waage AS, Vardund T, Lund V, et al. Detection of low numbers of pathogenic *Yersinia enterocolitica* in environmental water and sewage samples by nested polymerase chain reaction. *J Appl Microbiol* 1999;**87**:814–21.
226. Wang X, Yang L, Jing H, et al. Complete genome sequence of a *Yersinia enterocolitica* "Old World" (3/O:9) strain and comparison with the "New World" (1B/O:8) strain. *J Clin Microbiol* 2011;**49**:1251–9.
227. Weber J, Finlayson NB, Mark JBD. Mesenteric lymphadenitis and terminal ileitis due to *Yersinia pseudotuberculosis*. *N Engl J Med* 1970;**283**:172–4.
228. Wilson HD, McCormick JS, Feely JC. *Yersinia enterocolitica* infection in a 4-month-old infant associated with infection in household dogs. *J Pediatr* 1976;**89**:767–9.
229. Winblad S. Erythema nodosum associated with infection with *Yersinia enterocolitica*. *Scand J Infect Dis* 1969;**1**:11–16.
230. Winblad S. Arthritis associated with *Yersinia enterocolitica* infections. *Scand J Infect Dis* 1975;**7**:191–5.
231. Winblad S, Nilehn B, Jonsson M. Two further cases, bacteriologically verified, of human infection with "Pasteurella X" (syn. *Yersinia enterocolitica*). *Acta Pathol Microbiol Scand* 1966;**67**:537–41.
232. Winblad S, Nilehn B, Sternby NH. *Yersinia enterocolitica* (Pasteurella X) in human enteric infections. *Br Med J* 1966;**2**:1363–6.
233. Young GM, Badger JL, Miller VL. Motility is required to initiate host cell invasion by *Yersinia enterocolitica*. *Infect Immun* 2000;**68**: 4323–6.
234. Zen-Yoji H, Maruyama T, Sakai S, et al. An outbreak of enteritis due to *Yersinia enterocolitica* occurring at a junior high school. *Jpn J Microbiol* 1973;**17**:220–2.
235. Zhang L, Radziejewska-Lebrecht J, Krajewska-Pietrasik D, et al. Molecular and chemical characterization of the lipopolysaccharide O-antigen and its role in the virulence of *Yersinia enterocolitica* serotype O:8. *Mol Microbiol* 1997;**23**:63–76.
236. Zhang Y, Romanov G, Bliska JB. Type III secretion system-dependent translocation of ectopically expressed Yop effectors into macrophages by intracellular *Yersinia pseudotuberculosis*. *Infect Immun* 2011;**79**:4322–31.

clinically.[41] *Salmonella* usually ferments mannitol, sorbitol, and rhamnose, however. The innate resistance of *Edwardsiella* to colistin also distinguishes it from *Salmonella*.[68] *E. tarda* grows well on usual differential media in the laboratory and produces smooth, glistening, semitranslucent colonies.

EPIDEMIOLOGY

E. tarda is an organism associated with fresh-water and marine life and has been isolated from turtles, fish, pelicans, alligators, seals, and toads.[41] It also is found in snakes and lizards. Case reports of human disease have implicated ornamental fish,[93] pet turtles,[69] snakes,[80] and catfish[7,17,35] and injuries in fresh and brackish water.[86]

Patients with chronic liver disease, chronic ethanol abuse, corticosteroid therapy, and hemoglobinopathy particularly are prone to developing infection with *E. tarda*.[59] Any condition associated with iron overload also is a potential risk factor. Serious infections with *E. tarda* in otherwise well individuals also can occur.[86]

Infection with *E. tarda* is global but occurs more commonly in tropical and subtropical climates, particularly Southeast Asia, Africa, and Latin America.[49] The elderly and the very young seem to be at increased risk for developing severe illness.[11] Asymptomatic carrier states have been documented well,[74] but no true epidemic has been reported, and person-to-person transmission has not been established directly.[24]

PATHOPHYSIOLOGY

Invasiveness of the organism in HeLa and HEp-2 cells,[77] siderophore production, the elaboration of a cell-associated hemolysin,[37] and resistance to complement-mediated lysis may contribute to virulence, although no clear associations have been made.[45] Hemolytic activity requires the presence of two genes, *ethA* and *ethB*. The *ethA* gene codes for the hemolysin, whereas the *ethB* gene codes for an activation/secretion protein, which is necessary for activation of the hemolysin. Transcription of the *ethB* gene is regulated by iron, which may explain the association between iron overload states and severe *E. tarda* infection.[37] Hemolysin activity seems to be necessary for cell entry and cytotoxicity.[89] *E. tarda* produces an iron-cofactored superoxide dismutase that inhibits macrophage-mediated innate immune responses.[15] *E. tarda* has both type III and type VI secretion systems; expression of these gene products is regulated by phosphorus and iron concentrations.[14]

CLINICAL MANIFESTATIONS

Infections with *E. tarda* can be divided broadly into two types: intestinal and extraintestinal. Approximately 80 percent of infections are intestinal.[86] Gastrointestinal infection usually causes self-limited enteritis, with intermittent watery diarrhea and low-grade fever.[40] Nausea and vomiting usually are not seen. Occasionally, enterocolitis or a dysentery-like illness is noted.[61] Because most laboratories do not culture stool specimens specifically for *Edwardsiella*, most cases are unrecognized.

Wound infection is the most common extraintestinal infection. Most of the wounds are caused by fish fins or snakes; wounds sustained in automobile accidents also have been implicated.[42] Cellulitis or abscesses may be produced. Coinfection with other organisms, particularly *Aeromonas hydrophila*, is common.[35,86] One case of necrotizing fasciitis and myonecrosis in an immunocompetent man has been reported. Cultures obtained at surgical débridement yielded a pure growth of *E. tarda*.[86]

Septicemia with *E. tarda* is a rare but serious infection that carries a mortality rate of approximately 45 percent. Most patients with septicemia have underlying conditions, such as liver disease, iron overload, diabetes mellitus, and immunosuppression. Septicemia occasionally follows a mild diarrheal illness.[17] Infants without other risk factors have been reported.[95] Septicemia manifests as high fever, shock, and, often, disseminated intravascular coagulation. Meningitis and multiple brain abscesses also have been reported.[83,90,97] Death sometimes occurs despite administration of appropriate antimicrobial therapy.

Other syndromes associated with *E. tarda* infection include an enteric fever–like illness,[17] multiple or solitary liver abscesses,[50,102] osteomyelitis,[80] septic arthritis,[7] cellulitis,[31] myonecrosis,[86] necrotizing fasciitis,[64] endocarditis,[70] uterine pyomyoma,[100] tubo-ovarian abscess,[78] and peritonitis.[17] A case of puerperal intrauterine infection in a woman without obvious risk factors has been reported; her infant was not affected.[66] In another case, neonatal sepsis resulted from maternal chorioamnionitis.[36] Patients with sickle-cell disorders may be predisposed to bony infection with *E. tarda*, as they are with *Salmonella*.[83,99] Reported cases all are in patients with sickle-cell hemoglobinopathy, rather than homozygous sickle-cell disease. Osteomyelitis has also been reported in X-linked chronic granulomatous disease.[47]

DIAGNOSIS AND TREATMENT

Diagnosis rests on identification of *E. tarda* in culture. The major pitfall is mistaking it for *Salmonella*.

E. tarda is sensitive in vitro to most antibiotics used routinely in the treatment of gram-negative infections, including β-lactams, cephalosporins, aminoglycosides, and fluoroquinolones.[16] It also is sensitive to chloramphenicol, trimethoprim-sulfamethoxazole, and tetracycline.[88] Resistance to polymyxin B, colistin, and, occasionally, penicillin has been shown.[16,17] The organism almost universally elaborates a β-lactamase, but no resistance to β-lactams other than penicillin[16] and oxacillin[88] has been reported.

Gastrointestinal disease usually does not require treatment. Severe disease, such as septicemia or meningitis, probably should be treated with the combination of a β-lactam and an aminoglycoside,[41] even though synergy has not been shown.

HAFNIA ALVEI

BACTERIOLOGY

H. alvei is a facultatively anaerobic, gram-negative bacillus, formerly referred to as *Enterobacter hafnia*.[84] This indole-negative, catalase-positive, and oxidase-negative organism is positive for lysine and ornithine decarboxylases. It is motile at lower temperatures but may be immotile at temperatures of 35° C (95° F) and greater. *H. alvei* ferments mannitol, maltose, and sucrose. It grows well on blood or MacConkey agar as a nonlactose fermenter, producing gray-white, slightly elevated, glistening colonies.[26] For a long time *H. alvei* was thought to be the only species within the genus *Hafnia;* however, in 2010, members of DNA hybridization group 2 were reclassified as *H. paralvei* sp. nov.[38]

EPIDEMIOLOGY

H. alvei has been found in soil, dairy products, sewage, and the feces of humans and animals. Some question exists as to whether it is part of the endogenous microflora of the gut[81] or whether an asymptomatic "carrier" state exists. One study from Japan cultured *H. alvei* from 13 percent of healthy subjects[63]; other epidemiologic surveys have shown the incidence to be less than 2 percent.[82] Long regarded as a nonpathogen, *H. alvei* now has been associated clearly with enteritis and rarely has been isolated in pure culture from other sites, including blood, cerebrospinal fluid, peritoneal fluid, and urine. Infection seems to be opportunistic.

PATHOPHYSIOLOGY

The pathophysiology of *H. alvei* has been investigated most thoroughly with regard to its production of gastrointestinal symptoms,[3] although whether *H. alvei* is actually a diarrheagenic pathogen remains controversial. Albert and associates[4] showed that although *H. alvei* elaborated neither enterotoxins nor a Shiga-like toxin and was not invasive in HeLa cell assays or by Sereny test it did produce diarrhea in experimental animals whether given parenterally or by mouth. Sections of intestines from infected animals showed lesions indistinguishable from those caused by enteropathogenic *Escherichia coli.* "Diarrheagenic strains" of *H. alvei* also were thought to differ from other strains in that they possessed the *eaeA* gene.[40] Janda and colleagues[44] first presented data suggesting that diarrheagenic *H. alvei* isolates were actually either unusual biotypes of *E. coli* or a new species in the genus *Escherichia.* Subsequently, the same group showed that organisms originally called *H. alvei* reported to produce attaching-effacing lesions and to possess the *eaeA* gene are actually a novel species, *Escherichia albertii.*[39] *H. alvei* does cause damage to intestinal epithelial cells but does not use the attaching-effacing mechanism, nor does it use the same mechanism as *E. coli* O157:H7.[25] Information about the pathophysiology of extraintestinal infection is sparse.[43]

CLINICAL MANIFESTATIONS

Cases of diarrhea caused by *H. alvei* (possibly *E. albertii*) have been reported primarily in children.[3,4] Most patients with gastroenteritis secondary to *H. alvei/E. albertii* report 6 to 12 episodes of watery diarrhea per day, low-grade or no fever, and nausea with or without vomiting. Mucus sometimes is found in stools, but blood is not.[81,98] In most patients, symptoms last for a few days, but in some patients, symptoms persist for more than a week.[81] One patient with a reactive arthritis from *H. alvei/E. albertii* enteritis has been reported.[71] A unique case has been reported of an 11-year-old girl who had a 3-day history of red lesions on the forehead, neck, and trunk associated with abdominal pain, emesis, and decreased urinary output, with one bloody diarrheal stool the day before admission who then developed hemolytic-uremic syndrome. Stool cultures repeatedly were negative for *E. coli* O157:H7 but positive for a toxigenic strain of *H. alvei* from two consecutive cultures.[19]

Traditionally, extraintestinal *H. alvei* infections were thought to occur mostly in hospitalized patients and to be hospital acquired. In one large series, the organism was isolated from 80 samples collected from 61 patients; 57 (93%) patients had underlying illnesses, most commonly malignancies.[34] Nearly half of isolates were from the respiratory tract. Other sites included blood, skin, wounds, urine, and intra-abdominal abscesses. In 60 (75%) samples, other organisms were cultured concomitantly. In a larger study from Canada, two thirds of 138 isolates were of community onset, 112 (81%) were from the urinary tract, and 94 (68%) were from monomicrobial infections.[51] Older age and female gender were risk factors for development of infection.

One 20-day-old premature infant with necrotizing enterocolitis grew *H. alvei* from blood and stool.[32] Yeager and associates[101] reported four cases of pneumatosis intestinalis in patients after they had undergone bone marrow transplantation, and in one of the four cases *H. alvei* was grown in pure culture from blood. An 8-year-old boy with acquired immunodeficiency syndrome (AIDS) developed recurrent episodes of *H. alvei* bacteremia.[18] Both episodes were associated with fever and diarrhea; the second episode also was associated with pneumonia and pleural effusion, the cause of which is unclear. A well-documented case of pneumonia in a 54-year-old woman with AIDS has been reported in which the pleural fluid grew a pure culture of *H. alvei.*[28] A patient with graft-versus-host disease developed *H. alvei* colonization with mild symptoms suggesting infection.[85] Pyelonephritis[55] and gangrenous cholecystitis[76] have been reported. A 39-day-old infant without obvious risk factors developed urosepsis.[54] A single case report of *H. paralvei*

bacteremia, in an 85-year-old man, exists. His culture was also positive for an enterococcus.[75]

Wound abscess caused by *H. alvei* has been reported.[1] A 2-year-old liver transplant recipient developed hepatic abscess and bacteremia with *H. alvei* and *Enterococcus faecalis*.[8] One case of meningitis in a 1-year-old infant without known predisposing risk factors has been reported.[67] A case of a woman with rheumatoid arthritis who contracted endophthalmitis with *H. alvei* in mixed culture has been described; the woman used snake powder as a food seasoning.[13] Two case reports are described in the older literature of persistent bacteremia with this organism, alone and in mixed cultures. One of these patients was a previously well 13-year-old girl.[33]

DIAGNOSIS AND TREATMENT

Diagnosis is established by isolation of the organism from stools or from normally sterile body fluids. Gunthard and Pennekamp[34] reported the susceptibility results of 80 *H. alvei* isolates recovered from 61 patients over a 2½-year period. All isolates tested were susceptible to ciprofloxacin. Isolates also were generally susceptible to tobramycin (99%), imipenem (99%), piperacillin (92%), trimethoprim-sulfamethoxazole (90%), ceftriaxone (88%), and ceftazidime (88%). Nearly all isolates were resistant to ampicillin and to amoxicillin-clavulanate.

H. alvei is constitutively cephalothin resistant.[98] One report describes an inducible β-lactamase that rendered the isolate ceftazidime-resistant.[91] In a study of 17 isolates from a single institution in France, 6 were noted to have a high-level constitutive, inducible cephalosporinase production profile similar to that of *Enterobacter* spp.[50] In one study, the antimicrobial susceptibility of *H. alvei* was compared with that of *E. albertii*, the organism with which *H. alvei* is confused most frequently. *E. albertii* isolates were naturally susceptible to all β-lactams except for penicillin and oxacillin; they also were susceptible to azithromycin. In contrast, *H. alvei* strains were naturally resistant to tetracycline, amoxicillin, amoxicillin-clavulanate, ampicillin-sulbactam, azithromycin, and the narrow-spectrum cephalosporins.[88]

Treatment probably is unnecessary for most cases of gastroenteritis. Treatment of invasive infection should be based on susceptibility testing. Empirical therapy with a third-generation cephalosporin and an aminoglycoside is reasonable pending susceptibility results.

PANTOEA AGGLOMERANS

First identified as a plant pathogen and named after Erwin Smith in 1917, the genus *Erwinia* has an extended domain as an infectious microbe, including production of disease in humans.[12,79,87] A member of this group of organisms, *Pantoea agglomerans (Enterobacter agglomerans, Erwinia herbicola)* has been established as a cause of conjunctivitis,[12] central nervous system infections,[12,84] urinary tract infections,[78,83] pneumonia,[2] and nosocomial infections secondary to contaminated intravenous fluids.[31,48]

BACTERIOLOGY

Species definition within *Erwinia* has been controversial and confusing. It has been suggested to rename the anaerogenic clinically relevant *Erwinia herbicola-lathryi* group *E. agglomerans* or *P. agglomerans*.[27,41,87] In clinical microbiology, both of the latter terms tend to be used interchangeably. This species consists of facultatively anaerobic, fermentative, hydrogen sulfide–negative, gram-negative rods; they do not possess oxidase, phenylalanine deaminase, proteinase, or arginine dehydrolase.[27] None decarboxylates ornithine.[41] They are motile, have peritrichous flagella, and produce a yellow pigment. Strains grow well at 37°C (98.6°F) on standard agar. When colonies are viewed microscopically after growth for 18 to 20 hours, characteristic biconvex spindle-shaped bodies and bacterial aggregates often can be seen.[12,84] Almost all strains isolated from clinical specimens and grown on agar slants show characteristic elongated, spheroidal aggregates,[86] termed *symplasmata* by Cruickshank,[20] who first described them.

EPIDEMIOLOGY

Microorganisms of the genus *Erwinia* long have been recognized as phytopathogens producing dry necrosis, wilts, and soft rots in plants; and more recently they have been associated with disease in trout and leafhoppers.[79,87] *P. agglomerans (E. herbicola)* first was isolated in humans from stool specimens of patients with typhoid fever in the 1920s and given the name *Bacterium typhi flavum* because of the organism's alleged capacity to be transformed into *Salmonella typhi* in subculture. Identification of saprophytic human strains followed, and the biochemical and cultural identity of *B. typhi flavum* with the *E. herbicola-lathryi* group finally was established.[27]

The first reports of *P. agglomerans* as a human pathogen appeared in the 1960s. Subsequently, a nationwide outbreak of infection caused by this organism was traced to contaminated liners from caps of parenteral solution bottles.[57,58] The importance of this organism as a nosocomial cause of bacteremia is emphasized by the 17 percent mortality rate in patients receiving an infusion with the contaminated intravenous fluid.[57] A trend was noted for an increased mortality rate in infected individuals younger than 20 years old. In neonatal intensive care units, the situation seems to be even more dire; in one study, the mortality rate for infants with blood cultures

positive for *P. agglomerans* was 88 percent (seven of eight).[94] Lipid-based medications support rapid bacterial growth at room temperature and, in the absence of strict aseptic handling, have been implicated in more recent nosocomial *P. agglomerans* bloodstream infections.[10] *Pantoea* outbreaks also have been traced to contaminated blood products; prolonged storage of packed red blood cells at 4° C provides conditions that allow these organisms to grow and subsequently produce high concentrations of endotoxin.[6] Cotton used to filter heroin has been implicated as a source of infection ("cotton fever") in intravenous drug users.[29]

Outbreaks in pediatric hospitals and urgent care centers caused by contaminated intravenous solutions[62] or tubing[9] also have been described. At least one retrospective review of *Erwinia* organisms isolated from clinical specimens suggested a predisposition to infection in the pediatric age group[12]; however, other studies have noted no preference based on season, sex, age, or residence in hospitals.[87] A large pseudo-outbreak traced to contamination of cotton pledgets also has been reported.[48]

PATHOPHYSIOLOGY

The true incidence of clinical infection caused by *P. agglomerans* is difficult to ascertain because of the common association of this microbe with other organisms when obtained from clinical specimens. Nonetheless, the accumulation of case reports in which this organism is isolated in pure culture from infected material leaves little doubt that *P. agglomerans* can be a human pathogen, apart from its role as a nosocomial contaminant. This organism has little inherent invasiveness. Evidence for animal pathogenicity of *Erwinia* strains isolated from plants and humans is limited[53]; 10^{13} washed organisms injected intraperitoneally into mice or guinea pigs do not cause symptoms, whereas inoculation of 10^{27} organisms leads to death within 36 hours.[20]

Most strains seem to act as saprophytes in humans,[96] but the organism has been isolated from purulent wounds of the extremities acquired through lacerations or thorn pricks, which suggests agricultural injury as a possible mode of infection.[96] Thorn injuries also have led to septic arthritis and osteomyelitis.[21] A delay of 4 to 6 weeks often occurs between the injury and the bone or joint infection. Most serious infections have occurred in individuals with a breach of host defenses (e.g., immunocompromised individuals or patients who received contaminated intravenous fluid).

CLINICAL MANIFESTATIONS

P. agglomerans bacteremia often is associated with fever, shaking chills, and systemic toxicity characteristic of gram-negative sepsis. These symptoms frequently have been misinterpreted, however, in hospitalized patients who unknowingly were administered contaminated intravenous fluids.[58] Premature neonates with sepsis owing to *P. agglomerans* tend to have a rapidly progressive sepsis with prominent pulmonary involvement, including hemorrhage and acute respiratory distress syndrome. A case series of five premature infants with *P. agglomerans* bacteremia has been reported; all infants had a birth weight of less than 1500 g, and in all cases the onset of sepsis was after age 7 days. Lethargy, mottling, and bradycardia were common; patients had desaturations but no evidence of pulmonary disease. Thrombocytopenia developed in four of the five infants, with metabolic acidosis in two and jaundice in two.[5] In the largest pediatric series reported to date, 21 of 53 (about 40%) *P. agglomerans* isolates from normally sterile sites were grown from central venous lines; of these, two thirds were polymicrobial.[21]

Eye and skin infections caused by *P. agglomerans* are particularly prominent. Bottone and Schneierson[12] included six cases of conjunctivitis, five of which occurred in infants, from whom this organism was isolated. Only two of the isolates were in pure culture, however, and a description of the clinical course of these children was not included in the report. *Erwinia* endophthalmitis has been associated with foreign-body penetration of the eye in a 14-year-old boy.[72]

Skin infection in association with a casted fracture has been described in an elderly patient,[96] and wound infections from which this organism was isolated have been described subsequently, most often in association with agricultural injury.[96] Four isolates were obtained in mixed cultures from skin lesions of children younger than 5 years old, but their possible role in those infections was not confirmed.[12] In a recent series, 14 cases of abscesses required drainage; all 14 were polymicrobial.[21] *P. agglomerans* was the only organism isolated from six consecutive blood cultures in a 9-year-old boy with osteomyelitis.[60] A 13-year-old boy developed septic arthritis caused by *P. agglomerans* 1 month after sustaining a plant thorn injury to his knee.[22] A case of *P. agglomerans* spondylodiscitis has been reported.[79] In many cases of *P. agglomerans* osteomyelitis or septic arthritis, the patient is afebrile and blood cultures are negative. In addition, measures of inflammation and joint fluid findings may be largely unremarkable.[21] A high index of suspicion of *P. agglomerans* must be maintained when bone or joint infection follows plant thorn injury.

Primary lung disease caused by these bacteria is a rare occurrence; it has been reported in an adult with chronic bronchitis[5] and also in a heart-lung transplant recipient.[5] This organism is a very rare cause of meningitis. A contaminated incubator has been implicated in two cases of neonatal central nervous system infection,[92] and a cisternal tap revealed the presence of *P. agglomerans* in an unrelated case in a newborn whose clinical course was not described.[12] A 57-year-old man with tetralogy of Fallot and cyanosis had a brain abscess in which *P. agglomerans* organisms grew in pure culture.[97] Presenting manifestations included headaches, seizures, and left-sided weakness, which occurred over a 2-week period before admission. The patient recovered after undergoing drainage of the abscess and gentamicin therapy. Several cases of peritonitis in patients on continuous ambulatory peritoneal dialysis have been reported[30,46,56]; in one case the infection was thought to be secondary to a rose thorn injury.[52]

DIAGNOSIS

Difficulty in identifying this organism is common; in March 1972, as part of a quality control program, *P. agglomerans* was sent as an unknown organism to 250 U.S. hospitals and was identified incorrectly 45 percent of the time.[58] Even when fully identified in isolates from human sources, *P. agglomerans* often is considered a contaminant or saprophyte. The organisms have been identified mistakenly as *Citrobacter*, *Escherichia coli*, *Flavobacterium*, and *Klebsiella*. In addition to routine microbiologic studies, identification of yellow-pigmented colonies and observation of the characteristic spindle-shaped bodies and symplasmata can aid in this differentiation. Symplasmata (elongated, spheroid aggregates) are seen best in the condensation water of slant cultures.[96] Spindle-shaped bodies termed *Wetzsteinformen* by German authors are observed best on standard agar with a low-power microscope lens.[87] Generally, speciation based on metabolic profile is problematic. A multilocus gene sequencing method for species identification and strain tracking has been described.[23]

TREATMENT

Most localized infections respond to treatment that includes an aminoglycoside. The presence of persistent localized infection with this organism should prompt a search for an organic foreign body, given the organism's tendency to live as a saprophyte or as a pathogen in vegetable material. In addition to appropriate antimicrobial therapy, treatment of bacteremia should include removal of any potentially contaminated intravenous access. The physician should be alert to the possibility of a common source of infection in nosocomial outbreaks.[58] In view of the rarity of bacteremia caused by *P. agglomerans*, single sporadic cases should be investigated, and clusters of two or more cases should lead to immediate inquiry into possible sources of contamination. Formal surveillance programs have been the key to early recognition and abortion of epidemics.[58]

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

5. Aly NY, Salmeen HN, Lila RA, et al. *Pantoea agglomerans* bloodstream infection in preterm neonates. *Med Pinc Pract* 2008;**17**:500–3.
10. Bicudo EL, Macedo VO, Carrara MA, et al. Nosocomial outbreak of *Pantoea agglomerans* in a pediatric urgent care center. *Braz J Infect Dis* 2007;**11**:281–4.
14. Chakrabory S, Sivaraman J, Leung KY, et al. Two-component PhoB-PhoR regulatory system and ferric uptake regulator sense phosphate and iron to control virulence genes in type III and type VI secretion systems of *Edwardsiella tarda*. *J Biol Chem* 2011;**286**:39417–30.
15. Cheng S, Zhang M, Sun L, et al. The iron-cofactored superoxide dismutase of *Edwardsiella tarda* inhibits macrophage-mediated innate immune response. *Fish Shellfish Immunol* 2010;**29**:972–8.
23. Deletoile A, Decre D, Courant S, et al. Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. *J Clin Microbiol* 2009;**47**:300–10.
25. Donato KA, Zareie M, Jassem AN, et al. *Escherichia albertii* and *Hafnia alvei* are candidate enteric pathogens with divergent effects on intercellular tight junctions. *Microb Pathog* 2008;**45**:377–85.
30. Ferrantino M, Navaneethan SD, Sloand JA. *Pantoea agglomerans*: an unusual inciting agent in peritonitis. *Perit Dial Int* 2008;**28**:428–30.
36. Hashavya S, Averbuch D, Berger I, et al. Neonatal sepsis following maternal amnionitis by *Edwardsiella tarda*: a case report and a review of the literature. *Eur J Pediatr* 2011;**170**:111–13.
38. Huys G, Crockaert M, Abbott SL, Janda JM, Vandamme P. *Hernia paralvei* sp. nov, formerly known as *Hafnia alvei* hybridization group 2. *Int J Syst Evol Microbiol* 2010;**60**:1725–8.
46. Kahveci A, Asicioglu E, Tigen E, et al. Unusual causes of peritonitis in a peritoneal dialysis patient: *Alcaligenes faecalis* and *Pantoea agglomerans*. *Ann Clin Microbiol Antimicrob* 2011;**10**:12.
47. Kawai T, Kusakabe H, Seki A, et al. Osteomyelitis due to trimethoprim/sulfamethoxazole-resistant *Edwardsiella tarda* infection in a patient with X-linked chronic granulomatous disease. *Infection* 2011;**39**:171–3.
50. Lancelevee J, Bret L, David K, et al. Antibiotic resistance and adherence properties of *Hafnia alvei* clinical isolates: a 19-month study in the hospital of Orleans, France. *J Chemother* 2007;**19**:677–81.
54. Liu CH, Lin WJ, Wang CC, et al. Young-infant sepsis combined with urinary tract infection due to *Hafnia alvei*. *J Formos Med Assoc* 2007;**106**:S39–43.
55. Loulergue P, Lortholary O, Mainardi JL, et al. *Hafnia alvei* endocarditis following pyelonephritis in a permanent pacemaker carrier. *Clin Infect Dis* 2007;**44**:621.
56. Magnette C, Tintillier M, Horlait G, et al. Severe peritonitis due to *Pantoea agglomerans* in a CCPD patient. *Perit Dial Int* 2008;**28**:207–8.
90. Takeuchi H, Fujita Y, Ogawa H, et al. Multiple brain abscesses in neonate caused by *Edwardsiella tarda*: case report. *Neurol Med Chir (Tokyo)* 2009;**49**:85–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agustin ET, Cunha BA. Buttock abscess due to *Hafnia alvei* [Letter]. *Clin Infect Dis* 1995;**20**:1426.
2. Al-Damluji S, Dickinson CM, Beck A. *Enterobacter agglomerans*: a new cause of primary pneumonia. *Thorax* 1982;**37**:865–6.
3. Albert MJ, Alam K, Islam M, et al. *Hafnia alvei*, a probable cause of diarrhea in humans. *Infect Immun* 1991;**59**:1507–13.
4. Albert MJ, Faruque SM, Ansaruzzaman M, et al. Sharing of virulence-associated properties at the phenotypic and genetic levels between enteropathogenic *Escherichia coli* and *Hafnia alvei*. *J Med Microbiol* 1992;**37**:310–14.
5. Aly NY, Salmeen HN, Lila RA, Nagaraja PA. *Pantoea agglomerans* bloodstream infection in preterm neonates. *Med Pinc Pract* 2008; **17**:500–3.
6. Arduino MJ, Bland LA, Tipple MA, et al. Growth and endotoxin production of *Yersinia enterocolitica* and *Enterobacter agglomerans* in packed erythrocytes. *J Clin Microbiol* 1989;**27**:1483–5.
7. Ashford RU, Sargeant PD, Lum GD. Septic arthritis of the knee caused by *Edwardsiella tarda* after a catfish puncture wound. *Med J Aust* 1998;**168**:443–4.
8. Barry JW, Dominguez EA, Boken DJ, et al. *Hafnia alvei* infection after liver transplantation. *Clin Infect Dis* 1997;**24**:1263–4.
9. Bennett SN, McNeil MM, Bland LA, et al. Postoperative infections traced to contamination of an intravenous anesthetic, propofol. *N Engl J Med* 1995;**333**:147–54.
10. Bicudo EL, Macedo VO, Carrara MA, et al. Nosocomial outbreak of *Pantoea agglomerans* in a pediatric urgent care center. *Braz J Infect Dis* 2007;**11**:281–4.
11. Bockemuhl J, Pan-Urai R, Burkhardt F. *Edwardsiella tarda* associated with human disease. *Pathol Microbiol* 1971;**37**:393–401.
12. Bottone E, Schneierson SS. *Erwinia* species: an emerging human pathogen. *Am J Clin Pathol* 1972;**57**:400–5.
13. Caravalho J Jr, McMillan VM, Ellis RB, et al. Endogenous endophthalmitis due to *Salmonella arizonae* and *Hafnia alvei*. *South Med J* 1990;**83**:325–7.
14. Chakrabory S, Sivaraman J, Leung KY, et al. Two-component PhoB-PhoR regulatory system and ferric uptake regulator sense phosphate and iron to control virulence genes in type III and type VI secretion systems of *Edwardsiella tarda*. *J Biol Chem* 2011; **286**:39417–30.
15. Cheng S, Zhang M, Sun L, et al. The iron-cofactored superoxide dismutase of *Edwardsiella tarda* inhibits macrophage-mediated innate immune response. *Fish Shellfish Immunol* 2010;**29**:972–8.
16. Clark RB, Lister PD, Janda JM. In vitro susceptibilities of *Edwardsiella tarda* to 22 antibiotics and antibiotic-beta-lactamase-inhibitor agents. *Diagn Microbiol Infect Dis* 1991;**14**:173–5.
17. Clarridge JE, Musher DM, Fainstein V, et al. Extraintestinal human infection caused by *Edwardsiella tarda*. *J Clin Microbiol* 1980;**11**:511–14.
18. Conte M, Castagnola E, Venzano P, et al. Bacteremia caused by *Hafnia alvei* in a human immunodeficiency virus–infected child [Letter]. *Pediatr Infect Dis J* 1996;**15**:182–3.
19. Crandall C, Abbott SL, Zhao YQ, et al. Isolation of toxigenic *Hafnia alvei* from a probable case of hemolytic uremic syndrome. *Infection* 2006;**34**:227–9.
20. Cruickshank JC. A study of the so-called bacterium *Typhi flavum*. *J Hyg* 1935;**35**:354–71.
21. Cruz AT, Cazacu AC, Allen CH. *Pantoea agglomerans*, a plant pathogen causing human disease. *J Clin Microbiol* 2007;**45**:1989–92.
22. De Champs C, Le Seaux S, Dubost JJ, et al. Isolation of *Pantoea agglomerans* in two cases of septic monoarthritis after plant thorn and wood sliver injuries. *J Clin Microbiol* 2000;**38**: 460–1.
23. Deletoile A, Decre D, Courant S, et al. Phylogeny and identification of *Pantoea* species and typing of *Pantoea agglomerans* strains by multilocus gene sequencing. *J Clin Microbiol* 2009;**47**: 300–10.
24. Desenclos JC, Conti L, Junejo S, et al. A cluster of *Edwardsiella tarda* infection in a day-care center in Florida. *J Infect Dis* 1990; **162**:782.
25. Donato KA, Zareie M, Jassem AN, et al. *Escherichia albertii* and *Hafnia alvei* are candidate enteric pathogens with divergent effects on intercellular tight junctions. *Microb Pathog* 2008;**45**: 377–85.
26. Englund GW. Persistent septicemia due to *Hafnia alvei*: report of a case. *Am J Clin Pathol* 1969;**51**:717–19.
27. Ewing WH, Fife MA. *Enterobacter agglomerans* (Beijerinck) comb. nov. (the *herbicola-lathryi* bacteria). *Int J Syst Bacteriol* 1972;**22**: 4–11.
28. Fazal BA, Justman JE, Turett GS, et al. Community-acquired *Hafnia alvei* infection. *Clin Infect Dis* 1997;**24**:527–8.
29. Ferguson R, Feeney C, Chirurgi VA. *Enterobacter agglomerans*–associated cotton fever. *Arch Intern Med* 1993;**153**:2381–2.
30. Ferrantino M, Navaneethan SD, Sloand JA. *Pantoea agglomerans*: an unusual inciting agent in peritonitis. *Perit Dial Int* 2008;**28**: 428–30.
31. Fournier S, Pialoux G, Feuillie V, et al. *Edwardsiella tarda* septicemia with cellulitis in a patient with AIDS [Letter]. *Eur J Clin Microbiol Infect Dis* 1997;**16**:551–3.
32. Ginsberg HG, Goldsmith JP. *Hafnia alvei* septicemia in an infant with necrotizing enterocolitis. *J Perinatol* 1988;**8**:122–3.
33. Grajupa LA, Mukhopadhyay D, Grossman BJ. Chronic polymicrobial bacteremia. *Clin Pediatr* 1975;**14**:280–3.
34. Gunthard H, Pennekamp A. Clinical significance of extraintestinal *Hafnia alvei* isolates from 61 patients and review of the literature. *Clin Infect Dis* 1996;**22**:1040–5.
35. Hargreaves JE, Lucey DR. Life-threatening *Edwardsiella tarda* soft-tissue infection associated with catfish puncture wound. *J Infect Dis* 1990;**162**:1416–17.
36. Hashavya S, Averbuch D, Berger I, et al. Neonatal sepsis following maternal amnionitis by *Edwardsiella tarda*: a case report and a review of the literature. *Eur J Pediatr* 2011;**170**:111–13.
37. Hirono I, Tange N, Aoki T. Iron-regulated haemolysin gene from *Edwardsiella tarda*. *Mol Microbiol* 1997;**24**:851–6.
38. Huys G, Crockaert M, Abbott SL, et al. *Hernia paralvei* sp. nov, formerly known as *Hafnia alvei* hybridization group 2. *Int J Syst Evol Microbiol* 2010;**60**:1725–8.
39. Huys G, Crockaert M, Janda JM, et al. *Escherichia albertii* sp. nov.: a diarrhoeogenic species isolated from stool specimens of Bangladeshi children. *Int J Syst Evol Microbiol* 2003;**53**:807–10.
40. Ismaili A, Bourke B, de Azavedo JC, et al. Heterogeneity in phenotypic and genotypic characteristics among strains of *Hafnia alvei*. *J Clin Microbiol* 1996;**34**:2973–9.
41. Janda JM, Abbott SL. Infections associated with the genus *Edwardsiella*: the role of *Edwardsiella tarda* in human disease. *Clin Infect Dis* 1993;**17**:742–8.
42. Janda JM, Abbott SL. Unusual food-borne pathogens: *Listeria monocytogenes*, *Aeromonas*, *Plesiomonas*, and *Edwardsiella* species. *Clin Lab Med* 1999;**19**:553–82.
43. Janda JM, Abbott SL. The genus *Hafnia*: from soup to nuts. *Clin Microbiol Rev* 2006;**19**:12–18.
44. Janda JM, Abbott SL, Albert MJ. Prototypal diarrheagenic strains of *Hafnia alvei* are actually members of the genus *Escherichia*. *J Clin Microbiol* 1999;**37**:2399–401.
45. Janda JM, Abbott SL, Kroske-Bystrom S, et al. Pathogenic properties of *Edwardsiella* species. *J Clin Microbiol* 1991;**29**:1997–2001.
46. Kahveci A, Asicioglu E, Tigen E, et al. Unusual causes of peritonitis in a peritoneal dialysis patient: *Alcaligenes faecalis* and *Pantoea agglomerans*. *Ann Clin Microbiol Antimicrob* 2011;**10**:12.
47. Kawai T, Kusakabe H, Seki A, et al. Osteomyelitis due to trimethoprim/sulfamethoxazole-resistant *Edwardsiella tarda* infection in a patient with X-linked chronic granulomatous disease. *Infection* 2011;**39**:171–3.
48. Koo HS, Kim JS, Eom JS, et al. Pseudooutbreak of *Pantoea* species bacteremia associated with contaminated cotton pledgets. *Am J Infect Control* 2006;**34**:443–6.
49. Kourany M, Vasquez MA, Saenz R. Edwardsiellosis in man and animals in Panama: clinical and epidemiological characteristics. *Am J Trop Med Hyg* 1977;**26**:1183–90.
50. Lancelevee J, Bret L, David K, et al. Antibiotic resistance and adherence properties of *Hafnia alvei* clinical isolates: a 19-month study in the hospital of Orleans, France. *J Chemother* 2007;**19**: 677–81.
51. Laupland KB, Church DL, Ross T, et al. Population-based laboratory surveillance of *Hafnia alvei* isolates in a large Canadian health region. *Ann Clin Microbiol Antimicrob* 2006;**5**:12.
52. Lim PS, Chen SL, Tsai CY, et al. *Pantoea* peritonitis in a patient receiving chronic ambulatory peritoneal dialysis. *Nephrology (Carlton)* 2006;**11**:97–9.

53. Lindh E, Kjaeldgaard P, Frederiksen W, et al. Phenotypical properties of *Enterobacter agglomerans* (*Pantoea agglomerans*) from human, animal and plant sources. *A P M I S* 1991;**99**:347–52.
54. Liu CH, Lin WJ, Wang CC, et al. Young-infant sepsis combined with urinary tract infection due to *Hafnia alvei*. *J Formos Med Assoc* 2007;**106**:S39–43.
55. Loulergue P, Lortholary O, Mainardi JL, et al. *Hafnia alvei* endocarditis following pyelonephritis in a permanent pacemaker carrier. *Clin Infect Dis* 2007;**44**:621.
56. Magnette C, Tintillier M, Horlait G, et al. Severe peritonitis due to *Pantoea agglomerans* in a CCPD patient. *Perit Dial Int* 2008;**28**:207–8.
57. Maki DG, Martin WT. Nationwide epidemic of septicemia caused by contaminated infusion products, part IV: Growth of microbial pathogens in fluids for intravenous infusions. *J Infect Dis* 1975; **131**:267–72.
58. Maki DG, Rhame FS, Mackel DC, et al. Nationwide epidemic of septicemia caused by contaminated intravenous products, part I: epidemiologic and clinical features. *Am J Med* 1976;**58**:471–85.
59. Manchanda V, Singh NP, Eideh HK, et al. Liver abscess caused by *Edwardsiella tarda* biogroup 1 and identification of its epidemiological triad by ribotyping. *Indian J Med Microbiol* 2006;**24**: 135–7.
60. Marklein G, Waschkowski G, Reichertz C. [Septicaemia caused by *Erwinia herbicola* in an 8-year-old boy (author's transl)]. *Klin Padiatr* 1981;**193**:394–7. In German.
61. Marsh PK, Gorbach SL. Invasive enterocolitis caused by *Edwardsiella tarda*. *Gastroenterology* 1982;**82**:336–8.
62. Matsaniotis NS, Syriopoulou VP, Theodoridou MC, et al. *Enterobacter* sepsis in infants and children due to contaminated intravenous fluids. *Infect Control* 1984;**5**:471–7.
63. Matsumoto H. Studies on the *Hafnia* isolated from normal human. *Jpn J Microbiol* 1963;**7**:105–14.
64. Matsushima S, Yajima S, Taguchi T, et al. [A fulminating case of *Edwardsiella tarda* septicemia with necrotizing fasciitis]. *Kansenshogaku Zasshi* 1996;**70**:631–6. In Japanese.
65. Mayer KH, Zinner SH. Bacterial pathogens of increasing significance in hospital-acquired infections. *Rev Infect Dis* 1985; **7**(Suppl. 3):S371–9.
66. Mikamo H, Ninomiya M, Sawamura H, et al. Puerperal intrauterine infection caused by *Edwardsiella tarda*. *J Infect Chemother* 2003; **9**:341–3.
67. Mojtabaee A, Siadati A. Enterobacter *Hafnia* meningitis. *J Pediatr* 1978;**93**:1062–3.
68. Muyembe T, Vandepitte J, Desmyter J. Natural colistin resistance in *Edwardsiella tarda*. *Antimicrob Agents Chemother* 1973;**4**:521–4.
69. Nagel P, Serritella A, Layden TJ. *Edwardsiella tarda* gastroenteritis associated with a pet turtle. *Gastroenterology* 1982;**82**:1436–7.
70. Nettles RE, Sexton DJ. Successful treatment of *Edwardsiella tarda* prosthetic valve endocarditis in a patient with AIDS. *Clin Infect Dis* 1997;**25**:918–19.
71. Newmark JJ, Hobbs WN, Wilson BE. Reactive arthritis associated with *Hafnia alvei* enteritis. *Arthritis Rheum* 1994;**37**:960.
72. Oesterle CS, Kronenberg HA, Peyman GA. Endophthalmitis caused by an *Erwinia* species. *Arch Ophthalmol* 1977;**95**:824–5.
73. Okubadejo OA, Alausa KO. Neonatal meningitis caused by *Edwardsiella tarda*. *BMJ* 1968;**3**:357–8.
74. Onogawa T, Terayama T, Zen-yoji H, et al. [Distribution of *Edwardsiella tarda* and hydrogen sulfide-producing *Escherichia coli* in healthy persons]. *Kansenshogaku Zasshi* 1976;**50**:10–17. In Japanese.
75. Osuka H, Hitomi S, Koganemaru H, et al. A case of bacteremia caused by *Hafnia paralvei*. *J Infect Chemother* 2011;**17**:855–7.
76. Palaniswamy C, Selvaraj DR, Selvaraj T. Gangrenous cholestitis caused by *Hafnia alvei*: a case report and review of literature. *J Am Med Dir Assoc* 2009;**15**:361–3.
77. Phillips AD, Trabulsi LR, Dougan G, et al. *Edwardsiella tarda* induces plasma membrane ruffles on infection of HEp-2 cells. *FEMS Microbiol Lett* 1998;**161**:317–23.
78. Pien FD, Jackson MT. Tuboovarian abscess caused by *Edwardsiella tarda*. *Am J Obstet Gynecol* 1995;**173**:964–5.
79. Porter P, Wray CC. *Enterobacter agglomerans* spondylodiscitis: a possible, unrecognized complication of tetracycline therapy. *Spine* 2000;**25**:1287–9.
80. Rao KR, Shah J, Rajashekaraiah KR, et al. *Edwardsiella tarda* osteomyelitis in a patient with SC hemoglobinopathy. *South Med J* 1981;**74**:288–92.
81. Reina J, Hervas J, Borrell N. Acute gastroenteritis caused by *Hafnia alvei* in children. *Clin Infect Dis* 1993;**16**:443.
82. Ridell J, Siitonen A, Paulin L, et al. *Hafnia alvei* in stool specimens from patients with diarrhea and healthy controls. *J Clin Microbiol* 1994;**32**:2335–7.
83. Sachs JM, Pacin M, Counts GW. Sickle hemoglobinopathy and *Edwardsiella tarda* meningitis. *Am J Dis Child* 1974;**128**: 387–8.
84. Sakazaki R. Genus IX: *Hafnia*. In: Garrity GM, editor. *Bergey's manual of systematic bacteriology*. New York: Springer; 1984. p. 484–6.
85. Savini V, DeBartolomeo E, Catavitello C, et al. Graft versus host disease–related *Hafnia alvei* colonization and probably infection. *J Med Microbiol* 2008;**57**:1167–9.
86. Slaven EM, Lopez FA, Hart SM, et al. Myonecrosis caused by *Edwardsiella tarda*: a case report and case series of extraintestinal *E. tarda* infections. *Clin Infect Dis* 2001;**32**:1430–3.
87. Starr MP, Chatterjee AK. The genus *Erwinia*: enterobacteria pathogenic to plants and animals. *Annu Rev Microbiol* 1972;**26**: 389–426.
88. Stock I, Wiedemann B. Natural antibiotic susceptibilities of *Edwardsiella tarda*, *E. ictaluri*, and *E. hoshinae*. *Antimicrob Agents Chemother* 2001;**45**:2245–55.
89. Strauss EJ, Ghori N, Falkow S. An *Edwardsiella tarda* strain containing a mutation in a gene with homology to shlB and hpmB is defective for entry into epithelial cells in culture. *Infect Immun* 1997;**65**:3924–32.
90. Takeuchi H, Fujita Y, Ogawa H, et al. Multiple brain abscesses in neonate caused by *Edwardsiella tarda*: case report. *Neurol Med Chir (Tokyo)* 2009;**49**:85–9.
91. Thomson KS, Sanders CC, Washington JA 2nd. Ceftazidime resistance in *Hafnia alvei*. *Antimicrob Agents Chemother* 1993;**37**: 1375–6.
92. Urmenyi AMC, Franklin AW. Neonatal death from pigmented coliform infection. *Lancet* 1961;**1**:313–15.
93. Vandepitte J, Lemmens P, de Swert L. Human edwardsiellosis traced to ornamental fish. *J Clin Microbiol* 1983;**17**:165–7.
94. Van Rostenberghe H, Noraida R, Wan Pauzi WI, et al. The clinical picture of neonatal infection with *Pantoea* species. *Jpn J Infect Dis* 2006;**59**:120–1.
95. Vohra K, Torrijos E, Jhaveri R, et al. Neonatal sepsis and meningitis caused by *Edwardsiella tarda*. *Pediatr Infect Dis J* 1988;**7**: 814–15.
96. von Graevenitz A. *Erwinia* species isolates. *Ann N Y Acad Sci* 1970;**174**:436–43.
97. Wechsler AM, Bottone E, Lasser R, et al. Brain abscess caused by an *Erwinia* species: report of a case and review of the literature. *Am J Med* 1971;**51**:680–4.
98. Westblom TU, Milligan TW. Acute bacterial gastroenteritis caused by *Hafnia alvei*. *Clin Infect Dis* 1992;**14**:1271–2.
99. Wilson JP, Waterer RR, Wofford JD Jr, et al. Serious infections with *Edwardsiella tarda*: a case report and review of the literature. *Arch Intern Med* 1989;**149**:208–10.
100. Yang CH, Wang CK. *Edwardsiella tarda* bacteraemia—complicated by acute pancreatitis and pyomyoma. *J Infect* 1999;**38**:124–6.
101. Yeager AM, Kanof ME, Kramer SS, et al. Pneumatosis intestinalis in children after allogeneic bone marrow transplantation. *Pediatr Radiol* 1987;**17**:18–22.
102. Zighelboim J, Williams TW Jr, Bradshaw MW, et al. Successful medical management of a patient with multiple hepatic abscesses due to *Edwardsiella tarda*. *Clin Infect Dis* 1992;**14**:117–20.

CHAPTER 115

Aeromonas

Santhosh Nadipuram • James D. Cherry

Aeromonas spp. were recognized for their role in disease first by Sanarelli in 1891. His findings associated aeromonads with bacteremic "red leg" disease in frogs.[154] Studies by Hill and associates in 1954 linked the first human disease, acute fulminating metastatic myositis, with *Aeromonas* infection.[29] Generally, *Aeromonas* spp. have been considered opportunistic pathogens for humans; however, these organisms have been identified with increasing frequency as primary pathogens in normal individuals as well as in compromised hosts.

Aeromonas bacteria are ubiquitous. They are found as normal flora in nonfecal sewage and are isolated from tap water, canals, streams, and rivers. They also have been isolated from clinical and food samples. Aeromonads are pathogens for cold-blooded animals (fish, shellfish, amphibians, and reptiles).

EPIDEMIOLOGY

Ewing and associates[55] and other investigators have isolated *Aeromonas* from tap water and from the water or sediments of rivers, especially during periods when the water temperature was relatively warm.[88,113,159] Hazen and colleagues[74] recovered *A. hydrophila* from 135 of 147 natural water sources in 30 states of the United States and reported that its density was higher in flowing (lotic) than in calm (lentic) water systems and lower in freshwater than in saline systems; *Aeromonas* could not be recovered from waters in which the saline content approached that of sea water or from extremely polluted waters. Leclerc and Buttiaux[97] found *Aeromonas* in 30 percent of more than 9000 samples of drinking water in France. Drinking water sampling by the U.S. Environmental Protection Agency (EPA) in 2011 revealed the presence of aeromonads in 42 of 293 (14.3%) of samples of public water systems in the United States.[49] *Aeromonas* spp. also have been recovered from hospital water supplies.[113,135,136] Pin and colleagues[138] isolated these organisms from clinical and food samples, including poultry, shellfish, pork, and beef, where they thrived even at low temperatures.

Aeromonas organisms survive readily on surfaces such as bench tops and moistened paper towels. Slotnick[166] recovered *Aeromonas* organisms from a moistened paper towel that had been allowed to dry 24 hours after the application of the organisms. When the towel was placed in a humidified closed environment, the period was extended to 2 weeks.

A. hydrophila may be found in the mouths of fish, alligators, turtles, tadpoles, and frogs.[78] Studies done in Thailand and Turkey have found high percentages of *Aeromonas* in uncooked commercially sold seafood (27% of samples from Thailand, 94% of fish from Turkey).[182,185] Another investigation of fish and animal meats bought at markets in northern India revealed *Aeromonas* species in 38 of 332 (11%) food samples.[163] It also has been found in the feces of guinea pigs and laboratory mice.[47] Ticks are another source of *Aeromonas*. Unusual sources of *Aeromonas* infection include contamination of home or hospital hemodialysis equipment,[83,143] tsunami-associated wound contamination,[77] and contamination of blood or blood products.[140,144] San Joaquin and colleagues[153] noted that *Aeromonas* organisms also have been found in ornamental aquaria belonging to patients with *Aeromonas*-associated gastroenteritis; however, they found that isolates from the aquaria differed in susceptibility testing from the gastrointestinal isolates, so the aquaria probably were not the sources of infection. A multicenter study of over 150 extraintestinal clinical isolates showed the presence of 22 isolates of *A. aquariorum*, a species once believed to be only virulent to ornamental aquaria.[58]

Aeromonas also have been isolated from a large proportion of ready-to-eat salads.[109] Of *Aeromonas* salad isolates, 35 percent were *A. hydrophila* or *A. sobria*. All isolates tested in this study had at least one marker of enteropathogenicity, including hemolysin and cytotoxin production. Nonetheless, few if any, cases of diarrhea have been associated with ingestion of these salads in immunocompetent hosts.

In 1988, California made infection by *Aeromonas* a reportable condition, permitting the first population-based study of the epidemiology of infection caused by this organism. The overall incidence rate for *Aeromonas* isolation was 10.6 cases per 1 million population. The gastrointestinal tract was the most common site of infection (81% of cases), followed by wounds (9%). Five (2%) of 219 patients with *Aeromonas* infection died; all had serious underlying medical conditions.[92] Studies of traveler's diarrhea revealed the presence of *Aeromonas* spp. in 8 of 56 (14%) stool samples in Thailand.[35] A retrospective review of diarrhea in neonates in Bangladesh showed the presence of *Aeromonas* spp. in 222 of 699 stool samples that were positive for bacteria (222 of 2511 overall), making it the second most common isolate after *Vibrio cholerae* in this cohort.[91] A study involving patients with diarrhea in Spain showed *Aeromonas* spp. in 32 of 800 (4%) diarrheal stools.[129] The bacteria isolated were found to be similar (by pulsed-field gel analysis) to isolates from drinking water isolates. Data from a study in southern Brazil using 680 stool samples from patients with

diarrhea compared with 300 healthy controls showed that 2.6 percent of the diarrheal samples contained *Aeromonas*, whereas only 0.3 percent of the control samples contained this organism.[172] *A. hydrophila* has been placed on the EPA's contaminant candidate list of emerging pathogens in drinking water because it has the potential to grow in water distribution systems and to be resistant to chlorination.[20]

ETIOLOGIC AGENT

Aeromonas organisms are motile, asporogenic, gram-negative rods that contain a single polar flagellum. The organisms are oxidase positive and catalase positive and produce acid or gas during carbohydrate fermentation. They can grow at temperatures between 0°C and 41°C. Growth may occur within a pH range of 5.5 to 9. Lipase, gelatinase, DNase, and other exoenzymes are formed by these organisms.[54,158,179]

Aeromonads grow well on blood agar; most strains produce a large zone of β-hemolysis on this medium, although nonhemolytic strains exist. *Aeromonas* colonies on blood agar have a ground-glass appearance and a fruity odor.[181] Aeromonads also grow on MacConkey, eosin-methylene blue, *Salmonella-Shigella*, and triple-sugar iron media.

The sensitivity and specificity of various media for detection of *Aeromonas* spp. in fecal specimens have been evaluated.[122,148,178] Isolation is achieved readily on any of the following: Pril-xylose-ampicillin agar, xylose-sodium, deoxycholate citrate agar, alkaline peptonic water, inositol–brilliant green bile salts agar, trypticase soy broth with ampicillin, and dextrin-fuchsin-sulfite agar.[178] The colonies appear almost colorless on these media, with the exception of growth on dextrin-fuchsin-sulfite agar, on which they appear dark red.

Mishra and associates[117] evaluated five selective agars. Of these, sheep blood agar with 30 mg of ampicillin per liter (ASBA 30) permitted the greatest number of *Aeromonas* colonies to grow while also inhibiting the competing fecal flora. These researchers recommend that ASBA 30 be used with DNase–toluidine blue agar (DNTA) to detect the ampicillin-susceptible strains and the nonhemolytic strains. The combination of ASBA 30 and DNTA allowed 98 percent of all isolates to be detected.

Strains of motile *Aeromonas* isolates can be identified to species level by use of the following tests: production of nitrate reductase; fermentation of D-glucose and trehalose; failure to use mucate; and the inability to produce acid from D-arabitol, dulcitol, erythritol, and xylose.[1] More recently, the use of restriction fragment length polymorphism analysis of the 16s RNA, along with gene sequencing of common genes has contributed to the speciation of *Aeromonas* isolates.[58] Detection of *Aeromonas* organisms by use of strain-specific fluorescent antibody has been described.[178] The technique is not satisfactory; only a small percentage of isolates react with prepared antisera, which suggests the presence of numerous serogroups. The use of a monoclonal antibody against *A. hydrophila* has been shown to overcome this problem, however.[46] This monoclonal antibody could prove beneficial in the future as a screening tool. Additionally, the study of selected housekeeping genes has been helpful in establishing phylogenetic relationships among members of the aeromonads.[169]

Aeromonas previously was considered a member of the Vibrionaceae, which included the genera *Vibrio* and *Plesiomonas*.[141] Molecular genetic techniques have identified that these three genera are not related evolutionarily, however, and aeromonads represent a family of their own.[41] *Aeromonas* have long been viewed as belonging to one of two major groups on the basis of their ability to grow at different temperatures. Mesophiles were strains that grew at 35°C to 37°C, and psychrophilic strains were more prevalent at lower temperatures (22°C to 28°C). These two groups could be distinguished not only by optimal growth conditions but also by elaboration of pigmentation on tyrosine agar and production of indole. The mesophilic strains are motile, and the psychrophilic strains are nonmotile.

Twenty-two *Aeromonas* spp. have been recognized, and this number is expected to increase as new strains are isolated and identified.[1,132] Although the number of strains of psychrophilic *Aeromonas* spp. has remained stable, the number of various strains of mesophilic species isolated has increased. Several classification schemes within the genus are in use. The strains are classified based on biochemical assays or the use of genotyping techniques such as DNA-DNA hybridization and restriction fragment length polymorphism of the 16S rRNA, or both. Genetic techniques have been used to differentiate the genus into genomospecies or hybridization groups (HG), allowing more precise identification.[21]

Abbott and associates[1] classified *Aeromonas* based on the results of the Møeller decarboxylase and dihydrolase reactions. They also were able to place the strains they studied into complexes based on certain biochemical reactions. The *Aeromonas* strains that produced elastase, pectinase, and stapholysin were grouped into the *A. hydrophila* complex (*A. hydrophila*, *A. bestiarum*, and *A. salmonicida*). Similarly, the *A. caviae* complex includes *A. caviae*, *A. media*, and *A. eucrenophil*, and the *A. sobria* complex comprises *A. veronii* HG8, *A. jandaei*, *A. schubertii*, and *A. trota*.

In addition, other species, including *Aeromonas media* (similar to *A. salmonicida*),[6] *A. veronii*,[76] *A. schubertii*,[28,75] *A. jandaei*,[27] *A. trota*,[26] *A. allosaccharophila*,[107] *A. encheleia*,[53] *A. bestiarum*,[8] *A. popoffii*,[81] and *A. eucrenophila*,[158] have been described. A new species, *A. culicicola*, was isolated more recently.[137] Other isolates include *A. enteropelogenes* and *A. ichthiosmia*, which seem to be similar to previously identified species.[40] Of the species isolated currently, *A. hydrophila*, *A. caviae*, and *A. veronii* have been associated most often with human infections.[115]

Aeromonas is confused most frequently with Enterobacteriaceae. The oxidase test always should be performed to aid in differentiation; *Aeromonas* organisms generally are oxidase positive, whereas the Enterobacteriaceae are oxidase negative. McGrath and associates[111] described oxidase-variable strains of *A. hydrophila*. These strains are oxidase positive when grown on nonselective media but oxidase negative when grown on differential or gram-negative media. Organic acid end-products of

lactose fermentation can inhibit the oxidase reaction. Paik[130] also has shown that a platinum wire loop should be used for oxidase testing; use of an iron-containing loop can cause false-positive oxidase results.

Aeromonas has traditionally shown susceptibility to ceftriaxone (8 μg/mL), chloramphenicol (2 to 4 μg/mL), gentamicin (0.5 to 2 μg/mL), streptomycin (16 μg/mL), trimethoprim-sulfamethoxazole (<0.5/9.5 μg/mL), and imipenem and meropenem.* Reinhardt and George[146] determined that the most active on a weight basis were ciprofloxacin, enoxacin, and norfloxacin; nalidixic acid and trimethoprim-sulfamethoxazole also possessed good activity. Neither sulfamethoxazole nor trimethoprim alone was active. In addition, these investigators did not note appreciable differences in susceptibility among species or in susceptibility between fecal versus nonenteric isolates. In contrast, Motyl and colleagues[121] noted higher levels of resistance to various antibiotics among *A. hydrophila* strains compared with *A. sobria* or *A. caviae*, as judged by minimal inhibitory concentration. Susceptibility to cephalothin may serve as a useful criterion in the identification of *A. sobria*.[121,153] Resistance to fluoroquinolones has been shown to be based on chromosomal mutations of the *gyrA* and *parC* genes,[2,7] along with a plasmid containing a *qnrS* gene[9,10,73] and a separate plasmid with the aac(6′)-Ib-cr.[57]

Aeromonas organisms consistently are resistant to penicillin, ampicillin, carbenicillin, cephalothin, erythromycin, clindamycin, and vancomycin. Sawai and associates[157] showed that resistance to β-lactam antibiotics is caused by the production of a species-specific, chromosome-mediated β-lactamase. Two studies of *Aeromonas* spp. cultures examining for the presence of the *cphA* gene encoding for metallo-β-lactamase have been published. In Brazil, 87 *Aeromonas* isolates recovered from environmental sources showed a prevalence of the *cphA* gene in 97.6 percent of *A. hydrophilia* isolates and 100 percent of *A. jandaei* isolates. Likewise, 51 consecutive blood isolates of *Aeromonas* spp. were examined and found that overall, *cphA* was present in 69 percent of these isolates. The rise of these resistant strains of *Aeromonas* spp. is very troubling.[12,183] Tetracycline resistance was found in environmental and fish isolates of *Aeromonas* by Han and colleagues.[72] This resistance was conferred by several *tetA* through *tetE* genes; *tetE* was found on a plasmid. It is not R plasmid mediated. In 1980, McNicol and coworkers[112] reported the recovery of *Aeromonas* isolates from the Chesapeake Bay that were resistant to tetracycline and polymyxin B. They also noted that 57 percent of isolates from Bangladesh had a multiple streptomycin-chloramphenicol-tetracycline resistance phenotype that correlated with the presence of a larger plasmid.

PATHOGENESIS

In addition to its pili, *A. hydrophila* produces numerous virulence factors, including extracellular toxins and enzymes that are involved in the pathogenesis of *A. hydrophila* infection. Some of the toxins elaborated by *A. hydrophila* include hemolysins, aerolysin, and enterotoxins.[15] Like many other gram-negative bacteria, *Aeromonas* spp. employ a type III secretion system to bind to host cells and deliver toxins.[2]

α-Hemolysin is released from cells during a stationary growth phase. It has a molecular weight of 50[101,102] to 65[101] kDa and is stable at room temperature between pH 3.5 and 9.5. It is heat labile and is cytotoxic to HeLa cells and human embryonic lung fibroblasts.[177] When injected into rabbit skin, it causes dermonecrosis. Intraperitoneal injection of α-hemolysin is lethal for rabbits and mice.

β-Hemolysin has a molecular weight of 49 to 53 kDa and is released near the end of the logarithmic phase of growth of the *Aeromonas* organism. It is heat labile and resists destruction by trypsin and pronase.[102] It causes dermonecrosis of rabbit skin and is lethal for rabbits, rats, and mice. It is cytotoxic to HeLa cells and to human diploid lung fibroblasts.[175] It also has been shown to induce impairment in the intestinal epithelial barrier and to stimulate active chloride secretion in human colon cell monolayers, contributing to enteritis.[52]

α-Hemolysin and β-hemolysin may be significant virulence factors. Most clinical isolates are β-hemolytic, and hemolysis is a common feature of infection caused by *Aeromonas* spp. α-Hemolysin and β-hemolysin in high concentrations produce hemorrhagic enteritis in a rabbit ileal loop; however, neither is clearly established as a virulence factor in diarrheal disease.[103] Antibodies to either hemolysin neutralize both toxins.

Aerolysin is a pore-forming toxin that shares some homology with β-hemolysin.[52] After inserting into its target cells, it disrupts ion gradients and membrane potentials, leading to cell lysis.[96] Caco-2 cells derived from human intestine have been shown to be very sensitive to the effects of aerolysin.[3]

In 1975, Sanyal and associates[155] showed that the enterotoxins from *A. hydrophila* were enterotoxigenic. Subsequently, enterotoxigenic *A. hydrophila* has been isolated from humans,[25,65-68,88,180] water sources,[15] fish, and pigs.[22,127] Sha and colleagues[160] have characterized three enterotoxins from *A. hydrophila.* The cytotoxic enterotoxin has hemolytic, cytotoxic, and enterotoxic activities and has a molecular weight of 52 kDa. It is a pore-forming toxin that has been shown to cause fluid secretion and tissue damage in mouse ileal loops.[96] The other two are cytotonic enterotoxins. One has a molecular weight of 44 kDa and is heat labile. The second has a predicted molecular weight of 71 kDa and is heat stable. These cytotonic enterotoxins cause elevation of cyclic adenosine monophosphate and prostaglandin levels in Chinese hamster ovary cells and fluid secretion in rabbit ileal loops.[160] All three enterotoxins have been implicated in *A. hydrophila*–induced gastroenteritis. *Aeromonas* spp. also produce proteinase A and B, endopeptidase, staphylolytic enzyme, fibrinolysin, and leukocidin.[101] The precise relationship of these toxins and enzymes to the pathogenesis of human infection is unclear.

Strains of *Aeromonas* produce proteases, DNase, lecithinase, and elastase.[4] These extracellular enzymes may have pathologic significance. *Aeromonas* strains that possess hemolytic and cytotoxic capabilities and that are characterized as HG1/BD-2 type strains have been

*References 12, 55, 56, 127, 128, 145, 178.

related strongly to patients with diarrheal disease.[95] Human *Aeromonas* isolates have been found also to express Shiga toxins (much like Shiga toxin–producing *Escherichia coli*).[9]

Antihemolysin and agglutinating and precipitating antibodies to *A. hydrophila* have been detected in patients with systemic *Aeromonas* infections but not in patients with superficial infections. Increases in the antihemolysin titer to 1:1280 and in the agglutinin titer to 1:640 have been noted.[30]

Burke and colleagues[24] found that many strains associated with diarrhea were able to hemagglutinate cells from human, horse, rat, and guinea pig. Of these strains, 68 percent displayed fucose-resistant hemagglutination. The investigators suggested that these properties may contribute to virulence. The ability of *Aeromonas* spp. to create biofilm allowing for colony protection and quorum sensing has been demonstrated.[33,50]

Ketover and associates[89] showed that normal serum promotes phagocytosis and intracellular killing of *Aeromonas* organisms by normal white blood cells. In contrast, sera from two patients with fatal *Aeromonas* infections failed to do so. One patient had an increase in the serum opsonic antibody titer from less than 1:5 in the acute stage of disease to 1:5120 in the convalescent stage. These studies suggest that a specific opsonizing antibody that is present in normal serum and normal bactericidal activity of neutrophils are required to prevent invasive *A. hydrophila* infections.

The flexible type IV pili of *Aeromonas* are the predominant pili expressed on fecal isolates of diarrhea-associated species of *Aeromonas.* They represent a family of type IV pili that has been designated Bfp (bundle-forming pili). Kirov and associates[93] have presented compelling evidence to support the concept that Bfp are important intestinal colonization factors. More recently, this group also showed that *Aeromonas* flagella are adhesins for human intestinal cells, playing a role in its enteropathogenicity.

Serogroup analysis may be helpful in establishing which *Aeromonas* isolates can cause human disease. Serogroups O:11, O:34, and O:16 predominate as causes of clinical infection.[82] Hadi and colleagues successfully isolated a 22-kb region of the *A. veronii* bv. *sobria* genome, demonstrating many functions of the Bfp subunits by mutations.[71]

Clear correlation between known virulence properties and enteropathogenicity in humans is lacking. This lack of a clear correlation has been attributed to the heterogeneity of *Aeromonas* spp., the great variety of *Aeromonas* virulence factors, and the loose association between virulence factors and the phenotype and genotype.[115] Consequently, many researchers have proposed a multifactorial pathogenesis with the involvement of numerous cellular and extracellular virulence factors.[36]

CLINICAL MANIFESTATIONS

Aeromonas spp. have been implicated as a cause of septicemia, gastroenteritis, peritonitis, skin and wound infections, osteomyelitis, septic arthritis, ocular infections, myositis, urinary tract infections, pneumonia, meningitis, and hemolytic-uremic syndrome in children. Most of these infections have been noted in normal and compromised hosts.[14,19,59,133,134]

Sepsis caused by *Aeromonas* spp. has been reported in children.* Because *Aeromonas* infection is not a reportable disease, the total number of affected children is unknown. Although septicemia caused by *Aeromonas* spp. has occurred in normal children, most children affected have had a disorder known to impair the normal host response to infection or a disorder in which the intact skin as a barrier to infection has been destroyed. *Aeromonas* infection has been noted in children with leukemia (particularly those with neutropenia), aplastic anemia, cirrhosis, hemoglobinopathies, malnutrition, burns, and renal failure. Neonates are susceptible to sepsis when exposed to contaminated water. Chaudhari and Todd detailed neonatal sepsis, necrotizing enterocolitis, and death associated with *A. hydrophilia* in a 27-week-gestation neonate born in a toilet.[34]

The clinical manifestations of septicemia are similar to the manifestations noted in other gram-negative enteric bloodstream infections with high fever and shock. During the course of septicemia in some of these patients, ecthyma gangrenosum also has been noted. The reported case-fatality rate of 50 percent despite administration of antibiotic therapy presumably is related to the severity of the underlying disorders and not to an unusual virulence of the organism. The propensity for infection in the compromised host suggests that *Aeromonas* organisms are of low virulence for the human host. Bacteremia with *A. sobria*, *A. punctate*, *A. veronii*, *A. caviae*, and *A. schubertii* also has been described.[37,83,87,144,165]

Meningitis caused by *Aeromonas* spp. also has been reported in children.[60,61,184] In one reported review of 21 years' experience with gram-negative bacillary meningitis, *Aeromonas* spp. accounted for 2 percent of the cases.[177] The course was fulminant, and the patients died despite having received antibiotic therapy. All of these children could be considered immunocompromised as a result of sickle-cell anemia (a 23-month-old child) or age (neonates).

Gastrointestinal infections caused by *Aeromonas* spp. have been reported with greater frequency in pediatric patients. *A. hydrophila*, *A. sobria*, *A. caviae*, and *A. punctata* have been recovered from stool specimens of patients with gastroenteritis.[25,32,62,83,120] In 1961, Martinez-Silva and colleagues[108] described an epidemic of enteritis in a newborn nursery affecting nine infants (eight as neonates and one at 7 days old). *Aeromonas* spp. were found in the stools of six of these infants. One neonate, the only patient with a pure growth of *Aeromonas* in a stool specimen, died. In 1964, Rosner[150] reported severe gastroenteritis in a child with growth of *A. hydrophila* from four stool cultures. In 1991, one study reported 224 cases of *Aeromonas* gastroenteritis in Iowa from January to June.[142] Taneja and associates[173] described an outbreak of diarrhea caused by *Aeromonas* spp. on a hematology-oncology ward in a pediatric hospital during March-April 2001 where 6 of 18 children were infected. *Aeromonas* spp.

*References 23, 39, 44, 69, 80, 89, 124, 133, 149, 156, 181, 184, 186.

were found to be the sole enteropathogen in five of the six children. Aeromonads have been detected worldwide as the sole pathogen causing diarrhea in 2 to 20 percent of affected children and in only up to 2 percent of children without diarrhea.[36,43,68,174]

In an attempt to assess the role of *Aeromonas* spp. in diarrheal disease, numerous investigators have evaluated the fecal carriage rate of the organism.[70] Freij[61] described a study performed by other investigators who recovered *Aeromonas* from none of 300 adults and from 31 of 4426 (0.7%) children younger than 2 years of age. Pitarangsi and coworkers[139] reported carrier rates of 16 to 27 percent in various districts in Thailand and noted the same frequency of *A. hydrophila* in stools of Thais with and without diarrhea. In contrast, *A. hydrophila* was recovered from the stools of American Peace Corps volunteers more frequently when they had diarrhea than when their stool frequency was normal.[35] Only three *Aeromonas* isolates were reported from 1685 rectal swab cultures obtained from 1217 children hospitalized for gastroenteritis in Manitoba, Canada. Bhat and colleagues[16] recovered *Aeromonas* organisms from 7 of 133 patients with acute diarrhea in a valley in India and found *Aeromonas* and *Plesiomonas shigelloides* in the well water commonly used by these individuals. Soltan Dallal and Moezardalan[170] isolated *Aeromonas* spp. from 14 of 310 Iranian children in a case-control study. A large, retrospective study on the island of Crete found a prevalence of *A. hydrophilia* to be 0.44 percent in submitted stool cultures from children over an 18-year period.[106]

Gracey and associates[66,67] described a prospective study in Australia of 1156 children with diarrhea and an equal number of age- and sex-matched control subjects. Enterotoxigenic *Aeromonas* organisms were isolated from 10.2 percent of children with diarrhea compared with 0.6 percent of healthy children. *Aeromonas* was the only potential pathogen recovered in 6.5 percent of children with diarrhea. Cases of *Aeromonas* infection peaked during the summer months. The mean duration of diarrhea was 15.3 days, and 33 percent of the children required hospitalization. These investigators described three clinical syndromes of *Aeromonas* gastroenteritis: (1) watery diarrhea, vomiting, and low-grade fever in 41 percent; (2) diarrhea with blood and mucus in 22 percent; and (3) prolonged diarrhea of more than 2 weeks' duration in 37 percent.

Many investigators[5,31,123,125,152] have attempted to describe gastrointestinal infections caused by *Aeromonas* spp. Gluskin and associates[63] performed a 15-year study of the rate of *Aeromonas* spp. in gastroenteritis in hospitalized children. A total of 146 strains of *Aeromonas* spp. were isolated from 32,810 fecal specimens from 13,820 hospitalized patients. These isolates constituted 4 percent of all pathogenic bacterial strains cultured. Most of the cases of diarrhea (94%) occurred in children younger than 3 years old. The peak incidence occurred in infants aged 2 to 6 months. Bloody diarrhea occurred in 7 percent of children. Several investigators[5,152] have detected a larger number of cases of *Aeromonas*-associated diarrhea in the summer months than in other months, whereas Challapali and colleagues[31] found no seasonal patterns of *Aeromonas* isolation. The greatest number of cases occurred in children younger than 12 months to 3 years of age.

Symptoms included diarrhea, bloody stools, vomiting, abdominal cramps, mild dehydration, and fever. *Aeromonas*-associated diarrhea resembled other types of bacterial diarrhea, except that fecal leukocytes were absent in children with *Aeromonas*-associated diarrhea; in contrast, fecal leukocytes were found in 60 percent of children with other types of bacterial enteritis.[31] A cholera-like illness caused by enterotoxigenic *A. sobria* has been described.[32] Gastroenteritis caused by *A. sobria* and *A. hydrophila* tended to be acute, whereas diarrhea associated with *A. caviae* frequently was chronic, lasting 4 to 6 weeks in untreated patients.

San Joaquin and Pickett[152] described three patients with *A. caviae* diarrhea who originally presented with failure to thrive presumed to be secondary to formula intolerance. Diarrhea in these patients improved after administration of trimethoprim-sulfamethoxazole. Moyer[123] noted that although *A. caviae* is considered nonpathogenic, five pediatric patients with otitis media who were treated with penicillin or ampicillin subsequently developed diarrhea in which *A. caviae* was the only potential enteric pathogen. The prior therapy with antibiotics to which *Aeromonas* spp. are known to be resistant probably contributed to colonization of the gastrointestinal tract with *A. caviae* and subsequent development of diarrhea. These four patients also were treated successfully with trimethoprim-sulfamethoxazole.

Outbreaks of diarrhea associated with *Aeromonas* in daycare centers have been described.[43] *A. caviae*, *A. hydrophila*, and *A. sobria* were the strains recovered most commonly.

Complications of *Aeromonas* intestinal infection included gram-negative bacteremia, intussusception, internal hernia strangulation, hemolytic-uremic syndrome,[19,118,147] and failure to thrive. Peritonitis has been reported in a 5-year-old patient with a ruptured appendix.[80] No additional clinical information about this patient was provided. Peritoneal dialysis catheter–related peritonitis was described in one patient.[100] The patient was involved in gardening and had poor adherence to peritoneal dialysis protocol and hand washing guidelines.

A. hydrophila has been recovered from the skin or from wound infections of children.* Most of these patients have been normal hosts; three had leukemia. In 40 percent of these cases, *Aeromonas* organisms were not recovered from the lesion in pure culture. The lower extremity was involved in 75 percent of these cases. Exposure to water was noted in 40 percent of these cases; alligator bites, snake bites, stepping on glass, and burns were other presumed predisposing factors. Clinical manifestations included cellulitis, hemorrhagic blebs, and purulent diarrhea with fever and leukocytosis. Secondary bacteremia and osteomyelitis have been reported.[18,79] In a study of neonates with omphalitis in Karachi, Pakistan, 19 of 583 (3.2%) of purulent secretions from umbilical stumps yielded a positive culture with *Aeromonas* spp. The clinical implications of the presence of *Aeromonas* in these neonates was not discussed.[116]

*References 13, 18, 45, 59, 80, 89, 110, 124, 134, 147, 161, 171, 178.

Aeromonas organisms have been recovered from skin lesions that have been the result of tick bites.* In each of the cases, a circumscribed area of purple discoloration has surrounded the bite and nonpurulent drainage from the center of the lesion yielded the organism. These patients sought medical attention because the local lesion had persisted or had increased in size over the course of 1 to 2 weeks. *A. schubertii* also has been isolated from traumatic wound infections.[28]

Ecthyma gangrenosum caused by *A. hydrophila* has been described in several children with leukemia.[89,124,161] Ecthyma gangrenosum has been seen in several children with *Aeromonas* septicemia who have had malignant or hepatobiliary disease. Clark and Chenoweth[38] isolated *A. hydrophila* from an 18-month-old boy and a 2-year-old boy, both of whom had liver transplants for biliary atresia and subsequently developed cholangitis. In both instances, these were polymicrobial infections.

Current practice by plastic surgeons to use medicinal leeches to control venous congestion after reimplantation of limbs or closure of skin flaps has led to sepsis and skin and soft tissue infection with *Aeromonas* spp. in adults.[99] This practice is now becoming more prevalent in children, and practitioners should be aware that therapy with leeches requires antibiotic prophylaxis. Leeches can harbor *Aeromonas* spp. resistant to the prophylaxis of choice.

Lopez and associates[104] described an 8-year-old child with acute myelogenous leukemia with bacteremia and osteomyelitis; *Aeromonas* organisms grew from a bone aspirate of this patient. Blatz[18] reported finding osteomyelitis and *Aeromonas* bacteremia in a previously healthy 16-year-old patient; a bone aspirate was not attempted. Septic arthritis caused by *A. hydrophila* also has been described in a child with leukemia; the organism was recovered from the second metacarpophalangeal joint at autopsy.[44] Elwitigala and colleagues[51] also recovered *A. hydrophila* in a previously healthy 13-year-old girl who developed severe septic arthritis of the knee after injury sustained in a private freshwater lake. This infection frequently is rapidly progressive.

Aeromonas has been recovered from the conjunctiva of a previously healthy 7-year-old boy whose eye had been penetrated by a safety pin[168] and from the anterior chamber of an 8-year-old boy who developed endophthalmitis after receiving a corneal laceration from a fishhook.[39] *A. sobria* endophthalmitis has been described in a 14-year-old patient after a penetrating eye injury occurred in which a cormorant pecked the patient's eye.[98]

Although numerous cases of *Aeromonas* urinary tract infections have been reported in adults, only a few cases have been reported in children. McCracken and Barkley[110] reported the recovery of *A. hydrophila* in pure culture from the urine of a 5-month-old boy with diarrhea. Bartolomé and colleagues[13] described a case of urinary tract infection associated with diarrhea in a male neonate with bilateral ureterohydronephrosis and bladder involvement from posterior urethral valves. Ojeda-Vargas and associates[126] reported the case of an 18-year-old girl with severe pyelonephritis requiring admission to an intensive care unit: *A. hydrophila* and *Pseudomonas* were isolated from her urine cultures. In a case series of seven patients with *Aeromonas* urinary tract infections in a hospital in Puducherry, India, one 4-year-old girl with chronic renal failure was found to grow *A. caviae* from a urine specimen.[105]

Myositis caused by *Aeromonas* also has been described in children. A 9-year-old girl and a 16-year-old boy required amputation of their legs as a result of *Aeromonas* myositis.[45,167] Another case of sepsis accompanied by myositis of the thigh was described by Papadakis and associates in a 2½-year-old girl with acute lymphoblastic leukemia; this child recovered well with prompt antibiotic therapy and surgical débridement.[131] Necrosis of muscle was noted in both cases, and gas was seen on the radiographs before amputation in the 9-year-old girl. Fatal myofascial necrosis also has been reported in a patient with a history of aplastic anemia.[64] *A. hydrophila* also has been isolated from abscesses caused by snake bites.[84]

Pneumonia caused by *A. hydrophila* is a rare event in children. Kao and associates[86] reported that there have been a total of seven case reports in the literature so far, and four patients died of fulminant disease. Of the seven patients, four had accompanying medical conditions, including acute leukemia (two patients), cavernous hemangioma with thrombocytopenia (one patient), and nephrotic syndrome (one patient). One patient was the victim of a near-drowning, and two patients were previously healthy.[86,145,164] One of the previously healthy patients was described by Kao and associates[86] in their case report and literature review. The patient was a 5-year-old girl who presented with fulminant sepsis and pneumonia and quickly died. *A. hydrophila* was recovered from her blood, endotracheal aspirate, and postmortem pleural fluid. Neither a bone marrow aspirate nor an immunologic workup was performed, so whether she had an underlying malignancy or immunodeficiency that might have contributed to her rapid demise is unknown. *A. hydrophila* has been isolated from lung abscess at autopsy of a 16-year-old girl with *A. hydrophila* septicemia and leukemia.[44]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Aeromonas should be considered a possible cause of infection in children with any of the disorders previously noted. In addition, *Aeromonas* infection should be considered in any child noted to be exposed to a contaminated water source (e.g., sewage, waste). It also always should be included as a possible cause of gastroenteritis, bacteremia, and skin infection in a compromised host. Generally, *Aeromonas* organisms are recognized only when they grow from body fluids or tissues that normally are sterile. Also, as noted earlier, *Aeromonas* infection should be suspected in ill children after the use of medicinal leech therapy in surgery or subsequent to an alligator bite. The best methods for isolation of *Aeromonas* organisms have been described (see the section on etiologic agent).

*Feigin RD, unpublished data.

A. hydrophila in food can be detected by an enzyme-linked immunosorbent assay and polymerase chain reaction.[17,114,176] These organisms also have been identified in environmental samples by use of 16S rDNA–targeted oligonucleotide primers.[48] Many sophisticated techniques, including rRNA sequencing, DNA-DNA reassociation techniques, and polymerase chain reaction, have been used to continue to identify and differentiate *Aeromonas* spp.[21,42,90,115]

TREATMENT AND PROGNOSIS

Penicillin-hydrolyzing β-lactamases have been detected in most strains of *Aeromonas*, rendering those strains resistant to ampicillin.[119] Piperacillin shows variable activity against *Aeromonas* spp., whereas ticarcillin-clavulanate generally is active. In vitro, *Aeromonas* organisms generally are susceptible to chloramphenicol, aminoglycosides, trimethoprim-sulfamethoxazole, aztreonam, quinolones, and the third-generation cephalosporins.[85,94,151] Great care must be taken to obtain minimal inhibitory concentrations on *Aeromonas* isolates, and carbapenems should be initiated when there is lack of clinical response, because extended-spectrum β-lactamase–producing organisms are known to occur both in the environment and in clinical isolates.[12] Likewise, rates of tetracycline, fluoroquinolone, and aminoglycoside resistance should be known before considering these agents.[2,10-12,73,162]

Initial empirical treatment with a third-generation cephalosporin is suggested. Following this, a drug to which the organism is sensitive should be provided (usually intravenously). The duration of administration depends on the site of infection and the clinical response to therapy. The occurrence of *Aeromonas* infections predominantly in compromised hosts accounts for the high case-fatality rate despite administration of an antibiotic agent to which the organism is susceptible.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abolghait SK, Iida T, Kodama T, et al. Recombinant AexU effector protein of *Aeromonas veronii* bv. *sobria* disrupts the actin cytoskeleton by downregulation of Rac1 and induces direct cytotoxicity to beta4-integrin expressing cell lines. *Microb Pathog* 2011;**51**:454–65.
7. Alcaide E, Blasco MD, Esteve C. Mechanisms of quinolone resistance in *Aeromonas* species isolated from humans, water and eels. *Res Microbiol* 2010;**161**:40–5.
9. Alperi A, Figueras MJ. Human isolates of *Aeromonas* possess Shiga toxin genes (stx1 and stx2) highly similar to the most virulent gene variants of *Escherichia coli*. *Clin Microbiol Infect* 2010; **16**:1563–7.
10. Arias A, Seral C, Gude MJ, et al. Molecular mechanisms of quinolone resistance in clinical isolates of *Aeromonas caviae* and *Aeromonas veronii* bv. *sobria*. *Int Microbiol* 2010;**13**:135–41.
11. Arias A, Seral C, Navarro F, et al. Plasmid-mediated QnrS2 determinant in an *Aeromonas caviae* isolate recovered from a patient with diarrhoea. *Clin Microbiol Infect* 2010;**16**:1005–7.
12. Balsalobre LC, Dropa M, Lincopan N, et al. Detection of metallo-beta-lactamases-encoding genes in environmental isolates of *Aeromonas hydrophila* and *Aeromonas jandaei*. *Lett Appl Microbiol* 2009;**49**:142–5.
14. Behera B, Bhoriwal S, Mathur P, et al. Post-traumatic skin and soft tissue infection due to *Aeromonas hydrophila*. *Indian J Crit Care Med* 2011;**15**:49–51.
15. Berg KA, Lyra C, Niemi RM, et al. Virulence genes of *Aeromonas* isolates, bacterial endotoxins and cyanobacterial toxins from recreational water samples associated with human health symptoms. *J Water Health* 2011;**9**:670–9.
33. Chan KG, Puthucheary SD, Chan XY, et al. Quorum sensing in *Aeromonas* species isolated from patients in Malaysia. *Curr Microbiol* 2011;**62**:167–72.
34. Chaudhari T, Todd DA. *Aeromonas hydrophila* sepsis in a preterm neonate. *Indian Pediatr* 2009;**46**:913–14.
35. Chongsuvivatwong V, Chariyalertsak S, McNeil E, et al. Epidemiology of travelers' diarrhea in Thailand. *J Travel Med* 2009; **16**:179–85.
37. Chuang HC, Ho YH, Lay CJ, et al. Different clinical characteristics among *Aeromonas hydrophila*, *Aeromonas veronii* biovar *sobria* and *Aeromonas caviae* monomicrobial bacteremia. *J Korean Med Sci* 2011;**26**:1415–20.
49. Egorov AI, Best JM, Frebis CP, et al. Occurrence of *Aeromonas* spp. in a random sample of drinking water distribution systems in the USA. *J Water Health* 2011;**9**:785–98.
50. Elhariry HM. Biofilm formation by *Aeromonas hydrophila* on green-leafy vegetables: cabbage and lettuce. *Foodborne Pathog Dis* 2011;**8**:125–31.
57. Figueira V, Vaz-Moreira I, Silva M, et al. Diversity and antibiotic resistance of *Aeromonas* spp. in drinking and waste water treatment plants. *Water Res* 2011;**45**:5599–611.
58. Figueras MJ, Alperi A, Saavedra MJ, et al. Clinical relevance of the recently described species *Aeromonas aquariorum*. *J Clin Microbiol* 2009;**47**:3742–6.
71. Hadi N, Yang Q, Barnett TC, et al. Bundle-forming pilus locus of *Aeromonas veronii* bv. *Sobria*. *Infect Immun* 2012;**80**:1351–60.
72. Han JE, Kim JH, Choresca CH Jr, et al. Prevalence of *tet* gene and complete genome sequencing of *tet* gene-encoded plasmid (pAHH01) isolated from *Aeromonas* species in South Korea. *J Appl Microbiol* 2012;**112**:631–8.
73. Han JE, Kim JH, Choresca CH Jr, et al. A small IncQ-type plasmid carrying the quinolone resistance (qnrS2) gene from *Aeromonas hydrophila*. *Lett Appl Microbiol* 2012;**54**:374–6.
79. Huang KF, Hung MH, Lin YS, et al. Independent predictors of mortality for necrotizing fasciitis: a retrospective analysis in a single institution. *J Trauma* 2011;**71**:467–73; discussion 473.
87. Kao TL, Kao ML. A fatal case of necrotizing *Aeromonas schubertii* fasciitis after penetrating injury. *Am J Emerg Med* 2012;**30**: 258, e253–5.
91. Khan AM, Hossain MS, Khan AI, et al. Bacterial enteropathogens of neonates admitted to an urban diarrhoeal hospital in Bangladesh. *J Trop Pediatr* 2009;**55**:122–4.
100. Liakopoulos V, Arampatzis S, Kourti P, et al. *Aeromonas hydrophila* as a causative organism in peritoneal dialysis-related peritonitis: case report and review of the literature. *Clin Nephrol* 2011;**75**(Suppl. 1):65–8.
105. Mandal J, Dhodapkar R, Acharya NS, et al. Urinary tract infection due to *Aeromonas* spp, a lesser known causative bacterium. *J Infect Dev Ctries* 2010;**4**:679–81.
106. Maraki S, Ladomenou F, Samonis G, et al. Long-term trends in the epidemiology and resistance of childhood bacterial enteropathogens in Crete. *Eur J Clin Microbiol Infect Dis* 2012;**31**:1889–94.
116. Mir F, Tikmani SS, Shakoor S, et al. Incidence and etiology of omphalitis in Pakistan: a community-based cohort study. *J Infect Dev Ctries* 2011;**5**:828–33.
118. Monforte Cirac ML, Palacian Ruiz MP, Aisa Iriarte ML, et al. [Aeromonas-induced hemolytic uremic syndrome: a case report]. *Rev Clin Esp* 2010;**210**:206–7.
129. Pablos M, Remacha MA, Rodriguez-Calleja JM, et al. Identity, virulence genes, and clonal relatedness of *Aeromonas* isolates from patients with diarrhea and drinking water. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1163–72.
131. Papadakis V, Poniros N, Katsibardi K, et al. Fulminant *Aeromonas hydrophila* infection during acute lymphoblastic leukemia treatment. *J Microbiol Immunol Infect* 2012;**45**:154–7.
162. Shak JR, Whitaker JA, Ribner BS, et al. Aminoglycoside-resistant *Aeromonas hydrophila* as part of a polymicrobial infection following a traumatic fall into freshwater. *J Clin Microbiol* 2011;**49**:1169–70.
163. Sharma I, Kumar A. Occurrence of enterotoxigenic *Aeromonas* species in foods of animal origin in North East India. *Eur Rev Med Pharmacol Sci* 2011;**15**:883–7.

172. Surek M, Vizzotto BS, Souza EM, et al. Identification and antimicrobial susceptibility of *Aeromonas* spp. isolated from stool samples of Brazilian subjects with diarrhoea and healthy controls. *J Med Microbiol* 2010;**59**:373–4.
182. Woodring J, Srijan A, Puripunyakom P, et al. Prevalence and antimicrobial susceptibilities of *Vibrio*, *Salmonella*, and *Aeromonas* isolates from various uncooked seafoods in Thailand. *J Food Prot* 2012;**75**:41–7.
183. Wu CJ, Chen PL, Wu JJ, et al. Distribution and phenotypic and genotypic detection of a metallo-beta-lactamase, CphA, among bacteraemic *Aeromonas* isolates. *J Med Microbiol* 2012;**61**:712–19.
185. Yucel N, Balci S. Prevalence of *Listeria*, *Aeromonas*, and *Vibrio* species in fish used for human consumption in Turkey. *J Food Prot* 2010;**73**:380–4.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 116

PASTEURELLA MULTOCIDA

Barbara W. Stechenberg • Kathy Pinkerman

In 1878, Kitt first isolated a bacterium of the *Pasteurella* group from wild hogs during an epidemic; 2 years later, Pasteur described the organism that causes fowl cholera. Since that time, the same organism has been implicated in rabbit septicemia, swine plague, hemorrhagic septicemia, and wildseuche (a fatal disease in deer). Hueppe applied the term *hemorrhagic septicemia* to this group of infectious diseases in lower animals because of the characteristic hemorrhagic areas scattered throughout most of the viscera. Original names for the causative organisms included *Pasteurella aviseptica*, *Pasteurella boviseptica*, *Pasteurella suiseptica*, and *Pasteurella lepiseptica*, but these organisms now are classified under the name *Pasteurella multocida*, a small, nonmotile, gram-negative rod.[40]

Although *P. multocida* primarily is a pathogen in animals, recognition of its potential for infection in humans has been increasing. Brugnatelli reported the first bacteriologically proven case in a human in 1913. Schipper[41] did an extensive review of the literature from 1930 through 1947 and reported only 40 cases of infection with *P. multocida*. Since then, an increasing number of human infections with *P. multocida* have been reported.[43]

THE ORGANISM

P. multocida generally appears as a short, ovoid, gram-negative rod; however, it also may appear as a coccobacillus with convex sides and rounded ends. The length ranges from 0.3 to 1.25 μm, and the diameter ranges from 0.15 to 0.25 μm. *P. multocida* may appear singly or in pairs, chains, or clusters. Healthy organisms stain easily with aniline dyes and are gram negative. They may show bipolar staining, especially when smears are made directly from animal tissue or fluids. They become increasingly pleomorphic on subculture and may resemble Enterobacteriaceae in broth. They do not grow on eosin–methylene blue, MacConkey, deoxycholate, or any other bile-containing agar. They are facultatively anaerobic and nonmotile. They do not require X or V factor for growth, an important differential point in distinguishing them from *Haemophilus influenzae*.

Colonies are nonhemolytic and translucent, usually 1 to 2 mm in diameter, and generally low, convex, and butyrous. Occasionally, they may be larger and mucoid or iridescent. Colonies on blood agar are smaller, opaque, and grayish white. In broth cultures, turbidity, often with a flocculent sediment, is present.

Cultures tend to autoagglutinate in saline and have a peculiar odor described as musty, similar to semen or burning hair. They are catalase positive, usually oxidase positive, and indole positive. They usually ferment galactose, glucose, fructose, mannitol, mannose, and sucrose without production of gas. Some variability occurs in the fermentation of other sugars. They reduce nitrates but have negative urease, methyl red, and Voges-Proskauer reactions. Using fermentation reactions, Oberhofer[33] developed a biotyping system in which correlation of biotype, 61 percent A and B, was found with cat-bite isolates but not with dog-bite isolates.

The pathogenicity of the organism varies; most mucoid and smooth colony-forming strains produce a capsule and usually are highly pathogenic for mice and rabbits. The capsule is antiphagocytic, resisting intracellular killing by neutrophils.

REFERENCES

1. Abbott SL, Cheung WK, Janda JM. The genus *Aeromonas:* biochemical characteristics, atypical reactions, and phenotypic identification schemes. *J Clin Microbiol* 2003;**41**:2348–57.
2. Abolghait SK, Iida T, Kodama T, et al. Recombinant AexU effector protein of *Aeromonas veronii* bv. *sobria* disrupts the actin cytoskeleton by downregulation of Rac1 and induces direct cytotoxicity to beta4-integrin expressing cell lines. *Microb Pathog* 2011;**51**: 454–65.
3. Abrami L, Fivaz M, Glauser PE, et al. Sensitivity of polarized epithelial cells to the pore-forming toxin aerolysin. *Infect Immun* 2003;**71**:739–46.
4. Agarwal RK, Kapoor KN, Kumar A. Virulence factors of aeromonads—an emerging food borne pathogen problem. *J Communicable Dis* 1998;**30**:71–8.
5. Agger WA, McCormick JD, Gurwith MJ. Clinical and microbiological features of *Aeromonas hydrophila*–associated diarrhea. *J Clin Microbiol* 1985;**21**:909–13.
6. Albert MJ, Ansaruzzaman M, Talukder KA, et al. Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhea, healthy controls, and the environment. *J Clin Microbiol* 2000;**38**:3785–90.
7. Alcaide E, Blasco MD, Esteve C. Mechanisms of quinolone resistance in *Aeromonas* species isolated from humans, water and eels. *Res Microbiol* 2010;**161**:40–5.
8. Ali A, Carnahan AM, Altwegg M, et al. *Aeromonas bestiarum* sp. nov. (formerly genomospecies DNA group 2 *A. hydrophilia*), a new species isolated from non-human sources. *Medical Microbiol Lett* 1996;**5**:156–65.
9. Alperi A, Figueras MJ. Human isolates of *Aeromonas* possess Shiga toxin genes (stx1 and stx2) highly similar to the most virulent gene variants of *Escherichia coli*. *Clin Microbiol Infect* 2010;**16**:1563–7.
10. Arias A, Seral C, Gude MJ, et al. Molecular mechanisms of quinolone resistance in clinical isolates of *Aeromonas caviae* and *Aeromonas veronii* bv. *sobria*. *Int Microbiol* 2010;**13**:135–41.
11. Arias A, Seral C, Navarro F, et al. Plasmid-mediated QnrS2 determinant in an *Aeromonas caviae* isolate recovered from a patient with diarrhoea. *Clin Microbiol Infect* 2010;**16**:1005–7.
12. Balsalobre LC, Dropa M, Lincopan N, et al. Detection of metallo-beta-lactamases-encoding genes in environmental isolates of *Aeromonas hydrophila* and *Aeromonas jandaei*. *Lett Appl Microbiol* 2009;**49**:142–5.
13. Bartolomé RM, Andreu A, Xercavins M, et al. Urinary tract infection by *Aeromonas hydrophila* in a neonate. *Infection* 1989;**17**: 172–3.
14. Behera B, Bhoriwal S, Mathur P, et al. Post-traumatic skin and soft tissue infection due to *Aeromonas hydrophila*. *Indian J Crit Care Med* 2011;**15**:49–51.
15. Berg KA, Lyra C, Niemi RM, et al. Virulence genes of *Aeromonas* isolates, bacterial endotoxins and cyanobacterial toxins from recreational water samples associated with human health symptoms. *J Water Health* 2011;**9**:670–9.
16. Bhat P, Shanthakumari S, Rajan D. The characterization and significance of *Plesiomonas shigelloides* and *Aeromonas hydrophila* isolated from an epidemic of diarrhoea. *Indian J Med Res* 1974; **62**:1051–60.
17. Bin Kingombe CI, Huys G, Howald D, et al. The usefulness of molecular techniques to assess the presence of *Aeromonas* spp. harboring virulence markers in foods. *Int J Food Microbiol* 2004;**94**: 113–21.
18. Blatz DJ. Open fracture of the tibia and fibula complicated by infection with *Aeromonas hydrophila:* a case report. *J Bone Joint Surg Am* 1979;**61**:790–1.
19. Bogdanovic R, Cobeljic M, Markovic M, et al. Haemolytic-uraemic syndrome associated with *Aeromonas hydrophila* enterocolitis. *Pediatr Nephrol* 1991;**5**:293–5.
20. Borchardt MA, Stemper ME, Standridge JH. *Aeromonas* isolates from human diarrheic stool and groundwater compared by pulsed-field gel electrophoresis. *Emerg Infect Dis* 2003;**9**:224–8.
21. Borrell N, Acinas SG, Figueras MJ, et al. Identification of *Aeromonas* clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. *J Clin Microbiol* 1997; **35**:1671–4.
22. Boulanger Y, Lallier R, Cousineau G. Isolation of enterotoxigenic *Aeromonas* from fish. *Can J Microbiol* 1977;**23**:1161–4.
23. Bulger RJ, Sherris JC. The clinical significance of *Aeromonas hydrophila*. Report of two cases. *Arch Intern Med* 1966;**118**:562–4.
24. Burke V, Cooper M, Robinson J. Haemagglutination patterns of *Aeromonas* spp. related to species and source of strains. *Aust J Exp Biol Med Sci* 1986;**64**(Pt 6):563–70.
25. Burke V, Gracey M, Robinson J, et al. The microbiology of childhood gastroenteritis: *Aeromonas* species and other infective agents. *J Infect Dis* 1983;**148**:68–74.
26. Carnahan AM, Chakraborty T, Fanning GR, et al. *Aeromonas trota* sp. nov, an ampicillin-susceptible species isolated from clinical specimens. *J Clin Microbiol* 1991;**29**:1206–10.
27. Carnahan AM, Fanning GR, Joseph SW. *Aeromonas jandaei* (formerly genospecies DNA group 9 *A. sobria*), a new sucrose-negative species isolated from clinical specimens. *J Clin Microbiol* 1991; **29**:560–4.
28. Carnahan AM, Marii MA, Fanning GR, et al. Characterization of *Aeromonas schubertii* strains recently isolated from traumatic wound infections. *J Clin Microbiol* 1989;**27**:1826–30.
29. Caselitz FH. How the *Aeromonas* story started in medical microbiology. *Med Microbiol Lett* 1996;**5**:46–54.
30. Caselitz FH, Freitag V, Jannasch G. [Demonstration of specific antibodies in sera of patients with infections caused by *Aeromonas hydrophila*]. *Zentralbl Bakteriol Orig A* 1975;**233**:437–454. In German.
31. Challapalli M, Tess BR, Cunningham DG, et al. *Aeromonas*-associated diarrhea in children. *Pediatr Infect Dis J* 1988;**7**:693–8.
32. Champsaur H, Andremont A, Mathieu D. Cholera-like illness due to *Aeromonas sobria*. *J Infect Dis* 1982;**145**:248–54.
33. Chan KG, Puthucheary SD, Chan XY, et al. Quorum sensing in *Aeromonas* species isolated from patients in Malaysia. *Curr Microbiol* 2011;**62**:167–72.
34. Chaudhari T, Todd DA. *Aeromonas hydrophila* sepsis in a preterm neonate. *Indian Pediatr* 2009;**46**:913–14.
35. Chongsuvivatwong V, Chariyalertsak S, McNeil E, et al. Epidemiology of travelers' diarrhea in Thailand. *J Travel Med* 2009;**16**: 179–85.
36. Chopra AK, Houston CW. Enterotoxins in *Aeromonas*-associated gastroenteritis. *Microbes Infect* 1999;**1**:1129–37.
37. Chuang HC, Ho YH, Lay CJ, et al. Different clinical characteristics among *Aeromonas hydrophila*, *Aeromonas veronii* biovar *sobria* and *Aeromonas caviae* monomicrobial bacteremia. *J Korean Med Sci* 2011;**26**:1415–20.
38. Clark NM, Chenoweth CE. *Aeromonas* infection of the hepatobiliary system: report of 15 cases and review of the literature. *Clin Infect Dis* 2003;**37**:506–13.
39. Cohen KL, Holyk PR, McCarthy LR, et al. *Aeromonas hydrophila* and *Plesiomonas shigelloides* endophthalmitis. *Am J Ophthalmol* 1983;**96**:403–4.
40. Collins MD, Martinez-Murcia AJ, Cai J. *Aeromonas enteropelogenes* and *Aeromonas ichthiosmia* are identical to *Aeromonas trota* and *Aeromonas veronii*, respectively, as revealed by small-subunit rRNA sequence analysis. *Int J Syst Bacteriol* 1993;**43**:855–6.
41. Colwell RR, MacDonell MT, De Ley J. Proposal to recognize the family Aeromonadaceae fam. nov. *Int J Syst Bacteriol* 1986;**36**: 473–7.
42. Davin-Regli A, Bollet C, Chamorey E, et al. A cluster of cases of infections due to *Aeromonas hydrophila* revealed by combined RAPD and ERIC-PCR. *J Med Microbiol* 1998;**47**:499–504.
43. De la Morena ML, Van R, Singh K, et al. Diarrhea associated with *Aeromonas* species in children in day care centers. *J Infect Dis* 1993;**168**:215–18.
44. Dean HM, Post RM. Fatal infection with *Aeromonas hydrophila* in a patient with acute myelogenous leukemia. *Ann Intern Med* 1967;**66**:1177–9.
45. Deepe GS Jr, Coonrod JD. Fulminant wound infection with *Aeromonas hydrophila*. *South Med J* 1980;**73**:1546–7.
46. Delamare AP, Echeverrigaray S, Duarte KR, et al. Production of a monoclonal antibody against *Aeromonas hydrophila* and its application to bacterial identification. *J Appl Microbiol* 2002;**92**: 936–40.
47. Dobrescu L. Enterotoxigenic *Aeromonas hydrophila* from a case of piglet diarrhoea. *Zentralbl Veterinarmed B* 1978;**25**:713–18.
48. Dorsch M, Ashbolt NJ, Cox PT, et al. Rapid identification of *Aeromonas* species using 16S rDNA targeted oligonucleotide primers: a molecular approach based on screening of environmental isolates. *J Appl Bacteriol* 1994;**77**:722–6.

49. Egorov AI, Best JM, Frebis CP, et al. Occurrence of *Aeromonas* spp. in a random sample of drinking water distribution systems in the USA. *J Water Health* 2011;**9**:785–98.
50. Elhariry HM. Biofilm formation by *Aeromonas hydrophila* on green-leafy vegetables: cabbage and lettuce. *Foodborne Pathog Dis* 2011;**8**:125–31.
51. Elwitigala JP, Higgs DS, Namnyak S, et al. Septic arthritis due to *Aeromonas hydrophila:* case report and review of the literature. *Int J Clin Pract Suppl* 2005;**147**:121–4.
52. Epple HJ, Mankertz J, Ignatius R, et al. *Aeromonas hydrophila* beta-hemolysin induces active chloride secretion in colon epithelial cells (HT-29/B6). *Infect Immun* 2004;**72**:4848–58.
53. Esteve C, Gutierrez MC, Ventosa A. *Aeromonas encheleia* sp. nov, isolated from European eels. *Int J Syst Bacteriol* 1995;**45**:462–6.
54. Ewing WH, Hugh R. Aeromonas. In: Lennette EH, Spaulding EH, Truant JP, editors. *Manual of clinical microbiology*. 2nd ed. Washington, D.C.: American Society for Microbiology; 1974. p. 230–7.
55. Ewing WH, Hugh R, Johnson JG. *Studies on the* Aeromonas *group*. Atlanta: Public Health Service, Communicable Disease Center; 1961. p. 1–8.
56. Fainstein V, Weaver S, Bodey GP. In vitro susceptibilities of *Aeromonas hydrophila* against new antibiotics. *Antimicrob Agents Chemother* 1982;**22**:513–14.
57. Figueira V, Vaz-Moreira I, Silva M, et al. Diversity and antibiotic resistance of *Aeromonas* spp. in drinking and waste water treatment plants. *Water Res* 2011;**45**:5599–611.
58. Figueras MJ, Alperi A, Saavedra MJ, et al. Clinical relevance of the recently described species *Aeromonas aquariorum*. *J Clin Microbiol* 2009;**47**:3742–6.
59. Fraire AE. *Aeromonas hydrophila* infection. *JAMA* 1978;**239**:192.
60. Freij BJ. *Aeromonas:* biology of the organism and diseases in children. *Pediatr Infect Dis* 1984;**3**:164–75.
61. Freij BJ. Human disease other than gastroenteritis caused by *Aeromonas* and *Plesiomonas.* Presented before the *Aeromonas* Symposium, Manchester, England, September 5-6, 1986. p. 19–20.
62. Fritsche D, Dahn R, Hoffmann G. [*Aeromonas punctata* subsp. *caviae* as the causative agent of acute gastroenteritis.] *Zentralbl Bakteriol Orig A* 1975;**233**:232–5. In German.
63. Gluskin I, Batash D, Shoseyov D, et al. A 15-year study of the role of *Aeromonas* spp. in gastroenteritis in hospitalised children. *J Med Microbiol* 1992;**37**:315–18.
64. González-Barca E, Ardanuy C, Carratalá J, et al. Fatal myofascial necrosis due to imipenem-resistant *Aeromonas hydrophila*. *Scand J Infect Dis* 1997;**29**:91–4.
65. Goodwin CS, Harper WE, Stewart JK, et al. Enterotoxigenic *Aeromonas hydrophila* and diarrhoea in adults. *Med J Aust* 1983;**1**: 25–6.
66. Gracey M, Burke V, Robinson J. *Aeromonas*-associated gastroenteritis. *Lancet* 1982;**2**:1304–6.
67. Gracey M, Burke V, Rockhill RC, et al. *Aeromonas* species as enteric pathogens. *Lancet* 1982;**1**:223–4.
68. Gracy M. Gastro-enteritis in Australian children: studies on the aetiology of acute diarrhoea. *Ann Trop Paediatr* 1988;**8**:68–75.
69. Gupta P, Ramachandran VG, Seth A. Early onset neonatal septicemia caused by *Aeromonas hydrophilia*. *Indian Pediatr* 1996;**33**: 703–4.
70. Gurwith MJ, Williams TW. Gastroenteritis in children: a two-year review in Manitoba, part I: etiology. *J Infect Dis* 1977;**136**: 239–47.
71. Hadi N, Yang Q, Barnett TC, et al. Bundle-forming pilus locus of *Aeromonas veronii* bv. *sobria*. *Infect Immun* 2012;**80**:1351–60.
72. Han JE, Kim JH, Choresca CH Jr, et al. Prevalence of *tet* gene and complete genome sequencing of *tet* gene-encoded plasmid (pAHH01) isolated from *Aeromonas* species in South Korea. *J Appl Microbiol* 2012;**112**:631–8.
73. Han JE, Kim JH, Choresca CH Jr, et al. A small IncQ-type plasmid carrying the quinolone resistance *(qnrS2)* gene from *Aeromonas hydrophila*. *Lett Appl Microbiol* 2012;**54**:374–6.
74. Hazen TC, Fliermans CB, Hirsch RP, et al. Prevalence and distribution of *Aeromonas hydrophila* in the United States. *Appl Environ Microbiol* 1978;**36**:731–8.
75. Hickman-Brenner FW, Fanning GR, Arduino MJ, et al. *Aeromonas schubertii,* a new mannitol-negative species found in human clinical specimens. *J Clin Microbiol* 1988;**26**:1561–4.
76. Hickman-Brenner FW, MacDonald KL, Steigerwalt AG, et al. *Aeromonas veronii,* a new ornithine decarboxylase-positive species that may cause diarrhea. *J Clin Microbiol* 1987;**25**:900–6.
77. Hiransuthikul N, Tantisiriwat W, Lertutsahakul K, et al. Skin and soft-tissue infections among tsunami survivors in southern Thailand. *Clin Infect Dis* 2005;**41**:e93–6.
78. Hird DW, Diesch SL, McKinnell RG, et al. *Aeromonas hydrophila* in wild-caught frogs and tadpoles *(Rana pipiens)* in Minnesota. *Lab Anim Sci* 1981;**31**:166–9.
79. Huang KF, Hung MH, Lin YS, et al. Independent predictors of mortality for necrotizing fasciitis: a retrospective analysis in a single institution. *J Trauma* 2011;**71**:467–73, discussion 473.
80. Hunter WF, Atkinson HM. Infection due to *Aeromonas hydrophila*. *Med J Aust* 1968;**1**:565.
81. Huys G, Kampfer P, Altwegg M, et al. *Aeromonas popoffii* sp. nov, a mesophilic bacterium isolated from drinking water production plants and reservoirs. *Int J Syst Bacteriol* 1997;**47**:1165–71.
82. Janda JM, Abbott SL, Khashe S, et al. Further studies on biochemical characteristics and serologic properties of the genus *Aeromonas*. *J Clin Microbiol* 1996;**34**:1930–3.
83. Janda JM, Bottone EJ, Reitano M. *Aeromonas* species in clinical microbiology: significance, epidemiology, and speciation. *Diagn Microbiol Infect Dis* 1983;**1**:221–8.
84. Jorge MT, Nishioka SDA, De Oliveira RB, et al. *Aeromonas hydrophila* soft-tissue infection as a complication of snake bite: report of three cases. *Ann Trop Med Parasitol* 1998;**92**:213–17.
85. Kampfer P, Christmann C, Swings J, et al. In vitro susceptibilities of *Aeromonas* genomic species to 69 antimicrobial agents. *Syst Appl Microbiol* 1999;**22**:662–9.
86. Kao HT, Huang YC, Lin TY. Fatal bacteremic pneumonia caused by *Aeromonas hydrophila* in a previously healthy child. *J Microbiol Immunol Infect* 2003;**36**:209–11.
87. Kao TL, Kao ML. A fatal case of necrotizing *Aeromonas schubertii* fasciitis after penetrating injury. *Am J Emerg Med* 2012;**30**: 258, e253–5.
88. Kaper JB, Lockman H, Colwell RR, et al. *Aeromonas hydrophila:* ecology and toxigenicity of isolates from an estuary. *J Appl Bacteriol* 1981;**50**:359–77.
89. Ketover BP, Young LS, Armstrong D. Septicemia due to *Aeromonas hydrophila:* clinical and immunologic aspects. *J Infect Dis* 1973; **127**:284–90.
90. Khan AA, Cerniglia CE. Rapid and sensitive method for the detection of *Aeromonas caviae* and *Aeromonas trota* by polymerase chain reaction. *Lett Appl Microbiol* 1997;**24**:233–9.
91. Khan AM, Hossain MS, Khan AI, et al. Bacterial enteropathogens of neonates admitted to an urban diarrhoeal hospital in Bangladesh. *J Trop Pediatr* 2009;**55**:122–4.
92. King GE, Werner SB, Kizer KW. Epidemiology of *Aeromonas* infections in California. *Clin Infect Dis* 1992;**15**:449–52.
93. Kirov SM, Barnett TC, Pepe CM, et al. Investigation of the role of type IV *Aeromonas pilus* (Tap) in the pathogenesis of *Aeromonas* gastrointestinal infection. *Infect Immun* 2000;**68**:4040–8.
94. Ko WC, Chiang SR, Lee HC, et al. In vitro and in vivo activities of fluoroquinolones against *Aeromonas hydrophila*. *Antimicrob Agents Chemother* 2003;**47**:2217–22.
95. Kuhn I, Albert MJ, Ansaruzzaman M, et al. Characterization of *Aeromonas* spp. isolated from humans with diarrhea, from healthy controls, and from surface water in Bangladesh. *J Clin Microbiol* 1997;**35**:369–73.
96. Laohachai KN, Bahadi R, Hardo MB, et al. The role of bacterial and non-bacterial toxins in the induction of changes in membrane transport: implications for diarrhea. *Toxicon* 2003;**42**:687–707.
97. Leclerc H, Buttiaux R. Fréquence des *Aeromonas* dans les eaux d'alimentation. *Ann Inst Pasteur* 1962;**103**:97–100.
98. Lee LR, O'Hagan S, Dal Pra M. *Aeromonas sobria* endophthalmitis. *Aust N Z J Ophthalmol* 1997;**25**:299–300.
99. Levine SM, Frangos SG, Hanna B, et al. *Aeromonas* septicemia after medicinal leech use following replantation of severed digits. *Am J Crit Care* 2010;**19**:469–71.
100. Liakopoulos V, Arampatzis S, Kourti P, et al. *Aeromonas hydrophila* as a causative organism in peritoneal dialysis-related peritonitis: case report and review of the literature. *Clin Nephrol* 2011; **75**(Suppl. 1):65–8.
101. Ljungh A, Wadstrom T. *Aeromonas* toxins. *Pharmacol Ther* 1982; **15**:339–54.

102. Ljungh A, Wadstrom T. Toxins of *Vibrio parahaemolyticus* and *Aeromonas hydrophila*. *J Toxicol Toxin Rev* 1982;**1**:257–307.
103. Ljungh A, Wadstrom T. *Aeromonas* and *Plesiomonas* as possible causes of diarrhoea. *Infection* 1985;**13**:169–73.
104. Lopez JF, Quesada J, Saied A. Bacteremia and osteomyelitis due to *Aeromonas hydrophila:* a complication during the treatment of acute leukemia. *Am J Clin Pathol* 1968;**50**:587–91.
105. Mandal J, Dhodapkar R, Acharya NS, et al. Urinary tract infection due to *Aeromonas* spp, a lesser known causative bacterium. *J Infect Dev Ctries* 2010;**4**:679–81.
106. Maraki S, Ladomenou F, Samonis G, et al. Long-term trends in the epidemiology and resistance of childhood bacterial enteropathogens in Crete. *Eur J Clin Microbiol Infect Dis* 2012;**31**:1889–94.
107. Martinez-Murcia AJ, Esteve C, Garay E, et al. *Aeromonas allosaccharophila* sp. nov, a new mesophilic member of the genus *Aeromonas*. *FEMS Microbiol Lett* 1992;**70**:199–205.
108. Martinez-Silva R, Guzmann-Urrego M, Caselitz FH. [On the problem of the significance of *Aeromonas* strains in enteritis in infants]. *Z Tropenmed Parasitol* 1961;**12**:445–51. In German.
109. Mattick KL, Donovan TJ. The risk to public health of *Aeromonas* in ready-to-eat salad products. *Commun Dis Public Health* 1998;**1**: 267–70.
110. McCracken AW, Barkley R. Isolation of *Aeromonas* species from clinical sources. *J Clin Pathol* 1972;**25**:970–5.
111. McGrath VA, Overman SB, Overman TL. Media-dependent oxidase reaction in a strain of *Aeromonas hydrophila*. *J Clin Microbiol* 1977;**5**:112–13.
112. McNicol LA, Aziz KM, Huq I, et al. Isolation of drug-resistant *Aeromonas hydrophila* from aquatic environments. *Antimicrob Agents Chemother* 1980;**17**:477–83.
113. Meeks MV. The genus *Aeromonas:* methods for identification. *Am J Med Technol* 1963;**29**:361–78.
114. Merino S, Camprubí S, Tomás JM. Detection of *Aeromonas hydrophila* in food with an enzyme-linked immunosorbent assay. *J Appl Bacteriol* 1993;**74**:149–54.
115. Michael Janda J, Abbott SL. Evolving concepts regarding the genus *Aeromonas:* an expanding panorama of species, disease presentations, and unanswered questions. *Clin Infect Dis* 1998;**27**: 332–44.
116. Mir F, Tikmani SS, Shakoor S, et al. Incidence and etiology of omphalitis in Pakistan: a community-based cohort study. *J Infect Dev Ctries* 2011;**5**:828–33.
117. Mishra S, Balakrish Nair G, Bhadra RK, et al. Comparison of selective media for primary isolation of *Aeromonas* species from human and animal feces. *J Clin Microbiol* 1987;**25**:2040–3.
118. Monforte Cirac ML, Palacian Ruiz MP, Aisa Iriarte ML, et al. [*Aeromonas*-induced hemolytic uremic syndrome: a case report]. *Rev Clin Esp* 2010;**210**:206–7. In Spanish.
119. Morita K, Watanabe N, Kurata S, et al. β-Lactam resistance of motile *Aeromona*s isolates from clinical and environmental sources. *Antimicrob Agents Chemother* 1994;**38**:353–5.
120. Motyl MR, Janda JM. *Aeromonas* gastroenteritis: a two-year survey. Presented at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Las Vegas, October 24-6, 1983.
121. Motyl MR, McKinley G, Janda JM. In vitro susceptibilities of *Aeromonas hydrophila*, *Aeromonas sobria*, and *Aeromonas caviae* to 22 antimicrobial agents. *Antimicrob Agents Chemother* 1985;**28**:151–3.
122. Moulsdale MT. Isolation of *Aeromonas* from faeces. *Lancet* 1983;**1**:351.
123. Moyer NP. Clinical significance of *Aeromonas* species isolated from patients with diarrhea. *J Clin Microbiol* 1987;**25**:2044–8.
124. Moyes CD, Sykes PA, Rayner JM. *Aeromonas hydrophila* septicaemia producing ecthyma gangrenosum in a child with leukaemia. *Scand J Infect Dis* 1977;**9**:151–3.
125. Nygaard GS, Bissett ML, Wood RM. Laboratory identification of aeromonads from man and other animals. *Appl Microbiol* 1970; **19**:618–20.
126. Ojeda-Vargas M, González-Fernández MA, Alfonso-Rodríguez O, et al. [Urinary tract infection caused by bacteria of the genus *Aeromonas*].. *Enferm Infecc Microbiol Clin* 2005;**23**:181–2. In Spanish.
127. Olivier G, Lallier R, Lariviere S. A toxigenic profile of *Aeromonas hydrophila* and *Aeromonas sobria* isolated from fish. *Can J Microbiol* 1981;**27**:330–3.
128. Overman TL, Janda JM. Antimicrobial susceptibility patterns of *Aeromonas jandaei*, *A. schubertii*, *A. trota*, and *A. veronii* biotype *veronii*. *J Clin Microbiol* 1999;**37**:706–8.
129. Pablos M, Remacha MA, Rodriguez-Calleja JM, et al. Identity, virulence genes, and clonal relatedness of *Aeromonas* isolates from patients with diarrhea and drinking water. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1163–72.
130. Paik G. Reagents, stains, and miscellaneous test procedures. In: Lennette EH, Balows A, Hausler WJ Jr, et al, editors. *Manual of clinical microbiology*. 3rd ed. Washington, D.C.: American Society for Microbiology; 1980. p. 1006–7.
131. Papadakis V, Poniros N, Katsibardi K, et al. Fulminant *Aeromonas hydrophila* infection during acute lymphoblastic leukemia treatment. *J Microbiol Immunol Infect* 2012;**45**:154–7.
132. Parker JL, Shaw JG. *Aeromonas* spp. clinical microbiology and disease. *J Infect* 2011;**62**:109–18.
133. Pearson TA, Mitchell CA, Hughes WT. *Aeromonas hydrophila* septicemia. *Am J Dis Child* 1972;**123**:579–82.
134. Phillips JA, Bernhardt HE, Rosenthal SG. *Aeromonas hydrophila* infections. *Pediatrics* 1974;**53**:110–12.
135. Picard B, Arlet G, Goullet P. Origin hydrique d'infections hôpitalières à *Aeromonas hydrophila*. *Presse Med* 1983;**12**:700.
136. Picard B, Goullet P. Seasonal prevalence of nosocomial *Aeromonas hydrophila* infection related to aeromonas in hospital water. *J Hosp Infect* 1987;**10**:152–5.
137. Pidiyar V, Kaznowski A, Narayan NB, et al. *Aeromonas culicicola* sp. nov, from the midgut of *Culex quinquefasciatus*. *Int J Syst Evol Microbiol* 2002;**52**:1723–8.
138. Pin C, Benito Y, Carcia ML, et al. Influence of temperature, pH, sodium chloride, and sodium nitrate on the growth of clinical and food motile *Aeromonas* sp. strains. *Arch Lebensmittelhyg* 1996; **47**:35–56.
139. Pitarangsi C, Echeverria P, Whitmire R, et al. Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides:* prevalence among individuals with and without diarrhea in Thailand. *Infect Immun* 1982;**35**:666–73.
140. Pittman M. A study of bacteria implicated in transfusion reactions and of bacteria isolated from blood products. *J Lab Clin Med* 1953;**42**:273–88.
141. Popoff M. Genus III: *Aeromonas* Kluyver and van Niel 1936. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*. Baltimore: Williams & Wilkins; 1984. p. 545.
142. Quinn JP. UHL enteric bacterial disease surveillance. *Lab Hotline* 1991;**28**:2.
143. Ramsay AM, Rosenbaum BJ, Yarbrough CL, et al. *Aeromonas hydrophila* sepsis in a patient undergoing hemodialysis therapy. *JAMA* 1978;**239**:128–9.
144. Raszeja S, Krynski S, Krueger A, et al. [Blood contamination with *Aeromonas hydrophilus* as a cause of lethal post-transfusion complications]. *Pol Tyg Lek* 1973;**28**:1159–62. In Polish.
145. Reines HD, Cook FV. Pneumonia and bacteremia due to *Aeromonas hydrophila*. *Chest* 1981;**80**:264–7.
146. Reinhardt JF, George WL. Comparative in vitro activities of selected antimicrobial agents against *Aeromonas species* and *Plesiomonas shigelloides*. *Antimicrob Agents Chemother* 1985;**27**:643–5.
147. Robson WL, Leung AK, Trevenen CL. Haemolytic-uraemic syndrome associated with *Aeromonas hydrophila* enterocolitis. *Pediatr Nephrol* 1992;**6**:221.
148. Rogol M, Sechter I, Grinberg L, et al. Pril-xylose-ampicillin agar, a new selective medium for the isolation of *Aeromonas hydrophila*. *J Med Microbiol* 1979;**12**:229–31.
149. Rosenthal SG, Bernhardt HE, Phillips JA 3rd. *Aeromonas hydrophila* wound infection. *Plast Reconstr Surg* 1974;**53**:77–9.
150. Rosner R. *Aeromonas hydrophila* as the etiologic agent in a case of severe gastroenteritis. *Am J Clin Pathol* 1964;**42**:402–4.
151. Sader HS, Jones RN. Antimicrobial susceptibility of uncommonly isolated non-enteric gram-negative bacilli. *Int J Antimicrob Agents* 2005;**25**:95–109.
152. San Joaquin VH, Pickett DA. *Aeromonas*-associated gastroenteritis in children. *Pediatr Infect Dis J* 1988;**7**:53–7.
153. San Joaquin VH, Pickett DA, Welch DF, et al. *Aeromonas* species in aquaria: a reservoir of gastrointestinal infections? *J Hosp Infect* 1989;**13**:173–7.
154. Sanarelli G. Über einem neuen Mikroorganismus des Wassers, welcher für Tieren mit veranderlichen und konstanter Temperatur

Pathogen ist. *Zentralbl Bakteriol Parasitenk* 1891;**9**:193–9, 222–8.
155. Sanyal SC, Singh SJ, Sen PC. Enteropathogenicity of *Aeromonas hydrophila* and *Plesiomonas shigelloides*. *J Med Microbiol* 1975;**8**: 195–8.
156. Sasu D, Apostica E. [On a strain of *Aeromonas liquefaciens* isolated from blood]. *Microbiol Parazitol Epidemiol (Bucur)* 1967;**12**:437–41. In Romanian.
157. Sawai T, Takahashi I, Nakagawa H, et al. Immunochemical comparison between an oxacillin-hydrolyzing penicillinase of *Aeromonas hydrophila* and those mediated by R plasmids. *J Bacteriol* 1978;**135**:281–2.
158. Schubert RH, Hegazi M. *Aeromonas eucrenophila* species nova *Aeromonas caviae* a later and illegitimate synonym of *Aeromonas punctata*. *Zentralbl Bakteriol Mikrobiol Hyg A* 1988;**268**:34–9.
159. Seidler RJ, Allen DA, Lockman H. Isolation, enumeration, and characterization of *Aeromonas* from polluted waters encountered in diving operations. *Appl Environ Microbiol* 1980;**39**:1010–18.
160. Sha J, Kozlova EV, Chopra AK. Role of various enterotoxins in *Aeromonas hydrophila*-induced gastroenteritis: generation of enterotoxin gene–deficient mutants and evaluation of their enterotoxic activity. *Infect Immun* 2002;**70**:1924–35.
161. Shackelford PG, Ratzan SA, Shearer WT. Ecthyma gangrenosum produced by *Aeromonas hydrophilia*. *J Pediatr* 1973;**83**:100–1.
162. Shak JR, Whitaker JA, Ribner BS, et al. Aminoglycoside-resistant *Aeromonas hydrophila* as part of a polymicrobial infection following a traumatic fall into freshwater. *J Clin Microbiol* 2011;**49**:1169–70.
163. Sharma I, Kumar A. Occurrence of enterotoxigenic *Aeromonas* species in foods of animal origin in North East India. *Eur Rev Med Pharmacol Sci* 2011;**15**:883–7.
164. Sirinavin S, Likitnukul S, Lolekha S. *Aeromonas* septicemia in infants and children. *Pediatr Infect Dis* 1984;**3**:122–5.
165. Skoll PJ, Hudson DA, Simpson JA. *Aeromonas hydrophila* in burn patients. *Burns* 1998;**24**:350–3.
166. Slotnick IJ. *Aeromonas* species isolates. *Ann N Y Acad Sci* 1970; **174**:503–10.
167. Smith JA. *Aeromonas hydrophila:* analysis of 11 cases. *Can Med Assoc J* 1980;**122**:1270–2.
168. Smith JA. Ocular *Aeromonas hydrophila*. *Am J Ophthalmol* 1980; **89**:449–51.
169. Soler L, Yanez MA, Chacon MR, et al. Phylogenetic analysis of the genus *Aeromonas* based on two housekeeping genes. *Int J Syst Evol Microbiol* 2004;**54**:1511–19.
170. Soltan Dallal MM, Moezardalan K. *Aeromonas* spp associated with children's diarrhoea in Tehran: a case-control study. *Annals of Tropical Paediatrics* 2004;**24**:45–51.
171. Stephen S, Rao KN, Dumar MS, et al. Letter: Human infection with *Aeromonas* species: varied clinical manifestations. *Ann Intern Med* 1975;**83**:368–9.
172. Surek M, Vizzotto BS, Souza EM, et al. Identification and antimicrobial susceptibility of *Aeromonas* spp. isolated from stool samples of Brazilian subjects with diarrhoea and healthy controls. *J Med Microbiol* 2010;**59**:373–4.
173. Taneja N, Khurana S, Trehan A, et al. An outbreak of hospital acquired diarrhea due to *Aeromonas sobria*. *Indian Pediatr* 2004;**41**: 912–16.
174. Teka T, Faruque AS, Hossain MI, et al. *Aeromonas*-associated diarrhoea in Bangladeshi children: clinical and epidemiological characteristics. *Ann Trop Paediatr* 1999;**19**:15–20.
175. Thelestam M, Ljungh A. Membrane-damaging and cytotoxic effects on human fibroblasts of alpha- and beta-hemolysins from *Aeromonas hydrophila*. *Infect Immun* 1981;**34**:949–56.
176. Ullmann D, Krause G, Knabner D, et al. Isolation and characterization of potentially human pathogenic, cytotoxin-producing aeromonas strains from retailed seafood in Berlin, Germany. *J Vet Med B Infect Dis Vet Public Health* 2005;**52**:82–7.
177. Unhanand M, Mustafa MM, McCracken GH Jr, et al. Gram-negative enteric bacillary meningitis: a twenty-one-year experience. *J Pediatr* 1993;**122**:15–21.
178. von Graevenitz A. *Aeromonas* and *Plesiomonas*. In: Lennette EH, Balows A, Hausler WH, et al, editors. *Manual of clinical microbiology*. 3rd ed. Washington, D.C.: American Society for Microbiology; 1980. p. 220–5.
179. Von Graevenitz A, Bucher C. Evaluation of differential and selective media for isolation of *Aeromonas* and *Plesiomonas* spp. from human feces. *J Clin Microbiol* 1983;**17**:16–21.
180. Wadstrom T, Aust-Kettis A, Habte D, et al. Enterotoxin-producing bacteria and parasites in stools of Ethiopian children with diarrhoeal disease. *Arch Dis Child* 1976;**51**:865–70.
181. Washington JA II. The role of *Aeromonas hydrophila* in clinical infection. In: Holloway WJ, editor. *Infectious disease reviews*, vol. 2. Mount Kisco, N.Y.: Futura Publishing; 1973. p. 75–86.
182. Woodring J, Srijan A, Puripunyakom P, et al. Prevalence and antimicrobial susceptibilities of *Vibrio*, *Salmonella*, and *Aeromonas* isolates from various uncooked seafoods in Thailand. *J Food Prot* 2012;**75**:41–7.
183. Wu CJ, Chen PL, Wu JJ, et al. Distribution and phenotypic and genotypic detection of a metallo-beta-lactamase, CphA, among bacteraemic *Aeromonas* isolates. *J Med Microbiol* 2012;**61**: 712–19.
184. Yadava R, Seeler RA, Kalelkar M, et al. Fatal *Aeromonas hydrophila* sepsis and meningitis in a child with sickle cell anemia. *Am J Dis Child* 1979;**133**:753–4.
185. Yucel N, Balci S. Prevalence of *Listeria*, *Aeromonas*, and *Vibrio* species in fish used for human consumption in Turkey. *J Food Prot* 2010;**73**:380–4.
186. Zajc-Satler J. Morphological and biochemical studies of 27 strains belonging to the genus *Aeromonas* isolated from clinical sources. *J Med Microbiol* 1972;**5**:263–5.

Nielsen and Rosdahl[31] have developed a bacteriophage typing system for typing toxigenic and nontoxigenic strains. DNA hybridization studies have led to a reclassification of the genus *Pasteurella*. *P. multocida* now includes three subspecies: *P. multocida* subsp. *multocida*, *P. multocida* subsp. *septica*, and *P. multocida* subsp. *gallicida*. Of 159 strains recovered from 46 infected humans, 95 were identified as *P. multocida* subsp. *multocida* and 21 were identified as *P. multocida* subsp. *septica;* the remainder were divided among multiple other species.[17] Other less common *Pasteurella* spp. include *P. canis*, *P. dagmatis*, and *P. stomatis*. The use of serology in conjunction with DNA fingerprinting can classify isolates for epidemiologic studies.[53]

Immunity to *P. multocida* can be shown in many animals, and vaccines have been developed with known efficacy, especially in birds and cattle. The precise mechanisms involved in this immunity and, more important, in natural immunity are being elucidated. Antibodies to somatic and capsular antigenic determinants develop within 2 weeks of development of clinical infection, with the capsular antibodies being longer lasting.[5] The precise role of these antibodies in human host defenses is unclear. Woolcock and Collins[55] developed models in pathogen-free mice that may help uncover these mechanisms. The efficacy of heat-killed vaccine has been shown to be considerable when multiple doses are used. With the use of an aerogenic mouse model for the stimulation of respiratory spread, however, the protection is reduced. Local instillation of the vaccine can be used with this model to develop local antibodies, but preliminary results show protection only with modest challenge doses.[3] A footpad inoculation model may help elucidate the mechanisms in local human infections.

TRANSMISSION

The organism is found in the oral flora of many different animals; 67 percent of cats may harbor this organism in their mouths or throats.[51] Smith[42] found that *P. multocida* could be recovered from the tonsils of 54 percent of dogs and from the nose of 10 percent of dogs. Schipper[41] grew *P. multocida* from 14 percent of wild rats trapped in the Baltimore area. Hansmann and Tully[16] showed that *P. multocida* may remain as a commensal for prolonged periods in the mouth of a cat. They described a patient who had been bitten on two occasions 3 years apart by the same healthy pet cat; each time, an abscess caused by *P. multocida* developed. *P. multocida* also has been isolated from the lion, tiger, panther, buffalo, mink, and opossum.[4,20]

Although verifying the mode of transmission has been easiest when an infection has been related specifically to a pet or farm animal, many cases of infection caused by *P. multocida* have been documented despite a negative history of such exposure. Respiratory tract infection with this organism has been described in veterinarians, farmers, milkmen, and individuals employed where animal tissues are processed. A recent case series was reported of life-threatening *P. multocida* respiratory tract infections in association with palliative pet care in the home.[30]

The possibility of a reservoir of infection in humans with resultant interhuman transmission rarely has been considered. In a study of veterinary students, Smith[42] described 2 of 71 with positive throat isolations. The organisms were present in 1 student for a few days and in the other for the full 4 months of the study; both patients were asymptomatic. Several other cases of isolation of the organism from the human respiratory tract, many without associated symptoms or known animal contact, have been made.

In addition, interhuman spread by nasopharyngeal excretions, feces, and urine may occur because the organism has been recovered from these sites. The female genital tract is another potential source, especially for cases of septicemia and meningitis that occur during pregnancy and in the newborn period.

Investigators of an outbreak in a chronic disease hospital showed that *P. multocida* may be viable on a hand towel for 24 hours. Although the source in this outbreak was not established, this finding may have implications for spread in other situations, particularly for pet owners.[22]

EPIDEMIOLOGY

P. multocida has been isolated from humans in all areas of North America and Europe, with some reports coming from other areas. In the United States, it is not a reportable organism, so incidence and prevalence data are unavailable. Lee and Buhr[26] have cited a seasonal variation in the number of reported cases related to dog bites, with the highest incidence being in the fall and winter months, possibly related to increased nasal carriage in dogs during that period. Other investigators have found no seasonal differences.[9,29]

No difference in attack rate has been found between the sexes. The attack rate is higher in individuals of both sexes in very young (birth to 4 years old) and in older (>55 years old) individuals.

PATHOGENESIS AND PATHOLOGY

In animals that are stressed, a benignly parasitic strain may invade the mucous membrane on which it is carried. With highly virulent strains, a picture of hemorrhagic septicemia may develop that may be characterized by high fever, cardiac weakness, toxemia, and early death. Organisms can be cultured from the blood; autopsy findings may be minimal or include petechial hemorrhages on mucosal and serosal surfaces and in various organs. Less acute forms, such as a pneumonia with serofibrinous exudate in the interlobular septa of the lungs, a hemorrhagic gastroenteritis, and subacute and chronic infections such as otitis in the rabbit, may occur. The mechanisms by which these bacteria invade the mucosa and cause systemic disease continue to unfold. Key virulence factors are the capsule and lipopolysaccharide, with others still being identified.[17]

In humans, three major types of infections occur.[52] In the first and most common type, local infection occurs after a cat bite or scratch, a dog bite, or, rarely, the bite of another animal. Fifteen to 20 percent of dog-bite wounds and more than 50 percent of cat-bite wounds become infected. Many cases have been associated with nonbite exposure, such as licking.[11] These cases usually are characterized by a rapidly progressive, acute cellulitis with lymphangitis, local lymphadenitis, or both. Cat-bite wounds may progress to osteomyelitis of the underlying bone. This development is not because of any known predilection of *P. multocida* for the bone but because the sharp fangs of a cat deposit the organism on or under the periosteum.

The second type includes cases of chronic pulmonary infection in which the organism may be the only isolate or one of several organisms. Cases of bronchiectasis and empyema have been reported, usually in patients with underlying pulmonary disease. In a series of 28 cases of bronchiectasis in which the organism was recovered, it usually appeared as a secondary invader. It seems to have low pathogenicity in the respiratory tract until some other infection or physiologic disturbance decreases the natural resistance of the host, which enables active infection, most commonly liver disease such as cirrhosis, to occur.

Pasteurella infection also may be septicemic or occur with meningitis. The pathology and pathogenesis are similar to those of other organisms.

CLINICAL MANIFESTATIONS

In cases of local infection from a scratch or bite, the usual clinical pattern shows swelling, erythema, and tenderness within a few hours of the bite; most symptoms occur within the first 24 hours. A gray serous or sanguinopurulent discharge from the puncture sites may be present. Signs of systemic toxic effects, such as chills and fever, may or may not be present; regional lymphadenopathy often is evident. Less commonly, the infection may be lower grade and smoldering.[37] As noted, osteomyelitis and tenosynovitis most often occur after cat bites because of the sharpness of cats' teeth.

Lee and Buhr[26] found *P. multocida* to be the most common infecting organism in a report of 69 dog bites that had been cultured; 20 of the bites became grossly infected, and *P. multocida* was isolated from 10. Of 30 wounds that were sutured, 14 (47%) were infected with *P. multocida*. Other unusual localized infections include chronic skin ulcers, secondary infection of a gouty joint, and infection of a compound fracture site and an amputation site.[48] Preexisting joint disease seems to be a risk.

Clinical manifestations of patients having respiratory complaints are not unusual. Most of the isolates have been associated with chronic bronchitis, bronchiectasis, chronic sinusitis or otitis media, and pneumonia. Several cases of massive pulmonary abscesses, pleural effusion, and empyema also have been reported. Larsen and Holden[25] described a 14-year-old girl with chronic otitis media for 2 years who developed a *P. multocida* cerebellar abscess. A case of epiglottitis caused by this organism has been reported in an adult.[23] A child developed Ludwig angina after having a nonbite exposure. A 15-year-old boy with Kikuchi disease and *P. multocida* bacteremia has been described.[54]

In a report of 136 cases of *P. multocida* infection that were not related to animal bites, the most common site of infection after the respiratory tract was the abdomen; the organism was recovered from 10 patients with appendicitis. Eight isolates were from the female reproductive tract, four from the urine, and one from a chronic sacral abscess.[21] Whether these cases are secondary to ingestion of the organism or to hematogenous spread has not been determined. Raffi and colleagues[36] described three children with appendiceal peritonitis associated with *P. multocida*. The organism also has been seen in cases of peritoneal dialysis–associated peritonitis secondary to puncture of dialysis tubing.[27,28]

The disseminated infections are the other major clinical group of *Pasteurella* infections. Isolated bacteremia may be present[35]; however, most of these cases have been meningitis, many of which were mistaken for *Haemophilus influenzae* or *Neisseria meningitidis* infection because of the morphologic similarities among these organisms. In a review of the subject in 1967, Controni and Jones[7] noted 14 confirmed cases of *Pasteurella* meningitis; 11 occurred in adults, and 3 occurred in children. Eight of the 14 patients had a history of accidental or surgical trauma. The mortality rate was 50 percent, but only five patients were treated with antibiotics. Evaluation of the cerebrospinal fluid showed white blood cell counts ranging from 580/mm^3 to 5200/mm^3, all with a predominance of polymorphonuclear leukocytes. More recently, Green and coworkers[15] reviewed 29 cases of *P. multocida* meningitis in adults.

The first reported neonate with *Pasteurella* meningitis died at 88 hours of age.[1] The mother had a fever in the postpartum period, but her pretreatment cultures were lost, so verification of the source was impossible.[7] Since then, a case of *Pasteurella* chorioamnionitis associated with premature delivery and neonatal sepsis and death within 1.5 hours of delivery has been reported.[44] Gingival cultures of a pet cat that had scratched a mother numerous times during pregnancy also yielded *P. multocida*. Subsequently, several young infants with septicemia and meningitis caused by this organism survived without apparent sequelae after treatment with penicillin or ampicillin and gentamicin.* Pizey[34] reported another case of neonatal infection in a 3-week-old infant with septic arthritis. *P. multocida* infection may take a rapidly fatal course even in an older infant.[41,46] Clapp and associates[6] described two infants whose disease was associated with nontraumatic facial licking by pets, an avoidable exposure. In another report, a case of in utero infection at 12 weeks' gestation was described.[50] A neonate with meningitis and cervical spine osteomyelitis with full recovery has been reported.[18,19] Other neonatal infections, primarily sepsis and meningitis, have been described with similar incidental exposure, usually licking by a pet.

*References 2, 10, 14, 24, 38, 47.

DIAGNOSIS AND TREATMENT

Although *P. multocida* is one of the more likely pathogens to cause infection of cat or dog bites, its clinical manifestations are not unusual. Diagnosis of *P. multocida* infection can be made definitively only by culture. It may resemble several other organisms morphologically, but identification should not be difficult to make. The fact that it does not require X and V factors for growth should distinguish it from *H. influenzae*. Its production of indole should differentiate it from the *Neisseria* group, and its inability to grow on MacConkey agar or a bile salt medium should distinguish it from *Acinetobacter* spp. and the enteric organisms.

The drug of choice for *P. multocida* infection is penicillin, to which the organism is exquisitely sensitive. This feature may be used as a rapid means of distinguishing it from *H. influenzae* or the Enterobacteriaceae. Rare strains producing β-lactamase and resistance to penicillins have been recovered.[39] The organism usually is sensitive to a wide variety of other antibiotics, including ampicillin, other broad-spectrum penicillins (e.g., ticarcillin, piperacillin, mezlocillin), amoxicillin/clavulanic acid,[12] tetracyclines, parenteral cephalosporins (particularly second-generation and third-generation agents),[32] cefuroxime, cefpodoxime, and chloramphenicol. Semisynthetic penicillins (e.g., nafcillin, dicloxacillin), erythromycin, some orally administered cephalosporins (cephalexin, cefaclor), clindamycin, and aminoglycosides have relatively low activity against *P. multocida*.[8,13,43] Azithromycin seems to have acceptable activity,[6] as does ciprofloxacin.[22] Trimethoprim-sulfamethoxazole may be an alternative, particularly for patients unable to take a β-lactam antibiotic.[40] Surgical drainage or débridement also may be necessary. The duration of treatment depends on the primary disease process. Seven to 10 days generally is adequate for local infections.

PROGNOSIS AND PREVENTION

Proper cleansing and débridement of wounds caused by animal bites or scratches is important in prevention of *P. multocida* infection. Lee and Buhr[26] found that suturing wounds caused by a dog bite was associated with a higher incidence of infection. Whether a wound is sutured often depends on the site and the potential cosmetic result. The use of prophylactic antibiotics to prevent infection after animal bites is controversial. Some experts recommend their use for facial bites, if a delay occurs in seeking medical assistance, for cat bites, or for patients with immunocompromising conditions.

Limiting contact with wild and domestic animals that may harbor the organism probably is the only definitive way to prevent infection. Teaching proper handling of pets and keeping pets from licking infants and young children, particularly on the face, may help. No vaccine for human use is available.

Prognosis depends on the particular site of infection. With appropriate treatment, resolution usually occurs, but the healing process may be very slow, particularly in local infections with extension to the bone or tendons.[9,49]

NEW REFERENCES SINCE THE SIXTH EDITION

24. Kobayaa H, Souki RR, Trust S, Domachowski JB. *Pasteurella multocida* meningitis in newborns after incidental animal exposure. *Pediatr Infect Dis* 2009;**10**:928–9.
30. Myers EM, Ward SL, Myers JP. Life-threatening respiratory pasteurellosis associated with palliative pet care. *Clin Infect Dis* 2012;**54**:e55–7.
54. Wong VK, Campion-Smith J, Smith S. Kikuchi disease in association with *Pasteurella multocida* infection. *Pediatrics* 2010;**125**: e679–82.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Bates HA, Controni G, Elliott N, et al. Septicemia and meningitis in a newborn due to *Pasteurella multocida*. *Clin Pediatr* 1965;**4**:668–70.
2. Bhave SA, Guy LM, Rycroft JA. *Pasteurella multocida* meningitis in an infant with recovery. *BMJ* 1977;**2**:741–2.
3. Branson D, Bunkfeldt F. *Pasteurella multocida* in animal bites of humans. *Am J Clin Pathol* 1967;**48**:552–5.
4. Burdge DR, Scheifele D, Speart DP. Serious *Pasteurella multocida* infections from lion and tiger bites. *JAMA* 1985;**253**:3296–7.
5. Choudat D, Paul G, Legoff C, et al. Specific antibody responses to *Pasteurella multocida*. *Scand J Infect Dis* 1987;**19**:453–7.
6. Clapp WC, Kleiman MB, Reynolds JK, et al. *Pasteurella multocida* meningitis in infancy. *Am J Dis Child* 1986;**140**:444–6.
7. Controni G, Jones RS. *Pasteurella* meningitis: a review of the literature. *Am J Med Technol* 1967;**33**:379–86.
8. Fass RJ. Erythromycin, clarithromycin and azithromycin: use of frequency distribution curves, scattergrams and regression analyses to compare in vitro activities and describe cross-resistance. *Antimicrob Agents Chemother* 1993;**37**:2080–6.
9. Francis DP, Holmes MA, Brandon G. *Pasteurella multocida*: infection after domestic animal bites and scratches. *JAMA* 1975;**233**:42–5.
10. Frutos AA, Levitsky D, Scott EG, et al. A case of septicemia and meningitis in an infant due to *Pasteurella multocida*. *J Pediatr* 1978; **92**:853.
11. Goldstein EJC. Bite wounds and infection. *Clin Infect Dis* 1991; **14**:633–40.
12. Goldstein EJC, Citron DM. Comparative activities of cefuroxime, amoxicillin-clavulanic acid, ciprofloxacin, enoxacin, and ofloxacin against aerobic and anaerobic bacteria isolated from bite wounds. *Antimicrob Agents Chemother* 1988;**32**:1144–8.
13. Goldstein EJC, Citron DM, Rechwald GA. Lack of in vitro efficacy of oral forms of certain cephalosporins, erythromycin and oxacillin against *Pasteurella multocida*. *Antimicrob Agents Chemother* 1988; **32**:213–15.
14. Goytia VK, Demmler GJ, Pannaraj PS, et al. An unusual cause of sepsis and meningitis in a neonate. *Semin Pediatr Infect Dis* 2006;**187**:225–7.
15. Green BT, Ramsey KM, Nolan PE. *Pasteurella multocida* meningitis: case report and review of the last 11 y. *Scand J Infect Dis* 2002;**34**: 213–17.
16. Hansmann GH, Tully M. Cat bite and scratch wounds with consequent *Pasteurella* infection of man. *Am J Clin Pathol* 1945;**15**: 312–18.
17. Harper M, Boyce JD, Adler B. *Pasteurella multocida* pathogenesis: 125 years after Pasteur. *FEMS Microbiol Lett* 2006;**265**:1–10.
18. Hirsh D, Farrel K, Reilly C, Dobson S. *Pasteurella multocida* meningitis and cervical spine osteomyelitis in a neonate. *Pediatr Infect Dis J* 2004;**23**:1063–5.
19. Holst E, Rollof J, Larsson L, et al. Characterization and distribution of *Pasteurella* species recovered from humans. *J Clin Microbiol* 1992;**30**:2984–7.
20. Hubbert WT, Rosen MN. *Pasteurella multocida* infection due to animal bite. *Am J Public Health* 1970;**60**:1103–8.
21. Hubbert WT, Rosen MN. *Pasteurella multocida* infection in man unrelated to animal bite. *Am J Public Health* 1970;**60**:1109–17.
22. Itoh M, Tierno PM, Milstoc M, et al. A unique outbreak of *Pasteurella multocida* in a chronic disease hospital. *Am J Public Health* 1980;**70**:1170–3.
23. Johnson RH, Rumans LW. Unusual infections caused by *Pasteurella multocida*. *JAMA* 1977;**237**:146–7.
24. Kobayaa H, Souki RR, Trust S, Domachowski JB. *Pasteurella multocida* meningitis in newborns after incidental animal exposure. *Pediatr Infect Dis* 2009;**10**:928–9.
25. Larsen TE, Holden FA. Isolation of *Pasteurella multocida* from an otogenic cerebellar abscess. *Can Med Assoc J* 1969;**101**:629–30.
26. Lee ML, Buhr AJ. Dog bites and local infection with *Pasteurella septica*. *BMJ* 1960;**1**:169–71.
27. Loghman-Adham M. *Pasturella multocida* peritonitis in patients undergoing peritoneal dialysis. *Pediatr Nephrol* 1997;**11**:353–4.
28. Malik A, Aly A, Mailey KS, Bastani B. *Pasteurella multocida* peritoneal dialysis–associated peritonitis: a report of two cases and review of the literature. *J Nephrol* 2005;**18**:791–3.
29. Mortensen JE, Giger O, Rodgers GL. In vitro activity of oral antimicrobial agents against clinical isolates of *Pasturella multocida*. *Diagn Microbiol Infect Dis* 1998;**30**:99–102.
30. Myers EM, Ward SL, Myers JP. Life-threatening respiratory pasteurellosis associated with palliative pet care. *Clin Infect Dis* 2012; **54**:e55–7.
31. Nielsen JP, Rosdahl VT. Development and epidemiological applications of a bacteriophage typing system for typing *Pasteurella multocida*. *J Clin Microbiol* 1990;**28**:103–7.
32. Noel GT, Teele DW. In vitro activities of selected new and long-acting cephalosporins against *Pasteurella multocida*. *Antimicrob Agents Chemother* 1986;**29**:344–5.
33. Oberhofer TR. Characteristics and biotypes of *Pasteurella multocida* isolated from humans. *J Clin Microbiol* 1981;**13**:566–77.
34. Pizey NCD. Infection with *Pasteurella septica* in a child aged three weeks. *Lancet* 1953;**2**:324–6.
35. Raffi F, Barrier J, Baron D, et al. *Pasteurella multocida* bacteremia: report of thirteen cases over twelve years and review of the literature. *Scand J Infect Dis* 1987;**19**:385–93.
36. Raffi F, David A, Mouzard A, et al. *Pasteurella multocida* appendiceal peritonitis: report of three cases and review of the literature. *Pediatr Infect Dis* 1986;**5**:695–8.
37. Reinert P, Canet J, Pesnel G, et al. Une cause souvent ignorée d'arthrite subaiguë chez l'enfant; la pasteurellose à *P. multocida*. *Arch Fr Pediatr* 1972;**29**:99–104.
38. Repice JP, Neter E. *Pasteurella multocida* meningitis in an infant with recovery. *J Pediatr* 1975;**86**:91–3.
39. Rosenau A, Labigne A, Escande F, et al. Plasmid-mediated ROB-1 β-lactamase in *Pasteurella multocida* from a human specimen. *Antimicrob Agents Chemother* 1991;**35**:2419–22.
40. Sands M, Ashley R, Brown R. Trimethoprim-sulfamethoxazole therapy of *Pasteurella multocida* infection. *J Infect Dis* 1989;**160**: 353–4.
41. Schipper GJ. Unusual pathogenicity of *Pasteurella multocida* isolated from the throats of common wild rats. *Bull Johns Hopkins Hosp* 1947;**81**:333–56.
42. Smith JE. Studies on *Pasteurella septica*, part I: Occurrence in nose and tonsils of dogs. *J Comp Pathol* 1955;**65**:239–45.
43. Stevens DL, Higbee JW, Oberhofer TR, et al. Antibiotic susceptibilities of human isolates of *Pasteurella multocida*. *Antimicrob Agents Chemother* 1979;**16**:322–4.
44. Strand CL, Helfman L. *Pasteurella multocida* chorioamnionitis associated with premature delivery and neonatal sepsis and death. *Am J Clin Pathol* 1971;**55**:713–16.
45. Swartz MN, Kunz LJ. *Pasteurella multocida* infection in man. *N Engl J Med* 1959;**261**:889–93.
46. Tessin I, Brorson JE, Trollfors B. Rapidly fatal *Pasteurella multocida* septicemia in infant following cat scratch. *Pediatr Infect Dis* 1987;**6**: 425–6.
47. Thompson CM, Pappu L, Levkoff AH, et al. Neonatal septicemia and meningitis due to *Pasteurella multocida*. *Pediatr Infect Dis* 1984;**3**:559–61.
48. Tindall JP, Harrison CM. *Pasteurella multocida* infections following animal injuries, especially cat bites. *Arch Dermatol* 1972;**105**: 412–16.
49. Torphy DE, Ray CG. *Pasteurella multocida* in dog and cat bite infections. *Pediatrics* 1969;**43**:295–7.
50. Waldor M, Roberts D, Kazanjian P. In utero infections due to *Pasteurella multocida* in the first trimester of pregnancy: case report and review. *Clin Infect Dis* 1992;**14**:497–500.
51. Weber DJ, Hausen AR. Infections resulting from animal bites. *Infect Dis Clin North Am* 1991;**5**:663–80.
52. Weber DJ, Wolfson JS, Swartz MN, et al. *Pasteurella multocida* infections: report of 34 cases and review of the literature. *Medicine (Baltimore)* 1984;**63**:133–54.
53. Wilson MA, Rimbler RB, Hoffman LJ. Comparison of DNA fingerprints and somatic serotypes of serogroup B and E *Pasteurella multocida* isolates. *J Clin Microbiol* 1992;**30**:1518–24.
54. Wong VK, Campion-Smith J, Smith S. Kikuchi disease in association with *Pasteurella multocida* infection. *Pediatrics* 2010;**125**: e679–82.
55. Woolcock JB, Collins FM. Immune mechanism in *Pasteurella multocida*–infected mice. *Infect Immun* 1976;**13**:949–58.

disproportionately affects low-income countries.[86] Epidemic, endemic, and a mixture of the two forms occur. Epidemic cholera typically occurs in populations with little preexisting immunity and affects both children and adults. Endemic cholera tends to affect children with a higher incidence and severity because of lower levels of acquired immunity relative to that in adults.[21]

The incidence of cholera has increased since 2000.[98] In 2010, nearly 50 countries, mostly in Sub-Saharan Africa and Asia, reported 317,534 cases, 7543 deaths, and a case-fatality rate of 2.4 percent.[98] The United States reported 15 cases in 2010, all imported.[98] The disease burden of cholera is considered vastly underreported, and the actual number of cases is estimated at 3 to 5 million annually, with more than 100,000 deaths.[94] Recent outbreaks occurred in the Democratic Republic of Congo, the Republic of Congo, Cameroon, Chad, Niger, Iraq, Nigeria, Sudan, Somalia, Angola, Zimbabwe, and Haiti.[95] In 2011, Haiti reported over 500,000 cholera cases and 7000 deaths shortly after the October 2010 outbreak,[70] likely from a South Asian variant.[17] The case fatality rate during the recent Haitian outbreak began at 7 percent, one of the highest recorded rates in recent history, and then fell to 1.3 percent, just slightly above the generally accepted benchmark of 1 percent.[23] Many organizations advocate for an integrated and comprehensive approach to the control and prevention of this global public health problem.

HISTORY

Cholera has long been indigenous to the Ganges River valley in northern India; however, the modern history of cholera is described in seven discrete pandemics, all originating from Asia and all presumably caused by the O1 serogroup.[6] The first six pandemics, caused by the classic biotype, occurred between 1817 and 1923. The ongoing seventh pandemic began in Indonesia and South East Asia in 1961, and then spread throughout Asia and Africa. By 1991, it appeared in Peru and spread throughout Latin America. This most recent pandemic was caused by the El Tor biotype, which has almost completely replaced the classic biotype and is the longest and most widespread of all pandemics to date.[6] Several atypical El Tor variants containing phenotypic and genetic characteristics of the classic biotype were recently recognized, suggesting the emergence of hybrids.[78] In 1992, the first non-O1 serogroup isolated in association with an epidemic was reported.[77] This new O139 Bengal serogroup emerged in India and Bangladesh, raising concern for an eighth pandemic, but has not spread beyond the region to date.

The disastrous saga of cholera over the past 2 centuries helped shape public health as a discipline. Beginning in 1826, during the second cholera pandemic, many jurisdictions were induced to establish local sanitation and health boards, eventually evolving into the first organized public health apparatus. During the London cholera epidemic of 1854, investigations by Dr. John Snow demonstrated that transmission of the disease was associated with consumption of contaminated drinking water from the city's infamous Broad Street pump; this episode is considered the first scientific public health investigation and gave rise to modern epidemiology.[29] In 1866, an epidemic in New York City led to the creation of the first Board of Health in the United States, and cholera became the first reportable disease.

The Italian scientist Fillipo Pacini identified the comma-shaped organism in 1854, but this discovery was overlooked until Robert Koch identified the cholera bacillus in 1883 during the fifth pandemic. Koch's cholera discoveries contributed to his pioneering work that began the field of Medical Microbiology.[29] The addition of glucose to enhance the absorption of sodium in oral rehydration therapy, the cornerstone of cholera treatment, has saved the lives of millions and is heralded as one of the most important medical advancements of the 20th century.[29]

MICROBIOLOGY

V. cholerae is a curved gram-negative rod classified by biochemical tests and subdivided into serogroups based on the somatic O antigen. It belongs to the family Vibrionaceae, which differs from the related family Enterobacteriaceae in being oxidase-positive and motile with a single flagellum. Vibrios are fermentative and oxidative in metabolism. They have few nutritional requirements and can grow in glucose as the sole source of carbon and energy. Most vibrios are marine organisms, and most grow best in 2 to 3 percent sodium chloride under aerobic conditions. Although neither the typical comma shape of *V. cholerae* nor its motility is seen on Gram stain, both are apparent in wet mount even on fresh fecal samples. If motility stops after adding anti-*Vibrio* antiserum, this establishes a presumptive diagnosis of the homologous organism.

Numerous *Vibrio* spp. exist, most of which are nonpathogenic in humans. *V. cholerae* spp. includes more than 200 serogroups classified according to the antigenic characteristics of the lipopolysaccharide O antigen.[9] To date, only *V. cholerae* organisms carrying the somatic O antigens O1 and O139 are associated with epidemic disease. Some non-O1 *V. cholerae* serogroups cause sporadic diarrheal illness, which can be severe and sometimes invasive and inflammatory.

The O1 serogroup is divided further into two biotypes, classic and El Tor, based on biochemical properties and phage susceptibilities. The classic biotype traditionally causes a more severe disease, but the El Tor biotype may be better adapted to persist in the environment and establish epidemics. The classical biotype expresses genes conferring human virulence more than the El Tor biotype, which shows enhanced expression of genes for biofilm formation, chemotaxis, and transport of amino acids, peptides, and iron.[9] Classic and El Tor biotypes are divided into three serotypes (i.e., Ogawa, Inaba, and Hikojima) based on their dominant heat-stable lipopolysaccharide somatic antigens. The O139 serogroup comprises several genetically diverse strains, including nontoxigenic variants, and is genetically related to the O1 El Tor biotype.[27] Media used to isolate cholera organisms

in stool include thiosulfate citrate bile salts sucrose (TCBS) agar and tellurite taurocholate gelatin agar. The appearance of yellow ("popcorn") colonies on TCBS agar is considered highly suggestive of *V. cholerae*. The bacterium is confirmed further by its ability to grow in the presence (or absence) of salt and by serology.

PATHOGENESIS

The pathogenic paradigm of *V. cholerae* comprises colonization of the small bowel mucosa followed by release of one or more enterotoxins, the most potent of which is the oligomeric cholera toxin itself. *V. cholerae* attaches to the intestinal mucosa by virtue of proteinaceous hairlike fimbriae, the most important being the toxin-coregulated pilus.[77] These pili mediate microcolony formation of the intestinal epithelium. Colonization is facilitated by a protease that degrades mucin[82] and epithelial tight junction–associated proteins and a single flagellum, which accelerates approach to the intestinal mucosa in conjunction with chemotactic receptors and intracellular sensor molecules.[13]

V. cholerae organisms shed in the stool of an infected individual act more virulently than strains acquired from an environmental source. Serial passage through multiple patients may augment virulence further, partly accounting for the violent epidemics occurring throughout history.[57] On intestinal passage, genes required for acquiring nutrients and motility are upregulated, whereas genes required for chemotaxis are downregulated. This increased virulence was confirmed in an animal model in which *V. cholerae* shed by mice has a 10-fold lower infectious dose than organisms grown in vitro.[57] Reduced chemotaxis prevents dispersion in environmental waters and increases clumping of the bacteria[14]; the resulting increased concentration of organisms also may contribute to the vigor of cholera outbreaks.

Cholera toxin is composed of five identical binding (B) subunits and one active (A) subunit. Similar in structure and function to the heat-labile toxin produced by enterotoxigenic *Escherichia coli*,[62] cholera toxin binds to the GM_1 ganglioside receptor on the surface of intestinal epithelial cells. The A subunit is internalized into the cell, where it activates adenylate cyclase by ADP-ribosylation of the $G_{s\alpha}$ protein. This results in upregulation of cyclic adenosine monophosphate, causing an increase in chloride secretion from the intestinal crypt cells via the cystic fibrosis transmembrane conductance regulator chloride channel and a decrease in sodium chloride absorption by the villous cells. In addition to this mechanism, cholera toxin may have effects on prostaglandins and the enteric nervous system that could contribute to the voluminous diarrhea characteristic of the disease.[47] Water follows the loss of electrolytes along the osmotic gradient into the intestinal lumen. The dramatic loss of fluid and electrolytes rapidly leads to decreased intravascular volume and the complications of hypovolemic shock and metabolic acidosis. Although cholera toxin is responsible for much of the pathogenicity associated with the organism, strains mutated in cholera toxin still elicit mild diarrhea.[47] Several other toxins have been identified, but their exact role in pathogenesis and immune modulation is not completely understood. These include the zonula occludens toxin, which increases small intestinal mucosa permeability through the modulation of internal tight junctions, and the accessory cholera enteroxin, an integral membrane protein thought to function as an ion-permeable pore.[16,28,90] The multifunctional cholera toxin protein is also a powerful immunomodulator and may have potential applications in immunotherapeutics and as a mucosal or possibly epicutaneous adjuvant.[10,68]

The genetics of *V. cholerae* provide insight into the organism's pathogenicity and spread. The genome of 4 million DNA base pairs comprises two circular chromosomes,[22,40] the larger of which contains genes for growth and pathogenicity and the smaller contains elements of metabolism and regulation. The genes required for virulence can propagate laterally and among different strains. Required for pathogenicity are the cholera toxin genetic element and the *Vibrio* pathogenicity island, which encodes toxin-coregulated pilus and the critical regulator ToxT, the primary direct transcriptional activator of the virulence genes. The pathogenicity island can be moved between strains by phage transduction and chitin-induced transformation. Toxigenic strains emerge through the acquisition of the bacteriophage CTXΦ, which harbors the *ctxAB* gene encoding cholera toxin.[56] The complex regulatory system controlling expression of virulence genes includes the ToxT/ToxR regulator proteins, quorum sensing, and small RNA molecule, and differs among in vivo and in vitro life stages[12,42,56] and between clinical and environmental isolates.[59]

EPIDEMIOLOGY

Cholera is classically described as a waterborne illness, but it can also be transmitted via contaminated food. The incubation period depends on the inoculum, with lower inocula corresponding to a longer incubation period and decreased stool volumes.[47] Incubation generally is 1 to 3 days, with a range of several hours to 5 days. Symptoms usually last 2 to 3 days, and patients treated with antibiotics may be infectious for several days. Patients who are untreated remain infectious for 1 to 2 weeks. Although waterborne transmission is typical in developing countries of Asia and Africa, *V. cholerae* found in marine environments can be transmitted via undercooked shellfish.[20] The role of household animals in transmission is probably minimal,[81] and person-to-person transmission is rare, owing to the high infectious dose.[63]

Seasonal patterns in endemic areas have long been recognized for cholera outbreaks, with a predilection for warmer months. Studies conducted during interepidemic periods suggest a complex interaction between humans and the environment. *V. cholerae* exists in saline aquatic environments in a viable but nonculturable state, mostly in the form of metabolically dormant biofilm associated with zooplankton.[44,87] This latent form originates from human stool, survives gastric acidity, and causes infection. When human cases begin to occur in a community, direct contamination of food and water may occur, circumventing the need for the aquatic reservoir.[24] Seasonal

epidemics are associated with other factors that affect the environmental reservoir, including changes in zooplankton blooms, the abundance of chitin, and rainfall. Several cholera prediction models have been developed based on these factors.[45,73,74] Recent evidence suggests that increases in vibrio-infecting bacteriophages may be associated with the end of an outbreak and interepidemic periods in Bangladesh.[25,26]

Host factors associated with susceptibility to *V. cholerae* include age, immunity, blood type, and reduced gastric acid production. Age groups commonly affected by cholera typically include young children because susceptibility depends on preexisting immunity. In endemic areas such as Bangladesh, cases of cholera are concentrated in the 2- to 9-year-old age category, followed by women of childbearing age.[47] The increased susceptibility of children is further supported by a more recent prospective study in Kolkata, India, where the burden of cholera was greatest in children younger than 2 years old and cases were more likely where a household member had diarrhea.[37,83] Mucosal humoral immune responses in both children and adults to *V. cholerae* may be decreased with intestinal helminth coinfection.[37] Breast-feeding protects against disease in children colonized with *V. cholerae*.[31] Acholorhydric patients are at higher risk for acquiring infection, because bacteria are more likely to survive the gastric environment and colonize the small intestine.[65] Observations of increased cholera severity among people with blood type O were confirmed through challenge and cohort studies,[36,52] presumably because of binding of blood type O antigen to cholera toxin.[39] For reasons that are not completely understood, individuals with the Lewis blood group Le(a+b–) phenotype may also be at increased risk for symptomatic disease in contrast to those with Le(a–b+) and Le(a–b–).[4] Other host genetic factors likely also play a role, as evidenced by familial clustering of cases and gene association studies.[33,51,71]

CLINICAL MANIFESTATIONS

Most individuals infected with *V. cholerae* have only mild symptoms, indistinguishable from symptoms caused by other enteric infections. Individuals who develop disease urgently need fluid resuscitation and replacement of ongoing losses. Of all causes of infectious diarrhea, the stool volumes passed and the rapidity by which dehydration develops are greatest with cholera.[80] In severe disease, stool volume can exceed 250 mL/kg body weight in a 24-hour period.[60,72]

The most distinctive clinical feature of cholera is the painless passage of voluminous, watery stools. Termed "rice water stools" because of the watery, colorless diarrhea dotted with mucus, cholera stools may have a fishy smell or may be nearly odorless. Other common symptoms include abdominal cramping and vomiting. Abdominal cramping is probably due to increased abdominal secretions and resulting small bowel distention. Vomiting commonly occurs a few hours after the onset of diarrhea, but it also occurs later in the course of the illness.[15,38,64]

Complications of cholera relate mostly to the massive loss of intravascular volume and electrolytes. If cholera is not treated promptly, diarrhea and vomiting eventually lead to dehydration, which is most often isotonic. Patients with severe cholera may experience vascular collapse, shock, and death rates of 50 percent, starting within hours after the onset of diarrhea.[77] Related to the massive loss of intravascular volume and hypoperfusion, bicarbonate losses in stool and hyperphosphatemia, acidosis also may develop.[91,93] Compensatory tachypnea may be seen in patients with acidosis. In addition to bicarbonate losses in stool, potassium is also lost and may not be reflected in serum potassium levels; acidosis induces the shift of intracellular potassium into the intravascular space in exchange for hydrogen ions, resulting in decreased total body potassium and potentially normal serum potassium values. As the acidosis is corrected, serum hypokalemia may result. In cases complicated by severe malnutrition, in which body potassium stores already are depleted, hypokalemia may be quite severe and manifest as paralytic ileus. This hypokalemia is rarely associated with severe cardiac arrhythmias, but it may manifest as changes on electrocardiogram.

After dehydration, hypoglycemia is the second most common cause of death in pediatric patients with cholera.[8] In one study, the mortality rate in pediatric patients with hypoglycemia caused by cholera was 15 percent in contrast to 1 percent mortality in patients with normoglycemia.[8] Although the precise mechanism of hypoglycemia is unknown, malnourished children who are acutely ill have lower glycogen stores and impaired gluconeogenesis. Special attention should be given to monitor patients with concomitant malnutrition and cholera for hypoglycemia.

Severe cholera is diagnosed in individuals who present with clinical signs and symptoms of severe dehydration (loss of ≥15% of total body water or loss of ~10% of body weight). Common signs of severe volume depletion include a combination of decreased skin turgor, depressed anterior fontanelle in infants, lack of tears and sunken eyes, dry mouth, anuria, tachycardia, decreased peripheral perfusion, and hypotension (Fig. 117-1). In some cases, mental status changes may be a sign of volume

FIGURE 117-1 ■ An adolescent girl with severe dehydration from cholera. Characteristic features include obtundation, sunken eyes, and "tenting" of the abdominal skin and subcutaneous tissues after firmly pinching the abdomen.

depletion, but more common are somnolence, restlessness, and lethargy.

LABORATORY FINDINGS AND DIAGNOSIS

Laboratory abnormalities in patients with cholera relate to the massive loss of volume and electrolytes. The hypovolemic state can lead to hemoconcentration, causing increased hematocrit, serum specific gravity, serum protein, serum creatinine, and blood urea nitrogen.[91,93] Because of the loss of sodium that accompanies water loss, serum sodium is either slightly low or normal.[47,72] As noted earlier, although potassium loss occurs in the stool, serum potassium may be normal, whereas total body potassium may be decreased. Acidosis caused by loss of bicarbonate in stool and lactic acidosis can result in a serum bicarbonate concentration of less than 15 mmol/L.[91,93] Serum creatinine and blood urea nitrogen also are elevated as a result of a decreased glomerular filtration rate. The common abnormalities of sodium, potassium, bicarbonate, and glucose mandate early determination of serum glucose and electrolytes as a guide to therapy.

Although presumptive diagnosis is made on the basis of typical oxidase-positive colonies from stool samples that agglutinate with O1 or O139 antiserum, conventional culture methods remain the gold standard. The characteristically motile organisms can be visualized directly using darkfield microscopy,[7] but few laboratories except those in cholera-endemic regions are experienced with this technique. If cholera is suspected, special culture media, such as TCBS agar, should be used to culture the organism.

In epidemic settings or in areas of intense transmission, a rapid vibrio dipstick test may be used. This is a one-step immunochromatographic test developed by the Institut Pasteur for rapid detection of cholera O1 and O139 from stool samples. The method has been tested in Madagascar, Bangladesh, and Mozambique, where the specificity ranged from 84 to 100 percent and sensitivity ranged from 94 to 100 percent.[66,92] It is especially advantageous because it requires minimal technical skill, can be read in 10 minutes,[66] and detects cholera from rectal swabs.[11,92] Confirmation of *V. cholerae* in stool also can be done using polymerase chain reaction amplification or oligoprobes.[43,58,89]

TREATMENT

The cornerstone of cholera management is fluid resuscitation. When fluid resuscitation is initiated, previous losses must be estimated based on the degree of dehydration and replaced and ongoing losses must be quantified and replenished. Replacement fluids in children with cholera should be similar to fluids that are lost, such as lactated Ringer solution (Table 117-1).[41,96] The ideal solution contains amounts of bicarbonate and potassium sufficient to replace what is lost in stool.

TABLE 117-1 **Electrolyte Concentration in Cholera Stool and in Fluids Used for Rehydration and Replacement of Stool Losses**

	Electrolyte Concentration (mmol/L)				
	Na^+	Cl^-	K^+	HCO_3^-	Osmolality
Cholera Stool					
Adults	130	100	20	44	300*
Infants and children	100	90	33	30	300*
Hydration Solutions					
WHO oral rehydration	75	65	20	10†	170‡
Intravenous					
Dhaka	133	98	13	48	273
Lactated	130	109	4	28§	251
Ringer					
5:4:1	129	97	11	44	281

*Osmolality includes unmeasured osmotically active molecules (primarily organic acids) in addition to electrolytes.
†As citrate.
‡From electrolytes only; also contains 75 mmol/L of glucose.
§As lactate.
WHO, World Health Organization.

At the International Centre for Diarrhoeal Disease Research, Bangladesh, where thousands of cholera patients are treated annually, patients of all ages are seen with severe dehydration of rapid onset. Many arrive for treatment in hypovolemic shock. The extent of dehydration is determined, one or two intravenous catheters are inserted, and isotonic resuscitation solution is administered rapidly. The composition of the preferred solution at the Center (Dhaka solution) is listed in Table 117-1. The solution contains Na^+, Cl^-, K^+, and HCO_3^-, but no glucose. The complete estimated deficit is administered over 4 hours, whereupon ongoing losses and maintenance fluids are provided. Serum glucose and electrolytes are determined rapidly, with administration of glucose as needed based on these results. Because severely dehydrated patients characteristically are anuric, concern may exist over early administration of potassium. The rapid onset of dehydration in cholera patients reduces this risk, however, and acute tubular necrosis rarely is seen.[72,93] A greater concern may be the rapid shift of potassium from the circulation on correction of acidosis. Patients treated in this manner respond dramatically. After the 4-hour resuscitation phase, most patients can begin to accept oral rehydration solution. Close attention to fluids and electrolytes is required for the duration of the diarrhea.

Although oral and intravenous rehydration methods are acceptable in theory for the management of cholera, the severity of losses in severe cholera renders intravenous rehydration the mainstay for treatment initiation

FIGURE 117-2 ■ A cholera cot: A simple folding cot covered with plastic that has a hole and bucket for collecting the stool output, as used for the care of cholera patients at the International Centre for Diarrhoeal Disease Research, Bangladesh. The bucket is calibrated, and the volume of stool (and replacement fluids required) can be calculated easily. The plastic sheet is cleaned daily and between patients.

in these cases. Not only do intravenous fluids allow predictable and rapid expansion of intravascular fluid volume to occur, they also ensure timely replacement of ongoing losses that may be challenging because of excessive purging.[69] Because massive volumes of fluid are lost in severe cholera, the volume deficit is frequently underestimated and rehydration therapy is often inadequate. Treatment of severe cases is best carried out by those with adequate training and experience in the management of these cases. Mild cases can be managed with oral rehydration solution (see later discussion). Guidelines for the initial management of cholera in an outbreak setting have been published by the World Health Organization (WHO) Global Task Force on Cholera Control.[97]

The use of a "cholera cot" is extremely helpful in quantifying ongoing fluid loss and for patient comfort (Fig. 117-2) and can easily be set up in cholera treatment centers. It is constructed of a simple cot with a hole in the center through which a plastic sheet drains into a graduated bucket below via a funnel. This allows for accurate quantification of ongoing losses and permits the patient to remain horizontal, an important advantage if the patient is initially hypotensive. Attention given to tachycardia and signs of hypoperfusion in prostrate patients is essential.

The WHO-approved oral rehydration salts (see Table 117-1) are recommended for patients with no detectable dehydration or mild dehydration, provided that the patient can drink sufficient quantities to replace the deficit and keep pace with ongoing losses. Oral rehydration solution can also be used for patients with severe dehydration to replace ongoing losses after initial intravenous replacement therapy. For cholera, oral rehydration solution that is made with rice rather than with glucose is preferred, because carbohydrate reduces the purging rate.[34,61,99] Food should be offered to patients as soon as they are able to eat and should not be restricted. Supplementation with zinc reduces the duration of diarrhea and the volume of stool output in children with cholera.[76] Effective use of oral rehydration solution requires extra effort in treating pediatric patients with cholera. Parents need instruction to encourage and supervise oral rehydration solution intake by the child, including taking small amounts frequently. Parents also need reassurance that oral rehydration is still effective despite occasional vomiting.

A short course of antibiotics should be given to children with clinically significant cholera. This approach reduces the amount of diarrhea, the duration of illness, and the infectious dose in stools.[32,77] Antibiotic therapy may also favorably affect the dynamics of transmission and hence have public health implications.[67] Because of high resistance rates in some areas, the organism should be isolated and tested for susceptibility using standard methods for gram-negative enteric bacteria. On average, patients not treated with antibiotics may continue to shed organisms for 5 or more days.[53]

Treatment options for cholera in pediatric patients are listed in Table 117-2. First-line therapies for *V. cholerae* in children include single-dose azithromycin or erythromycin for 3 days in younger children, and a single dose of doxycycline may be given to children older than 8 years. Tetracycline and doxycycline are generally not recommended in children younger than 8 years because of the risk for dental staining. In addition, ciprofloxacin is also effective against susceptible strains.[67] Trimethoprim-sulfamethoxazole has demonstrated efficacy in children,[46] although resistance may be present among O139 strains.[1] Both single-dose azithromycin 20 mg/kg and single dose ciprofloxacin 20 mg/kg achieved clinical success rates similar to a those of a 12-dose course of erythromycin in children 15 years of age or younger, but ciprofloxacin was less effective in eradicating *V. cholerae* from the stool.[49,79] Recently, single-dose azithromycin showed superiority to single-dose ciprofloxacin in children.[48]

Multidrug-resistant strains of *V. cholerae* have been isolated from both clinical and environmental settings, and the number of pathogenic strains resistant to one or more antibiotics is increasing.[50] Several different mechanisms of drug resistance have been reported and include the production of extended-spectrum β-lactamases, enhanced multidrug efflux pump activity, plasmid-mediated quinolone and fluoroquinolone resistance and chromosomal mutations.[5,30,35,88] The horizontal transfer of resistance determinants with mobile genetic elements such as integrons and the integrating conjugative elements assist in the dissemination of drug resistance.[30] SXT, first identified in a *V. cholerae* O139 strain from India, is an integrating conjugative element carrying a resistance gene for trimethoprim-sulfamethoxazole, streptomycin, and chloramphenicol.[30] Appropriate antibiotics, usually prescribed before obtaining sensitivity testing results, should be administered according to local resistance patterns and should be monitored for changes in sensitivity.

TABLE 117-2 **Antimicrobial Therapy of Cholera***

Agent	Single Dose	Multiple Dose
Azithromycin	20 mg/kg; maximal dose 1 g	
Erythromycin	Not evaluated	50 mg/kg/day erythromycin divided into four doses for 3 days; maximal dose 2 g/day
Doxycycline	2-4 mg/kg; maximal dose 300 mg	
Tetracycline	Not evaluated	50 mg/kg/day divided into four doses for 3 days; maximal dose 2 g/day
Ciprofloxacin‡	20 mg/kg; maximal dose 1 g	30 mg/kg/day divided into 2 doses for 3 days; maximal dose 1 g/day
Trimethoprim-sulfamethoxazole	Not evaluated	10 mg trimethoprim/50 mg sulfamethoxazole/kg/day divided into 2 doses for 5 days; maximal doses 320 mg trimethoprim and 1.6 g sulfamethoxazole daily
Ampicillin	Not evaluated	50 mg/kg/day divided into four doses for 3 days; maximal dose 2 g/day
Furazolidone	7 mg/kg; maximal dose 300 mg†	5 mg/kg/day divided into four doses for 3 days; maximal dose 400 mg/day

*Antimicrobial therapy is an adjunct to fluid therapy for cholera and is not an essential component. It reduces diarrhea volume and duration, however, by approximately 50 percent. The choice of antimicrobial agent is determined by the susceptibility pattern of local strains of *Vibrio cholerae* O1 or O139. Resistance to all agents has been reported and is common in some areas.
†Single-dose therapy with these drugs has not been evaluated systematically in children, and recommendations are extrapolated from experience in adults.
‡The fluoroquinolones, such as ciprofloxacin, are only approved for use in children <18 years old in the United States for special indications because of concerns about arthropathy; multi-dose therapy has not been evaluated systematically in children, and recommendations are extrapolated from experience in adults.

PREVENTION

Because contaminated food and water are the main routes of transmission of pathogenic *V. cholerae*, strategies to improve access to safe water sources and a clean food supply are the best methods for prevention. Proper sanitation methods also play a key role. During outbreaks, measures to increase awareness of cholera transmission and means to decrease spread should be communicated to the public. These measures include proper hand washing (especially after defecating), proper cooking of food, and identifying ill individuals so they can be treated.

Vaccination is another method of preventing cholera. Whole-cell, inactivated parenteral vaccines have been superseded by inactivated oral vaccines and are currently the only cholera vaccines commercially available and are used mostly as travel vaccines. The whole-cell plus B subunit vaccine (WC-rBS; Dukoral) consists of approximately 1×10^{11} inactivated whole cells of the classic and El Tor biotypes of *V. cholerae* O1, serotypes Inaba and Ogawa with 1 mg of recombinant cholera toxin B subunit. The vaccine is prequalified by the WHO and approved for children older than 24 months of age. It is supplied as 3-mL single-dose vials, each provided with a sachet of sodium bicarbonate buffer. The buffer solution is prepared by dissolving the sachet in 150 mL of water immediately before consumption. The vaccine must be refrigerated during shipping. Two doses administered at least 15 days apart are recommended for adults and three doses for children younger than 5 years of age. A large field trial in Matlab, Bangladesh showed protective efficacy of approximately 85 percent during the first 6 months after vaccination among women and children over the age of 2 years,[18] but this protection was not sustained in children younger than 5 years.[19] In Beira, Mozambique, one or more doses of WC-rBS vaccine during a mass campaign protected nearly 80 percent of children and adults against culture-confirmed cholera in a setting where the human immunodeficiency virus seroprevalence rate is 20 to 30 percent.[54] All confirmed cases were due to an O1 El Tor Ogawa variant strain expressing the classic cholera toxin, indicating that the vaccine also provided protection against these newer strains of cholera.[21] The WC-rBS vaccine also may provide herd immunity in endemic settings.[2]

Two closely related oral vaccines are also available (mORCVAX and Shanchol). These killed bivalent (O1 and O139) vaccines do not contain the B subunit, do not require an oral buffer, and appear safe and immunogenic in trials conducted in both Vietnam and India.[3,55] Protective efficacy after two doses was about 60 percent and remained near 50 percent 3 to 5 years after vaccination.[84] The Vietnamese vaccine (mORCVAX) is licensed and used nationally. The Indian vaccine (Shanchol) is prequalified by the WHO and licensed for use in adults and children older than 1 year of age. Two doses are administered in a 2-week interval, with a booster dose recommended 2 years after primary vaccination.[21]

The live, attenuated cholera vaccine, CVD 103-HgR, is genetically engineered from an O1 classic Inaba strain by deletion of the *ctxA* subunit and insertion of a marker

mercury ion receptor gene into the chromosomal *hlyA* locus. Protection of 100 percent was seen against the homologous strain in challenge studies of North American volunteers and lasts at least 6 months.[85] It was previously licensed in the 1990s, but subsequently did not demonstrate protection in children or adults living in an endemic area,[75] potentially as a result of both direct and indirect protection.[2] In an outbreak setting, however, a single dose provided 79 percent protection.[14] Although CVD 103-HgR is not currently available, the U.S. Food and Drug Administration accepted an investigational new drug application to begin additional clinical trials in North America. Several other candidate oral cholera vaccines, mostly live, attenuated, are under development, including Peru-15, a genetically engineered strain derived from an O1 El Tor Inaba strain.[21]

Cholera vaccines should be used in conjunction with other prevention and control strategies in areas where the disease is endemic and considered in areas at risk for outbreaks.[98] The impact of reactive vaccination in response to outbreaks is less clear, although recent mathematical models predict a substantial benefit with prompt institution.[21]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Arifuzzaman M, Ahmed T, Rahman MA, et al. Individuals with Le(a+b–) blood group have increased susceptibility to symptomatic vibrio cholerae O1 infection. *PLoS Negl Trop Dis* 2011; **5**:e1413.
10. Bharati K, Ganguly NK. Cholera toxin: a paradigm of a multifunctional protein. *Indian J Med Res* 2011;**133**:179–87.
12. Bradley ES, Bodi K, Ismail AM, et al. A genome-wide approach to discovery of small RNAs involved in regulation of virulence in *Vibrio cholerae*. *PLoS Pathog* 2011;**7**:e1002126.
16. Chatterjee T, Mukherjee D, Dey S, et al. Accessory cholera enterotoxin, Ace, from *Vibrio cholerae:* structure, unfolding, and virstatin binding. *Biochemistry* 2011;**50**:2962–72.
17. Chin CS, Sorenson J, Harris JB, et al. The origin of the Haitian cholera outbreak strain. *N Engl J Med* 2011;**364**:33–42.
21. Desai SN, Clemens JD. An overview of cholera vaccines and their public health implications. *Curr Opin Pediatr* 2012;**24**:85–91.
23. Farmer P, Almazor CP, Bahnsen ET, et al. Meeting cholera's challenge to Haiti and the world: a joint statement on cholera prevention and care. *PLoS Negl Trop Dis* 2011;**5**:e1145.
29. Ghose AC. Lessons from cholera & *Vibrio cholerae*. *Indian J Med Res* 2011;**133**:164–70.
30. Ghosh A, Ramamurthy T. Antimicrobials & cholera: are we stranded? *Indian J Med Res* 2011;**133**:225–31.
33. Guimaraes CP, Carette JE, Varadarajan M, et al. Identification of host cell factors required for intoxication through use of modified cholera toxin. *J Cell Biol* 2011;**195**:751–64.
37. Harris JB, Podolsky MJ, Bhuiyan TR, et al. Immunologic responses to *Vibrio cholerae* in patients co-infected with intestinal parasites in Bangladesh. *PLoS Negl Trop Dis* 2009;**3**:e403.
39. Heggelund JE, Haugen E, Lygren B, et al. Both El Tor and classical cholera toxin bind blood group determinants. *Biochem Biophys Res Commun* 2012;**418**:731–5.
42. Holmgren J, Kaper JB. Oral cholera vaccines. In: Levine MM, Dougan G, Good MF, et al, editors. *New Generation Vaccines*. 4th ed. New York: Informa Healthcare; 2010. p. 506–15.
45. Jutla AS, Akanda AS, Islam S. Tracking cholera in coastal regions using satellite observations. *J Am Water Resour Assoc* 2010;**46**: 651–62.
48. Kaushik JS, Gupta P, Faridi MM, et al. Single dose azithromycin versus ciprofloxacin for cholera in children: a randomized controlled trial. *Indian Pediatr* 2010;**47**:309–15.
50. Kitaoka M, Miyata ST, Unterweger D, et al. Antibiotic resistance mechanisms of *Vibrio cholerae*. *J Med Microbiol* 2011;**60**:397–407.
51. Larocque RC, Sabeti P, Duggal P, et al. A variant in long palate, lung and nasal epithelium clone 1 is associated with cholera in a Bangladeshi population. *Genes Immun* 2009;**10**:267–72.
55. Mahalanabis D, Lopez AL, Sur D, et al. A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. *PLoS One* 2008;**3**:e2323.
59. Mohammadi-Barzelighi H, Bakhshi B, Rastegar Lari A, et al. Characterization of pathogenicity island prophage in clinical and environmental strains of *Vibrio cholerae*. *J Med Microbiol* 2011;**60**: 1742–9.
67. Nelson EJ, Nelson DS, Salam MA, et al. Antibiotics for both moderate and severe cholera. *N Engl J Med* 2011;**364**:5–7.
68. Olvera-Gomez I, Hamilton SE, Xiao Z, et al. Cholera toxin activates nonconventional adjuvant pathways that induce protective CD8 T-cell responses after epicutaneous vaccination. *Proc Natl Acad Sci U S A* 2012;**109**:2072–7.
70. Ministère de la Santé Publique et de la Population. *Rapports journaliers du MSPP sur l'évolution du choléra en Haiti (in French). 25* December 2011. http://www.mspp.gouv.ht/site/downloads/Rapport%20journalier%20MSPP%20du%2025%20decembre%202011.pdf.
71. Rahman KM, Duggal P, Harris JB, et al. Familial aggregation of *Vibrio cholerae*-associated infection in Matlab, Bangladesh. *J Health Popul Nutr* 2009;**27**:733–8.
73. Reiner RC Jr, King AA, Emch M, et al. Highly localized sensitivity to climate forcing drives endemic cholera in a megacity. *Proc Natl Acad Sci U S A* 2012;**109**:2033–6.
74. Reyburn R, Kim DR, Emch M, et al. Climate variability and the outbreaks of cholera in Zanzibar, East Africa: a time series analysis. *Am J Trop Med Hyg* 2011;**84**:862–9.
76. Roy SK, Hossain MJ, Khatun W, et al. Zinc supplementation in children with cholera in Bangladesh: randomised controlled trial. *BMJ* 2008;**336**:266–8.
78. Safa A, Nair GB, Kong RY. Evolution of new variants of *Vibrio cholerae* O1. *Trends Microbiol* 2010;**18**:46–54.
84. Sur D, Kanungo S, Sah B, et al. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. *PLoS Negl Trop Dis* 2011;**5**:e1289.
86. Talavera A, Perez EM. Is cholera disease associated with poverty? *J Infect Dev Ctries* 2009;**3**:408–11.
90. Walia K, Ganguly N. Toxins of *Vibrio cholerae* and their role in inflammation, pathogenesis and immunomodulation. In: Ramamurthy T, Bhattacharya SK, editors. *Epidemiological and Molecular Aspects on Cholera*. New York: Springer Science+Business Media; 2011. p. 259–75.
94. World Health Organization. Cholera vaccines: WHO position paper. *Wkly Epidemiol Rec* 2010;**85**:117–28.
95. World Health Organization. *Global alert and response: cholera*. http://www.who.int/csr/don/archive/disease/cholera/en/.
97. World Health Organization. *First steps for managing an outbreak of acute diarrhoea*. 2009. http://www.who.int/topics/cholera/publications/first_steps/en/.
98. World Health Organization. Cholera, 2010. *Wkly Epidemiol Rec* 2011;325–40.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Albert MJ. *Vibrio cholerae* O139 Bengal. *J Clin Microbiol* 1994;**32**:2345–9.
2. Ali M, Emch M, von Seidlein L, et al. Herd immunity conferred by killed oral cholera vaccines in Bangladesh: a reanalysis. *Lancet* 2005;**366**:44–9.
3. Anh DD, Canh do G, Lopez AL, et al. Safety and immunogenicity of a reformulated Vietnamese bivalent killed, whole-cell, oral cholera vaccine in adults. *Vaccine* 2007;**25**:1149–55.
4. Arifuzzaman M, Ahmed T, Rahman MA, et al. Individuals with Le(a+b–) blood group have increased susceptibility to symptomatic vibrio cholerae O1 infection. *PLoS Negl Trop Dis* 2011;**5**:e1413.
5. Baranwal S, Dey K, Ramamurthy T, et al. Role of active efflux in association with target gene mutations in fluoroquinolone resistance in clinical isolates of *Vibrio cholerae*. *Antimicrob Agents Chemother* 2002;**46**:2676–8.
6. Barua D. History of cholera. In: Barua D, Greenhough WG III, editors. *Cholera*. New York, London: Plenum; 1992. p. 1–36.
7. Benenson AS, Islam MR, Greenough WB 3rd. Rapid identification of *Vibrio cholerae* by darkfield microscopy. *Bull World Health Organ* 1964;**30**:827–31.
8. Bennish ML. Cholera: Pathophysiology, clinical features and treatment. In: Wachsmuth IK, Blake PA, Olsvik O, editors: *Vibrio cholerae and Cholera: Molecular to Global Perspectives*. Washington, D.C.: American Society of Microbiology; 1994. p. 229–55.
9. Beyhan S, Tischler AD, Camilli A, et al. Differences in gene expression between the classical and El Tor biotypes of *Vibrio cholerae* O1. *Infect Immun* 2006;**74**:3633–42.
10. Bharati K, Ganguly NK. Cholera toxin: a paradigm of a multifunctional protein. *Indian J Med Res* 2011;**133**:179–87.
11. Bhuiyan NA, Qadri F, Faruque AS, et al. Use of dipsticks for rapid diagnosis of cholera caused by Vibrio cholerae O1 and O139 from rectal swabs. *J Clin Microbiol* 2003;**41**:3939–41.
12. Bradley ES, Bodi K, Ismail AM, et al. A genome-wide approach to discovery of small RNAs involved in regulation of virulence in *Vibrio cholerae*. *PLoS Pathog* 2011;**7**:e1002126.
13. Butler SM, Camilli A. Going against the grain: chemotaxis and infection in *Vibrio cholerae*. *Nat Rev Microbiol* 2005;**3**:611–20.
14. Butler SM, Nelson EJ, Chowdhury N, et al. Cholera stool bacteria repress chemotaxis to increase infectivity. *Mol Microbiol* 2006;**60**:417–26.
15. Cash RA, Music SI, Libonati JP, et al. Response of man to infection with *Vibrio cholerae*. I. Clinical, serologic, and bacteriologic responses to a known inoculum. *J Infect Dis* 1974;**129**:45–52.
16. Chatterjee T, Mukherjee D, Dey S, et al. Accessory cholera enterotoxin, Ace, from *Vibrio cholerae:* structure, unfolding, and virstatin binding. *Biochemistry* 2011;**50**:2962–72.
17. Chin CS, Sorenson J, Harris JB, et al. The origin of the Haitian cholera outbreak strain. *N Engl J Med* 2011;**364**:33–42.
18. Clemens JD, Sack DA, Harris JR, et al. Field trial of oral cholera vaccines in Bangladesh. *Lancet* 1986;**2**:124–7.
19. Clemens JD, Sack DA, Harris JR, et al. Field trial of oral cholera vaccines in Bangladesh: results from three-year follow-up. *Lancet* 1990;**335**:270–3.
20. Colwell RR, Seidler RJ, Kaper J, et al. Occurrence of *Vibrio cholerae* serotype O1 in Maryland and Louisiana estuaries. *Appl Environ Microbiol* 1981;**41**:555–8.
21. Desai SN, Clemens JD. An overview of cholera vaccines and their public health implications. *Curr Opin Pediatr* 2012;**24**:85–91.
22. Dziejman M, Balon E, Boyd D, et al. Comparative genomic analysis of *Vibrio cholerae:* genes that correlate with cholera endemic and pandemic disease. *Proc Natl Acad Sci U S A* 2002;**99**:1556–61.
23. Farmer P, Almazor CP, Bahnsen ET, et al. Meeting cholera's challenge to Haiti and the world: a joint statement on cholera prevention and care. *PLoS Negl Trop Dis* 2011;**5**:e1145.
24. Faruque SM, Biswas K, Udden SM, et al. Transmissibility of cholera: in vivo-formed biofilms and their relationship to infectivity and persistence in the environment. *Proc Natl Acad Sci U S A* 2006;**103**:6350–5.
25. Faruque SM, Islam MJ, Ahmad QS, et al. Self-limiting nature of seasonal cholera epidemics: role of host-mediated amplification of phage. *Proc Natl Acad Sci U S A* 2005;**102**:6119–24.
26. Faruque SM, Naser IB, Islam MJ, et al. Seasonal epidemics of cholera inversely correlate with the prevalence of environmental cholera phages. *Proc Natl Acad Sci U S A* 2005;**102**:1702–7.
27. Faruque SM, Sack DA, Sack RB, et al. Emergence and evolution of *Vibrio cholerae* O139. *Proc Natl Acad Sci U S A* 2003;**100**:1304–9.
28. Fasano A. Intestinal zonulin: open sesame! *Gut* 2001;**49**:159–62.
29. Ghose AC. Lessons from cholera & Vibrio cholerae. *Indian J Med Res* 2011;**133**:164–70.
30. Ghosh A, Ramamurthy T. Antimicrobials & cholera: are we stranded? *Indian J Med Res* 2011;**133**:225–31.
31. Glass RI, Svennerholm AM, Stoll BJ, et al. Protection against cholera in breast-fed children by antibodies in breast milk. *N Engl J Med* 1983;**308**:1389–92.
32. Greenough WB 3rd, Gordon RS Jr, Rosenberg IS, et al. Tetracycline in the treatment of cholera. *Lancet* 1964;**1**:355–7.
33. Guimaraes CP, Carette JE, Varadarajan M, et al. Identification of host cell factors required for intoxication through use of modified cholera toxin. *J Cell Biol* 2011;**195**:751–64.
34. Hahn S, Kim S, Garner P. Reduced osmolarity oral rehydration solution for treating dehydration caused by acute diarrhoea in children. *Cochrane Database Syst Rev* 2002;CD002847.
35. Hall RM, Collis CM. Mobile gene cassettes and integrons: capture and spread of genes by site-specific recombination. *Mol Microbiol* 1995;**15**:593–600.
36. Harris JB, Khan AI, LaRocque RC, et al. Blood group, immunity, and risk of infection with *Vibrio cholerae* in an area of endemicity. *Infect Immun* 2005;**73**:7422–7.
37. Harris JB, Podolsky MJ, Bhuiyan TR, et al. Immunologic responses to *Vibrio cholerae* in patients co-infected with intestinal parasites in Bangladesh. *PLoS Negl Trop Dis* 2009;**3**:e403.
38. Harvey RM, Enson Y, Lewis ML, et al. Hemodynamic studies on cholera: effects of hypovolemia and acidosis. *Circulation* 1968;**37**:709–28.
39. Heggelund JE, Haugen E, Lygren B, et al. Both El Tor and classical cholera toxin bind blood group determinants. *Biochem Biophys Res Commun* 2012;**418**:731–5.
40. Heidelberg JF, Eisen JA, Nelson WC, et al. DNA sequence of both chromosomes of the cholera pathogen *Vibrio cholerae*. *Nature* 2000;**406**:477–83.
41. Hirschhorn N. The treatment of acute diarrhea in children: an historical and physiological perspective. *Am J Clin Nutr* 1980;**33**:637–63.
42. Holmgren J, Kaper JB. Oral cholera vaccines. In: Levine MM, Dougan G, Good MF, et al, editors. *New Generation Vaccines*. 4th ed. New York: Informa Healthcare; 2010. p. 506–15.
43. Hoshino K, Yamasaki S, Mukhopadhyay AK, et al. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139. *FEMS Immunol Med Microbiol* 1998;**20**:201–7.
44. Huq A, Colwell RR, Rahman R, et al. Detection of *Vibrio cholerae* O1 in the aquatic environment by fluorescent-monoclonal antibody and culture methods. *Appl Environ Microbiol* 1990;**56**:2370–3.
45. Jutla AS, Akanda AS, Islam S. Tracking cholera in coastal regions using satellite observations. *J Am Water Resour Assoc* 2010;**46**:651–62.
46. Kabir I, Khan WA, Haider R, et al. Erythromycin and trimethoprim-sulphamethoxazole in the treatment of cholera in children. *J Diarrhoeal Dis Res* 1996;**14**:243–7.
47. Kaper JB, Morris JG Jr, Levine MM. Cholera. *Clin Microbiol Rev* 1995;**8**:48–86.
48. Kaushik JS, Gupta P, Faridi MM, et al. Single dose azithromycin versus ciprofloxacin for cholera in children: a randomized controlled trial. *Indian Pediatr* 2010;**47**:309–15.
49. Khan WA, Saha D, Rahman A, et al. Comparison of single-dose azithromycin and 12-dose, 3-day erythromycin for childhood cholera: a randomised, double-blind trial. *Lancet* 2002;**360**:1722–7.
50. Kitaoka M, Miyata ST, Unterweger D, et al. Antibiotic resistance mechanisms of *Vibrio cholerae*. *J Med Microbiol* 2011;**60**:397–407.
51. Larocque RC, Sabeti P, Duggal P, et al. A variant in long palate, lung and nasal epithelium clone 1 is associated with cholera in a Bangladeshi population. *Genes Immun* 2009;**10**:267–72.
52. Levine MM, Nalin DR, Rennels MB, et al. Genetic susceptibility to cholera. *Ann Hum Biol* 1979;**6**:369–74.
53. Lindenbaum J, Greenough WB, Islam MR. Antibiotic therapy of cholera. *Bull World Health Organ* 1967;**36**:871–83.
54. Lucas ME, Deen JL, von Seidlein L, et al. Effectiveness of mass oral cholera vaccination in Beira, Mozambique. *N Engl J Med* 2005;**352**:757–67.

55. Mahalanabis D, Lopez AL, Sur D, et al. A randomized, placebo-controlled trial of the bivalent killed, whole-cell, oral cholera vaccine in adults and children in a cholera endemic area in Kolkata, India. *PLoS One* 2008;**3**:e2323.
56. Matson JS, Withey JH, DiRita VJ. Regulatory networks controlling *Vibrio cholerae* virulence gene expression. *Infect Immun* 2007;**75**: 5542–9.
57. Merrell DS, Butler SM, Qadri F, et al. Host-induced epidemic spread of the cholera bacterium. *Nature* 2002;**417**:642–5.
58. Miyagi K, Matsumoto Y, Hayashi K, et al. Successful application of enzyme-labeled oligonucleotide probe for rapid and accurate cholera diagnosis in a clinical laboratory. *Microbiol Immunol* 1994;**38**:301–4.
59. Mohammadi-Barzelighi H, Bakhshi B, Rastegar Lari A, et al. Characterization of pathogenicity island prophage in clinical and environmental strains of *Vibrio cholerae*. *J Med Microbiol* 2011; **60**:1742–9.
60. Molla AM, Rahman M, Sarker SA, et al. Stool electrolyte content and purging rates in diarrhea caused by rotavirus, enterotoxigenic *E. coli*, and *V. cholerae* in children. *J Pediatr* 1981;**98**: 835–8.
61. Molla AM, Sarker SA, Hossain M, et al. Rice-powder electrolyte solution as oral-therapy in diarrhoea due to *Vibrio cholerae* and *Escherichia coli*. *Lancet* 1982;**1**:1317–19.
62. Moseley SL, Falkow S. Nucleotide sequence homology between the heat-labile enterotoxin gene of *Escherichia coli* and *Vibrio cholerae* deoxyribonucleic acid. *J Bacteriol* 1980;**144**:444–6.
63. Mosley WH, Alvero MG, Joseph PR, et al. Studies of cholera El Tor in the Philippines. IV. Transmission of infection among neighbourhood and community contacts of cholera patients. *Bull World Health Organ* 1965;**33**:651–60.
64. Nalin DR, Levine MM, Hornick RB, et al. The problem of emesis during oral glucose-electrolytes therapy given from the onset of severe cholera. *Trans R Soc Trop Med Hyg* 1979;**73**:10–14.
65. Nalin DR, Levine MM, Rhead J, et al. Cannabis, hypochlorhydria, and cholera. *Lancet* 1978;**2**:859–62.
66. Nato F, Boutonnier A, Rajerison M, et al. One-step immunochromatographic dipstick tests for rapid detection of *Vibrio cholerae* O1 and O139 in stool samples. *Clin Diagn Lab Immunol* 2003;**10**: 476–8.
67. Nelson EJ, Nelson DS, Salam MA, et al. Antibiotics for both moderate and severe cholera. *N Engl J Med* 2011;**364**:5–7.
68. Olvera-Gomez I, Hamilton SE, Xiao Z, et al. Cholera toxin activates nonconventional adjuvant pathways that induce protective CD8 T-cell responses after epicutaneous vaccination. *Proc Natl Acad Sci U S A* 2012;**109**:2072–7.
69. Palmer DL, Koster FT, Islam AF, et al. Comparison of sucrose and glucose in the oral electrolyte therapy of cholera and other severe diarrheas. *N Engl J Med* 1977;**297**:1107–10.
70. Ministère de la Santé Publique et de la Population. *Rapports journaliers du MSPP sur l'évolution du choléra en Haiti (in French)*. 25 December 2011. http://www.mspp.gouv.ht/site/downloads/Rapport%20journalier%20MSPP%20du%2025%20decembre%202011.pdf.
71. Rahman KM, Duggal P, Harris JB, et al. Familial aggregation of *Vibrio cholerae*-associated infection in Matlab, Bangladesh. *J Health Popul Nutr* 2009;**27**:733–8.
72. Rahman O, Bennish ML, Alam AN, et al. Rapid intravenous rehydration by means of a single polyelectrolyte solution with or without dextrose. *J Pediatr* 1988;**113**:654–60.
73. Reiner RC Jr, King AA, Emch M, et al. Highly localized sensitivity to climate forcing drives endemic cholera in a megacity. *Proc Natl Acad Sci U S A* 2012;**109**:2033–6.
74. Reyburn R, Kim DR, Emch M, et al. Climate variability and the outbreaks of cholera in Zanzibar, East Africa: a time series analysis. *Am J Trop Med Hyg* 2011;**84**:862–9.
75. Richie EE, Punjabi NH, Sidharta YY, et al. Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. *Vaccine* 2000;**18**:2399–410.
76. Roy SK, Hossain MJ, Khatun W, et al. Zinc supplementation in children with cholera in Bangladesh: randomised controlled trial. *BMJ* 2008;**336**:266–8.
77. Sack DA, Sack RB, Nair GB, et al. Cholera. *Lancet* 2004;**363**: 223–33.
78. Safa A, Nair GB, Kong RY. Evolution of new variants of *Vibrio cholerae* O1. *Trends Microbiol* 2010;**18**:46–54.
79. Saha D, Khan WA, Karim MM, et al. Single-dose ciprofloxacin versus 12-dose erythromycin for childhood cholera: a randomised controlled trial. *Lancet* 2005;**366**:1085–93.
80. Sanchez JL, Vasquez B, Begue RE, et al. Protective efficacy of oral whole-cell/recombinant-B-subunit cholera vaccine in Peruvian military recruits. *Lancet* 1994;**344**:1273–6.
81. Sanyal SC, Singh SJ, Tiwari IC, et al. Role of household animals in maintenance of cholera infection in a community. *J Infect Dis* 1974;**130**:575–9.
82. Silva AJ, Leitch GJ, Camilli A, et al. Contribution of hemagglutinin/protease and motility to the pathogenesis of El Tor biotype cholera. *Infect Immun* 2006;**74**:2072–9.
83. Sur D, Deen JL, Manna B, et al. The burden of cholera in the slums of Kolkata, India: data from a prospective, community based study. *Arch Dis Child* 2005;**90**:1175–81.
84. Sur D, Kanungo S, Sah B, et al. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. *PLoS Negl Trop Dis* 2011;**5**:e1289.
85. Tacket CO, Losonsky G, Nataro JP, et al. Onset and duration of protective immunity in challenged volunteers after vaccination with live oral cholera vaccine CVD 103-HgR. *J Infect Dis* 1992; **166**:837–41.
86. Talavera A, Perez EM. Is cholera disease associated with poverty? *J Infect Dev Ctries* 2009;**3**:408–11.
87. Tamplin ML, Gauzens AL, Huq A, et al. Attachment of *Vibrio cholerae* serogroup O1 to zooplankton and phytoplankton of Bangladesh waters. *Appl Environ Microbiol* 1990;**56**:1977–80.
88. Thungapathra M, Amita, Sinha KK, et al. Occurrence of antibiotic resistance gene cassettes aac(6′)-Ib, dfrA5, dfrA12, and ereA2 in class I integrons in non-O1, non-O139 *Vibrio cholerae* strains in India. *Antimicrob Agents Chemother* 2002;**46**:2948–55.
89. Varela P, Pollevick GD, Rivas M, et al. Direct detection of *Vibrio cholerae* in stool samples. *J Clin Microbiol* 1994;**32**:1246–8.
90. Walia K, Ganguly N. Toxins of *Vibrio cholerae* and their role in inflammation, pathogenesis and immunomodulation. In: Ramamurthy T, Bhattacharya S, editors. *Epidemiological and Molecular Aspects on Cholera*. New York: Spring Science+Business Media; 2011. p. 259–75.
91. Wang F, Butler T, Rabbani GH, et al. The acidosis of cholera: contributions of hyperproteinemia, lactic acidemia, and hyperphosphatemia to an increased serum anion gap. *N Engl J Med* 1986; **315**:1591–5.
92. Wang XY, Ansaruzzaman M, Vaz R, et al. Field evaluation of a rapid immunochromatographic dipstick test for the diagnosis of cholera in a high-risk population. *BMC Infect Dis* 2006;**6**:17.
93. Watten RH, Morgan FM, Yachai Na S, et al. Water and electrolyte studies in cholera. *J Clin Invest* 1959;**38**:1879–89.
94. World health Organization. Cholera vaccines: WHO position paper. *Wkly Epidemiol Rec* 2010;**85**:117–28.
95. World Health Organization. *Global alert and response: cholera*. http://www.who.int/csr/don/archive/disease/cholera/en/.
96. World Health Organization. *Programme for Control of Diarrhoeal Disease: Guidelines for Cholera Control*. Geneva: World Health Organization; 1991.
97. World Health Organization. *First steps for managing an outbreak of acute diarrhoea*. 2009. http://www.who.int/topics/cholera/publications/first_steps/en/.
98. World Health Organization. Cholera, 2010. *Wkly Epidemiol Rec* 2011;325–40.
99. Zaman K, Yunus M, Rahman A, et al. Efficacy of a packaged rice oral rehydration solution among children with cholera and cholera-like illness. *Acta Paediatr* 2001;**90**:505–10.

CHAPTER 118

Vibrio parahaemolyticus

David Y. Hyun

Vibrio parahaemolyticus is recognized worldwide as a cause of foodborne disease associated with consumption of seafood, in particular crustaceans and mollusks, and occasionally as a cause of wound infections and sepsis in immunocompromised hosts. *V. parahaemolyticus* was isolated first in Japan during a foodborne outbreak in 1951. Almost 10 years after it was first described, the organism was classified correctly in the genus *Vibrio*,[9] and its distribution in coastal waters and estuaries of temperate climates around the world was recognized. The regional differences in the incidence of this disease are related to patterns of consumption of marine products and practices of food preparation. In Japan and Taiwan, where fish and shellfish are a major source of dietary protein and frequently consumed raw, *V. parahaemolyticus* accounts for 10 to 40 percent of foodborne outbreaks.[2,66] In the United States, the first confirmed foodborne outbreak occurred in Maryland in 1971, secondary to consumption of contaminated crabmeat.[22] Since then, sporadic outbreaks have been reported,[6,7,14,22,24] showing the potential risk for development of infectious and toxic syndromes from marine products in the United States.[29]

BACTERIOLOGY

V. parahaemolyticus is a gram-negative, non–spore-forming, curved rod with rounded ends that possess a polar flagellum.[54] On surfaces or in viscous environments, *V. parahaemolyticus* can differentiate into swarmer cells equipped with additional shorter lateral peritrichous flagella.[53] *V. parahaemolyticus* is a facultative anaerobe with respiratory and fermentative metabolisms. Similar to most vibrios, it is an oxidase-positive nonfermenter of lactose.[8] It is arginine dihydrolase–negative and ornithine decarboxylase–positive. The requirement of sodium and enhancement of growth in a specific range of concentration differentiate it from *Vibrio cholerae* and *Vibrio mimicus*. The optimal sodium concentration range is 2 to 4 percent; a concentration greater than 8 percent inhibits its growth.[82]

V. parahaemolyticus produces round, blue-green colonies on the widely used *Vibrio*-selective thiosulfate citrate bile salt sucrose (TCBS) agar. This medium inhibits most fecal flora by the presence of bile salts and a highly alkaline pH. Direct plating on TCBS agar may be used for feces and other clinical specimens, but food samples that are not heavily contaminated require enrichment either in alkaline peptone water supplemented with 3 percent sodium chloride or in a strongly selective medium, such as Glucose Salt Teepol Broth.[82] The optimal pH for growth is in the neutral range, but *V. parahaemolyticus* can survive in alkaline media. Optimal growth temperatures are between 35° and 39°C (95° to 102.2°F).[86] Growth is remarkable in conventional media and foodstuff, with generation times of only 8 minutes[43]; a significant number of organisms can be found after a short period of inappropriate storage. Growth is inhibited at temperatures of 44°C (111.2°F) or greater. The degree of inactivation is greater with increased temperatures; a million-fold decline occurs in viable bacteria in shrimp homogenate kept at 100°C (212°F) for only 1 minute.[80] In laboratory conditions with low temperature and nutrient supply, *V. parahaemolyticus* has been shown to enter a viable but nonculturable state, during which the organism no longer can be cultured on routine growth media, but its viability can be confirmed using direct microscopic assays.[31,42,84]

The genome of *V. parahaemolyticus* consists of two circular chromosomes. Genes for a type III secretion system (TTSS) were identified in a pathogenicity island on chromosome 2.[51] Two types of nonredundant TTSS have been identified.[63] TTSS, which is considered a central virulence factor in salmonellae and shigellae, possesses needle-like structures that facilitate direct injections of bacterial proteins into a target host cell and is related strongly to the pathogenicity of inflammatory diarrhea seen with *V. parahaemolyticus* infections. This finding suggests that the presence of TTSS in *V. parahaemolyticus* differentiates its pathogenicity from that of *V. cholerae*, which does not carry the genes.[51]

EPIDEMIOLOGY

In the United States, *V. parahaemolyticus* is found along the East, West, and Gulf coasts.[25,43] Water, sediments, suspended particulates, plankton, fish, and shellfish have been shown to harbor the organism.[5,56] The organisms are present in highest numbers in water at temperatures between 17° and 35°C (62.6° to 95°F) and containing 0.5 to 2.5 percent salinity.[41] Marked seasonal and geographic variations occur, with maximal mean concentrations occurring during late summer and spring along the Gulf Coast.[25] The seasonal distribution correlates with outbreaks, predominantly between June and October.[4]

Finfish and all types of shellfish products, including oysters, clams, crabs, and shrimp may be involved in the transmission of the infection (Table 118-1).[41] The risk for acquiring infection is higher with molluscan shellfish because of their ability to concentrate contaminants and bacteria by filter feeding.[5,31,86] In oysters, the density of

TABLE 118-1 **Selected Outbreaks of *Vibrio Parahaemolyticus* Infection**

Setting	Persons Ill/ Exposed	Symptoms (%)	Incubation	Resolution	Medical Attention/ Hospitalization (%/%)	Vehicle	Risk Factor
Picnic[19]	351/631 (56)	D (95), C (82), N (68), V (61), HA (41), F (27)	16 hr (4-42 hr)	4 days (<1-10 days)	60/2	Steamed crab	Cross-contamination, unrefrigerated food
Chronic hospital[19]	24/100 (24)	D (100), C (89), N (72), V (44), HA (56), F (33)	18 hr (4-60 hr)	3 days (<1-7 days)	No data available	Crab salad	No data available
Shrimp boil[57]	~600/1200 (50)	D, C, V, HA, F	23 hr (5-92 hr)	(few hr-1 wk)	1/0	Boiled shrimp	Storage temperature
International flight[51]	12/134 (9)	D, C, V	(8-20 hr)	No data available	40/25	Cooked crab	Cross-contamination
Parish dinner[12]	~1000/1700 (59)	D (95), C (92), N (72), HA (47), F (47), V (12)	16 hr (3-76 hr)	4.6 days (<1-8 days)	26/7.4	Shrimp	Cross-contamination, storage temperature
Pacific Northwest[55]	209 ill	D (99), C (88), N (52), V (39), F (33)	15 hr (4-96 hr)	3 days mean	2 hospitalized, 1 death	Raw oysters	Uncooked oysters

C, Cramps; *D,* diarrhea; *F,* fever; *HA,* headache; *N,* nausea; *V,* vomiting.

V. parahaemolyticus may be 100 times greater than in the surrounding water.[25] The levels of contamination of seafood generally are low in freshly collected oysters, with the highest mean density of only 160 bacteria/g found in the United States.[25] In Japan, higher counts (up to 10^3 to 10^4/g) have been reported in market shellfish.[18] The minimum infective dose is thought to be in the range of 10^5 to 10^7 organisms, based on volunteer feeding studies.[27] More recent outbreaks of illness caused by oysters that met bacteriologic safety standards have raised questions about current recommendations, however.[23]

During the period 1973 to 1987, only 23/1869 foodborne disease outbreaks with known bacterial etiology reported to the Centers for Disease Control and Prevention (CDC) were caused by *V. parahaemolyticus*.[6] In 18 of these outbreaks, a shellfish was recognized as the food vehicle. No fatal cases were reported. Contributing factors included inadequate cooking (92%), improper holding temperature (75%), and food acquired from an unsafe source (75%).[6,7] Most of the affected individuals were adults, and no patterns of unusual susceptibility by age group or gender were noted. No evidence of secondary spread was found among family members. Between 1988 and 1997, 345 sporadic cases were reported to the CDC. Most infections were related to eating raw oysters. Most of these infections (59%) were gastroenteritis.[24] In the early summer of 2006, outbreaks of *V. parahaemolyticus* associated with consumption of contaminated shellfish harvested from a common area were reported across states of New York, Oregon, and Washington. Seventy-eight percent of the isolates tested had a subtype of O4:K12.[16]

Until more recently, *V. parahaemolyticus* infections were causally associated with multiple serotypes and occurred as sporadic cases or localized outbreaks.[58] In 1996, a sudden increase in incidence was seen in India, and 50 to 80 percent of the cases were attributed to a single serotype, O3:K6.[64] Shortly thereafter, O3:K6 strains were isolated from clinical samples from other countries in Asia.[59] In 1998, two outbreaks of gastroenteritis in the United States were associated with O3:K6.[15,24] By 2005, this pandemic clone was found in patients from Europe, South America, and Africa,[3,35,52] and other clones that genetically diverged from O3:K6, such as O4:K68 and O1:KUT, were isolated from clinical cases.[21] Although rates may have stabilized in some regions during the late 2000s,[33] cases and outbreaks associated with these pandemic clones continue to be reported in high numbers globally.[65,83]

PATHOGENESIS

The ability of certain strains of *V. parahaemolyticus* to produce β-hemolysis on Wagatsuma agar, known as the Kanagawa phenomenon (KP), was first epidemiologically associated with human pathogenicity.[55] KP is observed in 88 to 96 percent of strains from clinical specimens and in 1 to 2 percent of strains from environmental sources.[27,43,78] Thermostable direct hemolysin (TDH) produces KP.[77] TDH has a molecular weight of 42,000, consisting of two subunits of 21,000,[56] and, in contrast to other hemolysins produced by *V. parahaemolyticus*, is not inactivated by heating at 100°C (212°F) for 10 minutes. It is a pore-forming toxin that causes hemolysis by binding to and disrupting erythrocyte membranes.[37] TDH also has been shown to have enterotoxic activities, such as causing accumulation of fluid in rabbit ileal loop assays, invading intestinal mucosa,[20,81] and increasing intracellular calcium concentrations that

trigger secretion of chloride by intestinal cells.[71,75,76] TDH is cytotoxic, lethal to small experimental animals, and cardiotoxic.[88]

Certain KP-negative isolates from outbreaks have been shown to produce TDH-related hemolysin (TRH).[39] Although TRH is heat-labile, it shares similarities with TDH, such as hemolysis, production of fluid accumulation in the rabbit ileal loop model, and induction of chloride secretion in human colonic epithelial cells.[38,69] Likewise, some variants closely related to the original TDH by molecular analysis (Vp TDH/I and Vp TDH/II) have been isolated from KP-negative strains.[57]

When the *tdh* and *trh* genes had been cloned and sequenced, researchers realized that certain strains possess the genetic material but are phenotypically incomplete. Strains with a typical hemolysin-positive phenotype carried two chromosome gene copies, whereas *tdh* gene–positive strains with weakly positive or negative hemolysin phenotype possessed only a single chromosome gene copy.[60] Fewer than 50 percent of *trh* gene–positive strains produce TRH when examined by enzyme-linked immunosorbent assay.[73] Low-level expression of the *tdh* genes may be the reason for the KP-negative phenotype.[61] The expression of the *tdh* genes is controlled by the Vp-toxRS operon. The basal production of mRNA and the degree of transcriptional activation seem to be related to differences in the nucleotide sequences and strength of the promoter region.[61]

Some studies suggest that virulence mechanisms in addition to TDH and TRH exist and that the mere presence of the *tdh* or *trh* genes does not reliably correlate with virulence of a given strain.[81,86] One study found that 5.6 percent of their clinical isolates were toxin negative by PCR.[50] *V. parahaemolyticus* requires intestinal colonization factors to cause disease. A variety of pili and other potential colonization factors are present, but the evidence is too poor to implicate any of the candidate adhesins in virulence.[18] Adherence of *V. parahaemolyticus* to intestinal epithelial cells of rabbits[19] and human small intestine[34,85] seems to require binding to complex carbohydrates or hemagglutinins of the host. Also, *V. parahaemolyticus* appears to disrupt epithelial barrier function, regardless of TDH and TRH production.[50]

The occurrence of cases with grossly bloody stools[14,40] is indirect evidence of either invasiveness or production of cytotoxin. Invasion of Caco-2 cells in culture has been shown in approximately 20 percent of strains.[1] KP-positive and KP-negative strains from clinical specimens can invade and colonize the mucosal cells of the rabbit ileum, producing acute inflammation, degeneration, and erosion of the villi.[8] Vibrios can be cultured from tissue specimens of spleen, liver, and heart in experimental animals, indicating spread through the lymphatic or circulatory systems.[11]

CLINICAL MANIFESTATIONS

The spectrum of intestinal disease varies from mild gastroenteritis to dysenteric syndrome. The incubation period typically is 15 to 24 hours (range, 4 to 96 hours)[4,74]; the variability presumably is related to the number of organisms ingested. Resolution is expected to occur in approximately 3 days,[56] but it varies from several hours to more than 10 days.[4,12] Fatigue may persist for a few days. Diarrhea (96%) and abdominal pain (95%) are the most frequent and earliest symptoms, accompanied by nausea, vomiting, and headache in 40 to 70 percent of cases. Chills and moderate fever are less frequent (20%) manifestations.[4,22] Diarrhea is watery and explosive,[13] with patients having up to 15 stools during the first day. Shock caused by loss of fluid is an exceptional event.[25] Mucus is observed frequently, but grossly bloody stool is seen less commonly.[9,40,49] Small superficial ulcerations on sigmoidoscopy may be present.[9] No difference in symptoms exists in cases associated with KP-negative strains.[37]

Extraintestinal infections occur. During the Vibrio Surveillance Program that started in 1989 in four Gulf Coast states, 18 to 34 percent of *V. parahaemolyticus* isolates were found to come from wound infections, and 3 to 5 percent were found to be associated with septicemia.[23,49] In 2009, 11 percent of isolates from the United States were obtained from wounds and 2 percent of isolates from blood cultures.[17] Wound infection occurs after contamination of skin lacerations with seawater or after direct trauma with pieces of shellfish, fishhooks, or utensils contaminated with seawater.[10] Superficial infection can extend to deeper soft tissue and in some cases lead to fatal necrotizing fasciitis.[67] Septicemia is a concern in immunocompromised patients, particularly patients with leukemia[27] and patients with liver disease.[26,36] Bacteremia may develop after either wound infections[10,27] or ingestion of seafood.[36,70] Skin bullae,[36] intravascular hemolysis,[27] and disseminated intravascular coagulation[36] may complicate wound and bacteremic infections.

COMPLICATIONS

An acute diarrhea episode requires medical attention in 25 to 50 percent of cases during an outbreak, but less frequently requires hospitalization.[12,22,49,74] Children younger than 5 years of age are more likely to require hospitalization in contrast to individuals older than 5 years.[79] Usually, no long-term sequelae occur. Severe dehydration, shock,[40,68] and death (0.04% of cases in Japan) can occur.[8,82] Primary septicemia,[47,49,70] septicemia secondary to wound infection,[10,27,47] and septicemia secondary to gastroenteritis[36,47] have higher mortality rates, especially in immunosuppressed patients or in patients with liver diseases.[10,30]

DIAGNOSIS

Epidemiologic data are the basis for the presumptive diagnosis. In a patient with compatible symptoms, a history of recent consumption of seafood should suggest this diagnosis. Leukocytosis and fecal leukocytes may be found.[9,40] A positive stool culture on selective media, such

as TCBS agar, can confirm the clinical impression. Routine use of TCBS agar is not cost-effective, even in coastal areas, unless an appropriate clinical setting is available.[10,49,54] The use of a transport medium, such as Cary-Blair, is necessary if a delay in processing the sample is expected. Isolation of more than 10^5 *V. parahaemolyticus* from epidemiologically implicated food supports the diagnosis and identifies the vehicle.[54] A correlation between the serotype of the food and isolates from patients is not always present because multiple strains can contaminate a single food.[4] Adding an enrichment broth to the processing increases the yield of isolation.

For testing blood specimens, use of routine culture media followed by selective media is appropriate.[36,70] Immunoassays (enzyme-linked immunosorbent assay, immunoprecipitation in agar medium, reversed passive latex agglutination) are available in research laboratories and commercially in Japan (KAP-RPLA, Denka Seiken, Tokyo; BT test, Nissui Pharmaceutical, Tokyo) to detect the TDH. Strains producing TRH may be detected by cross reactions.[87] Serologic methods (slide agglutination) can detect H antigens in lateral flagella.[72] Gene probe hybridization[62] and polymerase chain reaction with a sequence of a highly conserved DNA fragment[48] or targeting the *tdh* or *trh* genes can be used to detect low numbers of the organism in environmental and clinical specimens,[45,46] but these methods are usually limited to research laboratory settings. Histologic findings from duodenal and rectal biopsy samples from patients infected with *V. parahaemolyticus* were consistent with acute inflammatory response, showing epithelial degradation, polymorphonuclear neutrophil infiltration of villi and crypt cells, and hemorrhage.[69]

TREATMENT

Only supportive therapy and careful control of the fluid and electrolyte balances are required for the management of gastroenteritis. Oral rehydration usually is appropriate, although intravenous fluids may be required if massive losses of fluid occur.[9,40,68] Antibiotic therapy is unnecessary for this short-lived disease. In the unusual protracted episode, tetracycline or a fluoroquinolone may be beneficial for adults and older children.[8,9,43]

For wound infections and septicemia, antibiotics are always indicated. Patients with septicemia should be treated with a tetracycline plus a third-generation cephalosporin. *V. parahaemolyticus* usually is susceptible to tetracycline. In addition, only a few clinical strains are resistant to chloramphenicol, trimethoprim-sulfamethoxazole, third-generation cephalosporins, aztreonam, imipenem, fluoroquinolones, and aminoglycosides.[10,27,32,36] Penicillins are ineffective because of the presence of β-lactamases in 50 percent of isolates.[32,44] The older cephalosporins also have poor activity.[44]

PREVENTION AND CONTROL

Although ensuring the lack of contamination of seafood is impossible, avoiding food-handling errors should diminish the risk for infection. A recommendation for an acceptable upper limit of 100 colony-forming units per gram of *V. parahaemolyticus* in raw shrimp has been made by the International Commission on Microbiological Specification for Foods.[41] Thorough cooking eliminates the organism.[80] Heating seafood to 60°C (156.2°F) for 15 minutes[28] or boiling for 7 minutes[12] seems to be adequate to reduce the risk for infection. When undercooked or raw seafood is consumed, adequate prior refrigeration to preclude multiplication of organisms is important. During the preparation of seafood, special attention should be paid to possible cross-contamination. Use of the same utensils, board surfaces, or containers for fresh and recently cooked seafood should be avoided.[68,80] Raw seafood consumption should be discouraged, particularly for individuals at high risk for development of septicemia.[26,47,49]

NEW REFERENCES SINCE THE SIXTH EDITION

16. Centers for Disease Control and Prevention. *Vibrio parahaemolyticus* infections associated with consumption of raw shellfish: three States, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:854–6.
21. Chowdhury NR, Chakraborty S, Ramamurthy T, et al. Molecular evidence of clonal *Vibrio parahaemolyticus* pandemic strains. *Emerg Infect Dis* 2000;**6**:631–6.
30. Fernando RR, Krishnan S, Fairweather MG, et al. *Vibrio parahaemolyticus* septicaemia in a liver transplant patient: a case report. *J Med Case Rep* 2011;**6**:171.
33. Garcia K, Torres R, Uribe P, et al. Dynamics of clinical and environmental *Vibrio parahaemolyticus* strains during seafood-related summer diarrhea outbreaks in southern Chile. *Appl Environ Microbiol* 2009;**75**:7482–7.
45. Kadhim HM, Miah A, Munn CB, et al. Development of a polymerase chain reaction (PCR) test for the detection of virulent forms of *Vibrio parahaemolyticus*. *Eur J Clin Microbiol Infect Dis* 2012;**31**:431–9.
46. Kang MH, Kim IW, Lee DW, et al. Development of a rapid detection method to detect *tdh* gene in *Vibrio parahaemolyticus* using 2-step ultrarapid real-time polymerase chain reaction. *Diagn Microbiol Infect Dis* 2011;**69**:21–9.
63. Noriea NF 3rd, Johnson CN, Griffitt KJ, et al. Distribution of type III secretion systems in *Vibrio parahaemolyticus* from the northern Gulf of Mexico. *J Appl Microbiol* 2010;**109**:953–62.
65. Ottaviani D, Leoni F, Rocchegiani E, et al. *Vibrio parahaemolyticus*-associated gastroenteritis in Italy: persistent occurrence of O3:K6 pandemic clone and emergence of O1:KUT serotype. *Diagn Microbiol Infect Dis* 2010;**66**:452–5.
67. Payinda G. Necrotizing fasciitis due to *Vibrio parahaemolyticus*. *N Z Med J* 2008;**121**:99–101.
68. Peffers AS, Bailey J, Barrow GI, et al. *Vibrio parahaemolyticus* gastroenteritis and international air travel. *Lancet* 1973;**1**:143–5.
83. Velazquez-Roman J, Leon-Sicairos N, Flores-Villasenor H, et al. Association of pandemic *Vibrio parahaemolyticus* O3:K6 present in the coastal environment of Northwest Mexico with cases of recurrent diarrhea between 2004 and 2010. *Appl Environ Microbiol* 2012;**78**:1794–803.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Akeda Y, Nagayama K, Yamamoto K, et al. Invasive phenotype of *Vibrio parahaemolyticus*. *J Infect Dis* 1997;**176**:822–4.
2. *Vibrio parahaemolyticus*, Japan, 1996–1998. *Wkly Epidemiol Rec* 1999; **74**:361–3.
3. Ansaruzzaman M, Lucas M, Deen JL, et al. Pandemic serovars (O3:K6 and O4:K68) of *Vibrio parahaemolyticus* associated with diarrhea in Mozambique: spread of the pandemic into the African continent. *J Clin Microbiol* 2005;**43**:2559–62.
4. Barker WH Jr. *Vibrio parahaemolyticus* outbreaks in the United States. *Lancet* 1974;**1**:551–4.
5. Baross J, Liston J. Occurrence of *Vibrio parahaemolyticus* and related hemolytic vibrios in marine environments of Washington State. *Appl Microbiol* 1970;**20**:179–86.
6. Bean NH, Griffin PM. Foodborne disease outbreaks in the United States, 1973–1987: pathogens, vehicles and trends. *J Food Protect* 1990;**53**:804–17.
7. Bean NH, Griffin PM, Goulding JS, et al. Foodborne disease outbreaks, 5-year summary, 1983–1987. MMWR. CDC. *Surveill Summ* 1990;**39**:15–57.
8. Blake PA. Disease of humans (other than cholera) caused by vibrios. *Annu Rev Microbiol* 1980;**34**:341–67.
9. Bolen JL, Zamiska SA, Greenough WB. Clinical features in enteritis due to *Vibrio parahaemolyticus*. *Am J Med* 1974;**57**:638–41.
10. Bonner JR, Coker AS, Berryman CR, et al. Spectrum of *Vibrio* infections in a Gulf Coast community. *Ann Intern Med* 1983;**99**: 464–9.
11. Boutin BK, Townsend SF, Scarpino PV, et al. Demonstration of invasiveness of *Vibrio parahaemolyticus* in adult rabbits by immunofluorescence. *Appl Environ Microbiol* 1979;**37**:647–53.
12. Caraway CT, Gregg J, McFarland L. *Vibrio parahaemolyticus* foodborne outbreak, Louisiana. *MMWR Morb Mortal Wkly Rep* 1978;**27**: 345–6.
13. Carpenter CJ. More pathogenic vibrios. *N Engl J Med* 1979;**300**: 39–41.
14. Centers for Disease Control and Prevention. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters: Pacific Northwest, 1997. *MMWR Morb Mortal Wkly Rep* 1998;**47**: 457–62.
15. Centers for Disease Control and Prevention. Outbreak of *Vibrio parahaemolyticus* infection associated with eating raw oysters and clams harvested from Long Island Sound: Connecticut, New Jersey, and New York, 1998. *MMWR Morb Mortal Wkly Rep* 1999;**48**: 48–51.
16. Centers for Disease Control and Prevention. *Vibrio parahaemolyticus* infections associated with consumption of raw shellfish: three states, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:854–6.
17. Centers for Disease Control and Prevention. *Cholera and other* Vibrio *illness (COVIS) annual summary, 2009*. Centers for Disease Control and Prevention (CDC) and US Department of Health and Human Services; 2011.
18. Chai TJ, Pace J. Vibrio parahaemolyticus. In: Hui Y, Gorham JR, Murrel KD, editors. *Foodborne Disease Handbook*. New York: Marcel Dekker; 1994. p. 395–425.
19. Chakrabarti MK, Sinha AK, Biswas T. Adherence of *Vibrio parahaemolyticus* to rabbit intestinal epithelial cells in vitro. FEMS. *Microbiol Lett* 1991;**84**:113–18.
20. Chatterjee BD, Mukherjee A, Sanyal SN. Enteroinvasive model of *Vibrio parahaemolyticus*. *Indian J Med Res* 1984;**79**:151–8.
21. Chowdhury NR, Chakraborty S, Ramamurthy T, et al. Molecular evidence of clonal *Vibrio parahaemolyticus* pandemic strains. *Emerg Infect Dis* 2000;**6**:631–6.
22. Dadisman TA, Nelson R, Molenda JR, et al. *Vibrio parahaemolyticus* gastroenteritis in Maryland. I. Clinical and epidemiological aspects. *Am J Epidemiol* 1972;**96**:414–26.
23. Daniels NA, MacKinnon L, Bishop R, et al. *Vibrio parahaemolyticus* infections in the United States, 1973–1998. *J Infect Dis* 2000;**181**: 1661–6.
24. Daniels NA, Ray B, Easton A, et al. Emergence of a new *Vibrio parahaemolyticus* serotype in raw oysters: a prevention quandary. *JAMA* 2000;**284**:1541–5.
25. DePaola A, Hopkins LH, Peeler JT, et al. Incidence of *Vibrio parahaemolyticus* in U.S. coastal waters and oysters. *Appl Environ Microbiol* 1990;**56**:2299–302.
26. Desenclos JA, Klontz KC, Wolfe LE, et al. The risk of *Vibrio* illness in the Florida raw oyster eating population, 1981–1988. *Am J Epidemiol* 1991;**134**:290–7.
27. Dobroszycki J, Sklarin NT, Szilagy G, et al. *Vibrio parahaemolyticus* septicemia in a patient with neutropenic leukemia. *Clin Infect Dis* 1992;**15**:738–9.
28. Doyle MP. Pathogenic *Escherichia coli*, *Yersinia enterocolitica*, and *Vibrio parahaemolyticus*. *Lancet* 1990;**336**:1111–15.
29. Eastaugh J, Shepherd S. Infectious and toxic syndromes from fish and shellfish consumption. *Arch Intern Med* 1989;**149**:1735–40.
30. Fernando RR, Krishnan S, Fairweather MG, et al. *Vibrio parahaemolyticus* septicaemia in a liver transplant patient: a case report. *J Med Case Reports* 2011;**6**:171.
31. Franca SM, Gibbs DL, Samuels P, et al. *Vibrio parahaemolyticus* in Brazilian coastal waters. *JAMA* 1980;**244**:587–8.
32. French GL, Woo ML, Hui YW, et al. Antimicrobial susceptibilities of halophilic vibrios. *J Antimicrob Chemother* 1989;**24**:183–94.
33. Garcia K, Torres R, Uribe P, et al. Dynamics of clinical and environmental *Vibrio parahaemolyticus* strains during seafood-related summer diarrhea outbreaks in southern Chile. *Appl Environ Microbiol* 2009;**75**:7482–7.
34. Gingras SP, Howard LV. Adherence of *Vibrio parahaemolyticus* to human epithelial cell lines. *Appl Environ Microbiol* 1980;**39**: 369–71.
35. Gonzalez-Escalona N, Cachicas V, Acevedo C, et al. *Vibrio parahaemolyticus* diarrhea, Chile, 1998 and 2004. *Emerg Infect Dis* 2005; **11**:129–31.
36. Hally RJ, Rubin RA, Fraimow HS, et al. Fatal *Vibrio parahaemolyticus* septicemia in a patient with cirrhosis. *Dig Dis Sci* 1995;**40**: 1257–60.
37. Honda SI, Goto I, Minematsu I, et al. Gastroenteritis due to Kanagawa negative *Vibrio parahaemolyticus*. *Lancet* 1987;**1**:331–2.
38. Honda T, Ni Y, Miwatani T. Purification and characterization of a hemolysin produced by a clinical isolate of Kanagawa phenomenon-negative *Vibrio parahaemolyticus* and related to the thermostable direct hemolysin. *Infect Immun* 1988;**56**:961–5.
39. Honda T, Ni Y, Miwatani T, et al. The thermostable direct hemolysin of *Vibrio parahaemolyticus* is a pore-forming toxin. *Can J Microbiol* 1992;**38**:1175–80.
40. Hughes JM, Boyce JM, Aleem AR, et al. *Vibrio parahaemolyticus* enterocolitis in Bangladesh: Report of an outbreak. *Am J Trop Med Hyg* 1978;**27**:106–12.
41. Janda JM, Powers C, Bryant RG, et al. Current perspective on the epidemiology and pathogenesis of clinically significant *Vibrio* spp. *Clin Microbiol Rev* 1988;**1**:245–67.
42. Jiang X, Chai TJ. Survival of *Vibrio parahaemolyticus* at low temperatures under starvation conditions and subsequent resuscitation of viable, nonculturable cells. *Appl Environ Microbiol* 1996;**62**: 1300–5.
43. Joseph SW, Colwell RR, Kaper JB. *Vibrio parahaemolyticus* and related halophilic vibrios. *Crit Rev Microbiol* 1983;**10**:77–124.
44. Joseph SW, DeBell RM, Brown WP. In vitro response to chloramphenicol, tetracycline, ampicillin, gentamicin and beta-lactamase production by halophilic vibrios from human and environmental sources. *Antimicrob Agents Chemother* 1978;**13**:244–8.
45. Kadhim HM, Miah A, Munn CB, et al. Development of a polymerase chain reaction (PCR) test for the detection of virulent forms of *Vibrio parahaemolyticus*. *Eur J Clin Microbiol Infect Dis* 2012;**31**: 431–9.
46. Kang MH, Kim IW, Lee DW, et al. Development of a rapid detection method to detect *tdh* gene in *Vibrio parahaemolyticus* using 2-step ultrarapid real-time polymerase chain reaction. *Diagn Microbiol Infect Dis* 2011;**69**:21–9.
47. Klontz KC. Fatalities associated with *Vibrio parahaemolyticus* and *Vibrio cholerae* non-O1 infections in Florida (1981 to 1988). *South Med J* 1990;**83**:500–2.
48. Lee CY, Pan SF, Chen CH. Sequence of a cloned pR72H fragment and its use for detection of *Vibrio parahaemolyticus* in shellfish with the PCR. *Appl Environ Microbiol* 1995;**61**:1311–17.
49. Levine WC, Griffin PM. *Vibrio* infections on the Gulf Coast: results of first year of regional surveillance. *J Infect Dis* 1993;**167**: 479–83.
50. Lynch T, Livingstone S, Buenaventura E, et al. *Vibrio parahaemolyticus* disruption of epithelial cell tight junctions occurs independently of toxin production. *Infect Immun* 2005;**73**:1275–83.

51. Makino K, Oshima K, Kurokawa K, et al. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet* 2003;**361**:743–9.
52. Martinez-Urtaza J, Simental L, Velasco D, et al. Pandemic *Vibrio parahaemolyticus* O3 : K6, Europe. *Emerg Infect Dis* 2005;**11**:1319–20.
53. McCarter LL. Dual flagellar systems enable motility under different circumstances. *J Mol Microbiol Biotechnol* 2004;**7**:18–29.
54. McLaughlin JC. Vibrio. In: Murray PR, Baron EJ, Pfaller MA, editor. *Manual of Clinical Microbiology*. 6th ed. Washington, D.C.: American Society for Microbiology; 1995. p. 465–76.
55. Miyamoto Y, Kato T, Obara Y, et al. In vitro hemolytic characteristic of *Vibrio parahaemolyticus*: its close correlation with human pathogenicity. *J Bacteriol* 1969;**100**:1147–9.
56. Morris Jr JG, Black RE. Cholera and other vibrios in the United States. *N Engl J Med* 1985;**312**:343–50.
57. Nagayama K, Yamamoto K, Mitawani T, et al. Characterization of a haemolysin related to Vp-TDH produced by a Kanagawa phenomenon-negative clinical isolate of *Vibrio parahaemolyticus*. *J Med Microbiol* 1995;**42**:83–90.
58. Nair GB, Hormazabal JC. The *Vibrio parahaemolyticus* pandemic. *Rev Chilena Infectol* 2005;**22**:125–30.
59. Nasu H, Iida T, Sugahara T, et al. A filamentous phage associated with recent pandemic *Vibrio parahaemolyticus* O3 : K6 strains. *J Clin Microbiol* 2000;**38**:2156–61.
60. Nishibuchi M, Kaper JB. Duplication and variation of the thermostable direct haemolysin *(tdh)* gene in *Vibrio parahaemolyticus*. *Mol Microbiol* 1990;**4**:87–99.
61. Nishibuchi M, Kaper JB. Thermostable direct hemolysin gene of *Vibrio parahaemolyticus*: a virulence gene acquired by a marine bacterium. *Infect Immun* 1995;**63**:2093–9.
62. Nishibuchi M, Ishibashi M, Takeda Y, et al. Detection of the thermostable direct hemolysin gene and related DNA sequences in *Vibrio parahaemolyticus* and other *Vibrio* species by the DNA colony hybridization test. *Infect Immun* 1985;**49**:481–6.
63. Noriea 3rd NF, Johnson CN, Griffitt KJ, et al. Distribution of type III secretion systems in *Vibrio parahaemolyticus* from the northern Gulf of Mexico. *J Appl Microbiol* 2010;**109**:953–62.
64. Okuda J, Ishibashi M, Hayakawa E, et al. Emergence of a unique O3 : K6 clone of *Vibrio parahaemolyticus* in Calcutta, India, and isolation of strains from the same clonal group from Southeast Asian travelers arriving in Japan. *J Clin Microbiol* 1997;**35**:3150–5.
65. Ottaviani D, Leoni F, Rocchegiani E, et al. *Vibrio parahaemolyticus*-associated gastroenteritis in Italy: persistent occurrence of O3:K6 pandemic clone and emergence of O1:KUT serotype. *Diagn Microbiol Infect Dis* 2010;**66**:452–5.
66. Pan T, Wang T, Lee C, et al. Foodborne disease outbreaks due to bacteria in Taiwan. *J Clin Microbiol* 1997;**35**:1260–2.
67. Payinda G. Necrotizing fasciitis due to *Vibrio parahaemolyticus*. *N Z Med J* 2008;**121**:99–101.
68. Peffers AS, Bailey J, Barrow GI, et al. *Vibrio parahaemolyticus* gastroenteritis and international air travel. *Lancet* 1973;**1**:143–5.
69. Qadri F, Alam MS, Nishibuchi M, et al. Adaptive and inflammatory immune responses in patients infected with strains of *Vibrio parahaemolyticus*. *J Infect Dis* 2003;**187**:1085–96.
70. Rabinowitch BL, Nam MH, Levy CS, et al. *Vibrio parahaemolyticus* septicemia associated with water skiing. *Clin Infect Dis* 1993;**16**: 339–40.
71. Raimondi F, Kao JP, Fiorentini C, et al. Enterotoxicity and cytotoxicity of *Vibrio parahaemolyticus* thermostable direct hemolysin in in vitro systems. *Infect Immun* 2000;**68**:3180–5.
72. Shinoda S, Nakahara N, Ninomiya Y, et al. Serological method for identification of *Vibrio parahaemolyticus* from marine samples. *Appl Environ Microbiol* 1983;**45**:148–52.
73. Shirai H, Ito H, Hirayama T, et al. Molecular epidemiologic evidence for association of thermostable direct hemolysin (TDH) and TDH-related hemolysin of *Vibrio parahaemolyticus* with gastroenteritis. *Infect Immun* 1990;**58**:3568–73.
74. Spearman JG, Tronca EL, Nichlos EM, et al. Epidemiologic notes and reports, *Vibrio parahaemolyticus*, Louisiana. MMWR. *Morb Mortal Wkly Rep* 1972;**21**:341–3.
75. Takahashi A, Kenjyo N, Imura K, et al. Cl^- secretion in colonic epithelial cells induced by the *Vibrio parahaemolyticus* hemolytic toxin related to thermostable direct hemolysin. *Infect Immun* 2000; **68**:5435–8.
76. Takahashi A, Sato Y, Shiomi Y, et al. Mechanisms of chloride secretion induced by thermostable direct haemolysin of *Vibrio parahaemolyticus* in human colonic tissue and a human intestinal epithelial cell line. *J Med Microbiol* 2000;**49**:801–10.
77. Takea Y. Thermostable direct hemolysin of *Vibrio parahaemolyticus*. *Pharmacol Ther* 1983;**19**:123–46.
78. Thompson CA, Vanderzant C. Serological and hemolytic characteristics of *Vibrio parahaemolyticus* from marine sources. *J Food Sci* 1976;**41**:204–5.
79. Tuyet DT, Thiem VD, Von Seidlein L, et al. Clinical, epidemiological, and socioeconomic analysis of an outbreak of *Vibrio parahaemolyticus* in Khanh Hoa Province, *Vietnam J Infect Dis* 2002; **186**:1615–20.
80. Twedt RM. Vibrio parahaemolyticus. In: Doyle MP, editor. *Foodborne Bacterial Pathogens*. New York: Marcel Dekker; 1989. p. 543–68.
81. Twedt RM, Peerler JT, Spaulding PL. Effective ileal loop dose of Kanagawa-positive *Vibrio parahaemolyticus*. *Appl Environ Microbiol* 1980;**40**:1012–16.
82. Varmam AH. Vibrio. In: Varmam AH, Evans MG, editors. *Foodborne Pathogens*. London: Wolfe Publishing; 1991. p. 157–83.
83. Velazquez-Roman J, Leon-Sicairos N, Flores-Villasenor H, et al. Association of pandemic *Vibrio parahaemolyticus* O3:K6 present in the coastal environment of Northwest Mexico with cases of recurrent diarrhea between 2004 and 2010. *Appl Environ Microbiol* 2012;**78**:1794–803.
84. Wong HC, Wang P. Induction of viable but nonculturable state in *Vibrio parahaemolyticus* and its susceptibility to environmental stresses. *J Appl Microbiol* 2004;**96**:359–66.
85. Yamamoto T, Yokota T. Adherence targets of *Vibrio parahaemolyticus* in human small intestines. *Infect Immun* 1989;**57**:2410–19.
86. Yeung PSM, Boor KJ. Epidemiology, pathogenesis, and prevention of foodborne *Vibrio parahaemolyticus* infections. *Foodborne Pathog Dis* 2004;**1**:74–88.
87. Yoh M, Kawakami N, Funakoshi Y, et al. Evaluation of two assay kits for thermostable direct hemolysin (TDH) as an indicator of TDH-related hemolysin (TRH) produced by *Vibrio parahaemolyticus*. *Microbiol Immunol* 1995;**39**:157–9.
88. Zhang XH, Austin B. Haemolysins in *Vibrio* species. *J Appl Microbiol* 2005;**98**:1011–19.

CHAPTER 119

Vibrio vulnificus

Randall G. Fisher

BACTERIOLOGY

Vibrio vulnificus is a small, curvilinear, gram-negative rod of the family Vibrionaceae.[7] This facultative anaerobe is oxidase-positive and lysine-positive, similar to other species of the genus *Vibrio*. Its major difference is that it ferments lactose, a feature that accounts for its original name, Lac + *Vibrio*. *V. vulnificus* is arginine-negative and ornithine-variable. This halophilic (salt-loving) organism grows in concentrations of sodium chloride of from 1 to 8 percent and seems to grow best at approximately 3 percent.[37]

EPIDEMIOLOGY

V. vulnificus, similar to *Vibrio parahaemolyticus*, is a marine organism that is a common inhabitant of off-shore waters, especially estuarial waters.[57] It has been isolated from sediment, plankton, water, finfish, crabs, and oysters.[65] Peak recovery occurs in the summer and early fall,[18] possibly because at higher water temperatures *V. vulnificus* is released and rises to surface waters. There it attaches to plankton and shore fish and then is taken up and concentrated by filter-feeding mollusks and crustaceans.

V. vulnificus has been isolated from wild and commercial oysters throughout the world. It especially is prevalent in warm coastal waters. Reports about the survival of the organism in oysters stored at low temperatures are conflicting.[31] Nilsson and associates[55] revealed, however, that even if *V. vulnificus* is rendered nonculturable by storage at low temperatures, it can be "resuscitated" by allowing the oysters to warm up to room temperature; this cycle could be carried out twice without any reduction in bacterial count. Because of the ubiquity of the organism in the marine environment and its ability to thrive under even the most careful conditions of sanitation, storage, and transport,[17,35] ensuring that commercial shellfish do not contain viable *V. vulnificus* is very difficult.

So-called heat shock shucking, during which the internal meat temperature exceeds 50° C (122° F) for 1 to 4 minutes, reduces the *V. vulnificus* and total bacterial levels in oysters 10-fold to 10,000-fold compared with conventional processing.[23] A separate study confirms that low-temperature pasteurization, which brings oyster temperatures to 50° C for 10 to 15 minutes, reduces colony counts from greater than 100,000/g of oyster meat to undetectable levels.[2] Ice immersion for 3 hours is ineffective at reducing *V. vulnificus* and actually increases coliform counts in oysters.[56,60] High-pressure processing is a promising new approach to eliminating *V. vulnificus* from oysters; in preliminary studies, *V. vulnificus* could be reduced to undetectable levels by processing at 586 MPa with a 7-minute "come up" time.[38]

Disease in humans is initiated by contact with the organism. Contact occurs either through marine contamination of a wound or in the gastrointestinal tract after ingestion of the organism in raw shellfish, most commonly oysters.[7]

PATHOPHYSIOLOGY

V. vulnificus causes three distinct diseases in humans: wound infection and gastrointestinal infection, which may be self-limited or progress to septicemia. Local infection occurs after a wound is exposed to contaminated sea water. Wound infections with *V. vulnificus* are marked by rapid spread, the formation of bullae, and necrosis of involved tissues.[7] Marked edema and vascular thromboses occur in experimental and natural infection. *V. vulnificus* elaborates a cytolysin,[21,22,37,40] a collagenase,[63] and a protease,[50] which enhance its rapid spread in tissues. The protease activates the plasma kallikrein-kinin system to produce bradykinin[49]; histamine also is released locally. These factors account for the intense inflammatory reaction seen in these lesions. One group of researchers constructed an isolate that lacked a flagellum and found that its ability to adhere to cells was decreased, as was its lethality in mice.[42] Hemorrhagic complications may be due to the presence of a metalloprotease that interferes with blood homeostasis through prothrombin activation and fibrinolysis.[11]

In otherwise healthy individuals, a mild, self-limited gastrointestinal illness similar to that caused by *V. parahaemolyticus* has been described.[36] The most serious infection produced by *V. vulnificus* is primary septicemia, which occurs most commonly in patients with liver disease, after they have ingested the organism in raw shellfish.[7,58,59]

For patients with hepatic disease, the risk for acquiring septicemia is 40 to 80 times greater, and the case-fatality rate is 2.5 times higher than for otherwise healthy individuals.[25] *V. vulnificus*, similar to many other gram-negative organisms, requires iron for growth and grows better in an excess of iron.[73] It is able to extract iron from hemoglobin, even if it is complexed completely to

haptoglobin.[73,76] Liver damage, excess iron, and deferoxamine therapy have been shown to decrease the median lethal dose (LD_{50}) of *V. vulnificus* in experimental animals. Deferoxamine alone decreased the LD_{50} by four orders of magnitude.[9] Iron overload almost certainly underlies *V. vulnificus* septicemia in patients who require repeated transfusions and deferoxamine therapy for anemia.[30,75] Some evidence indicates that alcoholism, even in the absence of demonstrable liver disease, is a risk factor for developing sepsis.[58] Fatal septicemia has been reported in patients with other chronic diseases, such as diabetes mellitus and lymphomas.[59]

Virulence factors of the organism have been described.[74] Virulent strains are resistant to the bactericidal activity of human serum,[39] probably because of the presence of a polysaccharide capsule.[1] Poor uptake of virulent strains into phagocytes[66] and opaqueness of the colony on agar have been correlated with the presence of the capsule.[62,74] RTX toxin is a cytolysin that also may be important in protecting the organism from phagocytosis.[44] No difference has been shown in the lipopolysaccharides of virulent versus avirulent strains.[5] The production of recalcitrant shock in patients with septicemia is secondary to toxins, loss of vascular tone, capillary leak, and possibly negative inotropy, as in other forms of gram-negative sepsis.

CLINICAL MANIFESTATIONS

Patients commonly have wound infection or primary septicemia. Wound infection occurs after injury in sea water or after the contamination of a recently acquired wound with sea water. Often, the wound is caused by the shell of a crustacean or mollusk. Many patients with wound infections work in shellfish-related industries. Apparently, wound infection with *V. vulnificus* can develop from superficial wounds acquired within 24 hours of "uneventful" fish handling; the physiologic characteristics of human sweat may be conducive to the survival of the organism on the skin, allowing for later inoculation.[10] Cellulitis may develop and spread rapidly. Overlying skin often is covered with tense bullae. At débridement, the extent of necrosis may exceed presurgical expectations.[72] Primary wound infection may progress to systemic infection; for this reason, wound infection with *V. vulnificus* carries a mortality rate of 7 to 24 percent.[7,36] Findings associated with mortality in patients with necrotizing fasciitis include low white blood cell counts, low platelet counts, hypoalbuminemia, high immature neutrophil percentages, and hypotension at presentation.[69]

Patients with primary septicemia generally give a history of recent (i.e., 6 to 72 hours) consumption of raw seafood. Illness is marked by the rapid onset of fever, hypotension, and septic shock. Prodromal symptoms, such as malaise, chills, and fever, are common manifestations. Vomiting and diarrhea are seen in approximately 20 percent of patients.[6] Shock progresses quickly and is difficult to reverse. Secondary skin lesions develop in approximately half of patients[7] and may be bullous, petechial, or maculopapular. *V. vulnificus* frequently is isolated from cultures of secondary lesions, providing evidence of septicemic spread to those sites.[7] The mortality rate from primary septicemia has been reported to be 46 to 79 percent.[6,58]

In addition to causing the described syndromes, *V. vulnificus* has been reported as a cause of corneal ulcer,[19] myositis,[32] adult epiglottitis,[46] osteomyelitis,[70] endocarditis,[68] peritonitis,[27] tubo-ovarian abscess,[47] and meningitis.[30,58] In one case, a patient had endogenous endophthalmitis without systemic symptoms that developed after ingestion of raw seafood.[29] A case of fatal septicemia in which the presenting symptom was a compartment syndrome of the forearm has been described.[28] A 9-year-old girl with thalassemia and iron overload had septic arthritis as the presenting feature of her septicemia.[41] Ninety percent of reported patients are 40 years old or older.[7,58] Childhood cases of septicemia have been associated with thalassemia major,[29] for which frequent transfusions and deferoxamine therapy are required. Wound infections in previously healthy children and adolescents have been reported. *V. vulnificus* also has been isolated from a premature infant's stool sample obtained on the infant's first day of life; the infant's mother worked as an oyster shucker.[4]

DIAGNOSIS

The diagnosis of *V. vulnificus* infection is made by isolating the organism from blood or tissue culture. It also may be recovered from stool specimens.[4,59] Agars designed specifically to aid in the growth and identification of *V. vulnificus* have been developed; cellobiose-colistin agar out-performs thiosulfate citrate bile salts sucrose agar (TCBS) for recovery of *V. vulnificus* from environmental samples.[26] From a practical standpoint, however, most hospital microbiology laboratories do not stock cellobiose-colistin agar. Of the commercially available media, *V. vulnificus* tends to grow best in TCBS,[7] but it also may be recovered from ordinary blood agar plates[3,61] or MacConkey plates.[7]

A very sensitive nested polymerase chain reaction technique that is capable of detecting 1 pg of bacterial DNA and one colony-forming unit of *V. vulnificus* has been described; it was positive in 94 percent of clinical samples that grew the bacteria in culture and in 42 percent of culture-negative samples from patients with suspected *V. vulnificus* infection.[24,43] A newer multiplex PCR was developed that had 100 percent sensitivity and specificity for identification and differentiation of *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* in validation tests with 488 strains.[53] An enzyme immunoassay also has been developed.[66] These methods are experimental and not yet available for routine clinical use.

The diagnosis of *V. vulnificus* wound infection or septicemia can be suspected on clinical grounds and appropriate therapy initiated while awaiting culture results. A history of ingestion of raw shellfish or contamination of a wound with either sea water or brackish inland waters[64] should be sought. *V. vulnificus* infection should be given high consideration in patients with hemosiderosis, anemia with transfusion therapy, liver disease, or other chronic diseases.

TREATMENT

For severe wound infections, performing surgical therapy as rapidly as possible is paramount. For primary septicemia, support of the patient's airway, along with aggressive pressor support and other adjunctive therapies for severe septic shock, is of primary concern. Secondarily, appropriate antibiotic treatment for *V. vulnificus* should be started as early as possible.[51]

In vitro, the organism is susceptible to many antibiotics, including ampicillin, third-generation cephalosporins, tetracycline, chloramphenicol, and gentamicin.[7] Bowdre and associates[8] reported that in mice the minimum inhibitory concentrations obtained in the laboratory did not seem to correspond with response to therapy. In particular, the organism seemed to be exquisitely sensitive to cefotaxime in vitro (minimum inhibitory concentration 0.06 μg/mL); however, 9/10 mice treated with cefotaxime died of overwhelming infection. Anecdotal evidence indicates that this puzzling phenomenon may occur in humans as well; 4/5 patients reported by Chuang and associates[15] died, despite receiving therapy with third-generation cephalosporins at appropriate dosages. Similarly, although the organism is almost universally sensitive to gentamicin in the laboratory, case reports show a lack of response to this antibiotic. Of all the antibiotics to which the organism was sensitive in vitro, only tetracycline led to survival of mice in Bowdre's study; all 12 of the mice treated with tetracycline survived the infection.[8]

Case reports also show favorable outcomes with tetracycline[20] or doxycycline[15] and chloramphenicol[15] and ciprofloxacin.[45] More recently, the combination of minocycline and cefotaxime has been shown to be synergistic in vitro, with the combination reducing the growth of *V. vulnificus* by six orders of magnitude in contrast to either drug alone.[14] Enhanced survival of mice infected with *V. vulnificus* and treated with minocycline plus cefotaxime provides an in vivo correlate of the in vitro results.[16] No data are available comparing the activity of minocycline and that of tetracycline; nonetheless, the previously mentioned evidence is sufficiently strong to suggest that either tetracycline or minocycline together with cefotaxime should be the treatment of choice for known or suspected *V. vulnificus* infection. Fluoroquinolones also may have a role in treatment. In vitro synergy studies show that the combination of ciprofloxacin and cefotaxime is synergistic for *V. vulnificus*,[34] and in a murine model, the newer fluoroquinolones (e.g., moxifloxacin) worked as well as combination therapy in preventing lethality in mice.[13] As yet, no human clinical data are available regarding the efficacy of third-generation fluoroquinolones. In vitro, the addition of the iron chelator deferasirox to antimicrobial treatment (in this study, ciprofloxacin) resulted in synergistic antimicrobial activity.[54] One case has been reported of a patient iron-overloaded from hemochromatosis who survived after treatment with the combination of ciprofloxacin and deferasirox.[33]

The addition of modified Dakin solution (0.025% sodium hypochlorite) may have some utility in the treatment of skin and wound infections. An in vitro study of eight topical antibiotic preparations showed that *V. vulnificus* was most sensitive to modified Dakin solution.[48] Although no controlled trial has been performed, a series has been reported of 10 patients with culture-proved *V. vulnificus* wound infections treated with doxycycline and topical modified Dakin solution, none of whom experienced progression of infection or required surgical débridement.[71] Autologous platelet concentrate spray was used successfully as adjunctive wound therapy in a renal transplant patient.[67]

PREVENTION

Patients with severe anemia, liver disease, hemosiderosis, or other debilitating chronic diseases and patients on deferoxamine therapy should be advised against eating raw seafood of any kind. Awareness of the disease has been shown to be low in high-risk patients; physicians need to educate at-risk patients.[52] Patients with open wounds probably should avoid contact with sea water or brackish inland waters. In a mouse model, intramuscular VvpE (a recombinant metalloprotease antigen of *V. vulnificus*) elicits a good antibody response and confers protection against lethal challenge.[12]

Acknowledgments

The author thanks Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

12. Chen YC, Chang CC, Chang SY, Su JH. A recombinant metalloprotease antigen of *Vibrio vulnificus* elicits protective antibodies in a murine model. *Lett Appl Microbiol* 2010;**50**:168–72.
33. Kim DM, Cho HS, Kang JI, Kim HS, Park CY. Deferasirox plus ciprofloxacin combination therapy after rapid diagnosis of *Vibrio vulnificus* sepsis using real-time polymerase chain reaction. *J Infect* 2008;**57**:489–92.
41. Kuo CH, Dai ZK, Wu J, et al. Septic arthritis as the initial manifestation of fatal *Vibrio vulnificus* septicemia in a patient with thalassemia and iron overload. *Pediatr Blood Cancer* 2009;**53**:1156–8.
44. Lo HR, Lin JH, Chen YH, et al. TRX toxin enhances the survival of *Vibrio vulnificus* during infection by protecting the organism from phagocytosis. *J Infect Dis* 2011;**15**:1866–74.
52. Nagao Y, Matsuoka H, Seike M, et al. Knowledge of *Vibrio vulnificus* infection among Japanese patients with liver diseases: a prospective multicenter study. *Med Sci Monit* 2009;**15**:115–20.
53. Neogi SB, Chowdhury N, Asakura M, et al. A highly sensitive and specific multiplex PCR assay for simultaneous detection of *Vibrio cholera*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. *Lett Appl Microbiol* 2010;**51**:293–300.
54. Neupane GP, Kim DM. In vitro time-kill activities of ciprofloxacin alone and in combination with the iron chelator deferasirox against *Vibrio vulnificus*. *Eur J Clin Microbiol Infect Dis* 2010;**29**:407–10.
67. Thodis E, Kriki P, Kakagia D, et al. Rigorous *Vibrio vulnificus* soft tissue infection of the lower leg in a renal transplant patient managed by vacuum therapy and autologous growth factors. *J Cutan Med Surg* 2009;**13**:209–14.
69. Tsai YH, Hsu RW, Huang KC, Huang TJ. Laboratory indicators for early detection and surgical treatment of vibrio necrotizing fasciitis. *Clin Orthop Relat Res* 2010;**468**:2230–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Amako K, Okada K, Miake S. Evidence for the presence of a capsule in *Vibrio vulnificus*. *J Gen Microbiol* 1984;**130**:2741–3.
2. Andrews LS, Park DL, Chen YP. Low temperature pasteurization to reduce the risk of vibrio infections from raw shell-stock oysters. *Food Addit Contam* 2000;**17**:787–91.
3. Armstrong CW, Lake JL, Miller Jr GB. Extraintestinal infections due to halophilic *Vibrios*. *South Med J* 1983;**76**:571–4.
4. Bachman B, Boyd Jr WP, Lieb S, et al. Marine noncholera *Vibrio* infections in Florida. *South Med J* 1983;**76**:296–9.
5. Bahrani K, Oliver JD. Studies on the lipopolysaccharide of a virulent and an avirulent strain of *Vibrio vulnificus*. *Biochem Cell Biol* 1990;**68**:547–51.
6. Blake PA, Weaver RE, Hollis DG. Disease of humans (other than cholera) caused by vibrios. *Ann Rev Microbiol* 1980;**34**:341–67.
7. Blake PA, Merson MH, Weaver RE, et al. Disease caused by a marine *Vibrio*: clinical characteristics and epidemiology. *N Engl J Med* 1979;**300**:1–5.
8. Bowdre JH, Hull JH, Cocchetto DM. Antibiotic efficacy against *Vibrio vulnificus* in the mouse: superiority of tetracycline. *J Pharmacol Exp Ther* 1983;**225**:595–8.
9. Brennaman B, Soucy D, Howard RJ. Effect of iron and liver injury on the pathogenesis of *Vibrio vulnificus*. *J Surg Res* 1987;**43**: 527–31.
10. Calif E, Shalom S. Hand infections caused by delayed inoculation of *Vibrio vulnificus*: does human skin serve as a potential reservoir of vibrios? *Hand Surg* 2004;**9**:39–44.
11. Chang AK, Kim HY, Park JE, et al. *Vibrio vulnificus* secretes a broad-specificity metalloprotease capable of interfering with blood homeostasis through prothrombin activation and fibrinolysis. *J Bacteriol* 2005;**187**:6909–16.
12. Chen YC, Chang CC, Chang SY, Su JH. A recombinant metalloprotease antigen of *Vibrio vulnificus* elicits protective antibodies in a murine model. *Lett Appl Microbiol* 2010;**50**:168–72.
13. Chiang SR, Chuang YC. *Vibrio vulnificus* infection: clinical manifestations, pathogenesis, and antimicrobial therapy. *J Microbiol Immunol Infect* 2003;**36**:81–8.
14. Chuang YC, Liu JW, Ko WC, et al. In vitro synergism between cefotaxime and minocycline against *Vibrio vulnificus*. *Antimicrob Agents Chemother* 1997;**41**:2214–17.
15. Chuang YC, Yuan CY, Lin CY, et al. *Vibrio vulnificus* infection in Taiwan: report of 28 cases and review of clinical manifestations and treatment. *Clin Infect Dis* 1992;**115**:271–6.
16. Chuang YW, Ko WC, Wang ST, et al. Minocycline and cefotaxime in the treatment of experimental murine *Vibrio vulnificus* infection. *Antimicrob Agents Chemother* 1998;**42**:1319–22.
17. Cook DW. Effect of time and temperature on multiplication of *Vibrio vulnificus* in postharvest Gulf Coast shellstock oysters. *Appl Environ Microbiol* 1994;**60**:3483–4.
18. DePaola A, Capers GM, Alexander D. Densities of *Vibrio vulnificus* in the intestines of fish from the U.S. Gulf Coast. *Appl Environ Microbiol* 1994;**60**:984–8.
19. DiGaetano M, Ball SF, Straus JG. *Vibrio vulnificus* corneal ulcer: case reports. *Arch Ophthalmol* 1989;**107**:323–4.
20. Fang FC. Use of tetracycline for treatment of *Vibrio vulnificus* infections. *Clin Infect Dis* 1992;**15**:1071–2.
21. Gray LD, Kreger AS. Purification and characterization of an extracellular cytolysin produced by *Vibrio vulnificus*. *Infect Immun* 1985; **48**:62–72.
22. Gray LD, Kreger AS. Mouse skin damage caused by cytolysin from *Vibrio vulnificus* and by *Vibrio vulnificus* infection. *J Infect Dis* 1987; **155**:236–41.
23. Hesselman DM, Motes ML, Lewis JP. Effects of a commercial heat-shock process on *Vibrio vulnificus* in the American oyster, *Crassostrea virginica*, harvested from the Gulf Coast. *J Food Prot* 1999;**62**:1266–9.
24. Hill WE, Keasler SP, Trucksess MW, et al. Polymerase chain reaction identification of *Vibrio vulnificus* in artificially contaminated oysters. *Appl Environ Microbiol* 1991;**57**:707–11.
25. Hlady WG, Mullen RC, Hopkin RS. *Vibrio vulnificus* from raw oysters: leading cause of reported deaths from foodborne illness in Florida. *J Fla Med Assoc* 1993;**80**:536–8.
26. Hoi L, Dalsgaard I, Dalsgaard A. Improved isolation of *Vibrio vulnificus* from seawater and sediment with cellobiose-colistin agar. *Appl Environ Microbiol* 1998;**64**:1721–4.
27. Holcombe DJ. *Vibrio vulnificus* peritonitis: a unique case. *J La State Med Soc* 1991;**143**:27–8.
28. Hui KC, Zhang F, Komorowska–Timek E, et al. Compartment syndrome of the forearm as the initial symptom of systemic *Vibrio vulnificus* infection. *J Hand Surg Am* 1999;**24**:715–17.
29. Jung SI, Shin DH, Park KH, et al. *Vibrio vulnificus* endophthalmitis occurring after ingestion of raw seafood. *J Infect* 2005;**51**:e281–3.
30. Katz BZ. *Vibrio vulnificus* meningitis in a boy with thalassemia after eating raw oysters. *Pediatrics* 1988;**82**:784–6.
31. Kaysner CA, Tamplin ML, Wekell MM, et al. Survival of *Vibrio vulnificus* in shellstock and shucked oysters (*Crassostrea gigas* and *Crassostrea virginica*) and effects of isolation medium on recovery. *Appl Environ Microbiol* 1989;**55**:3072–9.
32. Kelly MT, McCormick WF. Acute bacterial myositis caused by *Vibrio vulnificus*. *JAMA* 1981;**246**:72–3.
33. Kim DM, Cho HS, Kang JI, Kim HS, Park CY. Deferasirox plus ciprofloxacin combination therapy after rapid diagnosis of *Vibrio vulnificus* sepsis using real-time polymerase chain reaction. *J Infect* 2008;**57**:489–92.
34. Kim DM, Lym Y, Jang SJ, et al. In vitro efficacy of the combination of ciprofloxacin and cefotaxime against *Vibrio vulnificus*. *Antimicrob Agents Chemother* 2005;**49**:3489–91.
35. Kizer KW. *Vibrio vulnificus* hazard in patients with liver disease. *West J Med* 1994;**161**:64–5.
36. Klontz KC, Lieb S, Schreiber M, et al. Syndromes of *Vibrio vulnificus* infections: clinical and epidemiologic features in Florida cases, 1981–1987. *Ann Intern Med* 1988;**109**:318–23.
37. Koga T, Kawata T. Composition of the major outer membrane proteins of *Vibrio vulnificus* isolates: effect of different growth media and iron deficiency. *Microbiol Immunol* 1986;**30**:193–201.
38. Koo J, Jahncke ML, Reno PW, et al. Inactivation of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in phosphate-buffered saline and in inoculated whole oysters by high-pressure processing. *J Food Prot* 2006;**69**:596–601.
39. Kreger A, DeChatelet L, Shirley P. Interaction of *Vibrio vulnificus* with human polymorphonuclear leukocytes: association of virulence with resistance to phagocytosis. *J Infect Dis* 1981;**144**: 244–8.
40. Kreger AS, Kothary MH, Gray LD. Cytolytic toxins of *Vibrio vulnificus* and *Vibrio damsela*. *Methods Enzymol* 1988;**165**:176–89.
41. Kuo CH, Dai ZK, Wu J, et al. Septic arthritis as the initial manifestation of fatal *Vibrio vulnificus* septicemia in a patient with thalassemia and iron overload. *Pediatr Blood Cancer* 2009;**53**: 1156–8.
42. Lee JH, Rho JB, Park KJ, et al. Role of flagellum and motility in pathogenesis of *Vibrio vulnificus*. *Infect Immun* 2004;**72**:4905–10.
43. Lee SE, Kim SY, Kim SJ, et al. Direct identification of *Vibrio vulnificus* in clinical specimens by nested PCR. *J Clin Microbiol* 1998; **36**:2887–92.
44. Lo HR, Lin JH, Chen YH, et al. TRX toxin enhances the survival of *Vibrio vulnificus* during infection by protecting the organism from phagocytosis. *J Infect Dis* 2011;**15**:1866–74.
45. Meadors MC, Pankey GA. *Vibrio vulnificus* wound infection treated successfully with oral ciprofloxacin. *J Infect* 1990;**20**:88–9.
46. Mehtar S, Bangham L, Kalmanovitch D, et al. Adult epiglottitis due to *Vibrio vulnificus*. *Br Med J Clin Res* 1988;**296**:827–8.
47. Midturi J, Baker D, Winn R, Fader R. Tubo-ovarian abscess caused by *Vibrio vulnificus*. *Diagn Microbiol Infect Dis* 2005;**51**:131–3.
48. Milner SM, Heggers JP. The use of a modified Dakin's solution (sodium hypochlorite) in the treatment of *Vibrio vulnificus* infection. *Wild Environ Med* 1999;**10**:10–12.
49. Miyoshi N, Miyoshi S, Sugiyama K, et al. Activation of the plasma kallikrein-kinin system by *Vibrio vulnificus* protease. *Infect Immun* 1987;**55**:1936–9.
50. Miyoshi S, Shinoda S. Role of the protease in the permeability enhancement by *Vibrio vulnificus*. *Microbiol Immunol* 1988;**32**:1025–32.
51. Morris Jr JG, Tenney J. Antibiotic therapy for *Vibrio vulnificus* infection. *JAMA* 1985;**253**:1121–2.
52. Nagao Y, Matsuoka H, Seike M, et al. Knowledge of *Vibrio vulnificus* infection among Japanese patients with liver diseases: a prospective multicenter study. *Med Sci Monit* 2009;**15**:115–20.
53. Neogi SB, Chowdhury N, Asakura M, et al. A highly sensitive and specific multiplex PCR assay for simultaneous detection of *Vibrio cholera*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. *Lett Appl Microbiol* 2010;**51**:293–300.

54. Neupane GP, Kim DM. In vitro time-kill activities of ciprofloxacin alone and in combination with the iron chelator deferasirox against *Vibrio vulnificus*. *Eur J Clin Microbiol Infect Dis* 2010;**29**: 407–10.
55. Nilsson L, Oliver JD, Kjelleberg S. Resuscitation of *Vibrio vulnificus* from the viable but nonculturable state. *J Bacteriol* 1991;**173**: 5054–9.
56. Oliver JD. Lethal cold stress of *Vibrio vulnificus* in oysters. *Appl Environ Microbiol* 1981;**41**:710–17.
57. Oliver JD, Warner RA, Cleland DR. Distribution of *Vibrio vulnificus* and other lactose-fermenting vibrios in the marine environment. *Appl Environ Microbiol* 1983;**45**:985–98.
58. Park SD, Shon HS, Joh NJ. *Vibrio vulnificus* septicemia in Korea: clinical and epidemiologic findings in seventy patients. *J Am Acad Dermatol* 1991;**24**:397–403.
59. Pollak SJ, Parrish III EF, Barrett TJ, et al. *Vibrio vulnificus* septicemia: isolation of organism from stool and demonstration of antibodies by indirect immunofluorescence. *Arch Intern Med* 1983;**143**: 837–8.
60. Quevedo AC, Smith JG, Rodrick GE, Wright AC. Ice immersion as a postharvest treatment of oysters for the reduction of *Vibrio vulnificus*. *J Food Prot* 2005;**68**:1192–7.
61. Saraswathi K, Barve SM, Deodhar LP. Septicaemia due to *Vibrio vulnificus*. *Trans R Soc Trop Med Hyg* 1989;**83**:714.
62. Simpson LM, White VK, Zane SF, et al. Correlation between virulence and colony morphology in *Vibrio vulnificus*. *Infect Immun* 1987;**55**:269–72.
63. Smith GC, Merkel JR. Collagenolytic activity of *Vibrio vulnificus*: potential contribution to its invasiveness. *Infect Immun* 1982;**35**: 1155–6.
64. Tacket CO, Barrett TJ, Mann JM, et al. Wound infections caused by *Vibrio vulnificus*, a marine *Vibrio*, in inland areas of the United States. *J Clin Microbiol* 1984;**19**:197–9.
65. Tamplin ML, Martin AL, Ruple AD, et al. Enzyme immunoassay for identification of *Vibrio vulnificus* in seawater, sediment, and oysters. *Appl Environ Microbiol* 1991;**57**:1235–40.
66. Tamplin ML, Specter S, Rodrick GE, et al. *Vibrio vulnificus* resists phagocytosis in the absence of serum opsonins. *Infect Immun* 1985; **49**:715–18.
67. Thodis E, Kriki P, Kakagia D, et al. Rigorous *Vibrio vulnificus* soft tissue infection of the lower leg in a renal transplant patient managed by vacuum therapy and autologous growth factors. *J Cutan Med Surg* 2009;**13**:209–14.
68. Truwit JD, Badesch DB, Savage AM, et al. *Vibrio vulnificus* bacteremia with endocarditis. *South Med J* 1987;**80**:1457–9.
69. Tsai YH, Hsu RW, Huang KC, Huang TJ. Laboratory indicators for early detection and surgical treatment of vibrio necrotizing fasciitis. *Clin Orthop Relat Res* 2010;**468**:2230–7.
70. Vartian CV, Septimus EJ. Osteomyelitis caused by *Vibrio vulnificus*. *J Infect Dis* 1990;**161**:363.
71. Wilhelmi BJ, Calianos 2nd TA, Appelt EA, et al. Modified Dakin's solution for cutaneous vibrio infections. *Ann Plast Surg* 1999;**43**: 386–9.
72. Woo ML, Patrick WG, Simon MT, et al. Necrotising fasciitis caused by *Vibrio vulnificus*. *J Clin Pathol* 1984;**37**:1301–4.
73. Wright AC, Simpson LM, Oliver JD. Role of iron in the pathogenesis of *Vibrio vulnificus* infections. *Infect Immun* 1981;**34**:503–7.
74. Yoshida S, Ogawa M, Mizuguchi Y. Relation of capsular materials and colony opacity to virulence of *Vibrio vulnificus*. *Infect Immun* 1985;**47**:446–51.
75. Yoshida S, Tanabe T, Chiba S, et al. Fatal *Vibrio vulnificus* infection in a patient with aplastic anemia. *Sangyo Ika Daigaku Zasshi* 1983;**5**: 95–100.
76. Zakaria–Meehan Z, Massad G, Simpson LM, et al. Ability of *Vibrio vulnificus* to obtain iron from hemoglobin-haptoglobin complexes. *Infect Immun* 1988;**56**:275–7.

CHAPTER 120

Miscellaneous Non-Enterobacteriaceae Fermentative Bacilli

Randall G. Fisher

Fermentative bacilli that are not of the family Enterobacteriaceae are discussed in this chapter. Specifically examined are *Chromobacterium violaceum*, *Plesiomonas shigelloides*, and *Pasteurella* organisms other than *Pasteurella multocida*.

CHROMOBACTERIUM VIOLACEUM

C. violaceum is a facultatively anaerobic, gram-negative rod that is a saprophyte of soil and water and especially is found in tropical and subtropical climates. It causes occasional illness in animals and, rarely, in humans. Infection with *C. violaceum*, when it does occur, is a serious disease with a high mortality rate.

Bacteriology

C. violaceum is a long, motile, gram-negative bacillus that appears singly or in pairs on Gram stain. It has a polar flagellum and one to four subpolar or lateral flagella that antigenically are distinct from the polar flagellum.[85] Most isolates produce an insoluble pigment, violacein. This pigment is intense and makes colonies appear dark purple to black, especially on blood agar. Violacein induces apoptotic death in certain cell types in vitro, including leukemia and lymphoma cells.[65] The therapeutic potential of violacein and structural analogs is being explored. Violacein also has antibacterial, antitrypanosomic, antimalarial,[58] and weak antiviral effects.[8] In a rat model, violacein displays immunomodulatory, analgesic, and antipyretic effects[9]; in addition, it protects against gastric ulcers and is an effective treatment for diarrhea.[10] *C. violaceum* grows readily on standard agar (any medium that contains tryptophan supports growth). The colonies are low convex, violet, smooth, and not gelatinous. Colonies produce hydrogen cyanide, so a faint almond odor may be present.[85]

C. violaceum is catalase-positive and oxidase-positive, although the latter may be difficult to interpret because of the production of pigment. Growing the organism anaerobically inhibits pigment formation.[52] Pigment also may be lost on subculture[75] or as effective treatment is initiated. *C. violaceum* has a fermentative, not oxidative, attack on carbohydrates.

Epidemiology

C. violaceum commonly is found in soil and water of areas with tropical or subtropical weather patterns. It also has been recovered from soil as far north as New Jersey.[23] Ten of the first 12 cases reported in the United States occurred in Florida; the other two were in Louisiana. Subsequently, one case from New Jersey[70] and one from Ohio[87] have been reported, along with more from southeastern states. All but one case occurred during summer.[75] Patients tend to be young, with a median age of 14 years.[75]

Pathophysiology

Although *C. violaceum* is a common inhabitant of soil and water, human infection is rare. Disorders of neutrophil function are important risk factors. A disproportionately high number of *C. violaceum* infections have occurred in patients with chronic granulomatous disease.[60] *C. violaceum* infection in a child with another disorder of neutrophil function, polymorphonuclear leukocyte glucose-6-phosphate dehydrogenase deficiency, has been reported.[61] Organisms may show variable virulence, and differences in endotoxin activity, resistance to phagocytosis, and production of catalase and hydrogen peroxide have been observed between clinical and soil isolates.[67] An elastase activity, expressed through the entire life cycle and produced by a zinc metalloproteinase, may account for the propensity of *C. violaceum* to cause abscesses.[102] Because *C. violaceum* is a free-living organism that is exposed to diverse environmental conditions, it exploits a wide range of energy resources and is able to thrive in aerobic and anaerobic conditions.[22] It is equipped to be tolerant of harsh conditions, including acid and ultraviolet stress, temperature stress, heavy metal exposure, and antibiotic exposure.[44] The complete sequencing of its genome has allowed researchers to identify putative virulence genes that are likely involved in host cell adhesion, cell invasion, and cytolysis.[15] Cytotoxicity is caused by pore formation on host cell membranes; this is mediated by a type 3 secretion system product of pathogenicity island 1.[66] A *Serratia*-type hemolysin has also been described.[17] *C. violaceum* is able to produce an exopolysaccharide biofilm.[12]

The organism usually gains entrance to the body through cuts or abrasions that come in contact with contaminated soil or water. After entrance, a localized infection usually develops at or around the site of entry, which is commonly followed by dissemination of infection via the bloodstream to distant sites. Rapid débridement and appropriate antibiotic therapy may arrest the infection at the wound stage.[55] Two cases of systemic infection occurred in near-drowning victims. One case report describes fulminant infection and death in an immunocompetent host from *C. violaceum* sepsis that began as conjunctivitis after a fall that splattered mud into the patient's eye.[28] Numerous microabscesses are found in multiple organs, especially liver, lung, and kidneys. Spread to bone, joints, and the central nervous system also has been described.

Clinical Manifestations

The pattern of illness in all reported patients is similar, with a contaminated inoculation site, localized disease, regional lymphadenopathy, and hematogenous spread to visceral organs. Progression of symptoms tends to be rapid after a variable incubation period.

Most patients have cutaneous lesions,[60] which are described as being nodular or pustular and sometimes with surrounding cellulitis.[93] They may progress to suppuration and drainage or to ulceration. Occasionally, classic ecthyma gangrenosum lesions have been described.[16] Regional lymphadenopathy is common; some of the nodes suppurate and require surgical drainage or removal.

Severe disease is heralded by high fever (39° C to 41° C [102.2° F to 105.8° F]), confusion or lethargy, abdominal pain, headaches, nausea and vomiting, and sometimes myalgias. Patients with systemic illness appear toxic. Hepatosplenomegaly develops frequently, and jaundice may be present. Progression from high fever and moderate toxicity to septic shock with disseminated intravascular coagulation and multisystem organ failure is precipitous. After the liver, the lung is the most common site of dissemination of infection, and evidence of pneumonia often is found. Adult respiratory distress syndrome is rare.[56] Brain and liver abscesses have been noted.[68] The overall mortality rate is 65 percent.[60] Rarely, there may be recurrence, which can prove fatal.[52,75] A review of all 25 pediatric cases reported between 1971 and 2005 revealed, counterintuitively, that the case-fatality rate was 12 of 16 (75%) in patients without chronic granulomatous disease and in none of 9 in patients with a known diagnosis of chronic granulomatous disease.[83] One case in a patient with chronic granulomatous disease who suffered three separate episodes of *C. violaceum* sepsis has been reported.[89]

A review of all cases reported between 1952 and 2009 (n = 106) found that all patients had fever. Sepsis developed in 82 percent of patients. Localized abscesses were found in about half of the patients, with the liver being the most common site. Relapse occurred in 6.6 percent, with a median interval of 135 days. Risk factors for mortality were young age at diagnosis, the presence of a localized abscess, a shorter clinical course, and inappropriate initial antimicrobial therapy.[99]

Diagnosis

Diagnosis is made by recovery of the organism from blood, lymph nodes, skin lesions, or abscesses. Gram-negative bacilli sometimes can be seen in smears of material from skin lesions. Laboratory values reveal either very low or very high white blood cell counts with marked left shifts. Mild to moderate anemia is common. Serum levels of liver enzymes may be elevated, and evidence of early renal failure sometimes is present. The organism grows readily and is easy to identify if it produces the characteristic violet pigment. Nonpigmented forms exist in soil and have similar virulence in mice[84] but are recovered only rarely from clinical specimens.[87] Other cases may have been missed, however, because the nonpigmented forms often are misidentified as *Aeromonas hydrophila* or as pseudomonads.[84] Reports that *C. violaceum* infection causes a melioidosis-like illness were probably due to misidentification of *Burkholderia pseudomallei* by the API 20NE system.[45]

A clinical history of contamination of a wound with water or soil, especially in the southeastern United States or in Southeast Asia, with subsequent local infection, lymph node suppuration, and lack of response to conventional antibiotic therapy, should arouse suspicion of *C. violaceum* infection. The clinician particularly should be aware of the possibility of *C. violaceum* infection in patients with chronic granulomatous disease.

Treatment

C. violaceum is sensitive in vitro to chloramphenicol, gentamicin, fluorinated quinolones, tetracyclines, imipenem, trimethoprim-sulfamethoxazole, and semisynthetic penicillins. *C. violaceum* often is resistant to cephalosporins, penicillin, ampicillin, and the antistaphylococcal penicillins. All isolates are resistant to vancomycin and rifampin.[5] Some strains have been shown to elaborate a β-lactamase, which is chromosomal and inducible in vitro.[27] At least one case report gives in vivo evidence of inducible resistance to ceftazidime.[93] Laboratory evidence of susceptibility to erythromycin cannot be relied on. In vitro, the fluoroquinolones have the highest activity.[5] Clinical experience with the fluoroquinolones in *C. violaceum* infection is scarce; an impressive case of a 4-month-old infant with severe disseminated *C. violaceum* infection who survived after therapy with trimethoprim-sulfamethoxazole and ciprofloxacin has been reported.[68] The review of 25 cases mentioned earlier found that all survivors had been given one or a combination of the following agents: ciprofloxacin, trimethoprim-sulfamethoxazole, chloramphenicol, or imipenem.[83]

Because infection with *C. violaceum* is rare and often rapidly fatal, optimal antimicrobial therapy is unknown. Duration of therapy also is unknown, but because of late recurrences, some authors recommend 3 to 4 weeks of intravenous therapy followed by 1 month or more of oral trimethoprim-sulfamethoxazole.[75]

PLESIOMONAS SHIGELLOIDES

Plesiomonas shigelloides is the only species for the genus *Plesiomonas.* These organisms are facultatively anaerobic, motile, gram-negative rods that are common inhabitants of surface water and fish. They have been implicated in gastrointestinal infections and, rarely, have been recovered from extraintestinal sites.

Bacteriology

P. shigelloides is a member of the Vibrionaceae, although some authorities suggest it is related more closely to Enterobacteriaceae.[59] The organisms are motile by means of a polar flagellum. They are lysine positive, ornithine positive, and arginine decarboxylase positive. They can be distinguished from Enterobacteriaceae by oxidase positivity. They also are catalase positive and indole positive. They grow well on MacConkey agar but not on thiosulfate citrate bile salts sucrose (TCBS) agar. Growth may be enhanced by the use of selective media, such as trypticase soy broth with ampicillin[76] and inositol brilliant green–bile salts agar. Growth is maximal at 40° C to 44° C and completely inhibited at 8° C. Usually within 24 hours, 1.0- to 1.5-mm grayish, shiny, opaque colonies with a slightly raised center and a smooth surface are visible. A few isolates of *P. shigelloides* share a common O antigen with *Shigella sonnei.*

Epidemiology

The organism is a ubiquitous freshwater inhabitant at temperatures greater than 8° C. It sometimes also is found in estuarial waters in temperate or tropical climates and can exist in sea water during the summer. It has been cultured from finfish, shellfish, pigs, birds, and dogs.[97] Although infection with *P. shigelloides* has been associated with ingestion of raw or improperly cooked fish (especially oysters), it is unknown what role, if any, other animals play in the ecology of the organism. Asymptomatic carriage of *P. shigelloides* is rare in developed countries[79] but may be 15 percent in some parts of China.[97]

Pathophysiology

Despite the ubiquity of the organism in nature, human infection is uncommon and has been recognized only more recently. Most often, infection is associated with gastroenteritis. Evidence for the role of *P. shigelloides* in the production of gastrointestinal symptoms is that it has been isolated much more frequently from patients with diarrhea than from healthy control subjects[42]; there have been some outbreaks, especially in Japan; it often is the only organism detected in the stools of patients with gastroenteritis[64]; and patients who have *P. shigelloides* growing from a stool culture recover more quickly with than without antibiotic therapy.[49] Acquisition of disease has been linked specifically to consumption of raw seafood or untreated water and to foreign travel, especially to Mexico.[39,63]

The mechanism by which the organism produces disease has been elusive. It is not enteroinvasive by laboratory tests, most investigators fail to find either a Shiga toxin or an enterotoxin,[1,39] no animal model of gastrointestinal disease has been found,[14] patients in the recovery phase do not show serologic evidence of infection, and inoculation of volunteers fails to produce illness.[39] In a suckling gnotobiotic piglet model in which the animals became septic, histology of the gastrointestinal tract showed neither destruction of cells nor invasion of tissues.[39] Neonatal BALB/c mice became chronically infected with *P. shigelloides*, however, and histopathologic findings included some cases of necrosis of the mucosal surface of the ileum and colon.[96]

Some potential virulence factors (i.e., a cholera-like toxin, a weak cytolysin, serum resistance, and a large [>150-kDa] plasmid) have been described, but their exact roles in pathogenesis are uncertain; attempts to correlate these features with virulence have been fruitless.[1] Investigators have characterized a cytotoxin derived from *P. shigelloides* using strains from patients with a diarrheal illness. The cytotoxin is a complex of three lipopolysaccharide-binding proteins and lipopolysaccharide in a 6:5 ratio.[72] Cytotoxic activity was inhibited 80 percent in vitro by proteinase K or when incubated with anticholera toxin antibody. This cytotoxin produced a positive reaction in the suckling mouse assay, whereas the purified lipopolysaccharide exhibited almost no cytotoxicity.[72]

Transmission electron microscopy has provided investigators with the first definitive proof that *P. shigelloides* can adhere to and invade eukaryotic intestinal cells. Organisms attached to microvilli and plasma membranes of the cells; they also were seen within vacuoles in the cell cytoplasm, suggesting a phagocytotic entry mechanism.[91] In one in vitro system using Caco-2 cells, adherence occurred within 10 minutes and internalization occurred within 60 minutes. Cytotoxicity was due to the induction of apoptosis.[94] In vitro, cell-free and cell-associated hemolysis is demonstrable,[35] and low levels of elastase, proteinase, histidine decarboxylase, and moderate levels of triacylglycerol lipase activity have been found.[21] Culture filtrates are capable of inducing vacuolation of a variety of mammalian cells in vitro.[26]

P. shigelloides rarely, but more clearly, is a pathogen in extraintestinal sites. Osteomyelitis, endophthalmitis, cholecystitis, pseudoappendicitis, spontaneous peritonitis,[4] meningitis,[90] and septicemia have been reported sporadically.[14] Most patients with septicemia have been immunocompromised hosts, but the organism has been isolated from blood cultures in otherwise healthy individuals.[46,74] The mode of infection in extraintestinal sites is unclear, but most cases are thought to arise from the gastrointestinal tract. The case of a newborn with *P. shigelloides* meningitis, septicemia, and endophthalmitis who was born to a mother who reported severe diarrhea after eating raw oysters 2 weeks before delivery raises the possibility of transplacental transmission.[62]

Clinical Manifestations

Patients with *P. shigelloides* gastroenteritis complain of diarrhea, crampy abdominal pain, nausea and vomiting, headache, and fever. Symptoms usually begin 24 hours to

4 days after contact with the organism.[42] Diarrhea tends to be secretory, although some patients have symptoms more consistent with colitis.[49] Passage of blood, mucus, or both in the stools is a common manifestation, as is the presence of white blood cells by Wright stain.[42] Patients with *P. shigelloides* gastroenteritis tend to have disease that is more acute, associated with more severe abdominal pain, and of longer duration than patients with diseases caused by other enteropathogens.[49]

In one case-control study, 76 percent of patients were sick for more than 2 weeks and 32 percent were sick for more than a month.[49] In contrast, a large Japanese study of returning travelers suggested that for most patients the symptoms abated within approximately 3 days.[82] In a series of 38 pediatric cases in Bangladesh, 84 percent had secretory diarrhea and 71 percent had associated emesis. Only three patients (8%) had fever, and only five (13%) had diarrhea for 14 days or longer.[54] In a separate report, one child developed migratory polyarthritis during an otherwise typical case of culture-proven gastrointestinal infection. All symptoms and signs of arthritis disappeared when the gastrointestinal infection was treated with antibiotics.[36]

Localized extraintestinal infections have been reported but are rare. Two cases of severe polymicrobial endophthalmitis resulting from fishhook trauma progressed to enucleation.[18] Two cases of peritonitis associated with continuous ambulatory peritoneal dialysis were reported from Hong Kong. Both patients recovered after 10 days of therapy with intraperitoneal cefazolin and tobramycin.[98] In one odd case, a woman had pyosalpinx from *P. shigelloides* thought to have been acquired by swimming in contaminated water.[80] A cavitary pneumonia was found in a 78-year-old woman who underwent gastrectomy and esophagojejunostomy because of adenocarcinoma of the stomach. Biopsy showed granulomatous changes.[81] One patient developed a skin abscess after incurring trauma in fresh water.[40]

Septicemia, meningitis, or both usually occur in immunocompromised hosts. Severe, rapidly progressive sepsis complicated by disseminated intravascular coagulation, adult respiratory distress syndrome, renal insufficiency, hepatic dysfunction, adrenal hemorrhage, and splenic abscess has occurred in patients with hemoglobinopathies and absence of splenic function, including thalassemia intermedia and sickle-cell disease.[7,95] Unusual sites of infection sometimes are noted in immunocompromised individuals, such as epididymo-orchitis in a patient with human immunodeficiency virus infection.[101] Newborns constitute most of the reported cases of *P. shigelloides* meningitis, in whom the mortality rate is 80 percent.[1] Cerebrospinal fluid parameters may be normal, even when the culture is positive.[73] Septicemia also has a high mortality rate in adults, although otherwise well patients may recover with appropriate antimicrobial therapy.

Diagnosis

A clinical history of foreign travel or of ingestion of raw seafood or untreated water should raise suspicion of possible *P. shigelloides* infection, especially when the clinical illness matches the description just mentioned. Oxidase tests should be done on any predominant or solitary organisms to distinguish them from Enterobacteriaceae.[42] The organisms can be shown not to be aeromonads or pseudomonads by production of ornithine decarboxylase and fermentation of inositol. Selective medium can be used if the index of suspicion is high. A new indicator medium (UNISC agar) for the identification of *Aeromonas* and *Plesiomonas* has been developed; colonies turn yellow if xylose is fermented and turn blue if it is not.[78]

Treatment

Most strains of *P. shigelloides* produce a β-lactamase,[77] which seems to be specific for the penicillins. In one study, all isolates were resistant to ampicillin, ticarcillin, carbenicillin, and piperacillin.[32] *P. shigelloides* is universally susceptible to trimethoprim-sulfamethoxazole, the fluoroquinolones, most cephalosporins, carbapenems,[88] tetracycline,[88] and chloramphenicol. It is variably susceptible to the aminoglycosides and mostly resistant to erythromycin.[48]

P. shigelloides gastroenteritis resolves without therapy, but the illness may be prolonged. Treatment seems to shorten the course.[49] Extraintestinal infections carry a poor prognosis and should be treated aggressively. For meningitis, cephalosporins and carbapenems have good cerebrospinal fluid penetration and are effective therapy against most isolates.

OTHER PASTEURELLA ORGANISMS

The genus *Pasteurella* consists of a group of pleomorphic, gram-negative coccobacilli that are part of the normal flora of many animals. These organisms are frequent animal pathogens. *P. multocida* is a common human pathogen and is discussed in Chapter 116. The other species of the genus *Pasteurella* are rare but occasionally cause serious infection in humans.

Bacteriology

These organisms, similar to *P. multocida*, grow readily on most common laboratory media, including blood agar. Most of the species do not grow on MacConkey agar. They are non–spore forming, nonmotile, aerobic, and facultatively anaerobic. These glucose-fermenting organisms all are oxidase positive. Most are nitrate positive and catalase positive, and all except *Avibacterium gallinarum* (formerly *Pasteurella gallinarum*, see later) produce indole. They are small, coccoid or rod-shaped bacilli that may show prominent bipolar staining on Gram stain. Colonies are small, translucent, and gray and may be smooth or rough. A browning discoloration may develop around them, and they are nonhemolytic and have a distinctive musty or "mushroom" odor.[20]

The taxonomy of these organisms is confusing and continues to be revised. *Pasteurella ureae* and *Pasteurella pneumotropica* have been moved into the genus *Actinobacillus*. *Pasteurella haemolytica* has been reclassified into a new

genus, *Mannheimia*.[11] *Pasteurella dagmatis* is the name now given to what formerly was called *P.* new species, or P. "gas."[69] *Pasteurella gallinarum*, *Pasteurella avium*, and *Pasteurella volantium* have 96.8 percent sequence similarity and are phenotypically separate from other *Pasteurella* spp.; this feature has led to a proposal that they be moved into a new genus, *Avibacterium*.[13] Most experts agree that the genus *Pasteurella sensu stricto* should include *P. multocida*, *Pasteurella canis*, *Pasteurella stomatis*, and *P. dagmatis*.[50] *Pasteurella* spp. B has now been reclassified as *P. oralis* sp nov.[19] Clinically recovered species other than *P. multocida* include *Actinobacillus ureae*, *Mannheimia haemolytica*, *Actinobacillus pneumotropica*, *P. dagmatis*, *P. canis*, *Pasteurella aerogenes*, *Pasteurella bettyae*, *P. "SP"* group, and *P. stomatis*. *Pasteurella caballi* has caused wound infections after horse bites.[24] *Avibacterium gallinarum* is an extremely rare pathogen in humans.

Pathophysiology

Infection with *Pasteurella* spp. has been divided clinically into three types of infection: (1) from animal bites, (2) from animal contact,[31] and (3) without known animal contact.[22] Infections from animal bites include cellulitis, abscesses, tenosynovitis, and bone and joint infection, but infection can become generalized, especially in patients who are immunocompromised. Infections caused by animal contact can be similar to infections described earlier and are caused by animals licking broken skin or wounds. Sometimes pulmonary infections occur, possibly related to aerosolization of organisms. In some cases, these organisms have been reported as respiratory flora in patients with pets, but at present no proof exists that such colonization is an antecedent of infection.[29] Cases without known animal contact history constitute 3 to 30 percent of all cases.[43,47]

Infection usually occurs when the organisms are inoculated into deeper tissues either on animal teeth that break the skin or in animal saliva that comes in contact with nonintact skin. Bite wound infections often are polymicrobial. Infection in cases without known animal contact is harder to explain but definitely happens.[51] Most cases of serious infection occur in patients with underlying diseases, such as diabetes mellitus, chronic alcoholism, and other types of liver disease.[71] Central nervous system infection with these organisms has occurred after head trauma or neurosurgery in 10 of 11 reported cases.[51] One intrauterine death of the fetus of a 20-year-old woman who worked at a pig farm has been attributed to *P. aerogenes*.[92] Case reports of unusual infections with rare species of *Pasteurella*, such as fatal sepsis and meningitis in a 4-day-old infant caused by *A. gallinarum*,[2] should be considered doubtful unless molecular identification methods have been used.[38]

Clinical Manifestations

Pasteurella infections produce pain, swelling, pus, and sometimes abscess formation at the site of inoculation, beginning within 24 to 36 hours. Clinically, these infections are indistinguishable from wound infections with *Staphylococcus aureus* or other gram-positive organisms. Gram stain may show the characteristic pleomorphic bacilli with bipolar staining. Growth on standard agar is rapid.

Patients with peritonitis,[4,71] meningitis,[51] osteomyelitis,[32] or infectious endocarditis[6] have symptoms typical of these diagnoses. Risk factors, such as household pet exposure, animal contact, animal bites, and comorbid conditions, should heighten suspicion of possible *Pasteurella* infection. One report describes a previously healthy 21-month-old girl who developed *P. canis* bacteremia after exposure to rabbit saliva.[100] Bacteremia due to *P. canis* has also been described in a cirrhotic patient whose leg wound was licked by a dog.[3]

Diagnosis

Establishing the diagnosis of *Pasteurella* infection can be difficult, not because the organism is fastidious or slow growing but because it often is misidentified. Other organisms of the same family (i.e., *Actinobacillus* spp. and *Haemophilus* spp.) have similar biochemical profiles and can be misidentified by commonly used systems, such as API. Lester and associates[57] reported that of 30 species firmly identified as *Pasteurella* by biochemical means, only 3 were identified correctly by the API 20E system. *Actinobacillus* (*Haemophilus) aphrophilus* and *Actinobacillus actinomycetemcomitans* are sometimes misidentified as *Avibacterium gallinarum* by commercial systems.[30] Additionally, Hamilton-Miller[37] reported that four strains of *Haemophilus influenzae* and three strains of *Haemophilus parainfluenzae* were identified falsely as *Pasteurella* spp. by API and suggested that if the clinical history renders *Pasteurella* infection unlikely then tests for X and V factor requirements should be performed (see Chapter 133). Clinical case reports corroborate these laboratory observations.[25,71,86] Notifying the bacteriology laboratory of suspicion of *Pasteurella* infection is helpful.

Treatment

Penicillin has been considered the drug of choice for *Pasteurella* infection in the past and, despite some reports of penicillin resistance, still is effective against most strains. *Pasteurella* spp. also are susceptible to ampicillin, β-lactamase–inhibitor combination drugs, tetracycline, and chloramphenicol. Truncated but fully functional tetracycline resistance genes have been identified in isolates from animal sources.[53] The aminoglycosides, erythromycin, clindamycin, cefadroxil, and cefaclor are not recommended. Dicloxacillin and cephalexin have poor activity against *Pasteurella* spp. and should not be used as monotherapy for animal bite wounds.[33] In one study of bite wound infections, ertapenem was found to be active against all *Pasteurella* spp.[34] Of fluoroquinolones tested in vitro against 75 clinical isolates of *Pasteurella* spp., levofloxacin was the most active.[41]

Acknowledgments

I would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Albert TJ, Stevens DL. The first case of *Pasteurella canis* bacteremia: a cirrhotic patient with an open leg wound. *Infection* 2010; **38**:483–5.
9. Antonisamy P, Ignacimuthu S. Immunomodulatory, analgesic and antipyretic effect of violacein isolated from *Chromobacterium violaceum*. *Phytomedicine* 2010;**17**:300–4.
10. Antonisamy P, Kannan P, Ignacimuthu S. Anti-diarrhoeal and ulcer-protective effects of violacein isolated from *Chromobacterium violaceum* in Wistar rats. *Fundam Clin Pharmacol* 2009;**23**: 483–90.
12. Becker S, Soares C, Porto LM. Computational analysis suggests that virulence of *Chromobacterium violaceum* might be linked to biofilm formation and poly-NAG biosynthesis. *Genet Mol Biol* 2009;**32**:640–4.
19. Christensen H, Bertelsen MF, Bojesen AM, Bisgaard M. Classification of *Pasteurella* sp. B as *Pasteurella oralis* sp nov. *Int J Syst Evol Microbiol* 2012;**62**(Pt 6):1396–401.
58. Lopes SC, Blanco YC, Justo GZ, et al. Violacein extracted from *Chromobacterium violaceum* inhibits *Plasmodium* growth in vitro and in vivo. *Antimicrob Agents Chemother* 2009;**53**:2149–52.
66. Miki T, Iguchi M, Akiba K, et al. *Chromobacterium* pathogenicity island 1 type III secretion system is a major virulence determinant for *Chromobacterium violaceum*-induced cell death in hepatocytes. *Mol Microbiol* 2010 [Epub ahead of print].
73. Ozdemir O, Sari S, Terzioglu S, Zenciroglu A. *Plesiomonas shigelloides* sepsis and meningoencephalitis in a surviving neonate. *J Microbiol Immunol Infect* 2010;**43**:344–6.
78. Rocha MP, Scheid LA, Alves SH. New selective indicator medium for detection of *Aeromonas* and *Plesiomonas:* UNISC agar. *Rev Soc Bras Med Trop* 2008;**41**:431–3.
81. Schneider F, Lang N, Reibke R, et al. *Plesiomonas shigelloides* pneumonia. *Med Mal Infect* 2009;**39**:397–400.
89. Sureisen M, Choon SK, Tai CC. Recurrent *Chromobacterium violaceum* infection in a patient with chronic granulomatous disease. *Med J Malaysia* 2008;**63**:346–7.
99. Yang CH, Li YH. *Chromobacterium violaceum* infection: a clinical review of an important but neglected infection. *J Chin Med Assoc* 2011;**74**:435–41.
100. Yefet E, Abolzaid S, Nasser W, et al. Unusual infection—*Pasteurella canis* bacteremia in a child after exposure to rabbit secretions. *Harefuah* 2011;**150**:13–15.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 121

Acinetobacter

Armando G. Correa

First recognized as a human pathogen in 1908,[79] the ubiquitous organism *Acinetobacter* has emerged as a rather common cause of nosocomial infections in immunocompromised hosts.[8] Some of the confusion regarding this organism may be attributed to the many changes in nomenclature that the members of this genus have undergone over the years. Names used in the past to identify this genus include *Herellea*, *Bacterium*, *Mima*, *Achromobacter*, *Alcaligenes*, *Neisseria*, *Micrococcus*, *Diplococcus*, *Moraxella*, and *Cytophaga*. Treatment of infection caused by *Acinetobacter* is complicated by its widespread multidrug resistance and the difficulty encountered in eradicating the organism.

THE ORGANISM

The genus *Acinetobacter* belongs to the Neisseriaceae, which also includes the *Neisseria*, *Moraxella*, and *Kingella* genera. *Acinetobacter* is a gram-negative bacterium that typically appears as a rod 0.9 to 1.6 μm in diameter and 1.5 to 2.5 μm in length, but it may become spherical in the stationary phase of growth. It frequently occurs in pairs or short chains. Many strains are encapsulated. The organism has a strictly aerobic respiratory metabolism and does not grow under anaerobic conditions. It does not form spores or exhibit swimming mobility. *Acinetobacter* grows well in all common complex media between 20° C and 30° C, with optimal growth occurring between 33° C and 35° C, and it has no growth factor requirements.

Convex, grayish white colonies 1.0 to 2.5 mm in diameter are typical findings. The colonies may appear mucoid if the strain is encapsulated. *Acinetobacter* is catalase positive and may be differentiated readily from other closely related genera by virtue of its negative reaction to oxidase.

Formerly, the genus *Acinetobacter* contained the single species *Acinetobacter calcoaceticus* subdivided into two subspecies or biovars: *anitratus* and *lwoffii*.[37] However, in 1986, the taxonomy of the genus *Acinetobacter* was changed extensively on the basis of DNA hybridization studies,[13] and at least 32 genomic species have been

REFERENCES

1. Abbott SL, Kokka RP, Janda JM. Laboratory investigations on the low pathogenic potential of *Plesiomonas shigelloides*. *J Clin Microbiol* 1991;**29**:148–53.
2. Ahmed K, Sein PP, Shahnawaz M, Hoosen AA. *Pasteurella gallinarum* neonatal meningitis. *Clin Microbiol Infect* 2002;**8**:55–7.
3. Albert TJ, Stevens DL. The first case of *Pasteurella canis* bacteremia: a cirrhotic patient with an open leg wound. *Infection* 2010;**38**: 483–5.
4. Alcaniz JP, de Cuenca Moron B, Gomez Rubio M, et al. Spontaneous bacterial peritonitis due to *Plesiomonas shigelloides*. *Am J Gastroenterol* 1995;**90**:1529–30.
5. Aldridge KE, Valainis GT, Sanders CV. Comparison of the in vitro activity of ciprofloxacin and twenty-four other antimicrobial agents against clinical strains of *Chromobacterium violaceum*. *Diagn Microbiol Infect Dis* 1988;**10**:31–8.
6. al-Fadel Saleh M, al-Madan MS, Erwa HH, et al. First case of human infection caused by *Pasteurella gallinarum* causing infective endocarditis in an adolescent ten years after surgical correction for truncus arteriosus. *Pediatrics* 1995;**95**:944–8.
7. Ampofo K, Graham P, Ratner A, et al. *Plesiomonas shigelloides* sepsis and splenic abscess in an adolescent with sickle-cell disease. *Pediatr Infect Dis J* 2001;**20**:1178–9.
8. Andrighetti-Frohner CR, Antonio RV, Creczynski-Pasa TB, et al. Cytotoxicity and potential antiviral evaluation of violacein produced by *Chromobacterium violaceum*. *Mem Inst Oswaldo Cruz* 2003; **98**:843–8.
9. Antonisamy P, Ignacimuthu S. Immunomodulatory, analgesic and antipyretic effect of violacein isolated from *Chromobacterium violaceum*. *Phytomedicine* 2010;**17**:300–4.
10. Antonisamy P, Kannan P, Ignacimuthu S. Anti-diarrhoeal and ulcer-protective effects of violacein isolated from *Chromobacterium violaceum* in Wistar rats. *Fundam Clin Pharmacol* 2009;**23**: 483–90.
11. Angen O, Mutters R, Caugant DA, et al. Taxonomic relationships of the [*Pasteurella*] *haemolytica* complex as evaluted by DNA-DNA hybridizations and 16S rRNA sequencing with proposal of *Mannheimia haemolytica* gen. *nov*., comb. *nov*., *Mannheimia granulomatis* comb. *nov*., *Mannheimia glucosida* sp. *nov*., *Mannheimia ruminalis* sp. *nov*. and *Mannheimia varigena* sp. *nov*. *Int J Syst Bacteriol* 1999;**49**:67–86.
12. Becker S, Soares C, Porto LM. Computational analysis suggests that virulence of *Chromobacterium violaceum* might be linked to biofilm formation and poly-NAG biosynthesis. *Genet Mol Biol* 2009;**32**:640–4.
13. Blackall PJ, Christensen H, Beckenham T, et al. Reclassification of *Pasteurella gallinarum*, [*Haemophilus*] *paragallinarum*, *Pasteurella avium* and *Pasteurella volantium* as *Avibacterium gallinarum* gen. *nov*., comb. *nov*., *Avibacterium paragallinarum* comb. *nov*., *Avibacterium avium* comb. *nov.* and *Avibacterium volantium* comb. *nov*. *Int J Syst Evol Microbiol* 2005;**55**:353–62.
14. Brendan RA, Miller MA, Janda JM. Clinical disease spectrum and pathogenic factors asssociated with *Plesiomonas shigelloides* infections in humans. *Rev Infect Dis* 1988;**10**:303–16.
15. Brito CF, Carvalho CB, Santos F, et al. *Chromobacterium violaceum* genome: molecular mechanisms associated with pathogenicity. *Genet Mol Res* 2004;**31**:148–61.
16. Brown KL, Stein A, Morrell DS. Ecthyma gangrenosum and septic shock syndrome secondary to *Chromobacterium violaceum*. *J Am Acad Dermatol* 2006;**54**(Suppl. 5):S224–8.
17. Brumbach KC, Eason BD, Anderson LK. The *Serratia*-type hemolysin of *Chromobacterium violaceum*. *FEMS Microbiol Lett* 2007;**267**:243050.
18. Butt AA, Figueroa J, Martin DH. Ocular infection caused by three unusual marine organisms. *Clin Infect Dis* 1997;**24**:740–1.
19. Christensen H, Bertelsen MF, Bojesen AM, Bisgaard M. Classification of *Pasteurella* sp. B as *Pasteurella oralis* sp nov. *Int J Syst Evol Microbiol* 2012;**62**(Pt 6):1396–401.
20. Citron DM, Edelstein MA, Garcia LS, et al. *Pasturella* and similar organisms. In: Baron EJ, Peterson LR, Finegold SM, editors. *Bailey and Scott's diagnostic microbiology*. 9th ed. St. Louis: Mosby; 1994. p. 420–3.
21. Ciznar I, Hostacka A, Gonzalez-Rey C, Krovacek K. Potential virulence-associated properties of *Plesiomonas shigelloides* strains. *Folia Microbiol* 2004;**49**:543–8.
22. Creczynski-Pasa TB, Antonio RV. Energetic metabolism of *Chromobacterium violaceum*. *Genet Mol Res* 2004;**31**:162–6.
23. Duma RJ. Aztreonam, the first monobactam. *Ann Intern Med* 1987;**106**:766.
24. Escande F, Vallee E, Aubart F. *Pasteurella caballi* infection following a horse bite. *Zentralbl Bakteriol* 1997;**285**:440–4.
25. Fajfar-Whetsone CJT, Coleman L, Biggs DR, et al. *Pasteurella multocida* septicemia and subsequent *Pasteurella dagmatis* septicemia in a diabetic patient. *J Clin Microbiol* 1995;**33**:202–4.
26. Falcon R, Carbonell GV, Figueredo PM, et al. Intracellular vacuolation induced by culture filtrates of *Plesiomonas shigelloides* isolated from environmental sources. *J Appl Microbiol* 2003;**95**: 273–8.
27. Farrar Jr WE, O'Dell NM. Beta-lactamase activity in *Chromobacterium violaceum*. *J Infect Dis* 1976;**134**:290–3.
28. Feldman RB, Stern GA, Hood I. *Chromobacterium violaceum* infection of the eye. *Arch Ophthalmol* 1984;**102**:711–13.
29. Frebourg NB, Berthelot G, Hocq R, et al. Septicemia due to *Pasteurella pneumotropica*: 16srRNA sequencing for diagnosis confirmation. *J Clin Microbiol* 2002;**40**:687–9.
30. Fredericksen W, Tonning B. Possible misidentification of *Haemophilus aphrophilus* as *Pasteurella gallinarum*. *Clin Infect Dis* 2001; **32**:987–8.
31. Furie RA, Cohen RP, Hartman BJ, et al. *Pasteurella multocida* infection: report in urban setting and review of spectrum of human disease. *N Y State Med J* 1980;**80**:1597–602.
32. Gadberry JL, Zipper R, Taylor JA, et al. *Pasteurella pneumotropica* isolated from bone and joint infections. *J Clin Microbiol* 1984; **19**:926–7.
33. Goldstein EJ Citron DM. Comparative activities of cefuroxime, amoxicillin-clavulanic acid, ciprofloxacin, enoxacin, and ofloxacin against aerobic and anaerobic bacteria isolated from bite wounds. *Antimicrob Agents Chemother* 1988;**32**:1143–8.
34. Goldstein EJ, Citron DM, Merriam CV, et al. Comparative in vitro activity of ertapenem and 11 other antimicrobial agents against aerobic and anaerobic pathogens isolated from skin and soft tissue animal and human bite wound infections. *J Antimicrob Chemother* 2001;**48**:641–51.
35. Gonzalez-Rodriguez N, Santos JA, Otero A, Garcia-Lopez ML. Cell-associated hemolytic activity in environmental strains of *Plesiomonas shigelloides* expressing cell-free, iron-influenced extracellular hemolysin. *J Food Prot* 2007;**70**:885–90.
36. Gupta S. Migratory polyarthritis associated with *Plesiomonas shigelloides* infection. *Scand J Rheumatol* 1995;**24**:323–5.
37. Hamilton-Miller JM. A possible pitfall in the identification of *Pasteurella* spp. with the API system. *J Med Microbiol* 1993; **39**:78–9.
38. Hamilton-Miller JM. Distinguishing *Pasteurella* spp. from *Haemophilus* spp.: the problem revisited. *Clin Microbiol Infect* 2003; **9**:243.
39. Herrington DA, Tzipori S, Robins-Browne RM, et al. In vitro and in vivo pathogenicity of *Plesiomonas shigelloides*. *Infect Immun* 1987; **55**:979–85.
40. Herve V, Bigaillon C, Duhamel P, et al. [Cutaneous abscess due to *Plesiomonas shigelloides* consecutive to a trauma in fresh water.] *Med Mal Infect* 2007;**37**:840. In French.
41. Heurtin C, Desbordes L, Travert MF, et al. Comparative study of the bacteriostatic and bactericidal activity of levofloxacin against *Pasteurella* strains isolated from man. *Pathol Biol (Paris)* 2001;**49**: 606–11.
42. Holmberg SD, Wachsmuth K, Hickman-Brenner FW, et al. *Plesiomonas* enteric infections in the United States. *Ann Intern Med* 1986;**105**:690–4.
43. Holst E, Rollof J, Larsson L, et al. Characterization and distribution of *Pasteurella* species recovered from infected humans. *J Clin Microbiol* 1992;**30**:2984–7.
44. Hungria M, Nicolas MF, Guimaraes CT, et al. Tolerance to stress and environmental adaptability of *Chromobacterium violaceum*. *Genet Mol Res* 2004;**31**:102–16.
45. Inglis TJ, Chiang D, Lee GS, Chor-Kiang L. Potential misidentification of *Burkholderia pseudomallei* by API 20NE. *Pathology* 1998;**30**:62–4.
46. Ingram CW, Morrison Jr AJ, Levitz RE. Gastroenteritis, sepsis, and osteomyelitis caused by *Plesiomonas shigelloides* in an immunocompetent host: case report and review of the literature. *J Clin Microbiol* 1987;**25**:1791–3.

47. Jones Jr FL, Smull CE. Infections in man due to *Pasteurella multocida*: importance of human carrier. *Pa Med J* 1985;**76**:41–4.
48. Kain KC, Kelly MT. Antimicrobial susceptibilities of *Plesiomonas shigelloides* from patients with diarrhea. *Antimicrob Agents Chemother* 1989;**33**:1609–10.
49. Kain KC, Kelly MT. Clinical features, epidemiology, and treatment of *Plesiomonas shigelloides* diarrhea. *J Clin Microbiol* 1989;**27**: 998–1001.
50. Kainz A, Lubitz W, Busse HJ. Genomic fingerprints, ARDRA profiles and quinone systems for classification of *Pasteurella sensu stricto*. *Syst Appl Microbiol* 2000;**23**:494–503.
51. Kaka S, Lunz R, Klugman KP. *Actinobacillus (Pasteurella) ureae* meningitis in an HIV-positive patient. *Diagn Microbiol Infect Dis* 1994;**20**:105–7.
52. Kaufman SC, Ceraso D, Schugurensky A. First case report from Argentina of fatal septicemia caused by *Chromobacterium violaceum*. *J Clin Microbiol* 1986;**23**:956–8.
53. Kehrenberg C, Schwartz S. Identification of a truncated, but functionally active tet(H) tetracycline resistance gene in *Pasteurella aerogenes* and *Pasteurella multocida*. *FEMS Microbiol Lett* 2000; **188**:191–5.
54. Khan AM, Faruque AS, Hossain MS, et al. *Plesiomonas shigelloides*–associated diarrhea in Bangladeshi children: a hospital-based surveillance study. *J Trop Pediatr* 2004;**50**:354–6.
55. Lee J, Kim JS, Nahm CH, et al. Two cases of *Chromobacterium violaceum* infection after injury in a subtropical region. *J Clin Microbiol* 1999;**37**:2068–70.
56. Leet S, Wright BD. Fulminating chromobacterial septicemia presenting as respiratory distress syndrome. *Thorax* 1981;**36**: 557–9.
57. Lester A, Jarlov JO, Westh H, et al. *Pasteurella haemolytica* diagnosis questioned. *J Infect* 1992;**25**:334–5.
58. Lopes SC, Blanco YC, Justo GZ, et al. Violacein extracted from *Chromobacterium violaceum* inhibits *Plasmodium* growth in vitro and in vivo. *Antimicrob Agents Chemother* 2009;**53**:2149–52.
59. MacDonell MT, Colwell RR. Phylogeny of the Vibrionaceae, and recommendation for two new genera, *Listonella* and *Shewanella*. *Syst Appl Microbiol* 1985;**6**:171–82.
60. Macher AM, Casale TB, Fauci AS. Chronic granulomatous disease of childhood and *Chromobacterium violaceum* infections in the southeastern United States. *Ann Intern Med* 1982;**97**:51–5.
61. Mamlok RJ, Mamlok V, Mills GC, et al. Glucose-6-phosphate dehydrogenase deficiency, neutrophil dysfunction, and *Chromobacterium violaceum* sepsis. *J Pediatr* 1987;**111**:852–4.
62. Marshman WE, Lyons CJ. Congenital endophthalmitis following maternal shellfish ingestion. *Aust N Z J Ophthalmol* 1998;**26**: 161–3.
63. Martin DL, Gustafson TL. *Plesiomonas* gastroenteritis in Texas. *JAMA* 1985;**15**:2063.
64. McNeely D, Ivy P, Craft JC, et al. *Plesiomonas*: biology of the organism and disease in children. *Pediatr Infect Dis* 1984;**3**: 176–81.
65. Melo PS, Maria SS, Vidal BC, et al. Violacein cytotoxicity and induction of apoptosis in V79 cells. *In Vitro Cell Dev Biol Anim* 2000;**36**:539–43.
66. Miki T, Iguchi M, Akiba K, et al. *Chromobacterium* pathogenicity island 1 type III secretion system is a major virulence determinant for *Chromobacterium violaceum*-induced cell death in hepatocytes. *Mol Microbiol* 2010 [Epub ahead of print].
67. Miller DP, Blevins WT, Steele DB, et al. A comparative study of virulent and avirulent strains of *Chromobacterium violaceum*. *Can J Microbiol* 1988;**32**:249–55.
68. Moore CC, Lane JE, Stephens JL. Successful treatment of an infant with *Chromobacterium violaceum* sepsis. *Clin Infect Dis* 2001;**32**:e107–10.
69. Mutters R, Ihm P, Pohl S, et al. Reclassification of the genus *Pasteurella* Trivisan 1887 on the basis of deoxyribonucleic acid homology, with proposals for the new species *Pasteurella dagmatis*, *Pasteurella canis*, *Pasteurella stomatis*, *Pasteurella anatis*, and *Pasteurella langaa*. *Int J Syst Bacteriol* 1985;**35**:309.
70. Myers J, Ragasa DA, Eisole C. *Chromobacterium violaceum* septicemia in New Jersey. *J Med Soc N J* 1982;**79**:213–14.
71. Noble RC, Marek BJ, Overman SB. Spontaneous bacterial peritonitis caused by *Pasteurella ureae*. *J Clin Microbiol* 1987;**25**: 442–4.
72. Okawa Y, Ohtomo Y, Tsugawa H, et al. Isolation and characterization of a cytotoxin produced by *Plesiomonas shigelloides* P-1 strain. *FEMS Microbiol Lett* 2004;**239**:125–30.
73. Ozdemir O, Sari S, Terzioglu S, Zenciroglu A. *Plesiomonas shigelloides* sepsis and meningoencephalitis in a surviving neonate. *J Microbiol Immunol Infect* 2010;**43**:344–6.
74. Paul R, Siitonen A, Karkkainen P. *Plesiomonas shigelloides* bacteremia in a healthy girl with mild gastroenteritis. *J Clin Microbiol* 1990;**28**:1445–6.
75. Ponte R, Jenkins SG. Fatal *Chromobacterium violaceum* infections associated with exposure to stagnant waters. *Pediatr Infect Dis J* 1992;**11**:583–6.
76. Rahim Z, Kay BA. Enrichment for *Plesiomonas shigelloides* from stools. *J Clin Microbiol* 1988;**26**:789–90.
77. Reinhardt JF, George WL. Comparative in vitro activities of selected antimicrobial agents against *Aeromonas* species and *Plesiomonas shigelloides*. *Antimicrob Agents Chemother* 1985;**27**: 643–5.
78. Rocha MP, Scheid LA, Alves SH. New selective indicator medium for detection of *Aeromonas* and *Plesiomonas:* UNISC agar. *Rev Soc Bras Med Trop* 2008;**41**:431–3.
79. Rolston KVI, Hopfer RL. Diarrhea due to *Plesiomonas shigelloides* in cancer patients. *J Clin Microbiol* 1984;**20**:597–8.
80. Roth T, Hentsch C, Erard P, Tschantz P. Pyosalpinx: Not always a sexual transmitted disease? Pyosalpinx caused by *Plesiomonas shigelloides* in an immunocompetent host. *Clin Microbiol Infect* 2002;**8**:803–5.
81. Schneider F, Lang N, Reibke R, et al. *Plesiomonas shigelloides* pneumonia. *Med Mal Infect* 2009;**39**:397–400.
82. Shigematsu M, Kaufmann ME, Charlett A, et al. An epidemiologic study of *Plesiomonas shigelloides* diarrhea among Japanese travellers. *Epidemiol Infect* 2000;**125**:523–30.
83. Sirinavin S, Techasaensiri C, Benjaponpitak S, et al. Invasive *Chromobacterium violaceum* infection in children: case report and review. *Pediatr Infect Dis J* 2005;**24**:559–61.
84. Sivendra R, Tan SH. Pathogenicity of unpigmented cultures of *Chromobacterium violaceum*. *J Clin Microbiol* 1977;**5**:514–16.
85. Sneath PHA. Genus *Chromobacterium*. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*. Baltimore: Williams & Wilkins; 1984. p. 580–2.
86. Sorbello AF, O'Donnell J, Kaiser-Smith J, et al. Infective endocarditis due to *Pasteurella dagmatis*: case report and review. *Clin Infect Dis* 1994;**18**:336–8.
87. Sorenson RU, Jacobs MR, Shurin SB. *Chromobacterium violaceum* adenitis acquired in the northern United States as a complication of chronic granulomatous disease. *Pediatr Infect Dis J* 1985;**4**: 701–2.
88. Stock I, Wiedemann B. Natural antimicrobial susceptibilities of *Plesiomonas shigelloides* strains. *J Antimicrob Chemother* 2001;**48**: 803–11.
89. Sureisen M, Choon SK, Tai CC. Recurrent *Chromobacterium violaceum* infection in a patient with chronic granulomatous disease. *Med J Malaysia* 2008;**63**:346–7.
90. Terpeluk C, Goldman A, Bartmann P, et al. *Plesiomonas shigelloides* sepsis and meningoencephalitis in a neonate. *Eur J Pediatr* 1992; **151**:499–501.
91. Theodoropoulos C, Wong TH, O'Brien M, Stenzel D. *Plesiomonas shigelloides* enters polarized human intestinal Caco-2 cells in an in vitro model system. *Infect Immun* 2001;**69**:2260–9.
92. Thorsen P, Moller BR, Arpi M, et al. *Pasteurella aerogenes* isolated from stillbirth and mother. *Lancet* 1994;**343**:485–6.
93. Ti TY, Tan WC, Chong APY, et al. Nonfatal and fatal infections caused by *Chromobacterium violaceum*. *Clin Infect Dis* 1993;**17**: 505–7.
94. Tsugawa H, Ono T, Murakami H, Okawa Y. Invasive phenotype and apoptosis induction of *Plesiomonas shigelloides* P-1 strain to Caco-2 cells. *J Appl Microbiol* 2005;**99**:1235–443.
95. Tzanetea R, Konstantopoulos K, Xanthaki A, et al. *Plesiomonas shigelloides* sepsis in a thalassemia intermedia patient. *Scand J Infect Dis* 2002;**34**:687–9.
96. Vitovec J, Aldova E, Vladik P, Krovacek K. Enteropathogenicity of *Plesiomonas shigelloides* and *Aeromonas* spp. in experimental mono- and coinfection with *Cryptosporidium parvum* in the intestine of neonatal BALB/c mice. *Comp Immunol Microbiol Infect Dis* 2001;**24**:39–55.

97. Wang S. A study of the ecology of *Plesiomonas shigelloides* (Chinese). Chung-Hua Liu Hsing Ping Hsueh Tsa Chih Chin. *J Epidemiol* 1991;**12**:295–8.
98. Woo PC, Lau SK, Wong SS, Yuen KY. Two cases of continuous ambulatory peritoneal dialysis–associated peritonitis due to *Plesiomonas shigelloides*. *J Clin Microbiol* 2004;**42**:933–5.
99. Yang CH, Li YH. *Chromobacterium violaceum* infection: a clinical review of an important but neglected infection. *J Chin Med Assoc* 2011;**74**:435–41.
100. Yefet E, Abolzaid S, Nasser W, et al. Unusual infection—*Pasteurella canis* bacteremia in a child after exposure to rabbit secretions. *Harefuah* 2011;**150**:13–15.
101. Young AZ, Neujahr D, Estok L. Case report: epididymo-orchitis and bacteremia caused by *Plesiomonas shigelloides* in an HIV-infected patient. *AIDS Read* 2001;**11**:617–19.
102. Zims MM, Zimprich CA, Petermann SR, Rust L. Expression and partial characterization of an elastase from *Chromobacterium violaceum*. *Vet Microbiol* 2001;**80**:63–74.

proposed, of which 21 have been assigned species names.[19,51,52] At least 15 of these nomenspecies have been isolated from human specimens, including: *Acinetobacter baumannii*, *A. calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter johnsonii*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Acinetobacter radioresistens*, *Acinetobacter parvus*, *Acinetobacter schindleri*, and *Acinetobacter ursingi*.[17] Several genospecies that have been isolated from human clinical samples and had been given the provisional designation of genomic species 3, 13TU, 10, and 11 have now been named as *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter bereziniae*, and *Acinetobacter guillouiae*, respectively [51,52] *Acinetobacter soli*, a species previously isolated only from soil, has recently been implicated in an outbreak in a neonatal care unit.[57] These species may be difficult to differentiate in the clinical laboratory on the basis of their growth characteristics and biochemical activity. Because of their clinical relevance and antimicrobial resistance, four of these species, *A. baumannii*, *A. calcoaceticus*, *A. pittii* (formerly genomic species 3), and *Acinetobacter nosocomialis* (formerly genomic species 13TU), are grouped under the term *A. baumannii-calcoaceticus* complex. Under the new classification, most *A. baumannii* strains represent organisms that were classified formerly as biovar *anitratus*, whereas *A. junii* and *A. lwoffii* previously were listed under the biovar *lwoffii*.

EPIDEMIOLOGY

Acinetobacter strains are distributed widely in nature and can be found in soil, fresh water, and sewage.[10,31] *Acinetobacter* also can be isolated from many animals, fresh meats, poultry, contaminated milk, and frozen foods.[10,31] The organism can be part of the bacterial flora of the skin in healthy individuals,[10,15] and the skin frequently becomes a reservoir for *Acinetobacter* in hospitalized patients and the health care staff.[8,10] It occasionally forms part of the normal flora of the oral cavity and the upper respiratory, genital, and lower gastrointestinal tracts.[10,15] Colonization by *Acinetobacter* is particularly common in patients who have undergone a tracheostomy.[63] The organism frequently can be found in the hospital environment, particularly in moist areas, such as in humidifiers, water sinks, and ventilators.[10] Nosocomial outbreaks have been linked to colonized medical equipment such as ventilator tubing and other respiratory equipment,[1,29] intravenous catheters,[10] hygroscopic bandages,[73] gloves,[56] and mattresses.[71]

The frequency of occurrence of nosocomial infections by *Acinetobacter* is not easy to assess because the pathogenic role of this organism often has been underestimated. However, a national surveillance study conducted from 1974 to 1977 identified *Acinetobacter* as a pathogen in 0.76 percent of nosocomial infections.[60] The estimated rate of nosocomial infections caused by this organism was 3.11 per 10,000 patients discharged, and approximately 15 percent of 1372 reported episodes occurred in the pediatric age group.[60] By 1978, this rate increased by 14 percent and accounted for 1 percent of the bacterial isolates associated with nosocomial infections.[15] An unusual seasonal pattern was observed, with most infections occurring in late summer.[15,60] The cause of this increase is unknown. In a national prospective survey of U.S. hospitals from 1995 through 2002, *Acinetobacter* spp. accounted for 1.3 percent of all nosocomial bloodstream infections and was associated with a crude mortality rate of 34 percent.[83] By 2003, the percentage almost doubled to 2.4 percent of nosocomial bloodstream infections from the same surveillance system.[25] When comparing data from 1986 to 2003, *Acinetobacter* spp. were the only gram-negative pathogens associated with a consistently increasing proportion of hospital-acquired pneumonias, as well as surgical site, urinary tract, and bloodstream infections.[25] Data from SENTRY, an international surveillance program that monitors the frequency of occurrence and antimicrobial susceptibility of bacterial pathogens, revealed a significantly greater frequency of *Acinetobacter* infections in Latin America than in all other regions.[24] A higher prevalence rate of *Acinetobacter* infections was noted in patients ranging in age from birth to 10 years.[24] In the pediatric age group, at least 20 outbreaks of invasive nosocomial infections due to *Acinetobacter* have been reported in the past 3 decades[33,34,73]; the vast majority of them were concentrated in neonatal intensive care units (Table 121-1).[34,49,73]

More recently, *Acinetobacter* emerged as a particularly important pathogen in unusual situations such as earthquakes and war zones.[36] This prevalence was illustrated by reports of outbreaks of multidrug-resistant *Acinetobacter* infections associated with the U.S.-Iraq conflict.[68]

PATHOGENESIS

Limited information is available regarding the pathogenesis of *Acinetobacter* infections. The lipopolysaccharide of *Acinetobacter*, a normal constituent of the outer membrane of gram-negative bacteria, is capable of eliciting multiple pathogenic host responses. Strains producing exopolysaccharide have been shown to be more pathogenic than are nonproducers.[36] Nonspecific adherence factors, such as fimbriae, also have been described in *Acinetobacter*.[36] The role of quorum sensing as a regulatory mechanism for autoinduction of multiple virulence factors in *Acinetobacter* is under investigation.[36]

In animal models, *Acinetobacter* can enhance the virulence of other bacteria in mixed infections, perhaps by slime-induced inhibition of neutrophils.[54] Researchers have speculated that the ability of this organism to grow in an acidic pH at lower temperatures may enhance its ability to invade devitalized tissue.[2] The organism also may survive in a dry environment for as long as a week.[16]

CLINICAL MANIFESTATIONS

Acinetobacter can cause suppurative infection of virtually any organ, and the clinical manifestations typically are similar to those seen with other bacterial infections because no unique features are suggestive of *Acinetobacter* infection. The clinical manifestations also may depend on the underlying immune status of compromised hosts.

TABLE 121-1 **Illustrative Nosocomial Clusters of *Acinetobacter* Infection in Pediatric Patients over the Past 3 Decades**

Country	Year	Type of Unit	Infected Children	Colonized Children	Presentation	Mortality (%)	Suspected Source
United Kingdom[49]	1981	NICU	4	0	Meningitis	0	None identified
United Kingdom[74]	1983	NICU	9	1	Pulmonary infection	22	Ambu bag
India[38]	1988	Oncology	8	N/A	Meningitis	38	Intrathecal needle
Germany[67]	1988	NICU	3	41	Sepsis	100	Humidifier
Israel[59]	1988-1990	NICU	9	N/A	Sepsis	44	None identified
India[48]	1995	NICU	79	N/A	Sepsis	14	None identified
Bahamas[47]	1996	NICU	8	1	Sepsis	37	Air conditioner
South Africa[58]	1997	NICU	9	N/A	Sepsis	22	Suction catheters
Taiwan[35]	2000	NICU	9	N/A	Sepsis	0	HCWs hands
USA[73]	2004	NICU	4	3	Sepsis	57	Referring hospital
Brazil[57]	2005	NICU	5	N/A	Sepsis	20	Intravenous fluids
Turkey[33]	2006-2007	NICU	64	N/A	Sepsis	83	Multiple sources

HCWs, Health care workers; N/A, data not available; NICU, neonatal intensive care unit.

Infections caused by *Acinetobacter* are rare occurrences in normal children[20] and have mostly occurred in tropical areas.

Intracranial Infection

Most cases of *Acinetobacter* meningitis are the result of a penetrating injury or occur after a neurosurgical procedure, although sporadic cases of meningitis have been reported in the absence of these factors. A cluster of eight children in whom *Acinetobacter* meningitis developed after the administration of intrathecal methotrexate was reported.[38] All patients had fever, headache, nausea, and vomiting; and lumbar puncture revealed cerebrospinal fluid (CSF) pleocytosis.

The earlier literature contains several reports of *Acinetobacter* meningitis developing in apparently normal children.[17,18,32,76,80] CSF pleocytosis with a predominance of segmented forms occurred commonly.[18] Because as many as 30 percent of these patients had a petechial rash and gram-negative diplococci on CSF smears, the diagnosis of meningococcal meningitis was made erroneously in most of these cases, thereby leading to a delay in the institution of appropriate therapy and possibly contributing to a mortality rate as high as 27 percent.[18]

Siegman-Igra and associates,[72] in a review of 25 cases of *Acinetobacter* meningitis secondary to invasive procedures that included some children, found that fever, leukocytosis, and neck stiffness, along with other clinical signs of central nervous system (CNS) infection, were common features. The CSF in these patients showed pleocytosis with a predominance of polymorphonuclear leukocytes, elevated protein concentration, and a low glucose level. Most of the infections were associated with indwelling ventriculostomy tubes or a fistula into the CSF space. A case series from Pakistan[65] reported 8 cases of *Acinetobacter* meningitis that occurred over the course of a 4-year period in children who had undergone neurosurgical procedures. Mortality in this series was 25 percent, and the use of intrathecal antibiotics was explored as an adjunctive therapy.[65]

Researchers have suggested that an inherited or acquired complement deficiency may be associated with meningitis caused by *Acinetobacter*[22] because it is seen with *Neisseria meningitidis* and other related species. Treatment of CNS infections caused by *Acinetobacter* requires a minimum of 3 weeks of parenteral antibiotics.

Bacteremia

Acinetobacter bacteremia may occur as an isolated event or may be secondary to a primary infected site, such as the respiratory tract or urinary tract or a wound. Primary bacteremia appears to occur more commonly in immunocompromised neonates, and clinical manifestations can vary from an absence of clinical signs of infection to fulminant septic shock and disseminated intravascular coagulation.[39,67] Thrombocytopenia has been reported to be a prominent feature in these neonates.[35,53,59] Pneumonia has been seen more commonly in early-onset sepsis.[48] Predisposing factors include low birth-weight,[64,67] previous antibiotic therapy,[59,64,67] and the presence of indwelling catheters.[35,47,64]

Acinetobacter bacteremia in children with malignant diseases also has been noted to occur rarely. Fuchs and colleagues[23] reported 29 episodes of sepsis caused by this organism over the course of a 12-year period in an oncology center. All these children were febrile and appeared ill at the time of diagnosis, and a high association of *Acinetobacter* sepsis with the presence of intravascular catheters was noted. Surprisingly, no connection was found with the level of neutropenia.[23]

Respiratory Tract Infections

Because *Acinetobacter* may be a transient colonizer of the pharynx in 7 percent of healthy children[7] and adults[26] and this rate is increased in hospitalized patients, the

relative importance of *Acinetobacter* in comparison with other potential pathogens isolated from sputum is difficult to ascertain. The tracheobronchitis and pneumonia attributed to *Acinetobacter* are mostly nosocomial infections associated with the presence of an endotracheal tube or tracheostomy.[26] Pneumonias frequently are multilobar and occasionally may lead to cavitary destruction or pleural empyema.[26]

Community-acquired pneumonia caused by *A. baumannii* has been reported to occur in adults in the Northern Territory of Australia and other tropical regions.[5] This entity generally is seen in patients with diminished host defenses caused by alcoholism, cigarette smoking, or underlying pulmonary disease and is characterized by the rapid onset of fever, dyspnea, pleuritic chest pain, and purulent sputum. The mortality rate has been as high as 53 to 64 percent.[5] A case of necrotizing pneumonia due to *A. lwoffii* in a previously healthy toddler has been reported.[50]

Miscellaneous Infections

Urinary tract infections occur almost exclusively in patients with indwelling bladder catheters, usually are limited to the bladder, and generally are mild.[26] Burns, as well as traumatic and surgical wounds, frequently become colonized by *Acinetobacter* as a result of the ability of this organism to thrive on compromised tissue and foreign material.[26] Bacteremia may occur as a consequence of this colonization, which is often polymicrobial. *Acinetobacter* is a prominent cause of peritonitis in children undergoing peritoneal dialysis when gram-negative organisms are involved.[81]

Other rare infections caused by *Acinetobacter* that have been reported include suppurative otitis media,[61,76] cellulitis (frequently in association with trauma, a foreign body, or an animal bite),[26,61] synergistic necrotizing fasciitis,[4] native-valve and prosthetic-value endocarditis,[28] septic arthritis,[61] osteomyelitis,[76] and liver abscesses.[26] Ocular infections also have been documented.[45] A case of osteomyelitis occurring after a hamster bite in a child has been described.[46]

DIAGNOSIS

The diagnosis of *Acinetobacter* infection is made by culture of appropriate body fluids or tissue specimens. No serologic or antigen-detection tests are available. A selective medium containing MacConkey agar with cephaloridine has been used to culture skin specimens during investigation of outbreaks[74] because of its ability to inhibit most of the skin flora but not *Acinetobacter*.

Biotyping, phage typing, electrophoretic analysis of isoenzyme and cell wall proteins, plasmid analysis, polymerase chain reaction–based DNA fingerprinting, and restriction endonuclease digestion of DNA have been used for investigation of nosocomial outbreaks.[9] Antibiogram typing no longer is considered an effective method in the investigation of *Acinetobacter* epidemics because the susceptibility pattern may change rapidly within the same outbreak.[9,14]

TREATMENT

As with many other opportunistic gram-negative organisms, treatment of infections caused by *Acinetobacter* spp., particularly those of the *A. baumannii-calcoaceticus* complex,[69,78] have become more complicated by the rapid increase in resistance to the antibiotics used commonly in hospitals. Selection of an antibiotic regimen should be based on in vitro susceptibility testing and ideally should include both a β-lactam antibiotic and an aminoglycoside, which may have synergistic activity[26] and prevent the emergence of resistance.[8]

Species of the *A. baumannii-calcoaceticus* complex have shown decreased susceptibility to ampicillin, broad-spectrum penicillins, cephalosporins, aminoglycosides, and ciprofloxacin.[24,69] Resistance to extended-spectrum cephalosporins may be the result of the presence of cephalosporinases (particularly the chromosomally encoded cephalosporinase AmpC),[12] other broad-spectrum β-lactamases, or changes in the outer-membrane porins and penicillin-binding proteins.[12,55] Resistance to aminoglycosides is mediated by aminoglycoside-modifying enzymes.[78] The carbapenems imipenem and meropenem appear to be the most active agents against *A. baumannii*,[72,78] but reports have found more than 10 percent of such strains to be resistant to these antibiotics in some areas,[24,44] and increasing numbers of nosocomial outbreaks of carbapenem-resistant *Acinetobacter* infections have been reported.[27,77,85] The incidence of carbapenem-resistant *Acinetobacter* strains has been particularly high in Latin America, Africa, Europe, and the Asian Pacific rim region.[11,24,43] Resistance to the carbapenems has been associated with the presence of serine and metallo-β-lactamases (carbapenemases). Of particular concern are the numerous carbapenem-hydrolyzing OXA enzymes that have emerged in *A. baumannii*.[12] Caution should be exercised when using imipenem in high doses in children for the treatment of meningitis (i.e., 100 mg/kg/day) because of an unusually high rate of seizures.[84] Thus, meropenem is recommended for this indication. Doripenem, a newer carbapenem antibiotic, has more potent in vitro activity against *Acinetobacter* species compared with other carbapenems,[21] but its safety and effectiveness in pediatric patients have not been established. On the other hand, ertapenem has poor in vitro activity against *Acinetobacter*[41] and should not be used for the treatment of these infections. Combinations of a β-lactam antibiotic with a β-lactamase inhibitor, such as ampicillin-sulbactam, piperacillin-tazobactam, or ticarcillin-clavulanate, have been used for infections caused by carbapenem-resistant strains.[77,85]

The fluoroquinolones, in particular ciprofloxacin, have been used successfully to treat infections caused by multidrug-resistant *Acinetobacter* in children[48,58] and adults. Although ciprofloxacin has been approved for the treatment of complicated urinary tract infections in children and levofloxacin for postexposure treatment of inhalation anthrax in the pediatric age group, the other systemic fluoroquinolones are not approved for use in children younger than 16 years of age because of the theoretic concern for damage to growth cartilage. Of the

quinolones, gatifloxacin exhibits the best in vitro activity against *A. baumannii*, followed by levofloxacin and trovafloxacin.[30] Although 76 to 86 percent of sporadic isolates of *A. baumannii* are susceptible to the fluoroquinolones, only 32 to 55 percent of outbreak-related strains remain susceptible to this antibiotic class.[30] Topoisomerase mutations in both *gyrA* and *parC* have been identified in quinolone-resistant *A. baumannii*.[12] In some cases, the polymyxin drugs polymyxin B and colistin are the only therapeutic options for the treatment of multidrug-resistant *Acinetobacter* infection.[27,40] Efflux pumps capable of conferring resistance to multiple antibiotic classes have been identified in *Acinetobacter* spp.[12,43,62]

Tigecycline is the first member of the glycylcycline antimicrobial class to gain approval in the United States. Because this agent demonstrates in vitro activity against multidrug-resistant strains of *A. baumannii*, its potential use as an alternative therapy for severe infections caused by this organism is being investigated.[62] Data regarding the use of this agent in pediatric patients are lacking. The development of resistance to tigecycline among clinical isolates of *Acinetobacter* has been documented and appears to be increasing in some regions.[3,43]

Imipenem, meropenem, amikacin, ciprofloxacin, ceftazidime, and ceftriaxone have exhibited good in vitro activity against isolates identified as species other than the *A. baumannii-calcoaceticus* complex.[69] In addition to antimicrobial therapy, prompt drainage of focal suppurative sites and removal of infected indwelling catheters are essential. Intraventricular administration of amikacin, polymixin B, or colistin has been used in the treatment of CNS infections caused by this organism.[65,82] Similarly, the use of adjunctive aerosolized therapy with colistin or tobramycin has been explored in adults with pulmonary infections.[6]

PROGNOSIS

Because *Acinetobacter* strains often are resistant to the antibiotics used commonly, prompt recognition of the specific cause and institution of effective antibiotic therapy are critical to achieving a successful outcome. The reported mortality rate in a series of pediatric patients ranged from 0 percent to more than 50 percent (see Table 121-1), and the outcome appeared to correlate more closely with the underlying condition than with other factors such as polymicrobial bacteremia.[75] In a series of 58 infections caused by this organism that occurred over the course of a 2-year period from 1973 through 1974 at the Massachusetts General Hospital in Boston, the mortality rate was 23 percent.[26] A 1999 prospective study in the Slovak Republic revealed that the case-fatality rate for 157 episodes of *A. baumannii* bacteremia was significantly higher in adults than in children (34% vs. 12%).[39] A recent systematic review that included a total of 469 children from 51 outbreaks and case series of invasive *Acinetobacter* infections revealed an attributable mortality of 14.5 percent.[34]

The nosocomial acquisition of multiresistant *A. baumannii-calcoaceticus* complex has been associated with high mortality rates and prolonged hospitalization in adult patients in intensive care units,[42,66] in contrast to the more benign clinical outcome usually seen with other species of *Acinetobacter*.[70]

PREVENTION

Nosocomial acquisition of *Acinetobacter* by high-risk compromised hosts can be prevented by placing emphasis on the control measures routinely used for endemic infections, such as careful hand washing by personnel, limitation of the frequency and duration of use of devices, proper isolation of colonized and infected patients, application of strict techniques for invasive procedures, restricted use of antibiotics, and implementation of antibiotic stewardship programs.[16,42,43,73]

NEW REFERENCES SINCE THE SIXTH EDITION

3. Al-Sweih NA, Al-Hubail MA, Rotimi VO. Emergence of tigecycline and colistin resistance in *Acinetobacter* species isolated from patients in Kuwait hospitals. *J Chemother* 2011;**23**:13–16.
6. Arnold HM, Sawyer AM, Kollef MH. Use of adjunctive aerosolized antimicrobial therapy in the treatment of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ventilator-associated pneumonia. *Respir Care* 2012;**57**(8):1226–33.
11. Bertrand X, Dowzicky MJ. Antimicrobial susceptibility among gram-negative isolates collected from intensive care units in North America, Europe, the Asia-Pacific rim, Latin America, the Middle East, and Africa between 2004 and 2009 as part of the Tigecycline Evaluation and Surveillance Trial. *Clin Ther* 2012;**34**:124–37.
21. Fera MT, Zummo S, De Sarro A. In vitro activity studies of doripenem and two other carbapenems tested against *Pseudomonas aeruginosa* and other non-fermentative bacilli. *J Chemother* 2011; **23**:335–40.
33. Hosoglu S, Hascuhadar M, Yasar E, et al. Control of an *Acinetobacter baumannii* outbreak in a neonatal ICU without suspension of service: a devastating outbreak in Diyarbakir, Turkey. *Infection* 2012;**40**:11–18.
34. Hu J, Robinson JL. Systematic review of invasive *Acinetobacter* infections in children. *Can J Infect Dis Med Microbiol* 2010;**21**: 83–8.
43. Manchanda V, Sanchaita S, Singh NP. Mutidrug resistant *Acinetobacter*. *J Glob Infect Dis* 2010;**2**:291–304.
50. Moreira Silva G, Morais L, Marques L, Senra V. *Acinetobacter* community-acquired pneumonia in a healthy child. *Rev Port Pneumol* 2012;**18**(2):96–8.
51. Nemec A, Krizova L, Maixnerova M, et al. Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). *Res Microbiol* 2011;**162**:393–404.
52. Nemec A, Musilek M, Sedo O, et al. *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. *Int J Syst Evol Microbiol* 2010;**60**:896–903.
57. Pellegrino FL, Vieira VV, Baio PV, et al. *Acinetobacter soli* as a cause of bloodstream infection in a neonatal intensive care unit. *J Clin Microbiol* 2011;**49**:2283–5.
65. Saleem AF, Shah MS, Shaikh AS, et al. *Acinetobacter* species meningitis in children: a case series from Karachi, Pakistan. *J Infect Dev Ctries* 2011;**5**:809–14.
73. Simmonds A, Munoz J, Aguero-Rosenfeld M, et al. Outbreak of *Acinetobacter* infection in extremely low birth weight neonates. *Pediatr Infect Dis J* 2009;**28**:210–14.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ahmed J, Brutus A, D'Amato RF, et al. *Acinetobacter calcoaceticus anitratus* outbreak in the intensive care unit traced to a peak flow meter. *Am J Infect Control* 1994;**22**:319–21.
2. Allen DM, Hartman BJ. *Acinetobacter* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas and Bennett's principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 2009–13.
3. Al-Sweih NA, Al-Hubail MA, Rotimi VO. Emergence of tigecycline and colistin resistance in *Acinetobacter* species isolated from patients in Kuwait hospitals. *J Chemother* 2011;**23**:13–16.
4. Amsel MB, Horrilleno E. Synergistic necrotizing fasciitis: a case of polymicrobial infection with *Acinetobacter calcoaceticus*. *Curr Surg* 1985;**42**:370–2.
5. Anstey NM, Currie BJ, Withnall KM. Community-acquired *Acinetobacter* pneumonia in the Northern Territory of Australia. *Clin Infect Dis* 1992;**14**:83–91.
6. Arnold HM, Sawyer AM, Kollef MH. Use of adjunctive aerosolized antimicrobial therapy in the treatment of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* ventilator-associated pneumonia. *Respir Care* 2012;**57**(8):1226–33.
7. Baltimore RS, Duncan RL, Shapiro ED, et al. Epidemiology of pharyngeal colonization of infants with aerobic gram-negative rod bacteria. *J Clin Microbiol* 1989;**27**:91–5.
8. Bergogne-Berezin E. *Acinetobacter* spp., saprophytic organisms of increasing pathogenic importance. *Zentrabl Bakteriol* 1994;**81**: 389–405.
9. Bergogne-Berezin E, Joly-Guillou ML. Hospital infection with *Acinetobacter* spp.: an increasing problem. *J Hosp Infect* 1991;**18**(Suppl. A):250–5.
10. Bergogne-Berezin E, Joly-Guillou ML, Vieu JF. Epidemiology of nosocomial infections due to *Acinetobacter calcoaceticus*. *J Hosp Infect* 1987;**10**:105–13.
11. Bertrand X, Dowzicky MJ. Antimicrobial susceptibility among gram-negative isolates collected from intensive care units in North America, Europe, the Asia-Pacific rim, Latin America, the Middle East, and Africa between 2004 and 2009 as part of the Tigecycline Evaluation and Surveillance Trial. *Clin Ther* 2012;**34**: 124–37.
12. Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2006;**43**(Suppl. 2):S49–56.
13. Bouvet PJM, Grimont PAD. Taxonomy of the genus *Acinetobacter* with the recognition of *Acinetobacter baumannii* sp. nov., *Acinetobacter haemolyticus* sp. nov., *Acinetobacter johnsonii* sp. nov., and *Acinetobacter junii* sp. nov. and the emended descriptions of *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii*. *Int J Syst Bacteriol* 1986; **36**:228–40.
14. Carlquist JF, Conti M, Burke JP. Progressive resistance in a single strain of *Acinetobacter calcoaceticus* recovered during a nosocomial outbreak. *Am J Infect Control* 1982;**10**:43–8.
15. Centers for Disease Control and Prevention. Nosocomial infections caused by *Acinetobacter calcoaceticus*: United States, 1978. *MMWR Morb Mortal Wkly Rep* 1979;**28**:177–9.
16. Crombach WHJ, Dijkshoorn L, van Noort-Klaassen M, et al. Control of an epidemic spread of a multi-resistant strain of *Acinetobacter calcoaceticus* in a hospital. *Intensive Care Med* 1989; **15**:166–70.
17. DeBord GG. *Mima polymorpha* in meningitis. *J Bacteriol* 1948; **55**:764–5.
18. Donald WD, Doak WM. Mimeae meningitis and sepsis. *JAMA* 1967;**200**:111–13.
19. Dortet L, Legrand P, Soussy CJ, Cattoir V. Bacterial identification, clinical significance, and antimicrobial susceptibilities of *Acinetobacter ursingii* and *Acinetobacter schindleri*, two frequently misidentified opportunistic pathogens. *J Clin Microbiol* 2006;**44**:4471–8.
20. Feigin RD, Shearer WT. Opportunistic infection in children, part III: in the normal host. *J Pediatr* 1975;**87**:852–66.
21. Fera MT, Zummo S, De Sarro A. In vitro activity studies of doripenem and two other carbapenems tested against *Pseudomonas aeruginosa* and other non-fermentative bacilli. *J Chemother* 2011; **23**:335–40.
22. Fijen CAP, Kuijper EJ, Tjia HG, et al. Complement deficiency predisposes for meningitis due to nongroupable meningococci and *Neisseria*-related bacteria. *Clin Infect Dis* 1994;**18**:780–4.
23. Fuchs GJ, Jaffe N, Pickering LK. *Acinetobacter calcoaceticus* sepsis in children with malignancies. *Pediatr Infect Dis* 1986;**5**:545–9.
24. Gales AC, Jones RN, Forward KR, et al. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY antimicrobial surveillance program (1997-1999). *Clin Infect Dis* 2001; **32**(Suppl. 2):104–13.
25. Gaynes R, Edwards JR, and the National nosocomial Infections Surveillance System. Overview of nosocomial infections caused by gram-negative bacilli. *Clin Infect Dis* 2005;**41**:848–54.
26. Glew RH, Moellering RC, Kunz LJ. Infections with *Acinetobacter calcoaceticus (Herellea vaginicola)*: clinical and laboratory studies. *Medicine (Baltimore)* 1977;**56**:79–97.
27. Go ES, Urban C, Burns J, et al. Clinical and molecular epidemiology of *Acinetobacter* infections sensitive only to polymyxin B and sulbactam. *Lancet* 1994;**344**:1329–32.
28. Gradon JD, Chapnick EK, Lutwick LI. Infective endocarditis of a native valve due to *Acinetobacter*: case report and review. *Clin Infect Dis* 1992;**14**:1145–8.
29. Hartstein AI, Rashad AL, Liebler JM, et al. Multiple intensive care unit outbreak of *Acinetobacter calcoaceticus* subspecies *anitratus* respiratory infection and colonization associated with contaminated, reusable ventilator circuits and resuscitation bags. *Am J Med* 1988; **85**:624–31.
30. Heinemann B, Wisplinghoff H, Edmond M, Seifert H. Comparative activities of ciprofloxacin, clinafloxacin, gatifloxacin, gemifloxacin, levofloxacin, moxifloxacin, and trovafloxacin against epidemiologically defined *Acinetobacter baumannii* strains. Antimicrob. *Agents Chemother* 2000;**44**:2211–13.
31. Henriksen SD. *Moraxella*, *Acinetobacter*, and the Mimeae. *Bacteriol Rev* 1973;**37**:522–61.
32. Hermann G, Melnick T. *Mima polymorpha* meningitis in the young. *Am J Dis Child* 1965;**110**:315–18.
33. Hosoglu S, Hascuhadar M, Yasar E, et al. Control of an *Acinetobacter baumannii* outbreak in a neonatal ICU without suspension of service: a devastating outbreak in Diyarbakir, Turkey. *Infection* 2012;**40**:11–18.
34. Hu J, Robinson JL. Systematic review of invasive *Acinetobacter* infections in children. *Can J Infect Dis Med Microbiol* 2010;**21**: 83–8.
35. Huang YC, Su LH, Wu TL, et al. Outbreak of *Acinetobacter baumanii* bacteremia in a neonatal intensive care unit: clinical implications and genotyping analysis. *Pediatr Infect Dis J* 2002;**21**: 1105–9.
36. Joly-Guillou ML. Clinical impact and pathogenicity of *Acinetobacter*. *Clin Microbiol Infect* 2005;**11**:868–73.
37. Juni E. *Acinetobacter*. In: Krieg NR, editor. *Bergey's manual of systematic bacteriology*. Baltimore: Williams & Wilkins; 1984. p. 303–6.
38. Kelkar R, Gordon SM, Giri N, et al. Epidemic iatrogenic *Acinetobacter* spp. meningitis following administration of intrathecal methotrexate. *J Hosp Infect* 1989;**14**:233–43.
39. Koprnova J, Svetlansky I, Bilikova E, et al. *Acinetobacter baumannii* bacteremia in children. *Pediatr Infect Dis J* 2001;**20**:1183.
40. Levin AS, Barone AA, Penco J, et al. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin Infect Dis* 1999;**28**: 1008–11.
41. Livermore DM, Carter MW, Bagel S, et al. In vitro activity of ertapenem (MK-0826) against recent clinical bacteria collected in Europe and Australia. *Antimicrob Agents Chemother* 2001;**45**: 1860–7.
42. Lortholary O, Fagon JY, Hoi AB, et al. Nosocomial acquisition of multiresistant *Acinetobacter baumannii*: risk factors and prognosis. *Clin Infect Dis* 1995;**20**:790–6.
43. Manchanda V, Sanchaita S, Singh NP. Mutidrug resistant *Acinetobacter*. *J Glob Infect Dis* 2010;**2**:291–304.
44. Manikal VM, Landman D, Saurina G, et al. Endemic carbapenem-resistant *Acinetobacter* species in Brooklyn, New York: Citywide prevalence, interinstitutional spread, and relation to antibiotic usage. *Clin Infect Dis* 2000;**31**:101–6.
45. Marcovich A, Levartovsky S. *Acinetobacter* exposure keratitis. *Br J Ophthalmol* 1994;**78**:489–90.
46. Martin RW, Martin DL, Levy CS. *Acinetobacter* osteomyelitis from a hamster bite. *Pediatr Infect Dis J* 1988;**5**:364–5.

47. McDonald LC, Walker M, Carson L, et al. Outbreak of *Acinetobacter* spp. bloodstream infections in a nursery associated with contaminated aerosols and air conditioners. *Pediatr Infect Dis J* 1998; **17**:716–22.
48. Mishra A, Mishra S, Jaganath G, et al. *Acinetobacter* sepsis in newborns. *Indian Pediatr* 1998;**35**:2732.
49. Morgan ME, Hart CA. *Acinetobacter* meningitis: acquired infection in a neonatal intensive care unit. *Arch Dis Child* 1982;**657**: 557–9.
50. Moreira Silva G, Morais L, Marques L, Senra V. *Acinetobacter* community-acquired pneumonia in a healthy child. *Rev Port Pneumol* 2012;**18**(2):96–8.
51. Nemec A, Krizova L, Maixnerova M, et al. Genotypic and phenotypic characterization of the *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex with the proposal of *Acinetobacter pittii* sp. nov. (formerly *Acinetobacter* genomic species 3) and *Acinetobacter nosocomialis* sp. nov. (formerly *Acinetobacter* genomic species 13TU). *Res Microbiol* 2011;**162**:393–404.
52. Nemec A, Musilek M, Sedo O, et al. *Acinetobacter bereziniae* sp. nov. and *Acinetobacter guillouiae* sp. nov., to accommodate *Acinetobacter* genomic species 10 and 11, respectively. *Int J Syst Evol Microbiol* 2010;**60**:896–903.
53. Ng PC, Herrington RA, Beane CA, et al. An outbreak of *Acinetobacter* septicaemia in a neonatal intensive care unit. *J Hosp Infect* 1989;**14**:363–8.
54. Obana Y. Pathogenic significance of *Acinetobacter calcoaceticus*: analysis of experimental infection in mice. *Microbiol Immunol* 1986; **30**:645–57.
55. Obara M, Nakae T. Mechanisms of resistance to β-lactam antibiotics in *Acinetobacter calcoaceticus*. *J Antimicrob Chemother* 1991;**28**: 791–800.
56. Patterson JE, Vecchio J, Pantelick EL, et al. Association of contaminated gloves with transmission of *Acinetobacter calcoaceticus* var. *anitratus* in an intensive care unit. *Am J Med* 1991;**91**:479–83.
57. Pellegrino FL, Vieira VV, Baio PV, et al. *Acinetobacter soli* as a cause of bloodstream infection in a neonatal intensive care unit. *J Clin Microbiol* 2011;**49**:2283–5.
58. Pillay T, Pillay DG, Adhikari M, Sturm AW. An outbreak of neonatal infection with *Acinetobacter* linked to contaminated suction catheters. *J Hosp Infect* 1999;**43**:299–304.
59. Regev R, Dolfin T, Zelig I, et al. *Acinetobacter* septicemia: a threat to neonates? Special aspects in a neonatal intensive care unit. *Infection* 1993;**21**:394–6.
60. Retailliau HF, Hightower AW, Dixon RE, et al. *Acinetobacter calcoaceticus*: a nosocomial pathogen with an unusual seasonal pattern. *J Infect Dis* 1979;**139**:371–5.
61. Reynolds RC, Cluff LE. Infection of men with Mimeae. *Ann Intern Med* 1963;**58**:759–67.
62. Rice LB. Challenges in identifying new antimicrobial agents effective for treating infections with *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2006;**43**(Suppl. 2):S100–5.
63. Rosenthal SL. Sources of *Pseudomonas* and *Acinetobacter* species found in human culture materials. *Am J Clin Pathol* 1974;**62**: 807–11.
64. Sakata H, Fujita K, Maruyama S, et al. *Acinetobacter calcoaceticus* biovar *anitratus* septicaemia in a neonatal intensive care unit: epidemiology and control. *J Hosp Infect* 1989;**14**:15–22.
65. Saleem AF, Shah MS, Shaikh AS, et al. *Acinetobacter* species meningitis in children: a case series from Karachi, Pakistan. *J Infect Dev Ctries* 2011;**5**:809–14.
66. Scerpella EG, Wanger AR, Armitige L, et al. Nosocomial outbreak caused by a multiresistant clone of *Acinetobacter baumannii*: Results of the case-control and molecular epidemiologic investigations. *Infect Control Hosp Epidemiol* 1995;**16**:92–7.
67. Schloesser RL, Laufkoetter EA, Lehners T, et al. An outbreak of *Acinetobacter calcoaceticus* infection in a neonatal care unit. *Infection* 1990;**18**:230–3.
68. Scott P, Deye G, Srinivasan A, et al. An outbreak of multidrug-resistant *Acinetobacter baumannii-calcoaceticus* complex infection in the US Military Health Care System associated with military operations in Iraq. *Clin Infect Dis* 2007;**44**:1577–84.
69. Seifert H, Baginski R, Schulze A, et al. Antimicrobial susceptibility of *Acinetobacter* species. *Antimicrob Agents Chemother* 1993;**37**: 750–3.
70. Seifert H, Strate A, Schulze A, et al. Bacteremia due to *Acinetobacter* species other than *Acinetobacter baumannii*. *Infection* 1994;**22**:379–85.
71. Sherertz RJ, Sullivan ML. An outbreak of infections with *Acinetobacter calcoaceticus* in burn patients: contamination of patients' mattresses. *J Infect Dis* 1985;**151**:252–8.
72. Siegman-Igra Y, Bar-Yosef S, Gorea A, et al. Nosocomial *Acinetobacter* meningitis secondary to invasive procedures: report of 25 cases and review. *Clin Infect Dis* 1993;**17**:843–9.
73. Simmonds A, Munoz J, Aguero-Rosenfeld M, et al. Outbreak of *Acinetobacter* infection in extremely low birth weight neonates. *Pediatr Infect Dis J* 2009;**28**:210–14.
74. Stone JW, Das BC. Investigation of an outbreak of infection with *Acinetobacter calcoaceticus* in a special care baby unit. *J Hosp Infect* 1985;**6**:42–8.
75. Tilley PAG, Roberts FJ. Bacteremia with *Acinetobacter* species: risk factors and prognosis in different clinical settings. *Clin Infect Dis* 1994;**18**:896–900.
76. Torregrosa MV, Ortiz A. Severe infections in children due to rare gram-negative bacilli *(Mima polymorpha* and *Bacillus anitratum)*. *J Pediatr* 1961;**59**:35–41.
77. Urban C, Go E, Mariano N, et al. Effect of sulbactam on infections caused by imipenem-resistant *Acinetobacter calcoaceticus* biotype *anitratus*. *J Infect Dis* 1993;**167**:448–51.
78. Vila J, Marcos A, Marco F, et al. In vitro antimicrobial production of β-lactamases, aminoglycoside-modifying enzymes, and chloramphenicol acetyltransferase by and susceptibility of clinical isolates of *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 1993;**37**: 138–41.
79. Von Lingelsheim W. Beitrage zur Aetiologie der epidemischen Genickstarre nach den Ergebnissen der letzten Jahre. *Z Hyg Infektionskr* 1908;**59**:457–60.
80. Waite CL, Kline AH. *Mima polymorpha* meningitis. *Am J Dis Child* 1959;**98**:121–6.
81. Warady BA, Campoy SF, Gross SP, et al. Peritonitis with continuous ambulatory peritoneal dialysis and continuous cycling peritoneal dialysis. *J Pediatr* 1984;**105**:726–9.
82. Wirt TC, McGee ZA, Oldfield EH, et al. Intraventricular administration of amikacin for complicated gram-negative meningitis and ventriculitis. *J Neurosurg* 1979;**50**:95–9.
83. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004;**39**: 309–17.
84. Wong VK, Wright HT, Ross LA, et al. Imipenem/cilastatin treatment of bacterial meningitis in children. *Pediatr Infect Dis J* 1991;**10**:122–5.
85. Wood CA, Reboli AC. Infections caused by imipenem-resistant *Acinetobacter calcoaceticus* biotype *anitratus*. *J Infect Dis* 1993;**168**: 1602–3.

CHAPTER 122

Achromobacter (Alcaligenes)

Randall G. Fisher

Organisms of the genus *Achromobacter* are gram-negative bacilli that live in aqueous environments. Originally considered commensals, they increasingly are recognized as important, although rare, hospital pathogens. *Achromobacter* can be especially problematic in immunocompromised patients and in neonates, in whom infection can be life-threatening. These organisms have been isolated from such diverse clinical specimens as sputum, urine, feces, blood, cerebrospinal fluid, cornea, and peritoneal and pleural fluids.

BACTERIOLOGY

Achromobacter spp. are gram-negative, motile, indole-negative, obligate aerobes that are oxidase- and catalase-positive. They are considered to be nonfermenters because of their extremely limited action on carbohydrates. Most ferment xylose, and some ferment glucose. All reduce nitrate to nitrite. They are urease-, lysine-, and ornithine-negative. They grow well on both blood and MacConkey agar and produce colonies that are smooth and glistening and have a distinct edge. *Achromobacter* spp. alkalinize organic salts and amides, a property that led to the name *Alcaligenes*, which means *alkali producing*. *Alcaligenes faecalis* has a distinct, sweet odor that has been described as resembling that of green apples.[29]

Bacteriologically, these bacilli may be confused with other nonfermenting gram-negative organisms, especially *Pseudomonas* spp. Morphologically, however, they can be distinguished easily from pseudomonads by the presence of peritrichous flagella: *Pseudomonas* spp. have polar flagella.[25]

The taxonomy of these organisms is confusing and undergoes frequent changes. They were classified as *Achromobacter* spp., then reclassified as *Alcaligenes*, and have been reassigned the name *Achromobacter*. The genera *Achromobacter* and *Alcaligenes* are closely related and comprise many species, but clinically important ones are as follows: (1) *Achromobacter xylosoxidans*, which has two subspecies: *xylosoxidans* and *dentrificans*, the former of which is the most common cause of clinically recognizable infection; (2) *A. faecalis*, which is a less common pathogen but has a distinct antimicrobial susceptibility pattern[6]; (3) and *Achromobacter piechaudii*, which has been isolated from clinical specimens[42] but is of doubtful significance. In the discussion that follows, the abbreviation *A. xylosoxidans* refers to *Achromobacter xylosoxidans* subspecies *xylosoxidans*.

EPIDEMIOLOGY

Like *Pseudomonas* spp., *Achromobacter* are water organisms and prefer aqueous environments and moist soil. They do not survive long on porous surfaces or fomites or if they become desiccated.[46] They also may be part of the normal flora of the ear and of the gastrointestinal and respiratory tracts of some people. These organisms establish a niche within the hospital environment and have been recovered from ventilators, humidifiers, "sterile" saline, intravenous fluids, and irrigation and dialysis solutions. *Achromobacter* spp. also have been recovered from infant formula,[18] children's soap bubbles,[38] well water,[52] and swimming pools.[27] Organisms also survive many disinfectants and have been cultured from chlorhexidine,[49] 1 percent eosin,[7] and alcohol- or quaternary amine–containing compounds.[46,49] Shigeta and associates[49] reported an outbreak of *A. xylosoxidans* ventriculitis secondary to contaminated chlorhexidine used on a surgical ward. Foley and colleagues[21] reported an outbreak accompanied by deaths in a neonatal intensive care unit secondary to contamination of saline used as an eyewash. Boukadida and coworkers[7] reported a neonatal death caused by meningitis contracted by dissemination after treatment of a diaper rash with 1 percent eosin. An outbreak of 37 cases (with two fatalities) that was caused by bacterial contamination of deionized water in a hemodialysis system was described by Reverdy and associates.[43] Surgical wound infection also has occurred in which infection was suspected to be secondary to contaminated irrigation fluids used in surgery.[59] One outbreak of four cases in a hemodialysis unit was linked to an atomizer of 2.5 percent chlorhexidine used to disinfect the skin.[55] Three kinds of pseudo-outbreaks have been described: in one, seven patients in a pediatric hospital were infected with *A. xylosoxidans*, but restriction fragment-length polymorphism analysis proved they all were genetically unrelated[5]; in another, *A. xylosoxidans* was isolated from three different clinical specimens but was later proved to be a contaminant of the saline used in their processing[24]; in a third, blood cultures were positive when drawn on the night shift because the nurses were cleaning the top of the blood culture bottles with a contaminated disinfectant.[51]

Studies have documented that *A. xylosoxidans* colonization of patients with cystic fibrosis is on the rise. In one large study, *A. xylosoxidans* was isolated from the sputum culture of 52/595 patients (8.7%).[8] In other studies, isolates of *A. xylosoxidans* at a single cystic fibrosis center

were not genetically related, a finding implying no common source of infection and little or no patient-to-patient spread within the center.[16,58] However, colonization of multiple patients with identical strains also is well documented.[44] Colonization rates as high as 17.6 percent have been reported; many of these patients were shown to be chronically colonized.[31] Most investigators report no significant changes in forced expiratory volume in 1 second (FEV_1) or growth parameters in *A. xylosoxidans*–colonized patients.[31] Environmental cultures at one cystic fibrosis center were positive for *A. xylosoxidans* only 0.8 percent of the time, whereas 22.8 percent of such cultures were positive for *Pseudomonas aeruginosa*.[20]

PATHOPHYSIOLOGY

Achromobacter spp. are weakly virulent bacteria. Medical care commonly provides the conduit through which organisms are introduced into their host, by way of indwelling catheters, endotracheal tubes, and so forth. The bacteria may take advantage of a weakened immune system and disseminate, causing sepsis, meningitis, and death. Preterm or term infants who are small for gestational age are at particular risk for acquiring such severe *Achromobacter* infections.[21] Although most neonatal infections are considered to be nosocomial, vertical transmission from mother to baby may occur.[25] An increased incidence of infection has been reported for patients with neoplasms[32] and those receiving long-term steroid therapy.[28] Sporadic cases of *Achromobacter* infection in patients with idiopathic immunoglobulin (Ig)M deficiency,[17] Waldenström macroglobulinemia,[53] and systemic lupus erythematosus have been reported.[45] My colleagues and I have seen one boy with hyper-IgM syndrome in whom 14 separate episodes of *Achromobacter* bacteremia occurred; an exhaustive environmental search for a source was fruitless. The source was eventually proved to be deep infection of a cervical lymph node, and the episodes ceased when the node was removed.[60] *Achromobacter* infections occur in patients with acquired immunodeficiency syndrome (AIDS),[9,23] but whether this syndrome is an independent risk factor for infection is unclear.

In one study, patients with cystic fibrosis and colonized with *A. xylosoxidans* tended to be older (mean age, 20 years) and at baseline had worse lung function than did noncolonized controls; however, over the course of the study, the rate of decline of lung function did not differ between cases and controls.[11] In a second similar case-control study, lung function decline and growth parameters generally were not affected by colonization; however, a subset of patients had rapidly increasing antibody titers against *A. xylosoxidans*, and this group did experience more accelerated decline.[44]

In unusual circumstances, patients with neither overt underlying disease nor obvious immune deficiency will develop infection with *Achromobacter* spp. Most of these cases involve penetrating trauma. One case of corneal infection complicating epidemic keratoconjunctivitis in a normal host has been described.[39] Eye infections, including conjunctivitis and keratitis, also have occurred in patients who wear contact lenses; the organism was also recovered from contact lens cases and solutions.[30,35] Severe infection requiring corneal transplantation occurred two months after LASIK.[34]

CLINICAL MANIFESTATIONS

Signs of sepsis or meningitis caused by *A. xylosoxidans* in the newborn are difficult to differentiate from other causes of bacterial sepsis. However, some babies may develop a distinctive rash in association with this infection, in which 1- to 2-cm, sharply demarcated red patches appear, especially in the head and neck region. This rash was noted in 29/33 newborns with *A. xylosoxidans* infection reported by Doxiadis and associates in 1960[14] and was seen again in a case reported in 1993.[7] *A. xylosoxidans* sepsis and meningitis tend to manifest later in life than do infections with the usual vertically acquired pathogens and may have a more insidious onset.[36] In some cases, cerebrospinal fluid profiles may resemble those usually associated with viral meningitis, with white blood cell counts in the hundreds, and with monocytic predominance.[47] Neonatal *Achromobacter* sepsis or meningitis has an extremely poor prognosis; one series noted a mortality rate that approached 75 percent, and 36 percent of survivors had severe neurologic deficits.[21] The incidence of intracranial hemorrhage also was high.

Three large series of bloodstream infections with *A. xylosoxidans* all revealed a similar story; most cases are nosocomial, most patients have an underlying malignant disease, many are neutropenic or receiving high-dose steroids, and many have indwelling vascular catheters.[2,22,48] Polymicrobial bacteremia was not rare in these series. Mortality rates ranged from 15[22] to 48 percent.[48] Risk factors for mortality were age older than 65 years at diagnosis, neutropenia, nosocomial acquisition, and polymicrobial bacteremia. An earlier review of reported cases revealed that the case-fatality rate was lowest in patients with catheter-associated bacteremia and highest (65%) in those with pneumonia, meningitis, or endocarditis.[15]

An outbreak of urinary tract infection was traced to ultrasound gel used in transrectal ultrasound-guided prostate biopsies.[40] Urinary tract infections are generally seen in elderly patients with underlying malignancies or urologic abnormalities.[54]

One child developed osteomyelitis caused by *A. xylosoxidans* after stepping on a nail through old sneakers (a clinical situation classically associated with *Pseudomonas* infection)[26,27]; another developed *Achromobacter* infection as a consequence of a gunshot wound.[10] In the setting of a patient with an artificial heart valve, *A. xylosoxidans* endocarditis has been described.[41] In another case, endocarditis was associated with an abandoned pacemaker lead.[1] A case series of liver abscesses included three patients from whom *A. xylosoxidans* was isolated, all of whom shared a clinical pattern—history of cholecystectomy, "coral-like" multilobulated appearance on computed tomography, and epithelioid granulomas at the periphery of the abscesses.[3] *A. xylosoxidans* infection in older patients usually is not suspected on clinical grounds but rather in the context of a common-source outbreak

or because of microbiologic clues. *A. faecalis* infection is less common and usually is part of a polymicrobial process.

DIAGNOSIS AND TREATMENT

Generally, the diagnosis of *Achromobacter* infections rests on recovery of the organism from clinical samples, although newer methods sometimes have been employed. In one case of culture-negative endophthalmitis, fluid obtained from the anterior chamber was subjected to polymerase chain reaction (PCR) using a 16S rDNA primer set, finding a 214 base pair sequence from *A. xylosoxidans*.[57] These organisms often are mistaken for pseudomonads, and the clinician should suspect *A. xylosoxidans* when the laboratory reports an organism as a *Pseudomonas* spp. that is resistant to all aminoglycosides.[46] Key differentiation features include the antibiogram and the morphology of the organism, with its distinctive peritrichous flagella. The organism can be identified in the sputum of cystic fibrosis patients using a PCR for the bla(OXA-114-like) gene, which is constitutive in *A. xylosoxidans*.[56] Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) also has been used to identify nonfermenting gram-negative rods in cystic fibrosis sputum with great accuracy, correctly identifying 549/559 strains.[13]

Achromobacter spp. typically are resistant to a large number of antibiotics, including ampicillin, aztreonam, aminoglycosides, first- and second-generation cephalosporins, tetracyclines, and rifampin. They variably are resistant to chloramphenicol, fluoroquinolones, macrolides, ureidopenicillins, and β-lactamase combination drugs.[6] *Achromobacter* spp. have been shown to produce β-lactamases, some of which are chromosomal, constitutive, and inducible[12] and some of which are on plasmids.[33] Some isolates overproduce β-lactamase,[12] which stoichiometrically can render β-lactamase inhibitors useless. In addition, their porins are small, thus rendering antibiotic entry difficult. More recently, a multidrug efflux pump of the RND-type has been described.[4] Although there is no antibiotic to which all isolates have been shown to be sensitive,[41] most are sensitive in vitro to trimethoprim-sulfamethoxazole, piperacillin-tazobactam, imipenem, ceftazidime, and cefoperazone. Imipenem-resistant strains also have been discovered. These strains produced VIM-2, OXA-30, and a chromosomal AmpC β-lactamase.[50] Two case reports describe treatment failures of ceftazidime[37] and piperacillin[12] in clinical isolates that were sensitive at the time of isolation but developed resistance during the course of therapy.

Because resistance patterns vary across isolates, the combination of a third-generation cephalosporin, piperacillin (or piperacillin-tazobactam), or imipenem with trimethoprim-sulfamethoxazole is reasonable empirical therapy for suspected *Achromobacter* infection, pending susceptibility results. In general, in vitro susceptibilities seem to correlate well with in vivo results,[32] but the risk for inducible resistance to β-lactam antibiotics should be acknowledged. One report described synergy in microbial killing with an aminoglycoside, even though the isolate was resistant to the same aminoglycoside when it was tested alone.[9] This phenomenon was confirmed by two-disk Kirby-Bauer approximation methods using 11 clinical blood culture isolates; all were resistant to gentamicin, but 10/11 were inhibited synergistically when gentamicin was added to ticarcillin-clavulanate, and 9/11 displayed synergy when gentamicin was added to piperacillin or ceftazidime.[15]

Removal of infected catheters may speed recovery, although some patients have been treated successfully through indwelling lines.[9] Because of a high recurrence rate, experts in the care of patients with renal failure treated with continuous ambulatory peritoneal dialysis recommend removal of peritoneal catheters in patients who develop peritonitis with *A. xylosoxidans*.[19]

Acknowledgments

I would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Bador J, Amoureux L, Duez JM, et al. First description of an RND-type multidrug efflux pump in *Achromobacter xylosoxidans*, AxyABM. *Antimicrob Agents Chemother* 2011;**55**:4912–14.
13. Degand N, Carbonnelle E, Dauphin B, et al. Matrix-associated laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. *J Clin Microbiol* 2008;**46**:3361–7.
30. Kiernan DF, Chin EK, Sclafani LA, Saidel MA. Multiple drug-resistant *Alcaligenes xylosoxidans* keratitis in a sanitation worker. *Eye Contact Lens* 2009;**35**:212–14.
31. Lambiase A, Catania MR, Del Pezzo M, et al. *Achromobacter xylosoxidans* respiratory tract infection in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 2011;**30**:973–80.
34. Linke SJ, Skevas C, Richard G, Katz T. Bilateral *Achromobacter xylosoxidans* keratitis after laser in situ keratomileusis. *J Cataract Refract Surg* 2010;**36**:1045–7.
35. Lucatelli JF, Cantarelli VV, Picoli SU. Conjunctivitis due to *Achromobacter xylosixidans*: case report. *Arg Bras Oftalmol* 2009;**72**:261–3.
40. Olshtain-Pops K, Block C, Temper V, et al. An outbreak of *Achromobacter xylosoxidans* associated with ultrasound gel used during transrectal ultrasound guided prostate biopsy. *J Urol* 2011;**185**:144–7.
55. Tena D, Gonzalez-Praetorius A, Perez-Balsalobre M, et al. Urinary tract infection due to *Achromobacter xylosoxidans*: report of 9 cases. *Scand J Infect Dis* 2008;**40**:84–7.
56. Turton JF, Mustafa N, Shah J, et al. Identification of *Achromobacter xylosoxidans* by detection of the bla(OXA-114-like) gene intrinsic in this species. *Diagn Microbiol Infect Dis* 2011;**70**:408–11.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ahn Y, Kim NH, Shin DH, et al. Pacemaker lead endocarditis caused by *Achromobacter xylosoxidans*. *J Korean Med Sci* 2004;**19**: 291–3.
2. Aisenberg G, Rolston KV, Safdar A. Bacteremia caused by *Achromobacter* and *Alcaligenes* species in 46 patients with cancer (1989–2003). *Cancer* 2004;**101**:2134–40.
3. Asano K, Tada S, Matsumoto T, et al. A novel bacterium *Achromobacter xylosoxidans* as a cause of liver abscess: three case reports. *J Hepatol* 2005;**43**:362–5.
4. Bador J, Amoureux L, Duez JM, et al. First description of an RND-type multidrug efflux pump in *Achromobacter xylosoxidans*, AxyABM. *Antimicrob Agents Chemother* 2011;**55**:4912–14.
5. Benaoudia F, Bengen E. Evidence for the genetic unrelatedness of nosocomial *Alcaligenes xylosoxidans* strains in a pediatric hospital. *Infect Control Hosp Epidemiol* 1997;**18**:132–4.
6. Bizet C, Tekaia F, Phillipon A. In vitro susceptibility of *Alcaligenes faecalis* compared with those of other *Alcaligenes* species to antimicrobial agents including seven beta-lactams. *J Antimicrob Chemother* 1993;**32**:907–10.
7. Boukadida J, Monastiri K, Snoussi N, et al. Nosocomial neonatal meningitis by *Alcaligenes xylosoxidans* transmitted by aqueous eosin. *Pediatr Infect Dis J* 1993;**12**:696–7.
8. Burns JL, Emerson J, Stapp JR, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. *Clin Infect Dis* 1998;**27**:158–63.
9. Cieslak TJ, Raszka WV. Catheter-associated sepsis due to *Alcaligenes xylosoxidans* in a child with AIDS. *Clin Infect Dis* 1993;**16**: 592–3.
10. D'Amato RF, Salemi M, Mathews A, et al. *Achromobacter xylosoxidans* (*Alcaligenes xylosoxidans* subsp. *xylosoxidans*) meningitis associated with a gunshot wound. *J Clin Microbiol* 1988;**26**:2425–6.
11. De Baets F, Schelstraete P, Van Daele S, et al. *Achromobacter xylosoxidans* in cystic fibrosis: prevalence and clinical relevance. *J Cyst Fibros* 2007;**6**:75–8.
12. Decre D, Arlet G, Danglot C, et al. A beta-lactamase overproducing strain of *Alcaligenes dentrificans* subsp. *xylosoxidans* isolated from a case of meningitis. *J Antimicrob Chemother* 1992;**30**:769–79.
13. Degand N, Carbonnelle E, Dauphin B, et al. Matrix-associated laser desorption ionization-time of flight mass spectrometry for identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patients. *J Clin Microbiol* 2008;**46**:3361–7.
14. Doxiadis SA, Pavlatou M, Chryssostomidou O. *Bacillus foecalis alcaligenes* septicemia in the newborn. *J Pediatr* 1960;**56**:648–54.
15. Duggan JM, Goldstein SJ, Chenoweth CE, et al. *Achromobacter xylosoxidans* bacteremia: report of four cases and review of the literature. *Clin Infect Dis* 1996;**23**:569–76.
16. Dunne Jr WM, Maisch S. Epidemiological investigation of infections due to *Alcaligenes* species in children and patients with cystic fibrosis: use of repetitive-element-sequence polymerase chain reaction. *Clin Infect Dis* 1995;**20**:836–41.
17. Dworzack DL, Murray CM, Hodges GR, et al. Community acquired bacteremic *Achromobacter xylosoxidans* type IIIa: pneumonia in a patient with idiopathic IgM deficiency. *Am J Clin Pathol* 1978;**70**:712–17.
18. Edwards LD, Tan-Gatue LG, Levin S, et al. The problem of bacteriologically contaminated infant formulas in a newborn nursery. *Clin Pediatr* 1974;**13**:63–5.
19. El-Shahawy MA, Kim D, Gadallah MF. Peritoneal dialysis–associated peritonitis caused by *Alcaligenes xylosoxidans*. *Am J Nephrol* 1998;**18**:452–5.
20. Festini F, Taccetti G, Mannini C, et al. Patient risk of contact with respiratory pathogens from inanimate surfaces in a cystic fibrosis outpatient clinic: a prospective study over a four-year period. *Pediatr Pulmonol* 2007;**42**:779–84.
21. Foley JF, Gravelle CR, Englehard WE, et al. *Achromobacter* septicemia fatalities in prematures. *Am J Dis Child* 1961;**101**: 279–88.
22. Gomez-Cerezo J, Suarez I, Rios JJ, et al. *Achromobacter xylosoxidans* bacteremia: a 10-year analysis of 54 cases. *Eur J Clin Microbiol Infect Dis* 2003;**22**:360–3.
23. Gradon JD, Mayrev AR, Hayes J. Pulmonary abscess associated with *Alcaligenes xylosoxidans* in a patient with AIDS. *Clin Infect Dis* 1993;**17**:1071–2.
24. Granowitz EV, Keenholtz SL. A pseudoepidemic of *Alcaligenes xylosoxidans* attributable to contaminated saline. *Am J Infect Control* 1998;**83**:284–5.
25. Hearn YR, Gander RM. *Achromobacter xylosoxidans*: an unusual neonatal pathogen. *Am J Clin Pathol* 1991;**96**:211–14.
26. Hoddy DM, Barton LL. Puncture wound–induced *Achromobacter xylosoxidans* osteomyelitis of the foot. *Am J Dis Child* 1991;**145**: 599–600.
27. Holmes B, Snell JJS, Lapage SP. Strains of *Achromobacter xylosoxidans* from clinical material. *J Clin Pathol* 1977;**30**:595–601.
28. Igra-Siegman Y, Chmel H, Cobbs C. Clinical and laboratory characteristics of *Achromobacter xylosoxidans* infection. *J Clin Microbiol* 1980;**11**:141–5.
29. Kersters K, DeLey J. Genus *Alcaligenes*. In: Krieg NR, Holt JG, editors. *Bergey's Manual of Systematic Bacteriology*. Baltimore: Williams & Wilkins; 1984. p. 361–73.
30. Kiernan DF, Chin EK, Sclafani LA, Saidel MA. Multiple drug-resistant *Alcaligenes xylosoxidans* keratitis in a sanitation worker. *Eye Contact Lens* 2009;**35**:212–14.
31. Lambiase A, Catania MR, Del Pezzo M, et al. *Achromobacter xylosoxidans* respiratory tract infection in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 2011;**30**:973–80.
32. Legrand C, Anqissie E. Bacteremia due to *Achromobacter xylosoxidans* in patients with cancer. *Clin Infect Dis* 1992;**14**:479–84.
33. Levesque R, Royu PH, Letarte R, et al. A plasmid-mediated cephalosporinase from *Achromobacter* species. *J Infect Dis* 1982;**145**: 753–61.
34. Linke SJ, Skevas C, Richard G, Katz T. Bilateral *Achromobacter xylosoxidans* keratitis after laser in situ keratomileusis. *J Cataract Refract Surg* 2010;**36**:1045–7.
35. Lucatelli JF, Cantarelli VV, Picoli SU. Conjunctivitis due to *Achromobacter xylosixidans*: case report. *Arg Bras Oftalmol* 2009;**72**: 261–3.
36. Mandell WF, Garvey GJ, Neu HC. *Achromobacter xylosoxidans* bacteremia. *Rev Infect Dis* 1987;**9**:1001–5.
37. Manjra AI, Moosa A, Bhamjee A. Fatal neonatal meningitis and ventriculitis caused by multi-resistant *Achromobacter xylosoxidans*: a case report. *S Afr Med J* 1989;**76**:571–3.
38. McGarrity GJ, Coriell LL. Bacterial contamination of children's soap bubbles. *Am J Dis Child* 1973;**125**:224–6.
39. Oh JY, Shin YJ, Wee WR. A case of epidemic keratoconjunctivitis complicated by *Alcaligenes xylosoxidans* infection. *Korean J Ophthalmol* 2005;**19**:233–4.
40. Olshtain-Pops K, Block C, Temper V, et al. An outbreak of *Achromobacter xylosoxidans* associated with ultrasound gel used during transrectal ultrasound guided prostate biopsy. *J Urol* 2011; **185**:144–7.
41. Olson DA, Hoeprich PD. Postoperative infection of an aortic prosthesis with *Achromobacter xylosoxidans*. *West J Med* 1982;**136**: 153–7.
42. Peel MM, Hibberd AJ, King BM, et al. *Alcaligenes piechaudii* from chronic ear discharge. *J Clin Microbiol* 1988;**26**:1580–1.
43. Reverdy ME, Freney J, Fleurette J, et al. Nosocomial colonization and infection by *Achromobacter xylosoxidans*. *J Clin Microbiol* 1984; **19**:140–3.
44. Ronne Hansen C, Pressler T, Hoiby N, Gormsen M. Chronic infection with *Achromobacter xylosoxidans* in cystic fibrosis patients: a retrospective case control study. *J Cyst Fibros* 2006;**5**:245–51.
45. San-Miguel VV, Lavery JP, York JC, et al. *Achromobacter xylosoxidans* septic arthritis in a patient with systemic lupus erythematosus. *Arthritis Rheum* 1991;**34**:1484–5.
46. Schoch PE, Cunha BA. Nosocomial *Achromobacter xylosoxidans* infections. *Infect Control Hosp Epidemiol* 1988;**9**:84–7.
47. Sepkowitz DV, Bostic DE, Maslow MJ. *Achromobacter xylosoxidans* meningitis: case report and review of the literature. *Clin Pediatr* 1987;**26**:483–5.
48. Shie SS, Huang CT, Leu HS. Characteristics of *Achromobacter xylosoxidans* bacteremia in northern Taiwan. *J Microbiol Immunol Infect* 2005;**38**:277–82.
49. Shigeta S, Yasunaga Y, Honsumi K, et al. Cerebral ventriculitis associated with *Achromobacter xylosoxidans*. *J Clin Pathol* 1978;**70**: 712–17.
50. Shin KS, Han K, Lee J, et al. Imipenem-resistant *Achromobacter xylosoxidans* carrying blaVIM-2–containing class 1 integrin. *Diagn Microbiol Infect Dis* 2005;**53**:215–20.

51. Siebor E, Llanes C, Lafon I, et al. Presumed pseudobacteremia outbreak resulting from contamination of proportional disinfectant dispenser. *Eur J Clin Microbiol Infect Dis* 2007;**26**:195–8.
52. Spear JB, Fuhrer J, Kirby BD. *Achromobacter xylosoxidans* (*Alcaligenes xylosoxidans* subsp. *xylosoxidans*) bacteremia associated with well-water source: case report and review of the literature. *J Clin Microbiol* 1988;**26**:598–9.
53. Taylor P, Fischbein L. Prosthetic knee infection due to *Achromobacter xylosoxidans*. *J Rheumatol* 1992;**19**:992–3.
54. Tena D, Gonzalez-Praetorius A, Perez-Balsalobre M, et al. Urinary tract infection due to *Achromobacter xylosoxidans*: report of 9 cases. *Scand J Infect Dis* 2008;**40**:84–7.
55. Tena D, Carranza R, Barbera JR, et al. Outbreak of long-term intravascular catheter-related bacteremia due to *Achromobacter xylosoxidans* subspecies *xylosoxidans* in a hemodialysis unit. *Eur J Clin Microbiol Infect Dis* 2005;**24**:727–32.
56. Turton JF, Mustafa N, Shah J, et al. Identification of *Achromobacter xylosoxidans* by detection of the bla(OXA-114-like) gene intrinsic in this species. *Diagn Microbiol Infect Dis* 2011;**70**:408–11.
57. Uy HS, Matias R, de la Cruz F, Natividad F. *Achromobacter xylosoxidans* endophthalmitis diagnosed by polymerase chain reaction and gene sequencing. *Ocul Immunol Inflamm* 2005;**13**:463–7.
58. Vu-Thien H, Moissenet D, Valcin M, et al. Molecular epidemiology of *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Alcaligenes xylosoxidans* in a cystic fibrosis center. *Eur J Clin Microbiol Infect Dis* 1996;**15**:876–9.
59. Walsh RD, Klein NC, Cunha BA. *Achromobacter xylosoxidans* osteomyelitis. *Clin Infect Dis* 1993;**16**:176–8.
60. Weitkamp JH, Tang YW, Haas DW, et al. Recurrent *Achromobacter xylosoxidans* bacteremia associated with persistent lymph node infection in a patient with hyper-immunoglobulin M syndrome. *Clin Infect Dis* 2000;**31**:1183–7.

CHAPTER 123

Eikenella corrodens

Randall G. Fisher

Eikenella corrodens is a facultatively anaerobic, fastidious gram-negative rod that is part of the normal flora of the mouth and the gastrointestinal and genitourinary tracts. Long regarded as a commensal, its pathogenicity no longer is in doubt. It frequently is a pathogen of periodontitis in both adults and children and is a common isolate from wounds that have been contaminated by oral secretions. It also has been recovered from pleuropulmonary infections, central nervous system infections, orbital cellulitis, peritonsillar abscesses, abdominal infections, osteomyelitis, and bloodstream infections, including endocarditis.

BACTERIOLOGY

In 1948, Hendriksen[18] described the organism and called it the "corroding bacillus" because it pitted the agar. It was characterized more fully in 1958 by Eiken,[10] who named it *Bacteroides corrodens*. In 1972, Jackson and Goodman[19] separated two species of corroding bacteria; the strict anaerobe kept the name *B. corrodens* (now called *Bacteroides ureolyticus*), and the facultative anaerobe was classified as *Eikenella*. It is a small, straight, nonmotile gram-negative rod that occasionally is coccobacillary. It is oxidase positive and catalase negative. Most strains are positive for lysine and ornithine decarboxylase. The organism is nonfermentative, reduces nitrate to nitrite, and is negative for urease and indole. *E. corrodens* cell surface components vary from isolate to isolate; these differences probably relate to virulence.[6]

E. corrodens will grow either aerobically or anaerobically, but its growth is not rapid. Growth can be enhanced by 3 to 10 percent carbon dioxide. It grows on blood or chocolate agar but poorly or not at all on MacConkey agar. Selective medium, which contains clindamycin, may increase the yield. Colonies are small and grayish. They look slightly yellow when they are old. Although *E. corrodens* is nonhemolytic, a faint green appearance may be seen on blood agar. Approximately 50 percent will produce the characteristic pitting. They elaborate an odor that resembles that of bleach or hypochlorite.[19]

E. corrodens is a member of the so-called HACEK family of organisms, which have the following in common: slow growth, a requirement for carbon dioxide, and a predilection for infecting heart valves. The other members of the family are *Aggregatibacter*[33] (formerly *Haemophilus*) *aphrophilus*, *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans*, *Cardiobacterium hominis*, and *Kingella kingae*.

EPIDEMIOLOGY

Infection with *E. corrodens* occurs when mucosal or skin barriers are disrupted and the organism gains access to deeper tissues. Puncture of the skin with forks[35] or toothpicks[41] may result in deep-seated infections. One case of vertebral osteomyelitis that occurred after a woman accidentally inoculated the organism into the paravertebral space by penetration of a fish bone through the posterior pharynx has been reported.[30] In a similar case, a fish bone stuck in the throat for 2 months eventually led to a spinal epidural abscess.[23] Infection commonly occurs after clenched-fist injury as a result of fistfighting.[14] Hand infections in children are more likely to be secondary to digital biting or sucking.[16] Intravenous drug abusers are at risk for injection-site and soft tissue abscesses,[15] bacteremia, and endocarditis.[9,34] Elderly persons and patients with advanced carcinomas are the other high-risk groups. However, children are at particularly high risk for serious *E. corrodens* infections.[40] Reports of thyroid abscesses[7,49] and purulent thyroiditis[39] all have been in children. In one review,[25] more than 20 percent of *E. corrodens* pleuropulmonary infections occurred in children younger than 14 years of age and more than 50 percent of abdominal infections were reported in patients younger than 25 years of age.[8] *E. corrodens* orbital cellulitis,[17] empyema,[46] peritonsillar abscess,[27] paronychia,[1] and osteomyelitis[37,43] have been observed in children. A review of 54 cases of *E. corrodens* infection in children and adolescents revealed that 41 percent of pediatric infections occurred in the head and neck. The most common single site was the thyroid gland.[35]

PATHOPHYSIOLOGY

E. corrodens infections often are polymicrobial[47] and may include other anaerobes or gram-negative rods. However, *E. corrodens* is accompanied most frequently by recovery of α-hemolytic streptococci. In most reports, the streptococci were not speciated further but Jacobs and associates[21] made a case for the *Streptococcus anginosus* group because of similarities between the two organisms (i.e., both are found in the mouth and gastrointestinal tract, both produce local suppurative infection, and both thrive in carbon dioxide–rich, oxygen-poor environments). Brooks and colleagues[4] also reported synergy of the two organisms in a rabbit model of skin infection. In vitro studies of *E. corrodens* cocultivated with members of the *S. anginosus* group showed that a significant degree of

coaggregation occurs. Additionally, exponential growth of *Streptococcus constellatus* and *Streptococcus intermedius* occurs 6 hours into incubation when these species are grown in the presence of *E. corrodens;* in its absence, exponential growth does not occur until 25 hours after inoculation.[51]

The possible role of *E. corrodens* in periodontitis has not been delineated precisely. However, soluble products of *E. corrodens* induce gene expression and protein production of vascular endothelial growth factor and cause phosphorylation of mitogen-activated protein kinase in vitro, a process that leads to increased production of interleukin-8 and adhesion molecules.[52] This cascade of inflammatory responses could promote chronic periodontitis.

E. corrodens has a propensity toward formation of an abscess in any location, whether alone or in concert with other organisms. Such formation is a hallmark of central nervous system infection.[3] Of intra-abdominal infections reported by Danziger and associates,[8] 15 of 19 patients had abscesses. In two cases of orbital cellulitis reported by Hemady and coworkers,[17] both patients had subperiosteal abscesses. Deep or superficial skin abscesses reported in drug addicts[15] or in clenched-fist injury from fistfighting[14] often recur, even after presumed adequate drainage.[38]

CLINICAL MANIFESTATIONS

Infections with *E. corrodens* are indolent. The time from inoculation to onset of symptoms generally is 1 week or longer.[4] Many cases show initial improvement with treatment but relapse days later, even with appropriate therapy.[16,27,36,40] The head and neck are the most common sites of infection at all ages.[35]

Infection of periodontal sites may be associated with rapid progression and bone resorption thought to be secondary to surface-associated materials of *E. corrodens* and other organisms of periodontitis.[31] Craniofacial and neck infections tend to have prolonged morbidity; many require repeated drainage procedures and long courses of antimicrobial agents.[40] Central nervous system infections often are preceded by sinus infections but also have been seen in children with congenital heart disease.[2,48]

Pleuropulmonary infections are marked by fever, cough, and chest pain. Necrotizing pneumonia with multiple abscesses sometimes is seen. Effusions or empyema are noted in 30 percent, and cavitation is seen in 8 percent. Children with a predisposition toward aspiration may be at higher risk.[25] In one case, a bedridden 16-year-old girl developed a bronchopleural-cutaneous fistula complicating necrotizing pneumonia with empyema.[50]

Endocarditis is associated with large, friable vegetations and frequent emboli and often requires valve replacement.[11] Intravenous drug use has been implicated in approximately half of reported cases.

Abdominal *E. corrodens* infections are seen most commonly as complications of ruptured appendicitis but also have been associated with abdominal trauma and surgery. The clinical course is protracted.[8]

Chorioamnionitis leading to premature delivery has been documented.[12,22] In one case, the infection precipitated the birth of twins at 23 weeks' gestation and led to the demise of one twin.[24,26,45]

Soft tissue infections tend to be severe. Many require wide débridement and skin grafting. Infection of underlying joints, tendons, or bones is not infrequent and can be necrotizing and even lead to amputation.[38]

DIAGNOSIS

Definitive diagnosis rests on recovery of *E. corrodens* in culture, which can be a difficult task because of the organism's slow growth. *Eikenella* tends to be overgrown by hardier species when it is part of a polymicrobial process and may be missed, especially if the isolate does not pit the agar. All the HACEK organisms can pit agar,[5] although not with the regularity of *E. corrodens.*

Many bacteriology laboratories have difficulty identifying and separating catalase-negative, oxidase-positive, gram-negative rods. Not surprisingly, one report noted that of 100 isolates of *E. corrodens* identified by the National Collection of Type Cultures, only 21 were sent in as probable *E. corrodens.*[5] Organisms that *E. corrodens* may be mistaken for include the other HACEK organisms, *Aggregatibacter* (formerly *Haemophilus*) *paraphrophilus*, *Moraxella atlantae*, and *Actinobacillus ureae.*

TREATMENT

E. corrodens has a very unusual antimicrobial susceptibility pattern: although most isolates are sensitive to penicillin and ampicillin, they are resistant to semisynthetic penicillins, such as methicillin and nafcillin.[44] Additionally, these organisms uniformly are resistant to clindamycin and metronidazole,[20] drugs commonly used to treat anaerobic infections. They also variably are resistant to aminoglycosides.

Most isolates are sensitive to piperacillin, second- and third-generation cephalosporins, carbapenems, second- and third-generation fluoroquinolones, and tetracycline. In one report, all of 31 *Eikenella* isolates derived from normal oral flora were sensitive to azithromycin in vitro.[32] Although penicillin often is cited as the drug of choice, some strains produce β-lactamases. One report associated the β-lactamase with a transposon[29] and another with a plasmid[42]; one report found a chromosomal enzyme that was not inducible.[28] In addition, there are reports of intermediate resistance to penicillin, even in isolates that do not produce a β-lactamase.[13]

Incision and drainage of abscesses and débridement of necrotic tissue are essential to recovery from these infections. Therapy should be prolonged after patients apparently have recovered because early cessation of antibiotic therapy tends to be associated with relapse. If patients continue to have fever or other signs of infection days after appropriate therapy has been started, obtaining

more images of the infected area may be prudent to detect early reaccumulation of purulence.

Acknowledgments

I would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCE SINCE THE SIXTH EDITION

12. Garnier F, Masson G, Bedu A, et al. Maternofetal infections due to *Eikenella corrodens*. *J Med Microbiol* 2009;**58**:273–5.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 124

Elizabethkingia and *Chryseobacterium* Species

Randall G. Fisher

Members of the genus *Chryseobacterium* (formerly *Flavobacterium*) seldom are associated with human infection. Most disease occurs after exposure to a contaminated environmental source. In 1944, Shulmann and Johnson[52] reported a case of meningitis caused by a previously unidentified, gram-negative bacillus isolated from a 9-day-old premature infant. The term *Flavobacterium meningosepticum* was proposed for this organism by King[31] in 1959, based on her studies of bacterial isolates associated primarily with neonatal meningitis and septicemia. Although neonatal meningitis is the most common manifestation of human disease caused by this genus,[15,58] sepsis,[14,23,44,55] endocarditis, pneumonia,[46] septic arthritis with penetrating trauma or prosthesis,[22,32] and skin infection[19] (including one case of necrotizing fasciitis)[33] occur in individuals beyond the newborn period.[3]

BACTERIOLOGY

The taxonomy of these organisms is confusing because of frequent changes. All but one of the clinically relevant species of the original genus *Flavobacterium* were reclassified to the new genus *Chryseobacterium* in 1994.[62] Phylogenetic analysis based on 16S rRNA sequencing demonstrated that the organisms formerly classified as *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* represent a separate lineage. Researchers proposed that these two organisms be moved to a new genus, named *Elizabethkingia* after the microbiologist who first described them.[30] The former *Flavobacterium odoratum*, rarely responsible for human disease, has been divided into two species (*odoratus* and *odoratimimus*) reclassified as members of the genus *Myroides*.[61] *Chryseobacterium indologenes* is the species isolated most frequently from human specimens,[28] but it usually is not associated with significant disease. Therefore, this chapter focuses mainly on *Elizabethkingia meningosepticum*, which can cause severe infections, especially in newborns. These organisms are long, thin, catalase-positive, gram-negative rods with slightly swollen ends; they are nonmotile, oxidase-positive, weakly fermentative, and proteolytic and grow on solid agar as 1- to 2-mm, convex, glistening colonies of buttery consistency.[31] Yellow pigmentation is seen occasionally. Colonies do not demonstrate hemolysis on blood agar but may produce a lavender-green color in the surrounding media as a result of extensive proteolytic enzyme activity. *Elizabethkingia* is unable to grow on *Salmonella-Shigella* agar or Simmons citrate and lacks motility. These characteristics distinguish *Elizabethkingia* from *Pseudomonas*, with which it often is confused.[15] Similarly, use of glucose in an open tube of oxidation-fermentation media distinguishes *Elizabethkingia* from *Achromobacter faecalis*.[15] The clinically relevant species *E. meningosepticum* (also referred to interchangeably in the current literature by its old name *Chryseobacterium meningosepticum* and in the older literature as *Flavobacterium meningosepticum*) and *Flavobacterium IIB* (renamed *Flavobacterium balustinum*) are differentiated by the former's consistent liquefaction of gelatin and early use of

REFERENCES

1. Barton LL, Anderson LE. Paronychia caused by HB-1 organisms. *Pediatrics* 1974;**54**:372–3.
2. Brill CB, Pearlstein LS, Kaplan M, et al. Central nervous system infections caused by *Eikenella corrodens*. *Arch Neurol* 1982;**39**: 431–2.
3. Bronitsky R, Heim CR, McGee ZA. Multifocal brain abscesses: combined medical and neurosurgical therapy. *South Med J* 1982; **75**:1261–3.
4. Brooks GF, O'Donoghue JM, Rissing JP. *Eikenella corrodens:* a recently recognized pathogen. Infections in medical-surgical patients and in association with methylphenidate abuse. *Medicine (Baltimore)* 1974;**53**:325–42.
5. Chadwick PR, Malnick H, Ebizie AO. *Haemophilus paraphrophilus* infection: a pitfall in laboratory diagnosis. *J Infect* 1995;**30**:67–9.
6. Chen CKC, Wilson ME. Outer membrane protein and lipopolysaccharide heterogeneity among *Eikenella corrodens* isolates. *J Infect Dis* 1990;**162**:664–71.
7. Cheng AF, Man DWK, French GL. Thyroid abscess caused by *Eikenella corrodens*. *J Infect* 1988;**16**:181–5.
8. Danziger LH, Schoonover LL, Kale P, et al. *Eikenella corrodens* as an intra-abdominal pathogen. *Am Surg* 1994;**60**:296–9.
9. Decker MD, Graham BS, Hunter EB, et al. Endocarditis and infections of intravascular devices due to *Eikenella corrodens*. *Am J Med Sci* 1986;**292**:209–12.
10. Eiken M. Studies on an anaerobic, rod-shaped, gram-negative micro-organism: *Bacteroides corrodens*. *Acta Pathol Microbiol Scand* 1958;**43**:391–406.
11. Ellner JJ, Rosenthal MS, Lerner PI, et al. Infectious endocarditis caused by slow-growing, fastidious, gram negative bacteria. *Medicine (Baltimore)* 1979;**58**:145–58.
12. Garnier F, Masson G, Bedu A, et al. Maternofetal infections due to *Eikenella corrodens*. *J Med Microbiol* 2009;**58**:273–5.
13. Goldstein EJC, Citron DM. Sensitivity of *Eikenella corrodens* to penicillin, apalcillin, and twelve new cephalosporins. *Antimicrob Agents Chemother* 1984;**26**:947–8.
14. Goldstein EJC, Miller TA, Citron DM, et al. Infections following clenched-fist injury: a new perspective. *J Hand Surg* 1978;**3**: 455–7.
15. Gonzalez MH, Garst J, Nourbush P, et al. Abscesses of the upper extremities from drug abuse by injection. *J Hand Surg Am* 1993; **18**:868–70.
16. Harness N, Blazar PE. Causative microorganisms in surgically treated pediatric hand infections. *J Hand Surg Am* 2005;**30**: 1294–7.
17. Hemady R, Zimmerman A, Katzen BW, et al. Orbital cellulitis caused by *Eikenella corrodens*. *Am J Ophthalmol* 1992;**114**:584–8.
18. Hendriksen SD. Studies in gram-negative anaerobes, part II: gram-negative anaerobic rods with spreading colonies. *Acta Pathol Microbiol Scand* 1948;**25**:368–75.
19. Jackson FL, Goodman YE. Transfer of the facultatively anaerobic organism *Bacteroides corrodens Eiken* to a new genus, *Eikenella*. *Int J Syst Bacteriol* 1972;**22**:73–7.
20. Jackson FL, Goodman YE. Genus *Eikenella*. In: Krieg NR, Holt JG, editors. *Bergey's manual of systematic bacteriology*. Baltimore: Williams & Wilkins; 1984. p. 591–7.
21. Jacobs JA, Algie GD, Cie GH, et al. Association between *Eikenella corrodens* and streptococci. *Clin Infect Dis* 1993;**16**:173.
22. Jadhav AR, Belfort MA, Dildy 3rd GA. *Eikenella corrodens* chorioamnionitis: modes of infection. *Am J Obstet Gynecol* 2009;**200**: e4–5.
23. Jeon SH, Han DC, Lee SG, et al. *Eikenella corrodens* cervical spine epidural abscess induced by a fish bone. *J Korean Med Sci* 2007; **22**:380–2.
24. Jeppson KG, Reimer LG. *Eikenella corrodens* amnionitis. *Obstet Gynecol* 1991;**78**:503–5.
25. Joshi N, O'Bryan T, Appelbaum PC. Pleuropulmonary infections caused by *Eikenella corrodens*. *Rev Infect Dis* 1991;**13**:1207–12.
26. Kostadinov S, Pinar H. Amniotic fluid infection syndrome and neonatal mortality caused by *Eikenella corrodens*. *Pediatr Dev Pathol* 2005;**8**:489–92.
27. Knudsen TD, Simke EJ. *Eikenella corrodens:* an unexpected pathogen causing a persistent peritonsillar abscess. *Ear Nose Throat J* 1995;**74**:114–17.
28. Lacroix JM, Walker CB. Characterization of a beta-lactamase found in *Eikenella corrodens*. *Antimicrob Agents Chemother* 1991;**35**: 886–91.
29. Lacroix JM, Walker CB. Identification of a streptomycin resistance gene and a partial Tn3 transposon coding for a beta-lactamase in a periodontal strain of *Eikenella corrodens*. *Antimicrob Agents Chemother* 1992;**36**:740–3.
30. Lehman CR, Deckey JE, Hu SS. *Eikenella corrodens* vertebral osteomyelitis secondary to direct inoculation: a case report. *Spine* 2000;**25**:1185–7.
31. Meghji S, Wilson M, Barber P, et al. Bone resorbing activity of surface-associated material from *Actinobacillus actinomycetemcomitans* and *Eikenella corrodens*. *J Med Microbiol* 1994;**41**:197–203.
32. Merriam CV, Citron DM, Tyrrell KL, et al. In vitro activity of azithromycin and nine comparator agents against 296 strains of oral anaerobes and 31 strains of *Eikenella corrodens*. *Int J Antimicrob Agents* 2006;**28**:244–8.
33. Nørskov-Lauritsen N, Kilian M. Reclassification of *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter actinomycetemcomitans* gen. nov., comb. nov., *Aggregatibacter aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include V factor–dependent and V factor–independent isolates. *Int J Syst Evol Microbiol* 2006;**56**:2135–46.
34. Patrick WD, Brown WD, Bowmer MI, et al. Infectious endocarditis due to *Eikenella corrodens:* case report and review of the literature. *Can J Infect Dis* 1990;**1**:139–42.
35. Paul K, Patel SS. *Eikenella corrodens* infections in children and adolescents: case reports and review of the literature. *Clin Infect Dis* 2001;**33**:54–61.
36. Perez-Pomata MT, Dominguez J, Hercajo P, et al. Spleen abscess caused by *Eikenella corrodens*. *Eur J Clin Microbiol Infect Dis* 1992;**11**: 162–3.
37. Polin K, Shulman ST. *Eikenella corrodens* osteomyelitis. *Pediatrics* 1982;**70**:462–3.
38. Pollner JH, Khan A, Tuazon CU. Severe soft tissue infection caused by *Eikenella corrodens*. *Clin Infect Dis* 1992;**15**:740–1.
39. Queen JS, Clegg HW, Council JC, et al. Acute suppurative thyroiditis caused by *Eikenella corrodens*. *J Pediatr Surg* 1988;**23**:359–61.
40. Raffensperger JG. *Eikenella corrodens* infections in children. *J Pediatr Surg* 1986;**21**:644–6.
41. Robinson LG, Kourtis AP. Tale of a toothpick: *Eikenella corrodens* osteomyelitis. *Infection* 2000;**28**:332–3.
42. Rotger RE, Garcia-Valdes E, Trallero EP. Characterization of a beta-lactamase–specifying plasmid isolated from *Eikenella corrodens* and its relationship to a commensal *Neisseria* plasmid. *Antimicrob Agents Chemother* 1986;**30**:508–9.
43. Sagerman SD, Lourie GM. *Eikenella* osteomyelitis in a chronic nail biter: a case report. *J Hand Surg Am* 1995;**20**:71–2.
44. Sofianou D, Kolokotronis A. Susceptibility of *Eikenella corrodens* to antimicrobial agents. *J Chemother* 1990;**2**:156–8.
45. Sporken JM, Muytjens HL, Vemer HM. Intrauterine infection due to *Eikenella corrodens*. *Acta Obstet Gynecol Scand* 1985;**64**:683–4.
46. St John A, Belda AA, Matlow A, et al. *Eikenella corrodens* empyema in children. *Am J Dis Child* 1981;**135**:415–17.
47. Suwanagool S, Rothkopf MM, Smith SM, et al. Pathogenicity of *Eikenella corrodens* in humans. *Arch Intern Med* 1983;**143**:2265–8.
48. Swanston WH, Cameron ES, Ramchaunder V. *Eikenella corrodens* brain abscess in a child with congenital heart disease. *West Indian Med J* 1988;**37**:243–5.
49. Vichyanond P, Howard CP, Olson LC. *Eikenella corrodens* as a cause of thyroid abscess. *Am J Dis Child* 1983;**137**:971–3.
50. Wong KS, Huang YC. Bronchopleural cutaneous fistula due to *Eikenella corrodens*. *J Pediatr (Rio J)* 2005;**81**:265–7.
51. Young KA, Allaker RP, Hardie JM, Whiley RA. Interactions between *Eikenella corrodens* and "*Streptococcus milleri*-group" organisms: possible mechanisms of pathogenicity in mixed infections. *Antonie Van Leeuwenhoek* 1996;**69**:371–3.
52. Yumoto H, Yamada M, Shinohara C, et al. Soluble products from *Eikenella corrodens* induce cell proliferation and expression of interleukin-8 and adhesion molecules in endothelial cells via mitogen-activated protein kinase pathways. *Oral Microbiol Immunol* 2007;**22**:36–45.

mannitol and maltose and the latter's lack of these abilities.[31] *Myroides odoratus* and *Myroides odoratimimus (F. odoratum)*, which have been identified most commonly as saprophytes in skin wounds,[26] characteristically are nonsaccharolytic and produce a fruity odor when they are grown on standard media.

EPIDEMIOLOGY

Elizabethkingia spp. and chryseobacteria are distributed widely as saprophytes in fresh and salt water, as well as in the soil. *E. meningosepticum* has been identified as a pathogen in birds.[60] In hospitals, these organisms have been found to be ubiquitous colonizers of the patient's environment and have been isolated from flower vases,[57] ice machines,[55] vials of intravenous drugs,[44] and nebulizers.[16] In addition, tap water,[16] eyewashes,[47] tube feedings,[16] sink traps,[8] and hand cultures of hospital personnel[16] have yielded this organism. In some instances, these reservoirs have been implicated in nosocomial outbreaks of patient colonization and invasive disease.

Neonatal infection caused by *E. meningosepticum* has been reported frequently in the literature, often in association with nursery epidemics.[2,7,49] As with other neonatal pathogens, infants who are premature and small for gestational age seem to be at particular risk for infection. More than 50 percent of infected infants weigh less than 2500 g. Almost all cases occur within 3 weeks of birth, and more than 50 percent of these infants manifest illness before they are 7 days old.[15]

Nosocomial epidemics have occurred sporadically since the nursery outbreak reported by Brody and colleagues in 1958.[2,5,7,8] Cabrera and Davis[8] reported such an outbreak in detail in 1960. During a 3-month period, the bacteria were isolated from a total of 44 infants, of whom 14 had overt infection. Most colonized infants had organisms isolated from the nasopharynx. The only reservoir of infectious bacteria discovered was a faulty sink trap, beneath which cleaning materials for the nursery were stored. Repair of the defective trap and thorough cleansing and repainting of the nursery coincided with termination of the epidemic. In one situation, respiratory tract colonization developed in four premature neonates, but none of them developed disease; routine infection control measures ensured that no further infants were colonized.[40]

Nursery outbreaks of *E. meningosepticum* have been traced to saline used to flush infants' eyes after administration of silver nitrate,[47] and organisms have been recovered from washbasins, sinks, disinfectants, and suction devices in other epidemics.[8] Colonization of patients in a surgical intensive care unit was associated with tap water, sinks, ice machines, and washbasins yielding the bacteria.[16] Ribotyping and random amplified polymorphic DNA fingerprinting (RAPD) offer promise for more precise characterization of epidemics.[12,13] Use of these techniques has demonstrated, in some cases, that isolates in what appears to be an outbreak may be unrelated. In one case, isolates from the surrounding environment were also unrelated to the types found in the patients.[64]

PATHOPHYSIOLOGY

Elizabethkingia spp. and *Chryseobacterium* spp. generally are of low virulence. Rabbits administered 1-mL intravenous infections of 24-hour-old broth cultures demonstrated no mortality or morbidity; death rates were less than 30 percent in mice inoculated intracerebrally with "barely turbid" preparations.[31]

Most cases of invasive human disease are thought to be caused by environmental contamination with high numbers of *E. meningosepticum*, with spread to the compromised newborn or debilitated older patient. Some neonatal infections may be caused by colonization of the infant during passage through the birth canal of a colonized mother.[13] Intrapartum infection is supported by occurrence of symptoms as early as 10 hours after birth.[15] However, only 0.3 percent of genital swabs submitted from patients with suspected venereal disease yielded the organism.[43] Continuing reports of *E. meningosepticum* as a cause of neonatal infection in developing countries have been speculated to be related to use of contaminated groundwater for bathing of newborn infants and feminine genital hygiene.[15] The propensity for this organism to produce meningitis in the newborn is not understood, but infection may occur in association with heavy nasopharyngeal colonization, leading to subsequent bacteremia and seeding of the meninges.

Cases in older children have been related to insulin-dependent diabetes mellitus,[9] thalassemia major with splenectomy,[45] and oncologic disease with indwelling catheters.[42] Peritonitis in patients undergoing continuous ambulatory peritoneal dialysis, including a case series of 30 patients, also has been reported.[46]

In older individuals, *E. meningosepticum* and chryseobacteria primarily play the role of opportunists.[39] Heavy nosocomial colonization combined with a blunted immune response probably accounts for the immunocompromised patient's poor capacity to handle this otherwise noninvasive bacterium.

CLINICAL MANIFESTATIONS

Neonatal sepsis and meningitis caused by *E. meningosepticum* share signs and symptoms in common with other forms of neonatal bacterial infection. However, the development of meningitis may be insidious, and several days of illness often pass before its presentation[15,49]; this factor is consistent with the low virulence of *E. meningosepticum* in contrast to other agents of neonatal sepsis. Prognosis is extremely poor, and mortality rates may exceed 60 percent.[34] Fifty percent of survivors develop significant neurologic complications, often in association with hydrocephalus.[35]

These organisms are uncommon pathogens in adults, and childhood disease occurring beyond the newborn period is extremely rare. Among the 24 initial isolates of *E. meningosepticum* identified by King,[31] organisms were identified in a throat culture from an adult patient and in cerebrospinal fluid (CSF) from an 8-month-old infant. Bacteria formerly classified as *Flavobacterium IIB*

(now known as *F. balustinum*) were isolated from the blood and CSF of several adult patients without clinical information.[27] Since their initial identification in 1959, these agents have been implicated as causes of meningitis,[39,49] postoperative bacteremia,[3,43] bacterial endocarditis,[65] pneumonia,[56,57] catheter-associated infection, septic arthritis, and skin infection.[19] *E. meningosepticum* is the clinically relevant species most commonly isolated, but *C. balustinum*, *M. odoratus*, and *M. odoratimimus (F. odoratum)* and other *Chryseobacterium* spp. have been implicated in human disease. *Elizabethkingia* and *Chryseobacterium* spp. accounted for 0.25 percent of infections in a large consecutive series of patients with human immunodeficiency virus infection. Risk factors included low CD4 counts and leukopenia.[38]

E. meningosepticum meningitis beyond the neonatal period typically occurs in immunocompromised patients. Adults with preexisting leukemia,[49] glomerulonephritis,[39] and squamous cell carcinoma[23] have been described as having meningitis caused by this organism. In a 56-year-old woman, meningitis with *E. meningosepticum* developed after she underwent transsphenoidal hypophysectomy[10]; in an 8-month-old male child with preceding severe neurologic damage, meningitis developed with bacteria designated by the Centers for Disease Control and Prevention as *Flavobacterium*-like organisms (IIE). In a 6-week-old infant, *E. meningosepticum* bacteremia and meningitis developed in association with a strangulated hernia.[15]

Elizabethkingia and *Chryseobacterium* spp. were isolated frequently from tracheal aspirates of patients in intensive care during a 70-month observation period; yet during that time, none of more than 2000 critically ill patients developed pneumonia attributable to these microbes.[16] However, true respiratory tract infection was identified in an intubated pediatric patient and in adults receiving aerosolized medications.[6,57]

Sporadic cases of bacteremia have been reported in adult patients.[23,36] Two reviews of adult bacteremia in Taiwan have been published. The largest of these included 118 patients at a single center over a 7-year period. The incidence of *E. meningosepticum* bacteremia increased from 7.5/100,000 admissions in 1999 to 35.6/100,000 in 2006. The vast majority of these infections were nosocomial, and 60 percent were acquired in intensive care units. Approximately a third of the patients had malignancy, and a fourth suffered from diabetes mellitus. The 14-day mortality rate was 23.4 percent. The main risk factor for mortality was acquisition of infection in an intensive care unit setting (odds ratio, 4.23). Rapid institution of appropriate antibiotic therapy reduced mortality.[29] Infection in immunocompromised patients can occur as a complication of relatively benign invasive procedures or as a localized infection.[36,53] Endocarditis was documented in intravenous drug abusers and patients undergoing dialysis.[18,65] Postoperative bacteremia in eight adult patients was linked to contaminated intravenous medications infused during anesthesia.[44] Contaminated arterial catheters were implicated in an epidemic of *Chryseobacterium* bacteremia.[55] Four patients, including a 7-year-old boy, became bacteremic in an outbreak associated with contamination at the time of intracardiac surgery.[3] This organism also has been associated with bacteremia in pediatric burn patients.[51]

Chryseobacterium spp. also have been isolated from infected skin lesions manifesting as papules, sheetlike lesions, plaques, and deep panniculitis.[19] One fatal case of necrotizing fasciitis in a patient with diabetes mellitus and chronic heart failure was reported.[33] Infection may have been related to wound contamination during repair of an orthopedic injury. A woman with pemphigus vulgaris developed cellulitis and sepsis.[54] Chryseobacteria have been isolated from amputation stumps, but the bacteria may play a largely saprophytic role at these sites.[26]

DIAGNOSIS

Rapid identification of infection with these organisms is urgent, not only to ensure providing proper therapy for the patient, but also to hasten initiating appropriate infection control measures to forestall epidemic outbreaks. Identification of *E. meningosepticum* is hindered by characteristically long periods required for oxidation of carbohydrates and weak or delayed indole production. Cultures may be misidentified as species of *Achromobacter* or *Pseudomonas*.[15] Clinical isolation of an unidentified gram-negative rod that is catalase- and oxidase-positive and that shows multiple antibiotic resistance should raise suspicion of *E. meningosepticum* infection. Cultures should be kept for several days for observation for typical carbohydrate reactions, which confirm the diagnosis.[15,48] Pulse-field gel electrophoresis of DNA was used successfully to identify recurrence of intravenous catheter-associated infection in a 6-year-old boy with non-Hodgkin lymphoma.[4,50]

TREATMENT

Unfortunately, treatment of *E. meningosepticum* meningitis represents an especially difficult challenge for the physician. The organism is paradoxically likely to be sensitive to drugs commonly employed against gram-positive organisms (i.e., rifampin, vancomycin, clindamycin, and trimethoprim-sulfamethoxazole) and resistant to those used to treat gram-negative infections (i.e., aminoglycosides, cephalosporins, tetracyclines, and carbapenems). Most *Elizabethkingia* and *Chryseobacterium* spp. produce broad-spectrum metallo–β-lactamases. In *E. meningosepticum*, the enzyme is highly efficient at hydrolyzing carbapenems. Inactivation of cephalosporins displays a remarkable variability that is based on the affinity of the enzyme for the different compounds.[63] Delay in establishing a specific identification of the organism occurs commonly, so the physician knows only that the organism is gram-negative, often resulting in prolonged periods of suboptimal therapy because recommended empirical antimicrobial treatment of gram-negative neonatal meningitis usually consists of a third-generation cephalosporin or ampicillin and an aminoglycoside, drugs to which *E. meningosepticum* almost uniformly is resistant. Moreover, antimicrobial susceptibilities determined by disk diffusion must be interpreted with caution. Aber and

associates[1] found clinical isolates in which specific strains were sensitive to gentamicin and rifampin by disk diffusion but were resistant by agar gel dilution susceptibility testing. Therefore, more direct methods of measuring the minimal inhibitory concentration than disk diffusion sensitivity should be used to determine the optimal microbial agents for therapy.

Probably as a consequence of difficulties encountered in providing rapid and effective antibacterial therapy, these organisms frequently persist for prolonged periods in CSF. Average persistence of *E. meningosepticum* in CSF is 19 days,[15] in contrast to the 3.9 days described by McCracken[41] for most cases of gram-negative neonatal bacterial meningitis.

Studies of in vitro susceptibility data forced a reappraisal of the idea that vancomycin is a good first-line choice for the treatment of chryseobacterial infection. In one report, a literature review showed that only 65 percent of historic isolates were susceptible to vancomycin[4]; in another, a thorough search for a susceptible organism among 58 clinical isolates met with failure.[20] Ninety-seven percent of *E. meningosepticum* isolates were susceptible to rifampin. Clinically, drugs that have been used alone or in combination with some success have included erythromycin, vancomycin, trimethoprim-sulfamethoxazole, fluoroquinolones (especially sparfloxacin and levofloxacin), and rifampin.[59] Some of these agents have the potential disadvantage of poor penetration of CSF. Combined use of many of these drugs renders interpreting therapeutic response difficult. The 3 survivors among the 12 patients reported by George and associates[21] all received vancomycin intravenously, intrathecally, or both, as a part of their regimen. Hawley and Gump[24] reported a case of *E. meningosepticum* meningitis in a neonate who responded to systemic vancomycin after unsuccessful treatment with multiple antibiotics, including erythromycin.

Intraventricular erythromycin[17,49] or rifampin[11,34,35,49] has been used in conjunction with systemic administration of these drugs. In particular, Lee and associates[35] reported no deaths in seven infants with *E. meningosepticum* meningitis who were treated with intraventricular rifampin through an Ommaya reservoir at a dosage of 2 to 5 mg every 24 hours combined with 40 mg/kg/day administered intravenously. Intraventricular administration continued until the CSF was sterile. However, colonization of the Ommaya reservoir was a common finding, and formation of a porencephalic cyst occurred in one patient. Chandrika and Adler[11] reported sterilization of ventricles in one afflicted neonate within 48 hours after institution of therapy with intraventricular and intravenous rifampin. Erythromycin has been used intraventricularly with limited success at 5 to 10 mg/day.[17,49] Rios and associates[49] reported the successful addition of intraventricular rifampin to a failing regimen of intravenous and intraventricular erythromycin. Development of resistance during therapy has been demonstrated with erythromycin and rifampin[17,49]; persistence of organisms in the CSF despite administration of presumably adequate therapy should alert the physician to test for this possibility. Addition of trimethoprim-sulfamethoxazole may be of benefit with such an occurrence; this agent affected a bacteriologic cure in eight of nine infants with meningitis.[37] This agent usually is not recommended in the neonatal period because of possible displacement of bilirubin from albumin-binding sites. Bacterial eradication was achieved in 48 hours in two patients with meningitis who were treated with clindamycin, rifampin, and cefotaxime systemically and rifampin intraventricularly.[7]

As with other types of gram-negative meningitis, antimicrobial therapy should be continued for at least 2 weeks after sterilization of ventricular fluid has been achieved. Time to sterilization is frequently prolonged; most patients, therefore, require 4 to 6 weeks of antimicrobial therapy. Complications of hydrocephalus and the potential use of intraventricular therapy render the neurosurgeon an essential part of the management team. Historically, mortality has been in excess of 70 percent, no doubt in part because of delays in identifying the organism and the limited antibiotic spectrum available for effective therapy. More recent series of patients, although small, suggest some improvement in this statistic, but the rate of morbidity of hydrocephalus and neurologic deficits remains high.

Recovery has been the rule in immunocompetent older individuals infected with contaminated materials, often despite treatment with antibiotics to which the organism was insensitive, a finding highlighting the very low virulence of these organisms in immunocompetent adults.[39] However, significant mortality and morbidity often occur in immunocompromised individuals with bacteremia or meningitis. Use of chloramphenicol, vancomycin, levofloxacin, erythromycin, or rifampin has achieved some success in these patients, but the choice of antibiotic must be based on a detailed examination of the organism's susceptibility.[25,51]

Acknowledgments

I would like to thank Dr. William Gruber and Dr. Thomas Boyce for their invaluable assistance with earlier versions of this chapter.

NEW REFERENCES SINCE THE SIXTH EDITION

29. Hus MS, Liao CH, Huang YT, et al. Clinical features, antimicrobial susceptibilities, and outcomes of *Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) bacteremia at a medical center in Taiwan, 1997-2006. *Eur J Clin Microbiol Infect Dis* 2011;**30**:1271–8.
40. Maraki S, Soulica E, Manoura A, et al. A *Chryseobacterium meningosepticum* colonization outbreak in a neonatal intensive care unit. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1415–19.
54. Sood S, Nerurkar V, Malvankar S. *Chryseobacterium meningosepticum* cellulitis and sepsis in an adult female with pemphigus vulgaris. *Indian J Med Microbiol* 2010;**28**:268–9.
64. Weaver KN, Jones RC, Albright R, et al. Acute emergence of *Elizabethkingia meningoseptica* infection among mechanically ventilated patients in a long-term acute care facility. *Infect Control Hosp Epidemiol* 2010;**31**:54–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aber RC, Wennersten C, Moellering Jr RC. Antimicrobial susceptibility of flavobacteria. *Antimicrob Agents Chemother* 1978;**14**: 483–7.
2. Abrahamsen TG, Finne PH, Lingaas E. *Flavobacterium meningosepticum* infections in a neonatal intensive care unit. *Acta Paediatr Scand* 1989;**78**:51–5.
3. Berry WB, Morrow AG, Harrison DC, et al. *Flavobacterium* septicemia following intracardiac operations. *J Thorac Cardiovasc Surg* 1963;**45**:476–81.
4. Bloch KC, Nadarajah R, Jacobs R. *Chryseobacterium meningosepticum*: an emerging pathogen among immunocompromised adults. *Medicine (Baltimore)* 1997;**76**:30–41.
5. Brody JA, Moore H, King EO. Meningitis caused by an unclassified gram-negative bacterium in newborn infants. *Am J Dis Child* 1958;**96**:1–5.
6. Brown RB, Phillips D, Barker MJ, et al. Outbreak of nosocomial *Flavobacterium meningosepticum* respiratory infections associated with use of aerosolized polymyxin B. *Am J Infect Control* 1989;**17**: 121–5.
7. Bruun B, Jensen ET, Lundstrom K, et al. *Flavobacterium meningosepticum* infection in a neonatal ward. *Eur J Clin Microbiol Infect Dis* 1989;**8**:509–14.
8. Cabrera HA, Davis GH. Epidemic meningitis of the newborn caused by flavobacteria. I. Epidemiology and bacteriology. *Am J Dis Child* 1961;**101**:289–95.
9. Cascio A, Stassi G, Costa GB, et al. Chryseobacterium indologenes bacteraemia in a diabetic child. *J Med Microbiol* 2005;**54**:677–80.
10. Chan KH, Chau PY, Wang RYC, et al. Meningitis caused by *Flavobacterium meningosepticum* after transsphenoidal hypophysectomy with recovery. *Surg Neurol* 1983;**20**:294–6.
11. Chandrika T, Adler SP. A case of neonatal meningitis due to *Flavobacterium meningosepticum* successfully treated with rifampin. *Pediatr Infect Dis* 1982;**1**:40–1.
12. Chiu CH, Waddingdon M, Greenberg D, et al. Atypical *Chryseobacterium meningosepticum* and meningitis and sepsis in newborns and the immunocompromised. *Taiwan Emerg Infect Dis* 2000;**6**: 481–6.
13. Colding H, Bangsborg J, Fiehn NE, et al. Ribotyping for differentiating *Flavobacterium meningosepticum* isolates from clinical and environmental sources. *J Clin Microbiol* 1994;**32**:501–5.
14. Coyle-Gilchrist MM, Crew P, Roberts G. *Flavobacterium meningosepticum* in the hospital environment. *J Clin Pathol* 1976;**29**: 824–6.
15. Dooley JR, Nims LJ, Lipp VH, et al. Meningitis of infants caused by *Flavobacterium meningosepticum*. *J Trop Pediatr* 1980;**26**:24–30.
16. du Moulin GC. Airway colonization by *Flavobacterium* in an intensive care unit. *J Clin Microbiol* 1979;**10**:155–60.
17. Ferlauto JJ, Wells DH. *Flavobacterium meningosepticum* in the neonatal period. *South Med J* 1981;**74**:757–9.
18. Ferrer C, Jakob E, Pastorino G, et al. Right-sided bacterial endocarditis due to *Flavobacterium odoratum* in a patient on chronic hemodialysis. *Am J Nephrol* 1995;**15**:82–4.
19. Findlay GH, Hull PR, Smith HE, et al. Cutaneous flavobacteriosis: polymorphous skin granulomas from *Flavobacterium capsulatum*. *S Afr Med J* 1983;**64**:247–50.
20. Fraser SL, Jorgensen JH. Reappraisal of the antimicrobial susceptibilities of *Chryseobacterium* and *Flavobacterium* species and methods for reliable susceptibility testing. *Antimicrob Agents Chemother* 1997; **41**:2738–41.
21. George RM, Cochran CP, Wheeler WE. Epidemic meningitis of the newborn caused by flavobacteria. II. Clinical manifestations and treatment. *Am J Dis Child* 1961;**101**:296–304.
22. Gunnarsson G, Baldursson H, Hilmarsdottir I. Septic arthritis caused by *Chryseobacterium meningosepticum* in an immunocompetent male. *Scand J Infect Dis* 2002;**34**:299–300.
23. Harrington SP, Perlino CA. *Flavobacterium meningosepticum* sepsis: disease due to bacteria with unusual antibiotic susceptibility. *South Med J* 1981;**74**:764–6.
24. Hawley HB, Gump DW. Vancomycin therapy of bacterial meningitis. *Am J Dis Child* 1973;**126**:261–4.
25. Hirsh BE, Wong B, Kiehn TE, et al. *Flavobacterium meningosepticum* bacteremia in an adult with acute leukemia: use of rifampin to clear persistent infection. *Diagn Microbiol Infect Dis* 1986;**4**:65–9.
26. Holmes B, Owen RJ, McMeekin TA. Genus *Flavobacterium*. In: Krieg NR, Holt JG, editors. *Bergey's Manual of Systematic Bacteriology*. Vol. 1. Baltimore: Williams & Wilkins; 1984. p. 353–60.
27. Holmes B, Snell JJS, Lapage SP. *Flavobacterium odoratum*: a species resistant to a wide range of antimicrobial agents. *J Clin Pathol* 1979;**32**:73–7.
28. Hsueh PR, Hsiue TR, Wu JJ, et al. *Flavobacterium indologenes* bacteremia: clinical and microbiological characteristics. *Clin Infect Dis* 1996;**23**:550–5.
29. Hus MS, Liao CH, Huang YT, et al. Clinical features, antimicrobial susceptibilities, and outcomes of *Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) bacteremia at a medical center in Taiwan, 1997-2006. *Eur J Clin Microbiol Infect Dis* 2011;**30**: 1271–8.
30. Kim KK, Kim MD, Lim JH, et al. Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. *Int J Syst Evol Microbiol* 2005;**55**: 1287–93.
31. King EO. Studies on a group of previously unclassified bacteria associated with meningitis in infants. *Am J Clin Pathol* 1959;**31**: 241–7.
32. Kumar R, Stephens JL. Septic arthritis caused by *Chryseobacterium meningosepticum* in an elbow joint prosthesis. *South Med J* 2004; **97**:74–6.
33. Lee CC, Chen PL, Wang LR, et al. Fatal case of community-acquired bacteremia and necrotizing fasciitis caused by *Chryseobacterium meningosepticum:* case report and review of the literature. *J Clin Microbiol* 2006;**44**:1181–3.
34. Lee EL, Robinson MJ, Thong ML, et al. Rifamycin in neonatal flavobacteria meningitis. *Arch Dis Child* 1976;**51**:209–13.
35. Lee EL, Robinson MJ, Thong ML, et al. Intraventricular chemotherapy in neonatal meningitis. *J Pediatr* 1977;**91**:991–5.
36. Lee M, Munoz J. Septicemia occurring after colonoscopic polypectomy in a splenectomized patient taking corticosteroids. *Am J Gastroenterol* 1994;**89**:2245–6.
37. Linder N, Korman SH, Eyal F, et al. Trimethoprim-sulphamethoxazole in neonatal *Flavobacterium meningosepticum* infection. *Arch Dis Child* 1984;**59**:582–4.
38. Manfredi R, Nanetti A, Ferri M, et al. *Flavobacterium* spp. organisms as opportunistic bacterial pathogens during advanced HIV disease. *J Infect* 1999;**39**:146–52.
39. Mani RM, Kuruvila KC, Batliwala PM, et al. *Flavobacterium meningosepticum* as an opportunist. *J Clin Pathol* 1978;**31**:220–2.
40. Maraki S, Soulica E, Manoura A, et al. A *Chryseobacterium meningosepticum* colonization outbreak in a neonatal intensive care unit. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1415–19.
41. McCracken Jr GH. New developments in the management of children with bacterial meningitis. *Pediatr Infect Dis* 1984;**3**(Suppl.): S32–4.
42. Nulens E, Bussels B, Bols A, et al. Recurrent bacteremia by *Chryseobacterium indologenes* in an oncology patient with a totally implanted intravascular device. *Clin Microbiol Infect* 2001;**7**: 391–3.
43. Olsen H, Raun T. *Flavobacterium meningosepticum* isolated from the genitals. *Acta Pathol Microbiol Immunol Scand* 1971;**79**:102–6.
44. Olsen H, Frederiksen WC, Siboni KE. *Flavobacterium meningosepticum* in 8 non-fatal cases of postoperative bacteraemia. *Lancet* 1965;**1**:1294–6.
45. Ozkalay N, Anil M, Agus N, et al. Community-acquired meningitis and sepsis caused by *Chryseobacterium meningosepticum* in a patient diagnosed with thalassemia major. *J Clin Microbiol* 2006;**44**: 3037–9.
46. Perera S, Palasuntheram C. *Chryseobacterium meningosepticum* infections in a dialysis unit. *Ceylon Med J* 2004;**49**:57–60.
47. Plotkin SA, McKitrick JC. Nosocomial meningitis of the newborn caused by *Flavobacterium*. *JAMA* 1966;**198**:662–4.
48. Ratner H. Flavobacterium meningosepticum. *Infect Control* 1984; **5**:237–9.
49. Rios I, Klimek JJ, Maderazo E, et al. *Flavobacterium meningosepticum* meningitis: report of selected aspects. *Antimicrob Agents Chemother* 1978;**14**:444–7.
50. Sader HS, Jones RN, Pfaller MA. Relapse of catheter-related *Flavobacterium meningosepticum* bacteremia demonstrated by DNA macrorestriction analysis. *Clin Infect Dis* 1995;**21**:997–1000.

51. Sheridan RL, Ryan CM, Pasternack MS, et al. Flavobacterial sepsis in massively burned pediatric patients. *Clin Infect Dis* 1993;**17**: 185–7.
52. Shulmann BH, Johnson MS. A case of meningitis in a premature infant due to a proteolytic gram-negative bacillus. *J Lab Clin Med* 1944;**29**:500–7.
53. Skapek SX, Jones WS, Hoffman KM, et al. Sinusitis and bacteremia caused by *Flavobacterium meningosepticum* in a sixteen-year-old with Shwachman Diamond syndrome. *Pediatr Infect Dis* 1992;**11**: 411–13.
54. Sood S, Nerurkar V, Malvankar S. *Chryseobacterium meningosepticum* cellulitis and sepsis in an adult female with pemphigus vulgaris. *Indian J Med Microbiol* 2010;**28**:268–9.
55. Stamm WF, Colella JJ, Anderson RL, et al. Indwelling arterial catheters as a source of nosocomial bacteremia. *N Engl J Med* 1975; **292**:1099–102.
56. Sundin D, Gold BD, Berkowitz FE, et al. Community-acquired *Flavobacterium meningosepticum* meningitis, pneumonia and septicemia in a normal infant. *Pediatr Infect Dis* 1991;**10**:73–6.
57. Teres D. ICU-acquired pneumonia due to *Flavobacterium meningosepticum*. *JAMA* 1974;**228**:732.
58. Thong WL, Puthucheary SD, Lee EL. *Flavobacterium meningosepticum* infection: An epidemiological study in a newborn nursery. *J Clin Pathol* 1981;**34**:429–33.
59. Tizer KB, Cervia JS, Dunn AM, et al. Successful combination vancomycin and rifampin therapy in a newborn with community-acquired *Flavobacterium meningosepticum* neonatal meningitis. *Pediatr Infect Dis J* 1995;**14**:916–17.
60. Vancanneyt M, Segers P, Hauben L, et al. *Flavobacterium meningosepticum*, a pathogen in birds. *J Clin Microbiol* 1994;**32**:2398–403.
61. Vancanneyt M, Segers P, Torck U, et al. Reclassification of *Flavobacterium odoratum* (Stutzer 1929) strains to a new genus, *Myroides*, as *Myroides odoratus* comb. nov. and *Myroides odoratimumus* sp. nov. *Int J Syst Bacteriol* 1996;**46**:926–32.
62. Vandamme P, Bernardet JF, Segers P, et al. New perspectives in the classification of the flavobacteria: description of *Chryseobacterium* gen. Nov., *Bergeyella* gen. nov., and *Empedobacter* nom. rev. *Int J Syst Bacteriol* 1994;**44**:474–81.
63. Vessillier S, Docquier JD, Rival S, et al. Overproduction and biochemical characterization of the *Chryseobacterium meningosepticum* BlaB metallo-beta-lactamase. *Antimicrob Agents Chemother* 2002;**46**: 1921–7.
64. Weaver KN, Jones RC, Albright R, et al. Acute emergence of *Elizabethkingia meningoseptica* infection among mechanically ventilated patients in a long-term acute care facility. *Infect Control Hosp Epidemiol* 2010;**31**:54–8.
65. Werthamer S, Weiner M. Subacute bacterial endocarditis due to *Flavobacterium meningosepticum*. *Am J Clin Pathol* 1972;**57**:410–12.

CHAPTER 125

Pseudomonas and Related Genera

Michael T. Brady • Mario J. Marcon

The genus *Pseudomonas* contains a large number of bacterial species, most of which are found in the soil or water as saprophytes or are plant pathogens and not associated with animal or human infections. Early attempts at better understanding the taxonomy of this diverse group of organisms led to placement of *Pseudomonas* spp. into five species clusters of genetically related organisms, also known as ribosomal RNA (rRNA) homology groups (designated groups I to V).[141] This genus since has undergone extensive revision using DNA-DNA hybridization, 16S rRNA sequencing, and related techniques, with four of the five homology groups reclassified into different genera (Table 125-1).[205] The taxon *Pseudomonas* was retained for organisms in rRNA homology group I with *P. aeruginosa* as the type species. Although they can cause infections in otherwise healthy individuals, they are more commonly opportunistic human pathogens causing disease in patients with burn wounds, cystic fibrosis, malignancy, or immunodeficiency, as well as in individuals who are malnourished, are receiving immunosuppressive therapy, or have indwelling devices. The most important species by far relating to human disease is *P. aeruginosa;* however, some other related organisms are less commonly associated with specific clinical syndromes in both children and adults. This chapter will focus on *Pseudomonas* spp. (classic rRNA group I organisms) and *Burkholderia*, *Ralstonia*, *Cupriavidus*, and *Pandoraea* spp. (classic rRNA group II and related organisms) of primary clinical significance in pediatric patients.

ETIOLOGY

Pseudomonas spp. and related genera (pseudomonads) are nonfastidious, nonfermentative, obligately aerobic (i.e., metabolize carbohydrates oxidatively and thus require molecular oxygen as the terminal hydrogen/electron acceptor), catalase positive, generally cytochrome oxidase positive and motile, non–spore-forming, gram-negative bacilli. Some species, including *P. aeruginosa*, may grow anaerobically in the presence of nitrates or other nonorganic compounds as terminal hydrogen/electron acceptors. They lack the phosphoenolpyruvate-hexose-phosphotransferase system of glycolysis and catabolize carbohydrates by the Entner-Doudoroff (aerobic, oxygen-requiring) pathway. Because pseudomonads can metabolize a wide variety of carbon sources, including simple and complex carbohydrates, alcohols, and amino acids, they can survive and multiply in almost any moist environment containing minimal amounts of organic compounds.

It is useful to discuss the *Pseudomonas* spp. (former rRNA homology group I organisms) as members of either the "fluorescent pseudomonad group" or the "nonfluorescent pseudomonad group." The fluorescent pseudomonads include *P. aeruginosa*, *P. putida*, *P. fluorescens*, and less commonly encountered species; the nonfluorescent group includes *P. stutzeri* and *P. mendocina* (stutzeri group), *P. alcaligenes* and *P. pseudoalcaligenes* (alcaligenes group), and *P. luteola* and *P. oryzihabitans* (yellow-pigmented group).

The fluorescent pseudomonads generally produce the water-soluble yellow-green fluorescent pigment pyoverdin (fluorescein), whereas many clinical isolates of *P. aeruginosa* also produce the water-soluble blue phenazine pigment pyocyanin. These pigments diffuse into and color the medium surrounding the colonies. The combination of pyoverdin and pyocyanin produced by *P. aeruginosa* combine to give the bright-green to blue-green color characteristic of this organism on certain clear laboratory media, particularly Mueller-Hinton agar. Some strains of *P. aeruginosa* may produce other pigments, such as dark red (pyorubin) or brown-black (pyomelanin) pigment.

P. aeruginosa is readily recovered from clinical specimens and usually is recognized easily on laboratory media such as sheep blood agar by its characteristic flat-spreading colonies with a metallic-sheen, an irregular edge, and production of β-hemolysis; however, atypical colony types such as smooth-round, mucoid, or small colony variants may occur, particularly in patients with cystic fibrosis. These atypical colony types are likely due to the impact of previous antibiotic therapy on synthetic biochemical pathways. It is common to recover a mixture of colony types in such patients. On selective and differential MacConkey agar, *P. aeruginosa* produces colorless colonies because lactose is not used as a sole carbohydrate source and there is no change in pH of the medium. In some situations, additional selective media containing inhibitors such as cetrimide and acetamide may be used to improve the isolation of *P. aeruginosa* from clinical specimens with mixed bacterial flora. However, in practice, such media are not commonly employed because *P. aeruginosa* is not likely to be overgrown by other organisms in patient specimens. When pigment production and typical metallic-sheen colony type are combined with a positive cytochrome oxidase test, *P. aeruginosa* can be identified with high certainty without further testing. Many laboratories rely on commercial biochemical identification systems for identifying gram-negative rods suspected of being *P. aeruginosa*, particularly those with atypical colony morphology. Most such

TABLE 125-1 Taxonomic Changes Associated with *Pseudomonas Spp* and Related Organisms Causing Disease in Humans

Rrna Homology Group	Previous Species Designation	Current Species Designation
I		*Pseudomonas aeruginosa*
		Pseudomonas fluorescens
		Pseudomonas putida
		Pseudomonas stutzeri
		Pseudomonas mendocina
		Pseudomonas pseudoalcaligenes
		Pseudomonas alcaligenes
	Chryseomonas luteola	*Pseudomonas luteola*
	Flavimonas oryzihabitans	*Pseudomonas oryzihabitans*
II	*Pseudomonas cepacia*	*Burkholderia cepacia*
	Pseudomonas gladioli	*Burkholderia gladioli*
	Pseudomonas mallei	*Burkholderia mallei*
	Pseudomonas pickettii	*Ralstonia pickettii*
	Pseudomonas pseudomallei	*Burkholderia pseudomallei*
III	*Pseudomonas acidovorans*	*Delftia acidovorans*
	Pseudomonas testosteroni	*Comamonas testosteroni*
	Pseudomonas delafieldii	*Acidovorax delafieldii*
	Hydrogenomonas facilis	*Acidovorax facilis*
IV	*Pseudomonas diminuta*	*Brevundimonas diminuta*
	Pseudomonas vesicularis	*Brevundimonas vesicularis*
V	*Xanthomonas maltophilia*	*Stenotrophomonas maltophilia*

From Henry DA, Speert DP. Pseudomonas. In: Versalovic J, Carroll KC, G Funke, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.

systems are highly accurate (>90%) for identification of *P. aeruginosa*, but less accurate for identification of other *Pseudomonas* spp. Such systems for identification of *P. aeruginosa* isolates from patients with cystic fibrosis are less accurate, particularly for slow-growing or variant colony types. Some systems may have difficulty separating the three closely related fluorescent pseudomonads, and thus definitive identification may require additional testing. Although growth is optimal at 35° to 37°C (95° to 98.6°F), *P. aeruginosa* grows at 42°C (107.6°F) and *P. fluorescens* and *P. putida* do not. In addition, most isolates of *P. aeruginosa* reduce nitrogen to gas, whereas the other two fluorescent *Pseudomonas* spp. do not. Additional biochemical characteristics of *P. aeruginosa* include a reaction of alkaline over no change in triple sugar iron agar; production of acid oxidatively from glucose and xylose but not lactose, sucrose, or maltose; and a positive reaction for arginine dihydrolase. In practice, these latter tests are generally not used because they do not clearly differentiate among the fluorescent pseudomonads. Most strains are motile by one or more polar flagella and also display surface pili. Most isolates also carry the genes required to synthesize the extracellular mucopolysaccharide alginate, whose overproduction results in the mucoid colony type; however, such colony types are almost exclusively isolated from the respiratory tract of patients with cystic fibrosis.[222]

Among the nonfluorescent group *Pseudomonas* spp., *P. luteola* and *P. oryzihabitans* are somewhat unique because they are cytochrome oxidase negative and produce an intracellular yellow, nondiffusible pigment. Most isolates of *P. stutzeri* produce a distinctive dry, wrinkled colony on agar media, and the colonies can adhere tenaciously to the agar. In contradistinction, *P. mendocina* produces smooth colonies with a brown-yellow pigment. Isolates of *P. alcaligenes* and *P. pseudoalcaligenes* do not produce distinctive colony types and are rarely encountered in clinical specimens. As a group, these organisms are identified with variable accuracy by commercial biochemical identification systems. Depending on the clinical situation, an identification of *Pseudomonas*-like gram-negative rod or *Pseudomonas* spp., not *aeruginosa*, with a request to contact the laboratory for further identification and antimicrobial susceptibility testing may be sufficient. When definitive identification is required, nucleic acid–based methods for identification of culture isolates of *Pseudomonas* spp. have proved useful. These methods include sequencing of the genes coding for 16S rRNA or the use of specific nucleic acid probes or polymerase chain reaction (PCR) assays, such as assays targeting the exotoxin A or other *P. aeruginosa*–specific gene sequences. Peptide nucleic acid fluorescence in situ hybridization has also been shown to be sensitive and specific for identification of *P. aeruginosa*, including identification in positive blood culture bottles.

The former rRNA homology group 2 and related organisms currently comprise a diverse group of bacteria in the genera *Burkholderia*, *Ralstonia*, *Cupriavidus*, and *Pandoraea*. Like the *Pseudomonas* spp., they are found in a wide variety of environmental habitats as saprophytes, plant pathogens, and opportunistic animal and human pathogens and are nutritionally versatile. The more well-studied organisms in the group belong to the genus *Burkholderia*, which now contains about 60 species. Members of this genus can be divided into two major groups—the *B. cepacia* complex and non–*B. cepacia* complex organisms. There are currently 17 named *B. cepacia* complex organisms with all but one having been recovered from human specimens. The most important species in the complex are *B. multivorans* and *B. cenocepacia* because of their role as colonizers and opportunistic pathogens in patients with cystic fibrosis. Of the non–*B. cepacia* complex *Burkholderia* spp., those of human clinical significance include *B. gladioli* and *B. pseudomallei*. *B. gladioli* is an important

agent of respiratory tract colonization and infection in patients with cystic fibrosis.[300] *B. pseudomallei* causes melioidosis and has also been recovered from the respiratory tract of patients with cystic fibrosis. *Burkholderia mallei* is an important animal pathogen causing glanders in horses.

The genera *Ralstonia*, *Cupriavidus*, and *Pandoraea* all contain species of clinical significance, but they are recovered in culture at lower rates than *Burkholderia* spp. The genus *Ralstonia* includes at least five species, with the following species known to cause human infections: *R. pickettii*, *R. insidiosa*, and *R. mannitolitytica*.[72,73] The genus *Cupriavidus* contains at least 11 species, including *C. gilardii*, *C. respiraculi*, *C. taiwanensis*, and *C. pauculus*, the latter of which have been recovered from patients with cystic fibrosis.[333] The genus *Pandoraea* contains at least five named species (i.e., *P. apista*, *P. pulmonicola*, *P. pnomenusa*, *P. sputorum*, and *P. norimbergensis*) and several unnamed species.[74] *P. apista*, *P. pnomenusa*, and *P. sputorum* have been recovered from patients with cystic fibrosis.[108] As a group, these organisms are of low virulence and infection in healthy individuals is uncommon. They have been associated with catheter-related bloodstream infections and other health care–associated infections in immunocompromised patients and with colonization and infection in those with cystic fibrosis.

As a group, these organisms grow well but slowly on most standard laboratory media such as sheep blood, chocolate, and MacConkey agars and appear as small, smooth, raised colonies. They may appear as colorless colonies or, with prolonged incubation, pink to dark-red colonies because of the slow usage of lactose, as is usually the case with *B. cepacia* complex organisms. They are all cytochrome oxidase weak or strong positive, gram-negative, motile rods, except for *B. gladioli* and *Pandoraea* spp., which are oxidase variable. A majority of *B. cepacia* complex isolates are lysine decarboxylase positive, a helpful feature in identification of this group. Isolation of *B. cepacia* complex organisms from clinical specimens with mixed bacterial flora, particularly the respiratory tract of patients with cystic fibrosis, is enhanced by using selective and differential media with the ability to inhibit the growth of *P. aeruginosa* and other gram-negative rods. In addition, recovery may be improved if culture plates are incubated at least 3 days at 35° to 37° C (95° to 98.6° F). Several selective agar media are commercially available, including oxidative-fermentative base–polymyxin B–bacitracin–lactose (OFPBL),[346] "*P*". *cepacia* (PC), and *B. cepacia*–selective (BCSA) agars.[140,359] These media take advantage of the fact that *Burkholderia* spp. and some related organisms are generally resistant in vitro to the polymixin antibiotics, whereas *P. aeruginosa* and most commonly isolated enteric bacteria are susceptible (*Serratia* spp. are a notable exception) and therefore inhibited on such media. Several studies have shown differences in the ability of these media to recover *B. cepacia* complex organisms or to inhibit the growth of other bacterial flora, with BCSA being equivalent or superior to the other media for this purpose.[140,359] Some of these media are also useful for the recovery of *B. gladioli* from patients with cystic fibrosis.

B. pseudomallei grows on agar media commonly used for isolation of gram-negative bacteria. The colonies develop slowly (over a week) and have a characteristic "daisy-head" appearance. α-Hemolysis is noted on sheep blood agar. Ashdown selective medium, which contains crystal violet and gentamicin, can increase the recovery rate of *B. pseudomallei* from clinical specimens containing mixed bacterial flora, including throat, rectal, and sputum specimens.[15] *B. pseudomallei* produces a dry, wrinkled, violet-purple colony with a pungent, earthy odor on Ashdown medium.[205] Other selective media, including a recently described *B. pseudomallei*–selective agar (BPSA) and selective media for *B. cepacia* (BCSA, PC, and OFPBL), have been evaluated against Ashdown medium for the recovery of *B. pseudomallei*.[130,253] Ashdown medium remains the recommended medium for recovery of *B. pseudomallei* in endemic areas, but commercially available selective agars for *B. cepacia*, particularly PC agar, are acceptable media in nonendemic areas. Selective enrichment broths also have been developed for the recovery of *B. pseudomallei* from superficial sites. Such broths have been shown to improve the recovery of the organism when used in addition to direct plating onto Ashdown medium. Because of the potential of *B. pseudomallei* as an agent of bioterrorism via inhalation, isolation of the organism from patients with no travel history to endemic sites should immediately be reported to public health.

Accurate identification of the rRNA group II and related organisms may be difficult using either traditional biochemical or commercially available biochemical test systems.[121,182,273,276] Few of these species are found in the databases of such systems, and none of these systems are able to clearly differentiate among the various species in the *B. cepacia* complex. As with *P. aeruginosa*, molecular methods including amplification of the 16S rRNA gene followed by sequencing have been most widely used for this purpose; however, such methods may not always discriminate among closely related pseudomonads. Special emphasis should be given to accurate identification of such organisms when recovered from patients with cystic fibrosis, because of the clinical significance and patient management implication of a *B. cepacia* complex or related organisms in such patients. An initial, probably biochemical, identification of *B. cepacia* complex for a patient with cystic fibrosis should be confirmed with a molecular method capable of determining the exact species identification. To this end, the Cystic Fibrosis Foundation established a *B. cepacia* reference laboratory at the University of Michigan for phenotypic characterization and genotypic species identification for suspected *B. cepacia* complex and related organisms. Recently, matrix-assisted laser desorption ionization–time of flight mass (MALDI-TOF) spectrometry has received increasing attention as a method for rapid and accurate identification of bacteria, including difficult to identify organisms recovered from the respiratory tract of patients with cystic fibrosis.[215] MALDI-TOF allows for the analysis of biomolecules such as nucleic acids, proteins, and sugars, as well as other large organic molecules that may be fragile and fragment when ionized by more conventional ionization methods. The method allows for very rapid characterization of bacteria at the genus, species, and possibly the strain level.

EPIDEMIOLOGY

P. aeruginosa is a ubiquitous environmental organism found in soil, water, and on vegetation, including the surface of many raw fruits and vegetables. Its minimal nutritional requirements and ability to grow in a wide variety of physical environments enhance the organism's ability to survive in numerous ecologic niches. *P. aeruginosa* may be found as colonizing flora in a small percentage of healthy humans.[137] Sites of colonization include moist body sites and mucosal surfaces such as throat, nasal mucosa, axillae, and perineum. Carriage rates in the gastrointestinal tract have been reported as high as 24 percent based on selective culture of stool. The gastrointestinal tract may be the site of transient colonization after ingestion of food or liquids containing *P. aeruginosa*. Despite the ability to colonize skin and mucosal surfaces, it rarely results in persistent colonization. The respiratory tract is a common site of colonization of patients who have been hospitalized for extended periods, have foreign bodies in place (i.e., endotracheal tubes or tracheostomies), have poor mucociliary clearance, or have received broad-spectrum antibiotics or chemotherapy.

P. aeruginosa is best classified as an opportunistic pathogen whose pathogenic potential is manifest in situations in which the host's immune defenses are diminished or lacking. Such situations include children with poor mucociliary clearance (cystic fibrosis), neutropenia (secondary to cancer chemotherapy), or damaged skin barrier (burn wound).The organism generally does not invade intact skin or mucous membranes.

P. aeruginosa frequently enters the hospital environment on the clothes and shoes of patients, visitors, or hospital personnel and also from the same individuals with skin, gastrointestinal tract, or respiratory tract colonization. It may also enter the hospital on plants or flowers. As a result, the organism may be found growing in any moist hospital environment, including hospital kitchens and laundries, mops, shower heads, whirlpools, antiseptic solutions, eye drops, distilled water sources, irrigation fluid, dialysis fluid, and equipment used for dialysis and respiratory care or inhalation therapy. It tolerates elevated temperatures and can grow in distilled water sources containing only inorganic compounds. *P. aeruginosa* commonly is found on the surface of many types of plants and flowers, raw fruits, and vegetables. Consumption of these foods by profoundly immunosuppressed children can result in colonization of the gastrointestinal tract and potentially can lead to invasive disease. In hospitalized patients, the likelihood of colonization increases with duration of hospitalization. Transmission of *P. aeruginosa* from patient to patient or from hospital personnel to patient often is assumed but rarely documented.[12,13,92,350] Although clear evidence exists of patient-to-patient transmission of "epidemic strains" of *P. aeruginosa* among the cystic fibrosis population, the relative importance of this mode of acquisition as opposed to acquisition from environmental sources is unclear.[3,132,204] Environmental sources of *P. aeruginosa* outside the hospital that may result in colonization with subsequent infection include swimming pools, water slides, hot tubs, contact lens solutions, cosmetics, illicit injectable drugs, and the inner soles of sneakers.

Various typing systems have been used to characterize strains of *P. aeruginosa* for epidemiologic purposes; these can be classified as either phenotypic or genotypic typing methods. Historically, the phenotypic method of serologic typing based on the O-polysaccharide component of lipopolysaccharide was used most often, but some strains of *P. aeruginosa* lack the O-polysaccharide. Other phenotypic typing methods include phage and pyocin typing. These methods have limited ability to differentiate strains, particularly from chronic infections because of the variability of gene expression in this organism. Phenotypic methods have been replaced by genotypic methods, including restriction fragment length polymorphism (RFLP) analysis, pulsed-field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) analysis, multilocus sequence typing (MLST), and others.[77,315] Each of these methods has certain advantages and disadvantages in terms of cost, complexity, and discriminatory power. They have proved helpful in demonstrating, for example, that patients with cystic fibrosis were usually infected with unique strains of *P. aeruginosa* that they harbored for extended periods rather than being replaced by other strains.

The environmental distribution of the other *Pseudomonas* spp. is similar to that of *P. aeruginosa*, and hospitalized as well as nonhospitalized individuals can become transiently colonized. Infection may infrequently occur, particularly in individuals who are immunocompromised. *P. fluorescens* can be isolated from the skin.[298] *P. fluorescens* has been responsible for transfusion-associated bacteremia in blood recipients, likely as a result of contamination during blood collection.[298] In addition, contamination of blood with *P. fluorescens* during the blood culture collection process has been responsible for outbreaks of pseudobacteremia.[177,306] *Pseudomonas pseudoalcaligenes* has one of the more unusual environmental habitats—industrial metalworking fluid consisting of a mixture of water and petroleum products. Although metalworkers with long-term exposure to aerosols containing high concentrations ($>10^5$ organisms/mL) of *P. pseudoalcaligenes* develop high antibody titers to the organism, they do not have clinical evidence of acute or chronic respiratory or systemic disease.[216] Limited data exist on typing systems for *Pseudomonas* spp. other than *P. aeruginosa*.

B. cepacia complex and related organisms are ubiquitous in nature and can be found in water, soil, decaying organic matter, and as plant pathogens.[153,195,334] Unlike *P. aeruginosa*, *B. cepacia* complex organisms are infrequently encountered in the hospital environment or nonhospital sites such as swimming pools, sinks and showers. As a group, these organisms are intrinsically resistant to disinfectants and a wide range of antimicrobial agents, including the aminoglycosides and carboxypenicillins (e.g., ticarcillin and carbenicillin). Moreover, resistance can be acquired to quinolones, extended-spectrum penicillin/β-lactamase inhibitor compounds, third- and fourth-generation cephalosporins and carbapenem antibiotics. *B. cepacia* can survive for extended periods in moist environments, even in the presence of disinfectants, including povidone-iodine.[8] These organisms have

been identified as agents of sporadic nosocomial outbreaks of infection in medical intensive care units. When such outbreaks occur, they have been traced to contaminated instruments and solutions, such as in automated peritoneal dialysis machines, blood gas analyzers, mouthwashes, ultrasound gels, medications, and skin antiseptics, including povidone-iodine and chlorhexidine.[27,28,139,311] In patients with cystic fibrosis, colonization of the respiratory tract, sometimes associated with lower respiratory tract and disseminated infection, is of major clinical significance because of the association of this organism group with increased morbidity and mortality.[132,154,205] Although acquisition of *B. cepacia* complex organisms from the environment is the more common mode of transmission, as evidenced by the fact that the majority of infected patients harbor genetically distinct strains, epidemiologic investigations have clearly demonstrated "epidemic strains" and interpatient transmission in the cystic fibrosis population.[20,32] Most data suggest that once colonized with a specific strain, replacement of that strain with another strain occurs infrequently.[29] *B. cepacia* complex organisms also have become more important as a cause of infection and complications in patients with chronic granulomatous disease[316] and sickle-cell hemoglobinopathies.[30]

B. gladioli, like *B. cepacia* complex organisms, is a plant pathogen found in water and soil. It is associated with infections in immunocompromised patients, including patients with chronic granulomatous disease and in patients with cystic fibrosis. In fact, it is recovered more frequently from patients with cystic fibrosis than most *B. cepacia* complex species other than *B. cenocepacia* and *B. multivorans*. *B. pseudomallei*, the causative agent of melioidosis, and the closely related species *B. thailandensis*, are found in soil and surface water in the tropical and subtropical areas of Southeast Asia and northern Australia, including rice paddy surface waters in rice-growing areas of Thailand, Cambodia, Vietnam, and Laos.[63,81,312] *Ralstonia*, *Cupriavidus*, and *Pandoraea* spp. are also found in soil and water sources, but their epidemiology has not been well studied. Unlike some *B. cepacia* complex species, person-to-person transmission of other *Burkholderia* spp. or *Ralstonia*, *Cupriavidus*, and *Pandoraea* spp. has been rarely reported.

Genotypic typing systems have been applied to epidemiologic investigations of *B. cepacia* complex organisms, other *Burkholderia* spp., and species of other genera in this group.

PATHOGENESIS

Among the *Pseudomonas* spp., the pathogenesis of infections with *P. aeruginosa* has been well studied. Pathogenesis is multifactorial,[24,208] and the outcome of *P. aeruginosa*–host encounters depends on a variety of organism-specific and host factors. To establish lung infection, for example, *P. aeruginosa* must contend with the mucociliary clearance mechanism and innate immunity, including antibacterial peptides such as lactoferrin, and the action of phagocytic cells.[285] The large genome of the organism provides it with the metabolic flexibility to adapt to various host environments. A large number of varied and redundant virulence factors include cell-associated (bound to the bacterial cell surface) and extracellular proteins and other macromolecules, which are either injected directly into the host cell cytoplasm or secreted into the general extracellular environment.[335] These factors, along with intrinsic and acquired resistance to antimicrobial agents, allow the organism to colonize, resist host defense mechanisms, produce local tissue damage, and in some situations invade and result in disseminated infection despite the host immune response.[351]

Expression of virulence factors is under tight regulatory control and depends on the body site and type of infection. Different factors may be expressed in acute versus chronic infections. Two major types of regulatory systems promote organism survival and proliferation. The first system type involves one- or two-component transcriptional regulatory secretion of exoproteins. One-step systems promote direct delivery of preformed exoproteins, whereas two-step systems synthesize exoproteins as precursor molecules requiring cleavage before exporting outside the cell.[131] In either case, exoproteins are delivered across the inner and outer cell membranes and peptidoglycan cell wall layer to the periplasmic space. The second regulatory system type involves quorum-sensing regulatory systems that help coordinate gene expression throughout the bacterial population to aid the organism in adapting to environmental conditions. Quorum-sensing systems involve cell-to-cell signaling by production of small diffusible molecules known as autoinducers. Quorum sensing plays an important role in promoting the formation of biofilms on mucosal surfaces and internalized medical devices. Such biofilms are important for survival of the organism and contribute to persistent and chronic infections. Interestingly, macrolide antibiotics, which do not inhibit growth of *P. aeruginosa* in vitro, may inhibit quorum sensing and biofilm formation.[328,335]

P. aeruginosa displays virtually all known classes of microbial virulence factors. Cell-associated virulence factors include flagella, pili, and endotoxin (lipopolysaccharide). Flagella and pili play an important role in establishment of initial infection because of their functions of providing motility and binding to specific epithelial cell surface receptors. *P. aeruginosa* has one or more polar flagella that allow for motility and appear to be important in initial stages of lung colonization. The flagella themselves do not appear to enhance binding to epithelial cells, but they do bind to respiratory mucin produced in response to infection and may contribute to other cell-binding mechanisms. Decreased clearance of mucin in the lung of some individuals may contribute to establishment of infection. Flagellar proteins contribute to excess activation of proinflammatory cytokines. Once infection is established in the lung, flagella are generally no longer produced, and they are usually not found in organisms associated with biofilms. Strains of *P. aeruginosa* that lack flagella are less capable of dissemination from wounds and less likely to cause bacteremia and pneumonia in animal models.[14,94,106] Pili, like flagella, are also found at the poles of the *P. aeruginosa* rod. They promote

adherence to mucosal epithelial cells by binding to specific cell-surface glycolipids.[338] They also mediate twitching motility, which appears to be important in biofilm formation, as demonstrated by in vitro models.

Other cell surface adhesions likely exist, including outer membrane proteins and oligosaccharides that work in complement with flagella and pili to promote initial colonization of cell surfaces by *P. aeruginosa*. The cystic fibrosis transmembrane conductance regulator (CFTR) protein also may play an important role as an epithelial cell receptor for *P. aeruginosa*. The CFTR protein appears to be involved with bacterial internalization and clearance from the lung.[297] Diminished internalization of bacteria in murine and primate models of cystic fibrosis might provide some basis for hypersusceptibility of cystic fibrosis patients to infections with *P. aeruginosa*.[297]

P. aeruginosa endotoxin is not as potent as are the endotoxins produced by other gram-negative organisms, including *Escherichia coli* (2 to 3 mg is needed to kill a 20-g mouse). The oligosaccharide side chain of *P. aeruginosa* endotoxin prevents lysis by complement and the lipid moiety triggers cytokine pathways that lead to the sepsis syndrome and shock. The virulence of *P. aeruginosa* in a mouse burn model was found to be related directly to lipopolysaccharide integrity.[79] Deficiency of the O side chain of lipopolysaccharide reduced virulence markedly.[260]

Extracellular virulence factors of *P. aeruginosa* include numerous exotoxins with proteolytic or lipolytic activity. These include the elastolytic proteases LasA protease and elastase (LasB), collagenase, gelatinase, caseinase, alkaline protease, fibrinolysin, phospholipase C (a thermolabile hemolysin), lecithinase, exotoxin A, and a group of exoenzymes including ExoS, ExoT, ExoU, and ExoY. The proteolytic enzymes may be responsible for localized necrosis of the skin or lung tissue and for corneal ulceration. The various proteases also can degrade plasma proteins, including complement and coagulation factors.[358] Solubilization and destruction of lecithin (surfactant) may play a role in the atelectasis seen in pulmonary infections caused by *P. aeruginosa*. Fibronectin protects epithelial cells from bacterial attachment, but its ability to do so is reduced in patients with cystic fibrosis because of the high level of proteases in respiratory secretions and also after cellular injury from trauma, intubation, or postviral infection.

Exotoxin A is an adenosine-5′-diphosphate ribosyltransferase enzyme that inhibits eukaryotic cell protein synthesis by a mechanism similar to the action of diphtheria toxin. Specific exotoxin A–deficient mutants of *P. aeruginosa* have reduced virulence in mouse and rat models of corneal and lung infection.[244,356] Exotoxin A also diminishes the activity of host phagocytes. Passive or active immunization against exotoxin A significantly protects against experimental infection with exotoxin A–producing strains of *P. aeruginosa*. Phospholipase C degrades phospholipids, which are plentiful in eukaryotic but not prokaryotic cell membranes. The hemolysis produced by *P. aeruginosa* may be caused by heat-labile phospholipase C and by a heat-stable moiety.

The exoenzymes S, T, U, and Y have multiple enzymatic and chemical functions. Their secretion is regulated by a type III secretion system commonly found in other bacterial pathogens. This system is associated with acute infections, requires pilin-mediated contact with target epithelial cells, and allows the organism to directly inject these products into the host cell cytoplasm rather than simply into the general environment. ExoS and ExoT are bifunctional proteins that display GTPase activation and ADP-ribosyltransferase activity. They confer antiphagocytic activity by depolarizing actin filaments and thus disrupting normal cytoskeletal organization. ExoS also inhibits interleukin production by alveolar macrophages and induces tumor necrosis factor-α production. Production of ExoS is associated with invasive disease in burn patients. *P. aeruginosa* growing on the burn wound but not on intact skin produces ExoS,[238] and ExoS can be found in the blood of patients who develop *P. aeruginosa* sepsis before the bacteria can be detected. *P. aeruginosa* strains that do not produce ExoS are less able to cause invasive disease.[291] ExoU is a potent cytotoxin possessing phospholipase A2 activity and causing rapid damage to cell membranes and cell death. ExoY has adenylate cyclase activity, but its specific role in virulence is unclear.

The importance of type III secretion proteins in the pathogenicity of *P. aeruginosa* has been demonstrated in animal model systems. Neutralization of these proteins has been shown to prevent septic shock and improve survival in a mouse model system. Similarly, antibodies against PcrV, a protein involved in translocation of the exoenzymes, resulted in complete survival of the animals. Rabbit model studies of *P. aeruginosa*–induced septic shock after lung injury also showed the protective effect of antibodies to PcrV.

Among the vast array of proteases produced by *P. aeruginosa*, the elastolytic enzymes, LasA and LasB, cause direct damage to lung tissue (elastin accounts for 30% of the protein of lung tissue) and can also interfere with immune clearance of *P. aeruginosa* from the lungs. Berger and associates[25] noted that elastase treatment of isolated polymorphonuclear leukocytes severely impaired their ability to kill opsonized *P. aeruginosa*. They demonstrated proteolytic degradation of C3b receptors and suggested that it may contribute to the inability of patients with cystic fibrosis to eradicate *P. aeruginosa* from their lungs. Because several cell types, including macrophages, monocytes, B-lymphocytes, and some T-lymphocytes, carry the same C3b receptor, the proteolytic activity may cleave this molecule from all these cells, thereby decreasing the phagocytic activity of monocytes and macrophages in these patients. Berger and colleagues[25] also demonstrated that optimal interaction between the complement-derived opsonic ligands C3b and iC3b and their respective receptors does not occur in the milieu of the lungs of patients with cystic fibrosis who are infected with *P. aeruginosa*. They suggested that both *P. aeruginosa* and host proteases may contribute to the initiation of a cycle of events in which neutrophils entering the infected lung actually impair phagocytosis rather than eradicate the source of infection. Breakdown of elastin in the walls of blood vessels might be responsible for the intrapulmonary hemorrhage noted in individuals with cystic fibrosis.

P. aeruginosa also produces siderophores, including pyoverdin and pyochelin; these are iron-scavenging compounds that allow the organism to replicate in an iron-deficient environment. Iron in host tissue is tightly bound to transferrin or lactoferrin in the lungs. Siderophores bind iron efficiently and then are taken up by the bacterium through cell-specific receptors. Siderophores also play a role in control of expression of other virulence factors.

Mucociliary clearance is impeded in patients with cystic fibrosis, and, as a consequence, they fail to cleanse the bronchopulmonary epithelium of inhaled particles, including bacteria.[185,227] *P. aeruginosa* adherence to the airways of children with cystic fibrosis may be enhanced by the acidic environment that results from the hyperacidification of the trans-Golgi network from the dysfunctional CFTR protein in lung epithelial cells.[264] The specific colonizing strain or strains of *P. aeruginosa* may reflect exposure to other colonized individuals in health care facilities, cystic fibrosis camps, or at home, as well as by selective pressure by the use of mist tents, inhalation therapy, and continuous broad-spectrum antibiotic therapy.[184,373]

The evolution of colonization from the nonmucoid to the mucoid phenotype of *P. aeruginosa* can be correlated with the patient's age, clinical score, extent of pulmonary function abnormalities, severity of changes on chest radiographs, and serum immunoglobulin levels.[67,68] The specific lung environment of a patient with cystic fibrosis is believed to trigger a switch to a cluster of genes that code for abundant production of alginate, thereby giving rise to the mucoid phenotype.[67,318] Strains of *P. aeruginosa* expressing the mucoid phenotype are rarely recovered from patients without cystic fibrosis (0.5% to 1.7%). Chronic infection of the lungs in patients with cystic fibrosis selects for additional genetic adaptations by *P. aeruginosa*, including the loss of genes coding for virulence factors (primarily proteins) required for initiation of acute infections.[308] The loss of such virulence factors may contribute to a decrease in the immunogenicity of the organism, a diminished effective immune response, and the inability of the host to eradicate *P. aeruginosa* from the airways.

Persistence of *P. aeruginosa* within the respiratory tract is aided by growth of the organism in microcolonies embedded in a biofilm of alginate.[145,148,254] This biofilm allows nutrients to pass while protecting the organism from host defense mechanisms, antibodies, and antibiotics.[186] In addition, investigators have noted that rabbit alveolar macrophages fail to phagocytize and kill the organism in the presence of serum from patients with cystic fibrosis. This phenomenon suggests that these patients have a specific, local defect in pulmonary resistance to *P. aeruginosa*.

The mucoid strains of *P. aeruginosa* isolated from the respiratory secretions of patients with cystic fibrosis produce large quantities of the mucopolysaccharide alginate, which is composed of acetylated D-mannuronic acid and L-glucuronic acid.[67,317] This polysaccharide polymer not only gives *P. aeruginosa* a mucoid appearance on agar medium but also has antiphagocytic activity. Alginate elicits a significant inflammatory immune response in the lungs of patients with cystic fibrosis and may contribute to the lung damage that occurs in chronic *P. aeruginosa* lung infection.[219] Because of its viscous nature, alginate contributes to the thick bronchial secretions in the lungs of children with cystic fibrosis; these secretions obstruct small airways and impair mucociliary clearance and movement of phagocytic cells. Production of alginate by *P. aeruginosa* is regulated and inducible. Mucoid isolates of *P. aeruginosa* lose the mucoid trait when serially cultured on laboratory media. Nonmucoid isolates convert to the mucoid phenotype when inoculated into the lung in a rat model.[291] Alginate also inhibits the action of antibiotics.[146]

The predilection for *P. aeruginosa* to colonize and infect the respiratory tract of patients with cystic fibrosis may partially be the result of decreased oxygen tension within the hyperviscous mucous layer.[357] Epithelial cells in individuals with cystic fibrosis have increased oxygen usage, which is responsible for the decreased oxygen tension.[357] This local hypoxia results in a change in the phenotype of *P. aeruginosa* resulting in alginate production, decreased motility (loss of flagella), and biofilm production.[357] Once these phenotypic changes occur, eradication of *P. aeruginosa* from the lungs of individuals with cystic fibrosis is nearly impossible.

The pathogenicity of *P. aeruginosa* also depends on its ability to resist or overwhelm host cell defense mechanisms. Fick and Reynolds[110,111] noted that in patients with cystic fibrosis, the opsonic function of IgG was reduced as a result of a molecular change in the Fc portion of the immunoglobulin (Ig)G molecule. This deficit was magnified in the lungs of patients with cystic fibrosis infected with *P. aeruginosa* because bacterial proteases can fragment IgG and further impair its opsonic activity. The persistence of *P. aeruginosa* in the lungs of patients with cystic fibrosis also may be related to the presence of one or more factors in their respiratory secretions that interfere with the bactericidal activity of fresh normal human serum against *P. aeruginosa*. These "blocking factors" have been shown to be IgG antibody that inhibit the normal bactericidal activity of human sera IgM antibody.[256,296]

Concentrations of IgG subclass immunoglobulins have been studied in patients with cystic fibrosis and compared with values obtained in age-matched healthy children and adults. Pressler and associates[265] noted that in 52 percent of patients with cystic fibrosis, at least one of the four IgG subclasses had an elevated serum concentration in contrast to controls. A significant correlation of elevated serum concentrations of IgG2 (and to a lesser extent IgG3) with decreased forced expiratory volume at 1 second was noted. Moss[228] found that patients with cystic fibrosis who are infected with *P. aeruginosa* have markedly elevated serum concentrations of IgG antibodies to the opsonic immunodeterminant, type-specific lipopolysaccharide. This elevation was distributed among all four IgG subclasses with a significant shift toward IgG3. Sera from patients with cystic fibrosis who were colonized with *P. aeruginosa* had diminished opsonic capacity, but complement-dependent human neutrophil phagocytosis was not impaired. Serum concentrations of IgG4 but not IgG1, IgG2, or IgG3 are correlated

inversely with opsonic capacity. On the basis of these data, Moss[228] suggested that high levels of IgG4 antibody to opsonic immunodeterminants may inhibit normal pulmonary clearance of *P. aeruginosa* by pulmonary macrophages in vivo. In addition to alterations in humoral immune responses to *P. aeruginosa*, it appears that dysfunctional CFTR also alters the bacterial activity of macrophages against *P. aeruginosa*.[87]

Infection of the lower respiratory tract in patients with cystic fibrosis results in massive infiltration of lung tissue with neutrophils.[75,93] The neutrophils release elastolytic enzymes that overwhelm the ability of protease-inhibiting compounds of the lung to protect against tissue damage. This results in destruction of lung tissue and accumulation of large amounts of extracellular nucleic acid and cell cytosol matrix, which contributes to airway viscosity.[85]

Virulence factors of other *Pseudomonas* spp., *B. cepacia* complex, and related organisms have been less well studied but include their ability to adhere to indwelling devices and elaboration of exoenzymes such as elastase and gelatinase.[231] *B. cepacia* complex has numerous important virulence factors, some of which play a specific role in the clinical syndromes seen in patients with cystic fibrosis. *P. aeruginosa* enhances subsequent adhesion to epithelial surfaces by *B. cepacia*,[288] suggesting a synergistic relationship between the two bacterial pathogens, particularly in patients with cystic fibrosis.[314] Additionally, *B. cepacia* complex strains from cystic fibrosis patients with the most severe progression bind most avidly to mucin.[289] *B. cepacia* also resists nonoxidative neutrophil killing.[316] These organisms are intrinsically resistant to many antimicrobial agents. The following host-specific factors increase the risk for colonization with these organisms, as well as progression from colonization to infection: (1) prolonged hospitalization, especially in intensive care settings[216]; (2) administration of broad-spectrum antibiotics[318]; (3) malignancy, particularly if associated with immunosuppressive therapy and neutropenia[179,318]; and (4) breaks in mucocutaneous defense barriers, chiefly by instrumentation or the use of invasive devices.[179]

As with most pathogens, the pathogenicity of *B. pseudomallei* is related to both virulence factors of the organism and the host's immune response to infection and in many ways resembles the types of infections and clinical outcomes associated with *P. aeruginosa* and to some extent *B. cepacia* complex organisms. As a saprophyte associated with soil and surface waters, the organism can survive harsh environmental conditions, including extremes of temperature, pH, and levels of moisture.[63] Moreover, the organism also resists the action of many antiseptics, disinfectants, and antibiotics. Altered phenotypes of the organism may be found in culture of clinical material, including small-colony variants with increased antibiotic resistance, as are seen in chronic infection with *P. aeruginosa*.

B. pseudomallei produces a variety of exoenzymes secreted into the environment, including proteases, lipases, lecithinase, phospholipase C, and hemolysins.[63] The organism also has type III secretion systems, as are found in many pathogens, including *P. aeruginosa*. These systems allow for delivery of effector molecules directly into host cells and may allow the organism to survive in phagosomes and promote tissue invasion. Interestingly, the closely related *B. thailandensis* does not have type III secretion systems and is significantly less virulent. Cell-associated virulence factors include extracellular polysaccharide capsule, lipopolysaccharide, and outer membrane proteins and proteins associated with flagella and pili. As a group, these factors contribute to attachment to epithelial cells and protection against host immune responses, and they may contribute to biofilm formation. Notwithstanding a wide array of virulence factors, *B. pseudomallei* generally has a low potential for causing disease in humans, as evidenced by the high rates of seropositivity in asymptomatic individuals in endemic areas and the increased likelihood of causing disease in individuals with predisposing factors.[63]

CLINICAL MANIFESTATIONS

Previously Healthy Children

P. aeruginosa can produce disease in previously healthy, immunocompetent children.* When such infections occur, the source of the organism is water, soil, or vegetative or related contaminated material, and the organism has gained entry though the skin or mucous membrane by a minor or penetrating wound. After entry, the organism may produce a localized infection with cellulitis that may progress to abscess formation and may be associated with a green or blue-green exudate. The skin lesions, whether caused by direct inoculation or secondary to septicemia, begin as pink macules that progress to small cutaneous hemorrhagic nodules and eventually to areas of necrosis, with eschar formation surrounded by an intense red areola (ecthyma gangrenosum, Fig. 125-1).[230,271] In addition to skin infections, previously healthy children may experience localized and systemic infections, including corneal infection, dacryocystitis,[374] otitis externa, mastoiditis, mastitis, urinary tract infection, septicemia, endocarditis, bone and joint infections, peritonitis, meningitis, pneumonia, and necrotizing fasciitis.[232]

Outbreaks of dermatitis (folliculitis; "hot-tub syndrome"), plantar nodules ("hot-foot syndrome"), otitis externa, mastitis, and urinary tract infections caused by *P. aeruginosa* have been reported in normal, healthy children after the use of community swimming pools, water slides, recreational whirlpools, or family-owned hot tubs.† Pruritic or painful skin lesions (5 to 30 mm) develop several hours to 5 days or longer (mean, 48 hours) after contact with these water sources. Subcutaneous involvement, including mastitis, has been reported.[122] Skin lesions may be erythematous, macular, or pustular. In some cases, very tender nodules have been observed.[113] Illness may vary from a few scattered lesions in some patients to extensive truncal involvement in others. The rash is most severe in areas occluded by snug-fitting bathing suits. In some children, malaise, fever, otitis

*References 7, 8, 105, 245, 305, 336, 352, 360, 365.
†References 104, 105, 113, 133, 290, 309, 341.

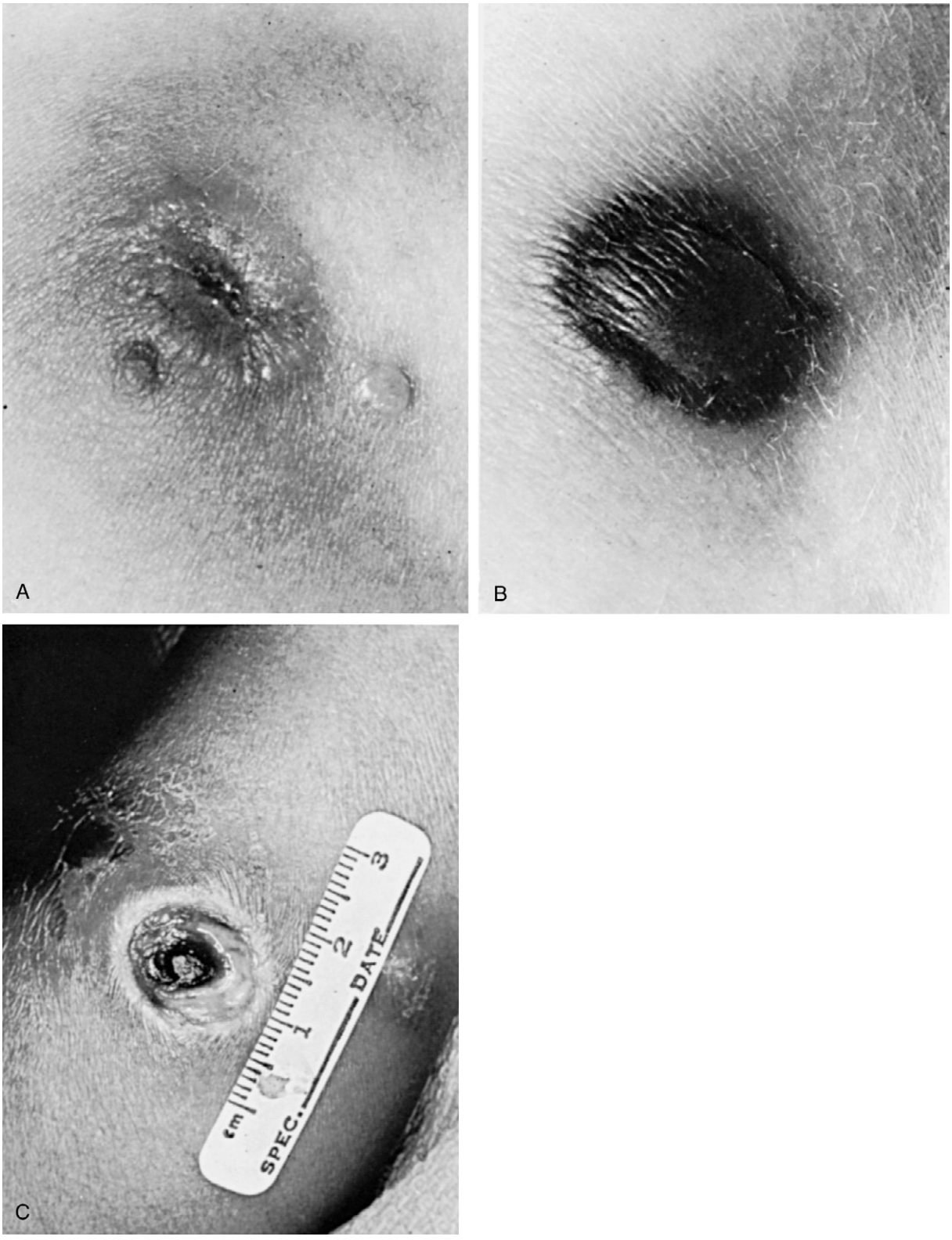

FIGURE 125-1 ■ **A,** Skin lesion caused by septicemic *Pseudomonas aeruginosa* infection. **A,** A large macule has begun to undergo central necrosis and is surrounded by two smaller macules. **B,** Small cutaneous nodule representing the skin lesion of septicemic *P. aeruginosa*. **C,** A final stage of ecthyma gangrenosum in which a cutaneous hemorrhagic nodule has undergone central necrosis and eschar formation.

externa, vomiting, sore throat, conjunctivitis, rhinitis, pyuria, abdominal cramps, and swollen breasts may be associated with the dermal lesions. Rarely, this recreational water-associated *P. aeruginosa* infection can present with an acute onset of exquisitely painful plantar nodules.[113,369] Skin biopsy of the tender nodules reveals perivascular and perieccrine neutrophilic infiltrate and microabscesses.[113] Laboratory evaluations reveal leukocytosis with increased proportions of neutrophils and elevations of the sedimentation rate and C-reactive protein.[113]

Multiple serotypes of *P. aeruginosa* have been associated with these outbreaks. The use of whirlpool baths usually involves soaking in water for variable periods. Superhydration of skin and exposure to *P. aeruginosa* result in primary cutaneous infection.[146] Whirlpool water is heated to temperatures above 37.8° C (100° F) and frequently is not filtered, thereby allowing for the persistence of desquamated skin. Both of these factors are conducive to growth of *P. aeruginosa*.

Otitis externa caused by *P. aeruginosa* has been reported in healthy competitive swimmers who swim repetitively in a pool contaminated with *P. aeruginosa*.[270] The organism also has been associated with a more malignant form of otitis externa manifested as high fever, necrosis of portions of the external ear, facial nerve paralysis, mastoiditis, and osteomyelitis of the temporal bone and basilar skull.[151,233] Rarely, *P. aeruginosa* meningitis results from progression of this infection.[262] Malignant otitis externa usually is associated with predisposing factors such as malnutrition, leukopenia (a disorder of leukocyte function), malignancy, or diabetes mellitus. Successful management of malignant otitis externa requires aggressive surgical débridement in addition to appropriate systemic antibiotic therapy.

P. aeruginosa is a common agent of chronic suppurative otitis media (with or without cholesteatoma), and acute and chronic mastoiditis.[56,175,240] Chronic suppurative otitis media is a complication of inadequately treated acute otitis media and is manifested as a perforated tympanic membrane with persistent otorrhea. Chronic suppurative otitis media also occurs in children with surgically induced perforations of the tympanic membrane by tympanostomy tubes and incompletely or inadequately treated otitis media. The microbial agents of acute otitis media in children with tympanostomy tubes differ from those in children with intact tympanic membranes. *P. aeruginosa* has been recovered from 12 percent of children with acute otitis media and tympanostomy tubes.[278] When tympanostomy tubes become colonized with *P. aeruginosa*, eradication may be complicated by the production of a biofilm.[39] The presence of a biofilm will reduce effectiveness of conventional systemic and topical antimicrobial therapy. Outpatient therapy with oral antibiotics frequently is unsuccessful because of a lack of oral antimicrobial agents with antipseudomonal activity. Topical ciprofloxacin with or without dexamethasone has been shown to be effective and superior to oral amoxicillin–clavulanic acid in the treatment of acute otitis media in children with otorrhea through tympanostomy tubes.[91] Intravenous antibiotics targeting the bacterial agents isolated from middle ear aspirates may be needed to cure chronic suppurative otitis media. This therapy may preclude the necessity of performing tympanomastoid surgery, which becomes essential in those with extensive granulation tissue and osteitis in the mastoid.[51]

P. aeruginosa wound infections of the ear have occurred after commercial ear piercing, resulting in perichondritis.[171] Upper ear cartilage is more commonly involved than lobe piercing. In one report reuse of a "single-use" disinfectant bottle contaminated at a sink harboring *P. aeruginosa* was implicated.[171]

P. aeruginosa infections of the eye usually occur after minor trauma or surgery, with deposition of a large inoculum topically, or by hematogenous spread. The use of contaminated contact lens solution or the use of tap water during contact lens care, as well as endotracheal suctioning without covering the eyes of sedated or comatose patients have been implicated in dacryocystitis, keratitis, and blepharoconjunctivitis.[46,143,147,149] Use of topical steroids, exposure to contaminated ocular medications, and prior ocular irradiation can predispose to keratitis. Infection of the cornea can result in ulceration, which may progress to more invasive disease, including endophthalmitis. Most cases of endophthalmitis in children result from penetrating ocular injuries or as a postoperative complication of surgery. Loss of vision may result, even if appropriate antimicrobial therapy is administered promptly.

Urinary tract infection (UTI) caused by *P. aeruginosa* occurs rarely in healthy children. More typically, pseudomonal UTI occurs in children who are catheterized or have had instrumentation or surgery on their urinary tract or who were immunocompromised.[246] UTI resulting from *P. aeruginosa* primarily occurs as a result of ascending infection; however, the urinary tract may become infected secondary to bacteremia. The symptoms of pseudomonal UTI are not usually different from those of UTI caused by other bacteria. Because of differing antibiotic susceptibilities of *P. aeruginosa* and the underlying medical condition of the child with a pseudomonal UTI, antimicrobial therapy and clinical response to therapy may differ.

P. aeruginosa is the most common cause of osteomyelitis after puncture wounds of the foot and is responsible for more than 90 percent of cases.[43,102,225] The calcaneus or metatarsal bones commonly are affected.[78] Symptoms may be present for 2 to 40 days (mean, 9 days) before diagnosis is made and hospitalization is needed.[78] Pain and swelling are the most common symptoms; fever and wound drainage rarely are noted. Leukocytosis (white blood cell count >10,000/mm^3) and an elevated erythrocyte sedimentation rate are present in most patients. Radiographs of the affected foot usually show evidence of osteomyelitis at some time during the period of evaluation and treatment. Bone scan results almost universally are abnormal and frequently yield evidence of osteomyelitis before positive findings on radiographs. The inner pad of sneakers has been implicated as a possible source of *P. aeruginosa* in these patients[115]; however, *P. aeruginosa* osteomyelitis of the foot bones has developed when the puncture occurred through other types of footwear or while the child was barefoot.[114]

Other *P. aeruginosa* infections of bones and joints are uncommon findings in children. When they do occur,

they are the result of hematogenous spread of *P. aeruginosa* in patients who are intravenous drug abusers or who have urinary tract or pelvic infections. Although any bone or joint may be associated with a bacteremic episode, *P. aeruginosa* has a unique predilection for the vertebrae, sternoarticular joints, pelvis, and symphysis pubis. The clinical course of *P. aeruginosa* osteomyelitis or septic arthritis is more indolent than that occurring after infection with *Staphylococcus aureus*. Contiguous spread can occur after penetrating trauma, surgery, or overlying soft tissue infections, especially decubitus ulcers.

P. aeruginosa may produce serious infections in healthy neonates. Septicemia may be noted in the earliest hours of life and is associated with high morbidity and mortality. In utero acquisition of the organism resulting in intraamniotic infection (amnionitis), neonatal sepsis, and death has been described.[241,282,331,370] The clinical course is similar to that of any other form of gram-negative septicemia, with hypotension, respiratory distress, and skin lesions being the predominant manifestations. Mortality rates may be as high as 80 percent.[160] Late-onset neonatal *P. aeruginosa* infection usually occurs as a nosocomial infection (i.e., bacteremia, urinary tract infection, and pneumonia) associated with a foreign body (e.g., indwelling urinary or vascular catheter or endotracheal tube) in hospitalized infants. An outbreak in a neonatal intensive care unit of *P. aeruginosa* pneumonia and bloodstream infection was determined to be associated with intermittent otitis externa in a health care worker.[371] Maternal colonization after the use of a hot tub for relaxation during labor was responsible for *P. aeruginosa* meningitis and bacteremia in an 11-day-old infant.[340]

Other pseudomonads (except *B. pseudomallei*) rarely cause disease in healthy persons. Reports in normal, healthy children, particularly when the children have been hospitalized in an intensive care unit,[35] include pneumonia, keratitis,[203] and abscesses caused by *B. cepacia* complex organisms; abscesses caused by *P. fluorescens;* and otitis media, pneumonia, and osteomyelitis[53,280] caused by *P. stutzeri.* A penetrating lawn dart resulted in a mixed flora brain abscess with *Pseudomonas paucimobilis.*[329] Peritonitis and septicemia caused by *B. cepacia* complex organisms have been associated with contamination of equipment used for peritoneal dialysis.[27]

Melioidosis is a rare disease of Southeast Asia and northern Australia that increased in frequency in the United States when Americans returned from Vietnam, Honduras,[58] or El Salvador[56] and, rarely, after immigration by Southeast Asians.[81,250] The causative agent is *B. pseudomallei*, an environmental saprophyte of soil and water in the tropics, particularly in rice paddies.[190] Infection occurs after contact of abrasions or wounds with contaminated soil or water, inhalation of contaminated dust, or ingestion of contaminated water. Patients with poorly controlled diabetes, renal disease, or immunocompromised conditions caused by collagen vascular disease, hematologic malignancies, or immunosuppressive therapy appear to have an increased risk for development of disease after infection with *B. pseudomallei*.[201]

Transmission most commonly occurs after percutaneous inoculation. Inhalation and ingestion are less common modes of transmission. Hematogenous spread occurs after local infection. Transmission from animals to humans has not been reported. The rat flea *(Xenopsylla cheopis)* and the *Aedes aegypti* mosquito have been reported to infect animals with *B. pseudomallei*, but it is unclear whether this route of infection is associated with human cases.[248] Human-to-human transmission has occurred during sexual or prolonged close contact.[120] Two cases of mother-to-child transmission of *B. pseudomallei* occurred through breast milk.[267] One mother had obvious mastitis.[267] An outbreak of melioidosis in Australia was traced to a potable water source from a water treatment plant that had irregularities in purification.[153]

Most infections with *B. pseudomallei* remain subclinical.[201] Melioidosis can have a broad spectrum of clinical signs, and symptoms may be latent for months or years before the disease becomes clinically apparent. The initial clinical finding may be a single primary skin lesion (i.e., vesicle, pustule, bulla, or urticaria) in a patient with no underlying disease. Septicemia occasionally occurs, and multiple abscesses may be noted in every organ of the body. The mortality rate associated with fulminant sepsis approaches 90 percent.[188] Meningitis; encephalitis; arthritis; nodular pulmonary densities; abscesses of liver,[11] lung, spleen, kidney, and bones; and endophthalmitis have been observed in both normal and compromised hosts after or concomitant with an episode of septicemia. The acute septicemic illness is indistinguishable from other types of septicemia caused by gram-negative organisms.

B. pseudomallei can cause myocarditis, pericarditis, endocarditis, intestinal abscess, cholecystitis, acute gastroenteritis, UTI, septic arthritis, paraspinal abscess, osteomyelitis, hilar lymphadenopathy, and cervical lymphadenopathy. Parotitis was documented in 38 percent of 126 children with melioidosis in Thailand.[82] None of the 126 children with melioidosis had any apparent predisposition to infection.

Subacute melioidosis generally is characterized by an illness lasting weeks to months. Pulmonary infection in this form of disease is a common occurrence and may mimic tuberculosis. The disease can vary from mild bronchitis to severe, fulminant pneumonitis, and lung consolidation and cavitations occur frequently.[188,221]

Neonatal melioidosis has been reported in Thailand.[213] Infants with neonatal septicemia, meningitis, or both caused by *B. pseudomallei* have been described. The mode of transmission of this organism to these newborn infants is not always clear. A clear case of mother-to-child transmission of *B. pseudomallei* has been reported.[2] The mother had acute melioidosis treated with ofloxacin at 32 weeks' gestation. Consolidation of the right lung of the infant occurred, and an abscess eventually developed. Postpartum cultures of the mother's cervix yielded *B. pseudomallei*.

Chronic melioidosis occurs more frequently in whites than in Asians.[217] Chronic melioidosis may involve every organ in the body, including the brain.[188] Melioidosis may become dormant, with exacerbations occurring years after primary infection when host defenses are impaired as a result of steroid use, burns, diabetes mellitus, or other processes. The longest latent period (24 years) was reported by Kingston.[181]

Melioidosis should be considered in any person who has been to Southeast Asia or northern Australia at any time and has fever of unknown origin, overwhelming sepsis, single or multiple abscesses, or any tuberculosis-like illness. The diagnosis is established by culture of blood, skin lesions, or purulent material from an abscess cavity or from other sites of infection.[62]

Burn Wounds

The surface of burn wounds and skin grafts are frequently colonized by pseudomonads and other gram-negative organisms.[105] Colonization does not always progress to infection, but it is a necessary prerequisite to development of invasive disease. Septicemia with *P. aeruginosa* is a major problem in burn patients, with the mortality rates of burn wound sepsis approaching 78 percent.[220] Systemic involvement may be related to multiplication of organisms in devitalized surface areas with subsequent invasion of healthy tissue and dissemination, or it can be associated with prolonged use of intravenous or urinary catheterization required for the care of these patients. The use of antibiotics for prophylaxis may diminish the susceptible microbiologic flora but select for resistant strains of *P. aeruginosa*. In addition, the hydrotherapy that is commonly provided to burn patients promotes colonization of the burned area and other body sites.[330]

In burn patients, abnormalities in neutrophil function that precede the onset of septicemia have been described.[57] Killing of *P. aeruginosa* by neutrophils is impaired. Burn injury is also associated with abnormal responses to microbial antigens, delayed rejection of homografts, abnormal vascular responses, impaired delayed hypersensitivity responses, diminished uptake of foreign substances particles by the reticuloendothelial system, and altered antimicrobial pharmacokinetics. Contamination of wounds with high concentrations of bacteria in excess of 10^5 colony-forming units per gram of tissue impedes contraction and healing of the wound.[252] In addition, *P. aeruginosa* produces numerous substances that can further inhibit the natural healing process of burns and wounds. Exogenous plasminogen activators and proteases break down proteins such as fibrin and halt the contraction process.[258] *P. aeruginosa* ExoA, a protein synthesis inhibitor, also contributes to retardation of wound healing.[138] Secondary bacterial infection results in nearly 25 percent of skin graft loss after full- or split-thickness skin grafts.[332] *P. aeruginosa* was responsible for nearly 60 percent of the infections that resulted in skin graft loss. Skin graft loss was more common when the skin grafts were performed on the lower extremities and when grafts were applied at multiple sites.[332] Skin graft infections with *P. aeruginosa* were more severe and resulted in a fourfold increase in reoperation rates.[332]

Cystic Fibrosis

Cystic fibrosis is one of the most common lethal inherited diseases of children.[105,229] It is a generalized disorder of salt and water transport that affects the exocrine glands and is caused by mutations in the CFTR gene.[186,279] The course and prognosis of disease are determined largely by chronic infections of the airways with opportunistic bacteria and death usually resulting from destructive pneumonitis and chronic obstructive pulmonary disease. *P. aeruginosa* is the most common respiratory bacterial pathogen recovered from patients with cystic fibrosis. A 2008 U.S. surveillance study reported that slightly more than 50 percent of patients with cystic fibrosis had *P. aeruginosa* recovered from sputum cultures.[22,204] The prevalence varied by age, ranging from 25 percent for children younger than 5 years of age to 80 percent for adults in the 25- to 34-year-old group.[204,272] Initial recovery of *P. aeruginosa* from the respiratory tract of a child with cystic fibrosis may represent colonization and not infection. Colonization rates are higher in such children, who require pancreatic enzyme supplementation. Colonization is followed by infection, which is intermittent and usually associated with multiple strains of *P. aeruginosa*. At some point, colonization and subsequent infections by a single strain that expresses the mucoid phenotype is the norm, and eradication of the organism with antimicrobial therapy is impossible.[21,112,128,304]

Infection in patients with cystic fibrosis is limited almost entirely to the respiratory tract, and pulmonary exacerbations with endobronchial disease are a common finding.[84,144] Infection of the pulmonary parenchyma rarely occurs. Rather, the epithelium of the airways and the submucosa are edematous and contain infiltrates of chronic inflammatory cells. Documentation of a pulmonary exacerbation in cystic fibrosis relies heavily on clinical impression (e.g., increase in the frequency of productive cough; increase in volume or a change in characteristics of the sputum; increase in the respiratory rate or dyspnea; and decrease in appetite, activity, or exercise tolerance). Fever and leukocytosis are present in a minority of patients and are associated with poorer pulmonary function test results and a worse prognostic score.[307] Concentrations of *P. aeruginosa*, host cell DNA (derived from polymorphonuclear leukocytes and to a lesser extent from respiratory epithelial cells), and total protein in sputum are increased during pulmonary exacerbations and decrease significantly after antimicrobial therapy.[307] Bronchitis, bronchiolitis, and bronchiectasis can occur. Eventually, local necrotizing pneumonitis may be noted, in contrast to the overwhelming generalized necrotizing pneumonitis seen in immunosuppressed patients. Septicemia is a rare occurrence, but *P. aeruginosa* bacteremia may develop in patients with indwelling venous catheters. Colonization of lungs with *P. aeruginosa* after lung transplantation in patients with cystic fibrosis is common and occurs in the early posttransplantation period.[187]

B. cepacia complex organisms have emerged as agents of asymptomatic colonization, pneumonia, and septicemia in patients with cystic fibrosis. Although *B. cenocepacia* previously was the most common *B. cepacia* complex species isolated from patients with cystic fibrosis in the United States, *B. multivorans* now is the most common species, accounting for more than one third of clinical isolates and responsible for over half of new *Burkholderia* spp. infections.[204] Some members of the complex are more commonly associated with acute clinical deterioration

(cepacia syndrome), in particular *B. cenocepacia*[52,162,211,214]; however, other species, such as *B. multivorans*, have also been associated with the cepacia syndrome[34] and *B. dolosa*, an uncommonly encountered species, is associated with a rapid deterioration in lung function and decreased survival.[52,167]

The prevalence of infection with *B. cepacia* complex organisms in the United States generally ranges from 3 to 4 percent, with rates as high as 8 percent having been reported.[204] The higher frequency of colonization of the respiratory tract with *B. cepacia* complex in patients with cystic fibrosis has been associated with increased morbidity and mortality rates in some cystic fibrosis centers since the 1980s. The risk for colonization with *B. cepacia* complex increases with the severity of underlying disease and increasing age.[326] The source or mode of transmission of the organism has not been defined adequately[206,207,327]; but person-to-person transmission has been documented.[163] Nosocomial transmission (i.e., patient-to-patient and contaminated inhalation therapy equipment-to-patient) within cystic fibrosis centers and social contact, particularly at summer camps, is one mode of acquisition of new infection. Nonetheless, the majority of new infections with *B. cepacia* complex organisms are likely to be the result of acquisition of unrelated strains from the natural environment.

Once colonization with *B. cepacia* complex has been identified in patients with cystic fibrosis, three distinct clinical patterns have been noted: (1) chronic asymptomatic carriage, usually in association with *P. aeruginosa;* (2) progressive deterioration over many months with recurrent acute pulmonary exacerbations accompanied by fever, progressive weight loss, leukocytosis, and an elevated erythrocyte sedimentation rate; and (3) rapid, usually fatal deterioration in pulmonary function associated with necrotizing pneumonia and sometimes bacteremia ("cepacia syndrome").[155,211] The last complication can occur even in patients whose respiratory status was affected only mildly before acquiring *B. cepacia* complex infection.

Malignancy, Immunosuppression, and Other Predisposing Conditions

Children with leukemia, particularly those receiving immunosuppressive therapy and those who are neutropenic, are extremely susceptible to septicemia caused by *P. aeruginosa* and other pseudomonads.[105] Some pseudomonads, including *P. putida*, have been reported as causes of septicemia in children with malignancy.[6] Infection generally results from invasion of the bloodstream by a colonizing *Pseudomonas* spp. (e.g., from the gastrointestinal tract). Anorexia, malaise, nausea, vomiting, diarrhea, and fever may be noted. Generalized vasculitis develops, and hemorrhagic necrotic lesions can be found in all organs, including the skin, where they appear as purple nodules or ecchymotic areas that become gangrenous.[269] Other invasive *P. aeruginosa* infections noted in children receiving immunosuppressive chemotherapy include hemorrhagic gangrenous perirectal cellulitis or abscess, typhlitis, preseptal cellulitis, and necrotizing fasciitis.[209,223,337]

Children undergoing treatment for malignancies are particularly vulnerable to bacterial infection. Chemotherapy and radiation therapy can disrupt mucocutaneous barriers and result in moderate to severe immunosuppression. Fergie and associates[107] described 98 children and adolescents with cancer in whom *P. aeruginosa* bacteremia developed; the rate of bacteremia was highest in patients with leukemia. Most cases occurred when patients had absolute neutrophil counts of less than 100/mm^3. Mortality associated with *P. aeruginosa* bacteremia was higher in patients with solid tumors, an absolute neutrophil count of less than 100/mm,[5] perineal skin lesions, and bacteremia during remission or induction therapy rather than during a relapse.

The single most important factor that predisposes children with cancer to infection is granulocytopenia. The bactericidal capacity of children with leukemia and other neoplasms also may be impaired. Heat-stable opsonins specific for *P. aeruginosa* likewise may fall precipitously in children with acute leukemia who are receiving intensive combination chemotherapy. Fatal infections with *P. aeruginosa* may be related in part to deficiency of this specific opsonin.

Immunosuppressive agents may be used in the management of malignancies, transplantation, or collagen vascular disease. The location of the infectious process and the type of causative organisms depend somewhat on the underlying disease. Infection by *P. aeruginosa*, particularly pneumonia and septicemia, occurs more commonly in children receiving immunosuppressive therapy than in the normal, healthy population.

P. aeruginosa is a major cause of health care–associated infections in children and is particularly associated with the use of implantable medical devices. Infections of intrathecal baclofen pumps used to manage spasticity have been associated with wound infections and meningitis.[361] Delayed infections in cochlear implant recipients with *P. aeruginosa* have been reported.[125] *P. aeruginosa* is a leading cause of nosocomial respiratory tract infection in children undergoing mechanical ventilation or receiving inhalation therapy.[186] Asymptomatic colonization of the upper and lower airways occurs commonly and should be distinguished from respiratory tract disease, tracheitis, and pneumonia. A predominance of gram-negative bacilli with an abundance of polymorphonuclear leukocytes on Gram stain of lower respiratory tract secretions, in conjunction with a positive culture for *P. aeruginosa*, strongly supports the role of *P. aeruginosa* as the causative agent for the lower respiratory tract infection. Absence of *P. aeruginosa* from lower respiratory tract secretions markedly reduces the likelihood that *P. aeruginosa* is in the lower respiratory tract. In these cases of device-associated infection, removal of the device is important for successful management of the infection.

Septicemia resulting from *P. aeruginosa* occurs with increased frequency in children with indwelling vascular or urinary catheters.[322] Urinary tract infections also have been associated with cystoscopic examination. In addition, septicemia may occur in children with congenital or acquired neutropenia or in persons with a functional deficit in polymorphonuclear leukocyte function. *P. aeruginosa* is a common cause of abscesses and meningitis in

children with dermoid sinus tracts or dermoids extending down to or communicating with the meninges or neural tissue and in children with meningomyelocele.

Endocarditis caused by *P. aeruginosa* must be considered in children and adolescents with prosthetic valves or other intracardiac synthetic material. The organism also may produce acute or subacute endocarditis in children with congenital cardiac lesions before or after cardiac surgery and on both native and prosthetic heart valves. Tricuspid valve involvement occurs most commonly, but involvement of multiple valves is possible with *P. aeruginosa;* the manifestations are typical of subacute endocarditis. If the left side of the heart is involved (i.e., aortic or mitral valves), the patient has acute and more fulminant disease. Fever and heart murmur are almost universal findings. Fortunately, endocarditis is an uncommon occurrence in the pediatric population because its usual setting is in adolescents and adults who inject illicit drugs. Other *Pseudomonas* spp. have been rarely associated with post–cardiac surgery infections.[115]

P. aeruginosa needs to be considered in children and adolescents at risk for acquiring human immunodeficiency virus (HIV) infection, primarily after severe immunodeficiency has occurred.[116,277,313] Risks for acquiring *P. aeruginosa* infection in HIV-infected individuals include hospital exposure, declining $CD4^+$ cell count, and the use of dapsone or trimethoprim-sulfamethoxazole (TMP-SMX); azithromycin use was protective.[264,313] Bacteremia may occur with or without the presence of an indwelling vascular catheter. Fever, hypotension, skin lesions (i.e., papules or ecthyma gangrenosum), and pneumonia are common manifestations.[116] Mortality rates can be high, particularly when empiric antimicrobial therapy is inadequate for the treatment of severe, invasive *P. aeruginosa* infection. *P. aeruginosa* supraglottitis has been reported in a 6-month-old child with severe combined immunodeficiency syndrome.[194]

B. cepacia complex organisms can cause opportunistic infections in patients other than those with cystic fibrosis, primarily in hospitalized or immunocompromised patients. These include nosocomial pneumonia in patients on ventilation, bacteremia, wound infections, urinary tract infections, meningitis, endocarditis, and skin lesions. *B. cepacia* complex organisms are also an important cause of infections in patients with chronic granulomatous disease (CGD) because of the organism's ability to resist killing by defective neutrophils using nonoxidative pathways. Bacteria that cause most infections in CGD are catalase-positive organisms. The CGD-affected neutrophil can kill numerous microorganisms despite its defects because most microorganisms endogenously produce hydrogen peroxide, which the CGD-affected neutrophil can modify and use against the organism in the phagosome. Whereas both *P. aeruginosa* and *B. cepacia* are catalase-positive organisms, the former is a rare pathogen in CGD because CGD neutrophils can kill *P. aeruginosa* organisms by means of nonoxidative mechanisms. Fortunately, all of these infections are uncommon findings in children.

P. putida has been primarily associated with health care–associated infection, particularly in the neonatal intensive care unit. Most cases present as bacteremia or as cutaneous infections.* Cutaneous manifestations reported in neonates with *P. putida* have an appearance similar to that of staphylococcal scalded skin syndrome.[193] Mortality rates after infection with *P. putida* are high because of vulnerability of the patients and inappropriate empirical antibiotic therapy.[193] *Pseudomonas putida* is typically susceptible to antipseudomonal β-lactam antibiotics and carbapenems but not reliably susceptible to aminoglycosides.[193]

P. fluorescens causes disease primarily in immunocompromised patients who have been inadvertently exposed to contaminated infusion fluids, disinfectants, and drinking water.[101,126,150,178,353] Most infections result in fever; systemic reactions of sepsis and mortality have been reported but are not typical. *P. fluorescens* is usually susceptible to carbapenems, aminoglycosides, and tetracycline[150]; however, *P. fluorescens* is less likely to be susceptible to third-generation cephalosporins and trimethoprim.[150] Mupirocin, a topical antibiotic, is produced through fermentation of *P. fluorescens*.

P. stutzeri has been responsible for a diverse group of clinical conditions, with wound infections being the most common presentation.[237] Bacteremia is the second most common presentation and usually results from exposure to contaminated infusion fluids.[239] Pneumonia, UTI, and skin and eye infections have also been reported. Most patients with presumed *P. stutzeri* infections are hospitalized and have predisposing conditions that make them vulnerable to opportunistic organism.[237] *P. stutzeri* are typically susceptible to antipseudomonal penicillins and cephalosporins, aminoglycosides, and TMP-SMX.

P. mendocina is a recently described opportunistic pathogen.[239] Bacteremia, osteomyelitis, subphrenic abscess, meningitis, and wound infections have been reported.[66,159,239] *P. mendocina* is typically susceptible to carbapenems, third-generation cephalosporins, aminoglycosides, and ciprofloxacin[239]; however, resistance to some or all of these antibiotics has been reported.[239]

Ralstonia spp., especially *R. pickettii*, have caused infections in immunocompromised patients and in those with cystic fibrosis.† *Ralstonia* spp. infections such as catheter-associated bloodstream infections or health care–associated pneumonia have been reported in association with contamination of infusion fluids or respiratory equipment.‡ *Ralstonia* spp. have been reported to be susceptible to antipseudomonal β-lactams, ciprofloxacin, and TMP-SMX, and some isolates susceptible to aminoglycosides.[85,320,372] However, some strains may be resistant to antibiotics typically considered susceptible.[85,372] Susceptibility testing on clinical organisms is important to guide appropriate therapy. Catheter-associated bloodstream infections may require catheter removal to affect a cure.[183]

Most infections with *Cupriavidus* and *Pandoraea* have occurred in immunocompromised individuals or in those with cystic fibrosis. *Cupriavidus* spp. has resulted in bacteremia, skin and wound infections, and respiratory

*References 40, 88, 193, 259, 364, 366.
†References 37, 55, 57, 85, 157, 173, 180, 183, 191, 255, 287, 320, 339, 345, 355.
‡References 55, 57, 157, 173, 180, 191, 255, 287.

tract colonization.[168,170,196,321] *Pandoraea* spp. have been associated with severe lung disease and systemic infection in persons with cystic fibrosis.[161,164,324] Transmission among patients with cystic fibrosis has been documented.[161,164,324]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

The diagnosis of *P. aeruginosa* and other pseudomonad infections depends on recovery of the organism from a normally sterile body site such as blood, cerebrospinal fluid (CSF), joint fluid, peritoneal or peritoneal dialysis fluid, or deep tissue obtained in a manner that avoids contamination by cutaneous flora (see previous discussion on etiology). Diagnosis of UTI often requires suprapubic aspiration or urethral catheterization for young children. Purulent material associated with subcutaneous abscesses or areas of cellulitis should be obtained by needle aspiration. A diagnosis of *P. aeruginosa* pneumonia can be made by needle aspiration of the lung and, less convincingly, by recovery of the organism from sputum obtained by postural drainage of a child with cystic fibrosis. Recovery of the organism from the surface of the skin or the throat, a tracheal aspirate, or bronchial secretions may reflect colonization and is not necessarily diagnostic of infection. The validity of a positive culture is enhanced when it is associated with a typical clinical syndrome (e.g., *P. aeruginosa* recovered from a skin lesion typical of "whirlpool" folliculitis). Isolation of *P. aeruginosa* from the respiratory tract, particularly when obtained from an endotracheal tube in an intubated patient, is not an unusual occurrence. Differentiating colonization from infection is clinically important. Gram stain of respiratory secretions obtained by endotracheal suction typically reveals abundant gram-negative rods and polymorphonuclear leukocytes in the setting of a true lower respiratory tract infection caused by *P. aeruginosa* (tracheitis or pneumonitis). An absence of gram-negative rods or the presence of squamous epithelial cells rather than polymorphonuclear leukocytes indicates that either the patient does not have a lower respiratory tract infection or, if an infection is present, the etiologic agent is not likely to be *P. aeruginosa*.

The presence of bluish, nodular skin lesions and the ulcers with ecchymotic and gangrenous centers and bright areolae (ecthyma gangrenosum) have been considered virtually pathognomonic of *P. aeruginosa* infection. Rarely, skin lesions that are clinically indistinguishable from those caused by *P. aeruginosa* develop after septicemia secondary to *Aeromonas hydrophila*.[302] Cutaneous or disseminated infections with *Aspergillus* and *Fusarium* spp. in immunocompromised patients also can cause the necrotic skin lesions of ecthyma gangrenosum.

Isolation of *Pseudomonas*, *Burkholderia*, and other related species other than *P. aeruginosa* from clinical specimens occurs far less frequently. Recovery of these organisms from sites that normally are sterile, such as blood or blood product containers, always should be considered clinically significant unless proved otherwise.

Aside from culture diagnosis, serologic tests have been investigated for the diagnosis of *P. aeruginosa* infection, but these are generally not recommended. Although specific IgG and IgA serum antibodies to *P. aeruginosa* surface antigens, including outer membrane proteins, alginate, and lipopolysaccharide, may be detected by enzyme-linked immunosorbent assay (ELISA), immunoblotting,[45,303] and other methods, serodiagnosis is not clinically useful for diagnosis of acute *P. aeruginosa* infection. Antibody titer increases are associated with chronic, active disease in patients with cystic fibrosis, and titers returned to baseline when *P. aeruginosa* infection was controlled by effective antimicrobial therapy. Nonetheless, little consensus exists regarding the clinical value of their measurement in cystic fibrosis, except perhaps in patients in whom the initial appearance of antibodies predates culture positivity[347] and a positive serology indicates the onset of *P. aeruginosa* infection. Moreover, no standardized assay protocols exist and it is difficult to determine the relative positive and negative predictive values of such assays.

In contrast to serologic diagnosis of *P. aeruginosa* infection, serologic tests may be useful in establishing the diagnosis of melioidosis, particularly in latent or asymptomatic forms of this disease.[4,235] Hemagglutination, indirect hemagglutination, complement fixation, ELISA, and immunoblot assays have been used.* Diagnostic sensitivity of these serologic tests is highly variable. High rates of seropositivity in populations in endemic areas also limit specificity.[16,65,362] Hemagglutination A antibodies generally are present within 7 to 14 days after onset of the illness, whereas the complement fixation test yields positive results in 4 to 6 weeks. Maximal titers for both tests are reached in 4 to 6 months, and detectable antibodies may persist for months to years after initial infection. The indirect hemagglutination test, although not currently available commercially, is used by the Centers for Disease Control and Prevention (CDC) for the diagnosis of melioidosis.[632] The test uses sheep red blood cells sensitized with *B. pseudomallei* antigens in a microwell format.

The interpretation of all serologic tests for melioidosis is complicated by the fact that there are no generally accepted cutoff values defining acute infection or exposure. Serologic tests are of limited value in patients living in endemic areas because of the rate of seropositivity of such individuals. Serology is valuable in individuals who do not reside in but have recently traveled to endemic areas. Paired sera should be tested whenever possible, but a single high-titer serum sample collected from a patient with a relevant travel history and a clinically consistent presentation may be helpful. Because some individuals with culture-proved melioidosis do not have detectable antibodies, a negative serologic test does not rule out infection.[136]

Several direct antigen detection assays have been developed for the rapid detection of *B. pseudomallei* in clinical specimens. These include latex agglutination, ELISA, or direct fluorescent antibody detection[10,310,363] on purulent discharges, blood, or body fluid specimens.

*References 17, 64, 135, 192, 202, 354.

As with many other antigen detection assays, the sensitivity of these assays precludes their use as stand-alone tests. Reagents for antigen detection tests for *B. pseudomallei* are not available commercially. Gram stain and immunofluorescent stains are simpler and cheaper than antigen detection assays.

PCR and other molecular methods have been used to detect *P. aeruginosa* directly in clinical specimens, particularly in respiratory specimens from patients with cystic fibrosis.[89,210] Some studies suggest that PCR may be useful in detecting *P. aeruginosa* in sputa of cystic fibrosis patients several months before culture diagnosis and at a time before establishment of chronic infection. This would allow for earlier aggressive antibiotic therapy to eradicate the organisms at a time when eradication may be possible. Other studies have suggested that PCR performed directly on clinical specimens does not significantly increase detection of *P. aeruginosa* in contrast to culture. In addition, it is difficult to interpret the clinical significance of a positive PCR and a negative culture on the same sample; a false-positive PCR result cannot easily be ruled out. Subsequent culture-confirmed colonization occurring weeks to months later may or may not be due to colonization by the same strain initially "detected" by PCR.

PCR-based and other molecular assays also have been applied to direct detection of pseudomonads other than *P. aeruginosa*, including *B. cepacia* complex organisms and *B. pseudomallei*. The same difficulty in interpreting PCR results for *P. aeruginosa* in patients with cystic fibrosis applies to *B. cepacia* complex organisms. There have been more studies on the diagnostic accuracy of nucleic acid amplification methods for direct detection of *B. pseudomallei* in clinical specimens.[60,119,166,325] Sensitivity of PCR is highest when performed on specimen types such as body fluids and purulent discharges than on blood, reflective of the bacterial load in the specimen. PCR, when available, may be used as an adjunct to but not a replacement for culture. At this point, molecular assays for the pseudomonads must be considered only investigational.

TREATMENT

Selection of Antimicrobial Therapy

Suspected or proved systemic infections with *P. aeruginosa* should be treated promptly with empirical therapy to include an aminoglycoside (i.e., gentamicin, tobramycin, or amikacin) combined with a β-lactam antibiotic (i.e., antipseudomonal penicillin, third- or fourth-generation cephalosporin, or carbapenem) pending in vitro susceptibility test results.[9,33,176] The combination of an aminoglycoside and a β-lactam antibiotic may be synergistic against the organism. However, because of increasing antibiotic resistance, dual therapy is typically used in empirical therapy to improve the opportunity that the antibiotic regimen contains at least one antibiotic to which the *P. aeruginosa* is susceptible. Rifampin is synergistic in vitro with the antipseudomonal penicillin and aminoglycoside combination therapy and may be added if the clinical response is not adequate. Antibiotic therapy should be adjusted once the results of in vitro susceptibility tests are known.[127]

Community-acquired *P. aeruginosa* infections are typically susceptible to antipseudomonal penicillins, including penicillin and β-lactamase inhibitor combinations; ceftazidime; cefepime; carbapenem antibiotics, including meropenem, imipenem, and doripenem, but not ertapenem; aminoglycosides; and quinolone antibiotics, including ciprofloxacin and levofloxacin.[172] Susceptibility is less predictable for aztreonam, a monobactam. Strains of *P. aeruginosa* causing health care–associated infections are more likely to be antibiotic-resistant than strains causing community-acquired infections. Response to treatment may be impaired when systemic infection occurs in an immunocompromised host, thus requiring a prolonged course of therapy.

Prior use of a specific antibiotic for treatment of infections caused by *P. aeruginosa* may be associated with emergence of resistance.[100,249] Specifically, monotherapy with antipseudomonal β-lactam antibiotics (i.e., antipseudomonal penicillins, cephalosporins, and carbapenems), as well as with the fluoroquinolones, has resulted in selection of resistant mutants during therapy,[23,299] including selection of multidrug-resistant mutants.[86,249] Combination therapy to include an aminoglycoside, although unlikely to have a significant effect on initial clinical response, has been suggested as a means for reducing the development of resistance while on treatment, and some studies support the use of combination therapy for this reason.[95,286,342] Some studies found that the use of antipseudomonal β-lactam antibiotics alone, which are rapidly bactericidal to *P. aeruginosa*,[118] is as effective as use of an antipseudomonal β-lactam antibiotic and aminoglycoside combination regimen for preventing the emergence of antibiotic resistance.[251] Combination therapy with two β-lactam antibiotics is inappropriate for serious *Pseudomonas* infections because the induction of β-lactamase by one of the agents may result in resistance to both antibiotics.

Table 125-2 provides dosages of some of the more commonly prescribed antipseudomonal antibiotics.[41] These dosages are only guidelines, because the doses of some of these antibiotics may vary with different clinical situations and patient populations. Once-daily administration of an aminoglycoside may decrease nephrotoxicity and improve clinical efficacy. Aminoglycoside doses must be decreased, preferably by increasing the dosing interval, in patients with diminished creatinine clearance (e.g., renal impairment, neonates). Significantly higher doses (e.g., gentamicin or tobramycin 7 to 12 mg/kg/day) may be required for patients with increased total plasma clearance, such as those with cystic fibrosis and burns. Therefore, aminoglycoside therapy must be individualized and doses guided by pharmacokinetic information. "Extended-infusion" (4-hour infusion every 8 hours) of β-lactam antibiotics (e.g., piperacillin-tazobactam) has been used to enhance the duration of drug concentration that remains above the minimum inhibitory concentration (MIC) of *P. aeruginosa*.[212]

Endocarditis caused by *P. aeruginosa* requires aggressive medical and surgical therapy. Despite combination therapy with maximal β-lactam and aminoglycoside

TABLE 125-2 **Dosages of Commonly Prescribed Antipseudomonal Antibiotics**

Generic Name	Dosage (mg/kg/day)	Route	Dosing Interval	Pediatric Precautions
Antipseudomonal Penicillins				
Ticarcillin-clavulanate	200-300 (ticarcillin) (max 12-18 g/day)	IV	q4-6h	
Piperacillin-tazobactam	240-300 (piperacillin) (max 12-16 g/day)	IV	q6h	PDNE
Cephalosporins				
Ceftazidime	200-300	IV, IM	q8h	
Cefepime	100-150 (max 2-6 g/day)	IV, IM	q8-12h	
Aminoglycosides				
Gentamicin sulfate	3-7.5 (7-10 for patients with cystic fibrosis)	IV, IM	q8h; or q24h	
Tobramycin sulfate	3-7.5 (7-10 for patients with cystic fibrosis)	IV, IM	q8h; or q24h	
Amikacin sulfate	15-22.5	IV, IM	q8h; or q24h	
Quinolones				
Ciprofloxacin	40 for PO 20-30 for IV (max 3-6 g/day)	PO, IV	q12h	>18 yr
Levofloxacin	Respiratory tract infections	PO, IV	q12h	
Moxifloxacin	<5 yr: 20 ≥5 yr:10	PO, IV	q24h	
Monobactams				
Aztreonam	90-120 (max 3-6 g/day)	IV, IM	q6-8h	
Aztreonam (75-mg solution)	≥7 yr: 75 mg/dose via altered nebulizer	Inhaled	q8h	
Carbapenems				
Imipenem-cilastatin sodium	60-100	IV IM (≥12y)	q6h	SNE
Meropenem	60 (max 3 g/day) Meningitis: 120 (max 6 g/day)	IV	q8h	
Other				
Colistimethate	2.5-5 (based on ideal body weight) (max 5-7 based on ideal body weight)	IV, IM	q8h	

IM, Intramuscular; *IV,* intravenous; *PDNE,* pediatric dose not yet established; *SNE,* safety in children not yet established.

antibiotics, valve replacement (i.e., native and prosthetic) frequently is required for cure. When gentamicin or tobramycin is administered in three divided doses per day, peak concentrations should be maintained between 12 to 15 μg/mL. Quinolones should be reserved for patients who are intolerant of aminoglycosides or whose bacteria are resistant to aminoglycosides or for long-term suppression of *P. aeruginosa* prosthetic valve endocarditis.

Meningitis or brain abscess resulting from *P. aeruginosa* should be treated with ceftazidime 200 to 300 mg/kg/day in four divided doses every 6 hours and an aminoglycoside given intravenously. The initial empirical choice of the aminoglycoside should be guided by local susceptibility patterns. Concomitant intraventricular or intrathecal treatment with gentamicin may be required if initial intravenous therapy fails to sterilize the CSF. Gentamicin can be placed into the ventricular or lumbar CSF in a total dose of 1 to 5 mg once each day (the dose is independent of body weight). Meropenem has good penetration into CSF and can be used when the *P. aeruginosa* is ceftazidime-resistant. Meropenem is the preferred carbapenem because the high doses of imipenem required to treat central nervous system (CNS) infections may be associated with CNS toxicity. Fluoroquinolones such as parenteral ciprofloxacin or pefloxacin and aztreonam are possible alternatives in the treatment of *P. aeruginosa* CNS infections if more conventional therapy has failed; however, experience with these agents for CNS infections is limited.[181,200,238] Ocular infection with *P. aeruginosa* can be serious and sight-threatening. *P. aeruginosa* corneal ulcerations or keratitis may be seen in contact lens wearers or in intubated, sedated patients in intensive care units. Topical therapy with ticarcillin, piperacillin, tobramycin, gentamicin, amikacin, ciprofloxacin, or ofloxacin may be used and is effective. Clinical efficacy is improved by frequently clearing inflammatory debris and topical application of antibiotics. Initially, antibiotic solutions should be administered

every 15 to 30 minutes. The frequency can be decreased gradually to four to six times per day when clinical improvement is apparent.

P. aeruginosa endophthalmitis frequently occurs after invasive eye surgery or penetrating injuries. Systemic, topical, and intraocular (i.e., anterior chamber and vitreous cavity) routes all are required. Even with aggressive medical intervention, return of retinal function is not a common occurrence. The prognosis is worse when initiation of therapy is delayed. Ceftazidime, imipenem, and ciprofloxacin have greater intraocular penetration than do the aminoglycosides.[368] Initial therapy for *P. aeruginosa* endophthalmitis should include ceftazidime and an aminoglycoside (e.g., tobramycin or amikacin).[42] Cefepime, meropenem, or imipenem would be potential alternatives to ceftazidime if susceptibility testing suggest that they would provide an advantage over ceftazidime. However, no clinical data support the use of these alternative antibiotics.[42]

Chronic suppurative otitis media may be due to a variety of organisms, including *P. aeruginosa*. Obtaining cultures from the middle ear is extremely valuable in determining optimal antimicrobial therapy. In most cases, therapy with a fluoroquinolone available as a topical otic compound (i.e., ciprofloxacin, ofloxacin, or besifloxacin) with or without steroids is effective.[42] Because middle ear drainage typically represents perforation of the tympanic membrane, aminoglycoside including neomycin-containing topical therapy should not be used because of potential aminoglycoside ototoxicity. Malignant otitis externa and chronic mastoiditis caused by *P. aeruginosa* require systemic antipseudomonal antibiotic therapy. Surgical débridement also may be required for effective management.

P. aeruginosa folliculitis and plantar nodules generally are self-limited and do not usually require specific antimicrobial therapy. More severe cases can be treated by the topical application of any of the following: 2.5 percent acetic acid compresses (vinegar is 5% acetic acid), gentamicin ointment, topical 0.1 percent polymyxin B, or silver sulfadiazine (Silvadene).

Skin abscesses or abscesses in other locations caused by *P. aeruginosa* should be incised and drained.[269] Failure to do so may result in a poor response despite prolonged systemic antibiotic treatment. *P. aeruginosa* may be part of multiorganism infections causing necrotizing fasciitis. Empirical therapy for necrotizing fasciitis should include antibiotics with activity against *P. aeruginosa*. Skin lesions consistent with ecthyma gangrenosum always require empirical therapy to contain antipseudomonal antibiotics.

Osteomyelitis of foot bones from *P. aeruginosa* (e.g., osteochondritis after a puncture wound) requires surgical débridement in every case. Appropriate antibiotics for 7 to 10 days after surgery appears to be adequate if surgery has removed the infected tissue effectively. Oral ciprofloxacin is an appropriate alternative antibiotic to complete antimicrobial therapy after surgery and evidence of *P. aeruginosa* as a causative agent. The adequacy of surgical débridement of the foot bones and clinical improvement can be monitored by serial sedimentation rates.[78] *P. aeruginosa* infection of foreign bodies (e.g., vascular, peritoneal, and CNS catheters) may require removal of the foreign material to cure the infection, particularly if a tunnel or exit-site infection exists.

Management of UTIs caused by *P. aeruginosa* will depend on the clinical situation of the infected child, that is, presence or absence of septicemia, instrumentation, and the anatomic location of the infection. Symptomatic pseudomonal UTIs should be treated. In patients who are clinically stable and not immunocompromised, a single antibiotic is usually adequate. For patients who might be exhibiting signs of sepsis or for those who are immunocompromised, empirical therapy typically should include two antipseudomonal antibiotics to increase the likelihood that the choices will provide adequate antimicrobial coverage and potentially take advantage of antibiotic synergy. Antibiotic choice should be guided by antimicrobial susceptibilities. Extended-spectrum cephalosporins, aminoglycosides, carbapenems, aztreonam, and fluoroquinolones are reasonable antibiotic options. In children, more experience exists with extended-spectrum cephalosporins and aminoglycosides. Oral fluoroquinolones may be considered in situations with no systemic infection and an interest in providing nonparenteral therapy. Duration of appropriate antibiotic therapy should be at least 7 to 10 days. Longer duration of therapy may be needed in immunocompromised patients, presence of systemic infection, presence of a urinary catheter or other dwelling instrument, pyelonephritis, or patients with anatomic or functional abnormalities.

P. aeruginosa may be responsible for lower respiratory tract infections in neonates, infants, children, and adolescents in a variety of settings. The most common manifestations are involvement of the airways in patients with cystic fibrosis and health care–associated tracheitis or pneumonitis in intubated patients. *P. aeruginosa* also may be responsible for pneumonia in immunocompromised children, particularly those with neutropenia, and rarely as a cause of bacterial tracheitis after viral laryngotracheobronchitis.[42]

In patients with cystic fibrosis experiencing an exacerbation of pulmonary disease and in critically ill intubated patients in whom *P. aeruginosa* infection is suspected, combination therapy with a β-lactam antibiotic and an aminoglycoside is recommended as empirical treatment. Combination therapy has the potential for synergistic activity and has superior clinical efficacy in contrast to monotherapy.[142] Quinolones do not appear to be synergistic with either β-lactam antibiotics or aminoglycosides.[174] Quinolones could be used in postpubertal children as combination therapy when antibiotic resistance reduces the potential benefit of either the β-lactam or the aminoglycoside.[156] The choice of which β-lactam antibiotic and which aminoglycoside to use should be based on the patient's previous antibiotic experience and knowledge of the usual antibiotic susceptibility pattern in the patient's clinical environment. Once susceptibility testing results are known, at least two effective antibiotics should be included in the patient's regimen. The dose and pharmacotherapeutics of the chosen antibiotics should be optimized. If the patient fails to respond to appropriate antibiotic therapy, as evidenced by clinical deterioration,

acquisition of antibiotic resistance should be anticipated and additional bacterial cultures should be obtained; one or more of the newer antibiotics should be substituted pending additional susceptibility test results.

After years of antibiotic exposure, patients with cystic fibrosis commonly are infected with strains of *P. aeruginosa* resistant in vitro to achievable blood concentrations of many if not all of the common classes of antimicrobial agents. Moreover, penetration of aminoglycosides from blood into respiratory secretions is poor.[26] The administration of antibiotics by aerosolization, particularly the aminoglycosides through an endotracheal tube, provides much higher concentrations in respiratory secretions. This approach results in more rapid bacteriologic eradication, but clinical efficacy has varied across studies.[47] Administration of aerosolized aminoglycosides to patients with cystic fibrosis resulted in improvement in pulmonary function tests, decreased concentrations of *P. aeruginosa* in sputum, and no significant apparent toxicity. This aerosol treatment did not increase the isolation of *B. cepacia* complex, *Stenotrophomonas maltophilia*, or *Alcaligenes xylosoxidans;* however, isolation of *Candida albicans* and *Aspergillus* spp. increased.[49]

Aerosolized tobramycin may be used to yield concentrations in respiratory secretions in the range of 100 to 200 μg/mL. Some strains of *P. aeruginosa* resistant to blood concentrations of tobramycin may be susceptible to achievable concentrations in respiratory secretions. In vitro susceptibility testing of these organisms may be performed to determine the exact minimal inhibitory concentrations (MIC).[129,226] Limited data exist on the correlation of such in vitro test results and clinical outcome; therefore, in practice, such testing is not commonly performed. In addition to management of resistant *P. aeruginosa*, aerosolized tobramycin given over a prolonged period (>1 year) may have the potential to eradicate *P. aeruginosa* temporarily from cystic fibrosis patients who have newly acquired this organism.[268]

More recently, inhaled aztreonam has been shown to provide benefit to some patients with cystic fibrosis.[218,243,343] Inhaled administration of aztreonam can provide local concentrations of aztreonam in pulmonary mucus that is more than 100 times the MIC50. of the patient's *P. aeruginosa* isolates.[18] Confirmation of the efficacy of prolonged antibiotic aerosol therapy may prove valuable because recent data indicate a poor outcome in children with cystic fibrosis who acquire *P. aeruginosa* by the age of 7 years.[236] Although aztreonam is not approved for use in pediatric patients, inhaled aztreonam has recently been shown to improve lung function (improvements in FEV_1), health-related quality of life, and weight in patients with cystic fibrosis who are colonized with *P. aeruginosa*.[218,243,343] Reductions also occurred in bacterial density in sputum.[218,343] Inhalation of aztreonam had minimal effect of susceptibility of the patient's *P. aeruginosa* isolates to aztreonam after a course of treatment[18,242]; however, increases in susceptibility to tobramycin were observed.[242]

Increasing rates of respiratory and invasive infections with multidrug resistant *P. aeruginosa* in cystic fibrosis and immunodeficient patients, particularly those with malignancies, have resulted in the need to expand antimicrobial options, including reconsideration of older antibiotics. Although the use of polymyxin B and colistin (polymyxin E) has been superseded largely by less toxic agents, they may be useful in selected patients who are infected with strains resistant to the other agents. *P. aeruginosa* is almost always susceptible to these agents (*B. cepacia* complex organisms are resistant). Colistin is available as colistin sulfate, a topical agent or oral preparation (poorly absorbed from the gastrointestinal tract) and colistimethate sodium for parenteral administration. Colistin has retained antimicrobial activity against some multidrug-resistant gram-negative bacteria, including *P. aeruginosa*. During early use of colistin, high rates of nephrotoxicity dampened interest in the drug[97,103]; however, more recent studies have documented lower rates of nephrotoxicity.[261] Studies in adults have shown safety and efficacy in selected populations against *P. aeruginosa* and other multidrug-resistant gram-negative bacteria.*

In the United States, quinolone usage is limited to specific infections until after puberty because these antibiotics may bind cartilage and produce growth arrest. Fluoroquinolones are approved for use in patients younger than 18 years of age to treat complicated urinary tract infections, pyelonephritis, and postexposure treatment for inhalation anthrax. The information available from clinical trials in Europe suggests that ciprofloxacin and the other quinolones may not be as harmful in children as they are in other juvenile animal species.[69,293,294]

Among the quinolones, ciprofloxacin and levofloxacin are considered antipseudomonal and have been evaluated most often for the treatment of acute and chronic *P. aeruginosa* infection in adolescents and adults with cystic fibrosis.[158,281,319,323] Ciprofloxacin, which may be given orally or intravenously, proved to be effective, as judged by clinical scores and results of pulmonary function tests. Ciprofloxacin may be considered in selected children when the risks associated with the use of this antibiotic are outweighed by the potential benefits associated with its clinical efficacy. Such situations include the treatment of multiresistant strains of *P. aeruginosa* and the substitution of an oral quinolone to avoid long-term intravenous therapy requiring an indwelling catheter. If ciprofloxacin is used in a patient younger than 18 years of age for infections other than those for which it is approved, the balance of the risks and benefits of administering ciprofloxacin and alternatives should be explained to the patient and parents or guardians. Ciprofloxacin may be administered in a dosage of 15 mg/kg every 12 hours orally or 10 to 15 mg/kg twice per day intravenously. The oral dosage should not exceed 1000 mg/day in patients who weigh less than 40 kg or 1500 mg/day in patients who weigh more than 40 kg.

Determining the optimal antibiotic therapy for patients with cystic fibrosis may be problematic. Respiratory cultures often yield different colony morphotypes of *P. aeruginosa*. The various morphotypes may have significantly different antibiotic susceptibility patterns. It is common practice for laboratories supporting the

*References 31, 38, 120, 134, 169, 247, 275, 283.

management of patients with cystic fibrosis to individually test multiple morphotypes to include both mucoid and nonmucoid strains.

Antibiotic therapy for *B. cepacia* complex infection is challenging and should be guided by results of in vitro susceptibility testing. Unfortunately, *B. cepacia* is frequently resistant to many commonly used antipseudomonal antibiotic agents, particularly the aminoglycosides. Antibiotics that may have activity against *B. cepacia* include TMP-SMX, minocycline, ceftazidime, antipseudomonal penicillins, antipseudomonal quinolones, and carbapenems; meropenem has greater in vitro activity against *B. cepacia* than imipenem.[98] Typically, *B. cepacia* isolates from patients with cystic fibrosis are more antibiotic-resistant than isolates from other patient populations. Combination therapy with two or three antibiotics may be required to achieve a clinical response. Combinations of β-lactam agents with aminoglycosides might provide synergy clinically, even when the *B. cepacia* strain isolated is aminoglycoside-resistant. *B. cepacia* may be sensitive to minocycline.[189] Minocycline may be considered to have an adjunctive role in the management of *B. cepacia* infection in patients with cystic fibrosis.

The most active antibiotics against *B. pseudomallei* are imipenem, piperacillin-tazobactam, piperacillin, ceftazidime, ticarcillin-clavulanate, ampicillin-sulbactam, tetracycline, and chloramphenicol.[312] Piperacillin, ceftazidime, and imipenem are not bactericidal in vitro.[312] Ciprofloxacin seems to be of limited value because of a high rate of resistance.

For acute systemic melioidosis, ceftazidime 150 mg/kg/day divided every 8 hours or meropenem 60 mg/kg/day divided every 8 hours are preferred.[42] These medications are administered for 7 to 14 days. Eradication requires additional therapy with TMP-SMX, with 8 mg/kg/day of TMP, orally divided every 12 hours for 12 to 24 weeks.[42] Some eradication regimens might use amoxicillin-clavulanate with 90 mg/kg/day of amoxicillin, divided every 8 hours, instead of TMP-SMX, or may add doxycycline 4 mg/kg/day divided every 12 hours to the TMP-SMX. When third-generation cephalosporins have been used, cefoperazone and ceftazidime have shown greater activity against *B. pseudomallei* than have other third-generation cephalosporin agents. Ceftazidime was compared with chloramphenicol, doxycycline, and TMP-SMX for the treatment of severe melioidosis.[349] Ceftazidime in a dosage of 150 mg/kg/day intravenously in three divided doses every 8 hours was associated with a 50 percent lower overall mortality rate in contrast to that of other forms of therapy. These results suggest that ceftazidime combined with an aminoglycoside and sulfisoxazole now should be considered the treatment of choice for severe melioidosis. Soft tissue infections should be treated for 4 to 6 months with tetracycline in children older than 8 years, provided in a dosage of 50 mg/kg/day in four divided doses. In younger children, TMP-SMX, with 8 mg/kg/day of TMP and 40 mg/kg/day of SMX, in two divided doses may be used. Most penicillins are ineffective.[99,312] The duration of therapy must be guided by clinical and laboratory findings; therapy for 4 weeks to many months may be required in patients with osteomyelitis. Relapses are common occurrences and should be treated as one would treat the first episode.[61]

Chronic melioidosis can be treated with chloramphenicol over a period of many months or with tetracycline or doxycycline. TMP-SMX was recommended previously, but most strains currently are resistant.

Mechanisms of Antibiotic Resistance and In Vitro Susceptibility Testing

Antibiotic resistance is an important factor in management of patients with serious pseudomonal infections that do not respond to antibiotic therapy. *P. aeruginosa* shows intrinsic resistance to some antibiotics generally effective against enteric gram-negative rods. This is partly due to the relative impermeability of the outer membrane of *P. aeruginosa*. This membrane is a selective barrier to the uptake of antibiotics. The major porin channel of *P. aeruginosa*, OprF, has a limited number of large channels and other porins, including OprB and OPrD, and limits the rate of passage of molecules the size of antibiotics.[249] Aside from the importance of porin channels in resistance or reduced susceptibility to antibiotics, *P. aeruginosa* may show intrinsic resistance to a wide variety of antibiotic classes by the presence of multiple efflux pumps, particularly the resistance modulation systems MexAB-OprM and MexXY-OprM. Finally, upregulation and overexpression of the intrinsic, inducible chromosomal AmpC β-lactamase renders the organism resistant to first- and second-generation cephalosporins, as well as cefotaxime and ceftriaxone. Thus, the high level of intrinsic resistance to antimicrobial agents in *P. aeruginosa* is a combination of several mechanisms acting concurrently.[44,335]

Aside from intrinsic resistance, *P. aeruginosa* may acquire resistance genes by horizontal transfer from other bacteria by conjugation, transformation, or transduction, as well as by mutational events in the bacterial genome.[76] For example, *P. aeruginosa* may acquire resistance via acquisition of plasmid-mediated β-lactamases, including extended-spectrum β-lactamases such as the TEM, SHV, VEB, GES, and PER types of enzymes, and metallo-β-lactamases (carbapenemases) such as the IMP and VIM types of enzymes or aminoglycoside-inactivating enzymes (AAC [6′]-I and APH [3′]-II). Similarly, mutational events in the genome of *P. aeruginosa* may lead to clinical resistance. Hyperproduction of AmpC β-lactamase (encoded by the *ampC* gene) may result from a mutation in the *ampD* gene, whose protein product controls the activity of the AmpR regulator protein. Mutations in genes coding for antibiotic target enzymes such as DNA gyrase/topoisomerase II (*gryA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) may yield enzymes with reduced fluoroquinolone-binding activity and clinical resistance. Furthermore, overexpression of the MexAB-OprM efflux pump as a result of a mutation in the *mexZ* gene may lead to clinical resistance to aminoglycoside, fluoroquinolone, and select antipseudomonal β-lactam antibiotics.[263,368]

The most clinically relevant β-lactamases produced by *P. aeruginosa* are chromosomally encoded Ambler class C cephalosporinases,[274] whose synthesis may be increased

on exposure to a number of β-lactam antibiotics (derepression of the *ampC* gene).[292] The propensity to induce *ampC* β-lactamase varies among the β-lactam antibiotics, with both cefoxitin and imipenem being strong inducers.[19,80,96] All antipseudomonal penicillins, cephalosporins (including cephamycins such as cefoxitin), and aztreonam are inactivated to varying degrees by the AmpC β-lactamase produced by *P. aeruginosa*, but cefepime and to a greater extent the carbapenems are relatively stable to inactivation.[234] *P. aeruginosa* strains harboring the *ampC* gene may produce only low levels of β-lactamase in vitro on initial isolation, and such strains may appear susceptible to β-lactam antibiotics on initial in vitro testing. Once patients colonized or infected with these strains are exposed to β-lactam antibiotics during therapy, induction of β-lactamase production may lead to emergence of resistance. For this reason, repeat in vitro susceptibility testing of additional clinical isolates of *P. aeruginosa* obtained after 48 to 72 hours of appropriate therapy is warranted.

Clavulanate and tazobactam are β-lactamase inhibitors effective against plasmid-encoded Ambler class A β-lactamases, including extended-spectrum β-lactamases found in *P. aeruginosa* and other gram-negative bacteria. However, these β-lactamase inhibitors are not only ineffective against the common AmpC β-lactamase of *P. aeruginosa*, but also are potent β-lactamase inducers. Use of these β-lactamase inhibitors will not enhance the activity of ticarcillin-clavulanate or piperacillin-tazobactam against *P. aeruginosa* and might actually increase the likelihood of emergence of resistant strains.

Whereas imipenem, meropenem, and doripenem resist the β-lactamases commonly produced by *P. aeruginosa*, resistance to carbapenems can result through the loss of an outer-membrane porin that allows carbapenems to enter *Pseudomonas*.[50] The permeability of other β-lactam antibiotics also may be reduced when this outer-membrane porin is lost. Although chemically distinct from imipenem or meropenem, fluoroquinolones such as ciprofloxacin may induce decreased permeability to both antibiotics.[266] Plasmid-mediated metallo-β-lactamases that confer resistance to imipenem have been described.[224,301,344] Fortunately, these metallo-β-lactamases are identified only rarely in *P. aeruginosa*.

Ciprofloxacin and levofloxacin are the most effective of the quinolones against *P. aeruginosa*. Alteration of the binding site of DNA gyrase and decreased penetration of ciprofloxacin through the *Pseudomonas* cell membrane can result in resistant strains.[96,199]

Tobramycin is the most active aminoglycoside against *P. aeruginosa*, and amikacin induces the lowest frequency of resistant strains. Resistance to aminoglycosides usually results from enzyme-mediated antibiotic modification.[90] The various aminoglycoside-modifying enzymes have different substrate affinities. Therefore, resistance to one aminoglycoside through aminoglycoside-modifying enzymes does not predict resistance to others necessarily. Resistance is less common to amikacin than to other aminoglycosides.[124,367] Aminoglycoside-modifying enzymes usually are coded by plasmid-mediated genes, but they occasionally can be coded by genes on the bacterial chromosome.[90] Plasmid-encoded resistance supports rapid transference among strains within an institution. *P. aeruginosa* also can become resistant to aminoglycosides by decreasing the intracellular uptake of aminoglycosides or by modification of intracellular ribosomal attachment.[83,96] These mechanisms of resistance generally cause cross resistance for all aminoglycosides.

In addition to antibiotic resistance, other clinical factors can adversely affect aminoglycoside activity against *Pseudomonas*. The acidic environment in tissue infected with *P. aeruginosa* can inactivate aminoglycosides.[36] Aminoglycosides may fail to reach therapeutic tissue levels because of poor penetration into bronchial secretions and lung tissue.[26] For patients with tracheitis (e.g., intubated patients) or endobronchial disease (e.g., cystic fibrosis), aminoglycosides and, less frequently, colistin have been administered by aerosol.[295] This route of delivery allows for greater availability of the antibiotic at the site of the infection, with enhanced safety because of negligible absorption into the systemic circulation. Doses of gentamicin and tobramycin of 2.5 to 8 mg/kg can be given safely by aerosol three times per day, with a maximum of 300 mg/dose. Resistance may emerge after prolonged courses.

Some studies have reported on the accuracy of automated or semiautomated antimicrobial susceptibility test systems as to the potential for reporting errors for susceptibility of *P. aeruginosa* to various antibiotics, particularly β-lactam agents.[77,165,284] The problem appears to be more pronounced with rapid systems that generate a susceptibility result in less than the 18- to 24-hour incubation period used in conventional testing. Such systems have difficulty in detecting resistance against β-lactam agents that may require a minimum time for expression of resistance to a level detectable by phenotypic methods relying on detection of growth. In general, more traditional antimicrobial susceptibility test systems, which include incubation for at least 18 to 24 hours, such as disk diffusion, broth or agar dilution, or epsilometer test (E-test), produce more reliable results. In one large multicenter study, the agar diffusion methods of disk diffusion and E-test performed acceptably in contrast to a reference broth microdilution method for both mucoid and nonmucoid isolates of *P. aeruginosa* from patients with cystic fibrosis.[48] In the same study, mucoid isolates were generally found to be more susceptible than nonmucoid isolates. This finding agreed with several other, but not all, studies. The clinical significance of these in vitro findings is difficult to interpret given that the mucoid phenotype is thought to be primarily associated with biofilms where antibiotic penetration is compromised. In fact, the role of routine in vitro antibiotic susceptibility testing in children with cystic fibrosis as it relates to clinical response and short-term outcomes has been questioned.[152] Not surprisingly, the role of in vitro antibiotic synergy testing for multidrug-resistant *P. aeruginosa* has also been questioned because of the lack of standardized testing methods and good outcome data.[1,117]

There are several recent Clinical Laboratory Standards Institute (CLSI)[71] changes to disk diffusion and MIC interpretative standards (MIC breakpoints) for susceptibility to *P. aeruginosa*, specifically affecting the

breakpoints for piperacillin, ticarcillin, piperacillin-tazobactam, and ticarcillin-clavulanate and breakpoints for the antipseudomonal carbapenem antibiotics—imipenem, meropenem, and doripenem. The MIC breakpoints for the antipseudomonal penicillin and penicillin and inhibitor combination antibiotics have been changed from the previous breakpoints of ≤64 μg/mL = susceptible (S) and ≥128 μg/mL = resistant (R) to ≤16 = S, 32 to 64 = intermediate (I), and ≥128 μg/mL or greater = R. This change was prompted by studies indicating that the likelihood of achieving blood levels of these drugs above 32 or 64 μg/mL for 50 percent of the time between doses was zero.[209] Indeed, some studies suggest that monotherapy for serious *P. aeruginosa* infection is often ineffective with organisms with such elevated MICs.[59,253] Similarly, the MIC breakpoints against *P. aeruginosa* for imipenem and meropenem have been changed from the previous breakpoints of ≤4 = S, 8 = I, and ≥16 μg/mL = R to ≤2 = S, 4 = I, and ≥8 μg/mL = R. The same revised breakpoints are applicable to doripenem. The revised and more conservative carbapenem breakpoints reportedly allow for better detection of "nonsusceptibility" among isolates of *P. aeruginosa*.

It is important to note that CLSI has published separate MIC breakpoints for other *Pseudomonas* spp. and nonfastidious, glucose-nonfermenting, gram-negative rods, as well as specific MIC breakpoints for *B. cepacia*, *B. pseudomallei*, and selected other related organisms.[70] In most cases, breakpoints are available only for a limited number of antibiotics and may not include disk diffusion breakpoints. In general, MIC methods are preferred for such organisms.

PREVENTION

Prevention of infection with pseudomonads depends in part on a continuous surveillance program of the hospital environment that is designed to identify and subsequently eradicate sources of pseudomonads as quickly as possible. Transmission of health care–associated *P. aeruginosa* can frequently be traced back to colonization of the health care institution's water distribution system. Biofilm colonization of faucets can be particularly problematic. However, eradication of *P. aeruginosa* from the water distribution system has not been successful even after using high water temperatures, copper and silver ionization, microfilters at faucets, or removal of colonized faucets. Because it is not possible to eradicate *P. aeruginosa* from the environment, other infection control measures, especially good hand hygiene and the use of sterile water to wash patients and patient supplies are needed. Pseudomonads can grow to a concentration of 10^6 organisms per milliliter in distilled water that appears to be perfectly clear. Growth of pseudomonads in distilled water, disinfectants, and medications is the factor most commonly incriminated in single-source outbreaks of *Pseudomonas* infection in hospitals. Prevention of the follicular dermatitis caused by *P. aeruginosa* contamination of whirlpools or hot tubs should be possible by maintaining the pool water at a pH of 7.2 to 7.8 and free allowable chlorine concentrations at 0.4 to 1.5 ppm.[54]

Outbreaks of *Pseudomonas* infection in newborn nurseries have been reported.[35] Generally, infection has been transmitted by the hands of personnel from washbasin surfaces and suction catheter rinse solution to the newborn infants. Strict attention to hand washing, particularly with a liquid iodophor hand washing agent before and between contact with newborn infants, may prevent or interdict epidemic disease. Growth of *Pseudomonas* on suction catheters can be prevented by rinsing the catheter in an acetic acid solution.

Daily replacement of all apparatus used for intravenous administration greatly reduces the hazard of extrinsic contamination by *Pseudomonas* and other gram-negative organisms. When intravenous administration is indicated, a small metal needle is preferable to a plastic catheter because these needles have been associated with a lower rate of septicemia and phlebitis.

Meticulous care is required in the preparation of solutions for total parenteral alimentation and in the insertion and care of catheters.

The risk for developing *Pseudomonas* infection in a burn patient also can be minimized by careful protective isolation and by the topical application of silver nitrate (0.5%) solution or 10 percent mafenide acetate cream. Débridement for removal of devitalized tissue is also imperative.

Pseudomonas infection of dermal abnormalities that communicate with the cerebrospinal axis can be prevented by careful evaluation and early surgical repair. Providing antibiotic prophylaxis of *Pseudomonas* urinary tract infection is difficult without a suitable oral antipseudomonal antibiotic for children. Identification and surgical correction of obstructive lesions of the urinary tract minimize or prevent the development of *Pseudomonas* infection of the urinary tract.

Cohorting plus isolation of patients with cystic fibrosis who are colonized with multiresistant strains of *P. aeruginosa* or *B. cepacia* has been suggested as a means of reducing nosocomial transmission of these organisms.[109] However, the proper manner of handling patients with these organisms has not been established. Any attempt to reduce transmission should be based on measures with proved efficacy and must also consider the potential consequences of strict isolation or segregation on this population, which spends so much time at health care facilities.

In patients with cystic fibrosis and certain immunocompromised patients, high rates of *P. aeruginosa* colonization and infection and ever-increasing antibiotic resistance render active or passive immunization (or both) against *P. aeruginosa* desirable. In the last 2 decades, an understanding of the human immune response to *P. aeruginosa* and the immune responses that may provide protection against infection or disease has increased considerably. Naturally occurring immunity generally is ineffective. Certain naturally generated antibodies may even be detrimental.[348] These antibodies may form antigen-antibody complexes that increase pulmonary inflammation and direct lung damage. Even if neutralizing antibodies could be administered passively or developed after vaccine administration, the large quantities of mucoid exopolysaccharide produced by *P. aeruginosa* may

mask many of the antigens targeted for antibody neutralization or opsonization.

Despite the previously mentioned difficulties, numerous investigations of candidates for *P. aeruginosa* vaccines are ongoing. Purified bacterial proteins, including flagellar antigen, outer-membrane proteins, lipopolysaccharide-O, several inactivated bacterial toxins, high-molecular-weight polysaccharide antigen and glycoconjugate, and killed whole-cell vaccine preparations, have been tested. Many of the candidate vaccines have been shown to be safe, immunogenic, and capable of generating protective immunity in various animal systems.[123] To date, evidence of protective efficacy in humans has not been established definitively for any of these vaccines. Some studies have demonstrated the efficacy of active immunization of burned patients with specific strains of *Pseudomonas* or the administration of hyperimmune globulin in the prevention of *Pseudomonas* septicemia.[5,167] *P. aeruginosa* vaccine also has been suggested as a possible method for preventing or delaying this disease in patients with acute leukemia or cystic fibrosis.[197,257]

The route of vaccine delivery may be important in creating the optimal immune response. Data from experimental animals suggest that vaccine antigens administered parenterally stimulate IgG and IgM antibodies and antigens administered orally induce IgA and IgG at mucosal surfaces. For patients whose disease occurs after mucosal colonization (e.g., cystic fibrosis), oral administration of a *P. aeruginosa* vaccine might be the preferred route of administration to develop the optimal immune response at the site of *P. aeruginosa* infection.

Purified bacterial proteins and lipopolysaccharide from *B. cepacia* are in the initial vaccine research and development stages. Whether these *B. cepacia* vaccine candidates will enter preclinical or phase I clinical trials is yet to be determined.

PROGNOSIS

The prognosis depends largely on the nature of the underlying disease process. Septicemia is the leading cause of death in children with leukemia; *Pseudomonas* is responsible for half of these deaths. Four variables independently influence the outcome of *Pseudomonas* septicemia: (1) the development of septic shock, (2) inappropriate antibiotic therapy, (3) granulocyte counts less than 500/mm,[5] and (4) the development of septic metastases.[24] Most deaths in children with cystic fibrosis are caused by pulmonary insufficiency. *Pseudomonas* can be recovered from the lungs of almost every one of these patients and, in many, has been responsible for their deaths.

NEW REFERENCES SINCE THE SIXTH EDITION

18. Assael BM. Aztreonam inhalation solution for suppressive treatment of chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis. *Expert Rev Anti Infect Ther* 2011;**9**:967–73.
19. Balasubramanian D, Schneper L, Merighi M, et al. The regulatory repertoire of *Pseudomonas aeruginosa* AmpC ss-lactamase regulator AmpR includes virulence genes. *PLoS One* 2012;**7**: e34067.
37. Bonatti H, Stelzmueller I, Laimer I, Obwegeser A. *Ralstonia pickettii* meningitis in a child with hydrocephalus. *Eur J Pediatr Surg* 2009;**19**:341–2.
44. Breidenstein EB, de la Fuente-Nunez C, Hancock RE. *Pseudomonas aeruginosa:* all roads lead to resistance. *Trends Microbiol* 2011; **19**:419–26.
62. Cheng AC. Melioidosis: advances in diagnosis and treatment. *Curr Opin Infect Dis* 2010;**23**:554–9.
70. Clinical and Laboratory Standards Institute. Methods for *antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria: approved guidelines*. 2nd ed. Informational Supplement. CLSI document M45-A22. Wayne, Pa: Clinical and Laboratory Standards Institute; 2010.
75. Cohen TS, Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. *Nat Med* 2012;**18**:509–19.
77. Cramer N, Wiehlmann L, Tummler B. Clonal epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis. *Int J Med Microbiol* 2010;**300**:526–33.
87. Del Porto P, Cifani N, Guarieri S, et al. Dysfunctional CFTR alters the bactericidal activity of human macrophages against *Pseudomonas aeruginosa*. *PLoS One* 2011;**6**:e19970.
89. Deschaght P, Van Daele S, DeBaets F, Vaneechotte M. PCR and the detection of *Pseudomonas aeruginosa* in respiratory samples of CF patients: a literature review. *J Cyst Fibros* 2011;**10**:293–7.
93. Downey DG, Bell SC, Elborn JS. Neutrophils in cystic fibrosis. *Thorax* 2009;**64**:81–8.
97. Durakovic N, Radojcic C, Boban A, et al. Efficacy and safety of colistin in the treatment of infections caused by multidrug-resistant *Pseudomonas aeruginosa* in patients with hematologic malignancy: a matched pair analysis. *Intern Med* 2011;**50**: 1009–13.
108. Fernandez-Olmos A, Morosini MI, Lamas A, et al. Clinical and microbiological features of a cystic fibrosis patient chronically colonized with *Pandoraea sputorum* identified by combining 16S rRNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2012;**50**: 1096–8.
117. Foweraker JE, Laughton CR, Brown DF, et al. Comparison of methods to test antibiotic combinations against heterogeneous populations of multiresistant *Pseudomonas aeruginosa* from patients with acute infective exacerbations in cystic fibrosis. *Antimicrob Agents Chemother* 2009;**53**:4809–15.
122. George SM, Rattan J, Walker K, Garg A, et al. *Pseudomonas* mastitis caused by hot tub exposure. *Pediatr Infect Dis J* 2011;**30**:816.
130. Glass MB, Beesley CA, Wilkins PP, Hoffmaster AR, et al. Comparison of four selective media for the isolation of *Burkholderia mallei* and *Burkholderia pseudomallei*. *Am J Trop Med Hyg* 2009; **80**:1023–8.
131. Gooderham WJ Hancock RE. Regulation of virulence and antibiotic resistance by two-component regulatory systems in Pseudomonas aeruginosa. *FEMS Microbiol Rev* 2009;**33**:279–94.
135. Harris PN, Ketheesan N, Owens L, Norton RE. Clinical features that affect indirect-hemagglutination-assay responses to *Burkholderia pseudomallei*. *Clin Vaccine Immunol* 2009;**16**:924–30.
141. Henry DA, Speert DP. *Pseudomonas*. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.
145. Høiby N, Ciofu O, Bjarnsholt T. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. *Future Microbiol* 2010;**5**:1663–74.
146. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 2010;**35**: 322–32.
152. Hurley MN, Ariff AH, Bertenshaw C, et al. Results of antibiotic susceptibility testing do not influence clinical outcome in children with cystic fibrosis. *J Cyst Fibros* 2012;**11**:288–92.
166. Kaestli M, Richardson LJ, Colman RE, et al. Comparison of TaqMan PCR assays for detection of the melioidosis agent *Burkholderia pseudomallei* in clinical specimens. *J Clin Microbiol* 2012; **50**:2059–62.
168. Kalka-Moll WM, LiPuma JJ, Accurso FJ, et al. Airway infection with a novel *Cupriavidus* species in persons with cystic fibrosis. *J Clin Microbiol* 2009;**47**:3026–8.
170. Karafin M, Romagnoli M, Fink DL, et al. Fatal infection caused by *Cupriavidus gilardii* in a child with aplastic anemia. *J Clin Microbiol* 2010;**48**:1005–7.

187. Kovats Z, Sütto Z, Muraközy G, et al. Airway pathogens during the first year after lung transplantation: a single-center experience. *Transplant Proc* 2011;**43**:1290–1.
196. Langevin S, Vincelette J, Bekal S, Gaudreau C. First case of invasive human infection caused by *Cupriavidus metallidurans*. *J Clin Microbiol* 2011;**49**:744–5.
201. Limmathurotsakul D, Peacock SJ. Melioidosis: a clinical overview. *Br Med Bull* 2011;**99**:125–39.
202. Limmathurotsakul D, Chantratita N, Teerawattanasook N, et al. Enzyme-linked immunosorbent assay for the diagnosis of melioidosis: better than we thought. *Clin Infect Dis* 2011;**52**:1024–8.
204. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;**23**:299–323.
205. LiPuma J, Currie B, et al. *Burkholderia*, *Stenotrophomonas*, *Ralstonia*, *Cupriavidas*, *Pandoraea*, *Brevundimonas*, *Comamonas*, *Delfia*, and *Acidovoax*. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.
210. Logan C, Habington A, Lennon G, Cronin F, O'Sullivan N, et al. Evaluation of the efficacy of real-time polymerase chain reaction for the routine early detection of *Pseudomonas aeruginosa* in cystic fibrosis sputum and throat swab specimens. *Diagn Microbiol Infect Dis* 2010;**68**:358–65.
215. Marko DC, Saffert RT, Cunningham SA, et al. Evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of nonfermenting gram-negative bacilli isolated from cultures from cystic fibrosis patients. *J Clin Microbiol* 2012;**50**:2034–9.
221. Meumann EM, Cheng AC, Ward L, Currie BJ. Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. *Clin Infect Dis* 2012; **54**:362–9.
239. Nseir W, Taha H, Abid A, Kjateeb J. *Pseudomonas mendocina* sepsis in a healthy man. *Isr Med Assoc J* 2011;**13**:375–6.
242. Oermann CM, McCoy KS, Retsch-Bogart GZ, et al. Pseudomonas aeruginosa antibiotic susceptibility during long-term use of aztreonam for inhalation solution (AZLI). *J Antimicrob Chemother* 2011;**66**:2398–404.
243. Oermann CM, Retsch-Bogart GZ, Quittner AL, et al. An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr Pulmonol* 2010; **45**:1121–34.
300. Segonds C, Clavel-Batut P, Thouverez M, et al. Microbiological and epidemiological features of clinical respiratory isolates of *Burkholderia gladioli*. *J Clin Microbiol* 2009;**47**:1510–16.
321. Stovall SH, Wisdom C, McKamie W, et al. Nosocomial transmission of *Cupriavidus pauculus* during extracorporeal membrane oxygenation. *ASAIO J* 2010;**56**:486–7.
335. Veesenmeyer JL, Hauser AR, Lisboa T, Rello J, et al. *Pseudomonas aeruginosa* virulence and therapy: evolving translational strategies. *Crit Care Med* 2009;**37**:1777–86.
343. Wainwright CE, Quittner AL, Geller DE, et al. Aztreonam for inhalation solution (AZLI) in patients with cystic fibrosis, mild lung impairment, and *P. aeruginosa*. *J Cyst Fibros* 2011;**10**: 234–42.
351. Williams BJ, Dehnbostel J, Blackwell TS. *Pseudomonas aeruginosa:* host defence in lung diseases. *Respirology* 2010;**15**:1037–56.
353. Wong V, Levi K, Baddal B, Turton J, Boswell TC, et al. Spread of *Pseudomonas fluorescens* due to contaminated drinking water in a bone marrow transplant unit. *J Clin Microbiol* 2011;**49**: 2093–6.
366. Yoshino Y, Kitazawa T, Kamimura M, et al. *Pseudomonas putida* bacteremia in adult patients: five case reports and a review of the literature. *J Infect Chemother* 2011;**17**:278–82.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 126

Stenotrophomonas (Xanthomonas) maltophilia

Carlos A. Sattler

Stenotrophomonas maltophilia is a gram-negative bacillus that previously belonged to the *Pseudomonas* and subsequently the *Xanthomonas* genus. In 1993, the new genus *Stenotrophomonas* was proposed, resulting in the most recent reclassification of this bacterium.[93] *S. maltophilia* is an opportunistic pathogen that has emerged as a significant cause of nosocomially acquired infection. It rarely is a cause of infection in healthy, immunocompetent children.

BACTERIOLOGY

S. maltophilia is an aerobic, nonfermentative, gram-negative bacillus that is lysine decarboxylase–positive and oxidase-negative, although recently, oxidase-positive isolates have been described.[15] In addition, key features that allow identification of *S. maltophilia* include oxidation of glucose and maltose, as well as a positive DNase reaction.[49] Optimal growth occurs at 35°C (95°F); on sheep

REFERENCES

1. Aaron SD. Antibiotic synergy testing should not be routine for patients with cystic fibrosis who are infected with multiresistant bacterial organisms. *Paediatr Respir Rev* 2007;**8**:256–61.
2. Abbink FC, Orendi JM, de Beaufort AJ. Mother-to-child transmission of *Burkholderia pseudomallei*. *N Engl J Med* 2001;**344**:1171–2.
3. Al-Aloul M, Crawley J, Winstanley C, et al. Increased morbidity associated with chronic infection by an epidemic *Pseudomonas aeruginosa* strain in CF patients. *Thorax* 2004;**59**:334–6.
4. Alexander AD, Huxsoll DL, Warner AR Jr, Shepler V, Dorsey A, et al. Serological diagnosis of human melioidosis with indirect hemagglutination and complement fixation tests. *Appl Microbiol* 1970;**20**:825–33.
5. Alexander JW, Fisher MW. Immunization against *Pseudomonas* in infection after thermal injury. *J Infect Dis* 1974;**130**:152–8.
6. Anaissie E, Fainstein V, Miller P, et al. *Pseudomonas putida:* newly recognized pathogen in patients with cancer. *Am J Med* 1987;**82**: 1191–4.
7. Ancel AM, Borque C, Castello FD. *Pseudomonas* sepsis in children without previous medical problems. *Pediatr Infect Dis J* 1993;**12**: 58–60.
8. Anderson RL, Vess RW, Carr JH, et al. Investigations of intrinsic *Pseudomonas cepacia* contamination in commercially manufactured povidone-iodine. *Infect Control Hosp Epidemiol* 1991;**12**:297–302.
9. Anonymous. *Pseudomonas aeruginosa* infections: persisting problems and current research to find new therapies. *Ann Intern Med* 1975;**82**:819–31.
10. Anuntagool A, Intachote P, Naigowit P, Sirisinha S. Rapid antigen detection assay for identification of *Burkholderia (Pseudomonas) pseudomallei* infection. *J Clin Microbiol* 1996;**34**:975–6.
11. Apisarnthanarak A, Apisarnthanarak P, Mundy LM. Computed tomography characteristics of *Burkholderia pseudomallei* liver abscess. *Clin Infect Dis* 2006;**42**:989–93.
12. Armstrong D, Bell S, Robinson M, et al. Evidence for spread of a clonal strain of *Pseudomonas aeruginosa* among cystic fibrosis clinics. *J Clin Microbiol* 2003;**41**:2266–7.
13. Armstrong DS, Nixon GM, Carzino R, et al. Detection of a widespread clone of *Pseudomonas aeruginosa* in a pediatric cystic fibrosis clinic. *Am J Respir Crit Care Med* 2002;**166**:983–7.
14. Arora SK, Neely AN, Blair B, Lory S, Ramphal R, et al. Role of motility and flagellin glycosylation in the pathogenesis of *Pseudomonas aeruginosa* burn wound infections. *Infect Immun* 2005; **73**:4395–8.
15. Ashdown LR. An improved screening technique for isolation of *Pseudomonas pseudomallei* from clinical specimens. *Pathology* 1979; **11**:293–7.
16. Ashdown LR, Guard RW. The prevalence of human melioidosis in Northern Queensland. *Am J Trop Med Hyg* 1984;**33**:474–8.
17. Ashdown LR, Johnson RW, Koehler JM, Cooney CA, et al. Enzyme-linked immunosorbent assay for the diagnosis of clinical and subclinical melioidosis. *J Infect Dis* 1989;**160**:253–60.
18. Assael BM. Aztreonam inhalation solution for suppressive treatment of chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis. *Expert Rev Anti Infect Ther* 2011;**9**:967–73.
19. Balasubramanian D, Schneper L, Merighi M, et al. The regulatory repertoire of *Pseudomonas aeruginosa* AmpC ss-lactamase regulator AmpR includes virulence genes. *PLoS One* 2012;**7**:e34067.
20. Baldwin A, Mahenthiralingam E, Drevinek P, et al. Environmental *Burkholderia cepacia* complex isolates in human infections. *Emerg Infect Dis* 2007;**13**:458–61.
21. Bauernfeind A, Bertele RM, Harms K, et al. Qualitative and quantitative microbiological analysis of sputa of 102 patients with cystic fibrosis. *Infection* 1987;**15**:270–7.
22. Baussano I, Tardivo I, Bellezza-Fontana R, et al. Neonatal screening for cystic fibrosis does not affect time to first infection with Pseudomonas aeruginosa. *Pediatrics* 2006;**118**:888–95.
23. Bell SM, Pham JN, Lanzarone JY. Mutation of *Pseudomonas aeruginosa* to piperacillin resistance mediated by beta-lactamase production. *J Antimicrob Chemother* 1985;**15**:665–70.
24. Bergan T. Pathogenetic factors of *Pseudomonas aeruginosa*. *Scand J Infect Dis Suppl* 1981;**29**:7–12.
25. Berger M, Sorensen RU, Tosi MF, Dearborn DG, Döring G. Complement receptor expression on neutrophils at an inflammatory site, the *Pseudomonas*-infected lung in cystic fibrosis. *J Clin Invest* 1989;**84**:1302–13.
26. Bergogne-Berezin E. Pharmacokinetics of antibiotics in respiratory secretions. In: Pennington JE, editor. *Respiratory infection: diagnosis and management*. New York: Raven; 1989. p. 608.
27. Berkelman RL, Godly J, Weber JA, et al. *Pseudomonas cepacia* peritonitis associated with contamination of automatic peritoneal dialysis machines. *Ann Intern Med* 1982;**96**:456–8.
28. Berkelman RL, Lewin S, Allen JR, et al. Pseudobacteremia attributed to contamination of povidone-iodine with *Pseudomonas cepacia*. *Ann Intern Med* 1981;**95**:32–6.
29. Bernhardt SA, Spilker T, Coffey T, LiPuma JJ. *Burkholderia cepacia* complex in cystic fibrosis: frequency of strain replacement during chronic infection. *Clin Infect Dis* 2003;**37**:780–5.
30. Berry MD, Asmar BI. *Pseudomonas cepacia* bacteremia in children with sickle cell hemoglobinopathies. *Pediatr Infect Dis J* 1991;**10**:696–9.
31. Betrosian AP, Frantzeskaki F, Xanthaki A, Douzinas EE. Efficacy and safety of high-dose ampicillin/sulbactam vs. colistin as monotherapy for the treatment of multidrug resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *J Infect* 2008;**56**:432–6.
32. Biddick R, Spilker T, Martin A, LiPuma JJ, et al. Evidence of transmission of *Burkholderia cepacia*, *Burkholderia multivorans* and *Burkholderia dolosa* among persons with cystic fibrosis. *FEMS Microbiol Lett* 2003;**228**:57–62.
33. Bisbe J, Gatell JM, Puig J, et al. *Pseudomonas aeruginosa* bacteremia: univariate and multivariate analyses of factors influencing the prognosis in 133 episodes. *Rev Infect Dis* 1988;**10**:629–35.
34. Blackburn L, Brownlee K, Conway S, Denton M, et al. 'Cepacia syndrome' with *Burkholderia multivorans*, 9 years after initial colonization. *J Cyst Fibros* 2004;**3**:133–4.
35. Bobo RA, Newton EJ, Jones LF, Farmer LH, Farmer JJ 3rd. Nursery outbreak of *Pseudomonas aeruginosa:* epidemiological conclusions from five different typing methods. *Appl Microbiol* 1973; **25**:414–20.
36. Bodem CR, Lampton LM, Miller DP, Tarka EF, Everett ED. Endobronchial pH. Relevance of aminoglycoside activity in gram-negative bacillary pneumonia. *Am Rev Respir Dis* 1983;**127**:39–41.
37. Bonatti H, Stelzmueller I, Laimer I, Obwegeser A. *Ralstonia pickettii* meningitis in a child with hydrocephalus. *Eur J Pediatr Surg* 2009;**19**:341–2.
38. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992;**101**:1644–55.
39. Bothwell MR, Smith AL, Phillips T. Recalcitrant otorrhea due to *Pseudomonas* biofilm. *Otolaryngol Head Neck Surg* 2003;**129**:599–601.
40. Bouallegue O, Mzoughi R, Weill FX, et al. Outbreak of *Pseudomonas putida* bacteraemia in a neonatal intensive care unit. *J Hosp Infect* 2004;**57**:88–91.
41. Bradley JS, Nelson JD, Kimberlin DW, et al. Alphabetic listing of antimicrobials. In *Nelson's Pediatric Antimicrobial Therapy*. 19th ed. Elk Grove Village, IL; 2012. pp 137–63.
42. Bradley JS, Nelson JD, Kimberlin DW, et al. Preferred therapy for specific bacterial and mycobacterial pathogens. In *Nelson's Pediatric Antimicrobial Therapy*. 19th ed. Elk Grove Village, IL; 2012. pp 85–99.
43. Brand RA, Black H. *Pseudomonas* osteomyelitis following puncture wounds in children. *J Bone Joint Surg Am* 1974;**56**:1637–42.
44. Breidenstein EB, de la Fuente-Nunez C, Hancock RE. *Pseudomonas aeruginosa:* all roads lead to resistance. *Trends Microbiol* 2011;**19**:419–26.
45. Brett MM, Ghoneim AT, Littlewood JM. Prediction and diagnosis of early *Pseudomonas aeruginosa* infection in cystic fibrosis: a follow-up study. *J Clin Microbiol* 1988;**26**:1565–70.
46. Briscoe D, Rubowitz A, Assia E. Changing bacterial isolates and antibiotic sensitivities of purulent dacryocystitis. *Orbit* 2005;**24**: 29–32.
47. Brown RB, Kruse JA, Counts GW, et al. Double-blind study of endotracheal tobramycin in the treatment of gram-negative bacterial pneumonia. The Endotracheal Tobramycin Study Group. *Antimicrob Agents Chemother* 1990;**34**:269–72.
48. Burns JL, Saiman L, Whittier S, et al. Comparison of agar diffusion methodologies for antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients. *J Clin Microbiol* 2000;**38**:1818–22.
49. Burns JL, Van Dalfsen JM, Shawar RM, et al. Effect of chronic intermittent administration of inhaled tobramycin on respiratory

microbial flora in patients with cystic fibrosis. *J Infect Dis* 1999; **179**:1190–6.
50. Buscher KH, Mann W, Dick W, Opferkuch W. Imipenem resistance in *Pseudomonas aeruginosa* resulting from diminished expression of an outer membrane protein. *Antimicrob Agents Chemother* 1987;**31**:703–8.
51. Butbul-Aviel Y, Miron D, Halevy R, Koren A, Sakran W. Acute mastoiditis in children: *Pseudomonas aeruginosa* as a leading pathogen. *Int J Pediatr Otorhinolaryngol* 2003;**67**:277–81.
52. Caraher E, Duff C, Mullen T, et al. Invasion and biofilm formation of *Burkholderia dolosa* is comparable with *Burkholderia cenocepacia* and *Burkholderia multivorans*. *J Cyst Fibros* 2007;**6**:49–56.
53. Carratala J, Salazar A, Mascaro J, Santin M. Community-acquired pneumonia due to *Pseudomonas stutzeri*. *Clin Infect Dis* 1992;**14**:792.
54. Centers for Disease Control and Prevention. *Swimming Pools: safety and disease control through proper design and operation*. DHHS Publication. Washington DC: Centers for Disease Control and Prevention; 1979. p. 98–411.
55. Centers for Disease Control and Prevention. Nosocomial *Ralstonia pickettii* colonization associated with intrinsically contaminated saline solution: Los Angeles, California, 1998. *MMWR Morb Mortal Wkly Rep* 1998;**47**:285–6.
56. Centers for Disease Control and Prevention. Laboratory exposure to *Burkholderia pseudomallei:* Los Angeles, California, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:988–90.
57. Centers for Disease Control and Prevention. *Ralstonia* associated with Vapotherm oxygen delivery device: United States, 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1052–3.
58. Centers for Disease Control and Prevention. Imported melioidosis: South Florida, 2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**: 873–6.
59. Chamot E, Boffi El Amari E, Rohner P, Van Delden C. Effectiveness of combination antimicrobial therapy for *Pseudomonas aeruginosa* bacteremia. *Antimicrob Agents Chemother* 2003;**47**:2756–64.
60. Chantratita N, Meumann E, Thanwisai A, et al. Loop-mediated isothermal amplification method targeting the TTS1 gene cluster for detection of *Burkholderia pseudomallei* and diagnosis of melioidosis. *J Clin Microbiol* 2008;**46**:568–73.
61. Chaowagul W, Suputtamongkol Y, Dance DA, et al. Relapse in melioidosis: incidence and risk factors. *J Infect Dis* 1993;**168**: 1181–5.
62. Cheng AC. Melioidosis: advances in diagnosis and treatment. *Curr Opin Infect Dis* 2010;**23**:554–9.
63. Cheng AC, Currie BJ. Melioidosis: epidemiology, pathophysiology, and management. *Clin Microbiol Rev* 2005;**18**:383–416.
64. Cheng AC, O'Brien M, Freeman K, et al. Indirect hemagglutination assay in patients with melioidosis in northern Australia. *Am J Trop Med Hyg* 2006;**74**:330–4.
65. Cheng AC, Wuthiekanun V, Limmathurotsakul D, Chierakul W, Peacock SJ. Intensity of exposure and incidence of melioidosis in Thai children. *Trans R Soc Trop Med Hyg* 2008;**102**(Suppl. 1): S37–9.
66. Chi CY, Lai CH, Fung CP, Wang JH. *Pseudomonas mendocina* spondylodiscitis: a case report and literature review. *Scand J Infect Dis* 2005;**37**:950–3.
67. Chitnis CE, Ohman DE. Genetic analysis of the alginate biosynthetic gene cluster of *Pseudomonas aeruginosa* shows evidence of an operonic structure. *Mol Microbiol* 1993;**8**:583–93.
68. Chusid MJ, Hillmann SM. Community-acquired *Pseudomonas* sepsis in previously healthy infants. *Pediatr Infect Dis J* 1987;**6**: 681–4.
69. Chysky V, Kapila K, Hullmann R, et al. Safety of ciprofloxacin in children: worldwide clinical experience based on compassionate use: emphasis on joint evaluation. *Infection* 1991;**19**:289–96.
70. Clinical and Laboratory Standards Institute. Methods for *Antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria; approved guidelines*. 2nd ed. Informational Supplement. CLSI document M45-A22. Wayne, Pa: Clinical and Laboratory Standards Institute; 2010.
71. Clinical and Laboratory Standards Institute. *Performance standards for antimircrobial susceptibility testing: twenty-second informational supplement*. CLSI document M100-S22. Wayne, Pa: Clinical and Laboratory Standards Institute; 2012.
72. Coenye T, Falsen E, Vancanneyt M, et al. Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* 1999;**49**(Pt 2):405–13.
73. Coenye T, Vandamme P, LiPuma JJ. Infection by *Ralstonia* species in cystic fibrosis patients: identification of *R. pickettii* and *R. mannitolilytica* by polymerase chain reaction. *Emerg Infect Dis* 2002;**8**:692–6.
74. Coenye T, Liu L, Vandamme E, LiPumma JJ. Identification of *Pandoraea* species by 16S ribosomal DNA-based PCR assays. *J Clin Microbiol* 2001;**39**:4452–5.
75. Cohen TS, Prince A. Cystic fibrosis: a mucosal immunodeficiency syndrome. *Nat Med* 2012;**18**:509–19.
76. Craig WA, Ebert SD. Antimicrobial therapy in *Pseudomonas aeruginosa* infections. In: Baltch AL, Smith RP, editors. Pseudomonas aeruginosa *infections and treatment*. New York: Marcel Dekker; 1994.
77. Cramer N, Wiehlmann L, Tummler B. Clonal epidemiology of *Pseudomonas aeruginosa* in cystic fibrosis. *Int J Med Microbiol* 2010;**300**:526–33.
78. Crosby LA, Powell DA. The potential value of the sedimentation rate in monitoring treatment outcome in puncture-wound-related *Pseudomonas osteomyelitis*. *Clin Orthop Relat Res* 1984:168–72.
79. Cryz SJ Jr, Pitt TL, Fürer E, Germanier R. Role of lipopolysaccharide in virulence of *Pseudomonas aeruginosa*. *Infect Immun* 1984; **44**:508–13.
80. Cullman W, Buscher KH, Dick W. Selection and properties of *Pseudomonas aeruginosa* variants resistant to beta-lactam antibiotics. *Eur J Clin Microbiol* 1987;**6**:467–73.
81. Dance DA. Melioidosis: the tip of the iceberg? *Clin Microbiol Rev* 1991;**4**:52–60.
82. Dance DA, Davis TM, Wattanagoon Y, et al. Acute suppurative parotitis caused by *Pseudomonas pseudomallei* in children. *J Infect Dis* 1989;**159**:654–60.
83. Davis BD. Mechanism of bactericidal action of aminoglycosides. *Microbiol Rev* 1987;**51**:341–50.
84. Davis PB. Pathophysiology of the lung disease in cystic fibrosis. In: Davis PB, editor. *Cystic fibrosis*. New York: Marcel Dekker; 1993.
85. Daxboeck F, Stadler M, Assadian O, et al. Characterization of clinically isolated *Ralstonia mannitolilytica* strains using random amplification of polymorphic DNA (RAPD) typing and antimicrobial sensitivity, and comparison of the classification efficacy of phenotypic and genotypic assays. *J Med Microbiol* 2005;**54** (Pt 1):55–61.
86. Defez C, Fabbro-Peray P, Bouziges N, et al. Risk factors for multidrug-resistant *Pseudomonas aeruginosa* nosocomial infection. *J Hosp Infect* 2004;**57**:209–16.
87. Del Porto P, Cifani N, Guarnieri S, et al. Dysfunctional CFTR alters the bactericidal activity of human macrophages against *Pseudomonas aeruginosa*. *PLoS One* 2011;**6**:e19970.
88. Dervisoglu E, Dundar DO, Yegenaga I, Willke A. Peritonitis due to *Pseudomonas putida* in a patient receiving automated peritoneal dialysis. *Infection* 2008;**36**:379–80.
89. Deschaght P, Van Daele S, De Baets F, Vaneechoutte M. PCR and the detection of *Pseudomonas aeruginosa* in respiratory samples of CF patients: a literature review. *J Cyst Fibros* 2011;**10**:293–7.
90. Dever LA, Dermody TS. Mechanisms of bacterial resistance to antibiotics. *Arch Intern Med* 1991;**151**:886–95.
91. Dohar J, Giles W, Richard P, et al. Topical ciprofloxacin/dexamethasone superior to oral amoxicillin/clavulanic acid in acute otitis media with otorrhea through tympanostomy tubes. *Pediatrics* 2006;**118**:e561–9.
92. Doring G, et al. Molecular epidemiology of *Pseudomonas aeruginosa* in an intensive care unit. *Epidemiol Infect* 1993;**110**: 427–36.
93. Downey DG, Bell SC, Elborn JS. Neutrophils in cystic fibrosis. *Thorax* 2009;**64**:81–8.
94. Drake D, Montie TC. Flagella, motility and invasive virulence of *Pseudomonas aeruginosa*. *J Gen Microbiol* 1988;**134**:43–52.
95. Drusano GL, et al. Impact of pharmacodynamic dosing of meropenem on emergence of resistance during treatment of ventilator-associated pneumonia: a prospective clinical trial [abstract K-127]. Program and abstracts of the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society of Microbiology. Washington DC, December 16-9, 2005.
96. Dunn M, Wunderink RG. Ventilator-associated pneumonia caused by *Pseudomonas* infection. *Clin Chest Med* 1995;**16**: 95–109.

97. Durakovic N, Radojcic V, Boban A, et al. Efficacy and safety of colistin in the treatment of infections caused by multidrug-resistant *Pseudomonas aeruginosa* in patients with hematologic malignancy: a matched pair analysis. *Intern Med* 2011;**50**:1009–13.
98. Edwards JR, Turner PJ. Laboratory data which differentiate meropenem and imipenem. *Scand J Infect Dis Suppl* 1995;**96**: 5–10.
99. Eickhoff TC, Ett JV, Hayes PS, Feeley J. *Pseudomonas pseudomallei:* susceptibility to chemotherapeutic agents. *J Infect Dis* 1970; **121**:95–102.
100. El Amari EB, Chamot E, Auckenthaler R, Pechère JC, Van Delden C. Influence of previous exposure to antibiotic therapy on the susceptibility pattern of *Pseudomonas aeruginosa* bacteremic isolates. *Clin Infect Dis* 2001;**33**:1859–64.
101. Elting LS, Bodey GP. Septicemia due to *Xanthomonas* species and non-aeruginosa *Pseudomonas* species: increasing incidence of catheter-related infections. *Medicine (Baltimore)* 1990;**69**:296–306.
102. Faden H, Grossi M. Acute osteomyelitis in children. Reassessment of etiologic agents and their clinical characteristics. *Am J Dis Child* 1991;**145**:65–9.
103. Falagas ME, Kasiakou SK. Toxicity of polymyxins: a systematic review of the evidence from old and recent studies. *Crit Care* 2006;**10**:R27.
104. Feder HM Jr, Grant-Kels JM, Tilton RC. *Pseudomonas* whirlpool dermatitis: report of an outbreak in two families. *Clin Pediatr (Phila)* 1983;**2** :638–42.
105. Feigin RD, Shearer WT. Opportunistic infection in children. III. In the normal host. *J Pediatr* 1975;**87**(6 Pt 1):852–66.
106. Feldman M, Bryan R, Rajan S, et al. Role of flagella in pathogenesis of *Pseudomonas aeruginosa* pulmonary infection. *Infect Immun* 1998;**66**:43–51.
107. Fergie JE, Shema SJ, Lott L, Crawford R, Patrick CC. *Pseudomonas aeruginosa* bacteremia in immunocompromised children: analysis of factors associated with a poor outcome. *Clin Infect Dis* 1994;**18**:390–4.
108. Fernandez-Olmos A, Morosini MI, Lamas A, et al. Clinical and microbiological features of a cystic fibrosis patient chronically colonized with *Pandoraea sputorum* identified by combining 16S rRNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J Clin Microbiol* 2012;**50**: 1096–8.
109. Festini F, Buzzetti R, Bassi C, et al. Isolation measures for prevention of infection with respiratory pathogens in cystic fibrosis: a systematic review. *J Hosp Infect* 2006;**64**:1–6.
110. Fick RB Jr. Pathogenetic mechanisms in cystic fibrosis lung disease: a paradigm for inflammatory airways disease. *J Lab Clin Med* 1993;**121**:632–4.
111. Fick RB, Reynolds HY. *Pseudomonas* respiratory infection in cystic fibrosis: a possible defect in opsonic IgG antibody? *Bull Eur Physiopathol Respir* 1983;**19**:151–61.
112. Fick RB Jr, Sonoda F, Hornick DB. Emergence and persistence of *Pseudomonas aeruginosa* in the cystic fibrosis airway. *Semin Respir Infect* 1992;**7**:168–78.
113. Fiorillo L, Zucker M, Sawyer D, Lin AN, et al. The pseudomonas hot-foot syndrome. *N Engl J Med* 2001;**345**:335–8.
114. Fisher MC, Goldsmith JF, Gilligan PH. Sneakers as a source of *Pseudomonas aeruginosa* in children with osteomyelitis following puncture wounds. *J Pediatr* 1985;**106**:607–9.
115. Fisher MC, Long SS, Robers EM, Dunn JM, Balsara RK. *Pseudomonas maltophilia* bacteremia in children undergoing open heart surgery. *JAMA* 1981;**246**:1571–4.
116. Flores G, Stavola JJ, Noel GJ. Bacteremia due to *Pseudomonas aeruginosa* in children with AIDS. *Clin Infect Dis* 1993;**16**: 706–8.
117. Foweraker JE, Laughton CR, Brown DF, et al. Comparison of methods to test antibiotic combinations against heterogeneous populations of multiresistant *Pseudomonas aeruginosa* from patients with acute infective exacerbations in cystic fibrosis. *Antimicrob Agents Chemother* 2009;**53**:4809–15.
118. Fox RC, Williams GJ, Wunderlink RG, et al. Followup bronchoscopy predicts therapeutic outcome in ventilated patients with nosocomial pneumonia. *Am Rev Resp Dis* 1991;**143**:A109.
119. Gal D, Mayo M, Spencer E, Cheng AC, Currie BJ. Short report: application of a polymerase chain reaction to detect *Burkholderia pseudomallei* in clinical specimens from patients with suspected melioidosis. *Am J Trop Med Hyg* 2005;**73**:1162–4.
120. Garnacho-Montero J, Ortiz-Leyba C, Jiménez-Jiménez FJ, et al. Treatment of multidrug-resistant *Acinetobacter baumannii* ventilator-associated pneumonia (VAP) with intravenous colistin: a comparison with imipenem-susceptible VAP. *Clin Infect Dis* 2003;**36**:1111–18.
121. Geiss HK, Geiss M. Evaluation of a new commercial system for the identification of Enterobacteriaceae and non-fermentative bacteria. *Eur J Clin Microbiol Infect Dis* 1992;**11**:610–16.
122. George SM, An J, Walker K, Garg A, et al. *Pseudomonas* mastitis caused by hot tub exposure. *Pediatr Infect Dis J* 2011;**30**:816.
123. Gerber MA, editor. *Pseudomonas aeruginosa*, *in The Jordan Report 2000: accelerated development of vaccines*. Bethesda, Md: U.S. Dept. of Health and Human Services; 2000. p. 60–2.
124. Gerding DN, Larson TA. Aminoglycoside resistance in gram-negative bacilli during increased amikacin use: Comparison of experience in 14 United States hospitals with experience in the Minneapolis Veterans Administration Medical Center. *Am J Med* 1985;**79**(1A):1–7.
125. Germiller JA, El-Kashlan HK, Shah UK. Chronic *Pseudomonas* infections of cochlear implants. *Otol Neurotol* 2005;**26**:196–201.
126. Gershman MD, Kennedy DJ, Noble Wang J, et al. Multistate outbreak of *Pseudomonas fluorescens* bloodstream infection after exposure to contaminated heparinized saline flush prepared by a compounding pharmacy. *Clin Infect Dis* 2008;**47**:1372–9.
127. Giamarellou H, Kanellakopoulou K. Current therapies for *Pseudomonas aeruginosa*. *Crit Care Clin* 2008;**24**:261–78, viii.
128. Gilligan PH. Microbiology of airway disease in patients with cystic fibrosis. *Clin Microbiol Rev* 1991;**4**:35–51.
129. Gilligan PH. Report on the consensus document for microbiology and infectious diseases in cystic fibrosis. *Clin Microbiol Newslett* 1996;**11**:83–7.
130. Glass MB, Beesley CA, Wilkins PP, Hoffmaster AR. Comparison of four selective media for the isolation of *Burkholderia mallei* and *Burkholderia pseudomallei*. *Am J Trop Med Hyg* 2009;**80**: 1023–8.
131. Gooderham WJ, Hancock RE. Regulation of virulence and antibiotic resistance by two-component regulatory systems in *Pseudomonas aeruginosa*. *FEMS Microbiol Rev* 2009;**33**:279–94.
132. Govan JR, Brown AR, Jones AM. Evolving epidemiology of *Pseudomonas aeruginosa* and the *Burkholderia cepacia* complex in cystic fibrosis lung infection. *Future Microbiol* 2007;**2**:153–64.
133. Gustafson TL, Band JD, Hutcheson RH Jr, Schaffner W. *Pseudomonas* folliculitis: an outbreak and review. *Rev Infect Dis* 1983; **5**:1–8.
134. Hachem RY, Chemaly RF, Ahmar CA, et al. Colistin is effective in treatment of infections caused by multidrug-resistant *Pseudomonas aeruginosa* in cancer patients. *Antimicrob Agents Chemother* 2007;**51**:1905–11.
135. Harris PN, Ketheesen N, Owens L, et al. Clinical features that affect indirect-hemagglutination-assay responses to *Burkholderia pseudomallei*. *Clin Vaccine Immunol* 2009;**16**:924–30.
136. Harris PN, Williams NL, Morris JL, et al. Evidence of *Burkholderia pseudomallei*-specific immunity in patient sera persistently nonreactive by the indirect hemagglutination assay. *Clin Vaccine Immunol* 2011;**18**:1288–91.
137. Hatchette TF, Gupta R, Marrie TJ. *Pseudomonas aeruginosa* community-acquired pneumonia in previously healthy adults: case report and review of the literature. *Clin Infect Dis* 2000;**31**: 1349–56.
138. Heggers JP, Haydon S, Ko F, Hayward PG, Carp S, Robson MC. *Pseudomonas aeruginosa* exotoxin A: its role in retardation of wound healing: the 1992 Lindberg Award. *J Burn Care Rehabil* 1992;**13**: 512–18.
139. Henderson DK, Baptiste R, Parrillo J, Gill VJ. Indolent epidemic of *Pseudomonas cepacia* bacteremia and pseudobacteremia in an intensive care unit traced to a contaminated blood gas analyzer. *Am J Med* 1988;**84**:75–81.
140. Henry D, Ell M, McGimpsey C, et al. Comparison of isolation media for recovery of *Burkholderia cepacia* complex from respiratory secretions of patients with cystic fibrosis. *J Clin Microbiol* 1999;**37**:1004–7.
141. Henry DA, Speert DP. *Pseudomonas*. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.

142. Hilf M, Yu VL, Sharp J, Zuravleff JJ, Korvick JA, Muder RR. Antibiotic therapy for *Pseudomonas aeruginosa* bacteremia: outcome correlations in a prospective study of 200 patients. *Am J Med* 1989;**87**:540–6.
143. Hilton E, Adams AA, Uliss A, et al. Nosocomial bacterial eye infections in intensive-care units. *Lancet* 1983;**1**:1318–20.
144. Høiby N, Koch C. Cystic fibrosis. I. *Pseudomonas aeruginosa* infection in cystic fibrosis and its management. *Thorax* 1990;**45**: 881–4.
145. Høiby N, Ciofu O, Bjarnsholt T. *Pseudomonas aeruginosa* biofilms in cystic fibrosis. *Future Microbiol* 2010;**5**:1663–74.
146. Høiby N, Bjarnsholt T, Givskov M, et al. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 2010;**35**:322–32.
147. Hojyo-Tomoka MT, Marples RR, Kligman AM. *Pseudomonas* infection in superhydrated skin. *Arch Dermatol* 1973;**107**:723–7.
148. Holland SP, Pulido JS, Shires TK, et al. *Pseudomonas aeruginosa* ocular infections. In: Fick RB, editor. Pseudomonas aeruginosa: *the opportunist*. Boca Raton, Fla: CRC Press; 1993. p. 159–76.
149. Horn KL, Gherini S. Malignant external otitis of childhood. *Am J Otol* 1981;**2**:402–4.
150. Hsueh PR, Teng LJ, Pan HJ, et al. Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. *J Clin Microbiol* 1998;**36**:2914–17.
151. Huang YC, Lin TY, Wang CH. Community-acquired *Pseudomonas aeruginosa* sepsis in previously healthy infants and children: analysis of forty-three episodes. *Pediatr Infect Dis J* 2002;**21**: 1049–52.
152. Hurley MN, Ariff AH, Bertenshaw C, Bhatt J, Smyth AR. Results of antibiotic susceptibility testing do not influence clinical outcome in children with cystic fibrosis. *J Cyst Fibros* 2012;**11**:288–92.
153. Inglis TJ, Garrow SC, Henderson M, et al. *Burkholderia pseudomallei* traced to water treatment plant in Australia. *Emerg Infect Dis* 2000;**6**:56–9.
154. Isles A, Maclusky I, Corey M, et al. *Pseudomonas cepacia* infection in cystic fibrosis: an emerging problem. *J Pediatr* 1984;**104**: 206–10.
155. Jacobs RF, McCarthy RE, Elser JM. *Pseudomonas* osteochondritis complicating puncture wounds of the foot in children: a 10-year evaluation. *J Infect Dis* 1989;**160**:657–61.
156. Jensen T, Pedersen SS, Nielsen CH, Høiby N, Koch C. The efficacy and safety of ciprofloxacin and ofloxacin in chronic *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Antimicrob Chemother* 1987;**20**:585–94.
157. Jhung MA, Sunenshine RH, Noble-Wang J, et al. A national outbreak of *Ralstonia mannitolilytica* associated with use of a contaminated oxygen-delivery device among pediatric patients. *Pediatrics* 2007;**119**:1061–8.
158. Jiang JH, Iu NC, Huang FY, et al. Neonatal sepsis in the neonatal intensive care unit: characteristics of early versus late onset. *J Microbiol Immunol Infect* 2004;**37**:301–6.
159. Johansen HK, Kjeldsen K, Høiby N. *Pseudomonas mendocina* as a cause of chronic infective endocarditis in a patient with situs inversus. *Clin Microbiol Infect* 2001;**7**:650–2.
160. John M, Ecclestone E, Hunter E, Couroux P, Hussain Z. Epidemiology of *Pseudomonas cepacia* colonization among patients with cystic fibrosis. *Pediatr Pulmonol* 1994;**18**:108–13.
161. Johnson LN, Han JY, Moskowitz SM, et al. *Pandoraea* bacteremia in a cystic fibrosis patient with associated systemic illness. *Pediatr Infect Dis J* 2004;**23**:881–2.
162. Jones AM, Dodd ME, Govan JR, et al. *Burkholderia cenocepacia* and *Burkholderia multivorans:* influence on survival in cystic fibrosis. *Thorax* 2004;**59**:948–51.
163. Jones CE, Alexander JW, Fisher M. Clinical evaluation of *Pseudomonas* hyperimmune globulin. *J Surg Res* 1973;**14**:87–96.
164. Jorgensen IM, Johansen HK, Frederiksen B, et al. Epidemic spread of *Pandoraea apista*, a new pathogen causing severe lung disease in cystic fibrosis patients. *Pediatr Pulmonol* 2003;**36**: 439–46.
165. Juretschko S, Labombardi VJ, Lerner SA, Schreckenberger PC; Pseudomonas AST Study Group. Accuracies of beta-lactam susceptibility test results for *Pseudomonas aeruginosa* with four automated systems (BD Phoenix, MicroScan WalkAway, Vitek, and Vitek 2). *J Clin Microbiol* 2007;**45**:1339–42.
166. Kaestli M, Richardson LJ, Colman RE, et al. Comparison of TaqMan PCR assays for detection of the melioidosis agent *Burkholderia pseudomallei* in clinical specimens. *J Clin Microbiol* 2012; **50**:2059–62.
167. Kalish LA, Waltz DA, Dovey M, et al. Impact of *Burkholderia dolosa* on lung function and survival in cystic fibrosis. *Am J Respir Crit Care Med* 2006;**173**:421–5.
168. Kalka-Moll WM, LiPuma JJ, Accurso FJ, et al. Airway infection with a novel *Cupriavidus* species in persons with cystic fibrosis. *J Clin Microbiol* 2009;**47**:3026–8.
169. Kallel H, Hergafi L, Bahloul M, et al. Safety and efficacy of colistin compared with imipenem in the treatment of ventilator-associated pneumonia: a matched case-control study. *Intensive Care Med* 2007;**33**:1162–7.
170. Karafin M, Romagnoli M, Fink DL, et al. Fatal infection caused by *Cupriavidus gilardii* in a child with aplastic anemia. *J Clin Microbiol* 2010;**48**:1005–7.
171. Keene WE, Markum AC, Samadpour M. Outbreak of *Pseudomonas aeruginosa* infections caused by commercial piercing of upper ear cartilage. *JAMA* 2004;**291**:981–5.
172. Kemmerich B, Small GJ, Pennington JE. Comparative evaluation of ciprofloxacin, enoxacin, and ofloxacin in experimental *Pseudomonas aeruginosa* pneumonia. *Antimicrob Agents Chemother* 1986; **29**:395–9.
173. Kendirli T, Ciftçi E, Ince E, et al. *Ralstonia pickettii* outbreak associated with contaminated distilled water used for respiratory care in a paediatric intensive care unit. *J Hosp Infect* 2004;**56**: 77–8.
174. Kenna MA, Bluestone CD, Reilly JS, Lusk RP. Medical management of chronic suppurative otitis media without cholesteatoma in children. *Laryngoscope* 1986;**96**:146–51.
175. Kercsmar CM, Stern RC, Reed MD, et al. Ceftazidime in cystic fibrosis: pharmacokinetics and therapeutic response. *J Antimicrob Chemother* 1983;**12**(Suppl. A):289–95.
176. Kerr KG, Anson JJ, Patmore R, Smith G. Intravenous line infections. *J Hosp Infect* 1994;**26**:73–5.
177. Keys TF, Melton LJ 3rd, Maker MD, Ilstrup DM. A suspected hospital outbreak of pseudobacteremia due to *Pseudomonas stutzeri*. *J Infect Dis* 1983;**147**:489–93.
178. Khabbaz RF, Arnow PM, Highsmith AK, et al. *Pseudomonas fluorescens* bacteremia from blood transfusion. *Am J Med* 1984; **76**:62–8.
179. Kilpatrick M, Girgis N, Farid Z, Bishay E. Aztreonam for treating meningitis caused by gram-negative rods. *Scand J Infect Dis* 1991;**23**:125–6.
180. Kimura AC, Calvet H, Higa JI, et al. Outbreak of *Ralstonia pickettii* bacteremia in a neonatal intensive care unit. *Pediatr Infect Dis J* 2005;**24**:1099–103.
181. Kingston CW. Chronic or latent melioidosis. *Med J Aust* 1971; **2**:618–21.
182. Kiska DL, Kerr A, Jones MC, et al. Accuracy of four commercial systems for identification of *Burkholderia cepacia* and other gram-negative nonfermenting bacilli recovered from patients with cystic fibrosis. *J Clin Microbiol* 1996;**34**:886–91.
183. Kismet E, Atay AA, Demirkaya E. Two cases of *Ralstonia pickettii* bacteremias in a pediatric oncology unit requiring removal of the Port-A-Caths. *J Pediatr Hematol Oncol* 2005;**27**:37–8.
184. Klinger JD, Thomassen MJ. Occurrence and antimicrobial susceptibility of gram-negative nonfermentative bacilli in cystic fibrosis patients. *Diagn Microbiol Infect Dis* 1985;**3**:149–58.
185. Koch C, Høiby N. Pathogenesis of cystic fibrosis. *Lancet* 1993;**341**:1065–9.
186. Kollef MH, Silver P, Murphy DM, Trovillion E. The effect of late-onset ventilator-associated pneumonia in determining patient mortality. *Chest* 1995;**108**:1655–62.
187. Kovats Z, Sütto Z, Muraközy G, et al. Airway pathogens during the first year after lung transplantation: a single-center experience. *Transplant Proc* 2011;**43**:1290–1.
188. Kunakorn M, Petchclai B, Khupulsup K, Naigowit P. Gold blot for detection of immunoglobulin M (IgM)- and IgG-specific antibodies for rapid serodiagnosis of melioidosis. *J Clin Microbiol* 1991;**29**:2065–7.
189. Kurlandsky LE, Fader RC. In vitro activity of minocycline against respiratory pathogens from patients with cystic fibrosis. *Pediatr Pulmonol* 2000;**29**:210–12.
190. Kurukularatne C, Barzaga R. Melioidosis. *Infect Dis Pract Clinicians* 2000;**24**:37–40.

191. Labarca JA, Trick WE, Peterson CL, et al. A multistate nosocomial outbreak of *Ralstonia pickettii* colonization associated with an intrinsically contaminated respiratory care solution. *Clin Infect Dis* 1999;**29**:1281–6.
192. Lacroix J, Er M, Lapointe N, et al. *Pseudomonas aeruginosa* supraglottitis in a six-month-old child with severe combined immunodeficiency syndrome. *Pediatr Infect Dis J* 1988;**7**:739–41.
193. Ladhani S, Bhutta ZA. Neonatal *Pseudomonas putida* infection presenting as staphylococcal scalded skin syndrome. *Eur J Clin Microbiol Infect Dis* 1998;**17**:642–4.
194. Laing FP, Ramotar K, Read RR, et al. Molecular epidemiology of *Xanthomonas maltophilia* colonization and infection in the hospital environment. *J Clin Microbiol* 1995;**33**:513–18.
195. Lang AB, Rüdeberg A, Schöni MH, et al. Vaccination of cystic fibrosis patients against *Pseudomonas aeruginosa* reduces the proportion of patients infected and delays time to infection. *Pediatr Infect Dis J* 2004;**23**:504–10.
196. Langevin S, Vincelette J, Bekal S, Gaudreau C. First case of invasive human infection caused by *Cupriavidus metallidurans*. *J Clin Microbiol* 2011;**49**:744–5.
197. Leelarasamee A, Bovornkitti S. Melioidosis: review and update. *Rev Infect Dis* 1989;**11**:413–25.
198. Legakis NJ, Tzouvelekis LS, Makris A, Kotsifaki H. Outer membrane alterations in multiresistant mutants of *Pseudomonas aeruginosa* selected by ciprofloxacin. *Antimicrob Agents Chemother* 1989; **33**:124–7.
199. Lentnek AL, Williams RR. Aztreonam in the treatment of gram-negative bacterial meningitis. *Rev Infect Dis* 1991;**13**(Suppl. 7): S586–90.
200. Levy JH, Katz HR. *Pseudomonas cepacia* keratitis. *Cornea* 1989;**8**:67–71.
201. Limmathurotsakul D, Peacock SJ. Melioidosis: a clinical overview. *Br Med Bull* 2011;**99**:125–39.
202. Limmathurotsakul D, Chantratita N, Teerawattanasook N, et al. Enzyme-linked immunosorbent assay for the diagnosis of melioidosis: better than we thought. *Clin Infect Dis* 2011;**52**:1024–8.
203. LiPuma JJ. Burkholderia and emerging pathogens in cystic fibrosis. *Semin Respir Crit Care Med* 2003;**24**:681–92.
204. Lipuma JJ. The changing microbial epidemiology in cystic fibrosis. *Clin Microbiol Rev* 2010;**23**:299–323.
205. LiPuma J, Currie B, et al. *Burkholderia*, *Stenotrophomonas*, *Ralstonia*, *Cupriavidas*, *Pandoraea*, *Brevundimonas*, *Comamonas*, *Delfia*, and *Acidovoax*. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.
206. LiPuma JJ, Mortensen JE, Dasen SE, et al. Ribotype analysis of *Pseudomonas cepacia* from cystic fibrosis treatment centers. *J Pediatr* 1988;**113**:859–62.
207. LiPuma JJ, Spilker T, Gill LH, et al. Disproportionate distribution of *Burkholderia cepacia* complex species and transmissibility markers in cystic fibrosis. *Am J Respir Crit Care Med* 2001;**164**: 92–6.
208. Lo WT, Cheng SN, Wang CC, Chu ML. Extensive necrotising fasciitis caused by *Pseudomonas aeruginosa* in a child with acute myeloid leukaemia: case report and literature review. *Eur J Pediatr* 2005;**164**:113–14.
209. Lodise TP Jr, Lomaestro B, Drusano GL. Piperacillin-tazobactam for *Pseudomonas aeruginosa* infection: clinical implications of an extended-infusion dosing strategy. *Clin Infect Dis* 2007;**44**: 357–63.
210. Logan C, Habington A, Lennon G, Cronin F, O'Sullivan N. Evaluation of the efficacy of real-time polymerase chain reaction for the routine early detection of *Pseudomonas aeruginosa* in cystic fibrosis sputum and throat swab specimens. *Diagn Microbiol Infect Dis* 2010;**68**:358–65.
211. Lui PV. Biology of *Pseudomonas aeruginosa*. *Hosp Pract* 1976;**11**: 139–47.
212. Lumbiganon P, Pengsaa K, Puapermpoonsiri S, Puapairoj A. Neonatal melioidosis: a report of 5 cases. *Pediatr Infect Dis J* 1988;**7**:634–6.
213. Malizia WF, West GA, Brundage WG, Walden DC. Melioidosis-laboratory studies. *Health Lab Sci* 1969;**6**:27–39.
214. Manno G, Dalmastri C, Tabachioni S, et al. Epidemiology and clinical course of *Burkholderia cepacia* complex infections, particularly those caused by different *Burkholderia cenocepacia* strains, among patients attending an Italian Cystic Fibrosis Center. *J Clin Microbiol* 2004;**42**:1491–7.
215. Marko DC, Saffert RT, Cunningham SA, et al. Evaluation of the Bruker Biotyper and Vitek MS matrix-assisted laser desorption ionization-time of flight mass spectrometry systems for identification of nonfermenting gram-negative bacilli isolated from cultures from cystic fibrosis patients. *J Clin Microbiol* 2012;**50**:2034–9.
216. Mattsby-Baltzer I, Edebo L, Järvholm B, Lavenius B. Serum antibodies to *Pseudomonas pseudoalcaligenes* in metal workers exposed to infected metal-working fluids. *Int Arch Allergy Appl Immunol* 1989;**88**:304–11.
217. Mayer JH, Finnlayson MH. Chronic melioidosis: a case with bone and pulmonary lesions. *S Afr Med J* 1944;**18**:109.
218. McCoy KS, Quittner AL, Oermann CM, et al. Inhaled aztreonam lysine for chronic airway *Pseudomonas aeruginosa* in cystic fibrosis. *Am J Respir Crit Care Med* 2008;**178**:921–8.
219. McCubbin M, Fick RB. Pathogenesis of *Pseudomonas* lung disease in cystic fibrosis. In: Fick RB, editor. Pseudomonas aeruginosa: *the opportunist*. CRC Press: Boca Raton, Fla; 1993. p. 189–211.
220. McManus AT, Mason AD Jr, McManus WF, Pruitt BA Jr. Twenty-five year review of *Pseudomonas aeruginosa* bacteremia in a burn center. *Eur J Clin Microbiol* 1985;**4**:219–23.
221. Meumann EM, Cheng AC, Ward L, Currie BJ. Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. *Clin Infect Dis* 2012; **54**:362–9.
222. Miller MB, Gilligan PH. Laboratory aspects of management of chronic pulmonary infections in patients with cystic fibrosis. *J Clin Microbiol* 2003;**41**:4009–15.
223. Milstone AM, Ruff AJ, Yeamans C, Higman MA. *Pseudomonas aeruginosa* pre-septal cellulitis and bacteremia in a pediatric oncology patient. *Pediatr Blood Cancer* 2005;**45**:353; discussion 354.
224. Minami S, Akama M, Araki H, et al. Imipenem and cephem resistant *Pseudomonas aeruginosa* carrying plasmids coding for class B beta-lactamase. *J Antimicrob Chemother* 1996;**37**:433–44.
225. Minnefor AB, Olson MI, Carver DH. *Pseudomonas* osteomyelitis following puncture wounds of the foot. *Pediatrics* 1971;**47**:598–601.
226. Morosini MI, García-Castillo M, Loza E, Pérez-Vázquez M, Baquero F, Cantón R. Breakpoints for predicting *Pseudomonas aeruginosa* susceptibility to inhaled tobramycin in cystic fibrosis patients: use of high-range Etest strips. *J Clin Microbiol* 2005;**43**: 4480–5.
227. Mortensen J, Hansen A, Falk M, Nielsen IK, Groth S. Reduced effect of inhaled beta 2-adrenergic agonists on lung mucociliary clearance in patients with cystic fibrosis. *Chest* 1993;**103**:805–11.
228. Moss RB. The role of IgG subclass antibodies in chronic infection: the case of cystic fibrosis. *N Engl Reg Allergy Proc* 1988;**9**:57–61.
229. Moss RB. Cystic fibrosis: pathogenesis, pulmonary infection, and treatment. *Clin Infect Dis* 1995;**21**:839–49; quiz 850-1.
230. Mull CC, Scarfone RJ, Conway D. Ecthyma gangrenosum as a manifestation of *Pseudomonas* sepsis in a previously healthy child. *Ann Emerg Med* 2000;**36**:383–7.
231. Nelson JW, Butler SL, Krieg D, et al. Virulence factors of *Burkholderia cepacia*. *FEMS Immunol Med Microbiol* 1994;**8**:89–97.
232. Neo EN, Haritharan T, Thambidorai CR, Suresh V. *Pseudomonas* necrotizing fasciitis in an immunocompetent infant. *Pediatr Infect Dis J* 2005;**24**:942–3.
233. Neu HC. The role of Pseudomonas aeruginosa in infections. *J Antimicrob Chemother* 1983;**11**(Suppl. B):1–13.
234. Neu HC. Carbapenems: special properties contributing to their activity. *Am J Med* 1985;**78**(6A):33–40.
235. Nigg C, Johnston MM. Complement fixation test in experimental clinical and subclinical melioidosis. *J Bacteriol* 1961;**82**:159–68.
236. Nixon GM, Armstrong DS, Carzino R, et al. Clinical outcome after early *Pseudomonas aeruginosa* infection in cystic fibrosis. *J Pediatr* 2001;**138**:699–704.
237. Noble RC, Overman SB. *Pseudomonas stutzeri* infection: a review of hospital isolates and a review of the literature. *Diagn Microbiol Infect Dis* 1994;**19**:51–6.
238. Norrby SR. 4-Quinolones in the treatment of infections of the central nervous system. *Rev Infect Dis* 1988;**10**(Suppl. 1):S253–5.
239. Nseir W, Taha H, Abid A, Khateeb J, et al. *Pseudomonas mendocina* sepsis in a healthy man. *Isr Med Assoc J* 2011;**13**:375–6.

240. Nussinovitch M, Yoeli R, Elishkevitz K, Varsano I. Acute mastoiditis in children: epidemiologic, clinical, microbiologic, and therapeutic aspects over past years. *Clin Pediatr (Phila)* 2004;**43**:261–7.
241. O'Boyle JD, Heslinger KA, Hier-Duffin SL. *Pseudomonas aeruginosa* as an unusual cause of intraamniotic infection, fulminant neonatal sepsis and neonatal death: a case report. *J Reprod Med* 1996;**41**:534–6.
242. Oermann CM, McCoy KS, Retsch-Bogart GZ, et al. *Pseudomonas aeruginosa* antibiotic susceptibility during long-term use of aztreonam for inhalation solution (AZLI). *J Antimicrob Chemother* 2011;**66**:2398–404.
243. Oermann CM, Retsch-Bogart GZ, Quittner AL, et al. An 18-month study of the safety and efficacy of repeated courses of inhaled aztreonam lysine in cystic fibrosis. *Pediatr Pulmonol* 2010;**45**:1121–34.
244. Ohman DE, Burns RP, Iglewski BH. Corneal infections in mice with toxin A and elastase mutants of *Pseudomonas aeruginosa*. *J Infect Dis* 1980;**142**:547–55.
245. Orti A, Escrig R, Pérez-Tamarit D, et al. *Pseudomonas aeruginosa* infection in a previously healthy infant. *Clin Pediatr (Phila)* 2002;**41**:525–8.
246. Ozumba UC. Urinary tract infections in children due to *Pseudomonas aeruginosa* in Enugu, Nigeria. *J Trop Pediatr* 1998;**44**:315–16.
247. Papagelopoulos PJ, Mavrogenis AF, Giannitsioti E, et al. Management of a multidrug-resistant *Pseudomonas aeruginosa* infected total knee arthroplasty using colistin: a case report and review of the literature. *J Arthroplasty* 2007;**22**:457–63.
248. Patamasucon P, Schaad UB, Nelson JD, Melioidosis. *J Pediatr* 1982;**100**:175–82.
249. Paterson DL. The epidemiological profile of infections with multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter species*. *Clin Infect Dis* 2006;**43**(Suppl. 2):S43–8.
250. Pattamasukon P, Pichyangkura C, Fischer GW. Melioidosis in childhood. *J Pediatr* 1975;**87**:133–6.
251. Paul M, Silbiger I, Grozinsky S, Soares-Weiser K, Leibovici L. Beta lactam antibiotic monotherapy versus beta lactam-aminoglycoside antibiotic combination therapy for sepsis. *Cochrane Database Syst Rev* 2006(1):CD003344.
252. Peacock EE. *Wound repair*. 3rd ed. Philadelphia: W.B. Saunders; 1984. p. 38–55.
253. Peacock SJ, Chieng G, Cheng AC, et al. Comparison of Ashdown's medium, *Burkholderia cepacia* medium, and *Burkholderia pseudomallei* selective agar for clinical isolation of *Burkholderia pseudomallei*. *J Clin Microbiol* 2005;**43**:5359–61.
254. Pedersen SS. Lung infection with alginate-producing, mucoid *Pseudomonas aeruginosa* in cystic fibrosis. *APMIS Suppl* 1992;**28**:1–79.
255. Pellegrino FL, Schirmer M, Velasco E, et al. *Ralstonia pickettii* bloodstream infections at a Brazilian cancer institution. *Curr Microbiol* 2008;**56**:219–23.
256. Penketh AR, Pitt TL, Hodson ME, Batten JC. Bactericidal activity of serum from cystic fibrosis patients for *Pseudomonas aeruginosa*. *J Med Microbiol* 1983;**16**:401–8.
257. Pennington JE, Reynolds HY, Wood RE, Robinson RA, Levine AS. Use of a *Pseudomonas aeruginosa* vaccine in pateints with acute leukemia and cystic fibrosis. *Am J Med* 1975;**58**:629–36.
258. Perry AW, Sutkin HS, Gottlieb LJ, Stadelmann WK, Krizek TJ. Skin graft survival: the bacterial answer. *Ann Plast Surg* 1989;**22**:479–83.
259. Perz JF, Craig AS, Stratton CW, et al. *Pseudomonas putida* septicemia in a special care nursery due to contaminated flush solutions prepared in a hospital pharmacy. *J Clin Microbiol* 2005;**43**:5316–18.
260. Pier GB. *Pseudomonas aeruginosa* lipopolysaccharide: a major virulence factor, initiator of inflammation and target for effective immunity. *Int J Med Microbiol* 2007;**297**:277–95.
261. Pintado V, San Miguel LG, Grill F, Mejía B, et al. Intravenous colistin sulphomethate sodium for therapy of infections due to multidrug-resistant gram-negative bacteria. *J Infect* 2008;**56**:185–90.
262. Pollack M. *Pseudomonas aeruginosa*. In: Mandell GL, Douglas RG, Bennett JE, editors. *Principles and practice of infectious diseases*. New York: Churchill Livingstone; 1990. p. 1673–91.
263. Poole K. Bacterial multidrug resistance: emphasis on efflux mechanisms and Pseudomonas aeruginosa. *J Antimicrob Chemother* 1994;**34**:453–6.
264. Poschet JF, Boucher JC, Tatterson L, et al. Molecular basis for defective glycosylation and *Pseudomonas* pathogenesis in cystic fibrosis lung. *Proc Natl Acad Sci U S A* 2001;**98**:13972–7.
265. Pressler T, Mansa B, Jensen T, et al. Increased IgG2 and IgG3 concentration is associated with advanced *Pseudomonas aeruginosa* infection and poor pulmonary function in cystic fibrosis. *Acta Paediatr Scand* 1988;**77**:576–82.
266. Radberg G, Nilsson LE, Svensson S. Development of quinolone-imipenem cross resistance in *Pseudomonas aeruginosa* during exposure to ciprofloxacin. *Antimicrob Agents Chemother* 1990;**34**:2142–7.
267. Ralph A, McBride J, Currie BJ. Transmission of *Burkholderia pseudomallei* via breast milk in northern Australia. *Pediatr Infect Dis J* 2004;**23**:1169–71.
268. Ratjen F, Doring G, Nikolaizik WH. Effect of inhaled tobramycin on early *Pseudomonas aeruginosa* colonisation in patients with cystic fibrosis. *Lancet* 2001;**358**:983–4.
269. Reed RK, Larter WE, Sieber OF Jr, John TJ. Peripheral nodular lesions in *Pseudomonas* sepsis: the importance of incision and drainage. *J Pediatr* 1976;**88**:977–9.
270. Reid TM, Porter IA. An outbreak of otitis externa in competitive swimmers due to *Pseudomonas aeruginosa*. *J Hyg (Lond)* 1981;**86**:357–62.
271. Reymond D, Frey B, Birrer P. [Invasive *Pseudomonas aeruginosa* and Ecthyma gangrenosum infection in a child without risk factors]. *Arch Pediatr* 1996;**3**:569–72.
272. Reynolds HY, Di Sant'Agnese PA, Zierdt CH. Mucoid *Pseudomonas aeruginosa:* a sign of cystic fibrosis in young adults with chronic pulmonary disease? *JAMA* 1976;**236**:2190–2.
273. Rhoads S, Marinelli L, Imperatrice CA, Nachamkin I. Comparison of MicroScan Walk Away system and Vitek system for identification of gram-negative bacteria. *J Clin Microbiol* 1995;**33**:3044–6.
274. Richmond MH, Sykes RB. The beta-lactamases of gram-negative bacteria and their possible physiological role. *Adv Microb Physiol* 1973;**9**:31–88.
275. Rios FG, Luna CM, Maskin B, et al. Ventilator-associated pneumonia due to colistin susceptible-only microorganisms. *Eur Respir J* 2007;**30**:307–13.
276. Robinson A, McCarter YS, Tetreault J. Comparison of crystal enteric/nonfermenter system, API 20E system, and Vitek AutoMicrobic system for identification of gram-negative bacilli. *J Clin Microbiol* 1995;**33**:364–70.
277. Roilides E, Butler KM, Husson RN, et al. *Pseudomonas* infections in children with human immunodeficiency virus infection. *Pediatr Infect Dis J* 1992;**11**:547–53.
278. Roland PS, Parry DA, Stroman DW. Microbiology of acute otitis media with tympanostomy tubes. *Otolaryngol Head Neck Surg* 2005;**133**:585–95.
279. Romling U, Fiedler B, Bosshammer J, et al. Epidemiology of chronic *Pseudomonas aeruginosa* infections in cystic fibrosis. *J Infect Dis* 1994;**170**:1616–21.
280. Rowley AH, Dias LD, Chadwick EG, Shulman ST, et al. *Pseudomonas stutzeri:* an unusual cause of calcaneal *Pseudomonas* osteomyelitis. *Pediatr Infect Dis J* 1987;**6**:296–7.
281. Rubio TT. Ciprofloxacin: comparative data in cystic fibrosis. *Am J Med* 1987;**82**(4A):185–8.
282. Ruvalo C, Bauer CR. Intrauterinely acquired *Pseudomonas* infection in the neonate. *Clin Pediatr (Phila)* 1982;**21**:664–7.
283. Sabuda DM, Laupland K, Pitout J, et al. Utilization of colistin for treatment of multidrug-resistant *Pseudomonas aeruginosa*. *Can J Infect Dis Med Microbiol* 2008;**19**:413–18.
284. Sader HS, Fritsche TR, Jones RN. Accuracy of three automated systems (MicroScan WalkAway, VITEK, and VITEK 2) for susceptibility testing of *Pseudomonas aeruginosa* against five broad-spectrum beta-lactam agents. *J Clin Microbiol* 2006;**44**:1101–4.
285. Sadikot RT, Blackwell TS, Christman JW, Prince AS. Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. *Am J Respir Crit Care Med* 2005;**171**:1209–23.
286. Safdar N, Handelsman J, Maki DG. Does combination antimicrobial therapy reduce mortality in Gram-negative bacteraemia? A meta-analysis. *Lancet Infect Dis* 2004;**4**:519–27.
287. Saiman L. Providing a safety net for children: investigating a multistate outbreak of *Ralstonia mannitolilytica* related to a contaminated reusable device. *Pediatrics* 2007;**119**:1207–9.

288. Saiman L, Cacalano G, Prince A. *Pseudomonas cepacia* adherence to respiratory epithelial cells is enhanced by *Pseudomonas aeruginosa*. *Infect Immun* 1990;**58**:2578–84.
289. Sajjan US, Corey M, Karmali MA, Forstner JF. Binding of *Pseudomonas cepacia* to normal human intestinal mucin and respiratory mucin from patients with cystic fibrosis. *J Clin Invest* 1992;**89**: 648–56.
290. Salmen P, Dwyer DM, Vorse H, Kruse W. Whirlpool-associated *Pseudomonas aeruginosa* urinary tract infections. *JAMA* 1983;**250**: 2025–6.
291. Salyers AA, Whitt DD. *Pseudomonas aeruginosa*. In: Salyers AA, Whitt DD, editors. *Bacterial pathogenesis: a molecular approach*. Washington, DC: American Society for Microbiology Press; 1994. p. 260–72.
292. Sanders CC, Sanders WE Jr. Type I beta-lactamases of gram-negative bacteria: interactions with beta-lactam antibiotics. *J Infect Dis* 1986;**154**:792–800.
293. Schaad UB, Sander E, Wedgewood J, et al. Morphologic studies for skeletal toxicity after prolonged ciprofloxacin therapy in two juvenile cystic fibrosis patients. *Pediatr Infect Dis J* 1992;**11**: 1047–9.
294. Schaad UB, Tschaeppeler H, Vock P. Clinical, radiologic and magnetic resonance monitoring for skeletal toxicity in pediatric patients with cystic fibrosis receiving a three-month course of ciprofloxacin. *Pediatr Infect Dis J* 1991;**10**:723–9.
295. Schaad UB, Wedgewood-Krucko J, Suter S, et al. Efficacy of inhaled amikacin as adjunct to intravenous combination therapy (ceftazidime and amikacin) in cystic fibrosis. *J Pediatr* 1987; **111**:599–605.
296. Schiller NL, Millard RL. *Pseudomonas*-infected cystic fibrosis patient sputum inhibits the bactericidal activity of normal human serum. *Pediatr Res* 1983;**17**:747–52.
297. Schroeder TH, Reiniger N, Meluleni G, et al. Transgenic cystic fibrosis mice exhibit reduced early clearance of *Pseudomonas aeruginosa* from the respiratory tract. *J Immunol* 2001;**166**:7410–18.
298. Scott J, Boulton FE, Govan JR, et al. A fatal transfusion reaction associated with blood contaminated with *Pseudomonas fluorescens*. *Vox Sang* 1988;**54**:201–4.
299. Scully BE, Neu HC, Parry MF, Mandell W. Oral ciprofloxacin therapy of infections due to *Pseudomonas aeruginosa*. *Lancet* 1986; **1**:819–22.
300. Segonds C, Clavel-Batut P, Thouverez M, et al. Microbiological and epidemiological features of clinical respiratory isolates of *Burkholderia gladioli*. *J Clin Microbiol* 2009;**47**:1510–16.
301. Senda K, Arakawa Y, Nakashima K, et al. Multifocal outbreaks of metallo-beta-lactamase-producing *Pseudomonas aeruginosa* resistant to broad-spectrum beta-lactams, including carbapenems. *Antimicrob Agents Chemother* 1996;**40**:349–53.
302. Shackelford PG, Ratzan SA, Shearer WT. Ecthyma gangrenosum produced by *Aeromonas hydrophilia*. *J Pediatr* 1973;**83**:100–1.
303. Shand GH, Pedersen SS, Tilling R, Brown MR, Høiby N. Use of immunoblot detection of serum antibodies in the diagnosis of chronic *Pseudomonas aeruginosa* lung infection in cystic fibrosis. *J Med Microbiol* 1988;**27**:169–77.
304. Sharma GD, Tosi MF, Stern RC, Davis PB. Progression of pulmonary disease after disappearance of *Pseudomonas* in cystic fibrosis. *Am J Respir Crit Care Med* 1995;**152**:169–73.
305. Simon PR. *Pseudomonas* bacteraemia in a previously healthy 2-year-old boy. *Clin Pediatr (Phila)* 1989;**28**:339.
306. Simor AE, Ricci J, Lau A, Bannatyne RM, Ford-Jones L. Pseudobacteremia due to *Pseudomonas fluorescens*. *Pediatr Infect Dis* 1985; **4**:508–12.
307. Smith AL, Redding G, Doershuk C, et al. Sputum changes associated with therapy for endobronchial exacerbation in cystic fibrosis. *J Pediatr* 1988;**112**:547–54.
308. Smith EE, Buckley DG, Wu Z, et al. Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc Natl Acad Sci U S A* 2006;**103**:8487–92.
309. Smith GL. Methods for preventing pseudomonas folliculitis. *Cutis* 1982;**29**:378, 381.
310. Smith MD, Wuthiekanun V, Walsh AL, et al. Latex agglutination for rapid detection of *Pseudomonas pseudomallei* antigen in urine of patients with melioidosis. *J Clin Patbol* 1995;**48**:174–6.
311. Sobel JD, Hashman N, Reinherz G, Merzbach D. Nosocomial *Pseudomonas cepacia* infection associated with chlorhexidine contamination. *Am J Med* 1982;**73**:183–6.
312. Sookpranee T, Sookpranee M, Mellencamp MA, Preheim LC. *Pseudomonas pseudomallei*, a common pathogen in Thailand that is resistant to the bactericidal effects of many antibiotics. *Antimicrob Agents Chemother* 1991;**35**:484–9.
313. Sorvillo F, Beall G, Turner PA, et al. Incidence and determinants of *Pseudomonas aeruginosa* infection among persons with HIV: association with hospital exposure. *Am J Infect Control* 2001;**29**: 79–84.
314. Speert DP. Understanding *Burkholderia cepacia:* epidemiology, genomovars and virulence. *Infect Med* 2001;**18**:49–56.
315. Speert DP. Molecular epidemiology of Pseudomonas aeruginosa. *Front Biosci* 2002;**7**:e354–61.
316. Speert DP, Bond M, Woodman RC, et al. Infection with *Pseudomonas cepacia* in chronic granulomatous disease: role of nonoxidative killing by neutrophils in host defense. *J Infect Dis* 1994; **170**:1524–31.
317. Speert DP, Farmer SW, Campbell ME, et al. Conversion of *Pseudomonas aeruginosa* to the phenotype characteristic of strains from patients with cystic fibrosis. *J Clin Microbiol* 1990;**28**: 188–94.
318. Spencer RC. The emergence of epidemic, multiple-antibiotic-resistant *Stenotrophomonas (Xanthomonas) maltophilia* and *Burkholderia (Pseudomonas) cepacia*. *J Hosp Infect* 1995;**30**(Suppl.):453–64.
319. Steen HJ, Scott EM, Stevenson MI, et al. Clinical and pharmacokinetic aspects of ciprofloxacin in the treatment of acute exacerbations of pseudomonas infection in cystic fibrosis patients. *J Antimicrob Chemother* 1989;**24**:787–95.
320. Stelzmueller I, Biebl M, Wiesmayr S, et al. *Ralstonia pickettii:* innocent bystander or a potential threat? *Clin Microbiol Infect* 2006;**12**:99–101.
321. Stovall SH, Wisdom C, McKamie W, et al. Nosocomial transmission of *Cupriavidus pauculus* during extracorporeal membrane oxygenation. *ASAIO J* 2010;**56**:486–7.
322. Strand CL, Bryant JK, Morgan JW, et al. Nosocomial *Pseudomonas aeruginosa* urinary tract infections. *JAMA* 1982;**248**:1615–18.
323. Strandvik B, Hjelte L, Lindblad A, et al. Comparison of efficacy and tolerance of intravenously and orally administered ciprofloxacin in cystic fibrosis patients with acute exacerbations of lung infection. *Scand J Infect Dis Suppl* 1989;**60**:84–8.
324. Stryjewski ME, LiPuma JJ, Messier RH Jr, Reller LB, Alexander BD. Sepsis, multiple organ failure, and death due to *Pandoraea pnomenusa* infection after lung transplantation. *J Clin Microbiol* 2003;**41**:2255–7.
325. Supaprom C, Wang D, Leelayuwat C, et al. Development of real-time PCR assays and evaluation of their potential use for rapid detection of *Burkholderia pseudomallei* in clinical blood specimens. *J Clin Microbiol* 2007;**45**:2894–901.
326. Tablan OC, Martone WJ, Doershuk CF, et al. Colonization of the respiratory tract with *Pseudomonas cepacia* in cystic fibrosis: risk factors and outcomes. *Chest* 1987;**91**:527–32.
327. Tablan OC, Martone WJ, Jarvis WR. The epidemiology of *Pseudomonas cepacia* in patients with cystic fibrosis. *Eur J Epidemiol* 1987;**3**:336–42.
328. Tateda K, Ii Y, Kimura S, et al. Suppression of *Pseudomonas aeruginosa* quorum-sensing systems by macrolides: a promising strategy or an oriental mystery? *J Infect Chemother* 2007;**13**:357–67.
329. Tiffany KK, Kline MW. Mixed flora brain abscess with *Pseudomonas paucimobilis* after a penetrating lawn dart injury. *Pediatr Infect Dis J* 1988;**7**:667–9.
330. Tredget EE, Shankowsky HA, Joffe AM, et al. Epidemiology of infections with *Pseudomonas aeruginosa* in burn patients: the role of hydrotherapy. *Clin Infect Dis* 1992;**15**:941–9.
331. Ugwumadu AH, Manyonda IT, Hay PE. Chorioamnionitis due to *Pseudomonas aeruginosa:* a complication of prolonged antibiotic therapy for premature rupture of membranes. *Br J Obstet Gynaecol* 1996;**103**:1054–5; author reply 1055–6.
332. Unal S, Ersoz G, Demirkan F, et al. Analysis of skin-graft loss due to infection: infection-related graft loss. *Ann Plast Surg* 2005;**55**:102–6.
333. Vandamme P, Coenye T. Taxonomy of the genus *Cupriavidus:* a tale of lost and found. *Int J Syst Evol Microbiol* 2004; **54**(Pt 6):2285–9.
334. Vartivarian SE, Anaissie E. *Stenotrophomonas maltophilia* and *Burkholderia cepacia*. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. Philadelphia: Churchill Livingstone; 2000.

335. Veesenmeyer JL, Hauser AR, Lisboa T, Rello J. *Pseudomonas aeruginosa* virulence and therapy: evolving translational strategies. *Crit Care Med* 2009;**37**:1777–86.
336. Viola L, Langer A, Pulitanò S, et al. Serious *Pseudomonas aeruginosa* infection in healthy children: case report and review of the literature. *Pediatr Int* 2006;**48**:330–3.
337. Virgili A, Colombo E, Serino R, Pedretti S, Corazza M. Necrotizing fasciitis from *Pseudomonas aeruginosa* in infantile acute lymphoblastic leukaemia. *Acta Derm Venereol* 2005;**85**:538–9.
338. Vishwanath S, Ramphal R. Adherence of *Pseudomonas aeruginosa* to human tracheobronchial mucin. *Infect Immun* 1984;**45**:197–202.
339. Vitaliti SM, Maggio MC, Cipolla D, Corsello G, Mammina C. Neonatal sepsis caused by *Ralstonia pickettii*. *Pediatr Infect Dis J* 2008;**27**:283.
340. Vochem M, Vogt M, Doring G. Sepsis in a newborn due to *Pseudomonas aeruginosa* from a contaminated tub bath. *N Engl J Med* 2001;**345**:378–9.
341. Vogt R, LaRue D, Parry MF, et al. *Pseudomonas aeruginosa* skin infections in persons using a whirlpool in Vermont. *J Clin Microbiol* 1982;**15**:571–4.
342. Wade JC. Antibiotic therapy for the febrile granulocytopenic cancer patient: combination therapy vs. monotherapy. *Rev Infect Dis* 1989;**11**(Suppl. 7):S1572–81.
343. Wainwright CE, Quittner AL, Geller DE, et al. Aztreonam for inhalation solution (AZLI) in patients with cystic fibrosis, mild lung impairment, and *P. aeruginosa*. *J Cyst Fibros* 2011;**10**:234–42.
344. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991;**35**:147–51.
345. Wauters G, Claeys G, Verschraegen G, et al. Case of catheter sepsis with *Ralstonia gilardii* in a child with acute lymphoblastic leukemia. *J Clin Microbiol* 2001;**39**:4583–4.
346. Welch DF, Muszynski MJ, Pai CH, et al. Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis. *J Clin Microbiol* 1987;**25**:1730–4.
347. West SE, Zeng L, Lee BL, et al. Respiratory infections with *Pseudomonas aeruginosa* in children with cystic fibrosis: early detection by serology and assessment of risk factors. *JAMA* 2002;**287**:2958–67.
348. Wheeler WB, Williams M, Matthews WJ Jr, Colten HR. Progression of cystic fibrosis lung disease as a function of serum immunoglobulin G levels: a 5-year longitudinal study. *J Pediatr* 1984;**104**:695–9.
349. White NJ, Dance DA, Chaowagul W, et al. Halving of mortality of severe melioidosis by ceftazidime. *Lancet* 1989;**2**:697–701.
350. Widmer AF, Zel RP, Trilla A, et al. Outbreak of *Pseudomonas aeruginosa* infections in a surgical intensive care unit: probable transmission via hands of a health care worker. *Clin Infect Dis* 1993;**16**:372–6.
351. Williams BJ, Dehnbostel J, Blackwell TS. *Pseudomonas aeruginosa:* host defence in lung diseases. *Respirology* 2010;**15**:1037–56.
352. Wong SN, Tam AY, Yung RW, Kwan EY, Tsoi NN. *Pseudomonas septicaemia* in apparently healthy children. *Acta Paediatr Scand* 1991;**80**:515–20.
353. Wong V, Levi K, Baddal B, Turton J, Boswell TC. Spread of *Pseudomonas fluorescens* due to contaminated drinking water in a bone marrow transplant unit. *J Clin Microbiol* 2011;**49**:2093–6.
354. Wongratanacheewin S, Tattawasart U, Lulitanond V. An avidin-biotin enzyme-linked immunosorbent assay for the detection of *Pseudomonas pseudomallei* antigens. *Trans R Soc Trop Med Hyg* 1990;**84**:429–30.
355. Woo PC, Wong SS, Yuen KY. *Ralstonia pickettii* bacteraemia in a cord blood transplant recipient. *New Microbiol* 2002;**25**:97–102.
356. Woods DE, Cryz SJ, Friedman RL, Iglewski BH. Contribution of toxin A and elastase to virulence of *Pseudomonas aeruginosa* in chronic lung infections of rats. *Infect Immun* 1982;**36**:1223–8.
357. Worlitzsch D, Tarran R, Ulrich M, et al. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. *J Clin Invest* 2002;**109**:317–25.
358. Wretlind B, Pavlovskis OR. The role of proteases and exotoxin A in the pathogenicity of *Pseudomonas aeruginosa* infections. *Scand J Infect Dis Suppl* 1981;**29**:13–19.
359. Wright RM, Moore JE, Shaw A, et al. Improved cultural detection of *Burkholderia cepacia* from sputum in patients with cystic fibrosis. *J Clin Pathol* 2001;**54**:803–5.
360. Wu BY, Peng CT, Tsai CH, Chiu HH. Community-acquired *Pseudomonas aeruginosa* bacteremia and sepsis in previously healthy infants. *Acta Paediatr Taiwan* 1999;**40**:233–6.
361. Wunderlich CA, Krach LE. Gram-negative meningitis and infections in individuals treated with intrathecal baclofen for spasticity: a retrospective study. *Dev Med Child Neurol* 2006;**48**:450–5.
362. Wuthiekanun V, Chierakul W, Langa S, et al. Development of antibodies to *Burkholderia pseudomallei* during childhood in melioidosis-endemic northeast Thailand. *Am J Trop Med Hyg* 2006;**74**:1074–5.
363. Wuthiekanun V, Desakom V, Wongsuvan G, et al. Rapid immunofluorescence microscopy for diagnosis of melioidosis. *Clin Diagn Lab Immunol* 2005;**12**:555–6.
364. Yang CH, Young T, Peng MY, Weng MC. Clinical spectrum of *Pseudomonas putida* infection. *J Formos Med Assoc* 1996;**95**:754–61.
365. Yeung CK, Lee KH. Community acquired fulminant *Pseudomonas* infection of the gastrointestinal tract in previously healthy infants. *J Paediatr Child Health* 1998;**34**:584–7.
366. Yoshino Y, Kitazawa T, Kamimura M, et al. *Pseudomonas putida* bacteremia in adult patients: five case reports and a review of the literature. *J Infect Chemother* 2011;**17**:278–82.
367. Young EJ, Sewell CM, Koza MA, Clarridge JE. Antibiotic resistance patterns during aminoglycoside restriction. *Am J Med Sci* 1985;**290**:223–7.
368. Yu VL Paterson DL. *Pseudomonas aeruginosa*. In: Yu VL, Merigan TC, Barriere SL, editors. *Antimicrobial therapy and vaccines*. Baltimore: Williams & Wilkins; 1999. p. 348–58.
369. Yu Y, Cheng AS, Wang L, Dunne WM, Bayliss SJ. Hot tub folliculitis or hot hand-foot syndrome caused by *Pseudomonas aeruginosa*. *J Am Acad Dermatol* 2007;**57**:596–600.
370. Zachariah M, Vyjayanthi S, Bell-Thomas S. Umbilical vein varix thrombosis: a rare pathology. *J Obstet Gynaecol* 2004;**24**:581.
371. Zawacki A, O'Rourke E, Potter-Bynoe G, et al. An outbreak of *Pseudomonas aeruginosa* pneumonia and bloodstream infection associated with intermittent otitis externa in a healthcare worker. *Infect Control Hosp Epidemiol* 2004;**25**:1083–9.
372. Zellweger C, Bodmer T, Täuber MG, Mühlemann K. Failure of ceftriaxone in an intravenous drug user with invasive infection due to *Ralstonia pickettii*. *Infection* 2004;**32**:246–8.
373. Zimakoff J, Høiby N, Rosendal K, Guilbert JP. Epidemiology of *Pseudomonas aeruginosa* infection and the role of contamination of the environment in a cystic fibrosis clinic. *J Hosp Infect* 1983;**4**:31–40.
374. Zomorrodi A, Wald ER. Ecthyma gangrenosum: considerations in a previously healthy child. *Pediatr Infect Dis J* 2002;**21**:1161–4.

blood agar, colonies appear rough and lavender green and have a distinct ammonia-like odor. Morphologically, the organism is a straight bacillus, 0.7 to 1.8 μm long, that is motile by means of multiple polar flagella. It grows well on standard culture media, including blood agar and chocolate agar, as well as in broth blood culture systems, within the standard 5-day incubation period. Because of the hardy nature of this organism, standard collection, transport, and storage procedures are sufficient. The use of selective media such as MacConkey agar allows for *S. maltophilia* to be isolated from polymicrobial specimens, although for highly contaminated samples such as feces, the addition of antibiotics such as imipenem, vancomycin, and amphotericin B to the media should be considered.[68] New diagnostic techniques for the identification of *S. maltophilia* are being developed, but further experience with these methodologies is needed.[80,88]

EPIDEMIOLOGY

S. maltophilia is a free-living, ubiquitous organism; the natural habitats for it include water, soil, and plants. The ability to survive in an aqueous milieu has allowed *S. maltophilia* to occupy a niche in the hospital environment. It has been cultured from contaminated dialysis fluids[10] and intraocular rinsing solution,[60] as well as ventilators and other respiratory equipment,[30,69,101] and preoperative surgical brushes.[92] Several reports have linked nosocomial outbreaks of *S. maltophilia* to contamination of hospital water sources such as faucet aerators,[121] taps,[102] and sinks,[69] as well as disinfectant solutions.[124] Intestinal colonization with *S. maltophilia* has been described in hospitalized oncology patients with diarrhea and could represent a potential source of nosocomial infection.[4] Other reports have described cases of nosocomial cross infection occurring in neonatal and pediatric intensive care units.[45,54,73] In addition, cases of "pseudoinfection" caused by contamination of blood collection tubes have been described.[105]

Predisposing factors associated with colonization and infection by *S. maltophilia* include the presence of a severe, debilitating underlying illness, particularly malignancy; immune suppression (including human immunodeficiency virus infection); hematopoietic stem cell transplantation; exposure to broad-spectrum antibiotics (particularly to carbapenems, quinolones, or broad-spectrum cephalosporins); prolonged exposure to antibiotics; the presence of a central venous catheter; neutropenia; severe mucositis; prolonged hospital stay; stay in an intensive care unit; tracheostomy; mechanical ventilation; chronic obstructive pulmonary disease; or a combination of these factors.*

Among children, predisposing factors are similar to those found in adults.[104] Infection with *S. maltophilia* generally is hospital acquired.[46,104] Interestingly, a recent retrospective case-control study in children found that patients with *S. maltophilia* bloodstream infection were more likely to develop their infection in the home setting. However, almost all patients had a central venous catheter in place; thus, the cases represented health care–associated infections.[64] *S. maltophilia* is being isolated from clinical samples with increasing frequency.* This increase probably is related to advances in medical care that allow for a rise in the survival rate of severely ill patients, who are at highest risk for acquiring infection with this organism and who require more frequent use of invasive medical equipment, including indwelling lines, as well as broad-spectrum antimicrobials to which the organism is not susceptible.

PATHOPHYSIOLOGY

Like many pseudomonads, *S. maltophilia* is an organism with low virulence and limited invasiveness. An intact host immune system is an important deterrent to acquisition of a severe and even life-threatening infection; septicemia and death occur in patients with underlying debilitating illnesses. *S. maltophilia* elaborates a wide range of extracellular enzymes, including DNase, RNase, fibrinolysin, lipase, hyaluronidase, protease, and elastase, which may play a role in the pathogenesis of disease processes associated with *S. maltophilia*.[29] The pathogenesis of *S. maltophilia* infection also may be related to the development of cytotoxic activity.[41] In addition, *S. maltophilia* has been found to adhere to plastic materials, including intravenous catheters, and to produce biofilm that limits access to both phagocytes and antibiotics, properties that may account for the relatively high incidence of catheter-related bloodstream infections caused by this organism.[25,29,37] *S. maltophilia* is inherently resistant to several classes of antibiotics. Colonization with *S. maltophilia*, especially in the respiratory tract, is not an uncommon finding. In debilitated patients, exposure to broad-spectrum antimicrobials, many of which are ineffective against this bacterium, may allow overgrowth of colonizing organisms that subsequently gain access to sterile body sites and cause infection. Portals of infection include indwelling devices such as central venous catheters, peritoneal dialysis or urinary tract catheters, the respiratory tract, and the gastrointestinal tract.

CLINICAL MANIFESTATIONS

S. maltophilia once was regarded as a microorganism with very limited pathogenicity that was unlikely to cause infection except in the most debilitated patients.[46] However, the incidence as well as the severity of infection and the spectrum of clinical manifestations caused by this bacterium have increased. The most common site of isolation of *S. maltophilia* is the respiratory tract, particularly in hospitalized, mechanically ventilated patients. Attributing a causative role to *S. maltophilia* occasionally is difficult when the organism is isolated only from

*References 5, 6, 13, 14, 27, 36, 44, 82, 90, 91, 112, 117, 126

*References 5, 35, 70, 81, 85, 104.

respiratory secretions, particularly as part of a mixed culture. Most critically ill, mechanically ventilated patients have abnormal chest radiographs, and differentiating infectious from noninfectious infiltrates may be very difficult. Nonetheless, *S. maltophilia* has unequivocally been associated with pneumonia, which may be severe and which may occur in patients who are not receiving mechanical ventilation.[1,42,48] Occasionally, the pathogen may be associated with massive, fatal pulmonary hemorrhage in adult patients with malignant disease,[34] and it is a cause of ventilator-associated pneumonia.[78] In adults with pneumonia, the development of secondary bacteremia signals a grave prognosis.[35]

S. maltophilia bloodstream infections usually are catheter-associated.[11,69,71,86,116] In a series of 32 episodes of bacteremia in children, all were related to the presence of a central venous catheter, although in 10 episodes, the primary source was not the catheter.[104] In a series of 217 episodes of *S. maltophilia* bloodstream infections occurring in patients with cancer who had central venous catheters, secondary bacteremia generally occurred in patients who were neutropenic, had concurrent pneumonia, or were critically ill, and it was associated with a worse outcome in contrast to patients who had central venous catheter–related bacteremia.[11] Frequently, *S. maltophilia* is isolated as part of a polymicrobial bloodstream infection, particularly when the central venous catheter is the origin of the infection. In children, the severity of illness appears to be similar regardless of whether *S. maltophilia* is isolated in a monomicrobial or mixed blood culture.[104] Malignant disease is the most common underlying illness in patients with bacteremia, and many patients have concomitant neutropenia. Bloodstream infections caused by *S. maltophilia* can be severe. In a pediatric series, 31 percent of the patients initially were seen in septic shock, and the attributable mortality rate was 6.3 percent.[104] In another pediatric series of 32 episodes of bacteremia occurring in 31 children, the crude mortality rate was 40.6 percent.[125] However, the death rate associated with *S. maltophilia* bloodstream infection is, in general, higher in adults.[69,70,83,86]

S. maltophilia endocarditis is a rare occurrence. It has been associated with intravenous drug abuse and frequently occurs after replacement of a prosthetic valve.[7,57,87,127] Surgical therapy often is required, although cure with medical treatment alone has been reported. No cases of endocarditis caused by this organism in children have been reported.

Urinary tract infections caused by *S. maltophilia* almost invariably occur in patients with structural abnormalities of the urinary tract, indwelling urinary catheters, or underlying illnesses. Infection may be severe and associated with sepsis and septic shock.[114]

Skin and soft tissue infections caused by *S. maltophilia* have occurred in patients who have had work-related injuries and wounds contaminated with soil and plant material, such as occur in injuries associated with use of lawn mowers.[23,33,51] In the hospital setting, *S. maltophilia* frequently is cultured from wounds and surgical sites, although determining the clinical significance of this organism in children, particularly when it is isolated in mixed culture, often is difficult.[104] In adult patients with cancer, tender, erythematous nodular skin lesions have been described in association with *S. maltophilia* bacteremia and probably represent metastatic infectious foci.[115] Cases of metastatic soft tissue involvement, including nodular skin lesions and pyomyositis, in pediatric patients with bacteremia have also been described.[108,125] All of these patients had severe underlying diseases, particularly hematologic malignancies, associated with neutropenia. *S. maltophilia* bacteremia and sepsis as a complication in burn patients have also occurred.[75,110]

S. maltophilia has been implicated in the development of ocular infections, including conjunctivitis and keratitis in the setting of ocular surface compromise resulting from trauma, the use of soft contact lenses, or previous infection with human herpes simplex virus.[97] Endophthalmitis and scleritis occurring after ophthalmologic surgical interventions and trauma also have been described.[18,19,59,65,99]

Meningitis caused by *S. maltophilia* is an extremely rare occurrence and usually is nosocomial and associated with neurosurgical procedures and infection of intraventricular devices.[96] However, spontaneous meningitis in four infants was reported.[77,89,103]

S. maltophilia is the fourth most common organism isolated from the bronchial secretions of patients with cystic fibrosis, after *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Haemophilus influenzae*.[111] The incidence and prevalence of *S. maltophilia* isolated from the respiratory secretions of patients with cystic fibrosis are increasing.[26,67,100,107] *S. maltophilia* is recovered from the sputum of an estimated 12 percent of patients with cystic fibrosis in the United States and from as many as 25 percent of such patients in Europe.[100,118] The origin of the bacterium is uncertain; evidence suggests that it may be acquired both in the hospital and in the community.[27] Colonization may be transient or persistent, with chronic colonization occurring more frequently in older patients.[111] Case-control studies identified greater exposure to antipseudomonal antibiotics, oral ciprofloxacin, inhaled aminoglycosides, and oral corticosteroids, as well as more hospitalization days, and isolation of *Aspergillus fumigatus* from the sputum as risk factors for colonization with *S. maltophilia*.[32,79,107] Unlike with *Burkholderia cepacia* infection, no evidence of patient-to-patient transmission of *S. maltophilia* has been found, nor does the organism appear to be associated with rapid deterioration of pulmonary function in patients with cystic fibrosis.[31,52] The clinical significance of isolation of *S. maltophilia* from the sputum of patients with cystic fibrosis and its role in the deterioration in lung function are unclear. Its presence may represent more a marker of severe, advanced disease than causally related respiratory deterioration.[28,53] However, recent data suggest that chronic infection with *S. maltophilia* is an independent risk factor for pulmonary exacerbation,[120] but chronic infection does not appear to affect subsequent lung function recovery.[119] Cases of *S. maltophilia* bacteremia have been rarely reported in patients with cystic fibrosis.[66]

Other infections caused by *S. maltophilia* include peritoneal catheter exit-site infections and peritonitis in

patients undergoing peritoneal dialysis,[24,106] as well as cholangitis,[94] osteochondritis,[8] mastoiditis,[58] bursitis,[95] sinusitis,[56] liver abscess,[98] osteomyelitis,[72] and necrotizing ulcerative gingivitis.[84]

DIAGNOSIS

The diagnosis of infection is established by isolating the organism from normally sterile sites in the presence of a compatible clinical picture. However, growth of *S. maltophilia* from normally sterile samples actually may represent an episode of pseudoinfection caused by the ability of this organism to contaminate and survive in hospital equipment such as blood collection tubes and antiseptic solutions. Because *S. maltophilia* is a common colonizer of hospitalized patients, isolation from nonsterile sites such as sputum or wounds is more difficult to interpret, particularly when the organism is isolated in mixed culture. Because nosocomial outbreaks have been reported, isolation of *S. maltophilia* from other patients in a hospital setting should alert the physician to the possibility of *Stenotrophomonas* infection. Infection control offices should be notified after the organism has been isolated.

Occasionally, *B. cepacia* is misidentified as *S. maltophilia*, especially in patients with cystic fibrosis.[12] *S. maltophilia* is oxidase-negative and DNase-positive. These tests should be repeated when identification of the organism is in doubt. Because of the serious clinical implications of misidentification in this group of patients, molecular analysis of the isolates should be considered if results remain uncertain.[123]

TREATMENT

Providing antibiotic therapy for *S. maltophilia* is difficult for several reasons. This organism displays multiple intrinsic resistance determinants that confer broad antibiotic resistance, including resistance to β-lactam antibiotic agents.[22] Mechanisms of resistance include the production of two chromosomally encoded, inducible β-lactamases designated L1 and L2. The former is a β-lactamase inhibitor–resistant metalloenzyme that hydrolyzes a broad range of β-lactam antibiotics, including carbapenems such as imipenem and meropenem. L2 is a cephalosporinase and, unlike L1, is susceptible to β-lactamase inhibitors.[29] Other mechanisms of resistance include reduced antibiotic uptake, the main mechanism providing aminoglycoside resistance, and an antibiotic efflux system that confers multidrug resistance.[3,129] Resistance to trimethoprim-sulfamethoxazole mediated by mobile genetic elements *sul1* and *sul2* has recently been reported.[109] In addition, in vitro antibiotic susceptibility testing is plagued by numerous methodologic problems. Several factors, including the time of incubation and the composition of the medium, affect the interpretation of test results. Furthermore, poor reproducibility among different testing methods has been described.[16,55] The Clinical and Laboratory Standards Institute (CLSI) has established standards for antibiotic susceptibility testing, including testing by both disk diffusion and broth or agar dilution (minimum inhibitory concentration [MIC] evaluation).[21] For the disk diffusion method, CLSI recommends testing only trimethoprim-sulfamethoxazole, levofloxacin, and minocycline. In the case of broth or agar dilution, CLSI recommends testing trimethoprim-sulfamethoxazole (susceptible: ≤2/38 μg/mL; resistant: ≥4/76 μg/mL), ceftazidime (susceptible: ≥8 μg/mL; intermediate: 16 μg/mL; resistant: ≥32 μg/mL), chloramphenicol (susceptible: ≤8 μg/mL; intermediate: 16 μg/mL; resistant: ≥32 μg/mL), levofloxacin (susceptible: ≤2 μg/mL; intermediate: 4 μg/mL; resistant: ≥8 μg/mL), minocycline (susceptible: ≤4 μg/mL; intermediate: 8 μg/mL; resistant: ≥16 μg/mL), and ticarcillin-clavulanate (susceptible: ≤16/2 μg/mL; intermediate: 32/2 to 64/2 μg/mL; resistant: ≥128/2 μg/mL). Regardless, no controlled clinical studies have determined the most effective antibiotic regimen or duration of treatment.

Antibiotic susceptibility studies and clinical observation suggest that the most active antibiotic against *S. maltophilia* is trimethoprim-sulfamethoxazole, and most authorities agree that it is the drug of choice. However, it is bacteriostatic—a property that may have clinical implications in immunosuppressed patients. In addition, resistant strains are being reported with increasing frequency,[2,83,113] although this is not a uniform finding.[76] Other antibiotics that have shown in vitro activity include ticarcillin-clavulanate, doxycycline, minocycline, and colistin, although clinical experience with the latter three drugs is limited.[29,38,39,62] The activity of early fluoroquinolones such as ciprofloxacin and ofloxacin varies widely, and emergence of resistance during treatment has been reported.[20] Newer quinolones, including levofloxacin and gatifloxacin, appear to have better activity in vitro against *S. maltophilia*,[40,63,74,122] but their activity decreases against multiresistant strains, and the development of resistance during the course of treatment has been described.[9] In addition, geographic variations in antibiotic resistance rates have been reported.[43] Tigecycline is emerging as a potential therapeutic option for *S. maltophilia*. However, clinical experience, particularly in children, is limited, and the proportion of susceptible isolates varies across studies.[61,39,62,50]

Bloodstream infections can be severe, particularly in immunosuppressed patients. Combination antibiotic therapy with trimethoprim-sulfamethoxazole and ticarcillin-clavulanate for bloodstream infections by isolates that are susceptible has been advocated by some authorities and is supported by in vitro pharmacodynamic model data.[86,113,128] Other combinations have shown varying efficacy and correlation between in vitro and in vivo findings, which have not always been consistent.[29] In cases of catheter-related bloodstream infection, removal of the catheter has been associated with improved outcome, in some circumstances irrespective of the appropriateness of antibiotic therapy, and should be considered for severely ill patients.[17,35,104,126] Nonetheless, successful treatment without removal of the catheter[86] as well as an association of inappropriate antibiotic therapy and death,[83] has been described. In cases of serious infection such as bacteremia or severe pneumonia, combination antibiotic therapy should be considered when in vitro and in vivo evidence

suggests that rapid emergence of resistance may occur during treatment.[47]

Appropriate management of patients from whom *S. maltophilia* is isolated from nonsterile sites, particularly in mixed culture, is not always clear because some isolates may represent colonization or contamination of the sample. In a pediatric study describing nonrespiratory infections in children, 6/16 patients with *S. maltophilia* that was cultured from sites other than the bloodstream were treated with antibiotics not active in vitro, and 5 of these children were "cured."[104] Nonetheless, in adults, *S. maltophilia* has been associated unquestionably with severe infection at sites other than the bloodstream and death caused by *S. maltophilia* infection has been associated with inappropriate initial antibiotic therapy.[90]

NEW REFERENCES SINCE THE SIXTH EDITION

6. Araoka H, Fujii T, Izutsu K, et al. Rapidly progressive fatal hemorrhagic pneumonia caused by *Stenotrophomonas maltophilia* in hematologic malignancy. *Transpl Infect Dis* 2012;**0**:1–9.
15. Carmody LA, Spilker T, LiPuma JJ. Reassessment of *Stenotrophomonas maltophilia* phenotype. *J Clin Microbiol* 2011;**49**:1101–3.
17. Chaplow R, Palmer B, Heyderman R, et al. *Stenotrophomonas maltophilia* bacteraemia in 40 haematology patients: risk factors, therapy and outcome. *Bone Marrow Transplant* 2010;**45**:1109–10.
18. Chen KJ, Wang NK, Sun MH, et al. Endophthalmitis caused by *Stenotrophomonas maltophilia*. *Ophthalmic Surg Lasers Imaging* 2010;**22**:e555–61.
21. Clinical and Laboratory Standards Institute (CLSI). *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement. CLSI document M100-S21*. Wayne, Pa: CLSI; 2011.
22. Crossman LC, Gould VC, Dow JM, et al. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. *Genome Biol* 2008;**9**:R74.
26. de Vrankrijker AMM, Wolfs TFW, van der Ent CK. Challenging and emerging pathogens in cystic fibrosis. *Paediatr Respir Rev* 2010;**11**:246–54.
38. Entenza JM, Moreillon P. Tigecycline in combination with other antimicrobials: a review of in vitro, animal and case report studies. *Int J Antimicrob Agents* 2009;**34**(1):8.e1–9.
39. Farrell DJ, Sader HS, Jones RN. Antimicrobial susceptibilities of a worldwide collection of *Stenotrophomonas maltophilia* isolates tested against tigecycline and agents commonly used for *S. maltophilia* infections. *Antimicrob Agents Chemother* 2010;**54**:2735–7.
44. Garazi M, Singer C, Tai J, Ginocchio CC. Bloodstream infections caused by *Stenotrophomonas maltophilia:* a seven-year review. *J Hosp Infect* 2012;**81**:114–18.
50. Goncalves-Vidigal P, Grosse-Onnebrink J, Mellies U, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: improved detection by the use of selective agar and evaluation of antimicrobial resistance. *J Cyst Fibros* 2011;**10**:422–7.
55. Gülmez D, Cakar A, Sener B, et al. Comparison of different antimicrobial susceptibility testing methods for *Stenotrophomonas maltophilia* and results of synergy testing. *J Infect Chemother* 2010;**16**:322–8.
60. Horster S, Bader L, Seybold U, et al. *Stenotrophomonas maltophilia* induced post-cataract-surgery endophthalmitis: outbreak investigation and clinical courses of 26 patients. *Infection* 2009;**37**:117–22.
62. Jacquier H, Le Monnier A, Carbonnelle E, et al. In vitro antimicrobial activity of "last-resort" antibiotics against unusual nonfermenting gram-negative bacilli clinical isolates. *Microb Drug Resist* 2012;**18**:396–401.
64. Kagen J, Zaoutis TE, McGowa KL, et al. Bloodstream infection caused by *Stenotrophomonas maltophilia* in children. *Pediatr. Infect Dis J* 2007;**26**:508–12.
75. Leung C, Drew P, Azzopardi EA. Extended multidrug-resistant *Stenotrophomonas maltophilia* septicemia in a severely burnt patient. *J Burn Care Res* 2010;**31**:966.
76. Livermore DM, Hope R, Brick G, et al. Non-susceptibility trends among *Pseudomonas aeruginosa* and other non-fermentative Gram-negative bacteria from bacteraemias in the UK and Ireland, 2001-06. *J Antimicrob Chemother* 2008;**62**(Suppl. 2):ii55–63.
80. Marko DC, Saffert RT, Cunningham SA, et al. Evaluation of the Bruker Biotyper and VITEK® MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Systems for the identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patient cultures. *J Clin Microbiol* 2012;**50**:2034–9.
88. Nakamura A, et al. Diagnostic value of PCR analysis of bacteria and fungi from blood in empiric-therapy-resistant febrile neutropenia. *J Clin Microbiol* 2010;**48**:2030–6.
99. Ramos-Esteban JC, Jeng BH. Posttraumatic *Stenotrophomonas maltophilia* infectious scleritis. *Cornea* 2008;**27**:232–5.
100. Razvi S, Quittell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. *Chest* 2009;**136**:1554–60.
102. Sakhnini E, Weissmann A, Oren I. Fulminant *Stenotrophomonas maltophilia* soft tissue infection in immunocompromised patients: an outbreak transmitted via tap water. *Am J Med Sci* 2002;**323**:269–72.
109. Toleman MA, Bennett PM, Bennett DM, et al. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of *sul* genes. *Emerg Infect Dis* 2007;**13**:559–65.
110. Tsai WP, Chen CL, Ko WC, Pan SC. *Stenotrophomonas maltophilia* bacteremia in burn patients. *Burns* 2006;**32**:155–8.
119. Waters V, Atenafu EG, Salazar JG, et al. Chronic *Stenotrophomonas maltophilia* infection and exacerbation outcomes in cystic fibrosis. *J Cyst Fibros* 2012;**11**:8–13.
120. Waters V, Yau Y, Prasad S, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. *Am J Respir Crit Care Med* 2011;**183**:635–40.
126. Yeshurun M, Gafter-Gvili A, Thaler M, et al. Clinical characteristics of *Stenotrophomonas maltophilia* infection in hematopoietic stem cell transplantation recipients: a single center experience. *Infection* 2010;**38**:211–15.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aisenberg G, Rolston KV, Dickey BF, et al. *Stenotrophomonas maltophilia* pneumonia in cancer patients without traditional risk factors for infection, 1997-2004. *Eur J Clin Microbiol Infect Dis* 2007;**26**:13–20.
2. Al-Jasser AM. *Stenotrophomonas maltophilia* resistant to trimethoprim-sulfamethoxazole: an increasing problem. *Ann Clin Microbiol Antimicrob* 2006;**5**:23.
3. Alonso A, Martinez JL. Expression of multidrug efflux pump SmeDEF by clinical isolates of *Stenotrophomonas maltophilia*. *Antimicrob Agents Chemother* 2001;**45**:1879–81.
4. Apisarnthanarak A, Fraser VJ, Dunne WM, et al. *Stenotrophomonas maltophilia* intestinal colonization in hospitalized oncology patients with diarrhea. *Clin Infect Dis* 2003;**37**:1131–5.
5. Apisarnthanarak A, Mayfield JL, Garison T, et al. Risk factors for *Stenotrophomonas maltophilia* bacteremia in oncology patients: a case-control study. *Infect Control Hosp Epidemiol* 2003;**24**:269–74.
6. Araoka H, Fujii T, Izutsu K, et al. Rapidly progressive fatal hemorrhagic pneumonia caused by Stenotrophomonas maltophilia in hematologic malignancy. *Transpl Infect Dis* 2012;**0**: 1–9.
7. Aydin K, Koksal I, Kaygusuz S, et al. Endocarditis caused by *Stenotrophomonas maltophilia*. *Scand J Infect Dis* 2000;**32**:427–30.
8. Baltimore RS, Jenson HB. Puncture wound osteochondritis of the foot caused by *Pseudomonas maltophilia*. *Pediatr Infect Dis J* 1990; **9**:143–4.
9. Bellido JL, Hernandez FJ, Zufiaurre MN, Garcia-Rodriguez JA. In vitro activity of newer fluoroquinolones against *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 2000;**46**:334–5.
10. Berbari N, Johnson DH, Cunha BA. *Xanthomonas maltophilia* peritonitis in a patient undergoing peritoneal dialysis. *Heart Lung* 1993;**22**:282–3.
11. Boktour M, Hanna H, Ansari S, et al. Central venous catheter and *Stenotrophomonas maltophilia* bacteremia in cancer patients. *Cancer* 2006;**106**:1967–73.
12. Burdge DR, Noble MA, Campbell ME, et al. *Xanthomonas maltophilia* misidentified as *Pseudomonas cepacia* in cultures of sputum from patients with cystic fibrosis: a diagnostic pitfall with major clinical implications. *Clin Infect Dis* 1995;**20**:445–8.
13. Calza L, Manfredi R, Chiodo F. *Stenotrophomonas (Xanthomonas) maltophilia* as an emerging opportunistic pathogen in association with HIV infection: a 10-year surveillance study. *Infection* 2003; **31**:155–61.
14. Carmeli Y, Samore MH. Comparison of treatment with imipenem vs. ceftazidime as a predisposing factor for nosocomial acquisition of *Stenotrophomonas maltophilia*: A historical cohort study. *Clin Infect Dis* 1997;**24**:1131–4.
15. Carmody LA, Spilker T, LiPuma JJ. Reassessment of *Stenotrophomonas maltophilia* phenotype. *J Clin. Microbiol* 2011;**49**:1101–3.
16. Carroll KC, Cohen S, Nelson R, et al. Comparison of various in vitro susceptibility methods for testing *Stenotrophomonas maltophilia*. *Diagn Microbiol Infect Dis* 1998;**32**:229–35.
17. Chaplow R, Palmer B, Heyderman R, et al. *Stenotrophomonas maltophilia* bacteraemia in 40 haematology patients: risk factors, therapy and outcome. *Bone Marrow Transplant* 2010;**45**:1109–10.
18. Chen KJ, Wang NK, Sun MH, et al. Endophthalmitis caused by *Stenotrophomonas maltophilia*. *Ophthalmic Surg Lasers Imaging* 2010;**22**:e555–61.
19. Chen S, Stroh EM, Wald K, Jalkh A. *Xanthomonas maltophilia* endophthalmitis after implantation of sustained-release ganciclovir. *Am J Ophthalmol* 1992;**114**:772–3.
20. Cheng AF, Li MK, Ling TK, French GL. Emergence of ofloxacin-resistant *Citrobacter freundii* and *Pseudomonas maltophilia* after ofloxacin therapy. *J Antimicrob Chemother* 1987;**20**:283–5.
21. Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement. CLSI document M100-S21*. Wayne, Pa: CLSI; 2011.
22. Crossman LC, Gould VC, Dow JM, et al. The complete genome, comparative and functional analysis of *Stenotrophomonas maltophilia* reveals an organism heavily shielded by drug resistance determinants. *Genome Biol* 2008;**9**:R74.
23. Daley AJ, McIntyre PB. *Stenotrophomonas maltophilia* and lawn mower injuries in children. *J Trauma* 2000;**48**:536–7.
24. Dapena F, Selgas R, Garcia-Perea A, et al. Clinical significance of exit-site infections due to *Xanthomonas* in CAPD patients: a comparison with *Pseudomonas* infection. *Nephrol Dial Transplant* 1994;**9**:1774–7.
25. de Oliveira-Garcia D, Dall'Agnol M, Rosales M, et al. Fimbriae and adherence of *Stenotrophomonas maltophilia* to epithelial cells and to abiotic surfaces. *Cell Microbiol* 2003;**5**:625–36.
26. de Vrankrijker AMM, Wolfs TFW, van der Ent CK. Challenging and emerging pathogens in cystic fibrosis. *Paediatr Respir Rev* 2010;**11**:246–54.
27. del Toro MD, Rodríguez-Bano J, Herrero M, et al. Clinical epidemiology of *Stenotrophomonas maltophilia* colonization and infection: a multicenter study. *Medicine (Baltimore)* 2002;**81**:228–39.
28. Demko CA, Stern RC, Doershuk CF. *Stenotrophomonas maltophilia* in cystic fibrosis: incidence and prevalence. *Pediatr Pulmonol* 1998;**25**:304–8.
29. Denton M, Kerr KG. Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. *Clin Microbiol Rev* 1998;**11**:57–80.
30. Denton M, Rajgopal A, Mooney L, et al. *Stenotrophomonas maltophilia* contamination of nebulizers used to deliver aerosolized therapy to inpatients with cystic fibrosis. *J Hosp Infect* 2003;**55**: 180–3.
31. Denton M, Todd NJ, Kerr KG, et al. Molecular epidemiology of *Stenotrophomonas maltophilia* isolated from clinical specimens from patients with cystic fibrosis and associated environmental samples. *J Clin Microbiol* 1998;**36**:1953–8.
32. Denton M, Todd NJ, Littlewood JM. Role of anti-pseudomonal antibiotics in the emergence of *Stenotrophomonas maltophilia* in cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 1996;**15**: 402–5.
33. Dyte PH, Gillians JA. *Pseudomonas maltophilia* infection in an abattoir worker. *Med J Aust* 1977;**1**:444–5.
34. Elsner HA, Duhrsen U, Hollwitz B, et al. Fatal pulmonary hemorrhage in patients with acute leukemia and fulminant pneumonia caused by *Stenotrophomonas maltophilia*. *Ann Hematol* 1997;**74**: 155–61.
35. Elting LS, Bodey GP. Septicemia due to *Xanthomonas* species and non-*aeruginosa Pseudomonas* species: increasing incidence of catheter-related infections. *Medicine (Baltimore)* 1990;**69**:296–306.
36. Elting LS, Khardori N, Bodey GP, Fainstein V. Nosocomial infection caused by *Xanthomonas maltophilia*: a case-control study of predisposing factors. *Infect Control Hosp Epidemiol* 1990;**11**: 134–8.
37. Elvers KT, Leeming K, Moore CP, Lappin-Scott HM. Bacterial-fungal biofilms in flowing water photo-processing tanks. *J Appl Microbiol* 1998;**84**:607–18.
38. Entenza JM, Moreillon P. Tigecycline in combination with other antimicrobials: a review of in vitro, animal and case report studies. *Int J Antimicrob Agents* 2009;**34**:8.e1–9.
39. Farrell DJ, Sader HS, Jones RN. Antimicrobial susceptibilities of a worldwide collection of *Stenotrophomonas maltophilia* isolates tested against tigecycline and agents commonly used for *S. maltophilia* infections. *Antimicrob Agents Chemother* 2010;**54**:2735–7.
40. Fedler KA, Biedenbach DJ, Jones RN. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. *Diagn Microbiol Infect Dis* 2006;**56**: 427–36.
41. Figueiredo PM, Furumura MT, Santos AM, et al. Cytotoxic activity of clinical *Stenotrophomonas maltophilia*. *Lett Appl Microbiol* 2006;**43**:443–9.
42. Fujita J, Yamadori I, Xu G, et al. Clinical features of *Stenotrophomonas maltophilia* pneumonia in immunocompromised patients. *Respir Med* 1996;**90**:35–8.
43. Gales AC, Jones RN, Forward KR, et al. Emerging importance of multidrug-resistant *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in seriously ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance Program (1997-1999). *Clin Infect Dis* 2001;**32**(Suppl. 2):104–13.
44. Garazi M, Singer C, Tai J, Ginocchio CC. Bloodstream infections caused by *Stenotrophomonas maltophilia:* a seven-year review. *J Hosp Infect* 2012;**81**:114–18.

45. Garcia de Viedma D, Marin M, Cercenado E, et al. Evidence of nosocomial *Stenotrophomonas maltophilia* cross-infection in a neonatology unit analyzed by three molecular typing methods. *Infect Control Hosp Epidemiol* 1999;**20**:816–20.
46. Gardner P, Griffin WB, Swartz MN, et al. Nonfermentative gram negative bacilli of nosocomial interest. *Am J Med* 1970;**48**:735–49.
47. Garrison MW, Anderson DE, Campbell DM, et al. *Stenotrophomonas maltophilia*: emergence of multidrug-resistant strains during therapy and in an in vitro pharmacodynamic chamber model. *Antimicrob Agents Chemother* 1996;**40**:2859–64.
48. Gasparetto EL, Bertholdo DB, Davaus T, et al. *Stenotrophomonas maltophilia* pneumonia after bone marrow transplantation: case report with emphasis on the high-resolution CT findings. *Br J Radiol* 2007;**80**:e19–e20.
49. Gilligan PH, Whittier S. Burkholderia, Stenotrophomonas, Ralstonia, Brevundimonas, Comamonas, Acidovorax. In: Murray PR, Baron EJ, Pfaller MA, et al, editors. *Manual of Clinical Microbiology*. 7th ed. Washington, D.C.: American Society for Microbiology; 1999. p. 526–38.
50. Goncalves-Vidigal P, Grosse-Onnebrink J, Mellies U, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: improved detection by the use of selective agar and evaluation of antimicrobial resistance. *J Cyst Fibros* 2011;**10**:422–7.
51. Gordon G, Indeck M, Bross J, et al. Injury from silage wagon accident complicated by mucormycosis. *J Trauma* 1988;**28**: 866–7.
52. Goss CH, Mayer-Hamblett N, Aitken ML, et al. Association between *Stenotrophomonas maltophilia* and lung function in cystic fibrosis. *Thorax* 2004;**59**:955–9.
53. Goss CH, Otto K, Aitken ML, et al. Detecting *Stenotrophomonas maltophilia* does not reduce survival of patients with cystic fibrosis. *Am J Respir Crit Care Med* 2002;**166**:356–61.
54. Gulcan H, Kuzucu C, Durmaz R. Nosocomial *Stenotrophomonas maltophilia* cross-infection: three cases in newborns. *Am J Infect Control* 2004;**32**:365–8.
55. Gülmez D, Cakar A, Sener B, et al. Comparison of different antimicrobial susceptibility testing methods for *Stenotrophomonas maltophilia* and results of synergy testing. *J Infect Chemother* 2010;**16**:322–8.
56. Gunnarsson G, Steinsson K. Sinusitis due to *Stenotrophomonas maltophilia*. *Scand J Infect Dis* 2001;**34**:136–7.
57. Gutierrez Rodero F, Masia MM, Cortes J, et al. Endocarditis caused by *Stenotrophomonas maltophilia*: case report and review. *Clin Infect Dis* 1996;**23**:1261–5.
58. Harlowe HD. Acute mastoiditis following *Pseudomonas maltophilia* infection: case report. *Laryngoscope* 1972;**82**:882–3.
59. Horio N, Horiguchi M, Murakami K, et al. *Stenotrophomonas maltophilia* endophthalmitis after intraocular lens implantation. *Graefes Arch Clin Exp Ophthalmol* 2000;**238**:299–301.
60. Horster S, Bader L, Seybold U, et al. Stenotrophomonas maltophilia induced post-cataract-surgery endophthalmitis: outbreak investigation and clinical courses of 26 patients. *Infection* 2009; **37**:117–22.
61. Insa R, Cercenado E, Goyanes MJ, et al. In vitro activity of tigecycline against clinical isolates of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 2007;**59**: 583–5.
62. Jacquier H, Le Monnier A, Carbonnelle E, et al. In vitro antimicrobial activity of "last-resort" antibiotics against unusual nonfermenting gram-negative bacilli clinical isolates. *Microb Drug Resist* 2012;**18**:396–401.
63. Jones RN, Sader HS, Beach ML. Contemporary in vitro spectrum of activity summary for antimicrobial agents tested against 18569 strains non-fermentative gram-negative bacilli isolated in the SENTRY Antimicrobial Surveillance Program (1997-2001). *Int J Antimicrob Agents* 2003;**22**:551–6.
64. Kagen J, ZaoutisTE, McGowa KL, et al. Bloodstream infection caused by *Stenotrophomonas maltophilia* in children. *Pediatr Infect Dis J* 2007;**26**:508–12.
65. Kaiser GM, Tso PC, Morris R, McCurdy D. *Xanthomonas maltophilia* endophthalmitis after cataract extraction. *Am J Ophthalmol* 1997;**123**:410–11.
66. Kanj SS, Tapson V, Davis RD, et al. Infections in patients with cystic fibrosis following lung transplantation. *Chest* 1997;**112**: 924–30.
67. Karpati F, Malmborg AS, Alfredsson H, et al. Bacterial colonisation with *Xanthomonas maltophilia:* a retrospective study in a cystic fibrosis patient population. *Infection* 1994;**22**:258–63.
68. Kerr KG, Denton M, Todd N, et al. A new selective differential medium for isolation of *Stenotrophomonas maltophilia*. *Eur J Clin Microbiol Infect Dis* 1996;**15**:607–10.
69. Khardori N, Elting L, Wong E, et al. Nosocomial infections due to *Xanthomonas maltophilia (Pseudomonas maltophilia)* in patients with cancer. *Rev Infect Dis* 1990;**12**:997–1003.
70. Krcmery V Jr, Pichna P, Oravcova E, et al. *Stenotrophomonas maltophilia* bacteraemia in cancer patients: report on 31 cases. *J Hosp Infect* 1996;**34**:75–7.
71. Lai CH, Wong WW, Chin C, et al. Central venous catheter-related *Stenotrophomonas maltophilia* bacteraemia and associated relapsing bacteraemia in haematology and oncology patients. *Clin Microbiol Infect* 2006;**12**:986–91.
72. Landrum ML, Conger NG, Forgione MA. Trimethoprim-sulfamethoxazole in the treatment of *Stenotrophomonas maltophilia* osteomyelitis. *Clin Infect Dis* 2005;**40**:1551–2.
73. Lanotte P, Cantagrel S, Mereghetti L, et al. Spread of *Stenotrophomonas maltophilia* colonization in a pediatric intensive care unit detected by monitoring tracheal bacterial carriage and molecular typing. *Clin Microbiol Infect* 2003;**9**:1142–7.
74. Lanzafame A, Bonfiglio G, Santini L, et al. In vitro activity of levofloxacin against recent gram-negative nosocomial pathogens. *Chemotherapy* 2005;**51**:44–50.
75. Leung C, Drew P, Azzopardi EA. Extended multidrug-resistant *Stenotrophomonas maltophilia* septicemia in a severely burnt patient. *J Burn Care Res* 2010;**31**:966.
76. Livermore DM, Hope R, Brick G, et al. Non-susceptibility trends among *Pseudomonas aeruginosa* and other non-fermentative Gram-negative bacteria from bacteraemias in the UK and Ireland, 2001-06. *J Antimicrob Chemother* 2008;**62**(Suppl. 2):ii55–63.
77. Lo WT, Wang CC, Lee CM, et al. Successful treatment of multiresistant *Stenotrophomonas maltophilia* meningitis with ciprofloxacin in a preterm infant. *Eur J Pediatr* 2002;**161**:680–2.
78. Maningo E, Watanakunakorn C. *Xanthomonas maltophilia* and *Pseudomonas cepacia* in lower respiratory tracts of patients in critical care units. *J Infect* 1995;**31**:89–92.
79. Marchac V, Equi A, Le Bihan-Benjamin C, et al. Case-control study of *Stenotrophomonas maltophilia* acquisition in cystic fibrosis patients. *Eur Respir J* 2004;**23**:98–102.
80. Marko DC, Saffert RT, Cunningham SA, et al. Evaluation of the Bruker Biotyper and VITEK® MS Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry Systems for the identification of nonfermenting gram-negative bacilli isolated from cystic fibrosis patient cultures. *J Clin Microbiol* 2012;**50**:2034–9.
81. Marshall WF, Keating MR, Anhalt JP, Steckelberg JM. *Xanthomonas maltophilia*: an emerging nosocomial pathogen. *Mayo Clin Proc* 1989;**64**:1097–104.
82. Metan G, Hayran M, Hascelik G, et al. Which patient is a candidate for empirical therapy against *Stenotrophomonas maltophilia* bacteraemia? An analysis of associated risk factors in a tertiary care hospital. *Scand J Infect Dis* 2006;**38**:527–31.
83. Micozzi A, Venditti M, Monaco M, et al. Bacteremia due to *Stenotrophomonas maltophilia* in patients with hematologic malignancies. *Clin Infect Dis* 2000;**31**:705–11.
84. Miyairi I, Franklin JA, Andreansky M, et al. Acute necrotizing ulcerative gingivitis and bacteremia caused by *Stenotrophomonas maltophilia* in an immunocompromised host. *Pediatr Infect Dis* 2005;**24**:181–3.
85. Morrison AJ Jr, Hoffmann KK, Wenzel RP. Associated mortality and clinical characteristics of nosocomial *Pseudomonas maltophilia* in a university hospital. *J Clin Microbiol* 1986;**24**:52–5.
86. Muder RR, Harris AP, Muller S, et al. Bacteremia due to *Stenotrophomonas (Xanthomonas) maltophilia:* a prospective, multicenter study of 91 episodes. *Clin Infect Dis* 1996;**22**:508–12.
87. Munter RG, Yinnon AM, Schlesinger Y, Hershko C. Infective endocarditis due to *Stenotrophomonas (Xanthomonas) maltophilia*. *Eur J Clin Microbiol Infect Dis* 1998;**17**:353–6.
88. Nakamura A, Sugimoto Y, Ohishi K, et al. Diagnostic value of PCR analysis of bacteria and fungi from blood in empiric-therapy-resistant febrile neutropenia. *J Clin Microbiol* 2010;**48**:2030–6.
89. Nguyen MH, Muder RR. Meningitis due to *Xanthomonas maltophilia:* case report and review. *Clin Infect Dis* 1994;**19**:325–6.

90. Nseir S, Di Pompeo C, Brisson H, et al. Intensive care unit-acquired *Stenotrophomonas maltophilia*: incidence, risk factors, and outcome. *Crit Care* 2006;**10**:1–9.
91. Nseir S, Di Pompeo C, Cavestri B, et al. Multiple-drug-resistant bacteria in patients with severe acute exacerbation of chronic obstructive pulmonary disease: prevalence, risk factors, and outcome. *Crit Care Med* 2006;**34**:2959–66.
92. Oie S, Kamiya A. Microbial contamination of brushes used for preoperative shaving. *J Hosp Infect* 1992;**21**:103–10.
93. Palleroni NJ, Bradbury JF. *Stenotrophomonas*, a new bacterial genus for *Xanthomonas maltophilia* (Hugh 1980) Swings et al. 1983. *Int J Syst Bacteriol* 1993;**43**:606–9.
94. Papadakis KA, Vartivarian SE, Vassilaki ME, Anaissie EJ. *Stenotrophomonas maltophilia:* an unusual cause of biliary sepsis. *Clin Infect Dis* 1995;**21**:1032–4.
95. Papadakis KA, Vartivarian SE, Vassilaki ME, Anaissie EJ. Septic prepatellar bursitis caused by *Stenotrophomonas (Xanthomonas) maltophilia*. *Clin Infect Dis* 1996;**22**:388–9.
96. Papadakis KA, Vartivarian SE, Vassilaki ME, Anaissie EJ. *Stenotrophomonas maltophilia* meningitis: report of two cases and review of the literature. *J Neurosurg* 1997;**87**:106–8.
97. Penland RL, Wilhelmus KR. *Stenotrophomonas maltophilia* ocular infections. *Arch Ophthalmol* 1996;**114**:433–6.
98. Petri A, Tiszlavicz L, Nagy E, et al. Liver abscess caused by *Stenotrophomonas maltophilia:* report of a case. *Surg Today* 2003;**33**:224–8.
99. Ramos-Esteban JC, Jeng BH. Posttraumatic *Stenotrophomonas maltophilia* infectious scleritis. *Cornea* 2008;**27**:232–5.
100. Razvi S, Quittell L, Sewall A, et al. Respiratory microbiology of patients with cystic fibrosis in the United States, 1995 to 2005. *Chest* 2009;**136**:1554–60.
101. Rogues AM, Maugein J, Allery A, et al. Electronic ventilator temperature sensors as a potential source of respiratory tract colonization with *Stenotrophomonas maltophilia*. *J Hosp Infect* 2001;**49**:289–92.
102. Sakhnini E, Weissmann A, Oren I. Fulminant *Stenotrophomonas maltophilia* soft tissue infection in immunocompromised patients: an outbreak transmitted via tap water. *Am J Med Sci* 2002;**323**:269–72.
103. Sarvamangala Devi JN, Venkatesh A, Shivananda PG. Neonatal infections due to *Pseudomonas maltophilia*. *Indian Pediatr* 1984;**21**:72–4.
104. Sattler CA, Mason EO Jr, Kaplan SL. Nonrespiratory *Stenotrophomonas maltophilia* infection at a children's hospital. *Clin Infect Dis* 2000;**31**:1321–30.
105. Semel JD, Trenholme GM, Harris AA, et al. *Pseudomonas maltophilia* pseudosepticemia. *Am J Med* 1978;**64**:403–6.
106. Szeto CC, Li PK, Leung CB, et al. *Xanthomonas maltophilia* peritonitis in uremic patients receiving continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1997;**29**:91–5.
107. Talmaciu I, Varlotta L, Mortensen J, Schidlow DV. Risk factors for emergence of *Stenotrophomonas maltophilia* in cystic fibrosis. *Pediatr Pulmonol* 2000;**30**:10–15.
108. Teo WY, Chan MY, Lam CM, et al. Skin manifestation of *Stenotrophomonas maltophilia* infection: a case report and review article. *Ann Acad Med Singapore* 2006;**35**:897–900.
109. Toleman MA, Bennett PM, Bennett DM, et al. Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of *sul* genes. *Emerg Infect Dis* 2007;**13**:559–65.
110. Tsai WP, Chen CL, Ko WC, Pan SC. *Stenotrophomonas maltophilia* bacteremia in burn patients. *Burns* 2006;**32**:155–8.
111. Valdezate S, Vindel A, Maiz L, et al. Persistence and variability of *Stenotrophomonas maltophilia* in cystic fibrosis patients, Madrid, 1991-1998. *Emerg Infect Dis* 2001;**7**:113–22.
112. Van Couwenberghe CJ, Farver TB, Cohen SH. Risk factors associated with isolation of *Stenotrophomonas (Xanthomonas) maltophilia* in clinical specimens. *Infect Control Hosp Epidemiol* 1997;**18**:316–21.
113. Vartivarian S, Anaissie E, Bodey G, et al. A changing pattern of susceptibility of *Xanthomonas maltophilia* to antimicrobial agents: implications for therapy. *Antimicrob Agents Chemother* 1994;**38**:624–7.
114. Vartivarian SE, Papadakis KA, Anaissie EJ. *Stenotrophomonas (Xanthomonas) maltophilia* urinary tract infection: a disease that is usually severe and complicated. *Arch Intern Med* 1996;**156**:433–5.
115. Vartivarian SE, Papadakis KA, Palacios JA, et al. Mucocutaneous and soft tissue infections caused by *Xanthomonas maltophilia:* a new spectrum. *Ann Intern Med* 1994;**121**:969–73.
116. Victor MA, Arpi M, Bruun B, et al. *Xanthomonas maltophilia* bacteremia in immunocompromised hematological patients. *Scand J Infect Dis* 1994;**26**:163–70.
117. Villarino ME, Stevens LE, Schable B, et al. Risk factors for epidemic *Xanthomonas maltophilia* infection/colonization in intensive care unit patients. *Infect Control Hosp Epidemiol* 1992;**13**:201–6.
118. Waters V, Ratjen F. Multidrug-resistant organisms in cystic fibrosis: management and infection-control issues. *Expert Rev Anti Infect Ther* 2006;**4**:807–19.
119. Waters V, Atenafu EG, Salazar JG, et al. Chronic *Stenotrophomonas maltophilia* infection and exacerbation outcomes in cystic fibrosis. *J Cyst Fibros* 2012;**11**:8–13.
120. Waters V, Yau Y, Prasad S, et al. *Stenotrophomonas maltophilia* in cystic fibrosis: serologic response and effect on lung disease. *Am J Respir Crit Care Med* 2011;**183**:635–40.
121. Weber DJ, Rutala WA, Blanchet CN, et al. Faucet aerators: a source of patient colonization with *Stenotrophomonas maltophilia*. *Am J Infect Control* 1999;**27**:59–63.
122. Weiss K, Restieri C, De Carolis E, et al. Comparative activity of new quinolones against 326 clinical isolates of *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* 2000;**45**:363–5.
123. Whitby PW, Carter KB, Burns JL, et al. Identification and detection of *Stenotrophomonas maltophilia* by rRNA-directed PCR. *J Clin Microbiol* 2000;**38**:4305–9.
124. Wishart MM, Riley TV. Infection with *Pseudomonas maltophilia* hospital outbreak due to contaminated disinfectant. *Med J Aust* 1976;**2**:710–12.
125. Wu PS, Lu CY, Chang LY, et al. *Stenotrophomonas maltophilia* bacteremia in pediatric patients: A 10-year analysis. *J Microbiol Immunol Infect* 2006;**39**:144–9.
126. Yeshurun M, Gafter-Gvili A, Thaler M, et al. Clinical characteristics of *Stenotrophomonas maltophilia* infection in hematopoietic stem cell transplantation recipients: a single center experience. *Infection* 2010;**38**:211–15.
127. Yu VL, Rumans LW, Wing EJ, et al. *Pseudomonas maltophilia* causing heroin-associated infective endocarditis. *Arch Intern Med* 1978;**138**:1667–71.
128. Zelenitsky SA, Iacovides H, Ariano RE, et al. Antibiotic combinations significantly more active than monotherapy in an in vitro infection model of *Stenotrophomonas maltophilia*. *Diagn Microbiol Infect Dis* 2005;**51**:39–43.
129. Zhang L, Li XZ, Poole K. Multiple antibiotic resistance in *Stenotrophomonas maltophilia*: involvement of a multidrug efflux system. *Antimicrob Agents Chemother* 2000;**44**:287–93.

SUBSECTION 5
GRAM-NEGATIVE COCCOBACILLI

CHAPTER 127

AGGREGATIBACTER ACTINOMYCETEMCOMITANS

Suzanne Whitworth • Richard F. Jacobs

Aggregatibacter actinomycetemcomitans, formerly *Actinobacillus actinomycetemcomitans*, is a fastidious, non–spore-forming, nonmotile, facultatively anaerobic gram-negative coccobacillus that frequently complicates actinomycosis caused by *Actinomyces israelii*. In addition to being associated with actinomycosis, it has been implicated as a pathogen in periodontal disease and is part of the oral flora. Infection by this bacterium often is not resolved by the normal host, possibly because of inefficient phagocytosis and the weak oxidative burst response of neutrophils.[10] In addition, the bacterium has several virulence factors, including induction of apoptosis and tissue destruction.[5] *A. actinomycetemcomitans* is characterized by slow growth in culture and a requirement for incubation in an atmosphere enhanced with carbon dioxide.[7] Other bacterial species concomitantly isolated in human actinomycosis are *Eikenella corrodens*, *Fusobacterium*, *Bacteroides*, *Capnocytophaga*, *Staphylococcus*, *Streptococcus*, and members of the family Enterobacteriaceae.

A. actinomycetemcomitans is a pathogen in at least 30 percent of actinomycotic infections.[7] Failure to recognize this organism and treat it adequately has resulted in clinical relapse and deterioration in patients infected with actinomycosis.[8,16] Severe forms of periodontitis, particularly localized aggressive periodontitis, also are associated with this pathogen, and studies have shown that it is related strongly to children in the pubertal age group.[1,12] It is characterized by loss of attachment of bone around the permanent incisors and permanent molars, which occurs rapidly. Treatment consists of local débridement and antibiotic therapy, usually metronidazole alone or in combination with amoxicillin. *A. actinomycetemcomitans* also is an important pathogen in Papillon-Lefèvre syndrome, an autosomal recessive disorder characterized by prepubertal periodontitis and palmar-plantar hyperkeratosis.[11] Additionally, it is one of the HACEK (HACEK includes *Haemophilus aphrophilus*, *Cardiobacterium hominis*, *E. corrodens*, and *Kingella kingae*) organisms that have a propensity for infecting heart valves. The endocarditis caused by this organism usually is insidious, with fever occurring in fewer than 50 percent of cases.[7] This organism has been reported to cause pericarditis, meningitis, brain abscess, parotitis, synovitis, osteomyelitis, urinary tract infection, pneumonia, and empyema.[7] Cases of endophthalmitis[2,14] and cavernous sinus infection also have been reported, as well as facial cellulitis.[6,15] "In recent years there has been increasing evidence of the potential association between periodontal and cardiovascular diseases. Periodontitis may be an independent risk factor for cardiovascular disease. A recent study revealed the presence of *A. actinomycetemcomitans* by PCR in atheromatous plaques obtained from carotid endarterectomies."[4]

A. actinomycetemcomitans can be cultured on blood and chocolate agar but grows poorly on MacConkey agar. Cultures require incubation in an enhanced carbon dioxide atmosphere. Growth of the organism in a blood culture may take as long as 9 days in patients with endocarditis, and thus cultures should be held longer. On Gram stain, the organism appears coccoid to coccobacillary. Molecular techniques based on nonamplification nucleic acid probes or on polymerase chain reaction can provide rapid and accurate identification of *A. actinomycetemcomitans*.[13] Because of the frequency of coinfection with this organism in cases of actinomycosis, attempts always should be made to isolate this organism in these patients.

A. actinomycetemcomitans is susceptible to the newer cephalosporins, rifampin, trimethoprim-sulfamethoxazole, aminoglycosides, quinolones, tetracycline, azithromycin, and chloramphenicol. It also is susceptible to penicillin and ampicillin in vitro, but test results do not necessarily correlate with clinical outcome. Vancomycin, erythromycin, and clindamycin have very little activity against this organism. Treatment of aggressive periodontal disease consists of local débridement and antibiotic therapy. Metronidazole and amoxicillin appear to be effective in suppressing *A. actinomycetemcomitans* to below the level of detection.[3] Endocarditis caused by this organism has been treated successfully with a combination of ampicillin and gentamicin.[9] Cefotaxime or ceftriaxone also is acceptable.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Alani A, Seymour R. Aggressive periodontitis: how does an understanding of the pathogenesis affect treatment? *Dent Update* 2011; **38**:511–21.
4. Figuero E, Sanchez-Beltran M, Cuesta-Frechoso S, et al. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol* 2011;**82**:1469–77.
6. Hsieh CJ, Hwang KP, Kuo KC, Hsueh PR. Facial cellulitis because of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* and *Capnocytophaga* species in an immunocompetent patient. *J Microbiol Immunol Infect* 2011;**44**:149–51.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 128

BRUCELLOSIS

Edward Young

Brucellosis is a disease of animals (zoonosis) that is transmittable to humans.[91] Humans are accidental hosts, and human-to-human transmission is rare.[106] Brucellosis is distributed worldwide, and it is estimated that 500,000 human cases occur annually.[38,94] Programs to eradicate the disease in animals have reduced the incidence of human infection in many countries; however, brucellosis remains enzootic in large parts of the world.[144] Once considered rare in childhood, it is now recognized that persons of all ages are susceptible, especially in areas where *Brucella melitensis* is endemic.[128]

HISTORY

Brucellosis was one of many indistinguishable fevers until 1859 when J.A. Marston, a Royal Army Medical Corps (RAMC) physician, provided the first accurate description of the disease among troops stationed on Malta during the Crimean War. He also noted that "…when this form of fever attacks children, (which it frequently does), it is apt to leave behind it an enlarged condition of the spleen, difficult to remove."[76] During the 19th century, brucellosis was known by various names, including Mediterranean fever, Malta fever, and undulant fever.[87] Although the disease caused considerable morbidity and mortality in British military personnel stationed throughout the Mediterranean, the cause was not immediately apparent. In 1886, David Bruce, another RAMC surgeon, isolated a microorganism from spleen tissue of victims of Malta fever.[22] Termed *Micrococcus* (later renamed *Brucella*) *melitensis*, the organism could be found in the blood, urine, and feces of Malta fever patients. Between 1904 and 1907, Bruce headed the Mediterranean Fever Commission that studied aspects of the disease in Malta. It was Themistocles Zammit, a Maltese physician working with the Commission, who first identified native goats as the reservoir of brucellosis and their unpasteurized milk as the vehicle of transmission to humans.[135] When fresh goat's milk was replaced with tinned condensed milk in the military mess, the incidence of brucellosis among military personnel declined precipitously.[122] In 1897, Almroth Wright applied the newly devised agglutination assay to the serologic diagnosis of Malta fever.[139]

Unlike *B. melitensis* that was initially isolated from human tissue, other *Brucella* species were recognized for the disease they caused in animals. In 1897, Bernhard Bang, a Danish veterinarian, isolated the "abortion bacillus" (later *Brucella abortus*) from placental tissue of cattle suffering contagious abortions.[18] *Brucella suis* was isolated from swine around 1914 by Jacob Traum at the Bureau of Animal Industry.[60] In 1918, the bacteriologist Alice Evans finally recognized the relatedness of these disparate bacteria.[48] After Evan's work was confirmed, K.F. Meyer and E.B. Shaw proposed the name *Brucella* for the genus to honor Bruce.[57]

Additional *Brucella* species were isolated from sheep *(B. ovis)* and from desert wood rats *(B. neotomae)*, but neither has been linked to disease in humans. In 1966, Carmichael[26] identified *Brucella canis* as a cause of abortions in kennel-bred dogs; however, it appears to be a rare cause of infection in humans.[136] Recently, previously unrecognized *Brucella* species have been recovered from marine mammals.[21,55,62] Tentatively named *Brucella cetaceae* (dolphin and porpoise isolates) and *Brucella pinnepediae* (seal isolates), their role as human pathogens remains to be clarified.[31,77,116] Another novel strain of *Brucella* was recently isolated from a woman with an infected breast implant and was tentatively named *Brucella inopinata*.[40,137]

REFERENCES

1. Alani A, Seymour R. Aggressive periodontitis: how does an understanding of the pathogenesis affect treatment? *Dent Update* 2011;**38**: 511–21.
2. Binder MI, Chua J, Kaiser PK, et al. *Actinobacillus actinomycetemcomitans* endogenous endophthalmitis: report of two cases and review of the literature. *Scand J Infect Dis* 2003;**35**:133–6.
3. Dorfer CE. Antimicrobials for the treatment of aggressive periodontitis. *Oral Dis* 2003;**9**(Suppl. 1):51–3.
4. Figuero E, Sanchez-Beltran M, Cuesta-Frechoso S, et al. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol* 2011;**82**:1469–77.
5. Henderson B, Wilson M, Sharp L, Ward JM. Actinobacillus actinomycetemcomitans. *J Med Microbiol* 2002;**51**:1013–20.
6. Hsieh CJ, Hwang KP, Kuo KC, Hsueh PR. Facial cellulitis because of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* and *Capnocytophaga* species in an immunocompetent patient: case report. *J Microbiol Immunol Infect* 2011;**44**:149–51.
7. McGowan JE, Steinberg JP. Other gram-negative bacilli. In: Mandell GL, Bennett JE, Dolin R, editors: *Principles and practices of infectious disease*. New York: Churchill Livingstone; 1995. p. 2106–7.
8. Morris JF, Sewell DL. Necrotizing pneumonia caused by mixed infection with *Actinobacillus actinomycetemcomitans* and *Actinomyces israelii:* case report and review. *Clin Infect Dis* 1994;**18**:450–2.
9. Paturel L, Casalta JP, Habib G, et al. *Actinobacillus actinomycetemcomitans* endocarditis. *Clin Microbiol Infect* 2004;**10**:98–118.
10. Permpanich P, Kowolik MJ, Galli DM. Resistance of fluorescent-labelled *Actinobacillus actinomycetemcomitans* strains to phagocytosis and killing by human neutrophils. *Cell Microbiol* 2006;**8**:72–84.
11. Rudiger S, Petersilka G, Flemmig TF. Combined systemic and local antimicrobial therapy of periodontal disease in Papillon-Lefevre syndrome. *J Clin Periodontol* 1999;**26**:847–54.
12. Savitt ED, Kent RL. Distribution of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J Periodontol* 1991;**62**:490–4.
13. Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease: introduction. *Periodontol* 2000 **20**:7–13, 1999.
14. Sullivan P, Clark WL, Kaiser PK. Bilateral endogenous endophthalmitis caused by HACEK microorganism. *Am J Ophthalmol* 2002;**133**:144–5.
15. Tobias S, Lee JH, Tomford JW. Rare *Actinobacillus* infection of the cavernous sinus causing painful ophthalmoplegia: case report. *Neurosurgery* 2002;**51**:807–9.
16. Tyrrell J, Noone P, Prichard JS. Thoracic actinomycosis complicated by *Actinobacillus actinomycetemcomitans:* case report and review of literature. *Respir Med* 1992;**86**:341–3.

ETIOLOGY

The brucellae are small, fastidious, nonmotile, non–spore-forming gram-negative coccobacilli that lack native plasmids. The outer cell membrane resembles other gram-negative bacilli with a dominant lipopolysaccharide (LPS) component. Their metabolism is oxidative, and all strains are aerobic. Some strains require carbon dioxide for growth, especially for primary isolation. Brucellae are always catalase positive, but oxidase activity varies among biovars. Most strains reduce nitrate to nitrite, but some do not. Production of hydrogen sulfide also varies, as does urease activity. A variety of media support the growth of brucellae, including chocolate, trypticase soy, and serum dextrose agars. Growth in vitro is characteristically slow and when brucellosis is suspected the cultures should not be discarded before 28 days.

Brucellae belong to the α2 subdivision of the Proteobacteria phylogenetically related to plant pathogens and symbionts such as *Agrobacterium* and *Rhizobium* and to intracellular animal parasites such as *Bartonella* and *Rickettsia*.[32] On the basis of DNA-DNA hybridization and genome sequencing, the genus *Brucella* comprises a single species.[41,58,95] Despite genetic homology greater than 90 percent, the *Brucella* nomenspecies show a remarkable preference for certain natural hosts. Consequently, the traditional nomenspecies classification is retained for epidemiologic purposes (Table 128-1).

EPIDEMIOLOGY

Historically in the United States brucellosis was linked to the livestock industry and *B. abortus* was the predominant species causing human illness. Farmers, ranchers, abattoir workers, and veterinarians were at highest risk for infection, usually through direct contact with infected animals. With the virtual elimination of bovine brucellosis by way of test-and-slaughter and immunization of susceptible cattle, the epidemiology of brucellosis changed, especially in states that border Mexico. *B. melitensis* replaced *B. abortus* as the major cause of human disease, and foodborne transmission replaced direct contact with animals as the primary mode of infection.[28,131] On the Mexican border, human brucellosis occurs at a rate eight times above the national average, and unpasteurized goat's milk cheese is a common vehicle of transmission.[46,144,149] Human infection caused by *B. suis* has traditionally been an abattoir-associated infection, but in recent years the hunting of feral swine has become a risk when hunters are contaminated with the blood of infected animals.[24,124] Personnel working in clinical or research laboratories are also at risk, and brucellosis continues to be the most commonly reported laboratory-associated bacterial infection.[102,140]

Brucellosis once was thought to be uncommon or mild in children, but susceptibility now is recognized in persons of all ages.[112-114] Conditions for the transmission of brucellosis from animals to humans vary among countries and cultures. Where farm animals traditionally are raised in the home, contact with children occurs frequently. Moreover, foodborne infection is not limited to any age or sex and can occur without direct contact with animals. Childhood brucellosis occurs more commonly in locations where *B. melitensis* is enzootic, and multiple cases often occur within families.[11,74,147]

The clinical manifestations of brucellosis in children do not differ from those in adults, although unfamiliarity with the disease can delay the diagnosis.[7,27,29,126] Human-to-human transmission of brucellosis is rare, but evidence for venereal transfer has been documented.[106,123] Rare cases associated with blood or bone marrow transplantation have been reported.[2,5,10] Interestingly, few cases of brucellosis have been reported in patients infected with the human immunodeficiency virus.[84]

Spontaneous abortion is a common manifestation of brucellosis in animals where brucellae localize within the reproductive organs of both sexes. Brucellosis in pregnant women can lead to abortion if unrecognized and untreated.[104] In a retrospective study from Saudi Arabia, intrauterine death and spontaneous abortion occurred in 46 percent of 92 women with brucellosis.[67] This rate was significantly higher than the rate of spontaneous abortion in women without brucellosis. When diagnosed early, pregnant women can be treated successfully without compromising their ability to carry to term or to conceive again.[68] On rare occasions, transplacental transmission can lead to neonatal brucellosis.[85]

TABLE 128-1 **Nomenspecies Classification of *Brucella* spp.**

Species	Biovars	Natural Hosts	Pathogenicity for Humans
B. abortus	1-6, 9	Cattle, other Bovidae	Yes
B. melitensis	1-3	Goats, sheep	Yes
B. suis	1-3	Swine	Yes
	4	Reindeer, caribou	Yes
	5	Rodents	Yes
B. canis	—	Dogs, other canines	Yes
B. neotomae	—	Desert wood rats	No
B. ovis	—	Sheep	No
*B. maris**			
*B. pinnipediae**		Pinnipeds	?
*B. cetaceae**		Cetaceans	?
*B. inopinata**		?	? (single isolate)

*Tentative designations.
?, still unresolved.

PATHOGENESIS

The incubation period for brucellosis varies, but symptoms generally appear within 2 to 3 weeks after infection. Disease caused by *B. melitensis* and *B. suis* tends to be

more severe than illness due to *B. abortus* or *B. canis*.[131] Morbidity can be considerable, but death occurs in only about 1 percent of cases, usually from complications such as neurobrucellosis or endocarditis.[145] Although no racial or genetic differences in susceptibility are known in humans, innate immunity has been demonstrated in various animals.[45]

The brucellae lack the usual virulence factors of other bacteria. Lipopolysaccharide (LPS), the immunedominant antigen of the cell wall, appears to be the principal mediator of pathogenesis of brucellosis.[25] Although 10 to 100 times less toxic than conventional enterobacterial endotoxins such as *Escherichia coli*, brucella LPS functions through the same Toll-like receptor 4 (TLR4) pathway and contributes to the organism's ability to survive and replicate within naïve mononuclear phagocytes.[71] By mechanisms not yet fully understood, the smooth LPS of the organism impairs host complement-mediated killing, blocks phagosome-lysosome fusion, impairs inflammatory cytokine expression, and inhibits the production of nitrogen intermediates to undergo a respiratory burst.[25] Although humoral antibodies play some role in resistance to infection, the principal mechanism of recovery involves cellular immunity.[115] Intracellular killing of brucellae occurs when macrophages become "activated" by specifically committed $CD4^+$ and $CD8^+$ T lymphocytes. This T-helper-1 (Th1) response involves cytokines, including interferon-gamma, interleukin-2, and tumor necrosis factor.[141]

CLINICAL MANIFESTATIONS

The spectrum of human brucellosis ranges from subclinical (diagnosed by serology) to chronic (characterized by recurrent symptoms over many years).[148] The disease is characterized by a plethora of nonspecific somatic complaints, such as fatigue, anorexia, nausea, weight loss, sweats, and depression. In contrast, there is a paucity of physical findings, notably fever and occasionally hepatosplenomegaly. The infection can involve any organ or organ system of the body. Sometimes symptoms related to a single organ predominate, in which the disease is termed *localized*.[35] Not unexpectedly, localization often involves organs rich in elements of the reticuloendothelial system.

Osteoarticular complications are the most frequent localized manifestations of brucellosis, and in children symptoms of monoarticular arthritis involving the hips, knees, ankles, and sacroiliac joint predominate.[19,128,130,152] Spondylitis, osteomyelitis, and inflammatory arthropathy have also been described but occur less often in children than adults.[36,118] Despite advances in imaging techniques, serology and culture remain important for diagnosis.[63]

Neurobrucellosis comprises a variety of nervous system complications, including meningitis/encephalitis, myelitis, radiculitis, peripheral and cranial neuropathies, and demyelinating syndromes.[51,56,78,109,133] Vasculitis is common,[150] and rare cases of brain abscess and intracerebral granuloma can mimic a neoplasm of the central nervous system.[47,110] Although the majority of patients complain of headache and weakness, direct invasion of the central nervous system occurs in less than 5 percent of cases.[20,86] Analysis of cerebrospinal fluid (CSF) in cases of *Brucella* meningitis reveals a lymphocytic pleocytosis, elevated protein levels, and normal or low glucose levels. Organisms are rarely seen on Gram stain or cultured from CSF; however, antibodies to *Brucella* are present and their finding in CSF is diagnostic.[12] When available, polymerase chain reaction (PCR) assay on CSF can also confirm the diagnosis.[34]

Gastrointestinal complaints are common in uncomplicated brucellosis and include anorexia, nausea, vomiting, abdominal discomfort, and weight loss.[83,113] Rare cases of ileitis,[97] colitis,[64,125] and peritonitis[3] have been reported. As the largest organ of the reticuloendothelial system, the liver is likely always involved in brucellosis even though transaminase levels are only mildly elevated and can be normal.[35,72] Rarely, acute hepatitis occurs with hepatic enzyme levels resembling viral hepatitis.[4] The liver histology in patients infected with *B. abortus* is characterized by epithelioid granulomas indistinguishable from sarcoidosis.[121] In contrast, infection with *B. melitensis* can result in a spectrum of lesions ranging from diffuse hepatitis to granulomas.[142,145] Infection with *B. suis* (and *B. melitensis*) can also result in a suppurative abscess that may become chronic.[17,37,134]

Genitourinary tract involvement usually presents as orchitis or epididymo-orchitis and can be mistaken for testicular cancer.[59,89] Rare cases of interstitial nephritis, membranous nephropathy, and glomerulonephritis also have been reported.[151]

Respiratory tract localization of brucellosis is reported in about 7 percent of cases.[90,93,129] Lung lesions attributed to *Brucella* include hilar adenopathy, lobar pneumonia, lung nodules, pleural effusion, and thoracic empyema.[73] Pleural fluid adenosine deaminase levels may be elevated but are difficult to distinguish from tuberculosis.[43]

Cardiovascular lesions of brucellosis include endocarditis, myocarditis, pericarditis, and aneurysms of the aorta and cerebral blood vessels.[100] Both native and prosthetic valve endocarditis have been described, with the aortic valve involved most often.[42,61] Delays in making a diagnosis can result in life-threatening complications, such as valve rupture, myocardial abscess, and sinus of Valsalva fistulas. Treatment of *Brucella* endocarditis generally requires the combination of antibiotics plus valve surgery.[66,111]

Ocular lesions, most notably uveitis, have been described in patients with brucellosis. Other eye lesions that have been reported include endophthalmitis, optic neuritis, episcleritis, nummular keratitis, and chorioretinitis. The pathogenesis of some of the lesions is a matter of conjecture because brucellae are rarely isolated from the eye.[65,103,127]

Cutaneous lesions attributed to brucellosis include contact dermatitis, rashes, abscess, and vasculitis.[81,82] On occasion, brucellae have been cultured from subcutaneous papules[74] and from abscesses.[101]

Hematologic abnormalities such as anemia, leukopenia, and thrombocytopenia are common in the course of brucellosis.[30,39] These changes are generally mild and

resolve promptly with antimicrobial therapy. On occasion, thrombocytopenia can be severe, resulting in hemorrhage into the skin and from mucosal sites.[150] The etiology of this complication is likely multifactorial, including hypersplenism, disseminated intravascular coagulation, bone marrow suppression and hemophagocytosis, and immune mechanisms.

DIAGNOSIS

The symptoms of brucellosis are nonspecific; therefore, the importance of obtaining a detailed history including occupation, avocations, travel, animal exposure, and food habits cannot be overemphasized. Routine laboratory tests are generally not helpful, but characteristic hematologic findings (normal or low white blood cell count) can suggest the possibility of brucellosis.[8]

A definitive diagnosis of brucellosis is made by isolating a specific species of *Brucella* from blood, bone marrow, or other tissue. The rate of positive blood cultures varies from 15 to 70 percent depending on the methods used and the period of in vitro incubation.[54,108] Bone marrow culture has been reported to be more sensitive than blood cultures prior to the introduction of newer isolation techniques.[44,80] Nucleic acid amplification tests using a variety of gene sequences are now being applied with some success to the rapid diagnosis of brucellosis.[6,53,88,99,138]

In the absence of bacteriologic confirmation, a presumptive diagnosis can be made by measuring the titer of specific antibodies in serum.[143] Human brucellosis is characterized by an initial production of IgM antibodies followed by a switch to IgG synthesis within the second week of infection. Treatment results in a gradual decline in both antibody isotypes; however, the persistence of IgG antibodies is associated with relapse or chronic infection.[96] Consequently, the pattern of immunoglobulin isotypes is important to differentiate active from treated disease.[50,143] This is accomplished by performing the serum agglutination test (SAT) in tandem with 2-mercaptoethanol (2ME) (or dithiothreitol DTT); the latter are disulfide-reducing agents used to block agglutinability by IgM while preserving agglutination by IgG.[23,143] Alternatively, a more direct way to measure IgM and IgG antibodies is the indirect enzyme-linked immunosorbent assay (ELISA) employing anti-IgM and anti-IgG conjugates.[75,79] Because of problems with commercial IgM enzyme assays, the Centers for Disease Control and Prevention recommends that positive ELISA results be confirmed by agglutination.

Most serologic tests for brucellosis employ smooth lipopolysaccharide (S-LPS) that is capable of detecting antibodies to all smooth species *(B. abortus*, *B. melitensis*, *B. suis)*. Because *B. canis* is naturally rough (lacks S-LPS), antibodies to this organism require a test using antigen prepared from a rough species *(B. canis* or *B. ovis)*.[98] In addition, a variety of other cell wall or cytoplasmic proteins have been studied as potential serodiagnostic antigens[52] but none has proven to be superior to LPS-based assays. Recently, a latex agglutination assay using S-LPS has been shown to be simple to run, to be rapid, and to have good sensitivity and specificity.[1]

TREATMENT

Antimicrobial therapy lessens morbidity, shortens the course of illness, and reduces the incidence of complications of brucellosis.[146] A variety of drugs are active against *Brucella* spp., but the results of in vitro sensitivity tests do not always correlate with clinical effectiveness. Because the rate of relapse with single-drug therapy is high, successful treatment requires combination therapy for a prolonged time.[119,146] The tetracyclines remain the most effective antibiotics against brucellae with minimal inhibitory concentrations less than 1 μg/mL. In vitro killing of brucellae by numerous antibiotics can be enhanced by the addition of an aminoglycoside (streptomycin or gentamicin).[107] Traditionally, the combination of tetracycline administered for 6 weeks plus streptomycin given for 2 to 3 weeks provided the highest cure rates in patients with brucellosis.[13] Currently, the preferred regimen is doxycycline 200 mg/day given orally for 45 days plus gentamicin 5 mg/kg/day as a single daily intramuscular dose for the first 7 to 10 days.[14,105,117]

Rifampin also shows good in vitro activity against *Brucella* spp., and in 1986 the World Health Organization recommended the combination of oral administration for 45 days of doxycycline 200 mg/day plus rifampin 600 to 900 mg/day as the treatment of choice. Although this regimen has been used successfully, subsequent studies have shown the superiority of the combination of doxycycline plus an aminoglycoside.[16,120]

Children older than 8 years can be treated similar to adults.[132] Because tetracycline is contraindicated for children younger than age 8 years and for pregnant women, alternative treatments have been sought. Trimethoprim-sulfamethoxazole (TMP/SMX, cotrimoxazole) in a fixed combination of 80 mg TMP/400 mg SMX twice daily for 45 days plus gentamicin 5 mg/kg/day as a single daily dose for 7 days has yielded satisfactory results.[113] Alternatively, the combination of TMP/SMX plus rifampin 15 mg/kg once daily administered for either 6 or 8 weeks gave comparable results.[105] Similarly, pregnant women can be treated successfully with TMP/SMX plus rifampin without adverse drug effects in the newborn.[67]

The quinolones are active against *Brucella* spp. in vitro, but the minimal inhibitory concentration values vary for each compound and, to some extent, for *Brucella* isolates[49]; in clinical practice, however, the results have been disappointing.[69,70,146] Consequently, quinolones are not recommended as monotherapy for treating brucellosis and their role in combination therapy awaits well-designed clinical trials.

The optimal treatment of complications of brucellosis such as meningitis and endocarditis has not been defined with certainty.[92] Doxycycline crosses the blood-brain barrier more effectively than generic tetracycline, and it has been used in combination with other drugs (e.g., rifampin, TMP/SMX) for neurobrucellosis.[78,86] Third-generation cephalosporins achieve high concentrations in CSF, but the sensitivity of brucellae varies and, in general, β-lactam drugs are not effective when used alone.[69] On occasion, patients with *Brucella* endocarditis have been treated successfully with the usual antibiotic

combination; however, most cases have required additional valve replacement surgery.[9]

RELAPSE AND CHRONIC BRUCELLOSIS

The vast majority of patients with brucellosis recover completely within a few weeks to months after receiving adequate therapy. Despite appropriate treatment, some patients suffer a relapse characterized by recurrence of symptoms and re-isolation of brucellae from their blood.[15] Obviously, relapse is more frequent if the full course of treatment is not completed, and taking oral antibiotics for 6 weeks can tax a patient's compliance. With few exceptions, relapse is *not* caused by antibiotic-resistant strains of *Brucella*.[13]

Even with appropriate treatment, some patients will experience a delayed recovery and will continue to have nonspecific complaints, notably fatigue. Such patients have no objective evidence of infection, such as fever, and their antibody titers will decline as expected. Whether this represents a variant of the chronic fatigue syndrome is not clear, but additional antibiotic therapy does not improve their recovery.[33] Rarely, chronic brucellosis occurs from a persisting focus of infection such as osteomyelitis or a deep tissue abscess. Such patients will have fever or other objective signs of infection, and levels of IgG antibodies in the serum remain elevated. Scanning techniques (e.g., scintigraphy with technetium-99m or gallium-67, computed tomography, or magnetic resonance imaging) can be useful in localizing an occult focus of infection.

NEW REFERENCES SINCE THE SIXTH EDITION

5. Al-Anazi KA, Abu Jafar S, Al-Jasser AM, et al. *Brucella* bacteremia in a recipient of an allogenic hematopoietic stem cell transplant: a case report. *Cases J* 2009;**2**:91.
27. Carrington M, Choe U, Ubillos S, et al. Fatal case of brucellosis misdiagnosed in early stage of *Brucella suis* infection in a 46-year-old patient with Marfan syndrome. *J Clin Microbiol* 2012;**50**:2173–5.
30. Citak EC, Citak FE, Tanyeri B, et al. Hematologic manifestations of brucellosis in children: 5 years experience of an Anatolian center. *J Pediatr Hematol Oncol* 2010;**32**:137–40.
36. Colmenero JD, Ruiz-Mesa JD, Plata A, et al. Clinical findings, therapeutic approach, and outcome of brucellar vertebral osteomyelitis. *Clin Infect Dis* 2008;**46**:426–33.
40. De BK, Stauffer L, Koylass MS, et al. Novel *Brucella* strain (BO1) associated with a prosthetic breast implant infection. *J Clin Microbiol* 2008;**46**:43–9.
47. Erdem M, Namiduru M, Karaoglan I, et al. Unusual presentation of neurobrucellosis: a solitary intracranial mass lesion mimicking a cerebral tumor: a case of encephalitis caused by *Brucella melitensis*. *J Infect Chemother* 2012;**18**(5):767–70.
53. Gopaul KK, Koylass MS, Smith C, et al. Rapid identification of *Brucella* isolates to the species level by real time PCR based single nucleotide polymorphism analysis. *BMC Microbiol* 2008;**8**:86.
59. Hasanjani Roushan MR, Baiani M, Javanian M, et al. Brucellar epididymo-orchitis: review of 53 cases in Babol, northern Iran. Scand. *J Infect Dis* 2009;**41**:440–4.
61. Inan MB, Eyileten ZB, Ozcinar E, et al. Native valve *Brucella* endocarditis. *Clin Cardiol* 2010;**33**:e20–e26.
63. Janmohammadi N, Hasanjani Roushan MR. False negative serological tests may lead to misdiagnosis and mismanagement in osteoarticular brucellosis. *Trop Doctor* 2009;**39**:88–90.
68. Kurdoglu M, Adali E, Kurdoglu Z, et al. Brucellosis in pregnancy: a 6-year clinical analysis. *Arch Gynecol Obstet* 2010;**281**:201–6.
101. Resendiz-Sánchez J, Contreras-Rodríguez A, López-Merino A, et al. Isolation of *Brucella melitensis* from an abscess on the left foot of a 3-year-old infant. *J Med Microbiol* 2009;**58**:267–9.
103. Rolando I, Olarte L, Vilchez G, et al. Ocular manifestations associated with brucellosis: a 26-year experience in Peru. *Clin Infect Dis* 2008;**46**:1338–45.
104. Roushan MRH, Baiani M, Asnafi N, et al. Outcomes of 19 pregnant women with brucellosis in Babol, northern Iran. *Trans R Soc Trop Med Hyg* 2011;**105**:540–2.
109. Sahin E, Yilmaz A, Ersöz G, et al. Multiple cranial nerve involvement caused by *Brucella melitensis*. *South Med J* 2009;**102**:855–7.
111. Sasmazel A, Baysal A, Fedakar A, et al. Treatment of *Brucella* endocarditis: 15 years of clinical and surgical experience. *Ann Thorac Surg* 2010;**89**:1432–6.
114. Shen MW. Diagnostic and therapeutic challenges of childhood brucellosis in a nonendemic country. *Pediatrics* 2008;**121**:e1179–e1183.
115. Skendros P, Pappas G, Boura P. Cell-mediated immunity in human brucellosis. *Microbes Infect* 2011;**13**:134–42.
127. Sungur GK, Hazirolan D, Gurbuz Y, et al. Ocular involvement in brucellosis. *Can J Ophthalmol* 2009;**44**:598–601.
128. Tanir G, Tufekci SB, Tuygun N. Presentation, complications, and treatment outcome of brucellosis in Turkish children. *Pediatr Int* 2009;**51**:114–19.
129. Theegarten D, Albrecht S, Tötsch M, et al. Brucellosis of the lung: case report and review of the literature. *Virchows Arch* 2008;**452**:97–101.
130. Thoma S, Patsiogiannis N, Dempegiotis P, et al. A report of two cases of brucellar sacroiliitis without systemic manifestations in Greece. *J Pediatr Orthop* 2009;**29**:375–9.
133. Türel O, Sanli K, Hatipoğlu N, et al. Acute meningoencephalitis due to *Brucella:* case report and review of neurobrucellosis in children. *Turk J Pediatr* 2010;**52**:426–9.
137. Whatmore AM. Current understanding of the genetic diversity of *Brucella*, an expanding genus of zoonotic pathogens. *Infect Genet Evolution* 2009;**9**:1168–84.
147. Young EJ. Family studies in brucellosis. *Infection* 2008;**36**:578–9.
148. Young EJ. *Brucella* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 2921–5.
152. Zamani A, Kooraki S, Mohazab AM, et al. Epidemiological and clinical features of *Brucella* arthritis in 24 children. *Ann Saudi Med* 2011;**31**:270–3.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdoel TH, Smits HL. Rapid latex agglutination test for the serodiagnosis of human brucellosis. *Diag Microbiol Infect Dis* 2007; **57**:123–8.
2. Akçakuş M, Esel D, Çetin N, et al. *Brucella melitensis* in blood cultures of two newborns due to exchange transfusion. *Turk J Pediatr* 2005;**47**:272–4.
3. Akritidis N, Pappas G. Ascites caused by brucellosis: a report of two cases. *Scand J Infect Dis* 2001;**36**:110–12.
4. Akritidis N, Tzivras M, Delladetsima I, et al. The liver in brucellosis. *Clin Gastroenterol Hepatol* 2007;**5**:1109–12.
5. Al-Anazi KA, Abu Jafar S, Al-Jasser AM, et al. *Brucella* bacteremia in a recipient of an allogenic hematopoietic stem cell transplant: a case report. *Cases J* 2009;**2**:91.
6. Al Dahouk S, Tomaso H, Prenger-Berninghoff E, et al. Identification of *Brucella* species and biotypes using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *Crit Rev Microbiol* 2005;**31**:191–6.
7. Al-Eissa Y. Unusual suppurative complications of brucellosis in children. *Acta Pediatr* 1993;**82**:987–92.
8. Al-Eissa Y, Al-Nasser M. Haematological manifestations of childhood brucellosis. *Infection* 1993;**21**:23–6.
9. Al-Kasab S, Al-Fagih MR, Al-Yousef S, et al. *Brucella* infective endocarditis: successful combined medical and surgical therapy. *J Thorac Surg* 1988;**95**:862–70.
10. Al-Kharfy TM. Neonatal brucellosis and blood transfusion: case report and review of the literature. *Ann Trop Paediatr* 2001;**21**: 349–52.
11. Al Shaalan M, Memish ZA, Al Mahmoud S, et al. Brucellosis in children: clinical observations in 115 cases. *Int J Infect Dis* 2002;**6**:182–6.
12. Araj GF, Lulu AR, Saadah MA, et al. Rapid diagnosis of central nervous system brucellosis by ELISA. *J Neuroimmunol* 1986;**12**: 173–82.
13. Ariza J, Bosch J, Gudiol F, et al. Relevance of in vitro antimicrobial susceptibility of *Brucella melitensis* to relapse rate in human brucellosis. *Antimicrob Agents Chemother* 1986;**30**:958–60.
14. Ariza J, Bosilkovski M, Cascio A, et al. Perspectives for the treatment of brucellosis in the 21st century: the Ioannina recommendations. *PLoS Med* 2007;**4**:e317.
15. Ariza J, Corredoira J, Pallares R, et al. Characteristics of and risk factors for relapse of brucellosis in humans. *Clin Infect Dis* 1995;**20**:1241–9.
16. Ariza J, Gudiol F, Pallares R, et al. Treatment of human brucellosis with doxycycline plus rifampin or doxycycline plus streptomycin: a randomized, double-blind study. *Ann Intern Med* 1992;**117**: 23–30.
17. Ariza J, Pigrau C, Cañas C, et al. Current understanding and management of chronic hepatosplenic suppurative brucellosis. *Clin Infect Dis* 2001;**32**:1024–33.
18. Bang B. Die Aetiologie des seuchenhaften ("infectiosen"). *Verwerfens Zeit Thiermed* 1897;**1**:241–78.
19. Bosilkovski M, Krteva L, Dimzova M, et al. Brucellosis in 418 patients from the Balkan peninsula: exposure-related differences in clinical manifestations, laboratory test results, and therapy outcome. *Int J Infect Dis* 2007;**11**:342–7.
20. Bouza E, Garcia-de-la-Torre M, Parras F, et al. Brucellar meningitis. *Rev Infect Dis* 1987;**9**:810–22.
21. Bricker BJ, Ewalt DR, MacMillan AP, et al. Molecular characterization of *Brucella* strains isolated from marine mammals. *J Clin Microbiol* 2000;**38**:1258–62.
22. Bruce D. Note on the discovery of a micro-organism in Malta fever. *Practitioner* 1887;**39**:161.
23. Buchanan TM, Faber LC. 2-Mercaptoethanol *Brucella* agglutination test: usefulness for predicting recovery from brucellosis. *J Clin Microbiol* 1980;**11**:691–3.
24. Buchanan TM, Faber LC, Feldman RA. Brucellosis in the United States, 1960-1972: an abattoir-associated disease, part I: clinical features and therapy. *Medicine (Balt)* 1974;**53**:403–13.
25. Cardoso PG, Macedo GC, Azevedo V, et al. *Brucella* spp noncanonical LPS: structure, biosynthesis, and interaction with host immune system. *Microbial Cell Factories* 2006;**5**:13.
26. Carmichael LE, Brunner DW. Characteristics of a newly recognized species of *Brucella* responsible for infectious canine abortion. *Cornell Vet* 1968;**58**:579–92.
27. Carrington M, Choe U, Ubillos S, et al. Fatal case of brucellosis misdiagnosed in early stage of *Brucella suis* infection in a 46-year-old patient with Marfan syndrome. *J Clin Microbiol* 2012;**50**: 2173–5.
28. Chomel BB, De Bess EE, Mangiamele DM, et al. Changing trends in the epidemiology of human brucellosis in California from 1973 to 1992: a shift toward foodborne transmission. *J Infect Dis* 1994; **170**:1216–23.
29. Chusid MJ, Perzigian RW, Dunne M, et al. Brucellosis: an unusual cause of a child's fever of unknown origin. *Wisc Med J* 1989;**88**: 11–13.
30. Citak EC, Citak FE, Tanyeri B, et al. Hematologic manifestations of brucellosis in children: 5 years experience of an Anatolian center. *J Pediatr Hematol Oncol* 2010;**32**:137–40.
31. Cloeckaert A, Grayon M, Grépinet O, et al. Classification of *Brucella* strains isolated from marine mammals by infrequent restriction site-PCR and development of specific PCR identification tests. *Microbes Infect* 2003;**5**:593–602.
32. Cloeckaert A, Vizcaino N. DNA polymorphism and taxonomy of *Brucella* species. In: López-Goñi I, Moriyón I, editors. *Brucella: molecular and cellular biology*. Norfolk, UK: Horizon Books; 2004. p. 1–24.
33. Cluff LE. Medical aspects of delayed convalescence. *Rev Infect Dis* 1991;**13**(Suppl. 1):138–40.
34. Colmenero JD, Queipo-Ortuño MI, Reguera JM, et al. Real time polymerase chain reaction: a new powerful tool for the diagnosis of neurobrucellosis. *J Neurol Neurosurg Psychiatry* 2005;**76**: 1025–7.
35. Colmenero JD, Regnera JM, Martos F, et al. Complications associated with *Brucella melitensis* infection: a study of 530 cases. *Medicine* 1996;**75**:195–211.
36. Colmenero JD, Ruiz-Mesa JD, Plata A, et al. Clinical findings, therapeutic approach, and outcome of brucellar vertebral osteomyelitis. *Clin Infect Dis* 2008;**46**:426–33.
37. Colmenero JD, Suarez-Muñoz MA, Queipo-Ortuño MI, et al. Late reactivation of calcified granuloma in a patient with chronic suppurative brucellosis. *Eur J Microbiol Infect Dis* 2002;**21**: 897–9.
38. Corbel MJ. Brucellosis: an overview. *Emerg Infect Dis* 1997;**3**: 213–21.
39. Crosby E, Liosa L, Miro Quesada M, et al. Hematologic changes in brucellosis. *J Infect Dis* 1984;**150**:419–24.
40. De BK, Stauffer L, Koylass MS, et al. Novel *Brucella* strain (BO1) associated with a prosthetic breast implant infection. *J Clin Microbiol* 2008;**46**:43–9.
41. Del Vecchio VG, Kapatral V, Redkar RJ, et al. The genome sequence of the facultative intracellular pathogen *Brucella melitensis*. *Proc Nat Acad Sci U S A* 2002;**99**:443–8.
42. Dhand A, Ross JJ. Implantable cardioverter-defibrillator infection due to *Brucella melitensis*: case report and review of brucellosis of cardiac devices. *Clin Infect Dis* 2007;**44**:e37–e39.
43. Dikensoy O, Namiduru M, Hocaoglu S, et al. Increased pleural fluid adenosine deaminase in brucellosis is difficult to differentiate from tuberculosis. *Respiration* 2002;**69**:556–9.
44. Doern GV. Detection of selected fastidious bacteria. *Clin Infect Dis* 2000;**30**:166–73.
45. Dornand J, Gross A, Lafont V, et al. The innate immune response against *Brucella* in humans. *Vet Microbiol* 2002;**90**:383–94.
46. Doyle TJ, Bryan RT. Infectious disease morbidity in the U.S. region bordering Mexico, 1990-1998. *J Infect Dis* 2000;**182**: 1503–10.
47. Erdem M, Namiduru M, Karaoglan I, et al. Unusual presentation of neurobrucellosis: a solitary intracranial mass lesion mimicking a cerebral tumor: a case of encephalitis caused by *Brucella melitensis*. *J Infect Chemother* 2012;**18**(5):767–70.
48. Evans AC. Further studies on *Bacterium abortus* and related bacteria, part II: a comparison of *Bacterium abortus* and *Bacterium bronchosepticus* and with the agent which causes Malta fever. *J Infect Dis* 1918;**22**:580–7.
49. Falagas M E, Bliziotis IA. Quinolones for treatment of human brucellosis: critical review of the evidence from microbiological and clinical studies. Antimicrob. *Agents Chemother* 2006;**50**:22–33.
50. Gazapo E, Lahoz JG, Subiza JL, et al. Changes in IgM and IgG antibody concentrations in brucellosis over time: importance for diagnosis and follow-up. *J Infect Dis* 1989;**159**:219–25.

51. Goksel BK, Yerdelen D, Karatas M, et al. Abducens nerve palsy and optic neuritis as initial manifestations in brucellosis. *Scand J Infect Dis* 2006;**38**:721–5.
52. Goldbaum FA, Velikovosky CA, Baldi PC, et al. The 18-kDa cytoplasmic protein of *Brucella* species—an antigen useful for diagnosis—is a lumazine synthetase. *J Med Microbiol* 1999;**48**: 833–9.
53. Gopaul KK, Koylass MS, Smith C, et al. Rapid identification of *Brucella* isolates to the species level by real time PCR based single nucleotide polymorphism analysis. *BMC Microbiol* 2008;**8**:86.
54. Gotuzzo E, Carrillo C, Guerra J, et al. An evaluation of diagnostic methods for brucellosis: the value of bone marrow culture. *J Infect Dis* 1986;**153**:122–5.
55. Groussaud P, Shankster SJ, Koylass MS, et al. Molecular typing divides marine mammal strains of *Brucella* into at least three groups with distinct host preferences. *J Med Microbiol* 2007;**56**: 1512–18.
56. Habeeb YKR, Al-Najdi AKN, Sadek SAH, et al. Paediatric neurobrucellosis: case report and literature review. *J Infect* 1998; **37**:59–86.
57. Hall WH. History of *Brucella* as a human pathogen. In: Young EJ, Corbel MJ, editors. *Brucellosis: clinical and laboratory aspects*. Boca Raton, Fla.: CRC Press; 1989. p. 1–9.
58. Halling SM, Paterson-Burch BD, Bricker BJ, et al. Completion of the genome sequence of *Brucella abortus* and comparison to the highly similar genomes of *Brucella melitensis* and *Brucella suis*. *J Bacteriol* 2005;**187**:2715–26.
59. Hasanjani Roushan MR, Baiani M, Javanian M, et al. Brucellar epididymo-orchitis: review of 53 cases in Babol, northern Iran. Scand. *J Infect Dis* 2009;**41**:440–4.
60. Hayes FM, Traum J. Preliminary report of abortion in swine caused by *Br. abortus* (Bang). *Mod Vet Pract* 1929;**1**:58–65.
61. Inan MB, Eyileten ZB, Ozcinar E, et al. Native valve *Brucella* endocarditis. *Clin Cardiol* 2010;**33**:e20–e26.
62. Jahans KL, Foster G, Broughton ES. The characterisation of *Brucella* strains isolated from marine mammals. *Vet Microbiol* 1997;**57**:373–82.
63. Janmohammadi N, Hasanjani Roushan MR. False negative serological tests may lead to misdiagnosis and mismanagement in osteoarticular brucellosis. *Trop Doctor* 2009;**39**:88–90.
64. Jorens PG, Michielsen PP, Van den Enden EJ, et al. A rare cause of colitis: *Brucella melitensis*. *Dis Colon Rectum* 1991;**34**:194–6.
65. Karapinar B, Yilmaz D, Vardar F, et al. Unusual presentation of brucellosis in a child: acute blindness. *Acta Paediatr* 2005;**94**: 378–80.
66. Kazaz H, Celkan MA, Ustunsoy H, et al. Mitral annuloplasty with biodegradable ring for infective endocarditis: a new tool for the surgeon for valve repair in childhood. *Interact Cardiovasc Thorac Surg* 2005;**4**:378–80.
67. Khan MY, Mah MM, Memish ZA. Brucellosis in pregnant women. *Clin Infect Dis* 2001;**32**:1172–7.
68. Kurdoglu M, Adali E, Kurdoglu Z, et al. Brucellosis in pregnancy: a 6-year clinical analysis. *Arch Gynecol Obstet* 2010;**281**:201–6.
69. Lang R, Dugan R, Potasman I, et al. Failure of ceftriaxone in the treatment of acute brucellosis. *Clin Infect Dis* 1992;**14**:506–9.
70. Lang R, Rubinstein E: Quinolones for the treatment of brucellosis. *J Antimicrob Chemother* 1992;**29**:357–63.
71. Lapaque N, Moriyon I, Moreno E, et al. *Brucella* lipopolysaccharide acts as a virulence factor. *Curr Opinion Microbiol* 2005;**8**: 60–6.
72. Losurdo G, Timitilli A, Tasso L, et al. Acute hepatitis due to *Brucella* in a 2-year-old child. *Arch Dis Child* 1994;**71**:387.
73. Lubani MM, Lulu AR, Araj GF, et al. Pulmonary brucellosis. *Q J Med* 1989;**71**:319–24.
74. Mantur BG, Akki AS, Mangalgi SS, et al. Childhood brucellosis—a microbiological, epidemiological and clinical study. *J Trop Pediatr* 2004;**50**:153–7.
75. Marrodan T, Nenova-Poliakova R, Rubio M, et al. Evaluation of three methods to measure anti-*Brucella* IgM antibodies and interference of IgA in the interpretation of mercaptan-based tests. *Diagn Microbiol* 2001;**50**:663–6.
76. Marston JA. *Report on fever (Malta):* Army Medical Dept. statistical, sanitary, and medical reports for the year 1861. London: Harrison & Sons; 1863. p. 486–521.
77. McDonald WL, Jamaludin R, Mackereth G, et al. Characterization of a *Brucella* sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. *J Clin Microbiol* 2006;**44**:4363–70.
78. McLean DR, Russell N, Khan MY. Neurobrucellosis: clinical and therapeutic features. *Clin Infect Dis* 1992;**15**:582–90.
79. Memish Z, Almuneef M, Mah MW, et al. Comparison of the *Brucella* standard agglutination test with the ELISA IgG and IgM in patients with brucella bacteremia. *Diag Microbiol Infect Dis* 2002;**44**:129–32.
80. Memish Z, Mah MW, Al Mahmoud S, et al. *Brucella* bacteremia: clinical and laboratory observations in 160 patients. *J Infect* 2000;**40**:59–63.
81. Metin A, Akdeniz H, Buzgan T, et al. Cutaneous findings encountered in brucellosis and review of the literature. *Intern J Dermatol* 2001;**40**:434–8.
82. Milionis H, Christou L, Elisaf M. Cutaneous manifestations in brucellosis: case report and review of the literature. *Infection* 2000;**28**:124–6.
83. Mohamed AES, Ven D, Madkour MM, et al. Alimentary tract presentations of brucellosis. *Ann Saudi Med* 1986;**6**:27–31.
84. Moreno S, Ariza J, Espinosa FJ, et al. Brucellosis in patients infected with the human immunodeficiency virus. *Eur J Clin Microbiol Infect Dis* 1998;**17**:319–26.
85. Mosayebi Z, Movahedian AH, Ghayomi A, et al. Congenital brucellosis in a preterm neonate. *Indian Pediatr* 2005;**42**:599–601.
86. Mousa ARM, Koshy TS, Araj GF, et al. *Brucella* meningitis: presentation, diagnosis, and treatment: a prospective study of ten cases. *Q J Med* 1986;**60**:873–85.
87. Naudi JR. *Brucellosis: the Malta experience, a celebration 1905-2005*. Malta: Publishers Enterprise Group, Ltd.; 2005.
88. Navarro E, Segura JC, Castaño MJ, et al. Use of real-time quantitative polymerase chain reaction to monitor the evolution of *Brucella melitensis* DNA load during therapy and post-therapy follow-up in patients with brucellosis. *Clin Infect Dis* 2006;**42**:1266–73.
89. Navarro-Martínez A, Solera J, Corredoira J, et al. Epididymoorchitis due to *Brucella melitensis*: a retrospective study of 59 patients. *Clin Infect Dis* 2001;**33**:2017–22.
90. Olukman, Ö. Pulmonary involvement in childhood brucellosis: a case report. *Vector Borne Zoonotic Dis* 2008;**8**(2):245–8.
91. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. Brucellosis. *N Engl J Med* 2005;**352**:2325–36.
92. Pappas G, Akritidis N, Christou L. Treatment of neurobrucellosis: what is known and what remains to be answered. *Exp Rev Anti-infect Ther* 2007;**6**:983–90.
93. Pappas G, Bosilkovski M, Akritidis N, et al. Brucellosis and the respiratory system. *Clin Infect Dis* 2003;**37**:95e–99e.
94. Pappas G, Papadimitriou P, Akritidis N, et al. The new global map of human brucellosis. *Lancet Infect Dis* 2006;**6**:91–9.
95. Paulsen IT, Seshadri R, Nelson KE, et al. The *Brucella suis* genome reveals fundamental similarities between animal and plant pathogens and symbionts. *Proc Nat Acad Sci U S A* 2002;**99**:13148–53.
96. Pellicer T, Ariza J, Foz A, et al. Specific antibodies detected during relapse of human brucellosis. *J Infect Dis* 1988;**157**:918–24.
97. Petrella R, Young EJ. Acute *Brucella* ileitis. *Am J Gastroenterol* 1988;**83**:80–2.
98. Polt SS, Schaefer JA. A microagglutination test for human *Brucella canis* antibodies. *Am J Clin Pathol* 1982;**77**:740–4.
99. Probert WS, Schrader KN, Khuong NY, et al. Real-time multiplex PCR assay for detection of *Brucella* spp., *B. abortus* and *B. melitensis*. *J Clin Microbiol* 2004;**42**:1290–3.
100. Reguera JM, Alarcon A, Miralles F, et al. *Brucella* endocarditis: clinical, diagnostic, and therapeutic approach. *Eur J Clin Microbiol Infect Dis* 2003;**22**:647–50.
101. Resendiz-Sánchez J, Contreras-Rodríguez A, López-Merino A, et al. Isolation of *Brucella melitensis* from an abscess on the left foot of a 3-year-old infant. *J Med Microbiol* 2009;**58**:267–9.
102. Robichaud S, Libman M, Behr M, et al. Prevention of laboratory-acquired brucellosis. *Clin Infect Dis* 2004;**38**:e119–e122.
103. Rolando I, Olarte L, Vilchez G, et al. Ocular manifestations associated with brucellosis: a 26-year experience in Peru. *Clin Infect Dis* 2008;**46**:1338–45.
104. Roushan MRH, Baiani M, Asnafi N, et al. Outcomes of 19 pregnant women with brucellosis in Babol, northern Iran. Trans. *R Soc Trop Med Hyg* 2011;**105**:540–2.
105. Roushan MRH, Mohaz M, Janmohammadi N, et al. Efficacy of cotrimoxazole and rifampin for 6 or 8 weeks of therapy in childhood brucellosis. *Pediatr Infect Dis J* 2006;**25**:544–5.

106. Rubin B, Band JD, Wong P, et al. Person-to-person transmission of *Brucella melitensis*. *Lancet* 1991;**337**:14–15.
107. Rubinstein E, Lang R, Shasha B, et al. In vitro susceptibility of *Brucella melitensis* to antibiotics. *Antimicrob Agents Chemother* 1991;**35**:1925–7.
108. Ruiz J, Lorente I, Pérez J, et al. Diagnosis of brucellosis by using blood cultures. *J Clin Microbiol* 1997;**35**:2417–18.
109. Sahin E, Yilmaz A, Ersöz G, et al. Multiple cranial nerve involvement caused by *Brucella melitensis*. *South Med J* 2009;**102**:855–7.
110. San Miguel P, Fernández G, Vasallo FJ, et al. Neurobrucellosis mimicking cerebral tumor: case report and literature review. *Clin Neurol Neurosurg* 2006;**108**:404–6.
111. Sasmazel A, Baysal A, Fedakar A, et al. Treatment of *Brucella* endocarditis: 15 years of clinical and surgical experience. *Ann Thorac Surg* 2010;**89**:1432–6.
112. Seçmeer G, Ecevit Z, Gülbulak B, et al. Splenic abscess due to *Brucella* in childhood: a case report. *Turk J Pediatr* 1995;**37**: 403–6.
113. Sharda DC, Lubani M. A study of brucellosis in childhood. *Clin Pediatr* 1986;**25**:492–5.
114. Shen MW. Diagnostic and therapeutic challenges of childhood brucellosis in a nonendemic country. *Pediatrics* 2008;**121**:e1179–e1183.
115. Skendros P, Pappas G, Boura P. Cell-mediated immunity in human brucellosis. *Microbes Infect* 2011;**13**:134–42.
116. Sohn AH, Probert WS, Glaser CA, et al. Human neurobrucellosis with intracerebral granuloma caused by a marine mammal *Brucella* spp. *Emerging Infect Dis* 2003;**9**:485–8.
117. Solera J, Geijo P, Largo J, et al. A randomized, double-blind study to assess the optimal duration of doxycycline treatment for human brucellosis. *Clin Infect Dis* 2004;**39**:1776–82.
118. Solera J, Lozano E, Martinez-Alfaro E, et al. Brucellar spondylitis: review of 35 cases and literature survey. *Clin Infect Dis* 1999;**29**: 1440–9.
119. Solera J, Martinez-Alfaro E, Espinosa A. Recognition and optimum treatment of brucellosis. *Drugs* 1997;**53**:245–56.
120. Solera J, Rodríguez-Zapata M, Geijo P, et al. Doxycycline-rifampin versus doxycycline-streptomycin in treatment of human brucellosis due to *Brucella melitensis*. *Antimicrob Agents Chemother* 1995;**39**:2061–7.
121. Spink WW, Hoffbauer FW, Walker WW, et al. Histopathology of the liver in human brucellosis. *J Lab Clin Med* 1949;**34**: 40–58.
122. Spink WW. *The nature of brucellosis*. Minneapolis: University of Minnesota Press; 1956.
123. Stantic-Pavlinic M, Cec V, Mehk J. Brucellosis in spouses and the possibility of interhuman infection. *Infection* 1983;**11**:313–14.
124. Starnes CT, Talwani R, Horvath JA, et al. Brucellosis in two hunt club members in South Carolina. *J SC Med Assoc* 2004;**100**: 113–15.
125. Stermer E, Levy N, Potasman I, et al. Brucellosis as a cause of severe colitis. *Am J Gastroenterol* 1991;**86**:917–19.
126. Street L, Grant WW, Alva JD. Brucellosis in childhood. *Pediatrics* 1975;**55**:416–21.
127. Sungur GK, Hazirolan D, Gurbuz Y, et al. Ocular involvement in brucellosis. *Can J Ophthalmol* 2009;**44**:598–601.
128. Tanir G, Tufekci SB, Tuygun N. Presentation, complications, and treatment outcome of brucellosis in Turkish children. *Pediatr Int* 2009;**51**:114–19.
129. Theegarten D, Albrecht S, Tötsch M, et al. Brucellosis of the lung: case report and review of the literature. *Virchows Arch* 2008;**452**:97–101.
130. Thoma S, Patsiogiannis N, Dempegiotis P, et al. A report of two cases of brucellar sacroiliitis without systemic manifestations in Greece. *J Pediatr Orthop* 2009;**29**:375–9.
131. Troy SB, Rickman LS, Davis CE. Brucellosis in San Diego: epidemiology and species-related differences in acute clinical presentation. *Medicine* 2005;**84**:174–87.
132. Tsolia M, Drakonaki S, Messaritaki A, et al. Clinical features, complications and treatment outcome of childhood brucellosis in Central Greece. *J Infect* 2002;**44**:257–62.
133. Türel O, Sanli K, Hatipoğlu N, et al. Acute meningoencephalitis due to *Brucella:* case report and review of neurobrucellosis in children. *Turk J Pediatr* 2010;**52**:426–9.
134. Vallejo JG, Stevens AM, Dutton RV, et al. Hepatosplenic abscesses due to *Brucella melitensis*: report of a case involving a child and review of the literature. *Clin Infect Dis* 1996;**22**:485–9.
135. Vassallo DJ. The saga of brucellosis: controversy over credit for linking Malta fever with goat's milk. *Lancet* 1996;**348**:804–8.
136. Wanke MM. Canine brucellosis. *Animal Reprod Sci* 2004; **82–83**:195–207.
137. Whatmore AM. Current understanding of the genetic diversity of *Brucella*, an expanding genus of zoonotic pathogens. *Infect Genet Evol* 2009;**9**:1168–84.
138. Whatmore AM, Murphy TJ, Shankster S, et al. Use of amplified fragment length polymorphism to identify and type *Brucella* isolates of medical and veterinary interest. *J Clin Microbiol* 2005;**43**: 761–9.
139. Wright AE, Semple D. On the employment of dead bacteria in the serum diagnosis of typhoid and Malta fever. *BMJ* 1897;**1**: 1214–15.
140. Yagupsky P, Baron EJ. Laboratory exposure to brucellae and implications for bioterrorism. *Emerging Infect Dis* 2005;**11**: 1180–5.
141. Yingst S, Hoover DL. T cell immunity to brucellosis. *Crit Rev Microbiol* 2003;**29**:313–31.
142. Young EJ. *Brucella melitensis* hepatitis: the absence of granulomas. *Ann Intern Med* 1979;**91**:414–15.
143. Young EJ. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. *Clin Infect Dis* 1991;**13**:359–72.
144. Young EJ. An overview of human brucellosis. *Clin Infect Dis* 1995;**21**:283–90.
145. Young EJ. Brucellosis. In: Connor DH, Chandler FW, Manz HJ, et al, editors. *Pathology of infectious diseases*. Stamford, CT: Appleton & Lange; 1997. p. 447–52.
146. Young EJ. *Brucella* species. In: Yu V, Weber R, Raoult D, editors. *Antimicrobial therapy and vaccines*, Vol I, Microbes. 2nd ed. New York: Apple Trees Productions; 2002. p. 121–40.
147. Young EJ. Family studies in brucellosis. *Infection* 2008;**36**:578–9.
148. Young EJ. *Brucella* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 2921–5.
149. Young EJ, Suvannoparrat U. Brucellosis outbreak attributed to ingestion of unpasteurized goat cheese. *Arch Intern Med* 1975; **135**:240–3.
150. Young EJ, Tarry A, Genta RM, et al. Thrombocytopenic purpura associated with brucellosis: report of 2 cases and literature review. *Clin Infect Dis* 2000;**31**:904–9.
151. Zaman F, Abreo K. *Brucella* glomerulonephritis. *South Med J* 2005;**98**:1165–6.
152. Zamani A, Kooraki S, Mohazab AM, et al. Epidemiological and clinical features of brucella arthritis in 24 children. *Ann Saudi Med* 2011;**31**:270–3.

CHAPTER 129

Pertussis and Other *Bordetella* Infections

James D. Cherry • Ulrich Heininger

Pertussis (whooping cough) is an acute infectious illness of the respiratory tract caused by *Bordetella pertussis* and, less frequently, by *Bordetella parapertussis* and *Bordetella holmesii*.* The illness occurs worldwide and affects all age-groups, but it is recognized primarily in children; it is most serious in young, unprotected infants.[122,226,333,373]

HISTORY

Unlike other severe epidemic infectious diseases of children (e.g., smallpox, poliomyelitis, and measles), pertussis lacks an ancient history.[243] The first observation of pertussis occurred in France in 1414, and the first epidemic was noted in Paris in 1578.[96,112,288] This epidemic and the clinical characteristics of the cases were described in 1640 by Guillaume de Baillou.

Pertussis was noted as *the kink* (a Scottish term synonymous with "fit" or "paroxysm") and *kindhoest* (a Teutonic word meaning "child's cough") in the Middle Ages.[86,243] In 1669, Sydenham named the illness *pertussis* (meaning "violent cough").[288] Isolation of *B. pertussis*, the main causative agent of pertussis, was reported by Bordet and Gengou in 1906.[42,43]

Vaccines consisting of killed whole *B. pertussis* organisms were developed shortly after the bacterium was isolated, and the first results of protection were reported by Madsen in 1925.[319] The mouse protection test, developed and reported by Kendrick and collaborators[274] in 1947, allowed vaccine production to be standardized. Comprehensive studies conducted by the British Medical Research Council[341] in the 1940s and 1950s demonstrated a correlation between the potency of pertussis vaccines as determined by the mouse protection test and their clinical efficacy in children. As a consequence, immunization against pertussis, most commonly in combination with diphtheria and tetanus toxoids (DTP), became part of routine vaccination programs in many countries throughout the world.

Concern about a relationship between pertussis vaccination and temporally associated serious adverse events (e.g., sudden infant death syndrome [SIDS] and a variety of neurologic illnesses) led to a sharp decline in vaccination rates in Japan and several European countries during the 1970s.[77,83,96,279] This concern, along with well-documented high rates of unpleasant local (e.g., swelling and induration) and systemic (mainly fever) reactions, led to the development of new acellular vaccines. These vaccines cause reactions less frequently and have been used in Japan since 1981 and in many developed countries since the mid 1990s.[63,146,220,279,515]

B. bronchiseptica, which causes illness with a cough in a number of animals as well as humans, was first isolated sometime around 1910 by Ferry,[159] McGowan, and perhaps others who were studying dogs with distemper.[333,337] *B. parapertussis* was isolated first from children with pertussis in the 1930s, and *Bordetella holmesii* was noted in nasopharyngeal specimens from patients with pertussis-like illnesses in Massachusetts from 1995 through 1998.[45,148,149,334,545]

MICROBIOLOGY

The genus *Bordetella* contains nine species: *B. pertussis*, *B. parapertussis*$_{hu}$ (adapted to humans), *B. parapertussis*$_{ov}$ (ovine-adapted *B. parapertussis*), *B. bronchiseptica*, *Bordetella avium*, *Bordetella hinzii*, *B. holmesii*, *Bordetella trematum*, and *Bordetella petrii*.[118,193,231,275,333] *B. pertussis* infects exclusively humans. *B. parapertussis* also is a human pathogen, but it has been recovered from sheep ("ovine") as well.[407] Both *B. pertussis* and *B. parapertussis* are respiratory pathogens. *B. bronchiseptica* primarily is an animal pathogen that causes atrophic rhinitis and pneumonia in pigs, kennel cough in dogs, pneumonia in cats, and respiratory illnesses in other animals.[118,182,478] This organism also is the occasional cause of respiratory illness in humans.* *B. avium* is an important cause of respiratory illness in turkeys and other birds.[276]

In addition to *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica*, four other species have been recognized to infect/colonize humans.† *B. holmesii* and *B. hinzii* have been isolated from blood cultures, primarily in patients with underlying chronic illness. *B. holmesii* also has been isolated from the human respiratory tract as has *B. hinzii* and *B. petrii* in patients with cystic fibrosis, and *B. trematum* has been found in wounds and ear infections. *B. petrii*

*References 77, 83, 96, 231, 333, 361, 385, 545.

*References 44, 107, 133, 195, 383, 462, 478, 479, 541.
†References 16, 116, 126, 140, 171, 172, 215, 253, 268, 270, 272, 290, 304, 308, 334, 359, 361, 377, 379, 400, 459, 461, 482, 497, 526, 545.

TABLE 129-1 **Selected Differential Characteristics of *Bordetella* Species That Cause Respiratory Illnesses in Humans**

	Characteristic*				
Organism	CATALASE	OXIDASE	NITRATE REDUCTION	UREASE PRODUCTION	MOTILITY
B. pertussis	+	+	–	–	–
B. parapertussis	+	–	–	+	–
B. bronchiseptica	+	+	+	+	+
B. holmesii	+†	–	–	–	–

*+, activity; –, not present.
†Ten to 89 percent of strains are positive.
From Wirsing von Konig CH, Riffelmann M, Coenye T. *Bordetella* and related genera. In: Versalovic, J., editor. *Manual of clinical microbiology*. Washington (DC): ASM Press; 1970, vol 1. p. 739–50.

has been isolated from the environment and is capable of anaerobic growth.[509]

The genus *Bordetella* consists of gram-negative, pleomorphic, aerobic bacilli that are grouped together on the basis of genotypic characteristics, and species are differentiated by phenotypic characteristics. Selected differential characteristics of the four *Bordetella* spp. that cause respiratory illnesses in humans are presented in Table 129-1. All species have relatively simple requirements, but *B. pertussis* is quite fastidious and is inhibited by constituents in common laboratory media such as fatty acids, metal ions, sulfides, and peroxides.[502,535] For laboratory growth, *B. pertussis* requires the addition of "protective substances" such as charcoal, blood, or starch, whereas the other species are less fastidious and may grow in blood or MacConkey agars.

The genomes of representative strains of *B. pertussis*, *B. parapertussis*, and *B. bronchiseptica* have been sequenced.[60,409] The genome sizes are as follows: *B. pertussis*, 4,086,186 base-pairs (bp); *B. parapertussis*, 4,773,551 bp; and *B. bronchiseptica*, 5,338,400 bp. *B. pertussis* has 3816 predicted genes, whereas *B. parapertussis* and *B. bronchiseptica* have 4,404 and 5,007 predicted genes, respectively. Pseudogenes occur most commonly in *B. pertussis* (358) and *B. parapertussis* (220) and are uncommon in *B. bronchiseptica* (18). *B. pertussis* has three insertion sequence (IS) elements: IS 481 (238 copies), IS 1002 (6 copies), and IS 1663 (17 copies). *B. parapertussis* has IS 1001 (22 copies) and IS 1002 (90 copies). The sequenced RB50 *B. bronchiseptica* strain contained no IS elements, but studies of other *B. bronchiseptica* strains found strains with IS 481, IS 1001, and IS 1663.[137,169,312,417,486]

Etiology of Pertussis (Whooping Cough)

B. pertussis and *B. parapertussis* are the usual etiologic agents of pertussis, but 86 to 95 percent of illnesses are caused by *B. pertussis*.[105,183,231,411] In rare instances, *B. bronchiseptica*, which normally is enzootic in pigs, dogs, cats, rodents, and other animals, has been isolated from humans with pertussis-like illnesses with a cough.* From 1995 to 1998, *B. holmesii* was isolated from the nasopharynx of 33 individuals in Massachusetts suspected of having pertussis, most of them adolescents and young adults.[334] In contrast to all other *Bordetella* spp., this organism is susceptible to cephalexin, an antibiotic that frequently is added to *Bordetella* culture media.[388] This addition may explain why the organism has not been noted in patients with pertussis in other culture-based laboratory studies.

Adenoviruses have been isolated from children with pertussis, and some researchers have suggested that several adenoviral types on occasion may cause a pertussis-like illness.[22,111,113,379] However, the data of Nelson and associates[379] and Baraff and coworkers,[22] as well as our own observations, lead us to suggest that mixed infections are occurring and that the classic symptoms are caused by *B. pertussis* and not infection with an adenovirus. Wirsing von König and associates noted pertussis-like illnesses caused by viral or *Mycoplasma pneumoniae* infections in 83 patients in whom pertussis laboratory studies were performed and were negative. These authors serologically identified 33 adenoviral illnesses, 18 illnesses caused by parainfluenza viruses, 15 illnesses caused by *M. pneumoniae*, and 14 caused by respiratory syncytial virus (RSV). Wang and colleagues[512] retrospectively studied prolonged illness with cough in 179 children and found that 37 percent had evidence of *B. pertussis* infection and 13 percent had *M. pneumoniae* infections. The cough duration in those with *M. pneumoniae* was of significantly shorter duration (median 39 days) than in children with *B. pertussis* infections (median 118 days). In young infants, coinfection with an adenovirus and perhaps RSV may lead to more severe disease.[30,296] Zouari and associates[550] noted *B. pertussis* and *M. pneumoniae* coinfections in three fatal cases in young infants. Infection with human bocavirus also may cause an illness with a paroxysmal cough.[15] Of 54 children with laboratory-confirmed bocavirus infections, 85 percent had cough and 19 percent had paroxysmal coughing episodes.

Physicians often suggest that *Chlamydia trachomatis* can cause a pertussis-like illness. However, in our opinion, the repetitive cough of *C. trachomatis* is distinctly different from the paroxysmal cough of *B. pertussis* infection, and thus illnesses caused by the two agents usually should not be confused clinically. Infections with *Chlamydia pneumoniae* and *M. pneumoniae* also cause long-lasting illness with cough.[125,204,536] Although infection with these agents in older children and adults can be confused with

*References 44, 107, 133, 195, 383, 462, 478, 479, 541.

TABLE 129-2 **Biologically Active and Antigenic Components of *Bordetella pertussis***

Component	Characteristic
Surface Proteins and Autotransporters	
Fimbriae (FIM)	Two serologic types (types 2 and 3); antibody to specific types causes agglutination of the organism; organisms may contain fimbriae 2, fimbriae 3, fimbriae 2 and 3, or neither fimbriae 2 nor fimbriae 3.
Filamentous hemagglutinin (FHA)	220-kDa surface-associated and secreted protein; highly immunogenic
Pertactin (PRN)	A 69-kDa outer-membrane protein that allows the organism to resist neutrophil-mediated clearance; antibody to pertactin causes agglutination of the organism.
Vag8	95-kDa outer-membrane protein
BrkA	73-kDa surface-associated N-terminal passenger domain with 30-kDa outer-membrane C-terminal protein; confers serum resistance and protection against antimicrobial peptides in *B. pertussis*
SphB1	Subtilisin-like Ser protease/lipoprotein required for FHA maturation
Tracheal colonization factor (TcfA)	60-kDa secreted protein
Toxins	
Pertussis toxin (PT; also called lymphocytosis-promoting factor)	A classic bacterial toxin with an enzymatically active A subunit and a B oligomer-binding protein; effects in an animal model system include histamine sensitization, promotion of lymphocytosis, stimulation of insulin secretion, and adjuvant and mitogenic activity; it is an envelope protein that is also an important adhesin; it adversely affects host immune cell function
Adenylate cyclase toxin (ACT)	Calmodulin-activated RTX family toxin with dual adenylate cyclase/hemolysin activity; acts as an antiphagocytic factor during infection
Dermonecrotic toxin (DNT)	160-kDa heat-labile secreted toxin; induces necrosis in vitro
Tracheal cytotoxin	Disaccharide-tetrapeptide monomeric byproduct of peptidoglycan synthesis; causes mitochondrial bloating, disruption of tight junctions, damage to cilia, and interleukin-1α and nitric oxide production in hamster tracheal and tissue cultures
Type III secretion system	Allows *Bordetella* to translocate effector proteins directly into host cells
Lipopolysaccharide (LPS) (endotoxin)	An envelope toxin with activities similar to endotoxins of other gram-negative bacteria; a significant cause of reactions to whole-cell pertussis vaccines; antibody to LPS causes agglutination of the organism.

B. pertussis infection, true paroxysms typical of pertussis rarely occur.

Antigenic and Biologically Active Components of *Bordetella pertussis*

B. pertussis contains a variety of components that are antigenic or biologically active (Table 129-2).* With the exception of tracheal cytotoxin (TCT), all known virulence factors produced by *B. pertussis* are regulated by the single genetic locus *bvgAS*.[118] Under certain conditions, such as an environmental temperature of 37° C, *bvgAS* is active, toxins and biologically active surface proteins are produced, and the organism is virulent in a mouse model (*bvg*$^+$ phase). In the *bvg*$^-$ phase, a different set of genes (*vrg*, *vir* repressed genes) are expressed, and *B. pertussis* is avirulent in mice in this phase.[520] The switch from *bvg*$^+$ to *bvg*$^-$ is a phenomenon common to all *Bordetella* spp. and is associated with a change in phenotype. An intermediate phase with reduced virulence and expression of specific proteins also has been characterized. Although still speculative, the intermediate phase may have some function in transmission of the organism.[118,468]

*References 1, 10, 11, 13, 24, 54, 80, 96, 118, 119, 157, 180, 213, 217, 220, 233, 236, 238-240, 257, 275, 309, 310, 316, 321, 326, 327, 331, 333, 351, 360, 380, 395, 440, 452, 492, 495, 505, 511, 521, 529, 549.

Fimbriae

Fimbriae are protein projections on the surface of *B. pertussis*.* They are highly immunogenic, and antibody to them, as well as to other antigens, causes agglutination of the organism. Two fimbrial antigens (FIM 2 and 3) are the main agglutinogens; endotoxin and pertactin (PRN) also are agglutinogens.[80,426]

In the past, typing of *B. pertussis* strains was based on the agglutination patterns noted with specific antisera.[80,147,411,426] Six specific agglutinogens were recognized, and typing was based on the presence or absence of agglutination by each specific antiserum. More recently, researchers recognized that two of the agglutinogens (agglutinogens 2 and 3) are fimbrial in location (FIM 2 and 3) and that agglutinogens 4, 5, and 6 are minor antigens.[411,426] All *B. pertussis* strains contain agglutinogen 1. The nature of this agglutinogen is not known.[426] It could be lipopolysaccharide (LPS) or PRN, but because the original serotyping scheme was based on heat-labile antigens, it probably was not LPS.[46,297,484]

The fimbriae function as adhesins, but some studies suggest that in infection they are not the primary adhesins but serve to sustain the attachment established by other attachment factors.[96,427,492,521] An in vitro study by Rodriguez and associates[428] suggested that the effect of

*References 333, 357, 362, 411, 426, 427.

fimbriae on attachment was the result of bacterial agglutination. In the mouse model system, immunization with purified fimbriae resulted in protection against infection when challenged with *B. pertussis*.[269] Data from two trials in which serologic correlates of immunity were studied indicated that antibody to fimbriae is important in protection.[97,472]

Filamentous Hemagglutinin

Filamentous hemagglutinin (FHA) is a component of the cell wall of all *Bordetella* spp.* It is highly immunogenic and is the dominant attachment factor for *Bordetella* in animal model systems.[333,440,451] However, because FHA is released in large amounts from the cell surface, its role as an adhesin must be questioned. Adhesins typically remain associated with the bacterial surface to promote maximum attachment. FHA is a component of most acellular component (DTaP and Tdap) vaccines.[145] However, the importance of antibody to FHA and protection from disease is not clear. Some data indicate that a vaccine containing both pertussis toxin (PT) and FHA had an efficacy greater than that of a vaccine containing only toxoided PT.[470] However, in two studies in which serologic correlates of immunity were evaluated, researchers found that FHA made no contribution to protection.[97,472] Finally, one whole-cell component DTP vaccine in which vaccinees did not mount an antibody response to FHA was, nonetheless, highly efficacious.[224,463]

Pertactin

PRN is a 69-kDa outer-membrane protein that plays an important role in *B. pertussis* pathogenesis by allowing the organism to resist neutrophil-mediated clearance.[37,257,294,388,389] Antibody to PRN has a strong protective effect in aerosol-challenge studies in mice.[76,452] In the two vaccine efficacy trials in which serologic correlates of immunity were evaluated, researchers found that antibody to PRN was most important in protection.[97,472] In addition, the vaccine efficacy trials conducted in the 1990s, in which mild disease and typical disease were evaluated, revealed that DTaP vaccines that contained PRN in addition to PT and FHA clearly were significantly more effective.[84,103,198,333] Another study revealed that anti-PRN antibodies were required for efficient phagocytosis of *B. pertussis* by host immune cells.[234]

Other Autotransporters

In addition to PRN are several other surface-associated proteins (Vag8, BrkA, SphB1, and TcfA) that are considered to belong to the autotransformer family.† All likely play an important active role in *B. pertussis* infection.

Pertussis Toxin

PT is an adenosine diphosphate–ribosylating toxin synthesized and secreted exclusively by *B. pertussis*. It is an A-B toxin with an enzymatically active A subunit (S_1) and a B oligomer ($S_{2\text{-}5}$) binding portion.* PT inactivates G proteins, a process resulting in disruption of signaling pathways and leading to histamine sensitization, enhancement of insulin secretion in response to regulatory signals, and both suppressive and stimulatory immunologic effects in animal model systems.[333] The various effects of PT in animal model systems and in humans were reviewed in depth previously by one of us (J.D.C.).[96,333] In contrast to animal studies, histamine sensitization does not appear to happen in children, but PT does cause an increase in plasma insulin levels.[96,333] PT also is responsible for leukocytosis with lymphocytosis in *B. pertussis* infections. PT is a strong adjuvant in several immunologic systems in animals, but in DTP-vaccinated persons (DTP vaccines contain small amounts of active toxin), only the enhancement of serum antibody responses to antigens of other vaccines has been demonstrated. In mouse and rat models, PT inhibits chemotaxis and migration of neutrophils, monocytes/macrophages, and lymphocytes to infection sites, and PT also functions as an adhesin in the adherence of *B. pertussis* to human macrophages and ciliated respiratory epithelial cells.

In 1979, researchers suggested that pertussis was a single-toxin disease, a suggestion that led to the idea that pertussis could be prevented by a PT vaccine in a manner similar to the success achieved with diphtheria toxoid in diphtheria.[80,404,405] Although convincing arguments to the contrary have been presented, this idea persists.[422,423,489] The most compelling evidence that pertussis from *B. pertussis* infection is not a PT disease is as follows: identical illness results from *B. parapertussis* infection, and this organism does not express PT.[80,231]

However, PT does contribute to morbidity in *B. pertussis* infections, as indicated by the severity of illness, which tends to be greater than that caused by *B. parapertussis* infection. In particular, the frequent finding of extreme leukocytosis caused by PT in neonates and young infants with fatal pertussis is noteworthy.

Adenylate Cyclase Toxin

Adenylate cyclase toxin (ACT) is an extracytoplasmic enzyme that impairs host immune cell function and may contribute to local tissue damage in the respiratory tract.† ACT enters phagocytic cells (particularly polymorphonuclear neutrophils) and, once inside, is activated by calmodulin and catalyzes the production of supraphysiologic amounts of cyclic adenosine monophosphate from adenosine triphosphate, which intoxicates these cells.[333]

Dermonecrotic Toxin

Dermonecrotic toxin (DNT) is a heat-labile toxin described by Bordet and Gengou[43] in 1909. This cytoplasmic protein causes skin necrosis in laboratory animals,[375] and it may contribute to local tissue damage in the respiratory tract.

*References 1, 37, 96, 118, 236, 278, 321, 333, 492, 505.
†References 24, 217, 326, 327, 333, 395, 529.

*References 10, 11, 13, 14, 55, 96, 118, 174, 258, 294, 310, 333, 404, 405, 494.
†References 54, 96, 118, 240, 277, 333, 505, 522.

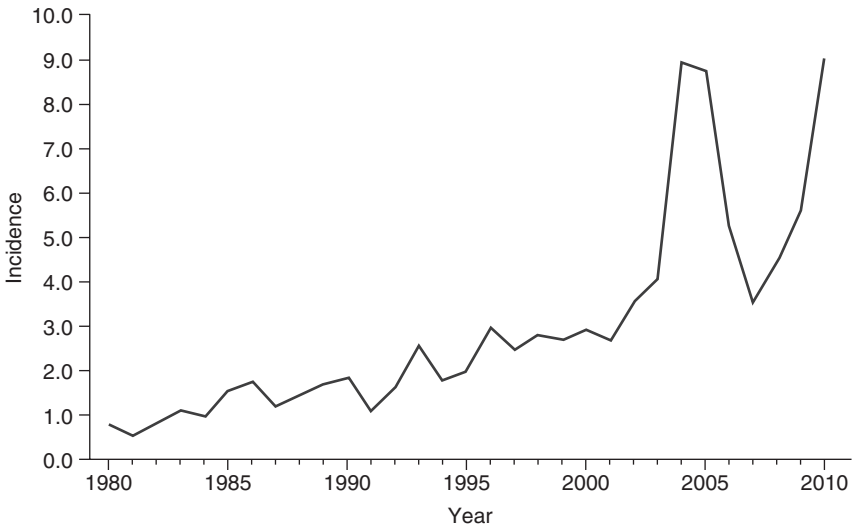

FIGURE 129-1 ■ Pertussis. Incidence per 100,000 population by year—United States, 1980 to 2010. (From Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.)

Tracheal Cytotoxin

TCT is a disaccharide-tetrapeptide monomer of peptidoglycan.[118,180] It causes local damage to respiratory epithelium in hamster tracheal organ cultures and in cultured hamster tracheal epithelial cells and may affect host neutrophil function adversely.[123,180] The cytopathology caused by TCT probably is the result of increases in nitric oxide.[118] It should be noted, however, that the ciliated epithelial cells of infants dying with *B. pertussis* pneumonia were quite well preserved.[399]

Type III Secretion System

The type III secretion system (TTSS) allows *Bordetella* to translocate effector proteins directly into the plasma membrane or cytoplasm of host cells.[157,307]

Lipopolysaccharide (Endotoxin)

The LPS of *B. pertussis* is similar to the endotoxins of other gram-negative bacteria.[74,96,118] Its function in disease is unknown, but it may act as an adhesin.[133] LPS is a major cause of reactions to whole-cell pertussis vaccines.[19] LPS is a significant agglutinogen. Antibody to LPS reduces colonization of *B. pertussis* in the lungs and trachea of mice after aerosol challenge.[370]

EPIDEMIOLOGY

Pertussis is an extremely infectious disease with an estimated R_0 of 12 to 17 (R_0 is the expected number of secondary cases produced by a typical primary case in an entirely susceptible population).[8,99,161]

Today, considerable misinformation is circulating with regard to the epidemiology of pertussis.* The main reason for misinformation is the failure to recognize the significant differences in the dynamics of reported pertussis compared with the dynamics of *B. pertussis* infections.[87,89] The two different epidemiologies are the (1) epidemiology of reported clinical pertussis and (2) the epidemiology of *B. pertussis* infection.

*References 48, 49, 293, 429, 430, 518.

Observed (Reported Pertussis)

Rates of reported pertussis depend on surveillance programs in the reporting countries, states, or counties.[480] For example, in Switzerland and three bordering countries (Germany, Italy, and Austria), the rates of reported pertussis for the period 1998 to 2002 varied by as much as 70-fold.[57] Because the immunization practices in the four countries were somewhat similar and because there is close proximity of the countries, the differences in the rates are explained by the rigorousness of the respective surveillance programs and not unique epidemiology by country.

Incidence

The incidence of reported pertussis and its mortality are affected markedly by the use of pertussis vaccine. In the prevaccine era in the United States, the average attack rate of reported pertussis was 157 per 100,000 population, versus 230 per 100,000 population in England and Wales.[77] Previous studies, however, suggested that reported cases represent only between 15 and 25 percent of cases that actually occur.[216,264,267,469]

With the introduction and widespread use of pertussis vaccines, the attack rate of reported pertussis in the United States fell approximately 150-fold from 1943 to 1976. For the 7-year period from 1976 to 1982, the attack rate in the United States remained between 0.5 and 1.0 per 100,000 population. From 1982 to 2010, the attack rate curve shifted modestly upward and reached a rate of 9.0 per 100,000 in 2010[73] (Fig. 129-1). Possible reasons for the resurgence of reported pertussis that have been suggested are (1) increased vaccine failures resulting from genetic changes in *B. pertussis*, (2) increased vaccine failures related to vaccines of lessened potency, (3) waning immunity, (4) greater awareness of pertussis, and (5) the

availability of better laboratory tests.* Of these possibilities, it is our opinion that greater awareness was initially the most important. Also of importance is the use of polymerase chain reaction (PCR) for diagnosis and that, in general, DTaP vaccines are less efficacious than are DTP vaccines, which, in particular, may be a contributing factor relating to the increase in reported pertussis in older children and adolescents. The number of cases and the attack rate in adolescents and adults will increase further when single serum serologic diagnosis becomes routine.[106] Finally, the data from California indicate that waning immunity is a major factor. In looking at Figure 129-1, there appears to be two periods. In the first period, the increase in cases is mainly due to increased awareness. In contrast, the sharp peaks in 2004 to 2005 and in 2010 are most likely due to DTaP vaccine failure, waning immunity, and PCR diagnosis.

In recent years, there has been considerable debate regarding the role of genetic change in the cause of vaccine failure.[106,130,362,390,446] Over time, it is clear that genetic changes in PT, PRN, and fimbriae have occurred. In fact, Preston[410] in 1965 noted that the British vaccines at the time contained only agglutinogen 2 (FIM 2) and that circulating *B. pertussis* strains contained agglutinogen 3 (FIM 3). However, we believe at the present time that genetic change has not contributed to vaccine failures. If genetic change were to lead to vaccine failure, one would expect it to occur first in Denmark where physicians have been using a single-component PT toxoid vaccine for 15 years.[92] At present, there are no data suggesting increased vaccine failure in Denmark.

Pertussis epidemics in the prevaccine era occurred at 2- to 5-year intervals (average, 3.2 years), and these cycles have continued in the vaccine era. As noted by Fine and Clarkson,[160,163] this continuation of the same cycles today as occurred in the prevaccine era indicates that, although immunization has controlled disease, it has not reduced transmission of the organism in the population.[77,82,85]

In the prevaccine era, the following percentages of cases by age were noted in Massachusetts: younger than 1 year, 7.5 percent; 1 to 4 years, 41.1 percent; 5 to 9 years, 46.0 percent; 10 to 14 years, 4.1 percent; and 15 years and older, 0.9 percent.[42] Associated with the marked reduction in reported cases of pertussis in the United States resulting from widespread pediatric immunization, a major shift occurred in the percentages by age category.[42] During the period from 1978 to 1981, the age distributions were as follows: younger than 1 year, 53.5 percent; 1 to 4 years, 26.5 percent; 5 to 9 years, 8.2 percent; 10 to 14 years, 5.4 percent; and 15 years or older, 6.5 percent. In contrast, U.S. data for 2010 revealed the following: younger than 1 year, 15 percent; 1 to 6 years, 22 percent; 7 to 10 years, 18 percent; 10 to 19 years, 20 percent; and 20 years or older, 25 percent. Today, pertussis in adolescents and adults is an important source of *B. pertussis* infection in unimmunized or partially immunized children.† Disease in adolescents and adults usually is not recognized as pertussis, even though the cough frequently is paroxysmal and the illness may persist for weeks.[85,132,325,408,447]

In a study in university students, members of our group found that 26 percent of students with an illness with cough of 6 days' duration or longer had *Bordetella* infections, none of which had been diagnosed correctly clinically.[356] The findings in this study led to the suggestion that *B. pertussis* infections are endemic in adults and are responsible for cyclic outbreaks in susceptible children. More recent studies in the United States, Germany, and elsewhere support this hypothesis.*

Morbidity and Mortality

During the first 30 years of the 20th century, pertussis was an important cause of death in the United States.[288] The number of deaths from pertussis in the United States between 1926 and 1930 was 36,013,[183] and most of these deaths occurred in children younger than 1 year of age. The pertussis death rate curve in the United States declined throughout the 20th century. In infants, the mortality rate decreased approximately fivefold from 1900 to 1944. During the next 35 years, it declined more than 85-fold.[367,368] Today, most of the deaths caused by pertussis occur in unimmunized infants younger than 6 months of age.† Currently in the United States, 10 to 20 deaths are reported each year.‡

Of importance is that deaths caused by pertussis frequently are misdiagnosed as deaths from other respiratory tract infectious illnesses.[77,228,384] For example, in England and Wales during the epidemic from 1977 to 1979 and at the beginning of the epidemic in 1982, 32 deaths were reported to be caused by pertussis.[77] However, when the excess deaths from other respiratory tract infectious illnesses were examined, approximately 362 additional deaths appeared to be caused by pertussis. Additional pertussis deaths were reported as SIDS.[384]

The morbidity caused by pertussis in recent years can be gleaned from numerous reports. The numbers of pertussis-related hospitalizations, complications, and deaths in the United States during 1990 to 1996 were reviewed in 1999 by Güris and colleagues.[197] Of the 31,837 cases analyzed, 31.7 percent of these patients were hospitalized, 9.5 percent had pneumonia, 1.4 percent had seizures, 0.2 percent had encephalopathy, and 0.2 percent died. The most severe morbidity occurred in infants younger than 6 months of age. Of this group, 72.2 percent were hospitalized, 17.3 percent had pneumonia, 2.1 percent had seizures, 0.5 percent had encephalopathy, and 0.5 percent died. During the period from 2000 to 2004, 12,174 cases in infants were reported.[70] Of this group, 62.8 percent were hospitalized, 55.8 percent had apnea, 12.7 percent had pneumonia, 1.5 percent had seizures, and 0.8 percent died.

In adolescents with reported pertussis, 0 to 2 percent are hospitalized and 2 percent have pneumonia.[69] In a review of data from seven studies of reported adult pertussis, the following findings were noted: hospitalization,

*References 87, 89, 103, 105, 109, 188, 198.
†References 39, 70, 85, 87, 89, 94, 287, 306, 307, 366, 378, 496, 524.

*References 38, 85, 87, 89, 135, 136, 362, 447, 534, 544.
†References 197, 201, 333, 373, 508, 543.
‡References 64, 66, 73, 197, 508, 543.

3 to 12 percent; seizures, 0 to 0.6 percent; rib fracture, 0 to 4 percent; and pneumonia, 0.6 to 8 percent.[70]

A pertussis surveillance system was introduced in conjunction with a large pertussis vaccine efficacy trial in several regions of Germany in 1990. The initial results of this ongoing surveillance study were published in 1993.[222] Of 601 culture-proven cases, 12.3 percent occurred in infants and 86.2 percent occurred in children younger than 6 years of age. Serious complications were reported in 22 of 275 patients with follow-up. These complications included pneumonia in 5.5 percent, apnea in 2.2 percent, and cardiorespiratory failure in 0.4 percent.

In a follow-up report of the same study, 1 of 185 culture-confirmed cases in infants was fatal.[226] In another German study from 1993 to 1996, Herzig and associates[178] noted 116 hospitalized pertussis patients with the following complications: pneumonia in 81 percent, apnea requiring assisted ventilation in 15 percent, seizures in 14 percent, encephalopathy in 5 percent, and death in 2 percent. In Canada from 1991 to 1997, Halperin and colleagues[211] analyzed the complications of pertussis in 1082 hospitalized children younger than 2 years of age and noted that 9.4 percent had pneumonia, 3 percent had atelectasis, 2.3 percent had seizures, 0.59 percent had encephalopathy, 0.8 percent had inguinal or umbilical hernias, 1.3 percent had more than a 5 percent weight loss, and 0.9 percent died.

Season, Geography, Race, Ethnicity, and Sex

Pertussis occurs throughout the world.[96] Historically, epidemic pertussis had no seasonal pattern.[77] However, in the present vaccine era in North America, pertussis usually occurs in the summer and fall.* The incidence of pertussis is usually greater in female than in male patients.[77,155,184,197,516] In 2004, 25,827 cases were reported in the United States, and of these patients, 11,199 (45%) were male and 13,879 (55%) were female (sex not identified in 749 cases).[65] In 2010, 55 percent of the reported cases were females. In the large study in Germany, 1,263 (50.7%) of the 2,493 subjects were female.[226]

Reported pertussis rates were higher in American Indians/Alaska Natives and whites than in Asians/Pacific Islanders and blacks in 2010.[73] Attack rates were also higher in Hispanics than in non-Hispanics.

Transmission

Transmission is thought to occur by droplets from a coughing patient that reach the upper respiratory tract of a susceptible person. Indirect spread also possibly occurs. A symptomatic patient could contaminate the environment with respiratory secretions. The hands of the new host-to-be make contact with the secretions and then may inoculate the respiratory tract.[96] Attack rates in susceptible household contacts range from 70 to 100 percent.[77,96] Antibody studies indicate that asymptomatic infections also occur in contacts.[135,313,314] These asymptomatic infections are likely to be short lived and probably are not important with regard to contagion. Transmissibility is greatest early in the illness, that is, during the catarrhal and early paroxysmal phases.

Bordetella pertussis Infection

In general, the cyclic pattern of reported pertussis is similar today to what it was in the prevaccine era.[333] This pattern is still different from that seen with other diseases that have been brought under control by universal immunization.[85,87,89,356] An example is measles. As this disease was brought under control, the interepidemic cycle lengthened because circulation of the measles virus in the population had been reduced. In contrast, with pertussis, immunization controlled disease but did not decrease the circulation of *B. pertussis*.[163,164] In the 1970s, Nelson[378] noted that the source of *B. pertussis* infection in hospitalized infants most often was an adult. This observation led many investigators to suspect that *B. pertussis* infections were endemic in adolescents and adults.[85,356] Sporadic infection in the adolescent and adult reservoir is the major source of *B. pertussis* infections in nonimmune children. The cyclic pattern occurs because it takes a few years for a significant number of susceptible members of the population to develop so that an outbreak will result from the sustained transmission.

Our present understanding of the importance of adolescent and adult *B. pertussis* infections has been the result of the ability to diagnose infection serologically by measuring immunoglobulin (Ig) G and IgA antibodies to PT by enzyme-linked immunosorbent assay (ELISA)*. During the past 25 years, numerous studies in adolescent and adult populations have contributed to our understanding of *B. pertussis* epidemiology.

Studies of prolonged illnesses with cough in adolescents and adults suggest that between 13 and 20 percent of the illnesses are caused by *B. pertussis* infection.† Many studies have looked at overall infection rates by determining significant IgG or IgA titer rises to PT in persons who have two or more serum samples collected over extended periods.‡ These studies suggest that between 1.0 and 6.7 percent of adolescents and adults have a *B. pertussis* infection each year. In another large study in the Netherlands, which used a combination of serologic surveys, an incidence of infection of 6.6 percent per year for persons between the ages of 3 and 79 years was estimated.[131] The incidence in children 3 to 4 years of age was 3.3 percent. It then increased gradually to a peak of 10.8 percent in those 20 to 24 years of age. Other age-specific incidences were 6.5 percent in persons 25 to 55 years old and 4.0 percent in those older than 55 years of age.

The specific rates of illnesses with cough caused by *B. pertussis* were determined in two prospective specific population-based studies.[475,513] In the first study the rate was 500 per 100,000 (0.5%), and in the second it was

*References 105, 155, 184, 197, 333, 378.

*References 87, 89, 106, 333, 356, 413

†References 38, 87, 89, 178, 265, 333, 356, 381, 433, 447, 475, 507, 534, 544.

‡References 87, 89, 120, 136, 241, 333, 514.

370 per 100,000 (0.37%). In addition, the retrospective serologic analysis of a respiratory disease study in adults older than 65 years suggested a rate as high as 1.5 percent.[241] This high value assumes that all respiratory illnesses with cough during the 4-month periods of seroconversion were the result of *B. pertussis* infection.

With increased clinical surveillance and more complete use of the laboratory (PCR and serologic study) and the diagnosis of mild cases in children, adolescents, and adults of all ages, the present cyclic pattern of reported pertussis will, for the most part, disappear. In fact, the pattern of reported pertussis has already lost some of its predictable regularity in some European countries with rigorous surveillance systems in place.

PATHOLOGY

Data on pathology of *B. pertussis* infections have been determined mainly by postmortem study in fatal cases.* However, information relating to uncomplicated pertussis can be gleaned from experiments using rhesus and ringtail monkeys.[288,442] In these studies, pure cultures of *B. pertussis* have been obtained from between the cilia of the smaller bronchi and bronchioli. Endobronchitis and peribronchitis are noted, and the bronchi contain leukocytes, mucus, and debris.

In a recent study of autopsy material from 15 infants (≤4 months old), the consistent histopathologic features were necrotizing bronchiolitis and pneumonia, intra-alveolar hemorrhage and fibrinous edema, and abundant intra-alveolar macrophages.[399] Angiolymphatic aggregates of mixed leukocytes in the intralobular septa and pleurae were seen in 86 percent of the specimens. Intact *Bordetella* were noted in cilia of the trachea, bronchi, and bronchioles and within airways and alveoli. They also were noted intracellularly in alveolar macrophages and respiratory epithelium. Despite the fact that all patients had severe pneumonia, the ciliated respiratory epithelial cells appeared relatively normal. Six of the respiratory specimens had evidence of coinfections with one or more other agents (cytomegalovirus, 1 child; RSV, 2 children; *Streptococcus pneumoniae*, 2 children; *Streptococcus pyogenes*, 2 patients; *Moraxella catarrhalis*, 1 child; and viridans streptococci, 1 child).

Pathologic changes in the brain and liver also have been described. Microscopic or gross cerebral hemorrhage may be noted, and cortical atrophy has been observed. These changes most likely are the result of anoxic brain damage. In some studies of pertussis encephalopathy, findings suggested meningoencephalitis, with perivascular cuffs of lymphocytes within cerebral gray matter and pleocytosis.[540] However, the studies in which inflammation was demonstrated were performed before modern virologic techniques became available. Probably, the neurologic findings in these instances were caused by interactions with neurotropic viruses or other infectious agents and were not the result of *B. pertussis* infection.[96] Fatty infiltration of the liver has been noted in patients with pertussis encephalopathy.

PATHOGENESIS AND IMMUNITY

After the patient is exposed to *B. pertussis*, the pathogenesis of infection depends on four important steps: attachment, evasion of host defenses, local damage, and systemic disease.* The biologically active antigenic components of *B. pertussis* listed in Table 129-2 have various roles in pathogenesis. During the past two decades, there have been literally hundreds of research publications exploring the effects of various *B. pertussis* proteins in animal model systems. These studies have revealed a large amount of information, but what is missing for the most part is the correlation or lack of correlation with pathogenesis in human infections.†

Infection is initiated in the respiratory tract by the attachment of *B. pertussis* organisms to the cilia of ciliated epithelial cells.[521] Adhesins (FHA, PT, FIM, LPS) facilitate this attachment.[412] Because of the redundancy of protein adhesins, determining the importance of the individual proteins and ascertaining a primary adhesin or adhesins have been difficult.[333] In animal model systems, FHA is an important adhesin; however, data from two vaccine efficacy trials in which serologic correlates of immunity were studied suggested that FHA may not be necessary if other proteins are present.[97,472] Data from one of these efficacy trials noted that a DTP vaccine that contained a minimal amount of FHA and generated a minimal anti-FHA response had greater efficacy than did a DTaP vaccine that contained a large amount of FHA and generated a vigorous antibody response to FHA.[224,463] However, in a study in which a PT toxoid vaccine was compared with a two-component PT/FHA vaccine, researchers found that the two-component vaccine was more efficacious.[2,470,471,473] This finding suggests that in the absence of antibody to other proteins, antibody to FHA contributes to protection.

Of the other attachment proteins, data relating to human infection are available for fimbriae only. In the two trials that looked at serologic correlates of immunity, researchers found in both that antibody to fimbriae was important.[94,472] Antibodies against fimbriae were shown to provide protection against colonization with *B. pertussis* in the murine respiratory tract.[528]

In many publications, PRN as well as other autotransporters are listed as being adhesins, which, for the most part, is incorrect. The reason for this belief is that antibody to PRN is associated with lack of attachment. However, PRN and other autotransporters have specific biologic activities in infection.‡ The two household contact studies of serologic correlates of protection both found that antibody to PRN was most important in preventing pertussis.[97,472]

*References 77, 96, 107, 288, 305, 322, 399, 456.

*References 96, 118, 180, 238, 333, 370, 372, 375, 411, 521.
†References 1, 10, 11, 24, 54, 55, 114, 118, 128, 158, 165, 175, 234, 236, 275, 326, 327, 333, 372, 387, 395, 411, 428, 492, 505, 521, 529, 546.
‡References 24, 234, 326, 327, 387, 395, 529.

Both ACT and PT adversely affect immune cell function and, therefore, allow infection, once initiated, to continue.* PT prevents migration of lymphocytes and macrophages to areas of infection and adversely affects phagocytosis and intracellular killing. ACT enters phagocytic cells and catalyzes excessive production of cyclic adenosine monophosphate, which intoxicates neutrophils and results in a decrease in phagocytosis.

TCT and DNT have been implicated as contributors to local tissue damage in the respiratory tract.[96,180,375] However, there are no data indicating tissue damage in human infections due to these two toxins.

Pertussis is a unique illness in that fever does not occur and it has only one manifestation of systemic disease in uncomplicated infection: leukocytosis with lymphocytosis caused by PT.[96,231,255] T and B lymphocytes increase to a similar extent in the circulation.[35] In contrast to the situation in *B. pertussis* infection, leukocytosis with lymphocytosis is not a characteristic of *B. parapertussis* infection because this organism does not liberate PT. Clinical experience and recent studies indicate that PT is the major causative factor in deaths in young infants.[373,399] PT inhibits G proteins, and this is the cause of the leukocytosis with lymphocytosis. It has been suggested that the extreme leukocytosis results in obstruction in small pulmonary vessels and this leads to severe pulmonary hypertension.[399] However, it is possible that the inhibition of G proteins in other cells in the lung or heart is the cause of death.[373] An important systemic complication of pertussis is encephalopathy, the mechanism for which is not known. The most likely explanation is anoxia associated with coughing paroxysms.

Some investigators have suggested that pertussis is a toxin-mediated disease caused by PT.[404,405] Although some researchers continue to entertain this idea,[422,423] little evidence supports it. Undoubtedly, PT is a fascinating protein with multiple activities in experimental animals, such as histamine sensitization, promotion of lymphocytosis, effects on glucose metabolism, and induction of adjuvant and mitogenic activity.[96,174,333,372] However, in infections in humans, the main effects that appear to be caused by PT are lymphocytosis and mild, compensated hyperinsulinemia. PT has been suggested to be the cause of the prolonged cough in pertussis. However, because persistent cough is a major manifestation of *B. bronchiseptica* infection in dogs and of *B. parapertussis* infection in children, and because neither organism liberates PT, this hypothesis should be refuted.[231,333,389]

The hallmark of *B. pertussis* illness is paroxysmal cough, and the cause is unknown. The paroxysmal cough is unique because of its long duration. In addition, there is memory for the cough so that it may return when people have subsequent respiratory tract viral infections. Recently, Hewitt and Canning[237] have suggested that bradykinin plays a role in the initiation of the cough. During *B. pertussis* infection, bradykinin production is increased and has a prolonged half-life in the airways and responsiveness to bradykinin reception activation is increased. Bradykinin activates sensory nerves implicated in cough. What is not known is the *Bordetella* protein or proteins that stimulate the unique coughing process.

Cell-mediated immune function is altered by *B. pertussis* infection. In some studies, cell-mediated immunity was depressed, whereas in others it was augmented.[396]

Various antibodies develop after exposure of the human host to infection with *B. pertussis*. The development of agglutinins, hemagglutination-inhibiting antibodies, and bactericidal antibodies has been described.[77] ELISA techniques have demonstrated class-specific antibodies (IgA, IgE, IgG, and IgM) to many of the specific proteins of *B. pertussis*.[219,323,397] These antibodies develop after infection and, with the exception of IgA antibodies, also after immunization. Neutralizing antibody to PT likewise develops after both infection and immunization.[207,397] Specific IgA antibodies to PT and FHA also can be demonstrated in nasopharyngeal secretions and saliva.[186,547]

At present, both *B. pertussis* infection and immunization with whole-cell or acellular pertussis vaccines clearly elicit protection of varying degree and duration against pertussis. The prevailing opinion throughout the past century was that immunity acquired from having *B. pertussis* infection is lifelong whereas vaccine-induced immunity is relatively short-lived. Although the latter clearly is true,[163] studies performed by members of our research group suggest that the former opinion regarding infection-induced immunity is wrong.* Proceeding from the knowledge that IgA antibodies to pertussis antigens (PT, FHA, and PRN) result from infection and not from primary vaccination, our group studied the prevalence of these antibodies in the sera of young German and American men of similar ages.[95] In Germany, routine childhood immunization was not carried out during the 1970s and 1980s and pertussis was epidemic. To our surprise, the rate and mean values of IgA antibodies in the two populations were similar, thus suggesting that adult infection rates were similar. In another study in Germany, we found that *Bordetella* infections were common occurrences in adults, often in persons with a known history of childhood pertussis.[447]

The nature of immunity in pertussis is not known. The consensus has been that serum antibodies greater than some unknown concentration to one or more of the pertussis antigens are responsible for protection.[135] Antibodies to PT, FHA, and PRN have been shown to be protective in animal model systems.[37,119,294,440,452] However, no serologic correlates of immunity had been established until relatively recently, although several large vaccine trials were carried out in the late 1980s and early 1990s.† In a nested household contact study, our group was able to evaluate the roles of IgG antibodies to PT, FHA, PRN, and FIM 2 by determining preexposure imputed values in children at the time of household exposure to *B. pertussis* infection by using both classification tree and logistic regression methods.[97] The imputed geometric mean antibody values to PT, PRN, and FIM 2 were higher in noncases than in cases. In the classification tree analysis, however, only antibodies against PRN contributed

*References 10, 11, 54, 96, 240, 372, 505.

*References 83, 86, 95, 333, 447, 504.
†References 2, 188, 198, 300, 454, 463, 490.

significantly to protection. Specifically, subjects with an imputed PRN value of less than 7 endotoxin units (EU)/mL had a 67 percent likelihood of infection. Logistic regression analysis also found that PRN values of 8 EU/mL or more were associated with prevention of illness after household exposure. In accordance with our findings, data from a Swedish study indicate that antibody values of 5 EU/mL or more to PRN and FIM 2/3 correlated with protection.[472] The data from these two studies indicate that antibody to PRN and FIM are most important in protection. However, what is difficult to understand is why some children get pertussis in spite of large amounts of antibody to either or both PRN and FIM.[100,489] Data from a study by Weiss and associates[99,523] suggested that high antibody values to PT may have a blocking effect on the protective effect of antibody to the more important antigens.

In addition to humoral responses to several *B. pertussis* antigens, evidence exists that cell-mediated immune responses to PT, FHA, and PRN also occur.* Studies in a murine respiratory tract infection model suggest that cellular immunity plays an important role in bacterial clearance and augments the effects of antibody by predominantly T-helper 1 (Th1) cell stimulation.[352,353] Studies in humans demonstrate a cellular immune response shortly after natural infection with *B. pertussis*, with PT, FHA, and PRN preferentially inducing the synthesis of Th1 cells.[527] Immunization with a whole-cell pertussis vaccine results in a Th1 response, whereas the response to acellular vaccines is more heterogeneous and involves both Th1 and Th2 cells.[61] Persistent memory T and B cells and anamnestic antibody responses are important in long-term immunity.[320]

Immunity developed after having *B. pertussis* infection or receiving vaccination with a whole-cell pertussis vaccine does not protect against illness caused by *B. parapertussis*, and, similarly, infection with *B. parapertussis* does not induce protection against disease caused by *B. pertussis*.[289,483] However, in our vaccine efficacy trial in Germany, the results showed some evidence that the acellular pertussis multicomponent vaccine, which contained a large amount of FHA, offered some protection against *B. parapertussis* infections, whereas the whole-cell vaccine, which contained minimal amounts of FHA, did not.[229] Although *B. pertussis* infection is a localized infection involving ciliated cells of the respiratory tract, bacteremia has been noted in immunocompromised adults.[68,263,491]

CLINICAL MANIFESTATIONS

The clinical manifestations of *B. pertussis* infection have considerable variation that depends on age, previous immunization or infection, the presence of passively acquired antibody, and perhaps other factors such as the degree of exposure, host genetic and acquired factors, and the genotype of the organism. The incubation period for pertussis generally varies between 6 and 20 days; most cases have an onset 7 to 10 days after exposure. However, after household exposure, 22 percent of secondary cases were noted to have an onset more than 4 weeks after the onset of illness in the primary case.[224]

Classic Illness

Classic illness occurs as a primary infection in unimmunized children between the ages of 1 and 10 years.[86,96,106,183,396] The illness usually lasts 6 to 12 weeks and has three stages: catarrhal, paroxysmal, and convalescent. The initial illness is characterized by rhinorrhea, lacrimation, and mild cough suggesting a common cold. Body temperature usually is normal. The severity of the cough gradually increases over a period of 1 to 2 weeks, but pertussis usually is not suspected until the cough becomes paroxysmal.

After the catarrhal period, the coughs increase in severity and number. Repetitive series of 5 to 10 or more forceful coughs during a single expiration can be noted. These paroxysms are followed by a sudden massive inspiratory effort, and a characteristic whoop may occur as air is forcefully inhaled through a narrowed glottis. Cyanosis, bulging eyes, protrusion of the tongue, salivation, lacrimation, and distention of neck veins occur during paroxysms. Several paroxysmal coughing episodes with their associated massive inspiratory effort may occur sequentially until the child succeeds in dislodging the obstructing mucus. The production of purulent sputum does not occur. Posttussive vomiting is a common occurrence. Paroxysms may strike several times per hour, and they occur during both day and night.

The paroxysmal episodes are exhausting, and patients frequently appear dazed and apathetic. Weight loss may occur as a result of vomiting and also because eating and drinking may be resisted, given that they trigger attacks. Attacks also may be triggered by yawning, sneezing, or physical exertion. Between attacks, the patient may appear normal and usually is in no distress.

Common and important complications of classic pertussis include pneumonia, otitis media, seizures, and encephalopathy. Pneumonia may be caused by *B. pertussis* or secondary bacterial invaders. Atelectasis may develop as a result of the mucus plugs. The forcefulness of the paroxysms can cause rupture of the alveoli and can produce interstitial or subcutaneous emphysema.

Otitis media is a common occurrence and frequently is caused by *S. pneumoniae*. Pertussis also has been associated with activation of latent tuberculosis. Convulsions and coma may be observed. These findings may be a reflection of cerebral hypoxia related to asphyxia. Rarely, subarachnoid and intraventricular hemorrhage may occur. Tetanic seizures may be associated with the severe alkalosis that results from the loss of gastric contents caused by persistent vomiting.

Other complications that have been noted include ulcer of the frenulum of the tongue, epistaxis, melena, subconjunctival hemorrhage, subdural hematoma, spinal epidural hematoma, rupture of the diaphragm, umbilical hernia, inguinal hernia, rectal prolapse, dehydration, meningoencephalitis, the syndrome of inappropriate

*References 17, 320, 352-354, 436,437, 488, 527, 548.

secretion of antidiuretic hormone, apnea, rib fracture, and nutritional disturbances.*

The convalescent stage, which usually lasts 1 to 2 weeks, is characterized by a decreasing frequency and severity of coughing episodes, whooping, and vomiting. All patients with classic pertussis caused by primary infection have leukocytosis associated with lymphocytosis. Fever and pharyngitis are not usual manifestations in pertussis, and, therefore, a search for a secondary cause should be undertaken when these findings occur. Except for the observation of typical paroxysms, physical examination in pertussis usually is unrewarding. Diffuse rhonchi may be noted on auscultation.

Infection with *B. parapertussis* causes an illness that is similar to that caused by *B. pertussis*, but generally it is less severe and of shortened duration.[230,301,330]

Mild Illness and Asymptomatic Infection

Mild, nonclassic illness is common in *B. pertussis* infection.[135,222,226,444] It occurs in previously vaccinated individuals and also as a primary infection in nonvaccinated children. In a study in which physicians were encouraged to send nasopharyngeal specimens from ill children with cough, regardless of whether the illness was typical of pertussis, to our laboratory,[222] 247 culture-positive cases were noted. Of these patients, 47 percent had a total duration of cough of 28 days or less. In 26 percent, the duration of cough was less than 3 weeks. Most of these cases occurred in unvaccinated children. In a 6-year, similar study involving 2592 culture-positive, previously unvaccinated children, 38 percent had a duration of cough of 28 days or less and in 17 percent it was 21 days or less.[226] In a study in which both culture and PCR were used for establishing the diagnosis of *B. pertussis* infection, many mild cases were found to be PCR positive and culture negative.[444] Of the patients with these cases, only 68 percent had a cough lasting 4 weeks or longer and only 57 percent and 32 percent had paroxysmal cough and whoop, respectively.

In studies of household contacts, asymptomatic infections in family members are common occurrences.[135,313,314] Deen and associates[135] found that 52 (46%) of 114 household contacts who remained well had laboratory evidence of *B. pertussis* infection. In another study, 21 of 399 healthy infants who were control subjects had PCR-positive nasopharyngeal samples.[225]

Infants

Pertussis in infancy is a unique experience.[333] Its spectrum of clinical manifestations varies by age, immunization, and the presence or absence of transplacentally acquired antibody.† Most deaths resulting from *B. pertussis* infection occur in neonates and early infancy, and morbidity is most severe in infants.‡ From 1997 through 2000, 8276 cases of pertussis were reported in infants in the United States.[64] Of this group, 59 percent of the infants were hospitalized, 11 percent had pneumonia, 1 percent had seizures, 0.2 percent had encephalopathy, and 0.7 percent died. Eighty-seven percent of these cases occurred in children younger than 6 months of age.

B. pertussis infection in neonates is particularly severe, with a death rate of between 1 and 3 percent.* Clinical characteristics of pertussis in early infancy are presented in Table 129-3. A common initial finding is apnea, and typical coughing may not be observed. Seizures in association with apnea caused by hypoxia occur frequently. Severe pulmonary hypertension is a relatively common problem in pertussis in the first 4 months of life.† The severity of disease and the risk for death correlate directly with the white blood cell (WBC) count and, in particular, the number of lymphocytes.‡ WBC counts in the range of 30,000 to more than 100,000 cells/mm^3 are common findings. A recent California Department of Public Health study provided useful information relating to risk factors for pulmonary hypertension and death.[373] This first study involved 31 infants 90 days of age or younger with pertussis admitted to a pediatric intensive care unit in Southern California. Infants with pulmonary hypertension or who died had higher WBC counts, had more rapid pulse and respiratory rates, and were more likely to have pneumonia than those who did not have pulmonary hypertension or died. In addition, in those with pulmonary hypertension or who died, the WBC count rose more rapidly and the pulse and respiratory rates attained higher levels sooner than in those without pulmonary hypertension or who died.

Coinfection with respiratory viruses (RSV, adenovirus, influenza viruses) and respiratory bacterial pathogens

TABLE 129-3 **Clinical Features of Pertussis in Young Infants**

Initially infant looks deceptively well; coryza, sneezing, clearing throat, no fever, mild cough
Paroxysmal stage: gagging, gasping, eye bulging, bradycardia (tachycardia occurs in severe illness), cyanosis, vomiting
Leukocytosis with lymphocytosis
Apneic episodes
Seizures
Respiratory distress
Pneumonia and pulmonary hypertension
Shock/hypotension with renal failure
Death associated with pulmonary hypertension and pneumonia
Adenovirus or respiratory syncytial virus coinfection can confuse picture

*References 58, 86, 260, 332, 408, 458, 517.
†References 30, 77, 96, 108, 151, 155, 228, 284, 338, 378, 384.
‡References 25, 30, 56, 108, 121, 122, 127, 161, 177, 185, 194, 197, 201-203, 225, 228, 230, 248, 296, 333, 336, 338, 346, 373, 376, 378, 384, 399, 402, 406, 431, 435, 443, 455-457, 460, 481, 508, 530.

*References 30, 108, 197, 228, 230, 248, 303, 338.
†References 127, 139, 185, 194, 203, 399, 402, 460, 530.
‡References 30, 108, 127, 194, 228, 338, 346, 399, 402.

(Streptococcus pneumoniae, Haemophilus influenzae) are relatively frequent.*

B. pertussis infection has been noted in association with SIDS, but whether a cause-and-effect relationship exists is not clear.[225,230,303,384] Nichol and Gardner,[384] in a study in England, found that many deaths attributed to SIDS were, in fact, related to *B. pertussis* infection. Using PCR, we found *B. pertussis* DNA in nasopharyngeal specimens from 9 (18%) of 51 infants who had sudden, unexpected deaths.[230] In a subsequent study, we collected specimens for PCR from 254 infants who experienced sudden, unexplained deaths and from 441 healthy matched controls.[225] The rate of PCR-positive results in the sudden death cases was 5.1 percent; it was 5.3 percent in the controls. In a careful follow-up histopathologic study with unique immunohistochemical staining of specimens from a subset of these fatal cases, we could find no evidence of specific *B. pertussis* pulmonary infection or pathologic features.[104]

The source of infection in infants usually is a family member.† In a study of 616 infant cases, the source was identified in 43 percent.[39] A family member was the source 75 percent of the time, and the mother was the most common source (32%). Of the source persons, 56 percent were adults and 20 percent were 10 to 19 years of age.

Adults

Increased awareness of adult pertussis has occurred since the 1970s.‡ Unrecognized pertussis cases in adults often are the source from which infants and children become infected.§ All adults previously have been exposed to *B. pertussis* antigens by immunization, infection, or both,[95,135,136,333] and this exposure tends to modify their illness. In a U.S. study, the following cough characteristics were noted in 31 university students with laboratory evidence of *Bordetella* infection: the median duration was 21 days before initial evaluation, 94 percent had one or more coughing episodes per hour, and 90 percent of the coughs had a staccato or paroxysmal quality.[356] Despite these findings, pertussis was not suspected in any of the students; and clinical diagnoses by the primary care providers included upper respiratory tract infection (39%), bronchitis (48%), and other diagnoses (16%). Although specific records were not available, most of these students probably were vaccinated as children and almost certainly had previous, unrecognized infections.[95,135]

In contrast to these findings in the United States, in a study in Germany adults were found to be more likely to have typical pertussis, even though the epidemiologic data suggested that all had a previous infection and 26 percent of 64 patients with laboratory evidence of infection recalled having had pertussis during childhood.[95,447] Rates of clinical manifestations in the 64 laboratory-confirmed cases were as follows: paroxysms, 70 percent; whoop, 38 percent; post-tussive phlegm, 66 percent; and posttussive vomiting, 17 percent. The clinical diagnosis in 39 percent was definite or probable pertussis; only 14 percent were thought not to have pertussis. Of note is that the clinical diagnosis was not made by primary care physicians but by a small team of specially trained central investigators with high awareness for pertussis.

Similar findings were noted in a German household contact study: 80 percent of 79 adults with laboratory-confirmed *B. pertussis* infection coughed for 3 or more weeks and 63 percent had spasmodic cough for 3 or more weeks.[408] In addition, in 53 percent of patients, coughing was followed by choking or vomiting. However, only 8 percent of the adults in this study had whoops. Complications that were observed included pneumonia, rib fracture, inguinal hernia, and severe weight loss. Unique sweating episodes are reported in approximately 5 percent of adults, and fainting can occur in association with coughing.[132,408]

DIFFERENTIAL DIAGNOSIS

In typical pertussis, the clinical diagnosis should be apparent based on the paroxysmal cough with posttussive vomiting and whooping and lack of significant fever. However, the cause of the illness can be *B. pertussis*, *B. parapertussis*, *B. holmesii*, and perhaps *B. bronchiseptica*. A history of contact with a known case will help to establish the diagnosis in a patient with mild or atypical illness. The presence of leukocytosis with lymphocytosis in a child with a cough or the presence of apnea in an infant is a strong indication that the illness is caused by *B. pertussis* and not another *Bordetella* spp. In a matched-control comparison, none of 11 children with culture-proven *B. parapertussis* infection versus 7 of 22 (32%) with *B. pertussis* infection had lymphocytosis of 10,000 cells/mm^3 or more.[231] Lymphocytosis of 10,000 cells/mm^3 or more is observed in few other illnesses that manifest with a cough.

Many other infectious agents cause illnesses with prolonged and repetitive cough that can be confused with *B. pertussis* infections.[333] In particular, *M. pneumoniae*, *C. pneumoniae*, adenoviruses, bocavirus, and other respiratory viruses all can cause a prolonged cough.* In addition, coughing episodes may be seen in children with asthma, bronchiolitis, bacterial pneumonia, cystic fibrosis, *Coccidioides immitis* infection, and other fungal pulmonary infections and tuberculosis. Another problem is the cough associated with sinusitis, which can be confused with *B. pertussis* infection. The cough associated with gastroesophageal reflux also can be confused with pertussis, as can cough associated with a foreign body in the airway.

DIAGNOSIS

A laboratory diagnosis of pertussis caused by *B. pertussis* or other *Bordetella* spp. can be made by culturing the

*References 12, 30, 117, 122, 124, 283, 296, 358, 399, 456.
†References 25, 39, 122, 135, 151, 284, 523.
‡References 38, 39, 65, 66, 69, 70, 85, 87, 89, 91, 99, 106, 131, 132, 135, 136, 155, 178, 197, 241, 262, 265, 287, 333, 355, 356, 378, 381, 408, 425, 433, 447, 475, 487, 514, 534, 544.
§References 30, 39, 85, 108, 228, 338, 378, 447, 524.

*References 15, 22, 77, 96, 111, 113, 125, 204, 379, 536.

organisms on appropriate media, by PCR, and by demonstrating the presence of specific antibodies. *Bordetella* spp. can be recovered from nasopharyngeal specimens, with the highest rate of isolation occurring within the first 3 weeks of cough.[222,474] Specimens for culture can be collected by swabbing the nasopharynx, by nasopharyngeal washing, or by nasopharyngeal aspiration.[96,205] In general, nasopharyngeal aspiration gives the highest yield of positive cultures and nasopharyngeal wash is the least sensitive.

B. pertussis and *B. parapertussis* are recovered most easily by direct plating of the specimen from the patient onto selective media.[96,98,245,311] Specific swabs (calcium alginate or Dacron) and media (Regan-Lowe or Bordet-Gengou agar and modified Stainer-Scholte broth) are required, and laboratory personnel should be experienced in isolating the organisms. If cultures cannot be inoculated directly, the use of Regan-Lowe transport medium is recommended. In classic disease, the culture will be positive in approximately 80 percent of cases if the specimen is obtained within 2 weeks of the onset of cough and antibiotics have not been administered previously.[223,397] Direct fluorescent antibody testing has been used for the diagnosis of pertussis caused by *B. pertussis* or *B. parapertussis* during the past 45 years.[333,371,464] It is rapid and inexpensive, but it lacks sensitivity and specificity; it does not employ amplification for sensitivity, and it lacks specificity because of cross reactions with other organisms of the nasopharyngeal flora.[153] Because of the lack of both sensitivity and specificity, its use is no longer recommended for diagnosis.[106,489,535]

Since the late 1980s, numerous PCR assays with primers derived from many different chromosomal regions have been developed for the diagnosis of *B. pertussis*, *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica* infections, and they have been evaluated in multiple studies by comparison with culture and clinically typical pertussis.*

PCR has the advantage of having much higher sensitivity than that of conventional culture. In a prospective study in which swabs for PCR and culture were obtained simultaneously from 555 ill subjects with cough, the use of PCR increased the identification of *B. pertussis* infection almost fourfold, from 28 to 111.[444] Only a few studies have been reported in which the sensitivity and the specificity of PCR for the diagnosis of *B. pertussis* infection were determined by comparison with serologically identified cases.[227,299,302,418,500] In a study that our group performed, we compared PCR results with serologic diagnosis and found that PCR had a sensitivity of 61 percent and a specificity of 88 percent.[163] Similar findings have been noted in other studies.[299,302,419,500] At present, the most commonly used primers for the diagnosis of pertussis include IS 481 and IS 1001.[105,312,333,486] IS 481 occurs in the genomes of *B. pertussis* (>50 copies) and *B. holmesii* (8 to 10 copies) but not in *B. parapertussis*. IS 1001 occurs in the genomes of *B. parapertussis* and *B. holmesii* (~20 copies) but not in *B. pertussis*. The genome of *B. bronchiseptica* generally contains neither IS 481 nor IS 1001. However, in recent studies, *B. bronchiseptica* strains were found to contain IS 1001 in their genomes (in the form of an IS 1001-like element, called hIS 1001).[169,417,486] False-positive results are a potential problem with the use of PCR for establishing the diagnosis of pertussis and other respiratory illnesses.[4,339,371] False-positive results can occur if specimens are opened in the pertussis laboratory before transport to the PCR laboratory.[333] False-positive results also can result from contamination of the air in a room in which the previous patient had pertussis and in a room where pertussis vaccine was administered.[324,485] Moreover, as noted earlier, *B. bronchiseptica* can be mistaken for *B. parapertussis* when the IS 1001 primer is used.[169,417] Therefore, rigorous internal and external laboratory controls are necessary. In addition, because insertion sequence elements are somewhat promiscuous, nucleic acid amplification tests should, whenever possible, be accompanied with conventional culture.

Obtaining a routine laboratory diagnosis of *B. pertussis* infection in adults or in other atypical cases is hampered by the problem that medical care usually is not sought until the third or fourth week of the illness and antibiotics frequently have been administered before the possibility of pertussis was considered.[106,356,447] During the past 25 years, the most significant advance in the diagnosis of pertussis has been the development of ELISA.[333]

Natural infection with *B. pertussis* is followed by a rise in serum concentrations of IgA, IgG, and IgM antibodies to specific antigens of the organism, as well as to preparations of the whole organism.* In contrast to natural infection, primary immunization of children induces mainly IgM and IgG antibodies.

Serologic testing for *B. pertussis* infection in the clinical setting is not well standardized but is widely available in Europe and North America.[106] In the research setting, the use of ELISA contributed significantly to establishing the diagnosis of *B. pertussis* infection in many patients with negative cultures.† Most useful was the determination of IgG and IgA antibodies to PT and FHA. The most reliable proof of acute infection is the demonstration of a significant increase in antibody values between acute-phase and convalescent-phase serum specimens. Frequently, because collection of acute-phase specimens is delayed and, therefore, the acute-phase values already are elevated, significant increases between first and second serum specimens cannot be demonstrated. However, a diagnosis can usually be established on the basis of a high value or values on a single serum specimen.[135,356,447,533,544] Because *B. parapertussis* infection induces cross-reacting antibodies to *B. pertussis* FHA, the use of this antigen alone cannot differentiate *B. pertussis* from *B. parapertussis* infection.[187,463]

Measurement of agglutinating antibodies also is useful for establishing the diagnosis of *B. pertussis* infection, and, because the test is simple, inexpensive, and accurate, it

*References 9, 105, 106, 141, 152, 156, 179, 191, 200, 218, 227, 252, 280, 298, 299, 302, 312, 324, 329, 339, 392, 417-419, 432, 444, 445, 485, 486, 489, 499-501, 535.

*References 115, 170, 209, 323, 345, 356, 397, 463, 506.
†References 136, 206, 313, 314, 355, 356, 447, 544.

can be used in the clinical setting.[135,232,356,397,447] Unfortunately, its sensitivity is low.

Today in clinical practice, the laboratory diagnosis of pertussis should be approached as follows: In all cases in which the cough is of less than 2 weeks' duration in adolescents and adults or 3 weeks' duration in children, a nasopharyngeal specimen should be obtained for culture or PCR. In adults who have had cough for more than 2 weeks' duration, single serum ELISA is the preferred method provided they have not been immunized against pertussis in the previous 12 months.[106] This method also can be used in children if they have not been immunized within a year. At present, many commercial laboratories offer single serum diagnostic tests for *B. pertussis*, and almost all the offered tests lack specificity. Any test that employs the whole organism in the test is fraught with false-positive results. Tests that report specific IgM antibodies also are unreliable. The greatest sensitivity and specificity for the serologic diagnosis of *B. pertussis* infection are achieved by ELISA or an ELISA-like test with the measurement of IgG and IgA antibodies to PT. Single high values of IgG or IgA antibodies to PT are indicative of infection.[333] A sensitive and specific test in North America is available from Focus Diagnostics, Cypress, California.[412] The performances of a number of European commercial serum ELISA kits for the diagnosis of *B. pertussis* illness has been evaluated by Riffelmann and associates.[421] They found that three kits that used PT as the antigen had specificities and sensitivities from 80 percent to 90 percent.

Not all infected persons develop antibody responses to PT. In children, approximately 25 percent lack an adequate response, as do approximately 10 percent of adolescents and adults.[93,463]

An ELISA also has been developed for the detection of IgA antibody to *B. pertussis* in nasopharyngeal secretions and as an indicator of recent infection.[181,186,547] *B. pertussis* IgA appears in nasopharyngeal secretions during the second or third week of illness and persists for at least 3 months.[181] However, the appearance of secretory IgA may be delayed in children younger than 1 year of age.[374] This antibody is not induced by primary parenteral *B. pertussis* vaccination. Detection with the use of ELISA of *B. pertussis* IgA in secretions may be a diagnostic aid in culture-negative patients whose symptoms have persisted for longer than 3 weeks. To our knowledge, this test is not clinically available.

TREATMENT

Several antibiotics have in vitro efficacy against *B. pertussis*.* The first choice for treatment since the 1970s has been oral erythromycin; this ameliorates the symptoms if it is given early during the course of the illness and eliminates the organism from the nasopharynx within a few days, thereby shortening the period of contagiousness.[34] The dose for children is 40 to 50 mg/kg/day given every 6 hours for 14 days; the dose for adults is 2 g/day given every 6 hours for 14 days. A 7-day course of erythromycin estolate was shown in a large study in Canada to be as efficacious as 14 days of treatment.[208] The newer macrolides, azithromycin 10 mg/kg on day 1 and 5 mg/kg on days 2 to 5 as a single dose for 5 days for children and 500 mg on day 1 and 250 mg on days 2 to 5 for adults or clarithromycin 15 to 20 mg/kg/day in two divided doses for 7 days for children and 1 g/day in two doses for 7 days for adults, also can be expected to be effective.[7,67] Although rare, the use of erythromycin in young infants is associated with hypertrophic pyloric stenosis, so parents need to be educated about the symptoms of this potential risk.[62,244,365] Because of this risk, the Centers for Disease Control and Prevention recommends treating neonates with azithromycin rather than erythromycin.[67] Trimethoprim-sulfamethoxazole can be used as an alternative agent in those who cannot tolerate erythromycin.[250] The first erythromycin-resistant strain of *B. pertussis* was isolated from a 2-month-old male infant in Yuma County, Arizona, in June 1994.[296] The isolate was highly resistant, with a minimal inhibitory concentration (MIC) greater than 64 μg/mL (the usual MIC of erythromycin is 0.02 to 0.1 μg/mL). Four more resistant *B. pertussis* strains were recovered from cases in California, Utah, and Minnesota.[196,282,292] In France, macrolide resistance developed during illness in a neonate. In Japan, six *B. pertussis* strains were found to have high level resistance to nalidixic acid and decreased susceptibilities to fluoroquinolones.[391] At present, no evidence of a pattern of emerging macrolide resistance has been seen, but because PCR rather than culture is becoming the diagnostic method of choice in many laboratories, the chance of missing resistant strains is a possible problem.

Patients infected with *B. parapertussis* and *B. holmesii* also can be treated with macrolides, but *B. bronchiseptica* usually is resistant to erythromycin so that alternative therapy is necessary.[333] *B. bronchiseptica* strains usually are sensitive to aminoglycosides, extended-spectrum third-generation penicillins, tetracyclines, quinolones, and trimethoprim-sulfamethoxazole.

Historically, no infectious disease has a greater list of remedies lauded as beneficial but without objective evidence of effectiveness.[36,90] Supportive care includes avoidance of factors that provoke attacks of coughing and maintenance of hydration and nutrition. In the hospital, gentle suction to remove secretions and well-humidified oxygen may be required, particularly in infants with pneumonia and significant respiratory distress. In severe infections in neonates and young infants, assisted ventilation is often necessary.

However, infants who develop pulmonary hypertension with respiratory and cardiovascular failure respond poorly to aggressive therapy (pulmonary artery vasodilators and extracorporeal membrane oxygenation [ECMO]) and have high mortality rates. Because data suggest that the refractory pulmonary hypertension results from the extreme leukocytosis with lymphocytosis, which is always present in fatal cases, our opinion is that leukocyte-reducing measures such as exchange transfusion should be implemented.[138,194,402,431] Exchange transfusion may be

*References 22, 27-29, 34, 67, 246, 249-251.

better than leukofiltration because it removes PT as well as reducing the WBC count.

The use of corticosteroids has received attention in the treatment of pertussis.[36,551] Cortisone treatment in the murine model of pertussis increased the mortality rate.[256] In a study by Roberts and associates,[424] dexamethasone treatment did not shorten the course of hospitalization compared with untreated controls.

The use of salbutamol has also been suggested as having some value, but no benefit was noted in three studies reviewed by Bettiol and coworkers.[36,47] Pillay and Swingler[403] reviewed the symptomatic treatment of pertussis and found no statistically significant benefit for the use of diphenhydramine, dexamethasone, or salbutamol.

PROGNOSIS

The prognosis in pertussis is related to the patient's age. In older children and adults, the prognosis is good, but infants have a significant risk for death and development of encephalopathy.* In addition, long-term follow-up suggests that apnea or seizures at the time of disease in children may be associated with subsequent intellectual impairment.[477] The present availability of pediatric intensive care units and assisted ventilation has reduced the rate of mortality in infants who receive medical care.[228] Unfortunately, many deaths occur outside the hospital. No evidence has shown that pertussis impairs ventilatory function later in life.[266]

PREVENTION

Vaccine Efficacy

Whole-Cell Vaccines

The first pertussis vaccines were developed in the 1920s, and effective vaccines have enjoyed worldwide use since the 1940s.[77,94,96,333] After World War II, extensive vaccine trials were organized by the British Medical Research Council.[340-342] Based on these studies, as well as smaller studies in England, the United States, and other countries, DTP vaccine use became routine in many countries.[164]

The pertussis attack rate was relatively constant in the United States in the prevaccine era between 1922 and 1942. From 1943 to 1976, a 150-fold reduction in the attack rate was noted in association with widespread childhood pertussis immunization.

The existence of a relationship between vaccine use and disease control also was supported by data from England and Wales. The pertussis attack rate declined between 1958 and 1973 and increased dramatically between 1977 and 1983 after a marked decrease occurred in the number of vaccinations administered, beginning in 1974.[77] The attack rate decreased with the widespread use of vaccine and increased when vaccine use decreased. Moreover, the attack rate after the decrease in vaccine use was increased most markedly in the newly susceptible cohort of children younger than 4 years of age.

In the 1980s, numerous household contact studies were performed.[59-61,135,398] In a study from 1982 to 1983 involving 440 household contacts aged 6 months to 9 years, the secondary attack rate in unvaccinated contacts was compared with the rate in children who had received three or more DTP doses. Vaccine efficacy was found to be 91.4 percent. A similar study conducted during the period 1979 to 1981 revealed an efficacy of 82.4 percent.

In another study, Onorato and associates[398] noted that the calculated efficacy varied markedly according to the clinical case definition. Efficacy against any cough was 63 percent, whereas it was 83 percent if a cough duration of 21 or more days was required.

In the 1990s, the most definitive studies of vaccine efficacy of both DTP and DTaP were performed in four countries.* In these trials, the efficacy of the candidate DTaP vaccines was compared with the efficacy of DTP vaccines; the controls were subjects who received DT. With the exception of one lot of one DTP vaccine (Connaught, USA), which had poor immunogenicity, the DTP vaccines were more efficacious than were the DTaP vaccines. The poor efficacy of the Connaught DTP vaccine probably was the result of an unusual low potency lot of this vaccine rather than a generic problem with this vaccine.[333] In a case-control study in the United States, the efficacy of the Connaught vaccine was similar to that of the Wyeth-Lederle DTP vaccine, which, in the controlled trial in Germany, had a high level of efficacy.[40] Finally, in a large comparative trial, the DTP vaccine from England (Evans vaccine) had efficacy greater than those of the three DTaP vaccines with which it was compared.[394]

Acellular Vaccines

Research in the 1970s showed that three *B. pertussis* antigens (PT, FHA, LPS) were liberated into the medium during culture and that these antigens could be concentrated and separated by density gradient centrifugation.[96,238] This finding allowed for the development and production of vaccines by six manufacturers in Japan.[102,279,386,441] All six vaccines had minimal or no endotoxin but different amounts of PT and FHA. In addition, some of the vaccines were found to contain FIM 2 and PRN.

Despite limited proof of efficacy, the six vaccines were put into routine use in Japan in 1981, and they have controlled, to some degree, reported epidemic pertussis during the ensuing decades. However, because adequate data were not available on any single vaccine or on vaccine use in young infants, many extensive trials were performed subsequently in Europe, Africa, and Japan.†

After extensive analysis of the data was conducted on the original efficacy trial in Sweden in the mid 1980s, calculated efficacy was found to vary significantly,

*References 25, 30, 108, 121, 122, 154, 155, 177, 194, 197, 248, 336, 338, 346, 373, 376, 406, 431, 435, 443, 455, 456, 481, 508.

*References 188, 198, 224, 302, 450, 454, 463.
†References 2, 188, 198, 224, 300, 369, 454, 463.

TABLE 129-4 **Pertussis Antigens in Nine Diphtheria-Tetanus-Acellular Pertussis Vaccines Evaluated in Eight Efficacy Trials (1990 to 1997)**

Vaccine Product	Pertussis Toxin (μg/Dose)	Filamentous Hemagglutinin (μg/Dose)	Pertactin (μg/Dose)	Fimbriae (μg/Dose)
Certiva*	40			
Tripedia*†	23.4	23.4		
Triavax	25	25		
SKB-2‡	25	25		
Acelluvax	5	2.5	2.5	
INFANRIX*	25	25	8	
Acel-Immune*	3.5	35	2	0.8§
Daptacel*	10	5	3	5‖
CCL DTaP5¶	20	20	3	5‖

*Licensed in the United States.
†This vaccine was subsequently found to contain both pertactin and fimbriae
‡No product name.
§Fimbriae 2.
‖Fimbriae 2 and 3.
¶No product name; however, this vaccine is available in Canada with IPV as Quadracel and in Canada and the United States with IPV and Hib as Pentacel.

depending on the clinical case definition and the laboratory methods.* Therefore, researchers decided that a universal primary case definition should be developed for use in all subsequent efficacy trials so that different vaccines in different trials could be compared.

A World Health Organization (WHO) committee met in Geneva in January 1991, and a primary case definition was developed.[542] This definition and minor variations of it were used in the efficacy trials in the 1990s. The WHO case definition is as follows: (1) an illness with 21 days or more of spasmodic cough and either culture-confirmed infection with *B. pertussis* or serologic evidence of infection with *B. pertussis* as indicated by a significant rise in IgA or IgG antibody by ELISA against PT or FHA in paired sera or (2) contact with a case of culture-confirmed pertussis in the household with onset within 28 days before or after the onset of cough in the study vaccinee. Not all members of the WHO committee, including one of us (J.D.C.), agreed with this primary case definition because its use results in the elimination of many laboratory-confirmed cases from efficacy calculations.† With this definition, vaccines that lessen the severity of disease but are poor at preventing mild disease will be overrated.

In 1994 and 1995, seven efficacy trials with candidate DTaP vaccines in four countries were completed,‡ and an additional trial in Sweden was completed in 1997.[394] As noted in Table 129-4, the nine vaccines are different in the number of antigens that they contain, as well as in the concentrations of the specific antigens. In all efficacy studies, confounding factors may affect the results. In general, double-blind studies with placebo and whole-cell vaccine controls are ideal. However, placebo control was not ethical in countries in which DTP vaccine was recommended. Therefore, studies in Germany and Senegal used various methods to obtain efficacy data in spite of the lack of a blinded diphtheria-tetanus (DT) toxoid group. Observer bias can affect the results of all studies, including those with double-blind control. For example, a less efficacious vaccine that prevents typical disease but not mild disease can be determined to be more efficacious than it actually is if the study observers believe they "know pertussis" and dismiss atypical cases as being other respiratory tract illnesses and do not obtain cultures or conduct prospective follow-up.[101]

In general, household contact studies, unless they are nested analyses in prospective cohort studies, also are subject to observer bias, and case-control studies result in significantly inflated efficacy percentages.* In cohort studies, observer bias by parents can be reduced by frequent prospective telephone contact with study families. Finally, serologic diagnosis, as well as diagnosis by culture, increases the identification of mild cases, which are more likely to occur in vaccinees than in control subjects.

A summary of the efficacy data for 10 acellular pertussis vaccines evaluated in the 8 trials performed in the 1990s and the earlier 1980s Swedish trial is presented in Table 129-5. The data in this table indicate that three- and four-component vaccines (vaccines containing PRN and FIM as well as PT and FHA) have greater efficacy against *B. pertussis* illness (mild and typical) than do the PT or PT/FHA vaccines. The apparent high efficacy of the two-component vaccine (Tripedia) in the Munich study[300] can be explained by the type of study (case-control study), significant observer bias, the vaccine containing PRN and FIM as well as PT and FHA, and the lack of serologic diagnosis. The final study, done in Stockholm, was a comparative study without a DT

*References 2, 41, 206, 470, 471, 473.
†References 84, 222, 226, 470, 471, 473.
‡References 188, 198, 300, 450, 454, 463, 490.

*References 84, 101, 103, 162, 164, 369.

TABLE 129-5 **Vaccine Efficacy Data for 10 Acellular Pertussis Vaccines Evaluated in Eight Trials Performed in the 1990s and the Earlier 1980s Swedish Trials**

Location	Design	Vaccine	Schedule	Efficacy	
				TYPICAL PERTUSSIS (%)	MILD AND TYPICAL PERTUSSIS (%)
Sweden, Stockholm[1,29,368]	Double-blind prospective cohort	JNIH-6	2 doses (2-3 mo apart starting at 5-11 mo of age)	84*	42
		JNIH-7		90	–7
Sweden, Göteborg†[385]	Double-blind prospective cohort	Certiva	3 doses (3, 5, 12 mo)	71	54
Sweden, Stockholm[146]	Double-blind prospective cohort	SKB-2	3 doses (2, 4, 6 mo)	59	42
		Daptacel		85	78
Italy, Rome[139]	Double-blind prospective cohort	Acelluvax	3 doses (2, 4, 6 mo)	84	71
		INFANRIX		84	71
Germany, Erlangen[362]	Prospective cohort	Acel-Immune	4 doses (3, 4½, 6, 15-18 mo)	83	72
Germany, Mainz[351]	Household contact	INFANRIX	3 doses (3, 4, 5 mo)	89	81
Germany, Munich‡[231]	Case control	Tripedia	4 doses (2, 4, 6, 15-25 mo)	80, 93	—
Senegal§[357]	Household contact	Triavax	3 doses	31, 74	—

*Efficacy against typical pertussis based on positive culture without serologic analysis.
†Significant observer bias occurred in this trial.[56]
‡Laboratory diagnosis based on culture only; 80 percent efficacy was against illness with cough of 21 or more days, and 93 percent efficacy was against the World Health Organization (WHO) case definition.
§Thirty-one percent efficacy based on 21 days or more of illness with cough; 74 percent efficacy was against the WHO case definition.

control group.[393] In this trial, in which vaccines were administered to children at 3, 5, and 12 months of age, the efficacy of CCL DTaP5 (a five-component vaccine) was significantly better than that of Acelluvax (a three-component vaccine) and was not significantly different from that of the comparative DTP vaccine (Evans vaccine). Acelluvax had greater efficacy than did the two-component vaccine SKB-2.

Follow-up studies of the various efficacy trials have been done in Sweden, Italy, and Germany, and all suggest sustained protection.[199,315,393,438,439] Of particular importance in this regard is the most recent report by Gustafsson and associates,[198] in which members of the large cohort vaccinated at 3, 5, and 12 months in the 1993 to 1996 trial were observed from October 1997 to September 2004. Overall, good protection lasted for approximately 5 years. The tabular data in this report suggest that Acelluvax had efficacy similar to that of CCL DTaP5. However, when all the data from the original trial and the present follow-up data are combined, the overall attack rate in those children who had received CCL DTaP5 was lower (47.5/100,000) than that in children who had received Acelluvax (59.7/100,000). This difference was not significantly different, however. Most notable is that the Evans DTP vaccine had greater sustained efficacy than did either Acelluvax or CCL DTaP5. In summary, it appears that DTaP vaccines that contain PRN have relatively good efficacy for approximately 5 years but that (with the exception of one lot of one DTP vaccine), in general, DTP vaccines are more efficacious.*

Around 2005, two acellular pertussis component, diphtheria, and tetanus toxoid vaccines (Tdap vaccines) became available for use in adolescents and adults.† These two vaccines are Adacel and BOOSTRIX, and their components are presented in Table 129-6. Both these vaccines elicit vigorous antibody responses to the antigens that they contain after a single dose. These responses are significantly greater than those observed in infants at 7 months of age after they have received three doses of the DTaP vaccines (Daptacel and INFANRIX) of the two manufacturers.

In a double-blind efficacy trial in adolescents and adults, an acellular pertussis vaccine (without diphtheria and tetanus toxoids) with the same concentrations of PT, FHA, and PRN as BOOSTRIX had an efficacy of 92 percent.[513] However, the data safety monitoring board allowed the removal of one case in a vaccinee. If this case is included, the efficacy is approximately 78 percent. No similar efficacy trial was done with Adacel or its acellular pertussis components. However, extensive epidemiologic data from Canada, where Adacel has been used since 2003, show a marked reduction of pertussis in adolescents.[271]

*References 188, 198, 300, 394, 438, 450, 454, 463.
†References 69, 70, 88, 167, 168, 221

TABLE 129-6 **Antigenic Composition of Adacel and BOOSTRIX per 0.5 mL**

Antigen	Adacel	BOOSTRIX
Diphtheria toxoid	2 Lf	2.5 Lf
Tetanus toxoid	5 Lf	5 Lf
Pertussis toxin toxoid (PT)	2.5 µg	8 µg
Filamentous hemagglutinin (FHA)	5 µg	8 µg
Pertactin (PRN)	3 µg	2.5 µg
Fimbriae 2/3 (FIM 2, 3)	5 µg	—

Lf, flocculation units.

The 2010 epidemic of pertussis in California, as well as more recent outbreaks in other states and Australia, has led to a number of studies of Tdap vaccine failure and vaccine effectiveness.[72,414,519,537] In a study by Wei and associates, Tdap vaccine effectiveness in school children 11 years of age and older was 66 percent.[519] However, the sample in this study was small so the result was not statistically significant. In a study of adolescents, Tdap effectiveness was found to be 78 percent.[415]

Adverse Events

Whole-Cell Vaccines

Local reactions and relatively mild systemic complaints are frequent occurrences after pertussis immunization. Less commonly, severe neurologic illness and death have been noted in temporal association with DTP immunization.

The largest study in the United States designed to assess the risk for relatively common and uncommon reactions to pertussis vaccine was performed by Baraff and associates.[18-21,110] This study was conducted between January 1978 and December 1979. Reactions in children who received either DTP or DT immunization were compared. A total of 15,752 DTP immunizations and 784 DT immunizations were given to children aged birth to 6 years of age. These children were evaluated for reactions that occurred within 48 hours of vaccine administration. All common local and systemic reactions occurred more frequently in the DTP recipients than in the DT group. Differences between the common reactions in the two groups were all highly significant ($p < .005$).

Redness at the injection site occurred in 37.4 percent of DTP recipients and in 7.6 percent of DT vaccinees. Fever (≥38° C [100.4° F]) was noted in 46.5 percent of DTP recipients. A temperature of 39° C (102.2° F) or higher occurred in 6.1 percent of DTP recipients, but in only 0.7 percent of DT recipients. Drowsiness, fretfulness, vomiting, anorexia, and persistent crying were other reactions recorded in 3.1 percent (persistent crying) to 53.4 percent (fretfulness) of DTP recipients versus 0.7 percent (persistent crying) to 22.6 percent (fretfulness) of DT vaccinees. In general, rates of local reactions, but not those of systemic reactions (except fever), increase from dose to dose in an immunization series.

In addition to these reactions, 0.1 percent of DTP recipients in this study were reported by the parents to have a high-pitched, unusual cry; 0.06 percent had convulsions, and 0.06 percent had hypotonic-hyporesponsive episodes (shock, collapse). No children in the control group (DT recipients) had similar reactions; however, the control group was of modest size (784 DT recipients), so statistical significance could not be assigned to any of these relatively uncommon events.

Because convulsions in young children are the result of many different etiologic factors, the cause-and-effect relationship with pertussis vaccine is less clear. However, inasmuch as fever develops in almost half of all DTP vaccinees and febrile convulsions are not uncommon events, a reasonable assumption is that many convulsions that occur in temporal association with DTP vaccination are induced by the immunization. Three studies have noted a significant association between pertussis immunization and febrile convulsions.[23,453,510] Approximately 1 per 1000 vaccinees older than 6 months of age will have a first febrile seizure after receiving pertussis immunization. The concomitant use of acetaminophen (15 mg/kg per dose at the time of immunization and every 4 hours for 24 hours) and DTP vaccine has been suggested as a means of reducing the incidence of febrile convulsions in vaccinees.[295]

Neurologic disease and death occurring in temporal association with pertussis immunization have been of major concern throughout the vaccine era. During the past 60 years of the 20th century, several case series and individual cases of neurologic illness occurring after receipt of pertussis immunization were reported; by 1979, more than 1000 cases of alleged neurologic damage induced by pertussis vaccine were reported.* Few of these reports had evidence of an adequate search for other possible causes of the neurologic disease, and in none were data available for rate calculations.

From 1967 to 1980, several attempts were made to determine the frequency of neurologic disease after receipt of pertussis immunization.† However, because controls were not included in any of the population evaluations, all rate estimates included children with temporally related events that had other causes.

A carefully designed prospective case-control study (National Childhood Encephalopathy Study [NCES]) of all hospital admissions of children aged 2 to 35 months with acute serious neurologic disorders in England, Wales, and Scotland was undertaken between 1976 and 1979.‡ The results of this study for the first time revealed an apparent statistical association between pertussis immunization and neurologic illness. Researchers found that a child who had received DTP vaccine within the previous 3 to 7 days was two to five times more likely to have neurologic disease than was a child who was not immunized during the same interval. The causal relationship between DTP immunization and neurologic illness noted in this study must be questioned, however,

*References 33, 53, 75, 77, 285, 328, 531.
†References 3, 77, 143, 144, 166, 214, 467, 476.
‡References 5, 31, 318, 347-350.

because both cases and controls had an equal frequency of immunization during the month preceding the index date. A more appropriate interpretation of the results is that they do not indicate cause and effect; rather, the DTP immunization calls attention to or brings out something that will occur anyway but just moves it forward in time ("trigger effect").[78,79]

Cases of infantile spasms, an identifiable seizure disorder of infancy, usually occur in the 6-month period from 2 to 7 months of age; therefore, that some cases occur after DTP immunization is not surprising. Simple calculations indicate that approximately 12 percent of all patients destined to have infantile spasms between 2 and 7 months of age will have an onset of illness within 7 days after receiving DTP immunization. The temporal association between DTP immunization and infantile spasms has led many people to assume a cause-and-effect relationship. However, controlled data from the NCES in Great Britain provide strong evidence against a causative role for pertussis vaccine in infantile spasms.[31] In another study, Melchior[343] in Denmark noted that the time of onset of infantile spasms was not altered when the time of pertussis immunization was changed from 5, 6, 7, and 15 months of age to 5 weeks, 9 weeks, and 10 months of age. In both periods, 42 percent of patients had their onset during the first 4 months of life.

Data from the NCES were reanalyzed with the exclusion of cases of infantile spasms.[349,350] From these analyses, the risk for permanent brain damage occurring from pertussis immunization has been suggested to be 1 per 330,000 vaccine doses and the risk for any encephalopathy has been estimated to be 1 per 140,000 vaccinations. However, a review of the NCES data by other investigators indicates that both rate estimates are incorrect. Specifically, Stephenson[466] showed that the 1 per 140,000 rate for all encephalopathy is an artifact resulting from the inclusion of 9 children with febrile convulsions. Similarly, MacRae[317] noted that the increased relative risk that was observed within 7 days of receiving immunization (which was used to calculate the risk for brain damage of 1 per 330,000 immunizations) was offset by a decreased relative risk over the subsequent 3-week period. This finding, similar to the original study data and the infantile spasm data, indicates not a cause-and-effect relationship but rather a redistribution of events over time.

In the United States, the major neurologic illness that was noted in temporal association with DTP immunization was the first seizure of what turned out to be severe epilepsy. By chance alone, this association may occur 400 times a year in the United States. Four carefully performed studies that included approximately 330,000 children and 1 million immunizations have examined the possibility that pertussis immunization is a causative factor in epilepsy; no evidence of a causative role has been found.* More recently, two large studies in the United States and Canada found no evidence of a causal association with DTP immunization and encephalopathy.[363,416]

Similar to infantile spasms, SIDS also occurs in early life; therefore, that cases are noted to occur after administration of DTP immunization again is not surprising. Hoffman and associates[242] performed an extensive prospective case-control study of risk factors in SIDS from October 1978 through December 1979. In this study of 800 cases, investigators found that DTP immunization was not a risk factor for development of the syndrome. Other good, controlled studies have yielded similar results.[96,189] No evidence has demonstrated that DTP vaccinees have an increased risk for developing asthma later in life.[235]

Acellular Vaccines

An extensive amount of reactogenicity information was generated in phase 2 and phase 3 studies with all licensed DTaP vaccines.* Because endotoxin has been removed from all DTaP vaccines, one is not surprised that all are less reactogenic than are DTP vaccines. In a double-blind study, the reactogenicity of 13 DTaP vaccines was presented and was compared with the reactogenicity of a DTP vaccine.[134] This study involved 2200 infants; 113 to 217 received an acellular product, and 370 received the whole-cell vaccine. Study participants received three doses of vaccine at 2, 4, and 6 months of age. Overall, all monitored reactions except vomiting occurred less frequently and were less severe in DTaP recipients than in DTP recipients. Specific results from this study are presented in Table 129-6. As can be seen, local redness and swelling and fever increased in frequency from the first to the third dose, whereas the complaint of drowsiness decreased.

In our efficacy trial in Germany with Acel-Immune, we monitored reactions in more than 8000 children after receipt of four doses of vaccine at 3, 4½, 6, and 15 to 18 months.[448,493] For the first three vaccine doses, the findings were similar to those noted in Table 129-7. After the fourth dose, the frequency of occurrence of local erythema and induration and fever increased considerably in comparison with their frequencies after the third dose. Ten percent of DTaP recipients had local erythema of 2.4 cm or greater, and 28 percent had temperatures of 38° C or higher. Other investigators also noted an increased frequency and severity of local reactions occurring after administration of the fourth and fifth doses of DTaP vaccines.[223,261,401,420,449] Of particular concern is the observation of extensive swelling of the thigh with booster doses of some DTaP vaccines.[420,449] Rennels and associates[420] found that this swelling occurred more commonly after immunization with DTaP vaccines containing high amounts of diphtheria toxoid. With Acel-Immune, a vaccine with a low diphtheria toxoid content, we noted that 15.4 percent of subjects had induration of more than 5 cm but less than 10 cm after receiving a fifth dose; swelling of the entire limb was not noted.[223]

In five of the 1990s efficacy trials, the occurrence of less common, more severe events (persistent crying, seizures, and hypotonic-hyporesponsive episodes) was monitored. A summary of these data is presented in Table 129-8. As can be seen, temporally related persistent crying, hypotonic-hyporesponsive episodes, and seizures were rare events after receipt of immunization with DTaP

*References 79, 176, 190, 453, 510.

*References 134, 188, 198, 300, 420, 449, 493.

TABLE 129-7 **Summary of Reactogenicity Data from the Nationwide Multicenter Acellular Pertussis Trial**

	DTaP			*DTP*		
Event	1st dose (%)	2nd dose (%)	3rd dose (%)	1st dose (%)	2nd dose (%)	3rd dose (%)
Local						
Redness	13.5	17.1	21.5	49.4	47.7	47.6
Swelling	8.7	12.1	13.3	39.7	34.1	35.7
Pain	3.8	2.0	2.1	27.3	18.7	15.8
Systemic						
Fever (Temp. ≥38.6°C [100.1°F])	4.2	11.3	15.8	27.3	34.1	37.7
Fussiness	6.6	7.7	6.7	20.1	23.5	17.3
Drowsiness	29.9	17.6	12.9	43.5	31.0	24.6
Anorexia	9.3	8.9	8.9	19.5	16.5	14.3
Vomiting	6.3	4.5	4.2	7.0	4.5	5.3
Use of antipyretic	39.3	36.7	36.3	60.5	59.8	61.4

DTaP, diphtheria-tetanus-acellular pertussis; DTP, diphtheria-tetanus-pertussis.
Data from Decker MD, Edwards KM, Steinhoff MC, et al. Comparison of 13 acellular pertussis vaccines: adverse reactions. *Pediatrics* 1995;**96**:557–66.

TABLE 129-8 **Rates* of Severe Events After Diphtheria-Tetanus-Acellular Pertussis Vaccines in the 1990s Efficacy Trials**

Vaccine	Persistent Crying (≥3 hr)	Hypotonic- Hyporesponsive Episodes	Seizures
Certiva	0	0	0.4
Tripedia	0.1	0.05	0.02
SKB-2	0.8	0	0.3
Acelluvax	1.9	0.07	0
INFANRIX	1.3	0	0.07
Acel-Immune	2.0	0	0.1
Daptacel	1.5	0.1	0

*Rates per 1000 doses.
Data from references 139, 146, 231, 385, and 388.

vaccines. In a large active surveillance program (IMPACT), researchers found that risks of having febrile seizures and hypotonic-hyporesponsive episodes after receiving pertussis-containing vaccines decreased significantly after the introduction of DTaP vaccines in Canada.[291] In a Vaccine Safety Datalink study, Huang and coworkers[254] did not observe an increased risk for seizures in children 6 weeks to 23 months of age after DTaP vaccination.

Schedules and Contraindications

Immunization schedules with whole-cell vaccines have varied throughout the world and to some measure were determined by concern relating to true and perceived reactions.[77,96,146]An immunization schedule involving only three doses given to infants at 2, 3, and 4 months of age was quite effective in controlling pertussis-related morbidity and mortality in the United Kingdom.[498] However, the five-dose schedule used in the United States resulted in lower attack rates in preschool- and school-aged children.[77,96]

The recommendation for the DTaP vaccine in the United States is that it be given in the same five-dose schedule as previously recommended for DTP vaccines.[7,63] However, follow-up data from the 1990s efficacy trials, as well as reactogenicity data, suggested that use of this "one size fits all" approach could be modified.* Our findings with Acel-Immune suggested that the present five-dose schedule was appropriate.[315,463] Conversely, data from other trials suggested that administration of the fourth dose of some vaccines could be postponed until the child was 4 to 6 years of age, or the third dose could be moved back to the child's second year of life.† These changes would not be expected to decrease efficacy but would decrease troubling local reactions with booster doses. It should be pointed out, however, that the follow-up data in the just-mentioned studies employed passive surveillance techniques and therefore pertussis attack rates in DTaP recipients were

*References 188, 198, 393, 438, 439, 449, 484, 490.
†References 199, 393, 394, 438, 439, 484, 490.

likely underestimated. Recent data from the 2010 California epidemic show a yearly increase in vaccine failures after the fifth dose of DTaP.[92,532]

Acellular pertussis component quadrivalent (DTaP, IPV), pentavalent (DTaP, IPV, HBV; DTaP, IPV, Hib), and hexavalent (DTaP, IPV, HBV, Hib) vaccines have been developed and are available in many countries.[146] In the United States, the following multicomponent vaccines are available: DTaP/Hib (TriHIBit for the fourth dose of DTaP and Hib series), DTaP/IPV/HBV (PEDIARIX), and DTaP, IPV, Hib (Pentacel). In general, multicomponent vaccine use should be encouraged to reduce the number of injections that a child receives.[7]

In addition to providing childhood immunization, the availability of adolescent and adult Tdap vaccines allows the vaccination of adolescents and adults to be conducted.* At present, the schedule for the use of Tdap vaccines varies in different countries.[167,168,221] The most common recommendation is for universal immunization of preadolescents and adolescents and the selective immunization of adults. However, our opinion has been that a universal program involving all preadolescents or adolescents and adults every 10 years will be necessary to prevent the transmission of *B. pertussis* effectively to unimmunized infants and to prevent the continued circulation of *B. pertussis* in a population. Because at present no country has an effective program for universal immunization of adults and no country is likely to achieve a universal Tdap booster program with reasonable compliance and because adults are the main source of pertussis in young infants, various cocooning programs have been or are being established.[7,71,192] These programs include postpartum immunization of the mother and, if possible, the father, attempts to vaccinate grandparents, and immunization of mothers-to-be during the second or third trimesters of pregnancy. Also considered is lowering the age of infant immunization, including a dose shortly after birth.† All of these cocooning programs have numerous logistical difficulties.

We believe the two most promising approaches are immunization of the mother-to-be in the second and third trimesters of pregnancy and starting infant immunization at birth. Immunizing the pregnant woman should be easy because it fits into routine obstetric care. However, a problem arises in the woman who has multiple pregnancies because repeated doses with a vaccine (Tdap) with full-strength tetanus toxoid could cause significant reactions.

The immunization of infants at birth followed by an accelerated schedule should provide some early protection and prevent deaths. Because some studies of early immunization have shown a suppression of the overall response to the vaccine antigens compared with a more delayed schedule, early immunization has, in recent years, not been looked upon favorably.[20,51] However, recent epidemics suggest that there may be reason for change.

Over the years, pertussis vaccine recommendations have undergone many changes. In particular, contraindications to pertussis immunization are evolving continually. An important note, however, is that few scientific data support any of the present contraindications. The primary goal of national immunization programs is to vaccinate all infants, children, and now adolescents and adults. If excessive contraindications and their overinterpretation lead to a large number of unimmunized persons, the programs will fail and the children in greatest need of protection will contract pertussis. In the United States, the most recent recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics generally should be followed.[7] However, individual case-by-case decisions often need to be made.

*References 69, 70, 81, 82, 85, 87-89, 167, 168, 210, 221, 273, 333, 434.
†References 20, 26, 32, 51, 52, 212, 281, 538, 539.

Isolation and Prophylactic Measures

Erythromycin, azithromycin, or clarithromycin treatment in the index case shortens the duration of communicability of the organisms and thus limits spread of the disease. During the first few days of treatment, contact with susceptible persons should be avoided. In general, close contacts (household members, those in daycare centers, playmates) of the index case should be protected from infection. Such protection can be implemented by the prophylactic use of erythromycin for 14 days, azithromycin for 5 days, or clarithromycin for 7 days.[7,67,333,465] Active immunization of all exposed persons (children, adolescents, and adults) who are not adequately vaccinated also should be conducted.

The use of prophylactic antibiotics in adolescents and adults in exposure situations such as classrooms and hospital settings frequently is recommended. This approach often involves many people and considerable expense.

In our experience, the side effects of erythromycin and other macrolides are such that adult compliance is poor. Therefore, our opinion is that erythromycin and other macrolides should generally not be used prophylactically in these large group settings but only for treatment at the first sign of respiratory illness in those exposed.

OTHER *BORDETELLA* INFECTIONS

Bordetella parapertussis Infection

B. parapertussis infection in children can cause unrecognized infection, mild pertussis, or typical pertussis.[333] We studied 38 children with *B. parapertussis* illnesses and compared their illnesses with those occurring in 76 children with *B. pertussis* illnesses.[231] The results were as follows *(B. pertussis/B. parapertussis)*, in percentages: cough for longer than 4 weeks, 57 percent/37 percent ($p = .06$); whooping, 80 percent/59 percent ($p = .07$); whooping for longer than 2 weeks, 26 percent/18 percent ($p = .05$); paroxysms, 90 percent/83 percent; posttussive vomiting, 47 percent/42 percent; and mean leukocyte and lymphocyte counts, 12,500/mm^3 and 7,600/mm^3, and 7,800/mm^3 and 3,500/mm^3 ($p < .0001$), respectively. In another study in Italy, children with *B. parapertussis* infection had the following rates of findings: cough, 100 percent; paroxysms, 76 percent; whooping, 33 percent; posttussive vomiting, 42 percent; apnea, 29 percent; and cyanosis, 12 percent.[330] All of these rates except cough

and paroxysms were lower in children with *B. parapertussis* infections than in children with *B. pertussis* infections. Concomitant infections with *B. pertussis* and *B. parapertussis* are not rare.[247,259,344]

Before and during the early pertussis vaccine era, pertussis caused by *B. parapertussis* was considerably less common than that caused by *B. pertussis*.[333] For example, during a 16-year period in the Grand Rapids area of Michigan, 4483 cases of pertussis were caused by *B. pertussis* and 106 cases were caused by *B. parapertussis*.[150] More recently, during the DTaP vaccine efficacy trials in Europe, the comparative rates of illness caused by *B. pertussis* or *B. parapertussis* were examined. In our trial in Germany, the rate of pertussis caused by *B. parapertussis* infection in control children was 0.9 cases per 100 person-years.[463] Of 130 culture-confirmed cases, 21 percent were caused by *B. parapertussis*. The percentage of cases caused by *B. parapertussis* in five other trials varied from 2.1 to 20 percent.[330]

Until recently, reported pertussis caused by *B. parapertussis* has been an uncommon finding in the United States. However, during 2004 and 2005, 493 culture-positive cases of pertussis were noted by the Wisconsin State Laboratory of Hygiene.[382] Of these cases, 14 percent were caused by *B. parapertussis*. During the period 2008 to 2010 clinical specimens from 9 states were tested by PCR for *B. pertussis* and *B. parapertussis* in a commercial laboratory.[105] Of the positive samples, 13.99 percent were identified as *B. parapertussis*. The *B. parapertussis*–positive samples had no seasonal periodicity, whereas the *B. pertussis*–positive samples occurred between weeks 22 and 38 each year. In contrast to the findings in the United States, Kurova and associates[286] noted *B. parapertussis* cyclic peaks in 1995, 2000, 2003, and 2007.

Bordetella bronchiseptica Infection

B. bronchiseptica causes respiratory tract infections in at least 18 different mammals.[118] Most notable are atrophic rhinitis in pigs, kennel cough (rhinotracheitis) in dogs, and bronchopneumonia in rabbits and other laboratory animals.

In 1911, McGowan[337] observed that laboratory workers exposed to various animals with *B. bronchiseptica* infections on occasion had respiratory tract illness. In 1926, a 5-year-old girl with a pertussis-like illness was found to be infected with *B. bronchiseptica*.[50] Her illness began 10 to 12 days after she had been given a rabbit with mild "snuffles." Otherwise healthy children who became infected with *B. bronchiseptica* after being exposed to farm animals or pets usually have pertussis-like illnesses.[333]

In 2008, Rath and coworkers[415] described an infant who had recurrent episodes of pertussis-like illness due to *B. bronchiseptica* that cleared after imipenem therapy. In another recent study, another immunocompetent infant with recurrent pertussis-like illness was described.[129]

Occasional infections in humans have been noted during the past 40 years, with the majority occurring in immunocompromised adults, including patients with acquired immunodeficiency syndrome.[6,142,525,541] Respiratory tract infections have ranged from mild upper respiratory tract illnesses to pneumonia. In patients with acquired immunodeficiency syndrome, the pneumonia frequently is cavitary. Sinusitis and bronchitis also occur.

Bordetella hinzii Infection

B. hinzii has, on occasion, been recovered from sputum cultures from patients with cystic fibrosis.[173,459] The role of these isolates in pulmonary disease in cystic fibrosis is unknown. Some isolates are likely transient colonization,[459] but one adult patient had the organism recovered during pulmonary exacerbations throughout a 3-year period.[173] *B. hinzii* has been found to cause septicemia and cholangitis in adult patients with immunodeficiencies.[16,116,171,253,272]

Bordetella holmesii Infection

In 1999, Yih and associates[545] reported the isolation of *B. holmesii* from the nasopharyngeal specimens of 33 patients suspected of having pertussis. Twenty-three of the cases were investigated further, and 19 (82%) of these patients were adolescents, 2 (9%) were adults, and 2 (9%) were children. All had cough: 61 percent had paroxysms, 26 percent had posttussive vomiting, and 9 percent had whoop. More recently, *B. holmesii* has been isolated from nasopharyngeal specimens from patients with pertussis-like illnesses in France, Canada, and the Netherlands.[361,385] The incidence of pertussis-like illness due to *B. holmesii* is unknown. *B. holmesii* contains IS 481 and IS 1001. These two insertion sequence elements are routinely used to diagnose *B. pertussis* and *B. parapertussis*, respectively.[105,310,486] If both IS 481 and IS1001 are positive, then it is thought the illness is due to *B. holmesii*. However, because infection with both *B. pertussis* and *B. parapertussis* can occur, dual positives can be either *B. holmesii* or mixed infections with *B. pertussis* and *B. parapertussis*.[105]

B. holmesii has been noted as a cause of meningitis in a 12-year-old anorectic girl, the cause of bacteremia in four asplenic children, the cause of pneumonia in a 15-year old with relapsing nephrotic syndrome, and the cause of septic arthritis in a 15-year-old boy with chronic hemolytic anemia.[140,359,400,497] This agent has also been a cause of bacteremia in children with sickle-cell disease.[335]

B. holmesii also has been isolated from a 10-month-old boy with bacteremia and from patients with septicemia, endocarditis, and respiratory failure.[364,482] In adults, *B. holmesii* has been a cause of prosthetic valve endocarditis, pericarditis in a patient with lymphoma, and meningitis in an asplenic patient with systemic lupus erythematosus.[268,308,377]

Bordetella trematum Infection

B. trematum has been isolated from wounds and ear infections.[126,503]

Bordetella petrii Infection

In adults, *Bordetella petrii* has been associated with suppurative mastoiditis, bronchiectasis, and mandibular osteomyelitis.[172,290,461]

Bordetella avium Infection

Bordetella avium is an avian pathogen, but recently it has been recovered from an adult with pneumonia. Additionally, a *B. avium*-like strain was recovered from an adult with a 4-month history of productive cough.[215]

NEW REFERENCES SINCE THE SIXTH EDITION

7. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering L, Baker C, Kimberlin D, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.
9. Andre P, Caro V, Njamkepo E, et al. Comparison of serological and real-time PCR assays to diagnose *Bordetella pertussis* infection in 2007. *J Clin Microbiol* 2008;**46**:1672–7.
10. Andreasen C, Carbonetti NH. Pertussis toxin inhibits early chemokine production to delay neutrophil recruitment in response to *Bordetella pertussis* respiratory tract infection in mice. *Infect Immun* 2008;**76**:5139–48.
11. Andreasen C, Powell DA, Carbonetti NH. Pertussis toxin stimulates IL-17 production in response to *Bordetella pertussis* infection in mice. *PLoS One* 2009;**4**:e7079.
26. Barrett CD, McLean IW, Molner JG, et al. Multiple antigen immunization of infants against poliomyelitis, diphtheria, pertussis, and tetanus: an evaluation of antibody responses of infants one day old to seven months of age at start of inoculations. *Pediatrics* 1962;**30**:720–36.
36. Bettiol S, Thompson MJ, Roberts NW, et al. Symptomatic treatment of the cough in whooping cough. *Cochrane Database Syst Rev* 2010;(**1**):CD003257.
49. Broutin H, Viboud C, Grenfell BT, et al. Impact of vaccination and birth rate on the epidemiology of pertussis: a comparative study in 64 countries. *Proc Biol Sci* 2010;**277**:3239–45.
54. Carbonetti NH. Pertussis toxin and adenylate cyclase toxin: key virulence factors of *Bordetella pertussis* and cell biology tools. *Future Microbiol* 2010;**5**:455–69.
56. Castagnini LA, Munoz FM. Clinical characteristics and outcomes of neonatal pertussis: a comparative study. *J Pediatr* 2010;**156**:498–500.
59. Centers for Disease Control and Prevention. Pertussis epidemic—Washington, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:517–26.
71. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged < 12 months—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1424–6.
73. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
91. Cherry JD. The present and future control of pertussis. *Clin Infect Dis* 2010;**51**:663–7.
92. Cherry JD. Why do pertussis vaccines fail? *Pediatrics* 2012;**129**:968–70.
99. Cherry JD, Harriman K. *Why do vaccine-preventable disease outbreaks occur in the United States?* Infectious Diseases Special Edition, 2012. http://www.idse.net/download/VPD_IDSE12_WM.pdf. Accessed June 7, 2013.
100. Cherry JD, Heininger U, Richards DM, et al. Antibody response patterns to *Bordetella pertussis* antigens in vaccinated (primed) and unvaccinated (unprimed) young children with pertussis. *Clin Vaccine Immunol* 2010;**17**:741–7.
104. Cherry JD, Paddock CD, Greer PW, et al. The respiratory pathology in infants with sudden unexpected deaths in whom respiratory specimens were initially PCR-positive or PCR-negative for *Bordetella pertussis*. *Infection* 2011;**39**:545–8.
105. Cherry JD, Seaton BL. Patterns of *Bordetella parapertussis* respiratory illnesses: 2008-2010. *Clin Infect Dis* 2012;**54**:534–7.
106. Cherry JD, Tan T, Wirsing von Konig CH, et al. Clinical definitions of pertussis: summary of a global pertussis initiative roundtable meeting, February 2011. *Clin Infect Dis* 2012;**54**:1756–64.
114. Conover MS, Redfern CJ, Ganguly T, et al. BpsR modulates *Bordetella* biofilm formation by negatively regulating the expression of the Bps polysaccharide. *J Bacteriol* 2012;**194**:233–42.
117. Cosnes-Lambe C, Raymond J, Chalumeau M, et al. Pertussis and respiratory syncytial virus infections. *Eur J Pediatr* 2008;**167**:1017–9.
128. de Gouw D, Diavatopoulos DA, Bootsma HJ, et al. Pertussis: a matter of immune modulation. *FEMS Microbiol Rev* 2011;**35**:441–74.
129. de la Torre MJ, de la Fuente CG, de Alegria CR, et al. Recurrent respiratory infection caused by *Bordetella bronchiseptica* in an immunocompetent infant. *Pediatr Infect Dis J* 2012;**31**(9):981–3.
146. Edwards K, Decker MD. Pertussis vaccines. In: Plotkin S, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders; 2008.
154. Falcon M, Rafael M, Garcia C, et al. Increasing infant pertussis hospitalization and mortality in south Texas, 1996 to 2006. *Pediatr Infect Dis J* 2010;**29**:265–7.
157. Fennelly NK, Sisti F, Higgins SC, et al. *Bordetella pertussis* expresses a functional type III secretion system that subverts protective innate and adaptive immune responses. *Infect Immun* 2008;**76**:1257–66.
175. Gaillard ME, Bottero D, Castuma CE, et al. Laboratory adaptation of *Bordetella pertussis* is associated with the loss of type three secretion system functionality. *Infect Immun* 2011;**79**:3677–82.
192. Grizas AP, Camenga D, Vazquez M. Cocooning: a concept to protect young children from infectious diseases. *Curr Opin Pediatr* 2012;**24**:92–7.
193. Gross R, Keidel K, Schmitt K. Resemblance and divergence: the "new" members of the genus *Bordetella*. *Med Microbiol Immunol* 2010;**199**:155–63.
196. Guillot S, Descours G, Gillet Y, et al. Macrolide-resistant *Bordetella pertussis* infection in newborn girl, France. *Emerg Infect Dis* 2012;**18**:966–8.
200. Guthrie JL, Robertson AV, Tang P, et al. Novel duplex real-time PCR assay detects *Bordetella holmesii* in specimens from patients with pertussis-like symptoms in Ontario, Canada. *J Clin Microbiol* 2010;**48**:1435–7.
201. Haberling DL, Holman RC, Paddock CD, et al. Infant and maternal risk factors for pertussis-related infant mortality in the United States, 1999 to 2004. *Pediatr Infect Dis J* 2009;**28**:194–8.
215. Harrington AT, Castellanos JA, Ziedalski TM, et al. Isolation of *Bordetella avium* and novel *Bordetella* strain from patients with respiratory disease. *Emerg Infect Dis* 2009;**15**:72–4.
217. Hayes JA, Erben E, Lamberti Y, et al. Identification of a new protective antigen of *Bordetella pertussis*. *Vaccine* 2011;**29**:8731–9.
236. Henderson MW, Inatsuka CS, Sheets AJ, et al. Contribution of *Bordetella* filamentous hemagglutinin and adenylate cyclase toxin to suppression and evasion of interleukin-17-mediated inflammation. *Infect Immun* 2012;**80**:2061–75.
237. Hewitt M, Canning BJ. Coughing precipitated by *Bordetella pertussis* infection. *Lung* 2010;**188**(Suppl 1):S73–9.
253. Hristov AC, Auwaerter PG, Romagnoli M, et al. *Bordetella hinzii* septicemia in association with Epstein-Barr virus viremia and an Epstein-Barr virus–associated diffuse large B-cell lymphoma. *Diagn Microbiol Infect Dis* 2008;**61**:484–6.
254. Huang WT, Gargiullo PM, Broder KR, et al. Lack of association between acellular pertussis vaccine and seizures in early childhood. *Pediatrics* 2010;**126**:263–9.
257. Inatsuka CS, Xu Q, Vujkovic-Cvijin I, et al. Pertactin is required for *Bordetella* species to resist neutrophil-mediated clearance. *Infect Immun* 2010;**78**:2901–9.
268. Jonckheere S, De Baere T, Schroeyers P, et al. Prosthetic valve endocarditis caused by *Bordetella holmesii*, an *Acinetobacter* lookalike. *J Med Microbiol* 2012;**61**:874–7.
270. Kamiya H, Otsuka N, Ando Y, et al. Transmission of *Bordetella holmesii* during Pertussis Outbreak, Japan. *Emerg Infect Dis* 2012;**18**:1166–9.
281. Knuf M, Schmitt HJ, Wolter J, et al. Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. *J Pediatr* 2008;**152**:655–60, 660 e651.
286. Kurova N, Njamkepo E, Brun D, et al. Monitoring of *Bordetella* isolates circulating in Saint Petersburg, Russia between 2001 and 2009. *Res Microbiol* 2010;**161**:810–5.

308. Livovsky DM, Leibowitz D, Hidalgo-Grass C, et al. *Bordetella holmesii* meningitis in an asplenic patient with systemic lupus erythematosus. *J Med Microbiol* 2012;**61**:1165–7.
310. Locht C, Coutte L, Mielcarek N. The ins and outs of pertussis toxin. *FEBS J* 2011;**278**:4668–82.
312. Loeffelholz M. Towards improved accuracy of *Bordetella pertussis* nucleic acid amplification tests. *J Clin Microbiol* 2012;**50**: 2186–90.
324. Mandal S, Tatti KM, Woods-Stout D, et al. Pertussis pseudo-outbreak linked to specimens contaminated by *Bordetella pertussis* DNA from clinic surfaces. *Pediatrics* 2012;**129**:e424–30.
326. Marr N, Oliver DC, Laurent V, et al. Protective activity of the *Bordetella pertussis* BrkA autotransporter in the murine lung colonization model. *Vaccine* 2008;**26**:4306–11.
327. Marr N, Shah NR, Lee R, et al. *Bordetella pertussis* autotransporter Vag8 binds human C1 esterase inhibitor and confers serum resistance. *PLoS One* 2011;**6**:e20585.
335. McCavit TL, Grube S, Revell P, et al. *Bordetella holmesii* bacteremia in sickle cell disease. *Pediatr Blood Cancer* 2008;**51**:814–6.
358. Miron D, Srugo I, Kra-Oz Z, et al. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? *Pediatr Infect Dis J* 2010;**29**:e7–10.
359. Moissenet D, Leverger G, Merens A, et al. Septic arthritis caused by *Bordetella holmesii* in an adolescent with chronic haemolytic anaemia. *J Med Microbiol* 2011;**60**:1705–7.
361. Mooi FR, Bruisten S, Linde I, et al. Characterization of *Bordetella holmesii* isolates from patients with pertussis-like illness in The Netherlands. *FEMS Immunol Med Microbiol* 2012;**64**:289–91.
362. Mooi FR, van Loo IH, van Gent M, et al. *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. *Emerg Infect Dis* 2009;**15**:1206–13.
373. Murray EL, Nieves D, Bradley JS, et al. Characteristics of severe *Bordetella pertussis* infection among infants ≤90 days of age admitted to pediatric intensive care units—Southern California, September 2009-June 2011. *J Pediatric Infectious Disease Society* 2013; **2**:1–6
385. Njamkepo E, Bonacorsi S, Debruyne M, et al. Significant finding of *Bordetella holmesii* DNA in nasopharyngeal samples from French patients with suspected pertussis. *J Clin Microbiol* 2011;**49**: 4347–8.
387. Noofeli M, Bokhari H, Blackburn P, et al. BapC autotransporter protein is a virulence determinant of *Bordetella pertussis*. *Microb Pathog* 2011;**51**:169–77.
390. Octavia S, Sintchenko V, Gilbert GL, et al. Newly emerging clones of *Bordetella pertussis* carrying prn2 and ptxP3 alleles implicated in Australian pertussis epidemic in 2008-2010. *J Infect Dis* 2012;**205**:1220–4.
391. Ohtsuka M, Kikuchi K, Shimizu K, et al. Emergence of quinolone-resistant *Bordetella pertussis* in Japan. *Antimicrob Agents Chemother* 2009;**53**:3147–9.
400. Panagopoulos MI, Saint Jean M, Brun D, et al. *Bordetella holmesii* bacteremia in asplenic children: report of four cases initially misidentified as *Acinetobacter lwoffii*. *J Clin Microbiol* 2010;**48**: 3762–4.
413. Prince HE, Lieberman JM, Cherry JD. Age-related differences in patterns of increased *Bordetella pertussis* antibodies. *Clin Vaccine Immunol* 2012;**19**:545–50.
414. Rank C, Quinn HE, McIntyre PB. Pertussis vaccine effectiveness after mass immunization of high school students in Australia. *Pediatr Infect Dis J* 2009;**28**:152–3.
415. Rath BA, Register KB, Wall J, et al. Persistent *Bordetella bronchiseptica* pneumonia in an immunocompetent infant and genetic comparison of clinical isolates with kennel cough vaccine strains. *Clin Infect Dis* 2008;**46**:905–8.
421. Riffelmann M, Thiel K, Schmetz J, et al. Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. *J Clin Microbiol* 2010;**48**: 4459–63.
423. Robbins JB, Schneerson R, Keith JM, et al. Pertussis vaccine: a critique. *Pediatr Infect Dis J* 2009;**28**:237–41.
429. Rohani P, Drake JM. The decline and resurgence of pertussis in the US. *Epidemics* 2011;**3**:183–8.
430. Rohani P, Zhong X, King AA. Contact network structure explains the changing epidemiology of pertussis. *Science* 2010;**330**:982–5.
432. Roorda L, Buitenwerf J, Ossewaarde JM, et al. A real-time PCR assay with improved specificity for detection and discrimination of all clinically relevant *Bordetella* species by the presence and distribution of three insertion sequence elements. *BMC Res Notes* 2011;**4**:11.
435. Rowlands HE, Goldman AP, Harrington K, et al. Impact of rapid leukodepletion on the outcome of severe clinical pertussis in young infants. *Pediatrics* 2010;**126**:e816–27.
443. Sawal M, Cohen M, Irazuzta JE, et al. Fulminant pertussis: a multi-center study with new insights into the clinico-pathological mechanisms. *Pediatr Pulmonol* 2009;**44**:970–80.
446. Schmidtke AJ, Boney KO, Martin SW, et al. Population diversity among *Bordetella pertussis* isolates, United States, 1935-2009. *Emerg Infect Dis J* 2012;**18**(8):1248–55.
459. Spilker T, Liwienski AA, LiPuma JJ. Identification of *Bordetella* spp. in respiratory specimens from individuals with cystic fibrosis. *Clin Microbiol Infect* 2008;**14**:504–6.
485. Tatti KM, Slade B, Patel M, et al. Real-time polymerase chain reaction detection of *Bordetella pertussis* DNA in acellular pertussis vaccines. *Pediatr Infect Dis J* 2008;**27**:73–4.
486. Tatti KM, Sparks KN, Boney KO, et al. Novel multitarget real-time PCR assay for rapid detection of *Bordetella* species in clinical specimens. *J Clin Microbiol* 2011;**49**:4059–66.
489. Tondella ML, Carlone GM, Messonnier N, et al. International *Bordetella pertussis* assay standardization and harmonization meeting report. Centers for Disease Control and Prevention, Atlanta, Georgia, United States, 19-20 July 2007. *Vaccine* 2009;**27**: 803–14.
497. Van Balen T, Nieman AE, Hermans MH, et al. *Bordetella holmesii* meningitis in a 12-year-old anorectic girl. *Pediatr Infect Dis J* 2012;**31**:421–2.
512. Wang K, Chalker V, Bermingham A, et al. *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough in England: a retrospective analysis. *Pediatr Infect Dis J* 2011;**30**: 1047–51.
518. Wearing HJ, Rohani P. Estimating the duration of pertussis immunity using epidemiological signatures. *PLoS Pathog* 2009;**5**: e1000647.
519. Wei SC, Tatti K, Cushing K, et al. Effectiveness of adolescent and adult tetanus, reduced-dose diphtheria, and acellular pertussis vaccine against pertussis. *Clin Infect Dis* 2010;**51**:315–21.
525. Wernli D, Emonet S, Schrenzel J, et al. Evaluation of eight cases of confirmed *Bordetella bronchiseptica* infection and colonization over a 15-year period. *Clin Microbiol Infect* 2010;**17**:201–3.
529. Williams CL, Haines R, Cotter PA. Serendipitous discovery of an immunoglobulin-binding autotransporter in *Bordetella* species. *Infect Immun* 2008;**76**:2966–77.
537. Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. *Clin Infect Dis* 2012;**54**: 1730–5.
538. Wood N, McIntyre P, Marshall H, et al. Acellular pertussis vaccine at birth and one month induces antibody responses by two months of age. *Pediatr Infect Dis J* 2010;**29**:209–15.
539. Wood N, Siegrist CA. Neonatal immunization: where do we stand? *Curr Opin Infect Dis* 2011;**24**:190–5.
550. Zouari A, Touati A, Smaoui H, et al. Dual infection with *Bordetella pertussis* and *Mycoplasma pneumoniae* in three infants: case reports. *Infection* 2012;**40**:213–7.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abramson T, Kedem H, Relman DA. Proinflammatory and pro-apoptotic activities associated with *Bordetella pertussis* filamentous hemagglutinin. *Infect Immun* 2001;**69**:2650–8.
2. Ad Hoc Group for the Study of Pertussis Vaccines. Placebo-controlled trial of two acellular pertussis vaccines in Sweden: protective efficacy and adverse events. *Lancet* 1988;**1**:955–60.
3. Advisory Panel of the Committee on Safety of Medicines. *The collection of data relating to adverse reactions in pertussis vaccine: Whooping Cough: reports from the Committee on Safety of Medicines and the Joint Committee on Vaccination and Immunisation*. London: Department of Health and Social Security, Her Majesty's Stationery Office; 1981. p. 27.
4. Aintablian N, Walpita P, Sawyer MH. Detection of *Bordetella pertussis* and respiratory synctial virus in air samples from hospital rooms. *Infect Control Hosp Epidemiol* 1998;**19**:918–23.
5. Alderslade R, Bellman MH, Rawson NSB, et al. *The National Childhood Encephalopathy Study: Whooping Cough: reports from the Committee on Safety of Medicines and the Joint Committee on Vaccination and Immunisation*. London: Department of Health and Social Security, Her Majesty's Stationery Office; 1981. p. 7.
6. Amador C, Chiner E, Calpe JL, et al. Pneumonia due to *Bordetella bronchiseptica* in a patient with AIDS. *Rev Infect Dis* 1991;**13**: 771–2.
7. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering L, Baker C, Kimberlin D, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.
8. Anderson RM, May RM. Vaccination and herd immunity to infectious diseases. *Nature* 1985;**318**:323–9.
9. Andre P, Caro V, Njamkepo E, et al. Comparison of serological and real-time PCR assays to diagnose *Bordetella pertussis* infection in 2007. *J Clin Microbiol* 2008;**46**:1672–7.
10. Andreasen C, Carbonetti NH. Pertussis toxin inhibits early chemokine production to delay neutrophil recruitment in response to *Bordetella pertussis* respiratory tract infection in mice. *Infect Immun* 2008;**76**:5139–48.
11. Andreasen C, Powell DA, Carbonetti NH. Pertussis toxin stimulates IL-17 production in response to *Bordetella pertussis* infection in mice. *PLoS One* 2009;**4**:e7079.
12. Aoyama T, Ide Y, Watanabe J, et al. Respiratory failure caused by dual infection with *Bordetella pertussis* and respiratory syncytial virus. *Acta Paediatr Jpn* 1996;**38**:282–5.
13. Arciniega JL, Shahin RD, Burnette WN, et al. Contribution of the B oligomer to the protective activity of genetically attenuated pertussis toxin. *Infect Immun* 1991;**59**:3407–10.
14. Arico B, Rappuoli R. *Bordetella parapertussis* and *Bordetella bronchiseptica* contain transcriptionally silent pertussis toxin genes. *J Bacteriol* 1987;**169**:2847–53.
15. Arnold JC, Singh KK, Spector SA, et al. Human bocavirus: prevalence and clinical spectrum at a children's hospital. *Clin Infect Dis* 2006;**43**:283–8.
16. Arvand M, Feldhues R, Mieth M, et al. Chronic cholangitis caused by *Bordetella hinzii* in a liver transplant recipient. *J Clin Microbiol* 2004;**42**:2335–7.
17. Ausiello CM, Lande R, Urbani F, et al. Cell-mediated immunity and antibody responses to *Bordetella pertussis* antigens in children with a history of pertussis infection and in recipients of an acellular pertussis vaccine. *J Infect Dis* 2000;**181**:1989–95.
18. Baraff LJ, Cherry JD. Nature and rates of adverse reactions associated with pertussis immunization. In: Manclark CR, Hill JC, editors. *International Symposium on Pertussis*. Publication No. (N. I. H.) 79-1830. Washington, D.C.: U.S. Dept. of Health, Education, and Welfare, U.S. Government Printing Office; 1979. p. 291.
19. Baraff LJ, Cody CL, Cherry JD. DTP-associated reactions: an analysis by injection site, manufacturer, prior reactions, and dose. *Pediatrics* 1984;**73**:31–6.
20. Baraff LJ, Leake RD, Burstyn DG, et al. Immunologic response to early and routine DTP immunization in infants. *Pediatrics* 1984;**73**:37–42.
21. Baraff LJ, Manclark CR, Cherry JD, et al. Analyses of adverse reactions to diphtheria and tetanus toxoids and pertussis vaccine by vaccine lot, endotoxin content, pertussis vaccine potency and percentage of mouse weight gain. *Pediatr Infect Dis J* 1989;**8**: 502–7.
22. Baraff LJ, Wilkins J, Wehrle PF. The role of antibiotics, immunizations, and adenoviruses in pertussis. *Pediatrics* 1978;**61**:224–30.
23. Barlow WE, Davis RL, Glasser JW, et al. The risk of seizures after receipt of whole-cell pertussis or measles, mumps, and rubella vaccine. *N Engl J Med* 2001;**345**:656–61.
24. Barnes MG, Weiss AA. BrkA protein of *Bordetella pertussis* inhibits the classical pathway of complement after C1 deposition. *Infect Immun* 2001;**69**:3067–72.
25. Baron S, Njamkepo E, Grimprel E, et al. Epidemiology of pertussis in French hospitals in 1993 and 1994: thirty years after a routine use of vaccination. *Pediatr Infect Dis J* 1998;**17**: 412–8.
26. Barrett CD, McLean IW, Molner JG, et al. Multiple antigen immunization of infants against poliomyelitis, diphtheria, pertussis, and tetanus: an evaluation of antibody responses of infants one day old to seven months of age at start of inoculations. *Pediatrics* 1962;**30**:720–736.
27. Bass JW. Pertussis: current status of prevention and treatment. *Pediatr Infect Dis* 1985;**4**:614–9.
28. Bass JW. Erythromycin for treatment and prevention of pertussis. *Pediatr Infect Dis* 1986;**5**:154–7.
29. Bass JW, Klenk EL, Kotheimer JB, et al. Antimicrobial treatment of pertussis. *J Pediatr* 1969;**75**:768–81.
30. Beiter A, Lewis K, Pineda EF, et al. Unrecognized maternal peripartum pertussis with subsequent fatal neonatal pertussis. *Obstet Gynecol* 1993;**82**:691–3.
31. Bellman MH, Ross EM, Miller DL. Infantile spasms and pertussis immunisation. *Lancet* 1983;**1**:1031–4.
32. Belloni C, De Silvestri A, Tinelli C, et al. Immunogenicity of a three-component acellular pertussis vaccine administered at birth. *Pediatrics* 2003;**111**:1042–5.
33. Berg JM. Neurological complications of pertussis immunization. *BMJ* 1958;**2**:24–7.
34. Bergquist SO, Bernander S, Dahnsjo H, et al. Erythromycin in the treatment of pertussis: a study of bacteriologic and clinical effects. *Pediatr Infect Dis J* 1987;**6**:458–61.
35. Bernales R, Eastman J, Kaplan J. Quantitation of circulating T and B lymphocytes in children with whooping cough. *Pediatr Res* 1976;**10**:965–7.
36. Bettiol S, Thompson MJ, Roberts NW, et al. Symptomatic treatment of the cough in whooping cough. *Cochrane Database Syst Rev* 2010;(**1**):CD003257.
37. Bhargava A, Leininger E, Roberts M, et al. Filamentous hemagglutinin and the 69-kDa protein, pertactin, promote adherence of *Bordetella pertussis* to epithelial cells and macrophages. Paper presented at the Sixth International Symposium on Pertussis, Bethesda, MD, September 26-28, 1990. p. 137–8.
38. Birkebaek NH, Kristiansen M, Seefeldt T, et al. *Bordetella pertussis* and chronic cough in adults. *Clin Infect Dis* 1999;**29**:1239–42.
39. Bisgard KM, Pascual FB, Ehresmann KR, et al. Infant pertussis: who was the source? *Pediatr Infect Dis J* 2004;**23**:985–9.
40. Bisgard KM, Rhodes P, Connelly BL, et al. Pertussis vaccine effectiveness among children 6 to 59 months of age in the United States, 1998-2001. *Pediatrics* 2005;**116**:e285–294.
41. Blackwelder WC, Storsaeter J, Olin P, et al. Acellular pertussis vaccines: efficacy and evaluation of clinical case definitions. *Am J Dis Child* 1991;**145**:1285–9.
42. Bordet J, Gengou O. Le microbe de la coqueluche. *Ann Inst Pasteur (Paris)* 1906;**20**:48–68.
43. Bordet J, Gengou O. L'endotoxin coquelucheuse. *Ann Inst Pasteur (Paris)* 1909;**23**:415–9.
44. Borras Sans M, Bonal J, Bonet J, et al. *Bordetella bronchiseptica* septicemia in a hemodialysis patient. *Nephron* 1991;**59**:676.
45. Bradford WL, Slavin B. An organism resembling *Hemophilus* pertussis: with special reference to color changes produced by its growth upon certain media. *Am J Public Health Nations Health* 1937;**27**:1277–82.
46. Brennan MJ, Li ZM, Cowell JL, et al. Identification of a 69-kilodalton nonfimbrial protein as an agglutinogen of *Bordetella pertussis*. *Infect Immun* 1988;**56**:3189–95.
47. Broomhall J, Herxheimer A. Treatment of whooping cough: the facts. *Arch Dis Child* 1984;**59**:185–7.
48. Broutin H, Guegan JF, Elguero E, et al. Large-scale comparative analysis of pertussis population dynamics: periodicity, synchrony, and impact of vaccination. *Am J Epidemiol* 2005;**161**:1159–67.

49. Broutin H, Viboud C, Grenfell BT, et al. Impact of vaccination and birth rate on the epidemiology of pertussis: a comparative study in 64 countries. *Proc Biol Sci* 2010;**277**:3239–45.
50. Brown JH. *Bacillus bronchisepticus* infection in a child with symptoms of pertussis. *Bull Johns Hopkins Hosp* 1926;**38**:147–53.
51. Burstyn DG, Baraff LJ, Peppler MS, et al. Serological response to filamentous hemagglutinin and lymphocytosis-promoting toxin of *Bordetella pertussis*. *Infect Immun* 1983;**41**:1150–6.
52. Butler NR, Wilson BD, Benson PF, et al. Response of infants to pertussis vaccine at one week and to poliomyelitis, diphtheria, and tetanus vaccine at six months. *Lancet* 1962;**2**:112–14.
53. Byers RK, Moll FC. Encephalopathies following prophylactic pertussis vaccine. *Pediatrics* 1948;**1**:437–57.
54. Carbonetti NH. Pertussis toxin and adenylate cyclase toxin: key virulence factors of *Bordetella pertussis* and cell biology tools. *Future Microbiol* 2010;**5**:455–69.
55. Carbonetti NH, Artamonova GV, Van Rooijen N, et al. Pertussis toxin targets airway macrophages to promote *Bordetella pertussis* infection of the respiratory tract. *Infect Immun* 2007;**75**:1713–20.
56. Castagnini LA, Munoz FM. Clinical characteristics and outcomes of neonatal pertussis: a comparative study. *J Pediatr* 2010;**156**:498–500.
57. Celentano LP, Massari M, Paramatti D, et al. Resurgence of pertussis in Europe. *Pediatr Infect Dis J* 2005;**24**:761–5.
58. Celermajer JM, Brown J. The neurological complications of pertussis. *Med J Aust* 1966;**1**:1066–9.
59. Centers for Disease Control and Prevention. Pertussis surveillance, 1979-1981. *MMWR Morb Mortal Wkly Rep* 1982;**31**:333–6.
60. Centers for Disease Control and Prevention. Pertussis: Maryland, 1982. *MMWR Morb Mortal Wkly Rep* 1983;**32**:297–300.305.
61. Centers for Disease Control and Prevention. Pertussis: United States, 1982 and 1983. MMWR. *Morb Mortal Wkly Rep* 1984;**33**:573–5.
62. Centers for Disease Control and Prevention. Hypertrophic pyloric stenosis in infants following pertussis prophylaxis with erythromycin—Knoxville, Tennessee, 1999. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1117–20.
63. Centers for Disease Control and Prevention. Use of diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine as a five-dose series: supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2000;**49**:1–8.
64. Centers for Disease Control and Prevention. Pertussis—United States, 1997-2000. *MMWR Morb Mortal Wkly Rep* 2002;**51**:73–6.
65. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:1–80.
66. Centers for Disease Control and Prevention. Pertussis—United States, 2001-2003. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1283–6.
67. Centers for Disease Control and Prevention. Recommended antimicrobial agents for the treatment and postexposure prophylaxes of pertussis: 2005 CDC guidelines. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1–16.
68. Centers for Disease Control and Prevention. Fatal case of unsuspected pertussis diagnosed from a blood culture: Minnesota, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:131–2.
69. Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adolescents: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccines. *MMWR Recomm Rep* 2006;**55**(RR–3):1–34.
70. Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine. *MMWR Recomm Rep* 2006;**55**(RR–17):1–37.
71. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged < 12 months—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1424–6.
72. Centers for Disease Control and Prevention. Pertussis Epidemic—Washington, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:517–26.
73. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
74. Chaby R, Caroff M. Lipopolysaccharides of *Bordetella pertussis* endotoxin. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 247–72.
75. Chakravorty AP. Blindness after use of triple antigen. *BMJ* 1963;**1**:105.
76. Charles IG, Li JL, Roberts M, et al. Identification and characterization of a protective immunodominant B cell epitope of pertactin (P.69) from *Bordetella pertussis*. *Eur J Immunol* 1991;**21**:1147–53.
77. Cherry JD. The epidemiology of pertussis and pertussis immunization in the United Kingdom and the United States: a comparative study. *Curr Probl Pediatr* 1984;**14**:1–78.
78. Cherry JD. Pertussis and the vaccine controversy. In: Root RK, Griffiss JM, Warren KS, et al, editors. *Immunization*. New York: Churchill Livingstone; 1989. p. 47–63.
79. Cherry JD "Pertussis vaccine encephalopathy": it is time to recognize it as the myth that it is. *JAMA* 1990;**263**:1679–80.
80. Cherry JD. Pertussis: the trials and tribulations of old and new pertussis vaccines. *Vaccine* 1992;**10**:1033–8.
81. Cherry JD. Acellular pertussis vaccines—a solution to the pertussis problem. *J Infect Dis* 1993;**168**:21–4.
82. Cherry JD. *Strategies for diphtheria, tetanus, and pertussis (DTP) immunization: report of the 104th Ross Conference on Pediatric Research*. Columbus, Ohio: Ross Products Division, Abbott Laboratories; 1994. p. 218–25.
83. Cherry JD. Historical review of pertussis and the classical vaccine. *J Infect Dis* 1996;**174**:259–63.
84. Cherry JD. Comparative efficacy of acellular pertussis vaccines: an analysis of recent trials. *Pediatr Infect Dis J* 1997;**16**:90–6.
85. Cherry JD. Epidemiological, clinical, and laboratory aspects of pertussis in adults. *Clin Infect Dis* 1999;**28**:112–7.
86. Cherry JD. Pertussis in the preantibiotic and prevaccine era, with emphasis on adult pertussis. *Clin Infect Dis* 1999;**28**:107–11.
87. Cherry JD. The epidemiology of pertussis: a comparison of the epidemiology of the disease pertussis with the epidemiology of *Bordetella pertussis* infection. *Pediatrics* 2005;**115**:1422–7.
88. Cherry JD. Pertussis vaccines for adolescents and adults. *Pediatrics* 2005;**116**:755–6.
89. Cherry JD. Epidemiology of pertussis. *Pediatr Infect Dis J* 2006;**25**:361–2.
90. Cherry JD. Historical perspective on pertussis and use of vaccines to prevent it. *Microbe* 2007;**2**:139–44.
91. Cherry JD. The present and future control of pertussis. *Clin Infect Dis* 2010;**51**:663–7.
92. Cherry JD. Why do pertussis vaccines fail? *Pediatrics* 2012;**129**:968–70.
93. Cherry JD. Unpublished observation.
94. Cherry JD, Baraff LJ, Hewlett E. The past, present, and future of pertussis: the role of adults in epidemiology and future control. *West J Med* 1989;**150**:319–28.
95. Cherry JD, Beer T, Chartrand SA, et al. Comparison of values of antibody to *Bordetella pertussis* antigens in young German and American men. *Clin Infect Dis* 1995;**20**:1271–4.
96. Cherry JD, Brunell PA, Golden GS, et al. Report of the Task Force on Pertussis and Pertussis Immunization: 1988. *Pediatrics* 1988;**81**:939–84.
97. Cherry JD, Gornbein J, Heininger U, et al. A search for serologic correlates of immunity to *Bordetella pertussis* cough illnesses. *Vaccine* 1998;**16**:1901–6.
98. Cherry JD, Grimprel E, Guiso N, et al. Defining pertussis epidemiology: clinical, microbiologic and serologic perspectives. *Pediatr Infect Dis J* 2005;**24**:S25–34.
99. Cherry JD, Harriman K. *Why do vaccine-preventable disease outbreaks occur in the United States?* Infectious Diseases Special Edition, 2012. http://www.idse.net/download/VPD_IDSE12_WM.pdf. Accessed June 7, 2013.
100. Cherry JD, Heininger U, Richards DM, et al. Antibody response patterns to *Bordetella pertussis* antigens in vaccinated (primed) and unvaccinated (unprimed) young children with pertussis. *Clin Vaccine Immunol* 2010;**17**:741–7.
101. Cherry JD, Heininger U, Stehr K, et al. The effect of investigator compliance (observer bias) on calculated efficacy in a pertussis vaccine trial. *Pediatrics* 1998;**102**:909–12.

102. Cherry JD, Mortimer EA Jr. Acellular and whole-cell pertussis vaccines in Japan: report of a visit by US scientists. *JAMA* 1987;**257**:1375–6.
103. Cherry JD, Olin P. Commentaries: The science and fiction of pertussis vaccines. *Pediatrics* 1999;**104**:1381–3.
104. Cherry JD, Paddock CD, Greer PW, et al. The respiratory pathology in infants with sudden unexpected deaths in whom respiratory specimens were initially PCR-positive or PCR-negative for *Bordetella pertussis*. *Infection* 2011;**39**:545–8.
105. Cherry JD, Seaton BL. Patterns of *Bordetella parapertussis* respiratory illnesses: 2008-2010. *Clin Infect Dis* 2012;**54**:534–7.
106. Cherry JD, Tan T, Wirsing von Konig CH, et al. Clinical definitions of pertussis: summary of a global pertussis initiative roundtable meeting, February 2011. *Clin Infect Dis* 2012;**54**:1756–64.
107. Choy KW, Wulffraat NM, Wolfs TF, et al. *Bordetella bronchiseptica* respiratory infection in a child after bone marrow transplantation. *Pediatr Infect Dis J* 1999;**18**:481–3.
108. Christie CD, Baltimore RS. Pertussis in neonates. *Am J Dis Child* 1989;**143**:1199–202.
109. Christie CD, Marx ML, Marchant CD, et al. The 1993 epidemic of pertussis in Cincinnati. Resurgence of disease in a highly immunized population of children. *N Engl J Med* 1994;**331**:16–21.
110. Cody CL, Baraff LJ, Cherry JD, et al. Nature and rates of adverse reactions associated with DTP and DT immunizations in infants and children. *Pediatrics* 1981;**68**:650–60.
111. Collier AM, Connor JD, Irving WR Jr. Generalized type 5 adenovirus infection associated with the pertussis syndrome. *J Pediatr* 1966;**69**:1073–8.
112. Cone TC Jr. Whooping cough is first described as a disease sui generis by Baillou in 1640. *Pediatrics* 1970;**46**:522.
113. Connor JD Evidence for an etiologic role of adenoviral infection in pertussis syndrome. *N Engl J Med* 1970;**283**:390–4.
114. Conover MS, Redfern CJ, Ganguly T, et al. BpsR modulates *Bordetella* biofilm formation by negatively regulating the expression of the Bps polysaccharide. *J Bacteriol* 2012;**194**:233–42.
115. Conway SP, Balfour AH, Ross H. Serologic diagnosis of whooping cough by enzyme-linked immunosorbent assay. *Pediatr Infect Dis J* 1988;**7**:570–4.
116. Cookson BT, Vandamme P, Carlson LC, et al. Bacteremia caused by a novel *Bordetella* species, "*B. hinzii*". *J Clin Microbiol* 1994; **32**:2569–71.
117. Cosnes-Lambe C, Raymond J, Chalumeau M, et al. Pertussis and respiratory syncytial virus infections. *Eur J Pediatr* 2008;**167**: 1017–19.
118. Cotter PA, Miller JF. Bordetella. In: Groisman EA, editor. *Principles of bacterial pathogenesis*. San Diego: Academic Press; 2001. p. 619–74.
119. Cowell JL, Sato Y, Sato H, et al. Separation, purification and properties of the filamentous hemagglutinin and the leukocytosis promoting factor-hemagglutinin from *Bordetella pertussis*. In: Robbins JB, Hill JC, Sadoff G, editors. Seminars in infectious disease. *Bacterial vaccines: International Symposium on Bacterial Vaccines*, Vol. IV. New York: Thieme-Stratton; 1982. p. 371–9.
120. Cromer BA, Goydos J, Hackell J, et al. Unrecognized pertussis infection in adolescents. *Am J Dis Child* 1993;**147**:575–7.
121. Crowcroft NS, Andrews N, Rooney C, et al. Deaths from pertussis are underestimated in England. *Arch Dis Child* 2002;**86**: 336–8.
122. Crowcroft NS, Booy R, Harrison T, et al. Severe and unrecognised: pertussis in UK infants. *Arch Dis Child* 2003;**88**:802–6.
123. Cundell DR, Kanthakumar K, Taylor GW, et al. Effect of tracheal cytotoxin from *Bordetella pertussis* on human neutrophil function in vitro. *Infect Immun* 1994;**62**:639–43.
124. Dagan R, Hall CB, Menegus MA. Atypical bacterial infections explained by a concomitant virus infection. *Pediatrics* 1985;**76**: 411–14.
125. Davis SF, Sutter RW, Strebel PM, et al. Concurrent outbreaks of pertussis and *Mycoplasma pneumoniae* infection: clinical and epidemiological characteristics of illnesses manifested by cough. *Clin Infect Dis* 1995;**20**:621–8.
126. Daxboeck F, Goerzer E, Apfalter P, et al. Isolation of *Bordetella trematum* from a diabetic leg ulcer. *Diabet Med* 2004;**21**: 1247–8.
127. De Berry BB, Lynch JE, Chung DH, et al. Pertussis with severe pulmonary hypertension and leukocytosis treated with extracorporeal membrane oxygenation. *Pediatr Surg Int* 2005;**21**:692–4.
128. de Gouw D, Diavatopoulos DA, Bootsma HJ, et al. Pertussis: a matter of immune modulation. *FEMS Microbiol Rev* 2011;**35**: 441–74.
129. de la Torre MJ, de la Fuente CG, de Alegria CR, et al. Recurrent Respiratory Infection Caused by *Bordetella bronchiseptica* in an Immunocompetent Infant. *Pediatr Infect Dis J* 2012;**31**(9):981–3.
130. de Melker HE, Schellekens JF, Neppelenbroek SE, et al. Reemergence of pertussis in the highly vaccinated population of the Netherlands: observations on surveillance data. *Emerg Infect Dis* 2000;**6**:348–57.
131. de Melker HE, Versteegh FG, Schellekens JF, et al. The incidence of *Bordetella pertussis* infections estimated in the population from a combination of serological surveys. *J Infect* 2006;**53**:106–13.
132. De Serres G, Shadmani R, Duval B, et al. Morbidity of pertussis in adolescents and adults. *J Infect Dis* 2000;**182**:174–9.
133. Decker GR, Lavelle JP, Kumar PN, et al. Pneumonia due to *Bordetella bronchiseptica* in a patient with AIDS. *Rev Infect Dis* 1991;**13**:1250–1.
134. Decker MD, Edwards KM, Steinhoff MC, et al. Comparison of 13 acellular pertussis vaccines: adverse reactions. *Pediatrics* 1995; **96**:557–66.
135. Deen JL, Mink CA, Cherry JD, et al. Household contact study of *Bordetella pertussis* infections. *Clin Infect Dis* 1995;**21**:1211–19.
136. Deville JG, Cherry JD, Christenson PD, et al. Frequency of unrecognized *Bordetella pertussis* infections in adults. *Clin Infect Dis* 1995;**21**:639–42.
137. Diavatopoulos DA, Cummings CA, Schouls LM, et al. *Bordetella pertussis*, the causative agent of whooping cough, evolved from a distinct, human-associated lineage of *B. bronchiseptica*. *PLoS Pathog* 2005;**1**:0373–83.
138. Donoso AF, Cruces PI, Camacho JF, et al. Exchange transfusion to reverse severe pertussis-induced cardiogenic shock. *Pediatr Infect Dis J* 2006;**25**:846–8.
139. Donoso AF, Leon J, Ramirez M, et al. Pertussis and fatal pulmonary hypertension: a discouraged entity. *Scand J Infect Dis* 2005; **37**:145–8.
140. Dorbecker C, Licht C, Korber F, et al. Community-acquired pneumonia due to *Bordetella holmesii* in a patient with frequently relapsing nephrotic syndrome. *J Infect* 2007;**54**:e203–5.
141. Douglas E, Coote JG, Parton R, et al. Identification of *Bordetella pertussis* in nasopharyngeal swabs by PCR amplification of a region of the adenylate cyclase gene. *J Med Microbiol* 1993;**38**: 140–4.
142. Dworkin MS, Sullivan PS, Buskin SE, et al. *Bordetella bronchiseptica* infection in human immunodeficiency virus-infected patients. *Clin Infect Dis* 1999;**28**:1095–9.
143. Edsall G. Present status of pertussis vaccination. *Practitioner* 1975;**215**:310–14.
144. Edsall G. Comment. In: *International Symposium on Pertussis, Bilthoven, 1969: immunobiologic standards*, vol. 13. New York: S. Karger; 1979. p. 170.
145. Edwards KM, Decker MD. Pertussis vaccine. *Vaccines* 2004;**4**: 484–5.
146. Edwards KM, Decker MD. Pertussis vaccines. In: Plotkin S, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders; 2008.
147. Eldering G, Hornbeck C, Baker J. Serological study of *Bordetella pertussis* and related species. *J Bacteriol* 1957;**74**:133–6.
148. Eldering G, Kendrick P. A group of cultures resembling both *Bacillus pertussis* and *Bacillus bronchisepticus* but identical with neither. *J Bacteriol* 1937;**33**:71.
149. Eldering G, Kendrick P. *Bacillus para-pertussis*: a species resembling both *Bacillus pertussis* and *Bacillus bronchisepticus* but identical with neither. *J Bacteriol* 1938;**35**:561–72.
150. Eldering G, Kendrick PL. Incidence of parapertussis in the Grand Rapids area as indicated by 16 years' experience with diagnostic cultures. *Am J Public Health Nations Health* 1952;**42**: 27–31.
151. Elliott E, McIntyre P, Ridley G, et al. National study of infants hospitalized with pertussis in the acellular vaccine era. *Pediatr Infect Dis J* 2004;**23**:246–52.
152. Erlandsson A, Backman A, Nygren M, et al. Quantification of *Bordetella pertussis* in clinical samples by colorimetric detection of competitive PCR products. *APMIS* 1998;**106**:1041–8.
153. Ewanowich CA, Chui LW, Paranchych MG, et al. Major outbreak of pertussis in northern Alberta, Canada: analysis of discrepant

direct fluorescent-antibody and culture results by using polymerase chain reaction methodology. *J Clin Microbiol* 1993;**31**:1715–25.
154. Falcon M, Rafael M, Garcia C, et al. Increasing infant pertussis hospitalization and mortality in south Texas, 1996 to 2006. *Pediatr Infect Dis J* 2010;**29**:265–7.
155. Farizo KM, Cochi SL, Zell ER, et al. Epidemiological features of pertussis in the United States, 1980-1989. *Clin Infect Dis* 1992;**14**:708–19.
156. Farrell DJ, McKeon M, Daggard G, et al. Rapid-cycle PCR method to detect *Bordetella pertussis* that fulfills all consensus recommendations for use of PCR in diagnosis of pertussis. *J Clin Microbiol* 2000;**38**:4499–502.
157. Fennelly NK, Sisti F, Higgins SC, et al. *Bordetella pertussis* expresses a functional type III secretion system that subverts protective innate and adaptive immune responses. *Infect Immun* 2008;**76**:1257–66.
158. Fernandez RC, Weiss AA. Cloning and sequencing of a *Bordetella pertussis* serum resistance locus. *Infect Immun* 1994;**62**:4727–38.
159. Ferry NS. *Bacillus bronchisepticus (bronchicanis)*: the cause of distemper in dogs and a similar disease in other animals. *Vet J* 1912;**68**:376–91.
160. Fine PE. Epidemiological considerations for whooping cough eradication. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 451–67.
161. Fine PE. Herd immunity: history, theory, practice. *Epidemiol Rev* 1993;**15**:265–302.
162. Fine PE. Implications of different study designs for the evaluation of acellular pertussis vaccines. *Dev Biol Stand* 1997;**89**:123–33.
163. Fine PE, Clarkson JA. The recurrence of whooping cough: possible implications for assessment of vaccine efficacy. *Lancet* 1982;**1**:666–9.
164. Fine PE, Clarkson JA. Reflections on the efficacy of pertussis vaccines. *Rev Infect Dis* 1987;**9**:866–83.
165. Finn TM, Stevens LA. Tracheal colonization factor: a *Bordetella pertussis* secreted virulence determinant. *Mol Microbiol* 1995;**16**:625–34.
166. Foege WH. *Statement read before the Subcommittee on Investigations and General Oversight Committee on Labor and Human Resources*. Washington, D.C.: United States Senate; 1982.
167. Food and Drug Administration. *Product approval information-licensing action, package insert: BOOSTRIX. Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine, adsorbed. GlaxoSmith-Kline Biologicals*. Available at http://www.fda.gov/cber/label/tdapgla122905LB.pdf. Rockville, MD: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research; 2005.
168. Food and Drug Administration. *Product approval information-licensing action, package insert: Tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine adsorbed ADACEL. Sanofi Pasteur*. Available at http://www.fda.gov/cber/label/tdapave012306LB.pdf. Rockville, MD: U.S. Department of Health and Human Services, Food and Drug Administration, Center for Biologics Evaluation and Research; 2006.
169. Friedman LE, Messina MT, Santoferrara L, et al. Characterization of *Bordetella bronchiseptica* strains using phenotypic and genotypic markers. *Vet Microbiol* 2006;**117**:313–20.
170. Friedman RL. Pertussis: the disease and new diagnostic methods. *Clin Microbiol Rev* 1988;**1**:365–76.
171. Fry NK, Duncan J, Edwards MT, et al. A UK clinical isolate of *Bordetella hinzii* from a patient with myelodysplastic syndrome. *J Med Microbiol* 2007;**56**:1700–3.
172. Fry NK, Duncan J, Malnick H, et al. *Bordetella petrii* clinical isolate. *Emerg Infect Dis* 2005;**11**:1131–3.
173. Funke G, Hess T, von Graevenitz A, et al. Characteristics of *Bordetella hinzii* strains isolated from a cystic fibrosis patient over a 3-year period. *J Clin Microbiol* 1996;**34**:966–9.
174. Furman BL, Sidey FM, Smith M. Metabolic disturbances produced by pertussis toxin. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 147–72.
175. Gaillard ME, Bottero D, Castuma CE, et al. Laboratory adaptation of *Bordetella pertussis* is associated with the loss of type three secretion system functionality. *Infect Immun* 2011;**79**:3677–82.
176. Gale JL, Thapa PB, Wassilak SG, et al. Risk of serious acute neurological illness after immunization with diphtheria-tetanus-pertussis vaccine: a population-based case-control study. *JAMA* 1994;**271**:37–41.
177. Gil A, Oyaguez I, Carrasco P, et al. Hospital admissions for pertussis in Spain, 1995-1998. *Vaccine* 2001;**19**:4791–4.
178. Gilberg S, Njamkepo E, Du Chatelet IP, et al. Evidence of *Bordetella pertussis* infection in adults presenting with persistent cough in a French area with very high whole-cell vaccine coverage. *J Infect Dis* 2002;**186**:415–18.
179. Glare EM, Paton JC, Premier RR, et al. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. *J Clin Microbiol* 1990;**28**:1982–7.
180. Goldman WE. Tracheal cytotoxin of *Bordetella pertussis*. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 237–46.
181. Goodman YE, Wort AJ, Jackson FL. Enzyme-linked immunosorbent assay for detection of pertussis immunoglobulin A in nasopharyngeal secretions as an indicator of recent infection. *J Clin Microbiol* 1981;**13**:286–92.
182. Goodnow RA. Biology of *Bordetella bronchiseptica*. *Microbiol Rev* 1980;**44**:722–38.
183. Gordon JE, Hood RI. Whooping cough and its epidemiological anomalies. *Am J Med Sci* 1951;**222**:333–61.
184. Gordon M, Davies HD, Gold R. Clinical and microbiologic features of children presenting with pertussis to a Canadian pediatric hospital during an eleven-year period. *Pediatr Infect Dis J* 1994;**13**:617–22.
185. Goulin GD, Kaya KM, Bradley JS. Severe pulmonary hypertension associated with shock and death in infants infected with *Bordetella pertussis*. *Crit Care Med* 1993;**21**:1791–4.
186. Granstrom G, Askelof P, Granstrom M. Specific immunoglobulin A to *Bordetella pertussis* antigens in mucosal secretion for rapid diagnosis of whooping cough. *J Clin Microbiol* 1988;**26**:869–74.
187. Granstrom M, Lindberg AA, Askelof P, et al. Detection of antibodies in human serum against the fimbrial haemagglutinin of *Bordetella pertussis* by enzyme-linked immunosorbent assay. *J Med Microbiol* 1982;**15**:85–96.
188. Greco D, Salmaso S, Mastrantonio P, et al. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. *N Engl J Med* 1996;**334**:341–8.
189. Griffin MR, Ray WA, Livengood JR, et al. Risk of sudden infant death syndrome after immunization with the diphtheria-tetanus-pertussis vaccine. *N Engl J Med* 1988;**319**:618–23.
190. Griffin MR, Ray WA, Mortimer EA, et al. Risk of seizures and encephalopathy after immunization with the diphtheria-tetanus-pertussis vaccine. *JAMA* 1990;**263**:1641–5.
191. Grimprel E, Begue P, Anjak I, et al. Comparison of polymerase chain reaction, culture, and western immunoblot serology for diagnosis of *Bordetella pertussis* infection. *J Clin Microbiol* 1993;**31**:2745–50.
192. Grizas AP, Camenga D, Vazquez M. Cocooning: a concept to protect young children from infectious diseases. *Curr Opin Pediatr* 2012;**24**:92–7.
193. Gross R, Keidel K, Schmitt K. Resemblance and divergence: the "new" members of the genus *Bordetella*. *Med Microbiol Immunol* 2010;**199**:155–63.
194. Grzeszczak MJ, Churchwell KB, Edwards KM, et al. Leukopheresis therapy for severe infantile pertussis with myocardial and pulmonary failure. *Pediatr Crit Care Med* 2006;**7**:580–2.
195. Gueirard P, Weber C, Le Coustumier A, et al. Human *Bordetella bronchiseptica* infection related to contact with infected animals: persistence of bacteria in host. *J Clin Microbiol* 1995;**33**:2002–6.
196. Guillot S, Descours G, Gillet Y, et al. Macrolide-resistant *Bordetella pertussis* infection in newborn girl, France. *Emerg Infect Dis* 2012;**18**:966–8.
197. Guris D, Strebel PM, Bardenheier B, et al. Changing epidemiology of pertussis in the United States: increasing reported incidence among adolescents and adults, 1990-1996. *Clin Infect Dis* 1999;**28**:1230–7.
198. Gustafsson L, Hallander HO, Olin P, et al. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. *N Engl J Med* 1996;**334**:349–55.

199. Gustafsson L, Hessel L, Storsaeter J, et al. Long-term follow-up of Swedish children vaccinated with acellular pertussis vaccines at 3, 5, and 12 months of age indicates the need for a booster dose at 5 to 7 years of age. *Pediatrics* 2006;**118**:978–84.
200. Guthrie JL, Robertson AV, Tang P, et al. Novel duplex real-time PCR assay detects *Bordetella holmesii* in specimens from patients with pertussis-like symptoms in Ontario, Canada. *J Clin Microbiol* 2010;**48**:1435–7.
201. Haberling DL, Holman RC, Paddock CD, et al. Infant and maternal risk factors for pertussis-related infant mortality in the United States, 1999 to 2004. *Pediatr Infect Dis J* 2009;**28**:194–8.
202. Hackman R, Perrin DG, Karmali M, et al. Fatal *Bordetella pertussis* infection: report of two cases with novel pathologic findings. *Pediatr Pathol Lab Med* 1996;**16**:643–53.
203. Halasa NB, Barr FE, Johnson JE, et al. Fatal pulmonary hypertension associated with pertussis in infants: does extracorporeal membrane oxygenation have a role? *Pediatrics* 2003;**112**:1274–8.
204. Hallander HO, Gnarpe J, Gnarpe H, et al. *Bordetella pertussis*, *Bordetella parapertussis*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae* and persistent cough in children. *Scand J Infect Dis* 1999; **31**:281–6.
205. Hallander HO, Reizenstein E, Renemar B, et al. Comparison of nasopharyngeal aspirates with swabs for culture of *Bordetella pertussis*. *J Clin Microbiol* 1993;**31**:50–2.
206. Hallander HO, Storsaeter J, Mollby R. Evaluation of serology and nasopharyngeal cultures for diagnosis of pertussis in a vaccine efficacy trial. *J Infect Dis* 1991;**163**:1046–54.
207. Halperin SA, Bortolussi R, Kasina A, et al. Use of a Chinese hamster ovary cell cytotoxicity assay for the rapid diagnosis of pertussis. *J Clin Microbiol* 1990;**28**:32–8.
208. Halperin SA, Bortolussi R, Langley JM, et al. Seven days of erythromycin estolate is as effective as fourteen days for the treatment of *Bordetella pertussis* infections. *Pediatrics* 1997;**100**:65–71.
209. Halperin SA, Bortolussi R, MacLean D, et al. Persistence of pertussis in an immunized population: results of the Nova Scotia Enhanced Pertussis Surveillance Program. *J Pediatr* 1989;**115**:686–93.
210. Halperin SA, Smith B, Russell M, et al. Adult formulation of a five component acellular pertussis vaccine combined with diphtheria and tetanus toxoids and inactivated poliovirus vaccine is safe and immunogenic in adolescents and adults. *Pediatr Infect Dis J* 2000;**19**:276–83.
211. Halperin SA, Wang EE, Law B, et al. Epidemiological features of pertussis in hospitalized patients in Canada, 1991-1997: report of the Immunization Monitoring Program—Active (IMPACT). *Clin Infect Dis* 1999;**28**:1238–43.
212. Halsey N, Galazka A. The efficacy of DPT and oral poliomyelitis immunization schedules initiated from birth to 12 weeks of age. *Bull World Health Organ* 1985;**63**:1151–69.
213. Hannah JH, Menozzi FD, Renauld G, et al. Sulfated glycoconjugate receptors for the *Bordetella pertussis* adhesin filamentous hemagglutinin (FHA) and mapping of the heparin-binding domain on FHA. *Infect Immun* 1994;**62**:5010–9.
214. Hannik CA, Cohen H. Pertussis vaccine experience in the Netherlands. In: Manclark CR, Hill JC, editors. *International Symposium on Pertussis*. Publication No. (N. I. H.) 79-1830. Washington, D.C.: U.S. Dept. of Health, Education, and Welfare, U.S. Government Printing Office; 1979. p. 279.
215. Harrington AT, Castellanos JA, Ziedalski TM, et al. Isolation of *Bordetella avium* and novel *Bordetella* strain from patients with respiratory disease. *Emerg Infect Dis* 2009;**15**:72–4.
216. Haward RA. Scale of undernotification of infectious diseases by general practitioners. *Lancet* 1973;**1**:873–4.
217. Hayes JA, Erben E, Lamberti Y, et al. Identification of a new protective antigen of *Bordetella pertussis*. *Vaccine* 2011;**29**:8731–9.
218. He Q, Mertsola J, Soini H, et al. Sensitive and specific polymerase chain reaction assays for detection of *Bordetella pertussis* in nasopharyngeal specimens. *J Pediatr* 1994;**124**:421–6.
219. Hedenskog S, Bjorksten B, Blennow M, et al. Immunoglobulin E response to pertussis toxin in whooping cough and after immunization with a whole-cell and an acellular pertussis vaccine. *Int Arch Allergy Appl Immunol* 1989;**89**:156–61.
220. Heininger U. Recent progress in clinical and basic pertussis research. *Eur J Pediatr* 2001;**160**:203–13.
221. Heininger U, Cherry JD. Pertussis immunization in adolescents and adults: *Bordetella pertussis* epidemiology should guide vaccination recommendations. *Expert Opin Biol Ther* 2006;**6**:1–13.
222. Heininger U, Cherry JD, Eckhardt T, et al. Clinical and laboratory diagnosis of pertussis in the regions of a large vaccine efficacy trial in Germany. *Pediatr Infect Dis J* 1993;**12**:504–9.
223. Heininger U, Cherry JD, Lugauer S, et al. Reactogenicity data following fourth and fifth doses of the Wyeth-Lederle Takeda acellular pertussis component vaccine: The Erlangen trial. Paper presented at the Acellular Pertussis Vaccine Conference, Bethesda, Md, November 12–4, 2000.
224. Heininger U, Cherry JD, Stehr K, et al. Comparative efficacy of the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine and Lederle whole-cell component DTP vaccine in German children after household exposure. Pertussis Vaccine Study Group. *Pediatrics* 1998;**102**:546–53.
225. Heininger U, Kleemann WJ, Cherry JD. A controlled study of the relationship between *Bordetella pertussis* infections and sudden unexpected deaths among German infants. *Pediatrics* 2004;**114**: e9–15.
226. Heininger U, Klich K, Stehr K, et al. Clinical findings in *Bordetella pertussis* infections: results of a prospective multicenter surveillance study. *Pediatrics* 1997;**100**:E10.
227. Heininger U, Schmidt-Schlapfer G, Cherry JD, et al. Clinical validation of a polymerase chain reaction assay for the diagnosis of pertussis by comparison with serology, culture, and symptoms during a large pertussis vaccine efficacy trial. *Pediatrics* 2000;**105**: E31.
228. Heininger U, Stehr K, Cherry JD. Serious pertussis overlooked in infants. *Eur J Pediatr* 1992;**151**:342–3.
229. Heininger U, Stehr K, Christenson P, et al. Evidence of efficacy of the Lederle/Takeda acellular pertussis component diphtheria and tetanus toxoids and pertussis vaccine but not the Lederle whole-cell component diphtheria and tetanus toxoids and pertussis vaccine against *Bordetella parapertussis* infection. *Clin Infect Dis* 1999;**28**:602–4.
230. Heininger U, Stehr K, Schmidt-Schlapfer G, et al. *Bordetella pertussis* infections and sudden unexpected deaths in children. *Eur J Pediatr* 1996;**155**:551–3.
231. Heininger U, Stehr K, Schmitt-Grohe S, et al. Clinical characteristics of illness caused by *Bordetella parapertussis* compared with illness caused by *Bordetella pertussis*. *Pediatr Infect Dis J* 1994;**13**: 306–9.
232. Heininger U, Stehr K, Schmitt-Grohe S, et al. [The microagglutination test—a simple and sensitive procedure for serodiagnosis of pertussis infections]. *Klin Padiatr* 1995;**207**:277–80.
233. Heiss LN, Lancaster JR Jr, Corbett JA, et al. Epithelial autotoxicity of nitric oxide: role in the respiratory cytopathology of pertussis. *Proc Natl Acad Sci U S A* 1994;**91**:267–70.
234. Hellwig SM, Rodriguez ME, Berbers GA, et al. Crucial role of antibodies to pertactin in *Bordetella pertussis* immunity. *J Infect Dis* 2003;**188**:738–42.
235. Henderson J, North K, Griffiths M, et al. Pertussis vaccination and wheezing illnesses in young children: prospective cohort study. The Longitudinal Study of Pregnancy and Childhood Team. *BMJ* 1999;**318**:1173–6.
236. Henderson MW, Inatsuka CS, Sheets AJ, et al. Contribution of *Bordetella* filamentous hemagglutinin and adenylate cyclase toxin to suppression and evasion of interleukin-17-mediated inflammation. *Infect Immun* 2012;**80**:2061–75.
237. Hewitt M, Canning BJ. Coughing precipitated by *Bordetella pertussis* infection. *Lung* 2010;**188**(Suppl 1):S73–9.
238. Hewlett EL, Cherry JD. New and improved vaccines against pertussis. In: Woodrow GC, Levine MM, editors. *New generation vaccines*. New York: Marcel Dekker; 1990. p. 231–50.
239. Hewlett EL, Cherry JD. New and improved vaccines against pertussis. In: Levine MM, Woodrow GC, Kaper JB, Cobon GS, editors. *New generation vaccines*. 2nd ed. New York: Marcel Dekker; 1997. p. 387–416.
240. Hewlett EL, Gordon VM. Adenylate cyclase toxin of *Bordetella pertussis*. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 193–209.
241. Hodder SL, Cherry JD, Mortimer EA Jr, et al. Antibody responses to *Bordetella pertussis* antigens and clinical correlations in elderly community residents. *Clin Infect Dis* 2000;**31**:7–14.
242. Hoffman HJ, Hunter JC, Damus K, et al. Diphtheria-tetanus-pertussis immunization and sudden infant death: results of the National Institute of Child Health and Human Development

Cooperative Epidemiological Study of Sudden Infant Death Syndrome Risk Factors. *Pediatrics* 1987;**79**:598–611.
243. Holmes WH. *Bacillary and rickettsial infections acute and chronic, a textbook: black death to white plague*. New York: Macmillan; 1940. p. 395–414.
244. Honein MA, Paulozzi LJ, Himelright IM, et al. Infantile hypertrophic pyloric stenosis after pertussis prophylaxis with erythromcyin: a case review and cohort study. *Lancet* 1999;**354**:2101–5.
245. Hoppe JE. Methods for isolation of *Bordetella pertussis* from patients with whooping cough. *Eur J Clin Microbiol Infect Dis* 1988;**7**:616–20.
246. Hoppe JE. State of art in antibacterial susceptibility of *Bordetella pertussis* and antibiotic treatment of pertussis. *Infection* 1998;**26**: 242–6.
247. Hoppe JE. Update on respiratory infection caused by *Bordetella parapertussis*. *Pediatr Infect Dis J* 1999;**18**:375–81.
248. Hoppe JE. Neonatal pertussis. *Pediatr Infect Dis J* 2000;**19**: 244–7.
249. Hoppe JE, Eichhorn A. Activity of new macrolides against *Bordetella pertussis* and *Bordetella parapertussis*. *Eur J Clin Microbiol Infect Dis* 1989;**8**:653–4.
250. Hoppe JE, Halm U, Hagedorn HJ, et al. Comparison of erythromycin ethylsuccinate and co-trimoxazole for treatment of pertussis. *Infection* 1989;**17**:227–31.
251. Hoppe JE, Haug A. Antimicrobial susceptibility of *Bordetella pertussis*, part I. *Infection* 1988;**16**:126–30.
252. Houard S, Hackel C, Herzog A, et al. Specific identification of *Bordetella pertussis* by the polymerase chain reaction. *Res Microbiol* 1989;**140**:477–87.
253. Hristov AC, Auwaerter PG, Romagnoli M, et al. *Bordetella hinzii* septicemia in association with Epstein-Barr virus viremia and an Epstein-Barr virus–associated diffuse large B-cell lymphoma. *Diagn Microbiol Infect Dis* 2008;**61**:484–6.
254. Huang WT, Gargiullo PM, Broder KR, et al. Lack of association between acellular pertussis vaccine and seizures in early childhood. *Pediatrics* 2010;**126**:263–9.
255. Hudnall SD, Molina CP. Marked increase in L-selectin–negative T cells in neonatal pertussis: the lymphocytosis explained? *Am J Clin Pathol* 2000;**114**:35–40.
256. Iida T, Kunitani A, Komase Y, et al. Studies on experimental infection with *Bordetella pertussis*: effect of cortisone on the infection and immunity in mice. *Jpn J Exp Med* 1983;**33**:283–95.
257. Inatsuka CS, Xu Q, Vujkovic-Cvijin I, et al. Pertactin is required for *Bordetella* species to resist neutrophil-mediated clearance. *Infect Immun* 2010;**78**:2901–9.
258. Irons LI, Gorringe AR. Pertussis toxin: production, purification, molecular structure, and assay. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 95–120.
259. Iwata S, Aoyama T, Goto A, et al. Mixed outbreak of *Bordetella pertussis* and *Bordetella parapertussis* in an apartment house. *Dev Biol Stand* 1991;**73**:333–41.
260. Jackson FE. Spontaneous spinal epidural hematoma coincident with whooping cough: case report. *J Neurosurg* 1963;**20**:715–17.
261. Jackson LA, Carste BA, Malais D, et al. Retrospective population-based assessment of medically attended injection site reactions, seizures, allergic responses and febrile episodes after acellular pertussis vaccine combined with diphtheria and tetanus toxoids. *Pediatr Infect Dis J* 2002;**21**:781–6.
262. Jackson LA, Cherry JD, Wang SP, et al. Frequency of serological evidence of *Bordetella* infections and mixed infections with other respiratory pathogens in university students with cough illnesses. *Clin Infect Dis* 2000;**31**:3–6.
263. Janda WM, Santos E, Stevens J, et al. Unexpected isolation of *Bordetella pertussis* from a blood culture. *J Clin Microbiol* 1994;**32**: 2851–3.
264. Jenkinson D. Whooping cough: what proportion of cases is notified in an epidemic? *BMJ* 1983;**287**:183–5.
265. Jensen DL, Gray GC, Putnam SD, et al. Evaluation of pertussis in U.S. Marine Corps trainees. *Clin Infect Dis* 1997;**25**:1099–107.
266. Johnston ID, Strachan DP, Anderson HR. Effect of pneumonia and whooping cough in childhood on adult lung function. *N Engl J Med* 1998;**338**:581–7.
267. Joint Committee on Vaccination and Immunisation. The whooping cough epidemic, 1977-79. In: *Whooping cough: reports from the Committee on Safety in Medicine and the Joint Committee on Vaccination and Immunisation*. London: Department of Health and Social Security, Her Majesty's Stationery Office; 1981. p. 170.
268. Jonckheere S, De Baere T, Schroeyers P, et al. Prosthetic valve endocarditis caused by *Bordetella holmesii*, an *Acinetobacter* lookalike. *J Med Microbiol* 2012;**61**:874–7.
269. Jones DH, McBride BW, Jeffery H, et al. Protection of mice from *Bordetella pertussis* respiratory infection using microencapsulated pertussis fimbriae. *Vaccine* 1995;**13**:675–81.
270. Kamiya H, Otsuka N, Ando Y, et al. Transmission of *Bordetella holmesii* during pertussis outbreak, Japan. *Emerg Infect Dis* 2012; **18**:1166–9.
271. Kandola K, Lea A, Santos M. Pertussis rates in Northwest Territories after introducing adult formulation acellular vaccine [abstract]. *Can J Infect Dis Med Microbiol* 2004;**15**:351.
272. Kattar MM, Chavez JF, Limaye AP, et al. Application of 16S rRNA gene sequencing to identify *Bordetella hinzii* as the causative agent of fatal septicemia. *J Clin Microbiol* 2000;**38**:789–94.
273. Keitel WA, Muenz LR, Decker MD, et al. A randomized clinical trial of acellular pertussis vaccines in healthy adults: dose-response comparisons of 5 vaccines and implications for booster immunization. *J Infect Dis* 1999;**180**:397–403.
274. Kendrick PL, Eldering G, et al. Mouse protection tests in the study of pertussis vaccine; a comparative series using the intracerebral route for challenge. *Am J Public Health Nations Health* 1947;**37**:803–10.
275. Kerr JR, Matthews RC. *Bordetella pertussis* infection: pathogenesis, diagnosis, management, and the role of protective immunity. *Eur J Clin Microbiol Infect Dis* 2000;**19**:77–88.
276. Kersters K, Hinz KH, Hertle A, et al. *Bordetella avium* sp. nov., isolated from the respiratory tracts of turkeys and other birds. *Int J Syst Bacteriol* 1984;**34**:56–70.
277. Khelef N, Sakamoto H, Guiso N. Both adenylate cyclase and hemolytic activities are required by *Bordetella pertussis* to initiate infection. *Microb Pathog* 1992;**12**:227–35.
278. Kimura A, Mountzouros KT, Relman DA, et al. *Bordetella pertussis* filamentous hemagglutinin: evaluation as a protective antigen and colonization factor in a mouse respiratory infection model. *Infect Immun* 1990;**58**:7–16.
279. Kimura M, Kuno-Sakai H. Pertussis vaccines in Japan. *Acta Paediatr Jpn* 1988;**30**:143–53.
280. Knorr L, Fox JD, Tilley PAG, et al. Evaluation of real-time PCR for diagnosis of *Bordetella pertussis* infection. *BMC Infect Dis* 2006;**6**:1–12.
281. Knuf M, Schmitt HJ, Wolter J, et al. Neonatal vaccination with an acellular pertussis vaccine accelerates the acquisition of pertussis antibodies in infants. *J Pediatr* 2008;**152**:655–60, 660 e651.
282. Korgenski EK, Daly JA. Surveillance and detection of erythromycin resistance in *Bordetella pertussis* isolates recovered from a pediatric population in the Intermountain West region of the United States. *J Clin Microbiol* 1997;**35**:2989–91.
283. Korppi M, Hiltunen J. Pertussis is common in nonvaccinated infants hospitalized for respiratory syncytial virus infection. *Pediatr Infect Dis J* 2007;**26**:316–18.
284. Kowalzik F, Barbosa AP, Fernandes VR, et al. Prospective multinational study of pertussis infection in hospitalized infants and their household contacts. *Pediatr Infect Dis J* 2007;**26**:238–42.
285. Kulenkampff M, Schwartzman JS, Wilson J. Neurological complications of pertussis inoculation. *Arch Dis Child* 1974;**49**:46–9.
286. Kurova N, Njamkepo E, Brun D, et al. Monitoring of *Bordetella* isolates circulating in Saint Petersburg, Russia between 2001 and 2009. *Res Microbiol* 2010;**161**:810–15.
287. Kurt TL, Yeager AS, Guenette S, et al. Spread of pertussis by hospital staff. *JAMA* 1972;**221**:264–7.
288. Lapin JH. *Whooping cough*. Springfield, Ill.: Charles C Thomas; 1943.
289. Lautrop H. Observations on parapertussis in Denmark, 1950-1957. *Acta Pathol Microbiol Scand* 1958;**43**:255–66.
290. Le Coustumier A, Njamkepo E, Cattoir V, et al. *Bordetella petrii* infection with long-lasting persistence in human. *Emerg Infect Dis* 2011;**17**:612–18.
291. Le Saux N, Barrowman NJ, Moore DL, et al. Decrease in hospital admissions for febrile seizures and reports of hypotonic-hyporesponsive episodes presenting to hospital emergency departments since switching to acellular pertussis vaccine in Canada: a report from IMPACT. *Pediatrics* 2003;**112**:348–53.

292. Lee B. Progressive respiratory distress in an infant treated for presumed pertussis. *Pediatr Infect Dis J* 2000;**19**(475):492–3.
293. Lee GM, Lebaron C, Murphy TV, et al. Pertussis in adolescents and adults: should we vaccinate? *Pediatrics* 2005;**115**:1675–84.
294. Leininger E, Kenimer JG, Brennan MJ. Surface proteins of *Bordetella pertussis*: role in adherence. Paper presented at the Sixth International Symposium on Pertussis. Bethesda, Md., September 26-28, 1990, p. 25–6.
295. Lewis K, Cherry JD, Sachs MH, et al. The effect of prophylactic acetaminophen administration on reactions to DTP vaccination. *Am J Dis Child* 1988;**142**:62–5.
296. Lewis K, Saubolle MA, Tenover FC, et al. Pertussis caused by an erythromycin-resistant strain of *Bordetella pertussis*. *Pediatr Infect Dis J* 1995;**14**:388–91.
297. Li ZM, Cowell JL, Brennan MJ, et al. Agglutinating monoclonal antibodies that specifically recognize lipooligosaccharide A of *Bordetella pertussis*. *Infect Immun* 1988;**56**:699–702.
298. Li ZM, Jansen DL, Finn TM, et al. Identification of *Bordetella pertussis* infection by shared-primer PCR. *J Clin Microbiol* 1994; **32**:783–9.
299. Lichtinghagen R, Diedrich-Glaubitz R, von Horsten B. Identification of *Bordetella pertussis* in nasopharyngeal swabs using the polymerase chain reaction: evaluation of detection methods. *Eur J Clin Chem Clin Biochem* 1994;**32**:161–7.
300. Liese JG, Meschievitz CK, Harzer E, et al. Efficacy of a two-component acellular pertussis vaccine in infants. *Pediatr Infect Dis J* 1997;**16**:1038–44.
301. Liese JG, Renner C, Stojanov S, et al. Clinical and epidemiological picture of *B. pertussis* and *B. parapertussis* infections after introduction of acellular pertussis vaccines. *Arch Dis Child* 2003;**88**: 684–7.
302. Lind-Brandberg L, Welinder-Olsson C, Lagergard T, et al. Evaluation of PCR for diagnosis of *Bordetella pertussis* and *Bordetella parapertussis* infections. *J Clin Microbiol* 1998;**36**:679–83.
303. Lindgren C, Milerad J, Lagercrantz H. Sudden infant death and prevalence of whooping cough in the Swedish and Norwegian communities. *Eur J Pediatr* 1997;**156**:405–9.
304. Lindquist SW, Weber DJ, Mangum ME, et al. *Bordetella holmesii* sepsis in an asplenic adolescent. *Pediatr Infect Dis J* 1995;**14**: 813–15.
305. Linnemann CC Jr. Host-parasite interactions in pertussis. In: Manclark CR, Hill JC, editors. *International Symposium on Pertussis*. Publication No. (N. I. H.) 79-1830. Washington, D.C.: U.S. Dept. of Health, Education, and Welfare, U.S. Government Printing Office; 1979. p. 3–18.
306. Linnemann CC Jr, Nasenbeny J. Pertussis in the adult. *Annu Rev Med* 1977;**28**:179–85.
307. Linnemann CC Jr, Ramundo N, Perlstein PH, et al. Use of pertussis vaccine in an epidemic involving hospital staff. *Lancet* 1975;**2**:540–3.
308. Livovsky DM, Leibowitz D, Hidalgo-Grass C, et al. *Bordetella holmesii* meningitis in an asplenic patient with systemic lupus erythematosus. *J Med Microbiol* 2012;**61**:1165–7.
309. Locht C, Bertin P, Menozzi FD, et al. The filamentous haemagglutinin, a multifaceted adhesion produced by virulent *Bordetella spp*. *Mol Microbiol* 1993;**9**:653–60.
310. Locht C, Coutte L, Mielcarek N. The ins and outs of pertussis toxin. *FEBS J* 2011;**278**:4668–82.
311. Loeffelholz M. Bordetella. In: Murray PR, Baron EJ, Jorgensen JH, editors. *Manual of clinical microbiology*. 8th ed. Washington, D.C.: American Society for Microbiology; 2003. p. 780–8.
312. Loeffelholz M. Towards improved accuracy of *Bordetella pertussis* nucleic acid amplification tests. *J Clin Microbiol* 2012;**50**:2186–90.
313. Long SS, Lischner HW, Deforest A, et al. Serologic evidence of subclinical pertussis in immunized children. *Pediatr Infect Dis J* 1990;**9**:700–5.
314. Long SS, Welkon CJ, Clark JL. Widespread silent transmission of pertussis in families: antibody correlates of infection and symptomatology. *J Infect Dis* 1990;**161**:480–6.
315. Lugauer S, Heininger U, Cherry JD, et al. Long-term clinical effectiveness of an acellular pertussis component vaccine and a whole cell pertussis component vaccine. *Eur J Pediatr* 2002;**161**: 142–6.
316. Luker KE, Collier JL, Kolodziej EW, et al. *Bordetella pertussis* tracheal cytotoxin and other muramyl peptides: distinct structure-activity relationships for respiratory epithelial cytopathology. *Proc Natl Acad Sci U S A* 1993;**90**:2365–9.
317. MacRae KD. Epidemiology, encephalopathy, and pertussis vaccine. In: FEMS-Symposium Pertussis: proceedings of a conference organized by the Society of Microbiology and Epidemiology of the GDR. Berlin, April 20-2, 1988.
318. Madge N, Diamond J, Miller D, et al. The National Childhood Encephalopathy study: a 10-year follow-up. A report on the medical, social, behavioural and educational outcomes after serious, acute, neurological illness in early childhood. *Dev Med Child Neurol Suppl* 1993;**68**:1–118.
319. Madsen T. Whooping cough: its bacteriology, diagnosis, prevention and treatment. *Boston Med Surg J* 1925;**192**:50–60.
320. Mahon BP, Brady MT, Mills KH. Protection against *Bordetella pertussis* in mice in the absence of detectable circulating antibody: implications for long-term immunity in children. *J Infect Dis* 2000;**181**:2087–91.
321. Makhov AM, Hannah JH, Brennan MJ, et al. Filamentous hemagglutinin of *Bordetella pertussis:* a bacterial adhesin formed as a 50-nm monomeric rigid rod based on a 19-residue repeat motif rich in beta strands and turns. *J Mol Biol* 1994;**241**:110–24.
322. Mallory FB, Hornor AA. Pertussis: the histological lesion in the respiratory tract. *J Med Res* 1912;**27**:115–23.
323. Manclark CR, Meade BD, Burstyn DG. Serological response to *Bordetella pertussis*. In: Rose NR, Friedman H, Fahey JL, editors. *Manual of clinical laboratory immunology*. 3rd ed. Washington, D.C.: American Society for Microbiology; 1986. p. 388–94.
324. Mandal S, Tatti KM, Woods-Stout D, et al. Pertussis pseudo-outbreak linked to specimens contaminated by *Bordetella pertussis* DNA from clinic surfaces. *Pediatrics* 2012;**129**:e424–30.
325. Mannerstedt G. Pertussis in adults. *J Pediatr* 1934;**5**:596–600.
326. Marr N, Oliver DC, Laurent V, et al. Protective activity of the *Bordetella pertussis* BrkA autotransporter in the murine lung colonization model. *Vaccine* 2008;**26**:4306–11.
327. Marr N, Shah NR, Lee R, et al. *Bordetella pertussis* autotransporter Vag8 binds human C1 esterase inhibitor and confers serum resistance. *PLoS One* 2011;**6**:e20585.
328. Martin GI, Weintraub MI. Brachial neuritis and seventh nerve palsy—a rare hazard of DPT vaccination. *Clin Pediatr (Phila)* 1973;**12**:506–7.
329. Mastrantonio P, Stefanelli P, Giuliano M. Polymerase chain reaction for the detection of *Bordetella pertussis* in clinical nasopharyngeal aspirates. *J Med Microbiol* 1996;**44**:261–6.
330. Mastrantonio P, Stefanelli P, Giuliano M, et al. *Bordetella parapertussis* infection in children: epidemiology, clinical symptoms, and molecular characteristics of isolates. *J Clin Microbiol* 1998;**36**: 999–1002.
331. Masure HR. The adenylate cyclase toxin contributes to the survival of *Bordetella pertussis* within human macrophages. *Microb Pathog* 1993;**14**:253–60.
332. Matherne P, Matson J, Marks MI. Pertussis complicated by the syndrome of inappropriate antidiuretic hormone secretion: pathophysiology and management. *Clin Pediatr (Phila)* 1986;**25**: 46–8.
333. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella pertussis* and other *Bordetella* subspecies. *Clin Microbiol Rev* 2005; **18**:326–82.
334. Mazengia E, Silva EA, Peppe JA, et al. Recovery of *Bordetella holmesii* from patients with pertussis-like symptoms: use of pulsed-field gel electrophoresis to characterize circulating strains. *J Clin Microbiol* 2000;**38**:2330–3.
335. McCavit TL, Grube S, Revell P, et al. *Bordetella holmesii* bacteremia in sickle cell disease. *Pediatr Blood Cancer* 2008;**51**:814–16.
336. McEniery JA, Delbridge RG, Reith DM. Infant pertussis deaths and the management of cardiovascular compromise. *J Paediatr Child Health* 2004;**40**:230–2.
337. McGowan JP. Some observations on a laboratory epidemic, principally among dogs and cats, in which the animals affected presented the symptoms of the disease called "distemper." *J Pathol Bacteriol* 1911;**15**:372–430.
338. McGregor J, Ogle JW, Curry-Kane G. Perinatal pertussis. *Obstet Gynecol* 1986;**68**:582–6.
339. Meade BD, Bollen A. Recommendations for use of the polymerase chain reaction in the diagnosis of *Bordetella pertussis* infections. *J Med Microbiol* 1994;**41**:51–5.

340. Medical Research Council. The prevention of whooping cough by vaccination. *BMJ* 1951;**1**:1463–71.
341. Medical Research Council. Vaccination against whooping cough: relation between protection in children and results of laboratory tests. *BMJ* 1956;**2**:454–62.
342. Medical Research Council. Vaccination against whooping cough. *BMJ* 1959;**1**:994–1000.
343. Melchior JC. Infantile spasms and early immunization against whooping cough. Danish survey from 1970 to 1975. *Arch Dis Child* 1977;**52**:134–7.
344. Mertsola J. Mixed outbreak of *Bordetella pertussis* and *Bordetella parapertussis* infection in Finland. *Eur J Clin Microbiol* 1985;**4**: 123–8.
345. Mertsola J, Ruuskanen O, Kuronen T, et al. Serologic diagnosis of pertussis: evaluation of pertussis toxin and other antigens in enzyme-linked immunosorbent assay. *J Infect Dis* 1990;**161**: 966–71.
346. Mikelova LK, Halperin SA, Scheifele D, et al. Predictors of death in infants hospitalized with pertussis: a case-control study of 16 pertussis deaths in Canada. *J Pediatr* 2003;**143**:576–81.
347. Miller D, Madge N, Diamond J, et al. Pertussis immunisation and serious acute neurological illnesses in children. *BMJ* 1993;**307**: 1171–6.
348. Miller D, Wadsworth J, Diamond J, et al. Pertussis vaccine and whooping cough as risk factors in acute neurological illness and death in young children. *Dev Biol Stand* 1985;**61**:389–94.
349. Miller D, Wadsworth J, Ross E. Severe neurological illness: further analyses of the British National Childhood Encephalopathy Study. *Tokai J Exp Clin Med* 1988;**13**(Suppl):145–55.
350. Miller DL, Ross EM, Alderslade R, et al. Pertussis immunisation and serious acute neurological illness in children. *BMJ (Clin Res Ed)* 1981;**282**:1595–9.
351. Miller JJ Jr, Silverberg RJ, Saito TM, et al. An agglutinative reaction for *Hemophilus pertussis*, part II: its relation to clinical immunity. *J Pediatr* 1943;**22**:644–51.
352. Mills KH, Barnard A, Watkins J, et al. Cell-mediated immunity to *Bordetella pertussis*: role of Th1 cells in bacterial clearance in a murine respiratory infection model. *Infect Immun* 1993;**61**:399–410.
353. Mills KH, Redhead K. Cellular immunity in pertussis. *J Med Microbiol* 1993;**39**:163–4.
354. Minh NNT, He Q, Edelman K, et al. Cell-mediated immune responses to antigens of *Bordetella pertussis* and protection against pertussis in school children. *Pediatr Infect Dis J* 1999;**18**:366–70.
355. Mink CA, Sirota NM, Nugent S. Outbreak of pertussis in a fully immunized adolescent and adult population. *Arch Pediatr Adolesc Med* 1994;**148**:153–7.
356. Mink CM, Cherry JD, Christenson P, et al. A search for *Bordetella pertussis* infection in university students. *Clin Infect Dis* 1992;**14**: 464–71.
357. Mink CM, O'Brien CH, Wassilak S, et al. Isotype and antigen specificity of pertussis agglutinins following whole-cell pertussis vaccination and infection with *Bordetella pertussis*. *Infect Immun* 1994;**62**:1118–20.
358. Miron D, Srugo I, Kra-Oz Z, et al. Sole pathogen in acute bronchiolitis: is there a role for other organisms apart from respiratory syncytial virus? *Pediatr Infect Dis J* 2010;**29**:e7–10.
359. Moissenet D, Leverger G, Merens A, et al. Septic arthritis caused by *Bordetella holmesii* in an adolescent with chronic haemolytic anaemia. *J Med Microbiol* 2011;**60**:1705–7.
360. Mooi FA, van der Heide HGJ, Wellems R, et al. *Bordetella pertussis* fimbriae: role in pathogenesis and mechanism of phase variation. Paper presented at the Sixth International Symposium on Pertussis, Bethesda, Md., September 26-28, 1990, p. 63.
361. Mooi FR, Bruisten S, Linde I, et al. Characterization of *Bordetella holmesii* isolates from patients with pertussis-like illness in The Netherlands. *FEMS Immunol Med Microbiol* 2012;**64**:289–91.
362. Mooi FR, van Loo IH, van Gent M, et al. *Bordetella pertussis* strains with increased toxin production associated with pertussis resurgence. *Emerg Infect Dis* 2009;**15**:1206–13.
363. Moore DL, Le Saux N, Scheifele D, et al. Lack of evidence of encephalopathy related to pertussis vaccine: active surveillance by IMPACT, Canada, 1993-2002. *Pediatr Infect Dis J* 2004;**23**: 568–71.
364. Morris JT, Myers M. Bacteremia due to *Bordetella holmesii*. *Clin Infect Dis* 1998;**27**:912–13.
365. Morrison W. Infantile hypertrophic pyloric stenosis in infants treated with azithromycin. *Pediatr Infect Dis J* 2007;**26**:186–8.
366. Mortimer EA Jr. Pertussis and its prevention: a family affair. *J Infect Dis* 1990;**161**:473–9.
367. Mortimer EA Jr, Jones PK. An evaluation of pertussis vaccine. *Rev Infect Dis* 1979;**1**:927–34.
368. Mortimer EA Jr, Jones PK. Pertussis vaccine in the United States: the benefit-risk ratio. In: Manclark CR, Hill JC, editors. *International Symposium on Pertussis*. Publication No. (N. I. H.) 79-1830. Washington, D.C.: U.S. Dept. of Health, Education, and Welfare, U.S. Government Printing Office; 1979. p. 250.
369. Mortimer EA Jr, Kimura M, Cherry JD, et al. Protective efficacy of the Takeda acellular pertussis vaccine combined with diphtheria and tetanus toxoids following household exposure of Japanese children. *Am J Dis Child* 1990;**144**:899–904.
370. Mountzouros KT, Kimura A, Cowell JL. A bactericidal monoclonal antibody specific for the lipooligosaccharide of *Bordetella pertussis* reduces colonization of the respiratory tract of mice after aerosol infection with *B. pertussis*. *Infect Immun* 1992;**60**:5316–18.
371. Muller FM, Hoppe JE, Wirsing von Konig CH. Laboratory diagnosis of pertussis: state of the art in 1997. *J Clin Microbiol* 1997;**35**:2435–43.
372. Munoz JJ. Action of pertussigen (pertussis toxin) on the host immune system. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 173–92.
373. Murray EL, Nieves D, Bradley JS, et al. Characteristics of severe *Bordetella pertussis* infection among infants ≤90 days of age admitted to pediatric intensive care units—Southern California, September 2009-June 2011. *J Pediatric Infectious Disease Society* 2013; **2**:1–6
374. Nagel J, Poot-Scholtens EJ. Serum IgA antibody to *Bordetella pertussis* as an indicator of infection. *J Med Microbiol* 1983;**16**: 417–26.
375. Nakase Y, Endoh M. Heat-labile toxin of *Bordetella pertussis*. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 217–29.
376. Namachivayam P, Shimizu K, Butt W. Pertussis: severe clinical presentation in pediatric intensive care and its relation to outcome. *Pediatr Crit Care Med* 2007;**8**:207–11.
377. Nei T, Hyodo H, Sonobe K, et al. First report of infectious pericarditis due to *Bordetella holmesii* in an adult patient with malignant lymphoma. *J Clin Microbiol* 2012;**50**:1815–17.
378. Nelson JD. The changing epidemiology of pertussis in young infants: the role of adults as reservoirs of infection. *Am J Dis Child* 1978;**132**:371–3.
379. Nelson KE, Gavitt F, Batt MD, et al. The role of adenoviruses in the pertussis syndrome. *J Pediatr* 1975;**86**:335–41.
380. Nencioni L, Pizza MG, Volpini G, et al. Properties of the B oligomer of pertussis toxin. *Infect Immun* 1991;**59**:4732–4.
381. Nennig ME, Shinefield HR, Edwards KM, et al. Prevalence and incidence of adult pertussis in an urban population. *JAMA* 1996;**275**:1672–4.
382. Newman A, Davis JP, Warshaner D. Personal communication. 2006.
383. Ng VL, Boggs JM, York MK, et al. Recovery of *Bordetella bronchiseptica* from patients with AIDS. *Clin Infect Dis* 1992;**15**: 376–7.
384. Nicoll A, Gardner A. Whooping cough and unrecognised postperinatal mortality. *Arch Dis Child* 1988;**63**:41–7.
385. Njamkepo E, Bonacorsi S, Debruyne M, et al. Significant finding of *Bordetella holmesii* DNA in nasopharyngeal samples from French patients with suspected pertussis. *J Clin Microbiol* 2011;**49**: 4347–8.
386. Noble GR, Bernier RH, Esber EC, et al. Acellular and whole-cell pertussis vaccines in Japan: report of a visit by US scientists. *JAMA* 1987;**257**:1351–6.
387. Noofeli M, Bokhari H, Blackburn P, et al. BapC autotransporter protein is a virulence determinant of *Bordetella pertussis*. *Microb Pathog* 2011;**51**:169–77.
388. Novotny P. Pathogenesis in *Bordetella* species. *J Infect Dis* 1990; **161**:581–3.
389. Novotny P, Chubb AP, Cownley K, et al. Biologic and protective properties of the 69-kDa outer membrane protein of *Bordetella pertussis*: a novel formulation for an acellular pertussis vaccine. *J Infect Dis* 1991;**164**:114–22.

390. Octavia S, Sintchenko V, Gilbert GL, et al. Newly emerging clones of *Bordetella pertussis* carrying prn2 and ptxP3 alleles implicated in Australian pertussis epidemic in 2008-2010. *J Infect Dis* 2012;**205**:1220–4.
391. Ohtsuka M, Kikuchi K, Shimizu K, et al. Emergence of quinolone-resistant *Bordetella pertussis* in Japan. *Antimicrob Agents Chemother* 2009;**53**:3147–9.
392. Olcen P, Backman A, Johansson B, et al. Amplification of DNA by the polymerase chain reaction for the efficient diagnosis of pertussis. *Scand J Infect Dis* 1992;**24**:339–45.
393. Olin P, Gustafsson L, Barreto L, et al. Declining pertussis incidence in Sweden following the introduction of acellular pertussis vaccine. *Vaccine* 2003;**21**:2015–21.
394. Olin P, Rasmussen F, Gustafsson L, et al. Randomised controlled trial of two-component, three-component, and five-component acellular pertussis vaccines compared with whole-cell pertussis vaccine. Ad Hoc Group for the Study of Pertussis Vaccines. *Lancet* 1997;**350**:1569–77.
395. Oliver DC, Fernandez RC. Antibodies to BrkA augment killing of *Bordetella pertussis*. *Vaccine* 2001;**20**:235–41.
396. Olsen LC. Pertussis. *Medicine (Baltimore)* 1975;**54**:427–69.
397. Onorato IM, Wassilak SG. Laboratory diagnosis of pertussis: the state of the art. *Pediatr Infect Dis J* 1987;**6**:145–51.
398. Onorato IM, Wassilak SG, Meade B. Efficacy of whole-cell pertussis vaccine in preschool children in the United States. *JAMA* 1992;**267**:2745–9.
399. Paddock CD, Sanden GN, Cherry JD, et al. Pathology and pathogenesis of fatal *Bordetella pertussis* infection in infants. *Clin Infect Dis* 2008;**47**:328–38.
400. Panagopoulos MI, Saint Jean M, Brun D, et al. *Bordetella holmesii* bacteremia in asplenic children: report of four cases initially misidentified as *Acinetobacter lwoffii*. *J Clin Microbiol* 2010;**48**: 3762–4.
401. Pichichero ME, Edwards KM, Anderson EL, et al. Safety and immunogenicity of six acellular pertussis vaccines and one whole-cell pertussis vaccine given as a fifth dose in four- to six-year-old children. *Pediatrics* 2000;**105**:e11.
402. Pierce C, Klein N, Peters M. Is leukocytosis a predictor of mortality in severe pertussis infection? *Intensive Care Med* 2000;**26**: 1512–14.
403. Pillay V, Swingler G. Symptomatic treatment of the cough in whooping cough. *Cochrane Database Syst Rev* (4):CD003257, 2003.
404. Pittman M. Pertussis toxin: the cause of the harmful effects and prolonged immunity of whooping cough: a hypothesis. *Rev Infect Dis* 1979;**1**:401–12.
405. Pittman M. The concept of pertussis as a toxin-mediated disease. *Pediatr Infect Dis* 1984;**3**:467–86.
406. Pooboni S, Roberts N, Westrope C, et al. Extracorporeal life support in pertussis. *Pediatr Pulmonol* 2003;**36**:310–15.
407. Porter JF, Connor K, Donachie W. Isolation and characterization of *Bordetella parapertussis*-like bacteria from ovine lungs. *Microbiology* 1994;**140**(Pt 2):255–61.
408. Postels-Multani S, Schmitt HJ, Wirsing von Konig CH, et al. Symptoms and complications of pertussis in adults. *Infection* 1995;**23**:139–42.
409. Preston A, Parkhill J, Maskell DJ. The Bordetellae: lessons from genomics. *Nat Rev Microbiol* 2004;**2**:379–90.
410. Preston NW. Effectiveness of pertussis vaccines. *BMJ* 1965;**2**: 11–13.
411. Preston NW. Pertussis today. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 1–18.
412. Prince HE, Lape-Nixon M, Matud J. Evaluation of a tetraplex microsphere assay for *Bordetella pertussis* antibodies. *Clin Vaccine Immunol* 2006;**13**:266–70.
413. Prince HE, Lieberman JM, Cherry JD. Age-related differences in patterns of increased *Bordetella pertussis* antibodies. *Clin Vaccine Immunol* 2012;**19**:545–50.
414. Rank C, Quinn HE, McIntyre PB. Pertussis vaccine effectiveness after mass immunization of high school students in Australia. *Pediatr Infect Dis J* 2009;**28**:152–3.
415. Rath BA, Register KB, Wall J, et al. Persistent *Bordetella bronchiseptica* pneumonia in an immunocompetent infant and genetic comparison of clinical isolates with kennel cough vaccine strains. *Clin Infect Dis* 2008;**46**:905–8.
416. Ray P, Hayward J, Michelson D, et al. Encephalopathy after whole-cell pertussis or measles vaccination: lack of evidence for a causal association in a retrospective case-control study. *Pediatr Infect Dis J* 2006;**25**:768–73.
417. Register KB, Sanden GN. Prevalence and sequence variants of IS481 in *Bordetella bronchiseptica*: implications for IS481-based detection of *Bordetella pertussis*. *J Clin Microbiol* 2006;**44**:4577–83.
418. Reizenstein E, Johansson B, Mardin L, et al. Diagnostic evaluation of polymerase chain reaction discriminative for *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica*. *Diagn Microbiol Infect Dis* 1993;**17**:185–91.
419. Reizenstein E, Lindberg L, Mollby R, et al. Validation of nested *Bordetella* PCR in pertussis vaccine trial. *J Clin Microbiol* 1996; **34**:810–15.
420. Rennels MB, Deloria MA, Pichichero ME, et al. Extensive swelling after booster doses of acellular pertussis-tetanus-diphtheria vaccines. *Pediatrics* 2000;**105**:e12.
421. Riffelmann M, Thiel K, Schmetz J, et al. Performance of commercial enzyme-linked immunosorbent assays for detection of antibodies to *Bordetella pertussis*. *J Clin Microbiol* 2010;**48**:4459–63.
422. Robbins JB, Pittman M, Trollfors B, et al. Primum non nocere: a pharmacologically inert pertussis toxoid alone should be the next pertussis vaccine. *Pediatr Infect Dis J* 1993;**12**:795–807.
423. Robbins JB, Schneerson R, Keith JM, et al. Pertussis vaccine: a critique. *Pediatr Infect Dis J* 2009;**28**:237–41.
424. Roberts I, Gavin R, Lennon D. Randomized controlled trial of steroids in pertussis. *Pediatr Infect Dis J* 1992;**11**:982–3.
425. Robertson PW, Goldberg H, Jarvie BH, et al. *Bordetella pertussis* infection: a cause of persistent cough in adults. *Med J Aust* 1987;**146**:522–5.
426. Robinson A, Ashworth LA, Irons LI Serotyping *Bordetella pertussis* strains. *Vaccine* 1989;**7**:491–4.
427. Robinson A, Irons LI, Seabrook RN, et al. Structure-function studies of *Bordetella pertussis* fimbriae. In: Manclark CR, editor. *Proceedings of the Sixth International Symposium on Pertussis*. DHHS. publication No. (FDA) 90. Bethesda, Md.: Department of Health and Human Services, U.S. Public Health Service; 1990. p. 126–35.
428. Rodriguez ME, Hellwig SM, Perez Vidakovics ML, et al. *Bordetella pertussis* attachment to respiratory epithelial cells can be impaired by fimbriae-specific antibodies. *FEMS Immunol Med Microbiol* 2006;**46**:39–47.
429. Rohani P, Drake JM. The decline and resurgence of pertussis in the US. *Epidemics* 2011;**3**:183–8.
430. Rohani P, Zhong X, King AA. Contact network structure explains the changing epidemiology of pertussis. *Science* 2010;**330**:982–5.
431. Romano MJ, Weber MD, Weisse ME, et al. Pertussis pneumonia, hypoxemia, hyperleukocytosis, and pulmonary hypertension: improvement in oxygenation after a double volume exchange transfusion. *Pediatrics* 2004;**114**:e264–266.
432. Roorda L, Buitenwerf J, Ossewaarde JM, et al. A real-time PCR assay with improved specificity for detection and discrimination of all clinically relevant *Bordetella* species by the presence and distribution of three Insertion Sequence elements. *BMC Res Notes* 2011;**4**:11.
433. Rosenthal S, Strebel P, Cassiday P, et al. Pertussis infection among adults during the 1993 outbreak in Chicago. *J Infect Dis* 1995; **171**:1650–2.
434. Rothstein EP, Anderson EL, Decker MD, et al. An acellular pertussis vaccine in healthy adults: safety and immunogenicity. Pennridge Pediatric Associates. *Vaccine* 1999;**17**:2999–3006.
435. Rowlands HE, Goldman AP, Harrington K, et al. Impact of rapid leukodepletion on the outcome of severe clinical pertussis in young infants. *Pediatrics* 2010;**126**:e816–27.
436. Ryan M, Murphy G, Gothefors L, et al. *Bordetella pertussis* respiratory infection in children is associated with preferential activation of type 1 T helper cells. *J Infect Dis* 1997;**175**:1246–50.
437. Ryan M, Murphy G, Ryan E, et al. Distinct T-cell subtypes induced with whole cell and acellular pertussis vaccines in children. *Immunology* 1998;**93**:1–10.
438. Salmaso S, Mastrantonio P, Tozzi AE, et al. Sustained efficacy during the first 6 years of life of 3-component acellular pertussis vaccines administered in infancy: the Italian experience. *Pediatrics* 2001;**108**:1–7.

439. Salmaso S, Mastrantonio P, Wassilak SG, et al. Persistence of protection through 33 months of age provided by immunization in infancy with two three-component acellular pertussis vaccines. Stage II Working Group. *Vaccine* 1998;**16**:1270–5.
440. Sato H, Sato Y. *Bordetella pertussis* infection in mice: correlation of specific antibodies against two antigens, pertussis toxin, and filamentous hemagglutinin with mouse protectivity in an intracerebral or aerosol challenge system. *Infect Immun* 1984;**46**:415–21.
441. Sato Y, Kimura M, Fukumi H. Development of a pertussis component vaccine in Japan. *Lancet* 1984;**1**:122–6.
442. Sauer LW, Hambrecht L. Experimental whooping cough. *Am J Dis Child* 1929;**37**:732–44.
443. Sawal M, Cohen M, Irazuzta JE, et al. Fulminant pertussis: a multi-center study with new insights into the clinico-pathological mechanisms. *Pediatr Pulmonol* 2009;**44**:970–80.
444. Schlapfer G, Cherry JD, Heininger U, et al. Polymerase chain reaction identification of *Bordetella pertussis* infections in vaccinees and family members in a pertussis vaccine efficacy trial in Germany. *Pediatr Infect Dis J* 1995;**14**:209–14.
445. Schlapfer G, Senn HP, Berger R, et al. Use of the polymerase chain reaction to detect *Bordetella pertussis* in patients with mild or atypical symptoms of infection. *Eur J Clin Microbiol Infect Dis* 1993;**12**:459–63.
446. Schmidtke AJ, Boney KO, Martin SW, et al. Population diversity among *Bordetella pertussis* isolates, United States, 1935-2009. *Emerg Infect Dis J* 2012;**18**(8):1248–55.
447. Schmitt-Grohe S, Cherry JD, Heininger U, et al. Pertussis in German adults. *Clin Infect Dis* 1995;**21**:860–6.
448. Schmitt-Grohe S, Stehr K, Cherry JD, et al. Minor adverse events in a comparative efficacy trial in Germany in infants receiving either the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine, the Lederle whole-cell component DTP (DTP) or DT vaccine. The Pertussis Vaccine Study Group. *Dev Biol Stand* 1997;**89**:113–18.
449. Schmitt HJ, Beutel K, Schuind A, et al. Reactogenicity and immunogenicity of a booster dose of a combined diphtheria, tetanus, and tricomponent acellular pertussis vaccine at fourteen to twenty-eight months of age. *J Pediatr* 1997;**130**:616–23.
450. Schmitt HJ, von Konig CH, Neiss A, et al. Efficacy of acellular pertussis vaccine in early childhood after household exposure. *JAMA* 1996;**275**:37–41.
451. Shahin RD, Amsbaugh DF, Leef MF. Mucosal immunization with filamentous hemagglutinin protects against *Bordetella pertussis* respiratory infection. *Infect Immun* 1992;**60**:1482–8.
452. Shahin RD, Brennan MJ, Li ZM, et al. Characterization of the protective capacity and immunogenicity of the 69-kD outer membrane protein of *Bordetella pertussis*. *J Exp Med* 1990;**171**:63–73.
453. Shields WD, Nielsen C, Buch D, et al. Relationship of pertussis immunization to the onset of neurologic disorders: a retrospective epidemiologic study. *J Pediatr* 1988;**113**:801–5.
454. Simondon F, Preziosi MP, Yam A, et al. A randomized double-blind trial comparing a two-component acellular to a whole-cell pertussis vaccine in Senegal. *Vaccine* 1997;**15**:1606–12.
455. Skladal D, Horak E, Fruhwirth M, et al. Successful treatment of ARDS and severe pulmonary hypertension in a child with *Bordetella pertussis* infection. *Wien Klin Wochenschr* 2004;**116**:760–2.
456. Smith C, Vyas H. Early infantile pertussis; increasingly prevalent and potentially fatal. *Eur J Pediatr* 2000;**159**:898–900.
457. Somerville RL, Grant CC, Grimwood K, et al. Infants hospitalised with pertussis: estimating the true disease burden. *J Paediatr Child Health* 2007;**43**:617–22.
458. Southall DP, Thomas MG, Lambert HP. Severe hypoxaemia in pertussis. *Arch Dis Child* 1988;**63**:598–605.
459. Spilker T, Liwienski AA, LiPuma JJ. Identification of *Bordetella* spp. in respiratory specimens from individuals with cystic fibrosis. *Clin Microbiol Infect* 2008;**14**:504–6.
460. Sreenan CD, Osiovich H. Neonatal pertussis requiring extracorporeal membrane oxygenation. *Pediatr Surg Int* 2001;**17**:201–3.
461. Stark D, Riley LA, Harkness J, et al. *Bordetella petrii* from a clinical sample in Australia: isolation and molecular identification. *J Med Microbiol* 2007;**56**:435–7.
462. Stefanelli P, Mastrantonio P, Hausman SZ, et al. Molecular characterization of two *Bordetella bronchiseptica* strains isolated from children with coughs. *J Clin Microbiol* 1997;**35**:1550–5.
463. Stehr K, Cherry JD, Heininger U, et al. A comparative efficacy trial in Germany in infants who received either the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine, the Lederle whole-cell component DTP vaccine, or DT vaccine. *Pediatrics* 1998;**101**:1–11.
464. Steketee RW, Burstyn DG, Wassilak SG, et al. A comparison of laboratory and clinical methods for diagnosing pertussis in an outbreak in a facility for the developmentally disabled. *J Infect Dis* 1988;**157**:441–9.
465. Steketee RW, Wassilak SG, Adkins WN Jr, et al. Evidence for a high attack rate and efficacy of erythromycin prophylaxis in a pertussis outbreak in a facility for the developmentally disabled. *J Infect Dis* 1988;**157**:434–40.
466. Stephenson JB. A neurologist looks at neurological disease temporally related to DTP immunization. *Tokai J Exp Clin Med* 1988;**13**(Suppl):157–64.
467. Stewart GT. Vaccination against whooping-cough: efficacy versus risks. *Lancet* 1977;**1**:234–7.
468. Stockbauer KE, Fuchslocher B, Miller JF, et al. Identification and characterization of BipA, a *Bordetella* Bvg-intermediate phase protein. *Mol Microbiol* 2001;**39**:65–78.
469. Stocks P. *Studies in the population of England and Wales 1944-47: Studies on medical and population subjects*. No. 2. London: His Majesty's Stationery Office; 1949.
470. Storsaeter J, Blackwelder WC, Hallander HO. Pertussis antibodies, protection, and vaccine efficacy after household exposure. *Am J Dis Child* 1992;**146**:167–72.
471. Storsaeter J, Hallander H, Farrington CP, et al. Secondary analyses of the efficacy of two acellular pertussis vaccines evaluated in a Swedish phase III trial. *Vaccine* 1990;**8**:457–61.
472. Storsaeter J, Hallander HO, Gustafsson L, et al. Levels of anti-pertussis antibodies related to protection after household exposure to *Bordetella pertussis*. *Vaccine* 1998;**16**:1907–16.
473. Storsaeter J, Olin P. Relative efficacy of two acellular pertussis vaccines during three years of passive surveillance. *Vaccine* 1992;**10**:142–4.
474. Strebel P, Cochi S, Farizo K, et al. Pertussis in Missouri: evaluation of nasopharyngeal culture, direct fluorescent antibody testing, and clinical case definitions in the diagnosis of pertussis. *Clin Infect Dis* 1993;**16**:276–85.
475. Strebel P, Nordin J, Edwards K, et al. Population-based incidence of pertussis among adolescents and adults, Minnesota, 1995-1996. *J Infect Dis* 2001;**183**:1353–9.
476. Strom J. Further experience of reactions, especially of a cerebral nature, in conjunction with triple vaccination: a study based on vaccinations in Sweden 1959-65. *BMJ* 1967;**4**:320–3.
477. Swansea Research Unit of the Royal College of General Practitioners. Study of intellectual performance of children in ordinary schools after certain serious complications of whooping cough. *BMJ* 1987;**295**:1044–7.
478. Switzer WP, Mare CJ, Hubbard ED. Incidence of *Bordetella bronchiseptica* in wildlife and man in Iowa. *Am J Vet Res* 1966;**27**:1134–6.
479. Tamion F, Girault C, Chevron V, et al. *Bordetella bronchoseptica* pneumonia with shock in an immunocompetent patient. *Scand J Infect Dis* 1996;**28**:137–8.
480. Tan T, Trindade E, Skowronski D. Epidemiology of pertussis. *Pediatr Infect Dis J* 2005;**24**:S10–18.
481. Tanaka M, Vitek CR, Pascual FB, et al. Trends in pertussis among infants in the United States, 1980-1999. *JAMA* 2003;**290**:2968–75.
482. Tang YW, Hopkins MK, Kolbert CP, et al. *Bordetella holmesii*-like organisms associated with septicemia, endocarditis, and respiratory failure. *Clin Infect Dis* 1998;**26**:389–92.
483. Taranger J, Trollfors B, Lagergard T, et al. Parapertussis infection followed by pertussis infection. *Lancet* 1994;**344**:1703.
484. Taranger J, Trollfors B, Lagergard T, et al. Unchanged efficacy of a pertussis toxoid vaccine throughout the two years after the third vaccination of infants. *Pediatr Infect Dis J* 1997;**16**:180–4.
485. Tatti KM, Slade B, Patel M, et al. Real-time polymerase chain reaction detection of *Bordetella pertussis* DNA in acellular pertussis vaccines. *Pediatr Infect Dis J* 2008;**27**:73–4.
486. Tatti KM, Sparks KN, Boney KO, et al. Novel multitarget real-time PCR assay for rapid detection of *Bordetella* species in clinical specimens. *J Clin Microbiol* 2011;**49**:4059–66.
487. Thomas PF, McIntyre PB, Jalaludin BB. Survey of pertussis morbidity in adults in western Sydney. *Med J Aust* 2000;**173**:74–6.

488. Tomoda T, Ogura H, Kurashige T. Immune responses to *Bordetella pertussis* infection and vaccination. *J Infect Dis* 1991;**163**: 559–63.
489. Tondella ML, Carlone GM, Messonnier N, et al. International *Bordetella pertussis* assay standardization and harmonization meeting report. Centers for Disease Control and Prevention, Atlanta, Georgia, United States, 19-20 July 2007. *Vaccine* 2009; **27**:803–14.
490. Trollfors B, Taranger J, Lagergard T, et al. A placebo-controlled trial of a pertussis-toxoid vaccine. *N Engl J Med* 1995;**333**: 1045–50.
491. Troseid M, Jonassen TO, Steinbakk M. Isolation of *Bordetella pertussis* in blood culture from a patient with multiple myeloma. *J Infect* 2006;**52**:e11–13.
492. Tuomanen E. *Bordetella pertussis* adhesins. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 75–94.
493. Uberall MA, Stehr K, Cherry JD, et al. Severe adverse events in a comparative efficacy trial in Germany in infants receiving either the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine, the Lederle whole-cell component DTP (DTP) or DT vaccine. The Pertussis Vaccine Study Group. *Dev Biol Stand* 1997;**89**:83–9.
494. Ui M. The multiple biological activities of pertussis toxin. In: Wardlaw AC, Parton R, editors. *Pathogenesis and immunity in pertussis*. New York: John Wiley & Sons; 1988. p. 121–46.
495. Urisu A, Cowell JL, Manclark CR. Filamentous hemagglutinin has a major role in mediating adherence of *Bordetella pertussis* to human WiDr cells. *Infect Immun* 1986;**52**:695–701.
496. Valenti WM, Pincus PH, Messner MK. Nosocomial pertussis: possible spread by a hospital visitor. *Am J Dis Child* 1980;**134**: 520–1.
497. Van Balen T, Nieman AE, Hermans MH, et al. *Bordetella holmesii* meningitis in a 12-year-old anorectic girl. *Pediatr Infect Dis J* 2012;**31**:421–2.
498. Van Buynder PG, Owen D, Vurdien JE, et al. *Bordetella pertussis* surveillance in England and Wales: 1995-7. *Epidemiol Infect* 1999;**123**:403–11.
499. van der Zee A, Agterberg C, Peeters M, et al. Polymerase chain reaction assay for pertussis: simultaneous detection and discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. *J Clin Microbiol* 1993;**31**:2134–40.
500. van der Zee A, Agterberg C, Peeters M, et al. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. *J Infect Dis* 1996;**174**:89–96.
501. van Kruijssen AM, Templeton KE, van der Plas RN, et al. Detection of respiratory pathogens by real-time PCR in children with clinical suspicion of pertussis. *Eur J Pediatr* 2007;**166**: 1189–91.
502. Vancanneyt M, Vandamme P, Kersters K. Differentiation of *Bordetella pertussis*, *B. parapertussis*, and *B. bronchiseptica* by whole-cell protein electrophoresis and fatty acid analysis. *Int J Syst Bacteriol* 1995;**45**:843–7.
503. Vandamme P, Heyndrickx M, Vancanneyt M, et al. *Bordetella trematum* sp. nov., isolated from wounds and ear infections in humans, and reassessment of Alcaligenes denitrificans Ruger and Tan 1983. *Int J Syst Bacteriol* 1996;**46**:849–58.
504. Versteegh FG, Schellekens JF, Nagelkerke AF, et al. Laboratory-confirmed reinfections with *Bordetella pertussis*. *Acta Paediatr* 2002;**91**:95–7.
505. Vidakovics MLA, Lamberti Y, van der Pol WL, et al. Adenylate cyclase influences filamentous haemagglutinin-mediated attachment of *Bordetella pertussis* to epithelial alveolar cells. *FEMS Immunol Med Microbiol* 2006;**48**:140–7.
506. Viljanen MK, Ruuskanen O, Granberg C, et al. Serological diagnosis of pertussis: IgM, IgA and IgG antibodies against *Bordetella pertussis* measured by enzyme-linked immunosorbent assay (ELISA). *Scand J Infect Dis* 1982;**14**:117–22.
507. Vincent JM, Cherry JD, Nauschuetz WF, et al. Prolonged afebrile nonproductive cough illnesses in American soldiers in Korea: a serological search for causation. *Clin Infect Dis* 2000;**30**:534–9.
508. Vitek CR, Pascual FB, Baughman AL, et al. Increase in deaths from pertussis among young infants in the United States in the 1990s. *Pediatr Infect Dis J* 2003;**22**:628–34.
509. von Wintzingerode F, Schattke A, Siddiqui RA, et al. *Bordetella petrii* sp. nov., isolated from an anaerobic bioreactor, and emended description of the genus *Bordetella*. *Int J Syst Evol Microbiol* 2001; **51**:1257–65.
510. Walker AM, Jick H, Perera DR, et al. Neurologic events following diphtheria-tetanus-pertussis immunization. *Pediatrics* 1988;**81**: 345–9.
511. Walker KE, Weiss AA. Characterization of the dermonecrotic toxin in members of the genus *Bordetella*. *Infect Immun* 1994;**62**: 3817–28.
512. Wang K, Chalker V, Bermingham A, et al. *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough in England: a retrospective analysis. *Pediatr Infect Dis J* 2011;**30**:1047–51.
513. Ward JI, Cherry JD, Chang SJ, et al. Efficacy of an acellular pertussis vaccine among adolescents and adults. *N Engl J Med* 2005;**353**:1555–63.
514. Ward JI, Cherry JD, Chang SJ, et al. *Bordetella Pertussis* infections in vaccinated and unvaccinated adolescents and adults, as assessed in a national prospective randomized Acellular Pertussis Vaccine Trial (APERT). *Clin Infect Dis* 2006;**43**:151–7.
515. Warngard O, Nilsson L, Fahraeus C, et al. Catch-up primary vaccination with acellular pertussis vaccines in 3-4-year-old children—reactogenicity and serological response. *Vaccine* 1998; **16**:480–4.
516. Washburn TC, Medearis DN Jr, Childs B. Sex differences in susceptibility to infections. *Pediatrics* 1965;**35**:57–64.
517. Watts CC, Acosta C. Pertussis and bilateral subdural hematomas. *Am J Dis Child* 1969;**118**:518–9.
518. Wearing HJ, Rohani P. Estimating the duration of pertussis immunity using epidemiological signatures. *PLoS Pathog* 2009;**5**: e1000647.
519. Wei SC, Tatti K, Cushing K, et al. Effectiveness of adolescent and adult tetanus, reduced-dose diphtheria, and acellular pertussis vaccine against pertussis. *Clin Infect Dis* 2010;**51**:315–21.
520. Weiss AA, Falkow S. Genetic analysis of phase change in *Bordetella pertussis*. *Infect Immun* 1984;**43**:263–9.
521. Weiss AA, Hewlett EL. Virulence factors of *Bordetella pertussis*. *Annu Rev Microbiol* 1986;**40**:661–86.
522. Weiss AA, Hewlett EL, Myers GA, et al. Pertussis toxin and extracytoplasmic adenylate cyclase as virulence factors of *Bordetella pertussis*. *J Infect Dis* 1984;**150**:219–22.
523. Weiss AA, Patton AK, Millen SH, et al. Acellular pertussis vaccines and complement killing of *Bordetella pertussis*. *Infect Immun* 2004;**72**:7346–51.
524. Wendelboe AM, Njamkepo E, Bourillon A, et al. Transmission of *Bordetella pertussis* to young infants. *Pediatr Infect Dis J* 2007; **26**:293–9.
525. Wernli D, Emonet S, Schrenzel J, et al. Evaluation of eight cases of confirmed *Bordetella bronchiseptica* infection and colonization over a 15-year period. *Clin Microbiol Infect* 2010;**17**:201–3.
526. Weyant RS, Hollis DG, Weaver RE, et al. *Bordetella holmesii* sp. nov., a new gram-negative species associated with septicemia. *J Clin Microbiol* 1995;**33**:1–7.
527. Wiertz EJ, Loggen HG, Walvoort HC, et al. In vitro induction of antigen specific antibody synthesis and proliferation of T lymphocytes with acellular pertussis vaccines, pertussis toxin and filamentous haemagglutinin in humans. *J Biol Stand* 1989;**17**: 181–90.
528. Willems RJ, Kamerbeek J, Geuijen CA, et al. The efficacy of a whole cell pertussis vaccine and fimbriae against *Bordetella pertussis* and *Bordetella parapertussis* infections in a respiratory mouse model. *Vaccine* 1998;**16**:410–6.
529. Williams CL, Haines R, Cotter PA. Serendipitous discovery of an immunoglobulin-binding autotransporter in *Bordetella* species. *Infect Immun* 2008;**76**:2966–77.
530. Williams GD, Numa A, Sokol J, et al. ECLS in pertussis: does it have a role? *Intensive Care Med* 1998;**24**:1089–92.
531. Wilson GS. *The hazards of immunization*. London: Althone Press; 1967.
532. Winter K, Harriman K, Zipprich J, et al. California pertussis epidemic, 2010. *J Pediatr* 2012;**16**:1091–6.
533. Wirsing von Konig CH, Gounis D, Laukamp S, et al. Evaluation of a single-sample serological technique for diagnosing pertussis in unvaccinated children. *Eur J Clin Microbiol Infect Dis* 1999; **18**:341–5.

534. Wirsing von Konig CH, Postels-Multani S, Bock HL, et al. Pertussis in adults: frequency of transmission after household exposure. *Lancet* 1995;**346**:1326–9.
535. Wirsing von Konig CH, Riffelmann M, Coenye T. *Bordetella* and related genera. In: Versalovic J, editor. *Manual of clinical microbiology*, vol. 1. Washington, D.C.: ASM Press; 1970.
536. Wirsing von Konig CH, Rott H, Bogaerts H, et al. A serologic study of organisms possibly associated with pertussis-like coughing. *Pediatr Infect Dis J* 1998;**17**:645–9.
537. Witt MA, Katz PH, Witt DJ. Unexpectedly limited durability of immunity following acellular pertussis vaccination in preadolescents in a North American outbreak. *Clin Infect Dis* 2012;**54**: 1730–5.
538. Wood N, McIntyre P, Marshall H, et al. Acellular pertussis vaccine at birth and one month induces antibody responses by two months of age. *Pediatr Infect Dis J* 2010;**29**:209–15.
539. Wood N, Siegrist CA. Neonatal immunization: where do we stand? *Curr Opin Infect Dis* 2011;**24**:190–5.
540. Woolf AL, Caplin H. Whooping cough encephalitis. *Arch Dis Child* 1956;**31**:87–91.
541. Woolfrey BF, Moody JA. Human infections associated with *Bordetella bronchiseptica*. *Clin Microbiol Rev* 1991;**4**:243–55.
542. World Health Organization: *World Health Organization Meeting on Case Definition of Pertussis, Geneva, January 10-1, 1991, MIM/EPI/PERT/9.1*. Geneva: World Health Organization; 1991.
543. Wortis N, Strebel PM, Wharton M, et al. Pertussis deaths: report of 23 cases in the United States, 1992 and 1993. *Pediatrics* 1996; **97**:607–12.
544. Wright SW, Edwards KM, Decker MD, et al. Pertussis infection in adults with persistent cough. *JAMA* 1995;**273**:1044–6.
545. Yih WK, Silva EA, Ida J, et al. *Bordetella holmesii*-like organisms isolated from Massachusetts patients with pertussis-like symptoms. *Emerg Infect Dis* 1999;**5**:441–3.
546. Yuk MH, Harvill ET, Miller JF. The BvgAS virulence control system regulates type III secretion in *Bordetella bronchiseptica*. *Mol Microbiol* 1998;**28**:945–59.
547. Zackrisson G, Lagergard T, Trollfors B, et al. Immunoglobulin A antibodies to pertussis toxin and filamentous hemagglutinin in saliva from patients with pertussis. *J Clin Microbiol* 1990;**28**: 1502–5.
548. Zepp F, Knuf M, Habermehl P, et al. Pertussis-specific cell-mediated immunity in infants after vaccination with a tricomponent acellular pertussis vaccine. *Infect Immun* 1996;**64**:4078–84.
549. Zhang YL, Sekura RD. Purification and characterization of the heat-labile toxin of *Bordetella pertussis*. *Infect Immun* 1991;**59**: 3754–9.
550. Zouari A, Touati A, Smaoui H, et al. Dual infection with *Bordetella pertussis* and *Mycoplasma pneumoniae* in three infants: case reports. *Infection* 2012;**40**:213–17.
551. Zoumboulakis D, Anagnostakis D, Albanis V, et al. Steroids in treatment of pertussis. a controlled clinical trial. *Arch Dis Child* 1973;**48**:51–4.

CHAPTER 130

Calymmatobacterium granulomatis

Mariam R. Chacko

Calymmatobacterium granulomatis has been renamed *Klebsiella granulomatis* and is the causative organism for the genital ulcer disease granuloma inguinale.[8,51] Granuloma inguinale has been known through the years by many different names, the most common being granuloma inguinale, donovanosis, and granuloma venereum.[35]

THE ORGANISM

In 1905, *C. granulomatis* was isolated in India by Donovan. It is an encapsulated gram-negative rod measuring 1.5 × 0.7 μm. *C. granulomatis* was considered a unique species distinct from other related organisms belonging to the subclass Proteobacteria.[24,34,35] However, because of difficulties in growing the organism the biochemical and bacteriologic characteristics of the organism had not been identified.[34,35] In recent studies using DNA hybridization techniques and 16S rDNA sequencing, the position of *C. granulomatis* within the *Klebsiella* genus closest to *K. pneumoniae* has been confirmed. Thus, the organism has now been reclassified under the genus *Klebsiella*.

Study of the organism reveals a complex cell envelope. In addition to having regular bacterial structures such as a mesosome, ribosomes, and nuclear material, the cytoplasm contains electron-dense granules; transmission electron microscopy shows an outer membrane, middle electron-opaque layer, and an inner plasma membrane. The organisms are enclosed mainly in large histiocytic cells and occasionally in polymorphonuclear cells and plasma cells. They multiply intracellularly to approximately 30 in number and eventually cause cell rupture.[9,35]

EPIDEMIOLOGY

Granuloma inguinale occurs predominantly in young and older adults, 20 to 40 years of age, and is a cause of genital ulcerative disease in tropical and subtropical regions of the world; it is now mainly seen in sporadic cases in Papua New Guinea, South Africa, India, Brazil, and Australia.[10,33,49] In the preantibiotic era granuloma inguinale was prevalent in many areas of the world.[33] As with all genital ulcerative diseases, granuloma inguinale has been given greater attention as a risk factor for development of human immunodeficiency virus (HIV) infection.[41] In South Africa, the prevalence of granuloma inguinale in patients with genital ulcer disease is reported to be approximately 10 percent.[38] A resurgence of the disease was reported in the late 1980s in Durban, South Africa. After a rapid test for donovanosis was introduced in the early 1990s, the number of cases reported by the Durban Health Department in South Africa increased substantially.[31] As a result of the availability of the rapid test and implementation of an HIV/sexually transmitted infections (STIs) initiative, the number of new cases of donovanosis in Australia decreased dramatically; five cases were reported in 2005.[3] In 1994, after 4 decades, a case of granuloma inguinale was reported in China.[16]

The prevalence of granuloma inguinale increased in developed countries in the 1990s, especially among migrant and poor people living in metropolitan areas. For example, three cases of granuloma inguinale were reported in Rome between 1993 and 2001, with most of them diagnosed in 2000 and 2001.[30] In 1992, three unrelated cases of granuloma inguinale, seen within a period of a few weeks, were reported in Toronto; two occurred in immigrants, and one occurred in a native-born Canadian. The first patient was a recent immigrant from El Salvador, and the second patient had immigrated several years previously and had a history of sexual activity with a Jamaican who had immigrated recently. The sexual partner of the third case had come from Turkey.[21] In 2007, donovanosis and syphilis were diagnosed in a single genital ulcer at a London clinic in a Jamaican heterosexual male known to have herpes.[45]

In 1947, the estimated population prevalence of granuloma inguinale in the United States was 5000 to 10,000[35]; more than 1000 cases per year were reported to the Centers for Disease Control and Prevention (CDC). Since the antibiotic era, the prevalence of this disease has declined rapidly and no cases have been reported in recent years through standard surveillance reports by the CDC. During the past 2 decades, granuloma inguinale has been reported predominantly through case reports; Growden and colleagues,[19] in a 1984 case report, provided national data obtained from the local health departments; 75 cases were reported in 1977 and 17 cases in 1982. This report described a case of a white 18-year-old woman in California in 1985 with a 10-month history of vulvar ulceration and a Giemsa stain positive for Donovan bodies.[19] In 1991, nongenital granuloma inguinale was diagnosed in a man in Texas with testicular carcinoma.[29] More recent case reports have been published from Chicago in 2006[43] and Charlottesville, Virginia in 2009.[1] Although the prevalence of the disease is low, this disease needs to be suspected in North America and Europe because of international travel and immigration.

Granuloma inguinale is a rare occurrence in children. In the early 1950s, 4 percent of 1- to 4-year-old children in a Papua New Guinea population were found to have granuloma inguinale. Although the mode of transmission

in adolescents and adults is considered to be sexual, the mode of transmission of the disease in these children was thought to be by skin-to-skin contact from sitting on the laps of infected adults.[35] Cases published since 1997 report otitis media, mastoiditis, neck mass, and cervical lymphadenopathy as the initial manifestations of donovanosis in infants and children.[4,17,18] The modes of transmission in these cases are thought to be perinatal and by skin-to-skin contact.[4,17,18]

Granuloma inguinale usually occurs more commonly in male than in female patients. However, in adolescents, the disease can develop more commonly in girls than in boys; a reflection of sexual activity between adolescent girls and adult men.[32] The risk factors and mode of transmission of granuloma inguinale are not clear. The disease generally is considered sexually transmitted and has been diagnosed in 12 to 50 percent of marital or steady sexual partners.[35,38] However, in many cases, granuloma inguinale cannot be detected in the sexual partners of infected persons. Anal intercourse also has been associated with rectal and penile lesions of granuloma inguinale.[26] O'Farrell and associates[39] studied the patterns of sexual behavior in Zulu men and women with genital ulcer disease and found that patients with granuloma inguinale and secondary syphilis, despite the presence of ulcers, were more likely than were patients with other genital ulcer diseases to have had sexual intercourse. Studies reported from India and South Africa have noted a preponderance of granuloma inguinale cases in uncircumcised male patients with poor genital hygiene.[32,35]

Coexistence of granuloma inguinale with other STIs occurs commonly. Syphilis has been described in as many as 23 percent of patients with granuloma inguinale. In one report, HIV-1 antibodies were found in as many as 8 percent of male patients with granuloma inguinale.[29,41]

PATHOGENESIS AND PATHOLOGY

The primary lesion in granuloma inguinale is an indurated nodule that erodes through the skin and becomes a granulomatous heaped ulcer. Adjacent lesions form and eventually coalesce, especially in the perineal area. Secondary infection of lesions may occur and may aggravate the tissue destruction and cause scarring. *C. granulomatis* organisms invade mononuclear endothelial cells. Extensive acanthosis and dense dermal infiltrates of mainly plasma cells and histiocytes have been observed in the indurated nodules. Polymorphonuclear cell infiltration also occurs, but lymphocytes are rare findings when secondary infection develops. The pathognomonic feature of granuloma inguinale is a large, infected mononuclear cell, 25 to 90 μm in diameter that contains many intracytoplasmic cysts filled with deep-staining Donovan bodies. Metastatic spread to the bones, joints, liver, and lymphatics occasionally occurs.[35]

A possible link between human leukocyte antigen (HLA)-B57 and granuloma inguinale infection may exist; class I, class II, and DQ antigens have been detected in the genital ulcers of individuals with granuloma inguinale.[36] Circulating lymphocytes and tissue-level lymphocyte subpopulations in granuloma inguinale have been studied.[47,48] T-lymphocyte and B-lymphocyte infiltration in tissues is almost identical, without any significant difference in ulcerogranulomatous and hypertrophic variants of granuloma inguinale. Total leukocyte, absolute lymphocyte, and total T lymphocytes counts; CD4, CD8, and CD22 levels; and the CD4/CD8 ratio are all increased significantly in the ulcerogranulomatous variant of granuloma inguinale. In contrast, the hypertrophic variant causes a significant elevation only in the CD4/CD8 ratio. This finding suggests a greater cell-mediated immune response in the ulcerogranulomatous variant of granuloma inguinale and is consistent with the paucity of Donovan bodies in smears obtained from patients with this variant.[47,48]

CLINICAL MANIFESTATIONS

The incubation period of granuloma inguinale usually is less than 2 weeks but may be as long as 3 months. The disease begins as one or more subcutaneous nodules that erode through the skin to produce clean, large, beefy-red, granulomatous ulcers that bleed easily. The lesions are sharply defined and painless, and the ulcers feel hard when palpated. The disease usually is limited to local tissue; therefore, constitutional symptoms are unlikely to be present. Autoinoculation is a common manifestation that produces "kissing" lesions. The genitalia are involved in 90 percent of cases, the inguinal region in 10 percent, the anal region in 5 to 10 percent, and extragenital sites, usually involving the head and neck, in 1 to 5 percent. Regional (inguinal) lymphadenopathy is not associated with granuloma inguinale.

The morphology of the cutaneous lesions of granuloma inguinale can vary, depending on the stage of the disease. The exuberant or hypertrophic stage appears before secondary infection develops. It consists of large, vegetating masses with overgrowth of granulation tissue, usually in the perianal region. The ulcerative stage is accompanied by secondary infection. In this stage, large, spreading, shallow necrotic ulcers with a foul odor may be noted. The cicatricial stage results after prolonged healing and is characterized by fibrosis, scarring, depigmentation, keloid formation, elephantiasis, and stenosis of the vagina, urethra, and anus. In patients in Durban, ulcerogranulomatous lesions occurred far more commonly than did hypertrophic and necrotic lesions. Although lymphadenopathy is an unusual occurrence in granuloma inguinale, pseudobuboes and pseudoelephantiasis of the genitals (massive edema) may be seen. Pseudobuboes are the result of deep, inguinal granulomata; pseudoelephantiasis is caused by cutaneous extension of lesions and inflammation.[13,29,35]

In male patients, lesions usually occur on the prepuce and also can occur on the coronal sulcus and frenulum of the penis.[10,34,35] The most common clinical manifestation is vulvar ulceration in pregnant (88%) and nonpregnant women (46%).[2] Genital tract bleeding is the next most common finding in nonpregnant women (20%). Multiple sites of genital ulceration (i.e., vulva, vagina, and cervix) have been noted only in nonpregnant women (Fig. 130-1).[1,13,32,34] Extragenital sites may be involved in up to 6

FIGURE 130-1 ■ Granuloma inguinale.

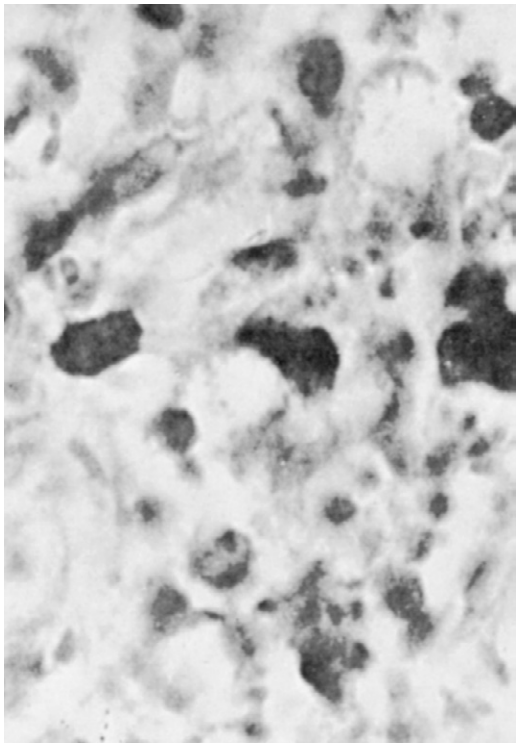

FIGURE 130-2 ■ Donovan bodies.

percent of cases, through hematogenous spread, and have been reported in the mouth, chin, axillae, abdomen, pelvic cavity, and foot.* These distant sites usually are associated with a primary lesion in the genital area.[35] Lesions in the oral cavity have been described after apparently successful treatment of genital lesions.[12] Donovanosis ulcers may take longer to heal in HIV-positive individuals, and greater destruction of tissue may be noted as well.[34,35]

In endemic areas, unusual manifestations of granuloma inguinale may cause confusion with a wide variety of diseases and can result in misdiagnosis. The differential diagnosis includes carcinoma, secondary syphilis, necrotic ulcerations of amebiasis, tuberculosis, and filariasis. In addition, secondary infection can confuse the diagnosis.[20,35]

DIAGNOSIS

Generally, granuloma inguinale is diagnosed on clinical grounds. The accuracy of a clinical diagnosis of granuloma inguinale can be as high as 63 percent in male patients and 83 percent in female patients. In contrast to other genital ulcers, the ulcers are larger in granuloma inguinale, are painless, bleed easily to touch, and usually are not associated with inguinal lymphadenopathy.[40,44]

The diagnosis of granuloma inguinale can be confirmed by identification of dark-staining Donovan bodies in large, histiocytic cells on a stained crush specimen from the lesion (Fig. 130-2). The common stain used to identify Donovan bodies is Wright-Giemsa or Warthin-Starry blue-black stain. A modified Giemsa stain (Rapi-Diff) can yield rapid results in a busy clinic.[35] Donovan bodies have been identified on Papanicolaou smears from the cervix.[11] Examination of appropriately stained specimens from active lesions remains the most reliable diagnostic test. Light-microscopic examination of biopsy specimens that have been fixed in formalin and embedded in wax is less reliable. Donovan bodies rarely are seen by this method.[35,40]

If donovanosis is suspected, a swab for this condition should be taken first, before swabs for other organisms, so that an adequate amount of cellular material can be obtained.[23,35,37] When *C. granulomatis* organisms are likely to be scarce or when smear or crush specimens are likely to be nondiagnostic, obtaining a biopsy of the lesion should be considered. Accordingly, a biopsy specimen, preferably stained with Giemsa or silver, is recommended in very early, very sclerotic, or heavily superinfected specimens.[23,35,37]

Successful isolation of *C. granulomatis* by culture on human epithelial cell lines with a modified *Chlamydia* culture technique has been reported from South Africa and Australia.[5] Polymerase chain reaction (PCR) techniques for *C. granulomatis* also have been developed and further refined into a colorimetric detection system for use in diagnostic laboratories. A genital ulcer disease multiplex PCR test has been developed in Australia using an in-house nucleic acid amplification technique that includes *C. granulomatis* primers.[35] However, no FDA-cleared molecular tests for detection of *C. granulomatis* DNA exist yet in the United States but can be in a laboratory that has conducted a *Clinical Laboratories Improvement Amendments* verification study.[8]

Serologic and skin tests for granuloma inguinale are highly sensitive but not specific. A serologic test using

*References 12, 14, 25, 27, 35, 42, 46, 50.

the indirect immunofluorescence technique has been evaluated and found to have a sensitivity of 100 percent, a specificity of 98 percent, a positive predictive value of 89 percent, and a negative predictive value of 100 percent in diagnosing granuloma inguinale. In the absence of culture methods for *C. granulomatis*, this test may prove helpful in the diagnosis of established lesions and not early ulcers.[15]

The CDC STI surveillance case definition for granuloma inguinale of a confirmed case is a clinically compatible case that is laboratory confirmed. The clinical description of a clinically compatible case is a slowly progressive ulcerative disease of the skin and lymphatics of the genital and perianal area caused by infection with *C. granulomatis*. A clinically compatible case would have one or more painless or minimally painful granulomatous lesions in the anogenital area. The laboratory criteria for diagnosis are demonstration of intracytoplasmic Donovan bodies in Wright- or Giemsa-stained smears or biopsies of granulation tissue.[7]

TREATMENT

Antibiotics with strong activity against *C. granulomatis* are those effective in the treatment of gram-negative bacilli or those whose lipid solubility ensures good intracellular penetration. The CDC recommends treatment of adults and adolescents with doxycycline 100 mg orally twice per day for a minimum of 3 weeks and until lesions are healed.[8] Alternative regimens include the following: azithromycin 1 g orally weekly for at least 3 weeks, ciprofloxacin 750 mg orally twice per day for at least 3 weeks, erythromycin, 500 mg orally four times per day for at least 3 weeks, or trimethoprim-sulfamethoxazole one double-strength tablet (160 mg/800 mg) orally twice per day for at least 3 weeks.[8] Children can be treated with a short course of oral azithromycin suspension 20 mg/kg/day, trimethoprim-sulfamethoxazole (10 mg/kg/day trimethoprim), or erythromycin 30 to 50 mg/kg/day.[8] Clinical response to antibiotics should be noted within a week of treatment; the lesions should become paler and less friable. After a week of treatment, the lesions become smaller, and total healing of the area takes 3 to 5 weeks. Relapse occurs in approximately 10 percent of cases, especially if use of the antibiotic is discontinued before the primary lesion has healed completely. Donovan bodies may reappear within 7 to 10 days.[35]

Pregnant and lactating adolescents should be treated with an erythromycin regimen and consideration given to adding a parenteral aminoglycoside such as gentamicin. Although no published data exist, azithromycin also may be an appropriate antibiotic during pregnancy. Doxycycline and trimethoprim-sulfamethoxazole are contraindicated during pregnancy.[8]

The treatment regimen for an HIV-positive person is the same as for an HIV-negative person. Treatment can fail in patients with coexisting HIV infection and immunosuppression; in contrast to HIV-negative patients, the mean duration to complete ulcer healing in these patients is significantly longer—25.7 days versus 16.8 days.[22] More intensive and prolonged antibiotic therapy such as an aminoglycoside (e.g., gentamicin 1 mg/kg intravenously every 8 hours) may need to be added.

Merianos and associates[28] reported the possible effectiveness of ceftriaxone for chronic, recurrent granuloma inguinale. Patients in this study had the disease for 1 to 5 years and had received 4 to 10 courses of a single daily intramuscular infection of 1 g of ceftriaxone diluted in 2 mL of 1 percent lidocaine.

PROGNOSIS

Healing is complete in patients who seek treatment early in the course of the disease and who comply with their medication and follow-up regimens.[28,32] One third of patients are reported to recover completely without a recurrence after receiving daily doses of ceftriaxone for 7 to 10 days. Patients with mild recurrences responded to additional ceftriaxone or short courses of oral antibiotics.[28] O'Farrell[32] noted complete healing of lesions in 24 percent of patients who complied with follow-up. Complications of granuloma inguinale include pseudoelephantiasis, urethral stricture, and pelvic abscess, which may require surgery. Vulvectomy is reserved for infections that have not responded to antibiotic treatment or for patients with severe vulvar elephantiasis.[13,35]

The severe, mutilating complications of granuloma inguinale are primarily a result of delayed treatment or poor compliance with medication. In Durban, South Africa, almost half of the male patients had ulcerations for 1 to 6 months before they sought medical care, and 16 percent had ulcerations for 1 to 3 weeks. In contrast, approximately 25 percent of female patients had ulcerations for 1 to 6 months, and 50 percent had ulcerations for 1 to 3 weeks.[28] Delayed medical attention may be related to limited education, ignorance of STIs, absence of suitable medical facilities, or embarrassment in seeking treatment because of extensive genital lesions. Another complication is the acquisition of HIV infection, especially when patients with ulcerations that are left untreated for a prolonged period have sexual contact with an HIV-positive partner.[28,35]

PREVENTION

Sexual partners of persons with the disease should be traced, examined, and treated. Treatment of granuloma inguinale when the nodule first appears is associated with a benign course. Community-based eradication that targets men with granuloma inguinale in endemic areas should be implemented. Programs should be aimed at early suspicion and identification of lesions, access to care, provision of early treatment, and prevention of severe complications. Teaching the importance of personal genital hygiene, such as instruction on simple retraction of the foreskin in men and cleansing the penis with soap and water, also is effective.[32,35] The CDC and the U.S. Public Health Service provide technical instructions on required examinations and standard treatment regimens to panels of physicians and consular officers

evaluating the health of aliens applying for entry into the United States.[6]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Barroso LF, Wispelwey B. Donovanosis presenting as a pelvic mass mimicking ovarian cancer. *South Med J* 2009;**102**:104–5.
6. Centers for Disease Control and Prevention. *Immigrant and refugee health: technical instructions for medical examinations of aliens*. http://www.cdc.gov/immigrantrefugeehealth/exams/ti/panel/technical-instructions/panel-physicians/other-stds.html#stds.
7. Centers for Disease Control and Prevention. *STD surveillance case definitions. II. Case definitions for nationally notifiable infectious diseases, granuloma inguinale*, 2011: http://www.cdc.gov/std/stats09/app-casedef-2.htm#a3.
8. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines. 2010. 59(No.RR-12):1-109. *Morb Mortal Wkly Rep* 2010; http://www.cdc.gov/std/treatment/2010/genital-ulcers.htm.
10. Chandra Gupta TS, Rayudu T, Murthy SV. Donovanosis with auto-amputation of penis in a HIV-2 infected person. *Ind J Dermatol Venereol Lepr* 2008;**74**:49–492.
25. Kirkpatrick DJ. Donovanosis (granuloma inguinale): a rare cause of osteolytic bone lesions. *Clin Radiol* 1970;**21**:101–5.
43. Rashid RM, Janjua SA, Khachemoune A. Granuloma inguinale: a case report. *Dermatole online J* 2006;**12**:14.
44. Richens J. The diagnosis and treatment of donovanosis (granuloma inguinale). *Genitourin Med* 1991;**67**:441–52.
45. Samuel M, Aderogba K, Dutt N, Lambert JS, Taylor C. A hat trick of ulcerating pathogens in a single genital lesion. *Int J STD AID* 2007;**8**:65–6.
49. Taneja S, Jena A, Tangri R, Sekhon R. MR appearance of cervical donovanosis mimicking carcinoma of the cervix. *Br J Radiol* 2008;**81**:e170–2.
51. World Health Organization. *Sexually transmitted infections*. http://www.who.int/mediacentre/factsheets/fs110/en/.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 131

Campylobacter jejuni

Ramia Zakhour • Gloria P. Heresi • James R. Murphy

Campylobacter jejuni is a frequent cause of enteritis and less often of extraintestinal infection in humans. Since it first was recognized as a common human pathogen in the 1970s, appreciation of this agent's importance as a cause of disease has increased steadily. *C. jejuni* is one of the most frequent bacterial causes of human enteritis in the United States and is a leading cause of bacterial foodborne diarrheal disease throughout the world.[6,39,273] Immunoreactive complications may develop after infection.[214,228]

Although they are not isolated as frequently as *C. jejuni* and *Campylobacter coli*, other *Campylobacter* spp. are gaining recognition as human pathogens. Currently at least 25 species of *Campylobacter* have been identified.[169] *Campylobacter fetus*, a classic cause of perinatal infection, also is an infrequent cause of bacteremia in immunocompromised hosts. *Campylobacter upsaliensis*, *Campylobacter lari*, *Campylobacter hyointestinalis*, and *Campylobacter concisus* are associated primarily with diarrheal disease. Both normal and immunosuppressed hosts are affected by these species. Other less common *Campylobacter* spp. have been isolated from blood or stool or involved in periodontal disease. The clinical spectrum of these organisms is expanding as the testing for them becomes more widely available.

HISTORY

The pathologic consequences of infection with *Campylobacter* were recognized first in 1909 from studies of abortions in sheep,[174] although it may have been identified earlier, in 1886, when Theodore Escherich described nonculturable spiral-shaped bacteria.[249] A few years later, Smith[259] reported their association with bovine abortion, as well, and gave them the name *Vibrio fetus*. Although never confirmed microbiologically, these organisms are thought to have been *C. fetus* according to current nomenclature.[148] In 1947 the sheep abortion–associated organism *V. fetus* was isolated from a blood culture from a pregnant woman who had an influenza-like illness and delivered a stillborn infant with a necrotic, infarcted placenta.[300] In 1957, King[152,153] hypothesized that *V. fetus*–related organisms could be associated with human enteric disease. In addition to pregnancy, gastrectomy, tooth extraction, heart disease, diabetes, and cirrhosis were predisposing conditions in King's 1957 review of 15 patients with *V. fetus* bacteremia.[153] Butzler and colleagues[35] showed that bacteria similar to *V. fetus* were present in the stools of children with diarrhea. This observation was confirmed rapidly and repeatedly.[23,36,93,255,275] Major differences in biochemical activities, growth characteristics,

REFERENCES

1. Barroso LF, Wispelwey B. Donovanosis presenting as a pelvic mass mimicking ovarian cancer. *South Med J* 2009;**102**:104–5.
2. Bassa AG, Hoosen AA, Moodley J, et al. Granuloma inguinale (donovanosis) in women: an analysis of 61 cases from Durban, South Africa. *Sex Transm Dis* 1993;**20**:164–7.
3. Bowden FJ. National Donovanosis Eradication Advisory Committee: Donovanosis in Australia: going, going. *Sex Transm Infect* 2005; **81**:365–6.
4. Bowden FJ, Bright A, Rode JW, Brewster D. Donovanosis causing cervical lymphadenopathy in a five-month-old boy. *Pediatr Infect Dis* 2000;**19**:167–9.
5. Carter J, Hutton S, Sriprakash KS, et al. Culture of the causative organism of donovanosis (*Calymmatobacterium granulomatis*) in MEP-2 cells. *J Clin Microbiol* 1997;**35**:2915–17.
6. Centers for Disease Control and Prevention. *Immigrant and refugee health: technical instructions for medical examinations of aliens*. http://www.cdc.gov/immigrantrefugeehealth/exams/ti/panel/technical-instructions/panel-physicians/other-stds.html#stds.
7. Centers for Disease Control and Prevention. *STD surveillance case definitions. II. Case definitions for nationally notifiable infectious diseases, granuloma inguinale*. 2011: http://www.cdc.gov/std/stats09/app-casedef-2.htm#a3.
8. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines. 2010. 59(No.RR-12):1-109. *Morbid Mortal Wkly Rep* 2010; http://www.cdc.gov/std/treatment/2010/genital-ulcers.htm.
9. Chandra M, Jain AK. Fine structure *of Calymmatobacterium granulomatis* with particular reference to the surface structure. *Indian J Med Res* 1991;**93**:225–31.
10. Chandra Gupta TS, Rayudu T, Murthy SV. Donovanosis with autoamputation of penis in a HIV-2 infected person. *Ind J Dermatol Venereol Lepr* 2008;**74**: 49–492.
11. DeBoer A, DeBoer F, Van Der Merwe J. Cytologic identification of Donovan bodies in granuloma inguinale. *Acta Cytol* 1984;**28**:126–8.
12. Doddridge M, Muirhead R. Donovanosis of the oral cavity: case report. *Aust Dent J* 1994;**39**:203–5.
13. Faro S. Lymphogranuloma venereum, chancroid, and granuloma inguinale. *Obstet Gynecol Clin North Am* 1989;**16**:517–30.
14. Fletcher HM, Rattray CA, Hanchard B, et al. Disseminated donovanosis (granuloma inguinale) with osteomyelitis of both wrists. *West Indian Med J* 2002;**51**:194–6.
15. Freinkel AL, Dangor Y, Koornhof HJ, et al. A serological test for granuloma inguinale. *Genitourin Med* 1992;**68**:269–72.
16. Gao Y, Ni K, Hu B, Zheng K. Granuloma inguinale: first case reported in the last four decades in China. *Int J Dermatol* 1996; **35**:758–9.
17. Govender D, Hadley GP, Donnellan R. Granuloma inguinale (donovanosis) as a neck mass in an infant. *Pediatr Surg Int* 1999;**15**: 129–31.
18. Govender D, Naidoo K, Chetty R. Granuloma inguinale (donovanosis): an usual cause of otitis media and mastoiditis in children. *Am J Clin Pathol* 1997;**108**:510–14.
19. Growden WA, Lebherz TB, Moore JG, et al. Granuloma inguinale in a white teenager: a diagnosis easily forgotten, poorly pursued. *West J Med* 1985;**143**:105–8.
20. Gupta S, Ajith C, Kanwar AJ, et al. Genital elephantiasis and sexually transmitted infections revisited. *Int J STD AIDS* 2006;**17**: 157–66.
21. Hacker P, Fisher BK, Dekoven J, et al. Granuloma inguinale: three cases diagnosed in Toronto, Canada. *Int J Dermatol* 1992;**31**:696–9.
22. Jamkhedkar PP, Hira SK, Shroff HJ, et al. Clinico-epidemiologic features of granuloma inguinale in the era of acquired immune deficiency syndrome. *Sex Transm Infect* 1998;**25**:196–200.
23. Joseph AK, Rosen T. Laboratory techniques used in the diagnosis of chancroid, granuloma inguinale and lymphogranuloma venereum. *Dermatol Clin* 1994;**12**:1–8.
24. Kharsany AB, Hoosen AA, Kiepala P, et al. Phylogenetics analysis of *Calymmatobacterium granulomatis* based on 16S sequence. *J Med Microbiol* 1999;**48**:841–7.
25. Kirkpatrick DJ. Donovanosis (granuloma inguinale): a rare cause of osteolytic bone lesions. *Clin Radiol* 1970;**21**:101–5.
26. Marmell M. Donovanosis of the anus in the male: an epidemiologic consideration. *Br J Vener Dis* 1958;**34**:213–18.
27. Mein J, Russell C, Know J, et al. Intrapelvic donovanosis presenting as a psoas abscess in two patients. *Sex Transm Infect* 1999;**75**:75–6.
28. Merianos A, Gilles M, Chuah J. Ceftriaxone in the treatment of chronic donovanosis in central Australia. *Genitourin Med* 1994;**70**: 84–9.
29. Morris LF, Cohen PR, Dodd LG. Nongenital granuloma inguinale in an oncology patient. *Am J Clin Oncol* 1994;**17**:456–60.
30. Morrone A, Toma L, Franco G, et al. Donovanosis in developed countries: neglected or misdiagnosed disease? *Int J STD AIDS* 2003;**14**:288–89.
31. O'Farrell N. Trends in reported cases of donovanosis in Durban, South Africa. *Genitourin Med* 1992;**68**:366–9.
32. O'Farrell N. Clinico-epidemiological study of donovanosis in Durban, South Africa. *Genitourin Med* 1993;**69**:108–11.
33. O'Farrell N. Global eradication of donovanosis: an opportunity for limiting the spread of HIV-1 infection. *Genitourin Med* 1995; **71**:27–31.
34. O'Farrell N. Donovanosis: an update. *Int J STD AIDS* 2001; **12**:423–7.
35. O'Farrell N. Donovanosis. In: Holmes KK, Sparling PF, Stamm WE, et al., editors. *Sexually Transmitted Diseases*. New York: McGraw-Hill Medical; 2008. p. 701–8.
36. O'Farrell N, Hammond M. HLA antigens in donovanosis (granuloma inguinale). *Genitourin Med* 1991;**67**:400–2.
37. O'Farrell N, Hoosen AA, Coetzee KD, et al. A rapid stain for the diagnosis of granuloma inguinale. *Genitourin Med* 1990;**66**: 200–1.
38. O'Farrell N, Hoosen AA, Coetzee KD, et al. Genital ulcer disease in men in Durban, South Africa. *Genitourin Med* 1991;**67**:327–30.
39. O'Farrell N, Hoosen AA, Coetzee KD, et al. Sexual behavior in Zulu men and women with genital ulcer disease. *Genitourin Med* 1992;**68**:245–8.
40. O'Farrell N, Hoosen AA, Coetzee KD, et al. Genital ulcer disease: accuracy of clinical diagnosis and strategies to improve control in Durban, South Africa. *Genitourin Med* 1994;**70**:7–11.
41. O'Farrell N, Windsor I, Becker P. HIV-1 infection among heterosexual attenders at a sexually transmitted diseases clinic in Durban. *S Afr Med J* 1991;**80**:17–20.
42. Rao MV, Thappa DM, Jaisaukar TJ, Ratnakar C. Extragenital donovanosis of the foot. *Sex Transm Infect* 1998;**74**:298–9.
43. Rashid RM, Janjua SA, Khachemoune A. Granuloma inguinale: a case report. *Dermatole online J* 2006;**12**:14.
44. Richens J. The diagnosis and treatment of donovanosis (granuloma inguinale). *Genitourin Med* 1991;**67**:441–52.
45. Samuel M, Aderogba K, Dutt N, Lambert JS, Taylor C. A hat trick of ulcerating pathogens in a single genital lesion. *Int J STD AID* 2007;**18**:65–6.
46. Sanders CT. Extragenital donovanosis in a patient with AIDS. *Sex Transm Infect* 1998;**74**:142–3.
47. Sehgal VN, Gupta MM, Jain VK. Tissue level lymphocyte subpopulations in donovanosis. *Int J Dermatol* 1991;**30**:857–9.
48. Sehgal VN, Sharma HK, Sharma VK. Characterization of circulating lymphocytes by monoclonal antibodies in donovanosis. *J Dermatol* 1991;**18**:181–3.
49. Taneja S, Jena A, Tangri R, Sekhon R. MR appearance of cervical donovanosis mimicking carcinoma of the cervix. *Br J Radiol* 2008; **81**:e170–72.
50. Veeranna S, Raghu TY. Oral donovanosis. *Int J STD AIDS* 2002;**13**:855–6.
51. World Health Organization. *Sexually transmitted infections*. http://www.who.int/mediacentre/factsheets/fs110/en/.

and DNA base nucleotide content between true vibrio and *V. fetus* led to the establishment of the new genus *Campylobacter* by Sebald and Vernon in 1967.[298] The genome of a representative *C. jejuni* was published in 2000.[210] Information gleaned from this and comparative analyses of additional isolates have markedly advanced knowledge of these agents.[88,117]

Many reports describing new *Campylobacter* spp. were published in the 1980s and early 1990s. *C. upsaliensis* was reported to be a pathogen in dogs and humans in 1983 and 1985, respectively.[210,238] *C. lari*, a common isolate from healthy seagulls, was found to be a cause of gastrointestinal and extraintestinal disease in humans.[17,274] Originally identified in the intestines of swine with proliferative ileitis, *C. hyointestinalis* was reported first as a human pathogen in a homosexual man with proctitis.[71] The hydrogen-requiring campylobacters *C. concisus*,[295] *C. rectus*,[226] and *Campylobacter curvus*, as well as *Campylobacter showae* and *Campylobacter gracilis*, have been associated with periodontal disease. *Campylobacter sputorum* has been identified in abscesses,[189] as well as in bacteremia[50,119] and gastroenteritis,[169] and *Campylobacter mucosalis* was reported in two children with diarrhea.[75] *Campylobacter ureolyticus*, initially known as *Bacteroides ureolyticus*, was first described associated with genital tract infections and only more recently found to be isolated from stools.[31,78] Once called *Campylobacter*-like organisms, *Helicobacter cinaedi* and *Helicobacter fennelliae* are now classified in the *Helicobacter* genus.[199] These pathogens cause enteritis and proctocolitis in homosexual men and, on occasion, bacteremia.[209,289] Two former *Campylobacter* spp., *Arcobacter butzleri* and *Arcobacter cryaerophilus*, are associated with abortion and enteritis in cattle and pigs, in addition to bacteremia and diarrhea in humans.[151]

THE ORGANISM

Campylobacter is a Greek word meaning "curved rod." Members of this genus are fastidious, non–spore forming gram-negative rods that are curved, S-shaped, or spiral (Fig. 131-1) and may vary in width from 0.2 to 0.9 μm and in length from 0.5 to 5.0 μm.[294] Although most grow at 37°C (98.6°F), *C. jejuni* grows best in cultures maintained at 42°C (107.6°F). Most *Campylobacter* spp. require microaerophilic growth conditions (5% to 10% oxygen). *Campylobacter* spp. usually are motile, with either a single polar flagellum or two flagella, one at each end of the rod. Exceptions include *C. gracilis*, which is nonmotile, and *C. showae*, which has multiple flagella. Campylobacters are oxidase-positive and reduce nitrates but do not ferment or oxidize carbohydrates.[212]

Some species, including *C. sputorum*, *C. concisus*, *C. mucosalis*, *C. curvus*, *C. rectus*, and *C. hyointestinalis*, may require hydrogen for primary isolation and growth. Many media selective for isolation of *Campylobacter* spp. have been developed, but because of species differences in antibiotic resistance patterns, no single formulation allows isolation of all species of clinical importance.[100] For example, *C. jejuni* and *Campylobacter coli* are resistant to cephalothin, whereas *C. fetus* is susceptible. A filtration method with nonselective media may be used to complement direct culture on selective media for the detection of antibiotic-susceptible *Campylobacter* spp. Because of their small size and motility, campylobacters may pass through filters with pores of 0.45 to 0.65 μm, whereas other enteric flora are retained.[7,189]

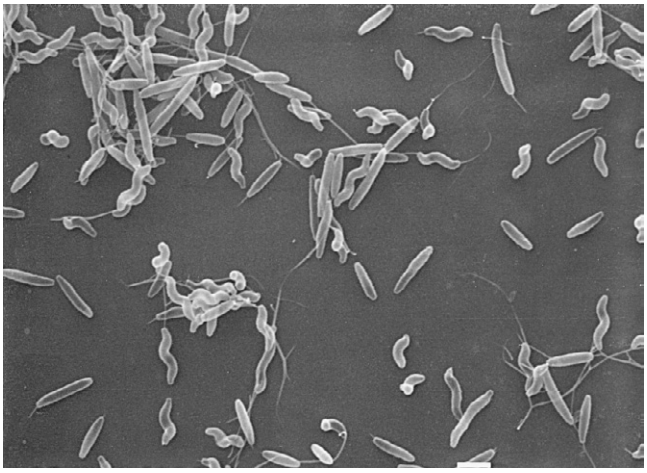

FIGURE 131-1 ■ Scanning electron microscopy of *Campylobacter jejuni* strain 20-01. (From Baqar S, Rice B. *Campylobacter jejuni* enteritis. *Clin Infect Dis* 2001;**33**:901–5.)

Although colonies may appear on plates within 24 to 48 hours, growth of campylobacters from stool may take as long as 72 to 96 hours. Primary isolation from blood may require 2 weeks.[7] Gram stains of young cultures reveal vibrioid forms, and longer incubation may yield spherical or coccoid bodies. *Campylobacter* spp. usually can be distinguished from one another on the basis of biochemical tests and growth characteristics (Table 131-1). Although the significant pathogens *C. jejuni*, *C. coli*, and *C. lari* cannot be discriminated reliably by the use of 16S rDNA data, these sequences provide a substantially improved basis for the identification and differentiation of *Campylobacter* spp.[97] Many investigators have applied molecular techniques to identify enteric campylobacters directly from stool.[293]

C. jejuni belongs to rRNA superfamily VI, a specialized subgroup of gram-negative bacteria that also includes *Arcobacter* and *Helicobacter*. The species *C. jejuni* has two subspecies, *C. jejuni jejuni* and *C. jejuni doylei*. *C. jejuni doylei* can be differentiated from *C. jejuni jejuni* by the failure of *C. jejuni doylei* to grow at 42°C (107.6°F), lack of nitrate reduction, and sensitivity to cephalothin. For brevity, we refer to *C. jejuni jejuni* as *C. jejuni*.

C. jejuni has a circular chromosome of 1.64 million base pairs (30.6% guanosine and cytosine).[208] Marked differences in pathogenic surrogates (i.e., motility, colonization of chicks, invasion, and translocation of cultured cells) are associated with variable chromosomal sequences within different passage sequences for a single isolate, and unique chromosomal sequences are associated with known pathogenic isolates of *C. jejuni*. Genomic analyses demonstrate that substantial differences exist among organisms classified by conventional means and that some of these differences are associated with the capacity to cause disease in humans.[88,117,207] Genomic sequence analyses have yielded a catalog of phase-variable surface

TABLE 131-1 Growth and Biochemical Characteristics of Species of the Genus *Campylobacter*

Species	Growth							
	25°C	37°C	42°C	Anaerobically	In CO_2 Inhibitor	Glycine 1%	Bile 1%	Charcoal Casein Deoxycholate
C. lari	–	+	+	+	+	+	+	+
C. upsaliensis	–	+	+	+	+	–	+	+
C. fetus	+	+	(–)	–	+	+	–	–
C. hyointestinalis	(+)	+*	+	+	+	+	NA	NA
C. concisus	–	+	+	+	+	+	NA	NA
C. mucosalis	+	+	+	+	+	+	NA	NA
C. sputorum†	–	+	+	+	+	+	+	+

Modified from Ruiz-Palacios G, Pickering LK. *Campylobacter* and *Helicobacter* infections. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases.* 3rd ed. Philadelphia: Saunders; 1992. p. 1072-2084.
*Best at 35°C (95°F).
†*C. sputorum* has three biovars with different biochemical characteristics.
+, Positive; –, negative; (+), most strains positive; (–), most strains negative; *NA,* data not available or found; *R,* resistant; *S,* susceptible; *TSI,* triple sugar iron.

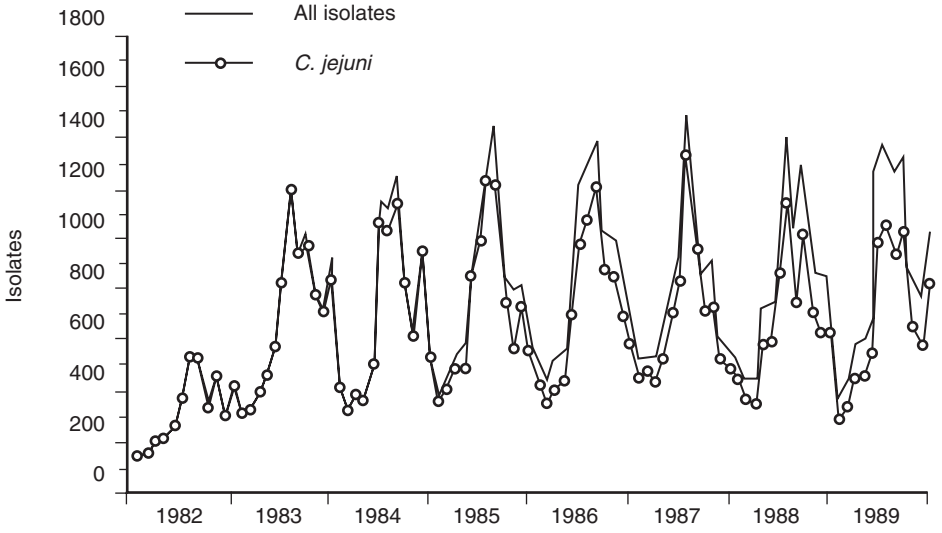

FIGURE 131-2 ■ Reported human *Campylobacter* isolates by month in the United States, 1982 to 1989. (From Tauxe RV. Epidemiology of *Campylobacter jejuni* infections in the United States and other industrialized countries. In: Nachamkin I, Blaser MJ, Tompkins LS, editors. Campylobacter jejuni: *Current Strategy and Future Trends.* Washington [DC]: American Society for Microbiology; 1992, p. 9–19.)

structures (including lipo-oligosaccharide and capsule) that appear to relate to pathogenesis and immunity,[79,208] a lack of classical operon structure, repetitive DNA, and an unexpected capacity to produce polysaccharide.[208] Populations of *C. jejuni* are nonclonal, and interstrain exchange of genetic material occurs.[50]

EPIDEMIOLOGY

Human infection with *C. jejuni* occurs worldwide.[20] *C. jejuni* persists in zoonotic niches (Table 131-2), and most human infections are thought to arise from these reservoirs. Chickens and contaminated water are the main sources of campylobacteriosis in developed countries. The organism is a common commensal of the gastrointestinal tract of cattle, pigs, dogs, cats, and most birds used as human food,[24,250] and transmission from these sources occurs. Fresh-water bathing sites may yield *Campylobacter* organisms that are epidemiologically linked to human infections,[197,245,246] and waterborne outbreaks have been reported.[146] In the United States, *C. jejuni* infection peaks in late summer and early fall (Fig. 131-2). In a study from Philadelphia, June and July were shown to be the 2 months with the highest incidence of disease. Overall increase in temperature and humidity are associated with higher risk for disease. Interestingly, cooler river water was associated with higher disease. Lower water temperature has been associated with longer environmental persistence of *Campylobacter*.[305] Dairy cows in the United

						Susceptibility	
Oxidase	**Catalase**	**Urease**	**Hippurate**	**Nitrate**	**H2S (TSI)**	**Nalidixic acid**	**Cephalothin**
+	+	–	–	+	–	R	R
+	(–)	–	–	+	–	S	S
+	+	–	–	+	–	R	S
+	+	–	–	+	+	R	S
+	–	–	–	+	+	R	R
+	–	–	–	+	+	R	S
+	–	–	–	+	+	R	S

TABLE 131-2 **Illustrative Studies of Isolation of *Campylobacter jejuni* from Animal Sources**

Animal	Sample	Location and Reference	Sample Size	Positive*
Chicken	Processing plants, shops	Japan[286]	156	67.9
	Flocks on farms	England[125]	49	76.0
	Flocks on farms	Russia[266]	370	31.5
	Giblets	Egypt[150]	50	23.5
	Eggs from 23 farms	United States[13]	276	0.0
	Live birds	United States[13]	10	90.0
	Processing plants, carcasses	United States[260]	325	78.5
Duck	Giblets	Egypt[150]	50	19.0
	At reservoir	United States[202]	113	73.0
Goose	At reservoir	United States[202]	94	5.0
Turkey	Giblets	Egypt[150]	50	14.5
	Feces	United Kingdom[247]	5000	100.0
Squab	Giblets	Egypt[150]	50	4.0
Crane	At reservoir	United States[202]	91	81.0
Pig	Pork at processing plants, shops	Japan[285]	94	2.1
Cow	Beef at processing plants, shops	Japan	52	0.0
	Rectal swabs	United Kingdom[124]	668	72.0
	Farms	Canada[301]	78	13.0
	Milk cows	United States[60]	78	68.0
	Milk, bulk tanks	United States[60]	108	0.9
	Milk, bulk tanks	Netherlands	19	84.0
	Housed indoors, feces	Switzerland[32]	395	38.5
	Outdoors, feces	Switzerland[32]	395	13.3
Goat	Rectal swabs	Ghana[2]	72	33.3
Sheep	Rectal swabs	Ghana[2]	13	23.0
Sheep	Liver	New Zealand[46]	272	66.2
Cat	Domestic, rectal swabs	United States[91]	430	1.0
	Zoo, rectal swab (species-positive)	United States[91]	15	6.7
	Feces	United States[115]	206	1.0
Monkey	Stool	United States[185]	50	77.0
	Stool	Indonesia[185]	50	36.0
Dog	Rectal swabs of puppies	Denmark[106]	72	22.2
	Fecal culture of puppies	United States[307]	4	100.0
Birds	Feces of migrating passerines	Sweden[205]	101	3.0
	Gulls	Scotland	990	4.8
	Wild birds feces from children's playgrounds	New Zealand[174]	192	12.5
	Crane feces and contaminated peas	Alaska[87]	16	44.4
Pigeons, feral	Rectal swabs, feces	Norway[165]	200	3.0
Penguin	Feces	South Georgia Island[28]	100	3.0
Zoo animals	Tamarins, tapirs, maned wolf, red panda, red kangaroo feces	United Kingdom[267]	44	25.0

*Percent positive. In instances in which studies reported ranges of percent positive, the highest rate is recorded.

TABLE 131-3 **Selected Longitudinal Studies of Frequency of *Campylobacter jejuni* Infection in Children**

Location	No. of Children	Isolation of *C. jejuni* from Children*	
		With Diarrhea	Without Diarrhea
Guatemala[225]	321	12.1	8.1
Czechoslovakia[118]	5831	10.1	NR
Mexico[36]	179	0.4	1.7
Thailand[277]	411	0.4	1.1

*Results are reported as percent of stools positive.
NR, Not reported.

TABLE 131-4 **Selected Cross-Sectional Studies of Frequency of *Campylobacter jejuni* Infection in Children**

Location	Frequency of Isolation of *C. jejuni* from Children: Percentage of Stools with *C. jejuni* (Number of Children Studied)	
	With Diarrhea No. (%)	Without Diarrhea No. (%)
South Africa[26]	35.0 (78)	16.0 (63)
Zaire[52]	14.4 (416)	3.0 (200)
Rwanda[51]	9.3 (150)	0.0 (58)
Zaire[35]	8.6 (70)	0.0 (30)
Cameroon[156]	7.7 (272)	3.2 (157)
Bangladesh[109]	25.5 (102)	8.6 (93)
China[312]	18.7 (48)	8.6 (104)
China[58]	11.9 (303)	4.6 (953)
Kuwait[248]	7.0 (621)	0.0 (152)
India[192]	4.0 (607)	0.9 (529)
Saudi Arabia[44]	1.0 (7369)	0.1 (1130)
Belgium[35]	5.1 (800)	1.3 (1000)
Canada[203]	4.3 (1004)	0.0 (176)
Chile[74]	10.0 (299)	6.0 (304)
Pakistan[261]	7.0 (8032)	NA
Canada[142]	32.8 (5068)	NA
Uganda[188]	9.3 (226)	NA
Bulgaria[132]	3.6 (51,607)	NA
Korea[43]	0.1 (84,406)	3 (58)
Canada[130]	29 (442)	NA
Taiwan[311]	6.8 (894)	1.6 (256)
South Africa[236]	12.8 (39)	NA
Guatemala[137]	30.8 (289)	NA
Palestine[3]	5 (150)	

Kingdom have higher concentrations of *Campylobacter* organisms in spring and autumn, but the concentration of *Campylobacter* organisms in beef cattle at slaughter does not change with season; as many as 90 percent of beef cattle may be stool-positive for *Campylobacter*.[265] In subtropical areas, the peak incidence of isolation of *C. jejuni* often is associated with the rainy season. In most tropical climates, rates of isolation are similar year-round. Studies in volunteers show an incubation period of 2 to 4 days after challenges ranging from 800 to 100 million colony-forming units.[19] Fecal shedding of *C. jejuni* by humans may last a median of 2 to 3 weeks, with a range of 3 days to several months.[36,148,203]

The distribution of *C. jejuni* infections within populations is linked to the level of industrialization. In industrialized countries, *C. jejuni* infection is often found in children and adults with enteritis and seldom in healthy individuals.[271] In less industrialized areas, *C. jejuni* is isolated frequently from children, even in the absence of enteritis (Tables 131-3 and 131-4). In industrialized countries, *C. jejuni* has been isolated from between 1 and 13 percent of children with diarrhea, and the prevalence of infection in healthy individuals has been reported to be between 0 and 1.5 percent.[23,35,229,255,273] A 5-year, laboratory-based national surveillance of *Campylobacter* spp. showed an isolation rate of 5.5/100,000 person-years (with *C. jejuni* accounting for 99% of the *Campylobacter* isolates; Fig. 131-3).[277] Population-based isolation rates of *Campylobacter* in the United States range from 28 to 1560/100,000 per year.[275,276] The rate of death attributable to *Campylobacter* is estimated between 100 and 124 per year in the United States.[292,293] The case mortality rate is between 0.10[175] and 0.23 percent.[113] FoodNet published that between 1996 and 2005, *Campylobacter* accounted for 9 percent of 552 deaths caused by bacterial pathogens transmitted through food, with a case fatality rate of 0.1 percent; 46,354 laboratory-confirmed *Campylobacter* infections were reported during that period (38% of all bacterial food-transmitted infections and the most common).[15] A registry-based study demonstrated about fivefold and twofold increases in mortality rates for the first 30 days or first year, respectively, after developing *Campylobacter* infection.[113] A Canadian report showed a 5.1 percent hospitalization rate for *Campylobacter* infections between 2001 and 2004, with highest rates at extremes of age (<1 year and >59 years). Population-based studies in England, the United States, and Sweden showed a bimodal age distribution, with a peak of illness occurring in children younger than 5 years and a second peak at 15 to 29 years of age.[233,275] The highest isolation rate occurs in the first year of life (see Fig. 131-3). Recent reports from the United Kingdom suggest a decrease in incidence of campylobacteriosis in the younger age groups in more recent years versus an increase in the population older than 60 years of age.[92] A male predominance of campylobacteriosis is seen, which has been reproducible in an animal model, suggesting physiologic differences in response to infection between the genders.[268]

In industrialized regions, most sporadic cases occur because of handling, preparation, and consumption of contaminated raw or undercooked poultry or beef.* Raw milk and contaminated water are less frequent sources (see Table 131-2).[121,127,178,278,303] Poor kitchen hygiene plays a role in transmission[56,86]; the risk for acquiring infection is inversely related to the frequency of using

*References 23, 34, 55, 86, 248, 258, 275.

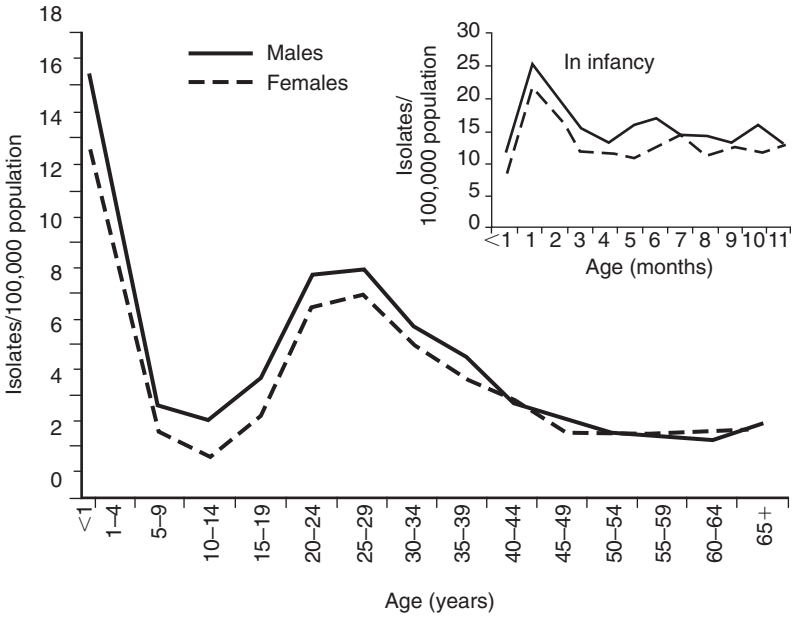

FIGURE 131-3 ■ Annual isolation rates of *Campylobacter* by age and sex, United States, 1982 to 1986. (From Tauxe RV, Hargrett-Bean N, Patton CM, et al. *Campylobacter* isolates in the United States, 1982-1986. *MMWR CDC Surveill Summ* 1988;**37**:1–13.)

soap to clean cutting boards.[275] Barbecues represent a special hazard because they permit easy transfer of bacteria from raw meat to hands and other food and from there to the mouth.[36] Other identified risk factors are eating at restaurants and takeaways.[48,56,86] Sporadic cases of *C. jejuni* infection occur much more frequently than outbreaks. Hospital outbreaks associated with food provided at the hospital kitchen have been reported.[136] Rural setting and higher income have been associated with higher numbers of infections.[18,76]

In less industrialized regions, *Campylobacter* is found in association with childhood diarrhea in 8 to 45 percent of cases, but it is isolated at similar rates from healthy children.[36,47,89] The highest rates of *Campylobacter* isolation are in children younger than 5 years of age.[36] As many as 75 percent of *Campylobacter* infections occurring during the first year of life are asymptomatic.[47]

Usually, a contaminated food or water vehicle transmits *Campylobacter*. However, direct transmission of the agent may occur, usually to individuals with direct exposure to reservoir animals or to those who process contaminated animal products.[31] Person-to-person spread may occur in areas of high contamination, such as where diapered children are present.[211] In addition, intrapartum transmission is documented.[33,299] Asymptomatic mothers may transmit the infection to their newborns.[33]

In developing countries, transmission is multifactorial. Free-roaming poultry, toddlers, unsafe water supply, and lack of adequate disposal of excreta are documented sources.[98] However, proving that *Campylobacter* causes disease in regions where the agent is environmentally pervasive is difficult. Confounders include statistically indistinguishable rates of isolation from diarrhea cases and matched controls (see Table 131-3), and direct evidence that the presence in a household of *Campylobacter* shared between humans and chickens is associated with protection from diarrhea. Improved knowledge of the immune responses that protect from *Campylobacter* infection and disease and knowledge of the components of *Campylobacter* required to cause disease are needed to understand the agent's epidemiology better.

Because of the biochemical inactivity of *Campylobacter* organisms, discriminating species and subspecies by conventional chemotaxonomic methods is problematic. The consequences of this difficulty include ambiguities in epidemiologic investigations and low resolution in studies of mechanisms of virulence and immunity. Effort is being invested in devising genotyping schemes.[59,198,304]

Much needs to be learned about the epidemiology of *Campylobacter* spp. other than *C. jejuni* and *C. coli*, a group of organisms that made up only about 1 percent of *Campylobacter* spp. reported to the Centers for Disease Control and Prevention (CDC) from 1982 to 1986.[275] These organisms are gaining ground as etiologic agents of undiagnosed gastroenteritis. Non-*jejuni*, non-*coli* *Campylobacter* spp. also have been associated with extraintestinal disease, especially bacteremia in patients with comorbidities affecting the immune system.

Age-specific isolation rates of *C. fetus* parallel those of *C. jejuni* and *C. coli;* rates peak in infancy and increase in young adulthood, and *C. fetus* increases substantially in elderly persons. Seasonal distribution patterns of *C. jejuni*, *C. coli*, and *C. fetus* are similar, with peaks occurring in warm months. In a surveillance study, *C. jejuni* and *C. coli* isolates were predominantly from stool, whereas 54 percent of *C. fetus* isolates with a known source were from blood.[275] The epidemiology and antimicrobial susceptibilities of 111 *C. fetus* strains isolated from 103 patients from 1983 to 2000 in Quebec, Canada, were determined.[291] The isolation site was blood in 69 percent of the patients; stool in 20 percent; and other body fluids, including fluid surrounding the aorta, bile, synovial fluid, cerebrospinal fluid, tubo-ovarian abscess, ascites, and pleural fluid, in 11 percent of these patients.[291] *C. fetus*, an important cause of sporadic abortion in cattle and sheep, may be isolated from the intestines and genital tracts of these animals.[212] Contaminated food and water are the suspected sources of infection for sheep, cattle,

and other animals, including goats, pigs, cats, dogs, hamsters, guinea pigs, antelopes, chickens, and turkeys.[81] *C. fetus* bacteremia generally occurs in immunosuppressed hosts (especially elderly men), pregnant women, and neonates.[229,287] Predisposing conditions include alcoholism or cirrhosis, diabetes mellitus, heart disease, malignancy, splenectomy, and corticosteroid or other immunosuppressive therapy.[61,129,231] *C. fetus* is not considered a major cause of gastroenteritis, perhaps because of the failure of this organism to grow in stool specimens evaluated by routine laboratory methods for *C. jejuni* and *C. coli*.[212,275] In addition to raw milk,[306] raw beef liver,[262] and nutritional therapy (i.e., raw fruit, vegetable juice, calves' liver, coffee enemas) are associated with *C. fetus* infection.[38]

First associated with human disease in a homosexual man with proctitis, *C. hyointestinalis* (*hyos*, "hog"; *intestinalis*, "pertaining to the intestines") originally was isolated from the intestines of swine with proliferative ileitis.[71,212] *C. hyointestinalis* also has been isolated from the stool of persons with nonbloody, watery diarrhea.[65]

C. lari is frequently isolated from apparently healthy seagulls, although seagulls do not play a direct role in its epidemiology.[276] Epidemiologically and microbiologically similar to *C. jejuni*, *C. lari* has been reported to cause enteritis in patients with and without a history of animal exposure and bacteremia in two elderly patients with multiple myeloma and permanent pacemakers.[183,191,252,276] A waterborne outbreak of *C. lari*–associated gastroenteritis also has been reported.[27]

C. upsaliensis group is associated with gastroenteritis, breast abscess, spontaneous abortion, and bacteremia in normal hosts, as well as opportunistic infections in immunocompromised persons.[105,107,210] Conditions predisposing to *C. upsaliensis* bacteremia include gallbladder surgery, ectopic pregnancy, kwashiorkor, and acquired immunodeficiency syndrome (AIDS).[210] Young puppies and kittens are potential transmitters of *C. upsaliensis*,[106] and an outbreak in a child care center suggesting direct transmission between humans has been reported.[94,139]

C. concisus has been implicated in gastrointestinal disease. In contrast to *C. jejuni*, disease distribution was year-round rather than higher in certain seasons. Patients most commonly affected were children younger than 1 year of age and elderly people.

PATHOLOGY

Most *C. jejuni* infections are not associated with illness or notable pathologic features. When illness does occur, watery diarrhea, invasive enteritis, or systemic infection may result. The spectrum of pathology reflects this range of manifestations. Acute watery diarrhea may occur in the absence of grossly visible pathologic features. Acute inflammation of the colon and rectum is the hallmark of *C. jejuni* invasive enteritis,[168] although hemorrhagic jejunitis and ileitis may also occur.[68,148,152,257] In patients who have undergone proctoscopy, normal mucosa is found in approximately 50 percent; in the remainder, mucosal edema, congestion, friability, and granularity are seen. The spectrum of histologic changes ranges from minimal edema with acute and chronic inflammatory cells and no vascular congestion, to moderate inflammation and cryptitis, to crypt abscess formation. Acute appendicitis, mesenteric lymphadenitis, and ileocolitis have been reported in patients who have undergone appendectomies while they were infected with *C. jejuni*.[19] *C. jejuni* infection is at low frequency a precursor to immunoreactive complications in which disease results from immunologic effectors generated in response to infection that damages neurons. The demonstration that, after adjustments for comorbidities, *Campylobacter* infection is associated with increased mortality over the course of a 1-year period after infection suggests that as yet unidentified severe late disease occurs.[113] *C. jejuni* is one of a few microbes that have been hypothesized to trigger a common mechanism leading to lymphomas.[104]

PATHOGENESIS

The mechanisms by which *C. jejuni* causes diarrhea, dysentery, and, less frequently, systemic disease are not well understood. Campylobacters have been shown to invade different cell lines. Motility, adherence, and invasion of cells and ability to produce toxins have been associated with capacity to cause disease,* but the pattern of their expression in disease-associated isolates is not uniform. Dogs,[224] rhesus monkeys,[77] rabbits,[264] mice,[77] and hamsters[123] have been evaluated as models of *Campylobacter* enteritis, but none faithfully reproduces the disease seen in humans. A mouse intranasal challenge model[14] has been developed for use in studies of *C. jejuni* invasiveness, and a ferret model[16] has been developed for studies of *C. jejuni*–induced diarrhea. Studies in mice mainly have served as disease models associated with other *Campylobacter* spp.[169] As few as 500 to 800 colony-forming units have been shown to have the potential to cause disease.[135]

Capsular polysaccharides produced by *C. jejuni* have been shown to increase adherence to human intestinal epithelia and provide resistance against some epithelial antimicrobial peptides, including β-defensin and lysozyme. Flagella help overcome peristalsis and allow entry into the mucous layer. Secreted flagellar and nonflagellar proteins are required for adhesion and invasion. Absence of flagellin A (flaA) has been shown to be associated with decreased motility and virulence. Cytolethal distending toxin (CDT) is the best-characterized toxin associated with *Campylobacter*. It comprises three subunits: *cdt*A and *cdt*C play a role in binding to host cells and allowing delivery of *cdt*B, which has a DNAse I–like activity and plays a role in destruction of the host cells. Types IV and VI secretion systems have been described associated with various *Campylobacter* species. *C. fetus* and *C. rectus* have a surface layer that makes them more resistant to lysis and phagocytosis than other *Campylobacter* spp. Survival of *Campylobacter* spp. is facilitated by the ability to form biofilms.

In vitro studies demonstrate a capacity to disrupt tight junctions of intestinal epithelial cell monolayers and preferentially induce proinflammatory cytokine responses,

*References 11, 12, 83, 84, 103, 114, 145, 169, 182, 249, 316.

through activation of nuclear factor κB (NF-κB) and production of interleukin (IL)-18. These capacities and the demonstrated microtubule-dependent eukaryotic cell evasion mechanism[154] may contribute to establishment and maintenance of *C. jejuni* in the gut.[42]

IMMUNITY

The preponderance of evidence of protective acquired immune responses to *C. jejuni* comes from studies of children in developing countries. Such children have more frequent symptomatic infections at younger ages; with increasing age, the rate of symptomatic infection decreases. The number of *C. jejuni* organisms excreted per gram of stool of infected individuals also declines with increasing age,[279] as does the duration of excretion of the organism.[277] These phenomena parallel increasing titers of *Campylobacter*-specific antibodies.* Serum *Campylobacter* IgA and IgM increase within a week of the infection and disappear within 2 to 3 months, whereas IgG levels increase several weeks after infection and last significantly longer.[112] Specific IgA can be isolated from the stools of infected subjects, as well. Additional inferential evidence includes that breast-feeding is associated with reduced frequency of *C. jejuni* diarrhea.[235,288] Adult volunteers who became ill after a first challenge were protected from illness after repeat challenge with an homologous strain[19] and resistance related to the presence of anti-*Campylobacter* antibodies. A Canadian study showed recurrence rate of *C. jejuni* infection within 5 years after an initial infection to be 1.2 percent, suggesting that immunity against *C. jejuni*, at least in developed countries where the environmental load of *Campylobacter* may not be very high, is not persistent or sufficient to protect from subsequent infections.[10] Prolonged, severe, and sometimes recurrent infections occur in immunodeficient patients.[172,176,218] Hypogammaglobulinemic patients have difficulty clearing *Campylobacter* organisms. Patients with late-stage acquired immunodeficiency syndrome (AIDS) are at increased risk for acquiring severe relapsing *Campylobacter* infection; patients with early stages of human immunodeficiency virus (HIV) infection and high CD4 counts are not at risk. Immunity to disease may not protect against asymptomatic colonization. The bacterial components against which the protective immune responses are directed are not known, although several potential immunogenic antigens and virulence factors have been investigated.[196] The sum of this evidence forms the basis for attempts to develop *C. jejuni* vaccines.

Very little information is available on cellular immune responses to *C. jejuni*. Cellular responses may play an important role in facilitating the formation of antibody and in clearing intracellular *C. jejuni* from eukaryotic cells.[135]

A recent in vitro organ culture model system using human gut has shown microcolonies of *C. jejuni* to adhere more frequently to small intestine tissue than to colon tissue, despite similar cytokine responses in the tissues. Significant increase in production of interferon (IFN)-γ and IL-22 was noticed in the presence of *C. jejuni*, as well as IL-23 and to a lesser extent IL-17 production, inferring the presence of an innate and adaptive Th1/Th17 immune response to *C. jejuni*. It was also suggested in mice models that innate immunity and nitric oxide production play an important role in response to *C. jejuni* infection.[271]

Despite the mass of evidence indicating protective roles for immune responses, the *Campylobacter* components against which protective immune responses are targeted are not known. Similarly, attempts to relate classic serotyping schemes, flagellar types, or presence or absence of virulence factors with disease-causing capacity have yielded associations of poor strength. The completion of the genome sequence of *C. jejuni*, NCTC 11168, in 2000[208] enabled studies to be pursued that are beginning to explain the relationships between bacterial components and immune responses. The *Campylobacter* glycome is remarkably plastic,[147] and *Campylobacter* spp. produce a surprising variety of carbohydrates (~8% of the genome is dedicated to the biosynthesis of surface carbohydrates). Furthermore, multiple mechanisms exist for sequence variation for genes encoding the glycome. In a clinical trial, researchers demonstrated that passage of *C. jejuni* in volunteers can be associated with phase variation in lipo-oligosaccharide.[223]

CLINICAL MANIFESTATIONS

C. jejuni produces a spectrum of manifestations, the most common of which is enteritis. Bacteremia, other systemic manifestations, and perinatal infections occur infrequently.

Enteritis

Children with *Campylobacter* enteritis may have unformed stools, watery diarrhea, inflammatory diarrhea, or a combination of these.* Inflammatory diarrhea can be so severe that it is misdiagnosed as inflammatory bowel disease.[255] Inflammatory diarrhea is a more common occurrence in industrialized countries, and secretory watery diarrhea more typically occurs in developing areas. Patients developing diarrhea abroad tend to keep the presentation that is common in their country of origin.[315] Patients with ciprofloxacin-resistant strains have a longer duration of diarrhea than patients infected with sensitive strains,[194] although a recent study from Finland shows opposite results.[72]

Most cases of enteric illness subside within 7 days, although 20 to 30 percent last for 2 weeks and a few (5% to 10%) may persist longer, with a relapsing course lasting for weeks.[148,203] In one third to half of patients, the initial symptoms are periumbilical cramping, intense abdominal pain, malaise, myalgia, and headache. An acute abdomen or appendicitis may be suspected at first[40]

*References 22, 24, 141, 171, 269, 279, 284.

*References 7, 23, 21, 36, 148, 203, 255.

because acute abdominal pain occasionally may be the only initial symptom; pseudoappendicitis or mesenteric adenitis and terminal ileitis can be found.[215] The pain may be mild and intermittent for several weeks, and vomiting is a common occurrence. Secretory diarrhea with 10 or more profuse, watery stools per day may be present. Because this course occurs commonly in younger children, dehydration frequently (10%) is an outcome. Relapse of symptoms may occur.

The symptoms of inflammatory diarrhea are similar to those caused by *Shigella*, invasive *Escherichia coli*, and *Salmonella* and consist of generalized malaise, fever, abdominal cramps, tenesmus, bloody stools, and the presence of fecal leukocytes on light microscopy.[168] Fever without other symptoms may develop and can be associated with febrile seizures.[309] Toxic megacolon with massive bleeding may occur.[101,143,243] In neonates, blood-streaked formed stools or hematochezia may be associated with the isolation of *C. jejuni*.[101,143] The abdomen is tender, especially in the right lower quadrant. Splenomegaly occurs rarely.

C. upsalensis and *C. concisus* are the two most common emerging campylobacters to cause gastroenteritis, although reported cases of gastroenteritis associated with *C. fetus*, *C. hyointestinalis*, *C. insulaenigrae*, *C. lari*, *C. mucosalis*, *C. sputorum*, and *C. ureolyticus* exist.[169] These emerging *Campylobacter* spp. may have an important role in gastroenteritis without an identifiable etiology.

C. upsaliensis was the only organism isolated from 83 patients in a large stool culture survey using a filtration system for *Campylobacter* in Belgium.[96] Ninety-two percent of patients had diarrhea, which was of acute onset. Vomiting (14%) and fever (7%) were uncommon occurrences, and symptoms generally abated in less than a week. Gross or occult blood was identified in 25 percent of cases, and neutrophils were seen on fecal smear in approximately 20 percent. Erythromycin (11 patients) or amoxicillin (2 patients) eradicated the organism, with resolution of symptoms in all 13 patients treated with antibiotics.[96] An Australian study identified *C. upsaliensis* in 19 (0.1%) of 18,516 stool specimens from August 1992 to March 1999 at the Royal Children's Hospital in Melbourne.[139] Infection with *C. upsaliensis* was associated with milder disease than was infection with *C. jejuni;* patients with *C. upsaliensis* infection had significantly less fever, diarrhea, and rectal bleeding.

C. concisus has been associated with gastroenteritis in children. In an older study, *C. concisus* was isolated from 13.2 percent of stools of 174 children with diarrhea versus 9 percent of 958 children without gastrointestinal manifestations.[169] A more recent study from Denmark showed *C. concisus* to be the second most common bacterial isolate from stools of 8302 patients studied, and the annual incidence was reported to be 35/100,000 inhabitants. In this study, healthy controls were not found to have *C. concisus* in their stools, which contradicts previous studies that questioned the true pathologic role of *C. concisus*, especially in children. Four genomospecies of *C. concisus* exist that may have different virulence and clinical manifestations.[160,195]

Six clinical *C. lari* isolates were referred to the national *Campylobacter* reference laboratory at the CDC in 1982 and 1983.[276] Clinical illness associated with these isolates included enteritis in four patients, severe crampy abdominal pain in a 7-year-old girl, and terminal bacteremia in a 71-year-old man with multiple myeloma and chronic renal failure. The ages of the four patients with enteritis were 8 months, 3 years, 22 years, and 39 years. Diarrhea was watery or mucoid, and fever was an unusual occurrence. Potential exposure included consuming chicken, having contact with house pets, drinking untreated surface water, and eating raw oysters. *C. lari* colitis also developed in an HIV-infected woman.[69]

C. hyointestinalis has been isolated from the stool specimens of adult and pediatric patients experiencing nonbloody, watery diarrhea[65] and a rectal culture from a homosexual man with proctitis.[71] Over a 4-year period, 20 strains of *C. curvus* were isolated from two separate and distinct clinical settings, a hospital survey of infectious causes of bloody diarrhea and an outbreak of Brainerd (chronic) diarrhea in northern California.[1]

A study from Ireland was the first to report isolation of *C. ureolyticus* from diarrheal stools; the organism accounted for 23.8 percent of 349 stool samples that were *Campylobacter* positive. Of 8 patients who were followed clinically, only 2 were pediatric patients aged 1 and 6 years. All 8 patients presented with abdominal cramping and had symptoms of more than 5 days' duration. Three of the patients were immunosuppressed (2 diabetic and 1 with HIV-associated lymphoma).[31]

Extraintestinal Infections

Bacteremia with *C. jejuni* occurs much less commonly than enteritis. Bacteremia was recognized first in malnourished children, patients with chronic illness or immunodeficiency, and patients at the extremes of age.[4,102,176,227] Cirrhosis, cancer, immunosuppressive therapy, and HIV infection commonly are underlying conditions in patients with bacteremia.[220] These findings led to the view that *C. jejuni* bacteremia was a disease of the relatively immunocompromised patient. However, most *C. jejuni* blood isolates are from healthy individuals who often have histories of recent gastrointestinal disease.[256] The average incidence of *Campylobacter* bacteremia in England and Wales is 1.5/1000 intestinal *Campylobacter* infections. The CDC reports that only 0.4 percent of *C. jejuni* isolates in the United States are from blood cultures.[275] Most *C. jejuni* strains are susceptible to killing by serum, a finding that perhaps explains the transient nature of the bacteremia and its tendency to resolve without specific therapy. In HIV-infected patients, *Campylobacter* bacteremia occurs more frequently and with increased morbidity and higher mortality rates,[170,220,280] although these numbers have improved after the era of combined antiretroviral therapy. Fatal cases have been reported in the absence of enteric disease.[170] In a Danish study reporting 46 cases of *Campylobacter* bacteremia over a 10-year period, the incidence of bacteremia was estimated at 2.9/1 million person-years. Only one case was reported in a child. Patients with bacteremia tended to be older than patients with isolated enteritis, and enteritis was detected in 59 percent of patients with bacteremia. *C. jejuni* accounted for 37 cases; *C. coli*, *C. fetus*, and *C.*

lari were responsible for the rest. The mortality rate associated with bacteremia was 4 percent. Patients who survived had no recurrence within a 365-day follow-up period. A study from Spain reported an incidence of 4.7/1 million person-years over a 23-year period (71 episodes of bacteremia). Of these events, 31 percent originated extraintestinally and 26 percent were primary infections. Isolates included *C. jejuni* (66%), *C. coli* (19%), and *C. lari* (12%). The mortality rate was 16.4 percent. The recurrence rate was 5 percent. In both studies patients had comorbidities, including major gastrointestinal surgeries, alcoholism, HIV, immunodeficiency, and malignancy.[73] In the largest recent series of *Campylobacter* bacteremia, 183 cases were reported from France in 178 patients in a 5-year period. Only 7 patients were younger than 15 years of age. Fever was the only presenting symptom in 42 percent of cases. *C. fetus* accounted for 57 percent of cases. Other isolated *Campylobacter* spp. included *C. jejuni*, *C. coli*, and *C. lari*.[201]

Bacteremia is one of the most common presentations of uncommon *Campylobacter* species (Table 131-5).

The main reason for the increased recognition of extraintestinal *Campylobacter* infections appears to be the growing application of appropriate microbiologic culture methods. The incidence of *C. jejuni* bacteremia probably remains underestimated. Typically, blood cultures are not performed in individuals with the primary complaint of diarrhea. Rarely,[222] cholecystitis, urinary tract infection,[49] pancreatitis,[85] hepatitis,[155] and meningitis[61,284] can result from *Campylobacter* infection.

C. fetus is the non-*jejuni*, non-*coli Campylobacter* most commonly described to cause disease in humans. It is known to cause perinatal disease but is otherwise rare in healthy children. More commonly it can cause bacteremia in adults with other comorbidities, especially immune suppression. Typically, illness begins with fever, malaise, and headache. Chills and night sweats are prominent, as is weight loss in prolonged illness. Diarrhea, nausea, vomiting, and abdominal pain occur in as many as 38 percent of cases, and hepatosplenomegaly or jaundice develops in two thirds.[102,310] Pulmonary involvement is a rare finding.[25,102]

C. fetus also has a tropism to the vessels. Three patterns of invasive *C. fetus* disease have been described.[231] Clinical manifestations of the first localized infection accompanied by septicemia include meningitis,[29,163] endocarditis,[70] pericarditis,[184] thrombophlebitis,[37] mycotic aneurysm,[231] cellulitis,[37,80] gluteal abscess,[53] septic arthritis,[159] salpingitis,[29] and peritonitis.[272] The second form is transient asymptomatic bacteremia, which may be self-limited.[102,229,232] Prolonged and recurrent bacteremia, with waxing and waning symptoms as spontaneous relapses and remissions occur, is the third pattern of invasive *C. fetus* infection.[25,45,133,233,310]

C. curvus also was reported to be associated with polymicrobial liver and lung abscesses in two patients with cancer. *C. rectus* was reported in a polymicrobial breast abscess in a patient with lymphoma.[1] *C. concisus*, *C. curvus*, *C. gracilis*, *C. lari*, *C. mucosalis*, *C. rectus*, *C. showae*, *C. sputorum*, and *C. ureolyticus* can be isolated from the oral cavity. *C. rectus* and *C. gracilis* are thought to be associated with periodontitis.[169]

The extraintestinal clinical features of "other" *Campylobacter* spp. are presented in Table 131-5.

TABLE 131-5 **Clinical Features Associated with Extraintestinal Infection by "Atypical" *Campylobacter* Species**

Species	Extraintestinal Clinical Features
C. fetus	Bacteremia, sepsis, meningitis, vascular infections, septic abortion
C. upsaliensis	Bacteremia, abscesses, abortion, hemolytic-uremic syndrome
C. lari	Bacteremia
C. hyointestinalis	Bacteremia
C. sputorum	Pulmonary, perianal, groin, knee, and axillary abscesses, bacteremia
C.concisus	Periodontitis, brain abscess[54]
C. curvus	Liver abscess, bronchial abscess, bacteremia
C. gracilis	Periodontitis, brain abscess[54] head and neck infection
C. rectus	Periodontitis, necrotizing soft tissue infections, empyema thoracis,[160] vertebral abscess[54]
C. showae	Intraorbital abscess[54]
C. ureolyticus	Oral and perianal abscesses, soft tissue and bone infections
C. insulaenigrae	Bacteremia
C.hominis	Bacteremia

Perinatal Infections

Occasionally, abortion or stillbirth, premature labor, neonatal sepsis, and meningitis caused by *C. jejuni* have been described, although these manifestations have been more commonly associated with *C. fetus*.[213] *Campylobacter*-associated second-trimester abortion generally is preceded by mild gastroenteritis.[186,253] The placenta may have areas of necrosis, infarction, microabscesses, and inflammation. The most likely route of placental or fetal infection is through the bloodstream, although a case with possible ascending spread has been reported.[57] Maternal blood, placenta, cervix, vaginal, and stool cultures have yielded *C. fetus* in reported perinatal cases.[163,253,308] Infected infants often are premature. Illness in neonates generally is mild or asymptomatic, but symptomatic gastroenteritis and asymptomatic bloody diarrhea caused by *C. jejuni* have been reported in newborn infants.[30,203] Bacteremia and meningitis also may occur.[95,284] Hemorrhagic infarction and necrosis, as well as cystic degeneration of the cerebral cortex, are the cerebral lesions most commonly reported in *Campylobacter* meningitis.[308] The source of the organism usually has been the mother, who may be symptomatic or asymptomatic at the time of delivery.[30,251]

Immunoreactive Complications

An episode of *C. jejuni* infection may be followed by immunoreactive complications such as Guillain-Barré

syndrome (GBS),[110,111,142,179,230] Reiter syndrome,[140,217,221] reactive arthritis,[64,222,241] and erythema nodosum.[9,82,263,264] A preceding *C. jejuni* infection has been documented by serologic methods or stool culture in 12 to 60 percent of patients with GBS.* *C. jejuni* infection is the most common identified causal factor for GBS.[190,228] However, the risk for development of this syndrome after having a *C. jejuni* infection is less than 1 percent.[6] *Campylobacter*-associated GBS is associated with axonal degeneration and poorer outcome than in GBS associated with other causes.[228] During the 2 months after a symptomatic episode of *C. jejuni* infection, the likelihood of GBS is approximately 100 times (30.4/100,000) higher than the risk in the general population (0.3/100,000).[173] In a nested case-control study from 1991 to 2001 using data from the United Kingdom General Practice Research Database, 20 percent of GBS cases were attributable to *Campylobacter*.[270] A case-control study from India showed 27.7 percent of patients with GBS to have evidence of a recent *Campylobacter* infection.[144] A study from Bangladesh showed higher rates of *Campylobacter* infection preceding GBS with positive serology in 57 percent of cases. Patients with *Campylobacter* infection were more likely to have axonal variant, greater disability, and higher mortality; they also had higher antibodies to gangliosides GM_1 and CD1a.[131] GBS syndrome appears to be an age-related risk; one study found no cases in patients younger than 20 years of age, 14/100,000 in patients aged 20 to 59 years, and 248/100,000 infections in those older than 60 years of age.[173] Certain serotypes of *C. jejuni* are associated more frequently with the subsequent development of GBS.[8,157]

Molecular mimicry between G_{M1} ganglioside and *C. jejuni* lipo-oligosaccharide is established as one of the triggers of GBS.[313] A positive correlation of serologic evidence of *C. jejuni* and the presence of antibody to G_{M1} has been described.[99,134] Lipopolysaccharide extracted from *C. jejuni* was found to have core oligosaccharide resembling human ganglioside G_{M1}. Pure motor neuropathy with a tendency for more distal weakness and sparing of the cranial nerves has been associated with *C. jejuni* infection in patients with anti-G_{M1} antibody.[6,283] *Campylobacter* carrying the *cst-II* sialy transferase gene is associated with the development of GBS and Fisher syndrome, and gene polymorphisms may determine which syndrome develops after having a case of *C. jejuni* enteritis.[313] Cases of Miller-Fisher syndrome, a polyneuritis variant characterized by ophthalmoplegia, areflexia, and cerebellar ataxia, as well as Bickerstaff's brainstem encephalitis (Miller-Fisher variant with impaired consciousness), also have been reported in association with *C. jejuni* infection.[128,314] These patients often have antibodies to ganglioside G_{Q1b}.

Reactive arthritis may be associated with *Campylobacter* enteritis, especially in adults with human leukocyte antigen (HLA)-B27.[64,241]

Campylobacter-reactive arthritis may occur in 1 to 5 percent of patients infected.[222] The arthritis starts a few days to several weeks after the episode of diarrhea. Involvement of joints can be monarticular or multiple, as well as migratory, and both large and small joints can be affected. Synovial fluid is sterile, and fever and leukocytosis are absent; the duration ranges from 1 week to several months. The course is self-limited, and the prognosis is good.[108] A study from Oregon including 6379 culture-confirmed *Campylobacter*, *Salmonella*, *Yersinia*, *Shigella*, and *E. coli* O157 infections showed an incidence of 2.1/100,000 of reactive arthritis after *Campylobacter* infection. Women, adults, and patients with more severe illness were found to be at higher risk for developing this complication. Interestingly, no association with HLA-B27 was found.[290]

C. jejuni and more recently other campylobacters have been suggested to be associated with the development of inflammatory bowel disease in children, although Jess and colleagues[138] relate increased rates of *Campylobacter* infections around the first episode of inflammatory bowel disease to a possible detection bias. In one study, *C. concisus* was isolated from 65 percent (35/54) of stools from children with Crohn disease and 12 other samples were positive for other non-*jejuni Campylobacter* (i.e., *rectus*, *gracilis*, *showae*).[169]

It has also been postulated that *Campylobacter* enteritis can be followed by development of functional gastrointestinal disorders in children, including irritable bowel syndrome and dyspepsia.[241]

Severe, persistent, and relapsing *C. jejuni* infections have been reported in patients with immune deficiencies, including congenital and acquired hypogammaglobulinemia and malnutrition.[4,120,176] In patients with AIDS, increased frequency and severity of *C. jejuni* infection have been reported; the severity correlates inversely with the CD4 count.[164,216,218]

DIAGNOSIS

The initial characteristics of *C. jejuni* enteritis are not sufficiently unique to permit the diagnosis to be established on clinical grounds. The differential diagnosis should include *Shigella*, *Salmonella*, invasive *E. coli*, *E. coli* O157:H7, *Yersinia enterocolitica*, *Aeromonas*, *Vibrio parahaemolyticus*, amebiasis, and pseudomembranous colitis caused by *Clostridium difficile*. Fecal leukocytes are found in as many as 75 percent of cases of *Campylobacter* enteritis; gross or occult fecal blood is present in 50 percent.[19,23,168] White blood cell counts usually are normal, although a shift to the left may occur. Mild elevations in alanine aminotransferase, alkaline phosphatase, and the sedimentation rate are observed in as many as 25 percent of patients.

Methods for demonstrating *C. jejuni* include direct microscopy,[204,206] bacteriologic culture, antigen detection by electroimmunoassay (EIA) or enzyme-linked immunosorbent assay (ELISA),[116,242,286] DNA probes,[281] polymerase chain reaction (PCR),[134,161,200,297] and serology.[7] Detection of antigen by EIA is nearly as sensitive and specific as culture. ELISA using recombinant P18 and P39 as antigens has demonstrated 91.9 percent sensitivity and 99 percent specificity.[242] PCR enables some culture-negative *Campylobacter* infections to be diagnosed.[161,162]

*References 5, 110, 111, 122, 149, 179.

Serologic tests appear useful for epidemiologic investigations but are not recommended for routine diagnosis.

C. jejuni can be detected by darkfield and phase-contrast examination of fresh suspensions of stool. The distinguishing characteristic of *Campylobacter* is darting motility. Gram stain of stool showing *Vibrio* forms is said to be useful in making a presumptive diagnosis.[240] Using the direct carbol-fuchsin Gram-stain method, Wang and Murdoch[302] reported 89 percent sensitivity and 99.7 percent specificity when examining stool samples of *Campylobacter*-infected patients. The indirect fluorescent antibody test can be used for identification of *Campylobacter* on smears; however, standardized reagents for this procedure are not available from commercial sources.

Establishing a definitive diagnosis of *Campylobacter* infection requires the demonstration of the organism in stool or in a tissue sample. Unfortunately, not all laboratories culture for *Campylobacter*, despite its frequency, and not all laboratories use the same culture technique.[167] Culture of *Campylobacter* from stool requires special methods and media. It can be accomplished with media that contain antibiotics[66] to which *Campylobacter* organisms are resistant. If culturing is to be done on medium free of antibiotics, diluted stool samples should be passed through a cellulose acetate membrane filter with a pore size of 0.65 μm to reduce the number of other enteric microorganisms.[66] Inoculated plates should be incubated in 5 percent oxygen and 10 percent carbon dioxide at 37° to 42° C (98.6° to 107.6° F). Colony formation may not be grossly visible until 72 hours after plating. Identification of colonies as *C. jejuni/coli* is based on a Gram stain showing characteristic morphology and positive catalase and oxidase reactions. Hydrolysis of hippurate establishes an isolate as meeting the conventional inclusion criteria for *C. jejuni*. Routine media usually are adequate for isolation of *Campylobacter* from normally sterile body fluids and tissues. PCR analysis performed on fixed, routinely processed colon biopsies is an excellent diagnostic method for detecting *Campylobacter* from focal active colitis cases. An advantage of the PCR on tissue is that it can be done retrospectively from the paraffin block.[244]

TREATMENT

Most *C. jejuni* organisms are susceptible to macrolides, aminoglycosides, fluoroquinolones, chloramphenicol, imipenem, and clindamycin and are resistant to cephalosporins, tetracyclines, rifampin, penicillins, trimethoprim, and vancomycin.[254,296] However, the development of resistance to macrolides is starting to emerge, with case reports worldwide.[90] Patterns of antibiotic resistance in *C. jejuni* show regional differences. In the past, quinolones were used as empirical therapy for adult traveler's diarrhea because of their good microbiologic activity against *Campylobacter*, *Shigella*, and *Salmonella* strains.[6,62,239] However, since the late 1980s, *Campylobacter* strains have become increasingly resistant to fluoroquinolones worldwide, except in Australia (Table 131-6). The emergence of resistance has been associated with the use of quinolones such as sarafloxacin, difloxacin, and enrofloxacin in veterinary medicine in Europe and the United States.[66,67,126,257] The association of human infection with fluoroquinolone-resistant *Campylobacter* spp. and consumption of poultry prompted the U.S. Food and Drug Administration (FDA) to withdraw enrofloxacin for use in poultry, effective September 2005.[193] Despite this measure, it will take time for fluoroquinolone-sensitive *Campylobacter* spp. to be reestablished in the environment. A study comparing infection with erythromycin-resistant and erythromycin-susceptible isolates in children did not show any clinical difference in course of disease or presentation. Although fluoroquinolone-resistant *Campylobacter* is generally thought to cause more severe disease, recent studies show opposite results.

Fluoroquinolone resistance in *C. jejuni* appears to be related to mutations in the genes encoding subunits of DNA gyrase.[119] The frequency of erythromycin-resistant *Campylobacter* isolates is low (see Table 131-6).[237] Azithromycin is considered the drug of choice in adults and children.[63,158] Most patients with *C. jejuni* enteritis have mild symptoms and do not require antibiotic therapy. For these patients, oral rehydration and replacement of

TABLE 131-6 **Antibiotic Resistance Pattern of *Campylobacter jejuni* Isolates**

			Percentage of Isolates Resistant to			
Location	**Study Years**	**No. Tested**	**Erythromycin**	**Fluoroquinolones**	**Tetracycline**	**Gentamicin**
Netherlands[242]	1994-1997	1315	2	11-29	7-15	ND
Minnesota[233]	1994-1998	4953	ND	1.3-10.2	ND	ND
Spain, Barcelona[203]	1995-1998	909	5	81	72	1
Canada[67]	1995-1997	158	0	12.7	56	ND
Spain[216]	1997-1998	537	3.2	75	ND	0.4
Taiwan[311]	1994-1996	93	10	52	95	1
Thailand[150]	1995	57	ND	84	ND	ND
South Africa[236]	2003-2005	98	95	11	35	21
Taiwan[313]	2003-2005	61	3.3	90.2	93.4	ND
Cambodia[177]	2004-2006	87	2	31	25	ND
Portugal	2003-2007	123	ND	80.5	ND	ND
Chile[74]	2002-2007	73	0	32.4	ND	ND
Poland [234]	2000-2007	251	0.4	49.5	17.5	ND

ND, No data.

electrolytes are sufficient. Patients who may benefit from antibiotic therapy are those with extraintestinal infections, fever, bloody stool, and symptoms lasting longer than a week.[7] Patients with HIV infection or other immunodeficiency syndromes should be treated.[219]

Data on antibiotic treatment are controversial. A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by *Campylobacter* spp. looked at 11 randomized control trials. It concluded that antibiotic therapy (with erythromycin, ciprofloxacin, or norfloxacin) shortened the duration of intestinal symptoms by less than 2 days, especially if these drugs were given early in the course of disease.[282] In addition, antibiotic therapy shortened the excretion of *Campylobacter* spp. from feces.[282] All immunocompromised and bacteremic patients with *C. jejuni* infections should be treated with an appropriate antibiotic such as gentamicin, imipenem, or both drugs.[7]

Synergistic combination therapy is indicated for patients with meningitis and endocarditis, in which bactericidal activity is critical.[7,181] Patients with *Campylobacter* in their stool who are being treated for an extraintestinal *Campylobacter* infection with gentamicin should be prescribed supplemental oral therapy because gentamicin is ineffective against *Campylobacter* in the gut.[7]

Prolonged antimicrobial therapy and follow-up blood cultures are warranted for patients with *C. fetus* bacteremia because of the relapsing nature of the illness.[104,184] Chloramphenicol should be used with caution in treating *C. fetus* meningitis because clinical outcome and in vitro susceptibility results for this drug have been disappointing.[163,181]

PREVENTION

Tactics for prevention of campylobacteriosis include breast-feeding and avoidance of raw food and food that has been cooked under conditions that permit the survival of bacteria or that has been handled in such a way that bacterial contamination may occur. Risks for foodborne illnesses, including campylobacteriosis transmitted by restaurant-prepared meals, may be reduced by mandating that food-service employees obtain training in food safety (e.g., thorough hand washing with soap and water after handling raw poultry or meat, cooking poultry to 82°C [180°F] or until meat is no longer pink and juices run clear, and separating raw poultry from other foods during preparation).[39]

A 24-month (2002 to 2004), population-based surveillance case-control study by FoodNet in the United States evaluated infants with laboratory-confirmed *Campylobacter* infection and identified risk factors associated with their infection. Identified risk factors include drinking well water, eating fruits and vegetables prepared at home, having a pet with diarrhea in the home, visiting or living on a farm, riding in a shopping cart next to meat or poultry, and traveling outside the United States.[181] Prevention measures should then be targeted at the potential source. An interesting finding is that attendance at a daycare center did not pose an increased risk in this study.

Selected microorganisms may displace *Campylobacter* from its ecologic niches. This finding may prove useful in reducing *Campylobacter* contamination of food animals.[41,166,180,187] Attempts to develop vaccines against *Campylobacteriosis* continue, but no product is in advanced development yet.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Abu-Elamreen FH, Abed AA, Sharif FA. Viral, bacterial and parasitic etiology of pediatric diarrhea in Gaza, Palestine. *Med Princ Pract* 2008;**17**:296–301.
10. Arsenault J, Ravel A, Michel P, et al. Do patients with recurrent episodes of campylobacteriosis differ from those with a single disease event? *BMC Public Health* 2011;**11**:32.
15. Barton Behravesh C, Jones TF, Vugia DJ, et al. Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996-2005. *J Infect Dis* 2011;**204**:263–7.
18. Bessell PR, Matthews L, Smith-Palmer A, et al. Geographic determinants of reported human *Campylobacter* infections in Scotland. *BMC Public Health* 2010;**10**:423.
31. Bullman S, Corcoran D, O'Leary J, et al. *Campylobacter ureolyticus:* an emerging gastrointestinal pathogen? *FEMS Immunol Med Microbiol* 2011;**61**:228–30.
43. Cho SH, Shin HH, Choi YH, et al. Enteric bacteria isolated from acute diarrheal patients in the Republic of Korea between the year 2004 and 2006. *J Microbiol* 2008;**46**:325–30.
48. Danis K, Di Renzi M, O'Neill W, et al. Risk factors for sporadic infection: an all-Ireland case-control study. *Euro Surveill* 2009;**14**:19123.
54. de Vries JJ, Arents NL, Manson WL. *Campylobacter* species isolated from extra-oro-intestinal abscesses: a report of four cases and literature review. *Eur J Clin Microbiol Infect Dis* 2008;**27**:1119–23.
56. Denno DM, Keene WE, Hutter CM, et al. Tri-county comprehensive assessment of risk factors for sporadic reportable bacterial enteric infection in children. *J Infect Dis* 2009;**199**:467–76.
72. Feodoroff FB, Lauhio AR, Sarna SJ, et al. Severe diarrhoea caused by highly ciprofloxacin-susceptible *Campylobacter* isolates. *Clin Microbiol Infect* 2009;**15**:188–92.
73. Fernandez-Cruz A, Munoz P, Mohedano R, et al. *Campylobacter* bacteremia: clinical characteristics, incidence, and outcome over 23 years. *Medicine (Baltimore)* 2010;**89**:319–30.
76. Fitzenberger J, Uphoff H, Gawrich S, et al. Urban-rural differences of age- and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005-June 2006. *Eur Surveill* 2010;**15**:19693.
86. Gallay A, Bousquet V, Siret V, et al. Risk factors for acquiring sporadic *Campylobacter* infection in France: results from a national case-control study. *J Infect Dis* 2008;**197**:1477–84.
87. Gardner TJ, Fitzgerald C, Xavier C, et al. Outbreak of campylobacteriosis associated with consumption of raw peas. *Clin Infect Dis* 2011;**53**:26–32.
92. Gillespie I. Population-attributable risk estimates for *Campylobacter* infection, Australia. *Emerg Infect Dis* 2009;**15**:850–1; author reply 851-2.
112. Havelaar AH, van Pelt W, Ang CW, et al. Immunity to *Campylobacter*: its role in risk assessment and epidemiology. *Crit Rev Microbiol* 2009;**35**:1–22.
130. Inglis GD, Boras VF, Houde A. Enteric campylobacteria and RNA viruses associated with healthy and diarrheic humans in the Chinook health region of southwestern Alberta, Canada. *J Clin Microbiol* 2010;**49**:209–19.
131. Islam Z, Jacobs BC, van Belkum A, et al. Axonal variant of Guillain-Barré syndrome associated with *Campylobacter* infection in Bangladesh. *Neurology* 2010;**74**:581–7.
132. Ivanova K, Marina M, Petrov P, et al. Campylobacteriosis and other bacterial gastrointestinal diseases in Sofia, Bulgaria for the period 1987-2008. *Euro Surveill* 2010;**15**:19474.
135. Janssen R, Krogfelt KA, Cawthraw SA, et al. Host-pathogen interactions in *Campylobacter* infections: the host perspective. *Clin Microbiol Rev* 2008;**21**:505–18.

136. Jelovcan S, Schmid D, Lederer I, et al. Cluster of nosocomial campylobacteriosis, Austria 2006. *J Hosp Infect* 2008;**69**: 97–8.
137. Jensen LA, Marlin JW, Dyck DD, et al. Prevalence of multigastrointestinal infections with helminth, protozoan and *Campylobacter* spp. in Guatemalan children. *J Infect Dev Countries* 2009;**3**: 229–34.
138. Jess T, Simonsen J, Nielsen NM, et al. Enteric *Salmonella* or *Campylobacter* infections and the risk of inflammatory bowel disease. *Gut* 2011;**60**:318–24.
144. Kalra V, Chaudhry R, Dua T, et al. Association of *Campylobacter jejuni* infection with childhood Guillain-Barré syndrome: a case-control study. *J Child Neurol* 2009;**24**:664–8.
146. Karagiannis I, Sideroglou T, Gkolfinopoulou K, et al. A waterborne *Campylobacter jejuni* outbreak on a Greek island. *Epidemiol Infect* 2010;**138**:1726–34.
160. Lastovica AJ. Clinical relevance of *Campylobacter concisus* isolated from pediatric patients. *J Clin Microbiol* 2009;**47**:2360.
167. M'Ikanatha NM, Dettinger LA, Perry A, et al. Culturing stool specimens for *Campylobacter* spp., Pennsylvania, USA. *Emerg Infect Dis* 2012;**18**:484–7.
169. Man SM. The clinical importance of emerging *Campylobacter* species. *Nat Rev Gastroenterol Hepatol* 2011;**8**:669–85.
177. Meng CY, Smith BL, Bodhidatta L, et al. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. *Pediatr Infect Dis J* 2011;**30**:331–5.
188. Mshana SE, Joloba M, Kakooza A, et al. *Campylobacter* spp among children with acute diarrhea attending Mulago hospital in Kampala, Uganda. *Afr Health Sci* 2009;**9**:201–5.
195. Nielsen H, Steffensen R, Ejlertsen T. Risk and prognosis of campylobacteriosis in relation to polymorphisms of host inflammatory cytokine genes. *Scand J Immunol* 2012;**75**:449–54.
196. Nielsen LN, Luijkx TA, Vegge CS, et al. Identification of immunogenic and virulence-associated *Campylobacter jejuni* proteins. *Clin Vaccine Immunol* 2011;**19**:113–19.
201. Pacanowski J, Lalande V, Lacombe K, et al. *Campylobacter* bacteremia: clinical features and factors associated with fatal outcome. *Clin Infect Dis* 2008;**47**:790–6.
219. Pickering LK. Antimicrobial resistance among enteric pathogens. *Adv Exp Med Biol* 2008;**609**:154–63.
234. Rozynek E, Dzierzanowska-Fangrat K, Szczepanska B, et al. Trends in antimicrobial susceptibility of *Campylobacter* isolates in Poland (2000-2007). *Pol J Microbiol* 2009;**58**:111–15.
236. Samie A, Guerrant RL, Barrett L, et al. Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarroeal human stools from Vhembe district, South Africa. *J Health Popul Nutr* 2009;**27**:739–45.
239. Saps M, Pensabene L, Di Martino L, et al. Post-infectious functional gastrointestinal disorders in children. *J Pediatr* 2008;**152**: 812–16, 816 e811.
249. Silva J, Leite D, Fernandes M, et al. *Campylobacter* spp. as a foodborne pathogen: a review. *Front Microbiol* 2011;**2**:200.
261. Soofi SB, Habib MA, von Seidlein L, et al. A comparison of disease caused by *Shigella* and *Campylobacter* species: 24 months community based surveillance in 4 slums of Karachi, Pakistan. *J Infect Public Health* 2011;**4**:12–21.
267. Stirling J, Griffith M, Blair I, et al. Prevalence of gastrointestinal bacterial pathogens in a population of zoo animals. *Zoonoses Public Health* 2008;**55**:166–72.
268. Strachan NJ, Watson RO, Novik V, et al. Sexual dimorphism in campylobacteriosis. *Epidemiol Infect* 2008;**136**:1492–5.
271. Tarantino M, Dionisi AM, Pistoia C, et al. Involvement of nitric oxide in the control of a mouse model of *Campylobacter jejuni* infection. *FEMS Immunol Med Microbiol* 2009;**56**:98–101.
290. Townes JM, Deodhar AA, Laine ES, et al. Reactive arthritis following culture-confirmed infections with bacterial enteric pathogens in Minnesota and Oregon: a population-based study. *Ann Rheum Dis* 2008;**67**:1689–96.
305. White AN, Kinlin LM, Johnson C, et al. Environmental determinants of campylobacteriosis risk in Philadelphia from 1994 to 2007. *Ecohealth* 2009;**6**:200–8.
311. Yang JR, Wu HS, Chiang CS, et al. Pediatric campylobacteriosis in northern Taiwan from 2003 to 2005. *BMC Infect Dis* 2008; **8**:151.
316. Zilbauer M, Dorrell N, Wren BW, et al. *Campylobacter jejuni*-mediated disease pathogenesis: an update. *Trans R Soc Trop Med Hyg* 2008;**102**:123–9.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 132

TULAREMIA

Gordon E. Schutze

Tularemia is an acute febrile illness caused by *Francisella tularensis*. Although it is primarily a disease of animals, humans also are highly susceptible hosts.

HISTORY

McCoy[101] published the first documented evidence of tularemia in 1911 when he described a plaguelike disease in ground squirrels *(Citellus beecheyi)* that occurred in Tulare County, California. Within 2 years, McCoy and Chapin[102] isolated and characterized the organism *Bacterium tularense* from naturally infected ground squirrels. They also detailed the pathologic process produced in ground squirrels, defined the susceptibility of other animal species to *B. tularense*, and identified fleas as vectors for the plaguelike disease in 1912.

The first description of tularemia in humans may be that by Homma Soken, a court physician in eastern Japan.[122] In 1837, he described an illness as "hare meat

REFERENCES

1. Abbott SL, Waddington M, Lindquist D, et al. Description of *Campylobacter curvus* and *C. curvus*-like strains associated with sporadic episodes of bloody gastroenteritis and Brainerd's diarrhea. *J Clin Microbiol* 2005;**43**:585–8.
2. Abrahams CA, Agbodaze D, Nakano T, et al. Prevalence and antibiogram of *Campylobacter jejuni* in domestic animals in rural Ghana. *Arch Environ Health* 1990;**45**:59–62.
3. Abu-Elamreen FH, Abed AA, Sharif FA. Viral, bacterial and parasitic etiology of pediatric diarrhea in Gaza, Palestine. *Med Princ Pract* 2008;**17**:296–301.
4. Ahnen DJ, Brown WR. *Campylobacter* enteritis in immune-deficient patients. *Ann Intern Med* 1982;**96**:187–8.
5. Allos BM. Association between *Campylobacter* infection and Guillain-Barré syndrome. *J Infect Dis* 1997;**176**(Suppl. 2):S125–128.
6. Allos BM. *Campylobacter jejuni* infections: update on emerging issues and trends. *Clin Infect Dis* 2001;**32**:1201–6.
7. Allos BM, Blaser MJ. *Campylobacter jejuni* and the expanding spectrum of related infections. *Clin Infect Dis* 1995;**20**:1092–9; quiz 1100–1.
8. Allos BM, Lippy FT, Carlsen A, et al. *Campylobacter jejuni* strains from patients with Guillain-Barré syndrome. *Emerg Infect Dis* 1998;**4**:263–8.
9. Anton E. [Reactive arthritis and erythema nodosum caused by *Campylobacter jejuni*]. *Enferm Infecc Microbiol Clin* 1998;**16**:387–8.
10. Arsenault J, Ravel A, Michel P, et al. Do patients with recurrent episodes of campylobacteriosis differ from those with a single disease event? *BMC Public Health* 2011;**11**:32.
11. Bacon DJ, Alm RA, Burr DH, et al. Involvement of a plasmid in virulence of *Campylobacter jejuni* 81-176. *Infect Immun* 2000;**68**:4384–90.
12. Bacon DJ, Alm RA, Hu L, et al. DNA sequence and mutational analyses of the pVir plasmid of *Campylobacter jejuni* 81-176. *Infect Immun* 2002;**70**:6242–50.
13. Baker RC, Paredes MD, Qureshi RA. Prevalence of *Campylobacter jejuni* in eggs and poultry meat in New York State. *Poult Sci* 1987;**66**:1766–70.
14. Baqar S, Bourgeois AL, Schultheiss PJ, et al. Safety and immunogenicity of a prototype oral whole-cell killed *Campylobacter* vaccine administered with a mucosal adjuvant in non-human primates. *Vaccine* 1995;**13**:22–8.
15. Barton Behravesh C, Jones TF, Vugia DJ, et al. Deaths associated with bacterial pathogens transmitted commonly through food: foodborne diseases active surveillance network (FoodNet), 1996-2005. *J Infect Dis* 2011;**204**:263–7.
16. Bell JA, Manning DD. A domestic ferret model of immunity to *Campylobacter jejuni*-induced enteric disease. *Infect Immun* 1990;**58**:1848–52.
17. Benjamin J, Leaper S, Owen RJ, et al. Description of *Campylobacter laridis*, a new species comprising the nalidixic acid resistant thermophilic *Campylobacter* (NARTC) group. *Curr Microbiol* 1983;**8**:231–8.
18. Bessell PR, Matthews L, Smith-Palmer A, et al. Geographic determinants of reported human *Campylobacter* infections in Scotland. *BMC Public Health* 2010;**10**:423.
19. Black RE, Levine MM, Clements ML, et al. Experimental *Campylobacter jejuni* infection in humans. *J Infect Dis* 1988;**157**:472–9.
20. Blaser MJ. Epidemiologic and clinical features of *Campylobacter jejuni* infections. *J Infect Dis* 1997;**176**(Suppl. 2):S103–105.
21. Blaser MJ, Parsons RB, Wang WL. Acute colitis caused by *Campylobacter fetus* ss. *jejuni*. *Gastroenterology* 1980;**78**:448–53.
22. Blaser MJ, Taylor DN, Echeverria P. Immune response to *Campylobacter jejuni* in a rural community in Thailand. *J Infect Dis* 1986;**153**:249–54.
23. Blaser MJ, Berkowitz ID, LaForce FM, et al. *Campylobacter* enteritis: clinical and epidemiologic features. *Ann Intern Med* 1979;**91**:179–85.
24. Blaser MJ, Duncan DJ, Osterholm MT, et al. Serologic study of two clusters of infection due to *Campylobacter jejuni*. *J Infect Dis* 1983;**147**:820–3.
25. Bokkenheuser V. *Vibrio fetus* infection in man. I. Ten new cases and some epidemiologic observations. *Am J Epidemiol* 1970;**91**:400–9.
26. Bokkenheuser VD, Richardson NJ, Bryner JH, et al. Detection of enteric campylobacteriosis in children. *J Clin Microbiol* 1979;**9**:227–32.
27. Broczyk A, Thompson S, Smith D, et al. Water-borne outbreak of *Campylobacter laridis*-associated gastroenteritis. *Lancet* 1987;**1**:164–5.
28. Broman T, Bergstrom S, On SL, et al. Isolation and characterization of *Campylobacter jejuni* subsp. *jejuni* from macaroni penguins *(Eudyptes chrysolophus)* in the subantarctic region. *Appl Environ Microbiol* 2000;**66**:449–52.
29. Brown WJ, Sautter R. *Campylobacter fetus* septicemia with concurrent salpingitis. *J Clin Microbiol* 1977;**6**:72–5.
30. Buck GE, Kelly MT, Pichanick AM, et al. *Campylobacter jejuni* in newborns: a cause of asymptomatic bloody diarrhea. *Am J Dis Child* 1982;**136**:744.
31. Bullman S, Corcoran D, O'Leary J, et al. *Campylobacter ureolyticus:* an emerging gastrointestinal pathogen? *FEMS Immunol Med Microbiol* 2011;**61**:228–30.
32. Busato A, Hofer D, Lentze T, et al. Prevalence and infection risks of zoonotic enteropathogenic bacteria in Swiss cow-calf farms. *Vet Microbiol* 1999;**69**:251–63.
33. Butzler JP. *Campylobacter*, from obscurity to celebrity. *Clin Microbiol Infect* 2004;**10**:868–76.
34. Butzler JP, Skirrow MB. *Campylobacter* enteritis. *Clin Gastroenterol* 1979;**8**:737–65.
35. Butzler JP, Dekeyser P, Detrain M, et al. Related vibrio in stools. *J Pediatr* 1973;**82**:493–5.
36. Calva JJ, Ruiz-Palacios GM, Lopez-Vidal AB, et al. Cohort study of intestinal infection with campylobacter in Mexican children. *Lancet* 1988;**1**:503–6.
37. Carbone KM, Heinrich MC, Quinn TC. Thrombophlebitis and cellulitis due to *Campylobacter fetus* ssp. *Fetus:* report of four cases and a review of the literature. *Medicine (Baltimore)* 1985;**64**:244–50.
38. Centers for Disease Control and Prevention. *Campylobacter* sepsis associated with "nutritional therapy": California. *MMWR Morb Mortal Wkly Rep* 1981;**30**:294–5.
39. Centers for Disease Control and Prevention. Preliminary FoodNet data on the incidence of infection with pathogens transmitted commonly through food: 10 sates, 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:336–9.
40. Chan FT, Stringel G, Mackenzie AM. Isolation of *Campylobacter jejuni* from an appendix. *J Clin Microbiol* 1983;**18**:422–4.
41. Chang MH, Chen TC. Reduction of *Campylobacter jejuni* in a simulated chicken digestive tract by lactobacilli cultures. *J Food Prot* 2000;**63**:1594–7.
42. Chen ML, Ge Z, Fox JG, et al. Disruption of tight junctions and induction of proinflammatory cytokine responses in colonic epithelial cells by *Campylobacter jejuni*. *Infect Immun* 2006;**74**:6581–9.
43. Cho SH, Shin HH, Choi YH, et al. Enteric bacteria isolated from acute diarrheal patients in the Republic of Korea between the year 2004 and 2006. *J Microbiol* 2008;**46**:325–30.
44. Chowdhury MN, al-Eissa YA. *Campylobacter* gastroenteritis in children in Riyadh, Saudi Arabia. *J Trop Pediatr* 1992;**38**:158–61.
45. Collins HS, Blevins A, Benter E. Protracted bacteremia and meningitis due to *Vibrio fetus*. *Arch Intern Med* 1964;**113**:361–4.
46. Cornelius AJ, Nicol C, Hudson JA. *Campylobacter* spp. in New Zealand raw sheep liver and human campylobacteriosis cases. *Int J Food Microbiol* 2005;**99**:99–105.
47. Cravioto A, Reyes RE, Trujillo F, et al. Risk of diarrhea during the first year of life associated with initial and subsequent colonization by specific enteropathogens. *Am J Epidemiol* 1990;**131**:886–904.
48. Danis K, Di Renzi M, O'Neill W, et al. Risk factors for sporadic infection: an all-Ireland case-control *study Eur Surveill* 2009;**14**:19123.
49. Davies JS, Penfold JB. *Campylobacter* urinary infection. *Lancet* 1979;**1**:1091–2.
50. de Boer P, Wagenaar JA, Achterberg RP, et al. Generation of *Campylobacter jejuni* genetic diversity in vivo. *Mol Microbiol* 2002;**44**:351–9.
51. De Mol P, Bosmans E. *Campylobacter* enteritis in Central Africa. *Lancet* 1978;**1**:604.
52. De Mol P, Brasseur D, Lauwers S. An important enteropathogen in a tropical area. In 20th Interscience Conference on

Antimicrobial Agents and Chemotherapy. American Society of Microbiology. New Orleans, La, September 22-4, 1980.
53. de Otero J, Pigrau C, Buti M, et al. Isolation of *Campylobacter fetus* subspecies *fetus* from a gluteal abscess. *Clin Infect Dis* 1994;**19**: 557–8.
54. de Vries JJ, Arents NL, Manson WL. *Campylobacter* species isolated from extra-oro-intestinal abscesses: a report of four cases and literature review. *Eur J Clin Microbiol Infect Dis* 2008;**27**: 1119–23.
55. Deming MS, Tauxe RV, Blake PA, et al. *Campylobacter* enteritis at a university: transmission from eating chicken and from cats. *Am J Epidemiol* 1987;**126**:526–34.
56. Denno DM, Keene WE, Hutter CM, et al. Tri-county comprehensive assessment of risk factors for sporadic reportable bacterial enteric infection in children. *J Infect Dis* 2009;**199**:467–76.
57. Denton KJ, Clarke T. Role of *Campylobacter jejuni* as a placental pathogen. *J Clin Pathol* 1992;**45**:171–2.
58. Desheng L, Zhixin C, Bolun W. Age distribution of diarrhoeal and healthy children infected with *Campylobacter jejuni*. *J Trop Med Hyg* 1992;**95**:218–20.
59. Dingle KE, Colles FM, Wareing DR, et al. Multilocus sequence typing system for *Campylobacter jejuni*. *J Clin Microbiol* 2001;**39**: 14–23.
60. Doyle MP, Roman DJ. Prevalence and survival of *Campylobacter jejuni* in unpasteurized milk. *Appl Environ Microbiol* 1982;**44**: 1154–8.
61. Dronda F, Garcia-Arata I, Navas E, et al. Meningitis in adults due to *Campylobacter fetus* subspecies *fetus*. *Clin Infect Dis* 1998; **27**:906–7.
62. DuPont HL. Use of quinolones in the treatment of gastrointestinal infections. *Eur J Clin Microbiol Infect Dis* 1991;**10**:325–9.
63. DuPont HL. Therapy for and prevention of traveler's diarrhea. *Clin Infect Dis* 2007;**45**(Suppl. 1):S78–84.
64. Ebright JR, Ryan LM. Acute erosive reactive arthritis associated with *Campylobacter jejuni*-induced colitis. *Am J Med* 1984;**76**: 321–3.
65. Edmonds P, Patton CM, Griffin PM, et al. *Campylobacter hyointestinal*is associated with human gastrointestinal disease in the United States. *J Clin Microbiol* 1987;**25**:685–91.
66. Endtz HP, Ruijs GJ, Zwinderman AH, et al. Comparison of six media, including a semisolid agar, for the isolation of various *Campylobacter* species from stool specimens. *J Clin Microbiol* 1991; **29**:1007–10.
67. Engberg J, Aarestrup FM, Taylor DE, et al. Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli:* resistance mechanisms and trends in human isolates. *Emerg Infect Dis* 2001; **7**:24–34.
68. Evans RG, Dadswell V. Human vibriosis. *Br Med* 1967;**3**:240.
69. Evans TG, Riley D. *Campylobacter laridis* colitis in a human immunodeficiency virus-positive patient treated with a quinolone. *Clin Infect Dis* 1992;**15**:172–3.
70. Farrugia DC, Eykyn SJ, Smyth EG. *Campylobacter fetus* endocarditis: two case reports and review. *Clin Infect Dis* 1994;**18**:443–6.
71. Fennell CL, Rompalo AM, Totten PA, et al. Isolation of "*Campylobacter hyointestinalis*" from a human. *J Clin Microbiol* 1986;**24**: 146–8.
72. Feodoroff FB, Lauhio AR, Sarna SJ, et al. Severe diarrhoea caused by highly ciprofloxacin-susceptible *Campylobacter* isolates. *Clin Microbiol Infect* 2009;**15**:188–92.
73. Fernandez-Cruz A, Munoz P, Mohedano R, et al. *Campylobacter* bacteremia: clinical characteristics, incidence, and outcome over 23 years. *Medicine (Baltimore)* 2010;**89**:319–30.
74. Figueroa G, Galeno H, Troncoso M, et al. Prospective study of *Campylobacter jejuni* infection in Chilean infants evaluated by culture and serology. *J Clin Microbiol* 1989;**27**:1040–4.
75. Figura N, Guglielmetti P, Zanchi A, et al. Two cases of *Campylobacter mucosalis* enteritis in children. *J Clin Microbiol* 1993;**31**: 727–8.
76. Fitzenberger J, Uphoff H, Gawrich S, et al. Urban-rural differences of age- and species-specific campylobacteriosis incidence, Hesse, Germany, July 2005-June 2006. *Euro Surveill* 2010;**15**:19693.
77. Fitzgeorge RB, Baskerville A, Lander KP. Experimental infection of rhesus monkeys with a human strain of *Campylobacter jejuni*. *J Hyg (Lond)* 1981;**86**:343–51.
78. Fontaine EA, Bryant TN, Taylor-Robinson D, et al. A numerical taxonomic study of anaerobic gram-negative bacilli classified as *Bacteroides ureolyticus* isolated from patients with non-gonococcal urethritis. *J Gen Microbiol* 1986;**132**:3137–46.
79. Fouts DE, Mongodin EF, Mandrell RE, et al. Major structural differences and novel potential virulence mechanisms from the genomes of multiple campylobacter species. *PLoS Biol* 2005;**3**:e15.
80. Francioli P, Herzstein J, Grob JP, et al. *Campylobacter fetus* subspecies fetus bacteremia. *Arch Intern Med* 1985;**145**:289–92.
81. Franklin B, Ulmer DD. Human infection with *Vibrio fetus*. *West J Med* 1974;**120**:200–4.
82. Frohli P, Hanselmann R, Koelz HR. [Erythema nodosum in *Campylobacter jejuni* colitis]. *Schweiz Med Wochenschr* 1990;**120**: 946–7.
83. Fry BN, Feng S, Chen YY, et al. The *galE* gene of *Campylobacter jejuni* is involved in lipopolysaccharide synthesis and virulence. *Infect Immun* 2000;**68**:2594–601.
84. Fullerton KE, Ingram LA, Jones TF, et al. Sporadic campylobacter infection in infants: a population-based surveillance case-control study. *Pediatr Infect Dis J* 2007;**26**:19–24.
85. Gallagher P, Chadwick P, Jones DM, et al. Acute pancreatitis associated with campylobacter infection. *Br J Surg* 1981;**68**:383.
86. Gallay A, Bousquet V, Siret V, et al. Risk factors for acquiring sporadic *Campylobacter* infection in France: results from a national case-control study. *J Infect Dis* 2008;**197**:1477–84.
87. Gardner TJ, Fitzgerald C, Xavier C, et al. Outbreak of campylobacteriosis associated with consumption of raw peas. *Clin Infect Dis* 2011;**53**:26–32.
88. Gaynor EC, Cawthraw S, Manning G, et al. The genome-sequenced variant of *Campylobacter jejuni* NCTC 11168 and the original clonal clinical isolate differ markedly in colonization, gene expression, and virulence-associated phenotypes. *J Bacteriol* 2004;**186**:503–17.
89. Georges-Courbot MC, Beraud-Cassel AM, Gouandjika I, et al. Prospective study of enteric *Campylobacter* infections in children from birth to 6 months in the Central African Republic. *J Clin Microbiol* 1987;**25**:836–9.
90. Gibreel A, Taylor DE. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J Antimicrob Chemother* 2006;**58**: 243–55.
91. Gifford DH, Shane SM, Smith RE. Prevalence of *Campylobacter jejuni* in Felidae in Baton Rouge, Louisiana. *Int J Zoonoses* 1985; **12**:67–73.
92. Gillespie I. Population-attributable risk estimates for *Campylobacter* infection, Australia. *Emerg Infect Dis* 2009;**15**:850–1; author reply 851-2.
93. Glass RI, Stoll BJ, Huq MI, et al. Epidemiologic and clinical features of endemic *Campylobacter jejuni* infection in Bangladesh. *J Infect Dis* 1983;**148**:292–6.
94. Goossens H, Giesendorf BA, Vandamme P, et al. Investigation of an outbreak of *Campylobacter upsaliensis* in day care centers in Brussels: analysis of relationships among isolates by phenotypic and genotypic typing methods. *J Infect Dis* 1995;**172**:1298–305.
95. Goossens H, Henocque G, Kremp L, et al. Nosocomial outbreak of *Campylobacter jejuni* meningitis in newborn infants. *Lancet* 1986;**2**:146–9.
96. Goossens H, Vlaes L, De Boeck M, et al. Is "*Campylobacter upsaliensis*" an unrecognised cause of human diarrhoea? *Lancet* 1990; **335**:584–6.
97. Gorkiewicz G, Feierl G, Schober C, et al. Species-specific identification of campylobacters by partial 16S rRNA gene sequencing. *J Clin Microbiol* 2003;**41**:2537–46.
98. Grados O, Bravo N, Black RE, et al. Paediatric campylobacter diarrhoea from household exposure to live chickens in Lima, Peru. *Bull World Health Organ* 1988;**66**:369–74.
99. Gregson NA, Koblar S, Hughes RA. Antibodies to gangliosides in Guillain-Barré syndrome: specificity and relationship to clinical features. *Q J Med* 1993;**86**:111–17.
100. Griffiths PL, Park RW. Campylobacters associated with human diarrhoeal disease. *J Appl Bacteriol* 1990;**69**:281–301.
101. Guandalini S, Cucchiara S, de Ritis G, et al. *Campylobacter* colitis in infants. *J Pediatr* 1983;**102**:72–4.
102. Guerrant RL, Lahita RG, Winn WC Jr, et al. Campylobacteriosis in man: pathogenic mechanisms and review of 91 bloodstream infections. *Am J Med* 1978;**65**:584–92.
103. Guerry P, Ewing CP, Hickey TE, et al. Sialylation of lipooligosaccharide cores affects immunogenicity and serum resistance of *Campylobacter jejuni*. *Infect Immun* 2000;**68**:6656–62.

104. Guidoboni M, Ferreri AJ, Ponzoni M, et al. Infectious agents in mucosa-associated lymphoid tissue-type lymphomas: pathogenic role and therapeutic perspectives. *Clin Lymphoma Myeloma* 2006; **6**:289–300.
105. Gurgan T, Diker KS. Abortion associated with *Campylobacter upsaliensis*. *J Clin Microbiol* 1994;**32**:3093–4.
106. Hald B, Madsen M. Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. *J Clin Microbiol* 1997;**35**:3351–2.
107. Hanna JN, Enbom RM, Murphy DM. *Campylobacter upsaliensis* bacteraemia in an aboriginal child. *Med J Aust* 1994;**160**:655–6.
108. Hannu T, Kauppi M, Tuomala M, et al. Reactive arthritis following an outbreak of *Campylobacter jejuni* infection. *J Rheumatol* 2004;**31**:528–30.
109. Haq JA, Rahman KM. *Campylobacter jejuni* as a cause of acute diarrhoea in children: a study at an urban hospital in Bangladesh. *J Trop Med Hyg* 1991;**94**:50–4.
110. Hartung HP, Pollard JD, Harvey GK, et al. Immunopathogenesis and treatment of the Guillain-Barré syndrome. I. *Muscle Nerve* 1995;**18**:137–53.
111. Hartung HP, Pollard JD, Harvey GK, et al. Immunopathogenesis and treatment of the Guillain-Barré syndrome. II. *Muscle Nerve* 1995;**18**:154–64.
112. Havelaar AH, van Pelt W, Ang CW, et al. Immunity to *Campylobacter*: its role in risk assessment and epidemiology. *Crit Rev Microbiol* 2009;**35**:1–22.
113. Helms M, Vastrup P, Gerner-Smidt P, et al. Short and long term mortality associated with foodborne bacterial gastrointestinal infections: registry based study. *BMJ* 2003;**326**:357.
114. Hendrixson DR, DiRita VJ. Identification of *Campylobacter jejuni* genes involved in commensal colonization of the chick gastrointestinal tract. *Mol Microbiol* 2004;**52**:471–84.
115. Hill SL, Cheney JM, Taton-Allen GF, et al. Prevalence of enteric zoonotic organisms in cats. *J Am Vet Med Assoc* 2000;**216**: 687–92.
116. Hindiyeh M, Jense S, Hohmann S, et al. Rapid detection of *Campylobacter jejuni* in stool specimens by an enzyme immunoassay and surveillance for *Campylobacter upsaliensis* in the greater Salt Lake City area. *J Clin Microbiol* 2000;**38**:3076–9.
117. Hofreuter D, Tsai J, Watson RO, et al. Unique features of a highly pathogenic *Campylobacter jejuni* strain. *Infect Immun* 2006;**74**: 4694–707.
118. Hofstetr A, Dvorakova A, Nikodymova I, et al. [A 3-year follow-up study of the incidence of campylobacteriosis in a pediatric population]. *Cesk Pediatr* 1990;**45**:651–4.
119. Hooper DC. Emerging mechanisms of fluoroquinolone resistance. *Emerg Infect Dis* 2001;**7**:337–41.
120. Hossain MA, Kabir I, Albert MJ, et al. *Campylobacter jejuni* bacteraemia in children with diarrhoea in Bangladesh: report of six cases. *J Diarrhoeal Dis Res* 1992;**10**:101–4.
121. Hudson PJ, Vogt RL, Brondum J, et al. Isolation of *Campylobacter jejuni* from milk during an outbreak of campylobacteriosis. *J Infect Dis* 1984;**150**:789.
122. Hughes RA, Rees JH. Guillain-Barré syndrome. *Curr Opin Neurol* 1994;**7**:386–92.
123. Humphrey CD, Montag DM, Pittman FE. Experimental infection of hamsters with *Campylobacter jejuni*. *J Infect Dis* 1985;**151**: 485–93.
124. Humphrey TJ, Beckett P. *Campylobacter jejuni* in dairy cows and raw milk. *Epidemiol Infect* 1987;**98**:263–9.
125. Humphrey TJ, Henley A, Lanning DG. The colonization of broiler chickens with *Campylobacter jejuni:* some epidemiological investigations. *Epidemiol Infect* 1993;**110**:601–7.
126. Humphrey TJ, Jorgensen F, Frost JA, et al. Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrob Agents Chemother* 2005;**49**:690–8.
127. Hutchinson DN, Bolton FJ, Hinchliffe PM, et al. Evidence of udder excretion of *Campylobacter jejuni* as the cause of milk-borne *Campylobacter* outbreak. *J Hyg (Lond)* 1985;**94**:205–15.
128. Ichikawa H, Sugita K, Fukui T, et al. [Fisher's syndrome following *Campylobacter jejuni* enteritis: a case report and review of the literature]. *Rinsho Shinkeigaku* 1995;**35**:391–5.
129. Ichiyama S, Hirai S, Minami T, et al. *Campylobacter fetus* subspecies *fetus* cellulitis associated with bacteremia in debilitated hosts. *Clin Infect Dis* 1998;**27**:252–5.
130. Inglis GD, Boras VF, Houde A. Enteric campylobacteria and RNA viruses associated with healthy and diarrheic humans in the Chinook health region of southwestern Alberta, Canada. *J Clin Microbiol* 2010;**49**:209–19.
131. Islam Z, Jacobs BC, van Belkum A, et al. Axonal variant of Guillain-Barré syndrome associated with *Campylobacter* infection in Bangladesh. *Neurology* 2010;**74**:581–7.
132. Ivanova K, Marina M, Petrov P, et al. Campylobacteriosis and other bacterial gastrointestinal diseases in Sofia, Bulgaria for the period 1987-2008. *Euro Surveill* 2010;**15**:19474.
133. Jackson JF, Hinton P, Allison F, Jr. Human vibriosis: report of a patient with relapsing febrile illness due to *Vibrio fetus*. *Am J Med* 1960;**28**:986–94.
134. Jacobs BC, van Doorn PA, Schmitz PI, et al. *Campylobacter jejuni* infections and anti-GM1 antibodies in Guillain-Barré syndrome. *Ann Neurol* 1996;**40**:181–7.
135. Janssen R, Krogfelt KA, Cawthraw SA, et al. Host-pathogen interactions in *Campylobacter* infections: the host perspective. *Clin Microbiol Rev* 2008;**21**:505–18.
136. Jelovcan S, Schmid D, Lederer I, et al. Cluster of nosocomial campylobacteriosis, Austria 2006. *J Hosp Infect* 2008;**69**:97–8.
137. Jensen LA, Marlin JW, Dyck DD, et al. Prevalence of multigastrointestinal infections with helminth, protozoan and *Campylobacter* spp. in Guatemalan children. *J Infect Dev Countries* 2009;**3**: 229–34.
138. Jess T, Simonsen J, Nielsen NM, et al. Enteric *Salmonella* or *Campylobacter* infections and the risk of inflammatory bowel disease. *Gut* 2011;**60**:318–24.
139. Jimenez SG, Heine RG, Ward PB, et al. *Campylobacter upsaliensis* gastroenteritis in childhood. *Pediatr Infect Dis J* 1999;**18**:988–92.
140. Johnsen K, Ostensen M, Melbye AC, et al. HLA-B27-negative arthritis related to *Campylobacter jejuni* enteritis in three children and two adults. *Acta Med Scand* 1983;**214**:165–8.
141. Jones DM, Eldridge J, Dale B. Serological response to *Campylobacter jejuni/coli* infection. *J Clin Pathol* 1980;**33**:767–9.
142. Kaldor J, Speed BR. Guillain-Barré syndrome and *Campylobacter jejuni:* a serological study. *Br Med J (Clin Res Ed)* 1984;**288**: 1867–70.
143. Kalkay MN, Ayanian ZS, Lehaf EA, et al. Campylobacter-induced toxic megacolon. *Am J Gastroenterol* 1983;**78**:557–9.
144. Kalra V, Chaudhry R, Dua T, et al. Association of *Campylobacter jejuni* infection with childhood Guillain-Barré syndrome: a case-control study. *J Child Neurol* 2009;**24**:664–8.
145. Kanipes MI, Holder LC, Corcoran AT, et al. A deep-rough mutant of *Campylobacter jejuni* 81-176 is noninvasive for intestinal epithelial cells. *Infect Immun* 2004;**72**:2452–5.
146. Karagiannis I, Sideroglou T, Gkolfinopoulou K, et al. A waterborne *Campylobacter jejuni* outbreak on a Greek island. *Epidemiol Infect* 2010;**138**:1726–34.
147. Karlyshev AV, Ketley JM, Wren BW. The *Campylobacter jejuni* glycome. *FEMS Microbiol Rev* 2005;**29**:377–90.
148. Karmali MA, Fleming PC. *Campylobacter* enteritis. *Can Med Assoc J* 1979;**120**:1525–32.
149. Kendall EJ, Tanner EI. *Campylobacter* enteritis in general practice. *J Hyg (Lond)* 1982;**88**:155–63.
150. Khalafalla FA. *Campylobacter jejuni* in poultry giblets. *Zentralbl Veterinarmed B* 1990;**37**:31–4.
151. Kiehlbauch JA, Brenner DJ, Nicholson MA, et al. *Campylobacter butzleri* sp. nov. isolated from humans and animals with diarrheal illness. *J Clin Microbiol* 1991;**29**:376–85.
152. King E. The laboratory recognition of *Vibrio fetus* and a closely related *Vibrio* isolated from cases of human vibriosis. *Ann N Y Acad Sci* 1962;**98**:700–11.
153. King EO. Human infections with *Vibrio fetus* and a closely related *Vibrio*. *J Infect Dis* 1957;**101**:119–28.
154. Kopecko DJ, Hu L, Zaal KJ. *Campylobacter jejuni:* microtubule-dependent invasion. *Trends Microbiol* 2001;**9**:389–96.
155. Korman TM, Varley CC, Spelman DW. Acute hepatitis associated with *Campylobacter jejuni* bacteraemia. *Eur J Clin Microbiol Infect Dis* 1997;**16**:678–81.
156. Koulla-Shiro S, Loe C, Ekoe T. Prevalence of *Campylobacter* enteritis in children from Yaounde (Cameroon). *Cent Afr J Med* 1995;**41**:91–4.
157. Kuroki S, Saida T, Nukina M, et al. *Campylobacter jejuni* strains from patients with Guillain-Barré syndrome belong mostly to

Penner serogroup 19 and contain beta-N-acetylglucosamine residues. *Ann Neurol* 1993;**33**:243–7.
158. Kuschner RA, Trofa AF, Thomas RJ, et al. Use of azithromycin for the treatment of *Campylobacter* enteritis in travelers to Thailand, an area where ciprofloxacin resistance is prevalent. *Clin Infect Dis* 1995;**21**:536–41.
159. Kutner LJ, Arnold WD. Septic arthritis due to *Vibrio fetus:* report of a case. *J Bone Joint Surg Am* 1970;**52**:161–4.
160. Lastovica AJ. Clinical relevance of *Campylobacter concisus* isolated from pediatric patients. *J Clin Microbiol* 2009;**47**:2360.
161. Lawson AJ, Logan JM, O'Neill GL, et al. Large-scale survey of *Campylobacter* species in human gastroenteritis by PCR and PCR-enzyme-linked immunosorbent assay. *J Clin Microbiol* 1999; **37**:3860–4.
162. Lawson AJ, Shafi MS, Pathak K, et al. Detection of campylobacter in gastroenteritis: comparison of direct PCR assay of faecal samples with selective culture. *Epidemiol Infect* 1998;**121**:547–53.
163. Lee MM, Welliver RC, La Scolea LJ, Jr. *Campylobacter* meningitis in childhood. *Pediatr Infect Dis* 1985;**4**:544–7.
164. Leyes M, Vara F, Reina J, et al. [*Campylobacter* gastroenteritis in patients with human immunodeficiency virus infection]. *Enferm Infecc Microbiol Clin* 1994;**12**:332–6.
165. Lillehaug A, Monceyron Jonassen C, Bergsjo B, et al. Screening of feral pigeon *(Colomba livia)*, mallard *(Anas platyrhynchos)* and graylag goose *(Anser anser)* populations for *Campylobacter* spp., *Salmonella* spp., avian influenza virus and avian paramyxovirus. *Acta Vet Scand* 2005;**46**:193–202.
166. Ljungh A, Wadstrom T. Lactic acid bacteria as probiotics. *Curr Issues Intest Microbiol* 2006;**7**:73–89.
167. M'Ikanatha NM, Dettinger LA, Perry A, et al. Culturing stool specimens for *Campylobacter* spp., Pennsylvania, USA. *Emerg Infect Dis* 2012;**18**:484–7.
168. Maki M, Maki R, Vesikari T. Faecal leucocytes in *Campylobacter*-associated diarrhoea in infants. *Acta Paediatr Scand* 1979;**68**: 271–2.
169. Man SM. The clinical importance of emerging *Campylobacter* species. *Nat Rev Gastroenterol Hepatol* 2011;**8**:669–85.
170. Manfredi R, Nanetti A, Ferri M, et al. Fatal *Campylobacter jejuni* bacteraemia in patients with AIDS. *J Med Microbiol* 1999;**48**: 601–3.
171. Martin PM, Mathiot J, Ipero J, et al. Immune response to *Campylobacter jejuni* and *Campylobacter coli* in a cohort of children from birth to 2 years of age. *Infect Immun* 1989;**57**:2542–6.
172. Martinez RM, Figueras MP, Ramos C, et al. [*Campylobacter jejuni* and HIV infection]. *Enferm Infecc Microbiol Clin* 1994;**12**:90–4.
173. McCarthy N, Giesecke J. Incidence of Guillain-Barré syndrome following infection with *Campylobacter jejuni*. *Am J Epidemiol* 2001;**153**:610–14.
174. McFadyean F, Stockman S. *Report to the Departmental Committee Appointed by the Board of Agriculture and Fisheries to Inquire into Epizootic Abortion. III. Abortion in sheep*. In London: His Majesty's Stationary Office; 1913.
175. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis* 1999;**5**:607–25.
176. Melamed I, Bujanover Y, Igra YS, et al. *Campylobacter* enteritis in normal and immunodeficient children. *Am J Dis Child* 1983;**137**: 752–3.
177. Meng CY, Smith BL, Bodhidatta L, et al. Etiology of diarrhea in young children and patterns of antibiotic resistance in Cambodia. *Pediatr Infect Dis J* 2011;**30**:331–5.
178. Mentzing LO. Waterborne outbreaks of *Campylobacter* enteritis in central Sweden. *Lancet* 1981;**2**:352–4.
179. Mishu B, Blaser MJ. Role of infection due to *Campylobacter jejuni* in the initiation of Guillain-Barré syndrome. *Clin Infect Dis* 1993; **17**:104–8.
180. Morishita TY, Aye PP, Harr BS, et al. Evaluation of an avian-specific probiotic to reduce the colonization and shedding of *Campylobacter jejuni* in broilers. *Avian Dis* 1997;**41**:850–5.
181. Morooka T, Oda T, Shigeoka H. In vitro evaluation of antibiotics for treatment of meningitis caused by *Campylobacter fetus* subspecies fetus. *Pediatr Infect Dis J* 1989;**8**:653–4.
182. Morooka T, Umeda A, Amako K. Motility as an intestinal colonization factor for *Campylobacter jejuni*. *J Gen Microbiol* 1985;**131**: 1973–80.
183. Morris CN, Scully B, Garvey GJ. *Campylobacter lari* associated with permanent pacemaker infection and bacteremia. *Clin Infect Dis* 1998;**27**:220–1.
184. Morrison VA, Lloyd BK, Chia JK, et al. Cardiovascular and bacteremic manifestations of *Campylobacter fetus* infection: case report and review. *Rev Infect Dis* 1990;**12**:387–92.
185. Morton WR, Bronsdon M, Mickelsen G, et al. Identification of *Campylobacter jejuni* in *Macaca fascicularis* imported from Indonesia. *Lab Anim Sci* 1983;**33**:187–8.
186. Moscuna M, Gross Z, Korenblum R, et al. Septic abortion due to *Campylobacter jejuni*. *Eur J Clin Microbiol Infect Dis* 1989;**8**: 800–1.
187. Moyen EN, Bonneville F, Fauchere JL. [Modification of intestinal colonization and translocation of *Campylobacter jejuni* by erythromycin and an extract of *Lactobacillus acidophilus* in axenic mice]. *Ann Inst Pasteur Microbiol* 1986;**137A**:199–207.
188. Mshana SE, Joloba M, Kakooza A, et al. *Campylobacter* spp among children with acute diarrhea attending Mulago hospital in Kampala: Uganda. *Afr Health Sci* 2009;**9**:201–5.
189. Nachamkin I. *Campylobacter* and *Arcobacter*. In: Murray PR, Baron E, Pfaller MA, editors. *Manual of clinical microbiology*. 6th ed. Washington, D.C.: ASM Press; 1995. p. 483–91.
190. Nachamkin I, Allos BM, Ho T. *Campylobacter* species and Guillain-Barré syndrome. *Clin Microbiol Rev* 1998;**11**:555–67.
191. Nachamkin I, Stowell C, Skalina D, et al. *Campylobacter laridis* causing bacteremia in an immunosuppressed patient. *Ann Intern Med* 1984;**101**:55–7.
192. Nath G, Shukla BN, Reddy DC, et al. A community study on the aetiology of childhood diarrhoea with special reference to *Campylobacter jejuni* in a semiurban slum of Varanasi, India. *J Diarrhoeal Dis Res* 1993;**11**:165–8.
193. Nelson JM, Chiller TM, Powers JH, et al. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clin Infect Dis* 2007;**44**:977–80.
194. Nelson JM, Smith KE, Vugia DJ, et al. Prolonged diarrhea due to ciprofloxacin-resistant *Campylobacter* infection. *J Infect Dis* 2004;**190**:1150–7.
195. Nielsen H, Steffensen R, Ejlertsen T. Risk and prognosis of campylobacteriosis in relation to polymorphisms of host inflammatory cytokine genes. *Scand J Immunol* 2012;**75**:449–54.
196. Nielsen LN, Luijkx TA, Vegge CS, et al. Identification of immunogenic and virulence-associated *Campylobacter jejuni* proteins. *Clin Vaccine Immunol* 2011;**19**:113–19.
197. Obiri-Danso K, Jones K. Distribution and seasonality of microbial indicators and thermophilic campylobacters in two freshwater bathing sites on the River Lune in northwest England. *J Appl Microbiol* 1999;**87**:822–32.
198. On SL. Identification methods for campylobacters, helicobacters, and related organisms. *Clin Microbiol Rev* 1996;**9**:405–22.
199. Orlicek SL, Welch DF, Kuhls TL. Septicemia and meningitis caused by *Helicobacter cinaedi* in a neonate. *J Clin Microbiol* 1993; **31**:569–71.
200. Oyofo BA, Soderquist R, Lesmana M, et al. Norwalk-like virus and bacterial pathogens associated with cases of gastroenteritis onboard a US Navy ship. *Am J Trop Med Hyg* 1999;**61**:904–8.
201. Pacanowski J, Lalande V, Lacombe K, et al. *Campylobacter* bacteremia: clinical features and factors associated with fatal outcome. *Clin Infect Dis* 2008;**47**:790–6.
202. Pacha RE, Clark GW, Williams EA, et al. Migratory birds of central Washington as reservoirs of *Campylobacter jejuni*. *Can J Microbiol* 1988;**34**:80–2.
203. Pai CH, Sorger S, Lackman L, et al. *Campylobacter* gastroenteritis in children. *J Pediatr* 1979;**94**:589–91.
204. Paisley JW, Mirrett S, Lauer BA, et al. Dark-field microscopy of human feces for presumptive diagnosis of *Campylobacter fetus* subsp. *jejuni* enteritis. *J Clin Microbiol* 1982;**15**:61–3.
205. Palmgren H, Sellin M, Bergstrom S, et al. Enteropathogenic bacteria in migrating birds arriving in Sweden. *Scand J Infect Dis* 1997;**29**:565–8.
206. Park CH, Hixon DL, Polhemus AS, et al. A rapid diagnosis of *Campylobacter* enteritis by direct smear examination. *Am J Clin Pathol* 1983;**80**:388–90.
207. Parker CT, Quinones B, Miller WG, et al. Comparative genomic analysis of *Campylobacter jejuni* strains reveals diversity due to

genomic elements similar to those present in *C. jejuni* strain RM1221. *J Clin Microbiol* 2006;**44**:4125–35.
208. Parkhill J, Wren BW, Mungall K, et al. The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* 2000;**403**:665–8.
209. Pasternak J, Bolivar R, Hopfer RL, et al. Bacteremia caused by *Campylobacter*-like organisms in two male homosexuals. *Ann Intern Med* 1984;**101**:339–41.
210. Patton CM, Shaffer N, Edmonds P, et al. Human disease associated with "*Campylobacter upsaliensis*" (catalase-negative or weakly positive *Campylobacter* species) in the United States. *J Clin Microbiol* 1989;**27**:66–73.
211. Pearson AD, Healing TD. The surveillance and control of campylobacter infection. *Commun Dis Rep CDR Rev* 1992;**2**:R133–9.
212. Penner JL. The genus *Campylobacter:* a decade of progress. *Clin Microbiol Rev* 1988;**1**:157–72.
213. Pepersack F, Prigogyne T, Butzler JP, et al. *Campylobacter jejuni* post-transfusional septicaemia. *Lancet* 1979;**2**:911.
214. Perera VN, Nachamkin I, Ung H, et al. Molecular mimicry in *Campylobacter jejuni:* role of the lipo-oligosaccharide core oligosaccharide in inducing anti-ganglioside antibodies. *FEMS Immunol Med Microbiol* 2007;**50**:27–36.
215. Perkins DJ, Newstead GL. *Campylobacter jejuni* enterocolitis causing peritonitis, ileitis and intestinal obstruction. *Aust N Z J Surg* 1994;**64**:55–8.
216. Perlman DM, Ampel NM, Schifman RB, et al. Persistent *Campylobacter jejuni* infections in patients infected with the human immunodeficiency virus (HIV). *Ann Intern Med* 1988;**108**:540–6.
217. Peterson MC. Rheumatic manifestations of *Campylobacter jejuni* and *C. fetus* infections in adults. *Scand J Rheumatol* 1994;**23**:167–70.
218. Peterson MC, Farr RW, Castiglia M. Prosthetic hip infection and bacteremia due to *Campylobacter jejuni* in a patient with AIDS. *Clin Infect Dis* 1993;**16**:439–40.
219. Pickering LK. Antimicrobial resistance among enteric pathogens. *Adv Exp Med Biol* 2008;**609**:154–63.
220. Pigrau C, Bartolome R, Almirante B, et al. Bacteremia due to *Campylobacter* species: clinical findings and antimicrobial susceptibility patterns. *Clin Infect Dis* 1997;**25**:1414–20.
221. Ponka A, Martio J, Kosunen TU. Reiter's syndrome in association with enteritis due to *Campylobacter fetus* ssp. *jejuni*. *Ann Rheum Dis* 1981;**40**:414–15.
222. Pope JE, Krizova A, Garg AX, et al. *Campylobacter* reactive arthritis: a systematic review. *Semin Arthritis Rheum* 2007;**37**:48–55.
223. Prendergast MM, Tribble DR, Baqar S, et al. In vivo phase variation and serologic response to lipooligosaccharide of *Campylobacter jejuni* in experimental human infection. *Infect Immun* 2004;**72**:916–22.
224. Prescott JF, Barker IK, Manninen KI, et al. *Campylobacter jejuni* colitis in gnotobiotic dogs. *Can J Comp Med* 1981;**45**:377–83.
225. Ramiro Cruz J, Cano F, Bartlett AV, et al. Infection, diarrhea, and dysentery caused by *Shigella* species and *Campylobacter jejuni* among Guatemalan rural children. *Pediatr Infect Dis J* 1994;**13**:216–23.
226. Rams TE, Feik D, Slots J. *Campylobacter rectus* in human periodontitis. *Oral Microbiol Immunol* 1993;**8**:230–5.
227. Reed RP, Friedland IR, Wegerhoff FO, et al. *Campylobacter* bacteremia in children. *Pediatr Infect Dis J* 1996;**15**:345–8.
228. Rees JH, Soudain SE, Gregson NA, et al. *Campylobacter jejuni* infection and Guillain-Barré syndrome. *N Engl J Med* 1995;**333**:1374–9.
229. Rettig PJ. *Campylobacter* infections in human beings. *J Pediatr* 1979;**94**:855–64.
230. Rhodes KM, Tattersfield AE. Guillain-Barré syndrome associated with *Campylobacter* infection. *Br Med J (Clin Res Ed)* 1982;**285**:173–4.
231. Righter J, Woods JM. *Campylobacter* and endovascular lesions. *Can J Surg* 1985;**28**:451–2.
232. Righter J, Wells WA, Hart GD, et al. Relapsing septicemia caused by *Campylobacter fetus* subsp. *fetus*. *Can Med Assoc J* 1983;**128**:686–9.
233. Riley LW, Finch MJ. Results of the first year of national surveillance of *Campylobacter* infections in the United States. *J Infect Dis* 1985;**151**:956–9.
234. Rozynek E, Dzierzanowska-Fangrat K, Szczepanska B, et al. Trends in antimicrobial susceptibility of *Campylobacter* isolates in Poland (2000-2007). *Pol J Microbiol* 2009;**58**:111–15.
235. Ruiz-Palacios GM, Calva JJ, Pickering LK, et al. Protection of breast-fed infants against *Campylobacter* diarrhea by antibodies in human milk. *J Pediatr* 1990;**116**:707–13.
236. Samie A, Guerrant RL, Barrett L, et al. Prevalence of intestinal parasitic and bacterial pathogens in diarrhoeal and non-diarroeal human stools from Vhembe district, South Africa. *J Health Popul Nutr* 2009;**27**:739–45.
237. Sanchez R, Fernandez-Baca V, Diaz MD, et al. Evolution of susceptibilities of *Campylobacter* spp. to quinolones and macrolides. *Antimicrob Agents Chemother* 1994;**38**:1879–82.
238. Sanstedt K, Ursing J, Walder M. Thermotolerant *Campylobacter* with no or weak catalase activity isolated from dogs. *Curr Microbiol* 1983;**8**:209.
239. Saps M, Pensabene L, Di Martino L, et al. Post-infectious functional gastrointestinal disorders in children. *J Pediatr* 2008;**152**:812–16, 816 e811.
240. Sazie ES, Titus AE. Rapid diagnosis of *Campylobacter* enteritis. *Ann Intern Med* 1982;**96**:62–3.
241. Schaad UB. Reactive arthritis associated with *Campylobacter* enteritis. *Pediatr Infect Dis* 1982;**1**:328–32.
242. Schmidt-Ott R, Brass F, Scholz C, et al. Improved serodiagnosis of *Campylobacter jejuni* infections using recombinant antigens. *J Med Microbiol* 2005;**54**:761–7.
243. Schneider A, Runzi M, Peitgen K, et al. *Campylobacter jejuni*-induced severe colitis: a rare cause of toxic megacolon. *Z Gastroenterol* 2000;**38**:307–9.
244. Schneider EN, Havens JM, Scott MA, et al. Molecular diagnosis of *Campylobacter jejuni* infection in cases of focal active colitis. *Am J Surg Pathol* 2006;**30**:782–5.
245. Schonberg-Norio D, Sarna S, Hanninen ML, et al. Strain and host characteristics of *Campylobacter jejuni* infections in Finland. *Clin Microbiol Infect* 2006;**12**:754–60.
246. Schonberg-Norio D, Takkinen J, Hanninen ML, et al. Swimming and *Campylobacter* infections. *Emerg Infect Dis* 2004;**10**:1474–7.
247. Schumer W, Wong TP, Wallace S, et al. Standard linear plans in single channel high dose rate brachytherapy: a dosimetric analysis. *Med Dosim* 1998;**23**:307–10.
248. Shanker S, Rosenfield JA, Davey GR, et al. *Campylobacter jejuni:* incidence in processed broilers and biotype distribution in human and broiler isolates. *Appl Environ Microbiol* 1982;**43**:1219–20.
249. Silva J, Leite D, Fernandes M, et al. *Campylobacter* spp. as a foodborne pathogen: a review. *Front Microbiol* 2011;**2**:200.
250. Simbert R. Genus *Campylobacter*. In: Krieg NR, Holt H, editors. *Manual of systematic bacteriology*, vol. 1. Baltimore: Williams & Wilkins; 1984.
251. Simor AE, Ferro S. *Campylobacter jejuni* infection occurring during pregnancy. *Eur J Clin Microbiol Infect Dis* 1990;**9**:142–4.
252. Simor AE, Wilcox L. Enteritis associated with *Campylobacter laridis*. *J Clin Microbiol* 1987;**25**:10–12.
253. Simor AE, Karmali MA, Jadavji T, et al. Abortion and perinatal sepsis associated with *Campylobacter* infection. *Rev Infect Dis* 1986;**8**:397–402.
254. Sjögren E, Kaijser B, Werner M. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* isolated in Sweden: a 10-year follow-up report. *Antimicrob Agents Chemother* 1992;**36**:2847–9.
255. Skirrow MB. *Campylobacter* enteritis: a "new" disease. *Br Med J* 1977;**2**:9–11.
256. Skirrow MB, Jones DM, Sutcliffe E, et al. *Campylobacter* bacteraemia in England and Wales, 1981-91. *Epidemiol Infect* 1993;**110**:567–73.
257. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. Investigation Team. *N Engl J Med* 1999;**340**:1525–32.
258. Smith MV, 2nd, Muldoon PJ. *Campylobacter fetus* subspecies *jejuni (Vibrio fetus)* from commercially processed poultry. *Appl Microbiol* 1974;**27**:995–6.
259. Smith T. Spirilla associated with disease of the fetal membranes in cattle (infectious abortion). *J Exp Med* 1918;**28**:701–19.
260. Son I, Englen MD, Berrang ME, et al. Prevalence of *Arcobacter* and *Campylobacter* on broiler carcasses during processing. *Int J Food Microbiol* 2007;**113**:16–22.

261. Soofi SB, Habib MA, von Seidlein L, et al. A comparison of disease caused by *Shigella* and *Campylobacter* species: 24 months community based surveillance in 4 slums of Karachi, Pakistan. *J Infect Public Health* 2011;**4**:12–21.
262. Soonattrakul W, Andersen BR, Bryner JH. Raw liver as a possible source of *Vibrio fetus* septicemia in man. *Am J Med Sci* 1971;**261**: 245–9.
263. Sota Busselo I, Onate Vergara E, Perez-Yarza EG, et al. [Erythema nodosum: etiological changes in the last two decades]. *An Pediatr (Barc)* 2004;**61**:403–7.
264. Spira WM, Sack RB, Froehlich JL. Simple adult rabbit model for *Vibrio cholerae* and enterotoxigenic *Escherichia coli* diarrhea. *Infect Immun* 1981;**32**:739–47.
265. Stanley KN, Wallace JS, Currie JE, et al. The seasonal variation of thermophilic campylobacters in beef cattle, dairy cattle and calves. *J Appl Microbiol* 1998;**85**:472–80.
266. Stern NJ, Bannov VA, Svetoch EA, et al. Distribution and characterization of *Campylobacter* spp. from Russian poultry. *J Food Prot* 2004;**67**:239–45.
267. Stirling J, Griffith M, Blair I, et al. Prevalence of gastrointestinal bacterial pathogens in a population of zoo animals. *Zoonoses Public Health* 2008;**55**:166–72.
268. Strachan NJ, Watson RO, Novik V, et al. Sexual dimorphism in campylobacteriosis. *Epidemiol Infect* 2008;**136**:1492–5.
269. Svedhem A, Gunnarsson H, Kaijser B. Diffusion-in-gel enzyme-linked immunosorbent assay for routine detection of IgG and IgM antibodies to *Campylobacter jejuni*. *J Infect Dis* 1983;**148**: 82–92.
270. Tam CC, O'Brien SJ, Petersen I, et al. Guillain-Barré syndrome and preceding infection with campylobacter, influenza and Epstein-Barr virus in the general practice research database. *PLoS One* 2007;**2**:e344.
271. Tarantino M, Dionisi AM, Pistoia C, et al. Involvement of nitric oxide in the control of a mouse model of *Campylobacter jejuni* infection. *FEMS Immunol Med Microbiol* 2009;**56**:98–101.
272. Targan SR, Chow AW, Guze LB. Spontaneous peritonitis of cirrhosis due to *Campylobacter fetus*. *Gastroenterology* 1976;**71**: 311–13.
273. Tauxe RV. *Campylobacter jejuni:* current strategy and future trends. In Nachamkin I, Blaser MJ, Tompkins LS, editors. *Epidemiology of Campylobacter jejuni infections in the United States and other industrialized countries*. Washington, D.C.: American Society for Microbiology; 1992. p. 9–19.
274. Tauxe RV, Deming MS, Blake PA. *Campylobacter jejuni* infections on college campuses: a national survey. *Am J Public Health* 1985; **75**:659–60.
275. Tauxe RV, Hargrett-Bean N, Patton CM, et al. *Campylobacter* isolates in the United States, 1982-1986. *MMWR CDC Surveill Summ* 1988;**37**:1–13.
276. Tauxe RV, Patton CM, Edmonds P, et al. Illness associated with *Campylobacter laridis*, a newly recognized *Campylobacter* species. *J Clin Microbiol* 1985;**21**:222–5.
277. Taylor DN, Echeverria P, Pitarangsi C, et al. Influence of strain characteristics and immunity on the epidemiology of *Campylobacter* infections in Thailand. *J Clin Microbiol* 1988;**26**:863–8.
278. Taylor DN, McDermott KT, Little JR, et al. *Campylobacter* enteritis from untreated water in the Rocky Mountains. *Ann Intern Med* 1983;**99**:38–40.
279. Taylor DN, Perlman DM, Echeverria PD, et al. *Campylobacter* immunity and quantitative excretion rates in Thai children. *J Infect Dis* 1993;**168**:754–8.
280. Tee W, Luppino M, Rambaldo S. Bacteremia due to *Campylobacter sputorum* Biovar sputorum. *Clin Infect Dis* 1998;**27**:1544–5.
281. Tenover FC, Carlson L, Barbagallo S, et al. DNA probe culture confirmation assay for identification of thermophilic *Campylobacter* species. *J Clin Microbiol* 1990;**28**:1284–7.
282. Ternhag A, Asikainen T, Giesecke J, et al. A meta-analysis on the effects of antibiotic treatment on duration of symptoms caused by infection with *Campylobacter* species. *Clin Infect Dis* 2007;**44**:696–700.
283. Teunis PF, Nagelkerke NJ, Haas CN. Dose response models for infectious gastroenteritis. *Risk Anal* 1999;**19**:1251–60.
284. Thomas K, Chan KN, Ribeiro CD. *Campylobacter jejuni/coli* meningitis in a neonate. *Br Med J* 1980;**280**:1301–2.
285. Tokumaru M, Konuma H, Umesako M, et al. Rates of detection of *Salmonella* and *Campylobacter* in meats in response to the sample size and the infection level of each species. *Int J Food Microbiol* 1991;**13**:41–6.
286. Tolcin R, LaSalvia MM, Kirkley BA, et al. Evaluation of the Alexon-trend ProSpecT *Campylobacter* microplate assay. *J Clin Microbiol* 2000;**38**:3853–5.
287. Torphy DE, Bond WW. *Campylobacter fetus* infections in children. *Pediatrics* 1979;**64**:898–903.
288. Torres O, Cruz JR. Protection against *Campylobacter* diarrhea: role of milk IgA antibodies against bacterial surface antigens. *Acta Paediatr* 1993;**82**:835–8.
289. Totten PA, Fennell CL, Tenover FC, et al. *Campylobacter cinaedi* (sp. nov.) and *Campylobacter fennelliae* (sp. nov.): two new *Campylobacter* species associated with enteric disease in homosexual men. *J Infect Dis* 1985;**151**:131–9.
290. Townes JM, Deodhar AA, Laine ES, et al. Reactive arthritis following culture-confirmed infections with bacterial enteric pathogens in Minnesota and Oregon: a population-based study. *Ann Rheum Dis* 2008;**67**:1689–96.
291. Tremblay C, Gaudreau C, Lorange M. Epidemiology and antimicrobial susceptibilities of 111 *Campylobacter fetus* subsp. *fetus* strains isolated in Quebec, Canada, from 1983 to 2000. *J Clin Microbiol* 2003;**41**:463–6.
292. U.S. Department of Agriculture. Campylobacter *Infections*. Washington, DC: U.S. Department of Agriculture; 2005.
293. U.S. Department of Agriculture. *Foodborne illness and Diseases*. Washington, DC: U.S. Department of Agriculture; 2007.
294. Vandamme P, De Ley J. Proposal for a new family, Campylobacteraceae. *Int J Syst Bacteriol* 1991;**41**:451–5.
295. Vandamme P, Falsen E, Pot B, et al. Identification of EF group 22 campylobacters from gastroenteritis cases as *Campylobacter concisus*. *J Clin Microbiol* 1989;**27**:1775–81.
296. Vanhoof R, Gordts B, Dierickx R, et al. Bacteriostatic and bactericidal activities of 24 antimicrobial agents against *Campylobacter fetus* subsp. *jejuni*. *Antimicrob Agents Chemother* 1980;**18**:118–21.
297. Vanniasinkam T, Lanser JA, Barton MD. PCR for the detection of *Campylobacter* spp. in clinical specimens. *Lett Appl Microbiol* 1999;**28**:52–6.
298. Vernon M, Chatelain R. Taxonomic study of the genus *Campylobacter* and designation of the neotype strain for the type species, *Campylobacter fetus*. *Int J Syst Bacteriol* 1973;**23**:122–34.
299. Vesikari T, Huttunen L, Maki R. Perinatal *Campylobacter fetus* ss jejuni enteritis. *Acta Paediatr Scand* 1981;**70**:261–3.
300. Vinzent R, Dumas J, Picard N. Septicemia grave au cours de la grossesse due a vibrion: Avortement consecutif. *Bull Acad Natl Med* 1947;**131**:122–34.
301. Waltner-Toews D, Martin SW, Meek AH. An epidemiological study of selected calf pathogens on Holstein dairy farms in southwestern Ontario. *Can J Vet Res* 1986;**50**:307–13.
302. Wang H, Murdoch DR. Detection of *Campylobacter* species in faecal samples by direct Gram stain microscopy. *Pathology* 2004;**36**:343–4.
303. Warner DP, Bryner JH, Beran GW. Epidemiologic study of campylobacteriosis in Iowa cattle and the possible role of unpasteurized milk as a vehicle of infection. *Am J Vet Res* 1986;**47**:254–8.
304. Wassenaar TM, Newell DG. Genotyping of *Campylobacter* spp. *Appl Environ Microbiol* 2000;**66**:1–9.
305. White AN, Kinlin LM, Johnson C, et al. Environmental determinants of campylobacteriosis risk in Philadelphia from 1994 to 2007. *Ecohealth* 2009;**6**:200–8.
306. Willis MD, Austin WJ. Human *Vibrio fetus* infection: report of two dissimilar cases. *Am J Dis Child* 1966;**112**:459–62.
307. Wolfs TF, Duim B, Geelen SP, et al. Neonatal sepsis by *Campylobacter jejuni:* genetically proven transmission from a household puppy. *Clin Infect Dis* 2001;**32**:E97–9.
308. Wong SN, Tam AY, Yuen KY. *Campylobacter* infection in the neonate: case report and review of the literature. *Pediatr Infect Dis J* 1990;**9**:665–9.
309. Wright EP, Seager J. Convulsions associated with *Campylobacter* enteritis. *Br Med J* 1980;**281**:454.
310. Wyatt RA, Younoszai K, Anuras S, et al. *Campylobacter fetus* septicemia and hepatitis in a child with agammaglobulinemia. *J Pediatr* 1977;**91**:441–2.
311. Yang JR, Wu HS, Chiang CS, et al. Pediatric campylobacteriosis in northern Taiwan from 2003 to 2005. *BMC Infect Dis* 2008;**8**:151.
312. Young DM, Biao J, Zheng Z, et al. Isolation of *Campylobacter jejuni* in Hunan, the People's Republic of China: epidemiology and

comparison of Chinese and American methodology. *Diagn Microbiol Infect Dis* 1986;**5**:143–9.
313. Yuki N, Koga M. Bacterial infections in Guillain-Barré and Fisher syndromes. *Curr Opin Neurol* 2006;**19**:451–7.
314. Yuki N, Ichikawa H, Doi A. Fisher syndrome after *Campylobacter jejuni* enteritis: human leukocyte antigen and the bacterial serotype. *J Pediatr* 1995;**126**:55–7.
315. Zilbauer M, Dorrell N, Elmi A, et al. A major role for intestinal epithelial nucleotide oligomerization domain 1 (NOD1) in eliciting host bactericidal immune responses to *Campylobacter jejuni*. *Cell Microbiol* 2007;**9**:2404–16.
316. Zilbauer M, Dorrell N, Wren BW, et al. *Campylobacter jejuni*-mediated disease pathogenesis: an update. *Trans R Soc Trop Med Hyg* 2008;**102**:123–9.

poisoning." Nearly a century later, Vail[148] and Wherry and Lamb[155] independently reported the first etiologically proved case of tularemia in humans in 1914. Thereafter, knowledge of the organism, susceptible hosts, modes of transmission, and clinical manifestations of disease was acquired rapidly, and retrospective information was assessed in light of this new information. Much of the current understanding of the disease in humans originated from the work of Edward Francis,[51,52] a U.S. Public Health Service surgeon. Intrigued by the new diseases, which were called "deer fly fever" by Pearse[114] in 1911 and "plaguelike disease" by McCoy, Francis relocated to Utah in 1919 and established his laboratory in an unused coal shed.[52-55] Soon thereafter, he recognized the singular cause of these two diseases and renamed them "tularemia" because of isolation of *B. tularense* from blood.[122] He isolated the organism from humans and jackrabbits[51] and demonstrated transmission of the organism by the deer fly.[53]

For a more complete historical review, the reader is referred to the classic paper by Dr. Edward Francis,[52] "A Summary of the Present Knowledge of Tularemia." Almost 9 decades later, this article is accurate and contains most of the knowledge essential for understanding tularemia.

ETIOLOGY

The causative agent of tularemia is *F. tularensis*, named in honor of Dr. Edward Francis. *F. tularensis* is a small (0.2 to 1.0 × 1 to 3 μm), nonmotile, non–spore-forming, pleomorphic gram-negative coccobacillus. It is a strict anaerobe that infects as a facultative intracellular bacterium.[73]

Hesselbrock and Foshay[66] described the morphology as resembling that of the pleuropneumonia group of organisms; the usual coccoid form is a spheroidal cystic structure with a delicate, transparent cell wall. Electron-microscopic studies confirmed the presence of a delicate, almost transparent cell wall, as well as the presence of pili on the surface of the organism.

The outstanding growth characteristic of these fastidious organisms is their requirement for cysteine or sulfhydryl compounds in amounts exceeding those usually present in nutrient media.[44] Although *F. tularensis* grows best on cysteine-glucose-blood agar and on coagulated egg yolk medium and less well in thioglycolate broth, it can be isolated in routine cultures and on enriched chocolate agar.[90,105] Hornick[70] suggested that the addition of cycloheximide and penicillin facilitates isolation of the organism from the respiratory tract or skin ulcers. In a study by Johansson and colleagues,[79] an Amies agar medium with charcoal and a Thayer-Martin medium with antibiotics were equal in preserving bacterial viability for 1 week in contrast to saline or Stuart medium.

F. tularensis is killed readily by heat. Exposure to a temperature of 56°C (132.8°F) for 10 minutes is sufficient for killing. The organisms are not destroyed by freezing and may remain viable in frozen animal carcasses for as long as 3 years. However, adequate cooking renders the meat of game birds and animals harmless. Treatment with tricresol solution (1%) for 2 minutes also kills organisms in tissue; organisms from cultures are killed in 24 hours by 0.1 percent formalin.

Despite serologic homogeneity, four distinct subspecies of tularemia have been identified thus far. *F. tularensis* subsp. *tularensis* (type A) accounts for approximately 90 percent of organisms isolated in North America and has been seen in Europe as well.[61] The less virulent strain *F. tularensis* subsp. *holarctica* (type B) is found primarily in Europe and Asia. These two strains have been found to coexist in the same ecosystem.[98] *F. tularensis* subsp. *mediasiatica* has been isolated primarily in central Asian republics of the former Soviet Union,[112] whereas the subsp. *novicida*, which is isolated rarely, can be found worldwide. Through the use of molecular typing techniques, type A isolates can be divided into two distinct clades, A1 and A2.[133] These clades have been further subdivided (A1a, A1b, A2a, and A2b) based on data from pulsed-field gel electrophoresis and global single-nucleotide polymorphism analysis.[86,89,113] No differences have been noted between infections caused by A2a and A2b; therefore, only three type A genotypes (A1a, A1b, and A2) appear to be clinically important. A1 isolates are mainly found in the eastern United States, and A2 strains occur in the western United States. Infections with A1b have significantly higher mortality rates than do infections from A1a, A2, or B.[89] *F. tularensis* is able to survive and replicate within macrophages in part because of two proteins (VgrG and Ig1I) secreted by the *Francisella* pathogenicity island (FPI).[8] This ability to survive and replicate within the macrophage has been correlated with the organism's high level of virulence. In addition, the organism has been demonstrated to be able to invade human erythrocytes, which may serve to shield the pathogen from antimicrobials.[71] *F. tularensis* does not produce any exotoxin, but pharmacologic tests indicate that the organism contains a lipopolysaccharide (LPS) similar to that of gram-negative bacilli. Unlike LPS of other gram-negative enteric organisms, the LPS of *F. tularensis* is 1000-fold less potent and does not activate macrophages or endothelial cells to release proinflammatory cytokines through Toll-like receptors.[3,123,142,152] The O-antigens of the major subspecies *F. tularensis* that are pathogenic to humans are identical in their carbohydrate content and structure, a finding implying that the differences in virulence among subspecies probably is not the result of the O-antigen.[121,144,151,153]

An electron-dense surface material resembling a capsule has been demonstrated with electron microscopy. This capsule-like complex is not always visible, suggesting it may be upregulated under specific conditions.[7,20]

EPIDEMIOLOGY

Tularemia has been reported throughout the United States, in East Asia, and in Europe.[51,69,99,108-110,137] *F. tularensis* has been found in Canada and Mexico but has not been reported in South America or Africa. Within the United States, the disease most commonly occurs in the south central region. From 1990 to 2000, three states

(i.e., Arkansas, Missouri, and Oklahoma) accounted for approximately half of all reported cases.[17]

In the United States, the incidence of tularemia has been recorded since 1927. From 1990 to 2004, the incidence of tularemia was 0 to 0.06 cases/100,000 persons.[17,19] Recent data from a highly endemic region found an average annual incidence of 0.4 cases/100,000.[18] The number of human cases declined steadily from 1939 (2291 cases[76]) until 1975 (129 cases). Since then, the number of reported cases of tularemia has remained consistent, with a range between 90 and 310 cases annually.[19] This range probably is a gross underestimate of the actual incidence. In 1968, in a serologic survey of 1936 subjects in California, approximately 1 percent of this population had antibody against *F. tularensis*.[42] Using skin test antigens, Casper and Phillip[16] showed that 6.6 percent of 365 persons in eastern Montana had evidence of previous infection with *F. tularensis*. Of the persons in whom skin test results were positive, 80 percent had no previous history of the disease, a finding indicating a high number of subclinical or self-limited infections. The mortality rate for tularemia is 0 to 4 cases per year.[19]

Persons especially at risk are hunters, trappers, meat processors, muskrat farmers,[157] cooks, lawn mowers, and brush cutters,[47] sheep herders and shearers,[77] and laboratory workers.[128,149] The incidence of tularemia among American Indians/Native Alaskans (0.5/100,000) is significantly higher than that in whites (0.04) and African Americans (<.01). In all age groups, boys and men have a higher incidence of tularemia than girls and women.[17] During 1990 to 2000, the average annual incidence of tularemia ranged from 0.06 to 0.1/100,000 in boys (0.03 to 0.06 in girls) 0 to 14 years of age, and the highest incidence occurred in boys aged 5 to 9 years (0.1 to 0.06 for girls). In comparison, adults older than 75 years of age have an incidence of tularemia higher than that of children.[17] Children acquire tularemia by the same routes as adults—vector bites, animal bites, and ingestion of infected, inadequately cooked meat.

Tularemia occurs year-round. However, peaks occur in the summer and winter months, depending on the region. May through August accounted for 70 percent of all cases of tularemia onset during 1990 to 2000.[17] Tularemia occurs more commonly in the central and southern states during the summer months, when ticks are more prevalent. The incidence in the northern and eastern states peaks in the winter months during the hunting season.

The most common sources of human infection are contact with infected animals or their carcasses and bites by ticks or tabanid flies (deer flies). Less commonly, people acquire the disease from the bite of a diseased animal or one in which the mouth has been contaminated by the ingestion of a diseased animal. Numerous outbreaks of human tularemia have occurred by water transmission,[11,21,54,78,83] in water contaminated by voles, beavers, lemmings, and muskrats. Infection also may occur by aerosolization of the organisms, especially in laboratory workers,[84,149] lawn mowers and brush cutters,[47] farm workers (inhalation of dust and threshing material contaminated by voles and other rodents)[34,69] and cleaning infected animals.[65] Person-to-person transmission has not been documented.

Hopla,[69] in describing the ecology of tularemia, states that "rarely does one encounter a zoonotic disease of such complexity." Indeed, to understand the development of epizootics and transmission to humans, one must consider the role of numerous vertebrates (i.e., humans and other animals, both wild and domestic) and many invertebrates.

Approximately 100 species of wild mammals, 9 species of domestic animals, 25 species of birds, and several species of fish and amphibians have been found to be infected naturally,[111] but probably fewer than a dozen species of mammals are important in the transmission of *F. tularensis*.[69] Lagomorphs (rabbits and hares) and some rodents (muskrats, voles) are highly susceptible to *F. tularensis;* sheep and domestic rabbits are susceptible but have low sensitivity to the organism; and cats, cattle, dogs, and horses are virtually insusceptible to infection. Vertebrate animals are called *reservoirs*, but they rarely are true reservoirs because most of them become sick and either die or recover, with elimination of the organism. The varying hare (snowshoe rabbit) is less susceptible to *F. tularensis* and may serve as a reservoir because of its high natural resistance and carrier state.[50] Protozoa, which serve as reservoirs for many other intracellular pathogens, may do the same for *F. tularensis*.[1]

Many invertebrates can be infected experimentally by *F. tularensis*, but relatively few are infected naturally. In 1924, ticks were first described as vectors for *F. tularensis* infection in guinea pigs. However, the role of ticks in the spread of the human disease was not recognized until 1949, when Washburn and Tuohy[154] reported that 56 percent (391/704) of tularemia cases in Arkansas were associated with exposure to ticks. To date, ticks remain the most common vectors for *F. tularensis* in the United States and can serve as reservoirs because the organism can be transmitted from generation to generation by transovarian passage.[69]

At least 13 species of ticks have been found to be infected naturally by *F. tularensis*.[69] The species involved in transmission to humans are *Dermacentor andersoni* (wood tick), *Dermacentor variabilis* (dog tick), and *Amblyomma americanum* (Lone Star tick). A fourth tick, *Haemaphysalis leporispalustris* (rabbit tick), is important in the epidemiology of tularemia. Although this tick does not transmit tularemia directly to humans, it perpetuates the cycle of infection by acting as the sylvatic vector between rabbits.[64]

When the tick feeds on an infected animal, the organisms penetrate the gut into the hemolymph and are disseminated throughout the body, including the salivary glands. The tick then transmits the organisms by injecting them, along with saliva, when it feeds on another animal. In addition, transmission by tick fecal contamination also is likely. Ticks, biting flies (i.e., deer flies), and fleas probably are responsible for the continuing endemic disease in susceptible animals and for epizootic disease. Infrequently, mosquitoes and mites may transport *F. tularensis* mechanically from animal to animal or from animal to human.[69] Humans act as terminal hosts of *F. tularensis*

because they do not transmit it to other humans or to other mammals.

A few cases of tularemia acquired by cat bite have been reported.[5,15,154,158] Because cats rarely are infected by *F. tularensis*, such transmission probably is the result of mechanical transmission from the contaminated teeth or claws of a cat that has come into contact with or has fed on an infected animal. Cat bites may be an important source of disease in children.

Ecogenetic analysis of *F. tularensis* in North America shows that the subspecies have specific geographic distributions across the continent.[46,133] *F. tularensis* subsp. *holartica* was found along the northwestern coast and along the tributaries of the Mississippi River. In contrast, the subspecies *tularensis* spanned the region from the east coast to Arkansas, Oklahoma, Texas, and Louisiana in the south; South Dakota in the north; and the Sierra Nevada in the west. Genetic analysis of the subspecies *tularnsis* in this vast region revealed that type A1 is found primarily in the eastern United States, whereas most type A2 clusters to the West.[86,89,133] This spatial separation of subspecies *tularensis* clades A1 and A2 coincides with the distribution of vectors *A. americanum* and *D. variabilis* in the East and *D. andersoni* and *Chrysops discalis* (deer fly) in the West. The spatial clustering of A1 and A2 also coincides with the distribution of an important host, cottontail rabbits, *Sylvilagus floridanus* and *Sylvilagus nuttallii*, respectively.[46]

PATHOGENESIS

Bacterial and Host Interactions

Much of the knowledge regarding interactions between *F. tularensis* and the host was derived from experimental studies using live attenuated strains. During World War II, live attenuated strains of *F. tularensis* were developed in the Soviet Union in an effort to produce a vaccine.[119,146] In 1956, a mixture of attenuated strains of *F. tularensis* was transferred from the Soviet Union to the United States. From this mixture, a strain of suitable virulence was selected, tested for safety and efficacy, and designated *F. tularensis* live vaccine strain (LVS).[36,38,75] This strain has been used extensively in experimental studies on the development of immunity in humans.

Capsulated *F. tularensis* organisms are thought to be opsonized by human macrophages and dendritic cells through the activation of complement, especially C3, and binding to the complement receptor 3 (CD11b/CD18).[10] The interaction of complement-coated *Francisella* with the macrophage membrane initiates an actin-dependent process then engulfs the bacterium into a unique asymmetric spacious pseudopod loop that forms the phagosome.[24,145] The organism is able to survive and replicate within macrophages in part because of two proteins (VgrG and Ig1I) that are secreted by the FPI.[8] The phagosomes are surrounded by a dense fibrillar coating that begins to disintegrate approximately 3 to 4 hours after infection, a process that allows bacteria free access to the cytoplasm.[23] Approximately 24 hours after infection, replication of cytosolic *Francisella* induces apoptosis that leads to cell death and release of bacteria. Tularemia is followed by effective immunospecific protection of the host; reinfection has been documented.[14] Studies in which the infecting bacteria were suppressed by the use of a bacteriostatic agent such as tetracycline showed that protective immunity seems to be activated approximately 2 weeks after onset of the disease. When tetracycline treatment was initiated on the day of onset of tularemia and was given for 10 days, early relapses were common occurrences. When tetracycline treatment was administered for 14 days, relapses occurred less frequently, thus indicating that protective immunity had begun to arise.[125] Innate immunity involving interferon (IFN)-γ, tumor necrosis factor (TNF) and interleukin (IL)-12 and cells that produce these cytokines (i.e., natural killer cells, neutrophils, and macrophages) are thought to help control the initial phase of primary infection by curtailing the replication of *F. tularensis*.[25] Approximately 2 to 3 weeks after infection, *F. tularensis* elicits both humoral and cell-mediated immune responses. Both responses reach maximal levels during the second week after infection.[22] Because *F. tularensis* is described as a facultative intracellular parasite, the general consensus is that long-term control and clearance ultimately depend on cell-mediated immunity, just as in infections with *Listeria monocytogenes* and *Mycobacterium tuberculosis*.[87,136,159] This finding was confirmed by several trials showing that vaccination with killed tularemia preparations, despite inducing agglutinating serum antibodies, provided poor protection.[14,75,124] Numerous studies showed that passive transfer of mononuclear leukocytes from infected animals to noninfected animals conferred resistance when the nonimmunized animals were challenged with a virulent strain.[129] Ultimately, the presence of tularemia-specific $CD4^+$ and $CD8^+$ T lymphocytes is responsible for clearance of the bacteria.[29,31,40,156] Infection with LVS disrupts the production of proinflammatory cytokines from human macrophages and endothelial cells.[49,141] This event is consistent with the lack of increase in proinflammatory cytokines, with the exception of a slight increase in IFN-γ during the early phase of the infection, in humans with ulceroglandular disease.

The importance of the humoral immune response in tularemia has only recently been considered important.[87] Although the presence of immunoglobulin A (IgA) antibodies in nasal secretions correlates with resistance to infection by aerosolized organisms, earlier work by Bellanti and associates[9] showed that humoral immunity plays a an important role, especially in pulmonary tularemia. Specific antibodies probably are involved initially in delaying the infection, thereby allowing time for the induction of cytokine production and specific T-cell–mediated immunity. This suggestion is consistent with the observation that although neutrophils have only a minor role in resistance to infection, mice depleted of $CD4^+$ T cells or $CD8^+$ T cells, or both, remained capable of controlling and partly resolving a primary sublethal *F. tularensis* infection.[29] Antibodies may function as opsonins for macrophages and neutrophils, but the level of antitularemia antibody does not correlate with protection.[140] Elkins and associates[39] showed that mice deficient in B cells do not succumb to sublethal doses of tularemia

during a primary infection. However, in secondary infection, the presence of cytokines from B cells modulates the response of neutrophils against tularemia-infected hepatocytes and prevents the uncontrolled degranulation of neutrophils that may lead to severe tissue damage and poor clinical outcome.[13,28,33]

The role of neutrophils in the control of *Francisella* infection is thought to be minor but necessary. McCaffrey and Allen[100] showed that neutrophils, like macrophages and dendritic cells, rapidly opsonize *Francisella* but are prevented from mounting the respiratory burst to kill the organism. The organisms then escape to the cytosol of the neutrophils and replicate. However, studies in which neutrophils were depleted from mice showed that mice given a sublethal dose of the LVS or mice chronically infected with LVS became susceptible to infection and died.[129] Although the mechanism of action of neutrophils in these cases is unclear, it may involve direct lysis of LVS-infected hepatocytes.[28]

Persistence of immunity to *F. tularensis* depends on the T-cell response to a variety of antigens; this response may not be noted for as long as 2 weeks after exposure. Poquet and associates[120] showed that virulent strains of *F. tularensis* produce high levels of phosphoantigens that cause a proliferation in γ/δ T cells to a greater extent than does exposure to the LVS. The reduced number of this T-cell subtype in vaccinated individuals could explain the lack of complete protection seen in vaccinated laboratory workers. As with membrane proteins stimulating an α/β T-cell response, heat shock protein chaperones such as DnaK and Cpn60 also create a nonimmunodominant proliferation of T cells in immunized individuals.[43] Denaturation of the protein or carbohydrate of a macromolecular antigen of *F. tularensis* has shown that T-cell reactivity is associated with only protein determinants, whereas human immune serum reacts mostly with carbohydrate determinants of the organism.[2,37,144]

Invasion and Disease Production

A single *F. tularensis* organism of a virulent strain can produce fatal infection in susceptible animals such as mice, guinea pigs, and hamsters.[160] As few as 10 organisms of a virulent strain injected intradermally or 25 organisms given by aerosol may produce systemic disease in human volunteers.[75] The organism may gain access to the human body through the skin, conjunctiva, oropharynx, respiratory tract, or gastrointestinal tract. It spreads by the lymphatics or hematogenously, and bacteremia usually develops during the first week of infection (3 to 12 days).[84] Infection commonly involves the skin, regional lymph nodes, liver, spleen, and lungs. Rarely, the gastrointestinal tract and the central nervous system also are involved.

Histopathologic examination of mammals experimentally infected with *F. tularensis* indicates that the organism disseminates and causes cellular changes in a manner typical of intracellular parasites. After the organism is introduced into a susceptible host, multiplication occurs locally, with early spread to regional lymph nodes occurring within 48 to 96 hours.[38,48] The developing cutaneous ulcer or soft tissue focus goes through a series of changes, in which polymorphonuclear leukocytes are replaced by macrophages. In time, necrosis, epithelioid cell infiltrates, giant-cell formation, and true granulomata may develop.[62,103,127] Organisms are difficult to demonstrate in tissue but occasionally are found at the periphery of lesions. In addition to the development of classic necrotic and granulomatous lesions, degeneration of the parenchyma in the liver and spleen may occur, as may marked hyperplasia of the reticuloendothelial system. These granulomata can organize to form microabscesses and abscesses. The pace of the illness depends on the virulence of the strain, as well as the inoculum size, portal of entry, and immune status of the host.[68] When organisms enter the blood circulation, typical endotoxemia may ensue, sometimes in association with acute rhabdomyolysis.[82]

Autopsies of fatal cases of tularemia in humans have confirmed the findings in experimental animals.[59,94,116] Lymph nodes from patients with nonfatal disease have shown follicular hyperplasia with conglomerates of macrophages and caseating granulomata.[87,94,96,133] These findings are similar to those seen in miliary tuberculosis. In fact, tularemia and tuberculosis may be histopathologically distinguishable only because of the difference in timing of the development of tissue changes,[88] a difference related to the rapid replication of *F. tularensis*.

CLINICAL MANIFESTATIONS

Regardless of the portal of entry, the mode of onset of tularemia and the general features of the disease are the same. The usual incubation period of tularemia is 3 to 4 days, with a range of 1 to 21 days. The onset of symptoms is abrupt. Symptoms include fever, with a temperature usually higher than 39.4°C (103°F), chills, headache, myalgia, anorexia, vomiting, and occasionally photophobia. Fever may be continuous or biphasic, with an intermittent period of defervescence. In untreated patients, fever may persist for longer than 3 weeks. Physical findings usually include lymphadenopathy, hepatosplenomegaly, pharyngitis, and skin lesions. Temperature–pulse dissociation has been described in as many as 42 percent of cases.[45,56] A variety of skin rashes (e.g., maculopapular, vesicular, and pustular rashes; erythema nodosum,[117] and erythema multiforme) may appear during the second week of illness and can last for a few days to several weeks.[6,84,138]

Laboratory studies, including a complete blood count, erythrocyte sedimentation rate, and urinalysis, usually are not helpful in diagnosing tularemia. Sterile pyuria has been reported in patients with tularemia, 22 percent in one study[45] and 32 percent in another.[115] Sterile pyuria combined with a history of fever, dysuria, or low back pain led to an erroneous diagnosis of urinary tract infection in 11 percent of the patients reported by Evans and associates.[45] Increased liver function test values with jaundice and atypical lymphocytosis also have been reported.[57,60] The six clinical syndromes of tularemia can be classified by the portal of entry: ulceroglandular, glandular, oculoglandular, typhoidal, oropharyngeal, and pneumonic.

Ulceroglandular Tularemia

Ulceroglandular tularemia, the most common form of the disease, accounts for more than 75 percent of all cases (in adults and children).[93] The organism gains access through the skin. Approximately 2 days after the onset of general symptoms, the patient reports tender, swollen lymph nodes, most commonly in the axillary or inguinal areas. Within 24 hours, the portal of entry may become evident when a painful swollen papule develops distal to the regional node. This papule ruptures and leaves a punched-out ulcer with raised edges. The ulcer is indolent and, in untreated cases, may persist for longer than a month. The skin over the involved nodes may be inflamed. In untreated cases, approximately 50 percent of the lymph nodes suppurate and drain. In other cases, the nodes remain firm, enlarged, and tender for several months. Mild, generalized lymphadenopathy and enlargement of the liver and spleen may be present.

Glandular Tularemia

The glandular form of tularemia accounts for 15 percent of all cases.[93] Glandular tularemia is almost identical to the ulceroglandular form, except that the portal of entry cannot be identified.[26] The consensus is that the portal is an insignificant break in the skin. Isolated cases of cervical lymphadenopathy have been related causally to *F. tularensis* infection.

Oropharyngeal Tularemia

The oropharyngeal form, which accounts for fewer than 5 percent of all tularemia cases,[93] resembles the ulceroglandular form. The infection is introduced into the oropharyngeal mucosa through infected, inadequately cooked meat. The organisms enter through abrasions, or aerosolization may occur during chewing. Local involvement consists of acute tonsillitis with cervical adenitis.[12] The tonsils may be covered by an exudate or membrane that extends in all directions and may resemble a diphtheritic membrane. Complaints of sore throat usually are out of proportion to the visible pathologic features, but ulcers sometimes are present. The cervical lymph nodes may suppurate. Infrequently, *F. tularensis* invades the lower portions of the gastrointestinal tract, in which case vomiting, diarrhea, and abdominal pain are prominent symptoms. Awareness of the ability of *F. tularensis* to cause oropharyngeal involvement is important for physicians caring for children.[72,85,92]

Oculoglandular Tularemia

In oculoglandular tularemia, which accounts for approximately 1 percent of all cases,[93] the portal of entry is the conjunctival sac, which may become inoculated by rubbing with contaminated fingers, having contact with infected water, splashing of infected liquids, or inhaling infected aerosols. The eyelids may become edematous and the conjunctivae inflamed and painful. Numerous small, sharply defined, yellowish nodules and ulcers may be present on the palpebral conjunctivae.[132] In some cases, corneal ulceration occurs. As seen in cat-scratch disease, the Parinaud complex of unilateral preauricular lymph node involvement and conjunctivitis can result.[132,134] The regional lymph nodes, the preauricular nodes, and the submaxillary and cervical nodes are swollen, tender, and painful.[117] In severe cases, the axillary nodes may be involved.[52]

Typhoidal Tularemia

Typhoidal tularemia is manifested as fever of unknown cause. The symptoms are those of acute septicemia, with no localized skin lesions and often without lymphadenopathy. Patients are seriously ill, and shock may develop. The symptoms and signs of typhoidal tularemia are toxemia, continuous fever, myalgia, and severe headache. Patients may be delirious and may exhibit meningismus. Patients often complain of having severe pharyngeal pain, but pharyngeal lesions may not be evident. Diarrhea occurs in this form of tularemia in both adults and children. Sometimes, a dry cough and retrosternal pain are present. Pleuropulmonary involvement is a common finding in adults with typhoidal tularemia.[35] In children, typhoidal tularemia can be the result of ingestion of the causative agent, and necrotic lesions may be present throughout the bowel.[35] Inhalation of aerosolized organisms is a more common mode of acquisition in adults (e.g., laboratory workers and farmers).

Pneumonic Tularemia

The pneumonic form of the disease occurs most commonly in laboratory workers and is the most severe and lethal form.[75] Pneumonic tularemia may be acquired by the aerogenic route,[34,47,143] or pulmonary disease may be associated with other forms of tularemia, particularly the typhoidal type. Dienst[35] and Miller and Bates[105] published outstanding descriptions of pleuropulmonary tularemia. Miller and Bates stressed that the symptoms and signs of pleuropulmonary tularemia are nonspecific and vary with the location and degree of pulmonary involvement. The variable radiographic features may be confused with those of tuberculosis, mycotic infection, common bacterial pneumonia, lymphoma, or carcinoma of the lung. In the series of 29 patients by Miller and Bates,[105] pleural effusion developed in 6 patients. Pleural effusions have been found to contain more than 3 g of protein/100 mL and more than 1000 white blood cells/mm^3, with a lymphocytic predominance.[58] Because of the necrotizing nature of the pathologic process, the lung may heal with residual fibrosis or calcification. In other instances, the disease is so fulminant that death occurs before the pathologic features can progress fully.

Additional Clinical Manifestations

All large series of reported cases have included patients with subcutaneous nodules resembling those seen in sporotrichosis. The nodules usually are distributed on the anterior or posterior surface of the arm and may extend from the primary lesion to the regional lymph nodes. Initially, these nodules are firm and movable, but later

they become fixed to the skin and may suppurate. They vary in size from less than a centimeter to more than several centimeters, if they become confluent. As many as 30 single nodules may be present. Other unusual manifestations of tularemia include pericarditis, appendicitis, peritonitis, liver abscess, cerebellitis with ataxia, meningitis, encephalitis, osteomyelitis, rhabdomyolysis, and venous thrombosis.[30,67]

The incidence of these forms in children is somewhat different from that in adults. Although the pneumonic form previously was thought to be relatively rare in children,[92] more recent studies on the changing epidemiology and clinical manifestations of this disease showed that the occurrence of pneumonic tularemia is not uncommon.[74] In a 1985 study by Jacobs and associates,[74] 14 percent of tularemia cases in children were the pneumonic form. This percentage contrasts with the absence of pulmonary involvement in a series of 48 cases of tularemia in children reported by Levy and associates[92] in 1950. The distribution of these forms of tularemia also varies with the geographic location. In a report of 67 children with tularemia in Finland, 79 percent of the cases were of the ulceroglandular form and 8 percent were glandular.[147] This distribution is significantly different from that in the United States. In the study by Jacobs and associates,[74] who reported on 28 cases of tularemia in children, 45 percent were ulceroglandular, and 25 percent were glandular. More recently from the same region, of the 30 cases described, 57 percent were ulceroglandular disease.[131] The authors of the Finnish study attributed the higher proportion of the ulceroglandular form in their study to heightened awareness of the disease and early diagnosis. In addition, the differences also could be explained by the different strains of *F. tularensis* and different vectors in the two regions.

F. tularensis also can occur in the setting of an immunocompromised host. The pneumonic form of tularemia was reported in a patient with chronic granulomatous disease requiring lobectomy.[97] Pneumonic tularemia also was seen in a patient who previously had undergone peripheral blood stem cell transplantation for acute myelogenous leukemia (it resulted in a solitary pulmonary nodule) and in a patient infected with human immunodeficiency virus (HIV).[60,106] In addition, pneumonic tularemia was noted in a patient with a ventriculoperitoneal shunt.[118] Impairment of normal immunity could prevent adequate protection from *F. tularensis* and could necessitate more prolonged therapy.

DIAGNOSIS

The diagnosis of tularemia is established by a thorough history of possible exposure, clinical manifestations, and serial serologic tests. Obtaining a careful family history often is rewarding because several family members commonly are infected simultaneously. In some instances, however, a positive history never is elicited. Tularemia has no absolute pathognomonic features. In diagnosing tularemia, the physician must account for the endemic rate of the disease in the area, the season of the year, the clinical manifestations of the disease, the precise epidemiologic setting,[135] and the unresponsiveness of the disease to antibiotics that are not effective against tularemia.

The diagnosis is confirmed by the standard agglutination test, which is available commercially and is reliable. Unfortunately, it may not provide an early diagnosis because agglutinating antibodies may not be detectable until the second week of illness. Occasionally, seroconversion is not confirmed until the patient has experienced 4 to 6 weeks of illness. In rare cases, agglutinating antibody may never be detected. A fourfold increase in convalescent titer confirms the diagnosis (with an initial minimum titer of 1:160), but a presumptive diagnosis should be considered in patients with acute titers of 1:160 or greater. This titer may indicate current or past infection, but in a clinically suspicious case, it should be considered an indication for presumptive therapy. Titers of 1:1280 or greater often develop in patients with active disease as the initial manifestation of seroconversion. The agglutination test is specific, but cross reaction with *Brucella* spp., *Legionella* spp. or other gram-negative bacteria can occur. Antibiotic therapy does not prevent the development of agglutinating antibodies. A single titer of 1:128 or greater determined by microagglutination or with a fourfold increase with convalescent testing is also considered positive.

Polymerase chain reaction (PCR)–based assays have been used for early detection of *F. tularensis* infection.[81,95] The PCR test has been shown to be effective in establishing the diagnosis of tularemia even when samples of a tularemic ulcer were obtained 1 to 14 days after infection.[130] When PCR and culture of the wound and blood were compared with agglutination tests, the sensitivity of PCR was 75 percent, and the sensitivity of culture was 62 percent.[79] In addition to increasing the safety for laboratory personnel, the PCR method was more sensitive and could detect infection at earlier stages. In contrast to conventional PCR-based assays, real-time PCR (RT-PCR) has been shown to be more specific and 10 times more sensitive.[150] These assays are not widely commercially available at this time.

In a Norwegian field study, a rapid immunochromatography assay proved inexpensive, time efficient, and portable in contrast to PCR and enzyme-linked immunosorbent assay (ELISA), but this method was less sensitive than PCR.[11] This assay could accelerate identification and treatment decisions in clinical practice.

Gram-stained smears of patient specimens such as exudate and sputum usually do not reveal the organism. However, collecting the specimens poses no danger, and examination of direct smears helps rule out other causative agents. Specimens should not be cultured for *F. tularensis* in the usual hospital or diagnostic laboratory because isolation of these organisms in facilities other than a level P-3 laboratory is hazardous to laboratory personnel. If confirmation by culture is indicated, physicians should notify the laboratory of the potential for the specimen to contain *F. tularensis*, so that appropriate laboratory precautions can be taken.

The differential diagnosis of tularemia depends on the clinical form of the disease. Ulceroglandular and glandular tularemia must be differentiated from disease caused

by ordinary bacterial pathogens such as *Streptococcus* and *Staphylococcus*, from disease caused by *M. tuberculosis* and atypical mycobacteria such as *Mycobacterium marinum*, and from anthrax, HIV infection, and cat-scratch disease. In older patients with inguinal lymphadenopathy, lymphogranuloma venereum, granuloma inguinale, and other sexually transmitted infections should be considered. Occasionally, sporotrichosis and infectious mononucleosis are diagnosed in these patients. Oculoglandular fever is somewhat more distinctive, but the possibility of disease caused by common bacterial pathogens, *L. monocytogenes* infection, herpes zoster, inclusion conjunctivitis, and keratoconjunctivitis must not be ruled out. Oropharyngeal tularemia must be differentiated from streptococcal tonsillopharyngitis and corynebacterial disease. Typhoidal tularemia can be confused with ordinary bacteremia and must be differentiated from the more common bacterial and enteric disease, as well as from malaria, miliary tuberculosis, brucellosis, and typhoid fever. Tularemic pneumonia must be differentiated from other bacterial as well as nonbacterial pneumonia, including tuberculosis, *Mycoplasma* infection, legionnaires' disease, psittacosis, viral pneumonia, Q fever, fungal infection, and chemical pneumonitis.

TREATMENT

Gentamicin is recommended for the treatment of tularemia. The dosage for children is 5 mg/kg daily in two divided doses either intravenously or intramuscularly. The duration of therapy is usually 7 to 10 days, with more severe disease requiring a longer duration of treatment. In mild to moderate cases in which patients become afebrile within 48 hours of starting antimicrobial therapy, a 5- to 7-day course can be used. Defervescence and alleviation of other signs and symptoms occur promptly, usually within several days. Response may be delayed if the lymph nodes have progressed to suppuration requiring drainage

If available, streptomycin given intramuscularly also can be used. The recommended dose of streptomycin is 30 to 40 mg/kg/day administered intramuscularly in two divided doses for 7 days. If a patient has mild symptoms initially or responds dramatically to therapy, an alternative streptomycin regimen of 30 to 40 mg/kg/day for 3 days followed by 15 to 20 mg/kg/day for 4 days may be given intramuscularly. In severe cases or if a child does not become afebrile and asymptomatic within a few days of therapy, extension of treatment beyond 7 days is indicated. Streptomycin-resistant strains of tularemia are reported, but they are rare. Other aminoglycosides (e.g., tobramycin) are ineffective in treating tularemia and should not be used.

Bacteriostatic agents such as tetracycline and chloramphenicol have been used to treat tularemia, with cure rates of 88 percent and 77 percent, respectively. However, these agents are considered suboptimal for the treatment of tularemia because of a high incidence of relapse after therapy is stopped (i.e., 12% for tetracycline and 22% for chloramphenicol).[41]

Although in vitro susceptibility testing indicates that the third-generation cephalosporins may be effective against *F. tularensis*, one report showed treatment failure in 8 children given ceftriaxone.[32] Treatment with other antibiotics has been reported sporadically in the literature.[63,91] Fluoroquinoles have been used with success when treating infections caused by the subspecies *holartica*.[4,104,106,126,139] An efficacy trial in Sweden of ciprofloxacin demonstrated successful outpatient management of *F. tularensis* in 10 of 12 patients 1 to 10 years of age.[80] The dosage used was 15 to 20 mg/kg/day in two divided doses for a 10- to 14-day course of therapy. Defervescence occurred on the fourth day of treatment. The lack of successful treatment data for the subspecies *tularensis* and the issues related to the use of fluoroquinolones in children younger than 18 years of age limit the use of these medications in North American children at this time.

Hearing screening should be considered before initiation of streptomycin or gentamicin therapy. If the child has preexisting hearing loss, the alternative streptomycin regimen or gentamicin therapy can be considered, with close monitoring of serum levels in severe cases. In these cases, audiologic evaluation is indicated after therapy is initiated.

Bed rest and supportive therapy are, of course, indicated. In a severely ill patient who shows signs of endotoxic shock, appropriate monitoring and admission to an intensive care unit are indicated and corticosteroid therapy should be considered. Suppurative lymph nodes may require surgical drainage. Complicated cases (e.g., meningitis) may require treatment with more than one antimicrobial agent.

Before the advent of effective antimicrobial therapy, tularemia often was a protracted illness that lasted weeks or months. Subsequently, a long period of convalescence was necessitated by debility. Antibiotics have interrupted the natural history of the disease. When the disease is diagnosed promptly, the course generally is less than a month. As a result of administering appropriate antibiotic therapy, the mortality rate has declined from 5 to 30 percent to less than 1 percent, except in cases of fulminant pneumonic and typhoidal disease.

PREVENTION

Prevention of human tularemia depends on prevention of exposure to either the vectors or contaminated animal tissue. Children living in areas of tick endemicity should have their skin and hair checked frequently for ticks. Ticks should be removed carefully with tweezers (not with fingernails) by pulling perpendicular to the skin where the ticks have attached.[107] Care should be taken not to squeeze the ticks between the fingers. Persons living in tick-infested areas should wear clothing with tightly fitting cuffs at the wrists and ankles when staying outdoors. Tick repellents should be used with caution on children. Prophylaxis with antimicrobial agents has not proved effective in preventing disease in children with tick bites.

Children should be warned against handling sick or dead rodents or rabbits. Incineration or burial should be

used to dispose of rabbits caught by household pets. Rubber gloves should be worn for preparing game animals, and the meat should be cooked thoroughly before eating. Hunters, especially rabbit hunters, should take precautions against contracting tularemia. A wide range of approaches to vaccine development are being evaluated, but no vaccine against tularemia is currently licensed.[27]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Aranda EA. Treatment of tularemia with levofloxacin. *Clin Microbiol Infect* 2001;**7**:167–8.
6. Asano S, Mori K, Yamazaki K, et al. Temporal differences of onset between primary skin lesions and regional lymph node lesions for tularemia in Japan: a clinicopathologic and immunohistochemical study of 19 skin cases and 54 lymph node cases. *Virchows Arch* 2012;**460**:651–8.
7. Bandara AB, Champion AE, Wang X, et al. Isolation and mutagenesis of a capsule-like complex (CLC) from *Francisella tularensis*, and contribution of the CLC to *F. tularensis* virulence in mice. *Plos One* 2011;**6**:e19003.
8. Barker JR, Chong A, Wehrly TD, et al. The *Francisella tularensis* pathogenicity island encodes a secretion system that is required for phagosome escape and virulence. *Mol Microbiol* 2009;**74**: 1459–70.
18. Centers for Disease Control and Prevention. Tularemia: Missouri, 2000-2007. *MMWR Morb Mortal Wkly Rep* 2009;**58**:744–8.
20. Cherwonogrodzky JW, Knodel MH, Spence MR. Increased encapsulation and virulence of *Francisella tularensis* live vaccine strain (LVS) by subculturing on synthetic medium. *Vaccine* 1994; **12**:773–5.
21. Chitzade N, Kuchuloria T, Clark DV, et al. Water-borne outbreak of oropharyngeal and glandular tularemia in Georgia: investigation and follow-up. *Infection* 2009;**37**:514–21.
27. Conlan JW. Tularemia vaccines: recent developments and remaining hurdles. *Future Microbiol* 2011;**6**:391–405.
30. Contentin L, Soret J, Zamfir O, et al. *Francisella tularensis* meningitis. *Med Mal Infect* 2011;**41**:556–8.
65. Hauri AM, Hofstetter I, Seibold E, et al. Investigating an airborne tularemia outbreak, Germany. *Emerg Infect Dis* 2010;**16**:238–43.
67. Holfinger DM, Cardona L, Mertz GJ, Davis LE. Tularemic meningitis in the United States. *Arch Neurol* 2009;**66**:523–7.
71. Horzempa J, O'Dee DM, Stolz DB, et al. Invasion of erythrocytes by *Francisella tularensis*. *J Infect Dis* 2011;**204**:51–9.
85. Kaya A, Deveci K, Uysal IO, et al. Tularemia in children: evaluation of clinical, laboratory and therapeutic features of 27 tularemia cases. *Turk J Pediatr* 2012;**54**:105–12.
86. Keim P, Johansson A, Wagner DM. Molecular epidemiology, evolution, and ecology of *Francisella*. *Ann NY Acad Sci* 2007; **1105**:30–66.
87. Kirimanjeswara GS, Olmos S, Bakshi CS, Metzger DW. Humoral and cell-mediated immunity to intracellular pathogen *Francisella tularensis*. *Immunol Rev* 2008;**225**:244–55.
89. Kugeler KJ, Mead PS, Janusz AM, et al. Molecular epidemiology of *Francisella tularensis* in the United States. *Clin Infect Dis* 2009; **48**:863–70.
99. Maurin M, Pelloux I, Brion JP, et al. Human tularemia in France, 2006-2010. *Clin Infect Dis* 2011;**53**:e133–41.
104. Meric M, Willke A, Finke EJ, et al. Evaluation of clinical, laboratory, and therapeutic features of 145 tularemia cases: the role of quinolones in oropharyngeal tularemia. *APMIS* 2008;**116**:66–73.
113. Pandya GA, Holmes MH, Petersen JM, et al. Whole genome single nucleotide polymorphism based phylogeny of *Francisella tularensis* and its application to the development of a strain typing assay. *BMC Microbiol* 2009;**7**:213.
131. Snowden J, Stovall S. Tularemia: Retrospective review of 10 years' experience in Arkansas. *Clin Pediatr* 2011;**50**:64–8.
158. Yuen JC, Malotky MV. *Francisella tularensis* osteomyelitis of the hand following a cat bite: a case of clinical suspicion. *Plast Reconstruct Surg* 2011;**128**:e37–8.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 133

Haemophilus influenzae

Stephen J. Barenkamp

Relatively few species of the genus *Haemophilus* are pathogenic to humans, and nearly all that cause human disease are either encapsulated or unencapsulated strains of *H. influenzae*. These latter bacteria are small, gram-negative pleomorphic coccobacilli that generally are considered to be normal constituents of the microbial flora of the upper respiratory tract of humans.[231,262] Strains without polysaccharide capsules often cause infections of mucosal surfaces, such as otitis media, bronchitis, conjunctivitis, sinusitis, and types of pneumonia.[286,291] Encapsulated strains, especially *H. influenzae* type b (Hib), cause invasive diseases such as septicemia, meningitis, septic arthritis, cellulitis, epiglottitis, pneumonia, and empyema. Before Hib vaccines became widely available, *H. influenzae* was the leading cause of bacterial meningitis in the United States and most other countries and it also was an important cause of other bacteremic illnesses, primarily in young children.[50,252,459]

The organism now known as *H. influenzae* first was described in 1892 by Robert Pfeiffer, who isolated it from the lungs and sputum of patients during the 1889 to 1892 pandemic of influenza. He proposed that the organism

REFERENCES

1. Abd H, Johansson T, Golovliov I, et al. Survival and growth of *Francisella tularensis* in *Acanthamoeba castellanii*. *Appl Environ Microbiol* 2003;**69**:600–6.
2. Allen WP. Immunity against tularemia: passive protection of mice by transfer of immune tissues. *J Exp Med* 1962;**115**:411–20.
3. Ancuta P, Pedron T, Girard R, et al. Inability of the *Francisella tularensis* lipopolysaccharide to mimic or to antagonize the induction of cell activation by endotoxins. *Infect Immun* 1996;**64**: 2041–6.
4. Aranda EA. Treatment of tularemia with levofloxacin. *Clin Microbiol Infect* 2001;**7**:167–8.
5. Arav-Boger R. Cat-bite tularemia in a seventeen-year-old girl treated with ciprofloxacin. *Pediatr Infect Dis J* 2000;**19**:583–4.
6. Asano S, Mori K, Yamazaki K, et al. Temporal differences of onset between primary skin lesions and regional lymph node lesions for tularemia in Japan: a clinicopathologic and immunohistochemical study of 19 skin cases and 54 lymph node cases. *Virchows Arch* 2012;**460**:651–8.
7. Bandara AB, Champion AE, Wang X, et al. Isolation and mutagenesis of a capsule-like complex (CLC) from *Francisella tularensis*, and contribution of the CLC to *F. tularensis* virulence in mice. *Plos One* 2011;**6**:e19003.
8. Barker JR, Chong A, Wehrly TD, et al. The *Francisella tularensis* pathogenicity island encodes a secretion system that is required for phagosome escape and virulence. *Mol Microbiol* 2009;**74**:1459–70.
9. Bellanti JA, Buescher EL, Brandt WE, et al. Characterization of human serum and nasal hemagglutinating antibody in *Francisella tularensis*. *J Immunol* 1967;**98**:171–8.
10. Ben Naser A, Haithcoat J, Masterson JE, et al. Critical role for serum opsonins and complement receptors CR3 (CD11b/CD18) and CR4 (CD11c/CD18) in phagocytosis of *Francisella tularensis* by human dendritic cells (DC): uptake of *Francisella* leads to activation of immature DC and intracellular survival of the bacteria. *J Leukoc Biol* 2006;**80**:774–86.
11. Berdal B, Mehl R, Haaheim H, et al. Field detection of *Francisella tularensis*. *Scand J Infect Dis* 2000;**32**:287–91.
12. Blanco JR, Gutierrez C, Zabalza M, et al. Clinical microbiological case: sore throat and painful bilateral lymph nodes. *Eur J Clin Microbiol Infect Dis* 2001;**7**:637–8.
13. Bosio CM, Elkins KL. Susceptibility to secondary *Francisella tularensis* live vaccine strain infection in B-cell–deficient mice is associated with neutrophilia but not with defects in specific T-cell–mediated immunity. *Infect Immun* 2001;**69**:194–203.
14. Burke DS. Immunization against tularemia: analysis of the effectiveness of live *Francisella tularensis* vaccine in prevention of laboratory-acquired tularemia. *J Infect Dis* 1977;**135**:55–60.
15. Callaway GD, Peterson SS, Good JT. Tularemia in southwest Missouri. *Mo Med* 1954;**51**:906–9.
16. Casper EA, Phillip RN. A skin test survey of tularemia in a Montana sheep-raising county. *Public Health Rep* 1969;**84**:611–15.
17. Centers for Disease Control and Prevention. Tularemia: United States, 1990-2000. *M MWR Morb Mortal Wkly Rep* 2002;**51**: 181–4.
18. Centers for Disease Control and Prevention. Tularemia: Missouri, 2000-2007. *MMWR Morb Mortal Wkly Rep* 2009;**58**:744–8.
19. Centers for Disease Control and Prevention. Summary of notifiable disease: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–811.
20. Cherwonogrodzky JW, Knodel MH, Spence MR. Increased encapsulation and virulence of *Francisella tularensis* live vaccine strain (LVS) by subculturing on synthetic medium. *Vaccine* 1994; **12**:773–5.
21. Chitzade N, Kuchuloria T, Clark DV, et al. Water-borne outbreak of oropharyngeal and glandular tularemia in Georgia: investigation and follow-up. *Infection* 2009;**37**:514–21.
22. Claflin JL, Larson CL. Infection-immunity in tularemia: specificity of cellular immunity. *Infect Immun* 1972;**5**:311–17.
23. Clemens DL, Lee BY, Horwitz MA. Virulent and avirulent strains of *Francisella tularensis* prevent acidification and maturation of their phagosomes and escape into the cytoplasm in human macrophages. *Infect Immun* 2004;**72**:3204–17.
24. Clemens DL, Lee BY, Horwitz MA. *Francisella tularensis* enters macrophages via a novel process involving pseudopod loops. *Infect Immun* 2005;**73**:5892–902.
25. Cole LE, Elkins KL, Michalek SM, et al. Immunologic consequences of *Francisella tularensis* live vaccine strain infection: role of the innate immune response infection and immunity. *J Immunol* 2006;**176**:6888–99.
26. Collison PJ, Adams B. Glandular tularaemia in a Native American child. *Ear Nose Throat J* 2003;**82**:851–4.
27. Conlan JW. Tularemia vaccines: recent developments and remaining hurdles. *Future Microbiol* 2011;**6**:391–405.
28. Conlan JW, North RJ. Early pathogenesis of infection in the liver with the facultative intracellular bacteria *Listeria monocytogenes*, *Francisella tularensis*, and *Salmonella typhimurium* involves lysis of infected hepatocytes by leukocytes. *Infect Immun* 1992;**60**:5164–71.
29. Conlan JW, Sjostedt A, North RJ. $CD4^+$ and $CD8^+$ T-cell–dependent and independent host defense mechanisms can operate to control and resolve primary and secondary *Francisella tularensis* LVS infection in mice. *Infect Immun* 1994;**62**:5603–7.
30. Contentin L, Soret J, Zamfir O, et al. *Francisella tularensis* meningitis. *Med Mal Infect* 2011;**41**:556–8.
31. Cowley SC, Elkins KL. Multiple T cell subsets control *Francisella tularensis* LVS intracellular growth without stimulation through macrophage interferon gamma receptors. *J Exp Med* 2003;**198**: 379–89.
32. Cross JT, Jacobs RF. Tularemia: treatment failures with outpatient use of ceftriaxone. *Clin Infect Dis* 1993;**17**:76–80.
33. Culkin SJ, Rhinehart-Jones T, Elkins KL. A novel role for B cells in early protective immunity to an intracellular pathogen, *Francisella tularensis* strain LVS. *J Immunol* 1997;**158**:3277–84.
34. Dahlstrand S, Ringertz O, Zetterberg B. Airborne tularemia in Sweden. *Scand J Infect Dis* 1971;**3**:7–16.
35. Dienst FT Jr. Tularemia: a perusal of three hundred thirty-nine cases. *J La State Med Soc* 1963;**115**:114–27.
36. Eigelsbach HT, Hornick RB, Tulis JJ. Recent studies on live tularemia vaccine. *Med Ann D C* 1967;**36**:282–6.
37. Eigelsbach HT, Hunter DH, Janssen WA, et al. Murine model for study of cell-mediated immunity: protection against death from fully virulent *Francisella tularensis* infection. *Infect Immun* 1915; **12**:999–1005.
38. Eigelsbach HT, Tulis JJ, McGavran MH, et al. Live tularemia vaccine: host-parasite relationship in monkeys vaccinated intracutaneously or aerogenically. *J Bacteriol* 1962;**84**:1020–7.
39. Elkins KL, Bosio CM, Rhinehart-Jones TR. Importance of B cells, but not specific antibodies, in primary and secondary protective immunity to the intracellular bacterium *Francisella tularensis* live vaccine strain. *Infect Immun* 1999;**67**:6002–7.
40. Elkins KL, Rhinehart-Jones TR, Culkin SJ, et al. Minimal requirements for murine resistance to infection with *Francisella tularensis* LVS. *Infect Immun* 1996;**64**:3288–93.
41. Enderlin G, Morales L, Jacobs RF, et al. Streptomycin and alternative agents for the treatment of tularemia: review of the literature. *Clin Infect Dis* 1994;**19**:42–7.
42. Engelfried JJ. Antibodies to *Pasteurella tularensis* in a selected human population. *Mil Med* 1968;**135**:723–6.
43. Ericsson M, Golovliov I, Sandstrom G, et al. Characterization of the nucleotide sequence of the groE operon encoding heat shock proteins chaperone-60 and -10 of *F. tularensis* and determination of the T-cell response to the proteins in individuals vaccinated with *F. tularensis*. *Infect Immun* 1997;**65**:1824–9.
44. Evans ME. *Francisella tularensis*. *Infect Control* 1985;**6**:381–3.
45. Evans ME, Gregary DW, Schaffner W, et al. Tularemia: a 30-year experience with 88 cases. *Medicine (Baltimore)* 1985;**64**:251–69.
46. Farlow J, Wagner DM, Dukerich M, et al. *Francisella tularensis* in the United States. *Emerg Infect Dis* 2005;**11**:1835–41.
47. Feldman KA, Enscore R, Lathrop S, et al. Outbreak of primary pneumonic tularemia on Martha's Vineyard. *N Engl J Med* 2001; **345**:1601–6.
48. Finegold MJ, Pulliam JD, Landay ME, et al. Pathological changes in rabbits injected with *Pasteurella tularensis* killed by ionizing radiation. *J Infect Dis* 1969;**119**:635–40.
49. Forestal CA, Benach JL, Carbonara C, et al. *Francisella tularensis* selectively induces proinflammatory changes in endothelial cells. *J Immunol* 2003;**171**:2563–70.
50. Foshay L. Tularemia. *Annu Rev Microbiol* 1950;**4**:313–30.
51. Francis E. A new disease of man. *JAMA* 1922;**78**:1015.
52. Francis E. A summary of the present knowledge of tularemia. *Medicine (Baltimore)* 1928;**7**:411–32.

53. Francis E. Sources of infection and seasonal incidence of tularemia in man. *Public Health Rep* 1937;**52**:103–13.
54. Francis E. Oculoglandular tularemia. *Arch Ophthalmol* 1942;**28**: 711–41.
55. Francis E, Mayne B. *Public Health Rep* 1921;**36**:1938–46.
56. Fredricks D, Remington J. Tularemia presenting as community-acquired pneumonia. *Arch Intern Med* 1996;**156**:2137–40.
57. Gelfand MS, Mehra N, Simmons BP. Tularemia and atypical lymphocytosis. *J Tenn Med Assoc* 1989;**82**:417–18.
58. Gill V, Cunha B. Tularemia pneumonia. *Semin Respir Infect* 1997;**12**:61–7.
59. Goodpasture EW, House SJ. The pathologic anatomy of tularemia in man. *Am J Pathol* 1928;**4**:213–16.
60. Gries D, Fairchok M. Typhoidal tularemia in a human immunodeficiency virus–infected adolescent. *Pediatr Infect Dis J* 1996;**15**: 838–9.
61. Gurycova D. First isolation of *F. tularensis* subsp. *tularensis* in Europe. *Eur J Epidemiol* 1998;**14**:797–802.
62. Hall WC, Kovatch RM, Schricker RL. Tularemic pneumonia: pathogenesis of the aerosol-induced disease in monkeys. *J Pathol* 1973;**110**:193–201.
63. Harrell RE Jr, Simmons HF. Pleuropulmonary tularemia: successful treatment with erythromycin. *South Med J* 1990;**83**:1363–4.
64. Harwood RF, James MT. *Entomology in human and animal health*. New York: Macmillan; 1973. p. 403–4.
65. Hauri AM, Hofstetter I, Seibold E, et al. Investigating an airborne tularemia outbreak. *Germany Emerg Infect Dis* 2010;**16**:238–43.
66. Hesselbrock WB, Foshay L. The morphology of *Bacterium tularense*. *J Bacteriol* 1945;**49**:209–31.
67. Holfinger DM, Cardona L, Mertz GJ, Davis LE. Tularemic meningitis in the United States. *Arch Neurol* 2009;**66**:523–7.
68. Hood AM. Virulence factors of *Francisella tularensis*. *J Hyg (Lond)* 1977;**79**:47–60.
69. Hopla CE. The ecology of tularemia. *Adv Vet Sci Comp Med* 1974;**18**:25–53.
70. Hornick RB. Tularemia. In: Hoeprich PD, editor. *Infectious diseases*. New York: Harper & Row; 1972. p. 1043–9.
71. Horzempa J, O'Dee DM, Stolz DB, et al. Invasion of erythrocytes by *Francisella tularensis*. *J Infect Dis* 2011;**204**:51–9.
72. Hughes WT Jr, Etteldorf JN. Oropharyngeal tularemia. *J Pediatr* 1957;**51**:363–72.
73. Jacobs R. Tularemia. *Adv Pediatr Infect Dis* 1997;**12**:55–69.
74. Jacobs R, Condrey YM, Yamauchi T. Tularemia in adults and children: a changing presentation. *Pediatrics* 1985;**76**:818–22.
75. Jaslaw S, Eigelsback HT, Prior JA, et al. Tularemia vaccine study. I. Intracutaneous challenge. *Arch Intern Med* 1961;**107**:689–701.
76. Jellison WL. *Tularemia in North America, 1930-1974*. Missoula, Mont: University of Montana; 1974.
77. Jellison WL, Kohls GM. Tularemia in sheep in sheep industry workers in western United States. *Public Health Monogr* 1955; **28**:1–19.
78. Jellison WL, Epler DC, Kunns E, et al. Tularemia in man from a domestic rural water supply. *Public Health Rep* 1950;**65**:1219–26.
79. Johansson A, Berglund L, Eriksson U, et al. Comparative analysis of PCR versus culture for diagnosis of ulceroglandular tularemia. *J Clin Microbiol* 2000;**38**:22–6.
80. Johansson A, Berglund L, Gothefors L, et al. Ciprofloxacin for the treatment of tularemia in children. *Pediatr Infect Dis J* 2000; **19**:449–53.
81. Johansson A, Forsman M, Sjostedt A. The development of tools for diagnosis of tularemia and typing of *Francisella tularensis*. *APMIS* 2004;**112**:898–907.
82. Kaiser AB, Rieves O, Price AH, et al. Tularemia and rhabdomyolysis. *JAMA* 1985;**253**:241–3.
83. Karpoff SP, Antonoff NI. The spread of tularemia through water, as a new factor in its epidemiology. *J Bacteriol* 1936;**32**:243–58.
84. Kavanaugh CN. Tularemia: A consideration of one hundred and twenty three cases with observations at autopsy in one. *Arch Intern Med* 1935;**55**:61–85.
85. Kaya A, Deveci K, Uysal IO, et al. Tularemia in children: evaluation of clinical, laboratory and therapeutic features of 27 tularemia cases. *Turk J Pediatr* 2012;**54**:105–12.
86. Keim P, Johansson A, Wagner DM. Molecular epidemiology, evolution, and ecology of *Francisella*. *Ann NY Acad Sci* 2007;**1105**: 30–66.
87. Kirimanjeswara GS, Olmos S, Bakshi CS, Metzger DW. Humoral and cell-mediated immunity to intracellular pathogen *Francisella tularensis*. *Immunol Rev* 2008;**225**:244–55.
88. Kitamura S, Fukada M, Takeda H, et al. Pathology of tularemia. *Acta Pathol Jpn* 1956;**6**(Suppl.):719–64.
89. Kugeler KJ, Mead PS, Janusz AM, et al. Molecular epidemiology of *Francisella tularensis* in the United States. *Clin Infect Dis* 2009; **48**:863–70.
90. Larson BW, Jacobson HJ. Tularemia with unusual laboratory characteristics in South Dakota children. *S D J Med* 1984;**37**: 5–10.
91. Lee HC, Horowitz E, Linder W. Treatment of tularemia with imipenem/cilastatin sodium. *South Med J* 1991;**84**:1277–8.
92. Levy HS, Webb CH, Wilkinson JD. Tularemia as a pediatric problem. *Pediatrics* 1950;**6**:113–22.
93. Levy PD, Chiang WK. Update on emerging infections: news from the Centers for disease control and prevention. *Ann Emerg Med* 2002;**40**:356–60.
94. Lillie RD, Francis G. The pathology of tularemia in man (*Homo sapiens*). In: *The pathology of tularemia*. National Institutes of Health Bulletin No. 167. Washington, D.C.: Public Health Service; 1936. p. 1–81.
95. Long GW, Oprandy JJ, Narayanan RB, et al. Detection of *Francisella tularensis* in blood by polymerase chain reaction. *J Clin Microbiol* 1993;**31**:152–4.
96. Ludmerer KM, Kissane JM. Fever, leukopenia, acute renal failure and death in a 65-year-old man. *Am J Med* 1984;**77**:117–24.
97. Maranan M, Schiff D, Johnson D, et al. Pneumonic tularemia in a patient with chronic granulomatous disease. *Clin Infect Dis* 1997; **25**:630–3.
98. Markwitl LE, Hynes NA, de la Cruz P, et al. Tick-borne tularemia: an outbreak of lymphadenopathy in children. *JAMA* 1985;**254**:2922–5.
99. Maurin M, Pelloux I, Brion JP, et al. Human tularemia in France, 2006-2010. *Clin Infect Dis* 2011;**53**:e133–41.
100. McCaffrey RL, Allen LH. Pivotal advance: *Francisella tularensis* LVS evades killing by human neutrophils via inhibition of the respiratory burst and phagosome escape. *J Leukoc Biol* 2007;**81**: 1–7.
101. McCoy GW. A plague-like disease in rodents. *Public Health Bull* 1911;**43**:53.
102. McCoy GW, Chapin CW. Further observations on a plague-like disease of rodents with a preliminary note on the causative agent: *Bacterium tularense*. *J Infect Dis* 1912;**10**:61–72.
103. McGowran MH, White JD, Eigelsbach HT, et al. Morphologic and immunohistochemical studies of the pathogenesis of infection and antibody formation subsequent to vaccination of macacavirus with an attenuated strain of *Pasteurella tularensis*. I. Intracutaneous vaccination. *Am J Pathol* 1962;**41**:259–71.
104. Meric M, Willke A, Finke EJ, et al. Evaluation of clinical, laboratory, and therapeutic features of 145 tularemia cases: the role of quinolones in oropharyngeal tularemia. *APMIS* 2008;**116**:66–73.
105. Miller RP, Bates JH. Pleuropulmonary tularemia. *Am Rev Respir Dis* 1969;**99**:31–41.
106. Naughton M, Brown R, Adkins D, DiPersio J. Tularemia: an unusual cause of a solitary pulmonary nodule in the post-transplant setting. *Bone Marrow Transplant* 1999;**24**:197–9.
107. Needham GR. Evaluation of five popular methods for tick removal. *Pediatrics* 1985;**75**:997–1002.
108. Ohara H. Jikken Dobutsu. *Exp Amin* 1925;**11**:508–23.
109. Ohara H. Human innoculation experiment with a disease of wild rabbits, with a bacteriologic study. *Kinsei Igaku* 1925;**12**:401–10.
110. Ohara H. Studies on Ohare's disease. *Jpn J Exp Med* 1954;**24**: 69–79.
111. Olsen PF. Tularemia. In: Hubbert WT, McCullock WF, Schnurrenberger PR, editors. *Diseases transmitted from animals to man*. Springfield, Ill: Charles C Thomas; 1975. p. 191–223.
112. Olsufjev NG, Meshcherayakova IS. Subspecies taxonomy of *Francisella tularensis*. *Int J Syst Bacteriol* 1983;**33**:872–4.
113. Pandya GA, Holmes MH, Petersen JM, et al. Whole genome single nucleotide polymorphism based phylogeny of *Francisella tularensis* and its application to the development of a strain typing assay. *BMC Microbiol* 2009;**7**:213.
114. Pearse RA. Insect bite. *Northwest Med* 1911;**3**:81.
115. Penn RL, Kinasewitz GT. Factors associated with a poor outcome in tularemia. *Arch Intern Med* 1987;**147**:265–8.

116. Permar HH, Maclachlan WWG. Tularemic pneumonia. *Ann Intern Med* 1931;**5**:687–98.
117. Peter R, Banyai T. Erythema nodosum revealing oculoglandular tularemia. *Dermatology* 2001;**202**:79–80.
118. Pittman T, Williams D, Friedman A. A shunt infection caused by *F. tularensis*. *Pediatr Neurosurg* 1996;**24**:50–1.
119. Pollitzer R. *History and incidence of tularemia in the Soviet Union: a review*. Bronx, NY: Institute of Contemporary Russian Studies, Fordham University; 1967.
120. Poquet Y, Kroca M, Halary F, et al. Expansion of Vγ9 Vδ2 T cells is triggered by *F. tularensis*–derived phosphoantigens in tularemia but not after tularemia vaccination. *Infect Immun* 1998;**66**:2107–14.
121. Prior JL, Prior RG, Hitchen PG, et al. Characterization of the O antigen gene cluster and structural analysis of the O antigen of *Francisella tularensis* subsp. *tularensis*. *J Med Microbiol* 2003;**52**: 845–51.
122. Rockwood SW. Tularemia: what's in a name? *Am Soc Microbiol* 1983;**49**:63–5.
123. Sandstrom G, Sjostedt A, Johansson T, et al. Immunogenicity and toxicity of lipopolysaccharide from *Francisella tularensis* LVS. *FEMS Microbiol Immunol* 1992;**5**:201–10.
124. Saslaw S, Eigelsbach HT, Prior JA, et al. Tularemia vaccine study. II. Respiratory challenge. *Arch Intern Med* 1961;**107**:702–14.
125. Sawyer WD, Dangerfield HG, Hogge AL, et al. Antibiotic prophylaxis and therapy of airborne tularemia. *Bacteriol Rev* 1965; **30**:542–50.
126. Scheel O, Reiersen R, Hoel T. Treatment of tularemia with ciprofloxacin. *Eur J Clin Microbiol Infect Dis* 1992;**11**:447–8.
127. Schricker RL, Eigelsbach HT, Mitten JQ, et al. Pathogenesis of tularemia in monkeys aerogenically exposed to *Francisella tularensis* 425. *Infect Immun* 1972;**5**:734–44.
128. Shapiro DS, Schwartz DR. Exposure of laboratory workers to *Francisella tularensis* despite a bioterrorism procedure. *J Clin Microbiol* 2002;**40**:2278–81.
129. Sjostedt A, Conlan JW, North RJ. Neutrophils are critical for host defense against primary infection with the facultative intracellular bacterium *Francisella tularensis* in mice and participate in defense against reinfection. *Infect Immun* 1994;**62**:2779–83.
130. Sjostedt A, Eriksson U, Berglund L, Tarnvik A. Detection of *Francisella tularensis* in ulcers of patients with tularemia by PCR. *J Clin Microbiol* 1997;**35**:1045–8.
131. Snowden J, Stovall S. Tularemia: retrospective review of 10 years' experience in Arkansas. *Clin Pediatr* 2011;**50**:64–8.
132. Stacy T, Omphroy L, Oetting T. Parinaud's oculoglandular syndrome attributable to an encounter with a wild rabbit. *Am J Ophthalmol* 2001;**131**:283–4.
133. Staples JE, Kubota KA, Chalcraft LG, et al. Epidemiologic and molecular analysis of human tularemia, United States, 1964-2000. *Emerg Infect Dis* 2006;**12**:1113–18.
134. Steinmann T, Sheikholeslami M, Brown H, Bradsher R. Oculoglandular tularemia. *Arch Ophthalmol* 1999;**117**:132–3.
135. Stewart S. Tularemia: association with hunting and farming. *FEMS Immunol Med Microbiol* 1996;**13**:197–9.
136. Suter E. Passive transfer of acquired resistance to infection with *Mycobacterium tuberculosis* by means of cells. *Am Rev Respir Dis* 1961;**83**:535–43.
137. Suvorov SV, Volfertz AA, Voronkova MM. Plague like lymphadenitis of the Astrikhansky region. *Vestn Mikrobiol* 1928;**7**:293–9.
138. Syrjala H, Karvonen J, Salminen A. Skin manifestations of tularemia: a study of 88 cases in northern Finland during 16 years (1967-1983). *Acta Dermatol Venereol* 1984;**64**:513–16.
139. Syrjala H, Schildt R, Raijainen S. In vitro susceptibility of *Francisella tularensis* to fluoroquinolones and treatment of tularemia with norfloxacin and ciprofloxacin. *Eur J Clin Microbiol Infect Dis* 1991;**10**:68–70.
140. Tarnvik A. Nature of protective immunity to *Francisella tularensis*. *Rev Infect Dis* 1989;**11**:440–51.
141. Telepnev M, Golovilov I, Sjostedt A. *Francisella tularensis* LVS initially activates but subsequently down-regulates intracellular signaling and cytokine secretion in mouse monocytic and human peripheral blood mononuclear cells. *Microb Pathog* 2005;**38**:239–47.
142. Telepnev M, Golovliov I, Grundstrom T, et al. *Francisella tularensis* inhibits toll-like receptor-mediated activation of intracellular signaling and secretion of TNF-alpha and IL-1 from murine macrophages. *Cell Microbiol* 2003;**5**:41–51.
143. Teutsch SM, Martone WJ, Brink EW, et al. Pneumonic tularemia on Martha's Vineyard. *N Engl J Med* 1979;**301**:826–8.
144. Thirumalapura NR, Goad DW, Mort A, et al. Structural analysis of the O-antigen of *Francisella tularensis* subspecies *tularensis* strain OSU 10. *J Med Microbiol* 2005;**54**:693–5.
145. Thorpe BD, Marcus S. Phagocytosis and intracellular fate of *Pasteurella tularensis*. III. In vivo studies with passively transferred cells and sera. *J Immunol* 1965;**94**:578–85.
146. Tigertt WD. Soviet viable *Pasteurella tularensis* vaccines: a review of selected articles. *Bacteriol Rev* 1962;**26**:254–373.
147. Uhari M, Syrjala H, Salminen A. Tularemia in children caused by *Francisella tularensis* biovar *palaearctica*. *Pediatr Infect Dis J* 1990;**9**:80–3.
148. Vail DT. *Ophthalmic Res* 1914;**23**:487.
149. Van Metre TE Jr, Kadull PJ. Laboratory-acquired tularemia in vaccinated individuals: a report of 62 cases. *Ann Intern Med* 1959;**50**:621–32.
150. Versage JL, Severin DD, Chu MC, Petersen JM. Development of a multitarget real-time TaqMan PCR assay for enhanced detection of *Francisella tularensis* in complex specimens. *J Clin Microbiol* 2003;**41**:5492–9.
151. Vinogradov E, Perry MB. Characterization of the core part of the lipopolysaccharide O-antigen of *Francisella novicida* (U112). *Carbohydr Res* 2004;**339**:1643–8.
152. Vinogradov E, Perry MB, Conlan JW. Structural analysis of *Francisella tularensis* lipopolysaccharide. *Eur J Biochem* 2002;**269**: 6112–18.
153. Vinogradov EV, Shashkov AS, Knirel YA, et al. Structure of the O-antigen of *Francisella tularensis* strain 15. *Carbohydr Res* 1991; **214**:289–97.
154. Washburn AM, Tuohy JR. The changing picture of tularemia in Arkansas. *South Med J* 1949;**42**:60–2.
155. Wherry WB, Lamb BH. Infection of man with *Bacterium tularense*. *J Infect Dis* 1914;**15**:331–40.
156. Yee D, Rhinehart-Jones TR, Elkins KL. Loss of either $CD4^+$ or $CD8^+$ T cells does not affect the magnitude of protective immunity to an intracellular pathogen, *Francisella tularensis* strain LVS. *J Immunol* 1996;**157**:5042–8.
157. Young LS, Bicnell DS, Arecher BG, et al. Tularemia epidemic: Vermont, 1968. Forty seven cases linked to contact with muskrats. *N Engl J Med* 1969;**280**:1253–60.
158. Yuen JC, Malotky MV. *Francisella tularensis* osteomyelitis of the hand following a cat bite: a case of clinical suspicion. *Plast Reconstruct Surg* 2011;**128**:e37–8.
159. Zinkernagel RM. Restriction by H-2 gene complex of transfer of cell-mediated immunity to *Listeria monocytogenes*. *Nature* 1974; **251**:230–3.
160. Zinsser H. *Francisella*. In: Joklich WK, Willett HP, Amos DB, editors. *Zinsser microbiology*. 18th ed. Norwalk, Conn: Appleton-Century-Crofts; 1984. p. 649–55.

was the cause of influenza, and it initially was known as the Pfeiffer influenza bacillus.[329] The bacteria were difficult to culture on routine media until investigators appreciated that supplementation with X (hemin) and V (nicotinamide adenine dinucleotide [NAD]) factors was required for its growth. By the turn of the century, the organism had been recovered from the blood and cerebrospinal fluid (CSF) of young children with meningitis. Although doubts remained about the etiologic role of the Pfeiffer bacillus as the cause of influenza, not until the influenza pandemic of 1918 was its etiologic role questioned seriously. In 1920, the organism was renamed *Haemophilus influenzae* to acknowledge its inappropriate historic association with influenza and to emphasize its requirement of blood factors for growth (from the Greek *haemophilus*, or "blood loving").[461] In 1933, the viral etiology of influenza was discovered, which refuted any remaining confusion about the erroneous association between *H. influenzae* and influenza virus.

Key concepts relevant to the development of treatment and prevention modalities derive from the pioneering work of Margaret Pittman in the early 1930s.[333,334] Paralleling earlier research on the pneumococcus, she defined two major categories of *H. influenzae*: encapsulated and unencapsulated strains. Among the encapsulated strains, she characterized six distinct serotypes (designated a through f), which now are known to differ biochemically in the composition of their polysaccharide capsules (Table 133-1). She observed that Hib strains were recovered primarily from the blood and CSF of young patients with meningitis and that unencapsulated strains and other *H. influenzae* serotypes were recovered primarily from respiratory tract secretions. Furthermore, she demonstrated that antibody to Hib capsule conferred type-specific protection against lethal infection in rabbits. This observation led to the use of antiserum prepared by immunization with formalin-killed Hib, initially in horses and later in rabbits, as the first treatment of disease. Before this development, Hib meningitis and other forms of invasive Hib disease were almost always fatal.[418] However, not until the late 1930s did treatment of meningitis with both Hib antiserum and sulfonamides substantially reduce the case-fatality rate in children.[7,8]

In 1933, Fothergill and Wright[130] described the age-related risk for acquiring *H. influenzae* meningitis, which affected mostly children younger than 5 years of age. Importantly, they noted the correlation between the age-related risk for disease and the absence of bactericidal antibodies. Later researchers identified antibody to Hib capsule as the major antibody contributing to the protective activity of bactericidal serum.[199,372] These observations suggested that naturally acquired type b anticapsular antibody is protective and that early stimulation of protective immunity with vaccines might be possible. Unfortunately, the advent of effective antimicrobial agents focused attention away from the need for primary prevention. Even with effective antimicrobial therapies and excellent hospital care, significant mortality (approximately 5%) and neurologic morbidity (approximately 20%) remained.[97,98] Ultimately, appreciation that the morbidity and mortality of the disease could never be eliminated completely by treatment gave impetus to the development of vaccines for prevention.

In the early 1970s, investigators purified and characterized the type b capsular polysaccharide (polyribosylribitol phosphate [PRP]) and proposed it as a potential vaccine candidate. Subsequently, the protective efficacy of a PRP vaccine against invasive Hib disease was demonstrated in older children in a 1974 field trial conducted in Finland.[321] This and other studies culminated in the licensure of PRP vaccine in the United States in 1985, thus rendering it the first vaccine available for the prevention of Hib disease.[65] Unfortunately, this vaccine induced equivocal immune responses and incomplete protection in older children and provided no protection for young infants, those at greatest risk for acquiring Hib disease.[447] Improved vaccines used polysaccharide-protein conjugate techniques, and four such vaccines were licensed for use in children. Three of these vaccines were shown to protect young infants against Hib disease[38,362,430]; and with the universal immunization of young infants, invasive Hib disease nearly has been eliminated in many populations.[4,37,322] Unfortunately, Hib disease continues to be a significant cause of morbidity and mortality among children in a number of resource-poor countries around the world where access to Hib vaccines is still limited.[277,435,450]

TABLE 133-1 Structures of *Haemophilus influenzae* Capsular Polysaccharides

Type	Structure
a	4)-β-D-Glc-(1 → 4)-D-ribitol-5-(PO4 →
b	3)-β-D-Rib-(1 → 1)-D-ribitol-5-(PO4 →
c	4)-β-D-GlcNAc-(1→ 3)-α-D-Gal-1-(PO4 → 3 ↑ R = OAc | H R
d	4)-β-D-GlcNAc-(1→ 3)-β-D-ManANAc-(1 → 6 ↑ | R = L-serine | L-threonine R L-alanine
e	3)-β-D-GlcNAc-(1→ 4)-β-D-ManANAc-(1 →
e′	3)-β-D-GlcNAc-(1→ 4)-β-D-ManANAc-(1 → 3 ↑ 2 β-D-fructose
f	3)-β-D-GalNAc-(1→ 4)-α-D-GalNAc-1-(PO4 → 3 ↑ OAc

MICROBIOLOGY

Morphologic and Cultural Characteristics

H. influenzae is a small gram-negative coccobacillus that in clinical specimens can appear filamentous or

pleomorphic, especially when obtained from patients who previously have received antibiotics. The organism is nonmotile, non–spore forming, and facultatively anaerobic and requires two supplemental factors for in vitro growth.[231] The X factor (hemin) is a heat-stable, iron-containing protoporphyrin essential for activity of the electron transport chain, which is important for aerobic growth. The heat-labile V factor (NAD) is a coenzyme. Both factors are present within erythrocytes and are released by appropriate heating or enzyme lysis of the red blood cells, which permits growth on chocolate agar. The requirement for these factors for growth remains the primary basis for the laboratory differentiation of *H. influenzae* from other *Haemophilus* species.[21,231]

The growth of *H. influenzae* is fastidious, and clinical specimens need to be inoculated promptly onto appropriate media, such as chocolate agar.[231] The organism can be grown in most enriched liquid or solid media supplemented with X and V factors. Although not mandatory for growth, 5 to 10 percent carbon dioxide makes some strains grow better. In blood or liquid media, *H. influenzae* may not grow to sufficient quantity to result in visual turbidity; therefore, to detect positive cultures, blood and CSF cultures should be assayed for release of carbon dioxide or routinely subcultured at 24 to 48 hours. After overnight incubation on solid media, colonies appear that are 0.5 to 1.5 mm in diameter and usually rough or granular. Encapsulated strains generally produce slightly larger colonies that are mucoid or glistening. Fermentation reactions and other metabolic activities are variable and, therefore, not particularly useful for identification. However, a biotyping scheme based on the metabolism of indole, urea, and ornithine decarboxylase activity has been used to subtype strains.[231]

Capsular Polysaccharides

Several surface structures of *H. influenzae* contribute to the organism's pathogenicity, but one of the most important is the capsular polysaccharide. Strains can express one of six unique polysaccharide capsules (a, b, c, d, e, or f) (see Table 133-1) or lack capsule expression completely (nontypeable strains). The Hib capsule is of particular clinical, pathogenic, and immunologic importance because Hib accounts for 95 percent of all strains that cause invasive disease in nonimmunized populations.[50,252,259] The Hib polysaccharide consists of a repeating polymer of ribosyl and ribitol phosphate (PRP) that has a 1-1 linkage.[102] The genes involved in production of the Hib capsule have been cloned and consist of two repeating 17-kb DNA fragments separated by a 1-kb bridge region *(bex A)*[188,219]; 98 percent of Hib organisms tested contain this duplication.[219] Encapsulation often is unstable, with loss of capsule production associated with loss of one 17-kb repeat.[218] These strains produce type b capsule, but in barely detectable amounts. Of importance is that the release of Hib capsular antigen in the body fluids of infected individuals can be detected by specific immunologic techniques (i.e., latex agglutination) that are useful for rapid diagnosis.[204,449] The other capsular serotypes are composed of hexose rather than pentose sugars,[102,103,422,423] and these strains also occasionally result in invasive disease.[149,178,227]

Noncapsular Cell Wall Antigens: Proteins

The cell envelope of *H. influenzae* is typical of other gram-negative bacteria, consisting of a cytoplasmic membrane, a peptidoglycan layer, and an outer membrane that represents the outermost layer of the envelope. The outer membrane contains an array of proteins, lipopolysaccharides, and phospholipids, many of which serve important functional roles for the organisms. Over the past 2 decades, significant advances have been made in our understanding of these many outer-membrane components and their contributions to the pathogenesis of disease.[105,286,291]

Early laboratory investigations of *H. influenzae* identified and characterized a number of major and minor outer-membrane proteins of the organism.* However, an understanding of the functional roles of most of these proteins was lacking. Subsequent studies have helped to clarify the roles in disease pathogenesis of an increasing number of these proteins. Several proteins have been shown to be important for attachment of *H. influenzae* to host respiratory mucosal tissues.[55,61,85,175,281] Other surface proteins have been shown to play a central role in bacterial acquisition of iron, a phenotypic trait critical for survival of the organism in the human host.† Other studies have identified bacterial surface proteins important in resistance of *H. influenzae* to components of the host innate immune system, the first line of defense in the nonimmune host.[92,171,172,174,260] The availability for study of the completed genomes of several representative *H. influenzae* strains[182,187,284,383] has also helped to increase the rate of discovery of other proteins important in disease pathogenesis.

Noncapsular Cell Wall Antigens: Lipo-oligosaccharide

Early studies of *H. influenzae* lipo-oligosaccharide demonstrated that although it differed chemically and structurally from the lipopolysaccharide of Enterobacteriaceae, it shared a number of biologic properties with the latter molecule. Lipo-oligosaccharide purified from Hib produced a dermal Schwartzman reaction, was lethal to mice, caused a febrile response in rabbits, evoked polyclonal B-cell activation, and had limulus lysate activity.[128] In addition, human leukocytes incubated with *H. influenzae* lipo-oligosaccharide generated potent procoagulant activity,[275] a property that may be relevant to understanding the mechanisms responsible for intravascular coagulation in severe infection.

Over the past 20 years, knowledge concerning the genetics, structure, biosynthesis, and role in pathogenesis of the *H. influenzae* lipo-oligosaccharide has increased

*References 31, 283, 285, 290, 305, 387.
†References 6, 196, 212, 261, 278, 466.

greatly. *H. influenzae* lipo-oligosaccharide consists of a conserved triheptose inner core linked via a single 2-keto-3-deoxy-octulosonic acid molecule to lipid A from which there are oligosaccharide extensions (outer core), mainly hexose sugars, that vary from one strain to another.[169,256,257,408] *H. influenzae* lipo-oligosaccharide lacks the repetitive side chains typical of gram-negative enteric bacteria but displays great complexity in its outer core sugars and other substituents such as phosphorylcholine[451,452] and sialic acid.[10,47] Several of these sugars and nonsugar substituents are phase variable.[167,168,452] Phase variation is mediated by translational switching of genes that contain tetranucleotide repeats within the 5′ ends of the respective reading frames.[169,192,452] Phase-variable expression of several of these lipopolysaccharide components has been shown to contribute to the pathogenesis of disease (see later).

IgA Proteases

IgA proteases are bacterial enzymes whose only known substrate is human IgA1. These secreted enzymes cleave the IgA heavy chain at specific sites.[215] IgA proteases are regarded as potentially important virulence factors because mucosal defense is in part IgA mediated. *H. influenzae* produces at least two distinct types of IgA protease that cleave different peptide bonds within the IgA1 hinge region.[282,335] *H. influenzae* strains isolated from patients with disease express higher levels of IgA protease than strains isolated from asymptomatic carriers,[434] again suggesting that IgA protease is an important virulence factor for *H. influenzae*. In more recent work, investigators have identified and characterized another IgA protease gene that is present in about a third of *H. influenzae* strains.[120] This latter gene, designated *igaB*, is distinct from the previously characterized IgA protease gene, and its presence appears to be associated with higher levels of IgA protease expression and with a greater likelihood of causing disease,[120] particularly in patients with chronic obstructive pulmonary disease.[292]

Population Structure

A number of powerful typing techniques have been developed for identifying individual *H. influenzae* strains and defining the relationships between strains.[270,300,328] These techniques have provided valuable information for a variety of epidemiologic studies and have served to define the population structure for *H. influenzae* organisms as a whole.[207,374] Encapsulated *H. influenzae* can be grouped into two distinct primary phylogenetic divisions.[300] Furthermore, the population structure of encapsulated *H. influenzae* is clonal. Most of the invasive disease worldwide is caused by serotype b strains from a limited number of clones.[219] Strains producing serotype c, e, and f capsules belong to single divisions and have no close genetic relationships to strains of other serotypes.[300] Serotype a and b strains occur in both primary phylogenetic divisions, probably as a result of transfer and recombination of serotype-specific sequences of the cap region between clonal lineages.[220,300] In contrast to the encapsulated strains, the unencapsulated or nontypeable *H. influenzae* strains demonstrate substantial genetic diversity.[207,299,328,374] Contributing to the genetic diversity of the nontypeable *H. influenzae* are apparently rather high rates of recombination between individual bacterial strains.[82,142,270,409]

Antibiotic Resistance

Another important feature of *H. influenzae* has been the development of antibiotic resistance. Resistance to a wide variety of antibiotics (sulfonamides, trimethoprim-sulfamethoxazole, erythromycin, tetracycline, penicillin) has been described, but these antibiotics are not essential for therapy. Of greater importance is resistance to ampicillin, first noted in the mid 1970s,[259] because it was the primary antibiotic used for the treatment of disease. Since then, ampicillin resistance has become widespread, with between 5 and 40 percent of all isolates in various parts of the world now resistant.[27,87,184,389] The mechanism of resistance usually involves the production of plasmid-mediated β-lactamase enzyme, and resistant strains often are characterized by their plasmid or β-lactamase enzyme content.[202] Recently, non–β-lactamase–mediated ampicillin and ampicillin–clavulanic acid resistance have been reported among an increasing percentage of isolates from around the world, particularly among nontypeable *H. influenzae*.[87,149,389,420] The resistance in these stains appears to be mediated primarily by alterations in specific penicillin-binding proteins.[27,409,420] Resistance to chloramphenicol usually is mediated by the enzyme chloramphenicol acetyltransferase.[352,439] Although chloramphenicol-resistant strains are rare in the United States, they are more prevalent in some areas of the world, and strains resistant to both ampicillin and chloramphenicol have been reported.[64,316,425] Currently, third-generation cephalosporins are the mainstays of therapy for invasive disease; concern about the potential for increasing resistance to these highly effective agents further emphasizes the need for means to prevent disease.[420]

PATHOGENESIS

Acquisition and Carriage of Organisms

Illness caused by *H. influenzae* infection results from a series of pathogenic events beginning with exposure to the organism followed by colonization of respiratory mucosal membranes.[131] Under natural conditions, *H. influenzae* is exclusively a pathogen of humans, and it usually is transmitted asymptomatically from person to person by transfer of respiratory secretions.[184,314,319] The incubation period is unknown because many transmission cycles may occur before a susceptible person becomes ill. Furthermore, in some individuals, the organism can be carried in the upper respiratory tract for many months before it causes disease.[88,342,392]

Both typeable and nontypeable organisms may be present as part of the normal flora of the upper

respiratory tract.[195] Nearly all individuals (as many as 80%) are colonized at some point with nontypeable strains.[184,319,342] In the prevaccine era, Hib carriage rates were lowest in adults and young infants and highest in preschool-aged children.[424] In a prevaccine era study conducted at a daycare center in Dallas where no invasive infections occurred, the average rate of colonization with Hib was 10 percent.[293] During the 18 months of the study, 71 percent of the children 18 to 35 months of age and 48 percent of the children 36 to 71 months of age were colonized at some time. Carriage rates were substantially higher in households or daycare centers in which a case occurred. For example, colonization prevalence rates among children in daycare centers where a case of invasive disease had occurred were as high as 58 to 91 percent.[158,446] Likewise, within families in which a case of invasive disease occurred, rates of colonization of 60 to 70 percent in siblings and 20 percent in parents were observed.[57,446] Whether the high carriage rates in these exposed semi-closed populations were the cause or the result of disease was not clear.[262,273] Close contact among exposed susceptible individuals, as occurs within families and daycare centers, facilitated the risk for transmission and acquisition of disease.

Despite a low point prevalence of Hib pharyngeal carriage (1% to 5%) in the prevaccine era, most young children became colonized with Hib during the first 2 to 5 years of life.[262,424] Consequently, specific Hib immunity developed in these colonized children.[164,372] Hib strains were demonstrated to persist in the nasopharynx for months[273,293] and often were not eliminated by treatment with antimicrobial agents that did not penetrate into respiratory secretions.[381,382] The relationship between carriage of Hib and the subsequent development of disease and immunity is not well understood. Factors that influence the efficiency of transmission and the ability of the organism to establish colonization also are understood poorly. Two factors that probably potentiate the risk for acquiring infection and invasive disease are the size of the bacterial inoculum[403] and the presence of a concomitant viral infection.[217]

Data derived from animal models of infection may be of some benefit in understanding the dynamic nature of the colonization process. Inoculation of Hib organisms into the nose of infant animals results in local infection.[279,355] In this model system, rhinorrhea was not noted but nasopharyngeal washings revealed numerous polymorphonuclear leukocytes and organisms that reached maximal density in approximately 24 hours. Shortly thereafter, bacteremia could be detected in the animals. With colonization of humans, Hib is found on the surface of the respiratory mucosa. Rarely, an organism can be observed by electron microscopy penetrating through a nasal mucosal epithelial cell.[403] An acute inflammatory response to the submucosal bacteria occurs, but it is not marked. The exact mode of entrance of the organisms into the vascular compartment is unknown, but it is assumed that they enter via lymphatics, probably carried by phagocytic cells, which are found in the submucosa. In support of this hypothesis, Rubin and Moxon[355] detected early transient bacteremia in rats after nasal inoculation.

Pathogenesis of Mucosal Infections

Mucosal colonization and mucosal infections occur much more frequently than do invasive bacteremic infections, particularly in those populations where Hib vaccine is in widespread use. Mucosal infections, most of which are caused by nontypeable *H. influenzae*, still cause considerable morbidity and are associated with substantial health care costs.[363,404,412] Mucosal infections generally involve direct movement of organisms through the nasal ostia to the sinuses, up the eustachian tubes, where they cause otitis media, and down the bronchi, where they cause bronchitis and pneumonia. Bacteremia rarely is involved, and such infections generally are not life threatening. These infections appear to be enhanced by antecedent viral infection,[74,318] eustachian tube malfunction,[406] foreign bodies, or mucosal damage from smoking or other irritants.[359]

A considerable amount of recent research has focused on better understanding of the molecular pathogenesis of mucosal surface infections caused by nontypeable *H. influenzae*. The same principles almost certainly apply to the early steps in mucosal colonization by encapsulated *H. influenzae*. The following model has been proposed to explain early events in the process.[181] After transmission from an *H. influenzae* carrier to a new host,[88] bacteria first come in contact with the upper respiratory tract. Here they must overcome the nonspecific mucociliary defenses if colonization is to persist.[377] *H. influenzae* major outer-membrane proteins, P2 and P5, and probably other factors promote bacterial binding to mucus and help establish an early foothold for the bacteria in the upper respiratory tract.[343,403] Local elaboration of lipopolysaccharide and other bacterial products then causes damage to ciliated cells and further impairs mucociliary function.[442] Subsequently, adhesins including HMW1 and HMW2, Hia, Hap, pili, protein E, and others help mediate direct adherence to nonciliated epithelial cells.* Cleavage of IgA1,[120,292,434] invasion into cells and the subepithelial space,[129,403,407] and phase and antigenic variation of surface antigens facilitate evasion of local immune mechanisms.[107,192,452] Binding and uptake of iron and heme allow organisms to persist on the respiratory mucosa despite the relative scarcity of these nutrients.[261,376,457] In the setting of a viral infection, allergic disease, or exposure to cigarette smoke, bacteria spread from the nasopharynx to other sites within the respiratory tract and produce symptomatic disease.[74,318,406]

Pathogenesis of Invasive Disease

Once mucosal colonization with Hib and other potentially invasive strains has been established, dissemination may occur from the upper respiratory tract mucosa to the bloodstream and then elsewhere in the body.[355] The incidence of invasive disease is a small fraction of the carrier rate for Hib.[293] When dissemination does occur, the organism appears to invade the mucosa by separating the

*References 23, 32, 125, 145, 146, 390, 400.

apical tight junctions of the columnar epithelium and moving intercellularly.[403] The resulting bacteremia initially is low in concentration but steadily increases over the course of hours.[315] The dynamics between bacterial proliferation and clearance are influenced by antibody, complement, and phagocytes, all of which have an effect on the magnitude of the bacteremia.[454] The polysaccharide capsule of Hib is antiphagocytic and a major virulence factor. In the absence of anticapsular antibody, bacteremia increases steadily over a period of hours.[166] When the bacterial concentration exceeds 10^4 organisms/mL, metastatic seeding occurs, especially to the meninges via the choroid plexus. Although meningitis is the most frequently recognized manifestation of invasive Hib disease, other potential metastatic sites include the lungs, joint synovium, pleura, peritoneum, and pericardium.[50,418]

The pathogenic events that lead to pneumonia, cellulitis, and epiglottitis are less well understood, even though these invasive infections are associated with bacteremia. Presumably, pneumonia occurs after the aspiration of a critical number of virulent organisms, epiglottitis involves focal infection of the epiglottis, and cellulitis occurs by secondary seeding of deep subcutaneous tissues via the bloodstream.[147] With all forms of invasive Hib disease, invasion of the bloodstream occurs as either a primary or a secondary event.

Viral interactions enhance the pathogenesis of Hib.[303,411] Influenza virus infection has been shown to reduce neutrophil chemotaxis, bacterial killing, systemic macrophage function, the number of circulating T cells, T-cell blastogenesis, and expression of delayed cutaneous hypersensitivity.[1,2,83] Reduced bacterial killing may be due to a defect in phagosome-lysosome fusion that is maximal 5 to 7 days after viral infection and inoculation of Hib.

Bacteremia in Animal Models

During bacteremia, organisms are cleared continually from the vascular compartment by antibody, complement, and the reticuloendothelial system.[280,355] The balance of these processes determines the magnitude and duration of the bacteremia. If bacterial clearance is stopped by reticuloendothelial blockade, bacterial densities increase to a maximum and death quickly ensues, presumably because of the effects of endotoxin.

Initially, bacterial concentrations are very low (approximately 100 organisms/mL). They then steadily increase in density over the course of the next 24 hours and reach a plateau value.[355,356] In young animals, this plateau level is 10^7 organisms/mL and usually is associated with the features of human sepsis. In older animals, the plateau value is lower: approximately 10^4 organisms/mL. These animals have low-grade fever but a relative paucity of symptoms. This primary bacteremia leads to seeding of serous surfaces, such as the peritoneum, diarthrodial joints, pleura, pericardium, and meninges. Early in the infectious process, organisms can be obtained from all these surfaces, but without an observable inflammatory response; later in the process, an inflammatory response ensues. In experimental animals, the first evidence of an immune response is antibody directed against somatic antigens.

Strains of Hib vary in virulence potential. Furthermore, the other five capsular types or nonencapsulated strains result in only transient, low-level, or undetectable bacteremia, even with large inocula (>10^7 colony-forming units).[470] Complement and the spleen are critical factors for host defense in the rat. Rats depleted of C3 and splenectomized (or with iatrogenic splenic congestion caused by hemolytic anemia) have an increased incidence and magnitude of bacteremia.[71,470] These studies suggest the importance of the alternative pathway of complement (opsonic antibody) and reticuloendothelial phagocytes as determinants of intravascular clearance.

Meningitis

Bacteremia precedes the development of meningitis, except for rare situations in which direct extension of infection from adjacent sinuses or an ear infection occurs. Data from experimental studies in infant rats and infant monkeys support this hypothesis.[279] Both the magnitude and duration of bacteremia are probably the primary determinants of invasion of the central nervous system (CNS). After a critical bacterial concentration is exceeded in blood, Hib appears to enter the CNS via the choroid plexus.[395] This theory is supported by the following: (1) the earliest histopathologic lesion seen in the CNS is choroid plexitis, (2) the choroid plexus is one of the foci seeded from the bloodstream, and (3) bacterial density early in infection is greater in the lateral cerebral ventricles than in other CSF compartments.[395] Furthermore, pulse-chase experiments using tracer strains show that organisms enter the CSF through the choroid plexus and that inflammation of the choroid plexus is a uniform feature of meningitis. Subsequently, organisms infect the CSF and the arachnoid villi of the leptomeninges and cause blockage of CSF return, thereby increasing bacterial density and CSF pressure.[395] Generally, the magnitude of CSF bacterial density correlates with the severity of clinical illness.[117,367] Egress of CSF from the subarachnoid space is by flow through the subarachnoid villi, and bacterial density in CSF can be increased or decreased by manipulating CSF egress, which occurs in meningitis via inflammatory responses. The inflammatory response of the choroid plexus is followed by pachymeningitis, which also inhibits CSF reabsorption and increases pressure. Phlebitis of the cerebral blood vessels and thrombosis can occur. All these events contribute to decreased blood flow to the cortex. The resulting increased bacterial density, inflammation, edema, cranial nerve damage, and overall increased CSF pressure are responsible for the morbidity and mortality associated with meningitis.[98,116,395] Parenchymal invasion of the brain rarely occurs.

IMMUNOLOGY

Resistance to *H. influenzae* infection depends on successful integration of a wide variety of host defenses, including (1) mucosal factors that prevent the organism from attaching and penetrating the respiratory epithelium[232,396,401]; (2) activation of the alternative and classical complement pathways, which leads to killing

of the organism and initiation of other inflammatory responses[402,460]; (3) induction of antibody formation[156,160]; (4) phagocytosis and killing by macrophages and polymorphonuclear cells in tissues, the circulation, and the reticuloendothelial system[35,298,462]; and (5) cell-mediated immunity.[100] Assessing the role of each of these immunologic mechanisms independently or determining which mechanisms are most important in host defense is difficult. Although antibodies are not the sole defense against bacteremia, it has been the research emphasis of vaccine development.[156] The goal has been to induce antibodies that are bactericidal, opsonophagocytic, and ultimately protective. Although usually only antibody is measured in these studies, other immune factors are induced and probably play important roles in protection.

Anticapsular Antibody

Initially, antibody activity was assessed by measuring agglutinin and bactericidal titers of serum. In 1933, Fothergill and Wright[130] suggested that bactericidal activity was responsible for immunity to Hib meningitis and that acquisition of this immunity correlated with the age of the individual. Although antibodies to several surface antigens of *H. influenzae* play roles in conferring immunity to Hib[384], antibody to Hib capsular polysaccharide appears to be of primary importance.[351] Newborns and young infants are at low risk for the development of infection, presumably because they have maternally acquired antibody. Young children at highest risk for acquiring disease have low or undetectable levels of antibody, whereas older children at lower risk have higher antibody levels. By 5 years of age, most children have naturally acquired anticapsular antibody that appears to provide protection,[164,372] although natural exposure also induces antibodies to outer-membrane proteins, lipopolysaccharide, and other surface antigens of the bacteria that contribute to natural immunity. The evidence that anticapsular antibodies protect humans from the acquisition of invasive Hib disease is considerable: they activate complement,[402,460] are opsonophagocytic[306] and bactericidal,[130,306] and protect animals from lethal Hib challenge.[405] Moreover, passive prophylaxis with serum preparations containing anticapsular antibody protects agammaglobulinemic patients[351] and high-risk children from the acquisition of invasive Hib disease.[361] Furthermore, in the preantibiotic era, immune serum was an effective therapy for Hib disease.[7,333] However, the most compelling evidence for the protective efficacy of PRP antibody is the clinical protection achieved in older children vaccinated with purified PRP vaccine[321] and younger infants immunized with Hib conjugate vaccines.[252,322] Induction of antibody to Hib polysaccharide is the immunologic basis of all Hib vaccines.

A precise minimal level of anti-PRP antibody that is protective has not been established.[156] Data from passive protection of agammaglobulinemic children, challenge experiments in infant rats, and studies of naturally acquired antibody levels in healthy individuals of various ages suggest that the minimal serum concentration of anti-PRP antibody that provides protection ranges from 0.05 μg/mL in animals[373] to 0.15 to 1.00 μg/mL in humans.[210,321] Such estimates are crude and do not take into account the different functional properties of different immunoglobulins[160,247] or the contribution of antibodies to other Hib antigens. In addition, antibody levels decline over time and a given peak level may not reflect levels at the time of exposure, which would predict long-term protection better.[16,321] In a Finnish PRP vaccine trial, an antibody level greater than 1.0 μg/mL 1 month after immunization correlated with clinical protection for a minimum of 1 year.[321] However, this antibody level might not be extrapolated readily to the immunogenicity data evaluated in different studies or with different Hib conjugate vaccines.

Class and Subclass-Specific Antibody

Several studies have shown variable immunoglobulin class, isotype, idiotype, and IgG subclass responses to PRP polysaccharide after natural Hib exposure, disease, and immunization.[211,373] Most individuals respond with IgG antibodies after receiving PRP immunization, although some children have predominantly IgA or IgM responses.[211] Schreiber and associates[373] showed that IgG antibody is bactericidal, opsonic for polymorphonuclear leukocytes in the presence of complement, and protective for animals. IgM antibody is equally protective and more bactericidal than IgG in the presence of complement, but it opsonizes poorly. IgA antibody is not bactericidal, opsonic, or protective for animals. Some researchers have hypothesized that IgA-specific antibody blocks the activity of other more functional antibodies and thereby may depress immunity.[297]

Data from experiments in mice and humans suggest that polysaccharide antigens induce restricted IgG subclass responses.[349] The findings of increased susceptibility to Hib disease in IgG subclass–deficient patients (predominantly IgG2 and IgG4 deficiencies)[317,375] and the low levels of IgG2 in children younger than 2 years of age[380] suggest that differences exist in the role of subclass-specific anticapsular antibodies. In adults, natural exposure or immunization with PRP vaccine results in a predominantly IgG2 subclass response.[340] In children, IgG1 and IgG2 antibodies develop after PRP immunization, but IgG1 antibodies predominate after immunization with Hib conjugate vaccines.[12,189,208] Human anti-PRP antibodies express predominantly kappa light chains[414] and may be grouped into a few restricted clonotypes. These clonotypes and antibody specificities have been characterized by idiotype analysis[245,248,388] and amino acid sequencing of the immunoglobulin light chain.[414] Individuals of different ages produce different proportions or repertoires of antibody.[246] Some differences in binding specificity and affinity have been described with the different anti-PRP antibodies,[247,248,369] but whether the antibodies have different degrees of protective potency or are substantially different in proportion in individuals given different vaccines is not clear.

The role of mucosal immunity in killing Hib or inhibiting adherence or penetration of the mucosa is understood poorly, although studies of secretory IgA antibody to the Hib capsule have been conducted.[331,332] Moreover, Hib strains produce IgA proteases that can inactivate

mucosal antibody.[120,282] The observation of reduced carriage of Hib in children given Hib conjugate vaccines[121,195,295,311,410] suggests that mucosal immunity may be important in reducing transmission of the disease.

Cellular Immune Responses

Most of our understanding of the interactions of B cells, T cells, and antigen-presenting cells (macrophages) is derived from extensive research in mice.[91] Based on T-cell involvement in antibody synthesis, antigens can be classified as T-dependent (thymus-dependent) or T-independent immunogens. Most protein antigens induce helper T-cell regulation of antibody synthesis and, therefore, are considered T dependent. These antigens first are recognized and processed by macrophages and then presented to both T and B cells. The activated T cells induce proliferation and differentiation of specific antigen-reactive B-cell subpopulations. They also retain the memory necessary for subsequent booster responses.[33] Through the release of cytokines, helper T cells appear to regulate (1) the magnitude of the immune response, especially in young infants; (2) the switch in immunoglobulin classes (IgM to IgG); (3) the functional activity of antibody; and (4) the capacity to elicit immunologic memory.

Polysaccharides consist of repeating oligosaccharide units and elicit weak immune responses involving minimal T-cell influences.[33] These T-independent antigens elicit antibody responses primarily by direct stimulation of B cells. In general, polysaccharide vaccines have the following T-independent immunologic characteristics: (1) delayed ontogeny of immune responsiveness in the young, (2) limited and variable quantitative immune responses, (3) restricted isotype (predominantly IgM) and IgG subclass responses, and (4) lack of a booster or anamnestic response with secondary antigenic challenge. The quest for a Hib vaccine that is immunogenic and protective in young infants has involved attempts to convert the PRP antigen from a T-independent to a T-dependent antigen by using the carrier-hapten principles first defined by Landsteiner in the first half of the 20th century.[228] PRP can be considered a hapten that is linked covalently to a T-dependent immunogen, a carrier, to form a conjugate vaccine. The Hib conjugate vaccines, which demonstrate markedly enhanced immunogenicity, are described in a subsequent section.

Genetic Factors

When compared with protein vaccines, the immune responses to most polysaccharide antigens are variable and may be influenced by genetic factors. Several studies have shown associations between the immune responses to PRP vaccine and genetically determined factors such as red cell antigens, human leukocyte antigen, or immunoglobulin allotypes.[11,162,379,456] However, because many factors influence immunogenicity, whether these associations have relevance is not known, and establishing controls for them is difficult. In addition, whether the antibody differences are important clinically also is not known. No single genetic relationship regulating susceptibility or the immune responses to polysaccharide antigens has been demonstrated convincingly.

Complement

The importance of complement components in host defense against Hib is substantiated by elimination of the bactericidal activity of serum via heat, by the susceptibility of complement-depleted animals to Hib disease, and by the increased susceptibility of patients with specific congenital complement deficiencies.[86,402,460] Hib is capable of activating both the classical and the alternative complement pathways,[86,402] thereby initiating opsonophagocytosis and cell killing and eliciting other inflammatory responses. Whereas the alternative pathway probably is most important early in the course of infection in a nonimmune host, the antibody-dependent classical complement pathway is more likely to predominate as a defense mechanism at a later stage of infection.[460] Both encapsulated and unencapsulated organisms activate complement, thus underscoring the importance of noncapsular antigens in host defense.[296,298] Although the Hib capsule is a poor activator of the alternative complement pathway, antibody to the capsule activates both the classical and the alternative pathways.[402] Other cell wall antigens activate the alternative pathway, and antibody to these antigens activates the classical pathway.[86,402,460] Thus, antibodies to both capsular and noncapsular antigens activate the complement system, primarily via the classical pathway. Activation of the terminal complement components mediates the bactericidal activity of serum.

Later studies explored the contribution of lipopolysaccharide structure to the susceptibility of *H. influenzae* to complement-dependent serum bactericidal activity.[122,169,250,451] Phase-variable structural modifications to the core lipopolysaccharide structure including additions of phosphorylcholine,[77,452] sialic acid,[378] and other carbohydrate moieties[106,467] all have been shown to influence the susceptibility of these organisms to complement-mediated killing. Related studies in animal models of infection have also demonstrated the importance of these phase-variable lipopolysaccharide modifications to disease pathogenesis in vivo.*

More recent work has focused on the ability of *H. influenzae*, particularly nontypeable strains, to modulate activation of the complement cascade by interacting with human complement regulatory proteins.[174] In the presence of human serum, nontypeable *H. influenzae* strains are able to bind the C4B binding protein,[172] the human complement inhibitor factor H,[177] and vitronectin.[176,391] The presence of these proteins on the bacterial surface protects the organisms against complement-mediated attack and contributes to the survival of *H. influenzae* in the presence of human serum and serum components.[173]

Phagocytosis

Opsonization leading to phagocytosis and killing of *H. influenzae* also is an important determinant of host

*References 47, 122, 197, 229, 419, 451.

defense.[296,298,405,462,464] Impairment of phagocytic function or a reduction in the number of phagocytes results in increased susceptibility to disease, as does loss of the spleen or impairment of its function (e.g., hemoglobinopathies).[72,337] The opsonic activity of serum is influenced greatly by the roles of complement and antibody. Opsonization and phagocytosis of Hib appear to be dependent on (1) IgG binding, (2) antibody activation of the classical complement pathway with deposition of C3b on the bacterial surface, and (3) direct bacterial activation of the alternative complement pathway.[308] Relatively little is known about direct cell-mediated killing of *H. influenzae*.[100]

EPIDEMIOLOGY

Haemophilus influenzae type b

Humans are the only natural host for *H. influenzae*, and asymptomatic nasopharyngeal carriage, usually by unencapsulated strains, is a common finding.[113,262] In the era before widespread use of Hib conjugate vaccines, nasopharyngeal acquisition of Hib strains was also quite common and was shown to increase after infancy and to persist for weeks to months. Most children were colonized at some time during the first 5 years of life.[262] Colonization rates greater than 70 percent occurred after recent exposure in closed populations, such as among family members or daycare center contacts of a patient with disease.[293] Person-to-person transmission occurred via respiratory droplets, and fomites were also thought to play a role. As Hib conjugate vaccine administration has become part of the routine infant immunization schedule in many countries, there has been an associated and pronounced drop in the rate of nasopharyngeal carriage of Hib strains.[195,244,294,311,410]

Before the development of immunization, invasive Hib disease was a leading infectious disease problem worldwide that affected primarily young children (Table 133-2).[440,455,459] Hib strains are responsible for more than 95 percent of invasive infections in children. According to population-based studies, an 20,000 to 25,000 persons acquired invasive Hib disease annually in the United States, 85 percent of which occurred in children younger than 5 years of age.[50] The incidence of Hib meningitis and all invasive Hib disease was 40 to 69 and 67 to 130 cases per 100,000 children younger than 5 years, respectively. Invasive Hib disease developed in an estimated 1 in every 200 children in the United States during the first 5 years of life.[80] Hib pneumonia was estimated to cause as many as 15 percent of cases of ambulatory pneumonia in children younger than 6 years old, but the true incidence was unknown because microbiologic diagnosis is difficult. Overall, the incidence of Hib disease in the prevaccine era was similar to that of paralytic poliomyelitis during its peak epidemic years before immunization. A bimodal seasonal pattern had been observed in several studies, with one peak occurring between September and December and a second peak between March and May.[344,455] The attack rate of Hib was slightly higher in boys.[254]

TABLE 133-2 **Worldwide Incidence of Invasive *Haemophilus influenzae* Type b Disease Before the Use of Hib Vaccines in Children Younger Than Age 5 Years**

Region	Years	Hib Meningitis*	All Hib Disease*
Australia/ New Zealand	1985-1987	25-53	39-92
U.S./Canada	1959-1991	40-69	67-130
Europe	1985-1990	15-26	33-60
Israel	1985-1990	18	34
Africa	1980s	36-60	NA
South America	1989-1990	15-25	21-43
Asia	1990s	1.3-1.9	1.9-2.7

Hib, *Haemophilus influenzae* type b; NA, not available.
*Annual incidence per 100,000 population younger than 5 years of age.
Adapted from Vadheim CM, Ward JI. Epidemiology in developed countries. In: Ellis RW, Granoff DM, editors. *Development and clinical uses of Haemophilus b conjugate vaccines.* New York: Marcel Dekker; 1994, p. 231–45; and Bijlmer HA. Epidemiology of *Haemophilus influenzae* invasive disease in developing countries and intervention strategies. In: Ellis RW, Granoff DM, editors. *Development and clinical uses of Haemophilus b conjugate vaccines.* New York: Marcel Dekker; 1994, p. 247–64. Courtesy Marcel Dekker, Inc.

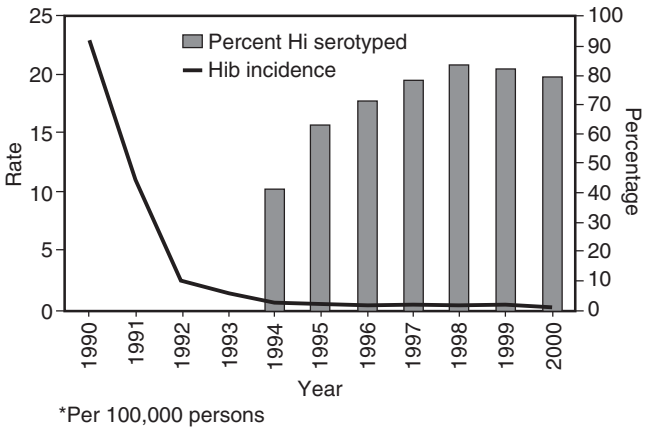

FIGURE 133-1 ■ Incidence rate of *Haemophilus influenzae* type b (Hib) invasive disease per 100,000 persons and the percentage of *H. influenzae* (Hi) isolates serotyped in children younger than 5 years—United States, 1990-2000. (From Centers for Disease Control and Prevention. Progress towards elimination of *Haemophilus influenzae* type b invasive disease among infants and children—United States, 1998-2000. *MMWR Morb Mortal Wkly Rep* 2002;**51**:234–37.)

The widespread use of Hib conjugate vaccines altered the epidemiology of invasive Hib disease dramatically (Fig. 133-1).[252] The first Hib conjugate vaccine was licensed in the United States in 1987 for use in children 18 months or older.[66] Subsequently, decreases in the incidence of Hib disease were seen in older children and, unexpectedly, in unimmunized infants and children as well. This finding was attributed to a direct effect of vaccination on nasopharyngeal carriage, thus decreasing the

environmental burden of Hib infection and, ultimately, the transmission of disease. In late 1990, Hib conjugate vaccines were approved for use in infants beginning at 2 months of age, which heralded a dramatic decline in the incidence of invasive Hib disease in all children.[67] In populations in which immunization rates are high, the development of invasive Hib disease has been eliminated almost completely.[224,252]

Some population-based studies of the incidence of *H. influenzae* disease have been conducted outside the United States, primarily in Western Europe, and they showed an incidence approximately one third to two thirds that in the United States.[323,360,398] The incidence of Hib disease is especially high in certain ethnic groups, including Aboriginal children in central Australia[180,195]; Navajo Indians; Alaskan natives; and Apache, Yakima, Athabascan, and Canadian Indians.[243,252,274,448,469] *H. influenzae* appears to rank as a leading cause of bacterial meningitis in some developing countries as well.[9,56,397,443,468] As seen in the United States, a substantial reduction in Hib disease has occurred with the implementation of widespread vaccination programs.

Children between the ages of 6 and 18 months are at highest risk for acquiring invasive Hib disease[254]; however, the age distribution of specific clinical syndromes of the disease varies. The peak incidence of Hib meningitis occurs in children 6 to 9 months of age and declines markedly after 2 years of age.[134,413] Hib cellulitis tended to occur during the first year of life, whereas epiglottitis generally occurs in unimmunized children older than 2 years of age. Invasive *H. influenzae* disease was seen much less frequently in adults because of the development of protective antibodies over time, but it occurred more frequently in immunocompromised patients. Nonetheless, Hib caused pneumonia and meningitis in adults[114,326] and disease caused by other serotypes and nontypeable strains, especially pneumonia, otitis media, bronchitis, and sinusitis, are common findings in all age groups.[203,224,252,265] Most adults in whom invasive *H. influenzae* disease develops were not immunized as children and have an underlying condition such as chronic obstructive pulmonary disease, human immunodeficiency virus (HIV) infection, alcoholism, pregnancy, or malignancy.[51,114,326]

The development of invasive Hib disease in a given individual is the consequence of a complex interaction of a variety of factors, including the risk for exposure, the characteristics of the organism, and the host.[132] In populations that experienced a high incidence of disease, such as American Indians, the age-specific incidence peaked in a younger age group (<6 months), presumably because of early or intense exposure to Hib at home or in the community.[249] Several factors that may reflect environmental exposure to the organism, such as household size,[157] crowding,[427] attendance at daycare centers,[81,193] low family income,[26,133,134] and low parental education level,[133,134] have been shown to be risk factors for the acquisition of disease, whereas breast-feeding appears to be protective.[327] Several underlying medical conditions are associated with an increased risk for the acquisition of Hib disease, including HIV infection,[62,368] sickle-cell anemia,[337] asplenia or splenectomy,[72] antibody[115] and complement deficiency syndromes,[123] and malignancy.[453]

Although the direct contagiousness of invasive Hib disease is limited, small outbreaks and direct secondary transmission of disease can occur.[57,445,446] Numerous studies have estimated the risk for developing secondary disease in household contacts in the 30 days after the onset of disease in an index case. Overall, the attack rate for contacts of all ages in the prevaccine era was 0.3 percent, which represents a risk approximately 600-fold higher than the age-adjusted risk in the general population.[57,445] However, attack rates varied inversely with age, with children younger than 4 years being at greatest risk. Among household contacts, nearly two thirds of secondary cases occurred within the first week after the onset of disease in the index patient.[413] Controversy exists about the degree of risk for the development of secondary Hib disease in daycare center contacts exposed to a child with invasive Hib disease. Such risk in unimmunized children younger than 2 years of age ranged up to 3.2 percent,[26,314] whereas for those older than 2 years it was less than 1 percent.

A trend causing concern several years ago was the reappearance or persistence of Hib disease in highly immunized populations.[22,195,226,252,393] The factors contributing to these problems are still the subject of debate, but with the reemergence of Hib disease in the United Kingdom in the late 1990s, attention focused on the lack of a booster dosing of infants as part of the regular immunization series and the decreased immunogenicity of combination vaccines in which the Hib conjugate vaccine was combined with acellular pertussis vaccine components.[226,268] The Alaskan experience raised different concerns.[393] However, in this latter population unique characteristics of Hib nasopharyngeal carriage in the rural population and variability in the antibody response of young infants to different Hib vaccines appeared to be the factors most critical to explaining persistent Hib disease in Alaskan natives.[252,392,393]

Non–type b Encapsulated *Haemophilus influenzae*

H. influenzae organisms expressing one of the five non–type b capsular types are also carried in the nasopharynx of a small percentage of the population.[88,262] With the success of the Hib conjugate vaccine in preventing invasive disease caused by type b organisms, there was concern that disease caused by other encapsulated strains might become more prevalent.[67] There were several reported clusters of invasive disease caused by *H. influenzae* type a over the past decade,[5,53,205,326,347] as well as occasional cases of disease caused by other serotypes.[203,224,313,426] However, surveillance studies for the most part have failed to document a sustained increase in the incidence of disease caused by non–type b serotypes after introduction of the Hib conjugate vaccines.[203,224,252,265] These data suggest that the concern about serotype replacement may be more theoretical than real.

Nontypeable *Haemophilus influenzae*

Asymptomatic colonization of the mucous membranes of the upper respiratory tract by nontypeable *H. influenzae*

strains is very common, occurring in 60 to 90 percent of young children.[221] Infants often become colonized with nontypeable *H. influenzae* early during the first year of life.[111] Nasopharyngeal colonization is a dynamic process characterized by relatively rapid turnover of individual strains and, not infrequently, simultaneous carriage of several distinct clones.[28,88,113,223,374] With increasing age, nasopharyngeal carriage of nontypeable *H. influenzae* becomes less frequent, but it is still present in a significant percentage of the population.[221,374] Nontypeable *H. influenzae* organisms are only rarely associated with invasive disease[28,108,309,385,433] but are a common cause of mucosal surface infections such as otitis media, sinusitis, bronchitis, and occasionally pneumonia.[234,286,291,330]

CLINICAL MANIFESTATIONS

Bacteremia

Occult bacteremia (i.e., not associated with a primary focus of infection) is not a common occurrence with Hib, but bacteremia does precede essentially all invasive Hib infections. In the prevaccine era, Hib was the second leading cause, after *Streptococcus pneumoniae*, of occult bacteremia primarily affecting children 6 to 36 months of age.[15] Most children initially have fever and peripheral leukocytosis. Unlike *S. pneumoniae* bacteremia, the condition is not benign. In 30 to 50 percent of children with occult Hib bacteremia, a focal infection such as meningitis, pneumonia, or cellulitis develops.[84,216]

Meningitis

Meningitis is the most serious manifestation of invasive Hib disease; however, no clinical feature differentiates Hib meningitis from other causes of bacterial meningitis in children.[116] The onset of disease can be fulminant,[194] but more commonly the signs and symptoms are nonspecific (particularly in young infants) and may include irritability, fever, lethargy, poor feeding, or vomiting. Children younger than 18 months often do not have nuchal rigidity. Older children are more likely to have findings of headache, photophobia, and meningismus. With fulminant Hib meningitis, very rapid neurologic deterioration may occur with increased intracranial pressure, seizures, coma, and respiratory arrest.[116] Ten to 20 percent of children with meningitis will have other foci of infection, such as cellulitis, arthritis, or pneumonia,[25,124] and essentially all have concomitant bacteremia.

CSF examination typically reveals a pleocytosis (mean of 4000 to 5000 white blood cells/mm^3) with a predominance of polymorphonuclear leukocytes.[98] Approximately 75 percent of patients have hypoglycorrhachia, and around 90 percent have an elevated CSF protein concentration. Eighty percent of meningitis cases caused by Hib will have a positive CSF Gram stain. As with other types of bacterial meningitis, however, previous antimicrobial therapy significantly decreases the concentration of Hib organisms in the CSF and diminishes the sensitivity of Gram staining.[39] Previous treatment does not affect the total blood cell count and differential count, glucose value, or protein level substantially, thus permitting a diagnosis of meningitis. In more than 90 percent of cases, capsular antigen can be detected in CSF or serum.[449] Anemia, leukocytosis, thrombocytosis, and thrombocytopenia also are observed frequently.[116]

Complications of Hib meningitis include seizures, cerebral edema, subdural effusions or empyema, inappropriate secretion of antidiuretic hormone, cortical infarction (often manifested by focal neurologic abnormalities), cerebritis, intracerebral abscess, hydrocephalus, and, rarely, cerebral herniation.[44,98,116,336] Computed tomography and magnetic resonance imaging of the head should not be performed routinely for Hib meningitis, but these studies may be helpful if focal neurologic findings are present or if the clinical course becomes complicated. Small subdural effusions are common findings but are not usually of clinical significance.[116]

Even with prompt intensive care, the mortality from Hib meningitis is approximately 5 percent, and significant long-term morbidity, including sensorineural hearing loss, delay in language acquisition, developmental delay, gross motor abnormalities, vision impairment, and behavior abnormalities, may occur in 15 to 30 percent of survivors.[97,98,116,336,415] A substantial proportion of such abnormalities may resolve over time, thus emphasizing the need for long-term monitoring of these patients.[116]

Pneumonia

Hib pneumonia is clinically indistinguishable from other bacterial pneumonias.[148,438] Most patients have a preceding upper respiratory tract infection, fever, and cough accompanied by peripheral leukocytosis with a predominance of polymorphonuclear leukocytes. Radiologically, Hib pneumonia may be segmental, lobar, interstitial, or diffuse, and many cases have evidence of pleural or pericardial involvement on radiographic examination. Cavitation and pneumatoceles are rare occurrences but have been reported.[14,73] Computed tomography or magnetic resonance imaging can be useful adjuncts to the evaluation of complicated disease caused by Hib.[79] Blood, pleural fluid, tracheal aspirate, and lung aspirate cultures are positive in 75 to 90 percent of cases. Detection of capsular polysaccharide in pleural fluid, serum, or urine can establish the diagnosis, particularly if antimicrobial therapy had been instituted previously. Although fever may persist for several days while the patient is receiving adequate therapy, uncomplicated Hib pneumonia rarely is associated with long-term pulmonary dysfunction. In the developing world, in addition to disease caused by Hib, pneumonia caused by nontypeable *H. influenzae* is a continuing problem[140,233] that is also associated with significant morbidity and mortality.

Epiglottitis

Acute upper airway obstruction caused by Hib infection of the epiglottis and supraglottic tissues occurred in the prevaccine era primarily in children 2 to 7 years of age.[34,269] Its onset usually is abrupt, with high fever, sore throat, dysphagia, and sepsis. Antecedent upper respiratory tract infection with cough occurs in approximately

50 percent of patients.[49,242] The child may drool because of an inability to swallow oropharyngeal secretions, and progressive respiratory distress with tachypnea, stridor, cyanosis, and retractions may develop over a period of hours. The child usually is agitated and may sit forward with the chin extended to maintain an open airway. In children younger than 2 years of age, Hib epiglottitis may be manifested in an atypical fashion, with low-grade fever and a cough suggestive of croup.[242]

The most important aspect of the management of a child with epiglottitis is maintenance of a patent airway. Nasotracheal intubation is preferable to tracheostomy because it is equally effective, is not permanent or disfiguring, and has fewer inherent risks.[34] Seventy to 90 percent of patients with epiglottitis will have positive blood cultures; tissue for culture of the inflamed epiglottis should be obtained only after the airway has been secured. A lateral neck radiograph revealing dilatation of the hypopharynx and the "thumbprint" sign (swollen epiglottis)[46] can be helpful if the clinical findings are subtle, but in most cases diagnostic studies should not delay intubation and direct inspection of the epiglottis in controlled surroundings. The mortality rate is 5 to 10 percent, and death is almost always caused by abrupt airway obstruction.

Joint Infection

Before the routine use of Hib conjugate vaccines, Hib was the leading cause of septic arthritis in children younger than 2 years of age.[124] It affects a single large joint at the knee, ankle, elbow, or hip in more than 90 percent of cases. Contiguous osteomyelitis occurs in 10 to 20 percent.[124,230] No feature clearly distinguishes septic arthritis caused by Hib from that of other bacterial causes. Patients initially are febrile, and more than two thirds have decreased range of motion, local warmth, and swelling.[89,124,230] Usually, pain, swelling, and erythema of the involved joint are preceded by a nonspecific upper respiratory tract illness. The clinical features may be subtle, with only decreased range of motion of the joint or abnormal gait as the initial finding. Hib capsular antigen concentrations are very high in the infected joint fluid of children with septic arthritis, and rapid detection of antigen is useful diagnostically. Septic arthritis of the hip requires surgical drainage, and resolution generally is rapid, but long-term cartilage damage may result from Hib arthritis despite adequate therapy.[415]

Cellulitis

Cellulitis is a relatively uncommon form of Hib disease and was seen in the prevaccine era almost exclusively in children younger than 2 years of age. Most cellulitis (74%) is located in the cheek (buccal cellulitis), the periorbital region, and the neck[89] and rarely on the extremities. Facial cellulitis occurs most commonly in infants and is manifested as acute fever and a unilateral, raised, warm, tender, and indurated area that may progress to a violaceous hue, although this finding is not unique to Hib disease.[417] Aspirate cultures of the point of maximal swelling usually yield the organism, and bacteremia is a typical finding.[126,191] A secondary focus (including meningitis) may be present in 10 to 15 percent of patients.[25] Orbital cellulitis generally is a complication of ethmoidal sinusitis and, consequently, can be caused by non-Hib strains. An etiologic diagnosis can be established by blood culture or aspiration of subcutaneous tissues. Antibiotics always are indicated, but the need for surgical drainage depends on the degree of involvement of the tissues within the orbit.

Pericarditis

Hib pericarditis usually is a complication of adjacent pneumonia and is characterized by fever, an ill appearance, respiratory distress, and tachycardia.[70,101,348] The radiographic or clinical diagnosis can be confirmed by two-dimensional echocardiography[465] and may be suggested by finding capsular polysaccharide in serum or pericardial fluid or in Gram-stained pericardial fluid. Cultures of pericardial fluid are positive in more than 70 percent of cases.[89] Early drainage is an important part of the management of this illness, and early pericardiectomy or pericardiostomy is preferred over repeated pericardiocentesis.[276,348]

Neonatal Disease

H. influenzae causes 2 to 8 percent of neonatal early-onset sepsis.[58,186,441] Most of these cases are caused by nontypeable strains, most of which are concordant with those isolated from the maternal genital tract.[441] The precise pathogenesis is unknown, but neonatal disease often is associated with prematurity, low birth weight, premature rupture of membranes, and maternal chorioamnionitis; several cases have occurred after cesarean delivery, thus suggesting in utero transmission.[357] Clinical manifestations include pneumonia, bacteremia, and conjunctivitis. More than two thirds of neonatal *H. influenzae* disease occurs on the first day of life, with an overall mortality rate of 55 percent.[137]

Other Invasive Infections

Other invasive Hib infections include endophthalmitis,[416] CSF shunt infections,[236,345] necrotizing fasciitis, pyomyositis,[302] peritonitis,[76,136] scrotal abscess,[241] brain abscess,[119] polyserositis,[271] tenosynovitis,[301] epididymitis,[165] lung abscess,[240] periappendiceal abscess,[264] and bacterial tracheitis.[59] Invasive disease also may be characterized by fever alone, fever with petechiae, or fever of unknown origin.[52,432]

Diseases Caused by Non–type b *H. influenzae*

Respiratory tract infections associated with nontypeable *H. influenzae* are a major cause of morbidity and mortality today in both developed and nonindustrialized nations.[233,440] Previously, invasive disease caused by nontypeable *H. influenzae* was rare and generally associated

with underlying medical conditions such as prematurity, malignancy, cystic fibrosis, asthma, leakage of CSF, CNS shunts, congenital heart disease, lymphoproliferative disorders, and immunoglobulin deficiency.[112,143,307] More recently, with a decline in the rate of infections caused by Hib, other *H. influenzae* serotypes such as types a, e, and f, and nontypeable strains have become relatively more common causes of both mucosal surface infection and invasive disease,[203,205,224,265,421] although it is unclear that the absolute incidence of disease has increased.[252] There have been several reports of invasive disease clusters caused by *H. influenzae* type a.* One such report suggested that a IS1016-bexA deletion present in the chromosome of the recovered type a strains, a deletion similar to that present in many Hib strains,[219] might be a marker for more virulent type a clones.[5] Other serotypes have also been reported as causing cases of non–type b invasive disease.[203,224,252,313] Urwin and associates[426] identified 91 cases of invasive *H. influenzae* type f disease in a multistate area over the course of a 6-year period. The incidence of invasive type f disease was 0.5 cases per 1 million population in 1989 and 1.9 cases per 1 million population in 1994. In children, pneumonia and meningitis each accounted for 40 percent of the total cases. Overall, mortality from type f *H. influenzae* disease was 21 percent in children. Serotype f also has been reported as a cause of endocarditis[135] and septic arthritis[150] in healthy, normal children.

Invasive disease in children caused by nontypeable *H. influenzae* has also been reported.[60,108,307,433] Heath and associates[185] conducted an active prospective surveillance study of *H. influenzae* infections in the United Kingdom and Republic of Ireland. During the study period (October 1992 to December 1998), 102 cases of invasive disease caused by nontypeable *H. influenzae* were reported. Children with nontypeable *H. influenzae* infection were compared with those who had Hib disease. The former were more likely to be younger (16 vs. 22 months) and more likely to have pneumonia and bacteremia (p <.001) than were those with Hib disease. In 1998, the incidence of non–type b *H. influenzae* disease in all children younger than 5 years of age in the United Kingdom was 1.3 per 100,000 versus 0.6 per 100,000 population for Hib disease. Most non–type b strains isolated were nontypeable.

Mucosal Infections

Unencapsulated or non-Hib strains cause a variety of mucosal infections, including otitis media, sinusitis, conjunctivitis, and bronchitis.[214,234,291] Nontypeable *H. influenzae* otitis media has clinical features similar to otitis media caused by other common middle ear pathogens including fever, symptoms referable to the upper respiratory tract, and nonspecific symptoms such as irritability, vomiting, and diarrhea.[235] Recent data suggest that nontypeable *H. influenzae* is the most common cause of acute otitis media and persistent or recurrent otitis media in children who have received the pneumococcal conjugate vaccine.* Sinusitis caused by nontypeable *H. influenzae* may be manifested as common cold symptoms that are persistent or more severe than usual.[436,437] Older children and adults are more likely to complain of headache and paranasal, dental, or facial pain. Other common symptoms are a daytime cough that may worsen at night or reactive airway disease unresponsive to therapy. Rarely, chronic otitis or sinusitis may result in the development of mastoiditis or a parameningeal abscess.[206] Conjunctivitis usually is bilateral and purulent and may occur in outbreaks.[41-43] Although common, these infections rarely are life threatening and are not associated with bacteremia.

DIAGNOSIS

A high index of suspicion for the possibility of Hib disease must be maintained when evaluating children with appropriate clinical manifestations and findings. The primary criterion for the diagnosis of *H. influenzae* infection is Gram stain or isolation of the organism from an infected focus (e.g., CSF, pleural fluid, sputum, or blood). Because most invasive Hib disease is associated with bacteremia, blood cultures should be performed for any febrile child with potential Hib disease.[216] The specimens need to be processed immediately because the organism is fastidious. Although most commercial blood culture media support the growth of *H. influenzae*, the inoculum should be applied, when possible, directly to chocolate agar or a semisynthetic medium containing hemin and NAD.[21,139,231,325] In liquid medium, growth may not be sufficient to result in turbidity; therefore, for blood cultures, advanced detection systems or subcultures should be performed.[231] In addition, selective media that suppress the growth of gram-positive organisms may increase the recovery of *H. influenzae* from upper respiratory tract specimens.[69] Identification of an isolate as *H. influenzae* relies on its dependence on heme and NAD.[231] Determination of the capsular type expressed by *H. influenzae* is important both for clinical and epidemiologic purposes. Traditionally, this has been performed by slide agglutination with capsular type-specific antisera and suspensions of bacterial organisms.[231] Recently, polymerase chain reaction–based techniques that target the capsular gene locus have been developed that accurately identify strains expressing the six recognized capsular types.[251,304]

Other techniques that may assist in microbiologic diagnosis include rapid antigen detection, staining techniques, and immunofluorescence.[204,258,449] Such techniques are useful in the context of a patient whose cultures are sterile because of previous antibiotic therapy, or they can confirm the clinical diagnosis before bacterial growth occurs. The three techniques most commonly used in the past for antigen detection were latex particle agglutination (LPA), countercurrent immunoelectrophoresis, and coagglutination. False-positive results on CSF were rare for all three tests but did occur when testing serum and

*References 5, 53, 178, 179, 205, 313.

*References 40, 63, 200, 324, 330, 458.

urine. LPA appeared to be the most sensitive of the three tests and is the only one that remains in common use today. False-positive results can occur in the urine of children with nasopharyngeal carriage of the organism or, more commonly, for several days after immunization with Hib conjugate vaccine.[399] Acridine orange is a fluorescent stain that binds to cellular nucleic acids and may be useful in situations in which smaller bacterial concentrations are present.[213] Immunofluorescent staining of purulent specimens from patients with partially treated disease also has been useful.[78]

TREATMENT

Invasive Disease

Bacteremia plays a central role in the pathogenesis of invasive Hib disease; therefore, occult invasion of the CNS always must be considered in the context of any manifestation of Hib infection.[147,216] In addition, severe Hib disease often is fatal if not treated adequately. Therefore, elimination of Hib bacteremia and its complications requires antimicrobial therapy that will (1) penetrate the blood-brain barrier to achieve bactericidal concentrations and (2) be of adequate duration to sterilize the primary and potential secondary foci. The choice of specific antibiotic therapy must take into account the local antibiotic susceptibility patterns of invasive isolates (Table 133-3). *H. influenzae* resistance to several antimicrobial agents, including ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, rifampin, and certain second-generation cephalosporins, has been increasing in several areas of the world.[99,183,202,346,420]

For proven or suspected Hib meningitis, cefotaxime or ceftriaxone is recommended until the antibiotic susceptibility of the organism is known or an alternative diagnosis is established.[13,116] Both antibiotics have bactericidal activity against Hib strains, including those that produce β-lactamase, and they penetrate well into infected CSF and are well tolerated. Cefuroxime should not be used for the treatment of *H. influenzae* meningitis because delayed sterilization of CSF may be at least twofold more common than with ampicillin plus chloramphenicol or with the third-generation cephalosporins.[19,394] Moreover, ampicillin, formerly a mainstay of therapy for this infection, should not be used empirically to treat infections caused by Hib because as many as 50 percent of Hib isolates in the United States are resistant, usually via plasmid-mediated β-lactamase production.[202,231] Chloramphenicol, another medication that frequently was used in the past to treat Hib disease,[272,358] now rarely is used because safer antibiotics with greater activity are available. Although adequate bactericidal blood and CNS levels of chloramphenicol can be achieved, even with oral administration,[118,364] its use requires monitoring of drug levels. Dose-dependent, yet reversible, bone marrow toxicity may occur, particularly in neonates, in patients with liver disease, or in those who require prolonged treatment.[272] Idiosyncratic aplastic anemia, a dose-independent complication of chloramphenicol use, is an extremely rare occurrence.

TABLE 133-3 **Selected Antimicrobial Agents for Treatment of *Haemophilus influenzae* Infections**

Antimicrobial Agent	Total Daily Dose (mg/kg)	Dose Frequency
Parenteral*		
Ampicillin†	200-400	Every 4-6 hr
Cefuroxime‡	100-200	Every 8 hr
Cefotaxime	200-225	Every 6-8 hr
Ceftriaxone	50-100	Every 12-24 hr
Oral		
Amoxicillin§	40-90	Three times a day/twice a day
Amoxicillin-clavulanate	40-90	Three times a day/twice a day
Erythromycin-sulfisoxazole§	40 (erythromycin)	Four times a day
Trimethoprim-sulfamethoxazole§	8 (trimethoprim)	Twice a day
Clarithromycin§	15	Twice a day
Azithromycin§	10	Daily
Cefuroxime axetil	30	Twice a day
Cefprozil	30	Twice a day
Cefpodoxime	10	Twice a day
Cefixime	8	Daily
Cefdinir	14	Daily

*Other parenteral agents may be used in special circumstances, but they should not be considered first-line therapy. Such agents include ureidopenicillins, carbapenems, and fluoroquinolones.
†Not to be used presumptively because of the prevalence of resistant strains.
‡Not to be used for suspected meningitis.
§Do not use these oral agents if resistance is suspected or prevalent in your area.

Cefuroxime has good activity against *H. influenzae* and is useful for empirical treatment of nonmeningitic infections such as pneumonia, periorbital cellulitis, and septic arthritis.[431] Other parenteral agents that have activity against *H. influenzae* include meropenem, ampicillin-sulbactam, aztreonam, and other third-generation cephalosporins such as ceftazidime.[231,263] In general, the spectrum of activity of these agents is too broad for routine use in pediatric infections in which Hib is an important pathogen.

Children with occult Hib bacteremia need to be reevaluated carefully because focal disease may develop in 30 to 50 percent of such patients who are clinically well.[84,216] The duration of therapy is determined by the site of infection and the clinical response. Children with uncomplicated Hib meningitis can be treated for 10 days.[13,116] Children with Hib cellulitis can be switched to oral therapy after several days of parenteral therapy, provided that they have had a satisfactory clinical response and do not have meningitis. Patients with septic arthritis should receive at least 10 to 14 days of therapy,[124] whereas children with pericarditis, empyema, or osteomyelitis may require longer courses of antibiotic treatment (3 to 6 weeks).[70,124] These patients often can be switched to oral antibiotics after documenting susceptibility, a good therapeutic response, and adequate antimicrobial blood levels and ensuring compliance.

Equally important in the overall management of a child with invasive Hib disease is supportive care. For Hib meningitis, several studies have shown that adjunctive therapy with dexamethasone moderates the inflammatory cascade and may decrease the likelihood of hearing loss.[310] The recommended dose is 0.6 mg/kg/day given every 6 hours for 4 days, with the first dose administered just before or concurrently with the first antibiotic dose.[13,310,338] Management of a child with meningitis requires continuing careful evaluation for complications such as the development of shock, inappropriate secretion of antidiuretic hormone, seizures, subdural empyema, and secondary foci of infection.[97,98,336] Prolonged fever is a common occurrence, with approximately 10 percent of children remaining febrile for at least 10 days.[116] Repeat lumbar puncture to document sterility of the CSF is not necessary in uncomplicated cases.

In children with epiglottitis, protection of the airway is the most important component of therapy and should be initiated even before the administration of antimicrobial agents.[46] Endotracheal intubation or tracheostomy is performed optimally in the operating room by personnel experienced with these procedures in children. Before establishing the airway, care should be taken not to precipitate laryngospasm by attempting to examine the epiglottis or by performing procedures such as venipuncture.[34,46] Blood should be obtained for culture and intravenous antibiotic therapy initiated as soon as possible after the airway has been secured.

Patients with subdural empyema, pericarditis, or pleural empyema usually require percutaneous or surgical drainage.[70,98] Infected joint fluid should be aspirated from children with septic arthritis to confirm the diagnosis and reduce pressure.[124] Repeated aspirations or placement of a surgical drain also may be needed. Infection of the hip requires surgical incision and drainage to decompress the joint; failure to do so may result in avascular necrosis of the femoral head.[124]

Noninvasive Disease

Noninvasive *H. influenzae* infections, usually caused by nontypeable strains, include otitis media, sinusitis, conjunctivitis, bronchitis, and pneumonia. Numerous orally administered antimicrobial agents are available to treat these infections (see Table 133-3). Despite the increasing prevalence of β-lactamase–producing organisms, amoxicillin remains the drug of choice for empirical treatment of otitis media in most areas because of its low cost and proven safety.[13,239] Several other antimicrobial agents with activity against *H. influenzae* are available and include amoxicillin-clavulanate; trimethoprim-sulfamethoxazole; erythromycin-sulfisoxazole; other macrolides such as clarithromycin and azithromycin; and second- and third-generation cephalosporins such as cefuroxime axetil, cefixime, cefpodoxime, cefdinir, and cefprozil.[231] The quinolone antibiotics, such as ciprofloxacin, also are active but are not recommended routinely for use in children younger than 18 years of age because of a quinolone-induced arthropathy seen in juvenile animals. In the context of poor clinical response or isolation of β-lactamase–producing organisms, amoxicillin-clavulanate, cefixime, and cefpodoxime appear to be the most useful.[13,239]

Most mucosal infections are treated presumptively without obtaining definitive cultures; consequently, the duration of therapy and the need for alternative antibiotics are based on assessment of the clinical response. Otitis media usually is treated for 7 to 10 days, and sinusitis should be treated for at least 1 week beyond the resolution of symptoms.[13,239,436] If resistance is suspected and leads to failure of treatment, an empirical change in antibiotics is indicated.[239]

PREVENTION

The near elimination of invasive Hib disease in the United States and other countries is a direct result of the routine use of Hib conjugate vaccines and represents a remarkable success story.[252] Prevention with hyperimmunoglobulin also has been shown to be effective in high-risk populations, but it is costly, of short-lived benefit, and not licensed for general use.[361] Antimicrobial prophylaxis is effective for the prevention of secondary Hib disease, but it represents only a minor proportion of the overall disease burden.[13,26]

Active Immunization

The first Hib vaccine was the PRP polysaccharide vaccine, which is composed of purified Hib capsular polysaccharide PRP.[20,353] In 1985, in children older than 18 months of age, it became the first vaccine to be licensed for the prevention of Hib disease. Children younger than 18 months of age had inadequate immune responses with PRP vaccination.[17,209] PRP vaccine has immunologic

properties similar to those of some other polysaccharide vaccines, which generally are considered to be T-independent immunogens.[3] As a result, antibody responses are limited and of short duration, particularly in young children, and no booster response occurs with repeated administration of the vaccine. Furthermore, the induced antibody may have reduced functional qualities (i.e., primarily IgM, low avidity).[350,380]

Licensure of PRP vaccine was based on the findings of a large randomized clinical trial conducted in Finland in 1974[320,321] that suggested a protective efficacy of 90 percent for children who were immunized between 18 and 71 months of age. Subsequent to licensure of this vaccine in the United States, it became apparent that routine use of the vaccine resulted in efficacy less than that determined in the Finnish vaccine trial—between 0 and 88 percent.[447] This vaccine is mainly of historic significance because its role has been supplanted by development and licensure of the PRP-protein conjugate vaccines.

Hib conjugate vaccines were developed in an effort to enhance immune responses to the PRP antigen. Basic to all conjugate vaccines is the use of a covalently linked (conjugated) immunogenic protein carrier that confers on the PRP polysaccharide hapten recognition by T cells and macrophages and stimulation of T-dependent immunity.[228,447] Four Hib conjugate vaccines have been developed and evaluated in infants, and all use PRP polysaccharide or oligosaccharides derived from it as the primary immunogen (Table 133-4): PRP-D (diphtheria toxoid), HbOC (mutant diphtheria toxin), PRP-OMP (major outer-membrane protein of *Neisseria meningitidis* serogroup B), and PRP-T (tetanus toxoid). The immune response after administration of Hib conjugate vaccination has the following general characteristics: (1) it is quantitatively enhanced, particularly in younger infants; (2) repeat administration of vaccine elicits booster responses; and (3) maturation of class-specific immunity with a predominance of IgG antibody and probably enhanced functional properties occurs.

The first Hib conjugate vaccine licensed was PRP-D, but it was less immunogenic than are the conjugate vaccines developed subsequently and is not currently used in the United States. In children 15 months of age or older who received PRP-D, high antibody concentrations were achieved with a single dose.[36,189] In infants, however, antibody levels greater than 1 μg/mL developed in less than half, even after receiving three doses.[93,208] Numerous case-control studies have demonstrated that a single dose of vaccine was at least 80 percent efficacious in preventing disease in children 18 months or older. In infants who received vaccine at 3, 4, 6, and 14 to 18 months of age, the protective efficacy after three doses was 94 percent in Finland.[109] In contrast, a trial conducted in Alaska native infants found no evidence of protection in that high-risk population.[444]

A single dose of HbOC is highly immunogenic in children older than 18 months of age,[189,253] and after the administration of three doses in infancy, high antibody levels are achieved.[93,354] Two prospective clinical studies demonstrated that two or three doses of HbOC administered in the first 6 months of life provided a high degree of protective efficacy. In the Kaiser Permanente northern California region population, HbOC was 100 percent efficacious after two doses administered in infancy.[38] The vaccine also was evaluated in Finland in infants who received vaccine at 4, 6, and 14 to 18 months of age, and efficacy was 95 percent after two doses.[110] Data from a third post-licensure study in infants in Los Angeles County also suggested a protective efficacy of 89 percent after two doses and 94 percent after three doses.[429] Although used for a number of years, the HbOC vaccine is no longer available for use in children in the United States.

PRP-OMP induces an immune response that is less age dependent than is the response to the other Hib conjugate vaccines. Adults and children respond to a single vaccine dose with high antibody levels.[354] In infants as young as 6 to 8 weeks, a single dose of PRP-OMP induces a good antibody response.[54] In addition, the antibody levels achieved after two doses are higher than those after two doses of any of the other conjugate vaccines[54,93] and a third dose does not enhance the response. PRP-OMP was evaluated in a randomized, double-blind, placebo-controlled trial involving high-risk Navajo Indian infants[362] who received vaccine at 2 and 4 months of age; an overall efficacy of 95 percent was reported. Additional data from a population-based case-control study in Los Angeles County suggested a level of effectiveness similar to that seen for HbOC.[428]

TABLE 133-4 **Characteristics of *Haemophilus influenzae* Type b Conjugate Vaccines**

Vaccine	Polysaccharide Size	Protein Carrier	Linkage	Trade Name*
PRP-D	Medium	Diphtheria toxoid	Protein with 6-carbon spacer	ProHIBiT
HbOC	Small	CRM_{197} (mutant diphtheria toxin)	PS without a spacer	HibTITER
PRP-OMP	Medium	*Neisseria meningitidis* outer-membrane protein complex	Protein and PS with bigeneric spacer	PedvaxHIB
PRP-T	Large	Tetanus toxoid	PS with 6-carbon spacer	ActHIB
PRP-T	Large	Tetanus toxoid	PS with 6-carbon spacer	Hiberix

*HbOC, PRP-OMP, and PRP-T also are available in combination with diphtheria-tetanus-pertussis, diphtheria-tetanus–acellular pertussis, and hepatitis B vaccines.

HbOC, mutant diphtheria toxin; PRP-D, diphtheria toxoid; PRP-OMP, major outer-membrane protein of *N. meningitidis* serogroup B; PRP-T, tetanus toxoid; PS, polysaccharide.

PRP-T is highly immunogenic in adults and older children,[75,371] and high concentrations of antibody are achieved in infants with a three-dose immunization series at 2, 4, and 6 months of age.[93] The protective efficacy of PRP-T was evaluated in two large prospective randomized trials that were terminated prematurely because of the licensure of other Hib conjugate vaccines for infants. More than 12,000 infants were enrolled in studies in southern California and North Carolina, and no cases of invasive Hib disease occurred in the vaccinated children versus five cases in the control groups.[138,430] Efficacy subsequently was shown in a study in England; after three doses in infancy, the efficacy of PRP-T was estimated to be 100 percent.[45] In Finland, during the first 2 years of its general use, more than 100,000 infants were immunized and only two cases of invasive Hib disease occurred in vaccinees, both after a single dose.

Direct comparisons of Hib conjugate vaccines must be considered in the context of the varying study designs, differences in vaccine lots, and different laboratory and statistical methodologies that were used in the clinical trials. Despite these uncertainties, certain principles are evident: (1) all Hib conjugate vaccines are safe in infants; (2) PRP-D is the least immunogenic conjugate vaccine; (3) only PRP-OMP induces a good immune response after one dose in young infants, but antibody levels are lower than those induced by multiple doses of HbOC and PRP-T vaccine; and (4) PRP-OMP, HbOC, and PRP-T appear to be efficacious, but no direct comparison of the protective efficacy of these vaccines has ever been completed. Mixed administration sequences of Hib vaccines (e.g., PRP-OMP followed by HbOC or PRP-T) may enhance the antibody response,[154,163] and simultaneous or prior receipt of other non-Hib vaccines also may impact the antibody response,[159,161,238] presumably secondary to effects of the carrier proteins. Better understanding of the biologic basis for these observations must await future studies.

Investigation of the feasibility of administering Hib vaccines in combination with other vaccines has been the focus of much research effort over the past decade. Early studies by Schmitt and associates[370] assessed the impact and effectiveness of DTaP (IPV)-Hib combination vaccines in Germany. Although combination DTaP-Hib vaccines elicited lower anti-Hib titers than separate vaccines, the investigators were able to show that combination vaccines were effective in reducing the incidence of Hib disease.[370] Subsequent data from Canada also suggested that a multicomponent Hib-containing combination vaccine was safe and effective in preventing invasive type b *Haemophilus* disease in young children.[365,366] These studies and others led to the subsequent licensing of a five-component combination vaccine (DTaP-IPV-Hib) that has been approved for use in the primary Hib vaccination series for U. S. children since 2008.[68,153]

The phenomenal success of the Hib vaccination strategy has been related to the effectiveness of Hib conjugate vaccines in preventing colonization. Fernandez and associates[121] reported that the anti-Hib capsular polysaccharide antibody concentration required to prevent colonization was greater than that needed for protection against invasive disease. Whether concentrations in the range that Fernandez and associates suggested was important (>5 μg/mL) can be achieved and sustained by using conjugate vaccines in all population groups remains a subject of investigation. Studies in some high-risk population groups (Alaska natives) have documented that invasive disease is prevented best by the use of PRP-OMP Hib vaccine,[392,393] at least for the first dose of vaccine, and that even the use of PRP-OMP Hib vaccine in these individuals may not eliminate carriage of the organism in the population.[95,141] Use of combination vaccines in this population is unlikely to be as effective as use of PRP-OMP Hib conjugate vaccine.[393]

To date, essentially all *H. influenzae* vaccines are based on immunity to the Hib capsule. Antibodies to other components of the bacterium also have been shown to be bactericidal, opsonophagocytic, and protective in animal studies.* Vaccines containing other bacterial antigens could in theory provide supplemental protection against Hib, although this protection does not appear to be necessary in most individuals based on the efficacy of the available Hib conjugate vaccines. Even so, such alternative vaccines could provide immunity to non-Hib strains, which are ubiquitous colonizers of the upper respiratory tract of humans.[113,221] One of the basic problems that must be overcome in the development of such vaccines is the known diversity of many of the candidate vaccine antigens.[30,144] Because of the known strain-to-strain variability in a number of these antigens,[96] finding an antigen relevant to all or most strains has been a challenge.[30,287] Even so, recent human trials have reported success in preventing some cases of *H. influenzae* otitis media with a novel pneumococcal polysaccharide–*H. influenzae* protein D conjugate vaccine[339] and a number of other candidate vaccine antigens remain the subject of active preclinical research.[29,94,222,463,464]

Passive Immunization

Although active immunization clearly is preferred for the control of Hib disease, passive immunization has potential utility in the following settings: (1) selected high-risk groups with a risk for acquiring disease soon after birth and too young to respond to vaccination (i.e., Alaska natives and American Indians), (2) functionally asplenic patients, (3) immunocompromised patients, and (4) prevention of secondary disease in households, daycare centers, or institutions. A human hyperimmunoglobulin from adult Hib-immunized donors called bacterial polysaccharide immunoglobulin has been prepared,[386] but it is not commercially available. Pharmacologic studies show that high levels of antibody can be achieved after intramuscular injection, and significant protective efficacy against invasive Hib disease has been demonstrated in Apache children given three doses during the first year of life.[361] The use of concurrent active immunization with bacterial polysaccharide immunoglobulin also may be an effective strategy.[237] Another possible approach in such groups would be maternal Hib immunization[151] to induce

*References 24, 30, 94, 104, 170, 222, 285, 288, 289, 462.

transplacental antibody, but questions remain regarding the safety and acceptability of vaccinating pregnant women and the inability to immunize women who do not receive prenatal care.

Impact of *H. influenzae* Type b Vaccination

The impact of widespread vaccination with Hib conjugate vaccine has been dramatic and reproduced in many areas of the United States[4,67,252,295] and many other countries throughout the world.[48,127,201,312,322] In general, the decrease in the incidence of disease has exceeded expectations given the estimated proportion of the population completely immunized. In addition, in essentially all these populations, a significant decrease in the incidence of disease was observed in infants before the licensure and recommended use of vaccines in that age group. These findings probably are explained by a reduction in Hib carriage as a result of vaccination[295,410] and, consequently, decreased transmission from immunized children to unimmunized young children and infants.

The immunization schedules vary in different countries to parallel immunization practices for other vaccine-preventable diseases. Also the age-specific incidence of disease varies in different countries. Initially in the United Kingdom the occurrence of disease was such that physicians felt justified to use only a three-dose schedule at 2, 3, and 4 months of age and not to provide a booster dose in the second year of life. As alluded to earlier, this schedule, introduced in the United Kingdom in 1993, appeared to coincide with the reappearance of type b disease in older children observed in the late 1990s.* This experience emphasizes the importance of giving a fourth dose when children are between 12 and 18 months of age.

Chemoprophylaxis

Secondary disease accounts for less than 2 percent of all cases of invasive Hib disease. Chemoprophylaxis, however, can protect susceptible persons from acquiring Hib by eliminating Hib colonization in close contacts. Children younger than 4 years of age have a 600-fold increased risk for acquiring Hib disease after household contact with a case.[445] Risk also appears to be increased in daycare center settings, but this risk is less well defined.[155,314] In addition, adults and older children who are colonized can transmit Hib to susceptible children even though they have little risk for acquiring invasive disease themselves.

Antimicrobial agents effective for chemoprophylaxis must achieve bactericidal levels intracellularly and in mucosal secretions. Rifampin, which achieves high concentrations in respiratory secretions,[266] is the most effective antimicrobial agent for eradicating Hib from the nasopharynx. Rifampin 20 mg/kg once daily (maximal daily dose, 600 mg) for 4 days eradicates Hib carriage in 95 percent or more of household[26,152] or daycare center[158] contacts of a case. Cohort studies have shown the effectiveness of rifampin prophylaxis in preventing secondary Hib disease in household and daycare center attendees.[152,158,255] Antimicrobial agents effective in the treatment of Hib disease, such as ampicillin, trimethoprim-sulfamethoxazole, erythromycin-sulfisoxazole, and cefaclor, have been shown to be ineffective for antimicrobial prophylaxis.[190] However, they eliminated Hib carriage in less than 70 percent of culture-positive contacts and, therefore, are not recommended for chemoprophylaxis.

Both the U.S. Public Health Service Advisory Committee on Immunization Practices[66] and the American Academy of Pediatrics Committee on Infectious Diseases[13] recommend rifampin prophylaxis for all household contacts, including adults, and for the index patient (therapeutic antibiotics do not eradicate Hib from the nasopharynx consistently) if the household has a contact younger than 4 years who is not fully immunized. Prophylaxis should be instituted as soon as possible because the risk for acquiring secondary disease is greatest during the few days after the onset of disease in the index patient and within 2 weeks of the onset of disease. With regard to the daycare center setting, no consensus exists concerning the need for chemoprophylaxis because of uncertainty about the magnitude of the risk for acquiring secondary Hib disease in this setting. Some authors recommend chemoprophylaxis if classroom contacts include those younger than 2 years of age, whereas others think that recommendations should be individualized.[90,314] However, virtually all experts recommend prophylaxis if two or more cases of Hib disease have occurred among attendees within 60 days.[13,66]

In all situations in which the potential for secondary disease exists, this risk should be explained to families, with an emphasis on the importance of seeking prompt medical attention for febrile illnesses. Clinicians should not obtain pharyngeal cultures to determine whether prophylaxis should be administered because doing so only delays the prompt administration of chemoprophylaxis.

CONCLUSION

The perspective on *Haemophilus* disease has changed dramatically in recent years. Before the availability of Hib conjugate vaccines, invasive Hib was one of the most important bacterial pathogens of children. It was the leading cause of bacterial meningitis and an important cause of other bacteremic illnesses. The spectrum of illness is broad, morbidity and mortality are significant, and subtleties in making an early diagnosis and instituting appropriate management exist. Currently, when one considers that most antibiotic use worldwide is for upper respiratory tract infections, including otitis media, and that *H. influenzae* causes a significant proportion of these illnesses, it still must be considered a very important pediatric pathogen.

Although various aspects of disease caused by *H. influenzae* have been reviewed in this chapter, what is clear is that the most important aspect has been disease prevention by routine immunization of infants with

*References 18, 198, 225, 226, 267, 341.

polysaccharide-protein conjugate vaccines. This achievement is the culmination of more than 100 years of research on *H. influenzae*. Although historically there were many technologic problems and misunderstandings about the organism and its pathogenesis, much has been accomplished in recent years. The impact of routine infant immunizations with Hib conjugate vaccines is relatively recent and very dramatic. The public health benefits parallel the eradication of polio and the control of other vaccine-preventable childhood diseases. Widespread Hib immunization virtually has eliminated Hib disease in the United States and in many developed countries where it is used routinely. The degree of disease control exceeded all expectations and is in excess of what the known levels of immunization would have predicted. Unfortunately, Hib conjugate vaccines are currently still not used routinely in many developing countries, and this remains one of the biggest challenges in preventing and eliminating disease worldwide.[277,312]

Some progress has been made at the preclinical level in the development of vaccines against other *H. influenzae* serotypes and nontypeable strains. Control of infection caused by these organisms will have an important public health impact, and the use of such vaccines may have a role in adolescents and adults. The technologies that led to the development of Hib conjugate vaccines served as a prototype for vaccines to prevent disease caused by other encapsulated bacteria including the pneumococcus, meningococcus, and group B streptococcus. As such, the lessons learned in the quest to eliminate Hib disease have had important implications in the control of many other bacterial diseases.

NEW REFERENCES SINCE THE SIXTH EDITION

13. American Academy of Pediatrics. *Haemophilus influenzae* infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 345–52.
27. Barbosa AR, Giufre M, Cerquetti M, Bajanca-Lavado MP. Polymorphism in *fts*I gene and beta-lactam susceptibility in Portuguese *Haemophilus influenzae* strains: clonal dissemination of beta-lactamase-positive isolates with decreased susceptibility to amoxicillin/clavulanic acid. *J Antimicrob Chemother* 2011;**66**:788–96.
48. Braikat M, Barkia A, El Mdaghri N, et al. Vaccination with *Haemophilus influenzae* type b conjugate vaccine reduces bacterial meningitis in Morocco. *Vaccine* 2012;**30**:2594–9.
53. Bruce MG, Deeks SL, Zulz T, et al. Epidemiology of *Haemophilus influenzae* serotype a, North American Arctic, 2000-2005. *Emerg Infect Dis* 2008;**14**:48–55.
61. Carruthers MD, Tracy EN, Dickson AC, et al. Biological roles of nontypeable *Haemophilus influenzae* type IV pilus proteins encoded by the *pil* and *com* operons. *J Bacteriol* 2012;**194**:1927–33.
68. Centers for Disease Control and Prevention. Licensure of a *Haemophilus influenzae* type b (Hib) vaccine (Hiberix) and updated recommendations for use of Hib vaccine. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1008–9.
77. Clark SE, Snow J, Li J, et al. Phosphorylcholine allows for evasion of bactericidal antibody by *Haemophilus influenzae*. *PLoS Pathog* 2012;**8**:e1002521.
87. Dabernat H, Delmas C. Epidemiology and evolution of antibiotic resistance of *Haemophilus influenzae* in children 5 years of age or less in France, 2001-2008: a retrospective database analysis. *Eur J Clin Microbiol Infect Dis* 2012;**31**:2745–53.
127. Fleming JA, Dieye Y, Ba O, et al. Effectiveness of *Haemophilus influenzae* type b conjugate vaccine for prevention of meningitis in Senegal. *Pediatr Infect Dis J* 2011;**30**:430–2.
142. Gawthorne JA, Beatson SA, Srikhanta YN, et al. Origin of the diversity in DNA recognition domains in phasevarion-associated modA genes of pathogenic *Neisseria* and *Haemophilus influenzae*. *PLoS One* 2012;**7**:e32337.
149. Giufre M, Cardines R, Caporali MG, et al. Ten years of Hib vaccination in Italy: prevalence of non-encapsulated *Haemophilus influenzae* among invasive isolates and the possible impact on antibiotic resistance. *Vaccine* 2011;**29**:3857–62.
171. Hallstrom T, Blom AM, Zipfel PF, Riesbeck K. Nontypeable *Haemophilus influenzae* protein E binds vitronectin and is important for serum resistance. *J Immunol* 2009;**183**:2593–601.
173. Hallstrom T, Resman F, Ristovski M, Riesbeck K. Binding of complement regulators to invasive nontypeable *Haemophilus influenzae* isolates is not increased compared to nasopharyngeal isolates, but serum resistance is linked to disease severity. *J Clin Microbiol* 2010;**48**:921–7.
174. Hallstrom T, Riesbeck K. *Haemophilus influenzae* and the complement system. *Trends Microbiol* 2010;**18**:258–65.
175. Hallstrom T, Singh B, Resman F, et al. *Haemophilus influenzae* protein E binds to the extracellular matrix by concurrently interacting with laminin and vitronectin. *J Infect Dis* 2011;**204**:1065–74.
177. Hallstrom T, Zipfel PF, Blom AM, et al. *Haemophilus influenzae* interacts with the human complement inhibitor factor H. *J Immunol* 2008;**181**:537–45.
184. Hashida K, Shiomori T, Hohchi N, et al. Nasopharyngeal *Haemophilus influenzae* carriage in Japanese children attending daycare centers. *J Clin Microbiol* 2008;**46**:876–81.
195. Jacups SP, Morris PS, Leach AJ. *Haemophilus influenzae* type b carriage in indigenous children and children attending childcare centers in the Northern Territory, Australia, spanning pre- and post-vaccine eras. *Vaccine* 2011;**29**:3083–8.
197. Jenkins GA, Figueira M, Kumar GA, et al. Sialic acid mediated transcriptional modulation of a highly conserved sialometabolism gene cluster in *Haemophilus influenzae* and its effect on virulence. *BMC Microbiol* 2010;**10**:48.
200. Jokinen J, Palmu AA, Kilpi T. Acute otitis media replacement and recurrence in the Finnish Otitis Media (FinOM) vaccine trial. *Clin Infect Dis* 2012;**55**:1673–6.
203. Kalies H, Siedler A, Grondahl B, et al. Invasive *Haemophilus influenzae* infections in Germany: impact of non-type b serotypes in the post-vaccine era. *BMC Infect Dis* 2009;**9**:45.
207. Kaur R, Chang A, Xu Q, et al. Phylogenetic relatedness and diversity of non-typable *Haemophilus influenzae* in the nasopharynx and middle ear fluid of children with acute otitis media. *J Med Microbiol* 2011;**60**:1841–8.
223. Lacross NC, Marrs CF, Patel M, et al. High genetic diversity of nontypeable *Haemophilus influenzae* isolates from two children attending a day care center. *J Clin Microbiol* 2008;**46**:3817–21.
224. Ladhani S, Slack MP, Heath PT, et al. Invasive *Haemophilus influenzae* disease, Europe, 1996-2006. *Emerg Infect Dis* 2010;**16**:455–63.
225. Ladhani S, Slack MP, Heys M, et al. Fall in *Haemophilus influenzae* serotype b (Hib) disease following implementation of a booster campaign. *Arch Dis Child* 2008;**93**:665–9.
226. Ladhani SN. Two decades of experience with the *Haemophilus influenzae* serotype b conjugate vaccine in the United Kingdom. *Clin Ther* 2012;**34**:385–99.
227. Ladhani SN, Collins S, Vickers A, et al. Invasive *Haemophilus influenzae* serotype e and f disease, England and Wales. *Emerg Infect Dis* 2012;**18**:725–32.
229. Langereis JD, Stol K, Schweda EK, et al. Modified lipooligosaccharide structure protects nontypeable *Haemophilus influenzae* from IgM-mediated complement killing in experimental otitis media. *MBio* 2012;**3**:e00079–12.
231. Ledeboer NA, Doern GV. Haemophilus. In: Versalovic R, Carroll KC, Funke G, et al., editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: American Society for Microbiology; 2012. p. 588–602.
244. Lowther SA, Shinoda N, Juni BA, et al. *Haemophilus influenzae* type b infection, vaccination, and *H. influenzae* carriage in children in Minnesota, 2008-2009. *Epidemiol Infect* 2012;**140**:566–74.
252. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* disease—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.

261. Mason KM, Raffel FK, Ray WC, Bakaletz LO. Heme utilization by nontypeable *Haemophilus influenzae* is essential and dependent on Sap transporter function. *J Bacteriol* 2011;**193**:2527–35.
277. Morris SK, Moss WJ, Halsey N. *Haemophilus influenzae* type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008;**8**:435–43.
278. Morton DJ, Seale TW, Vanwagoner TM, et al. The *dppBCDF* gene cluster of *Haemophilus influenzae*: role in heme utilization. *BMC Res Notes* 2009;**2**:166.
291. Murphy TF, Faden H, Bakaletz LO, et al. Nontypeable *Haemophilus influenzae* as a pathogen in children. *Pediatr Infect Dis J* 2009;**28**:43–8.
292. Murphy TF, Lesse AJ, Kirkham C, et al. A clonal group of nontypeable *Haemophilus influenzae* with two IgA proteases is adapted to infection in chronic obstructive pulmonary disease. *PLoS One* 2011;**6**:e25923.
304. Nelson KL, Smith AL. Determination of capsulation status in *Haemophilus influenzae* by multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2010;**66**:235–40.
311. Oh SY, Griffiths D, John T, et al. School-aged children: a reservoir for continued circulation of *Haemophilus influenzae* type b in the United Kingdom. *J Infect Dis* 2008;**197**:1275–81.
312. Ojo LR, O'Loughlin RE, Cohen AL, et al. Global use of *Haemophilus influenzae* type b conjugate vaccine. *Vaccine* 2010;**28**:7117–22.
330. Pichichero ME, Casey JR, Hoberman A, Schwartz R. Pathogens causing recurrent and difficult-to-treat acute otitis media, 2003-2006. *Clin Pediatr* 2008;**47**:901–6.
346. Resman F, Ristovski M, Forsgren A, et al. Increase of β-lactam–resistant invasive *Haemophilus influenzae* in Sweden, 1997 to 2010. *Antimicrob Agents Chemother* 2012;**56**:4408–15.
374. Schumacher SK, Marchant CD, Loughlin AM, et al. Prevalence and genetic diversity of nontypeable *Haemophilus influenzae* in the respiratory tract of infants and primary caregivers. *Pediatr Infect Dis J* 2012;**31**:145–9.
385. Shuel M, Law D, Skinner S, et al. Characterization of nontypeable *Haemophilus influenzae* collected from respiratory infections and invasive disease cases in Manitoba, Canada. *FEMS Immunol Med Microbiol* 2010;**58**:277–84.
390. Singh B, Brant M, Kilian M, et al. Protein E of *Haemophilus influenzae* is a ubiquitous highly conserved adhesin. *J Infect Dis* 2010;**201**:414–19.
391. Singh B, Jalalvand F, Morgelin M, et al. *Haemophilus influenzae* protein E recognizes the C-terminal domain of vitronectin and modulates the membrane attack complex. *Mol Microbiol* 2011;**81**:80–98.
412. Talbird SE, Taylor TN, Caporale J, et al. Residual economic burden of *Streptococcus pneumoniae*- and nontypeable *Haemophilus influenzae*–associated disease following vaccination with PCV-7: a multicountry analysis. *Vaccine* 2010;**28**(Suppl 6):G14–22.
433. van Wessel K, Rodenburg GD, Veenhoven RH, et al. Nontypeable *Haemophilus influenzae* invasive disease in The Netherlands: a retrospective surveillance study 2001-8. *Clin Infect Dis* 2011;**53**:e1–7.
435. von Gottberg A, Cohen C, Whitelaw A, et al. Invasive disease due to *Haemophilus influenzae* serotype b ten years after routine vaccination, South Africa, 2003-2009. *Vaccine* 2012;**30**:565–71.
450. Watt JP, Wolfson LJ, O'Brien KL, et al. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet* 2009;**374**:903–11.
458. Wiertsema SP, Kirkham LA, Corscadden KJ, et al. Predominance of nontypeable *Haemophilus influenzae* in children with otitis media following introduction of a 3+0 pneumococcal conjugate vaccine schedule. *Vaccine* 2011;**29**:5163–70.
462. Winter LE, Barenkamp SJ. Antibodies specific for the Hia adhesion proteins of nontypeable *Haemophilus influenzae* mediate opsonophagocytic activity. *Clin Vaccine Immunol* 2009;**16**:1040–6.
467. Wong SM, St. Michael F, Cox A, et al. ArcA-regulated glycosyltransferase lic2B promotes complement evasion and pathogenesis of nontypeable *Haemophilus influenzae*. *Infect Immun* 2011;**79**:1971–83.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abramson JS, Lyles DS, Heller KA, Bass DA. Influenza A virus–induced polymorphonuclear leukocyte dysfunction. *Infect Immun* 1982;**37**:794–9.
2. Abramson JS, Wagner MP, Ralston EP, et al. The ability of polymorphonuclear leukocyte priming agents to overcome influenza A virus-induced cell dysfunction. *J Leukoc Biol* 1991;**50**:160–6.
3. Ada G. Vaccines and vaccination. *N Engl J Med* 2001;**345**: 1042–53.
4. Adams WG, Deaver KA, Cochi SL, et al. Decline of childhood *Haemophilus influenzae* type b (Hib) disease in the Hib vaccine era. *JAMA* 1993;**269**:221–6.
5. Adderson EE, Byington CL, Spencer L, et al. Invasive serotype a *Haemophilus influenzae* infections with a virulence genotype resembling *Haemophilus influenzae* type b: emerging pathogen in the vaccine era? *Pediatrics* 2001;**108**:E18.
6. Adhikari P, Kirby SD, Nowalk AJ, et al. Biochemical characterization of a *Haemophilus influenzae* periplasmic iron transport operon. *J Biol Chem* 1995;**270**:25142–9.
7. Alexander HE, Heidelberger M, Leidy G. The protective or curative element in type b *H. influenzae* rabbit serum. *Yale J Biol Med* 1944;**16**:425–40.
8. Alexander HE, Leidy G, MacPherson C. Production of types a, b, c, d, e, and f *H. influenzae* antibody for diagnostic and therapeutic purposes. *J Immunol* 1946;**54**:207–11.
9. All India Institute of Medical Sciences. Are *Haemophilus influenzae* infections a significant problem in India? A prospective study and review. *Clin Infect Dis* 2002;**34**:949–57.
10. Allen S, Zaleski A, Johnston JW, et al. Novel sialic acid transporter of *Haemophilus influenzae*. *Infect Immun* 2005;**73**:5291–300.
11. Ambrosino DM, Schiffman G, Gotschlich EC, et al. Correlation between G2m(n) immunoglobulin allotype and human antibody response and susceptibility to polysaccharide encapsulated bacteria. *J Clin Invest* 1985;**75**:1935–42.
12. Ambrosino DM, Sood SK, Lee MC, et al. IgG1, IgG2 and IgM responses to two *Haemophilus influenzae* type b conjugate vaccines in young infants. *Pediatr Infect Dis J* 1992;**11**:855–9.
13. American Academy of Pediatrics. *Haemophilus influenzae* infections. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 345–52.
14. Amitai I, Mogle P, Godfrey S, Aviad I. Pneumatocele in infants and children: report of 12 cases. *Clin Pediatr (Phila)* 1983;**22**: 420–2.
15. Anderson AB, Ambrosino DM, Siber GR. *Haemophilus influenzae* type b unsuspected bacteremia. *Pediatr Emerg Care* 1987;**3**: 82–5.
16. Anderson P. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1984;**149**:1034–5.
17. Anderson P, Smith DH, Ingram DL, et al. Antibody of polyribophate of *Haemophilus influenzae* type b in infants and children: effect of immunization with polyribophosphate. *J Infect Dis* 1977; **136**(Suppl):S57–62.
18. Aracil B, Slack M, Perez-Vazquez M, et al. Molecular epidemiology of *Haemophilus influenzae* type b causing vaccine failures in the United Kingdom. *J Clin Microbiol* 2006;**44**:1645–9.
19. Arditi M, Herold BC, Yogev R. Cefuroxime treatment failure and *Haemophilus influenzae* meningitis: case report and review of literature. *Pediatrics* 1989;**84**:132–5.
20. Argaman M, Liu TY, Robbins JB. Polyribitol-phosphate: an antigen of four gram-positive bacteria cross-reactive with the capsular polysaccharide of *Haemophilus influenzae* type b. *J Immunol* 1974;**112**:649–55.
21. Artman M, Domenech E, Weiner M. Growth of *Haemophilus influenzae* in simulated blood cultures supplemented with hemin and NAD. *J Clin Microbiol* 1983;**18**:376–9.
22. Baggett HC, Hennessy TW, Bulkow L, et al. Immunologic response to *Haemophilus influenzae* type b (Hib) conjugate vaccine and risk factors for carriage among Hib carriers and noncarriers in Southwestern Alaska. *Clin Vaccine Immunol* 2006;**13**:620–6.
23. Bakaletz LO, Baker BD, Jurcisek JA, et al. Demonstration of type IV pilus expression and a twitching phenotype by *Haemophilus influenzae*. *Infect Immun* 2005;**73**:1635–43.
24. Bakaletz LO, Leake ER, Billy JM, Kaumaya PT. Relative immunogenicity and efficacy of two synthetic chimeric peptides of fimbrin as vaccinogens against nasopharyngeal colonization by nontypeable *Haemophilus influenzae* in the chinchilla. *Vaccine* 1997;**15**:955–61.
25. Baker RC, Bausher JC. Meningitis complicating acute bacteremic facial cellulitis. *Pediatr Infect Dis* 1986;**5**:421–3.
26. Band JD, Fraser DW, Ajello G. Prevention of *Hemophilus influenzae* type b disease. *JAMA* 1984;**251**:2381–6.
27. Barbosa AR, Giufre M, Cerquetti M, Bajanca-Lavado MP. Polymorphism in *fts*I gene and beta-lactam susceptibility in Portuguese *Haemophilus influenzae* strains: clonal dissemination of beta-lactamase–positive isolates with decreased susceptibility to amoxicillin/clavulanic acid. *J Antimicrob Chemother* 2011;**66**: 788–96.
28. Barbosa-Cesnik C, Farjo RS, Patel M, et al. Predictors for *Haemophilus influenzae* colonization, antibiotic resistance and for sharing an identical isolate among children attending 16 licensed day-care centers in Michigan. *Pediatr Infect Dis J* 2006;**25**:219–23.
29. Barenkamp SJ. Immunization with high-molecular-weight adhesion proteins of nontypeable *Haemophilus influenzae* modifies experimental otitis media in chinchillas. *Infect Immun* 1996;**64**: 1246–51.
30. Barenkamp SJ. Rationale and prospects for a nontypable *Haemophilus influenzae* vaccine. *Pediatr Infect Dis J* 2004;**23**:461–2.
31. Barenkamp SJ, Munson RS Jr, Granoff DM. Subtyping isolates of *Haemophilus influenzae* type b by outer-membrane protein profiles. *J Infect Dis* 1981;**143**:668–76.
32. Barenkamp SJ, StGeme JW, 3rd. Identification of a second family of high-molecular-weight adhesion proteins expressed by nontypable *Haemophilus influenzae*. *Mol Microbiol* 1996;**19**:1215–23.
33. Barrett DJ. Human immune responses to polysaccharide antigens: an analysis of bacterial polysaccharide vaccines in infants. *Adv Pediatr* 1985;**32**:139–58.
34. Baugh R, Baker SR. Epiglottitis in children: review of 24 cases. *Otolaryngol Head Neck Surg* 1982;**90**:157–62.
35. Berenson CS, Garlipp MA, Grove LJ, et al. Impaired phagocytosis of nontypeable *Haemophilus influenzae* by human alveolar macrophages in chronic obstructive pulmonary disease. *J Infect Dis* 2006; **194**:1375–84.
36. Berkowitz CD, Ward JI, Meier K, et al. Safety and immunogenicity of *Haemophilus influenzae* type b polysaccharide and polysaccharide diphtheria toxoid conjugate vaccines in children 15 to 24 months of age. *J Pediatr* 1987;**110**:509–14.
37. Black SB, Shinefield HR. Immunization with oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine on a large health maintenance organization population: extended follow-up and impact on *Haemophilus influenzae* disease epidemiology. The Kaiser Permanente Pediatric Vaccine Study Group. *Pediatr Infect Dis J* 1992;**11**:610–13.
38. Black SB, Shinefield HR, Fireman B, et al. Efficacy in infancy of oligosaccharide conjugate *Haemophilus influenzae* type b (HbOC) vaccine in a United States population of 61,080 children. The Northern California Kaiser Permanente Vaccine Study Center Pediatrics Group. *Pediatr Infect Dis J* 1991;**10**:97–104.
39. Blazer S, Berant M, Alon U. Bacterial meningitis: effect of antibiotic treatment on cerebrospinal fluid. *Am J Clin Pathol* 1983;**80**: 386–7.
40. Block SL, Hedrick J, Harrison CJ, et al. Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. *Pediatr Infect Dis J* 2004;**23**:829–33.
41. Bodor FF. Conjunctivitis-otitis syndrome. *Pediatrics* 1982;**69**: 695–8.
42. Bodor FF. Systemic antibiotics for treatment of the conjunctivitis-otitis media syndrome. *Pediatr Infect Dis J* 1989;**8**:287–90.
43. Bodor FF, Marchant CD, Shurin PA, Barenkamp SJ. Bacterial etiology of conjunctivitis-otitis media syndrome. *Pediatrics* 1985; **76**:26–8.
44. Bonadio WA. Cerebral herniation syndrome as the presenting sign of *Haemophilus influenzae* meningitis. *Pediatr Emerg Care* 1987;**3**:253–5.
45. Booy R, Moxon ER, MacFarlane JA, et al. Efficacy of *Haemophilus influenzae* type b conjugate vaccine in Oxford region. *Lancet* 1992;**340**:847.

46. Bottenfield GW, Arcinue EL, Sarnaik A, Jewell MR. Diagnosis and management of acute epiglottitis: report of 90 consecutive cases. *Laryngoscope* 1980;**90**:822–5.
47. Bouchet V, Hood DW, Li J, et al. Host-derived sialic acid is incorporated into *Haemophilus influenzae* lipopolysaccharide and is a major virulence factor in experimental otitis media. *Proc Natl Acad Sci U S A* 2003;**100**:8898–903.
48. Braikat M, Barkia A, El Mdaghri N, et al. Vaccination with *Haemophilus influenzae* type b conjugate vaccine reduces bacterial meningitis in Morocco. *Vaccine* 2012;**30**:2594–9.
49. Brilli RJ, Benzing G, 3rd, Cotcamp DH. Epiglottitis in infants less than two years of age. *Pediatr Emerg Care* 1989;**5**:16–21.
50. Broome CV. Epidemiology of *Haemophilus influenzae* type b infections in the United States. *Pediatr Infect Dis J* 1987;**6**:779–82.
51. Broome CV, Schlech WFI. Recent developments in the epidemiology of bacterial meningitis. In: Sande MA, Smith A, Root RD, editors. *Bacterial meningitis*. Edinburgh: Churchill Livingstone; 1985. p. 1–10.
52. Broughton RA, Edwards MS, Taber LH, Baker CJ. Systemic *Haemophilus influenzae* type b infection presenting as fever of unknown origin. *J Pediatr* 1981;**98**:925–8.
53. Bruce MG, Deeks SL, Zulz T, et al. Epidemiology of *Haemophilus influenzae* serotype a, North American Arctic, 2000-2005. *Emerg Infect Dis* 2008;**14**:48–55.
54. Bulkow LR, Wainwright RB, Letson GW, et al. Comparative immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in Alaska Native infants. *Pediatr Infect Dis J* 1993;**12**: 484–92.
55. Buscher AZ, Burmeister K, Barenkamp SJ, StGeme JW, 3rd. Evolutionary and functional relationships among the nontypeable *Haemophilus influenzae* HMW family of adhesins. *J Bacteriol* 2004; **186**:4209–17.
56. Cadoz M, Prince-David M, Diop Mar I, Denis F. [Epidemiology and prognosis of *Haemophilus influenzae* meningitis in Africa (901 cases)]. *Pathol Biol (Paris)* 1983;**31**:128–33.
57. Campbell LR, Zedd AJ, Michaels RH. Household spread of infection due to *Haemophilus influenzae* type b. *Pediatrics* 1980;**66**: 115–17.
58. Campognone P, Singer DB. Neonatal sepsis due to nontypable *Haemophilus influenzae*. *Am J Dis Child* 1986;**140**:117–21.
59. Cant AJ, Gibson PJ, West RJ. Bacterial tracheitis in Down's syndrome. *Arch Dis Child* 1987;**62**:962–3.
60. Cardines R, Giufre M, Mastrantonio P, et al. Nontypeable *Haemophilus influenzae* meningitis in children: phenotypic and genotypic characterization of isolates. *Pediatr Infect Dis J* 2007; **26**:577–82.
61. Carruthers MD, Tracy EN, Dickson AC, et al. Biological roles of nontypeable *Haemophilus influenzae* type IV pilus proteins encoded by the pil and com operons. *J Bacteriol* 2012;**194**:1927–33.
62. Casadevall A, Dobroszycki J, Small C, Pirofski LA. *Haemophilus influenzae* type b bacteremia in adults with AIDS and at risk for AIDS. *Am J Med* 1992;**92**:587–90.
63. Casey JR, Pichichero ME. Changes in frequency and pathogens causing acute otitis media in 1995-2003. *Pediatr Infect Dis J* 2004;**23**:824–8.
64. Centers for Disease Control and Prevention. Ampicillin and chloramphenicol resistance in systemic *Haemophilus influenzae* disease. *MMWR Morb Mortal Wkly Rep* 1984;**33**:35–7.
65. Centers for Disease Control and Prevention. Polysaccharide vaccine for prevention of *Haemophilus influenzae* type b disease. *MMWR Morb Mortal Wkly Rep* 1985;**34**:201–5.
66. Centers for Disease Control and Prevention. Recommendations for use of *Haemophilus* b conjugate vaccines and a combined diphtheria, tetanus, pertussis, and *Haemophilus* b vaccine. Recommendations of the advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1993;**42**:1–15.
67. Centers for Disease Control and Prevention. Progress toward elimination of *Haemophilus influenzae* type b invasive disease among infants and children—United States, 1998-2000. *MMWR Morb Mortal Wkly Rep* 2002;**51**:234–7.
68. Centers for Disease Control and Prevention. Licensure of a *Haemophilus influenzae* type b (Hib) vaccine (Hiberix) and updated recommendations for use of Hib vaccine. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1008–9.
69. Chapin KC, Doern GV. Selective media for recovery of *Haemophilus influenzae* from specimens contaminated with upper respiratory tract microbial flora. *J Clin Microbiol* 1983;**17**:1163–5.
70. Cheatham JE Jr, Grantham RN, Peyton MD, et al. *Hemophilus influenzae* purulent pericarditis in children: diagnostic and therapeutic considerations. *J Thorac Cardiovasc Surg* 1980;**79**:933–6.
71. Chen LT, Moxon ER. Effect of splenic congestion associated with hemolytic anemia on mortality of rats challenged with *Haemophilus influenzae* b. *Am J Hematol* 1983;**15**:117–21.
72. Chilcote RR, Baehner RL, Hammond D. Septicemia and meningitis in children splenectomized for Hodgkin's disease. *N Engl J Med* 1976;**295**:798–800.
73. Chitayat D, Diamant S, Lazevnick R, Spirer Z. *Hemophilus influenzae* type b pneumonia with pneumatocele formation. *Clin Pediatr (Phila)* 1980;**19**:151–2.
74. Chonmaitree T, Heikkinen T. Viruses and acute otitis media. *Pediatr Infect Dis J* 2000;**19**:1005–7.
75. Claesson BA, Trollfors B, Lagergard T, et al. Clinical and immunologic responses to the capsular polysaccharide of *Haemophilus influenzae* type b alone or conjugated to tetanus toxoid in 18- to 23-month-old children. *J Pediatr* 1988;**112**:695–702.
76. Clark JH, Fitzgerald JF, Kleiman MB. Spontaneous bacterial peritonitis. *J Pediatr* 1984;**104**:495–500.
77. Clark SE, Snow J, Li J, Zola TA, Weiser JN. Phosphorylcholine allows for evasion of bactericidal antibody by *Haemophilus influenzae*. *PLoS Pathog* 2012;**8**:e1002521.
78. Clausen CR. Detection of bacterial pathogens in purulent clinical specimens by immunofluorescence techniques. *J Clin Microbiol* 1981;**13**:1119–21.
79. Cleveland RH, Foglia RP. CT in the evaluation of pleural versus pulmonary disease in children. *Pediatr Radiol* 1988;**18**:14–19.
80. Cochi SL, Broome CV. Vaccine prevention of *Haemophilus influenzae* type b disease: past, present and future. *Pediatr Infect Dis* 1986;**5**:12–19.
81. Cochi SL, Fleming DW, Hightower AW, et al. Primary invasive *Haemophilus influenzae* type b disease: a population-based assessment of risk factors. *J Pediatr* 1986;**108**:887–96.
82. Cody AJ, Field D, Feil EJ, et al. High rates of recombination in otitis media isolates of non-typeable *Haemophilus influenzae*. *Infect Genet E* 2003;**3**:57–66.
83. Colamussi ML, White MR, Crouch E, Hartshorn KL. Influenza A virus accelerates neutrophil apoptosis and markedly potentiates apoptotic effects of bacteria. *Blood* 1999;**93**:2395–403.
84. Cortese MM, Goepp J, Almeido-Hill J, et al. Children with *Haemophilus influenzae* bacteremia initially treated as outpatients: outcome in 85 American Indian children. *Pediatr Infect Dis J* 1992;**11**:521–5.
85. Cotter SE, Yeo HJ, Juehne T, St. Geme JW, 3rd. Architecture and adhesive activity of the *Haemophilus influenzae* Hsf adhesin. *J Bacteriol* 2005;**187**:4656–64.
86. Crosson FJ Jr, Winkelstein JA, Moxon ER. Participation of complement in the nonimmune host defense against experimental *Haemophilus influenzae* type b septicemia and meningitis. *Infect Immun* 1976;**14**:882–7.
87. Dabernat H, Delmas C. Epidemiology and evolution of antibiotic resistance of *Haemophilus influenzae* in children 5 years of age or less in France, 2001-2008: a retrospective database analysis. *Eur J Clin Microbiol Infect Dis* 2012;**31**:2745–53.
88. Dabernat H, Plisson-Saune MA, Delmas C, et al. *Haemophilus influenzae* carriage in children attending French day care centers: a molecular epidemiological study. *J Clin Microbiol* 2003;**41**: 1664–72.
89. Dajani AS, Asmar BI, Thirumoorthi MC. Systemic *Haemophilus influenzae* disease: an overview. *J Pediatr* 1979;**94**:355–64.
90. Daum RS, Granoff DM, Gilsdorf J, et al. *Haemophilus influenzae* type b infections in day care attendees: implications for management. *Rev Infect Dis* 1986;**8**:558–67.
91. Davie JM. Antipolysaccharide immunity in man and animals. In: Sell SH, Wright PF, editors. *Haemophilus influenzae*. New York: Elsevier Science; 1982.
92. De Souza-Hart JA, Blackstock W, Di Modugno V, et al. Two-component systems in *Haemophilus influenzae*: a regulatory role for ArcA in serum resistance. *Infect Immun* 2003;**71**:163–72.
93. Decker MD, Edwards KM, Bradley R, Palmer P. Comparative trial in infants of four conjugate *Haemophilus influenzae* type b vaccines. *J Pediatr* 1992;**120**:184–9.

94. DeMaria TF, Murwin DM, Leake ER. Immunization with outer membrane protein P6 from nontypeable *Haemophilus influenzae* induces bactericidal antibody and affords protection in the chinchilla model of otitis media. *Infect Immun* 1996;**64**:5187–92.
95. Dentinger CM, Hennessy TW, Bulkow LR, et al. Immunogenicity and reactogenicity to *Haemophilus influenzae* type b (Hib) conjugate vaccine among rural Alaska adults. *Hum Vaccin* 2006;**2**: 24–8.
96. Dixon K, Bayliss CD, Makepeace K, et al. Identification of the functional initiation codons of a phase-variable gene of *Haemophilus influenzae*, *lic2A*, with the potential for differential expression. *J Bacteriol* 2007;**189**:511–21.
97. Dodge PR, Davis H, Feigin RD, et al. Prospective evaluation of hearing impairment as a sequela of acute bacterial meningitis. *N Engl J Med* 1984;**311**:869–74.
98. Dodge PR, Swartz MN. Bacterial meningitis: a review of selected aspects—special neurologic problems, postmeningitic complications and clinicopathological correlations. *N Engl J Med* 1965; **272**:1003–10.
99. Doern GV, Jones RN, Pfaller MA, Kugler K. *Haemophilus influenzae* and *Moraxella catarrhalis* from patients with community-acquired respiratory tract infections: Antimicrobial susceptibility patterns from the SENTRY antimicrobial Surveillance Program (United States and Canada, 1997). *Antimicrob Agents Chemother* 1999;**43**:385–9.
100. Drexhage HA, van de Plassche EM, Kokje M, Leezenberg HA. Abnormalities in cell-mediated immune functions to *Haemophilus influenzae* chronic purulent infections of the upper respiratory tract. *Clin Immunol Immunopathol* 1983;**28**:218–28.
101. Echeverria P, Smith EW, Ingram D, et al. *Hemophilus influenzae* b pericarditis in children. *Pediatrics* 1975;**56**:808–18.
102. Egan W, Schneerson R, Werner KE, Zon G. Structural studies and chemistry of bacterial capsular polysaccharides: investigations of phosphodiester-linked capsular polysaccharides isolated from *Haemophilus influenzae* types a, b, c and f: NMR spectroscopic identification and chemical modification of endgroups and the nature of base-catalyzed hydrolytic depolymerization. *J Am Chem Soc* 1982;**104**:2898–910.
103. Egan W, Tsui FP, Schneerson R. Structural studies of the *Haemophilus influenzae* type f capsular polysaccharide. *Carbohydr Res* 1980;**79**:271–7.
104. El-Adhami W, Kyd JM, Bastin DA, Cripps AW. Characterization of the gene encoding a 26-kilodalton protein (OMP26) from nontypeable *Haemophilus influenzae* and immune responses to the recombinant protein. *Infect Immun* 1999;**67**:1935–3997.
105. Eldika N, Sethi S. Role of nontypeable *Haemophilus influenzae* in exacerbations and progression of chronic obstructive pulmonary disease. *Curr Opin Pulm Med* 2006;**12**:118–24.
106. Erwin AL, Allen S, Ho DK, et al. Role of *lgtC* in resistance of nontypeable *Haemophilus influenzae* strain R2866 to human serum. *Infect Immun* 2006;**74**:6226–35.
107. Erwin AL, Bonthuis PJ, Geelhood JL, et al. Heterogeneity in tandem octanucleotides within *Haemophilus influenzae* lipopolysaccharide biosynthetic gene *losA* affects serum resistance. *Infect Immun* 2006;**74**:3408–14.
108. Erwin AL, Nelson KL, Mhlanga-Mutangadura T, et al. Characterization of genetic and phenotypic diversity of invasive nontypeable *Haemophilus influenzae*. *Infect Immun* 2005;**73**:5853–63.
109. Eskola J, Kayhty H, Takala AK, et al. A randomized, prospective field trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* type b disease. *N Engl J Med* 1990;**323**:1381–7.
110. Eskola J, Peltola H, Kayhty H, et al. Finnish efficacy trials with *Haemophilus influenzae* type b vaccines. *J Infect Dis* 1992; **165**(Suppl 1):S137–138.
111. Faden H, Duffy L, Williams A, et al. Epidemiology of nasopharyngeal colonization with nontypeable *Haemophilus influenzae* in the first 2 years of life. *J Infect Dis* 1995;**172**:132–5.
112. Falla TJ, Dobson SR, Crook DW, et al. Population-based study of non-typable *Haemophilus influenzae* invasive disease in children and neonates. *Lancet* 1993;**341**:851–4.
113. Farjo RS, Foxman B, Patel MJ, et al. Diversity and sharing of *Haemophilus influenzae* strains colonizing healthy children attending day-care centers. *Pediatr Infect Dis J* 2004;**23**:41–6.
114. Farley MM, Stephens DS, Brachman PS Jr, et al. Invasive *Haemophilus influenzae* disease in adults: a prospective, population-based surveillance. CDC Meningitis Surveillance Group. *Ann Intern Med* 1992;**116**:806–12.
115. Farrand RJ. Recurrent *Haemophilus* septicaemia and immunoglobulin deficiency. *Arch Dis Child* 1970;**45**:582–4.
116. Feigin RD, McCracken GH Jr, Klein JO. Diagnosis and management of meningitis. *Pediatr Infect Dis J* 1992;**11**:785–814.
117. Feldman WE, Ginsburg CM, McCracken GH Jr, et al. Relation of concentrations of *Haemophilus influenzae* type b in cerebrospinal fluid to late sequelae of patients with meningitis. *J Pediatr* 1982; **100**:209–12.
118. Feldman WE, Manning NS. Effect of growth phase on the bactericidal action of chloramphenicol against *Haemophilus influenzae* type b and *Escherichia coli* K-1. *Antimicrob Agents Chemother* 1983;**23**:551–4.
119. Feldman WE, Schwartz J. *Haemophilus influenzae* type b brain abscess complicating meningitis: case report. *Pediatrics* 1983;**72**: 473–5.
120. Fernaays MM, Lesse AJ, Cai X, Murphy TF. Characterization of *igaB*, a second immunoglobulin A1 protease gene in nontypeable *Haemophilus influenzae*. *Infect Immun* 2006;**74**:5860–70.
121. Fernandez J, Levine OS, Sanchez J, et al. Prevention of *Haemophilus influenzae* type b colonization by vaccination: correlation with serum anti-capsular IgG concentration. *J Infect Dis* 2000;**182**: 1553–6.
122. Figueira MA, Ram S, Goldstein R, et al. Role of complement in defense of the middle ear revealed by restoring virulence of *siaB* mutants of nontypeable *Haemophilus influenzae*. *Infect Immun* 2007;**75**:325–33.
123. Figueroa JE, Densen P. Infectious diseases associated with complement deficiencies. *Clin Microbiol Rev* 1991;**4**:359–95.
124. Fink CW, Nelson JD. Septic arthritis and osteomyelitis in children. *Clin Rheum Dis* 1986;**12**:423–35.
125. Fink DL, Buscher AZ, Green B, et al. The *Haemophilus influenzae* Hap autotransporter mediates microcolony formation and adherence to epithelial cells and extracellular matrix via binding regions in the C-terminal end of the passenger domain. *Cell Microbiol* 2003;**5**:175–86.
126. Fleisher G, Ludwig S, Campos J. Cellulitis: bacterial etiology, clinical features, and laboratory findings. *J Pediatr* 1980;**97**: 591–3.
127. Fleming JA, Dieye Y, Ba O, et al. Effectiveness of *Haemophilus influenzae* type b conjugate vaccine for prevention of meningitis in Senegal. *Pediatr Infect Dis J* 2011;**30**:430–2.
128. Flesher AR, Insel RA. Characterization of lipopolysaccharide of *Haemophilus influenzae*. *J Infect Dis* 1978;**138**:719–30.
129. Forsgren J, Samuelson A, Ahlin A, et al. *Haemophilus influenzae* resides and multiplies intracellularly in human adenoid tissue as demonstrated by in situ hybridization and bacterial viability assay. *Infect Immun* 1994;**62**:673–9.
130. Fothergill LD, Wright J. Influenzal meningitis: the relation of age incidence to the bactericidal power of blood against the causal organism. *J Immunol* 1933;**24**:273–84.
131. Foxwell AR, Kyd JM, Cripps AW. Nontypeable *Haemophilus influenzae*: pathogenesis and prevention. *Microbiol Mol Biol Rev* 1998;**62**:294–308.
132. Fraser DW. *Haemophilus influenzae* in the community and in the home. In: Sell SH, Wright PF, editors. *Haemophilus influenzae*. New York: Elsevier; 1982. p. 11–22.
133. Fraser DW, Geil CC, Feldman RA. Bacterial meningitis in Bernalillo County, New Mexico: a comparison with three other American populations. *Am J Epidemiol* 1974;**100**:29–34.
134. Fraser DW, Henke CE, Feldman RA. Changing patterns of bacterial meningitis in Olmsted County, Minnesota, 1935-1970. *J Infect Dis* 1973;**128**:300–7.
135. Frayha HH, Kalloghlian AK, deMoor MM. Endocarditis due to *Haemophilus influenzae* serotype f. *Clin Infect Dis* 1996;**23**:401–2.
136. Freij BJ, Votteler TP, McCracken GH Jr. Primary peritonitis in previously healthy children. *Am J Dis Child* 1984;**138**:1058–61.
137. Friesen CA, Cho CT. Characteristic features of neonatal sepsis due to *Haemophilus influenzae*. *Rev Infect Dis* 1986;**8**:777–80.
138. Fritzell B, Plotkin S. Efficacy and safety of a *Haemophilus influenzae* type b capsular polysaccharide-tetanus protein conjugate vaccine. *J Pediatr* 1992;**121**:355–62.
139. Fuller DD, Davis TE, Kibsey PC, et al. Comparison of BACTEC Plus 26 and 27 media with and without fastidious organism

supplement with conventional methods for culture of sterile body fluids. *J Clin Microbiol* 1994;**32**:1488–91.
140. Funkhouser A, Steinhoff MC, Ward J. *Haemophilus influenzae* disease and immunization in developing countries. *Rev Infect Dis* 1991;**13**(Suppl 6):S542–554.
141. Galil K, Singleton R, Levine OS, et al. Reemergence of invasive *Haemophilus influenzae* type b disease in a well-vaccinated population in remote Alaska. *J Infect Dis* 1999;**179**:101–6.
142. Gawthorne JA, Beatson SA, Srikhanta YN, et al. Origin of the diversity in DNA recognition domains in phasevarion associated *modA* genes of pathogenic *Neisseria* and *Haemophilus influenzae*. *PLoS One* 2012;**7**:e32337.
143. Gilsdorf JR. *Haemophilus influenzae* non-type b infections in children. *Am J Dis Child* 1987;**141**:1063–5.
144. Gilsdorf JR, Marrs CF, Foxman B. *Haemophilus influenzae*: genetic variability and natural selection to identify virulence factors. *Infect Immun* 2004;**72**:2457–61.
145. Gilsdorf JR, McCrea KW, Marrs CF. Role of pili in *Haemophilus influenzae* adherence and colonization. *Infect Immun* 1997;**65**: 2997–3002.
146. Gilsdorf JR, Tucci M, Marrs CF. Role of pili in *Haemophilus influenzae* adherence to, and internalization by, respiratory cells. *Pediatr Res* 1996;**39**:343–8.
147. Ginsburg CM. *Hemophilus influenzae* type b buccal cellulitis. *J Am Acad Dermatol* 1981;**4**:661–4.
148. Ginsburg CM, Howard JB, Nelson JD. Report of 65 cases of *Haemophilus influenzae* b pneumonia. *Pediatrics* 1979;**64**:283–6.
149. Giufre M, Cardines R, Caporali MG, et al. Ten years of Hib vaccination in Italy: prevalence of non-encapsulated *Haemophilus influenzae* among invasive isolates and the possible impact on antibiotic resistance. *Vaccine* 2011;**29**:3857–62.
150. Glatman-Freedman A, Litman N. Septic arthritis caused by an unusual type of *Haemophilus influenzae*. *J Infect* 1996;**32**: 143–5.
151. Glezen WP, Englund JA, Siber GR, et al. Maternal immunization with the capsular polysaccharide vaccine for *Haemophilus influenzae* type b. *J Infect Dis* 1992;**165**(Suppl 1):S134–136.
152. Glode MP, Daum RS, Halsey NA, et al. Rifampin alone and in combination with trimethoprim in chemoprophylaxis for infections due to *Haemophilus influenzae* type b. *Rev Infect Dis* 1983; **5**(Suppl 3):S549–555.
153. Gold R, Barreto L, Ferro S, et al. Safety and immunogenicity of a fully liquid vaccine containing five-component pertussis-diphtheria-tetanus-inactivated poliomyelitis-*Haemophilus influenzae* type b conjugate vaccines administered at two, four, six and 18 months of age. *Can J Infect Dis Med Microbiol* 2007;**18**:241–8.
154. Goldblatt D, Fairley CK, Cartwright K, Miller E. Interchangeability of conjugated *Haemophilus influenzae* type b vaccines during primary immunisation of infants. *BMJ* 1996;**312**:817–18.
155. Goodman RA, Osterholm MT, Granoff DM, Pickering LK. Infectious diseases and child day care. *Pediatrics* 1984;**74**:134–9.
156. Granoff DM. Assessing efficacy of *Haemophilus influenzae* type b combination vaccines. *Clin Infect Dis* 2001;**33**(Suppl 4):S278–287.
157. Granoff DM, Basden M. *Haemophilus influenzae* infections in Fresno County, California: a prospective study of the effects of age, race, and contact with a case on incidence of disease. *J Infect Dis* 1980;**141**:40–6.
158. Granoff DM, Gilsdorf J, Gessert C, Basden M. *Haemophilus influenzae* type b disease in a day care center: Eradication of carrier state by rifampin. *Pediatrics* 1979;**63**:397–401.
159. Granoff DM, Holmes SJ, Belshe RB, et al. Effect of carrier protein priming on antibody responses to *Haemophilus influenzae* type b conjugate vaccines in infants. *JAMA* 1994;**272**:1116–21.
160. Granoff DM, Lucas AH. Laboratory correlates of protection against *Haemophilus influenzae* type b disease: Importance of assessment of antibody avidity and immunologic memory. *Ann N Y Acad Sci* 1995;**754**:278–88.
161. Granoff DM, Rathore MH, Holmes SJ, et al. Effect of immunity to the carrier protein on antibody responses to *Haemophilus influenzae* type b conjugate vaccines. *Vaccine* 1993;**11**(Suppl 1):S46–51.
162. Granoff DM, Shackelford PG, Pandey JP, Boies EG. Antibody responses to *Haemophilus influenzae* type b polysaccharide vaccine in relation to Km(1) and G2m(23) immunoglobulin allotypes. *J Infect Dis* 1986;**154**:257–64.
163. Greenberg DP, Lieberman JM, Marcy SM, et al. Enhanced antibody responses in infants given different sequences of heterogeneous *Haemophilus influenzae* type b conjugate vaccines. *J Pediatr* 1995;**126**:206–11.
164. Greenfield S, Peter G, Howie VM, et al. Acquisition of type-specific antibodies to *Hemophilus influenzae* type b. *J Pediatr* 1972; **80**:204–8.
165. Greenfield SP. Type b *Hemophilus influenzae* epididymo-orchitis in the prepubertal boy. *J Urol* 1986;**136**:1311–13.
166. Gregorius FK, Johnson BL Jr, Stern WE, Brown WJ. Pathologenesis of hematogenous bacterial meningitis in rabbits. *J Neurosurg* 1976;**45**:561–7.
167. Griffin R, Bayliss CD, Herbert MA, et al. Digalactoside expression in the lipopolysaccharide of *Haemophilus influenzae* and its role in intravascular survival. *Infect Immun* 2005;**73**: 7022–6.
168. Griffin R, Cox AD, Makepeace K, et al. The role of *lex2* in lipopolysaccharide biosynthesis in *Haemophilus influenzae* strains RM7004 and RM153. *Microbiology* 2003;**149**:3165–75.
169. Griffin R, Cox AD, Makepeace K, et al. Elucidation of the monoclonal antibody 5G8-reactive, virulence-associated lipopolysaccharide epitope of *Haemophilus influenzae* and its role in bacterial resistance to complement-mediated killing. *Infect Immun* 2005; **73**:2213–21.
170. Gu XX, Sun J, Jin S, et al. Detoxified lipooligosaccharide from nontypeable *Haemophilus influenzae* conjugated to proteins confers protection against otitis media in chinchillas. *Infect Immun* 1997;**65**:4488–93.
171. Hallstrom T, Blom AM, Zipfel PF, Riesbeck K. Nontypeable *Haemophilus influenzae* protein E binds vitronectin and is important for serum resistance. *J Immunol* 2009;**183**:2593–601.
172. Hallstrom T, Jarva H, Riesbeck K, Blom AM. Interaction with C4b-binding protein contributes to nontypeable *Haemophilus influenzae* serum resistance. *J Immunol* 2007;**178**:6359–66.
173. Hallstrom T, Resman F, Ristovski M, Riesbeck K. Binding of complement regulators to invasive nontypeable *Haemophilus influenzae* isolates is not increased compared to nasopharyngeal isolates, but serum resistance is linked to disease severity. *J Clin Microbiol* 2010;**48**:921–7.
174. Hallstrom T, Riesbeck K. *Haemophilus influenzae* and the complement system. *Trends Microbiol* 2010;**18**:258–65.
175. Hallstrom T, Singh B, Resman F, et al. *Haemophilus influenzae* protein E binds to the extracellular matrix by concurrently interacting with laminin and vitronectin. *J Infect Dis* 2011;**204**: 1065–74.
176. Hallstrom T, Trajkovska E, Forsgren A, Riesbeck K. *Haemophilus influenzae* surface fibrils contribute to serum resistance by interacting with vitronectin. *J Immunol* 2006;**177**:430–6.
177. Hallstrom T, Zipfel PF, Blom AM, et al. *Haemophilus influenzae* interacts with the human complement inhibitor factor H. *J Immunol* 2008;**181**:537–45.
178. Hammitt LL, Block S, Hennessy TW, et al. Outbreak of invasive *Haemophilus influenzae* serotype a disease. *Pediatr Infect Dis J* 2005;**24**:453–6.
179. Hammitt LL, Hennessy TW, Romero-Steiner S, Butler JC. Assessment of carriage of *Haemophilus influenzae* type a after a case of invasive disease. *Clin Infect Dis* 2006;**43**:386–7.
180. Hansman D, Hanna J, Morey F. High prevalence of invasive *Haemophilus influenzae* disease in central Australia, 1986. *Lancet* 1986;**2**:927.
181. Hardy GG, Tudor SM, StGeme JW, 3rd. The pathogenesis of disease due to nontypeable *Haemophilus influenzae*. *Methods Mol Med* 2003;**71**:1–28.
182. Harrison A, Dyer DW, Gillaspy A, et al. Genomic sequence of an otitis media isolate of nontypeable *Haemophilus influenzae*: comparative study with *H. influenzae* serotype d, strain KW20. *J Bacteriol* 2005;**187**:4627–36.
183. Hasegawa K, Chiba N, Kobayashi R, et al. Rapidly increasing prevalence of beta-lactamase–nonproducing, ampicillin-resistant *Haemophilus influenzae* type b in patients with meningitis. *Antimicrob Agents Chemother* 2004;**48**:1509–14.
184. Hashida K, Shiomori T, Hohchi N, et al. Nasopharyngeal *Haemophilus influenzae* carriage in Japanese children attending day-care centers. *J Clin Microbiol* 2008;**46**:876–81.
185. Heath PT, Booy R, Azzopardi HJ, et al. Non-type b *Haemophilus influenzae* disease: clinical and epidemiologic characteristics in the

Haemophilus influenzae type b vaccine era. *Pediatr Infect Dis J* 2001;**20**:300–5.
186. Hershckowitz S, Elisha MB, Fleisher-Sheffer V, et al. A cluster of early neonatal sepsis and pneumonia caused by nontypable *Haemophilus influenzae*. *Pediatr Infect Dis J* 2004;**23**:1061–2.
187. Hogg JS, Hu FZ, Janto B, et al. Characterization and modeling of the *Haemophilus influenzae* core and supragenomes based on the complete genomic sequences of Rd and 12 clinical nontypeable strains. *Genome Biol* 2007;**8**:R103.
188. Hoiseth SK, Moxon ER, Silver RP. Genes involved in *Haemophilus influenzae* type b capsule expression are part of an 18-kilobase tandem duplication. *Proc Natl Acad Sci U S A* 1986;**83**:1106–10.
189. Holmes SJ, Murphy TV, Anderson RS, et al. Immunogenicity of four *Haemophilus influenzae* type b conjugate vaccines in 17- to 19-month-old children. *J Pediatr* 1991;**118**:364–71.
190. Horner DB, McCracken GH Jr, Ginsburg CM, Zweighaft TC. A comparison of three antibiotic regimens for eradication of *Haemophilus influenzae* type b from the pharynx of infants and children. *Pediatrics* 1980;**66**:136–8.
191. Howe PM, Eduardo Fajardo J, Orcutt MA. Etiologic diagnosis of cellulitis: comparison of aspirates obtained from the leading edge and the point of maximal inflammation. *Pediatr Infect Dis J* 1987;**6**:685–6.
192. Humphries HE, High NJ. The role of *licA* phase variation in the pathogenesis of invasive disease by *Haemophilus influenzae* type b. *FEMS Immunol Med Microbiol* 2002;**34**:221–30.
193. Istre GR, Conner JS, Broome CV, et al. Risk factors for primary invasive *Haemophilus influenzae* disease: increased risk from day care attendance and school-aged household members. *J Pediatr* 1985;**106**:190–5.
194. Jacobs RF, Hsi S, Wilson CB, et al. Apparent meningococcemia: clinical features of disease due to *Haemophilus influenzae* and *Neisseria meningitidis*. *Pediatrics* 1983;**72**:469–72.
195. Jacups SP, Morris PS, Leach AJ. *Haemophilus influenzae* type b carriage in indigenous children and children attending childcare centers in the Northern Territory, Australia, spanning pre- and post-vaccine eras. *Vaccine* 2011;**29**:3083–8.
196. Jarosik GP, Maciver I, Hansen EJ. Utilization of transferrin-bound iron by *Haemophilus influenzae* requires an intact tonB gene. *Infect Immun* 1995;**63**:710–13.
197. Jenkins GA, Figueira M, Kumar GA, et al. Sialic acid mediated transcriptional modulation of a highly conserved sialometabolism gene cluster in *Haemophilus influenzae* and its effect on virulence. *BMC Microbiol* 2010;**10**:48.
198. Johnson NG, Ruggeberg JU, Balfour GF, et al. *Haemophilus influenzae* type b reemergence after combination immunization. *Emerg Infect Dis* 2006;**12**:937–41.
199. Johnston RB Jr, Anderson P, Rosen FS, Smith DH. Characterization of human antibody to polyribophosphate, the capsular antigen of *Hemophilus influenzae*, type b. *Clin Immunol Immunopathol* 1973;**1**:234–40.
200. Jokinen J, Palmu AA, Kilpi T. Acute otitis media replacement and recurrence in the Finnish Otitis Media (FinOM) vaccine trial. *Clin Infect Dis* 2012;**55**:1673–6.
201. Jonsdottir KE, Steingrimsson O, Olafsson O. Immunisation of infants in Iceland against *Haemophilus influenzae* type b. *Lancet* 1992;**340**:252–3.
202. Jorgensen JH. Update on mechanisms and prevalence of antimicrobial resistance in *Haemophilus influenzae*. *Clin Infect Dis* 1992;**14**:1119–23.
203. Kalies H, Siedler A, Grondahl B, et al. Invasive *Haemophilus influenzae* infections in Germany: impact of non-type b serotypes in the post-vaccine era. *BMC Infect Dis* 2009;**9**:45.
204. Kaplan SL. Antigen detection in cerebrospinal fluid—pros and cons. *Am J Med* 1983;**75**:109–18.
205. Kapogiannis BG, Satola S, Keyserling HL, Farley MM. Invasive infections with *Haemophilus influenzae* serotype a containing an IS1016-bexA partial deletion: possible association with virulence. *Clin Infect Dis* 2005;**41**:e97–103.
206. Katz A, Leibovitz E, Greenberg D, et al. Acute mastoiditis in Southern Israel: a twelve year retrospective study (1990 through 2001). *Pediatr Infect Dis J* 2003;**22**:878–82.
207. Kaur R, Chang A, Xu Q, et al. Phylogenetic relatedness and diversity of non-typable *Haemophilus influenzae* in the nasopharynx and middle ear fluid of children with acute otitis media. *J Med Microbiol* 2011;**60**:1841–8.
208. Kayhty H, Eskola J, Peltola H, et al. Immunogenicity in infants of a vaccine composed of *Haemophilus influenzae* type b capsular polysaccharide mixed with DPT or conjugated to diphtheria toxoid. *J Infect Dis* 1987;**155**:100–6.
209. Kayhty H, Karanko V, Peltola H, Makela PH. Serum antibodies after vaccination with *Haemophilus influenzae* type b capsular polysaccharide and responses to reimmunization: no evidence of immunologic tolerance or memory. *Pediatrics* 1984;**74**:857–65.
210. Kayhty H, Peltola H, Karanko V, Makela PH. The protective level of serum antibodies to the capsular polysaccharide of *Haemophilus influenzae* type b. *J Infect Dis* 1983;**147**:1100.
211. Kayhty H, Schneerson R, Sutton A. Class-specific antibody response to *Haemophilus influenzae* type b capsular polysaccharide vaccine. *J Infect Dis* 1983;**148**:767.
212. Kirby SD, Gray-Owen SD, Schryvers AB. Characterization of a ferric-binding protein mutant in *Haemophilus influenzae*. *Mol Microbiol* 1997;**25**:979–87.
213. Kleiman MB, Reynolds JK, Watts NH, et al. Superiority of acridine orange stain versus Gram stain in partially treated bacterial meningitis. *J Pediatr* 1984;**104**:401–4.
214. Klein JO. Role of nontypeable *Haemophilus influenzae* in pediatric respiratory tract infections. *Pediatr Infect Dis J* 1997;**16**:S5–8.
215. Koomey JM, Falkow S. Nucleotide sequence homology between the immunoglobulin A1 protease genes of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Haemophilus influenzae*. *Infect Immun* 1984;**43**:101–7.
216. Korones DN, Marshall GS, Shapiro ED. Outcome of children with occult bacteremia caused by *Haemophilus influenzae* type b. *Pediatr Infect Dis J* 1992;**11**:516–20.
217. Krasinski K, Nelson JD, Butler S, et al. Possible association of mycoplasma and viral respiratory infections with bacterial meningitis. *Am J Epidemiol* 1987;**125**:499–508.
218. Kroll JS, Hopkins I, Moxon ER. Capsule loss in *H. influenzae* type b occurs by recombination-mediated disruption of a gene essential for polysaccharide export. *Cell* 1988;**53**:347–56.
219. Kroll JS, Loynds BM, Moxon ER. The *Haemophilus influenzae* capsulation gene cluster: a compound transposon. *Mol Microbiol* 1991;**5**:1549–60.
220. Kroll JS, Moxon ER, Loynds BM. Natural genetic transfer of a putative virulence-enhancing mutation to *Haemophilus influenzae* type a. *J Infect Dis* 1994;**169**:676–9.
221. Kuklinska D, Kilian M. Relative proportions of *Haemophilus* species in the throat of healthy children and adults. *Eur J Clin Microbiol* 1984;**3**:249–52.
222. Kyd JM, Cripps AW, Novotny LA, Bakaletz LO. Efficacy of the 26-kilodalton outer membrane protein and two P5 fimbrin-derived immunogens to induce clearance of nontypeable *Haemophilus influenzae* from the rat middle ear and lungs as well as from the chinchilla middle ear and nasopharynx. *Infect Immun* 2003;**71**:4691–9.
223. Lacross NC, Marrs CF, Patel M, et al. High genetic diversity of nontypeable *Haemophilus influenzae* isolates from two children attending a day care center. *J Clin Microbiol* 2008;**46**:3817–21.
224. Ladhani S, Slack MP, Heath PT, et al. Invasive *Haemophilus influenzae* disease, Europe, 1996-2006. *Emerg Infect Dis* 2010;**16**:455–63.
225. Ladhani S, Slack MP, Heys M, et al. Fall in *Haemophilus influenzae* serotype b (Hib) disease following implementation of a booster campaign. *Arch Dis Child* 2008;**93**:665–9.
226. Ladhani SN. Two decades of experience with the *Haemophilus influenzae* serotype b conjugate vaccine in the United Kingdom. *Clin Ther* 2012;**34**:385–99.
227. Ladhani SN, Collins S, Vickers A, et al. Invasive *Haemophilus influenzae* serotype e and f disease, England and Wales. *Emerg Infect Dis* 2012;**18**:725–32.
228. Landsteiner K. *The specificity of serologic reactions*. Cambridge: Harvard University Press; 1962.
229. Langereis JD, Stol K, Schweda EK, et al. Modified lipooligosaccharide structure protects nontypeable *Haemophilus influenzae* from IgM-mediated complement killing in experimental otitis media. *MBio* 2012;**3**:e00079–12.
230. Lebel MH, Nelson JD. *Haemophilus influenzae* type b osteomyelitis in infants and children. *Pediatr Infect Dis J* 1988;**7**:250–4.
231. Ledeboer NA, Doern GV. Haemophilus. In: Versalovic R, Carroll KC, Funke G, et al., editors. *Manual of clinical microbiology*.

10th ed. Washington, D.C.: American Society for Microbiology; 2012. p. 588–602.
232. Lee HY, Andalibi A, Webster P, et al. Antimicrobial activity of innate immune molecules against *Streptococcus pneumoniae*, *Moraxella catarrhalis* and nontypeable *Haemophilus influenzae*. *BMC Infect Dis* 2004;**4**:12.
233. Lehmann D. Epidemiology of acute respiratory tract infections, especially those due to *Haemophilus influenzae*, in Papua New Guinean children. *J Infect Dis* 1992;**165**(Suppl 1):S20–25.
234. Leibovitz E, Jacobs MR, Dagan R. *Haemophilus influenzae*: a significant pathogen in acute otitis media. *Pediatr Infect Dis J* 2004;**23**:1142–52.
235. Leibovitz E, Satran R, Piglansky L, et al. Can acute otitis media caused by *Haemophilus influenzae* be distinguished from that caused by *Streptococcus pneumoniae*? *Pediatr Infect Dis J* 2003;**22**:509–15.
236. Lerman SJ. *Haemophilus influenzae* infections of cerebrospinal fluid shunts: report of two cases. *J Neurosurg* 1981;**54**:261–3.
237. Letson GW, Santosham M, Reid R, et al. Comparison of active and combined passive/active immunization of Navajo children against *Haemophilus influenzae* type b. *Pediatr Infect Dis J* 1988;**7**:747–52.
238. Lieberman JM, Greenberg DP, Wong VK, et al. Effect of neonatal immunization with diphtheria and tetanus toxoids on antibody responses to *Haemophilus influenzae* type b conjugate vaccines. *J Pediatr* 1995;**126**:198–205.
239. Lieberthal A, Ganiats T, and Subcommittee on management of acute otitis media. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.
240. Liechty E, Kleiman MB, Ballantine TV, Grosfeld JL. Primary *Hemophilus influenzae* lung abscesses with bronchial obstruction. *J Pediatr Surg* 1982;**17**:281–4.
241. Lin YC, King DR, Birken GA, Barson WJ. Acute scrotum due to *Haemophilus influenzae* type b. *J Pediatr Surg* 1988;**23**:183–4.
242. Losek JD, Dewitz-Zink BA, Melzer-Lange M, Havens PL. Epiglottitis: comparison of signs and symptoms in children less than 2 years old and older. *Ann Emerg Med* 1990;**19**:55–8.
243. Losonsky GA, Santosham M, Sehgal VM, et al. *Haemophilus influenzae* disease in the White Mountain Apaches: molecular epidemiology of a high risk population. *Pediatr Infect Dis* 1984;**3**:539–47.
244. Lowther SA, Shinoda N, Juni BA, et al. *Haemophilus influenzae* type b infection, vaccination, and *H. influenzae* carriage in children in Minnesota, 2008-2009. *Epidemiol Infect* 2012;**140**:566–74.
245. Lucas AH. Expression of crossreactive idiotypes by human antibodies specific for the capsular polysaccharide of *Hemophilus influenzae* b. *J Clin Invest* 1988;**81**:480–6.
246. Lucas AH, Azmi FH, Mink CM, Granoff DM. Age-dependent V region expression in the human antibody response to the *Haemophilus influenzae* type b polysaccharide. *J Immunol* 1993; **150**:2056–61.
247. Lucas AH, Granoff DM. Functional differences in idiotypically defined IgG1 anti-polysaccharide antibodies elicited by vaccination with *Haemophilus influenzae* type b polysaccharide-protein conjugates. *J Immunol* 1995;**154**:4195–202.
248. Lucas AH, McLean GR, Reason DC, et al. Molecular ontogeny of the human antibody repertoire to the *Haemophilus influenzae* type b polysaccharide: expression of canonical variable regions and their variants in vaccinated infants. *Clin Immunol* 2003; **108**:119–27.
249. Lucher LA, Reeves M, Hennessy T, et al. Reemergence, in southwestern Alaska, of invasive *Haemophilus influenzae* type b disease due to strains indistinguishable from those isolated from vaccinated children. *J Infect Dis* 2002;**186**:958–65.
250. Lysenko E, Richards JC, Cox AD, et al. The position of phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* affects binding and sensitivity to C-reactive protein-mediated killing. *Mol Microbiol* 2000;**35**:234–45.
251. Maaroufi Y, De Bruyne JM, Heymans C, Crokaert F. Real-time PCR for determining capsular serotypes of *Haemophilus influenzae*. *J Clin Microbiol* 2007;**45**:2305–8.
252. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* disease—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.
253. Madore DV, Johnson CL, Phipps DC, et al. Safety and immunogenicity of *Haemophilus influenzae* type b oligosaccharide-CRM197 conjugate vaccine in infants aged 15 to 23 months. *Pediatrics* 1990;**86**:527–34.
254. Makela PH, Takala AK, Peltola H, Eskola J. Epidemiology of invasive *Haemophilus influenzae* type b disease. *J Infect Dis* 1992;**165**(Suppl 1):S2–6.
255. Makintubee S, Istre GR, Ward JI. Transmission of invasive *Haemophilus influenzae* type b disease in day care settings. *J Pediatr* 1987;**111**:180–6.
256. Mansson M, Bauer SH, Hood DW, et al. A new structural type for *Haemophilus influenzae* lipopolysaccharide: structural analysis of the lipopolysaccharide from nontypeable *Haemophilus influenzae* strain 486. *Eur J Biochem* 2001;**268**:2148–59.
257. Mansson M, Hood DW, Moxon ER, Schweda EK. Structural characterization of a novel branching pattern in the lipopolysaccharide from nontypeable *Haemophilus influenzae*. *Eur J Biochem* 2003;**270**:2979–91.
258. Marcon MJ, Hamoudi AC, Cannon HJ. Comparative laboratory evaluation of three antigen detection methods for diagnosis of *Haemophilus influenzae* type b disease. *J Clin Microbiol* 1984;**19**: 333–7.
259. Mason EO Jr, Kaplan SL, Lamberth LB, et al. Serotype and ampicillin susceptibility of *Haemophilus influenzae* causing systemic infections in children: 3 years of experience. *J Clin Microbiol* 1982;**15**:543–6.
260. Mason KM, Munson RS Jr, Bakaletz LO. A mutation in the *sap* operon attenuates survival of nontypeable *Haemophilus influenzae* in a chinchilla model of otitis media. *Infect Immun* 2005;**73**: 599–608.
261. Mason KM, Raffel FK, Ray WC, Bakaletz LO. Heme utilization by nontypeable *Haemophilus influenzae* is essential and dependent on Sap transporter function. *J Bacteriol* 2011;**193**:2527–35.
262. Masters PL, Brumfitt W, Mendez RL, Likar M. Bacterial flora of the upper respiratory tract in Paddington families: 1952-4. *BMJ* 1958;1200–5.
263. Mathai D, Lewis MT, Kugler KC, et al. Antibacterial activity of 41 antimicrobials tested against over 2773 bacterial isolates from hospitalized patients with pneumonia, part I: results from the SENTRY Antimicrobial Surveillance Program (North America, 1998). *Diagn Microbiol Infect Dis* 2001;**39**:105–16.
264. McCarthy LG. *Haemophilus influenzae* associated with periappendiceal abscess. *Am J Gastroenterol* 1981;**76**:157–9.
265. McConnell A, Tan B, Scheifele D, et al. Invasive infections caused by *Haemophilus influenzae* serotypes in twelve Canadian IMPACT centers, 1996-2001. *Pediatr Infect Dis J* 2007;**26**:1025–31.
266. McCracken GH Jr, Ginsburg CM, Zweighaft TC, Clahsen J. Pharmacokinetics of rifampin in infants and children: relevance to prophylaxis against *Haemophilus influenzae* type b disease. *Pediatrics* 1980;**66**:17–21.
267. McVernon J, Andrews N, Slack MP, Ramsay ME. Risk of vaccine failure after *Haemophilus influenzae* type b (Hib) combination vaccines with acellular pertussis. *Lancet* 2003;**361**:1521–3.
268. McVernon J, Howard AJ, Slack MP, Ramsay ME. Long-term impact of vaccination on *Haemophilus influenzae* type b (Hib) carriage in the United Kingdom. *Epidemiol Infect* 2004;**132**:765–7.
269. McVernon J, Slack MP, Ramsay ME. Changes in the epidemiology of epiglottitis following introduction of *Haemophilus influenzae* type b (Hib) conjugate vaccines in England: a comparison of two data sources. *Epidemiol Infect* 2006;**134**:570–2.
270. Meats E, Feil EJ, Stringer S, et al. Characterization of encapsulated and noncapsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing. *J Clin Microbiol* 2003;**41**:1623–36.
271. Mehl AL. *Haemophilus influenzae* polyserositis. *J Pediatr* 1988; **112**:160–1.
272. Meissner HC, Smith AL. The current status of chloramphenicol. *Pediatrics* 1979;**64**:348–56.
273. Michaels RH, Norden CW. Pharyngeal colonization with *Haemophilus influenzae* type b: a longitudinal study of families with a child with meningitis or epiglottitis due to *H. influenzae* type b. *J Infect Dis* 1977;**136**:222–8.
274. Michaels RH, Schultz WF. The frequency of *Haemophilus influenzae* infections: analysis of racial and environmental factors. In: Sell SH, Karzon DT, editors. *Haemophilus influenzae*. Nashville, Tenn.: Vanderbilt University Press; 1973. p. 243–50.
275. Miragliotta G, Colucci M, Semeraro N. Platelet injury and stimulation of leukocyte procoagulant activity in vitro by a lipopolysaccharide from *Haemophilus influenzae* type b. *Microbiologica* 1981; **4**:173–80.

276. Morgan RJ, Stephenson LW, Woolf PK, et al. Surgical treatment of purulent pericarditis in children. *J Thorac Cardiovasc Surg* 1983; **85**:527–31.
277. Morris SK, Moss WJ, Halsey N. *Haemophilus influenzae* type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008;**8**: 435–43.
278. Morton DJ, Seale TW, Vanwagoner TM, et al. The *dppBCDF* gene cluster of *Haemophilus influenzae*: role in heme utilization. *BMC Res Notes* 2009;**2**:166.
279. Moxon ER. Experimental studies of *H. influenzae* in a rat model. In: Sell SH, Wright PF, editors. *Haemophilus influenzae: epidemiology, immunology, and prevention of disease*. New York: Elsevier; 1982.
280. Moxon ER. Molecular basis of invasive *Haemophilus influenzae* type b disease. *J Infect Dis* 1992;**165**(Suppl 1):S77–81.
281. Mu XQ, Egelman EH, Bullitt E. Structure and function of Hib pili from *Haemophilus influenzae* type b. *J Bacteriol* 2002;**184**: 4868–74.
282. Mulks MH, Kornfeld SJ, Frangione B, Plaut AG. Relationship between the specificity of IgA proteases and serotypes in *Haemophilus influenzae*. *J Infect Dis* 1982;**146**:266–74.
283. Munson RS Jr, Granoff DM. Purification and partial characterization of outer membrane proteins P5 and P6 from *Haemophilus influenzae* type b. *Infect Immun* 1985;**49**:544–9.
284. Munson RS Jr, Harrison A, Gillaspy A, et al. Partial analysis of the genomes of two nontypeable *Haemophilus influenzae* otitis media isolates. *Infect Immun* 2004;**72**:3002–10.
285. Munson RS Jr, Shenep JL, Barenkamp SJ, Granoff DM. Purification and comparison of outer membrane protein P2 from *Haemophilus influenzae* type b isolates. *J Clin Invest* 1983;**72**:677–84.
286. Murphy TF. Respiratory infections caused by non-typeable *Haemophilus influenzae*. *Curr Opin Infect Dis* 2003;**16**:129–34.
287. Murphy TF. Vaccine development for nontypeable *Haemophilus influenzae* and *Moraxella catarrhalis*: progress and challenges. *Expert Rev Vaccines* 2005;**4**:843–53.
288. Murphy TF, Bartos LC. Human bactericidal antibody response to outer membrane protein P2 of nontypeable *Haemophilus influenzae*. *Infect Immun* 1988;**56**:2673–9.
289. Murphy TF, Bartos LC, Rice PA, et al. Identification of a 16,600-dalton outer membrane protein on nontypeable *Haemophilus influenzae* as a target for human serum bactericidal antibody. *J Clin Invest* 1986;**78**:1020–7.
290. Murphy TF, Dudas KC, Mylotte JM, Apicella MA. A subtyping system for nontypable *Haemophilus influenzae* based on outer-membrane proteins. *J Infect Dis* 1983;**147**:838–46.
291. Murphy TF, Faden H, Bakaletz LO, et al. Nontypeable *Haemophilus influenzae* as a pathogen in children. *Pediatr Infect Dis J* 2009;**28**:43–8.
292. Murphy TF, Lesse AJ, Kirkham C, et al. A clonal group of nontypeable *Haemophilus influenzae* with two IgA proteases is adapted to infection in chronic obstructive pulmonary disease. *PLoS One* 2011;**6**:e25923.
293. Murphy TV, Granoff D, Chrane DF, et al. Pharyngeal colonization with *Haemophilus influenzae* type b in children in a day care center without invasive disease. *J Pediatr* 1985;**106**:712–16.
294. Murphy TV, Pastor P, Medley F, et al. Decreased *Haemophilus* colonization in children vaccinated with *Haemophilus influenzae* type b conjugate vaccine. *J Pediatr* 1993;**122**:517–23.
295. Murphy TV, White KE, Pastor P, et al. Declining incidence of *Haemophilus influenzae* type b disease since introduction of vaccination. *JAMA* 1993;**269**:246–8.
296. Musher D, Goree A, Murphy T, et al. Immunity to *Haemophilus influenzae* type b in young adults: correlation of bactericidal and opsonizing activity of serum with antibody to polyribosylribitol phosphate and lipooligosaccharide before and after vaccination. *J Infect Dis* 1986;**154**:935–43.
297. Musher DM, Goree A, Baughn RE, Birdsall HH. Immunoglobulin A from bronchopulmonary secretions blocks bactericidal and opsonizing effects of antibody to nontypable *Haemophilus influenzae*. *Infect Immun* 1984;**45**:36–40.
298. Musher DM, Hague-Park M, Baughn RE, et al. Opsonizing and bactericidal effects of normal human serum on nontypable *Haemophilus influenzae*. *Infect Immun* 1983;**39**:297–304.
299. Musser JM, Barenkamp SJ, Granoff DM, Selander RK. Genetic relationships of serologically nontypable and serotype b strains of *Haemophilus influenzae*. *Infect Immun* 1986;**52**:183–91.
300. Musser JM, Kroll JS, Granoff DM, et al. Global genetic structure and molecular epidemiology of encapsulated *Haemophilus influenzae*. *Rev Infect Dis* 1990;**12**:75–111.
301. Mustafa MM, Lebel MH, McCracken GH Jr. Tenosynovitis and transient arthritis associated with *Haemophilus influenzae* type b bacteremia. *Pediatr Infect Dis J* 1988;**7**:517–19.
302. Mustafa MM, Scarvey L, Rollins N, Siegel JD. Primary suppurative myositis associated with *Haemophilus influenzae* type b septicemia. *Pediatr Infect Dis J* 1988;**7**:815–17.
303. Myerowitz RL, Michaels RH. Mechanism of potentiation of experimental *Haemophilus influenzae* type b disease in infant rats by influenza A virus. *Lab Invest* 1981;**44**:434–41.
304. Nelson KL, Smith AL. Determination of capsulation status in *Haemophilus influenzae* by multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2010;**66**:235–40.
305. Nelson MB, Munson RS Jr, Apicella MA, et al. Molecular conservation of the P6 outer membrane protein among strains of *Haemophilus influenzae*: analysis of antigenic determinants, gene sequences, and restriction fragment length polymorphisms. *Infect Immun* 1991;**59**:2658–63.
306. Newman SL, Waldo B, Johnston RB Jr. Separation of serum bactericidal and opsonizing activities for *Haemophilus influenzae* type b. *Infect Immun* 1973;**8**:488–90.
307. Nizet V, Colina KF, Almquist JR, et al. A virulent nonencapsulated *Haemophilus influenzae*. *J Infect Dis* 1996;**173**:180–6.
308. Noel GJ, Mosser DM, Edelson PJ. Role of complement in mouse macrophage binding of *Haemophilus influenzae* type b. *J Clin Invest* 1990;**85**:208–18.
309. O'Neill JM, St. Geme JW, 3rd, Cutter D, et al. Invasive disease due to nontypeable *Haemophilus influenzae* among children in Arkansas. *J Clin Microbiol* 2003;**41**:3064–9.
310. Odio CM, Faingezicht I, Paris M, et al. The beneficial effects of early dexamethasone administration in infants and children with bacterial meningitis. *N Engl J Med* 1991;**324**:1525–31.
311. Oh SY, Griffiths D, John T, et al. School-aged children: a reservoir for continued circulation of *Haemophilus influenzae* type b in the United Kingdom. *J Infect Dis* 2008;**197**:1275–81.
312. Ojo LR, O'Loughlin RE, Cohen AL, et al. Global use of *Haemophilus influenzae* type b conjugate vaccine. *Vaccine* 2010;**28**: 7117–22.
313. Omikunle A, Takahashi S, Ogilvie CL, et al. Limited genetic diversity of recent invasive isolates of non–serotype b encapsulated *Haemophilus influenzae*. *J Clin Microbiol* 2002;**40**:1264–70.
314. Osterholm MT, Pierson LM, White KE, et al. The risk of subsequent transmission of *Hemophilus influenzae* type b disease among children in day care: results of a two-year statewide prospective surveillance and contact survey. *N Engl J Med* 1987;**316**:1–5.
315. Ostrow PT, Moxon ER, Vernon N, Kapko R. Pathogenesis of bacterial meningitis: studies on the route of meningeal invasion following *Hemophilus influenzae* inoculation of infant rats. *Lab Invest* 1979;**40**:678–85.
316. Overturf GD, Cable D, Ward J. Ampicillin-chloramphenicol-resistant *Haemophilus influenzae*: plasmid-mediated resistance in bacterial meningitis. *Pediatr Res* 1987;**22**:438–41.
317. Oxelius VA. Quantitative and qualitative investigations of serum IgG subclasses in immunodeficiency diseases. *Clin Exp Immunol* 1979;**36**:112–16.
318. Patel JA, Nguyen DT, Revai K, Chonmaitree T. Role of respiratory syncytial virus in acute otitis media: implications for vaccine development. *Vaccine* 2007;**25**:1683–9.
319. Peerbooms PG, Engelen MN, Stokman DA, et al. Nasopharyngeal carriage of potential bacterial pathogens related to day care attendance, with special reference to the molecular epidemiology of *Haemophilus influenzae*. *J Clin Microbiol* 2002;**40**:2832–6.
320. Peltola H, Kayhty H, Sivonen A, Makela H. *Haemophilus influenzae* type b capsular polysaccharide vaccine in children: a double-blind field study of 100,000 vaccinees 3 months to 5 years of age in Finland. *Pediatrics* 1977;**60**:730–7.
321. Peltola H, Kayhty H, Virtanen M, Makela PH. Prevention of *Hemophilus influenzae* type b bacteremic infections with the capsular polysaccharide vaccine. *N Engl J Med* 1984;**310**:1561–6.
322. Peltola H, Kilpi T, Anttila M. Rapid disappearance of *Haemophilus influenzae* type b meningitis after routine childhood immunisation with conjugate vaccines. *Lancet* 1992;**340**:592–4.
323. Peltola H, Virtanen M. Systemic *Haemophilus influenzae* infection in Finland. *Clin Pediatr (Phila)* 1984;**23**:275–80.

324. Pelton SI. Modifying acute otitis media: implications of immunization with pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 2004;**23**:839–41.
325. Pennekamp A, Zbinden R, von Graevenitz A. Detection of *Haemophilus influenzae* and *Haemophilus parainfluenzae* from body fluids in blood culture bottles. *J Microbiol Methods* 1996;**25**: 303–7.
326. Perdue DG, Bulkow LR, Gellin BG, et al. Invasive *Haemophilus influenzae* disease in Alaskan residents aged 10 years and older before and after infant vaccination programs. *JAMA* 2000;**283**: 3089–94.
327. Petersen GM, Silimperi DR, Chiu CY, Ward JI. Effects of age, breast feeding, and household structure on *Haemophilus influenzae* type b disease risk and antibody acquisition in Alaskan Eskimos. *Am J Epidemiol* 1991;**134**:1212–21.
328. Pettigrew MM, Foxman B, Ecevit Z, et al. Use of pulsed-field gel electrophoresis, enterobacterial repetitive intergenic consensus typing, and automated ribotyping to assess genomic variability among strains of nontypeable *Haemophilus influenzae*. *J Clin Microbiol* 2002;**40**:660–2.
329. Pfeiffer R. Vorlaufige mit Heilungen uber die Erreger der Influenzae. *Dtsch Med Wochenschr* 1892;**18**:28–34.
330. Pichichero ME, Casey JR, Hoberman A, Schwartz R. Pathogens causing recurrent and difficult-to-treat acute otitis media, 2003-2006. *Clin Pediatr* 2008;**47**:901–6.
331. Pichichero ME, Hall CB, Insel RA. A mucosal antibody response following systemic *Haemophilus influenzae* type b infection in children. *J Clin Invest* 1981;**67**:1482–9.
332. Pichichero ME, Insel RA. Mucosal antibody response to parenteral vaccination with *Haemophilus influenzae* type b capsule. *J Allergy Clin Immunol* 1983;**72**:481–6.
333. Pittman M. The action of type-specific *H. influenzae* antiserum. *J Exp Med* 1933;**58**:583–706.
334. Pittman M. Variation and type specificity in the bacterial species: *H. influenzae*. *J Exp Med* 1931;**53**:471–95.
335. Plaut AG. The IgA1 proteases of pathogenic bacteria. *Annu Rev Microbiol* 1983;**37**:603–22.
336. Pomeroy SL, Holmes SJ, Dodge PR, Feigin RD. Seizures and other neurologic sequelae of bacterial meningitis in children. *N Engl J Med* 1990;**323**:1651–7.
337. Powars D, Overturf G, Turner E. Is there an increased risk of *Haemophilus influenzae* septicemia in children with sickle cell anemia? *Pediatrics* 1983;**71**:927–31.
338. Prober CG. The role of steroids in the management of children with bacterial meningitis. *Pediatrics* 1995;**95**:29–31.
339. Prymula R, Peeters P, Chrobok V, et al. Pneumococcal capsular polysaccharides conjugated to protein D for prevention of acute otitis media caused by both *Streptococcus pneumoniae* and nontypable *Haemophilus influenzae*: a randomised double-blind efficacy study. *Lancet* 2006;**367**:740–8.
340. Ramadas K, Petersen GM, Heiner DC, Ward JI. Class and subclass antibodies to *Haemophilus influenzae* type b capsule: comparison of invasive disease and natural exposure. *Infect Immun* 1986;**53**:486–90.
341. Ramsay ME, McVernon J, Andrews NJ, et al. Estimating *Haemophilus influenzae* type b vaccine effectiveness in England and Wales by use of the screening method. *J Infect Dis* 2003;**188**: 481–5.
342. Raymond J, Armand-Lefevre L, Moulin F, et al. Nasopharyngeal colonization by *Haemophilus influenzae* in children living in an orphanage. *Pediatr Infect Dis J* 2001;**20**:779–84.
343. Reddy MS, Bernstein JM, Murphy TF, Faden HS. Binding between outer membrane proteins of nontypeable *Haemophilus influenzae* and human nasopharyngeal mucin. *Infect Immun* 1996; **64**:1477–9.
344. Redmond SR, Pichichero ME. *Hemophilus influenzae* type b disease: an epidemiologic study with special reference to day-care centers. *JAMA* 1984;**252**:2581–4.
345. Rennels MB, Wald ER. Treatment of *Haemophilus influenzae* type b meningitis in children with cerebrospinal fluid shunts. *J Pediatr* 1980;**97**:424–6.
346. Resman F, Ristovski M, Forsgren A, et al. Increase of β-lactam–resistant invasive *Haemophilus influenzae* in Sweden, 1997 to 2010. *Antimicrob Agents Chemother* 2012;**56**:4408–15.
347. Ribeiro GS, Reis JN, Cordeiro SM, et al. Prevention of *Haemophilus influenzae* type b (Hib) meningitis and emergence of serotype replacement with type a strains after introduction of Hib immunization in Brazil. *J Infect Dis* 2003;**187**:109–16.
348. Ricketts RR, Ilbawi MN, Idriss FS. Management of *Hemophilus influenza* pericarditis. *J Pediatr Surg* 1982;**17**:285–9.
349. Riesen WF, Skvaril F, Braun DG. Natural infection of man with group A streptococci: levels, restriction in class, subclass, and type, and clonal appearance of polysaccharide-group-specific antibodies. *Scand J Immunol* 1976;**5**:383–90.
350. Robbins JB, Parke JC Jr, Schneerson R, Whisnant JK. Quantitative measurement of "natural" and immunization-induced *Haemophilus influenzae* type b capsular polysaccharide antibodies. *Pediatr Res* 1973;**7**:103–10.
351. Robbins JB, Schneerson R, Pittman M. *H. influenzae* type b infections. In: Germanier R, editor. *Bacterial vaccines*. Orlando, Fla.: Academic Press; 1984. p. 290–313.
352. Roberts MC, Swenson CD, Owens LM, Smith AL. Characterization of chloramphenicol-resistant *Haemophilus influenzae*. *Antimicrob Agents Chemother* 1980;**18**:610–15.
353. Rodrigues LP, Schneerson R, Robbins JB. Immunity to *Hemophilus influenzae* type b, part I: the isolation, and some physicochemical, serologic and biologic properties of the capsular polysaccharide of *Hemophilus influenzae* type b. *J Immunol* 1971;**107**:1071–80.
354. Rowe JE, Messinger IK, Schwendeman CA, Popejoy LA. Three-dose vaccination of infants under 8 months of age with a conjugate *Haemophilus influenzae* type b vaccine. *Mil Med* 1990;**155**:483–6.
355. Rubin LG, Moxon ER. Pathogenesis of bloodstream invasion with *Haemophilus influenzae* type b. *Infect Immun* 1983;**41**:280–4.
356. Rubin LG, Zwahlen A, Moxon ER. Role of intravascular replication in the pathogenesis of experimental bacteremia due to *Haemophilus influenzae* type b. *J Infect Dis* 1985;**152**:307–14.
357. Rusin P, Adam RD, Peterson EA, et al. *Haemophilus influenzae*: an important cause of maternal and neonatal infections. *Obstet Gynecol* 1991;**77**:92–6.
358. Sack CM, Koup JR, Smith AL. Chloramphenicol pharmacokinetics in infants and young children. *Pediatrics* 1980;**66**:579–84.
359. Saez-Llorens X. Pathogenesis of acute otitis media. *Pediatr Infect Dis J* 1994;**13**:1035–8.
360. Salwen KM, Vikerfors T, Olcen P. Increased incidence of childhood bacterial meningitis: a 25-year study in a defined population in Sweden. *Scand J Infect Dis* 1987;**19**:1–11.
361. Santosham M, Reid R, Ambrosino DM, et al. Prevention of *Haemophilus influenzae* type b infections in high-risk infants treated with bacterial polysaccharide immune globulin. *N Engl J Med* 1987;**317**:923–9.
362. Santosham M, Wolff M, Reid R, et al. The efficacy in Navajo infants of a conjugate vaccine consisting of *Haemophilus influenzae* type b polysaccharide and *Neisseria meningitidis* outer-membrane protein complex. *N Engl J Med* 1991;**324**:1767–72.
363. Schappert SM. National Ambulatory Medical Care Survey: 1992 summary. *Adv Data* 1994;1–20.
364. Schauf V, Green DC, van der Stuyf L, Riff L. Chloramphenicol kills *Haemophilus influenzae* more rapidly than does ampicillin or cefamandole. *Antimicrob Agents Chemother* 1983;**23**:364–8.
365. Scheifele D, Halperin S, Law B, et al. Invasive *Haemophilus influenzae* type b infections in vaccinated and unvaccinated children in Canada, 2001-2003. *CMAJ* 2005;**172**:53–6.
366. Scheifele DW, Halperin SA, Rubin E, et al. Safety and immunogenicity of a pentavalent combination vaccine (diphtheria, tetanus, acellular pertussis, polio, and *Haemophilus influenzae* type b conjugate) when administered as a fourth dose at 15 to 18 months of age. *Hum Vaccin* 2005;**1**:180–6.
367. Scheld WM, Park TS, Dacey RG, et al. Clearance of bacteria from cerebrospinal fluid to blood in experimental meningitis. *Infect Immun* 1979;**24**:102–5.
368. Schlamm HT, Yancovitz SR. *Haemophilus influenzae* pneumonia in young adults with AIDS, ARC, or risk of AIDS. *Am J Med* 1989;**86**:11–14.
369. Schlesinger Y, Granoff DM. Avidity and bactericidal activity of antibody elicited by different *Haemophilus influenzae* type b conjugate vaccines. The Vaccine Study Group. *JAMA* 1992; **267**:1489–94.
370. Schmitt HJ, von Kries R, Hassenpflug B, et al. *Haemophilus influenzae* type b disease: impact and effectiveness of diphtheria-tetanus toxoids-acellular pertussis (-inactivated poliovirus)/*H. influenzae* type b combination vaccines. *Pediatr Infect Dis J* 2001;**20**:767–74.

371. Schneerson R, Robbins JB, Parke JC Jr, et al. Quantitative and qualitative analyses of serum antibodies elicited in adults by *Haemophilus influenzae* type b and pneumococcus type 6A capsular polysaccharide-tetanus toxoid conjugates. *Infect Immun* 1986;**52**: 519–28.
372. Schneerson R, Rodrigues LP, Parke JC Jr, Robbins JB. Immunity to disease caused by *Hemophilus influenzae* type b, part II: specificity and some biologic characteristics of "natural," infection-acquired, and immunization-induced antibodies to the capsular polysaccharide of *Hemophilus influenzae* type b. *J Immunol* 1971; **107**:1081–9.
373. Schreiber JR, Barrus V, Cates KL, Siber GR. Functional characterization of human IgG, IgM, and IgA antibody directed to the capsule of *Haemophilus influenzae* type b. *J Infect Dis* 1986;**153**: 8–16.
374. Schumacher SK, Marchant CD, Loughlin AM, et al. Prevalence and genetic diversity of nontypeable *Haemophilus influenzae* in the respiratory tract of infants and primary caregivers. *Pediatr Infect Dis J* 2012;**31**:145–9.
375. Schur PH, Borel H, Gelfand EW, et al. Selective gamma-g globulin deficiencies in patients with recurrent pyogenic infections. *N Engl J Med* 1970;**283**:631–4.
376. Seale TW, Morton DJ, Whitby PW, et al. Complex role of hemoglobin and hemoglobin-haptoglobin binding proteins in *Haemophilus influenzae* virulence in the infant rat model of invasive infection. *Infect Immun* 2006;**74**:6213–25.
377. Sethi S. Bacterial infection and the pathogenesis of COPD. *Chest* 2000;**117**:286S–91S.
378. Severi E, Randle G, Kivlin P, et al. Sialic acid transport in *Haemophilus influenzae* is essential for lipopolysaccharide sialylation and serum resistance and is dependent on a novel tripartite ATP-independent periplasmic transporter. *Mol Microbiol* 2005;**58**: 1173–85.
379. Shackelford PG, Granoff DM, Nahm MH, et al. Relation of age, race, and allotype to immunoglobulin subclass concentrations. *Pediatr Res* 1985;**19**:846–9.
380. Shackelford PG, Granoff DM, Nelson SJ, et al. Subclass distribution of human antibodies to *Haemophilus influenzae* type b capsular polysaccharide. *J Immunol* 1987;**138**:587–92.
381. Shapiro ED. Persistent pharyngeal colonization with *Haemophilus influenzae* type b after intravenous chloramphenicol therapy. *Pediatrics* 1981;**67**:435–7.
382. Shapiro ED, Wald ER. Efficacy of rifampin in eliminating pharyngeal carriage of *Haemophilus influenzae* type b. *Pediatrics* 1980; **66**:5–8.
383. Shen K, Antalis P, Gladitz J, et al. Identification, distribution, and expression of novel genes in 10 clinical isolates of nontypeable *Haemophilus influenzae*. *Infect Immun* 2005;**73**:3479–91.
384. Shenep JL, Munson RS Jr, Barenkamp SJ, Granoff DM. Further studies of the role of noncapsular antibody in protection against experimental *Haemophilus influenzae* type b bacteremia. *Infect Immun* 1983;**42**:257–63.
385. Shuel M, Law D, Skinner S, et al. Characterization of nontypeable *Haemophilus influenzae* collected from respiratory infections and invasive disease cases in Manitoba, Canada. *FEMS Immunol Med Microbiol* 2010;**58**:277–84.
386. Siber GR, Ambrosino DM, McIver J, et al. Preparation of human hyperimmune globulin to *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis*. *Infect Immun* 1984; **45**:248–54.
387. Sikkema DJ, Murphy TF. Molecular analysis of the P2 porin protein of nontypeable *Haemophilus influenzae*. *Infect Immun* 1992;**60**:5204–11.
388. Silfverdal SA, Bodin L, Ulanova M, et al. Expression of idiotypic antibodies-1 and -2 and breastfeeding in relation to antibody levels against *Haemophilus influenzae* type b in children. *Scand J Immunol* 2006;**63**:371–5.
389. Sill ML, Law DK, Zhou J, et al. Population genetics and antibiotic susceptibility of invasive *Haemophilus influenzae* in Manitoba, Canada, from 2000 to 2006. *FEMS Immunol Med Microbiol* 2007; **51**:270–6.
390. Singh B, Brant M, Kilian M, et al. Protein E of *Haemophilus influenzae* is a ubiquitous highly conserved adhesin. *J Infect Dis* 2010;**201**:414–19.
391. Singh B, Jalalvand F, Morgelin M, et al. *Haemophilus influenzae* protein E recognizes the C-terminal domain of vitronectin and modulates the membrane attack complex. *Mol Microbiol* 2011;**81**: 80–98.
392. Singleton R, Bulkow LR, Levine OS, et al. Experience with the prevention of invasive *Haemophilus influenzae* type b disease by vaccination in Alaska: the impact of persistent oropharyngeal carriage. *J Pediatr* 2000;**137**:313–20.
393. Singleton R, Hammitt L, Hennessy T, et al. The Alaska *Haemophilus influenzae* type b experience: lessons in controlling a vaccine-preventable disease. *Pediatrics* 2006;**118**:e421–429.
394. Sirinavin S, Chiemchanya S, Visudhipan P, Lolekha S. Cefuroxime treatment of bacterial meningitis in infants and children. *Antimicrob Agents Chemother* 1984;**25**:273–5.
395. Smith AL, Daum RS, Scheifele D. Pathogenesis of *H. influenzae* meningitis. In: Sell SH, Wright PF, editors. *Haemophilus influenzae: epidemiology, immunology, and prevention of disease*. New York: Elsevier; 1982. p. 89–109.
396. Solzbacher D, Hanisch FG, van Alphen L, et al. Mucin in middle ear effusions inhibits attachment of *Haemophilus influenzae* to mucosal epithelial cells. *Eur Arch Otorhinolaryngol* 2003;**260**: 141–7.
397. Sow SO, Diallo S, Campbell JD, et al. Burden of invasive disease caused by *Haemophilus influenzae* type b in Bamako, Mali: impetus for routine infant immunization with conjugate vaccine. *Pediatr Infect Dis J* 2005;**24**:533–7.
398. Spanjaard L, Bol P, Ekker W, Zanen HC. The incidence of bacterial meningitis in the Netherlands—a comparison of three registration systems, 1977-1982. *J Infect* 1985;**11**:259–68.
399. Spinola SM, Sheaffer CI, Philbrick KB, Gilligan PH. Antigenuria after *Haemophilus influenzae* type b polysaccharide immunization: a prospective study. *J Pediatr* 1986;**109**:835–8.
400. StGeme JW, 3rd, Falkow S, Barenkamp SJ. High-molecular-weight proteins of nontypable *Haemophilus influenzae* mediate attachment to human epithelial cells. *Proc Natl Acad Sci U S A* 1993;**90**:2875–9.
401. Starner TD, Swords WE, Apicella MA, McCray PB Jr. Susceptibility of nontypeable *Haemophilus influenzae* to human beta-defensins is influenced by lipooligosaccharide acylation. *Infect Immun* 2002;**70**:5287–9.
402. Steele NP, Munson RS Jr, Granoff DM, et al. Antibody-dependent alternative pathway killing of *Haemophilus influenzae* type b. *Infect Immun* 1984;**44**:452–8.
403. Stephens DS, Farley MM. Pathogenic events during infection of the human nasopharynx with *Neisseria meningitidis* and *Haemophilus influenzae*. *Rev Infect Dis* 1991;**13**:22–33.
404. Stool S, Berg A, Berman S, et al. *Otitis media with effusion in young children. No. 12 of Clinical practice guideline*. Rockville, MD: Department of Health and Human Services; 1994. DHHS publication No. (AHCPR) 94-0622.
405. Stull TL, Jacobs RF, Haas JE, et al. Human serum bactericidal activity against *Haemophilus influenzae* type b. *J Gen Microbiol* 1984;**130**:665–72.
406. Suzuki K, Bakaletz LO. Synergistic effect of adenovirus type 1 and nontypeable *Haemophilus influenzae* in a chinchilla model of experimental otitis media. *Infect Immun* 1994;**62**:1710–18.
407. Swords WE, Buscher BA, Ver Steeg Ii K, et al. Non-typeable *Haemophilus influenzae* adhere to and invade human bronchial epithelial cells via an interaction of lipooligosaccharide with the PAF receptor. *Mol Microbiol* 2000;**37**:13–27.
408. Swords WE, Jones PA, Apicella MA. The lipo-oligosaccharides of *Haemophilus influenzae*: an interesting array of characters. *J Endotoxin Res* 2003;**9**:131–44.
409. Takahata S, Ida T, Senju N, et al. Horizontal gene transfer of *fts*I, encoding penicillin-binding protein 3, in *Haemophilus influenzae*. *Antimicrob Agents Chemother* 2007;**51**:1589–95.
410. Takala AK, Eskola J, Leinonen M, et al. Reduction of oropharyngeal carriage of *Haemophilus influenzae* type b (Hib) in children immunized with an Hib conjugate vaccine. *J Infect Dis* 1991;**164**: 982–6.
411. Takala AK, Meurman O, Kleemola M, et al. Preceding respiratory infection predisposing for primary and secondary invasive *Haemophilus influenzae* type b disease. *Pediatr Infect Dis J* 1993;**12**: 189–95.
412. Talbird SE, Taylor TN, Caporale J, et al. Residual economic burden of *Streptococcus pneumoniae*- and nontypeable *Haemophilus influenzae*–associated disease following vaccination with PCV-7: a multicountry analysis. *Vaccine* 2010;**28**(Suppl 6):G14–22.

413. Tarr PI, Peter G. Demographic factors in the epidemiology of *Hemophilus influenzae* eningitis in young children. *J Pediatr* 1978; **92**:884–8.
414. Tarrand JJ, Scott MG, Takes PA, Nahm MH. Clonal characterization of the human IgG antibody repertoire to *Haemophilus influenzae* type b polysaccharide: demonstration of three types of V regions and their association with H and L chain isotypes. *J Immunol* 1989;**142**:2519–26.
415. Taylor HG, Mills EL, Ciampi A, et al. The sequelae of *Haemophilus influenzae* meningitis in school-age children. *N Engl J Med* 1990;**323**:1657–63.
416. Taylor JR, Cibis GW, Hamtil LW. Endophthalmitis complicating *Haemophilus influenzae* type b meningitis. *Arch Ophthalmol* 1980; **98**:324–6.
417. Thirumoorthi MC, Asmar BI, Dajani AS. Violaceous discoloration in pneumococcal cellulitis. *Pediatrics* 1978;**62**:492–3.
418. Todd JK, Bruhn FW. Severe *Haemophilus influenzae* infections. *Am J Dis Child* 1975;**129**:607–11.
419. Tong HH, Blue LE, James MA, et al. Evaluation of phase variation of nontypeable *Haemophilus influenzae* lipooligosaccharide during nasopharyngeal colonization and development of otitis media in the chinchilla model. *Infect Immun* 2000;**68**:4593–7.
420. Tristram S, Jacobs MR, Appelbaum PC. Antimicrobial resistance in *Haemophilus influenzae*. *Clin Microbiol Rev* 2007;**20**:368–89.
421. Tsang RS, Mubareka S, Sill ML, et al. Invasive *Haemophilus influenzae* in Manitoba, Canada, in the postvaccination era. *J Clin Microbiol* 2006;**44**:1530–5.
422. Tsui FP, Schneerson R, Boykins RA, et al. Structural and immunological studies of the *Haemophilus influenzae* type d capsular polysaccharide. *Carbohydr Res* 1981;**97**:293–306.
423. Tsui FP, Schneerson R, Egan W. Structural studies of the *Haemophilus influenzae* type e capsular polysaccharide. *Carbohydr Res* 1981;**88**:85–92.
424. Turk DC. Naso-pharyngeal carriage of *Haemophilus influenza* type b. *J Hyg (Lond)* 1963;**61**:247–56.
425. Uchiyama N, Greene GR, Kitts DB, Thrupp LD. Meningitis due to *Haemophilus influenzae* type b resistant to ampicillin and chloramphenicol. *J Pediatr* 1980;**97**:421–4.
426. Urwin G, Krohn JA, Deaver-Robinson K, et al. Invasive disease due to *Haemophilus influenzae* serotype f: clinical and epidemiologic characteristics in the *H. influenzae* serotype b vaccine era. the *Haemophilus influenzae* Study Group. *Clin Infect Dis* 1996;**22**: 1069–76.
427. Vadheim CM, Greenberg DP, Bordenave N, et al. Risk factors for invasive *Haemophilus influenzae* type b in Los Angeles County children 18-60 months of age. *Am J Epidemiol* 1992;**136**:221–35.
428. Vadheim CM, Greenberg DP, Eriksen E, et al. Eradication of *Haemophilus influenzae* type b disease in southern California. Kaiser-UCLA Vaccine Study Group. *Arch Pediatr Adolesc Med* 1994;**148**:51–6.
429. Vadheim CM, Greenberg DP, Eriksen E, et al. Protection provided by *Haemophilus influenzae* type b conjugate vaccines in Los Angeles County: a case-control study. *Pediatr Infect Dis J* 1994;**13**:274–80.
430. Vadheim CM, Greenberg DP, Partridge S, et al. Effectiveness and safety of an *Haemophilus influenzae* type b conjugate vaccine (PRP-T) in young infants. Kaiser-UCLA Vaccine Study Group. *Pediatrics* 1993;**92**:272–9.
431. Vallejo JG, Kaplan SL, Mason EO Jr. Treatment of meningitis and other infections due to ampicillin-resistant *Haemophilus influenzae* type b in children. *Rev Infect Dis* 1991;**13**:197–200.
432. Van Nguyen Q, Nguyen EA, Weiner LB. Incidence of invasive bacterial disease in children with fever and petechiae. *Pediatrics* 1984;**74**:77–80.
433. van Wessel K, Rodenburg GD, Veenhoven RH, et al. Nontypeable *Haemophilus influenzae* invasive disease in The Netherlands: a retrospective surveillance study 2001-8. *Clin Infect Dis* 2011;**53**: e1–7.
434. Vitovski S, Dunkin KT, Howard AJ, Sayers JR. Nontypeable *Haemophilus influenzae* in carriage and disease: a difference in IgA1 protease activity levels. *Jama* 2002;**287**:1699–705.
435. von Gottberg A, Cohen C, Whitelaw A, et al. Invasive disease due to *Haemophilus influenzae* serotype b ten years after routine vaccination, South Africa, 2003-2009. *Vaccine* 2012;**30**:565–71.
436. Wald ER. Sinusitis in children. *Pediatr Infect Dis J* 1988;**7**:S150–153.
437. Wald ER, Byers C, Guerra N, et al. Subacute sinusitis in children. *J Pediatr* 1989;**115**:28–32.
438. Wald ER, Levine MM. *Haemophilus influenzae* type b pneumonia. *Arch Dis Child* 1978;**53**:316–18.
439. Walker CW, Brown DF. The reliability of methods for detecting chloramphenicol resistance in *Haemophilus influenzae*. *J Antimicrob Chemother* 1988;**22**:905–10.
440. Wall RA, Corrah PT, Mabey DC, Greenwood BM. The etiology of lobar pneumonia in the Gambia. *Bull World Health Organ* 1986;**64**:553–8.
441. Wallace RJ Jr, Baker CJ, Quinones FJ, et al. Nontypable *Haemophilus influenzae* (biotype 4) as a neonatal, maternal, and genital pathogen. *Rev Infect Dis* 1983;**5**:123–36.
442. Wang B, Cleary PP, Xu H, Li JD. Up-regulation of interleukin-8 by novel small cytoplasmic molecules of nontypeable *Haemophilus influenzae* via p38 and extracellular signal-regulated kinase pathways. *Infect Immun* 2003;**71**:5523–30.
443. Wang YJ, Vuori-Holopainen E, Yang Y, et al. Relative frequency of *Haemophilus influenzae* type b pneumonia in Chinese children as evidenced by serology. *Pediatr Infect Dis J* 2002;**21**:271–7.
444. Ward J, Brenneman G, Letson GW, Heyward WL. Limited efficacy of a *Haemophilus influenzae* type b conjugate vaccine in Alaska Native infants. The Alaska *H. influenzae* Vaccine Study Group. *N Engl J Med* 1990;**323**:1393–401.
445. Ward JI, Fraser DW, Baraff LJ, Plikaytis BD. *Haemophilus influenzae* meningitis: a national study of secondary spread in household contacts. *N Engl J Med* 1979;**301**:122–6.
446. Ward JI, Gorman G, Phillips C, Fraser DW. *Hemophilus influenzae* type b disease in a day-care center: report of an outbreak. *J Pediatr* 1978;**92**:713–17.
447. Ward JI, Lieberman JM, Cochi SL. *Haemophilus influenzae* vaccines. In: Plotkin SA, Mortimer EA, editors. *Vaccines*. Philadelphia: WB Saunders; 1994. p. 337–86.
448. Ward JI, Lum MK, Hall DB, et al. Invasive *Haemophilus influenzae* type b disease in Alaska: background epidemiology for a vaccine efficacy trial. *J Infect Dis* 1986;**153**:17–26.
449. Ward JI, Siber GR, Scheifele DW, Smith DH. Rapid diagnosis of *Hemophilus influenzae* type b infections by latex particle agglutination and counterimmunoelectrophoresis. *J Pediatr* 1978; **93**:37–42.
450. Watt JP, Wolfson LJ, O'Brien KL, et al. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet* 2009;**374**:903–11.
451. Weiser JN, Pan N, McGowan KL, et al. Phosphorylcholine on the lipopolysaccharide of *Haemophilus influenzae* contributes to persistence in the respiratory tract and sensitivity to serum killing mediated by C-reactive protein. *J Exp Med* 1998;**187**: 631–40.
452. Weiser JN, Shchepetov M, Chong ST. Decoration of lipopolysaccharide with phosphorylcholine: a phase-variable characteristic of *Haemophilus influenzae*. *Infect Immun* 1997;**65**:943–50.
453. Weitzman S, Aisenberg AC. Fulminant sepsis after the successful treatment of Hodgkin's disease. *Am J Med* 1977;**62**:47–50.
454. Weller PF, Smith AL, Smith DH, Anderson P. Role of immunity in the clearance of bacteremia due to *Haemophilus influenzae*. *J Infect Dis* 1978;**138**:427–36.
455. Wenger JD, Hightower AW, Facklam RR, et al. Bacterial meningitis in the United States, 1986: report of a multistate surveillance study. The Bacterial Meningitis Study Group. *J Infect Dis* 1990; **162**:1316–23.
456. Whisnant JK, Rogentine GN, Mann DL, Robbins JB. Human cell-surface structures related to *Haemophilus influenzae* type b disease. *Lancet* 1971;**2**:895–8.
457. Whitby PW, Sim KE, Morton DJ, et al. Transcription of genes encoding iron and heme acquisition proteins of *Haemophilus influenzae* during acute otitis media. *Infect Immun* 1997;**65**:4696–700.
458. Wiertsema SP, Kirkham LA, Corscadden KJ, et al. Predominance of nontypeable *Haemophilus influenzae* in children with otitis media following introduction of a 3+0 pneumococcal conjugate vaccine schedule. *Vaccine* 2011;**29**:5163–70.
459. Wilfert CM. Epidemiology of *Haemophilus influenzae* type b infections. *Pediatrics* 1990;**85**:631–5.
460. Winkelstein JA, Moxon ER. The role of complement in the host's defense against *Haemophilus influenzae*. *J Infect Dis* 1992; **165**(Suppl 1):S62–65.

461. Winslow CE, Broadhurst J, Buchanan RE, et al. The families and genera of the bacteria: final report of the Committee of the Society of American Bacteriologists on Characterization and Classification of Bacterial Types. *J Bacteriol* 1920;**5**:191–229.
462. Winter LE, Barenkamp SJ. Human antibodies specific for the high-molecular-weight adhesion proteins of nontypeable *Haemophilus influenzae* mediate opsonophagocytic activity. *Infect Immun* 2003;**71**:6884–91.
463. Winter LE, Barenkamp SJ. Antibodies specific for the high-molecular-weight adhesion proteins of nontypeable *Haemophilus influenzae* are opsonophagocytic for both homologous and heterologous strains. *Clin Vaccine Immunol* 2006;**13**:1333–42.
464. Winter LE, Barenkamp SJ. Antibodies specific for the Hia adhesion proteins of nontypeable *Haemophilus influenzae* mediate opsonophagocytic activity. *Clin Vaccine Immunol* 2009;**16**:1040–6.
465. Wolf WJ. Echocardiographic features of a purulent pericardial peel. *Am Heart J* 1986;**111**:990–2.
466. Wong JC, Holland J, Parsons T, et al. Identification and characterization of an iron-regulated hemopexin receptor in *Haemophilus influenzae* type b. *Infect Immun* 1994;**62**:48–59.
467. Wong SM, St. Michael F, Cox A, et al. ArcA-regulated glycosyltransferase lic2B promotes complement evasion and pathogenesis of nontypeable Haemophilus influenzae. *Infect Immun* 2011;**79**:1971–83.
468. World Health Organization. Review panel on *Haemophilus influenzae* type b (Hib) disease burden in Bangladesh, Indonesia and other Asian countries, Bangkok, 28-29 January 2004. *Wkly Epidemiol Rec* 2004;**79**:173–5.
469. Wotton KA, Stiver HG, Hildes JA. Meningitis in the central Arctic: a 4-year experience. *Can Med Assoc J* 1981;**124**:887–90.
470. Zwahlen A, Winkelstein JA, Moxon ER. Surface determinants of *Haemophilus influenzae* pathogenicity: comparative virulence of capsular transformants in normal and complement-depleted rats. *J Infect Dis* 1983;**148**:385–94.

CHAPTER 134

Other *Haemophilus* Species (*ducreyi*, *haemolyticus*, *influenzae* Biogroup *aegyptius*, *parahaemolyticus*, and *parainfluenzae*) and *Aggregatibacter (Haemophilus) aphrophilus*

Stephen J. Barenkamp

Most serious pediatric infections caused by organisms of the *Haemophilus* genus are caused by *H. influenzae*. However, other *Haemophilus* spp. can cause disease occasionally. Among the other species that have been reported to cause disease in pediatric and adolescent patients are *H. aegyptius*, *H. ducreyi*, and *H. parainfluenzae*. *Aggregatibacter aphrophilus*, until recently known as *Haemophilus aphrophilus*, is also an occasional cause of infections in pediatric patients. *H. aegyptius* is currently classified as a member of the species *H. influenzae* (*H. influenzae* biogroup aegyptius), but given the unique characteristics of this organism and the distinctive illness with which it is associated, it will be discussed in this chapter. Other *Haemophilus* spp., such as *H. haemolyticus* and *H. parahaemolyticus*, rarely cause illness in the pediatric population, so they will not be discussed in detail here. Of note, however, *H. haemolyticus* has been the subject recent papers in the literature because nonhemolytic strains of *H. haemolyticus* are sometimes misidentified as *H. influenzae* unless careful and thorough microbiologic testing is performed.[133,144,198]

Members of the *Haemophilus* genus are gram-negative coccobacillary bacteria that are facultatively anaerobic. They demonstrate optimal growth when they are incubated in a humid atmosphere containing 5 to 10 percent carbon dioxide.[105,115,143] Most species have fastidious nutritional requirements and require special media, supplements, or both for optimal growth.[105,115] X factor (hemin), V factor (nicotinamide adenine dinucleotide), or both are required for in vitro growth.[154] Organisms with the "para-" prefix require V factor only. The specific nutritional requirements of *Haemophilus* isolates are one characteristic used to subclassify these organisms into the different species (Table 134-1).

AGGREGATIBACTER (HAEMOPHILUS) APHROPHILUS

Bacteriology

H. aphrophilus was described first by Khairat[104] in 1940 in association with a fatal case of endocarditis. He suggested the species name *aphrophilus* because the organism required relatively high concentrations of carbon dioxide for isolation on the usual media. In earlier times, a well-known manifestation of carbon dioxide was the formation of bubbles of gas in fermenting wine (i.e., froth, or *aphros*).[24,104] Although the organism originally was classified as a *Haemophilus* spp. because of the growth requirement for X factor, more recent studies suggest that it can grow independent of this factor.[105,149,196] Given this latter characteristic and based on other, more recent analyses,[49] the organism has now been placed in the genus *Aggregatibacter* and is known as *A. aphrophilus*.[147,226]

Epidemiology and Pathogenesis

A. aphrophilus is a component of the normal oral flora. Using a selective medium, Kraut and coworkers[108] isolated the organism from gingival scrapings and interdental material of one third of the healthy adults they examined. With respect to disease pathogenesis, it has been suggested that dental disease or manipulation predisposes to transient bacteremia, which results in seeding of distant tissue sites where localized infection subsequently develops.[24,76,146,149,172] In addition, several case reports of patients with *A. aphrophilus* disease reported an association between human infection and contact with or

TABLE 134-1 **Differential Characteristics of *Haemophilus* Species**

Species	Factor Requirement		Hemolysis of Horse Blood	Fermentation of:				Presence of Catalase	CO_2 Enhancement of Growth
	X*	V		Glucose	Sucrose	Lactose	Mannose		
H. influenzae†	+	+	–	+	–	–	–	+	+
H. haemolyticus	+	+	+	+	–	–	–	+	-
H. ducreyi	+	–	–	–	–	–	–	–	-
H. parainfluenzae	–	+	–	+	+	–	+	D	D
H. parahaemolyticus	–	+	+	+	+	–	–	+	-
A. aphrophilus	–	–	–	+	+	+	+	–	+

*As determined by the porphyrin test.
†Includes biogroup aegyptius.
D, Differences encountered.

bites from dogs and cats.[64,93,146,149,209] However, a causal relationship has been difficult to confirm, and the human mouth and respiratory tract probably are the portals of entry for the great majority of infections.

Clinical Manifestations

A. aphrophilus is a relatively rare cause of infection and disease in pediatric patients, with fewer than 50 cases reported in the literature.* Brain abscesses and endocarditis are the pediatric infections reported most commonly.† Other reported sites of infection in children include the oropharynx,[24] the abdominal cavity,[24,102] and various superficial soft tissue sites.[149,216,217] Infections with *A. aphrophilus* do not appear to be associated with any distinctive clinical features in contrast to infection caused by other organisms at the same sites. However, *A. aphrophilus* infections frequently are associated with underlying conditions predisposing the host to infection, such as congenital heart disease, trauma, and immunosuppression.‡

Treatment

Antimicrobial susceptibility testing has not been well standardized for this organism, and disk-diffusion testing, in particular, has been found to be unreliable.[24,88,149,196] Tube or agar dilution testing generally is considered to be the preferred testing method. Older reports found that *A. aphrophilus* was uniformly susceptible to some antibiotics, including chloramphenicol and the aminoglycosides.[24,149,196] Susceptibility to penicillins has been variable. Even so, penicillin has been used successfully for treatment of susceptible organisms,[24,76,149,209] at times in combination with aminoglycosides and other antimicrobials. Some reports document the usefulness of ampicillin or ceftriaxone for treatment of infection.[20,137,146] Several different antibiotics probably can be used to treat *H. aphrophilus* infections successfully. The choice for treatment should be guided by appropriate in vitro susceptibility testing.

*References 12, 20, 24, 58, 64, 92, 93, 102, 140, 156, 216, 217.
†References 20, 24, 58, 64, 93, 140, 149, 156.
‡Reference 24, 60, 64, 89, 140, 149, 158, 216.

HAEMOPHILUS DUCREYI

Bacteriology

In 1889, Ducrey first identified *H. ducreyi* in purulent material recovered from genital ulcers of patients with soft chancre or chancroid.[51] Although unable to culture the organism in vitro, Ducrey was able to establish the specificity of the infectious agent by serial cutaneous inoculations.[51,141] *H. ducreyi* originally was assigned to the *Haemophilus* genus because of its requirement for hemin (X-factor) and a guanosine plus cytosine content within the expected range for *Haemophilus* spp.[8,204] However, more recent studies, including genetic transformation and DNA hybridization analyses, suggest that *H. ducreyi* is not related to the true haemophili, such as *H. influenzae*, and possibly should be placed in a separate genus.[8,204]

The last two decades have seen significant advances in the understanding of bacterial components that contribute to disease pathogenesis. Two cytotoxins, a hemolysin, a diffusible cytolethal distending toxin, and two classes of serum resistance proteins have been identified, their respective genes have been cloned, and their potential contributions to disease pathogenesis have been investigated.* The lipooligosaccharide of *H. ducreyi* has been characterized in much greater detail, and relevant biosynthetic genes have been cloned and studied.† Genes encoding the major outer membrane proteins of the organism have been cloned and the corresponding proteins characterized,[106] and a novel class of pili expressed by *H. ducreyi* has been identified.[34] *H. ducreyi* requires heme for growth and several of the critical molecules required for heme acquisition and use have been identified over the last several years.[54,55,118,192,199] The knowledge gained from molecular characterization of these important bacterial components should enhance efforts to better understand the pathogenesis of disease.

Epidemiology

Chancroid is a genital ulcerative disease found throughout the world. It is seen frequently in Africa, Asia, and

*References 1, 10, 41, 43, 94, 118, 119, 150, 153, 208.
†References 3, 15, 62, 124, 136, 160, 195, 187, 205, 223.

Latin America, where it may be a more common cause of genital ulcer disease than syphilis.[8,21-23,141,202,204] Although generally considered a relatively uncommon cause of illness in the United States, chancroid continues to be diagnosed, particularly in large urban areas among patients, including adolescents, who have genital ulcer disease.* Furthermore, recent data suggest that chancroid may be more common in the United States than suspected because it can be difficult to diagnose correctly using traditional clinical and laboratory means.[50,142,204] Symptomatic disease among patients in the United States has been reported most commonly among nonwhite heterosexual males.[26,65,81,141] However, symptomatic disease is not restricted to men, and female prostitutes have been implicated as important sources of infection in several of the outbreaks reported in the United States.[26,81,141]

H. ducreyi has been the subject of renewed medical and scientific interest since the early 1980s. This interest followed from epidemiologic studies, primarily from Africa, demonstrating that the presence of genital ulcer disease (much of which was chancroid) was strongly associated with an increased risk for heterosexual transmission of HIV infection.† Mechanisms proposed to explain this enhanced transmission included an increased shedding of HIV through the ulcers,[109,204] an increased number of HIV-susceptible cells (e.g., CD4 T lymphocytes) in genital ulcers of a person being exposed to HIV,[188,204] or an increased viral load in the blood and semen of individuals coinfected with HIV and *H. ducreyi*.[53]

Pathogenesis

As noted previously, significant progress has been made over the past 20 years in better understanding the pathogenesis of *H. ducreyi* infection.[4,95,184,204] Several useful in vivo models of *H. ducreyi* infection have been developed. These include a temperature-dependent rabbit model of dermal infection,[161] a primate model of genital chancroid infection in adult pigtailed macaques,[203] a swine model of dermal infection,[90] and an experimental model of human infection.‡ Putative virulence factors have been identified, and their contributions to the pathogenesis of disease are being investigated using several in vitro and in vivo model systems. Some of the molecules and characteristics studied include bacterial lipooligosaccharide,[16,36,124,188,225] pili,§ the cytolethal distending toxin,¶ a hemolysin,‖ serum resistance proteins,[1,41,116,117] lectin binding proteins,[1,97,208,211] and the ability to adhere specifically to epithelial cells of genital origin.[9,114] The contribution of these proposed virulence factors to the pathogenesis of natural infection in humans is still being defined.[95] However, it is known that strains deficient in expression of several of these factors are attenuated in their ability to cause disease in the experimental model of human infection.** Ongoing investigations of *H. ducreyi* undoubtedly will define the virulence mechanisms of the organism more clearly and should enhance efforts to develop protective vaccines.*

Clinical Manifestations

The incubation period of chancroid usually is between 4 and 7 days. It rarely is less than 3 days or more than 10 days.[11,141,170,171] Typically, the first lesion noted is a small inflammatory papule surrounded by a zone of erythema. Within 2 or 3 days, a pustule forms that soon ruptures, leaving a sharply circumscribed ulcer with ragged undermined edges *without* induration.[121,141,170] The base of the ulcer usually has a granular appearance and always is painful. In males, the most common sites of appearance of the ulcers are on the distal prepuce, on the mucosal surface of the prepuce on the frenulum, and in the coronal sulcus. In females, most lesions are at the entrance to the vagina.[121,141,170] Painful, tender inguinal adenopathy is present in up to 50 percent of patients and usually is unilateral. The involved lymph nodes rapidly may become fluctuant and rupture, with the formation of inguinal ulcers.[141,170]

The combination of a painful ulcer with tender inguinal adenopathy is suggestive of chancroid and, when accompanied by suppurative inguinal adenopathy, is almost pathognomonic.[220] However, a significant percentage of patients with *H. ducreyi* infection may have ulcers that can be confused with other genital ulcer diseases, such as herpes or syphilis.[72,121,141,170] Furthermore, as many as 10 percent of patients with chancroid may be coinfected with *Treponema pallidum* or herpes simplex virus.[220] Thus, it becomes mandatory to establish a definitive diagnosis by laboratory means if the diagnosis is to be made with confidence. Recently, chancroid has been described as a cause for chronic lower extremity skin ulcers in children in the South Pacific area,[207] but how common such a presentation may be is unclear.

Diagnosis

As noted earlier, diagnosing chancroid on clinical grounds alone is difficult because the clinical presentation often is not classic and many clinicians do not have a great deal of experience with the disease.[204,220] Definitive diagnosis of chancroid requires isolation of the organism from a genital ulcer or from involved lymph nodes. However, the organism is fastidious and is difficult to isolate, even under the best of circumstances.[141] To obtain specimens for culture, a swab should be used to obtain material from the purulent base of an ulcer or a fluctuant inguinal lymph node should be aspirated directly. Gram stain of purulent material may reveal gram-negative rods in the characteristic "school-of-fish" pattern, but this appearance probably is more characteristic of in vitro propagated organisms.[141] However, even with use of the selective media now recommended for isolation of *H. ducreyi*, it has been estimated that the sensitivity of culture is no higher than 80 percent.[121,220]

Given the low sensitivity of culture, alternative non–culture-based diagnostic tests have been evaluated. Early

*References 26, 81, 87, 138, 139, 179.
†References 23, 98, 109, 110, 142, 159, 212.
‡References 6, 7, 17, 18, 97, 152, 184, 189.
§References 3, 4, 19, 62, 63, 75, 177, 185, 194, 205.
¶References 44, 45, 69, 73, 77, 113, 122, 126, 162, 163, 191, 222.
‖References 10, 52, 151, 153, 201, 219, 224.
**References 5, 30, 67, 70, 95-97, 186.

*References 2, 6, 42, 47, 71, 83, 119.

studies examined the utility of diagnosing chancroid serologically with an enzyme-linked immunosorbent assay (ELISA) using either an outer-membrane protein preparation or a lipooligosaccharide preparation of *H. ducreyi*.[11] More recent studies have employed slightly altered antigen preparations and serum preparation techniques meant to improve the sensitivity and specificity of the assay.[38] Although these modifications did lead to improvement in the performance characteristics of the assay, the ability of the modified assay to aid in the diagnosis of acute infection remains limited because many patients do not develop a serum antibody response until several weeks after onset of infection.[38,202] Recent work has focused on the use of recombinant proteins as test antigens for a serologic test.[56] This latter assay does appear to show promise for seroprevalence studies, but its utility in the diagnosis of acute infection has yet to be demonstrated. Another recently described diagnostic test relies on detection of *H. ducreyi* with monoclonal antibodies directed against the hemoglobin receptor[155] but it has yet to undergo extensive field testing.

Nucleotide-based diagnostic methods also have been described in recent years.[99,173,204] Perhaps most promising are the polymerase chain reaction (PCR)-based techniques. These assays demonstrate high sensitivity and appear to identify patients with chancroid from whom bacterial cultures for *H. ducreyi* are negative.[202,204] Multiplex PCR assays that can amplify and subsequently detect DNA from *H. ducreyi*, *T. pallidum*, and herpes simplex virus from genital ulcer specimens simultaneously are undergoing field trials and have shown early promise.[129,148,204]

Even if chancroid is diagnosed definitively, it is recommended that patients also be tested for human immunodeficiency virus (HIV) at the time of diagnosis. In addition, it must be remembered that up to 10 percent of patients with chancroid may be coinfected with *T. pallidum* or herpes simplex virus.[220] Appropriate testing for these other pathogens should be strongly considered when a patient presents with any form of genital ulcer disease.

Treatment and Prevention

Successful antimicrobial treatment of genital ulcers caused by *H. ducreyi* cures infection, resolves clinical symptoms, and prevents transmission to others. However, in cases of extensive ulcerative disease, scarring may result, despite successful antimicrobial therapy.[220] The Centers for Disease Control and Prevention (CDC) currently recommends one of four antibiotic regimens for treatment of chancroid in adolescents and adults.[121,178,220] These regimens are (1) azithromycin 1 g orally in a single dose, (2) ceftriaxone 250 mg intramuscularly in a single dose, (3) ciprofloxacin 500 mg orally twice per day for 3 days, or (4) erythromycin base 500 mg orally three times per day for 7 days.[178,220] All four regimens are generally effective for treatment of chancroid among patients without HIV infection.[178] A successful response to therapy usually is apparent within 48 to 72 hours, as shown by decreased ulcer tenderness and pain.[170,178,220] Complete healing of ulcers may take up to 28 days but often is achieved in 7 to 14 days.[170] Healing of fluctuant adenopathy is slower than that of the ulcers and may require needle aspiration through adjacent intact skin or incision and drainage to achieve a successful response to therapy.[59,178]

Patients with HIV infection must be monitored closely because they may require longer courses of antimicrobial agents than the standard regimens just outlined.[178,220] Treatment failures have been noted with several of these regimens,[29,178,206] and some suggestion has been made that persons who are most immunosuppressed are at the greatest risk for failure of standard regimens.[206] Some experts recommend using the erythromycin 7-day regimen for treating all HIV-infected patients because of good experience with this regimen in the HIV-infected population and limited successful experience with the alternative regimens.[178]

It is critical to identify all sexual contacts of infected persons to prevent further spread of *H. ducreyi* disease. The CDC recommends that all persons who have had sexual contact with a patient with proved *H. ducreyi* infection within the 10 days before onset of the patient's symptoms be examined and treated.[220] Contacts should be examined and treated even in the absence of symptoms.

In the longer term, alternative strategies for control of chancroid should be examined. If feasible, vaccination for prevention of *H. ducreyi* infection would be a worthy goal. Data generated in animal models of infection are somewhat encouraging.[2,47,83] Protective immunity to both homologous and heterologous challenge has been reported after immunization of rabbits with cell-surface extracts of *H. ducreyi*.[83] In other work, a purified pilus preparation also was reported to induce immunity in that same model.[47] More recently, immunization with the hemoglobin receptor protein was reported to protect against infection in the swine model of chancroid.[2,71,119] These data suggest that prevention of chancroid by vaccination may be an achievable goal. However, data from the experimental human model of infection suggest that development of a protective vaccine for human use may not be a straightforward process.[6,96]

HAEMOPHILUS INFLUENZAE BIOGROUP AEGYPTIUS *(HAEMOPHILUS AEGYPTIUS)*

Bacteriology

H. influenzae biogroup aegyptius *(H. aegyptius)* was described originally by Koch[107] in 1883 in Egyptian patients with conjunctivitis. A more detailed description of the organism and the clinical characteristics of disease followed 3 years later in the work of Weeks.[214] The Koch-Weeks bacillus has continued to be an important cause of conjunctivitis since these initial reports. Because of several reportedly unique characteristics,[132] the Koch-Weeks bacillus originally was designated as a unique species of the *Haemophilus* genus *(H. aegyptius)* distinct from *H. influenzae*. However, more recent phenotypic and phylogenetic studies, including DNA relatedness

analyses, have raised questions about the validity of this separation.[33,197] The organism is known currently as *H. influenzae* biogroup aegyptius, although debate continues in the literature as to the appropriateness of this designation.[125]

This organism became the subject of intense scientific study in the 1980s as a result of its association with a newly described fulminant and often fatal disease called Brazilian purpuric fever.[31,32] *H. influenzae* biogroup aegyptius was isolated from nine blood cultures and one hemorrhagic cerebrospinal fluid culture collected from 10 clinically ill children in Serrana, São Paulo State, Brazil.[32] The *H. influenzae* biogroup aegyptius strains causing Brazilian purpuric fever were felt initially to be members of a single virulent clone.[33,197] This clone was characterized by the presence of several unique features, including a 24-MDa plasmid with a characteristic restriction endonuclease pattern,[33] a unique multilocus electrophoretic enzyme typing pattern,[145] one of two rRNA gene restriction fragment length polymorphisms,[33,48] a single sodium dodecyl sulfate–polyacrylamide gel electrophoresis profile for whole-cell lysates,[33] specific reactivity with monoclonal antibodies recognizing epitopes unique to Brazilian purpuric fever strains,[120,197] agglutination with antisera specific for the Brazilian purpuric fever clone,[33] and conservation of certain major outer membrane proteins.[167] Although most Brazilian purpuric fever–associated strains of *H. influenzae* biogroup aegyptius appeared to be members of a unique clone, a few Brazilian purpuric fever–associated strains were identified that lacked some of the defining characteristics.[197,200] Furthermore, two cases of Brazilian purpuric fever reported from Australia were associated with strains clearly distinct from the Brazilian purpuric fever clone.[125,197] An excellent review of the epidemiologic, clinical, and laboratory aspects of the disease and its investigation was recently published, and the reader is referred to that work for additional information.[85]

Epidemiology

In the United States, *H. influenzae* biogroup aegyptius has remained an important cause of conjunctivitis, with disease most commonly reported from the southern states.[86] The United States experienced only one reported case of possible Brazilian purpuric fever.[210] In Brazil, the epidemiology of Brazilian purpuric fever became more clearly defined with time.[85,86] The median age of patients with Brazilian purpuric fever was 2 to 3 years, with an overall range of ages of 3 months to 10 years.[31,32] Brazilian purpuric fever appeared to occur with the onset of warmer temperatures and was less likely during the Brazilian winter.[85] Furthermore, it appeared to be more common in small agricultural towns than in larger cities.[31,32,86] Case-control studies attempting to identify risk factors for development of disease identified a history of preceding conjunctivitis as being associated strongly with the development of Brazilian purpuric fever[31,32,86] (although many of the controls also gave a history of conjunctivitis) and suggested that daycare center attendance was an additional risk factor.[32] No additional cases of Brazilian purpuric fever have been reported since the early 1990s. The reasons for the apparent disappearance of the disease remain unclear.[85]

Pathogenesis

Efforts to identify unique virulence factors of *H. influenzae* biogroup aegyptius responsible for the fulminant nature of Brazilian purpuric fever have been ongoing since the initial descriptions of the illness. Progress to date has been limited,[37,85] but recent genome-based studies have demonstrated clear differences between *H. influenzae* biogroup aegyptius prototype strains and other *H. influenza* strains.[123,135,183] Brazilian purpuric fever clone strains express novel or unique surface molecules or secreted proteins that theoretically could result in enhanced virulence.* Distinctive lipooligosaccharide phenotypes,[37,175] IgA proteases,[37,125,134] pili,[15,165,166,218] secreted proteins,[218] and adhesins[180,193] have been reported for Brazilian purpuric fever strains. However, none has been shown to have a specific dominant role in bacterial virulence. One study suggested that the risk for developing Brazilian purpuric fever correlated with the lack of serum bactericidal activity against the Brazilian purpuric fever clone, but this observation needs further confirmation.[174]

Both in vitro and in vivo models were developed for further investigating the pathogenesis of Brazilian purpuric fever.[164,175,215] In these model systems, Brazilian purpuric fever–associated strains demonstrated increased virulence in contrast to control strains not associated with Brazilian purpuric fever, but, again, the specific molecular correlates of this increased pathogenicity have yet to be identified clearly.[164,175]

Clinical Manifestations

The clinical presentation of Brazilian purpuric fever is distinctive and dramatic.[31,32,85,86] The syndrome initially manifests as a purulent conjunctivitis without distinguishing characteristics. Symptoms of Brazilian purpuric fever typically appear 3 to 15 days later, after the conjunctivitis has resolved. The affected children experience the acute onset of fever, which may be associated with vomiting and abdominal pain. Death frequently ensues within 48 hours after the development of disseminated purpura, vascular collapse, and hypotensive shock. The precise pathophysiologic mechanisms responsible for progression from conjunctivitis because of the Brazilian purpuric fever clone to full-blown Brazilian purpuric fever are unknown. The overall case-fatality rate since Brazilian purpuric fever first was recognized is estimated to be 70 percent.[86] Children may develop conjunctivitis with the Brazilian purpuric fever clone and, after recovery from the conjunctivitis, have no further problems.[86] The risk factors that predispose only some children to develop Brazilian purpuric fever are not well understood.

Treatment

Data from the limited number of Brazilian purpuric fever cases studied suggest that early antimicrobial therapy

*References 33, 37, 120, 125, 175, 218.

may improve survival.[32] One suggested regimen is high-dose ampicillin and chloramphenicol. The small number of patients treated to date does not permit a comparison of the efficacy of different antibiotic regimens.[32] Most of the patients who developed Brazilian purpuric fever in the Brazilian studies were treated with topical antimicrobials for conjunctivitis, yet still developed systemic disease.[32] This suggests that local topical therapy is ineffective in eradicating the organism from the host. One study examined the relative efficacy of oral rifampin and topical chloramphenicol in eradicating conjunctival carriage of the Brazilian purpuric fever clone.[157] Although the number of patients who actually carried the Brazilian purpuric fever clone was small, rifampin was shown to be significantly better than topical chloramphenicol in eradicating carriage of the Brazilian purpuric fever clone.

HAEMOPHILUS PARAINFLUENZAE

Bacteriology

H. parainfluenzae was identified first as a species distinct from *H. influenzae* by Rivers[169] in 1922. Both organisms are fastidious, gram-negative coccobacilli, but with in vitro culture, *H. parainfluenzae* can be propagated on nutrient agar plates with supplemental factor V alone (thus the "para-" designation), rather than with both factor X and factor V, which are required by *H. influenzae* isolates (see Table 134-1). Testing for hemolysis on blood-containing media differentiates *H. parainfluenzae* from hemolysis-producing species, such as *H. haemolyticus* and *H. parahaemolyticus*.[105,115] Recovery of *H. parainfluenzae* organisms from blood cultures is enhanced by routine subculturing of all specimens. The organisms tend to grow as small colonies along the side walls of the blood bottles or in the red cell mass, leaving the broth clear. Routine subculturing to supplemented chocolate agar and incubation with supplemental carbon dioxide should allow recovery of any *H. parainfluenzae* organisms that are present.[40,80]

Epidemiology and Pathogenesis

H. parainfluenzae is found commonly in the oropharyngeal flora of normal children.[79,112,128] The organism can be recovered from oropharyngeal cultures of one quarter or more of healthy children. Of children who develop serious invasive disease caused by *H. parainfluenzae*, more than half give histories of identifiable preceding illnesses, such as upper respiratory tract infection, otitis media, or dental infection,[13,25,79,91] suggesting that local inflammation in the upper respiratory tract may predispose to transient bacteremias with this organism, which allows for seeding of other sites, such as the meninges and the heart valves. No specific virulence factors of the organism have been identified to date.

Clinical Manifestations

H. parainfluenzae remains an uncommon cause of infection in pediatric patients, but an increasing number of cases have been reported since the 1970s.[25] The most commonly reported infection is meningitis.* The clinical courses of the patients described with *H. parainfluenzae* meningitis are not remarkably different from those typical of acute bacterial meningitis caused by other organisms. However, the average age of affected children is 2.2 years,[25] an age significantly greater than that of the typical pediatric patient with bacterial meningitis.

The next most commonly reported infection is endocarditis.† *H. parainfluenzae* endocarditis has several unique features. The reported cases of pediatric endocarditis usually occur in adolescents and involve females more commonly than males.[25] The clinical presentation often is subacute and frequently is not associated with localizing signs on physical examination (i.e., pathologic murmurs), at least initially.[40,127,128] Another unique feature of *H. parainfluenzae* endocarditis is the high incidence of major arterial occlusion secondary to release of large emboli from the heart.[25,40,74,127,190] This high incidence of embolization is thought to be due to the particularly friable nature of the vegetations.[25,74] Another characteristic feature noted by several authors is the relatively slow and variable response to antimicrobial therapy of endocarditis caused by this organism.[25,40,127,128]

Other *H. parainfluenzae* infections reported in pediatric patients include brain abscesses,[79,130] septic arthritis,[25] urinary tract infection,[25,84] peritonitis,[131] and sepsis in neonates.[39,168]

TREATMENT

H. parainfluenzae usually is susceptible in vitro to multiple antibiotics, including chloramphenicol, aminoglycosides, trimethoprim-sulfamethoxazole, and third-generation cephalosporins.[25,40,100,128] Although the majority of isolates in the past were susceptible to penicillins, more recent studies have documented an increasing incidence of β-lactamase–producing strains resistant to penicillin and ampicillin.[25,176] For β-lactamase–negative, penicillin-susceptible organisms, administration of ampicillin with an aminoglycoside has been recommended for serious *H. parainfluenzae* infections.[40,82] Individual case reports document successful treatment with a variety of other antimicrobials, including ampicillin alone, cephalosporins, chloramphenicol, and trimethoprim-sulfamethoxazole.[35,40,101,128] At present, pending results of susceptibility testing, it would be reasonable to initiate therapy for serious *H. parainfluenzae* infections with a third-generation cephalosporin, perhaps in combination with an aminoglycoside.

NEW REFERENCES SINCE THE SIXTH EDITION

49. Di Bonaventura MP, DeSalle R, Pop M, et al. Complete genome sequence of *Aggregatibacter* (*Haemophilus*) *aphrophilus* NJ8700. *J Bacteriol* 2009;**191**:4693–4.
71. Fusco WG, Afonina G, Nepluev I, et al. Immunization with the *Haemophilus ducreyi* hemoglobin receptor HgbA with adjuvant monophosphoryl lipid A protects swine from a homologous but not a heterologous challenge. *Infect Immun* 2010;**78**:3763–72.

*References 13, 14, 46, 66, 68, 78, 79, 91, 103, 111, 130, 182, 213, 221.
†References 25, 27, 28, 35, 57, 61, 74, 127, 128, 181.

84. Hansson S, Svedhem A, Wennerstrom M, Jodal U. Urinary tract infection caused by *Haemophilus influenzae* and *Haemophilus parainfluenzae* in children. *Pediatr Nephrol* 2007;**22**:1321–5.
86. Harrison LH, Simonsen V, Waldman EA. Emergence and disappearance of a virulent clone of *Haemophilus influenzae* biogroup aegyptius, cause of Brazilian purpuric fever. *Clin Microbiol Rev* 2008;**21**:594–605.
88. Hindler JA, Jorgensen JH. Susceptibility test methods: Fastidious bacteria. In: Versalovic R, Carroll KC, Funke G, et al, editors. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2012. p. 1180–203.
95. Janowicz DM, Li W, Bauer ME. Host-pathogen interplay of *Haemophilus ducreyi*. *Curr Opin Infect Dis* 2010;**23**:64–9.
96. Janowicz DM, Ofner S, Katz BP, Spinola SM. Experimental infection of human volunteers with *Haemophilus ducreyi*: ffteen years of clinical data and experience. *J Infect Dis* 2009;**199**:1671–9.
115. Ledeboer NA, Doern GV. Haemophilus. In: Versalovic R, Carroll KC, Funke G, et al, editors. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2012. p. 588–602.
116. Leduc I, Olsen B, Elkins C. Localization of the domains of the *Haemophilus ducreyi* trimeric autotransporter DsrA involved in serum resistance and binding to the extracellular matrix proteins fibronectin and vitronectin. *Infect Immun* 2009;**77**:657–66.
117. Leduc I, White CD, Nepluev I, et al. Outer membrane protein DsrA is the major fibronectin-binding determinant of *Haemophilus ducreyi*. *Infect Immun* 2008;**76**:1608–16.
118. Leduc I, Banks KE, Fortney KR, et al. Evaluation of the repertoire of the TonB-dependent receptors of *Haemophilus ducreyi* for their role in virulence in humans. *J Infect Dis* 2008;**197**:1103–9.
119. Leduc I, Fusco WG, Choudhary N, et al. Passive immunization with a polyclonal antiserum to the hemoglobin receptor of *Haemophilus ducreyi* confers protection against a homologous challenge in the experimental swine model of chancroid. *Infect Immun* 2011;**79**:3168–77.
124. Li W, Katz BP, Spinola SM. *Haemophilus ducreyi* lipooligosaccharides induce expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase via type I interferons and tumor necrosis factor alpha in human dendritic cells. *Infect Immun* 2011;**79**:3338–47.
126. Lundqvist A, Fernandez-Rodrigues J, Ahlman K, Lagergard T. Detoxified *Haemophilus ducreyi* cytolethal distending toxin and induction of toxin specific antibodies in the genital tract. *Vaccine* 2010;**28**:5768–73.
133. McCrea KW, Xie J, LaCross N, et al. Relationships of nontypeable *Haemophilus influenzae* strains to hemolytic and nonhemolytic *Haemophilus haemolyticus* strains. *J Clin Microbiol* 2008;**46**:406–16.
143. Murphy TF. *Haemophilus* species (including *H. influenzae* and chancroid). In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 2911–19.
144. Murphy TF, Brauer AL, Sethi S, et al. *Haemophilus haemolyticus*: a human respiratory tract commensal to be distinguished from *Haemophilus influenzae*. *J Infect Dis* 2007;**195**:81–9.
156. Pereira RM, Bucaretchi F, Tresoldi AT. Infective endocarditis due to *Haemophilus aphrophilus*: a case report. *J Pediatr (Rio J)* 2008;**84**:178–80.
180. Serruto D, Spadafina T, Scarselli M, et al. HadA is an atypical new multifunctional trimeric coiled-coil adhesin of *Haemophilus influenzae* biogroup aegyptius, which promotes entry into host cells. *Cell Microbiol* 2009;**11**:1044–63.
188. Spinola SM, Li W, Fortney KR, et al. Sialylation of lipooligosaccharides is dispensable for the virulence of *Haemophilus ducreyi* in humans. *Infect Immun* 2012;**80**:679–87.
193. Strouts FR, Power P, Croucher NJ, et al. Lineage-specific virulence determinants of *Haemophilus influenzae* biogroup aegyptius. *Emerg Infect Dis* 2012;**18**:449–57.
198. Theodore MJ, Anderson RD, Wang X, et al. Evaluation of new biomarker genes for differentiating *Haemophilus influenzae* from *Haemophilus haemolyticus*. *J Clin Microbiol* 2012;**50**:1422–4.
207. Ussher JE, Wilson E, Campanella S, Taylor SL, Roberts SA. *Haemophilus ducreyi* causing chronic skin ulceration in children visiting Samoa. *Clin Infect Dis* 2007;**44**:e85–87.
220. Workowski KA, Berman S; Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1–110.
226. Zbinden R, von Graevenitz A. *Actinobacillus*, *Capnocytophaga*, *Eikenella*, *Kingella*, *Pasteurella*, and other fastidious or rarely encountered Gram-negative rods. In: Versalovic R, Carroll KC, Funke G, et al, editors. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2012. p. 574–87.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 135

Helicobacter pylori

Aldo Maspons • Mark A. Gilger

The discovery of *Helicobacter pylori* in 1983 as the bacterial cause of peptic ulcer disease[163] revolutionized the understanding, diagnosis, and treatment of acid peptic disease. *H. pylori* appears to infect approximately 50 percent of the world's population, thus rendering it one of the most common bacterial infections in humans.[101,116] An understanding of *H. pylori* is important to pediatricians because this infection usually is acquired in childhood.[88] This chapter details current consensus and understanding of this important pathogen.[46,74,101]

BACKGROUND

The presence of bacteria in the gastric mucosa has been known for more than 100 years.[170] In 1874, Böttcher[18]

REFERENCES

1. Abdullah M, Nepluev I, Afonina G, et al. Killing of dsrA mutants of *Haemophilus ducreyi* by normal human serum occurs via the classical complement pathway and is initiated by immunoglobulin M binding. *Infect Immun* 2005;**73**:3431–9.
2. Afonina G, Leduc I, Nepluev I, et al. Immunization with the *Haemophilus ducreyi* hemoglobin receptor HgbA protects against infection in the swine model of chancroid. *Infect Immun* 2006;**74**: 2224–32.
3. Ahmed HJ, Frisk A, Mansson JE, Schweda EK, Lagergard T. Structurally defined epitopes of *Haemophilus ducreyi* lipooligosaccharides recognized by monoclonal antibodies. *Infect Immun* 1997; **65**:3151–8.
4. Al-Tawfiq JA, Bauer ME, Fortney KR, et al. A pilus-deficient mutant of *Haemophilus ducreyi* is virulent in the human model of experimental infection. *J Infect Dis* 2000;**181**:1176–9.
5. Al-Tawfiq JA, Fortney KR, Katz BP, et al. An isogenic hemoglobin receptor-deficient mutant of *Haemophilus ducreyi* is attenuated in the human model of experimental infection. *J Infect Dis* 2000;**181**: 1049–54.
6. Al-Tawfiq JA, Palmer KL, Chen CY, et al. Experimental infection of human volunteers with *Haemophilus ducreyi* does not confer protection against subsequent challenge. *J Infect Dis* 1999;**179**: 1283–7.
7. Al-Tawfiq JA, Thornton AC, Katz BP, et al. Standardization of the experimental model of *Haemophilus ducreyi* infection in human subjects. *J Infect Dis* 1998;**178**:1684–7.
8. Albritton WL. Biology of *Haemophilus ducreyi*. *Microbiol Rev* 1989; **53**:377–89.
9. Alfa MJ, DeGagne P, Hollyer T. *Haemophilus ducreyi* adheres to but does not invade cultured human foreskin cells. *Infect Immun* 1993;**61**:1735–42.
10. Alfa MJ, DeGagne P, Totten PA. *Haemophilus ducreyi* hemolysin acts as a contact cytotoxin and damages human foreskin fibroblasts in cell culture. *Infect Immun* 1996;**64**:2349–52.
11. Alfa MJ, Olson N, Degagne P, et al. Humoral immune response of humans to lipooligosaccharide and outer membrane proteins of *Haemophilus ducreyi*. *J Infect Dis* 1993;**167**:1206–10.
12. Arneborn P, Lindquist BL, Sjöberg L. Severe pulmonary infection by *Haemophilus aphrophilus* in a non-compromised child. *Scand J Infect Dis* 1985;**17**:327–9.
13. Bachman DS. *Hemophilus* meningitis: comparison of *H. influenzae* and *H. parainfluenzae*. *Pediatrics* 1975;**55**:526–30.
14. Barnshaw JA, Phillips CF. *Haemophilus parainfluenzae* meningitis in a 4-year-old boy. *Pediatrics* 1970;**45**:856–7.
15. Bauer BA, Lumbley SR, Hansen EJ. Characterization of a WaaF (RfaF) homolog expressed by *Haemophilus ducreyi*. *Infect Immun* 1999;**67**:899–907.
16. Bauer BA, Stevens MK, Hansen EJ. Involvement of the *Haemophilus ducreyi gmhA* gene product in lipooligosaccharide expression and virulence. *Infect Immun* 1998;**66**:4290–8.
17. Bauer ME, Goheen MP, Townsend CA, Spinola SM. *Haemophilus ducreyi* associates with phagocytes, collagen, and fibrin and remains extracellular throughout infection of human volunteers. *Infect Immun* 2001;**69**:2549–57.
18. Bauer ME, Spinola SM. Localization of *Haemophilus ducreyi* at the pustular stage of disease in the human model of infection. *Infect Immun* 2000;**68**:2309–14.
19. Bauer ME, Townsend CA, Ronald AR, Spinola SM. Localization of *Haemophilus ducreyi* in naturally acquired chancroidal ulcers. *Microbes Infect* 2006;**8**:2465–8.
20. Bayraktar M, Onal C, Durmaz B, Yakinci C, Sonmezgoz E. *Haemophilus aphrophilus* brain abscess in the first decade. *Indian J Med Microbiol* 2005;**23**:259–61.
21. Behets FM, Andriamiadana J, Randrianasolo D, et al. Chancroid, primary syphilis, genital herpes, and lymphogranuloma venereum in Antananarivo, Madagascar. *J Infect Dis* 1999;**180**:1382–5.
22. Behets FM, Brathwaite AR, Hylton-Kong T, et al. Genital ulcers: etiology, clinical diagnosis, and associated human immunodeficiency virus infection in Kingston, Jamaica. *Clin Infect Dis* 1999;**28**: 1086–90.
23. Behets FM, Liomba G, Lule G, et al. Sexually transmitted diseases and human immunodeficiency virus control in Malawi: a field study of genital ulcer disease. *J Infect Dis* 1995;**171**: 451–5.
24. Bieger RC, Brewer NS, Washington JA 2nd. *Haemophilus aphrophilus*: a microbiologic and clinical review and report of 42 cases. *Medicine (Baltimore)* 1978;**57**:345–55.
25. Black CT, Kupferschmid JP, West KW, Grosfeld JL. *Haemophilus parainfluenzae* infections in children, with the report of a unique case. *Rev Infect Dis* 1988;**10**:342–6.
26. Blackmore CA, Limpakarnjanarat K, Rigau-Perez JG, Albritton WL, Greenwood JR. An outbreak of chancroid in Orange County, California: descriptive epidemiology and disease-control measures. *J Infect Dis* 1985;**151**:840–4.
27. Blair DC, Weiner LB. Prosthetic valve endocarditis due to *Haemophilus parainfluenzae* biotype II. *Am J Dis Child* 1979;**133**: 617–18.
28. Blair DC, Walker W, Sodeman T, Pagano T. Bacterial endocarditis due to *Haemophilus parainfluenzae*. *Chest* 1977;**71**:146–9.
29. Bogaerts J, Kestens L, Martinez Tello W, et al. Failure of treatment for chancroid in Rwanda is not related to human immunodeficiency virus infection: in vitro resistance of *Haemophilus ducreyi* to trimethoprim-sulfamethoxazole. *Clin Infect Dis* 1995;**20**: 924–30.
30. Bong CT, Throm RE, Fortney KR, et al. DsrA-deficient mutant of *Haemophilus ducreyi* is impaired in its ability to infect human volunteers. *Infect Immun* 2001;**69**:1488–91.
31. Brazilian Purpuric Fever Study Group. Brazilian purpuric fever: epidemic purpura fulminans associated with antecedent purulent conjunctivitis. *Lancet* 1987;**2**:757–61.
32. Brazilian Purpuric Fever Study Group. *Haemophilus aegyptius* bacteraemia in Brazilian purpuric fever. *Lancet* 1987;**2**:761–3.
33. Brenner DJ, Mayer LW, Carlone GM, et al. Biochemical, genetic, and epidemiologic characterization of *Haemophilus influenzae* biogroup aegyptius (*Haemophilus aegyptius*) strains associated with Brazilian purpuric fever. *J Clin Microbiol* 1988;**26**:1524–34.
34. Brentjens RJ, Ketterer M, Apicella MA, Spinola SM. Fine tangled pili expressed by *Haemophilus ducreyi* are a novel class of pili. *J Bacteriol* 1996;**178**:808–16.
35. Calio AJ, Cusumano S, Ullman RF, Tjioe DY, Cunha BA. *Haemophilus parainfluenzae* endocarditis. *Heart Lung* 1987;**16**:222–3.
36. Campagnari AA, Wild LM, Griffiths GE, et al. Role of lipooligosaccharides in experimental dermal lesions caused by *Haemophilus ducreyi*. *Infect Immun* 1991;**59**:2601–8.
37. Carlone GM, Gorelkin L, Gheesling LL, et al. Potential virulence-associated factors in Brazilian purpuric fever. Brazilian Purpuric Fever Study Group. *J Clin Microbiol* 1989;**27**:609–14.
38. Chen CY, Mertz KJ, Spinola SM, Morse SA. Comparison of enzyme immunoassays for antibodies to *Haemophilus ducreyi* in a community outbreak of chancroid in the United States. *J Infect Dis* 1997;**175**:1390–5.
39. Chen RV, Bradley JS. *Haemophilus parainfluenzae* sepsis in a very low birth weight premature infant: a case report and review of the literature. *J Perinatol* 1999;**19**:315–17.
40. Chunn CJ, Jones SR, McCutchan JA, Young EJ, Gilbert DN. *Haemophilus parainfluenzae* infective endocarditis. *Medicine (Baltimore)* 1977;**56**:99–113.
41. Cole LE, Kawula TH, Toffer KL, Elkins C. The *Haemophilus ducreyi* serum resistance antigen DsrA confers attachment to human keratinocytes. *Infect Immun* 2002;**70**:6158–65.
42. Cole LE, Toffer KL, Fulcher RA, et al. A humoral immune response confers protection against *Haemophilus ducreyi* infection. *Infect Immun* 2003;**71**:6971–7.
43. Cope LD, Lumbley S, Latimer JL, et al. A diffusible cytotoxin of *Haemophilus ducreyi*. *Proc Natl Acad Sci U S A* 1997;**94**:4056–61.
44. Cortes-Bratti X, Chaves-Olarte E, Lagergard T, Thelestam M. The cytolethal distending toxin from the chancroid bacterium *Haemophilus ducreyi* induces cell-cycle arrest in the G2 phase. *J Clin Invest* 1999;**103**:107–15.
45. Cortes-Bratti X, Chaves-Olarte E, Lagergard T, Thelestam M. Cellular internalization of cytolethal distending toxin from *Haemophilus ducreyi*. *Infect Immun* 2000;**68**:6903–11.
46. Davis DJ. Haemophilus parainfluenzae. *Pediatr Infect Dis* 1982;**1**: 448–9.
47. Desjardins M, Filion LG, Robertson S, Cameron DW. Inducible immunity with a pilus preparation booster vaccination in an animal model of *Haemophilus ducreyi* infection and disease. *Infect Immun* 1995;**63**:2012–20.
48. Dewhirst FE, Paster BJ, Olsen I, Fraser GJ. Phylogeny of 54 representative strains of species in the family *Pasteurellaceae* as

determined by comparison of 16S rRNA sequences. *J Bacteriol* 1992;**174**:2002–13.
49. Di Bonaventura MP, DeSalle R, Pop M, et al. Complete genome sequence of *Aggregatibacter* (*Haemophilus*) *aphrophilus* NJ8700. *J Bacteriol* 2009;**191**:4693–4.
50. DiCarlo RP, Martin DH. The clinical diagnosis of genital ulcer disease in men. *Clin Infect Dis* 1997;**25**:292–8.
51. Ducrey A. Experimentelle untersuchungen uber den ansteckungsstoff des weichen schankers und uber die bobunen. *Monatschr Prakt Dermatol* 1889;**9**:387.
52. Dutro SM, Wood GE, Totten PA. Prevalence of, antibody response to, and immunity induced by *Haemophilus ducreyi* hemolysin. *Infect Immun* 1999;**67**:3317–28.
53. Dyer JR, Eron JJ, Hoffman IF, et al. Association of CD4 cell depletion and elevated blood and seminal plasma human immunodeficiency virus type 1 (HIV-1) RNA concentrations with genital ulcer disease in HIV-1-infected men in Malawi. *J Infect Dis* 1998;**177**:224–7.
54. Elkins C, Chen CJ, Thomas CE. Characterization of the *hgbA* locus encoding a hemoglobin receptor from *Haemophilus ducreyi*. *Infect Immun* 1995;**63**:2194–200.
55. Elkins C, Totten PA, Olsen B, Thomas CE. Role of the *Haemophilus ducreyi* Ton system in internalization of heme from hemoglobin. *Infect Immun* 1998;**66**:151–60.
56. Elkins C, Yi K, Olsen B, Thomas C, Thomas K, Morse S. Development of a serological test for *Haemophilus ducreyi* for seroprevalence studies. *J Clin Microbiol* 2000;**38**:1520–6.
57. Ellner JJ, Rosenthal MS, Lerner PI, McHenry MC. Infective endocarditis caused by slow-growing, fastidious, Gram-negative bacteria. *Medicine (Baltimore)* 1979;**58**:145–58.
58. Elster SK, Mattes LM, Meyers BR, Jurado RA. *Hemophilus aphrophilus* endocarditis: review of 23 cases. *Am J Cardiol* 1975;**35**: 72–9.
59. Ernst AA, Marvez-Valls E, Martin DH. Incision and drainage versus aspiration of fluctuant buboes in the emergency department during an epidemic of chancroid. *Sex Transm Dis* 1995;**22**: 217–20.
60. Farrington M, Eykyn SJ, Walker M, Warren RE. Vertebral osteomyelitis due to coccobacilli of the HB group. *Br Med J (Clin Res Ed)* 1983;**287**:1658–60.
61. Feder HM Jr, Roberts JC, Salazar JC, Leopold HB, Toro-Salazar O. HACEK endocarditis in infants and children: two cases and a literature review. *Pediatr Infect Dis J* 2003;**22**:557–62.
62. Filiatrault MJ, Munson RS Jr, Campagnari AA. Genetic analysis of a pyocin-resistant lipooligosaccharide (LOS) mutant of *Haemophilus ducreyi*: restoration of full-length LOS restores pyocin sensitivity. *J Bacteriol* 2001;**183**:5756–61.
63. Filiatrault MJ, Gibson BW, Schilling B, et al. Construction and characterization of *Haemophilus ducreyi* lipooligosaccharide (LOS) mutants defective in expression of heptosyltransferase III and beta1,4-glucosyltransferase: identification of LOS glycoforms containing lactosamine repeats. *Infect Immun* 2000;**68**: 3352–61.
64. Fischbein CA, Beckett KM, Rosenthal A. *Hemophilus aphrophilus* brain abscess associated with congenital heart disease. *J Pediatr* 1973;**83**:631–3.
65. Flood JM, Sarafian SK, Bolan GA, et al. Multistrain outbreak of chancroid in San Francisco, 1989-1991. *J Infect Dis* 1993;**167**: 1106–11.
66. Florman AL. An acute febrile illness with rash and leucopenia due to *Haemophilus parainfluenzae*. *J Pediatr* 1943;**22**:202–4.
67. Fortney KR, Young RS, Bauer ME, et al. Expression of peptidoglycan-associated lipoprotein is required for virulence in the human model of *Haemophilus ducreyi* infection. *Infect Immun* 2000;**68**:6441–8.
68. Frazier JP, Cleary TG, Pickering LK. Meningitis due to *Haemophilus parainfluenzae*: report of three cases and review of the literature. *Pediatr Infect Dis* 1982;**1**:117–19.
69. Frisan T, Cortes-Bratti X, Chaves-Olarte E, Stenerlow B, Thelestam M. The *Haemophilus ducreyi* cytolethal distending toxin induces DNA double-strand breaks and promotes ATM-dependent activation of RhoA. *Cell Microbiol* 2003;**5**:695–707.
70. Fulcher RA, Cole LE, Janowicz DM, et al. Expression of *Haemophilus ducreyi* collagen binding outer membrane protein NcaA is required for virulence in swine and human challenge models of chancroid. *Infect Immun* 2006;**74**:2651–8.
71. Fusco WG, Afonina G, Nepluev I, et al. Immunization with the *Haemophilus ducreyi* hemoglobin receptor HgbA with adjuvant monophosphoryl lipid A protects swine from a homologous but not a heterologous challenge. *Infect Immun* 2010;**78**:3763–72.
72. Gaisin A, Heaton CL. Chancroid: alias the soft chancre. *Int J Dermatol* 1975;**14**:188–97.
73. Gelfanova V, Hansen EJ, Spinola SM. Cytolethal distending toxin of *Haemophilus ducreyi* induces apoptotic death of Jurkat T cells. *Infect Immun* 1999;**67**:6394–402.
74. Geraci JE, Wilkowske CJ, Wilson WR, Washington JA 2nd. *Haemophilus* endocarditis: report of 14 patients. *Mayo Clin Proc* 1977;**52**:209–15.
75. Goon S, Schilling B, Tullius MV, Gibson BW, Bertozzi CR. Metabolic incorporation of unnatural sialic acids into *Haemophilus ducreyi* lipooligosaccharides. *Proc Natl Acad Sci U S A* 2003;**100**: 3089–94.
76. Gribble MJ, Hunter T. *Hemophilus aphrophilus* vertebral osteomyelitis: a case report and literature review. *Diagn Microbiol Infect Dis* 1987;**8**:189–91.
77. Guerra L, Teter K, Lilley BN, et al. Cellular internalization of cytolethal distending toxin: a new end to a known pathway. *Cell Microbiol* 2005;**7**:921–34.
78. Gullekson EH, Dumoff M. *Haemophilus parainfluenzae* meningitis in a newborn. *JAMA* 1966;**198**:1221.
79. Hable KA, Logan GB, Washington JA 2nd. Three *Hemophilus* species: pathogenic activity. *Am J Dis Child* 1971;**121**:35–7.
80. Hamed KA, Dormitzer PR, Su CK, Relman DA. *Haemophilus parainfluenzae* endocarditis: application of a molecular approach for identification of pathogenic bacterial species. *Clin Infect Dis* 1994;**19**:677–83.
81. Hammond GW, Slutchuk M, Scatliff J, Sherman E, Wilt JC, Ronald AR. Epidemiologic, clinical, laboratory, and therapeutic features of an urban outbreak of chancroid in North America. *Rev Infect Dis* 1980;**2**:867–79.
82. Hand WL. *Haemophilus* species (including chancroid). In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*. Philadelphia: Churchill Livingstone; 2000. p. 2378–82.
83. Hansen EJ, Lumbley SR, Richardson JA, et al. Induction of protective immunity to *Haemophilus ducreyi* in the temperature-dependent rabbit model of experimental chancroid. *J Immunol* 1994;**152**:184–92.
84. Hansson S, Svedhem A, Wennerstrom M, Jodal U. Urinary tract infection caused by *Haemophilus influenzae* and *Haemophilus parainfluenzae* in children. *Pediatr Nephrol* 2007;**22**:1321–5.
85. Harrison LH, Simonsen V, Waldman EA. Emergence and disappearance of a virulent clone of *Haemophilus influenzae* biogroup aegyptius, cause of Brazilian purpuric fever. *Clin Microbiol Rev* 2008;**21**:594–605.
86. Harrison LH, da Silva GA, Pittman M, et al. Epidemiology and clinical spectrum of Brazilian purpuric fever. Brazilian Purpuric Fever Study Group. *J Clin Microbiol* 1989;**27**:599–604.
87. Haydock AK, Martin DH, Morse SA, et al. Molecular characterization of *Haemophilus ducreyi* strains from Jackson, Mississippi, and New Orleans, Louisiana. *J Infect Dis* 1999;**179**:1423–32.
88. Hindler JA, Jorgensen JH. Susceptibility test methods: Fastidious bacteria. In: Versalovic R, Carroll KC, Funke G, et al, editors. *Manual of Clinical Microbiology*, 10th ed. Washington, DC: American Society for Microbiology; 2012. p. 1180–203.
89. Ho JL, Soukiasian S, Oh WH, Snydman DR. *Hemophilus aphrophilus* osteomyelitis. *Am J Med* 1984;**76**:159–61.
90. Hobbs MM, San Mateo LR, Orndorff PE, Almond G, Kawula TH. Swine model of *Haemophilus ducreyi* infection. *Infect Immun* 1995;**63**:3094–100.
91. Holt RN, Taylor CD, Schneider HJ, Hallock JA. Three cases of *Hemophilus parainfluenzae* meningitis. *Clin Pediatr (Phila)* 1974;**13**: 666–8.
92. Huang ST, Lee HC, Lee NY, Liu KH, Ko WC. Clinical characteristics of invasive *Haemophilus aphrophilus* infections. *J Microbiol Immunol Infect* 2005;**38**:271–6.
93. Isom JB, Gordy PD, Selner JC, Brown LJ, Willis M. Brain abscess due to *Haemophilus aphrophilus*. *N Engl J Med* 1964;**271**: 1059–61.
94. Janowicz D, Leduc I, Fortney KR, et al. A DltA mutant of *Haemophilus ducreyi* is partially attenuated in its ability to cause pustules in human volunteers. *Infect Immun* 2006;**74**:1394–7.

95. Janowicz DM, Li W, Bauer ME. Host-pathogen interplay of *Haemophilus ducreyi*. *Curr Opin Infect Dis* 2010;**23**:64–9.
96. Janowicz DM, Ofner S, Katz BP, Spinola SM. Experimental infection of human volunteers with *Haemophilus ducreyi*: fifteen years of clinical data and experience. *J Infect Dis* 2009;**199**:1671–9.
97. Janowicz DM, Fortney KR, Katz BP, et al. Expression of the LspA1 and LspA2 proteins by *Haemophilus ducreyi* is required for virulence in human volunteers. *Infect Immun* 2004;**72**: 4528–33.
98. Jessamine PG, Ronald AR. Chancroid and the role of genital ulcer disease in the spread of human retroviruses. *Med Clin North Am* 1990;**74**:1417–31.
99. Johnson SR, Martin DH, Cammarata C, Morse SA. Alterations in sample preparation increase sensitivity of PCR assay for diagnosis of chancroid. *J Clin Microbiol* 1995;**33**:1036–8.
100. Jorgensen JH, Howell AW, Maher LA. Antimicrobial susceptibility testing of less commonly isolated *Haemophilus* species using *Haemophilus* test medium. *J Clin Microbiol* 1990;**28**:985–8.
101. Julander I, Lindberg AA, Svanbom M. *Haemophilus parainfluenza:* an uncommon cause of septicemia and endocarditis. *Scand J Infect Dis* 1980;**12**:85–9.
102. Kaplan JM, McCracken GH Jr, Nelson JD. Infections in children caused by the HB group of bacteria. *J Pediatr* 1973;**82**: 398–403.
103. Kaufman SR, Hambly F, Dyke JW, Gordon RC. *Hemophilus parainfluenzae* meningitis. *Clin Pediatr (Phila)* 1974;**13**:661–3.
104. Khairat O. Endocarditis due to a new species of *Haemophilus*. *J Pathol Bacteriol* 1940;**50**:497–505.
105. Kilian M. A taxonomic study of the genus *Haemophilus*, with the proposal of a new species. *J Gen Microbiol* 1976;**93**:9–62.
106. Klesney-Tait J, Hiltke TJ, Maciver I, et al. The major outer membrane protein of *Haemophilus ducreyi* consists of two OmpA homologs. *J Bacteriol* 1997;**179**:1764–73.
107. Koch R. Bericht uber die thatigkeit der Deutschen Cholerakommission in Aegypten und *Ostindien. Wein Med Wochenschr* 1883; **33**:1548.
108. Kraut MS, Attebery HR, Finegold SM, Sutter VL. Detection of *Haemophilus aphrophilus* in the human oral flora with a selective medium. *J Infect Dis* 1972;**126**:189–92.
109. Kreiss JK, Coombs R, Plummer F, et al. Isolation of human immunodeficiency virus from genital ulcers in Nairobi prostitutes. *J Infect Dis* 1989;**160**:380–4.
110. Kreiss JK, Koech D, Plummer FA, et al. AIDS virus infection in Nairobi prostitutes: spread of the epidemic to East Africa. *N Engl J Med* 1986;**314**:414–18.
111. Krishnaswami R, Schwartz J, Boodish W. Pathogenicity of *H. parainfluenzae*. *Pediatrics* 1972;**50**:498–9.
112. Kuklinska D, Kilian M. Relative proportions of *Haemophilus* species in the throat of healthy children and adults. *Eur J Clin Microbiol* 1984;**3**:249–52.
113. Lagergard T, Purven M. Neutralizing antibodies to *Haemophilus ducreyi* cytotoxin. *Infect Immun* 1993;**61**:1589–92.
114. Lammel CJ, Dekker NP, Palefsky J, Brooks GF. In vitro model of *Haemophilus ducreyi* adherence to and entry into eukaryotic cells of genital origin. *J Infect Dis* 1993;**167**:642–50.
115. Ledeboer NA, Doern GV. Haemophilus. In: Versalovic R, Carroll KC, Funke G, et al, editors. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2012. p. 588–602.
116. Leduc I, Olsen B, Elkins C. Localization of the domains of the *Haemophilus ducreyi* trimeric autotransporter DsrA involved in serum resistance and binding to the extracellular matrix proteins fibronectin and vitronectin. *Infect Immun* 2009;**77**:657–66.
117. Leduc I, White CD, Nepluev I, et al. Outer membrane protein DsrA is the major fibronectin-binding determinant of *Haemophilus ducreyi*. *Infect Immun* 2008;**76**:1608–16.
118. Leduc I, Banks KE, Fortney KR, et al. Evaluation of the repertoire of the TonB-dependent receptors of *Haemophilus ducreyi* for their role in virulence in humans. *J Infect Dis* 2008;**197**:1103–9.
119. Leduc I, Fusco WG, Choudhary N, et al. Passive immunization with a polyclonal antiserum to the hemoglobin receptor of *Haemophilus ducreyi* confers protection against a homologous challenge in the experimental swine model of chancroid. *Infect Immun* 2011;**79**:3168–77.
120. Lesse AJ, Gheesling LL, Bittner WE, Myers SD, Carlone GM. Stable, conserved outer membrane epitope of strains of *Haemophilus influenzae* biogroup aegyptius associated with Brazilian purpuric fever. *Infect Immun* 1992;**60**:1351–7.
121. Lewis DA. Chancroid: clinical manifestations, diagnosis, and management. *Sex Transm Infect* 2003;**79**:68–71.
122. Li L, Sharipo A, Chaves-Olarte E, et al. The *Haemophilus ducreyi* cytolethal distending toxin activates sensors of DNA damage and repair complexes in proliferating and non-proliferating cells. *Cell Microbiol* 2002;**4**:87–99.
123. Li MS, Farrant JL, Langford PR, Kroll JS. Identification and characterization of genomic loci unique to the Brazilian purpuric fever clonal group of *H. influenzae* biogroup aegyptius: functionality explored using meningococcal homology. *Mol Microbiol* 2003;**47**:1101–11.
124. Li W, Katz BP, Spinola SM. *Haemophilus ducreyi* lipooligosaccharides induce expression of the immunosuppressive enzyme indoleamine 2,3-dioxygenase via type I interferons and tumor necrosis factor alpha in human dendritic cells. *Infect Immun* 2011;**79**: 3338–47.
125. Lomholt H, Kilian M. Distinct antigenic and genetic properties of the immunoglobulin A1 protease produced by *Haemophilus influenzae* biogroup aegyptius associated with Brazilian purpuric fever in Brazil. *Infect Immun* 1995;**63**:4389–94.
126. Lundqvist A, Fernandez-Rodrigues J, Ahlman K, Lagergard T. Detoxified *Haemophilus ducreyi* cytolethal distending toxin and induction of toxin specific antibodies in the genital tract. *Vaccine* 2010;**28**:5768–73.
127. Lutwick LI, Gradon JD, Chapnick EK, Hakim A, Benkel MM. *Haemophilus parainfluenzae* endocarditis treated with vegetectomy and complicated by late, fatal splenic rupture. *Pediatr Infect Dis J* 1991;**10**:778–81.
128. Lynn DJ, Kane JG, Parker RH. *Haemophilus parainfluenzae* and *influenzae* endocarditis: a review of forty cases. *Medicine (Baltimore)* 1977;**56**:115–28.
129. Mackay IM, Harnett G, Jeoffreys N, et al. Detection and discrimination of herpes simplex viruses, *Haemophilus ducreyi*, *Treponema pallidum*, and *Calymmatobacterium* (*Klebsiella*) *granulomatis* from genital ulcers. *Clin Infect Dis* 2006;**42**:1431–8.
130. Maller R, Ansehn S, Fryden A. *Haemophilus parainfluenzae* infection of the central nervous system: a report on two infants. *Scand J Infect Dis* 1977;**9**:241–2.
131. Mayer MP, Schweizer P. Primary peritonitis in a child caused by *Haemophilus parainfluenzae*. *Pediatr Surg Int* 2002;**18**:728–9.
132. Mazloum HA, Kilian M, Mohamed ZM, Said MD. Differentiation of *Haemophilus aegyptius* and *Haemophilus influenzae*. *Acta Pathol Microbiol Immunol Scand [B]* 1982;**90**:109–12.
133. McCrea KW, Xie J, LaCross N, et al. Relationships of nontypeable *Haemophilus influenzae* strains to hemolytic and nonhemolytic *Haemophilus haemolyticus* strains. *J Clin Microbiol* 2008;**46**: 406–16.
134. McGillivary G, Smoot LM, Actis LA. Characterization of the IgA1 protease from the Brazilian purpuric fever strain F3031 of *Haemophilus influenzae* biogroup aegyptius. *FEMS Microbiol Lett* 2005;**250**:229–36.
135. McGillivary G, Tomaras AP, Rhodes ER, Actis LA. Cloning and sequencing of a genomic island found in the Brazilian purpuric fever clone of *Haemophilus influenzae* biogroup aegyptius. *Infect Immun* 2005;**73**:1927–38.
136. Melaugh W, Campagnari AA, Gibson BW. The lipooligosaccharides of *Haemophilus ducreyi* are highly sialylated. *J Bacteriol* 1996;**178**:564–70.
137. Merino D, Saavedra J, Pujol E, et al. *Haemophilus aphrophilus* as a rare cause of arthritis. *Clin Infect Dis* 1994;**19**:320–2.
138. Mertz KJ, Trees D, Levine WC, et al. Etiology of genital ulcers and prevalence of human immunodeficiency virus coinfection in 10 US cities. The Genital Ulcer Disease Surveillance Group. *J Infect Dis* 1998;**178**:1795–8.
139. Mertz KJ, Weiss JB, Webb RM, et al. An investigation of genital ulcers in Jackson, Mississippi, with use of a multiplex polymerase chain reaction assay: high prevalence of chancroid and human immunodeficiency virus infection. *J Infect Dis* 1998;**178**: 1060–6.
140. Mesko ZG, Bauza J, Vinas C. Bacterial endocarditis due to *Hemophilus aphrophilus* with cerebral embolism. *J Pediatr* 1976;**89**: 1031–2.
141. Morse SA. Chancroid and *Haemophilus ducreyi*. *Clin Microbiol Rev* 1989;**2**:137–57.

142. Morse SA, Trees DL, Htun Y, et al. Comparison of clinical diagnosis and standard laboratory and molecular methods for the diagnosis of genital ulcer disease in Lesotho: association with human immunodeficiency virus infection. *J Infect Dis* 1997;**175**:583–9.
143. Murphy TF. *Haemophilus* species (including *H. influenzae* and chancroid). In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases*, 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 2911–19.
144. Murphy TF, Brauer AL, Sethi S, Kilian M, Cai X, Lesse AJ. *Haemophilus haemolyticus*: a human respiratory tract commensal to be distinguished from *Haemophilus influenzae*. *J Infect Dis* 2007;**195**: 81–9.
145. Musser JM, Selander RK. Brazilian purpuric fever: evolutionary genetic relationships of the case clone of *Haemophilus influenzae* biogroup aegyptius to encapsulated strains of *Haemophilus influenzae*. *J Infect Dis* 1990;**161**:130–3.
146. Nahass RG, Cook S, Weinstein MP. Vertebral osteomyelitis due to *Haemophilus aphrophilus*: treatment with ceftriaxone. *J Infect Dis* 1989;**159**:811–12.
147. Norskov-Lauritsen N, Kilian M. Reclassification of *Actinobacillus actinomycetemcomitans*, *Haemophilus aphrophilus*, *Haemophilus paraphrophilus* and *Haemophilus segnis* as *Aggregatibacter actinomycetemcomitans* gen. nov., comb. nov., *Aggregatibacter aphrophilus* comb. nov. and *Aggregatibacter segnis* comb. nov., and emended description of *Aggregatibacter aphrophilus* to include V factor-dependent and V factor-independent isolates. *Int J Syst Evol Microbiol* 2006;**56**:2135–46.
148. Orle KA, Gates CA, Martin DH, Body BA, Weiss JB. Simultaneous PCR detection of *Haemophilus ducreyi*, *Treponema pallidum*, and herpes simplex virus types 1 and 2 from genital ulcers. *J Clin Microbiol* 1996;**34**:49–54.
149. Page MI, King EO. Infection due to *Actinobacillus actinomycetemcomitans* and *Haemophilus aphrophilus*. *N Engl J Med* 1966;**275**: 181–8.
150. Palmer KL, Goldman WE, Munson RS Jr. An isogenic haemolysin-deficient mutant of *Haemophilus ducreyi* lacks the ability to produce cytopathic effects on human foreskin fibroblasts. *Mol Microbiol* 1996;**21**:13–19.
151. Palmer KL, Grass S, Munson RS Jr. Identification of a hemolytic activity elaborated by *Haemophilus ducreyi*. *Infect Immun* 1994;**62**: 3041–3.
152. Palmer KL, Schnizlein-Bick CT, Orazi A, et al. The immune response to *Haemophilus ducreyi* resembles a delayed-type hypersensitivity reaction throughout experimental infection of human subjects. *J Infect Dis* 1998;**178**:1688–97.
153. Palmer KL, Thornton AC, Fortney KR, et al. Evaluation of an isogenic hemolysin-deficient mutant in the human model of *Haemophilus ducreyi* infection. *J Infect Dis* 1998;**178**:191–9.
154. Parker RH, Hoeprich PD. Disk method for rapid identification of *Hemophilus* species. *Am J Clin Pathol* 1962;**37**:319–27.
155. Patterson K, Olsen B, Thomas C, et al. Development of a rapid immunodiagnostic test for *Haemophilus ducreyi*. *J Clin Microbiol* 2002;**40**:3694–702.
156. Pereira RM, Bucaretchi F, Tresoldi AT. Infective endocarditis due to *Haemophilus aphrophilus*: a case report. *J Pediatr (Rio J)* 2008; **84**:178–80.
157. Perkins BA, Tondella ML, Bortolotto IM, et al. Comparative efficacy of oral rifampin and topical chloramphenicol in eradicating conjunctival carriage of *Haemophilus influenzae* biogroup aegyptius. Brazilian Purpuric Fever Study Group. *Pediatr Infect Dis J* 1992;**11**:717–21.
158. Petty BG, Burrow CR, Robinson RA, Bulkley GB. *Hemophilus aphrophilus* meningitis followed by vertebral osteomyelitis and suppurative psoas abscess. *Am J Med* 1985;**78**:159–62.
159. Plummer FA, Simonsen JN, Cameron DW, et al. Cofactors in male-female sexual transmission of human immunodeficiency virus type 1. *J Infect Dis* 1991;**163**:233–9.
160. Post DM, Mungur R, Gibson BW, Munson RS Jr. Identification of a novel sialic acid transporter in *Haemophilus ducreyi*. *Infect Immun* 2005;**73**:6727–35.
161. Purcell BK, Richardson JA, Radolf JD, Hansen EJ. A temperature-dependent rabbit model for production of dermal lesions by *Haemophilus ducreyi*. *J Infect Dis* 1991;**164**:359–67.
162. Purven M, Frisk A, Lonnroth I, Lagergard T. Purification and identification of *Haemophilus ducreyi* cytotoxin by use of a neutralizing monoclonal antibody. *Infect Immun* 1997;**65**:3496–9.
163. Purven M, Lagergard T. *Haemophilus ducreyi*, a cytotoxin-producing bacterium. *Infect Immun* 1992;**60**:1156–62.
164. Quinn FD, Weyant RS, Worley MJ, et al. Human microvascular endothelial tissue culture cell model for studying pathogenesis of Brazilian purpuric fever. *Infect Immun* 1995;**63**:2317–22.
165. Read TD, Dowdell M, Satola SW, Farley MM. Duplication of pilus gene complexes of *Haemophilus influenzae* biogroup aegyptius. *J Bacteriol* 1996;**178**:6564–70.
166. Read TD, Satola SW, Opdyke JA, Farley MM. Copy number of pilus gene clusters in *Haemophilus influenzae* and variation in the hifE pilin gene. *Infect Immun* 1998;**66**:1622–31.
167. Reed RB, Frost JB, Kort K, Myers SD, Lesse AJ. DNA sequence analysis and restriction fragment length polymorphisms of the P1 gene of *Haemophilus influenzae* biogroup aegyptius associated with Brazilian purpuric fever. *Infect Immun* 1996;**64**:3666–72.
168. Rele M, Giles M, Daley AJ. Invasive *Haemophilus parainfluenzae* maternal-infant infections: an Australasian perspective and case report. *Aust N Z J Obstet Gynaecol* 2006;**46**:258–60.
169. Rivers TM. Influenza-like bacilli: Growth of influenza-like bacilli on media containing only an autoclave-labile substance as an accessory food factor. *Bull Johns Hopkins Hosp* 1922;**33**:429–31.
170. Ronald AR, Albritton WL. Chancroid and *Haemophilus ducreyi*. In: Holmes KK, Sparling PF, Mardh PA, editors. *Sexually Transmitted Diseases*. 3rd ed. New York: McGraw-Hill; 1999. p. 515–23.
171. Ronald AR, Plummer FA. Chancroid and *Haemophilus ducreyi*. *Ann Intern Med* 1985;**102**:705–7.
172. Root TE, Silva EA, Edwards LD, Topp JH. *Hemophilus aphrophilus* endocarditis with a probable primary dental focus of infection. *Chest* 1981;**80**:109–10.
173. Rossau R, Duhamel M, Jannes G, Decourt JL, Van Heuverswyn H. The development of specific rRNA-derived oligonucleotide probes for *Haemophilus ducreyi*, the causative agent of chancroid. *J Gen Microbiol* 1991;**137**:277–85.
174. Rubin LG, Peters VB, Ferez MC. Bactericidal activity of human sera against a Brazilian purpuric fever (BPF) strain of *Haemophilus influenzae* biogroup aegyptius correlates with age-related occurrence of BPF. *J Infect Dis* 1993;**167**:1262–4.
175. Rubin LG, St Geme JW 3rd. Role of lipooligosaccharide in virulence of the Brazilian purpuric fever clone of *Haemophilus influenzae* biogroup aegyptius for infant rats. *Infect Immun* 1993;**61**: 650–5.
176. Scheifele DW, Fussell SJ. Frequency of ampicillin-resistant *Haemophilus parainfluenzae* in children. *J Infect Dis* 1981;**143**: 495–8.
177. Schilling B, Gibson BW, Filiatrault M, Campagnari AA. Characterization of lipooligosaccharides from *Haemophilus ducreyi* containing polylactosamine repeats. *J Am Soc Mass Spectrom* 2002;**13**: 724–34.
178. Schmid GP. Treatment of chancroid, 1997. *Clin Infect Dis* 1999;**28**(suppl 1):S14–20.
179. Schmid GP, Sanders LL Jr., Blount JH, Alexander ER. Chancroid in the United States: reestablishment of an old disease. *JAMA* 1987;**258**:3265–8.
180. Serruto D, Spadafina T, Scarselli M, et al. HadA is an atypical new multifunctional trimeric coiled-coil adhesin of *Haemophilus influenzae* biogroup aegyptius, which promotes entry into host cells. *Cell Microbiol* 2009;**11**:1044–63.
181. Simon MW, Mitchell BL, O'Connor WN, et al. Glomerulonephritis, pulmonary hemorrhage and coagulopathy associated with *Haemophilus parainfluenzae* endocarditis. *Pediatr Infect Dis* 1985;**4**: 183–8.
182. Smith WK, Berger HW. *Hemophilus parainfluenzae* meningitis: a case in an adult. *Mt Sinai J Med* 1974;**41**:543–8.
183. Smoot LM, Franke DD, McGillivary G, Actis LA. Genomic analysis of the F3031 Brazilian purpuric fever clone of *Haemophilus influenzae* biogroup aegyptius by PCR-based subtractive hybridization. *Infect Immun* 2002;**70**:2694–9.
184. Spinola SM, Bauer ME, Munson RS Jr. Immunopathogenesis of *Haemophilus ducreyi* infection (chancroid). *Infect Immun* 2002;**70**: 1667–76.
185. Spinola SM, Castellazzo A, Shero M, Apicella MA. Characterization of pili expressed by *Haemophilus ducreyi*. *Microb Pathog* 1990;**9**:417–26.
186. Spinola SM, Wild LM, Apicella MA, Gaspari AA, Campagnari AA. Experimental human infection with *Haemophilus ducreyi*. *J Infect Dis* 1994;**169**:1146–50.

187. Spinola SM, Fortney KR, Katz BP, et al. *Haemophilus ducreyi* requires an intact flp gene cluster for virulence in humans. *Infect Immun* 2003;**71**:7178–82.
188. Spinola SM, Li W, Fortney KR, et al. Sialylation of lipooligosaccharides is dispensable for the virulence of *Haemophilus ducreyi* in humans. *Infect Immun* 2012;**80**:679–87.
189. Spinola SM, Orazi A, Arno JN, et al. Haemophilus ducreyi elicits a cutaneous infiltrate of CD4 cells during experimental human infection. *J Infect Dis* 1996;**173**:394–402.
190. Steckelberg JM, Murphy JG, Ballard D, et al. Emboli in infective endocarditis: the prognostic value of echocardiography. *Ann Intern Med* 1991;**114**:635–40.
191. Stevens MK, Latimer JL, Lumbley SR, et al. Characterization of a *Haemophilus ducreyi* mutant deficient in expression of cytolethal distending toxin. *Infect Immun* 1999;**67**:3900–8.
192. Stevens MK, Porcella S, Klesney-Tait J, et al. A hemoglobin-binding outer membrane protein is involved in virulence expression by *Haemophilus ducreyi* in an animal model. *Infect Immun* 1996;**64**:1724–35.
193. Strouts FR, Power P, Croucher NJ, et al. Lineage-specific virulence determinants of *Haemophilus influenzae* biogroup aegyptius. *Emerg Infect Dis* 2012;**18**:449–57.
194. Sun S, Scheffler NK, Gibson BW, Wang J, Munson RS Jr. Identification and characterization of the N-acetylglucosamine glycosyltransferase gene of *Haemophilus ducreyi*. *Infect Immun* 2002;**70**:5887–92.
195. Sun S, Schilling B, Tarantino L, et al. Cloning and characterization of the lipooligosaccharide galactosyltransferase II gene of *Haemophilus ducreyi*. *J Bacteriol* 2000;**182**:2292–8.
196. Sutter VL, Finegold SM. *Haemophilus aphrophilus* infections: clinical and bacteriologic studies. *Ann N Y Acad Sci* 1970;**174**:468–87.
197. Swaminathan B, Mayer LW, Bibb WF, et al. Microbiology of Brazilian purpuric fever and diagnostic tests. Brazilian Purpuric Fever Study Group. *J Clin Microbiol* 1989;**27**:605–8.
198. Theodore MJ, Anderson RD, Wang X, et al. Evaluation of new biomarker genes for differentiating *Haemophilus influenzae* from *Haemophilus haemolyticus*. *J Clin Microbiol* 2012;**50**:1422–4.
199. Thomas CE, Olsen B, Elkins C. Cloning and characterization of *tdhA*, a locus encoding a TonB-dependent heme receptor from *Haemophilus ducreyi*. *Infect Immun* 1998;**66**:4254–62.
200. Tondella ML, Quinn FD, Perkins BA. Brazilian purpuric fever caused by *Haemophilus influenzae* biogroup aegyptius strains lacking the 3031 plasmid. *J Infect Dis* 1995;**171**:209–12.
201. Totten PA, Norn DV, Stamm WE. Characterization of the hemolytic activity of *Haemophilus ducreyi*. *Infect Immun* 1995;**63**:4409–16.
202. Totten PA, Kuypers JM, Chen CY, et al. Etiology of genital ulcer disease in Dakar, Senegal, and comparison of PCR and serologic assays for detection of *Haemophilus ducreyi*. *J Clin Microbiol* 2000;**38**:268–73.
203. Totten PA, Morton WR, Knitter GH, et al. A primate model for chancroid. *J Infect Dis* 1994;**169**:1284–90.
204. Trees DL, Morse SA. Chancroid and *Haemophilus ducreyi*: an update. *Clin Microbiol Rev* 1995;**8**:357–75.
205. Tullius MV, Phillips NJ, Scheffler NK, et al. The *lbgAB* gene cluster of *Haemophilus ducreyi* encodes a beta-1,4-galactosyltransferase and an alpha-1,6-DD-heptosyltransferase involved in lipooligosaccharide biosynthesis. *Infect Immun* 2002;**70**:2853–61.
206. Tyndall M, Malisa M, Plummer FA, et al. Ceftriaxone no longer predictably cures chancroid in Kenya. *J Infect Dis* 1993;**167**:469–71.
207. Ussher JE, Wilson E, Campanella S, Taylor SL, Roberts SA. *Haemophilus ducreyi* causing chronic skin ulceration in children visiting Samoa. *Clin Infect Dis* 2007;**44**:e85–87.
208. Vakevainen M, Greenberg S, Hansen EJ. Inhibition of phagocytosis by *Haemophilus ducreyi* requires expression of the LspA1 and LspA2 proteins. *Infect Immun* 2003;**71**:5994–6003.
209. Varghese R, Melo JC, Barnwell P, Chun CH, Raff MJ. Endocarditis due to *Hemophilus aphrophilus*: report of a case with possible transmission from dog to man. *Chest* 1977;**72**:680–2.
210. Virata M, Rosenstein NE, Hadler JL, et al. Suspected Brazilian purpuric fever in a toddler with overwhelming Epstein-Barr virus infection. *Clin Infect Dis* 1998;**27**:1238–40.
211. Ward CK, Latimer JL, Nika J, et al. Mutations in the lspA1 and lspA2 genes of *Haemophilus ducreyi* affect the virulence of this pathogen in an animal model system. *Infect Immun* 2003;**71**:2478–86.
212. Wasserheit JN. Epidemiological synergy: interrelationships between human immunodeficiency virus infection and other sexually transmitted diseases. *Sex Transm Dis* 1992;**19**:61–77.
213. Watson KC, Grimstone J, O'Hare AE. Meningitis due to *Haemophilus parainfluenzae*. *J Infect* 1981;**3**:380–4.
214. Weeks JE. The bacillus of acute conjunctival catarrh, or "pink eye." *Arch Ophthalmol* 1886;**15**:441–51.
215. Weyant RS, Quinn FD, Utt EA, et al. Human microvascular endothelial cell toxicity caused by Brazilian purpuric fever-associated strains of *Haemophilus influenzae* biogroup aegyptius. *J Infect Dis* 1994;**169**:430–3.
216. White CB, Lampe RM, Copeland RL, Morrison RE. Soft tissue infection associated with *Haemophilus aphrophilus*. *Pediatrics* 1981;**67**:434–5.
217. White DR, Mukherji SK, Mangum ME, Hamrick HJ. Recurrent cervical lymphadenitis caused by *Haemophilus aphrophilus*. *Clin Infect Dis* 2000;**30**:627–9.
218. Whitney AM, Farley MM. Cloning and sequence analysis of the structural pilin gene of Brazilian purpuric fever-associated *Haemophilus influenzae* biogroup aegyptius. *Infect Immun* 1993;**61**:1559–62.
219. Wood GE, Dutro SM, Totten PA. Target cell range of *Haemophilus ducreyi* hemolysin and its involvement in invasion of human epithelial cells. *Infect Immun* 1999;**67**:3740–9.
220. Workowski KA, Berman S. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1–110.
221. Wort AJ. *Hemophilus parainfluenzae* meningitis. *Can Med Assoc J* 1975;**112**:606–7.
222. Xu T, Lundqvist A, Ahmed HJ, et al. Interactions of *Haemophilus ducreyi* and purified cytolethal distending toxin with human monocyte-derived dendritic cells, macrophages and CD4+ T cells. *Microbes Infect* 2004;**6**:1171–81.
223. Young RS, Filiatrault MJ, Fortney KR, et al. *Haemophilus ducreyi* lipooligosaccharide mutant defective in expression of beta-1,4-glucosyltransferase is virulent in humans. *Infect Immun* 2001;**69**:4180–4.
224. Young RS, Fortney KR, Gelfanova V, et al. Expression of cytolethal distending toxin and hemolysin is not required for pustule formation by *Haemophilus ducreyi* in human volunteers. *Infect Immun* 2001;**69**:1938–42.
225. Young RS, Fortney K, Haley JC, et al. Expression of sialylated or paragloboside-like lipooligosaccharides are not required for pustule formation by *Haemophilus ducreyi* in human volunteers. *Infect Immun* 1999;**67**:6335–40.
226. Zbinden R, von Graevenitz A. *Actinobacillus*, *Capnocytophaga*, *Eikenella*, *Kingella*, *Pasteurella*, and other fastidious or rarely encountered Gram-negative rods. In: Versalovic R, Carroll KC, Funke G, et al, editors. *Manual of Clinical Microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2012. p. 574–87.

observed bacteria in the human stomach. Bizzozero[12] described spirochetes in the stomach of dogs in 1893, and his observations were confirmed by Solomon[175] and by Kasai and Kobayashi[102] in the dog, cat, rat, and monkey. Muhlens[139] and later Luger and Neuberger[120] reported finding spiral organisms in ulcerating carcinomas of the stomach in humans. In 1938, Doenges[39] explored the issue of gastric spirochetes and their clinical relevance. He reported a 43 percent prevalence of spiral organisms in the human stomach in an autopsy review of 242 patients without known gastrointestinal disease. Specimen autolysis, however, rendered interpretation of the pathologic significance of the gastric spirochetes impossible. Freedburg and Barron[66] verified the findings of Doenges in 1940, when they identified spirochetes in the stomachs of 37 percent of patients who had undergone partial gastric resection for carcinoma or ulcer disease. Work on the clinical significance of gastric spiral bacteria continued until a report by Palmer[150] was published in 1954. In an attempt to confirm the findings of spirochetes in the gastric mucosa, Palmer performed an exhaustive review of gastric fundus biopsies from 1000 adult patients, 80 percent of whom were being evaluated for upper gastrointestinal complaints and 20 percent were healthy control volunteers. Palmer noted, "None of the 1180 specimens was found to contain spirochetes or any structure which could reasonably be considered to be of spirochetal nature." He concluded that spirochetes are not part of the human gastric mucosa in health or illness. Palmer, a prominent researcher in gastritis, inadvertently may have curtailed further research into the role of gastric spiral bacteria.

In 1975, Steer,[176] using electron microscopy, noted curved bacteria in stomach biopsy specimens from patients with gastric ulcers. A few years later, Warren, an Australian pathologist, noted the appearance of spiral bacteria overlying inflamed gastric mucosa. Warren noted that this organism looked like a *Campylobacter*. He and Marshall began a series of culture experiments using *Campylobacter*-specific methods. The story culminates in 1983 and 1984, respectively, with their reports of successful culture of the curved bacillus.[2,132] In an attempt to fulfill the remainder of Koch's postulates, Marshall and associates[131] and Morris and Nicholson[138] independently ingested pure cultures of *H. pylori*, and symptomatic acute gastritis developed in both. The organism subsequently was recovered from the gastric mucosa by endoscopy and successfully cultured, thus completing Koch's postulates.

MICROBIOLOGY AND PATHOPHYSIOLOGY

H. pylori initially was named *Campylobacter pyloridis* because of its resemblance to *Campylobacter* spp.[2] and later changed to *Campylobacter pylori*. However, it became clear that the bacterium did not belong to any known genus. An entirely new genus, *Helicobacter*, was created, with *helico* describing the spiral shape and *pylori* denoting its typical location. *H. pylori* is a spiral-shaped, gram-negative bacteria with four to seven unipolar sheathed flagella (Fig. 135-1).[13,148] It is 0.5 μm wide and 3 to 5 μm long and has a smooth surface.[3,136] It generally is "S"-shaped in vivo but can take on many forms, from "U"-shaped to cocci to rodlike.[134]

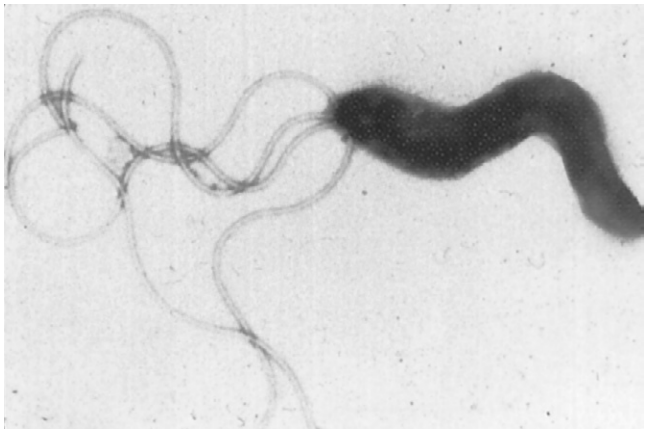

FIGURE 135-1 ■ Electron microscopy of *Helicobacter pylori* demonstrating the multipolar flagella and the typical spiral shape.

H. pylori inhabits a unique ecologic niche—the submucosa of gastric epithelium. Several attributes, including adherence, shape, microaerophilism, urease production, and motility, allow adaptation to the acidic gastric environment. *H. pylori* overlies the intercellular tight junctions of epithelial cells, beneath the mucous layer. Specific adhesion molecules appear to be tropic to gastric mucus-producing cells.[111] This adherence allows the organism to maintain colonization despite the rapid gastric cell turnover.[112] The spiral shape may allow the organism to corkscrew through the gastric mucus (see Fig. 135-1). This spiral movement has been demonstrated in vitro with methylcellulose solutions.[112] *H. pylori* is microaerophilic and grows slowly in culture media.[14,149] Translucent colonies of 1 mm grow after 5 to 7 days on blood-supplemented or serum-supplemented media with a low concentration of oxygen and carbon dioxide at a temperature of 37° C (98.6° F), which promotes optimal growth. This microaerophilism is well suited to the low oxygen levels of the gastric submucosa.[111] *H. pylori* is the most potent urease producer of any known microbe; researchers have estimated that as much as 10 percent of its total protein production is urease, and the organism appears to surround itself in a cloud of ammonia.[3,9,14,151] The urea is converted to ammonium and bicarbonate.[13,76] Little evidence supports the notion that the ammonium produced is cytotoxic to gastric epithelium.[92] The bicarbonate creates an alkaline environment in the gastric submucosa. *H. pylori* produces several other enzymes, including mucinase,[173] lipase,[171] catalase,[117] hemolysin, and cytopathic toxin.[115] The multiple, unipolar flagella (see Fig. 135-1) provide motility that may enable the organism to escape the acid lumen of the stomach[112] and evade host immune responses.[111]

H. pylori may be more akin to normal gastrointestinal flora because the bacterium and the host can coexist for decades without apparent problems.[81] *H. pylori* infection produces inflammation, although in most cases the host is asymptomatic.[16] The inflammatory response is

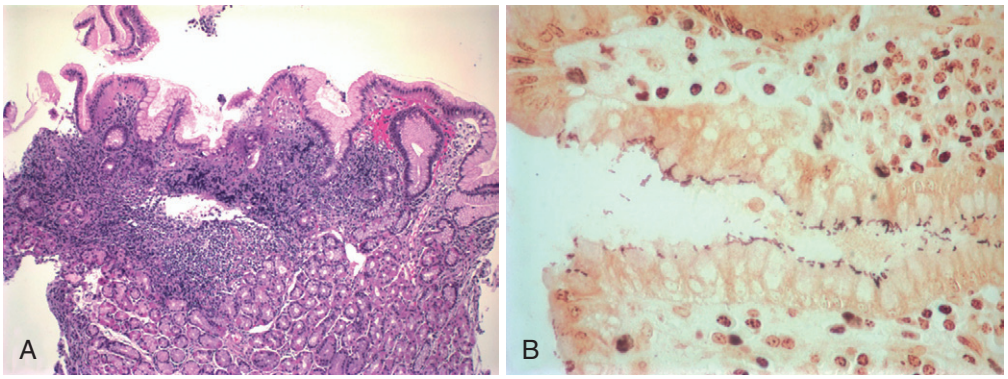

FIGURE 135-2 ■ A, Chronic active gastritis. Corpus mucosa displays a large lymphoid infiltrate (×100, hematoxylin-eosin). **B,** *Helicobacter pylori* are abundant at the surface and line the mucous glands of the gastric pit (×640, silver stain). (See companion Expert Consult website for color version.) (Courtesy Dr. Milton J. Finegold, Baylor College of Medicine, Texas Children's Hospital.)

characterized by infiltration of polymorphonuclear leukocytes, monocytes, lymphocytes, and plasma cells into the lamina propria (Fig. 135-2).[117] A significant immune response, including the production of antibodies, is produced by the host, but it does not clear the organism in most cases.[115] *H. pylori* produces a chronic infection that persists for decades, possibly for life.[117] This lifelong colonization is accomplished by the same features that allow survival in the gastric mucosa, namely, high urease production, flagellar motility, spiral shape, microaerophilism, and adherence.[117]

Numerous potential virulence factors, such as urease, catalase, cytotoxin, and lipopolysaccharide, have been identified, although whether any of these substances actually contribute to symptomatic disease is unknown. The urease of *H. pylori* is a potent antigen that induces elevations in antiurease immunoglobulin (Ig)G and IgA.[117] Interestingly, the ammonia produced by the urease does not appear to have a significant role in pathogenesis.[83] Catalase, another *H. pylori* enzyme, prevents the formation of oxygen metabolites from hydrogen peroxide in neutrophils,[50] which may provide an ability to evade host destruction. A vacuolating cytotoxin has been identified in many *H. pylori* strains,[50] although whether it is more common in patients with symptomatic disease is unclear. *H. pylori* has a peculiar lipopolysaccharide outer membrane. Unlike most gram-negative bacteria, its lipopolysaccharide coat is a significantly less potent inducer of the host complement cascade, some 1000-fold less potent than that of the Enterobacteriaceae.[117] This low biologic activity of the outer membrane of *H. pylori* may be another adaptive mechanism allowing gastric colonization.[15]

The most pathogenic strain of *H. pylori* contains a cytotoxin-associated gene pathogenicity island (Cag PAI).[167,168] The Cag PAI encodes a type IV secretion system with a pilus that directly injects Cag A into the host's cytoplasm. Cag A affects gastric epithelial functions that include cell proliferation, suppression of apoptosis, as well as affecting intercellular junctions.[7] Cag A–positive strains are considered more virulent because of Cag A involvement in the intestinal cell transdifferentiation of gastric epithelial cells—a precursor to carcinogenesis.[141,142]

EPIDEMIOLOGY

H. pylori is one of the most common bacterial infections in humans, with an estimated 1 billion people in the world infected.[101,116] It is estimated that in developing countries more than 80 percent of the population is infected with *H. pylori*, whereas in industrialized countries the infection rate remains under 40 percent (Fig. 135-3).[82,154,155,160,180] Transmission appears to be from person to person.[113,133,182] Transmission via the fecal-oral route is the primary mode of infection.* The presence of pets may be a risk factor for *H. pylori* infection, given that the bacterium has been isolated from domestic animals.[20,21,42,93] Interestingly, breast-feeding appears to increase the risk for acquiring *H. pylori* infection, possibly secondary to close contact with infected mothers, and breast milk does not protect against infection.[41,166,193] *H. pylori* usually is acquired in childhood,[33,116] and humans appear to be a natural reservoir for infection.[85] Environmental sources of *H. pylori* infection, such as municipal water sources, have been confirmed in Peru and Mexico.[119,135]

Children are important factors in the transmission of *H. pylori* infection, which clusters within families with children.[48,126,134,146,189] Family clustering emphasizes the role of crowding and personal hygiene in the person-to-person spread of infection. The prevalence of infection increases with age.[181] In the United States, for example, only about 20 percent of individuals younger than 30 years of age are infected, in contrast to 50 percent of those older than 60 years.[130] Infection in young children is a rare event in the United States and developed nations.[117] The phenomenon of increasing prevalence with age most likely reflects a cohort effect; that is, the high prevalence in older persons simply reflects a higher childhood infection rate.[117] This cohort effect may be explained best by a lower standard of living of older persons during childhood. Socioeconomic status varies inversely with the prevalence of infection, with low-income individuals having the highest rates of infection.[60] Conversely, Ozen and associates[149] found that low

*References 19, 51, 57, 127, 143, 192.

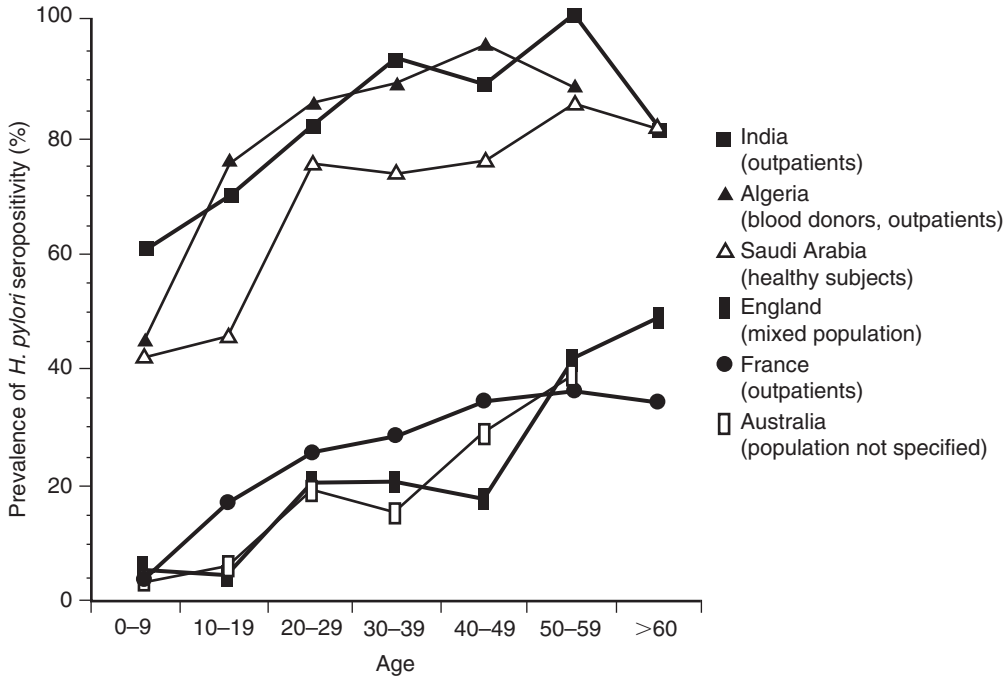

FIGURE 135-3 ■ Seroepidemiology of *Helicobacter pylori* infection demonstrating the difference in disease prevalence between developing and developed countries. (Data from Graham DY, Adam E, Reddy GT, et al. Seroepidemiology of *Helicobacter pylori* infection in India: comparison of developing and developed countries. *Dig Dis Sci* 1991;**36**:1084–8.)

household population and a history of antibiotic use during the previous 6 months served as protective factors against infection. A higher prevalence of infection exists in developing countries than in developed countries (see Fig. 135-3),[134] and the prevalence of infection varies greatly around the world. In Nigeria, for example, 58 percent of children younger than 1 year of age and 91 percent of children older than 10 years were infected.[95] Such differences have been attributed to socioeconomic factors, hygiene, and the number of household occupants.[91] *H. pylori* is more prevalent in blacks and Hispanics than in whites in the United States.[174]

The rate of *H. pylori* reinfection in the pediatric population ranges from 2.4 percent per year to about 20 percent per year.[103,145,146,162,166] Magista and colleagues[125] demonstrated that the difference in reinfection rate depends primarily on the socioeconomic status of the family and the local prevalence of *H. pylori*. They and others[58,59] also noted that living in an area with a high prevalence of *H. pylori* resulted in a fourfold higher risk of reinfection. Although *H. pylori* infection remains common worldwide, the seroprevalence of *H. pylori* infection in U.S. children has dropped significantly in all ethnic groups.[75]

CLINICAL MANIFESTATIONS

Gastritis develops in most *H. pylori*–infected patients, but complications associated with infection develop in only a few.[101] For example, the lifetime risk for developing peptic ulcer disease is estimated to be 10 to 15 percent, whereas gastric cancer will develop in less than 1 percent of *H. pylori*–infected persons.[102] This finding is especially true in children, in whom peptic ulcer is considerably less common than in adults. To confound matters, children have no specific symptoms of *H. pylori* infection.* Symptoms such as epigastric pain, nighttime awakening with abdominal pain, hematemesis, and recurrent vomiting are suggestive but in no way predictive of infection.[47,105,144] Because these clinical symptoms are typical of peptic ulcer disease, testing for *H. pylori* infection alone has no relevance but rather should be done to determine the presence of an underlying condition.[110] Such symptoms possibly are present only if *H. pylori* infection is found with a duodenal ulcer. Furthermore, the presence of *H. pylori* infection alone in both adults and children usually is asymptomatic.†

The association of *H. pylori* infection with recurrent abdominal pain of childhood remains a topic of ongoing debate. The critical issue is whether *H. pylori* antral gastritis is a source of abdominal pain. The issue remains unresolved.‡ A logical conclusion for the pediatrician would be that a child with active upper gastrointestinal tract complaints in whom *H. pylori* infection is suspected deserves either treatment of the infection or diagnostic upper endoscopy to determine the presence of ulcer disease. *H. pylori* infection also has been associated with protein-losing enteropathy, as well as iron deficiency anemia.§ Indeed, successful treatment of *H. pylori* infection improves iron stores.[23]

*References 72, 77, 79, 89, 124, 161, 163
†References 38, 40, 41, 46, 78, 86, 157, 159.
‡References 73, 122, 123, 146, 164, 182, 184, 185.
§References 21, 26, 27, 29, 32, 94, 198.

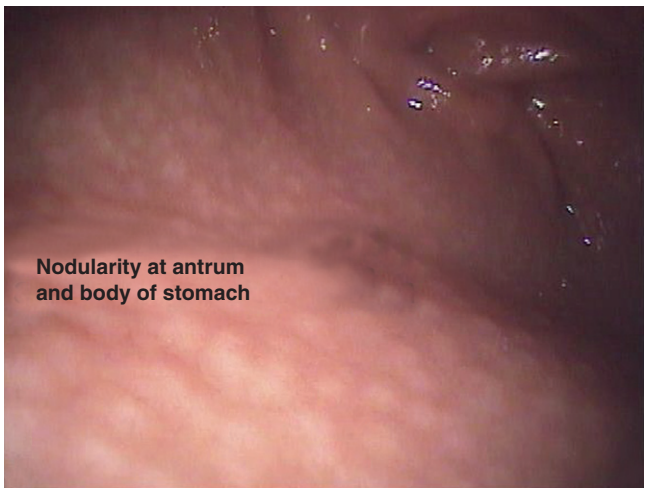

FIGURE 135-4 ■ Endoscopic view of the gastric antrum and body demonstrating marked nodularity of the gastric surface. (See companion Expert Consult website for color version.)

DIAGNOSIS

The gold standard for diagnosis of *H. pylori* infection is culture of the organism from gastric biopsy samples or histologic review.[2] Upper endoscopy or other invasive means are necessary to obtain such biopsy specimens. In children, upper endoscopy can be quite helpful in that it often reveals a distinct nodularity at the antrum (Fig. 135-4), but it also may appear normal. This nodularity is found in two thirds of infected children but only rarely in adults.[22,90,91] Histologically, prominent lymphoid follicles are seen. Routine staining with hematoxylin and eosin may be adequate but must be performed properly to see the bacteria clearly. Alternative staining techniques, such as acridine orange, silver stains, and the "triple" stain, improve visualization.[68,108,124] The triple, or Genta, stain (Steiner hematoxylin-eosin alcian blue) may offer some distinct advantages because it is significantly more sensitive than hematoxylin-eosin staining and is particularly useful for the detection of small numbers of bacteria, which often is the case in children (see Fig. 135-2). Although culture and histology require the need for endoscopy, culture may be important, especially when considering the increase in antibiotic resistance worldwide.[6,37] In cases of recurrent *H. pylori* infection in which antibiotic resistance is suspected, culture allows in vitro antibiotic sensitivities to be determined.

Noninvasive methods for detection of *H. pylori* infection are detailed in Table 135-1 and include serology, varying methods for detection of urease production, detection of salivary antibody, stool culture, stool antigen, polymerase chain reaction (PCR), and urine ammonia production.[158] The 13C-urea breath test is considered the best available noninvasive diagnostic test in children.[53,101] In 2012, it was approved by the U.S. Food and Drug Administration to detect and monitor *H. pylori* infection in pediatric patients 3 to 17 years of age.[53] Serology, on the other hand, is not useful. Measurement of *H. pylori* stool antigen can be performed by either a polyclonal or monoclonal antibody technique.[52,104] This test detects the presence of antigen, thus providing evidence of active infection for both primary diagnosis or proof of cure. It appears to be of equal accuracy to the urea breath test.

H. pylori infection induces a vigorous neutrophilic and lymphocytic (T-cell and B-cell) response that fails to clear the infection. The humoral immune response produces antibodies that can be detected in gastric secretions, as well as systemically. Some *H. pylori* antigens have been determined with the use of bacterial cell wall sonicates, urease, or membrane extracts as the capture antigen.* Some commercial assays have been found to have inappropriate positive and negative cutoff values for use in children.[31,34] Care must be taken to ensure that the serologic tests performed have been verified for use in children. Although detection of IgA, polymeric IgA, and IgM antibodies against haptoglobin can be performed, such detection is not reproducible and thus not of any useful clinical value. Serologic diagnosis, though simple and widely available, does not indicate symptomatic disease.

H. pylori is a vigorous producer of urease. This characteristic has been used to create a variety of tests to detect the presence of urease. The urea breath test uses a labeled carbon of urea, either radioactive carbon-14 (^{14}C)[11] or a nonradioactive, stable isotope, carbon-13 (^{13}C).[84] Patients fast 4 to 8 hours and then drink the labeled carbon accompanied by a meal to delay gastric emptying; then the amount of labeled carbon dioxide in the breath is measured. Only those with gastric *H. pylori* present (and thus gastric urease activity to degrade the labeled urea) will be identified. Urea breath testing is useful for both initial diagnosis and determination of successful treatment because the results rapidly return to normal after eradication. The radioactive ^{14}C method does not deserve consideration for use in children. The stable isotope ^{13}C methodology currently is the simplest and most accurate noninvasive test available method for detection of *H. pylori* infection. Its usefulness remains limited, however, because of the need for specialized equipment for analysis.

The urease production of *H. pylori* can be measured directly in gastric biopsy specimens with a variety of commercial assays.[3,132] A portion of the gastric biopsy specimen is placed into a urea medium, and hydrolysis of urea leads to a color change in the medium from tan to pink. False-negative results have been noted in children because of the low numbers of organisms.[49] Such testing is useful for establishing a rapid diagnosis during endoscopy but requires gastric biopsy. Urease activity also can be detected by measurement of another nonradioactive, stable isotope, ammonium, in the urine after oral ingestion.[100] This noninvasive test has not proved to be accurate. *H. pylori* has been identified in saliva and dental plaque by culture[109] and PCR.[28] Others have identified salivary antibody to *H. pylori* with an indirect immunofluorescence assay.[96] The saliva test is not accurate in young children and cannot be recommended.[70] Viable *H. pylori* organisms have been cultured from feces in Gambian children.

*References 30, 34, 36, 55, 172, 183.

TABLE 135-1 **Summary of Methods for Detecting *Helicobacter Pylori***

Method	Sensitivity (%)	Specificity (%)	Advantages	Disadvantages
Invasive				
Histology	93-99	95-99	Widely available; detection best with special stains; can evaluate underlying mucosal damage; gold standard	Expensive; at least two biopsies required; observer error; recent antibiotics or proton pump inhibitor use can lead to false-negative results
Culture of biopsy specimens	77-92	100	In vitro antibiotic susceptibility can be determined	Expensive; organism requires special transfer and culture technique; requires up to 1 wk for results; recent antibiotics or proton pump inhibitor use can lead to false-negative results
Rapid urease test CLO test hpFast PyloriTek	89-98	93-98	Rapid results; easy to perform; less expensive than other invasive techniques	Formalin, simethicone, local anesthetic spray, recent antibiotics, bismuth, or proton pump inhibitor use can lead to false-negative results; poor technique or handling will affect results
Noninvasive				
Urea breath test ^{13}C ^{14}C	90-100	89-100	Inexpensive, represents entire mucosa (not subject to biopsy sampling bias)	Antibiotics or proton pump inhibitor use can lead to false-negative results; presence of ulcer disease not determined; can be difficult to collect in children younger than 2 yr
Serology (ELISA) HM-CAP Pylori.STAT Rapid serology FlexSure QuickVue	44-99	89-95	Widely available, inexpensive	Not useful after treatment
Stool antigen testing (HpSA)	85-94	97.7	Inexpensive, more accurate than serology; may become the test of choice in children	Stool must be collected
Saliva	71-93	82-92	Easy to collect; inexpensive	Low sensitivity, especially in children younger than 2 yr
Urine (Anti-H. *pylori* IgG antibodies in urine)	90	100	Rapid results, easy to perform, does not require large bacterial load	Length of time needed to prove eradication not known

Data from references 70, 80, 121, 147, 165.
ELISA, Enzyme-linked immunosorbent assay.

TREATMENT

Cure of *H. pylori* infection associated with peptic ulcer disease in both children and adults significantly reduces the likelihood of recurrence of peptic ulceration.[98,130,161,197] The first-line therapy for *H. pylori* infection in children has long been a twice-daily, three-drug regimen that includes amoxicillin, clarithromycin, and a proton pump inhibitor given for 14 days.[25,69,101,185,195] Table 135-2 presents a variety of suggested regimens for the treatment of *H. pylori* infection in children. *H. pylori* infection is difficult to cure.

Confirmation of successful treatment has been defined as absence of detectable organisms by tissue biopsy or the urea breath test at least 1 month after completion of treatment.[10] Because tissue biopsy is invasive, urea breath testing or stool antigen detection is helpful for determination of successful treatment. Serologic tests are not useful for detection of cure because of the prolonged elevation of titers, which remain elevated for 6 months to 1 year or longer after treatment.

A growing body of evidence indicates that acquisition of *H. pylori* infection in childhood is a significant risk factor for the development of gastric cancer such as adenocarcinoma (Fig. 135-5).[97,152,182,187] An estimated twofold to sixfold increased risk for the development of gastric cancer exists among *H. pylori*–infected patients.[45,63,186] A current concern is whether *H. pylori* treatment decreases the risk for development of gastric cancer.[179] Multiple studies have demonstrated that no significant difference exists between treated groups and placebo groups. Patients in whom gastric cancer develops appear to already have atrophic gastritis or metaplasia at baseline.[114,137,194] This finding argues for treatment of *H. pylori* infection in childhood, before the onset of atrophic gastritis. The location of the infection within the stomach may influence the outcome. For example, antral-predominant infection more commonly leads to increased acid secretion, which results in duodenal peptic ulcers and a low risk for gastric cancer. In contrast, fundus-predominant infection leads to decreased acid secretion, which results in atrophic gastritis and a higher risk for gastric cancer.[114] Treatment of gastric lymphoma with antibiotics directed at *H. pylori* has resulted in regression of the tumor.[5,195]

Some researchers argue that the risk for developing either lymphoma or adenocarcinoma provides cause for

TABLE 135-2 **Suggested Treatment Regimens for *Helicobacter Pylori* Infection in Children**

Medications	Dosage	Duration
Triple Therapy		
Amoxicillin	50 mg/kg/day twice daily (maximum, 1 g twice daily)	7-14 days
Clarithromycin	15 mg/kg/day twice daily (maximum, 50 mg)	7-14 days
Proton pump inhibitor* (i.e., omeprazole)	1 mg/kg/day twice daily	7-14 days
Amoxicillin	50 mg/kg/day twice daily (maximum, 1 g twice daily)	7-14 days
Metronidazole	20 mg/kg/day twice daily (maximum, 500 mg twice daily)	7-14 days
Proton pump inhibitor* (i.e., omeprazole)	1 mg/kg/day	7-14 days
Quadruple Therapy		
Bismuth subsalicylate†	15 mL (17.5 mg/mL) four times daily	10-14 days
Metronidazole	20 mg/kg/day twice daily (maximum, 500 mg twice daily)	10-14 days
Tetracycline‡	50 mg/kg/day twice daily (maximum, 1 g twice daily)	10-14 days
Proton pump inhibitor* (i.e., omeprazole)	1 mg/kg/day (maximum, 20 mg twice daily)	10-14 days
Amoxicillin	50 mg/kg/day twice daily (maximum, 1 g twice daily)	10-14 days
Clarithromycin	15 mg/kg/day twice daily (maximum, 500 mg twice daily)	10-14 days
Metronidazole	20 mg/kg/day twice daily (maximum, 500 mg twice daily)	10-14 days
Proton pump inhibitor (i.e., omeprazole)	1 mg/kg/day	10-14 days
Sequential Therapy		
First 5 days		
Amoxicillin	50 mg/kg/day twice daily (maximum, 1 g twice daily)	
Proton pump inhibitor (i.e., omeprazole)	1 mg/kg/day	
Second 5 days		
Clarithromycin	15 mg/kg/day twice daily (maximum, 500 mg twice daily)	
Tinidazole	20 mg/kg/day bid (max, 500 mg bid)	
Proton pump inhibitor (i.e., omeprazole)	1 mg/kg/day	

Modified from Gilger MA. Treatment of *Helicobacter pylori* infection in children. *Curr Pharmaceut Dis* 2000;**6**:370–84; additional data from Gatta L, Vakil N, Leandro G, et al. Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children. *Am J Gastroenterol* 2009;**104**:3069–79.

*Comparable proton pump inhibitors such as lansoprazole and rabeprazole can be substituted at appropriate doses.

†Bismuth will produce black stools and has the potential complication of salicylate toxicity. Salicylates also pose the risk for Reye syndrome.

‡Tetracycline is not recommended for children younger than 8 years of age.

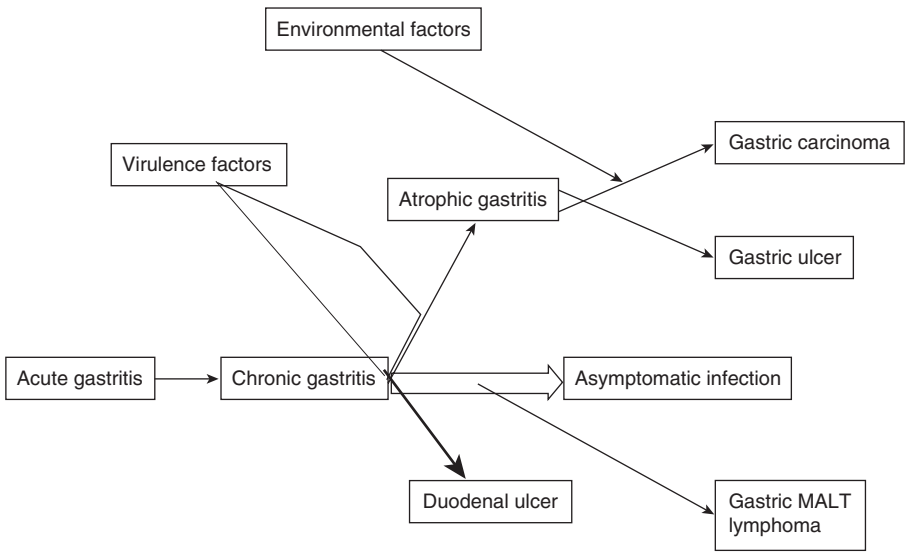

FIGURE 135-5 ■ Timeline of possible outcomes of *Helicobacter pylori* infection starting from the acute acquisition of infection and possible sequelae. *MALT,* Mucosa-associated lymphoid tissue. (Courtesy Dr. D. Graham, personal data.)

the treatment of all children infected with *H. pylori*. Because the risk for development of cancer cannot be predicted accurately and because most children with *H. pylori* infection have no clinical symptoms, no current rationale exists for treating all children infected with *H. pylori*.

In children, the most common anti-*Helicobacter* therapy is legacy triple therapy, which includes amoxicillin, clarithromycin, and a proton pump inhibitor (see Table 135-2).[87,101,129] Resistance to clarithromycin, however, has led to a decline in efficacy from 34 to 66 percent and eradication rates of less than 80 percent.[62,144,189] Eradication rates of greater than 90 percent, however, were demonstrated when *H. pylori* susceptibility testing was performed in children.[4,177] Antimicrobrial evasion to clarithromycin, for example, has been attributed to three point mutations—A2143G, A2142G, and A2142C in domain V of 23S ribosomal RNA.[65,188] Clarithromycin-containing therapy should be used if the area resistance rate is low (<10%),[106] and clarithromycin resistance rates of 15 to 20 percent should dissuade its use. Increasing resistance has prompted newer treatment regimens, such as sequential therapy, which was shown to be superior to legacy triple therapy in treatment-naïve cases, as well as in the clarithromycin-resistant and metronidazole-resistant cases.[65,67,71,99,199] Sequential therapy is 5 days of a proton pump inhibitor and one antibiotic, usually amoxicillin, followed by 5 days of a proton pump inhibitor and two other antibiotics, such as clarithromycin and tinidazole (see Table 135-2).

In patients allergic to penicillin, amoxicillin may be replaced with metronidazole. Metronidazole, to which the *H. pylori* organism has been highly sensitive, has a high rate of resistance because of its overuse for other infections.[54] A multicenter study in Europe found the overall resistance to be 25 percent for metronidazole, 24 percent for clarithromycin, and only 0.6 percent for amoxicillin.[107] In cases of antimicrobial resistance, the use of rifabutin, furazolidone,* and fluoroquinolones, such as ciprofloxacin, along with rifampin and streptomycin, has been reported as options in treating resistant *H. pylori* infection.[43,51,64,169] These second-line regimens have no particular advantage over the first-line antibiotics and should be considered only when resistance is suspected. No single antibiotic agent given alone is effective therapy against *H. pylori*.[130,151,161] Extensive study, including study of antibiotic resistance, should be used to determine the optimal treatment of *H. pylori*.

When cure of infection has been obtained, the long-term rates of reinfection are as low as 1 percent per year in developed countries but much higher in developing nations.[17,153] However, reinfection may be more likely to occur in families, especially when small children are the first infected.[8] Hence, in recurrence, all family members should be tested. No conclusive evidence currently exists, however, to recommend routine treatment of the entire family.[146]

Vaccination against *H. pylori* infection appears possible and also cost effective.[24,44] Vaccination has cleared *H. pylori* infection in animal models.[190] Human models, however, have proved more difficult. The inactivated whole-cell *H. pylori* vaccine failed to eradicate preexisting infection.[118] Although the live-vector, urease-based vaccines did not completely clear all of the preexisting infections, they showed promise and demonstrated a T-cell–mediated response to infection.[1,190] Success in human subjects was demonstrated with an antibody response to aluminium hydroxide–adjuvanted VacA-CagA-NAP vaccine[128] The protease-activated receptor 2 signaling, which is believed to regulate immune and inflammatory responses, has been shown to be important to the vaccine response.[190,191] Despite this progress, the cellular response to bacterial colonization is not completely understood.[140,178] Prophylactic and therapeutic vaccination is needed because current antibiotic therapy does not eradicate *H. pylori* infection in approximately 20 percent of affected patients. Despite vaccination successes in treatment and prevention, the failure to achieve sterilization immunity in animal models has led to continued pathology secondary to *H. pylori*. A truly effective vaccine has thus far remained elusive.[178]

FUTURE DIRECTIONS

H. pylori is a major cause of peptic ulcer disease in children and adults, which has caused a dramatic reappraisal of many previous notions about ulcer disease. Although the dictum "No acid, no ulcer" still holds, many other previous considerations, such as genetic predisposition, must be reexamined. Figure 135-5 demonstrates a proposed *H. pylori* timeline. It emphasizes the acquisition of an acute infection in childhood, persistent chronic gastritis, and the possible progression to ulcer, atrophic gastritis, and, potentially, gastric carcinoma and lymphoma.

As resistance rates of standard therapies for *H. pylori* rise, new therapies are needed. Such novel treatments are represented by benzimidazole derivatives, polycyclic compounds, pyloricidin, aryl thiazole, and plant extracts. New targets for therapy also are being identified. Genes needed for colonization, motility, and urease production, for example, represent such targets.[61]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Aebischer T, Bumann D, Epple HJ, et al. Correlation of T cell response and bacterial clearance in human volunteers challenged with *Helicobacter pylori* revealed by randomised controlled vaccination with Ty21a- based *Salmonella* vaccines. *Gut* 2008;**57**:1065–72.
4. Arenz T, Antos D, Russmann H, et al. Esomeprazole-based 1-week triple therapy directed by susceptibility testing for eradication of *Helicobacter pylori* infection in children. *J Pediatr Gastroenterol Nutr* 2006;**43**:180–4.
6. Ayala G, Galvan-Portillo M, Fierros G, et al. Resistance to antibiotics and characterization of *Helicobacter pylori* strains isolated from antrum and body from adults in Mexico. *Microb Drug Resist* 2011;**17**:149–55.
7. Backert. S, Selbach M. Role of type IV secretion in *Helicobacter pylori* pathogenesis. *Cell Microbiol* 2008;**10**:1573–81.
23. Cardenas VM, Prieto-Jimenez CA, Mulla ZD, et al. *Helicobacter pylori* eradication and change in markers of iron stores among non-iron-deficient children in El Paso, Texas: an etiologic intervention study. *J Pediatr Gastroenterol Nutr* 2011;**52**:326–32.

*References 35, 54, 56, 88, 155, 156, 196.

37. De Francesco V, Giorgio F, Hassan C, et al. Worldwide *H. pylori* antibiotic resistance: a systematic review. *J Gastrointestin Liver Dis* 2010;**19**:409–14.
53. Elitsur Y, Toia V, Gilger MA, et al. Urea breath test in children: the U.S. prospective, multicenter study. *Helicobacter* 2009;**14**: 134–40.
61. Fiorini G, Zullo A, Gatta L, et al. Newer agents for *Helicobacter pylori* eradication. *Clinical Experimental Gastroenterol* 2012;**5**: 109–12.
62. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*. *Aliment Pharmacol Ther* 2007;**26**: 343–57.
65. Francavilla R, Lionetti E, Castellaneta S, et al. Clarithromycin resistant genotypes and eradication of *Helicobacter pylori*. *J Pediatr* 2010;**157**:228–32.
67. Gatta L, Vakil N, Leandro G, et al. Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children. *Am J Gastroenterol* 2009;**104**:3069–79.
71. Gisbert J, Calvet X, O'Connor A, et al. Sequential therapy for *Helicobacter pylori* eradication: a critical review. *J Clin Gastroenterol* 2010;**44**:313–25.
99. Jafri N, Hornburg A, Howden C. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naïve. *Ann Intern Med* 2008;**148**:923–3.
106. Koletzko S, Jones N, Goodman K, et al. Evidence-based guidelines From ESPGHAN and NASPGHAN for *Helicobacter pylori* infection in children. *J Pediatr Gastroenterol Nutr* 2011;**53**: 230–43.
118. Losonsky GA, Kotloff KL, Walker RI. B-cell responses in gastric antrum and duodenum following oral inactivated *Helicobacter pylori* whole cell (HWC) vaccine and LT(R192G) in *H. pylori* seronegative individuals. *Vaccine* 2003;**21**:562–5.
128. Malfertheiner P, Schultze V, Rosenkranz B, et al. Safety and immunogenicity of an intramuscular *Helicobacter pylori* vaccine in noninfected volunteers: a Phase I study. *Gastroenterology* 2008;**135**: 787–95.
140. Muller A, Solnick JV. Inflammation, immunity, and vaccine development for *Helicobacter pylori*. *Helicobacter* 2011;**16**(suppl. 1): 26–32.
141. Murata-Kamiya N, Kurashima Y, Teishikata Y, et al. *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the b-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. *Oncogene* 2007;**26**:4617–26.
143. Odera G, Scherbakv P, Bontes P, et al. European Pediatric Task Force on *Helicobacter* pylori: results from the European Register for Treatment of the *Helicobacter pylori*. *Helicobacter* 2007;**12**: 150–6.
151. Parsonnet J. Cost-effectiveness of a potential prophylactic *Helicobacter pylori* vaccine in the United States. *J Infect Dis* 2009;**200**: 1311–7.
167. Ruggiero P. *Helicobacter pylori* infection: what's new. *Curr Opin Infect Dis* 2012;**25**:337–44.
174. Street M, Cellini L, Di Campli E, et al. Antibiotic resistance and antibiotic sensitivity based treatment in *Helicobacter pylori* infection: advantages and outcome. *Arch Dis Child* 2001;**84**:419–22.
175. Sutton P. Vaccinating against *Helicobacter pylori:* dissecting the mechanism. *Gastroenterology* 2011;**141**:1149–51.
185. Vakil N. *Helicobacter pylori* treatment: a practical approach. *Am J Gastroenterology* 2006;**101**:497–9.
186. Vakil N, Megraud F. Reviews in basic and clinical gastroenterology. *Gastroenterology* 2007;**133**; 985–1001.
187. Velin D, Michetti P. Advances in vaccination against *Helicobacter pylori*. *Expert Rev Gastroenterol Hepatol* 2010;**4**:157–66.
188. Velin D, Narayan S, Bernasconi E, et al. PAR2 promotes vaccine-induced protection against *Helicobacter* infection in mice. *Gastroenterology* 2011;**141**:1273–82.
196. Zullo A, De Francesco V, Hassan C. The sequential therapy regimen for *Helicobacter pylori* eradication: a pooled-data analysis. *Gut* 2007;**56**:1353–7.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 136

Kingella kingae

Eric A. Porsch • Kimberly F. Starr • Joseph W. St. Geme III

Kingella kingae is increasingly recognized as an important cause of invasive disease in young children, reflecting improved cultivation methods and new molecular diagnostics for this fastidious organism. Other members of the *Kingella* genus include *K. denitrificans*, *K. oralis*, and *K. potus*. *K. denitrificans* and *K. oralis* are commensals in the human oral cavity and are uncommon pathogens. *K. potus* is a commensal in the oral cavity of arboreal mammals and has been isolated from a wound caused by an animal bite.[33]

HISTORY

K. kingae was first isolated in 1960 from blood, joint fluid, bone exudates, and throat swabs by Elizabeth O. King, a bacteriologist at the Centers for Disease Control and Prevention. King's initial studies suggested that this organism was a novel *Moraxella* sp., prompting shipment of samples to Henriksen and Bovre at the University of Oslo, Norway for their opinion.[21] Work by these investigators confirmed King's initial impressions and led to a

REFERENCES

1. Aebischer T, Bumann D, Epple HJ, et al. Correlation of T cell response and bacterial clearance in human volunteers challenged with *Helicobacter pylori* revealed by randomised controlled vaccination with Ty21a- based *Salmonella* vaccines. *Gut* 2008;**57**: 1065–72.
2. Anonymous. Unidentified curved bacilli on gastric epithelium in active chronic gastritis. *Lancet* 1993;**1**:1273–5.
3. Antonescu CG, Marshall BJ. *Helicobacter pylori:* a potentially curable form of peptic ulcer disease. *Gastroenterol J Club* 1990;**2**:3–9, 12.
4. Arenz T, Antos D, Russmann H, et al. Esomeprazole-based 1-week triple therapy directed by susceptibility testing for eradication of *Helicobacter pylori* infection in children. *J Pediatr Gastroenterol Nutr* 2006;**43**:180–4.
5. Ashorn P, Lahde PL, Ruuska T, et al. Gastric lymphoma in an 11 Y/O boy: a case report. *Med Pediatr Oncol* 1994;**22**:66–7.
6. Ayala G, Galvan-Portillo M, Fierros G, et al. Resistance to antibiotics and characterization of *Helicobacter pylori strains* isolated from antrum and body from adults in Mexico. *Microb Drug Resist* 2011;**17**:149–55.
7. Backert. S. Selbach M. Role of type IV secretion in *Helicobacter pylori* pathogenesis. *Cell Microbiol* 2008;**10**:1573–81.
8. Bamford KB, Bickley J, Collins JS. *Helicobacter pylori:* comparison of DNA fingerprints provides evidence for intrafamilial infection. *Gut* 1993;**34**:1348–50.
9. Bauerfiend P, Garner R, Dunn BE, et al. Synthesis of *Helicobacter pylori* urease and catalase at low pH. *Gut* 1997;**40**:25–30.
10. Bell GD. Anti–*Helicobacter pylori* therapy: clearance, elimination, or eradication? *Lancet* 1991;**337**:310–1.
11. Bell GD, Weil J, Garrison G, et al. 14C-urea breath analysis: a noninvasive test for *Campylobacter pylori* in the stomach. *Lancet* 1987;**1**:1367–8.
12. Bizzozero G. Über die schlauchformigen Drusen des magen Darmkanals und die Bezienhungen ihres Epithels zu dem ober Flachenepithel der Schleimhaut. *Arch Mikr Anast* 1893;**42**:82.
13. Blakeley RL, Hinds JA, Kunze HE, et al. Jack bean urease (EC 3.5.1.5): demonstration of a carbamoyl-transfer reaction and inhibition by hydroxamic acids. *Biochemistry* 1969;**8**:1991–2000.
14. Blaser MJ. Gastric *Campylobacter*-like organisms, gastritis and peptic ulcer disease. *Gastroenterology* 1987;**96**:615–25.
15. Blaser MJ. *Helicobacter pylori:* microbiology of a "slow" bacterial infection. *Trends Microbiol* 1993;**1**:255–60.
16. Blaser MJ. The 10 most common questions about *Helicobacter pylori*. *Infect Dis Clin Pract* 1993;**2**:439–40.
17. Borody TJ, Cole P, Noonan S. Recurrence of duodenal ulcer and *Campylobacter pylori* infection after eradication. *Med J Aust* 1989; **151**:431–5.
18. Böttcher A. Zur Genese des perforierenden Magengeschwürs. *Dorpater Med Z* 1874;**5**:148.
19. Brown LM. *Helicobacter pylori:* epidemiology and routes of transmission. *Epidemiol Rev* 2000;**22**:283–97.
20. Brown LM, Thomas TL, Ma JL, et al. *Helicobacter pylori* infection in rural China: exposure to domestic animals during childhood and adulthood. *Scand J Infect Dis* 2001;**33**:686–91.
21. Brown LM, Thomas TL, Ma JL, et al. *Helicobacter pylori* infection in rural China: demographic, lifestyle and environmental factors. *Int J Epidemiol* 2002;**31**:638–46.
22. Bujanover Y, Konikoff F, Baratz M. Nodular gastritis and *Helicobacter pylori*. *J Pediatr Gastroenterol Nutr* 1990;**11**:41–4.
23. Cardenas VM, Prieto-Jimenez CA, Mulla ZD, et al. *Helicobacter pylori* eradication and change in markers of iron stores among non-iron-deficient children in El Paso, Texas: an etiologic intervention study. *J Pediatr Gastroenterol Nutr* 2011;**52**:326–32.
24. Chen M, Lee A, Haze S, et al. Immunization against gastric infection with *Helicobacter* species: first step in the prophylaxis of gastric cancer. *Zentralbl Bakteriol* 1993;**290**:155–65.
25. Chiba N, Rao BV, Rademaker JW. Meta-analysis of the efficacy of antibiotic therapy in eradicating *Helicobacter pylori*. *Am J Gastroenterol* 1992;**87**:1716–20.
26. Choe YH, Kim SK, Hong YC. The relationship between *Helicobacter pylori* infection and iron deficiency: a seroprevalence study in 937 prepubescent children. *Arch Dis Child* 2003;**88**:178.
27. Choe YH, Kim SK, Son BK, et al. Randomized placebo-controlled trial of *Helicobacter pylori* eradication for iron-deficiency anemia in preadolescent children and adolescents. *Helicobacter* 1999;**4**: 135–9.
28. Clayton C, Kleanthous H, Coates PJ, et al. Sensitive detection of *Helicobacter pylori* by using polymerase chain reaction. *J Clin Microbiol* 1992;**30**:192–200.
29. Cohen HA, Shapiro P, Frydman M, et al. Childhood protein-losing enteropathy associated with *Helicobacter pylori* infection. *J Pediatr Gastroenterol Nutr* 1991;**13**:201–3.
30. Correa P, Fox J, Fontham E, et al. *Helicobacter pylori* and gastric carcinoma: serum antibody prevalence in populations with contrasting cancer risks. *Cancer* 1990;**66**:2569–74.
31. Crabtree JE, Mahoney MJ, Taylor JD, et al. Immune responses to *Helicobacter pylori* in children with recurrent abdominal pain. *J Clin Pathol* 1991;**44**:768–73.
32. Crone J, Gold BJ. *Helicobacter pylori* infection in pediatrics. *Helicobacter* 2004;**9**:s49–56.
33. Cullen DJ, Collins BJ, Christiansen KJ, et al. When is *Helicobacter pylori* acquired? *Gut* 1993;**34**:1681–2.
34. Czinn SJ, Carr HS, Speck WT. Diagnosis of gastritis caused by *Helicobacter pylori* in children by means of an ELISA. *Rev Infect Dis* 1991;**13**(suppl. 8):S700.
35. Dariani NE, Mirmomen S, Ghanaei FM, et al. The efficacy of furazolidone-based quadruple therapy for eradication of *Helicobacter pylori* infection in Iranian patients resistant to metronidazole-based quadruple therapy. *Med Sci Monit* 2003;**9**:105–8.
36. De Giacomo C, Lisato L, Negrini R, et al. Serum antibody response to *Helicobacter pylori* in children: epidemiologic and clinical applications. *J Pediatr* 1991;**119**:205–10.
37. De Francesco V, Giorgio F, Hassan C, et al. Worldwide *H. pylori* antibiotic resistance: a systematic review. *J Gastrointestin Liver Dis* 2010;**19**:409–14.
38. Demers B, Karmali M, Sherman P. Seroprevalence of *Helicobacter pylori* IgG antibodies in Canadian children. *Ir J Med Sci* 1992; **161**(suppl. 10):78.
39. Doenges JL. Spirochetes in the gastric glands of Macacas, rhesus, and humans without definite history of related disease. *Proc Soc Exp Med Biol* 1938;**38**:536–8.
40. Dooley CP, Cohen H, Fitzgibbons PL, et al. Prevalence of *Helicobacter pylori* infection and histologic gastritis in asymptomatic persons. *N Engl J Med* 1989;**321**:1562–6.
41. Dore MP, Malaty HM, Graham DY, et al. Risk factors associated with *Helicobacter pylori* infection among children in a defined geographic area. *Clin Infect Dis* 2002;**35**:240–5.
42. Dore MP, Sepulveda AR, El-Zimaity H, et al. Isolation of *Helicobacter pylori* from sheep: implications for transmission to humans. *Am J Gastroenterol* 2001;**96**:1396–401.
43. Dresner D, Coyle W, Nemec R, et al. Efficacy of ciprofloxacin in the eradication of *Helicobacter pylori*. *South Med J* 1996;**89**: 775–8.
44. Drumm B. *Helicobacter pylori* in the pediatric patient. *Gasterol Clin North Am* 1991;**22**:169–82.
45. Drumm B, Day S, Gold B, et al. *Helicobacter pylori* and peptic ulcer: Working Group Report of the Second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2004;**39**:S626–31.
46. Drumm B, Koletzko S, Oderda G, et al. *Helicobacter pylori* infection in children: a consensus statement. *J Pediatr Gastroenterol Nutr* 2000;**30**:207–13.
47. Drumm B, O'Brien A, Cutz E, et al. *Campylobacter pylori*–associated primary gastritis in children. *Pediatrics* 1987;**80**:192–5.
48. Drumm B, Perez-Perez GL, Blaser MJ, et al. Intrafamilial clustering of *Helicobacter pylori* infection. *N Engl J Med* 1990;**322**: 359–63.
49. Drumm B, Sherman P, Cutz E, et al. Rapid diagnosis of *Campylobacter pylori* infection. *Lancet* 1986;**1**:149.
50. Dunn BE. Pathogenic mechanisms of *Helicobacter pylori*. *Gastroenterol Clin North Am* 1993;**22**:43–57.
51. Elitsur Y, Yahav J. *Helicobacter pylori* infection in pediatrics. *Helicobacter* 2005;**10**(suppl. 1):47–53.
52. Elitsur Y, Lawrence Z, Hill I. Stool antigen test for the diagnosis of *Helicobacter pylori* infection in children with symptomatic disease: a prospective study. *J Pediatr Gastroenterol Nutr* 2004;**20**:264–7.
53. Elitsur Y, Toia V, Gilger MA, et al. Urea breath test in children: the U.S. prospective, multicenter study. *Helicobacter* 2009;**14**: 134–40.

54. European Study Group on Antibiotic Susceptibility of *Helicobacter pylori:* results of a multicentre European survey in 1991 of metronidazole resistance in *Helicobacter pylori*. *Eur J Clin Microbiol Infect Dis* 1992;**11**:777–81.
55. Evans DJ, Evans DE, Graham DY, et al. A sensitive and specific serology test for detection of *Campylobacter pylori* infection. *Gastroenterology* 1989;**96**:1004–8.
56. Fakheri H, Merat S, Hosseini V, et al. Low-dose furazolidone in triple and quadruple regimens for *Helicobacter pylori* eradication. *Aliment Pharmacol Ther* 2004;**1**:89–93.
57. Farrell S, Doherty GM, Milliken I, et al. Risk factors for *Helicobacter pylori* infection in children: an examination of the role played by intrafamilial bed sharing. *Pediatr Infect Dis J* 2005;**24**:149–52.
58. Farrell S, Milliken I, Doherty GM, et al. Total family unit *Helicobacter pylori* eradication and pediatric re-infection rates. *Helicobacter* 2004;**9**:285–8.
59. Feydt-Schmidt A, Kindermann A, Konstantopoulos N, et al. Reinfection rate in children after successful *Helicobacter pylori* eradication. *Eur J Gastroenterol Hepatol* 2002;**14**:1119–23.
60. Fiedorak SC, Malaty HM, Evans DL, et al. Factors influencing the epidemiology of *Helicobacter pylori* infection in children. *Pediatrics* 1991;**88**:578–82.
61. Fiorini G, Zullo A, Gatta L, et al. Newer agents for *Helicobacter pylori* eradication. *Clin Experimental Gastroenterol* 2012;**5**: 109–12.
62. Fischbach L, Evans EL. Meta-analysis: the effect of antibiotic resistance status on the efficacy of triple and quadruple first-line therapies for *Helicobacter pylori*. *Aliment Pharmacol Ther* 20071;**26**: 343–57.
63. Forman D. The prevalence of *Helicobacter pylori* infection in gastric cancer. *Aliment Pharmacol Ther* 1995;**9**:s71–6.
64. Forsmark CE, Wilcox CM, Cello JP, et al. Ciprofloxacin in the treatment of *Helicobacter pylori* in patients with gastritis and peptic ulcer. *J Infect Dis* 1990;**162**:998–9.
65. Francavilla R, Lionetti E, Castellaneta S, et al. Clarithromycin resistant genotypes and eradication of *Helicobacter pylori*. *J Pediatr* 2010;**157**:228–32.
66. Freedburg AS, Barron LE. The presence of spirochetes in human gastric mucosa. *Am J Dig Dis* 1940;**7**:443–5.
67. Gatta L, Vakil N, Leandro G, et al. Sequential therapy or triple therapy for *Helicobacter pylori* infection: systematic review and meta-analysis of randomized controlled trials in adults and children. *Am J Gastroenterol* 2009;**104**:3069–79.
68. Genta RM, Robason GO, Graham DY. Simultaneous visualization of *Helicobacter pylori* and gastric morphology: a new stain. *Hum Pathol* 1994;**25**:221–6.
69. Gessner BD, Bruce MG, Parkinson AJ, et al. A randomized trial of triple therapy for pediatric *Helicobacter pylori* infection and risk factors for treatment failure in a population with a high prevalence of infection. *Clin Infect Dis* 2005;**41**:1261–8.
70. Gilger MA, Tolia V, Johnson A, et al. The use of an oral fluid immunoglobulin G ELISA for the detection of *Helicobacter pylori* infection in children. *Helicobacter* 2003;**7**:105.
71. Gisbert J, Calvet X, O'Connor A, et al. Sequential therapy for *Helicobacter pylori* eradication: a critical review. *J Clin Gastroenterol* 2010;**44**:313–25.
72. Glassman M, Schwartz S, Medow M, et al. *Campylobacter pylori*–related gastrointestinal disease in children: incidence and clinical findings. *Dig Dis Sci* 1989;**34**:1501–4.
73. Glassman MS. *Helicobacter pylori* infection in children: a clinical overview. *Clin Pediatr* 1992;**31**(suppl.):481–7.
74. Gold BD, Colletti RB, Abbott M, et al. *Helicobacter pylori* infection in children: recommendations for diagnosis and treatment. *J Pediatr Gastroenterol Nutr* 2000;**31**:490–7.
75. Gold BD, Kruzon-Moran D, Sobel J, et al. Decreasing seroprevalence of *Helicobacter pylori* infection in US children aged 6-19 years. *J Pediatr Gastroenterol Nutr* 2003;**37**:360.
76. Gorin G. On the mechanism of urease action. *Biochim Biophys Acta* 1959;**34**:268–9.
77. Gormally S, Drumm B. *Helicobacter pylori* and gastrointestinal symptoms. *Arch Dis Child* 1994;**70**:165–6.
78. Gormally S, Sherman P, Drumm B. Clinical syndromes of *Helicobacter pylori* infection in children. In: Goodwin CS, editor. *Helicobacter pylori: biology and clinical practice*. Boca Raton, Fla: CRC Press; 1993. p. 85.
79. Gormally SM, Prakash N, Durnin MT, et al. Association of symptoms with *Helicobacter pylori* infection in children. *J Pediatr* 1995; **126**:753–6.
80. Graham DY. *Helicobacter today*. Attrincham, Cheshire, UK: Norris Communications; 1995. p. 5.
81. Graham DY, Blaser MJ, Soll AH. *Helicobacter pylori: pathophysiology and epidemiology*. Deerfield, Ill: Discovery International; 1995. p. 9.
82. Graham DY, Adam E, Reddy GT, et al. Seroepidemiology of *Helicobacter pylori* infection in India: comparison of developing and developed countries. *Dig Dis Sci* 1991;**36**:1084–8.
83. Graham DY, Go M, Evans D Jr. Urease, gastric ammonium/ ammonia and *Helicobacter pylori:* the past, the present, and recommendations for future research. *Aliment Pharmacol Ther* 1992;**6**: 659–69.
84. Graham DY, Klein PD, Evans DG Jr, et al. *Campylobacter pylori* detected noninvasively by the 13C-urea breath test. *Lancet* 1987;**2**: 1174–7.
85. Graham DY, Klein PD, Evans DG Jr, et al. *Helicobacter pylori:* epidemiology, relationship to gastric cancer and the role of infants in transmission. *Eur J Gastroenterol Hepatol* 1992;**4**(suppl. 1): S1–6.
86. Graham DY, Malaty HM, Evans DG, et al. Epidemiology of *Helicobacter pylori* in an asymptomatic population in the United States: effect of age, race and socioeconomic status. *Gastroenterology* 1991;**100**:1495–501.
87. Grayson ML, Eliopoulos GM, Ferraro MJ, et al. Effect of varying pH on the susceptibility of *Campylobacter pylori* to antimicrobial agents. *Eur J Clin Microbiol Infect Dis* 1989;**8**:888–9.
88. Guo CY, Wu YB, Liu HL, et al. Clinical evaluation of four one-week triple therapy regimens in eradicating *Helicobacter pylori* infection. *World J Gastroenterol* 2004;**10**:747–9.
89. Hardikar W, Davidson PM, Cameron DJ, et al. *Helicobacter pylori* infection in children. *J Gastroenterol Hepatol* 1991;**6**:450–4.
90. Hassell E, Dimmick JE. Unique features of *Helicobacter pylori* disease in children. *Dig Dis Sci* 1991;**36**:417–23.
91. Hazell SL. *H. pylori* in developing countries. In: Hunt RH, Tygat GNJ, editors. *Helicobacter pylori: basic mechanisms to clinical cure*. Dordrecht, Netherlands: Kluwer Academic; 1994. p. 85–94.
92. Hazell SL, Lee A. *Campylobacter pyloridis*, urease hydrogen ion back diffusion and gastric ulcers. *Lancet* 1986;**2**:15–7.
93. Herbarth O, Krmbiegel P, Fritz GJ. *Helicobacter pylori* prevalences and risk factors among school beginners in a German urban center and its rural county. Environ. *Health Perspect* 2001;**109**:573–7.
94. Hill ID, Sinclair-Smith C, Lastorica AJ, et al. Transient protein enteropathy associated with acute gastritis and *Campylobacter pylori* gastritis. *Arch Dis Child* 1987;**62**:1215–9.
95. Holcombe C, Tsimuri S, Eldridge J, et al. Prevalence of antibody to *Helicobacter pylori* in children in northern Nigeria. *Trans R Soc Trop Med Hyg* 1994;**87**:19–21.
96. Husson MO, Gottrand F, Truck D. et al. Detection of *H. pylori* in saliva using a monoclonal antibody. *Zentralbl Bakteriol* 1993;**279**: 466–71.
97. Isaacson PG. Gastric lymphoma and *Helicobacter pylori*. *N Engl J Med* 1994;**330**:1310–1.
98. Israel DM, Hassall E. Treatment and long-term follow up of *Helicobacter pylori*–associated duodenal ulcer disease in children. *J Pediatr* 1993;**123**:53–9.
99. Jafri N, Hornburg A, Howden C. Meta-analysis: sequential therapy appears superior to standard therapy for *Helicobacter pylori* infection in patients naive. *Ann Intern Med* 2008;**148**(12): 923–3.
100. Jicong W, Guolong L, Zhentua Z, et al. 15NH4+ excretion test: a new method for detection of *Helicobacter pylori* infection. *J Clin Microbiol* 1992;**30**:181–4.
101. Jones NL, Sherman P, Fallone CA, et al. Canadian Helicobacter Study Group consensus conference: update on the approach to *Helicobacter pylori* infection if children and adolescents—an evidence-based evaluation. *Can J Gastroenterol* 2005;**19**:399–408.
102. Kasai K, Kobayashi R. Stomach spirochetes occurring in mammals. *J Parasitol* 1919;**6**:1–11.
103. Kato S, Abukawa D, Furuyama N, et al. *Helicobacter pylori* reinfection rates in children after eradication therapy. *J Pediatr Gastroenterol Nutr* 1998;**27**:54–546.

104. Kato S, Nakayama K, Minoura T, et al. Comparison between the 13C-urea breath test and stool antigen test for the diagnosis of childhood *Helicobacter pylori* infection. *J Gastroenterol* 2004;**39**: 1045–50.
105. Kilbridge PM, Dahms BB, Czinn SJ. *Campylobacter pylori*–associated gastritis and peptic ulcer disease in children. *Am J Dis Child* 1988;**142**:1149–52.
106. Koletzko S, Jones N, Goodman K, et al. Evidence-based guidelines from ESPGHAN and NASPGHAN for *Helicobacter pylori* infection in children. *J Pediatr Gastroenterol Nutr* 2011;**53**: 230–43.
107. Koletzko S, Richy F, Bontems P, et al. Prospective multicentre study on antibiotic resistance of *Helicobacter pylori* strains obtained from children living in Europe. *Gut* 2006;**55**:1711–6.
108. Kradjen S, Sherman P. *Helicobacter* (*Campylobacter*) *pylori* and acid peptic diseases. *Can J Gastroenterol* 1990;**4**:237–41.
109. Kradjen S, Fuska M, Anderson J, et al. Examination of human stomach biopsies, saliva and dental plaque for *Campylobacter pylori*. *J Clin Microbiol* 1989;**27**:1397–8.
110. Kusters JG, Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* 2006;**19**:449–90.
111. Lee A. The microbiology and epidemiology of *Helicobacter pylori* infection. *Scand J Gastroenterol* 1994;**29**(suppl. 201):2–6.
112. Lee A, Mitchell H. Basic bacteriology of *H. pylori: H. pylori* colonization factors. In: Hunt RH, Tygat, GNJ, editors. *Helicobacter pylori: Basic Mechanisms to Clinical Cure*. Dordrecht, Netherlands: Kluwer Academic; 1994. p. 59–72.
113. Lee A, Fox J, Hazell S. Pathology of *Helicobacter pylori:* a perspective. *Infect Immun* 1993;**61**:1601–10.
114. Leung WK, Lin SR, Ching JY, et al. Factors predicting progression of gastric intestinal metaplasia: results of a randomized trial on *Helicobacter pylori* infection. *Gut* 2004;**53**:1244–9.
115. Leunk RD, Johnson PT, David BC, et al. Cytoxic activity in broth-culture filtrates of *Campylobacter pylori*. *J Med Microbiol* 1988;**26**: 93–9.
116. Levine TS, Price AB. *Helicobacter pylori:* enough to give anyone an ulcer. *Br J Clin Pract* 1993;**47**:328–32.
117. Lior H, Johnson WM. Catalase, peroxidase and superoxide dismutase activities in *Campylobacter* spp. In: Pearson AD, Skirrow MB, Lior H, et al, editors. *Campylobacter III*. London: Health Laboratory Service; 1985. p. 226–7.
118. Losonsky GA, Kotloff KL, Walker RI. B-cell responses in gastric antrum and duodenum following oral inactivated *Helicobacter pylori* whole cell (HWC) vaccine and LT(R192G) in *H. pylori* seronegative individuals. *Vaccine* 2003;**21**.562–5.
119. Lu Y, Redlinger TE, Avitia R. Isolation and genotyping of *Helicobacter pylori* from untreated municipal wastewater. *Appl Environ Microbiol* 2002;**68**:1436–9.
120. Luger A, Neuberger HZ. Über Spirochätenbefunde im Magensaft und deren diagnostische Bedeutung für des Carcinoma ventriculi. *Klin Med* 1921;**92**:54–75.
121. Luzza F, Oderda G, Maletta M, et al. Salivary immunoglobulin G assay to diagnose Helicobacter pylori infection in children. *J Clin Microbiol* 1997;**35**:3358–60.
122. Macarthur C. *Helicobacter pylori* infection and childhood recurrent abdominal pain: lack of evidence for a causal relationship. *Can J Gastroenterol* 1999;**13**:607.
123. Macarthur C, Saunders N, Feldman W. *Helicobacter pylori*, gastroduodenal disease and recurrent abdominal pain in children. *JAMA* 1995;**273**:729–34.
124. Maden E, Kemp J, Westblom TU, et al. Evaluation of staining methodologies for identifying *Campylobacter pylori*. *Am J Clin Pathol* 1988;**90**:450–3.
125. Magista AM, Ierardi E, Castellaneta S, et al. *Helicobacter pylori* status and symptom assessment two years after eradication in pediatric patients from a high prevalence area. *J Pediatr Gastroenterol Nutr* 2005;**40**:312–8.
126. Malaty H, Graham D, Klein P, et al. Transmission of *Helicobacter pylori* infection: studies in families of healthy individuals. *Scand J Gastroenterol* 1991;**26**:927–32.
127. Malcolm CA, MacKay WG, Weaver LT. *Helicobacter pylori* in children is strongly associated with poverty. *Scott Med J* 2004;**49**: 136–8.
128. Malfertheiner P, Schultze V, Rosenkranz B, et al. Safety and immunogenicity of an intramuscular *Helicobacter pylori* vaccine in noninfected volunteers: a Phase I study. *Gastroenterology* 2008;**135**: 787–95.
129. Marshall BJ. Epidemiology of *H. pylori* in Western countries. In: Hunt RH, Tygat GNJ, editors. *Helicobacter pylori: basic mechanisms to clinical cure*. Dordrecht, Netherlands: Kluwer Academic; 1995. p. 75–84.
130. Marshall BJ, Goodwin CS, Warren JR. Prospective double-blind trial of duodenal ulcer relapse after eradication of *Campylobacter pylori*. *Lancet* 1988;**2**:1437–42.
131. Marshall BJ, Armstrong JA, McGechie DB, et al. Attempt to fulfill Koch's postulates for pyloric *Campylobacter*. *Med J Aust* 1985;**125**: 436–44.
132. Marshall BJ, Royce H, Annear DI, et al. Original isolation of *Campylobacter pyloridis* from human gastric mucosa. *Microbios* 1984;**25**:83–8.
133. McNutty CAM, Wise R. Rapid diagnosis of *Campylobacter*-associated gastritis. *Lancet* 1985;**1**:1443–4.
134. Megraud F. Epidemiology of *Helicobacter pylori* infection. *Gastroenterol Clin North Am* 1993;**22**:73–88.
135. Megraud R, Bonnet F, Garnier M, et al. Characterization of "*Campylobacter pyloridis*" by culture, enzymatic profile, and protein content. *J Clin Microbiol* 1985;**22**:1007–10.
136. Mendall M. Natural history and mode of transmission. In: Northfield T, Mendall M, Goggin P, editors. *Helicobacter pylori infection: pathophysiology, epidemiology and management*. Boston: Kluwer Academic; 1993. p. 21–3.
137. Mera R, Fontham ET, Bravo LE, et al. Long-term follow up of patients treated for *Helicobacter pylori* infection. *Gut* 2005;**54**: 1536–40.
138. Morris A, Nicholson G. Ingestion of *Campylobacter pyloridis* causes gastritis and raised fasting gastric pH. *Am J Gastroenterol* 1987;**32**: 192–9.
139. Muhlens PZ. Vergleichende spirochetenstudien. *Z Hyg Infekt* 1907;**57**:405–16.
140. Muller A, Solnick JV. Inflammation, immunity, and vaccine development for *Helicobacter pylori*. *Helicobacter* 2011;**16**(suppl. 1): 26–32.
141. Murata-Kamiya N, Kurashima Y, Teishikata Y, et al. *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the b-catenin signal that promotes intestinal. transdifferentiation in gastric epithelial cells. *Oncogene* 2007;**26**:4617–26.
142. Ndip RN, Malange AE, Akoachere JF, et al. *Helicobacter pylori* antigens in the faeces of asymptomatic children in the Buea and Limbe health districts of Cameroon: a pilot study. *Trop Med Int Health* 2004;**9**:1036–40.
143. Odera G, Scherbakv P, Bontes P, et al. European Pediatric Task Force on *Helicobacter pylori*: results from the European Register for Treatment of the *Helicobacter pylori*. *Helicobacter* 2007;**12**: 150–6.
144. Oderda G, Dell'Olio D, Travassoli K. *Campylobacter pylori* gastritis and peptic ulcer disease in children. *Am J Dis Child* 1989;**143**:877.
145. Oderda G, Vaira D, Ainley C. Eighteen-month follow-up of *Helicobacter pylori*–positive children treated with amoxicillin and tinidazole. *Gut* 1992;**33**:1328–30.
146. Oderda G, Vaira D, Holton J, et al. *Helicobacter pylori* in children with peptic ulcer and their families. *Dig Dis Sci* 1991;**36**:572–6.
147. Opekun AR, Luu P, Gotschall AB, et al. Point-of-care Helicobacter pylori urine antibody detection in a multi-ethnic adult population in the United States. *Translational Research* 2006;**148**: 13–8.
148. Ormand JE, Talley NJ. *Helicobacter pylori:* controversies and an approach to management. *Mayo Clin Proc* 1990;**65**:414–26.
149. Ozen A, Ertem D, Pehlivanoglu E. Natural history and symptomatology of *Helicobacter pylori* in childhood and factors determining the epidemiology of infection. *J Pediatr Gastroenterol Nutr* 2006; **42**:398–404.
150. Palmer ED. Investigation of the gastric mucosa spirochetes of the human. *Gastroenterology* 1954;**27**:218–20.
151. Parsonnet J. Cost-effectiveness of a potential prophylactic *Helicobacter pylori* vaccine in the United States. *J Infect Dis* 2009;**200**: 1311–7.
152. Parsonnet J, Friedman GD, Vandersteen DP. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**:1127–31.

153. Penston JG. *Helicobacter pylori* eradication: understandable caution but no excuse for inertia. *Aliment Pharmacol Ther* 1994;**8**: 369–89.
154. Perez GI, Rothenbacher D, Brenner H. Epidemiology of *Helicobacter pylori* infection. *Helicobacter* 2004;**9**:1–6.
155. Perri F, Festa V, Andriulli A. Treatment of antibiotic-resistant *Helicobacter pylori* infection. *N Engl J Med* 1998;**339**:53.
156. Perri F, Festa V, Clemente R. Rifabutin-based "rescue therapy" for *Helicobacter pylori* infected patients after failure of standard regimens. *Aliment Pharmacol Ther* 2000;**14**:311–6.
157. Peterson W. *Helicobacter pylori* and peptic ulcer disease. *N Engl J Med* 1991;**324**:1043–8.
158. Pezzi JS, Shiau YF. *Helicobacter pylori* and gastrointestinal disease. *Am Fam Physician* 1995;**52**:1717–24.
159. Pounder RE, Ng D. The prevalence of *Helicobacter pylori* infection in different countries. *Aliment Pharmacol Ther* 1995;**9**:33–19.
160. Prieto G, Polanco I, Larrauri J, et al. *Helicobacter pylori* infection in children: clinical endoscopic and histologic correlations. *J Pediatr Gastroenterol Nutr* 1992;**14**:420–5.
161. Rauws EAJ, Tygat GNJ. Cure of duodenal ulcer associated with eradication of *Helicobacter pylori*. *Lancet* 1990;**335**:1233–9.
162. Raymond J, Bergeret M, Benhamou H, et al. A two-year study of *Helicobacter pylori* in children. *J Clin Microbiol* 1994;**32**:461–3.
163. Reifen R, Rasooly I, Drumm B, et al. *Helicobacter pylori* infection in children: is there specific symptomatology? *Dig Dis Sci* 1994;**39**:1488–92.
164. Rosioru C, Glassman MS, Berezin SH, et al. Treatment of *Helicobacter pylori*–associated gastroduodenal disease in children: clinical evaluation of antisecretory vs. antibacterial therapy. *Dig Dis Sci* 1993;**38**:123–8.
165. Rothenbacher D, Inceoglu J, Brenner H. Acquisition of Helicobacter pylori infection in a high risk population occurs within the first 2 years of life. *J Pediatr* 2000;**136**:744–8.
166. Rothenbacher D, Winkler M, Gonser T, et al. History of breastfeeding and *Helicobacter pylori* infection in preschool children: result of a population-based study from Germany. *Int J Epidemiol* 2002;**31**:632–7.
167. Rowland M, Kumar D, Daly D, et al. Low rate of *Helicobacter pylori* reinfection in children. *Gastroenterology* 1998;**117**:336–41.
168. Ruggiero P. *Helicobacter pylori* infection: what's new. *Curr Opin Infect Dis* 2012;**25**:337–44.
169. Sanaka M, Kuyama Y, Yamanaka M. Decrease in serum concentrations of *Helicobacter pylori* IgG antibodies during antituberculosis therapy: the possible eradication by rifampicin and streptomycin. *Am J Gastroenterol* 1999;**94**:1983–4.
170. Sarosiek J, Slomiany A, Van Horn K, et al. Lipolytic activity of *Campylobacter pylori:* effect of sofalcone. *Gastroenterology* 1988;**94**: A399.
171. Schwartz K. Über penbtrierende magenund jejunal-geschwüre. *Beltr Ucin Chir* 1910;**67**:96–128.
172. Sherman P. Peptic ulcer disease in children: diagnosis, treatment and the implication of *Helicobacter pylori*. *Gastroenterol Clin North Am* 1994;**23**:707–25.
173. Slomiany BL, Bliski J, Sarosiek J, et al. *Campylobacter pyloridis* degrades mucin and undermines gastric mucosal integrity. *Biochem Biophys Res Commun* 1987;**144**:307–14.
174. Smoak BL, Kelley PW, Taylor DN. Seroprevalence of *Helicobacter pylori* infections in a cohort of U.S. Army recruits. *Am J Epidemiol* 1994;**139**:513–9.
175. Solomon H. Über das Spirilllim des Saugetiermagens und sein Verhalten zu den Belegzelen. *Centrallbl Bakt* 1896;**19**:433.
176. Steer HW. Ultrastructure of cell migration through the gastric epithelium and its relationship to bacteria. *J Clin Pathol* 1975;**28**: 639–46.
177. Street M, Cellini L, Di Campli E, et al. Antibiotic resistance and antibiotic sensitivity based treatment in *Helicobacter pylori* infection: advantages and outcome. *Arch Dis Child* 2001;**84**: 419–22.
178. Sutton P. Vaccinating against *Helicobacter pylori:* dissecting the mechanism. *Gastoenterology* 2011;**141**:1149–51.
179. Take S, Mizuno M, Ishiki K, et al. The effect of eradicating *Helicobacter pylori* on the development of gastric cancer in patients with peptic ulcer disease. *Am J Gastroenterol* 2005;**100**: 1037–42.
180. Talley MJ. Epidemiology of *Helicobacter pylori* infection. Presented at the National Institutes of Health Consensus Development Conference on *Helicobacter pylori* in Peptic Ulcer Disease, Bethesda, Md, 1994, p. 7–9.
181. Taylor D, Blaser MJ. The epidemiology of *Helicobacter pylori* infection. *Epidemiol Rev* 1991;**13**:42–B59.
182. Thomas JE, Gibson GR, Darboe MK, et al. Isolation of *Helicobacter pylori* from human faeces. *Lancet* 1992;**340**:1194–5.
183. Thomas JE, Whatmore AM, Barer MR, et al. Serodiagnosis of *Helicobacter pylori* infection in childhood. *J Clin Microbiol* 1990;**28**: 2641–5.
184. Tolia V. *Helicobacter pylori* in pediatric nonulcer dyspepsia: pathogen or commensal. *Am J Gastroenterol* 1995;**90**:865–8.
185. Tygat GNJ Treatments that impact favorably upon the eradication of *Helicobacter pylori* and ulcer recurrence. *Aliment Pharmacol Ther* 1994;**8**:359–68.
186. Uemura N. The magnitude of association between *Helicobacter pylori* infection and the development of gastric cancer. *Scand J Gastroenterol* 2002;**37**:869–70.
187. Uemura N, Okamoto S, Yamamoto S, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;**345**:784.
188. Vakil N. *Helicobacter pylori* treatment: a practical approach. *Am J Gastroenterol* 2006;**101**: 497–9.
189. Vakil N, Megraud F. Reviews in basic and clinical gastroenterology. *Gastroenterology* 2007;**133**:985–1001.
190. Velin D, Michetti P. Advances in vaccination against *Helicobacter pylori*. *Expert Rev Gastroenterol Hepatol* 2010;**4**:157–66.
191. Velin D, Narayan S, Bernasconi E, et al. PAR2 promotes vaccine-induced protection against *Helicobacter* infection in mice. *Gastroenterology* 2011;**141**:1273–82.
192. Vincent P, Gottand F, Pernes P, et al. High prevalence of *Helicobacter pylori* infection in cohabiting children: epidemiology of a cluster with special emphasis on molecular typing. *Gut* 1994;**35**: 313–6.
193. Wewer V, Kalach N. *Helicobacter pylori* infection in pediatrics. *Helicobacter* 2003;**8**:s61–7.
194. Wong BC, Lam SK, Wong WM. *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China. *JAMA* 2004;**291**:187–94.
195. Wotherspoon AC, Doglioni E, Diss TC. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 1993;**342**:575–8.
196. Xiao SD, Liu WZ, Xia DH, et al. The efficacy of furazolidone and metronidazole in the treatment of chronic gastritis associated with *Helicobacter* (*Campylobacter*) *pylori*—a randomized double-blind placebo-controlled clinical trial. *Hepatogastroenterology* 1990;**37**:503–6.
197. Yeung CK, Fu KH, Yeun KY. *Helicobacter pylori* and associated duodenal ulcer. *Arch Dis Child* 1990;**65**:1212–5.
198. Yip R, Limburg PJ, Ahlquist DA, et al. Pervasive occult gastrointestinal bleeding in an Alaska Native population with prevalent iron deficiency: role of *Helicobacter pylori* gastritis. *J A M A* 1997; **277**:1135–9.
199. Zullo A, De Francesco V, Hassan C, et al. The sequential therapy regimen for *Helicobacter pylori* eradication: a pooled-data analysis. *Gut* 2007;**56**:1353–7.

publication describing the new species, which they named *Moraxella kingii* in honor of King.[21] Additional studies in the late 1960s and 1970s established significant differences between *M. kingii* and other members of the *Moraxella* genus and resulted in reclassification as a novel genus and renaming as *Kingella kingae* in 1976.[5,22]

Until the 1990s, *K. kingae* was largely ignored as a human pathogen because of its infrequent recovery in association with disease, generally in young patients with septic arthritis, osteomyelitis, or endocarditis.* As novel culture-based isolation techniques were developed in the early 1990s,[62] the frequency of reported infections caused by *K. kingae* increased. Numerous studies using PCR-based diagnostic techniques have shown that *K. kingae* is present in a significant percentage of culture-negative joint fluid and bone samples from patients with suspected osteoarticular infection.† Recent evidence indicates that *K. kingae* is the leading cause of septic arthritis in children 6 to 36 months of age, more common even than *Staphylococcus aureus*.[14]

MICROBIOLOGY

K. kingae is a gram-negative bacterium that belongs to the *Neisseriaceae* family and is only distantly related to other members of this family. It is a facultative anaerobic, β-hemolytic, small bacillus (0.6 to 1 μm × 1 to 3 μm) that appears as pairs or short chains with tapered ends and often resists decolorization, sometimes resulting in misidentification as a gram-positive organism.[21,55] *K. kingae* produces acid from glucose and maltose but not other sugars and is oxidase-positive and catalase-, urease-, and indole-negative. Growth is supported on blood and chocolate agar but not on Krigler or MacConkey agar. Optimal growth occurs at 37° C (98.6° F) in a 5 to 10 percent carbon dioxide–enhanced atmosphere, but few strains are truly capnophilic.[55] Colonies from growth on solid agar appear as three distinct types: spreading/corroding, nonspreading/noncorroding, and domed.[25,28,55] The spreading/corroding colony type produces marked indentations on the agar surface, develops a peripheral fringe around the small central raised colony, and is associated with high-density piliation. The nonspreading/noncorroding colony has a smaller fringe with a large, flat, central colony and is associated with low-density piliation. The domed colony produces no fringe and is nonpiliated.[28] *K. kingae* is most easily differentiated from *K. denitrificans*, *K. oralis*, *K. potus*, and *Neisseria* spp. by a distinct narrow zone of β-hemolysis surrounding colonies on blood agar plates.

To date, *K. kingae* has never been isolated from a nonhuman source, suggesting that the organism is human specific.

EPIDEMIOLOGY

Carriage and Transmission

K. kingae is a human commensal that colonizes the posterior pharynx asymptomatically in a subset of the population and occasionally invades the bloodstream to produce invasive disease. Using a novel vancomycin-containing medium developed in the early 1990s for isolation of *K. kingae* from the pharynx,[62] several studies examined carriage rates in the healthy population.[10,55,59,63,66] Based on studies conducted primarily in Israel and Europe, colonization with *K. kingae* begins around 6 months of age and increases to a rate of 9 to 12 percent in children 12 to 24 months of age.[10,59,63] The carriage rate gradually decreases in older children and adults, suggesting that immune system development in healthy individuals may be responsible for upper respiratory tract clearance.[59,63]

To achieve a better understanding of the transmission of *K. kingae* strains circulating in the community, pulsefield gel electrophoresis genotyping studies have been performed.[63] In one study, upper respiratory tract isolates from a cohort of healthy individuals were compared to a set of invasive isolates over a 15-year period.[63] Interestingly, this analysis revealed clustering of genotypically indistinguishable strains in geographic regions and households, suggesting person-to-person transmission among family members and close contacts. In addition, many strains circulating among healthy individuals matched historical strains recovered from sites of invasive disease, indicating that certain strains may persist in the population.[63]

In a recent study, Yagupsky and colleagues[64] examined paired isolates from the pharynx and bloodstream in three children with *K. kingae* bacteremia and found that the pharyngeal and blood isolates were genotypically identical in each case, providing evidence that colonization of the posterior pharynx is a precursor to invasive disease. Similarly, Basmaci and associates[1] reported isolation of *K. kingae* from the pharynx in children with confirmed *K. kingae* septic arthritis.

Invasive Disease

The most comprehensive study to date on the epidemiology of invasive disease was a multicenter study in Israel that examined a total of 321 cases of *K. kingae* disease.[17] In this report, 296 of the 312 pediatric cases involved children younger than 4 years of age, representing an annual incidence of 9.4/100,000 children birth to 4 years of age. Of note, only 4 cases involved children younger than 6 months of age.[17] Children younger than 4 years of age diagnosed with *K. kingae* disease were generally otherwise healthy,[17] whereas older children and adults who developed *K. kingae* disease usually had underlying predisposing conditions, including malignancy, immunosuppression, or cardiac valve pathological processes. The 321 total cases included 140 cases of septic arthritis, 19 cases of osteomyelitis, 7 cases of osteomyelitis with septic arthritis of the adjacent joint, and 3 cases of tenosynovitis, for a total 169 skeletal infections (53%/321). Among the remaining cases were 140 cases of bacteremia without a focus (44%/321), 4 cases of with bacteremia and lower respiratory tract infection, and 8 cases of endocarditis.[17] Other reports have described *K. kingae* as a rare etiology of pericarditis,[35] meningitis,[50] peritonitis,[4] urinary tract infection,[40] and ocular infection.[6]

*References 15, 16, 29, 32, 37, 41, 45, 49, 53.
†References 7, 8, 12, 14, 23, 36, 48, 51, 54.

In Israel, invasive *K. kingae* disease occurs throughout the year and is most common during November and December and least common between February and April.[17,18] In contrast, the occurrence of carriage is more uniform throughout the year, suggesting that the seasonal distribution of invasive *K. kingae* disease cannot be readily explained by the epidemiology of pharyngeal carriage. At the time of presentation with *K. kingae* disease, approximately 50 percent of children display symptoms of upper respiratory tract infection, 15 to 20 percent have stomatitis, and 15 to 20 percent have diarrhea, raising the possibility that concomitant viral infection contributes to development of invasive disease.[63]

Daycare Facility Attendance

A longitudinal study in Israel in the mid-1990s aimed to evaluate the transmission of *K. kingae* strains among daycare facility attendees.[47] Fifty isolates were collected over an 11-month period by sampling two cohorts of daycare facility attendees every 2 weeks. These isolates were compared to a collection of 60 epidemiologically diverse respiratory tract and invasive isolates. Based on a variety of typing techniques, children were found to routinely carry the same strain continuously or intermittently for weeks or months. Different strains were able to periodically replace the originally detected strain. Interestingly, a single strain represented 28 percent of the isolates and was most prevalent over the first 6 months of the study and a different strain represented 46 percent of the isolates over the remaining 5 months.[47] Epidemiologically unrelated strains showed much greater variability in relatedness than isolates from the daycare facility cohorts.

To date, three reports of daycare facility outbreaks of invasive *K. kingae* disease have been described.[30,44,60] In Minnesota in 2003, three cases of osteomyelitis/septic arthritis in children from the same classroom of 16- to 24-month olds were diagnosed.[30] Carriage analysis of other attendees of the same facility revealed an overall colonization rate of 13 percent (15/122) and a peak colonization rate of 45 percent (9/20) in the classroom of 16- to 24-month-olds. All three children with invasive disease had concurrent or antecedent upper respiratory tract illness. In Israel in 2005, one confirmed and two suspected cases of *K. kingae* skeletal infections were diagnosed in children from the same daycare facility.[60] Evaluation of the other children at the same facility established that 4/11 were colonized. Pulse-field gel electrophoresis demonstrated that the pharyngeal and invasive isolates from the source daycare facility were genotypically identical and pharyngeal isolates from a control facility where genotypically distinct.[60] Finally, in North Carolina in 2007, a case of confirmed *K. kingae* endocarditis and potential meningitis and a case of suspected intervertebral discitis and osteomyelitis were identified in a 2-week period in children who attended the same daycare facility.[44]

PATHOGENESIS AND IMMUNITY

The pathogenesis of disease caused by *K. kingae* is believed to begin with colonization of the posterior pharynx.[55,65]

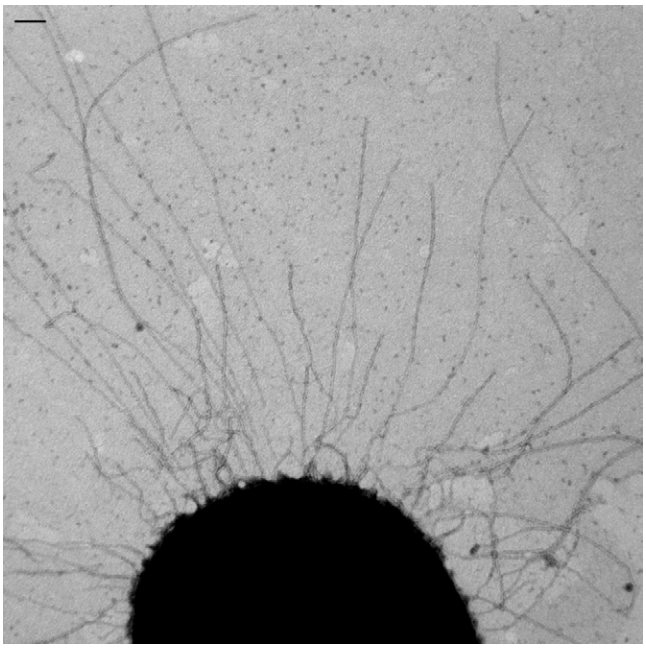

FIGURE 136-1 ■ Transmission electron micrograph of type IV pili emanating from the surface of *K. kingae* strain 269-492, a septic arthritis isolate. The sample was negatively stained with uranyl acetate. Scale bar = 100 nm.

The process of colonization likely involves adherence to respiratory epithelial cells, which is mediated in vitro in part by polymeric surface pili (Fig. 136-1).[26-28] *K. kingae* pili are classified as type IV pili and are composed primarily of a major pilin subunit, PilA1.[26] In addition, *K. kingae* pili contain two pilus-associated proteins, PilC1 and PilC2, that appear to play complementary roles in promoting pilus assembly and in mediating adherence to respiratory epithelial cells.[26] Consistent with the conclusion that pili promote *K. kingae* colonization, examination of a collection of pharyngeal isolates of *K. kingae* demonstrated that the majority are piliated and express abundant pili.[28]

K. kingae also produces a nonpilus adhesive protein, Knh, which is essential for full-level adherence to cultured respiratory epithelial cells.[39] Knh shares homology with the *N. meningitidis* NhhA adhesin and belongs to the trimeric autotransporter family of proteins. Like other trimeric autotransporters, Knh has an N-terminal signal sequence, a characteristic C terminus, and a modular internal passenger domain that is ultimately surface localized. The passenger domain contains a series of 19 YadA-like head domains with similarity to the β-roll that has been implicated in the adhesive activity of the *Yersinia* YadA trimeric autotransporter and a stalk that contains 7 Trp ring domains, 3 neck domains, 3 KG domains, and an iSneck2 domain. Disruption of the gene encoding Knh results in an approximately 50 percent decrease in adherence in in vitro assays.[39]

After colonization of the posterior pharynx, *K. kingae* must translocate across the pharyngeal epithelium to enter the bloodstream. Given that patients with invasive *K. kingae* disease frequently have symptoms of a viral respiratory tract infection, evidence of herpetic gingivostomatitis, or signs of aphthous stomatitis, it is possible

that viral-induced damage to the respiratory mucosa facilitates *K. kingae* invasion of the bloodstream.[55,65] Beyond the role of viral coinfection, *K. kingae* produces a potent extracellular toxin that belongs to the RTX family of toxins and is capable of lysing epithelial, synovial, and macrophage cells.[25] This toxin may facilitate disruption of the respiratory epithelium, perhaps with an increased effect in the setting of viral coinfection. The *K. kingae* RTX locus resembles the RTX locus in *Mycobacterium bovis* and *Neisseria meningitidis* and was likely acquired through horizontal transfer.[25]

Once in the bloodstream, *K. kingae* must be able to evade a variety of host defense mechanisms. Similar to other bacterial pathogens that produce bacteremic disease in children, *K. kingae* produces an extracellular polysaccharide capsule.[2,39] By analogy to other encapsulated bacteria, encapsulation of *K. kingae* may facilitate resistance to serum killing activity and opsonophagocytosis and thereby promote intravascular survival and dissemination to distant sites.[65] At this point, the level of heterogeneity of the polysaccharide capsule among diverse isolates of *K. kingae* remains unclear.

In patients in whom *K. kingae* is able to persist in the bloodstream, the organism may produce uncomplicated bacteremia or instead disseminate to joints, bones, or the endocardium.[55,65] Analysis of a large collection of *K. kingae* systemic isolates demonstrated that strains recovered from the blood of patients with uncomplicated bacteremia were generally piliated but typically expressed relatively few pili.[25] In contrast, strains recovered from joint fluid, bone aspirates, or the blood of patients with endocarditis were generally nonpiliated.[28] It is possible that low-density piliation facilitates a tropism for joints, bones, and the endocardium and potentiates an inflammatory response, which then selects against piliated organisms. Consistent with this possibility, pili promote efficient adherence to cultured synovial cells. Similar to type IV pili expressed by other pathogens, *K. kingae* pili are regulated by a transcription factor called σ54 and by a two-component sensor/regulator system termed PilS/PilR.[27] Mutations in the PilS sensor result in a reduced density of pili, similar to the relative decrease in density of pili in isolates recovered from the bloodstream in contrast to the posterior pharynx. In contrast, mutations in the PilR response regulator result in elimination of piliation, similar to the absence of pili in isolates from joints and bones.[27] It is unclear which environmental factors influence σ54, PilS, and PilR activity and control density of *K. kingae* piliation. The three different colony morphologies that are detectable on agar plates and correlate with the level of piliation may reflect mutations in PilS and PilR.[28]

To investigate the role of the host immune system in development of *K. kingae* disease in healthy individuals, Slonim and colleagues[46] examined serum immunoglobulin (Ig)A and IgG levels against outer membrane proteins in 19 children with invasive disease. As expected, children convalescing from invasive disease had significant increases in IgA and IgG levels. Further analysis has demonstrated that the age incidence of disease is inversely correlated with serum IgA and IgG levels in healthy individuals. Infants younger than 6 months of age have undetectable serum IgA but high serum IgG levels, suggesting that protection from invasive disease in this age group is due to maternally derived IgG.[46] Children between 6 and 18 months of age have the highest age incidence of disease and the lowest serum IgG levels.[17] Children 24 months of age and older have a progressively decreasing incidence of disease and progressively increasing IgG and IgA levels, suggesting that carriage or exposure to *K. kingae* during the first 2 years of life may be an immunizing event. However, *K. kingae* outer membrane protein epitopes are polymorphic from strain to strain, raising the possibility that the immune response is strain specific and may not prevent recolonization by an antigenically distinct strain.[57]

CLINICAL MANIFESTATIONS

Osteoarticular Infections

Septic arthritis caused by *K. kingae* is monoarticular and most often involves the knee, hip, or ankle.[55] Other joints that may be affected include the wrist, shoulder, elbow, sternoclavicular, metacarpophalangeal, and tarsal joints. Fever is present in approximately 85 percent of patients with *K. kingae* arthritis. Arthrocentesis reveals purulent synovial fluid with a mean white blood cell count of approximately 130,000/μL (range ~5000 to ~300,000/μL) and a differential that contains over 90 percent neutrophils.[18] Other laboratory measurements include a mean peripheral white blood cell count of 14,800/mm^3 (range 6000 to 28,450/mm^3), a mean C-reactive protein of 3.7 mg/dL (range, 0.2 to 17.0 mg/dL), and a mean erythrocyte sedimentation rate of 44.1 mm/hr (range, 5 to 140 mm/hr).[17]

Osteomyelitis resulting from *K. kingae* usually involves long bones such as the femur and tibia.[55] However, *K. kingae* also has an unusual predilection to produce disease in small bones, including the calcaneus and talus.[17,55] Children with *K. kingae* osteomyelitis often present with a limp and have a significantly more prolonged course of symptoms before presentation in contrast to patients with septic arthritis (9.2 ± 9.4 vs. 3.2 ± 3.0 days). Laboratory measurements include a mean peripheral white blood cell count of 13,888/mm^3 (range, 5860 to 2,000/mm^3), a mean C-reactive protein of 1.8 mg/dL (range, 0.2 to 4.3 mg/dL), and a mean erythrocyte sedimentation rate of 40.0 mm/hour (range, 12 to 85 mm/hr).[17]

Among patients younger than 4 years of age with spondylodiscitis, at least one quarter have *K. kingae* infection.[9,11,19] In order of frequency, infection involves the lumbar, thoracolumbar, cervical, thoracic, and lumbosacral intervertebral spaces. Patients with *K. kingae* spondylodiscitis usually have a limp, back pain, refusal to sit or walk, or neurologic symptoms. Fever is present in two thirds of patients but rarely exceeds 39°C (102.2°F). Other skeletal infections caused *by K. kingae* include tenosynovitis[55] and dactylitis.[13]

K. kingae bacteremia without evidence of endocarditis or other localizing signs is being increasingly recognized in young children. The duration of symptoms before diagnosis ranges from 1 to 7 days, and the mean maximal

fever is 39°C (102.2°F).[18,55] Occasional patients have a maculopapular rash resembling the rash in early meningococcal or gonococcal infection.[20,49] Laboratory evaluation often reveals an elevated peripheral white blood cell count (mean, 12,700/μL; range, 7120 to 47,040/μL), modest elevation of C-reactive protein (mean, 2.2 mg/dL, range 0.6 to 13.4 mg/dL), and significant elevation of erythrocyte sedimentation rate (mean 50 mm/hr; range, 14 to 115 mm/hr).[17]

K. kingae is included in the HACEK (*Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, and *Kingella* spp.) group of bacteria accountable for 3 to 5 percent of cases of bacterial endocarditis. In contrast to other *K. kingae* infections, *K. kingae* endocarditis is most common in older children and adults.[55] In approximately 50 percent of cases, infection involves a native valve, often related to a predisposing cardiac malformation or rheumatic heart disease.* Disease usually involves the left side of the heart, most often the mitral valve.[55,65] Patients with *K. kingae* endocarditis typically have fever and elevated C-reactive protein and erythrocyte sedimentation rate.[17] Common complications include congestive heart failure, septic shock, mycotic aneurysm, pulmonary infarction, meningitis, and stroke, and overall mortality is approximately 16 percent.[3,42,44,52,67]

DIAGNOSIS

The recovery of *K. kingae* from purulent specimens seeded onto solid culture media is poor, often resulting in a lack of growth. The yield of cultures is improved significantly by inoculating specimens into Isolator 1.5 microbial tubes or aerobic blood culture vials from a variety of automated blood culture systems such as BacTec, BacT/Alert, and Hemoline DUO.[55] When positive blood culture vials are subcultured onto routine solid media, *K. kingae* grows readily, suggesting that exudates may inhibit growth and that dilution of purulent material in a large volume of broth may decrease the concentration of inhibitory factors, improving the recovery of this fastidious organism.[55] In studies in which blood culture vials were routinely used for culturing synovial fluid aspirates from young children with suspected joint infections, *K. kingae* was isolated in almost half of the patients who ultimately had culture-proved bacterial disease.[36,58]

Studies published over the past decade have demonstrated that polymerase chain reaction (PCR) dramatically enhances detection of *K. kingae* in bone and joint fluid samples in contrast to routine cultures and blood culture vials. Some studies have used universal 16S rRNA primers,[48] and others have used specific primers that amplify either the *K. kingae cpn60* gene or the *K. kingae rtxA* gene.[8,12,23,34] As an example, Chometon and coworkers[14] sequentially analyzed bone and joint samples using blood culture vials, conventional PCR with universal primers, and real-time PCR with primers derived from the *K. kingae cpn60* gene and achieved a detection rate that increased from 29 to 41 to 45 percent of microbiologically proved cases, accounting for approximately 80 percent of cases in children younger than 3 years of age.[14]

Plain radiographs often complement culture and PCR-based diagnostic methods, demonstrating soft tissue swelling and joint effusions in approximately 50 percent of patients with septic arthritis and showing lytic lesions or disk space narrowing in 95 percent of cases of osteomyelitis or spondylodiscitis.[55] Technetium-99m bone scans may be helpful in diagnosing *K. kingae* osteomyelitis during the early course of disease, before abnormalities are present on plain radiographs.[19,20] Echocardiography is helpful in diagnosing *K. kingae* endocarditis and often reveals large vegetations, especially in patients who have had symptoms for a prolonged period or have clinical evidence of embolization.

TREATMENT

K. kingae is typically highly susceptible to penicillins and cephalosporins, although in vitro susceptibility to oxacillin is relatively reduced and β-lactamase production with low-level activity has been reported.[24,31,59] In addition, *K. kingae* is almost always susceptible to aminoglycosides, macrolides, trimethoprim-sulfamethoxazole, tetracyclines, fluoroquinolones and chloramphenicol.[24,31,55,61] Between 40 and 100 percent of isolates are resistant to clindamycin,[61] and virtually all isolates are resistant to trimethoprim and glycopeptide antibiotics, including vancomycin.[18,61]

Given the lack of controlled studies, no detailed guidelines for preferred antibiotic and length of treatment course have been developed for *K. kingae* infections. Typically, treatment varies based on accepted measures for other pathogens. Empiric treatment with intravenous oxacillin/nafcillin or a second-generation or third-generation cephalosporin is appropriate for suspected skeletal infections while laboratory results are pending.[55] In areas where methicillin-resistant *Staphylococcus aureus* is common, addition of vancomycin is recommended.[43] Once *K. kingae* is identified and β-lactam resistance is excluded, antibiotic therapy is commonly changed to amoxicillin or cefuroxime. The switch from intravenous to oral antibiotic therapy should be evaluated based on clinical response and assessment of acute-phase reactants such as C-reactive protein.

Length of antibiotic treatment for *K. kingae* disease has varied based on specific type of infection. Treatment has generally varied from 2 to 3 weeks for *K. kingae* arthritis, from 3 to 6 weeks for *K. kingae* osteomyelitis, and from 3 to 12 weeks for *K. kingae* spondylodiscitis.[55] Patients with septic arthritis usually respond well to antibiotic therapy and do not require repeated joint aspirations or invasive surgical procedures.[55]

Patients with *K. kingae* bacteremia typically have a relatively benign course and are usually treated initially with an intravenous β-lactam antibiotic and then switched to oral therapy once the clinical condition has improved. Antibiotic therapy is generally continued for a total of 1 to 2 weeks.[56]

Children with *K. kingae* native valve endocarditis are usually treated intravenously with a β-lactam alone or in combination with an aminoglycoside.[55] Typically length

*References 3, 38, 42, 52, 55, 67.

of treatment is 4 to 7 weeks.[55] Due to the potential risk for life-threatening valve damage resulting from endocarditis, patients who do not respond to antibiotic therapy may require early surgical intervention.[42,52,67]

PREVENTION

In the three reported cases of daycare outbreaks, oral antibiotics were administered to contacts of the infected children to prevent further spread. The antibiotic courses varied and included either rifampin 10 mg/kg up to 600 mg per dose twice daily for 2 days[30] or a combination of rifampin 20 mg/kg twice daily for 2 days and amoxicillin 80 mg/kg/day for 2 days[44] or 4 days.[60] *K. kingae* carriage was monitored in colonized contacts who received antibiotic prophylaxis. In the first report, 67 percent of colonized contacts became culture negative after prophylaxis.[60] In the second report, 80 percent of colonized individuals became culture negative after prophylaxis.[30] In the third report, carriage rates were low and prevented analysis of colonization at the end of antibiotic treatment.[44]

Currently no measures are available to prevent *K. kingae* infection in the general population. With recent evidence that *K. kingae* expresses a capsular polysaccharide,[2,39] it is possible that a polysaccharide-conjugate vaccine would be a viable approach to prevention of disease, similar to the situation with *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, and *N. meningitidis*. Further research is necessary to define the heterogeneity of *K. kingae* polysaccharide capsule types and to determine whether selected capsule types are associated with invasive disease and thus represent attractive vaccine antigens.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Basmaci R, Ilharreborde B, Bidet P, et al. Isolation of *Kingella kingae* in the oropharynx during *K. kingae* arthritis in children. *Clin Microbiol Infect* 2012;**18**:E134–6.
2. Bendaoud M, Vinogradov E, Balashova NV, et al. Broad-spectrum biofilm inhibition by *Kingella kingae* exopolysaccharide. *J Bacteriol* 2011;**193**:3879–86.
4. Bofinger JJ, Fekete T, Samuel R. Bacterial peritonitis caused by *Kingella kingae*. *J Clin Microbiol* 2007;**45**:3118–20.
7. Ceroni D, Cherkaoui A, Combescure C, et al. Differentiating osteoarticular infections caused by *Kingella kingae* from those due to typical pathogens in young children. *Pediatr Infect Dis J* 2011;**30**:906–9.
8. Ceroni D, Cherkaoui A, Ferey S, et al. *Kingella kingae* osteoarticular infections in young children: clinical features and contribution of a new specific real-time PCR assay to the diagnosis. *J Pediatr Orthop* 2010;**30**:301–4.
9. Ceroni D, Cherkaoui A, Kaelin A, et al. *Kingella kingae* spondylodiscitis in young children: toward a new approach for bacteriological investigations? A preliminary report. *J Child Orthop* 2010;**4**:173–5.
10. Ceroni D, Dubois-Ferriere V, Anderson R, et al. Small risk of osteoarticular infections in children with asymptomatic oropharyngeal carriage of *Kingella kingae*. *Pediatr Infect Dis J* 2012;**31**:983–5.
12. Cherkaoui A, Ceroni D, Emonet S, et al. Molecular diagnosis of *Kingella kingae* osteoarticular infections by specific real-time PCR assay. *J Med Microbiol* 2009;**58**:65–8.
14. Chometon S, Benito Y, Chaker M, et al. Specific real-time polymerase chain reaction places *Kingella kingae* as the most common cause of osteoarticular infections in young children. *Pediatr Infect Dis J* 2007;**26**:377–81.
17. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
18. Dubnov-Raz G, Scheuerman O, Chodick G, et al. Invasive *Kingella kingae* infections in children: clinical and laboratory characteristics. *Pediatrics* 2008;**122**:1305–9.
19. Garron E, Viehweger E, Launay F, et al. Nontuberculous spondylodiscitis in children. *J Pediatr Orthop* 2002;**22**:321–8.
23. Ilharreborde B, Bidet P, Lorrot M, et al. New real-time PCR-based method for *Kingella kingae* DNA detection: application to samples collected from 89 children with acute arthritis. *J Clin Microbiol* 2009;**47**:1837–41.
25. Kehl-Fie TE, St Geme JW 3rd. Identification and characterization of an RTX toxin in the emerging pathogen *Kingella kingae*. *J Bacteriol* 2007;**189**:430–6.
26. Kehl-Fie TE, Miller SE, St Geme JW 3rd. *Kingella kingae* expresses type IV pili that mediate adherence to respiratory epithelial and synovial cells. *J Bacteriol* 2008;**190**:7157–63.
27. Kehl-Fie TE, Porsch EA, Miller SE, et al. Expression of *Kingella kingae* type IV pili is regulated by sigma54, PilS, and PilR. *J Bacteriol* 2009;**191**:4976–86.
28. Kehl-Fie TE, Porsch EA, Yagupsky P, et al. Examination of type IV pilus expression and pilus-associated phenotypes in *Kingella kingae* clinical isolates. *Infect Immun* 2010;**78**:1692–9.
31. Kugler KC, Biedenbach DJ, Jones RN. Determination of the antimicrobial activity of 29 clinically important compounds tested against fastidious HACEK group organisms. *Diagn Microbiol Infect Dis* 1999;**34**:73–6.
32. La Selve H, Berard J, Barbe G, et al. Osteoarthritis and osteomyelitis due to *Kingella kingae* in children: apropos of 5 cases and review of the literature. *Pediatrie* 1986;**41**:297–304.
34. Lehours P, Freydiere AM, Richer O, et al. The *rtxA* toxin gene of *Kingella kingae*: a pertinent target for molecular diagnosis of osteoarticular infections. *J Clin Microbiol* 2011;**49**:1245–50.
35. Matta M, Wermert D, Podglajen I, et al. Molecular diagnosis of *Kingella kingae* pericarditis by amplification and sequencing of the 16S rRNA gene. *J Clin Microbiol* 2007;**45**:3133–4.
38. Odum L, Jensen KT, Slotsbjerg TD. Endocarditis due to *Kingella kingae*. *Eur J Clin Microbiol* 1984;**3**:263–6.
39. Porsch EA, Kehl-Fie TE, St Geme JW 3rd. Modulation of *Kingella kingae* adherence to human epithelial cells by type IV pili, capsule, and a novel trimeric autotransporter. *MBio* 2012;**3**(5):e00372–12.
40. Ramana K, Mohanty S. An adult case of urinary tract infection with *Kingella kingae*: a case report. *J Med Case Rep* 2009;**3**:7236.
42. Rotstein A, Konstantinov IE, Penny DJ. *Kingella*-infective endocarditis resulting in a perforated aortic root abscess and fistulous connection between the sinus of Valsalva and the left atrium in a child. *Cardiol Young* 2010;**20**:332–3.
43. Saphyakhajon P, Joshi AY, Huskins WC, et al. Empiric antibiotic therapy for acute osteoarticular infections with suspected methicillin-resistant *Staphylococcus aureus* or *Kingella*. *Pediatr Infect Dis J* 2008;**27**:765–7.
44. Sena AC, Seed P, Nicholson B, et al. *Kingella kingae* endocarditis and a cluster investigation among daycare attendees. *Pediatr Infect Dis J* 2010;**29**:86–8.
53. Woolfrey BF, Lally RT, Faville RJ. Intervertebral diskitis caused by *Kingella kingae*. *Am J Clin Pathol* 1986;**85**:745–9.
54. Yagupsky P. Diagnosis of *Kingella kingae* arthritis by polymerase chain reaction analysis. *Clin Infect Dis* 1999;**29**:704–5.
55. Yagupsky P. *Kingella kingae*: from medical rarity to an emerging paediatric pathogen. *Lancet Infect Dis* 2004;**4**:358–67.
58. Yagupsky P, Bar-Ziv Y, Howard CB, et al. Epidemiology, etiology, and clinical features of septic arthritis in children younger than 24 months. *Arch Pediatr Adolesc Med* 1995;**149**:537–40.
64. Yagupsky P, Porat N, Pinco E. Pharyngeal colonization by *Kingella kingae* in children with invasive disease. *Pediatr Infect Dis J* 2009;**28**:155–7.
65. Yagupsky P, Porsch E, St Geme JW 3rd. *Kingella kingae*: an emerging pathogen in young children. *Pediatrics* 2011;**127**:557–65.
66. Yagupsky P, Weiss-Salz I, Fluss R, et al. Dissemination of *Kingella kingae* in the community and long-term persistence of invasive clones. *Pediatr Infect Dis J* 2009;**28**:707–10.
67. Youssef D, Henaine R, Di Filippo S. Subtle bacterial endocarditis due to *Kingella kingae* in an infant: a case report. *Cardiol Young* 2010;**20**:448–50.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Basmaci R, Ilharreborde B, Bidet P, et al. Isolation of *Kingella kingae* in the oropharynx during *K. kingae* arthritis in children. *Clin Microbiol Infect* 2012;**18**:E134–6.
2. Bendaoud M, Vinogradov E, Balashova NV, et al. Broad-spectrum biofilm inhibition by *Kingella kingae* exopolysaccharide. *J Bacteriol* 2011;**193**:3879–86.
3. Berkun Y, Brand A, Klar A, et al. *Kingella kingae* endocarditis and sepsis in an infant. *Eur J Pediatr* 2004;**163**:687–8.
4. Bofinger JJ, Fekete T, Samuel R. Bacterial peritonitis caused by *Kingella kingae*. *J Clin Microbiol* 2007;**45**:3118–20.
5. Bovre K, Henriksen SD, Jonsson V. Correction of specific epithet *kingii* in combinations *Moraxella kingii* Henriksen and Bovre 1968 and *Pseudomonas kingii* Jonsson 1970 to *Kingae*. *Int J Syst Bacteriol* 1974;**24**:307.
6. Carden SM, Colville DJ, Gonis G, et al. *Kingella kingae* endophthalmitis in an infant. *Aust N Z J Ophthalmol* 1991;**19**: 217–20.
7. Ceroni D, Cherkaoui A, Combescure C, et al. Differentiating osteoarticular infections caused by *Kingella kingae* from those due to typical pathogens in young children. *Pediatr Infect Dis J* 2011;**30**: 906–9.
8. Ceroni D, Cherkaoui A, Ferey S, et al. *Kingella kingae* osteoarticular infections in young children: clinical features and contribution of a new specific real-time PCR assay to the diagnosis. *J Pediatr Orthop* 2010;**30**:301–4.
9. Ceroni D, Cherkaoui A, Kaelin A, et al. *Kingella kingae* spondylodiscitis in young children: toward a new approach for bacteriological investigations? A preliminary report. *J Child Orthop* 2010;**4**: 173–5.
10. Ceroni D, Dubois-Ferriere V, Anderson R, et al. Small risk of osteoarticular infections in children with asymptomatic oropharyngeal carriage of *Kingella kingae*. *Pediatr Infect Dis J* 2012;**31**:983–5.
11. Chanal C, Tiget F, Chapuis P, et al. Spondylitis and osteomyelitis caused by *Kingella kingae* in children. *J Clin Microbiol* 1987;**25**: 2407–9.
12. Cherkaoui A, Ceroni D, Emonet S, et al. Molecular diagnosis of *Kingella kingae* osteoarticular infections by specific real-time PCR assay. *J Med Microbiol* 2009;**58**:65–8.
13. Chiquito PE, Elliott J, Namnyak SS. *Kingella kingae* dactylitis in an infant. *J Infect* 1991;**22**:102–3.
14. Chometon S, Benito Y, Chaker M, et al. Specific real-time polymerase chain reaction places *Kingella kingae* as the most common cause of osteoarticular infections in young children. *Pediatr Infect Dis J* 2007;**26**:377–81.
15. Clement JL, Berard J, Cahuzac JP, et al. *Kingella kingae* osteoarthritis and osteomyelitis in children. *J Pediatr Orthop* 1988;**8**: 59–61.
16. de Groot R, Glover D, Clausen C, et al. Bone and joint infections caused by *Kingella kingae*: six cases and review of the literature. *Rev Infect Dis* 1988;**10**:998–1004.
17. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
18. Dubnov-Raz G, Scheuerman O, Chodick G, et al. Invasive *Kingella kingae* infections in children: clinical and laboratory characteristics. *Pediatrics* 2008;**122**:1305–9.
19. Garron E, Viehweger E, Launay F, et al. Nontuberculous spondylodiscitis in children. *J Pediatr Orthop* 2002;**22**:321–8.
20. Goutzmanis JJ, Gonis G, Gilbert GL. *Kingella kingae* infection in children: ten cases and a review of the literature. *Pediatr Infect Dis J* 1991;**10**:677–83.
21. Henriksen SD, Bovre K. *Moraxella kingii* sp. nov., a haemolytic, saccharolytic species of the genus *Moraxella*. *J Gen Microbiol* 1968; **51**:377–85.
22. Henriksen SD, Bovre K. Transfer of *Moraxella-kingae Kingella*-Henriksen and Bovre to Genus gen-nov in family Neisseriaceae. *Int J Syst Bacteriol* 1976;**26**:447–50.
23. Ilharreborde B, Bidet P, Lorrot M, et al. New real-time PCR-based method for *Kingella kingae* DNA detection: application to samples collected from 89 children with acute arthritis. *J Clin Microbiol* 2009;**47**:1837–41.
24. Jensen KT, Schonheyder H, Thomsen VF. In-vitro activity of beta-lactam and other antimicrobial agents against *Kingella kingae*. *J Antimicrob Chemother* 1994;**33**:635–40.
25. Kehl-Fie TE, St Geme JW 3rd. Identification and characterization of an RTX toxin in the emerging pathogen *Kingella kingae*. *J Bacteriol* 2007;**189**:430–6.
26. Kehl-Fie TE, Miller SE, St Geme JW 3rd. *Kingella kingae* expresses type IV pili that mediate adherence to respiratory epithelial and synovial cells. *J Bacteriol* 2008;**190**:7157–63.
27. Kehl-Fie TE, Porsch EA, Miller SE, et al. Expression of *Kingella kingae* type IV pili is regulated by sigma54, PilS, and PilR. *J Bacteriol* 2009;**191**:4976–86.
28. Kehl-Fie TE, Porsch EA, Yagupsky P, et al. Examination of type IV pilus expression and pilus-associated phenotypes in *Kingella kingae* clinical isolates. *Infect Immun* 2010;**78**:1692–9.
29. Kennedy CA, Rosen H. *Kingella kingae* bacteremia and adult epiglottitis in a granulocytopenic host. *Am J Med* 1988;**85**:701–2.
30. Kiang KM, Ogunmodede F, Juni BA, et al. Outbreak of osteomyelitis/septic arthritis caused by *Kingella kingae* among child care center attendees. *Pediatrics* 2005;**116**:e206–13.
31. Kugler KC, Biedenbach DJ, Jones RN. Determination of the antimicrobial activity of 29 clinically important compounds tested against fastidious HACEK group organisms. *Diagn Microbiol Infect Dis* 1999;**34**:73–6.
32. La Selve H, Berard J, Barbe G, et al. Osteoarthritis and osteomyelitis due to *Kingella kingae* in children: apropos of 5 cases and review of the literature. *Pediatrie* 1986;**41**:297–304.
33. Lawson PA, Malnick H, Collins MD, et al. Description of *Kingella potus* sp. nov., an organism isolated from a wound caused by an animal bite. *J Clin Microbiol* 2005;**43**:3526–9.
34. Lehours P, Freydiere AM, Richer O, et al. The *rtxA* toxin gene of *Kingella kingae*: a pertinent target for molecular diagnosis of osteoarticular infections. *J Clin Microbiol* 2011;**49**:1245–50.
35. Matta M, Wermert D, Podglajen I, et al. Molecular diagnosis of *Kingella kingae* pericarditis by amplification and sequencing of the 16S rRNA gene. *J Clin Microbiol* 2007;**45**:3133–4.
36. Moumile K, Merckx J, Glorion C, et al. Osteoarticular infections caused by *Kingella kingae* in children: contribution of polymerase chain reaction to the microbiologic diagnosis. *Pediatr Infect Dis J* 2003;**22**:837–9.
37. Noftal F, Mersal A, Yaschuk Y, et al. Osteomyelitis due to *Kingella kingae* infection. *Can J Surg* 1988;**31**:21–2.
38. Odum L, Jensen KT, Slotsbjerg TD. Endocarditis due to *Kingella kingae*. *Eur J Clin Microbiol* 1984;**3**:263–6.
39. Porsch EA, Kehl-Fie TE, St Geme JW 3rd. Modulation of *Kingella kingae* adherence to human epithelial cells by type IV pili, capsule, and a novel trimeric autotransporter. *MBio* 2012;**3**(5):e00372–12.
40. Ramana K, Mohanty S. An adult case of urinary tract infection with *Kingella kingae*: a case report. *J Med Case Rep* 2009;**3**:7236.
41. Raymond J, Bergeret M, Bargy F, et al. Isolation of two strains of *Kingella kingae* associated with septic arthritis. *J Clin Microbiol* 1986;**24**:1100–1.
42. Rotstein A, Konstantinov IE, Penny DJ. *Kingella*-infective endocarditis resulting in a perforated aortic root abscess and fistulous connection between the sinus of Valsalva and the left atrium in a child. *Cardiol Young* 2010;**20**:332–3.
43. Saphyakhajon P, Joshi AY, Huskins WC, et al. Empiric antibiotic therapy for acute osteoarticular infections with suspected methicillin-resistant *Staphylococcus aureus* or *Kingella*. *Pediatr Infect Dis J* 2008;**27**:765–7.
44. Sena AC, Seed P, Nicholson B, et al. *Kingella kingae* endocarditis and a cluster investigation among daycare attendees. *Pediatr Infect Dis J* 2010;**29**:86–8.
45. Shelton MM, Nachtigal MP, Yngve DA, et al. *Kingella kingae* osteomyelitis: report of two cases involving the epiphysis. *Pediatr Infect Dis J* 1988;**7**:421–4.
46. Slonim A, Steiner M, Yagupsky P. Immune response to invasive *Kingella kingae* infections, age-related incidence of disease, and levels of antibody to outer-membrane proteins. *Clin Infect Dis* 2003; **37**:521–7.
47. Slonim A, Walker ES, Mishori E, et al. Person-to-person transmission of *Kingella kingae* among day care center attendees. *J Infect Dis* 1998;**178**:1843–6.
48. Stahelin J, Goldenberger D, Gnehm HE, et al. Polymerase chain reaction diagnosis of *Kingella kingae* arthritis in a young child. *Clin Infect Dis* 1998;**27**:1328–9.
49. Toshniwal R, Draghi TC, Kocka FE, et al. Manifestations of *Kingella kingae* infections in adults: resemblance to neisserial infections. *Diagn Microbiol Infect Dis* 1986;**5**:81–5.

50. Van Erps J, Schmedding E, Naessens A, et al. *Kingella kingae*, a rare cause of bacterial meningitis. *Clin Neurol Neurosurg* 1992;**94**: 173–5.
51. Verdier I, Gayet-Ageron A, Ploton C, et al. Contribution of a broad range polymerase chain reaction to the diagnosis of osteoarticular infections caused by *Kingella kingae*: description of twenty-four recent pediatric diagnoses. *Pediatr Infect Dis J* 2005;**24**:692–6.
52. Wells L, Rutter N, Donald F. *Kingella kingae* endocarditis in a sixteen-month-old-child. *Pediatr Infect Dis J* 2001;**20**:454–5.
53. Woolfrey BF, Lally RT, Faville RJ. Intervertebral diskitis caused by *Kingella kingae*. *Am J Clin Pathol* 1986;**85**:745–9.
54. Yagupsky P. Diagnosis of *Kingella kingae* arthritis by polymerase chain reaction analysis. *Clin Infect Dis* 1999;**29**:704–5.
55. Yagupsky P. *Kingella kingae*: from medical rarity to an emerging paediatric pathogen. *Lancet Infect Dis* 2004;**4**:358–67.
56. Yagupsky P, Dagan R. *Kingella kingae* bacteremia in children. *Pediatr Infect Dis J* 1994;**13**:1148–9.
57. Yagupsky P, Slonim A. Characterization and immunogenicity of *Kingella kingae* outer-membrane proteins. *FEMS Immunol Med Microbiol* 2005;**43**:45–50.
58. Yagupsky P, Bar-Ziv Y, Howard CB, et al. Epidemiology, etiology, and clinical features of septic arthritis in children younger than 24 months. *Arch Pediatr Adolesc Med* 1995;**149**:537–40.
59. Yagupsky P, Dagan R, Prajgrod F, et al. Respiratory carriage of *Kingella kingae* among healthy children. *Pediatr Infect Dis J* 1995;**14**: 673–8.
60. Yagupsky P, Erlich Y, Ariela S, et al. Outbreak of *Kingella kingae* skeletal system infections in children in daycare. *Pediatr Infect Dis J* 2006;**25**:526–32.
61. Yagupsky P, Katz O, Peled N. Antibiotic susceptibility of *Kingella kingae* isolates from respiratory carriers and patients with invasive infections. *J Antimicrob Chemother* 2001;**47**:191–3.
62. Yagupsky P, Merires M, Bahar J, et al. Evaluation of novel vancomycin-containing medium for primary isolation of *Kingella kingae* from upper respiratory tract specimens. *J Clin Microbiol* 1995;**33**:1426–7.
63. Yagupsky P, Peled N, Katz O. Epidemiological features of invasive *Kingella kingae* infections and respiratory carriage of the organism. *J Clin Microbiol* 2002;**40**:4180–4.
64. Yagupsky P, Porat N, Pinco E. Pharyngeal colonization by *Kingella kingae* in children with invasive disease. *Pediatr Infect Dis J* 2009;**28**:155–7.
65. Yagupsky P, Porsch E, St Geme JW 3rd. *Kingella kingae*: an emerging pathogen in young children. *Pediatrics* 2011;**127**:557–65.
66. Yagupsky P, Weiss-Salz I, Fluss R, et al. Dissemination of *Kingella kingae* in the community and long-term persistence of invasive clones. *Pediatr Infect Dis J* 2009;**28**:707–10.
67. Youssef D, Henaine R, Di Filippo S. Subtle bacterial endocarditis due to *Kingella kingae* in an infant: a case report. *Cardiol Young* 2010;**20**:448–50.

CHAPTER 137

Legionnaires' Disease, Pontiac Fever, and Related Illnesses

Paul H. Edelstein • Wilbert H. Mason

Legionnaires' disease is an acute pneumonic illness caused by gram-negative bacilli of the genus *Legionella.* Pontiac fever is a febrile, nonpneumonic, systemic illness closely associated with, if not caused by, *Legionella* spp. infection.

HISTORY

Legionnaires' disease (LD) first was recognized as a distinct clinical entity when it caused an epidemic of pneumonia at an American Legion convention in Philadelphia in 1976, where 221 people were affected and 34 died.[74] Investigators were unable to determine the exact cause of the outbreak immediately, and this mystery provoked considerable fear and widespread speculation about the cause. Approximately 6 months later, two investigators at the Centers for Disease Control and Prevention (CDC), Joseph McDade and Charles Shepard, announced that they had discovered the etiologic agent, a fastidious gram-negative bacillus.[128] Researchers determined subsequently that both the organism and the disease had been studied previously as long ago as the 1940s but had been forgotten.[40,57,168] Because of the historical association with the American Legion convention, this disease now is called LD and the etiologic agents belong to the family Legionellaceae.

ETIOLOGIC AGENT

Legionella is the only genus in the family Legionellaceae. More than 50 different species of *Legionella* now are recognized (Table 137-1). The closest pathogenic relative of the Legionellaceae is *Coxiella burnetii*, the agent of Q fever. *Legionella pneumophila* is responsible for the 1976 Philadelphia epidemic and causes most cases of LD.* *L. pneumophila* serogroup 1 is estimated to cause 70 to 90 percent of all cases of LD in previously healthy people.[47,124] *Legionella* spp. other than *L. pneumophila*, and serogroups other than *L. pneumophila* serogroup 1, cause 25 to 40 percent of nosocomial outbreaks of LD; of these *L. pneumophila* serogroups 4 and 6, *Legionella micdadei*, and *Legionella dumoffii* are the most common.[92,185] *L. pneumophila* and *Legionella longbeachae* are the only two *Legionella* spp. that normally infect nonimmunocompromised patients.

The Legionellaceae are obligately aerobic, mesophilic, motile, L-cysteine auxotrophic gram-negative bacilli with variable oxidase and catalase reactions.[56] Genomes of six different *L. pneumophila* serogroup 1 strains have been sequenced, revealing that these bacteria harbor a number of eukaryotic-like genes, a number of secretion systems, remarkable plasticity, and the presence of mobile elements.[79,119] About 10 percent of the approximately 3.5-mb size genome is devoted to strain-specific genes.[20,32,34]

Antigenic relationships among different *Legionella* spp. and among serotypes of the same species are complex.[168] The serologic typing scheme is based on surface antigens (O antigens), which primarily are lipopolysaccharides. These surface antigens are shared by different species and, in rare cases, by other gram-negative bacilli, such as *Pseudomonas* spp. Identifying an unknown strain by use of serologic methods alone occasionally leads to a false or misleading identification. Cross reactions also may be observed in testing for human antibodies to *Legionella* spp. Patients with infections caused by some *Pseudomonas* spp. strains, *Campylobacter jejuni*, and other gram-negative bacilli may develop antibodies to *Legionella* spp.[56]

Identification of *Legionella* spp. other than *L. pneumophila* often requires the capabilities of a research, or national reference, laboratory that performs molecular-based identification.[15] However, isolation and presumptive identification of *L. pneumophila* should be within the capabilities of most clinical laboratories.[56]

Legionella bacteria are ubiquitous in the aqueous environment and can be found, often in high concentrations, in lake water, ponds, bathing water, hot-water tanks, hot-water plumbing, and air-conditioning cooling towers.[168] Optimal growth temperature ranges from 28° C to 40° C (82° F to 104° F). The natural hosts and reservoirs of environmental *Legionella* bacteria are freshwater amebae, such as *Acanthamoeba* and *Hartmannella*.[69] Factors that promote the environmental growth of *Legionella* include the presence of other microorganisms, use of plumbing materials that promote bacterial growth, stagnation, and warm temperatures.[168] In the home, older municipal water distribution systems, use of electric rather than gas water heaters, and use of well water all appear to promote the presence of *Legionella* in water.[69,164] In the particular case of *L. longbeachae*, amebae-laden potting soil exposed to warm temperatures allows multiplication of the bacterium to high concentrations.[181] Most newly discovered *Legionella* spp. have been environmental isolates not associated with clinical illness.[69]

A large number of bacterial virulence factors have been described, the most important of which is the Dot/Icm complex. This complex comprises 26 genes that form a type IV secretion system that is required for *L.*

*References 40, 45, 57, 110, 118, 168.

TABLE 137-1 ***Legionella* Species and Serogroups**

Species	No. of Serogroups	Implicated in Human Disease
adelaidensis	1	No
anisa	1	Yes
beliardensis	1	No
birminghamensis	1	Yes
bozemane	2	Yes
brunensis	1	No
busanensis	Unknown	No
cherrii	1	No
cincinnatiensis	1	Yes
drancourtii	Unknown	No
dresdenensis	1	No
drozanskii	Unknown	No
dumoffii	1	Yes
erythra	2	No
fairfieldensis	1	No
falloni	Unknown	No
feeleii	2	Yes
geestiana	1	No
gormanii	1	Yes
gratiana	1	No
gresilensis	1	No
hackeliae	2	Yes
impletisoli	Unknown	No
israelensis	1	No
jamestownensis	1	No
*"jeonii"**	Unknown	No
jordanis	1	Yes
lansingensis	1	Yes
londiniensis	1	No
longbeachae	2	Yes
lytica	Unknown	Yes
maceachernii	1	Yes
micdadei	1	Yes
moravica	1	No
nagasakiensis	1	Yes
nautarum	1	No
oakridgensis	1	No
parisiensis	1	Yes
pneumophila	16	Yes
quateirensis	1	No
quinlivanii	2	No
rowbothamii	Unknown	No
rubrilucens	1	No
sainthelensi	2	Yes
sainticrucis	1	No
shakespearei	1	No
spiritensis	2	No
steelei	Unknown	No†
steigerwaltii	1	No
taurinensis	1	No
tucsonensis	1	Yes
wadsworthii	1	Yes
waltersii	1	No
worsleiensis	1	No
yabuuchiae	Unknown	No

**L. "jeonii"* is not a validly named species.
†Isolated from humans but unlikely to have caused disease.

pneumophila growth within macrophages. *L. pneumophila* is not killed actively by macrophages. The Dot/Icm complex allows the bacterium to evade normal phagolysosomal trafficking and bacterial destruction, by injecting effectors into the host macrophage within minutes of the time of initial bacterial-macrophage contact. Several hundred injected effectors have been described, but only a few have been fully characterized functionally.[72,97,139] The end result is that the normal host processes are hijacked into providing an intracellular compartment for the bacterium that associates with endoplasmic reticulum.[36,135,154] Other bacterial virulence factors include a variety of enzymes, iron-binding proteins, miscellaneous proteins enhancing intracellular growth, and survival or invasion.[36] An important virulence factor for *L. pneumophila* serogroup 1 is a lipopolysaccharide 8-O-acetylase of unknown pathogenic mechanism of action; bacterial strains with this acetylase constitute the majority of *L. pneumophila* clinical isolates yet are uncommon in the environment.[31]

Availability of intracellular iron appears to play a key role in the growth of intracellular *L. pneumophila*. Interferon-γ, the production of which is increased during *L. pneumophila* infection of macrophages, downregulates the number of iron-binding receptors on macrophages, which has the effect of decreasing the availability of intracellular iron and, hence, limiting intracellular growth of the bacterium.[24]

L. pneumophila is serum resistant.[168] High-titer antibody promotes phagocytosis but may not enhance killing. Macrophage activation factors possibly are more important than is antibody in promoting phagocytosis and bacterial killing.[95]

EPIDEMIOLOGY

Incidence and Frequency

National passive LD surveillance has been initiated through the CDC by using serologic, culture and mainly urine antigen testing. Cases reported to the CDC from 1990 through 2005 included a total of 23,076 cases, of which there were 375 pediatric cases or 1.7 percent of the total number of cases. Most pediatric cases, (44.3%) were in the 15- to 19-year age group, followed by those younger than age 1 year (18.1%).

Pediatric LD rarely appears to be the cause of pneumonia in otherwise healthy children.* Fewer than 15 culture-confirmed cases of LD have been reported in otherwise normally healthy children, and fewer than 70 culture-confirmed cases have been reported in immunosuppressed children.†

A CDC study on the epidemiology of LD in children from 1984 through 2004 found that 66 of 6575 (1%) of cases of LD reported during the period occurred in children younger than 19 years. The median age was 11 years, and 72 percent reported a health care exposure, 30 percent had at least one risk factor for the infection; 26

*References 4, 10, 28, 38, 44, 75, 93, 96, 114, 131, 147, 160, 167.
†References 1, 7, 18, 27, 28, 38, 41, 61, 67, 81, 82, 100, 107, 109, 111, 116, 117, 138, 145, 149, 161, 171, 175, 182.

percent of cases were associated with an outbreak, and 22 percent died.[2] The very low frequency of LD in U.S. children has also been observed in children with pneumonia in Gabon and Germany.[112,180]

A retrospective serosurvey of 500 patients with pneumonia, 83 percent of whom did not need hospitalization, found that only 5 of the 132 patients younger than 15 years of age had significant antibody titers against *L. pneumophila* and none developed seroconversion.[73] The estimated incidence of LD in the overall population was approximately 12 per 100,000 per year. Several prospective serologic studies of children with pneumonia have found that LD constitutes around 1 percent of the diagnosed causes of pneumonia.[38,141,152] This rate is approximately the same as the 0.5 to 4 percent frequency found in the adult population.[40,124,168] LD is not a major cause of pneumonia in either children or adults.

The frequency of LD in immunosuppressed children is unknown, although it is clear that immunosuppressed children can be at high risk for this disease. LD has been reported in children with acute leukemia, chronic granulomatous disease, after bone marrow or solid-organ transplantation, or after being treated with corticosteroids for other reasons.* Nosocomial or community-acquired LD has been reported in several "immunologically normal" infants, who may have had some degree of immunocompromise that predisposed them to this disease.† Neonates appear to be at increased risk for acquiring nosocomial, water-birthing related, and community-acquired LD.[82,117,158,173]

More than 100 cases of Pontiac fever in children have been reported in outbreaks of this disease.[22,78,98,101,120] The risk for acquiring Pontiac fever was found to be greater for younger people in one outbreak,[88,98] which was just the opposite finding of another outbreak investigation.[101] The true frequency of Pontiac fever in children is difficult to know because the diagnosis is impossible to make with certainty in the sporadic form. One hotel hot tub–related outbreak of both LD and Pontiac fever in families and attendees of a youth athletic event found that the median ages of those with LD and Pontiac fever were 6.5 and 15 years, respectively.[22]

Several serosurveys found asymptomatic elevations of *L. pneumophila* antibody in widely varying frequency. In one study, about half of 52 children studied developed an increase in antibody titer unrelated to acute illness over a period of several years.[4] Another study found that 15 percent of 126 children sampled had antibody titers to *L. pneumophila* of 256 or higher, which was not significantly different from those seen with an adult control population.[134] The striking finding was that the antibody titers rose with age, doubling each year until age 3 years, when the values plateaued; this finding held even when patients with pneumonia were excluded. The significance of these elevated antibody titers in children without documented pneumonia is unclear and can be interpreted as cross reactions to other colonizing or infecting bacteria, asymptomatic infection, or atypical disease.

Several investigators have examined the question of whether atypical disease caused by *Legionella* occurs in children using poorly specific serologic tests. Italian physicians found that two children with acute, reversible cerebellar ataxia developed significant antibody titer changes to *L. pneumophila*.[140] Another Italian study found two children with pericarditis who had significant antibody changes.[162] None of 140 British infants with sudden death had measurable antibodies to *L. pneumophila*, despite suggestions that they might be linked.[178] Three studies have shown that children with cystic fibrosis have higher antibody levels to *Legionella* than do normal children, although these data are suspect because of known cross reactions between *Legionella* and *Pseudomonas aeruginosa*.[37,59,104] Thus, despite several epidemiologic surveys, the prevalence of atypical *Legionella* infections in children is unclear.

In adults, LD incidence figures vary widely, most likely related to the insensitivity of passive reporting systems and the vigor of diagnostic testing. The highest reported incidence rates, between 187 to 375 LD cases per million population per year, were reported from a 2008 prospective study of community-acquired pneumonia in Germany, where special efforts were made to capture all cases and to use sensitive diagnostic tests.[176] Significantly lower incidence has been reported in France (20/million/year), where surveillance is passive but disease reporting is mandatory.[26] The reported rate in the United States, 11/million/year, is lower, most likely owing to a reliance on passive surveillance and limited diagnostic testing for the disease.[33] Estimates based on serologic surveys range from 20 to 200 cases per million population per year.[40,150,168] A prospective study in Ohio found that the incidence of LD requiring hospitalization was approximately 60 per million per year, in what is thought to be a geographic region with an above-average frequency of the disease.[125] Estimates of the proportion of adult pneumonias caused by LD range from 1 to 25 percent, with a reasonable mean of 3 percent.[124] Mild or atypical *Legionella* infection certainly occurs in adults, although the exact incidence is unknown. Asymptomatic infection may occur in adults, based on serosurveys. Neither oropharyngeal colonization nor a carrier state has been documented.[19]

LD, although often thought of as an epidemic disease, is actually more common as a sporadic disease. A recent European study showed that sporadic cases represented about 92 percent of all 5960 reported LD cases from 2007 through 2008, similar to the findings in the United States.[33,102]

Risk factors for acquisition of LD can be divided into two main categories: those that increase exposure to contaminated water and those that suppress pulmonary defense mechanisms.[40,168] Included in the former category are occupational or residential exposure to warm or stagnant water, traveling and residence in hotels, and stays in hospitals with contaminated water distribution systems; a risk factor for residential acquisition of disease is use of well water.[164] Weather may be a risk factor in some regions, with increased humidity or rain implicated in a few studies.[71] Included in risk factors that suppress pulmonary defense mechanisms are surgical procedures

*References 41, 61, 87, 100, 107, 109, 115, 145, 146, 155, 157, 165, 169.

†References 39, 68, 75, 93, 94, 103, 114, 138, 149.

requiring general anesthesia, administration of glucocorticosteroids, cigarette smoking, chronic lung disease, and diseases or therapy that compromise the innate or cellular immune systems. Use of biological response modifiers, especially tumor necrosis factor antagonists, is a significant risk factor for LD.[122,172] Alemtuzumab treatment is also a likely significant risk factor for LD. Males are more than twice as likely as females to develop the disease, perhaps as a result of the greater male prevalence of cigarette smoking and chronic lung disease. Middle-aged and older people also are at significantly higher risk than are younger people, as exemplified by recent data from the United States; the incidence rates (per million/year) were 0.2, 0.3, 1.3, 3.6, 8.1, 14.4, and 26.6 for people aged younger than 9, 10 to 19, 20 to 29, 30 to 39, 40 to 49, 50 to 59, and greater than 80 years, respectively.[33] Other than exposure to aerosols of contaminated water, no particular predisposing factors for the development of Pontiac fever have been identified. LD is not contagious and transmission from patients to staff or other patients has never been documented.

Disease Outbreaks

Multiple epidemics of LD and Pontiac fever have been recognized.[18,40,78,168] In the case of LD, most outbreaks have occurred in residents, employees, or visitors to large buildings or in urban settings with exposure to aerosolized water from fountains and cooling towers. Hotels, hospitals, museums, factories, retail stores, office buildings, and housing complexes have all been implicated in LD epidemics. Nosocomial LD has been reported in hospitals throughout the world. Disease reservoirs and disseminators have been potable water distribution systems, air-conditioning cooling towers, hot tub spas, decorative fountains, industrial water systems, and others.[40,168] Outbreaks have been ended by disinfection of water and cooling tower systems by hyperchlorination or pasteurization.[40,168] In some of these outbreaks, disease occurred for many years until effective disinfection procedures were utilized. Attack rates in LD epidemics consistently have been less than 5 percent overall. Incubation periods ranging from 2 to 19 days have been observed, with median incubation periods of 4 to 6 days and average ranges from 2 to 10 days.

Pontiac fever outbreaks generally have been associated with exposure to an aerosol of warm water contaminated with *Legionella*.[77,106,168] Examples include whirlpool baths, an engine assembly plant using contaminated water to cool machine lathes, and a health department building in which condensate in the air-conditioning system was contaminated. The attack rates in Pontiac fever outbreaks have been high, in the range of 95 to 100 percent. The incubation period is short at 12 to 36 hours.[77]

PATHOLOGY, PATHOGENESIS, AND IMMUNITY

Very good, but indirect, epidemiologic and pathologic evidence suggests that LD infection results from inhalation of an aerosol.[40,168] The bacterial form that causes infection is unknown, with possibilities including a sporelike form, intra-amebal bacteria, bacteria enmeshed in biofilm, and bacteria released from biofilm.[69,76,113] Some patients have acquired LD after aspiration of *Legionella*-contaminated tap water, rather than via aerosol inhalation.[16,99,123,174]

In guinea pigs infected by the aerosol route, multiplication of *L. pneumophila* begins within 16 hours.[42] Bacterial multiplication occurs within the alveolar macrophage, although growth in respiratory epithelial cells may also occur. Cell culture studies show that multiplication occurs only in cells and not in the extracellular tissue culture medium.[95] This intracellular location of bacteria protects them from serum factors such as antibody and complement as well as from the effects of those antimicrobial agents that are not concentrated in cells. Killing of *L. pneumophila* within macrophages in cell culture is limited by failure of phagolysosomal acidification and fusion.[95]

L. pneumophila avoids macrophage killing by injecting phagosomal maturation-altering compounds into its host cell within minutes of initial contact using the Dot/Icm type IV secretion system.[97]An additional mechanism involves shedding of bacterial flagellin into the host cytosol, which acts via the innate immune system to alter macrophage killing of the bacteria.[151] In the late phases of bacterial multiplication in macrophages, the bacteria change phenotype to enable infection of the next susceptible macrophage, becoming highly motile, quite small, and more resistant to antimicrobial agents.[8,132] The *L. pneumophila*–containing vacuole becomes huge, occupying almost the entire volume of the macrophage, over a period of a few days. The bacteria effect escape from the presumably nutrient-depleted macrophage by inducing cellular apoptosis and can then infect available uninfected macrophages.[21] Innate macrophage resistance to bacterial infection may be mediated by Toll-like receptors, in particular TLR-2 and TLR-5, and perhaps TLR-4.[88-90] Acquired macrophage resistance to infection derives from activation by interferon-γ and the resultant downregulation of available iron stores.[23] Tumor necrosis factor-α plays a very important role in host defense to infection.[129]

Polymorphonuclear leukocytes neither ingest nor kill the organism effectively in vitro, although animal studies suggest that they may form the bulwark of initial host defenses.[42] Clinical evidence suggests that leukopenic hosts without concomitant macrophage dysfunction are not high-risk candidates for LD, although they may be at higher risk than is the normal population.[29,57]

Lung histopathologic findings in LD show intense intra-alveolar inflammation.[168] Airways larger than the terminal bronchioles are not affected. The interstitial spaces generally are uninvolved, although necrosis of alveoli may bridge the interstitial spaces. The alveoli contain a variable mixture of polymorphonuclear leukocytes, alveolar macrophages, and necrotic debris. Hemorrhage is observed, as are microabscesses. Later, fibrin formation and a histiocytic predominance occur.

Gross lung changes evolve in the classic pattern of lobar pneumonia, with first red and then gray hepatization.[168] Lymph nodes are involved occasionally. The lung

segments involved often are subpleural, a finding that sometimes suggests septic or bland infarction to the clinician. The pleural space is involved variably and seemingly is more prone to infection in immunosuppressed patients. One of the most striking pulmonary findings is the usual absence of significant intrabronchial exudate. This absence of intrabronchial exudate, and lack of bronchial inflammation, may mislead clinicians to exclude bacterial pneumonia.

Despite frequent signs and symptoms of extrapulmonary disease in patients with LD, no specific extrapulmonary pathologic findings have been identified.[168] In fatal cases, organisms often can be recovered or detected in the liver or spleen. Occasionally, patients have nonbacterial endocardial vegetations. Some patients have hilar and paratracheal adenitis. Patients may have metastatic foci with abscesses in almost any location, including the myocardium, pericardium, peritoneum, brain, kidney, bowel wall, perirectal region, prosthetic and native heart valves, muscle and skin, and hemodialysis shunts (Table 137-2).[57,143,168] Bacteremia is rarely detected, although molecular studies and clinical findings suggest that it is common.[57,136,168] Elaboration of toxins by *Legionella* has been postulated to account for some aspects of the systemic disease, but supporting evidence is not convincing.[168]

Host cytokine production in response to *L. pneumophila* infection likely causes most of the acute toxicity of the disease. Cell culture and animal experiments show that tumor necrosis factor, interferon-γ, and other cytokines are produced during *L. pneumophila* infection.[11,183] Humans infected with *L. pneumophila* have been shown to produce a number of cytokines during infection, as well as inflammatory markers such as procalcitonin, neopterin, and C-reactive protein.[43,66,166]

TABLE 137-2 **Diseases Caused by *Legionella***

Pneumonia (Legionnaires' Disease)

Associated with Pneumonia

Bacteremia
Bowel wall abscess
Brain abscess
Colitis
Endocarditis
Hemodialysis shunt infection
Myopericarditis
Myositis, cellulitis
Peritonitis
Perirectal abscess
Pleural empyema
Renal abscess

Not Associated with Pneumonia

Colitis
Lymphadenitis
Peritoneal dialysis-related peritonitis
Pleural empyema
Endocarditis
Sepsis syndrome
Sinusitis
Soft tissue infections
Wound infection

Pontiac Fever (? Toxin-Mediated)

Episodes of recurrent or relapsing LD in patients with elevated antibody titers support experimental evidence suggesting that the humoral immune system plays a minor role in this disease.[57] Also supporting this evidence is the rarity of LD case reports in patients with hypogammaglobulinemia or other diseases in which the humoral immune system primarily is deficient. The rise of specific antibody levels does not seem to have any clinical correlates, nor does the absolute antibody level.[57] Patients may recover from LD without any significant increase in antibody levels, again providing indirect evidence of the limited role of the humoral immune system.

Several studies have shown that passive or active immunization is protective against intraperitoneal and subcutaneous chamber infection in rats, mice, and guinea pigs.[5,60,91,153] Preopsonization of *L. pneumophila* before intratracheal inoculation of hamsters was found to be partially protective, but several investigators have shown that active immunization fails to protect against pneumonia after intratracheal, aerosol, or intranasal inoculation of large numbers of *L. pneumophila* bacteria.[9,48,60] Protective immunity to pulmonary challenge in the guinea pig can be achieved after sublethal infection or infection with an avirulent mutant strain and by vaccination with an *L. pneumophila*–derived metalloprotease or outer-membrane protein.[12-15,179]

Evidence for the importance of the cellular immune system in preventing infection largely is indirect and based on the greater prevalence of LD in immunosuppressed patients with cellular immunodeficiencies.[57,168] Curiously, LD in patients with acquired immunodeficiency syndrome (AIDS) has been reported infrequently, although one epidemiologic survey showed that patients with AIDS had a disease attack rate approximately 40 times greater than that in the normal population.[14,15,124,179]

The pathogenesis of Pontiac fever is not understood. No differences have been detected between an *L. pneumophila* strain isolated from an outbreak of Pontiac fever and strains isolated from outbreaks of LD; these studies have included examination of virulence in an animal model, toxin production, and biochemical characteristics.[168] Detailed clinical studies of patients with Pontiac fever have not been performed, which renders producing an experimental model of this disease difficult. Because it has a short incubation time, which can be as brief as 12 hours, this disease likely does not represent widespread bacterial multiplication within the body, and more likely it represents a toxin-induced or allergic disease. In fact, the link between *Legionella* and Pontiac fever is circumstantial; entirely possible is that other microbes, or their toxins, coexisting with *Legionella* may cause the disease.[77,106] The clinical syndromes most like Pontiac fever are bathwater fever, humidifier fever, and extrinsic allergic alveolitis; these syndromes are thought to be caused either by the direct toxic activity of inhaled endotoxin or by an allergic reaction to microorganisms, most particularly amebae such as *Naegleria* spp.[58,133,156] *L. pneumophila* makes a poorly active endotoxin, suggesting that if inhaled endotoxin is the cause of Pontiac fever then the endotoxin comes from non-*Legionella* bacteria. Support for inhaled endotoxin as the cause of Pontiac fever is found

in two studies of endotoxin present at outbreaks of the disease.[30,70]

CLINICAL MANIFESTATIONS OF LEGIONNAIRES' DISEASE

Signs and Symptoms

Legionnaires' disease usually presents as pneumonia.[57] The pneumonia is atypical in that usual pathogenic bacteria generally are not isolated from respiratory tract secretions or blood and patients do not respond, except fortuitously, to antimicrobial agents commonly used to treat pneumonia in adults (e.g., penicillins, cephalosporins, and aminoglycosides). Beyond this, considerable speculation and controversy continue over whether a distinct clinical syndrome exists. Several prospective studies of both community-acquired and nosocomial LD have failed to demonstrate any clinical, radiographic, or nonspecific laboratory features that distinguish LD from other common causes of pneumonia.[50,65,84] The classic clinical findings are reviewed because many physicians consider that they are distinctive for *Legionella* pneumonias. Whether LD in children mimics the clinical findings in adults is unknown because of the rare instances of well-documented pediatric cases and the possibility of spectrum bias.

The onset of pneumonia may be either insidious or abrupt. Recurrent chills, abdominal pains, myalgia, headache, malaise, anorexia, and severe fatigue are common findings. Diarrhea, consisting of loose, nonbloody stools several times a day, occurs in 30 to 40 percent of cases. Fever may be low grade or absent initially. Over the course of a day to several days, these nonspecific symptoms gradually worsen, often resulting in severe debilitation. These symptoms gradually increase in severity, as measured by the median time of 4 to 5 days to present for hospital care, at which time the patient can be quite ill. Noteworthy is the frequent absence of symptoms referable to the respiratory system. Rash, splenomegaly, adenopathy, and rhinorrhea are exceptionally uncommon findings. Physical examination early in the illness generally is remarkable for a paucity of localizing findings and the frequent impression that the patient has an influenzal or typhoidal illness.

Within a day to several days after onset, the patient usually, but not always, develops high fever. Respiratory complaints, especially dyspnea and pleuritic chest pains, may become prominent. Cough usually is not a major complaint, although it is common. The sputum almost never is frankly purulent; blood-streaked sputum or frank hemoptysis is observed in 20 to 30 percent of patients. Many patients experience confusion, cerebellar ataxia, lethargy, agitation, or some other neurologic disorder. Severe abdominal or back pain may occur, sometimes with localization. Physical examination at this time reveals a "toxic" febrile patient with apparent multisystem disease. Chest examination usually discloses findings of consolidating pneumonia. Depending on the location and stage of consolidation, rales may or may not be heard. Pleural friction rubs or signs of pleural effusion can be observed. Despite frequent symptoms of abdominal pain, signs of peritoneal irritation, such as decreased bowel sounds or rebound tenderness, seldom are detected. Signs of meningeal irritation occur rarely.

Most previously healthy patients recover without specific therapy, usually by day 7 to 10 of illness. Patients with mild disease not requiring hospital admission appear to do quite well without antibiotic therapy.[176] Those who do not recover usually die of progressive respiratory failure, along with failure of other organ systems. Empyema, pulmonary cavitation, renal failure, memory loss, fatigue, and neurologic disorders all are potential complications and may persist for weeks to months after onset of the disease. A variety of extrapulmonary diseases may very rarely occur as the result of LD, most common of which are pancreatitis,[130] hepatitis, and myopericarditis.[6,126] Myositis, as measured by elevated serum creatine kinase levels occurs commonly, with severe muscle disease such as rhabdomyolysis being a rare complication.[105,177] Most of these extrapulmonary diseases improve rapidly with resolution of the pneumonia.

Focal infection without pneumonia is a very rare manifestation of *Legionella* spp. infections, with only a handful of cases reported (Table 137-2). Direct inoculation of bacteria into surgical or traumatic wounds is the usual mode of pathogenesis, although bacteremia and bacteremic metastatic infection have been reported.[127,148] *Legionella* spp. have recently been implicated by polymerase chain reaction (PCR) testing as a cause of persistent cervical lymphadenitis in children, often after hot tub exposure. Because the involved lymph nodes were stain and culture negative for *Legionella* and because the patients failed to improve with macrolide therapy, the study needs verification.[35]

Radiographic Findings

The radiographic hallmark of LD is an acinar filling pattern with consolidation.[40,57,168] There is no distinctive predilection for any lung region; pleural-based consolidation and bilateral infiltrates may occur. Nodular infiltrates may be seen, as may cavitation in the areas of original consolidation. Purely interstitial infiltrates are distinctly uncommon findings in established disease but rarely occur very early in the disease process; these interstitial infiltrates rapidly progress to consolidating ones within a day or so. High-resolution computed tomography (CT) of lungs of patients with LD often reveals a mixture of ground-glass opacities and consolidation, and in the convalescent phase an organizing pneumonia pattern may be seen.[86,184] Pleural effusion, with or without parenchymal infiltrates, is a common finding in chest CT scans and less commonly visualized in plain films. Pleural effusion has been documented as the only chest finding in patients treated early with specific antibiotic therapy.

Laboratory Findings

Nonspecific abnormal laboratory results are common in patients with LD.[40,57] Hematologic abnormalities may include leukocytosis or leukopenia, usually with a left shift; lymphopenia; thrombocytosis; and disseminated

intravascular coagulation. Proteinuria and pyuria are common findings; myoglobinuria also may be present. Hyponatremia and hypophosphatemia commonly occur, as do elevations of aminotransferase enzymes, bilirubin, alkaline phosphatase, lactate dehydrogenase, and creatine kinase. Elevated ferritin is also seen. Severe azotemia occurs, although rarely. Elevation of cold agglutinin titers, cold agglutinin–induced hemolytic anemia, and elevation of complement fixation titers to *Mycoplasma pneumoniae* may also occur. Arterial oxygenation usually is depressed in relation to the extent of pneumonia.

Laboratory diagnosis of LD is best accomplished by urine antigen testing, but this test can be falsely negative. Recovery of *Legionella* from sputum or other lower respiratory tract secretions or tissues requires specialized laboratory testing and is insensitive.[56] Other specific diagnostic methods are detection of *L. pneumophila* antigen by immunofluorescent microscopy and detection of *Legionella* DNA in respiratory tract specimens using the PCR assay. Serologic diagnosis is of uncertain value in children, but it is useful in adults.

Sputum is plated on buffered charcoal yeast extract medium supplemented with α-ketoglutaric acid (BCYE-α) and on BCYE-α supplemented with antibiotics.[56] *Legionella* organisms grow on these media 3 to 7 days after inoculation and incubation at 35° C in air. Culture diagnosis has a higher yield than does immunofluorescent microscopy or serology and yields no false-positive results. Sputum culture yield depends on the severity of illness; it is close to 100 percent for LD affecting immunocompromised patients and patients with ventilator-dependent pneumonia but can be as low as 10 to 25 percent in mild LD. Blood and pleural fluid cultures have very low yield, less than 5 to 10 percent, with blood culture only positive in severely ill, usually immunocompromised, patients.

Immunofluorescent microscopy for *L. pneumophila* is a rapid and highly specific (99.9%), but insensitive (50% to 70% sensitivity), technique for diagnosis.[56] It can be performed on sputum or lung tissue in 1 to 2 hours after receipt of the specimen. The test is technically demanding, requiring extensive expertise and training; use of the test by inexperienced technologists yields more false- than true-positive findings. Because of these difficulties few laboratories perform this testing.

Serologic testing is of most value in epidemiologic studies and of least value in the acute diagnosis of sporadic cases.[54] As many as 25 percent of patients with culture-documented disease fail to undergo seroconversion against the homologous serotype; this failure is not related solely to early treatment or immunosuppression, although these factors may cause failure of antibody formation. As long as 3 months may be required for antibody levels to increase after onset of illness; the median time is approximately 2 weeks. Also, as with any other means of immunologic diagnosis of this disease, the multiplicity of antigenic types renders serologic testing extremely cumbersome. Because between 5 and more than 25 percent of the normal population have antibody titers as high as 1:512 to *Legionella*, only a fourfold rise in titer is considered significant. The specificity is highest for *L. pneumophila* serogroup 1 and much lower for other species. Commercial testing kits can yield false-positive results and may also be insensitive in comparison to reference laboratory tests. Only paired samples, drawn 3 to 6 weeks apart, and tested in parallel, should be tested. Specimens taken up to 9 to 12 weeks after onset of the disease should be tested if earlier samples reveal no changes. A negative serologic result does not exclude disease.

The specificity of seroconversion to *L. pneumophila* serogroup 1 ranges from 95 to 99 percent.[54] Cross-reactive antibodies may be found in the serum of patients with leptospirosis, melioidosis, *Bacteroides fragilis* infections, *P. aeruginosa* infections, *C. jejuni* enteritis, and possibly *Haemophilus influenzae* or enteric bacterial infections. However, even 99 percent specificity is not sufficient for certainty of diagnosis of a sporadic case. If the estimated 1 percent prevalence of LD in children is correct, fewer than 50 percent of all seroconversions yield truly positive results (positive predictive accuracy of 45%). This observation, combined with the studies cited previously showing age-related elevations of anti-*Legionella* antibody in young asymptomatic children, renders serologic diagnosis of pediatric LD highly suspect.

Detection of soluble bacterial antigen in urine can be used to diagnose *L. pneumophila* serogroup 1 infections successfully and is the most sensitive and most rapid test for community-acquired LD.[53,56] The major drawback of this test is that it preferentially detects only *L. pneumophila* serogroup 1 infections. Otherwise, it has an excellent sensitivity (90% to 99% vs. culture and 60% to 70% in patients with community-acquired LD) and extraordinary specificity (at least 99.9%). The antigen test may be positive when sputum culture for *L. pneumophila* serogroup 1 is negative, especially in previously treated patients and in epidemics of LD. In nosocomial LD, and in LD of immunocompromised patients the antigen test is less sensitive, because other serogroups and other *Legionella* spp. become likely in these groups. Although the antigen test detects 60 to 70 percent of those with community-acquired LD, fewer than 40 percent of patients with nosocomial cases test positive. A negative urine antigen test should not be used as the sole reason to stop therapy for LD.[49]

The PCR test has been used to detect *L. pneumophila* in sputum, serum, and urine. Whereas both sensitive and specific PCR tests are available, none is commercially available in the United States. Several U.S. reference laboratories perform home-brew PCR assays. PCR testing appears to add about 11 percent additional yield to that obtained by urine antigen testing and may not be cost effective.[46]

With the exception of the urine antigen test, none of the nonculture tests is as sensitive as is culture diagnosis. Culture should be performed in every case; if desired, the other tests can be used to provide same day answers. Because none of the *Legionella*-specific tests is 100 percent sensitive, the clinician sometimes must treat for LD in the absence of confirmatory laboratory tests. One or 2 days of therapy with erythromycin apparently does not affect the sensitivity of the diagnostic tests, although therapy with azithromycin or levofloxacin can affect culture yield. Therapy should not be withheld pending results of laboratory tests.

TREATMENT

No adequately sized prospective clinical studies of antimicrobial therapy for LD have been performed. All recommendations regarding therapy are based on retrospective and small prospective clinical studies, as well as laboratory studies.

Because of the superiority of newer fluoroquinolone agents and azithromycin in experimental nonhuman studies, and on the basis of uncontrolled trials of these agents in adults with LD, the current recommendation is that adults with severe LD and immunocompromised patients with the disease be given either azithromycin or fluoroquinolone therapy, rather than erythromycin or clarithromycin therapy. In one prospective observational study in adults that compared levofloxacin-treated patients with those who received the less-effective macrolides, erythromycin and clarithromycin, those who received levofloxacin had a significantly shorter time to defervescence and a shorter length of hospital stay than those treated with erythromycin or clarithromycin.[137] However, other studies have shown no significant differences in outcome for patients treated with clarithromycin or levofloxacin.[63,83] Nonhospitalized patients with mild LD can be treated equally well with any of the specific therapies, including doxycycline, newer fluoroquinolones, azithromycin, clarithromycin, or erythromycin.[51,52,121] No inherent reason, other than age-specific drug toxicity, precludes these guidelines for children.

Fluoroquinolone antimicrobial agents generally are contraindicated in children, although the evidence suggests that the risk for toxicity from short courses of therapy is low. The scope of this chapter does not allow the discussion of the use of these drugs in children, but in some cases the benefit of using these drugs in severe LD may be greater than the potential risk for development of drug side effects.

Clarithromycin 7.5 mg/kg every 12 hours may be used in place of erythromycin, although there are good laboratory and reasonable clinical data showing that this drug is no more effective for LD than is erythromycin.[17,144] Clarithromycin is not FDA approved for the treatment of LD. Azithromycin has largely replaced erythromycin as the drug of choice for the treatment of LD in children, primarily because of the adverse effects associated with erythromycin, especially gastrointestinal effects and severe phlebitis when given intravenously.

Azithromycin, which is approved for use in children in the United States, is as active against *Legionella* bacteria as are the newer fluoroquinolone agents, making it a candidate for the drug of choice for severe pediatric LD or for LD in immunocompromised children. The drug dose for children for this type of pneumonia has not been studied, but based on extrapolation from adults, a dose of 10 mg/kg (maximum, 500 mg) given once daily for 3 to 5 days should be sufficient in most cases, with extended duration of therapy (7 to 10 days) for severely immunocompromised patients. Azithromycin is available in both intravenous and oral forms and is approved by the FDA for use in adults with LD.

Newer fluoroquinolone agents, along with azithromycin, are considered to be the drugs of choice for the treatment of severe LD in adults. Levofloxacin is used commonly in the United States to treat this disease, in a dosage of 500 mg daily for 10 to 14 days. No dosage guidelines exist for the treatment of pediatric LD because of the potential for drug toxicity in this population. Levofloxacin is the only available quinolone agent approved by the FDA for the treatment of LD in adults; note that neither ciprofloxacin nor moxifloxacin is approved for this indication. Ciprofloxacin appears to be effective for this indication on the basis of case reports, and moxifloxacin may be effective based on limited laboratory studies. Combining azithromycin with levofloxacin has been reported to be more effective than levofloxacin alone in a very small inadequately controlled study; until this is confirmed in larger studies, use of this combination should be avoided.[36]

Alternative drugs to macrolides or quinolones include doxycycline and possibly co-trimoxazole. Neither of these agents has been approved for this use by the FDA, although limited clinical and experimental data support their effectiveness, more so for doxycycline than for co-trimoxazole. Co-trimoxazole should only be used if there is no possibility of using a macrolide, tetracycline, or fluoroquinolone drug. Doxycycline, because of its high lipid solubility, may be more effective than is tetracycline, although whether this is true remains conjecture. The dosage of doxycycline used in children is 2 to 4 mg/kg/day, given in one or two doses. Use of tetracyclines is associated with the risk for dental staining in children younger than 8 years of age. The daily co-trimoxazole dosage is 15 to 20 mg/kg of the trimethoprim component and 75 to 100 mg/kg of the sulfamethoxazole component in three divided doses; cotrimoxazole use in newborns may cause kernicterus. Duration of therapy for these drugs is the same as that for erythromycin.

Rifampin combined with erythromycin is a less favorable alternative to the use of azithromycin by itself. However, if erythromycin is a preferred therapy for reasons of cost, toxicity, or availability, then rifampin probably should be added in the case of severe disease or in immunocompromised patients. The dosage is 16 to 20 mg/kg/day, given in two divided doses for the first 2 days of therapy. This drug rapidly diminishes bacterial counts in experimental disease and has been effective in combination with other drugs in patients in whom erythromycin therapy has failed. Use of this drug for treatment of LD is not approved by the FDA. A recent small nonrandomized study has shown that adult patients treated with combination rifampin and clarithromycin had significantly longer hospital lengths of stay than did those treated with clarithromycin alone, with a trend to worse outcome in other outcome measures in those given combined therapy. Because the largest effect of rifampin in experimental animals is in the first 1 or 2 days of therapy, giving this drug for more than 2 days may do more harm than good. The best policy would be to use an alternative to erythromycin or clarithromycin in severe illness, and thus to avoid the question of combination therapy.[80] There is no good clinical or laboratory evidence that combining rifampin with either azithromycin or a fluoroquinolone is beneficial.

Treatment of extrapulmonary foci of infection does not appear to differ from treatment of LD without

extrapulmonary disease. The duration of therapy and indications for surgical drainage need to be assessed individually in these cases.

Response to Treatment

Most patients improve dramatically within a few days after initiation of specific therapy.[40,50,57,168] Response may be as rapid as 6 hours after administration of the first dose of therapy. Patients regain their appetite, lose symptoms of myalgia and fatigue, and feel better overall within a few days. Up to a week may be required for a patient to become completely afebrile, with longer periods sometimes required for severely immunosuppressed patients. The chest radiograph changes slowly and even may appear to worsen despite overall clinical improvement; progressive consolidation after 3 to 4 days of intravenous antimicrobial therapy is unusual. Because very severe immunocompromise can impair the ability of the cellular immune system to completely clear infection, some of these patients are prone to relapses of disease once antimicrobial therapy has been stopped, even after a long course of therapy.[85,159] The relapses can be insidious and manifest only as renewed immunosuppression. Profoundly immunosuppressed patients with LD who do not have recovery of immune function may benefit from prolonged suppressive antimicrobial therapy until they recover sufficient cellular immune function.

Failure to respond to appropriate therapy can be due to severe underlying disease, advanced pneumonia at presentation, coinfection with another microorganism, mistaken diagnosis, and complications of pneumonia. These complications can include pulmonary embolism, empyema, pancreatitis, myopericarditis, hepatitis, and soft tissue infections.

The mortality rate in otherwise healthy adults who are treated promptly is approximately 5 percent, whereas in treated immunosuppressed patients it is approximately 20 percent. Untreated fatality rates range from 15 to 20 percent in normally healthy patients and upward of 80 percent in immunosuppressed patients. However, very mild community-acquired LD in healthy adults that does not require hospitalization appears to have negligible mortality and may not require specific antibiotic therapy, although such a course may not be advisable.[176] Even in previously healthy patients, delayed therapy and development of respiratory failure are exceptionally poor prognostic factors.[62,142,170]

DIFFERENTIAL DIAGNOSIS

Other causes of atypical, or culture-negative, pneumonia may closely resemble LD. Mycoplasmal pneumonia usually is a milder illness not requiring hospitalization. Cough is a prominent symptom in mycoplasmal pneumonia, whereas it is not in LD. Neither rash nor otitis is found in LD. Laboratory abnormalities also are found more commonly in LD. Serologic testing may provide positive results for both diseases, a confusing finding that can be clarified by performing specific laboratory testing. Fortunately the treatment is the same for both diseases.

Psittacosis and Q fever may closely resemble LD. A history of bird or cattle exposure may be helpful, but its absence does not exclude either of these zoonoses. An interstitial rather than an acinar-filling infiltrate on chest radiograph would be point against LD. Pathogen-specific laboratory tests help in this differential diagnosis. A tetracycline can be used successfully for all three of these diseases.

Early in their evolution, some diseases may resemble LD. They include typhoid fever, acute coccidioidomycosis, influenza, the typhus or spotted fevers, and leptospirosis. Distinguishing these diseases on the basis of their clinical evolution, the laboratory results, and the exposure or travel history generally is easy.

Tularemia may pose a problem in differential diagnosis because immunologic test results for LD can be falsely positive in tularemia and because some of the growth characteristics of *Francisella tularensis* closely resemble those of *Legionella*. In regions endemic for tularemia, clinicians must work closely with the laboratory to facilitate this differential diagnosis. One case record of tularemia misdiagnosed as LD reported that the patient responded to erythromycin therapy.

Dual infection sometimes occurs in LD. Coexistence of LD with pneumonia caused by *Mycobacterium tuberculosis*, pneumococcus, *H. influenzae*, *Neisseria meningitidis*, *Pneumocystis jiroveci*, *Moraxella catarrhalis*, and various viral agents has been reported. Thus, dual infection should be suspected in patients not responding to therapy for pneumonia. Pathogen-specific laboratory tests often are useful in these cases.

CLINICAL SYNDROMES CAUSED BY OTHER *LEGIONELLA* SPECIES

Relatively few cases have been reported of disease caused by the non–*L. pneumophila Legionella* spp., with the major exception of *L. longbeachae*.[3,64,181] These reported cases have few differences in clinical findings, diagnostic methods, or treatment. Almost all cases of LD due to *Legionella* spp. other than *L. pneumophila* or *L. longbeachae*, have been reported to occur in immunocompromised patients; and because of these patients' underlying diseases and immunocompromise, the pneumonia severity and rapidity of response to therapy may be compromised. The lower frequency of these infections in normal hosts is most likely due to decreased virulence of the bacteria. *L. longbeachae* has a different mechanism of transmission than does *L. pneumophila* because *L. longbeachae* infections do not appear to be transmitted from water sources but rather from nonsterile potting soil.[25,108,163] Exposure to potting soil that has been exposed to high ambient temperatures, not washing hands after using the soil, and cigarette smoking are the major risk factors for this disease. The mode of spread and nosocomial reservoirs of the *Legionella* spp. other than *L. pneumophila* and *L. longbeachae* are not well defined. These infections are unlikely to be diagnosed by immunologic means, except for *L. longbeachae* infection in endemic regions (Australia and New Zealand), because few laboratories routinely test for all possible species. As with *L. pneumophila*, and even

more so for the non–*L. pneumophila Legionella* spp., treatment sometimes must be based solely on clinical suspicion without the benefit of confirmatory laboratory tests.

PONTIAC FEVER

Clinical Manifestations

Fever, myalgia, malaise, chills, and headache are the most common symptoms of Pontiac fever.[77] The symptoms may begin suddenly or have a more gradual onset for several hours. Many symptoms are respiratory, such as dry nonproductive cough, chest pain, and pharyngitis. Nausea is a common manifestation, but diarrhea and vomiting are less frequent. Neurologic symptoms including dizziness, confusion, and poor coordination also have been reported. Pontiac fever symptoms usually are at their worst within a day after onset of illness and gradually resolve over a 2- to 7-day period. Physical examination shows only tachycardia and fever. Leukocytosis has been the only laboratory abnormality reported. Chest radiographs show no abnormalities. Pulmonary function testing has not been performed in patients with Pontiac fever. Rechallenge by return to the contaminated building in the original Pontiac outbreak produced only a mild illness compared with first exposure; the length of time between first and second exposure was not stated clearly.

Diagnosis

The diagnosis of Pontiac fever primarily is one of exclusion.[40,168] Significant rises in anti-*Legionella* antibody level, combined with characteristic symptoms, and isolation of *Legionella* from an aerosol source are the diagnostic criteria. Rarely, *L. pneumophila* urinary antigen may be detected.[55] Detailed epidemiologic and environmental studies must be performed to diagnose Pontiac fever specifically. This is because of the nonspecificity of the symptoms and the ubiquitous presence of environmental *Legionella*. Definitive diagnosis of sporadic cases is difficult.

Treatment

Antimicrobial therapy does not appear to be effective for the treatment of Pontiac fever. Removal of the patient from the area of the contaminated water source appears to be the best means of management.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Alexander NT, Fields BS, Hicks LA. Epidemiology of reported pediatric Legionnaires' disease (LD) in the United States, 1980–2004 [abstract]. Presented before the 48th annual meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, 2008.
3. Amodeo MR, Murdoch DR, Pithie AD. Legionnaires' disease caused by *Legionella longbeachae* and *Legionella pneumophila:* comparison of clinical features, host-related risk factors, and outcomes. *Clin Microbiol Infect* 2010;**16**:1405–7.
26. Campese C, Bitar D, Jarraud S, et al. Progress in the surveillance and control of *Legionella* infection in France, 1998–2008. *Int J Infect Dis* 2011;**15**:e30–7.
31. Cazalet C, Jarraud S, Ghavi-Helm Y, et al. Multigenome analysis identifies a worldwide distributed epidemic *Legionella pneumophila* clone that emerged within a highly diverse species. *Genome Res* 2008;**18**:431–41.
33. Centers for Disease Control and Prevention. Legionellosis—United States, 2000–2009. *MMWR Morb Mortal Wkly Rep* 2011; **60**:1083–6.
35. Choi P, Qin X, Chen EY, et al. Polymerase chain reaction for pathogen identification in persistent pediatric cervical lymphadenitis. *Arch Otolaryngol Head Neck Surg* 2009;**135**:243–8.
43. de Jager CP, de Wit NC, Weers-Pothoff G, et al. Procalcitonin kinetics in *Legionella pneumophila* pneumonia. *Clin Microbiol Infect* 2009;**15**:1020–5.
56. Edelstein PH. Legionella. In: Versalovic J, Carroll K, Funke G, et al, editors. *Manual of clinical microbiology*, vol. 10. Washington, D.C.: ASM Press; 2011. p. 770–85.
72. Fontana MF, Banga S, Barry KC, et al. Secreted bacterial effectors that inhibit host protein synthesis are critical for induction of the innate immune response to virulent *Legionella pneumophila*. *PLoS Pathog* 2011;**7**:e1001289.
79. Gomez-Valero L, Rusniok C, Buchrieser C. *Legionella pneumophila:* population genetics, phylogeny and genomics. *Infect Genet Evol* 2009;**9**:727–39.
83. Griffin AT, Peyrani P, Wiemken T, et al. Macrolides versus quinolones in *Legionella pneumonia:* results from the Community-Acquired Pneumonia Organization international study. *Int J Tuber Lung Dis* 2010;**14**:495–9.
85. Han JH, Nguyen JC, Harada S, et al. Relapsing *Legionella pneumophila* cellulitis: a case report and review of the literature. *J Infect Chemother* 2010;**16**:439–42.
86. Haroon A, Higa F, Hibiya K, et al. Organizing pneumonia pattern in the follow-up CT of *Legionella*-infected patients. *J Infect Chemother* 2011;**17**:493–8.
97. Hubber A, Roy CR. Modulation of host cell function by *Legionella pneumophila* type IV effectors [review]. *Annu Rev Cell Dev Biol* 2010;**26**:261–83.
108. Kozak NA, Buss M, Lucas CE, et al. Virulence factors encoded by *Legionella longbeachae* identified on the basis of the genome sequence analysis of clinical isolate D-4968. *J Bacteriol* 2010;**192**: 1030–44.
112. Lassmann B, Poetschke M, Ninteretse B, et al. Community-acquired pneumonia in children in Lambarene, Gabon. *Am J Trop Med Hyg* 2008;**79**:109–14.
113. Lau HY, Ashbolt NJ. The role of biofilms and protozoa in *Legionella* pathogenesis: implications for drinking water. *J Appl Microbiol* 2009;**107**:368–78.
119. Lurie-Weinberger MN, Gomez-Valero L, Merault N, et al. The origins of eukaryotic-like proteins in *Legionella pneumophila*. *IJMM Int J Med Microbiol* 2010;**300**:470–81.
122. Mariette X, Gottenberg JE, Ravaud P, et al. Registries in rheumatoid arthritis and autoimmune diseases: data from the French registries. *Rheumatology* 2011;**50**:222–9.
139. Neunuebel MR, Chen Y, Gaspar AH, et al. De-AMPylation of the small GTPase Rab1 by the pathogen *Legionella pneumophila*. *Science* 2011;**333**:453–6.
143. Pearce MM, Theodoropoulos N, Noskin GA, et al. Native valve endocarditis due to a novel strain of *Legionella*. *J Clin Microbiol* 2011;**49**:3340–2.
158. Shachor-Meyouhas Y, Kassis I, Bamberger E, et al. Fatal hospital-acquired *Legionella pneumonia* in a neonate. *Pediatr Infect Dis J* 2010;**29**:280–1.
173. Unit for Surveillance and Control of Communicable Diseases. Legionnaires disease in a neonatal unit of a private hospital, Cyprus, December 2008: preliminary outbreak report. *Euro Surveill* 2009:**14**:pii: 19090.
176. von Baum H, Ewig S, Marre R, et al. Community-acquired *Legionella* pneumonia: new insights from the German competence network for community acquired pneumonia. *Clin Infect Dis* 2008;**46**:1356–64.
181. Whiley H, Bentham R. *Legionella longbeachae* and legionellosis. *Emerg Infect Dis* 2011;**17**:579–83.
184. Yu H, Higa F, Hibiya K, et al. Computed tomographic features of 23 sporadic cases with *Legionella pneumophila* pneumonia. *Eur J Radiol* 2010;**74**:e73–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abernathy-Carver KJ, Fan LL, Boguniewicz M, et al. *Legionella* and *Pneumocystis* pneumonias in asthmatic children on high doses of systemic steroids. *Pediatr Pulmonol* 1994;**18**:135–8.
2. Alexander NT, Fields BS, Hicks LA. Epidemiology of reported pediatric Legionnaires' disease (LD) in the United States, 1980–2004 [abstract]. Presented before the 48th annual meeting of the Interscience Conference on Antimicrobial Agents and Chemotherapy, 2008.
3. Amodeo MR, Murdoch DR, Pithie AD. Legionnaires' disease caused by *Legionella longbeachae* and *Legionella pneumophila:* comparison of clinical features, host-related risk factors, and outcomes. *Clin Microbiol Infect* 2010;**16**:1405–7.
4. Andersen RD, Lauer BA, Fraser DW, et al. Infections with *Legionella pneumophila* in children. *J Infect Dis* 1981;**143**:386–90.
5. Arko RJ, Wong KH, Feeley JC. Immunologic factors affecting the in-vivo and in-vitro survival of the legionnaires' disease bacterium. *Ann Intern Med* 1979;**90**:680–3.
6. Armengol S, Domingo C, Mesalles E. Myocarditis: a rare complication during *Legionella* infection. *Int J Cardiol* 1992;**37**: 418–20.
7. Aubert G, Bornstein N, Rayet I, et al. Nosocomial infection with *Legionella pneumophila* serogroup 1 and 8 in a neonate. *Scand J Infect Dis* 1990;**22**:367–70.
8. Barker J, Scaife H, Brown MR. Intraphagocytic growth induces an antibiotic-resistant phenotype of *Legionella pneumophila*. *Antimicrob Agents Chemother* 1995;**39**:2684–8.
9. Baskerville A, Fitzgeorge RB, Gibson DH, et al. Pathological and bacteriological findings after aerosol *Legionella pneumophila* infection of susceptible, convalescent, and antibiotic-treated animals. In: Thornsberry C, Balows A, Feeley JC, et al, editors. *Legionella. Presented before the Proceedings of the 2nd International Symposium*. Washington, D.C.: American Society for Microbiology; 1984. p. 131–2.
10. Beyer P, Kahn D, Horbach J, et al. Unusual progression of a *Legionella pneumophila* infection in a young child. *Eur J Pediatr* 1984;**141**:173–5.
11. Blanchard DK, Friedman H, Klein TW, et al. Induction of interferon-gamma and tumor necrosis factor by *Legionella pneumophila:* augmentation of human neutrophil bactericidal activity. *J Leukoc Biol* 1989;**45**:538–45.
12. Blander SJ, Breiman RF, Horwitz MA. A live avirulent mutant *Legionella pneumophila* vaccine induces protective immunity against lethal aerosol challenge. *J Clin Invest* 1989;**83**:810–5.
13. Blander SJ, Horwitz MA. Vaccination with the major secretory protein of *Legionella pneumophila* induces cell-mediated and protective immunity in a guinea pig model of legionnaires' disease. *J Exp Med* 1989;**169**:691–705.
14. Blander SJ, Horwitz MA. Vaccination with *Legionella pneumophila* membranes induces cell-mediated and protective immunity in a guinea pig model of legionnaires' disease. Protective immunity independent of the major secretory protein of *Legionella pneumophila*. *J Clin Invest* 1991;**87**:1054–9.
15. Blander SJ, Horwitz MA. Major cytoplasmic membrane protein of *Legionella pneumophila*, a genus common antigen and member of the hsp 60 family of heat shock proteins, induces protective immunity in a guinea pig model of legionnaires' disease. *J Clin Invest* 1993;**91**:717–23.
16. Blatt SP, Parkinson MD, Pace E, et al. Nosocomial legionnaires' disease: aspiration as a primary mode of disease acquisition. *Am J Med* 1993;**95**:16–22.
17. Blázquez Garrido RM, Espinosa Parra FJ, Alemany Francés L, et al. Antimicrobial chemotherapy for legionnaires disease: levofloxacin versus macrolides. *Clin Infect Dis* 2005;**40**:800–6.
18. Brady MT. Nosocomial legionnaires disease in a children's hospital. *J Pediatr* 1989;**115**:46–50.
19. Bridge JA, Edelstein PH. Oropharyngeal colonization with *Legionella pneumophila*. *J Clin Microbiol* 1983;**18**:1108–12.
20. Bruggemann H, Cazalet C, Buchrieser C. Adaptation of *Legionella pneumophila* to the host environment: role of protein secretion, effectors and eukaryotic-like proteins. *Curr Opin Microbiol* 2006;**9**: 86–94.
21. bu-Zant A, Jones S, Asare R, et al. Anti-apoptotic signalling by the Dot/Icm secretion system of *L. pneumophila*. *Cell Microbiol* 2007;**9**:246–64.
22. Burnsed LJ, Hicks LA, Smithee LM, et al. A large, travel-associated outbreak of legionellosis among hotel guests: utility of the urine antigen assay in confirming Pontiac fever. *ClinInfect Dis* 2007;**44**:222–8.
23. Byrd TF, Horwitz MA. Interferon gamma–activated human monocytes downregulate transferrin receptors and inhibit the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron. *J Clin Invest* 1989;**83**:1457–65.
24. Byrd TF, Horwitz MA. Chloroquine inhibits the intracellular multiplication of *Legionella pneumophila* by limiting the availability of iron: a potential new mechanism for the therapeutic effect of chloroquine against intracellular pathogens. *J Clin Invest* 1991; **88**:351–7.
25. Cameron S, Roder D, Walker C, et al. Epidemiological characteristics of *Legionella* infection in South Australia: implications for disease control. *Aust NZ J Med* 1991;**21**:65–70.
26. Campese C, Bitar D, Jarraud S, et al. Progress in the surveillance and control of *Legionella* infection in France, 1998–2008. *Int J Infect Dis* 2011;**15**:e30–7.
27. Campins M, Ferrer A, Callis L, et al. Nosocomial legionnaire's disease in a children's hospital. *Pediatr Infect Dis J* 2000;**19**: 228–34.
28. Carlson NC, Kuskie MR, Dobyns EL, et al. Legionellosis in children: an expanding spectrum. *Pediatr Infect Dis J* 1990;**9**: 133–7.
29. Carratala J, Gudiol F, Pallares R, et al. Risk factors for nosocomial *Legionella pneumophila* pneumonia. *Am J Respir Crit Care Med* 1994;**149**:625–9.
30. Castor ML, Wagstrom EA, Danila RN, et al. An outbreak of Pontiac fever with respiratory distress among workers performing high-pressure cleaning at a sugar-beet processing plant. *J Infect Dis* 2005;**191**:1530–7.
31. Cazalet C, Jarraud S, Ghavi-Helm Y, et al. Multigenome analysis identifies a worldwide distributed epidemic *Legionella pneumophila* clone that emerged within a highly diverse species. *Genome Res* 2008;**18**:431–41.
32. Cazalet C, Rusniok C, Bruggemann H, et al. Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nat Genet* 2004;**36**:1165–73.
33. Centers for Disease Control and Prevention. Legionellosis—United States, 2000–2009. *MMWR Morb Mortal Wkly Rep* 2011; **60**:1083–6.
34. Chien M, Morozova I, Shi S, et al. The genomic sequence of the accidental pathogen *Legionella pneumophila*. *Science* 2004;**305**: 1966–8.
35. Choi P, Qin X, Chen EY, et al. Polymerase chain reaction for pathogen identification in persistent pediatric cervical lymphadenitis. *Arch Otolaryngol Head Neck Surg* 2009;**135**:243–8.
36. Cianciotto NP, Abu Kwaik Y, Edelstein PH, et al. Legionella: *state of the art 30 years after its recognition*. Washington, D.C.: ASM Press; 2006.
37. Collins MT, McDonald J, Hoiby N, et al. Agglutinating antibody titers to members of the family Legionellaceae in cystic fibrosis patients as a result of cross-reacting antibodies to *Pseudomonas aeruginosa*. *J Clin Microbiol* 1984;**19**:757–62.
38. Couvreur J, Dournon E, Garcia J, et al. [Legionnaires' disease in children. Epidemiological survey with a new case report and review of the literature]. *Ann Pédiatr (Paris)* 1986;**33**:379–84. In French.
39. Couvreur J, Khiati M, Petiot A, et al. [Legionella pneumophila pneumopathy in a 4½-month-old infant]. *Arch Fr Pédiatr* 1983; **40**:649–52. In French.
40. Cunha BA. *Seminars in respiratory infections: legionnaires' disease*. Philadelphia: Grune and Stratton; 1987.
41. Cutz E, Thorner PS, Rao CP, et al. Disseminated *Legionella pneumophila* infection in an infant with severe combined immunodeficiency. *J Pediatr* 1982;**100**:760–2.
42. Davis GS, Winn WC Jr, Gump DW, et al. The kinetics of early inflammatory events during experimental pneumonia due to *Legionella pneumophila* in guinea pigs. *J Infect Dis* 1983;**148**:823–35.
43. de Jager CP, de Wit NC, Weers-Pothoff G, et al. Procalcitonin kinetics in *Legionella pneumophila* pneumonia. *Clin Microbiol Infect* 2009;**15**:1020–5.
44. Della Santa L, Crimaldi G, Pellegrini V, et al. [Pulmonary infections in children, part I: pneumonia due to Legionella pneumophila]. *Minerva Pediatr* 1994;**46**:89–97. In Italian.

45. Dennis PJ, Brenner DJ, Thacker WL, et al. Five new *Legionella* species isolated from water. *Int J Syst Bacteriol* 1993;**43**:329–37.
46. Diederen BM, Kluytmans JA, Vandenbroucke-Grauls CM, et al. Utility of real-time PCR for diagnosis of legionnaires' disease in routine clinical practice. *J Clin Microbiol* 2008;**46**:671–7.
47. Dournon E, Bibb WF, Rajagopalan P, et al. Monoclonal antibody reactivity as a virulence marker for *Legionella pneumophila* serogroup 1 strains. *J Infect Dis* 1988;**157**:496–501.
48. Drutz DJ, Demarsh P, Edelstein P, et al. *Legionella pneumophila* pneumonia in athymic nude mice. In: Thornsberry C, Balows A, Feeley JC, et al, editors. *Legionella. Proceedings of the 2nd International Symposium*. Washington, D.C.: American Society for Microbiology; 1984. p. 134–5.
49. Dumoff K, McGovern PC, Edelstein PH, et al. *Legionella maceachernii* pneumonia in a patient with HIV infection. *Diagn Microbiol Infect Dis* 2004;**50**:141–5.
50. Edelstein PH. Legionnaires' disease. *Clin Infect Dis* 1993;**16**: 741–9.
51. Edelstein PH. Antimicrobial chemotherapy for legionnaires' disease: a review. *Clin Infect Dis* 1995;**21**:S265–76.
52. Edelstein PH. Antimicrobial chemotherapy for legionnaires disease: time for a change. *Ann Intern Med* 1998;**129**:328–30.
53. Edelstein PH. Urinary antigen detection for *Legionella* spp. In: Isenberg HD, editor. *Clinical microbiology procedures handbook*, vol. 2. Washington: ASM Press; 2004. p. 11.14.11–16.
54. Edelstein PH. Detection of antibodies to *Legionella*. In: Detrick B, Hamilton RG, Folds JD, editors. *Manual of molecular and clinical laboratory immunology*, vol. 7. Washington, D.C.: ASM Press; 2006. p. 468–76.
55. Edelstein PH. Urine antigen tests positive for Pontiac fever: implications for diagnosis and pathogenesis. *Clin Infect Dis* 2007;**44**:229–31.
56. Edelstein PH. Legionella. In: Versalovic J, Carroll K, Funke G, et al, editors. *Manual of clinical microbiology*, vol. 10. Washington, D.C.: ASM Press, 2011. p. 770–85.
57. Edelstein PH, Meyer RD. Legionnaires' disease: a review. *Chest* 1984;**85**:114–20.
58. Edwards JH, Harbord P, Skidmore JW, et al. Humidifier fever. *Thorax* 1977;**32**:653–63.
59. Efthimiou J, Hodson ME, Taylor P, et al. Importance of viruses and *Legionella pneumophila* in respiratory exacerbations of young adults with cystic fibrosis. *Thorax* 1984;**39**:150–4.
60. Eisenstein TK, Tamada R, Meissler J, et al. Vaccination against *Legionella pneumophila:* serum antibody correlates with protection induced by heat-killed or acetone- killed cells against intraperitoneal but not aerosol infection in guinea pigs. *Infect Immun* 1984;**45**:685–91.
61. Ephros M, Engelhard D, Maayan S, et al. *Legionella gormanii* pneumonia in a child with chronic granulomatous disease. *Pediatr Infect Dis J* 1989;**8**:726–7.
62. Falcó V, Fernández de Sevilla T, Alegre J, et al. *Legionella pneumophila:* a cause of severe community-acquired pneumonia. *Chest* 1991;**100**:1007–11.
63. Falcó V, Molina I, Juste C, et al. [Treatment for Legionnaires' disease. Macrolides or quinolones?]. *Enferm Infecc Microbiol Clin* 2006;**24**:360–4. In Spanish.
64. Fang GD, Yu VL, Vickers RM. Disease due to the Legionellaceae (other than *Legionella pneumophila*): historical, microbiological, clinical, and epidemiological review. *Medicine (Baltimore)* 1989;**68**: 116–32.
65. Fernández-Sabé N, Rosón B, Carratalà J, et al. Clinical diagnosis of *Legionella* pneumonia revisited: evaluation of the Community-Based Pneumonia Incidence Study Group scoring system. *Clin Infect Dis* 2003;**37**:483–9.
66. Fernandez-Serrano S, Dorca J, Coromines M, et al. Molecular inflammatory responses measured in blood of patients with severe community-acquired pneumonia. *Clin Diagn Lab Immunol* 2003; **10**:813–20.
67. Ferrer A, Elcuaz RI, Giménez-Pérez M, et al. [Childhood legionellosis]. *Enferm Infecc Microbiol Clin* 1990;**8**:278–81. In Spanish.
68. Ferrer Marcelles A, Garcia Hernández F, Elcuaz Romano R, et al. [Pneumonia caused by *Legionella* in a newborn infant]. *An Español Pediatr* 1989;**30**:213–4, In Spanish.
69. Fields BS, Benson RF, Besser RE. *Legionella* and legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 2002;**15**: 506–26.
70. Fields BS, Haupt T, Davis JP, et al. Pontiac fever due to *Legionella micdadei* from a whirlpool spa: possible role of bacterial endotoxin. *J Infect Dis* 2001;**184**:1289–92.
71. Fisman DN, Lim S, Wellenius GA, et al. It's not the heat, it's the humidity: wet weather increases legionellosis risk in the greater Philadelphia metropolitan area. *J Infect Dis* 2005;**192**: 2066–73.
72. Fontana MF, Banga S, Barry KC, et al. Secreted bacterial effectors that inhibit host protein synthesis are critical for induction of the innate immune response to virulent *Legionella pneumophila*. *PLoS Pathog* 2011;**7**:e1001289.
73. Foy HM, Broome CV, Hayes PS, et al. Legionnaires' disease in a prepaid medical-care group in Seattle 1963–75. *Lancet* 1979;**1**: 767–70.
74. Fraser DW, Tsai TR, Orenstein W, et al. Legionnaires' disease: description of an epidemic of pneumonia. *N Engl J Med* 1977; **297**:1189–97.
75. Fuchs GJ, LaRocco M, Robinson A, et al. Fatal legionnaires' disease in an infant. *Pediatr Infect Dis* 1986;**5**:377–9.
76. Garduño RA, Garduño E, Hiltz M, et al. Intracellular growth of *Legionella pneumophila* gives rise to a differentiated form dissimilar to stationary-phase forms. *Infect Immun* 2002;**70**: 6273–83.
77. Glick TH, Gregg MB, Berman B, et al. Pontiac fever: an epidemic of unknown etiology in a health department, part I: clinical and epidemiologic aspects. *Am J Epidemiol* 1978;**107**:149–60.
78. Goldberg DJ, Wrench JG, Collier PW, et al. Lochgoilhead fever: outbreak of non-pneumonic legionellosis due to *Legionella micdadei*. *Lancet* 1989;**1**:316–8.
79. Gomez-Valero L, Rusniok C, Buchrieser C. *Legionella pneumophila:* population genetics, phylogeny and genomics. *Infect Genet Evol* 2009;**9**:727–39.
80. Grau S, Antonio JM, Ribes E, et al. Impact of rifampicin addition to clarithromycin in *Legionella pneumophila* pneumonia. *Int J AntimicrobAgents* 2006;**28**:249–52.
81. Green M, Wald ER, Dashefsky B, et al. Field inversion gel electrophoretic analysis of *Legionella pneumophila* strains associated with nosocomial legionellosis in children. *J Clin Microbiol* 1996; **34**:175–6.
82. Greenberg D, Chiou CC, Famigilleti R, et al. Problem pathogens: paediatric legionellosis—implications for improved diagnosis. *Lancet Infect Dis* 2006;**6**:529–35.
83. Griffin AT, Peyrani P, Wiemken T, et al. Macrolides versus quinolones in *Legionella* pneumonia: results from the Community-Acquired Pneumonia Organization international study. *Int J Tuber Lung Dis* 2010;**14**:495–9.
84. Gupta SK, Imperiale TF, Sarosi GA. Evaluation of the Winthrop-University Hospital criteria to identify *Legionella* pneumonia. *Chest* 2001;**120**:1064–71.
85. Han JH, Nguyen JC, Harada S, et al. Relapsing *Legionella pneumophila* cellulitis: a case report and review of the literature. *J Infect Chemother* 2010;**16**:439–42.
86. Haroon A, Higa F, Hibiya K, et al. Organizing pneumonia pattern in the follow-up CT of *Legionella*-infected patients. *J Infect Chemother* 2011;**17**:493–8.
87. Hartemann E, Berthier JC, Barrois S, et al. [Severe *Legionella* pneumopathy in an immunologically normal infant]. *Pediatrie* 1983;**38**:393–9. In French.
88. Hawn TR, Smith KD, Aderem A, et al. Myeloid differentiation primary response gene (88)- and Toll-like receptor 2-deficient mice are susceptible to infection with aerosolized *Legionella pneumophila*. *J Infect Dis* 2006;**193**:1693–702.
89. Hawn TR, Verbon A, Janer M, et al. Toll-like receptor 4 polymorphisms are associated with resistance to legionnaires' disease. *Proc Natl Acad Sci U S A* 2005;**102**:2487–9.
90. Hawn TR, Verbon A, Lettinga KD, et al. A common dominant TLR5 stop codon polymorphism abolishes flagellin signaling and is associated with susceptibility to legionnaires' disease. *J Exp Med* 2003;**198**:1563–72.
91. Hedlund KW, McGann VG, Copeland DS, et al. Immunologic protection against the legionnaires' disease bacterium in the AKR/J mouse. *Ann Intern Med* 1979;**90**:676–9.
92. Helbig JH, Bernander S, Castellani PM, et al. Pan-European study on culture-proven legionnaires' disease: distribution of *Legionella pneumophila* serogroups and monoclonal subgroups. *Eur J Clin Microbiol Infect Dis* 2002;**21**:710–6.

93. Hervás JA, Lopez P, de la Fuente A, et al. Multiple organ system failure in an infant with *Legionella* infection. *Pediatr Infect Dis J* 1988;**7**:671–3.
94. Horie H, Kawakami H, Minoshima K, et al. Neonatal legionnaires' disease: histopathological findings in an autopsied neonate. *Acta Pathol Jpn* 1992;**42**:427–31.
95. Horwitz MA. Interactions between macrophages and *Legionella pneumophila*. *Curr Top MicrobiolImmunol* 1992;**181**:265–82.
96. Hsu CM, Huang LM, Kao YF, et al. Prevalence of *Legionella pneumophila* infection in children and its role in pediatric community-acquired atypical pneumonia. *Acta Paediatr Sinica* 1996;**37**:188–92.
97. Hubber A, Roy CR. Modulation of host cell function by *Legionella pneumophila* type IV effectors [Review]. *Annu Rev Cell Dev Biol* 2010;**26**:261–83.
98. Huhn GD, Adam B, Ruden R, et al. Outbreak of travel-related pontiac fever among hotel guests illustrating the need for better diagnostic tests. *J Travel Med* 2005;**12**:173–9.
99. Johnston JM, Latham RH, Meier FA, et al. Nosocomial outbreak of legionnaires' disease: molecular epidemiology and disease control measures. *Infect Control* 1987;**8**:53–8.
100. Joly JR, Déry P, Gauvreau L, et al. Legionnaires' disease caused by *Legionella dumoffii* in distilled water. *Can Med Assoc J* 1986; **135**:1274–7.
101. Jones TF, Benson RF, Brown EW, et al. Epidemiologic investigation of a restaurant-associated outbreak of Pontiac fever. *Clin Infect Dis* 2003;**37**:1292–7.
102. Joseph CA, Ricketts KD. European Working Group for Legionella, part I.: legionnaires disease in Europe 2007–8. *Euro Surveill* 2010;**15**:19493.
103. Joseph CA, Watson JM, Harrison TG, et al. Nosocomial legionnaires' disease in England and Wales, 1980–92. *Epidemiol Infect* 1994;**112**:329–45.
104. Katz SM, Holsclaw DS Jr. Serum antibodies to *Legionella pneumophila* in patients with cystic fibrosis. *JAMA* 1982;**248**: 2284–8.
105. Kaufman D, Weber K, Gradon JD. *Legionella* pneumonia: an unusual cause of rhabdomyolysis and acute renal failure. *South Med J* 2002;**95**:660.
106. Kaufmann AF, McDade JE, Patton CM, et al. Pontiac fever: isolation of the etiologic agent *(Legionella pneumophila)* and demonstration of its mode of transmission. *Am J Epidemiol* 1981;**114**: 337–47.
107. Kovatch AL, Jardine DS, Dowling JN, et al. Legionellosis in children with leukemia in relapse. *Pediatrics* 1984;**73**:811–5.
108. Kozak NA, Buss M, Lucas CE, et al. Virulence factors encoded by *Legionella longbeachae* identified on the basis of the genome sequence analysis of clinical isolate D-4968. *J Bacteriol* 2010;**192**: 1030–44.
109. Kugler JW, Armitage JO, Helms CM, et al. Nosocomial legionnaires' disease: occurrence in recipients of bone marrow transplants. *Am J Med* 1983;**74**:281–8.
110. La Scola B, Birtles RJ, Greub G, et al. *Legionella drancourtii* sp. nov., a strictly intracellular amoebal pathogen. *Int J Syst Evol Microbiol* 2004;**54**:699–703.
111. La Scola B, Michel G, Raoult D. Isolation of *Legionella pneumophila* by centrifugation of shell vial cell cultures from multiple liver and lung abscesses. *J Clin Microbiol* 1999;**37**: 785–7.
112. Lassmann B, Poetschke M, Ninteretse B, et al. Community-acquired pneumonia in children in Lambarene, Gabon. *Am J Trop Med Hyg* 2008;**79**:109–14.
113. Lau HY, Ashbolt NJ. The role of biofilms and protozoa in *Legionella* pathogenesis: implications for drinking water. *J Appl Microbiol* 2009;**107**:368–78.
114. Lavocat MP, Berthier JC, Rousson A, et al. [Pulmonary legionnaires' disease in a child following drowning in fresh water]. *Presse Med* 1987;**16**:780. In French.
115. Lefrançois C, Casadevall I, Betremieux P, et al. [Fatal legionellosis in an infant treated with ACTH]. *Arch Fr Pédiatr* 1989;**46**:591–3. In French.
116. Leluc O, Doucet V, Petit P, et al. [*Legionella pneumophila:* unusual lung and hepatic manifestations]. *J Radiol* 2000;**81**:241–2. In French.
117. Levy I, Rubin LG. *Legionella* pneumonia in neonates: a literature review. *J Perinatol* 1998;**18**:287–90.
118. LoPresti F, Riffard S, Meugnier H, et al. *Legionella gresilensis* sp. nov. and *Legionella beliardensis* sp. nov., isolated from water in France. *Int J Syst Evol Microbiol* 2001;**51**:6–57.
119. Lurie-Weinberger MN, Gomez-Valero L, Merault N, et al. The origins of eukaryotic-like proteins in *Legionella pneumophila*. *IJMM Int J Med Microbiol* 2010;**300**:470–81.
120. Lüttichau HR, Vinther C, Uldum SA, et al. An outbreak of Pontiac fever among children following use of a whirlpool. *Clin Infect Dis* 1998;**26**:1374–8.
121. Mandell LA, Bartlett JG, Dowell SF, et al. Update of practice guidelines for the management of community-acquired pneumonia in immunocompetent adults. *Clin Infect Dis* 2003;**37**: 1405–33.
122. Mariette X, Gottenberg JE, Ravaud P, et al. Registries in rheumatoid arthritis and autoimmune diseases: data from the French registries. *Rheumatology* 2011;**50**:222–9.
123. Marrie TJ, Haldane D, MacDonald S, et al. Control of endemic nosocomial legionnaires' disease by using sterile potable water for high risk patients. *Epidemiol Infect* 1991;**107**:591–605.
124. Marston BJ, Lipman HB, Breiman RF. Surveillance for legionnaires' disease: Risk factors for morbidity and mortality. *Arch Intern Med* 1994;**154**:2417–22.
125. Marston BJ, Plouffe JF, File TM Jr, et al. Incidence of community-acquired pneumonia requiring hospitalization: results of a population-based active surveillance study in Ohio. The Community-Based Pneumonia Incidence Study Group. *Arch Intern Med* 1997;**157**:1709–18.
126. Mayock R, Skale B, Kohler RB. *Legionella pneumophila* pericarditis proved by culture of pericardial fluid. *Am J Med* 1983;**75**: 534–6.
127. McClelland MR, Vaszar LT, Kagawa FT. Pneumonia and osteomyelitis due to *Legionella longbeachae* in a woman with systemic lupus erythematosus. *Clin Infect Dis* 2004;**38**:e102–6.
128. McDade JE, Shepard CC, Fraser DW, et al. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. *N Engl J Med* 1977;**297**:1197–203.
129. McHugh SL, Newton CA, Yamamoto Y, et al. Tumor necrosis factor induces resistance of macrophages to *Legionella pneumophila* infection. *Proc Soc Exp Biol Med* 2000;**224**:191–6.
130. Megarbane B, Montambault S, Chary I, et al. Acute pancreatitis caused by severe *Legionella pneumophila* infection. *Infection* 2000; **28**:329–31.
131. Millunchick EW, Floyd J, Blanks J. Legionnaires' disease in an immunologically normal child. *Am J Dis Child* 1981;**135**: 1065–6.
132. Molofsky AB, Swanson MS. Differentiate to thrive: lessons from the *Legionella pneumophila* life cycle. *Mol Microbiol* 2004;**53**: 29–40.
133. Muittari A, Kuusisto P, Virtanen P, et al. An epidemic of extrinsic allergic alveolitis caused by tap water. *Clin Allergy* 1980;**10**: 77–90.
134. Muldoon RL, Jaecker DL, Kiefer HK. Legionnaires' disease in children. *Pediatrics* 1981;**67**:329–32.
135. Murata T, Delprato A, Ingmundson A, et al. The *Legionella pneumophila* effector protein DrrA is a Rab1 guanine nucleotide-exchange factor. *Nat Cell Biol* 2006;**8**:971–7.
136. Murdoch DR. Diagnosis of *Legionella* infection. *Clin Infect Dis* 2003;**36**:64–9.
137. Mykietiuk A, Carratalà J, Fernández-Sabé N, et al. Clinical outcomes for hospitalized patients with *Legionella* pneumonia in the antigenuria era: the influence of levofloxacin therapy. *Clin Infect Dis* 2005;**40**:794–9.
138. Nègre V, Chevallier B, Dournon E, et al. [Nosocomial Legionnaires' disease in children. Preventive measures]. *Arch Fr Pédiatr* 1990;**47**:43–5. In French.
139. Neunuebel MR, Chen Y, Gaspar AH, et al. De-AMPylation of the small GTPase Rab1 by the pathogen *Legionella pneumophila*. *Science* 2011;**333**:453–6.
140. Nigro G, Pastoris MC, Fantasia MM, et al. Acute cerebellar ataxia in pediatric legionellosis. *Pediatrics* 1983;**72**:847–9.
141. Orenstein WA, Overturf GD, Leedom JM, et al. The frequency of *Legionella* infection prospectively determined in children hospitalized with pneumonia. *J Pediatr* 1981;**99**:403–6.
142. Pachon J, Prados MD, Capote F, et al. Severe community-acquired pneumonia: etiology, prognosis, and treatment. *Am Rev Respir Dis* 1990;**142**:369–73.

143. Pearce MM, Theodoropoulos N, Noskin GA, et al. Native valve endocarditis due to a novel strain of *Legionella*. *J Clin Microbiol* 2011;**49**:3340–2.
144. Pedro-Botet L, Yu VL. *Legionella:* macrolides or quinolones? *Clin Microbiol Infect* 2006;**12**(Suppl. 3):25–30.
145. Peerless AG, Liebhaber M, Anderson S, et al. *Legionella* pneumonia in chronic granulomatous disease. *J Pediatr* 1985;**106**:783–5.
146. Peeters M, Cornu G, De Meyer R. Legionnaires' disease in an immunosuppressed child. *Acta Paediatr Belg* 1980;**33**:189–93.
147. Peliowski A, Finer NN. Intractable seizures in legionnaires disease. *J Pediatr* 1986;**109**:657–8.
148. Qin X, Abe PM, Weissman SJ, et al. Extrapulmonary *Legionella micdadei* infection in a previously healthy child. *Pediatr Infect Dis J* 2002;**21**:1174–6.
149. Quagliano PV, Das Narla L. *Legionella pneumonia* causing multiple cavitating pulmonary nodules in a 7-month-old infant. *AJR Am J Roentgenol* 1993;**161**:367–8.
150. Reingold AL. Role of legionellae in acute infections of the lower respiratory tract. *Rev Infect Dis* 1988;**10**:1018–28.
151. Ren T, Zamboni DS, Roy CR, et al. Flagellin-deficient *Legionella* mutants evade caspase-1- and Naip5-mediated macrophage immunity. *PLoS Pathog* 2006;**2**:e18.
152. Renner ED, Helms CM, Hierholzer WJ Jr, et al. Legionnaires' disease in pneumonia patients in Iowa: a retrospective seroepidemiologic study, 1972–1977. *Ann Intern Med* 1979;**90**:603–6.
153. Rolstad B, Berdal BP. Immune defenses against *Legionella pneumophila* in rats. *Infect Immun* 1981;**32**:805–12.
154. Roy CR, Salcedo SP, Gorvel JP. Pathogen-endoplasmic-reticulum interactions: in through the out door. *Nat Rev Immunol* 2006;**6**:136–47.
155. Ryan ME, Feldman S, Pruitt B, et al. Legionnaires' disease in a child with cancer. *Pediatrics* 1979;**64**:951–3.
156. Rylander R, Haglind P. Airborne endotoxins and humidifier disease. *Clin Allergy* 1984;**14**:109–12.
157. Schmid H, Henze G, Schwerdtfeger R, et al. Fractionated total body irradiation and high-dose VP-16 with purged autologous bone marrow rescue for children with high risk relapsed acute lymphoblastic leukemia. *Bone Marrow Transplant* 1993;**12**: 597–602.
158. Shachor-Meyouhas Y, Kassis I, Bamberger E, et al. Fatal hospital-acquired *Legionella* pneumonia in a neonate. *Pediatr Infect Dis J* 2010;**29**:280–1.
159. Sheldon PA, Tight RR, Renner ED. Fatal legionnaires' disease coincident with initiation of immunosuppressive therapy. *Arch Intern Med* 1985;**145**:1138–9.
160. Simpson RM, Cogswell JJ, Mitchell ER, et al. Legionnaires' disease in an infant. *Lancet* 1980;**2**:740–1.
161. Skogberg K, Nuorti JP, Saxen H, et al. A newborn with domestically acquired legionnaires disease confirmed by molecular typing. *Clin Infect Dis* 2002;**35**:e82–5.
162. Spanò C, Menozzi M. Legionnaire's disease in Palermo and possible involvement of *Legionella pneumophila* in cases of pericardial effusion. *Infection* 1982;**10**:103–4.
163. Steele TW, Moore CV, Sangster N. Distribution of *Legionella longbeachae* serogroup 1 and other legionellae in potting soils in Australia. *Appl Environ Microbiol* 1990;**56**:2984–8.
164. Straus WL, Plouffe JF, File TM Jr, et al. Risk factors for domestic acquisition of legionnaires disease. Ohio Legionnaires Disease Group. *Arch Intern Med* 1996;**156**:1685–92.
165. Sturm R, Staneck JL, Myers JP, et al. Pediatric legionnaires' disease: diagnosis by direct immunofluorescent staining of sputum. *Pediatrics* 1981;**68**:539–43.
166. Tateda K, Matsumoto T, Ishii Y, et al. Serum cytokines in patients with *Legionella* pneumonia: relative predominance of Th1-type cytokines. *Clin Diagn Lab Immunol* 1998;**5**:401–3.
167. Thompson JE. Community acquired pneumonia in northeastern Australia—a hospital based study of aboriginal and non-aboriginal patients. *Aust NZ J Med* 1997;**27**:59–61.
168. Thornsberry C, Balows A, Feeley JC, et al. Legionella: *Proceedings of the 2nd International Symposium*. Washington, D.C.: American Society for Microbiology; 1984.
169. Tokunaga Y, Concepcion W, Berquist WE, et al. Graft involvement by *Legionella* in a liver transplant recipient. *ArchSurg* 1992;**127**:475–7.
170. Torres A, Serra-Batlles J, Ferrer A, et al. Severe community-acquired pneumonia: epidemiology and prognostic factors. *Am Rev Respir Dis* 1991;**144**:312–8.
171. Trubel HK, Meyer HG, Jahn B, et al. Complicated nosocomial pneumonia due to *Legionella pneumophila* in an immunocompromised child. *Scand J Infect Dis* 2002;**34**:219–21.
172. Tubach F, Ravaud P, Salmon-Ceron D, et al. Emergence of *Legionella pneumophila* pneumonia in patients receiving tumor necrosis factor-alpha antagonists. *Clin Infect Dis* 2006;**43**:e95–100.
173. Unit for Surveillance and Control of Communicable Diseases. Legionnaires disease in a neonatal unit of a private hospital, Cyprus, December 2008: preliminary outbreak report. *Euro Surveill* 2009;**14**:pii: 19090.
174. Venezia RA, Agresta MD, Hanley EM, et al. Nosocomial legionellosis associated with aspiration of nasogastric feedings diluted in tap water. *Infect Control Hosp Epidemiol* 1994;**15**:529–33.
175. Visca P, Goldoni P, Luck PC, et al. Multiple types of *Legionella pneumophila* serogroup 6 in a hospital heated-water system associated with sporadic infections. *J Clin Microbiol* 1999;**37**: 2189–96.
176. von Baum H, Ewig S, Marre R, et al. Community-acquired *Legionella* pneumonia: new insights from the German competence network for community acquired pneumonia. *Clin Infect Dis* 2008;**46**:1356–64.
177. Warner CL, Fayad PB, Heffner RR Jr. *Legionella* myositis. *Neurology* 1991;**41**:750–2.
178. Watson KC, Bain AD, Bartholomew SE. Legionellosis and sudden infant death syndrome. *Lancet* 1983;**2**:1312.
179. Weeratna R, Stamler DA, Edelstein PH, et al. Human and guinea pig immune responses to *Legionella pneumophila* protein antigens OmpS and Hsp60. *Infect Immun* 1994;**62**:3454–62.
180. Weigl JA, Puppe W, Meyer CU, et al. Ten years' experience with year-round active surveillance of up to 19 respiratory pathogens in children. *Eur J Pediatr* 2007;**166**:957–66.
181. Whiley H, Bentham R. *Legionella longbeachae* and legionellosis. *Emerg Infect Dis* 2011;**17**:579–83.
182. Winn WC Jr. *Legionella* and the clinical microbiologist. *Infect Dis Clin North Am* 1993;**7**:377–92.
183. Yamamoto Y, Retzlaff C, He P, et al. Quantitative reverse transcription-PCR analysis of *Legionella pneumophila*-induced cytokine mRNA in different macrophage populations by high-performance liquid chromatography. *Clin Diagn Lab Immunol* 1995;**2**:18–24.
184. Yu H, Higa F, Hibiya K, et al. Computed tomographic features of 23 sporadic cases with *Legionella pneumophila* pneumonia. *Eur J Radiol* 2010;**74**:e73–8.
185. Yu VL, Plouffe JF, Pastoris MC, et al. Distribution of *Legionella* species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. *J Infect Dis* 2002;**186**:127–8.

CHAPTER 138

Streptobacillus moniliformis (Rat-Bite Fever)

Kara Dubray • Carol A. Glaser

Rat bite fever is an acute febrile illness usually acquired by humans from the bite of a rat. The common term *rat bite fever* may be misleading because the disease actually encompasses two different etiologies and disease syndromes. Both *Streptobacillus moniliformis* and *Spirillum minus* are causative agents of rat bite fever; however, these organisms have different epidemiology, incubation periods, and clinical presentation. Streptobacillary rat bite fever, caused by *S. moniliformis*, is the focus of this chapter; *S minus* is discussed in Chapter 142.

Rat-bite fever caused by *Streptobacillus* is typically characterized by fever, rash, and arthritis. Although it is often acquired from the bite of a rat, other animals may be infected and modes of transmission other than a bite have been documented. Haverhill fever, for example, is acquired after ingestion of contaminated food or water. *S. moniliformis* is a zoonosis with worldwide distribution[41,119] and is the leading cause of rat bite fever in the United States.

HISTORY

The first description of rat bite fever is found in the 2500-year-old Indian *Compendium of Medicine*, the *Susruta Samhita*.[21] In this text, a description of the illness is given that remains valid today: "The blood of any part of the human body coming in contact with the semen of rats or scratched with their nails or teeth is vitiated and gives rise to the appearance of nodes, swellings, eruptions of circular erythematous patches on skin, pustules, violent and acute erysipelas, breaking pain in the joints, extreme pain in the body, fever, anemia, aversion to food, shivering, and horripilation." The disease also was known in ancient Japan, where treatment consisted of the local application of herbs and dynamite to cause an "explosion in the wound."[21]

The first modern accounts of the disease are found in a lecture by Professor Eli Ives at Yale University in 1831.[38] In 1900, Miyake[66] gave a detailed description of the disease, which he named *sodoku*, from the Japanese *so* ("rat") and *doku* ("poison"). The first case report was published in 1839.[116] Schottmüller,[96] Blake,[12] and Levaditi and colleagues[58] were the first to isolate and describe the causative agent of streptobacillary rat bite fever in 1914, 1916, and 1925, respectively. The organism has been known as *Streptothrix muris ratti* and *Streptobacillus muris minus* but currently is referred to as *S. moniliformis*.[50]

Haverhill fever was reported first in 1926 after an outbreak of epidemic illness in Haverhill, Massachusetts. In that outbreak, 86 cases developed and were traced to contaminated, unpasteurized milk.[75] The illness was referred to as *erythema arthriticum epidemicum*. Although this was the first published outbreak, in 1925 approximately 400 cases of a similar illness were reported in Chester, Pennsylvania.[33] The causative organism was isolated, described in detail by Parker and Hudson[70] in 1926, and given the name *Haverhillia multiformis*. It was later shown to be identical to *S. moniliformis*. The first outbreak in Britain was described in 1983 at a boarding school in Essex and affected more than 200 students; it was also believed to be related to contaminated raw milk.[72,100]

EPIDEMIOLOGY

Rats are estimated to compose one third of the mammalian population of the world, with numbers of approximately 10 billion.[41] More than 2 million animal bites are reported annually in the United States, and rat bites account for a small number (<1%), although the incidence is probably underreported.[31,41,44] A review of animal bites in Maryland during a 3-year period found that approximately 4.7 percent were caused by rats or lagomorphs.[25] According to another report, 40,000 rat bites are reported annually.[22] Among bitten patients, the risk for rat bite fever developing is significant, with reported rates between 4 and 11 percent.[83,115]

Between 10 and 100 percent of laboratory rats and 50 to 100 percent of wild rats carry *S. moniliformis* as normal nasopharyngeal flora and excrete it in their urine.[4,41,105] The disease also has been transmitted to humans from mice,[81] squirrels,[64] weasels,[27] gerbils,[117] and rat eating carnivores, such as cats,[43,65] dogs,[71,84] and pigs.[114] In addition, *S. moniliformis* can cause disease in turkeys,[67] guinea pigs,[56] koalas,[91] spinifex hopping mice,[52] and nonhuman primates (rhesus monkey).[109] In one study looking at colonization in dogs with confirmed rat contact, 3/10 animals had evidence of *S. moniliformis* by polymerase chain reaction (PCR) using mouth swabs.[118] Because many of these animals prey on rodents, they could pose a potential source of infection for humans.

Given the changing perception of rats and breeding as family pets, rat bite fever in the United States is shifting from a disease of poverty to a disease of pet owners, pet store workers, and laboratory workers. Children are

generally reported to account for at least 50 percent of rat bite fever cases and, indeed, half of all *S. moniliformis* isolates identified at the California state Microbial Diseases Laboratory between 1970 and 1998 involved children younger than 9 years of age.[47] Not surprisingly, children living in crowded urban centers or rural impoverished areas continue to be at greatest risk.[25,51,80,112] Recently, however, reports of rat bite fever are emerging among individuals who keep rats as pets.[4,8,24,110] Most of the other reported cases involve laboratory personnel who handle rats.[7]

Rat-bite fever usually is transmitted through the bite of a rat, but it also may be transmitted by rat scratches, contact with a break in the skin, mucosal contact (kissing a pet rat), or handling a dead animal.[17,24,36,92] Unlike typical rat bite fever, Haverhill fever is transmitted by the ingestion of food or water contaminated by rats, with previous outbreaks involving unpasteurized milk, ice cream (made from raw milk), and water.[62,75] Rat-bite fever was reported after varicella in a child who handled pet rats frequently while she had open skin lesions,[77] and septic arthritis caused by *S. moniliformis* was reported in a child who kissed pet rats.[49] One review of 12 pediatric cases documented 5 patients with exposure to pet rats.[69] In another study of 16 cases of *S. moniliformis* septic arthritis, the majority of patients with rat exposure involved rats as household pets.[26] A review of 65 detailed cases in the English literature from 1938 until 2005 showed 26 (40%) with wild rat exposure, 8 (12%) with laboratory rat exposure, and 3 (5%) with pet shop rat exposure. Twenty-two (34%) of the patients described an exposure not relating to a bite or a rat.[33]

Rat-bite fever has been reported worldwide; however, it is not a reportable disease, and the actual incidence is unknown. A report from the California state Microbial Disease Laboratory from 2001 reported an incidence of approximately 1.5 cases/year from 1970 to 1998, with more than half of the reported cases occurring after 1990. The report cited a possible increase in incidence after 1990; however, this is largely speculative because the numbers represent only confirmed cases.[47] Difficulty in isolating the organism, improved detection techniques, and an increase in physician awareness of the disease all may influence the reported rates. The mortality rate of rat bite fever has been reported to be 7 to 13 percent if left untreated[26,33,41]; however, the number of patients with mild or subclinical disease is not known.

BACTERIOLOGY

S. moniliformis is a pleomorphic, microaerophilic, nonmotile, nonencapsulated, non–acid-fast, gram-negative bacillus. It measures 1 to 5 μm in length.[50] The organism is oxidase- and catalase-negative and will ferment glucose, maltose, fructose, galactose, and salicin.[50] *S. moniliformis* is facultatively anerobic[35,41] and is the only species in its genus.

The organism is fastidious and requires special handling for isolation. Optimal growth is achieved in trypticase soy agar or broth supplemented with 20 percent horse or rabbit serum.[70] Alternatively, brain-heart infusion broth supplemented with "Panmede" (a papain digest of ox liver) also has been shown to support the growth of *S. moniliformis*.[101] Sodium polyanethol sulfonate (SPS), which is added to most aerobic blood culture bottles at a concentration of 0.05 percent, will inhibit the growth of *S. moniliformis* at concentrations as low as 0.0125 percent.[101] Blood culture bottles without SPS added should be used for primary isolation of *S. moniliformis* when rat bite fever is suspected. Another option is to inoculate twice the amount of blood than recommended by the manufacturer into the blood culture bottle.[42] Material from lymph nodes and abscesses also may be useful material for isolation of the organism.[42] One other alternative is to inoculate citrated blood directly into broth. Cultures should be incubated at 35° to 37°C (95° to 98.6°F) in a humid environment with a partial pressure of carbon dioxide between 8 and 10 percent.

The morphologic characteristics of the bacterium depend on the environment.[69] In favorable media, the typical appearance is that of short rods that may grow in chains. In other conditions, the organism tends to grow in long, interwoven filaments that commonly contain beaded and fusiform swellings throughout their length. The Latin term *moniliformis* translates to "in the form of a necklace." In broth culture, colonies usually appear in 2 to 10 days.[50] The colonies are white, soft "puffballs" measuring 1 to 2 mm in diameter (Fig. 138-1).[4] On blood agar plates, the colonies are round, gray, and glistening and measure 1 to 2 mm in diameter after 2 to 3 days of incubation.[50]

Stable L-forms of the organism develop spontaneously in vivo or in vitro. These cell wall–deficient forms have a "fried egg" appearance with dark centers and lacy edges when grown on solid media.[50,73] The L form is considered

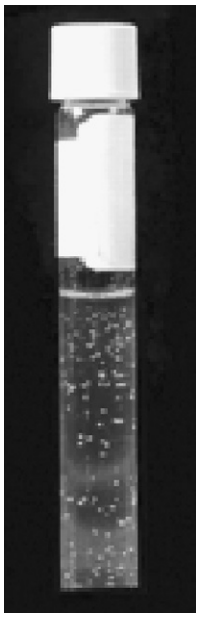

FIGURE 138-1 ■ The colonies are white, soft "puffballs" 1 to 2 mm in diameter. (From Albedwawi S, LeBlanc C, Shaw A, et al. A teenager with fever, rash and arthritis. *CMAJ* 2006;**175**:354.)

nonpathogenic, even though spontaneous conversion between the two forms has been reported in vitro and may be responsible for relapses and resistance to therapy, although this is not certain.[33,40,98] L forms are resistant to penicillin and other antibiotics active against the bacterial cell wall. These variants may be deposited in tissues and prolong the symptoms of illness.[29]

CLINICAL MANIFESTATIONS

Streptobacillary rat bite fever often has an incubation period of less than a week, with the usual range from 3 to 10 days. Incubation has been documented up to 3 weeks.[3] Young children may have shorter incubation times. Typically the bite site, if known, is well healed (as opposed to spirillary rat bite fever) and often is not evident on examination. Most individuals have evidence of contact with a rat, although not necessarily a bite history. Up to 30 percent of individuals are reported to have no history of a bite or scratch.[18]

A review of 65 patients showed an age range of 2 months to 87 years. Symptoms described included fever (92%), polyarthralgias (66%), rash (61%), nausea and vomiting (40%), headache (34%), myalgias (29 %), and sore throat (17%). White blood cell counts were often normal, with polymorphonuclear cell and band form predominance. Only 5 patients (8%) demonstrated white blood cell counts higher than 15,000/mm^3.[33]

Rash develops usually within a few days of fever onset (range, 1 to 8 days) in approximately 75 percent of patients. The rash may be generalized, but more often is prominent on the extremities and frequently involves the palms and soles (Figs. 138-2 and 138-3).[1,24,61,73] Rash has been described as maculopapular, petechial, purpuric, pustular, hemorrhagic, vesicular, and bullous.[3,33,41,53] Desquamation is reported in approximately 20 percent and the rash may take several weeks to resolve.[41]

Arthralgia or arthritis with or without joint effusion also develops in more than 50 percent of patients classically within the first week of illness. Arthritis usually involves large joints, but also may affect small joints, and tends to be migratory, nonsymmetric, and extremely painful.[6,36,51,97] Arthritis may be monoarticular or polyarticular and acute or subacute. Although multiple joints are usually affected, at least two cases of monoarticular involvement in children have been described involving the hip joint.[30,48] With untreated infection, persistent or recurrent episodes of fever and arthritis may be experienced.[37,107]

Isolation of *Streptobacillus* from joint fluid is not common and the arthritis from rat bite fever is considered to be nonsuppurative with sterile joint fluid cultures. It has been hypothesized that *Streptobacillus* may cause a direct infective arthritis and an immune-mediated phenomena, although this is not well understood.[26] At least two recent reviews of septic arthritis caused by *S. moniliformis* (defined by isolation from joint or synovial fluid) with at least 17 individual cases have been reported.[26,113] Rash was present in a minority of patients only. Arthritis may present as a septic hip in children and may mimic rheumatoid arthritis in older patients with peripheral joints affected.[57] Patients with underlying osteoarthritis may be predisposed to this complication. The prognosis is quite favorable overall, with all patients in these series recovering with treatment.[26,113]

Complications of *S. moniliformis* infection include anemia,[86] cutaneous or subcutaneous abscess formation,[16,48,73,111] interstitial pneumonia,[63,98] mastoiditis,[74] meningitis,[8,98] pericardial effusion,[15] pancreatitis, prostatitis,[119] septic arthritis,* and overwhelming sepsis.[98] Abscess formation in several different organs, including the brain,[28] the female genital tract,[73] and the spleen,[20] has been reported. Unusual and potentially devastating complications include chorioamnionitis[34] and periarteritis nodosa,[79] and endocarditis.

The most frequent serious complication is bacterial endocarditis, with nearly 50 percent mortality.† At least

FIGURE 138-2 ■ Within 1 to 8 days after the onset of fever, a pink-red maculopapular rash develops in approximately 75 percent of patients and frequently involves the palms and soles. (From Albedwawi S, LeBlanc C, Shaw A, et al. A teenager with fever, rash and arthritis. *CMAJ* 2006;**175**:354.)

FIGURE 138-3 ■ An 8-year-old child with fever, rash, and arthritis, with confirmed rat bite fever from contact with a pet rat. (See companion Expert Consult website for color version.) (Courtesy S. Islam, A. Petru, and A. LaBeaud, Children's Hospital & Research Center, Oakland, Calif.)

*References 26, 49, 59, 89, 93, 113.
†References 9, 19, 61, 82, 85, 90, 103.

20 cases of endocarditis have been reported in the literature with over half of the cases reported prior to 1968.[19,33,90,102] Many patients reported had underlying valvular disease.[33] In a review of 17 cases, the majority of patients (12) had systemic symptoms, including fever, rash, arthritis.[90] Of 14 cases in which clinical symptoms were reported, murmur was noted in 100 percent, petechiae in 13 percent, Osler's nodes in 13 percent, hepatosplenomegaly in 33 percent, anemia in 33 percent, and cardiac dysrhythmia in 13 percent.[90] At least 6 cases have involved children younger than 18 years, with the youngest just 2 months of age.* Of the 6 children, 5 died during the acute illness.

At least seven fatal pediatric cases have been reported, the majority with endocarditis. Most deaths were described in the 1930s and 1940s among older children with endocarditis. In the 1980s, two separate cases of infant deaths related to rat bite fever were described. One case was a 3-month-old infant with endocarditis, and the other was a 2-month-old infant with disseminated disease. In 2011, another fatal case in a 14-month-old infant with disseminated disease was described with autopsy findings similar to those of the 2-month-old infant, including interstitial pneumonitis, meningitis (with isolation of the organism from cerebrospinal fluid [CSF]), and hepatomegaly.[10,50,69,86]

Haverhill fever is similar to streptobacillary rat bite fever, with an abrupt onset of fever and chills (100%), rash (95%), and arthritis (97%).[2,62] Generally, the incubation period is 1 to 3 days. Upper respiratory and gastrointestinal problems are common. Multiple recurrences of fever are found rarely, and the rash tends to be small and uniform in size.[63]

PATHOPHYSIOLOGY AND PATHOLOGY

Factors influencing the virulence of *S. moniliformis* are not well described. The organism has an affinity for synovial tissue in both animals and humans, but the mechanisms by which *S. moniliformis* produces arthritis are unknown.† Studies performed in mice indicate that *S. moniliformis* is only slightly immunogenic and produces mild leukocytosis and minimal homologous antibody production.[94,95] In addition, the organism is resistant to phagocytic destruction.[94] These factors may allow chronic infection to develop. Again the organism may spontaneously convert to the cell wall–deficient form (or L form), and this has been speculated to be responsible for relapsing disease.

The pathologic features of streptobacillary rat bite fever have been described in a limited number of autopsy reports. Common features include ulcerative endocarditis with secondary septic embolization in the liver and spleen, septic arthritis, and interstitial pneumonia.[12,98] Mononuclear meningitis and erythrophagocytosis also have been reported.[98] In a pediatric autopsy report, in which *Streptobacillus* grew from the CSF, mild flattening of the cerebral gyri with mild collapse of the ventricular system was seen, but no major histopathologic changes were noted in the brain. The kidneys had findings consistent with disseminated intravascular coagulopathy. The small and large intestine, liver, thymus, thyroid gland, larynx, and heart showed mild to moderate autolysis, but no other major histopathologic changes.[10] In most reports, the site of the bite reveals little histologic evidence of inflammation.[63]

DIAGNOSIS

Two recent fatal cases reported in the Centers for Disease Control and Prevention (CDC) *Morbidity and Mortality Weekly Report* highlight the importance of considering rat bite fever in the differential.[18] The diagnosis of rat bite fever requires a high index of suspicion on the part of the physician. The diagnosis is suggested in a febrile patient with a history of exposure to rats, but in most clinical settings, the exposure history is not elucidated until after the diagnosis is made. It should be considered in patients who present with fever, rash, and arthritis.[104]

Nonspecific laboratory findings may include an elevation in the white blood cell count,[12] usually in the range of 10,000 to 30,000 cells/mm^3 with a left shift, mild anemia, and a false-positive serologic test for syphilis, which may occur in 25 percent of patients.[86] Direct visualization of the organism on Giemsa stain of blood or joint fluid may suggest the diagnosis. Serologic assays are not currently available for humans. An enzyme-linked immunosorbent assay has been developed to monitor infection in rodent colonies.[14]

Molecular techniques may be an effective means for detection of *S. moniliformis* from humans and animals and may offer an alternative to traditional culture methods.[5,11,13,38] Broad-range PCR amplification of a part of the 16S rRNA gene followed by sequencing allowed the detection and identification of *S. moniliformis* in blister fluid of a patient reported in 1991.[11] A PCR assay specific for *S. moniliformis* was described by Boot and colleagues[14] using primers designed on the basis of 16S rRNA gene base sequence data of human and rodent strains of *S. moniliformis*, and similar assays have been used subsequently.[68,118] At least one report described a positive PCR obtained from a bite site.[68] PCR testing is becoming more refined, with a 2008 publication describing new primers to amplify the 16S rRNA gene of *Streptobacillus* spp. more specifically than prior tests.[55] Currently PCR testing is not widely available and is not yet validated for diagnostic use, but may be requested from the CDC and reference laboratories.

S. moniliformis has been cultured from blood, joint fluid, abscesses, pericardial fluid, meninges, and tissues obtained at autopsy.[10,17] The organism has strict growth requirements, and the choice of culture media and technique is of critical importance for optimal growth of the bacterium (see the discussion of bacteriology). Whenever this agent is suspected, it is helpful to alert the laboratory that will be performing isolation. In general, routine aerobic blood cultures are not satisfactory for isolation. If the organism is isolated, it may be identified rapidly by gas-liquid chromatography. *S. moniliformis* has a

*References 63, 78, 87, 90, 98, 103, 106.
†References 45, 46, 51, 54, 59, 95, 108.

characteristic fatty acid profile, with major peaks being palmitic, linoleic, oleic, and stearic acid.[73,88] Electrophoretic protein patterns also have been described that can distinguish Haverhill fever strains from rat bite fever strains.[23]

The differential diagnosis for streptobacillary rat bite fever includes illness caused by *S. minus* (see Chapter 142). It also includes all relapsing fevers, such as *Borrelia recurrentis*, malaria, and typhoid. Rocky Mountain spotted fever must be considered,[60] as well as other infectious entities, including leptospirosis, Lyme disease, disseminated gonococcal infection, meningococcemia, brucellosis, and syphilis. Viral infections also may be mistaken for rat bite fever. Acute rheumatic fever should be considered as well. The frequent symmetric involvement of small joints of the hands can be particularly suggestive of rheumatoid arthritis.[51] Other noninfectious entities include drug reactions, collagen vascular disease, and other rheumatologic conditions.

TREATMENT AND PREVENTION

Although fatal in some cases, streptobacillary rat bite fever is a very treatable disease usually responsive to antibiotics. The treatment of choice for *S. moniliformis* is penicillin G. Either procaine penicillin intramuscularly or aqueous penicillin intravenously is acceptable.[3,79] Tests of *S. moniliformis* antibiotic susceptibility by the disk diffusion method usually demonstrate sensitivity to penicillins, cephalosporins, carbapenems, aztreonam, clindamycin, erythromycin, nitrofurantoin, bacitracin, tetracycline, teicoplanin, and vancomycin; intermediate susceptibility to aminoglycosides, fluoroquinolones, amikacin, and chloramphenicol; and resistance to trimethoprim-sulfamethoxazole, polymyxin B, and nalidixic acid.[119] Only one penicillin-resistant *S. moniliformis* strain has been demonstrated, and that was more than 50 years ago.[33] A 1965 study noted the rapid response to penicillin was in stark contrast to other regimens.[79]

In adults, the dosage of penicillin should be no less than 400,000 to 600,000 IU/day of IV aqueous penicillin G continued for at least 7 days and up to 10 to 14 days.[33,39,53,69,85] Treatment with penicillin for up to 28 days or more has been reported in cases of presumed septic arthritis.[113] If no response is seen within 2 days, the dosage should be increased to 1.2 million IU/day.[79] Children have been treated successfully with 20,000 to 50,000 IU/kg/day of intravenous penicillin, up to a maximum of 1.2 million IU/day.[99] No controlled studies have been done on duration of intravenous versus oral therapy. In children, some suggest after 5 to 7 days of intravenous therapy with good response, treatment may be completed with oral penicillin.[18,33,53] For mild cases, oral penicillin V 1 to 2 g/day in divided doses may be given.[39,99]

In penicillin-allergic adults, both streptomycin and oral tetracycline have been effective.[18,76,90,97] The suggested dose is oral tetracycline 500 mg 4 times per day or intramuscular streptomycin 7.5 mg/kg twice daily.[75] In children, streptomycin and oral erythromycin have been used, but treatment failures with oral erythromycin have been reported.[48] Clarithromycin has been used successfully to treat an adult with rat bite fever.[110] Antibiotics, including cefuroxime, cefotaxime, gentamicin, and ciprofloxacin, have good activity against *S. moniliformis* in vitro.[32] Several reports document successful treatment with cephalosporins, including cefuroxime and ceftriaxone.[17,24,49,85] At least one case of suspected rat bite fever is reported to have been treated successfully with amoxicillin.[17]

Optimal therapy for *S. moniliformis* endocarditis is based on case reports given the rarity of disease. High-dose aqueous intravenous penicillin G or procaine penicillin intramuscularly is the treatment of choice. Some experts suggest using penicillin in combination with streptomycin or gentamicin, although limited data exist.[33] For adults, daily administration of intramuscular procaine penicillin G at 4.8 million IU has been recommended if the isolate is susceptible (0.1 μg/mL).[33,90] If the isolate is more resistant, intravenous penicillin G 20 million IU/day should be used.[90] In children, the dosage is intravenous aqueous penicillin 160,000 to 240,000 IU/kg/day (96 to 144 mg/kg/day), up to the adult maximum of 20 million IU/day.[33,90,99] Treatment should be continued for at least 4 weeks, although up to 6 weeks may be considered based on treatment of other types of bacterial endocarditis.[33] In a review of 16 patients with endocarditis, 4 patients who did not receive specific antimicrobial therapy died, as did 3 patients who received less than 1 million units of penicillin per day. Only 2 patients who received more than 1 million units of penicillin per day died, and one of those patients died suddenly 4 months after valve replacement.[90]

Antibiotic susceptibility testing of the organism should be performed and minimum inhibitory and bactericidal concentrations for several antimicrobial agents determined. It is desirable to have a peak serum minimum bactericidal concentration of at least 1:8. Recently, successful treatment of endocarditis with ceftriaxone in combination with gentamicin was reported.[85] Penicillin G is also the drug of choice for Haverhill fever. Most individuals can be treated as outpatients.

PREVENTION

Rat-bite fever can be prevented by controlling rodents in urban areas, properly handling rodents, and avoiding unpasteurized milk products. Individuals who own rats and those who work with rodents should receive anticipatory guidance regarding the handling of these animals and the signs and symptoms of rat bite fever. If a rat bites an individual, prophylactic antibiotics, such as amoxicillin or amoxicillin-clavulanic acid, may prevent rat bite fever, but current data do not support the routine use of antibiotics after all rat bites. Some researchers suggest that because of the serious nature of rat bite fever in young infants, infants younger than 3 months who are bitten by a rat should receive antibiotic prophylaxis.[60]

NEW REFERENCES SINCE THE SIXTH EDITION

3. American Academy of Pediatrics. Rat bite fever. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book: 2009 report of the*

Committee on Infectious Diseases. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 559–60.
9. Balakrishnan N, Menon T, Shanmugasundaram S, et al. *Streptobacillus moniliformis* endocarditis. *Emerg Infect Dis* 2006;**12**: 1037–8.
10. Banerjee P, Ali Z, Fowler D. Rat bite fever, a fatal case of *Streptobacillus moniliformis* infection in a 14-month-old boy. *J Forensic Sci* 2011;**56**:531–3.
19. Chen P-L, Lee N-Y, Yan J-J, et al. Prosthetic valve endocarditis caused by *Streptobacillus moniliformis:* a case of rat bite fever. *J Clin Microbiol* 2007;**45**:3125–6.
22. Committee on Urban Pest Management. *Urban Pest Management*. Washington, DC: National Academies Press; 1980.
26. Dendle C, Woolley IJ, Korman TM. Rat bite fever septic arthritis: illustrative case and literature review. *Eur J Clin Microbiol InfectDis* 2006;**25**:791–7.
30. Downing ND, Dewnany GD, Radford PJ. A rare and serious consequence of a rat bite. *Ann R Coll Surg Engl* 2001;**83**:279–80.
33. Elliott SP. Rat bite fever and *Streptobacillus moniliformis*. *ClinMicrobiol Rev* 2007;**20**:13–22.
35. Fleming MP. *Streptobacillus moniliformis* isolations from cervical abscesses of guinea pigs. *Vet Rec* 1976;**99**:256.
39. Freels LK, Elliott SP. Rat bite fever: three case reports and a literature review. *Clin Pediatr* 2004;**43**:291–5.
40. Freundt EA. Experimental investigations into the pathogenicity of the L-phase variant of *Streptobacillus moniliformis*. *Acta Pathol Microbiol Scand* 1956;**38**:246–58.
41. Gaastra W, Boot R, Hoa TKH, et al. Rat bite fever. *Vet Microbiol* 2009;**133**:211–28.
42. Garcia LS. *Clinical Microbiology Procedures Handbook*, vols. 1-3, 3rd ed. American Society for Microbiology; 2010. p. 3.4.1.
44. Glaser C, Lewis P, Wong S. Pet-, animal-, and vector-borne infections. *Pediatr Rev* 2000;**21**:219–95.
53. Islam S, Cooney T, Singh A, et al. Painful arthritis and extremity rash in an 8 year old boy. *Clin Infect Dis* 2012;**54**:1473, 1514–15.
55. Kimura M, Tanikawa T, Suzuki M, et al. Detection of *Streptobacillus* spp. in feral rats by specific polymerase chain reaction. *Microbiol Immunol* 2008;**52**:9–15.
68. Nakagomi D, Deguchi N, Yagasaki A, et al. Rat-bite-fever identified by polymerase chain reaction detection of *Streptobacillus moniliformis* DNA. *J Dermatol* 2008;**35**: 667–70.
69. Ojukwu IC, Christy C. Rat bite fever in children: case report and review. *Scand J Infect Dis* 2002;**34**:474–7.
72. Pilsworth R. Haverhill fever. *Lancet* 1983;**2**:336–7.
113. Wang TKF, Wong SSY. *Streptobacillus moniliformis* septic arthritis: a clinical entity distinct from rat bite fever? *BMC Infect Dis* 2007;**7**:56.
118. Wouters E, Ho HTK, Lipman L, et al. Dogs as vectors of *Streptobacillus moniliformis* infection? *Vet Microbiol* 2008;**128**:419–22.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 139

Bartonella Infections

Leigh M. Howard • Kathryn M. Edwards • Barbara Stechenberg

DEFINITION

Bartonella species cause infection in both zoonotic and human hosts and give rise to a wide spectrum of acute or chronic disease symptoms. The development of different manifestations of *Bartonella* disease depends on the immune status of the host. Until 1993, only three human diseases were known to be caused by *Bartonella* species.[3] However, advances in molecular techniques have led to the reclassification of several pathogens, with the family Bartonellaceae, genus *Bartonella*, expanding to include more than 30 validated species.[11] Several of these species have been associated with human disease (Table 139-1). The current *Bartonella* genus was created by merging with the genus *Rochalimaea* based on both DNA-DNA hybridization data and comparison of 16s rRNA gene sequences.[12] Of the members of the genus *Rochalimaea* that have been reclassified as *Bartonella* spp., *Bartonella henselae*, the cause of cat-scratch disease, is the most common *Bartonella*-associated infection in children. *Bartonella* infections other than cat-scratch disease seldom occur in children and are only discussed briefly later in the chapter.

MICROBIOLOGY

Comparisons of the 16s rRNA-encoding genes have shown that the *Bartonella* genus is most closely related to *Brucella* and *Agrobacterium*. *Bartonella* are small, gram-negative, fastidious coccobacilli or slightly curved rods that grow best on enriched blood–containing media in 5 percent carbon dioxide. Colony morphology varies from small, dry, gray-white colonies to smooth, creamy yellow colonies. Although the optimal growth temperature for *B. bacilliformis* is 28°C, optimal temperatures for other *Bartonella* species range from 35 to 37°C. Even with optimal conditions, these organisms require 1 to 6 weeks to grow in culture.[13] Increasingly, the presence of *Bartonella* organisms is detected by molecular methods.

REFERENCES

1. Albedwawi S, LeBlanc C, Shaw A, et al. A teenager with fever, rash and arthritis. *CMAJ* 2006;**175**:354.
2. Albritten F, Sheely R, Jeffers W. *Haverhillia multiformis* septicemia: etiologic and clinical relationship to rat-bite fever. *JAMA* 1940;**175**:2360–3.
3. American Academy of Pediatrics. Rat bite fever. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 559–60.
4. Anderson LC, Leary SL, Manning PJ. Rat-bite fever in animal research laboratory personnel. *Lab Anim Sci* 1983;**33**:292–4.
5. Andre JM, Freydiere AM, Benito Y, et al. Rat bite fever caused by *Streptobacillus moniliformis* in a child: human infection and rat carriage diagnosed by PCR. *J Clin Pathol* 2005;**58**:1215–16.
6. Anglada A, Comas L, Euras JM, et al. [Arthritis caused by *Streptobacillus moniliformis:* a case of fever induced by a rat bite]. *Med Clin (Barc)* 1990;**94**:535–7.
7. Arkless HA. Rat-bite fever at Albert Einstein Medical Center. *Pa Med* 1970;**73**:49.
8. Atala A, Correa CN, Correa WM, et al. [Meningitis caused by *Streptobacillus moniliformis*]. *Rev Paul Med* 1973;**82**:175–8.
9. Balakrishnan N, Menon T, Shanmugasundaram S, et al. *Streptobacillus moniliformis* endocarditis. *Emerg Infect Dis* 2006;**12**: 1037–8.
10. Banerjee P, Ali Z, Fowler DR. Rat bite fever, a fatal case of *Streptobacillus moniliformis* infection in a 14-month-old boy. *J Forensic Sci* 2011;**56**:531–3.
11. Berger C, Altwegg M, Meyer A, et al. Broad range polymerase chain reaction for diagnosis of rat-bite fever caused by *Streptobacillus moniliformis*. *Pediatr Infect Dis J* 2001;**20**:1181–2.
12. Blake FG. The etiology of rat-bite fever. *J Exp Med* 1916; **23**:39–60.
13. Boot R, Oosterhuis A, Thuis HC. PCR for the detection of *Streptobacillus moniliformis*. *Lab Anim* 2002;**36**:200–8.
14. Boot R, Bakker RH, Thuis H, et al. An enzyme-linked immunosorbent assay (ELISA) for monitoring rodent colonies for *Streptobacillus moniliformis* antibodies. *Lab Anim* 1993;**27**:350–7.
15. Carbeck RB, Murphy JF, Britt EM. Streptobacillary rat-bite fever with massive pericardial effusion. *JAMA* 1967;**201**:703–4.
16. Centers for Disease Control and Prevention. Rat-bite fever in a college student: California. *MMWR Morb Mortal Wkly Rep* 1984;**33**:318–20.
17. Centers for Disease Control and Prevention. Rat-bite fever: New Mexico, 1996. *MMWR Morb Mortal Wkly Rep* 1998;**47**:89–91.
18. Centers for Disease Control and Prevention. Fatal rat-bite fever: Florida and Washington, 2003. *MMWR Morb Mortal Wkly Rep* 2005;**53**:1198–202.
19. Chen PL, Lee NY, Yan JJ, et al. Prosthetic valve endocarditis caused by *Streptobacillus moniliformis:* a case of rat bite fever. *J Clin Microbiol* 2007;**45**:3125–6.
20. Chulay JD, Lankerani MR. Splenic abscess: report of 10 cases and review of the literature. *Am J Med* 1976;**61**:513–22.
21. Cohen H. Rat-bite fever: contributions to its history and war significance. *Bull Hist Med* 108–15. 1944;
22. Committee on Urban Pest Management. *Urban Pest Management*. Washington, DC: National Academies Press; 1980.
23. Costas M, Owen RJ. Numerical analysis of electrophoretic protein patterns of *Streptobacillus moniliformis* strains from human, murine and avian infections. *J Med Microbiol* 1987;**23**:303–11.
24. Cunningham BB, Paller AS, Katz BZ. Rat bite fever in a pet lover. *J Am Acad Dermatol* 1998;**38**:330–2.
25. De Hoff JB, Ross L. Animal bites. *Md State Med J* 1981;**30**: 35–45.
26. Dendle C, Woolley IJ, Korman TM. Rat-bite fever septic arthritis: illustrative case and literature review. *Eur J Clin Microbiol Infect Dis* 2006;**25**:791–7.
27. Dick G, Turncliff R. *Streptothrix* isolated from the blood of a patient bitten by a weasel. *J Infect Dis* 1918;**23**:183–7.
28. Dijkmans BA, Thomeer RT, Vielvoye GJ, et al. Brain abscess due to *Streptobacillus moniliformis* and *Actinobacterium meyerii*. *Infection* 1984;**12**:262–4.
29. Dolman CE, Kerr DE, Chang H, et al. Two cases of rat bite fever due to *Streptobacillus moniliformis*. *Can J Public Health* 1951;**42**: 228–41.
30. Downing ND, Dewnany GD, Radford PJ. A rare and serious consequence of a rat bite. *Ann R Coll Surg Engl* 2001;**83**:279–80.
31. Edwards M. Animal Bites. In: Feigin R, Cherry J, editors. *Textbook of Pediatric Infectious Diseases*, vol. 2. Philadelphia: WB Saunders; 1998. p. 2248–53.
32. Edwards R, Finch RG. Characterisation and antibiotic susceptibilities of *Streptobacillus moniliformis*. *J Med Microbiol* 1986;**21**: 39–42.
33. Elliott SP. Rat bite fever and *Streptobacillus moniliformis*. *Clin Microbiol Rev* 2007;**20**:13–22.
34. Faro S, Walker C, Pierson RL. Amnionitis with intact amniotic membranes involving *Streptobacillus moniliformis*. *Obstet Gynecol* 1980;**55**:9S–11S.
35. Fleming MP. *Streptobacillus moniliformis* isolations from cervical abscesses of guinea-pigs. *Vet Rec* 1976;**99**:256.
36. Fordham JN, McKay-Ferguson E, Davies A, et al. Rat bite fever without the bite. *Ann Rheum Dis* 1992;**51**:411–12.
37. Francis E. Rat-bite fever and relapsing fever in the United States. *Trans Assoc Am Physicians* 1932;**47**:143–51.
38. Fredricks DN, Relman DA. Application of polymerase chain reaction to the diagnosis of infectious diseases. *Clin Infect Dis* 1999; **29**:475–86; quiz 487.
39. Freels LK, Elliott SP. Rat bite fever: three case reports and a literature review. *Clin Pediatr (Phila)* 2004;**43**:291–5.
40. Freundt EA. Experimental investigations into the pathogenicity of the L-phase variant of *Streptobacillus moniliformis*. *Acta Pathol Microbiol Scand* 1956;**38**:246–58.
41. Gaastra W, Boot R, Ho HT, et al. Rat bite fever. *Vet Microbiol* 2009;**133**:211–28.
42. Garcia LS. *Clinical Microbiology Procedures Handbook*, vols. 1-3. 3rd ed. Washington, DC: American Society for Microbiology; 2010.
43. Gascard E, Vignoli R, Moulard JC, et al. [Case of febrile eruption after a cat bite: *Streptobacillus moniliformis* septicemia?]. *Mars Med* 1967;**104**:861–4.
44. Glaser C, Lewis P, Wong S. Pet-, animal-, and vector-borne infections. *Pediatr Rev* 2000;**21**:219–32.
45. Glastonbury JR, Morton JG, Matthews LM. *Streptobacillus moniliformis* infection in Swiss white mice. *J Vet Diagn Invest* 1996; **8**:202–9.
46. Glunder G, Hinz KH, Stiburek B. [Joint disease in turkeys caused by *Streptobacillus moniliformis* in Germany]. *Dtsch Tierarztl Wochenschr* 1982;**89**:367–70.
47. Graves MH, Janda JM. Rat-bite fever *(Streptobacillus moniliformis):* a potential emerging disease. *Int J Infect Dis* 2001;**5**:151–5.
48. Hagelskjaer L, Sorensen I, Randers E. *Streptobacillus moniliformis* infection: 2 cases and a literature review. *Scand J Infect Dis* 1998;**30**:309–11.
49. Hockman DE, Pence CD, Whittler RR, et al. Septic arthritis of the hip secondary to rat bite fever: a case report. *Clin Orthop Relat Res* 2000:173–6.
50. Holmes B, Pickett M, Hollis D. Streptobacillus moniliformis. In: Murray P, editor: *Manual of Clinical Microbiology*. Washington, D.C.: ASM Press; 1995. p. 506–7.
51. Holroyd KJ, Reiner AP, Dick JD. Streptobacillus moniliformis polyarthritis mimicking rheumatoid arthritis: an urban case of rat bite fever. *Am J Med* 1988;**85**:711–14.
52. Hopkinson WI, Lloyd JM. *Streptobacillus moniliformis* septicaemia in spinifex hopping mice *(Notomys alexis)*. *Aust Vet J* 1981; **57**:533–4.
53. Islam S, Cooney T, Singh A, et al. Photo quiz. Painful arthritis and extremity rash in an 8-year-old boy. *Clin Infect Dis* 2012; **54**:1473, 1514–1475.
54. Kaspareit-Rittinghausen J, Wullenweber M, Deerberg F, et al. [Pathological changes in *Streptobacillus moniliformis* infection of C57bl/6J mice]. *Berl Munch Tierarztl Wochenschr* 1990;**103**:84–7.
55. Kimura M, Tanikawa T, Suzuki M, et al. Detection of *Streptobacillus* spp. in feral rats by specific polymerase chain reaction. *Microbiol Immunol* 2008;**52**:9–15.
56. Kirchner BK, Lake SG, Wightman SR. Isolation of *Streptobacillus moniliformis* from a guinea pig with granulomatous pneumonia. *Lab Anim Sci* 1992;**42**:519–21.
57. Legout L, Senneville E, Mulleman D, et al. Rat bite fever mimicking rheumatoid arthritis. *Scand J Infect Dis* 2005;**37**:532–3.
58. Levaditi C, Nicolau S, Poindoux P. Sur le role etiologique de *Streptobacillus moniliformis* (nov. spec.) dans l'erytheme polymorphe aigu septicemique. *Compt Rend Acad Sci* 1925;**180**:1188–90.

59. Mandel DR. Streptobacillary fever: an unusual cause of infectious arthritis. *Cleve Clin Q* 1985;**52**:203–5.
60. Mathiasen T, Rix M. [Rat-bite: an infant bitten by a rat]. *Ugeskr Laeger* 1993;**155**:1475–6.
61. McCormack RC, Kaye D, Hook EW. Endocarditis due to *Streptobacillus moniliformis*. *JAMA* 1967;**200**:77–9.
62. McEvoy MB, Noah ND, Pilsworth R. Outbreak of fever caused by *Streptobacillus moniliformis*. *Lancet* 1987;**2**:1361–3.
63. McHugh TP, Bartlett RL, Raymond JI. Rat bite fever: report of a fatal case. *Ann Emerg Med* 1985;**14**:1116–18.
64. McMillan B, Boulger LR. Squirrel-bite fever. *Trans R Soc Trop Med Hyg* 1968;**62**:567.
65. Mock H, Morrow A. Rat-bite fever transmitted from a cat bite. *Ill Med J* 1932;**61**:67–70.
66. Miyake H. Gerber die rattenbiskrankheit. *Mitt Grenzgeb Med Chir* 1900;**5**:231.
67. Mohamed YS, Moorhead PD, Bohl EH. Natural *Streptobacillus moniliformis* infection of turkeys, and attempts to infect turkeys, sheep, and pigs. *Avian Dis* 1969;**13**:379–85.
68. Nakagomi D, Deguchi N, Yagasaki A, et al. Rat-bite fever identified by polymerase chain reaction detection of *Streptobacillus moniliformis* DNA. *J Dermatol* 2008;**35**:667–70.
69. Ojukwu IC, Christy C. Rat-bite fever in children: case report and review. *Scand J Infect Dis* 2002;**34**:474–7.
70. Parker F, Hudson NP. The etiology of Haverhill fever (erythema arthriticum epidemicum). *Am J Pathol* 1926;**2**:357–80, 357.
71. Peel MM. Dog-associated bacterial infections in humans: isolates submitted to an Australian reference laboratory, 1981-1992. *Pathology* 1993;**25**:379–84.
72. Pilsworth R. Haverhill fever. *Lancet* 1983;**2**:336–7.
73. Pins MR, Holden JM, Yang JM, et al. Isolation of presumptive *Streptobacillus moniliformis* from abscesses associated with the female genital tract. *Clin Infect Dis* 1996;**22**:471–6.
74. Pirodda E. [Atypical otomastoiditis due to *Haverhillia multiformis (Streptobacillus moniliformis)*]. *Otorinolaringol Ital* 1965;**34**:23–32.
75. Place E, Sutton H, Willner O. Erythema arthriticum epidemicum: preliminary report. *Boston Med Surg J* 1926;**194**:285–7.
76. Portnoy BL, Satterwhite TK, Dyckman JD. Rat bite fever misdiagnosed as Rocky Mountain spotted fever. *South Med J* 1979;**72**: 607–9.
77. Prager L, Frenck RW Jr. *Streptobacillus moniliformis* infection in a child with chickenpox. *Pediatr Infect Dis J* 1994;**13**:417–18.
78. Priest WS, Smith JM, Mc GC. Penicillin therapy of subacute bacterial endocarditis: a study of the end results in 34 cases, with particular reference to dosage, methods of administration, criteria for judging adequacy of treatment and probable reasons for failures. *Arch Intern Med (Chic)* 1947;**79**:333–59.
79. Prouty M, Schafer EL. Periarteritis nodosa associated with ratbite fever due to streptobacillus moniliformis (erythema arthriticum epidemicum). *J Pediatr* 1950;**36**:605–13.
80. Raffin BJ, Freemark M. Streptobacillary rat-bite fever: a pediatric problem. *Pediatrics* 1979;**64**:214–17.
81. Reitzel R, Haim A, Prindle K. Rat bite fever from a field mouse. *JAMA* 1936;**106**:1090.
82. Rey JL, Laurans G, Pleskof A, et al. [*Streptobacillus moniliformis* endocarditis: apropos of 2 cases]. *Ann Cardiol Angeiol (Paris)* 1987;**36**:297–300.
83. Richter C. Incidence of rat bites and rat-bite fever in Baltimore. *JAMA* 1945;**128**:324.
84. Ripley H, Van Sant H. Rat-bite fever acquired from a dog. *JAMA* 1934;**102**:1917.
85. Rordorf T, Zuger C, Zbinden R, et al. *Streptobacillus moniliformis* endocarditis in an HIV-positive patient. *Infection* 2000;**28**: 393–4.
86. Roughgarden JW. Antimicrobial therapy of ratbite fever. a review. *Arch Intern Med* 1965;**116**:39–54.
87. Roundtree P, Rohan M. A fatal human infection with *Streptobacillus moniliformis*. *Med J Aust* 1941;**1**:359.
88. Rowbotham TJ. Rapid identification of *Streptobacillus moniliformis*. *Lancet* 1983;**2**:567.
89. Rumley RL, Patrone NA, White L. Rat-bite fever as a cause of septic arthritis: a diagnostic dilemma. *Ann Rheum Dis* 1987;**46**: 793–5.
90. Rupp ME. *Streptobacillus moniliformis* endocarditis: case report and review. *Clin Infect Dis* 1992;**14**:769–72.
91. Russell EG, Straube EF. Streptobacillary pleuritis in a koala *(Phascolarctos cinereus)*. *J Wildl Dis* 1979;**15**:391–4.
92. Rygg M, Bruun CF. Rat bite fever (*Streptobacillus moniliformis*) with septicemia in a child. *Scand J Infect Dis* 1992;**24**:535–40.
93. Saez Villaverde R, Alvarez Lario B, Alegre Lopez J, et al. [*Streptobacillus moniliformis* septic oligoarthritis (fever caused by rat bite)]. *Rev Clin Esp* 1996;**196**:413–15.
94. Savage NL. Host-parasite relationships in experimental *Streptobacillus moniliformis* arthritis in mice. *Infect Immun* 1972;**5**:183–90.
95. Savage NL, Joiner GN, Florey DW. Clinical microbiological, and histological manifestations of *Streptobacillus moniliformis*-induced arthritis in mice. *Infect Immun* 1981;**34**:605–9.
96. Schottmüller H. Zur aetiologie und Klinik der Bisskrankheit. *Dermatol Wochenschr* 1914;**58**(Suppl.):77–103.
97. Schuurman B, van Griethuysen AJ, Marcelis JH, et al. [Rat bite fever after a bite from a tame pet rat]. *Ned Tijdschr Geneeskd* 1998;**142**:2006–9.
98. Sens MA, Brown EW, Wilson LR, et al. Fatal *Streptobacillus moniliformis* infection in a two-month-old infant. *Am J Clin Pathol* 1989;**91**:612–16.
99. Shackelford P. Rat-bite fever. In: Kaplan S, editor. *Current Therapy in Pediatric Infectious Disease*. 3rd ed. St. Louis: Mosby–Year Book; 1993. p. 235.
100. Shanson DC, Pratt J, Greene P. Comparison of media with and without 'Panmede' for the isolation of *Streptobacillus moniliformis* from blood cultures and observations on the inhibitory effect of sodium polyanethol sulphonate. *J Med Microbiol* 1985;**19**:181–6.
101. Shanson DC, Gazzard BG, Midgley J, et al. *Streptobacillus moniliformis* isolated from blood in four cases of Haverhill fever. *Lancet* 1983;**2**:92–4.
102. Shvartsblat S, Kochie M, Harber P, et al. Fatal rat bite fever in a pet shop employee. *Am J Ind Med* 2004;**45**:357–60.
103. Simon MW, Wilson HD. *Streptobacillus moniliformis* endocarditis: a case report. *Clin Pediatr (Phila)* 1986;**25**:110–11.
104. Stehle P, Dubuis O, So A, et al. Rat bite fever without fever. *Ann Rheum Dis* 2003;**62**:894–6.
105. Strangeways W. Rats as carriers of *Streptobacillus moniliformis*. *J Pathol Bacteriol* 1933;**37**:45–51.
106. Stuart-Harris C, Wells A, Rosher H. Four cases of infective endocarditis due to organisms similar to *Haemophilus parainfluenza* and one case due to pleomorphic *Streptobacillus J Pathol Bacteriol* 1935;**41**:407–21.
107. Taber LH, Feigin RD. Spirochetal infections. *Pediatr Clin North Am* 1979;**26**:377–413.
108. Taylor JD, Stephens CP, Duncan RG, et al. Polyarthritis in wild mice (Mus musculus) caused by *Streptobacillus moniliformis*. *Aust Vet J* 1994;**71**:143–5.
109. Valverde CR, Lowenstine LJ, Young CE, et al. Spontaneous rat bite fever in non-human primates: a review of two cases. *J Med Primatol* 2002;**31**:345–9.
110. van Nood E, Peters SH. Rat-bite fever. *Neth J Med* 2005;**63**: 319–21.
111. Vasseur E, Joly P, Nouvellon M, et al. Cutaneous abscess: a rare complication of *Streptobacillus moniliformis* infection. *Br J Dermatol* 1993;**129**:95–6.
112. Vasseur E, Joly P, Nouvellon M, et al. [*Streptobacillus moniliformis:* a rare anthropozoonosis sometimes observed in urban area]. *Ann Dermatol Venereol* 1993;**120**:813–14.
113. Wang TK, Wong SS. *Streptobacillus moniliformis* septic arthritis: a clinical entity distinct from rat-bite fever? *BMC Infect Dis* 2007; **7**:56.
114. Washburn R. *Streptobacillus moniliformis* (rat-bite fever). In: Mandell G, Bennett J, Dolin R, editors. *Principles and Practice of Infectious Diseases*, vol. 2. New York: Churchill Livingstone; 1995. p. 2084–6.
115. Watkins CG. Ratbite fever. *J Pediatr* 1946;**28**:429–48.
116. Wilcox W. Violent symptoms from the bite of a rat. *Am J Med Sci* 1839;**26**:245–6.
117. Wilkins EG, Millar JG, Cockcroft PM, et al. Rat-bite fever in a gerbil breeder. *J Infect* 1988;**16**:177–80.
118. Wouters EG, Ho HT, Lipman LJ, et al. Dogs as vectors of *Streptobacillus moniliformis* infection? *Vet Microbiol* 2008;**128**:419–22.
119. Wullenweber M. *Streptobacillus moniliformis*: a zoonotic pathogen—taxonomic considerations, host species, diagnosis, therapy, geographical distribution. *Lab Anim* 1995;**29**:1–15.

TABLE 139-1 ***Bartonella* Species Causing Human Disease**

Bartonella Species	Country of First Cultivation	Year of Description	Reservoir Host/Vector	Human Disease
B. alsatica	France	1999	Rabbit	Endocarditis, lymphadenopathy
B. bacilliformis		1909	Human/sand fly	Carrión disease, Oroya fever, verruga peruana
B. clarridgeiae		1996	Cat/cat flea	Cat-scratch disease
B. elizabethae	United States	1993	Rat	Endocarditis, neuroretinitis
B. grahamii	United Kingdom	1995	Rat, insectivore	Neuroretinitis
B. henselae		1990	Cat/cat flea	Cat-scratch disease, endocarditis, bacillary angiomatosis, bacillary peliosis hepatis, Parinaud oculoglandular syndrome, neuroretinitis, osteomyelitis, arthropathy, bacteremia with fever
B. koehlerae	United States	1999	Cat	Endocarditis
B. mayotimonensis	United States	2009	Unknown	Endocarditis
B. melophagi	United States	2006	Unknown	Bacteremia
B. quintana		1920	Human/body louse	Trench fever, endocarditis, bacillary angiomatosis
B. rochalimae	United States	2007		Bacteremia, fever, splenomegaly
B. tamiae	Thailand	2008		Febrile illness
B. vinsonii subsp. *arupensis*	United States	1999	Dog, rodent/ticks	Bacteremia with fever, endocarditis
B. vinsonii subsp. *berkhoffii*	United States	1999	Dog	Endocarditis
B. washoensis		2000	Ground squirrel	Myocarditis

Adapted from Rolain JM, Rauoult D. *Bartonella* infections. In: Goldman L, Schafer AI, et al, editors. *Cecil medicine.* 24th ed. Philadelphia: Saunders; 2012, p 1907.

Organisms of the *Bartonella* genus may colonize human endothelial cells or may persist as intraerythrocytic parasites in the host.

CAT-SCRATCH DISEASE

Cat-scratch disease (CSD), caused by *Bartonella henselae*, is an acute, self-limited infection that begins as a papule or nodule at the site of a scratch or bite by a cat, followed by regional lymphadenopathy. In a small number of patients, more serious systemic complications result, including involvement of the central nervous system, liver, spleen, bone, heart, eyes, or skin. Although CSD was recognized as a clinical entity in the 1930s, the first written report, by Debré from France, was not published until 1950.

Etiology

For more than 40 years, numerous unsuccessful attempts were made to isolate an organism from nodes of patients with CSD. The first clue emerged in 1983, when Wear and colleagues,[73] using Warthin-Starry silver stain, demonstrated small, pleomorphic, gram-negative bacilli in lymph nodes and skin papules from patients with CSD. Then, in 1988, an organism was isolated by English and associates[23] from the lymph nodes of patients with CSD and subsequently termed *Afipia felis*. However, the discovery of bacillary angiomatosis and its association with *B.* (formerly *Rochalimaea*) *henselae* suggested that it might be the agent responsible for CSD.[59] In 1992, Regnery and coworkers,[58] using indirect fluorescent antibody (IFA) assays, demonstrated elevated antibodies to *B. henselae* in serum samples from patients with suspected CSD. We and others using enzyme-linked immunosorbent assays (ELISAs) demonstrated that patients with CSD had significant serologic responses to *B. henselae* and *Bartonella quintana* but not to *A. felis*. These data further supported that the causative agent of CSD was related antigenically to the *Bartonella* genus and not to *Afipia*.[70] In a physician survey to identify cases of CSD occurring during a 13-month period in cat owners in Connecticut, Zangwill and colleagues[75] demonstrated that of 45 patients with clinical CSD, 38 had antibody titers of 1:64 or higher for *B. henselae* as compared with only 4 of 112 samples from controls (p <.001). Finally, the use of polymerase chain reaction (PCR) confirmed the causative agent of CSD as *B. henselae*.[2] Eventually, PCR amplification was used to detect *B. henselae* not only in purulent material aspirated from involved nodes but also in CSD skin test material.[1,32,66] Subsequently, *B. henselae* has been cultured from lymph nodes obtained from patients with CSD.[29]

Transmission

Domesticated house cats are healthy carriers of *B. henselae*, and cat fleas *(Ctenocephalides felis)* play a major role in cat-to-cat transmission. Approximately 90 percent of patients with CSD have a history of exposure to cats, particularly a scratch or bite from a kitten with fleas. Although cats remain asymptomatic, they serve as a persistent reservoir for *B. henselae*. Reported *B. henselae* seroprevalence rates in cats vary. The southeastern United States, Hawaii, and coastal California have the highest seroprevalence in cats (40% to 54.7%), whereas rates are much lower (3.7% to 6.7%) in the Rocky Mountain and Midwestern regions.[39] Blood samples obtained from

domestic and impounded cats in the San Francisco Bay region grew *B. henselae* 41 percent of the time. *B. henselae* also was detected in fleas from infected cats by both direct culture and PCR, although no data support flea-to-human transmission. Interestingly, Chang and colleagues[18] found that *Ixodes pacificus* and *Dermacentor* ticks from five of six California counties were also PCR positive for *B. henselae*. Coinfections with *B. henselae* and *Borrelia burgdorferi* have been described in three patients in New Jersey.[24] *Ixodes* ticks obtained from two of the three households of patients with CSD were PCR positive for *B. henselae*. Thus, these observations should alert clinicians to consider *Bartonella* infection in humans after tick bites, although its frequency of transmission is unknown.

Epidemiology

CSD most commonly affects children and young adults worldwide, with regional variations. The Centers for Disease Control and Prevention estimates that, overall, more than 22,000 cases of CSD occur each year in the United States. Using a national inpatient database, Reynolds and coworkers estimated that 437 pediatric hospitalizations for CSD (0.60 per 100,000 population) occurred in 2000, with 60 percent of them occurring between July and October. Rates were higher in children younger than 5 years and higher in the southern states, underscoring the regional differences. Zangwill and colleagues[75] suggested that the seasonality of CSD could be explained by the breeding patterns of cats and fleas. Cats often become pregnant in late spring or early fall, and after a 9-week gestation period, kittens are birthed in early fall or midwinter. Flea breeding peaks during the late summer months.

Pathobiology

Histopathologic examination of the primary inoculation lesion demonstrates dermal necrosis, with variable numbers of histiocytes and occasional multinucleated giant cells accompanied by scattered microabscesses with neutrophils, eosinophils, lymphocytes, and plasma cells. The epidermal changes are nonspecific and consist of parakeratosis, hyperkeratosis, edema, and exocytosis of inflammatory cells. Characteristic findings in the lymph nodes, similar to the primary lesion, include follicular hyperplasia, focal cortical necrosis, and necrotizing granulomas with central microabscesses and palisading histiocytes. A perivascular neutrophilic infiltrate may also be present. Subsequently, the lesions progress to "stellate microabscesses" within small cortical granulomas. Warthin-Starry or Steiner silver impregnation stains may reveal pleomorphic bacilli in clusters or short chains within the areas of central necrosis or around small vessels, particularly in early lesions. Granulomas with microabscess formation also can be found in the liver, spleen, and bone. Because other organisms such as tularemia, fungi, and mycobacteria may have similar histopathologic characteristics, the histopathologic findings must be correlated with the clinical findings and other laboratory studies, including serology, culture, or PCR.

In vitro studies have identified several additional properties of *B. henselae*. When *B. henselae* infects endothelial cells, it induces activation of nuclear factor kappa B and expression of adhesion molecules, an important step in the pathogenesis of CSD and bacillary angiomatosis.[18,30] Animal studies have shown that interferon-γ–mediated activation of macrophages is involved in clearing *B. henselae* infection and that microbicidal activity is mediated to a large extent by nitric oxide.[50] A role for cell-mediated immunity in CSD is suggested by the positive skin test reaction to injected material aspirated from cat-scratch nodes and the granulomatous lesions noted in biopsy specimens.

Clinical Manifestations and Course

The most common clinical manifestation, often referred to as *typical CSD*, is a gradually enlarging regional lymph node occurring 1 to 3 weeks after a scratch or bite from a cat. An inoculation site, which often appears as a red papule 3 to 5 mm in diameter, can be detected in two thirds of patients. Constitutional symptoms of malaise, fatigue, anorexia, emesis, and headache are common but usually are mild. Fever occurs in fewer than half the patients with typical CSD, thus rendering the common name "cat-scratch fever" inexact. Physical examination generally reveals a skin papule at the inoculation site and regional lymphadenopathy (Fig. 139-1). In approximately 50 percent of cases, lymphadenopathy is the only manifestation of the disease and occurs most commonly in the upper extremity (axillary or epitrochlear lymph nodes, 46.1%), the head and neck area (cervical or submandibular, 26.1%), and the lower extremities (femoral or inguinal, 17.5%).[15] Supraclavicular lymphadenopathy, which often raises concern for malignant neoplasm, occurs rarely (2% of typical CSD). Suppuration of the involved lymph nodes occurs in about 10 percent of patients (Fig. 139-2). More unusual manifestations of CSD include neuroretinitis, subacute iritis, optic neuritis, focal chorioretinitis, and the oculoglandular syndrome of Parinaud (Fig. 139-3). Patients with this syndrome have painful conjunctivitis without eye drainage and preauricular adenopathy. Recovery is spontaneous without sequelae within 2 to 4 months after onset.

Hepatosplenic CSD is an important cause of prolonged fever in children and should be included in the differential diagnosis of fever of unknown origin. Often these children will have fever for several weeks (mean, 3 weeks), abdominal pain (occurring in about two thirds of patients),[5,22] joint pain, headache, weight loss, and chills. Significant lymphadenopathy is lacking in more than 50 percent of cases, thus rendering the diagnosis problematic. Hepatic or splenic enlargement (or both) is found in half of these children, but hepatic transaminase levels usually are normal. Erythrocyte sedimentation rates invariably are elevated and frequently are greater than 100 mm/hr. Abdominal imaging usually demonstrates typical granulomatous lesions in the liver or spleen that often are diagnostic of this entity (Fig. 139-4). Although both ultrasonography and computed tomography (CT) will demonstrate these lesions, a number of reports suggest that the ultrasound evaluation is more sensitive

FIGURE 139-1 ■ Cat-scratch disease involving an axillary lymph node with a primary granuloma of the upper part of the arm. Note that the primary lesion is within the line of a healed scratch. (Courtesy Hugh A. Carithers, MD.)

FIGURE 139-2 ■ Purulent material aspirated from an epitrochlear lymph node of a child with cat-scratch disease. The primary lesion was on the index finger.

than CT.[43,46] As is the case with other infections causing hepatosplenic granulomas, late calcification has been described.

Neurologic complications have been reported in approximately 2 percent of CSD cases with an estimated 51 pediatric hospitalizations in 2000.[62] Encephalopathy, the most common manifestation, typically occurs 1 to 6 weeks after the onset of lymphadenopathy. Fever and seizures develop abruptly, followed by confusion, disorientation, and, occasionally, combativeness. Status epilepticus is well described. Cerebrospinal fluid examination frequently is normal, but occasional mild pleocytosis or elevated protein levels are seen. Electroencephalographic findings frequently are abnormal, with diffuse slowing or focal abnormalities. CT of the head generally is normal. Resolution of seizures and normalization of mental status often occur as suddenly as does the onset. Neurologic recovery commonly occurs within weeks and almost always is complete within a year. Persistent deficits or a need for prolonged anticonvulsant therapy have been reported in only a few cases.[34,60] Two immunocompetent children have been reported to have central nervous system infection associated with *B. quintana* with a similar symptom constellation.[52]

Osteomyelitis caused by *B. henselae* is well described in the literature, with one report documenting its occurrence in 2 of 1200 cases. With improved imaging, serologic methods, and PCR for diagnosis, it is now clear that *B. henselae* osteomyelitis can occur as part of a disseminated infection, in the setting of otherwise typical CSD, or as a seemingly isolated manifestation.[37,72] The pathogenesis is not clear, but it probably represents

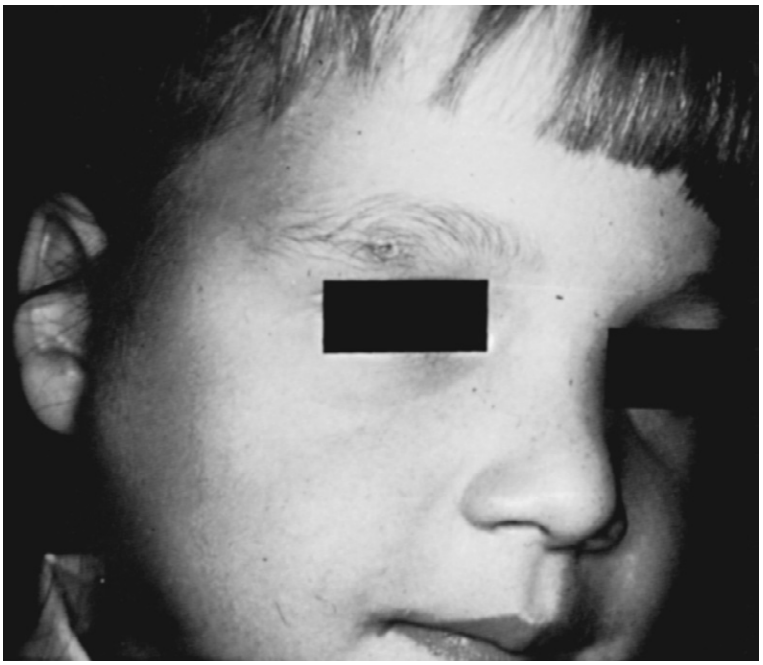

FIGURE 139-3 ■ Child with the oculoglandular syndrome of Parinaud as a manifestation of cat-scratch disease. Note the parotid swelling and the primary site in the right eyebrow. (Courtesy James D. Cherry, MD.)

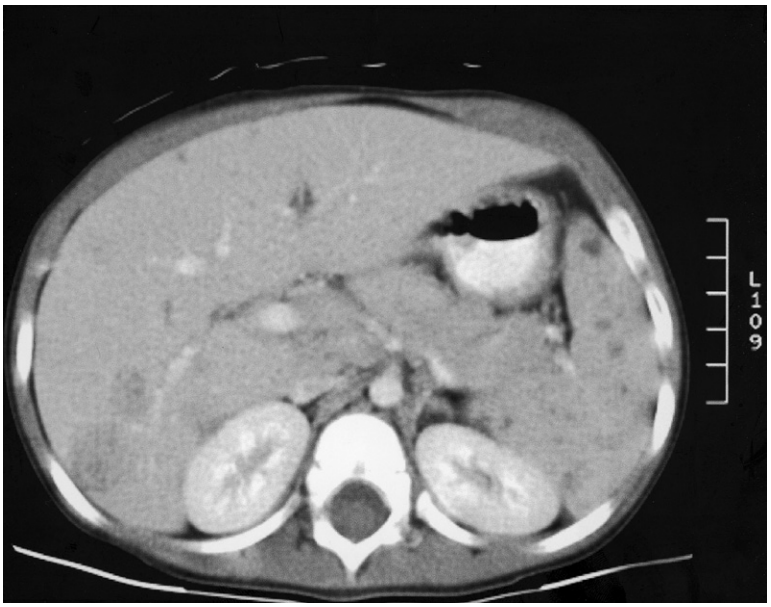

FIGURE 139-4 ■ Multiple hypodense lesions in the liver and spleen seen on CT in a child with prolonged fever, abdominal pain, and positive *Bartonella henselae* serology.

hematogenous spread, but local spread from a lymph node or abscess has been reported. Although the presentation of osteomyelitis may be acute, many patients have weeks to months of pain and intermittent fever before the diagnosis is made. A high proportion of reported cases involve the axial skeleton, most commonly the vertebral column. In a review of 47 cases by Hajjaji and colleagues,[35] lesions in the vertebrae or pelvic girdle were present in more than 50 percent of cases. Involvement of the long bones of the limbs, the sternum, and the skull has also been described. Although single-site involvement is typical, multifocal involvement was present in 28 percent of the cases in Hajjaji and colleagues' review.[35] Most commonly, the bony lesions identified by plain film, CT, or magnetic resonance imaging (MRI) are osteolytic. MRI also may show periosteal reactions, marginal sclerosis, and bone marrow lesions without cortical destruction, which are seen as hyperintense foci on T2-weighted images.

Patients with CSD also may have skin lesions other than at the inoculation site, including maculopapular rash, erythema nodosum, and petechiae due to thrombocytopenia. Molecular techniques have also documented hepatitis, hemolytic anemia, atypical pneumonia, pulmonary nodules, an infectious mononucleosis–like syndrome, and disseminated bartonellosis. Bacillary angiomatosis (mixed neovascular and inflammatory lesions in the skin or viscera), bacillary peliosis (dilated

capillaries or multiple blood-filled cavernous spaces in the liver, spleen, or lymph nodes), and relapsing fever with bacteremia due to *B. henselae* also can develop in immunocompromised subjects.[48]

ENDOCARDITIS

Epidemiology

Bartonella spp. have been reported as the cause of endocarditis in more than 100 cases,[56] with most cases in adults and several in adolescents. Often the cultures are negative.[7,54,55,68] In a series from a large referral laboratory in France *Bartonella* species accounted for 28 percent of culture-negative endocarditis, while in another report *Bartonella* accounted for only 3 percent of the culture-negative cases. *B. quintana* predominates and accounts for roughly 75 percent of the cases of endocarditis. *B. henselae* causes most of the remaining cases, and other species (*B. elizabethae* and *B. vinsonii*) have been reported in isolated cases. The epidemiologic characteristics of the patients differ considerably between the main two causative species. *B. quintana* endocarditis is highly associated with homelessness, chronic alcoholism, and body lice and is less likely to occur in the setting of cat exposure and underlying valvular heart disease. Endocarditis cases due to *B. quintana* also have been described in persons immunocompromised by infection with human immunodeficiency virus (HIV).[55,67] In contrast, *B. henselae* endocarditis occurs most often in patients with underlying valvulopathy (90%) and clearly is linked to exposure to cats or fleas.[27]

Clinical Manifestations

Bartonella endocarditis has a subacute presentation with fever, heart murmur, and often evidence of heart failure. Prolonged fever, night sweats, and profound weight loss also may occur. The aortic valve usually is affected; right ventricular or prosthetic valve involvement is an unusual finding. Valvular surgery is needed in many cases, often owing to delay in diagnosis.

BARTONELLOSIS (CARRIÓN DISEASE)

Bartonellosis is a term that has been used to describe a distinct disease seen in Peru, Ecuador, and Colombia caused by *Bartonella bacilliformis*. *B. bacilliformis* causes two distinct illnesses: Oroya fever, a severe febrile hemolytic anemia, and verruga peruana, an eruption of hemangioma-like lesions.

The first written account of bartonellosis is attributed to Gago de Vadillo, who published a treatise on the subject in 1630. Then, in 1764, Cosme Bueno first described the vector of this disease and of cutaneous leishmaniasis as the uta or sand fly.[36] In the mid 1800s an epidemic occurred that took the lives of hundreds.[65]

In 1885, a Peruvian medical student (Carrión) was collecting data on the geographic distribution and clinical features of verruga peruana. To study disease symptoms in the pre-eruption period of verruga, he inoculated himself with material taken from a patient with verruga. Within 21 days after inoculation he became symptomatic, developed classic Oroya fever, and died. In 1905, Alberto Barton, a Peruvian physician, described the etiologic agent *(B. bacilliformis)*, but several years passed before this organism was accepted as the cause of Oroya fever and named in his honor.

Epidemiology

Historically, the distribution of bartonellosis has been restricted to the mountain valleys of the Andes Mountains in Peru, Ecuador, and Colombia between the altitudes of 500 and 3200 meters above sea level and primarily in valleys that are at right angles to the prevailing wind. The distribution of disease reflects the presence of the sand fly vector *Lutzomyia verrucarum*, which is seen only at these altitudes. The disease is usually acquired at twilight or soon thereafter because of the feeding habits of the insects. Within the region, the disease is endemic, but with sporadic epidemic outbreaks.[33,42]

Pathobiology

After inoculation by the sand fly, *Bartonella* organisms enter the endothelial cells of the blood vessels, where they proliferate. Microscopically, masses of organisms are noted within the cytoplasm of the cells lining the blood vessels and lymph channels, causing the lumen of the vessel to bulge. The organisms may also be found in reticuloendothelial cells in the lymph nodes, liver, and spleen, as well as within the bone marrow, kidneys, adrenals, pancreas, and rarely, the skin, heart, and lungs of infected patients.[9] They also parasitize erythrocytes, causing indentations and deformation of the erythrocyte membrane, membrane fusion, and entrance of the organisms into intracellular vacuoles, where they replicate.[10] Severe anemia results from destruction of the parasitized cells, with as many as 90 percent of the erythrocytes involved. However, all parasitized cells are not destroyed and no hemolysins or agglutinins have been recovered.[61] Cuadra and Takano[21] have shown that the parasites are located predominantly within cells, rather than on the surface. In the recovery phase, the rod-shaped organisms change to a more coccoid form and rapidly disappear from the blood.

A patient who survives the acute phase of Oroya fever may or may not experience cutaneous manifestations of the disease, which appear as nodular, hemangiomatous lesions ranging from a few millimeters to several centimeters. Light microscopy reveals angioblastic and histiocytic hyperplasia of the dermis. Numerous newly formed small vessels with endothelial cell proliferation are found. Mast cells, lymphocytes, and macrophages are present.[4] Electron microscopy demonstrates that the bacterial organisms are located within the verruga, extracellular in the fine fibrous interstitium.[57] In vitro studies have shown that *B. bacilliformis* stimulates endothelial cells, and in vivo studies have shown angiogenic activity. This finding may explain the similar pathologic findings of verruca and bacillary angiomatosis produced by other *Bartonella* spp.[4]

Clinical Manifestations

The incubation period of bartonellosis varies from 2 to 14 weeks, with a mean of 3 weeks. Some patients are totally asymptomatic, and disease is detected only by blood culture or on serologic survey. In a population-based cohort study, 0.5 percent of participants had asymptomatic bacteremia.[17] Other patients only develop headache, malaise, and occasional fever, with *B. bacilliformis* recovered from blood cultures. Still others have severe anemia (Oroya fever), are febrile, and are deeply apathetic with a peculiar discoloration of their skin and sclerae.[63] Tachycardia and soft murmurs are noted; occasionally, peripheral vascular collapse occurs. Clouding of the sensorium and delirium are common findings; these effects usually are mild but may progress to overt psychosis. The body temperature usually fluctuates between 37.5°C and 38.5°C (99.5°F and 101.3°F). Physical examination discloses generalized lymphadenopathy and nonpainful hepatomegaly.[45]

Laboratory studies reveal anemia that is macrocytic and hypochromic, with anisocytosis and poikilocytosis. The erythrocyte count may drop to as low as 500,000/mm^3 in the first 2 to 4 weeks of illness, with reticulocytes increasing to 50 percent. The pathognomonic sign of the disease is the presence of *B. bacilliformis* within Giemsa-stained erythrocytes as red-violet rods. The leukocyte count may be normal, low, or elevated.

The "critical stage" of the anemia is the period of transition when the organism suddenly disappears from the erythrocytes. In as many as 40 percent of patients, the illness becomes more severe, with the development of an intercurrent infection, particularly with *Salmonella*.[20] In the pre-eruptive stage, patients may complain of pain in their joints, bones, and muscles, as well as cramps and paresthesias, but the anemia and lymphadenopathy of the invasive stage disappear.

The appearance of red cutaneous nodules, or verruga, is pathognomonic of the disease in the eruptive stage. Usually, these nodules are present in the skin, but they may also be found in mesenchymatous tissue. They vary greatly in number and size, from small asymptomatic nodules to disfiguring zonular (hemangioma-like) lesions. This stage may last from several months to a year and may be the sole manifestation of the disease, particularly in school-aged children in endemic areas.

TRENCH FEVER

Epidemiology

Trench fever (from *B. quintana*) was first described in Russia during World War I when more than 1 million troops were infected.[9] The human body louse, *Pediculus humanus* var. *corporis*, is the vector, with humans the only known reservoir.

Clinical Manifestations

After an incubation period that is extremely variable (from 4 to 35 days with an average of 22 days), one of four major fever patterns results: (1) a single febrile episode; (2) a single febrile period lasting 4 to 5 days; (3) three to eight recurrent febrile episodes, each lasting 4 to 5 days (sometimes for a year or more); or (4) persistent fever lasting 2 to 6 weeks.[26,69] Associated signs and symptoms include conjunctival injection, retro-orbital pain, myalgias, arthralgias, headache, bone pain (especially in the shins), and splenomegaly. Chronic bacteremia following clinical improvement is common.[26]

BACILLARY ANGIOMATOSIS AND BACILLARY PELIOSIS HEPATIS

Etiology

Both *B. henselae* and *B. quintana* can cause vasculoproliferative disorders, which typically occur in immunocompromised persons, primarily adults with acquired immunodeficiency syndrome (AIDS) and with very low CD4$^+$ counts, and, to a lesser extent, in adults with cancer or recipients of organ transplants. Although these infections are exceptionally rare in children, reports exist describing bacillary angiomatosis in a child infected with HIV,[19] a child undergoing chemotherapy,[51] and, more rarely still, children who are thought to be immunocompetent.[76] Vasculoproliferation can also manifest as cutaneous or subcutaneous lesions (bacillary angiomatosis, caused by *B. henselae* or *B. quintana*) and pathologically resemble the verruga of *B. bacilliformis* or solid organ lesions (bacillary peliosis hepatis, caused only by *B. henselae*).

Clinical Manifestations

Characteristically, the vasoproliferative lesions of bacillary angiomatosis are red with a collarette of scale, but the clinical findings can be diverse. Deep soft tissue masses may develop, and trauma may result in ulceration or bleeding. Osseous lesions occur in the long bones and can be very painful. The central nervous system, bone marrow, and mucosal surfaces of the gastrointestinal and respiratory tracts may also be involved.

Bacillary peliosis hepatis is seen primarily in HIV-infected adults who have fever and abdominal pain. In these patients, vascular proliferative lesions develop, primarily in the liver and spleen, although the abdominal lymph nodes and bone marrow may also be involved. This disease has not been reported in children.

DIAGNOSIS

Cat-Scratch Disease

Until recently, the clinical diagnosis of CSD required the presence of at least three of the following: a history of contact with a cat and the presence of a scratch or a primary lesion, a positive skin test using material obtained from a purulent node from a patient with CSD, regional lymphadenopathy with negative studies for other potential causes of lymphadenopathy, and characteristic histopathologic findings on a biopsy specimen. The difficulty in isolating organisms from routine cultures of lymph nodes or other tissue specimens from patients with

suspected CSD required the use of this strict clinical definition. In addition, an intradermal skin test composed of heated purulent lymph node material from patients with CSD was used for many years as a skin test reagent. Although the skin test was 90 to 98 percent sensitive and specific for the diagnosis of CSD, the material was not readily available, was not standardized, and was not licensed for routine use. In addition, the potential for transmission of other infectious agents with this test currently prohibits its use.

Specific diagnostic tests to assess serum antibodies against *B. henselae* such as IFA and ELISA and PCR amplification to identify *B. henselae* DNA sequences in tissues have reduced the need for the skin test and for invasive diagnostic procedures. Giladi and colleagues[31] developed a new enzyme immunoassay (EIA) for IgM and IgG that uses *N*-lauroylsarcosine–insoluble outer-membrane antigens from agar-grown *B. henselae* and demonstrated increased sensitivity. This study determined the EIA sensitivity to be 75 percent for anti–*B. henselae* IgG alone, 48 percent for IgM alone, and 85 percent overall when positive IgG, IgM, or both were accepted as diagnostic. Another study[49] evaluated the kinetics of anti–*B. henselae* IgM and IgG antibodies using EIA. Forty-eight (92%) of the 52 IgM-positive patients became IgM negative within 3 months after the onset of disease. Two hundred eight (93%) of 223 serum samples tested positive for anti–*B. henselae* IgG. Beyond 6, 12, and 24 months, 35 percent, 25 percent, and 25 percent of serum samples (and patients), respectively, remained IgG positive. Some authors have suggested that a single IgG titer of greater than 1:256 can reliably diagnose acute infection. For any manifestation of CSD, detection of a significant rise in titer between acute and convalescent sera is confirmatory. However, cross reactions between antibodies directed toward *B. henselae*, *B. quintana*, and *Chlamydia* spp. can render interpretation problematic. Cross adsorption and Western immunoblotting can overcome this problem.[38]

Histopathologic examination of cat-scratch tissue includes granuloma formation with microabscesses and follicular hyperplasia. A Warthin-Starry stain and culture (ideally plated on both chocolate and rabbit blood agar and cocultivated with eukaryotic cells) may be performed, although neither has high sensitivity. Very strain-specific assays involving PCR amplification of the 16S rRNA gene, the citrase synthase gene *(gltA)*, or the *htrA* gene of *B. henselae* offer improved sensitivity over culture and can be useful in establishing a diagnosis when purulent lymph node aspirates or other infected tissue are obtained.[25]

The differential diagnosis of CSD includes other forms of bacterial adenitis, typical or atypical mycobacterial infections, lymphogranuloma venereum, infectious mononucleosis, tularemia, plague, sporotrichosis, blastomycosis, histoplasmosis, syphilis, human immunodeficiency virus infection, neoplasm, or sarcoidosis.

Endocarditis

The diagnosis of endocarditis is generally made with the use of serology and/or PCR techniques; however, among patients with culture-negative endocarditis, a single IgG titer of 1:800 or higher has a 95.5 percent positive predictive value for *Bartonella* as the cause.[28] Because PCR of blood rarely is positive, PCR of valvular tissue is the most sensitive method to diagnose endocarditis. Special culturing techniques, including the use of Isolator tubes, and prolonged incubation may be helpful, in addition to serology, histopathology of valvular tissue, and PCR.[77]

Bartonellosis

The diagnosis of bartonellosis is based on clinical manifestations, on Giemsa-stained blood smears showing typical organisms, or on positive blood cultures. The presence of typical verruga in patients from an endemic area is pathognomonic of the disease. IgM antibody may be present in both stages of the disease, as well as in some asymptomatic persons.[33,40] Persons with typical Oroya fever treated with antibiotics may not have an antibody response.[33,40] An indirect fluorescent antibody assay has shown promise for evaluating patients in both the acute and convalescent phases of the disease.[16] The differential diagnosis in the initial phase includes typhoid fever, malaria, tuberculosis, leptospirosis, brucellosis, and meningitis, as well as hematologic malignancy and aplastic or hemolytic anemia. The eruptive phase may resemble hemangiomas, bacillary angiomatosis, Kaposi sarcoma, and other nodular diseases.

Trench Fever

In a nonepidemic situation, establishing the diagnosis of trench fever is very difficult because the manifestations are not distinctive. The relapsing form can mimic malaria or *Borrelia recurrentis* relapsing fever. A history of body louse infestation or association with an epidemic should heighten suspicion. *B. quintana* can be cultured from blood by using a modification that includes culturing on epithelial cells. Serologic testing is available; however, cross reactions with *B. henselae* occur.

Bacillary Angiomatosis and Bacillary Peliosis Hepatis

Given the difficulty with culture and the poor reliability of serologic studies, the diagnosis of these diseases relies on direct visualization of the organisms in tissue samples on electron microscopy or with Warthin-Starry stain or, increasingly, on detection of the organism in the affected tissue by PCR. The differential diagnosis of bacillary angiomatosis includes Kaposi sarcoma, pyogenic granuloma, verruga peruana, and multiple other opportunistic infections. The differential diagnosis of bacillary peliosis hepatis includes hepatic Kaposi sarcoma, lymphoma, extrapulmonary pneumocystosis, and infection with *Mycobacterium avium-intracellulare*.

TREATMENT

Cat-Scratch Disease

In an immunocompetent host, typical CSD is self-limited and resolves spontaneously in 1 to 2 months without the

use of antibiotics. Application of moist soaks and local heat, use of analgesics, limitation of activity of the affected limb, and aspiration of fluctuant material in the nodes (in some instances, done serially) may relieve the pain and resolve the inflammation. Excisional biopsy of chronically involved lymph nodes also can be performed occasionally to rule out other treatable causes. However, conflicting data exist in the literature regarding whether antibiotic therapy is ever helpful in normal hosts. Macrolides, fluoroquinolones, trimethoprim-sulfamethoxazole (TMP-SMX), doxycycline, and β-lactam agents all have in vitro activity against the organism, but gentamicin and rifampin appear to be the only bactericidal agents. Because in vitro susceptibility testing is technically challenging, lacks standards for interpretation of results, and produces minimum inhibitory concentrations that have correlated poorly with clinical efficacy, it often is not useful.[64] In Margileth's retrospective review of 202 patients treated with antibiotics for CSD,[47] rifampin was more effective than ciprofloxacin, gentamicin, or TMP-SMX. Another prospective, randomized, double-blind, placebo-controlled study using oral azithromycin for 5 days was effective in reducing lymph node volume measured by three-dimensional ultrasonography.[8] Adults and children weighing more than 100 lb were given an initial single dose of 500 mg azithromycin on day 1 of treatment and 250 mg on days 2 to 5 as single daily doses. Patients weighing less received the liquid preparation of 10 mg/kg on day 1 and 5 mg/kg on days 2 to 5.

No randomized, prospective, controlled studies have evaluated therapy for the systemic manifestations of CSD. In one retrospective review, children with hepatosplenic CSD who were given rifampin 20 mg/kg/day in two doses for 14 days, either alone or in combination with another agent, experienced a decreased duration of fever.[5] In the same retrospective review, gentamicin or TMP-SMX also led to defervescence within 5 days, but penicillins, cephalosporins, tetracycline, and erythromycin had minimal or no clinical efficacy. In 2004, evidence-based treatment recommendations for *Bartonella* infections were published and no recommendations for typical CSD, hepatosplenic CSD, or osteomyelitis were presented.[64] Outcomes of hepatosplenic disease and osteomyelitis caused by *B. henselae* generally have been excellent with or without antibiotic therapy or surgical intervention. Suggested therapy for central nervous system disease includes doxycycline and rifampin; however, no data suggest that antibiotic treatment of CSD encephalopathy is beneficial. Reports of the duration of antibiotic therapy have varied from 5 days in immunocompetent patients to 6 weeks in patients with atypical CSD or in immunocompromised patients.

Corticosteroid therapy has been reported in a small number of cases, including patients with typical CSD, retinal involvement, hepatosplenic disease, or encephalopathy, as well as in a single immunocompromised patient with fever and diffuse lymphadenopathy.[14,15,44,74] Because the pathogenesis of CSD with prolonged symptoms may include a postinfectious, inflammatory process,[14,64] corticosteroids appear to have some theoretical role. Despite the reported anecdotal successes, however, corticosteroids are not recommended for routine treatment of any manifestation of CSD.

Endocarditis

Initial empirical treatment of culture-negative endocarditis with ceftriaxone and gentamicin is effective for *Bartonella* organisms. A retrospective analysis of 101 adult patients with endocarditis showed a benefit with the use of an aminoglycoside as part of a treatment regimen for a minimum of 14 days. If *Bartonella* is proven, doxycycline plus gentamicin is recommended. The recommended duration of ceftriaxone or doxycycline is 6 weeks, with gentamicin combination therapy for the first 14 days.[6]

Bartonellosis

B. bacilliformis is sensitive to many antibiotics, including penicillin, tetracycline, streptomycin, and chloramphenicol. With treatment, the fever usually abates by 24 hours; the rod-shaped organisms change to more coccoid forms and soon disappear from the blood. The choice of antibiotic may be guided by considerations other than simple eradication of *B. bacilliformis*, including the risk for developing intercurrent infection. Chloramphenicol is considered to be the drug of choice because it also is useful in the treatment of salmonellosis.[71] Occasionally patients need the addition of another antibiotic, usually a β-lactam.[64] Blood transfusions may be helpful during the period of severe anemia, especially if blood is obtained from patients who have recently recovered from the disease.[61]

Treatment of verruga peruana usually is not necessary unless particularly large zonular lesions interfere with function; in these persons, surgery may be necessary. Treatment is considered when there are more than 10 cutaneous lesions, if the lesions are particularly erythematous or violaceous, or if the onset of lesions was earlier than 1 month before presentation. Oral rifampin, ciprofloxacin (not approved for children), or tetracycline with or without gentamicin may be used to aid in healing of the cutaneous lesions.[11,46,64]

Trench Fever

No controlled trials of treatment of trench fever have been performed, but dramatic defervescence has been noted with the use of tetracycline and chloramphenicol.[41,69] Recommended therapy for *B. quintana* bacteremia is doxycycline in combination with gentamicin.[11]

Bacillary Angiomatosis and Bacillary Peliosis Hepatis

Both of these diseases have been treated successfully with antimicrobial therapy, including erythromycin or newer macrolides such as azithromycin or clarithromycin for 3 months for bacillary angiomatosis or 4 months for bacillary peliosis hepatis.[41,53,69] The duration of therapy is critical to prevent relapses. Doxycycline may be used as an alternative regimen.

PROGNOSIS AND PREVENTION

Arthropod control should be recommended for prevention of transmission of *Bartonella* species to humans, particularly with regard to pet ownership for immunocompromised patients. Routine veterinary visits, control of flea and tick infestations, and declawing of young kittens are the current practical preventive measures for CSD. Currently, no vaccine is available to prevent *Bartonella* infections in animals or humans.

Typically, the prognosis of CSD is excellent without any sequelae. Although patients with atypical findings and involvement of other organs may have a prolonged course and antibiotics may be used, the prognosis is good. Reinfection occurs only rarely. Although the mortality rate in patients with *Bartonella* endocarditis has been reported to be as high as 30 percent, more recent series have shown lower rates (7%). The mortality rate in untreated bartonellosis has in the past been estimated at approximately 40 percent. However, the fatality rate has been reported as approximately 9 percent in hospitalized patients and 0.7 percent in a population-based study.[40,42] Intercurrent *Salmonella* infection increases the mortality rate. Treatment with chloramphenicol improves prognosis, and permanent immunity develops in most patients. Treatment of trench fever with antimicrobial therapy improves outcomes and prevents progression to more severe disease, such as endocarditis. Antibiotics are usually effective for bacillary angiomatosis and bacillary peliosis hepatis, although prolonged courses are needed.

NEW REFERENCES SINCE THE SIXTH EDITION

11. Biswas S, Rolain JM. *Bartonella* infection: treatment and drug resistance. *Future Microbiol* 2010;**5**:1719–31.
25. Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infection. *Pediatrics* 2008;**121**:e1413–25.
76. Zarraga M, Rosen L, Herschthal D. Bacillary angiomatosis in an immunocompetent child: a case report and review of the literature. *Am J Dermatopathol* 2011;**33**:513–15.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 6 *TREPONEMATACEAE*

CHAPTER 140

Borrelia

140A ■ LYME DISEASE

Sunil K. Sood • Peter J. Krause

Lyme borreliosis, or Lyme disease, is caused by a group of spirochetal bacteria (genus *Borrelia*) that are transmitted by several hard-bodied ticks of the genus *Ixodes*. It is found in the temperate zones of North America and Eurasia and is the most common tick-borne disease in these regions. The name Lyme disease dates from the late 1970s, but the first description of the disease was published in 1883, when a German physician described the chronic skin manifestation later named acrodermatitis chronica atrophicans (ACA).[13,80] The hallmark expanding erythema chronicum migrans (ECM) rash of acute Lyme disease was first reported by a Swedish physician in 1909.[2] About half a century later, a Wisconsin physician provided the first description of the disease in the New World and included successful treatment with penicillin.[66] A small cluster of cases of ECM observed in 1975 in Connecticut and Massachusetts occurred in the same year the Connecticut Health Department and Yale University rheumatologists responded to reports from the public of several children with arthritis in and around the towns of Lyme, Old Lyme, and East Haddam in southeastern Connecticut.[35,50] Ultimately 51 cases of arthritis (39 children and 12 adults) associated with ECM rash were documented and given the name Lyme arthritis.[85] Lyme arthritis was subsequently recognized to be part of a multisystem disease that was named Lyme disease.

REFERENCES

1. Anderson B, Kelly C, Threlkel R, Edwards K. Detection of *Rochalimaea henselae* in cat-scratch disease skin test antigens. *J Infect Dis* 1993;**168**:1034–6.
2. Anderson B, Sims K, Regnery R, et al. Detection of *Rochalimaea henselae* DNA in specimens from cat scratch disease patients by PCR. *J Clin Microbiol* 1994;**32**:942–8.
3. Anderson BE, Neuman MA. *Bartonella* spp. as emerging human pathogens. *Clin Microbiol Rev* 1997;**10**:203–19.
4. Arias-Stella J, Lieberman PH, Erlandson RA, Arias-Stella J Jr. Histology, immunohistochemistry, and ultrastructure of the verruga in Carrion's disease. *Am J Surg Pathol* 1986;**10**:595–610.
5. Arisoy ES, Correa AG, Wagner ML, Kaplan SL. Hepatosplenic cat-scratch disease in children: selected clinical features and treatment. *Clin Infect Dis* 1999;**28**:778–84.
6. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications: a statement for healthcare professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association: endorsed by the Infectious Diseases Society of America. *Circulation* 2005;**111**:e394–434.
7. Baorto E, Payne RM, Slater LN, et al. Culture-negative endocarditis caused by *Bartonella henselae*. *J Pediatr* 1998;**132**:1051–4.
8. Bass JW, Freitas BC, Freitas AD, et al. Prospective randomized double blind placebo-controlled evaluation of azithromycin for treatment of cat-scratch disease. *Pediatr Infect Dis J* 1998;**17**: 447–52.
9. Bass JW, Vincent JM, Person DA. The expanding spectrum of *Bartonella* infections, part I: bartonellosis and trench fever. *Pediatr Infect Dis J* 1997;**16**:2–10.
10. Benson LA, Kar S, McLaughlin G, Ihler GM. Entry of *Bartonella bacilliformis* into erythrocytes. *Infect Immun* 1986;**54**:347–53.
11. Biswas S, Rolain JM. *Bartonella* infection: treatment and drug resistance. *Future Microbiol* 2010;**5**:1719–31.
12. Brenner DJ, O'Connor SP, Winkler HH, Steigerwalt AG. Proposals to unify the genera *Bartonella* and *Rochalimaea*, with descriptions of *Bartonella quintana* comb. nov., *Bartonella vinsonii* comb. nov., *Bartonella henselae* comb. nov., and *Bartonella elizabethae* comb. nov., and to remove the family Bartonellaceae from the order Rickettsiales. *Int J Syst Bacteriol* 1993;**43**:777–86.
13. Brenner SA, Rooney JA, Manzewitsch P, Regnery RL. Isolation of *Bartonella (Rochalimaea) henselae:* effects of methods of blood collection and handling. *J Clin Microbiol* 1997;**35**:544–7.
14. Bryant K, Marshall GS. Hepatosplenic cat scratch disease treated with corticosteroids. *Arch Dis Child* 2003;**88**:345–6.
15. Carithers HA. Cat-scratch disease: an overview based on a study of 1,200 patients. *Am J Dis Child* 1985;**139**:1124–33.
16. Chamberlin J, Laughlin L, Gordon S, et al. Serodiagnosis of *Bartonella bacilliformis* infection by indirect fluorescence antibody assay: test development and application to a population in an area of bartonellosis endemicity. *J Clin Microbiol* 2000;**38**: 4269–71.
17. Chamberlin J, Laughlin LW, Romero S, et al. Epidemiology of endemic *Bartonella bacilliformis:* a prospective cohort study in a Peruvian mountain valley community. *J Infect Dis* 2002;**186**: 983–90.
18. Chang CC, Hayashidani H, Pusterla N, et al. Investigation of *Bartonella* infection in ixodid ticks from California. *Comp Immunol Microbiol Infect Dis* 2002;**25**:229–36.
19. Chitsike I, Muronda C. Bacillary angiomatosis in an HIV positive child. First case report in Zimbabwe. *Cent Afr J Med* 1997;**43**: 238–9.
20. Cuadra M. Salmonellosis complication in human bartonellosis. *Tex Rep Biol Med* 1956;**14**:97–113.
21. Cuadra M, Takano J. The relationship of *Bartonella bacilliformis* to the red blood cell as revealed by electron microscopy. *Blood* 1969;**33**:708–16.
22. Dunn MW, Berkowitz FE, Miller JJ, Snitzer JA. Hepatosplenic cat-scratch disease and abdominal pain. *Pediatr Infect Dis J* 1997; **16**:269–72.
23. English CK, Wear DJ, Margileth AM, et al. Cat-scratch disease: isolation and culture of the bacterial agent. *JAMA* 1988;**259**: 1347–52.
24. Eskow E, Rao RV, Mordechai E. Concurrent infection of the central nervous system by *Borrelia burgdorferi* and *Bartonella henselae:* evidence for a novel tick-borne disease complex. *Arch Neurol* 2001;**58**:1357–63.
25. Florin TA, Zaoutis TE, Zaoutis LB. Beyond cat scratch disease: widening spectrum of *Bartonella henselae* infection. *Pediatrics* 2008;**121**:e1413–25.
26. Foucault C, Brouqui P, Raoult D. *Bartonella quintana* characteristics and clinical management. *Emerg Infect Dis* 2007;**12**:217–23.
27. Fournier PE, Lelievre H, Eykyn SJ, et al. Epidemiologic and clinical characteristics of *Bartonella quintana* and *Bartonella henselae* endocarditis: a study of 48 patients. *Medicine (Baltimore)* 2001;**80**: 245–51.
28. Fournier PE, Mainardi JL, Raoult D. Value of microimmunofluorescence for diagnosis and follow-up of *Bartonella* endocarditis. *Clin Diagn Lab Immunol* 2002;**9**:795–801.
29. Fournier PE, Robson J, Zeaiter Z, et al. Improved culture from lymph nodes of patients with cat scratch disease and genotypic characterization of *Bartonella henselae* isolates in Australia. *J Clin Microbiol* 2002;**40**:3620–24.
30. Fuhrmann O, Arvand M, Göhler A, et al. *Bartonella henselae* induces NF-kappaB-dependent upregulation of adhesion molecules in cultured human endothelial cells: possible role of outer membrane proteins as pathogenic factors. *Infect Immun* 2001;**69**:5088–97.
31. Giladi M, Kletter Y, Avidor B, et al. Enzyme immunoassay for the diagnosis of cat-scratch disease defined by polymerase chain reaction. *Clin Infect Dis* 2001;**33**:1852–8.
32. Goral S, Anderson B, Hager C, Edwards KM. Detection of *Rochalimaea henselae* DNA by polymerase chain reaction from suppurative nodes of children with cat-scratch disease. *Pediatr Infect Dis J* 1994;**13**:994–7.
33. Gray GC, Johnson AA, Thornton SA, et al. An epidemic of Oroya fever in the Peruvian Andes. *Am J Trop Med Hyg* 1990;**42**: 215–21.
34. Hahn JS, Sum JM, Lee KP. Unusual MRI findings after status epilepticus due to cat-scratch disease. *Pediatr Neurol* 1994;**10**: 255–8.
35. Hajjaji N, Hocqueloux L, Kerdraon R, Bret L. Bone infection in cat-scratch disease: a review of the literature. *J Infect* 2007;**54**: 417–21.
36. Herrer A, Christensen HA. Implication of *Phlebotomus* sand flies as vectors of bartonellosis and leishmaniasis as early as 1764. *Science* 1975;**190**:154–5.
37. Hipp SJ, O'Shields A, Fordham LA, et al. Multifocal bone marrow involvement in cat-scratch disease. *Pediatr Infect Dis J* 2005;**24**: 472–4.
38. Houpikian P, Raoult D. Blood culture-negative endocarditis in a reference center: etiologic diagnosis of 348 cases. *Medicine (Baltimore)* 2005;**84**:162–73.
39. Jameson P, Greene C, Regnery R, et al. Prevalence of *Bartonella henselae* antibodies in pet cats throughout regions of North America. *J Infect Dis* 1995;**172**:1145–9.
40. Knobloch J, Solano L, Alvarez O, Delgado E. Antibodies to *Bartonella bacilliformis* as determined by fluorescence antibody test, indirect haemagglutination and ELISA. *Trop Med Parasitol* 1985; **36**:183–85.
41. Koehler JE. *Bartonella* infections. *Adv Pediatr Infect Dis* 1996;**11**: 1–27.
42. Kosek M, Lavarello R, Gilman RH, et al. Natural history of infection with *Bartonella bacilliformis* in a nonendemic population. *J Infect Dis* 2000;**182**:865–72.
43. Larsen CE, Patrick LE. Abdominal (liver, spleen) and bone manifestations of cat scratch disease. *Pediatr Radiol* 1992;**22**:353–5.
44. Lerdluedeeporn P, Krogstad P, Roberts RL, Stiehm ER. Oral corticosteroids in cat-scratch disease. *Clin Pediatr (Phila)* 2003;**42**: 71–3.
45. Maguina C, Garcia PJ, Gotuzzo E, et al. Bartonellosis (Carrion's disease) in the modern era. *Clin Infect Dis* 2001;**33**:772–9.
46. Maguina C, Gotuzzo E. Bartonellosis: new and old. *Infect Dis Clin North Am* 2000;**14**:1–22, vii.
47. Margileth AM. Antibiotic therapy for cat-scratch disease: clinical study of therapeutic outcome in 268 patients and a review of the literature. *Pediatr Infect Dis J* 1992;**11**:474–8.
48. Massei F, Gori L, Macchia P, Maggiore G. The expanded spectrum of bartonellosis in children. *Infect Dis Clin North Am* 2005;**19**: 691–711.

49. Metzkor-Cotter E, et al. Long-term serological analysis and clinical follow-up of patients with cat scratch disease. *Clin Infect Dis* 2003;**37**:1149–54.
50. Musso T, Badolato R, Ravarino D, et al. Interaction of *Bartonella henselae* with the murine macrophage cell line J774: infection and proinflammatory response. *Infect Immun* 2001;**69**:5974–80.
51. Myers SA, Prose NS, Garcia JA, et al. Bacillary angiomatosis in a child undergoing chemotherapy. *J Pediatr* 1992;**121**:574–8.
52. Parrott JH, Dure L, Sullender W, et al. Central nervous system infection associated with *Bartonella quintana:* a report of two cases. *Pediatrics* 1997;**100**:403–8.
53. Perkocha LA, Geaghan SM, Yen TS, et al. Clinical and pathological features of bacillary peliosis hepatis in association with human immunodeficiency virus infection. *N Engl J Med* 1990;**323**: 1581–86.
54. Posfay Barbe K, Jaeggi E, Ninet B, et al. *Bartonella quintana* endocarditis in a child. *N Engl J Med* 2000;**342**:1841–2.
55. Raoult D, Fournier PE, Drancourt M, et al. Diagnosis of 22 new cases of *Bartonella* endocarditis. *Ann Intern Med* 1996;**125**:646–52.
56. Raoult D, Fournier PE, Vandenesch F, et al. Outcome and treatment of *Bartonella* endocarditis. *Arch Intern Med* 2003;**163**: 226–30.
57. Recavarren S, Lumbreras H. Pathogenesis of the verruga of Carrion's disease: ultrastructural studies. *Am J Pathol* 1972;**66**: 461–70.
58. Regnery RL, Olson JG, Perkins BA, Bibb W. Serological response to "*Rochalimaea henselae*" antigen in suspected cat-scratch disease. *Lancet* 1992;**339**:1443–5.
59. Relman DA, Loutit JS, Schmidt TM, et al. The agent of bacillary angiomatosis: an approach to the identification of uncultured pathogens. *N Engl J Med* 1990;**323**:1573–80.
60. Revol A, Vighetto A, Jouvet A, et al. Encephalitis in cat scratch disease with persistent dementia. *J Neurol Neurosurg Psychiatry* 1992;**55**:133–35.
61. Reynafarje C, Ramos J. The hemolytic anemia of human bartonellosis. *Blood* 1961;**17**:562–78.
62. Reynolds MG, Holman RC, Curns AT, et al. Epidemiology of cat-scratch disease hospitalizations among children in the United States. *Pediatr Infect Dis J* 2005;**24**:700–4.
63. Ricketts WE. Clinical manifestations of Carrion's disease. *Arch Intern Med (Chicago)* 1949;**84**:751–81.
64. Rolain JM, Brouqui P, Koehler JE, et al. Recommendations for treatment of human infections caused by *Bartonella* species. *Antimicrob Agents Chemother* 2004;**48**:1921–33.
65. Schultz MG. A history of bartonellosis (Carrion's disease). *Am J Trop Med Hyg* 1968;**17**:646–652.
66. Scott MA, et al. Cat scratch disease: detection of *Bartonella henselae* DNA in archival biopsies from patients with clinically, serologically, and histologically defined disease. *Am J Pathol* 1996;**149**:2161–67.
67. Spach DH, Callis KP, Paauw DS, et al. Endocarditis caused by *Rochalimaea quintana* in a patient infected with human immunodeficiency virus. *J Clin Microbiol* 1993;**31**:692–4.
68. Spach DH, Kanter AS, Daniels NA, et al. *Bartonella (Rochalimaea)* species as a cause of apparent "culture-negative" endocarditis. *Clin Infect Dis* 1995;**20**:1044–7.
69. Spach DH, Koehler JE. *Bartonella*-associated infections. *Infect Dis Clin North Am* 1998;**12**:137–55.
70. Szelc-Kelly CM, Goral S, Perez-Perez GI, et al. Serologic responses to *Bartonella* and *Afipia* antigens in patients with cat scratch disease. *Pediatrics* 1995;**96**:1137–42.
71. Urteaga O, Payne EH. Treatment of the acute febrile phase of Carrion's disease with chloramphenicol. *Am J Trop Med Hyg* 1955;**4**:507–11.
72. Verdon R, Geffray L, Collet T, et al. Vertebral osteomyelitis due to *Bartonella henselae* in adults: a report of 2 cases. *Clin Infect Dis* 2002;**35**:e141–44.
73. Wear DJ, Margileth AM, Hadfield TL, et al. Cat scratch disease: a bacterial infection. *Science* 1983;**221**:1403–5.
74. Weston KD, Tran T, Kimmel KN, Maria BL. Possible role of high-dose corticosteroids in the treatment of cat-scratch disease encephalopathy. *J Child Neurol* 2001;**16**:762–3.
75. Zangwill KM, Hamilton DH, Perkins BA, et al. Cat scratch disease in Connecticut: epidemiology, risk factors, and evaluation of a new diagnostic test. *N Engl J Med* 1993;**329**:8–13.
76. Zarraga M, Rosen L, Herschthal D. Bacillary angiomatosis in an immunocompetent child: a case report and review of the literature. *Am J Dermatopathol* 2011;**33**:513–15.
77. Zeaiter Z, Fournier PE, Greub G, Raoult D. Diagnosis of *Bartonella* endocarditis by a real-time nested PCR assay using serum. *J Clin Microbiol* 2003;**41**:919–25.

TABLE 140A-1 **Principal Vector Ticks and Spirochetes Associated with Lyme Borreliosis**

Tick Species	Distribution	Genotype of *B. burgdorferi*
Ixodes scapularis	Eastern North America	*B. burgdorferi* sensu stricto
Ixodes pacificus	Western North America	*B. burgdorferi* sensu stricto
Ixodes ricinus	Western and Central Europe*	*B. garinii, B. afzelii, B. burgdorferi* sensu stricto
Ixodes persulcatus	Central Europe and Asia	*B. garinii, B. afzelii*

**B. spielmanii, B. lusitaniae,* and *B. valaisiana* occasionally isolated from humans; *B. garinii* serotype 4, also known as *B. bavariensis.*

From Piesman J, Humair P-F. The spirochetes and vector ticks of Lyme borreliosis in nature. In: Sood SK, editor. Lyme borreliosis in Europe and North America. *Hoboken (NJ): John Wiley & Sons; 2011.*

THE ORGANISM

The microbial etiology of Lyme disease was established in 1983 when Willy Burgdorfer discovered spirochetes in *Ixodes* ticks from Shelter Island, New York[14] and when the same organism was isolated from cultures of blood, skin lesions, and cerebrospinal fluid (CSF) of patients from Connecticut and Long Island, New York.[11,84] The pathogen was named *Borrelia burgdorferi* and was soon linked to the ECM rash in European patients, which is now called erythema migrans.[40] The name *Borrelia burgdorferi* sensu lato (*sensu lato* meaning "in a broad sense") indicates that other genospecies are included under this group name (Table 140A-1). Two other genospecies (*B. afzelii* and *B. garinii*) cause Lyme disease in Eurasia and account for variation in the clinical presentation on that continent. *B. afzelii* is strongly associated with ACA and is a common cause of erythema migrans, and *B. garinii* is associated with neurologic infection. In North America, Lyme disease is caused solely by the genospecies *B. burgdorferi* "sensu stricto" (meaning "in a narrow sense"). *B. burgdorferi* is a slow-growing bacterium with a cell membrane that is covered by a loosely associated outer membrane and by flagella that account for the coiled shape of the microorganism and provide motility.

EPIDEMIOLOGY

Lyme disease–endemic areas in the United States are primarily in the Northeast and northern Midwest (Fig. 140A-1A). In 2011, 96 percent of all cases were reported from 13 states in these regions.[17] Far fewer cases are reported from the northern Pacific Coast region. In 2010, 22,561 confirmed and 7597 probable cases were reported to the Centers for Disease Control and Prevention (CDC) (7.3 cases/100,000 population). This represents an approximate doubling of cases since 2000, but the actual number of infections is estimated at 10- to 12-fold higher.[43,51,82]

In Europe the incidence varies among countries, in part because of different reporting methods and travel. The highest incidence is generally in the temperate zone between latitudes 42 and 62 degrees north.[80] Lyme disease is more common among children than adults, presumably because of their increased exposure to *Ixodes* ticks, with about a quarter of all reported cases occurring in children younger than 14 years of age.[9,77]

Lyme disease is a zoonotic infection that involves multiple hosts and reservoirs.[48] *Ixodes* ticks take a blood meal from a vertebrate host in each of the three active stages during their 2-year life cycle (i.e., larva, nymph, and adult). Spirochetes are ingested during a blood meal from an asymptomatic infected reservoir host and transmitted to other reservoir hosts or to humans during a subsequent blood meal. The primary reservoir hosts are the white-footed mouse *(Peromyscus leucopus)* in the Northeast and Midwest and the western gray squirrel and western fence lizard in the Pacific Coast region. Other small mammals such as chipmunks and some species of birds also may serve as reservoir hosts.[57] White-tailed deer *(Odocoileus virginianus)* provide an important habitat for adult ticks during the winter season. They do not become infected with *B. burgdorferi* but amplify the tick population by providing a blood meal for adult ticks. An increase in the deer population over the past few decades is an important factor in the increase in the *I. scapularis* population and in the number of Lyme disease cases in the United States.[60] Domestic animals such as the dog may carry adult *Ixodes* ticks but do not appear to be important reservoir hosts for the tick, even though dogs can be infected with *B. burgdorferi*. The tick vectors in Europe have a greater variety of reservoir hosts, including ground-feeding birds such as blackbirds, small mammals such as *Apodemus* mice, and medium-sized mammals such as hedgehogs.[80]

PATHOGENESIS

Borrelia spirochetes are deposited in the skin during tick feeding. The organism does not produce toxins, so disease is primarily a result of the immunologic host response. Major antigens of the bacteria include the outer surface lipoproteins (Osps) A, B, and C and the flagellar protein. Osps are important for survival in both the tick and the mammalian hosts.[59,90] Innate immune activation begins with spirochete binding to pattern recognition receptors such as Toll-like receptors on dendritic cells and macrophages in the dermis.[12,36,47,64] This initiates signal transduction pathways that result in production of chemokines and cytokines by macrophages and dendritic cells.[47,65] Spirochetes are ingested by macrophages and neutrophils and degraded within phagocytic vacuoles.[65] Degradation products bind to intercellular pattern recognition receptors that further amplify the inflammatory response.[65] *B. burgdorferi* also produces specific proteins that inhibit complement activity.[46]

Adaptive immune responses also help clear spirochetes. *Borrelia*-specific T cells are activated. These aid

FIGURE 140A-1 ■ **A,** Erythema migrans scalp lesion only partially visible on the face. **B,** Erythema migrans oval lesion. **C,** Erythema migrans dark annular lesion. **D,** Multiple erythema migrans. **E,** Borrelial lymphocytoma. Five-year-old girl from southern California who, while visiting Norway in August 2006, had a tick removed from her left ear lobe. (See companion Expert Consult website for color version.) (**A** to **C,** Courtesy Vijay Sikand, MD; **D,** courtesy Lorry Rubin, MD; **E,** courtesy Mark Salzman, MD. All images reproduced from Sood SK, editor. *Lyme Borreliosis in Europe and North America.* Hoboken [NJ]: John Wiley and Sons; 2011.)

in macrophage killing and help B cells differentiate into antibody-producing plasma cells.[59] Patients with Lyme arthritis typically have higher *Borrelia*-specific antibody titers than do patients with other manifestations of Lyme disease.[22] Despite a vigorous immune response, the spirochetes are not immediately killed, partly because of immune evasion strategies, such as downregulation of expression of Osps, and antigenic variation from the variable major protein-like sequence expression locus (VlsE).[24,59] Surviving *Borrelia* organisms can disseminate from the skin to other tissues through blood and possibly also through tissue planes. During dissemination within humans, *B. burgdorferi* attach to certain host integrins. This elicits a proinflammatory response that includes production of both matrix glycosaminoglycans and extracellular-matrix proteins, which may explain the organism's tropism for particular tissues (e.g., collagen fibrils in the extracellular matrix of the heart, nervous system, and joints).[59] Dissemination appears to be genotype or strain dependent, with some strains more likely to disseminate than others.[94,95] Furthermore, infection with specific *B. burgdorferi* genotypes may result in unique clinical manifestations of disease. For example, only the European species *B. afzelii* causes chronic skin lesions of ACA. Long-term immunity is incomplete because recurrent episodes of Lyme disease may occur in some people. Reinfection has been found in about 3 percent of children and 10 percent of adults.[31,45a,51]

Several lines of evidence suggest that persistent symptoms do not result from active infection. Synovial tissue from patients with Lyme arthritis typically shows hypertrophy, vascular proliferation, and a marked mononuclear cell infiltrate, but joint cultures in patients with recurrent arthritis are negative.[12,83] Some adult patients with Lyme

arthritis, particularly those with human leukocyte antigen (HLA)-DRB1 alleles, will develop chronic, antibiotic refractory, autoimmune arthritis.[83] Antibiotic-refractory Lyme arthritis may be due to *B. burgdorferi* breakdown components that can persist near cartilaginous tissue and induce an inflammatory response after treatment is completed.[12] Meningitis results from direct invasion of the subarachnoid space by *B. burgdorferi*, as shown by the presence of spirochetes in cultures of CSF. *B. burgdorferi* infiltrated the connective tissue sheath surrounding each bundle of peripheral nerve fibers in the peripheral nervous system in a monkey model.[63] No evidence suggests that long-term neurologic symptoms are due to persistent *Borrelia* infection, however.

CLINICAL MANIFESTATIONS

The clinical presentation of Lyme disease in children comprises a few well-characterized manifestations that are classified as early or late. Early manifestations are defined as those that begin within 8 weeks after the tick bite and are further characterized as early localized or early disseminated.[92] In North America, approximately 90 percent of children have erythema migrans, either as a single lesion or multiple erythema migrans lesions. The remainder present with arthritis, facial palsy, aseptic meningitis, or carditis.[31] Some children with early Lyme disease have more than one manifestation. In Europe, neurologic manifestations are significantly more common in children (17% to 38% of cases) than in adults, and Bannwarth polyradiculoneuritis is more common.[18,77] Borrelial lymphocytoma occurs only in Europe and is common in children.[52,58]

Early Localized Disease

Erythema Migrans

The initial manifestation of Lyme disease is a single erythema migrans lesion that begins at the site of the tick bite and appears after an incubation of about 1 to 31 days (mean, 10 days) (see Fig. 140A-1A-C). This generally consists of a flat red rash that expands over days. The "bullseye" shape with central clearing is firmly entrenched in both the lay and medical literature but occurs in only two thirds of skin biopsy–proved or culture-proved erythema migrans.[54] Although the lesion is usually painless, mild stinging, warmth, pruritus, hyperesthesia, or dysesthesia may be present. Vesicular, urticarial, scaling, purpuric, and noncircular variants are well documented.[54,73] Alternating rings of lighter and darker erythema or triangular lesions may be seen in areas such as the inguinal region. Patients with erythema migrans often have mild constitutional symptoms, including low-grade fever. Because small, ring-shaped lesions from insect bites are common in children, practitioners should not rush to diagnose erythema migrans in every child with a focal rash. Close follow-up will establish the diagnosis if the lesion attains a diameter of at least 5 cm, the minimum diameter required to meet the CDC case definition of erythema migrans. Rashes that suggest erythema migrans have been observed in nonendemic southern states and recently in a child on Long Island, New York, after bites of Lone Star ticks.[15,27] This entity has been given the name southern tick-associated rash illness (STARI), and although not resulting from *B. burgdorferi* infection, the causative organism is unknown.

Early Disseminated Disease

Spirochetemia can occur shortly after the tick bite, leading to disseminated skin, neurologic, cardiac, and musculoskeletal manifestations.[96]

Multiple Erythema Migrans

Multiple erythema migrans are the most common form of early-disseminated Lyme disease (see Fig. 140A-1D).[6,74] Lesions can be distinguished from erythema multiforme by the fact that all are morphologically similar, except they vary in size. A single secondary lesion may occur at the site of a primary lesion that has disappeared. The child also may report constitutional symptoms.

Neurologic Manifestations

Acute neurologic Lyme disease is another common form of early-disseminated infection, almost always presenting as cranial nerve VII palsy, lymphocytic meningitis, or both.[10,75] These manifestations mimic Bell palsy and viral meningitis, respectively. Facial palsy should always raise the diagnostic possibility of Lyme disease. Children with facial palsy should be assessed for meningitis and the need for lumbar puncture, especially when neck stiffness or severe headache occur.[70] If a lumbar puncture is performed and shows CSF pleocytosis consistent with meningitis, treatment should consist of an antibiotic administered intravenously rather than orally.

Because Lyme meningitis and enteroviral meningitis are common in Lyme disease–endemic areas, it is important to appreciate characteristic clinical and laboratory features of each, in the absence of an erythema migrans rash. The presentation of Lyme meningitis is more indolent.[25,68] Children with Lyme meningitis often experience several days of headache, lethargy, and neck pain symptoms before seeing a physician. Evidence of meningismus, such as Kernig and Brudzinski signs, is less pronounced. The cerebrospinal pleocytosis consists predominantly of mononuclear cells (mostly lymphocytes) in contrast to the relative prominence of neutrophils early in the course of viral meningitis. In one study, the negative predictive value for Lyme meningitis was 99 percent when neutrophils accounted for more than 10 percent of the cells in the CSF.[68] Clinical prediction algorithms based on these features have been developed to help in the diagnosis of Lyme meningitis in the emergency department setting.[8,19] One of these was termed the "rule of 7s."[19] Children with meningitis in whom all three of the following were present: 7 days or longer of headache, 70 percent or greater mononuclear cells, and presence of seventh (or other) cranial nerve palsy, had a 77 percent likelihood of Lyme meningitis, whereas

absence of these predictors was highly sensitive (96%) for identifying a low risk for Lyme meningitis. Another important difference between Lyme meningitis and viral meningitis is the more frequent complication of increased intracranial pressure that is associated with Lyme meningitis. This is important because such elevated intracranial pressure is sometimes associated with transient or permanent loss of vision.[62] All published reports of raised intracranial pressure in Lyme disease have been in children, and the complication has been observed in the absence of meningitis.[41,77] Headache is prominent in these children. Although this is a rare complication of Lyme disease, it is important to examine the optic discs of every child with acute disseminated Lyme disease for papilledema. Opening CSF pressure should be measured as part of the lumbar puncture, and, if elevated, treatment to lower intracranial pressure should be initiated immediately.

Other neurologic signs or symptoms are very uncommon in children. Bannwarth syndrome, a neurologic manifestation of Lyme disease in Europe, consists of inflammation of peripheral nerves.[56] It should be considered in children who have recently traveled to Europe and have neuralgia, paresthesia, and motor or sensory impairment, mostly affecting cervical and thoracic dermatomes. The association of Lyme disease with optic neuritis, Guillain-Barré–like syndrome, or acute meningoencephalitis in published reports is uncertain.

Carditis

Lyme carditis is a very uncommon early dissemination manifestation of Lyme disease. It usually occurs in association with involvement of the skin and nervous system, but may be the sole manifestation, presenting with syncope and malaise. Abnormalities include first-, second-, or third-degree atrioventricular conduction block or bundle branch block.[32] An electrocardiogram should be considered in a child with acute disseminated Lyme disease because cardiac conduction abnormalities often are asymptomatic. Rare presentations of Lyme carditis are myocarditis, myopericarditis, left ventricular dysfunction, or cardiomegaly. Patients with Lyme carditis almost invariably have positive *B. burgdorferi* serologic findings acutely or at follow-up.[5] The outcome in children who receive appropriate antibiotic therapy for Lyme carditis is excellent.

Acute Generalized Illness

Investigators in Connecticut tested the hypothesis that early Lyme disease can present with a nonspecific illness without focal manifestations. Of 24 untreated children who presented to their primary care physicians with "flulike illness" of less than 3 weeks duration without identifiable cause, 5 were found to have serologic evidence of acute *B. burgdorferi* infection.[26] Fewer than 10 percent of children with *B. burgdorferi* infection are thought to be identified in this way. Antibiotic treatment should not be prescribed for children with nonspecific illness without *B. burgdorferi*–specific laboratory confirmation of Lyme disease. Because babesiosis, human granulocytic anaplasmosis, ehrlichiosis, or *Borrelia miyamotoi* infection can have a similar generalized clinical presentation, these infections also should be considered in evaluating any child thought to have tick-borne illness who has nonspecific findings.[42a,44,57a]

Late Disseminated Disease

Arthritis

Arthritis develops as the first symptom of Lyme disease weeks to months after the tick bite in a small proportion of children. The mean incubation period is about 4 months.[89] Arthritis usually presents in children who do not receive appropriate antibiotics for acute Lyme disease because the tick bite or rash go unnoticed. Many children report intermittent and migratory arthralgias or mild periarticular inflammation in the weeks leading up to development of frank arthritis. Lyme arthritis presents as a monoarthritis in two thirds of cases and an oligoarthritis (fewer than four joints) in the remainder.[30] The knee is the most common presenting joint and is involved in 90 percent of the cases. Usually obvious joint swelling and effusion are seen, with stiffness out of proportion to the mild to moderate pain. Children most often continue to walk and are not immediately taken for emergency care. It is not unusual for the swelling to be attributed to an acute traumatic event and for a child to be initially evaluated by an orthopedic surgeon. These clinical features distinguish Lyme arthritis from septic arthritis, despite similar laboratory findings of neutrophil-predominant exudate in synovial fluid and elevated acute-phase reactants. An effusion typically reaccumulates if arthrocentesis is performed, consistent with the pathogenesis of a reactive arthritis. Active inflammation persists for several weeks even after appropriate treatment. Both the severity and duration of arthritis is increased in adolescents.[37] Recurrent episodes can involve the same joint or a different joint, another pattern that is consistent with reactive arthritis.

Late Neurologic Disease

Follow-up studies of children who previously had Lyme disease have demonstrated an excellent neuropsychological outcome.[1] Although low-grade encephalopathy and sensorineural peripheral neuropathy have occurred as late sequelae of Lyme disease in adults, late neurologic disease is rarely, if ever, encountered in children. Assays of CSF for culture, *B. burgdorferi* DNA, antibody, or antigen detection are not useful because they have very poor specificity and sensitivity.[33] Very little evidence exists that incidentally detected nonspecific white matter changes on magnetic resonance imaging of the brain are due to Lyme disease. Occasionally some cognitive disorders, processing deficits on a battery of neuropsychological tests, or persistent fatigue in children are attributed to Lyme disease. The detection of antibodies to *B. burgdorferi* in a child suspected of having late neurologic Lyme disease usually is proof of previously resolved

infection, however, that has no relation to current symptoms.

Other Manifestations

Asymptomatic Seroconversion

Asymptomatic seroconversion refers to the development of antibodies to *B. burgdorferi* infection in the absence of a recognized illness. This is well documented and common in endemic areas. The ratio of symptomatic infection to asymptomatic Lyme disease was about 9:1 in a prospective study in an endemic area.[86] Children generally remain well, indicating successful clearance of the spirochete rather than latent infection.

Acrodermatitis Chronica Atrophicans

ACA is a late chronic skin manifestation of Lyme borreliosis that occurs in Europe. In the majority of cases it is caused by *B. afzelii*.[52] The lesions are painful, bluish-red discolored areas of swelling on the extremities, usually accompanied by a sensory peripheral neuropathy. Although rarely encountered in children, it is important to include ACA in the differential diagnosis of discolored skin lesions so that atrophic and neural sequelae can be minimized by early treatment.[97]

Borrelial Lymphocytoma

Borrelial lymphocytoma is a localized manifestation of disseminated Lyme disease that is seen in Europe. It mimics a benign tumor of the skin, usually on the ear lobes or breast areolae.[20] The lesion is a painless, solitary, bluish-red plaque or nodule 1 to 5 cm in diameter that develops 1 to 6 months after the tick bite (see Fig. 140A-1E).

Coinfection with Other *Ixodes* Tick-Borne Pathogens

Human babesiosis and human granulocytic anaplasmosis are tick-borne diseases that may be cotransmitted with *B. burgdorferi* in areas where these infections are endemic. Between 3 and 13 percent of Lyme disease patients may experience babesiosis, human granulocytic anaplasmosis coinfection, or both in areas where these infections are endemic.[43,44,45] Coinfection with babesiosis or human granulocytic anaplasmosis is associated with an increase in acute symptoms in contrast to Lyme disease alone and may result in a more extended period of illness. Physicians caring for patients with moderate to severe Lyme disease should consider obtaining diagnostic tests for babesiosis and anaplasmosis in regions where these diseases are zoonotic. This is especially important when the response to antibiotic treatment is inadequate. No data exist to date indicating that Lyme disease complications are increased as a result of coinfection.

Lyme Disease in Pregnancy

Concern has been raised that untreated Lyme disease during pregnancy could result in fetal or neonatal disease, even though maternal to fetal transmission appears to be very rare, if it occurs at all. Large epidemiologic investigations have failed to show an association between maternal exposure during pregnancy and fetal death, prematurity, or congenital malformations.[29,87,88] To date, no proved cases of *B. burgdorferi* causing fetal wastage, fetal malformations or persistent neonatal infection have been reported.[23,49]

DIAGNOSIS

Most persons who acquire Lyme disease do not recall a tick bite, because it is painless and the tick falls off after feeding. Lyme disease is primarily diagnosed by clinical manifestations, most commonly an erythema migrans rash, along with supportive epidemiologic evidence, and laboratory confirmation for manifestations other than erythema migrans. The CDC developed a clinical case definition for Lyme disease that was initially intended for epidemiologic surveillance purposes. When used in conjunction with the Food and Drug Administration (FDA) guidelines for diagnostic tests, this case definition has been widely accepted as a way to standardize the clinical diagnosis of Lyme disease. A patient is considered to have Lyme disease by the CDC case definition if the patient has an erythema migrans rash or at least one objective sign of early disseminated or late disease (i.e., musculoskeletal, nervous, or cardiovascular signs), in addition to a positive laboratory test result that confirms infection with *B. burgdorferi*.

Erythema migrans is defined as a skin lesion that begins as a red macule or papule and expands over a period of days to weeks to form a large round lesion, often with partial central clearing. For CDC surveillance purposes a single primary lesion must reach 5 cm or greater in size. Epidemiologic evidence includes visiting a wooded, brushy, or grassy area where Lyme disease is endemic, 1 to 32 days before onset of the rash. A deer tick bite at the site of the rash may or may not have been identified. Positive *B. burgdorferi* serologic findings are not necessary for the diagnosis of erythema migrans. Early disseminated neurologic manifestations include cranial neuritis (particularly facial palsy) or lymphocytic meningitis, alone or in combination. Early disseminated cardiovascular system manifestations include acute onset of a high-grade second- or third-degree atrioventricular conduction defect that resolves in days to weeks and is sometimes associated with myocarditis. Late musculoskeletal manifestations consist of objective joint swelling in one or a few joints.

Serology is the primary laboratory test used to confirm the diagnosis of Lyme disease because unlike most other bacterial illnesses, Lyme disease is very seldom diagnosed by culture or microscopic visualization of the organism.[93] Antibodies to *B. burgdorferi* begin to develop during early localized infection. The immune response intensifies in patients with early disseminated or late disease.[5] A patient with erythema migrans may become *B. burgdorferi* antibody positive during therapy. This does not constitute treatment failure, because approximately one quarter of patients with erythema migrans are antibody positive

when diagnosed and an additional half become positive during therapy. In contrast to syphilis, in which a falling Venereal Disease Research Laboratory (VDRL) titer indicates successful therapy, *B. burgdorferi* antibodies may remain elevated for years in a successfully treated patient. Serial *B. burgdorferi* serologic findings should not be used to monitor effectiveness of treatment of Lyme disease.

A two-tier approach is standard for the detection of antibodies to *B. burgdorferi*.[5,16] The first test consists of one of several FDA-approved enzyme-linked immunosorbent assay (ELISA) tests for total IgM and IgG antibodies to the antigens of *B. burgdorferi*. The ELISA is a very sensitive assay and false-positive results are common, primarily from cross-reacting antibodies against microorganisms that share antigenically similar proteins with *B. burgdorferi*. Therefore, sera that are positive or equivocal by ELISA are then confirmed using separate IgM and IgG western immunoblot assays. The western immunoblot is more specific than the ELISA because it identifies antibodies in the patient's serum to individual polypeptides of *B. burgdorferi*. They are visualized and reported as "bands" according to their molecular weight expressed in kilodaltons (kDa or K). Based on extensive laboratory testing, the CDC has defined a positive *B. burgdorferi* IgM immunoblot result as the presence of at least 2 of the 3 following bands: 23, 39, and 41 kDa. A blot for IgG is considered positive if at least 5 of the following 10 bands are present: 18, 23, 28, 30, 39, 41, 45, 58, 66, and 93 kD.[16] Only the IgG response should be used to support the diagnosis after the first month of infection. After that time, an IgM response alone is likely to represent a false-positive result.[67] Immunoblot interpretation should be performed only in qualified laboratories that follow CDC-recommended evidence-based guidelines on immunoblot interpretation. The use of immunoblot alone for diagnosis of Lyme disease is not recommended.

As with any diagnostic test, the predictive value of serologic tests for Lyme disease depends on the probability that the patient has Lyme disease, based on epidemiologic and clinical history and the physical examination (the "pretest probability" of Lyme disease). Use of serologic tests to rule out Lyme disease in patients with a low probability of the illness will result in a very high proportion of test results that are falsely positive.[39] Therefore, antibody tests for Lyme disease should not be used as screening tests, as has become common practice in children with nonspecific fevers, arthralgias, headaches, or fatigue. Even in areas with a high prevalence of Lyme disease, patients with only nonspecific signs and symptoms are not likely to have Lyme disease.[28,71] Physicians should avoid requesting *B. burgdorferi* serologic findings on such patients because they have a very low pretest probability of disease and false-positive results cause patient anxiety, unnecessary antibiotic treatment, and the possibility of missing another diagnosis.[28]

Patients with erythema migrans should not have serologic testing because they frequently are seronegative at this early stage of the infection and a typical rash is pathognomonic for Lyme disease. If the rash is atypical, the patient is not treated, and serologic examination is negative or equivocal, a second specimen should be obtained within 2 to 4 weeks. Patients with early neurologic manifestations of Lyme disease have demonstrable serum antibodies to *B. burgdorferi*, but the response may initially be limited to IgM antibodies.[19,38] Patients with Lyme carditis almost always have positive *B. burgdorferi* serologic findings acutely or at follow-up.[4,5] Patients with Lyme arthritis invariably have positive *B. burgdorferi* serologic findings for IgG antibodies.[5] The presence of a positive serologic result does not guarantee that a medical condition is due to *B. burgdorferi* infection, however, because the positive serologic examination may be due to previous infection or to a false-positive test result. The background *B. burgdorferi* seropositivity in areas that are highly endemic for Lyme disease may exceed 4 percent.

Additional tests to confirm *B. burgdorferi* infection in clinical specimens have been used primarily in research settings. For CSF, these include measurement of CSF to serum antibody ratios to document specific intrathecal antibody production, *B. burgdorferi* DNA detection by polymerase chain reaction (PCR), and *B. burgdorferi* culture of CSF, skin biopsies, and joint fluid.[93] Because of limited availability, expense, poor sensitivity, and insufficient evidence of value in the management of most patients, these tests should be reserved for special situations in which the pretest probability of Lyme disease is high but manifestations are atypical. Tests to detect antigens of *B. burgdorferi* in urine are not FDA approved, and they have no current role in the diagnosis or management of Lyme disease.

TREATMENT AND PROGNOSIS

The current standard for treatment of Lyme disease in children is the evidence-based Infectious Diseases Society of America Practice Guideline.[94] Treatment recommendations are listed in Table 140A-2, and selected treatment principles are discussed later. *B. burgdorferi* is highly susceptible to β-lactam antibiotics and tetracyclines. Consequently, treatment of Lyme disease is effective at all stages of the infection and treatment failures are very uncommon. First-line antibiotics are β-lactams or doxycycline, the latter drug being effective for treatment of human granulocytic anaplasmosis (although not for babesiosis) that may simultaneously occur with Lyme disease.

Doxycycline and amoxicillin are equivalent choices for treatment of early Lyme disease. Doxycycline is prescribed for children 8 years or older and amoxicillin for children younger than 8 years. Ceftriaxone is the treatment of choice for early disseminated Lyme disease presenting as meningitis or advanced atrioventricular heart block. If a child with Lyme facial palsy is assessed for concomitant meningitis, presence of CSF pleocytosis would indicate the use of parenteral ceftriaxone rather than an oral antibiotic. Children who have second- or third-degree atrioventricular block and those with first-degree heart block when the PR interval is prolonged to 30 milliseconds or greater should be hospitalized and continuously monitored. This is because the degree of block may fluctuate and worsen very rapidly. Patients experiencing advanced heart block should have a consultation with a cardiologist. A temporary pacemaker may be required.

TABLE 140A-2 **Treatment of Lyme borreliosis**

Manifestation	First-Line Drugs	Equivalent Alternative Drugs	Second-Line Drugs
Early localized EM	Amoxicillin PO 10-21 days or doxycycline PO 10-21 days	Cefuroxime axetil PO* 10-21 days	Erythromycin PO 10-21 days or clarithromycin 10-21 days
Early Disseminated			
Multiple EM	Amoxicillin PO 21-28 days or doxycycline PO 21-28 days	Cefuroxime axetil PO* 21-28 days or ceftriaxone IV 14 days or cefotaxime IV 14 days	Erythromycin PO 21-28 days or clarithromycin PO 21-28 days
Facial palsy	Amoxicillin PO 21-28 days or doxycycline PO 21-28 days	Ceftriaxone IV 14-28 days or cefotaxime 14-28 days	Erythromycin PO 21-28 days or clarithromycin PO 21-28 days
Meningitis/facial palsy with meningitis/polyneuropathy	Ceftriaxone IV 14-28 days or cefotaxime IV 14-28 days	Doxycycline PO 28 days	Penicillin IV 14-28 days
Cardiac†	Amoxicillin PO 21 days, or doxycycline PO 21 days, or cefotaxime IV 14 days		Erythromycin PO 21 days or clarithromycin PO 21 days or penicillin IV 14 days
Late			
Arthritis	Amoxicillin PO 28 days or doxycycline PO 28 days	Ceftriaxone‡ IV 14-28 days or cefotaxime‡ IV 14-28 days	Penicillin IV 14-28 days
Neurologic	Ceftriaxone or cefotaxime IV 14-28 days	Doxycycline PO 28 days	Penicillin IV 14-28 days
Other			
Seroconversion (asymptomatic or with acute generalized illness)	Amoxicillin PO 21-28 days or doxycycline PO 21-28 days	Cefuroxime axetil PO* 21-28 days	Erythromycin PO 21-28 days or clarithromycin PO 21-28 days

*Cefuroxime axetil was tested in children 12 years and older.
†First-degree block with PR interval <0.3 sec, PO therapy; PR >0.3 sec or higher grade, IV initially, then PO if responds rapidly.
‡For oral therapy failures only.
Doses:
- Doxycycline PO 2-4 mg/kg/day divided into two doses, up to adult dose of 100 mg twice daily.
- Amoxicillin PO 40-50 mg/kg/day divided into three doses, up to adult dose of 2 g/day.
- Cefuroxime axetil PO 30 mg/kg/day divided into two doses, up to adult dose of 500 mg twice daily.
- Erythromycin PO 30-40 mg/kg/day divided into four doses, up to adult dose of 250 mg four times daily.
- Clarithromycin PO 15 mg/kg/day divided into 4 doses, up to adult dose of 500 mg twice daily.
- Ceftriaxone IV 100 mg/kg/day once daily, up to adult dose of 2 g/day.
- Cefotaxime IV 180 mg/kg/day, up to adult dose of 2 g three times daily.
- Penicillin IV 200,000-400,000 units/kg/day, divided into four doses, up to adult dose of 24 million U/day.

EM, Erythema migrans.

Children with Lyme arthritis are treated with doxycycline or amoxicillin. For those who have persistent or recurrent joint swelling after antibiotic therapy, repeat treatment with a 4-week course of oral antibiotics or with a 2- to 4-week course of ceftriaxone should be considered. If persistent synovitis is associated with significant pain or limitation of function, nonsteroidal anti-inflammatory agents, intra-articular corticosteroids, disease-modifying antirheumatic drugs such as hydroxychloroquine, or arthroscopic synovectomy provide symptomatic relief.[91] Ongoing knee pain often is due to patellofemoral joint disease rather than arthritis.

The prognosis for children given appropriate antibiotic therapy for Lyme disease is excellent. This includes children with arthritis, even though recurrent episodes of arthritis can occur.[91] In one study, parents were telephoned 2 to 12 years after their child had experienced Lyme disease. Of 90 children, 4 had ongoing musculoskeletal complaints but none had evidence of arthritis.[30] The incidence of chronic arthritis was higher in children in Germany, where about half of 55 children treated for a previous diagnosis of Lyme arthritis had chronic arthritis either at onset or during their follow-up.[78] A poorer outcome was associated with older age and female sex.

Despite resolution of the objective manifestations of Lyme disease, children on rare occasions may continue to experience fatigue, musculoskeletal pain, or difficulties with concentration or short-term memory. No evidence indicates that these are attributable to persistent *B. burgdorferi* infection.[69] The pathogenesis of these symptoms is not well defined, but is probably similar to other postinfectious conditions in which persistent immune-mediated inflammation occurs in the absence of active infection. No evidence has been reported that prolonged courses of antibiotic treatment that exceed 1 month are effective for adults with ongoing symptoms after Lyme disease, based on at least three prospective controlled antibiotic trials.[28] Also, no data suggest that children differ from adults in this regard. As with adults, it is not appropriate to prescribe an antibiotic for a child

who has positive serologic findings in the absence of accompanying or recent specific clinical manifestations. The term *chronic Lyme disease* has been used by a small number of practitioners to describe patients whom they believe have persistent *B. burgdorferi* infection, a condition they suggest requires long-term antibiotic treatment and even may be incurable.[28] The assumption that chronic, subjective symptoms are caused by persistent infection with *B. burgdorferi* is not supported by carefully conducted laboratory studies or by controlled treatment trials.[7]

Prevention and Management of *Ixodes* Tick Bites

Prevention of Tick Bites

It is obviously best to keep free of tick-infested vegetation such as brush and wooded areas, but recreational, occupational, and residential habits make it difficult to avoid tick exposure for most people living in endemic areas.[34] Ticks are often present in grassy areas and in leaf litter on personal property and can attach during even brief exposure. Use of repellents is very helpful in preventing tick bites. Light-colored clothes (to make crawling ticks more visible), long-sleeved clothing, and socks tucked or taped into pants are somewhat effective and more so if used in conjunction with repellents. The repellent N,N-diethyl-m-toluamide or N,N-diethyl-3-methylbenzamide (DEET) is the most effective, but is underused because of exaggerated concerns about potential toxicity. Very little DEET is absorbed after topical application, and it is rapidly metabolized and eliminated. In very young children, care should be taken to avoid application near mucosal surfaces. Certain DEET-sunscreen combinations and topical retinoids can enhance the absorption of DEET, however, and the use of combination DEET-sunscreen preparations is not recommended.[42,61] A 30 percent formulation provides about 6 hours of protection. The use of higher concentrations has been discontinued in Canada. Permethrin is a highly effective acaricide and repellent that can be applied to or impregnated in clothing, shoes, and fomites.[42]

Tick checks under bright lighting should be performed at the end of each day of exposure. Use of a washcloth or tweezers with special attention to armpit, groin, back, and scalp areas may dislodge unattached ticks. Studies have demonstrated that early removal of an *Ixodes* tick minimized the risk for acquiring *B. burgdorferi* infection.[53,79]

The geographic range of *Ixodes* tick species is expanding beyond previously endemic regions, and practitioners should be knowledgeable about the incidence of Lyme disease where they practice. Most Lyme disease occurs in the peridomestic environment. Landscape-management measures, such as keeping grass mowed, removing leaf litter, using plantings that do not attract deer, deer fences, spraying areas of high tick density with acaricidal formulations, and using dry pebble or wood chip borders between lawns and surrounding vegetation, may help reduce the risk for tick-borne infections.[34] The marked reduction of deer populations sharply reduces the risk for infection but is difficult to implement. Public education about the risks for and characteristic symptoms of tick-borne diseases is an important component of prevention.

Preventing Infection After an Ixodes *Tick Bite*

The risk for acquiring *B. burgdorferi* infection after an *Ixodes* tick bite is low, even in endemic areas. The incidence has been shown to be 1.2 to 4.4 percent in U.S. studies.[3,21,72] Nevertheless, considerable apprehension accompanies tick bites, and practitioners tend to overprescribe antibiotic prophylaxis. The use of an evidence-based approach will serve to allay anxiety, decrease indiscriminate use of antibiotics, and prevent some cases of *B. burgdorferi* infection. A tick should be removed with an ordinary pair of thin-tipped tweezers or forceps. The tick should be grasped by the mouth-end and gently pulled straight upward. No evidence suggests that residual embedded mouth parts or crushing the tick increase risk for transmission. Other removal techniques are ineffective and can be dangerous.[55] Most importantly, the patient should save the tick in any dry vessel for identification, because almost one third of suspected deer ticks are a different species or not ticks at all.[79] By referring to images on the Internet, a practitioner should be able to distinguish a six-legged insect from an eight-legged tick, a dog tick or a Lone Star tick from a deer.[76] Most commercial laboratories and health departments in endemic areas perform tick identification. Patient requests to have a tick analyzed for *B. burgdorferi* DNA should be discouraged because this testing may be unreliable and does not correlate with transmission risk. More important are the developmental stage of the tick and the duration of attachment. Most cases of Lyme disease are transmitted by nymphal deer ticks.[53] Duration of attachment is critical because there is a 36- to 72-hour delay between the onset of feeding and the migration of spirochetes from the tick midgut to saliva and transmission to the host. The incidence of infection increases 20-fold from ticks attached for 72 hours or longer than for those attached for less than 72 hours.[79] Tick identification and assessment of engorgement can be used to help define a small, high-risk subset of people who should benefit from antibiotic prophylaxis.

A single dose of doxycycline was shown to be 87 percent effective in prevention of Lyme borreliosis in a study in a Lyme disease–hyperendemic area of the United States, where an *I. scapularis* tick was analyzed within 72 hours of the bite and duration of attachment of 72 hours was documented by an entomologist.[53] Adverse effects occurred in 30 percent of doxycycline recipients, however. If the tick species, stage, and duration of attachment cannot be determined, a "wait and watch" approach is appropriate, given the overall low risk for Lyme borreliosis. This is the standard approach in Europe.[81] Serologic testing does not benefit management decisions after a tick bite and is not recommended. In sum, routine use of antibiotics to prevent Lyme disease in persons who are bitten by a deer tick is not recommended, even in highly

endemic areas. Persons who have removed attached ticks from themselves should be monitored closely for signs and symptoms of Lyme disease (and other tick-borne diseases) for a month. Those who develop a rash at the site of the tick bite or multiple skin lesions elsewhere, or a generalized nonspecific illness, should promptly seek medical attention.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Afzelius A. Verhandlungen der Dermatologischen Gesellschaft zu Stockholm, 28 Oct 1909. *Arch Dermatol Syph* 1910;**101**:404.
3. Agre F, Schwartz R. The value of early treatment of deer tick bites for the prevention of Lyme disease. *Am J Dis Child* 1993;**147**: 945–7.
4. Aguero-Rosenfeld ME, Nowakowski J, McKenna DF, et al. Serodiagnosis in early Lyme disease. *J Clin Microbiol* 1993;**31**:3090–5.
5. Aguero-Rosenfeld ME, Wang G, Schwartz I, et al. Diagnosis of lyme borreliosis. *Clin Microbiol Rev* 2005;**18**:484–509.
6. Arnez M, Pleterski-Rigler D, Ahcan J, et al. Demographic features, clinical characteristics and laboratory findings in children with multiple erythema migrans in Slovenia. *Wien Klin Wochenschr* 2001; **113**:98–101.
7. Auwaerter PG, Bakken JS, Dattwyler RJ, et al. Scientific evidence and best patient care practices should guide the ethics of Lyme disease activism. *J Med Ethics* 2011;**37**:68–73.
9. Bacon RM, Kugeler KJ, Mead PS. Surveillance for Lyme disease: United States, 1992-2006. *MMWR Surveill Summ* 2008;**57**:1–9.
10. Belman AL, Iyer M, Coyle PK, et al. Neurologic manifestations in children with North American Lyme disease. *Neurology* 1993; **43**:2609–14.
12. Bockenstedt LK, Gonzalez DG, Haberman AM, et al. Spirochete antigens persist near cartilage after murine Lyme borreliosis therapy. *J Clin Invest* 2012;**122**:2652–60.
13. Buchwald A. Ein Fall von diffuser idiopathischer Haut-Atrophie. *Arch Derm Syphilol* 1883;**10**:553–6.
15. Campbell GL, Paul WS, Schriefer ME, et al. Epidemiologic and diagnostic studies of patients with suspected early Lyme disease, Missouri, 1990-1993. *J Infect Dis* 1995;**172**:470–80.
17. Centers for Disease Control and Prevention. Reported cases of Lyme Disease by Year, United States. 2002-2011. Accessed July 13, 2013, at http://www.cdc.gov/lyme/stats/chartstables/casesbyyear.html.

17a. Chowdri HR, Gugliotta JL, Berardi VP, et al. *Borrelia miyamotoi* presenting as human granulocytic anaplasmosis. A case report. *Ann Intern Med* 2013;**159**:21–7.

18. Christen HJ, Hanefeld F, Eiffert H, et al. Epidemiology and clinical manifestations of Lyme borreliosis in childhood: a prospective multicentre study with special regard to neuroborreliosis. *Acta Paediatr Suppl* 1993;**386**:1–75.
19. Cohn KA, Thompson AD, Shah SS, et al. Validation of a clinical prediction rule to distinguish Lyme meningitis from aseptic meningitis. *Pediatrics* 2012;**129**:e46–53.
20. Colli C, Leinweber B, Mullegger R, et al. *Borrelia burgdorferi*-associated lymphocytoma cutis: clinicopathologic, immunophenotypic, and molecular study of 106 cases. *J Cutan Pathol* 2004;**31**:232–40.
23. Elliott DJ, Eppes SC, Klein JD. Teratogen update: Lyme disease. *Teratology* 2001;**64**:276–81.
24. Embers ME, Liang FT, Howell JK, et al. Antigenicity and recombination of VlsE, the antigenic variation protein of *Borrelia burgdorferi*, in rabbits, a host putatively resistant to long-term infection with this spirochete. *FEMS Immunol Med Microbiol* 2007;**50**:421–9.
27. Feder HM Jr, Hoss DM, Zemel L, et al. Southern tick-associated rash illness (STARI) in the North: STARI following a tick bite in Long Island, New York. *Clin Infect Dis* 2011;**53**:e142–6.
28. Feder HM Jr, Johnson BJ, O'Connell S, et al. A critical appraisal of "chronic Lyme disease." *N Engl J Med* 2007;**357**:1422–30.
31. Gerber MA, Shapiro ED, Burke GS, et al. Lyme disease in children in southeastern Connecticut. Pediatric Lyme Disease Study Group. *N Engl J Med* 1996;**335**:1270–4.

31a. Gugliotta JL, Goethert HK, Berardi VP, et al. Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N Engl J Med* 2013;**368**:240–5.

32. Haddad FA, Nadelman RB. Lyme disease and the heart. *Front Biosci* 2003;**8**:s769–82.
33. Halperin JJ. Nervous system lyme disease: diagnosis and treatment. *Rev Neurol Dis* 2009;**6**:4–12.
34. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med* 2003;**348**:2424–30.
35. Hazard GW, Leland K, Mathewson HO. Erythema chronicum migrans and "Lyme arthritis." *JAMA* 1976;**236**:2392.
36. Hirschfeld M, Kirschning CJ, Schwandner R, et al. Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J Immunol* 1999;**163**:2382–6.
37. Huppertz HI, Karch H, Suschke HJ, et al. Lyme arthritis in European children and adolescents. The Pediatric Rheumatology Collaborative Group. *Arthritis Rheum* 1995;**38**:361–8.
38. Jain VK, Hilton E, Maytal J, et al. Immunoglobulin M immunoblot for diagnosis of *Borrelia burgdorferi* infection in patients with acute facial palsy. *J Clin Microbiol* 1996;**34**:2033–5.
39. Johnson BJB, Aguero-Rosenfeld ME, Wilske B. Serodiagnosis of Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 185–212.
40. Johnson RC, Hyde FW, Steigerwalt AG, Brenner DJ. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *Int J Syst Bacteriol* 1984;**34**:496–7.
41. Kan L, Sood SK, Maytal J. Pseudotumor cerebri in Lyme disease: a case report and literature review. *Pediatr Neurol* 1998;**18**:439–41.
42. Katz TM, Miller JH, Hebert AA. Insect repellents: historical perspectives and new developments. *J Am Acad Dermatol* 2008;**58**: 865–71.

42a. Krause PJ, Narasimhan S, Wormser GP, et al. Human *Borrelia miyamotoi* infection in the United States. *N Engl J Med* 2013;**368**:291–3.

43. Krause PJ, McKay K, Gadbaw J, et al. Increasing health burden of human babesiosis in endemic sites. *Am J Trop Med Hyg* 2003;**68**: 431–6.
45. Krause PJ, Telford SR 3rd, Spielman A, et al. Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness. *JAMA* 1996;**275**:1657–60.

45a. Krause PJ, Foley DT, Burke GS, et al. Reinfection and relapse in early Lyme disease. *Am J Trop Med Hyg* 2006;**75**:1090–4.

46. Kurtenbach K, Hanincova K, Tsao JI, et al. Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol* 2006;**4**:660–9.
47. Liu N, Montgomery RR, Barthold SW, et al. Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in *Borrelia burgdorferi*-infected mice. *Infect Immun* 2004;**72**:3195–203.
48. LoGiudice K, Ostfeld RS, Schmidt KA, et al. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc Natl Acad Sci U S A* 2003;**100**:567–71.
50. Mast WE, Burrows WM. Erythema chronicum migrans and "Lyme arthritis." *JAMA* 1976;**236**:2392.
51. Meek JI, Roberts CL, Smith EV Jr, et al. Underreporting of Lyme disease by Connecticut physicians, 1992. *J Public Health Manag Pract* 1996;**2**:61–5.
52. Mullegger RR. Dermatological manifestations of Lyme borreliosis. *Eur J Dermatol* 2004;**14**:296–309.
54. Nadelman RB, Nowakowski J, Forseter G, et al. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. *Am J Med* 1996;**100**:502–8.
55. Needham GR. Evaluation of five popular methods for tick removal. *Pediatrics* 1985;**75**:997–1002.
56. Pfister HW, Einhaupl K, Preac-Mursic V, et al. The spirochetal etiology of lymphocytic meningoradiculitis of Bannwarth (Bannwarth's syndrome). *J Neurol* 1984;**231**:141–4.
57. Piesman J, Eisen L. Prevention of tick-borne diseases. *Annu Rev Entomol* 2008;**53**:323–43.

57a. Platonov AE, Karan LS, Kolyasnikova NM, et al. Humans infected with the relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emer Inf Dis* 2011;**17**:1816–23.

58. Pohl-Koppe A, Wilske B, Weiss M, et al. *Borrelia* lymphocytoma in childhood. *Pediatr Infect Dis J* 1998;**17**:423–6.
59. Radolf JD, Caimano MJ, Stevenson B, et al. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol* 2012;**10**:87–99.
60. Rand PW, Lubelczyk C, Holman MS, et al. Abundance of *Ixodes scapularis* (Acari: Ixodidae) after the complete removal of deer from an isolated offshore island, endemic for Lyme Disease. *J Med Entomol* 2004;**41**:779–84.

61. Ross EA, Savage KA, Utley LJ, et al. Insect repellent [correction of repellant] interactions: sunscreens enhance DEET (N,N-diethyl-m-toluamide) absorption. *Drug Metab Dispos* 2004;**32**:783–5.
63. Rupprecht TA, Koedel U, Fingerle V, et al. The pathogenesis of lyme neuroborreliosis: from infection to inflammation. *Mol Med* 2008;**14**:205–12.
64. Salazar JC, Duhnam-Ems S, La Vake C, et al. Activation of human monocytes by live *Borrelia burgdorferi* generates TLR2-dependent and -independent responses which include induction of IFN-beta. *PLoS Pathog* 2009;**5**:e1000444.
65. Salazar JC, Pope CD, Sellati TJ, et al. Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. *J Immunol* 2003;**171**:2660–70.
66. Scrimenti RJ. Erythema chronicum migrans. *Arch Dermatol* 1970;**102**:104–5.
67. Seriburi V, Ndukwe N, Chang Z, et al. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clin Microbiol Infect* 2012;**18**:1236–40.
68. Shah SS, Zaoutis TE, Turnquist J, et al. Early differentiation of Lyme from enteroviral meningitis. *Pediatr Infect Dis J* 2005;**24**:542–5.
69. Shapiro E, Sood SK. Prognosis of persons with Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 213–23.
70. Shapiro ED, Gerber MA. Lyme disease and facial nerve palsy. *Arch Pediatr Adolesc Med* 1997;**151**:1183–4.
71. Shapiro ED, Gerber MA. Lyme disease: fact versus fiction. *Pediatr Ann* 2002;**31**:170–7.
73. Sikand V, Mullegger R. Early lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 53–79.
75. Sood SK. Lyme disease. *Pediatr Infect Dis J* 1999;**18**:913–25.
76. Sood SK. Effective retrieval of Lyme disease information on the Web. *Clin Infect Dis* 2002;**35**:451–64.
77. Sood SK. What we have learned about Lyme borreliosis from studies in children. *Wien Klin Wochenschr* 2006;**118**:638–42.
78. Sood SK, Ilowite NT. Lyme arthritis in children: is chronic arthritis a common complication? *J Rheumatol* 2000;**27**:1836–8.
79. Sood SK, Salzman MB, Johnson BJ, et al. Duration of tick attachment as a predictor of the risk of Lyme disease in an area in which Lyme disease is endemic. *J Infect Dis* 1997;**175**:996–9.
80. Sood SK, O'Connell S, Weber K. The emergence and epidemiology of Lyme borreliosis in Europe and North America. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 1–35.
81. Stanek G, Kahl O. Chemoprophylaxis for Lyme borreliosis? *Zentralbl Bakteriol* 1999;**289**:655–65.
82. Steere AC. Lyme borreliosis in 2005, 30 years after initial observations in Lyme Connecticut. *Wien Klin Wochenschr* 2006;**118**:625–33.
83. Steere AC, Glickstein L. Elucidation of Lyme arthritis. *Nat Rev Immunol* 2004;**4**:143–52.
86. Steere AC, Sikand VK, Schoen RT, et al. Asymptomatic infection with *Borrelia burgdorferi*. *Clin Infect Dis* 2003;**37**:528–32.
90. Tilly K, Rosa PA, Stewart PE. Biology of infection with *Borrelia burgdorferi*. *Infect Dis Clin North Am* 2008;**22**:217–34.
91. Tory HO, Zurakowski D, Sundel RP. Outcomes of children treated for Lyme arthritis: results of a large pediatric cohort. *J Rheumatol* 2010;**37**:1049–55.
92. Wormser GP. Clinical practice: early Lyme disease. *N Engl J Med* 2006;**354**:2794–801.
94. Wormser GP, Liveris D, Nowakowski J, et al. Association of specific subtypes of *Borrelia burgdorferi* with hematogenous dissemination in early Lyme disease. *J Infect Dis* 1999;**180**:720–5.
95. Wormser GP, McKenna D, Carlin J, et al. Brief communication: hematogenous dissemination in early Lyme disease. *Ann Intern Med* 2005;**142**:751–5.
96. Wormser GP, Wang G. The role of culture and nucleic acid amplification in diagnosis of Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 159–83.
97. Zalaudek I, Leinweber B, Kerl H, et al. Acrodermatitis chronica atrophicans in a 15-year-old girl misdiagnosed as venous insufficiency for 6 years. *J Am Acad Dermatol* 2005;**52**:1091–4.

The full reference list for this chapter is available at expertconsult.com.

140B ■ RELAPSING FEVER

Peter J. Krause

Relapsing fever is an arthropod-borne infection caused by several species of spirochetes in the genus *Borrelia*.* Two major types of relapsing fever are classified according to their arthropod vector—tick-borne relapsing fever (TBRF, endemic) and louse-borne relapsing fever (LBRF, epidemic). Both arthropod vectors transmit a different group of *Borrelia* spp. that cause a similar clinical syndrome. Illness is characterized by recurring episodes of fever and nonspecific symptoms, including headache, shaking chills, myalgia, arthralgia, and abdominal distress separated by periods of well-being or minor symptoms.[21,23,46] The characteristic relapse of symptoms is caused by recurrent spirochetemia and the associated immune responses. After a *Borrelia* relapsing fever strain infects a person, its proliferation in blood is curtailed by antibody and other immune factors. The *Borrelia* subsequently change surface antigens so that a new variant (serotype) emerges and the cycle is repeated.† A single organism can produce as many as 40 antigenic variant membrane proteins, and six or more relapses may occur over several months.[21] The clinical presentation is highly variable, with some patients experiencing a flulike illness, some gastrointestinal symptoms, and others meningitis. Although the disease may be severe, the fatality rate in patients with treated TBRF is less than 5 percent.[22] The fatality rate for patients with treated LBRF is approximately 5 percent. Overall mortality rates may exceed 40 percent in large LBRF epidemics among nutritionally and immunosuppressed populations caught up in war or famine.[11,22,55] During the last great LBRF epidemic in North Africa and Europe during World War II, an estimated 50,000 patients died of the illness.[11,22,26] Both TBRF and LBRF occur worldwide, but only TBRF is found in the United States.

THE ORGANISM

Borrelia are spirochetal bacteria whose spiral shape enhances their motility and whose loose coiling helps distinguish them from other spirochetes such as

*References 2, 11, 21-23, 25, 26, 30-32, 35, 47, 64.
†References 2, 4-6, 39, 49, 57.

REFERENCES

1. Adams WV, Rose CD, Eppes SC, et al. Cognitive effects of Lyme disease in children: a 4 year followup study. *J Rheumatol* 1999;**26**:1190–4.
2. Afzelius A. Verhandlungen der Dermatologischen Gesellschaft zu Stockholm, 28 Oct 1909. *Arch Dermatol Syph* 1910;**101**:404.
3. Agre F, Schwartz R. The value of early treatment of deer tick bites for the prevention of Lyme disease. *Am J Dis Child* 1993;**147**:945–7.
4. Aguero-Rosenfeld ME, Nowakowski J, McKenna DF, et al. Serodiagnosis in early Lyme disease. *J Clin Microbiol* 1993;**31**:3090–5.
5. Aguero-Rosenfeld ME, Wang G, Schwartz I, et al. Diagnosis of lyme borreliosis. *Clin Microbiol Rev* 2005;**18**:484–509.
6. Arnez M, Pleterski-Rigler D, Ahcan J, et al. Demographic features, clinical characteristics and laboratory findings in children with multiple erythema migrans in Slovenia. *Wien Klin Wochenschr* 2001;**113**:98–101.
7. Auwaerter PG, Bakken JS, Dattwyler RJ, et al. Scientific evidence and best patient care practices should guide the ethics of Lyme disease activism. *J Med Ethics* 2011;**37**:68–73.
8. Avery RA, Frank G, Glutting JJ, et al. Prediction of Lyme meningitis in children from a Lyme disease-endemic region: a logistic-regression model using history, physical, and laboratory findings. *Pediatrics* 2006;**117**:e1–7.
9. Bacon RM, Kugeler KJ, Mead PS. Surveillance for Lyme disease: United States, 1992-2006. *MMWR Surveill Summ* 2008;**57**:1–9.
10. Belman AL, Iyer M, Coyle PK, et al. Neurologic manifestations in children with North American Lyme disease. *Neurology* 1993;**43**:2609–14.
11. Benach JL, Bosler EM, Hanrahan JP, et al. Spirochetes isolated from the blood of two patients with Lyme disease. *N Engl J Med* 1983;**308**:740–2.
12. Bockenstedt LK, Gonzalez DG, Haberman AM, et al. Spirochete antigens persist near cartilage after murine Lyme borreliosis therapy. *J Clin Invest* 2012;**122**:2652–60.
13. Buchwald A. Ein Fall von diffuser idiopathischer Haut-Atrophie. *Arch Derm Syphilol* 1883;**10**:553–6.
14. Burgdorfer W, Barbour AG, Hayes SF, et al. Erythema chronicum migrans: a tickborne spirochetosis. *Acta Trop* 1983;**40**:79–83.
15. Campbell GL, Paul WS, Schriefer ME, et al. Epidemiologic and diagnostic studies of patients with suspected early Lyme disease, Missouri, 1990-1993. *J Infect Dis* 1995;**172**:470–80.
16. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *MMWR Morb Mortal Wkly Rep* 1995;**44**:590–1.
17. Centers for Disease Control and Prevention. Reported cases of Lyme Disease by Year, United States, 2002-2011. Accessed July 13, 2013, at http://www.cdc.gov/lyme/stats/chartstables/casesbyyear.html.

17a. Chowdri HR, Gugliotta JL, Berardi VP, et al. *Borrelia miyamotoi* presenting as human granulocytic anaplasmosis. A case report. *Ann Intern Med* 2013;**159**:21–7.

18. Christen HJ, Hanefeld F, Eiffert H, et al. Epidemiology and clinical manifestations of Lyme borreliosis in childhood: a prospective multicentre study with special regard to neuroborreliosis. *Acta Paediatr Suppl* 1993;**386**:1–75.
19. Cohn KA, Thompson AD, Shah SS, et al. Validation of a clinical prediction rule to distinguish Lyme meningitis from aseptic meningitis. *Pediatrics* 2012;**129**:e46–53.
20. Colli C, Leinweber B, Mullegger R, et al. *Borrelia burgdorferi*-associated lymphocytoma cutis: clinicopathologic, immunophenotypic, and molecular study of 106 cases. *J Cutan Pathol* 2004;**31**:232–40.
21. Costello CM, Steere AC, Pinkerton RE, et al. A prospective study of tick bites in an endemic area for Lyme disease. *J Infect Dis* 1989;**159**:136–9.
22. Dressler F, Whalen JA, Reinhardt BN, et al. Western blotting in the serodiagnosis of Lyme disease. *J Infect Dis* 1993;**167**:392–400.
23. Elliott DJ, Eppes SC, Klein JD. Teratogen update: Lyme disease. *Teratology* 2001;**64**:276–81.
24. Embers ME, Liang FT, Howell JK, et al. Antigenicity and recombination of VlsE, the antigenic variation protein of *Borrelia burgdorferi*, in rabbits, a host putatively resistant to long-term infection with this spirochete. *FEMS Immunol Med Microbiol* 2007;**50**:421–9.
25. Eppes SC, Nelson DK, Lewis LL, et al. Characterization of Lyme meningitis and comparison with viral meningitis in children. *Pediatrics* 1999;**103**:957–60.
26. Feder HM Jr, Gerber MA, Krause PJ, et al. Early Lyme disease: a flu-like illness without erythema migrans. *Pediatrics* 1993;**91**:456–9.
27. Feder HM Jr, Hoss DM, Zemel L, et al. Southern tick-associated rash illness (STARI) in the North: STARI following a tick bite in Long Island, New York. *Clin Infect Dis* 2011;**53**:e142–6.
28. Feder HM Jr, Johnson BJ, O'Connell S, et al. A critical appraisal of "chronic Lyme disease." *N Engl J Med* 2007;**357**:1422–30.
29. Gerber MA, Zalneraitis EL. Childhood neurologic disorders and Lyme disease during pregnancy. *Pediatr Neurol* 1994;**11**:41–3.
30. Gerber MA, Zemel LS, Shapiro ED. Lyme arthritis in children: clinical epidemiology and long-term outcomes. *Pediatrics* 1998;**102**:905–8.
31. Gerber MA, Shapiro ED, Burke GS, et al. Lyme disease in children in southeastern Connecticut. Pediatric Lyme Disease Study Group. *N Engl J Med* 1996;**335**:1270–4.

31a. Gugliotta JL, Goethert HK, Berardi VP, et al. Meningoencephalitis from *Borrelia miyamotoi* in an immunocompromised patient. *N Engl J Med* 2013;**368**:240–5.

32. Haddad FA, Nadelman RB. Lyme disease and the heart. *Front Biosci* 2003;**8**:s769–82.
33. Halperin JJ. Nervous system lyme disease: diagnosis and treatment. *Rev Neurol Dis* 2009;**6**:4–12.
34. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med* 2003;**348**:2424–30.
35. Hazard GW, Leland K, Mathewson HO. Erythema chronicum migrans and "Lyme arthritis." *JAMA* 1976;**236**:2392.
36. Hirschfeld M, Kirschning CJ, Schwandner R, et al. Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J Immunol* 1999;**163**:2382–6.
37. Huppertz HI, Karch H, Suschke HJ, et al. Lyme arthritis in European children and adolescents. The Pediatric Rheumatology Collaborative Group. *Arthritis Rheum* 1995;**38**:361–8.
38. Jain VK, Hilton E, Maytal J, et al. Immunoglobulin M immunoblot for diagnosis of *Borrelia burgdorferi* infection in patients with acute facial palsy. *J Clin Microbiol* 1996;**34**:2033–5.
39. Johnson BJB, Aguero-Rosenfeld ME, Wilske B. Serodiagnosis of Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 185–212.
40. Johnson RC, Schmid GP, Hyde FW, Steigerwalt AG, Brenner DJ. *Borrelia burgdorferi* sp. nov.: etiologic agent of Lyme disease. *Int J Syst Bacteriol* 1984;**34**:496–7.
41. Kan L, Sood SK, Maytal J. Pseudotumor cerebri in Lyme disease: a case report and literature review. *Pediatr Neurol* 1998;**18**:439–41.
42. Katz TM, Miller JH, Hebert AA. Insect repellents: historical perspectives and new developments. *J Am Acad Dermatol* 2008;**58**:865–71.

42a. Krause PJ, Narasimhan S, Wormser GP, et al. Human *Borrelia miyamotoi* infection in the United States. *N Engl J Med* 2013;**368**:291–3.

43. Krause PJ, McKay K, Gadbaw J, et al. Increasing health burden of human babesiosis in endemic sites. *Am J Trop Med Hyg* 2003;**68**:431–6.
44. Krause PJ, McKay K, Thompson CA, et al. Disease-specific diagnosis of coinfecting tickborne zoonoses: babesiosis, human granulocytic ehrlichiosis, and Lyme disease. *Clin Infect Dis* 2002;**34**:1184–91.
45. Krause PJ, Telford SR 3rd, Spielman A, et al. Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness. *JAMA* 1996;**275**:1657–60.

45a. Krause PJ, Foley DT, Burke GS, et al. Reinfection and relapse in early Lyme disease. *Am J Trop Med Hyg* 2006;**75**:1090–4.

46. Kurtenbach K, Hanincova K, Tsao JI, et al. Fundamental processes in the evolutionary ecology of Lyme borreliosis. *Nat Rev Microbiol* 2006;**4**:660–9.
47. Liu N, Montgomery RR, Barthold SW, et al. Myeloid differentiation antigen 88 deficiency impairs pathogen clearance but does not alter inflammation in *Borrelia burgdorferi*-infected mice. *Infect Immun* 2004;**72**:3195–203.

48. LoGiudice K, Ostfeld RS, Schmidt KA, et al. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. *Proc Natl Acad Sci U S A* 2003;**100**:567–71.
49. Markowitz LE, Steere AC, Benach JL, et al. Lyme disease during pregnancy. *JAMA* 1986;**255**:3394–6.
50. Mast WE, Burrows WM. Erythema chronicum migrans and "Lyme arthritis." *JAMA* 1976;**236**:2392.
51. Meek JI, Roberts CL, Smith EV Jr, et al. Underreporting of Lyme disease by Connecticut physicians, 1992. *J Public Health Manag Pract* 1996;**2**:61–5.
52. Mullegger RR. Dermatological manifestations of Lyme borreliosis. *Eur J Dermatol* 2004;**14**:296–309.
53. Nadelman RB, Nowakowski J, Fish D, et al. Prophylaxis with single-dose doxycycline for the prevention of Lyme disease after an Ixodes scapularis tick bite. *N Engl J Med* 2001;**345**:79–84.
54. Nadelman RB, Nowakowski J, Forseter G, et al. The clinical spectrum of early Lyme borreliosis in patients with culture-confirmed erythema migrans. *Am J Med* 1996;**100**:502–8.
55. Needham GR. Evaluation of five popular methods for tick removal. *Pediatrics* 1985;**75**:997–1002.
56. Pfister HW, Einhaupl K, Preac-Mursic V, et al. The spirochetal etiology of lymphocytic meningoradiculitis of Bannwarth (Bannwarth's syndrome). *J Neurol* 1984;**231**:141–4.
57. Piesman J, Eisen L. Prevention of tick-borne diseases. *Annu Rev Entomol* 2008;**53**:323–43.
57a. Platonov AE, Karan LS, Kolyasnikova NM, et al. Humans infected with the relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emer Inf Dis* 2011;**17**:1816–23.
58. Pohl-Koppe A, Wilske B, Weiss M, et al. Borrelia lymphocytoma in childhood. *Pediatr Infect Dis J* 1998;**17**:423–6.
59. Radolf JD, Caimano MJ, Stevenson B, et al. Of ticks, mice and men: understanding the dual-host lifestyle of Lyme disease spirochaetes. *Nat Rev Microbiol* 2012;**10**:87–99.
60. Rand PW, Lubelczyk C, Holman MS, et al. Abundance of *Ixodes scapularis* (Acari: Ixodidae) after the complete removal of deer from an isolated offshore island, endemic for Lyme Disease. *J Med Entomol* 2004;**41**:779–84.
61. Ross EA, Savage KA, Utley LJ, et al. Insect repellent [correction of repellant] interactions: sunscreens enhance DEET (N,N-diethyl-m-toluamide) absorption. *Drug Metab Dispos* 2004;**32**:783–5.
62. Rothermel H, Hedges TR 3rd, Steere AC. Optic neuropathy in children with Lyme disease. *Pediatrics* 2001;**108**:477–81.
63. Rupprecht TA, Koedel U, Fingerle V, et al. The pathogenesis of lyme neuroborreliosis: from infection to inflammation. *Mol Med* 2008;**14**:205–12.
64. Salazar JC, Duhnam-Ems S, La Vake C, et al. Activation of human monocytes by live Borrelia burgdorferi generates TLR2-dependent and -independent responses which include induction of IFN-beta. *PLoS Pathog* 2009;**5**:e1000444.
65. Salazar JC, Pope CD, Sellati TJ, et al. Coevolution of markers of innate and adaptive immunity in skin and peripheral blood of patients with erythema migrans. *J Immunol* 2003;**171**:2660–70.
66. Scrimenti RJ. Erythema chronicum migrans. *Arch Dermatol* 1970;**102**:104–5.
67. Seriburi V, Ndukwe N, Chang Z, et al. High frequency of false positive IgM immunoblots for *Borrelia burgdorferi* in clinical practice. *Clin Microbiol Infect* 2012;**18**:1236–40.
68. Shah SS, Zaoutis TE, Turnquist J, et al. Early differentiation of Lyme from enteroviral meningitis. *Pediatr Infect Dis J* 2005;**24**:542–5.
69. Shapiro E, Sood SK. Prognosis of persons with Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 213–23.
70. Shapiro ED, Gerber MA. Lyme disease and facial nerve palsy. *Arch Pediatr Adolesc Med* 1997;**151**:1183–4.
71. Shapiro ED, Gerber MA. Lyme disease: fact versus fiction. *Pediatr Ann* 2002;**31**:170–7.
72. Shapiro ED, Gerber MA, Holabird NB, et al. A controlled trial of antimicrobial prophylaxis for Lyme disease after deer-tick bites. *N Engl J Med* 1992;**327**:1769–73.
73. Sikand V, Mullegger R. Early Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 53–79.
74. Smith RP, Schoen RT, Rahn DW, et al. Clinical characteristics and treatment outcome of early Lyme disease in patients with microbiologically confirmed erythema migrans. *Ann Intern Med* 2002;**136**:421–8.
75. Sood SK. Lyme disease. *Pediatr Infect Dis J* 1999;**18**:913–25.
76. Sood SK. Effective retrieval of Lyme disease information on the Web. *Clin Infect Dis* 2002;**35**:451–64.
77. Sood SK. What we have learned about Lyme borreliosis from studies in children. *Wien Klin Wochenschr* 2006;**118**:638–42.
78. Sood SK, Ilowite NT. Lyme arthritis in children: is chronic arthritis a common complication? *J Rheumatol* 2000;**27**:1836–8.
79. Sood SK, Salzman MB, Johnson BJ, et al. Duration of tick attachment as a predictor of the risk of Lyme disease in an area in which Lyme disease is endemic. *J Infect Dis* 1997;**175**:996–9.
80. Sood SK, O'Connell S, Weber K. The emergence and epidemiology of Lyme borreliosis in Europe and North America. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 1–35.
81. Stanek G, Kahl O. Chemoprophylaxis for Lyme borreliosis? *Zentralbl Bakteriol* 1999;**289**:655–65.
82. Steere AC. Lyme borreliosis in 2005, 30 years after initial observations in Lyme Connecticut. *Wien Klin Wochenschr* 2006;**118**:625–33.
83. Steere AC, Glickstein L. Elucidation of Lyme arthritis. *Nat Rev Immunol* 2004;**4**:143–52.
84. Steere AC, Grodzicki RL, Kornblatt AN, et al. The spirochetal etiology of Lyme disease. *N Engl J Med* 1983;**308**:733–40.
85. Steere AC, Malawista SE, Snydman DR, et al. Lyme arthritis: an epidemic of oligoarticular arthritis in children and adults in three Connecticut communities. *Arthritis Rheum* 1977;**20**:7–17.
86. Steere AC, Sikand VK, Schoen RT, et al. Asymptomatic infection with *Borrelia burgdorferi*. *Clin Infect Dis* 2003;**37**:528–32.
87. Strobino B, Abid S, Gewitz M. Maternal Lyme disease and congenital heart disease: a case-control study in an endemic area. *Am J Obstet Gynecol* 1999;**180**:711–16.
88. Strobino BA, Williams CL, Abid S, et al. Lyme disease and pregnancy outcome: a prospective study of two thousand prenatal patients. *Am J Obstet Gynecol* 1993;**169**:367–74.
89. Szer IS, Taylor E, Steere AC. The long-term course of Lyme arthritis in children. *N Engl J Med* 1991;**325**:159–63.
90. Tilly K, Rosa PA, Stewart PE. Biology of infection with *Borrelia burgdorferi*. *Infect Dis Clin North Am* 2008;**22**:217–34.
91. Tory HO, Zurakowski D, Sundel RP. Outcomes of children treated for Lyme arthritis: results of a large pediatric cohort. *J Rheumatol* 2010;**37**:1049–55.
92. Wormser GP. Clinical practice: early Lyme disease. *N Engl J Med* 2006;**354**:2794–801.
93. Wormser GP, Wang G. The role of culture and nucleic acid amplification in diagnosis of Lyme borreliosis. In: Sood SK, editor. *Lyme Borreliosis in Europe and North America*. Hoboken, NJ: John Wiley & Sons; 2011. p. 159–83.
94. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006;**43**:1089–134.
95. Wormser GP, Liveris D, Nowakowski J, et al. Association of specific subtypes of *Borrelia burgdorferi* with hematogenous dissemination in early Lyme disease. *J Infect Dis* 1999;**180**:720–5.
96. Wormser GP, McKenna D, Carlin J, et al. Brief communication: hematogenous dissemination in early Lyme disease. *Ann Intern Med* 2005;**142**:751–5.
97. Zalaudek I, Leinweber B, Kerl H, et al. Acrodermatitis chronica atrophicans in a 15-year-old girl misdiagnosed as venous insufficiency for 6 years. *J Am Acad Dermatol* 2005;**52**:1091–4.

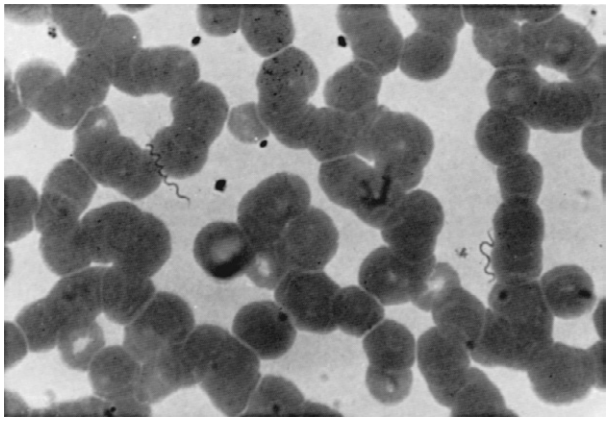

FIGURE 140B-1 ■ *Borrelia hermsii* spirochetes in the peripheral blood smear of a 20-year-old patient with tick-borne relapsing fever (Wright stain, ×1000). (From Boyer KM, Munford RS, Maupin GO, et al. Tick-borne relapsing fever: an interstate outbreak originating at Grand Canyon National Park. *Am J Epidemiol* 1977;**105**: 469–79.)

Leptospira and *Treponema* (Fig. 140B-1).[2,25,31,64] They have inner and outer membranes and several internal flagella that are not surface exposed. The flagella are responsible for shape and motility. *Borrelia* spp. are divided into two major groups—the Lyme borreliosis group transmitted by hard-bodied ticks and the relapsing fever group primarily transmitted by soft-bodied ticks but also by hard-bodied ticks and lice.[2,25,64]

The genome of the relapsing fever *Borrelia hermsii* contains a linear chromosome of about 1 million base pairs and one copy each of several types of linear and circular plasmids. Each organism contains 5 to 10 identical genomes.[2,7] Numerous surface lipoproteins of the outer membrane—the variable major proteins (Vmps)—determine variant or serotype identity.[3] A change in the expression of Vmp proteins allows the relapsing fever *Borrelia* spp. to survive in the highly diverse environments of mammals and ticks and allows the organism to escape immune attack within mammalian hosts.*

Relapsing fever *Borrelia* spp. are microaerophilic and can be cultivated in cell-free media but have complex nutritional requirements for growth.[2,64] They may no longer be able to infect laboratory animals after several passages in vitro and lose their ability to infect ticks after prolonged passage in laboratory animals.

TRANSMISSION

Relapsing fever is transmitted in almost all cases by an infected tick or louse. Rare causes of transmission include blood transfusion, intravenous drug use, laboratory worker accidents, and transplacental transmission from mother to fetus.[2,27,56,64]

Most ticks that transmit relapsing fever *Borrelia* spp. are soft-bodied *(Ornithodoros)* ticks. Transmission begins within minutes of the start of a blood meal that takes from 15 to 90 minutes.[2,22] The ticks usually feed at night, and most relapsing fever patients are unaware of being bitten.

*References 3, 4, 7, 39, 43, 49, 57.

The *Borrelia* spp. and the ticks are maintained in a tick-mammalian host life cycle in which rodents and other small mammals become asymptomatically infected and provide a blood meal for the ticks.[21,22,25] Nymphal and adult ticks are the most common source of transmission, but in some tick species larvae may rarely be infected through transovarial transmission in which an adult female transmits infection to her eggs.[22] Humans are a dead-end host for TBRF and do not contribute to the transmission cycle. The recently discovered *Borrelia miyamotoi* has been found in hard-bodied (ixodid) ticks in North America and Eurasia.[28,44,54] Approximately 15 percent of the spirochetes found in *Ixodes scapularis* (the tick vector for Lyme disease, babesiosis, and anaplasmosis) are *B. miyamotoi*.[54]

LBRF occurs when an infected louse feeds on a human, the sole known host.[32] In contrast to TBRF, humans are the reservoir host for LBRF and are responsible for perpetuating the infection cycle. Transmission occurs when the louse is wounded or crushed by scratching, and infected hemolymph or feces contaminate the bite site. LBRF is most common when large numbers of people gather in one area and hygiene is compromised.

EPIDEMIOLOGY

Eleven different species of *Borrelia* have been shown to cause TBRF (Table 140B-1).[2] In the United States, the most common species is *B. hermsii*, followed by *Borrelia turicatae* and *Borrelia parkeri*.[2,22] All cases occur west of Texas, Oklahoma, and Kansas. Cases most commonly occur in the summertime among people who have slept in rustic cabins in forested areas.[1,8,17-19,59,60] TBRF is very widespread outside the United States, and cases have been described in Canada, Mexico, Central and South America, Africa, Asia, and Europe.[1,22] Thus far, human cases of *B. miyamotoi* relapsing fever have been described in central Russia and the northeastern United States,[17a,31a,42a,57a] but the discovery of this spirochete in a variety of ixodid ticks greatly expands the potential geographic distribution of relapsing fever.[44,54] In contrast, LBRF is caused by a single species *Borrelia recurrentis*. It has been found worldwide but is currently only significantly endemic in Ethiopia and Sudan.[46,47]

PATHOGENESIS AND PATHOLOGY

Relapsing fever *Borrelia* organisms enter the bloodstream after tick transmission and multiply rapidly. Based on animal experiments and human studies, it is estimated that bacterial concentrations reach 100,000 to 1 million spirochetes per milliliter of blood during symptomatic disease.[11,22,57] *Borrelia* are initially phagocytized in the bloodstream by neutrophils, macrophages, and dendritic cells that cause the release of cytokines and other immune factors, resulting in symptoms.[14,15,45,62,65] Antibody (especially immunoglobulin [Ig]M) plays a central role in bacterial eradication and most *Borrelia* organisms are cleared from the bloodstream after 2 to 3 days.[22,57] Surviving organisms are able to change outer membrane proteins so that the original IgM antibodies are no longer effective. A new antigenic variant emerges and causes

TABLE 140B-1 **Important Borreliae Causing Relapsing Fever, Their Vectors, and Their Current Geographic Distribution**

Borrelia Species	Vector	Geographic Distribution
B. recurrentis	*Pediculus humanus humanus*	Ethiopia, Sudan
B. caucasica	*Ornithodoros verrucosus*	Iraq, central Russia
B. crocidurae	*Ornithodoros erraticus*(small variant)	North Africa, Middle East
B. duttonii	*Ornithodoros moubata*	Eastern and Central Africa
B. hermsii	*Ornithodoros hermsi*	Western United States and Canada
B. hispanica	*Ornithodoros erraticus* (large variant)	Spain, western North Africa
B. latyschewii	*Ornithodoros tartakowskyi*	Iran, central Asia
B. mazzottii	*Ornithodoros talaje*	Mexico, Central America
B. miyamotoi	Ixodid ticks	central Russia, northeastern United States
B. parkeri	*Ornithodoros parkeri*	Western United States
B. persica	*Ornithodoros tholozani*	Middle East, western Asia
B. turicatae	*Ornithodoros turicata*	Southwestern United States, Mexico
B. venezuelensis	*Ornithodoros rudis*	Northern South America

TABLE 140B-2 **Characteristics of Tick-Borne Relapsing Fever and Lyme Disease**

Characteristic*	Fever	Lyme Disease
Location in the United States	Northeast, West	Northeast, Northern Midwest, Pacific coast
Fever relapses	Common	Rare/none
Fever ≥39° C (102.2° F)	Common	Rare
Focal skin rash	Rare	Common
Arthritis	Rare/none	Common
Neurologic involvement	Occasional	Occasional
Spirochetes on blood smear	Common	Rare/none
Jarisch-Herxheimer reaction	Common	Occasional

*Clinical characteristics of untreated patients.

recurrence of fever and other symptoms.* The same process repeats itself for several cycles in untreated patients. Because some antibody cross reactivity occurs, subsequent spirochetemias and clinical manifestations usually are milder.[9] *Borrelia* antigenic variation is a result of new Vmp outer surface proteins that are encoded by alternative *vmp* genes found on linear plasmids.[3,4,7,51] A copy of silent and expressed genes are found on the same or different plasmids within each borrelial microorganism. Antigenic switching occurs when a silent *vmp* gene begins to encode for protein while the existing *vmp* gene is shut down. The stimulus for antigenic switching is unknown. Recent data suggest that the antigenic switching that occurs when relapsing *Borrelia* organisms move from mouse to tick is induced by the temperature change from the warm-blooded host to the cooler tick vector.[51]

Relapsing fever *Borrelia* spp. are not confined to the bloodstream but also infect the nervous system, eyes, lung, liver, kidney, heart, and spleen.[20,22,23,26,32] They may persist in these tissues.[2] Evidence in animal models demonstrates that the same serotype antigenic variation seen in the blood also occurs in brain tissue and *Borrelia* spp. have been shown to persist for up to 3 years.[2] Tissue damage in fatal human cases has been noted in the central nervous system (meningitis, degenerative lesions, hemorrhage, and perivascular infiltrates), heart (myocarditis), lung (pneumonia), gastrointestinal tract (hemorrhage), liver (enlargement, hepatic necrosis), and spleen (splenic abscess).[26,32] Relapsing fever *Borrelia* spp. can cross the placenta or be transmitted during birth, causing abortion, premature birth, or severe perinatal infection.[27,56]

CLINICAL MANIFESTATIONS

Initial clinical manifestations may consist of the characteristic recurring high fever and accompanying symptoms of headache, chills, and fatigue or may reflect rapid dissemination to the central nervous system, gastrointestinal tract, or lungs.* Symptom type and severity depend on the species and strain of infecting *Borrelia* organisms and the age and immune status of the host. Although TBRF and LBRF have similar clinical manifestations, tick-borne illness generally is less severe (Table 140B-2). *B. recurrentis* (LBRF) and *Borellia duttonii* (TBRF) are associated with severe disease. Pregnant women often experience severe disease.[21,27,56]

For TBRF, the mean incubation period is 7 days, with a range of 4 to 18 days. Symptoms generally last 3 days to 6 days (range, 12 hours to 17 days) for the first episode in untreated patients. This is followed by a week (range, 3 to 36 days) of well-being or mild nonspecific symptoms before the next febrile episode.[22] The duration of symptoms and asymptomatic intervals are several days longer for LBRF. As many as six relapses have been noted in TBRF patients but the average number is three to five in untreated patients.[8,21,22,44] The duration of symptoms decreases, and the time between relapses increases with each episode, although this is not always the case.[59]

TBRF may be associated with fatal hypotension and shock, usually at the end of the first episode of fever. Gastrointestinal manifestations (e.g., nausea, vomiting,

*References 3, 4, 7, 34, 39, 49, 51, 57.

*References 8, 11, 16, 20-23, 30, 34, 35, 46, 59, 60, 64.

diarrhea, hepatomegaly, or splenomegaly) occur in more than half of cases; dry cough in about a quarter of cases; a macular, papular, or petechial rash in about a fifth of cases; and meningitis in about 3 percent of cases.[21,22] Ocular manifestations are seen in fewer than 10 percent of TBRF patients and include eye pain, photophobia, and conjunctival injection. Pregnant women are subject to more severe disease with high fever and associated miscarriage and congenital infection. The clinical manifestations of Ixodid relapsing fever described in central Russia and in the northeastern United States appear to be similar to those of TBRF transmitted by soft-bodied ticks.[17a,31a,42a,44]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

A history of travel to wooded areas, especially rustic cabins with exposure to small rodents and ticks, is helpful in making the diagnosis, but a definitive diagnosis generally is made by examination of a Wright-stained thick or thin blood smear (see Fig. 140B-1). Use of a buffy coat smear with Giemsa stain, acridine orange stain, or dark-field examination increases diagnostic sensitivity.[53] *Borrelia* organisms are rarely found during afebrile periods.

An initial enzyme-linked immunosorbent assay (ELISA) or immunofluorescent antibody assay followed by an immunoblot blot is often used to test for antibody in sera samples from patients with acute illness and convalescent patients.[48] Serology testing is compromised by alternating outer surface protein (i.e., antigenic switching) and cross reactivity of relapsing fever borrelia antibody against other spirochetes, such as the causative agents of Lyme disease and syphilis. Glycophosphoryl diester phosphodiesterase (GlpQ) protein (produced with recombinant DNA technology) has been identified in relapsing fever spirochetes but not in Lyme disease or syphilis spirochetes.[52] GlpQ protein–based ELISA assays are superior to those that use whole-cell lysate of cultured bacteria such as *B. hermsii* but are not yet widely available.[22,42a,52] Other tests that have been developed to diagnose TBRF include *B. hermsii* and *B. miyamotoi* PCR, in vitro cultivation, or amplification of organisms by mouse inoculation followed by microscopic visualization.[33,44,61]

TBRF and LBRF are frequently misdiagnosed. The differential diagnosis is broad and includes enterovirus infection, influenza, malaria, hepatitis, leptospirosis, dengue, typhoid fever, and tick-borne illnesses, including Colorado tick fever, Lyme disease, babesiosis, anaplasmosis, ehrlichiosis, and Rocky Mountain spotted fever. Periodic fever syndromes such as periodic fever, apthous ulcers, pharyngitis, and adenitis (PFAPA); hyper-IgD syndrome; and familial Mediterranean fever, have longer intervals between febrile episodes.[22,36,40]

TREATMENT

Several antibiotics have been shown to be effective in treatment of relapsing fever, including doxycycline, tetracycline, erythromycin, penicillin, and chloramphenicol.[5,37,41,42,50] Erythromycin is the drug of choice for children younger than 8 years of age, because penicillin is probably less effective, doxycycline and tetracycline are associated with teeth staining, and chloramphenicol may suppress the bone marrow. For older children, tetracycline and doxycycline are the antimicrobial therapies of choice. A proposed management approach for febrile children is to substitute oral phenoxymethyl penicillin (a single low dose of 7.5 mg/kg) or intravenous penicillin G 10,000 U/kg infused over a period of 30 minutes as initial treatment on the first day to minimize the potential problem of the Jarisch-Herxheimer reaction.[12,49,62] Patients with this reaction experience an acute exacerbation of symptoms after the first dose of antibiotic.* Data suggest that large numbers of bacteria are abruptly fragmented and killed with antibiotic therapy, exposing antigen that elicits an intense cytokine response. Penicillin may result in less rapid destruction of spirochetes than tetracycline or doxycycline. In one large study, 54 percent of patients treated for TBRF experienced the Jarisch-Herxheimer reaction, with more than half experiencing high fever (≥40° C [104° F]), chills, rigors, diaphoresis, and hypotension.[22] Associated symptoms are even worse with LBRF. Fortunately, children generally experience milder reactions than adults. Regardless, however, they still should be monitored closely for the first 12 hours after the initial dose of antibiotic in the emergency room or hospital with intravenous access in place. Therapy consists of supportive measures, such as volume expansion and antipyretics. A 10-day course of oral erythromycin or tetracycline (40 mg/kg/day of either drug divided every 6 hours) should then be given to eradicate tissue spirochetes and prevent relapse. Preventive measures include the use of doxycycline tick-bite prophylaxis and acaricidal spraying of tick-infested bedding and sleeping areas.[8,29]

NEW REFERENCES SINCE THE SIXTH EDITION

16. Cadavid D, Barbour AG. Neuroborreliosis during relapsing fever: review of the clinical manifestations, pathology, and treatment of infections in humans and experimental animals. *Clin Infect Dis* 1998;**26**:151–64.
22. Dworkin MS, Schwan TG, Anderson DE, et al. Tick-borne relapsing fever. *Infect Dis North Am* 2008;**22**:449–68.
28. Fukunaga M, Takahashi Y, Tsuruta Y, et al. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *Int J Syst Bacteriol* 1995;**45**:804–10.
44. Platonov AE, Karan LS, Kolyasnikova NM, et al. Humans infected with the relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerg Infect Dis* 2011;**17**:1816–23.
47. Ramos JM, Malmierca E, Reyes F, et al. Results of a 10-year survey of louse-borne relapsing fever in southern Ethiopia: a decline in endemnicity. *Ann Trop Med Parsitol* 2008;**102**:467–9.
52. Schwan TG, Schrumpf ME, Hinnebusch BJ, et al. GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. *J Clin Microbiol* 1996;**34**:2483–92.
54. Scoles GA, Papero M, Beati L, Fish D. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. *Vector Borne Zoonotic Dis* 2001;**1**:21–34.
65. Woodman ME, Cooley AE, Avdiushko R, et al. Roles for phagocytic cells and complement in controlling relapsing fever infection. *J Leukoc Biol* 2009;**86**:727–36.

The full reference list for this chapter is available at expertconsult.com.

*References 10, 12, 13, 24, 38, 58, 63.

REFERENCES

1. Banerjee SN, Banerjee M, Fernando K, et al. Tick-borne relapsing fever in British Columbia, Canada: first isolation of *Borrelia hermsii*. *J Clin Microbiol* 1998;**36**:3505–8.
2. Barbour AG, Hayes SF. Biology of *Borrelia* species. *Microbiol Rev* 1986;**50**:381–400.
3. Barbour AG, Barrera O, Judd RC. Structural analysis of the variable major proteins of *Borrelia hermsii*. *J Exp Med* 1983;**158**:2127–40.
4. Barbour AG, Carter CJ, Sohaskey CD. Surface protein variation by expression site switching in the relapsing fever agent *Borrelia hermsii*. *Infect Immun* 2000;**68**:7114–21.
5. Barbour AG, Todd WJ, Stoenner HG. Action of penicillin on *Borrelia hermsii*. *Antimicrob Agents Chemother* 1982;**21**:823–9.
6. Barbour AG, Carter CJ, Burman N, et al. Tandem insertion sequence–like elements define the expression site for variable antigen genes of *Borrelia hermsii*. *Infect Immun* 1991;**59**:390–7.
7. Barbour AG, Dai Q, Restrepo BI, et al. Pathogen escape from host immunity by a genome program for antigenic variation. *Proc Natl Acad Sci* 2006;**103**:18290–5.
8. Boyer KM, Munford RS, Maupin GO, et al. Tick-borne relapsing fever: an interstate outbreak originating at Grand Canyon National Park. *Am J Epidemiol* 1977;**105**:469–79.
9. Breinl A, Dutton JE, Kinghorn A, et al. *An experimental study of the parasite of the African tick fever*. Memoir XXI of the Liverpool School of Tropical Medicine. London: Williams & Norgate; 1906.
10. Bryceson ADM. Clinical pathology of the Jarisch-Herxheimer reaction. *J Infect Dis* 1976;**133**:696–704.
11. Bryceson ADM, Parry EHO, Perine PL, et al. Louse-borne relapsing fever. *Q J Med* 1970;**39**:129–70.
12. Butler T. Relapsing fever: new lessons about antibiotic action. *Ann Intern Med* 1985;**102**:397–9.
13. Butler T, Jones PK, Wallace CK. *Borrelia recurrentis* infection: Single-dose antibiotic regimens and management of the Jarisch-Herxheimer reaction. *J Infect Dis* 1978;**137**:573–7.
14. Butler T, Aikawa M, Habte-Michael A, et al. Phagocytosis of *Borrelia recurrentis* by blood polymorphonuclear leukocytes is enhanced by antibiotic treatment. *Infect Immun* 1980;**28**:1009–13.
15. Butler T, Spagnuolo PJ, Goldsmith GH, et al. Interaction of *Borrelia* spirochetes with human mononuclear leukocytes causes production of leukocytic pyrogen and thromboplastin. *J Lab Clin Med* 1982;**99**:709–21.
16. Cadavid D, Barbour AG. Neuroborreliosis during relapsing fever: review of the clinical manifestations, pathology, and treatment of infections in humans and experimental animals. *Clin Infect Dis* 1998;**26**:151–64.
17. Centers for Disease Control and Prevention. Common source outbreak of relapsing fever: California. *MMWR Morb Mortal Wkly Rep* 1990;**39**:579, 585–6.
18. Centers for Disease Control and Prevention. Outbreak of relapsing fever: Grand Canyon National Park, Arizona, 1991. *MMWR Morb Mortal Wkly Rep* 1991;**40**:296–7, 303.
19. Centers for Disease Control and Prevention. Tick-borne relapsing fever outbreak after a family gathering: New Mexico, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:809–12.
20. Centers for Disease Control and Prevention. Tick-borne relapsing fever: Acute respiratory distress syndrome in persons with tick-borne relapsing fever: 3 states, 2004-2005. *MMWR Morb Mortal Wkly Rep* 2007;**56**:1073–6.
21. Dworkin MS, Anderson DE Jr, Schwan RG, et al. Tick-borne relapsing fever in the northwestern United States and southwestern Canada. *Clin Infect Dis* 1998;**26**:122–31.
22. Dworkin MS, Schwan TG, Anderson DE, et al. Tick-borne relapsing fever. *Infect Dis North Am* 2008;**22**:449–68.
23. Edall TA, Emerson JK, Maupin GO, et al. Tick-borne relapsing fever in Colorado: historical review and report of cases. *JAMA* 1979;**241**:2279–82.
24. Fekade D, Knox K, Hussein K, et al. Prevention of Jarisch-Herxheimer reactions by treatment with antibodies against tumor necrosis factor α. *N Engl J Med* 1996;**335**:311–15.
25. Felsenfeld O. *Borrelia*, human relapsing fever, and parasite-vector-host relationships. *Bacteriol Rev* 1965;**29**:46–74.
26. Felsenfeld O. *Borrelia: Strains, Vectors, Human and Animal Borreliosis*. St. Louis: Warren H. Green; 1971.
27. Fuchs PC, Oyama AA. Neonatal relapsing fever due to transplacental transmission of *Borrelia*. *JAMA* 1969;**208**:690–2.
28. Fukunaga M, Takahashi Y, Tsuruta Y, et al. Genetic and phenotypic analysis of *Borrelia miyamotoi* sp. nov., isolated from the ixodid tick *Ixodes persulcatus*, the vector for Lyme disease in Japan. *Int J Syst Bacteriol* 1995;**45**:804–10.
29. Hasin T, Davidovitch N, Cohen R, et al. Treatment with doxycycline for the prevention of tick-borne relapsing fever. *N Engl J Med* 2006;**355**:148–55.
30. Horton JM, Blaser MJ. The spectrum of relapsing fever in the Rocky Mountains. *Arch Intern Med* 1985;**145**:871–5.
31. Hyde FW, Johnson RC. Genetic relationship of Lyme disease spirochetes to *Borrelia*, *Treponema*, and *Leptospira* spp. *J Clin Microbiol* 1984;**20**:151–4.
32. Judge DM, Samuel I, Perine PL, et al. Louse-borne relapsing fever in man. *Arch Pathol* 1974;**97**:136–40.
33. Kelly RT. Cultivation of *Borrelia hermsii*. *Science* 1971;**173**:443–4.
34. Kitten T, Barbour AG. Juxtaposition of expressed variable antigen genes with a conserved telomere in the bacterium *Borrelia hermsii*. *Proc Natl Acad Sci U S A* 1990;**87**:6077–81.
35. Le CT. Tick-borne relapsing fever in children. *Pediatrics* 1980;**66**:963–6.
36. Miller LC, Sisson BA, Tucker LB, et al. Prolonged fevers of unknown origin in children: patterns of presentation and outcome. *J Pediatr* 1996;**129**:419–23.
37. Nassif X, Dupont B, Fleury J, et al. Ceftriaxone in relapsing fever. *Lancet* 1988;**2**:394.
38. Negussie Y, Remick DG, DeForge LE, et al. Detection of plasma tumor necrosis factor, interleukins 6 and 8 during the Jarisch-Herxheimer reaction of relapsing fever. *J Exp Med* 1992;**175**:1207–12.
39. Norris SJ. Antigemic variation with a twist: the *Borrelia* story. *Mol Microbiol* 2006;**60**:1319–22.
40. Padeh S. Periodic fever syndromes. *Pediatr Clin North Am* 2005;**52**:577–609.
41. Perine PL, Tekly B. Antibiotic treatment of louse-borne relapsing fever in Ethiopia: a report of 377 cases. *Am J Trop Med Hyg* 1983;**32**:1096–100.
42. Perine PL, Krause DW, Awoke S, et al. Single-dose doxycycline treatment of louse-borne relapsing fever and endemic typhus. *Lancet* 1974;**2**:742–4.
43. Plasterk RHA, Simon MI, Barbour AG. Transposition of structural genes to an expression sequence on a linear plasmid causes antigenic variation in the bacterium *Borrelia hermsii*. *Nature* 1985;**318**:257–63.
44. Platonov AE, Karan LS, Kolyasnikova NM, et al. Humans infected with the relapsing fever spirochete *Borrelia miyamotoi*, Russia. *Emerg Inf Dis* 2011;**17**:1816–23.
45. Radolph JD, Norgard MV, Brandt ME, et al. Lipoproteins of *Borrelia burgdorferi* and *Treponema pallidum* activate cachectin/tumor necrosis factor synthesis: analysis using a CAT reporter construct. *J Immunol* 1991;**147**:1968–74.
46. Ramos JM, Malmierca E, Reyes F, et al. Characteristics of louse-borne relapsing fever in Ethiopian children and adults. *Ann Trop Med Parasitol* 2004;**98**:191–6.
47. Ramos JM, Malmierca E, Reyes F, et al. Results of a 10-year survey of louse-borne relapsing fever in southern Ethiopia: a decline in endemnicity. *Ann Trop Med Parasitol* 2008;**102**:467–9.
48. Rath PU, Ragler G, Schonberg A, et al. Relapsing fever and its serological discrimination from Lyme borreliosis. *Infection* 1992;**20**:283–6.
49. Restrepo BI, Barbour AG. Antigenic diversity in the bacterium *B. hermsii* through "somatic mutations" in rearranged vmp genes. *Cell* 1994;**78**:867–76.
50. Salih SY, Mustafa D. Louse-borne relapsing fever. II. Combined penicillin and tetracycline therapy in 160 Sudanese patients. *Trans R Soc Trop Med Hyg* 1977;**71**:49–51.
51. Schwan TG, Hinnebusch BJ. Bloodstream- versus tick-associated variants of a relapsing fever bacterium. *Science* 1998;**280**:1938–40.
52. Schwan TG, Schrumpf ME, Hinnebusch BJ, et al. GlpQ: an antigen for serological discrimination between relapsing fever and Lyme borreliosis. *J Clin Microbiol* 1996;**34**:2483–92.
53. Sciotto CG, Lauer BA, White WL, et al. Detection of *Borrelia* in acridine orange–stained blood smears by fluorescence microscopy. *Arch Pathol Lab Med* 1983;**107**:384–6.
54. Scoles GA, Papero M, Beati L, Fish D. A relapsing fever group spirochete transmitted by *Ixodes scapularis* ticks. *Vector Borne Zoonotic Dis* 2001;**1**:21–34.

55. Southern PM, Sanford JP. Relapsing fever: A clinical and microbiological review. *Medicine (Baltimore)* 1969;**48**:129–49.
56. Steenbarger JR. Congenital tick-borne relapsing fever: report of a case with first documentation of transplacental transmission. *Birth Defects* 1982;**18**:39–45.
57. Stoenner HG, Dodd T, Larsen C. Antigenic variation of *Borrelia hermsii*. *J Exp Med* 1982;**156**:1297–311.
58. Teklu B, Habte-Michael A, Warrell DA, et al. Meptazinol diminishes the Jarisch-Herxheimer reaction of relapsing fever. *Lancet* 1983;**1**:835–9.
59. Thompson RS, Burgdorfer W, Russell R, et al. Outbreak of tick-borne relapsing fever in Spokane County, Washington. *J A M A* 1969;**310**:1045–50.
60. Trevejo RT, Schreifer ME, Gage KL, et al. An interstate outbreak of tick-borne relapsing fever among vacationers at a Rocky Mountain cabin. *Am J Trop Med Hyg* 1998;**58**:743–7.
61. Uhlmann EJ, Seed PC, Schwan TG, et al. Tick-borne relapsing fever: polymerase chain reaction of tick-borne relapsing fever caused by *Borrelia hermsii*. *Pediatr Infect Dis J* 2007;**26**:267–9.
62. Vidal V, Scragg IG, Cutler SJ, et al. Variable major lipoprotein is a principal TNF-inducing factor of louse-borne relapsing fever. *Nat Med* 1998;**4**:1416–20.
63. Warrell DA, Perine PL, Krause DW, et al. Pathophysiology and immunology of the Jarisch-Herxheimer reaction in louse-borne relapsing fever: comparison of tetracycline and slow release penicillin. *J Infect Dis* 1983;**147**:898–909.
64. Wilske B, Schreifer ME. Borrelia. In: Murray PR, Baron EJ, Jorgensen J, et al, editors. *Manual of Clinical Microbiology*. Washington, D.C.: American Society for Microbiology; 2003. p. 937–54.
65. Woodman ME, Cooley AE, Avdiushko R, et al. Roles for phagocytic cells and complement in controlling relapsing fever infection. *J Leukoc Biol* 2009;**86**:727–36.

CHAPTER 141

Leptospirosis

Delma J. Nieves

HISTORY

Weil[257] is credited with providing the first description of leptospirosis in 1886. Not until 1915, however, was the causal agent, originally known as *Spirochaeta icterohaemorrhagiae*, identified by Inada and associates.[117] Eight years earlier, Stimson[220] unknowingly had identified the same organism within sections of kidney obtained from a patient in whom yellow fever had been diagnosed incorrectly.

Inada and associates demonstrated the role of the rat as a carrier in 1916.[117] Noguchi[166] first recovered this organism from a Norway rat in 1917; in 1922, the first case of Weil disease in a person associated with rat exposure was identified.[252] For many years the rat was considered the only animal host of *Leptospira icterohaemorrhagiae*. Then, in 1944, Randall and Cooper[180] isolated this agent from a naturally infected dog, and *L. icterohaemorrhagiae* subsequently has been associated with many animal hosts, including goats, swine, cattle, and hamsters.

In 1938 and 1939, Meyer and associates[149] popularized the concept that infection with *L. canicola* caused disease in dogs and humans in the United States. "Canicola fever" first was reported in Great Britain in 1946[18]; in 1951, 40 percent of the dogs in Great Britain were noted to be seropositive.[42] Surveys have confirmed the presence of *L. canicola* infection in species other than dogs.[199,245]

In 1950, Gochenour and colleagues[98] identified *L. pomona* as the agent responsible for leptospirosis in cows. Widespread *L. pomona* infection among cattle in the United States was recognized quickly, and in time this finding stimulated extensive epidemiologic investigations in livestock. Infections of cattle with *L. hebdomadis* and *L. grippotyphosa* were identified, and, concomitantly, infections of swine and horses with *L. pomona* were documented.[112] In Europe, *L. pomona* was identified as the agent responsible for "swineherd disease," and it was recovered from other domestic animals as well. In 1951, the first human cases of *L. pomona* infection were identified.

Many new serotypes of leptospires were recognized in the early 1950s after the establishment of serologic diagnostic services for leptospires by the Centers for Disease Control and Prevention (CDC) and the Walter Reed Army Institute of Research. Along with the identification of additional leptospiral serotypes, the spectrum of clinical disease associated with infection by leptospires was elucidated. Patients with autumnal fever (a disease in Japanese peasants and potters) and Fort Bragg fever (a febrile illness associated with pretibial eruptions described in army recruits) were shown to suffer from leptospirosis caused by *L. autumnalis*.[97,194] "Mud fever," "pea-pickers disease," and "European swamp fever," names that were used to describe a disease of undetermined etiology in eastern Germany, the Far East, and western Poland, respectively, were shown to be examples of leptospirosis caused by *L. grippotyphosa*.[48] Seven-day fever in Japan, Wycon fever and Bushy Creek fever in the United States, cane field fever in Australia, and swineherd disease in Europe were identified as examples of infection caused by *L. hebdomidis*, *L. canicola*, *L. pomona*, *L. australis*, and *L. pomona*, respectively.[24,30,36,59,112]

Leptospirosis is a disease now thought to be caused by a single family of organisms that has multiple serogroups and serotypes[4] and is characterized by a broad spectrum of clinical findings.[174] More than 300 strains, called serovars (serotypes), which are divided into serogroups on the basis of common antigens, have been identified. These are divided into two species: *L. interrogans* sensu lato, which contains the pathogenic strains and *L. biflexa* sensu lato, which contains the saprophytic strains.[109] New classification based on DNA relatedness suggests that the genus *Leptospira* can be classified into 20 species. Serovars of the same group may be distributed between different species.[50] Leptospirosis is now often described as the most widespread and prevalent zoonotic disease worldwide. The organism's ability to survive outside the host as well as establish a chronic carrier state in rodents promotes its survival and promulgation. Global warming and increased rainfall are predictors of increased incidence. In 2003, the World Health Organization (WHO) reported a worldwide incidence of 0.1 to 1 cases per 100,000 population per year in temperate climates, more than 10 cases per 100,000 population in humid subtropical region, and more than 100 cases per 100,000 population during outbreaks.[109] However, the difficulty in diagnosing and identifying chronic infection leads to underestimation.[3] Outbreaks in poor urban slums with rat infestations have contributed to its title as a neglected infection of poverty in the United States.[113] It is also considered an infection of adventure seekers with outdoor recreational exposure and international travel.[132] WHO has established the Leptospirosis Burden Epidemiology Reference Group, which is focused on better estimating the global burden of disease.[260]

EPIDEMIOLOGY

Animal Reservoirs

Among mammals, rodents are the most important reservoir of leptospires, but nearly all mammals may be infected and can transmit the disease.[233] Leptospires also

have been isolated in reptiles and birds, but the epidemiologic significance of these animals in terms of maintenance of the organism in nature or transmission of disease to humans is not clear. For many species, infectivity rates of 10 to 50 percent have been reported frequently.[112,184] During epizootics, the circulation of leptospires among many species of animals living within a given biocenosis has been well recognized.[67]

The failure of leptospires to elicit a significant systemic antibody response in certain animal species may be due to the development of local immunity within their kidney tubules.[19,227] Some animals fail to develop homologous antibody titers but harbor leptospires in their kidneys for extended periods.[227] Thus, lack of a positive titer to leptospires, as determined during the course of serologic surveys of animal populations, does not indicate absence of infection. For this reason, serologic surveys of animal populations cannot reflect the true incidence of leptospiral infection accurately.

A particular host species may serve as a reservoir for one or more serotypes of leptospires, and a particular serotype may be hosted by many different animal species. Turner[239,241] stressed that a particular animal species commonly serves as a reservoir for selected serotypes but temporarily may be infected and serve as an incidental host for other serotypes with which it usually is not infected. Although any animal susceptible to infection by leptospires may become a urinary "shedder" of the organisms temporarily, only selected animal species with "biologic sympathy" for a particular strain of leptospires can become a principal or maintenance host of the serotype.[19]

Transmission of Leptospires to Humans

Leptospires are transmitted to humans either by contact with blood, urine, tissues, or organs of infected animals or by exposure to an environment that has been contaminated by leptospires. Humans usually represent a dead end in the chain of infection because although person-to-person transmission is possible theoretically it is rare. After direct exposure of humans to infected animals, leptospires may enter breaks in the skin or may penetrate the mucous membranes, including the conjunctiva, nasopharynx, and vagina.[24,70,193,241] Human-to-human transmission via human milk has been reported in a breast-fed infant from a lactating mother who was infected with *L. interrogans.*[34]

Indirect transmission of leptospires to humans (from soil or water) depends on the presence of an environment that favors the survival of leptospires outside the animal host. A warm climate (25°C [77°F]), the presence of moisture, and pH values of soil or surface water between 6.2 and 8.0 are optimal conditions for survival of leptospires. These conditions prevail in many tropical regions throughout the year and in temperate climates during the late spring, summer, and autumn. Smith and Self[209] demonstrated survival of leptospires in cultures of infected soil for 43 days. In a survey of leptospirosis in Hawaii from 1999 to 2008, among 177 cases where exposure was determined, 45 percent were from recreational exposure (freshwater swimming, hiking, camping), 44 percent were occupational (taro farming), and 11 percent were from exposure around the home (gardening).[126] A study of human leptospirosis in the Caribbean from 1997 to 2005 found that in cases of suspected leptospirosis the highest seropositivity was found among 1- to 20-year-old and 31- to 40-year-old males and its occurrence was higher during the wet season.[5]

Ingestion or exposure from contaminated water sources, such as via infected rat or cattle urine, is a well-described source of leptospirosis in humans.[44,96,241] The importance of occupation as related to the risk for leptospirosis was emphasized in 1965.[112] Disease appeared to occur most frequently in people with occupations that required exposure to cattle or swine or to water contaminated by rat urine. The urine of infected cows may contain as many as 100 million leptospires per milliliter. If conditions are favorable, surface water contaminated by the urine of infected cattle may remain infectious for several weeks.[45] The largest reported outbreak of leptospirosis in the United States occurred in 1998 in Springfield, Illinois, after a triathlon that was preceded by heavy rains. There were 66 laboratory-confirmed cases of leptospirosis after the event, which included a swimming portion in a lake that received runoff from nearby livestock farms. Fifty-two of these cases occurred in triathlon participants, and 11 cases involved nonparticipants who came in contact with lake water near the event.[156] In a study performed in Detroit, 90 percent of rats carried *L. icterohemorrhagiae.*[73] Strain-specific tests comparing antibody titers in the sera of inner-city and suburban children were performed. Thirty-one percent of inner-city children had antibodies against *L. icterohaemorrhagiae;* 10 percent of suburban children also had antibodies to this organism. Lau and colleagues have studied the patterns of global climate change, flooding, and urbanization to predict that an increase in leptospirosis can be expected.[131]

Venereal transmission of leptospirosis is important in rodents and can occur in livestock. Leptospires have been recovered from the semen of bulls and have been transmitted by artificial insemination and by coitus. The possibility of seminal transmission in humans remains speculative. Transplacental infection of the fetus in utero is well documented in livestock and other animals and may occur in humans.*

During the past several decades, the number of cases acquired during outdoor recreation has increased. In developed countries, an increasing percentage of cases of leptospirosis are from travelers returning from areas of high endemicity and are often related to water activities.[132] In California, from 1982 to 2001, 59 percent of reported cases of leptospirosis were due to recreational exposure (often to contaminated freshwater), increasing to 85 percent in the 1997 to 2001 period.[147]

The dog, including the immunized pet dog, has been incriminated as an important vector and as a reservoir of leptospirosis.[100,226] Although immunization of dogs against leptospirosis is possible, an important note to remember is that (1) immunization may not prevent the dog from having renal carriage and excreting the organism, (2) canine immunity after immunization may wane

*References 47, 56, 60, 70, 241, 243.

after just 1 year, and (3) immunity, when established, is effective only for serotypes present in the canine vaccine.[19] In one survey of suburban and urban areas, however, between 15 and 40 percent of dogs were found to be infected.[29] Harkin and coworkers found that irrespective of health status, 8.2 percent of dogs were shedding pathogenic leptospires.[108] Rojas and colleagues found that 7.05 percent of dogs had urinary shedding.[187] Dogs were implicated in 58 percent of the 820 known cases of leptospirosis reported between 1962 and 1971.[49]

Asymptomatic renal colonization in humans has been shown. Using a 16S rDNA hybridization assay technique to identify *Leptospira* DNA in urine, Ganoza and associates identified a long-term renal shedder group among persons asymptomatically infected with pathogenic and intermediately pathogenic *Leptospira*. Almost 5 percent of healthy people living in a rural Amazonian community were urinary shedders of *Leptospira* but did not have serologic or clinical evidence of recent infection.[94]

PATHOPHYSIOLOGY

After almost 100 years since the discovery of the causative organism of leptospirosis, the understanding of mechanisms of pathogenesis has come a long way. Leptospires are thin and highly motile spirochetes with two periplasmic flagella responsible for their movement.[84] They have a double-membrane structure, making it unique and similar to both gram-positive and gram-negative bacteria. Describing the membrane from inside out, leptospires have an inner cytoplasmic membrane, a peptidoglycan cell well, periplasm, outer membrane with outer-membrane proteins (OMPs), phospholipids, and lipopolysaccharide (LPS).[65,66]

The era of genomics has provided new insights into potential virulence factors as well as host response to disease. Studying the unique characteristics of pathogenic versus saprophytic leptospires as well as comparing the host-pathogen interaction in acute, chronic, and carrier states has become the focus of many researchers. Valuable information has been obtained by the use of animal models of acute and chronic disease, by observing the interactions of leptospires with various cell lines, and by employing molecular genetic tools. After penetrating the skin or mucous membranes, leptospires invade the bloodstream and spread throughout the body to produce the protean manifestation of the disease.[82,83,216] Because of their inherent ability to revolve rapidly in a corkscrew fashion, it had been suggested in early studies that the organism bores through connective tissue.[216] Since then, the specific factors responsible for invasiveness of leptospires have been extensively studied and are better understood.[81,90,217] In vitro studies have shown leptospires bind to a wide range of extracellular matrix components. They have been shown to bind to several different cells, including endothelial cells, monocytes, macrophages, kidney epithelial cells, and fibroblasts.[40] They can translocate through polarized MDCK cells, suggesting a possible intracellular component to its life cycle.[26] Leptospiral immunoglobulin-like proteins (Lig), surface proteins found in pathogenic leptospires, are believed to be involved in cell adhesion[87] and are among those that have been studied as potential vaccine candidates.[155]

Specific factors responsible for the virulence of leptospires, although better understood, still remain unclear. Some of these virulence factors that continue to be a subject of investigation include LPS, hemolysins, outer-membrane proteins, surface proteins, and adhesion molecules.[81] The completion of the *Leptospira* genome sequence has revealed several proteins suspected to play a role in pathogenesis.[6,51,182] Comparative analysis of *Leptospira* proteomes looking at different serovars of pathogenic organisms, nonpathogenic organisms, clinical isolates, and acute lethal infection versus chronic carrier infection has elucidated potential virulence factors and vaccine candidates.[51,65,164,165,231] Hemolysin-like proteins have been identified, including sphingomyelinases that are present in pathogenic but not saprophytic leptospires. Surface-exposed proteins that might have potential roles in adhesion and pathogenesis include OmpL36, OmpL37, OmpL47, and OmpL54.[175] Outer surface proteins LigA, LigB, and LigC contain immunoglobulin-like domains[142] found in virulence factors such as intimin and invasin. Leptospiral endostatin-like proteins (Len) have been identified that have been shown to be involved in the organism's ability to evade the complement system.[217] LipL32 is an outer-membrane protein shown to bind collagens, laminin, and fibronectin and is highly conserved in pathogenic species.[106,111,238] Loa22 is a lipoprotein that is upregulated during acute infection and appears to be necessary for virulence in animal models of disease.[165,186] However, the specific roles and functions in pathogenesis for many of these molecules are not entirely understood. A significant amount of redundancy in functional virulence proteins is suspected.

The possible role played by animal hosts in determining the virulence of leptospires for humans remains speculative. Faine[82] compared the fate of virulent and nonvirulent strains of *L. icterohaemorrhagiae* in guinea pigs. Both strains behaved similarly after intraperitoneal infection, but virulent organisms survived and multiplied whereas avirulent strains did not. Both virulent and avirulent strains were taken up by fixed phagocytes in reticuloendothelial tissues in vivo. Faine[83] also showed that the severity of lesions correlated positively with the number of organisms present and that a discrete number of organisms were required to cause death. The logarithm of the dose for strains of a given virulence was related constantly to survival time after ingestion. This relationship was correlated with the growth rate in vivo. Faine[82] also suggested that the low proportion of virulent organisms within avirulent strains permits modification of the disease process by antibodies that have sufficient time to develop before the disease process becomes irreversible. He hypothesized that virulence results from the selective multiplication of virulent leptospires in vivo. This hypothesis was supported by the fact that maximum virulence can be regained after a single animal passage that follows isolation in culture. Virulence may be lost in culture by mutation to nonvirulent forms.

The humoral response is the main mechanism of resistance against leptospirosis. IgG and IgM antibodies are detected in patients who have recovered from infection.

Antibodies are produced against leptospiral LPS. LPS vaccines are effective, but immunity is limited to homologous serovars.[81] Wang and associates[253] demonstrated that polymorphonuclear leukocytes are not an efficient defense factor for pathogenic leptospires in nonimmune hosts. The virulence of leptospires appears to be related to their ability to resist killing both by serum and by neutrophils.

A toxic and pathogenic potential in vivo for lipid products of leptospiral metabolism has been suggested.[2] The cell wall of the leptospire is high in lipid content; component fatty acids vary among leptospiral strains. Lipids are used as a source of energy by leptospires.[2] Saprophytic leptospires invariably possess lipase activity, whereas pathogenic leptospires may be lipase positive or lipase negative.[2] Kasarov and Addamiano[125] investigated the lipolytic activity of leptospires on serum lipoproteins. On the basis of their ability to attack these lipoproteins, leptospires can be divided into three groups: (1) strains that degrade lecithin and sphingomyelin, (2) strains that degrade neither lecithin nor sphingomyelin, and (3) strains that degrade lecithin but not sphingomyelin. Virulent leptospires behaved as group 1 and 2 strains, whereas saprophytic leptospires behaved as a group 3 strain.

Hemolytic Anemia, Jaundice, and Hepatic Injury

The development of hemolytic anemia and jaundice in patients with leptospirosis has suggested a role for hemolysis in the pathogenesis of this disease. Alexander and associates[8] reported the presence of a heat-labile, oxygen-stable, nondialyzable hemolysin in the supernate of leptospiral cultures; subsequently, Russell[189] noted that this hemolysin could be inhibited by leptospiral antiserum. Hemolysis may persist during leptospirosis despite the development of serum antibodies, which suggests that circulating hemolysin is adsorbed by erythrocytes early in the course of leptospirosis and that the erythrocytes lyse subsequently[28,114,158] despite the presence of circulating antibody. Lee and colleagues[135] cloned a hemolysin of *L. interrogans* serovar Lai and demonstrated cell membrane pore-forming activity in vitro, in contrast to the sphingomyelinase and phospholipase activity demonstrated by other leptospiral hemolysins. The precise role of hemolysins in human disease remains unclear.

A prominent feature of experimental leptospirosis is a hemorrhagic diathesis that increases in severity before death.[13] Many investigators have attributed bleeding to depletion of serum prothrombin, to thrombocytopenia, or to both.[58,103,179] Rishavy and colleagues[185] sequenced an orthologue of vitamin K–dependent carboxylase from a pathogenic leptospiral strain and proposed that this acquired activity may result in depletion of host vitamin K. Prothrombin activity can be corrected in children and adults with leptospirosis by the administration of vitamin K; however, the overall severity of the hemorrhagic diathesis is unchanged.[13,95,220] Moreover, thrombocytopenia is not a consistent concomitant in patients who bleed during the course of leptospirosis. For these reasons, the hemorrhagic diathesis most likely reflects widespread damage to the capillary endothelium.[48,77,253] The precise mechanism of capillary injury is uncertain, but the damage has been suggested to be induced by toxin.[13] Generally, hemorrhage is restricted to the skin or mucosal surfaces, but rarely death may follow the occurrence of a massive gastrointestinal hemorrhage or bleeding into a vital organ.[103]

In humans, profound derangement in hepatic function has been demonstrated. Liver cell necrosis, however, occurs infrequently; thus, the activity of aspartate aminotransferase and alanine aminotransferase generally is elevated only slightly. The most striking clinical manifestation of hepatic dysfunction is jaundice. Laboratory evidence of hepatic involvement in human leptospirosis includes the following: impaired bromosulfophthalein excretion, positive cephalin flocculation reactions, reduced esterification of cholesterol, abnormal galactose tolerance test results, impaired production of the clotting factors dependent on vitamin K, decreased serum albumin, and increased serum globulins. These abnormalities have been noted in icteric and anicteric patients with leptospirosis.

Several theories attempt to explain the jaundice of leptospirosis. Early investigation suggested that hyperbilirubinemia was the result of hemolytic anemia.[27] Considerable evidence does not support the hemolytic theory; attempts to demonstrate elaboration of hemolysin by *L. icterohaemorrhagiae*, a serotype associated frequently with hyperbilirubinemia in humans, have failed repeatedly.[13] Variability in the presence of anemia, the poor temporal association between anemia (when present) and the development of icterus, the absence of hemoglobinuria and reticulocytosis, and generally normal fecal urobilinogen values provide evidence that hemolysis is not the cause of jaundice in many patients with leptospirosis.[179] On the other hand, a significant hemolytic process can be documented in selected patients.[230] Hemoglobinuria has been documented early in the course of leptospirosis, even before the development of jaundice.[11,119] Significant anemia is a feature only of icteric cases of leptospirosis. Most likely, hemolysis occurs in selected cases of severe leptospirosis in humans, and it may contribute to the development of jaundice in some cases.[77,230]

One must conclude that the hepatic manifestations of leptospirosis, including jaundice, most likely are the result of hepatocellular injury because hemolysis is not a consistent finding and neither intrahepatic nor extrahepatic biliary stasis has been observed morphologically or clinically.[13,179,252] Although hepatocellular injury occurs, hepatocellular destruction is not significant, as reflected by complete recovery without residual hepatic dysfunction, even in survivors of severe icteric leptospirosis. Histologic changes that have been observed consistently include disorganization of liver cell plates; variations in the shape and size of parenchymal cells; large numbers of binucleated, trinucleated, and multinucleated cells with bizarre nuclei; proliferation of Kupffer cells with erythrophagocytosis; and cholestasis associated with scant infiltrates of round cells in the periportal spaces.[14,32,77] These changes also have been observed in anicteric patients.[32] Electron microscopy has demonstrated alterations in cell membranes, alteration or destruction of mitochondria, and a predominance of smooth over rough

endoplasmic reticulum in hepatocytes because of altered protein turnover.[72]

Additional evidence of hepatocellular damage is provided by the histochemical demonstration of reduced activity of succinic, isocitric, glutamic, and lactate dehydrogenase concomitant with functional alteration,[15] findings suggesting that the fundamental hepatic lesion is subcellular and that critical cellular enzyme systems somehow are affected. Presumably, the hepatocellular damage is not caused by the direct action of leptospiral organisms because the most severe pathologic changes are noted at a time when leptospires are difficult to demonstrate in tissue section.[102] Moreover, leptospires rarely have been identified in sections of hepatic tissue. Elaboration of one or more toxins by leptospires or release of various products after lysis that may be injurious to hepatocytes is the most plausible explanation for hepatic injury at this time. The completion of the genomic sequence of a virulent *L. interrogans* revealed various genes possibly related to adhesion, invasion, and the hematologic changes seen in leptospirosis.[182]

Renal Injury

Renal failure is an important cause of death in patients with leptospirosis. In patients who have died during the first week of disease, renal changes included cloudy swelling or isolated tubular epithelial cell necrosis previously involving the distal convoluted tubule and the ascending loop of Henle, isolated foci of acute vasculitis, segmental thickening of the basement membrane, and isolated areas of mild interstitial edema with lymphocytic infiltrates. In patients who have died during the second week of the illness, numerous foci of tubular epithelial necrosis have been apparent. Interstitial edema and infiltrates of lymphocytes, monocytes, plasma cells, and neutrophils are more prominent. When patients die after day 12 of illness, the inflammatory infiltrate is widespread and involves the medulla, as well as the cortex. Foci of tubular necrosis and interstitial inflammation are large, irregular, and packed densely with plasma cells, monocytes, lymphocytes, and neutrophils. Cells lining the lumen of the renal tubules are distended and disorganized and contain hyaline, granular, epithelial, and even bile casts. The glomeruli show mesangial hyperplasia, focal fusion of the foot processes, moderate cloudy swelling of the epithelium in the Bowman capsule, and thickening of the basement membrane.* The changes seen in epithelial cells may be responsible for the protein leak observed clinically as proteinuria.[57] Leptospires have been demonstrated in the liver or renal tubules and less frequently in the interstices of the renal cortex.[14,77,130]

Hypokalemia may be noted in patients with leptospirosis and acute renal failure. Studies of Abdulkader and associates[1] suggest that these findings are a result of renal potassium wasting facilitated by increased secretion of aldosterone and cortisol.

Although some investigators[116] have emphasized that interstitial nephritis is the fundamental lesion of leptospirosis, renal failure primarily is the result of tubular damage. Interstitial nephritis occurs primarily in patients who have survived until inflammation has had an opportunity to develop. Interstitial nephritis frequently is absent in patients with fulminant disease.[14] Tubulointerstitial nephritis is the most common lesion associated with chronic infection and may lead to fibrosis and subsequent renal failure. Several hypotheses exist to explain how leptospires evade the renal immune system, including absence of complement, downregulation of antigens, delayed lymphocyte infiltration, and delayed colonization of renal tubules.[154] Monahan and colleagues studied the rat model of chronic infection to confirm that differential protein expression by *Leptospira* during chronic infection facilitates its persistence in the presence of a specific host antibody response.[153]

Hypoxia may contribute significantly to the pathogenesis of renal dysfunction in leptospirosis. The focal distribution of the lesions suggests a relationship to impaired renal blood flow. Even in relatively mild cases of leptospirosis in which glomerular function remains unaffected, tubular function as measured by the excretion of *p*-aminohippurate is reduced markedly.[62] In severe cases, the tubular maximum for *p*-aminohippurate becomes negligible and glomerular filtration drops precipitously. On the basis of observations of this type, researchers have concluded that impaired renal blood flow is the fundamental alteration in the nephropathy of leptospirosis. Histochemical and enzymatic studies also demonstrate hypoxic damage and suggest renal ischemia.[15] Diminution of renal perfusion in leptospirosis also is suggested by the clinical occurrence of hypovolemia, hypotension, and circulatory collapse.[32,80,92,133] The reversible oliguria frequently observed during the course of leptospirosis has been attributed to reduced renal blood flow resulting from hypotension, a deficit of extracellular fluid, or both.[77] During periods of oliguria, decreased glomerular filtration rates have been noted. Renal function recovers first by restitution of glomerular function; subsequently, and more slowly, renal tubular function improves.[13]

The host response to infection may play a crucial role in the hepatic and renal damage seen in leptospirosis. Toll-like receptors (TLR) have been implicated in this response. The LPS of typical gram-negative organisms activates TLR4, but leptospiral LPS is unique. By using in vitro studies, it has been shown that LPS from *Leptospira* uses TLR2 instead of TLR4 to stimulate tumor necrosis factor production in a human monocyte-like cell line.[259] By using animal models of disease, it has been shown that, in circulation, B cells are activated via TLR4 to produce IgM against leptospiral LPS. In the liver, infection leads to TLR2- and TLR4-dependent production of interferon-γ (IFN-γ). In kidneys, leptospires activate T cells and parenchymal cells through TLR2 and TLR4 to produce IFN-γ and inducible nitric oxide synthase (iNOS). B cells and T cells can also induce inflammation in the liver and kidneys via a TLR-independent pathway that can contribute to organ damage.[258] TLR2, TLR4, and TLR5 have been shown to play a role in the response to viable pathogenic leptospires in a human whole-blood model.[101] Blocking nitric oxide production in infected hamsters accelerated the mortality rate to 100 percent by 1 day and increased the rate from 20 percent

*References 14, 15, 62, 72, 116, 130, 207, 261.

to 60 percent at day 14 of infection. It led to increased bacterial burden in kidney tissue, aggravated tubulointerstitial nephritis, and reduced the specific humoral response against *Leptospira*.[177]

Pulmonary Involvement

Pulmonary lesions in leptospirosis generally are the result of hemorrhage rather than acute inflammation. In selected cases, acute inflammation is noted but usually reflects a secondary pyogenic infection. Localized or confluent hemorrhagic pneumonitis may be noted, and petechial and ecchymotic hemorrhages are seen throughout the lungs, pleura, and tracheobronchial tree.[204] Acute hemorrhagic lobar pneumonia and massive hemoptysis have been observed in fatal cases.[16,172,262] Silverstein[204] suggested that pulmonary capillary damage was the result of a toxin because leptospires had not been demonstrated in the lung, although Yang and Hsu[262] subsequently reported a fatal case of pulmonary hemorrhage in which leptospires were visualized directly in the lung parenchyma by silver stain techniques. Alveolar septal deposition of immunoglobulin and complement may play a role in the pathogenesis of pulmonary hemorrhage.[163,262]

Cardiovascular Findings

Hypovolemia or hypotension in patients with leptospirosis may reflect dehydration secondary to vomiting, increased insensible water loss, diminished intake of fluid, and, rarely, massive gastrointestinal or pulmonary hemorrhage.[17,77,88,172,262] A decrease in intravascular volume caused by a shift of fluid from the intravascular to the extracellular spaces as a result of severe endothelial injury also may occur.

Rarely during human leptospirosis, adrenal insufficiency occurs after hemorrhagic infarction of the adrenal glands.[135] The vascular collapse observed terminally in fatal cases may reflect, in part, adrenal insufficiency secondary to hemorrhage. However, it cannot be the cause of the reversible state of shock that is noted early during the course of leptospirosis.

Cardiac dysfunction also may lead to hypoperfusion in severe leptospirosis. Focal hemorrhagic myocarditis, acute coronary arteritis, pericarditis, aortitis, and cardiac arrhythmias have been documented. Rarely, sudden death results from congestive heart failure or arrhythmias.[14,71,202,212,225] Cardiac malfunction also may develop secondary to hypertension, hypovolemia, electrolyte imbalance, or uremia. Peripheral vascular collapse in leptospirosis most often occurs irrespective of any cardiac involvement, obvious dehydration, or massive hemorrhage. Regardless of the etiologic factor, shock is a common occurrence in the course of severe leptospirosis.

The epicardium, endocardium, and myocardium all may be involved during leptospirosis. Arean[13] described focal or diffuse epicardial hemorrhages with or without lymphocytic and monocytic infiltrates in 10 fatal cases. In four patients, mesothelial desquamation and fibrin formation in the pericardial cavity also were noted. Myocardial changes included focal or diffuse lesions characterized by interstitial edema with fragmented fibers and infiltrates of monocytes, lymphocytes, and plasma cells. Neutrophilic infiltrates also were observed in most necrotic foci. Aortic insufficiency caused by focal endocarditis involving the aortic valves likewise was noted.[14] Patchy interstitial edema, cellular infiltrates, necrosis, and focal hemorrhagic lesions have been detected in other patients.[102,230] In most cases, these findings are not mirrored by the clinical findings. Rarely, leptospires have been demonstrated in myocardium.[77]

Nervous System Findings

Central or peripheral nervous system involvement may be striking. Most investigators agree that signs of meningeal inflammation cannot be attributed to invasion of the meninges by leptospires. Leptospires frequently are isolated from cerebrospinal fluid (CSF) that otherwise is normal; thus, reaction to the presence of leptospires in the meninges appears to be minimal. The leptospires disappear rapidly after the onset of meningeal signs, usually during the second week of disease. Because meningeal reaction occurs only after the development of antibody, leptospiral meningitis has been suggested to be a reflection of an antigen-antibody reaction.[19,103] Meningitis as a result of hypersensitivity may explain the absence of pleocytosis in the early stages of meningeal involvement, the abrupt onset of meningitis at the end of the first week of leptospiral disease, and the good prognosis of patients with leptospirosis who have involvement of the central nervous system. Pathologic examination of the meninges may reveal nothing[14] or may show thickening of the meninges, a slight increase in the number of arachnoid cells, and a predominance of mononuclear cells in the exudate.[16]

Uncommon features of leptospirosis include encephalitis, myelitis, radiculitis, and peripheral neuritis. When present, they occur during the second week of illness.[77] These neurologic findings also may be the result of hypersensitivity reactions similar to those seen in other postinfectious encephalitis syndromes.[150,151] Koppisch and Bond,[130] however, demonstrated perivascular infiltration of blood vessels in the spinal cord, basal ganglia, hippocampus, and white matter of the cerebellum and in the subcortical areas of the cerebrum; these changes are not pathologic features of postinfectious viral encephalitis.[14,99] In certain cases, neurologic manifestations have been attributed to subarachnoid, peripapillary, and subdural hemorrhages.[43,45]

Ocular Involvement

The aqueous humor provides a protective environment for leptospires; despite the development of high antibody titers in serum, leptospires may remain viable in the anterior chamber of the eye for many months.[7] Persistence of leptospires in the aqueous humor may be responsible for the recurrent, chronic, or latent uveitis syndromes that have been seen in patients with leptospirosis. The acute ocular inflammatory response that occurs during the leptospiremic phase of the disease generally disappears without complications and with little or no opacification of the vitreous. Chronic ocular involvement

occurs less commonly but is more significant because anterior uveal inflammation and vitreous opacification may occur. The development of hypopyon during the course of leptospirosis may be followed by loss of vision. Verma and coworkers have identified two lens proteins and a retinal protein that react with antiserum directed against LruA and LruB, which are leptospiral proteins expressed in uveitic eyes.[247] These cross-reacting antibodies are believed to play a role in *Leptospira*-associated recurrent uveitis.

Musculoskeletal Findings

The myalgia reported so frequently in patients with leptospirosis most likely relates to the pathologic process that has been noted.[133] Biopsy specimens obtained early in the course of leptospirosis have vacuoles within the cytoplasm of the myofibril. Subsequent focal cytoplasmic changes include fragmentation and loss of cellular detail, which results in homogeneous or irregular acidophilic masses. The polymorphonuclear infiltrates that may be seen in affected areas are minimal, even in muscle fibers that are affected severely. Infiltration by sarcoblasts, with new myofibril formation, ultimately leads to healing without significant fibrosis.[33,119] Histologic changes in the muscles of patients with mild infection generally are minimal.[77] Pathologic evidence of myopathy resolves completely and promptly in most cases; pathologic changes usually are absent in the muscles of patients dying in the second week of disease.[230]

The selective involvement of certain muscle groups in some patients with leptospirosis is not explained. Any or all muscles may be affected; generalized myalgia occurs commonly. Myalgia is an early clinical feature concurrent with leptospirosis, and these clinical findings are correlated with the timing of the histologic changes in muscle. Generally, muscle pain subsides promptly as leptospiral agglutinin titers develop and the septicemic stage of leptospirosis ends. These observations are consistent with active invasion of skeletal muscle by leptospires rather than a toxin-related effect.[133,213] Antigens of leptospires have been demonstrated by fluorescent antibody techniques in patients infected with *L. icterohemorrhagiae*.[203] Bone involvement in leptospirosis is not a significant feature clinically or pathologically, and no explanation accounts for the apparent failure of leptospires to proliferate in bone.[118]

Except for focal hemorrhages, no characteristic lesions have been noted in the adrenal glands, lymph nodes, spleen, gastrointestinal tract, pancreas, ureter, or bladder. Interstitial edema with monocytic and lymphocytic infiltrates has been found in testicular tissue associated with impaired spermatogenesis.[99]

CLINICAL MANIFESTATIONS

Leptospirosis is an acute systemic infection characterized by extensive vasculitis. Serologic surveys in human populations indicate that a large number of subclinical infections also occur. Surveys of veterinarians and packinghouse and abattoir workers reveal positive leptospiral titers in 5 to 16 percent of people tested.[120,152,157,232] A low index of suspicion for this disorder by physicians, coupled with the diversity and nonspecificity of its manifestations, accounts for the significant number of cases that go unrecognized. In one series of 483 proven cases, only 17 percent were diagnosed initially as leptospirosis.[30]

The incubation period generally is 7 to 12 days, but a range of 2 to 20 days has been noted.[197,216,240] The incubation period does not vary significantly among serotypes and is not of prognostic significance. Variability in incubation period may be attributed to the dose of virulent organisms to which the host is exposed and to the portal of entry of the organism.[197,216,240]

The clinical course of leptospirosis varies, but generally it is predictable: both anicteric and icteric leptospirosis follow a biphasic course (Fig. 141-1).

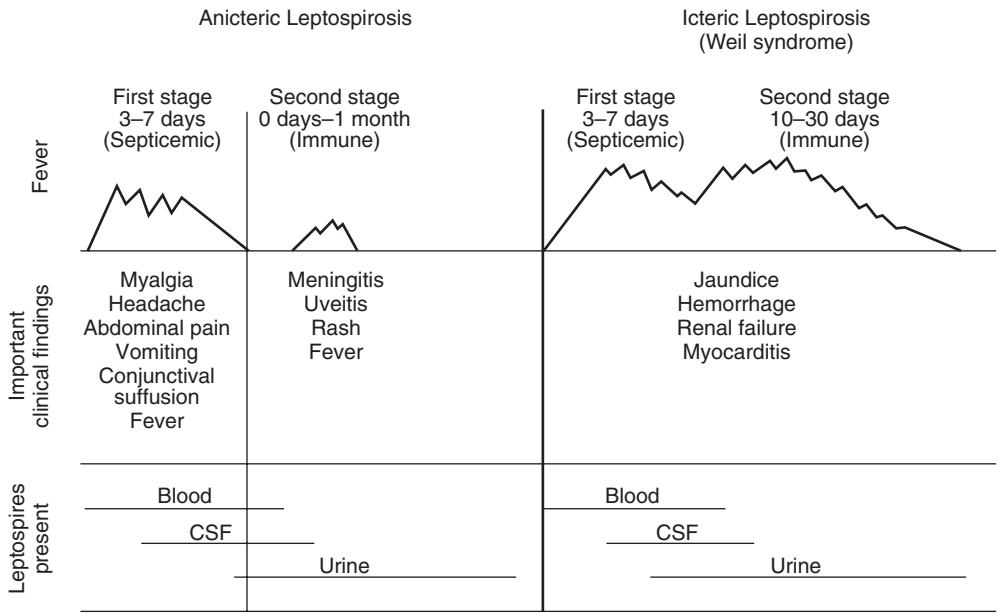

FIGURE 141-1 ■ Clinical course of leptospirosis: anicteric and icteric disease.

The first stage (septicemic phase) is characterized by acute systemic infection. The onset of symptoms is abrupt. This phase terminates after 4 to 7 days; symptomatic improvement and defervescence coincide with disappearance of leptospires from the blood, CSF, and all other tissues, with the exception of the aqueous humor and renal parenchyma. Antibody titers to leptospires develop rapidly; this immune response heralds the second, or "immune," stage of the illness, which lasts 4 to 30 days. Leptospiruria is prevalent and continues for 1 week to 1 month; generally, it is unaffected by antibiotic therapy. Meningitis or hepatic or renal manifestations, when present, reach their peak intensity during this stage of the disease.

There is also a more recently described severe pulmonary form of leptospirosis that may occur in the absence of jaundice or as an elevated Weil syndrome and carries a high mortality (see later).[140]

Leptospirosis manifestations in children are similar and can be very severe as well.* During the 2001 outbreak of leptospirosis in Mumbai, India, 32 percent of children admitted in a 2-month period had leptospirosis.[124] A year earlier in the same city, following floods, leptospirosis was found in 34 percent of children screened who had abrupt onset of fever, contact with flood water, conjunctival suffusion, abdominal pain, or rash.[123]

Anicteric Leptospirosis

Ninety percent or more of all patients with leptospirosis are anicteric. They frequently escape definitive diagnosis because jaundice and azotemia are absent. The onset of the septicemic phase of anicteric leptospirosis is abrupt[78] and heralded by fever, malaise, headache, myalgia, and occasionally prostration and circulatory collapse.[144] Chills, remittent fever, headaches, severe myalgia, and abdominal pain are prominent for 4 to 7 days. Fever defervesces by lysis, and other symptoms resolve. Death is an extraordinarily rare occurrence in the first stage of anicteric illness. Some patients with anicteric leptospirosis do not experience a biphasic illness and remain asymptomatic after the first week.[78]

The second phase of anicteric disease may be characterized by fever, uveitis, rash, headache, and meningitis. If present, fever usually is of brief duration and has a lower peak than that of the septicemic phase.[77,78,112] Maximum temperatures range from 38.2°C to 40.6°C (100.8°F to 105°F), with one or more daily peaks. Recurrence of fever 2 or 3 weeks after leptospirosis resolves is not unusual, but no reports of the isolation of leptospires from blood document relapse on these occasions. Relapse generally occurs when the immune response of the host is peaking and at a time of maximal leptospiruria, which suggests an allergic or immune basis for the febrile episodes.[32] Headache may be intense and usually is not controlled well by analgesics. Typically, it is frontal in distribution and characterized as bitemporal or occipital. It may be associated with retrobulbar pain.[61,77,78] Persistence or recurrence of headache after termination of the septicemic phase of disease generally indicates the onset of meningitis. The factors responsible for headache in the septicemic phase of leptospirosis are unknown.

Restlessness, nocturnal confusion, mood disturbances, and mild alterations in consciousness usually occur briefly and commonly in both stages of leptospirosis.[77,112] Delirium, hallucinations, psychotic behavior, and suicidal tendencies have been reported.[77,112,201]

Anorexia, nausea, vomiting, and abdominal pain may be reported in both stages of anicteric disease. Constipation, diarrhea, and gastrointestinal hemorrhage also have been documented.[77,144,166] Generally, hemorrhagic complications are associated exclusively with icteric disease.

Physical examination during the septicemic stage may reveal dehydration, muscle tenderness, conjunctival suffusion, generalized lymphadenopathy, hepatosplenomegaly, and rashes that may be macular, maculopapular, erythematous, urticarial, petechial, purpuric, hemorrhagic, or desquamating. Skin lesions are most prominent over the trunk, but any area of the body may be affected. Pretibial eruptions have been noted in patients with infection caused by *L. autumnalis*, but other serotypes also may cause disease with pretibial eruptions. Recurrent, transient urticarial eruptions have appeared for many days after resolution of the other manifestations of leptospirosis. Pharyngitis, rales, arthritis, and nonpitting edema occur less commonly.* Tachycardia is a common occurrence, and cardiac arrhythmias are noted occasionally.[171,212] Hypotension rarely occurs in anicteric leptospirosis.[78]

Muscle pain and tenderness may be generalized, but the muscles of the calf, lumbosacral spine, and abdomen are affected most frequently. Tenderness and rigidity of the abdominal wall may suggest the possibility of an acute surgical abdomen. Tenderness of the muscles adjacent to the cervical spine often causes nuchal rigidity in patients without meningeal involvement. The muscle tenderness usually subsides with termination of the septicemic stage of the disease.

Conjunctival suffusion, photophobia, ocular pain, and conjunctival hemorrhage are more specifically helpful diagnostic signs. Chemosis and inflammatory exudates generally are absent despite marked conjunctival infection. In anicteric disease, conjunctival infection involves primarily the bulbar conjunctiva only. It appears by the third day of illness and disappears 3 days to 3 weeks later.

Abdominal pain and tenderness, when associated with vomiting and hypoactive bowel sounds, clearly suggest the possibility of a surgical abdomen and present a challenging diagnostic problem because acute intra-abdominal catastrophes may complicate the natural history of this disease. Nonobstructive toxic dilation of the gallbladder requiring cholecystotomy has been noted repeatedly in children with leptospirosis (Fig. 141-2). Pain of this type must be differentiated from myositis, subperitoneal or subserosal hemorrhage, abdominal wall causalgia, or pancreatitis, all of which may occur in some children with anicteric or icteric disease.

Pulmonary involvement may be observed in anicteric patients, generally during the septicemic phase, and usually is manifested as a dry, hacking cough, occasionally

*References 55, 123, 124, 237, 247-249.

*References 9, 14, 31, 61, 78, 112, 119, 144, 190, 235, 240.

FIGURE 141-2 ■ Radiograph demonstrating a dilated, opaque gallbladder protruding from the inferior margin of the liver. Nonobstructed toxic dilation of the gallbladder is present in a child with leptospirosis.

productive of blood-stained sputum, or by the finding of infiltrates on a chest radiograph.[31,176,204] Hemoptysis, chest pain, respiratory distress, and cyanosis appear rarely during anicteric disease.[176] Hemoptysis, when present, clears in 3 to 5 days. Physical examination of the chest may reveal rales, evidence of consolidation, or a pleural or pericardial friction rub.

Chest radiographs may show (1) confluent infiltrates or massive consolidation representing larger areas of pulmonary hemorrhage; (2) small, patchy, snowflake-like lesions in the periphery of the lung fields that are restricted to a few intercostal spaces or disseminated widely; and (3) solitary, patchy lesions with ill-defined margins.[176,254] Of these radiographic appearances, the second is most common. Small pleural effusions are rare occurrences in anicteric disease,[254] and hilar adenopathy has not been described. Although the chest radiograph may help delineate the extent of pulmonary disease, it does not provide information that could be considered pathognomonic of leptospirosis.

Other signs and symptoms of the septicemic phase of anicteric leptospirosis that have been reported include parotitis,[27] orchitis,[221] epididymitis,[112] prostatitis,[112] otitis media,[31] arthralgia,[31,77,112] and monoarticular or polyarticular arthritis.[72]

The hallmark of the immune phase of anicteric leptospirosis is meningitis, and it is reflected by CSF pleocytosis with or without meningeal symptoms or signs. During the leptospiremic phase, leptospires may be found in the subarachnoid space unassociated with the presence of inflammatory cells. As an antibody titer develops, leptospires are cleared rapidly from CSF, and an inflammatory response develops.[77] If CSF is examined during the second week of illness in all patients with anicteric leptospirosis, a meningeal reaction can be demonstrated in more than 80 percent, but only 50 percent of these patients have clinical signs and symptoms of meningitis.[46,77] The severity of meningitis varies and does not correlate with the severity of other clinical manifestations of leptospirosis. Symptoms referable to the nervous system usually subside within 1 or 2 days but rarely persist for 2 or 3 weeks. The CSF pleocytosis may persist for 2 to 3 months but generally disappears within 7 to 21 days.[77] In some cases, patients are asymptomatic during the septicemic phase of leptospirosis but seek medical attention during the immune phase because of headache, vomiting, and nuchal rigidity. Papilledema rarely has been observed in patients with leptospirosis.[43]

Lumbar puncture may reveal CSF pressures varying from normal to 350 mm H_2O. Mean values generally are less than 200 mm H_2O.[115] Cell counts within CSF vary from normal to more than 500 cells/mm^3; generally, fewer than 500 cells have been reported.[77,112] Polymorphonuclear leukocytes predominate early in the immune phase, but mononuclear cells subsequently predominate. Protein concentrations within CSF range from normal to 300 mg/dL. In some cases, protein values have been elevated in the absence of pleocytosis. Abnormal values may persist for several weeks after the clinical symptoms resolve.[31,46,112,145,146] Glucose concentrations within CSF generally are normal.[46,78,145]

Encephalitis, focal weakness, spasticity, paralysis, nystagmus, peripheral neuritis, cranial nerve paralysis, seizures, radiculitis, visual disturbances, myelitis, Guillain-Barré syndrome, or acute disseminated encephalomyelitis may appear with or subsequent to the immune

stage of anicteric disease.* Generally, these symptoms resolve, but complete resolution may require several weeks to months. Neurologic sequelae secondary to central nervous system hemolysis may occur.[43,45]

The anterior uveal tract may be affected as early as the third week of illness, but symptoms may be found up to 1 year after the onset of leptospirosis. Conjunctival suffusion (characteristic during the septicemic phase) is not found in the immune stage of the disease. Rather, iritis, iridocyclitis, and, occasionally, chorioretinitis are noted.[30,77,78,112] Uveal involvement may be unilateral or bilateral and may occur as a single, self-limited episode, as recurrent episodes, or as a chronic unrelenting process.[38,77] The severity of the uveitis does not correlate with the severity of other clinical manifestations. When uveitis is transient or self-limited, complete healing is the rule; however, in some cases, blindness and cataract formation are noted.

The precise incidence of involvement of the uveal tract is unclear because symptoms may be minimal or may not appear until after other clinical manifestations have resolved completely. The generally benign course of uveitis may be attributable to the capacity of leptospires to survive in the aqueous humor without eliciting an intense inflammatory response.[78] Despite the presence of high titers of specific antibodies to leptospires in serum, antibodies to leptospires are absent or found in low titer in the aqueous humor.

Leptospiruria is the rule during the immune stage of anicteric leptospirosis, and it is not associated with impaired renal function. In contrast to many animal species, humans do not serve as a reservoir for leptospires; leptospiruria is transient. In anicteric patients, proteinuria, pyuria, microscopic hematuria, and mild to moderate azotemia may be observed.[31]

The white blood cell count may be low, normal, or elevated. Neutrophilia is the rule, regardless of the total white blood cell count. Leukocytosis generally is associated with hepatic involvement. Anemia is an inconsistent finding; when present, it may be attributable to blood loss, vascular damage, or hemolysis. In the absence of blood loss, significant anemia is not a manifestation of anicteric cases. The erythrocyte sedimentation rate is elevated consistently.

Icteric Leptospirosis (Weil Syndrome)

The term *Weil syndrome* should be applied to define a form of leptospirosis that is distinctive in clinical expression but nonspecific with respect to serotypic etiologic agents. In addition to having the symptoms and signs of anicteric leptospirosis, Weil syndrome is set apart by the presence of impaired hepatic and renal function, vascular collapse, hemorrhage, severe alterations in consciousness, and a high mortality rate.

Weil syndrome may be heterogeneous in its manifestations, and the course may be dominated by symptoms of renal, hepatic, or vascular dysfunction. Jaundice and azotemia may be so severe that the biphasic course of illness is not observed. Fever may persist without defervescence between the septicemic and immune stages and is more prominent and of longer duration during the immune stage than in anicteric cases. The mortality rate, despite adequate supportive care, is between 5 and 10 percent.

Jaundice remains the hallmark of Weil syndrome. The intensity of jaundice varies; a maximum total serum bilirubin concentration in the range of 60 to 80 mg/dL has been reported.[145] Usually, the bilirubin concentration is less than 20 mg/dL. Both direct- and indirect-reacting bilirubin levels increase, but an increase in the direct fraction usually accounts for most of the elevation in bilirubin.[218] Jaundice may occur as early as the third day of illness or may not appear until the second week.[16,78] The serum bilirubin concentration peaks within the first 7 days after the onset of jaundice in 85 percent of cases.[179]

Modest elevations in serum alkaline phosphatase level and depressed activity of plasma prothrombin are noted occasionally.[179] Hypoprothrombinemia responds uniformly to parenteral administration of vitamin K. The serum albumin level may be depressed; concentrations of 2.0 to 2.5 g are not uncommon.[218] Aspartate aminotransferase and alanine aminotransferase values are elevated minimally, rarely exceeding 100 and 200 units, respectively. The urine may contain bilirubin and urobilinogen.

Hepatomegaly is found in approximately 24 percent of patients, a frequency that is no greater than that in anicteric cases.[77] Transient biliary obstruction, probably intrahepatic, may occur, but no evidence that obstructive phenomena are the primary mechanism of impaired hepatic function exists. Even in severely icteric cases, acholic stools generally are not observed.[77,112] Pruritus has been reported rarely in patients with leptospirosis.[77] The presence of abnormal urinary urobilinogen values in the absence of acholic stools suggests patency of the biliary tract in most cases.[179]

In some reports of children with leptospirosis, acalculous cholecystitis has been seen in 55 percent of cases.[219] In these patients, right upper quadrant pain, tenderness, and a palpable mass were present. Abdominal radiographs confirmed the presence of a mass in the region of the gallbladder. When cholecystotomy was performed, a massively distended gallbladder containing colorless bile was noted. Routine aerobic and anaerobic cultures of bile were negative, but cultures for leptospires were positive.

Hepatic dysfunction is not an important cause of death in patients with leptospirosis. It is present, however, in most patients who die of this disease; and, conversely, a fatal outcome is extremely rare in the absence of hepatic dysfunction.[31] Renal dysfunction, cardiovascular collapse, and hemorrhagic complications occur most often in patients whose icterus is most prominent.

Renal dysfunction may be observed in all forms of leptospirosis, regardless of the severity of disease or the serotype causing infection.[31,77,112] Symptoms attributable to functional renal impairment generally are observed only during icteric leptospirosis.[16,31,77,78,240] In the leptospiremic phase, abnormal urinalysis results are noted in as many as 80 percent of cases.[31,77,219] Proteinuria is the most frequent abnormality and generally is mild. Hyaline

*References 10, 23, 52, 77, 80, 112, 145, 150, 246.

or granular casts and cellular elements (red and white blood cells) may be found in the urinary sediment. Microscopic or gross hematuria is seen in many patients and most likely reflects the presence of a hemorrhagic diathesis rather than glomerular injury.[246]

Fatal cases of icteric leptospirosis in which urinalysis results were normal have been reported.[16] The abnormalities in urinary sediment and proteinuria may persist for weeks in patients without significant azotemia.[25]

Oliguria or anuria may be noted as early as the third day of illness but occurs more commonly after the first week. Generally, blood urea nitrogen values remain below 100 mg/dL, but values may exceed 300 mg/dL in some cases.[112,167] The height of the blood urea nitrogen value is not of prognostic value in individual cases, although in groups of patients it correlates well with outcome.[230]

Azotemic patients with leptospirosis can be divided into two groups: (1) those with decreased renal perfusion (ratio of urine osmolality [U_{Osm}] to plasma osmolality [P_{Osm}] of about 2 : 1) and a good response to fluid administration and (2) those with a U_{Osm}-to-P_{Osm} ratio close to 1 : 1, with impaired resorption of sodium and water from the renal tubules and no response to administration of fluids. The manifestations of the second group of patients are those of acute tubular necrosis. The factors responsible for oliguria in the first group of patients (those with prerenal azotemia), including hypotension, shock, and volume depletion, if uncorrected, ultimately may progress to acute tubular necrosis as well.

Anuria is an ominous sign, whereas diuresis is a good prognostic omen.[16] The impairment in renal function may persist, and fatalities have been recorded after the onset of diuresis.[167] Hyposthenuria can persist for months in some cases.[78] Some evidence of renal disease has been demonstrated by renal function tests and renal biopsies for as long as 6 months after the onset of leptospirosis.[21] Renal failure is the principal cause of death in patients with leptospirosis, but it generally is reversible in time. Usually, renal function improves by 6 months after acute infection.[68]

Cardiac involvement is a relatively infrequent occurrence, but when it is present congestive heart failure and cardiovascular collapse may occur.[78,119] Electrocardiographic changes are seen in all forms of leptospirosis.[171] In one series of patients, electrocardiograms obtained during the first week of illness were abnormal in 90 percent of patients at a time when none had signs or symptoms of congestive failure, pericarditis, or hypotension.[171] The electrocardiographic abnormalities disappeared by 10 days in most cases. The electrocardiographic changes that have been described often are nonspecific findings common to many infectious diseases or attributable to fever alone.[171,191]

Cerebrovascular accidents may occur in patients with leptospirosis.[136] In a study of 21 cases in which postmortem examination was performed, subarachnoid hemorrhage was described in 1 case, cerebral hemorrhage in 2, and recent cerebral infarction in 1.

Hyponatremia is a rather consistent finding in patients with severe icteric leptospirosis. The hyponatremia appears to be the result of (1) failure of the sodium pump, which causes sodium to move intracellularly in exchange for potassium, and (2) redistribution of fluid such that the extracellular fluid space is expanded at the expense of the intracellular space. Hyponatremia in these patients may be unresponsive to either sodium replacement or fluid restriction. It is treated best by fluid restriction, which can be continued unless systemic blood pressure falls. Clinical improvement in the patient generally follows a spontaneous increase in serum sodium level, which may occur before any other evidence of clinical improvement is noted.

The majority of patients with leptospirosis recover without long-term sequelae.[196] However, given the evidence of prolonged urinary shedding, it has been speculated that there may be a chronic form of leptospirosis.[113] A prospective study in São Paulo, Brazil, followed patients who had been hospitalized with leptospirosis[214]: 2 of 47 patients reported continuing symptoms, 1 with profound malaise without a defined reason and 1 with new diagnosis of panic disorder.

Severe Pulmonary Form of Leptospirosis

A recently described severe pulmonary form of leptospirosis may occur in the absence of jaundice or as an elevated Weil syndrome. It carries a mortality greater than 50 percent[140] and manifests as dyspnea, chest pain, and cough with hemoptysis.

The chest radiograph shows small nodular opacities that may progress into larger coalescent areas of consolidation.[74] High-resolution computed tomography shows bilateral ground-glass opacities, areas of consolidation, airspace nodules, and small pleural effusions. On gross pathology, extensive pulmonary hemorrhage with numerous bleeding foci of different sizes can be seen. Histologic sections usually demonstrate pulmonary congestion, pulmonary edema, and several foci of interstitial and intra-alveolar bleeding, with varying degrees of severity.[140] Treatment involves maintaining hemodynamic stability and respiratory support. Corticosteroids and platelet transfusions have been used.[173] Because the pathogenesis of SPFL is believed to likely be immune mediated, immunosuppression has been used in its treatment, including plasma exchange, cyclophosphamide, desmopressin, dexamethasone, and recombinant factor.[140]

INDICATORS OF PROGNOSIS

Attempts have been made to identify prognostic factors associated with leptospirosis. Such studies, which aimed to evaluate the prognostic significance of certain clinical and laboratory variables at different stages of disease, have produced variable results. Hypotension, oliguria, hyperkalemia, the development of pulmonary rales, or rhonchi, and electrocardiographic repolarization abnormalities each have been associated with a poor prognosis,[69,76,139,169,229] although conflicting data exist about the implications of hypotension.[76] In a small series of 12 patients, Truccolo and colleagues[236] reported death of all patients with a leptospiral load greater than 10^4 organisms/

mL as determined by quantitative polymerase chain reaction (PCR). Chang and colleagues,[53] in a series of 11 patients with late leptospirosis, reported mortality in all patients with hepatitis and a disproportionate aspartate aminotransferase level, as defined by a ratio of peak aspartate aminotransferase to peak alanine aminotransferase greater than 3. Respiratory failure, hemoptysis, metabolic acidosis, and thrombocytopenia also have been associated with increased mortality rates.[229] In Marotto and coworkers' prediction model, serum potassium, serum creatinine, respiratory rate, presenting shock, and Glasgow Coma Scale score less than 15 were independently associated with development of leptospirosis-associated pulmonary hemorrhage syndrome.[141] Advancing age, renal involvement, pulmonary involvement, and multiple organ dysfunction have been repeatedly found to be associated with increased mortality.[178]

LABORATORY DIAGNOSIS

Whenever possible, the physician should use laboratory facilities in which culture and serologic tests for leptospirosis are performed routinely. It is recommended that specimens be sent to the standard reference laboratory, the National Leptospirosis Laboratory at the CDC in Atlanta. Despite proper collection and handling of specimens, obtaining laboratory confirmation of cases of leptospirosis may be difficult, even for facilities with skill in this area.

A confirmed case of leptospirosis, as defined by the U.S. Department of Health and Human Services, fulfills one of the following criteria: (1) clinical specimens that are culture positive for leptospires or (2) clinical symptoms compatible with leptospirosis and either seroconversion or a fourfold or greater rise in the microscopic agglutination titer between acute and convalescent serum specimens obtained 2 or more weeks apart and studied at the same laboratory. PCR has been shown to be effective for detecting pathogenic *Leptospira* but may not be commercially available.

Presumptive leptospirosis is defined as the presence of clinical symptoms that are compatible with leptospirosis and a microscopic agglutination titer of 1:100 or greater, a positive microscopic agglutination slide test reaction on a single serum specimen obtained after the onset of symptoms, or a stable microscopic agglutination titer of 1:100 or greater in two or more serum specimens obtained after the onset of symptoms.

Identification by Culture

Leptospires can be recovered from blood or CSF obtained from patients during the septicemic stage of illness or from urine during the immune stage. Other than these body fluids, only tissue sections obtained by biopsy or at necropsy are sources from which organisms can be recovered. Rarely, organisms are isolated from intraocular fluid obtained during convalescence.[7]

Media for the cultivation of leptospires generally contain a buffered solution, with or without peptone and with or without 0.1 to 0.2 percent agar to which rabbit serum has been added to provide a final concentration in the medium of 5 to 10 percent. In addition, a pH between 7.2 and 7.8 appears to be essential. Clinical material obtained for culture frequently is contaminated; antimicrobial agents, including neomycin, vancomycin, or bacitracin, added to leptospiral media in low concentration have been found to be effective in reducing contamination and exert little if any effect on leptospires.

For routine use, Fletcher semisolid medium[89] or EMJH semisolid medium[121,216] is recommended. Stuart medium[222] has been used to prepare and maintain antigens for serologic tests. Tween 80–albumin medium (OAC) was developed not long ago and is available commercially. This medium seems to be superior for primary isolation of leptospires.

Several solid media are available but appear to be most useful for isolation and purification of leptospires from contaminated natural material such as water.[223,243] Preparation, use, and maintenance of these solid media and other media are described in other works.[148,223,243]

Multiple cultures should be obtained from patients with leptospirosis because the concentration of organisms in blood at any point in time is low.[121] Freshly drawn blood is most desirable, but leptospires may remain viable in anticoagulated blood for as long as 11 days.[222] Blood should be inoculated into several tubes of semisolid media. The number of drops of blood placed into each tube should be varied (one to four drops). Excessive amounts of blood inhibit the growth of leptospires; hence, a small inoculum yields the best results.[89] Cultures are incubated at 28°C to 30°C in the dark for 6 weeks or longer.

In semisolid media, leptospires grow in a concentrated ring about 0.5 to 1 cm below the surface. Growth may not be detected in Fletcher semisolid medium for several weeks but may occur earlier in polysorbate medium.

Contaminated specimens or suspensions of primary cultures in which contamination is suspected may be inoculated into hamsters. When an animal dies, phlebotomy or necropsy is performed and sections of liver, kidney, and brain then are recultured in appropriate semisolid media.

If collected during the septicemic phase, CSF may be cultured in the same manner as blood.

Urine serves as the main source from which leptospires can be isolated during the immune and convalescent phases of leptospirosis. Prolonged urinary shedding after infection is possible in humans. Chow and coworkers reported a 10-year-old girl who shed leptospires in urine for 6 weeks after exposure.[55] Clean-voided urine may be inoculated directly into an appropriate semisolid medium. Urine specimens must be diluted with sterile, buffered saline solution to ensure growth.[230] Best results are obtained by adding 0.1 mL of urine to 0.9 mL of buffered saline before inoculation into 5 mL of semisolid medium. This procedure can be continued with four additional dilutions. Other bacterial contaminants that may be present in undiluted urine cultures generally do not survive in these cultures after dilution.[223]

Identification by Means Other Than Culture

The morphologic appearance of all members of the genus *Leptospira* is similar. They are slender, threadlike organisms about 0.1 μm in diameter and 6 to 12 μm in length, tightly coiled on their long axis. Like other spirochetes, they cannot be seen in wet preparations by lightfield microscopy but on darkfield examination they may be observed readily. For the detection of one leptospire per high-power field by darkfield examination, a concentration of 10,000 to 20,000 leptospires per milliliter of fluid is needed.[243] At best, darkfield examination should be considered an aid that may suggest but not establish a diagnosis of leptospirosis.

Leptospires can be stained by several silver impregnation techniques.[41,128,148,223,243] The modified method of Van Orden has been used at the CDC for demonstrating organisms in sections of liver, kidney, or other tissues. Infecting serotypes cannot be differentiated by silver impregnation techniques. Leptospiral antigen also has been detected with the use of an immunoperoxidase staining procedure.[86]

Fluorescent antibody techniques may be applied successfully to the detection of leptospires in urine or tissue.[159,160,223,243] This test is based on specific antigen-antibody reactions using fluorescence-tagged antisera. In theory, the fluorescent antibody reaction should demonstrate distorted and fragmented, as well as whole, organisms, but caution is required. Control specimens that have been treated with unlabeled antiserum before the addition of fluorescein-labeled antiserum should be used.[148] The control specimen should not fluoresce. The fluorescent antibody technique may provide the physician with useful information in the course of the disease in some patients. Positive results, however, are considered only presumptive evidence of infection.

In addition to this technique, DNA hybridization techniques or nucleic acid amplification procedures, including PCR protocols using leptospiral-specific cDNA probes or oligonucleotide primers, can be used to detect the presence of leptospires in body fluids or culture supernatants.[35] These techniques are being developed in the laboratories of the Leptospirosis Branch at the CDC, but proof of their superiority in terms of sensitivity or specificity in detecting leptospiral organisms in body fluids or other clinical samples has not been established.

The reference method for serovar identification is considered to be the cross-agglutinin absorption test (CAAT) but requires maintenance of large panels of reference antisera and live antigens. Multilocus sequence typing has been applied to provide strain information but is limited to a few species.[162] Pulse-field gel electrophoresis can be used to identify *Leptospira* to the serovar level, is applicable to all pathogenic species, and can rapidly identify new serovars.[91]

Serologic Tests

Evaluation of serologic findings to supplement clinical and epidemiologic information generally is recommended as a first step in establishing a diagnosis of leptospirosis. One of the most widely used specific serologic tests for leptospirosis has been the microscopic agglutination test (MAT), in which live antigen is used. This test is time consuming and potentially hazardous to the technician but is considered the reference test against which all other tests are evaluated. Formalinized antigens can be used for the MAT, and they are preferred in some laboratories, but the titers obtained are lower than those obtained with live antigen, and more cross reactions with heterologous serotypes occur. Generally, serum is used for the MAT or other agglutination tests, but CSF, urine, bile, or aqueous humor may be used as well.[148]

The CDC uses a standard panel of commonly occurring serovars representing 20 serogroups for routine performance of the MAT. However, because of geographic differences in serovar distribution, serovars are added or changed depending on the region where exposure occurred and the location from which specimens were obtained. Killed antigens remain stable for at least 12 months and are available commercially either individually or in pools. Sulzer and associates[223] have provided detailed descriptions of methods for performance of the MAT. Modifications of the MAT have been developed and include the semi-micromethod and microtiter techniques.[93]

A newer serologic test involving diffusion in gel, the enzyme-linked immunosorbent assay (ELISA), has been compared with the MAT for the serologic diagnosis of leptospirosis.[67,251] The results suggest that this test is a viable alternative to the MAT because of its sensitivity, potential for standardization, and simplicity. Variations of this test have been developed; rapid serodiagnosis of leptospirosis with the use of an IgM-specific dot ELISA has proved to be as sensitive and specific as MATs.[250,255] The dot ELISAs are inexpensive and simple to perform, and they use minute volumes of leptospiral antigens.

The IgM-PK ELISA, an assay for IgM using a proteinase K–treated antigen, was compared with the Leptoteste-S macroagglutination test and with the MAT for the diagnosis of leptospirosis. All three tests were comparable in their ability to detect the presence of leptospirosis. Both the IgM-PK ELISA and the Leptoteste-S differed statistically from the MAT in terms of the positivity of acute-phase sera. Thirty-eight percent of patients with leptospirosis were identified earlier with either test than when the MAT was used. The IgM-PK ELISA, which had a sensitivity of 89.9 percent and a specificity of 97.4 percent, has been suggested as the test of choice for laboratories that are equipped to perform ELISA.[183]

A slide agglutination test available commercially has been compared with the ELISA IgM and has yielded equivalent results for the diagnosis of leptospirosis. The slide agglutination test is inexpensive and can be performed more quickly and more easily than ELISA, thus rendering it a useful test for laboratories that are less well equipped than those in which IgM ELISA currently is performed.[39]

The Lepto Dipstick, a dipstick assay for detection of *Leptospira*-specific IgM antibodies in serum, has been studied as a method for the diagnosis of leptospirosis in situations in which laboratory facilities may not be available.[210] This test's results correlated well (80% to 96.7%

observed agreement, κ value of 0.62 to 0.92) with results obtained by the IgM ELISA leptospiral antigen detection method.[22,105] A 93.2 percent agreement (κ value of 0.66) was reported between the Lepto Dipstick and MAT, along with a high number of false-positive results as well.[22] The dipstick test is a valuable and useful tool for rapid screening for leptospirosis and may be useful in the field for detecting and monitoring outbreaks of leptospirosis.

Other tests that may be used for the serologic diagnosis of leptospirosis include a complement-fixation assay,[54] a hemolytic test,[54] an indirect immunofluorescent test,[234] an erythrocyte-sensitizing substance test,[54] countercurrent immunoelectrophoresis,[161] and flow cytometry light scatter analysis.[263] These tests are genus specific and may yield positive results earlier in the course of leptospirosis than the agglutination tests do. Their results also revert to negative earlier; therefore, these tests are of little value for serologic surveys. They may be of value in distinguishing current from past infections when agglutination test results are equivocal.[222,223] Specific details concerning the use of these techniques are presented elsewhere.[54,64,181,234,243]

An indirect hemagglutination test offers the advantage of detecting antibodies as early as the fourth day after the onset of illness. It is genus specific, is less time consuming, and requires just one antigen in the test system. It has excellent sensitivity and specificity, and some investigators have suggested that it may replace the MAT as the screening test of choice.[223] Effler and colleagues,[79] however, reported discouraging results when this test was used for the diagnosis of leptospirosis in Hawaii.

Agglutination tests have been considered to be serotype specific. Because of the antigenic complexity of leptospires, however, cross-agglutination reactions occur; serotypes that belong to the same serogroup cross react at high titers. Early in the course of leptospirosis, heterologous reactions may be stronger than homologous reactions. Because of these paradoxical cross reactions, one should not depend on serologic determination alone to define the infecting serotype. When agglutination tests are performed on serial specimens over the course of time, the homologous reaction becomes the dominant one in most cases. Performance of agglutination absorption studies may be necessary to define the infecting serotype in some cases. The antigen (serotype) that absorbs out agglutinin to all the serotypes in a serogroup most likely is the infecting serotype.[148,218,242]

A passive microcapsule agglutination test that uses chemically stable microcapsules instead of sheep erythrocytes has been developed.[16] When compared with the MAT, the passive microcapsule agglutination test showed a relatively greater degree of genus specificity and 4- to 32-fold higher titers. The sensitized microcapsules were stable for at least 1 year. This test is simple to perform and reproducible and can be used readily in the routine laboratory. Moreover, the test appears to be more sensitive than is the MAT in the early stages of leptospirosis.[200]

A positive leptospiral agglutination reaction generally is not found until the 6th to 12th day of illness, and maximal levels are reached between 21 and 28 days. After recovery, low titers may persist for many years. One blood sample should be obtained early in the course of illness, and a second one should be obtained at the end of 1 month. Negative reactions in serial samples do not exclude the possibility of leptospirosis because patients may be infected with a serotype not included in the battery of test antigens or with a previously unrecognized serotype. Moreover, the titer may have peaked before the acute-phase specimen was collected. Antibiotic therapy also may suppress the development of positive titers or delay their appearance.[104,148] Peak microscopic agglutination titers of 1:3,000 to 1:100,000 usually are reached during the third week of illness.[77,242] An unchanging titer of 1:100 on two successive serum specimens has been defined as sufficient for making a presumptive diagnosis of leptospirosis. A fourfold increase in titer between acute and convalescent sera is indisputable evidence of active leptospirosis.

Leptospira DNA may be detected by PCR at a minimum detection limit of two to three cells per sample.[138,211] Nonradioactive, arbitrarily primed PCR assays can be used to discriminate species of *Leptospira*.[188] Romero and colleagues[188] reported that PCR was more likely to facilitate the early diagnosis of leptospiral aseptic meningitis than was either the IgM ELISA or the MAT test.

PCR, IgM ELISA, latex agglutination, the Lepto Dipstick assay, and an antibody-based urine antigen assay each may be more sensitive than the MAT early in the course of disease.[127,168,192]

TREATMENT

To be of maximum therapeutic benefit, an antimicrobial agent would have to be administered before invading organisms damage the endothelium of blood vessels and various organs or tissues. One of the problems in evaluating the efficacy of treatment is the fact that generally leptospirosis is a self-limited disease with a favorable prognosis. Even patients with severe icteric leptospirosis may recover without specific treatment.

Most claims of the beneficial value of antimicrobial agents in human leptospirosis are based on the response of individual patients rather than on controlled studies. Hall and associates[107] compared the effects of penicillin, chloramphenicol, chlortetracycline, and oxytetracycline with placebo in 67 confirmed cases of leptospirosis. No appreciable effect of antibiotics could be demonstrated on the duration or severity of illness or on the prevention or amelioration of central nervous system, hepatic, renal, or hemorrhagic complications of this disease. Moreover, the duration of leptospiremia and the persistence of organisms in CSF were not altered by treatment. Kocen[129] compared the effects of penicillin administered on the fourth day of illness to 28 patients with a control group of 33 who were given only supportive care and reported that the duration of fever was shorter and the incidence of jaundice, meningismus, renal involvement, and hemorrhagic manifestations was diminished in the treated group.[129] None of these controlled studies was entirely satisfactory with respect to randomization of patients.

McClain and associates[143] studied the therapeutic efficacy of doxycycline in military recruits who contracted leptospirosis while training at the Jungle Operations Training Center in Panama. Twenty-nine patients with anicteric disease were treated in a randomized, double-blinded fashion with doxycycline 100 mg orally twice a day or with placebo. Therapy was administered for 7 days in the hospital, after which patients were monitored for 3 weeks. The duration of illness before therapy and the severity of illness were similar in both study groups. Doxycycline shortened the duration of illness by 2 days and favorably influenced fever, malaise, headache, and myalgias. Treatment also prevented leptospiruria, and no significant adverse effects of doxycycline administration were observed.

In another randomized, double-blinded, placebo-controlled field trial at the same military training site, Takafuji and associates[228] demonstrated that doxycycline in a 200-mg oral dose given weekly or at the completion of jungle training was highly effective in preventing the onset of clinical leptospirosis. Twenty cases of disease were documented in the placebo group (attack rate, 4.2%) as opposed to only one case in the treatment group (attack rate, 0.2%), findings supporting the prophylactic utility of doxycycline in this setting.

Watt and associates[256] reported the results of a trial in which a 7-day course of intravenous penicillin with 6 million units per day was compared with placebo in a randomized, double-blinded trial involving 42 patients. All the patients had severe, advanced disease. Every measurable aspect of the disease was affected favorably by penicillin. The duration of fever was shortened significantly ($p < .005$) in the group receiving penicillin. Penicillin therapy decreased the number of days of hospitalization and prevented the development of leptospiruria. These investigators concluded that intravenous penicillin should be given to patients with severe leptospirosis, even if therapy can be initiated only late in the course of their disease. Subsequently, Costa and associates[63] reported the contrary. Two hundred fifty-three patients with leptospirosis and longer than 4 days of symptoms were randomized to receive 6 million units of penicillin for 7 days or placebo (mean pretreatment duration of symptoms, 6.6 days and 6.5 days, respectively). Neither the number of days of hospitalization nor the in-hospital case-fatality rate differed significantly between the two groups.

Limited data exist comparing the use of alternative antimicrobial agents with penicillin. Panaphut and colleagues[170] reported no difference in time to resolution of fever, mortality, or duration of organ dysfunction between patients randomized to receive a 7-day course of penicillin or intravenous ceftriaxone. A subsequent study[224] comparing penicillin, doxycycline, and cefotaxime reported no significant differences among the three groups with regard to mortality, time to defervescence, or time to resolution of abnormal laboratory test results.

Nonetheless, I favor treatment with penicillin or tetracycline (to be avoided in children younger than 9 years) *early* in the course of disease if a diagnosis of leptospirosis is suspected. Parenteral aqueous penicillin G 6 to 8 million units/m^2/day in six divided doses should provide optimal blood and tissue concentrations of penicillin. For patients sensitive to penicillin, tetracycline 25 to 50 mg/kg/day orally in four divided doses (up to 3 grams per day) or doxycycline 5 mg/kg/day orally in two divided doses (up to 200 mg per day) for 1 week, should be provided.

Management of leptospirosis requires careful attention to supportive care. Fluid and electrolyte balance requires meticulous attention. Dehydration, cardiovascular collapse, and acute renal failure may necessitate prompt and specific treatment. In some cases, acute renal failure may be prevented by ensuring adequate renal perfusion and appropriate fluid administration early in the course of disease, when prerenal azotemia and shock may be seen.[25,205] If prerenal azotemia is suspected, diuresis should be attempted promptly with administration of a fluid or colloid load designed to expand extracellular volume and replace extracellular fluid deficits.[25] In patients who do not respond to such therapy, acute tubular necrosis may be suspected and appropriate fluid restriction should be initiated. If azotemia is severe or prolonged, peritoneal dialysis or hemodialysis should be instituted.[12,85,244] The use of exchange transfusion has been suggested in patients with marked hyperbilirubinemia and acute renal failure and may be associated with lower mortality.[160,167,178,206]

The use of corticosteroids in the treatment of severe cases has not been evaluated critically, but their use in patients with impending hepatic coma has been suggested.[78] Anecdotal reports also suggest that corticosteroids may be of value in patients with profound hypotension or shock.

PREVENTION

Prevention of leptospirosis, as with many other infections, invokes hygienic practices, immunizations, and prophylaxis after potential exposures. Benches in rat-infested, fish-gutting sheds and sewers may be decontaminated. Hygienic conditions should be encouraged in slaughterhouses, farmyard buildings, and bathing pools. Public health prevention campaigns in Australia emphasize the "cover-wash-clean up" strategy for reducing the incidence of leptospirosis. "Cover" exposed skin and mucous membranes that might come in contact with animals or animal secretions, "wash" hands or shower after a potential contamination, and "clean up" areas that might be contaminated or might attract rodents.[208] Given the rise of leptospirosis acquired during recreation or international travel, travelers should be advised about preventive measures such as avoiding flood waters, wearing protective clothing and boots, and covering up cuts and abrasions on their skin when engaging in outdoor activities.[132] Monitoring water for infection, whether for consumption or recreation could help prevent exposure. Zhou and colleagues describe a PCR-based DNA microarray system for waterborne pathogen detection in water samples, including *L. interrogans*.[264] Identifying contaminated water sources can aid in the prevention of further spread.

In addition to hygiene, prevention of leptospirosis primarily depends on immunization of animals. Immunization of workers at high risk for acquiring leptospirosis has been used successfully in mines in Japan and Poland and

in rice fields in Italy and Spain.[241] Leptospire bacterins are available commercially and have been evaluated for safety and efficacy in laboratory animals and domestic livestock.[37,122,195,215] The degree of protection attained depends largely on the antigenic potential of the immunizing agent. Requirements for the *L. pomona* vaccine used in cattle are such that not more than one eight-hundredth of the dose recommended for cattle must protect 80 percent of hamsters challenged intraperitoneally 14 to 18 days after vaccination with a dose of 100 hamster LD_{50}s. In contrast, most dogs are immunized with a vaccine that is but one tenth of the potency of that used for cattle. Most dogs thus immunized have been protected against disease but not necessarily from carrying and excreting leptospires in their urine. Trends documenting that many cases of leptospirosis in children have been associated with contact with dogs suggest that more stringent requirements for the immunization of pet dogs are needed. The challenge in vaccine efforts has been production of a safe and efficacious vaccine that promotes cross protection against various pathogenic serovars that will eliminate infection and prevent chronic renal carriage.[265] Much of leptospirosis research efforts continue to focus on identification of potential vaccine candidates. The availability of the genome sequence of *Leptospira* has facilitated this search and provided grounds for continued attempts at finding the ideal vaccine.[110,155]

A randomized trial of doxycycline versus placebo was undertaken to assess the efficacy of doxycycline prophylaxis in the prevention of infection and disease caused by leptospires during outbreaks in North Andaman.[198] Leptospiral infection was not prevented, but the patients who received doxycycline and in whom disease subsequently developed had lower morbidity and mortality rates.

Additional resources are provided elsewhere.[20,37,75,134,137]

NEW REFERENCES SINCE THE SIXTH EDITION

3. Abela-Ridder B, Sikkema R, Hartskeerl RA. Estimating the burden of human leptospirosis. *Int J Antimicrob Agents* 2010;**36S**: S5–7.
5. Adesiyun AA, Baboolal S, Suepaul S, et al. Human leptospirosis in the Carribean, 1997-2005: characteristics and serotyping of clinical samples from 14 countries. *Rev Panam Salud Publica* 2011;**29**:350–7.
6. Adler B, Lo M, Seemann T, et al. Pathogenesis of leptospirosis: the influence of genomics. *Vet Microbiol* 2011;**153**:73–81.
35. Bourhy P, Bremont S, Zinini F, et al. Comparison of real-time PCR assays for detection of pathogenic *Leptospira* spp. in blood and identification of variations in target sequences. *J Clin Microbiol* 2011;**49**:2154–60.
40. Breiner D, Fahey M, Salvdor R, et al. *Leptospira interrogans* binds to human cell surface receptors including proteoglycans. *Infect Immun* 2009;**77**:5528–36.
50. Cerqueira GM, Picardeau M. A century of *Leptospira* strain typing. *Infect Genet E* 2009;**9**:760–8.
51. Cinco M. New insights into the pathogenicity of leptospires: evasion of host defenses. *New Microbiol* 2010;**33**:283–92.
55. Chow E, Deville J, Nally J, et al. Prolonged *Leptospira* urinary shedding in a 10-year-old girl. *Case Rep Pediatr* 2012; **2012**:169013.
81. Evangelista KV, Coburn J. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiol* 2010;**5**:1413–25.
87. Figueira CP, Croda J, Choy HA, et al. Heterologous expression of pathogen-specific genes ligA and ligB in the saprophyte *Leptospira biflexa* confers enhanced adhesion to cultured cells and fibronectin. *BMC Micriobiol* 2011;**11**:129.
90. Fraga TR, Barbosa AS, Isaac L. Leptospirosis: aspects of innate immunity, immunopathogenesis and immune evasion from the complement system. *Scand J Immunol* 2011;**73**:408–19.
91. Galloway RL, Levett PN. Application and validation of PFGE for serovar identification of *Leptospira* clinical isolates. *PLoS Negl Trop Dis* 2010;**4**:e824.
94. Ganoza CA, Matthias MA, Saito M, et al. Asymptomatic renal colonization of humans in the peruvian Amazon by *Leptospira*. *PLoS Negl Trop Dis* 2010;**4**:e612.
100. Goldstein RE. Canine leptospirosis. *Vet Clin North Am Small Anim Pract* 2010;**40**:1091–101.
101. Goris MGA, Wagenaar JFP, Hartskeerl RA, et al. Potent innate immune response to pathogenic *Leptospira* in human whole blood. *PLoS ONE* 2011;**6**:e18279.
109. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin Microbiol Infect* 2011;**17**:494–501.
110. Hartwig DD, Seixas FK. Characterization of the immunogenic and antigenic potential of putative lipoproteins from *Leptospira* interrogans. *Curr Microbiol* 2011;**62**:1337–41.
111. Hauk P, Macedo F, Romero EC, et al. In LipL32, the major leptospiral lipoprotein, the C-terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin. *Infect Immun* 2008;**76**:2646–50.
113. Hotez PJ. Neglected infections of poverty in the United States of America. *PLoS Negl Trop Dis* 2008;**2**:e256.
126. Katz AR, Buchholz AE, Hinson K, et al. Leptospirosis in Hawaii, USA, 1999-2008. *Emerg Infect Dis* 2011;**17**:221–6.
131. Lau CL, Smythe LD, Craig SB, et al. Climate change, flooding, urbanization and leptospirosis: fuelling the fire? *Trans R Soc Trop Med Hyg* 2010;**104**:631–8.
132. Lau C, Smythe LD, Weinstein P. Leptospirosis: an emerging disease in travelers. *Travel Med Infect Dis* 2010;**8**:33–9.
140. Marchiori E, Lourenço S, Setúbal S, et al. Clinical and imaging manifestations of hemorrhagic pulmonary leptospirosis: a state-of-the-art review. *Lung* 2011;**189**:1–9.
141. Marotto PCF, Ko AI, Murta-Nascimento C, et al. Early identification of leptospirosis-associated pulmonary hemorrhage syndrome by use of a validated prediction model. *J Infect* 2010;**60**:218–23.
153. Monahan AM, Callanan JJ, Nally JE. Proteomic analysis of *Leptospira interrogans* shed in urine of chronically infected hosts. *Infect Immun* 2008;**76**:4952–8.
154. Monahan AM, Callanan JJ, Nally JE. Host-pathogen interactions in the kidney during chronic leptospirosis. *Vet Pathol* 2009;**46**: 792–9.
155. Monte LG, Conceição FR, Coutinho ML, et al. Monoclonal antibodies against the leptospiral immunoglobulin-like proteins A and B conserved regions. *Comp Immun Microbiol Infect Dis* 2011;**34**: 441–6.
162. Nalam K, Ahmed A, Devi SM, et al. Genetic affinities within a large global collection of pathogenic *Leptospira*: implications for strain identification and molecular epidemiology. *PLoS One* 2010; **5**:e12637.
175. Pinne M, Haake DA. A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of *Leptospira* interrogans. *PLoS One* 2009;**4**:e6071.
177. Prêtre G, Olivera N, Cédola M, et al. Role of inducible nitric oxide synthase in the pathogenesis of experimental leptospirosis. *Microb Pathog* 2011;**51**:203–8.
178. Rajapakse S, Rodrigo C, Haniffa R. Developing a clinically relevant classification to predict mortality in severe leptospirosis. *J Emerg Trauma Shock* 2010;**3**:213–19.
187. Rojas P, Monahan AM, Schuller S, et al. Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1305–9.
196. Schelotto F, Hernandez E, Gonzalez S, et al. A ten-year follow up of human leptospirosis in Uruguay: an unresolved health problem. *Rev Inst Med Trop* 2012;**54**:69–75.
208. Slack A. Leptospirosis. *Aust Fam Phys* 2010;**30**:495–8.
214. Spichler A, Athanazio D, Seguro AC, et al. Outpatient follow-up of patients hospitalized for acute leptospirosis. *Int J Infect Dis* 2011;**15**:e486–90.
226. Sykes JE, Hartmann K, Lunn KF, et al. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *J Vet Intern Med* 2011;**25**:1–13.

231. Thongboonkerd V, Chiangjong W, Saetun P, et al. Analysis of different proteomes in pathogenic and non-pathogenic *Leptospira:* potential pathogenic and virulence factors. *Proteomics* 2009;**13**: 3522–34.
237. Tullu MS, Karande S. Leptospirosis in children: a review for family physicians. *Indian J Med Sci* 2009;**63**:368–78.
238. Tung J, Yang C, Chou S, et al. Calcium binds to LipL32, a lipoprotein from pathogenic *Leptospira*, and modulates fibronectin binding. *J Biol Chem* 2010;**285**:3245–52.
247. Verma A, Kumar P, Babb K, et al. Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins. *PLoS Negl Trop Dis* 2010; **4**:e778.
258. Werts C. Leptospirosis: a toll road from B lymphocytes. *Chang Gung Med J* 2010;**33**:591–601.
260. World Health Organization. *Zoonosis and veterinary public health*. Leptospirosis Burden Epidemiology Reference Group (LERG). Last reviewed/updated 13 July 2010 [accessed 16 July 16 2012]. Available at http://www.who.int/zoonoses/diseases/lerg/en/
264. Zhou G, Wen S, Liu Y, et al. Development of a DNA microarray for detection and identification of *Legionella pneumophila* and ten other pathogens in drinking water. *Int J Food Microbiol* 2011; **145**:293–300.
265. Zuerner RL, Alt DP, Palmer MV, et al. A *Leptospira borgpetersenii* serovar *Hardjo* vaccine induces a Th1 response, activates NK cells, and reduces renal colonization. *Clin Vaccine Immunol* 2011;**18**: 684–91.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 142

Spirillum minus (Rat-Bite Fever)

Kara Dubray • Carrie L. Byington

Spirillum minus is one of the causative agents of rat-bite fever, also known as *sodoku*, from the Japanese *so* (rat) and *doku* (poison).[13] Rat-bite fever also may be caused by *Streptobacillus moniliformis* (see Chapter 138). *S. minus* is a spirochete that was isolated first by Futaki and associates[5] in 1917. The original name for the organism was *Spirochaeta morsus muris*. *S. minus* is reported to be a common cause of rat-bite fever in Asia, although few reports exist in the English literature to confirm its prevalence.[9] Only one case of spirillary rat-bite fever has been reported in the American literature in the past 4 decades, with more cases reported in the 1920s to 1940s.[3,13] Many reports discuss both etiologies simultaneously, although the two types of rat-bite fever are distinct with different epidemiology and clinical presentations.

BACTERIOLOGY

S. minus is a short, rigid, aerobic, gram-negative, flagellated spirochete measuring 2 to 5 μm in length. The organism is thicker than *Treponema pallidum* and usually contains two to five regular sharp turns along its length. On darkfield microscopy, the organism moves quickly with the use of a terminal flagellum.[5] *S. minus*, like other spirochetes, does not grow on artificial media and requires animal inoculation for successful isolation.[15] However, two reports in the literature describe the isolation of *S. minus* in broth,[8,14] but these cases may have represented other infectious illnesses.

EPIDEMIOLOGY AND PATHOLOGY

Like streptobacillary infection, disease appears to be transmitted primarily through rat bites. Approximately 25 percent of rats are carriers of *S. minus* in the nasopharynx, and rats with conjunctivitis have been shown to have *S. minus* in the eye discharge that drains into their mouths.[11] Disease has not been reported after oral ingestion of the organism. Human-to-human transmission has not been reported but conceivably could occur during blood transfusions.

Gunning[6] has given an excellent description of the pathologic changes associated with *S. minus* infection. The infection provokes edema, mononuclear leukocyte infiltration, and necrosis at the site of inoculation. Regional lymph nodes are hyperplastic. The relapsing symptoms are associated with invasion of the blood by spirilla. Toxic, hemorrhagic, or necrotic changes may occur in the liver and kidney.

CLINICAL MANIFESTATIONS

The incubation period of spirillary rat-bite fever typically is longer than that of the streptobacillary form, with an average of 14 to 18 days and a range of 1 to 36 days.[2] The disease is heralded by the appearance of an indurated lesion at the site of the initially healed bite, which coincides with the onset of fever and chills. Chancre formation or ulceration may occur at the site, and regional

REFERENCES

1. Abdulkader RC, Seguro AC, Malheiro PS, et al. Peculiar electrolytic and hormonal abnormalities in acute renal failure due to leptospirosis. *Am J Trop Med* 1996;**54**:1–6.
2. Abdussalam M, Alexander AD, Babudieri B, et al. Research needs in leptospirosis. *Bull W H O* **47**:113–22, 1971–1972.
3. Abela-Ridder B, Sikkema R, Hartskeerl RA. Estimating the burden of human leptospirosis. *Int J Antimicrob Agents* 2010;**36S**: S5–7.
4. Addamiano L. [Serological classification of strains of Leptospira from Indonesia]. *Rend 1st Sup Sanit* **22**:5–12, 1959. In Italian.
5. Adesiyun AA, Baboolal S, Suepaul S, et al. Human leptospirosis in the Carribean, 1997-2005: characteristics and serotyping of clinical samples from 14 countries. *Rev Panam Salud Publica* 2011; **29**:350–7.
6. Adler B, Lo M, Seemann T, et al. Pathogenesis of leptospirosis: the influence of genomics. *Vet Microbiol* 2011;**153**:73–81.
7. Alexander A, Baer A, Fair JP, et al. Leptospiral uveitis: report of bacteriologically verified cases. *Arch Ophthalmol* 1952;**48**:292–7.
8. Alexander AD, Smith OH, Hiatt CW, et al. Presence of hemolysin in cultures of pathogenic leptospires. *Proc Soc Exp Biol Med* 1956; **91**:205–11.
9. Allen GL, Weber DR, Russell PK. The clinical picture of leptospirosis in American soldiers in Vietnam. *Mil Med* 1968;**133**: 275–80.
10. Alonso-Valle H, Munoz R, Hernandez JL, Matorras P. Acute disseminated encephalomyelitis following *Leptospira* infection. *Eur Neurol* 2001;**46**:104–5.
11. Alston JM, Broom JC. *Leptospirosis in man and animals*. Edinburgh: E. & S. Livingston; 1958.
12. Andrade L, Cleto S, Seguro AC. Door-to-door time and daily hemodialysis in patients with leptospirosis: impact on mortality. *Clin J Am Soc Nephrol* 2007;**2**:739–44.
13. Arean VM. Studies on the pathogenesis of leptospirosis, part II: A clinico-pathologic evaluation of hepatic and renal function in experimental leptospiral infections. *Lab Invest* 1962;**11**:273–88.
14. Arean VM. The pathologic anatomy and pathogenesis of fatal human leptospirosis (Weil's disease). *Am J Pathol* 1962;**40**: 393–414.
15. Arean VM, Henry JB. Studies on the pathogenesis of leptospirosis, part IV: the behavior of transaminases and oxidative enzymes in experimental leptospirosis: a histochemical and biochemical assay. *Am J Trop Med Hyg* 1964;**13**:430–42.
16. Arimitsu Y, Kobayashi S, Akama K, et al. Development of a simple serological method for diagnosing leptospirosis: a microcapsule agglutination test. *J Clin Microbiol* 1982;**15**:835–41.
17. Arokianathan D, Trower K, Pooboni S, et al. Leptospirosis: a case report of a patient with pulmonary haemorrhage successfully managed with extra corporeal membrane oxygenation. *J Infect* 2005;**50**:158–62.
18. Baber MD, Stuart RD. Leptospirosis canicola: case treated with penicillin. *Lancet* 1946;**2**:594–6.
19. Babudieri B. Animal reservoirs of leptospires. *Ann N Y Acad Sci* 1958;**70**:393–413.
20. Babudieri B. Laboratory diagnosis of leptospirosis. *Bull W H O* 1961;**24**:45–58.
21. Bain BJ, Ribush NT, Nicoll P, et al. Renal failure and transient paraproteinemia due to *Leptospira pomona*. *Arch Intern Med* 1973;**131**:740–5.
22. Bajani MD, Ashford DA, Bragg SL, et al. Evaluation of four commercially available rapid serologic tests for diagnosis of leptospirosis. *J Clin Microbiol* 2003;**41**:803–9.
23. Bal AM, Bharadwaj RS, Gita N, et al. Guillain-Barré syndrome in a pediatric patient following infection due to *Leptospira*. *Jpn J Infect Dis* 2003;**56**:29–31.
24. Barciscewski M, Domanski E. [Case of Weil's disease acquired in a laboratory]. *Pol Typ Lek* 1951;**6**:1550–1. In Polish.
25. Barrett-Connor E, Child CM, Carter MJ. Renal failure in leptospirosis. *South Med J* 1970;**63**:580–3.
26. Barrocchi MA, Ko AI, Reis MG, et al. Rapid translocation of polarized MDCK cell monolayers by *Leptospira interrogans*, an invasive but nonintracellular pathogen. *Infect Immun* 2002;**70**: 6926–32.
27. Basile C. Pathology and pathogenesis of spirochetosis icterohemorrhagiae. *J Pathol Bacteriol* 1921;**24**:277–85.
28. Bauer DC, Eames LN, Sleight SD, et al. The significance of leptospiral hemolysin in the pathogenesis of *Leptospira pomona* infections. *J Infect Dis* 1961;**108**:229–36.
29. Beck A, Barbehenn KR. *The status of leptospirosis in the St. Louis Area: final report to the Commissioners of Health*. St. Louis City and University City: Missouri; 1974.
30. Beeson PB, Hankey DD, Cooper CF Jr. Leptospiral iridocyclitis: evidence of human infection with *Leptospira pomona* in the United States. *JAMA* 1951;**145**:229–30.
31. Berman SJ, Tsai C, Holmes K, et al. Sporadic anicteric leptospirosis in South Vietnam: a study in 150 patients. *Ann Intern Med* 1973;**79**:167–73.
32. Bhamarapravati N, Boonyapaknavig V, Viranuvatti V, et al. Liver changes in leptospirosis: a study of needle biopsies in twenty-two cases. *Am J Proctol* 1966;**17**:480–7.
33. Blake FG. Weil's disease in the United States: report of a case in Connecticut. *N Engl J Med* 1940;**223**:5561–5.
34. Bolin CA, Koellner P. Human-to-human transmission of *Leptospira interrogans* by milk. *J Infect Dis* 1988;**158**:246–7.
35. Bourhy P, Bremont S, Zinini F, et al. Comparison of real-time PCR assays for detection of pathogenic *Leptospira* spp. in blood and identification of variations in target sequences. *J Clin Microbiol* 2011;**49**:2154–60.
36. Bowdoin CD. New disease entity. *J Med Assoc Ga* 1942; **31**:437.
37. Bramel RG, Scheidy SF. The effect of revaccination of horses and cattle with *Leptospira pomona* bacterin. *J Am Vet Med Assoc* 1956;**128**:399–400.
38. Brand N, Moshe HB. Human leptospirosis associated with eye complications. *Isr Med J* 1963;**22**:182–4.
39. Brandão AP, Camargo ED, Emilson D, et al. Macroscopic agglutination test for rapid diagnosis of human leptospirosis. *J Clin Microbiol* 1998;**36**:3138–42.
40. Breiner D, Fahey M, Salvdor R, et al. *Leptospira interrogans* binds to human cell surface receptors including proteoglycans. *Infect Immun* 2009;**77**:5528–36.
41. Bridges CH, Luna L. Kerr's improved Warthin-Starry technic. *Lab Invest* 1957;**6**:357–67.
42. Broom JC. Canicola fever in Great Britain. *Monthly Bull Ministry Health* 1951;**10**:258–65.
43. Buzzard EM, Wylie JAH. Meningitis leptospirosa. *Lancet* 1947; **2**:417–20.
44. Cacciapuoti B, Ciceroni L, Maffei C, et al. A waterborne outbreak of leptospirosis. *Am J Epidemiol* 1987;**126**:535–45.
45. Carayon A, Fouin G. Encephalité pseudotumorale et leptomeningité hémorragique en "plaques" sousdurales, complication tardive d'une leptospirose icterohemorrhagique en Extreme-Orient. *Med Trop* 1953;**13**:698–702, and Bull Hyg 1954;29: 1226–7.
46. Cargill WH Jr, Beeson PB. The value of spinal fluid examination as a diagnostic procedure in Weil's disease. *Ann Intern Med* 1947;**27**:396–400.
47. Carles G, Montoya E, Joly F, Peneau C. Leptospirosis and pregnancy: eleven cases in French Guyana. *J Gynecol Obstet Biol Reprod (Paris)* 1995;**24**:418–21.
48. Carrasco ED, Dunkelberg WE. European swamp fever: leptospirosis due to *Leptospira grippotyphosa*: A case report. *Mil Med* 1962;**127**:569–70.
49. Centers for Disease Control and Prevention. *Annual summary of leptospirosis for 1972*. Atlanta: CDC; 1974.
50. Cerqueira GM, Picardeau M. A century of *Leptospira* strain typing. *Infect Genet E* 2009;**9**:760–8.
51. Cinco M. New insights into the pathogenicity of leptospires: evasion of host defenses. *New Microbiol* 2010;**33**:283–92.
52. Chandra SR, Kalpana D, Anilkumar TV, et al. Acute disseminated encephalomyelitis following leptospirosis. *J Assoc Physicians India* 2004;**52**:327–9.
53. Chang ML, Yang CW, Chen JC, et al. Disproportional exaggerated aspartate transaminase is a useful prognostic parameter in late leptospirosis. *World J Gastroenterol* 2005;**11**:5553–6.
54. Chang RS, Smith OJW, McComb DC, et al. The use of erythrocyte-sensitizing substance in the diagnosis of leptospirosis. *Am J Trop Med Hyg* 1957;**6**:101–7.
55. Chow E, Deville J, Nally J, et al. Prolonged *Leptospira* urinary shedding in a 10-year-old girl. *Case Rep Pediatr* 2012;**2012**: 169013.

56. Chung HL, Ts'ao WC, Mo PS, et al. Transplacental or congenital infection of leptospirosis: clinical and experimental observations. *Chin Med J (Peking)* 1963;**82**:777–82.
57. Chung J. Electron microscopic aspects of renal pathology. In: Becker EL, editor. *Structural basis of renal disease*. New York: Harper & Row; 1968. p. 132–96.
58. Clapper M, Myers GB. Clinical manifestations of Weil's disease with particular reference to meningitis. *Arch Intern Med* 1943;**72**:18–30.
59. Cockburn TA, Vavra JD, Spencer SS, et al. Human leptospirosis associated with a swimming pool, diagnosed after eleven years. *Am J Hyg* 1954;**60**:1–7.
60. Coghlan JD, Norval J. Canicola fever in man from contact with infected pigs: further observations. *BMJ* 1960;**1**:1711–13.
61. Cohn AP, Howard AA. Common characteristics of leptospirosis: a report of 11 cases. *Ann Intern Med* 1961;**54**:57–65.
62. Cora D. La funzionalita renale nel morbo di Weil. *G Clin Med* 1956;**37**:1295.
63. Costa E, Lopes AA, Sacramento E, et al. Penicillin at the late stage of leptospirosis: a randomized controlled trial. *Rev Inst Med Trop Sao Paulo* 2003;**45**:141–5.
64. Cox DC. Hemolysis of sheep erythrocytes sensitized with leptospiral extracts. *Proc Soc Exp Biol Med* 1955;**90**:610–15.
65. Cullen PA, Cordwell SJ, Bulach DM, et al. Global analysis of outer membrane proteins from *Leptospira interrogans* serovar *Lai*. *Infect Immun* 2002;**70**:1211–18.
66. Cullen PA, Haake DA, Adler B. Outer membrane proteins of pathogenic spirochetes. *FEMS Microbiol Rev* 2004;**28**:291–318.
67. Cursons R, Pyke P. Diffusion in gel-enzyme–linked immunosorbent assay: a new serological test for leptospirosis. *J Clin Pathol* 1981;**34**:1128–31.
68. Daher EF, Zanetta DM, Abdulkader RC. Pattern of renal function recovery after leptospirosis acute renal failure. *Nephron Clin Pract* 2004;**98**:c8–14.
69. Daher EF, Zanetta DM, Cavalcante MB, Abdulkader RC. Risk factors for death and changing patterns in leptospirosis acute renal failure. *Am J Trop Med Hyg* 1999;**61**:630–4.
70. Daniels WB, Grennan HA. Pretibial fever, an obscure disease. *JAMA* 1943;**122**:361–5.
71. de Brito T, Morais CF, Yasuda PH, et al. Cardiovascular involvement in human and experimental leptospirosis: pathologic findings and immunohistochemical detection of leptospiral antigen. *Ann Trop Med Parasitol* 1987;**81**:207–14.
72. de Brito T, Penna DO, Pereira VC, et al. Kidney biopsies in human leptospirosis: a biochemical and electron microscopy study. *Virchows Arch [A]* 1967;**343**:124–35.
73. Demers RY, Thiermann A, Demers P, et al. Exposure to *Leptospira icterohaemorrhagiae* in inner city and suburban children: a serologic comparison. *J Fam Pract* 1983;**17**:1007–11.
74. Dolhnikoff M, Mauad T, Bethlem EP, et al. Pathology and pathophysiology of pulmonary manifestations in leptospirosis. *Braz J Infect Dis* 2007;**11**:142–8.
75. Doudier B, Garcia S, Quennee V, et al. Prognostic factors associated with severe leptospirosis. *Clin Microbiol Infect* 2006;**12**: 299–300.
76. Dupont H, Dupont-Perdrizet D, Perie JL, et al. Leptospirosis: prognostic factors associated with mortality. *Clin Infect Dis* 1997; **25**:720–4.
77. Edwards GA, Domm BM. Human leptospirosis. *Medicine (Baltimore)* 1960;**39**:117–56.
78. Edwards GA, Domm BM. Leptospirosis, part II. *Med Times* 1966; **94**:1086–95.
79. Effler PV, Domen HY, Bragg SL, et al. Evaluation of the indirect hemagglutination assay for diagnosis of acute leptospirosis in Hawaii. *J Clin Microbiol* 2000;**38**:1081–4.
80. Elian M, Tamir M, Bornstein B. Unusual case of brachial plexitis in relation to leptospires and Coxsackie virus. *Confin Neurol* 1965;**26**:1–6.
81. Evangelista KV, Coburn J. *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. *Future Microbiol* 2010;**5**:1413–25.
82. Faine S. Virulence in *Leptospira*, part I: reactions of guinea pigs to experimental infection with *Leptospira icterohemorrhagiae*. *Br J Exp Pathol* 1957;**38**:1–8.
83. Faine S. Virulence in *Leptospira*, part II: the growth in vivo of virulent *Leptospira icterohemorrhagiae*. *Br J Exp Pathol* 1957;**38**:8–14.
84. Faine S, Adler B, Bolin C, et al. *Leptospira and leptospirosis*. Melbourne: MedScience; 1999.
85. Feigin RD, Lobes LA, Anderson DC, et al. Human leptospirosis from immunized dogs. *Ann Intern Med* 1973;**79**:777–85.
86. Ferreira Alvas VA, Vianna MR, Yasuda PH, et al. Detection of leptospiral antigens in the human liver and kidney using an immunoperoxidase staining procedure. *J Pathol* 1987;**115**: 125–31.
87. Figueira CP, Croda J, Choy HA, et al. Heterologous expression of pathogen-specific genes *ligA* and *ligB* in the saprophyte *Leptospira biflexa* confers enhanced adhesion to cultured cells and fibronectin. *BMC Microbiol* 2011;**11**:129.
88. Finco DR, Low DG. Water, electrolyte, and acid-base alterations in experimental canine leptospirosis. *Am J Vet Res* 1968;**29**: 1799–807.
89. Fletcher W. Recent work on leptospirosis, tsutsugamushi disease and tropical typhus in the Federated Malay States. *Trans R Soc Trop Med Hyg* 1928;**21**:265–88.
90. Fraga TR, Barbosa AS, Isaac L. Leptospirosis: aspects of innate immunity, immunopathogenesis and immune evasion from the complement system. *Scand J Immunol* 2011;**73**:408–19.
91. Galloway RL, Levett PN. Application and validation of PFGE for serovar identification of *Leptospira* clinical isolates. *PLoS Negl Trop Dis* 2010;**4**:e824.
92. Galton MM, Menges RW, Shotts EB Jr, et al. *Leptospirosis. PHS Publication No. 951*. Washington, D.C.: U.S. Government Printing Office; 1962.
93. Galton MM, Sulzer CR, Santa Rosa A, et al. Application of a microtechnique to the agglutination test for leptospiral antibodies. *Appl Microbiol* 1965;**13**:81–5.
94. Ganoza CA, Matthias MA, Saito M, et al. Asymptomatic renal colonization of humans in the peruvian Amazon by *Leptospira*. *PLoS Negl Trop Dis* 2010;**4**:e612.
95. Geszti O, Chung HL, Ts'ao WC. Studies on blood coagulation disorders in experimental leptospirosis. *Chin Med J* 1957;**75**: 603–15.
96. Gillespie RW, Ryn J. Epidemiology of leptospirosis. *Am J Public Health* 1963;**53**:950–5.
97. Gochenour WS Jr, Smadel JE, Jackson EB, et al. Leptospiral etiology of Fort Bragg fever. *Public Health Rep* 1952;**67**:811–13.
98. Gochenour WS Jr, Yager RH, Wetmore PW. Antigenic similarity of bovine strains of leptospira (United States) and *Leptospira pomona*. *Proc Soc Exp Biol Med* 1950;**74**:199–202.
99. Goebel A, Koburg E. Experimentelle Untersuchungen zur Frage der Nierenveranderungen bei respirattorescher Insuffizienz [Experimental studies on the problem of kidney changes in respiratory insufficiency]. *Beitr Pathol Anat* 1959;**120**:111–24.
100. Goldstein RE. Canine leptospirosis. *Vet Clin North Am Small Anim Pract* 2010;**40**:1091–101.
101. Goris MGA, Wagenaar JFP, Hartskeerl RA, et al. Potent innate immune response to pathogenic *Leptospira* in human whole blood. *PLoS ONE* 2011;**6**:e18279.
102. Gourley IM. *Studies of experimental canine leptospirosis*. Thesis. Minneapolis: University of Minnesota Graduate School; 1962.
103. Gsell O. *Leptospirosis*. Bern: Hans Huber Verlag; 1952.
104. Gsell O. Epidemiology of the leptospiroses. In: *Symposium of the Leptospiroses, December 11-12, 1952*. Medical Science Publication No. 1. Washington, D.C.: U.S. Government Printing Office; 1953. p. 4–23.
105. Gussenhoven GC, van der Hoorn MA, Goris MG, et al. LEPTO dipstick, a dipstick assay for detection of *Leptospira*-specific immunoglobulin M antibodies in human sera. *J Clin Microbiol* 1997;**35**:92–7.
106. Haake DA, Chao G, Zuerner RL, et al. The leptospiral major outer membrane protein LipL32 is a lipoprotein expressed during mammalian infection. *Infect Immun* 2000;**68**:2276–85.
107. Hall HE, Hightower JA, Rivera RD, et al. Evaluation of antibiotic therapy in human leptospirosis. *Ann Intern Med* 1951;**35**: 981–98.
108. Harkin KR, Roshto YM, Sullivan JT, et al. Comparison of polymerase chain reaction assay, bacteriologic culture, and serologic testing in assessment of prevalence of urinary shedding of leptospires in dogs. *J Am Vet Med Assoc* 2003;**222**:1230–3.
109. Hartskeerl RA, Collares-Pereira M, Ellis WA. Emergence, control and re-emerging leptospirosis: dynamics of infection in the changing world. *Clin Microbiol Infect* 2011;**17**:494–501.

110. Hartwig DD, Seixas FK. Characterization of the immunogenic and antigenic potential of putative lipoproteins from *Leptospira* interrogans. *Curr Micriobiol* 2011;**62**:1337–41.
111. Hauk P, Macedo F, Romero EC, et al. In LipL32, the major leptospiral lipoprotein, the C-terminus is the primary immunogenic domain and mediates interaction with collagen IV and plasma fibronectin. *Infect Immun* 2008;**76**:2646–50.
112. Heath CW Jr, Alexander AD, Galton MM. Leptospirosis in the United States. *N Engl J Med* 1965;**273**:857–922.
113. Hotez PJ. Neglected infections of poverty in the United States of America. *PLoS Negl Trop Dis* 2008;**2**:e256.
114. Hubbart WR. *Immunologic studies on leptospirosis*. Thesis. Los Angeles: University of California Graduate School; 1964.
115. Hubbert WT, Humphrey GL. Epidemiology of leptospirosis in California: a cause of aseptic meningitis. *Calif Med* 1968; **108**:113–16.
116. Hutchison JH, Poppard JS, White MHG, et al. Outbreak of Weil's disease in the British army in Italy; clinical study; post-mortem and histological findings. *BMJ* 1946;**1**:81–3.
117. Inada R, Ido Y, Hoki R, et al. The etiology, mode of infection and specific therapy of Weil's disease (spirochaetosis icterohaemorrhagica). *J Exp Med* 1916;**23**:377–402.
118. Jacobs JH. Spondylitis following Weil's disease. *Ann Rheum Dis* 1951;**10**:61–3.
119. Jeghers HJ, Houghton JD, Foley JA. Weil's disease: report of case with postmortem observations and review of recent literature. *Arch Pathol* 1935;**20**:447–76.
120. Johnson DW. The Australian leptospiroses. *Med J Aust* 1950;**2**:724–31.
121. Johnson RC, Harris VG. Differentiation of pathogenic and saprophytic leptospires, part I: growth at low temperatures. *J Bacteriol* 1967;**94**:27–31.
122. Kahrs RF, Baker JA. Combined vaccines for dairy cattle. In: Proceedings of the 69th Annual Meeting of the U.S. Livestock Sanitary Association, 1966. p. 177.
123. Karande S, Bhatt M, Kelkar A, et al. An observational study to detect leptospirosis in Mumbai, India, 2000. *Arch Dis Child* 2003;**88**:1070–5.
124. Karande S, Kulkarni H, Kukrni M, et al. Leptospirosis in children in Mumbai slums. *Indian J Pediatr* 2002;**69**:855–8.
125. Kasarov LB, Addamiano L. Metabolism of the lipoproteins of serum by leptospires: degradation of the triglycerides. *J Med Microbiol* 1969;**2**:165–8.
126. Katz AR, Buchholz AE, Hinson K, et al. Leptospirosis in Hawaii, USA, 1999-2008. *Emerg Infect Dis* 2011;**17**:221–6.
127. Kemapunmanus M, Sretrirutchai S, Khuntikij P, et al. A prospective evaluation of four immunodiagnostic assays for human leptospirosis. *Southeast Asian J Trop Med Public Health* 2004;**35**:863–7.
128. Kerr DA. Improved Warthin-Starry technique of staining spirochetes in tissue section. *Am J Clin Pathol* 1938;**2**(Tech. Suppl.): 63–7.
129. Kocen RS. Leptospirosis, a comparison of symptomatic and penicillin therapy. *BMJ* 1962;**1**:1181–3.
130. Koppisch E, Bond WM. The morbid anatomy of human leptospirosis: a report on thirteen fatal cases. In: *Symposium on the Leptospiroses, December 11-12, 1952*. Medical Science Publication No. 1. Washington, D.C.: U.S. Government Printing Office; 1953. p. 83–105.
131. Lau CL, Smythe LD, Craig SB, et al. Climate change, flooding, urbanization and leptospirosis: fuelling the fire? *Trans R Soc Trop Med Hyg* 2010;**104**:631–8.
132. Lau C, Smythe LD, Weinstein P. Leptospirosis: an emerging disease in travelers. *Travel Med Infect Dis* 2010;**8**:33–9.
133. Laurain AR. Lesions of skeletal muscle in leptospirosis: review of reports and an experimental study. *Am J Pathol* 1955;**31**:501–14.
134. Lawson JH. Leptospirosis in the west of Scotland. *Scott Med J* 1972;**1**:220–4.
135. Lee SH, Kim S, Park SC, Kim MJ. Cytotoxic activities of *Leptospira interrogans* hemolysin SphH as a pore-forming protein on mammalian cells. *Infect Immun* 2002;**70**:315–22.
136. Lessa I, Cortes E. Cerebrovascular accident as a complication of leptospirosis. *Lancet* 1981;**2**:1113.
137. Letocart M, Baranton G, Perolat P. Rapid identification of pathogenic *Leptospira* species (*Leptospira interrogans*, *L. borgpetersenii*, and *L. kirschneri*) with species-specific DNA probes produced by arbitrarily primed PCR. *J Clin Microbiol* 1997;**35**:248–53.
138. Levett PN, Morey RE, Galloway RL, et al. Detection of pathogenic leptospires by real-time quantitative PCR. *J Med Microbiol* 2005;**54**:45–9.
139. Lopes AA, Costa E, Costa YA, et al. The association between serum potassium at hospital admission and the case-fatality rate of leptospirosis in men. *Rev Inst Med Trop Sao Paulo* 2001;**43**: 217–20.
140. Marchiori E, Lourenço S, Setúbal S, et al. Clinical and imaging manifestations of hemorrhagic pulmonary leptospirosis: a state-of-the-art review. *Lung* 2011;**189**:1–9.
141. Marotto PCF, Ko AI, Murta-Nascimento C, et al. Early identification of leptospirosis-associated pulmonary hemorrhage syndrome by use of a validated prediction model. *J Infect* 2010;**60**:218–23.
142. Matsunaga J, Barocchi MA, Croda C, et al. Pathogenic *Leptospira* species express surface-exposed proteins belonging to the bacterial immunoglobulin superfamily. *Mol Microbiol* 2003;**49**:929–49.
143. McClain JB, Ballou WR, Harrison SM, et al. Doxycycline therapy for leptospirosis. *Ann Intern Med* 1984;**100**:696–8.
144. McCrumb FR Jr, Stockard JL, Robinson CR, et al. Leptospirosis in Malaya, part I: sporadic cases among military and civilian personnel. *Am J Trop Med Hyg* 1952;**6**:238–56.
145. McCulloch WF, Braun JL, Robinson RG. Leptospiral meningitis. *J Iowa Med Soc* 1962;**52**:728–31.
146. McNee JW. Spirochaetal jaundice: the morbid anatomy and mechanism of production of the icterus. *J Pathol Bacteriol* 1919;**23**:342–9.
147. Meites E, Jay MT, Deresinski S, et al. Reemerging leptospirosis, California. *Emerg Infect Dis* 2004;**10**:406–12.
148. Menges RW, Galton MM, Hall AD. Diagnosis of leptospirosis from urine specimens by direct culture following bladder tapping. *J Am Vet Med Assoc* 1958;**132**:58–60.
149. Meyer KF, Anderson-Stewart B, Eddie B. Canine leptospirosis in the United States. *J Am Vet Med Assoc* 1939;**95**:710–29.
150. Middleton JE. Canicola fever with neurological complications. *BMJ* 1955;**2**:25–6.
151. Miller HG, Stanton JB, Gibbons JL. Parainfectious encephalomyelitis and related syndromes: a critical review of the neurological complication of certain specific fevers. *Q J Med* 1956;**25**:427.
152. Miller NG, Wilson RB. In vivo and in vitro observations of *Leptospira pomona* by electron microscopy. *J Bacteriol* 1962;**84**: 569–76.
153. Monahan AM, Callanan JJ, Nally JE. Proteomic analysis of *Leptospira interrogans* shed in urine of chronically infected hosts. *Infect Immun* 2008;**76**:4952–8.
154. Monahan AM, Callanan JJ, Nally JE. Host-pathogen interactions in the kidney during chronic leptospirosis. *Vet Pathol* 2009;**46**: 792–9.
155. Monte LG, Conceição FR, Coutinho ML, et al. Monoclonal antibodies against the leptospiral immunoglobulin-like proteins A and B conserved regions. *Comp Immun Microbiol Infect Dis* 2011;**34**: 441–6.
156. Morgan J, Bornstein SL, Karpati AM, et al. Leptospirosis Working Group: Outbreak of leptospirosis among triathlon participants and community residents in Springfield, Illinois, 1998. *Clin Infect Dis* 2002;**34**:1593–9.
157. Morse EV, Allen V, Worley G Jr. Brucellosis and leptospirosis serological test results on serums of Wisconsin veterinarians. *J Am Vet Med Assoc* 1955;**126**:59.
158. Morse EV, Morter RL, Langham RF, et al. Experimental bovine leptospirosis, *Leptospira pomona* infection. *J Infect Dis* 1957;**101**: 129–36.
159. Moulton JE, Howarth JA. The demonstration of *Leptospira canicola* in hamster kidneys by means of fluorescent antibody. *Cornell Vet* 1957;**47**:524–32.
160. Murphy KJ. Exchange transfusion and albumin infusion for severe leptospiral jaundice. *Med J Aust* 1969;**1**:1299–300.
161. Myers DM. Serodiagnosis of human leptospirosis by countercurrent immunoelectrophoresis. *J Clin Microbiol* 1987;**25**:897–9.
162. Nalam K, Ahmed A, Devi SM, et al. Genetic affinities within a large global collection of pathogenic *Leptospira*: implications for strain identification and molecular epidemiology. *PLoS One* 2010;**5**:e12637.
163. Nally JE, Chantranuwat C, Wu XY, et al. Alveolar septal deposition of immunoglobulin and complement parallels pulmonary hemorrhage in a guinea pig model of severe pulmonary leptospirosis. *Am J Pathol* 2004;**164**:1115–27.

164. Nally JE, Whittelegge R, Aguilera MM, et al. Purification and proteomic analysis of outer membrane vesicles from a clinical isolate of *Leptospira interrogans* serovar *Copenhageni*. *Proteomics* 2005;**5**:144–52.
165. Nally JE, Whitelegge R, Bassilian S, et al. Characterization of the outer membrane proteome of *Leptospira interrogans* expressed during acute lethal infection. *Infect Immun* 2007;**75**:766–73.
166. Noguchi H. *Spirochaeta icterohaemorrhagiae* in American wild rats and its relation to the Japanese and European strains. *J Exp Med* 1917;**25**:755–63.
167. Ooi BS, Chen BT, Tan KK, et al. Human renal leptospirosis. *Am J Trop Med Hyg* 1972;**21**:336–41.
168. Ooteman MC, Vago AR, Koury MC. Evaluation of MAT, IgM ELISA and PCR methods for the diagnosis of human leptospirosis. *J Microbiol Methods* 2006;**65**:247–57.
169. Panaphut T, Domrongkitchaiporn S, Thinkamrop B. Prognostic factors of death in leptospirosis: a prospective cohort study in Khon Kaen, Thailand. *Int J Infect Dis* 2002;**6**:52–9.
170. Panaphut T, Domrongkitchaiporn S, Vibhagool A, et al. Ceftriaxone compared with sodium penicillin G for treatment of severe leptospirosis. *Clin Infect Dis* 2003;**36**:1507–13.
171. Parsons M. Electrocardiographic changes in leptospirosis. *BMJ* 1965;**2**:201–3.
172. Pea L, Roda L, Boussaud V, Lonjon B. Desmopressin therapy for massive hemoptysis associated with severe leptospirosis. *Am J Respir Crit Care Med* 2003;**167**:726–8.
173. Pereira da Silva JJ, Dalston MO, Manhães de Carvalho JE, et al. Clinicopathological and immunohistochemical features of the severe pulmonary form of leptospirosis. *Rev Soc Bras Med Trop* 2002;**35**:395–9.
174. Peter G. Leptospirosis: a zoonosis of protean manifestations. *Pediatr Infect Dis* 1982;**1**:282–8.
175. Pinne M, Haake DA. A comprehensive approach to identification of surface-exposed, outer membrane-spanning proteins of *Leptospira interrogans*. *PLoS One* 2009;**4**:e6071.
176. Poh SC, Soh CS. Lung manifestations in leptospirosis. *Thorax* 1970;**25**:751–5.
177. Prêtre G, Olivera N, Cédola M, et al. Role of inducible nitric oxide synthase in the pathogenesis of experimental leptospirosis. *Microb Pathog* 2011;**51**:203–8.
178. Rajapakse S, Rodrigo C, Haniffa R. Developing a clinically relevant classification to predict mortality in severe leptospirosis. *J Emerg Trauma Shock* 2010;**3**:213–19.
179. Ramos-Morales F, Diaz-Rivera RS, Cintron-Rivera AA, et al. The pathogenesis of leptospiral jaundice. *Ann Intern Med* 1959;**51**: 861–78.
180. Randall R, Cooper HR. Golden hamster *(Cricetus auratus)* as test animal for diagnosis of leptospirosis. *Science* 1944;**100**:133–4.
181. Randall R, Wetmore PW, Warner AR. Sonic-vibrated leptospirae as antigens in complement fixation test for diagnosis of leptospirosis. *J Lab Clin Med* 1949;**34**:1411–15.
182. Ren S, Fu G, Jiang X, et al. Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. *Nature* 2003;**422**:888–93.
183. Ribeiro MA, Brando AP, Romero EC. Evaluation of diagnostic tests for human leptospirosis. *Braz J Med Biol Res* 1996;**29**: 773–7.
184. Richardson DJ, Gauthier JL. A serosurvey of leptospirosis in Connecticut peridomestic wildlife. *Vector Borne Zoonotic Dis* 2003;**3**: 187–93.
185. Rishavy MA, Hallgren KW, Yakubenko AV, et al. The vitamin K–dependent carboxylase has been acquired by *Leptospira pathogens* and shows altered activity that suggests a role other than protein carboxylation. *J Biol Chem* 2005;**280**:34870–7.
186. Ristow P, Bourhy P, McBride FW, et al. The OmpA-like protein Loa22 is essential for leptospiral virulence. *PLoS Pathog* 2007;**3**:e97.
187. Rojas P, Monahan AM, Schuller S, et al. Detection and quantification of leptospires in urine of dogs: a maintenance host for the zoonotic disease leptospirosis. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1305–9.
188. Romero EC, Billerbeck AE, Lando VS, et al. Detection of *Leptospira* DNA in patients with aseptic meningitis by PCR. *J Clin Microbiol* 1998;**36**:1453–5.
189. Russell CM. A hemolysin associated with leptospirae. *J Immunol* 1956;**77**:405–9.
190. Russell RW. Clinical features of tropical leptospirosis. *Ann Trop Med Parasitol* 1959;**53**:416–20.
191. Sacramento E, Lopes AA, Costa E, et al. Electrocardiographic alterations in patients hospitalized with leptospirosis in the Brazilian city of Salvador. *Arq Bras Cardiol* 2002;**78**:267–70.
192. Saengjaruk P, Chaicumpa W, Watt G, et al. Diagnosis of human leptospirosis by monoclonal antibody–based antigen detection in urine. *J Clin Microbiol* 2002;**40**:480–9.
193. Sarasin G, Tucker DN, Arean VM. Accidental laboratory infection caused by *Leptospira icterohaemorrhagiae*: report of a case. *Am J Clin Pathol* 1963;**40**:146–50.
194. Schaeffer M. Leptospiral meningitis: investigation of a waterborne epidemic due to *L. pomona*. *J Clin Invest* 1951;**30**:670–1.
195. Scheidy SF. Leptospirosis vaccination studies in cattle, swine, sheep, and horses. *J Am Vet Med Assoc* 1957;**131**:366–8.
196. Schelotto F, Hernandez E, Gonzalez S, et al. A ten-year follow up of human leptospirosis in Uruguay: an unresolved health problem. *Rev Inst Med Trop* 2012;**54**:69–75.
197. Schuffner W. Recent work on leptospirosis. *Trans R Soc Trop Med Hyg* 1934;**28**:7–37.
198. Sehgal SC, Sugunan AP, Murhekar MV, et al. Randomized controlled trial of doxycycline prophylaxis against leptospirosis in an endemic area. *Int J Antimicrob Agents* 2000;**13**:249–55.
199. Seiler HE, Noval J, Coghlan JD. Leptospirosis in piggery workers. *Nature* 1956;**177**:1042.
200. Seki M, Sato T, Arimitsu Y, et al. One-point method for serological diagnosis of leptospirosis: a microcapsule agglutination test. *Epidemiol Infect* 1987;**99**:399–405.
201. Semiz UB, Turhan V, Basoglu C, et al. Leptospirosis presenting with mania and psychosis: four consecutive cases seen in a military hospital in Turkey. *Int J Psychiatry Med* 2005;**35**:299–305.
202. Senekjie HA. The clinical manifestations of leptospirosis in Louisiana. *JAMA* 1944;**126**:5–10.
203. Sheldon WH. Lesions of muscle in spirochetal jaundice (Weil's disease; spirochetoses icterohemorrhagica). *Arch Intern Med* 1945;**75**:119–24.
204. Silverstein CM. Pulmonary manifestations of leptospirosis. *Radiology* 1953;**61**:327–34.
205. Sitprija V. Renal involvement in human leptospirosis. *BMJ* 1968;**2**:656–8.
206. Sitprija V, Chusilp S. Renal failure and hyperbilirubinemia in leptospirosis: treatment with exchange transfusion. *Med J Aust* 1973;**1**:171–3.
207. Sitprija V, Evans H. The kidney in human leptospirosis. *Am J Med* 1970;**49**:780–8.
208. Slack A. Leptospirosis. *Aust Fam Phys* 2010;**30**:495–8.
209. Smith DJW, Self HR. Observations on the survival of *Leptospira australis* A in soil and water. *J Hyg (Lond)* 1955;**53**:436–44.
210. Smits HL, Ananyina YV, Chereshsky A, et al. International multicenter evaluation of the clinical utility of a dipstick assay for detection of *Leptospira*-specific immunoglobulin M antibodies in human serum specimens. *J Clin Microbiol* 1999;**37**:2904–9.
211. Smythe LD, Smith IL, Smith GA, et al. A quantitative PCR (TaqMan) assay for pathogenic *Leptospira* spp. *B M C Infect Dis* 2002;**2**:13.
212. Sodeman WA, Kilough JH. Cardiac manifestations of Weil's disease. *Am J Trop Med* 1951;**31**:479–88.
213. Solbrig AU, Sher JH, Kula RW. Rhabdomyolysis in leptospirosis (Weil's disease). *J Infect Dis* 1987;**156**:692–3.
214. Spichler A, Athanazio D, Seguro AC, et al. Outpatient follow-up of patients hospitalized for acute leptospirosis. *Int J Infect Dis* 2011;**15**:e486–90.
215. Stalheim OH, Wilson JB. Antigenicity and immunogenicity of leptospires grown in chemically characterized medium. *Am J Vet Res* 1964;**25**:1277–80.
216. Stavitsky AB. Studies on the pathogenesis of leptospirosis. *J Infect Dis* 1945;**76**:179–92.
217. Stevenson B, Choy HA, Pinne M, et al. *Leptospira interrogans* endostatin-like outer membrane proteins bind host fibronectin, laminin and regulators of complement. *PloS One* 2007;**2**:e1188.
218. Sterling K. Hepatic function in Weil's disease. *Gastroenterology* 1950;**15**:52–8.
219. Stiles WW, Goldstein JD, McCann WS. Leptospiral nephritis. *JAMA* 1946;**131**:1271–4.
220. Stimson AM. Note on an organism found in yellow-fever tissue. *Public Health Rep* 1907;**22**:541.

221. Stoenner HG, Marlean D. *Leptospirosis ballum* contracted from Swiss albino mice. *Arch Intern Med* 1958;**101**:606–10.
222. Stuart RD. Transport problems in public health bacteriology: the use of transport media and other devices to maintain the viability of bacteria in specimens. *Can J Public Health* 1956;**47**:115–22.
223. Sulzer CR, Glosser JW, Rogers F, et al. Evaluation of an indirect hemagglutination test for the diagnosis of human leptospirosis. *J Clin Microbiol* 1975;**2**:218–21.
224. Suputtamongkol Y, Niwattayakul K, Suttinont C, et al. An open, randomized, controlled trial of penicillin, doxycycline, and cefotaxime for patients with severe leptospirosis. *Clin Infect Dis* 2004;**39**:1417–24.
225. Sutliff WD, Shepard R, Dunham WB. Acute *Leptospira pomona* arthritis and myocarditis. *Ann Intern Med* 1953;**39**:134–40.
226. Sykes JE, Hartmann K, Lunn KF, et al. 2010 ACVIM small animal consensus statement on leptospirosis: diagnosis, epidemiology, treatment, and prevention. *J Vet Intern Med* 2011;**25**: 1–13.
227. Tabel H, Karstad L. The renal carrier state of experimental *Leptospira pomona* infections in skunks *(Mephitis mephitis)*. *Am J Epidemiol* 1967;**85**:9–15.
228. Takafuji ET, Kirkpatrick JW, Miller RN, et al. An efficacy trial of doxycycline chemoprophylaxis against leptospirosis. *N Engl J Med* 1984;**310**:497–500.
229. Tantitanawat S, Tanjatham S. Prognostic factors associated with severe leptospirosis. *J Med Assoc Thailand* 2003;**86**:925–31.
230. Thomson JG. Fatal Weil's disease with myocarditis in South Africa. *S Afr Med J* 1964;**38**:696–700.
231. Thongboonkerd V, Chiangjong W, Saetun P, et al. Analysis of different proteomes in pathogenic and non-pathogenic *Leptospira:* potential pathogenic and virulence factors. *Proteomics* 2009;**13**: 3522–34.
232. Tobie JE, McCullough NB. Serologic evidence of *Leptospira pomona* infections in meat inspectors. *J Am Vet Med Assoc* 1961;**138**:434–6.
233. Torten M, Birnbaum S, Klingberg MA, et al. Epidemiologic investigation of an outbreak of leptospirosis in the Upper Galilee, Israel. *Am J Epidemiol* 1970;**91**:52–8.
234. Torten M, Shenberg E, van der Hoeden J. The use of immunofluorescence in the diagnosis of human leptospirosis by a genus-specific antigen. *J Infect Dis* 1966;**116**:537–43.
235. Trimble AP. Clinical aspects of leptospirosis in Malaya. *Proc R Soc Med* 1957;**50**:125–8.
236. Truccolo J, Serais O, Merien F, Perolat P. Following the course of human leptospirosis: evidence of a critical threshold for the vital prognosis using a quantitative PCR assay. *FEMS Microbiol Lett* 2001;**204**:317–21.
237. Tullu MS, Karande S. Leptospirosis in children: a review for family physicians. *Indian J Med Sci* 2009;**63**:368–78.
238. Tung J, Yang C, Chou S, et al. Calcium binds to LipL32, a lipoprotein from pathogenic *Leptospira*, and modulates fibronectin binding. *J Biol Chem* 2010;**285**:3245–52.
239. Turner LH. Leptospirosis. *BMJ* 1969;**1**:231–5.
240. Turner LH. Leptospirosis. *BMJ* 1973;**1**:537–40.
241. Turner LH. Leptospirosis, part I. *Trans R Soc Trop Med Hyg* 1967;**61**:842–55.
242. Turner LH. Leptospirosis, part II. *Trans R Soc Trop Med Hyg* 1968;**62**:880–99.
243. Turner LH. Leptospirosis, part III. Maintenance, isolation and demonstration of leptospires. *Trans R Soc Trop Med Hyg* 1970;**64**:623–46.
244. Valek K, Neuwirtova R, Chytil M. Treatment of acute renal failure in the course of Weil's disease by the artificial kidney. *Rev Czech Med* 1959;**5**:32–9.
245. van der Hoeden J, Shenberg E, Torten M. The epidemiological complexity of *Leptospira canicola* infection of man and animals in Israel. *Isr J Med Sci* 1967;**3**:880–4.
246. Van Thiele PH. *The leptospiroses*. Leiden: Universitaire Pers Leiden; 1948.
247. Verma A, Kumar P, Babb K, et al. Cross-reactivity of antibodies against leptospiral recurrent uveitis-associated proteins A and B (LruA and LruB) with eye proteins. *PLoS Negl Trop Dis* 2010;**4**: e778.
248. Verma B, Daga SR, Sawant D. Leptospirosis in children. *Indian Pediatr* 2003;**40**:1081–3.
249. Vijayachari P, Sugunan AP, Murhekar MV, et al. Leptospirosis among schoolchildren of the Andaman & Nicobar Islands, India: low levels of morbidity and mortality among pre-exposed children during an epidemic. *Epidemiol Infec* 2004;**132**:1115–20.
250. Vijayachari P, Sugunan AP, Sehgal SC. Evaluation of Lepto Dri Dot as a rapid test for the diagnosis of leptospirosis. *Epidemiol Infect* 2002;**129**:617–21.
251. Vitale G, La Russa C, Galioto A, et al. Evaluation of an IgM-ELISA test for the diagnosis of human leptospirosis. *New Microbiol* 2004;**27**:149–54.
252. Wadsworth A, Langworthy HV, Stewart FO, et al. Infectious jaundice occurring in New York State: preliminary report of an investigation, with report of a case of accidental infection of the human subject with *Leptospira icterohaemorrhagiae* from the rat. *JAMA* 1922;**78**:1120.
253. Wang B, Sullivan J, Sullivan GW, et al. Interaction of leptospires with human polymorphonuclear neutrophils. *Infect Immun* 1984; **44**:459–64.
254. Wang CP, Chi CW, Lu FL. Studies on anicteric leptospirosis, part III: roentgenologic observations of pulmonary changes. *Chin Med J (Peking)* 1965;**84**:298–306.
255. Watt G, Alquiza LM, Padre LP, et al. The rapid diagnosis of leptospirosis: a prospective comparison of the DOT enzyme-linked immunosorbent assay and the genus-specific microscopic agglutination test at different stages of illness. *J Infect Dis* 1988;**157**:840–2.
256. Watt G, Padre LP, Tuazon ML, et al. Placebo-controlled trial of intravenous penicillin for severe and late leptospirosis. *Lancet* 1988;**1**:433–5.
257. Weil A. Ueber eine Eigenthumliche, mit Milztumor, Icterus und Nephritis einhergehende, acute Infectionskrankheit. *Dtsch Arch Klin Med* 1886;**39**:209–32.
258. Werts C. Leptospirosis: a toll road from B lymphocytes. *Chang Gung Med J* 2010;**33**:591–601.
259. Werts C, Tapping RI, Mathison JC, et al. Leptospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* 2001;**2**:346–52.
260. World Health Organization. *Zoonosis and veterinary public health*. Leptospirosis Burden Epidemiology Reference Group (LERG). Last reviewed/updated 13 July 2010 [accessed 16 July 16 2012]. Available at http://www.who.int/zoonoses/diseases/lerg/en/
261. Wylie JAH. Relative importance of renal and hepatic lesions in experimental leptospirosis icterohemorrhagiae. *J Pathol Bacteriol* 1946;**58**:351–8.
262. Yang GG, Hsu YH. Nitric oxide production and immunoglobulin deposition in leptospiral hemorrhagic respiratory failure. *J Formos Med Assoc* 2005;**104**:759–63.
263. Yitzhaki S, Barnea A, Keysary A, Zahavy E. New approach for serological testing for leptospirosis by using detection of *Leptospira* agglutination by flow cytometry light scatter analysis. *J Clin Microbiol* 2004;**42**:1680–5.
264. Zhou G, Wen S, Liu Y, et al. Development of a DNA microarray for detection and identification of *Legionella pneumophila* and ten other pathogens in drinking water. *Int J Food Microbiol* 2011;**145**: 293–300.
265. Zuerner RL, Alt DP, Palmer MV, et al. A *Leptospira borgpetersenii* serovar *Hardjo* vaccine induces a Th1 response, activates NK cells, and reduces renal colonization. *Clin Vaccine Immunol* 2011;**18**: 684–91.

lymphadenopathy is a common finding. The temperature may reach 41° C (105.8° F) in a stepwise fashion over the course of 2 to 4 days and then fall abruptly.[6]

Six to eight regularly occurring relapses of fever may occur, separated by afebrile periods lasting 3 to 7 days. During febrile periods, the patient also may experience myalgia, headache, and vomiting. In approximately 50 percent of patients, a purple to red-brown rash develops and consists of large macules with occasional indurated erythematous plaques or urticarial lesions.[15] In contrast to streptobacillary rat-bite fever, joint manifestations are rare occurrences. In untreated cases, the illness may persist for 3 to 8 weeks, but relapses have occurred after months or years.[13]

Spontaneous cure is the general rule, but several untreated cases have persisted for more than 1 year.[15] The untreated mortality rate is reported to be 6.5 percent.[2] In protracted, untreated cases, severe complications include endocarditis,[8] meningitis,[10] myocarditis, hepatitis, and nephritis.[4] Anemia, weight loss, and severe diarrhea are common complications in infants and children. Epididymitis, nuchal rigidity, headache, pleurisy, pleural effusion, and splenomegaly also have been reported.[12] Mortality has been noted to be significantly lower than in streptobacillary rat-bite fever.[15] Herxheimer reactions have been reported in patients treated with arsenic-based therapies used before penicillin.[13]

The differential diagnosis includes streptobacillary rat-bite fever, as well as many other infectious and noninfectious diseases (see Chapter 138).

DIAGNOSIS

Definitive diagnosis requires isolation and identification of the spirochete. Although the organism rarely may be seen on a peripheral blood smear[1] or darkfield examination of ulcer exudate,[7] animal inoculation usually is required for isolation. Blood or wound aspirates are injected intraperitoneally into guinea pigs or mice. The spirochetes then may be recovered in 5 to 15 days from the animals' blood, which is examined under darkfield microscopy. This process is time consuming and may not be available in most centers. In addition, the inoculated animals must be screened carefully for previous *Spirillum* carriage.

Nonspecific diagnostic criteria include a false-positive test for syphilis in 50 percent of patients, white blood cell count between 10,000 and 20,000 cells/mm,[3] and moderate anemia.[2,15] No specific serologic tests are available for *S. minus*. Molecular methods, such as polymerase chain reaction, may offer hope in the future for detection of *S. minus*.[4]

TREATMENT

Before penicillin, *S. minus* was treated successfully with arsenic therapy; however, response was slower, with reported relapses and even death.[13] In general, *S. minus* is considered more sensitive to penicillin than *S. moniliformis* and penicillin is the drug of choice for both forms of the disease. Doses as low as 24,000 U/day for 5 days have been shown to be effective.[13] However, because distinguishing spirillary disease from streptobacillary disease may be difficult, it is important to treat initially with higher dosages effective against *S. moniliformis* (see Chapter 138).

REFERENCES

1. Bhatt KM, Mirza NB. Rat bite fever: a case report of a Kenyan. *East Afr Med J* 1992;**69**:542–3.
2. Brown T, Nunemaker J. Rat-bite fever: review of American cases with reevaluation of its etiology and report of cases. *Bull Johns Hopkins Hosp* 1942;**70**:201–2.
3. Cole JS, Stoll RW, Bulger RJ. Rat-bite fever: report of three cases. *Ann Intern Med* 1969;**71**:979–81.
4. Fredricks DN, Relman DA. Application of polymerase chain reaction to the diagnosis of infectious diseases. *Clin Infect Dis* 1999;**29**:475–88.
5. Futaki K, Takaki I, Taniguchi T. *Spirochaeta morsus muris* n. sp.: the cause of rat-bite fever. *J Exp Med* 1917;**25**:33–44.
6. Gunning J. Rat-bite fever. In: Hunter G, Swartzwelder J, Clyde D, editors. *Tropical medicine*. Philadelphia: W. B. Saunders; 1976. p. 245–7.
7. Hinrichsen SL, Ferraz S, Romeiro M, et al. [Sodoku: a case report.] *Rev Soc Bras Med Trop* 1992;**25**:135–8.
8. Hitzig W, Liebman A. Subacute endocarditis associated with a spirillum: report of a case with repeated isolation of the organism from the blood. *Arch Intern Med* 1944;**73**:415.
9. Ito A. [Rat-bite fever.] *Ryoikibetsu Shokogun Shirizu* 1999;**24**:300.
10. Kiefer H, Froscher W, Mohr HP. [Rat bite disease with meningoencephalitic involvement.] *Med Klin* 1981;**76**:653–5.
11. McDermott E. Rat-bite fever: study of experimental disease with critical review of the literature. *Q J Med* 1928;**21**:433–58.
12. Robertson A. *Spirillum minus*: etiological agent of rat-bite fever. *Ann Trop Med* 1930;**24**:367.
13. Roughgarden J. Antimicrobial therapy of rat-bite fever. *Arch Intern Med* 1965;**116**:39–53.
14. Shwartzman G, Florman A, Bass M. Repeated recovery of a spirillum by blood culture from two children with prolonged and recurrent fevers. *Pediatrics* 1951;**8**:227.
15. Taber LH, Feigin RD. Spirochetal infections. *Pediatr Clin North Am* 1979;**26**:377–413.

CHAPTER 143

SYPHILIS

Simon R. Dobson • Pablo J. Sánchez

Syphilis was recognized first at the end of the 15th century in Europe, where it appeared seemingly as an emerging infectious disease. It rapidly spread to reach epidemic proportions. Its European origins are a mystery that will not be solved easily, with theories ranging from its being a disease introduced from the New World by the returning crew of Columbus to its being a virulent form of the endemic yaws and bejel of African peoples that appeared later as virulent syphilis in a susceptible European population.

Syphilis initially was called the *Italian disease*, the *French disease*, and the *great pox* (as distinguished from smallpox). Its venereal transmission was not recognized until the 18th century. Delineation of characteristics of syphilis was hindered by the confusion of its symptoms with those of gonorrhea. In 1767, Hunter, a great English experimental biologist and physician, inoculated himself with urethral exudate from a patient with gonorrhea. The patient also had syphilis, and the subsequent symptoms experienced by Hunter convinced two generations of physicians of the unity of gonorrhea and syphilis. The separate natures of gonorrhea and syphilis were shown in 1838 by Ricord, who reported his observations on more than 2500 human inoculations. Recognition of the stages of syphilis followed; and in 1905, Schaudinn and Hoffman discovered the causative agent. The following year, Wassermann introduced the diagnostic blood test that bears his name.

THE ORGANISM

Morphologic characteristics are the primary features by which members of the family Spirochaetaceae are placed into a single taxon. Spirochetes are helix-shaped, heterotrophic bacteria. These organisms are slender, coiled, and flexible, with one or more complete turns in the helix. Spirochetes are motile, their corkscrew motility resulting from the action of axial fibrils known as *endoflagella*. The family Spirochaetaceae has five genera, of which only *Treponema*, *Borrelia*, and *Leptospira* spp. cause major human illnesses. Differentiation among genera of the family Spirochaetaceae is based primarily on the morphology of the organism.

The name *Treponema* is derived from the Greek words meaning, "turning thread." Individual organisms are 5 to 15 μm in length and 0.092 to 0.5 μm in diameter and have finely tapered ends. Whole cells appear to have a flat wave with one or more planes per cell, giving the appearance of a helical coil. Each cell has 8 to 14 evenly distributed waves. They exhibit a sluggish mobility, with drifting motion and graceful flexuous movements.[73] The internal structure of *Treponema pallidum* subsp. *pallidum* generally is similar to that of other spirochetes. An outermost thin three-layered membrane surrounds the protoplasmic cylinder of the cell. Intracytoplasmic microtubules have been described, and such structures may be specific for *Treponema* spp.

The six axial fibrils or endoflagella are long, flagella-like, intracellular organelles that originate at either end of the cell from knoblike structures and extend toward the other end. Axial fibrils vary in length but overlap one another near the middle of the cell. These fibrils are thought to determine the spiral shape of the cells and are responsible for the characteristic motility along the longitudinal axis exhibited by members of Spirochaetaceae.

The complete genome of *T. pallidum* subsp. *pallidum* (Nichols strain) was sequenced by the whole-genome rapid sequencing method.[49] The *T. pallidum* genome is a circular chromosome and is small compared with most bacteria at 1.14 Mb encoding 1041 predicted proteins. By polyacrylamide gel electrophoresis technique, the major constituent proteins of *T. pallidum* have been characterized and include integral membrane proteins with apparent molecular masses of 47 kDa, 34 kDa, 17 kDa, and 15 kDa.[100] These lipoprotein antigens are highly immunogenic in laboratory animals and humans.[7,68]

Virulent strains of *T. pallidum* are propagated by intratesticular inoculation of rabbits, and the inability to propagate the organisms in vitro has hampered study of these microorganisms. The time required for division of the organism to occur in rabbits is slow, at approximately 30 hours. Velocity sedimentation employing discontinuous gradients of radiocontrast medium (Hypaque) successfully has purified and concentrated treponemes extracted from infected rabbit testes. These organisms retain the antigens for the fluorescence test, although motility is lost.

Limited cultivation of *T. pallidum* on monolayers of baby hamster kidney cells in 7 percent carbon dioxide has been reported. Research with in vitro characteristics of these organisms also has resulted in the description of adherence of virulent *T. pallidum* to primary cell cultures of rabbit testicular cells and to an established continuous line of human epithelial cells (HEp-2). To date, however, direct in vitro culture for establishing the diagnosis of *T. pallidum* disease has not been possible. In vitro growth is hindered by the reliance of *T. pallidum* on glycolysis alone for adenosine triphosphate synthesis, its sensitivity to oxygen, and its sensitivity to growth temperature.[73]

Other pathogenic *Treponema* organisms cause characteristic clinical diseases and now are known to be genetically different from *T. pallidum* subsp. *pallidum*. These are *T. pallidum* subsp. *carateum* (pinta), *T. pallidum* subsp.

endemicum (bejel or endemic syphilis), and *T. pallidum* subsp. *pertenue* (yaws). Several nonpathogenic treponemes also inhabit the oral cavity and intestinal tract.[18]

TRANSMISSION

Acquired Syphilis

Syphilis is not a highly contagious disease. An individual who has had sexual contact with an infected partner has approximately a 30 percent chance of acquiring disease. The median infective dose to humans experimentally has been estimated to be 57 organisms. *T. pallidum* has the capability to invade the intact mucous membrane or microabrasions of the skin. Direct inoculation from contact with an infected individual is necessary for infection to occur because survival of the organism outside the host is very limited. The organism is killed easily by heat, drying, soap, and water. Sexual contact is the common method of transmission of acquired disease, and the site of inoculation usually is on the genital organs—the vagina or cervix in females and the penis in males. Other sites include lips, breast, tongue, and abraded areas of the skin. Examining physicians and pathologists may be infected by contact if appropriate barrier protection is not used.

Congenital Syphilis

Congenital syphilis usually results from transplacental infection of the developing fetus from spirochetes in the maternal bloodstream. *T. pallidum* becomes widely disseminated to many tissues in an adult soon after initial infection occurs even if clinical manifestations are delayed by months. Maternal spirochetemia and placental infection are not surprising findings.[10] A neonate occasionally may be infected at delivery by contact with an infectious lesion present in the birth canal or perineum. Intrauterine transmission is supported by isolation of the organism from umbilical cord blood and amniotic fluid,[54,98,124,150] detection of spirochetes in the placenta and umbilical cord in association with typical histopathologic changes,[43,48,109] and detection of specific IgM antibody to *T. pallidum* in neonatal serum obtained at birth.[81,90,122,124] The isolation of *T. pallidum* from 74 percent of amniotic fluid specimens obtained from women with early syphilis also suggests that the organism is capable of traversing the fetal membranes, gaining access to the amniotic fluid and resulting in fetal infection.[82] Breast-feeding is not associated with transmission of syphilis, unless the mother has a chancre on her breast.

Transmission of syphilis to the fetus can occur throughout pregnancy. Occasional reports in the literature describing treponemes in fetal tissue or placentas before the fifth month of gestation were disputed for decades.[42] The thought was that the Langhans cell layer of the cytotrophoblast formed a placental barrier against treponemal invasion of the fetus. Researchers subsequently showed, however, that the layer of Langhans cells in the placenta persisted throughout pregnancy.

In 1976, Harter and Benirschke[59] visualized spirochetes by Warthin-Starry silver stain and immunofluorescence in tissue from two aborted fetuses at gestational ages of 9 and 10 weeks. The expected inflammatory response was not observed in these two fetuses, but such changes have been found in infected fetuses after the 15th week of pregnancy. These investigators noted that researchers describing syphilitic fetuses or placentas in the older literature worked with the products of spontaneous abortions. Such fetal loss caused by syphilis occurred only after 18 weeks of gestation, implying that the fetal loss was a reflection of damage incurred as a result of the host response to the organism. The observation of sequential acquisition by the fetus of the ability to respond to a variety of antigens suggests that inflammation can be present only after the fetus acquires the immunologic ability to recognize the treponeme.[134] The more recent detection of spirochetes in amniotic fluid obtained by amniocentesis from a woman with early syphilis at 14 weeks of pregnancy has shown that the fetus can be infected with *T. pallidum* in early pregnancy.[97] The rate of vertical transmission does increase with advancing gestation, however.

Vertical transmission is related directly to the maternal stage of syphilis, with early syphilis resulting in significantly higher transmission rates than late latent infection. Generally, the greater the time that has elapsed since the woman's primary or secondary infection, the less likely she is to transmit disease to the fetus. Ingraham[64] reported in 1950 that among 251 women with syphilis having a duration of 4 years or less, 41 percent of their infants were born alive and had congenital syphilis; 25 percent were stillborn; 14 percent died in the neonatal period; 21 percent had low birth weight, but no evidence of syphilis; and 18 percent were normal full-term infants. In contrast, only 2 percent of infants born to mothers with late disease had congenital syphilis. In 1952, Fiumara and colleagues[46] reported that untreated primary or secondary syphilis resulted in 50 percent of infants having congenital syphilis, whereas the other half were stillborn or premature or died in the neonatal period. With early latent infection, 40 percent of the infants had congenital syphilis, whereas only 10 percent with late latent disease developed syphilis. These data are supported by a more recent study of Sheffield and colleagues,[130] in which mothers with primary, secondary, early latent, and late latent infection had transmission rates of 29, 59, 50, and 13 percent.

Syphilis is an ulcer-causing disease that is associated with increased sexual transmission of human immunodeficiency virus (HIV). In this regard, increasing numbers of neonates who are infected with congenital syphilis also are born to mothers with HIV, and vice versa. The contribution of maternal coinfection with *T. pallidum* and HIV to vertical transmission of either syphilis or HIV has not been elucidated fully. Virulent *T. pallidum* can promote the induction of HIV gene expression in macrophages, possibly resulting in increased systemic HIV levels and more rapid progression of the HIV infection.[144] A more recent study noted higher rates of congenital syphilis in infants born to coinfected mothers, but the diagnosis of congenital syphilis was based on a surveillance definition used by the Centers for Disease Control and Prevention (CDC) and not on strict diagnostic criteria.[128]

EPIDEMIOLOGY

The introduction of penicillin in 1942, and its subsequent widespread use in the 1950s, resulted in a marked decline in the occurrence of syphilis in the United States. After an increase in the number of cases occurred in the early 1960s, the rates again declined in the 1970s, only to have a dramatic resurgence from 1986 to 1991.[20,31,115] The rapid increase during these years in the rate and numbers of cases of primary and secondary syphilis in adults, specifically among women of child-bearing age, resulted in a significant increase in rates of congenital syphilis. Rates of syphilis were greatest among blacks and Hispanics residing in large urban centers. The disease was centered in populations in which substance abuse, particularly crack cocaine, was common and where there was involvement with the sex trade industry often in exchange for drugs.[20]

Other factors that may have contributed to the increased rates of syphilis included underfunded and overwhelmed public health resources; use of spectinomycin for treatment of penicillinase-producing *Neisseria gonorrhoeae* because spectinomycin is ineffective against incubating syphilis; and failure to implement safer sexual practices, especially among adolescents and young adults. Generally, individuals who acquire syphilis characteristically are young and often have had contact with an average of five individuals during the incubation period. Because of the high rate of dual infection (8%) with *N. gonorrhoeae*, some individuals identified and treated for gonorrhea with ceftriaxone also are treated for syphilis while in the pre-primary stage of disease.

Strenuous attempts were made to control syphilis, and the lowest rate ever reported in the United States was reached in 2000, with the rate of primary and secondary syphilis declining by 90 percent between 1990 and 2000 to 2.1 per 100,000 population.[22] The reasons for this decline included (1) wider screening practices secondary to medical and public awareness of the syphilis epidemic of the late 1980s that led to identification and treatment of infected individuals; (2) increased state and local funding for syphilis control programs such as partner notification, community-based screening and presumptive treatment strategies, and risk-reduction counseling; (3) introduction of HIV prevention programs that target prevention of other sexually transmitted diseases (STDs); and (4) decrease in crack cocaine use and trading sex for drug behaviors; and possibly (5) development of acquired immunity to syphilis among high-risk populations that resulted in less reacquisition of infectious syphilis.

The concentration of cases in the United States to a few geographic areas, especially in the southeastern United States, led to an optimistic "National Plan to Eliminate Syphilis" announced by the CDC in 1999.[138] The plan had to be restated in 2006 in the presence of resurgent rates.[22] A gender gap has been noted, with rates of primary and secondary syphilis in men increasing from 3 per 100,000 in 2001 to 5.7 per 100,000 in 2006, whereas a sustained increase in rates in women has been from 0.8 per 100,000 in 2004 to 1 per 100,000 in 2006. The increase in male rates has been explained partly by cases in men who have sex with men and attributed to high-risk sexual behaviors and coinfection with HIV. With the rates also increasing in heterosexuals, the rates of congenital syphilis also have rebounded.

In 1987, the reported rate of congenital syphilis was 10.5 cases per 100,000 live births. By 1991, there were 4398 cases of congenital syphilis (107 per 100,000 live births). These rates reflect a reporting case definition that was changed in 1988[20,155] and resulted in an almost fourfold increase in the number of cases reported to the CDC.[31] There also was a genuine increase, however, in the number of actual cases and case rates secondary to the increase in early syphilis among women.[70] Case rates of congenital syphilis were greatest in the Northeast (186.2 cases per 100,000) and least in the Midwest (54.8 per 100,000).[41] Canadian rates have followed a similar trend where it was notable that delayed access to antenatal care was a key factor, in that all women who were delivered of infants with congenital syphilis had histories of substance abuse and street involvement.[135]

In 1988, investigators recognized that prior definitions of congenital syphilis that had been used for surveillance and for treatment decisions had been difficult to apply to the clinical setting because they required a diagnosis that often could be established only over weeks or months. During that period, many children were lost to follow-up and were neither treated nor reported.[31] Because of the high incidence of congenital disease in infants born to inadequately treated mothers, current definitions of congenital syphilis for a presumptive case (which should be reported and treated) require only (1) that the infant be born to a mother with untreated or inadequately treated syphilis or (2) that the child have physical or laboratory signs of congenital syphilis. A summary of the surveillance case definition used since 1988 is provided in Table 143-1.

Minor differences exist among case definitions of congenital syphilis as formulated by several agencies and experts.[120] Some experts would consider as a presumptive case a neonate who is well clinically but was born to a mother who had contact within 90 days before delivery with an individual with primary or secondary syphilis and who had not been treated or had been treated inadequately, even if the mother has nonreactive serology. Although recommendations for therapy commonly have assumed that adequate therapy given to a mother with primary or secondary syphilis during pregnancy would prevent congenital syphilis with a high degree of reliability, reasons to doubt this premise have emerged.[3,87,154] In particular, treatment failures in which the infant developed syphilis despite maternal therapy have been reported when the mother was treated first within 30 days of delivery.[12,14,86,99,119]

An infant born to a mother who was treated within 30 days of delivery is considered to have been treated inadequately, and the infant should receive appropriate therapy. Table 143-2 lists the various circumstances in which maternal therapy may be presumed to be subtherapeutic. The consequences of inadequate therapy for the mother are shown in data in Table 143-3. In that experience, 13 percent of children born to inadequately treated mothers had congenital syphilis, all of which was neurosyphilis.[118]

TABLE 143-1 Surveillance Case Definition for Congenital Syphilis

A *confirmed case* of congenital syphilis is when *Treponema pallidum* is identified by darkfield microscopy, fluorescent antibody, or other specific stains in specimens from lesions, placenta, umbilical cord, amniotic fluid, or autopsy material.

A *presumptive case* of congenital syphilis can occur in either of the following:

A. Any infant whose mother had untreated or inadequately treated* syphilis at delivery, regardless of findings in the infant *or*

B. Any infant or child who has a reactive treponemal test for syphilis and any one of the following:
   1. Any evidence of congenital syphilis on physical examination *or*
   2. Any evidence of congenital syphilis on long bone radiograph *or*
   3. Reactive cerebrospinal fluid VDRL *or*
   4. Elevated cerebrospinal fluid cell count or protein (without other cause) *or*
   5. Quantitative nontreponemal serologic titers that are fourfold higher than the mother's (both drawn at birth) *or*
   6. Reactive test for FTA-ABS-19S-IgM antibody

A *syphilitic stillbirth* is defined as a death of a fetus weighing >500 g or having a gestational age >20 weeks in which the mother had untreated or inadequately treated syphilis at delivery.

FTA-ABS, fluorescent treponemal antibody absorption; VDRL, Venereal Disease Research Laboratory.

*Inadequate treatment consists of any nonpenicillin therapy or penicillin given <30 days before delivery.

Modified from Centers for Disease Control. Congenital syphilis, New York City, 1986-1988. *MMWR Morb Mortal Wkly Rep* 1989;**38**:828.

TABLE 143-2 Circumstances in Which Maternal Therapy for Syphilis May Be Subtherapeutic or Inadequate

Treatment with a nonpenicillin regimen (including macrolide antibiotics)

History of maternal treatment was not documented fully or verifiable

Treatment during the month before delivery

Treatment in HIV-infected women

Serial posttherapy assays of maternal nontreponemal antibody titers were not performed

Serial posttherapy assays of maternal nontreponemal antibody titers did not show a fourfold decline in titers after treatment of early syphilis, not permitting assessment of adequacy of therapy, and suggesting possibility of failure to eradicate infection

Serial posttherapy assays of maternal nontreponemal antibody titers show a fourfold increase in titers, suggesting reinfection or relapse

HIV, human immunodeficiency virus.

Substantial underreporting of infected infants did occur previously,[38] but the revised surveillance definition does not represent diagnostic criteria. Rather, it reflects the public health burden of the disease because these infants require medical and public health interventions.

TABLE 143-3 Clinical Findings of 148 Infants Whose Mothers Had Syphilis and Whose Treatment Was Adequate, Inadequate, or Not Provided

	Maternal Treatment		
Infant Findings	NONE (N = 72)	INADEQUATE (N = 31)	ADEQUATE (N = 45)
Clinical disease	3	0	0
Positive CSF-VDRL test	7	4	0
Abnormal bone radiograph	6	0	0
Stillbirth	6	0	0
Total (any abnormality)	22 (31%)	4 (13%)	0

CSF, cerebrospinal fluid; VDRL, Venereal Disease Research Laboratory.

From Reyes MP, Hunt N, Ostrea EM, et al. Maternal/congenital syphilis in a large tertiary care urban hospital. *Clin Infect Dis* 1993;**17**:1041-6.

Notwithstanding this change in reporting guidelines, the number of cases of congenital syphilis had continued to decline from a peak of 107 per 100,000 live births in 1991; after 14 consecutive years of decline, the rate increased between 2005 and 2008 (from 8.2 to 10.1 cases per 100,000 live births) but has since declined to 8.7 cases per 100,000 live births in 2010, reflecting a similar decline in primary and secondary syphilis in women.[23]

Worldwide syphilis remains a considerable public health problem. Russia reported 730 cases in 2001, an increase from 15 cases in 1991. Russia and countries of the former Soviet Union have rates of syphilis in pregnancy of 1 to 2 percent. In the Caribbean and Latin America, these rates are 2 to 7 percent, but they are even worse in Southern Africa, with 2 to 17 percent of all pregnancies occurring in women with syphilis.[146] The toll of untreated maternal syphilis on the outcomes of pregnancy is severe. An estimated one fourth of all stillbirths and 10 percent of neonatal deaths in Southern Africa are caused by congenital syphilis.[143] China has the highest, recent documented increase in acquired and congenital syphilis, where the incidence of congenital syphilis increased with an average yearly rise of 71.9 percent, from 0.01 cases in 1991 to 19.68 cases per 100,000 live births in 2005. This likely still underestimates the total burden of congenital syphilis in China, because it does not take into account stillbirths.[28] The World Health Organization (WHO) estimates that every year 1 million pregnancies are complicated by syphilis, with half resulting in early pregnancy abortion or perinatal death, a fourth resulting in low birth weight or premature birth, and a final fourth resulting in overt congenital syphilis.[146]

Congenital syphilis is a disease that should be amenable almost fully to eradication if currently available prenatal health measures were implemented completely. Table 143-4 presents two studies in the United States of

TABLE 143-4 **Prenatal Care and the Occurrence* of Congenital Syphilis in Infants**

Prenatal Care	Mascola et al., 1984[84] (n = 50)	Coles et al., 1995[30] (n = 318)
No prenatal care	56	46
First prenatal test negative; testing not repeated in late pregnancy	6	14
Medical mismanagement	6	10
Failure of conventional prenatal syphilis treatment of mother	8	5
Negative maternal syphilis test at delivery	14	—
Infection late in pregnancy or no prenatal care until late in pregnancy	—	20
Mother not tested	8	3
Laboratory error	2	—

*Numbers represent percentage of total cases in study.
Modified from Gutman LT. Congenital syphilis. In: Mandell GL, editor. *Atlas of infectious disease. Vol 5. Sexually transmitted diseases.* Philadelphia: Current Medicine; 1996.

prenatal care of women who were delivered of infants with congenital syphilis and indicates the hurdles that control of the disease will have to overcome.[32,87] Lack of prenatal care is the most important one.[36,89,148] National surveillance data analyzed by the CDC in 2002 highlight the fact that cases of congenital syphilis still occur even when prenatal care is implemented if the mother's response to treatment is inadequate or if the infant's evaluation is not thorough (Fig. 143-1).[21]

Penicillin was a major reason for the decline of the treponematoses seen in the early 1950s.[43] Other factors must be considered in the attempted eradication of these infections. Nonvenereal endemic treponemal infections (endemic syphilis, bejel, yaws, pinta) have continued to flourish in underdeveloped areas of the world where hygiene is poor, despite the introduction of penicillin. Progress in control of these diseases requires that mass treatment programs be coupled with efforts to improve local living conditions. In areas where this goal could be accomplished, a resulting decline in endemic treponematoses occurred.

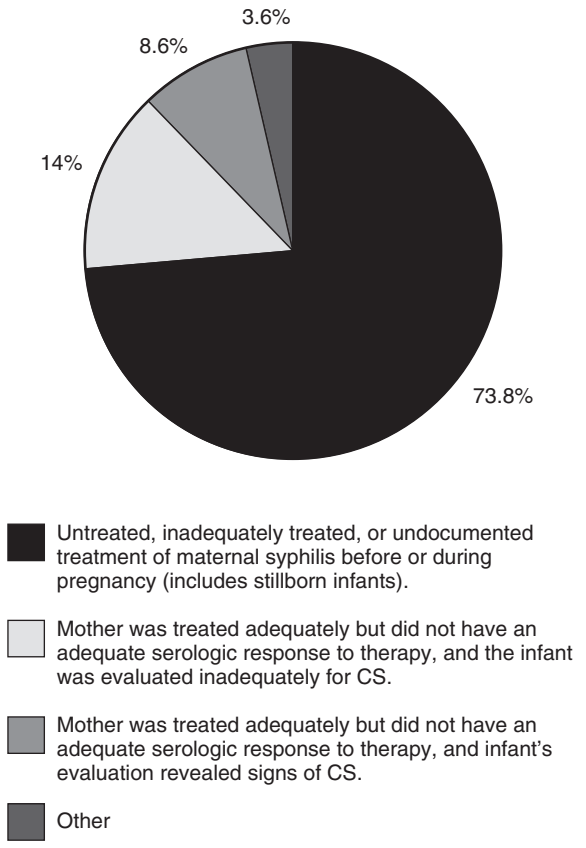

FIGURE 143-1 ■ **Maternal treatment history of 451 cases of congenital syphilis (CS) reported in 2002.** (From Centers for Disease Control and Prevention. Congenital syphilis—United States, 2002. *MMWR Morb Mortal Wkly Rep* 2004;**53**:716-9.)

PATHOLOGY

Syphilis often is a lifelong infection that progresses in three clear characteristic stages (Fig. 143-2). After initial invasion occurs through mucous membranes or skin, the organism undergoes rapid multiplication and is disseminated widely. Spread through the perivascular lymphatics and then through the systemic circulation probably occurs even before the clinical development of the primary lesion. Ten to 90 days later, usually within 3 to 4 weeks, the patient manifests an inflammatory response to the infection at the site of the inoculation. The resulting lesion, the chancre, is characterized by the profuse discharge of spirochetes; accumulation of mononuclear leukocytes, lymphocytes, and plasma cells; and the swelling of capillary endothelia. The regional lymph nodes are enlarged, and the cellular infiltrate resembles that of the primary lesions. Resolution of the primary lesion occurs by fibrosis.

Secondary lesions develop when tissues of ectodermal origin, such as skin, mucous membranes, and central nervous system (CNS), participate in an inflammatory response. Mucous patches in the mouth are caused by local vasculitis. The cellular infiltrate resembles that of the primary lesion, with the predominance of plasma cells. Little or no necrosis is present, and healing is without scarring but may include pigmentary changes.

Tertiary syphilis may involve any organ system and often is asymmetric. Gummas are lesions typified by extensive necrosis, a few giant cells, and a paucity of

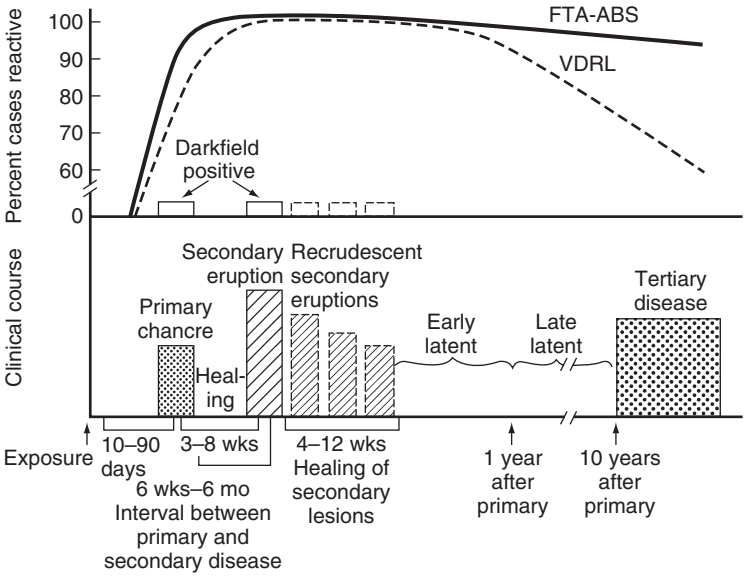

FIGURE 143-2 ■ The course of untreated syphilis. FTA-ABS, fluorescent treponemal antibody absorption; VDRL, Venereal Disease Research Laboratory.

organisms. They commonly occur in internal organs, bone, and skin. The other major form of tertiary lesion is a diffuse chronic inflammation, with plasma cells and lymphocytes but without caseation, which may result in an aneurysm of the aorta, paralytic dementia, or tabes dorsalis. Chronic swelling of the capillary endothelium and fibrosis result in the characteristic tissue changes.

Congenital syphilis is a result of hematogenous infection and the disseminated involvement of almost all viscera. The intense inflammatory response occurs in the perivascular framework and interstitial stroma, rather than in the parenchyma.[96] Bone, liver, pancreas, intestine, kidney, and spleen are involved most reproducibly and severely. Other tissues, such as the brain, pituitary gland,[14] lymph nodes, and lungs, also may be infected. The gastrointestinal tract shows a pattern of mononuclear cell infiltration in the mucosa and submucosa, with subsequent widening of the submucosa by the ensuing fibrosis. This event is most prominent in the small bowel. In the kidney, a perivascular inflammation, particularly in the juxtamedullary region, is evident. The basic architecture of the tissue influences the ultimate pattern of involvement. The deposition of collagen around arteries of the spleen produces a typical onionskin appearance. Periosteum and epiphyses are the most affected portions of bone, and syphilitic granulation tissue may interfere with bone formation. Pancreatitis also is observed, with typical inflammation and fibrosis.

The fetus or neonate shows diffuse extramedullary hematopoiesis in many tissues. The placenta of infants with congenital syphilis often is large, thick, and pale. Three histopathologic features commonly are seen: enlarged and hypercellular villi, proliferative fetal vascular changes, and acute and chronic inflammation of the villi.[52,130] Erythroblastosis involving the placenta has been seen more frequently among stillborn infants with congenital syphilis than in liveborn infants with or without syphilis.[130]

Spirochetes may be identified in placental tissue using conventional staining, although they may be difficult to visualize. Nucleic acid amplification methods also have been used to identify *T. pallidum* genome in involved placental specimens.[52] In addition, the umbilical cord may exhibit significant inflammation, with abscess-like foci of necrosis located within Wharton jelly and centered around the umbilical vessels, which is termed *necrotizing funisitis*.[48,131] Macroscopically, the umbilical cord resembles a "barber's pole"; the edematous portions have a spiral striped zone of red and pale blue discoloration, interspersed with streaks of chalky white. Histochemical staining may show spirochetes within the wall of the umbilical vessels. Placental and umbilical cord histopathology should be performed on every case of suspected syphilis.[131]

PATHOGENESIS AND IMMUNE RESPONSE

The host immune response to syphilis is vigorous and is responsible for many of the clinical manifestations seen in the disease. Despite this immune response, the infection may persist for life. In addition, prior infection does not confer resistance to reinfection. Researchers continue to try to explain this contradiction.[104]

Treponemal Virulence–Associated Factors

Virulent *T. pallidum* attach to host cells during parasitism and are oriented by the proximal hook to the host cell surface. There seems to be a ligand-receptor adherence mechanism involving the treponemal outer-membrane proteins. Virulent strains attach to metabolically active mammalian cells, and treponemes are capable

of multiplication only during attachment. Fetal and infant cells seem to support treponemal growth maximally, and capillary cells are the prime target of parasitism. Virulent treponemal strains produce hyaluronidase, which may facilitate the perivascular infiltration that is apparent by histopathologic study. These strains also may invade the ground substance that joins capillary endothelial cells.

Virulent *T. pallidum* is coated with fibronectin having host origin. This coating seems to protect the organism from antibody-mediated phagocytosis; allows the organism to adhere to the surface of host phagocytes with only limited ingestion of the organisms; may block complement-mediated lysis of the coated treponemes; and, finally, may allow the treponemes additionally to acquire physiologically active host proteins, such as ceruloplasmin and transferrin, on which they are dependent.[105]

Host Response

Treponemes seem to persist in extracellular loci with little or no inflammatory response elicited. Polymorphonuclear leukocytes ingest virulent treponemes, incorporate them into phagocytic vacuoles, degranulate, and digest *T. pallidum*. In addition, phagocytosis occurs slowly and is facilitated by the presence of immune serum.[2] Large numbers of treponemes are needed to elicit this response, however, and small numbers may escape recognition.[93] Although there are few surface-exposed transmembrane proteins, one more recently described, Tpr K, seems to act as a porin.[26] It has been shown to elicit an opsonic antibody response in the rabbit model. Variable regions of the protein are explained by diversity in the encoding genes. This antigenic variation could explain the success of the treponeme in evading the host response.[25,77,91] Innate immunity through Toll-like receptor 4 is activated by lipoproteins under the outer membrane and may be important in orchestrating the overall immune response to *T. pallidum*.[16,111,121]

Alterations in host cell-mediated immunity occur during primary and secondary stages of syphilis. During early stages of disease, all aspects of cell-mediated immunity, including responses to nonspecific T-cell mitogens, are suppressed. Blastogenesis to treponemal antigens is not demonstrable until late in the course of secondary disease. Natural killer cell activity is increased during early primary syphilis and depressed in secondary and latent syphilis.[67] Some evidence supports the idea that lymphocytes from previously infected animals confer moderate protection to nonimmune animals. In human disease, $CD4^+$ T cells and macrophages predominate in primary chancres, whereas $CD8^+$ cells predominate in secondary syphilis lesions. In primary and secondary syphilis lesions, T helper cell type 1 (Th1) cytokines are present.[107,121,145] Some investigators have suggested that increased apoptosis of peripheral blood lymphocytes and $CD4^+$ cells by a Fas-mediated pathway that occurs during the secondary stage of infection may explain the failure to clear the organism and set up a chronic infection.[40] Alterations in the immune environment of the mother during pregnancy may alter the balance of containment of the organism, allowing transplacental passage to the fetus.[153]

During the initial infection with *T. pallidum*, humoral IgG and IgM antibodies are detectable by the time the chancre appears. In primary syphilis, the main immunoglobulin (Ig) G subclass is IgG1, whereas IgG1 and IgG3 predominate in secondary syphilis.[9] If the patient is treated adequately, IgM antibody declines during the next 1 to 2 years, but IgG antibody usually persists through the lifetime of the patient. The stages of syphilis evolve, despite humoral antibody response.

Individuals with untreated syphilis have only a relative resistance to reinfection, so the development of a chancre with reinfection is unusual and probably depends on the challenge inoculum. After reexposure, untreated individuals may develop an increased humoral antibody level.

In individuals who have been treated for syphilis, especially if treatment was provided during the secondary or earlier stages, the protective effect of prior disease is minor, and active disease frequently occurs after reinfection, regardless of whether the patient maintains a reactive nontreponemal antibody test (serofast) or is serononreactive. Patients who have been treated for congenital syphilis also may acquire symptomatic disease.[45,103] Although active or prior syphilis modifies the response of a patient to subsequent reinfection, protection is only relative and is unreliable.

Secondary syphilis and congenital infection may be accompanied by the nephrotic syndrome. The nephrosis characteristically responds rapidly to penicillin, and light microscopy reveals membranous glomerulonephritis with glomerular mesangial cell proliferation. The subendothelial basement membrane deposits contain IgG and C3 or only globulin. Acute syphilitic glomerulonephritis seems to be an immune complex disease.[15,50,69] In infants with congenital syphilis, analysis of circulating immune complexes using immunoblotting methods showed the presence of an 83-kDa *T. pallidum* antigen.[37] Findings have been similar with secondary syphilis.

CLINICAL MANIFESTATIONS

The course of untreated syphilis characteristically progresses through three or four stages over many years. Information on the natural history of untreated syphilis comes primarily from follow-up studies of almost 2000 untreated syphilitic patients who were seen initially between 1891 and 1910 in the clinic of Boeck. The subsequent studies of these patients by Bruusgaard and by other epidemiologists later provided the basis for most of our concepts of the consequences of syphilis. Clark and Danbolt[30] published a summary of some of these studies. Figure 143-2 depicts some of the characteristics of an untreated course of disease.

Acquired Syphilis

Most recognized syphilitic disease in children is congenital. Acquired syphilis in prepubertal children seldom is reported and is assumed to resemble the clinical course of acquired syphilis in adulthood. Children with acquired syphilis should be assumed to have been infected through

contact during child sexual abuse, unless another method of transmission is identified. All patients with syphilis should be tested for HIV infection.[24]

The decision to screen for syphilis during the medical evaluation of a child who is suspected of having been sexually abused should be made on an individual basis because the prevalence of STDs is low in these circumstances. Situations in which screening is recommended include the following: the perpetrator has an STD or is at high risk for having one, such as residing in a community or being of a social setting in which the prevalence of STDs is high; multiple perpetrators are involved; the child is infected with another STD, such as HIV infection, or has physical signs of sexual abuse; STDs have been diagnosed in siblings, other children, or adults in the household; the patient is postpubertal; or screening is a matter of patient or family preference.[11,74,132,133] A difficult aspect of the follow-up is the requirement of repeat serologic assays in 3 to 6 months because the initial test may not detect incubating syphilis.

Primary Disease

The chancre of primary syphilis typically is a single reddish lesion, nontender and firm, with a clean surface and raised border. It may be overlooked by women because it frequently is situated on the cervix or vaginal wall. Systemic signs or symptoms are absent, but adjacent lymph nodes frequently are enlarged and nontender. Gutman[56] provides depictions of children with acquired primary syphilis.

Secondary Disease

The patient may experience secondary disease 2 to 10 weeks after the primary lesions manifest. Prominent findings include fever, sore throat, muscle aches, generalized lymphadenopathy, headache, and rash. The palms and soles frequently are involved, in contrast to many other dermatologic conditions. On mucous membranes, the lesions may appear as white mucous patches. Condylomata lata occur around moist areas, such as the anus and vagina. All secondary lesions of the skin and mucous membranes are highly infectious. In their review, Baughn and Musher[10] give a full description of the manifestations of secondary syphilis. Acquired syphilis in early childhood may reveal minimal dermal findings.[1]

Latent Disease

After the clinical signs of secondary disease resolve, the patient enters the stage of latent disease. *Latent syphilis* refers to infection in individuals who have reactive serologic tests for syphilis but no clinical manifestations. The first year after infection is considered *early latent infection*, and the subsequent period is considered *late latent syphilis*. Early latent syphilis originally was classified as syphilis occurring within 4 years of the acquisition of infection, based on the time when mucocutaneous lesions may recur. More recent classification of early latent syphilis is based on the time period of highest communicability, which is in the first year after infection is acquired. Treponemes still can be present in the blood intermittently, however, and pass across the placenta to the fetus during latent syphilis in a pregnant woman.

If the duration of infection with syphilis cannot be determined, the disease is classified as syphilis of unknown duration; clinical management and treatment should be the same as that for late latent infection. If therapy for syphilis is given first during the latent stage, the patient is unlikely to show regression of nontreponemal antibody determinations. Approximately 60 percent of untreated patients in the late latent stage continue to have an asymptomatic course, whereas 30 to 40 percent develop symptoms of late or tertiary disease. Progression of disease from late latent to late symptomatic syphilis usually is prevented if appropriate antimicrobial therapy is given at this stage. The reader is referred to the article by Gutman[56] for depictions of children with acquired secondary syphilis.

Tertiary Disease

Three to 10 years after the last evidence of secondary disease has occurred, the patient may develop nonprogressive, localized nodules of the dermal elements or supporting structures of the body, called *gummas*. These nodules are granulomatous and can have central necrosis. Because these lesions are relatively quiescent, the term *benign tertiary syphilis* often is used. Spirochetes are extremely sparse or absent. The gummatous reaction is primarily a pronounced immunologic reaction of the host.

Neurosyphilis

During the early stage of syphilis, approximately one third of all patients have involvement of the CNS. If untreated, only half of these patients develop late neurosyphilis. The interval between primary disease and late neurosyphilis usually is more than 5 years. Of the few children recognized to have acquired (vs. congenital) late neurosyphilis, several have developed symptomatic disease at a very early age. It is possible that the disease progresses more rapidly in children than in adults.

Late neurosyphilis may be asymptomatic or if symptomatic may occur in a variety of ways. Classic presentations include paralytic dementia, tabes dorsalis, amyotrophic lateral sclerosis, meningovascular syphilis, seizures, optic atrophy, and gummatous changes of the cord. Neurosyphilis may resemble virtually any other neurologic disease.

Cardiovascular Syphilis

Ten to 40 years after having primary syphilis, an untreated patient may develop signs of cardiovascular involvement. Most frequently involved are the great vessels of the heart, where syphilitic aortic and pulmonary arteritis develop. One complication of this development is aortic regurgitation. The inflammatory reaction also may cause stenosis of the coronary ostia, with resulting angina, myocardial insufficiency, and death.

Syphilis in Pregnancy

Pregnancy has no known effect on the clinical course of syphilis. Untreated syphilis can affect profoundly the outcome of the pregnancy, however, resulting in spontaneous abortion, stillbirth, nonimmune hydrops fetalis,[8,17] premature delivery, perinatal death, or two characteristic syndromes of congenital disease, early and late congenital syphilis (see later discussion).[55,123] The outcome of untreated fetal infection varies. Intrauterine death occurs in an estimated 25 percent of infections, with abortion usually occurring after the first trimester. Perinatal death may occur in another 25 to 30 percent of untreated infected infants.[96] In a study of the perinatal outcome of congenital syphilis, the fatality rate was 464 per 1000 infected births. Of the fatalities, 27 percent were neonatal deaths and 73 percent were stillbirths.[119] Currently, most infected infants, if not hydropic at birth, survive the neonatal period, most likely because of early identification and treatment of infected infants and improved neonatal care.

The investigations of Taber and Huber[141] illustrate the importance in pregnant women of recognition of the disease and administration of appropriate therapy. In this study, 22 women with syphilis were undiagnosed during pregnancy and did not receive prenatal therapy. Eleven infants were followed without initial therapy. At delivery, all were asymptomatic clinically, 4 had a reactive Venereal Disease Research Laboratory (VDRL) test, 5 had a nonreactive VDRL test, and 2 were not tested. All these infants were readmitted with obvious disease involving multisystem infection, 3 with proven spirochetal hepatitis, 2 with nephrotic syndrome, and 3 with CNS involvement. This experience emphasizes the importance of recognition of transmission of infection late in gestation, leading to delivery of infants who are well clinically but whose disease emerges in the weeks after delivery if untreated.[39]

Congenital Syphilis

At the onset of congenital syphilis, *T. pallidum* is liberated directly into the circulation of the fetus, resulting in spirochetemia with widespread dissemination. The clinical, laboratory, and radiographic abnormalities of congenital syphilis are a consequence of active infection with *T. pallidum* and the resultant inflammatory response induced in various body organs and tissues. The severity of these manifestations varies and can range from overwhelming involvement of multiple organs and body systems, as is seen in fetal hydrops, to only laboratory or radiographic abnormalities. Most of the infants born to mothers with untreated syphilis seem completely normal at birth and have no clinical or laboratory evidence of infection at birth. These infants may develop manifestations of disease several months to years later if left untreated.[29] The signs and symptoms of congenital syphilis are divided arbitrarily into early manifestations, which appear in the first 2 years of life, and late manifestations, which emerge at any time thereafter, usually near puberty.

TABLE 143-5 **Findings in 310 Cases of Early Congenital Syphilis**

Findings	No. Patients
Hepatomegaly	100
Skeletal abnormalities	91
Birth weight <2500 g	51
Skin lesions	45
Hyperbilirubinemia	40
Pneumonia	51
Splenomegaly	56
Severe anemia, hydrops, edema	50
Snuffles, nasal discharge	27
Painful limbs	22
Pancreatitis	14
Cerebrospinal fluid abnormalities	21
Nephritis	11
Failure to thrive	10
Testicular mass	1
Chorioretinitis	1
Hypoglobulinemia	1

Data from references 43, 75, 88, 90, and 94-97.

Early Congenital Syphilis

The abnormal physical and laboratory findings in early congenital syphilis are varied and unpredictable.[112] Table 143-5 lists the major physical and laboratory findings from 310 reported cases of early congenital syphilis.* These results undoubtedly are minimal rates for each finding. The onset occurs between birth and approximately 3 months of age, with most cases occurring within the first 5 weeks of life.

Skeletal System. Bone involvement is the most common manifestation of congenital syphilis, occurring in 60 to 80 percent of infants with clinical signs of syphilis. In addition, it may be the only abnormality seen in infants born to mothers with untreated syphilis. Because of their frequency and early appearance, the radiographic changes in the bones, termed *osteochondritis* and *periostitis*, are of diagnostic value.[34] The femur and humerus are involved most often. Radiographically, accumulating calcified matrix is seen at the epiphyseal margin, which may be smooth or serrated. The serrated appearance is known as *Wegner sign* and represents points of calcified cartilage along the nutrient cartilage canal.

A zone of rarefaction at the metaphysis, which represents syphilitic granulation tissue containing a few scattered calcified remnants and a mass of connective tissue containing areas of perivascular infiltration of small round cells, may be seen. Irregular areas of increased density and rarefaction produce the motheaten appearance of the radiograph. The demineralization and osseous destruction of the upper medial tibial metaphysis are called *Wimberger sign*. Previously thought to be specific for syphilis, Wimberger sign also may occur in osteomyelitis and hyperparathyroidism. Epiphyseal separation

*References 17, 34, 69, 76, 101, 119, 141, 142.

may occur as a result of a fracture of the brittle layer of calcified cartilage. Irregular periosteal thickening also is a common finding. The changes usually are present at birth but may appear in the first few weeks of life. The bony changes are self-limited and usually are healed in the first 6 months, even in the absence of specific therapy. They may be painful lesions, and pain on motion often leads an affected infant to appear to have a limb paralysis, termed *pseudoparalysis of Parrot.*

Rhinitis. Rhinitis, coryza, or snuffles are likely to mark the onset of congenital syphilis. Usually, it appears in the first week of life and seldom later than the third month. The snuffles are more severe and persist longer than the common cold. The nasal discharge is white and often bloody, and the snuffles frequently are associated with laryngitis. Secondary bacterial superinfection may result in a purulent appearance. The nasal discharge is teeming with spirochetes and should be examined by darkfield microscopy to confirm its diagnosis.

Rash. The syphilitic rash usually appears 1 to 2 weeks after rhinitis manifests. The typical eruption is maculopapular and consists of small spots that are dark red-copper. If the rash is present at birth, it often is widely disseminated and bullous and is called *pemphigus syphiliticus.* It is most severe on the hands and feet. The bullous fluid contains many spirochetes. The rash erupts slowly, taking 1 to 3 weeks, and is followed by desquamation and crusting. As the rash fades, the lesions become coppery or dusky red, and pigmentation may persist. The reader is referred to the article by Gutman[56] for depictions of a variety of cutaneous manifestations of early congenital syphilis.

Fissures and Mucous Patches. Fissures and mucous patches are not seen often but are highly characteristic features of congenital syphilis. The fissures develop around the lips, nares, and anus. They bleed readily and heal with scarring. A cluster of scars radiating around the mouth is called *rhagades* and is a characteristic of late congenital syphilis. Mucous patches may be found on any of the mucous membranes, especially in the mouth and genitalia. Condylomata lata are raised, moist lesions appearing on areas of the skin where there is moisture or friction. They are highly infectious because they contain many spirochetes.

Hematologic Findings. Congenital syphilis is characterized by anemia, thrombocytopenia, hemolytic processes, and leukopenia and leukocytosis.[76] The hemolytic process is Coombs test negative and often accompanied by cryoglobulinemia, immune complex formation, and macroglobulinemia. The hemolysis, similar to the liver disease, is refractory to therapy and may persist for weeks. Paroxysmal nocturnal hemoglobinuria is a late manifestation of congenital syphilis.

Central Nervous System Involvement. In the era before the introduction of penicillin therapy, approximately 15 percent of infants with congenital syphilis developed manifestations of meningovascular disease. These findings included meningitis, meningeal irritation, bulging fontanelle, cranial nerve palsies, seizures, and hydrocephalus.[63] In addition, involvement of the pituitary gland in congenital syphilis is a common manifestation, occurring in approximately 40 percent of autopsy cases,[101] and consists of interstitial inflammation and fibrosis with formation of gumma in the anterior lobe. Clinical disease in affected infants is manifested by persistent hypoglycemia and diabetes insipidus.[35,99]

Most infants infected with *T. pallidum* are completely asymptomatic, and involvement of the CNS is inferred from abnormalities of the cerebrospinal fluid (CSF), such as reactivity on a VDRL test, pleocytosis, and elevated protein content. Because of the wide range of normal values for CSF protein, red blood cells, and white blood cells in the neonatal period, defining the proportion of infants with congenital syphilis who have abnormalities of these laboratory values has been difficult. Current consensus identifies an abnormal CSF white blood cell count in infants being evaluated for possible congenital syphilis as greater than 25 cells/mm^3 and protein as greater than 150 mg/dL (>170 mg/dL if infant is premature). A reactive CSF VDRL test is considered to be specific for neurosyphilis in older children and adults. In neonates, the significance of a reactive CSF VDRL test is suspect, however, because maternal nontreponemal IgG antibodies can pass from maternal serum to fetal and neonatal serum and then diffuse into the CSF. Children may fail to have a reactive VDRL test on initial examination and still develop later signs of neurosyphilis.

Using rabbit infectivity testing, which involves the inoculation of CSF into rabbits to determine the presence of the spirochete in the CSF specimen, Michelow and coworkers[90] found that invasion of the CNS with *T. pallidum* occurs in 41 percent of infants who have clinical, laboratory, or radiographic abnormalities of congenital syphilis. None of these infants had clinical signs of neurologic disease. Compared with isolation of spirochetes in CSF by rabbit inoculation, the sensitivity and specificity in CSF of a reactive VDRL test, elevated white blood cell count, and elevated protein were 54 percent and 90 percent, 38 percent and 88 percent, and 56 percent and 78 percent, respectively. These investigators also documented CNS infection in three infants who had normal CSF studies. Using current methods, the best indicator of CNS infection in neonates is an abnormal evaluation consisting of abnormal findings on physical examination, anemia, thrombocytopenia, CSF abnormalities, and abnormal bone radiographs. A normal evaluation renders CNS infection rare.

Pneumonia. Syphilitic pneumonia is a common occurrence in congenital syphilis, particularly in developing countries. The classic radiographic appearance is one of complete opacification of both lung fields and is termed *pneumonia alba.* More commonly today, a fluffy, diffuse infiltrate involving all lung areas is seen on the chest radiograph. At autopsy, pneumonia alba consists of a focal obliterative fibrosis with scarring and thickening of alveolar walls with loss of alveolar spaces. Follow-up evaluation of children who have recovered from congenital syphilis has shown that at least 10 percent may have

chronic pulmonary disease, particularly if they were premature and required mechanical ventilation.

Hepatosplenomegaly. Hepatomegaly is the most common clinical sign in congenital syphilis. Its occurrence in the fetus has been documented by ultrasonography, and it may be a marker of inadequate treatment of the fetal infection despite maternal treatment during pregnancy.[60] Hepatosplenomegaly often is caused by extramedullary hematopoiesis. Hepatomegaly may occur in the absence of splenomegaly; but in contrast to congenital cytomegalovirus, the reverse does not. Neonatal syphilitic hepatitis is associated with visible spirochetes on biopsy specimens of liver tissue, jaundice, and cholestasis. Aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferase determinations often are elevated, and direct hyperbilirubinemia is a common finding. The prothrombin time may be delayed. The liver disease often resolves slowly, even after administration of apparently adequate therapy, and may be exacerbated by penicillin therapy before improving.[129]

Ectodermal Changes. Ectodermal changes in syphilitic infants include suppuration and exfoliation of the nails, loss of hair and eyebrows, choroiditis, and iritis.

Other Findings. Other clinical manifestations of congenital syphilis include nonimmune fetal hydrops, possibly intrauterine growth restriction, generalized lymphadenopathy, fever, failure to thrive, nephrotic syndrome, and myocarditis. Involvement of the eyes has been manifested by chorioretinitis, cataract, glaucoma, and chancre of the eyelid. Gastrointestinal presentations include rectal bleeding caused by syphilitic ileitis, necrotizing enterocolitis, malabsorption secondary to fibrosis of the gastrointestinal tract, and fetal bowel dilation seen on antenatal ultrasonography.[114,119] Some children with symptomatic congenital syphilis also may have sepsis caused by other bacteria, including *Escherichia coli*, group B streptococci, and *Yersinia* spp. Neonates and infants with congenital syphilis may resemble infants with other illnesses peculiar to neonates, including toxoplasmosis, rubella, cytomegalovirus infection, herpes simplex virus infection, "sepsis" of the newborn, blood group incompatibilities, battered child syndrome, "periostitis" of prematurity, neonatal hepatitis, and osteomyelitis.

Late Congenital Syphilis

Late manifestations or stigmata of congenital syphilis are the result of scarring from the early systemic disease or reactions to persistent inflammation and include involvement of the teeth, bones, eyes, and eighth cranial nerve; gummas in the viscera, skin, or mucous membranes; and neurosyphilis (Table 143-6).[47] Approximately 40 percent of surviving and untreated infected infants, as reported in the early literature, develop late manifestations of infection. Some of these changes can be prevented by treating the mother during pregnancy or the infant before the child reaches 3 months of age. In contrast, treatment of 15 children at 4 months of age or later showed 7 with dental changes.[108] Other stigmata (e.g., keratitis, saber shins) may occur or progress, despite the infants being administered appropriate therapy. Nonspecific sequelae of congenital syphilis have not been described well because follow-up studies of children with congenital syphilis are minimal. It has been the experience of one of the authors (P.J.S.), however, that infants who are diagnosed with congenital syphilis and treated appropriately in the neonatal period generally do well at least through early childhood. There is every reason to be hopeful and optimistic with the families of these children, even if they are symptomatic and have CNS involvement at the time of diagnosis.

TABLE 143-6 **Stigmata of Late Congenital Syphilis***

Stigmata	Percent of Total Patients
Frontal boss of Parrott	87
Short maxilla	84
High palatal arch	76
Hutchinson triad	75
Higouménakis sign	73
Relative protuberance of mandible	65
Saddle nose	63
Interstitial keratitis	39
Rhagades	26
Mulberry molars	9
Saber shin	7
Eighth nerve deafness	4
Hutchinson teeth	3
Scaphoid scapulae	0.7
Clutton joint	0.3

*An analysis of 271 patients.
Modified from Fiumara NJ, Lessell S. Manifestations of late congenital syphilis. *Arch Dermatol* 1970;**102**:78-83. Copyright, American Medical Association.

Dentition. Characteristic changes are found in the permanent upper central incisors, which have a notched appearance of the biting edges; radiographic study leads to diagnosis, even while deciduous teeth are in place. These are Hutchinson teeth, which also are small and hypoplastic and widely spaced with abnormal enamelization. If first molars show maldevelopment of the cusps, the finding is called *mulberry* or *moon molars*.

Interstitial Keratitis. Interstitial keratitis is the most common late lesion. It may appear at any age between 4 and 30 years or later but characteristically appears when the patient is close to puberty. A ground-glass appearance may develop in the cornea, accompanied by vascularization of the adjacent sclera. These changes become bilateral and usually lead to blindness. It has been seen in adolescent patients despite their having received previous and appropriate penicillin therapy during infancy. At the time of its occurrence, keratitis is not affected by penicillin therapy, but it may respond transiently to corticosteroid treatment.

Central Nervous System. The same manifestations of neurosyphilis seen in acquired syphilis may occur in congenital syphilis. Paresis is seen more frequently and tabes dorsalis less frequently in the congenital form than in the acquired form of the disease. Cranial nerve palsies and optic atrophy are prominent.

Eighth Cranial Nerve Deafness. Hearing loss usually is sudden and occurs when the child reaches 8 to 10 years of age. It often accompanies interstitial keratitis. The constellation of eighth cranial nerve deafness, interstitial keratitis, and Hutchinson teeth is called the *Hutchinson triad.* The hearing loss usually involves the higher frequencies, and normal conversational tones become affected later. This hearing loss may respond to long-term corticosteroid treatment.

Bone and Joint Changes. Bone changes include the sclerosing lesions, saber shin and frontal bossing, which are sequelae of periostitis that involved the frontal bone and tibia. Periosteal reaction of the sternoclavicular portion of the clavicle may lead to swelling, known as the Higouménakis sign. The gummatous or destructive lesions include saddle-nose deformity, which occurs as a sequela of rhinitis. Perforation of the hard palate is almost pathognomonic of congenital syphilis. Clutton joints are uncommon findings and represent painless arthritis of the knees and, rarely, other joints.

Cutaneous Lesions. Rhagades represent scars resulting from persistent rhinitis during infancy and rarely are seen today.

DIAGNOSIS

The vagaries of the maternal history and signs or lack of signs in the infant at birth (i.e., timing of the mother's initial syphilis infection before or during pregnancy, adequacy and documentation of her treatment, an infant who is infected and symptomatic at birth, an infant who is asymptomatic at birth but may not remain so because infection has occurred, and an infant who is asymptomatic and not infected) demand a "safety first" approach to diagnosis and treatment. Efforts to diagnose infectious syphilis suffer from the lack of a method to culture the organisms on laboratory media. Methods that are used in establishing the diagnosis of syphilis include (1) direct visualization of the organism by darkfield microscopy or fluorescent antibody technique of infected fluids or lesions, (2) demonstration of the organism by special stains on histopathologic examination of tissue,[58,117] (3) animal inoculation (rabbit infectivity test), (4) demonstration of serologic reactions typical of syphilis, and (5) detection of *T. pallidum* DNA in a clinical specimen.[19]

The rabbit infectivity test is still the gold standard for the identification of viable *T. pallidum* in clinical specimens, having a sensitivity of less than 10 organisms.[83,84] It involves intratesticular inoculation of the specimen into a rabbit and awaiting serologic seroconversion and orchitis with subsequent visualization of motile spirochetes by darkfield microscopy in testicular tissue. The rabbit infectivity test is performed only in research laboratories and may take several months for identification of the organism. *T. pallidum* DNA has been detected by polymerase chain reaction (PCR) in body fluids such as amniotic fluid and infant blood, CSF, and endotracheal aspirate.[54,90] The new genetic information about *T. pallidum* may lead to development of novel diagnostic tools.

In the clinical setting, the diagnosis of syphilis is established by visualization of the spirochete by darkfield microscopy or special staining and serology. Patients with a primary syphilis lesion (chancre) and with active secondary lesions may be diagnosed by darkfield microscopy. Because this diagnosis depends on direct visualization of motile spirochetes, the organisms must be active and viable. Prior use of many antibiotics rapidly destroys the motility of the organisms, as do many topical disinfectants. Serous fluid from the base of the lesion should be collected for darkfield examination. Syphilitic lesions of the mouth may harbor indigenous treponemes, of which the morphologic similarity to pathogenic species can confuse the interpretation of findings.

Direct darkfield examination is particularly helpful in establishing a diagnosis early in the course of the disease, before the development of seroreactivity. If a darkfield microscope is unavailable, a direct fluorescent antibody stain for *T. pallidum* may be made.[17] Exudate is collected in capillary tubes or slides and stained with specific antibody. Amniotic fluid may be examined for the presence of spirochetes using darkfield microscopy or fluorescent antitreponemal staining; the finding of spirochetes may be a marker for more severe fetal disease.[53,149] On a practical basis, however, the diagnosis is established by serologic methods.[80,152]

Serologic Tests

The two types of serologic tests for syphilis are the nontreponemal antibody tests and the treponemal antibody tests (Table 143-7). Although the latter tests indicate experience with a treponemal infection in the past, they cross react with the antigens of other treponemal diseases, such as the antigens causing yaws and pinta. No test is specific for syphilis, and no test is completely sensitive. Efforts to produce a more sensitive and more specific test are continuous. A promising approach is the use of recombinant clones expressing immunogenic proteins of *T. pallidum* to investigate pathogen-specific antigens.[65] Some of these products are being investigated for use as diagnostic material.[124]

Nontreponemal Tests

The original test for syphilis, as described by Wassermann, used syphilitic tissue as complement-fixing antigen to detect the presence of antibody (reagin) that is induced by *T. pallidum.* Extracts of other normal tissue, such as beef heart, had similar properties, and purification and standardization of these materials led to the use as antigen of a preparation containing cardiolipin and lecithin in cholesterol.

Two tests currently using cardiolipin, lecithin, and cholesterol are the VDRL and rapid plasma reagin (RPR)

TABLE 143-7 **Standard Serologic Tests for Syphilis**

Type	Test	Percent Reactivity During		
		PRIMARY STAGE	SECONDARY STAGE	TERTIARY STAGE
Nontreponemal				
Extracts of tissue (cardiolipin-lecithin-cholesterol)	VDRL	78	95	71
	RPR	86	98	73
Treponemal				
Treponema pallidum	MHA-TP*	76	100	94
	FTA-ABS	84	100	97

*MHA-TP has been replaced with *Treponema pallidum* particle agglutination (TP-PA) test.
FTA-ABS, fluorescent treponemal antibody absorption; MHA-TP, micro-hemagglutination assay–*Treponema pallidum;* RPR, rapid plasma reagin; VDRL, Venereal Disease Research Laboratory.

tests. These tests measure mostly IgG, but also IgM antibodies nondiscriminatively. The tests provide similar clinical information and have similar advantages. The RPR titer often is one to two dilutions higher than the titer obtained using the VDRL test, so caution must be exercised when making clinical decisions on the basis of results obtained from these two tests performed on the same patient. Both are inexpensive to perform and show increasing and decreasing antibody titers that often correlate with adequacy of therapy and the clinical status of a patient. A fourfold increase in RPR or VDRL titers indicates active disease, whereas a fourfold decrease suggests adequate therapy. Disadvantages include a high proportion of biologic acute and chronic false-positive reactors and an increasing proportion of false-negative reactions in the later stages of untreated syphilis. The main technical difficulty is a negative reaction owing to the prozone phenomenon, which occurs in 1 to 2 percent of individuals, usually patients with secondary syphilis, and is due to an excess amount of reagin antibody present in the patient's undiluted serum that prevents flocculation. Diluting the serum sample before testing overcomes the inhibition and results in the positive reaction. Because the RPR test generally is more sensitive than is the VDRL test, it is preferred for screening pregnant women. The VDRL test is recommended for use on CSF, however.

The RPR test is used frequently to screen pregnant women for possible infection with *T. pallidum*. Transplacental passage of IgG antibodies to the infant means that mothers with positive RPR tests usually transmit these antibodies to their infants. Taber and Huber[90] reviewed the relationship of maternal to newborn VDRL tests in their study of mothers who were undiagnosed and untreated. Although 12 of 22 mothers had VDRL test results two to four times that of their infants, 6 of these women and their infants had nonreactive serology at the time of delivery. These and other data showed that it was unusual for an infant to have a VDRL test of greater titer than the mother's, even if the infant was incubating congenital syphilis.[139] Nonetheless, the finding of a titer in the infant serum fourfold higher than that seen in the maternal serum when both are obtained at the same time is indicative of active infection in the infant. Even if the infant is asymptomatic, a 10-day course of penicillin therapy should be administered.

Treponemal Tests

The most significant development of the past 3 decades in the serologic study of syphilis was the detection of treponemal antibody by fluorescein-labeled antihuman antibody. These tests are used to confirm the validity of a positive nontreponemal test and to diagnose late stages of syphilis. The tests are sensitive and reliable.

Fluorescent treponemal antibody absorption (FTA-ABS) tests use lyophilized Nichols strain organisms as antigen and measure IgG and IgM antibodies. Antigen is fixed to a slide, and the test serum is applied, allowing reaction of antitreponemal antibody with antigen to occur. The slide is layered with fluorescein isothiocyanate–labeled antihuman gamma-globulin, and the presence or absence of antibody is determined by fluorescent microscopy.

Test sera are preabsorbed with sorbent to eliminate group-reactive antibody. The test is rendered specific for disease with virulent treponemal species, usually *T. pallidum*. The FTA-ABS test is expensive and time consuming, however. It is recommended not for general screening but for confirmation of positive nontreponemal tests and for establishing the diagnosis of later stages of syphilis in which the results of nontreponemal tests may be negative.

Microhemagglutination tests, and specifically the *T. pallidum* particle agglutination (TP-PA) test, depend on the passive hemagglutination of erythrocytes or latex particles that have been sensitized with Nichols strain *T. pallidum*. The test has been automated and is easy to perform technically and inexpensively. The TP-PA test has largely replaced the FTA-ABS test as the most efficient specific test for antibody to *T. pallidum*. The TP-PA test is as sensitive as is the FTA-ABS test, except in primary syphilis. The test is positive in 75 to 85 percent of patients with primary syphilis and in 100 percent of patients with secondary syphilis. Similar to the FTA-ABS test, the TP-PA test is unlikely to revert to a nonreactive

state after treatment of the patient, unless treatment is given very early.

IgM Tests

In the diagnosis of congenital syphilis, having a means to differentiate between passive transplacental transfer of maternal antibody to the fetus and production by the fetus of endogenous antitreponemal antibody would be most helpful. Because antibodies of the IgM class do not cross the placenta, detection of IgM antibody in the fetal or neonatal serum would indicate antibody production by the fetus because of active fetal infection. No IgM assay currently is commercially available that is sufficiently sensitive and specific to recommend for routine use in the evaluation of infants born to mothers with syphilis. The fluorescent treponemal antibody that measures IgM antitreponemal antibodies, the IgM-FTA-ABS test, has been associated with false-positive and false-negative results. When the test was refined further by use of the IgM fraction of neonatal serum only, it had a sensitivity of 73 percent.[139] The test also is insensitive when the onset of disease is delayed. Occasional false-positive IgM-FTA-ABS tests occur because of the presence of an IgM anti-IgG antibody or rheumatoid factor. For these reasons, the CDC has recommended that the IgM-FTA-ABS test be suspended for diagnostic testing of neonates, and the test is available only as a provisional test.

Efforts to develop a sensitive and specific serologic test for congenital syphilis have led to the identification of antigenic components of *T. pallidum* that are epitopes for the immune response. A 34-kDa membrane protein is a dipoprotein[140] and the target of IgM antibody formation in the sera of some congenitally infected infants.[38] The Captia Syphilis M test is an enzyme-linked immunosorbent assay that uses this treponemal protein; it is commercially available but showed a sensitivity of only 88 percent among infants with clinical and laboratory findings of congenital syphilis.[78,139]

Immunoblotting has been used to characterize the specific neonatal IgM antibody responses to *T. pallidum*. Specific IgM antibodies directed against *T. pallidum* antigens with apparent molecular masses of 72 kDa, 47 kDa, 45 kDa, 42 kDa, 37 kDa, 17 kDa, and 15 kDa have been detected in sera of fetuses[62] and infants with clinical findings of congenital syphilis and in 20 to 40 percent of asymptomatic infants born to mothers with untreated syphilis.* IgM reactivity against the 47-kDa antigen, a membrane lipoprotein of the organism, has been the most consistent finding. Similar reactivities also have been seen in CSF of infants with congenital syphilis.[81,126] A more recent study showed that a reactive serum IgM immunoblot identified all 17 infants in whom spirochetes were detected in their CSF by rabbit infectivity testing.[90] Efforts to develop rapid diagnostic tests based on these findings are in progress.[75] In addition, immunoblotting has been used to detect similar IgA reactivities among infants with congenital syphilis.[127]

*References 38, 81, 90, 122, 124, 127.

Polymerase Chain Reaction Assay

The PCR assay has been used on neonatal blood and CSF for establishing the diagnosis of congenital syphilis.[54,90,124] Compared with isolation of the organism by rabbit infectivity testing, the sensitivity and specificity of PCR on CSF was 65 to 71 percent and 97 to 100 percent, respectively.[90,124] Among 17 infants who had spirochetes detected in their CSF by rabbit inoculation, blood PCR test was the best predictor of CNS infection with *T. pallidum*.[90] This finding supports further the concept that spirochetes gain access to the CNS by a hematogenous route.

False-Positive Reactions

All of the available serologic tests for syphilis produce occasional reactive results in patients who have no other evidence of syphilitic infection. These reactions usually are called *biologic false-positive* (BFP) reactions and are distinct from positive reactions owing to technical errors. Most BFP reactions occur with nontreponemal tests; approximately 1 percent of normal adults have a BFP reaction by nontreponemal antigen tests. These reactions probably are not more common in pregnant women than in the general population. Reaginic antibody is reactive with at least 200 antigens other than those of *T. pallidum*, and although the specific stimulus for this antibody in syphilis and other diseases is unknown, it may represent antibody to cellular lipoidal antigens of the host that are liberated during various diseases. For clinical purposes, BFP reactions may be classified as acute, in which the reactivity resolves within 6 months, or chronic, in which reactivity is persistent.

Acute Biologic False-Positive Reactions. Most BFP reactions are detected by nontreponemal tests and occur in patients with other acute illnesses, especially pneumonia, hepatitis, and viral exanthematous disease, or after receiving vaccinations. The prognosis for the patient's health is not affected by the finding. The titer of antibody usually is low (<1 : 8), and in most instances the FTA-ABS is nonreactive. Approximately two thirds of patients with BFP reactions have acute reactions, and reactivity subsides within 6 months.

Chronic Biologic False-Positive Reactions. Many patients with chronic BFP reactions have or develop systemic disease. Drug addiction, chronic hepatitis, old age, leprosy, and collagen vascular disease, especially systemic lupus erythematosus, are associated highly with chronic BFP reactions. A familial predisposition to this finding may exist. The antibody detected by the VDRL test in chronic BFP reactions predominantly is IgM, whereas it mainly is IgG in syphilis. Patients with chronic BFP reactions and systemic lupus erythematosus commonly also have a reactive FTA-ABS test.[79] The triosephosphate isomerase test may be helpful in the differential diagnosis in these instances. A finding of particular concern is that there seems to be a relative increase in acute and chronic BFP reactions in women who are infected with HIV.

Evaluation and Diagnosis of Early Congenital Syphilis

The diagnosis of congenital syphilis is suggested by results of serologic tests, physical examination, laboratory tests including CSF examination, and radiographs of long bones and is established by the observation of spirochetes in body fluids or tissue. Examination of the placenta or umbilical cord using specific fluorescent antitreponemal antibody staining is recommended.[52,109] The decision to evaluate and ultimately to treat an infant for congenital syphilis is based on clinical, serologic, and epidemiologic considerations. The evaluation includes an assessment of the mother for general risk factors for increased rates of syphilis (Table 143-8), followed by an evaluation of the mother's current known serologic status (Table 143-9). If the mother has been treated, the clinician must assess the adequacy of the therapy.

TABLE 143-8 General Maternal Risk Factors Associated with Increased Rates of Early Syphilis in Pregnancy

Infection with human immunodeficiency virus
Adolescent or unmarried status
History of sexually transmitted disease
Substance abuse, especially cocaine
Inadequate or absent prenatal care
Prostitution or promiscuity
Localized populations or geographic areas
Treatment of gonorrhea with ciprofloxacin or spectinomycin
Poor communication among medical personnel regarding maternal/infant status

Data from references 44, 48, 50, and 51.

TABLE 143-9 Components of Epidemiologic Evaluation of an Infant's Mother for Possible Syphilis That Aid in Evaluation and Treatment of Infant

Evaluate mother for prior history of syphilis or major risk factors for syphilis (see Table 143-8).
If mother received treatment for syphilis, evaluate course of therapy for adequacy in the treatment of congenital syphilis (see Table 143-2).
Evaluate current maternal status with a nontreponemal test (e.g., Venereal Disease Research Laboratory, rapid plasma reagin) and, if reactive, a treponemal test (e.g., fluorescent treponemal antibody absorption, *Treponema pallidum* particle agglutination test).
If mother is identified clinically or through contact tracing as having early syphilis during the 3 mo after delivery, reevaluate the infant and consider therapy at that time.
Higher maternal titers to nontreponemal tests are associated with failure of maternal therapy to prevent congenital disease.[88]
Unknown duration of maternal syphilis is associated with failure of maternal therapy to prevent congenital disease.[88]

If the mother's serologic assays have been positive, the infant must be assessed for clinically apparent disease. The nontreponemal (RPR) and treponemal (TP-PA) tests measure IgG antibody and do not distinguish disease of the infant from maternally derived antibody. Many infants are born to women who have had syphilis in the past, received therapy, and remained seroreactive. Their infants also are seroreactive. Ensuring that the infant does not have congenital disease in the immediate neonatal period may be impossible.

Figure 143-2 and Table 143-10 present an approach to the evaluation of infants born to mothers with reactive serologic tests for syphilis. Testing of all pregnant women with syphilis for coinfection with HIV is recommended strongly, even though infants born to coinfected mothers do not require any different evaluation for syphilis.[78] All infants born to mothers with reactive serologic tests for syphilis should have a serum quantitative nontreponemal test performed and a thorough physical examination that focuses on finding evidence of congenital syphilis. The nontreponemal test that is performed on the infant should be the same as the test done on the mother to be able to compare serologic titers. Although it is an uncommon finding, a serum quantitative nontreponemal titer that is fourfold or greater than the corresponding maternal titer is diagnostic of congenital syphilis; when it has occurred, one of the authors (P.J.S.) has isolated spirochetes from blood or CSF in all infants.

The CDC recommends that the infant's serologic test be performed on serum and not umbilical cord blood because false-positive test results have been reported with the use of umbilical cord blood secondary to contamination of the specimen with maternal blood and Wharton jelly. Some clinicians continue to use umbilical cord blood, however, because it is readily available and easily collected; appropriate care should be taken during collection to minimize contamination with maternal blood. In addition, false-negative test results may occur when the maternal titer is low dilution, which argues for maternal screening rather than screening of infant serum.

Infants who have (1) an abnormal physical examination that is consistent with congenital syphilis, (2) a serum quantitative nontreponemal serologic titer that is fourfold or greater than the mother's, or (3) a positive darkfield or fluorescent antibody test of body fluid should have a complete blood cell count and platelet count performed and examination of the CSF for cell count, protein content, and VDRL test. Other tests, such as bone and chest radiographs, liver function tests, cranial ultrasonography, ophthalmologic examination, and auditory brain stem response should be performed as clinically indicated. These infants are considered to have *proved or highly probable disease;* spirochetemia with invasion of the CNS occurs in 40 to 50 percent of these infants.[16] Although these infants must receive a full course of penicillin therapy, which treats possible neurosyphilis, it is beneficial for follow-up purposes to establish CNS abnormalities at presentation. Nonetheless, the diagnosis of congenital neurosyphilis is difficult to establish (see Clinical Manifestations). The presence of red blood cells in the CSF as a result of a traumatic lumbar puncture can produce a false-positive serologic reaction. Examination

TABLE 143-10 **Treatment Guidelines for Congenital Syphilis**

Scenario	Maternal Stage/Treatment	Recommended Evaluation	Regimen
Infant with proven or highly probable disease	Any or none	CSF analysis: VDRL, cell count, protein CBC and differential; platelet count. As clinically indicated: radiographs of long bones, liver function tests, cranial ultrasonography, eye examination, hearing evaluation	Aqueous penicillin G 50,000 units/kg IV every 12 hr (≤1 wk old), every 8 hr (>1 wk old, ≤4 wk old), every 6 hr (>4 wk old) × 10 days *or*
(a) Abnormal physical examination			Procaine penicillin G 50,000 units/kg IM × 10 days (≤4 wk old)
(b) Abnormal evaluation*			
(c) Serum nontreponemal titer ≥ 4 times maternal titer			
(d) Visualization of spirochete in clinical specimen			
Infant with *normal* physical examination and serum nontreponemal titer <4 times maternal titer	Any stage of infection *and*	CSF analysis: VDRL, cell count, protein; CBC and differential; platelet count; radiographs of long bones	Treatment A: benzathine penicillin G† 50,000 units/kg IM × 1
	No treatment *or*	If *all* normal: treatment A	Treatment B: aqueous penicillin G 50,000 units/kg IV every 12 hr (≤1 wk old), every 8 hr (>1 wk old, ≤4 wk old), every 6 hr (>4 wk old) × 10 days *or*
	Inadequate or undocumented treatment *or*	If *any* abnormal or not done‡: treatment B	Procaine penicillin G 50,000 units/kg IM × 10 days (≤4 wk old)
	Erythromycin or nonpenicillin treatment *or*		
	Therapy ≤4 wk before delivery *or*		
	Adequate therapy >1 mo before delivery, but maternal nontreponemal titers have not decreased fourfold after treatment for early syphilis (primary, secondary, early latent syphilis)		
	Adequate therapy >1 mo before delivery, and appropriate for stage of infection *or*	No evaluation	Benzathine penicillin G 50,000 units/kg IM × 1
	Maternal nontreponemal titer decreased fourfold after treatment for early syphilis *or*		
	Maternal nontreponemal titers remained stable and low for late syphilis *and* no evidence of reinfection or relapse	No evaluation	Benzathine penicillin G 50,000 units/kg IM × 1
	Adequate therapy before pregnancy and stable nontreponemal titers (VDRL ≤1:2 or RPR ≤1:4) throughout pregnancy		

TABLE 143-10 **Treatment Guidelines for Congenital Syphilis (Continued)**

Scenario	Maternal Stage/Treatment	Recommended Evaluation	Regimen
Congenital syphilis in infant >28 days old	Any or none	CSF analysis: VDRL, cell count, protein CBC and differential; platelet count. As clinically indicated: radiographs of long bones, liver function tests, cranial ultrasonography, eye examination, hearing evaluation	Aqueous penicillin G, 50,000 units/kg every 4-6 hours × 10 days§

*CSF examination (VDRL test, cell count, protein), bone radiographs, CBC, platelets, umbilical cord or serum VDRL/RPR (same test as performed on maternal serum).
†If the infant's nontreponemal test is nonreactive, no evaluation is required, but the infant should receive treatment A.
‡Clinical and serologic follow-up must be certain.
§Some experts prefer prolonged therapy by administration of a single dose of benzathine penicillin G after the 10-day course of IV aqueous penicillin G.
From American Academy of Pediatrics. Syphilis. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 690-703; and Centers for Disease Control and Prevention; Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1-110.

of the CSF for *T. pallidum* DNA by PCR may prove more useful for diagnosing congenital neurosyphilis.[54,90,124]

For infants who have a normal physical examination and a serum quantitative nontreponemal test that is less than fourfold the maternal titer, further evaluation and treatment depend on the maternal treatment history and stage of infection (see Fig. 143-2 and Table 143-10). Whether to perform a complete evaluation (lumbar puncture, radiographs of the long bones, and complete blood cell and platelet counts) on the infant depends on the maternal treatment history and planned treatment of the infant (see Treatment). If the mother did not receive any prior treatment for syphilis, or the treatment was inadequate, a complete evaluation must be performed and be normal if a single intramuscular dose of benzathine penicillin G therapy is administered. Although it is preferred to help establish a diagnosis of congenital syphilis, a complete evaluation is unnecessary if a full 10-day course of parenteral penicillin is provided because such therapy would treat for the possibility of congenital infection.[13,92]

The need to perform a lumbar puncture has been questioned because the yield of abnormal findings from examination of the CSF has been very low in some experiences and appreciable in others.[12,44,118] A primary benefit of obtaining CSF studies from infants who are receiving a 10-day parenteral course of aqueous or procaine penicillin therapy is identification of infants for whom follow-up of abnormal CSF results should be done. Likewise, radiographs of long bones are abnormal in approximately 65 percent of infants with clinical findings of syphilis but only in a few asymptomatic infants. The finding of osteochondritis or periostitis in an infant born to a mother with reactive serologic tests for syphilis indicates congenital syphilis, and the infant requires a full course of penicillin therapy for highly probable disease. Such an infant is likely to have CNS infection, however, even in the absence of clinical signs of neurosyphilis, and close clinical and serologic follow-up would be indicated.[90]

After the neonatal period, children with syphilis should have a lumbar puncture performed for evaluation of the CSF to detect asymptomatic neurosyphilis. In addition, birth and maternal records should be reviewed to assess whether such children have acquired or congenital syphilis.[29] Children with acquired syphilis should be evaluated for the possibility of sexual abuse.[72]

TREATMENT

T. pallidum is extremely sensitive to penicillin, as defined by experimental animal work. The minimal inhibitory concentration of penicillin is approximately 0.004 U/mL (or 0.0025 μg/mL). There is no evidence of increasing resistance to penicillin by the spirochetes, but such evidence would come only from the recognition of therapeutic failures. Effective treatment of syphilis must maintain a minimal inhibitory concentration of 0.03 U/mL of penicillin in serum (or CSF) for 7 to 10 days. Therapy is designed to achieve and maintain several times the necessary inhibiting levels and to avoid penicillin-free intervals during therapy. Penicillin remains the drug of first choice because of its established efficacy and minimal toxicity.[22]

Acquired Syphilis

Early syphilis (i.e., primary, secondary, and early latent infection) is treated with benzathine penicillin G, 50,000 units/kg up to the adult dose of 2.4 million units intramuscularly in a single dose. Late latent syphilis requires benzathine penicillin G, 150,000 units/kg up to the adult dose of 7.2 million units, administered as

50,000 units/kg up to the adult dose of 2.4 million units at 1-week intervals for 3 weeks. When the duration of infection is unknown, the patient should be treated as for late latent disease.

All patients with syphilis should be tested for HIV infection at diagnosis, and if they have early syphilis, they should be retested 3 to 6 months later. Because neurosyphilis may be asymptomatic and can be defined accurately only by examining the CSF, the CDC recommends performing a lumbar puncture in the following situations: (1) neurologic or ophthalmic signs or symptoms, (2) active tertiary syphilis, (3) treatment failure, and (4) HIV infection with late latent syphilis or syphilis of unknown duration.[24] A lumbar puncture is not recommended routinely for patients with primary or secondary syphilis. Benzathine penicillin does not produce inhibitory CSF levels of penicillin reliably.[137] Shorter-acting penicillins must be employed for neurosyphilis. In evaluating a patient for neurosyphilis, a CSF specimen without contamination by peripheral blood is needed.[66]

The recommended therapy for neurosyphilis is aqueous crystalline penicillin G, 18 to 24 million units/day, administered as 3 to 4 million units intravenously every 4 hours or continuous infusion for 10 to 14 days. An alternative regimen consists of procaine penicillin, 2.4 million units intramuscularly once daily, plus probenecid, 500 mg orally four times per day, for 10 to 14 days. Because these regimens are of shorter duration than is the regimen used for late latent syphilis, some experts recommend the additional administration of benzathine penicillin G, 2.4 million units intramuscularly once per week for up to 3 weeks after completion of the 10- to 14-day course. Older children with definite acquired syphilis and a normal neurologic and CSF examination may be treated with benzathine penicillin G, 50,000 units/kg intramuscularly to a maximal dose of 2.4 million units. Children with neurosyphilis should receive aqueous penicillin G, 200,000 to 300,000 units/kg/day intravenously administered as 50,000 units/kg every 4 to 6 hours for 10 days. Similar to the procedure with adults, an additional dose of benzathine penicillin G, 50,000 units/kg intramuscularly, may be given following the 10-day intravenous penicillin treatment.

The evaluation and treatment of early syphilis in HIV-infected individuals are the same as those for individuals not infected with HIV except that they may be at increased risk for developing neurologic complications and have a higher risk for experiencing treatment failure. HIV-infected individuals who have late latent syphilis or syphilis of unknown duration should have their CSF examined for evidence of neurosyphilis before receiving treatment; further management and treatment depend on CSF findings. Close serologic follow-up of HIV-infected individuals is mandatory to detect treatment failure and the need for examination of CSF and re-treatment.

Data to support the use of alternatives to penicillin in the treatment of syphilis are limited. Any history of penicillin allergy should be documented clearly, and, whenever possible, patients should receive skin testing, desensitization, and therapy with penicillin.[24] Doxycycline 100 mg orally twice daily and tetracycline 500 mg four times daily have been used for 14 days for treatment of early syphilis and 28 days for late latent infection and syphilis of unknown duration. Close serologic follow-up is mandatory. Neither of these therapies is recommended for children younger than 8 years of age. Ceftriaxone 100 mg/kg/day (maximum of 1 g daily) given either intravenously or intramuscularly for 8 to 10 days may be effective for treating early syphilis, but neither the optimal dose nor the duration of treatment has been defined.[61] Preliminary data also suggest that azithromycin as a single dose of 2 g may be effective in adults, but treatment failures have been described, as has emergence of resistance.[24] Because experience with these alternative antibiotics in HIV-infected individuals is limited, they should be used with caution.

Two to 12 hours after receiving treatment for syphilis, a variable proportion of patients develop an acute systemic (Jarisch-Herxheimer) reaction usually consisting of headache, malaise, fever (≥38° C [100.4° F]), and resolution within 1 day. The reaction is observed most frequently in the early stages of syphilis, probably represents a reaction to liberated endotoxin,[51] and does not affect the course of recovery. In the later stages of syphilis, fewer than one in four patients develops the reaction. Most reactions in late syphilis clinically are insignificant, but an occasional reaction may produce damage to the CNS or cardiovascular system.

Syphilis in Pregnancy

Pregnant women with reactive serologic tests for syphilis should be considered infected unless an adequate treatment history is documented, and sequential nontreponemal antibody titers have declined. They should receive the penicillin regimen appropriate for the stage of syphilis.[24,152] For early syphilis, many experts recommend that an additional dose of benzathine penicillin G be provided 1 week after the initial dose. Management and treatment decisions may be guided further by the use of fetal ultrasonography. Evidence of fetal infection may require additional doses of benzathine penicillin G until resolution of fetal abnormalities has been achieved. The Jarisch-Herxheimer reaction may complicate treatment and result in preterm labor and fetal decelerations, although concern for its occurrence should not delay initiation of treatment.[71,95] No alternative to penicillin is available; pregnant women who have a history of penicillin allergy should undergo skin testing, desensitization, and treatment with penicillin.[151] Erythromycin is not recommended; infants have been born with congenital syphilis after maternal treatment with erythromycin. Any therapy other than penicillin is considered to be inadequate fetal therapy.[43,106]

Women who are coinfected with syphilis and HIV should receive treatment regimens corresponding to their stage of syphilis. The follow-up on treated HIV-infected women must be thorough and frequent.[57,94]

Patients who received therapy for gonorrhea with ceftriaxone have a high rate of cure of pre-primary syphilis, but failures have occurred, and efficacy in pregnancy is not well studied.[61] This regimen cannot be assumed to have provided adequate therapy for syphilis in pregnancy.

Congenital Syphilis

Infants with Proven or Highly Probable Disease

Infants who have findings on physical examination that are consistent with congenital syphilis—a quantitative nontreponemal serologic titer that is at least fourfold greater than the mother's titer, or spirochetes visualized in body fluids—should be treated with aqueous crystalline penicillin G, 50,000 units/kg/dose intravenously every 12 hours during the first 7 days of life and every 8 hours thereafter for 10 days, or with procaine penicillin G, 50,000 units/kg/dose administered intramuscularly in a single daily dose for 10 days (see Table 143-10).[24] Although the levels of penicillin that are achieved in the CSF after procaine therapy are lower than the levels seen with intravenous penicillin treatment, the clinical significance is unclear.[6] No treatment failures have been reported after procaine penicillin therapy. If more than 1 day of penicillin therapy is missed, the entire course should be restarted.

During a penicillin shortage in the United States, ampicillin was recommended as an alternative agent, although data are insufficient for routine administration. When possible, a full 10-day course of penicillin is preferred even if ampicillin was provided initially for possible sepsis. Data also are lacking for the use of ceftriaxone in these infants. Infants born to mothers coinfected with syphilis and HIV do not require more intense or prolonged treatment for syphilis than is recommended for all infants.

Infants with a Normal Physical Examination and a Serum Quantitative Nontreponemal Serologic Titer That Is the Same or Less than Fourfold the Maternal Titer

Treatment decisions for "asymptomatic" infants are based on the maternal history of syphilis and past treatment. The following situations are associated with a high likelihood that the infant may be infected with *T. pallidum* and should receive treatment: (1) mother was not treated, was inadequately treated, or has no documentation of having received treatment[110]; (2) mother was treated with erythromycin or other nonpenicillin regimen; (3) mother received treatment *less than* 4 weeks before delivery[14]; and (4) mother has early syphilis and has a nontreponemal titer that either has not decreased fourfold or has increased fourfold. If the infant's nontreponemal test is reactive, the infant should be evaluated with a complete blood cell count and differential; platelet count; radiographs of long bones; and CSF analysis for VDRL test, cell count, and protein content (Fig. 143-3 and Table 143-10).[24] If the entire evaluation is normal and follow-up is certain, the infant may receive benzathine penicillin G, 50,000 units/kg as a single intramuscular dose.

If any part of the evaluation is abnormal or not done, or follow-up is uncertain, treatment should consist of aqueous penicillin G or procaine penicillin G (see earlier discussion) for 10 days. If the infant's nontreponemal serum test is nonreactive, the evaluation may be omitted, but the infant should be treated with a single intramuscular dose of benzathine penicillin for the possibility of incubating syphilis. Some experts prefer that all infants born to mothers with untreated syphilis receive parenteral penicillin therapy for 10 days, even if the evaluation is normal, because many of these infants are likely to be infected with *T. pallidum*, especially if the mother has secondary syphilis at delivery or seroconverted during the pregnancy.[5,24]

More recent studies have reevaluated the efficacy of treatment regimens for asymptomatic infants who are born to mothers in whom the treatment for possible syphilis was suboptimal.[102,110] Paryani and colleagues[102] randomly assigned 152 infants to receive either one injection of benzathine penicillin or a 10-day course of parenteral procaine penicillin. All study infants were asymptomatic on physical examination and had normal CSF evaluation, normal radiographic studies of long bones, and no visceral abnormalities. The results of both forms of therapy were excellent, with no treatment failures. This study indicates that single-dose therapy may have a high rate of success when an infant has negative studies and is asymptomatic.

Failure of such therapy in three infants has been reported; however, the frequency seems to be low, although it is unknown.[12,154] None of the three infants who developed clinical signs of congenital syphilis several weeks after having received a single intramuscular dose of benzathine penicillin was fully evaluated for congenital syphilis at birth. The concern has been that infected but asymptomatic infants may have CNS infection and fail therapy with benzathine penicillin because benzathine penicillin does not achieve treponemicidal concentrations in CSF.[137] Using rabbit inoculation, Michelow and colleagues[16] showed that invasion of the CNS is an infrequent occurrence among these infants with normal results on clinical, laboratory, and radiographic evaluations.

An infant does not require any evaluation in the following situations: (1) mother was treated during the pregnancy, treatment was appropriate for the stage of infection, and treatment was administered more than 4 weeks before delivery; (2) mother's nontreponemal titers decreased fourfold after administration of appropriate therapy for early syphilis or remained stable and low for late syphilis; and (3) mother has no evidence of reinfection or relapse. The CDC recommends, however, that these infants receive a single dose of benzathine penicillin G, 50,000 units/kg intramuscularly.[5,24] Infection of the fetus may occur despite administration of appropriate maternal therapy during pregnancy; failure rates of maternal treatment for prevention of fetal infection have been reported to be 2 to 14 percent and are more common with maternal secondary syphilis.[4,33,97,130] Treating the infant at birth may prevent the development of clinical disease if these infants were infected in utero. Some specialists would not treat the infant, however, but provide close serologic follow-up only.

If the mother's treatment was adequate before pregnancy, and the mother's nontreponemal serologic titer remained low and stable during pregnancy and at delivery, no evaluation or treatment is recommended by the CDC. Some specialists would provide a single intramuscular dose of benzathine penicillin, however, if follow-up is uncertain for the remote possibility that the mother

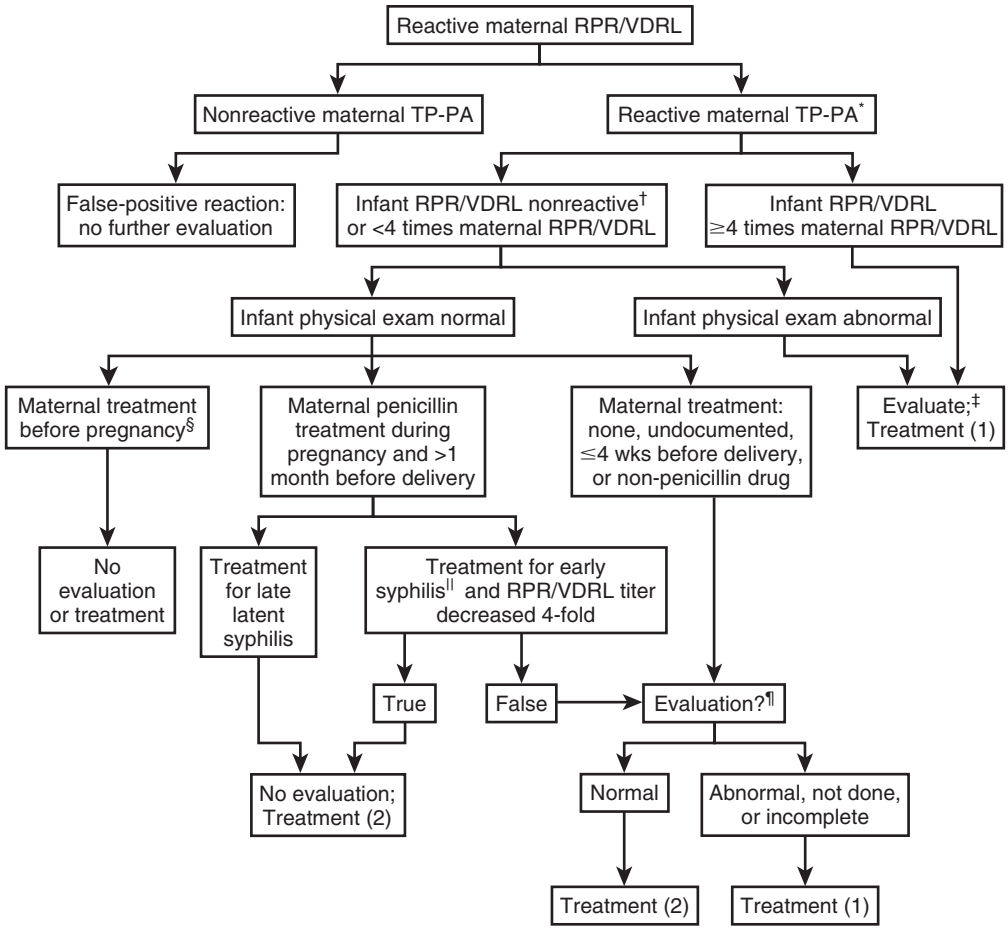

FIGURE 143-3 ■ Algorithm for evaluation and treatment of infants born to mothers with reactive serologic tests for syphilis. *Test for human immunodeficiency virus (HIV) antibody. Infants of HIV antibody–positive mothers do not require different evaluation or treatment. †Infant's RPR may be nonreactive because of low maternal RPR titer or recent maternal infection. If the mother has untreated or inadequately treated syphilis, and the infant's physical examination is normal, treat infant with a single intramuscular injection of benzathine penicillin (50,000 units/kg). No further evaluation is needed. ‡Evaluation consists of complete blood cell count; platelet count; and cerebrospinal fluid examination for cell count, protein, and quantitative VDRL. Other tests as clinically indicated include radiographs of the long bones, cranial ultrasonography, auditory brain stem response testing, eye examination, chest radiograph, liver function tests, and urine or meconium toxicology. §Women who maintain a VDRL titer ≤1:2 (RPR ≤1:4) >1 year after successful treatment are considered serofast. ||Early syphilis: primary, secondary, or early latent infection. ¶Complete blood cell count; platelet count; cerebrospinal fluid examination for cell count, protein, and quantitative VDRL; long bone radiographs. Treatment regimens are as follows: (1) aqueous penicillin G 50,000 units/kg IV every 12 hours (≤1 week old) or every 8 hours (>1 week old) or procaine penicillin G 50,000 units/kg IM single daily dose ×10 days. (2) Benzathine penicillin G 50,000 units/kg IM ×1 dose. RPR/VDRL, rapid plasma reagin/Venereal Disease Research Laboratory; TP-PA, *T. pallidum* particle agglutination.

was reinfected. These infants are at very low risk for having congenital syphilis; for the most part, these mothers are serofast.

After the neonatal period, all children who are diagnosed with congenital syphilis should be evaluated with a complete blood cell count and differential, platelet count, and examination of the CSF. Other tests, such as auditory brain stem responses, should be performed as clinically indicated. These children and children with late congenital syphilis should be treated with 200,000 to 300,000 units/kg/day of aqueous crystalline penicillin G, given as 50,000 units/kg every 4 to 6 hours for a minimum of 10 to 14 days.

The Jarisch-Herxheimer reaction may complicate the treatment of congenital syphilis. It rarely is seen in newborns, although it occurs more commonly when syphilis is treated later in infancy. It is manifested usually by fever, but cardiovascular collapse and seizures also have been reported.

FOLLOW-UP EVALUATION

Table 143-11 summarizes recommendations concerning follow-up of infants after they have been treated for congenital syphilis and of seroreactive infants who were not treated because of the presumed adequacy of the management of the mother's serologic status.[62,63,113] Infants born to mothers with syphilis should have serial quantitative nontreponemal tests performed until the test results show nonreactivity.[58,116] Similarly, infants who are seronegative but whose mothers acquired syphilis late in gestation should be followed with serial testing after penicillin therapy is instituted.

TABLE 143-11 Recommended Follow-up of Infants Born to Mothers with Syphilis

1. Nontreponemal test (e.g., RPR):
   a. *Treated infant:* Perform at 2, 4, 6, 12, 15, and 24 months old (or post therapy) until nonreactive. Nontreponemal test titer should decrease fourfold within 6 mo after treatment and be nonreactive by 6-12 mo. If test remains reactive ≥12 mo after treatment, consider reevaluation and re-treatment. If nontreponemal test titer increases fourfold, full reevaluation and re-treatment for proven disease is required.
   b. *Untreated infant:* Perform at 1, 2, 4, 6, 12, 15, and 24 months old (or post therapy) until nonreactive. Nontreponemal test titer should decrease fourfold within 6 mo after treatment and be nonreactive by 6 mo. If nontreponemal assay is reactive at 6 mo, reevaluate child (including CSF examination) and treat. If nontreponemal test titer increases fourfold, full evaluation and treatment for proven disease are required.
2. Treponemal test (e.g., TP-PA): Perform at 12 mo. If reactive, repeat at ages 18 and 24 mo.*
3. Abnormal CSF examination at diagnosis: Repeat CSF examination (VDRL, cell count, protein content) at age 6 mo (or post therapy). If abnormal and no other explanation found, re-treat infant.
4. Monitor yearly for neurologic, hearing, and ophthalmic disorders.
5. Monitor developmental status and assess school function.

*Approximately 30% of infected children maintain a reactive treponemal test beyond age 18 months. This finding confirms the diagnosis of congenital syphilis. If child was not previously treated, treatment for proven congenital syphilis is mandatory.

CSF, cerebrospinal fluid; RPR, rapid plasma reagin; TP-PA, *T. pallidum* particle agglutination; VDRL, Venereal Disease Research Laboratory.

Modified from Ikeda MK, Jenson HB. Evaluation and treatment of congenital syphilis. *J Pediatr* 1990;**117**:843-52; Rathbun KC. Congenital syphilis: a proposal for improved surveillance, diagnosis and treatment. *Sex Transm Dis* 1983;**10**:102-7; and Ingall D, Sánchez PJ. Syphilis. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* 5th ed. Philadelphia: WB Saunders, 2001, p. 643-81.

Follow-up for infants who received appropriate penicillin therapy for possible syphilis can be incorporated into routine pediatric care at 2, 4, 6, 12, 15, and 24 months. These infants should show a fourfold decrease in nontreponemal serologic titers. In infants with congenital syphilis, nontreponemal serologic tests become nonreactive within 6 to 12 months after they have received appropriate treatment. Uninfected infants usually become seronegative by the time they reach 6 months of age.[27] Infants with persistently low, stable titers of nontreponemal tests beyond 1 year of age may require re-treatment.

Untreated infants may benefit from having a nontreponemal test performed at 1 month of age. Untreated infants who are not seronegative by the time they are 6 months old should be reevaluated clinically and treated. If the nontreponemal titer increases fourfold in any infant during follow-up, full evaluation and treatment are indicated. All infants and children should be evaluated thoroughly for the extent of disease if there is serologic evidence of failure of treatment or of recurrent disease. At a minimum, such evaluation should consist of CSF examination and complete blood cell count and platelet count. Other tests, such as long bone radiographs, liver function tests, hearing evaluation, and ophthalmologic evaluation, can be performed as clinically indicated.

A reactive treponemal test in an infant who is older than 18 months of age and has lost all maternal antibody confirms the diagnosis of congenital syphilis. This finding occurs, however, in only a few infants with congenital syphilis, as documented by isolation of spirochetes by rabbit inoculation of infant blood or CSF. If the child did not receive treatment previously, a full evaluation and treatment are indicated.

Infants with abnormal CSF findings should have a repeat lumbar puncture performed at 6 months after therapy. A reactive CSF VDRL test result or an abnormal protein content or cell count at that time is an indication for re-treatment.

Adults and older children with primary or secondary syphilis should be examined clinically and serologically at least at 6 and 12 months after treatment. Failure of nontreponemal titers to decrease fourfold within 6 months of treatment indicates probable treatment failure. These individuals should be reevaluated for HIV infection, and consideration should be given to performance of a lumbar puncture for detection of unrecognized neurosyphilis and re-treatment. For latent syphilis, subsequent follow-up with quantitative serology at 6, 12, and 24 months is recommended. Failure of a fourfold decrease in initially elevated nontreponemal serologic titers (≥1:32) within 12 to 24 months of therapy is an indication for examination of CSF and re-treatment. Re-treatment for syphilis also is recommended when clinical signs or symptoms persist or recur, or if there is a sustained fourfold increase in titer of a nontreponemal serologic test. Patients with neurosyphilis require examinations and repeat CSF examinations every 6 months until the cell count is normal. If the cell count is not decreased after 6 months or the CSF is not normal after 2 years, re-treatment should be considered.

In most patients receiving appropriate therapy during the primary or secondary stages, active disease is arrested totally and permanently. Persistent seroreactivity as measured by the FTA-ABS test may be avoided if treatment is given during the pre-primary stage, but seldom thereafter. Nonetheless, progression to tertiary disease seldom, if ever, occurs. Similarly, therapy administered during early or late latent syphilis averts the development of symptomatic tertiary disease. Antimicrobial therapy for symptomatic neurosyphilis, optic neuritis, and cardiovascular syphilis may not be followed by significant clinical improvement, and established damage to vital organs may fail to resolve.

PREVENTION

Methods to control the spread of syphilis have relied extensively on treatment of case contacts. Individuals with active syphilis are interviewed to identify all sexual

contacts that may have occurred at (1) 3 months plus duration of symptoms for primary syphilis, (2) 6 months plus duration of symptoms for secondary syphilis, and (3) 1 year for early latent syphilis. The contacts are examined, tested serologically, and treated appropriately even if they are seronegative. Advantage is taken of the long incubation period of syphilis by preventing disease in contacts before they can transmit infection.

Adverse outcomes of pregnancy and congenital infection can be prevented effectively by performing routine prenatal serologic screening of and providing penicillin treatment to infected women and their sexual partners.[136,147] All pregnant women should have a nontreponemal serologic test for syphilis at the first prenatal visit. For communities and populations in which the prevalence of syphilis is high or for patients at increased risk for contracting disease, serologic testing should be performed at 28 weeks' gestation and at delivery because some women may acquire syphilis during pregnancy.[2] In the absence of clinical symptoms or proven exposure to an active case and with a negative treponemal test, treatment may be withheld and studies repeated in 4 weeks because this probably represents a BFP result. When epidemiologic, clinical, or serologic evidence of infection is present, or the diagnosis cannot be excluded, treatment should be instituted.

Serologic screening tests should be performed on maternal serum and not on the infant's serum or umbilical cord blood.[114] An infant's serologic titer usually is one to two dilutions less than that of the mother's titer; an infant may have a nonreactive serologic test for syphilis when the maternal titer is of low dilution. Some of these infants, especially infants born to mothers with no prior treatment for syphilis, require treatment for prevention of late-onset disease. They would be missed if only infant screening is performed.

Limitations of current screening practices also exist. A false-negative nontreponemal test can occur from a prozone phenomenon. Also, a negative maternal nontreponemal test at delivery would not exclude incubating syphilis or even primary syphilis if nontreponemal and treponemal antibodies have not yet reached detectable concentrations.[39,125] Infants born to such mothers may be infected and develop clinical disease in the ensuing 3 to 14 weeks. Repeat screening of mothers who reside in areas with a high prevalence of syphilis or engage in high-risk behaviors may be advisable at the first postpartum visit.

It is a policy statement of the CDC that no infant should leave the hospital without the serologic status of the infant's mother having been documented at least once during pregnancy and preferably again at delivery.[24,85] In this era of early and very early discharge from the hospital after deliveries, fulfilling the policy goal may require careful planning and advocacy by the clinician.

All syphilis cases must be reported to the local public health department. This practice allows for contact investigation, appropriate follow-up, and identification of core environments and populations in which the greatest transmission of syphilis is occurring in a particular community. Despite great progress in recent years, the prevention, control, and even elimination of endemic syphilis in the United States remains an elusive goal in public health policy and on a global scale in countries where resources may be limited and the practicalities of serologic screening and treatment in prenatal care settings are challenging.

NEW REFERENCES SINCE THE SIXTH EDITION

5. American Academy of Pediatrics. Syphilis. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 690–703.
20. Centers for Disease Control and Prevention (CDC). Congenital syphilis—United States, 2003-2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:413–17.
24. Centers for Disease Control and Prevention; Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1–110.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 144

Nonvenereal Treponematoses

David C. Hilmers

Pinta, yaws, and endemic syphilis (bejel) are the three chronic granulomatous diseases that constitute the pathogenic nonvenereal treponematoses of humans. These diseases are caused by morphologically indistinguishable treponemes found almost exclusively in underdeveloped areas of the tropics and bordering arid regions. There has been recent sequencing of the yaws genome that has identified targets for molecular diagnostics that could differentiate venereal syphilis from yaws.[7-10,18,72] The pathogenic treponemes have been given subspecies

REFERENCES

1. Ackerman AB, Goldfaden G, Cosmides JC. Acquired syphilis in early childhood. *Arch Dermatol* 1972;**106**:92–3.
2. Alder JD, Friess L, Tengowski M, et al. Phagocytosis of opsonized *Treponema pallidum* subsp. *pallidum* proceeds slowly. *Infect Immun* 1990;**58**:1167–73.
3. Alexander JM, Sheffield JS, Sanchez PJ, et al. Efficacy of treatment for syphilis in pregnancy. *Obstet Gynecol* 1999;**93**:5–8.
4. Al-Salihi FL, Curran JP, Shteir OA. Occurrence of fetal syphilis after a nonreactive early gestational serologic test. *J Pediatr* 1971;**78**:121–3.
5. American Academy of Pediatrics. Syphilis. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 690–703.
6. Azimi PH, Janner D, Berne P, et al. Concentrations of procaine and aqueous penicillin in the cerebrospinal fluid of infants treated for congenital syphilis. *J Pediatr* 1994;**124**:649–53.
7. Baker-Zander SA, Hook EW III, Bonin P, et al. Antigens of *Treponema pallidum* recognized by IgG and IgM antibodies during syphilis in humans. *J Infect Dis* 1985;**151**:264–72.
8. Barton JR, Thorpe EM, Shaver DC, et al. Nonimmune hydrops fetalis associated with maternal infection with syphilis. *Am J Obstet Gynecol* 1992;**167**:56–8.
9. Baughn RE, Jorizzo JL, Adams CB, et al. Ig class and IgG subclass responses to *Treponema pallidum* in patients with syphilis. *J Clin Immunol* 1988;**8**:128–39.
10. Baughn RE, Musher DM. Secondary syphilitic lesions. *Clin Microbiol Rev* 2005;**18**:205–16.
11. Bays J, Chadwick D. The serologic test for syphilis in sexually abused children and adolescents. *Adolesc Pediatr Gynecol* 1991;**4**: 148–51.
12. Beck-Sague C, Alexander R. Failure of benzathine penicillin G treatment in early congenital syphilis. *Pediatr Infect Dis* 1987;**6**: 1061–4.
13. Beeram MR, Chopde N, Dawood Y. Lumbar puncture in the evaluation of possible asymptomatic congenital syphilis in neonates. *J Pediatr* 1996;**128**:125–9.
14. Benzick AE, Wirthwein DP, Weinberg A, et al. Pituitary gland gumma in congenital syphilis after failed maternal treatment: a case report. *Pediatrics* 1999;**104**:e1–4.
15. Braunstein GD, Lewis EJ, Galvanek EG, et al. The nephrotic syndrome associated with secondary syphilis. *Am J Med* 1970;**48**: 643–8.
16. Brightbill HD, Libraty DH, Krutzik SR, et al. Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 1999;**285**:732–6.
17. Bromberg K, Rawstron S, Tannis G. Diagnosis of congenital syphilis by combining *Treponema pallidum*–specific IgM detection with immunofluorescent antigen detection of *T. pallidum*. *J Infect Dis* 1993;**168**:238–42.
18. Bruckner DA. Nomenclature for aerobic and anaerobic bacteria. In: Feigin RD, Cherry JD, Demmler GJ, et al, editors. *Textbook of pediatric infectious diseases*. 5th ed. Philadelphia: W. B. Saunders; 2004. p. 1082–99.
19. Burstain JM, Grimprel E, Lukehart SA, et al. Sensitive detection of *Treponema pallidum* by using the polymerase chain reaction. *J Clin Microbiol* 1991;**29**:62–9.
20. Centers for Disease Control and Prevention. Congenital syphilis—New York City, 1986-1988. *MMWR Morb Mortal Wkly Rep* 1989; **38**:825–9.
21. Centers for Disease Control and Prevention. Congenital syphilis—United States, 2002. *MMWR Morb Mortal Wkly Rep* 2004;**53**: 716–19.
22. Centers for Disease Control and Prevention. *Sexually transmitted disease surveillance 2006 supplement, syphilis surveillance report*. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2007. Available at: http://www.cdc.gov/std/Syphilis2006/Syphilis2006Short.pdf. Accessed March 13, 2008.
23. Centers for Disease Control and Prevention. Congenital syphilis—United States, 2003-2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**: 413–17.
24. Centers for Disease Control and Prevention; Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1–110.
25. Centurion-Lara A, Castro C, Barrett L, et al. *Treponema pallidum* major sheath protein homologue Tpr K is a target of opsonic antibody and the protective immune response [erratum appears in *J Exp Med* 1999;**189**(11):following 1852]. *J Exp Med* 1999; **189**:647–56.
26. Centurion-Lara A, LaFond RE, Hevner K, et al. Gene conversion: a mechanism for generation of heterogeneity in the *tprK* gene of *Treponema pallidum* during infection. *Mol Microbiol* 2004;**52**: 1579–96.
27. Chang SN, Chung KY, Lee MG, et al. Seroreversion of the serological tests for syphilis in the newborns born to treated syphilic mothers. *Genitourin Med* 1995;**71**:68–70.
28. Chen ZQ, Zhang GC, Gong XD, et al. Syphilis in China: results of a national surveillance programme. *Lancet* 2007;**369**:132–8.
29. Christian CW, Lavelle J, Bell LM. Preschoolers with syphilis. *Pediatrics* 1999;**103**:e4.
30. Clark EG, Danbolt N. The Oslo study of the natural course of untreated syphilis: an epidemiologic investigation based on a re-study of the Boeck-Bruusgaard material. *Med Clin North Am* 1964;**48**:613–23.
31. Cohen DA, Boyd D, Pabhudas I, et al. The effects of case definition, maternal screening, and reporting criteria on rates of congenital syphilis. *Am J Public Health* 1990;**80**:316–17.
32. Coles FB, Hipp SS, Silberstein GS, et al. Congenital syphilis surveillance in upstate New York, 1989-1992: implications for prevention and clinical management. *J Infect Dis* 1995;**171**: 732–5.
33. Conover CS, Rend CA, Miller GB Jr, et al. Congenital syphilis after treatment of maternal syphilis with a penicillin regimen exceeding CDC guidelines. *Infect Dis Obstet Gynecol* 1998;**6**: 134–7.
34. Cremin BJ, Fisher RM. The lesions of congenital syphilis. *Br J Radiol* 1970;**43**:333–41.
35. Daaboul JJ, Kartchner W, Jones KL. Neonatal hypoglycemia caused by hypopituitarism in infants with congenital syphilis. *J Pediatr* 1993;**123**:983–5.
36. Desenclos J-CA, Scaggs M, Wroten JE. Characteristics of mothers with live infants with congenital syphilis in Florida, 1987-1989. *Am J Epidemiol* 1992;**136**:657–61.
37. Dobson SRM, Taber LH, Braughn RE. Characterization of the components in circulating immune complexes from infants with congenital syphilis. *J Infect Dis* 1988;**158**:940–7.
38. Dobson SRM, Taber LH, Braughn RE. Recognition of *Treponema pallidum* antigens by IgM and IgG antibodies in congenitally affected newborns and their mothers. *J Infect Dis* 1988;**157**: 903–10.
39. Dorfman DH, Glaser JH. Congenital syphilis presenting in infants after the newborn period. *N Engl J Med* 1990;**323**: 1299–302.
40. Dorman HG, Sahyun BF. Identification and significance of spirochetes in placenta: report of 105 cases with positive findings. *Am J Obstet Gynecol* 1937;**33**:954–67.
41. Dunn RA, Webster LA, Nakashima AK, et al. Surveillance for geographic and secular trends in congenital syphilis—United States, 1983-1991. *MMWR CDC Surveill Summ* 1993;**42**:59–71.
42. Fan Y-M, Zeng W-J, Wu Z-H, et al. Immunophenotypes, apoptosis, and expression of Fas and Bcl-2 from peripheral blood lymphocytes in patients with secondary early syphilis. *Sex Transm Dis* 2004;**31**:221–4.
43. Fenton LJ, Light IJ. Congenital syphilis after maternal treatment with erythromycin. *Obstet Gynecol* 1976;**47**:492–4.
44. Finelli L, Crayne EM, Spitalny KG. Treatment of infants with reactive syphilis serology, New Jersey. *Pediatrics* 1998;**102**:e27.
45. Fiumara NJ. Acquired syphilis in three patients with congenital syphilis. *N Engl J Med* 1974;**290**:1110–20.
46. Fiumara NJ, Fleming WL, Downing JG, et al. The incidence of prenatal syphilis at the Boston City Hospital. *N Engl J Med* 1952;**247**:48–52.
47. Fiumara NJ, Lessell S. Manifestations of late congenital syphilis. *Arch Dermatol* 1970;**102**:78–83.
48. Fojaca RM, Hensely GT, Moskowitz L. Congenital syphilis and necrotizing funisitis. *JAMA* 1989;**12**:1788.
49. Fraser CM, Morris SJ, Weinstock GM, et al. Complete genome sequence of *Treponema pallidum*, the syphilis spirochete. *Science* 1998;**281**:375–88.
50. Gamble CN, Reardan JB. Immunopathogenesis of syphilitic glomerulonephritis. *N Engl J Med* 1975;**292**:449–54.

51. Gelfand JA, Elin RJ, Berry FW Jr, et al. Endotoxemia associated with the Jarisch-Herxheimer reaction. *N Engl J Med* 1976;**295**: 211–13.
52. Genest DR, Choi-Hong SR, Tate JE, et al. Diagnosis of congenital syphilis from placental examination: comparison of histopathology, Steiner stain, and polymerase chain reaction for *Treponema pallidum* DNA. *Hum Pathol* 1996;**27**:366–72.
53. Glover DD, Winter CA, Charles D, et al. Diagnostic considerations in intra-amniotic syphilis. *Sex Transm Dis* 1985;**12**:145–9.
54. Grimprel E, Sanchez PJ Jr, Wendel GD Jr, et al. Use of polymerase chain reaction and rabbit infectivity testing to detect *Treponema pallidum* in amniotic fluids, fetal and neonatal sera, and cerebrospinal fluid. *J Clin Microbiol* 1991;**29**:1711–18.
55. Gust DA, Levine WC, St. Louis ME, et al. Mortality associated with congenital syphilis in the United States, 1992-1998. *Pediatrics* 2002;**109**:e79.
56. Gutman LT. Congenital syphilis. In: Mandell GL, editor. *Atlas of infectious disease. Vol. 5. Sexually transmitted diseases*. Philadelphia: Current Medicine; 1996.
57. Haas JS, Bolan G, Larsen SA, et al. Sensitivity of treponemal tests for detecting prior treated syphilis during human immunodeficiency virus infection. *J Infect Dis* 1990;**162**:862–6.
58. Hardy JB, Hardy PH, Oppenheimer EH. Failure of penicillin in a newborn with congenital syphilis. *JAMA* 1970;**212**:1345–9.
59. Harter CA, Benirschke K. Fetal syphilis in the first trimester. *Am J Obstet Gynecol* 1976;**124**:705–11.
60. Hollier LM, Harstad TW, Sanchez PJ. Fetal syphilis: clinical and laboratory characteristics. *Obstet Gynecol* 2001;**97**:947–53.
61. Hook EW, Roddy RE, Hardsfield HH. Ceftriaxone therapy for incubating and early syphilis. *J Infect Dis* 2001;**158**:881–4.
62. Ikeda MK, Jenson HB. Evaluation and treatment of congenital syphilis. *J Pediatr* 1990;**117**:843–52.
63. Ingall D, Sanchez PJ. Syphilis. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 5th ed. Philadelphia: W. B. Saunders; 2001. p. 643–81.
64. Ingraham NR. The value of penicillin alone in the prevention and treatment of congenital syphilis. *Acta Derm Venereol* 1950; **31**(Suppl. 24);60–87.
65. Isaacs RD, Radolf JD. Molecular approaches to improved syphilis serodiagnosis. *Serodiagn Immunother Infect Dis* 1989;**3**:299–306.
66. Izzat NN, Bartruff JK, Glicksman JM. Validity of the VDRL test on cerebrospinal fluid contaminated by blood. *Br J Vener Dis* 1971;**47**:162–4.
67. Jensen JR, Thestrup-Pedersen K, From E. Fluctuations in natural killer cell activity in early syphilis. *Br J Vener Dis* 1983;**59**:30–2.
68. Jones SA, Marchitto KS, Miller JN. Monoclonal antibody with hemagglutination, immobilization, and neutralization activities defines an immunodominant, 47,000 mol wt, surface-exposed immunogen of *Treponema pallidum* (Nichols). *J Exp Med* 1984;**160**: 1404–20.
69. Kaplan BS, Wiglesworth FW, Marks MI. The glomerulopathy of congenital syphilis: an immune deposit disease. *J Pediatr* 1972; **81**:1154–6.
70. Klass PE, Brown ER, Pelton SI. The incidence of prenatal syphilis at the Boston City Hospital: a comparison across four decades. *Pediatrics* 1994;**94**:24–8.
71. Klein VR, Cox SM, Mitchell MD, et al. The Jarisch-Herxheimer reaction complicating syphilotherapy in pregnancy. *Obstet Gynecol* 1990;**92**:375–80.
72. Knight J, Richardson AC, White KC. The role of syphilis serology in the evaluation of suspected sexual abuse. *Pediatr Infect Dis J* 1992;**11**:125–7.
73. Lafond RE, Lukehart SA. Biological basis for syphilis. *Clin Microbiol Rev* 2006;**19**:29–49.
74. Lande MB, Richardson AC, White KC. The role of syphilis in the evaluation of suspected sexual abuse. *Pediatr Infect Dis J* 1992; **11**:125–7.
75. Larsen SA, Steiner BM, Rudolph AH. Laboratory diagnosis and interpretation of tests for syphilis. *Clin Microbiol Rev* 1995;**8**: 1–21.
76. Lascari AD, Diamond J, Nolan BE. Anemia as the only presenting manifestation of congenital syphilis. *Clin Pediatr* 1976;**15**:90–1.
77. Leader BT, Hevner K, Molini BJ, et al. Antibody responses elicited against the *Treponema pallidum* repeat proteins differ during infection with different isolates of *Treponema pallidum* subsp. *pallidum*. *Infect Immun* 2003;**71**:6054–7.
78. Lefevre JC, Betrand MA, Bauriaud R. Evaluation of the Captia enzyme immunoassays for detection of immunoglobulins G and M to *Treponema pallidum* in syphilis. *J Clin Microbiol* 1990;**28**: 1704–7.
79. Lesser RP, O'Connell EJ. Positive fluorescent treponemal antibody test in systemic lupus erythematosus in childhood: report of a case. *J Pediatr* 1971;**79**:1006–8.
80. Lewis LL. Congenital syphilis: serologic diagnosis in the young infant. *Infect Dis Clin North Am* 1992;**6**:31–9.
81. Lewis LL, Taber LH, Baughn RE. Evaluation of immunoglobulin M Western blot analysis in the diagnosis of congenital syphilis. *J Clin Microbiol* 1990;**28**:296–302.
82. Lucas MJ, Theriot SK, Wendel GD. Doppler systolic-diastolic ratios in pregnancies complicated by syphilis. *Obstet Gynecol* 1991;**77**:217–22.
83. Lukehart SA, Hook EW, Baker-Sander SA, et al. Invasion of the central nervous system by *Treponema pallidum*: implications for diagnosis and therapy. *Ann Intern Med* 1988;**109**:855–62.
84. Magnuson HJ, Eagle H, Fleischman R. The minimal infectious inoculum of *Spirochaeta pallida* (Nichols strain), and a consideration of its rate of multiplication in vivo. *Am J Syphilis Gonorrhea Vener Dis* 1948;**32**:1–18.
85. Martin D, Bertrand J, McKegney C. Congenital syphilis surveillance and newborn evaluation in a low-incidence state. *Arch Pediatr Adolesc Med* 2001;**155**:140–4.
86. Mascola L, Pelosi R, Alexander CE. Inadequate treatment of syphilis in pregnancy. *Am J Obstet Gynecol* 1984;**150**:945–7.
87. Mascola L, Pelosi R, Blount JH. Congenital syphilis: why is it still occurring? *JAMA* 1984;**252**:1719–22.
88. Mascola L, Pelosi R, Blount JH. Congenital syphilis revisited. *Am J Dis Child* 1985;**139**:575–80.
89. McFarlin BL, Bottoms SF, Dock BS. Epidemic syphilis: maternal factors associated with congenital infection. *Am J Obstet Gynecol* 1994;**170**:535–40.
90. Michelow IC, Wendel GD, Norgard MV. Central nervous system infection in congenital syphilis. *N Engl J Med* 2002;**346**:1792–8.
91. Morgan CA, Lukehart SA, Van Voorhis WC. Protection against syphilis correlates with specificity of antibodies to the variable regions of *Treponema pallidum* repeat protein K. *Infect Immun* 2003;**71**:5605–12.
92. Moyer VA, Schneider V, Yetman R. Contribution of long-bone radiographs to the management of congenital syphilis in the newborn infant. *Arch Pediatr Adolesc Med* 1998;**152**:353–7.
93. Musher DM, Hague-Park M, Gyorkey F. The interaction between *Treponema pallidum* and human polymorphonuclear leukocytes. *J Infect Dis* 1983;**147**:77–86.
94. Musher DM, Hamill RJ, Baughn RE. Effect of human immunodeficiency virus (HIV) infection on the course of syphilis and on the response to treatment. *Ann Intern Med* 1990;**113**:872–81.
95. Myles TD, Elam G, Park-Hwang E, et al. The Jarisch-Herxheimer reaction and fetal monitoring changes in pregnant women treated for syphilis. *Obstet Gynecol* 1998;**92**:859–64.
96. Nabarro JND. *Congenital syphilis*. Baltimore: Williams & Wilkins; 1954.
97. Nathan L, Bohman VR, Sánchez PJ, et al. In utero infection with *Treponema pallidum* in early pregnancy. *Prenat Diagn* 1997;**17**: 119–23.
98. Nathan L, Twickler DM, Peters MT, et al. Fetal syphilis: correlation of sonographic findings and rabbit infectivity testing of amniotic fluid. *J Ultrasound Med* 1993;**2**:97–101.
99. Nolt D, Saad R, Kouatli A. Survival with hypopituitarism from congenital syphilis. *Pediatrics* 2002;**109**:e1–4.
100. Norris SJ, Alderete JE, Axelson NH. Identity of *Treponema pallidum* subsp. *pallidum* polypeptides: correlation of sodium dodecyl sulfate-polyacrylamide gel electrophoresis results from different laboratories. *Electrophoresis* 1987;**8**:77.
101. Oppenheimer EH, Hardy JB. Congenital syphilis in the newborn infant: clinical and pathological observations in recent cases. *Johns Hopkins Med J* 1971;**129**:63–82.
102. Paryani SG, Vaugh AJ, Crosby M. Treatment of asymptomatic congenital syphilis benzathine versus procaine penicillin G therapy. *J Pediatr* 1994;**125**:471–5.
103. Pavithran K. Acquired syphilis in a patient with late congenital syphilis. *Sex Transm Dis* 1987;**14**:119–21.
104. Peeling RW, Hook EW. The pathogenesis of syphilis: the great mimicker, revisited. *J Pathol* 2006;**208**:224–32.

105. Peterson K, Baseman JB, Alderete JF. *Treponema pallidum* receptor binding proteins interact with fibronectin. *J Exp Med* 1983;**157**: 1958.
106. Philipson A, Sabeth LD, Charles D. Transplacental passage of erythromycin and clindamycin. *N Engl J Med* 1973;**288**: 1219–21.
107. Podwinska J, Lusiak M, Zaba R, et al. The pattern and level of cytokines secreted by Th1 and Th2 lymphocytes of syphilitic patients correlate to the progression of the disease. *FEMS Immunol Med Microbiol* 2000;**28**:1–14.
108. Putkonen T. Does early treatment prevent dental changes in congenital syphilis? *Acta Derm Venereol* 1963;**43**:240–9.
109. Qureshi F, Jacques SM, Reyes MP. Placental histopathology in syphilis. *Hum Pathol* 1993;**24**:779–84.
110. Radcliffe M, Meyer M, Roditi M, et al. Single-dose benzathine penicillin in infants at risk of congenital syphilis—results of a randomised study. *S Afr Med J* 1997;**87**:62–5.
111. Radolf JD, Arndt LL, Akins DR, et al. *Treponema pallidum* and *Borrelia burgdorferi* lipoproteins and synthetic lipopeptides activate monocytes/macrophages. *J Immunol* 1995;**154**:2866–77.
112. Rathburn KC. Congenital syphilis. *Sex Transm Dis* 1983;**10**: 93–9.
113. Rathburn KC. Congenital syphilis: a proposal for improved surveillance, diagnosis and treatment. *Sex Transm Dis* 1983;**10**: 102–7.
114. Rawstron SA, Bromberg K. Comparison of newborn and maternal serologic tests for syphilis. *Am J Dis Child* 1991;**145**:1383–8.
115. Rawstron SA, Jenkins S, Blanchard S. Maternal and congenital syphilis in Brooklyn, NY: epidemiology, transmission, and diagnosis. *Am J Dis Child* 1993;**147**:727–31.
116. Rawstron SA, Mehta S, Marcellino L. Congenital syphilis and fluorescent treponemal antibody test reactivity after the age of 1 year. *Sex Transm Dis* 2001;**28**:412–16.
117. Rawstron SA, Vetrano J, Tannis G, et al. Congenital syphilis: Detection of *Treponema pallidum* in stillborns. *Clin Infect Dis* 1997;**24**:24–7.
118. Reyes MP, Hunt N, Ostrea EM. Maternal/congenital syphilis in a large tertiary care urban hospital. *Clin Infect Dis* 1993;**17**: 1041–6.
119. Ricci JM, Fojaco RM, O'Sullivan MJ. Congenital syphilis: the University of Miami/Jackson Memorial Medical Center experience, 1986-1988. *Obstet Gynecol* 1989;**74**:687–93.
120. Risser WL, Hwang LY. Problems in the current case definitions of congenital syphilis. *J Pediatr* 1996;**129**:499–505.
121. Salazar JC, Hazlett KRO, Radolf JD. The immune response to infection with *Treponema pallidum*, the stealth pathogen. *Microbes Infect* 2002;**4**:1133–40.
122. Sanchez PJ, McCracken GH, Wendel GD. Molecular analysis of the fetal IgM response to *Treponema pallidum* antigens: implications for improved serodiagnosis of congenital syphilis. *J Infect Dis* 1989;**159**:508–17.
123. Sanchez PJ, Wendel GD. Syphilis in pregnancy. *Clin Perinatol* 1997;**24**:71–90.
124. Sanchez PJ, Wendel GD, Grimprel E. Evaluation of molecular methodologies and rabbit infectivity testing for the diagnosis of congenital syphilis and central nervous system invasion by *Treponema pallidum*. *J Infect Dis* 1993;**167**:148–57.
125. Sanchez PJ, Wendel GD, Norgard MV. Congenital syphilis associated with negative results of maternal serologic tests at delivery. *Am J Dis Child* 1991;**145**:967–9.
126. Sanchez PJ, Wendel GD, Nørgard MV. IgM antibody to *Treponema pallidum* in cerebrospinal fluid of infants with congenital syphilis. *Am J Dis Child* 1992;**146**:1171–5.
127. Schmitz JL, Gertis KS, Mauney C. Laboratory diagnosis of congenital syphilis by immunoglobulin M (IgM) and IgA immunoblotting. *Clin Diagn Lab Immunol* 1994;**1**:32–7.
128. Schulte JM, Burkham S, Hamaker D. Syphilis among HIV-infected mothers and their infants in Texas from 1988 to 1994. *Sex Transm Dis* 2001;**28**:315–20.
129. Shah MC, Barton LL. Congenital syphilitic hepatitis. *Pediatr Infect Dis J* 1989;**8**:891–2.
130. Sheffield JS, Sanchez PJ, Morris G. Congenital syphilis after maternal treatment for syphilis during pregnancy. *Am J Obstet Gynecol* 2002;**186**:569–73.
131. Sheffield JS, Sanchez PJ, Wendel GD Jr, et al. Placental histopathology of congenital syphilis. *Obstet Gynecol* 2002;**100**:126–33.
132. Shew ML, Fortenberry JD. Syphilis screening in adolescents. *J Adolesc Health* 1992;**13**:303–5.
133. Silber TJ, Milard MF. The clinical spectrum of syphilis in adolescence. *Adolesc Health Care* 1984;**5**:196–7.
134. Silverstein AM. Congenital syphilis and the timing of immunogenesis in the human fetus. *Nature* 1962;**194**:196–7.
135. Singh AE, Sutherland K, Lee B, et al. Resurgence of early congenital syphilis in Alberta. *Can Med Assoc J* 2007;**177**:33–6
136. Southwick KL, Guidry HM, Weldon MM, et al. An epidemic of congenital syphilis in Jefferson County, Texas, 1994-1995: inadequate prenatal syphilis testing after an outbreak in adults. *Am J Public Health* 1999;**89**:557–60.
137. Speer ME, Taber LH, Clark DB. Cerebrospinal fluid levels of benzathine penicillin in the neonate. *J Pediatr* 1977;**91**:996–7.
138. St. Louis ME, Wasserheit JM. Elimination of syphilis in the United States. *Science* 1998;**281**:353–4.
139. Stoll BJ, Lee FK, Larsen SA. Clinical and serologic evaluation of neonates for congenital syphilis: a continuing diagnostic dilemma. *J Infect Dis* 1993;**167**:415–22.
140. Swancott MA, Radolf JD, Norgard MV. The 34-kilodalton membrane immunogen of *Treponema pallidum* is a lipoprotein. *Infect Immun* 1990;**58**:384–92.
141. Taber LH, Huber TW. Congenital syphilis. *Prog Clin Biol Res* 1975;**3**:183–90.
142. Tan KL. The re-emergence of early congenital syphilis. *Acta Pediatr* 1973;**62**:601–7.
143. Temmerman M, Mohamedali F, Fransen L. Syphilis prevention in pregnancy: an opportunity to improve reproductive and child health in Kenya. *Health Policy Plan* 1993;**8**:122–7.
144. Theus SA, Harrich DA, Gaynor R. *Treponema pallidum*, lipoproteins, and synthetic lipoprotein analogues induce human immunodeficiency virus type 1 gene expression in monocytes via NF-kB activation. *J Infect Dis* 1998;**177**:941–50.
145. Van Voorhis WC, Barrett LK, Koelle DM, et al. Primary and secondary syphilis lesions contain mRNA for Th1 cytokines. *J Infect Dis* 1996;**173**:491–5.
146. Walker DG, Walker GJ. Prevention of congenital syphilis—time for action. *Bull World Health Organ* 2004;**82**:401.
147. Warner L, Rochat RW, Fichtner RR. Missed opportunities for congenital syphilis prevention in an urban southeastern hospital. *Sex Transm Dis* 2001;**28**:92–8.
148. Webber MP, Lambert G, Bateman DA. Maternal risk factors for congenital syphilis: A case-control study. *Am J Epidemiol* 1993;**137**:415–22.
149. Wendel GD, Maberry MC, Christmas JT. Examination of amniotic fluid in diagnosing congenital syphilis with fetal death. *Obstet Gynecol* 1989;**74**:967–70.
150. Wendel GD, Sanchez PJ, Peters MT. Identification of *Treponema pallidum* in amniotic fluid and fetal blood from pregnancies complicated by congenital syphilis. *Obstet Gynecol* 1991;**78**:890–5.
151. Wendel GD, Sheffield JS, Hollier LM. Penicillin allergy and desensitization in serious infections during pregnancy. *N Engl J Med* 2002;**312**:1229.
152. Wendel GD Jr, Sheffield JS, Hollier LM, et al. Treatment of syphilis in pregnancy and prevention of congenital syphilis. *Clin Infect Dis* 2002;**35**(Suppl. 2):S200–9.
153. Wicher V, Wicher K. Pathogenesis of maternal-fetal syphilis revisited. *Clin Infect Dis* 2001;**33**:354–63.
154. Woolf A, Wilfert CM, Kelsey DB. Childhood syphilis in North Carolina. *N C Med J* 1980;**41**:443–9.
155. Zenker PN, Berman SM. Congenital syphilis: reporting and reality. *Am J Public Health* 1990;**80**:271–2.

TABLE 144-1 **Comparison of the Treponematoses**

	Syphilis	Yaws	Endemic Syphilis	Pinta
Organism	*T. pallidum* subsp. *pallidum*	*T. pallidum* subsp. *pertenue*	*T. pallidum* subsp. *endemicum*	*T. carateum*
Distribution	Worldwide	Tropics, Africa, Asia, Pacific	Arid areas of Africa, Arabia	Tropical Central, South America
Peak Ages Affected	All ages	1-12 yr	<15 yr	15-20 yr
Route of Entry	Mucous membranes	Skin	Oral mucosa, fomites	Skin
Incubation Period	2-10 wk	3-9 wk	3 wk	2-3 wk
Experimental Hosts	Rabbit, primate, guinea pig, hamster	Rabbit, hamster	Rabbit, hamster	Primate only
Sexual Transmission	Yes	No	No	No
Congenital Transmission	Yes	No	No	No
Skin Infection	Yes	Yes	Yes	Yes
Bone Involvement	5%-10%	20%-40%	20%-40%	0
Neurologic Involvement	Yes	No	Rare	No
Cardiologic Involvement	Yes	No	Rare	No

designations as follows: syphilis, *Treponema pallidum* subsp. *pallidum (T. pallidum)*; yaws, *T. pallidum* subsp. *pertenue (T. pertenue)*; and endemic syphilis, *T. pallidum* subsp. *endemicum (T. pallidum)*. The designation for the causative agent of pinta remains *Treponema carateum* (Table 144-1).

Although neither sexually nor congenitally acquired, agents responsible for the nonvenereal treponematoses are transmitted person to person by means of skin or mucous membrane contact. Close contact with children or young adults, traumatized areas of skin, lack of clothing, and poor personal hygiene are important factors in the transmission of these diseases. All three nonvenereal treponematoses are potentially debilitating diseases that have enormous social and economic implications within endemic areas.[23,24] Although their worldwide prevalence generally has been reduced considerably by means of seroepidemiologic and mass treatment campaigns sponsored by the World Health Organization (WHO) and the United Nations International Children's Emergency Fund (UNICEF) in the 1950s and 1960s, they remain a public health problem in several areas of the world where sporadic outbreaks continue to occur.[4]

Since the termination of these mass treatment campaigns, lack of attention to these diseases and diversion of resources to other public health problems, such as human immunodeficiency virus, malaria, and tuberculosis, have led to a resurgence in the nonvenereal treponematoses in some parts of the world. Because of a lack of vigilance and awareness of these diseases, they probably are underreported. Data reported by WHO in 1997 estimated that there were more than 2.5 million cases worldwide.[72] The potential effectiveness of a determined treatment and surveillance program was shown in a region of northern Ecuador where no new cases of yaws were reported from 1993 to 1998.[2] Prior to the inception of this program there was a prevalence of clinical cases of 16.5 percent in the area's population. A focused effort in India has also been successful, and no new cases have been reported since 2004.[4]

The history, development, and global spread of treponemal diseases have been the subject of widespread debate.[56] Because the primary reservoirs for these organisms are humans, their history is intertwined within the economic, cultural, and social framework of humanity. One theory postulates that all treponemal diseases originated from pinta or yaws, first found in Central Africa. This view states that treponemes spread widely from there, and the various disease processes that we know today are a result of mutations caused by the adaptation of the organisms to environmental and development changes in their hosts. Supporters of the pre-Columbian existence of syphilis in Europe point to radiocarbon dating of skeletons in 14th century England that appear to bear the stigmata of syphilis. Critics of this evidence point to possible inaccuracies in methods and lack of peer review.[3] The Columbian theory suggests that treponemes began as nonvenereal pathogens in Africa that spread to the Americas via Asia thousands of years ago while mutating into endemic syphilis (bejel) as it crossed Africa and Asia. In North America, it developed into the venereally transmitted form of disease caused by *T. pallidum* that we know today as syphilis. Syphilis was carried to Europe by Columbus and his men on his return voyage from the Americas. Supporters of this theory point to skeletal evidence of syphilis found in the Dominican Republic before the arrival of Columbus on the island and to evidence of nonvenereal treponemal infections, but not syphilis, in England during the same period.[54,73] More recent evidence from Guyana found an isolated tribe with treponemal infections that had characteristics of both yaws and syphilis. Phylogenetic analysis of this strain lends support of the Columbian theory of the origin of syphilis and the Old World roots of the nonvenereal species.[29,75,76]

On morphologic examination, the treponemes are thin, tightly coiled organisms that do not stain or stain poorly with the usual aniline dyes. They contain 4 to 14 spirals and are actively motile by virtue of periplasmic flagella. The organisms are quite fragile and sensitive to drying, atmospheric oxygen, and temperatures greater

than 35°C. The organisms can be stored in either 15 percent glycerol at −70°C or liquid nitrogen and still retain their virulence for long periods.[64] Although limited multiplication of *T. pallidum* subsp. *pallidum* has been achieved in a tissue-culture system,[18] it has not been accomplished as yet with the nonvenereal pathogenic treponemes. No human pathogenic treponemes have been cultured in or on artificial media. Because they measure 10 to 13 µm in length but only 0.15 µm in width, they are below the resolution of light microscopy and must be identified by darkfield microscopy in infected skin or mucous membrane lesions.

Although recent discoveries in the genomic variations of the species hold promise for future molecular diagnostic testing, little can currently be done to distinguish the various species of pathogenic treponemes. Clinical and epidemiologic features of each disease state from which the treponeme is recovered constitute the primary method of differentiation. Although not practical, further differentiation is based on the response of animal models to experimental infection.[28,32,54,60,61] Each of the pathogenic treponemes stimulates cross-reactive antibodies assayed in the serologic diagnosis of the human diseases by nontreponemal and treponemal tests. Various degrees of homologous resistance and cross immunity exist after infection occurs with any of these spirochetes.* Rabbit models have shown complete immunity against reinfection with homologous pathogens but only incomplete or nonexistent protection against other subspecies.[64] No specific antigenic differentiation has been found among species to explain these findings. Despite differences in methods of transmission, clinical disease, geographic distribution, and rare reports of resistant cases, all human treponemes remain sensitive to penicillin.[70]

PINTA

Pinta (mal del pinto, carate, enfermedad azul) is a chronic, contagious, nonvenereally transmitted treponemal disease that normally is the mildest of these disorders. Although it affects primarily patients in the first 2 decades of life, it can affect children and adults of all age groups, producing dyschromic skin lesions without pathogenic involvement of deeper organ structures. Pinta is not a fatal disease, but a disfiguring one, which results in social ostracism and an inability to adjust to an urban society.

Biology and Immunology

Pinta is caused by *T. carateum*. The organism is characterized primarily by its isolation from patients with clinically apparent pinta and by its lack of pathogenicity in small laboratory animals. Only chimpanzees have been shown to be susceptible to experimental infection.[33,34] Human studies by Medina[42] suggest that *T. carateum* induces significant protection against homologous reinfection and cross immunity against the treponemes of yaws and syphilis; however, these two subspecies of *T. pallidum* fail to confer cross protection against *T. carateum*.[42]

*References 28, 30, 37, 41, 42, 60, 65.

Epidemiology

Pinta is found primarily in tropical Central and South America among underprivileged Indians and in the Caribbean. It occurs in major river basins of Mexico, Venezuela, Colombia, Brazil, Peru, and Ecuador. Cases also have been reported in Argentina, Chile, Haiti, Guatemala, Dominican Republic, Honduras, Nicaragua, and Bolivia. Pinta rarely occurs at higher elevations in the mountain regions of these countries. In the early 1920s, approximately 500,000 cases occurred throughout Latin America; however, today, as a result of seroepidemiologic and mass treatment campaigns, few cases, if any, are being reported. The possibility of underreporting exists because a 20 percent clinical prevalence was reported in 1982 in a village in Panama.[52] A case of pinta in Austria in a visitor from Cuba was reported in 1999.[73]

The disease is found where poor hygienic and crowded conditions exist. Although endemic areas basically are rural, tribal tradition allows very close contact among its members to occur. These tropical societies, with their absence of shoes and clothes, continually subject exposed skin to trauma and to contact with infectious lesions. Transmission of *T. carateum* occurs primarily by intimate skin-to-skin contact; a break in the skin is required for invasion of the treponeme. Pinta is not acquired congenitally or by blood transfusion. Transmission of the disease by insect vectors has been proposed but not proven[42]; insects probably initiate disease by producing the necessary breaks in the skin.[15] Pinta is distributed equally between males and females and among all races in endemic areas. It is acquired primarily by individuals aged 15 to 20 years old,[53] frequently spreading to family members because of crowded living conditions.

Pathogenesis and Pathology

T. carateum penetrates skin through breaks in the epidermis, with resultant damage restricted to the dermal and epidermal tissues. The organisms multiply in these layers, eliciting cellular proliferation and plasma cell, lymphocyte, and Langerhans cell infiltration. The resulting primary lesions (one to three) may appear on any area of exposed skin but usually occur on the legs, the dorsum of the feet, the forearms, or the back of the hands.[53] A lesion enlarges as a result of continual progression and direct extension or by coalescence with adjacent primary lesions; occasionally, regional lymphadenopathy develops. Secondary lesions, known as *pintids*, may arise years later from treponemal dissemination and exhibit a cellular proliferation and infiltration similar to that of the primary lesion. In contrast to venereal syphilis, blood vessels remain intact and do not show proliferation. The dyschromic or multicolored nature (brown, slate blue, black, or gray) of the primary and secondary lesions is more characteristic of older than of younger lesions, in which pigmentary changes usually are minimal. The variously pigmented older lesions may show hyperkeratosis or parakeratosis. Large numbers of treponemes can be found throughout the dermis and epidermis in the highly contagious primary and secondary lesions.

Late pinta is characterized by pigmentary changes, from dyschromic treponeme-containing lesions to achromic treponeme-free lesions. This depigmentation process occurs at different rates even within the same lesion, which gives rise to different degrees of hypochromia and atrophy around dyschromic and achromic lesions.[53] A concomitant paucity of hair follicles and sebaceous glands may occur.[14]

Clinical Manifestations

Pinta is characterized by a continuing production of early infectious lesions from either direct extension or dissemination and by the concomitant presence of lesions in various stages of dyschromia and achromia. The lesions are not well delineated, often merge, and may be accompanied by regional lymphadenopathy. Primary, secondary, and tertiary stages may or may not occur simultaneously.

The early stage of pinta includes the initial lesions, occasional regional lymphadenopathy, and secondary lesions resulting from dissemination of treponemes. A primary lesion (not always evident) develops after an incubation period of 3 days to 2 months, usually 2 to 3 weeks. The primary lesion begins as one or more small erythematous papules that may appear scaly and indurated. The papules progressively enlarge over 1 to 3 months, often coalescing, becoming pigmented, more erythematous, and scaly while developing heaped-up margins. Occasionally, an accompanying regional lymphadenopathy is present. The lesion may disappear in several months or may continue to enlarge for several years, forming larger psoriasiform plaques that coalesce further.

The primary lesions of pinta generally overlap development of the secondary lesions or pintids, which appear months to years after infection occurs. During this stage, dissemination ensues and almost any area of the skin can be involved. The lesions occur typically on exposed areas of skin and are variously pigmented. The degree of pigmentation is related to the state of development of the lesion, the age of the lesion, the degree of exposure to sun, and the host's natural pigmentation. Pintids begin as small, scaly papules that gradually enlarge and coalesce. Several colors may exist within the same lesion. Initially, pintids usually are red to violaceous; later, they become slate blue, gray, or black as a result of photosensitization. Lesions on the legs typically are yellowish brown or dark brown.

The late stage of pinta is characterized by the development of depigmented lesions. Achromia usually begins several years after the onset of the disease but may appear 3 months after onset. Areas of depigmentation spread slowly, leaving large achromic lesions, reminiscent of vitiligo, as the end result. Early achromia tends to be asymmetric, whereas later depigmented lesions are symmetric. Characteristic of the Cuban form of pinta is the development of hyperkeratosis of the palms and soles. Many chronic dermatologic entities characterized by scaly, psoriasiform lesions or by dyschromia or depigmentation can be confused with pinta in endemic areas. Maculopapulosquamous diseases, including psoriasis, parapsoriasis, and lichen planus, and dyschromic dermatologic diseases such as vitiligo, ochronosis, and argyria must be taken into consideration.

Prognosis

Pinta is not a fatal disease; it produces changes related only to the skin. There is no involvement of the central nervous system, the cardiovascular system, or other internal organs. If the disease is not treated, the patient frequently develops large achromic cutaneous blemishes. The major resulting problem is one of social ostracism from the community. Infected individuals are removed from the urban society and find refuge in the rural areas. This isolation separates the patient farther from the principal sources of medical therapy.

YAWS

Yaws (frambesia, pian, buba, bouba) is a communicable, nonvenereally transmitted treponematosis of the tropical zones. The disease is characterized by its early acquisition (usually before puberty) and its chronic, relapsing pattern of early benign lesions separated by periods of latency that terminate with late destructive lesions of skin, bones, and cartilaginous tissues. A very prevalent disease in the early part of the 20th century, yaws was estimated to have affected more than 50 million people. Although seroepidemiologic and mass treatment campaigns sponsored by WHO and UNICEF have reduced its overall prevalence dramatically, endemic areas of concern continue to exist.[4,21,46,61,65]

Biology and Immunology

T. pallidum subsp. *pertenue*, the etiologic agent of yaws, is morphologically identical to other pathogenic treponemes. The presence of infection with either yaws or syphilis strains has previously been distinguishable only by epidemiologic and clinical means. The genomes of three strains of *T. pallidum* subsp. *pertenue* were completely sequenced and compared with four strains of *T. pallidum* in an attempt to find differences between the genomic structures of syphilis and yaws. The two genomes were found to have a 99.8 percent concordance. The differences were believed to be mostly located in genes that could be encoding virulence factors and have the potential for identifying specific targets for diagnostic testing. This may be important in differentiating between childhood congenital syphilis and yaws, a distinction that can be problematic.[8]

Although not clinically useful, animal models can be used to distinguish between syphilis and yaws. Rabbits and hamsters have been infected experimentally by both diseases, with resultant lymphadenopathy and visible lesions. Characteristic differences in the distribution, form, and number of lesions can be used to distinguish the identity of the infectious agent. On the basis of human and experimental studies, the organism stimulates a significant degree of homologous resistance to reinfection and superinfection and cross immunity to *T. pallidum* subsp. *pallidum* and *endemicum*.[28,32,60,64]

Epidemiology

Yaws is a disease of tropical countries found primarily among rural populations. It exists in the warm, moist, endemic areas of rural Africa, Southeast Asia, the Caribbean, South America, the Pacific, and central and southern Africa. As indicated, seroepidemiologic and mass treatment campaigns have resulted in a significant reduction in prevalence of the disease in most of these areas. The failure of many countries to integrate active control measures into the functions of local health services has led, however, to a gradual buildup and extension of reservoirs of yaws, with the emergence of significant numbers of new, active cases since 1974 in Ghana, Togo, Benin, Indonesia, Papua New Guinea, and the Ivory Coast.[4,71] In a WHO survey in the Central African Republic, Congo, and Gabon, clinical yaws was detected in more than 20 percent of the Pygmy population and reactive serologic tests were obtained in 80 percent.[71] Health care workers in the periurban settlements of Port Moresby, Papua New Guinea, identified 494 cases between April 2000 and September 2001. A 2001 survey in the Solomon Islands showed that yaws was the sixth leading cause of morbidity and that the prevalence was 4106 cases per 100,000 population.[63] In Latin America, cases still occur in Brazil, Colombia, Haiti, Venezuela, and Suriname.[16,43] Intense surveillance and eradication efforts in India, however, appear to have been successful with no new cases reported since 2004.[4]

Poor hygiene, close crowding among children (especially in sleeping areas), and lack of protective clothing facilitate transmission of the disease by direct skin-to-skin contact, whereby *T. pallidum* subsp. *pertenue* enters traumatized exposed areas from an infected lesion. The usual portals of entry are the lower extremities, head, face, and mouth. The presence of treponemal antibodies and the isolation of treponemes (most probably *T. pallidum* subsp. *pertenue* from West African monkeys[6,19]) suggest that primates in this area may act as a reservoir for spread of the disease.

Yaws usually is acquired before puberty and is most prevalent among children. Because such a large percentage of the population contracts the disease in infancy, young children represent the primary reservoir. Older children and adults generally are not infectious; therefore, congenital and sexual transmission does not occur. Boys and girls are affected equally.

Pathogenesis and Pathology

T. pallidum subsp. *pertenue* enters the host through abraded skin, most frequently below the knees. The organisms multiply locally and in the regional lymph nodes. Epithelial hyperplasia and plasma cell, lymphocytic, and macrophage infiltration are elicited. The end result is the formation of a primary lesion and lymphadenopathy containing numerous treponemes. Shortly after their introduction into the host, organisms are carried to skin, bone, and cartilage through the circulation. Disseminated treponemes in these tissues multiply, elicit a chronic inflammatory response similar to that seen in the primary lesion, and produce distant papillomas, lymphadenopathy, hyperkeratosis, and bone involvement that develop without interruption until they are reversed by either immune mechanisms or treatment. In contrast to those associated with venereal syphilis, vascular changes are discrete or do not occur in cutaneous yaws.[68]

Untreated primary and secondary cutaneous lesions generally heal with only minimal scarring, unless they are complicated by secondary bacterial infection. The healing process and maintenance of latency seem to involve humoral and cellular mechanisms of immunity.[59] Relapses may occur during latency and, together with late disease, may be caused by a breakdown in the immune state, the development of antigenic variation by the treponeme, or both.

The late gummatous lesions presumably are caused by a hypersensitivity-induced mechanism similar to that postulated for the syphilitic gumma. Bone changes in late stages of yaws most often involve the long bones and manifest as hypertrophic periostitis, gummatous periostitis, osteitis, and nodular or generalized osteomyelitis.[26] Juxta-articular nodules occur near major joints and are characterized as nonspecific granulomas.[13]

Clinical Manifestations

Yaws is a chronic, debilitating disease characterized by early infectious lesions; periods of latency and relapse; and late destructive lesions of cutaneous, subcutaneous, cartilaginous, and bony tissue. After an incubation period of 9 to 90 days (usually about 3 weeks), the primary lesion forms at the site of inoculation and is accompanied by regional lymphadenopathy. The lesion typically appears as a raised papule on the lower extremity that enlarges to become a hyperkeratotic papilloma measuring 2 to 5 cm in diameter, referred to as a *mother yaw*. It undergoes shallow ulceration and persists a few months to 3 years, at which time healing occurs with a resultant hypopigmented scar and surrounding dark margins.[13]

Before healing of the mother yaw or weeks to months thereafter, crops of secondary, generalized, nondestructive papular lesions appear together with lymphadenopathy and malaise. These lesions tend to form near areas with mucous membranes, such as the nose and mouth, and may resemble condylomata lata. Some papules fade, whereas others enlarge to become papillomatous lesions referred to as *satellite secondaries* or *daughter yaws*. The multiple papillomas are circular, raised, red-yellow lesions with a granular, lobulated, and verrucous surface. They contain numerous treponemes, usually measure 1 to 3 cm in diameter, and produce a yellow discharge that dries to form a black scab. Hyperkeratotic involvement of the palms and soles occurs commonly. Painful fissuring of the soles may cause the patient to walk on the sides of the feet, producing the characteristic gait of "crab" yaws. Bone pain may be severe, and nondestructive long bone lesions, including periostitis, osteitis, and osteomyelitis, may occur.[13,25]

Untreated secondary lesions may persist for longer than 6 months, at which time, owing to the development of host immune mechanisms, they usually heal without scars or residual defects, unless ulceration caused by

secondary bacterial infection occurs. During the dry season, early yaws lesions often are atypical, tending to be macular and fewer in number; papillomas, which are small, scanty, dry, flat, and grayish in appearance and of short duration (about 1 month), are confined mainly to the hidden, protected, moist skin folds.[48,53,66]

Despite healing, some treponemes evade the immune process and persist in the affected tissues (latency). Latency frequently is interrupted by relapses, which tend to occur several times over a 3- to 5-year period. Fewer lesions are produced with each relapse, and they tend to be localized to the periaxillary, perianal, or circumoral area.[53] Upon cessation of the relapses and usually after a latent period of 3 to 10 years, tertiary lesions occur in 10 percent of patients[53]; the latent state persists in the remaining patients for their lifetime.

The tertiary lesions characteristically are solitary and destructive; they involve skin, subcutaneous tissue, bone, or cartilage and most commonly occur after the onset of puberty. Painful hyperkeratosis of the palms and soles similar to that seen in early yaws frequently occurs. Other lesions develop as ulcerating, subcutaneous nodules and may heal spontaneously to form scars or extend widely from their margins. The scarring results in depigmentation and sometimes contractions. Bone deformities of late-stage yaws include chronic hypertrophic periostitis, osteitis, gummatous periostitis, and osteomyelitis, each of which may ulcerate through the skin. Gangosa, or rhinopharyngitis mutilans, the destructive gummatous ulceration of the skin and bones of the central face, and juxta-articular nodules, ganglions of tendon sheaths, and saber tibiae also may occur as manifestations of the late stages of the disease.[26] Gondou, a disfiguring hypertrophic osteitis of the frontal processes of the maxillae, previously reported among western Africans with yaws, seems to have disappeared.[38] Other diseases that must be differentiated from the skin lesions of yaws include vitamin deficiencies, early stages of leprosy, venereal syphilis, tinea versicolor, molluscum contagiosum, scabies, lichen planus, plantar warts, tungiasis, psoriasis, and cutaneous leishmaniasis. Sickle-cell disease, tuberculosis, and bacterial osteomyelitis may produce clinical manifestations that mimic the osseous lesions of yaws.

Prognosis

Yaws is not a benign disease. If left untreated, it can produce destructive, disfiguring lesions of the face, feet, and hands and painful and disabling lesions of the fingers and long bones. Ulcers near joints may result in crippling contractures. Secondary bacterial infection of ulcers and of protruding bone lesions can result in further permanent damage to skin and bone tissues. Fractures generally do not occur.

ENDEMIC SYPHILIS

Endemic syphilis (bejel, njovera, siti, dichuchwa) is a chronic, nonvenereally transmitted disease of prepubescent children. It occurs in the warm, dry, arid regions of the world[53]; lesions are confined to the skin, bone, and cartilage. The disease, known to exist for centuries in Africa, has been recorded in epidemic proportions in areas where conditions among children allow transmission. As with yaws, although seroepidemiologic and mass treatment campaigns have reduced its overall prevalence drastically,[67] endemic areas of concern continue to exist and include the region bordering the southern Sahara Desert, Botswana, and the Arabian Peninsula.[49,53,69]

Biology and Immunology

T. pallidum subsp. *endemicum*, the etiologic agent of endemic syphilis, morphologically is identical to the other pathogenic treponemes. *T. pallidum* subsp. *endemicum* exhibits similarity to syphilis and yaws by producing visible lesions and generalized lymphadenopathy in rabbits and hamsters experimentally infected by the intratesticular and intradermal routes.[28,61,64] The degree of induration of lesions, distribution and number of lesions, and the characteristic lymphadenopathy can be used to differentiate among the species. On the basis of experimental studies in inbred hamsters, *T. pallidum* subsp. *endemicum* stimulates a high degree of homologous resistance to reinfection and cross immunity to *T. pallidum* subsp. *pallidum* and *T. pallidum* subsp. *pertenue*.[61]

Epidemiology

Endemic syphilis continues to persist in the warm, drier desert areas bordering the tropical belt. It is prevalent primarily among the seminomadic rural populations in the Arabian peninsula and along the southern border of the Sahara Desert in Africa known as the Sahel region[49,53]; a significant resurgence occurred in Mali, Mauritania, Niger, and the upper Volta during the 1970s.[47,65] More than 1000 cases were reported in Senegal in 1980.[43] Although scattered endemic foci did exist in central Asia, Australia, Bosnia, and India, they have been virtually eliminated from these areas by mass treatment campaigns.[49,53]

As with pinta and yaws, endemic syphilis propagates under conditions of poor hygiene, crowding, and wearing of little or no clothing. Transmission by oral mucous membranes is favored through contaminated objects, such as drinking vessels and kitchen utensils, and through contact with saliva-contaminated fingers and mouth-to-mouth contact.[12,17,31] Fomites are thought to play a role. Transmission may occur through direct contact of the oral lesion to skin.[53] Occasionally, a previously uninfected nursing mother develops a primary lesion on or near the nipple after the transfer of treponemes from her infected infant.[21,69] Congenital transmission does not seem to occur. Recent analysis of pre-Columbian European skeletons found no bejel despite significant contact with cases during combat in the Crusades. The conclusions drawn from this analysis are that adults are not susceptible to transmission from other adults and furthermore that adults cannot transmit endemic syphilis.[57] The role of insect vectors, such as flies, has been suggested, but it may be caused by creating a portal of entry by breaks in the skin after itching. Endemic syphilis occurs predominantly

among children, with onset usually occurring in children younger than 15 years and with equal sex distribution. Spread occurs most commonly within the family, and active disease can be manifest in more than one family member at any given time.

Pathogenesis and Pathology

T. pallidum subsp. *endemicum* enters the host usually through the oral mucosa. The small number of treponemes introduced into a susceptible host usually precludes the local multiplication and host inflammatory response required to produce a visible primary buccal lesion.[21] When a primary lesion does occur, it appears as a papule or ulcer resulting from a chronic inflammatory response to the proliferating organisms consisting of plasma cell, lymphocytic, and macrophage infiltration. Endothelial cell swelling of small blood vessels also is evident.

The organisms are carried to the regional lymph nodes within a few hours of entry, commonly through the oral mucosa portal. They multiply and elicit epithelial hyperplasia and plasma cell, lymphocytic, and macrophage infiltration, with resultant lymphadenopathy. Dissemination occurs through the circulation, and the organisms are carried to the skin, bone, oral mucosa, axillae, and anogenital regions, where they multiply and elicit a chronic inflammatory response characterized by a cellular infiltration, as seen in the lymph nodes. Vascular changes and perivascular cuffing are prominent.

The untreated early lesions heal as a result of mechanisms thought to involve humoral and cellular immune responses by the host,[58] and the disease enters into a state of latency. Maintenance of latency is thought to involve similar mechanisms. The occurrence of infectious relapses during the latent period still is uncertain.

The late lesions of endemic syphilis are strikingly similar to those of late-stage yaws. Late-stage disease and relapses (if they occur) may be caused by a hypersensitivity-induced mechanism similar to that postulated for the syphilitic gumma.[20,21] Juxta-articular nodules may occur and represent a nonspecific granulomatous response occurring near major joints.[13] The rarity of cardiovascular and neurologic manifestations may be due to the slow acquisition of small numbers of organisms over a long time, which results in the immunologic protection of the heart and nervous system.[21,35]

Clinical Manifestations

Endemic syphilis is a chronic, often debilitating disease characterized by early infectious secondary lesions; variable periods of latency; and late destructive lesions of cutaneous, subcutaneous, and bone tissues. As mentioned, primary lesions are rare. They appear usually on the breast or nipple as a papule or shallow ulcer similar to that seen in primary venereal syphilis after an approximate 3-week incubation period[1]; they may persist for years before healing.[31]

Even without the appearance of a primary lesion, generalized infection occurs as a result of early dissemination. The onset begins after an incubation period of 3 to 6 months and is characterized by the presence of highly infectious, relatively painless, ulcerative patches on the oropharyngeal mucosa, including the tongue, lips, palate, tonsils, and larynx, with accompanying regional lymphadenopathy. Involvement of the larynx usually results in hoarseness.[13] Split papules or angular stomatitis occurs at the angles of the mouth. Osteoperiostitis of the long bones of the lower extremities, similar to that seen in yaws, is a common early manifestation causing nocturnal leg pains.[13] Occasionally, axillary and anogenital secondary-type lesions result and consist of condylomata similar to those of yaws or dry papillomatous annular patches, with accompanying axillary and inguinal lymphadenopathy. Disseminated papules that are indistinguishable from the papules seen in secondary venereal syphilis may occur. Other forms of cutaneous lesions can occur but are rare.

Untreated secondary lesions may persist for 6 to 9 months, at which time healing occurs because of the development of host immunity. This period of latency varies and, similar to yaws, may last for several years.

Most patients develop tertiary manifestations. Late lesions generally occur during adolescence or adult life and may resemble lesions seen in either late yaws or late-stage venereal syphilis. Gummata may affect any part of the body but commonly occur in the nasopharynx, skin, and bone, resulting in destructive, disfiguring, chronic ulcerations characteristic of gangosa or gangosa-like lesions. Late gummatous lesions can occur during childhood, possibly as a result of superinfection in an already infected host.[22] Bone involvement also is a common finding and results in painful lesions. This condition involves osteitis with gumma formation and, similar to yaws, periostitis affecting most frequently the long bones of the lower extremities. Bilateral synovitis, especially of the knees, occasionally may occur with concomitant juxta-articular nodules.[22] The cardiovascular and neurologic findings common to venereal syphilis rarely occur in endemic syphilis; when clinical manifestations do occur, they usually are atypical and very mild.[11,49]

Endemic syphilis reportedly has become "clinically attenuated" in Saudi Arabia.[49] Previously florid, the classic disease seems to have been replaced by a milder form in which the number, severity, and duration of early and late lesions are reduced and seroreactive latent infection is increased.[49] The most common late manifestation observed in this study was painful osteoperiostitis of the legs affecting mainly the tibia and fibula. Researchers have postulated that attenuation is a result of improvement in hygienic conditions, better nutrition, and medical care.[49] This indicates the importance of attention to public health measures and the use of antibiotics in the eradication of the disease.

Prognosis

The main complication of endemic syphilis is the destructive gummatous lesions of the face and bones. Severely disfiguring and disabling, these lesions prevent the patient from working effectively in the community. Many of the bone lesions are extremely painful and incapacitating.

DIAGNOSIS

In the nonvenereal treponematoses, until techniques based on molecular probes are developed a presumptive diagnosis is based largely on clinical presentation of the disease in an endemic area. Although darkfield examination of early lesions and lymph nodes permits visualization of treponemes, late lesions usually contain few, if any, organisms. Differentiating the treponematoses from one another solely on the basis of serologic testing is currently untenable because of shared antigens.[20,53] The diagnosis of yaws in nonendemic areas or during periods of latency is difficult to make. The coexistence of yaws and endemic syphilis in certain geographic locations, together with their often identical clinical manifestations, renders darkfield and serologic assays useless for differentiating these diseases in such areas.[53] Similar limitations are applicable in differentiating venereal syphilis from yaws or endemic syphilis in nonendemic areas.[53] Under these circumstances, the diagnosis can be based only on a careful history and epidemiologic data.[53]

In tropical areas, numerous other diseases may be confused with yaws.[27,53] Impetigo and chronic tropical ulcers are found frequently and may respond to penicillin therapy. Ecthyma may produce ulcers that occasionally are similar to the ulcerative papillomas of yaws.

The same serologic testing is used in the diagnosis of venereal and nonvenereal treponematoses. The rapid plasma reagin and Venereal Disease Research Laboratory tests are useful but may be nonreactive in early lesions. They are nonspecific nontreponemal studies using cardiolipin antigens that produce similar results in the presence of each disease and cannot be used to differentiate between them. Care must be taken in the interpretation of nontreponemal tests in establishing the diagnosis because of the possibility of obtaining false-positive reactions among patients with nontreponemal diseases or conditions.[45] These tests are quantitative, however, and false-positive results usually have a titer of less than 1:4. After treatment has been given, a twofold or greater drop in titers usually signifies a successful outcome.

The treponemal tests, such as the fluorescent treponemal antibody absorption test, the *T. pallidum* immobilization test, and the *T. pallidum* hemagglutination assay, are specific but normally remain positive for life and used alone may not be useful in diagnosing active cases. Combining nontreponemal and treponemal tests is helpful in establishing the diagnosis when there is a high pretest suspicion, when a false-positive nontreponemal test is likely when a treponemal test is negative, or when there is a low pretest probability. In pinta, both types of tests may not become reactive until 4 months after the development of the initial lesion, whereas in yaws and endemic syphilis, both tests become reactive within the first few weeks of illness.

Although it may be less practical than serologic testing in remote and resource-poor areas, darkfield examination of fluid obtained from the initial serous exudates from cutaneous or mucous membrane lesions generally reveals the presence of treponemes. As the lesions become more chronic, treponemes become difficult to find. Direct fluorescent antibody testing can detect treponemal species on a slide but generally is unavailable in the field. Silver impregnation of biopsy material from skin lesions or lymph nodes may reveal the organisms, but care must be taken in differentiating treponemes from tissue artifact.

Newer techniques such as polymerase chain reaction have been used to detect the presence of treponemes. Although polymerase chain reaction is highly sensitive and specific, until further research elucidates unique antigenic fingerprints, it cannot differentiate among species as yet and is not practical for field use in resource-poor endemic areas.[50]

TREATMENT AND PREVENTION

The treatment of choice for all treponemes remains benzathine penicillin G, although recent studies show promise for the use of macrolides in eradication programs.[46,47] Patients older than 10 years of age with clinical disease, those in a period of latency or in the incubatory stages, and contacts of cases should receive 1.2 million units of benzathine penicillin G intramuscularly in a single dose; children younger than 10 years of age should receive 600,000 units in a single dose by the same route.[53,71] Isolated cases of resistance have been reported in Papua New Guinea,[5] Ecuador,[2] and Guyana[62]; however, whether these cases represent true resistant strains, other concurrent infections, or problems in storage and administration of the medicine remains unclear. Penicillin continues to be considered effective for treatment and eradication. Oral penicillin was used to treat 17 active cases of yaws in Guyana in 2000 to 2001. All but one patient had complete resolution of the lesions. Although this development is promising because it would eliminate the need for refrigeration and intramuscular injections, more data on the efficacy and compliance with oral penicillin regimens are needed.[62]

A noninferiority trial of a single dose of 3 mg/kg of azithromycin versus benzathine penicillin in Papua New Guinea in children aged 6 months to 15 years with confirmed diagnoses of yaws showed that yaws can be cured in its early stages and can prevent transmission.[46,47] Renewal of flagging efforts toward eradication with a single pill overcomes some of the disadvantages of the standard treatment with benzathine penicillin, particularly in eradication campaigns. Sterile protocols for intramuscular injections are not always easy to follow in remote areas, trained medical personnel are needed to administer the injections, penicillin allergy is common, and penicillin requires refrigeration. Oral penicillin also is effective but requires 10 days of treatment, which makes compliance problematic. However, the emergence of cases of macrolide resistance and the induction of macrolide resistance to *Streptococcus pneumoniae* may temper some of the enthusiasm associated with the use of azithromycin.[36,39,40,74]

Tetracycline is an alternative treatment for penicillin-allergic patients older than 8 years of age and not pregnant. The recommended dosage is 500 mg by mouth four times daily for 15 days (total dose of 30 g); children aged 8 to 15 years old may be given half the dose.[53] Although

the newer tetracyclines have been used and are assumed to be equally effective, no information is available about their use. Studies to determine the efficacy of erythromycin have not been reported, but based on its use in syphilis, erythromycin is presumed to be another alternative for penicillin-allergic patients. The recommended dosage is 8 to 10 mg/kg up to the adult dosage of 250 mg four times daily.[53]

Therapy in yaws renders early lesions noninfectious in a few days, with complete healing achieved in 7 to 10 days. Recurrences after treatment may occur as a result of reinfection.[27] Late lesions heal more slowly after therapy and may require surgery. In endemic syphilis (bejel), infectious lesions disappear rapidly and relapses usually are prevented after therapy. The clinical response to therapy is remarkably slow in pinta. Primary and early secondary lesions take 4 to 6 months to disappear, whereas late secondary lesions require 6 to 12 months for complete healing to occur.[55] Hyperchromic lesions heal without residua, and hypochromic lesions often result in depigmented areas. Old achromic lesions usually remain intact without repigmentation.

In yaws and nontreponemal bejel, test titers may revert to nonreactive if the patient is treated early in the course of the disease. The longer the patient remains untreated, however, the more slowly conversion to seronegativity occurs, and treatment during the later stages may result in the persistence of high titers.[51] Generally, the serologic response to therapy is absent or slow in pinta; nontreponemal tests may take years to decline after adequate therapy has been given.[44] In each of the treponemal diseases, treponemal tests remain reactive for life after the patient has received adequate therapy.

Improvement of living conditions and the general hygiene of the community, mass treatment of patients and contacts, and seroepidemiologic campaigns contribute significantly to the prevention of yaws. WHO recommendations state that the entire population should be treated when the prevalence exceeds 10 percent. Where prevalences of 5 to 10 percent exist, all cases, close contacts, and all children younger than 15 years are treated. Where a prevalence of less than 5 percent exists, WHO recommends that cases, close contacts, and all members of the household be treated. Where implemented, this strategy has been remarkably effective, such as seen in an eradication program in Ecuador and the successful program in India.[2,4]

FUTURE CONSIDERATIONS

It is a tragedy that such easily curable diseases have yet to be eradicated. Little recent epidemiologic data are available because many governments have stopped collecting prevalence data since the presence of these diseases implies poor health conditions. Recent advances in molecular diagnostics hold promise that genomic or antigenic markers will soon be available and will simplify the diagnosis and differentiation between species. A renewed commitment by bodies such as WHO has been declared that it is hoped will bring the elimination of these diseases that have brought so much suffering and social ostracism. Simple measures, such as improved hygiene, education, and better access to health care, can ameliorate greatly the severity of disease. The effective eradication campaigns of the 1950s and 1960s need to be continued, along with awareness and vigilance on the part of health care professionals and health policy makers. Promising new tools, such as the use of single-dose azithromycin in endemic areas, may at last bring an end to these ancient scourges.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Armelagos GJ, Zuckerman MK, Harper KN. The science behind pre-Columbian evidence of syphilis in Europe: research by documentary. *Evol Anthropol* 2012;**21**(2):50–7.
4. Asiedu K, Amouzou B, Dhariwal A, et al. Yaws eradication: past efforts and future perspectives. *Bull World Health Organ* 2008; **86**(7):499–499A.
8. Cejková D, Zobaníková M, Chen L, et al. Whole genome sequences of three *Treponema pallidum* ssp. *pertenue* strains: yaws and syphilis treponemes differ in less than 0.2% of the genome sequence. *PLoS Negl Trop Dis* 2012;**6**(1):e1471.
36. Mabey D. Oral azithromycin for treatment of yaws. *Lancet* 2012; **379**(9813):295–7.
39. Martin IE, Gu W, Yang Y, Tsang RS. Macrolide resistance and molecular types of *Treponema pallidum* causing primary syphilis in Shanghai, China. *Clin Infect Dis* 2009;**49**(4):515–21.
40. Martin IE, Tsang RS, Sutherland K, et al. Molecular characterization of syphilis in patients in Canada: azithromycin resistance and detection of *Treponema pallidum* DNA in whole-blood samples versus ulcerative swabs. *J Clin Microbiol* 2009;**47**(6):1668–73.
46. Mitjà O, Hays R, Rinaldi AC, et al. New treatment schemes for yaws: the path toward eradication. *Clin Infect Dis* 2012;**55**(3): 406–12.
47. Mitjà O, Hays R, Ipai A, et al. Single-dose azithromycin versus benzathine benzylpenicillin for treatment of yaws in children in Papua New Guinea: an open-label, non-inferiority, randomized trial. *Lancet* 2012;**379**(9813):342–7.
74. Woznicová V, Matejková P, Flasarová M, et al. Clarithromycin treatment failure due to macrolide resistance in *Treponema pallidum* in a patient with primary syphilis. *Acta Derm Venereol* 2010;**90**(2): 206–7.
76. Zimmer C. Infectious diseases: isolated tribe gives clues to the origins of syphilis. *Science* 2008;**319**(5861):272.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Akrawi F. Is bejel syphilis? *Br J Vener Dis* 1949;**25**:115–23.
2. Anselmi M, Moreira J, Calcedo C, et al. Community participation eliminates yaws in Ecuador. *Trop Med Int Health* 2003;**8**:634–8.
3. Armelagos GJ, Zuckerman MK, Harper KN. The science behind pre-Columbian evidence of syphilis in Europe: research by documentary. *Evol Anthropol* 2012;**21**(2):50–7.
4. Asiedu K, Amouzou B, Dhariwal A, et al. Yaws eradication: past efforts and future perspectives. *Bull World Health Organ* 2008; **86**(7):499–499A.
5. Backhouse JL, Hudson BJ, Hamilton PA, et al. Failure of penicillin treatment of yaws on Karkar Island, Papua New Guinea. *Am J Trop Med Hyg* 1998;**59**:388–92.
6. Baylet R, Thivolet J, Sepetjian M, et al. La treponematose naturelle ouverte du singe Papio papio en Casamance. *Bull Soc Pathol Exot* 1971;**64**:842–6.
7. Cameron CE, Castro C, Lukehart SA, et al. Sequence conservation of glycerophosphodiester phosphodiesterase among *Treponema pallidum* strains. *Infect Immun* 1999;**67**:3168–70.
8. Cejková D, Zobaníková M, Chen L, et al. Whole genome sequences of three *Treponema pallidum* ssp. *pertenue* strains: yaws and syphilis treponemes differ in less than 0.2% of the genome sequence. *PLoS Negl Trop Dis* 2012;**6**(1):e1471.
9. Centurion-Lara A, Castro C, Castillo R, et al. The flanking region sequences of the 15-kDa lipoprotein gene differentiate pathogenic treponemes. *J Infect Dis* 1998;**17**:1036–40.
10. Centurion-Lara A, Sun LK, Barrett C, et al. Multiple alleles of *Treponema pallidum* Repeat Gene D in *Treponema pallidum* isolates. *J Bacteriol* 2000;**182**:2332–5.
11. Csonka GW. Clinical aspects of bejel. *Br J Vener Dis* 1953;**29**: 95–103.
12. Cutler JC. Endemic syphilis, yaws, and pinta. In: Johnson RC, editor. *The biology of parasitic spirochetes*. New York: Academic Press; 1976. p. 365–73.
13. Dooley JR, Binford CH. Treponematoses. In: Binford CH, Connor DH, editors. *Pathology of tropical and extraordinary diseases*, vol. 1. Washington, D.C.: Armed Forces Institute of Pathology; 1976. p. 10–117.
14. Edmundson WF. Pinta. In: Demis DJ, Dobson RC, McGuire J, editors. *Clinical dermatology*, vol. 3. New York: Harper & Row; 1976. p. 1–12.
15. Edmundson WF, Lopez Rico A, Olansky S. A study of pinta in the Tepalcatepec Basin, Michoacan, Mexico. *Am J Syph* 1953;**37**: 201–25.
16. Engelkens HJ, Vuzevski VD, Stolz E. Nonvenereal treponematoses in tropical countries. *Clin Dermatol* 1999;**17**:143–52; discussion 105–6.
17. Farnsworth N, Rosen T. Endemic treponematosis: review and update. *Clin Dermatol* 2006;**24**:181–90.
18. Fieldsteel AH, Cox DL, Moeckli RA. Cultivation of virulent *Treponema pallidum* in tissue culture. *Infect Immun* 1981;**32**:908–15.
19. Fribourg-Blanc A, Niel G, Mollaret HH. Note sur quelques aspects immunologiques du cynocephale african. *Bull Soc Pathol Exot* 1963;**56**:474–85.
20. Garner MF, Backhouse JL, Cook CA, et al. Fluorescent treponemal antibody absorption (FTA-ABS) test in yaws. *Br J Vener Dis* 1970; **46**:284–6.
21. Grin EI. Epidemiology and control of endemic syphilis. *W H O Monogr Ser* 1953;**11**.
22. Grin EI. Endemic syphilis (bejel). In: Demis DJ, Dobson RC, McGuire J, editors. *Clinical dermatology*, vol. 3. New York: Harper & Row; 1976. p. 1–7.
23. Guthe T, Luger A. Epidemiologic aspects of nonvenereal endemic syphilis. *Dermatologica* 1957;**115**:248–72.
24. Guthe T, Wilcox RR. Changing concepts in the epidemiology and control of the treponematoses. *Chron W H O*, Special Number 1954;**8**:33–69.
25. Hackett CJ. *Bone lesions of yaws in Uganda*. Oxford: Blackwell Scientific Publications; 1951.
26. Hackett CJ. Yaws. In: Demis DJ, Dobson RC, McGuire J, editors. *Clinical dermatology*, vol. 3. New York: Harper & Row; 1976. p. 1–19.
27. Hackett CJ, Loewenthal LJA. Differential diagnosis of yaws. *W H O Monogr Ser* 1960;**45**:1–88.
28. Hardy PH. Pathogenic treponemes. In: Johnson RC, editor. *The biology of parasitic spirochetes*. New York: Academic Press; 1976. p. 107–19.
29. Harper KN, Ocampo PS, Steiner BM, et al. On the origin of the treponematoses: a phylogenetic approach. *PLoS Negl Trop Dis* 2008;**2**(1):e148.
30. Hill KR. Non-specific factors in the epidemiology of yaws. *Bull W H O* 1953;**8**:17–47.
31. Hudson EH. *Non-venereal syphilis*. Edinburgh: E. & S. Livingstone; 1958.
32. Knox JM, Musher D, Guzick ND. The pathogenesis of syphilis and the related treponematoses. In: Johnson RC, editor. *The Biology of Parasitic Spirochetes*. New York: Academic Press; 1976. p. 249–59.
33. Kuhn USG III, Medina R, Cohen PG, et al. Inoculation pinta in chimpanzees. *Br J Vener Dis* 1970;**46**:311–12.
34. Kuhn USG III, Varela G, Chandler FW Jr, et al. Experimental pinta in the chimpanzee. *J A M A* 1968;**206**:829.
35. Luger A, Schmid EE. Immunity of the central nervous system in endemic syphilis. *Dermatol Wochenschr* 1961;**143**:617–37.
36. Mabey D. Oral azithromycin for treatment of yaws. *Lancet* 2012; **379**(9813):295–7.
37. Magnuson HJ, Thomas EW, Olansky MD, et al. Inoculation syphilis in human volunteers. *Medicine* 1956;**35**:33–82.
38. Marfart B. Goundou: a historical form of yaws. *Lancet* 2002;**360**: 1168–70.
39. Martin IE, Gu W, Yang Y, Tsang RS. Macrolide resistance and molecular types of *Treponema pallidum* causing primary syphilis in Shanghai, China. *Clin Infect Dis* 2009;**49**(4):515–21.
40. Martin IE, Tsang RS, Sutherland K, et al. Molecular characterization of syphilis in patients in Canada: azithromycin resistance and detection of *Treponema pallidum* DNA in whole-blood samples versus ulcerative swabs. *J Clin Microbiol* 2009;**47**:1668–73.
41. McLeod CP, Magnuson HJ. Study of cross-immunity between syphilis and yaws in treated rabbits. *J Vener Dis Infect* 1951;**32**: 305–9.
42. Medina R. Pinta in South America. In: *Fogarty International Center Symposium Documents: Yaws and other endemic treponematoses*. April 16-18, 1984.
43. Meheus A, Antal GM. The endemic treponematoses: not yet eradicated. *World Health Stat Q* 1992;**45**:228–37.
44. Mesa J, Restrepo A, Cortes A. A study of fluorescent treponemal antibody absorption (FTA-ABS) and VDRL tests in pinta. *Int J Dermatol* 1973;**12**:135–8.
45. Miller JN. Value and limitations of nontreponemal and treponemal tests in the laboratory diagnosis of syphilis. *Clin Obstet Gynecol* 1975;**18**:191–203.
46. Mitjà O, Hays R, Ipai A, et al. Single-dose azithromycin versus benzathine benzylpenicillin for treatment of yaws in children in Papua New Guinea: an open-label, non-inferiority, randomized trial. *Lancet* 2012;**379**(9813):342–7.
47. Mitjà O, Hays R, Rinaldi AC, et al. New treatment schemes for yaws: the path toward eradication. *Clin Infect Dis* 2012;**55**(3): 406–12.
48. Niemal PLA, Brunings EA, Menke HE. Attenuated yaws in Surinam. *Br J Vener Dis* 1979;**55**:99–101.
49. Pace JL, Csonka GW. Endemic non-venereal syphilis (bejel) in Saudi Arabia. *Br J Vener Dis* 1984;**60**:293–7.
50. Palmer HM, Higgins SP, Herring AJ, Kingston MA. Use of PCR in the diagnosis of early syphilis in the United Kingdom. *Sex Transm Infect* 2003;**79**:479–83.
51. Parish JL. Treponemal infections in the pediatric population. *Clin Dermatol* 2000;**8**:687–700.
52. Pecher SA. Immunologia da pinta terciaria. *Med Cutan Ibero Lat Am* 1998;**16**:111–14.
53. Perine PL, Hopkins DR, Niemel PLA, et al. *Handbook of endemic treponematoses*. Geneva: World Health Organization; 1984.
54. Pierce CS, Wicher K, Nakeeb S. Experimental syphilis: guinea pig model. *Br J Vener Dis* 1983;**59**:157–68.
55. Rein CR. Bacteriologic and serologic aspects of pinta. *Am J Syph* 1954;**38**:336–40.
56. Rothschild BM. History of syphilis. *Clin Infect Dis* 2005;**40**: 1453–63.
57. Rothschild BM, Rothschild C, Naples V., et al. Bejel: acquirable only in childhood? *Acta Trop* 2006;**99**(2-3):160–4.

58. Schell RF, Chan JK, LeFrock JL. Endemic syphilis: passive transfer of resistance with serum and cells in hamsters. *J Infect Dis* 1979; **140**:378–83.
59. Schell RF, LeFrock JL, Babu JP. Passive transfer of resistance to frambesial infection in hamsters. *Infect Immun* 1978;**21**:430–5.
60. Schell RF, LeFrock JL, Babu JP, et al. Use of CB hamster in the study of *Treponema pertenue*. *Br J Vener Dis* 1979;**55**:316–19.
61. Schell RF, LeFrock JL, Chan JK, et al. LSH hamster model of syphilitic infection. *Infect Immun* 1980;**28**:909–13.
62. Scolnik D, Aronson L, Lolvinsky R, et al. Efficacy of a targeted, oral penicillin-based yaws control program among children living in rural South America. *Clin Infect Dis* 2003;**36**:1232–8.
63. Solomon Islands Health Information System. *Annual Report 2001*. Statistics Unit, Policy, Planning and Research Division. Ministry of Health; 2001.
64. Turner TB, Hollander DH. *Biology of the treponematoses*. Geneva: World Health Organization; 1957.
65. Turner TB, McLeod CP, Updyke EL. Cross immunity in experimental syphilis, yaws, and venereal spirochetosis of rabbits. *Am J Hyg* 1947;**46**:287–95.
66. Vorst FA. *Attenuating endemic treponematoses*. Thesis, University of Amsterdam; 1974.
67. Walker SL, Hay RJ. Yaws—a review of the last 50 years. *Int J Dermatol* 2000;**39**:258–60.
68. Wicher K, Wicher V, Abbruscato F, Baughn R. *Treponema pallidum* subsp. *pertenue* displays pathogenic properties different from those of *T. pallidum* subsp. *pallidum*. *Infect Immun* 2000;**68**:3219–25.
69. Wilcox RR. Endemic syphilis in Africa. *S Afr Med J* 1951;**25**: 501–4.
70. Wilcox RR. Changing patterns of treponemal disease. *Br J Vener Dis* 1974;**50**:169–78.
71. W.H.O. Technical Report Series No. 674. *Treponemal infections*. Geneva: World Health Organization; 1982.
72. *World Health Report 1998—Life in the 21st century: a vision for all*. Geneva: World Health Organization; 1998.
73. Woltsche-Kahr I, Schmidt B, Aberer W, et al. Pinta in Austria (or Cuba?): import of an extinct disease? *Arch Dermatol* 1999;**135**: 685–8.
74. Woznicová V, Matejková P, Flasarová M, et al. Clarithromycin treatment failure due to macrolide resistance in *Treponema pallidum* in a patient with primary syphilis. *Acta Derm Venereol* 2010;**90**(2): 206–7.
75. Zimmer C. Can genes solve the syphilis mystery? *Science* 2001; **292**:1091.
76. Zimmer C. Infectious diseases: isolated tribe gives clues to the origins of syphilis. *Science* 2008;**319**(5861):272.

SUBSECTION 7
ANAEROBIC BACTERIA

CHAPTER 145

Clostridial Intoxication and Infection

Gary D. Overturf

Clostridia are obligate anaerobic, endospore-forming bacilli that usually are positive on Gram stain. Of the more than 200 species of *Clostridium*, at least 30 species are potential pathogens in humans, mostly by virtue of their biologically active proteins (toxins). More than 25 lethal toxins produced by species of *Clostridium* have been defined in humans, including neurotoxins, enterotoxins, cytotoxins, necrotizing toxins, and enzymatic toxins.[9,66] These ubiquitous organisms frequently are found in soil, sewage, decaying tissue, and the intestinal tract of humans and other animals.[15] The genus *Clostridium* has undergone significant revisions in taxonomy since 1994, which has resulted in a proposal for definitions of several clusters based on 16S rRNA gene sequences.[3] Human disease caused by *Clostridium tetani* is discussed in Chapter 147.

BOTULISM

Botulism is an acute descending flaccid paralysis that results from the neurotoxin of *Clostridium botulinum*, which blocks neuromuscular transmission. Four naturally occurring clinical forms of botulism occur: (1) classic foodborne botulism, an intoxication caused by the ingestion of preformed botulinal toxin (BoNT) in contaminated food; (2) wound botulism, which results from elaboration of botulinal toxin in vivo after the growth of *C. botulinum* in an infected wound; (3) infant botulism, in which botulinal toxin is produced in vivo in the gastrointestinal tract; and (4) botulism resulting from the intestinal colonization in children and adults.[3,21,40] Recently, the potential use of botulinum toxin as a bioterrorist agent has become an important concern. Infant botulism is discussed in Chapter 146.

EPIDEMIOLOGY AND ETIOLOGY

Antigenically distinct types of botulinum toxins are designated by the letters A through G. Disease in humans is caused by toxin types A, B, E, F (rarely), and possibly G.* Types C and D cause botulism in animals.[31,65] From 1973 to 1996, a median of 24 cases of foodborne botulism, three cases of wound botulism, and 71 cases of infant botulism were reported annually to the CDC.[63] This trend has continued recently; in 2009, the Center for Disease Control and Prevention reported 10 cases of foodborne, 83 infant, and 25 wound or other source botulism cases.[20] In the United States, approximately 50 percent of the foodborne cases are caused by toxin A and another 25 percent each by toxins E and B. Type A occurs primarily in the western states, and type B is seen more commonly in the eastern states. Type E outbreaks occur more frequently in Alaska and the Great Lakes region. Additionally unique strains of clostridial organisms, other than *C. botulinum*, can also produce botulinal neurotoxins, and include *C. butyricum*, *C. barati*, and *C. argentinense*.[68]

BoNT is considered the most potent and lethal of all naturally occurring compounds; the intravenous lethal dose for BoNT has been estimated to be 0.1 to 0.5 ng per kilogram of body weight, or an oral dose of 0.2 to 1 μg/kg. BoNT consists of a simple dichain composed of a heavy 100-kDa chain joined by a disulfide bond to a 50-kDa light chain. The toxin's light chain is a Zn^{2+}-containing endopeptidase that blocks acetylcholine-containing vesicles from fusing with the terminal membrane of the motor neuron, thereby resulting in flaccid paralysis.[43] It is heat-labile; 5 minutes of boiling destroys the toxin, and little remains after 30 minutes of exposure at 80° C (176° F). Neurotoxins are produced by

*References 2, 25, 31, 42, 43, 53, 63, 65.

C. botulinum at all temperatures at which growth occurs (3° to 48°C [37.4°F]). BoNT also is formed at all pH values at which growth occurs (pH 4.8 to 8.5), but it is unstable at pH values higher than 7.0. Toxin thus is produced only during active growth and released during lysis of the bacterial cells. The presence of organisms in improperly processed acidic food, however, can allow toxin production to occur.[30,65] Type E BoNT may be produced quickly in small fragments of fish exposed to air and at lower pH values and cooler temperatures than noted with other BoNT types.[31]

Most outbreaks of botulism in the United States are traceable to home-processed foods; however, in recent years the proportion of cases that result from restaurant-associated outbreaks has increased significantly.[63] The most important food vehicles are vegetables, fish, fruits, and condiments. Type E botulism almost always is traceable to fish and fish products, but types A and B also may be involved in outbreaks related to this type of food. Recent outbreaks have been traced to unusual foods, such as potato salad and sautéed onions served by restaurants and commercial frozen pot pies mishandled at home.

Wound botulism results from infection of traumatized tissue by *C. botulinum* type A or B and subsequent production of toxin.[53] In the United States, approximately 80 percent of cases are caused by toxin A and 20 percent by toxin B.[63] Although infrequently reported, wound infection is a disease of pediatric concern; approximately half of the cases in the United States that occurred before 1991 involved children and teenagers, most of whom were boys with compound extremity fractures. In recent years, however, most new cases have occurred in users of contaminated injectable black tar heroin.[3,23,63]

PATHOPHYSIOLOGY

Botulinum toxin is absorbed from the proximal part of the intestine or an infected wound and is distributed hematogenously to the neuromuscular junction of motor neurons. The toxin irreversibly binds to presynaptic membranes, where it penetrates the plasma membrane by receptor-mediated endocytosis and the light chains of toxin (50 kDa) is internalized into the nerve cell through a protein channel. BoNT specifically cleaves proteins involved in vesicle trafficking of neurotransmitters, and exocytosis of acetylcholine is prevented. The prevention of neurotransmission results in clinically flaccid paralysis.[30,31,65] The cranial nerves are affected earliest and often most severely. Death occurs mainly from respiratory muscle paralysis (asphyxia) or its complications, such as cardiac arrhythmia, aspiration, and pneumonia.[22] The clinical hallmark of botulism is an acute flaccid paralysis that begins with bilateral cranial nerve impairment of the muscles of the eye, face, head, and pharynx, descending symmetrically to involve muscles of the thorax and extremities.[71] BoNT, unlike tetanus toxin, does not enter the central nervous system (CNS). Recovery occurs by regeneration of terminal motor function and the formation of new motor end-plates. The lethal dose of botulinum toxin for humans is not known but can be estimated from primate studies; the dose of crystalline toxin lethal to a 70-kg human would be approximately 0.09 to 0.15 μg intravenously (or intramuscularly), 0.70 to 0.90 μg by inhalation, and 70 μg orally.[62]

CLINICAL MANIFESTATIONS

Because the pathogenesis of botulism involves the systemic absorption of BoNT and hematogenous dissemination with subsequent involvement of the cholinergic synapses, virtually all forms of botulism have identical neurologic signs. The signs and symptoms of botulism are summarized in Table 145-1.[6] The onset of illness varies from 12 to 72 hours after the implicated food is ingested, but it has been documented to occur as late as 108 hours after ingestion in some epidemics.[6]

Foodborne illness, or classic botulism, may be preceded by abdominal cramps, nausea, vomiting, or diarrhea. The neurologic illness begins as mentioned previously, as a descending symmetric motor paralysis first affecting muscles supplied by the cranial nerves.[6,31]

TABLE 145-1 **Symptoms and Signs of Foodborne Botulism Types A and B**

Sign or Symptoms	Percentage of Cases
Symptoms	
Dysphagia	96
Dry mouth	93
Double vision	91
Dysarthria	84
Fatigue	77
Arm weakness	73
Constipation	73
Leg weakness	69
Blurred vision	65
Nausea	64
Dyspnea	60
Vomiting	59
Dizziness	51
Sore throat	54
Abdominal cramps	42
Diarrhea	19
Paresthesia	14
Signs	
Alert mental status	90
Arm weakness	75
Ptosis	73
Leg weakness	69
Gaze paralysis	65
Facial palsy	63
Diminished gag reflex	58
Tongue weakness	58
Pupils dilated or fixed	44
Hyporeflexia or areflexia	40
Nystagmus	22
Ataxia	17

No sensory disturbance occurs, although vision may be impaired and hearing distorted because of cranial nerve involvement.

Mental processes remain clear, but anxiety and agitation may be present. Fever is absent unless a secondary bacterial infection occurs. The triad of bulbar palsies (including a sluggish or absent pupillary response to light), lucid sensorium, and absent fever always should bring botulism to mind.

Common symptoms include blurred vision, diplopia, dysarthria, and dysphagia. The degree of ocular involvement is quite variable; in severe cases, the pupils may become fixed and dilated. The mucous membranes of the mouth, tongue, and pharynx may be so dry that pain results, which may lead to the mistaken diagnosis of pharyngitis. Dizziness or vertigo may occur. Urinary retention may be seen, occasionally with stress incontinence.

Two thirds of patients have no gastrointestinal symptoms. In those who do, with type A or type B botulism the gastrointestinal manifestations are primarily abdominal pain, cramps, fullness, and diarrhea. However, after an initial period of diarrhea, constipation or obstipation may be noted and, indeed, is more typical of the disease. In contrast to those with the other types, most patients with type E botulism first have gastrointestinal symptoms, including nausea, vomiting, substernal burning or pain, abdominal distention, and decreased bowel sounds. The most common signs encountered in botulism are respiratory impairment; specific muscle weakness or paralysis; eye muscle involvement, including ptosis; dry throat, mouth, or tongue; dilated, fixed pupils; and ataxia. Respiratory involvement, even aside from aspiration pneumonia, is a fairly common occurrence. Vital capacity is a more sensitive indicator of respiratory compromise than is measurement of blood gas. Postural hypotension, nystagmus, and somnolence may be noted.

The usual interval between the ingestion of food and the onset of symptoms is 18 to 36 hours, but it may be as short as 2 hours or as long as 8 days. In general, patients with shorter incubation periods are affected more severely and have a poorer prognosis. The shortness of the incubation period and the severity of illness correlate with the amount of BoNT ingested. Although the syndrome is similar for each toxin type, type A toxin has been associated with more severe disease and a higher mortality rate than has either type B or type E toxin.[76]

The symptoms of wound botulism are similar to those of food botulism, but some important differences may occur.[53] Fever may or may not be present. Constipation occurs, but nausea and vomiting do not. Unilateral sensory changes in association with the trauma or infection may be noted. The wound itself may have grossly purulent drainage, but sometimes the wound shows no evidence of infection. The incubation period of wound botulism usually is 4 to 14 days.

In the event of intentional foodborne poisoning with BoNT, the signs and symptoms probably would resemble those of naturally occurring foodborne botulism.[6,63] If the aerosolized BoNT from a bioterrorist attack were to be inhaled, the incubation period might be slightly longer, and gastrointestinal symptoms might not occur.[6,63]

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of botulism includes myasthenia gravis, cerebrovascular accidents, Guillain-Barré syndrome (particularly the Miller-Fisher variant), tick paralysis, paralytic shellfish poisoning, chemical intoxication, diphtheritic polyneuritis, psychiatric disease, and the Eaton-Lambert syndrome.[31,65]

Ordinary bacterial food poisoning generally is not a problem in the differential diagnosis because of the absence of cranial nerve involvement. Chemical food poisoning may cause neurologic manifestations, but the signs almost always appear within minutes or at most hours after the consumption of contaminated food. Atropine poisoning has a very rapid onset and is distinctive because of facial flushing and hallucinations. Shellfish and fish poisonings have a rapid onset and often cause characteristic paresthesias, tremors, and other signs. Mushroom poisoning results in severe abdominal pain, violent vomiting, diarrhea, and coma.

Myasthenia gravis usually spares pupillary oculomotor function. An edrophonium (Tensilon) test should be performed. Guillain-Barré syndrome can mimic botulism but usually shows ascending peripheral paralysis and, later, cranial nerve involvement. Muscle cramps, paresthesias, and an elevated protein content in cerebrospinal fluid (CSF) in the absence of cells help distinguish this disease. Electromyography may be extremely useful in differentiating botulism from atypical cases of Guillain-Barré syndrome. Because BoNT does not enter the CNS, abnormalities of the CSF are virtually never present.

The problem of identifying a case is complicated by reports of patients with features not characteristic of either botulism or the action of botulinum toxin, such as paresthesias, asymmetric weakness of the extremities, asymmetric ptosis, slightly elevated CSF protein, and a positive response to edrophonium. Some of these symptoms may be a consequence of the high anxiety that prevails in persons who know that they have eaten a food containing BoNT.

SPECIFIC DIAGNOSIS

Confirmation of the diagnosis of botulism depends primarily on detection of botulinum toxin or the organism in the patient or in the implicated food or wound.[31,65] Specimens to be examined for botulinum toxin include serum, gastric contents or vomitus, feces (at least 20 to 50 g when possible), and exudates from wounds and tissues. These specimens, particularly blood specimens, should be obtained as soon as possible and before antitoxin is given. When feasible, at least 15 to 20 mL of blood should be drawn into a large vacuum tube and sent without separation of the serum to the nearest laboratory capable of performing the mouse neutralization test and other tests for toxin. On suspicion of clinical botulism, the state health departments and the Centers for Disease Control and Prevention (CDC) should be consulted immediately regarding appropriate specimen collection and transport and the appropiate laboratories to which samples can be sent. The CDC can be contacted for

general information at 1-800-232-4636 available Monday through Friday, 8 a.m. to 8 p.m. Eastern time, and the CDC Emergency Operations Center is also available 24/7 for consultation and provision of therapeutic materials at 1-770-488-7100 (current information, available at http://www.cdc.gov).

Specimens should be refrigerated (4° C [39.2° F]) and be examined as quickly as possible after collection. Whenever feasible, suspect food should be kept sealed in the original container. Sterile unbreakable containers should be used for other food samples. Specimens to be shipped to laboratories must be placed in leak-proof containers; packed with dry ice in a second leak-proof, insulated container; and marked "Danger, hazardous material." Extreme caution should be used in handling material that may contain botulinum toxin because even minute quantities of toxin acquired by ingestion, inhalation, or absorption through the eye or a break in the skin may cause profound intoxication and death.

Laboratory confirmation of suspected botulism should be attempted, even late in the clinical course. Detection of the organism itself may be achieved by culture or a variety of molecular methodologies. However the most reliable assay for BoNT remains the mouse bioassay with type-specific neutralization of toxin with specific antitoxins.[22]

Botulinum neurotoxin is detected in serum or stool specimens in approximately 46 percent of clinically diagnosed cases. When stool specimens also are cultured for *C. botulinum*, confirmatory evidence is obtained in approximately 70 percent of botulism cases.[63,76] Thus, detection of *C. botulinum* toxin or organisms in the stool of a symptomatic person should be considered diagnostic.

TREATMENT

The mainstay of therapy for all forms of botulism is meticulous supportive care, with particular attention paid to the respiratory and nutritional needs of the patient and to anticipation of potential complications with the purpose of preventing them.[30,31] Symptomatic persons known to have ingested toxin-containing food should be hospitalized, with careful monitoring of respiratory and cardiac function. Measurement of vital capacity has been a useful index of clinical status in adult patients and may be used to follow the recovery and response to treatment.

In cases of foodborne botulism, if the patient is seen early, protected induced emesis and gastric lavage should be used to reduce the amount of unabsorbed toxin; in the absence of ileus, cathartic agents may be helpful. Magnesium-containing cathartic agents should be avoided because they may enhance the action of botulinum toxin.

Heptavalent antitoxin (types A to G; i.e., H-BAT) of equine origin frequently is considered in the management of foodborne botulism in adults, although conclusive evidence of its efficacy is lacking.[31,71] In the past, trivalent (A, B, and C) and bivalent (A and B) were available, but currently only the hepatavalent material is provided by the CDC. Antitoxin is provided to neutralize circulating botulinum toxin molecules that are not yet bound to nerve endings. The dose currently recommended in adults is one vial per patient as a single dose.[63] Hypersensitivity reactions have been reported in approximately 9 percent of persons treated with equine sera, so skin testing should be performed before administration of the antitoxin (recommendations for testing are provided by the CDC). Treatment of a single patient with suspected or proved foodborne botulism requires immediate notification of state and federal (CDC) health officials, who also are the antitoxin source, and the food responsible for the index patient's illness still may be available to other persons. The appropriate initial contact is via the state health department. If this agency cannot be reached, the CDC should be contacted immediately (see the CDC numbers in the earlier discussion of diagnosis). Presently, a human-derived botulinum antitoxin is available in the United States exclusively for the treatment of infantile botulism and is discussed in Chapter 146.

In the event of a potential exposure to a suspected contaminated food, the following procedures should be implemented. Locate all persons who ate the suspect food and determine whether anyone has signs or symptoms of botulism.[31] If patients are seen soon after the suspect meal, the use of gastric lavage, emetics, and cathartics deserves consideration. Antitoxin should be made available. Any samples of the suspect food that may remain should be collected and refrigerated. The health authorities should assist the clinician with these tasks. The fecal and serum specimens needed to establish the diagnosis should be obtained. If neurologic signs are present, one should try to identify defective neuromuscular transmission by electromyelography. If neurologic signs are absent, the patient and family should be informed of the early signs of botulinum intoxication and be instructed to return at the first manifestation of ptosis, diplopia, blurred vision, dysphonia, dysarthria, or dysphagia. Because of the serious side effects of equine botulinum antitoxin, prophylactic administration of it to asymptomatic persons who have ingested food known to contain botulinum toxin generally is not recommended.[3]

When wound botulism is suspected, exploration and débridement of the site must be undertaken, ideally after the administration of antitoxin has begun. Arrangements should be made to obtain material for anaerobic culture in the operating room and antibiotic therapy begun there. High-dose penicillin 250,000 to 400,000 U/kg/day for 10 to 14 days is the drug of choice. Guanidine, aminopyridine, steroids, and intravenous immunoglobulin have been used to treat a small number of cases of foodborne and wound botulism, but convincing evidence of their efficacy is lacking. Metronidazole has been suggested as an alternative for penicillin, based on studies suggesting its enhanced efficacy in tetanus, but evidence for its effectiveness in botulism is not available.

PROGNOSIS

With an emphasis on mechanical ventilation and intensive supportive care, the mortality rate from botulism has decreased steadily from 60 percent in the 1950s to 3 to 5 percent, and a mortality of less than 1 percent among hospitalized patients. It is lower with type B and type C disease than with type A[13] and lower with any type in

individuals younger than 20 years. An important factor is the dose of toxin ingested as reflected by the length of the incubation period. The longer the incubation period, the better the prognosis, whereas shorter incubation periods are associated with ingestion of larger doses and higher rates of morbidity and mortality. Thus, if the index case in an outbreak can be detected early, other patients exposed to the same food will have a much better prognosis. Recovery may be prolonged, and some symptoms (e.g., fatigability) may persist for as long as 1 year. Most patients recover entirely.

PREVENTION

Local and state health authorities and the CDC should be notified immediately of all suspected cases of botulism so that timely investigations can be undertaken. Although commercial products still are responsible for botulism occasionally, control measures taken by the industry have done a great deal to prevent botulism from this source. Home canners still must be instructed regarding appropriate means for sterilizing containers and food preservation and for adequate cooking of food before serving.[31,65] In canning, a pressure cooker must be used to obtain temperatures well above boiling to destroy the spores of *C. botulinum* types A and B. For certain foods, a temperature of 116°C (240.8°F) is recommended. Spores of *C. botulinum* are destroyed at 120°C (248°F) after 30 minutes. Pressure cookers set at 15 lb will achieve this temperature, but correction for higher altitudes must be made. Home-canned foods should be boiled for 10 minutes before serving. Food containers that appear to bulge may contain gas produced by *C. botulinum* and should not be opened. A pentavalent botulinum toxoid vaccine has been used in military personnel to protect them from a biologic warfare assault. It is given in three subcutaneous doses and leads to detectable antibodies in 83 percent of subjects.[23]

CLOSTRIDIAL INFECTIONS

Clostridia are encountered less commonly in infections than are non–spore-forming anaerobic bacteria, but these spore formers rarely may produce devastating disease. Overall, clostridia are present in 5 to 10 percent of anaerobic or mixed (aerobic and anaerobic) infections.[13,26,31,73] Table 145-2 depicts the relative frequency of clostridial species from clinically significant infections at a single academic hospital.[3]

Clostridium perfringens is the species encountered most commonly. It may be isolated in pure culture but more commonly is part of a mixed flora involving other anaerobes and nonanaerobes. Other species that are important clinically or encountered commonly (or both) include *C. novyi*, *C. septicum*, *C. bifermentans*, *C. histolyticum*, and *C. sordellii* (together with *C. perfringens*, these species commonly are referred to as the "gas gangrene group"); *C. tetani* (see Chapter 147); *C. difficile*, a major pathogen in pseudomembranous colitis; and a group of clostridia important in infections other than gas gangrene or myonecrosis (wound infection, abscesses, bacteremia, etc.)—*C. perfringens*, *C. ramosum*, *C. bifermentans*, *C. sphenoides*, *C. sporogenes*, and others.

Clostridia may be involved in a wide variety of infections throughout the body. Certain of these infections have a distinctive clinical picture and are discussed in this chapter. Many other infections, including peritonitis, intra-abdominal infection, wound infection, soft tissue infection, and, occasionally, pleuropulmonary infection, CNS infection, and urinary tract infection, are not distinctive and will not be discussed specifically here. Emphysematous cholecystitis involving *C. perfringens* has distinctive features, but it is not encountered in the pediatric age group and thus is not discussed. Bacteremia involving *C. perfringens* may or may not have characteristic features. The distinctive intravascular hemolysis that may occur with *C. perfringens* bacteremia is discussed in connection with female genital tract infections caused by this organism.

Clostridial infections in children have revealed diverse species among 113 isolates in 107 specimens, including (in order of frequency) unidentified *Clostridium* spp. (33%), *C. perfringens* (12%), *C. inoculum* (5%), *C. ramosum* (4%), *C. botulinum* (3%), *C. difficile* (2%), and *C. butyricum* (2%), as well as single isolates of *C. bifermentans*, *C. costridioforme*, *C. limosum*, and *C. parputrifucum*.[14] Most were from abscesses, peritonitis, bacteremia, and chronic otitis media, and many children had underlying conditions such as immunodeficiency, malignancy, diabetes, trauma, presence of foreign bodies, and prior surgery. Clostridia were isolated from 7 percent of all specimens processed for anaerobic bacteria with frequent co-isolation of *Bacteroides* spp., *E. coli*, *Prevotella* spp., *Porphyromonas* spp., and *Fusobacterium* spp.

EPIDEMIOLOGY

The vast majority of clostridial infections are of endogenous origin. Even those secondary to trauma and contamination of a wound with foreign bodies usually involve *C. perfringens* or other clostridia from the host's flora, chiefly the intestinal tract. *Clostridium* spp. are ubiquitous in nature, with habitats in soil and the intestinal tracts of many animals, including humans and most other mammals. Clostridia even may be isolated from infant intestine as early as the first week of life, and by 6 to 20 months of age, the feces of infants contain approximately the same number of *C. perfrigens* organisms as that of adults.[3]

Pathophysiology

The principal sites of normal carriage of *C. perfringens* and many other clostridial species in humans are the colon and vagina.[3,30,31] These organisms may gain access to tissues through wounds, by perforation of abdominal viscera, or because of local disease such as tumor. The

TABLE 145-2 ***Clostridium* Species Most Frequently Encountered in Clinical Specimens at Indiana University Hospital Anaerobe Laboratory: 1989 through 2001***

Species†	Isolates	
	No.	% of Total
C. perfringens	515	20
C. clostridioforme	421	16
C. inoculum	380	15
C. ramosum	357	14
C. difficile	287	11
C. butyricum	113	4
C. cadaveris	99	4
C. bifermentans	53	2
C. sporogenes	49	2
C. glycolicum	44	2
C. septicum	42	2
C. tertium	39	2
C. sordelli, C. subterminale, C. paraputrificum, C. symbiosum, and *C. baratii*	15-30	1

*The 2555 isolates do not include 270 isolates that did not belong to a recognized species (total of 2825 isolates), nor do they include *C. difficile* from fecal specimens.

†Six or fewer isolates each of *C. beijernickii, C. botulinum, C. carnis, C. celatum, C. coccides, C. cochlearium, C. ghoni, C. hastiforme, C. histolyticum, C. indolis, C. limosum, C. malenominatum, C. novyi, C. putrefaciens, C. putrificum,* and *C. sphenoides.*

organism then may grow in tissues if the oxidation-reduction potential is low, host defense mechanisms are impaired, or both. Factors favoring anaerobic growth include the presence of abundant necrotic tissue, a poor blood supply, the presence of foreign bodies, or previous multiplication of other bacteria in the wound leading to a lowered oxidation-reduction potential.

As noted *Clostrium* spp. produced a wide range of toxins.[39,68] *C. perfringens* produces at least a dozen different extracellular toxins or other factors that account for its pathogenicity.[31] The most important of the five major lethal toxins is α-toxin, a lecithinase and the main toxin responsible for destruction of tissue, hemolysis, and death. Other important factors include collagenase, hyaluronidase, leukocidin, deoxyribonuclease, and fibrinolysin. The enterotoxins produced by some strains of *C. perfringens* and *C. difficile* are important in the pathogenesis of certain gastrointestinal diseases.[49,52] Gas gangrene, or clostridial myonecrosis, is characterized by profound toxicity, necrosis of muscle, edema, thrombosis of small vessels, gas bubbles in tissues, and minimal infiltration of leukocytes (probably caused by destruction of leukocytes at the site).

CLINICAL MANIFESTATIONS

Gas Gangrene or Myonecrosis

Although other clostridia also are involved in gas gangrene, *C. perfringens* is the single causative species in approximately 90 to 95 percent of cases associated with trauma. Gas gangrene usually results from contamination of open wounds with contiguous injured muscle. The tissue destruction in gas gangrene is related to the profound attenuation in blood flow as a result of activation of platelet responses by the α-toxin of *C. perfringens*.[16] Clostridial myonecrosis may occur in the absence of a traumatic wound, so-called *spontaneous myonecrosis*, and is caused by bacteremic seeding of muscle with either *C. perfringens* or *C. septicum*. The latter species is almost entirely associated with the spontaneous form of gas gangrene.[48] In spontaneous myonecrosis, the source of the organism typically is the bowel, and the usual predisposing factors are mucosal tumors of the bowel or ulcerations produced by cytotoxic chemotherapy.[31] Only a handful of cases of spontaneous myonecrosis in children have been described.[54]

The typical case of clostridial myonecrosis is manifested by the sudden appearance of pain in the region of a wound.[31] The pain increases in severity but remains localized to the infected area. Subsequently, local swelling and edema occur, and a thin hemorrhagic exudate appears. The pulse is very rapid, out of proportion to the mild elevation in temperature. Initially, the skin is tense, white, somewhat colder than normal, and very tender. Bronze discoloration appears and increases with time. The process extends and becomes more severe, and the patient becomes toxemic. The skin becomes dusky or bronzed, and bullae filled with dark-red or purple fluid appear. Crepitus caused by gas may be noted, but the amount of gas produced generally is small. A peculiar sweet smell may be noted in some cases. Occasionally, a fetid odor that probably reflects the presence of a *Clostridium* organism other than *C. perfringens* may be present.[31] Toxic delirium and, later, overwhelming prostration and toxemia may develop. Some patients are alert and apprehensive, whereas others are apathetic. Later in the course of the disease, shock supervenes. At surgery, early changes in muscle consist primarily of edema and pallor, but increased reddening with mottled purple is present. The consistency of the muscle may be pasty or mucoid, and contractility disappears. Eventually, the muscle becomes diffusely gangrenous, dark greenish-purple or black, friable and even liquefied. When bacteremia supervenes, mortality rates often exceed 50 percent.

Gram stain of the drainage or fluids or tissue obtained by biopsy is usually definitive, revealing gram-positive rods and a lack of inflammatory cells. However, recent studies indicate that the marked tissue destruction and absence of neutrophils with *C. perfringens* gas gangrene are caused by α-toxin–induced occlusion of blood vessels by platelets and neutrophils.[15] The margin between healthy and necrotic tissue often advances several inches per hour despite appropriate antibiotic therapy and radical amputation or débridement of necrotic infected tissue remains the best life-saving treatment.

Soft Tissue Infection

C. perfringens and other clostridia also may be involved in less dramatic and less serious infections ranging from minor superficial infections to anaerobic cellulitis and

necrotizing fasciitis.[31] Clostridial organisms in these infections may occur as single isolates, as part of a mixed flora consisting solely of anaerobic bacteria, or as part of a mixed anaerobic-aerobic bacterial flora. The clinical picture in these various infections is no different from that noted with other types of organisms and thus is not discussed further here.

Bacteremia and Sepsis

Clostridium spp. are important causes of sepsis, although infrequent in children. *C. septicum* is isolated only rarely from feces of healthy individuals but may be found in the appendixes of normal individuals, and more than 50 percent of persons whose blood is positive for this organism have some gastrointestinal anomaly such as colonic diverticulae or Meckle's diverticulum. Many of the conditions most frequently predisposing to clostridial sepsis, such as gastrointestinal malignancy, vascular insufficiency or atheroscleoris, cirrhosis, neutropenia and pancreatitis, or uncontrolled diabetes, are rare in children and therefore, clostridial sepsis is rare in children.

SEPTIC ABORTION AND PUERPERAL SEPSIS

C. perfringens infections of the uterus usually occur after incomplete abortions and are induced under nonsterile conditions.[31] More recently, *C. sordelli* has been more frequently associated with these infections. Occasionally, this type of infection will develop after spontaneous abortion, prolonged labor, ruptured membranes, or operative interference with pregnancy. Early symptoms include uterine bleeding, suprapubic and back pain, and chills, but often with little or no fever.[30,31] The incubation period after the precipitating event usually is several days, but it may be less than 24 hours. In addition to vaginal bleeding, a foul-smelling, brown vaginal discharge containing necrotic tissue often is present. The uterus is tender, and the lower abdominal wall may be tense. Nausea, vomiting, and diarrhea may occur. Generalized peritonitis may complicate the picture. The most striking systemic manifestation of the disease, however, is massive intravascular hemolysis with hemoglobinemia, hemoglobinuria, and jaundice. Shock and acute renal failure may complicate the picture. Intrauterine gas formation may be detected. Postnatal uterine infections resulting in gas gangrene of uterus are often rapidy fatal.

Neonatal Sepsis and Meningitis

A small number of cases of sepsis or meningitis (or both) caused by *Clostridium* spp. have been reported in newborns.[32,34,55,56,69] Although some of these neonatal cases have been associated with invasive procedures or devitalized tissue, others have not had an obvious source.[34,41,55] In several of these cases, the systemic infection involved both the mother and infant. The typical clinical picture in neonatal infections is one of fulminant septic shock, intravascular hemolysis, respiratory distress, and rapid death. Pneumatocephalus may occur in the presence of meningitis.

Pseudomembranous Colitis (*C. difficile*)

Clostridia difficile is an increasing pathogen in children despite high carriage rates in asymptomatic newborns and infants younger than 2 years. In addition, the epidemiology of *C. difficile* in children has been increasingly defined in recent years.[5,11,45] The organism is the major cause of antibiotic associated diarrhea and pseudomembranous colitis.[35] Pathogenesis of this colitis is via the production of two toxins, toxin A and B.[27,28,74] Treatment and management of the infections in children has been adapted from adult standards.[77] Please refer to Chapter 45 for a detailed description of the organism, current epidemiology in children, pathogenesis, and treatment of the disease in children.

Food Poisoning and Other Enteric Infections

A usually mild, self-limited, and very common form of food poisoning may be caused by *C. perfringens*. Meat and meat products are the most common vehicles for such outbreaks.[30,31,58] Food poisoning is caused by a heat-labile enterotoxin produced by *C. perfringens* type A. Disease is the result of the ingestion of 10^8 or more vegetative cells that sporulate in the alakaline environment of the small intestine, producing the enterotoxin. The incubation period after ingestion of the contaminated food varies from 6 to 30 hours but usually is 8 to 12 hours. The major symptoms are crampy abdominal pain and diarrhea. Stools are liquid but do not contain blood or mucus. Nausea may be noted on occasion, but vomiting and fever seldom are present. The illness typically lasts less than 24 hours but may be more severe in the very young or elderly.[12]

Rarely, *C. perfringens* also may produce a very severe type of enteritis known as *enteritis necroticans*. Although food poisoning is produced by *C. perfringens* type A, necrotizing enteritis involves the β-toxin of *C. perfringens* type C.[47] Consumption of excessive amounts of rich food by people normally on a low-protein diet who have decreased levels of digestive proteases seems to be an important background factor. Additionally, proteases may be blocked by ingestion of the trypsin inhibitors found in sweet potatoes. In some cases, consumption of contaminated canned meat is involved. In Papua New Guinea, where this condition is known as "pigbel," enteritis necroticans in children is associated with traditional pig-feasting activities in which large quantities of pork are consumed. The disease is characterized by abdominal cramps, vomiting, shock, diarrhea (sometimes bloody), and acute inflammation of the small intestine with areas of necrosis and gangrene, particularly in the jejunum. A 12-year-old diabetic boy in whom enteritis necroticans developed after the ingestion of pig intestines (chitterlings) has been reported.[60]

MISCELLANEOUS INFECTIONS CAUSED BY CLOSTRIDIA

Fewer than 40 cases of septic arthritis attributable to *Clostridium* spp., including several pediatric patients,

have been reported.[38] Anaerobic osteomyelitis caused by *Clostridium* also is a rare finding.[33] Aggressive surgical débridement and prolonged antibiotic therapy are warranted in the treatment of anaerobic musculoskeletal infections.[64]

The clinical spectrum of clostridial bacteremia ranges from an asymptomatic patient with an incidental positive blood culture to fulminant sepsis syndrome.[18] Bacteremia with *C. septicum* is considered a unique syndrome associated with malignancies, particularly leukemia and colon cancer, and it has a devastating clinical course.[36,50] Infections with this organism also have been linked to children with cyclic neutropenia.[3,7] In a recent review of 28 pediatric patients with *C. septicum* bacteremia, Caya[19] noted that all of them had underlying cancer or gastrointestinal disease. The overall mortality rate was 72 percent. Five cases of *C. septicum* infection occurring after *Escherichia coli* O157–induced hemolytic-uremic syndrome have been described.[4,8]

Clostridium sordelli infections have been associated with high fatality rates and most often have occurred after trauma, childbirth, and routine gynecologic procedures. They have been reported after medically induced abortions and intravenous drug injections. In one review of 45 cases, 8 (18%) occurred after normal childbirth, and 5 occurred (11%) after medically induced abortions, with a 100 percent case-fatality rate in this group.[1] Only four cases occurred in children: a 4-year-old child with arm trauma, a 17-year-old adolescent with omphalitis, a 12-year-old child with ear infection, and an 18-year-old adolescent with a medically induced abortion. Only the 12-year-old child survived; the other three patients died of their infection.

Panophthalmitis involving *C. perfringens* or, occasionally, other clostridia is secondary to penetrating injury, usually with retention of a foreign body.[31] Pain and loss of vision occur within 12 hours after the injury. By 18 hours, evidence of fulminating panophthalmitis is present and consists of chemosis and brawny swelling of the lids, proptosis, hypopyon, increased intraocular tension, gas bubbles in the anterior chamber, and necrosis of the wound margins. Intracranial infections involving *Clostridium*, usually as a result of penetrating trauma with items such as a lawn dart or an arrow, have been described.

Pneumatosis cystoides intestinalis can be produced in animals by *C. perfringens*, and this organism has been recovered from this process in humans.[30,31] Pneumatosis cystoides intestinalis may be found in conjunction with toxic megacolon and neonatal necrotizing enterocolitis and as a complication of ileal bypass for obesity.

SPECIFIC DIAGNOSIS

The diagnosis of gas gangrene must be made on clinical grounds. The presence of a gas-forming infection and recovery of *C. perfringens* from the wound do not establish the diagnosis of gas gangrene. The key to this diagnosis is demonstration of myonecrosis. Clostridial myonecrosis must be differentiated from other gas-forming soft tissue infections, which may or may not involve *C. perfringens*, and from other causes of myonecrosis. The sudden onset, extreme toxemia, and severe pain that are noted in clostridial myonecrosis represent important differential features. Entities such as anaerobic cellulitis and streptococcal myonecrosis have a gradual onset, slight toxemia, and less pain than seen with clostridial myonecrosis. Synergistic nonclostridial anaerobic myonecrosis is a severe infection characterized by discrete areas of blue-gray necrosis of skin, along with extensive involvement of the underlying soft tissue and muscle and foul "dishwater" pus. Anaerobic cellulitis typically has much more gas and does not involve muscle.

In streptococcal myonecrosis, edema of the muscle is present initially, and then the muscle has a hemorrhagic appearance.[31] Specimens for Gram staining and culture should be obtained from the involved muscle rather than from the surface of the wound. Large gram-positive rods will be demonstrated on Gram stain in clostridial myonecrosis; no white blood cells may be demonstrable, or the white blood cells present may be distorted significantly as a result of the toxin of *C. perfringens* acting on them. Anaerobic cellulitis typically shows a mixture of organisms, which may include *C. perfringens*. Streptococcal myonecrosis reveals anaerobic streptococci, sometimes along with group A streptococci, *S. aureus*, and other organisms.[24] In synergistic nonclostridial anaerobic myonecrosis, *Bacteroides* organisms seem to be key pathogens, together with anaerobic cocci and nonanaerobic gram-negative bacilli.[31] Of importance when obtaining material for culture is to place it under anaerobic conditions promptly for transport to the laboratory. Anaerobic blood cultures also should be performed.

Uterine infection by *C. perfringens* varies in severity from secondary invasion of necrotic material into the uterus or a dead fetus to invasion of intact uterine muscle producing myonecrosis and physometra.[31] Although bacteremia is a relatively uncommon finding in gas gangrene, uterine infection with *C. perfringens* frequently is accompanied by sepsis, which leads to the dramatic picture of intravascular hemolysis described earlier. Demonstration of severe hemolytic anemia in association with uterine infection essentially is diagnostic. Anaerobic blood cultures should be performed.

Clostridia, particularly *C. perfringens*, occasionally are isolated from the blood of a patient with a clinically benign course. The usual scenario is that a hospitalized patient has a single fever spike of unclear etiology and blood cultures are performed as part of the evaluation; by the time that the culture becomes positive, no evidence of an infectious process is present. This transient and benign bacteremia probably originates from the colonic flora.[11,17]

C. perfringens food poisoning usually is seen in the setting of a sizable outbreak.[31] The organism grows to high counts in the responsible food and then sets up an infection in the host, with production of enterotoxin in the colon of patients. Demonstration of large numbers ($>10^5$/g) of *C. perfringens* in the implicated food and demonstration of the same serotype of *C. perfringens* in the stool of affected individuals and in the food are important in documenting the nature of the food poisoning. Enterotoxin also may be found in the stool of affected individuals, and the *C. perfringens* recovered from stool or food

can be demonstrated to produce enterotoxin in vitro. Necrotizing enteritis caused by *C. perfringens* may be suspected by virtue of the dramatic clinical picture and confirmed by demonstration of *C. perfringens* type C in the stool or suspect food or demonstration of serum antibody to the β-toxin of the organism.

C. difficile–associated diarrhea should be suspected in children with diarrhea who have received antibiotics within the previous 2 months or whose diarrhea begins 72 hours after hospitalization.* Typically, toxin testing of a single stool specimen will establish the diagnosis, but, occasionally, repeat testing or endoscopy may need to be performed. The finding of exudative plaques (pseudomembranes) and a hyperemic, friable mucosa by direct endoscopic visualization suggests the diagnosis of pseudomembranous colitis. Although *C. difficile* is isolated conveniently with the use of a selective medium, distinction between toxigenic and nonpathogenic strains cannot be made. Its toxin B (cytotoxin) is identified most easily by tissue culture assay, which remains the gold standard for diagnosis of antibiotic-associated diarrhea.[31] A simple latex particle agglutination test for detection of *C. difficile* is available, although it is considered relatively insensitive and nonspecific and, for that reason, no longer is recommended.[52,70,72]

In recent years, commercially available enzyme immunoassay (EIA) kits that detect toxin B or both toxin A and B have become the preferred method of diagnosis.[10,72] The EIAs in general are easy to perform, relatively inexpensive, and specific. Polymerase chain reaction amplification tests for toxins A and B have been designed but are not available commercially.[28,61,74]

Interpreting the significance of finding *C. difficile* or its toxins in very young patients with diarrhea is difficult because infants and toddlers have such a high rate of asymptomatic carriage.[27,44,75] Quantitation of toxin or organisms has not correlated with the presence or absence of symptoms. Consequently, once the possible presence of other diarrhea-producing pathogens (e.g., rotavirus, *Salmonella*) has been excluded, effort should be made to stop the presumptive precipitating antibacterial agent. If diarrhea with mucus or blood persists, endoscopy should be considered and specific therapy begun.

TREATMENT

The most important aspect of treating clostridial myonecrosis is immediate surgical intervention consisting of radical débridement and drainage and decompression of the fascial compartments.[30,31] The wound should not be closed after this surgery. Of crucial importance is that all bits of necrotic muscle and other tissue be removed. Polyvalent gas gangrene antitoxin was never established firmly as being beneficial in the context of modern therapy, and this product no longer is available in the United States. Hyperbaric oxygen is recommended enthusiastically by some physicians, but no definitive evidence that its use reduces mortality rates has been shown. It does facilitate demarcation of a limb with impaired vascular supply, and it appears to slow or arrest local spread of the gangrenous infection. Clearly, however, hyperbaric oxygen must not be used as a substitute for any of the important principles of surgical management. Antimicrobial therapy also is important as an adjunct, with high-dose penicillin G (250,000 to 400,000 U/kg/day) being the drug of choice.[30,31] In individuals allergic to penicillin G, clindamycin or metronidazole may be used. In addition, chloramphenicol is routinely active against all clostridia. The combination of penicillin and clindamycin has been used widely on the basis of experimental animal models that have shown enhanced efficacy with such a combination.[67]

Clostridial cellulitis is treated by incision plus drainage and antimicrobial therapy. Radical débridement is not necessary, but it is important to lay the tissues open to effect proper drainage and permit removal of all necrotic tissue.[30,31]

Uterine curettage should be performed for diagnosis and treatment of postabortal or puerperal clostridial infections.[30] Hysterectomy may be required if the myometrium is involved and if the patient's condition is deteriorating. At times, perforation of the uterus may be present without the typical clinical findings. Exchange transfusion has been recommended for sepsis caused by *C. perfringens* when significant intravascular hemolysis is present.

Food poisoning caused by *C. perfringens* is self-limited and requires no therapy. Antitoxin to the β-toxin produced by *C. perfringens* type C has been of considerable benefit in the treatment of necrotizing enteritis caused by this organism.

In any serious infection caused by *C. perfringens* or other clostridia, the usual supportive measures for shock, dehydration, anemia, and renal insufficiency are implemented as indicated.

Treatment of *C. difficile*–associated disease involves discontinuation of the offending antibiotic regimen when feasible. Isolates of *C. difficile* are susceptible to metronidazole and vancomycin. Oral or intravenous metronidazole 30 mg/kg/day in four divided doses is considered the drug of choice. With the recent and rapid increase in the recovery of vancomycin-resistant enterococci, many experts and the CDC suggest that oral vancomycin 40 mg/kg/day in four divided doses be used to treat *C. difficile*–induced diarrhea only in patients who are critically ill or those who do not respond to metronidazole.[29,59,77] Intravenous vancomycin is not effective. Orally administered bacitracin also may be useful, although some experts suggest that it may be less effective than metronidazole or vancomycin. Antimicrobial agents typically are administered for 10 days, but as many as 20 percent of patients may experience a relapse requiring a second course of treatment.[51] The use of cholestyramine or probiotics, such as *Saccharomyces* or *Lactobacillus*, in adult patients with multiple relapses has been advocated by some physicians, but such treatment has not been evaluated in children with disease caused by *C. difficile*.

PROGNOSIS

The mortality rate in patients with gas gangrene varies between 15 and 35 percent and is worse when large

*References 5, 11, 29, 35, 36, 45, 46, 57.

muscle groups, such as those of the buttock, thigh, leg, and shoulder, are involved or when areas that are difficult to débride, such as the viscera and pelvis, are involved with disease. Clostridial cellulitis has a much better prognosis, but aggressive therapy still is important to minimize mortality rates. The mortality rate in postabortal clostridial sepsis remains 50 to 85 percent. *C. perfringens* food poisoning has an excellent prognosis, but mortality rates are significant with necrotizing enteritis caused by *C. perfringens* type C.

PREVENTION

Wounds involving areas with large muscle masses are particularly prone to gas gangrene, as are compound fractures, severe crush injuries, and injuries secondary to high-velocity missiles. Extensive laceration or devitalization of muscle tissue, impairment of the main blood supply to a limb or large muscle group, and contamination by dirt and particularly by bowel contents all predispose to the development of clostridial myonecrosis. The most important aspect of prophylaxis by far is early and adequate surgical management.[30,31] All devitalized tissue must be débrided; meticulous hemostasis is very important. Primary closure and tight packing of the wound should be avoided. All aspects of the wound must be drained adequately. If a cast must be applied, it should be bivalved from the outset. Hyperbaric oxygen is not indicated prophylactically. Antimicrobial prophylaxis, however, definitely is indicated. Penicillin is the drug of choice and should be given as early as possible after the injury occurs. Of emphasis is that antimicrobial prophylaxis is strictly adjunctive and far from adequate by itself. Bathing, particularly showering, and application of a compress wet with an iodophor for 15 minutes have been shown to reduce the skin count of *C. perfringens* significantly and minimize the likelihood of postoperative gas gangrene developing. This type of decontamination, of course, also may be useful in the management of traumatic wounds.

Prevention of clostridial uterine infection involves ensuring that all products of conception are removed during abortion and that retained portions of the placenta are removed immediately after the third stage of labor. Prolonged labor should be anticipated when possible and analgesics administered judiciously. During labor, particularly with ruptured membranes, pelvic and rectal examinations should be kept to a minimum. During delivery, trauma should be minimized and lacerations repaired according to accepted surgical principles.

Proper sanitation in food-preparing facilities and adequate refrigeration are important safeguards against the acquisition of *C. perfringens* food poisoning. In areas where necrotizing enteritis caused by *C. perfringens* type C is found with some frequency (such as in Papua New Guinea), a *C. perfringens* type C toxoid has been given with encouraging results.

Contact isolation for the duration of the illness is recommended for hospitalized children with *C. difficile*–associated diarrhea. Meticulous hand washing, the use of gloves for handling contaminated objects and fomites, environmental cleaning and disinfection, and limiting the use of antimicrobial agents have been advocated widely to control the transmission of *C. difficile* in health care facilities.[37]

NEW REFERENCES SINCE THE SIXTH EDITION

9. Barth H, Aktories K, Popoff MR, et al. Binary toxins: biochemistry, biology and applications of *Clostridium* and *Bacillus* proteins. *Microbiol Mol Biol Rev* 2004;**68**:373–402.
10. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008;**45**(Suppl. 1): S12–18.
12. Bos JL, Smithee B, McClane RF, et al. Fatal necrotizing colitis following a foodborne outbreak of enterotoxigenic *Clostridium perfringens* type A infection. *Clin Infect Dis* 2005;**40**:e78–383.
14. Brooke I. Clostridial infection in children. *J Med Microbiol* 1995;**42**:78–82.
16. Bryant AE, Chen RYZ, Nagata Y, et al. Clostridial gas gangrene II. Phospholipase C induced activation of platelet gpIIb/IIIa mediates vascular occulusion and myonecrosis in *C. perfringens* gas gangrene. *J Infect Dis* 2000;**182**:808–15.
20. Centers for Disease Control and Prevention. Botulism. *MMWR Recomm Rep* 1997;**46**(RR-10):1–55.
21. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep 2009* 2010;**58**:49–50.
23. Cherington M. Botulism: update and review. *Semin Neurol* 2004; **24**:155–63.
28. Fang F. Point–counterpoint: what is the current role of algorithmic approaches to the diagnosis of *Clostridium difficile* infection? *J Clin Microbiol* 2010;**48**:4347–53.
40. Hatheway CL. Botulism. In: Balows A, Hausler WJ, Ohashi M, et al, editors: *Laboratory diagnosis of infectious diseases*. New York: Springer; 2009. p. 111–13.
45. Kim J, Smathers SA, Prasad P, et al. Epidemiologic features of *Clostridium difficile*–associated disease among inpatients at children's hospitals in the United States 2001-2006. *Pediatrics* 2008;**122**: 1266–70.
48. Leal J, Gregson DB, Ross T, et al. Epidemiology of *Clostridium* species bacteremia in Calgary, Canada 2000-2006. *J Infect Dis* 2008;**57**:198–203.
49. Loo VG, Bourgault A-M, Poirier L, et al. Host and pathogen factors for *Clostridium difficile* infection and colonization. *New Engl J Med* 2011;**365**:1693–703.
51. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *New Engl J Med* 2011;**364**:422–32.
57. Nylund CN, Goudle A, Garza J, et al. *Clostridium difficile* infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011;**165**:451–7.
58. Olsen SJ, MacKinnon LC, Goulding NH, et al. Surveillance for foodborne disease outbreaks: United States 1993-1997. *MMWR Surveillance Summary* 2000;**49**:1–62.
66. Smith LDS. Clostridium. In: Smith LDS, editor. *The pathogenic anaerobic bacteria*. Springfield, Ill: Charles C Thomas; 2012. p. 109–14.
68. Stevens DL, Bryant AE, Berger A, et al. Clostridium. In: Versalovic J, Jorgensen JH, Landry ML, et al, editors: *The manual of clinical microbiology*. 2012. 10th ed. Washington, D.C.: American Society of Microbiology Press; 2011.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aldape MJ, Bryant AE, Stevens DL. *Clostridium sordelli* infection: epidemiology, clinical findings, and current perspectives on diagnosis and treatment. *Clin Infect Dis* 2006;**43**:1436–46.
2. Allen SD. Pig-bel and other necrotizing disorders of the gut involving *Clostridium perfrigens*. In: Connor DH, Chandler FW, Schwartz DA, et al, editors. *Pathology of infectious diseases*. Norwalk, Conn: Appleton & Lange; 1997. p. 717–24.
3. Allen SD, Emergy CL, Lyerly DM. *Clostridium*. In: Murray PL, Baron EJ, Jorgensen JH, et al, editors. *Manual of clinical microbiology*. 8th ed. Washington, D.C.: American Society of Microbiology; 2003. p. 835–56.
4. American Academy of Pediatrics: Clostridial infections. In: Pickering LK, editor. *2000 red book: report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2000. p. 212–19.
5. Archibald LK, Banerjee SH, Jarvis WR. Secular trends in hospital-acquired *Clostrium difficile* disease in the United States, 1987-2000. *J Infect Dis* 2004;**189**:1585–9.
6. Arnon SS, Schecter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA* 2001;**285**:1059–70.
7. Bar-Joseph G, Halberthal M, Sweed Y, et al. *Clostridium septicum* infection in children with cyclic neutropenia. *J Pediatr* 1997;**131**: 317–19.
8. Barnham M, Weightman N. *Clostridium septicum* infection and hemolytic-uremic syndrome. *Emerg Infect Dis* 1998;**4**:321–4.
9. Barth H, Aktories K, Popoff MR, et al. Binary toxins: biochemistry, biology and applications of *Clostridium* and *Bacillus* proteins. *Microbiol Mol Biol Rev* 2004;**68**:373–402.
10. Bartlett JG, Gerding DN. Clinical recognition and diagnosis of *Clostridium difficile* infection. *Clin Infect Dis* 2008;**45**(Suppl. 1): S12–18.
11. Benson L, Song X, Campos J, et al. Changing epidemiology of *Clostridium difficile*–associated disease in children. *Infect Control Epidemiol* 2007;**28**:1233–5.
12. Bos JL, Smithee B, McClane RF, et al. Fatal necrotizing colitis following a foodborne outbreak of enterotoxigenic *Clostridium perfringens* type A infection. *Clin Infect Dis* 2005;**40**:e78–383.
13. Brook I. *Anaerobic infections in childhood*. Boston: G. K. Hall; 1983.
14. Brooke I. Clostridial infection in children. *J Med Microbiol* 1995; **42**:78–82.
15. Bryant AE, Chen RYZ, Nagata Y, et al. Clostridial gas gangrene. I. Cellular and molecular mechanisms of microvascular dysfunction induced by exotoxins of *Clostridium perfringens*. *J Infect Dis* 2000; **182**:799–807.
16. Bryant AE, Chen RYZ, Nagata Y, et al. Clostridial gas gangrene II. Phospholipase C induced activation of platelet gpIIb/IIIa mediates vascular occulusion and myonecrosis in *C. perfringens* gas gangrene. *J Infect Dis* 2000;**182**:808–15.
17. Burgener D, Sirakas S, Eagles G, et al. A prospective study of *Clostridium difficile* infection and colonization in pediatric oncology patients. *Pediatr Infect Dis J* 1997;**16**:1131–4.
18. Caya JG. *Clostridium septicum* bacteremia in the pediatric population. *Arch Pathol Lab Med* 2000;**124**:1583.
19. Caya JG, Truant AL. Clostridial bacteremia in the non-infant pediatric population: A report of two cases and review of the literature. *Pediatr Infect Dis J* 1999;**18**:291–8.
20. Centers for Disease Control and Prevention. Botulism. *MMWR Recomm Rep* 1997;**46**(RR-10):1–55.
21. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States 2010. *MMWR 2009*. 2010;**58**:49–50.
22. Cherington M. Clinical spectrum of botulism. *Muscle Nerve* 1998;**21**:701–10.
23. Cherington M. Botulism: Update and review. *Semin Neurol* 2004; **24**:155–63.
24. Cohen SH, Tang YJ, Silva J. Analysis of the pathogenicity locus in *Clostridium difficile* strains. *J Infect Dis* 2000;**181**:659–63.
25. Dowell VR, Jr.: Botulism and tetanus: selected epidemiologic and microbiologic aspects. *Rev Infect Dis* 1984;**6**(Suppl. 1):202–7.
26. Dunkle LM, Brotherton TJ, Feigin RD. Anaerobic infections in children: a prospective study. *Pediatrics* 1976;**57**:311–20.
27. Eglow R, Pothoukalis C, Itzkowitz S. Diminished *Clostridium difficile* toxin A sensitivity in newborn rabbit ilium is associated with decreased toxin A receptor. *J Clin Invest* 1992;**90**:822–9.
28. Fang F. Point–counterpoint: what is the current role of algorithmic approaches to the diagnosis of *Clostridium difficile* infection? *J Clin Microbiol* 2010;**48**:4347–53.
29. Fekety R. Guidelines for the diagnosis and management of *Clostridium difficile*–associated diarrhea and colitis. *Am J Gastroenterol* 1997;**92**:739–50.
30. Finegold SM, George WL, editors. *Anaerobic infections in humans*. San Diego, Calif: Academic Press; 1989.
31. Finegold SM. *Anaerobic bacteria in human disease*. New York: Academic Press; 1977.
32. Freedman S, Hollander M. *Clostridium perfringens* septicemia as a postoperative complication of the newborn infant. *J Pediatr* 1967; **71**:576–8.
33. Gaglani MJ, Murray JC, Morad AB, et al. Chronic osteomyelitis caused by *Clostridium difficile* in an adolescent with sickle cell disease. *Pediatr Infect Dis J* 1996;**15**:1054–6.
34. Gallaher KJ, Marks KH. Clostridial infection as a cause of fulminant congenital sepsis neonatorum. *Am J Perinatol* 1991;**8**:370–2.
35. George WL. Antimicrobial agent–associated colitis and diarrhea: historical background and clinical aspects. *Rev Infect Dis* 1984; **6**(Suppl. 1):208–13.
36. Gerding DN. Clindamycin, cephalosporins, fluoroquinolones and *Clostridium difficile*–associated diarrhea: this is an antimicrobial resistance problem. *Clin Infect Dis* 2004;**38**:646–8.
37. Gerding DN, Johnson S, Peterson LR, et al. *Clostridium difficile*–associated diarrhea and colitis. *Infect Control Hosp Epidemiol* 1995; **16**:459–77.
38. Gredlein CM, Silverman ML, Downey MS. Polymicrobial septic arthritis due to *Clostridium* species: case report and review. *Clin Infect Dis* 2000;**30**:590–4.
39. Hatheway CL. Toxigenic clostridia. *Clin Microbiol Rev* 1990;**3**: 66–98.
40. Hatheway CL. Botulism. In: Balows A, Hausler WJ, Ohashi M, et al, editors. *Laboratory diagnosis of infectious diseases*. New York: Springer; 2009. p. 111–13.
41. Heidemann SM, Meert KL, Perrin E, et al. Primary clostridial meningitis in infancy. *Pediatr Infect Dis J* 1989;**8**:126–8.
42. Horowitz MA, Hughes JM, Merson MH, et al. Food-borne botulism in the United States, 1970-1975. *J Infect Dis* 1977;**136**: 153–9.
43. Hughes LM, Blumenthal JR, Merson MH, et al. Clinical features of types A and B food-borne botulism. *Ann Intern Med* 1981;**95**: 442–5.
44. Jarvis WR, Feldman RA. *Clostridium difficile* and gastroenteritis: how strong is the association in children? *Pediatr Infect Dis* 1984; **3**:4–6.
45. Kim J, Smathers SA, Prasad P, et al. Epidemiologic Features of *Clostridium difficile*–associated disease among inpatients at children's hospitals in the United States 2001-2006. *Pediatrics* 2008;**122**: 1266–70.
46. Klein EJ, Boster DR, Stapp JR, et al. Diarrhea etiology in a children's hospital emergency department: a prospective cohort study. *Clin Infect Dis* 2006;**43**:807–13.
47. Lawrence G, Walker PD. Pathogenesis of enteritis necroticans in Papua, New Guinea. *Lancet* 1976;**1**:125–6.
48. Leal J, Gregson DB, Ross T, et al. Epidemiology of *Clostridium* species bacteremia in Calgary, Canada 2000-2006. *J Infect Dis* 2008; **57**:198–203.
49. Loo VG, Bourgault A-M, Poirier L, et al. Host and pathogen factors for *Clostridium difficile* infection and colonization. *New Engl J Med* 2011;**365**:1693–703.
50. Lorber B. Gas gangrene and other *Clostridium*-associated diseases. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas and Bennett's principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 2549–61.
51. Louie TJ, Miller MA, Mullane KM, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection. *New Engl J Med* 2011; **364**:422–32.
52. McDonald LC, Killgore GE, Thompson A, et al. An epidemic toxin-gene-variant of *Clostridium difficile*. *N Engl J Med* 2005; **353**:2433–41.
53. Merson MH, Dowell VR, Jr.: Epidemiologic, clinical and laboratory aspects of wound botulism. *N Engl J Med* 1973;**289**:1005–10.
54. Minutti CZ, Immergluck LC, Schmidt ML. Spontaneous gas gangrene due to *Clostridium perfringens*. *Clin Infect Dis* 1999; **28**:159–60.

55. Montecucco C. Clostridial neurotoxins: the molecular pathogenesis of tetanus and botulism. *Curr Top Microbiol Immunol* 1995;**195**: 1–278.
56. Motz RA, James AG, Dove B. *Clostridium perfringens* meningitis in a newborn infant. *Pediatr Infect Dis J* 1996;**8**:708–10.
57. Nylund CN, Goudle A, Garza J, et al. *Clostridium difficile* infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011;**165**:451–7.
58. Olsen SJ, MacKinnon LC, Goulding NH, et al. Surveillance for foodborne disease outbreaks: United States 1993-1997, *MMWR Surveill Summ* 2000;**49**:1–62.
59. Owens RC. *Clostridium difficile*–associated disease: an emerging threat to patient safety. *Pharmacotherapy* 2006;**26**:299–311.
60. Petrillo TM, Beck-Sague CM, Songer JG, et al. Enteritis necroticans (pigbel) in a diabetic child. *N Engl J Med* 2000;**342**:1250–3.
61. Samore M, Kigore G, Johnson S, et al. Multicenter typing comparison of sporadic and outbreak *Clostridium difficile* isolates from geographically diverse hospitals. *J Infect Dis* 1997;**176**: 1233–8.
62. Schantz EJ, Johnson EA. Properties and use of botulinum toxin and other microbial neurotoxins in medicine. *Microbiol Rev* 1992;**56**: 80–99.
63. Shapiro RL, Hatheway C, Swerdlow DL. Botulism in the United States; a clinical and epidemiologic review. *Ann Intern Med* 1998; **129**:221–8.
64. Shetty AK, Heinrich SD, Steele RW. *Clostridium septicum* osteomyelitis: case report and review. *Pediatr Infect Dis J* 1998;**17**:927–8.
65. Smith L, Sugiyama H. *Botulism: The organism, its toxins, the disease*. 2nd ed. Springfield, IL: Charles C Thomas; 1977.
66. Smith LDS. Clostridium. In: Smith LDS, editor. *The pathogenic anaerobic bacteria*. Springfield, Ill: Charles C Thomas; 2012. p. 109–14.
67. Stevens DL, Laine BM, Mitten JE. Comparison of single and combination antimicrobial agents for prevention of experimental gas gangrene caused by *Clostridium perfringens*. *Antimicrob Agents Chemother* 1987;**31**:312–16.
68. Stevens DL, Bryant AE, Berger A, et al. Clostridium. In: Versalovic J, Jorgensen JH, Landry ML, Warnock DW, et al, editors. *The manual of clinical microbiology*. 2012. 10th ed. Washington, D.C.: American Society of Microbiology Press; 2011.
69. Stunden RJ, Brown RA, Rode H, et al. Umbilical gangrene in the newborn. *J Pediatr Surg* 1988;**23**:130–4.
70. Sunnenshine RH, McDonald LC. *Clostridia difficile*–associated disease: new challenges. *Cleve Clin J Med* 2006;**73**:187–97.
71. Tacket CO, Shandera WX, Mann JM, et al. Equine antitoxin use and other factors that predict outcome in type A food-borne botulism. *Am J Med* 1984;**76**:794–8.
72. Thielman NM. Antibiotic-associated colitis. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas and Bennett's principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 1111–26.
73. Thirumoorthia MC, Keen BM, Dajani AS. Anaerobic infections in children: a prospective study. *J Clin Microbiol* 1976;**3**:318–23.
74. Warny M, Pepin J, Fang A, et al. Toxin production by an emerging strain of *Clostridium difficile* associated with outbreaks of severe disease in North America and Europe. *Lancet* 2005;**366**:1079–84.
75. Welch DF, Marks MI. Is *Clostridium difficile* pathogenic in infants? *J Pediatr* 1982;**100**:393–5.
76. Woodruff BA, Griffin PM, McCroskey LM, et al. Clinical and laboratory comparison of botulism from toxin types A, B, and E in the United States, 1975-1988. *J Infect Dis* 1992;**166**:1281–6.
77. Zar FA, Backanagari SR, Moorthi LST, et al. A comparison of vancomycin and metronidazole for treatment of CDAD. *Clin Infect Dis* 2007;**45**:302–7.

CHAPTER 146

Infant Botulism

Stephen S. Arnon

Of the three main forms of human botulism (foodborne, wound, and infant), infant botulism is the most recently recognized (1976) and the most common form in the United States and some other countries. Now recognized globally,[58] infant botulism results from a unique pathogenesis. Ingested spores of *Clostridium botulinum* germinate, colonize the infant's colon, and produce botulinum neurotoxin within it. The toxin then is absorbed, binds to peripheral cholinergic synapses, and causes flaccid paralysis. Knowledge of this intestinal pathogenesis resulted in discovery of novel pathogenic strains *Clostridium baratii* and *Clostridium butyricum*, each of which makes a botulinum neurotoxin and causes infant botulism. Discovery of these strains enlarged the number of organisms known to cause the "intestinal toxemias of infancy," of which infant botulism is the prototype. Parenthetically, adults and older children rarely may become susceptible to infant-type (i.e., intestinal toxemia) botulism after broad-spectrum antibiotic treatment, intestinal surgery, or inflammatory bowel disease[6,31,41] or in association with a Meckel diverticulum[39,91] or bone marrow transplantation procedures.[97]

HISTORY

Infant botulism is not a new disease; rather, it was recognized relatively recently and may be considered an "emerging" infectious disease. The first laboratory-proven case of human infant botulism occurred in California in 1931, although it was misdiagnosed at the time.[15] Decades later and well before the etiology of the disease was apparent, the characteristic clinical features of infant botulism had become evident to discerning observers. In 1974, Grover and associates[46] described nine patients from Pennsylvania with a neurologic syndrome of undetermined cause that from today's perspective almost certainly was infant botulism. The same idiopathic syndrome was recognized in southern California and was reported by Ramseyer and colleagues[88] in 1976 to have a characteristic electromyographic pattern. A year later, Clay and associates[32] linked their eight southern California patients to infant botulism.

The first report of frank botulism in infancy was provided by Pickett and colleagues[87] in 1976. Although the source of botulinum neurotoxin for their two patients was undetermined, the possibility of its in vivo production was suggested.[69,87] The diagnosis of botulism in these and other California patients was established by identification of *C. botulinum* toxin and organisms in the infants' feces.[69] Evidence also was obtained that ingested spores of *C. botulinum* had produced the toxin in the infants' intestinal tract.[10,69,117]

In subsequent years, the clinical spectrum of infant botulism was found to include mild outpatient cases and, in some, but not all,[26] locations, sudden unexpected death indistinguishable from typical sudden infant death syndrome.[11,76,81,86] In 1985, a *C. baratii* strain that produced a type F botulinum neurotoxin was recognized belatedly as the true cause of a case of infant botulism that occurred in New Mexico in 1979,[47,51] and in 1986, a *C. butyricum* strain that produced a type E botulinum neurotoxin was recognized as the cause of two cases of infant botulism in Rome, Italy.[16] These latter two novel clostridia were discovered only because they caused human infant botulism; their existence suggests that others like them await discovery.

ETIOLOGIC AGENT

C. botulinum is a gram-positive, spore-forming, obligate anaerobe whose natural habitat worldwide is the soil. Consequently, *C. botulinum* is as ubiquitous as the dust on which it may travel; and, hence, its spores commonly are present on fresh fruits, vegetables, and other agricultural products such as honey. Members of the *C. botulinum* species are so diverse in their biochemical capabilities and nucleic acid profiles that they would not be grouped as a single species except for the similar neurotoxin molecule that each strain produces[50,101]; at present, the *C. botulinum* species is subdivided into four groups (I to IV) based on metabolic characteristics.[101] Almost all cases of infant botulism in the United States have been caused by group I proteolytic type A or type B strains. However, unusual strains of *C. baratii* and *C. butyricum* that make botulinum toxins E and F also cause infant botulism.* The entire 3.9-megabase (Mb) chromosome and a 16.3-kilobase (kb) plasmid within a *C. botulinum* type A strain were sequenced recently and found to contain 3650 and 19 predicted genes, respectively,[96] thereby launching the era of functional genomic studies of this special bacterium. The botulinum toxin gene cluster is a mobile genetic element that may be found in the bacterial chromosome, a plasmid, or a bacteriophage.[102]

In general, each vegetative cell of *C. botulinum* produces just one of seven distinguishable toxins, which arbitrarily have been assigned the letters A to G. The seven toxin types are operationally distinguished by polyclonal monovalent antitoxins raised against each of the toxin

*References 16,37,47,84,107,116.

types; each monovalent antitoxin does not protect against any of the other six toxin types. The different toxin types serve as convenient epidemiologic and clinical markers. Subtypes of several toxin types have been identified by immunologic methods,[59,111] by neutralization studies using monoclonal antibodies,[3,103] and by toxin gene nucleic acid investigations.[50] Each toxin molecule is a simple protein consisting of two polypeptide chains of approximately 100,000 (heavy chain) and 50,000 (light chain) daltons joined by a disulfide bond.

Botulinum toxin is the most poisonous substance known.[42] For this reason and because of the ease with which it may be produced, transported, and disseminated, the Centers for Disease Control and Prevention (CDC) has listed botulinum toxin as one of just six "category A (tier 1)" (i.e., most dangerous) potential bioweapon agents.[13] By extrapolation from studies involving adult primates, the lethal dose in the bloodstream of humans is approximately 1 ng/kg body weight.[13,42] Its potency for infants may be even higher because of the narrowness of their pharyngeal airway.[114]

The basis of the phenomenal potency of the botulinum (and tetanus) toxins is enzymatic. The light chain of each neurotoxin is a zinc-containing protease that hydrolyzes one or more of three intracellular proteins needed for vesicle fusion and release of acetylcholine into the synaptic cleft.[93] The specificity of the toxin for peripheral cholinergic neurons results from their expression of a lower-affinity ganglioside cell surface receptor to which the toxin attaches first, which then is followed by attachment to a second, higher-affinity protein receptor that uniquely appears from the interior of the synaptic vesicle when it fuses with the terminal membrane to release acetylcholine.[30,54,82]

PATHOGENESIS

Infant botulism is not the diminutive form of foodborne botulism, and, hence, the disease is not "infantile botulism." Rather, infant botulism results from a unique infectious disease pathway and was so named to emphasize that fact.[10,69,117] Ingested spores of *C. botulinum* germinate, colonize the infant's colon, and produce botulinum neurotoxin within it.[10,48,71,73,117] The toxin subsequently is absorbed and carried by the bloodstream to peripheral cholinergic synapses, where it binds irreversibly. The light chain is then internalized into the cytosol of the neuron, where it blocks the release of acetylcholine by enzymatic cleavage of "fusion complex" proteins.[93] Clinically, the most important of the peripheral cholinergic synapses is the neuromuscular junction; the toxin's action results in flaccid paralysis and hypotonia. Preganglionic cholinergic synapses in the autonomic nervous system also may be affected.[63,93]

By use of a mouse model system of intestinal colonization (in which the animals paradoxically remained free of symptoms), Sugiyama and colleagues[25,73,108] demonstrated that the intestinal microflora ("microbiome") of adult animals ordinarily prevents colonization of the gut by *C. botulinum*. Administration of 10^6 type A spores failed to colonize the intestine of normal adult mice, whereas after treatment for 2½ days with a combination of oral erythromycin and kanamycin, half the mice could be colonized by just 2×10^4 spores. When the antibiotic-treated mice were placed in cages with normal mice, they lost their susceptibility to intestinal colonization after 3 days.[25] (Mice normally exhibit coprophagia.) In addition, adult germ-free mice could be colonized intestinally by just 10 *C. botulinum* type A spores. When the germ-free adult animals were placed in a room with conventional mice (but not in the same cages), in 3 days the formerly germ-free animals became resistant to colonization by 10^5 spores.[73]

In contrast to the experimental work with adult mice, normal infant mice were susceptible to intestinal colonization by *C. botulinum* spores.[108] Like human infants, the normal infant mice were subject to colonization for only a limited period (7 to 13 days of age). Susceptibility of the infant mice peaked between days 8 and 11 in a pattern reminiscent of the peaking of susceptibility seen between 2 and 4 months of age in human infant botulism (Fig. 146-1).[8,108] The infective dose of spores for infant mice was much smaller than that of their antibiotic-treated adult counterparts; the 50 percent infective dose for normal infants was only 700 spores. In one experiment, just 10 spores were needed to colonize an infant mouse.[108] The minimum infective dose of *C. botulinum* spores for human infants is not known, but from exposure to spore-containing honey, it has been estimated to be as low as 10 to 100.[12]

Recognition of the central role of the host's intestinal microflora ("microbiome") in determining susceptibility or resistance to colonization by *C. botulinum* has directed attention to factors that may influence the composition of the normal microflora. Diet may be the most important of these factors. When compared with adult-type flora, the infant flora is simpler, with fewer genera and species. The dominant members vary, depending in part on whether the infant is fed only breast milk, only formula milk, or a mixture of the two.[105,106] In addition, the composition of the intestinal flora is changed if solid foods, such as cereals, become part of the infant's diet. The normal human infant microflora contain several bacterial species, mainly *Bifidobacterium* and *Bacteroides*, that in vitro can inhibit the multiplication of *C. botulinum*.[110]

The onset of infant botulism occurs at a significantly younger age in formula-fed infants (7.6 weeks) than in breast-fed infants (13.7 weeks),[9] perhaps reflecting the earlier availability in formula-fed infants of suitable ecologic niches[9,64,105,106] and the formula-fed infants' lack of the immune factors (e.g., secretory IgA, lactoferrin) contained in human milk.[4,5,44] Moreover, introduction of solid foods may "perturb" the intestinal microflora[105] and thereby aid colonization with *C. botulinum*.[4,8,64,105]

An additional physiologic risk factor for infant botulism is slower gut motility, as measured by the frequency of defecation before the onset of illness.[95,104] Less than one bowel movement per day is a risk factor for both breast-fed and formula-fed infants, but this factor occurred in just 50 percent of cases.[95] Whether a Meckel diverticulum may predispose to the development of infant botulism caused by *C. botulinum*, as it appears to

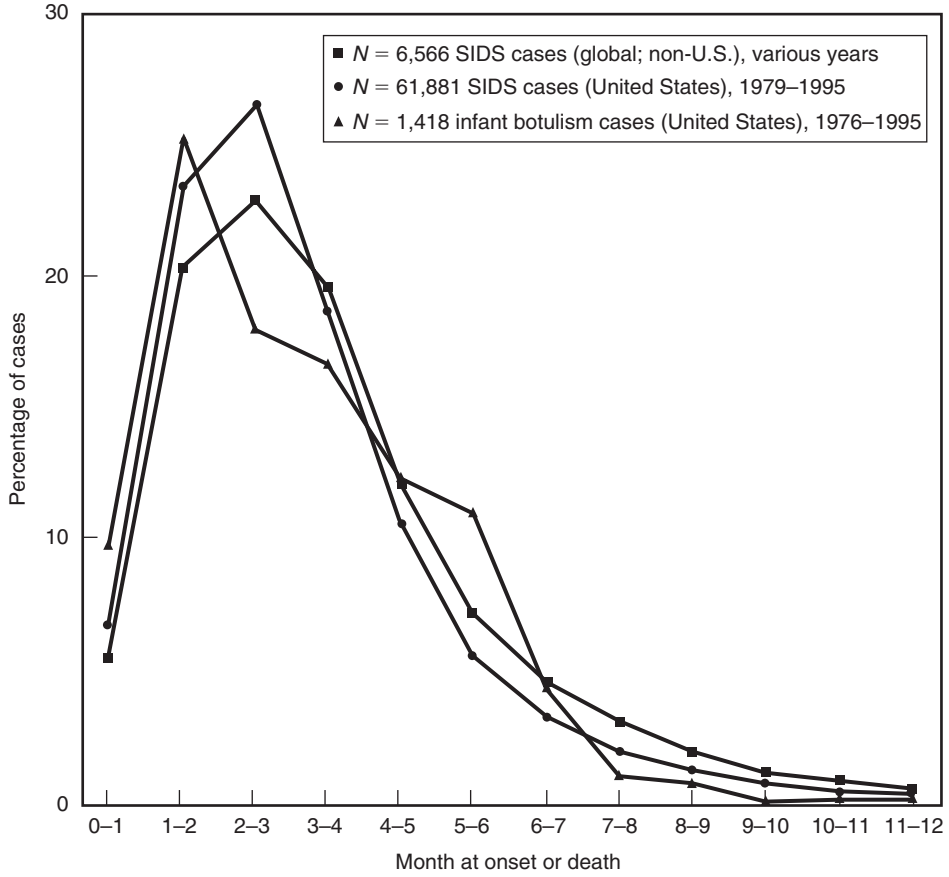

FIGURE 146-1 ■ Age distribution of infant botulism and sudden infant death syndrome (SIDS).

do for infant botulism caused by *C. butyricum*, is not known.[37,39,91]

EPIDEMIOLOGY

Any discussion of the epidemiology of infant botulism should be prefaced by the caveat that almost all presently available information is derived from study of only part of the clinical spectrum, namely, hospitalized patients. Accordingly, current perspectives may need to be modified as the outpatient and sudden death portions of the clinical spectrum become defined more fully. Furthermore, the perceived incidence remains more a reflection of physician awareness and access to diagnostic testing than the actual occurrence of disease. Somewhat less than half (39.2%) of U.S. cases have been reported from California, which has the largest number of births of any state. However, California does not have the highest incidence of infant botulism once adjustment is made for differences in annual births (Table 146-1). Notably, 8 of the 11 states with the highest incidence are located west of the Rocky Mountains, and 6 of the 8 are contiguous. The three eastern states with the highest incidence also are contiguous.

A unique epidemiologic feature of infant botulism is its age distribution, which, perhaps coincidentally, is virtually identical to the age distribution of sudden infant death syndrome (see Fig. 146-1).[8,11,27,104] Almost all U.S. cases (99.7%) of infant botulism reported to date have occurred in children younger than 1 year old. Some 90.1 percent of cases have occurred in the patients' first 6 months of life, 9.6 percent were distributed over the subsequent 6 months, and just 0.3 percent occurred between 53 and 72 weeks of age. The youngest known patient was just 38 hours old at onset (and had illness

TABLE 146-1 **Cases and Incidence of Infant Botulism: Top 11 States in Incidence, United States, 1977-2005**

State	Cases (*M*) 1977-2005	Incidence* 1977-2005
Delaware	41	13.4
Hawaii	46	8.6
Utah	92	7.5
California	927	6.3
Pennsylvania	282	6.2
Oregon	47	3.7
Washington	74	3.5
New Jersey	106	3.4
Idaho	17	3.2
New Mexico	24	3.1
Nevada	18	2.8

*Per 100,000 live births per year.

caused by *C. baratii* type F toxin),[20] whereas the oldest was 72 weeks old at onset. The illness has occurred in all major racial and ethnic groups and in equal proportions in males and females. A national seasonality is not evident. In California more cases have occur in the warmer, drier months (May to October, 60%) than in the cooler, wetter months (November to April, 40%).

Infant botulism has been reported from all inhabited continents except Africa. In the United States, with 40 exceptions (1.4% of cases), all hospitalized cases known as of December 2011 were caused by either type A or type B *C. botulinum* toxin. All 50 states now have reported infant botulism; California (1976) was the first, whereas Rhode Island (2008) was the last. In general, the distribution of cases by type of toxin has paralleled the distribution of toxin types in U.S. soil,[100] with type B cases predominating from the great plains eastward and type A cases from the Rocky Mountains westward. The 40 exceptional cases resulted from a variety of toxin types. Two cases in California and in Iowa and one each in Colorado, New Mexico, Ohio, Texas, Washington, and Wisconsin resulted from type F toxin produced by neurotoxigenic *C. baratii* strains.[20,47,51,84] Twenty-two cases were caused by a *C. botulinum* strain that produced mostly type B and some type A toxin (designated type Ba), and six cases resulted from Bf strains. One case caused by both *C. botulinum* type A and type B from today's perspective probably resulted from either a Ba or Ab strain. Three patients with Bf illness lived in California,[18] one lived in New Mexico, one lived in Texas, whereas the sixth patient had traveled from California through New Mexico to Texas immediately before onset of the illness. Two type Bf cases occurred in the United Kingdom.[99] The only *C. baratii* type F cases reported outside the United States occurred in Canada and Hungary.[116] In Illinois in 2008 a type E case caused by *C. botulinum* occurred,[66] and in Arizona in 2006 a type E case occurred, but neither *C. butyricum* nor *C. botulinum* could be isolated. Five European *C. butyricum* type E infant botulism cases were reported from Italy and two from the United Kingdom, and one was reported from Japan.[16,37-39]

As of December 2010, approximately one fifth (809/3612, 22.4%) of all known global infant botulism cases between 1976 and 2010 had been reported from 29 countries other than the United States.[58] Of these cases, Argentina has reported the largest number (605),[65] followed by Australia (32), Canada (32), Italy (30), Japan (26), Spain (13), United Kingdom (12), Germany (11), and France (8). The remaining countries have reported four or fewer cases each.[58] The non-U.S. cases occurred in 15 countries in Europe, Asia (3), the Middle East (5), North America (2), South America (3), and Australia.[67] The small number of reported cases from most non-U.S. countries most likely reflects limited physician awareness of the disease and limited access to specialized diagnostic testing laboratories, as well as actual variation in disease incidence.[58]

Geographic clustering has been noted. In Pennsylvania, 43 of 53 cases in the period 1977 to 1983 occurred in four suburban counties that form an arc bordering the city of Philadelphia.[62,64] In Colorado, three type A cases occurred in three separate families in a small town with approximately 300 annual births. Two of the infants had used the same crib sequentially; environmental samples, including the crib, soil, and household dust, yielded *C. botulinum* type A.[53] In California, two type A cases occurred 5 years apart in the children of two families who lived one house apart. In another California family, two successive infants each acquired type A infant botulism, but the third child born in sequence did not. Soil and dust specimens from the house where all three infants lived contained *C. botulinum* type A.

The role of breast-feeding and formula-feeding as factors possibly predisposing to illness remains unsettled. All studies to date have identified an association between being breast-fed and being hospitalized for infant botulism.* This finding has resulted in one perspective that holds that breast-feeding predisposes to the development of illness,[62-64,104] whereas the other perspective holds that breast-feeding slows its onset sufficiently to permit hospitalization to occur.[4,5,8,9] However, among hospitalized patients in California, the mean age at onset of botulism in formula-fed infants (8.6 weeks) was significantly younger and about half that of breast-fed infants (16.6 weeks). In addition, in California the patients with fulminant-onset infant botulism who stopped breathing and died at home all were formula fed.[9] The relative susceptibilities of formula-fed and breast-fed infants to infant botulism and the resultant severity of their disease possibly reflect differences in the availability of suitable ecologic niches for *C. botulinum* in the intestinal flora ("microbiome"), differences in the availability of immune factors (e.g., lactoferrin and secretory IgA) contained in human milk but not in formula milk,[44] or other differences not identified yet.

Probably few, if any, patients with infant botulism acquire *C. botulinum* spores from infant formula, despite isolation in the United Kingdom of *C. botulinum* type B from powdered infant formula consumed by a patient with type B infant botulism.[24,55] However, the capacity of powdered infant formula (a nonsterile product) to serve as a vehicle of clostridial spores was identified in a recent study of U.S. commercial powdered infant formulas that found a dozen different clostridial species in them, including *Clostridium sporogenes* (the functional equivalent of a toxigenic proteolytic *C. botulinum*).[19] In addition, the possibility that an infant patient may have foodborne botulism needs to be kept in mind because foodborne botulism caused by home-prepared baby food has been recognized.[2,21]

Honey is the one dietary reservoir of *C. botulinum* spores thus far definitively linked to infant botulism by both laboratory and epidemiologic evidence.† More than 35 instances worldwide are known in which *C. botulinum* spores have been found in the actual honey fed to the affected infant before the onset of illness. In each instance, the toxin type (A or B) of the spores in the honey matched the toxin type of the *C. botulinum* that caused the infant's illness; the probability that such perfect concordance occurred by chance is less than 1 in 10 billion. Occasionally, *C. botulinum* has been isolated from honey in which

*References 5, 9, 62-64, 74, 104, 113.
†References 8, 12, 17, 49, 52, 57, 70, 80, 90, 109.

the spore toxin type in the honey did not match the toxin type of the infant's illness[17,37]; in such instances, the conclusion is that the honey was not the source of the infective spores.

C. botulinum spores have been found in honey from the United States, Argentina, Australia, Canada, China (Taiwan also), Denmark, Finland, Italy, Norway, Spain, Japan, and Central America* but not in honey from the United Kingdom.[23]

In general, only low concentrations of *C. botulinum* spores have been found in honey (≤1 spore/g),[70,78] with the occasional higher concentrations (e.g., 36 to 60 spores/g) thought to result from multiplication of *C. botulinum* in dead bees and bee pupae.[77] Toxin type A, B, C, and F spores all have been found in honey, with some of these toxin types linked to the geographic origin of the honey.[78] For these reasons and because honey is not nutritionally essential, all major pediatric, public health, and honey industry agencies in the United States have joined in the recommendation that honey not be fed to infants. In 2000, several brands of honey sold in the United States began to carry a warning not to feed honey to infants; an equivalent label first appeared on British honey in 1996.

Discussion of the possible role of corn syrup in infant botulism is necessitated by two reports. In 1982, the U.S. Food and Drug Administration (FDA) found *C. botulinum* type B spores in approximately 0.5 percent (5 of 961) of previously unopened retail samples of light and dark corn syrup[58]; the manufacturer then made changes in the production process. In 1989, the federal CDC reported the results of a 2-year epidemiologic study of U.S. cases from all states except California.[104] By subgrouping patients by age and using logistic regression modeling techniques, a statistical association was obtained among the triad of exposure to corn syrup, breast-feeding, and age at onset of 2 months or older.[83,104]

In contrast to these reports, a 1988 Canadian survey found no *C. botulinum* spores in 43 samples of corn syrup.[49] A 1991 FDA market survey of 783 syrups (354 of which were light corn syrup and 271 were dark corn syrup) concluded that none contained *C. botulinum* spores.[60] A California study (unpublished) of 103 corn syrups, 72 of which had been fed to infants who subsequently became ill with infant botulism, did not find *C. botulinum* in any sample. Moreover, a 1979 epidemiologic study that simply compared rates of exposure to corn syrup in 41 cases and 107 control infants identified feeding of corn syrup as a significant protective factor against type A infant botulism.[12] The explanation offered for the latter observation was that if a parent chose corn syrup as a sweetener for the infant, the child was unlikely to have been fed honey as a second sweetener. Thus, on the basis of the available evidence, corn syrup appears not to constitute a source of *C. botulinum* spores or a risk factor for the development of infant botulism.

In addition to honey and syrup, hundreds of traditional and nontraditional infant food items, including formula milk, have been examined and found not to contain *C. botulinum*.[68] Also, in instances not associated with illness, *C. botulinum* spores have been found in raw sugar and molasses but not in refined sugar[79] and in herbal (chamomile) tea[22] and other herbal preparations.[58]

Potential environmental sources of *C. botulinum* spores have been identified in many locales. The soil in Pennsylvania,[64] soil and cistern water in Australia,[75] vacuum cleaner dust in Finland,[81] and the soil and vacuum cleaner dust in California[8] obtained from case homes were found to contain *C. botulinum*, with the toxin type (A or B) in each instance matching that of the ill infant. However, despite the foregoing, it deserves emphasis that for most cases of infant botulism, no source of *C. botulinum* spores ever is identified, even circumstantially. In these cases, illness probably was acquired by swallowing spores adherent to airborne microscopic (invisible) dust.

CLINICAL MANIFESTATIONS

Like other infectious diseases, infant botulism displays a spectrum of clinical severity.* To date, almost all patients with recognized disease have been sufficiently hypotonic and weak to need hospitalization. Consequently, the present picture of infant botulism is derived from hospitalized patients. However, outpatients who displayed only a few days of lethargy, poor feeding, and some decrease in frequency of bowel movement have been detected by alert physicians familiar with the more "classic" illness. At the opposite end of the clinical spectrum are patients whose "catastrophic manifestations" obscured and delayed establishment of the correct diagnosis[72] and those few cases for which the history and clinical findings were indistinguishable from typical cases of sudden infant death syndrome (SIDS, crib death),[11,81, 86] approximately 1 in 20 of which (in California) appears to result from fulminant infant botulism.[8,11]

The onset of infant botulism ranges from the insidious to the abrupt. At one extreme are patients who were nursing normally 6 hours before becoming so floppy that acute meningitis was the diagnosis at initial evaluation, and at the other extreme are patients who returned to their physicians four times in a week as the signs of illness gradually became apparent. Although rare, illness caused by *C. baratii* type F appears to be characterized by the triad of very young age at onset, rapid onset, and profound paralysis.[20,84] Equally rare, illness caused by *C. butyricum* type E may be manifested as a paradoxically rigid abdomen and associated bowel colonization with *Clostridium difficile*.[37,38]

In the "classic" case of infant botulism, the first sign of illness almost always is constipation (defined as 3 or more days without defecation in a previously regular infant), yet the constipation often is overlooked. A few patients (<5%) will not have a history of constipation. Usually, a mother first notices listlessness, lethargy, and poor feeding, together with breast engorgement if the infant had been nursing. The increasing weakness over the ensuing 1 to 4 days typically brings the infant to medical attention.

*References 12, 17, 49, 52, 57, 69, 77-80, 109.

*References 4, 8, 11, 63, 64, 72, 86, 94, 113.

FIGURE 146-2 ■ Mildly affected, 7-week-old infant with botulism. Note the minimal signs, including ptosis, mildly disconjugate gaze, expressionless face, slack jaw, and neck and arm hypotonia.

Botulism is manifested clinically as a symmetric, descending paralysis. Early in the course, weakness and hypotonia characterize the illness, and the remainder of the physical examination not involving the neuromuscular system is normal. The first signs of illness are found in the cranial nerves; a patient cannot have infant botulism without having bulbar palsies. The typical patient has an expressionless face, a feeble cry, ptosis (evident when the eyelids must work against gravity), poor head control, and generalized weakness and hypotonia (Fig. 146-2). Eye muscle paralysis varies, with the pupils often at mid position and initially briskly reactive. However, fatigability of the pupillary light reflex and extraocular motility is also often commonly present; these are pathognomonic signs and should be sought at presentation (Table 146-2).[4] The gag, suck, and swallow reflexes are impaired, as is the corneal reflex if it is tested repetitively. Deep tendon reflexes frequently are normal at initial evaluation and diminish subsequently as the paralysis extends and increases. The "frog's legs" sign often is seen. Patients are afebrile unless a secondary infection (e.g., aspiration pneumonia) is present.

TABLE 146-2 **Neurologic Signs Helpful in the Diagnosis of Infant Botulism**

1. Take the patient to a dark room. Shine a bright light into the eye; note the quickness of pupillary constriction. Remove the light when the constriction is maximal; let the pupil dilate again. Then immediately repeat the light, continuing thus for 1 to 3 minutes. The initially brisk pupillary response may become sluggish and the pupil unable to constrict maximally. *Fatigability with repetitive muscle contraction is the clinical hallmark of botulism.*
2. Shine a bright light onto the fovea and keep it there for 1 to 3 minutes, even if the infant tries to deviate the eyes. Latent ophthalmoplegia may be elicited, purposeful efforts to avoid the light may diminish, or both may occur.
3. Place a clean fifth finger in the infant's mouth while taking care to not obstruct the airway. Note the strength and duration of the reflex sucking. The suck is weak and poorly sustained. The gag reflex strength also may be quickly checked (if the infant has not been fed recently).

Modified from Arnon SS. Infant botulism. *Annu Rev Med* 1980;**31**:541-60, 1980. Reproduced with permission from Annual Reviews, Inc.

The results of most laboratory and clinical studies are normal. At admission, the child may have evidence of mild dehydration and fat mobilization because of diminished oral intake. Occasionally at admission, the protein concentration in cerebrospinal fluid (CSF) becomes elevated because of the mild dehydration. If infant botulism is suspected soon after the child is admitted, electroencephalography, computed tomography, and magnetic resonance imaging seldom are required but if they are performed these examinations yield nonspecific or normal results. Electromyography may offer rapid bedside confirmation of the clinical diagnosis (see later discussion of differential diagnosis and diagnosis).[33,36]

The definitive diagnostic laboratory study is examination of feces for the presence of *C. botulinum* organisms and toxin, which is the only certain way to identify the neurotoxin type (A, B, or other) responsible for the illness. Small amounts (<5 mouse LD_{50}/mL) of botulinum toxin sometimes can be identified in serum specimens if they are collected early in the course of the illness.* In one U.S. report, almost one patient in eight had toxin demonstrable in serum.[48] Clinically suspected cases that lack an identified toxin type are not included in official tallies of infant botulism.[28,29,58]

The usual hospital course of *untreated* infant botulism has certain general features.[4,56,64,94] After the increasing weakness has necessitated admission, the weakness and hypotonia continue to progress and usually become generalized. The deep tendon reflexes, which may be normal at admission, may diminish or disappear temporarily. The nadir of paresis and paralysis in untreated patients usually occurs within 1 to 2 weeks after admission; such patients often remain at their nadir for as long as 1 to 3 weeks before showing signs of improvement. However, once strength and tone begin to return, the improvement

*References 16, 37, 48, 65, 85, 112, 115.

continues steadily and gradually over the ensuing weeks in the absence of complications (Table 146-3). In contrast, patients treated with human botulism immune globulin have a mean hospital stay of approximately 2 weeks (see later discussion of treatment).[14]

In the California experience, infant botulism does not have a relapsing or biphasic course and perceived "relapses" have been found, in retrospect, to be an indication of premature discontinuation of gavage feeding or respiratory support, of the onset of a complication (see Table 146-3), or of premature discharge. However, the clinical experience elsewhere with regard to relapses has been different.[37,43,89,94] The patient is ready for discharge when gag, suck, and swallow are sufficiently strong both to protect the airway against accidental aspiration and to ensure adequacy of oral intake. Parents also may be taught to feed by gavage at home. In either situation, discharge may occur safely while head lag and constipation still are present.

TABLE 146-3 **Complications of Infant Botulism**

Adult respiratory distress syndrome
Anemia
Aspiration
Blood pressure instability
Clostridium difficile colitis
Fracture of the femur and humerus
Inappropriate secretion of antidiuretic hormone
Misplaced or plugged endotracheal tube
Necrotizing enterocolitis
Otitis media
Pneumonia
Recurrent atelectasis
Respiratory arrest
Seizures secondary to hyponatremia
Sepsis
Subglottic stenosis
Tension pneumothorax
Tracheal granuloma
Tracheal stenosis
Tracheitis
Tracheomalacia
Transfusion reaction
Urinary tract infection

DIFFERENTIAL DIAGNOSIS AND DIAGNOSIS

When initially brought to medical attention, patients with infant botulism often are so mildly weak and hypotonic that the illness is not considered. Even today, more than 30 years after the disease first was recognized, suspected sepsis remains the most common admission diagnosis for patients with infant botulism. A careful history (constipation commonly is overlooked) and physical examination (especially evaluation of cranial nerve function) usually can identify patients with infant botulism correctly and render unnecessary most additional testing for the other entities typically suspected (Table 146-4). A review of entities that so closely mimicked infant botulism that botulism immune globulin was administered soon after admission identified spinal muscular atrophy type I, mitochondrial disorders, and a small number of other conditions as the actual diagnoses (see Table 146-4).[40]

The diagnosis of infant botulism is established by identification of *C. botulinum* (or, rarely, of neurotoxigenic *C. butyricum* or *C. baratii*) organisms in the feces or enema effluent of an infant with clinical signs consistent with the paralyzing action of botulinum toxin.[28,56,69] Extensive studies have demonstrated that *C. botulinum* is not part of the normal resident flora of infants or adults.[8,48,105,106] If the fecal specimen is obtained sufficiently early in the course of the illness, it also will contain botulinum toxin. Because of the patient's constipation, an enema with sterile, nonbacteriostatic water (not saline) commonly is needed to obtain a fecal specimen for diagnostic examination. The mouse neutralization test remains the most robust, sensitive and specific assay for botulinum toxin.[29,61] Laboratory diagnosis that identifies the type of toxin responsible for the illness is essential for the case to be registered as infant botulism[58] and is important in determining the prognosis; mean hospital stay is

TABLE 146-4 **Working Differential Diagnosis of Infant Botulism**

Admission Diagnoses	Subsequent Working Diagnoses	Clinical Mimics*
Rule out sepsis	Hypothyroidism	Spinal muscular atrophy type I
Dehydration	Metabolic encephalopathy	Mitochondrial disorders
Viral syndrome	Amino acid metabolic disorder	Metabolic and amino acid disorders
Pneumonia	Heavy metal poisoning (Pb, Mg, As)	Assorted infectious disease disorders
Idiopathic hypotonia	Drug ingestion	Miscellaneous, including central nervous system disease
Failure to thrive	Poliomyelitis	Probable infant botulism lacking laboratory verification
	Medium-chain acyl-CoA dehydrogenase deficiency	
	Brain stem encephalitis	
	Myasthenia gravis	
	Viral polyneuritis	
	Guillain-Barré syndrome	
	Hirschsprung disease	
	Werdnig-Hoffmann disease	

*Patients so indistinguishable from infant botulism at admission that they were treated with human botulism immune globulin intravenous (BIG-IV).

From Francisco AM, Arnon SS. Clinical mimics of infant botulism. *Pediatrics* 2007;**119**:826-8.

significantly longer in untreated type A cases than in untreated type B cases (see section on Treatment).[4,14] Physicians are reminded that in all states, botulism or suspected botulism (all types) is an immediately reportable illness.

At the bedside, electromyography sometimes can be helpful in ambiguous situations, in that when a clinically weak muscle is tested, electromyography often discloses a pattern known by the acronym BSAP (*b*rief, *s*mall, *a*bundant motor unit *p*otentials).* The edrophonium (Tensilon) test is unnecessary because congenital myasthenia gravis can be excluded by the history and de novo myasthenia does not occur at this age because of the immaturity of an infant's immune system. Likewise, Guillain-Barré syndrome, which is well documented by finding a consistently elevated protein concentration in CSF, is of negligible occurrence in infancy. In infant botulism the protein concentration in CSF is normal, an occasional exception being that of a specimen collected while the child is mildly dehydrated.

TREATMENT

Specific therapy for infant botulism is now available. In California, a 5-year, randomized, double-blind, placebo-controlled treatment trial demonstrated the safety and efficacy of human-derived botulinum antitoxin, known formally as human botulism immune globulin intravenous (BIG-IV).[7,14] Use of BIG-IV reduced mean hospital stay per case from approximately 5.5 weeks to approximately 2.5 weeks (p <0.001) and reduced mean hospitalization cost per case by about $100,500 (2011 dollars; p <0.001). In a 6-year, follow-on, open-label study, treatment with BIG-IV within 7 days of hospital admission reduced mean hospital stay to 2.2 weeks. Treatment with BIG-IV should be started as early in the illness as possible to maximally neutralize the toxemia and should not be delayed for laboratory confirmation of the clinical diagnosis. BIG-IV may be obtained from the California Department of Public Health as a public service orphan drug.†

Successful management of infant botulism also depends on meticulous supportive care and the anticipation and avoidance of potentially fatal complications (see Table 146-3). Feeding and breathing generally require the most attention. At admission, patients should receive cardiac, respiratory, and transcutaneous blood gas monitoring (especially carbon dioxide pressure) until it is clear that the paralysis no longer is progressing. An endotracheal tube often is necessary to maintain and protect the airway, even in the absence of a need for mechanical ventilation. Particular care should be taken to avoid transmission of nosocomially acquired *C. difficile* colitis.[38,92]

A third cornerstone of management is forbearance. Antibiotics should be reserved to treat the principal secondary infections (pneumonia, urinary tract infection, otitis media) because their use may result in lysis of intraintestinal *C. botulinum* with liberation of intracellular neurotoxin into the gut lumen and absorption. This potential problem may be avoided by prompt use of BIG-IV because its long (28-day) half-life and substantial antitoxin content enable it to neutralize all absorbed and circulating toxin.

Tracheostomy is not necessary.[1] Improved management of the airway can be accomplished by two simple positioning measures. First, for expansion of the thoracic cage and assistance in diaphragmatic function, patients should be placed in an older-style crib, the rigid bottom mattress of which can be lifted to elevate the entire body to a 30-degree angle. Second, to tip the head back and to maintain normal curvature of neck and airway, a soft cloth should be rolled to the thickness of about three fingers and placed under just the child's neck. This maneuver allows oral secretions to drain away from the trachea and into the true posterior pharynx, where they are swallowed most easily.

Intravenous feeding (hyperalimentation) is discouraged because of its potential for secondary infection and because of the success obtained with nasogastric or nasojejunal tube feeding. Mother's milk is the nutritional fluid of choice. Isolation measures or "enteric precautions" are not required, but meticulous hand washing is. Soiled diapers should be autoclaved because they can contain botulinum neurotoxin as well as viable spores and vegetative cells of *C. botulinum*. For this reason, staff with open lesions on their hands should not handle the diapers.

In the untreated (placebo) group in the 5-year, randomized clinical trial of BIG-IV, when compared with the untreated type B patients the untreated type A patients had significantly longer mean hospital stays (6.7 vs. 4.2 weeks), mean stays in the intensive care unit (6.5 vs. 3.1 weeks), and mean time on a ventilator (6.4 vs. 2.2 weeks).[14] However, the distributions of the untreated type A and type B patients partially overlapped for all three parameters. Hence, untreated illness caused by type A toxin appears to be generally, but not invariably, more severe than that caused by type B toxin. With use of BIG-IV, the mean duration of hospital stay has been reduced to approximately 2.2 weeks for both type A and type B patients, with comparable decreases also in the duration of stay in the intensive care unit and time on a ventilator.[14]

OUTCOME AND PROGNOSIS

Recovery from infant botulism occurs through regeneration of the poisoned terminal unmyelinated nerve endings. The newly synthesized nerve twigs then induce the formation of new motor end plates that are indistinguishable functionally and morphologically from the original ones.[34,35] In experimental animals and in human infants, completion of this process takes several weeks.[35] Consequently, in the absence of hypoxic cerebral complications, full and complete recovery of strength and tone is the expected outcome of infant botulism. In addition, because botulinum toxin does not cross the blood-brain barrier to any functional degree, the child's intelligence and personality remain intact. Parents often need

*References 10, 33, 36, 45, 88, 94, 98.
†24-hour telephone: 510-231-7600; website: www.infantbotulism.org.

reassurance on this latter point. Reinfection with the same or a different toxin type of *C. botulinum* has not occurred. In the United States, the case-fatality ratio of hospitalized patients is less than 1 percent, a reflection of, and tribute to, the high quality of intensive care given to these critically ill infants. In other countries, the experience has not been as fortunate.[58]

PREVENTION

At present, the one known way to prevent infant botulism is not to feed honey to infants, and all major pediatric and public health agencies have endorsed this recommendation. Breast-feeding may help moderate the rapidity of onset and the severity of illness. Persuasive evidence that links infant botulism to the ingestion of corn or other syrup is lacking. In the pre–BIG-IV era, the patient with the most protracted illness was hospitalized for 10 months in 1988 at a cost of more than $1 million (2011 dollars). Mean hospital cost for the placebo-treated patients in the 1992 to 1997 randomized clinical trial of BIG-IV was $163,400, which was reduced in the BIG-IV group to $74,800, a net cost-savings of $88,600 (2004 dollars).[14] These economic facts combine with humanitarian considerations to make a compelling case for the prevention and effective treatment of infant botulism.

SUGGESTED READINGS

Peck MW. Biology and genomic analysis of *Clostridium botulinum*. *Adv Microb Physiol* 2009;**55**:183–265, 320.

Smith LD, Sugiyama H. *Botulism: the organism, its toxins, the disease*. 2nd ed. Springfield, Ill.: Charles C Thomas; 1988.

NEW REFERENCES SINCE THE SIXTH EDITION

19. Barash JR, Hsia JK, Arnon SS. Presence of soil-dwelling clostridia in commercial powdered infant formulas. *J Pediatr* 2010;**156**: 402–8.

22. Bianco MI, Lúquez C, de Jong LI et al. Presence of *Clostridium botulinum* spores in *Matricaria chamomilla* (chamomile) and its relationship with infant botulism. *Int J Food Microbiol* 2008;**121**: 357–60.

67. May ML, Corkeron MA, Stretton M. Infant botulism in Australia: availability of human botulinum antitoxin for treatment. *Med J Aust* 2010;**193**:614–15.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 147

TETANUS

James D. Cherry • Rick E. Harrison

Tetanus is caused by the anaerobic, spore-forming bacillus *Clostridium tetani*, an organism present in soil and in human and animal feces. The clinical symptoms are not caused by infection but result from a specific toxin, tetanospasmin, that is produced at the site of injury and acts primarily on the spinal cord but also on the brain, motor end-plates, and autonomic nerves. The disease may appear as a local form or as a more generalized syndrome. It is characterized by tonic spasms of skeletal muscles, little or no fever, and occasional spasms of the glottis and larynx. Bilateral trismus is the most common sign. Active immunization is highly protective. Although tetanus is controlled well today in developed countries throughout the world, it remains a major cause of death in developing countries.[26,28,112,122]

HISTORY

The first description of tetanus in recorded medical history probably was written by Hippocrates.[52] A vivid picture of the clinical course of the disease was recorded by Aretaeus the Cappadocian in the second century,[5] but very little of importance was added to this knowledge during the next 18 centuries. Transmissibility of tetanus was demonstrated by Carle and Rattone[22] in 1884. They reported that when the sciatic nerves of rabbits were injected with the contents of a human "pustule," the characteristic disease developed in the animals in 2 to 3 days; inoculation of tissue obtained from their nervous systems into healthy rabbits produced a similar syndrome. The role of soil in the pathogenesis of tetanus was demonstrated by Nicolaier[78] in 1884; although he saw the organism, he was unable to recover it.

The bacterium responsible for the disease was identified first by Rosenbach,[94] who described a bacillus containing a round terminal spore in pus obtained from a human case; tetanus developed when the purulent exudate was injected into animals. *C. tetani* was isolated by Kitasato,[59] who fulfilled Koch's postulates with it in 1889. One year later, von Behring and Kitasato[114] reported the appearance of specific antitoxin in the serum of animals

REFERENCES

1. Anderson TD, Shah UK, Schreiner MS, et al. Airway complications of infant botulism: ten-year experience with 60 cases. *Otolaryngol Head Neck Surg* 2002;**126**:234–9.
2. Armada M, Love S, Barrett E, et al. Foodborne botulism in a six-month-old infant caused by home-canned baby food. *Ann Emerg Med* 2003;**42**:226–9.
3. Arndt JW, Jacobson MJ, Abola EE, et al. A structural perspective of the sequence variability within botulinum neurotoxin subtypes A1-A4. *J Mol Biol* 2006;**362**:733–42.
4. Arnon SS. Infant botulism. *Annu Rev Med* 1980;**31**:541–60.
5. Arnon SS. Breast feeding and toxigenic intestinal infections: missing links in crib death? *Rev Infect Dis* 1984;**6**(Suppl. 1): 193–201.
6. Arnon SS. Botulism as an intestinal toxemia. In: Smith MJ, Ravdin PD, editors. *Infections of the gastrointestinal tract*. New York: Raven Press; 1995. p. 257–71.
7. Arnon SS. Creation and development of the public service orphan drug human botulism immune globulin. *Pediatrics* 2007;**119**: 785–9.
8. Arnon SS, Damus K, Chin J. Infant botulism: epidemiology and relation to sudden infant death syndrome. *Epidemiol Rev* 1981; **3**:45–66.
9. Arnon SS, Damus K, Thompson B, et al. Protective role of human milk against sudden death from infant botulism. *J Pediatr* 1982; **100**:568–73.
10. Arnon SS, Midura TF, Clay SA, et al. Infant botulism: epidemiological, clinical, and laboratory aspects. *JAMA* 1977;**237**: 1946–51.
11. Arnon SS, Midura TF, Damus K, et al. Intestinal infection and toxin production by *Clostridium botulinum* as one cause of sudden infant death syndrome. *Lancet* 1978;**1**:1273–7.
12. Arnon SS, Midura TF, Damus K, et al. Honey and other environmental risk factors for infant botulism. *J Pediatr* 1979;**94**:331–6.
13. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA* 2001;**285**:1059–70.
14. Arnon SS, Schechter R, Maslanka SE, et al. Human botulism immune globulin for the treatment of infant botulism. *N Engl J Med* 2006;**354**:462–71.
15. Arnon SS, Werner SB, Faber HK, et al. Infant botulism in 1931: discovery of a misclassified case. *Am J Dis Child* 1979;**133**: 580–2.
16. Aureli P, Fenicia L, Pasolini B, et al. Two cases of type E infant botulism caused by neurotoxigenic *Clostridium butyricum* in Italy. *J Infect Dis* 1986;**154**:207–11.
17. Aureli P, Franciosa G, Fenicia L. Infant botulism and honey in Europe: a commentary. *Pediatr Infect Dis J* 2002;**21**:866–8.
18. Barash JR, Arnon SS. Dual toxin-producing strain of *Clostridium botulinum* type Bf isolated from a California patient with infant botulism. *J Clin Microbiol* 2004;**42**:1713–15.
19. Barash JR, Hsia JK, Arnon SS. Presence of soil-dwelling clostridia in commercial powdered infant formulas. *J Pediatr* 2010;**156**: 402–8.
20. Barash JR, Tang TW, Arnon SS. First case of infant botulism caused by *Clostridium baratii* type F in California. *J Clin Microbiol* 2005;**43**:4280–2.
21. Berkes A, Szegedi I, Szikszay E, et al. [Botulism in infancy: survey of literature based on a case report.] *Orv Hetil* 2007;**148**:1117–25. In Hungarian.
22. Bianco MI, Lúquez C, de Jong LI, et al. Presence of *Clostridium botulinum* spores in *Matricaria chamomilla* (chamomile) and its relationship with infant botulism. *Int J Food Microbiol* 2008;**121**: 357–60.
23. Berry PR, Gilbert RJ, Oliver RW, et al. Some preliminary studies on low incidence of infant botulism in the United Kingdom. *J Clin Pathol* 1987;**40**:121.
24. Brett MM, McLauchlin J, Harris A, et al. A case of infant botulism with a possible link to infant formula milk powder: evidence for the presence of more than one strain of *Clostridium botulinum* in clinical specimens and food. *J Med Microbiol* 2005;**54**:769–76.
25. Burr DH, Sugiyama H. Susceptibility to enteric botulinum colonization of antibiotic-treated adult mice. *Infect Immun* 1982; **36**:103–6.
26. Byard RW, Moore L, Bourne AJ, et al. *Clostridium botulinum* and sudden infant death syndrome: a 10-year prospective study. *J Paediatr Child Health* 1992;**28**:156–7.
27. Centers for Disease Control and Prevention. Sudden infant death syndrome—United States, 1983-1994. *MMWR Morb Mortal Wkly Rep* 1996;**45**(40):859–63.
28. Centers for Disease Control and Prevention. Case definitions for infectious conditions under public health surveillance. *MMWR Recomm Rep* 1997;**46**(RR-10):1–55.
29. Centers for Disease Control and Prevention. *Botulism in the United States, 1899-1996: handbook for epidemiologists, clinicians, and laboratory workers*. Available at http://www.cdc.gov/ncidod/dbmd/diseaseinfo/files/botulism.pdf. Accessed September 1, 2012.
30. Chai Q, Arndt JW, Dong M, et al. Structural basis of cell surface receptor recognition by botulinum neurotoxin B. *Nature* 2006;**444**:1096–100.
31. Chia JK, Clark JB, Ryan CA, et al. Botulism in an adult associated with food-borne intestinal infection with *Clostridium botulinum*. *N Engl J Med* 1986;**315**:239–41.
32. Clay SA, Ramseyer JC, Fishman LS, et al. Acute infantile motor unit disorder: infantile botulism? Arch. *Neurol* 1977;**34**:236–43.
33. Cornblath DR, Sladky JT, Sumner AJ. Clinical electrophysiology of infantile botulism. *Muscle Nerve* 1983;**6**:448–52.
34. de Paiva A, Meunier FA, Molgo J, et al. Functional repair of motor endplates after botulinum neurotoxin type A poisoning: biphasic switch of synaptic activity between nerve sprouts and their parent terminals. *Proc Natl Acad Sci U S A* 1999;**96**:3200–5.
35. Duchen LW. Motor nerve growth induced by botulinum toxin as a regenerative phenomenon. *Proc R Soc Med* 1972;**65**:196–7.
36. Engel WK. Brief, small, abundant motor-unit action potentials: a further critique of electromyographic interpretation. *Neurology* 1975;**25**:173–6.
37. Fenicia L, Anniballi F, Aureli P. Intestinal toxemia botulism in Italy, 1984-2005. *Eur J Clin Microbiol Infect Dis* 2007;**26**:385–94.
38. Fenicia L, Da Dalt L, Anniballi F, et al. A case of infant botulism due to neurotoxigenic *Clostridium butyricum* type E associated with *Clostridium difficile* colitis. *Eur J Clin Microbiol Infect Dis* 2002; **21**:736–8.
39. Fenicia L, Franciosa G, Pourshaban M, et al. Intestinal toxemia botulism in two young people, caused by *Clostridium butyricum* type E. *Clin Infect Dis* 1999;**29**:1381–7.
40. Francisco AM, Arnon SS. Clinical mimics of infant botulism. *Pediatrics* 2007;**119**:826–8.
41. Freedman M, Armstrong RM, Killian JM, et al. Botulism in a patient with jejunoileal bypass. *Ann Neurol* 1986;**20**:641–3.
42. Gill DM. Bacterial toxins: a table of lethal amounts. *Microbiol Rev* 1982;**46**:86–94.
43. Glauser TA, Maguire HC, Sladky JT. Relapse of infant botulism. *Ann Neurol* 1990;**28**:187–9.
44. Goldman A, Goldblum R. Immunologic system in human milk: characteristics and effects. In Lebenthal, E., editor. *Textbook of gastroenterology and nutrition in infancy*. 2nd ed. New York: Raven Press; 1989. p. 135–42.
45. Graf WD, Hays RM, Astley SJ, et al. Electrodiagnosis reliability in the diagnosis of infant botulism. *J Pediatr* 1992;**120**:747–9.
46. Grover WD, Peckham GJ, Berman PH. Recovery following cranial nerve dysfunction and muscle weakness in infancy. *Dev Med Child Neurol* 1974;**16**:163–71.
47. Hall J, McCroskey LM, Pincomb BJ, et al. Isolation of an organism resembling *Clostridium barati* which produces type F botulinal toxin from an infant with botulism. *J Clin Microbiol* 1985;**21**: 654–5.
48. Hatheway CL, McCroskey LM. Examination of feces and serum for diagnosis of infant botulism in 336 patients. *J Clin Microbiol* 1987;**25**:2334–8.
49. Hauschild A, Hilsheimer R, Weiss K. *Clostridium botulinum* in honey, syrups and dry infant cereals. *J Food Prot* 1988;**51**:892–4.
50. Hill KK, Smith TJ, Helma CH, et al. Genetic diversity among botulinum neurotoxin–producing clostridial strains. *J Bacteriol* 2007;**189**:818–32.
51. Hoffman RE, Pincomb BJ, Skeels MR. Type F infant botulism. *Am J Dis Child* 1982;**136**:270–1.
52. Huhtanen C, Knox D, Shimanuki H. Incidence and origin of *Clostridium botulinum* spores in honey. *J Food Prot* 1981;**44**: 812–15.

53. Istre GR, Compton R, Novotny T, et al. Infant botulism: three cases in a small town. *Am J Dis Child* 1986;**140**:1013–14.
54. Jin R, Rummel A, Binz T, et al. Botulinum neurotoxin B recognizes its protein receptor with high affinity and specificity. *Nature* 2006;**444**:1092–5.
55. Johnson E, Tepp W, Bradshaw M, et al. Characterization of *Clostridium botulinum* strains associated with an infant botulism case in the United Kingdom. *J Clin Microbiol* 2005;**43**:2602–7.
56. Johnson RO, Clay SA, Arnon SS. Diagnosis and management of infant botulism. *Am J Dis Child* 1979;**133**:586–93.
57. Kautter DA, Lilly T, Solomon HM, et al. *Clostridium botulinum* spores in infant foods: a survey. *J Food Prot* 1982;**45**:1028–9.
58. Koepke R, Sobel J, Arnon SS. Global occurrence of infant botulism. *Pediatrics* 2008;**122**:e73–e82.
59. Kozaki S, Kamata Y, Nishiki T, et al. Characterization of *Clostridium botulinum* type B neurotoxin associated with infant botulism in Japan. *Infect Immun* 1998;**66**:4811–16.
60. Lilly T Jr, Rhodehamel EJ, Kautter DA, et al. *Clostridium botulinum* spores in corn syrup and other syrups. *J Food Prot* 1991; **54**:585–7.
61. Lindström M, Korkeala H. Laboratory diagnostics of botulism. *Clin Microbiol Rev* 2006;**19**(2):298–314.
62. Long SS. Epidemiologic study of infant botulism in Pennsylvania: report of the Infant Botulism Study Group. *Pediatrics* 1985;**75**: 928–34.
63. Long SS. Infant botulism and treatment with BIG-IV (BabyBIG). *Pediatr Infect Dis J* 2007;**26**:261–2.
64. Long SS, Gajewski JL, Brown LW, et al. Clinical, laboratory, and environmental features of infant botulism in southeastern Pennsylvania. *Pediatrics* 1985;**75**:935–41.
65. Lúquez C, Bianco M, Sagua M, et al. Relationship between the incidence of infant botulism and the presence of botulinum toxin-producing clostridia in the soil of Argentina from 1982-2005. *J Clin Neurol* 2007;**5**:1–8.
66. Lúquez C, Dykes JK, Yu PA, et al. First report worldwide of an infant botulism case due to *Clostridium botulinum* type E. *J Clin Microbiol* 2010;**48**:326–8. Erratum in: J Clin Microbiol 2010;48: 1017.
67. May ML, Corkeron MA, Stretton M. Infant botulism in Australia: availability of human botulinum antitoxin for treatment. *Med J Aust* 2010;**193**:614–15.
68. Midura TF. Laboratory aspects of infant botulism in California. *Rev Infect Dis* 1979;**1**:652–4.
69. Midura TF, Arnon SS. Infant botulism: identification of *Clostridium botulinum* and its toxins in faeces. *Lancet* 1976;**2**:934–6.
70. Midura TF, Snowden S, Wood RM, et al. Isolation of *Clostridium botulinum* from honey. *J Clin Microbiol* 1979;**9**:282–3.
71. Mills DC, Arnon SS. The large intestine as the site of *Clostridium botulinum* colonization in human infant botulism. *J Infect Dis* 1987;**156**:997–8.
72. Mitchell WG, Tseng-Ong L. Catastrophic presentation of infant botulism may obscure or delay diagnosis. *Pediatrics* 2005;**116**: e436–8.
73. Moberg LJ, Sugiyama H. Microbial ecological basis of infant botulism as studied with germfree mice. *Infect Immun* 1979;**25**: 653–7.
74. Morris JG Jr, Snyder JD, Wilson R, et al. Infant botulism in the United States: an epidemiologic study of cases occurring outside of California. *Am J Public Health* 1983;**73**:1385–8.
75. Murrell WG, Stewart BJ. Botulism in New South Wales, 1980-1981. *Med J Aust* 1983;**1**:13–17.
76. Murrell WG, Stewart BJ, O'Neill C, et al. Enterotoxigenic bacteria in the sudden infant death syndrome. *J Med Microbiol* 1993;**39**:114–27.
77. Nakano H, Kizaki H, Sakaguchi G. Multiplication of *Clostridium botulinum* in dead honey-bees and bee pupae, a likely source of heavy contamination of honey. *Int J Food Microbiol* 1994;**21**: 247–52.
78. Nakano H, Okabe T, Hashimoto H, et al. Incidence of *Clostridium botulinum* in honey of various origins. *Jpn J Med Sci Biol* 1990; **43**:183–95.
79. Nakano H, Yoshikuni Y, Hashimoto H, et al. Detection of *Clostridium botulinum* in natural sweetening. *Int J Food Microbiol* 1992;**16**:117–21.
80. Nevas M, Hielm S, Lindstrom M, et al. High prevalence of *Clostridium botulinum* types A and B in honey samples detected by polymerase chain reaction. *Int J Food Microbiol* 2002;**72**:45–52.
81. Nevas M, Lindstrom M, Virtanen A, et al. Infant botulism acquired from household dust presenting as sudden infant death syndrome. *J Clin Microbiol* 2005;**43**:511–13.
82. Nishiki T, Tokuyama Y, Kamata Y, et al. The high-affinity binding of *Clostridium botulinum* type B neurotoxin to synaptotagmin II associated with gangliosides GT1b/GD1a. *FEBS Lett* 1996;**378**: 253–7.
83. Olsen SJ, Swerdlow DL. Risk of infant botulism from corn syrup. *Pediatr Infect Dis J* 2000;**19**:584–5.
84. Paisley J, Lauer BA, Arnon SS. A second case of infant botulism type F caused by *Clostridium baratii*. *Pediatr Infect Dis J* 1995;**14**:912–14.
85. Paton JC, Lawrence AJ, Steven IM. Quantities of *Clostridium botulinum* organisms and toxin in feces and presence of *Clostridium botulinum* toxin in the serum of an infant with botulism. *J Clin Microbiol* 1983;**17**:13–15.
86. Peterson DR, Eklund MW, Chinn NM. The sudden infant death syndrome and infant botulism. *Rev Infect Dis* 1979;**1**:630–6.
87. Pickett J, Berg B, Chaplin E, et al. Syndrome of botulism in infancy: clinical and electrophysiologic study. *N Engl J Med* 1976;**295**:770–2.
88. Ramseyer J, Clay S, Fishman L. Electromyographic studies in acute infantile polyneuropathy. *Neurology* 1976;**26**:364.
89. Ravid S, Maytal J, Eviatar L. Biphasic course of infant botulism. *Pediatr Neurol* 2000;**23**:338–9.
90. Sakaguchi G, Sakaguchi S, Kamata Y, et al. Distinct characteristics of *Clostridium botulinum* type A strains and their toxin associated with infant botulism in Japan. *Int J Food Microbiol* 1990;**11**: 231–41.
91. Schechter R, Arnon SS. Commentary: where Marco Polo meets Meckel: type E botulism from *Clostridium butyricum*. *Clin Infect Dis* 1999;**29**:1388–93.
92. Schechter R, Peterson B, McGee J, et al. *Clostridium difficile* colitis associated with infant botulism: near-fatal case analogous to Hirschsprung's enterocolitis. *Clin Infect Dis* 1999;**29**:367–74.
93. Schiavo G, Matteoli M, Montecucco C. Neurotoxins affecting neuroexocytosis. *Physiol Rev* 2000;**80**:717–66.
94. Schreiner MS, Field E, Ruddy R. Infant botulism: a review of 12 years' experience at the Children's Hospital of Philadelphia. *Pediatrics* 1991;**87**:159–65.
95. Schwarz PJ, Arnon JM, Arnon SS. Epidemiological aspects of infant botulism in California, 1976-1991. In: DasGupta BR, editor. *Botulinum and tetanus neurotoxins: neurotransmission and biomedical aspects*. New York: Plenum Press; 1993. p. 503–4.
96. Sebaihia M, Peck MW, Minton NP, et al. Genome sequence of a proteolytic (group I) *Clostridium botulinum* strain Hall A and comparative analysis of the clostridial genomes. *Genome Res* 2007;**17**: 1082–92.
97. Shen WP, Felsing N, Lang D, et al. Development of infant botulism in a 3-year-old female with neuroblastoma following autologous bone marrow transplantation: potential use of human botulism immune globulin. *Bone Marrow Transplant* 1994;**13**: 345–7.
98. Sheth RD, Lotz BP, Hecox KE, et al. Infantile botulism: pitfalls in electrodiagnosis. *J Child Neurol* 1999;**14**:156–8.
99. Smith GE, Hinde F, Westmoreland D, et al. Infantile botulism. *Arch Dis Child* 1989;**64**:871–2.
100. Smith LD. The occurrence of *Clostridium botulinum* and *Clostridium tetani* in the soil of the United States. *Health Lab Sci* 1978;**15**:74–80.
101. Smith LD, Sugiyama H. *Botulism: the organism, its toxins, the disease*. 2nd ed. Springfield, Ill.: Charles C Thomas; 1988.
102. Smith TJ, Hill KK, Foley BT, et al. Analysis of the neurotoxin complex genes in *Clostridium botulinum* A1-A4 and B1 strains: BoNT/A3, /Ba4 and /B1 clusters are located within plasmids. *PLoS One* 2007;**2**:e1271.
103. Smith TJ, Lou J, Geren IN, et al. Sequence variation within botulinum neurotoxin serotypes impacts antibody binding and neutralization. *Infect Immun* 2005;**73**:5450–7.
104. Spika JS, Shaffer N, Hargrett-Bean N, et al. Risk factors for infant botulism in the United States. *Am J Dis Child* 1989;**143**:828–32.

105. Stark PL, Lee A. The microbial ecology of the large bowel of breast-fed and formula-fed infants during the first year of life. *J Med Microbiol* 1982;**15**:189–203.
106. Stark PL, Lee A. Clostridia isolated from the feces of infants during the first year of life. *J Pediatr* 1982;**100**:362–5.
107. Suen JC, Hatheway CL, Steigerwalt AG, et al. Genetic confirmation of identities of neurotoxigenic *Clostridium baratii* and *Clostridium butyricum* implicated as agents of infant botulism. *J Clin Microbiol* 1988;**26**:2191–2.
108. Sugiyama H, Mills DC. Intraintestinal toxin in infant mice challenged intragastrically with *Clostridium botulinum* spores. *Infect Immun* 1978;**21**:59–63.
109. Sugiyama H, Mills DC, Kuo C. Number of *Clostridium botulinum* spores in honey. *J Food Prot* 1978;**41**:848–50.
110. Sullivan H, Mills D, Riemann H, et al. Inhibition of growth of *Clostridium botulinum* by intestinal microflora isolated from healthy infants. *Microb Ecol Health Dis* 1988;**1**:179–92.
111. Tabita K, Sakaguchi S, Kozaki S, et al. Distinction between *Clostridium botulinum* type A strains associated with food-borne botulism and those with infant botulism in Japan in intraintestinal toxin production in infant mice and some other properties. *FEMS Microbiol Lett* 1991;**79**:251–6.
112. Takahashi M, Noda H, Takeshita S, et al. Attempts to quantify *Clostridium botulinum* type A toxin and antitoxin in serum of two cases of infant botulism in Japan. *Jpn J Med Sci Biol* 1990;**43**:233–7.
113. Thompson JA, Filloux FM, Van Orman CB, et al. Infant botulism in the age of botulism immune globulin. *Neurology* 2005;**64**:2029–32.
114. Tonkin S. Sudden infant death syndrome: hypothesis of causation. *Pediatrics* 1975;**55**:650–61.
115. Toyoguchi S, Tsugu H, Nariai A, et al. Infant botulism with Down syndrome. *Acta Paediatr Jpn* 1991;**33**:394–7.
116. Trethon A, Budai J, Herendi A, et al. [Botulism in infancy.] *Orv Hetil* 1995;**136**:1497–9. In Hungarian.
117. Wilcke BW Jr, Midura TF, Arnon SS. Quantitative evidence of intestinal colonization by *Clostridium botulinum* in four cases of infant botulism. *J Infect Dis* 1980;**141**:419–23.

1809 given injections of the tetanus toxin produced by the organism. This finding was followed, in 1926, by the development of toxoid, injections of which produced immunity.

MICROBIOLOGY

The vegetative form of *C. tetani* is a gram-positive, spore-forming, motile, anaerobic bacillus that measures 0.3 to 0.5 μm in width and 2.0 to 2.5 μm in length; in culture, long filament-like cells develop.[12,21,116,119] *C. tetani* is a strict anaerobe that grows best at 33° to 37°C (91.4° to 98.6°F); it can be cultured in many different routine media used for anaerobic organisms, such as thioglycolate, casein hydrolysate, and cooked meat. Enhanced growth occurs in medium supplemented with reducing substances and maintained at neutral or alkaline pH. With growth, gas with a fetid odor usually is produced.

The first step in the process of spore formation is the development of a bulge at one end of the organism; this bulge contains the spore and is responsible for the characteristic drumstick or tennis racquet appearance. As sporulation progresses, the organism decreases in length and the spores are extruded. They stain poorly by the Gram method. Sporulation occurs in tissues as well as in vitro and is dependent on the composition of the medium and the temperature of the culture. Enhanced sporulation occurs in the presence of oleic acid, phosphates, 1 to 2 percent sodium chloride, and manganese salts.[12,21,116] In vivo, sporulation is enhanced by lactic acid and other substances toxic to cells. Sporulation is inhibited by high and low temperatures (>41° and <25°C [>105.8° and <77°F), glucose, fatty acids, and potassium salts. The metabolic activity of *C. tetani* is limited; carbohydrates and proteins are digested poorly, and the vegetative, nonsporulated forms are killed easily by heat and numerous disinfecting agents. In contrast, the spores are resistant to boiling and phenol, cresol, 1:1000 bichloride of mercury, and other disinfectants; however, they are destroyed by heating at 120°C (248°F) for 15 to 20 minutes. If not exposed to sunlight, the spores may survive in soil for months to years. They also may constitute part of the normal intestinal microflora of some horses, cows, guinea pigs, sheep, dogs, cats, rats, chickens, and humans.

Three nonpathogenic clostridia are present in soil and in human and animal feces: *Clostridium tetanomorphum*, *Clostridium tertium*, and *Clostridium tetanoides*. Diagnostic confusion may occur because they are morphologically similar to the organism responsible for tetanus. *C. tetani* is recovered much more commonly from cultivated than from virgin or uncultivated soil. Rural dwellers and people engaged in agricultural occupations have a higher rate of intestinal, skin, and oral carriage of the organism than do city dwellers. Dust and dirt from houses, streets, and operating rooms, as well as solutions of heroin used by injection drug users, have been found to be contaminated with the organism.

EPIDEMIOLOGY

Source of Exposure

The predominant reservoir of *C. tetani* is the soil; it is also part of the normal flora of the intestinal tract of animals, both herbivores and omnivores.[116] Intestinal spores and bacilli are shed in the feces of animals and contribute to the soil reservoir.

The worldwide morbidity and mortality attributable to tetanus is related inversely to adequate immunization with tetanus toxoid and directly to suboptimal hygiene, childbirth practices, and wound care.[116] Throughout the world, tetanus has a seasonal trend; more cases occur in the summer or in "wet" seasons. Rates of illness are highest in countries that are located near the equator and have fertile soil.

Acute wounds, including relatively minor ones, are the site of most *C. tetani* infections leading to tetanus. In addition, infection may occur after parenteral drug use and surgical procedures. In many cases the source of exposure is unknown. The source of infection in neonatal tetanus is the umbilical cord or stump as a result of unsterile delivery conditions and unhygienic cultural rituals involving the umbilical stump.[53,54,87]

INCIDENCE

The incidence of tetanus in the vaccine era is related inversely to the degree to which effective immunization programs have been implemented in a population.[116] In the early 1980s an estimated 2 million cases of tetanus occurred throughout the world, with 1 million fatal neonatal cases and 122,000 to 300,000 non-neonatal fatal cases.[46,47]

Although neonatal tetanus is of very minor importance in developed areas of the world, it has been and still is a major cause of death in infants in developing countries.* In 1993, an estimated 515,000 deaths worldwide were caused by neonatal tetanus.[26] These deaths occurred predominantly in Southeast Asia (34.2%); Africa (28.2%); the western Pacific, including China (21.4%); and the eastern Mediterranean region (15.7%). The global mortality rate in 1993 was estimated to be 4.1/1000 live births. The very high frequency of neonatal tetanus in these areas probably was related to conditions surrounding the birth of infants.[45] Most babies were born in very unhygienic environments; delivery rarely took place in an adequate hospital. In addition, unclean instruments were used to sever the umbilical cord, rags—often contaminated with soil or feces—were used as dressings, and mud and manure were applied directly to the umbilical stump. In a study in Senegal that examined risk factors for the development of neonatal tetanus, the major source of *C. tetani* was found to be the hands of the birth attendant, and the mode of contamination of the infant was related to the method that the birth attendant and the mother used to dress the umbilical cord stump.[62] In rural

*References 26, 27, 61, 62, 70, 104, 112, 116, 122.

Pakistan, the ritual of bundling (wrapping the baby for prolonged periods in a sheepskin cover after the application of cow dung) is a significant risk factor for the acquisition of neonatal tetanus.[10] In a case-control study of risk factors for neonatal tetanus in Karachi, Pakistan, the following risks were identified: application of substances (i.e., mustard oil, ghee, or surma) to the umbilical cord, home delivery, and illiterate mothers.[87]

In 2002, deaths worldwide caused by tetanus were estimated at 213,000, with approximately 180,000 occurring in neonates and 15,000 to 30,000 maternal cases.[122] Most maternal cases are the result of unclean delivery or abortion practices. In 2008, 59,000 neonatal deaths occurred as a result of tetanus.[61]

The reported incidence of tetanus and tetanus-related deaths in the United States from 1947 to 2007 is presented in Figure 147-1, and the reported cases and fatal cases and their incidence are presented by age group for 1998 to 2000 in Figure 147-2.[29,80] From 1947 through 1976, the incidence of tetanus fell tenfold; since 1976, the continued decrease in incidence has been less than twofold. During the same period, mortality rates fell from 91 percent in 1947 to 44 percent in 1976 to 18 percent in 1998 to 2000.

In the period 1998 to 2000, 36 percent of the cases occurred in patients 60 years or older, 55 percent in patients aged 20 to 59 years, and only 9 percent in those younger than 20 years. During this period, the vaccination status was known in only 50 of the patients (38%). Of this group, eight (16%) had received three or more doses of tetanus toxoid, with the last dose being received less than 10 years before the onset of tetanus. None of

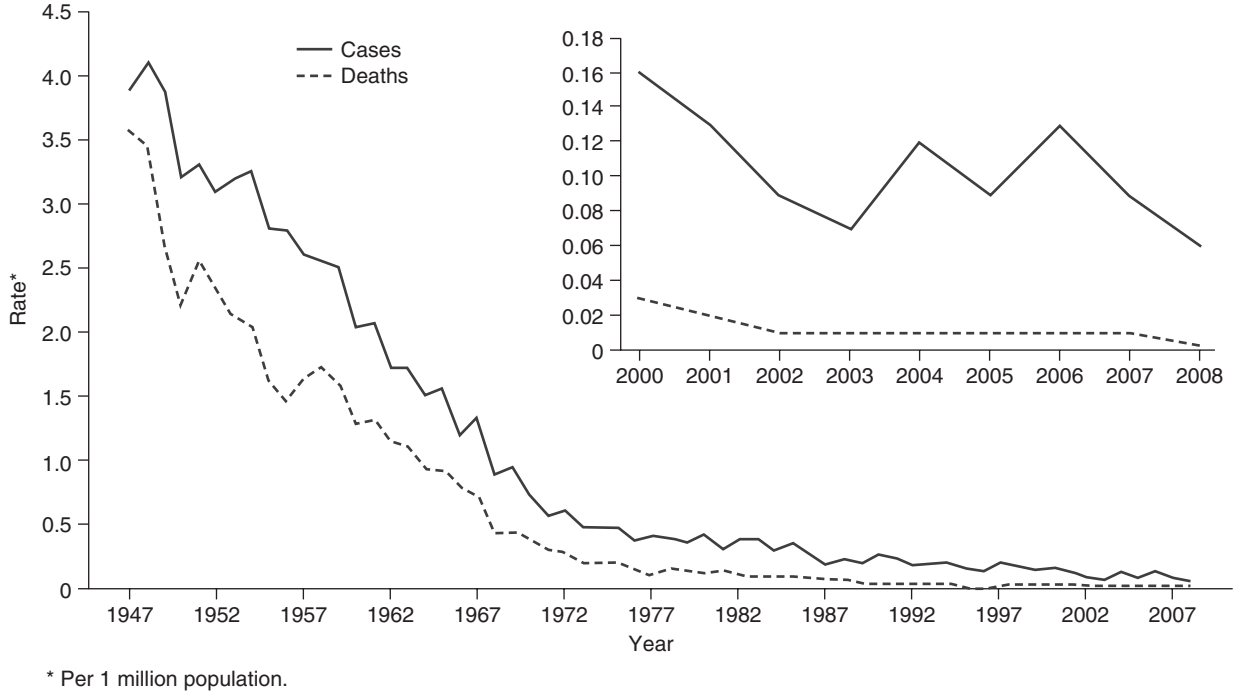

FIGURE 147-1 ■ Reported incidence of tetanus and tetanus-related deaths in the United States from 1947 to 2000. (Centers for Disease Control and Prevention. Tetanus surveillance: United States, 2001-2008. MMWR Morb Mortal Wkly Rep 2011;**60**:365-9.)

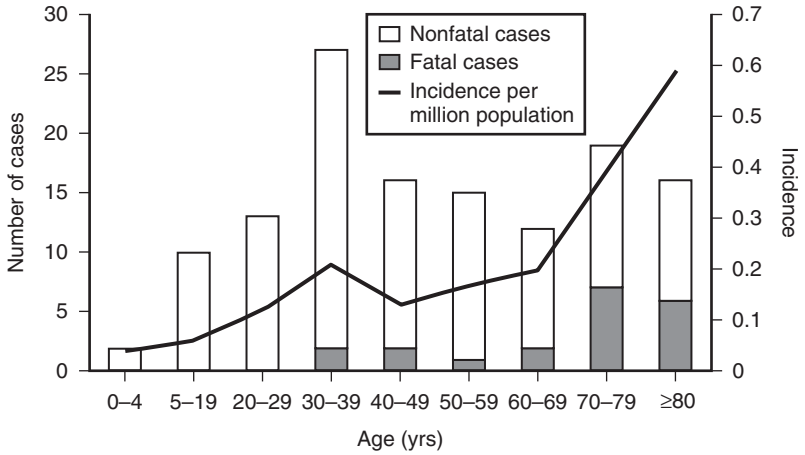

FIGURE 147-2 ■ Reported tetanus cases and incidence rates by age group in the United States in 1998 to 2000. (Pascual FB, McGinley EL, Zanardi LR, et al.: Tetanus surveillance: United States, 1998-2000. MMWR Surveill Summ 2003;**52**:1-8.)

these eight patients sought medical care for the injury before the onset of tetanus, and three were younger than 20 years. The outcome of the illness was available for only 18 of the 50 patients with a known immunization history, and one death occurred—in an injection drug user whose last dose of tetanus toxoid was 11 years before the onset of tetanus.

During the period 1998 to 2000, 60 percent of the cases occurred in males.

PATHOGENESIS

Tetanus is, by strictest definition, not a true infection. Spores introduced at a site of injury remain harmless until they are converted, after being stimulated by a variety of factors, to vegetative forms that multiply but do not produce injury to tissue or provoke an inflammatory response. The clinical syndrome is caused entirely by toxin elaborated in the area where the vegetative cells are growing. *C. tetani* produces two exotoxins: tetanolysin and tetanospasmin.[83] Tetanolysin induces hemolysis but plays no role in the disease. The activity of tetanospasmin is responsible for the clinical features of the disease. This toxin has a molecular weight of 150 kDa and is activated by proteolytic cleavage into two polypeptides, a heavy (100 kDa) and a light (50 kDa) chain that are linked by a disulfide bond.[20] One milligram contains 6,400,000 lethal doses for mice; 0.00001 mg may kill a 20-g mouse in 2 hours. With the exception of the toxin produced by *Clostridium botulinum*, tetanospasmin currently is the most potent known poison. Humans are most susceptible to this agent and require only $\frac{1}{2500}$ and $\frac{1}{350,000}$ of the dose that is fatal for cats and chickens, respectively.

C. tetani is introduced into an area of injury as the spore, the form usually present in soil and intestinal contents. Disease does not develop until the spores are converted to the toxin-elaborating bacillus, which does not follow the simple inoculation of spores. When these spores are injected with very sharp needles into animals so that tissue is not injured but merely separated, tetanus does not develop. However, the addition of a small quantity of calcium chloride to a suspension of spores before injection leads to development of the typical disease. Such development is not caused by a specific effect of the cation on conversion of the spore to the vegetative form of the organism but is related to the necrosis of tissue produced by it, which leads to a reduction in oxidation-reduction potential and oxygen tension, factors involved in vegetation of the spore; the same effects are produced by the presence of a foreign body, trauma, or a localized suppurative process.

The pathway by which toxin elaborated at the site at which *C. tetani* is multiplying reaches the central nervous system (CNS) has been a matter of controversy. Researchers first thought that tetanospasmin was absorbed chiefly by motor nerve endings and then traveled along axis cylinders to the anterior horn cells.[92] Symptoms of the disease were thought to appear only after the toxin had reached the nervous system, with the initial tetanic contractions occurring first in the injured extremity, then in the opposite limb, and finally, as the toxin diffused through the spinal cord, in all the muscles. A small amount of toxin was suggested to be absorbed into the lymph and carried to the bloodstream, from which it was taken up by motor nerve endings in various parts of the body.[72]

Abel and associates[1] postulated that toxin reached the nervous system via the arterial circulation and suggested the following series of events. Some of the toxin diffuses from the local site of injury into adjacent skeletal muscle and acts on neuromuscular organs to produce a state of maintained contraction—local tetanus. Some toxin also enters the lymphatics and bloodstream, from which it is taken up by specifically reactive cells in the spinal cord, medulla, and the motor end-organs of muscle.

Friedlander and colleagues[45] noted that tetanus toxin did not penetrate the blood-brain barrier, and therefore they questioned hematogenous transmission. This finding led researchers to focus attention on transport of tetanospasmin in nerves. Wright and coworkers[123] observed that direct injection of toxin into the vagal, facial, or hypoglossal nerves of rabbits led, within 24 hours, to the development of a syndrome characteristic of involvement of the brain stem, as indicated by the appearance of strabismus, immobility of hairs in the nasal cavity, salivation, bradycardia, and torticollis. Inoculation of the vagus nerve resulted in the successive development of these manifestations; those dependent on innervation from motor nuclei in close proximity to the nerve tended to appear early. This hypothesis is supported by observations indicating that a variety of substances, including India ink and radioactive compounds, migrate along peripheral nerves to the CNS after intraneural injection.[42] This observation, together with the demonstration by numerous investigators that toxin is present in the peripheral nerves closest to the site of inoculation, supports the concept of transport of tetanospasmin in the tissues of the nervous system.

Knowledge of the specific mechanisms involved in the absorption of tetanus toxin and its mechanisms of action on body cells is incomplete. Most of the evidence presently available indicates that some element of the peripheral nerve is involved. Whether transport occurs in the axis cylinder, the perineural space, or the lymphatics remains unsettled. Tetanus toxin is carried to the neurons of the sciatic nerve via the neurofibrillae in axis cylinders at a rate of approximately 3.35 mm/hr.[93]

PATHOPHYSIOLOGY

Tetanospasmin exerts its effects in four areas of the nervous system: (1) the motor end-plates in skeletal muscle, (2) the spinal cord, (3) the brain, and (4) the sympathetic nervous system (in some cases).*

Motor End-Plates in Skeletal Muscle

Tetanus toxin has been noted to interfere with neuromuscular transmission. Release of acetylcholine from the nerve terminals in muscle is inhibited. The presence of

*References 17-19, 23, 33, 55, 57, 71, 100, 111, 125.

tetanospasmin in the transverse and terminal sacs of the longitudinal elements of the sarcotubular system of skeletal muscles suggests that it acts by interfering with contraction coupling or with the mechanisms involved in contraction and relaxation. These phenomena probably are involved in the pathogenesis of local tetanus.

Spinal Cord

The effects of the toxin on the spinal cord are practically identical to those produced by strychnine. It does not act on reflex arcs that include only sensory and motor neurons (two-neuron or monosynaptic reflexes). However, the toxin profoundly alters the activity of the more complex polysynaptic reflexes involving interneurons, thereby leading to the inhibition of antagonists. Hyperpolarization of the membranes of neurons, a mechanism normally operating when direct inhibitory pathways are stimulated, is suppressed. The depolarization associated with excitation is not affected. Whether tetanospasmin blocks inhibitory synapses by preventing release of the inhibitory transmitter substance or by suppressing the action of this substance on the membrane of motor neurons is unknown. Selective blocking of inhibitory synapses in the CNS appears adequate to account for the primary phenomena of tetanus. Unchecked and uncoordinated excitatory impulses multiply and traverse reflex pathways to produce the characteristic tetanic spasms of muscles.

Brain

Some investigators have suggested that the action of tetanospasmin on the brain may be responsible for the typical seizures of tetanus.[17] This hypothesis is supported, in part, by the observation that cerebral gangliosides fix the toxin. The antidromic inhibition of evoked cortical activity is decreased. The effects of tetanospasmin on the brain are the same as those on the spinal cord, as well as those that occur after exposure to strychnine.

Sympathetic Nervous System

Manifestations indicating dysfunction of the sympathetic nervous system have been observed in some patients with tetanus. Signs and symptoms include profuse sweating, peripheral vasoconstriction, labile hypertension, cardiac arrhythmias, tachycardia, increased output of carbon dioxide, elevated urinary concentration of catecholamines, and hypotension late in the course of the disease.

CLINICAL MANIFESTATIONS

Although it may be as short as 1 day or as long as several months, the incubation period of tetanus usually is 3 to 21 days. A direct relationship may exist between the distance of the site of invasion by *C. tetani* from the CNS and the length of the interval between injury and the onset of disease; the greater the distance between the local area and the CNS, the longer, in general, the incubation period. Sustained or separate, repeated tonic spasms of isolated or multiple muscles are the characteristic clinical feature of the disease. Tetanus may appear in two forms: local and generalized.

Local Tetanus

The occurrence of local tetanus most likely is more common than recognized. Although local tetanus is thought to occur infrequently, it probably is so because it may be uncommon as an isolated syndrome or because by the time that diffuse involvement has occurred, the spasms in the muscles in the area of the site of entry of the organism cannot be separated from the generalized spasms. Local manifestations frequently precede development of the generalized disorder. The characteristic abnormality in local tetanus is unyielding, persistent, painful rigidity of the group of muscles that lie in close proximity to the site at which *C. tetani* was introduced. Local tetanus often is present when a dose of antitoxin has been given that is adequate to inactivate circulating toxin but insufficient to neutralize what has accumulated at the site of injury. Symptoms may persist for several weeks or months and finally disappear without leaving any residua.[73,88] Local tetanus may be the only manifestation, usually is mild, and has a fatality rate of approximately 1 percent.

Cephalic tetanus is a variant of local tetanus. It generally occurs after the introduction of *C. tetani* in the course of injuries to the scalp, eye, face, ear, or neck; in conjunction with chronic otitis media; and rarely, after tonsillectomy.[7] Insect bites on the face or head, especially if secondarily infected by pyogenic organisms, also may serve as portals of entry for the organism. The incubation period of this syndrome is short, frequently no more than 1 to 2 days. The principal distinctive clinical features are palsies of cranial nerves III, IV, VII, IX, X, and XII; they may be involved singly or in any combination. Dysfunction may persist for days or many months. In general, the prognosis for survival is poor. However, if death does not intervene, complete recovery without residual neurologic dysfunction is the rule. In some, but not all, instances, generalized tetanus may develop during the course of the cephalic form of the disease.

Generalized Tetanus

Generalized tetanus is the most common manifestation of the disease.[90,117] Despite the general impression that inoculation of the organism is associated most frequently with deep penetrating injury, the portal of entry in approximately 80 percent of cases is an insignificant wound.[85] Burns, injuries induced by blank cartridges, deep punctures, furunculosis, dental extraction, embedded splinters, decubitus ulcers, hypodermic injections, and compound fractures complicated by chronic active osteomyelitis are typical situations in which tetanus may develop because the environment in the tissues is optimal (decrease in oxidation-reduction potential and tension of oxygen) for conversion of spores to the toxin-producing vegetative organisms. Iatrogenic disease has occurred after the use of smallpox vaccine and surgical sutures contaminated with the spores of *C. tetani*. It also has occurred as a postoperative complication in patients

exposed to the organisms present in the dust in operating rooms.[86,89,99] Very minor injuries, such as penetration by "clean" sewing needles and bites by chiggers, bees, and scorpions, have been recorded as portals of entry. Tetanus also has occurred after induced abortions, usually performed under poor asepsis.

The initial manifestation in more than 50 percent of cases of generalized tetanus is trismus; it may be unilateral early in the disease but becomes bilateral within a short time. In some cases, it may be absent during the entire course of the illness or appear only after other abnormalities have become evident. In some instances, the only symptoms and signs may be irritability, restlessness, stiffness of the neck, difficulty swallowing, and rigidity of the abdominal or thoracic muscles; these symptoms may be present singly or in various combinations. The diagnostic importance of trismus cannot be overemphasized, despite the fact that it may be associated with numerous disorders unrelated to tetanus. Among these disorders are postmeasles encephalitis, trichinosis, suppurative and other types of parotitis, tender cervical lymphadenopathy, infected impacted upper molar teeth, and exposure to phenothiazine drugs.

As the activity of tetanospasmin persists, groups of muscles other than the masseters may become involved, including tonic spasms of the jaw, neck, back, and abdomen. Unrelenting trismus leads to the development of a characteristic facial expression, the sardonic smile (risus sardonicus). The abdominal and spinal muscles may become rigid. Intense and sustained contraction of the muscles of the chest and back results in persistent opisthotonos; in young children, this condition may be so severe that the youngster lies on one side with the soles of the feet resting on top of the head.

Generalized seizures, or tetanospasms, are unique in their appearance and peculiar to this disease. Characteristically, a sudden burst of tonic contractions of all groups of muscles occurs and leads to the development of opisthotonos, flexion and abduction of the arms, clenching of the fists on the chest, and extension of the legs. Pain in the spastic muscles usually is severe. Glottal and laryngeal spasm with the chest in the position of full inspiration may develop in some patients. During such an episode, the face becomes florid, the neck veins are distended markedly, and cyanosis develops. The features of this syndrome are consistent with an intense and sustained Valsalva maneuver; unless the tonic contraction of the glottis and larynx subsides or a tracheostomy is performed, death results. Dysphagia and hydrophobia may develop as isolated phenomena in the absence of a generalized tetanospasm. Dysuria or urinary retention also may occur.

Autonomic nervous system dysfunction commonly occurs in tetanus.[64,106,115] Manifestations include labile or persistent hypertension or hypotension, persistent sinus tachycardia, tachyarrhythmia or bradycardia alternating with tachycardia, and profuse sweating.

Electroencephalographic studies of patients have indicated involvement of the brain. Of 106 patients studied by Luisto,[66] 76 percent were found to have abnormal electroencephalograms. When patients of the same age and sex who had not had tetanus were compared with 40 individuals who had contracted the disease 7 years earlier, the latter were found to have more muscle fatigue and cramps; difficulty in speech, balance, and memory; and more peripheral paresis, muscular atrophy, decreased or absent tendon reflexes, and impaired mental capacity.[58]

The generalized seizures of tetanus often are triggered by very slight external stimuli, such as a light breeze, talking, a bump on the bed, or slight touching of the patient. The intense work generated by sustained or frequently repeated tetanospasms often leads to increases in body temperature of 1° to 3° C (33.8° to 37.4° F) or more.

The intense suffering of patients with the generalized tonic seizures of tetanus and the total frustration of the physician, who in ancient times had to stand by helplessly, unable to alter the course of the disease, were described best by Aretaeus[5] in the second century BC; no better description has ever appeared:

> *An inhuman calamity! An unseemly sight! A spectacle painful even to the beholder, an incurable malady! Owing to the distortion, not to be recognized by the dearest friends; and hence the prayers of the spectators, which formerly would have been reckoned not pious, now becomes good, that the patient may depart from life, or being a deliverance from the pains and unseemly evils attendant on it. But neither can the physician, though present and looking on, furnish any assistance, as regards life, relief from pain or from deformity. For if he should wish to straighten the limbs, he can do so only by cutting and breaking those of a living man. With them, then, who are overpowered by this disease, he can merely sympathize. This is the great misfortune of the physician.*

Injection Drug Users

When generalized tetanus develops in injection drug users, many of the clinical features differ from those present in people who do not use drugs.[63] Among such features are much higher levels of fever; absence of trismus at onset and throughout the course of the disease or its appearance at some time after other manifestations have been present; marked stiffness of the neck and back, which in the absence of trismus may be confused with meningitis (the normal cerebrospinal fluid in patients with tetanus excludes this possibility); and early onset of coma. Prophylaxis is less effective in injection drug users than in non–injection drug users. The fatality rate approaches 100 percent.

Neonatal Tetanus

Neonatal tetanus usually begins 3 to 14 days after birth and is characterized by poor sucking and excessive crying.[45,117] Manifestations include trismus, difficulty swallowing, other tetanic spasms, and, frequently, marked opisthotonos.

A study of the causes of death in patients with neonatal tetanus by Salimpour[95] indicated pulmonary disease to be most common. Bronchopneumonia or hemorrhage in the lungs (or both) was the most frequent finding at autopsy. Among the nonpulmonary disorders responsible for a fatal outcome were hepatitis, omphalitis, cerebral

hemorrhage, thrombosis, and rupture of the renal vein. As has been noted not only in infants but also in older children and adults with tetanus, any gross or histologic abnormalities are very uncommon findings. The complications described by Salimpour,[95] especially those involving the lungs, probably are related to aspiration associated with the laryngoglottal spasm characteristic of the disease. Newborns who survived tetanus in rural Kenya often had evidence of brain damage.[8] Indicators of a poor prognosis for neonates include age younger than 10 days when admitted to the hospital, symptoms of less than 5 days' duration, and the presence of risus sardonicus and fever.[68]

DIAGNOSIS

Differential Diagnosis

The differential diagnosis depends on the major clinical manifestations of the illness.[116] Cephalic tetanus may be confused with Bell palsy, trigeminal neuritis, and encephalitis, whereas generalized tetanus can be confused with rabies, strychnine poisoning, and phenothiazine reactions. An 11-year-old boy with neuromyotonia (Isaac syndrome) was diagnosed as having tetanus.[79] Trismus can result from dental problems, tonsillitis, peritonsillar abscess, temporomandibular joint dysfunction, and parotitis. Tetany caused by hypocalcemia or hyperventilation should be considered as well.

Specific Diagnosis

The diagnosis of tetanus is established on the basis of the history of an injury, particularly one in which either soil or fecal material has been introduced. In a small number of cases, especially those in which a relatively long incubation period has occurred, the site of entry of the organism may be healed and undetectable. In a review of 130 cases that occurred in the United States between 1998 and 2000, 94 (72%) were related to an acute injury[79]—47 puncture wounds, 31 lacerations, and 8 abrasions. In 34 cases not involving an acute injury, the following associations were noted: abscess, 7 cases; ulcer, 7 cases; gangrene, 2 cases; cellulitis, 1 case; gingivitis, 3 cases; and other infections, 2 cases. In 12 cases, no injury or other source location was identified. One of the 130 patients was a neonate, and 18 of the non–acute injury cases occurred in patients with a history of injection drug use. Laboratory studies are of little help. Peripheral leukocytosis may or may not be present. The characteristic gram-positive rods, some of which may contain subterminal spores, may be seen occasionally in stained material obtained from the wound that has served as the portal of entry. Anaerobic cultures of exudate or necrotic tissue may grow typical sporulated rods and vegetative forms, but the yield of positive findings tends to be low.[29]

Low or undetectable levels of serum antitoxin at the time of onset of the illness is supportive of the diagnosis, but, occasionally, serum antitoxin is present.[115] Rises in antitoxin titer occur uncommonly after infection, so the use of paired sera for retrospective diagnosis generally is not helpful; in addition, this form of serologic diagnosis is not possible when therapy included active immunization. In selected cases, electrophysiologic studies of the masseter muscle may be helpful.[9] A characteristic absence or shortening of the silent period occurs as a result of failure of Renshaw cell inhibition; exaggerated F responses indicating hyperexcitability also may be noted.

TREATMENT

Management of generalized tetanus is directed at the following goals:

1. Neutralization of toxin still present in the blood before it comes in contact with the nervous system. Neutralization is accomplished by the administration of antitoxin as soon as the possibility of the disease is suspected or confirmed.[4,12,84] However, no acceptable evidence has shown that toxin can be inactivated by antitoxin once the toxin is fixed to tissues. In fact, the effectiveness of even large doses of antitoxin in reducing the fatality rate has been questioned.
2. Surgical removal of the site of entry of the organism, when possible, to eliminate the "factory" in which tetanospasmin is being produced. Omphalectomy has been performed with good results in children with neonatal tetanus.[34] Hysterectomy has been recommended when the disease has complicated induced and often septic abortions; however, patients in whom the infected uterus is not removed occasionally may survive. When the surgical procedure may be mutilating, as is the case with lesions on the face, it should not be performed.
3. Constant and meticulous nursing care.
4. Close monitoring of fluid, electrolyte, and caloric balance because they frequently are abnormal, especially in patients with high temperature and repeated seizures, as well as in those unable to take food or liquids because of severe trismus, dysphagia, or hydrophobia.

The slightest external stimulus may precipitate potentially lethal seizures in persons with diffuse disease. For this reason, all therapeutic and other manipulations should be coordinated and carefully scheduled so that the risk for producing a tetanospasm is reduced to a minimum. All maneuvers are performed best after patients have received optimal sedation and relaxation. A quiet, darkened room in which the light is subdued and the doors padded, removed as far as possible from the mainstream of hospital traffic, is ideal, but at the same time the patient requires constant monitoring and observation in an intensive care environment.

The antitoxin of choice for the treatment of tetanus is human tetanus immune globulin (TIG) at a dose of 3000 to 6000 U intramuscularly; a second dose does not appear to be necessary. When TIG is not available, equine antitoxin (EA) may be used, but it should be avoided if at all possible. The dose of EA is 100,000 U; half is injected intramuscularly after appropriate tests to rule out sensitivity to horse serum have been performed. If EA is well tolerated, the remainder is given slowly intravenously. Patients sensitized to horse serum must be desensitized.

An intramuscular injection of 80,000 units of EA produces maximal concentrations of antibody in the blood in 48 to 72 hours; very good levels may be maintained for 7 days.[48,103,109,110] Intravenous administration of the same dose yields concentrations of 40 U or more of antitoxin per milliliter of serum after 6 hours; it may persist for approximately 48 hours. Essentially no difference in circulating quantities of antitoxin exists 7 days after it is given by either route. Local instillation of antitoxin around the known or suspected wound may be of value if excision cannot be performed; it also has been recommended before surgical removal of the injured area.

Intravenous immune globulin (IVIG) contains antibodies to tetanus toxin.[4] The Committee of Infectious Diseases of the American Academy of Pediatrics suggests using IVIG in a dose of 200 to 400 mg/kg if TIG is not available.

Although reports[77] have documented that intrathecal administration of antitetanus serum did not improve the rate of survival in neonatal tetanus, the results of a study by Mongi and colleagues[75] led them to recommend this form of therapy. They found that intrathecal serotherapy may be of great value in the management of tetanus. Their experience indicated that the death rate from this disease in patients given intrathecal therapy was 45 percent; in those who did not receive this agent, it was 82 percent. However, the results of a recent meta-analysis led Abrutyn and Berlin[2] to conclude that intrathecal therapy with either EA or TIG is not of proved benefit and, therefore, should be given only during well-designed, controlled therapeutic trials.

In a controlled trial in Brazil involving 120 adolescents and adults, 58 patients received TIG intrathecally and intramuscularly and 62 received TIG only by the intramuscular route.[74] The patients who received intrathecal and intramuscular treatment had better clinical progression than did those who were treated by only the intramuscular route. Most recently, a meta-analysis that involved 942 patients in 12 trials was reported.[56] In this analysis, the overall relative risk for mortality with intrathecal (antitetanus serum or TIG) versus intramuscular therapy was 0.71 (95% confidence interval, 0.62 to 0.81). Subcategory analyses for both adults and neonates and for high- and low-dose intrathecal therapy also indicated the beneficial effect of intrathecal therapy.

Penicillin kills the vegetative forms of *C. tetani*. Parenteral administration of penicillin G, 100,000 U/kg/day intravenously every 6 hours for 10 days, has been recommended in the past in all cases of tetanus. Observations have suggested that penicillin may act as an agonist to tetanospasmin by inhibiting the release of γ-aminobutyric acid (GABA).[116] Because of this possibility and the results of a controlled study in Indonesia, metronidazole has become the antimicrobial treatment of choice.[3,4] The metronidazole dosage is 30 mg/kg/day intravenously every 6 hours after an initial dose of 15 mg/kg. The usual duration of therapy is 10 to 14 days.

The spasticity and seizures of tetanus are caused by exaggerated reflex responses to afferent stimuli as a result of suppression of balancing central inhibition. Several classes of drugs that act at different sites along the reflex pathway are useful for control of these manifestations. Among them are hypnotics and sedatives, which reduce sensory input and generalized excitability; general anesthetics, which produce broad depression of the CNS; centrally acting muscle relaxants or spinal depressants, which lower reflex activity and decrease motor output from the spinal cord; and neuromuscular blocking agents, which inhibit the transmission of excess motor nerve activity to effector muscles.

Historically many different drugs have been used for the patient with tetanus. The use of diazepam was first reported in *The Lancet* in 1965, shortly after the drug was introduced clinically.[52] Since then the benzodiazepines have become the preferred drugs for the spasms and rigidity associated with tetanus.[32,51,78,105,113] They also have the advantage of being potent anticonvulsants and sedative hypnotic agents. Additionally, they are GABA agonists, perhaps partially overcoming the effect of tetanospasmin interfering with the normally inhibitory effect of GABA.[111] Diazepam (Valium) and lorazepam (Ativan) have been the most frequently used benzodiazepines for tetanus. Lorazepam has a somewhat longer half-life and may be preferred for this reason. Very large total daily doses (500 mg) of diazepam or lorazepam (200 mg) may be required.[100] Both drugs are formulated in propylene glycol solution for intravenous dosage, and the large doses required may result in significant propylene glycol toxicity, including a metabolic acidosis. For this reason, an enteral preparation that is free of propylene glycol should be given enterally if at all possible.[14]

Midazolam (Versed) is a short-acting benzodiazepine that is soluble in water and thus does not include propylene glycol in its parenteral formulation. Because of its short half-life, it should be administered as a continuous infusion and an initial infusion dose of 0.1 to 0.3 mg/kg/hr may be required. The benzodiazepines all induce tachyphylaxis and will require escalation of the dosage with time. The dosage should be titrated to prevent tetanic spasms and provide adequate sedation instead of being a specified dose. To avoid withdrawal symptoms after long-term use, the benzodiazepines should be tapered over the course of several weeks.

Magnesium sulfate has been advocated by some authors as first-line therapy for tetanus.[6,85,108] Magnesium was first reported as a therapy for tetanus, given intrathecally, over 100 years ago.[13] Magnesium is a muscle relaxant and has been well tolerated at high doses in the obstetric population. In addition, magnesium reduces catecholamine release and lowers heart rate and vascular tone, stabilizing some of the effects of autonomic instability seen in tetanus. The largest trial of magnesium therapy for severe tetanus was reported in *The Lancet*.[108] This was a randomized, double-blind, placebo-controlled trial of 256 Vietnamese patients over the age of 15 years. Magnesium was given as a loading dose of 40 mg/kg followed by an intravenous infusion titrated to maintain serum levels of 2 to 4 mmol/L. There was no difference in the requirement for mechanical ventilation, although the patients receiving magnesium required less benzodiazepine and neuromuscular blockade and also were less likely to require treatment for cardiovascular instability. A smaller, less well-controlled clinical trial of magnesium has reported a decreased incidence of the need for

mechanical ventilation.[101] Case reports in the pediatric population are somewhat limited,[24,85] although both reports advocate the use of magnesium as part of the therapeutic regimen for tetanus.

If the benzodiazepines and magnesium are unable to control the spasms and rigidity associated with tetanus, intrathecal baclofen should be considered. Baclofen is a GABA receptor agonist that directly stimulates the postsynaptic GABA β receptors on synapses blocked by tetanus toxin, thereby restoring physiologic inhibition of the α motor neuron. Numerous centers have described the efficacy of intrathecal baclofen for treating tetanus, although its safety and efficacy have not been established in children younger than 4 years.[15,37,38,97] When magnesium, benzodiazepines and intrathecal baclofen are unable to control the spasms and rigidity associated with tetanus, neuromuscular blockade is indicated. Although succinylcholine has been used in the past,[44] newer agents have supplanted it. Additionally, theoretical reasons provide the basis to avoid the use of succinylcholine. With functional denervation of the motor end-plate in neuromuscular junctions directly affected by tetanospasmin, succinylcholine may result in exaggerated release of potassium and hyperkalemia. This condition has been associated with cardiac arrhythmias and death in other denervating conditions. For this reason, nondepolarizing agents should be used. Potential agents include pancuronium, vecuronium, atracurium, cisatracurium, rocuronium, and pipecuronium—the agent of choice in a large tetanus treatment center in Vietnam.[108]

Neuromuscular blocking drugs should be used only by physicians experienced in their use in a critical care environment, typically anesthesiologists or intensivists.[60] Although neuromuscular blockers formerly were used at low doses to preserve diaphragmatic function and spontaneous respiration, current thought is that they should be administered in conjunction with tracheostomy or endotracheal intubation and ventilatory support. Remembering that neuromuscular blocking drugs have no effect on cortical function and have no sedative effect is critical. The benzodiazepines are good sedatives and have a significant amnestic effect. They are not, however, analgesics, and if pain is present (such as from previous muscle spasms), morphine sulfate is an effective analgesic. It also may be helpful in treating sympathetic hyperactivity. If sympathetic overactivity remains problematic, a combined α- and β-receptor blocker such as labetalol should be used. The use of a β blocker such as propranolol alone should be avoided because the unopposed α-mediated vasoconstriction could lead to significant hypertension. Clonidine and epidural anesthesia are alternative therapies for increased sympathetic discharge. A combination of epidural bupivacaine and sufentanil has been used by some physicians to treat the sympathetic overactivity.[11]

The management approach to a patient with tetanus spasms or rigidity should be one of escalation of therapy based on need. Benzodiazepines should be administered initially, with high doses often being required. If the benzodiazepines are not effective, infusion of magnesium to decrease muscle spasms and cardiovascular instability should be considered.[108] If control of the spasms or rigidity is still not achieved, intrathecal baclofen may be used.[37,38] If these therapies are inadequate or result in airway compromise or inadequate ventilation, endotracheal intubation should be performed with a nondepolarizing neuromuscular blocking agent.

Sixty-six percent of 103 children aged 1 to 12 years with severe tetanus studied by Wesley and Pathes[118] required management with total muscle paralysis and intermittent positive-pressure ventilation. The death rate in this group was 14.5 percent.

PROGNOSIS

The average fatality rate associated with tetanus ranges from 25 to 70 percent, but mortality rates can be reduced to 10 to 30 percent with modern intensive care.[91,116] The risk for death occurring in patients with tetanus neonatorum is particularly high; it was reported to be 99.5 percent in a group of 5794 infants in 1930.[52] With modern treatment and high-intensity supportive care, the mortality rate associated with neonatal tetanus can be reduced to approximately 25 percent.[116] Injection drug users are highly susceptible to the development of very severe disease and are likely to die.[58,63]

A variety of other factors play an important role in determining the outcome of tetanus.[50,107,115] Patients in the second and third decades of life have a higher rate of recovery than the elderly. An inverse relationship exists between the length of the incubation period and risk, as first pointed out by Hippocrates. The risk for death is approximately 58 percent when the interval between injury and the onset of tetanus is 2 to 10 days. When the interval is 11 to 22 days or longer, fatality rates have been 35 to 17 percent, respectively. A relationship also appears to exist between the period elapsing from the time of appearance of the first signs of tetanus and development of the first seizure or maximal intensity of the disease; the shorter this interval, the poorer is the prognosis.[31]

The clinical form of tetanus also influences the outcome.[65] Cephalic tetanus and tetanus neonatorum are associated with the highest incidence of death. In contrast, local tetanus, unless complicated by development of the generalized syndrome, has an excellent prognosis. Early administration of prophylactic antitoxin markedly increases the frequency of survival.

CAUSE OF DEATH

Because the clinical course of tetanus may be prolonged and the therapy used is complex and potentially dangerous, the cause of death often is not clear. Animals may die after the injection of toxin without any recognizable signs of the disease appearing.[43] Studies in parabiotic rats suggest that tetanospasmin exerts a lethal effect on the respiratory center.[98] Involvement of the medulla has been observed in experimental animals and humans, in whom episodes of respiratory failure, often in the absence of seizures, have been described.[60,123] The action of the toxin on brain tissue has been postulated to be the cause of hyperpyrexia, tachycardia, hypotension, bulbar palsy, and cardiac arrest.[76] Myocardial damage also may occur, and

both histologic and electrocardiographic abnormalities have been described.[82]

Death may occur during a seizure, but the specific mechanisms involved are not clear in all cases. Laryngospasm and disturbances in electrolyte balance may play important roles. Pneumonia complicating aspiration, induced by an inability to swallow and oversedation, occurs commonly. It may be directly responsible for death or may contribute to a fatal outcome by increasing the degree of anoxia of the respiratory center.

PREVENTION

As noted in Figure 147-1, tetanus in the United States has decreased dramatically, and this decline can be attributed to routine universal use of tetanus toxoid and improved wound management, including the use of tetanus prophylaxis in emergency departments.

Active Immunization

For complete information regarding tetanus immunization, the reader is referred to the most recent recommendations of the Advisory Committee on Immunization Practices of the U.S. Public Health Service,[25,28] the recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics,[4] and product information from vaccine manufacturers.

In the United States, primary immunization against tetanus is performed in conjunction with immunization against diphtheria and pertussis in the form of diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (DTaP). The schedule involves an initial series of three doses of vaccine at 2, 4, and 6 months of age; a reinforcing dose at 12 to 18 months of age; and a booster dose at 4 to 6 years of age. After the initial series, additional booster doses of adult-type diphtheria and tetanus toxoids adsorbed (Td) are recommended at 10-year intervals.[34,36] In 2005, two adult-type diphtheria and tetanus toxoids and acellular pertussis vaccine adsorbed (Tdap) were approved for use in the United States.[28] In adolescents and adults, Tdap should be the routine vaccine. At present, Tdap vaccines are approved for only one dose, but it is anticipated that a boosting schedule of Tdap will be similar to that recommended for Td (every 10 years). The minimal serum level of antitoxin needed for protection is 0.01 IU/mL.[35,67,68,102] After the initial three-dose series, the reinforcing dose, and the booster dose, levels of antitoxin 100- to 1000-fold higher than 0.01 IU/mL are attained, and levels higher than 0.01 IU/mL persist in nearly all vaccinees until the scheduled subsequent dose.

Tetanus Prophylaxis in Wound Management

Antimicrobial prophylaxis against tetanus is neither practical nor useful in managing wounds.[16,124] Cleaning of the wound, débridement when indicated, and proper immunization are important factors. The need for tetanus toxoid (active immunization), with or without TIG (passive immunization), depends on both the condition of the wound and the patient's vaccination history (Table 147-1).[4,25,30,69] Rarely has tetanus occurred in persons with documentation of having received a primary series of toxoid injections.

TABLE 147-1 **Summary Guide to Tetanus Prophylaxis in Routine Wound Management**

	Clean, Minor Wounds		All Other Wounds*	
History of Adsorbed Tetanus Toxoid (Doses)	**Td†**	**TIG**	**Td†**	**TIG**
Unknown or <3	Yes	No	Yes	Yes
≥3‡	No§	No	No‖	No

From Centers for Disease Control and Prevention (CDC). Diphtheria, tetanus and pertussis: recommendations for vaccine use and other preventive measures; recommendations of the Immunization Practices Advisory Committee (ACIP). MMWR Recomm Rep 1991;**40**(RR-10):2-28; CDC. Tetanus in epidemiology and prevention of vaccine-preventable diseases: the pink book—course textbook. 12th ed. Atlanta, 2012, CDC. Available at http://www.cdc.gov/vaccines/pubs/pinkbook/tetanus.html.

*Such as, but not limited to, wounds contaminated with dirt, feces, soil, and saliva; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns, and frostbite.

†For children younger than 7 years, DTaP (diphtheria-tetanus if pertussis vaccine is contraindicated) is preferred to tetanus toxoid alone. For persons 7 years or older, Tdap is preferred to tetanus toxoid alone.

‡If only three doses of fluid toxoid have been received, a fourth dose of toxoid, preferably an adsorbed toxoid, should be given.

§Yes, if longer than 10 years since the last dose.

‖Yes, if longer than 5 years since the last dose (more frequent boosters are not needed and can accentuate side effects).

Td, Adult-type diphtheria and tetanus toxoids; *TIG,* tetanus immune globulin.

A thorough attempt must be made to determine whether a patient has completed primary vaccination. Patients with unknown or uncertain previous vaccination histories should be considered to have had no previous tetanus toxoid doses. Patients who have not completed a primary series may require tetanus toxoid and passive immunization at the time of wound cleaning and débridement (see Table 147-1).

The evidence available indicates that complete primary vaccination with tetanus toxoid provides long-lasting protection for 10 or more years in most recipients. Consequently, after complete primary tetanus vaccination, boosters—even for wound management—need to be given only every 10 years when wounds are minor and uncontaminated. For other wounds, a booster is appropriate if the patient has not received tetanus toxoid within the preceding 5 years.[81] Antitoxin antibodies develop rapidly in persons who have received at least two doses of tetanus toxoid.

Tdap is the preferred preparation for active tetanus immunization in the wound management of patients aged 7 years or older. Because a large proportion of adults are susceptible, this plan enhances diphtheria protection.

Thus, by taking advantage of acute health care visits, such as for wound management, some patients can be protected who otherwise would remain susceptible. For routine wound management in children younger than 7 years of age who are not vaccinated adequately, DTaP should be used instead of single-antigen tetanus toxoid. Td may be used if pertussis vaccine is contraindicated. For patients of all ages who are inadequately vaccinated, completion of primary vaccination at the time of discharge or at follow-up visits should be ensured. Presently, Tdap rather than Td is recommended for wound management in adolescents and adults.[27]

If passive immunization is needed, human TIG is the product of choice. It provides protection longer than that of antitoxin of animal origin and causes fewer adverse reactions.[70,95,104] The TIG prophylactic dose currently recommended for wounds of average severity is 250 U intramuscularly. When tetanus toxoid and TIG are given concurrently, separate syringes and separate sites should be used.

Neonatal Tetanus

Several approaches have been taken to reduce the incidence and fatality rate of neonatal tetanus in developing areas of the world. Among these approaches are (1) educating pregnant women concerning the danger of using contaminated materials for cutting the umbilical cord and covering the stump, (2) training midwives in the application of modern techniques of obstetric asepsis, (3) developing hospitals in which babies are born under strict asepsis, and (4) immunizing all women of child-bearing age or, if such immunization is not possible, all who are pregnant. Studies performed by the World Health Organization (WHO) have demonstrated that such immunization is practical and leads to an appreciable reduction in the incidence of neonatal tetanus.[39,40,41,104,112,122] Babies born to mothers who have been immunized during pregnancy have adequate levels of circulating antibody and are protected against acquiring the disease. The level of protective antibody in newborns and the magnitude of the transfer rate of passive immunity to tetanus depend directly on the level of tetanus antitoxin in maternal serum. Mothers who had tetanus antitoxin levels of 1.28 IU/mL or greater could transfer protection to almost all the newborns (97% to 100%), irrespective of the doses of tetanus toxoid administered. Mothers who had received two doses of tetanus toxoid during pregnancy not only conferred good protection but also transferred high antitoxin levels to their newborns.[96]

In 1989, the WHO adopted a resolution to eliminate neonatal tetanus worldwide,[120,121] and in 1990, the World Summit for Children issued a declaration for global elimination of neonatal tetanus by the end of 1995.[26,122] In 1993, the WHO's goal was defined as elimination of neonatal tetanus as a public health problem by reducing its case incidence to less than 1/1000 live births for all health districts.[50]

From 1989 to 1993, the rate of vaccination coverage with two or more doses of tetanus toxoid administered to pregnant women in areas at risk increased from 27 to 45 percent. To achieve and maintain elimination of neonatal tetanus, 80 percent or more of infants need to be protected at birth through vaccination of their mothers with at least two doses of tetanus toxoid or through clean delivery and cord-care practices.[49] Considerable progress has been made in the reduction of maternal and neonatal tetanus, but the goal to reduce the rate of neonatal tetanus cases to less than 1/1000 live births in all geographic areas has not been achieved.[113] In 2003, 57 countries still remained in which neonatal tetanus had not been eliminated in all districts.

NEW REFERENCES SINCE THE SIXTH EDITION

4. American Academy of Pediatrics. Tetanus. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 707–12.
6. Attygalle D, Rodrigo N. New trends in the management of tetanus. *Expert Rev Anti Infect Ther* 2004;**2**:73–84.
13. Blake JA. The use of magnesium sulphate in the production of anaesthesia and in the treatment of tetanus. *Surg Gynecol Obstet* 1906;**2**:541–50.
24. Ceneviva GD, Thomas NJ, Kees-Folts D. Magnesium sulfate for control of muscle rigidity and spasms and avoidance of mechanical ventilation in pediatric tetanus. *Pediatr Crit Care Med* 2003;**4**: 480–4.
29. Centers for Disease Control and Prevention. Tetanus surveillance: United States, 2001-2008. *MMWR Morb Mortal Wkly Rep* 2011; **60**:365–9.
30. Centers for Disease Control and Prevention. Tetanus. In: Atkinson W, Hamborsky J, Wolfe S, editors. *Epidemiology and prevention of vaccine-preventable diseases*. 12th ed. Washington DC: Public Health Foundation; 2012. p. 291–300.
51. Hendrickse RG, Sherman PM. Therapeutic Trial of Diazepam in Tetanus. *Lancet* 1965;**1**:737–8.
61. Lawn JE, Kerber K, Enweronu-Laryea C, et al. 3.6 million neonatal deaths: what is progressing and what is not? *Semin Perinatol* 2010;**34**:371–86.
64. Lin TS, Chen LK, Lin TY, et al. Autonomic dysfunction because of severe tetanus in an unvaccinated child. *Pediatr Neonatol* 2011;**52**:169–71.
79. Paliwal VK. Neuromyotonia masquerading as tetanus. *J Clin Neurosci* 2010;**17**:814–15.
85. Puliyel MM, Pillai R, Korula S. Intravenous magnesium sulphate infusion in the management of very severe tetanus in a child: a descriptive case report. *J Trop Pediatr* 2009;**55**:58–9.
101. Sikendar RI, us Samad B, Ali S, et al. Post traumatic tetanus and role of magnesium sulphate. *J Ayub Med Coll Abbottabad* 2009; **21**:132–5.
108. Thwaites CL, Yen LM, Loan HT, et al. Magnesium sulphate for treatment of severe tetanus: a randomised controlled trial. *Lancet* 2006;**368**:1436–43.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abel JJ, Firor WM, Chalain W. Researches on tetanus. IX. Further evidence to show that tetanus toxin is not carried to central neurons by way of the axis cylinders of motor nerves. *Bull Johns Hopkins Hosp* 1938;**63**:373–403.
2. Abrutyn E, Berlin JA. Intrathecal therapy in tetanus: a meta-analysis. *JAMA* 1991;**266**:2262–7.
3. Ahmadsyah I, Salim A. Treatment of tetanus: an open study to compare the efficacy of procaine penicillin with metronidazole in the treatment of moderate tetanus. *Br Med J* 1985;**29**:640–50.
4. American Academy of Pediatrics. Tetanus. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 707–12.
5. Aretaeus. Tetanus. In: Major RH, editor. *Classic descriptions of disease*. 2nd ed. Springfield, Ill: Charles C Thomas; 1939. p. 148–9.
6. Attygalle D, Rodrigo N. New trends in the management of tetanus. *Expert Rev Anti Infect Ther* 2004;**2**:73–84.
7. Bagratuni L. Cephalic tetanus, with report of a case. *Br Med J* 1952;**1**:461–3.
8. Barlow JL, Mung'Ala-Odera V, Gona J, et al. Brain damage after neonatal tetanus in a rural Kenyan hospital. *Trop Med Int Health* 2001;**6**:305–8.
9. Bartlett JG. Clostridium tetani. In: Gorbac SL, Bartlett JG, Blacklow NR, editors. *Infectious diseases*. Philadelphia: W. B. Saunders; 1992. p. 1580–3.
10. Bennett J, Schooley M, Traverso H, et al. Bundling, a newly identified risk factor for neonatal tetanus: implications for global control. *Int J Epidemiol* 1996;**25**:879–84.
11. Bhagwanjee S, Bosenberg AT, Muckart DJ. Management of sympathetic overactivity in tetanus with epidural bupivacaine and sufentanil: experience with 11 patients. *Crit Care Med* 1999;**27**:1721–5.
12. Bizzini B. Tetanus. In: Germanier R, editor. *Bacterial vaccines*. Orlando, Fla: Academic Press; 1984. p. 38–68.
13. Blake JA. The use of magnesium sulphate in the production of anaesthesia and in the treatment of tetanus. *Surg Gynecol Obstet* 1906;**2**:541–50.
14. Bleck TP. Tetanus: pathophysiology, management, and prophylaxis. *Dis Mon* 1991;**37**:545–603.
15. Boots RJ, Lipman J, O'Callaghan J, et al. The treatment of tetanus with intrathecal baclofen. *Anaesth Intensive Care* 2000;**28**:438–42.
16. Botticelli JT, Waisbren BA. Tetanus in an urban community. *Am J Med Sci* 1961;**242**:44–50.
17. Bradley K, Easton DM, Eccles JC. An investigation of primary or direct inhibition. *J Physiol* 1953;**122**:474–88.
18. Brooks VB, Asanuma H. Action of tetanus toxin in the cerebral cortex. *Science* 1962;**137**:674–6.
19. Brooks VB, Curtis DR, Eccles JC. Mode of action of tetanus toxin. *Nature* 1955;**175**:120–1.
20. Bruggemann H, Gottschalk G. Insights in metabolism and toxin production from the complete genome sequence of *Clostridium tetani*. *Anaerobe* 2004;**10**:53–68.
21. Bytchenko B. Microbiology of tetanus. In Veronesi R, editor. *Tetanus: important new concepts*. Amsterdam: Excerpta Medica; 1981. p. 28–39.
22. Carle A, Rattone G. Studio esperimentale sull'eziologia del tetano. *G Acad Med Torino* 1884;**32**:174–80.
23. Carrea R, Lanari A. Chronic effect of tetanus toxin applied locally to the cerebral cortex of the dog. *Science* 1962;**137**:342–3.
24. Ceneviva GD, Thomas NJ, Kees-Folts D. Magnesium sulfate for control of muscle rigidity and spasms and avoidance of mechanical ventilation in pediatric tetanus. *Pediatr Crit Care Med* 2003;**4**:480–4.
25. Centers for Disease Control and Prevention. Diphtheria, tetanus and pertussis: recommendations for vaccine use and other preventive measures; recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991;**40**:2–28.
26. Centers for Disease Control and Prevention. Progress towards the global elimination of neonatal tetanus, 1989-1993. *MMWR Morb Mortal Wkly Rep* 1994;**43**:885–7, 893–84.
27. Centers for Disease Control and Prevention. Progress toward elimination of neonatal tetanus: Egypt, 1988-1994. *MMWR Morb Mortal Wkly Rep* 1996;**45**:89–92.
28. Centers for Disease Control and Prevention. Preventing tetanus, diphtheria, and pertussis among adolescents: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccines recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;**55**:1–37.
29. Centers for Disease Control and Prevention. Tetanus surveillance: United States, 2001-2008. *MMWR Morb Mortal Wkly Rep* 2011;**60**:365–9.
30. Centers for Disease Control and Prevention. Tetanus. In: Atkinson W, Hamborsky J, Wolfe S, editors. *Epidemiology and prevention of vaccine-preventable diseases*. 12th ed. Washington DC: Public Health Foundation; 2012. p. 291–300.
31. Christensen NA, Thurber DL. Clinical experience with tetanus: 91 cases. *Proc Staff Meet Mayo Clin* 1957;**32**:146–58.
32. Creech O Jr, Glover A, Ochsner A. Tetanus: evaluation of treatment at Charity Hospital, New Orleans, Louisiana. *Ann Surg* 1957;**146**:369–81; discussion 382–63.
33. Davies JR, Morgan RS, Wright EA, et al. The effect of local tetanus intoxication on the hind limb reflexes of the rabbit. *Arch Int Physiol* 1954;**62**:248–63.
34. Edsall G. Specific prophylaxis of tetanus. *J Am Med Assoc* 1959;**171**:417–27.
35. Edsall G. Problems in the immunology and control of tetanus. *Med J Aust* 1976;**2**:216–20.
36. Edsall G, Elliott MW, Peebles TC, et al. Excessive use of tetanus boosters. *JAMA* 1967;**202**:17–19.
37. Engrand N, Guerot E, Rouamba A, et al. The efficacy of intrathecal baclofen in severe tetanus. *Anesthesiology* 1999;**90**:1773–6.
38. Engrand N, Vilain G, Rouamba A, et al. [Value of intrathecal baclofen in the treatment of severe tetanus in the tropical milieu]. *Med Trop (Mars)* 2000;**60**:385–8.
39. Expanded Programme on Immunization. Reduction of neonatal deaths by immunizing women against tetanus. *Wkly Epidemiol Rec* 1981;**56**:185–6.
40. Expanded Programme on Immunization. Prevention of neonatal tetanus. *Wkly Epidemiol Rec* 1982;**57**:137–42.
41. Expanded Programme on Immunization. Global Advisory Group Meeting. Weekly Advisory Group Meeting. *Wkly Epidemiol Rec* 1983;**58**:15–18.
42. Fedinec AA, Matzke HA. The role of tissue spaces and nerve fibers in the spread of tetanus toxin in the rat. *Univ Kansas Sci Bull* 1958;**38**:1439–98.
43. Firor WM, Lamont A, Shumacker HB. Studies on the cause of death in tetanus. *Ann Surg* 1940;**111**:246–74.
44. Forrester AT. Treatment of tetanus with succinylcholine. *Br Med J* 1954;**2**:342–4.
45. Friedlander FC. Tetanus neonatorum. *J Pediatr* 1951;**39**:448–54.
46. Galazka A, Cook R. Neonatal tetanus today and tomorrow. Proceedings of the Seventh International Conference on Tetanus, Copanello, Rome, Italy, September 10-15, 1984. p. 350–63. Gangeni Publishing; 1985.
47. Galazka A, Kardymowicz B. Tetanus incidence and immunity in Poland. *Eur J Epidemiol* 1989;**5**:474–80.
48. Godfrey MP, Parsons V, Rawstron JR. Rapid destruction of antitetanus serum in a patient previously sensitised to horse serum. *Lancet* 1960;**2**:1229–30.
49. Group GA. Expanded Program on Immunization. Global Advisory Group. Part II. Achieving the major disease control goals. *Wkly Epidemiol Rec* 1994;**69**:29–31, 34–5.
50. Gurses N, Aydin M. Factors affecting prognosis of neonatal tetanus. *Scand J Infect Dis* 1993;**25**:353–5.
51. Hendrickse RG, Sherman PM. Therapeutic Trial of Diazepam in Tetanus. *Lancet* 1965;**1**:737–8.
52. Hippocrates. Tetanus. In: Major HH, editor: *Classic descriptions of disease*. 2nd ed. Springfield, Ill: Charles C Thomas; 1939. p. 148–149.
53. Hlady WG, Bennett JV, Samadi AR, et al. Neonatal tetanus in rural Bangladesh: risk factors and toxoid efficacy. *Am J Public Health* 1992;**82**:1365–9.
54. Howder CL. *Cardiopulmonary pharmacology*. Baltimore: Williams & Wilkins; 1996. p. 316.

55. Jenkins MT, Luhn NR. Active management of tetanus based on experiences of an anesthesiology department. *Anesthesiology* 1962; **23**:690–709.
56. Kabura L, Ilibagiza D, Menten J, et al. Intrathecal vs. intramuscular administration of human antitetanus immunoglobulin or equine tetanus antitoxin in the treatment of tetanus: a meta-analysis. *Trop Med Int Health* 2006;**11**:1075–81.
57. Kaeser HE, Saner A. Tetanus toxin, a neuromuscular blocking agent. *Nature* 1969;**223**:842.
58. Kerr JH, Corbett JL, Prys-Roberts C, et al. Involvement of the sympathetic nervous system in tetanus: studies on 82 cases. *Lancet* 1968;**2**:236–41.
59. Kitasato S. Über den tetanus Bacillus. *Z Hyg Infektkr* 1889; **7**:225–34.
60. Laurence DR, Webster RA. Pathologic physiology, pharmacology and therapeutics of tetanus. *Clin Pharmacol Ther* 1963;**4**:36–72.
61. Lawn JE, Kerber K, Enweronu-Laryea C, et al. 3.6 million neonatal deaths: what is progressing and what is not? *Semin Perinatol* 2010;**34**:371–86.
62. Leroy O, Garenne M. Risk factors of neonatal tetanus in Senegal. *Int J Epidemiol* 1991;**20**:521–6.
63. Levinson A, Marske RL, Shein MK. Tetanus in heroin addicts. *J Am Med Assoc* 1955;**157**:658–60.
64. Lin TS, Chen LK, Lin TY, et al. Autonomic dysfunction because of severe tetanus in an unvaccinated child. *Pediatr Neonatol* 2011;**52**:169–71.
65. Luisto M. Outcome and neurological sequelae of patients after tetanus. *Acta Neurol Scand* 1989;**80**:504–11.
66. Luisto M. Tetanus in Finland: diagnostic problems and complications. *Ann Med* 1990;**22**:15–19.
67. MacLennan R, Schofield FD, Pittman M, et al. Immunization against neonatal tetanus in New Guinea: antitoxin response of pregnant women to adjuvant and plain toxoids. *Bull World Health Organ* 1965;**32**:683–97.
68. McComb JA. The prophylactic dose of homologous tetanus antitoxin. *N Engl J Med* 1964;**270**:175–8.
69. McComb JA, Dwyer RC. Passive-active immunization with tetanus immune globulin (human). *N Engl J Med* 1963;**268**: 857–62.
70. Melgaard B, Mutie DM, Kimani G. A cluster survey of mortality due to neonatal tetanus in Kenya. *Int J Epidemiol* 1988;**17**:124–7.
71. Mellanby J, Vanheyningen WE, Whittaker VP. Fixation of tetanus toxin by subcellular fractions of brain. *J Neurochem* 1965;**12**: 77–9.
72. Meyer H, Ransom F. Untersuchungen über den Tetanus. *Arch Exp Pathol Pharmakol* 1903;**49**:369–416.
73. Millard AH. Local tetanus. *Lancet* 1954;**267**:844–6.
74. Miranda-Filho Dde B, Ximenes RA, Barone AA, et al. Randomised controlled trial of tetanus treatment with antitetanus immunoglobulin by the intrathecal or intramuscular route. *BMJ* 2004;**328**:615.
75. Mongi PS, Mbise RL, Msengi AE, et al. Tetanus neonatorum: experience with intrathecal serotherapy at Muhimbili Medical Centre, Dar es Salaam, Tanzania. *Ann Trop Paediatr* 1987;**7**: 27–31.
76. Montgomery RD. The cause of death in tetanus. *West Indian Med J* 1961;**10**:84–102.
77. Nicolaier A. Über infectiosen Tetanus. *Dtsch Med Wochenschr* 1884;**10**:842–84.
78. Okoromah CN, Lesi FE. Diazepam for treating tetanus. *Cochrane Database Syst Rev* 2004;(1):CD003954.
79. Paliwal VK. Neuromyotonia masquerading as tetanus. *J Clin Neurosci* 2010;**17**:814–15.
80. Pascual FB, McGinley EL, Zanardi LR, et al. Tetanus surveillance: United States, 1998–2000. *MMWR Surveill Summ* 2003;**52**:1–8.
81. Peebles TC, Levine L, Eldred MC, et al. Tetanus-toxoid emergency boosters: a reappraisal. *N Engl J Med* 1969;**280**:575–81.
82. Perez LR. The electrocardiogram in tetanus. *Rev Clin Esp* 1959; **75**:20.
83. Pillemer L, Wittler R, Grossberg DB. The isolation and crystallization of tetanal toxin. *Science* 1946;**103**:615.
84. Pratt EL. Clinical tetanus; a study of 56 cases, with special reference to methods of prevention and a plan for evaluating treatment. *JAMA* 1945;**129**:1243–7.
85. Puliyel MM, Pillai R, Korula S. Intravenous magnesium sulphate infusion in the management of very severe tetanus in a child: a descriptive case report. *J Trop Pediatr* 2009;**55**:58–9.
86. Ramon G, Zoeller C. L'immunite antitetanique par l'anatoxine chez l'homme. *Presse Med* 1926;**34**:485.
87. Raza SA, Akhtar S, Avan BI, et al. A matched case-control study of risk factors for neonatal tetanus in Karachi, Pakistan. *J Postgrad Med* 2004;**50**:247–51; discussion 251.
88. Risk WS, Bosch EP, Kimura J, et al. Chronic tetanus: clinical report and histochemistry of muscle. *Muscle Nerve* 1981;**4**:363–6.
89. Robinson DT, McLeod J, Downie AW. Dust in surgical theatres as a possible source of postoperative tetanus. *Lancet* 1946;**1**: 152–4.
90. Rocke DA, Wesley AG, Pather M, et al. Morphine in tetanus: the management of sympathetic nervous system overactivity. *S Afr Med J* 1986;**70**:666–8.
91. Romitti M, Romitti F, Banchini E. [Tetanus: physiopathology and intensive care treatment]. *Minerva Anestesiol* 2000;**66**:445–60.
92. Roofe PG. Role of the axis cylinder in transport of tetanus toxin. *Science* 1947;**105**:180–1.
93. Rosenbach AJF. Zur Aetiologie des Wundestarrkrampfes. Vorgetyrangen am 1. Sitzungslage des XV Congresses der Deutschen f. Chirurgie zu Berlin 7 April 1986. *Arch Klin Chir* 1887;**34**:306.
94. Rubbo SD, Suri JC. Passive immunization against tetanus with human immune globulin. *Br Med J* 1962;**2**:79–81.
95. Salimpour R. Cause of death in tetanus neonatorum: study of 233 cases with 54 necropsies. *Arch Dis Child* 1977;**52**:587–9.
96. Sangpetchsong V, Vichaikummart S, Vichitnant A, et al. Transfer rate of transplacental immunity to tetanus from non-immunized and immunized mothers. *Southeast Asian J Trop Med Public Health* 1984;**15**:275–80.
97. Santos ML, Mota-Miranda A, Alves-Pereira A, et al. Intrathecal baclofen for the treatment of tetanus. *Clin Infect Dis* 2004;**38**: 321–8.
98. Schellenberg DA, Matzke HA. Development of tetanus in parabiotic rats. *J Immunol* 1958;**80**:367–73.
99. Sevitt S. Source of two hospital-infected cases of tetanus. *Lancet* 1949;**2**:1075–8.
100. Sherrington CS. *The integrative action of the nervous system*. New York: Yale University Press; 1906. p. 303.
101. Sikendar RI, us Samad B, Ali S, et al. Post traumatic tetanus and role of magnesium sulphate. *J Ayub Med Coll Abbottabad* 2009;**21**:132–5.
102. Smith JW. Diphtheria and tetanus toxoids. *Br Med Bull* 1969;**25**: 177–82.
103. Smolens J, Vogt AB, Crawford MN, et al. The persistence in the human circulation of horse and human tetanus antitoxins. *J Pediatr* 1961;**59**:899–902.
104. Stanfield JP, Galazka A. Neonatal tetanus in the world today. *Bull World Health Organ* 1984;**62**:647–69.
105. Tallman JF, Gallager DW. The GABA-ergic system: a locus of benzodiazepine action. *Annu Rev Neurosci* 1985;**8**:21–44.
106. Thwaites CL, Yen LM, Cordon SM, et al. Urinary catecholamine excretion in tetanus. *Anaesthesia* 2006;**61**:355–9.
107. Thwaites CL, Yen LM, Glover C, et al. Predicting the clinical outcome of tetanus: the tetanus severity score. *Trop Med Int Health* 2006;**11**:279–87.
108. Thwaites CL, Yen LM, Loan HT, et al. Magnesium sulphate for treatment of severe tetanus: a randomised controlled trial. *Lancet* 2006;**368**:1436–43.
109. Turner TB, Stafford ES, Goldman L. Studies on the duration of protection afforded by active immunization against tetanus. *Bull Johns Hopkins Hosp* 1954;**94**:205–17.
110. Turner TB, Velasco-Joven EA, Prudovsky S. Studies on the prophylaxis and treatment of tetanus. II. Studies pertaining to treatment. *Bull Johns Hopkins Hosp* 1958;**102**:71–84.
111. Van Heyningen WE, Miller PA. The fixation of tetanus toxin by ganglioside. *J Gen Microbiol* 1961;**24**:107–19.
112. Vandelaer J, Birmingham M, Gasse F, et al. Tetanus in developing countries: an update on the Maternal and Neonatal Tetanus Elimination Initiative. *Vaccine* 2003;**21**:3442–5.
113. Vassa NT, Doshi HV, Yajnik VH, et al. Comparative clinical trial of diazepam with other conventional drugs in tetanus. *Postgrad Med J* 1974;**50**:755–8.

114. Von Behring E, Kitasato S. Über des Zustandekommen der Diphterie-Immunitat und der Tetanus-Immunitat bei Tieren. *Dtsch Med Wochenschr* 1890;**16**:1113–14.
115. Wasay M, Khealani BA, Talati N, et al. Autonomic nervous system dysfunction predicts poor prognosis in patients with mild to moderate tetanus. *BMC Neurol* 2005;**5**:2.
116. Wassilak SGF, Roper MH, Murphy TV, et al. Tetanus toxoid. In: Plotkin SA, Mortimer EA, editors. *Vaccines*. 4th ed. Philadelphia: W. B. Saunders; 2004. p. 745–81.
117. Weinstein L. Tetanus. *N Engl J Med* 1973;**289**:1293–6.
118. Wesley AG, Pather M. Tetanus in children: an 11-year review. *Ann Trop Paediatr* 1987;**7**:32–7.
119. Willis AT. *Clostridium:* The spore-bearing anaerobes. In: Wilson G, Miles A, Parker MT, editors. Topley and Wilson's principles of bacteriology, virology and immunity, vol. 2. Baltimore: Williams & Wilkins; 1983. p. 442–75.
120. World Health Assembly. *Expanded Program on Immunization*. Geneva: World Health Organization; May 19, 1989 (Resolution WHA42.32). 1989.
121. World Health Organization. *Revised plan of action for neonatal tetanus elimination*. Publication No. WHO/EPI/GEN/93.13. Geneva: World Health Organization, Expanded Program on Immunization; 1993.
122. World Health Organization. Tetanus vaccine. *Wkly Epidemiologic Rec* 2006;**81**:198–208.
123. Wright EA, Morgan RS, Wright GP. Tetanus intoxication of the brain stem in rabbits. *J Pathol Bacteriol* 1950;**62**:569–83.
124. Young LS, LaForce FM, Bennett JV. An evaluation of serologic and antimicrobial therapy in the treatment of tetanus in the United States. *J Infect Dis* 1969;**120**:153–9.
125. Zacks SI, Sheff MF. Tetanus toxin: fine structure localization of binding sites in striated muscle. *Science* 1968;**159**:643–4.

CHAPTER 148

Actinomycosis

Suzanne Whitworth • Richard F. Jacobs

Human actinomycosis is a clinical illness with typical histologic findings that is caused by a variety of pathogens. Actinomycetes are a group of aerobic and anaerobic bacteria in the order Actinomycetales. The name *actinomycosis* means "ray fungus," and the organisms may resemble fungi because of their filamentous appearance.[66] It often is polymicrobial, and *Aggregatibacter* (formerly *Actinobacillus*) *actinomycetemcomitans* frequently is a co-pathogen. It is endogenous worldwide, with sporadic cases reported annually. Occurrence of the disease is unrelated to age, sex, season, or occupation, although it is an uncommon event in the pediatric population. These organisms have the ability to spread locally without regard to fascial planes or other anatomic barriers. Actinomycosis is characterized by localized swelling with suppuration, abscess formation, and draining sinuses. The abscesses have fibrous walls and are filled with pus and characteristic sulfur granules. The three most common types of actinomycosis are oral and cervicofacial, abdominal, and pulmonary. However, involvement of the liver, female reproductive tract, and brain has been described. Definitive diagnosis of the infection rests on isolation of the organism from pus or identification of sulfur granules on histopathologic sections of biopsy material. Adequate treatment generally consists of surgical removal of the lesion, prolonged antibiotic therapy, or both.

MICROBIOLOGY

The clinical entity of actinomycosis is caused by species of the genera *Actinomyces* and *Propionibacterium*. Human actinomycosis most often is caused by *Actinomyces israelii*, although *Actinomyces gerensceriae* (formerly known as *A. israelii* serovar 2), *Actinomyces naeslundii*, *Actinomyces viscosus*, *Actinomyces odontolyticus*, *Actinomyces meyeri*, *Actinomyces radicidentis*, *Actinomyces neuii*, *Actinomyces radingae*, *Actinomyces turicensis*, and *Actinomyces graevenitzii* also are causes of human illness.[23,54] *Trueperella pyogenes* (previously in the *Arcanobacterium* genus) also causes human actinomycosis. *Propionibacterium propionica* is the only species in the *Propionibacterium* genus that is a cause of human actinomycosis. Some authors also include *Bifidobacterium dentium* as an etiologic agent of actinomycosis.[47]

These bacteria are irregular, non–spore-forming, non–acid-fast, nonmotile, gram-positive rods. They grow in most rich culture media and have various oxygen requirements. For example, *A. israelii* requires anaerobic conditions for growth. *A. viscosus*, however, grows in an aerobic environment with carbon dioxide. These species ferment carbohydrates as their source of energy for growth.[19] These organisms now are firmly classified as prokaryotic bacteria, although they originally were thought to be fungi because of the mycelial appearance of the organisms in sulfur granules and their branching morphology. However, neither genus contains chitin or glucans, which are characteristic macromolecules of fungi. In addition, they reproduce by bacillary fusion, are sensitive to antibiotics, and are resistant to antifungal agents. These organisms are members of the endogenous flora of mucous membranes. *A. israelii* always is found in the oral cavity when the appropriate anaerobic culture technique is used. It also has been found in the gastrointestinal tract, bronchi, and female genital tract.[53]

A. actinomycetemcomitans is a fastidious, non–spore-forming, nonmotile, facultatively anaerobic gram-negative coccobacillus that frequently complicates actinomycosis caused by *A. israelii*. In addition to being associated with actinomycosis, it has been implicated as a pathogen in periodontal disease and is part of the oral flora. Infection by this bacterium often is not resolved by the normal host, possibly because of inefficient phagocytosis and the weak oxidative burst response of neutrophils.[44] In addition, the bacterium has several virulence factors, including induction of apoptosis and tissue destruction.[21] *A. actinomycetemcomitans* is characterized by slow growth in culture and a requirement for incubation in an atmosphere enhanced with carbon dioxide.[36] Other bacterial species concomitantly isolated in human actinomycosis are *Eikenella corrodens*, *Fusobacterium*, *Bacteroides*, *Capnocytophaga*, *Staphylococcus*, *Streptococcus*, and members of the family Enterobacteriaceae.

PATHOGENESIS

The organisms that cause actinomycosis normally are found in the oral flora from infancy to adulthood. Actinomycetes are primary colonizers that can initiate the formation of plaque and set the stage for the development of infectious disease, as well as predispose to the formation of caries.[4,10] These bacteria adhere tenaciously to both the hard and soft tissue surfaces of the oral cavity.[71] Disruption of this mucous membrane probably is the initiating event for oral and cervicofacial disease. The organisms then invade locally and spread without regard to fascial planes. The exact mechanism of this spread is unknown but may be related to the ability of these organisms to suppress part of the host immune system. Organisms of the *Actinomyces* genus have been shown to be chemotactic, to activate lymphocyte blastogenesis, and to stimulate the release of lysosomal enzymes from

polymorphonuclear leukocytes and macrophages.[19] In addition, the co-pathogens involved in this infection may reduce local oxygen tension. Dental extractions are associated with mucosal breaks and tissue necrosis and may predispose to oral or cervicofacial actinomycosis.[46] Hematogenous dissemination can occur eventually but is an uncommon development. Gastrointestinal disease probably is associated with disruption of the mucosal barrier, similar to oral and cervicofacial disease.[53] Organisms causing pulmonary actinomycosis probably reach the lungs through aspiration. Thoracic actinomycosis usually is a complication of localized pulmonary parenchymal infection. Actinomycetes produce enzymes and thus spread by extension into the lungs, pleura, and chest wall without regard to tissue planes.[61] Numerous reports in the literature associate actinomycosis with intrauterine devices (IUDs), and some question exists regarding the association of foreign bodies with actinomycosis.[6,15]

FIGURE 148-1 ■ Cervicofacial disease with draining sinus tracts caused by *Actinomyces israelii*.

PATHOLOGY

Actinomycosis most commonly is manifested as a chronic infection with single or multiple indurated swellings. These lesions eventually soften, become fluctuant, and suppurate. The walls are fibrous and firm and often described as wooden, which frequently results in their confusion with neoplasms. Over the course of time, sinus tracts form and extend through the overlying skin or to adjacent bones or tissues. The overlying skin may have a bluish hue.[53]

Histologically, a typical lesion has a central purulent area containing neutrophils and sulfur granules, surrounded by an outer zone of granulation with collagen fibers and fibroblasts. The sulfur granules are firm, yellowish granules containing the organisms and are virtually diagnostic of actinomycosis, although they can be seen in nocardiosis and botryomycosis. In addition to neutrophils, lymphocytes and plasma cells frequently are seen in the lesions; eosinophils and multinucleated giant cells occasionally are seen.[53]

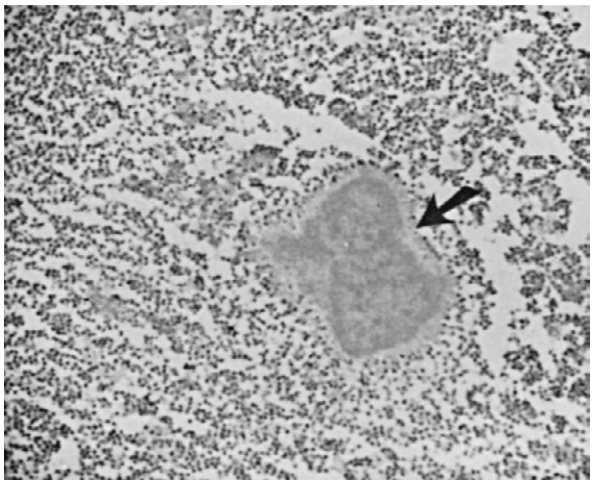

FIGURE 148-2 ■ Abscess containing an actinomycotic granule surrounded by purulent exudate. The peripheral clubbing *(arrow)* is stained by eosin. (Hematoxylin and eosin, ×52.)

CLINICAL MANIFESTATIONS

Actinomycotic infection occurs in three important sites. The order of frequency of occurrence is oral/cervicofacial, abdominal/pelvic, and pulmonary. Actinomycosis resembles several other chronic inflammatory diseases and must be differentiated from mycotic infections, tuberculosis, appendicitis, *Yersinia enterocolitica* pseudoappendicitis, osteomyelitis, amebiasis, hepatic abscess, and other chronic bacterial infections, including nocardiosis.

Because oral and cervicofacial actinomycosis occurs after disruption of the mucous membranes in the mouth or oropharynx, patients who have this type of actinomycosis may have a history of oral surgery, dental procedures, or trauma to the mouth. Clinical findings may include pain, trismus, firm swelling, and fistulas with drainage that contains the characteristic sulfur granules (Figs. 148-1 and 148-2). Patients most commonly have a chronic disease course but may be seen acutely with cellulitis. Infection may spread through the sinus tracts to the cranial bones, which gives rise to meningitis. Bone is not involved early in the disease, but periostitis may develop later. The marked ability of the organisms in this disease to burrow through tissue planes and even bone differentiates actinomycosis from nocardiosis and is an important characteristic of this infection. The cervicofacial type of actinomycosis, or "lumpy jaw," has the best prognosis. With surgical débridement and excision as an adjunct to proper antibiotic therapy, the disease usually is cured. At least two cases of thyroiditis secondary to actinomycosis have been reported in pediatric patients.[25,70]

Because abdominal actinomycosis also is the result of disruption of the mucosa of the gastrointestinal tract, patients may have a history of gastrointestinal surgery, diverticulitis, or appendicitis. The patient also may have a history of trauma to the abdomen. Patients may have chills, fever, night sweats, and weight loss. The course is indolent and similar to that of tuberculous peritonitis. Because appendicitis is the most common predisposing

event, on physical examination patients may have a hard, irregular mass in the ileocecal area that softens and then drains to the outside. Extension from such foci usually is by direct continuity (or, rarely, hematogenously) and involves any tissue or organ, including muscle, liver, spleen, kidney, fallopian tubes, ovaries, uterus, testes, bladder, esophagus, or rectum.* A delayed diagnosis of actinomycosis involving the abdomen or pelvis is typical and is frequently confused with malignancy.[28]

Hepatic involvement occurs in approximately 15 percent of cases of abdominal actinomycosis. Involvement of the liver can occur through direct extension from a subdiaphragmatic or subhepatic abscess. It also is a common finding in disseminated actinomycosis.[53] Occult disruption of the gastrointestinal mucosa with spread of organisms through the portal vein may provide a portal of entry for the organisms in cases of primary or isolated hepatic disease. Initial symptoms may include fever, abdominal pain, anorexia, weight loss, nausea, vomiting, shoulder pain, back pain, or diarrhea. The course usually is indolent, with 1 to 6 months of symptoms. On physical examination, the patient commonly has fever, abdominal tenderness, and hepatomegaly. A palpable abdominal mass, jaundice, or draining sinuses may be present. Disseminated intravascular coagulation has been reported.[65] Hepatic actinomycosis has been found in children who have undergone appendectomies.[37] Thrombotic thrombocytopenic purpura has been described in association with hepatic actinomycosis in an adult.[50] Other causes of liver masses included in the differential diagnosis are pyogenic abscess, amebiasis, and malignancy.

Women wearing IUDs are at risk for the development of pelvic actinomycosis. These patients may have vaginal discharge, pelvic pain, abdominal pain, menorrhagia, fever, a pelvic mass, a history of pelvic inflammatory disease, or a history of prolonged IUD use. The risk is more significant if the IUD has been in place for longer than 2 to 3 years. These devices are thought to cause an inflammatory response in the endometrium with focal necrosis. This anaerobic environment encourages the growth of *A. israelii*. In such patients, removal of the IUD plus treatment with antibiotics is necessary.[15] Pelvic actinomycosis can develop in both men and women; it can appear as a tumorlike mass and be confused with malignancy. A urachus or urachal remnant is also a risk factor.[35]

Establishing the diagnosis of pulmonary actinomycosis depends on having a high index of suspicion because neither the clinical nor the radiographic findings are specific. Patients may have a history of or risk factors for aspiration. Adults with thoracic actinomycosis usually have abnormal local defenses, such as chronic bronchitis, bronchiectasis, or emphysema; however, pediatric patients have been shown to have predisposing factors less often.[29,61] This type of actinomycosis also occurs after the introduction of a colonized foreign body. Patients with oral, cervicofacial, or abdominal actinomycotic infections are at risk for the development of pulmonary infection from direct or hematogenous spread. A history of these preexisting infections should heighten the index of suspicion. Pulmonary actinomycosis may be manifested as an endobronchial infection, a tumorlike lesion, diffuse pneumonia, or a pleural effusion.[9,12,45] The presence of chronic pleural effusion, underlying lung changes, and periosteal rib involvement is indicative of actinomycosis but seldom is reported.[68] The principal symptoms include chest pain, fever, productive cough, and weight loss. Hemoptysis has been reported.[48] The infection frequently dissects along tissue planes and may extend through the chest wall or diaphragm and produce multiple sinuses (Figs. 148-3 and 148-4). These characteristic sinus tracts contain small abscesses and purulent drainage. Empyema necessitans has been reported in one child.[62] The differential diagnosis of pulmonary actinomycosis includes tuberculosis, lung abscess, nocardiosis, fungal infection, and botryomycosis.

Laryngeal actinomycosis rarely has been reported in older teenagers.[39] Colonization of the oropharynx with these organisms may be involved in the development of obstructive tonsillar hypertrophy.[46] Several adult cases of

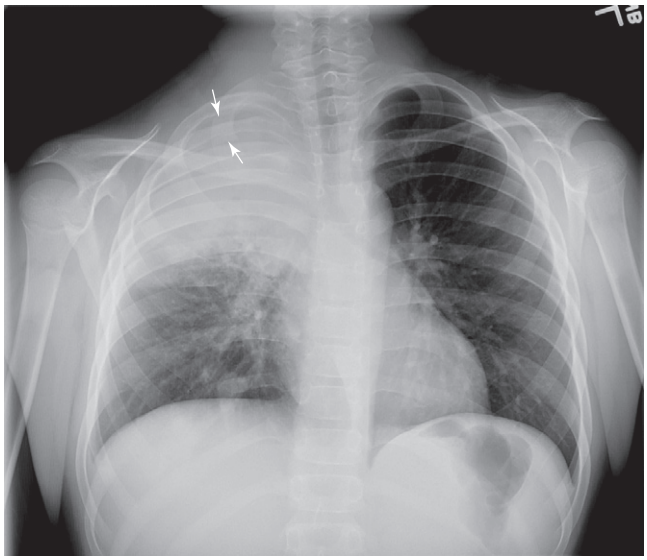

FIGURE 148-3 ■ Chest radiograph of a 10-year-old child with chronic pneumonia whose lung biopsy revealed sulfur granules and bacteria in the actinomycoses species.

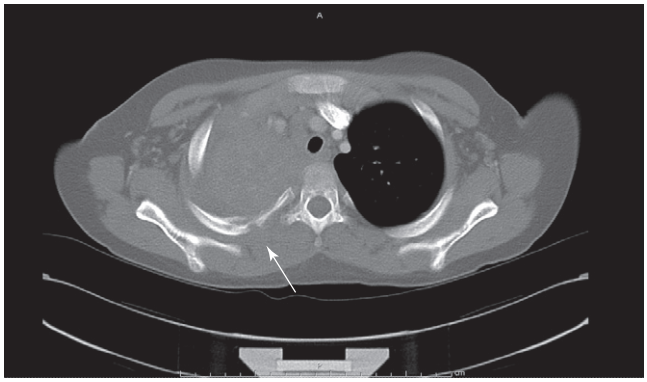

FIGURE 148-4 ■ Computed tomography of this child's chest revealing bony erosion *(arrow)* of the ribs next to the infiltrate.

*References 2, 3, 11, 27, 32, 50.

pericardial actinomycosis are reported in the literature.[16] Bacteremia is reported in all age groups, including neonates.[8,26,33] Septic arthritis has been reported.[30] Brain abscess has occurred in a child with complex congenital heart disease.[41] A case has been recently reported of purulent meningitis caused by *Actinomyces* spp.[24] Actinomycetes have been isolated from nearly every organ in the body, including the kidneys, brain, heart, breasts, mastoids, male genitourinary tract, and eyes. Osteomyelitis is reported in children, often involving the mandible, but other bones have been infected as well.[51] *T. pyogenes* has been implicated only rarely as a cause of human infection, but cases of septicemia, endocarditis, meningitis, arthritis, empyema, pneumonia, otitis media, cystitis, mastoiditis, appendicitis, and cutaneous infection have been reported.[14,18]

Actinomycosis does not seem to be associated commonly with human immunodeficiency virus infection, perhaps because these patients frequently are in the health care system and have close monitoring. They may be treated for other infections with intermittent antibiotic therapy that also treats subclinical actinomycosis. Thoracic, esophageal, oral, and anal cases have been reported, however, and all immunocompromised hosts should be monitored closely for this potential group of pathogens.[2,3,42,64] Actinomycosis has been reported in patients with chronic granulomatous disease, including cervicofacial, pulmonary, and hepatic infection.[49] Primary cutaneous actinomycosis caused by *A. bovis* has been reported in a patient with common variable immunodeficiency.[34]

*A. actinomycetemcomitan*s is a pathogen in at least 30 percent of actinomycotic infections[36] (see Chapter 127). Failure to recognize this organism and treat it adequately has resulted in clinical relapse and deterioration in patients infected with actinomycosis.[38,71] Severe forms of periodontitis, particularly localized aggressive periodontitis, also are associated with this pathogen, and studies have shown that it is related strongly to children in the pubertal age group.[1,56] It is characterized by loss of attachment of bone around the permanent incisors and permanent molars, which occurs rapidly. Treatment consists of local débridement and antibiotic therapy, usually metronidazole alone or in combination with amoxicillin. *A. actinomycetemcomitan*s also is an important pathogen in Papillon-Lefèvre syndrome, an autosomal recessive disorder characterized by prepubertal periodontitis and palmar-plantar hyperkeratosis.[52] Additionally, it is one of the HACEK (HACEK includes *Haemophilus aphrophilus*, *Cardiobacterium hominis*, *E. corrodens*, and *Kingella kingae*) organisms that have a propensity for infecting heart valves. The endocarditis caused by this organism usually is insidious, with fever occurring in fewer than 50 percent of cases.[36] This organism also has been reported to cause pericarditis, meningitis, brain abscess, parotitis, synovitis, osteomyelitis, urinary tract infection, pneumonia, and empyema.[36] Cases of endophthalmitis[5,67] and cavernous sinus infection also have been reported, as well as facial cellulitis.[22,69] In recent years there has been increasing evidence of the potential association between periodontal and cardiovascular diseases. Periodontitis may be an independent risk factor for cardiovascular disease. A recent study revealed the presence of *A. actinomycetemcomitans* by PCR in atheromatous plaques obtained from carotid endarterectomies.[17]

DIAGNOSIS

To establish a definitive diagnosis of actinomycosis, the clinician must isolate the causative organism from tissue or pus from a normally sterile body site, such as the lungs. Isolation of the organism from the oral cavity or the female genital tract without clinical evidence of disease, therefore, is not diagnostic. Because the organisms that cause actinomycosis are exquisitely sensitive to antibiotics, clinical specimens must be obtained before initiating their use. The specimens should be processed carefully to maintain anaerobic conditions. They should undergo routine Gram stain, which will reveal gram-positive rods that are not acid-fast and appear in diphtheroidal arrangements with or without branching.[19] Gram stain is more sensitive than is culture, particularly if the patient has been given antibiotics. Immunofluorescence is available for confirmation of organisms in biopsy specimens with suggestive Gram stains. Growth on media usually appears within 5 to 7 days but may take 2 to 4 weeks.[53] 16S rDNA sequencing and 16S rDNA–based nested polymerase chain reaction (PCR) have been used in some research laboratories for precise species identification.[50,57,60]

True microbiologic identification of these organisms occurs uncommonly, and the diagnosis most often rests on the clinical picture, along with identification of the characteristic sulfur granules. The sulfur granules may be found by drawing pus from a lesion, on the bandage covering the lesion, or in surgical specimens (see Fig. 148-2). Pus that is poured down the side of a glass will leave sulfur granules adhering to the sides so that the granules can be identified more easily. On hematoxylin and eosin stain, the granules are eosinophilic or variably surrounded by a radiating fringe of eosinophilic clubs. The formation of granules is a hallmark of actinomycosis and is related to a bacterial secretion that cements elements of *Actinomyces* spp. together. However, the formation of granules related to various nonfilamentous bacteria such as *Staphylococcus aureus* or *Pseudomonas* spp. is termed *botryomycosis* or *bacterial pseudomycosis*, and they can look grossly similar to the typical sulfur granules of actinomycosis.[12] Washed, crushed actinomycotic granules or well-mixed pus in the absence of granules is cultured on a rich medium, such as brain-heart infusion blood agar, and incubated anaerobically and aerobically with added carbon dioxide. Plates can be examined at 24 hours and after 5 to 7 days for the characteristic colonies of *Actinomyces* spp.[19] Currently, no skin tests are available for screening purposes, and no useful serologic tests are available for actinomycosis.

Various imaging modalities have been useful in diagnosing and characterizing actinomycosis. Computed tomography has been shown to help differentiate between inflammatory masses and tumors. Additionally, the location, extension, and relationship between the mass and surrounding structures can be defined better. Ultrasonography has been shown in one report to reveal a mass with an ill-defined margin that was hypoechoic

with intrinsic hyperechoic spots.[55] Magnetic resonance imaging has been used for diagnosing actinomycotic brain abscess, as well as actinomycosis of the chest, spine, soft tissues, and mandible.[29,41,51]

A. actinomycetemcomitans can be cultured on blood and chocolate agar but grows poorly on MacConkey agar. Cultures require incubation in an enhanced carbon dioxide atmosphere. Growth of the organism in a blood culture may take as long as 9 days in patients with endocarditis, and thus cultures should be held longer. On Gram stain, the organism appears coccoid to coccobacillary. Molecular techniques based on nonamplification nucleic acid probes or on PCR can provide rapid and accurate identification of *A. actinomycetemcomitans*.[31] Because of the frequency of coinfection with this organism in cases of actinomycosis, attempts always should be made to isolate this organism in these patients.

TREATMENT

The mainstays of therapy for actinomycosis remain surgical débridement or removal of the lesion and prolonged antimicrobial therapy. Most experts still recommend drainage of abscesses, fistulotomy, sinus tract excision, and debulking of large masses, although numerous successful outcomes with antimicrobial therapy alone are reported in the literature.[37,53] The option to treat medically and observe for clinical response seems reasonable in a stable, noncritical patient. The antibiotic of choice is penicillin. The recommended total duration of therapy ranges from 6 to 12 months. For patients who are allergic to penicillin, tetracycline or erythromycin is acceptable. Other alternatives are clindamycin, chloramphenicol, and the third-generation cephalosporins. Susceptibility breakpoints for linezolid, imipenem, meropenem, or piperacillin-tazobactam have not been established, but the minimal inhibitory concentrations appear favorable.[20,60] Most isolates are clearly resistant to ciprofloxacin and metronidazole.[20,60] Administration of daily ceftriaxone for 3 weeks followed by prolonged administration of ampicillin was effective treatment in one case.[61] Thoracic actinomycosis has been treated successfully with a total duration of therapy of only 4 months.[58] Two studies in adults with thoracic actinomycosis revealed good success rates with less than 14 days of intravenous therapy followed by prolonged oral treatments. Some of those patients required surgery.[7,63] Failure to respond to adequate treatment has been noted in patients with underlying malignancy. Because the disease is an uncommon occurrence in children and no significant randomized prospective treatment trials have been performed, most authors still recommend 4 to 6 weeks of intravenous therapy followed by oral therapy for a prolonged period. The total duration of therapy is based on clinical and radiographic follow-up. Numerous cases of actinomycosis in various forms that have been treated successfully with a total duration of 3 to 6 months of therapy are reported in the literature. Osteomyelitis may require longer therapy.[51] Certainly in cases for which surgical débridement or incision and drainage have been performed, a shorter duration of therapy would be reasonable provided that close clinical follow-up can be ensured.

A. actinomycetemcomitans is susceptible to the newer cephalosporins, rifampin, trimethoprim-sulfamethoxazole, aminoglycosides, quinolones, tetracycline, azithromycin, and chloramphenicol. It also is susceptible to penicillin and ampicillin in vitro, but test results do not necessarily correlate with clinical outcome. Vancomycin, erythromycin, and clindamycin have very little activity against this organism. Treatment of aggressive periodontal disease consists of local débridement and antibiotic therapy. Metronidazole and amoxicillin appear to be effective in suppressing *A. actinomycetemcomitans* to below the level of detection.[13] Endocarditis caused by this organism has been treated successfully with a combination of ampicillin and gentamicin.[43] Cefotaxime or ceftriaxone also is acceptable.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Alani A, Seymour R. Aggressive periodontitis: how does an understanding of the pathogenesis affect treatment? *Dent Update* 2011;**38**:511–21.
7. Choi J, Koh WJ, Kim TS, et al. Optimal duration of IV and oral antibiotics in the treatment of thoracic actinomycosis. *Chest* 2005;**128**:2211–17.
17. Figuero E, Sanchez-Beltran M, Cuesta-Frechoso S, et al. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol* 2011;**82**:1469–77.
22. Hsieh CJ, Hwang KP, Kuo KC, Hsueh PR. Facial cellulitis because of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* and *Capnocytophaga* species in an immunocompetent patient. *J Microbiol Immunol Infect* 2011;**44**:149–51.
23. Hwang SS, Park SD, Jang IH, et al. *Actinomyces graevenitzii* bacteremia in a patient with alcoholic liver cirrhosis. *Anaerobe* 2011;**17**:87–9.
24. Imamura K, Kamitani H, Nakayasu H, Asai Y, Nakashima K. Purulent meningitis caused by *Actinomyces* successfully treated with rifampicin: a case report. *Intern Med* 2011;**50**:1121–5.
34. Mansouri P, Farshi S, Khosravi A, Naraghi ZS. Primary cutaneous actinomycosis caused by *Actinomyces bovis* in a patient with common variable immunodeficiency. *J Dermatol* 2011;**38**:911–15.
49. Reichenbach J, Lopatin U, Mahlaoui M, et al. *Actinomyces* in chronic granulomatous disease: an emerging and unanticipated pathogen. *Clin Infect Dis* 2009;**49**:1703–10.
63. Song J, Park HY, Jeon K, et al. Treatment of thoracic actinomycosis: a retrospective analysis of 40 patients. *Ann Thorac Med* 2010;**5**:80–5.
66. Sullivan DC, Chapman SW. Bacteria that masquerade as fungi: actinomycosis/*Nocardia*. *Proc Am Thorac Soc* 2010;**7**:216–21.

The full reference list for this chapter is available at expertconsult.com

REFERENCES

1. Alani A, Seymour R. Aggressive periodontitis: how does an understanding of the pathogenesis affect treatment? *Dent Update* 2011; **38**:511–21.
2. Arora AK, Nord J, Olofinlade O, Javors B. Esophageal actinomycosis: a case report and review of the literature. *Dysphagia* 2003;**18**:27–31.
3. Bauer P, Sultan S, Atienza P. Perianal actinomycosis: diagnostic and management considerations—a review of six cases. *Gastroenterol Clin Biol* 2006;**30**:29–32.
4. Beighton D. The complex oral microflora of high-risk individuals and groups and its role in the caries process. *Commun Dent Oral Epidemiol* 2005;**33**:248–59.
5. Binder MI, Chua J, Kaiser PK, et al. *Actinobacillus actinomycetemcomitans* endogenous endophthalmitis: report of two cases and review of the literature. *Scand J Infect Dis* 2003;**35**:133–6.
6. Bugmann P, Birrauz J, Barrazzone C, et al. Severe bronchial synechia after removal of a long-standing bronchial foreign body: a case report to support control bronchoscopy. *J Pediatr Surg* 2003;**38**:E14.
7. Choi J, Koh WJ, Kim TS, et al. Optimal duration of IV and oral antibiotics in the treatment of thoracic actinomycosis. *Chest* 2005;**128**:2211–17.
8. Cone LA, Leung MM, Hirschberg J. *Actinomyces odontolyticus* bacteremia. *Emerg Infect Dis* 2003;**9**:1629–32.
9. Coodley EL, Yoshinaka R. Pleural effusion as the major manifestation of actinomycosis. *Chest* 1994;**106**:1615–17.
10. Corby PM, Lyons-Weiler J, Bretz WA, et al. Microbial risk indicators of early childhood caries. *J Clin Microbiol* 2005;**43**:5753–9.
11. Coremans G, Margaritis V, Van Poppel HP, et al. Actinomycosis, a rare and unsuspected cause of anal fistulous abscess: report of three cases and review of the literature. *Dis Colon Rectum* 2005; **48**:575–81.
12. de Montpreville V, Nashashibi N, Dulmet EM. Actinomycosis and other bronchopulmonary infections with bacterial granules. *Ann Diagn Pathol* 1999;**3**:67–74.
13. Dorfer CE. Antimicrobials for the treatment of aggressive periodontitis. *Oral Dis* 2003;**9**(Suppl. 1):51–3.
14. Drancourt M. Two cases of *Actinomyces pyogenes* infection in humans. *Eur J Microbiol Infect Dis* 1993;**12**:55–7.
15. Evans DPT. *Actinomyces israelii* in the female genital tract: a review. *Genitourin Med* 1993;**69**:54–9.
16. Fife TD, Finegold SM, Grennan T. Pericardial actinomycosis: case report and review. *Rev Infect Dis* 1991;**13**:120–6.
17. Figuero E, Sanchez-Beltran M, Cuesta-Frechoso S, et al. Detection of periodontal bacteria in atheromatous plaque by nested polymerase chain reaction. *J Periodontol* 2011;**82**:1469–77.
18. Gahrn-Hansen B, Frederiksen W. Human infections with *Actinomyces pyogenes* (*Corynebacterium pyogenes*). *Diagn Microbiol Infect Dis* 1992;**15**:349–54.
19. Gerencser MA. *Actinomyces*, *Arachnia*, and *Streptomyces*. In: Baron S, editor. *Medical microbiology*. 3rd ed. New York: Churchill Livingstone; 1991. p. 469–77.
20. Goldstein EJC, Citron DM, Merriam CV, et al. In vitro activities of the new semisynthetic glycopeptide telavancin (TD-6424), vancomycin, daptomycin, linezolid, and four comparator agents against anaerobic gram-positive species and *Corynebacterium* ssp. *Antimicrob Agents Chemother* 2004;**48**:2149–52.
21. Henderson B, Wilson M, Sharp L, Ward JM. Actinobacillus actinomycetemcomitans. *J Med Microbiol* 2002;**51**:1013–20.
22. Hsieh CJ, Hwang KP, Kuo KC, Hsueh PR. Facial cellulitis because of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* and *Capnocytophaga* species in an immunocompetent patient. *J Microbiol Immunol Infect* 2011;**44**:149–51.
23. Hwang SS, Park SD, Jang IH, et al. *Actinomyces graevenitzii* bacteremia in a patient with alcoholic liver cirrhosis. *Anaerobe* 2011; **17**:87–9.
24. Imamura K, Kamitani H, Nakayasu H, Asai Y, Nakashima K. Purulent meningitis caused by *Actinomyces* successfully treated with rifampicin: a case report. *Intern Med* 2011;**50**:1121–5.
25. Karatoprak N, Atay Z, Erol N, et al. Actinomycotic suppurative thyroiditis in a child. *J Trop Pediatr* 2005;**51**:383–5.
26. Knee DS, Christ MJ, Gries DM, Thompson MW. *Actinomyces* species and cerclage placement in neonatal sepsis: a case report. *J Perinatol* 2004;**24**:389–91.
27. Lakshmana Kumar YC, Javherani R, Malini A, Prasad SR. Primary hepatic actinomycosis. *Trans R Soc Trop Med Hyg* 2005;**99**: 868–70.
28. Latawiec-Mazurkiewicz I, Juszkiewicz P, Pacanowski J, et al. Tumour-like inflammatory abdominal conditions in children. *Eur J Pediatr Surg* 2005;**15**:38–43.
29. Lee JP, Rudoy R. Pediatric thoracic actinomycosis. *Hawaii Med J* 2003;**62**:30–2.
30. Lequerre T, Nouvellon M, Kraznowska K, et al. Septic arthritis due to *Actinomyces naeslundii*: report of a case. *Joint Bone Spine* 2002; **69**:499–501.
31. Leys JE, Griffen AL, Strong SJ, et al. Detection and strain identification of *Actinobacillus actinomycetemcomitans* by nested PCR. *J Clin Microbiol* 1994;**32**:1288–94.
32. Lin CH, Chen AC, Lin HC, et al. Abdominal actinomycosis complicated with hydronephrosis. *J Formos Med Assoc* 2005;**104**: 666–9.
33. Mann C, Dertinger S, Hartmann G, et al. *Actinomyces neuii* and neonatal sepsis. *Infection* 2002;**30**:178–80.
34. Mansouri P, Farshi S, Khosravi A, Naraghi ZS. Primary cutaneous actinomycosis caused by *Actinomyces bovis* in a patient with common variable immunodeficiency. *J Dermatol* 2011;**38**:911–15.
35. Marella VK, Hakimian O, Wise GJ, Silver DA. Pelvic actinomycosis: urologic perspective. *Clin Urol* 2004;**30**:367–76.
36. McGowan JE, Steinberg JP. Other gram-negative bacilli. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practices of infectious diseases*. New York: Churchill Livingstone; 1995. p. 2106–7.
37. Miyamoto MI, Fang FC. Pyogenic liver abscess involving *Actinomyces*: case report and review. *Clin Infect Dis* 1993;**16**:303–9.
38. Morris JF, Sewell DL. Necrotizing pneumonia caused by mixed infection with *Actinobacillus actinomycetemcomitans* and *Actinomyces israelii*: case report and review. *Clin Infect Dis* 1994;**18**:450–2.
39. Nelson EG, Tybor AG. Actinomycosis of the larynx. *Ear Nose Throat J* 1992;**71**:356–8.
40. Reference deleted in proofs.
41. Olah E, Berger C, Boltshauser E, Nadal D. Cerebral actinomycosis before adolescence. *Neuropediatrics* 2004;**35**:239–41.
42. Ossorio MA, Fields CL, Byrd RP, Roy TM. Thoracic actinomycosis and human immunodeficiency virus infection. *South Med J* 1997;**90**:1136–8.
43. Paturel L, Casalta JP, Habib G, et al. *Actinobacillus actinomycetemcomitans* endocarditis. *Clin Microbiol Infect* 2004;**10**:98–118.
44. Permpanich P, Kowolik MJ, Galli DM. Resistance of fluorescent-labelled *Actinobacillus actinomycetemcomitans* strains to phagocytosis and killing by human neutrophils. *Cell Microbiol* 2006;**8**:72–84.
45. Pinarli FG, Mutlu B, Celenk C, et al. Pulmonary actinomycosis mimicking chest wall tumor in a child. *Jpn J Infect Dis* 2005; **58**:247–9.
46. Pransky M, Feldman JI, Kearns DB, et al. Actinomycosis in obstructive tonsillar hypertrophy and recurrent tonsillitis. *Arch Otolaryngol* 1991;**117**:883–5.
47. Pulverer G, Schutt-Gerowitt H, Schaal KP. Human cervicofacial actinomycoses: microbiological data for 1997 cases. *Clin Infect Dis* 2003;**37**:490–7.
48. Reechaipichitkul W, Napaprasit T, Puapairoj A, Prathanee S. Pulmonary actinomycosis presenting with prolonged fever and massive hemoptysis: a case report. *Southeast Asian J Trop Med Public Health* 2005;**36**:1268–71.
49. Reichenbach J, Lopatin U, Mahlaoui M, et al. *Actinomyces* in chronic granulomatous disease: an emerging and unanticipated pathogen. *Clin Infect Dis* 2009;**49**:1703–10.
50. Riegert-Johnson DL. Thrombotic thrombocytopenic purpura associated with a hepatic abscess due to *Actinomyces turicensis*. *Clin Infect Dis* 2002;**35**:636–7.
51. Robinson JL, Vaudry WL, Dobrovolsky W. Actinomycosis presenting as osteomyelitis in the pediatric population. *Pediatr Infect Dis J* 2005;**24**:365–9.
52. Rudiger S, Petersilka G, Flemmig TF. Combined systemic and local antimicrobial therapy of periodontal disease in Papillon-Lefèvre syndrome. *J Clin Periodontol* 1999;**26**:847–54.
53. Russo TA. Agents of actinomycosis. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practices of infectious diseases*. New York: Churchill Livingstone; 1995. p. 2280–8.
54. Sabbe LJM, Van De Merwe D, Schouls L, et al. Clinical spectrum of infections due to the newly described *Actinomyces* species *A.*

turicensis, *A. radingae*, and *A. europaeus*. *J Clin Microbiol* 1999; **37**:8–13.
55. Sa'do B, Kazunori Y, Yuasa K, et al. Multimodality imaging of cervicofacial actinomycosis. *Oral Surg Oral Med Oral Pathol* 1993; **76**:772–8.
56. Savitt ED, Kent RL. Distribution of *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. *J Periodontol* 1991;**62**: 490–4.
57. Siqueira JF Jr, Rocas IN. Polymerase chain reaction detection of *Propionibacterium propionicus* and *Actinomyces radicidentis* in primary and persistent endodontic infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2003;**96**:215–22.
58. Skoutelis A, Petrochilow J, Bassaris H. Successful treatment of thoracic actinomycosis with ceftriaxone. *Clin Infect Dis* 1994;**19**: 161–2.
59. Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* in periodontal disease. *Periodontology* 1999;**20**:7–13.
60. Smith AJ, Hall V, Thakker B, Gemmell CG. Antimicrobial susceptibility testing of *Actinomyces* species with 12 antimicrobial agents. *J Antimicrob Chemother* 2005;**56**:407–9.
61. Snape PS. Thoracic actinomycosis: an unusual childhood infection. *South Med J* 1993;**86**:222–4.
62. Solomon N, Young M, Robinson J. Paraspinal mass in a seven-year-old girl. *Pediatr Infect Dis J* 2005;**24**:475–9.
63. Song J, Park HY, Jeon K, et al. Treatment of thoracic actinomycosis: a retrospective analysis of 40 patients. *Ann Thorac Med* 2010; **5**:80–5.
64. Spadari F, Tartaglia GM, Spadari E, Fazio N. Oral actinomycosis in acquired immunodeficiency syndrome. *Int J STD AIDS* 1998; **9**:424–6.
65. Sugano S, Matuda T, Suzuki T, et al. Hepatic actinomycosis: case report and review of the literature in Japan. *J Gastroenterol* 1997; **32**:672–6.
66. Sullivan DC, Chapman SW. Bacteria that masquerade as fungi: actinomycosis/*Nocardia*. *Proc Am Thorac Soc* 2010;**7**:216–21.
67. Sullivan P, Clark WL, Kaiser PK. Bilateral endogenous endophthalmitis caused by HACEK microorganism. *Am J Ophthalmol* 2002;**133**:144–5.
68. Sumoza D, Raad I, Douglas E. Differentiating thoracic actinomycosis from lung cancer. *Infect Med* 2000;**17**:695–8.
69. Tobias S, Lee JH, Tomford JW. Rare *Actinobacillus* infection of the cavernous sinus causing painful ophthalmoplegia: case report. *Neurosurgery* 2002;**51**:807–9; discussion 809-810.
70. Trites J, Evans M. Actinomycotic thyroiditis in a child. *J Pediatr Surg* 1998;**33**:781–2.
71. Tyrrell J, Noone P, Prichard JS. Thoracic actinomycosis complicated by *Actinobacillus actinomycetemcomitans*: case report and review of literature. *Respir Med* 1992;**86**:341–3.
72. Reference deleted in proofs.

CHAPTER 149

Bacteroides, *Fusobacterium*, and *Prevotella*

Steven C. Buckingham

Bacteroides, *Fusobacterium*, and *Prevotella*, along with *Porphyromonas*, are the most prominent genera of clinically important anaerobic gram-negative bacilli. These bacteria, like other anaerobes, constitute a significant portion of the normal flora of humans, who become colonized with these and other anaerobic flora as early as the neonatal period. Anaerobic gram-negative rods can cause infections of various body sites in children, although their importance as pediatric pathogens has historically been underappreciated. Many infections with these and other anaerobic pathogens are never diagnosed as such because clinicians fail to use appropriate methods for collection or transportation of specimens or because laboratories fail to use appropriate methods for the isolation and identification of anaerobic bacteria.

BACTERIOLOGY AND TAXONOMY

Bacteria belonging to the genera *Bacteroides*, *Fusobacterium*, *Prevotella*, and *Porphyromonas* are obligately anaerobic gram-negative bacilli. The taxonomic classification of these and other anaerobic bacteria has undergone considerable revision in recent decades, based largely on the characterization of the bacteria with the use of nucleic acid analyses.

Bacteroides Species

Bacteroides spp. are strictly anaerobic, nonmotile gram-negative rods, about 0.5 to 2.0 by 1.6 to 12 μm, with rounded ends. In general, these bacteria are nonpigmented, saccharolytic, and bile-resistant and on blood agar they form smooth, white to gray colonies that are 1 to 3 mm in diameter. As recently as the 1980s, the genus *Bacteroides* comprised a large and heterogeneous group of anaerobic gram-negative bacilli. Over the past 3 decades, many species formerly assigned to this genus have been reassigned to other genera.[101] The most notable taxonomic shift occurred between 1988 and 1990, when numerous *Bacteroides* spp. were reclassified into the newly created genera *Prevotella* and *Porphyromonas*.[98-100] Historically, the *Bacteroides* spp. most frequently recovered in clinical specimens have been collectively classified as the so-called "*Bacteroides fragilis* group," consisting of *Bacteroides fragilis*, *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, *Bacteroides vulgatus*, and others. Taxonomic revisions based on nucleic acid analyses, however, have disrupted even this close-knit group, because *B. distasonis*, *Bacteroides goldsteinii*, and *Bacteroides merdae* have been reclassified under the genus *Parabacteroides* and *Bacteroides forsythus* has been renamed *Tannerella forsythensis*.[96,97] Today, most previously recognized *Bacteroides* spp. that were not members of the *B. fragilis* group have been moved to other genera. One noteworthy taxonomic misfit that long awaited reassignment was *Bacteroides ureolyticus;* in 2010, this organism was reclassified as *Campylobacter ureolyticus*.[113]

Resistance to multiple classes of antibiotics is a hallmark of *Bacteroides* spp., and resistance even to drugs traditionally considered to have broad activity against anaerobes has increased substantially over the past 2 decades. Most *Bacteroides* spp., and virtually all members of the *B. fragilis* group, produce a class 2e cephalosporinase, encoded by the *cepA* gene, which confers broad resistance to penicillins and first- and third-generation cephalosporins. This β-lactamase, however, is uniformly neutralized by the β-lactamase inhibitors clavulanate, sulbactam, and tazobactam. Another, less frequently encountered β-lactamase, encoded by *cfxA*, confers resistance to cefoxitin. Resistance to β-lactams also can result, less commonly, from alterations in the penicillin-binding proteins, PB1 and PB2, or from porin mutations, which alter the permeability of the bacterial outer membrane to β-lactams.[52,63,80,101]

Resistance to carbapenems remains rare among *Bacteroides* spp., although recent reports from Turkey and Taiwan reported resistance rates of 6 percent to imipenem and 8 percent to meropenem among *B. fragilis* group isolates.[78,80,111] Carbapenem resistance results from production of a class B metallo-β-lactamase encoded by either *cfiA* or *ccrA*. Expression of these genes requires the presence of special insertion sequences upstream of the gene, which act as promoters. Ominously, the resulting β-lactamase renders the organism resistant not only to carbapenems but also to all β-lactams, including the penicillin/β-lactamase inhibitor combinations (i.e., amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin-clavulanate, and piperacillin-tazobactam).[63,80,112]

Resistance to clindamycin, which has been increasingly documented among *Bacteroides* isolates in recent decades, usually results from MLS-type ribosomal alterations coded by various *erm* genes, although other mechanisms apparently account for resistance in some isolates.[63,80] Metronidazole resistance, which remains rare, results from the production of nitroimidazole reductases encoded by any of seven known *nim* genes. Expression of these genes requires the presence of upstream

insertion sequences, which act as promoters. In some cases, metronidazole resistance has been documented in *Bacteroides* isolates lacking *nim* genes, following prolonged exposure to the drug.[52,63,80] Resistance to moxifloxacin, which has increased rapidly since the late 1990s, results either from alterations in the DNA gyrase genes *gyrA* and *gyrB* or from increased production of efflux pumps.[63,80] Most *B. fragilis* group isolates are resistant to tetracycline, owing to ribosomal alterations encoded by *tetQ*, *tetM*, or *tet36* genes. Tigecycline has much better activity against *Bacteroides* spp., although some isolates are resistant by virtue of their production of a tetracycline-degrading mono-oxygenase encoded by *tetX*.[80]

Prevotella Species and *Porphyromonas* Species

The genus *Prevotella* includes more than 40 recognized species, all of which are moderately saccharolytic, bile-sensitive gram-negative bacilli that were formerly classified under the genus *Bacteroides*. Some species are pigmented (e.g., *Prevotella corporis*, *Prevotella denticola*, *Prevotella intermedia*, *Prevotella loeschii*, *Prevotella melaninogenica*, and *Prevotella nigrescens*), whereas others are nonpigmented (e.g., *Prevotella bivia*, *Prevotella disiens*, *Prevotella oris*, *Prevotella buccae*, and *Prevotella heparinolytica*).[69,101]

Compared with the *B. fragilis* group, *Prevotella* spp. are generally more susceptible to antibiotics. Resistance to penicillin is frequently observed, owing to the production of β-lactamases coded by *cfxA* or other genes, although most isolates remain susceptible to third-generation cephalosporins, cefoxitin, carbapenems, and penicillin/β-lactamase inhibitor combinations.[63,80] Resistance to metronidazole, which is mediated by *nim*-encoded production of nitroimidazole reductases, occurs infrequently. Clindamycin resistance, which has increased in prevalence in recent years, is associated with *ermF*- or *ermG*-mediated ribosomal modification.[80] Data on moxifloxacin's activity against *Prevotella* spp. are sparse, but available studies indicate that most isolates are susceptible.[58,62,76,78,119] *Prevotella* spp. are frequently resistant to tetracycline, owing to ribosomal alterations encoded by the *tetQ*, *tetM*, or *tetW* genes. Available data suggest that tigecycline, however, is highly active against *Prevotella* strains.[80]

The genus *Porphyromonas* consists of pigmented or nonpigmented gram-negative bacilli that, like *Prevotella* spp., were formerly classified under the genus *Bacteroides*. Unlike *Prevotella* spp., *Porphyromonas* spp. are asaccharolytic or only weekly saccharolytic.[69,101] In contrast to *Bacteroides* and *Prevotella* spp., antibiotic resistance is unusual among *Porphyromonas* spp. Penicillin resistance, mediated by *cfxA*-encoded β-lactamase production, occurs in a minority of clinical specimens but is somewhat more frequent in animal-derived specimens.[63,80,101] Carbapenems and penicillin/β-lactamase inhibitor combinations are uniformly active against *Porphyromonas* spp. Resistance to clindamycin, associated with *ermF* and *ermG* genes, occurs in fewer than 10 percent of strains. Resistance to metronidazole and tetracycline, although described, is only rarely observed.[80]

Fusobacterium Species

Fusobacterium spp. are nonmotile, anaerobic gram-negative rods that metabolize peptones or carbohydrates to produce butyric acid. Since the early 1980s, several new *Fusobacterium* spp. have been described (*Fusobacterium periodonticum*, *Fusobacterium simiae*, *Fusobacterium ulcerans*, *Fusobacterium equinum*, and *Fusobacterium canifelinum*), whereas two long-standing members of the genus have been reclassified to other genera (*Fusobacterium plautii* and *Fusobacterium prausnitzii*). Moreover, three newly described species were subsequently reclassified to other genera (*Fusobacterium alocis*, *Fusobacterium sulci*, and *Fusobacterium polysaccharolyticum*), and another (*Fusobacterium pseudonecrophorum*) was described but found to be identical to *Fusobacterium varium*.[101] The type species, *Fusobacterium nucleatum*, is the one most frequently recovered from clinical specimens.[11]

Fusobacterium spp. are usually susceptible to penicillin, third-generation cephalosporins, clindamycin, and moxifloxacin, and more than 90 percent of strains are susceptible to cefoxitin, penicillin/β-lactamase inhibitor combinations, carbapenems, and metronidazole. In one study, all isolates of *F. nucleatum* and *Fusobacterium necrophorum* were susceptible to imipenem, amoxicillin-clavulanate, cefoxitin, and clindamycin. Isolates of the *Fusobacterium mortiferum/varium* group, however, demonstrated reduced susceptibility rates to imipenem (85%), amoxicillin-clavulanate (90%), and clindamycin (55%).[116] Tetracycline resistance, associated with *tetW* and *tetM* genes, is unusual among *Fusobacterium* spp. but has been described.[63,80] Erythromycin has unreliable activity against *Fusobacterium* spp., but limited data suggest that azithromycin is active against more than 90 percent of isolates in vitro.[57,61,69,75,114]

EPIDEMIOLOGY

A variety of aerobic and anaerobic bacteria normally colonize the skin and mucosal surfaces of humans. In the oral cavity, gastrointestinal tract, and female genital tract, anaerobes vastly outnumber aerobes; in the colon, where hundreds of different anaerobic species are found, anaerobes account for more than 99.9 percent of all bacteria present.[38,80]

The predominance of anaerobes among the normal microbial flora stands in stark contrast to the infrequency with which these organisms cause infections. Nonetheless, several types of infections routinely involve anaerobic bacteria, such as odontogenic infections, human and animal bite–associated infections, aspiration pneumonia, peritonitis in the presence of a ruptured viscus, amnionitis, endometritis, necrotizing infections of soft tissues, and abscesses of the brain, lung, tubo-ovarian tract, and perirectal area. Underlying conditions that predispose patients to anaerobic infections include trauma, foreign bodies, malignancy, recent surgery, shock, colitis, vascular disease, and current or recent infection with aerobic or facultative organisms.[38] In general, anaerobic infections occur less frequently in children than in adults, probably because children are

less likely than are adults to have these types of predisposing conditions.

Taxonomic revisions aside, organisms traditionally classified as belonging to the *B. fragilis* group comprise much of the normal human colonic flora and also form part of the normal flora of the female genital tract. *B. ureolyticus*, long recognized as a normal inhabitant of the oral cavity and intestinal tract (but not a member of the *B. fragilis* group), was reclassified in 2010 as *Campylobacter ureolyticus.*[113] *Bacteroides* spp., which are the most frequently recovered anaerobic bacteria from clinical specimens, are frequently found in intra-abdominal infections, especially abscesses, and occasionally in extra-abdominal infections, such as aspiration pneumonia, chronic otitis media, and brain abscesses.[57,69]

Prevotella spp. and *Porphyromonas* spp. are normal inhabitants of the oral cavity, gastrointestinal tract, and female urogenital tract of humans. These organisms are associated with infections in a diverse range of body sites, most commonly including abscesses of the head and neck region, chronic otitis media, sinusitis, aspiration pneumonia, and intra-abdominal infections. In a series of 504 pediatric clinical isolates of *Prevotella* and *Porphyromonas* spp., the most frequently recovered organisms were *Prevotella melaninogenica* (32%), *Prevotella intermedia* (21%), *Porphyromonas asaccharolytica* (17%), *Prevotella oris-buccae* (11%), and *Prevotella oralis* (11%).[31] *Prevotella bivia* and *Prevotella disiens* have been associated with genital tract infections in female adults, although they may also be recovered from oral infections. *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Porphyromonas endodontalis* are particularly involved in adult periodontal disease and various dental infections.[57,69]

Fusobacterium spp. also constitute part of the normal flora of the human oral cavity and gastrointestinal tract, and they are associated with a range of pediatric infections similar to those involving *Prevotella* spp. and *Porphyromonas* spp. The *Fusobacterium* spp. most commonly isolated from clinical specimens is *F. nucleatum*, followed by *F. necrophorum;* organisms of the *F. varium*-mortiferum *group* are rarely recovered from clinical specimens. The virulent pathogen *F. necrophorum* is occasionally isolated from peritonsillar abscesses and is the cause of Lemierre disease (see later discussion).[26,57,69,80]

PATHOGENESIS

For the most part, anaerobic bacteria and humans enjoy a mutually beneficial relationship. The colon, for example, provides a home for the billions of anaerobic bacteria that make up the normal intestinal flora of humans. In turn, the presence of *Bacteroides* spp. and other colonizing anaerobic flora serve beneficial functions for humans, through such mechanisms as occupying an ecologic niche that might otherwise be invaded by enteric pathogens (a process known as colonization resistance); inducing tolerance to microbial epitopes, thereby serving to regulate the immune system; and breaking down otherwise indigestible plant polysaccharides while also producing vitamin K. Infections with anaerobic bacteria usually arise either because mucosal surfaces become disrupted, allowing these opportunistic pathogens access to normally sterile tissues, or because anaerobes are directly inoculated into such tissues, as occurs with traumatic or bite wounds.[80,120] Pathologic characteristics of anaerobic infections include suppuration, abscess formation, tissue destruction, foul odor, and, occasionally, formation of gas.

The pathogenesis of anaerobic infections reflects a complex interaction among bacterial virulence factors, host defense mechanisms, and synergy with other organisms. The elucidation of the complete genome sequence of *B. fragilis*, *B. thetaiotaomicron*, *F. nucleatum*, and *Porphyromonas gingivalis* has facilitated the identification of a host of genes encoding putative virulence determinants. These include gene sequences encoding fimbriae, hemagglutinins, proteinases (e.g., immunoglobulin proteases and collagenases), and hemolysins, which collectively serve to enhance pathogenicity by facilitating adherence to other bacteria and to host cells, thus disrupting the integrity of host tissues and interfering with host immune responses.* Another key virulence factor for these bacteria is their possession of a capsule, which allows the organisms to evade phagocytosis and which is apparently important to abscess formation.[21,31,83,86] The genomes of *B. fragilis* and *Porphyromonas gingivalis* also include aerotolerance operons, which encode proteins that allow the organisms to survive despite exposure to limited quantities of oxygen.[83,108] Moreover, enterotoxin-producing *B. fragilis* isolates have been identified in livestock and humans, and the presence of these bacteria has been associated with cases of diarrhea in children and adults.[64,80,117]

Anaerobic infections are typically polymicrobial and often involve multiple species of both aerobic and anaerobic flora. Experiments in mice, which evaluated mixed aerobic-anaerobic infections involving *Bacteroides* spp., *Prevotella* spp., and *Porphyromonas* spp., demonstrated that while the growth of both aerobes and anaerobes is enhanced in such infections, the enhancement of the aerobes' growth is by far more pronounced. The factors responsible for aerobic-anaerobic synergism are not fully elucidated. Some have postulated that the bacterial species involved assist one another by providing essential growth factors, interfering with phagocytic uptake and killing, secreting β-lactamase into the extracellular milieu, or altering the oxidation-reduction potential of infected tissue.[20,31]

CLINICAL MANIFESTATIONS

Serious anaerobic infections are seen more frequently in compromised hosts or in neonates than in otherwise healthy children. Outcomes of severe anaerobic infections are tied to patients' underlying disease states and to the promptness with which appropriate antimicrobial therapy is started.

Bacteremia

Anaerobic bacteremia is unusual in children; and when it does occur, it usually arises from a primary focal

*References 46, 64, 70, 74, 83, 86, 120.

anaerobic or mixed aerobic-anaerobic infection. The risk for anaerobic bacteremia is elevated in patients compromised by a variety of conditions, including recent gastrointestinal or gynecologic surgery, dental manipulation, malignancy, immunosuppression, neonatal status, diabetes mellitus, and asplenia.[16,34,38,80] Among neonates, risk factors for anaerobic bacteremia include premature birth, maternal perinatal complications (e.g., premature rupture of membranes), and necrotizing enterocolitis.[25]

Overall, *B. fragilis* is the most frequently identified anaerobic bloodstream isolate. The specific anaerobes recovered from blood cultures vary depending on the entry portal: when the primary focus is in the gastrointestinal tract, *Bacteroides* spp. and *Clostridium* spp. predominate; in cases associated with oropharyngeal or pulmonary foci, pigmented *Prevotella* and *Porphyromonas* spp., *Fusobacterium* spp., and anaerobic gram-positive cocci are more likely. The presenting features of anaerobic bacteremia in children are similar to those associated with bacteremia caused by aerobic organisms. Fever, chills, and leukocytosis are frequently encountered, whereas hypotension, shock, and disseminated intravascular coagulation are less common findings. Mortality rates of 5 to 10 percent have been reported among children with anaerobic bacteremia.[16,34,38,80] Among neonates, reported mortality is higher among those with bacteremia caused by *Bacteroides* spp. (34%) than with other anaerobic species (17%).[25]

Head and Neck Infection

Anaerobes are usually involved in infections of dental origin, such as gingivitis, periodontitis, and periapical and dental abscesses. The most commonly implicated anaerobes in such infections are *Prevotella* spp. (particularly *P. melaninogenica*) and *Porphyromonas* spp. (particularly *P. gingivalis*).[38,80] Often these are found in mixed infections along with oral aerobic flora, including *Streptococcus salivarius* and microaerophilic streptococci. *Fusobacterium* spp. can also be recovered in odontogenic infections and are particularly associated with the ulcerative form of gingivitis known as Vincent angina (trench mouth).[38]

Prevotella and *Porphyromonas* spp. are also frequently recovered from various suppurative perioral infections, such as retropharyngeal and peritonsillar abscesses, cervical lymphadenitis, deep neck abscesses, thyroiditis, parotitis, infected cysts, and postsurgical wound infections involving the oral cavity. Occasionally, these infections also involve *Fusobacterium* spp., *Bacteroides* spp., and/or anaerobic gram-positive cocci, along with oral and nasopharyngeal aerobic flora. Anaerobes, including *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp., may be involved in the pathogenesis of recurrent tonsillitis, and production of β-lactamase by these organisms has been postulated to explain the failure of penicillin to eradicate pharyngeal carriage of *Streptococcus pyogenes* in some children.[38]

Rarely, pharyngotonsillitis and tonsillar abscesses can be complicated by Lemierre disease (also known as postanginal sepsis or human necrobacillosis), a syndrome characterized by *Fusobacterium* bacteremia, septic thrombophlebitis of the internal jugular and facial veins, and metastatic emboli to the lung and other distant organs. *F. necrophorum* is responsible for more than 80 percent of all cases. Occasionally, *F. nucleatum* or other *Fusobacterium* spp. are involved, often in combination with other anaerobic or aerobic flora. Lemierre disease is most common among adolescents and young adults but can occur in patients of any age. In the preantibiotic era, most patients with this syndrome died of overwhelming sepsis; more recently, mortality rates of 4 to 22 percent have been reported.[80,93,118]

Although anaerobic gram-negative bacilli have not been implicated in the pathogenesis of acute otitis media, these organisms are present in up to 50 percent of middle ear fluid cultures from patients with chronic suppurative otitis media or infected cholesteatomas and in nearly all patients with chronic mastoiditis. The microbial pathogens recovered from such patients include *Prevotella*, *Porphyromonas*, *Bacteroides*, and *Fusobacterium* spp., along with anaerobic gram-positive cocci and various aerobes, most notably *Staphylococcus aureus* and *Pseudomonas aeruginosa*.[38,40]

Anaerobes are not usually associated with acute bacterial rhinosinusitis, but they are frequently implicated as pathogens in children with chronic sinusitis (i.e., >30 days' symptoms). In one study, sinus aspirate cultures from 37 children with chronic sinusitis yielded an average of 2.7 anaerobes and 0.6 aerobes per patient. Anaerobic pathogens associated with chronic sinusitis include *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp., along with members of the *B. fragilis* group.[18,26,38]

Central Nervous System

Anaerobes can be isolated from the majority of intracranial abscesses that arise in association with sinusitis, otitis media, or dental infection. In one study, cultures of brain abscess specimens from 23 children yielded 11 aerobic and 44 anaerobic isolates and cultures of subdural empyema fluid from 16 children yielded 6 aerobic and 35 anaerobic isolates. Anaerobic gram-positive cocci were the anaerobes most frequently recovered from these patients, followed by *Fusobacterium* spp. (especially *F. nucleatum*), *Bacteroides* spp. (including *B. fragilis* group members), *Prevotella* spp. and *Actinomyces* species.[24] A similar range of anaerobes have been isolated, alone or along with aerobes, from epidural abscesses found in patients (primarily males aged 10 to 14 years) with frontal sinusitis complicated by a Pott puffy tumor.[4,5,55] *B. fragilis* group members have been particularly associated with temporal lobe abscesses arising from chronic otitis media.[2]

Anaerobic meningitis occurs rarely in children. The symptoms, signs, and laboratory characteristics of children with anaerobic meningitis are similar to those of children with meningitis caused by more common bacterial pathogens. Unlike most anaerobic infections, meningitis is often monomicrobial and mixed infections involving aerobes and anaerobes are unusual. The anaerobes most frequently recovered from cerebrospinal fluid cultures are *B. fragilis*, other *Bacteroides* spp., *F. necrophorum*, other *Fusobacterium* spp., and *Clostridium* spp. Meningitis caused by *Bacteroides* spp. generally occurs as a

complication of a preceding anaerobic infection of the oropharynx or lower respiratory tract or of an underlying gastrointestinal insult (e.g., recent surgery or necrotizing enterocolitis). Meningitis caused by *F. necrophorum* in children is frequently associated with an otogenic focus.[36]

Intra-abdominal Infections

Secondary peritonitis occurs when the intestinal wall is disrupted (e.g., by perforation of an inflamed appendix or surgical misadventure) and enteric bacteria consequently gain access to the peritoneal cavity. Over time the peritoneal infection may evolve, resulting in the formation of one or more intra-abdominal abscesses.[38,80] Secondary peritonitis and intra-abdominal abscesses are almost always polymicrobial processes involving a mixed flora of anaerobes and anaerobes. *B. fragilis* and other members of the *B. fragilis* group are recovered in most cultures of peritoneal or abscess fluid from patients with such infections; *Prevotella* and *Fusobacterium* spp. are also frequently implicated. Other anaerobic bacteria, including *Clostridium* spp., *Lactobacillus* spp. and anaerobic gram-positive cocci, are also commonly involved. The aerobic flora most frequently recovered in these infections include *Escherichia coli*, viridans streptococci, *Klebsiella* spp., *P. aeruginosa*, and *Enterococcus* species.[8,17,77,81,105]

Anaerobic gram-negative bacilli are also frequently present, as part of a mixed aerobic-anaerobic flora, in abscesses of the liver, spleen, and retroperitoneal space. The anaerobic bacteria most frequently recovered from liver abscesses are anaerobic gram-positive cocci, followed by *Bacteroides* spp., *Fusobacterium* spp., and *Prevotella* spp.[31,42,91] Splenic abscesses are associated with a similar range of anaerobic flora.[31] In a study of retroperitoneal abscess cultures in children, the most frequently isolated anaerobes were *Bacteroides* spp., followed by gram-positive cocci and *Prevotella* spp.[33]

Sexually active adolescent females are at risk for pelvic inflammatory disease and its complications (e.g., tubo-ovarian abscess). In addition to aerobic sexually transmitted organisms (e.g., *Chlamydia trachomatis* and *Neisseria gonorrhoeae*), these infections frequently involve anaerobic gram-negative bacilli, which arise from the normal vaginal flora and include *Prevotella* species (especially *P. bivia*, *P. disiens*, and *P. melaninogenica*), *Porphyromonas* spp. and *B. fragilis* group organisms. Anaerobic gram-positive cocci and *Clostridium* spp. may also be involved.[38,80]

Skin and Soft Tissue Infections

Cutaneous abscesses in children can be polymicrobial, involving both aerobic and anaerobic bacteria. Aerobes (particularly *S. aureus*) predominate in infections of the hand, neck, leg, and trunk regions, whereas anaerobes are more frequently recovered in infections of the vulvovaginal, buttocks, perirectal, finger, nail bed, and head regions.[41] A similar regional distribution of aerobes and anaerobes also occurs in cases of cellulitis.[28] The anaerobes most likely to be recovered from infections near the perirectal area include, not surprisingly, *B. fragilis* group and *Clostridium* spp.; infections closer to the oropharynx usually involve oral flora, such as *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp., and anaerobic gram-positive cocci. A wide variety of aerobic and anaerobic flora, including anaerobic gram-negative bacilli, can be isolated from sundry skin infections such as paronychia, infected burn wounds, pilonidal cysts, gastrostomy or tracheostomy site infections, and secondary infections of scabies lesions.*

Bacteria of the normal oral flora of humans and animals figure prominently in infected bite wounds. Such infections are frequently polymicrobial and usually involve a mix of aerobic and anaerobic bacteria. In infected human bite wounds, the most commonly involved anaerobes are *Fusobacterium* spp. (especially *F. nucleatum*) and *Prevotella* spp. (especially *P. melaninogenica*, *P. buccae*, and *P. intermedia*), whereas the predominant aerobic pathogens are *Streptococcus anginosus*, *Staphylococcus aureus*, and *Eikenella corrodens*.[106] In infections arising from dog and cat bite wounds, the most frequently isolated anaerobes are *Fusobacterium* spp. (especially *F. nucleatum*), *Bacteroides* spp. (especially *B. pyogenes*, previously designated *B. tectum*), *Porphyromonas* spp., and *Prevotella* spp. (especially *P. heparinolytica*), whereas the most frequently isolated aerobes are *Pasteurella* spp., *Staphylococcus* spp. and *Streptococcus* spp.[107]

Anaerobic gram-negative bacilli are occasionally involved in complicated skin and soft tissue infections. Crepitant cellulitis is a superficial infection that is associated with less systemic toxicity but more noticeable crepitus than clostridial myonecrosis. The usual causative pathogen is *Clostridium perfringens*, but crepitant cellulitis can also result from infection with combinations of aerobic bacteria with *B. fragilis* and/or other anaerobic pathogens. Necrotizing fasciitis is a dreaded soft tissue infection that can be rapidly progressive. In one type of necrotizing fasciitis, anaerobes such as *Bacteroides* spp. and anaerobic gram-positive cocci are frequently recovered, usually in association with various aerobic enteric flora. This type should be distinguished from necrotizing fasciitis caused by *S. pyogenes* (or, less frequently, by groups B, C or G streptococci), in which anaerobes are generally not involved.[50,80]

Bone and Joint Infections

Septic arthritis caused by anaerobic bacteria is rare in both children and adults and, unlike most anaerobic infections, is usually not polymicrobial. Most cases result from anaerobic bacteremia with gram-negative rods (especially *Fusobacterium* spp. or the *B. fragilis* group) or anaerobic gram-positive cocci. In some cases, the presence of anaerobes is suggested by foul-smelling synovial fluid, gas under pressure within the joint, negative synovial fluid cultures in the clinical picture of septic arthritis, or an anaerobic infection elsewhere in the patient.[35,80]

Anaerobic osteomyelitis is also quite uncommon in children. When it does occur, it generally results from direct extension from a contiguous focus, rather than via hematogenous seeding. In a series of 26 children with culture-proven anaerobic osteomyelitis, all had

*References 19, 27, 29, 38, 39, 43.

preexisting anaerobic infections at adjacent sites or other predisposing conditions for anaerobic infection. Culture results reflected those of the underlying anaerobic infection or of the normal flora of the nearest mucous membrane surface. Anaerobic gram-positive cocci were isolated most frequently, followed by pigmented *Prevotella* and *Porphyromonas* spp., *Fusobacterium* spp., *Clostridium* spp., *Prevotella oralis*, and *Bacteroides* spp. All but 2 patients had polymicrobial infections; in 10 patients, aerobic flora were also recovered. In contrast to acute hematogenous osteomyelitis, these cases presented insidiously; the mean duration of symptoms before presentation was 21 days.[22,35]

Pleuropulmonary Infections

Anaerobic infections of the lower respiratory tract are rare in healthy children. They are, however, relatively common in children with tracheoesophageal malformations or central nervous system abnormalities that impair cough reflexes. Such children are predisposed toward aspirating not only food, milk and vomitus but also their oropharyngeal secretions. Thus, aspiration pneumonia is usually a polymicrobial process involving both aerobic and anaerobic flora of the oral cavity. The risk for pneumonia is further elevated in children with poor oral hygiene, gingivitis, or periodontitis. If untreated, aspiration pneumonia can follow a progressive course with the development of single or multiple lung abscess and/or pleural empyema. The predominant anaerobes recovered from children with aspiration pneumonia (including those with and without associated lung abscesses) are anaerobic gram-positive cocci, followed by *Prevotella* and *Porphyromonas* spp., *F. nucleatum*, *B. fragilis* group members, and other *Bacteroides* spp. The major aerobic co-pathogens implicated in these infections include *P. aeruginosa*, *Streptococcus pneumoniae*, *E. coli*, *Klebsiella pneumoniae*, and *S. aureus*.[37,80]

Anaerobes are occasionally isolated from pleural fluid cultures of patients with pleural empyema, and it is possible that they account for at least some cases of empyema in which routine bacterial cultures are negative. In a study of 104 children with pleural empyema, anaerobes were isolated from five adolescents, in all cases along with other aerobic or anaerobic oral flora. Predisposing conditions in these patients were pneumonia in four and alcohol intoxication in one.[54] In another study of 72 neurologically impaired children with empyema, anaerobic bacteria were recovered in 24 percent of patients. Anaerobic pleural empyema was associated with the presence of other anaerobic infections in or near the respiratory tract, including aspiration pneumonia, lung abscess, subdiaphragmatic abscess, and dental or oropharyngeal abscess. Anaerobes isolated from these patients included *B. fragilis* group members, pigmented *Prevotella* and *Porphyromonas* spp., *Fusobacterium* spp., and anaerobic gram-positive cocci.[23,37]

Anaerobes are occasionally isolated from lower respiratory tract specimens of children with ventilator-associated pneumonia.[30,32] In one study, anaerobes were recovered from 9 of 10 sputum culture specimens obtained using protective brush catheters, either alone (3 children) or as part of a mixed infection with aerobic bacteria (6 children). The anaerobic flora recovered in these children included pigmented *Prevotella* and *Porphyromonas* spp. (5 children), anaerobic gram-positive cocci (4 children), *Fusobacterium* spp., and members of the *B. fragilis* group (2 children).[30]

DIAGNOSIS

As with other anaerobic infections, the diagnosis of infections caused by anaerobic gram-negative bacilli requires a high index of suspicion. For the most part, these infections must be suspected based on the clinical presentation and then diagnosed using microbiologic methods. When anaerobic infections are suspected, specimens for culture should be collected without contamination from the endogenous flora of adjacent mucous membranes. In general, acceptable specimens for anaerobic culture include blood; aspirates of normally sterile body fluids, abscesses, or deep wounds; and surgically obtained specimens. Aspirates and tissue are preferable to swabs. Gram-stained smears of aspirated material should be examined and interpreted in the context of culture results.

Specimens submitted for anaerobic culture should be rapidly transported to the laboratory in anaerobic transport devices and promptly inoculated into appropriate media in an oxygen-free environment (e.g., anaerobic jar or glove-box). Media that support cultivation of anaerobes are supplemented with reducing substances (e.g., thioglycolate, dithiothreitol, cysteine, iron shavings, or chopped meat) and antibiotics (to suppress the growth of facultative bacteria). Even before inoculation, such media must be prepared and stored under anaerobic conditions to prevent generation of superoxide or hydrogen peroxide.[38,87,109] Attention to detail is important. In one hospital, improvements in techniques for recovery of anaerobes (i.e., media and transport conditions) and education of clinicians and microbiologists were associated with dramatic reductions in health care costs and mortality among patients with anaerobic infections.[6]

Bacterial isolates that grow anaerobically should be tested for aerotolerance to confirm whether they are obligate or facultative anaerobes. This process necessarily lengthens the time to reporting of culture reports to the clinician; however, the morphologic characteristics of certain anaerobes (e.g., *B. fragilis* group, pigmented *Prevotella* and *Porphyromonas* spp.) are sufficiently distinctive to allow preliminary reporting of presumptive results before aerotolerance testing, at least in experienced laboratories. Definitive identification of anaerobic isolates is performed using biochemical tests, gas-liquid chromatography of fermentation reaction products, direct immunofluorescence, molecular genetic methods, or a combination of these.[38,109] At a minimum, anaerobic bacteria should be tested for the production of β-lactamase. More detailed antimicrobial susceptibility testing of anaerobes is possible using broth dilution, agar dilution, or E-test methods, but these procedures are not routinely performed in most hospital laboratories today.[38,60,80]

TABLE 149-1 **Percentages of Anaerobic Gram-Negative Bacilli Susceptible to Selected Antimicrobial Agents in Recent Reports**

	Bacteroides fragilis group*	*Prevotella* spp.†	*Fusobacterium* spp.‡	*Porphyromonas* spp.§
Penicillin G	0-13	16-69	85-100	74-100
Amoxicillin	0-21	58-88	80-100	88-100
Amoxicillin-clavulanate	82-90	93-100	89-100	100
Cefoxitin	60-90	93-100	100	95-100
Ceftriaxone	15-32	71-97	80-100	95-100
Chloramphenicol	97-100	100	96-100	
Clindamycin	48-80	71-97	85-100	90-100
Imipenem	94-100	94-100	92-100	100
Meropenem	92-100	99-100	92-100	100
Metronidazole	93-100	94-100	93-100	94-100
Moxifloxacin	52-89	71-88	80-88	96
Piperacillin-tazobactam	92-100	98-100	100	100
Tigecycline	73-100	100	100	

*References 1, 10, 56, 58, 62, 66, 71, 72, 76, 78, 81, 82, 84, 85, 94, 95, 103, 104, 111, 112, 116, 119.
†References 1, 3, 12, 13, 58, 62, 66, 68, 71-73, 75, 76, 78, 84, 85, 92, 94, 110, 114, 116, 119.
‡References 1, 12, 49, 58, 62, 65, 66, 71-73, 75, 78, 84, 94, 110, 114, 116, 119.
§References 1, 3, 62, 65-68, 71, 73, 75, 84, 85, 114, 116.

TREATMENT

Appropriate therapy for anaerobic infections is both surgical and medical. In general, abscesses should be drained (and their contents sent for routine and anaerobic cultures), and débridement of necrotic tissue is critical to survival in severe infections such as necrotizing fasciitis.[38,80] Hyperbaric oxygen has been used, in addition to débridement and antibiotics, in some children with clostridial myonecrosis or necrotizing fasciitis, but definitive recommendations as to its use cannot be made based on this limited experience.[38]

Patients with anaerobic infections should promptly receive appropriate antibiotic therapy (Table 149-1). Delays in the initiation of antianaerobic therapy have been associated with increased morbidity, mortality, and duration of hospitalization in patients with severe infections involving anaerobes.[52] In certain situations (e.g., peritonitis with a perforated viscus, brain abscess related to chronic mastoiditis), the clinician should assume that anaerobes are present and include agents active against these organisms in the initial antimicrobial regimen. For many anaerobic bacteria, there is no particular drug of choice, because many different antibiotics (alone or in combination) might be reasonable for a given patient. Because anaerobic infections often involve a mixed flora of aerobic and anaerobic bacteria, the selection of antimicrobial therapy must also take into account the likely aerobic bacteria involved in a given infection. Often it is this consideration that drives the selection of one antianaerobic drug over another, as in the choice of a meropenem or piperacillin-tazobactam (active against most aerobic Enterobacteriaceae as well as *B. fragilis* group organisms and other anaerobes) over metronidazole (active against most anaerobes but inactive against aerobes) for treatment of a complicated intra-abdominal infection. Other factors to consider include the toxicity, tolerability, tissue penetration, and cost of various available antibiotics.[38,80]

Further complicating matters, many anaerobic bacteria are now resistant to antibiotics once considered reliable against these organisms. This is particularly true of the anaerobic gram-negative bacilli covered in this chapter, all of which have demonstrated increasing rates of resistance to penicillin, clindamycin, and other drugs in recent decades. Furthermore, resistance among anaerobic bacteria has been identified as a risk factor for clinical failure in the treatment of these infections.[52,80] Because most hospital laboratories do not routinely perform antimicrobial susceptibility testing for anaerobes, and because local data on anaerobic susceptibility patterns are usually not available, clinicians often lack guidance (beyond that available in review articles and textbook chapters) as to the optimal selection of antibiotics for anaerobic infections in their patients. Nonetheless, some general principles still apply. For example, aminoglycosides, monobactams (e.g., aztreonam), and older fluoroquinolones all have uniformly poor activity against anaerobes, whereas macrolides and older tetracyclines are only modestly more reliable. Chloramphenicol remains highly active against anaerobes, including gram-negative bacilli, but rare instances of life-threatening blood dyscrasias essentially preclude its use. Several classes of antibiotics (see later discussion), traditionally considered as antianaerobic drugs, vary in their activity against different anaerobes, including *Bacteroides*, *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp. (see Table 149-1). Serious anaerobic infections should be treated with intravenous antimicrobial agents at maximum dosages to optimize their penetration into devitalized tissue and abscesses.[38,47,61]

Penicillins and Penicillin/β-Lactamase Inhibitor Combinations

Penicillin G, ampicillin, and amoxicillin are active against most anaerobes that do not produce β-lactamase, including most isolates of *F. nucleatum*, *F. necrophorum*, and

Porphyromonas spp. These drugs are less reliable against *Prevotella* spp. and virtually devoid of activity against *Bacteroides* spp., however, and thus should no longer be considered first-line agents for initial treatment of anaerobic infections at any body site. Their use should be limited to selected infections where source control has been achieved and involvement of β-lactamase–producing organisms (especially *B. fragilis* group members) has been excluded or where clinical suspicion of such organisms is low.

Combinations of penicillins with β-lactamase inhibitors, on the other hand, achieve broad-spectrum activity against anaerobic and aerobic bacteria, including anaerobic gram-negative bacilli. These agents are particularly well suited for treatment of intra-abdominal infections, in which β-lactamase–producing gram-negative bacilli are uniformly present. A clinical practice guideline, published in 2010, endorses (among other options) the use of piperacillin-tazobactam or ticarcillin-clavulanate for antimicrobial treatment of complicated intra-abdominal infections in children.[105] Amoxicillin-clavulanate is active against most anaerobic and aerobic oropharyngeal flora and is thus a suitable oral antibiotic for treatment of most anaerobic infections arising in the head, neck, and chest (although its use as a single agent may be limited by its lack of activity against methicillin-resistant *S. aureus*). In recent years, increasing resistance to amoxicillin-clavulanate and piperacillin-tazobactam among *B. fragilis* group isolates has been noted in Europe, indicating that continued vigilance for emerging resistance to this class of antibiotics will be required.[80]

Cephalosporins

First-generation cephalosporins have antianaerobic activity similar to that of penicillin G, and third-generation cephalosporins are only moderately more active. Ceftriaxone is active against most isolates of *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp., but most *B. fragilis* group isolates are resistant. The most active cephalosporins against anaerobes are the second-generation agents cefoxitin, cefotetan, and cefmetazole; of these, cefoxitin has the best activity against *B. fragilis* group members.[38] Cefoxitin has been recommended, among other options, as an appropriate single-agent therapy for adults with community-acquired complicated intra-abdominal infections of mild to moderate severity (but not for adults with severe disease or high-risk conditions).[105] Increasing resistance to cefoxitin has been documented in recent years, however, suggesting that its use as monotherapy for intra-abdominal infections should be approached with caution.[80] Cefoxitin remains useful for perioperative antimicrobial prophylaxis in surgical procedures involving the abdomen.[14] Moreover, it is recommended as a first-line agent, in combination with doxycycline, for parenteral therapy for pelvic inflammatory disease.[45]

Carbapenems

The carbapenems are parenterally administered β-lactams with broad coverage against both aerobes and anaerobes, including many cephalosporin-resistant gram-negative enteric agents. Because of this broad-spectrum activity, carbapenems are well suited for single-agent therapy for a variety of infections involving anaerobes, including complicated intra-abdominal infections.[105] Resistance to carbapenems has been documented among *B. fragilis* group organisms but remains rare, affecting less than 2 percent of isolates in most surveys. Resistance is exceptionally rare among isolates of *Prevotella* and *Fusobacterium* spp., and such resistant isolates have not been observed in the United States. Of the available carbapenems, meropenem is the preferred agent for use in children, because it is less likely to induce seizures than imipenem-cilastatin.[79,88]

Metronidazole

Metronidazole has excellent activity against anaerobic gram-negative bacilli, including *B. fragilis* group members that are frequently resistant to other classes of antibiotics. Resistance to this agent has begun to emerge among *B. fragilis* group strains and *Prevotella* spp. in the developing world and in Europe, but such isolates remain exceptionally rare in the United States.* Of note, metronidazole is less reliable in its activity against many gram-positive anaerobes, and virtually all aerobic bacteria are inherently resistant to it. Because most anaerobic infections are polymicrobial, metronidazole is generally not used alone but in combination with other antibiotics active against aerobic bacteria. For example, a recent practice guideline lists combination therapy with metronidazole plus either cefepime, a third-generation cephalosporin, or an aminoglycoside (with or without ampicillin) as an appropriate antimicrobial strategy for therapy of complicated intra-abdominal infections in children.[105] Among the many advantages of metronidazole are that it is generally well tolerated; penetrates well into many tissues, including those of the central nervous system; and can be administered orally.

Clindamycin

Over the past 2 decades, resistance to clindamycin has increased substantially among members of the *B. fragilis* group, with resistance rates exceeding 50 percent in some series.[58,66,76,80] Because clindamycin is now less reliable than carbapenems, penicillin/β-lactamase inhibitor combinations, or metronidazole against these organisms, it should no longer be considered a first-line agent for treatment of complicated intra-abdominal infections. However, clindamycin continues to have good activity against most other anaerobic bacteria, including most strains of *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp. and gram-positive anaerobes. Thus, today clindamycin is better suited for treatment of infections involving oropharyngeal anaerobes than for those involving intestinal flora.

Clindamycin achieves comparable serum levels whether administered orally or intravenously and penetrates well into most tissues and fluids, including bones, joints, and abscesses. Clindamycin does not penetrate

*References 1, 62, 78, 80, 85, 103.

well into cerebrospinal fluid and thus should not be used for treatment of central nervous system infections.[47] For treatment of polymicrobial infections, clindamycin should be combined with a β-lactam or other antibiotic active against aerobic gram-negative bacilli (e.g., *Pasteurella multocida* in animal bite wounds or *Eikenella corrodens* in human bite wounds). Although clindamycin has traditionally been particularly associated with *Clostridium difficile*–induced pseudomembranous colitis, many other antibiotics can also induce this complication.[38]

Quinolones

In contrast to earlier generations of fluoroquinolones, newer agents such as moxifloxacin possess significant in vitro activity against many anaerobic bacteria.[53,62] Unfortunately, resistance to moxifloxacin among *B. fragilis* group organisms rapidly emerged in the late 1990s, limiting the usefulness of newer quinolones for treatment of complicated intra-abdominal and other anaerobic infections.[9,59,80] Moxifloxacin's activity against other anaerobic gram-negative bacilli has not been as well studied, but available data suggest that resistance occurs among a substantial minority of *Prevotella* and *Fusobacterium* spp. isolates.

Therapeutic advantages of the quinolones include their wide distribution in tissues and fluids and their good absorption after oral administration.[89] Newer quinolones may provide options for treating anaerobic infections in selected children, such as older adolescents. Nonetheless, the quinolones' role in pediatrics remains limited because concern lingers over their potential toxicity, because other agents with established safety profiles have equal or better activity against anaerobes, and because bacterial resistance to quinolones will likely continue to rise, owing to their continued widespread use in adults.

Tetracyclines

Most *B. fragilis* group organisms are resistant to tetracycline, as are a substantial proportion of *Prevotella* and *Fusobacterium* spp. isolates. Tigecycline is a newer antibiotic, belonging to the glycylcycline class, which has vastly improved activity against anaerobic gram-negative bacilli. Tigecycline is listed, among other options, as an appropriate single-agent therapy for adults with community-acquired complicated intra-abdominal infections of mild to moderate severity (but not for adults with severe disease or high-risk conditions).[105] Tigecycline's safety and efficacy in children have not been established. In addition, the high rates of gastrointestinal side effects associated with this drug will likely limit any potential for its use in pediatric patients.[51]

Oxazolidinones

Linezolid possesses surprisingly good in vitro activity against anaerobic gram-negative bacilli. In a study of isolates in Belgium from 2003 through 2005, susceptibility rates to linezolid were 99 percent among *B. fragilis* group members, 98 percent among *Prevotella* spp., and 100 percent for *Fusobacterium* spp.[119] In another study from Germany, 100 percent of 80 *Fusobacterium* isolates were susceptible to linezolid.[49] Moreover, earlier studies also found overall excellent activity of oxazolidinones against many types of anaerobic bacteria, including *B. fragilis* group members.[7,48,90,121] Although experience with the clinical use of this agent for anaerobic infections is limited, a case report described microbiologic cure and clinical improvement with linezolid therapy in a patient with sepsis caused by a *B. fragilis* strain resistant to metronidazole, penicillin/β-lactamase inhibitors, carbapenems, and tetracyclines.[115] In another patient, an extensive soft tissue infection of the leg with *B. fragilis* resistant to clindamycin, metronidazole, cefoxitin, carbapenems, piperacillin-tazobactam, and tigecycline was successfully cured after combination therapy with linezolid and moxifloxacin.[102] The role of linezolid in therapy for complicated anaerobic infections merits further investigation.

PREVENTION

The prevention of infections caused by *Bacteroides*, *Prevotella*, *Porphyromonas*, and *Fusobacterium* spp. hinges on measures that serve to limit the introduction of these bacteria into healthy tissues. Such methods include, but are not limited to, cleaning and débriding wounds properly, removing foreign bodies promptly, maintaining good dental and oral hygiene, avoiding disruption of the intestinal wall during surgery, appropriately prescribing perioperative antibiotic prophylaxis, and following recommendations for prevention of nosocomial pneumonia.[14,15,38,44]

NEW REFERENCES SINCE THE SIXTH EDITION

10. Boente RF, Ferreira LQ, Falcão LS, et al. Detection of resistance genes and susceptibility patterns in *Bacteroides* and *Parabacteroides* strains. *Anaerobe* 2010;**16**:190–4.
13. Boyanova L, Kolarov R, Gergova G, et al. Trends in antibiotic resistance in *Prevotella* species from patients of the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria, in 2003-2009. *Anaerobe* 2010;**16**:489–92.
38. Brook I. Anaerobic infections in children. *Adv Exp Med Biol* 2011;**697**:117–52.
45. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**(No. RR-12):1–110, 2010.
52. Dubreuil L, Odou MF. Anaerobic bacteria and antibiotics: what kind of unexpected resistance could I find in my laboratory tomorrow? *Anaerobe* 2010;**16**:555–9.
56. Fernández-Canigia L, Litterio M, Legaria MC, et al. First national survey of antibiotic susceptibility of the *Bacteroides fragilis* group: emerging resistance to carbapenems in Argentina. *Antimicrob Agents Chemother* 2012;**56**:1309–14.
58. Glupczynski Y, Berhin C, Nizet H. Antimicrobial susceptibility of anaerobic bacteria in Belgium as determined by E-test methodology. *Eur J Clin Microbiol Infect Dis* 2009;**28**:261–7.
60. Goldstein EJ, Citron DM, Goldman PJ, Goldman RJ. National hospital survey of anaerobic culture and susceptibility methods, part III. *Anaerobe* 2008;**14**:68–72.
66. Jamal W, Shahin M, Rotimi VO. Surveillance and trends of antimicrobial resistance among clinical isolates of anaerobes in Kuwait hospitals from 2002 to 2007. *Anaerobe* 2010;**16**:1–5.
67. Japoni A, Vasin A, Noushadi S, et al. Antibacterial susceptibility patterns of *Porphyromonas gingivalis* isolated from chronic periodontitis patients. *Med Oral Patol Oral Cir Bucal* 2011;**16**: e1031–5.

76. Lee Y, Park Y, Kim MS, et al. Antimicrobial susceptibility patterns for recent clinical isolates of anaerobic bacteria in South Korea. *Antimicrob Agents Chemother* 2010;**54**:3993–7.
78. Liu CY, Huang YT, Liao CH, et al. Increasing trends in antimicrobial resistance among clinically important anaerobes and *Bacteroides fragilis* isolates causing nosocomial infections: emerging resistance to carbapenems. *Antimicrob Agents Chemother* 2008;**52**: 3161–8.
80. Nagy E. Anaerobic infections: update on treatment considerations. *Drugs* 2010;**70**:841–58.
81. Nagy E, Urbán E, Nord CE, ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria. Antimicrobial susceptibility of *Bacteroides fragilis* group isolates in Europe: 20 years of experience. *Clin Microbiol Infect* 2011;**17**:371–9.
82. Nakano V, Nascimento e Silva A, Merino VR, et al. Antimicrobial resistance and prevalence of resistance genes in intestinal *Bacteroidales* strains. *Clinics (São Paulo)* 2011;**66**:543–7.
86. Patrick S, Blakely GW, Houston S, et al. Twenty-eight divergent polysaccharide loci specifying within- and amongst-strain capsule diversity in three strains of *Bacteroides fragilis*. *Microbiology* 2010; **156**:3255–69.
92. Poeschl PW, Spusta L, Russmueller G, et al. Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; **110**:151–6.
93. Ridgway JM, Parikh DA, Wright R, et al. Lemierre syndrome: a pediatric case series and review of literature. *Am J Otolaryngol* 2010;**31**:38–45.
101. Shah HN, Olsen I, Bernard K, et al. Approaches to the study of the systematics of anaerobic, gram-negative non-sporeforming rods: current status and perspectives. *Anaerobe* 2009;**15**:179–94.
102. Sherwood JE, Fraser S, Citron DM, et al. Multi-drug resistant *Bacteroides fragilis* recovered from blood and severe leg wounds caused by an improvised explosive device (IED) in Afghanistan. *Anaerobe* 2011;**17**:152–5.
103. Snydman DR, Jacobus NV, McDermott LA, et al. Lessons learned from the Anaerobe Survey: historical perspective and review of the most recent data (2005-2007). *Clin Infect Dis* 2010;**50**(Suppl. 1): S26–33.
104. Snydman DR, Jacobus NV, McDermott LA. Update on resistance of *Bacteroides fragilis* group and related species with special attention to carbapenems 2006-2009. *Anaerobe* 2011;**17**:147–51.
105. Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**: 133–64.
111. Toprak NU, Uzunkaya OD, Sóki J, Soyletir G. Susceptibility profiles and resistance genes for carbapenems *(cfiA)* and metronidazole *(nim)* among *Bacteroides* species in a Turkish University Hospital. *Anaerobe* 2012;**18**:169–71.
112. Treviño M, Areses P, Peñalver MD, et al. Susceptibility trends of *Bacteroides fragilis* group and characterisation of carbapenemase-producing strains by automated REP-PCR and MALDI TOF. *Anaerobe* 2012;**18**:37–43.
113. Vandamme P, Debruyne L, De Brandt E, Falsen E. Reclassification of *Bacteroides ureolyticus* as *Campylobacter ureolyticus* comb. nov., and emended description of the genus *Campylobacter*. *Int J Syst Evol Microbiol* 2010;**60**:2016–22.
117. Wick EC, Sears CL. *Bacteroides* spp. and diarrhea. *Curr Opin Infect Dis* 2010;**23**:470–4.
118. Wright WF, Shiner CN, Ribes JA. Lemierre syndrome. *South Med J* 2012;**105**:283–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aldridge KE, Ashcraft D, Cambre K, et al. Multicenter survey of the changing in vitro antimicrobial susceptibilities of clinical isolates of *Bacteroides fragilis* group, *Prevotella*, *Fusobacterium*, *Porphyromonas*, and *Peptostreptococcus* species. *Antimicrob Agents Chemother* 2001;**45**:1238–43.
2. Ariza J, Casanova A, Fernandez Viladrich P, et al. Etiological agent and primary source of infection in 42 cases of focal intracranial suppuration. *J Clin Microbiol* 1986;**24**:899–902.
3. Austin MN, Meyn LA, Hillier SL. Susceptibility of vaginal bacteria to metronidazole and tinidazole. *Anaerobe* 2006;**12**:227–30.
4. Bair-Merritt MH, Shah SS, Zaoutis TE, et al. Suppurative intracranial complications of sinusitis in previously healthy children. *Pediatr Infect Dis J* 2005;**24**:384–6.
5. Bambakidis NC, Cohen AR. Intracranial complications of frontal sinusitis in children: Pott's puffy tumor revisited. *Pediatr Neurosurg* 2001;**35**:82–9.
6. Barenfanger J, Drake CA, Lawhorn J, et al. Outcomes of improved anaerobic techniques in clinical microbiology. *Clin Infect Dis* 2002;**35**(Suppl. 1):S78–83.
7. Behra-Miellet J, Calvet L, Dubreuil L. Activity of linezolid against anaerobic bacteria. *Int J Antimicrob Agents* 2003;**22**:28–34.
8. Bennion RS, Baron EJ, Thompson JE, et al. The bacteriology of gangrenous and perforated appendicitis—revisited. *Ann Surg* 1990;**211**:165–71.
9. Betriu C, Rodríguez-Avial I, Gómez M, et al. Changing patterns of fluoroquinolone resistance among *Bacteroides fragilis* group organisms over a 6-year period (1997-2002). *Diagn Microbiol Infect Dis* 2005;**53**:221–3.
10. Boente RF, Ferreira LQ, Falcão LS, et al. Detection of resistance genes and susceptibility patterns in *Bacteroides* and *Parabacteroides* strains. *Anaerobe* 2010;**16**:190–4.
11. Bolstad AI, Jensen HB, Bakken V. Taxonomy, biology, and periodontal aspects of *Fusobacterium nucleatum*. *Clin Microbiol Rev* 1996;**9**:55–71.
12. Boyanova L, Kolarov R, Gergova G, et al. Anaerobic bacteria in 118 patients with deep-space head and neck infections from the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria. *J Med Microbiol* 2006;**55**:1285–9.
13. Boyanova L, Kolarov R, Gergova G, et al. Trends in antibiotic resistance in *Prevotella* species from patients of the University Hospital of Maxillofacial Surgery, Sofia, Bulgaria, in 2003-2009. *Anaerobe* 2010;**16**:489–92.
14. Bratzler DW, Houck PM, Surgical Infection Prevention Guideline Writers Workgroup. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Am J Surg* 2005;**189**:395–404.
15. Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improve outcomes for patients having surgery. *Clin Infect Dis* 2006;**43**:322–30.
16. Brook I. Anaerobic bacteremia in children. *Am J Dis Child* 1980;**134**:1052–6.
17. Brook I. Bacterial studies of peritoneal cavity and postoperative surgical wound drainage following perforated appendix in children. *Ann Surg* 1980;**192**:208–12.
18. Brook I. Bacteriologic features of chronic sinusitis in children. *JAMA* 1981;**246**:967–9.
19. Brook I. Bacteriologic study of paronychia in children. *Am J Surg* 1981;**141**:703–5.
20. Brook I. Enhancement of growth of aerobic and facultative bacteria in mixed infections with *Bacteroides* species. *Infect Immun* 1985;**50**:929–31.
21. Brook I. Role of encapsulated anaerobic bacteria in synergistic infections. *Crit Rev Microbiol* 1987;**14**:171–93.
22. Brook I. Anaerobic osteomyelitis in children. *Pediatr Infect Dis* 1986;**5**:550–6.
23. Brook I. Microbiology of empyema in children and adolescents. *Pediatrics* 1990;**85**:722–6.
24. Brook I. Aerobic and anaerobic bacteriology of intracranial abscesses. *Pediatr Neurol* 1992;**8**:210–14.
25. Brook I. Anaerobic infections in the neonate. *Adv Pediatr* 1994;**41**:369–83.
26. Brook I. Fusobacterial infections in children. *J Infect* 1994;**28**:155–65.
27. Brook I. Aerobic and anaerobic microbiology of gastrostomy-site wound infections in children. *Clin Infect Dis* 1995;**20**(Suppl. 2):S257–8.
28. Brook I. Clinical features and aerobic and anaerobic microbiological characteristics of cellulitis. *Arch Surg* 1995;**130**:786–92.
29. Brook I. Microbiology of secondary bacterial infection in scabies lesions. *J Clin Microbiol* 1995;**33**:2139–40.
30. Brook I. Pneumonia in mechanically ventilated children. *Scand J Infect Dis* 1995;**27**:619–22.
31. Brook I. *Prevotella* and *Porphyromonas* infections in children. *J Med Microbiol* 1995;**42**:340–7.
32. Brook I. Aerobic and anaerobic microbiology of infections after trauma in children. *J Accid Emerg Med* 1998;**15**:162–7.
33. Brook I. Microbiology of retroperitoneal abscesses in children. *J Med Microbiol* 1999;**48**:697–700.
34. Brook I. Clinical review: bacteremia caused by anaerobic bacteria in children. *Crit Care* 2002;**6**:205–11.
35. Brook I. Joint and bone infections due to anaerobic bacteria in children. *Pediatr Rehabil* 2002;**5**:11–19.
36. Brook I. Meningitis and shunt infection caused by anaerobic bacteria in children. *Pediatr Neurol* 2002;**26**:99–105.
37. Brook I. Anaerobic pulmonary infections in children. *Pediatr Emerg Care* 2004;**20**:636–40.
38. Brook I. Anaerobic infections in children. *Adv Exp Med Biol* 2011;**697**:117–52.
39. Brook I, Anderson KD, Controni G, Rodriguez WJ. Aerobic and anaerobic bacteriology of pilonidal cyst abscess in children. *Am J Dis Child* 1980;**134**:679–80.
40. Brook I, Anthony BF, Finegold SM. Aerobic and anaerobic bacteriology of acute otitis media in children. *J Pediatr* 1978;**92**:13–16.
41. Brook I, Finegold SM. Aerobic and anaerobic bacteriology of cutaneous abscesses in children. *Pediatrics* 1981;**67**:891–5.
42. Brook I, Fraizer EH. Role of anaerobic bacteria in liver abscesses in children. *Pediatr Infect Dis J* 1993;**12**:743–7.
43. Brook I, Randolph JG. Aerobic and anaerobic bacterial flora of burns in children. *J Trauma* 1981;**21**:313–18.
44. Centers for Disease Control and Prevention. Guidelines for preventing health-care–associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Morb Mortal Wkly Rep* 2004;**53**(No. RR-3):1–36.
45. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR* 2010;**59**(No. RR-12):1–110, 2010.
46. Cerdeño-Tárraga AM, Patrick S, Crossman LC, et al. Extensive DNA inversions in the *B. fragilis* genome control variable gene expression. *Science* 2005;**307**:1463–5.
47. Chambers HF. Protein synthesis inhibitors and miscellaneous antibacterial agents. In: Brunton LL, Lazo JS, Parker KL, editors. *Goodman and Gilman's the pharmacological basis of therapeutics*. 11th ed. New York: McGraw-Hill; 2006. p. 1173–202.
48. Citron DM, Merriam CV, Tyrrell KL, et al. In vitro activities of ramoplanin, teicoplanin, vancomycin, linezolid, bacitracin, and four other antimicrobials against intestinal anaerobic bacteria. *Antimicrob Agents Chemother* 2003;**47**:2334–8.
49. Daeschlein G, Hoehne C, Assadian O, et al. In vitro activity of linezolid against clinical isolates of *Fusobacterium* spp. *J Antimicrob Chemother* 2006;**58**:789–93.
50. DiNubile MJ, Lipsky BA. Complicated infections of skin and skin structures: when the infection is more than skin deep. *J Antimicrob Chemother* 2004;**53**(Suppl. 2):ii37–50.
51. Doan TL, Fung HB, Mehta D, Riska PF. Tigecycline: a glycylcycline antimicrobial agent. *Clin Ther* 2006;**28**:1079–106.
52. Dubreuil L, Odou MF. Anaerobic bacteria and antibiotics: what kind of unexpected resistance could I find in my laboratory tomorrow? *Anaerobe* 2010;**16**:555–9.
53. Edmiston CE, Krepel CJ, Seabrook GR, et al. In vitro activities of moxifloxacin against 900 aerobic and anaerobic surgical isolates from patients with intra-abdominal and diabetic foot infections. *Antimicrob Agents Chemother* 2004;**48**:1012–16.
54. Fajardo JE, Chang MJ. Pleural empyema in children: a nationwide retrospective study. *South Med J* 1987;**80**:593–6.
55. Feder HM, Cates KL, Cementina AM. Pott puffy tumor: a serious occult infection. *Pediatrics* 1987;**79**:625–9.

56. Fernández-Canigia L, Litterio M, Legaria MC, et al. First national survey of antibiotic susceptibility of the *Bacteroides fragilis* group: emerging resistance to carbapenems in Argentina. *Antimicrob Agents Chemother* 2012;**56**:1309–14.
57. Finegold SM. Overview of clinically important anaerobes. *Clin Infect Dis* 1995;**20**(Suppl. 2):S205–7.
58. Glupczynski Y, Berhin C, Nizet H. Antimicrobial susceptibility of anaerobic bacteria in Belgium as determined by E-test methodology. *Eur J Clin Microbiol Infect Dis* 2009;**28**:261–7.
59. Golan Y, McDermott LA, Jacobus NV, et al. Emergence of fluoroquinolone resistance among *Bacteroides* species. *J Antimicrob Chemother* 2003;**52**:208–13.
60. Goldstein EJ, Citron DM, Goldman PJ, Goldman RJ. National hospital survey of anaerobic culture and susceptibility methods, part III. *Anaerobe* 2008;**14**:68–72.
61. Goldstein EJ, Citron DM, Merriam CV, et al. In vitro activities of telithromycin and 10 oral agents against aerobic and anaerobic pathogens isolated from antral puncture specimens from patients with sinusitis. *Antimicrob Agents Chemother* 2003;**47**:1963–7.
62. Goldstein EJ, Citron DM, Warren YA, et al. In vitro activity of moxifloxacin against 923 anaerobes isolated from human intra-abdominal infections. *Antimicrob Agents Chemother* 2006;**50**: 148–55.
63. Hecht DW. Anaerobes: antibiotic resistance, clinical significance, and the role of susceptibility testing. *Anaerobe* 2006;**12**:115–21.
64. Hofstad T. Virulence determinants in nonsporeforming anaerobic bacteria. *Scand J Infect Dis Suppl* 1989;**62**:15–24.
65. Jacinto RC, Gomes BP, Shah HN, et al. Incidence and antimicrobial susceptibility of *Porphyromonas gingivalis* isolated from mixed endodontic infections. *Int Endod J* 2006;**39**:62–70.
66. Jamal W, Shahin M, Rotimi VO. Surveillance and trends of antimicrobial resistance among clinical isolates of anaerobes in Kuwait hospitals from 2002 to 2007. *Anaerobe* 2010;**16**:1–5.
67. Japoni A, Vasin A, Noushadi S, et al. Antibacterial susceptibility patterns of *Porphyromonas gingivalis* isolated from chronic periodontitis patients. *Med Oral Patol Oral Cir Bucal* 2011;**16**: e1031–5.
68. Jaramillo A, Arce RM, Herrera D, et al. Clinical and microbiological characterization of periodontal abscesses. *J Clin Periodontol* 2005;**32**:1213–18.
69. Jousimies-Somer HR, Summanen PH, Wexler H, et al. *Bacteroides*, *Porphyromonas*, *Prevotella*, *Fusobacterium*, and other anaerobic gram-negative bacteria. In: Murray PR, Baron EJ, Jorgensen JH, et al, editors. *Manual of clinical microbiology*. Washington, DC: ASM Press; 2003. p. 880–901.
70. Kapatral V, Anderson I, Ivanova N, et al. Genome sequence and analysis of the oral bacterium *Fusobacterium nucleatum* strain ATCC 25586. *J Bacteriol* 2002;**18**:2005–18.
71. Koeth LM, Good CE, Appelbaum PC, et al. Surveillance of susceptibility patterns in 1297 European and U. S. anaerobic and capnophilic isolates to co-amoxiclav and five other antimicrobial agents. *J Antimicrob Chemother* 2004;**53**:1039–44.
72. Kommedal O, Nystad TW, Bølstad B, Digranes A. Antibiotic susceptibility of blood culture isolates of anaerobic bacteria at a Norwegian university hospital. *APMIS* 2007;**115**:956–61.
73. Kuriyama T, Williams DW, Yanagisawa M, et al. Antimicrobial susceptibility of 800 anaerobic isolates from patients with dentoalveolar infection to 13 oral antibiotics. *Oral Microbiol Immunol* 2007;**22**:285–8.
74. Kuwahara T, Yamashita A, Hirakawa H, et al. Genomic analysis of *Bacteroides fragilis* reveals extensive DNA inversions regulating cell surface adaptation. *Proc Natl Acad Sci U S A* 2004;**101**: 14919–24.
75. Lakhssassi N, Elhajoui N, Lodter JP, et al. Antimicrobial susceptibility variation of 50 anaerobic periopathogens in aggressive periodontitis: an interindividual variability study. *Oral Microbiol Immunol* 2005;**20**:244–52.
76. Lee Y, Park Y, Kim MS, et al. Antimicrobial susceptibility patterns for recent clinical isolates of anaerobic bacteria in South Korea. *Antimicrob Agents Chemother* 2010;**54**:3993–7.
77. Lin WJ, Lo WT, Chu CC, et al. Bacteriology and antibiotic susceptibility of community-acquired intra-abdominal infection in children. *J Microbiol Immunol Infect* 2006;**39**:249–54.
78. Liu CY, Huang YT, Liao CH, et al. Increasing trends in antimicrobial resistance among clinically important anaerobes and *Bacteroides fragilis* isolates causing nosocomial infections: emerging resistance to carbapenems. *Antimicrob Agents Chemother* 2008;**52**: 3161–8.
79. Lowe MN, Lamb HM. Meropenem: an updated review of its use in the management of intra-abdominal infections. *Drugs* 2000; **60**:619–46.
80. Nagy E. Anaerobic infections: update on treatment considerations. *Drugs* 2010;**70**:841–58.
81. Nagy E, Urbán E, Nord CE, ESCMID Study Group on Antimicrobial Resistance in Anaerobic Bacteria. Antimicrobial susceptibility of *Bacteroides fragilis* group isolates in Europe: 20 years of experience. *Clin Microbiol Infect* 2011;**17**:371–9.
82. Nakano V, Nascimento e Silva A, Merino VR, et al. Antimicrobial resistance and prevalence of resistance genes in intestinal *Bacteroidales* strains. *Clinics (São Paulo)* 2011;**66**:543–7.
83. Nelson KE, Fleischmann RD, DeBoy RT, et al. Complete genome sequence of the oral pathogenic bacterium *Porphyromonas gingivalis* strain W83. *J Bacteriol* 2003;**185**:5591–601.
84. Odou MF, Muller C, Calvet L, Dubreuil L. In vitro activity against anaerobes of retapamulin, a new topical antibiotic for treatment of skin infections. *J Antimicrob Chemother* 2007;**59**: 646–51.
85. Papaparaskevas J, Pantazatou A, Katsandri A, et al, Hellenic Study Group for Gram-Negative Anaerobic Bacteria. Multicentre survey of the in-vitro activity of seven antimicrobial agents, including ertapenem, against recently isolated gram-negative anaerobic bacteria in Greece. *Clin Microbiol Infect* 2005;**11**: 820–4.
86. Patrick S, Blakely GW, Houston S, et al. Twenty-eight divergent polysaccharide loci specifying within- and amongst-strain capsule diversity in three strains of *Bacteroides fragilis*. *Microbiology* 2010; **156**:3255–69.
87. Peterson LR. Effect of media on transport and recovery of anaerobic bacteria. *Clin Infect Dis* 1997;**25**(Suppl. 2):S134–6.
88. Petri WA. Penicillins, cephalosporins, and other beta-lactam antibiotics. In: Brunton LL, Lazo JS, Parker KL, editors. *Goodman and Gilman's the pharmacological basis of therapeutics*. 11th ed. New York: McGraw-Hill; 2006. p. 1127–54.
89. Petri WA. Sulfonamides, trimethoprim-sulfamethoxazole, quinolones, and agents for urinary tract infections. In: Brunton LL, Lazo JS, Parker KL, editors. *Goodman and Gilman's the pharmacological basis of therapeutics*. 11th ed. New York: McGraw-Hill; 2006. p. 1111–26.
90. Phillips OA, Rotimi VO, Jamal WY, et al. Comparative in vitro activity of PH-027 versus linezolid and other anti-anaerobic antimicrobials against clinical isolates of *Clostridium difficile* and other anaerobic bacteria. *J Chemother* 2003;**15**:113–17.
91. Pineiro-Carrero VM, Andres JM. Morbidity and mortality in children with pyogenic liver abscess. *Am J Dis Child* 1989;**143**: 1424–7.
92. Poeschl PW, Spusta L, Russmueller G, et al. Antibiotic susceptibility and resistance of the odontogenic microbiological spectrum and its clinical impact on severe deep space head and neck infections. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2010; **110**:151–6.
93. Ridgway JM, Parikh DA, Wright R, et al. Lemierre syndrome: a pediatric case series and review of literature. *Am J Otolaryngol* 2010;**31**:38–45.
94. Roberts SA, Shore KP, Paviour SD, et al. Antimicrobial susceptibility of anaerobic bacteria in New Zealand: 1999-2003. *J Antimicrob Chemother* 2006;**57**:992–8.
95. Rodríguez E, Gamboa MM, Rodríguez C, Vargas P. *Bacteroides fragilis* group in non-diarrheal human feces and its antimicrobial susceptibility. *Rev Esp Quimioter* 2006;**19**:357–62.
96. Sakamoto M, Benno Y. Reclassification of *Bacteroides distasonis*, *Bacteroides goldsteinii* and *Bacteroides merdae* as *Parabacteroides distasonis* gen. nov., comb. nov., *Parabacteroides goldsteinii* comb. nov. and *Parabacteroides merdae* comb. nov. *Int J Syst Evol Microbiol* 2006;**56**:1599–605.
97. Sakamoto M, Suzuki M, Umeda M, et al. Reclassification of *Bacteroides forsythus* (Tanner et al. 1986) as *Tannerella forsythensis* corrig., gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2002;**52**: 841–9.
98. Shah HN, Collins MD. Proposal for reclassification of *Bacteroides asaccharolyticus*, *Bacteroides gingivalis*, and *Bacteroides endodontalis* in a new genus, *Porphyromonas*. *Int J Syst Bacteriol* 1988;**38**: 128–31.

99. Shah HN, Collins MD. Proposal to restrict the genus *Bacteroides* (Castellani and Chalmers) to *Bacteroides fragilis* and closely related species. *Int J Syst Bacteriol* 1989;**39**:85–7.
100. Shah HN, Collins MD. *Prevotella*, a new genus to include *Bacteroides melaninogenicus* and related species formerly classified in the genus *Bacteroides*. *Int J Syst Bacteriol* 1990;**40**:205–8.
101. Shah HN, Olsen I, Bernard K, et al. Approaches to the study of the systematics of anaerobic, gram-negative non-sporeforming rods: current status and perspectives. *Anaerobe* 2009;**15**:179–94.
102. Sherwood JE, Fraser S, Citron DM, et al. Multi-drug resistant *Bacteroides fragilis* recovered from blood and severe leg wounds caused by an improvised explosive device (IED) in Afghanistan. *Anaerobe* 2011;**17**:152–5.
103. Snydman DR, Jacobus NV, McDermott LA, et al. Lessons learned from the Anaerobe Survey: historical perspective and review of the most recent data (2005-2007). *Clin Infect Dis* 2010;**50**(Suppl. 1):S26–33.
104. Snydman DR, Jacobus NV, McDermott LA. Update on resistance of *Bacteroides fragilis* group and related species with special attention to carbapenems 2006-2009. *Anaerobe* 2011;**17**:147–51.
105. Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intra-abdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**: 133–64.
106. Talan DA, Abrahamian FM, Moran GJ, et al. Clinical presentation and bacteriologic analysis of infected human bites in patients presenting to emergency departments. *Clin Infect Dis* 2003;**37**: 1481–9.
107. Talan DA, Citron DM, Abrahamian FM, et al. Bacteriologic analysis of infected dog and cat bites. Emergency Medicine Animal Bite Infection Study Group. *N Engl J Med* 1999;**340**:85–92.
108. Tang YP, Dallas MM, Malamy MH. Characterization of the Batl (*Bacteroides* aerotolerance) operon in *Bacteroides fragilis*: isolation of a *B. fragilis* mutant with reduced aerotolerance and impaired growth in in vivo model systems. *Mol Microbiol* 1999;**32**:139–49.
109. The anaerobic bacteria. In: Koneman EW, Allen SD, Janda WM, et al, editors. *Color atlas and textbook of diagnostic microbiology*. 5th ed. Philadelphia: Lippincott-Raven; 1997. p. 709–84.
110. Tomás I, Tomás M, Alvarez M, et al. Susceptibility of oral obligate anaerobes to telithromycin, moxifloxacin and a number of commonly used antibacterials. *Oral Microbiol Immunol* 2007;**22**: 298–303.
111. Toprak NU, Uzunkaya OD, Sóki J, Soyletir G. Susceptibility profiles and resistance genes for carbapenems *(cfiA)* and metronidazole *(nim)* among *Bacteroides* species in a Turkish University Hospital. *Anaerobe* 2012;**18**:169–71.
112. Treviño M, Areses P, Peñalver MD, et al. Susceptibility trends of *Bacteroides fragilis* group and characterisation of carbapenemase-producing strains by automated REP-PCR and MALDI TOF. *Anaerobe* 2012;**18**:37–43.
113. Vandamme P, Debruyne L, De Brandt E, Falsen E. Reclassification of *Bacteroides ureolyticus* as *Campylobacter ureolyticus* comb. nov., and emended description of the genus *Campylobacter*. *Int J Syst Evol Microbiol* 2010;**60**:2016–22.
114. van Winkelhoff AJ, Herrera D, Oteo A, Sanz M. Antimicrobial profiles of periodontal pathogens isolated from periodontitis patients in The Netherlands and Spain. *J Clin Periodontol* 2005; **32**:893–8.
115. Wareham DW, Wilks M, Ahmed D, et al. Anaerobic sepsis due to multidrug-resistant *Bacteroides fragilis:* microbiological cure and clinical response with linezolid therapy. *Clin Infect Dis* 2005;**40**: e67–8.
116. Wexler HM, Molitoris D, St. John S, et al. In vitro activities of faropenem against 579 strains of anaerobic bacteria. *Antimicrob Agents Chemother* 2002;**11**:3669–75.
117. Wick EC, Sears CL. *Bacteroides* spp. and diarrhea. *Curr Opin Infect Dis* 2010;**23**:470–4.
118. Wright WF, Shiner CN, Ribes JA. Lemierre syndrome. *South Med J* 2012;**105**:283–8.
119. Wybo I, Pierard D, Verschraegen I, et al. Third Belgian multicentre survey of antibiotic susceptibility of anaerobic bacteria. *J Antimicrob Chemother* 2007;**59**:132–9.
120. Xu J, Bjursell MK, Himrod J, et al. A genomic view of the human–*Bacteroides thetaiotaomicron* symbiosis. *Science* 2003;**299**:2074–6.
121. Yagi BH, Zurenko GE. In vitro activity of linezolid and eperezolid, two novel oxazolidinone antimicrobial agents, against anaerobic bacteria. *Anaerobe* 1997;**3**:301–6.

INDEX

A

Abacavir, 2613
Abdominal abscess, **704-705**
  anaerobic bacteria in, 1829
  clinical manifestations of, 704-705
  complications of, 705
  diagnosis of, 705
  Eikenella corrodens, 1577
  Escherichia coli, 1445
  fever of unknown origin in, 845
  treatment of, 705
Abdominal pain
  in appendicitis, 680-681
  in cholecystitis, 669
  in *Dientamoeba fragilis* infection, 2885
  differential diagnosis of, 681, 682t
  in enteroviral infection, 2070, 2072t
  Helicobacter pylori infection and, 1694
  in hepatitis, 648-649
  in pancreatitis, 689
  in peritonitis, 701
  in pyogenic liver abscess, 674
  in pyomyositis, 736-737
  in Whipple disease, 643
Abdominal wall, *Neisseria gonorrhoeae* infection of, 1288
Abortion
  enterovirus infection and, 2091-2092
  poliomyelitis and, 2100
  septic, 1797
Abscess
  abdominal. *See* Abdominal abscess
  abdominal wall, *Neisseria gonorrhoeae*, 1288
  adrenal, 706
  Bartholin duct, 579, 579f
  Bezold, 236, 239
  brain. *See* Brain abscess
  breast, **890**
  Brodie, 719
  epidural
    vs. acute transverse myelitis, 523
    cerebral, 443t, **470**
    spinal, 443t, **470-472**, 472f
  gallbladder, 670
  hair follicle, **776-777**
  head and neck. *See* Head and neck infection
  hepatic. *See* Liver abscess
  iliopsoas, 706-707, 707f
  intra-abdominal. *See* Abdominal abscess
  intraperitoneal, 704
  kidney. *See* Renal abscess
  liver. *See* Liver abscess
  lung. *See* Lung abscess
  muscle, **735-739**, 736t, 737f-738f. *See also* Myositis; Pyomyositis
Abscess *(Continued)*
  orbital, 800-801, 800f
  palatal, 141, 143f
  pancreatic, 696, 704-708
  parapharyngeal, 170f, **172-175**
  pelvic, **687**
  periapical, 141, 142f-143f, 144, **146**
  perinephric, 706
  peritoneal, 700-701. *See also* Peritonitis
  peritonsillar, **168-170**, 168f, 170f, 173-175
  psoas, 706
  renal, **553-558**, 555f-557f
  retroperitoneal, **706-709**, 707f
  retropharyngeal, **170-175**, 170f-171f
  scalp, 891, 1282-1283, 1295
  splenic, 673, 704-705
    in infective endocarditis, 355, 357
  submandibular, 149, 150f
  submental space, 142, 144f
  subperiosteal, mastoiditis and, 236, 239
  subphrenic, 704-705
  in toxic shock syndrome, 851t, 852
  tubo-ovarian, **590**
  visceral, 704
Absidia spp. infection
  after hematopoietic stem cell transplantation, 938
  after kidney transplantation, 1013
  neonatal, 899
ACAID (anterior chamber–associated immune deviation), 810
Acanthamoeba polyphaga mimivirus infection, **2048-2050**, 2049f-2050f
Acanthamoeba spp., 2977-2978, 2978f
  as pathogen reservoir, 2985
Acanthamoeba spp. infection (acanthamebiasis), **2975-2986**
  clinical manifestations of, 2979-2980
  CNS, 494t-498t, 501, 2975, 2979, 2981, 2984-2985
  contact lens–related, 1019, 2979-2980
  corneal, 808-809, 2977, 2979-2980, 2980f, 2985
  cutaneous, 2979, 2981, 2984
  diagnosis of, 2981-2983
  epidemiology of, 2975-2977
  neuroimaging in, 2980
  pathogenesis of, 2981
  pathology of, 2981
  prevention of, 2985-2986
  treatment of, 2983-2985
Acetaminophen
  in acute bronchitis, 264
  in common cold, 137
  in fever, 87
  vs. ibuprofen, 87-88
  ibuprofen with, 87
  in otitis media, 228
  in pharyngitis, 164
  in roseola infantum, 771
  in varicella, 85
Achromobacter spp., 1573
Achromobacter spp. infection, **1573-1575**
  clinical manifestations of, 1574-1575
  diagnosis of, 1575
  epidemiology of, 1573-1574
  pathophysiology of, 1574
  treatment of, 1575
Achromobacter xylosoxidans infection, in cystic fibrosis, 336, 341
Acid-base balance, 75-76
Acidosis
  lactic, in septic shock, 829, 832
  metabolic, 75-76
    in malaria, 2928
    in septic shock, 832
  respiratory, 75
Acinetobacter spp., 1568-1569
Acinetobacter spp. infection, **1568-1572**
  bloodstream, 1570
  burn wound, 1052-1053
  clinical manifestations of, 1569-1571
  diagnosis of, 1571
  epidemiology of, 1569, 1570t
  intracranial, 1570
  after lung transplantation, 962
  nosocomial, 1569
  pathogenesis of, 1569
  prevention of, 1572
  prognosis for, 1572
  respiratory tract, 1570-1571
  treatment of, 1571-1572
  urinary tract, 1571
Acoustic reflectometry, 218
Acquired immunodeficiency syndrome (AIDS). *See* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS)
Acremonium spp. infection, 479, **2857-2858**
Acrodermatitis, papular (Gianotti-Crosti syndrome), 649, 749, **766**, **783**, 783f
  in HAV infection, 2140
Acrodermatitis chronica atrophicans, in Lyme disease, 1734
ActA protein, 16
Acticoat, in burn injury, 1059
Actin, neutrophil, dysfunction of, 54
Actin tail
  of *Listeria monocytogenes*, 16
  of *Shigella flexneri*, 16
Actinomyces spp. infection (actinomycosis), **1820-1824**
  abdominal, 1821-1822
  bone, 725
  cervicofacial, 184, 1821, 1821f
  clinical manifestations of, 1821-1823, 1821f-1822f
  diagnosis of, 1823-1824
  hepatic, 1821-1822
  in immunocompromised host, 1823
  laryngeal, 1822-1823
  meningeal, 1822-1823

Page numbers followed by *f* indicate figures; t, tables; *b*, boxes. Page numbers in boldface refer to main discussions.

Actinomyces spp. infection (actinomycosis) *(Continued)*
parotid gland, 191-192
pathogenesis of, 1820-1821
pathology of, 1821, 1821f
pelvic, 1822
pulmonary, 1822, 1822f
retroperitoneal, 706-707, 709
treatment of, 1824
Acute disseminated encephalomyelitis (ADEM), 503, **513-520**
clinical manifestations of, 514
diagnosis of, 513-514, 514t, 515f
epidemiology of, 513
evaluation of, 518-519
immunologic factors in, 517-518
infection and, 515-516, 516t
measles virus, 2392
pathogenesis of, 518
prognosis for, 520
recurrence of, 514-515, 520
treatment of, 519-520
vaccine-associated, 516-517, 516t
variants of, 514-515
Acute hemorrhagic leukoencephalitis, 515
Acute lobar nephropathy, 539, 554, 555f, 556
Acute lung injury (ALI), 867. *See also* Acute respiratory distress syndrome (ARDS)
Acute lymphoblastic leukemia, human bocavirus infection in, 1861
Acute mountain sickness, 3118
Acute necrotizing ulcerative gingivitis, 147, 147f
Acute-phase reactants, 70
Acute-phase reaction, 65, 66f, 79-80. *See also* Metabolism
Acute respiratory distress syndrome (ARDS), **866-875**
clinical course of, 867t, 868-869
definition of, 867, 867t
etiology of, 867-868, 867t
exudative stage of, 869
fibrotic stage of, 870
immunomodulators in, 870
incidence of, 868
in influenza virus infection, 2352
mortality with, 869
pathology of, 869-870
pathophysiology of, 869-870
proliferative stage of, 869-870
treatment of, 870-872
corticosteroids in, 872-873
EMCO in, 873-874
fluid therapy in, 873
isolation in, 873
mechanical ventilation in, 870-872
nitric oxide in, 872
nutrition in, 873
oxygenation goals in, 871
prone positioning in, 873
rescue therapies in, 873-874
sedation and analgesia in, 873
surfactant replacement in, 872-873
Acute retroviral syndrome, 161
Acute transverse myelitis, **520-528**
clinical presentation of, 521-522
CSF examination in, 523
diagnosis of, 520, 521t
differential diagnosis of, 523-525, 523t, 524f
disability after, 528
disease-associated, 523-525, 525t-526t
epidemiology of, 521
idiopathic, 523, 527
Acute transverse myelitis *(Continued)*
imaging in, 522, 522f
immune system in, 527
infection and, 526-527, 526t
postinfectious, 526-527, 526t-527t
prognosis for, 528
radiation-induced, 524
recurrence of, 528
treatment of, 527-528
Acute urethral syndrome, 531
Acyclovir, 3277-3280, 3277f
adverse effects of, 3280
after bone marrow transplantation, 2032
in bone marrow transplantation, 1966
in CMV infection, 1984-1987, 1985f
in CMV infection prevention, 1988
dosage for, 3278t, 3280
in erythema multiforme, 1951
in heart transplantation, 949
in hematopoietic stem cell transplantation, 940-941
in herpes labialis, 155
in herpes zoster ophthalmicus, 804
in HSV infection, 1958-1964
indications for, 3278t, 3279-3280
in infectious mononucleosis, 2012
in kidney transplant–related VZV infection, 1009
in lung transplantation, 975
mechanism of action of, 3277-3278
in neonatal HSV infection, 819, 1962-1963
pharmacokinetics of, 3278-3279
during pregnancy, 1967
resistance to, 3277-3278
spectrum of activity of, 3277
structure of, 1985f
in VZV infection, 2028-2029
Adefovir dipivoxil, in HBV infection, 1922
Adenitis, *Streptococcus agalactiae*, 1161
Adeno-associated viruses, 1891
Adenoidectomy
in otitis media, 232
in otitis media with effusion, 229
in rhinosinusitis, 202
Adenosine deaminase
deficiency of, **48-49**
in severe combined immunodeficiency, 915
Adenotonsillectomy, 164
Adenovirus(es), 1888-1891
animal susceptibility of, 1891
antigenic composition of, 1890
classification of, 1888-1889, 1889t
multiplication of, 1890-1891
physical properties of, 1889-1890
tissue culture growth of, 1890
Adenovirus infection, **1888-1911**
age and, 1891
appendiceal, 1896t, 1902
bladder, 1901
bronchial, 262, 263t, 1896t, 1897
bronchiolitis obliterans in, 1896t, 1898
clinical manifestations of, 1895-1905, 1896t
CNS, 494t-498t, 1894, 1896t, 1903
coinfection with, 1893-1894
congenital, 1905
conjunctival, 802-803, 1896t, 1898-1899
cytokines in, 1895
diagnosis of, 1906-1907, 3611-3612, 3617
differential diagnosis of, 1905-1906
in dilated cardiomyopathy, 1902
Adenovirus infection *(Continued)*
encephalitic, 494t-498t, 1896t, 1903
vs. St. Louis encephalitis virus infection, 2259
epidemiology of, 1891-1893
exanthem in, 741, 742t-748t, 760, 1896t, 1900-1901
fecal virus excretion after, 1893
gastrointestinal tract, 607t, 608, 618-619, 1896t, 1901-1902
genitourinary tract, 1896t, 1901
hearing loss in, 1904
after heart transplantation, 955
after hematopoietic stem cell transplantation, 941-942
hemolytic-uremic syndrome in, 1901
hemorrhagic fever in, 1901
hepatic, 655, 1894, 1902
historical perspective on, 1888
host factors in, 1892
hyperlucent lung in, 1898
immune response to, 1894-1895
immunization against, 1908
in immunocompromised host, 1903-1904
after intestinal transplantation, 997t, 999
in intussusception, 1902
joint, 1904
keratoconjunctival, 1899-1900
after kidney transplantation, 1007, 1010-1011
laryngotracheal, 1897
after liver transplantation, 997t, 999
after lung transplantation, 981-982
meningeal, 488, 1896t, 1903
in mesenteric lymphadenitis, 1902
in military recruits, 1892, 1896-1898, 1896t
myocardial, 1896t, 1902
nasopharyngeal, 156, 157t, 158, 1895-1896, 1896t
neonatal, 1905
nosocomial, 1893, 3382
obesity and, 1905
ocular, 1896t, 1898-1900
oculogenital, 1901
pancreatic, 694
pathogenesis of, 1893-1894
pathology of, 1894-1895, 1894f
pericardial, 1902-1903
pertussis-like illness with, 1617, 1898
pharyngeal, 158-161, 159t-160t, 1895-1896, 1896t
pharyngoconjunctival fever in, 1893-1894, 1899, 1899t-1900t
in preseptal cellulitis, 799
prevalence of, 1891
prevention of, 1908
pulmonary, 284, 295-296, 1894, 1894f, 1896t, 1897-1898
renal, 1901
respiratory tract, 1895-1898, 1896t
roseola with, 769
seasonal patterns of, 1892
social factors in, 1892
testicular, 1901
thyroid, 1904
tonsillar, 1895-1896
transmission of, 1892-1893
treatment of, 1907-1908
Adenylate cyclase toxin, 17
of *Bordetella pertussis*, 1618t, 1619, 1624
Adhesins
nonpilus, 6-9, 7f-9f
pilus, 3-6, 4f-5f
Adhesions, peritoneal, 687-688

Adoptees. *See* International adoptees and refugees
Adrenal gland
  abscess of, 706
  in *Corynebacterium diphtheriae* infection, 1305
  hormones of, 81-82
  in *Leptospira* spp. infection, 1748
Adult respiratory distress syndrome. *See also* Acute respiratory distress syndrome (ARDS)
  in septic shock, 830, 832, 835
  in toxic shock syndrome, 858
Adult T-cell leukemia, HTLV-1 and, 2565-2569, 2566t-2567t
Aedes aegypti, 2243-2244
  in dengue, 2279-2280
  eradication and control of, 2289-2290
  in yellow fever, 2274, 2278
Aedes albopictus, 2242-2243
Aedes vigilax, 2248-2249
Aerobactin, 538
Aerococcus spp., 1248-1249
Aerococcus spp. infection, **1248-1250**
  clinical manifestations of, 1249
  diagnosis of, 1249
  epidemiology of, 1249
  pathophysiology of, 1249
  treatment of, 1249-1250
Aerolysin, 1540
Aeromonas caviae infection, 1542
Aeromonas hydrophila infection
  diagnosis of, 618, 1539-1540
  ecthyma gangrenosum with, 1543
  epidemiology of, 1538-1539
  gastrointestinal, 607t, 612, 616t, 623t, 1541-1542
  pathogenesis of, 1540-1541
  pulmonary, 1543
  urinary tract, 1543
  wound, 1542
Aeromonas spp., 1539-1540
  virulence factors of, 1540-1541
Aeromonas spp. infection, **1538-1545**
  clinical manifestations of, 1541-1543
  diagnosis of, 1539-1540, 1543-1544
  epidemiology of, 1538-1539
  pathogenesis of, 1540-1541
  treatment of, 1540, 1544
Aflatoxin, 2705
African sleeping sickness. *See Trypanosoma brucei gambiense* infection (African sleeping sickness); *Trypanosoma brucei rhodesiense* infection (African sleeping sickness)
Agammaglobulinemia, 907t
  X-linked, 46-47, **908-909**
    vs. chronic bronchitis, 267
    diarrhea in, 600
Age
  adenovirus infection and, 1891
  Babesia spp. infection and, 2918
  chronic fatigue syndrome and, 1087
  coronavirus infection and, 2489-2490
  Eastern equine encephalitis and, 2227
  in epidemiologic study, 108
  Escherichia coli infection and, 1450-1451, 1450t
  Haemophilus influenzae type b infection and, 1674
  HAV infection and, 2132-2133
  HSV infection and, 1936
  infant botulism and, 1803-1804, 1803f
  intestinal transplantation and, 990
Age *(Continued)*
  Kawasaki disease and, 1066-1067
  liver transplantation and, 990
  measles and, 2376, 2376t
  PANDAS and, 1145
  Paracoccidioides spp. infection and, 2781-2782, 2782t
  parainfluenza virus infection and, 2363-2364, 2363f
  rotavirus infection and, 2182
  RSV infection and, 2412-2413, 2413t, 2417f
  rubella virus infection and, 2200-2201, 2200t-2201t, 2201f
  Salmonella spp. infection and, 1493-1494
  St. Louis encephalitis virus infection and, 2257, 2257f, 2260
  Streptococcus pneumoniae infection and, 1199-1200, 1199f-1200f, 1202-1203, 1203f
  toxic shock syndrome and, 850, 850f
  tuberculosis and, 1342
Aggregatibacter actinomycetemcomitans infection, **1610-1611**
  in actinomycosis, 1820, 1823-1824
  periodontal, 140, 147, 1823
  treatment of, 1824
Aggregatibacter (Haemophilus) aphrophilus infection, **1685-1686**
Agranulocytosis, infantile (Kostmann syndrome), 917
AICDA, 909
AIDS (acquired immunodeficiency syndrome). *See* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS)
Air crescent sign, in *Aspergillus* spp. infection, 2712
Air filtration, in lung transplant–related *Aspergillus* infection prevention, 970
Air pollution, cough and, 268
AIRE, 915-916
Airplane travel, rhinosinusitis and, 195
Airway
  diameter of, bronchiolitis and, 276
  inhalation injury of, 1048-1049
  management of
    in epiglottitis, 257-258
    in laryngotracheitis, 257
    in septic shock, 835
  obstruction of
    in EBV infection, 2004
    in Ludwig angina, 149-150
Alanine aminotransferase (ALT)
  in HAV infection, 2130f, 2138
  in hepatitis, 650
Albaconazole, in *Scedosporium* spp. infection, 2850
Albendazole, 3347
  in *Echinococcus granulosus* infection, 3046
  in *Enterobius vermicularis* infection, 567, 3019
  in eosinophilic meningitis, 483-484
  in *Toxocara canis* infection, 3023
Albumin
  serum, in pyogenic liver abscess, 674
  urinary, in yellow fever, 2276
Alcohol use/abuse
  diphtheria and, 1303
  pancreatitis with, 691
Aldosterone, 81
Aleppo button. *See Leishmania* spp. infection (leishmaniasis), Old World
Alfuy virus infection, 2316t
Alice in Wonderland syndrome, 505, 2004
Alkalosis, respiratory, 75
Allergic bronchopulmonary aspergillosis, 2708-2709
  in cystic fibrosis, 341-342
  treatment of, 2721
Allergic rhinitis, vs. common cold, 136
Allergy/allergic reaction. *See also* Anaphylaxis
  cephalosporin, 1296, 3199
  croup and, 247
  egg, 3426, 3451
  vs. infection-related rash, 760
  influenza vaccine, 2352
  mumps vaccine, 2405-2406
  otitis media and, 211
  penicillin, 3189-3191, 3189t
  probiotics in, 3371
  rhinosinusitis and, 195, 201
  vs. roseola infantum, 771
Alligator bite, 3578
Alloiococcus otitidis infection, 214
Allopurinol, in visceral leishmaniasis, 2958
Alopecia areata, vs. tinea capitis, 785
Alveolar macrophages, in *Streptococcus pneumoniae* infection, 1225
Amantadine, 3268-3270, 3268f, 3268t
  in laryngotracheitis, 257
Amebiasis. *See Entamoeba histolytica* infection (amebiasis)
Ameboma, 2869-2870
Amikacin, 3208-3211, 3208t, 3231t-3234t
Amino acids
  metabolism of, 70-72
  requirements for, 71-72
Aminoglycosides, 3208-3211, 3208t, 3231t-3233t
  adverse effects of, 3210-3211
  indications for, 3210
  mechanisms of action of, 3208
  pharmacokinetics of, 3209-3210
  resistance to, 3149-3150, 3150f, 3150t, 3208-3209
  in vitro activity of, 3209
Aminopenicillins, 3183t-3184t, 3186-3187, 3231t-3233t
p-Aminosalicylic acid, in tuberculosis, 1367t, 1369
Aminotransferase, serum, in Kawasaki disease, 1076
Amodiaquine, in malaria, 2942
Amoxicillin, 3183t-3184t, 3186-3187, 3231t-3233t
  in chronic bronchitis, 269-270
  in odontogenic infection, 145
  in otitis media, 227, 228t, 231
  in rhinosinusitis, 200
Amoxicillin-clavulanate, 3187-3188
  Clostridium difficile–related infection and, 633-634
  in otitis media, 227-228, 228t
  in rhinosinusitis, 200
Amphotericin B, 3292f
  in *Aspergillus* spp. infection, 2717-2718
  in *Blastomyces dermatitidis* infection, 2732t, 2733
  in blastomycosis, 478
  in cancer patient, 928
  in *Candida* esophagitis, 597
  in *Candida* meningitis, 474-475
  in *Coccidioides* spp. infection, 477-478, 2777-2778
  in *Cryptococcus neoformans* infection, 475-476, 2804-2805
  in fungal endocarditis, 368
  in *Fusarium* spp. infection, 2848

Amphotericin B *(Continued)*
in *Histoplasma capsulatum* infection, 476-477
lipid formulations of, 3294-3298, 3296t, 3347
ABCD, 3296-3297, 3296t
ABLC, 3296t, 3297
in candidemia, 2747
in cardiac candidiasis, 2748
in chronic disseminated candidiasis, 2749
in CNS candidiasis, 2749
efficacy of, 3296
in endovascular candidiasis, 2748
indications for, 3298
L-AmB, 3296t, 3297-3298
in *Leishmania* spp. infection, 2951-2952, 2958
in neonatal *Candida* spp. infection, 2758
pharmacokinetics of, 3294-3296
safety of, 3296
in lung transplantation, 969
in *Mucor* spp. infection, 479, 2843-2844
in *Naegleria fowleri* infection, 2984
in neonatal zygomycosis, 899
in *Paecilomyces* spp. infection, 2857
in *Paracoccidioides* spp. infection, 2792-2793
in *Penicillium marneffei* infection, 2855
in *Rhizopus* spp. infection, 479, 2843-2844
in *Sporothrix schenckii* infection, 479, 2835
Amphotericin B deoxycholate, 3291-3294, 3347
adverse effects of, 3292-3293
drug interactions with, 3293-3294
in endovascular candidiasis, 2748
in *Histoplasma capsulatum* infection, 2825
indications for, 3294, 3294t-3295t
in lung transplant–related *Aspergillus* infection, 968
mechanism of action of, 3291
monitoring of, 3293
in neonatal *Candida* spp. infection, 2758
nephrotoxicity of, 3292-3293
in ocular candidiasis, 2749
in oropharyngeal candidiasis, 2747
in pericardial candidiasis, 2748
in peritoneal candidiasis, 2748
pharmacodynamics of, 3291-3292
pharmacokinetics of, 3292, 3293t
spectrum of activity of, 3291
Ampicillin, 3183t-3184t, 3186-3187, 3231t-3233t
in community-acquired pneumonia, 291, 291t
exanthematous reaction to, 2002, 2002f
in infectious mononucleosis, 2002, 2002f
in meningitis, 446, 450t
in odontogenic infection, 146t
Ampicillin-sulbactam, 3188, 3231t-3233t
Amylase
in mediastinitis, 420
in pancreatitis, 690, 692
Amyloidosis, in infective endocarditis, 355
Anal cancer, HPV and, 1873t, 1875, 1880-1881
Analgesia, in ARDS, 873
Anaphylaxis. *See also* Allergy/allergic reaction
animal serum and, 3493
HBV vaccine and, 1926
in IgA deficiency, 911
IGIM and, 3494
IVIG and, 3496
Anaphylaxis *(Continued)*
penicillin and, 3189-3190
vaccine and, 3426
Anaplasma spp. infection (anaplasmosis), 494t-498t, 751t-752t, **2661-2663**
Lyme disease and, 1734, 2663
Ancylostoma braziliense infection, 757t-758t, 3024
Ancylostoma duodenale infection, **3016-3018**, 3327t-3346t
Anemia, 77t, 78
aplastic, in infectious mononucleosis, 2002
in *Bartonella bacilliformis* infection, 1726
in congenital syphilis, 1770
hemolytic
in human erythrovirus B19 (human parvovirus B19) infection, 1855
in infectious mononucleosis, 2002
in *Leptospira* spp. infection, 1746-1747
in *Mycoplasma pneumoniae* infection, 2682
in rubella virus infection, 2212
in HIV infection, 2607-2608
in infective endocarditis, 358, 358t
in Kawasaki disease, 1075-1076
in malaria, 76, 78, 2926-2927
sickle-cell. *See* Sickle-cell disease
in Whipple disease, 644
Aneurysm, in Kawasaki disease, 1071, 1080-1084. *See also* Kawasaki disease
Angiography
in Kawasaki disease, 1083
in pyogenic liver abscess, 674
Angioneurotic edema, acute, 250t-251t, 253
Angiostrongylus cantonensis infection, **481-484**, 494t-498t, 3024. *See also* Meningitis, eosinophilic
Anidulafungin, 3308t, 3313f, 3317-3318, 3317t
in *Aspergillus* spp. infection, 968, 2718
Animal bite. *See* Bites, animal
Anisakis spp. infection, 3024
Anorectum, gonococcal infection of, 1287
Anoxia, hepatic injury with, 657
Ant bite, 758t
Anterior chamber–associated immune deviation (ACAID), 810
Anthrax, 602t-603t, **1311-1316**
bioterrorist, 3580-3582
clinical manifestations of, 753t-756t
cutaneous, 753t-756t, 1313-1314, 1313f, 3581-3582
vs. ecthyma, 776, 1315
diagnosis of, 1314-1315
differential diagnosis of, 1314-1315
epidemiology of, 1312
gastrointestinal, 1313-1314
historical perspective on, 1311-1312
inhalational, 1312-1314, 3581-3582, 3581f
meningeal, 1314
pathogenesis of, 1312-1313
postexposure treatment in, 3540t-3544t, 3582
prevention of, 1315-1316
prognosis for, 1315
transmission of, 1312
treatment of, 1315, 3503-3505
Anti-NMDAR (anti-*N*-methyl-D-aspartate receptor) encephalitis, 509
Antibiotic(s), **3182-3241.** *See also* Antibiotic prophylaxis; Antibiotic resistance *and specific antibiotic classes and agents*
in *Achromobacter* spp. infection, 1575
in *Acinetobacter* spp. infection, 1571-1572
Antibiotic(s) *(Continued)*
in *Actinomyces* spp. infection, 1824
administration of, 3230-3239
diffusion properties and, 3235
dosage schedules for, 3230, 3231t-3234t
drug-drug interactions with, 3239
duration of, 3235-3236
food-drug interactions in, 3234
in hepatic insufficiency, 3236
in home setting, 3239
intramuscular, 3234-3235
intravenous, 3234-3235, 3239, **3257-3265**, 3258t-3259t. *See also* Outpatient intravenous antimicrobial therapy
neonatal dosage schedules for, 3230, 3233t
oral vs. parenteral preparations in, 3234
pharmacokinetic-pharmacodynamic interface in, 3160-3163, 3166t. *See also at* Drug(s)
in renal insufficiency, 3236
restrictions on, 3238
in school/daycare setting, 3238
summary guidelines for, 3239-3240, 3240t
topical, 3236-3237
in *Aerococcus* spp. infection, 1249-1250
in *Aeromonas* spp. infection, 1540, 1544
in *Aggregatibacter actinomycetemcomitans* infection, 1610, 1824
in *Aggregatibacter (Haemophilus) aphrophilus* infection, 1686
in anthrax, 1315
in appendicitis, 686
in *Arcanobacterium haemolyticum* infection, 1322-1323, 1326
in *Babesia* spp. infection, 2919-2920
in *Bacillus cereus* infection, 1321
in bacterial meningitis, 446-449, 450t-451t, 1263-1266
in bacterial vaginosis, 578
in *Bacteroides* spp. infection, 1831-1833, 1831t
in *Bartonella bacilliformis* infection, 1728
in *Bartonella henselae* infection, 1727-1728
in *Bartonella quintana* infection, 1728
in *Bartonella* spp. endocarditis, 1728
in bite injury, 3575-3577, 3577t
in *Bordetella pertussis* infection, 1629-1630
in brain abscess, 465t, 468
in Brazilian purpuric fever, 1689-1690
in *Brucella* spp. infection, 1614-1615
in *Burkholderia cepacia* infection, 1601
in *Burkholderia pseudomallei* infection, 1601
in burn wound infection, 1060
in *Campylobacter jejuni* infection, 1655-1656
in catheter-related infection, 3389-3390
in cervical lymphadenitis, 185-188
in cervicitis, 585-586
in chemotherapy-associated infection, 925-928
in *Chlamydia pneumoniae* infection, 2644
in *Chlamydia psittaci* infection, 2639
in *Chlamydia trachomatis* infection, 2637-2638
in cholangitis, 663t
in cholera, 1553, 1554t
cholestasis with, 662
in *Chromobacterium violaceum* infection, 1564
in chronic bronchitis, 269-270
in chronic osteomyelitis, 726-727

Antibiotic(s) *(Continued)*
in *Chryseobacterium* spp. infection, 1580-1581
in *Citrobacter* spp. infection, 1431-1433
in *Clostridium difficile* infection, 637
in coagulase-negative staphylococcal infection, 1138-1139, 1138t
colitis with. *See* Colitis, antibiotic-associated
in community-acquired pneumonia, 289-292, 291t
in conjunctivitis, 802
in *Corynebacterium diphtheriae* infection, 1309-1310
in *Coxiella burnetii* infection, 2665
in craniofacial surgery–related infection, 1046
in croup syndromes, 256
in *Cryptosporidium* spp. infection, 2903
in CSF shunt infection, 1033-1036, 1033t, 1035f, 1037t
in cystic fibrosis–related infection, 333, 337-344
in cystitis, 546-548, 547t
in dacryoadenitis, 797-798
in dacryocystitis, 798
diarrhea with, 599. *See also* Colitis, antibiotic-associated
in diarrheogenic *Escherichia coli* infection, 1462-1463
in *Dientamoeba fragilis* infection, 2886, 2886t
in *Edwardsiella tarda* infection, 1533
in *Eikenella corrodens* infection, 1577-1578
in *Elizabethkingia* spp. infection, 1580-1581
in *Enterobacter* spp. infection, 1437-1438
in enterovirus infection, 2103
in epiglottitis, 259
in erysipelas, 776
in *Erysipelothrix rhusiopathiae* infection, 1328-1329
in *Escherichia coli* UTI, 1444t, 1446-1447
in fever without source, 839
in *Francisella tularensis* infection, 1664
in furunculosis, 777
in *Fusobacterium* spp. infection, 1831-1833, 1831t
in gas gangrene, 1799
in gastrointestinal tract infection, 620t, 622, 623t
in gonorrhea, 571-574
in group C streptococcal infection, 1152
in group G streptococcal infection, 1152
in *Haemophilus aegyptius* infection, 1689-1690
in *Haemophilus ducreyi* infection, 1688
in *Haemophilus influenzae* infection, 1678-1679, 1678t
in *Haemophilus parainfluenzae* infection, 1690
in *Hafnia alvei* infection, 1535
hearing loss and, 1664
in *Helicobacter pylori* infection, 1697t
in hordeolum, 796
in impetigo, 774-775
in inclusion conjunctivitis, 804
in infective endocarditis, 363-368, 364t-367t
in intra-abdominal abscess, 705
in intracranial pressure monitor–related infection, 1040
in intrathecal pump infusion device–related infection, 1041-1042
in keratitis, 807, 808t

Antibiotic(s) *(Continued)*
in *Kingella kingae* infection, 1703-1704
in *Klebsiella granulomatis (Calymmatobacterium granulomatis)* infection, 1643
in *Klebsiella* spp. infection, 1468
in *Legionella pneumophila* infection, 1712-1713
in *Leptospira* spp. infection, 1756-1757
in *Leuconostoc* spp. infection, 1247
in *Listeria monocytogenes* infection, 1333
in lung abscess, 319-321
in Lyme disease, 1735-1738, 1736t
in lymphogranuloma venereum, 584
in mandibular/maxillary osteomyelitis, 153-154
in mastoiditis, 238-240
in methicillin-resistant *Staphylococcus aureus* infection, 1125-1128, 1127f
in *Moraxella catarrhalis* infection, 1253
in *Morganella morganii* infection, 1470-1471
in *Mycobacterium ulcerans* infection, 1416
in *Mycoplasma pneumoniae* infection, 2686-2687
in *Mycoplasma* spp. infection, 2696-2697
in myositis, 739
in nasopharyngitis, 163-164
in necrotizing fasciitis, 779
in *Neisseria gonorrhoeae* infection, 1293-1297, 1293t-1294t
in *Neisseria meningitidis* infection, 1263-1266
in neonatal conjunctivitis, 806
in neonatal meningitis, 886
in neonatal musculoskeletal infection, 890
in neonatal parotitis, 891
in neonatal pneumonia, 892
in neonatal septic arthritis, 734
in neonatal urinary tract infection, 888-889
in *Nocardia* spp. infection, 1422-1423
in nontuberculous mycobacterial infection, 1387-1390, 1388t
normal flora effects of, 89-90
in oral infections, 144-145, 146t
in orbital cellulitis, 800
in *Orientia tsutsugamushi* infection (scrub typhus), 2661
in osteomyelitis, 717-719, 718t
in otitis externa, 206-207
in otitis media, 225-229, 225t, 227t-228t
pancreatitis with, 691-692, 691t
in *Pantoea agglomerans* infection, 1537
in parapharyngeal abscess, 173
in parotitis, 192
in *Pasteurella multocida* infection, 1548
in *Pediococcus* spp. infection, 1248
in pelvic inflammatory disease, 588-589, 589t, 1296
in pericarditis, 378-379, 378t
in peritonitis, 702-704, 703t
in peritonsillar abscess, 169, 174-175
pharmacokinetic-pharmacodynamic interface of. *See at* Drug(s)
in pharyngitis, 163-164
in *Plesiomonas shigelloides* infection, 1566
in pleural effusion, 313-314
in pneumococcal pneumonia, 1233, 1234t
in *Porphyromonas* spp. infection, 1831-1833, 1831t
in post–lung transplantation β-lactamase–producing multidrug-resistant infection, 962

Antibiotic(s) *(Continued)*
in post–lung transplantation *Pseudomonas aeruginosa* infection, 961-962
in preseptal cellulitis, 799
in *Prevotella* spp. infection, 1831-1833, 1831t
in prostatitis, 558-559
in *Proteus* spp. infection, 1473-1474
in *Providencia* spp. infection, 1475-1476
in *Pseudomonas* spp. infection, 1597-1601, 1598t
in pyogenic liver abscess, 675
in pyomyositis, 737-738
in relapsing fever, 1742
in renal abscess, 556-557
in retroperitoneal infection, 708-709
in retropharyngeal abscess, 171-172
in rheumatic fever, 414, 414f, 414t
in rhinosinusitis, 200-201
in *Rhodococcus equi* infection, 1427-1428
in *Rickettsia akari* infection, 2657
in *Rickettsia conorii* infection, 2656
in *Rickettsia prowazekii* infection, 2658-2659
in *Rickettsia typhi* infection, 2659
in Rocky Mountain spotted fever, 2654
in *Salmonella* ser. Typhi infection, 1505-1506, 1505t
in *Salmonella* spp. infection, 1504-1507, 1504t
in sepsis neonatorum, 883-884
in septic arthritis, 732-733
in septic shock, 833-836, 833t
in *Serratia* spp. infection, 1490-1491
in *Shigella* spp. infection, 1483-1485, 1484t
in spinal epidural abscess, 472
in *Spirillum minus* infection, 1760
in *Staphylococcus aureus* infection, 1123-1128, 1124t-1125t
in *Stenotrophomonas maltophilia* infection, 1608-1609
in *Streptobacillus moniliformis* infection, 1719
in *Streptococcus agalactiae* infection, 1154, 1163, 1164t
in *Streptococcus pneumoniae* infection, 1218-1219
in *Streptococcus pyogenes* infection, 1147-1149, 1148t
in *Streptococcus* viridans infection, 1194-1195
in subdural empyema, 470
subeschar, in burn wound infection, 1060
in syphilis, 1776t-1777t, 1777-1780, 1780f
topical, 3236-3237
in burn injury, 1059
in nasolacrimal duct obstruction, 797
in otitis externa, 206
in staphylococcal blepharitis, 794
in toxic shock syndrome, 856-857
in trachoma, 805
in traveler's diarrhea, 3115
in *Ureaplasma* spp. infection, 2696-2697
in urethritis, 533-534, 534t
in urinary catheter–related infection, 3394
in urinary tract infection, 546-548, 547t
in uvulitis, 166-167
in ventilator-associated pneumonia, 3392
in *Vibrio parahaemolyticus* infection, 1559
in *Vibrio vulnificus* infection, 1562
in Whipple disease, 645-646, 646t
in *Yersinia enterocolitica* infection, 1530-1531

Antibiotic(s) *(Continued)*
in *Yersinia pestis* infection, 1513
in *Yersinia pseudotuberculosis* infection, 1530-1531
Antibiotic prophylaxis, **3242-3257.** *See also* Postexposure prophylaxis
in asplenia, 3251-3252
in bacterial meningitis prevention, 1266-1267, 1266t, 3249
in bacterial meningitis prevention, 458-461
after bites, 781, 3251
in *Bordetella pertussis* infection prevention, 1636, 3249
in burn patient, 1060-1061
in cancer patient, 928-929
in cardiovascular surgery, 3255-3256
in catheter insertion, 3244
in cholangitis, 665
in chronic granulomatous disease, 918
in craniofacial surgery, 1045
in CSF leak, 3252
in CSF shunt infection prevention, 1038
in endocarditis prevention, 368-369, 369t, 417-418, 1195-1196, 3246-3247, 3246t-3248t
in epiglottitis prevention, 259
in *Escherichia coli* UTI prevention, 1446
in extracorporeal membrane oxygenation, 1023
in *Haemophilus influenzae* type b infection prevention, 1682, 3249-3250
in heart transplantation, 947
in hematopoietic stem cell transplantation, 935
in hemoglobinopathy, 3252
in IgA deficiency, 911
in IgG subclass deficiency, 911-912
in immunodeficiency disorders, 64
indications for, 3237-3238, 3237t
in intracranial pressure monitor–related infection prevention, 1040
in intrathecal pump infusion device–related infection prevention, 1042
in *Kingella kingae* infection prevention, 1704
in measles prevention, 2392-2393
in necrotizing enterocolitis, 3243-3244
in *Neisseria meningitidis* infection prevention, 1266-1267, 1266t, 3249
in neonate, 3242-3244
in neurosurgical procedures, 3253-3255
in nosocomial infection prevention, 3393
in otitis media prevention, 231, 3247-3248
in pancreatitis, 696
in *Pneumocystis* infection prevention, 3011t, 3012
principles of, 3242, 3243t
in rheumatic fever prevention, 3244-3246, 3245t, 3540t-3544t
in sickle-cell disease, 60, 3252
in *Streptococcus agalactiae* infection prevention, 1165-1168, 1166t, 1168f, 3243
in *Streptococcus pneumoniae* infection prevention, 1236
surgical, 3252-3256, 3253t-3254t
in cardiovascular procedures, 3255-3256
in general procedures, 3253
in neurosurgical procedures, 3253-3255
in type 1 leukocyte adhesion deficiency, 53
in urinary tract infection prevention, 548, 3248
Antibiotic prophylaxis *(Continued)*
in vesicoureteral reflux, 549-551, 549t, 550f-551f
in Wiskott-Aldrich syndrome, 913
in *Yersinia pestis* infection prevention, 1514
Antibiotic resistance, **3137-3159**
in *Achromobacter* spp. infection, 1575
in *Aeromonas* spp. infection, 1540
aminoglycoside, 3149-3150, 3150f, 3150t, 3208-3209
in *Arcanobacterium haemolyticum* infection, 1323
in *Bacteroides fragilis* infection, 1825-1826
in *Campylobacter jejuni* infection, 1655-1656, 1655t, 3155
carbapenem, 3139-3145
ceftaroline, 3153-3154
cellular mechanisms of, 3137-3139, 3138t
cephalosporin, 3139-3145
chloramphenicol, 3157-3158, 3215
in cholera, 1553
colistin, 3207
in cystic fibrosis, 3154
daptomycin, 3153
in *Enterobacter* spp. infection, 1435, 1437
in *Enterococcus* spp. infection, 1177-1182, 1179t. *See also Enterococcus* spp. infection, treatment of, resistance in
fluoroquinolone, 3226
glycopeptide, 3151-3154, 3152t
in *Haemophilus influenzae* infection, 1668
in *Hafnia alvei* infection, 1535
in *Klebsiella* spp. infection, 1468
β-lactam, 3139-3145, 3140f-3141f, 3140t, 3202-3203
lincosamide, 3145-3147, 3220
linezolid, 3153, 3223
macrolide, 3145-3147, 3211-3212
methicillin. *See Staphylococcus aureus* infection, methicillin-resistant
monobactam, 3139-3145
in *Neisseria gonorrhoeae* infection, 1291-1293, 1293f
in otitis media, 3147
penicillin, 3139-3145. *See also* Penicillin, resistance to
in pneumonia, 3147
in *Providencia* spp. infection, 1475-1476
in *Pseudomonas aeruginosa* infection, 1601-1603, 3154
quinolone, 3154-3157
in *Salmonella* ser. Typhi infection, 1495
in *Salmonella* spp. infection, 624, 1494
in *Serratia marcescens* infection, 1489
in *Shigella* spp. infection, 1484-1485, 3147-3148
in *Staphylococcus aureus* infection. *See Staphylococcus aureus* infection, methicillin-resistant
in *Stenotrophomonas maltophilia* infection, 1608-1609
in *Streptococcus pneumoniae* infection, 1219-1223, 1220t-1221t, 1222f, 3144-3145, 3155. *See also Streptococcus pneumoniae* infection, antibiotic resistance in
in *Streptococcus* viridans infection, 1194
streptogramin, 3145-3147
tetracycline, 3217-3218
trimethoprim-sulfamethoxazole, 3147-3149, 3148f, 3228
in urinary tract infection, 3148
vancomycin, 3151-3154, 3152t, 3204. *See also* Vancomycin, resistance to
in *Yersinia enterocolitica* infection, 1531
Antibody (antibodies). *See also specific immunoglobulins*
anti-GQib, 505
anti-*Toxoplasma*, 817
anti–streptolysin O, 1141, 1147
in common colds, 135-136
deficiencies of, 46-48
in IgA deficiency, 47
in IgG subclass deficiency, 47
IVIG in, 3497
laboratory evaluation of, 906
normal/elevated immunoglobulin levels and, 47-48
in transient hypogammaglobulinemia of infancy, **47**
in X-linked agammaglobulinemia, 46-47
in newborn, 42-44, 43f, 43t
TSST-I, 852, 856-857, 863-864
Anticancer agents. *See* Chemotherapy
Anticholinesterase insecticide poisoning, pancreatitis with, 692
Anticonvulsants
in brain abscess, 469
in meningitis-related seizures, 455
Antidiuretic hormone (ADH), inappropriate secretion of, 76, 81
in bacterial meningitis, 438, 453-454
Antiendotoxin therapy, in *Neisseria meningitidis* infection, 1264-1265, 1265t
Antifungal agents, **3291-3327**, 3292f. *See also specific antifungal agents*
in *Acremonium* spp. infection, 2858
in *Aspergillus* spp. infection, 2717-2721
in *Blastomyces dermatitidis* infection, 2732-2734, 2732t
in cancer patient, 928
in *Candida* spp. infection, 2746-2749, 2746t
in *Coccidioides* spp. infection, 2777-2778
in *Cryptococcus neoformans* infection, 2804-2805
in Entomophthorales infection, 2845
in *Fusarium* spp. infection, 2848
in *Histoplasma capsulatum* infection, 2824-2829, 2824t
in *Mucor* spp. infection, 2843-2844
in neonatal *Candida* spp. infection, 2757-2760
in *Paecilomyces* spp. infection, 2857
in *Paracoccidioides* spp. infection, 2792-2793
prophylactic, 2745-2746
in cancer patient, 929
in neonatal *Candida* spp. infection prevention, 2760-2765, 2764t
in *Rhizopus* spp. infection, 2843-2844
in *Scedosporium* spp. infection, 2850
in *Sporothrix schenckii* infection, 2835
topical, 3321t, 3324
in *Trichoderma longibrachiatum* infection, 2857
Antifungal azoles, 3301-3312, 3301f
mechanism of action of, 3302, 3302f
resistance to, 3302-3303
spectrum of activity of, 3302
Antigen
for delayed hypersensitivity skin test, 907
impaired response to. *See* Antibody (antibodies), deficiencies of
Antihistamines
in common cold, 137
in rhinosinusitis, 201
in rhinovirus infection, 2126

Antilymphocyte antibody therapy (OKT3), in kidney transplantation, 1007
Antimalarial agents, 2936-2943, 2937t-2940t
  prophylactic, 2943-2945, 2944t
Antimicrobial stewardship programs, 3414, 3414t
Antimonials, in visceral leishmaniasis, 2957
Antimotility agents
  in diarrheogenic *Escherichia coli* infection, 1463
  in traveler's diarrhea, 3114
Antioxidants, 77
Antiparasitic agents, **3327-3354**, 3327t-3346t, 3351t-3353t. *See also specific antiparasitic agents*
  adverse effects of, 3347
  in *Ancylostoma duodenale* infection, 3018
  in *Ascaris lumbricoides* infection, 3014-3015
  in *Babesia* spp. infection, 2919-2920
  in *Balantidium coli* infection, 2896
  in *Blastocystis hominis* infection, 2876
  in *Clonorchis sinensis* infection, 3052
  in *Cryptosporidium* spp. infection, 2903
  in *Dientamoeba fragilis* infection, 2886, 2886t
  in *Diphyllobothrium* spp. (fish tapeworm) infection, 3041
  in *Dipylidium caninum* (dog tapeworm) infection, 3041
  in *Echinococcus granulosus* infection, 3046
  in *Entamoeba coli* infection, 2877-2878
  in *Entamoeba histolytica* infection, 2873-2874, 2873t
  in *Enterobius vermicularis* infection, 3019
  in *Fasciola buski* infection, 3057
  in *Fasciola* spp. infection, 3053-3054
  in *Giardia lamblia* infection, 2882-2883, 2883t
  in *Hymenolepis nana* (dwarf tapeworm) infection, 3042
  manufacturers of, 3354
  in microsporidial infection, 2913
  in *Necator americanus* infection, 3018
  in *Onchocerca volvulus* infection, 3027
  in *Opisthorchis* spp. infection, 3052
  in *Paragonimus* spp. infection, 3056
  in pregnancy, 3351, 3351t-3353t
  in *Schistosoma* spp. infection, 3068
  in *Strongyloides fuelleborni kellyi* infection, 3021
  in *Strongyloides stercoralis* infection, 3021
  in *Taenia saginata* infection, 3032
  in *Taenia solium* infection, 3037-3039
  in *Toxocara canis* infection, 3023
  in *Toxoplasma gondii* infection, 817, 3000-3002, 3001t
  in *Trichomonas vaginalis* infection, 2892-2893, 2893t
  in *Trichuris trichiura* infection, 3016
  in *Trypanosoma brucei* infection, 2971-2973
  in *Trypanosoma cruzi* infection, 2966-2967
  in *Wuchereria bancrofti* infection, 3026
Antipyretics, **86-88**
  adverse effects of, 85
  indications for, 86
Antiretroviral agents, 600
  in adult T-cell leukemia, 2569
  in HIV-1 infection, 2593-2594, 2609-2617, 2610t, 2611f-2612f
  in HIV-2 infection, 2586-2588
  in HTLV-1–associated myelopathy, 2573
Anti–streptolysin O (ASO) antibodies/titer, 1141, 1147
  in rheumatic fever, 411
Antitoxin, 3491-3493
  in botulism, 1794, 3505
  in *Corynebacterium diphtheriae* infection, 1309-1310, 3506-3507
  in tetanus, 1815-1816, 3511-3512
Antituberculosis drugs, 1366-1369, 1367t
Antitussives, in common cold, 137
Antiviral agents, **3265-3290**, 3266t. *See also specific antiviral agents*
  in adenovirus infection, 1907-1908
  in arenavirus infection, 2475
  in BK virus infection, 1869
  in CMV infection, 1984-1987, 1985f, 2601-2602
  in Crimean-Congo hemorrhagic fever, 2533-2534
  in erythema multiforme, 1951
  in genital HSV infection, 1960-1961, 1960t
  in hantavirus infection, 2510-2511
  in HBV infection, 1920-1923, 1923t
  in HCV infection, 2323-2324
  in HHV-6 infection, 2019
  in HHV-8 infection, 2021
  historical perspective on, 3265-3268
  in hMPV infection, 2440
  in HPV infection, 1884
  in HSV infection, 1958-1964, 1961t
  in HSV infection prevention, 1966-1967
  in infectious mononucleosis, 2012
  in influenza virus infection, 2348-2351, 2349t
  in La Crosse virus encephalitis, 2524-2525
  mechanisms of action of, 3267, 3267f
  in monkeypox, 2044
  in post-transplant lymphoproliferative disease, 2012
  in rhinovirus infection, 2126
  in RSV infection, 2426
  stockpiles of, 2358
  in subacute sclerosing panencephalitis, 2390
  in VZV infection, 2028-2029
  in West Nile virus infection, 2272
Aortic regurgitation, in Kawasaki disease, 1081
Aphthous stomatitis, recurrent, 159t-160t, 161
Aplastic crisis
  diagnosis of, 1855
  in human erythrovirus B19 (human parvovirus B19) infection, 1849-1850
Apnea, in RSV infection, 2419
ApoA-I, 74
Apocrine glands, infection of, **777**
Apoi virus infection, 2316t
Appendectomy, 685-686
  malignancy and, 687-688
  in nonperforated appendicitis, 685
  in perforated appendicitis, 685-686
Appendicitis, **679-689**
  adenovirus, 1902
  bacteria in, 683t, 684
  clinical manifestations of, 680-681
  complications of
    early, 686-687
    late, 687-688
  coxsackievirus, 2070
  diagnosis of, 681-683
  differential diagnosis of, 682t
  epidemiology of, 679-680
  fungi in, 683t, 685
  historical perspective on, 679
  measles virus, 2392
Appendicitis *(Continued)*
  microbiology of, 683-685, 683t
  mortality with, 686
  neonatal, 681
  nonperforated, 685
  parasites in, 683t, 684-685
  Pasteurella multocida, 1547
  pathophysiology of, 680
  pelvic abscess and, **687**
  perforated, 701
    epidemiology of, 680
    treatment of, 685-686, 703, 703t
  in preschool-aged children, 681
  prognosis for, 686-687
  treatment of, 685-686
  viruses in, 683t, 685
  Yersinia enterocolitica, 1520, 1527
  Yersinia pseudotuberculosis, 1521, 1527
APTIMA test, in *Trichomonas vaginalis* infection, 2892
Aquacel, in burn injury, 1059
Arcanobacterium haemolyticum, 1322, 1323t
  toxin of, 1322
Arcanobacterium haemolyticum infection, **1322-1327**
  adult, 1325
  clinical manifestations of, 1324-1326, 1324f, 1324t
  CNS, 1325
  cutaneous, 753t-756t, 1325
  differential diagnosis of, 1326
  EBV infection and, 1324
  endocardial, 1325-1326
  epidemiology of, 1323
  exanthem in, 1324-1325, 1324f
  historical perspective on, 1322
  in Lemierre's disease, 1326
  pathogenesis of, 1323-1324
  pharyngeal, 1324-1325
  prognosis for, 1326
  thoracic, 1325
  treatment of, 1326
    antimicrobial susceptibility in, 1322-1323
  vancomycin-resistant, 1323
ARDS. *See* Acute respiratory distress syndrome (ARDS)
Area under the curve (AUC), 3161, 3161f
Arenavirus(es), 2467, 2468t, 2469f
Arenavirus infection, **2466-2477.** *See also* Lassa virus infection; Lymphocytic choriomeningitis virus (LCMV) infection
  bioterrorist, 3585
  clinical manifestations of, 2471-2472, 2471t
  diagnosis of, 2473-2475
  differential diagnosis of, 2475
  epidemiology of, 2467-2470, 2470t
  historical perspective on, 2466-2467
  nosocomial, 3401-3402
  pathogenesis of, 2472-2473
  pathology of, 2472-2473
  prevention of, 2476-2477
  prognosis for, 2475
  transmission of, 2470-2471
  treatment of, 2475, 3533-3534
  vaccine against, 2476-2477
Argentine hemorrhagic fever. *See* Junín virus infection
Argyll Robertson pupil, 812
Arlt lines, 804-805
Arrhythmias
  in myocarditis, 389, 397
  in pericarditis, 376

Artemether, 3347
Artemether-lumefantrine, 3347
  in malaria, 2937t-2940t, 2941
Arteriovenous fistula, endocarditis and, 352
Artesunate, 3347
  in malaria, 2942
Arthralgia
  in bacterial meningitis, 441
  in human erythrovirus B19 (human parvovirus B19) infection, 1851
  in Whipple disease, 643
Arthritis. *See also* Septic arthritis
  in adenovirus infection, 1904
  in bacterial meningitis, 441, 1261-1262
  Campylobacter jejuni, 1654
  Candida, 2742, 2749
  chikungunya virus, 2244, 2247
  Coccidioides spp., 2773-2774
  enterovirus, 2078
  in erythema infectiosum, 1849
  fungal, 730, **734**
  gonococcal, 729t, 730, 734
  HTLV-1, 2575-2576
  human erythrovirus B19 (human parvovirus B19), 1851
  in Kawasaki disease, 1074, 1082
  Lyme, 730, 812, 1731-1733, 1736
  Mycoplasma pneumoniae, 2683
  poststreptococcal, 412. *See also* Rheumatic fever
  reactive, 734
    with *Campylobacter jejuni* infection, 1654
    with *Shigella* spp. infection, 1481
    with *Yersinia enterocolitica* infection, 1528-1529
  Ross River virus, 2248-2251. *See also* Ross River virus arthritis
  rubella virus, 2208, 2211, 2219
  Sporothrix schenckii, 2834
  Streptobacillus moniliformis, 1717
  tuberculous, 1355-1356
Arthritis-dermatitis syndrome, in gonococcal infection, 1287, 1288f
Arthrocentesis, in Whipple disease, 643
Arthropod bites, 2862t-2863t, **3070-3079.** *See also specific arthropods*
  cutaneous manifestations of, 758t, 763t-764t
  vs. virus-induced exanthem, 762-763
Arthus reaction, penicillin-related, 3190
Ascaris lumbricoides infection (ascariasis), **3013-3015**
  appendiceal, 684
  biliary, 660, 3014
  clinical manifestations of, 757t-758t, 3014
  diagnosis of, 3014
  epidemiology of, 3013-3014
  neonatal, 3014
  ocular, 815
  pancreatic, 694, 694f, 707-708, 3014
  pathophysiology of, 3014
  prevention of, 3015
  prognosis for, 3015
  treatment of, 3014-3015, 3327t-3346t
Aschoff body, 408
Aspartate aminotransferase (AST), in hepatitis, 650, 2138
Aspergilloma, 2708
Aspergillus flavus, 2705
Aspergillus fumigatus, 2705
Aspergillus fumigatus infection, 2708-2709
  after heart transplantation, 953
  after lung transplantation, 965-970
Aspergillus spp., 2704-2705
Aspergillus spp. infection (aspergillosis), **2704-2722**
  bone, 726
  bronchopulmonary, allergic, 2708-2709
    in cystic fibrosis, 341-342
    treatment of, 2721
  burn wound, 1053
  in cancer patient, 922, 926-927, 927t
  cerebral, 2707, 2713-2714, 2714f
  chest radiography in, 2712
  chronic, 2708
  in chronic granulomatous disease, 56-57, 917, 918f, 2706, 2713f
  clinical manifestations of, 2705-2709
  corneal, 807-808
  CT in, 2712-2714, 2713f
  cutaneous, 791f, 2707-2708
  in cystic fibrosis, 336, 341-342, 2713f
  diagnosis of, 2711-2717
    antibody testing in, 2714
    bronchoalveolar lavage in, 2715-2716
    culture in, 2711-2712, 2712f
    G test in, 2716
    galactomannan antigen in, 926, 927t, 2714-2715
    imaging in, 2712-2714, 2713f-2714f
    PCR in, 2716-2717
  endocardial, 363
  epidemiology of, 2709-2711
  exanthem in, 757t
  after hematopoietic stem cell transplantation, 937-938, 2707-2711, 2713
  immune response to, 23, 2705
  after intestinal transplantation, 994-995, 995t
  after liver transplantation, 994, 995t
  after lung transplantation, 959-960, 965-970
  meningeal, 478-479
  neonatal, 899
  orbital, 801
  pancreatic, 695
  paranasal sinus, 196-197, 196t, 201, 2706, 2707f-2708f
  pulmonary, 2706
    CT in, 2712-2713, 2713f
    treatment of, 2717-2721, 3295t
  uveal, 813
Aspiration, prevention of, 3393
Aspiration (diagnostic/therapeutic)
  in brain abscess, 468
  in cervical lymphadenitis, 184
  in craniofacial surgery–related infection, 1046
  in CSF shunt–related infection, 1032
  in lung abscess, 319, 321
  in mastoiditis, 237-238
  in middle ear effusion, 218-220
  in osteomyelitis, 714-715
  in pericarditis, 378, 378t
  in peritonitis, 702
  in peritonsillar abscess, 169
  in prosthetic joint infection, 1028
  in septic arthritis, 731, 732t
Aspirin
  adverse effects of, 85
  in Kawasaki disease, 1078-1079, 1078t
  Reye syndrome and, 86-87, 657, **676-677**, 2351
Asplenia, 59
  antibiotic prophylaxis in, 3251-3252
  Streptococcus pneumoniae infection and, 1224, 1232
Asthma
  acetaminophen and, 87
  Chlamydia pneumoniae infection and, 2641-2642
  vs. chronic bronchitis, 266
  coronavirus infection and, 2491
  HIV-related, 2604
  hMPV infection and, 2439
  infectious, **271-282.** *See also* Bronchiolitis
    definition of, 271
    diagnosis of, 278
    differential diagnosis of, 278, 278t
    enterovirus in, 2066
    historical perspective on, 273
  vs. interstitial lung disease, 297
  Moraxella catarrhalis colonization and, 1252
  Mycoplasma pneumoniae infection and, 2679
  rhinosinusitis and, 197, 202
  rhinovirus infection and, 2123
  RSV infection and, 2421
  Schistosoma spp. infection and, 3063
  T lymphocytes in, 277-278
Astrovirus infection, 607t, 608
  in daycare facilities, 3558
Asunaprevir, in HCV infection, 2324
Asymmetric periflexural viral exanthem, **784**, 784f
Ataxia-telangiectasia, 50, 904-905, 907t, **914**
  vs. chronic bronchitis, 267
  clinical features of, 905f, 914
Atazanavir, 2614-2615
Atherosclerosis, CMV and, 1980
ATM gene, 50
Atopic disease, probiotics in, 3371
Atovaquone, 3347
  in hematopoietic stem cell transplantation, 938-939
Atovaquone-proguanil
  in malaria, 2937t-2940t, 2941
  in malaria prevention, 2944t, 2945
Atrial ectopic tachycardia, in myocarditis, 389
Attachment invasin locus (Ail) protein, 13, 1523-1524
Audiometry
  in mastoiditis, 239
  in otitis media, 218
Autism, vaccine and, 3429
Autoimmune polyendocrinopathy ectodermal dystrophy (APECED), 915-916
Autolysin, of *Streptococcus pneumoniae*, 1214
Autotransporter proteins, 6
  conventional, 6-7, 8f-9f
  trimeric, 6, 8f-9f
Axilla, temperature measurement by, 86
Azathioprine, in myocarditis, 396-397
Azithromycin, 3211-3215, 3213t, 3231t-3233t, 3347
  drug interactions with, 3215
  in heart transplant–related *Legionella pneumophila* infection, 949
  in *Naegleria fowleri* infection, 2984
  in nontuberculous mycobacterial lymphadenitis, 187
  in odontogenic infection, 146t
  topical, in neonatal conjunctivitis prevention, 1281
  in trachoma, 805
Aztreonam, 3200, 3231t-3233t
  in meningitis, 448

B

B lymphocytes, 40-42. *See also* Immunoglobulin(s)
  in burn injury, 1050
  in common variable immunodeficiency, 49
  in EBV infection, 1995
  fetal, 42
  in Kawasaki disease, 1077
  in RSV infection, 2415-2416
  in Wiskott-Aldrich syndrome, 50
B19 virus. *See* Human erythrovirus B19 (human parvovirus B19) infection
Babesia spp. infection (babesiosis), **2915-2920**
  clinical manifestations of, 2918
  diagnosis of, 2916f, 2918-2919
  epidemiology of, 2915-2917, 2916f
  immune response in, 2917-2918
  in immunocompromised host, 2918
  Lyme disease and, 1734
  pathogenesis of, 2917-2918
  prevention of, 2920
  transfusion-related transmission of, 2917
  treatment of, 2919-2920, 3327t-3346t
Bacillary angiomatosis, **1726**
  diagnosis of, 1727
  treatment of, 1728
Bacillary peliosis hepatis, **1726**
  diagnosis of, 1727
  treatment of, 1728
Bacille Calmette-Guérin (BCG), 3477-3478
  in HIV-infected patient, 1377
  in Kawasaki disease, 1074, 1077
  in *Mycobacterium leprae* infection, 1409-1410
  in *Mycobacterium tuberculosis* infection, 1376-1378, 1377t
  in *Mycobacterium ulcerans* infection, 1416-1417
Bacillus anthracis, 1312. *See also* Anthrax
Bacillus cereus, 1317
Bacillus cereus infection, **1316-1321**
  bloodstream, 1319
  clinical manifestations of, 1319-1320
  CNS, 1320
  complications of, 1320, 1321t
  diagnosis of, 618, 1320
  encephalopathy with, 677
  epidemiology of, 1317-1318
  gastrointestinal, 602t-603t, 607t, 613, 1319
  hepatic, 1320
  ocular, 820, 1319
  pathogenesis of, 1318-1319
  prevention of, 1321
  pseudoinfections with, 1320
  pulmonary, 1320
  skeletal, 1319
  soft tissue, 1319
  treatment of, 1320-1321, 1321t
Bacillus spp. infection
  burn wound, 1052
  endocardial, 362-363
Baclofen, in tetanus, 1817
Bacteremia, **824-836.** *See also* Septic shock
  Achromobacter xylosoxidans, 1574
  Acinetobacter spp., 1570
  Aerococcus spp., 1249
  Aeromonas spp., 1543
  Aggregatibacter (Haemophilus) aphrophilus, 1685-1686
  in appendicitis, 684
  Bacillus cereus, 1319
  Bacteroides fragilis, 1828
  Bordetella holmesii, 1637
Bacteremia *(Continued)*
  in burns, 1055
  Campylobacter spp., 1652-1653
  catheter-related, 3385-3391, 3386t-3388t, 3387f
  Chryseobacterium spp., 1580
  Citrobacter spp., 1431
  clinical presentation of, 831-833
  Clostridium perfringens, 1797
  Clostridium septicum, 1798
  Clostridium spp., 1798
  coagulase-negative staphylococci, 1133-1136, 1134t
  diagnosis of, 831-833
  Elizabethkingia meningosepticum, 1580
  Enterobacter spp., 1436
  Enterococcus spp., 1174-1177
  Escherichia coli, 1441
    clinical manifestations of, 1444
    epidemiology of, 1441
    pathogenesis of, 1443-1444
    treatment of, 1447
  Haemophilus influenzae, 1669-1670
  Haemophilus influenzae type b, 1675, 1678-1679
  Haemophilus parainfluenzae, 1690
  Hafnia alvei, 1534-1535
  after heart transplantation, 947-948
  after hematopoietic stem cell transplantation, 935-936, 1136
  incidence of, 824
  in infective endocarditis, 353-354, 353t
  after kidney transplantation, 1006
  Kingella kingae, 1700, 1702-1703
  Klebsiella pneumoniae, 1467-1468
  Leuconostoc spp., 1247
  after lung transplantation, 960
  in meningitis, 431-432, 432t
  Moraxella catarrhalis, 1252
  Morganella morganii, 1470
  in myositis, 739
  Neisseria gonorrhoeae, 1287
  Neisseria meningitidis, 1257
  Nocardia spp., 1421
  occult, 837-839, 840t, 1226-1228
  in osteomyelitis, 712
  Pantoea agglomerans, 1536
  Pasteurella multocida, 1547
  pathophysiology of, 825-831
  Pediococcus spp., 1248
  in pelvic abscess, 687
  in peritonitis, 702
  Proteus spp., 1473
  Providencia spp., 1475
  in pyogenic liver abscess, 672-673
  in pyomyositis, 735
  renal abscess and, 553
  Salmonella spp., 1500-1501, 1504-1505
  in septic arthritis, 728
  Serratia spp., 1490
  Shigella spp., 1481
  Staphylococcus aureus, 361-362, 824, 833-834, 1121
  Staphylococcus epidermidis, **1134-1136**, 1135t
  Stenotrophomonas maltophilia, 1607-1609
  Streptococcus agalactiae, 1160
  Streptococcus pneumoniae, 1226-1228, 1227t, 1235
  Streptococcus viridans, 1190
  treatment of, 833-836
  Vibrio parahaemolyticus, 1558
  in Whipple disease, 642-643
  without focus, 1227-1228
  Yersinia enterocolitica, 1528
Bacterial infection. *See also specific infections, infectious organisms, and sites of infection*
  adhesins in, 3-9, 4f-5f, 7f-9f
  antiphagocytic factors in, 18
  biofilms in, 11-12, 12f. *See also* Biofilm
  cell invasion in, 13-14, 14f
  cell-to-cell spread in, 16
  cold-like symptoms in, 132, 133t
  colonization in, 3-12. *See also* Colonization
  complement evasion in, 18
  encapsulation in, 19-20
  host response to, 23. *See also* Complement; Immunity; Metabolism
  immune system evasion in, 18-20
  intracellular pathogen survival in, 14-15
  laboratory diagnosis of, **3589-3595**
    culture in, 3589-3592, 3590t. *See also* Culture, bacterial
    mass spectroscopy in, 3594-3595
    microscopy in, 3592
    molecular techniques in, 3592-3594. *See also* Polymerase chain reaction (PCR)
    sequence-based technologies in, 3594
  nomenclature in, **1096-1112**, 1097t-1112t
  toxins in, 16-18. *See also* Toxin(s)
Bactericidal/permeability increasing (BPI) protein, 3365-3366
  recombinant, in *Neisseria meningitidis* infection, 1264-1265, 1265t, 3365-3366
Bacteriocins, of *Pediococcus* spp., 1248
Bacteriuria. *See also* Urinary tract infection (UTI)
  asymptomatic, 538-539
    Escherichia coli, 1441, 1443
    during pregnancy, 1154
  definition of, 542, 542t
  neonatal, 888-889
Bacteroides fragilis infection
  antibiotic resistance in, 1825-1826
  appendiceal, 684. *See also* Appendicitis
  bloodstream, 1828
  bone, 1829-1830
  CNS, 1828-1829
  diagnosis of, 1830
  endocardial, 367
  epidemiology of, 1827
  intra-abdominal, 1829
  joint, 1829-1830
  peritoneal, 700
  pleuropulmonary, 1830
  retroperitoneal, 706-707
  soft tissue, 1829
  treatment of, 1831-1833, 1831t
Bacteroides spp., 1825-1826
Bacteroides spp. infection, **1825-1834**
  bloodstream, 1827-1828
  bone, 1829-1830
  clinical manifestations of, 1827-1830
  CNS, 1828-1829
  cutaneous, 1829
  diagnosis of, 1830
  epidemiology of, 1826-1827
  head and neck, 1828
  intra-abdominal, 1829
  joint, 1829-1830
  pathogenesis of, 1827
  pleuropulmonary, 1830
  prevention of, 1833
  soft tissue, 1829
  treatment of, 1831-1833, 1831t
BAL. *See* Bronchoalveolar lavage (BAL)
Balamuthia mandrillaris, 2978-2979, 2978f

Balamuthia mandrillaris infection (balamuthiasis), **2975-2986**
clinical manifestations of, 2980
CNS, 494t-498t, 501, 511, 2975, 2980-2981, 2984-2985
diagnosis of, 2981-2983
epidemiology of, 2975-2977
neuroimaging in, 2980
pathology of, 2981
prevention of, 2985-2986
treatment of, 2983-2985
Balantidium coli infection (balantidiasis), **2894-2896**, 3327t-3346t
Balkan endemic nephropathy, 1867
Balloon catheter sinuplasty, 202
Bam H1A rightward transcript (BART), 1993-1994
Banna virus, 2175
Bannwarth syndrome, 1733
Banzi virus infection, 2316t
Barium x-ray
esophageal, 594-595, 595f
in *Shigella* spp. infection, 1483
in Whipple disease, 645
Barmah Forest fever, 2251-2252
Bartholin gland/duct
abscess of, 579, 579f
gonococcal infection of, 1284, 1285f
inflammation of, 579
Bartonella bacilliformis infection (bartonellosis, Carrión disease, Oroya fever), **1725-1726**
diagnosis of, 1727
exanthem in, 753t-756t, 1725-1726
prognosis for, 1729
treatment of, 1728
Bartonella henselae, 1721, 1721t
Bartonella henselae infection (cat-scratch disease), **1721-1725**
bone, 1723-1724
cervical lymphadenitis in, 178, 181
clinical manifestations of, 753t-756t, 1722-1725, 1723f-1724f
cutaneous lesions in, 753t-756t, 1724-1725
diagnosis of, 185, 1726-1727
differential diagnosis of, 1727
encephalitic, 494t-498t
endocardial, 363
epidemiology of, 1722
fever of unknown origin in, 844
hepatosplenic, 1722-1723, 1724f, 1728
lymphadenopathy in, 1722, 1723f
neurologic complications of, 1723
ocular manifestations of, 813
oculoglandular syndrome of Parinaud in, 1722, 1724f
pathobiology of, 1722
vs. plague, 1513
prevention of, 1729
prognosis for, 1729
vs. St. Louis encephalitis virus infection, 2259
transmission of, 1721-1722
treatment of, 187-188, 1727-1728
typical, 1722
vasculoproliferative, **1726-1728**
Bartonella quintana infection (trench fever), **1726**
diagnosis of, 1727
endocardial, 363
exanthem in, 753t-756t
ocular, 815
treatment of, 1728
vasculoproliferative, **1726-1728**
Bartonella spp., 1720-1721, 1721t
Bartonella spp. infection, **1720-1729.** *See also specific species infections*
Basic fibroblast growth factor, in *Streptococcus pneumoniae* infection, 1225
Basidiobolus spp., 2837
Basidiobolus spp. infection (entomophthoromycosis), **2844-2845**
Bat bite. *See* Rabies
Baylisascaris procyonis infection
encephalitic, 494t-498t
meningeal, **481-484**, 3023-3024. *See also* Meningitis, eosinophilic
ocular, 815
Beau lines, in Kawasaki disease, 1073-1074
Bed bugs, 758t, **3076-3077**
Bed rest
in *Corynebacterium diphtheriae* infection, 1310
in rheumatic fever, 413
Beef tapeworm *(Taenia saginata)* infection, **3030-3032**, 3327t-3346t
Behavior/behavioral disorders
congenital rubella and, 2217
infection and, 109-110
otitis media and, 222
urinary tract infection and, 536, 548
Behçet syndrome, 159t-160t, 161
Bejel, 1783t, **1787-1788**
Benznidazole, 3347
in *Trypanosoma cruzi* infection, 399, 2967
Benzodiazepines, in tetanus, 1816-1817
Benzyl alcohol, 3347
in lice, 3075
Bevacizumab, in West Nile virus infection, 2272
Bezold abscess, 236, 239
Bias, 102, 124-125
Bifidobacteria, in gastrointestinal tract infection, 626
Bile duct, common
infection of. *See* Cholangitis
obstruction of, 670
pancreatitis with, 692
Biliary atresia, 657
biloma in, 661-662
complications of, 664
hepatic portoenterostomy and, 658-659, 664-665
reovirus and, 2170-2171
Biliary stents, 667
Biliary system
infection of. *See* Cholangitis
obstruction of, 659. *See also* Cholangitis
surgical treatment of, 664
Bilirubin, serum
in HAV infection, 2138
in hepatitis, 650
in leptospirosis, 656
Biloma, 661-662
Biofilm, 11-12, 12f
in burn-related infection, 1050-1051
Citrobacter spp., 1430
of coagulase-negative staphylococci, 1132-1133, 1133f
in CSF shunt infection, 1030-1031
in implant-related infection, 1017
Neisseria gonorrhoeae, 1277
Pseudomonas aeruginosa, 11, 14f, 1588
Bipolaris spp. infection, **2855-2858**
Biopsy
in acute disseminated encephalomyelitis, 519
in *Aspergillus* spp. infection, 2711, 2712f
Biopsy *(Continued)*
in burn wound infection, 1056-1057, 1056f
in cervical lymphadenopathy, 185
in cholangitis, 661
in CMV infection, 1974, 1974f, 1984
in *Cryptosporidium* spp. infection, 2902
in *Entamoeba histolytica* infection, 2872
in HBV infection, 1919-1920, 1919f
in *Helicobacter pylori* infection, 1695
in HPV infection, 1878-1879, 1879f
in HSV encephalitis, 1948
in hypersensitivity pneumonitis, 304
in interstitial lung disease, 299-300
in *Leishmania* spp. infection, 2951
in *Leptospira* spp. infection, 1749
in *Mycoplasma pneumoniae* infection, 2673
in myocarditis, 393
in *Naegleria fowleri* infection, 2982, 2982f
in *Nocardia* spp. infection, 1421
in *Pneumocystis* infection, 3010
in Rocky Mountain spotted fever, 2653
Biostatistics, **120-125**
bias in, 102
categorical variables in, 101-102, 122-123, 123t
confidence intervals in, 123
confounding variables in, 123-124, 124t
continuous variables in, 100-101, 122
of diagnostic tests, 125-127, 126f, 126t-127t
frequency distribution in, 100-101, 100f
hypothesis testing in, 120-121
meta-analysis in, 124-125
multiple comparisons in, 121-122
statistical power in, 121
statistical significance in, 120, 122
summary statistics in, 99-102
type I error in, 121
type II error in, 121
of vaccine efficacy, 127-128, 127t
Bioterrorism, **3579-3585**, 3581t
anthrax, 3580-3582, 3581f. *See also* Anthrax
botulism, 1793, 3584-3585
epidemiology of, 3580
historical perspective on, 3579-3580
monkeypox, 2044
plague, 3583-3584
preparedness for, 3547-3548, 3585
smallpox, 2034, 3582-3583, 3583f
tularemia, 3584
viral hemorrhagic fever, 3585
Bird fancier's lung, 301, 302t-303t, 303f-305f
Bismuth subsalicylate
in gastrointestinal tract infection, 626
in rotavirus infection, 2186
in traveler's diarrhea, 3114
Bites, **3570-3579**
animal, 780-781, 780t. *See also* Cat bite; Dog bite; Rabies
complications of, 3572-3575, 3574f, 3574t
diagnosis of, 3575-3577
epidemiology of, 3570-3571
Erysipelothrix rhusiopathiae infection with, 1328
in *Francisella tularensis* infection, 1659-1660
microbiology of, 3571-3572, 3571t-3573t
polymicrobial, 1829
prevention of, 3578
treatment of, 3251, 3575-3577, 3576t-3577t

Bites *(Continued)*
arthropod, 2862t-2863t, **3070-3079**
cutaneous manifestations of, 758t, 763t-764t
vs. virus-induced exanthem, 762-763
in child abuse, 3571
in daycare facilities, 3571
historical perspective on, 3570
human, 780, 780t, 3573t
clinical manifestations of, 3574-3575
diagnosis of, 3575-3577
epidemiology of, 3571
microbiology of, 3572, 3573t
treatment of, 3251, 3575-3577, 3576t
reptile, 3578
tick. *See* Tick bite
Bithionol, 3348
BK virus, 1864-1865, 1864t
tumors and, 1868
BK virus infection, **1863-1870**
clinical manifestations of, 1866-1869
diagnosis of, 1868-1869, 3617
epidemiology of, 1865-1866
after hematopoietic stem cell transplantation, 942
after kidney transplantation, 1010
pulmonary, 1868
treatment of, 1869
urinary tract, 1867, 1867f
Black widow spider bite, **3077**
Bladder
infection of, 538. *See also* Urinary tract infection (UTI)
Schistosoma spp. infection of, 3064
suprapubic aspiration of, 541, 541f, 541t
Blastocystis hominis infection, **2875-2876**, 3327t-3346t
Blastomyces dermatitidis, 2723-2724, 2724f
Blastomyces dermatitidis infection (blastomycosis), **2723-2734**
bone, 726, 2728, 2729f-2730f
clinical manifestations of, 757t, 2727-2731, 2727f-2730f
CNS, 478, 480t, 494t-498t, 2729, 2730f
cutaneous, 757t, 2728, 2728f
diagnosis of, 2731-2732, 2731f
disseminated, 2727-2729, 2728f
epidemiology of, 2724-2726, 2724f, 2725t
historical perspective on, 2723
in immunocompromised host, 2729-2730
after kidney transplantation, 1013
after lung transplantation, 974
meningeal, 478, 480t
neonatal, 2730-2731
pathogenesis of, 2726-2727
pleural effusion in, 309-310
in pregnancy, 2730-2731, 2733
pulmonary, 2727, 2727f
treatment of, 2732-2734, 2732t, 3295t
Blau syndrome, 847-848
Bleach, in *Staphylococcus aureus* infection prevention, 1128-1129
Blepharitis. *See also* Eyelid infection
HSV, 1945
staphylococcal, 794
Blepharoconjunctivitis, *Pseudomonas aeruginosa*, 1591
Blistering distal dactylitis, **775**
Blood-brain barrier
in bacterial meningitis, 433-435, 434f
bacterial traversal of, 432-433, 433t
Blood group MSN, epiglottitis and, 249
Blood group O, cholera and, 1551
Blood group P, human erythrovirus B19 (human parvovirus B19) infection and, 1844-1845, 1854
Blood smear examination
in *Anaplasma* infection, 2662-2663
in *Babesia* spp. infection, 2918-2919
in *Ehrlichia* infection, 2662-2663
in immunodeficiency evaluation, 62
in malaria, 2930, 2931f-2934f
in parasite identification, 3625-3626
in *Trypanosoma cruzi* infection, 2959, 2960f, 2965-2966
Blood transfusion
CMV transmission with, 1972-1973
EBV transmission with, 1998, 2014-2015
HAV transmission with, 2135
HEV transmission with, 2163
HIV transmission with, 2592-2593
malaria transmission with, 2923
prion transmission with, 2622-2623
Trypanosoma cruzi transmission with, 2961
Yersinia enterocolitica transmission with, 1518
Blow dryer, in lice, 3075-3076
Boceprevir, in HCV infection, 2324
Body temperature, 83-84. *See also* Fever
Boerhaave syndrome, 419
Bolivian hemorrhagic fever. *See* Arenavirus infection
Bone
infarction of, 724
infection of. *See* Osteomyelitis
pancreatitis-related lesions of, 696
tuberculosis of, 1355-1356
Bone marrow
chloramphenicol-related suppression of, 3216-3217
examination of, in fever of unknown origin, 842-843
in *Paracoccidioides* spp. infection, 2784
Bone marrow transplantation (BMT). *See also* Hematopoietic stem cell transplantation (HSCT)
acyclovir in, 1966, 2032
in chronic granulomatous disease, 57
infection after
adenovirus, 1903-1904
Aspergillus spp., 2711
Hafnia alvei, 1534-1535
HSV, 1952, 1952f, 1954
IVIG prevention of, 3501
parainfluenza virus, 2368
Streptococcus pneumoniae, 1232-1233
in type 1 leukocyte adhesion deficiency, 53
Bone scan
in *Kingella kingae* infection, 1703
in osteomyelitis, 716-717, 716f
Bordetella avium, 1616
Bordetella bronchiseptica, 1616-1620, 1617t
Bordetella bronchiseptica infection, 1617, 1637
historical perspective on, 1616
after lung transplantation, 983
Bordetella hinzii, 1616-1617
Bordetella hinzii infection, 1637
Bordetella holmesii, 1616-1620, 1617t
Bordetella holmesii infection, 1617, 1637
historical perspective on, 1616
Bordetella parapertussis, 17, 1616-1620, 1617t
Bordetella parapertussis infection, 1617, 1626, 1636-1637
historical perspective on, 1616
immunization against, 1625
Bordetella pertussis, 1616-1620, 1617t
adenylate cyclase toxin of, 1618t, 1619, 1624
Bordetella pertussis (Continued)
dermonecrotic toxin of, 1618t, 1619, 1624
filamentous hemagglutinin of, 6, 7f, 1618t, 1619, 1623
fimbriae of, 1618-1619, 1618t
lipopolysaccharide of, 1618t, 1620
pertactin of, 1618t, 1619, 1623
pertussis toxin of, 1618t, 1619, 1624
toxins of, 17, 1618-1620, 1618t, 1623-1625
tracheal cytotoxin of, 1618t, 1620
type III secretion system of, 1618t, 1620
Bordetella pertussis infection (pertussis, whooping cough), **1616-1639**
adolescent/adult, 1622-1623, 1627
asymptomatic, 1626
clinical manifestations of, 1625-1627
coinfection with, 1617, 1626-1627
complications of, 1625-1626
cough of, 1624-1625
in daycare facilities, 3563
diagnosis of, 1627-1629, 3593
differential diagnosis of, 1627
epidemiology of, 1620-1623, 1620f
historical perspective on, 1616
immune response to, 1624-1625
immunization against, 1630-1636, 3457-3461
acellular vaccines for, 1630-1633, 1631t-1633t, 3458, 3458t
adverse events with, 1634-1635, 1635t
adverse events with, 1633-1635, 3459
contraindications to, 1636, 3460-3461
disease incidence and, 1620-1621, 1620f
efficacy of, 3459
encephalitis after, 502
failure of, 1620-1621
indications for, 3460
neurologic illness with, 3459
precautions for, 3460-3461
schedules for, 1635-1636
whole-cell vaccines for, 1630, 3458
adverse events with, 1633-1634
incidence of, 1620-1621, 1620f
in infant, 1626-1627, 1626t
isolation precautions in, 1636
mild, 1626
morbidity with, 1621-1622
mortality with, 1621-1622
neonatal, 891-892, 1626-1627, 1626t
neurologic, 502
nosocomial, 3382
pathogenesis of, 17, 1623-1625
pathology of, 1623
prevention of, 1630-1636, 3249
prognosis for, 1630
rate of, 1622-1623
transmission of, 1622
treatment of, 1629-1630, 1636, 3507
Bordetella petrii, 1616-1617
Bordetella petrii infection, 1637
Bordetella spp., 1616-1620, 1617t. *See also specific species infections*
Bordetella trematum, 1616-1617
Bordetella trematum infection, 1637
Bornholm disease, 739, 2067-2068
Borrelia burgdorferi, 1730, 1730t
antibodies to, 1734-1735
Borrelia burgdorferi infection. *See* Lyme disease
Borrelia spp., 1730t, 1739-1740
Borrelia spp. infection (relapsing fever), **1739-1742**
clinical manifestations of, 753t-756t, 1741-1742, 1741t

Borrelia spp. infection (relapsing fever) *(Continued)*
diagnosis of, 1742
differential diagnosis of, 1742
epidemiology of, 1730t, 1740
pathogenesis of, 1740-1741
transmission of, 1740
treatment of, 1742
Botryomycosis, 773, 1823
Botulism, 602t-603t
foodborne, **1791-1795**
bioterrorist, 1793, 3584-3585
CDC contact for, 1793-1794
clinical manifestations of, 1792-1793, 1792t
diagnosis of, 1793-1794
differential diagnosis of, 1793
epidemiology of, 1791-1792
pathophysiology of, 1792
prevention of, 1795
prognosis for, 1794-1795
reporting requirements for, 1794
treatment of, 1794, 3505
vaccine against, 1795
infant, **1801-1809**
age and, 1803-1804, 1803f
clinical manifestations of, 1805-1807, 1806f, 1806t-1807t
diagnosis of, 1807-1808, 1807t
epidemiology of, 1803-1805, 1803f, 1803t
historical perspective on, 1801
pathogenesis of, 1802-1803
prevention of, 1809
prognosis for, 1808-1809
risk factors for, 1802-1803
treatment of, 1808
wound, 1792
clinical manifestations of, 1793
treatment of, 1794, 3505
Botulism immune globulin, 1808, 3505
Bovine papular stomatitis virus infection, 2046
Bovine spongiform encephalopathy, 2621, 2623-2624
Bowman membrane, 806
Brain
Aspergillus spp. infection of, 2707, 2713-2714, 2714f
cryptococcoma of, 2803
lymphoma of, 2011, 2013
Naegleria fowleri–related pathology of, 2979f
tetanospasmin effects on, 1813
tuberculoma of, 1354
tumor of, 467
Brain abscess, **462-468**
Aggregatibacter (Haemophilus) aphrophilus, 1686
amebic, 2871
anticonvulsants in, 469
Arcanobacterium haemolyticum, 1325
Blastomyces dermatitidis, 2729, 2730f
Brucella spp., 1613
Candida, 2742-2743, 2757
Citrobacter spp., 1430-1431
clinical manifestations of, 463t, 464-466, 466t
corticosteroids in, 468-469
CSF examination in, 443t, 466
CT in, 466-467, 467f
culture in, 466
diagnosis of, 466-468, 467f
electroencephalography in, 467-468
gas within, 467
Brain abscess *(Continued)*
infective endocarditis and, 356-357
laboratory tests in, 466
mastoiditis and, 239
meningitis and, 457, 466
microorganisms in, 463-464, 465t
mortality with, 462
MRI in, 466-467
in neonatal meningitis, 885-887
Nocardia spp., 1421
odontogenic origin of, 151
otitis media and, 224-225, 225f
Pantoea agglomerans, 1536
pathogenesis of, 462-464, 463t
pathology of, 462-464, 463f-465f
Proteus spp., 1472-1473
Pseudomonas aeruginosa, 1598-1599
rhinosinusitis and, 202
rupture of, 463, 466
treatment of, 465t, 468
tuberculous, 1354
vs. tumor, 467
Brain stem encephalitis, 505
Brainerd diarrhea, 602
Branchial cleft cyst, 182
Brazilian hemorrhagic fever. *See* Arenavirus infection
Brazilian purpuric fever, **1688-1690**
BRCA1 gene, 50
Breast
abscess of, **890**
cancer of, 50
Breast-feeding
appendicitis and, 680
gastrointestinal tract infection and, 622, 626-627
Helicobacter pylori infection and, 1693
HEV infection and, 2163
infant botulism and, 1804
influenza virus infection and, 2356-2357
neonatal intestinal flora and, 89
otitis media and, 211
rotavirus infection and, 2182
RSV infection and, 2427
Salmonella spp. infection and, 1507
Shigella spp. infection and, 1486
Streptococcus pneumoniae infection and, 1204, 1236
West Nile virus infection and, 2264
Brodie abscess, 719
Bronchiectasis
in cystic fibrosis, 325, 325f
HTLV-1, 2576
Pasteurella multocida, 1547
Bronchiolitis, **271-282**
adenovirus, 1897
airway diameter and, 276
bacteria in, 272-273, 280
clinical presentation of, 274-275
in community-acquired pneumonia, 286
complications of, 281-282
definition of, 271
diagnosis of, 278
differential diagnosis of, 278, 278t
environmental factors in, 276, 276t
eosinophils in, 277-278
epidemiology of, 273-274, 273t
etiology of, 272-273, 272t-273t, 2066, 2122-2123
gender and, 274
historical perspective on, 272
hospitalization for, 274-275
human bocavirus, 1860
immunologic factors in, 277, 282
mortality with, 274, 281
Bronchiolitis *(Continued)*
Mycoplasma pneumoniae, 2679
necrotizing, 286
pathophysiology of, 275-278, 276t
patient positioning in, 280
prevention of, 280-281
prognosis for, 281-282
treatment of, 278-280
vitamin deficiency and, 280
wheezing with, 272, 273t, 274, 281-282
Bronchiolitis obliterans, 295-296
in adenovirus infection, 1898
in lung transplant recipient, 982
Bronchitis
acute, **262-265**
clinical presentation of, 263-264
diagnosis of, 264
differential diagnosis of, 264
epidemiology of, 262
etiology of, 262, 263t
pathology of, 262-263
pathophysiology of, 262-263
phases of, 264
prevention of, 265
prognosis for, 264-265
treatment of, 264
chronic, **265-271**
differential diagnosis of, 265-269, 266f
epidemiology of, 266t, 269
etiology of, 269, 269t
treatment of, 269-270, 270f
enterovirus, 2066
Mycoplasma pneumoniae, 2679
Bronchoalveolar lavage (BAL)
in *Aspergillus* spp. infection, 2715-2716
in *Blastomyces dermatitidis* infection, 2731
in burn patient, 1057
in cystic fibrosis, 325
galactomannan in, 967, 2715-2716
in *Histoplasma capsulatum* infection, 2820-2821
in HIV infection, 2599
in hypersensitivity pneumonitis, 304
in interstitial lung disease, 299
in interstitial pneumonitis, 948
in *Nocardia* spp. infection, 1421
in *Pneumocystis jiroveci* infection, 972-973
Bronchodilators
in bronchiolitis, 279
in chronic bronchitis, 269
in RSV infection, 2425-2426
Broncholithiasis, *Histoplasma capsulatum*, 2813
Bronchoscopy, in lung abscess, 318-319
Bronchus(i)
abscess rupture into, 318
tuberculosis-related obstruction of, 1345-1347, 1346f
Brown fat, 75, 84
Brown recluse spider bite, 758t, **3077**
Brucella abortus infection, 1612, 1612t
Brucella melitensis infection, 1612-1613, 1612t
pancreatic, 695
Brucella spp., 1612, 1612t
Brucella spp. infection (brucellosis), **1611-1615**
chronic, 1615
clinical manifestations of, 1613-1614
CNS, 1613-1615
cutaneous, 1613
diagnosis of, 1614
endocardial, 362, 1613-1615
epidemiology of, 1612
exanthem in, 753t-756t

Brucella spp. infection (brucellosis) *(Continued)*
fever of unknown origin in, 844
gastrointestinal tract, 602t-603t, 1613
genitourinary tract, 1613
hematologic abnormalities with, 1613-1614
historical perspective on, 1611
ocular, 813, 1613
osteoarticular, 725, 1613
pancreatic, 695
pathogenesis of, 1612-1613
in pregnancy, 1612
relapsing, 1615
respiratory tract, 1613
transmission of, 1612
treatment of, 1614-1615
Brudzinski sign, 439
Brugia spp. infection (filariasis), **3025-3026**, 3327t-3346t
Bruton disease. *See* Agammaglobulinemia, X-linked
BTK, 908-909
Bubonic plague. *See Yersinia pestis* infection (plague)
Buccal cellulitis, 151
Buckley syndrome (hyper-IgE syndrome), **55**, 919
Buffy coat test, in malaria, 2930-2935
Bulbar palsy, in infant botulism, 1806, 1806f, 1806t
Bulking agent injection therapy, in vesicoureteral reflux, 551
Burkholderia cepacia, 1583-1584
vs. *Stenotrophomonas maltophilia*, 1608
virulence factors of, 1589
Burkholderia cepacia infection
clinical manifestations of, 1592
in cystic fibrosis, 335-336, 335t, 341, 345t, 1593-1594
diagnosis of, 1597
epidemiology of, 1585-1586
after lung transplantation, 960-962
nosocomial, 1595
treatment of, 1601
vaccine against, 1604
Burkholderia gladioli infection, 1586
Burkholderia mallei infection, 753t-756t
Burkholderia pseudomallei, 1583-1584
virulence factors of, 1589
Burkholderia pseudomallei infection (melioidosis)
clinical manifestations of, 1592-1593
diagnosis of, 1596-1597
epidemiology of, 1586
treatment of, 1601
Burkholderia thailandensis infection, 1586
Burkitt lymphoma
EBV and, 1996, 2005, 2005f, 2013
reovirus and, 2171
treatment of, 2013
Burn(s), **1047-1062**
B lymphocytes in, 1050
bronchoalveolar lavage in, 1057
cell-mediated immunity in, 1050
circulatory impairment with, 1048
complement in, 1049-1050
cytokine response in, 1049
cytologic findings in, 1047
depth of, 1047, 1048f
dressing for, 1057-1059
excision and grafting in, 1061
first-degree, 1047, 1048f
fourth-degree, 1047, 1048f
gastrointestinal decontamination in, 1061
Burn(s) *(Continued)*
humoral immunity in, 1050
immune response in, 1049-1050
infection of, 3405
Acinetobacter spp., 1052-1053
Aspergillus spp., 1053
Bacillus spp., 1052
biomarkers in, 1055
biopsy of, 1056-1057, 1056f
bronchoalveolar lavage in, 1057
Candida spp., 1053, 1053t
clinical manifestations of, 1054-1056, 1055t
Clostridium tetani, 1052
complications of, 1055-1056
culture in, 1056-1057
Curvularia spp., 1053
Enterococcus spp., 1052, 1052t
fungal, 1053, 1053t
Fusarium spp., 1053
gram-negative bacteria in, 1052-1053, 1052t
gram-positive bacteria in, 1051-1052, 1052t, 1056f
histology of, 1057, 1057f, 1057t
HSV, 1053-1054, 1944, 1944f, 1953
IVIG use and, 1061, 3501, 3508-3509
local signs of, 1054, 1055t
microbiology of, 1050-1054, 1051t
Mucor spp., 1053
parasite, 1054
Penicillium spp., 1053
prevention of, 1057-1061
antibiotics in, 1060-1061
decontamination in, 1061
immunomodulators in, 1061
infection control in, 1061-1062
surgery in, 1061
Pseudomonas aeruginosa, 1052-1053, 1057f, 1593
Rhizomucor spp., 1053
Rhizopus spp., 1053
Staphylococcus aureus, 1051-1052, 1051t
Staphylococcus epidermidis, 1051-1052, 1051t
Streptococcus pyogenes, 1051-1052, 1051t
systemic signs of, 1054-1055, 1055t
treatment of, 1057-1061
antibiotics in, 1059-1060
cerium nitrate silver sulfadiazine in, 1058
chlorhexidine in, 1059
citric acid in, 1059
mafenide acetate in, 1058-1059
membrane dressings in, 1059
nystatin in, 1059
povidone-iodine in, 1059
silver nitrate in, 1058
silver sulfadiazine in, 1058
sodium hypochlorite in, 1059
subeschar antibiotic infusion in, 1060
topical antimicrobial agents in, 1058-1060
viral, 1053-1054, 1057, 1060
inflammation in, 1048-1050
inhalation injury with, 1048-1049
macrophages in, 1050
neutrophils in, 1049
PMN chemotaxis in, 56
second-degree, 1047, 1048f
T lymphocytes in, 1050
third-degree, 1047, 1048f
tissue changes in, 1048
toxic shock syndrome with, 860
Bursitis, septic, 732
Buruli ulcer, **1410-1417.** *See also* *Mycobacterium ulcerans* infection
clinical manifestations of, 1411f, 1413-1415, 1413f-1414f
diagnosis of, 1415
epidemiology of, 1411-1412
HIV infection and, 1415
pathology of, 1412-1413, 1412f
prevention of, 1416-1417
prognosis for, 1416
treatment of, 1416
Bussuquara virus infection, 2316t

C

C1, deficiency of, 51
C2, deficiency of, 51, 1225
C3, 29-31, 29f
deficiency of, 51, 55-56, 907
C3a, 31
C4, deficiency of, 51, 907
C4a, 31
C5, 29f
deficiency of, 51-52, 55-56
C5a, 29f, 31
C5a peptidase, 18
C5a-ase, of *Streptococcus agalactiae*, 1154, 1158
C6, deficiency of, 51-52
C7, deficiency of, 51-52
C8, deficiency of, 51-52
C9, 31
deficiency of, 429
C protein, of *Streptococcus agalactiae*, 1153-1154
C-reactive protein (CRP), 74
in burn wound infection, 1055
in craniofacial surgery–related infection, 1046
in CSF shunt–related infection, 1033
in fever of unknown origin, 842
in infective endocarditis, 358, 358t
in Kawasaki disease, 1075-1076
in meningitis, 444
in occult bacteremia, 838
in osteomyelitis, 719
in prosthetic joint infection, 1027
in sepsis neonatorum, 883
in *Streptococcus agalactiae* infection, 1162
in *Streptococcus pneumoniae* infection, 1224
in urinary tract infection, 543
Calcaneus, osteomyelitis of, 719-720
Calcification
in neurocysticercosis, 3034, 3038
in tuberculosis, 1347, 1348f-1349f
Calcitonin, 82
in toxic shock syndrome, 829-830
Calcium
intracellular, in cystic fibrosis, 331
loss of, 69
Calculi, gallbladder. *See* Gallstones
Calicivirus(es), 2147-2151, 2148f
antigenic properties of, 2150-2151
classification of, 2149, 2149t
genome of, 2149-2150, 2149f
recombination of, 2150, 2150f
structure of, 2148-2149
Calicivirus infection, **2147-2158.** *See also* *Norovirus* infection; *Sapovirus* infection
exanthem in, 749
historical perspective on, 2147-2148
Calymmatobacterium granulomatis. *See* *Klebsiella granulomatis*
CAM. *See* Complementary and alternative medicine (CAM)

Camelpox, **2046**
Campylobacter concisus infection, 1646t, 1650, 1652
Campylobacter curvus infection, 1653
Campylobacter fetus infection, 1646t, 1649-1650, 1653, 1653t
Campylobacter hyointestinalis infection, 1646t, 1650, 1652
Campylobacter jejuni, 1645-1646, 1645f
Campylobacter jejuni infection, 602t-603t, 609, **1644-1657**
  antibiotic resistance in, 1655-1656, 1655t, 3155
  clinical manifestations of, 1651-1654
  in daycare facilities, 3557
  diagnosis of, 618, 1654-1655
  differential diagnosis of, 1654
  epidemiology of, 1646-1650, 1646f, 1647t-1648t, 1653t
  extraintestinal, 1652-1653, 1653t
    treatment of, 1655-1656
  Guillain-Barré syndrome after, 1653-1654
  historical perspective on, 1644-1645
  immune response to, 1651
  immunoreactive complications of, 1653-1654
  neonatal, 888, 1653, 1656
  pathogenesis of, 1650-1651
  pathology of, 1650
  perinatal, 1653
  prevention of, 1656
  transmission of, 1649
  treatment of, 1655-1656, 1655t
Campylobacter lari infection, 1646t, 1650, 1652
Campylobacter spp., 1646t-1647t
Campylobacter spp. infection, 607t, 609, 753t-756t. *See also specific species infections*
  antibiotic resistance in, 3155
  treatment, 623-624, 623t
Campylobacter upsaliensis infection, 1646t, 1650, 1652
Campylobacter ureolyticus infection, 1652
Cancer. *See also* Leukemia; Lymphoma
  bacterial meningitis and, 437
  Blastomyces dermatitidis infection in, 2730
  breast, 50
  chemotherapy-associated infection in, **921-933**
    bacterial, 921-922, 922t
    chest radiography in, 925
    CMV, 1973
    fungal, 922, 2730
      evaluation of, 926-927, 927t
      risk stratification for, 924
      treatment of, 928
    hospital-acquired, 929
    laboratory evaluation of, 924-925
    occult, 923
    patient history in, 924
    physical examination in, 924
    prevention of, 928-929
    Pseudomonas spp., 1594-1596
    risk stratification for, 923-924
    treatment of, 925-928
    viral, 922-923, 923t
  EBV-related, 2005-2006, 2005f
    histopathology of, 1996-1997
    immunopathogenesis of, 1995-1996
    treatment of, 2012-2013
  fever without origin in, 840
  Helicobacter pylori infection and, 1696-1698, 1697f
  intestinal flora in, 94
  intravenous immunoglobulin in, 3499
Cancer *(Continued)*
  liver abscess with, 673
  vs. lung abscess, 319
  Mycoplasma spp. and, 2696
  nasopharyngeal, 1995-1996
  pleural effusion with, 313
  polyomaviruses and, 1868
  Pseudomonas spp. infection and, 1594-1596
  reovirus effect on, 2171
  Schistosoma spp. infection and, 3064
  septic arthritis with, 730
Candida albicans
  adhesins of, 9
  biofilm formation by, 11
  for delayed hypersensitivity skin test, 907
  Hwp1 of, 9
  INT1 of, 9
Candida albicans infection. *See also Candida* spp. infection (candidiasis)
  appendiceal, 685
  biliary, 660
  cutaneous, **787-789**, 788f
  endocardial, 354, 357-358, 363
  epidemiology of, 2736
  epiglottitis, 245
  exanthem in, 757t
  hepatic, 673-675
  in HIV infection, 148-149, 2602
  muscle, 739
  myocardial, 383f, 2740-2741, 2748
  neonatal, 892-893, 896, 2750. *See also Candida* spp. infection (candidiasis), neonatal
  ocular, 820
  oral, **787-788**, 787f
  pathogenesis of, 2737-2739
  treatment of, 788
  uvular, 167
  vulvovaginal, 569-570, 570f, 788
Candida glabrata infection, 2736. *See also Candida* spp. infection (candidiasis)
Candida krusei infection, 2736. *See also Candida* spp. infection (candidiasis)
Candida parapsilosis infection, 2736. *See also Candida* spp. infection (candidiasis)
Candida spp., 2735
  colonization by, 2751-2753
Candida spp. infection (candidiasis), 893f, **2735-2769**
  bloodstream, 2740
    epidemiology of, 2735-2737
    neonatal, 2754, 2759
    portal of entry in, 2738-2739
    treatment of, 2747-2748
  burn wound, 1053, 1053t
  in cancer patient, 922
  cardiac, 2740-2741
    treatment of, 2748
  clinical manifestations of, 2739-2744
  CNS, 2742-2743
    neonatal, 2757
    treatment of, 2749
  corneal, 807-808
    contact lens–related, 1019
  cutaneous, 2740
    neonatal, 893-894, 893f, 2753, 2754t, 2755f
    treatment of, 2747
  diagnosis of, 2735, 2744-2745
  disseminated, 2736-2744
    chronic, 2743-2744, 2749
    neonatal, 2755-2756, 2756f
  drug resistance of, 474
  endocardial, 357-358, 363, 2740, 2748
Candida spp. infection (candidiasis) *(Continued)*
  endovascular, 2740
    treatment of, 2748
  epidemiology of, 2735-2737
  esophageal, 593-597, 595f-596f, 2739
    treatment of, 2747
  exanthem in, 757t
  gallbladder, 2741
  gastrointestinal tract, 2741
  after heart transplantation, 948
  after hematopoietic stem cell transplantation, 936-937
  hepatic, 673-675
  hepatosplenic, 2743-2744
    treatment of, 2749
  host response to, 23
  immune response to, 2738-2739
  after intestinal transplantation, 994, 995t
  after kidney transplantation, 1013
  after liver transplantation, 993-994, 995t
  after lung transplantation, 970-971
  meningeal, 474-475, 480, 480t, 488, 2742-2743, 2749
    neonatal, 894-895, 897, 2754, 2757
  mucocutaneous, **787-789**, 788f
    chronic, 915-916, 2740
    neonatal, 893-894, 893f, 2753-2754
  mycetoma with, 2749
  neonatal, **892-897**, 893f, **2749-2766**
    acquired, 894
    bloodstream, 2754
    clinical manifestations of, 2753-2757
    CNS, 894, 2757
    congenital, 788-789, 894, 896
    cutaneous, 893-894, 893f, 2740, 2753-2754, 2754t, 2755f
    diagnosis of, 896, 2757
    disseminated, 894, 896-897, 2755-2756, 2756f
    drug-related risk factors in, 2765
    endocardial, 895, 897, 2756
    endophthalmic, 2756
    epidemiology of, 2749-2751, 2750f
    horizontal transmission in, 2752
    intra-abdominal, 895-897
    joint, 895, 897
    meningeal, 894-895, 897, 2754, 2757
    mortality with, 2766
    mucocutaneous, 893-894, 893f, 2753-2754
    neurodevelopmental impairment with, 2766, 2766f
    ocular, 895, 897, 2756
    oral, 896
    pathogenesis of, 2753
    peritoneal, 2754-2755
    prevention of, 897, 2760-2765
      fluconazole in, 2760-2761, 2764t
      lactoferrin in, 2765
      nystatin in, 2762-2763, 2764t
      recommendations for, 2763-2765, 2764t-2765t
    prognosis for, 2765-2766, 2766f
    pulmonary, 896
    renal, 894, 897, 2757
    risk factors for, 892, 2751-2753, 2752f
    treatment of, 896-897, 2757-2760
      adjunctive therapy in, 2759-2760
      amphotericin B in, 2758
      caspofungin in, 2758-2759
      central venous catheter and, 2759, 2765
      combination, 2759

Candida spp. infection (candidiasis) *(Continued)*
fluconazole in, 2758
voriconazole in, 2758
urinary tract, 2754
vertical transmission in, 2751
ocular, 2743
neonatal, 2756
treatment of, 2749
oral, **787-788**, 787f
in HIV infection, 148-149
in neonate, 892-893, 896
oropharyngeal, 159t-160t, 161, **2739**
immune response to, 2738
treatment of, 2746-2747
osteoarticular, 2742
treatment of, 2749
pathogenesis of, 2737-2739
peritoneal, 2741
treatment of, 2748
prevention of, 2745-2746
pseudomembranous, 2739
pulmonary, 2741
splenic, 673
treatment of, 2746-2749, 2746t, 3294t, 3321t
urinary tract, 540, 2741-2742
neonatal, 2754, 2757
treatment of, 2748-2749
uveal, 813, 820
vulvovaginal, 569-570, 570f, 788, 2739-2740
in pregnancy, 2765
treatment of, 2747
Candida tropicalis infection, 2736. *See also* *Candida* spp. infection (candidiasis)
Capillaria spp. infection, 3024, 3327t-3346t
Capillaritis, pulmonary, 297
Capnocytophaga, overgrowth of, 56
Capreomycin, in tuberculosis, 1367t
Capsule
Cryptococcus neoformans, 20
Haemophilus influenzae, 19-20
Staphylococcus aureus, 1113-1114, 1115f
Streptococcus agalactiae, 1154, 1158
Streptococcus pneumoniae, 1206t, 1211-1212, 1211f
Captopril, in murine myocarditis, 396
Carbacephems, 3199-3200
Carbamazepine, in chorea, 414
Carbapenem(s), 3200-3202
dosage schedule for, 3231t-3233t
resistance to, 3139-3145
Carbapenemases, 3142
Carbohydrates
hormone regulation of, 82
intestinal microflora effects on, 93-94
metabolism of, 72-73
Carbuncle, **776-777**
Cardiac tamponade, in pericarditis, 374
Cardiomyopathy
Chagas, 2963
dilated
adenovirus infection and, 1902
in enteroviral infection, 2075-2077
in myocarditis, 382, 387, 393
Cardiorespiratory arrest, in epiglottitis, 254
Cardioverter-defibrillator, implantable (ICD), infection with, 1025-1026
management of, 1026
microbiology of, 1025
Cardiovirus, 2051-2052. *See also* Saffold virus(es)
Carditis. *See also* Myocarditis
Lyme, 1733, 1735
Caries. *See also* Odontogenic infection
antifungal preparations and, 148-149
Garré sclerosing osteomyelitis with, 154, 154f-155f
in HIV infection, 149
nursing bottle, 146
pathogens in, 140, 1193
periapical abscess with, 141, 142f
Caroli disease, 666-667
Caroli syndrome, 666-667
Carpet cleaning, Kawasaki disease and, 1068
Carrión disease, **1725-1726**
diagnosis of, 1727
prognosis for, 1729
treatment of, 1728
Case-fatality rate, in epidemiologic study, 105
Caspofungin, 3308t, 3313f, 3315-3317
in *Aspergillus* spp. infection, 2718
in cancer patient, 928
dosing for, 3316-3317, 3316t
efficacy of, 3315-3316
in neonatal *Candida* spp. infection, 2758-2759
in oropharyngeal candidiasis, 2747
in transplant-related aspergillosis, 938, 968
Cassava poisoning, pancreatitis with, 692
Cat bite, 780-781, 780t
clinical manifestations of, 3572-3575
diagnosis of, 3575-3577
epidemiology of, 3570-3571
Erysipelothrix rhusiopathiae infection with, 1328
Francisella tularensis infection with, 1660
microbiology of, 3571-3572, 3571t-3572t
Pasteurella multocida infection with, 1546-1547, 1567
polymicrobial, 1829
prevention of, 3578
treatment of, 3251, 3575-3577, 3576t
Cat-scratch disease. *See Bartonella henselae* infection (cat-scratch disease)
Catalase
in chronic granulomatous disease, **56-58**
of *Helicobacter pylori*, 1693
Cataracts
in measles, 811
in rubella, 811, 818, 2213, 2214t-2216t, 2220-2221
surgery for, endophthalmitis after, 820
Catecholamines, plasma, 81-82
Cathelicidin, 24
Catheter-associated infection, 3385-3391, 3386t-3388t, 3387f
endocardial, 352
intestinal transplantation and, 991, 993
kidney transplantation and, 1006
Klebsiella spp., 1467-1468
liver transplantation and, 991, 993
Malassezia furfur, 2853
prevention of, 1139, 3244, 3390-3391
Proteus spp., 1472-1473
Providencia spp., 1475
Stenotrophomonas maltophilia, 1607-1609
treatment of, 3389-3390
Cavernous sinus thrombosis, vs. orbital cellulitis, 151
C4b binding protein, 18
CCAAT/enhancer binding protein epsilon (C/EBPe), 54
CD1 proteins, 37-38
CD11/CD18, in type 1 leukocyte adhesion deficiency, 53
CD18, in *Salmonella typhimurium* infection, 15
CD40L, 909-910
CD81, in hepatitis C virus infection, 11
Cdc42 protein, 14
Cefaclor, 3192t, 3193, 3231t-3233t
adverse effects of, 3199
Cefadroxil, 3191t-3192t, 3192-3193
Cefamandole, 3193
Cefazolin, 3192-3193, 3192t, 3231t-3233t
Cefdinir, 3192t, 3197-3198, 3231t-3233t
in rhinosinusitis, 200
Cefditoren, 3192t, 3198, 3231t-3233t
Cefepime, 3192t, 3198, 3231t-3233t
in meningitis, 448
Cefixime, 3192t, 3196, 3231t-3233t
Cefoperazone, in meningitis, 448
Cefotaxime, 3192t, 3195, 3231t-3233t
in meningitis, 447, 450t
in septic shock, 833-834, 833t
Cefoxitin, 3192t, 3193-3194, 3231t-3233t
in meningitis, 448
Cefpodoxime proxetil, 3192t, 3196-3197, 3231t-3233t
in rhinosinusitis, 200
Cefprozil, 3192t, 3194, 3231t-3233t
Ceftaroline, 3198-3199
resistance to, 3153-3154
Ceftaroline fosamil, 3238
Ceftazidime, 3192t, 3196, 3231t-3233t
in meningitis, 448, 450t
Ceftibuten, 3197, 3231t-3233t
Ceftizoxime, 3192t, 3195
Ceftriaxone, 3192t, 3195-3196, 3231t-3233t
biliary effects of, 662
in cervical lymphadenitis, 186
in meningitis, 447, 450t
pancreatitis with, 692
in septic shock, 833-834, 833t
Cefuroxime, 3192t, 3194, 3231t-3233t
in meningitis, 448
Cefuroxime axetil, 3194, 3231t-3233t
Cell cytotoxicity neutralization assay (CCNA), in *Clostridium difficile* infection, 635, 636t
Cell-mediated immunity. *See* Immunity, adaptive
Cellulitis, **777-778**
buccal, 151
Clostridium perfringens, 1796-1799
cochlear implant and, 1018
crepitant, 1829
facial, 440-441, 1231-1232
Haemophilus influenzae type b, 778, 778f, 1676
orbital. *See* Orbital cellulitis
periorbital (preseptal), 151, 798-801
peritonsillar, vs. abscess, 168-169
Streptococcus agalactiae, 1161, 1162f
Streptococcus pneumoniae, 1231-1232
supraglottic, 248
tissue expander and, 1017
Yersinia enterocolitica, 1528
Central line–associated bloodstream infection (CLABSI), 3385-3386, 3386t
Central nervous system (CNS). *See also* Brain; Spinal cord
dysfunction of, fever of unknown origin in, 846
immune activation in, 433-435, 434f
infection of. *See* Brain abscess; Encephalitis; Meningitis
in infective endocarditis, 356-357
progenitor cells of, 436
tuberculosis of, 1352-1354
in Whipple disease, 643, 644t, 646

Central venous catheter. *See also* Catheter-associated infection
endocarditis and, 352
infection with, 1136, 3385-3391, 3386t-3388t, 3387f
protocols for, 2765, 2765t
removal of, in neonatal *Candida* spp. infection, 2759
Centruroides spp. bite, **3077-3078**
Cephalexin, 3192-3193, 3192t, 3231t-3233t
in cervical lymphadenitis, 186
Cephalic pustulosis, 789, 789f
Cephalosporins, 3191-3199, 3191t
adverse effects of, 3199
allergy to, 1296
dosage schedules for, 3231t-3233t
fifth-generation, 3198-3199
first-generation, 3191t-3192t, 3192-3193, 3231t-3233t
fourth-generation, 3191t-3192t, 3198, 3231t-3233t
in neonatal meningitis, 886
in odontogenic infection, 145, 146t
pharmacokinetics of, 3191-3192, 3192t
resistance to, 3139-3145
second-generation, 3191t-3192t, 3193-3194, 3231t-3233t
in *Staphylococcus aureus* infection, 1124t, 1125
third-generation, 3191t-3192t, 3194-3198, 3231t-3233t
Cephalothin, 3192-3193, 3192t
Cephapirin, 3192-3193, 3192t
Cephradine, 3192-3193, 3192t
Cercopithecine herpesvirus 1 (herpes B virus) infection, 655
Cerebellar ataxia, acute, 505
Cerebellitis, EBV, 2004
Cerebral edema
at high-altitude illness, 3118
in meningitis, 439
Cerebral embolism, in infective endocarditis, 355
Cerebral infarction
in infective endocarditis, 356-357
in *Leptospira* spp. infection, 1753
in *Neisseria meningitidis* infection, 1260
Cerebrospinal fluid (CSF)
leakage of, 3252
peritoneal, 701
shunt for. *See* Cerebrospinal fluid (CSF) shunt
Cerebrospinal fluid (CSF) examination, 443t
in *Acanthamoeba* spp. infection, 2979
in acute disseminated encephalomyelitis, 519
in acute hemorrhagic encephalitis, 443t
in acute transverse myelitis, 523
in African sleeping sickness, 2970
in aseptic meningitis, 444-445, 490
in aspergillosis, 478-479
in *Bacillus anthracis* meningitis, 1314
in *Bacillus cereus* infection, 1320
in bacterial meningitis, 441-446, 443t-444t, 449, 451t, 455, 1262
in blastomycosis, 478, 480t
in brain abscess, 443t, 466
in *Brucella* spp. meningitis, 1613
in *Candida* spp. infection, 2743
in *Citrobacter* meningitis, 1430
in CMV infection, 1982
in *Coccidioides* spp. infection, 477, 480t, 2774, 2778
in congenital syphilis, 1777
in Creutzfeldt-Jakob disease, 2628
Cerebrospinal fluid (CSF) examination *(Continued)*
in cryptococcal meningitis, 443t, 475
in cryptococcal meningoencephalitis, 2801-2802
in Eastern equine encephalitis, 2228
in encephalitis, 506, 508-509
in enterovirus meningitis, 2089
in eosinophilic meningitis, 483
in *Escherichia coli* meningitis, 1446
in fungal meningitis, 479-480, 480t
Gram stain in, 442
in *Haemophilus influenzae* type b meningitis, 1675
in histoplasmosis, 476, 480t
in HSV encephalitis, 1946-1947
in HTLV-1 infection, 2571
in human monocytic ehrlichiosis meningitis, 2662
immunochromatographic membrane test in, in pneumococcal pneumonia, 1217
in intracranial pressure monitor–related infection, 1039-1040
in Japanese encephalitis virus infection, 2297
in La Crosse virus infection, 2522, 2522t
in *Leptospira* spp. infection, 1751
in Lyme disease, 1735
in mastoiditis, 238
in measles virus encephalitis, 2386
in mumps meningitis, 2401
in *Naegleria fowleri* infection, 2979
in *Neisseria meningitidis* infection, 1262
in neonatal *Candida* spp. infection, 894-895, 897
in neonatal HSV infection, 1955
in neonatal meningitis, 885-887, 1135
in orbital cellulitis, 800
in Oropouche fever virus meningitis, 2543
in *Pasteurella multocida* meningitis, 1547
in pneumococcal meningitis, 1228
in poliovirus infection, 2101
in rabies, 2450
in roseola infantum, 771
in shunt-related infection, 1032-1033, 1036, 1137
in spinal epidural abscess, 472
in St. Louis encephalitis virus infection, 2257
in *Streptococcus agalactiae* meningitis, 1162
in subacute sclerosing panencephalitis, 2389-2390
in subdural empyema, 443t, 469
in syphilis, 1770
in tick-borne encephalitis virus infection, 2309
transferrin band in, 441
in tuberculous meningitis, 1353
in *Ureaplasma* spp. infection, 2691-2692
VDRL test in, 819-820
in Venezuelan equine encephalitis, 2240
in viral meningitis, 489
in West Nile virus infection, 2270
in Western equine encephalitis, 2234-2235
Cerebrospinal fluid (CSF) shunt
endocarditis and, 352
infection with, 698, 700t, 701-703, **1029-1038**
clinical manifestations of, 1031-1032
coagulase-negative staphylococci in, 1136-1137
complications of, 1036
Cerebrospinal fluid (CSF) shunt *(Continued)*
CSF examination in, 1032-1033, 1036, 1137
diagnosis of, 1032-1033, 1137
epidemiology of, 1029-1030
etiology of, 1030, 1030t
Gram stain in, 1035, 1035f
hepatic, 673
pathogenesis of, 1030-1031
prevention of, 1038
prognosis for, 1037
third ventriculostomy in, 1034
treatment of, 1033-1036, 1033t, 1035f, 1037t
Cereolysin, 1318-1319
Cereulide, 1318
Cerium nitrate silver sulfadiazine, in burn injury, 1058
Ceruloplasmin, plasma, 79
Cerumen, 204
Cervical cancer, HPV and, 1873t, 1874-1875, 1877-1878
Cervical lymphadenitis, **175-189**
anatomy of, 176, 176f
biopsy in, 185
clinical presentation of, 179-181, 179f-180f, 183f
diagnosis of, 184-185
differential diagnosis of, 181-184, 182t-183t
epidemiology of, 176-178, 177t
etiology of, 176-178, 177t, 183, 183t
noninfectious, 182-183, 182t
pathophysiology of, 178-179
prevention of, 188
prognosis for, 188
treatment of, 185-188
Cervical lymphadenopathy
in infectious mononucleosis, 1996, 1999, 1999f, 1999t
in Kawasaki disease, 1074
Cervicitis, 584-586
Chlamydia trachomatis, 586
Neisseria gonorrhoeae, 586, 1284-1285, 1284f
Schistosoma spp., 3065
Cervicofacial actinomycosis, 184
Cesarean delivery, in neonatal HSV infection prevention, 1964-1965
CFTR (cystic fibrosis transmembrane conductance regulator) gene, 60
CH50 assay, 907
Chagas disease. *See Trypanosoma cruzi* infection (trypanosomiasis)
Chagoma, 2961, 2962f
Chalazion, **796-797**
Chancre, 1765, 1768
Chancroid, 583t, **1686-1688**
clinical manifestations of, 753t-756t, 1687
coinfection with, 1688
diagnosis of, 1687-1688
epidemiology of, 1686-1687
pathogenesis of, 1687
prevention of, 1688
treatment of, 1688
Chandipura virus infection, 500-501
Changuinola virus, 2176
Chapare virus infection, 2470t, 2471. *See also* Arenavirus infection
Charcot triad, 660, 660t
Chédiak-Higashi syndrome, **54**, 905-906, 919
Chemokine(s), 26t, 27-28
in bacterial meningitis, 434-435, 434f
in bronchiolitis, 278, 282

Chemokine(s) *(Continued)*
modulation of, 27-28
nomenclature for, 27
in phagocyte recruitment, 31-32
Chemokine receptors, 27
in HIV infection, 10-11
Chemotaxins, impaired generation of, 55-56
Chemotherapy
appendicitis and, 681
Clostridium difficile infection and, 634
fever and neutropenia with, **921-933.** *See also* Cancer, chemotherapy-associated infection in
epidemiology of, 921-923, 922t-923t
evaluation of, 924-925
persistent, 926-927, 927t
prevention for, 928-929
risk stratification for, 923-924
treatment of, 925-926
pancreatitis and, 691
Chest pain
in infective endocarditis, 356
in pericarditis, 374
in pneumonia, 288
Chest radiography
in *Actinomyces* spp. infection, 1822f
in acute eosinophilic pneumonia, 297
in ARDS, 868-869, 868f-869f
in *Aspergillus* spp. infection, 2712
in atypical measles, 2384, 2384f, 2392
in bronchiolitis, 275
in cancer patient, 925
in *Chlamydia pneumoniae* infection, 2641, 2642f
in *Chlamydia trachomatis* infection, 2633f
in chronic granulomatous disease, 918f
in CMV pneumonitis, 1976, 1976f
in *Coccidioides* spp. infection, 2773, 2773f
in congenital syphilis, 1770-1771
in *Cryptococcus neoformans* infection, 2800
in fever of unknown origin, 842
in hantavirus pulmonary syndrome, 2506, 2506f
in *Histoplasma capsulatum* infection, 2817-2819, 2818f
in hMPV infection, 2439f
in hypersensitivity pneumonitis, 303-304, 303f
in immunodeficiency evaluation, 906, 906f
in infectious mononucleosis, 2011
in influenza virus pneumonia, 2344f
in inhalational anthrax, 3581, 3581f
in *Leptospira* spp. infection, 1751, 1753
in lung abscess, 319, 319f
in mimivirus infection, 2048, 2050f
in *Mucor* spp. infection, 2842
in *Paracoccidioides* spp. infection, 2783, 2785f
in parapneumonic pleural effusion, 310, 311f
in *Pneumocystis* infection, 3009, 3009f
in recurrent respiratory papillomatosis, 1878-1879, 1878f
in *Rhizopus* spp. infection, 2842
in RSV infection, 2417-2418, 2417f
in severe combined immunodeficiency, 906f
Chest tube thoracostomy, in lung abscess, 321
Chi-square statistic, 122-123, 123t
Chickenpox. *See* Varicella (chickenpox)
Chigger bite, 758t
Chikungunya virus, 2242
Chikungunya virus infection, 500, 742t-748t, **2241-2248**
clinical manifestations of, 2244, 2245t-2246t
vs. dengue, 2282
diagnosis of, 2245-2247
epidemiology of, 2243-2244
geographic distribution of, 2243-2244
pathogenesis of, 2245
pathology of, 2245
prevention of, 2247
prognosis for, 2247
transmission of, 2242-2243
in traveler, 3116
treatment of, 2247
zoonotic, 2243
Child abuse
bites and, 3571
sexual. *See* Sexual abuse
Child care facilities. *See* Daycare facilities
Childhood-onset acute neuropsychiatric symptoms (CANS), 1146. *See also* PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection)
Chinese restaurant syndrome, 604, 604t
Chlamydia pneumoniae, 2639-2640, 2640f
Chlamydia pneumoniae (Chlamydophila pneumoniae) infection, 161, 292, **2639-2644**
atherogenesis and, 75
clinical manifestations of, 2641-2642, 2642f
diagnosis of, 2642-2644
endocardial, 363
epidemiology of, 2640-2641, 2641t
meningeal, 488
in otitis media, 214
in rhinosinusitis, 195-196
treatment of, 2644
Chlamydia psittaci, 2638
Chlamydia psittaci infection (psittacosis), **2638-2639**
clinical manifestations of, 2638-2639
diagnosis of, 2639, 2640t
endocardial, 363
epidemiology of, 2638
exanthem in, 750, 751t-752t
vs. *Legionella pneumophila* infection, 1713
treatment of, 2639
Chlamydia trachomatis, 2640f
Chlamydia trachomatis infection, 161, **2631-2638**
in adolescents, 2635
Bartholin duct, 579
blindness with, **2636**
cervical, 586
conjunctival, 804-805
neonatal, 805-806, 805t, 2633-2634
diagnosis of, 2636-2637, 3594
epidemiology of, 2631-2632
fallopian tube, **587-588**
in infants and children, 2634-2635
lymphogranuloma venereum with, **2635-2636**
Neisseria gonorrhoeae infection and, 1297
neonatal, 900-901, 2632-2633
conjunctival, 805-806, 805t, 2633-2634
diagnosis of, 2633-2634
epidemiology of, 2632-2633, 2632t
historical perspective on, 2632
prevention of, 2634
pulmonary, 2633, 2633f
treatment of, 2634
ocular, 804-805
Chlamydia trachomatis infection *(Continued)*
in otitis media, 214
pulmonary, 292, 1617-1618
neonatal, 2633, 2633f
treatment of, 2637-2638
urethral, **530-535**, 531f, 534t, 579
vulvovaginal, 572t-573t, 574-575, **584**, 584f
Chlamydophila pneumoniae. *See Chlamydia pneumoniae (Chlamydophila pneumoniae)* infection
Chloramphenicol, 3215-3217, 3231t-3233t
adverse effects of, 3216-3217
drug interactions with, 3217
indications for, 3216
mechanisms of action of, 3215
in meningitis, 446, 448, 450t
pharmacokinetics of, 3216
resistance to, 3157-3158, 3215
in Rocky Mountain spotted fever, 2654
in vitro activity of, 3215-3216
Chlorhexidine
in burn wound infection, 1059
in hematopoietic stem cell transplantation, 935
in *Staphylococcus aureus* infection prevention, 1128-1129
Chloroquine, 3348
in malaria, 2936-2941, 2937t-2940t
in malaria prevention, 2943-2944, 2944t
Chloroquine phosphate, 3348
N-Chlorotaurine (NCT), in adenovirus infection, 1908
Cholangiography, in *Clonorchis sinensis* infection, 3052
Cholangitis, **658-667**
biliary atresia and, 664-665
in Caroli disease, 666-667
choledochal cysts and, 666-667
clinical presentation of, 660-661, 660t
complications of, 664
congenital anomalies and, 666-667
diagnosis of, 660t, 661-662
differential diagnosis of, 662-663, 662t
echinococcal, 660
after endoscopic procedures, 667
etiology of, 659-660, 659t
fungal, 660
in immunocompromised host, 666
after liver transplantation, 665-666, 993
mycobacterial, 660
parasitic, 660, 664
pathogenesis of, 659-660
prevention of, 664
stent-related, 667
treatment of, 663-664, 663t
viral, 660
Cholecystectomy, 670
Cholecystitis, **667-671**
acalculous, 670-671, 670t
Candida, 2741
clinical presentation of, 669
complications of, 670-671
Escherichia coli, 1445-1446
etiology of, 667-669, 668t
evaluation of, 669
in *Leptospira* spp. infection, 1752
management of, 669-670
Choledochal cyst, 666-667
Choledocholithiasis, 670
Cholelithiasis. *See* Gallstones
Cholera, **611-612**, **1548-1555**
clinical manifestations of, 602t-603t, 607t, 1551-1552, 1551f
diagnosis of, 602t-603t, 616t, 618, 1552

Cholera *(Continued)*
epidemiology of, 611-612, 1550-1551
historical perspective on, 1549
host factors in, 1551
immunization against, 1554-1555, 3478-3479
nitrogen balance in, 68-69
pathogenesis of, 612, 1550
prevention of, 1554-1555
rapid dipstick test for, 1552
treatment of, 623t, 625, 1552-1553, 1552t, 1553f, 1554t
Cholestasis
drug-induced, 662
gallstones with, 668
sepsis-induced, 662
Cholesteatoma, 223, 233-234, 239
Cholesterol
bacterial infection and, 74
viral infection and, 73-74
Choline-binding protein A (CbpA), of *Streptococcus pneumoniae*, 1211, 1211f, 1213
Chorea, 413
in rheumatic fever, 410, 414
Choreiform movements, in PANDAS, 1145
Chorioamnionitis
Candida, 788-789
Eikenella corrodens, 1577
Ureaplasma spp., 2690
Chorioretinitis, 809
Ascaris lumbricoides, 815
Candida, 813
CMV, 1977
Coccidioides spp., 813-814
Cryptococcus neoformans, 814
Histoplasma capsulatum, 813
LCMV, 811-812
Loa loa, 815
in Q fever, 815
rickettsial, 815
Toxoplasma gondii, 2991, 2994, 2994f, 2999-3000
West Nile virus, 2268-2269, 2272
Choroid, tuberculosis of, 1352, 1357
Choroiditis, 809
Bartonella henselae, 813
Brucella, 813
Onchocerca, 815
Schistosoma, 815
Chromium, metabolism of, 77t
Chromobacterium violaceum, 1563
Chromobacterium violaceum infection, 776, **1563-1564**
clinical manifestations of, 1564
diagnosis of, 1564
epidemiology of, 1563
pathophysiology of, 1563-1564
treatment of, 1564
Chromoblastomycosis, 479, **789-790**, 790f
Chrondritis, suppurative, in burn patient, 1055
Chronic cavitary pulmonary aspergillosis, 2708
Chronic fatigue syndrome, **1085-1091**
cardiovascular factors in, 1088-1089
clinical manifestations of, 1086, 1088-1089
definition of, 1086, 1086t
diagnosis of, 1089-1090
EBV and, 2004
endocrine factors in, 1088
enterovirus and, 2091
epidemiology of, 1087, 1087t
etiology of, 1087-1088
Chronic fatigue syndrome *(Continued)*
genetic components in, 1088
historical perspective on, 1086-1087
human erythrovirus B19 (human parvovirus B19) infection and, 1854
immune dysfunction and, 1088
infection and, 1086-1087
management of, 1090, 1090t
neurologic factors in, 1088
prognosis for, 1091
psychological factors in, 1089
sleep disturbances in, 1089
Chronic granulomatous disease, **56-58**, 58f, 917-918
Aspergillus spp. infection in, 56-57, 917, 918f, 2706, 2713f
Burkholderia cepacia infection in, 1595
clinical features of, 917-918, 918f, 1122
diagnosis of, 907, 918
interferon-γ in, 3364
liver abscess in, 673
lung abscess in, 317
osteomyelitis in, 724
pathogenesis of, 907t, 918
treatment of, 918
X-linked, 56
Chronic necrotizing pulmonary aspergillosis, 2708
Chronic nonsuppurative sclerosing osteomyelitis (Garré sclerosing osteomyelitis), 154, 154f-155f
Chronic obstructive pulmonary disease (COPD), *Mycoplasma pneumoniae* infection and, 2679
Chronic progressive panencephalitis, in rubella virus infection, 2216
Chronic recurrent multifocal osteomyelitis (CRMO), 714
Chryseobacterium spp., 1578-1579
Chryseobacterium spp. infection, **1578-1581**
clinical manifestations of, 1579-1580
diagnosis of, 1580
epidemiology of, 1579
pathophysiology of, 1579
treatment of, 1580-1581
Churg-Strauss syndrome, 297
Cidofovir, 3278t, 3287-3289, 3287f
in adenovirus infection, 1907
in BK virus infection, 1869
in CMV infection, 1984-1986
in HPV infection, 1884
in kidney transplant–related CMV infection, 1008
in lung transplant–related adenovirus infection, 982
in monkeypox, 2044
Cigarette smoke exposure
cough and, 268
normal flora and, 89-90
otitis media and, 211
Streptococcus pneumoniae infection and, 1236
Ciguatera poisoning, 604, 605t
Ciliary dyskinesia, 60
Cimetidine, in papillomas, 1883
Cimex lectularius, 758t
Cimex lectularius infestation, **3076-3077**
Cineangiography, in infective endocarditis, 359
Ciprofloxacin, 3225-3227, 3231t-3233t
in anthrax, 1315
in odontogenic infection, 145
Circumcision, urinary tract infection and, 536, 552
Citric acid, in burn wound infection, 1059
Citrobacter diversus infection, 886
Citrobacter farmeri infection, 1431
Citrobacter freundii infection
diagnosis of, 1431
gastrointestinal tract, 1431
meningeal, 1429
Shiga toxins in, 17, 1430-1431
soft tissue, 1431
treatment of, 1431-1433
Citrobacter koseri infection
diagnosis of, 1431
meningeal, 1429-1431, 1432f
treatment of, 1432-1433
treatment of, 1431-1433
Citrobacter rodentium, 7-8
Citrobacter spp., 1429
Citrobacter spp. infection, **1429-1433**
clinical manifestations of, 1430-1431
diagnosis of, 1431
epidemiology of, 1429-1430
pathophysiology of, 1430
prevention of, 1433
in septic shock, 834
treatment of, 1431-1433
Cladophialophora bantiana infection, **2855-2858**
after lung transplantation, 974
Cladophialophora carrionii infection, **789-790**
Cladosporium spp. infection, after lung transplantation, 974
Clarithromycin, 3211-3215, 3213t, 3231t-3233t, 3348
in common cold, 138
drug interactions with, 3215
in nontuberculous mycobacterial lymphadenitis, 187
Claudin-I, 16
Clavicle, in congenital syphilis, 1772
Clenched-fist injury, 3572, 3575
Climate, infection and, 110
Clindamycin, 3220-3222, 3348
in cervical lymphadenitis, 186
in methicillin-resistant *Staphylococcus aureus* infection, 1127-1128
in odontogenic infection, 145, 146t
in rhinosinusitis, 200
in *Staphylococcus aureus* infection, 1125
in *Toxoplasma gondii* infection, 3001
Clinical trial, 96-97, 97t. *See also* Epidemiology
ethics in, 97
of vaccines, 127-128
Clitoris, infection of, 578-579
Clofazimine
in *Mycobacterium leprae* infection, 1407-1408, 1407t
in tuberculosis, 1367t
Clonorchis sinensis, 3049, 3050f-3051f
Clonorchis sinensis infection (clonorchiasis), **3049-3052**
clinical manifestations of, 3051
diagnosis of, 3051-3052
epidemiology of, 3050-3051
imaging in, 3052
pathogenesis of, 3050f, 3051
prevention, 3052
transmission of, 3050-3051
treatment of, 3052, 3327t-3346t
Clostridium baratii infection, 1801, 1804. *See also* Botulism, infant
Clostridium botulinum, 1791-1792, 1801-1802
in corn syrup, 1805
in honey, 1804-1805

Clostridium botulinum infection (botulism)
foodborne, **1791-1795**
clinical manifestations of, 602t-603t, 1792-1793
diagnosis of, 1793-1794
differential diagnosis of, 1793
epidemiology of, 1791-1792
pathophysiology of, 1792
prevention of, 1795
prognosis for, 1794-1795
treatment of, 1794
infant, **1801-1809**
clinical manifestations of, 1805-1807, 1806f, 1806t-1807t
diagnosis of, 1807-1808, 1807t
epidemiology of, 1803-1805, 1803f, 1803t
historical perspective on, 1801
pathogenesis of, 1802-1803
prevention of, 1809
prognosis for, 1808-1809
treatment of, 1808
wound, 1792
clinical manifestations of, 1793
treatment of, 1794
Clostridium butyricum infection, 1801, 1804. *See also* Botulism, infant
Clostridium difficile
colonization with, 633-635
NAP1 strain of, 632-633
toxins of, 634, 634f
transmission of, 635
vaccine against, 638
Clostridium difficile infection
antibiotic-associated, 599, **632-639**
amoxicillin-clavulanic acid and, 599
clindamycin and, 145
clinical manifestations of, 635
diagnosis of, 635-636, 636t, 1799, 3594
differential diagnosis of, 636-637
epidemiology of, 632-633
historical perspective on, 632
after kidney transplantation, 1006
pathogenesis of, 632-635, 634f
prevention of, 637-638, 1800
treatment of, 637, 1799
chemotherapy-associated, 599
in daycare facilities, 3558
gastrointestinal, 607t, 613, 616t, 618
after intestinal transplantation, 994
treatment of, 623t
after hematopoietic stem cell transplantation, 935
after intestinal transplantation, 994
IVIG in, 3505-3506
in necrotizing enterocolitis, 635
nosocomial, 3401
Clostridium perfringens, 1795-1796
Clostridium perfringens infection, **1795-1800**
diagnosis of, 618, 1798-1799
epidemiology of, 1795-1796, 1796t
gastrointestinal, 602t-603t, 607t, 613, 1797-1800
myonecrotic, 1796, 1798-1800, 3506
panophthalmic, 1798
pathophysiology of, 1795-1796
pneumatosis cystoides intestinalis in, 1798
prevention of, 1800
soft tissue, 1798-1799
uterine, 1797-1800
Clostridium septicum infection, 1797
bloodstream, 1798
Clostridium sordelli infection, 1797-1798
Clostridium spp., 1795, 1796t
nonpathogenic, 1810
Clostridium spp. infection, **1795-1800.** *See also specific species infections*
bloodstream, 1797-1798
bone, 1797-1798
clinical manifestations of, 1796-1797
diagnosis of, 1798-1799
enteric, 1797
epidemiology of, 1795-1796, 1796t
joint, 1797-1798
meningeal, 1797
myonecrotic, 1796
neonatal, 1797
pathophysiology of, 1795-1796
in pneumatosis cystoides intestinalis, 1798
prevention of, 1800
prognosis for, 1799-1800
soft tissue, 1796-1797
trauma-related, 1798
treatment of, 1799
uterine, 1797
Clostridium tetani, 1810, 1812
Clostridium tetani infection (tetanus), **1809-1819**
burn wound, 1052
cephalic, 1813
cerebral effects in, 1813
clinical manifestations of, 1813-1815
diagnosis of, 1815
differential diagnosis of, 1815
epidemiology of, 1810
generalized, 1813-1815
historical perspective on, 1809-1810
immunization against, 1818, 3470-3472
incidence of, 1810-1812, 1811f
in injection drug users, 1814
local, 1813
mortality with, 1817-1818
motor end-plate effects in, 1812-1813
neonatal, 1814-1815, 1819
pathogenesis of, 1812
pathophysiology of, 1812-1813
prevention of, 1818-1819
prognosis for, 1817
seizures in, 1814, 1816-1817
spinal cord effects in, 1813
sympathetic nervous system effects in, 1813
tetanus toxoid in, 1818-1819, 1818t
treatment of, 1815-1817
trismus in, 1814
Clotrimazole
in oropharyngeal candidiasis, 2746
in vulvovaginal fungal infection, 570
Cloxacillin, 3183t-3184t, 3185-3186, 3231t-3233t
in cervical lymphadenitis, 186
Clubbing (digital), in infective endocarditis, 357
Clue cell, 577, 577f
Clumping factor, 8-9, 1113, 1115f, 1118, 1129
Clutton joints, 1772
CMV. *See* Cytomegalovirus (CMV)
CMX001 (hexadecyloxypropyl-cidofovir)
in adenovirus infection, 1907
in BK virus infection, 1869
CNS. *See* Central nervous system (CNS)
Coagulopathy, in septic shock, 829
Cobalt, metabolism of, 77t
Coccidioides immitis, 2769-2770, 2770f
Coccidioides immitis infection (coccidioidomycosis), **2769-2779**
epidemiology of, 2770-2771
exanthem in, 757t, 762
Coccidioides immitis infection (coccidioidomycosis) *(Continued)*
extrapulmonary, 725-726
after intestinal transplantation, 1000
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 973
meningeal, 477-478, 480t, 483
ocular, 813-814
retropharyngeal, 174
Coccidioides spp. infection (coccidioidomycosis), **2769-2779**
bone, 2771, 2772f, 2773-2774
clinical manifestations of, 2772-2775, 2773f
culture in, 2775-2776, 2775f
cutaneous, 2773
diagnosis of, 2775-2777, 2775f
disseminated, 2771, 2774f
clinical manifestations of, 2773-2775
in immunosuppressed host, 2772
treatment of, 2777-2778
encephalitic, 494t-498t
epidemiology of, 2770-2771
exanthem in, 757t, 762, 2772-2773
granuloma in, 2769-2770, 2770f, 2773
in immunocompromised host, 2774-2775
meningeal, 477-478, 480t, 483, 2774
treatment of, 2778
pathogenesis of, 2771-2772, 2772f
pathology of, 2770f, 2771
in pregnancy, 2772, 2774
prevention of, 2779
prognosis for, 2778-2779
skin test in, 2776
in traveler, 3116-3117
treatment of, 2777-2778, 3295t
Cochlear implant–associated infection, 1017-1018
meningeal, 224, 437, 1017-1018, 1229
Coenurosis, 815, **3039-3040**
Cognitive development, otitis media and, 222, 222f
Cold, common. *See* Common cold
Cold agglutinins
in *Mycoplasma pneumoniae* infection, 2674, 2685
in pleural effusion, 313
Cold sore (herpes labialis), 154-155, 742t-748t, 1950, 1950f, 1952-1953, 1952f
Colic, probiotics in, 3371
Colicins, in urinary tract infection, 538
Colistin, 3207-3208, 3231t-3233t
Colitis. *See also* Gastrointestinal tract infection
antibiotic-associated, **632-639**
clinical manifestations of, 635, 3222
differential diagnosis of, 636-637
etiology of, 632-634
historical perspective on, 632
in *Klebsiella oxytoca* infection, 1468
laboratory studies in, 635-636, 636t
pathogenesis of, 634-635, 634f
prevention of, 637-638
treatment of, 637
differential diagnosis of, 2872
in *Entamoeba histolytica* infection, 2870, 2873
hemorrhagic, in *Escherichia coli* O157:H7 infection, 1454-1455
in *Trichuris* infection, 3016
Collagen-binding protein, of *Staphylococcus aureus*, 8-9

Colonization, 3-12
biofilms in, 11-12, 12f
Hwp1 protein in, 9
vs. infection, 103-104
INT1 protein in, 9
microbiome-related resistance to, 93
nonpilus adhesins in, 6-9, 7f-9f
normal flora, 89
pilus adhesins in, 3-6, 4f-5f
tissue tropism in, 10-11
Colonization factor antigens, in *Escherichia coli* infection, 1451, 1455, 1456f
Colonoscopy, in *Clostridium difficile* infection, 636
Colorado tick fever virus infection, **2173-2175**
clinical manifestations of, 742t-748t, 749, 2173-2174
diagnosis of, 2174-2175
differential diagnosis of, 2174
epidemiology, 2173, 2173f
pathophysiology of, 2174
prevention of, 2175
treatment of, 2175
Colposcopy, in HPV infection, 1882
Coltiviruses, 2172. *See also* Colorado tick fever virus infection
Common cold, **132-139**
age and, 2120, 2120f
clinical manifestations of, 136, 2121-2125, 2121t
complications of, 138-139
coronavirus in, 2490-2491
coxsackievirus A21 in, 2065
diagnosis of, 136-137, 2125, 2125t
differential diagnosis of, 136
enteroviruses in, 2064-2065
environmental temperature and, 2120
epidemiology of, 133-134, 133t, 2111-2113
etiology of, 132, 133t, 1895, 2064-2065, 2064t, 2110-2111, 2110f. *See also* Rhinovirus
gender and, 2120
historical perspective on, 132, 2109-2110
immune response in, 2118-2121
in immunocompromised host, 2125
in infants, 136
mucociliary transport in, 135
Mycoplasma pneumoniae in, 2678
pathogenesis of, 2118-2121
pathophysiology of, 134-136
prevention of, 139, 2117, 2117t, 2125-2126
prognosis for, 138-139
psychosocial factors in, 2120-2121
risk factors for, 134
seasonal distribution of, 134, 2112, 2113f
socioeconomic status and, 134
transmission of, 134, 2114-2118, 2114f, 2116f, 2117t
treatment of, 137-138, 2125-2126
Common variable immunodeficiency (CVID), 49, **910**
vs. chronic bronchitis, 267
diarrhea in, 600
furunculosis and, 777
IgA deficiency and, 911
Complement, **28-31**, 3365
activation of, 29-31, 29f
alternative pathway of, 29f, 30
in burns, 1049-1050
classical pathway of, 29-30, 29f
deficiencies of, 51-52, 429, **916-917**
clinical features of, 916
diagnosis of, 907, 917
Complement *(Continued)*
Neisseria meningitidis infection and, 1256-1257
pathogenesis of, 916-917
treatment of, 917
effector functions of, 30-31
evaluation of, 63
in *Haemophilus influenzae* infection, 1672
in immune regulation, 31
in inflammatory response, 31
in Kawasaki disease, 1077-1078
mannan-binding lectin pathway of, 29f, 30, 51
microbicidal activity of, 31
in *Neisseria* spp. infection, 18, 52, 429, 1279
in neonate, 44-45, 44t
opsonic activity of, 30-31
pathogen evasion of, 18
in septic shock, 829
in *Streptococcus pneumoniae* infection, 1209-1210, 1225
Complementary and alternative medicine (CAM), in common cold, 138
Computed tomography (CT)
in *Actinomyces* spp. infection, 1822f, 1823-1824
in acute disseminated encephalomyelitis, 518-519
in acute eosinophilic pneumonia, 297
in acute lobar nephropathy, 554, 555f, 556
in amebic CNS infection, 2980
in appendicitis, 682-683
in *Aspergillus* spp. infection, 2706, 2708f, 2712-2714, 2713f
in bacterial meningitis, 445-446, 446f, 454, 458
in *Bartonella henselae* infection, 1722-1723, 1724f
in brain abscess, 466-467, 467f
in chronic fatigue syndrome, 1088
in chronic osteomyelitis, 726, 726f
in *Citrobacter* spp. infection, 1431-1432, 1432f
in *Clostridium difficile* infection, 636
in *Cryptococcus neoformans* infection, 2797f, 2800
in cystic fibrosis, 325
in dental abscess, 152f
in Eastern equine encephalitis, 2228
in encephalitis, 506-507
in fungal infection, 926-927
high-resolution, in interstitial lung disease, 298-299
in *Histoplasma capsulatum* infection, 2817, 2818f
in HSV encephalitis, 1946-1947, 1947f
in hypersensitivity pneumonitis, 303-304, 303f
in intra-abdominal abscess, 705
in La Crosse virus encephalitis, 2522, 2523f
in *Legionella pneumophila* infection, 1710
in lung abscess, 319, 320f
in lung transplant–related *Aspergillus* infection, 966
in mastoiditis, 237-238, 238f
in mediastinitis, 420
in meningeal *Streptococcus agalactiae* infection, 1160, 1160f
in *Mycoplasma pneumoniae* infection, 2677-2678
in *Nocardia* spp. infection, 1421
Computed tomography (CT) *(Continued)*
in orbital cellulitis, 800, 800f
in osteomyelitis, 717
in pancreatitis, 690
in parapharyngeal abscess, 173
in parapneumonic pleural effusion, 310
in parotid gland enlargement, 192
in pericarditis, 377
in peritonsillar abscess, 169
in pyogenic liver abscess, 674, 674f
in recurrent respiratory papillomatosis, 1879f
in renal abscess, 555f, 556, 557f
in retroperitoneal infection, 708
in retropharyngeal abscess, 171, 171f
in rhinosinusitis, 199
in subacute sclerosing panencephalitis, 2389
in subdural empyema, 469, 470f
in tuberculous meningitis, 1353
in urinary tract infection, 546
Concentration quenching, 3174-3175, 3174f
Condoms, 1299
Conduction, in heat transfer, 84
Condyloma acuminata, 580-581, 580f-581f, 1873t, 1875-1877
Condylomata lata, 1770
Confidence interval, 123
Congenital heart disease
brain abscess and, 462
Candida spp. infection and, 2751
infective endocarditis with, 351, 351t
rubella virus–induced, 2214t-2216t, 2217, 2221
Congestive heart failure
in infective endocarditis, 356
in rheumatic fever, 414
Conidiobolus spp., 2837
Conidiobolus spp. infection (entomophthoromycosis), **2844-2845**
Conjunctiva (conjunctivae), 801
bulbar, in ataxia-telangiectasia, 905f
indigenous flora of, 90, 91t
infection of. *See* Conjunctivitis
Loa loa under, 3027
papilloma of, 1879-1880
Conjunctivitis, **801-806**
Achromobacter spp., 1574
adenoviral, 802-803, 1898-1899
Bacillus cereus, 1319
bacterial, 802
in bronchiolitis, 275
chemical, neonatal, 805-806, 805t
chlamydial, 804-805
neonatal, 805-806, 805t, 900-901, 2633-2634, 3242-3243
contact lens–related, 1019
Corynebacterium diphtheriae, 1305
EBV, 810
enteroviral, 810, 2073-2074, 2074t
gonococcal, 1288, 3242-3243. *See also* Ophthalmia neonatorum
Haemophilus aegyptius, 1689
hemorrhagic, acute, **810**
HSV, 803-804, 1945
inclusion, 804
in Kawasaki disease, 1073
Leishmania spp., 814
Leptospira spp., 1750-1752
Loa loa, 815
Lyme, 812
measles virus, 811
meningococcal, 1261
Moraxella catarrhalis, 1252

Conjunctivitis *(Continued)*
neonatal, 805t, 890, 3242-3243. *See also* Ophthalmia neonatorum
chlamydial, 805-806, 805t, 900-901, 2633-2634, 3242-3243
gonococcal, 805-806, **1280-1282**
Pantoea agglomerans, 1536
in Q fever, 815
rickettsial, 815
Rift Valley fever virus, 812
rubella virus, 811, 2212
Stenotrophomonas maltophilia, 1607
Streptococcus pneumoniae, 1231
in trench fever, 815
tuberculous, 1357
viral, 802-805
VZV, 804
Yersinia enterocolitica, 1528
Conjunctivitis-otitis syndrome, 212, 217
Connective tissue disease
fever of unknown origin in, 840
pulmonary manifestations of, 297
Constipation
in enteroviral infection, 2068t, 2070
in infant botulism, 1805
probiotics in, 3371
urinary tract infection and, 536
Contact dermatitis, vs. otitis externa, 208
Contact lenses, infection with, 1019, 2979-2980, 2980f
Contact precautions. *See also* Isolation precautions
in *Clostridium difficile* infection prevention, 637
Contagion, concept of, 103
Continuous ambulatory peritoneal dialysis (CAPD), infection with, 698, 700t, 701-703
Contraception, pelvic inflammatory disease and, 587
Convection, 84
Convulsions. *See* Seizures
Cooling therapy, in fever, 88
Copper, metabolism of, 77t, 79
Cor pulmonale, in *Schistosoma* spp. infection, 3065-3066
Corn syrup, infant botulism and, 1805
Cornea, 806
inflammation of. *See* Keratitis
Coronary artery (arteries)
aneurysm of, 1071, 1080-1084
atherosclerosis of, CMV and, 1980
vasculitis of, 1071-1072, 1074-1075. *See also* Kawasaki disease
Coronavirus, 2487-2488, 2487f
isolation of, 2494
Coronavirus infection, **2486-2495**
age and, 2489-2490
asthma and, 2491
asymptomatic, 2489
clinical manifestations of, 2490-2494
CNS, 2494
diagnosis of, 2494
enteric, 2493-2494
epidemiology of, 2488-2490, 2489f
exanthem in, 749
IgE in, 136
in immunocompromised host, 2491
Kawasaki disease and, 1070
lower respiratory tract, 2491
nosocomial, 3382
pathogenesis of, 2490
pulmonary, 284, 2491
transmission of, 2490
upper respiratory tract, 2490-2491
Corticosteroids
Acanthamoeba spp. infection and, 2985
in acute disseminated encephalomyelitis, 519
in acute transverse myelitis, 527-528
in ARDS, 872-873
Aspergillus infection and, 2710-2711
in bacterial meningitis, 451-453
in *Bartonella henselae* infection, 1728
in *Bordetella pertussis* infection, 1630
in brain abscess, 468-469
in bronchiolitis, 279
in chronic bronchitis, 269
in croup syndromes, 255-256
in cystic fibrosis, 344
in encephalitis, 510
in enterovirus infection, 2103
in eosinophilic meningitis, 483-484
in hematopoietic stem cell transplantation–related *Pneumocystis jiroveci* infection, 939
in hypersensitivity pneumonitis, 305
immunization and, 3451
infectious complications of, 255-256
in influenza virus infection, 2351
injection of, in chalazion, 796-797
in interstitial lung disease, 300
after Kasai procedure, 665
in Kawasaki disease, 1079-1080
in *Leptospira* spp. infection, 1757
in Murray Valley encephalitis, 2303
in *Mycoplasma pneumoniae* infection, 2687
in myocarditis, 396
in *Neisseria meningitidis* infection, 1264
in neonatal meningitis, 886
in orbital cellulitis, 800
in otitis media with effusion, 229
in parainfluenza virus infection, 2370
in peritonsillar abscess, 169
in PFAPA syndrome, 164
in pharyngitis, 164
in *Pneumocystis* infection, 3010
in rheumatic fever, 413
in rhinosinusitis, 201
in rhinovirus infection, 2126
in RSV infection, 2426
in *Salmonella* ser. Typhi infection, 1506
in *Schistosoma* spp. infection, 3068
in septic shock, 834-835
in toxic shock syndrome, 857
in tuberculosis, 1374-1375
in urinary tract infection, 548
Cortisol, plasma, 81
Corynebacterium diphtheriae, 1301-1302
carriage of, 1302-1303, 1309-1310
colony types of, 1302
toxin of, 1302, 1304
Corynebacterium diphtheriae infection, **1301-1311**
vs. *Arcanobacterium haemolyticum* infection, 1325
aural, 1305
cervical lymphadenopathy in, 177
clinical manifestations of, 1305-1306
conjunctival, 1305
cutaneous, 1305
in daycare facilities, 3560-3561
diagnosis of, 1306-1307
differential diagnosis of, 1307
endocardial, 362-363, 1303, 1306
epidemiology of, 1302-1304
exanthem in, 753t-756t
historical perspective on, 1301
immunization against, 1308-1309, 3430-3431
Corynebacterium diphtheriae infection *(Continued)*
joint, 1306
laryngeal, 243t, 250t-251t, 253, 1305, 1307
myocardial complications of, 1306
nasal, 1305, 1307
neurologic complications of, 1306
ocular complications of, 1306
vs. parainfluenza virus infection, 2369
pathogenesis of, 1304-1305
pharyngeal, 1305, 1307
during pregnancy, 1306
prevention of, 1308-1309
prognosis for, 1310
supportive treatment in, 1310
tonsillar, 1305, 1307
treatment of, 1309-1310, 3506-3507
vulvovaginal, 569, 1305
Corynebacterium jeikeium infection, 1423
clinical manifestations of, 1424
diagnosis of, 1426
epidemiology of, 1424
pathophysiology of, 1424
treatment of, 1426
Corynebacterium minutissimum infection, 1425
Corynebacterium pseudodiphtheriticum infection, 1425
Corynebacterium pseudotuberculosis infection, 1424-1425
Corynebacterium spp., 1423-1424
Corynebacterium spp. infection, **1423-1426.** *See also specific species infections*
clinical manifestations of, 1424-1426
diagnosis of, 1426
endocardial, 362-363, 1303, 1306
epidemiology of, 1424
pathophysiology of, 1424
treatment of, 1426
Corynebacterium striatum infection, 1425
Corynebacterium ulcerans, 1301, 1303
Corynebacterium ulcerans infection, 1425-1426
Corynebacterium urealyticum infection, 1424-1426
Corynebacterium xerosis infection, 1425
Cotton-wool spots, 811
Cough
in acute laryngotracheitis, 249, 250t-251t
air pollution–related, 268
in *Chlamydia trachomatis* infection, 1617-1618
in common cold, 137
in croup syndromes, 250t-251t
differential diagnosis of, 265-269, 266f, 1627
irritant-related, 268-269
in lung abscess, 318
in *Mycoplasma pneumoniae* infection, 1617-1618
in rhinosinusitis, 197
treatment of, 269-270, 270f
whooping. *See Bordetella pertussis* infection (pertussis, whooping cough)
Cowpox, **2044-2045**
Coxiella burnetii, 2663-2664
Coxiella burnetii infection (Q fever), **2663-2665**
chronic, 2664
clinical manifestations of, 2664
diagnosis of, 2664-2665
differential diagnosis of, 2665
encephalitic, 494t-498t
endocardial, 363, 2664

Coxiella burnetii infection (Q fever) *(Continued)*
epidemiology of, 2664
exanthem in, 750, 751t-752t
hepatic, 656-657
historical perspective on, 2663
vs. *Legionella pneumophila* infection, 1713
ocular, 815
pathology of, 2664
in pregnancy, 2664
treatment of, 2665
Coxsackie-adenovirus receptor (CAR), 11
Coxsackievirus
antigenic characteristics of, 2056
classification of, 2052-2053, 2052t-2053t
historical perspective on, 2051
host range of, 2056
prevalence of, 2057-2059, 2058t
replication of, 2053-2054, 2053t
Coxsackievirus infection, **2051-2109**
asymptomatic, 2063-2064, 2063t
in bronchiolitis, 2066
cerebellar ataxia and, 2088t, 2090
clinical manifestations of, 2063-2090
congenital, 2091-2092
in croup, 2066
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
in epididymitis, 2077
exanthems in, 742t-748t, 749, 760, 762, 764, 769, 2078-2086, 2079f, 2080t-2081t, 2082f-2083f, 2083t
gastrointestinal, 2068-2073, 2068t-2069t, 2071t-2072t
Guillain-Barré syndrome and, 2088t, 2090-2097
hematologic findings in, 2078
hemolytic-uremic syndrome in, 2078
hepatic, 2070-2072
herpangina in, 2066t
in immunocompromised host, 2091
meningeal, 485-487, 2086-2090, 2089t
muscle, 2078
myocardial, 384-387, 385f, 2075-2077, 2076t, 2104. *See also* Myocarditis
neonatal, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t-2089t
nonspecific febrile illness in, 2064, 2064t
in orchitis, 2077
paralytic, 2090
pericardial, **379-380**, 2075-2077, 2076t
peritoneal, 2070
pharyngeal, 2064t, 2065
pulmonary, 2067
respiratory manifestations of, 2064-2068, 2064t
rheumatic fever and, 2077
sudden infant death and, 2091
treatment of, 2103-2104
Coxsackievirus A
classification of, 2052-2053, 2052t-2053t
replication of, 2053-2054, 2053t
Coxsackievirus A infection
congenital, 2091-2092
conjunctival, 2073-2074, 2074t
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
gastrointestinal, 2068-2073, 2069t, 2071t
hematologic findings in, 2078
hemolytic-uremic syndrome in, 2078
hepatic, 2070-2072
Coxsackievirus A infection *(Continued)*
meningeal, 2089t
muscle, 2078
myocardial, 2075-2077, 2076t
neonatal, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t
nonspecific febrile illness in, 2064, 2064t
ocular, 2074-2075
parotid gland, 2066
pericardial, 2075-2077, 2076t
pharyngeal, 2064t, 2065
photophobia in, 2074
respiratory manifestations of, 2064-2068, 2064t
sudden infant death and, 2091
treatment of, 2103-2104
Coxsackievirus A2 infection, 2079, 2080t-2081t
Coxsackievirus A3 infection, 2079
Coxsackievirus A4 infection, 2061, 2065, 2079, 2079f, 2080t-2081t
Coxsackievirus A5 infection, 2079, 2080t-2081t
Coxsackievirus A6 infection, 762, 764, 2079, 2080t-2081t
Coxsackievirus A7 infection, 2061, 2079, 2080t-2081t
paralytic, 2090
Coxsackievirus A9 infection, 760, 2079-2082, 2080t-2081t, 2082f
photophobia in, 2074
pulmonary, 2067
Coxsackievirus A10 infection, 2080t-2081t, 2082
acute lymphonodular pharyngitis in, 2066
Coxsackievirus A16 infection
abortion and, 2091-2092
chronic, 2083
epidemiology of, 2057
exanthem in, 749, 762
hand-foot-and-mouth disease in, 2066, 2080t-2081t, 2082-2083, 2082f-2083f, 2083t
pathology of, 2055f
Coxsackievirus A21 infection, 2065
Coxsackievirus A24 infection, conjunctival, 810, 2073-2074
Coxsackievirus B
classification of, 2052-2053, 2052t-2053t
replication of, 2053-2054, 2053t
Coxsackievirus B infection
bronchial, 2066
congenital, 2091-2092
conjunctival, 2073-2074, 2074t
in croup, 2066
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
in epididymitis, 2077
gastrointestinal, 2068-2073, 2068t-2069t, 2071t
hemolytic-uremic syndrome in, 2078
hepatic, 655, 2070-2072
meningeal, 2086-2090, 2088t-2089t
muscle, 739
myocardial, 2075-2077, 2076t
myocardial infarction and, 2077
neonatal, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t
nonspecific febrile illness in, 2064, 2064t
Coxsackievirus B infection *(Continued)*
ocular, 2074-2075
in orchitis, 2077
pancreatic, 693, 2072
parotid gland, 2066
pathology of, 2061, 2062f
pericardial, 2075-2077, 2076t
peritoneal, 2070
pharyngeal, 2064t, 2065
respiratory manifestations of, 2064-2068, 2064t
sudden infant death and, 2091
treatment of, 2103-2104
Coxsackievirus B1 infection, 2055f, 2080t-2081t, 2083
peritoneal, 2070
pulmonary, 2067
Coxsackievirus B2 infection, 2080t-2081t, 2083
Coxsackievirus B3 infection, 2080t-2081t, 2083-2084
neonatal, 2093
Coxsackievirus B4 infection
CNS, 2061, 2062f
diabetes mellitus and, 2072-2073
exanthem in, 2080t-2081t, 2084
myocardial, 2061, 2062f
renal, 2077-2078
Coxsackievirus B5 infection, 760, 2080t-2081t, 2084
peritoneal, 2070
Coxsackievirus B6 infection, 2080t-2081t, 2084
pulmonary, 2067
CR3 (CD11b/CD18), 6
Craniofacial anomalies, in otitis media, 217
Craniofacial surgery, 1043-1046
evaluation for, 1045
infection with, **1044-1045**
evaluation of, 1045-1046
microbiology of, 1045
needle aspiration in, 1046
treatment of, 1046
irrigation during, 1045
meningitis with, 1044-1045
perioperative antibiotics in, 1045
reconstructive materials in, 1044
staging of, 1045
in Treacher Collins syndrome, 1044
Creatinine, urinary, 69
Creutzfeldt-Jakob disease (CJD), **2619-2629**
clinical manifestations of, 811, 2627-2628, 2627t
diagnosis of, 2628
epidemiology of, 2621-2624
pathogenesis of, 2624-2627
prevention of, 2628-2629
treatment of, 2628
Crigler-Najjar syndrome, 657
Crimean-Congo hemorrhagic fever, **2531-2535**
clinical manifestations of, 2532
diagnosis of, 2533
epidemiology of, 2531-2532, 2532f
pathogenesis of, 2533, 2533f
prognosis for, 2534
treatment of, 2533-2534
Crimean-Congo hemorrhagic fever virus, 2531
Crohn disease, 2872
appendicitis and, 687-688
vs. *Clostridium difficile* infection, 637
gallstones in, 668
vs. *Shigella* spp. infection, 1483

Cronobacter sakazakii infection, 1434, 1436, 1438
neonatal, 886
Cross-agglutinin absorption test (CAAT), in *Leptospira* spp. infection, 1755
Crotamiton, 3348
Croup, **241-260**, 242t
clinical presentation of, 249-253, 250t-251t
diagnosis of, 253
differential diagnosis of, 250t-251t, 253, 2369
epidemiology of, 245-246
etiology of, 242-244, 243t-244t, 2066
historical perspective on, 241-242
in *Mycoplasma pneumoniae* infection, 2679
pathogenesis of, 247-248
pathology of, 247-248
prevention of, 259
prognosis for, 259
spasmodic, 243t, 250t-251t, 252
etiology of, 242-244, 243t-244t
vs. laryngotracheitis, 253
treatment of, 254-257
terminology for, 242, 243t
treatment of, 254-257
Crowding, *Streptococcus pneumoniae* infection and, 1201, 1204
Cryoglobulinemia, in HAV infection, 2140
Cryopyrin-associated periodic fever syndromes, 847-848
Cryptococcal capsular polysaccharide antigen test, 475, 480
Cryptococcoma, 2803
Cryptococcus gattii, 2796-2797, 2798t, 2799
Cryptococcus neoformans, 2796-2797
capsule of, 20, 2796, 2797f
ecology of, 2797-2799, 2798t
extracellular enzymes of, 2797
melanin of, 2796-2797
virulence factors of, 2796
Cryptococcus neoformans infection (cryptococcosis), **2795-2806**
biliary, 660
bone, 726
CNS, 475-476, 480t, 2801-2803
CSF examination in, 443t, 475
treatment of, 2804-2805
congenital, 2804
cutaneous, 757t, 2804
diagnosis of, 2797f, 2798t, 2800-2802
epidemiology of, 2797-2799
host response to, 23
immune reconstitution inflammatory syndrome with, 972, 2803-2804
immune response to, 2799
interferon-γ in, 2805
after intestinal transplantation, 1000
intraparenchymal, 2803
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 971-972
neonatal, 897
pathogenesis of, 2799
prevention of, 2805
prostatic, 2804
pulmonary, 2800-2801
treatment of, 2804
secondary prophylaxis in, 2805
treatment of, 2804-2805
after lung transplantation, 971-972
uveal, 814
Cryptosporidium spp., 2896-2897, 2908f
life cycle of, 2897, 2898f
Cryptosporidium spp. infection (cryptosporidiosis), **2896-2905**
biliary, 660
clinical manifestations of, 602t-603t, 607t, 614-615, 617, 2900-2901, 2901t
in daycare facilities, 3556-3557
diagnosis of, 619, 2901-2902, 2902f, 2902t
epidemiology of, 2897-2899
extraintestinal, 2901
after hematopoietic stem cell transplantation, 943-944
immune response to, 2900
in immunocompromised host, 2898-2899, 2901, 2903
oral immunoglobulin in, 3498
pancreatic, 694-695
pathogenesis of, 2899-2900
pathology of, 2900
prevention of, 2903-2904
transmission of, 2899
treatment of, 625, 2903, 3327t-3346t, 3498
waterborne, 2899, 2904
CSF. *See* Cerebrospinal fluid (CSF)
Culex annulirostris, 2249, 2292
Culex tritaeniorhynchus, 2292
Culiseta melanura, 2226, 2229
Culiseta pipiens
in St. Louis encephalitis virus infection, 2254, 2254f
in West Nile virus infection, 2263
Culiseta quinquefasciatus, in St. Louis encephalitis virus infection, 2254, 2254f
Culiseta tarsalis, 2230-2231, 2237
in St. Louis encephalitis virus infection, 2254, 2254f
Cullen sign, in pancreatitis, 689-690
Culture
amebic
in *Acanthamoeba* spp. infection, 2982-2983
in *Balamuthia mandrillaris* infection, 2983
in *Naegleria fowleri* infection, 2982-2983
bacterial, 3589-3592, 3590t
in *Acinetobacter* spp. infection, 1571
in *Actinomyces* spp. infection, 1823
in *Aggregatibacter actinomycetemcomitans* infection, 1610, 1824
in *Arcanobacterium haemolyticum* infection, 1326
in *Aspergillus* spp. infection, 2712
in bloodstream infection, 3589-3590
in *Bordetella pertussis* infection, 1627-1629
in brain abscess, 466
in *Brucella* spp. infection, 1614
in burn wound, 1056-1057
in *Campylobacter* spp. infection, 1645, 1646t
in chemotherapy-associated infection, 924-925
in *Citrobacter* spp. infection, 1430
in CNS infection, 3591
in coagulase-negative staphylococcal infection, 1133-1134
in CSF shunt–related infection, 1033
in endophthalmitis, 820
in *Enterobacter* spp. infection, 1436-1437
in *Erysipelothrix rhusiopathiae* infection, 1328
in fever of unknown origin, 842
in gastrointestinal tract infection, 3592
in genital tract infection, 561
Culture *(Continued)*
in *Haemophilus influenzae* infection, 1667
in *Haemophilus influenzae* type b infection, 1677
in *Haemophilus parainfluenzae* infection, 1690
in *Kingella kingae* infection, 1703
in *Leptospira* spp. infection, 1754
in *Leuconostoc* spp. infection, 1247
in *Listeria monocytogenes* infection, 1332-1333
in meningitis, 442, 445
in *Neisseria gonorrhoeae* infection, 1274, 1289-1291, 1289t
in *Neisseria meningitis* infection, 1262
in neonatal *Candida* spp. infection, 896
in neonatal joint infection, 889
in neonatal osteomyelitis, 889
in *Pasteurella multocida* infection, 1545, 1548
in *Pseudomonas aeruginosa* infection, 1582-1583
in respiratory tract infection, 3590-3591
in rhinosinusitis, 199-200
in *Rhodococcus equi* infection, 1427
in *Salmonella* ser. Typhi infection, 1503
in *Salmonella* spp. infection, 1503
in sepsis neonatorum, 883
sputum
in *Blastomyces dermatitidis* infection, 2731
in *Cryptococcus neoformans* infection, 2800-2801
in cystic fibrosis–related infection, 332, 333t
in *Legionella pneumophila* infection, 1711
in *Staphylococcus aureus* infection, 1113, 1114f, 1123
in *Stenotrophomonas maltophilia* infection, 1605-1606, 1608
in *Streptobacillus moniliformis* infection, 1716, 1716f, 1718-1719
in streptococcal pharyngitis, 1147
in *Streptococcus pneumoniae* infection, 1215-1216
in *Streptococcus* viridans infection, 1194
in *Trichomonas vaginalis* infection, 2891
in urinary tract infection, 3591-3592
in *Vibrio parahaemolyticus* infection, 1559
in *Vibrio vulnificus* infection, 1561
in vulvovaginitis, 561
in *Yersinia enterocolitica* infection, 1530
in *Yersinia pestis* infection, 1509-1510
in *Yersinia pseudotuberculosis* infection, 1530
in *Chlamydia pneumoniae* infection, 2642-2643
in *Chlamydia trachomatis* infection, 2636
fungal, 3596, 3597t, 3599-3600, 3599t
in *Aspergillus* spp. infection, 2711-2712, 2712f
in *Blastomyces dermatitidis* infection, 2731, 2731f
in *Candida* spp. infection, 896, 2735, 2744
in *Coccidioides* spp. infection, 2770, 2775-2776, 2775f
in cryptococcal meningoencephalitis, 2802
in dermatophyte infection, 787
in *Histoplasma capsulatum* infection, 2820-2821
in *Paracoccidioides* spp. infection, 2788

Culture *(Continued)*
in *Mycoplasma pneumoniae* infection, 2686
stool. *See* Stool culture
in tuberculosis, 1364-1365
urine. *See* Urine culture
viral, 3605-3609, 3606t-3607t
in adenovirus infection, 1890
in CMV infection, 1981-1982
in Colorado tick fever virus infection, 2174-2175
in coronavirus infection, 2494
in dengue, 2283, 2285-2286
in EBV infection, 2011
in hantavirus infection, 2498-2499
in HSV infection, 1934, 1955-1956, 1956f
in influenza virus infection, 2345
in measles, 2374-2375, 2375f
in mimivirus infection, 2049-2050
in mumps, 2396
in rhinovirus infection, 2111, 2112f, 2125
in RSV infection, 2409, 2424
in rubella virus infection, 2197-2198
in West Nile virus infection, 2270
in yellow fever, 2277
Cupriavidus spp., 1584
Cupriavidus spp. infection, 1595-1596
Curvularia spp. infection, 1053
Cutaneous larva migrans, 3024, 3327t-3346t
Cutaneous T-cell lymphoma, 2569
Cyclitis, 809
Cycloserine, in tuberculosis, 1367t
Cyclospora cayetanensis, 2906
Cyclospora cayetanensis infection (cyclosporiasis), **2905-2907**
clinical manifestations of, 602t-603t, 607t, 615, 2906-2907
diagnosis of, 617, 2907, 2907f
epidemiology of, 2906
transmission of, 2906
treatment of, 625, 2907, 3327t-3346t
Cyclosporine, in heart transplantation, 955, 955t
Cyclosporine A, in HTLV-1–associated myelopathy, 2573
Cyst(s)
Bartholin, 579, 579f
branchial cleft, 182
choledochal, 666-667
hydatid. *See Echinococcus granulosus* infection (echinococcosis, hydatid disease)
thyroglossal duct, 182
Cystic fibrosis, **323-348**
Achromobacter spp. infection in, 336, 341, 1574
anti-inflammatory therapy in, 344
Aspergillus spp. infection in, 336, 341-342, 2713f
Bordetella hinzii infection in, 1637
bronchiectasis in, 325, 325f
Burkholderia spp. infection in, 335-336, 335t, 345t, 1593-1594
treatment of, 341
vs. chronic bronchitis, 267
clinical manifestations of, 323-326, 324t
diagnosis of, 326-327, 327t
gallstones in, 668
glucose metabolism in, 324
Haemophilus influenzae infection in, 334
hepatitis in, 657
immunization in, 344-345
immunodeficiency in, 60
Cystic fibrosis *(Continued)*
infection in, 331-332
age-specific prevalence of, 332
anti-inflammatory therapy in, 344
antibiotic resistance in, 3154
antimicrobial susceptibility testing in, 333
coronavirus, 2491
diagnosis of, 332-333, 333t
environmental prevention of, 345-346, 346t
epidemiology of, 331-332
immunization against, 344-345
inhaled antibiotic therapy in, 342-343
long-term suppressive therapy in, 342-343
macrolide antibiotics in, 343
molecular diagnostics in, 333
nosocomial, 3405-3406
pathogenesis of, 327-331, 329f-330f
prevention of, 344-346
treatment of, 337-344
IVIG in, 3508
liver disease in, 324-325
lung abscess and, 317
lung transplantation in, 343-344
mycobacterial infection in, 336-337, 1385-1386
nontuberculous *Mycobacterium* infection in, 341
pathogenesis of, 327-331, 329f-330f
Pseudomonas aeruginosa infection in, 60, 335, 1593-1594
antibiotic resistance in, 3154
clinical manifestations of, 1593-1594
diagnosis of, 1596-1597
eradication of, 337-338
pathogenesis of, 328-330, 329f, 1587-1589
prevention of, 337, 1603-1604
treatment of, 339-340, 1599-1601
viral infection and, 334
Scedosporium spp. infection in, 336
Staphylococcus aureus infection in, 334
eradication of, 337-338
methicillin-resistant, 338, 340-341
prevention of, 337
treatment of, 340-341
Stenotrophomonas maltophilia infection in, 336, 341, 1607
survival with, 326
viral infection in, 333-334
Cystic fibrosis transmembrane conductance regulator *(CFTR)* gene/protein, 267, 323, 327, 692, 1587
Cystic hygroma, 182
Cysticercosis. *See* Neurocysticercosis; *Taenia solium* infection (cysticercosis)
Cystitis, 538. *See also* Urinary tract infection (UTI)
chemical, 540
granulomatous, 540
hemorrhagic, in adenovirus infection, 1901
treatment of, 546-548, 547t
Cystoisospora belli, 2907-2909, 2908f
Cystoisospora belli infection, **2907-2911**
clinical manifestations of, 607t, 615, 2909
diagnosis of, 617, 2909-2910, 2910f
epidemiology of, 2909
pathophysiology of, 2909, 2909f
transmission of, 2909
treatment of, 2910-2911, 3327t-3346t
Cytochalasin D, 16
Cytokines, 25-27, 26t, 3357-3363, 3359t-3360t
in acute disseminated encephalomyelitis, 518
in adenovirus infection, 1895
in *Babesia* spp. infection, 2917
in bacterial meningitis, 433-435, 434f
in burns, 1049
in chronic fatigue syndrome, 1088
in *Cryptosporidium* spp. infection, 2900
in cystic fibrosis, 331
in influenza virus infection, 2338
in Kawasaki disease, 1076
in *Legionella pneumophila* infection, 1709
in malaria, 2926
in measles, 2379
measurement of, 80
in mycobacterial infection, 1382
in *Neisseria meningitidis* infection, 1257-1258, 1263
in *Pneumocystis* infection, 3008
proinflammatory, 65, 79-80
in rhinovirus infection, 2119, 2123
in RSV infection, 2416
in *Salmonella* spp. infection, 1498-1499, 1499f
in *Schistosoma* spp. infection, 3063
in septic shock, 827, 828f
in *Shigella* spp. infection, 1479
in *Streptococcus agalactiae* infection, 1158-1159
in *Streptococcus pneumoniae* infection, 1209-1210, 1224
T-cell secretion of, 39-40
Toll-like receptor effects on, 25
in tuberculosis, 1342
Cytolethal distending toxin, 1650
Cytomegalovirus (CMV), 1969-1970
antibody to, 1982-1983
detection of, 1981-1982
genotypes of, 1970
pp65 antigen of, 1982
Cytomegalovirus (CMV) infection, **1968-1991**
in adolescents, 1971-1972
autoimmune disease and, 1981
biliary, 660
in burn patient, 1053
clinical manifestations of, 742t-748t, 1975-1981
CNS, 1978-1980, 1979f
congenital, 881, 1970
clinical manifestations of, 1975
CNS, 1978-1979, 1979f
cutaneous, 1981
diagnosis of, 1983-1984
differential diagnosis of, 1975
hearing loss and, 1975, 1980
hepatitis in, 1978
ocular, 818, 881
treatment of, 1986-1987
cutaneous, 742t-748t, 1981
in daycare facilities, 3553-3554
diagnosis of, 951, 1981-1984
disseminated, 1974
endocrine, 1980-1981
epidemiology of, 1970-1974
esophageal, 594-597, 596f
fetal, 1975, 1983-1984
gastrointestinal tract, 1978
genitourinary, 1981
after heart transplantation, 950-952
after hematopoietic stem cell transplantation, 939-940
hepatic, **654**, 1977-1978

Cytomegalovirus (CMV) infection *(Continued)*
historical perspective on, 1969
in HIV-infected host, 811, 1979-1980, 2601-2602
immune response to, 1974-1975
in immunocompromised host, 1973-1975, 1984
immunomodulatory effects of, 974
after intestinal transplantation, 996-997, 997t, 1001
after kidney transplantation, 1007-1008
after liver transplantation, 996-997, 997t, 1001
after lung transplantation, 974-976
mononucleosis syndrome in, 1976
murine, 74
myelitic, 526
myocardial, 1980
neonatal, 811
treatment of, 1987
nosocomial, 1972-1973, 3383
ocular, 811, 1977
otic, 1980
pancreatic, 694
pathogenesis of, 1974-1975
pathology of, 1974, 1974f
perinatal, 1971
clinical manifestations of, 1975-1976
diagnosis of, 1984
pharyngeal, 159t-160t, 161
postnatal, 1971
CNS, 1979
diagnosis of, 1984
prevention of, 1987
during pregnancy, 1970, 1972, 1983, 1986, 1988
prevention of, 1987-1989
antiviral agents in, 1988-1989
immune globulin in, 1987-1988, 3520-3521
immunization in, 1989
perinatal, 3521
transplantation-related, 1987, 3520
pulmonary, 1976-1977, 1976f, 1984
retinal, 811, 1977
transmission of
blood transfusion, 1972-1973
intrafamilial, 1972
nosocomial, 1972-1973
sexual, 1972
treatment of, 1984-1987, 1985f, 3283-3289
Cytosine, 3298f

D

D test, 1126-1127, 1127f
Daclatasvir, in HCV infection, 2324
Dacryoadenitis, **797**
noninfectious, 797
Dacryocystitis, **797-798**
Pseudomonas aeruginosa, 1591
Dacryostenosis, 806
Dactylitis
distal, blistering, **775**
tuberculous, 1355
Dakin solution, in *Vibrio vulnificus* infection, 1562
Dane particle, 1912, 1915. *See also* Hepatitis B virus (HBV) infection
Dapsone, 3348
in hematopoietic stem cell transplantation, 938-939
Dapsone *(Continued)*
in *Mycobacterium leprae* infection, 1406, 1407t
in *Pneumocystis* infection, 3010
Daptomycin, 3206, 3238
in coagulase-negative staphylococcal infection, 1139
in methicillin-resistant *Staphylococcus aureus* infection, 1127
resistance to, 3153
in vancomycin-resistant *Enterococcus* spp. infection, 1184
Darunavir, 2615
Dawson inclusion body encephalitis. *See* Subacute sclerosing panencephalitis (SSPE)
Daycare facilities, **3550-3569**
Aeromonas spp. infection in, 1542
antibiotic administration in, 3238
astrovirus infection in, 3558
bites in, 3571
Campylobacter jejuni infection in, 3557
chickenpox in, 3562
Clostridium difficile infection in, 3558
CMV infection in, 1971, 3553-3554
common cold in, 133-134
Cryptosporidium infection in, 3556-3557
diarrheal disease in, 599
diphtheria in, 3560-3561
Escherichia coli infection in, 3558
Giardia lamblia infection in, 2879, 3556
Haemophilus influenzae type b infection in, 3561
hand, foot, and mouth disease in, 3555
HAV infection in, 2134-2135, 3564
HBV infection in, 3564-3565
head lice in, 3559
HIV infection in, 3560
HSV infection in, 3560
infection control in, 3565-3569
education for, 3566
exclusion policies and, 3566, 3567t-3568t
hygiene standards for, 3566
immunization in, 3565-3566
physical plant factors in, 3565
prophylaxis in, 3569
written policies for, 3565
infection patterns in, 3552-3553, 3553t
infection transmission in, 3548, 3550-3552, 3551t
airborne, 3552
bloodborne, 3552
contact-related, 3552
feces-related, 3552
food-related, 3552
saliva-related, 3552
sexual, 3552
influenza in, 3562
Kingella kingae infection in, 1701, 3555
measles in, 3562-3563
methicillin-resistant *Staphylococcus aureus* infection in, 3559
mumps in, 3564
Mycobacterium tuberculosis infection in, 3556
Neisseria meningitidis infection in, 3555-3556
norovirus infection in, 3558
otitis media in, 211, 3553, 3554t
parvovirus B19 infection in, 3554-3555
pertussis in, 3563
poliomyelitis in, 3563
rotavirus infection in, 3564
rubella in, 3563
Daycare facilities *(Continued)*
Salmonella spp. infection in, 1493-1494, 1507, 3557-3558
scabies in, 3559
Shigella infection in, 3557
Streptococcus pneumoniae infection in, 1201, 1204, 3561-3562
Streptococcus pyogenes infection in, 3555
upper respiratory tract infection in, 3553, 3554t
vaccine-preventable diseases in, 3560-3565
varicella in, 3562
Deafness. *See* Hearing loss
Débridement, in necrotizing fasciitis, 779
Decongestants, in common cold, 137
Decontamination, gastrointestinal tract, in burn patient, 1061
DEET (*N,N*-diethyl-3-metylbenzamide), 1737, 2175, 2526
neurotoxicity of, 2235-2236, 2311
precautions for, 2236, 2236t
Defecation, infection and, 109
Defective virus. *See* Hepatitis D virus (HDV) infection
Defensins, 24-25, 34t, 35, 3365-3366
Deferoxamine, mucormycosis and, 2840
Dehydration
in cholera, 1551-1552, 1551f
in gastrointestinal tract infection, 76, 620-622, 620t-621t
in rotavirus infection, 2186
Delayed fluorescence immune assay, in *Corynebacterium diphtheriae* infection, 1307
Delhi boil. *See Leishmania* spp. infection (leishmaniasis), Old World
Delta agent/virus. *See* Hepatitis D virus (HDV) infection
Delta toxin, of *Staphylococcus epidermidis*, 1133
Demeclocycline, 3217-3220
Demodex brevis infection, 795-796, 796f
Demodex folliculorum infection, 795-796, 796f
Dendritic cells
natural killer cell interaction with, 28
plasmacytoid, 38
Dengue, **2278-2291**
vs. chikungunya, 2244, 2245t-2246t, 2282
clinical manifestations of, 2281-2282
diagnosis of, 2284, 2285t
encephalitic, 500
epidemiology of, 2280
exanthem in, 742t-748t, 760, 762
geographic distribution of, 2280-2281
hemorrhagic diathesis in, 2281-2282
host range of, 2280
immunization against, 2289
laboratory studies in, 2285-2286
pathogenesis of, 2282-2284
pathology of, 2283
prevention of, 2289-2290
prognosis for, 2288-2289
reporting requirements for, 2288
severe, 2285, 2285t
transmission of, 2279-2280
in travel, 3115
treatment of, 2286
Dengue hemorrhagic fever/dengue shock syndrome, **2278-2291**
clinical manifestations of, 2282
diagnosis of, 2284-2285, 2284t
pathogenesis of, 2283-2284
prevention of, 2289-2290
prognosis for, 2288-2289
treatment of, 2286-2288

Dengue virus, 2279
identification of, 2285-2286
Dental caries. *See* Caries; Odontogenic infection
Dental procedures. *See also* Odontogenic infection
cervical lymphadenitis and, 183, 183f
cervicofacial actinomycosis and, 184
prophylactic antibiotics for, 368-369, 369t, 417-418, 1195-1196, 3246-3247, 3246t-3247t
rhinosinusitis and, 195
Depigmentation, in pinta, 1785
Depression, in chronic fatigue syndrome, 1089
Dermacentor andersoni tick, **3071-3072**
in Colorado tick fever virus infection, 2173-2174, 2173f
in Rocky Mountain spotted fever, 2651, 2654
Dermacentor variabilis tick, 2651, **3071-3072**
Dermatitis
allergic, 208
contact, 208
diaper, 788, 788f, 2740, 2747
infective, 742t-748t, 749-750
molluscum, 783
streptococcal, perianal, **775**
Dermatobia hominis infection, 757t-758t
Dermatoblepharitis, HSV, 803
Dermatophyte infection. *See at* Tinea
Dermatophytid reaction, 784-785, 785f
Dermonecrotic toxin, 1618t, 1619, 1624
Descemet's membrane, 806
Devic disease, 515
Device-related infection. *See* Prosthetic/implantable device–related infection
Dexamethasone. *See also* Corticosteroids
in bacterial meningitis, 451-453
in croup, 255-256
in encephalitis, 510
in septic arthritis, 733
Dextranomer/hyaluronic acid copolymer (Deflux), in vesicoureteral reflux, 551
Diabetes insipidus, fever of unknown origin in, 846
Diabetes mellitus
coxsackievirus B4 and, 2072-2073
cystic fibrosis and, 324
furunculosis and, 777
genetic susceptibility to, 72
immunization and, 3428-3429
liver abscess in, 673
mucormycosis and, 2840
mumps and, 2402
viral infection and, 72
Dialysis
in diarrheogenic *Escherichia coli* infection, 1462
infection with, 3406-3407
mucormycosis with, 2840
osteomyelitis with, 723
tuberculosis with, 1358
Diaper dermatitis, 788, 788f, 2740
treatment of, 2747
Diarrhea, 599-606. *See also* Gastrointestinal tract infection
acid-base disorders in, 76
acute, 606
antibiotic-associated, 599. *See also* Colitis, antibiotic-associated
in *Bacillus cereus* infection, 1319
bacterial infection and, 602t-603t, 607t, 608-613, 610t, 616t, 622, 623t
in *Balantidium coli* infection, 2895
Diarrhea *(Continued)*
Brainerd, 602
chronic, 606
in *Cryptosporidium* spp. infection, 2901
in daycare facilities, 599
dehydration in, 76, 620-622, 620t-621t
diagnostic evaluation of, 616-619, 616t, 620f
differential diagnosis of, 636-637
dysenteric, 606, 622-624. *See also Shigella* spp. infection (shigellosis)
in *Entamoeba histolytica* infection, 2870
in enteroviral infection, 2069-2070
in *Escherichia coli* infection. *See Escherichia coli* infection, diarrheogenic
in food poisoning, 601-606, 602t-605t
health care–related, 636-637. *See also* Colitis, antibiotic-associated
in immunosuppressed host, 599-600, 600t
inflammatory, 606
neonatal, 887-888
noninflammatory, 606
oral immunoglobulin in, 3498
parasitic infection and, 607t, **613-616**, 616t, 625
probiotics in, 3370
prolonged, 606
in rotavirus infection, 2183-2185
traveler's, 600-601, 1451, 1463, **3110-3115.** *See also* Enterotoxigenic *Escherichia coli* (ETEC) infection
epidemiology of, 3110-3112
management of, 3113-3115
prevention of, 3112-3113
treatment of, 619-626, 620t-621t, 623t
viral infection and, 606-608, 607t, 608f, 616t
in waterborne disease, 606
watery, 606
in Whipple disease, 643
Diazepam, in tetanus, 1816
Diazo reactants, urinary, 70
DIC. *See* Disseminated intravascular coagulation (DIC)
Dicloxacillin, 3183t-3184t, 3185-3186, 3231t-3233t
in cervical lymphadenitis, 186
Didanosine, 2612
Dideoxyinosine, pancreatitis with, 692
Dientamoeba fragilis, 2884, 2885f
Dientamoeba fragilis infection (dientamoebiasis), **2884-2887**
clinical manifestations of, 2885
diagnosis of, 2885-2886
epidemiology of, 2884-2885
treatment of, 2886, 2886t
Diet
infection and, 109
intestinal flora and, 89-90, 93
in pancreatitis, 695-696
Diethylcarbamazine citrate, 3348
Diffuse infiltrative lymphocytosis syndrome, 189, 191
Diffusely adherent *Escherichia coli* (DAEC) infection, 610t, 611, 1449
clinical manifestations of, 1450t, 1455
diagnosis of, 1461
epidemiology of, 1450t, 1453
pathogenesis of, 1460
prevention of, 1463-1464
treatment of, 1461-1463
DiGeorge syndrome, 48, 904-905, 907, **912-913**
clinical features of, 905f, 912
diagnosis of, 912-913
DiGeorge syndrome *(Continued)*
pathogenesis of, 907t, 912
treatment of, 913
Digital thermometer, 85
Digitalis, in myocarditis, 395
Diloxanide furoate, 3348
Diphtheria. *See Corynebacterium diphtheriae* infection
Diphyllobothrium spp. (fish tapeworm) infection, **3040-3041**, 3327t-3346t
Diplopia, in Whipple disease, 644
Dipylidium caninum (dog tapeworm) infection, **3041**, 3327t-3346t
Direct fluorescent antibody (DFA) test, 3606t-3607t, 3609-3610
in influenza virus infection, 2345
Dirofilaria immitis infection (dirofilariasis), **3028**
Disease
definition of, 112
epidemiology of. *See* Epidemiology
vs. infection, 103-104, 111
Diskitis, 720-721, 721f
Dispersin, 1460
Disseminated intravascular coagulation (DIC)
in influenza virus infection, 2345
in *Neisseria meningitidis* infection, 1258
phospholipids and, 75
in septic shock, 829
Diuretics, pancreatitis with, 691
Dobutamine, in myocarditis, 395
Dog, *Leptospira* infection of, 1744-1745, 1757-1758
Dog bite, 780-781, 780t. *See also* Rabies
clinical manifestations of, 3572-3575, 3574f
diagnosis of, 3575-3577
epidemiology of, 3570-3571
Erysipelothrix rhusiopathiae infection with, 1328
microbiology of, 3571-3572, 3571t-3572t
Pasteurella multocida infection with, 1547, 1567, 3574
polymicrobial, 1829
prevention of, 3578
treatment of, 3251, 3575-3577, 3576t
Dog tapeworm *(Dipylidium caninum)* infection, **3041**, 3327t-3346t
Donovan bodies, 1642, 1642f
Donovanosis. *See* Granuloma inguinale
Dopamine, in myocarditis, 395
Doripenem, 3238
Doxycycline, 3217-3220, 3218t, 3231t-3233t
in epidemic typhus, 2658-2659
in lymphogranuloma venereum, 584
in malaria, 2942
in malaria prevention, 2944t, 2945
in murine typhus, **2659-2660**
in Rocky Mountain spotted fever, 2654
in scrub typhus, 2661
Dracunculus medinensis infection, **3028**, 3327t-3346t
DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) syndrome, 657
Dressing, burn wound, 1057-1059
Drug(s), **3159-3181.** *See also* Antibiotic(s); Antifungal agents; Antiparasitic agents; Antiviral agents *and specific drugs and drug classes*
absolute bioavailability (F) of, 3160-3161
absorption of, 3161, 3161f
ontogeny effects on, 3164, 3165t
bioequivalence of, 3161

Drug(s) *(Continued)*
biopharmaceutics of, 3161
biotransformation of
ontogeny effects on, 3166-3167, 3168t
phase I pathways in, 3167, 3168t
phase II pathways in, 3167-3169, 3168t
cholestasis with, 662
clearance (Cl) of, 3161
compartment models of, 3161-3162
diffusion of, 3235
disposition of, 3162
distribution of, 3163
ontogeny effects on, 3164-3165, 3165f
dose-response curve of, 3162, 3162f
efficacy of, 3170-3174
combination drug regimens and, 3174, 3180
concentration above minimal inhibitory concentration and, 3171f, 3172-3173
inoculum size and, 3179-3180
minimal inhibitory concentration and, 3170, 3170f
pharmacodynamic determinants of, 3169-3180
pharmacogenetic determinants of, 3181
pharmacokinetic determinants of, 3169
subinhibitory concentrations and, 3175-3178
suprainhibitory concentrations and, 3174-3175, 3174f
time above minimal inhibitory concentration and, 3170-3172, 3171f
total-body exposure above minimal inhibitory concentration and, 3173-3174
elimination half-life of, 3162
ontogeny effects on, 3166, 3166t
fever of unknown origin with, 846
first-pass effect of, 3162
hepatitis with, 651t, 657
normal flora and, 89-90
pancreatitis with, 691-692, 691t, 696
pharmacokinetic-pharmacodynamic interface of, 3160-3163, 3166t
ontogeny effects on, 3163-3167, 3165f-3166f, 3165t-3166t, 3168t
postexposure effects of, 3178-3179
protein binding of, 3162
ontogeny effects on, 3165, 3166t
relative bioavailability of, 3162
renal excretion of, 3236
ontogeny effects on, 3165-3166, 3166f
steady state of, 3162
urethral symptoms with, 540
volume of distribution of, 3163
Drug abuse
endocarditis and, 352, 357
HAV infection and, 2135
tetanus and, 1814
Dubin-Johnson syndrome, 657
Dust, in hypersensitivity pneumonitis, 301, 302t-303t
Dust mites, Kawasaki disease and, 1068
Dwarf tapeworm *(Hymenolepis nana)* infection, **3042**, 3327t-3346t
Dysentery, 606, 622-624, 1476. *See also Shigella* spp. infection (shigellosis)
Dyspnea, in community-acquired pneumonia, 287
Dysrhythmias
in myocarditis, 389, 397
in pericarditis, 376
Dystonia, group A β-streptococcal infection and, 516
α-Dystroglycan, 2473
Dystrophin, in myocarditis, 387

E

E-test, in pneumococcal disease, 1218
Eagle effect, 3174-3175, 3174f
Ear(s)
external, 204
dermatologic disease of, 208
furunculosis of, 208
herpes zoster infection of, 208, 208f
inflammation of. *See* Otitis externa
normal flora of, 204-205
relapsing polychondritis of, 208
middle
effusion of, 209-210, 218-220. *See also* Otitis media with effusion
inflammation of. *See* Otitis media
tuberculosis of, 1357
pain in
in otitis externa, 205, 208
in otitis media, 217
in rhinosinusitis, 198
tropical. *See* Otitis externa
Eastern equine encephalitis, 494t-498t, **2226-2230**
age and, 2227
clinical manifestations of, 2227-2228
diagnosis of, 2228-2229
differential diagnosis of, 2229
ecology of, 2226
epidemiology of, 2226-2227, 2227f
pathogenesis of, 2228
pathology of, 2228
prevention of, 2229
prognosis for, 2228
sequelae of, 2228
treatment of, 2229
Eastern equine encephalitis virus, 2226
Ebola virus, 2478, 2479f
Ebola virus infection, **2479-2486**
bioterrorist, 3585
clinical manifestations of, 2483
diagnosis of, 2484
differential diagnosis of, 2483-2484
epidemiology of, 2479-2482, 2480t, 2481f
exanthem in, 742t-748t, 749
nosocomial, 3401-3402
pathology of, 2483
prevention of, 2485, 3534
treatment of, 2484-2485
EBV. *See* Epstein-Barr virus (EBV)
EBV-encoded RNA (EBER), 1993-1994
EBV nuclear antigen (EBNA), 1993-1994
Echinacea, in common cold, 138
Echinocandins, 3312-3319
adverse effects of, 3314-3315
in cancer patient, 928
drug interactions with, 3315
in hematopoietic stem cell transplantation–related aspergillosis, 938
in lung transplant–related aspergillosis, 968
in lung transplant–related candidiasis, 971
mechanism of action of, 3312-3313, 3313f
pharmacodynamics of, 3314
pharmacokinetics of, 3314, 3315t
resistance to, 3314
spectrum of activity of, 3313-3314
Echinococcus granulosus, 3044
Echinococcus granulosus infection (echinococcosis, hydatid disease), **3044-3047**
clinical manifestations of, 660, 757t-758t, 3044-3045
diagnosis of, 3045, 3046f
epidemiology of, 3044
myocardial, 400
ocular, 815
orbital, 801
PAIR treatment of, 3046-3047
pathogenesis of, 3044
prevention of, 3047
pulmonary, 3045, 3047
surgical treatment of, 3047
transmission of, 3044
treatment of, 3046-3047, 3327t-3346t
Echinococcus multilocularis infection, 660, 757t-758t, 3047
Echinococcus vogeli infection, 3047
Echocardiography
in endocarditis, 359-360
in Kawasaki disease, 1074-1075, 1080-1081
in myocarditis, 387, 388f, 392-393
in pacemaker-related infection, 1024
in pericarditis, 376-377, 376f
in rheumatic fever, 409, 411
Echovirus
antigenic characteristics of, 2056
classification of, 2052-2053, 2052t
host range of, 2056
prevalence of, 2057-2059, 2058t
Echovirus infection, **2051-2109**
asymptomatic, 2063-2064, 2063t
bronchial, 2066
in bronchiolitis, 2066
cerebellar ataxia and, 2088t, 2090
clinical manifestations of, 2063-2090
congenital, 2091-2092
conjunctival, 2073-2074, 2074t
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
exanthem in, 2078-2086, 2080t-2081t, 2083t
gastrointestinal, 2068-2073, 2069t, 2071t-2072t
Guillain-Barré syndrome and, 2088t, 2090-2097
hemolytic-uremic syndrome in, 2078
hepatic, 2070-2072
herpangina in, 2066t
historical perspective on, 2051
in immunocompromised host, 2091
joint, 2078
meningeal, 485-487, 2086-2090, 2088t-2089t
muscle, 2078
myocardial, 2075-2077, 2076t
neonatal, 2092-2097, 2094t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t
ocular, 2074-2075
oral cavity, 2066
pathology of, 2062
pericardial, 2075-2077, 2076t
peritoneal, 2070
pharyngeal, 2064t, 2065
photophobia in, 2074
pulmonary, 2067
stillbirth and, 2092
treatment of, 2103-2104
Echovirus 1 infection, 2080t-2081t, 2084

Echovirus 2 infection, 2080t-2081t, 2084
Echovirus 3 infection, 2080t-2081t, 2084
Echovirus 4 infection, 742t-748t, 749, 760, 2080t-2081t, 2084
Echovirus 5 infection, 2080t-2081t, 2084
Echovirus 6 infection, 742t-748t, 749, 2077-2078, 2080t-2081t, 2084
Echovirus 7 infection, 2080t-2081t, 2084
Echovirus 9 infection
  exanthem in, 742t-748t, 749, 760, 2080t-2081t, 2084-2085, 2085f, 2085t
  hepatic, 655
  oral cavity, 2066
  pathology of, 2062
Echovirus 11 infection
  in croup, 2066
  cytopathic effect of, 2054f
  epidemiology of, 2061
  exanthem in, 2080t-2081t, 2085
  neonatal, 2093
  oral cavity, 2066
  pathology of, 2062
Echovirus 13 infection, 2080t-2081t, 2085
Echovirus 14 infection, 2080t-2081t, 2085
Echovirus 16 infection, 760, 769, 2080t-2081t, 2085
Echovirus 17 infection, 2080t-2081t, 2085
Echovirus 18 infection, 2080t-2081t, 2085
Echovirus 19 infection, 2080t-2081t, 2085-2086
Echovirus 21 infection, 2080t-2081t, 2086
Echovirus 24 infection, 2080t-2081t, 2086
Echovirus 25 infection, 2080t-2081t, 2086
Echovirus 30 infection, 655, 2080t-2081t, 2086
Echovirus 32 infection, 2080t-2081t, 2086
Echovirus 33 infection, 2080t-2081t, 2086
ECMO. *See* Extracorporeal membrane oxygenation (ECMO)
Ecthyma contagiosum, 742t-748t
Ecthyma gangrenosum, **776**
  Aeromonas hydrophila, 1543
  Pseudomonas aeruginosa, 1590f, 1596
Eculizumab, in diarrheogenic *Escherichia coli* infection, 1463
Eczema herpeticum, 1943-1944, 1943f, 1953
Eczema vaccinatum, 2036, 2038-2039, 3532
Edema
  angioneurotic, 250t-251t, 253
  cerebral
    at high-altitude, 3118
    in meningitis, 439
  in cutaneous anthrax, 1303, 1313f
  infection-induced, 76
  palpebral
    in roseola infantum, 770
    in *Trypanosoma cruzi* infection, 2963
  pulmonary
    in epiglottitis-related intubation, 258
    at high-altitude, 3118
    in malaria, 2928
Edema factor, in anthrax, 1303
Edge Hill virus infection, 2316t
Edwardsiella tarda infection, **1532-1533**
Efavirenz, 2614
Effusion
  middle ear, 209-210, 218-220. *See also* Otitis media with effusion
  pericardial, 373-374, 376-377, 376f-377f
Eflornithine, 3348
  in *Trypanosoma brucei* infection, 2972-2973
Ehrlichia spp. infection (ehrlichiosis), 494t-498t, 751t-752t, **2661-2663**
Eicosanoids, 75
Eikenella corrodens, 1576
Eikenella corrodens infection, **1576-1578**
  clinical manifestations of, 1577
  diagnosis of, 1577
  epidemiology of, 1576
  in osteomyelitis, 725
  pathophysiology of, 1576-1577
  treatment of, 1577-1578
Electrical alternans, in pericarditis, 376
Electrocardiography (ECG)
  in endocardial fibroelastosis, 395
  in endocarditis, 359
  in infectious mononucleosis, 2002
  in Kawasaki disease, 1072, 1074-1075
  in *Leptospira* spp. infection, 1753
  in myocarditis, 389-392, 390f-392f, 394
  in pericarditis, 375-376
  in *Trypanosoma cruzi* infection, 2964-2965
  in visceral leishmaniasis, 2957
Electroencephalography (EEG)
  in brain abscess, 467-468
  in Creutzfeldt-Jakob disease, 2628
  in encephalitis, 507
  in HSV encephalitis, 1946-1947, 1947f
  in Japanese encephalitis virus infection in, 2296
  in La Crosse virus encephalitis, 2522
  in meningitis-related seizures, 455
  in St. Louis encephalitis virus infection, 2258
  in subacute sclerosing panencephalitis, 2389
  in tetanus, 1814
  in West Nile virus infection, 2270
Electrolytes, 75-76
Electromyography (EMG), in infant botulism, 1808
Electronic thermometer, 85
Electrophoresis
  HSV, 1957-1958, 1958f
  in meningitis, 442
  rotavirus, 2179, 2180f
  Staphylococcus aureus, 1119
Elek test, in *Corynebacterium diphtheriae* infection, 1307
Elizabethkingia spp., 1578-1579
Elizabethkingia spp. infection, **1578-1581**
  clinical manifestations of, 1579-1580
  diagnosis of, 1580
  epidemiology of, 1579
  pathophysiology of, 1579
  treatment of, 1580-1581
Embolism, in infective endocarditis, 355
Empyema
  anaerobic bacteria in, 1830
  in neonate, 892
  pulmonary, **306-315**
    diagnosis of, 310-313, 311f-312f
    epidemiology of, 307
    management of, 313-315
    microbiology of, 309-310, 309t
    pathophysiology of, 307-308, 308t
    prognosis for, 315
  subdural, **469-470**
    clinical manifestations of, 469
    CSF examination in, 443t, 469
    diagnosis of, 469, 470f
    treatment of, 470
Emtricitabine, 2613
Encephalitis, **492-512**
  adenovirus, 494t-498t, 1896t, 1903
  amebic, 494t-498t, 501
    Acanthamoeba spp., 494t-498t, 501, 2975, 2979, 2981, 2984-2985
    Balamuthia mandrillaris, 494t-498t, 501, 511, 2975, 2980-2981, 2984-2985
Encephalitis *(Continued)*
  anti-NMDAR, 509
  arbovirus, 494t-498t, 499-500, 510
  bacterial, 494t-498t, 501
  Banna virus, 2175
  brain stem, 505
  chronic, 503
  clinical manifestations of, 504-505
  CMV, 1979
  coxsackievirus B4, 493-499, 494t-498t, 2061, 2062f
  cysticercal, 3034, 3038
  diagnosis of, 507-509, 508t
  differential diagnosis of, 505-506
  Eastern equine, **2226-2230**, 2227f
  EBV, 2003-2004, 2011
  echovirus, 2088t, 2090
  EEG in, 507
  enterovirus, 493-499, 494t-498t
  enterovirus 71, 2062
  epidemiology of, 503
  etiology of, 493-503, 494t-498t
  evaluation of, 506-507
  fungal, 494t-498t, 501
  herpesvirus, 494t-498t, 499
  historical perspective on, 493
  hMPV, 494t-498t, 2439
  HSV, 494t-498t, 499, 505, 1945-1948, 1946t, 1949t
    acute retinal necrosis after, 1945
    clinical manifestations of, 1946, 1946t
    diagnosis of, 1946-1947, 1947f-1948f
    differential diagnosis of, 1948, 1949t
    vs. HSV meningitis, 1946
    pathogenesis of, 1939, 1946
    sequelae of, 1958
    vs. tick-borne encephalitis virus infection, 2310-2311
    treatment of, 1960
  human erythrovirus B19 (human parvovirus B19), 1853
  imaging in, 506-507
  influenza virus, 2352
  Japanese, 494t-498t, **2291-2301.** *See also* Japanese encephalitis virus infection
  La Crosse virus, 494t-498t, 500, 510, **2513-2527.** *See also* La Crosse virus (LACV) infection
  LCMV, 494t-498t, 500
  limbic, HHV-6 infection and, 2018
  measles virus, 494t-498t, 500, 2385-2386, 2391-2392
  mumps virus, 494t-498t, 500, 2401
  Murray Valley, **2301-2303**
  Mycoplasma pneumoniae, 494t-498t, 501-502, 2683-2684
  paramyxovirus, 494t-498t, 500-501
  parasite, 494t-498t, 501
  parechovirus, 493-499, 494t-498t
  pathogenesis of, 503-504
  pathology of, 504
  postimmunization, 502
  postinfectious, 493, **503**
    imaging in, 507
    pathogenesis of, 504
    pathology of, 504
  postvaccinial, 2038-2039
  prevention of, 511
  primary, 493
  prognosis for, 510-511
  reovirus, 2171
  rhabdovirus, 500-501
  Rocio virus, **2314-2315**
  Rocky Mountain spotted fever, 494t-498t, 501

Encephalitis *(Continued)*
in roseola infantum, 771
rotavirus, 2186
rubella virus, 494t-498t, 500, 2208, 2212
St. Louis encephalitis virus, **2253-2260.** *See also* St. Louis encephalitis virus infection
seizures in, 509-510
tick-borne, **2303-2313**, 2304f, 2306f. *See also* Tick-borne encephalitis virus infection
treatment of, 509-510
tuberculous, 494t-498t, 504
Venezuelan equine, **2237-2241**, 2238f, 2238t. *See also* Venezuelan equine encephalitis
viral, 493-501, 494t-498t
West Nile virus, **2261-2273.** *See also* West Nile virus infection
Western equine, **2230-2237**, 2231f-2233f, 2233t. *See also* Western equine encephalitis
Encephalitozoon bieneusi infection, 625
Encephalitozoon intestinalis infection, 625
Encephalomyelitis
disseminated, acute. *See* Acute disseminated encephalomyelitis (ADEM)
postherpetic, 1948
Encephalopathy, 493
in *Bacillus cereus* infection, 677
in *Bordetella pertussis* infection, 1623
DEET-related, 2235
evaluation of, 506-507
hepatic, probiotics in, 3371
HIV-related, 2597-2598, 2597f, 2604-2605
hMPV, 2439
influenza-associated, 502, 2343, 2352
in malaria, 2928
MMR-related, 2393-2394
pertussis-associated, 502
in Reye syndrome, 676-677
in septic shock, 830
toxic, in *Shigella* spp. infection, 1481
Endocardial fibroelastosis, vs. myocarditis, 395
Endocarditis. *See* Infective endocarditis
Endocrine disorders, CMV infection and, 1980-1981
Endometritis, **587**
Endophthalmitis, **820**
Aeromonas spp., 1543
Bacillus cereus, 1319
Candida, 813, 820, 936-937, 2743, 2749
neonatal, 895, 897, 2756
Coccidioides spp., 813-814
Cryptococcus spp., 814
Histoplasma capsulatum, 2816-2817
Morganella morganii, 1470
Plesiomonas shigelloides, 1566
Pseudomonas aeruginosa, 1591, 1599
Staphylococcus epidermidis in, 1138
Stenotrophomonas maltophilia, 1607
Endoscopic retrograde cholangiopancreatography (ERCP)
in cholangitis, 661-662
cholangitis after, 667
in pancreatitis, 690
Endoscopic sinus surgery, in rhinosinusitis, 201-202
Endoscopy
in *Aspergillus* spp. infection, 2706, 2707f
in *Candida* infection, 2739
Endoscopy *(Continued)*
in *Helicobacter pylori* infection, 1695, 1695f
in Whipple disease, 645
Endothelium, in phagocyte recruitment, 31-32
Endotoxin. *See* Lipopolysaccharide (LPS)
Enfuvirtide, 2611
Entamoeba coli infection, **2877-2878**
Entamoeba dispar, 2867
Entamoeba histolytica, 2867, 2868f
vs. *Entamoeba coli*, 2877
Entamoeba histolytica infection (amebiasis), 607t, 614, 662, **2866-2874**
ameboma in, 2869-2870
asymptomatic, 2873
biopsy in, 2872
cerebral, 2869, 2871
clinical manifestations of, 757t-758t, 2870-2871
colitis in, 2870, 2873
complications of, 2872-2873
cutaneous, 2871
diagnosis of, 2871-2872
diarrhea in, 2870
differential diagnosis of, 2870-2872
epidemiology of, 2867-2868, 2868t
extraintestinal, 2870-2871
diagnosis of, 2871-2872
treatment of, 2873-2874, 2873t
hepatic, 2869-2873
treatment of, 2873-2874
immune response to, 2869-2870
intestinal, 2870
treatment of, 2873, 2873t
metastatic, 2871
treatment of, 2873-2874, 2873t
ocular manifestations of, 814
pathogenesis of, 2868-2869, 2868f
pericardial, 2871
pleural, 309-310, 2869
prognosis for, 2874
vs. *Shigella* spp. infection, 1483
stool examination in, 2871
thoracic, 2871
treatment of, 625, 2873-2874, 2873t, 3327t-3346t
vulvovaginal, 569
Entecavir, in HBV infection, 1922-1923
Enteritis necroticans, 1797
Enteroaggregative *Escherichia coli* (EAEC) infection, 610t, 611, 1449
clinical manifestations of, 1450t, 1455
diagnosis of, 1460-1461, 1461f
epidemiology of, 1450t, 1453
pathogenesis of, 1460
prevention of, 1463-1464
prognosis for, 1461
treatment of, 1461-1463
Enterobacter spp., 1434
Enterobacter spp. infection, **1433-1439**
antibiotic-resistant, 1435, 1437
biliary, 659-660, 659t
bloodstream, 1436
clinical manifestations of, 1436
diagnosis of, 1436-1437
endocardial, 362
epidemiology of, 1434-1435
hospital-acquired, 1434
meningeal, 1435-1436
pathophysiology of, 1435-1436
risk factors for, 1434-1435
in septic shock, 834
treatment of, 1437-1438
Enterobactin, 538
Enterobius vermicularis infection (enterobiasis), 566-567, 566f, **3018-3019**
appendiceal, 684, 3019
bladder, 540
clinical manifestations of, 757t-758t, 3019
diagnosis of, 566, 566f, 3019
epidemiology of, 3018
pathophysiology of, 3019
treatment of, 3019, 3327t-3346t
vaginal, 3019
Enterococcus spp., 1170-1172, 1171f, 1172t
adherence properties of, 1173-1174
pili of, 5-6
typical vs. atypical, 1171, 1172t
virulence of, 1173-1174
Enterococcus spp. infection, **1170-1187**
bloodstream, 1174-1177
burn wound, 1052, 1052t
clinical manifestations of, 1174-1177
diagnosis of, 1177
endocardial, 361, 366, 366t-367t, 1174-1175
epidemiology of, 1172-1173
intra-abdominal, 1175
joint, 1177
meningeal, 1175-1176
neonatal, 882, 1176-1177
nosocomial, 1172, 1174, 3399-3400
prevention of, 1186
pathogenesis of, 1173-1174
peritoneal, 700
prevention of, 1185-1186
treatment of, 1182-1185
in antibiotic-resistant infection, 1183-1185
in antibiotic-susceptible infection, 1182-1183
resistance in, 1177-1182
acquired, 1178-1181, 1179t
aminoglycoside, 1178-1180
daptomycin, 1181
intrinsic, 1177-1178
β-lactam, 1177, 1179t, 1180
linezolid, 1181
prevention of, 1185-1186
quinupristin-dalfopristin, 1181
teicoplanin, 1180
testing for, 1181-1182
TMP-SMX, 1178
vancomycin, 1179t, 1180-1181
urinary tract, 1173-1174
vancomycin-resistant, 1170, 1179t, 1180-1181, 3151-3152
epidemiology of, 1172-1173
nosocomial, 3399-3400
prevention of, 1185-1186
treatment of, 1183-1185, 1185t
Enterocolitis. *See also* Gastrointestinal tract infection
necrotizing
Candida, 895-897
Clostridium difficile, 635
Cronobacter sakazakii, 1436
Klebsiella spp., 474
oral immunoglobulin in, 3498
platelet-activating factor and, 3366-3367
prevention of, 3243-3244
probiotics in, 3371-3372
Yersinia enterocolitica, 1526-1527
Enterohemorrhagic *Escherichia coli* (EHEC)
intimin of, 7-8, 1457
Shiga toxin of, 17, 1457-1459

Enterohemorrhagic *Escherichia coli* (EHEC) infection, 609-610, 610t, 616t, 619, 1449. *See also Escherichia coli* O157:H7 infection
  clinical manifestations of, 1450t, 1454-1455
  diagnosis of, 1460-1461
  epidemiology of, 1450t, 1451-1452
  food sources of, 1452
  hemolytic-uremic syndrome with, 17, 1452, 1454, 1463
  hemorrhagic colitis with, 1454
  pathogenesis of, 1457-1459
  prevention of, 1463-1464
  prognosis for, 1461
  vs. *Shigella* spp. infection, 1483
  treatment of, 624, 1461-1463
Enteroinvasive *Escherichia coli* (EIEC) infection, 602t-603t, 610t, 611, 1449
  clinical manifestations of, 1450t, 1455
  diagnosis of, 1460-1461
  epidemiology of, 1450t, 1452-1453
  pathogenesis of, 1459-1460, 1459f
  prevention of, 1463-1464
  prognosis for, 1461
  treatment of, 1461-1463
Enteropathogenic *Escherichia coli* (EPEC)
  attaching and effacing lesion of, 7-8, 9f
  atypical, 1451
  intimin of, 7-8, 9f
  locus of enterocyte effacement of, 7-8
  pili of, 5
  serotypes of, 1450t, 1451
Enteropathogenic *Escherichia coli* (EPEC) infection, 602t-603t, 610-611, 610t, 611f, 1449
  clinical manifestations of, 1450t, 1453-1454
  diagnosis of, 1460-1461
  epidemiology of, 1450t, 1451
  in neonate, 888
  pathogenesis of, 1457, 1457f-1458f
  prevention of, 1463-1464
  prognosis for, 1461
  treatment of, 1461-1463
Enterotoxigenic *Escherichia coli* (ETEC) infection, 602t-603t, 610t, 611, 1449
  clinical manifestations of, 1450t, 1453
  diagnosis of, 1460-1461
  epidemiology of, 1449-1451, 1450t
  pathogenesis of, 1455-1457, 1456f
  prevention of, 1463-1464
  prognosis for, 1461
  treatment of, 1461-1463
  vaccine against, 628, 1463-1464
Enterotoxins
  Aeromonas spp., 1540
  detection of, 1460-1461
  enteroaggregative *Escherichia coli*, 1460
  enterotoxigenic *Escherichia coli*, 1455-1457
  rotavirus, 2184
  Shigella spp., 1479
  Staphylococcal aureus, 862-863, 1117-1118
  Vibrio cholerae, 1550
  Yersinia enterocolitica, 1525
Enterovirus. *See also* Coxsackievirus; Echovirus; Poliovirus; Rhinovirus
  antigenic characteristics of, 2056
  classification of, 2052-2053, 2053t
  historical perspective on, 2051-2052
  host range of, 2056
  replication of, 2053-2054, 2053t
Enterovirus infection, **2051-2109.** *See also specific enterovirus infections*
  acute lymphonodular pharyngitis in, 2066
  asymptomatic, 2063-2064, 2063t
Enterovirus infection *(Continued)*
  bronchial, 2066
  cardiovascular manifestations of, 2075-2077, 2076t
  chronic fatigue syndrome and, 2091
  clinical manifestations of, 2063-2090, 2063t-2064t, 2066t, 2068t-2069t, 2071t-2072t, 2074t, 2076t
  in common cold, 2064-2065, 2064t
  congenital, 2091-2092
  conjunctival, 810, 2073-2075, 2074t
  in croup, 2066
  cytopathologic features of, 2054-2056, 2054f
  diabetes mellitus and, 2072-2073
  diagnosis of, 2101-2103
    clinical, 2101
    laboratory, 2101-2102
  differential diagnosis of, 2102-2103
  encephalitic, 493-499, 494t-498t, 2088t, 2090
  in epidemic pleurodynia, 2067-2068
  epidemiology of, 2056-2059
  in epididymitis, 2077
  exanthems in, 742t-748t, 749, 760, 762, 769, 2078-2086, 2079f, 2080t-2081t, 2082f-2083f, 2083t, 2085f, 2087t
  fibromyalgia and, 2091
  gastrointestinal manifestations of, 2068-2073, 2068t-2069t, 2071t-2072t
  genitourinary manifestations of, 2077-2078
  geographic features of, 2056-2057
  Guillain-Barré syndrome and, 2088t, 2090-2097
  hematologic manifestations of, 2078
  hemolytic-uremic syndrome in, 2078
  hepatic, **655**, 2070-2072
  herpangina in, 2065, 2066t
  historical perspective on, 2051-2052
  host factors in, 2060-2061
  in immunocompromised host, 2091
  intraoral manifestations of, 2065-2066, 2066t
  joint manifestations of, 2078
  meningeal, 485-487, 486t, 489, 2086-2090, 2088t-2089t
  muscle manifestations of, 2078
  myocardial, 384-387, 385f, 2075-2077, 2076t, 2104
  neonatal, 2092-2097, 2094t
    cardiovascular, 2096, 2096t
    clinical manifestations of, 2093-2097
    epidemiology of, 2092-2093
    exanthem in, 2097
    gastrointestinal, 2095-2096
    hepatic, 2096
    inapparent, 2093-2095
    IVIG in, 3513
    mild, 2095
    neurologic, 2097
    pancreatic, 2096
    pathogenesis of, 2092-2093
    respiratory, 2095
    sepsis-like, 2095
  neurologic manifestations of, 2086-2090, 2088t-2089t, 2104
  nonspecific febrile illness in, 2064, 2064t, 2066t, 2068t, 2103
  nosocomial, 3384
  ocular, 2073-2075, 2074t
  oral cavity, 2066
  in orchitis, 2077
  pancreatic, 2072
Enterovirus infection *(Continued)*
  parotid gland, 2066
  pathogenesis of, 2059-2063, 2059f-2060f
  pathology of, 2054-2056, 2055f, 2061, 2062f
  pericardial, 2076t
  pharyngeal, 2064t, 2065
  photophobia in, 2074
  prevalence of, 2057-2059, 2058t
  prevention of, 2105-2107
  prognosis for, 2104-2105
  pulmonary, 2067
  renal, 2077-2078
  respiratory manifestations of, 2064-2068, 2064t
  vs. Rocky Mountain spotted fever, 2653
  vs. St. Louis encephalitis virus infection, 2259
  stillbirth and, 2092
  sudden infant death and, 2091
  vs. tick-borne encephalitis virus infection, 2310-2311
  transmission of, 2056
  treatment of, 2103-2104
Enterovirus 68 infection, 2067
Enterovirus 70 infection, 810, 2073-2074
Enterovirus 71 infection
  cardiac, 2077
  CNS, 2062
  epidemiology of, 2057, 2061
  exanthem in, 764, 2080t-2081t, 2086
  paralytic, 2090
  pericardial, 2076t
Entomophthorales, 2837, 2838f
Entomophthorales infection (entomophthoromycosis), **2844-2845**
Environment, in epidemiologic study, 110-111, 114-116
Enzyme immunoassay (EIA)
  in *Bartonella* spp. infection, 1727
  in *Clostridium difficile* infection, 635, 636t
  in enterovirus infection, 2056
  in HIV infection, 2608, 2608t
  in *Norovirus* infection, 2153-2154
Enzyme-linked immunosorbent assay (ELISA), 3606t-3607t, 3610-3612
  in *Aspergillus* spp. infection, 2714
  in *Bordetella pertussis* infection, 1628-1629
  in *Brucella* spp. infection, 1614
  in *Campylobacter jejuni* infection, 1654-1655
  in Colorado tick fever virus infection, 2174-2175
  in *Corynebacterium diphtheriae* infection, 1307
  in *Cryptosporidium* spp. infection, 2902
  in dengue, 2285
  in Ebola virus infection, 2484
  in *Francisella tularensis* infection, 1663
  in *Giardia lamblia* infection, 2882
  in *Haemophilus ducreyi* infection, 1687-1688
  in HCV infection, 2321-2323
  in HEV infection, 2165
  in human erythrovirus B19 (human parvovirus B19) infection, 1856
  in influenza, 3610-3612, 3611t
  in Japanese encephalitis virus infection, 2298
  in LCMV infection, 2464-2465
  in *Leptospira* spp. infection, 1755
  in Lyme disease, 1735
  in mumps, 2403
  in *Mycoplasma pneumoniae* infection, 2685-2686

Enzyme-linked immunosorbent assay (ELISA) *(Continued)*
in *Paracoccidioides* spp. infection, 2791-2792
in relapsing fever, 1742
in Ross River virus arthritis, 2250
in rotavirus infection, 2183, 3611-3612
in RSV infection, 2424-2425, 3611, 3611t
in Toscana virus infection, 2549
in *Toxoplasma gondii* infection, 2994-2996, 2995t
in *Trypanosoma cruzi* infection, 2966
in Venezuelan equine encephalitis, 2240
in VZV infection, 2028
in West Nile virus infection, 2271
in Western equine encephalitis, 2235
Eosinophil(s)
in bronchiolitis, 277-278
CSF, 483
Eosinophilic meningitis. *See* Meningitis, eosinophilic
Eosinophilic pneumonia, acute, **297**
Ependymitis, in mumps meningitis, 2401
Epidemic pleurodynia, 739
Epidemiology, **95-102**
analytic study in, 95-96
case-control study in, 98-99, 98t-99t, 124t, 128
cohort study in, 97-99, 98t, 128
cross-sectional study in, 98-99
descriptive study in, 95
disease causation study in, 103-120. *See also* Infection, epidemiologic study of
experimental study in, 96-97
observational study in, 96-98, 128
study design in, 95-96
summary statistics in, 99-102, 100f. *See also* Biostatistics
validity in, 95
Epidermodysplasia verruciformis (Lewandowsky-Lutz dysplasia), 1871-1872, 1876
Epidermolysins, 1117
Epididymitis
enterovirus, 2077
after gonococcal urethritis, 1287
Epididymo-orchitis, 2401-2403
Epidural abscess
cerebral, 443t, **470**
spinal, **470-472**, 472f
clinical manifestations of, 471
diagnosis of, 443t, 471-472, 472f
treatment of, 472
Epiglottitis (supraglottitis), **241-260**, 243t
acute, 250t-251t
anatomy of, 248-249, 248f
clinical presentation of, 252-253
culture in, 254
diagnosis of, 253-254, 254f
epidemiology of, 246-247
etiology of, 244-245
genetic factors in, 249
Haemophilus influenzae type b, 1675-1676, 1679
vs. laryngotracheitis, 253-254
MSN erythrocyte antigens in, 249
necrotizing, 245
noninfectious, 245
pathophysiology of, 248-249
prevention of, 259
prognosis for, 259
radiography in, 254, 254f
tongue depressor examination in, 254
treatment of, 257-259
uvulitis and, 166
viral, 245
Epilepsy
fever of unknown origin in, 846
after pertussis immunization, 1634
Epinephrine
in animal serum–related anaphylaxis, 3493
in bronchiolitis, 279
in croup, 256-257
in parainfluenza virus infection, 2369-2370
plasma, 81-82
Epiphysis, osteomyelitis of, 719
Epithelium. *See also* Skin
barrier function of, 24-25
Epstein-Barr virus (EBV), 1992
antibodies against, 1997, 2007-2010, 2008t, 2009f, 2010t
cell transformation with, 1994
electron microscopy of, 2011
genome of, 1993
genotypes of, 1995
latency of, 1993-1994
nucleic acid testing for, 2010-2011
oropharyngeal shedding of, 1997-1998
replication of, 1993
structure of, 1993
tissue culture for, 2011
urinary shedding of, 1998
Epstein-Barr virus (EBV) infection, 158-161, **1992-2015.** *See also* Infectious mononucleosis
Arcanobacterium haemolyticum infection and, 1324
B-cell lymphoma and, 2013
Burkitt lymphoma and, 1996, 2005, 2005f
in burn patient, 1053
chronic, 2001, 2014
chronic fatigue syndrome and, 1086-1087
CNS, 1996, 2003-2004
corneal, 807
diagnosis of, 2007-2011, 2007f, 2008t, 3617
differential diagnosis of, 2011-2012
disseminated, 2000-2001
encephalitic, 494t-498t, 499, 505
epidemiology of, 1997-1998
exanthem in, 742t-748t, 749, 760
gastric carcinoma and, 2006
after heart transplantation, 952-953
after hematopoietic stem cell transplantation, 940-941
hemophagocytic lymphohistiocytosis and, 181, 1997, 2001, 2001f, 2014
hepatitis with, 654
histopathology of, 1996-1997
historical perspective on, 1992
HIV infection and, 2006-2007, 2014
Hodgkin disease and, 2005, 2013
immune response to, 2008-2010, 2009f, 2010t
immunopathogenesis of, 1995-1996
after intestinal transplantation, 997-998, 997t
after kidney transplantation, 1008-1009
after liver transplantation, 997-998, 997t
after lung transplantation, 976-978
lymphomatoid granulomatosis and, 2006
nasopharyngeal carcinoma and, 2005, 2013
pancreatitis with, 694
post-transplant lymphoproliferative disease and, 2005-2006, 2012-2013, 2015, 3521-3522
prevention of, 2014-2015, 3521-3522
prognosis for, 2014
Epstein-Barr virus (EBV) infection *(Continued)*
psychiatric features of, 2004
pyothorax-associated lymphoma and, 2006
rhinosinusitis with, 197
silent, 1998-1999
T-cell lymphoma and, 2006
transmission of, 1998
treatment of, 2012-2014
tumor-associated, 2005-2006, 2005f
histopathology of, 1996-1997
immunopathogenesis of, 1995-1996
treatment of, 2012-2013
uveal, 810
vaccine against, 2015
X-linked lymphoproliferative syndrome and, 2000-2001, 2013-2014
Equine antitoxin, in tetanus, 1815-1816
Errors, statistical, 121
Ertapenem, 3202, 3231t-3233t
Erwinia spp., 1535. *See also Pantoea agglomerans*
Erysipelas, 753t-756t, **776**, 1143
Erysipelothrix rhusiopathiae, 1327
Erysipelothrix rhusiopathiae infection, 753t-756t, **1327-1329**
clinical manifestations of, 1327-1328
cutaneous manifestations of, 1327-1328
diagnosis of, 1328
endocardial, 362-363
epidemiology of, 1327
pathophysiology of, 1327
treatment of, 1328-1329
Erythema chronicum migrans, 753t-756t
Erythema infectiosum, 742t-748t, 760
clinical manifestations of, 1847-1849, 1847f-1849f
diagnosis of, 1855-1856
differential diagnosis of, 1855
epidemiology of, 1845-1846
joint manifestations of, 1849, 1851
neurologic illness in, 1853
pathogenesis of, 1846-1847, 1846f
during pregnancy, 1852-1853
Erythema marginatum, in rheumatic fever, 409-410, 410f
Erythema migrans, 753t-756t, 1731f, 1732, 1734-1735
Erythema multiforme, **764**, 765t. *See also* Stevens-Johnson syndrome
in *Coccidioides* spp. infection, 2772-2773
HSV infection and, 764, 1951
urethritis and, 531
Erythema multiforme bullosum, in human erythrovirus B19 (human parvovirus B19) infection, 1849
Erythema nodosum, **764**, 765t, 1354-1355
in *Coccidioides* spp. infection, 2772-2773
after *Yersinia enterocolitica* infection, 1528-1529
Erythema nodosum leprosum, 1404-1405, 1404f
treatment of, 1409
Erythrocyte sedimentation rate (ESR)
in fever of unknown origin, 842
in infective endocarditis, 358, 358t
in Kawasaki disease, 1075-1076
in mastoiditis, 238
in *Mycoplasma pneumoniae* infection, 2678
in occult bacteremia, 838
in osteomyelitis, 719
in rheumatic fever, 410-411
in rhinosinusitis, 198
in sepsis neonatorum, 883

Erythromycin, 3211-3215, 3213t, 3231t-3233t
in chronic bronchitis, 269-270
drug interactions with, 3215
in heart transplant–related *Legionella pneumophila* infection, 949
in lymphogranuloma venereum, 584
in odontogenic infection, 145
topical
in inclusion conjunctivitis, 804
in neonatal conjunctivitis prevention, 805-806, 1281
in trachoma, 805
Erythromycin ethylsuccinate–sulfisoxazole acetyl, 3229
Erythrovirus infection. *See* Human erythrovirus B19 (human parvovirus B19) infection
Escherichia coli, 1439, 1440t
vs. *Entamoeba histolytica*, 2867
molecular tests on, 619, 620f
in toxic shock syndrome, 853
virulence factors of, 1443t, 1450t, 1460
Escherichia coli infection
appendiceal, 684. *See also* Appendicitis
biliary, 659-660, 659t, 663, 1445-1446
blood-brain barrier in, 432-433, 433t, 1443t, 1444
bloodstream, 432, 432t. *See also* Meningitis, bacterial
clinical manifestations of, 1444
epidemiology of, 1441
pathogenesis of, 1443-1444
treatment of, 1447
bone, 1441
CSF shunt, 1030, 1030t
diarrheogenic, 607t, **609-611**, 610t, 616t, **1449-1465.** *See also* Enteroaggregative *Escherichia coli* (EAEC) infection; Enterohemorrhagic *Escherichia coli* (EHEC) infection; Enteroinvasive *Escherichia coli* (EIEC) infection; Enteropathogenic *Escherichia coli* (EPEC) infection; Enterotoxigenic *Escherichia coli* (ETEC) infection
clinical manifestations of, 1453-1455
diagnosis of, 610t, 617, 619, 620f, 1460-1461, 1461f
epidemiology of, 1449-1453
pathogenesis of, 1455-1460, 1456f-1459f
prevention of, 1463-1464
prognosis for, 1461
transmission of, 1449-1453
treatment of, 623t, 624-625, 1461-1463
endocardial, 362, 1441
extraintestinal, **1439-1448**, 1440t
clinical manifestations of, 1444-1445
diagnosis of, 1445-1446
epidemiology of, 1439-1441
pathogenesis of, 1441-1444, 1442f, 1443t
prognosis for, 1447
treatment of, 1444t, 1446-1447
hepatic, 673
intra-abdominal, 1445
after lung transplantation, 962
meningeal, 435
clinical manifestations of, 1444-1445
diagnosis of, 1446
epidemiology of, 1441
pathogenesis of, 1444
peritoneal, 700, 1441
clinical manifestations of, 1445
Escherichia coli infection *(Continued)*
pulmonary, 1441
clinical manifestations of, 1445
diagnosis of, 1446
retroperitoneal, 706-709
in septic shock, 834
urinary tract, **537-538.** *See also* Urinary tract infection (UTI)
clinical manifestations of, 1444
diagnosis of, 1445-1446
epidemiology of, 1440-1441, 1440t
outcomes of, 1447
pathogenesis of, 1441-1443, 1442f
treatment of, 1444t, 1446-1447
uropathogenic. *See* Uropathogenic *Escherichia coli* (UPEC)
Escherichia coli O104:H4 infection, 1455
Escherichia coli O157:H7 infection, 624. *See also* Enterohemorrhagic *Escherichia coli* (EHEC) infection
in daycare facilities, 3558
diagnosis of, 1460
foodborne, 1452
hemolytic-uremic syndrome with, 1452, 1454, 1463
hemorrhagic colitis with, 1454-1455
Shiga toxin in, 17, 619
Esophagitis, **593-597**
bacterial, 593, 597
barium esophagography in, 594-595, 595f
Candida, 593-597, 595f-596f, 2739, 2747
clinical manifestations of, 593-594, 594t
CMV, 594-597, 596f
diagnosis of, 594-596
differential diagnosis of, 594
esophagoscopy in, 595-596, 595f-596f
etiology of, 593
fungal, 593, 597
in gastroesophageal reflux, 594
HSV, 594-597, 596f, 1948-1949, 1953
after kidney transplantation, 1006
pathophysiology of, 593
pill, 594
prognosis for, 597
treatment of, 596-597
viral, 596f, 597
Esophagography, 594-595, 595f
Esophagoscopy, 595-596, 595f-596f
Esophagus
enlargement of, in *Trypanosoma cruzi* infection, 2965
perforation of, mediastinitis with, 419-420
Estrogen, *Trichomonas vaginalis* infection and, 2889
Ethambutol, in tuberculosis, 1367t, 1368-1369
Ethics, in clinical trials, 97
Ethionamide, in tuberculosis, 1367t, 1369
Ethnicity
in epidemiologic study, 108, 113-114
in *Streptococcus pneumoniae* infection, 1203
Etravirine, 2614
European brown hare syndrome virus, 2155
Eustachian tube, in otitis media, 216, 216f
Evaporation, 84
Exanthem, **741-768.** *See also at specific infections*
blood-mediated, 750
diagnosis of, 766-767, 766t
differential diagnosis of, 766-767
epidemiology of, 750
etiology of, 741-750
arthropod, 758t, 763t-764t
bacterial, 753t-756t, 760t-766t
chlamydial, 751t-752t
Exanthem *(Continued)*
fungal, 757t
helminthic, 757t-758t, 761t, 763t
mycoplasmal, 751t-752t
protozoan, 757t-758t, 761t, 763t
rickettsial, 751t-752t, 760t-763t
viral, 742t-748t, 760t-766t, 782
historical perspective on, 741
immune-mediated, 750
in immunocompromised host, 766
macular, erythematous, 759-760, 760t
maculopapular, erythematous, 760-762, 761t
in meningitis, 765, 766t
nodular, 764, 764t
papular, 764, 764t
pathology of, 750-759
pathophysiology of, 750-759
petechial, 762-763, 763t. *See also* Petechiae
purpuric, 762-763, 763t. *See also* Purpura
in respiratory disease, 766, 766t
in toxic shock syndrome, 858
toxin-mediated, 750
ulcerative, 764, 764t. *See also* Ulcer(s)
urticarial, 763-764, 763t
vesicular, 762, 762t
viral, periflexural, **784**, 784f
Exanthem criticum. *See* Roseola infantum
Exanthem subitum. *See* Roseola infantum
Exchange transfusion, in septic shock, 836
Exfoliatins, 1117
Exophiala jeanselmei infection, after lung transplantation, 974
Exotic pet bite, 3578
External auditory canal, 204
inflammation of. *See* Otitis externa
Extracorporeal membrane oxygenation (ECMO)
antibiotic prophylaxis with, 1023
in ARDS, 873-874
Candida spp. infection and, 2751
infection with, 1022-1023
in septic shock, 836
Extrinsic allergic alveolitis. *See* Hypersensitivity pneumonitis
Eyach virus infection, vs. Colorado tick fever virus infection, 2174
Eye(s), **794-822.** *See also* Eyelid infection
Corynebacterium diphtheriae infection effects on, 1306
examination of
in infant botulism, 1806, 1806f, 1806t
in orbital cellulitis, 799-801
inflammation/infection of. *See* Endophthalmitis; Keratitis; Uveitis; Vitritis
Neisseria gonorrhoeae infection of, 1288. *See also* Ophthalmia neonatorum, gonococcal
presumed histoplasmosis of, 2816-2817
red. *See* Conjunctivitis
T cells of, 810
tuberculosis of, 1357
in Whipple disease, 644
Eyelid hygiene, in staphylococcal blepharitis, 794
Eyelid infection, **794-801**
anterior, 794-795
Demodex, 795-796, 796f
HSV, 1945
meibomian gland, **796-797**
molluscum contagiosum, 794-795, 795f
parasitic, 795-796, 795f
Phthirus pubis, 795, 795f

Eyelid infection *(Continued)*
posterior, 796-797
preseptal cellulitis with, 798-801
sebaceous gland, **796**
staphylococcal, 794

F

Face
cellulitis of. *See also* Orbital cellulitis; Periorbital cellulitis
in meningitis, 440-441
Streptococcus pneumoniae, 1231-1232
dermatophyte infection of, **785-786**, 785f
Haemophilus influenzae type b cellulitis of, 778, 778f
pustulosis of, 789, 789f
Facial nerve paralysis
mastoiditis and, 236, 239
otitis media and, 217, 224
Factitious fever, 846
Factor H, 18
Faget sign, 2276
Failure to thrive, immunodeficiency and, 904-905
Famciclovir, 3278t, 3282-3283
in HSV infection, 1959
in VZV infection, 2029
Familial dysautonomia (Riley-Day syndrome), fever of unknown origin in, 846
Familial granulocytopenia syndrome, 917
Familial Mediterranean fever, fever of unknown origin in, 847
Family, in infection transmission, 111, 3548
Fascial space infection
infraorbital space, 149
masticator space, 142, 148-149, 149f
submandibular space, 142, 149-150, 150f
Fasciitis, necrotizing. *See* Necrotizing fasciitis
Fasciola buski, 3050f, 3056
Fasciola buski infection (fasciolopsiasis), **3056-3057**
clinical manifestations of, 3057
diagnosis of, 3057
epidemiology of, 3056-3057
pathology of, 3057
prevention of, 3057
treatment of, 3057, 3327t-3346t
Fasciola gigantica, 3052
Fasciola hepatica, 3050f, 3052
Fasciola spp. infection (fascioliasis), **3052-3054**
clinical manifestations of, 3053
diagnosis of, 3051f, 3053
epidemiology of, 3052
imaging in, 3053
pathogenesis of, 3050f, 3053
prevention of, 3054
transmission of, 3052
treatment of, 3053-3054, 3327t-3346t
Fatal familial insomnia, 2620-2621, 2627t
Fatigue, chronic. *See* Chronic fatigue syndrome
Fecal leukocytes, 617
Fecal microbiota transplantation, in *Clostridium difficile* infection, 637
Feet
dermatophyte infection of, **786**
in Kawasaki disease, 1073-1074
puncture wound of, 724-725
Ferritin
CSF, in meningitis, 445
fever effects on, 85
Ferritin *(Continued)*
plasma, 78
serum, in septic shock, 829
Fertility, in cystic fibrosis, 325
Fetor hepaticus, 649
Fetus. *See also at* Newborn
antibody production by, 44
B cells in, 42
bacterial infection in, **881-892**
epidemiology of, 881-882, 881t
pathogenesis of, 881-882
CMV infection in, 1975, 1983-1984
EBV infection in, 1998
enterovirus infection in, 2091-2092
fungal infection in, **892-900**
HSV infection in, 1954-1955
human erythrovirus B19 (human parvovirus B19) infection of, 1847, 1852-1853
Mycoplasma pneumoniae infection in, 2684
poliovirus infection in, 2100-2101
rubella virus infection in, 2206-2208, 2213. *See also* Rubella virus infection, congenital
stillborn, enterovirus infection and, 2092
syphilis in, 1762, 1766. *See also* Syphilis, congenital
T cells in, 42
viral infection in, **877-881**, 878t
clinical manifestations of, 878-879
developmental malformation and, 877
diagnosis of, 878-880
laboratory diagnosis of, 880-881
pathogenesis of, 877-878, 878f
treatment of, 881
Fever, **83-88**. *See also* Fever of unknown origin (FUO); Fever without source (FWS)
adverse effects of, 85
antipyretics for, 86-88
in appendicitis, 681
in bacterial meningitis, 455, 1228-1229
beneficial effects of, 85
in brain abscess, 464
in bronchitis, 263
in croup syndromes, 250t-251t
in CSF shunt–related infection, 1032
drug, 846
effects of, 84-85
in epiglottitis, 250t-251t, 252
in *Escherichia coli* UTI, 1440-1441
external cooling for, 88
factitious, 846
in *Francisella tularensis* infection, 1661
hectic, 841
in infective endocarditis, 356
in influenza virus immunization, 2352
intermittent, 841
in Kawasaki disease, 1073, 1075
after kidney transplantation, 1007
in lung abscess, 318
in measles, 2381-2382
measurement of, 85-86
metabolic changes with. *See* Metabolism
in neutropenia, 921. *See also* Chemotherapy, fever and neutropenia with
nitrogen balance and, 67, 68f
in *Opisthorchis* spp. infection, 3051
in orbital cellulitis, 799
oxygen consumption with, 65
in parapneumonic effusion, 310
pathogenesis of, 84
periodic, 159t-160t, 162, 181
Fever *(Continued)*
pharyngoconjunctival, 1893-1894, 1899, 1899t-1900t
in pneumococcal meningitis, 1228-1229
relapsing, 841. *See also* Relapsing fever (*Borrelia* spp. infection)
remittent, 841
in renal abscess, 554
in retroperitoneal infection, 708
in rhinosinusitis, 197
in roseola infantum, 770
in rubella virus infection, 2211, 2218
seizures with, 85
after smallpox immunization, 2038
spiking, 841
in spinal epidural abscess, 471
sustained, 841
treatment of, 86-88
typhoid. *See Salmonella* ser. Typhi infection (typhoid fever)
in urinary tract infection, 536
Fever of unknown origin (FUO), **840-848**
in bacterial endocarditis, 845
in bone and joint infection, 845
in brucellosis, 844
in cancer patient, 923
in cat-scratch disease, 844
in central nervous system dysfunction, 846
in connective tissue disease, 840
definition of, 837, 840
in diabetes insipidus, 846
diagnosis of, 841-843, 843t
drug-related, 846
factitious, 846
in familial dysautonomia, 846
vs. fever without source, 837
in hemophagocytic lymphohistiocytosis, 846-847
in hepatic infection, 845
in immunodeficiency disorders, 845
in infantile cortical hyperostosis, 847
infectious causes of, 843t, 844-846
in inflammatory bowel disease, 847
in intra-abdominal abscess, 845
in juvenile idiopathic arthritis, 840, 847
laboratory evaluation in, 842-843
in leptospirosis, 844
in malaria, 844
in malignancy, 840
noninfectious causes of, 846-848
patient history in, 841
pattern of fever in, 841
in periodic fever disorders, 847-848
physical examination in, 842
pupillary constrictor response in, 842
in pyogenic liver abscess, 674
in salmonellosis, 844
in toxoplasmosis, 844
in tuberculosis, 844-845
in tularemia, 845
in upper respiratory tract infection, 845-846
in viral infection, 845
Fever therapy, 85
Fever without source (FWS), **837-840**
clinical assessment in, 839
clinical management of, 839-840
definition of, 837
vs. fever of unknown origin, 837
occult bacteremia and, 837-839, 840t
Fibromyalgia, enterovirus and, 2091
Fibronectin-binding protein A, of *Staphylococcus aureus*, 8-9
Fidaxomicin, 3225
in *Clostridium difficile* infection, 637

Fifth disease. *See* Erythema infectiosum
Filamentous hemagglutinin (FHA), 6, 7f
  of *Bordetella pertussis*, 6, 7f, 1618t, 1619, 1623
Filariasis, **3025-3028**
  Brugia spp., **3025-3026**
  Loa loa, 815, **3026-3027**
  Wuchereria bancrofti, 757t-758t, 815, **3025-3026**
FimH, 13, 14f
Finger(s)
  blistering infection of, **775**
  Candida paronychia of, 788, 788f
  desquamation of, in Kawasaki disease, 1073-1074
  herpetic whitlow of, 155, 1944-1945, 1944f-1945f
  paronychia of, 788, 788f, 3571-3572, 3574
Fingernails, dermatophyte infection of, **786**, 786f
Fire ant bite, 758t
Fish poisoning, 604, 605t
Fish tapeworm (*Diphyllobothrium* spp.) infection, **3040-3041**, 3327t-3346t
Fistula
  bronchopleural, 321-322
  tracheoesophageal, 268
Fite-Faraco stain, in *Mycobacterium leprae* infection, 1398, 1399f
Fitz-Hugh-Curtis syndrome, **589-590**
Flagella, *Pseudomonas aeruginosa*, 1586-1587
Flavobacterium meningosepticum infection, 448-449
Fleas, 758t
  in *Yersinia pestis* infection transmission, 1510-1511
Flexal virus infection, 2472. *See also* Arenavirus infection
Flora, normal, 88-89. *See also* Microbiome
  colonization with, 89
  exogenous influences on, 89-90
  external ear, 204-205
  in newborn, 89
  oral, 140, 141t
  resident vs. transient, 88
  site-specific, 90-93, 91t
Flow cytometry, in malaria, 2936
Fluconazole, 3301f, 3303-3305, 3348
  adverse effects of, 3304
  in *Blastomyces dermatitidis* infection, 478, 2733-2734
  in *Candida* esophagitis, 596-597
  in *Candida* meningitis, 475
  in candidemia, 2747
  in *Coccidioides* spp. infection, 477, 2778
  in *Cryptococcus neoformans* infection, 2804-2805
  drug interactions with, 3304
  in esophageal candidiasis, 2747
  in hematopoietic stem cell transplantation–related aspergillosis, 937
  in *Histoplasma capsulatum* infection, 2825
  indications for, 3294t-3295t, 3304-3305, 3321t
  in neonatal *Candida* spp. infection, 2758, 2760-2761, 2764t
  in oropharyngeal candidiasis, 2746-2747
  in osteoarticular candidiasis, 2749
  in *Paracoccidioides* spp. infection, 2793
  in peritoneal candidiasis, 2748
  pharmacodynamics of, 3303
  pharmacokinetics of, 3303-3304, 3304t
Fluconazole *(Continued)*
  prophylactic
    in hematopoietic stem cell transplantation, 936
    in neonatal *Candida* spp. infection, 897
  in tinea unguium, 787
  in urinary tract candidiasis, 2748-2749
  in vulvovaginal fungal infection, 570, 2747
Flucytosine, 3298-3300, 3298f, 3348
  adverse effects of, 3300
  in *Candida* meningitis, 474-475
  in *Cryptococcus neoformans* infection, 476, 2804-2805
  dosage for, 3300
  drug interactions with, 3300
  indications for, 3300
  pharmacodynamics of, 3299, 3299f
  pharmacokinetics of, 3299-3300, 3300t
  resistance to, 3298-3299
  spectrum of activity of, 3298-3299
Fluid overload, in dengue hemorrhagic fever/dengue shock syndrome, 2288
Fluid therapy
  in ARDS, 873
  in *Bacillus cereus* infection, 1320
  in cholera, 1552-1553, 1552t, 1553f
  in *Corynebacterium diphtheriae* infection, 1310
  in dengue hemorrhagic fever/dengue shock syndrome, 2286-2288
  in diarrheogenic *Escherichia coli* infection, 1461-1462
  in gastrointestinal tract infection, 620-622, 620t-621t
  in *Leptospira* spp. infection, 1757
  in neonatal diarrhea, 888
  in rotavirus infection, 2186
  in *Shigella* spp. infection, 1483
  in toxic shock syndrome, 857-858
  in *Vibrio parahaemolyticus* infection, 1559
Flukes, 3050f-3051f, 3327t-3346t
  intestinal, 2865, 3056-3058
  liver, 660, 2865-2866, 3049-3054
  lung, 2865-2866, 3054-3056
Fluorescent antibody to membrane antigen (FAMA), in VZV infection, 2028
Fluorescent treponemal antibody–antibody absorption (FTA-ABS) test, 819-820, 1773-1774, 1773t
Fluoridation, 1195
5-Fluorocytosine, in fungal endocarditis, 368
Fluoroquinolones, 3225-3227, 3231t-3233t
  adverse effects of, 3227
  indications for, 3226-3227
  mechanisms of action of, 3226
  pharmacokinetics of, 3226
  resistance to, 3226
  in vitro activity of, 3226
Fluorouracil, 3298-3300, 3298f
  in genital HPV infection, 581
Fly infestation (myiasis), 816, **3072-3074**
Focal neurologic signs
  in bacterial meningitis, 439, 440t
  in brain abscess, 463t, 464-466, 466t
Foix-Alajouanine syndrome, 523
Folinic acid, in *Toxoplasma gondii* infection, 3001t
Folliculitis, **776-777**
  Pseudomonas aeruginosa, 1599
Fomites, 107
Fonsecaea pedrosoi infection, **789-790**, 790f
Food(s)
  antimicrobial agent interaction with, 3234
  Clostridium difficile in, 635
Food(s) *(Continued)*
  contaminated, 109, 602-604, 3547. *See also* Food poisoning
  weaning, 627
Food poisoning, 601-606. *See also* Gastrointestinal tract infection *and specific infections*
  Aeromonas hydrophila, 602t-603t, 607t, 612, 616t
  Bacillus cereus, 602t-603t, 613, 1317-1320
  bacterial, 602-604, 602t-603t
  Campylobacter jejuni, 602t-603t, 609. *See also Campylobacter jejuni* infection
  chemical, 604, 604t
  clinical manifestations of, 601-602
  Clostridium botulinum, 602t-603t, **1791-1795.** *See also* Botulism
  Clostridium perfringens, 602t-603t, 613, 1797-1800
  Cryptosporidium spp., 602t-603t, 614-615, 2899. *See also Cryptosporidium* spp. infection (cryptosporidiosis)
  diagnosis of, 616-619, 616t. *See also* Stool culture; Stool specimen examination
  differential diagnosis of, 1793
  epidemiology of, 601
  Escherichia coli. *See Escherichia coli*, diarrheogenic
  HAV, 2134-2136
  HEV, 2163
  inorganic toxin, 604, 604t
  Listeria monocytogenes, 607t, 613, 618. *See also Listeria monocytogenes* infection (listeriosis)
  marine toxin, 604, 605t
  mushroom, 604, 604t
  parasite, 602-604, 602t-603t, 607t, **613-616**, 616t. *See also specific parasitic infections*
  Plesiomonas shigelloides, 607t, 612-613, 616t
  prevention of, 604-606, 626-628, 1321
  Salmonella spp., 602t-603t, 609, 1493. *See also Salmonella* spp. infection (salmonellosis)
  Shigella spp., 608-609. *See also Shigella* spp. infection (shigellosis)
  Staphylococcus aureus, 613. *See also Staphylococcus aureus* infection
  treatment of, 619-626, 620t-621t
  Vibrio cholerae, 602t-603t, **611-612.** *See also* Cholera
  Vibrio parahaemolyticus, 602t-603t, 612. *See also Vibrio parahaemolyticus* infection
  Vibrio vulnificus, 1560
  viral, 602-604, 602t-603t
  Yersinia enterocolitica, 602t-603t, 612. *See also Yersinia enterocolitica* infection (yersiniosis)
Foot (feet)
  dermatophyte infection of, **786**
  in Kawasaki disease, 1073-1074
  puncture wound of, 724-725
Foreign body
  esophageal, 419
  laryngeal, 250t-251t, 253
  pulmonary, 316-317
  in toxic shock syndrome, 852-853
  vaginal, 567-570, 567f
Foreign body reaction, implant-related, 1016
Fosamprenavir, 2615
Foscarnet, 3278t, 3286-3287, 3286f
  in acyclovir-resistant HSV infection, 1964
  in CMV infection, 1984-1987, 1985f
  in hematopoietic stem cell transplantation, 940

Foscarnet *(Continued)*
in kidney transplant–related CMV infection, 1008
structure of, 1985f
in VZV infection, 2029
FOXP3, 915-916
Fracture, osteomyelitis after, 719
Francisella tularensis, 1658
subspecies of, 1658, 1660
Francisella tularensis infection (tularemia), **1657-1665**
bioterrorist, 3584
clinical manifestations of, 753t-756t, 1661-1663
diagnosis of, 1663-1664
differential diagnosis of, 1663-1664
epidemiology of, 1658-1660
fever of unknown origin in, 845
glandular, 1662
historical perspective on, 1657-1658
host factors in, 1660-1661
immune response to, 1660-1661
vs. *Legionella pneumophila* infection, 1713
nodular, 1662-1663
oculoglandular, 1662
oropharyngeal, 1662
pathogenesis of, 1660-1661
prevention of, 1664-1665
pulmonary, 1662-1663
seasonality of, 1659
transmission of, 1659
treatment of, 1664
vs. tuberculosis, 1661
typhoidal, 1662
ulceroglandular, 1662
Free oxygen radicals, *Streptococcus pneumoniae* release of, 1214
Frequency distribution, 100-101, 100f
Friction rub, 374
Friedländer pneumonia, 1467
FTA-ABS (fluorescent treponemal antibody–antibody absorption) test, 819-820, 1773-1774, 1773t
Fuchs heterochromic uveitis, 2213
Fumagillin, 2913
Fungal infection. *See also specific infections*
appendiceal, 683t, 685
biliary, 660
bone, 725-726
burn wound, 1053, 1053t
in cancer patient, 922, 924, 928
corneal, 807-808
contact lens–related, 1019
CT in, 926-927
ear canal, **207**
encephalitic, 494t-498t, 501
endocardial, 352, 361t, 363, 367-368
esophageal, 593, 597
exanthem in, 757t
after hematopoietic stem cell transplantation, 936-939
hepatic, 651t, 657, 673-675
in HIV-infected host, 2602
host response to, 23. *See also* Immunity
after intestinal transplantation, 991
joint, 730, **734**
after kidney transplantation, 1006, 1012-1014
laboratory diagnosis of, **3596-3600**, 3599t
β-D-glucan assay in, 926, 938, 967, 2716, 2744-2745, 3597-3599, 3598t
culture in, 3596, 3597t, 3599-3600, 3599t
direct detection methods in, 3596-3599, 3598t
Fungal infection *(Continued)*
galactomannan in, 926, 927t, 3597-3599, 3598t
PCR in, 926
left ventricular assist device, 1022
after liver transplantation, 813, 991, 993-994, 995t
after lung transplantation, 959-960, 965-974
meningeal. *See* Meningitis, fungal
muscle, 739
neonatal, **898-899**
acquired, 898-899
congenital, 898
ocular, 820
orbital, 801
pancreatic, 695
pericardial, 373, 379
peritoneal, 701, 703
in rhinosinusitis, 196-197, 196t, 201
urinary tract, 540
uveal, 813-814
vulvovaginal, 569-570, 570f
Fungus (fungi), 2702-2704. *See also* Fungal infection
Funisitis, **890**
necrotizing, 1766
Furazolidone, 3348
Furunculosis, 208, **776-777**
Fusarium spp., 2846
Fusarium spp. infection (fusariosis), **2846-2852**
burn wound, 1053
clinical manifestations of, 2846-2847, 2847f
corneal, 807-808, 2846-2847
cutaneous, 757t, 2847, 2847f
diagnosis of, 2847-2848
disseminated, 2847, 2847f
after lung transplantation, 974
pathogenesis of, 2846
treatment of, 2847-2848
Fusobacterium spp., 1826
Fusobacterium spp. infection, **1825-1834**
Arcanobacterium haemolyticum infection with, 1326
bloodstream, 1827-1828
bone, 1829-1830
clinical manifestations of, 1827-1830
CNS, 1828-1829
cutaneous, 1829
diagnosis of, 1830
epidemiology of, 1826-1827
head and neck, 174, 1828
hepatic, 673
intra-abdominal, 1829
joint, 1829-1830
oral cavity, 140-141
in otitis media, 214
pathogenesis of, 1827
pleuropulmonary, 1830
prevention of, 1833
soft tissue, 1829
treatment of, 1831-1833, 1831t

G

Galactomannan, 938, 3597-3599, 3598t
in *Aspergillus* spp. infection, 926, 927t, 2714-2715
BAL fluid, 967, 2715-2716
in *Blastomyces dermatitidis* infection, 2731-2732
in *Coccidioides* spp. infection, 2777
in *Fusarium* spp. infection, 2847-2848
urine, 2731-2732
Gallbladder
inflammation of. *See* Cholecystitis
in Kawasaki disease, 1074, 1082
in *Leptospira* spp. infection, 1750, 1751f
perforation of, 670
stones of. *See* Gallstones
Gallbladder sludge, ceftriaxone therapy and, 447
Gallium, metabolism of, 77t
Gallstones, 667-669, 668t. *See also* Cholecystitis
cholesterol, 668
in cystic fibrosis, 324-325, 668
epidemiology of, 667
formation of, 667-668
pancreatitis with, 692
pigmented, 668
in sickle-cell disease, 670
Gamma globulin. *See* Immunoglobulin(s)
Ganciclovir, 3278t, 3283-3285, 3283f
adverse effects of, 3285
in CMV infection, 1984-1987, 1985f
in CMV infection prevention, 1988
dosage for, 3278t, 3284-3285
in heart transplant–related CMV infection, 951-952
in hematopoietic stem cell transplantation, 940
in hematopoietic stem cell transplantation–related CMV infection, 940
indications for, 3284
in kidney transplant–related CMV infection, 1008
in lung transplantation, 975-976
mechanism of activity of, 3283
pharmacokinetics of, 3283-3284
resistance to, 3283
spectrum of activity of, 3283
structure of, 1985f
Gangosa, 1787
Gangrene
in Kawasaki disease, 1082
pulmonary, 1467
Gardnerella vaginalis, vaginal colonization with, 562
Gardnerella vaginalis infection, 572t-573t, 577-578, 577f
Garré sclerosing osteomyelitis, 154, 154f-155f
Gas gangrene, 1796
diagnosis of, 1798
prevention of, 1800
prognosis for, 1799-1800
treatment of, 1799, 3506
Gastric acidity, *Yersinia enterocolitica* infection and, 1526
Gastric cancer
EBV infection and, 2006
Helicobacter pylori infection and, 1696-1698, 1697f
Gastric lavage, in botulism, 1794
Gastritis, 1694. *See also Helicobacter pylori* infection
Gastroenteritis. *See* Gastrointestinal tract infection
Gastroesophageal reflux
vs. chronic bronchitis, 268
esophagitis in, 594
Gastrointestinal tract
bleeding from, in Whipple disease, 643
decontamination of, in burn patient, 1061
indigenous flora of, 91t, 92-93

Gastrointestinal tract infection, **598-632**
adenovirus, 607t, 608, 618-619, 1896t, 1901-1902
Aeromonas spp., 607t, 612, 616t, 1541-1542
astroviral, 607t, 608
Bacillus anthracis, 1314
Bacillus cereus, 607t, 613, 1317-1320
bacterial, 607t, 608-613, 616t, 628
Balantidium coli, **2894-2896**, 3327t-3346t
Basidiobolus ranarum, 2844
Blastocystis hominis, **2875-2876**, 3327t-3346t
Brucella spp., 602t-603t, 1613
Campylobacter, 607t, 609, 616t, 623-624, 623t, **1644-1657.** *See also Campylobacter jejuni* infection
Candida, 2741
Citrobacter spp., 1431
Clostridium difficile, 607t, 613, 616t. *See also Clostridium difficile* infection, antibiotic-associated
Clostridium perfringens, 602t-603t, 607t, 613, 618, 1797-1800
CMV, 1978
coronavirus, 2493-2494
coxsackievirus, 2068-2073, 2068t-2069t, 2071t
Cryptosporidium spp., 614-615, 625, **2896-2905**, 2898f, 2901t-2902t
Cyclospora, 615, 625, **2905-2907**, 2907f
Cystoisospora belli, 607t, 615, 617, **2907-2911**, 2908f
diagnosis of, 616-619, 616t, 1503
coccidia stains in, 617
fecal leukocytes in, 617
immunologic tests in, 618-619
molecular tests in, 619, 620f
ova examination in, 617
stool culture in, 618. *See also* Stool culture
stool examination in, 616-617. *See also* Stool specimen examination
diarrhea in, 599-606, 600t. *See also* Diarrhea
Dientamoeba fragilis, **2884-2887**, 2885f, 2886t
echoviral, 2068-2073, 2069t, 2071t
Edwardsiella tarda, 1533, 2072t
Entamoeba coli, **2877-2878**
Entamoeba histolytica, 614, 625, **2866-2874.** *See also Entamoeba histolytica* infection (amebiasis)
Enterococcus spp., 1175
enterovirus, 2068-2073, 2068t-2069t, 2071t
epidemiology of, 598-606
Escherichia coli, 607t, **609-611**, 610t, 611f, 616t, 623t, 624-625, **1449-1465.** *See also Escherichia coli* infection, diarrheogenic *and specific species*
foodborne, 601-606, 602t-603t. *See also* Food poisoning
Giardia intestinalis, 602t-603t, 607t, 614, 625
Giardia lamblia, **2878-2883**, 2880f, 2881t, 2883t
Hafnia alvei, 1534
Histoplasma capsulatum, 2815
human bocavirus, 1860-1861
in international adoptees and refugees, 3128-3130
Listeria monocytogenes, 607t, 613
microsporidial, 607t, 615, 617, 625, 2911t, 2912
Mucor spp., 2842
Mycobacterium tuberculosis, 1357-1358
norovirus, 607-608, 607t, 616t, **2151-2154**, 2152f. *See also Norovirus* infection
nosocomial, 3382-3383
pancreatitis with, 695
parasitic, 607t, **613-616**, 616t, 625, 3012-3021. *See also specific parasitic infections*
diagnosis of, 617
parechoviral, 2068-2073, 2068t-2069t, 2071t
Plesiomonas shigelloides, 607t, 612-613, 616t, **1565-1566**
prevention of, 626-628
Rhizopus spp., 2842
rotavirus, 606-607, 607t, 608f, 616t, 627, **2176-2195.** *See also* Rotavirus infection
respiratory symptoms with, 158
Salmonella, 607t, 609, 616t, 623t, 624, **1491-1509.** *See also Salmonella* spp. infection (salmonellosis)
Schistosoma spp., 3065. *See also Schistosoma* spp. infection (schistosomiasis)
Shigella, 607t, 608-609, 616t, 623, 623t, **1476-1487**, 1478f, 1480t. *See also Shigella* spp. infection (shigellosis)
Staphylococcus aureus, 602t-603t, 607t, 613, 618
Strongyloides stercoralis, 615-616, 625. *See also Strongyloides stercoralis* infection (strongyloidiasis)
transmission of, 598-599
treatment of, 619-626, 620t
antimicrobials in, 620t, 622, 623t
antisecretory agents in, 626
fluid therapy in, 620-622, 620t-621t
nutritional management in, 620t, 622
probiotics in, 626
zinc in, 625-626
vaccines against, 627-628
Vibrio cholerae, 607t, **611-612**, 616t, 623t, 625, **1548-1555.** *See also* Cholera
Vibrio parahaemolyticus, 607t, 612, 616t, **1556-1559.** *See also Vibrio parahaemolyticus* infection
Vibrio vulnificus, **1560-1562**
viral, 606-608, 607t, 616t
waterborne, 606, 627, 3547
in Whipple disease, 642-643
Yersinia enterocolitica, 607t, 612, 616t, **1514-1532.** *See also Yersinia enterocolitica* infection (yersiniosis)
Gatifloxacin, pancreatitis with, 692
Gemella morbillorum infection, 362-363
Gene(s)
in primary immunodeficiency, 907, 907t
in septic shock, 831
virulence-associated, 3
Gene therapy
in chronic granulomatous disease, 57-58
in immunodeficiency disorders, 64
in severe combined immunodeficiency disease, 49, 915
in type 1 leukocyte adhesion deficiency, 53
Genital tract infection, **560-591.** *See also specific infections*
CMV, 1972
EBV, 1998
evaluation of, 560-561
HPV. *See* Human papillomavirus (HPV) infection, anogenital
HSV. *See* Herpes simplex virus (HSV) infection, genital
Mycobacterium tuberculosis, 1358
physical examination in, 561
ulcers with, 582-584, 583t
vaginal specimen collection in, 561, 561f
Genitourinary tract, indigenous flora of, 91t, 93
Genome
Blastomyces dermatitidis, 2723-2724
Bordetella spp., 1617
Borrelia hermsii, 1740
calicivirus, 2149-2150, 2149f
Campylobacter jejuni, 1645-1646
CMV, 1969
coronavirus, 2487
Corynebacterium diphtheriae, 1302
EBV, 1993
hantavirus, 2496-2497
HBV, 1912-1915
HCV, 2318-2319, 2318f
HDV, 1928
hepatitis A virus, 2128-2129, 2129f
HEV, 2159, 2159f
HHV-6, 2016
hMPV, 2435, 2435f
HPV, 1872
HSV, 1933
human bocavirus, 1858
human erythrovirus B19 (human parvovirus B19), 1844
influenza virus, 2327
measles virus, 2374
Mycobacterium leprae, 1393
Mycobacterium ulcerans, 1412
parainfluenza virus, 2361, 2362f
polyomavirus, 1864
rabies virus, 2445-2446
reovirus, 2168
retrovirus, 2553-2556, 2554f, 2581-2582
rotavirus, 2179, 2180f
RSV, 2408, 2409f
Staphylococcus aureus, 1120
Streptococcus pneumoniae, 1211-1212
Treponema pallidum, 1761
Trypanosoma brucei, 2968
Vibrio cholerae, 1550
Vibrio parahaemolyticus, 1556
VZV, 2022
West Nile virus, 2261, 2262f
Yersinia spp., 1515-1516
Gentamicin, 3208-3211, 3208t, 3231t-3234t
in burn wound infection, 1059
in coagulase-negative staphylococcal infection, 1138-1139
in meningitis, 450t
in neonatal conjunctivitis prevention, 1281
Germ theory of disease, 103
German measles. *See* Rubella virus infection
Gerstmann-Sträussler-Scheinker syndrome, 2620-2621, 2627t
Gianotti-Crosti syndrome (papular acrodermatitis), 649, 749, **766**, **783**, 783f
in HAV infection, 2140
Giant cells, in myocarditis, 383-384
Giardia intestinalis infection, 602t-603t, 607t, 614, 619
treatment of, 625
Giardia lamblia, 2878-2879
Giardia lamblia infection (giardiasis), **2878-2883**
clinical manifestations of, 757t-758t, 2881-2882, 2881t

Giardia lamblia infection (giardiasis) *(Continued)*
in daycare facilities, 3556
diagnosis of, 2882
epidemiology of, 2879
in immunocompromised hosts, 2881
pathogenesis of, 2879-2881, 2880f
retinal manifestations of, 814
treatment of, 2882-2883, 2883t, 3327t-3346t
Gilbert syndrome, 657
Gingiva, 146-147, 147f
inflammation of, 147, 147f
linear erythema of, 149
Gingivitis, 147, 147f
HIV (linear gingival erythema), 149
Gingivostomatitis, HSV, 154-155, 1940-1941, 1940f, 1959
Glanzmann thrombasthenia, 53-54
Glaucoma
vs. conjunctivitis, 806
in rubella, 818, 2213, 2214t-2216t, 2221
surgery for, endophthalmitis after, 820
Glioma, brain stem, 505
Global health, **3081-3091**
definition of, 3081-3082, 3082t
future directions for, 3087-3090, 3088f-3089f
goals for, 3084, 3085f, 3085t
historical perspective on, 3082-3083
neglected tropical diseases in, 3084-3087, 3085t, 3086f-3087f
social factors in, 3084, 3084t
Glomerulonephritis
in human erythrovirus B19 (human parvovirus B19) infection, 1855
infective endocarditis and, 354-355
membranoproliferative, HBV infection and, 1919
in syphilis, 1767
in Whipple disease, 644
Gloves and socks syndrome, 741, 742t-748t, 1849
Glucagon, 82
pancreatic, 72
β-D-Glucan assay, 926, 3597-3599, 3598t
in *Aspergillus* spp. infection, 938, 967, 2716
in *Candida* spp. infection, 936-937, 970, 2744-2745
in *Fusarium* spp. infection, 2847-2848
Glucocorticoids, plasma, 81
Glucose
in arthritides, 731, 732t
blood, 72
CSF, 885-886
release of, 82
Glucose-6-phosphate dehydrogenase (G6PD) deficiency, 58-59
HAV infection and, 2138
HEV infection and, 2164-2165
Glucose tolerance test, 82
Glutamate, in meningitis, 435
Glutathione peroxidase deficiency, 58-59
Glutathione synthetase deficiency, 59
Glycerol, in bacterial meningitis, 453
Glyceryl monolaurate, 864-865
Glycocalyces, 1017
Glycogen storage disease type 1B, 54-55
Glycyrrhizin, in HEV infection, 2166
Gnathostoma spinigerum infection, 3327t-3346t
encephalitic, 494t-498t
meningeal, **481-484.** *See also* Meningitis, eosinophilic
ocular, 815
Gongylonema spp. infection, 3327t-3346t
Gonococcal infection/gonorrhea. *See Neisseria gonorrhoeae* infection
Gradenigo syndrome, 224
Graft-versus-host disease
vs. chronic bronchitis, 267-268
in hematopoietic stem cell transplantation, 933
Gram-negative bacilli, 1097t-1112t. *See also specific organisms and infections*
anaerobic, 1097t-1112t
Gram-negative cocci, 1097t-1112t. *See also specific organisms and infections*
Gram-positive bacilli, 1097t-1112t. *See also specific organisms and infections*
Gram-positive cocci, 1097t-1112t. *See also specific organisms and infections*
Gram stain
in *Actinomyces* spp. infection, 1823
in *Aerococcus* spp. infection, 1249
in bacterial vaginosis, 578
in burn wound infection, 1056f
in *Clostridium perfringens* infection, 1796
CSF, 442, 1035, 1035f
in *Escherichia coli* pneumonia, 1446
in *Francisella tularensis* infection, 1663
in *Haemophilus ducreyi* infection, 1687
in *Listeria monocytogenes* infection, 1332
in meningitis, 445, 1262-1263
in *Neisseria gonorrhoeae* infection, 1290, 1290f
in *Neisseria meningitidis* infection, 1262-1263
in *Nocardia* spp. infection, 1419-1420
in staphylococcal infection, 1114f
in urethritis, 532-533, 532f
Granulocyte colony-stimulating factor (G-CSF), 3362-3363
glucose metabolism and, 73
in *Streptococcus pneumoniae* infection, 1225-1226
in type 1 leukocyte adhesion deficiency, 52-53
Granulocyte-macrophage colony-stimulating factor (GM-CSF), 3362-3363
in burns, 1049
Granuloma
in *Candida* infection, 2740
in *Coccidioides* spp. infection, 2769, 2770f, 2773
in *Histoplasma capsulatum* infection, 2811-2813, 2826-2827, 2826f
in *Paracoccidioides* spp. infection, 2785
in *Schistosoma* spp. infection, 3063
in *Toxocara* infection, 814
in Whipple disease, 642
Granuloma inguinale, 583t, **1640-1644**
clinical manifestations of, 1641-1642, 1642f
diagnosis of, 1642-1643, 1642f
epidemiology of, 1640-1641
pathogenesis of, 1641
prevention of, 1643-1644
prognosis for, 1643
treatment of, 1643
Granuloma venereum. *See* Granuloma inguinale
Granulomatous disease. *See* Chronic granulomatous disease
Granzymes, 28
Gray baby syndrome, 3217
Grey Turner sign, 689-690
Griseofulvin, 3319-3320, 3319f, 3321t
in tinea capitis, 787
Ground-glass hepatocytes, 1919, 1919f
Group A β-hemolytic streptococcal infection. *See Streptococcus pyogenes* infection
Group B β-hemolytic streptococcal infection. *See Streptococcus agalactiae* infection
Group C β-hemolytic streptococcal infection, 1150-1152
clinical manifestations of, 1151
epidemiology of, 1151
treatment of, 1152
Group G β-hemolytic streptococcal infection, 1150-1152
clinical manifestations of, 1151
epidemiology of, 1151
treatment of, 1152
Growth hormone (GH), plasma, 81
Growth restriction, in rubella virus infection, 2213, 2214t-2216t
Guanarito virus infection, 2469, 2470t. *See also* Arenavirus infection
Guillain-Barré syndrome
vs. acute transverse myelitis, 524
vs. botulism, 1793
after *Campylobacter jejuni* infection, 1653-1654
EBV infection and, 2004
enterovirus infection and, 2088t, 2090-2097
HAV infection and, 2140
HBV vaccine and, 1926
vs. infant botulism, 1808
rubella virus infection and, 2212
Guinea worm infection, **3028**
Gumma, 1765-1766, 1768, 1788
Gypsy moth rash, 758t

H

HA-1A antiendotoxin therapy, in *Neisseria meningitidis* infection, 1264-1265, 1265t
HAART. *See* Highly active antiretroviral therapy (HAART)
HACEK infection, endocardial, 362, 367, 367t, 1823
Haemophilus aegyptius, 1688-1689
Haemophilus aegyptius infection, **1688-1690**
clinical manifestations of, 1689
epidemiology of, 1689
pathogenesis of, 1689
treatment of, 1689-1690
Haemophilus ducreyi, 1686-1687, 1686t
Haemophilus ducreyi infection, **1686-1688**
clinical manifestations of, 753t-756t, 1687
coinfection with, 1688
diagnosis of, 1687-1688
epidemiology of, 1686-1687
pathogenesis of, 1687
prevention of, 1688
treatment of, 1688
Haemophilus influenzae, 1666-1668, 1686t
antibiotic resistance of, 1668
capsular polysaccharides of, 1666t, 1667
capsule of, 19-20
carriage of, 1668-1669
cell wall antigens of, 1667-1668
Hap protein of, 6-7
Hia adhesin of, 6-7, 9f
IgA proteases of, 1668
lipo-oligosaccharide of, 1667-1668
lipopolysaccharide of, 19
polysaccharides of, 1672
serotypes of, 19
typing of, 1668

Haemophilus influenzae infection, **1665-1684**
antibiotic resistance of, 1668
anticapsular antibody in, 1671
bloodstream, 1670
bronchial
acute, 262, 263t
chronic, 269, 269t
cellular immune response in, 1672
clinical manifestations of, 1675-1677
complement in, 1672
in cystic fibrosis, 334
diagnosis of, 1677-1678
endocardial, 362
epidemiology of, 1673-1675
exanthem in, 753t-756t
genetic factors in, 1672
historical perspective on, 1665-1666
IgG subclass-specific antibody in, 1671-1672
immune response to, 1670-1673
invasive, 1676-1677
pathogenesis of, 1669-1670
treatment of, 1678-1679, 1678t
mastoid, 234, 235t
meningeal, 429-430, 430f, 450t-451t, 460-461, 1670, 1675. *See also* Meningitis, bacterial
mucosal, 1669, 1677
treatment of, 1678t, 1679
nasopharyngeal, 158, 162-163
non–type b, 1674, 1676-1677
nontypeable strains in, 1674-1675, 1677
in otitis media, 212-215, 212t, 226
pathogenesis of, 1668-1670
phagocytosis in, 1672-1673
pharyngeal, 158
in rhinosinusitis, 195, 196t
treatment of, 1678-1679, 1678t
antibiotic resistance in, 1668
vulvovaginal, 568
Haemophilus influenzae type b
capsule of, 19-20
antibody against, 1666, 1671
polysaccharides of, 1667
carriage of, 1668-1669
Haemophilus influenzae type b infection, **1665-1684**
age and, 1674
bloodstream, 1675
bone, 711, 717-718
clinical manifestations of, 1675-1677
in daycare facilities, 3561
diagnosis of, 1677-1678
epidemiology of, 1673-1674, 1673t, 1678t
in epiglottitis, 244-249, 259, 1675-1676
immunization against, 1679-1682, 3431-3433, 3432t
active, 1679-1681, 1680t
disease reemergence and, 1674
in epiglottitis prevention, 259
in meningitis, 429, 461
passive, 1681-1682
in pneumonia prevention, 285, 292
results of, 1666, 1682
invasive, 1669-1670
joint, 728-730, 729t, 732-733, 1676
meningeal, 429-430, 430f, 450t-451t, 460-461, 1670, 1675. *See also* Meningitis, bacterial
mucosal, 1669
neonatal, 1676
pericardial, 372-373, 378, 1676
in preseptal cellulitis, 798
prevention of, 1679-1682, 3249-3250
pulmonary, 1675
Haemophilus influenzae type b infection *(Continued)*
secondary, 1674
soft tissue, 778, 778f, 1676
treatment of, 1678-1679, 1678t
prophylactic, 1682
uvular, 165, 167
viral infection and, 1670
Haemophilus parainfluenzae, 1686t, 1690
Haemophilus parainfluenzae infection, **1690**
Hafnia alvei infection, **1534-1535**
Hair follicles, infection of, **776-777**
Hair shaft, white piedra of, 790-791
Halo sign, in *Aspergillus* spp. infection, 2712
Halofantrine, in malaria, 2942
Hamman sign, 419-420
Hand(s)
in Kawasaki disease, 1073-1074
in sporotrichosis, 791f
tinea nigra of, 790, 790f
Hand and foot syndrome, vs. osteomyelitis, 724
Hand-foot-and-mouth disease (coxsackievirus A16 infection), 2066, 2082-2083, 2082f-2083f, 2083t
in daycare facilities, 3555
epidemiology of, 2057
exanthem in, 742t-748t, 749, 762, 764
ocular manifestations of, 2074-2075
Hand hygiene, 3411
in *Clostridium difficile* infection prevention, 638
in influenza virus infection prevention, 2355
Hansenula spp. infection. *See Pichia* spp. infection
Hantavirus, 2496-2500, 2498f, 2499t-2500t
tissue culture for, 2498-2499
Hantavirus infection, **2496-2513**
clinical manifestations of, 2503-2507
complications of, 2507-2508
diagnosis of, 2509-2510
differential diagnosis of, 2509
epidemiology of, 2497f, 2501-2503, 2501f
exanthem in, 742t-748t, 749-750
geographic distribution of, 2501-2502
hemorrhagic fever with renal syndrome in, 2496, 2503-2504, 2504t, 2510
historical perspective on, 2496
hospital infection control in, 2511-2512
immunization against, 2511
incidence of, 2502-2503
nephropathia epidemica in, 2496, 2504-2505
noncardiogenic pulmonary edema in, 2509
pathogenesis of, 2508-2509
pathology of, 2508-2509, 2508f
prevalence of, 2502
prevention of, 2511-2512
pulmonary syndrome in, 2505-2507, 2506f, 2510
seasonal patterns of, 2502
transmission of, 2500
treatment of, 2510-2511
Hantavirus pulmonary syndrome, 2505-2507, 2506f, 2510
differential diagnosis of, 2509
treatment of, 2510-2511
Haplorchis spp. infection (heterophyiasis), **3057-3058**
Harry Eagle effect, 739
HAV. *See* Hepatitis A virus (HAV)
Haverhill fever, 1715-1716, 1718. *See also Streptobacillus moniliformis* infection (rat-bite fever)
HBV. *See* Hepatitis B virus (HBV)
HDV. *See* Hepatitis D virus (HDV)
Head and neck cancer, HPV and, 1880
Head and neck infection, **167-175**
Bacteroides spp., 1828
epidemiology of, 167-168
Fusobacterium spp., 1828
in infectious mononucleosis, 2004
infraorbital space, 149
masticator space, 142, 148-149, 149f
microbiology of, 173-175
parapharyngeal, **172-173**
peritonsillar, **168-170**, 168f, 170f
Porphyromonas spp., 1828
Prevotella spp., 1828
retropharyngeal, **170-172**, 170f
submandibular space, 142, 149-150, 150f
Headache, in brain abscess, 464
Health care worker, infection in, 3412-3413
Health care–associated infection, **3377-3419**
Acinetobacter spp., 1569
adenovirus, 1893, 3382
Burkholderia cepacia, 1595
in burn injury, 1061-1062, 3405
in cancer patients, 929
Clostridium difficile, 3382, 3401
CMV, 1972-1973, 3383
coronavirus, 3382
in cystic fibrosis, 3405-3406
in dialysis, 3406-3407
Ebola virus, 3401-3402
Enterococcus spp., 1172, 1174, 1186
enterovirus, 3384
gastrointestinal tract, 3382-3383
Haemophilus influenzae, 3385
HAV, 2135, 3384
in health care worker, 3412-3413
historical perspective on, 3377-3378
HSV, 3383
human erythrovirus B19, 3384
in immunocompromised host, 3404-3405
influenza virus, 2352, 3380-3382
in intestinal transplantation, 993
intravascular catheter–associated, 3385-3391, 3386t-3388t, 3387f. *See also* Catheter-associated infection
Lassa fever virus, 2470-2471
lice, 3385
in liver transplantation, 991, 993
in long-term care facilities, 3407
measles, 3383-3384
methicillin-resistant *Staphylococcus aureus*, 3398-3399
Moraxella catarrhalis, 1252
multidrug-resistant gram-negative bacilli, 3400
mumps, 3383-3384
Neisseria meningitidis, 3385
in neonate, 3402-3404
norovirus, 3382
pertussis, 3382
plague, 3401
prevention of, 1061-1062, 3407-3414, 3409t
antimicrobial stewardship programs in, 3414, 3414t
hand hygiene in, 3411
isolation precautions in, 3411-3412
occupational health policies in, 3412-3413
outbreak investigations in, 3408, 3411t
policies and procedures for, 3408-3411, 3410t
Standard Precautions in, 3379, 3380t, 3412

Health care–associated infection *(Continued)*
surveillance in, 3408
Transmission-Based Precautions in, 3379, 3381t, 3412
visitor policies in, 3412
prion, 2622-2623
Pseudomonas aeruginosa, 1594
respiratory virus, 3379-3382
Rickettsia rickettsii, 2651
rotavirus, 3382
RSV, 2427-2428, 3379
rubella, 3383-3384
Salmonella spp., 1493, 1507
scabies, 3385
Serratia marcescens, 1488-1489
smallpox, 3401
Stenotrophomonas maltophilia, 1608
Streptococcus agalactiae, 1155
Streptococcus pneumoniae, 3385
surgery-associated, 3395-3398, 3396t
transmission of, 3378-3379
tuberculosis, 3384-3385
urinary tract, 3393-3395
vancomycin-resistant enterococci, 3399-3400
ventilator-associated, 3391-3393
VZV, 2025, 2030, 3383
Yersinia enterocolitica, 1519
Hearing loss
adenovirus infection and, 1904
antibiotics and, 1664, 3206, 3211
audiometric tests for, 218
CMV infection and, 1975, 1980
in Kawasaki disease, 1082
mastoiditis and, 237
meningitis and, 452, 457-458, 1259
mumps and, 2402
otitis media and, 217-218, 222
rubella and, 2213, 2214t-2216t, 2220-2221
syphilis and, 1772
Heart
infection of. *See* Infective endocarditis; Myocarditis; Pericarditis
rubella-related defects of, 2214t-2216t, 2217, 2221
syphilis of, 1768
tuberculosis of, 1351
in Whipple disease, 644
Heart block
in infective endocarditis, 359
in myocarditis, 389, 392f, 396
Heart disease
acquired. *See* Kawasaki disease
chagasic. *See Trypanosoma cruzi* infection (trypanosomiasis)
congenital, infective endocarditis with, 351, 351t
Heart failure
in infective endocarditis, 356
in rheumatic fever, 414
Heart murmurs
vs. friction rub, 374
in infective endocarditis, 356
in myocarditis, 389, 395
in rheumatic fever, 409
Heart sounds
in myocarditis, 389
in pericarditis, 374
Heart transplantation, **945-956**
antibiotic prophylaxis in, 947
evaluation for, 946-947, 946t
HBV infection and, 1924
immunization after, 955-956
immunosuppression in, 946, 955, 955t
Heart transplantation *(Continued)*
infection after, 946t, 947-950
adenovirus, 955, 1904
Aspergillus fumigatus, 953
CMV, 950-952
EBV, 952-953
erythrovirus B19 (parvovirus B19), 954
HSV, 949, 1952
influenza virus, 954
Legionella pneumophila, 949
mediastinal, 948-949
Nocardia asteroides, 953-954
Pneumocystic jiroveci, 954
RSV, 949-950
sternal, 948-949
Streptococcus pneumoniae, 954, 1233
Toxoplasma gondii, 953
VZV, 954-955
mortality with, 945-946
Heat energy, 84
Heat therapy, in *Sporothrix schenckii* infection, 2835
Heat transfer, 84
Heavy metal poisoning, 604, 604t
Heggers solution (0.025%), in burn wound infection, 1059
Helicobacter pylori, 1692-1693, 1692f-1693f
enzymes of, 1693
Helicobacter pylori infection, **1691-1699**
abdominal pain and, 1694
cancer and, 1696-1698, 1697f
clinical manifestations of, 1694, 1697f
diagnosis of, 1695, 1695f, 1696t
epidemiology of, 1693-1694, 1694f
historical perspective on, 1691-1692
immunization against, 1698
inflammatory response in, 1692-1693, 1693f
pancreatitis and, 695
probiotics in, 3370
recurrence of, 1694, 1698
transmission of, 1693-1694
treatment of, 1696-1698, 1697t
Helium-oxygen therapy, in RSV infection, 2425
Helminth infection, 2862t, **3012-3029.** *See also specific helminth infections*
cutaneous manifestations of, 757t-758t, 761t, 763t
encephalitic, 494t-498t
host response to, 23-24
HTLV-1 infection and, 2571
meningeal. *See* Meningitis, eosinophilic
uveal, 814-815
Helper virus. *See* Hepatitis D virus (HDV) infection
Hemangioendothelioma, 657
Hematoma, spinal, vs. acute transverse myelitis, 523
Hematopoietic stem cell transplantation (HSCT)
in adult T-cell leukemia, 2568-2569
graft-versus-host disease in, 933
infection after, **933-945**
Absidia, 938
adenovirus, 941-942, 1903-1904
Aspergillus, 937-938, 2707-2711, 2713
bacterial, 935-936
BK virus, 1867, 1867f, 1869
Candida spp., 936-937
Clostridium difficile, 935
CMV, 939-940, 3520-3521
Cryptosporidium spp., 943-944
EBV, 940-941
epidemiology of, 933-934
Hematopoietic stem cell transplantation (HSCT) *(Continued)*
fungal, 936-939
HHV-6, 941, 2017
HSV, 939
human metapneumovirus, 943
human polyomavirus type I, 942
influenza virus, 942
Mucor, 938
mycobacterial, 935-936
parainfluenza virus, 943
phase I (pre-engraftment), 934, 934f
phase II (early post-engraftment), 934, 934f
phase III (late post-engraftment), 934, 934f
Pneumocystis jiroveci, 938-939
protozoal, 943-944
rhinovirus, 943
Rhizopus, 938
RSV, 942-943
staphylococcal, 1136
Streptococcus pneumoniae, 935, 1232-1233
Strongyloides stercoralis, 943
Toxoplasma gondii, 943
viral, 939-943
VZV, 941
mortality with, 933
mumps immunization and, 2406
in severe combined immunodeficiency, 915
vaccination after, 944
in Wiskott-Aldrich syndrome, 913
Hematospermia, in *Schistosoma* spp. infection, 3065
Hemochromatosis, 657, 673
Hemolysins
Aeromonas spp., 1540
Staphylococcus aureus, 17, 1115-1117
Streptococcus agalactiae, 1154
Streptococcus pyogenes, 1141
in urinary tract infection, 538
Vibrio parahaemolyticus, 1557-1558
Hemolysis, in congenital syphilis, 1770
Hemolytic anemia
in human erythrovirus B19 (human parvovirus B19) infection, 1849-1850, 1855
in infectious mononucleosis, 2002
in *Leptospira* spp. infection, 1746-1747
in *Mycoplasma pneumoniae* infection, 2682
rubella virus infection and, 2212
Hemolytic-uremic syndrome (HUS), 17
in adenovirus infection, 1901
in enterohemorrhagic *Escherichia coli* infection, 1452, 1454, 1463
in enterovirus infection, 2078
in *Salmonella* ser. Typhi infection, 1501
in *Shigella* spp. infection, 1481
Hemophagocytic lymphohistiocytosis (hemophagocytic syndrome), 181
EBV and, 1997, 2001, 2001f, 2014
fever of unknown origin in, 846-847
HHV-8 and, 2021
treatment of, 2014
Hemorrhage, splinter, in infective endocarditis, 357
Hemorrhagic fever(s)
arenaviral. *See* Arenavirus infection
bioterrorist, 3585
Crimean-Congo, **2531-2535**, 2532f-2533f
Dengue. *See* Dengue hemorrhagic fever/dengue shock syndrome
filoviral. *See* Ebola virus infection; Marburg virus disease (Marburg fever, Marburg hemorrhagic fever)

Hemorrhagic fever(s) *(Continued)*
hepatitis with, 656
Omsk, **2316**
Hemorrhagic fever with renal syndrome
in adenovirus infection, 1901
in hantavirus infection, 2503-2504, 2504t, 2510-2511
Hendra virus infection, 494t-498t, 500-501
HEPA (high-efficiency particulate air) filtration, in lung transplant–related *Aspergillus* infection prevention, 970
Hepadnavirus type 1. *See* Hepatitis B virus (HBV) infection
Heparin, in *Neisseria meningitidis* infection, 1265
Hepatic abscess. *See* Liver abscess
Hepatic portoenterostomy, cholangitis and, 658-659, 664-665
Hepatic steatosis, 657-658
Hepatitis, **648-658**
adenovirus, 655, 1902
vs. anoxic injury, 657
autoimmune, 657
HAV infection and, 2141
bacterial, 650, 651t, 656-657
clinical manifestations of, 648-650
CMV, **654**, 1977-1978
coxsackievirus, 2070-2072
drug-related, 651t, 657
echovirus, 2070-2072
enterovirus, **655**
erythrovirus B19 (parvovirus B19), 655, 1854
fungal, 651t, 657
granulomatous, fever of unknown origin in, 845
hemorrhagic fever virus, 656
herpes B virus, 655
HHV-6, 654, 2017
HHV-7, 654
HHV-8, 654
historical perspective on, 648
HSV, 653-654, 1949, 1954
infectious, 650-657
isoniazid-related, 1366-1368
laboratory tests in, 649-650
Lassa virus, 2473
leptospiral, 656
macrolide-related, 3214-3215
measles virus, 655
noninfectious, 648, 651t, 657-658
parasite, 651t, 657
patient history in, 648-649
physical examination in, 649
rash in, 649
rickettsial, 656-657
rubella virus, 655-656, 2213
Shigella spp., 1481
syphilitic, 1771
treponemal, 656
viral, 650-656, 651f-652f, 651t. *See also specific hepatitis viruses*
laboratory tests in, 649-650, 662
pancreatitis in, 693-694, 693t
patient history in, 648-649
physical examination in, 649
serum iron in, 78
VZV, 654
Hepatitis A virus (HAV), 2128-2129
classification of, 2128
genome of, 2128-2129, 2129f
Hepatitis A virus (HAV) infection, 650, 651t, **2128-2147**
age and, 2132-2133
atypical, 2139-2141, 2139t
Hepatitis A virus (HAV) infection *(Continued)*
autoimmune hepatitis after, 2141
cholestatic, 2140-2141
clinical manifestations of, 2137-2139
community-wide epidemics of, 2134
cutaneous manifestations of, 2140
in daycare facilities, 2134-2135, 3564
diagnosis of, 2138-2139
epidemiology of, 2129-2134, 2131f-2132f
extrahepatic manifestations of, 2140
foodborne, 2134-2136
fulminant, 2139-2141
geographic patterns of, 2130-2134, 2131f
historical perspective on, 648, 2128
in illicit drug users, 2135
immune response to, 2136-2137
immunization against, 2142-2146, 2142t, 2145t, 3433-3437, 3434t, 3436t
immunoglobulin prevention of, 2141-2142
in international adoptees and refugees, 3122-3124
laboratory abnormalities in, 2138
laboratory tests in, 649-650, 651f
in MSM population, 2134-2135
neonatal, 2130
neurologic manifestations of, 2140
nosocomial, 2135, 3384
pancreatitis with, 693-694
pathogenesis of, 2136
pathology of, 2136
postexposure prophylaxis in, 2142, 2144, 3436-3437, 3436t
in pregnancy, 2130, 2140
prevention of, 2141-2146, 3514-3515
race/ethnicity and, 2133, 2133f
relapsing, 2139
in schools, 2135
transfusion-related, 2135
transmission of, 2129-2130, 2130f, 2134
travel-related, 2134-2135, 2141, 3515
treatment of, 2141
vascular manifestations of, 2140
Hepatitis B core antigen (HBcAg), 1912, 1918f-1919f, 1919
Hepatitis B e antigen (HBeAg), 1912, 1915, 1918f
Hepatitis B surface antigen (HBsAg), 1912, 1918f
Hepatitis B virus (HBV), 1911-1915
genome of, 1912-1915
genotypes of, 1913, 1916
life cycle of, 1913-1914, 1914f
particles of, 1912f, 1915, 1918f
surface antigens of, 1912, 1912f
Hepatitis B virus (HBV) infection, 650-652, **1911-1927**
acute, 1917-1918, 1918f
chronic, 1915, 1918-1919, 1918f
epidemiology of, 1917
follow-up for, 1927, 1927t
in daycare facilities, 3564-3565
epidemiology of, 1916-1917
exanthem in, 649, 742t-748t, 749
extrahepatic manifestations of, 1919
fulminant, 1916, 1920
HDV infection and, 1929-1931, 1930f-1931f
hepatocellular carcinoma and, 1916
histopathology of, 1919-1920, 1919f
historical perspective on, 648
HIV infection and, 1923-1924
immune response to, 1915-1916
Hepatitis B virus (HBV) infection *(Continued)*
immunization against, 1917, 1917t, 1925-1927, 1925t, 3437-3441, 3440t
hepatocellular carcinoma and, 1916
in international adoptees and refugees, 3124-3125
after kidney transplantation, 1007, 1014
Knodell-Ishak score in, 1919-1920
laboratory tests in, 649-650, 652f
natural history of, 1917-1919
neonatal, 1915, 1917, 1925-1926
pancreatitis with, 693-694
pathogenesis of, 1913-1916, 1914f
prevention of, 1917, 1917t, 1924-1927, 1925t, 3515-3519, 3518t
HBIG in, 1924-1925, 3516-3517
household precautions in, 1927
in liver transplantation, 3517-3519
perinatal, 3517-3518, 3518t
postexposure prophylaxis in, 3517-3519, 3518t
after sexual exposure, 3518-3519, 3518t
in solid-organ transplantation, 1924
transmission of, 1917
treatment of, 1920-1923, 1923t, 1927
adefovir dipivoxil in, 1922
entecavir in, 1922-1923
interferon-α in, 1920-1922
lamivudine in, 1921-1922
polymerase inhibitors in, 1921
tenofovir disoproxil fumarate in, 1922
Hepatitis C virus (HCV), 2318-2319, 2318f
genotypes of, 2319-2320
Hepatitis C virus (HCV) infection, 652-653, **2317-2326**
in burn patient, 1053-1054
chronic, 2320
clinical manifestations of, 2320-2321
diagnosis of, 2321-2323, 2322f
encephalitic, 494t-498t
epidemiology of, 2319
exanthem in, 742t-748t, 749
extrahepatic manifestations of, 2320-2321
genetic factors in, 2323
historical perspective on, 648, 2318
immune response to, 2321
in international adoptees and refugees, 3124-3125
after kidney transplantation, 1007, 1014-1015
laboratory tests in, 649-650
pathogenesis of, 11, 16, 20, 2319
prevention of, 2324-2325, 3519-3520
severity of, 2321-2323
sexual transmission of, 2320
transmission of, 2320
IVIG-related, 3496
treatment of, 2323-2324
vertical transmission of, 2320
Hepatitis D antigen (HDAg), 1928-1929
Hepatitis D virus (HDV), 1928
Hepatitis D virus (HDV) infection, 653, **1927-1933**
clinical manifestations of, 1929-1931
diagnosis of, 1929
epidemiology of, 1928
HBV infection with, 1929-1931, 1930f-1931f
historical perspective on, 648
immune response to, 1928-1929
laboratory tests in, 649-650
pathogenesis of, 1928-1929
prevention of, 1932
treatment of, 1931-1932

Hepatitis E virus (HEV), 2159-2160
classification of, 2160
genome of, 2159, 2159f
genotypes of, 2160
life cycle of, 2160
structure of, 2159-2160
Hepatitis E virus (HEV) infection, 653, **2158-2167**
breast-feeding and, 2163
clinical manifestations of, 2164-2165, 2165f
diagnosis of, 2165-2166
endemic, 2160-2162, 2161t
epidemic, 2162
epidemiology of, 2160-2163
extrahepatic manifestations of, 2164-2165
foodborne, 2163
historical perspective on, 648, 2158
immune response to, 2163, 2164f
immunization against, 2166-2167
laboratory tests in, 649-650
mortality in, 2164
pancreatitis with, 693-694
pathogenesis of, 2163
in pregnancy, 2163-2164
prevention of, 2166-2167
transfusion-related transmission of, 2163
treatment of, 2166
vaccine against, 2166-2167
vertical transmission of, 2163
zoonotic, 2162
Hepatobiliary scintigraphy (HIDA scan)
in cholangitis, 661
in cholecystitis, 669
Hepatoblastoma, 657
Hepatocellular carcinoma, 1916
Hepcidin, 78
Hernia, inguinal, 687-688
Herpangina, 2065, 2066t
vs. HSV gingivostomatitis, 1941
Herpes B virus infection, 494t-498t, 655, 812
Herpes gladiatorum, 1945
Herpes labialis, 154-155, 1950, 1950f, 1952-1953, 1952f
Herpes simplex virus (HSV), 1933-1934
antibodies against, 1957
detection of, 1955-1958, 1956f-1957f
electron microscopy of, 1956, 1957f
gel electrophoresis of, 1957-1958, 1958f
immediate early genes of, 20
latency of, 20, 1938
lytic replication cycle of, 20
Herpes simplex virus (HSV) infection, **1933-1968**
abrasion-related, 1944, 1944f
acyclovir-resistant, 1963-1964
anorectal, 1948-1949
burn-related, 1053-1054, 1944, 1944f, 1953
clinical manifestations of, 742t-748t, 1940-1955
CNS, 494t-498t, 499, 505, 1945-1948, 1946t, 1947f-1948f, 1949t
acute retinal necrosis after, 1945
in immunocompromised host, 1954
pathogenesis of, 1939
vs. tick-borne encephalitis virus infection, 2310-2311
treatment of, 1960
complications of, 1958
congenital, 818-819, 881, 1954
ocular manifestations of, 818-819
pathogenesis of, 1939-1940
conjunctival, 803-804
neonatal, 805-806, 805t
Herpes simplex virus (HSV) infection *(Continued)*
corneal, 803-804, 1945, 1959
in daycare facilities, 3560
diagnosis of, 1955-1958, 1956f-1957f
digital, 1944-1945, 1944f-1945f
disseminated, 1954
epidemiology of, 1935-1938
in epiglottitis, 245
erythema multiforme and, 764
esophageal, 594-597, 596f, 1948-1949, 1953
external auditory canal, 208
fetal, 1954-1955
gastrointestinal tract, 1948-1949
genital, 572t-573t, 582, 583t, 742t-748t, 1941-1943
clinical manifestations of, 1941-1943, 1942f
complications of, 1943
epidemiology of, 1936
psychological aspects of, 1943
recurrent, 1942f, 1950-1951, 1966-1967
treatment of, 1960-1961, 1960t
after heart transplantation, 949
after hematopoietic stem cell transplantation, 939
hepatic, 653-654, 1949
in HIV-infected host, 1943, 1952-1953, 1953f
immune response to, 1938-1939
in immunocompromised host, 1951-1955, 1951t, 1952f-1953f, 1961-1962, 1961t
after kidney transplantation, 1006-1007
labial, 1950, 1950f, 1952-1953, 1952f
after liver and intestinal transplantation, 998-999
after lung transplantation, 978
meningeal, 487, 1943, 1946
mucocutaneous, 1943-1945, 1943f-1944f, 1953
recurrence of, 1950
treatment of, 1961-1962
neonatal, 1935, 1954-1955
CNS, 1955
disseminated, 1954-1955
epidemiology of, 1936-1938
hepatic, 653-654
pathogenesis of, 1939-1940
prevention of, 1964-1966, 3522
recurrent, 1967
treatment of, 1962-1963
nosocomial, 3383
ocular, 810, 1945
treatment of, 1959
oral, 154-155, 742t-748t, 1940-1941, 1940f
in HIV infection, 149
recurrent, 1950, 1950f
treatment of, 1959
pathogenesis of, 1938-1940
pharyngeal, 159t-160t, 161, 1941
during pregnancy, 1936-1937, 1965, 1967
prevention of, 1964-1967, 3522
prognosis for, 1958
pulmonary, 1953-1954
recurrent, 1949-1951, 1950f
erythema multiforme with, 1951
pathogenesis of, 1938-1939
sequelae of, 1958
sports-related, 1945
transmission of, 1934-1935
treatment of, 1958-1964, 3279-3280, 3282-3283, 3288
acyclovir resistance and, 1963-1964
prophylactic, 1966-1967
Herpes simplex virus (HSV) infection *(Continued)*
renal dysfunction and, 1961, 1961t
susceptibility testing in, 1958
vaccine against, 1966
Herpes zoster, 742t-748t, **2021-2033**
clinical manifestations of, 2027-2028, 2027f
complications of, 2027-2028
diagnosis of, 2028
epidemiology of, 2023-2024
immunization against, 2023-2024
pathogenesis of, 2024-2025
prevention of, 2030-2032
prognosis for, 2029-2030
risk factors for, 2023-2024
after solid-organ transplantation, 979
transmission of, 2023
treatment of, 2028-2029
Herpes zoster ophthalmicus, 804, 810
Herpes zoster oticus, 208, 208f
Herpes zoster uveitis, 810
Herpesvirus simiae. *See* Herpes B virus infection
Herpetic whitlow, 155, 1944-1945, 1944f-1945f
Heterophyes spp. infection (heterophyiasis), **3057-3058**
Hexachlorophene, toxicity of, 1121
HHV-6 infection. *See* Human herpesvirus–6 (HHV-6) infection
HHV-7 infection. *See* Human herpesvirus–7 (HHV-7) infection
HHV-8 infection. *See* Human herpesvirus–8 (HHV-8) infection
Hia adhesin, 6-7, 9f
Hidradenitis suppurativa, **777**
High-altitude illness, 3118
Highlands J virus, 2231
Highly active antiretroviral therapy (HAART), 600, 2598
immune reconstitution inflammatory syndrome and, 1360-1361, 2601
postexposure, 2594
Higouménakis sign, 1772
Hip
dislocation of, 733
septic arthritis of, 730-732, 730t, 731f
toxic (transient) synovitis of, 732
tuberculosis of, 1355
Hippocampus, in bacterial meningitis, 435-436
Hirschsprung disease, *Clostridium difficile* infection and, 634
Histo spots, 813
Histocompatibility leukocyte antigen (HLA), in acute disseminated encephalomyelitis, 517
Histoplasma capsulatum, 2807-2808, 2808f
Histoplasma capsulatum infection (histoplasmosis), **2807-2832**
clinical manifestations of, 2811-2817, 2812t
CNS, 476-477, 480t, 494t-498t, 2811, 2816
treatment of, 2828
CT in, 2817, 2818f
cutaneous, 757t, 2813
diagnosis of, 2819-2824, 2819t-2820t
antigen test in, 2822-2823
complement fixation test in, 2821-2822
culture in, 2820-2821
histology in, 2819-2820, 2819f
immunodiffusion test in, 2822
molecular methods in, 2823-2824
serology in, 2821-2823
skin test in, 2823

Histoplasma capsulatum infection (histoplasmosis) *(Continued)*
disseminated, 2813-2817
in HIV-infected patient, 2602, 2815-2816, 2827-2828
in immunocompromised host, 2814-2815
in immunosuppressed host, 2827
in infant, 2814
treatment of, 2827
epidemiology of, 2808-2809
gastrointestinal tract, 2815
granuloma in, 2811-2813
immune response to, 23, 2810-2811
after intestinal transplantation, 1000
joint, 2828-2829
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 973-974
mediastinal, 422, 2826-2827, 2826f
mediastinal fibrosis in, 2813, 2817-2819
meningeal, 476-477, 480t, 2816, 2821, 2828
ocular, 813, 2816-2817
pathology of, 2811
pathophysiology of, 2809-2811
pericardial, 373, 377-378, 2813, 2828-2829
pleural, 309-310
prevention of, 2829-2830
prognosis for, 2829
pulmonary, 2812-2813
cavitary, 2813
treatment of, 2826
radiography in, 2817-2819, 2818f
in traveler, 3116-3117
treatment of, 2824-2829, 2824t, 3295t
surgical, 2829
Histoplasma capsulatum var. *duboisii*, 2808, 2817
HIV-1. *See* Human immunodeficiency virus type 1 (HIV-1)
HIV-2. *See* Human immunodeficiency virus type 2 (HIV-2)
HIV infection. *See* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS)
hMPV infection. *See* Human metapneumovirus (hMPV) infection
H1N1 influenza. *See* Influenza virus infection, H1N1
Hodgkin disease, 182-183, 182t
EBV and, 1997, 2005
reovirus and, 2171
Holocyclotoxin, 3071
Honey, infant botulism and, 1804-1805
Hookworm infection, **3016-3018**
clinical manifestations of, 3017-3018
diagnosis of, 3018
epidemiology of, 3017
pathophysiology of, 3017
prevention of, 3018
treatment of, 3018, 3327t-3346t
Hordeolum, **796**
Hormones
adrenal gland, 81-82
carbohydrate-regulating, 82
measurement of, 81-82
pituitary gland, 81
thyroid gland, 82
Hospitalization
infection during. *See* Health care–associated infection
normal flora effects of, 89-90
otitis media and, 212
Hot-foot syndrome, 1589-1591
Hot-tub syndrome, 776-777, 1589-1591
Howell-Jolly bodies, 906
HPV. *See* Human papillomavirus (HPV)
HSCT. *See* Hematopoietic stem cell transplantation (HSCT)
HSV. *See* Herpes simplex virus (HSV)
Human bite, 780, 780t, 3573t
clinical manifestations of, 3574-3575
diagnosis of, 3575-3577
epidemiology of, 3571
microbiology of, 3572, 3573t
treatment of, 3251, 3575-3577, 3576t
Human bocavirus (HBoV), 1858
historical perspective on, 1858
Human bocavirus (HBoV) infection, **1858-1863**
in common cold, 132
diagnosis of, 1861
epidemiology of, 1858-1859
exanthem in, 741, 742t-748t, 1861
gastrointestinal tract, 1860-1861
in immunocompromised host, 1861
Kawasaki disease and, 1861
pathogenesis of, 1859
pulmonary, 284, 1860
respiratory tract, 1859-1860
treatment of, 1861
Human erythrovirus B19 (human parvovirus B19), 1844-1845
historical perspective on, 1843-1844
Human erythrovirus B19 (human parvovirus B19) infection, **1843-1857.** *See also* Erythema infectiosum
aplastic crisis in, 1849-1850
clinical manifestations of, 1847-1855, 1847f, 1848t
CNS, 1853
in daycare facilities, 3554-3555
diagnosis of, 1855-1856
differential diagnosis of, 1855
epidemiology of, 1845-1846
erythema multiforme bullosum in, 1849
exanthem in, 741, 1847-1849, 1847f-1849f
fetal, 1847, 1852-1853, 1856
genotypes of, 1843
glove and sock syndrome in, 1849
after heart transplantation, 954
hematologic manifestations of, 1849-1850
hepatic, 655, 1854
historical perspective on, 1843-1844
immune response to, 1846
in immunocompromised host, 1847, 1851-1852, 1856
joint manifestations of, 1851
lymphadenitis in, 1854-1855
myocardial, 1853-1854, 1856
nosocomial, 3384
ocular, 811
pancytopenia in, 1850
pathogenesis of, 1846-1847, 1846f
prevention of, 1856-1857, 3527
prognosis for, 1856
red cell aplasia and, 1854
renal, 1855
thrombocytopenia in, 1850
transmission of, 1845-1846
treatment of, 1856
uveal, 811
Human fibroblast–derived interferon, in adenovirus infection, 1908
Human granulocytic anaplasmosis, **2661-2663**
Human herpesvirus–6 (HHV-6), 2016, 2017f
isolation of, 2019
Human herpesvirus–6 (HHV-6) infection, **2016-2019.** *See also* Roseola infantum
clinical manifestations of, 2016-2018
congenital, 2018
diagnosis of, 2019
encephalitic, 494t-498t, 499
epidemiology of, 2016
exanthem in, 742t-748t, 749, 765, 2017, 2017t
after hematopoietic stem cell transplantation, 941, 2018
hepatic, 654, 2017
after kidney transplantation, 1010, 2017
after liver transplantation, 999
after lung transplantation, 979
meningeal, 487, 2018
neurologic complications of, 2018
pathogenesis of, 2018-2019
posttransplantation acute limbic encephalitis and, 2018
treatment of, 2019
Human herpesvirus–7 (HHV-7) infection, **2016-2019.** *See also* Roseola infantum
clinical manifestations of, 2018
exanthem in, 742t-748t, 749
hepatic, 654
after kidney transplantation, 1010
after lung transplantation, 979
meningeal, 487, 2018
neurologic complications of, 2018
pathogenesis of, 2018-2019, 2019f
after transplantation, 999
Human herpesvirus–8 (HHV-8) infection, **2019-2021**
clinical manifestations of, 2020
diagnosis of, 2021
exanthem in, 742t-748t, 749
hemophagocytic lymphohistiocytosis and, 2021
hepatic, 654
after kidney transplantation, 1010
after lung transplantation, 979
transmission of, 2020-2021
after transplantation, 999
treatment of, 2021
Human immune globulin. *See* Immunoglobulin(s)
Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS), **2591-2619.** *See also* Retroviruses
acute retroviral syndrome in, 161
anemia in, 2607-2608
Bacille Calmette-Guérin vaccine complications in, 1377
BK virus infection in, 1867
Blastomyces dermatitidis infection in, 2729-2730
in burn patient, 1054
Campylobacter jejuni infection in, 1651
Candida infection in, 148-149, 474, 2602
caries in, 149
cervical lymphadenitis in, 177-178
cholangitis in, 666
clinical manifestations of, 2598-2603, 2598t
CMV infection in, 811, 1973, 1979-1980, 2601-2602
Coccidioides spp. infection in, 2775-2776
complications of, 2603-2608
cardiac, 2607
CNS, 2604-2605
gastrointestinal, 2605-2606
hematologic, 2607-2608
neurocognitive, 2605

Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS) *(Continued)*
oncologic, 2606-2607
psychosocial, 2605
pulmonary, 2603-2604, 2603f
renal, 2607
Cryptococcus infection in, 476, 2602, 2798
Cryptosporidium spp. infection in, 614-615, 2897-2898, 2901, 2903
in daycare facilities, 3560
definition of, 2591-2592
diagnosis of, 2608-2609, 2608t
EBV infection in, 2006-2007, 2014
epidemiology of, 2591-2595, 2592t
exanthem in, 742t-748t, 749-750
gastrointestinal tract infection in, 599-600, 600t
gonococcal infection and, 1296
Haemophilus ducreyi infection in, 1688
Hafnia alvei infection in, 1534-1535
HBV infection in, 1923-1924
Histoplasma capsulatum infection in, 476-477, 2602, 2815-2816, 2827-2828
HSV infection in, 1943, 1952-1953, 1953f, 2602
HTLV-1 infection and, 2577
immune dysfunction in, 2596-2598, 2597f
immune reconstitution inflammatory syndrome and, 1360-1361
in international adoptees and refugees, 3125
IVIG in, 3525-3526
Kaposi sarcoma in, 654, **2019-2021**, 2606
Klebsiella granulomatis (Calymmatobacterium granulomatis) infection in, 1643
leiomyoma in, 2606-2607
leiomyosarcoma in, 2606-2607
Leishmania spp. infection in, 2949, 2956-2957
linear gingival erythema in, 149
lymphocytic interstitial pneumonitis/hyperplasia in, 296, 1997, 2007
lymphoid interstitial pneumonitis in, 268
lymphoma in, 2006-2007, 2013
microsporidial infection in, 615
molluscum contagiosum in, 782-783, 795
monitoring of, 2609
mumps immunization in, 2406
mycobacterial infection in, 684
Mycobacterium avium-intracellulare complex infection in, 1384-1385, 2600-2601
Mycobacterium leprae infection in, 1403
Mycobacterium ulcerans infection in, 1415
myocarditis in, 398
neutropenia in, 2607
non-Hodgkin lymphoma in, 2606
nontuberculous mycobacterial infection in, 1384-1385
ocular manifestations of, 811
oral hairy leukoplakia in, 2007
oral manifestations of, 148-149
osteomyelitis in, 724
pancreatitis in, 692, 694
Paracoccidioides spp. infection and, 2787
parotid gland enlargement in, 191-192
pathogenesis of, 2595-2598, 2596f
chemokine receptors in, 10-11
pleural effusion in, 310
Pneumocystis infection in, 2599-2600, 2599f, 2600t, 3007-3009
postexposure prophylaxis in, 2594
probiotics in, 3372
prognosis for, 2617
progressive multifocal leukoencephalopathy in, 1869
Pseudomonas aeruginosa infection in, 1595
recalcitrant erythematous desquamating disorder and, 860
Rhodococcus equi infection and, 1427-1428
RSV infection in, 2421
selenium deficiency in, 79
Shigella spp. infection in, 1482
sinopulmonary infection in, 268
Sporothrix schenckii infection in, 2834
staging of, 2591-2592, 2592t
Streptococcus pneumoniae infection and, 1204-1205, 1232
syphilis and, 1762, 1778
Toxoplasma gondii infection in, 2993, 3003-3004
transmission of, 2592-2595
perinatal, 2593-2594
sexual, 2594
transfusion-related, 2592-2593
transplant-related, 1015
treatment of, 600, 2609-2617, 2610t, 2611f
immune-based, 2616
immune reconstitution inflammatory syndrome and, 1360-1361
integrase inhibitors in, 2615-2616
non-nucleoside reverse transcriptase inhibitors in, 2613-2614
nucleoside reverse transcriptase inhibitors in, 2612-2613, 2612f
nucleotide reverse transcriptase inhibitors in, 2613
protease inhibitors in, 2614-2615
supportive, 2616
Trichomonas vaginalis infection and, 2890
Trypanosoma cruzi infection in, 2965
tuberculosis in, 1336, 1339, 1360, 2601
vaccine against, 2616-2617
varicella immunization in, 2032
VZV infection in, 2026, 2602
Human immunodeficiency virus type 1 (HIV-1), 2581t, 2595, 2595f. *See also* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS); Retroviruses
HIV-2 infection and, 2583-2584
Human immunodeficiency virus type 2 (HIV-2), 2581-2582, 2581t. *See also* Retroviruses
Human immunodeficiency virus type 2 (HIV-2) infection, **2580-2588**
diagnosis of, 2585-2586
epidemiology of, 2582-2583
HIV-1 infection and, 2583-2584
monitoring of, 2588
natural history of, 2585-2586
perinatal transmission of, 2584-2585
sexual transmission of, 2584
transmission of, 2584-2585
treatment of, 2586-2588
Human leukocyte antigen (HLA)
in granuloma inguinale, 1641
in human erythrovirus B19 (human parvovirus B19) infection, 1845
in *Mycobacterium leprae* infection, 1395
in rheumatic fever, 406
in rubella virus infection, 2204
in Whipple disease, 641
Human metapneumovirus (hMPV), 2435, 2435f
strain variation in, 2436
Human metapneumovirus (hMPV) infection, **2434-2443**
asthma and, 2439
clinical manifestations of, 2437-2439
CNS, 494t-498t, 2439
coinfection with, 2438-2439
in common cold, 132
diagnosis of, 2440
differential diagnosis of, 2439-2440
epidemiology of, 2435-2437
exanthem in, 742t-748t, 749
geographic distribution of, 2435
after hematopoietic stem cell transplantation, 943
historical perspective on, 2434-2435
immune response to, 2437
in immunocompromised host, 2439
incidence of, 2436-2437
lower respiratory tract, 2438-2439, 2438t, 2439f
after lung transplantation, 980
otitis media and, 2438-2439
pathogenesis of, 2437
pathology of, 2437
prevalence of, 2436-2437
prevention of, 2440
prognosis for, 2440
pulmonary, 284, 285t, 2438-2439, 2438t, 2439f
seasonal pattern of, 2436
Streptococcus pneumoniae immunization and, 1230
treatment of, 2440
upper respiratory tract, 2438
vaccine against, 2440
Human monocytic ehrlichiosis, **2661-2663**
Human papillomavirus (HPV), 1871-1873, 1872t-1873t
Human papillomavirus (HPV) infection, **1871-1887**
anal cancer and, 1873t, 1875, 1880-1881
anogenital, 572t-573t, **580-581**, 580f-581f, 1873t, 1876-1877
colposcopy in, 1882
differential diagnosis of, 1877
epidemiology of, 1874-1876
female-specific, 1877-1878
male-specific, 1878
Pap smear for, 581, 1881-1882
cancer and
female-specific, 1873t, 1874-1875, 1877-1878
male-specific, 1873t, 1875-1876, 1878
clinical manifestations of, 1876-1881
conjunctival, 1879-1880
cutaneous, 742t-748t, 1873t, 1876
cytology of, 1881-1882
diagnosis of, 1881-1882
DNA assays in, 1882
epidemiology of, 1874-1876
gastrointestinal, 1880-1881
histology of, 1882
historical perspective on, 1871
immunization against, 1884-1886, 3441-3443
bivalent vaccine in, 1885-1886
quadrivalent vaccine in, 1884-1886
after kidney transplantation, 1010
molecular assay in, 1882
nasal, 1879
oropharyngeal cancer and, 1875-1876, 1880

Human papillomavirus (HPV) infection *(Continued)*
prevention of, 1884-1886
bivalent vaccine in, 1885-1886
quadrivalent vaccine in, 1884-1886
respiratory, 1878-1879, 1878f-1879f
surgical treatment of, 1882-1883
treatment of, 1882-1884
Human parvovirus B19 infection. *See* Human erythrovirus B19 (human parvovirus B19) infection
Human polyomaviruses. *See* BK virus; JC virus; Simian virus 40 (SV40)
Human T-cell lymphotropic virus type 1 (HTLV-1) infection, **2560.** *See also* Retroviruses
adult T-cell leukemia and, 2565-2569, 2566t-2567t
arthritis and, 2575-2576
bronchiectasis and, 2576
disease associations of, 2561t, 2564-2565
epidemiology of, 2560-2577
exanthem in, 742t-748t, 749-750
helminthic coinfection with, 2571
HIV-1 infection and, 2577
infective dermatitis and, 2574-2575, 2574t
lymphocytic pneumonitis and, 2576
myelopathy and, 2569-2573, 2570t
ocular, 811
parenteral transmission of, 2564
polymyositis and, 2576
sexual transmission of, 2562
Sjögren syndrome and, 2576
transmission of, 2562-2564
uveal, 2573-2574
vertical transmission of, 2562-2564
Human T-cell lymphotropic virus type 2 (HTLV-2) infection, **2577-2580.** *See also* Retroviruses
clinical manifestations of, 2579-2580
epidemiology of, 2578
pathogenesis of, 2577-2578
transmission of, 2579
Trypanosoma cruzi infection and, 388-389
Human T-cell lymphotropic virus type 3 (HTLV-3) infection, 2580
Human T-cell lymphotropic virus type 4 (HTLV-4) infection, 2580
Humerus, *Streptococcus agalactiae* infection of, 1161, 1161f
Hyaluronate lyase, of *Streptococcus pneumoniae*, 1211
Hyaluronidase, 17-18
of *Streptococcus pneumoniae*, 1214
Hydatid disease. *See Echinococcus granulosus* infection (echinococcosis, hydatid disease)
Hydrocephalus
in meningitis, 438, 445, 1260
otitic, 237
Hydrogen peroxide, in glutathione synthetase deficiency, 59
Hydrophobia, 2450
Hydrops fetalis, 1847, 1852, 1856, 2692
Hydrosalpinx, 589, 589f
Hydroxychloroquine, in interstitial lung disease, 300
17-Hydroxycorticosteroids, plasma, 81
Hygiene. *See also* Infection control practices
in *Clostridium difficile* infection prevention, 637-638
in CMV infection prevention, 1989
in HSV infection prevention, 1964-1966
infection and, 109-110
Hygiene *(Continued)*
in influenza virus infection prevention, 2355
in *Leptospira* spp. infection prevention, 1757
perineal, vulvovaginitis and, 564-566
in *Salmonella* spp. infection prevention, 1507
Hygroma, cystic, 182
Hylesia alinda (moth), 758t
Hymenolepis diminuta (rat tapeworm) infection, **3042**
Hymenolepis nana (dwarf tapeworm) infection, **3042**, 3327t-3346t
Hyper-IgE syndrome, **55**, 919
furunculosis and, 777
lung abscess and, 317
Hyper-IgM syndrome, **49-50**, 907t, **909-910**
vs. chronic bronchitis, 267
Cryptosporidium spp. infection and, 2900
Hyperbaric oxygen therapy
in gas gangrene, 1799
in mucormycosis, 2844
in necrotizing fasciitis, 150
Hyperbilirubinemia, differential diagnosis of, 662-663, 662t, 669
Hypercholesterolemia, after HIV infection treatment, 2606-2607
Hypercobaltemia, 77t
Hypercupremia, 77t
Hyperferremia, hepatitis-induced, 78
Hypergallemia, 77t
Hyperglycemia, 72
in septic shock, 829, 832
Hyperimmunoglobulinemia D with periodic fever syndrome, 847
Hyperimmunoglobulinemia E, **55**, 317, 919
Hyperinfection syndrome, *Strongyloides stercoralis*, 615-616
Hypermagnesemia, 77t
Hyperpigmentation, in Whipple disease, 644
Hyperreactive malarial syndrome, 2929
Hypersensitivity pneumonitis, 297, **301-305**
clinical presentation of, 301-303
diagnosis of, 303-304, 303f-305f
etiology of, 301, 302t-303t
forms of, 301
pathogenesis of, 301
prognosis for, 305
treatment of, 304-305
Hypersensitivity reaction
animal serum–related, 3493
cephalosporin-related, 3199
chloramphenicol-related, 3217
lincomycin-related, 3222
penicillin-related, 3189-3191, 3189t
TMP-SMX–related, 3229
Hypersideremia, 77t
Hypertriglyceridemia, 74
Hyperventilation, in septic shock, 831
Hyperzincemia, 77t
Hyphal wall protein 1 (Hwp1), 9
Hypoaminoacidemia, 70
Hypocalcemia
in septic shock, 829-830, 832
in toxic shock syndrome, 829-830
Hypochromia, 77t
Hypoferremia, 78
Hypogammaglobulinemia, transient, of infancy, **47**, **912**
Hypoglycemia, 72
in cholera, 1551
in malaria, 2928
in newborn, 72
in septic shock, 829, 832
Hypokalemia, in *Leptospira* spp. infection, 1747
Hyponatremia, 76
in *Leptospira* spp. infection, 1753
in septic shock, 832
Hypopyon, 809-810
Hyposideremia, 77t, 78
Hypotension
in chronic fatigue syndrome, 1088-1089
in *Leptospira* spp. infection, 1748
Hypothalamic-pituitary-adrenal axis, in chronic fatigue syndrome, 1088
Hypothesis testing, 120-121. *See also* Biostatistics
Hypoxemia, in community-acquired pneumonia, 287-288, 287t
Hypoxia, in *Leptospira* spp. infection, 1747
Hypozincemia, 77t, 79

I

Ibuprofen
vs. acetaminophen, 87-88
acetaminophen with, 87
in fever, 87
in otitis media, 228
Ichthyosis, PMN chemotaxis in, 56
IcsA protein, 16
IcsP protein, 16
Id reaction, 784-785, 785f
Ig. *See* Immunoglobulin(s)
Igbo-ora fever, 2251
IGIM. *See* Immunoglobulin(s), intramuscular (IGIM)
IGIV. *See* Immunoglobulin, intravenous (IVIG, IGIV)
IKBKG gene defect (NEMO defect), 916, 916f
Ilheus virus infection, 2316t
Imipenem, in meningitis, 448
Imipenem-cilastatin, 3200, 3231t-3233t
Imiquimod
in genital HPV infection, 581
in papillomas, 1883
Immotile cilia syndrome
vs. chronic bronchitis, 267
lung abscess and, 317
rhinosinusitis and, 195
Immune complexes
in infective endocarditis, 354-355, 358
in *Streptococcus agalactiae* infection, 1158
Immune globulin. *See* Immunoglobulin(s)
Immune reconstitution inflammatory syndrome (IRIS)
cryptococcosis and, 972, 2803-2804
HAART and, 1360-1361, 2601
tuberculosis and, 1360-1361
Immunity
adaptive, 35-36, 36f
antigen-presentation in, 36-38, 37f
B cells in, 40-42
in burns, 1050
CD1 antigen-presenting molecules in, 37-38
class I MHC in, 36-37, 38f
class II MHC in, 37, 38f
complement in, 31
deficiencies of, 46-48. *See also specific immunodeficiency disorders*
dendritic cells in, 38
immunoglobulins in, 40-41, 41f
pathogen evasion of, 19
T cells in, 38-40

Immunity *(Continued)*
concept of, 103
deficiencies in, **45-59.** *See also* Immunodeficiency disorders *and specific disorders*
fever effects on, 85
innate, 24-42, 35f
chemokines in, 27-28
complement in, **28-31**, 29f
cytokines in, 25-27, 26t
defensins in, 24-25
epithelium in, 24-25
natural killer cells in, 28
phagocytes in, 31-35, 32f-34f, 34t
Toll-like receptors in, 25, 25t
virus evasion of, 20
intestinal flora effects on, 93
in newborn, 42-45, 43f, 43t-44t
Immunization, **3420-3490.** *See also* Vaccine(s)
acute disseminated encephalomyelitis with, 516-517, 516t
adenovirus, 1908
anthrax, 1315-1316
arenavirus, 2476-2477
cholera, 1554-1555
Clostridium botulinum, 1795
CMV, 1989
combination vaccines for, 3476-3477, 3476t
contraindications to, 3426
Corynebacterium diphtheriae, 1308-1309
in cystic fibrosis, 344-345
dengue virus, 2289
diabetes mellitus and, 3428-3429
in DiGeorge syndrome, 913
dose for, 3422
DTaP/Tdap/DTP, 1630-1636, 3430-3431, 3457-3461
acellular vaccines for, 1630-1633, 1631t-1633t, 3458, 3458t
adverse events with, 1634-1635, 1635t
adverse events with, 1633-1635, 3430-3431, 3459
contraindications to, 1636, 3460-3461
disease incidence and, 1620f
efficacy of, 3459
encephalitis after, 502
failure of, 1620-1621
indications for, 3460
neurologic illness with, 3459
precautions for, 3460-3461
schedules for, 1635-1636
tolerance and, 44
whole-cell vaccines for, 1630, 3458
adverse events with, 1633-1634
efficacy of, 3420, 3421t
encephalitis after, 502
in endocarditis prevention, 369
group A streptococci, 406-407, 407f
Haemophilus ducreyi, 1688
Haemophilus influenzae type b, 461, 1679-1682, 3431-3433, 3432t
active, 1679-1681, 1680t
disease reemergence and, 1674
in epiglottitis prevention, 259
in meningitis, 429, 461
in meningitis prevention, 429
passive, 1681-1682
in pneumonia prevention, 285, 292
results of, 1666, 1682
hantavirus, 2511
HAV, 2142-2146, 2142t, 2145t, 3433-3437, 3434t, 3436t

Immunization *(Continued)*
HBV, 1917, 1917t, 1925-1927, 1925t, 3437-3441, 3440t
multiple sclerosis and, 3429
after heart transplantation, 955-956
Helicobacter pylori, 1698
HEV, 2166-2167
HPV, 1884-1886, 3441-3443
bivalent vaccine in, 1885-1886
quadrivalent vaccine in, 1884-1886
in immunodeficiency disorders, 63-64
in immunodeficiency evaluation, 62-63
implementation of, 3425-3426
influenza virus, 2353-2356, 3443-3448
adverse events with, 3445-3446
availability of, 2357
contraindications to, 3446-3448
economics of, 2356
efficacy of, 2355-2356, 3444-3445
encephalitis after, 502
after hematopoietic stem cell transplantation, 942
indications for, 3447f
maternal, 2356
in otitis media prevention, 231
precautions for, 3448f
precautions with, 3446-3448
preparations for, 3443-3444
production of, 2357
recommendations for, 2357, 3446
safety of, 2354-2355, 3445-3446
in international adoptees and refugees, 3130-3132, 3131t
for international traveler, 3094-3107, 3097t-3105t, 3427, 3428t
Japanese encephalitis virus, 2299-2300, 3106, 3479-3480
before kidney transplantation, 1003-1004
lapsed, 3423
Leptospira, for animals, 1757-1758
maternal, 44
measles, 2393-2394, 3448-3452
acute disseminated encephalomyelitis with, 517
adverse events with, 3449-3450
autism and, 3429
complications of, 2393-2394
contraindications to, 2393, 3450-3452
efficacy of, 3449
encephalitis after, 502
in HIV-infected host, 3429
immune globulin interaction with, 3451, 3452t
indications for, 3450
meningitis and, 488
precautions for, 2393, 3450-3452
preparations for, 3449
recommendations for, 2393
misconceptions about, 3426, 3427t
MMR/MMRV, 2031-2032, 2404-2405, 3472-3473
autism and, 3429
mumps, 2404-2405, 3455-3457
acute disseminated encephalomyelitis with, 517
adverse effects of, 2405, 3456
contraindications to, 2405-2406, 3457
in immunocompromised host, 2406
indications for, 3456
meningitis and, 488
precautions for, 3457
Mycobacterium leprae, 1409-1410
Mycobacterium tuberculosis, 1376-1378, 1377t
Mycoplasma pneumoniae, 2687
Neisseria gonorrhoeae, 1300

Immunization *(Continued)*
Neisseria meningitidis, 459-460, 460t, 1267-1270, 3452-3455
adverse events with, 3454
conjugate vaccine for, 1267-1270, 3454
contraindications to, 3455
polysaccharide vaccine for, 1267, 3453-3454
precautions for, 3455
recommendations for, 1268-1269, 1268t, 3454-3455
nitrogen balance and, 69, 69f
parainfluenza virus, 2370-2372
passive, **3491-3536.** *See also* Antitoxin; Immunoglobulin(s); Monoclonal antibodies; Passive immunization
for pediatric traveler, 3094-3107, 3097t-3105t
poliovirus, 2105-2106, 3463-3466
in premature infant, 44
protective efficacy of, 127-128, 127t
rabies, 781, 2458-2460, 2459t-2460t, 3480-3483
acute disseminated encephalomyelitis with, 516
encephalitis after, 502
meningitis and, 488
record keeping for, 3423-3424
rotavirus, 627, 2187-2191, 2187f, 3466-3468
adverse events with, 3467
contraindications to, 3468
efficacy of, 3466-3467
indications for, 3467-3468
non-live, 2191-2192
Rotarix, 2187f, 2190-2191, 2190f-2191f
RotaShield, 2187-2188, 2187f
RotaTeq, 2187f, 2188-2190, 2189f
safety of, 2191
schedule for, 2191
route of administration for, 3422, 3422t
rubella, 2221-2224, 3468-3470
acute disseminated encephalomyelitis with, 517
adverse reactions to, 2222-2223, 3469
contraindications to, 2223, 3469-3470
efficacy of, 3469
global results of, 2201-2203
inadvertent, 2223-2224
indications for, 3469
precautions for, 3469-3470
during pregnancy, 2223-2224
safety of, 3427-3429
Salmonella ser. Typhi, 1507
schedules for, 3420-3422, 3424-3425, 3424f-3425f, 3426t
for pediatric traveler, 3094-3107, 3097t-3105t
in sickle-cell disease, 60
simultaneous administration in, 3423, 3423t
smallpox, 749, 2036-2038
acute disseminated encephalomyelitis with, 517
complications of, 2037t, 2038-2039, 2039t
encephalitis after, 502
meningitis and, 488
myocarditis and, 382
Streptococcus agalactiae, 1168-1169
Streptococcus pneumoniae, 285, 292, 315, 1237-1244, 3461-3463
adverse events with, 3463
approval of, 97
conjugated vaccine for, 1238-1244, 1238f, 1238t, 1240f-1241f, 1240t

Immunization *(Continued)*
efficacy of, 3462-3463
human metapneumovirus infection and, 1230
indications for, 3463
in meningitis prevention, 458-459
in otitis media prevention, 230-231, 230t-231t
unconjugated vaccine for, 1237
tetanus, 1818-1819, 1818t, 3470-3472, 3472t
tick-borne encephalitis virus, 2311-2312, 2312f
typhoid, 1507, 3483-3485, 3484t
encephalitis after, 502
in urinary tract infection prevention, 552
vaccines for, 3476-3477, 3476t
Venezuelan equine encephalitis virus, 2240-2241
Vibrio cholerae, 1554-1555, 3478-3479
VZV, 2030-2032, 3472-3476
active, 2031-2032
adverse events with, 3473-3474
contraindications to, 3475-3476
efficacy of, 2031-2032, 3473
after hematopoietic stem cell transplantation, 941
indications for, 3474-3475
before kidney transplantation, 1009
before lung transplantation, 978-979
passive, 2030-2031
safety of, 2031
West Nile virus, 2272-2273
yellow fever, 2277-2278, 3485-3487
zoster, 2023-2024
Immunochromatographic membrane test, in pneumococcal pneumonia, 1217
Immunodeficiency disorders. *See also specific immunodeficiency disorders*
appendicitis in, 684
bacterial meningitis in, 436
cholangitis in, 666
dermatologic manifestations of, 62
diarrhea in, 599-600
evaluation for, 61-63
laboratory studies in, 62-63
patient history in, 25t, 61-62
physical examination in, 62
exanthems in, 766
fever of unknown origin in, 845
gene therapy in, 64
infection prevention in, 63-64
management of, 63-64
otitis media in, 211
pancreatitis in, 694
primary, **45-59**, **903-920.** *See also specific immunodeficiency disorders*
evaluation of, 903-908
cellular immunity in, 906-907
chest radiography in, 906, 906f
complement in, 907
delayed hypersensitivity skin tests in, 907
genetic testing in, 907, 907t
humoral immunity in, 906
laboratory tests in, 905-908, 906t
medical history in, 903-904, 904t
phagocyte function in, 907
physical examination in, 904-905, 905f
T cells in, 908
infection and, 903-904, 904t, 908, 908t
management of, 908, 908t, 3497, 3497t
X-linked, 904, 904t
secondary, 59-60, 903
Immunoglobulin(s), 40-41. *See also specific immunoglobulins*
aerosolized, 3498-3499
age-related levels of, 43t
anthrax, 3504
botulinum, 1808, 3505
CMV, 951, 1985-1986, 1988-1989, 3520-3521
in heart transplantation, 951-952
in lung transplantation, 975
in perinatal infection, 3521
in transplant recipient, 3520
HBV, 1924-1925, 3516-3517
in liver transplantation, 1924, 3517-3519
HCV, 3520
intramuscular (IGIM), 3493-3495
adverse effects of, 3494-3495
in antibody deficiency, 3494
in burn injury, 3508-3509
in HAV infection prevention, 2141-2142, 3514-3515
in HBV infection prevention, 3515-3516
in HCV infection prevention, 3519-3520
in infectious disease prevention, 3494
in measles prophylaxis, 2394-2395, 3526
pharmacology of, 3493-3494
in poliomyelitis prevention, 3512-3513
in respiratory infection, 3507-3509
in rubella prophylaxis, 2219-2220, 2224, 3531-3532
vaccine interactions with, 3496-3497
vaccinia, 3532-3533
in VZV infection, 3522
intrathecal, 3499
intratracheal, 3498-3499
intravenous (IVIG, IGIV), 3495-3497
administration of, 3495-3496
anaphylaxis with, 3496
in antibody deficiency, 3497
aseptic meningitis with, 3496
in burn injury, 1061, 3501, 3508-3509
in *Clostridium difficile* infection, 3505-3506
in CMV infection prevention, 3520-3521
in common variable immunodeficiency, 49, 910
in cystic fibrosis, 3508
cytokine effects of, 3361
in encephalitis, 510
in enteroviral meningoencephalitis, 3513
in enterovirus infection, 2103, 3513
in hematologic disease, 3499
hepatitis C transmission with, 3496
in HIV infection, 3525-3526
in HSV infection, 3522
in human erythrovirus B19 (human parvovirus B19) infection, 1852, 1854, 1856, 3527
in hyper-IgM syndrome, 50
in IgA deficiency, 47
in IgG subclass deficiency, 47
in immunoregulatory disorders, 3501-3502, 3502t
in intensive care patients, 3499-3500
in Kawasaki disease, 1073, 1078-1079, 1078t
in *Mycoplasma pneumoniae* infection, 2687
in myocarditis, 397
Immunoglobulin(s) *(Continued)*
in neonatal enteroviral infection, 3513
in neonatal staphylococcal infection, 3509-3510
in neurologic disorders, 3501-3502, 3502t
in oncologic disease, 3499
in otitis media, 3508
in PANDAS syndrome, 3511
pharmacology of, 3495
in pneumonia, 3508
in premature infant, 3500-3501
in primary immunodeficiency, 3497, 3497t
in protein-losing diseases, 3499
renal complications of, 3496
in respiratory infection, 3507-3509
in rheumatic fever, 413-414
in RSV infection, 2428-2429, 3530-3531
in secondary immunodeficiency, 3497t, 3499-3501
in sepsis, 3509
side effects of, 3496
in sinusitis, 3508
in staphylococcal infections, 3509-3510
in staphylococcal toxic shock syndrome, 835, 857, 3509
in *Staphylococcus aureus* infection, 3510
in streptococcal infections, 3510-3511
in streptococcal toxic shock syndrome, 861, 3511
in Sydenham chorea, 3511
thrombotic complications of, 3496
in transplantation patient, 3501
vaccine interactions with, 3496-3497
in VZV prophylaxis, 3523-3525
in West Nile virus infection, 2272
in X-linked agammaglobulinemia, 47, 909
isotypes of, 41-42
laboratory evaluation of, 62-63, 906
mumps, 3526
neonatal, 42-44, 43f, 43t
normal/elevated levels of, antibody deficiency with, 47-48
oral, 3498
in cryptosporidial infection, 3498
in diarrhea, 3498
in necrotizing enterocolitis, 3498
in rotavirus infection, 2186, 3498, 3531
paranasal sinus, 195
pertussis, 3507
rabies, 2454, 3527-3529
RSV, 2415, 2428-2429, 3529-3531
in lung transplantation, 980
structure of, 40-41, 41f
subcutaneous (IGSC), 3497-3498
tetanus, 1815-1816, 3511-3512
tick-borne encephalitis virus, 2312
vaccinia (VIG), 2036, 3532-3533
VZV, 2030, 3522-3525, 3524t
in hematopoietic stem cell transplantation, 941
West Nile virus, 2272
zoster, 3522-3523
Immunoglobulin A (IgA), 41
in adenovirus infection, 1894-1895
anaphylaxis with, 911
in *Bordetella pertussis* infection, 1622, 1628
in *Campylobacter jejuni* infection, 1651
deficiency of, 47, 911
vs. chronic bronchitis, 267
in *Helicobacter pylori* infection, 1695

Immunoglobulin A (IgA) *(Continued)*
in Kawasaki disease, 1070, 1072
in *Kingella kingae* infection, 1702
in *Mycobacterium leprae* infection, 1398
in *Mycoplasma pneumoniae* infection, 2673-2674, 2685-2686
in neonate, 43-44
in parainfluenza virus infection, 2366
polymeric, 1225
in rhinovirus infection, 2119
in rotavirus infection, 2180-2181
in RSV infection, 2415-2416
in rubella virus infection, 2204, 2206
secretory, 43-44
in gonococcal infection, 1280
in *Shigella* spp. infection, 1479
in *Streptococcus pneumoniae* infection, 1223
in *Toxoplasma gondii* infection, 2996
in *Trichomonas vaginalis* infection, 2890
in VZV infection, 2024
Immunoglobulin A (IgA) protease
of *Haemophilus influenzae*, 1668
of *Neisseria gonorrhoeae*, 1278
of *Streptococcus pneumoniae*, 1214
Immunoglobulin D (IgD), 41-42, 847
Immunoglobulin E (IgE), 41
in bronchiolitis, 277
in coronavirus infection, 136
increased levels of, **55**, 317, 777, 919
in Kawasaki disease, 1076-1077
in RSV infection, 2415-2416
in *Toxoplasma gondii* infection, 2996
Immunoglobulin G (IgG), 41-42
in *Acanthamoeba* spp. infection, 2983
in adenovirus infection, 1894-1895
in *Bartonella* spp. infection, 1727
in *Bordetella pertussis* infection, 1622, 1628
in *Brucella* spp. infection, 1614
in burns, 1050
in *Chlamydia pneumoniae* infection, 2642-2644
in CMV infection, 1982-1984
in *Coccidioides* spp. infection, 2776
complement activation by, 29-31
in congenital rubella, 2207, 2221
deficiency of, 62-63, 906
in common variable immunodeficiency, 910
in hyper-IgM syndrome, 909-910
in X-linked agammaglobulinemia, 909
in *Haemophilus influenzae* type b infection, 1671-1672
in HAV infection, 2130f, 2137-2138
in HEV infection, 2163, 2165
in HSV infection, 1957
in human erythrovirus B19 (human parvovirus B19) infection, 1846, 1855-1856
in hypersensitivity pneumonitis, 304, 304f
in infectious mononucleosis, 2008-2010, 2009f
in Kawasaki disease, 1076-1077
in *Kingella kingae* infection, 1702
in La Crosse virus infection, 2520-2521, 2521f
in LCMV infection, 2464
maternal, 42-44, 43f, 880
in measles, 2379
in mumps, 2400
in *Mycobacterium leprae* infection, 1398
in *Mycoplasma pneumoniae* infection, 2670, 2673-2674
in *Naegleria fowleri* infection, 2983
in pancreatitis, 692
in parainfluenza virus infection, 2366
Immunoglobulin G (IgG) *(Continued)*
in *Pseudomonas aeruginosa* infection, 1588-1589
in rhinovirus infection, 2119
in *Rickettsia prowazekii* infection, 2658
in rotavirus infection, 2180-2181
in RSV infection, 2415-2416, 2425
in rubella virus infection, 2204, 2206, 2218-2219
in *Shigella* spp. infection, 1479
in *Staphylococcus aureus* infection, 1129
in *Streptococcus pneumoniae* infection, 1223
in syphilis, 1767
in tick-borne encephalitis virus infection, 2310
in *Toxoplasma gondii* infection, 2994-2995, 2995t
in *Trichomonas vaginalis* infection, 2890
in VZV infection, 2024
in yellow fever, 2277
Immunoglobulin G1 (IgG1), deficiency of, 47, 911-912
Immunoglobulin G2 (IgG2), deficiency of, 47, 911-912
Immunoglobulin G3 (IgG3), deficiency of, 47, 911-912
Immunoglobulin G4 (IgG4), deficiency of, 47
Immunoglobulin G (IgG) subclass deficiency, 47
laboratory evaluation of, 62-63
Immunoglobulin M (IgM), 41
in *Acanthamoeba* spp. infection, 2983
in adenovirus infection, 1906-1907
anti-*Toxoplasma*, 817
in *Balamuthia mandrillaris* infection, 2983
in *Bartonella* spp. infection, 1727
in *Bordetella pertussis* infection, 1628
in *Brucella* spp. infection, 1614
in *Campylobacter jejuni* infection, 1651
in Chikungunya virus infection, 2245-2247
in *Chlamydia pneumoniae* infection, 2642-2644
in CMV infection, 1982-1983
in *Coccidioides* spp. infection, 2776
complement activation by, 29-30
in congenital rubella, 2207, 2218-2219
in dengue, 2285-2286
in Eastern equine encephalitis, 2229
in *Entamoeba histolytica* infection, 2869-2870
in enterovirus infection, 2056, 2102
in hantavirus infection, 2509-2510
in HAV infection, 2130f, 2137-2138
in *Helicobacter pylori* infection, 1695
in HEV infection, 2163, 2165
in HHV-6 infection, 2019
in human erythrovirus B19 (human parvovirus B19) infection, 1846-1847, 1855
increase in, **49-50**
vs. chronic bronchitis, 267
immunoglobulin deficiency with, 907t, **909-910**
in infectious mononucleosis, 2008-2010, 2009f
in Japanese encephalitis virus infection, 2298
in Kawasaki disease, 1076-1077
in La Crosse virus infection, 2520-2521
in *Leptospira* spp. infection, 1755
in Lyme disease, 1735
maternal, 880-881
in measles, 2379
in mumps, 2400, 2403
Immunoglobulin M (IgM) *(Continued)*
in *Mycobacterium leprae* infection, 1398
in *Mycoplasma pneumoniae* infection, 2673-2674, 2685-2686
in *Naegleria fowleri* infection, 2983
neonatal, 43-44
in parainfluenza virus infection, 2366
in Powassan virus infection, 2314
in rhinovirus infection, 2119
in Ross River virus arthritis, 2250
in rotavirus infection, 2180-2181
in RSV infection, 2425
in rubella virus infection, 2204, 2206, 2218-2219
in St. Louis encephalitis virus infection, 2258-2259
in syphilis, 1767, 1774
in tick-borne encephalitis virus infection, 2285, 2310
in *Toxoplasma gondii* infection, 2995-2996, 2995t
in *Trypanosoma brucei* infection, 2971
in Venezuelan equine encephalitis, 2240
in VZV infection, 2024, 2028
in West Nile virus infection, 2271
in Western equine encephalitis, 2235
in yellow fever, 2277
Immunoglobulin Y (IgY), in rotavirus infection, 2186
Immunoscintigraphy, in infective endocarditis, 359
Immunosuppressants. *See also* Corticosteroids
in heart transplantation, 946, 955, 955t
in intestinal/liver transplantation, 990-991
in lung transplantation, 958-959
in myocarditis, 396-397
Impetigo, 753t-756t, **772-775**
bullous, 753t-756t, 773-774, 774f-775f
nonbullous (simple), 772-773, 1143, 1149
epidemiology of, 773, 1141
transmission of, 1141
treatment of, 774-775
Implantable cardioverter-defibrillator (ICD)–associated infection, 1025-1026
management of, 1026
microbiology of, 1025
Implantable device–related infection. *See* Prosthetic/implantable device–related infection
Inappropriate secretion of antidiuretic hormone, 76, 81
in bacterial meningitis, 438, 453-454
Inclusion conjunctivitis, 804
Incontinentia pigmentosa with immunodeficiency, 916, 916f
Incubation period, 105-106, 106f
India ink staining, in cryptococcal meningoencephalitis, 2802
Indirect immunofluorescent antibody (IFA) assay
in *Anaplasma* infection, 2663
in *Babesia* spp. infection, 2918-2919
in *Ehrlichia* infection, 2663
in influenza virus infection, 2345
in La Crosse virus infection, 2520-2521, 2521f
in Mediterranean spotted fever, 2656
in *Naegleria fowleri* infection, 2982, 2982f
in Rocky Mountain spotted fever, 2653-2654
in scrub typhus, 2660
in *Toxoplasma gondii* infection, 2994-2996, 2995t
in typhus, 2658

Indium 111-labeled leukocyte scan
in appendicitis, 683
in osteomyelitis, 717
Infant. *See also* Newborn; Premature infant
Bordetella pertussis infection in, 1626-1627, 1626t
botulism in. *See* Botulism, infant
Campylobacter jejuni infection in, 888, 1653
cervical lymphadenitis in, 179
common cold in, 136
enterovirus infection of, 2092-2097, 2094t, 2096t
epidemic keratoconjunctivitis in, 1900
febrile seizures in, 85
fever without source in, 839-840
Histoplasma capsulatum infection in, 2814
Leishmania spp. infection in, 2955
Mycobacterium leprae infection in, 1394
necrotizing enterocolitis in, 635
nitrogen balance in, 69, 69f
nosocomial infection in, 3402-3404
Pasteurella multocida infection in, 1547
poliovirus infection in, 2100-2101
septic arthritis in, 730
streptococcosis syndrome of, 179
transient hypogammaglobulinemia of, **47**, **912**
tuberculosis in, 1358-1359
Infant formula, botulism and, 1804
Infantile cortical hyperostosis (Caffey disease), 847
Infantile epidemic keratoconjunctivitis, 1900
Infantile osteomyelitis, 153-154, 153f
Infantile periarteritis nodosa, 1071. *See also* Kawasaki disease
Infantile spasms, after pertussis immunization, 1634
Infarction
bone, vs. osteomyelitis, 724
cerebral
in infective endocarditis, 356-357
in *Leptospira* spp. infection, 1753
in *Neisseria meningitidis* infection, 1260
hepatic, 673
myocardial
vs. human erythrovirus B19 (human parvovirus B19) infection, 1854
in Kawasaki disease, 1081, 1083-1084
spinal cord, vs. acute transverse myelitis, 523, 524f
splenic, 357, 704, 2682
Infection. *See also specific infections, infectious agents, and sites of infection*
age patterns in, 108, 113
behavior and, 109-110
climate and, 110
cyclic variation in, 118-119
definition of, 103-104
in developing world, 112
vs. disease, 111
emerging, 119-120
endemic, 116-117
environment and, 110-111, 114-116
epidemic, 116-118
epidemiologic study of, 103-120
behavioral factors in, 109-110
biologic factors in, 108-109
environmental factors in, 110-111
historical perspective on, 103
host factors in, 107-108
infectious agents in, 104-107, 106f, 107t
population patterns in, 111-120, 116f-117f
ethnic patterns in, 108, 113-114
family episodes of, 114
Infection *(Continued)*
geographic factors and, 110-111, 114-116
geologic factors and, 110-111
global variation in, 115
health status and, 108-109
host factors in, 107-108
immunity and, 109. *See also* Immunity
in individual, 111-113
in kinships, 114
latent, 105-106, 106f
local patterns of, 115-116, 116f
long-term trends in, 119
monitoring of, 117, 117f
outbreak of, 117
pandemic, 117
persistent, 106
population occurrence of, 112-114, 116f-117f
racial patterns in, 108, 113-114
reporting requirements for, 112
seasonal variation in, 118-119
sex patterns in, 108, 113
socioeconomic patterns in, 110-111, 114
temporal patterns of, 116-120
time clusters of, 117-118
transmission of, 107, 107t
Infection control practices, 1061-1062, 3407-3414, 3409t
antimicrobial stewardship programs in, 3414, 3414t
in cancer patient care, 929
in daycare facilities, 3565-3569, 3567t-3568t
hand hygiene in, 3411
isolation precautions in, 3411-3412. *See also* Isolation precautions
occupational health policies in, 3412-3413
outbreak investigations in, 3408, 3411t
policies and procedures for, 3408-3411, 3410t
Standard Precautions in, 3379, 3380t, 3412
surveillance in, 3408
Transmission-Based Precautions in, 3379, 3381t, 3412
visitor policies in, 3412
Infectious mononucleosis, 158-161, **1999-2000.** *See also* Epstein-Barr virus (EBV)
acute phase of, 1999-2000, 1999f-2000f, 1999t
airway obstruction in, 2004
aplastic anemia in, 2002
cardiac manifestations of, 2002
complications of, 2001-2004
diagnosis of, 2007-2011, 2007f, 2008t
differential diagnosis of, 2011-2012
epidemiology of, 1997-1998
exanthem in, 742t-748t, 760, 1999-2000, 2002, 2002f
gastrointestinal manifestations in, 2003
hematologic manifestations of, 2002-2003
hemolytic anemia in, 2002
hepatic manifestations in, 2003
historical perspective on, 1992
imaging studies in, 2011
immune response in, 2008-2010, 2009f, 2010t
in immunodeficient host, 2006
immunopathogenesis of, 1995
neck abscess in, 2004
neurologic manifestations in, 2003-2004
neutropenia in, 2003
ocular involvement in, 810
pancreatitis with, 694
Infectious mononucleosis *(Continued)*
pancytopenia in, 2003
in pregnancy, 1998
prodrome in, 1999
pulmonary disease in, 2004
renal manifestations in, 2004
resolution phase of, 2000
respiratory manifestations in, 2004
splenic manifestations in, 2003
thrombocytopenia in, 2002-2003
transmission of, 1998
treatment of, 2012
in young children, 2000
Infective dermatitis, 742t-748t, 749-750, 2574-2575, 2574t
Infective endocarditis, **350-370**
Achromobacter xylosoxidans, 1574-1575
acute, 350
Aerococcus viridans, 1249
age-related incidence of, 350-351
Aggregatibacter actinomycetemcomitans, 1610, 1823
Aggregatibacter (Haemophilus) aphrophilus, 1686
Arcanobacterium haemolyticum, 1325-1326
arteriovenous fistula-related, 352
Bacillus spp., 362-363
bacteremia in, 353-354, 353t
Bacteroides fragilis, 367
Bartonella spp., 363, 1725, 1727-1729
Brucella spp., 362, 1613-1615
in burn patient, 1055
Candida, 354, 357-358, 363, 2740, 2748
neonatal, 895, 897, 2756
catheter-related, 352
Chlamydia spp., 363
clinical manifestations of, 356-357, 356t
CNS involvement in, 356-357
coagulation system in, 354
complications of, 355, 355f
congenital heart disease and, 351, 351t
Corynebacterium diphtheriae, 362-363, 1303, 1306
Coxiella burnetii, 363, 2664
CSF examination in, 443t
culture-negative, 358, 368
distant organ disease in, 355
Duke criteria for, 360, 360t-361t
echocardiography in, 359-360
Eikenella corrodens, 1577
emboli in, 355
enterococcal, 361, 366, 366t-367t
Enterococcus spp., 361, 366, 366t-367t, 1173-1175
epidemiology of, 350-352, 351t
Erysipelothrix rhusiopathiae, 362-363, 1328
Escherichia coli, 362, 1441
fever of unknown origin in, 845
fungal, 352, 361t, 363, 367-368
gram-negative, 361t, 362, 367
gram-positive bacilli, 362-363
HACEK, 362, 367, 367t, 1823
Haemophilus parainfluenzae, 1690
immunopathologic factors in, 354-355, 358
intravenous drug abuse and, 352, 357
Kingella kingae, 1703-1704
Klebsiella spp., 362, 367
laboratory evaluation in, 357-360, 358t, 360t-361t
Lactobacillus spp., 362-363
Legionella spp., 363
Listeria monocytogenes, 362-363
microbiology of, 360-363, 361t

Infective endocarditis *(Continued)*
mitral valve, 352
Neisseria gonorrhoeae, 362
neonatal, 357
pacemaker-related, 352
pathophysiology of, 352-355, 353t, 355f
pneumococcal, 361, 1232
prevention of, 368-369, 369t, 417-418, 1195-1196, 3246-3247, 3246t-3248t
prognosis for, 352
prophylactic antimicrobials against, 368-369, 369t, 417-418, 3246-3247, 3246t-3248t
prosthetic valves and, 351, 357, 365-366, 366t
coagulase-negative staphylococci in, 1137
Proteus spp., 1473
Pseudomonas aeruginosa, 362, 1595, 1597-1598
rheumatoid factor in, 355
serologic testing in, 358-359
staphylococcal, 361-362, 361t, 366-367, 367t, 1137
Stenotrophomonas maltophilia, 1607
Streptobacillus moniliformis, 1717-1719
streptococcal, 360-361, 361t, 365-366, 365t-366t, 1151-1152, 1232
Streptococcus viridans, 360-361, 361t, 365-366, 365t-366t, 1191-1192, 1195
subacute, 350, 355f
surgical treatment of, 368, 368t
treatment of, 363-368, 364t-368t
initiation of, 364
monitoring of, 364
vegetations in, 353-355, 355f
ventriculoatrial shunt–related, 352
von Reyn criteria for, 360
in Whipple disease, 644
Infectivity, 105
Infertility
appendicitis and, 687-688
cystic fibrosis and, 325
pelvic inflammatory disease and, 1286
Ureaplasma spp. infection and, 2689
Inflammation
in bacterial meningitis, 433-435, 434f
in burns, 1048
complement in, 31
cytokines in, 65, 79-80
joint. *See* Arthritis; Juvenile idiopathic (rheumatoid) arthritis; Rheumatoid arthritis
liver. *See* Hepatitis
metabolic changes with. *See* Metabolism
Inflammatory bowel disease (IBD), 2872
Campylobacter jejuni infection and, 1654
vs. *Clostridium difficile* infection, 637
fever of unknown origin in, 847
probiotics in, 3370-3371
Infliximab
adenovirus infection and, 1904
in Kawasaki disease, 1080
Influenza A virus, 2327-2338, 2327f-2328f. *See also* Influenza virus infection
avian, 2329-2330, 2334-2335
canine, 2329-2330
ecology of, 2329-2330
evolution of, 2331-2332
novel, 2334-2336
surveillance for, 2330-2332
swine, 2329-2330, 2335-2336
testing for, 2346-2348
transmission of, 2330, 2337-2338
Influenza B virus, 2327-2338, 2327f-2328f. *See also* Influenza virus infection
evolution of, 2331-2332
surveillance for, 2331
testing for, 2346-2348
transmission of, 2337-2338
Influenza C virus, 2327-2338
Influenza virus infection, **2326-2359**
in acute bronchitis, 262, 263t
avian subtypes in, 2334-2335
bacterial coinfection with, 2345, 2351-2352
bloodstream, 2345
cardiac complications of, 2343
chest radiography in, 2340, 2344f
clinical manifestations of, 2340-2345, 2342t-2343t
complications of, 2340
in daycare facilities, 3562
diagnosis of, 981, 2345-2358, 2348f, 3606t-3607t, 3610-3612, 3611t
disseminated intravascular coagulation with, 2345
economic impact of, 2333
encephalitic, 494t-498t, 502, 2343, 2352
epidemiology of, 2332-2334
exanthem in, 742t-748t, 749, 760-762
genetic factors in, 2338
global burden of, 2333-2334
after heart transplantation, 954
after hematopoietic stem cell transplantation, 942
hepatic complications of, 2344
in high-risk groups, 2333
historical perspective on, 2327
H1N1, 749, 981, 2330-2331, 2336-2337
H5N1, 2334-2335, 3115-3116
immune response to, 2338
immunization against, 2353-2356, 3443-3448
adverse events with, 3445-3446
availability of, 2357
contraindications to, 3446-3448
economics of, 2356
efficacy of, 2355-2356, 3444-3445
encephalitis after, 502
indications for, 3447f
maternal, 2356
precautions for, 3448f
precautions with, 3446-3448
preparations for, 3443-3444
production of, 2357
recommendations for, 2357, 3446
safety of, 2354-2355, 3445-3446
after intestinal transplantation, 997t, 999
after kidney transplantation, 1007
after liver transplantation, 997t, 999
after lung transplantation, 980-981
mortality with, 2333-2334
musculoskeletal complications of, 2344
in neonate, 2339
neurologic complications of, 2343
nonpharmaceutical prevention of, 2358
nosocomial, 2352, 3380-3382
novel viruses in, 2334-2336
ocular, 812
outbreaks of, 2352-2353
pandemic, 2330-2331, 2336-2337
preparedness for, 2358
pathogenesis of, 2338-2345
pathology of, 2338-2339, 2341f
during pregnancy, 2345
prevention of, 2352. *See also* Influenza virus infection, immunization against
breast-feeding and, 2356-2357
intrapartum, 2356-2357
Influenza virus infection *(Continued)*
pulmonary, 284, 2342-2343, 2344f
renal complications of, 2345, 2352
respiratory failure/distress with, 2352
seasonal, 2329, 2332-2334
sepsis with, 2345
Staphylococcus aureus infection and, 1122, 2338
Streptococcus pneumoniae infection and, 1215
sudden death with, 2345
surveillance for, 2331-2332
swine subtypes in, 2335-2336
transmission of, 2337-2338, 2345
in traveler, 3115-3116
treatment of, 2348-2351, 2349t
antiviral agents in, 2348-2351, 2349t, 3269-3270, 3272-3275
antiviral susceptibility testing in, 2345-2346
corticosteroids in, 2351
investigational therapies in, 2351
salicylates in, 2351
supportive care in, 2351
vertical transmission of, 2345
Infraorbital space, infection of, 149
Infrared thermometer, 85-86
Inhalation injury, 1048-1049. *See also* Burn(s)
bronchoalveolar lavage in, 1057
Inkoo virus, 2514, 2517
Innate immunity. *See* Immunity, innate
Inoculation arthritis, 728
Inoculation osteomyelitis, 724-725
Inosiplex, in subacute sclerosing panencephalitis, 2390
Insecticide poisoning, pancreatitis with, 692
Insulin, plasma/serum, 82
in septic shock, 829
INT1 protein, 9
Integrase inhibitors, 2615-2616
Integrin(s), in phagocyte recruitment, 31-32
Integrin activation deficiency defect, 53-54
Integrons, in antibiotic resistance, 3138-3139, 3138t
Interferon-alpha (INF-α), 80, 3363-3364
in congenital rubella, 2208
in HCV infection, 2323-2324
in HSV infection, 1966
pancreatitis with, 692
in papillomas, 1883
in rhinovirus infection prevention, 2125-2126
Interferon-alpha 2a (INF-α2a)
in HBV infection, 1920-1922
in HDV infection, 1931-1932
Interferon-alpha 2b (INF-α2b)
in common cold, 138
in HDV infection, 1920-1921, 1931-1932
in West Nile virus infection, 2272
Interferon-beta (INF-β), 3363-3364
in myocarditis, 397
Interferon-gamma (INF-γ), 3364
in chronic granulomatous disease, 57, 918
in *Cryptococcus neoformans* infection, 2805
defects in, 50-51
in mycobacterial infection, 1382
in *Mycobacterium leprae* infection, 1398
in *Trypanosoma cruzi* infection, 2967
in viral infection, 135
Interferon-gamma release (IGR) assay
in kidney transplantation, 1004, 1012
in *Toxoplasma gondii* infection, 2997
in tuberculosis, 1363-1364

Interleukin(s), 26t
Interleukin-1 (IL-1), 26-27, 26t, 80, 3358-3360, 3359t
  in burns, 1050
  in common cold, 135
Interleukin-1β (IL-1β), in bacterial meningitis, 434-435
Interleukin-2 (IL-2), 3359t, 3361
  in *Mycobacterium leprae* infection, 1398
  recombinant, in burns, 1050
Interleukin-4 (IL-4), 3362
Interleukin-6 (IL-6), 70, 3360t
Interleukin-8 (IL-8), 3359t, 3361-3362
  in upper respiratory tract infection, 135
Interleukin-9 (IL-9), 3361-3362
Interleukin-10 (IL-10), 3360t
  in septic shock, 827
Interleukin-12 (IL-12), 3359t, 3362
  defects in, 50-51
  in mycobacterial infection, 1382
Interleukin-17 (IL-17), 3359t, 3362
  in myocarditis, 387
Interleukin-18 (IL-18), 3362
Interleukin-1 (IL-1) receptor antagonist, 3360, 3360t
Internalins, 13
International adoptees and refugees, **3121-3133**, 3122f
  American trypanosomiasis in, 3129
  bacterial infection in, 3123t, 3126-3128
  blood lead levels in, 3130
  complete blood count in, 3130
  dermatologic diseases in, 3130
  enteric bacterial infections in, 3128
  hepatitis A in, 3122-3124, 3123t
  hepatitis B in, 3123t, 3124-3125
  hepatitis C in, 3123t, 3125
  HIV infection in, 3123t, 3125
  immunization of, 3130-3132, 3131t
  infectious disease screening in, 3122-3130, 3123t, 3128t
  intestinal parasites in, 3128-3130
  malaria in, 3129
  syphilis in, 3123t, 3126
  tissue parasites in, 3129
  tuberculosis in, 3126-3128, 3127f-3128f
International health, 3082t
International travel, **3091-3120**
  African trypanosomiasis and, 3116
  avian influenza A and, 3115-3116
  chikungunya and, 3116
  coccidioidomycosis and, 3116-3117
  dengue and, 3115
  diarrhea prevention and, **3110-3115**
  HAV infection and, 2134-2135, 2141
  health counseling for, 3117-3118, 3548-3549
  health kit for, 3118
  high-altitude destination and, 3118
  histoplasmosis and, 3116-3117
  information resources for, 3118, 3119t
  Japanese encephalitis virus and, 2294, 2295t-2296t, 3106
  leishmaniasis and, 3117
  leptospirosis and, 3117
  malaria prevention and, 2926, 3107-3110, 3109f, 3111t
  pretravel assessment for, 3092-3094
  rabies and, 3106-3107
  risks of, 3092
  schistosomiasis and, 3116
  sexually transmitted diseases and, 3117
  tick-borne encephalitis and, 3116
  tuberculosis and, 3116
  typhoid fever and, 3107
International travel *(Continued)*
  vaccination for, 3094-3107, 3097t-3105t, 3427, 3428t
Interstitial lung disease, **295-306**
  biopsy in, 299-300
  bronchoalveolar lavage in, 299
  classification of, 295-297, 296t
  clinical presentation of, 297-298
  CT in, 298-299
  diagnosis of, 298-300, 298t
  infection and, 295-296
  prognosis for, 300-301
  pulmonary function tests in, 298
  treatment of, 300
Intervertebral disk, infection of, 720-721, 721f
Intestinal transplantation, infection after, **989-1002**
  adenovirus, 997t, 999, 1903
  age and, 990
  bacterial, 994-995
  catheter and, 991, 993
  CMV, 990, 996-997, 997t, 1001
  coccidioidal, 1000
  cryptococcal, 1000
  donor-related factors in, 990
  early (0 to 30 days), 991-992, 991t
  EBV, 990, 997-998, 997t, 1001
  fungal, 991, 994-995, 995t
  HHV-6, 999
  HHV-7, 999
  Histoplasma capsulatum, 1000
  immunosuppression and, 990-991
  intermediate (31 to 180 days), 992, 992t
  intra-abdominal, 994
  intraoperative factors in, 990
  late (after 180 days), 992-993, 992t
  Mycobacterium tuberculosis, 1000
  nosocomial, 993
  Pneumocystis jiroveci, 1000
  post-transplant factors in, 990-991
  predisposing factors in, 989-991
  pretransplant evaluation in, 1000-1001
  pretransplant factors in, 989-990
  prevention of, 1001
  RSV, 997t, 999
  timing of, 991-993, 991t-992t
  viral, 995-1001, 997t
  wound, 994
Intimin, 7-8, 9f, 1457
Intracranial pressure (ICP)
  in cryptococcal meningoencephalitis, 2802-2803
  in meningitis, 438-439, 454
Intracranial pressure (ICP) monitor, infection with, 1038-1040
  clinical manifestations of, 1039
  diagnosis of, 1039-1040
  epidemiology of, 1038-1039, 1039t
  etiology of, 1039
  treatment of, 1040
Intraocular lenses, infection with, 1019
Intrathecal pump infusion device–associated infection, 1040-1042
  clinical manifestations of, 1041
  diagnosis of, 1041
  epidemiology of, 1041
  etiology of, 1041
  treatment of, 1041-1042
Intrathecal serotherapy, in neonatal tetanus, 1816
Intrauterine device (IUD), *Actinomyces* spp. infection and, 1822
Intravenous antimicrobial therapy. *See* Outpatient intravenous antimicrobial therapy
Intravenous immunoglobulin. *See* Immunoglobulin(s), intravenous (IVIG, IGIV)
Intussusception
  adenovirus and, 1902
  rotavirus infection and, 2186
Invasins, 13, 1523-1524
Iodine, metabolism of, 77t
Iodoquinol, 3348-3349
IpaB protein, 18
IPEX (immunodeficiency, polyendocrinopathy, enteropathy, X-linked) syndrome, 915-916
Ipratropium, in bronchiolitis, 279
Iquitos virus, 2543-2544
Iridocyclitis, 809
  Ascaris lumbricoides, 815
  coccidioidal, 813-814
  herpes zoster, 810
  HSV, 810
  Loa loa, 815
  rickettsial, 815
  Wuchereria bancrofti, 815
IRIS (immune reconstitution inflammatory syndrome), 972, 1360-1361
Iritis, 809
  Brucella, 813
  herpes zoster, 810
  HSV, 810
  mumps, 811
  tuberculous, 1357
Iron
  bacterial acquisition of, 538
  deficiency of, hookworm infection and, 3017
  metabolism of, 77t, 78
    in *Pseudomonas aeruginosa* infection, 1588
    in *Yersinia enterocolitica* infection, 1525
  Neisseria gonorrhoeae metabolism of, 1278
  serum, 78
    fever and, 85
    mucormycosis and, 2840
Iron transporters, of *Streptococcus pneumoniae*, 1214
Irrigation, nasal
  in common cold, 137
  in rhinosinusitis, 201
Irritable bowel syndrome
  probiotics in, 3371
  with *Shigella* spp. infection, 1481
Isocitrate lyase, 15
Isolation precautions, 3411-3412, 3546
  in ARDS, 873
  in *Bordetella pertussis* infection, 1636
  in congenital rubella, 2220
  in methicillin-resistant *Staphylococcus aureus* infection, 1126
  in monkeypox, 2044
  in *Shigella* spp. infection, 1486
  in tuberculosis, 1375
  in *Yersinia pestis* infection, 1514
Isoniazid, 1366-1369, 1367t
  before lung transplantation, 963-964
Isoproterenol, in myocarditis, 395
Isospora belli. *See Cystoisospora belli*
Itraconazole, 3301f, 3305-3308, 3349
  adverse effects of, 3307
  in *Blastomyces dermatitidis* infection, 478, 2732-2734, 2732t
  in *Candida* esophagitis, 596-597
  in *Coccidioides* spp. infection, 477
  drug interactions with, 3307
  in hematopoietic stem cell transplantation–related aspergillosis, 937

Itraconazole *(Continued)*
in *Histoplasma capsulatum* infection, 2825
indications for, 3294t-3295t, 3307-3308, 3321t
in *Paracoccidioides* spp. infection, 2793
pharmacodynamics of, 3305
pharmacokinetics of, 3305-3307, 3306t
in *Sporothrix schenckii* infection, 2835
in tinea capitis, 787
in tinea unguium, 787
Ivermectin, 3349
in lice, 3075
in onchocerciasis, 809
in scabies, 3074
in *Strongyloides stercoralis* infection, 3021
IVIG. *See* Immunoglobulin, intravenous (IVIG, IGIV)
Ixodes tick, **3071-3072**
in Lyme borreliosis, 1730, 1730t, 1737-1738
in Powassan virus infection, 2313
prevention of, 1737
in tick-borne encephalitis virus infection, 2304-2305, 2306f

J

Jamestown Canyon virus, 2514, 2520
Janeway lesion, 355, 357
Japanese encephalitis virus, 2291-2293, 2292f
Japanese encephalitis virus infection, 494t-498t, **2291-2301**
clinical manifestations of, 2294-2296, 2294f
complications of, 2297
diagnosis of, 2298
differential diagnosis of, 2298
epidemiology of, 2292f-2293f, 2293-2294
historical perspective on, 2291
immune response to, 2297
immunization against, 2299-2300, 3479-3480
pathology of, 2296-2297
pathophysiology of, 2297
in pregnancy, 2297
prevention of, 2299-2300
prognosis for, 2298-2299
relapse in, 2297
in travelers, 2294, 2295t-2296t
treatment of, 2298
Jarisch-Herxheimer reaction, 812
Jaundice
in cholecystitis, 669
in congenital rubella, 2220
differential diagnosis of, 662-663, 662t
in HAV infection, 2137, 2140-2141
in *Leptospira* spp. infection, 1746-1747, 1752
in *Paracoccidioides* spp. infection, 2786
in yellow fever, 2276
Jaw
lumpy, 184
osteomyelitis of, **152-154**, 152t
Garré, 154, 154f-155f
infantile, 153-154, 153f
suppurative, 153, 153f
JC virus, 1864-1865, 1864t
tumors and, 1868
JC virus infection, **1863-1870**
clinical manifestations of, 1866-1869
CNS, **1866-1867**, 1866f
diagnosis of, 1868-1869
epidemiology of, 1865-1866, 1865f
after kidney transplantation, 1010
treatment of, 1869
Jenner, Edward, 103
Jewelry, intraoral/perioral, 155
Job syndrome (hyper-IgE syndrome), **55**, 317, 777, 919, 3364
Joint(s)
bacterial infection of. *See* Septic arthritis
fungal infection of, **734**
inflammation of. *See* Arthritis; Juvenile idiopathic (rheumatoid) arthritis; Rheumatoid arthritis
prosthetic, infection with, **1026-1029**
clinical manifestations of, 1027
diagnosis of, 1027-1028
microbiology of, 1027
prevention of, 1029
risk factors for, 1026
treatment of, 1028-1029
synovial fluid of, 728
synovium of, 728
tuberculosis of, 1355-1356
viral infection of, 728
in Whipple disease, 643
Jugular vein, palpable "cord" at, 236
Junín virus infection, 2467-2468, 2470t, 2471, 2475. *See also* Arenavirus infection
immune plasma in, 3533-3534
vaccine against, 2476
Juvenile diabetes mellitus, 72
Juvenile idiopathic (rheumatoid) arthritis
aspirin in, 677
fever of unknown origin in, 840, 847
human parvovirus B19 and, 1851
joint fluid findings in, 732t
Juvenile-onset recurrent respiratory papillomatosis, 1878-1879
Juvenile periodontitis, 140, 147
PMN chemotaxis in, 56

K

K antigen
of *Klebsiella pneumoniae*, 1467
in urinary tract infection, 537-538
Kala-azar. *See Leishmania* spp. infection (leishmaniasis), visceral
Kanagawa phenomenon, in *Vibrio parahaemolyticus* infection, 1557-1558
Kanamycin, 3208-3211, 3208t, 3231t-3233t
in tuberculosis, 1367t
Kaposi sarcoma, 654, 742t-748t, 749, **2019-2021**, 2606
Kaposi sarcoma–associated herpesvirus infection. *See* Human herpesvirus–8 (HHV-8) infection
Kaposi varicelliform eruption, 1943-1944, 1943f, 1953
Karshi virus infection, 2316t
Kartagener syndrome, 195, 267
Kasai procedure, cholangitis and, 658-659, 664-665
Katayama fever, 3064
Kawasaki disease, 180, **1064-1085**
acute, 1072
adult, 1066
age and, 1066-1067
arthritis in, 1082
aspirin in, 677
autoantibodies in, 1077
carpet shampooing and, 1068
cervical lymphadenopathy in, 180
clinical manifestations of, 1072-1075, 1074t, 1081t
communicability of, 1068
complications of, 1081-1082, 1081t
Kawasaki disease *(Continued)*
coronary artery aneurysm in, 1071, 1080-1084
cutaneous manifestations of, 1073-1074
diagnosis of, 1065t, 1069f, 1073
epidemics of, 1067
epidemiology of, 1065-1068
ethnicity and, 1066
etiology of, 1068-1071
family cases of, 1067
gallbladder involvement in, 1074, 1082
gender ratio in, 1065-1066
genetics and, 1070-1071
geography and, 1067
hearing loss in, 1082
hemophagocytic syndrome in, 1082
hepatitis in, 657
historical perspective on, 1064-1065
human bocavirus infection and, 1861
immune response in, 1070, 1072
immunologic findings in, 1076-1078
incidence of, 1065
inclusion bodies in, 1070
incomplete, 1069f, 1075
laboratory findings in, 1075-1078, 1075t
vs. mastoiditis, 237
mitral regurgitation in, 1081
mortality in, 1066
myocardial infarction in, 1081, 1083-1084
pathology of, 1071-1072
peripheral artery aneurysm in, 1081-1082
peripheral gangrene in, 1082
pharyngeal involvement in, 159t-160t, 161
phases of, 1072-1075
prognosis for, 1082-1084
race and, 1066
rash in, 1074
recurrence of, 1067
renal pathology in, 1072
respiratory infection and, 1068
retrovirus and, 1069
seasonality of, 1067-1068
sequelae of, 1080-1081
subacute, 1072
treatment of, 1078-1081, 1078t
failure of, 1080
follow-up after, 1081, 1083-1084
rescue therapy in, 1080
risk assessment after, 1083-1084
urethritis in, 531
uvulitis in, 166
Kawasaki disease–like illness, in *Yersinia pseudotuberculosis* infection, 1529
Kemerovo virus, 2175
Keratitis, **806-809**
Acanthamoeba spp., 808-809, 2977, 2979-2980, 2980f, 2985, 3327t-3346t
Bacillus cereus, 1319
bacterial, 807, 808t
Citrobacter spp., 1431
contact lens–related, 1019
epithelial, 807
fungal, 807-808
contact lens–related, 1019
Fusarium spp., 2846-2847
HSV, 803-804, 1945, 1959
in Kawasaki disease, 1073
in leprosy, 813
microsporidia, 809, 2912-2913
vs. neonatal conjunctivitis, 806
protozoan, 808-809
Pseudomonas aeruginosa, 1591
Rhodococcus ruber, 1426
rubella virus, 2212
Stenotrophomonas maltophilia, 1607

Keratitis *(Continued)*
stromal (interstitial), 807, 812, 819
in congenital syphilis, 1771
syphilitic, 812
vaccinia, 3533
Keratoconjunctivitis
Achromobacter spp., 1574
adenovirus, 1894, 1899-1900, 1908
HSV, 803, 1945
microsporidia, 809, 2912-2913
Kernig sign, 439
Ketoconazole, 3349
in *Candida* esophagitis, 596-597
in *Histoplasma capsulatum* infection, 2825-2826
in *Paracoccidioides* spp. infection, 2793
in *Sporothrix schenckii* infection, 2835
Kidney(s)
abscess of. *See* Renal abscess
aminoglycoside toxicity to, 3210-3211
amphotericin B deoxycholate toxicity to, 3292-3293
Candida spp. infection of, 894, 897
cidofovir toxicity to, 3289
colistin toxicity to, 3208
in *Corynebacterium diphtheriae* infection, 1305
drug clearance by, 3236
in EBV infection, 2004
infection of, 706, 708. *See also* Pyelonephritis; Renal abscess; Urinary tract infection (UTI)
in influenza virus infection, 2345, 2352
radionuclide imaging of, in urinary tract infection, 544-545, 545f
scarring of, 538, 544-545, 545f, 551-552
Schistosoma spp. infection of, 3064
in toxic shock syndrome, 853, 858
transplantation of. *See* Kidney transplantation
tuberculosis of, 1358
ultrasonography of, in urinary tract infection, 544
in Whipple disease, 644
in yellow fever, 2276
Kidney failure
IVIG effects on, 3496
in *Leptospira* spp. infection, 1747-1748, 1752-1753, 1757
in malaria, 2928
in septic shock, 830, 832, 835
Kidney transplantation
antilymphocyte antibody therapy in, 1007
evaluation for, 1003-1004, 1003t
infection after, **1002-1015**
adenovirus, 1010-1011
BK virus, 1867-1869
catheter-related, 1006
CMV, 1007-1008, 3520-3521
early period, 1004-1007, 1005t
EBV, 1008-1009
fungal, 1012-1014
HBV, 1007, 1014
HCV, 1007, 1014-1015
HIV, 1015
HSV, 1006-1007, 1952
human erythrovirus, 1011
human herpesvirus, 1010, 2017
IVIG use and, 3501
late period, 1005t, 1014-1015
middle period, 1005t, 1007-1014
mycobacterial, 1011-1012
Mycobacterium tuberculosis, 1012, 1358
Mycoplasma, 1011
Nocardia spp., 1012
Kidney transplantation *(Continued)*
papillomavirus, 1010
parasitic, 1014
polyomavirus, 1010
pulmonary, 1006
respiratory, 1007, 1014
urinary tract, 1005-1006, 1014
viral, 1006-1007
VZV, 1009-1010
West Nile virus, 1011
wound, 1004-1005
rejection after, 1007
Kikuchi-Fujimoto disease, 181
Kindlin-3, 53-54
Kingella kingae, 1700
carriage of, 1700
Kingella kingae infection, **1699-1704**
bone, 711, 712t, 714, 717-718. *See also* Osteomyelitis
clinical manifestations of, 1702-1703
in daycare facilities, 1701, 3555
diagnosis of, 1703
endocardial, 362, 1703-1704
epidemiology of, 1700-1701
historical perspective on, 1699-1700
immune response to, 1702
joint, 728, 729t, 732-733
pathogenesis of, 1701-1702, 1701f
prevention of, 1704
transmission of, 1700
treatment of, 1703-1704
viral coinfection with, 1701-1702
KIPyV, 1864, 1864t, 1868
Kissing bug bite, 758t
Klebsiella granulomatis, 1640
Klebsiella granulomatis infection, **1640-1644**
clinical manifestations of, 753t-756t, 1641-1642, 1642f
diagnosis of, 1642-1643, 1642f
epidemiology of, 1640-1641
pathogenesis of, 1641
prevention of, 1643-1644
prognosis for, 1643
treatment of, 1643
Klebsiella oxytoca infection, 1468
Klebsiella planticola, 1465-1466
Klebsiella pneumoniae infection, **1465-1469**
antibiotic resistance in, 1466, 1468
biliary, 659-660, 659t, 663
bloodstream, 1467-1468
clinical manifestations of, 1467-1468
diagnosis of, 1468
epidemiology of, 1466
hepatic, 1467
after liver transplantation, 1468
after lung transplantation, 962
neonatal, 882
pathophysiology of, 1466-1467
pulmonary, 1466-1467
treatment of, 1468
vitamin C in, 77
Klebsiella spp., 1465-1466
K antigen of, 1467
Klebsiella spp. infection, **1465-1469.** *See also specific species infections*
antibiotic resistance in, 1468
clinical manifestations of, 1467-1468
CSF shunt, 1030, 1030t
diagnosis of, 1468
endocardial, 362, 367
epidemiology of, 1466
hepatic, 673
pathophysiology of, 1466-1467
peritoneal, 700
in septic shock, 834
Klebsiella spp. infection *(Continued)*
treatment of, 1468
uveitis after, 816
Knee, tuberculosis of, 1355
Koch, Robert, 103
Koilocytosis, in HPV infection, 1882
Kokobera virus infection, 2316t
Koplik spots, 161, 2378, 2381-2382, 2381f
Kostmann syndrome, 917
Koutango virus infection, 2316t
Kunjin virus infection, 742t-748t
Kuru, 2620-2622, 2627t
Kussmaul sign, 374, 374f
Kwashiorkor, 76
Kyasanur Forest disease, **2315-2316**

L

L-Selectin, in phagocyte recruitment, 31-32, 33f
La Crosse virus (LACV), 2514-2517, 2516f
La Crosse virus (LACV) infection, **2513-2527**
clinical manifestations of, 494t-498t, 500, 510, 2519-2520
diagnosis of, 2520-2522, 2521f, 2522t, 2523f
differential diagnosis of, 2522-2523
ecology of, 2516-2517, 2516f
epidemiology of, 2513-2514, 2514f, 2517-2519, 2518t
Glasgow Coma Scale in, 2524
meningeal, 487
pathogenesis of, 2519
pathology of, 2525-2526
prevention of, 2526
prognosis for, 2525
treatment of, 2523-2525
vaccine against, 2526
Laboratory tests, 125-127. *See also specific tests*
accuracy of, 125-126
in bacterial infection, **3589-3595**, 3590t. *See also* Culture, bacterial; Polymerase chain reaction (PCR)
in fungal infection, **3596-3600**, 3597t-3598t
normal vs. abnormal, 125-126, 126f
in parasitic infections, **3620-3627**, 3621t, 3623t, 3624f-3625f. *See also* Parasitic disease, laboratory diagnosis of
predictive value of, 126-127, 126t-127t
reproducibility of, 125
sensitivity of, 125-126
specificity of, 125-126
validity of, 125
in viral infection, **3600-3620.** *See also* Viral infection, laboratory diagnosis of
Labyrinthitis
mastoiditis and, 236
otitis media and, 224
Lacrimal gland, inflammation of, **797**
Lacrimal sac, infection of, **797-798**
β-Lactam antibiotics, 3182-3183. *See also* Penicillin(s)
resistance to, 3139-3145
β-lactamase production and, 3139-3143
carbapenemase production and, 3142
mechanism of, 3140-3143, 3140f-3141f, 3140t, 3202-3203
penicillin-binding protein alterations in, 3143-3145
β-Lactamase(s), 3139-3143, 3140f-3141f
extended-spectrum, 3141-3142, 3202-3203

β-Lactamase(s) *(Continued)*
group 1, 3140-3141, 3140t
group 2, 3140t, 3141-3142
group 3 (metalloenzymes), 3140t, 3142-3143
β-Lactamase inhibitors, 3187-3189
Lactate, CSF, 444
Lactate dehydrogenase, CSF, 444
Lactobacillus spp. infection
endocardial, 362-363
gastrointestinal tract, 626
Lactococcus spp., vs. *Enterococcus* spp., 1171
Lactoferrin
in neonatal *Candida* spp. infection prevention, 2765
in *Streptococcus pneumoniae* infection, 1226
Lactose intolerance, 622
Lagovirus, 2149, 2149f, 2149t
Lagovirus infection, **2154-2155**
Lamivudine, 2613
in HBV infection, 1921-1922
Lamotrigine, pancreatitis with, 691
Lampion (lantern) cells, 1846
Langat virus infection, 2316t
Language development, otitis media and, 222, 222f
Laninamivir, 3270-3273
Lantibiotics, of *Staphylococcus epidermidis*, 1133
Large intestine
enlargement of
in *Shigella* spp. infection, 1481
in *Trypanosoma cruzi* infection, 2965
indigenous flora of, 91t, 92-93
infection of. *See* Gastrointestinal tract infection
Laryngotracheitis, adenovirus, 1897
Laryngitis, 242t-243t, 250t-251t
etiology of, 242-244, 243t-244t
measles virus, 2385, 2392
treatment of, 257
Laryngotracheitis, 242t-243t, **249**, 250t-251t
vs. epiglottitis, 253-254
etiology of, 242-244
measles virus, 2385, 2392
pathogenesis of, 247-248
prognosis for, 259
vs. spasmodic croup, 253
treatment of, 254-257
Laryngotracheobronchitis, 242t-243t, 250t-251t, **252-253**
etiology of, 242-244, 243t-244t
treatment of, 257
Laryngotracheobronchopneumonitis, 242t-243t, 250t-251t, **252-253**
etiology of, 242-244, 243t-244t
treatment of, 257
Larynx, recurrent papillomatosis of, 1878-1879
Lassa virus infection, 656, 2467, 2470, 2470t. *See also* Arenavirus infection
bioterrorist, 3585
clinical manifestations of, 2471-2472, 2471t
diagnosis of, 2474
differential diagnosis of, 2475
exanthem in, 742t-748t, 749, 760
hepatitis in, 2473
immune response to, 2473
nosocomial, 2470-2471
prevention of, 2476-2477
treatment of, 2475
Latency, viral, 20
Latent membrane proteins, 1993-1994
Latent period, in infection, 105-106, 106f
Latex particle agglutination test
in *Haemophilus influenzae* type b infection, 1677-1678
in meningitis, 442, 1263
Lazy leukocyte syndrome, 56
LCMV. *See* Lymphocytic choriomeningitis virus (LCMV) infection
Lead poisoning
in international adoptees and refugees, 3130
vs. St. Louis encephalitis virus infection, 2259
Lebombo virus, 2176
Lecithin cholesterol acetyltransferase (LCAT), 74
Leech therapy, *Aeromonas* spp. infection with, 1543
Leflunomide, in hematopoietic stem cell transplantation–related CMV infection, 940
Left ventricular assist device (LVAD)–associated infection, 1020-1022, 1020f
microbiology of, 1022
Left ventricular dysfunction, in myocarditis, 387-389, 388f
Legionella longbeachae infection, 1713-1714
Legionella pneumophila, 1705-1706, 1706t
antibody to, 1707
Legionella pneumophila infection (Legionnaires' disease), **1705-1714**
clinical manifestations of, 753t-756t, 1710-1711
differential diagnosis of, 1713
dual infection in, 1713
epidemics of, 1708
epidemiology of, 1706-1708
extrapulmonary, 1709-1710, 1709t, 1712-1713
after heart transplantation, 949
historical perspective on, 1705
immune response to, 1709
in immunosuppressed host, 1707
laboratory findings in, 1710-1711
mortality with, 1713
vs. *Mycoplasma pneumoniae* infection, 2684
pathogenesis of, 1708-1710
pathology of, 1708-1709
radiography in, 1710
risk factors for, 1707-1708
treatment of, 1712-1713
urine antigen testing in, 1711
Legionella spp., 1705-1706, 1706t
Legionella spp. infection, **1705-1714.** *See also Legionella pneumophila* infection (Legionnaires' disease)
endocardial, 363
pancreatic, 695
Lemierre's disease, *Arcanobacterium haemolyticum* infection in, 1326
Leiomyoma, 2606-2607
Leiomyosarcoma, 2606-2607
Leishmania braziliensis infection, 757t-758t, 2952f, 2953
Leishmania donovani infection, 2954-2958, 2954f, 2956f
Leishmania mexicana infection, 757t-758t, 2952-2953, 2952f
Leishmania spp., 2948t, 2949
Leishmania spp. infection (leishmaniasis), **2947-2958**
cutaneous, 2948t
New World, 2948t, 2952-2954, 2953f
diagnosis of, 2953
L. braziliensis, 757t-758t, 2952f, 2953
Leishmania spp. infection (leishmaniasis) *(Continued)*
L. mexicana, 757t-758t, 2952-2953, 2952f
prevention of, 2954
treatment of, 2953-2954, 3327t-3346t
Old World, 2948t, 2950-2952
clinical manifestations of, 2950f-2951f, 2951
diagnosis of, 2951
epidemiology of, 2950
pathology of, 2950-2951
prevention of, 2952
treatment of, 2951-2952, 3327t-3346t
epidemiology of, 2949
ocular, 814
in traveler, 3117
visceral, 2948t, 2954-2958
clinical manifestations of, 2955-2956
diagnosis of, 2956-2957, 2956f
hormonal disturbances in, 2955-2956
infantile, 2955
pathology of, 2954-2955, 2954f
prevention of, 2958
prognosis for, 2957
treatment of, 2957-2958, 3327t-3346t
Leishmania tropica infection, 757t-758t, 2950-2952, 2950f-2951f, 2954f
Leishmanin skin test, 2951, 2957
Lemierre disease, 1828
Lemon eucalyptus oil repellent, 2236
Lenses
contact, infection with, 1019
intraocular, infection with, 1019
Lepromin test, 1396, 1397t, 1405-1406
Leprosy, **1392-1410**
in armadillos, 1394, 1396
Bacille Calmette-Guérin immunization in, 1409-1410
borderline, 1397t, 1399-1400, 1400f, 1402
classification of, 1397t, 1401
clinical manifestations of, 753t-756t, 1397t, 1398f-1400f, 1402f-1403f
diagnosis of, 1405-1406
differential diagnosis of, 1405-1406
elimination of, 1410
epidemiology of, 1392, 1394-1396
erythema nodosum in, 1404-1405, 1404f
treatment of, 1409
genetic factors in, 1395-1396
histoid, 1401, 1403, 1405-1406
histopathology of, 1397t, 1398-1401, 1399f-1401f
historical perspective on, 1392-1394
HIV infection and, 1403
human leukocyte antigens and, 1395
immunology of, 1396-1398, 1397t
indeterminate, 1397t, 1398, 1398f-1399f, 1401-1402
in infant, 1394
lepromatous, 1397t, 1400-1403, 1400f-1401f, 1403f-1404f
Lucio, 1403
mouse footpad assay in, 1405
multibacillary, 1401-1403
neuritic, 1403
ocular, 813, 1401
pathogenesis of, 1396-1401
pathology of, 1396-1401, 1396f
paucibacillary, 1401-1402
peripheral nerves in, 1401-1402, 1402f, 1405-1406
placental transmission of, 1393-1394
prevention of, 1409-1410
prognosis for, 1406

Leprosy *(Continued)*
reactions in, 1403-1405, 1404f
treatment of, 1408-1409
reversal reaction in, 1400f, 1404, 1404f
treatment of, 1408-1409
smear examination in, 1405
Toll-like receptors and, 1395-1396
transmission of, 1393-1394
treatment of, 1406-1409, 1407t
tuberculoid, 1397t, 1398-1399, 1399f, 1402, 1402f, 1404f
Lepto Dipstick, 1755-1756
Leptomeningitis. *See also* Meningitis
tuberculous, 1354
Leptospira spp., 1743
lipids of, 1746
virulence factors of, 1745
Leptospira spp. infection (leptospirosis), **1743-1759**
anicteric, 1750-1752, 1751f
animal immunization against, 1757-1758
animal reservoirs in, 1743-1744
cardiovascular findings in, 1748
clinical manifestations of, 753t-756t, 1749-1753, 1749f
diagnosis of, 1754-1756
epidemiology of, 1743-1745
fever of unknown origin in, 844
gallbladder in, 1750, 1751f
hemolytic anemia in, 1746-1747
hepatic, 656, 1746-1747
historical perspective on, 1743
icteric (Weil syndrome), 1749f, 1752-1753
immune response to, 1745-1746, 1750
jaundice in, 1746-1747
musculoskeletal findings in, 1749
nervous system findings in, 1748
ocular involvement in, 812-813, 1748-1749
pancreatitis in, 695
pathophysiology of, 1745-1749
prevention of, 1757-1758
prognosis for, 1753-1754
pulmonary involvement in, 1748, 1750-1751, 1753
renal injury in, 1747-1748
transmission of, 1744-1745
in traveler, 3117
treatment of, 1756-1757
Leptospiral endostatin-like proteins, 1745
Leptospiral immunoglobulin-like proteins, 1745
Lethal factor, in anthrax, 1303
Leuconostoc spp., 1246
vs. *Enterococcus* spp., 1171
Leuconostoc spp. infection, **1246-1247**
clinical manifestations of, 1247
diagnosis of, 1247
epidemiology of, 1246-1247
pathophysiology of, 1247
treatment of, 1247
Leucovorin, in *Toxoplasma gondii* infection, 3000
Leukemia
adult T-cell, 2565-2569, 2566t-2567t
Aspergillus infection and, 2711
Candida infection and, 2743-2744
coagulase-negative staphylococcal bacteremia in, 1135-1136
ecthyma in, 776
hematopoietic stem cell transplantation for. *See* Hematopoietic stem cell transplantation (HSCT)
HSV infection and, 1952
human bocavirus infection in, 1861
Leukemia *(Continued)*
intravenous immunoglobulin in, 3499
mumps immunization and, 2406
Pseudomonas spp. infection and, 1594-1596
VZV infection and, 2026, 2026f
Leukemoid reaction, in *Shigella* spp. infection, 1482
Leukocidins, of *Staphylococcus aureus*, 1115-1117
Leukocyte adhesion deficiency, 904-906, **918-919**
clinical features of, 905f, 918
diagnosis of, 919
pathogenesis of, 907t, 918-919
treatment of, 919
type 1, 52-53, 918-919
type 2, 53, 919
type 3, 53-54, 919
Leukocyte esterase, in urinary tract infection, 543
Leukocyte response integrin (LRI), filamentous hemagglutinin interaction with, 6
Leukotriene(s)
in burns, 1049
in *Streptococcus pneumoniae* infection, 1224-1225
Leukotriene B_4
in Kawasaki disease, 1078
of *Streptococcus agalactiae* infection, 1158
Leukotriene C_4, in bronchiolitis, 277
Levofloxacin
in odontogenic infection, 145
in tuberculosis, 1367t, 1369
Lice, 758t, **3074-3076**
body, 3075-3076
diagnosis of, 3075
disease transmission by, 3075
eyelid, 795, 795f
head, 3074-3076
in daycare facilities, 3559
nosocomial, 3385
pubic, 3075-3076
in relapsing fever, 1740, 1741t
treatment of, 3075-3076, 3327t-3346t
Lichtheimia spp., 2837
Light's criteria, 311-312
Lincomycin, 3220-3222
Lincosamides, 3220-3222, 3231t-3233t
adverse effect of, 3222
drug interactions with, 3222
indications for, 3221-3222
mechanisms of action of, 3220
pharmacokinetics of, 3221
resistance to, 3145-3147, 3220
in vitro activity of, 3220-3221
Lindane shampoo, 795
Linear gingival erythema, 149
Linezolid, 3222-3224, 3231t-3233t
adverse effects of, 3223-3224
in coagulase-negative staphylococcal infection, 1139
indications for, 3223
mechanisms of action of, 3223
in methicillin-resistant *Staphylococcus aureus* infection, 1127
in *Nocardia* spp. infection, 1422
pharmacokinetics of, 3223
resistance to, 3153, 3223
in tuberculosis, 1367t
in vancomycin-resistant *Enterococcus* spp. infection, 1183-1184
in vitro activity of, 3223
Lipase, serum, in pancreatitis, 690, 692
Lipids
eicosanoid, 75
energy metabolism and, 74-75
metabolism of, 73-75, 73t
plasma, in Kawasaki disease, 1076
Lipo-oligosaccharide
Haemophilus ducreyi, 1686
Haemophilus influenzae, 1667-1668
Moraxella catarrhalis, 1251
Neisseria gonorrhoeae, 1278
Neisseria meningitidis, 1254, 1257
Lipovnik virus, 2175
Lipopolysaccharide (LPS), 19, 24
in animal models, 825t, 826-827
Bordetella pertussis, 1618t, 1620
Brucella spp., 1613
components of, 826
Helicobacter pylori, 1693
in meningitis, 435
in septic shock, 827-831, 828f. *See also* Septic shock
Shigella, 1478-1479
in urinary tract infection, 537-538
Lipopolysaccharide-binding protein (LBP), 827, 828f
Lipoproteins, 73-74
high-density, 74
low-density, 74
Lipoteichoic acid, of *Streptococcus pneumoniae*, 1210, 1211f
Liquid crystal thermometer, 86
Listeria monocytogenes, 1329
ActA of, 16
carriage of, 1330
internalins of, 13
Listeria monocytogenes infection (listeriosis), **1329-1335**
clinical manifestations of, 753t-756t, 1331-1332, 1332f, 1332t
clinical manifestations of, 607t, 613
diagnosis of, 618, 1332-1333
endocardial, 362-363
epidemiology of, 1330, 1330f
exanthem in, 753t-756t, 1332, 1332f
immune response to, 1331
after kidney transplantation, 1011
neonatal, 882, 1331-1333, 1332t
pathogenesis of, 15-16, 1330-1331
prevention of, 1333-1334
prognosis for, 1333
transmission of, 1330
treatment of, 1333
Listeriolysin O, 15
Listeriopods, 16
LITH gene, 668-669
Liver. *See also at* Hepatic
abscess of. *See* Liver abscess
anoxic injury to, 657
bacterial infection of, 650, 651t, 656-657. *See also* Liver abscess
in congenital syphilis, 1771
in *Corynebacterium diphtheriae* infection, 1305
in cystic fibrosis, 324-325
drug metabolism by, 3236
Entamoeba histolytica infection of, 2869-2874
fatty infiltration of, 657-658
hydatid disease of. *See Echinococcus granulosus* infection (echinococcosis, hydatid disease)
infarction of, 673
in infectious mononucleosis, 1996, 2003
inflammation of. *See* Hepatitis
in influenza virus infection, 2344

Liver *(Continued)*
isoniazid effects on, 1366-1368
in *Leptospira* spp. infection, 1746-1747, 1752-1753
in *Mycoplasma pneumoniae* infection, 2682
Schistosoma spp. infection of, 3065
in septic shock, 830
in toxic shock syndrome, 853
transplantation of. *See* Liver transplantation
trauma to, abscess with, 673
tuberculosis of, 1357
tumors of, 657
viral infection of. *See* Hepatitis
in yellow fever, 2276
Liver abscess
Achromobacter xylosoxidans, 1574-1575
amebic, 662, 2869-2872
complications of, 2872-2873
differential diagnosis of, 2872
prognosis for, 2874
treatment of, 2873-2874, 2873t
differential diagnosis of, 2872
fever of unknown origin in, 845
Klebsiella pneumoniae, 1467
after liver transplantation, 993
pyogenic, 662, **672-675**, 674f
clinical manifestations of, 673-674
complications of, 675
cryptogenic, 673
diagnosis of, 674, 674f
drainage of, 674-675
epidemiology of, 672
microbiology of, 673
mortality with, 675
pathogenesis of, 672-673
prognosis for, 675
treatment of, 674-675
Liver failure
in *Bacillus cereus* infection, 1320
endogenous bacterial toxins in, 94
Liver flukes, 660, 2865-2866, 3049-3054, 3050f-3051f
Liver transplantation
in HAV infection, 2141
in HBV infection, 1924
in HDV infection, 1932
infection after, **989-1002**
adenovirus, 997t, 999, 1903
age and, 990
bacterial, 993-994
bile duct, 665-666
catheter and, 991, 993
CMV, 996-997, 997t, 1001, 3520-3521
coccidioidal, 1000
cryptococcal, 1000
donor-related factors in, 990
early (0 to 30 days), 991-992, 991t
EBV, 997-998, 997t, 1001
fungal, 813, 991, 993-994, 995t, 2745-2746
HBV, 1924, 3517-3519
HHV-6, 999
HHV-7, 999
Histoplasma capsulatum, 1000
immunosuppression and, 990-991
intermediate (31 to 180 days), 992, 992t
intraoperative factors in, 990
Klebsiella pneumoniae, 1468
late (after 180 days), 992-993, 992t
Mycobacterium tuberculosis, 1000
nosocomial, 991, 993
pancreatic, 692-693
Pneumocystis jiroveci, 1000
post-transplant factors in, 990-991
Liver transplantation *(Continued)*
predisposing factors in, 989-991
pretransplant evaluation in, 1000-1001
pretransplant factors in, 989-990
prevention of, 1001, 3517-3519
RSV, 997t, 999
timing of, 991-993, 991t-992t
viral, 995-1001, 997t
Ljungan virus infection, 2092
Loa loa filariasis (loiasis), 815, **3026-3027**, 3327t-3346t
Long-term care facilities, infection in, 3407
Loperamide
in diarrheogenic *Escherichia coli* infection, 1463
in gastrointestinal tract infection, 626
Lopinavir/ritonavir, 2615
Loracarbef, 3199-3200, 3231t-3233t
Louping ill virus infection, **2315-2316**
Louse. *See* Lice
Louse-borne relapsing fever, **1739-1742**, 1741t
Ludwig angina, 149-150, 150f
Lujo virus infection, 2470-2472, 2470t. *See also* Arenavirus infection
Lumbar puncture. *See* Cerebrospinal fluid (CSF) examination
Lumpy jaw, 184
Lung(s). *See also* Pneumonia
abscess of. *See* Lung abscess
acute injury to, 867. *See also* Acute respiratory distress syndrome (ARDS)
anthrax of, 1312-1314, 3581-3582, 3581f
Aspergillus spp. infection of, 2706, 2708, 2713f, 2717-2721
biopsy of
in *Aspergillus* spp. infection, 2711, 2712f
in hypersensitivity pneumonitis, 304
in interstitial lung disease, 299-300
in *Pneumocystis* infection, 3010
Blastomyces dermatitidis infection of, 2727, 2727f
Cryptococcus neoformans infection of, 2800-2801, 2804
EBV infection of, 2004
Histoplasma capsulatum infection of, 2812-2813, 2826
HIV-related disorders of, 2603-2604, 2603f
hyperlucent, unilateral, 1898
interstitial disease of. *See* Interstitial lung disease
shock, 830, 832
Sporothrix schenckii infection of, 2834
transplantation of. *See* Lung transplantation
in Whipple disease, 644
Lung abscess, **315-322**
age and, 316, 318
clinical features of, 317t, 318
diagnosis of, 319, 319f-320f
differential diagnosis of, 318-319
hematogenous seeding of, 316
immunodeficiency and, 317
Klebsiella spp., 1467
microbiology of, 317-318, 320t
mortality with, 315-316, 321
Mycoplasma pneumoniae, 2677
pathophysiology of, 316-317
primary, 316
prognosis for, 321
rupture of, 318
secondary, 316-317
surgery for, 321
tonsillectomy and, 316
Lung abscess *(Continued)*
treatment of, 319-321
viral infection and, 316
Lung flukes, 2865-2866, 3054-3056
Lung transplantation
in cystic fibrosis, 343-344
HBV infection and, 1924
historical perspective on, 957
indications for, 957
infection after, **957-988**
Acinetobacter, 962
adenovirus, 981-982
anastomotic, 959-960
Aspergillus spp., 959-960, 965-970
bacterial, 960-965
Blastomyces dermatitidis, 974
bloodstream, 960
Bordetella bronchiseptica, 983
bronchiolitis obliterans and, 982
Burkholderia cepacia, 960-962
Candida spp., 970-971
CMV, 974-976, 3520-3521
Coccidioides immitis, 973
Cryptococcus neoformans, 971-972
EBV, 976-978
Escherichia coli, 962
etiology of, 957, 958t
fungal, 959-960, 965-974
HHV-6, 979
HHV-7, 979
HHV-8, 979
Histoplasma capsulatum, 973-974
hMPV, 980
HSV, 978
immunosuppression and, 958-959
incidence of, 957
influenza virus, 980-981
Klebsiella pneumoniae, 962
LCMV, 983
Mycobacterium tuberculosis, 962-964
nontuberculous *Mycobacterium* spp., 964-965
parainfluenza virus, 980, 2368
Pneumocystis jiroveci, 972-973
Pseudomonas aeruginosa, 960-962
rabies, 983
risk factors for, 959
RSV, 979-980
sites of, 959-960
thoracic cavity, 959-960
timing of, 958-959, 958t-959t
treatment duration in, 959
viral, 957, 974-982
VZV, 978-979
West Nile virus, 983
zoonotic, 983
in interstitial lung disease, 300
mortality after, 957
Lupus pneumonitis, 297
Lupus vulgaris, 753t-756t
Lutein, 77
Lycopene, 77
Lyell syndrome, 753t-756t
Lymantria dispar. *See* Gypsy moth rash
Lyme disease, **1729-1739**
acrodermatitis chronic atrophicans in, 1734
Anaplasma spp. infection and, 1734, 2663
arthritis in, 730, 1731-1733, 1736
asymptomatic seroconversion in, 1734
Bannwarth syndrome in, 1733
cardiac manifestations of, 1733, 1735
chronic, 1736-1737
clinical manifestations of, 1731f, 1732-1734

Lyme disease *(Continued)*
coinfection in, 1734
diagnosis of, 1734-1735
epidemiology of, 1730
erythema migrans in, 753t-756t, 1731f, 1732, 1734-1735
historical perspective on, 1729
immune response to, 1730-1731
vs. infective endocarditis, 358-359
lymphocytoma in, 1731f, 1734
meningeal, 1732-1733
neurologic manifestations of, 494t-498t, 1732-1735
nonspecific illness in, 1733
ocular manifestations of, 812
pathogenesis of, 1730-1732
in pregnancy, 1734
vs. tick-borne encephalitis virus infection, 2311
vs. tick-borne relapsing fever, 1741t
treatment of, 1735-1738, 1736t
Lymph node(s)
cervical, 176, 176f. *See also* Cervical lymphadenitis
in infectious mononucleosis, 1996, 1999, 1999f, 1999t
in Kawasaki disease, 1074
in monkeypox, 2042
in *Paracoccidioides* spp. infection, 2784, 2784f-2785f, 2786
in rubella virus infection, 2210-2211, 2218
in *Toxoplasma gondii* infection, 2991
in Whipple disease, 644
Lymphadenitis
cervical. *See* Cervical lymphadenitis
in human erythrovirus B19 (human parvovirus B19) infection, 1854-1855
mesenteric
in adenovirus infection, 1902
in tuberculosis, 1357
in nontuberculous mycobacterial infection, 1383-1384
Streptococcus agalactiae, 1161
tuberculous, superficial, 1356
Lymphocyte. *See* B lymphocytes; T lymphocytes
Lymphocyte-specific protein-1 (LSP-1), 54
Lymphocyte transformation test, in *Toxoplasma gondii* infection, 2996
Lymphocytic choriomeningitis virus (LCMV) infection, 811-812, **2463-2466**
clinical manifestations of, 2464-2465
congenital, 817, 2465
diagnosis of, 2465
encephalitic, 494t-498t, 500
epidemiology of, 2463-2464
historical perspective on, 2463
after lung transplantation, 983
meningeal, 487
in transplant recipient, 2465
treatment of, 2465
Lymphocytic interstitial pneumonitis, 296, 1997, 2007
HIV-related, 2603-2604, 2603f
Lymphocytoma, borrelial, 1731f, 1734
Lymphogranuloma venereum, 582-584, 583t, 584f, **2635-2636**
vs. plague, 1513
Lymphohistiocytosis, hemophagocytic. *See* Hemophagocytic lymphohistiocytosis (hemophagocytic syndrome)
Lymphoid interstitial pneumonitis/hyperplasia, vs. chronic bronchitis, 268
Lymphokines, 3357-3362
Lymphoma
body cavity, 2007
Burkitt, 1996, 2005, 2005f, 2013
cerebral, 2011, 2013
coagulase-negative staphylococcal bacteremia in, 1135-1136
Hodgkin, 182-183, 182t, 1997, 2005, 2013
HTLV-1–associated, 2569
non-Hodgkin, 182-183, 182t, 2006-2007
pancreatic, 692
plasmablastic, 2006
pyothorax-associated, 2006
T-cell, 2006
in Whipple disease, 644
Lymphomatoid granulomatosis, 2006
Lymphoproliferative disease
post-transplant. *See* Post-transplant lymphoproliferative disease (PTLD)
X-linked, 915
Lysogeny, of *Streptococcus pneumoniae*, 1215
Lysosomal trafficking regulator (LYST), **54**
Lysozyme, 24, 34t, 35
Lyssavirus, 2445-2447, 2445f-2446f. *See also* Rabies

M

M protein, 10, 18
in rheumatic fever, 405-406, 405t, 407f
Streptococcus pyogenes, 1140
Machupo virus infection, 2466, 2468-2469, 2470t, 2471. *See also* Arenavirus infection
Macrolides, 3211-3215
adverse effects of, 3214-3215
dosage schedules for, 3231t-3233t
drug interactions with, 3215
indications for, 3213-3214
mechanisms of action of, 3211
pharmacokinetics of, 3212-3213, 3213t
resistance to, 3145-3147, 3211-3212
in vitro activity of, 3212
Macrophage(s)
in burns, 1050
in Whipple disease, 641-642, 642f, 645
Macrophage activation syndrome, in Kawasaki disease, 1082
Mafenide acetate, in burn injury, 1058-1059
Maggots, ocular, 816
Magnesium sulfate, in tetanus, 1816-1817
Magnetic resonance cholangiopancreatography (MRCP), 690
Magnetic resonance imaging (MRI)
in acute disseminated encephalomyelitis, 515f, 518-519
in acute transverse myelitis, 522, 522f
in amebic CNS infection, 2980
in *Aspergillus* spp. infection, 2707, 2713-2714, 2714f
in bacterial meningitis, 445-446, 454
in *Blastomyces dermatitidis* infection, 2730f
in brain abscess, 466-467
in chronic fatigue syndrome, 1088
in chronic osteomyelitis, 726, 726f
in Creutzfeldt-Jakob disease, 2628
in cryptococcal meningoencephalitis, 2801-2802
in Eastern equine encephalitis, 2228
in encephalitis, 506-507
in HSV encephalitis, 1948f
in HTLV-1 infection, 2571
in intra-abdominal abscess, 705
in Japanese encephalitis virus infection in, 2296
in myocarditis, 392-393
Magnetic resonance imaging (MRI) *(Continued)*
in necrotizing fasciitis, 779
in neurocysticercosis, 3035-3036, 3036f
in osteomyelitis, 715-716, 716f
in pericarditis, 377
in progressive multifocal leukoencephalopathy, 1866-1867, 1866f
in pyogenic liver abscess, 674
in pyomyositis, 737, 738f
in retroperitoneal infection, 707f, 708
in rhinosinusitis, 199
in *Schistosoma* spp. infection, 3067-3068
in spinal cord infarction, 524f
in spinal epidural abscess, 471-472, 472f
in subdural empyema, 469
in tick-borne encephalitis virus infection, 2285, 2308f
in urinary tract infection, 545-546
in vertebral osteomyelitis, 722
in West Nile virus infection, 2270
Magnetic resonance sialography, 192
Majeed syndrome, 714
Major histocompatibility complex (MHC)
class I, 36-37, 38f
class II, 37, 38f
Malaria, **2921-2947**
acid-base changes in, 2928
anemia in, 76, 78, 2926-2927
autochthonous, 2923
bloodborne, 2923
cerebral, 2928
chemoprophylaxis in, 2943, 2944t
in children, 2929
clinical manifestations of, 757t-758t, 2926-2929
congenital, 2924, 2929
cryptic, 2925
diagnosis of, 2930-2936
antibody detection in, 2936
antigen detection in, 2935
Buffy coat test in, 2930-2935
dipstick tests in, 2935
DNA probes in, 2935
flow cytometry in, 2936
microscopy in, 2930, 2931f-2934f
PCR in, 2935
EBV and, 1996
endemic, 2923
epidemiology of, 2923, 2924f-2925f, 3107-3108
etiology of, 2922-2923, 2922f, 2927t
fever of unknown origin in, 844
genetic factors in, 2926
glucose metabolism in, 72-73
historical perspective on, 2921-2922
host-parasite interaction in, 2925-2926
hyperactive malarial syndrome in, 2929
hyperlactatemia in, 75-76
hypoglycemia in, 2928
in international adoptees and refugees, 3129
metabolic acidosis in, 75-76
mortality from, 2922
mosquito bite in, 2922f, 2923, 2925-2926
myocarditis in, 400
nitrogen balance in, 68
ocular manifestations of, 814
pancreatitis in, 694-695
pathophysiology of, 2926, 2927t
personal protective measures in, 2943
in pregnancy, 2929-2930
prevention of, 2943-2946, 2944t
in travelers, 3108-3110, 3109f, 3111t

Malaria *(Continued)*
recurrent, 2926
renal complications of, 2928
respiratory complications of, 2928
Salmonella spp. infection and, 1498
Schistosoma spp. infection and, 3063-3064
serum iron in, 78
severe, 2927, 2928t
thyroid hormone levels in, 82
transmission of, 2923-2925
in travelers, 2926, 3108-3110, 3109f, 3111t
treatment of, 2936-2943, 2937t-2940t, 3327t-3346t
adjunctive, 2943
emergency, 2945-2946
supportive, 2942-2943
vaccine against, 2946
Malassezia spp. infection, **789**, 789f, **2852-2854**
neonatal, 789, 789f, 897-898
Malathion, 3349
in lice, 3075
Malignant otitis externa, 207-208
Malnutrition
bacterial meningitis and, 437
Cryptosporidium spp. infection and, 2901
hookworm infection and, 3017
murine tuberculosis and, 70
protein, 108-109
Mandible, osteomyelitis of. *See* Osteomyelitis, mandibular/maxillary
Manganese, metabolism of, 77t
Mannitol, in encephalitis, 510
Mannose-binding lectin (MBL)
deficiency/dysfunction of
furunculosis and, 777
Neisseria meningitidis infection and, 1257
in *Streptococcus pneumoniae* infection, 1205, 1226
Mansonella ozzardi infection, **3028**, 3327t-3346t
Mansonella perstans infection, **3028**, 3327t-3346t
Mantoux test. *See* Tuberculin skin test
Maraviroc, 2611
Marburg virus, 2478-2479
Marburg virus disease (Marburg fever, Marburg hemorrhagic fever), **2478-2486**
bioterrorist, 3585
clinical manifestations of, 2483
diagnosis of, 2484
differential diagnosis of, 2483-2484
epidemiology of, 2478-2479, 2479f, 2480t, 2482-2483
exanthem in, 742t-748t, 749, 760
pathology of, 2483
prevention of, 2485
treatment of, 2484-2485
Maribavir, in CMV infection, 1986
Marine toxins, 604, 605t
Mass spectroscopy, 3594-3595
Masticator space, infection of, 142, 148-149, 149f
Mastoid process, 224, 233-234
Mastoidectomy, 238-239
Mastoiditis, **233-240**
anatomy of, 233-234
chronic, 234, 236, 239
clinical presentation of, 234-236, 236f
coalescent, 233
complications of, 236-237
diagnosis of, 237-238, 238f
differential diagnosis of, 237
Mastoiditis *(Continued)*
historical perspective on, 233
microbiology of, 234, 235t
otitis media–related, 223-224
pathophysiology of, 233-234
prevention of, 240
prognosis for, 240
Streptococcus pneumoniae, 1231
treatment of, 238-240
Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry, in *Streptococcus pneumoniae* infection, 1216
Matrix metalloproteinase–9, in ARDS, 870
Maxilla, osteomyelitis of. *See* Osteomyelitis, mandibular/maxillary
Mayaro fever, 2252-2253
MCPyV, 1864, 1864t, 1867-1868
Measles, **2373-2395**
acute disseminated encephalomyelitis with, 516
appendiceal, 2392
aspirin in, 85
atypical, 2383-2384
clinical manifestations of, 2383-2384, 2384f
diagnosis of, 2391
exanthem in, 2384
historical aspects of, 2383
pathogenesis of, 2384
vs. Rocky Mountain spotted fever, 765
treatment of, 2392
cardiac involvement in, 2385
clinical manifestations of, 2381-2391
CNS, 494t-498t, 500, 2385-2386, 2391-2392
complications of, 2384-2386, 2392
corneal, 807
in daycare facilities, 3562-3563
in developing countries, 2390
diagnosis of, 2382, 2391
differential diagnosis of, 2391
epidemiology of, 2375-2377, 2375f, 2376t
eradication of, 2394
exanthem in, 742t-748t, 749, 2378, 2382, 2382f
fulminant, 2390
geographic distribution of, 2376
hepatitis with, 655
historical perspective on, 2373-2374
host factors in, 2376-2377
immune response to, 2379-2380, 2380t
immunization against, 2393-2395, 3448-3452
acute disseminated encephalomyelitis with, 517
adverse events with, 3449-3450
autism and, 3429
complications of, 2393-2394
contraindications to, 2393, 3450-3452
efficacy of, 3449
encephalitis after, 502
in HIV-infected host, 3429
immune globulin interaction with, 3451, 3452t
indications for, 2393, 3450
meningitis and, 488
precautions for, 2393, 3450-3452
preparations for, 3449
in immunocompromised host, 2390-2391
incidence of, 2376, 2376t
incubation period in, 2381
Koplik spots in, 2378, 2381-2382, 2381f
laboratory findings in, 2382
laryngitis in, 2385, 2392
modified, 2382-2383
Measles *(Continued)*
mutant, 2388-2389
nosocomial, 3383-3384
ocular manifestations of, 811
otitis media in, 2385, 2392
pathogenesis of, 2377-2378, 2378t
pathology of, 2378, 2378f
pharyngeal, 159t-160t, 161
prevalence of, 2375-2376, 2375f
prevention of, 2381
antibiotics in, 2392-2393
immunization in, 2393-2395
immunoglobulin in, 2394-2395, 3526
quarantine in, 2394
prodromal period in, 2381-2382, 2381f
pulmonary involvement in, 2384-2385, 2384f, 2391-2392
recovery from, 2380-2381
recurrent, 2383
reporting requirement for, 2394
respiratory tract involvement in, 2378, 2392
seasonal patterns of, 2376
subacute sclerosing panencephalitis after, **2386-2390**
thrombocytopenic purpura in, 2386
tracheitis in, 2385, 2392
transmission of, 2377
treatment of, 2391-2393
vitamin A deficiency and, 807, 2391-2392
Measles virus, 2374-2375
animal susceptibility of, 2375
antigenic composition of, 2374
classification of, 2374
isolation of, 2374-2375, 2375f
physical properties of, 2374, 2374f
Mebendazole, 3349
in *Echinococcus granulosus* infection, 3046
in *Enterobius vermicularis* infection, 567, 3019
Mechanical ventilation, 870-872
low tidal volume, 871
permissive hypercapnia with, 871-872
permissive hypoxemia with, 872
pneumonia with, 892, 3391-3393
Meconium ileus, in cystic fibrosis, 324-325
Mediastinitis, **419-423**
acute, 419-422, 420t
esophageal perforation and, 419-420
infection extension and, 420-421, 421f
postoperative, 421-422, 421t
chronic, 420t, 422-423
fibrosing, 422
in *Histoplasma capsulatum* infection, 2813, 2817-2819, 2828-2829
after heart transplantation, 948-949
postoperative, 421-422, 421t
retropharyngeal abscess and, 172
Mediastinum, 419
Mediterranean spotted fever, 2648t-2649t, **2655-2656**
Mefloquine, 3349
in malaria, 2937t-2940t, 2941
in malaria prevention, 2944t, 2945
Megacolon
toxic, with *Shigella* spp. infection, 1481
in *Trypanosoma cruzi* infection, 2965
Meglumine antimoniate, 3349
in visceral leishmaniasis, 2957
Meibomian gland
granulomatous inflammation of, **796-797**
infection of, **796**
Melanin, *Cryptococcus neoformans* production of, 2796-2797

Melarsoprol, 3349
in *Trypanosoma brucei* infection, 2972
Melioidosis. *See Burkholderia pseudomallei* infection (melioidosis)
Meningitest, 445
Meningitis
adrenal function in, 81
aseptic, **484-492**
adenovirus, 488, 1889t, 1903
arbovirus, 486t, 487
bacterial, 486t, 488-489
vs. bacterial meningitis, 444-445
C-reactive protein in, 444
Chlamydia pneumoniae, 488
clinical manifestations of, 489-490
diagnosis of, 490-491
differential diagnosis of, 490
drug-related, 488
EBV, 2003-2004
enterovirus, 485-487, 489, 2086-2090, 2088t-2089t
epidemiology of, 488-489
etiology of, 485-488, 486t
exanthem in, 765, 766t
fungal, 486t, 488
HHV-6, 487, 2018
HHV-7, 487, 2018
historical perspective on, 485
HIV-1, 487, 2605
HSV, 487, 1943
vs. HSV encephalitis, 1946
human erythrovirus B19 (human parvovirus B19), 1853
IVIG-related, 3496
La Crosse virus, 487
LCMV, 487
mumps virus, 488, 2401
vs. tick-borne encephalitis virus infection, 2310-2311
Mycobacterium tuberculosis, 488-489, 1352-1354, 1373
Oropouche fever virus, 2542-2543
parasite, 486t, 488
PCR in, 444
poliovirus, 2098. *See also* Poliovirus infection (poliomyelitis)
prognosis for, 491
recurrent, 489-490, 1946
reovirus, 2171
rotavirus, 2186
Toscana virus, 2548
treatment of, 491
VZV, 487, 2031
West Nile virus, 487, 489, **2261-2273.** *See also* West Nile virus infection
bacterial, **425-461**
Achromobacter spp., 1574
Acinetobacter spp., 1570
Actinomyces spp., 1822-1823
adjunctive therapy in, 449-453
Aerococcus viridans, 1249
Aeromonas spp., 1541
anti-inflammatory therapy in, 451
antidiuretic hormone in, 438, 453-454
arthralgia in, 441
arthritis in, 441
Bacillus anthracis, 1314
Bacillus cereus, 1320
bacteremia in, 431-432, 432t
Bacteroides spp., 1828-1829
blood-brain barrier in, 432-433, 433t
Bordetella holmesii, 1637
Borrelia spp., 1732-1733
brain abscess and, 466
Brucella spp., 1613-1615
Meningitis *(Continued)*
C-reactive protein in, 444
cancer and, 437
cellulitis in, 440-441
cerebral edema in, 439
chronic systemic disease and, 437
Citrobacter spp., 1429-1430, 1432-1433, 1432f
clinical manifestations of, 438-441, 439t-440t
clinical score in, 445
Clostridium spp., 1797
cochlear implant and, 437, 1017-1018, 1229
complications of, 1228-1229
corticosteroids in, 451-453
countercurrent immunoelectrophoresis in, 442
cranial nerve involvement in, 439
Cronobacter sakazakii, 1436, 1438
CSF examination in, 441-446, 443t-444t, 449, 451t, 455
vs. CSF shunt infection, 1030
CT in, 445-446, 446f, 454, 458
culture in, 442, 445
diagnosis of, 441-446, 443t-444t, 446f
differential diagnosis of, 441
Ehrlichia, 2662
Elizabethkingia meningosepticum, 1579-1581
Enterococcus spp., 1175-1176
epidemiology of, 425-430
Erysipelothrix rhusiopathiae, 1328
Escherichia coli, 1444-1445
epidemiology of, 1441
pathogenesis of, 1443t, 1444
postoperative, 1445
treatment of, 1447
exanthem in, 765, 766t
ferritin in, 445
focal neurologic signs in, 439, 440t
Fusobacterium spp., 1828-1829
genetic factors in, 436
glycerol in, 453
Gram stain in, 445
Haemophilus influenzae, 426t, 429-430, 430f, 450t-451t, 460-461, 1670, 1675, 1678-1679
Haemophilus parainfluenzae, 1690
Hafnia alvei, 1535
hearing loss and, 452, 457-458, 1259
humidified environment and, 431
hydrocephalus in, 438, 445
impaired consciousness in, 438
incidence of, 425-430, 426t
increased intracranial pressure in, 438-439, 454
inflammation in, 433-435, 434f
lactate dehydrogenase in, 444
lactate in, 444
latex particle agglutination test in, 442
Leptospira spp., 1748, 1751
Leuconostoc spp., 1247
malnutrition and, 437
mastoiditis and, 239
Meningitest in, 445
Moraxella catarrhalis, 1252-1253
Morganella morganii, 1470-1471
MRI in, 445-446, 454
mucosal colonization in, 431-432
Neisseria gonorrhoeae, 1288
Neisseria meningitidis, 426t, 428-429, 428f, 450t-451t, 459-460, 460t, 1258-1260. *See also Neisseria meningitidis* infection
Meningitis *(Continued)*
neonatal, 431, 436, 881-882, 885-887
neurologic sequelae of, 455-457, 456t-457t
neuronal injury in, 434f, 435-436
Nocardia spp., 1421
optic nerve involvement in, 440
otitis media and, 224
outpatient therapy in, 449
Pantoea agglomerans, 1536
Pasteurella multocida, 1547
pathogenesis of, 431-437, 446t
pathology of, 437-438, 437f
pathophysiology of, 431
pericardial effusion with, 441
persistent fever in, 455
Plesiomonas shigelloides, 1565-1566
polymerase chain reaction test in, 442-444
prevention of, 458-461, 3249
PRISM score in, 458
procalcitonin in, 444
prognosis for, 455-458
Proteus mirabilis, 1472-1474
Providencia spp., 1475
Pseudomonas aeruginosa, 1598-1599
radionuclide imaging in, 445
rapid antigen detection test in, 442
recurrent, 430-431, 441, 449
risk factors for, 436-437
route of infection in, 431
Salmonella spp., 1500-1501, 1505
seizures in, 439-440, 440t, 455
sequelae of, 455-458, 456t-457t
Serratia spp., 1490
shock in, 440, 454
vs. St. Louis encephalitis virus infection, 2259
Stenotrophomonas maltophilia, 1607
Streptococcus agalactiae, 1160, 1160f, 1162
Streptococcus pneumoniae, 426-427, 426t, 437, 450t-451t, 458-459, 1228-1229, 1235. *See also Streptococcus pneumoniae* infection
Streptococcus viridans, 1193
subdural effusion in, 438, 440, 454-455
supportive care in, 453-455
vs. tick-borne encephalitis virus infection, 2311
trauma-related, 432
treatment of, 446-455, 446t
anti-inflammatory therapy in, 451
antibiotics in, 446-449, 450t-451t
chemoprophylactic, 458-461
corticosteroids in, 451-453
CSF examination and, 442
fluid restriction in, 453-454
glycerol in, 453
immunoprophylactic, 458-460
supportive care in, 453-455
venous thrombosis in, 438
ventriculitis in, 437-438
vs. viral meningitis, 444-445
Yersinia pestis, 1512-1513
in burn patient, 1056
chemical, 431
cochlear implant and, 224, 437, 1017-1018, 1229
craniofacial surgery and, 1044-1045
eosinophilic, **481-484**
clinical manifestations of, 482-483
diagnosis of, 483
differential diagnosis of, 483
epidemiology of, 482

Meningitis *(Continued)*
etiology of, 481-482
pathogenesis of, 482
prevention of, 484
prognosis for, 484
treatment of, 483-484
fungal, **473-481**, 486t, 488
Acremonium spp., 479
Aspergillus spp., 478-479
Blastomyces dermatitidis, 478, 480t
Candida, 474-475, 480t, 2742-2743, 2749
neonatal, 894-895, 897, 2754, 2757
clinical manifestations of, 473-479
Coccidioides spp., 477-478, 480t, 483, 2774, 2778
Cryptococcus neoformans, 475-476, 480t
diagnosis of, 474, 479-480, 480t
epidemiology of, 473
Histoplasma capsulatum, 476-477, 480t, 2816, 2821, 2828
Mucor spp., 479
Sporothrix schenckii, 479
Mollaret, 489-490, 1946
Mycoplasma pneumoniae, 2683-2684
neonatal, 882
Candida, 894-895, 897
Clostridium spp., 1797
coagulase-negative staphylococci, 1135
Klebsiella pneumoniae, 882
Listeria monocytogenes, 882
Neisseria meningitidis, 1260
Streptococcus agalactiae, 881-882
Streptococcus viridans, 1192
noninfectious, 441
Meningococcal meningitis, 426t, 428-429, 428f, 450t-451t, 459-460, 460t, 1258-1260. *See also Neisseria meningitidis* infection
Meningococcemia, 432, 432t, 824, 829, 831, 1258-1260
chronic, 1260
cutaneous manifestations of, 1259, 1259f
differential diagnosis of, 1258t
treatment of, 833-834, 833t
Meningoencephalitis, **492-512.** *See also* Encephalitis
amebic, 501, 3327t-3346t
CMV, 1978-1980, 1979f
cryptococcal, 2801-2803
clinical manifestations of, 2801
complications of, 2802-2803
diagnosis of, 2801-2802
treatment of, 2804-2805
enteroviral, 2104, 3513
historical perspective on, 493
HSV, 1954
neonatal, 805
Morganella morganii, 1470
mumps, 2401, 2403
Naegleria fowleri, 2979, 2979f, 2981, 2984-2985, 3327t-3346t
rubella virus, 2213-2216, 2214t-2216t
Menstruation, *Staphylococcus aureus* infection and. *See* Toxic shock syndrome (TSS)
Mepitel, in burn injury, 1059
Merkel cell carcinoma, 1867-1868
Meropenem, 3201-3202, 3231t-3233t
in meningitis, 448, 450t
Mesenteric adenitis
Yersinia enterocolitica, 1520-1521, 1527
Yersinia pseudotuberculosis, 1521
Mesenteric lymphadenopathy, in *Yersinia enterocolitica* infection, 1527, 1527f
Meta-analysis, 124-125
Metabolic acidosis, in malaria, 2928
Metabolic disease
hepatitis with, 657
pancreatitis with, 691t, 692
vs. Reye syndrome, 677
Metabolism, **65-83**, 66f
acid-base, 75-76
amino acid, 70-72
carbohydrate, 72-73
copper, 79
electrolyte, 75-76
iron, 78
lipid, 73-75, 73t
nitrogen, 67-70, 68f-69f
selenium, 79
trace element, 77-79, 77t
vitamin, 76-77
zinc, 78-79
Metagonimus spp. infection (heterophyiasis), **3057-3058**
Metamorphopsia (Alice in Wonderland syndrome), 505, 2004
Metapneumoviruses. *See* Human metapneumovirus (hMPV)
Methicillin, 3183t-3184t, 3185-3186, 3231t-3233t
Methicillin-resistant *Staphylococcus aureus*. *See Staphylococcus aureus*, methicillin-resistant (MRSA)
3-Methylhistidine, 71
urinary, 69-70
Metorchis spp. infection (metorchiasis), **3058**, 3327t-3346t
Metronidazole, 3229-3230, 3231t-3233t, 3349
adverse effects of, 3230
in bacterial meningitis, 450t
in bacterial vaginosis, 578
in *Clostridium difficile* infection, 637
in odontogenic infection, 145, 146t
Mezlocillin, 3183t-3184t, 3187, 3231t-3233t
MHA-TP (microhemagglutination assay–*Treponema pallidum*), 1773-1774, 1773t
Micafungin, 3308t, 3313f, 3318-3319, 3319t
in *Aspergillus* spp. infection, 2718
in hematopoietic stem cell transplantation–related aspergillosis, 938
in lung transplant–related aspergillosis, 968
Miconazole, 3349
in *Naegleria fowleri* infection, 2984
Microbacterium spp. infection, in otitis externa, 206
Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), 8-9
Staphylococcus aureus, 1118
Microbiome, **88-94**
adverse effects of, 94
beneficial effects of, 93-94
exogenous influences on, 89-90
site-specific, 90-93, 91t
Microhemagglutination assay–*Treponema pallidum* (MHA-TP), 1773-1774, 1773t
Microscopic agglutination test (MAT), in *Leptospira* spp. infection, 1755
Microsporidia, 2864, 2911-2912, 2911t
Microsporidia infection, **2911-2913**
biliary tract, 2912
clinical manifestations of, 2912-2913
diagnosis of, 2913
disseminated, 2913
epidemiology of, 2912
Microsporidia infection *(Continued)*
gastrointestinal, 607t, 615, 2912
stains for, 617
treatment of, 625, 2913
ocular, 809, 2912-2913
treatment of, 2913, 3327t-3346t
Microsporum canis infection, 784
Midazolam, in tetanus, 1816
Military recruits
adenovirus infection in, 1892, 1896-1898, 1896t
viral pneumonia in, 1897-1898, 2026
Milk
infection and, 109, 3547
raw, 602
in tick-borne encephalitis virus infection, 2306-2307
Yersinia enterocolitica contamination of, 1518
Milker's nodules, 742t-748t
Miller-Fisher syndrome, after *Campylobacter jejuni* infection, 1654
Miltefosine, 3349
in *Leishmania* spp. infection, 2952, 2958
Mimivirus infection, **2048-2050**, 2049f-2050f
Minocycline, 3217-3220, 3218t
in *Nocardia* spp. infection, 1422
Mirizzi syndrome, 670
Mist therapy
in acute bronchitis, 264
in croup, 255
Mites, 758t, 795-796, 796f, **3073**
Mitoxantrone, in monkeypox, 2044
Mitral regurgitation, in Kawasaki disease, 1081
Modoc virus infection, 2316t
Mollaret meningitis, 489-490, 1946
Molluscum contagiosum, **782-783**
clinical manifestations of, 742t-748t, 782-783, 783f
epidemiology of, 782
eyelid, 794-795, 795f
genital, **581-582**
Molluscum contagiosum virus, 2047
Molluscum dermatitis, 783
Monkeypox, **2040-2044**
bioterrorist, 2044
clinical features of, 742t-748t, 749, 2042
congenital, 2042
diagnosis of, 2042-2043, 2043t
epidemiology of, 2040-2042
exanthem of, 742t-748t, 749, 2042
prevention of, 2043-2044
vs. smallpox, 2036
treatment of, 2043-2044
Monobactams, 3200, 3231t-3233t
resistance to, 3139-3145
Monoclonal antibodies, **3355-3357**, 3493t, **3502-3503**
adenovirus infection and, 1904
in diarrheogenic *Escherichia coli* infection, 1463
in heart transplant–related RSV infection, 950
in hematopoietic stem cell transplantation–related EBV infection, 940-941
in hematopoietic stem cell transplantation–related RSV infection, 943
in Kawasaki disease, 1080
in lung transplant–related RSV infection, 980

Monoclonal antibodies *(Continued)*
in post-transplant lymphoproliferative disease, 977-978, 2012-2013
in RSV infection, 2426-2427, 2429, 3503, 3529-3530
in septic shock, 835
in toxic shock syndrome, 857
in West Nile virus infection, 2272
Monokines, 3357-3362
Mononucleosis
CMV-induced, 1976, 1981
EPV-induced. *See* Infectious mononucleosis
Monosodium glutamate, 604, 604t
Montelukast, in RSV infection, 2426
Moon molars, 1771
Moraxella catarrhalis, 1251
lipo-oligosaccharide of, 1251
Moraxella catarrhalis infection, **1250-1253**
clinical manifestations of, 753t-756t, 1252-1253
epidemiology of, 1251-1252
immune response to, 1251
in mastoiditis, 234
nosocomial, 1252
in otitis media, 212-215, 212t
pathogenesis of, 1251
treatment of, 1253
vulvovaginal, 567
Moraxella spp. infection, conjunctival, 802
Morganella morganii infection, **1469-1471**
Morphine sulfate, in tetanus, 1817
Mosquito bite, 758t
in malaria, 2922f, 2923, 2925-2926
Motavizumab, in RSV infection, 2429
Moth-associated dermatitis, 758t
Motor neuron disease, enteroviral infection and, 2090-2091
Mouth. *See* Oral cavity
Moxifloxacin
in odontogenic infection, 145
in tuberculosis, 1369
MRSA. *See Staphylococcus aureus*, methicillin-resistant (MRSA)
MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), 8-9
of *Staphylococcus epidermidis*, 1132-1133
Mucocutaneous lymph node syndrome. *See* Kawasaki disease
Mucor spp., 2837, 2838f
Mucor spp. infection (mucormycosis), **2839-2844**
burn wound, 1053
clinical manifestations of, 2840-2842, 2840t-2841t
cutaneous, 791f, 2841, 2841f
diagnosis of, 2842-2843
disseminated, 2842
epidemiology of, 2837-2839
gastrointestinal, 2842
after hematopoietic stem cell transplantation, 938
after kidney transplantation, 1013
meningeal, 479
neonatal, 899
pathogenesis of, 2839-2840
pathology of, 2840
prevention of, 2844
prognosis for, 2844
pulmonary, 2841-2842
rhinocerebral, 2840
risk factors for, 2839, 2839t
treatment of, 2843-2844
Mucous membranes, bacterial colonization of, 431-432
Mucous patches, in congenital syphilis, 1765, 1770
Mulberry molars, 1771
Multiceps multiceps infection. *See Taenia multiceps* infection (coenurosis)
Multiple sclerosis, 515, 518, 520
HBV vaccine and, 1926, 3429
Mumps, 190-191, **2395-2407**
clinical manifestations of, 742t-748t, 2400-2403
containment of, 2406
in daycare facilities, 3564
deafness with, 2402
diabetes mellitus and, 2402
diagnosis of, 2403
differential diagnosis of, 2403
encephalitic, 494t-498t, 500, 2401
epidemiology of, 2396-2399, 2397f-2399f, 2397t
gonadal, 2401-2402
historical perspective on, 2395-2396
immune response to, 2400
immunization against, 2404-2405, 3455-3457
acute disseminated encephalomyelitis with, 517
adverse effects of, 2405, 3456
contraindications to, 2405-2406, 3457
in immunocompromised host, 2406
indications for, 3456
meningitis and, 488
precautions for, 3457
incidence of, 2396-2398, 2397f-2398f, 2397t
vs. mastoiditis, 237
meningeal, 488, 2401
vs. tick-borne encephalitis virus infection, 2310-2311
meningoencephalitic, 2401
morbidity with, 2398-2399
mortality with, 2398-2399
nephritis with, 2402
nosocomial, 3383-3384
ocular manifestations of, 811
pancreatic, 693, 2402
pathogenesis of, 2399-2400
pathology of, 2400
in pregnancy, 2402-2403, 2405
prevention of, 2404-2406, 3526
prognosis for, 2404
transmission of, 2399
treatment of, 2403
typical, 2400-2401, 2401f
Mumps virus, 2396
antigenic composition of, 2396
physical properties of, 2396
tissue culture of, 2396
Mupirocin
in burn wound infection, 1059
in impetigo, 774-775
in *Staphylococcus aureus* infection prevention, 1128-1129
Murphy sign, 669
Murray Valley encephalitis, 494t-498t, **2301-2303**
Muscles
abscess of, **735-739**, 736t. *See also* Myositis; Pyomyositis
catabolism of, 71-72
Mushroom poisoning, 604, 604t
Myalgia, in *Leptospira* spp. infection, 1749-1750
Myasthenia gravis, vs. botulism, 1793
Mycetoma
actinomycotic, 1393
Candida, 2749
Mycobacterial infection, **1381-1391.** *See also specific mycobacterial infections*
cervical lymphadenitis in, 177-178, 177t, 180, 183t, 187-188
cutaneous, 1386-1387
in cystic fibrosis, 336-337, 341
cytokines and, 1382
epidemiology of, 1381-1382
after hematopoietic stem cell transplantation, 935-936
HIV infection and, 1384-1385
immunodeficiency and, 916
interferon-γ receptor defects and, 50-51
interleukin-12 defects and, 50
after kidney transplantation, 1012
after lung transplantation, 964-965
lymphadenitis in, 1383-1384
microbiology of, 1382-1383, 1382t-1383t, 1389-1390
pulmonary, 1385-1386. *See also* Tuberculosis
treatment of, 1387-1390, 1388t
Mycobacterium abscessus infection, 1382, 1383t, 1389-1390
in cystic fibrosis, 1385-1386
after lung transplantation, 964-965
Mycobacterium avium-intracellulare complex infection, 1382t, 1387-1389
disseminated, 1388-1389
HIV infection and, 1384-1385, 2600-2601
after lung transplantation, 964-965
lymphadenitis in, 178-179, 1382
parotitis in, 191
peritoneal, 1389
pulmonary, 1385-1386, 1388
treatment of
in HIV-infected host, 1385
after lung transplantation, 965
Mycobacterium bolletii infection, 1387
Mycobacterium bovis infection, 700, 1382t
Mycobacterium chelonae infection, 1383t, 1390
in cervical lymphadenitis, 178
cutaneous, 1387
after lung transplantation, 964-965
Mycobacterium fortuitum infection, 1383t, 1390
cutaneous, 1387
hair follicle, 777
after lung transplantation, 964-965
Mycobacterium genavense infection, 1384-1385
Mycobacterium gordonae infection, 1382t
Mycobacterium haemophilum infection, 1387
Mycobacterium interjectum infection, 178
Mycobacterium kansasii infection, 1382, 1382t, 1389
HIV infection and, 1384
after lung transplantation, 964-965
pulmonary, 1385-1386
Mycobacterium lentiflavum infection, 178
Mycobacterium leprae, 1393, 1399-1400, 1399f-1401f. *See also* Leprosy
vaccines against, 1409-1410
Mycobacterium malmoense infection, 1389
Mycobacterium marinum infection, 1382, 1382t
cutaneous, 1386
after lung transplantation, 964-965
Mycobacterium massiliense infection, 1387, 1390
Mycobacterium mucogenicum infection, 1390
Mycobacterium neoaurum infection, 1390

Mycobacterium scrofulaceum infection, 178, 187, 1382, 1382t, 1389
HIV infection and, 1384
Mycobacterium septicum infection, 1390
Mycobacterium simiae infection, pulmonary, 1386
Mycobacterium smegmatis infection, 1390
Mycobacterium szulgai infection, 1382t, 1386, 1390
Mycobacterium tuberculosis, 1341. *See also* Tuberculosis
Mycobacterium ulcerans, 1381
Mycobacterium ulcerans infection, 1382t, 1386-1387
animal models of, 1413
bite-related, 780
clinical manifestations of, 1411f, 1413-1415, 1413f
complications of, 1415, 1415f
cutaneous, 1386-1387
diagnosis of, 1415
differential diagnosis of, 1415
edematous, 1413-1414, 1414f
epidemiology of, 1411-1412
HIV infection and, 1415
after lung transplantation, 964-965
nodular, 1412-1413
osseous, 1414-1416, 1414f-1415f
papular, 1413
pathogenesis of, 1412-1413
pathology of, 1412-1413, 1412f
plaque, 1413
prevention of, 1416-1417
prognosis for, 1416
scarring with, 1415, 1415f
surgical treatment of, 1416
transmission of, 1411-1412, 1411f
treatment of, 1416
ulcerative, 1411f, 1414
Mycobacterium wolinskyi infection, 1390
Mycobacterium xenopi infection, 1382t, 1386
Mycolactone, 1410, 1412
Mycoplasma fermentans, 2694
Mycoplasma genitalium, 2693-2694
Mycoplasma genitalium infection, **2693-2694**
clinical manifestations of, 532-533, 2694
differential diagnosis of, 2694
epidemiology of, 2694
treatment of, 2697
Mycoplasma hominis, 2692
Mycoplasma hominis infection, **2692-2693**
clinical manifestations of, 2693
differential diagnosis of, 2693
epidemiology of, 2692
after kidney transplantation, 1011
neonatal, 901, 2693
pharyngeal, 159t-160t, 161
treatment of, 2696-2697
Mycoplasma penetrans infection, **2694**
Mycoplasma pirum, 2695
Mycoplasma pneumoniae, 2669-2671
animal susceptibility to, 2671
antigenic composition of, 2670, 2674
Mycoplasma pneumoniae-associated mucositis, 2680, 2680t
Mycoplasma pneumoniae infection, **2669-2687**
vs. *Arcanobacterium haemolyticum* infection, 1326
asthma and, 2679
in bronchitis, 2679
in bullous hemorrhagic myringitis, 2678-2679
cardiac manifestations of, 2681-2682
clinical manifestations of, 2675-2685, 2676t
Mycoplasma pneumoniae infection *(Continued)*
CNS, 494t-498t, 501-502, 2683-2684
cold agglutinin antibodies in, 2674, 2685
cough with, 1617-1618
croup in, 2679
diagnosis of, 2685-2686
differential diagnosis of, 2685
enanthem in, 2679-2681
epidemiology of, 2671-2672
exanthem in, 750, 751t-752t, 764, 2679-2681, 2680t-2681t
fetal, 2684
gastrointestinal manifestations of, 2682-2683
hematologic manifestations of, 2682
hepatic manifestations of, 2682
immune response to, 2673-2674
immunization against, 2687
incubation period in, 2672
joint manifestations of, 2683
after kidney transplantation, 1011
meningeal, 488-489
mixed infections with, 2684
muscular manifestations of, 2683
nasopharyngeal, 2678
neonatal, 2685
in otitis media, 2678-2679
pancreatic, 695, 2682-2683
pathogenesis of, 2672-2675
pathology of, 2673
pertussis-like illness with, 1617
pharyngeal, 159t-160t, 161, 2678
prevention of, 2687
pulmonary, 285t, 287-289, 292, 2675-2678, 2676t. *See also* Pneumonia, community-acquired
vs. *Legionella pneumophila* infection, 1713
recurrent, 2684
in rhinosinusitis, 195
in sinusitis, 2679
splenic manifestations of, 2682
tonsillectomy and, 2684
treatment of, 2686-2687
upper respiratory tract, 2678
Mycoplasma salivarium, 2695
Mycoplasma spp., 1097t-1112t, 2668-2669, 2669t
as culture contaminant, 2696
Mycoplasma spp. infection, **2668-2700.** *See also specific species infections*
cancer and, 2696
historical perspective on, 2668
in HIV-infected host, 2696
in immunocompromised host, 2696
Neisseria meningitidis infection and, 1256
prevention of, 2697-2698
treatment of, 2696-2697
zoonotic, 2695-2696
MyD88 protein, in murine myocarditis, 386
Myelitis
CMV-associated, 526
transverse, acute. *See* Acute transverse myelitis
Myelopathy
HTLV-1– associated, 2569-2573, 2570t
HTLV-2– associated, 2580
Myeloperoxidase (MPO) deficiency, 59
Myiasis, 816, **3072-3074**
Myocardial infarction (MI)
coxsackievirus B infection and, 2077
vs. human erythrovirus B19 (human parvovirus B19) infection, 1854
in Kawasaki disease, 1081, 1083-1084
smallpox immunization and, 2039
Myocarditis, **381-400**
adenovirus, 1896t, 1902
autoantibodies in, 387
bacterial, 383
borderline, 393
Candida, 383f, 2740-2741, 2748
CMV, 1980
in congenital rubella, 2214t-2216t, 2217
after *Corynebacterium diphtheriae* infection, 1306
diagnosis of, 389-394
biomarkers in, 394
biopsy in, 393
electrocardiography in, 389-392, 390f-392f
PCR in, 382, 393-394
radiography in, 389, 389f
troponin T in, 394
virologic study in, 394
differential diagnosis of, 394-395
diphtheritic, 397
enterovirus, 384-387, 385f, 2075-2077, 2076t, 2104
epidemiology of, 381
etiology of, 381-382, 382t
giant cell, 383-384
in HIV infection, 398
human erythrovirus B19 (human parvovirus B19), 1853-1854, 1856
idiopathic dilated cardiomyopathy in, 382, 387, 393
immune factors in, 386-387
influenza virus, 2343
Leptospira spp., 1748
measles virus, 2385
Neisseria meningitidis, 1262
parasitic, 398-400
pathogenesis of, 384-387, 385f
pathology of, 383-384, 383f-385f
phases of, 384-386
prognosis for, 397
rubella virus, 2212
Shigella spp., 1481
smallpox vaccination and, 382
treatment of, 395-397
immune modulating agents in, 396-397
ventricular dysfunction in, 387-389, 388f
viral, **381-400**, 384f-385f
in Whipple disease, 644
Myocardium, in toxic shock syndrome, 853
Myonecrosis
Clostridium perfringens, 1796, 1798-1800
spontaneous, 1796
streptococcal, 1798
Myopathy, in Whipple disease, 644
Myopericarditis, smallpox immunization and, 2039
Myositis
Aeromonas spp., 1543
bacterial, **738-739.** *See also* Pyomyositis
clinical presentation of, 738-739
diagnosis of, 739
treatment of, 739
enterovirus, 2078
fungal, 736t, 739
in influenza virus infection, 2344
parasitic, 736t, 739
viral, 736t, 739
Myringitis, hemorrhagic, bullous, 2678-2679
Myringotomy, 218-220
in mastoiditis, 238
in otitis media, 232
in otitis media with effusion, 229

N

Naegleria fowleri, 2977, 2977f
Naegleria fowleri infection, **2975-2986**
  clinical manifestations of, 2979, 2979f
  CNS, 493-499, 494t-498t, 501-502
  diagnosis of, 2981-2983, 2982f
  epidemiology of, 2975-2977
  myocardial, 400
  neuroimaging in, 2980
  pathogenesis of, 2981
  pathology of, 2981
  prevention of, 2985-2986
  treatment of, 2983-2985
Nafcillin, 3183t-3184t, 3185-3186, 3231t-3233t
  in bacterial meningitis, 450t
  in septic shock, 833-834, 833t
Nagayama spots, 2017
Nanophyetus salmincola infection, 3327t-3346t
Naproxen, in rheumatic fever, 413
Narcolepsy, after influenza virus immunization, 2352-2353
Nasal polyps, rhinosinusitis and, 195
Nasal spray, in cold prevention, 139
Nasal tissues, virucidal, in cold prevention, 139, 2117, 2117t
Nasoantral window, in rhinosinusitis, 201
Nasolacrimal duct, obstruction of, 797
Nasopharyngeal carcinoma
  EBV infection and, 1995-1997, 2005
  treatment of, 2013
Nasopharyngitis, **156-158**
  adenovirus, 156, 157t, 158, 1895-1896, 1896t
  clinical presentation of, 158
  vs. common cold, 132
  diagnosis of, 163
  differential diagnosis of, 157t, 162-163
  epidemiology of, 156-158
  etiology of, 156, 157t
  Mycoplasma pneumoniae, 2678
  pathophysiology of, 158
  prevention of, 164
  prognosis for, 164
  treatment of, 163-164
Nasopharynx, indigenous flora of, 91t, 92
Nasotracheal intubation
  in epiglottitis, 257-258
  otitis media and, 211
Native Americans, diphtheria in, 1303
Natural killer cells, 28
  granzyme release by, 28
  in HIV infection, 2597-2598
  perforin release by, 28
  regulation of, 28
Nausea. *See also* Vomiting
  in appendicitis, 680-681
NBT dye test, in chronic granulomatous disease, 57, 58f
Nebovirus, 2148-2149, 2149t
Nebovirus infection, **2155**
Necator americanus infection, 757t-758t, **3016-3018**, 3327t-3346t
Neck abscess, **167-175.** *See also* Head and neck infection
  epidemiology of, 167-168
  microbiology of, 173-175
  parapharyngeal, **172-173**
  peritonsillar, **168-170**, 168f, 170f
  retropharyngeal, **170-172**, 170f
Necrotizing enteritis, *Clostridium perfringens*, 1797-1800
Necrotizing enterocolitis (NEC)
  Candida, 895-897
  Clostridium difficile, 635
Necrotizing enterocolitis (NEC) *(Continued)*
  Cronobacter sakazakii, 1436
  Klebsiella spp., 474
  oral immunoglobulin in, 3498
  platelet-activating factor and, 3366-3367
  prevention of, 3243-3244
  probiotics in, 3371-3372
Necrotizing epiglottitis, 245
Necrotizing fasciitis, **778-780**
  Bacteroides spp., 1829
  cervicofacial, 150, 151f
  Chryseobacterium spp., 1580
  clinical manifestations of, 778-779, 779f
  Clostridium perfringens, 1796-1797
  diagnosis of, 779
  Pseudomonas aeruginosa, 1599
  treatment of, 779-780
Necrotizing funisitis, 1766
Necrotizing lymphadenitis, 181
Necrotizing otitis externa, 207-208
Necrotizing ulcerative gingivitis (trench mouth), 147, 147f
Nedocromil sodium, in common cold, 138
Negishi virus infection, 2316t
Negri bodies, 504, 2453
Neisseria gonorrhoeae, 1273-1275, 1274t
  biofilm of, 1277
  DNA transformation of, 1274
  epithelial cell invasion by, 1276-1277, 1276f
  genetic characteristics of, 1274-1275
  IgA protease of, 1278
  iron metabolism by, 1278
  lipo-oligosaccharide of, 1278
  opacity-associated proteins of, 1277-1278
  peptidoglycan of, 1278-1279
  phagocytosis resistance of, 1276
  pili of, 5, 19, 1277
  porin protein of, 1278
  reduction modifiable protein of, 1279
  ribosomal protein L12 of, 1279
  strain typing of, 1275
  transglycosylases of, 1278-1279
  type IV secretion system of, 1279
  virulence factors of, 1277-1279
Neisseria gonorrhoeae infection, **1271-1301**
  abdominal wall, 1288
  anal, 586
  anorectal, 1287
  Bartholin gland/duct, 579, 1284, 1285f
  cervical, 586
  in child sexual abuse, 1297-1299, 1298t
  complement evasion in, 18
  conjunctival, 802
    neonatal, 805-806, 805t, **1280-1282**, 3242-3243
  cutaneous manifestations of, 1287, 1288f
  diagnosis of, 1274, 1288-1291, 1289t, 1290f, 3594
    medicolegal aspects of, 1291
    specimen collection for, 1291
  disseminated, 1279, 1287, 1288f
    neonatal, 1283, 1283t, 1295
  endocardial, 362
  endocervical, 1284-1285, 1284f
  epidemiology of, 1271-1273, 1272f-1273f, 1286t
  exanthem in, 753t-756t
  fallopian tube, **587-588**
  immune response to, 1279-1280
  joint, 729t, 730, 734
  meningeal, 1288
  ocular, 1288
  pathogenesis of, 18-19, 1275-1280, 1276f
Neisseria gonorrhoeae infection *(Continued)*
  pelvic, 1285-1286, 1286f
    treatment of, 1296
  perinatal, 1280-1283, 1280t, 1282f, 1283t. *See also* Ophthalmia neonatorum
    prevention of, 1299-1300
    treatment of, 1293-1295, 1293t
  pharyngeal, 1288
    treatment of, 1296
  prevention of, 1299-1300
  scalp wound, 1282-1283, 1295
  shunt-associated, 1288
  Skene's gland, 1284
  treatment of, 1293-1297, 1293t-1294t
    antibiotic resistance and, 1291-1293, 1293f
    cephalosporin allergy and, 1296
    in children and adolescents, 1294t, 1295-1297
    Chlamydia trachomatis coinfection and, 1297
    follow-up for, 1297
    in HIV-infected children, 1296
    in neonate, 1293t, 1294-1295
    during pregnancy, 1297
    syphilis infection and, 1297
  urethral, **530-535**, 532f, 534t, 1286-1287, 1287f, 1293f
  urinalysis in, 1285, 1291
  vaccine against, 1300
  vulvovaginal, 571-574, 572t-573t, 1283-1284, 1284f
Neisseria meningitidis, 1254-1255, 1274t
  DNA transformation of, 1254
  lipo-oligosaccharide of, 1254, 1257
  nasopharyngeal carriage of, 428, 432, 1256-1257
  phase variation in, 1254
  pili of, 1254
  porins of, 1254-1255
Neisseria meningitidis infection, **1253-1271**
  blood-brain barrier in, 432-433, 433t
  bloodstream, 432, 432t, 824, 829, 831, 1258-1260
    chronic, 1260
    cutaneous manifestations of, 1259, 1259f
    differential diagnosis of, 1258t
    treatment of, 833-834, 833t
  cerebral infarction with, 1260
  clinical manifestations of, 1258-1262
  complement deficiency and, 52, 429, 1256-1257
  conjunctival, 802, 1261
  cutaneous manifestations of, 750, 753t-756t, 762-763, 1258-1259, 1259f
  in daycare facilities, 3555-3556
  diagnosis of, 1262-1263
  empyema with, 1260
  epidemiology of, 1255-1257
  hearing loss after, 1259
  host genetic factors in, 1256-1257, 1263
  hydrocephalus with, 1260
  immunization against, 459-460, 460t, 1267-1270, 3452-3455
    adverse events with, 3454
    conjugate vaccine for, 1267-1270, 3454
    contraindications to, 3455
    polysaccharide vaccine for, 1267, 3453-3454
    precautions for, 3455
    recommendations for, 1268-1269, 1268t, 3454-3455

Neisseria meningitidis infection *(Continued)*
joint, 729t, 1261-1262
mannose binding lectin deficit and, 1257
meningeal, 428-429, 428f, 450t-451t, 459-460, 460t, 1258-1260. *See also* Meningitis, bacterial
mortality with, 1258, 1263
Mycoplasma infection and, 1256
myocardial, 1262
nasopharyngeal, 158, 162-163
neonatal, 1260
neurologic complications of, 1259
pathogenesis of, 1257-1258
pathology of, 1257-1258
pericardial, 373, 378-379, 1262
pharyngeal, 158, 1261
postexposure prophylaxis for, 3455
prevention of, 1266-1267, 1266t, 3249
prognosis for, 1263
properdin deficiency and, 1257
pulmonary, 1260-1261
vs. Rocky Mountain spotted fever, 2653
subdural effusion with, 1260
thrombosis with, 1260
treatment of, 446-455, 450t-451t, 1263-1266, 3365-3366
adjunctive/experimental, 446-455, 1264-1266, 1265t
viral infection and, 1256, 1260
vulvovaginal, 567
Neisseria sicca infection, 567, 1274t
Nelfinavir, 2615
Nematode infection, **3012-3029.** *See also specific nematode infections*
classification and nomenclature in, 2864-2865
hepatic, 673
NEMO defect, 916, 916f
Neomycin, 3208-3211, 3208t
Neonatal cephalic pustulosis, 789, 789f
Neonatal toxic shock syndrome–like exanthematous disease, 860-861
Neonate. *See* Newborn
Nephritis
adenovirus, 1901
CSF shunt–related, 1032
enterovirus, 2077-2078
interstitial
BK virus, 1867
Leptospira spp., 1747
mumps, 2402
postinfectious, in impetigo, 773
Nephronia, 706, 708
Nephropathia epidemica, 2496, 2504-2505
Nephropathy
HIV-associated, 2607
lobar, 539, 554, 555f, 556
Nephrotic syndrome
in malaria, 2928
in *Streptococcus pneumoniae* infection and, 1232
in syphilis, 1767
Netilmicin, 3208-3211, 3208t, 3231t-3233t
Neuraminidase, of *Streptococcus pneumoniae*, 1212
Neuraminidase inhibitors, 3270-3273, 3270f
adverse effects of, 3273
dosage for, 3272-3273
indications for, 3272
mechanism of action of, 3271
pharmacokinetics of, 3271-3272
resistance to, 3271
spectrum of activity of, 3270
Neuritis, rubella virus, 2212
Neuroblastoma, 182-183, 182t
Neurocysticercosis
calcification in, 3034, 3038
diagnosis of, 3035-3037, 3036f
epidemiology of, 3033
giant lesions in, 3035, 3039
MRI in, 3035-3036, 3036f
multiple lesions in, 3034, 3038
parenchymal, 3034, 3036f
pathogenesis of, 3033-3034
prevention of, 3039
prognosis for, 3039
single lesion in, 3034, 3037-3038
spinal, 3035
subarachnoid, 3035, 3036f, 3038
treatment of, 3037-3039
ventricular, 3034-3035, 3036f, 3037-3038
Neurodevelopment, neonatal *Candida* spp. infection and, 2766, 2766f
Neurologic disorders
in bacterial meningitis, 439, 440t, 455-457, 456t-457t
in *Bartonella henselae* infection, 1723
in *Bordetella pertussis* infection, 502
colistin-related, 3208
in coronavirus infection, 2494
in *Corynebacterium diphtheriae* infection, 1306
in coxsackievirus infection, 2086-2090, 2088t-2089t
DTP vaccine and, 3459
in echovirus infection, 2086-2090, 2088t
in enterovirus infection, 2086-2090, 2088t, 2097, 2104
in erythema infectiosum, 1853
in HHV-6 infection, 2018
in HHV-7 infection, 2018
in infectious mononucleosis, 2003-2004
in infective endocarditis, 356-357
in influenza virus infection, 2343
IVIG in, 3501-3502, 3502t
in Lyme disease, 1732-1735
metronidazole-related, 3230
in Murray Valley encephalitis, 2303
in *Neisseria meningitidis* infection, 1259
after pertussis immunization, 1633-1634
rabies, 2450
in rubella virus infection, 2212-2216, 2214t-2216t, 2221
in St. Louis encephalitis virus infection, 2257
in tick-borne encephalitis virus infection, 2311
Neurologic examination
in chronic fatigue syndrome, 1088
in St. Louis encephalitis virus infection, 2257
Neuromuscular blocking agents, in tetanus, 1817
Neuromyelitis optica, 515, 519, 525, 526t
Neuropathy
CMV, 1979-1980
Corynebacterium diphtheriae infection and, 1304-1306
Neuroretinitis
Baylisascaris procyonis, 815
in cat-scratch disease, 813
Neurosurgery, antibiotic prophylaxis in, 3253-3255
Neurosyphilis, 483, 812, 1768. *See also* Syphilis
congenital, 1770, 1772
treatment of, 1778
Neutropenia
burn-related, 1049
chemotherapy-associated, **921-933**
bacterial infection and, 921-922, 922t
epidemiology of, 921-923
evaluation of, 924-925
fungal infection and, 922, 924, 926, 927t, 928
occult infection and, 923
patient history in, 924
persistent, 926-927
physical examination in, 924
prevention of, 928-929
risk stratification for, 923-924
treatment of, 921, 925-928
viral infection and, 922-923, 923t
cyclic, 917
in infectious mononucleosis, 2003
in neonate, 45
Streptococcus pneumoniae infection and, 1205
Neutrophil(s), 3365
actin of, dysfunction of, 54
defective adhesion of, 52-55
defective chemotaxis of, 55
in hyper-IgE syndrome, 55
dysfunction of, *Streptococcus pneumoniae* infection and, 1205
in *Francisella tularensis* infection, 1661
glutathione synthetase deficiency in, 59
in glycogen storage disease type 1B, 54-55
granules of, 33-35, 34t
in Chédiak-Higashi syndrome, **54**
deficiency of, 54
in Kawasaki disease, 1077
in meningitis, 435
myeloperoxidase deficiency in, 59
Nevirapine, 2613-2614
Newborn. *See also* Infant; Premature infant
adenovirus infection in, 1905
antibiotic dosage schedule for, 3230, 3233t
antibody in, 42-44, 43f, 43t
appendicitis in, 681
Ascaris lumbricoides infection in, 3014
B cells in, 42
bacterial infection in, **881-892**
bloodstream, **882-885**, 1134-1135, 1135t
bone, **889-890**
breast, **890**
conjunctival, 890
epidemiology of, 881-882
gastrointestinal, 887-888
joint, **889-890**
meningeal, 885-887
orbital, 890
otic, 887
parotid gland, 890-891
pathogenesis of, 881-882, 881t
pulmonary, 891-892
scalp, 891
umbilical cord, **890**
umbilicus, **890**
urinary tract, 888-889
Blastomyces dermatitidis infection in, 2730-2731
Bordetella pertussis infection in, 1626-1627, 1626t
Burkholderia pseudomallei infection in, 1592
Campylobacter jejuni infection in, 888, 1653
Campylobacter spp. infection in, 1653
Candida spp. infection in, **892-897**, **2749-2766.** *See also Candida* spp. infection (candidiasis)
cell-mediated immunity in, 42

Newborn *(Continued)*
Chlamydia trachomatis infection in, 900-901, 2632-2633, 2633f
Clostridium difficile colonization in, 633-635
Clostridium spp. infection in, 1797
CMV hepatitis in, **654**
complement in, 44-45, 44t
conjunctivitis in, **805-806**, 805t
coronavirus infection in, 2491
Cryptococcus neoformans infection in, 2804
diarrhea in, 887-888
Elizabethkingia meningosepticum infection in, 1579
Enterococcus spp. infection in, 1176-1177
enterovirus infection in, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
Escherichia coli bacteremia in, 1441
fungal infection in, **898-899**
acquired, 898-899
Aspergillus, 899
congenital, 898
Zygomycete, 899
group B streptococcal infection in. *See* *Streptococcus agalactiae* infection
Haemophilus influenzae infection in, 1676
HAV infection in, 2130
HBV infection in, 1915, 1917, 1925-1926
HHV-6 infection in, 2018
host response of, 42-45
HSV infection in, 1954-1955. *See also* Herpes simplex virus (HSV), neonatal
hypoglycemia in, 72
indigenous flora of, 89
cutaneous, 90
intestinal, 93
respiratory, 92
infective endocarditis in, 357
influenza virus infection in, 2339
lincomycin-related gasping syndrome in, 3222
Listeria monocytogenes infection in, 1331-1333, 1332t
malaria in, 2924, 2929
monkeypox in, 2042
Mycobacterium leprae infection in, 1394
Mycoplasma hominis infection in, 901, 2693
Mycoplasma pneumoniae infection in, 2685
myocarditis in, 387-389
Neisseria gonorrhoeae infection in, 1280-1283, 1283t. *See also* Ophthalmia neonatorum
neutropenia in, 45
nosocomial infection in, 3402-3404
osteomyelitis in, 713, 713f, 723
otitis media in, 215
phagocytes in, 45
Pseudomonas aeruginosa infection in, 1592
Ritter disease in, 774, 775f
RSV infection in, 2411-2412, 2413t, 2418-2419
rubella virus infection in. *See* Rubella virus infection, congenital
sepsis in, **882-885**, 3509
septic arthritis in, 733-734
Shigella spp. infection in, 1481-1482
Staphylococcus epidermidis infection in, 882, 3509-3510
Streptococcus agalactiae infection in, 881-882, **1153-1169.** *See also* *Streptococcus agalactiae* infection
Streptococcus viridans infection in, 1192
T cells in, 42
tetanus in, 1814-1815, 1819, 3512
Newborn *(Continued)*
toxic shock syndrome–like exanthematous disease in, 860-861
Toxoplasma gondii infection in, **900**, 900t
Trypanosoma cruzi infection in, 2965
tuberculosis in, 1358-1359
Ureaplasma spp. infection in, 901, 2689-2690
vaginal discharge in, 563, 563f
viral infection in, **877-881**, 878t
clinical manifestations of, 879-880, 879t
developmental malformation and, 877
diagnosis of, 878-880
differential diagnosis of, 880
hospital-associated, 878
laboratory diagnosis of, 880-881
maternal evaluation in, 879
maternal infection and, 877
natal/perinatal, 877-878
pathogenesis of, 877-878, 878f
postnatal, 878
treatment of, 881
yeast infection in, **892-900.** *See also* Newborn, *Candida* spp. infection in
Cryptococcus neoformans, 897
Malassezia spp., 897-898
Pichia spp., 898
Rhodotorula spp., 898
Trichosporon spp., 898
Newbury-1 virus, 2155
Niclosamide, 3349
Nicotinamide adenine dinucleotide (NADH) oxidase, of *Streptococcus pneumoniae*, 1214
Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, 33-35, 34f
deficiency of, 58-59
Nifurtimox, in *Trypanosoma cruzi* infection, 399, 2966-2967
Nipah virus infection, 494t-498t, 500-501
Nitazoxanide, 3349
Nitrates, urinary, 70
Nitric oxide (NO), 3367
in ARDS, 872
biologic effects of, 70-71
in septic shock, 826
Nitrites, urinary, 543
Nitroblue tetrazolium (NBT) dye test, 907, 918
Nitrofurantoin, in burn wound infection, 1059
Nitrogen, 67-70
balance studies of, 67-69, 68f-69f
fecal, 69
urinary, 69-70
Nocardia farcinica infection, 1420
Nocardia spp., 1419-1420, 1421f
Nocardia spp. infection, **1418-1423**
bloodstream, 1421
clinical manifestations of, 1420-1422
CNS, 1421
cutaneous, 1419-1420
diagnosis of, 1421-1422
after heart transplantation, 953-954
immune response to, 1420
after kidney transplantation, 1012
pathogenesis of, 1419-1420
pathology of, 1420, 1421f
prognosis for, 1422-1423
pulmonary, 1419-1421
in rhinosinusitis, 197
risk of, 1420
traumatic, 1420-1421
treatment of, 1422-1423
Nodule(s), 764, 764t
Milker's, 742t-748t
Mycobacterium ulcerans, 1412-1413
in rheumatic fever, 410
Nomenclature
bacterial, 1097t-1112t
fungal, 2702-2704
parasite, 2861-2866, 2862t-2863t
viral, 1836-1843, 1837f, 1838t-1840t, 1841f-1842f
Non-Hodgkin lymphoma, 182-183, 182t
Non-nucleoside reverse transcriptase inhibitors, 2613-2614
Nonshivering thermogenesis, 84
Nonsteroidal anti-inflammatory drugs (NSAIDs)
pancreatitis with, 691
in rheumatic fever, 413
Norepinephrine, plasma, 81-82
Norovirus, 2149, 2149f, 2149t
Norovirus infection, 607-608, 607t, 616t, **2151-2154**
clinical manifestations of, 2153
in daycare facilities, 3558
diagnosis of, 2153-2154
differential diagnosis of, 2153
epidemiology of, 2151
immune response to, 2152-2153
nosocomial, 3382
pathogenesis of, 2152
pathology of, 2152
prognosis for, 2156
seasonal patterns of, 2151, 2152f
treatment of, 2155-2156
vaccine against, 2156
Norwalk agent/virus. *See Norovirus*
Nose
indigenous flora of, 91t, 92
infection of. *See* Rhinosinusitis
polyps of, 195
saline irrigation of
in common cold, 137
in rhinosinusitis, 201
surgery on, toxic shock syndrome after, 860
Nuchal rigidity, 439
Nuclear factor κB
in *Cryptosporidium* spp. infection, 2900
in gonococcal infection, 1277, 1279
Nuclear factor κB–enhancing modulator (NEMO) deficiency, 916, 916f
Nucleic acid amplification test (NAAT). *See also* Polymerase chain reaction (PCR)
in *Chlamydia trachomatis* infection, 2636-2637
in *Clostridium difficile* infection, 636, 636t
highly multiplexed, 3614-3616, 3615t
multiplexed, 3613t, 3614
in *Neisseria gonorrhoeae* infection, 1290-1291
quantitative, 3616-3617, 3616t
single-analyte, 3612-3614, 3613t
in viral infection, 3606t-3607t, 3608, 3612, 3613t, 3615t-3616t
Nucleic acid hybridization test (NAHT), in *Neisseria gonorrhoeae* infection, 1290-1291
Nucleoside reverse transcriptase inhibitors, 2612-2613, 2612f
pancreatitis with, 692
Nucleotide reverse transcriptase inhibitors, 2613
Nursing bottle caries, 146

Nutrition
in ARDS, 873
in gastrointestinal tract infection, 620t, 622
in *Shigella* spp. infection, 1485
Nystatin
in burn wound infection, 1059
in localized *Candida* spp. infection, 788
in neonatal *Candida* spp. infection, 2762-2763, 2764t
in vulvovaginal fungal infection, 570

O

Obesity, adenovirus infection and, 1905
Obsessive-compulsive disorder, in PANDAS, 1144-1145
Obturator sign, 730, 731f
Occludin-1, 16
Occupation, *Paracoccidioides* spp. infection and, 2782
Occupational health, 3412-3413
Ocular larva migrans, 3022-3023
Oculoglandular syndrome of Parinaud, 1722, 1724f
Oculorespiratory syndrome, after influenza virus immunization, 2352-2353
Odontogenic infection
cervical lymphadenitis and, 183, 183f
cervicofacial actinomycosis and, 184
complications of, 149-151, 149f-151f
Garré sclerosing osteomyelitis with, 154, 154f-155f
nursing bottle–related, 146
osteomyelitis with. *See* Osteomyelitis, mandibular/maxillary
pathogens in, 140-141, 141t
periapical, 141, 142f-143f, **146**
pericoronal, 141, 147-148, 148f
periodontal, 141, 146-147, 147f
vs. rhinosinusitis, 199
spread of, 141-142, 142f-144f
surgical treatment of, 144
treatment of, 142-149, 146t
Oesophagostomum spp. infection, 3024
Oestrus ovis infection, ocular (ophthalmomyiasis), 816
Omenn syndrome, 904-905
Omphalitis, **890**, 904
Omsk hemorrhagic fever, **2316**
Onchocerca volvulus infection, 757t-758t, 809, 815, **3027-3028**, 3327t-3346t
Onychomycosis, dermatophyte, **786-787**, 786f
O'nyong-nyong virus infection, 742t-748t, 2251
Oophoritis, **590**
mumps, 2401-2402
Opacity-associated proteins, of *Neisseria gonorrhoeae*, 1277-1278
Ophthalmia neonatorum, **805-806**, 805t
chlamydial, 805-806, 805t, 2633-2634
treatment of, 2634
gonococcal, **1280-1282**
clinical features of, 1282, 1282f
epidemiology of, 1280-1281
prevention of, 1281, 3242-3243
treatment of, 1293-1295, 1293t
Ophthalmomyiasis, 816
Ophthalmoplegia, in Whipple disease, 644
Opisthorchis spp., 3049, 3050f
Opisthorchis spp. infection, **3049-3052**
clinical manifestations of, 660, 3051
complications of, 3051
diagnosis of, 3051-3052
Opisthorchis spp. infection *(Continued)*
epidemiology of, 3050-3051
imaging in, 3052
pathogenesis of, 3051
prevention, 3052
transmission of, 3050-3051
treatment of, 3052, 3327t-3346t
Opsonins, 30-31
in phagocytosis, **32-33**, 33f
Optic nerve, in bacterial meningitis, 440
Optic neuritis
in acute disseminated encephalomyelitis, 514-515
CMV, 811
Oral cavity
cancer of, HPV infection and, 1873t, 1880
Candida spp. infection of, **787-788**, 787f
in HIV infection, 148-149
in neonate, 892-893, 896
treatment of, 788
flora of, 91t, 92, 94, 140, 141t, 1189-1190
HSV infection of, 154-155, 742t-748t, 1940-1941, 1940f
in HIV infection, 149
recurrent, 1950, 1950f
treatment of, 1959
infection of, **140-155**
anatomic considerations in, 141-142, 142f-144f
complications of, 149-151
enteroviral, 2066
etiology of, 140-141, 141t
examination for, 144
fascial space spread of, 149-150, 149f-150f
in HIV infection, 148-149
intracranial complications of, 145, 151
mediastinitis and, 420-421
necrotizing fasciitis with, 150, 151f
nursing bottle–related, 146
orbital complications of, 151
osteomyelitis with. *See* Osteomyelitis, mandibular/maxillary
periapical, 141, 142f-143f, **146**
pericoronal, 141, 147-148, 148f
periodontal, 141, 146-147, 147f
sinusitis with, 150-151, 152f
treatment of, 142-149, 146t
piercings of, 155
temperature measurement by, 86
trismus of, 144, 148-149, 168
tuberculosis of, 1357
Oral contraception
pelvic inflammatory disease and, 587
toxic shock syndrome and, 853
Oral hairy leukoplakia, 1997, 2007
Oral rehydration therapy
in cholera, 1552t, 1553
in gastrointestinal tract infection, 620t-621t, 621-622
in rotavirus infection, 2186
in *Shigella* spp. infection, 1483
in *Vibrio parahaemolyticus* infection, 1559
Orbit
hydatid cyst of, 801
infection of
implant-related, 1018-1019
odontogenic origin of, 151
Orbital cellulitis, **799-801**, 800f
vs. dacryoadenitis, 797
differential diagnosis of, 800
fungal, 801
Haemophilus influenzae, 1676
in infantile osteomyelitis, 153-154, 153f
neonatal, 890
Orbital cellulitis *(Continued)*
odontogenic origin of, 151
Streptococcus pneumoniae, 1231-1232
Orbiviruses, 2172, 2175-2176
Orchitis
adenovirus, 1901
enterovirus, 2077
mumps, 2401-2403
Orf virus infection, 742t-748t, **2046**
vs. ecthyma, 776
Organic acids, in cold prevention, 139
Oriental sore. *See Leishmania* spp. infection (leishmaniasis), Old World
Orientia tsutsugamushi infection (scrub typhus), 751t-752t, 2648t-2649t, **2660-2661**
Ornidazole, 3349
Ornithodoros tick, 1740
Oropharyngeal cancer, HPV and, 1875-1876, 1880
Oropharyngeal candidiasis, **2739**
immune response to, 2738
treatment of, 2746-2747
Oropharynx
flora of, 91t, 92
infection of. *See* Oral cavity, infection of; Pharyngitis
Oropouche fever, **2537-2544**
clinical manifestations of, 2542
diagnosis of, 2543
differential diagnosis of, 2543-2544
epidemiology of, 2539-2541
meningeal, 2542-2543
pathogenesis of, 2542-2543
prevention of, 2544
transmission of, 2541
treatment of, 2544
vs. Venezuelan equine encephalitis, 2240
Oropouche fever virus, 2538-2539, 2538f-2539f
Oroya fever, **1725-1726**
diagnosis of, 1727
prognosis for, 1729
treatment of, 1728
Orthopedic fixator devices, infection with, 725
Orungo virus, 2175-2176
Oseltamivir, 3268t, 3270-3273, 3270f
in community-acquired pneumonia, 290
in influenza virus infection, 2348-2351, 2349t
in laryngotracheitis, 257
Osler nodes, 355, 357
Ossicles, otitis media-related effects on, 223
Osteoarthritis, *Candida* spp., 895, 897
Osteochondritis
in congenital syphilis, 1769
of foot, 724-725
Osteomyelitis, **711-727**
Achromobacter xylosoxidans, 1574-1575
Actinomyces spp., 725
Aeromonas spp., 1543
anaerobic bacteria in, 711, 1829-1830
Bacillus cereus, 1319
Bartonella henselae, 1723-1724
Blastomyces dermatitidis, 2728, 2729f-2730f
Brucella spp., 725, 1613
C-reactive protein in, 719
Candida, 2742, 2749
cervical, retropharyngeal abscess and, 174
chronic, 726-727, 726f
Clostridium spp., 1797-1798
Coccidioides spp., 2771, 2772f, 2773-2774, 2774f
culture-negative, 714

Osteomyelitis *(Continued)*
epidemiology of, 711
Escherichia coli, 1441
etiology of, 711, 712t
fever of unknown origin in, 845
fungal, 725-726
Haemophilus influenzae type b, 1676
hematogenous, **711-724**
Brodie abscess in, 719
in chronic granulomatous disease, 56-57, 724
clinical manifestations of, 712-714, 713f, 713t
closed fracture and, 719
CT in, 717
diagnosis of, 714-717
differential diagnosis of, 714
epiphyseal, 719
in hemodialysis patient, 723
in hemoglobinopathy, 723-724
in HIV infection, 724
microbiology of, 714-715
MRI in, 715-716, 716f
neonatal, 713, 713f, 723
in nontubular bones, 719-720
pathogenesis of, 711-712, 712f
radiography in, 713, 715-717, 715f
radionuclide imaging in, 716-717, 716f
spinal, 720-723, 721f-722f
treatment of, 717-719, 718t
Kingella kingae, 1700, 1702
mandibular/maxillary, **152-154**, 152t
clinical findings in, 152-153
Garré, 154, 154f-155f
infantile, 153-154, 153f, 889
microbiology of, 152
predisposing factors in, 152
suppurative, 153, 153f
mastoiditis and, 237
multifocal, recurrent, chronic (CRMO), 714
Mycobacterium ulcerans, 1414-1416, 1414f
neonatal, 713, 713f, 723, **889-890**
mandibular/maxillary, 153-154, 153f, 889
nonhematogenous, **724-725**
contiguous sources of, 725
orthopedic devices and, 725
puncture wound, 724-725
Pantoea agglomerans, 1536
Proteus spp., 1473
Pseudomonas aeruginosa, 1591, 1599
rhinosinusitis and, 198
Salmonella spp., 1505
Sporothrix schenckii, 2834
Staphylococcus epidermidis, 1137
Streptococcus agalactiae, 1161
Streptococcus pneumoniae, 711, 712t
Streptococcus viridans, 1193
therapeutic drug monitoring in, 718-719
Otalgia, 208
differential diagnosis of, 220
in otitis externa, 205
in otitis media, 217
Otitic hydrocephalus, 237
Otitis externa, **203-209**
acute, 205-207
diagnosis of, 205, 205f
etiology of, 205-206, 206t
management of, 206-207
anatomy in, 204
chronic, 207
differential diagnosis of, 208, 208f
epidemiology of, 204
vs. mastoiditis, 237
Otitis externa *(Continued)*
necrotizing, 207-208
predisposing factors in, 204, 204t
prevention of, 208
protective mechanisms in, 204
Pseudomonas aeruginosa, 1591, 1599
Otitis media, **209-232**
acoustic reflectometry in, 218
adhesive, 223
anatomic abnormalities and, 211
antibiotic resistance in, 3147
audiometric test in, 218
behavioral disturbances with, 222
chemoprophylaxis in, 231
Chlamydia pneumoniae, 2642
cholesteatoma with, 223
clinical course of, 228
clinical presentation of, 217
complications of, 221-225, 221f
conjunctivitis with, 217
cost-related aspects of, 212
craniofacial anomalies and, 217
criteria for, 216-217
in daycare facilities, 3553, 3554t
developmental disorders and, 222, 222f
diagnosis of, 216-217
differential diagnosis of, 220
drug-resistant, 226
environmental risk factors for, 211-212
epidemiology of, 210-212
etiology of, 212-215, 212t-213t
eustachian tube in, 216, 216f
facial paralysis with, 217, 224
genetic factors in, 211
Haemophilus influenzae, 1677
hearing loss with, 217, 222
hMPV infection and, 2438-2439
immunodeficiency and, 211
intracranial complications of, 224-225, 225f
labyrinthitis with, 224
management of, 225-229, 225t, 227t-228t, 3508
mastoiditis with, 223-224
in measles, 2385, 2392
meningitis with, 224
Moraxella catarrhalis, 1252
Mycoplasma pneumoniae, 2678-2679
myringotomy in, 218-220
neonatal, 215, 887
observation for, 226-227, 227t
ossicular chain abnormalities with, 223
otalgia in, 217
otorrhea in, 217
otoscopy in, 217-218, 217f, 219f
parainfluenza virus infection and, 2367
pathogenesis of, 215
pathophysiology of, 215-216
petrositis with, 224
pneumococcal vaccine in, 230-231, 230t-231t
poverty and, 211
prevention of, 230-232, 230t-231t, 3247-3248
Proteus spp., 1473
Pseudomonas aeruginosa, 1591, 1599
radiography in, 220
recurrence of, 212, 3247-3248
risk factors for, 210-211, 210t
seasonal variation in, 211-212
Streptococcus pneumoniae, 1231, 1233-1236
suppurative, chronic, 212-213, 223
symptomatic therapy in, 228
tinnitus in, 217
tympanic membrane in, 215-216
tympanic membrane perforation with, 223
Otitis media *(Continued)*
tympanocentesis in, 218-220
tympanometry in, 218, 220f
tympanosclerosis with, 223
tympanostomy tubes in, 229, 232
vertigo in, 217, 222
viral infection and, 214, 2123-2124, 2169-2170
Otitis media with effusion, 209-210, **221**
etiology of, 212-213
management of, 229
pathogenesis of, 215, 215f
viral infection and, 2124
Otomycosis, **207**
Otorrhea, in otitis media, 217
Otoscopy, in otitis media, 217-218, 217f, 219f
Outpatient intravenous antimicrobial therapy, **3257-3265**
administration of, 3264
agent selection for, 3261-3263, 3262t-3263t
complications of, 3264-3265
evaluation of, 3257-3258, 3258t
indications for, 3261, 3262t
monitoring in, 3263
outcome analysis of, 3264-3265
program for, 3258-3261, 3259t, 3260f
Over-the-counter medications, in common cold, 137
Oxacillin, 3184t, 3185-3186, 3231t-3233t
in bacterial meningitis, 450t
Oxamniquine, 3350
Oxygen therapy
in bronchiolitis, 278-280
in croup, 255
hyperbaric
in gas gangrene, 1799
in mucormycosis, 2844
in necrotizing fasciitis, 150
in RSV infection, 2425
in septic shock, 835
Oxymetazoline, in rhinosinusitis, 200
Oxytetracycline, 3217-3220

P

P-Selectin, in phagocyte recruitment, 31-32, 33f
p value, 120
Pacemaker
infection with, 352, 1023-1025
clinical manifestations of, 1024
laboratory findings in, 1024
management of, 1024-1025
microbiology of, 1024
in myocarditis, 396
Pacifier thermometer, 86
Pacifier use, otitis media and, 211
Paecilomyces spp. infection, **2856-2857**
Pain
abdominal. *See* Abdominal pain
chest. *See* Chest pain
ear. *See* Otalgia
in herpes zoster, 2027-2028
in spinal epidural abscess, 471
Palivizumab
in heart transplant–related RSV infection, 950
in hematopoietic stem cell transplantation–related RSV infection, 943
in lung transplant–related RSV infection, 980
in RSV infection, 2426-2427, 2429, 3503, 3529-3530

Palpebral edema
in roseola infantum, 770
in *Trypanosoma cruzi* infection, 2963
Pancreas, abscess of, 696, 704-708. *See also* Pancreatitis
Pancreatic duct, obstruction of, 692
Pancreatic pseudocyst, 696
Pancreatitis, **689-697**
autoimmune disease-related, 691t, 692
bacterial, 693t, 695
chronic, 690, 692
clinical manifestations of, 689-690, 708
complications of, 696
coxsackievirus B, 2072
diagnosis of, 690, 707-708
drug-related, 691-692, 691t, 696
fibrosing, chronic, 692
fungal, 695
hereditary, 692
infectious, 693-695, 693t
metabolic disease-related, 691t, 692
mumps, 2402
mycoplasmal, 695, 2682-2683
noninfectious, 690-693, 691t
obstruction-related, 691t, 692, 707-708
osteolytic lesions after, 696
parasitic, 694-695, 694f
pathogenesis of, 695
pseudocyst in, 696
scoring system for, 690
trauma-related, 691, 691t
treatment of, 695-696, 709
vasculitis-related, 691t, 692
viral, 693-694, 693t
Pancytopenia
in human erythrovirus B19 (human parvovirus B19) infection, 1850
in infectious mononucleosis, 2003
PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection), 410, **1143-1146**
diagnosis of, 1144-1145
evidence for, 1146
pathogenesis of, 1144, 1144f
prevention of, 1145-1146
treatment of, 1145, 3511
Pandoraea spp., 1584
Pandoraea spp. infection, 1595-1596
Panophthalmitis
Bacillus cereus, 1319
Clostridium spp., 1798
Morganella morganii, 1470
Yersinia enterocolitica, 1528
Pantoea agglomerans, 1535
identification of, 1537
Pantoea agglomerans infection, **1535-1537**
clinical manifestations of, 1536
diagnosis of, 1537
epidemiology of, 1535-1536
pathophysiology of, 1536
treatment of, 1537
Panton-Valentine leukocidin, 1116-1117
Papanicolaou smear
in HPV infection, 581, 1881-1882
in HSV infection, 1956
in *Trichomonas vaginalis* infection, 2892
PapG adhesin, 537
Papillitis, 810
Papilloma. *See also* Human papillomavirus (HPV) infection; Warts
in yaws, 1786
Papillomatosis, respiratory, recurrent, 1878-1879, 1878f-1879f
Papillon-Lefèvre syndrome, 673, 1610
Papular acrodermatitis (Gianotti-Crosti syndrome), 649, 749, **766**, **783**, 783f
in HAV infection, 2140
Paracoccidioides spp., 2780-2781, 2783
identification of, 2787-2792, 2787f, 2789t-2790t
Paracoccidioides spp. infection (paracoccidioidomycosis), **2780-2795**
age and, 2781-2782, 2782t
bone involvement in, 2784, 2785f, 2787
bone marrow involvement in, 2784
clinical manifestations of, 2784f-2785f, 2786-2787, 2786t
diagnosis of, 2787-2792, 2787f, 2789t-2790t
epidemiology of, 2781-2783
gender and, 2782, 2782t
geographic distribution of, 2782-2783
after kidney transplantation, 1013
lymph node involvement in, 2784, 2784f-2785f, 2786
occupation and, 2782
pathogenesis of, 2783-2785
prognosis for, 2793-2794
race and, 2782
transmission of, 2781
treatment of, 2792-2793, 3295t
Paradoxical pulse, in pericarditis, 374, 375f
Paradoxical zone phenomenon, 3174-3175, 3174f
Paragonimus spp., 3050f-3051f, 3054
Paragonimus spp. infection (paragonimiasis), **3054-3055**
clinical manifestations of, 3055
CNS, 3055-3056
cutaneous, 3055
diagnosis of, 3051f, 3055-3056
eosinophilia in, 3056
epidemiology of, 3054-3055
pathogenesis of, 3050f, 3055
pleuropulmonary, 3055-3056
prevention of, 3056
transmission of, 3054-3055
treatment of, 3056, 3327t-3346t
Parainfluenza virus(es), 2361-2363
classification of, 2361-2363, 2361t, 2362f
isolation of, 2363
structure of, 2361-2363, 2362f
Parainfluenza virus (PIV) infection, **2360-2372**
in acute bronchitis, 262, 263t
age and, 2363-2364, 2363f
animal models of, 2365-2366
after bone marrow transplantation, 2368
clinical manifestations of, 2366-2368, 2367f
in common cold, 132
complications of, 2370
in croup syndromes, 242, 247-248
diagnosis of, 2368-2369
differential diagnosis of, 2369
encephalitic, 494t-498t
epidemiology of, 2363-2364
exanthem in, 742t-748t, 749, 760-762
after heart transplantation, 954
after hematopoietic stem cell transplantation, 943
historical perspective on, 2360-2361
immune response to, 2365-2366
in immunocompromised host, 2368
after intestinal transplantation, 997t, 999
after kidney transplantation, 1007
after liver transplantation, 997t, 999
after lung transplantation, 980, 2368
in otitis media, 2367
Parainfluenza virus (PIV) infection *(Continued)*
pathogenesis of, 2364-2366
pathology of, 2365
in pneumonia, 284
prevention of, 2370-2372
prognosis for, 2370
reinfection with, 2367-2368
seasonality of, 2364
transmission of, 2364
treatment of, 2369-2370
Paralysis
in enteroviral infection, 2090. *See also* Poliovirus infection (poliomyelitis)
in spinal epidural abscess, 471
Paramomycin, 3231t-3233t
Paranasal sinuses, 193-194
inflammation of. *See* Rhinosinusitis
Parapharyngeal abscess, 170f, **172-173**
clinical manifestations of, 172-173
microbiology of, 173-175
treatment of, 173
Parapoxviruses, 2041t, 2046
Parasitic disease. *See also specific parasite infections*
appendiceal, 683t, 684-685
biliary, 660, 664
in burn patient, 1054
classification and nomenclature in, 2861-2866, 2862t-2863t
encephalitic, 494t-498t, 501
eyelid, 795-796
gastrointestinal, 602-604, 602t-603t, **613-616**, 616t
stool examination in, 617
treatment of, 625
hepatic, 651t, 657
host response to, 23-24. *See also* Immunity
after kidney transplantation, 1014
laboratory diagnosis of, **3620-3627**, 3621t, 3624f-3625f
blood smear examination in, 3625-3626, 3625f
blood specimen for, 3624-3626, 3625f
duodenal specimen for, 3622
respiratory specimen for, 3622
serology in, 3626-3627
sigmoidoscopy specimen for, 3622
stool specimen for, 3622, 3623t. *See also* Stool specimen examination
tape-collected specimen for, 3622
urogenital specimen for, 3626-3627
meningeal, 488
muscle, 739
myocardial, 398-400
pancreatic, 694-695, 694f, 707-708
Paravaccinia virus infection (milker's nodule), 742t-748t
Parechovirus(es)
genome of, 2053
geographic distribution of, 2056-2057
host interaction with, 2054-2056
replication of, 2054
transmission of, 2056
Parechovirus infection, **2051-2109**
asymptomatic, 2063-2064
clinical manifestations of, 2063-2090
diagnosis of, 2101-2103
encephalitic, 493-499, 494t-498t
in epidemic pleurodynia, 2067-2068
exanthem in, 2080t-2081t, 2086
hemolytic-uremic syndrome in, 2078
herpangina in, 2065, 2066t
in immunocompromised host, 2091

Parechovirus infection *(Continued)*
myocardial, 2076t
neonatal, 2092-2097, 2094t
nonspecific febrile illness in, 2064t
respiratory manifestations of, 2064-2068, 2064t
treatment of, 2103-2104
uveal, 2074-2075
Parkinson disease, Western equine encephalitis virus infection and, 2235
Paromomycin, 3208-3211, 3208t, 3350
Paronychia, 3571-3572, 3574
Candida, 788, 788f
Parotid glands
in cat-scratch disease, 1722, 1724f
in HIV-infected host, 149, 191-192
infectious enlargement of. *See* Parotitis
noninfectious enlargement of, 192
Parotidectomy, 192
Parotitis, **189-193**
chronic, 189
clinical presentation of, 190-191
complications of, 192
diagnosis of, 190-191
differential diagnosis of, 192, 2403
enterovirus, 2066
epidemic, 189. *See also* Mumps
etiology of, 189-190, 190t
granulomatous, 189-191
in HIV infection, 191-192
neonatal, 890-891
pathophysiology of, 189, 190t
prevention of, 192-193
recurrent, 189, 191
suppurative, 189-190, 192-193
treatment of, 192
viral, 189-191, 190t. *See also* Mumps
Paroxysmal atrial tachycardia, in myocarditis, 389
Parrot, pseudoparalysis of, 1769-1770
PARV4 virus, 1843
Parvoviridae, 1838t-1840t. *See also* Human bocavirus (HBoV); Human erythrovirus B19 (human parvovirus B19) infection
genetic history of, 1845
Parvovirus B19. *See* Human erythrovirus B19 (human parvovirus B19) infection
Passive immunization, **3491-3536**
animal sera for, 3491-3493
antitoxin for, 3491-3493. *See also* Antitoxin
definition of, 3491, 3492t
human immune serum globulin, 3493-3499. *See also* Immunoglobulin(s)
monoclonal antibodies in, 3493. *See also* Monoclonal antibodies
Passive microcapsule agglutination test, in *Leptospira* spp. infection, 1756
Pasteur, Louis, 103
Pasteurella multocida, 1545-1546
Pasteurella multocida infection, **1545-1548**
clinical manifestations of, 1547, 3574
diagnosis of, 1548
epidemiology of, 1546
immune response to, 1546
pathogenesis of, 1546-1547
vs. plague, 1513
prevention of, 1548
transmission of, 1546
treatment of, 1548
Pasteurella spp., 1566-1567
Pasteurella spp. infection, **1566-1567**
Patella, osteomyelitis of, 725
Patent ductus arteriosus, in congenital rubella, 2214t-2216t, 2217
Pathogen, 3, 89. *See also* Microbiome *and specific organisms and infections*
in epidemiologic study, 104-107, 106f, 107t
facultative, 89, 91t
immunogenicity of, 105
infectivity of, 105
opportunistic, 89, 91t
pathogenicity of, 105
reservoirs for, 106-107
strict, 89
virulence of, 105
Pathogenicity, 105
Pathogenicity islands, 3
Helicobacter pylori, 1693
Salmonella spp., 1496-1497, 1496f
SPI-1, 13-14
SPI-2, 14-15
SPI-3, 15
Staphylococcus aureus, 1115, 1115t
Vibrio cholerae, 1550
Pattern recognition receptors, 433, 434f, 436
Pediatric acute-onset neuropsychiatric syndrome (PANS), 1146. *See also* PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection)
Pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection. *See* PANDAS (autoimmune neuropsychiatric disorder associated with streptococcal infection)
Pediatric Risk for Mortality (PRISM) score, 458
Pediculus humanus. *See* Lice
Pediococcus spp., 1247-1248
vs. *Enterococcus* spp., 1171
Pediococcus spp. infection, **1247-1248**
clinical manifestations of, 1248
diagnosis of, 1248
epidemiology of, 1248
pathophysiology of, 1248
treatment of, 1248
Pelvic inflammatory disease (PID), **586-589**, 588t-589t, 1285-1286, 1829
chlamydial infection and, 534-535
complications of, 589, 589f, 1286, 1286f
diagnosis of, 1285
risk factors for, 1286
treatment of, 1296
Trichomonas vaginalis infection and, 2891
Pelvis, osteomyelitis of, 720
Pemphigus syphiliticus, 1770
Penciclovir, 3278t, 3281-3282, 3281f
Penicillin(s), 3183-3191, 3183t
in acquired syphilis, 1777-1778
adverse effects of, 3189-3191, 3189t
in bacterial meningitis, 450t, 1263-1266
in cervical lymphadenitis, 185-186
in community-acquired pneumonia, 291
in congenital syphilis, 1776t-1777t, 1779-1780, 1780f
dosage schedules for, 3231t-3233t
extended-spectrum, 3183t-3184t, 3187, 3231t-3233t
Harry Eagle effect with, 739
in myositis, 739
in *Neisseria meningitidis* infection, 1263-1266
in odontogenic infection, 145, 146t
penicillinase-resistant, 3183t-3184t, 3185-3186, 3231t-3233t
in peritonsillar abscess, 174
Penicillin(s) *(Continued)*
resistance to, 3139-3145
in *Staphylococcus aureus* meningitis, 448-449
in *Streptococcus pneumoniae* mastoiditis, 234
in *Streptococcus pneumoniae* meningitis, 427, 447
in *Streptococcus* viridans infection, 1194
in rheumatic fever, 414, 414f, 414t
in rheumatic fever prevention, 415-418
in *Streptococcus pyogenes* infection, 1147-1149, 1148t
in syphilis, 812
in tetanus, 1816
in Whipple disease, 645-646, 646t
Penicillin-binding proteins, in antibiotic resistance, 3143-3145
Penicillin G, 3183-3185, 3184t, 3231t-3233t
Penicillin V, 3183-3185, 3184t, 3231t-3233t
Penicillium marneffei infection, **2855**, 2855f
Penicillium spp. infection, burn wound, 1053
Penis
cancer of, HPV and, 1873t, 1875, 1878
HSV infection of, 1942f. *See also* Herpes simplex virus (HSV) infection, genital
Pentamidine, 3350
in hematopoietic stem cell transplantation, 938-939
pancreatitis with, 691-692
in *Pneumocystis* infection, 691-692
in *Trypanosoma brucei* infection, 2971-2972
in visceral leishmaniasis, 2958
Pentoxifylline, 3360-3361
Peptic ulcer disease, 1691, 1694. *See also* *Helicobacter pylori* infection
Peptidoglycan
of *Neisseria gonorrhoeae*, 1278-1279
of *Streptococcus pneumoniae*, 1210, 1211f, 1214
Peptidoglycan *N*-acetylglucosamine deacetylase A, of *Streptococcus pneumoniae*, 1215
Peptostreptococcus spp. infection
peritoneal, 700
retroperitoneal, 706-707
Peramivir, 3270-3273
Perforin, 28
Perianal streptococcal dermatitis, **775**
Periarteritis nodosa, infantile, 1071. *See also* Kawasaki disease
Pericardial effusion
in meningitis, 441
in pericarditis, 373-374, 376-377, 376f-377f
Pericardial fluid, 371
blood in, 377-378
in pericarditis diagnosis, 377-378
Pericardial window, 378
Pericardiocentesis, 378, 378t
Pericarditis
adenovirus, 1902-1903
bacterial, **371-379**
clinical manifestations of, 372t, 374
diagnosis of, 375-378, 376f-377f
differential diagnosis of, 378
epidemiology of, 371, 372t
etiology of, 371-373, 372t
pathogenesis of, 373-374
prognosis for, 379
treatment of, 378-379, 378t
Candida, 2740-2741, 2748
Entamoeba histolytica, 2871
enterovirus, 2075-2077, 2076t
fungal, 373, 379

Pericarditis *(Continued)*
Haemophilus influenzae type b, 372-373, 378-379, 1676
Histoplasma capsulatum, 373, 377-378, 2813
influenza virus, 2343
measles virus, 2385
Morganella morganii, 1470
mortality with, 379
vs. myocarditis, 395
Neisseria meningitidis, 373, 378-379, 1262
noninfectious, 372t
pneumococcal, 373
Pseudomonas aeruginosa, 373
rubella virus, 2212
staphylococcal, 372, 379
streptococcal, 371-373, 379, 1232
surgical treatment of, 378
tuberculous, 373, 379, 1351
viral, **379-380**
clinical manifestations of, 379
diagnosis of, 380, 380f
etiology of, 372t, 379
prognosis for, 380
in Whipple disease, 644
Pericardium, 371
infection of. *See* Pericarditis
Pericoronitis, 147-148, 148f
Perihepatitis, **589-590**
Perineum, hygiene of, 564-566
Periodic fever, 159t-160t, 162, 181
Periodic fever disorders, 847-848
Periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome, 159t-160t, 162-164, 181, 848
Periodontitis, 146-147
Aggregatibacter actinomycetemcomitans, 140, 147, 1610, 1823
Eikenella corrodens, 1577
juvenile, 140, 147
PMN chemotaxis in, 56
in leukocyte adhesion deficiency, 904-905, 905f
pathogens in, 140
Periorbital cellulitis, 151, 798-801
Periostitis, in congenital syphilis, 1769, 1772
Peripheral neuropathy
CMV, 1979-1980
in *Corynebacterium diphtheriae* infection, 1304-1305
Peripherally inserted central catheter (PICC), infection with, 1136. *See also* Catheter-associated infection
Peritoneal cavity, 698, 699f
Peritoneal dialysis, 698, 700t, 701-703
catheter-related infection with, 1137
Peritoneal pseudocyst, in ventriculoperitoneal shunt, 1031-1032
Peritonitis, **698-704**
Aeromonas spp., 1542
anatomy of, 698, 699f
Candida, 2741, 2748, 2754-2755
CAPD-associated, 698, 700t, 701-703
chemical, 698
clinical manifestations of, 701
vs. *Clostridium difficile* colitis, 635
complications of, 704
coxsackievirus B, 2070
diagnosis of, 702
dialysis-related, 3406-3407
differential diagnosis of, 702
Escherichia coli, 1441, 1445
fungal, 701, 703
Mycobacterium avium-intracellulare complex, 1389
Peritonitis *(Continued)*
noninfectious, 698
vs. noninfectious diseases, 702
Pasteurella multocida, 1547
pathogenesis of, 698, 700t
Plesiomonas shigelloides, 1566
primary (spontaneous), 698-700, 700t, 702
secondary, 700-703, 700t
Stenotrophomonas maltophilia, 1607-1608
Streptococcus pneumoniae, 1232
tertiary (recurrent), 704
treatment of, 702-704, 703t
tuberculous, 700-701, 1357-1358
VP shunt-associated, 698, 700t, 701-703
Peritonsillar abscess, **168-170**, 170f
clinical manifestations of, 168-169, 168f
microbiology of, 173-175
treatment of, 169-170, 175
Perityphlitis, 679
Permethrin, 1737, 2175, 2236, 2526, 3350
in lice, 3075
in scabies, 3074
Pertactin, of *Bordetella pertussis*, 1618t, 1619, 1623
Pertussis. *See Bordetella pertussis* infection (pertussis, whooping cough)
Pertussis-like illness
in adenovirus infection, 1617, 1898
in *Mycoplasma pneumoniae* infection, 1617
Pertussis toxin, 17, 1618t, 1619, 1624
Petechiae, 762-763, 763t
in arenaviral hemorrhagic fevers, 2471
in infective endocarditis, 357
in meningococcal infection, 750, 753t-756t, 762-763, 1258-1259, 1259f
in septic shock, 831
Petrolatum jelly, in eyelid lice, 795
Petrositis, 224
mastoiditis and, 237
PFAPA (periodic fever, aphthous stomatitis, pharyngitis, and adenitis) syndrome, 159t-160t, 162-164, 181
pH
airway, in rhinovirus infection, 135
blood, in diarrheal disease, 76
CSF, in bacterial meningitis, 444
ear canal, in otitis externa, 207
fecal, in diarrheal disease, 76
Phaeoannellomyces werneckii infection, **790**, 790f
Phaeohyphomycosis, **2855-2858**, 2856f
Phagocyte(s), 31-35
disorders of, 52-59, 917-919, 917t
qualitative, 917t, **918-919**
quantitative, 917, 917t
evaluation of, 63, 907
microbicidal activity of, 33-35, 34f, 34t
defects of, 56-59
in newborn, 45
recruitment of, 31-32, 32f-33f
Phagocytosis, **32-33**, 33f
in *Haemophilus influenzae* infection, 1672-1673
Neisseria gonorrhoeae resistance to, 1276
pathogen evasion of, 18
in *Staphylococcus aureus* infection, 1122
in *Streptococcus pneumoniae* infection, 1223
Pharyngitis, **158-164**
adenovirus, 158-161, 159t-160t, 1895-1896
Arcanobacterium haemolyticum, 1324-1326, 1324f, 1324t
in bronchiolitis, 275
Citrobacter freundii, 1431
Pharyngitis *(Continued)*
clinical presentation of, 159t-160t, 161-162
complications of, 164
Corynebacterium diphtheriae, 1305, 1307
coxsackievirus A10, 2066
diagnosis of, 163
differential diagnosis of, 159t-160t, 162-163
enterovirus, 2064t, 2065
epidemiology of, 161
etiology of, 158-161, 159t-160t
fever in, 162
gonococcal, 1288, 1296
historical perspective on, 156
HSV, 1941
meningococcal, 1261
Mycoplasma pneumoniae, 2678
pathophysiology of, 161
prevention of, 164
prognosis for, 164
reovirus, 2169-2170
Saffold virus, 2065
streptococcal, 156, 159t-160t
clinical manifestations of, 161-162, 1142-1143
diagnosis of, 163, 1146-1147
epidemiology of, 1141
non–group A, 1151
pathogenesis of, 1142
prevention of, 1141-1142, 1150
rheumatic fever and, 408, 416. *See also* Rheumatic fever
tonsillectomy in, 1150
transmission of, 1141
treatment of, 163, 1147-1149, 1148t
treatment of, 163-164
uvulitis and, 166
Yersinia enterocolitica, 1528
Pharyngoconjunctival fever, 1893-1894, 1899, 1899t-1900t
Pharynx
hyperemia of, 842
infection of. *See* Pharyngitis
Phenol-soluble modulins, of *Staphylococcus epidermidis*, 1133
Phenylalanine, 71
serum, 830
urinary, 69
Phenylalanine-to-tyrosine ratio, 71
Phenylephrine hydrochloride, in rhinosinusitis, 200
Phialophora verrucosa infection, **789-790**
Phlebotomus fever, **2535-2537**, 2536f
Phlebovirus, 2535
Phosphoglucomutase, of *Streptococcus pneumoniae*, 1214
Phospholipase D, 1322
Phosphorylcholine esterase, of *Streptococcus pneumoniae*, 1213
Photophobia, in enterovirus infection, 2074
Phthirus pubis (crabs). *See* Lice
Physostigmine ointment, in eyelid lice, 795
Pichia spp. infection, 898
Piercings
intraoral, 155
perioral, 155
Pigbel, 1797-1800
Pilin protein, of *Streptococcus pneumoniae*, 1211
Pill esophagitis, 594
Pilosebaceous unit, of external ear, 204
Pilus (pili), 3-6
Aeromonas spp., 1541
antigenic variation in, 19

Pilus (pili) *(Continued)*
assembly of, 4-5, 4f-5f
chaperone-usher pathway of, 4-5, 4f-5f
donor-strand complementation in, 4-5, 5f
donor-strand exchange in, 4-5, 5f
enterotoxigenic *Escherichia coli*, 1455
Kingella kingae, 1701-1702, 1701f
Neisseria gonorrhoeae, 1277
Neisseria meningitidis, 1254
Pseudomonas aeruginosa, 1586-1587
Streptococcus agalactiae, 5-6, 1154
structure of, 3-4, 4f
type 4, 5
uropathogenic *Escherichia coli*, 3-4, 4f, 10
Pinchindé virus infection, 2472. *See also* Arenavirus infection
Pinta, 753t-756t, 1783t, **1784-1785**
diagnosis of, 1789
treatment of, 1789-1790
Pintids, 1785
Pinworms, 566-567, 566f, **3018-3019**
Scotch tape collection of, 566, 566f
Piperacillin, 3183t-3184t, 3187, 3231t-3233t
in meningitis, 450t
Piperacillin-tazobactam, 3188-3189
Pituitary gland, hormones of, 81
PIV infection. *See* Parainfluenza virus (PIV) infection
Placenta
in syphilis, 1766
in *Ureaplasma* spp. infection, 2690
Plague. *See Yersinia pestis* infection (plague)
Plaque reduction neutralization test
in Toscana virus infection, 2549
in yellow fever, 2277
Plasmapheresis
in acute disseminated encephalomyelitis, 519
in acute transverse myelitis, 527-528
in encephalitis, 510
in *Mycoplasma pneumoniae* infection, 2687
in septic shock, 836
Plasmid(s), in antibiotic resistance, 3137-3138, 3138t
Plasmid-encoded toxin, 1460
Plasminogen, *Streptococcus pneumoniae* binding of, 1214
Plasmodium spp., 2922-2923, 2927t. *See also* Malaria
antigen detection assays for, 2935
vs. *Babesia* spp, 2918-2919
DNA probes for, 2935
life cycle of, 2922-2923, 2922f
microscopy for, 2930, 2931f-2934f
PCR for, 2935
Platelet-activating factor, 3366-3367
Platelet-activating factor receptor, in *Streptococcus pneumoniae* infection, 1224
Platelet count, in Kawasaki disease, 1076
Platelet disorders, in Wiskott-Aldrich syndrome, 913
Pleconaril, 3276-3277, 3276f
in common cold, 138
in enterovirus infection, 2103
in rhinovirus infection, 2126
Plesiomonas shigelloides, 1565
Plesiomonas shigelloides infection, 607t, 612-613, 616t, **1565-1566**
Pleura, in Whipple disease, 644
Pleural effusion
bloody, 315
etiology of, 308, 308t
exudative, 308, 311
in *Francisella tularensis* infection, 1662
Light's criteria for, 311-312
Pleural effusion *(Continued)*
malignant, 313
parapneumonic, **306-315**
chest radiography in, 310, 311f
clinical presentation of, 310
CT in, 310
diagnosis of, 310-313
drainage of, 314-315
epidemiology of, 307
fluid analysis in, 310-313
historical perspective on, 307
management of, 312f, 313-315
microbiology of, 309-310, 309t
pathophysiology of, 307-308, 308t
prevention of, 315
prognosis for, 315
radiologist-directed thoracentesis for, 314
streptokinase for, 314-315
ultrasonography in, 310, 311f
video-assisted thoracoscopic surgery for, 314
pathophysiology of, 307-308, 308t
purulent, 306-308, 308t
transudative, 306-308, 308t, 311
in tuberculosis, 309, 1343f, 1347-1348, 1348f-1349f
Pleural fluid examination
in mediastinitis, 420
in parapneumonic effusion, 310-313
Pleurodynia, epidemic, 739, 2067-2068
Pneumatocele, pneumonia and, 322
Pneumatosis cystoides intestinalis, 1798
Pneumatosis oculi, 1431
Pneumococcal histidine-containing protein A (PhpA), of *Streptococcus pneumoniae*, 1213
Pneumococcal infection. *See Streptococcus pneumoniae* infection
Pneumococcal surface protein A (PspA), of *Streptococcus pneumoniae*, 1210-1211, 1211f, 1213
Pneumocystis pneumonia, **3005-3012**
clinical manifestations of, 3008-3009, 3009f
congenital immunodeficiency and, 3007
diagnosis of, 3009-3010
after heart transplantation, 954
after hematopoietic stem cell transplantation, 938-939
HIV infection and, 2599-2600, 2599f, 2600t, 3007
immunopathogenesis of, 3007-3008
after intestinal transplantation, 1000
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 972-973
pathology of, 3008
pentamidine isethionate in, 691-692
prevention of, 3010-3012
prognosis for, 3010
transmission of, 3006-3007
treatment of, 3010, 3011t, 3327t-3346t
Pneumocystis spp., 3006, 3006f, 3009f
Pneumolysin, *Streptococcus pneumoniae*, 1209-1210, 1212-1213, 1223-1224
Pneumonia
Acinetobacter spp., 1570-1571
adenovirus, 284, 295-296, 1894, 1894f, 1897-1898, 1900
Aeromonas hydrophila, 1543
antibiotic resistance in, 3147
atypical measles, 2384, 2384f
Bacillus cereus, 1320
Blastomyces dermatitidis, 2727, 2727f
Brucella spp., 1613
Pneumonia *(Continued)*
in burn patient, 1055
Candida, 2741
Chlamydia pneumoniae, 292, 1617-1618, **2639-2644**, 2641t, 2642f
Chlamydia trachomatis, 2633, 2633f
CMV, 1976-1977
community-acquired, **283-294**
abscess with, **315-322**, 317t, 319f-320f, 320t
Acinetobacter baumannii, 1571
auscultation in, 288
bacterial, 284-285, 285t
clinical manifestations of, 287-289, 287t
diagnosis of, 289
pathogenesis of, 286-287
prevention of, 292
treatment of, 289-292, 291t
clinical manifestations of, 287-289, 287t
clinical scores in, 290
comorbid conditions with, 290
complications of, **306-322**, 307t. *See also* Lung abscess; Pleural effusion, parapneumonic
diagnosis of, 289
empyema with, **306-315**, 308t-309t, 311f-312f
vs. eosinophilic pneumonia, **297**
epidemiology of, 285
etiology of, 283-285, 285t
hospitalization for, 290
management of, 289-292, 291t
mimivirus antibodies in, 2048-2049
pathogenesis of, 285-287
pleural effusion in. *See* Pleural effusion, parapneumonic
prevention of, 285, 292
rhinovirus infection and, 2123
vs. vasculitis, 297
viral, 284, 285t
clinical manifestations of, 288
diagnosis of, 289
pathogenesis of, 285-287
prevention of, 292
treatment of, 290
coronavirus, 284, 2491
coxsackievirus, 2067
echovirus, 2067
Eikenella corrodens, 1577
enterovirus, 2067
eosinophilic, **297**
Escherichia coli, 1441, 1445-1446
Francisella tularensis, 1662-1663
Haemophilus influenzae type b, 1675
health care–associated, 3391-3393
in neonate, 892
after heart transplantation, 947-948
hMPV, 284, 285t, 2438-2439, 2439f
HSV, 1953-1954
human bocavirus, 284, 1860
influenza virus, 284, 2340, 2344f
IVIG in, 3508
after kidney transplantation, 1005-1006
Klebsiella pneumoniae, 1466-1467
Legionella spp., **1705-1714.** *See also Legionella pneumophila* infection (Legionnaires' disease)
differential diagnosis of, 1713
measles virus, 2384-2385, 2384f, 2391-2392
in military recruits, 1898
Moraxella catarrhalis, 1252
Mycoplasma pneumoniae, 285t, 287-289, 292, 2675-2678, 2676t

Pneumonia *(Continued)*
necrotizing, 321
Neisseria meningitidis, 1260-1261
neonatal, 891-892
Plesiomonas shigelloides, 1566
pneumococcal, 284-285, 1229-1231, 1230f, 1230t. *See also Streptococcus pneumoniae* infection
diagnosis of, 1217-1218
treatment of, 1233, 1234t-1235t
Pneumocystis, **3005-3012**, 3006f, 3009f. *See also Pneumocystis* pneumonia
Pseudomonas aeruginosa, 1599
reovirus, 2170
Rhodococcus equi, 1427
RSV, 284, 290. *See also* Respiratory syncytial virus (RSV) infection
Stenotrophomonas maltophilia, 1606-1609
Streptococcus viridans, 1193
Treponema pallidum, 1770-1771
Ureaplasma spp., 296, 2690
ventilator-associated, 3391-3393
in neonate, 892
VZV, 2026
in Whipple disease, 643-644
Yersinia pestis, 1512-1513
Pneumonia alba, 1770-1771
Pneumonitis
CMV, 1974, 1976-1977, 1976f, 1984
after heart transplantation, 950-951
coxsackievirus, 2061-2062
hemorrhagic, in *Leptospira* spp. infection, 1748
hookworm, 3018
HSV, after kidney transplantation, 1006
hypersensitivity, 297, **301-305**, 302t-303t. *See also* Hypersensitivity pneumonitis
influenza virus, 2340
interstitial
after heart transplantation, 948
lymphocytic, 296
lupus, 297
Schistosoma spp., 3065-3066
Strongyloides stercoralis, 3020
Pneumothorax, pneumonia and, 322
Podophyllin, in genital HPV infection, 581
Poison ivy, vs. enteroviral infection, 762
Poisoning
food. *See* Food poisoning
heavy metal, 604, 604t
insecticide, 692
Poliovirus, 2052-2056, 2052t-2053t, 2059
Poliovirus infection (poliomyelitis), **2097-2101**
abortion and, 2100
congenital, 2100
in daycare facilities, 3563
epidemics of, 2057
epidemiology of, 119
extraneural, 2063
fetal, 2100-2101
global eradication of, 2106-2107
historical perspective on, 2051-2052
immunization against, 2105-2106, 3463-3466
meningeal, 488
minor, 2098
neonatal, 2100-2101
nonparalytic (aseptic), 2098
paralytic, 2098-2101, 2099t
passive immunization against, 3512-3513
pathogenesis of, 2060, 2060f
pathology of, 2062-2063
postnatal, 2101
Poliovirus infection (poliomyelitis) *(Continued)*
prevention of, 2105-2106
treatment of, 2104
Polyangiitis, microscopic, 297
Polychondritis, relapsing, vs. otitis externa, 208
Polymerase chain reaction (PCR)
in *Acanthamoeba* spp. infection, 2983
in *Achromobacter* spp. infection, 1575
in adenovirus infection, 982, 1906
in *Anaplasma* spp. infection, 2662-2663
in arenavirus infection, 2474
in *Aspergillus* spp. infection, 2716-2717
in bacterial meningitis, 442-444, 1263
in *Balamuthia mandrillaris* infection, 2983
in *Bartonella* spp. infection, 1727
in BK virus infection, 1868-1869
in *Bordetella* spp. infection, 1628, 3593
in *Burkholderia cepacia* infection, 1597
in *Campylobacter jejuni* infection, 1654-1655
in *Candida* spp. infection, 2757
in cervical lymphadenitis, 184
in *Chlamydia pneumoniae* infection, 2643-2644
in *Chlamydia psittaci* infection, 2639
in *Clostridium difficile* infection, 3594
in CMV infection, 1982
in Colorado tick fever virus infection, 2174-2175
in congenital rubella, 2219
in coronavirus infection, 2494
in *Corynebacterium diphtheriae* infection, 1307
in *Cryptosporidium* spp. infection, 2902
in Ebola virus infection, 2484
in EBV infection, 2010-2011
in *Ehrlichia* spp. infection, 2662-2663
in encephalitis, 507-508
in *Enterobacter* spp. infection, 1436-1437
in enterovirus infection, 2102
in *Fasciola* spp. infection, 3053
in *Francisella tularensis* infection, 1663
in fungal infection, 926
in gastroenteritis, 619, 620f
in *Giardia lamblia* infection, 2882
in *Haemophilus ducreyi* infection, 1688
in HAV infection, 2130, 2139
in HCV infection, 2321-2323
in HEV infection, 2165
in HHV-6 infection, 2019
in HIV infection, 2609
in hMPV infection, 2440
in HSV encephalitis, 1948
in HSV infection, 1935, 1956-1957
in human bocavirus infection, 1861
in influenza virus infection, 981, 2345-2346
in JC virus infection, 1869
in *Kingella kingae* infection, 1703
in *Klebsiella granulomatis* (*Calymmatobacterium granulomatis*) infection, 1642
in LCMV infection, 2465
in *Legionella pneumophila* infection, 1711
in *Leptospira* spp. infection, 1755-1756
in lung transplant–related *Aspergillus* infection, 967
in malaria, 2935
in microsporidial infection, 2913
in mycobacterial infection, 1383
in *Mycobacterium leprae* infection, 1405
in *Mycobacterium ulcerans* infection, 1415
in *Mycoplasma pneumoniae* infection, 2686
Polymerase chain reaction (PCR) *(Continued)*
in myocarditis, 382, 393-394
in *Naegleria fowleri* infection, 2983
in *Neisseria meningitidis* infection, 1263
in neonatal viral infection, 880
in *Norovirus* infection, 2153
in parainfluenza virus infection, 2368-2369
in parapneumonic pleural effusion, 313
in pneumococcal disease, 1217-1218
in *Pneumocystis* infection, 3010
in *Pseudomonas aeruginosa* infection, 1597
in rhinovirus infection, 2125
in Rift Valley fever, 2530
in rotavirus infection, 2182-2183
in RSV infection, 2425
in rubella virus infection, 2218
in *Shigella* spp. infection, 1482-1483
in smallpox, 2036
in *Streptobacillus moniliformis* infection, 1718
in *Streptococcus agalactiae* infection, 1162
in syphilis, 1774
in *Taenia saginata* infection, 3031-3032
in Toscana virus infection, 2550
in *Toxoplasma gondii* infection, 2996-2997
in *Trichomonas vaginalis* infection, 2892
in *Trypanosoma brucei* infection, 2971
in *Trypanosoma cruzi* infection, 2966
in tuberculosis, 1365-1366
in urethritis, 533
in *Vibrio vulnificus* infection, 1561
in viral infection, 3616-3617, 3616t
in viral meningitis, 444, 490
in VZV infection, 2028
in West Nile virus infection, 2270
in Whipple disease, 645
in yellow fever, 2277
in *Yersinia enterocolitica* infection, 1530
in *Yersinia pseudotuberculosis* infection, 1530
Polymethyl methacrylate (PMMA)
intraocular lenses, infection with, 1019
Polymorphonuclear leukocytes (PMNs), 31-35. *See also* Neutrophil(s)
defects in, 52-59
intrinsic, 52-55
secondary, 55-56
granules of, 34t
impaired adhesion of, 45
microbicidal activity of, 33-35, 34f, 34t
neonatal, 45
recruitment of, 31-32, 32f-33f
Polymyositis, HTLV-1, 2576
Polyomavirus infection, **1863-1870.** *See also* BK virus infection; JC virus infection; Simian virus 40 (SV40)
clinical manifestations of, 1866-1869
CNS, **1866-1867**, 1866f
cutaneous, 1864, 1864t, 1867-1868
diagnosis of, 1868-1869
historical perspective on, 1863-1864
malignancy and, 1868
pulmonary, 1868
retinal, 1868
treatment of, 1869
urinary tract, 1867, 1867f
Polyradiculopathy, CMV, 1979-1980
Polysaccharide intercellular adhesin (PIA), 1132-1133
Pontiac fever, **1714**
outbreaks of, 1708
pathogenesis of, 1709-1710
Population. *See also* Epidemiology
disease in, 111-120
household unit of, 111
Por1 protein, 18
Porcine circovirus, 2191

Porins
Neisseria gonorrhoeae, 1278
Neisseria meningitidis, 1254-1255
Pork, *Yersinia enterocolitica* contamination of, 1517-1518
Porphyromonas spp., 1826
Porphyromonas spp. infection, **1825-1834**
bloodstream, 1827-1828
bone, 1829-1830
CNS, 1828-1829
cutaneous, 1829
diagnosis of, 1830
epidemiology of, 1826-1827
head and neck, 1828
intra-abdominal, 1829
joint, 1829-1830
pathogenesis of, 1827
pleuropulmonary, 1830
prevention of, 1833
soft tissue, 1829
treatment of, 1831-1833, 1831t
Portosystemic shunting, in Caroli disease, 667
Posaconazole, 3301f, 3308-3310
adverse effects of, 3308
in *Aspergillus* spp. infection, 2718
in *Blastomyces dermatitidis* infection, 2733-2734
dosing for, 3309-3310
drug interactions with, 3309
efficacy of, 3309
in *Histoplasma capsulatum* infection, 2826
pharmacodynamics of, 3308
pharmacokinetics of, 3308, 3308t
in transplant-related aspergillosis, 937-938, 968
Positive end-expiratory pressure (PEEP), in ARDS, 871
Post-transplant lymphoproliferative disease (PTLD), 654, 976-978, 997-998, 1008
diagnosis of, 976-977
EBV and, 1996, 2005-2006, 2012
after heart transplantation, 952-953
management of, 998, 1009, 2012-2013, 3521-3522
prevention of, 1001, 2015
Postauricular lymphadenopathy, vs. mastoiditis, 237
Postexposure prophylaxis, 3248-3251, 3540t-3544t, 3546
in anthrax exposure, 3540t-3544t, 3582
in *Haemophilus influenzae* type b exposure, 3249-3250, 3540t-3544t
in HAV exposure, 2142, 2144, 3436-3437, 3436t, 3540t-3544t
in HBV exposure, 3517-3519, 3518t, 3540t-3544t
in HIV exposure, 2594
in *Neisseria meningitidis* exposure, 3249, 3455, 3540t-3544t
in pertussis exposure, 3249, 3540t-3544t
in rabies exposure, 2453-2458, 2456t-2457t, 3481-3483, 3482t, 3527-3529, 3540t-3544t
in tetanus-related wound management, 3471, 3472t
in tuberculosis exposure, 3250-3251, 3540t-3544t
in VZV exposure, 3475, 3540t-3544t
Postherpetic encephalomyelitis, 1948
Postherpetic neuralgia, 2027-2028
Postinfectious encephalitis, 493, **503**
imaging in, 507
pathogenesis of, 504
pathology of, 504
Postinfectious encephalomyelitis. *See* Acute disseminated encephalomyelitis (ADEM)
Post–kala-azar dermal leishmaniasis, 2956
Postpolio syndrome, 2104-2105
Posttransplantation acute limbic encephalitis (PALE), 2018
Postural orthostatic tachycardia syndrome (POTS), 1088-1089
Potassium, fecal, 76
Potassium hydroxide examination
in tinea nigra, 790, 790f
in white piedra, 791
Pott abscess, 1355
Povidone-iodine
in burn wound infection, 1059
in neonatal conjunctivitis prevention, 805-806, 1281
Powassan virus infection, 494t-498t, **2313-2314**
Power (statistical), 121
Poxviruses, 749, 2040, 2041t. *See also* Molluscum contagiosum; Monkeypox; Smallpox
identification of, 2047
Praziquantel, 3350
Prebiotics, 3369-3370
safety of, 3372
Prednisolone, in PFAPA syndrome, 164
Pregnancy. *See also* Newborn
antiparasitic agents in, 3351, 3351t-3353t
bacteruria in, 1154
Blastomyces dermatitidis infection during, 2730-2731, 2733
Brucella spp. infection during, 1612
CMV infection during, 1970, 1972, 1983, 1986, 1988
prevention of, 1989
Coccidioides spp. infection during, 2772, 2774
Colorado tick fever virus infection during, 2174
Corynebacterium diphtheriae infection during, 1306
Coxiella burnetii infection during, 2664
Eastern equine encephalitis virus infection during, 2228
EBV infection during, 1998
Edwardsiella tarda infection during, 1533
Eikenella corrodens infection during, 1577
gonococcal infection during, 1297
HAV infection during, 2130, 2140
HBV infection during, 1917, 1917t
HEV infection during, 2163-2164
HHV-6 infection during, 2018
HSV infection during, 653-654, 1936-1937, 1965, 1967. *See also* Herpes simplex virus (HSV) infection, neonatal
human erythrovirus B19 (human parvovirus B19) infection during, 1852-1853, 1856
inadvertent rubella virus immunization during, 2223-2224
inadvertent smallpox immunization during, 3533
influenza virus infection during, 2345, 2356-2357
Japanese encephalitis virus infection during, 2260, 2297
Lyme disease during, 1734
malaria during, 2929-2930
mumps during, 2402-2403, 2405
Neisseria gonorrhoeae infection during, 1280, 1280t, 1299
Pregnancy *(Continued)*
Pasteurella multocida infection during, 1547
rubella virus infection during, 2203, 2206-2207, 2219-2220. *See also* Rubella virus infection, congenital
Salmonella ser. Typhi infection during, 1502
Salmonella spp. infection during, 608
Streptococcus agalactiae infection during, 1154-1155, 1157-1158
syphilis during, 1769, 1778
Toxoplasma gondii infection during, 2990, 2992-2993, 2992t, 2997, 3002, 3004, 3004t
tuberculosis during, 1360
Ureaplasma spp. infection during, 2689
VZV infection during, 2026-2027
West Nile virus infection during, 2264
Western equine encephalitis virus infection during, 2234
Premature infant. *See also* Newborn
bacteremia in, 1134-1135, 1135t
enterovirus infection and, 2100
immunization in, 44
indigenous flora acquisition by, 89, 93
IVIG for, 3500-3501
maternal poliomyelitis and, 2100
sinopulmonary infection in, 268
Preseptal (periorbital) cellulitis, 151, 798-801
nontraumatic, 798-799
posttraumatic, 798
Presumed ocular histoplasmosis syndrome, 2816-2817
Prevotella spp., 1826
Prevotella spp. infection, **1825-1834**
bloodstream, 1827-1828
bone, 1829-1830
clinical manifestations of, 1827-1830
CNS, 1828-1829
cutaneous, 1829
diagnosis of, 1830
epidemiology of, 1826-1827
head and neck, 1828
intra-abdominal, 1829
joint, 1829-1830
pathogenesis of, 1827
pleuropulmonary, 1830
prevention of, 1833
soft tissue, 1829
treatment of, 1831-1833, 1831t
Primaquine, 3350
in malaria, 2937t-2940t, 2941-2942
in malaria prevention, 2945
Primary ciliary dyskinesia. *See* Immotile cilia syndrome; Kartagener syndrome
Primary sclerosing cholangitis, 662-663
Prion diseases. *See* Transmissible spongiform encephalopathies
PRISM score, 458
Probiotics, **3369-3373**
in allergic disease, 3371
in antibiotic-associated diarrhea, 3370
in atopic disease, 3371
in *Clostridium difficile* infection, 637
in cold prevention, 139
in constipation, 3371
definition of, 3369-3370, 3370t
in gastrointestinal tract infection, 626
in *Helicobacter pylori* infection, 3370
in hepatic encephalopathy, 3371
for HIV-infected host, 3372
in infant colic, 3371
in infectious diarrhea, 3370, 3371t

Probiotics *(Continued)*
in inflammatory bowel disease, 3370-3371
in irritable bowel syndrome, 3371
in necrotizing enterocolitis, 3371-3372
in neonatal gastroenteritis, 888
in neonatal sepsis, 3371-3372
in respiratory tract infections, 3371
in rotavirus infection, 2186
safety of, 3372
uses of, 3370-3372, 3371t
Procalcitonin, 82
in bacteremia, 832
in burn wound infection, 1055
in meningitis, 444
in pancreatitis, 690
in septic shock, 830, 832
in urinary tract infection, 543
Proctitis, HSV, 1948-1949, 1952-1953, 1953f
Progressive multifocal leukoencephalopathy (PML), 1010, **1866-1867**, 1866f
diagnosis of, 1869
historical perspective on, 1863
treatment of, 1869
Progressive outer retinal necrosis, 810
Proguanil, 3350
in malaria, 2945
Project Bioshield Act, 2036-2037
Prone positioning, in ARDS, 873
Properdin, deficiency of, 51-52
Neisseria meningitidis infection and, 1257
Propionibacterium propionica infection (actinomycosis), **1820-1824**
clinical manifestations of, 1821-1823
diagnosis of, 1823-1824
pathogenesis of, 1820-1821
pathology of, 1821
treatment of, 1824
Prostatitis, **558-560**
chronic, 559
organisms in, 558
treatment of, 558-559
Prosthetic heart valves, infective endocarditis with, 351, 357, 365-366, 366t, 1137
Prosthetic/implantable device–related infection, **1015-1043**, 1016t
cardioverter-defibrillator, 1025-1026
coagulase-negative staphylococci in, **1136-1137**
cochlear implant, 1017-1018
contact lens, 1019
CSF shunt, **1029-1038**, 1030t, 1033t, 1035f
extracorporeal membrane oxygenation circuit, 1022-1023
host factors in, 1016
intracranial pressure monitor, 1038-1040, 1039t
intraocular lens, 1019
joint prosthesis, **1026-1029**
left ventricular assist device, 1020-1022, 1020f
microbial factors in, 1016-1017
orbital implant, 1018-1019
pacemaker, 1023-1025
tissue expander, 1017
Protease, in *Pseudomonas aeruginosa* infection, 1587
Protease inhibitors, 2614-2615
Protein, CSF, in neonatal meningitis, 885
Protein A, of *Staphylococcus aureus*, 1114
Protein C
recombinant, in *Neisseria meningitidis* infection, 1265, 1265t
in septic shock, 829
Protein-calorie malnutrition, PMN chemotaxis in, 56
Protein F, 10
Protein-losing diseases, IVIG in, 3499
Proteus mirabilis infection
meningeal, 1472-1474
urinary tract, 1472-1474
Proteus morganii. *See Morganella morganii* infection
Proteus spp., 1471
Proteus spp. infection, **1471-1474**
clinical manifestations of, 1473
CSF shunt, 1030, 1030t
diagnosis of, 1473
epidemiology of, 1472
pathophysiology of, 1472-1473
treatment of, 1473-1474
Protozoan disease. *See also specific protozoan diseases*
appendiceal, 684-685
classification and nomenclature in, 2862-2864, 2862t
corneal, 808-809
cutaneous manifestations of, 757t-758t, 761t, 763t
encephalitic, 494t-498t, 501
after hematopoietic stem cell transplantation, 943-944
host response to, 23-24
uveal, 814
Providencia alcalifaciens infection, 1475
Providencia rettgeri infection, 1475
Providencia spp., 1474
Providencia spp. infection, **1474-1476**
Providencia stuartii infection, 1475
Pseudoappendicitis
in *Yersinia enterocolitica* infection, 1527
in *Yersinia pseudotuberculosis* infection, 1529
Pseudocowpox virus infection, 2046
Pseudocyst
pancreatic, 696
peritoneal, 701
Pseudohypoparathyroidism, HTLV-1 virus and, 2571
Pseudoinfection, *Bacillus cereus*, 1320
Pseudomonas aeruginosa
endotoxins of, 1587
exotoxins of, 1587
siderophores of, 1588
type III secretion system of, 1587
typing of, 1585
virulence factors of, 1586-1587
Pseudomonas aeruginosa infection, **1582-1605**
antibiotic resistance in, 1601-1603, 3149
biofilm formation in, 11, 14f, 1588
bloodstream, 1594-1595
bone, 1591, 1599
burn wound, 1052-1053, 1057f
clinical manifestations of, 1589-1596, 1590f
cutaneous manifestations of, 1589-1591, 1590f
in cystic fibrosis, 1593-1594. *See also* Cystic fibrosis, *Pseudomonas aeruginosa* infection in
diagnosis of, 1582-1583, 1596-1597
endocardial, 1595, 1597-1598
epidemiology of, 1585
exanthem in, 753t-756t, 1589-1591, 1590f
hair follicle, 776-777
joint, 730, 1591-1592
after lung transplantation, 960-962
meningeal, 1598-1599
neonatal, 1592
nosocomial, 1594
Pseudomonas aeruginosa infection *(Continued)*
ocular, 1591, 1598-1599
in osteochondritis, 724-725
in otitis externa, 1591, 1599
in otitis media, 1591, 1599
pathogenesis of, 1586-1589
pericardial, 373
peritoneal, 700
pili in, 5
prevention of, 1603-1604
in previously healthy children, 1589-1593
prognosis for, 1604
pulmonary, 1599
skin lesions in, 1589-1591, 1590f, 1596
treatment of, 1597-1603, 1598t
antibiotic resistance in, 1601-1603, 3149
susceptibility testing in, 1601-1603
urinary tract, 1591, 1599
vaccine against, 1604
Pseudomonas alcaligenes, 1583
Pseudomonas fluorescens infection, 1585, 1595
Pseudomonas luteola, 1583
Pseudomonas mendocina infection, 1595
Pseudomonas oryzihabitans, 1583
Pseudomonas pseudoalcaligenes infection, 1583, 1585
Pseudomonas putida infection, 1595
Pseudomonas spp., 1582-1584, 1583t
Pseudomonas spp. infection, **1582-1605.** *See also specific species infections*
antibiotic resistance in, 1601-1603
burn wound, 1593
clinical manifestations of, 1589-1596, 1590f
diagnosis of, 1596-1597
endocardial, 362, 1595, 1597-1598
epidemiology of, 1585-1586
in immunosuppressed patient, 1594-1596
malignancy-related, 1594-1596
in otitis externa, 205
pathogenesis of, 1586-1589
prevention of, 1603-1604
in previously healthy children, 1589-1593, 1590f
prognosis for, 1604
retroperitoneal, 706-709
treatment of, 1597-1603, 1598t
Pseudomonas stutzeri infection, 1583, 1595
Pseudoparalysis of Parrot, 1769-1770
Pseudorubella. *See* Roseola infantum
Psittacosis. *See Chlamydia psittaci* infection (psittacosis)
Psychological disorders/factors
in chronic fatigue syndrome, 1089
in common cold, 2120-2121
in congenital rubella, 2217
in EBV infection, 2004
in genital HSV infection, 1943
in HIV infection, 2605
Pterygomandibular space, infection of, 148-149, 149f
Public health, 3082t, **3538-3549**
consultation function of, 3545
definition of, 3538, 3539f
disease exposure analysis in, 3547-3548
disease spread analysis in, 3546-3547
disease transmission settings analysis in, 3548
disease vector analysis in, 3549
in international travel, 3548-3549
outbreak investigation function of, 3545-3546
prevention and control function of, 3546
surveillance function of, 3538-3545

Publication bias, 124-125
Puffer fish poisoning, 604, 605t
Pulmonary artery stenosis, in congenital rubella, 2214t-2216t, 2217
Pulmonary capillaritis, 297
Pulmonary edema
  in epiglottitis-related intubation, 258
  in malaria, 2928
Pulmonary function tests
  in bronchiolitis obliterans, 295
  in hypersensitivity pneumonitis, 303-304
  in interstitial lung disease, 298, 298t
Pulse oximetry
  in bronchiolitis, 275
  in community-acquired pneumonia, 288
Puncture wound, osteomyelitis with, 724-725
Pupil
  Argyll Robertson, 812
  constrictor response of, 842
Purple urine bag syndrome, 1475
Purpura, 762-763, 763t
  in meningococcal infection, 1258-1259, 1259f
  in septic shock, 831
Purpura fulminans
  in meningococcal infection, 1259
  in *Streptococcus pneumoniae* sepsis, 1228, 1228f
Pyelonephritis. *See also* Urinary tract infection (UTI)
  acute, 538, 544, 545f
    treatment of, 547-548, 547t
  Candida, 2748-2749
  xanthogranulomatous, 539-540, 554
Pyemotes herfsi infection, 3073
Pyomyositis, **735-738**
  clinical presentation of, 736-737
  diagnosis of, 737, 737f-738f
  pathophysiology of, 735-736, 736t
  Stenotrophomonas maltophilia, 1607
  treatment of, 737-738
Pyopneumothorax, in neonate, 892
Pyothorax, *Arcanobacterium haemolyticum*, 1325
Pyrantel pamoate, 3350
  in *Enterobius vermicularis* infection, 567, 3019
Pyrazinamide, in tuberculosis, 1367t, 1368
Pyrenochaeta romeroi infection, after lung transplantation, 974
Pyrimethamine, 3350
  in heart transplant–related toxoplasmosis, 953
  in *Toxoplasma gondii* infection, 3000, 3001t
Pyrimethamine-sulfadoxine, in malaria, 2942, 2945
Pyrogens, 84. *See also* Fever
Pyruvate oxidase, of *Streptococcus pneumoniae*, 1214
Pyuria
  in gonococcal urethritis, 1287
  in Kawasaki disease, 1074, 1076
  in urinary tract infection, 542-543

Q

Q fever. *See Coxiella burnetii* infection (Q fever)
Quarantine, 3546
Quinacrine, 3350
Quinidine, in malaria, 2937t-2940t, 2941
Quinine, 3350
  in malaria, 2937t-2940t, 2941
Quinolones, 1600
  in heart transplant–related *Legionella pneumophila* infection, 949
  resistance to, 3154-3157
    mechanisms of, 3155-3157
Quinsy, **168-170**, 168f
Quinupristin-dalfopristin, 3231t-3233t, 3238
  in coagulase-negative staphylococcal infection, 1139
  in methicillin-resistant *Staphylococcus aureus* infection, 1127
  in vancomycin-resistant *Enterococcus* spp. infection, 1184

R

Rabbit hemorrhagic disease virus, 2148, **2154-2155**
Rabbit infectivity test, 1772
Rabies, **2444-2462**
  in animals, 2449-2450
  clinical manifestations of, 494t-498t, 502, 504-505, 2450-2452, 2451t-2452t
  diagnosis of, 509, 2452-2453, 2453t
  differential diagnosis of, 2452
  epidemiology of, 2448-2449, 2448t
  furious, 2450
  historical perspective on, 2444-2445
  immunization against, 2458-2460, 2459t-2460t, 3480-3483
    acute disseminated encephalomyelitis with, 516
  in animal bite, 781
  meningitis and, 488
  after lung transplantation, 983
  pathogenesis of, 2448-2449
  postexposure prophylaxis for, 2453-2458, 2456t, 3481-3483, 3482t, 3527-3529
    cell culture vaccine in, 2455-2457
    duck embryo vaccine in, 2455
    indications for, 2457-2458, 2457t
    nerve tissue vaccine in, 2454-2455
  transmission of, 2447-2448
Rabies virus, 2445-2447, 2445f-2446f
Rac protein, 14
Race
  in epidemiologic study, 108, 113-114
  HAV infection and, 2133, 2133f
  Kawasaki disease and, 1066
  Paracoccidioides spp. infection and, 2782
  Streptococcus pneumoniae infection and, 1203
Racecadotril (acetorphan)
  in gastrointestinal tract infection, 626
  in rotavirus infection, 2186-2187
Radiation (heat transfer), 84
Radiation therapy, acute transverse myelitis after, 524
Radiography. *See also* Chest radiography
  in appendicitis, 681-682
  in cervical lymphadenitis, 184
  in *Clostridium difficile* infection, 636
  in croup syndromes, 250t-251t
  in diskitis, 720, 721f
  in epiglottitis, 254, 254f
  in esophageal perforation, 420
  in Garré osteomyelitis, 154, 154f-155f
  in idiopathic spontaneous focal intestinal perforation, 895-896
  in immunodeficiency evaluation, 62
  in infective endocarditis, 359
  in intra-abdominal abscess, 705
  in *Kingella kingae* infection, 1703
  in *Legionella pneumophila* infection, 1710
  in *Leptospira* spp. infection, 1751f
Radiography *(Continued)*
  in leukocyte adhesion deficiency, 905f
  in mandibular osteomyelitis, 153, 153f
  in mediastinitis, 420
  in *Mycobacterium ulcerans* infection, 1414f
  in *Mycoplasma pneumoniae* infection, 2677-2678
  in myocarditis, 389, 389f
  in neonatal bone infection, 889
  in neonatal joint infection, 889
  in neonatal pneumonia, 891
  in *Nocardia* spp. infection, 1421
  in osteomyelitis, 713, 715-717, 715f
  in otitis media, 220
  in pancreatitis, 690
  in *Paracoccidioides* spp. infection, 2786-2787
  in *Paragonimus* spp. infection, 3055
  in pericarditis, 375, 376f
  in peritonitis, 702
  in pneumococcal pneumonia, 1230, 1230f
  in prosthetic joint infection, 1027-1028
  in pyogenic liver abscess, 674
  in pyomyositis, 737
  in retropharyngeal abscess, 171
  in rheumatic fever, 411
  in rhinosinusitis, 198t, 199
  in septic arthritis, 730, 731f
  in septic shock, 832
  in *Streptococcus agalactiae* infection, 1160, 1161f
  in tuberculosis, 1345, 1346f, 1348f-1350f, 1352f
  in vertebral osteomyelitis, 722, 722f
  in Whipple disease, 645
  in *Yersinia enterocolitica* infection, 1527
Radionuclide imaging
  in bacterial meningitis, 445
  in osteomyelitis, 716-717, 716f
  in prosthetic joint infection, 1027-1028
  in pyomyositis, 737, 737f
  in urinary tract infection, 544-545, 545f
  in vertebral osteomyelitis, 722
Ralstonia pickettii infection, 1595
Ralstonia spp., 1584
Ralstonia spp. infection, 1595
Raltegravir, 2616
Ramichloridium mackenziei infection, 2856, 2856f
Ramsay Hunt syndrome, 208
Rapid antigen detection test
  in meningitis, 442
  in streptococcal pharyngitis, 1147
Rapid enzyme immunoassay, in *Corynebacterium diphtheriae* infection, 1307
Rapid immunochromatographic assay, in HEV infection, 2165
Rapid influenza diagnostic test (RIDT), 2345-2346
Rapid plasma reagin (RPR) test, 1772-1773, 1773t
Rash. *See* Exanthem *and at specific infections*
Rat bite, septic arthritis with, 730
Rat-bite fever
  vs. Rocky Mountain spotted fever, 765
  Spirillum minus, 753t-756t, **1759-1760**
  Streptobacillus moniliformis, **1715-1720**
    clinical manifestations of, 1717-1718, 1717f
    diagnosis of, 1718-1719
    differential diagnosis of, 1719
    epidemiology of, 1715-1716
    exanthem in, 753t-756t, 765, 1717, 1717f

Rat-bite fever *(Continued)*
historical perspective on, 1715
joint, 730
pathophysiology of, 1718
prevention of, 1719
treatment of, 1719
Rat tapeworm *(Hymenolepis diminuta)* infection, **3042**
Raxibacumab, 3503-3504
Reactive arthritis, 734
Reactive oxygen species, in meningitis, 435
Recalcitrant erythematous desquamating disorder, 860
Receptor(s)
chemokine, in HIV infection, 10-11
intimin, 7-8, 9f
microbial tropism for, 10-11
pattern recognition, 433, 434f, 436
Toll-like, 25, 25t, 3364-3365
in influenza virus infection, 2338
in *Leptospira* spp. infection, 1747-1748
Mycobacterium leprae infection and, 1395-1396
in myocarditis, 386
in parainfluenza virus infection, 2365
in rhinovirus infection, 2118
in RSV infection, 2415
Rectum
examination of
in appendicitis, 681
in genital tract infection, 561
temperature measurement by, 86
Recurrent aphthous stomatitis, 159t-160t, 161
Recurrent respiratory papillomatosis, 1878-1879, 1878f-1879f
retinoic acid in, 1883-1884
Red blood cells, CSF, 442
Red diaper syndrome, 1490
Red eye. *See* Conjunctivitis
Reduction modifiable protein, of *Neisseria gonorrhoeae*, 1279
Reduviid bugs, in trypanosomiasis, 2959-2961
Reed-Sternberg cells, 1997
Refugees. *See* International adoptees and refugees
Rehydration therapy. *See* Oral rehydration therapy
Reiter syndrome, 531
Relapsing fever (*Borrelia* spp. infection), **1739-1742**
clinical manifestations of, 753t-756t, 1741-1742, 1741t
diagnosis of, 1742
differential diagnosis of, 1742
epidemiology of, 1730t, 1740
pathogenesis of, 1740-1741
transmission of, 1740
treatment of, 1742
Renal abscess, **553-558**
bacteremia and, 553
clinical findings in, 554
diagnosis of, 554-556, 555f-557f
drainage of, 557
fungal, 554, 2757
organisms in, 553-554
treatment of, 556-557
tuberculous, 553
Reovirus, 2168-2169
anti-cancer activity of, 2171
Reovirus infection, **2168-2172**
biliary atresia and, 2170-2171
clinical manifestations of, 2169-2171
CNS, 2171
Reovirus infection *(Continued)*
in common cold, 132
diagnosis of, 2171-2172
epidemiology of, 2169, 2169t
exanthem in, 742t-748t, 749, 2171
gastrointestinal manifestations of, 2170-2171
historical perspective on, 2168
lymphoma and, 2171
pulmonary, 2170
upper respiratory tract, 2169-2170
Reptile bite, 3578
Reservoirs, of infectious agents, 106-107
Respiratory distress. *See also* Acute respiratory distress syndrome (ARDS)
in community-acquired pneumonia, 287, 287t
in congenital rubella, 2220
in septic shock, 830, 832, 835
Respiratory failure, in influenza virus infection, 2352
Respiratory rate
in bronchiolitis, 275
in community-acquired pneumonia, 287-288
Respiratory syncytial virus (RSV), 2408-2410
animal susceptibility to, 2409-2410
antigenic properties of, 2408-2409
classification of, 2408
isolation of, 2409, 2424
structure of, 2408-2409, 2409f
Respiratory syncytial virus (RSV) infection, **2407-2434**
in acute bronchitis, 262, 263t
in adults, 2422-2423, 2422f-2423f
antibody response to, 277
in ARDS, 867-868
asthma and, 2421
breast-feeding and, 2427
in bronchiolitis, 272t-273t, 273-274. *See also* Bronchiolitis
in burn patient, 1054
clinical manifestations of, 2416-2419, 2417f-2418f, 2417t
coinfection with, 2419
in common cold, 132
complications of, 2419
diagnosis of, 278, 2424-2425, 2424f, 3611, 3611t
epidemiology of, 2410-2413, 2410t, 2411f
exanthem in, 742t-748t, 749, 760-762
geographic distribution of, 2410
after heart transplantation, 949-950
after hematopoietic stem cell transplantation, 942-943
historical perspective on, 2408
in HIV-infected host, 2421
in hospitalized children, 2122, 2122t
immune response to, 2414-2416, 2414f
in immunocompromised host, 2420-2421
after intestinal transplantation, 997t, 999
after kidney transplantation, 1007
after liver transplantation, 997t, 999
after lung transplantation, 979-980
mortality with, 2412
neonatal, 2411-2412, 2413t, 2418-2419
nosocomial, 2427-2428, 2428t, 3379
pathogenesis of, 2413-2414
pathology of, 2413-2414, 2413f
prevention of, 280-281, 2427-2431
prognosis for, 2419-2423
prophylactic treatment for, 2428-2431, 3529-3531
pulmonary, 284, 290
Respiratory syncytial virus (RSV) infection *(Continued)*
pulmonary sequelae of, 2421
radiography in, 2417f, 2418
reactive airways disease in, 2419
recurrent, 2421-2423, 2422f
seasonal patterns of, 2410-2411, 2410t, 2411f
severe/fatal, 2420
strain variation in, 2411
transmission of, 2412-2413, 2413t
treatment of, 278-280, 2425-2427
palivizumab in, 2426-2427, 2429, 3503, 3529-3530
prophylactic, 2428-2431, 3529-3531
ribavirin in, 2426, 3274-3276
vaccine against, 277, 280, 2430-2431
Respiratory tract
indigenous flora of, 91t, 92
lower, infection of. *See* Bronchitis; Influenza virus infection; Pneumonia
upper, infection of. *See* Common cold; Epiglottitis (supraglottitis); Laryngitis; Laryngotracheitis; Rhinosinusitis; Tracheitis
Retina
BKV-associated necrosis of, 1868
cotton-wool spots of, 811
in giardiasis, 814
histo spots of, 813
HSV-related necrosis of, 1945
infection of, 816-820
CMV, 811, 818, 2601
in HIV infection, 811
HSV, 818-819
LCMV, 817
rubella virus, 817-818
Toxocara canis, 814
Toxoplasma gondii, 816-817
Treponema pallidum, 819-820
VZV, 810, 819
Retinal necrosis syndrome, 810, 812
Retinitis, 809
CMV, 1973
congenital, 818
in HIV infection, 811, 2601
herpes B virus, 812
HSV, 1945
congenital, 818-819
influenza A virus, 812
mumps virus, 811
necrotizing, 810, 812
in toxoplasmosis, 816-817
Onchocerca, 815
Rift Valley fever virus, 812
rubella virus, 811
in subacute sclerosing panencephalitis, 811
in trench fever, 815
VZV, 810
Retinochoroiditis, *Toxoplasma*, 817
Retinoic acid, in recurrent respiratory papillomatosis, 1883-1884
Retinopathy
in congenital rubella, 2213, 2214t-2216t, 2220-2221
pigmentary
in congenital rubella, 817
in congenital syphilis, 819
Retinopathy of prematurity, 2756
Retroperitoneal infection, **706-709**
anatomy of, 706, 707f
clinical manifestations of, 708
complications of, 706
diagnosis of, 708
differential diagnosis of, 708

Retroperitoneal infection *(Continued)*
drainage of, 708
microbiology of, 706-708
pathogenesis of, 706
prognosis for, 709
secondary, 706
treatment of, 708-709
Retropharyngeal abscess, **170-172**, 170f
clinical manifestations of, 171, 171f
microbiology of, 173-175
treatment of, 171-172
Retroviruses, **2552-2619.** *See also* Human immunodeficiency virus type 1 (HIV-1); Human immunodeficiency virus type 2 (HIV-2); Human T-cell lymphotropic virus type 1 (HTLV-1) infection; Human T-cell lymphotropic virus type 2 (HTLV-2) infection
accessory genes of, 2554-2556
classification of, 2552-2553
genome of, 2553-2556, 2554f
life cycle of, 2556-2559, 2557f
regulatory genes of, 2554-2556
replication of, 2556-2559
structure of, 2553-2556, 2553f
testing for, 2559-2560
Reversal reaction, in *Mycobacterium leprae* infection, 1400f, 1404, 1404f, 1408-1409
Reye syndrome, 86-87, **676-677**, 2351
clinical manifestations of, 676-677
diagnosis of, 676-677
epidemiology of, 676
hepatitis in, 657
vs. influenza-associated encephalopathy, 2340
pancreatitis with, 692
prevention of, 677
vs. St. Louis encephalitis virus infection, 2259
treatment of, 677
Rhabdomyolysis, in *Mycoplasma pneumoniae* infection, 2683
Rhabdovirus infection, 500-501
Rhagades, in congenital syphilis, 1770
Rheumatic fever, **401-418**, 1143. *See also* Pharyngitis, streptococcal
ASO titer in, 411
carditis in, 409, 415
clinical manifestations of, 404t, 408-410, 409t, 410f
coxsackievirus infection and, 2077
cutaneous manifestations of, 355, 357
in developing countries, 407-408, 407f
diagnosis of, 412-413
differential diagnosis of, 412-413
epidemiology of, 401-405, 402f-403f, 403t-404t
genetic factors in, 406
immunization against, 406-407, 407f
joint fluid findings in, 732t
Jones criteria for, 409, 409t, 411-412
laboratory findings in, 410-412
pathogenesis of, 405-406, 405t
pathology of, 408
prevention of, 415-418, 1147-1148, 1148t, 1150, 3244-3246, 3245t, 3540t-3544t
prognosis for, 415
recurrence of, 414f, 414t, 417
surgery in, 414-415
treatment of, 413-414, 414f, 414t
Rheumatoid arthritis. *See also* Juvenile idiopathic (rheumatoid) arthritis
human parvovirus B19 and, 1851
Proteus spp. in, 1473
Rheumatoid arthritis *(Continued)*
vs. rheumatic fever, 413
rubella virus and, 2211
septic arthritis with, 734
Rheumatoid factor, in infective endocarditis, 355, 358, 358t
Rhinitis, in congenital syphilis, 1770, 1772
Rhinopharyngitis mutilans, 1787
Rhinorrhea, in rhinosinusitis, 197-198
Rhinosinusitis, **193-203**
acute, 200
age-related symptoms of, 197
anatomy of, 193-194
Aspergillus spp., 2706, 2707f
in burn patient, 1055
chronic, 195, 198, 201-202
clinical presentation of, 197-198, 198t
complications of, 198
culture in, 199-200
diagnosis of, 199-200
differential diagnosis of, 199
epidemiology of, 197
etiology of, 195-197, 196t, 2124
Haemophilus influenzae, 1677
historical perspective on, 193
immunity in, 195
Moraxella catarrhalis, 1252
Mycoplasma pneumoniae, 2679
odontogenic, 150-151, 152f
orbital cellulitis and, 799
pathophysiology of, 194-195
prevention of, 202
prognosis for, 202
puncture and drainage in, 199-200
recurrent, 195, 201-202
rhinovirus, 2124
sphenoidal, 198
Streptococcus pneumoniae, 1231, 1233
subacute, 200
surgical treatment of, 201-202
treatment of, 200-202, 3508
Rhinovirus, 2110-2111, 2110f
in animals, 2111
antigenic properties of, 2111
host range of, 2111
life cycle of, 2110-2111
serotypes of, 2111-2112, 2112f
structure of, 2110
in tissue culture, 2111, 2112f
Rhinovirus infection, **2109-2127.** *See also* Common cold
age and, 2120, 2120f
in asthma, 2123
clinical manifestations of, 2121-2125, 2121t
diagnosis of, 2125, 2125t
environmental temperature and, 2120
epidemiology of, 2111-2113
exanthem in, 742t-748t, 749, 760-762
after hematopoietic stem cell transplantation, 943
historical perspective on, 2109-2110
in hospitalized children, 2122-2123, 2122t
immune response to, 2118-2121
in immunocompromised host, 2125
interleukin-1 in, 135
median infectious dose in, 2113
mortality with, 2121-2122
in otitis media, 2123-2124
pathogenesis of, 2118-2121
prevention of, 2125-2126
psychosocial factors and, 2120-2121
pulmonary, 284
replication of, 2111
resistance to, 2119-2120
Rhinovirus infection *(Continued)*
seasonal distribution of, 2112, 2113f
serotype circulation in, 2112
serotype predominance in, 2112-2113
in sinusitis, 2124
T cells in, 135
transmission of, 2114-2118, 2114f, 2116f, 2117t
treatment of, 2125-2126
Rhizomucor spp., 2837
Rhizomucor spp. infection. *See also Rhizopus* spp. infection (mucormycosis)
burn wound, 1053
after kidney transplantation, 1013
neonatal, 899
Rhizopus spp., 2837
Rhizopus spp. infection (mucormycosis), **2839-2844**
bone, 726
burn wound, 1053
clinical manifestations of, 2840-2842, 2840t-2841t
cutaneous, 2841, 2841f
diagnosis of, 2842-2843
disseminated, 2842
epidemiology of, 2837-2839
gastrointestinal, 2842
after hematopoietic stem cell transplantation, 938
after kidney transplantation, 1013
neonatal, 899
pathogenesis of, 2839-2840
pathology of, 2840, 2840f
prevention of, 2844
prognosis for, 2844
pulmonary, 2841-2842
rhinocerebral, 2840
treatment of, 2843-2844
Rhodococcus equi infection, **1426-1428**
clinical manifestations of, 1427
diagnosis of, 1427
epidemiology of, 1426
extrapulmonary, 1427
pathophysiology of, 1427
treatment of, 1427-1428
Rhodococcus ruber infection, 1426
Rhodococcus spp. infection, **1426-1428**
clinical manifestations of, 1427
diagnosis of, 1427
epidemiology of, 1426-1427
pathophysiology of, 1427
treatment of, 1427-1428
Rhodotorula spp. infection, 898, **2854-2855**
Ribavirin, 3268t, 3273, 3273f
in adenovirus infection, 1907
adverse effects of, 3275-3276
in bronchiolitis, 279-280
in community-acquired pneumonia, 290
in Crimean-Congo hemorrhagic fever, 2533-2534
dosage for, 3275
in hantavirus infection, 2510-2511
in HCV infection, 2323-2324
in heart transplant–related RSV infection, 950
in hematopoietic stem cell transplantation–related RSV infection, 942-943
in hepatitis E virus infection, 2166
in hMPV infection, 2440
indications for, 3274-3275
in La Crosse virus encephalitis, 2524-2525
in laryngotracheitis, 257
in LCMV infection, 2465
mechanism of action of, 3273-3274

Ribavirin *(Continued)*
pharmacokinetics of, 3274-3276
resistance to, 3273-3274
in RSV infection, 2426, 3274-3276
spectrum of activity of, 3273
in West Nile virus infection, 2272
Ribosomal protein L12, of *Neisseria gonorrhoeae*, 1279
Rickettsia africae infection, 751t-752t, 2657
Rickettsia akari infection (rickettsialpox), 751t-752t, 2648t-2649t, **2656-2657**
Rickettsia australis infection, 751t-752t, 2657
Rickettsia conorii infection (Mediterranean spotted fever), 751t-752t, 2648t-2649t, **2655-2656**
ocular, 815
Rickettsia felis infection, 2648t-2649t, 2657, **3078**
Rickettsia japonica infection, 2657
Rickettsia mooseri infection, 815
Rickettsia parkeri infection, 2657
Rickettsia prowazekii infection (epidemic typhus), 751t-752t, 2648t-2649t, **2657-2659**
Rickettsia rickettsii, 2648t-2649t, 2650-2651. *See also* Rocky Mountain spotted fever
Rickettsia sibirica infection, 751t-752t, 2657
Rickettsia slovaca infection, 2657
Rickettsia tsutsugamushi infection (scrub typhus), 751t-752t, 2648t-2649t, **2660-2661**
Rickettsia typhi infection (endemic typhus, murine typhus), 751t-752t, 2648t-2649t, **2659-2660**, **3078**
epidemiology of, 115-116, 116f
ocular, 815
Rickettsiae infection, **2647-2666**, 2648t-2649t. *See also specific rickettsial infections*
exanthem in, 750, 751t-752t, 760t-763t, 762
in hepatitis, 656-657
Rifabutin, 3224-3225
adverse effects of, 3225
indications for, 3225
in nontuberculous mycobacterial lymphadenitis, 187
Rifampin, 3224-3225, 3231t-3233t
adverse effects of, 3225
in bacterial meningitis, 449, 450t
in coagulase-negative staphylococcal infection, 1138-1139
in epiglottitis prevention, 259
indications for, 3224-3225
before lung transplantation, 963-964
in *Mycobacterium leprae* infection, 1406-1407, 1407t, 1409
in *Mycobacterium ulcerans* infection, 1416
in tuberculosis, 1367t, 1368
Rifamycins, 3224-3225
Rifaximin, 3224-3225
Rift Valley fever, **2528-2531**
clinical manifestations of, 2529-2530
diagnosis of, 2530
epidemiology of, 2529, 2529f
historical perspective on, 2528
ocular manifestations of, 812
prevention of, 2530
treatment of, 2530
Rift Valley fever virus, 2528-2529
Rimantadine, 3268-3270, 3268f, 3268t
in laryngotracheitis, 257
Ringworm, scalp (tinea capitis), **784-785**, 785f
Rio Bravo virus infection, 2316t
Ritonavir, 2614
Ritter disease, 753t-756t, 774, 775f
Rituximab
in hematopoietic stem cell transplantation–related EBV infection, 940-941
in post-transplant lymphoproliferative disease, 977-978, 2012-2013, 3521-3522
River blindness, 757t-758t, 809, 815, **3027-3028**
Rochalimaea quintana infection. *See Bartonella quintana* infection (trench fever)
Rocio encephalitis, **2314-2315**
Rocky Mountain spotted fever, 765, **2647-2654**, 2648t-2649t
vs. *Anaplasma* spp. infection, 2663
clinical manifestations of, 2652-2653
vs. Colorado tick fever virus infection, 2174
diagnosis of, 2653
differential diagnosis of, 2653-2654
vs. *Ehrlichia* spp. infection, 2663
encephalitic, 494t-498t, 501
epidemiology of, 2650-2651, 2650f
exanthem in, 751t-752t, 762, 2652-2653
historical perspective on, 2647-2650
immune response in, 2652
pathogenesis of, 2651-2652
prevention of, 2654
prognosis for, 2654
transmission of, 2651
treatment of, 2654
Romaña's sign, 2963
Rosacea, *Demodex* infection and, 795-796
Rose spots, 760, 1501
Rosenbach, erysipeloid of, 1327-1328
Roseola infantum, **768-771**
clinical presentation of, 742t-748t, 770-771, 2017, 2017t
complications of, 771
diagnosis of, 771
epidemiology of, 768-769
etiology of, 769-770
exanthem in, 742t-748t, 749, 765, 770
historical perspective on, 768
pathophysiology of, 770
treatment of, 771
white blood cell count in, 770-771
Roseola vaccinatum, 742t-748t
Ross River virus, 2249
Ross River virus arthritis, 2248-2251
clinical manifestations of, 2249
diagnosis of, 2250
epidemiology of, 2249
exanthem in, 742t-748t, 2249
pathogenesis of, 2250
pathology of, 2250
prevention of, 2251
transmission of, 2249
treatment of, 2250-2251
Rotavirus, 2177-2178
gel electrophoresis of, 2179, 2180f
genome of, 2179, 2180f
genotypes of, 2177-2178, 2179t
non–group A strains of, 2178
NSP4 toxin of, 2184
properties of, 2178-2182, 2179f-2180f
replication of, 2179-2180
Rotavirus infection, 602t-603t, 606-607, 607t, 608f, 616t, **2176-2195**
age and, 2182
breast-feeding and, 2182
clinical manifestations of, 2185-2186
CNS, 494t-498t, 2186
in daycare facilities, 3564
Rotavirus infection *(Continued)*
diagnosis of, 2182-2183, 3611-3612
diarrhea in, 2183-2185
epidemiology of, 2184-2185
exanthem in, 742t-748t, 749
historical perspective on, 2177
hospitalization for, 2185
immune response to, 2180-2182
immunization against, 627, 2187-2191, 2187f, 3466-3468
adverse events with, 3467
contraindications to, 3468
efficacy of, 3466-3467
indications for, 3467-3468
non-live, 2191-2192
Rotarix, 2187f, 2190-2191, 2190f-2191f
RotaShield, 2187-2188, 2187f
RotaTeq, 2187f, 2188-2190, 2189f
safety of, 2191
schedule for, 2191
intestinal villi changes in, 2183
intussusception and, 2186
after kidney transplantation, 1007
nosocomial, 3382
oral immunoglobulin in, 3498, 3531
pathology of, 2183-2184
respiratory symptoms with, 158
roseola with, 769
seasonality of, 2184-2185
transmission of, 2184
treatment of, 2186-2187, 3498
Roth spots, 357
Rotor syndrome, 657
RPR (rapid plasma reagin) test, 1772-1773, 1773t
RSV infection. *See* Respiratory syncytial virus (RSV) infection
Rubella virus, 2196-2198
animal susceptibility to, 2198
antibody response to, 2205-2207
antigenic composition of, 2197
classification of, 2196
E1 glycoprotein of, 2197
physical properties of, 2196-2197
tissue culture of, 2197-2198
Rubella virus infection, **2195-2225**
acute disseminated encephalomyelitis with, 516
age and, 2200-2201, 2200t-2201t, 2201f
antibody response in, 2205-2207
asymptomatic recurrence of, 2203
clinical manifestations of, 742t-748t, 749, 2208-2217, 2210f
complications of, 2211-2213, 2219
congenital, 817-818
agency resources in, 2221
auditory manifestations of, 2213, 2214t-2216t, 2220-2221
cardiovascular manifestations of, 2214t-2216t, 2217, 2220-2221
cataracts in, 2213, 2220-2221
clinical manifestations of, 2213-2217
cutaneous manifestations of, 2214t-2216t
delayed manifestations of, 2217
diagnosis of, 2218-2219
endocrine manifestations of, 2214t-2216t, 2217
epidemiology of, 2203
expanded (persistent), 2217
gastrointestinal manifestations of, 2214t-2216t
genetic manifestations of, 2214t-2216t
genitourinary manifestations of, 2214t-2216t

Rubella virus infection *(Continued)*
glaucoma in, 2213, 2221
growth restriction with, 2213
hematologic manifestations of, 2214t-2216t
hyperbilirubinemia in, 2220
immunologic manifestations of, 2207-2208, 2214t-2216t, 2221
isolation precautions in, 2220
malformations with, 2214t-2216t, 2217
musculoskeletal manifestations of, 2221
neurologic manifestations of, 2213-2216, 2214t-2216t, 2221
ocular manifestations of, 811, 817-818, 2213, 2214t-2216t, 2220-2221
orthopedic manifestations of, 2214t-2216t
pancreatitis with, 694
pathology of, 2208, 2209t
psychiatric manifestations of, 2217
pulmonary manifestations of, 2214t-2216t
respiratory distress in, 2220
retinopathy in, 2213, 2220-2221
treatment of, 2220-2221
in daycare facilities, 3563
diagnosis of, 2217-2219
differential diagnosis of, 2217-2218
encephalitic, 494t-498t, 500, 2208, 2212
epidemic, 2198-2200, 2198f-2199f
epidemiology of, 2198-2204, 2198f-2199f
vs. erythema infectiosum, 1855
exanthem in, 742t-748t, 749, 2210-2211, 2210f
fetal, 2206-2208
fever in, 2211, 2218
gender and, 2204
genetic factors in, 2204
geographic distribution of, 2204
hepatic, 655-656, 2213
historical perspective on, 2195-2196
immune response to, 2205-2206
immunization against, 2221-2224, 3468-3470
acute disseminated encephalomyelitis with, 517
adverse reactions to, 2222-2223, 3469
contraindications to, 2223, 3469-3470
efficacy of, 3469
global results of, 2201-2203
inadvertent, 2223-2224
indications for, 3469
precautions for, 3469-3470
during pregnancy, 2223-2224
incubation period in, 2208-2209, 2218
joint complications of, 2211
lymphadenopathy in, 2210-2211, 2218
myocarditis in, 2212
neurologic complications of, 2212
nosocomial, 3383-3384
ocular manifestations of, 811, 2212
pathogenesis of, 2204-2205, 2205t
pericarditis in, 2212
in pregnancy, 2203, 2206-2207, 2219-2220
prevention of, 2221-2224, 3531-3532
prodromal period in, 2209-2210
quarantine in, 2224
reporting requirements for, 2224
seasonality of, 2204
thrombocytopenic purpura in, 2212
transmission of, 2203
treatment of, 2219-2221
uncomplicated, 2219
uveitis in, 2213
Rubella virus infection *(Continued)*
white blood cell count in, 2211
without rash, 2210
Rug cleaning, Kawasaki disease and, 1068
Rupintrivir, in rhinovirus infection, 2126

S

Sabiá virus infection, 2467, 2470t. *See also* Arenavirus infection
Saccharomyces boulardii infection, 626
Saccharomyces cerevisiae, INT1 introduction into, 9
Sacroiliitis, 720, 731
Saffold virus(es), 2051-2052
geographic distribution of, 2056-2057
replication of, 2054
transmission of, 2056
Saffold virus infection, **2051-2109**
asymptomatic, 2063-2064
bronchial, 2066
cardiac, 2077
clinical manifestations of, 2063-2090
herpangina in, 2065, 2066t
pharyngeal, 2065
St. Anthony's fire, **776**
St. Louis encephalitis virus, 2254
ecology of, 2254, 2254f
St. Louis encephalitis virus infection, 494t-498t, 510-511, **2253-2260**
clinical manifestations of, 2257-2258, 2257f, 2257t
diagnosis of, 2258-2259
differential diagnosis of, 2259
enteroviral infection and, 2090
epidemiology of, 2255-2257, 2255f-2256f
immune response to, 2258
pathogenesis of, 2258
pathology of, 2258
prevention of, 2260
prognosis for, 2260
treatment of, 2259-2260
Salbutamol, in *Bordetella pertussis* infection, 1630
Salicylates
Reye syndrome and, 86-87, 657, **676-677**, 2351
in rheumatic fever, 413
Saline solution
in bronchiolitis, 280
inhalation therapy with, in RSV infection, 2425
nasal irrigation with
in common cold, 137
in rhinosinusitis, 201
Salivary glands
in HIV infection, 149, 191-192
infectious enlargement of. *See* Parotitis
noninfectious enlargement of, 192
Salmonella ser. Typhi infection (typhoid fever), 602t-603t, **1491-1509**
antibiotic resistance in, 1495, 1502
carrier state after, 1502, 1506-1507
clinical manifestations of, 1501-1502, 1502t
complications of, 1501, 1502t
diagnosis of, 1502-1503
differential diagnosis of, 1503-1504
epidemiology of, 1494-1495
exanthem in, 753t-756t, 760, 1501
hemolytic-uremic syndrome with, 1501
host factors in, 1497-1498
immune response to, 1498-1499
immunization against, 1507, 3483-3485, 3484t
encephalitis and, 502
Salmonella ser. Typhi infection (typhoid fever) *(Continued)*
pathophysiology of, 1495-1499, 1495t
in pregnancy, 1502
relapse of, 1501
reservoir for, 1494-1495
treatment of, 1505-1506, 1505t
Salmonella ser. Typhimurium
cell invasion by, 13-14
needle complex of, 13-14, 14f
SipB protein of, 18
SPI-1 of, 13-14
SPI-2 of, 14-15
SPI-3 of, 15
Salmonella ser. Typhimurium infection, 1492t, 1493. *See also Salmonella* spp. infection (salmonellosis)
CD18 in, 15
complications of, 1499
hypertriglyceridemia and, 74
pathogenicity islands in, 13-15
serum cholesterol and, 74
Salmonella spp., 1491-1492, 1492t
pathogenicity islands of, 1496-1497, 1496f
viability of, 1493
Salmonella spp. infection (salmonellosis), **1491-1509**
bloodstream, 1500-1501
treatment of, 1504-1505
bone, 711, 712t, 1500-1501, 1500t
in daycare facilities, 3557-3558
endocardial, 362
fever of unknown origin in, 844
focal, 1500-1501, 1500t
gastrointestinal, 602t-603t, 607t, 609, 616t, 618
age and, 1493-1494
animal reservoirs in, 1492-1493
antibiotic resistance in, 624, 1494
asymptomatic, 1499-1500
breast-feeding and, 1507
carrier state after, 1502, 1506-1507
clinical manifestations of, 1500
diagnosis of, 1502-1503
differential diagnosis of, 1503-1504
epidemiology of, 1492-1494
food sources in, 1492-1493
host factors in, 1497-1498, 1498t
human reservoir in, 1493
immune response to, 1498-1499
inoculum size in, 1494
nosocomial, 1493, 1507
pathophysiology of, 1495-1499, 1495t, 1496f
prevention of, 1507
prognosis for, 1507-1508
treatment of, 623t, 624, 1504, 1504t
interleukin-12 defects and, 50
joint, 728, 729t, 730
meningeal, 1500-1501, 1505
murine, 1499, 1499f
peritoneal, 700
Schistosoma spp. infection and, 1498, 1505, 3066
uveitis after, 816
Salpingitis, **587-588**, 1284, 1286, 1286f
Salt retention, 76
San Miguel sea lion virus infection, 749, 2154
Sandfly, in leishmaniasis, 2947, 2949, 2953f
Sandfly fever, **2535-2537**, 2536f
Sapovirus, 2148f-2149f, 2149, 2149t
Sapovirus infection, **2151-2154**
clinical manifestations of, 2153
diagnosis of, 2153-2154

Sapovirus infection *(Continued)*
differential diagnosis of, 2153
epidemiology of, 2151
immune response to, 2152-2153
pathogenesis of, 2152
pathology of, 2152
prognosis for, 2156
seasonal patterns of, 2151
treatment of, 2155-2156
Sappinia diploidea, 2975, 2979
Sarcoidosis, 443t
Sarcoptes scabiei infection, 758t, **3073**, 3327t-3346t
in daycare facilities, 3559
in health care facilities, 3385
Sashimi, 602-604
Saturated solution of potassium iodide (SSKI), in *Sporothrix schenckii* infection, 2835
Sauna baths, common cold and, 138
Scabies, 758t, **3073**, 3327t-3346t
in daycare facilities, 3559
nosocomial, 3385
Scalded skin syndrome, 753t-756t
Scalp
abscess of, 891, 1282-1283, 1295
pustulosis of, 789, 789f
ringworm of (tinea capitis), **784-785**, 785f
Scarlet fever, 1142-1143. *See also* Pharyngitis, streptococcal
vs. erythema infectiosum, 1855
exanthem in, 753t-756t, 760, 1142-1143
Scarring, in *Mycobacterium ulcerans* infection, 1415, 1415f
Scedosporium spp., 2848-2849
Scedosporium spp. infection, **2848-2850**
clinical manifestations of, 2849-2850
in cystic fibrosis, 336
diagnosis of, 2850
exanthem in, 757t
after lung transplantation, 974
pathogenesis of, 2848-2849
pathology of, 2849, 2849f
treatment of, 2850
Schick test, in *Corynebacterium diphtheriae* infection, 1307
Schistosoma spp. infection (schistosomiasis), **3060-3070**
acute (Katayama fever), 3064
chronic, 3063-3064
clinical manifestations of, 757t-758t, 3064-3066
CNS, 3066
diagnosis of, 3066-3068, 3067f
epidemiology of, 3061-3062, 3061f-3062f
genital, 3065
granuloma in, 3063
hepatosplenic, 3065-3066
imaging in, 3067-3068
immune response in, 3063
intestinal, 3065
ocular, 815
pathogenesis of, 74, 3062-3064, 3062f
prevention of, 3068-3069
pulmonary, 3065-3066
rash in, 3064, 3068
Salmonella spp. infection and, 1498, 1505, 3066
in traveler, 3116
treatment of, 3068, 3327t-3346t
urinary tract, 3064-3065
vaccine against, 3069
Schools, disease in, 3548. *See also at* Daycare facilities
Scintigraphy. *See* Radionuclide imaging
Scleritis, *Stenotrophomonas maltophilia*, 1607
Scombroid fish poisoning, 604, 605t
Scorpion sting, 692, **3077-3078**
Scotch tape, for specimen collection, 566, 566f, 3622
Scrapie, 2620-2621, 2624
Screening tests
in cystic fibrosis, 326-327
for international adoptees and refugees, 3122-3130
in severe combined immunodeficiency disease, 49
in syphilis, 1768
Scrofula, 177, 1356
Scrofuloderma, 1354
Scrum pox, 1945
Seadornaviruses, 2172, 2175
Seafood poisoning, 602t-603t, 604
Sealpox virus infection, 2046
Sebaceous glands, eyelid, infection of, **796**
Sedation, in ARDS, 873
Seizures
in bacterial meningitis, 439-440, 440t, 455
in brain abscess, 464-466, 469
in encephalitis, 509-510
febrile, 85
in HHV-6 infection, 2018
in HHV-7 infection, 2018
after influenza virus immunization, 2352
after MMR/MMRV immunization, 2031, 2393-2394
in neurocysticercosis, 3037
after pertussis immunization, 1634
in roseola infantum, 771
in *Shigella* spp. infection, 1480-1481
in tetanus, 1814, 1816-1817
in tick-borne encephalitis virus infection, 2309
Selectins, in phagocyte recruitment, 31-32, 33f
Selenium
deficiency of, 79
metabolism of, 79
Semliki Forest virus infection, 2253
Sepik virus infection, 2316t
Sepsis. *See also* Bacteremia; Septic shock
Achromobacter spp., 1574
Aeromonas spp., 1541, 1543
ARDS in, 868
Bacillus cereus, 1319
Clostridium spp., 1797
definition of, 825t
Edwardsiella tarda, 1533
Elizabethkingia meningosepticum, 1579
Escherichia coli, 1441
clinical manifestations of, 1444
epidemiology of, 1441
neonatal, 881-882
hepatic dysfunction in, 656
in influenza virus infection, 2345
IVIG administration in, 3500, 3509
jaundice in, 662
after kidney transplantation, 1006
Malassezia furfur, 789, 2853
Morganella morganii, 1470
neonatal, **882-885**, 3509
Clostridium spp., 1797
Klebsiella pneumoniae, 882
Neisseria gonorrhoeae, 1283
probiotics in, 3371-3372
Staphylococcus aureus, 882
Plesiomonas shigelloides, 1565-1566
procalcitonin in, 82
Serratia spp., 1490
severe, 825t
Sepsis *(Continued)*
Shigella spp., 1481
Streptococcus pneumoniae, 1228, 1228f
Streptococcus viridans, 1191-1192
Vibrio parahaemolyticus, 1558
Vibrio vulnificus, 1560-1561
Yersinia enterocolitica, 1518
Yersinia pestis, 1512
Yersinia pseudotuberculosis, 1529
Sepsis-like illness, enteroviral, 2095
Sepsis neonatorum, **882-885**
prevention of, 885
treatment of, 883-884
Septic arthritis, **727-734**
Aeromonas spp., 1543
anaerobic bacteria, 1829-1830
β-hemolytic streptococci in, 1151
clinical manifestations of, 730, 730t
Clostridium spp., 1797-1798
Corynebacterium diphtheriae, 1306
diagnosis of, 730-731, 730t
differential diagnosis of, 732
Enterococcus spp., 1177
epidemiology of, 727
Erysipelothrix rhusiopathiae, 1328
etiology of, 728-730, 729t
Haemophilus influenzae type b, 1676, 1679
inoculation in, 728
Kingella kingae, 1700, 1702
laboratory evaluation of, 731, 732t
Neisseria gonorrhoeae, 1283
neonatal, 733-734, **889-890**, 1283
Pantoea agglomerans, 1536
pathophysiology of, 728
postoperative, 728
prognosis for, 733
Pseudomonas aeruginosa, 1591
radiography in, 730-731, 731f
vs. reactive arthritis, 734
vs. retroperitoneal infection, 708
vs. rheumatic fever, 413
in rheumatoid arthritis, 734
Streptococcus agalactiae, 1161
Streptococcus pneumoniae, 1231
Streptococcus viridans, 1193
treatment of, 732-733
antibiotic, 732-733
surgical, 732
Septic bursitis, vs. septic arthritis, 732
Septic shock, **824-836**
central nervous system effects of, 830
clinical presentation of, 831-833
cutaneous manifestations of, 831
definition of, 825t
diagnosis of, 831-833
disseminated intravascular coagulation in, 829
genes in, 831
hematologic changes in, 829
in immunocompromised host, 824, 831, 833t, 834
interleukin-1 in, 27
in Kawasaki disease, 1075
liver function in, 830
metabolic alterations in, 829-830
mortality in, 825t, 836
myocardial function in, 828-829
nitric oxide in, 3367
organisms in, 824-825, 825t, 832-833
pathophysiology of, 826-831
prognosis for, 836
renal dysfunction in, 830, 832, 835
respiratory distress syndrome in, 830, 832, 835
Streptococcus viridans, 1192

Septic shock *(Continued)*
treatment of, 833-836, 833t, 3367
airway management in, 835
corticosteroids in, 834-835
fluid therapy in, 831
immunomodulating agents in, 835-836, 835t
parenteral alimentation in, 835
tumor necrosis factor-α in, 27
vascular resistance in, 827-828
Septicemia. *See also* Sepsis; Septic shock
in chronic granulomatous disease, 57
endophthalmitis in, 820
exanthem in, 753t-756t
Serratia marcescens infection
antibiotic resistance in, 1489
clinical manifestations of, 1489-1490
epidemiology of, 1488
nosocomial, 1488-1489
pathophysiology of, 1489
in septic shock, 834
treatment of, 1490-1491
Serratia spp., 1488
biotyping of, 1489
isolation of, 1490
Serratia spp. infection, **1488-1491**
clinical manifestations of, 1489-1490
diagnosis of, 1490
endocardial, 362
epidemiology of, 1488-1489
pathophysiology of, 1489
treatment of, 1490-1491
Serum amyloid A (SAA) protein, in Kawasaki disease, 1076
Serum sickness
cefaclor and, 3199
penicillin and, 3190
Severe acute respiratory infection (SARI), 2332
Severe acute respiratory syndrome (SARS), 2486-2487, 2491-2492
Severe combined immunodeficiency (SCID), **48-49**, **914-915**
chest radiography in, 906f
clinical features of, 914
diagnosis of, 915
gene defects in, 907, 907t
screening for, 908
treatment of, 49, 915
types of, 914, 915t
Sex, in epidemiologic study, 108
Sexual abuse
Chlamydia trachomatis infection with, 572t-573t, 574
evaluation for, 561
HPV infection with, 572t-573t, 580
HSV infection with, 582
Neisseria gonorrhoeae infection with, 571, 572t-573t, 1297-1299, 1298t
nonvenereal gonococcal infection and, 530, 534
syphilis screening in, 1768
Trichomonas vaginalis infection with, 572t-573t, 575-576
Sexual activity
EBV infection and, 1998
Escherichia coli UTI and, 1442
pelvic inflammatory disease and, 587
urinary tract infection and, 536-537
Sexually transmitted diseases (STDs), 570-579, 572t-573t. *See also specific infections*
sexual abuse and, 1297-1299, 1298t
in traveler, 3117
ulcers with, 582-584, 583t
Sézary syndrome, 2569
Shell vial assay, 3608-3609
Shellfish poisoning, 602-604, 605t. *See also Norovirus* infection
Shenton line, 731, 731f
Shiga toxin, 17, 619
Citrobacter freundii, 17, 1430-1431
detection of, 1460
enterohemorrhagic *Escherichia coli*, 17, 1457-1459
Shigella spp., 1479
Shigella boydii, 1476-1477
Shigella boydii infection, 1477
Shigella dysenteriae, 1476-1477
Shigella dysenteriae infection, 1477, 1479
Shigella flexneri, 16, 1476-1477
Shigella flexneri infection, 1477, 1479
vulvovaginal, 568-569
Shigella sonnei, 1476-1477
Shigella sonnei infection, 753t-756t, 1477
vulvovaginal, 568-569
Shigella spp., 1476-1477
invasiveness of, 1478-1479, 1478f
isolation of, 1482
toxins of, 1479
type III secretion system of, 1478
Shigella spp. infection (shigellosis), 602t-603t, 607t, 608-609, 616t, 618, **1476-1487**
clinical manifestations of, 1480-1482
complications of, 1480-1481, 1480t
in daycare facilities, 3557
diagnosis of, 1482-1483
differential diagnosis of, 1483
epidemiology of, 1477
extraintestinal manifestations of, 1480-1481
historical perspective on, 1476
in HIV-infected patient, 1482
immune response to, 1479-1480
inflammatory response in, 1479-1480
laboratory findings in, 1482
neonatal, 888, 1481-1482
pathogenesis of, 18, 1478-1480, 1478f
pathology of, 1480
prevention of, 1485-1486
prognosis for, 1485
transmission of, 1477, 1485-1486
treatment of, 623, 623t, 1483-1485
antibiotic resistance in, 1484-1485, 3147-3148
undernutrition and, 1480
vaccine against, 628, 1486
Shingles. *See* Herpes zoster
Shock. *See also* Septic shock
in bacterial meningitis, 440, 454
cardiogenic, 832
in dengue hemorrhagic fever, **2278-2291**, 2284t-2285t
distributive, 832
endotoxic
in animals, 826-827, 827t
in humans, 830-831
hypovolemic, 832
in myocarditis, 395
Shock lung, 830
Short stature, 904-905
Shunt. *See* Cerebrospinal fluid (CSF) shunt
Shwachman-Diamond syndrome, 56
Sialectasis, 191
Sialography, 192
Sic (streptococcal inhibitor of complement-mediated lysis) protein, 18
Sickle-cell disease
acute chest syndrome in, 2641
antibiotic prophylaxis in, 3252
Sickle-cell disease *(Continued)*
bone infarction in, 724
Edwardsiella tarda infection in, 1533
gallstones in, 670
human erythrovirus B19 (human parvovirus B19) infection in, 1849-1850
immunodeficiency in, 59-60
liver abscess in, 673
malaria and, 2926
osteomyelitis in, 723-724
pancreatitis in, 692
Salmonella spp. infection and, 1498, 1500
Streptococcus pneumoniae infection and, 1203-1204, 1205f, 1232
Sigmoidoscopy
in *Clostridium difficile* infection, 636
in *Shigella* spp. infection, 1483
Significance (statistical), 120
tests of, 122
Silver nitrate
in burn injury, 1058
prophylactic, 805-806, 805t, 1281-1282, 3242-3243
Silver sulfadiazine, in burn injury, 1058
Simian virus 40 (SV40), 1863-1866, 1864t
renal disease and, 1867
tumors and, 1868
Sin Nombre virus, 2496-2500, 2498f, 2499t-2500t. *See also* Hantavirus infection
Sindbis fever, 742t-748t, 2252
Single-photon emission computed tomography (SPECT)
in chronic fatigue syndrome, 1088
in encephalitis, 507
Sinusitis. *See* Rhinosinusitis
SipB protein, 18
Sitz bath, in nonspecific vulvovaginitis, 564-566
Sixth disease. *See* Roseola infantum
Sjögren syndrome, 192, 2576
Skene's gland, gonococcal infection of, 1284
Skin, 772
disorders of. *See also* Exanthem *and at specific infections*
of external ear, 208
in hepatitis, 649, 2140
in immunodeficiency disorders, 62
in Kawasaki disease, 1073-1074
in pinta, 1785
in rheumatic fever, 355, 357
in sepsis neonatorum, 883
in septic shock, 831
in systemic disease, 772, 773t
in toxic shock syndrome, 854, 855f, 858, 858f
in Whipple disease, 644
in yaws, 1786-1787
flora of, 90, 91t, 772
hexachlorophene absorption by, 1121
infection of. *See also specific infections*
bacterial, **772-781**, 773t
in Chédiak-Higashi syndrome, **54**
fungal, **784-792**
in glycogen storage disease type 1B, 54-55
in hyper-immunoglobulin E syndrome, **55**
in type 1 leukocyte adhesion deficiency, 52-53
viral, **782-784**
Skin test
in *Coccidioides* spp. infection, 2776
in *Histoplasma capsulatum* infection, 2823
in immunodeficiency evaluation, 907

Skin test *(Continued)*
in *Leishmania* spp. infection, 2951, 2957
in *Paracoccidioides* spp. infection, 2792
in penicillin allergy, 3190
in *Sporothrix schenckii* infection, 2835
in tuberculosis. *See* Tuberculin skin test
Skull, osteomyelitis of, 723
SLAM-associated protein, 915
Slapped cheek appearance, 1847-1848, 1847f
Sleep
disturbances of, in chronic fatigue syndrome, 1089
position for, otitis media and, 211
Sleeping sickness. *See Trypanosoma brucei gambiense* infection (African sleeping sickness); *Trypanosoma brucei rhodesiense* infection (African sleeping sickness)
Slit-lamp examination, in uveitis, 809-810
Small intestine, flora of, 91t, 92-94
Smallpox, **2033-2039**
bioterrorist, 2034, 3401, 3582-3583, 3583f
clinical manifestations of, 742t-748t, 749, 2035-2036
diagnosis of, 2036
differential diagnosis of, 2036
epidemiology of, 2034-2035
exanthem in, 2035
flat, 2035
hemorrhagic, 2035
historical perspective on, 2033-2034
immunization against, 2036-2038
acute disseminated encephalomyelitis with, 517
complications of, 2037t, 2038-2039, 2039t, 3533
encephalitis after, 502
meningitis and, 488
pre-event, 2038
mild (variola minor), 2035
pathology of, 2035
prevention of, 2036-2039, 2037t, 2039t, 3532-3533
reporting requirements for, 2037
risk for, 2037-2038
treatment of, 2036
Snake bite, 3578
Snow, John, 103
Snowshoe hare virus, 2514, 2517, 2520
Socioeconomic conditions
acute otitis media and, 211
infection and, 110-111
Sodium hypochlorite, in burn wound infection, 1059
Sodium stibogluconate, 3350
Soil-contaminated wound, 781, 781t
Solenopsis saevissima (fire ant), 758t
SopE protein, 14
Sore throat. *See* Pharyngitis
South American blastomycosis. *See Paracoccidioides* spp. infection (paracoccidioidomycosis)
spa typing, of *Staphylococcus aureus*, 1119
Specific granule deficiency, 54
Sphincter of Oddi, obstruction of, pancreatitis with, 692
Spider bite, 758t, **3077**
Spina ventosa, 1355
Spinal cord
CMV-associated inflammation of, 526
compression of, 523
infarction of, 523, 524f
inflammation of. *See* Acute transverse myelitis
tetanospasmin effects on, 1813
tumor of, 524
Spinal epidural abscess, 523
Spinal epidural hematoma, 523
Spine
Aerococcus urinae infection of, 1249
cysticercosis of, 3035
infection of, 720-723, 722f, 725
Kingella kingae infection of, 1702
tuberculosis of, 1355
in Whipple disease, 643
Spiramycin, 3350
in *Toxoplasma gondii* infection, 817, 3000, 3001t
Spirillum minus, 1759
Spirillum minus infection (rat-bite fever), 753t-756t, **1759-1760**
clinical manifestations of, 1759-1760
diagnosis of, 1760
epidemiology of, 1759
pathology of, 1759
treatment of, 1760
Spirometra spp. infection (sparganosis), **3043**
Spleen
abscess of, 673, 704-705
in infective endocarditis, 355, 357
absence of, 59
antibiotic prophylaxis in, 3251-3252
Streptococcus pneumoniae infection and, 1204, 1224, 1232
in congenital syphilis, 1771
dysfunction of, *Streptococcus pneumoniae* infection and, 1204
infarction of, 704
in infective endocarditis, 357
in *Mycoplasma pneumoniae* infection, 2682
rupture of, in infectious mononucleosis, 2003
Schistosoma spp. infection of, 3065
in *Streptococcus pneumoniae* infection, 1224
tuberculosis of, 1357
in Whipple disease, 644
Splenectomy, 59, 3251-3252
infection after, 436-437
in Wiskott-Aldrich syndrome, 913
Splenomegaly
in infectious mononucleosis, 1996, 1999-2000, 1999t, 2003
in infective endocarditis, 357
in malaria, 2929
Spondweni virus infection, 2316t
Spondylitis
tuberculous, 1355
in Whipple disease, 643
Spondylodiscitis
Aerococcus urinae, 1249
Kingella kingae, 1702
Spontaneous focal intestinal perforation, vs. necrotizing enterocolitis, 895-897
Sporadic fatal insomnia, 2620-2621, 2627t
Sporothrix schenckii, 2832
Sporothrix schenckii infection (sporotrichosis), **2832-2836**
asteroid body in, 2833
clinical manifestations of, 2833-2834
cutaneous, 757t, 791f, 2833-2834, 2833t-2834t, 2834f
diagnosis of, 2834-2835
epidemiology of, 2832-2833
extracutaneous, 2833t, 2834
granuloma in, 2833
in HIV-infected patient, 2834
immune response to, 2833
joint, 2834
meningeal, 479
pathogenesis of, 2833
Sporothrix schenckii infection (sporotrichosis) *(Continued)*
prevention of, 2836
prognosis for, 2835-2836
pulmonary, 2834
treatment of, 2835-2836, 3295t
uveal, 814
Sports, HSV infection and, 1945
SptP protein, 14
Sputum culture
in *Blastomyces dermatitidis* infection, 2731
in *Cryptococcus neoformans* infection, 2800-2801
in cystic fibrosis–related infection, 332, 333t
in *Legionella pneumophila* infection, 1711
ST-246, 2036
Stachybotrys chartarum infection, 296
Standard Precautions, 3379, 3380t, 3412
Staphylococcal cassette chromosome *mec* (SCC*mec*), 1119
Staphylococcus aureus, **1113-1130**
bacteriophage typing of, 1119
bound coagulase test of, 1113, 1114f
capsule of, 1113-1114, 1115f
carriage of, 1120-1121
clumping factor A of, 8-9
collagen-binding protein of, 8-9
culture of, 1113, 1114f
enzymes of, 1117
fibronectin-binding protein A of, 8-9
gene regulation of, 1118
genome of, 1120
Gram stain of, 1114f
hemolysins of, 17, 1115-1117
identification of, 1113, 1114f, 1114t
L-forms of, 1118
leukocidins of, 1115-1117
methicillin-resistant (MRSA), 1119-1120. *See also Staphylococcus aureus* infection, methicillin-resistant
genome sequencing of, 1120
identification of, 1119-1120
nasal carriage of, 1120
penicillin-binding protein–2 of, 1125
MSCRAMMs of, 1118
multilocus sequence typing of, 1119
opsonization of, 1122
pathogenicity islands of, 1115, 1115t
protein A of, 1114
pulsed-field gel electrophoresis of, 1119
small colony variants of, 1118
spa typing of, 1119
staphylococcal cassette chromosome *mec* of, 1119
structure of, 1113-1119
toxins of, 862, 1114-1115, 1115f. *See also* Toxic shock syndrome (TSS); Toxic shock syndrome toxin–I (TSST-I)
enterotoxin, 862-863, 1117-1118
epidermolytic, 1117
hemolytic, 1115-1117
leukocidin, 1115-1117
typing of, 1119-1120
Staphylococcus aureus infection, **1113-1130**
bloodstream, 361-362, 824, 831, 833-834, 1121
bone, 711-712, 712t, 718. *See also* Osteomyelitis
bronchial, 269, 269t
in bullous impetigo, 773-774, 774f
burn wound, 1051-1052, 1051t
in cervical lymphadenitis, 176-179, 179f, 185-186
in community-acquired pneumonia, 291

Staphylococcus aureus infection *(Continued)*
conjunctival, 802
neonatal, 805-806, 805t
CSF shunt, 1030-1031, 1030t
cutaneous, 750, 773-774. *See also* Impetigo
in cystic fibrosis, 334, 337-338
deep neck, 173-175
diagnosis of, 1123
endocardial, 354, 361-362, 361t, 366-367, 367t
epidemiology of, 1120-1121
eyelid, 794
gastrointestinal, 602t-603t, 607t, 613, 618
hair follicle, **776-777**
hepatic, 673
host defenses against, 1121-1123
immune response to, 1122
joint, 728, 729t, 732
after kidney transplantation, 1014
menstrual. *See* Toxic shock syndrome (TSS)
methicillin-resistant, 3144
antibiotic resistance in, 3144-3145
in bullous impetigo, 774-775
in cervical lymphadenitis, 177, 186
community-acquired, 1126-1127
in cystic fibrosis, 338, 340-341
D-test of, 1126-1127, 1127f
in daycare facilities, 3559
in deep neck abscess, 175
in furunculosis, 777
genome sequencing in, 1120
isolation in, 1126
neonatal, 882
nosocomial, 3398-3399
in osteomyelitis, 712, 714, 717-718
in otitis externa, 206
in pleural effusion, 310, 313-314
in pneumonia, 291
in preseptal cellulitis, 799
in retroperitoneal abscess, 709
in septic arthritis, 732
in septic shock, 824, 833-834
strain identification in, 1119-1120, 3144
TMP-SMX in, 3148
in toxic shock syndrome, 857
treatment of, 1125-1128
vancomycin-resistant, 1128
muscle, **735-738**
neonatal
conjunctival, 805-806, 805t
epidemiology of, 1121
methicillin-resistant, 882
prevention of, 1121
treatment of, 1125t
in otitis externa, 206
pathogenesis of, 1123
accessory gene regulator in, 1118
enterotoxins in, 1117-1118
enzymes in, 1117
hemolysins in, 1115-1117
leukocidins in, 1115-1117
MSCRAMMs in, 1118
protein A in, 1114
small colony variants in, 1118
staphylococcal cassette chromosome *mec* in, 1119
toxins in, 1114-1115, 1123
pelvic, 687
pericardial, 372, 379
prevention of, 1121, 1128-1129
renal, 556
retroperitoneal, 706-707, 709
in rhinosinusitis, 195, 196t
transmission of, 1120-1121
Staphylococcus aureus infection *(Continued)*
treatment of, 1123-1128, 1124t-1125t
antibiotics in, 1124-1125, 1124t
drainage in, 1123-1124
intravenous antibiotics in, 1124, 1124t
IVIG in, 3510
vancomycin-intermediate, 3151
vancomycin-resistant, 1128, 3151-3152
viral infection and, 1122, 2338
vulvovaginal, 568
Staphylococcus auricularis, 1132t
Staphylococcus capitis, 1132t
Staphylococcus capitis infection, 1030, 1030t
Staphylococcus caprae, 1132t
Staphylococcus caprae infection, 1138
Staphylococcus cohnii, 1132t
Staphylococcus epidermidis, 1131-1132, 1132t
enzymes of, 1133
ica operon of, 1133
identification of, 1114t
MSCRAMMs of, 1132-1133
neonatal colonization with, 1131-1132
phenol-soluble modulins of, 1133
polysaccharide intercellular adhesin of, 1132-1133
toxins of, 1133
Staphylococcus epidermidis infection
bloodstream, **1134-1136**, 1135t
bone, 1137
burn wound, 1051-1052, 1051t
catheter-related, 1136
in cervical lymphadenitis, 176-177
CSF shunt, 1030, 1030t
endocardial, 1137
eyelid, 794
neonatal, 882, 3509-3510
ocular, 820, 1138
in otitis externa, 206
peritoneal dialysis catheter–related, 1137
shunt-related, 1136-1137
surgical site, 1137
urinary tract, 1137-1138
Staphylococcus haemolyticus, 1131, 1132t
Staphylococcus hominis, 1132t
Staphylococcus hominis infection, 1030, 1030t
Staphylococcus lugdunensis, 1131-1132, 1132t
Staphylococcus lugdunensis infection
bone and joint, 1138
CSF shunt, 1030, 1030t
endocardial, 1137
Staphylococcus saccharolyticus, 1132t
Staphylococcus saprophyticus, 1131-1132, 1132t
genome of, 1133
identification of, 1114t
Staphylococcus saprophyticus infection, urinary tract, 1133, 1137-1138
Staphylococcus schleiferi, 1132t
Staphylococcus simulans, 1132t
Staphylococcus spp. infection. *See also specific species infections*
coagulase-negative, **1131-1139**
antibiotic-resistant, 1138-1139, 1138t
bloodstream, **1134-1136**, 1135t
bone, 1138
clinical manifestations of, 1133-1138, 1134t
device-related, **1136-1137**
endocardial, 362, 1137
epidemiology of, 1131-1132, 1132t
historical perspective on, 1131
microbiology of, 1131, 1132t
ocular, 1138
pathogenesis of, 1132-1133, 1133f
prevention of, 1139
surgical site, 1137
Staphylococcus spp. infection *(Continued)*
treatment of, 1138-1139, 1138t
urinary tract, 1137-1138
cutaneous, **772-775.** *See also* Impetigo
endocardial, 361-362, 361t, 366-367, 367t
soft tissue, **777-778**
Staphylococcus warneri, 1132t
Staphylococcus xylosus, 1132t
Statistics. *See* Biostatistics
Stavudine, 2613
STDs. *See* Sexually transmitted diseases (STDs)
Stenotrophomonas maltophilia, 1605-1606
Stenotrophomonas maltophilia infection, **1605-1609**
clinical manifestations of, 1606-1608
in cystic fibrosis, 336, 341
diagnosis of, 1608
epidemiology of, 1606
pathophysiology of, 1606
treatment of, 1608-1609
Sternotomy, mediastinitis with, 421-422, 421t, 948-949
Stevens-Johnson syndrome, 764
Stillbirth
bacterial infection and, 881
enterovirus infection and, 2092, 2100
listeriosis and, 1332, 1332f
Stomach. *See also at* Gastro-
flora of, 91t, 92-93
Stones, gallbladder. *See* Gallstones
Stool culture, 618, 3622-3624, 3623t, 3624f
in botulism, 1794
in *Campylobacter* spp. infection, 1645, 1655
in *Hafnia alvei* infection, 1535
in *Salmonella* spp. infection, 1502-1503
in *Shigella* spp. infection, 1482
in *Vibrio parahaemolyticus* infection, 1558-1559
in *Yersinia enterocolitica* infection, 1530
in *Yersinia pseudotuberculosis* infection, 1530
Stool specimen examination, 3622-3624, 3623t, 3624f. *See also* Stool culture
in *Ancylostoma duodenale* infection, 3018
in *Ascaris lumbricoides* infection, 3014
in *Balantidium coli* infection, 2895-2896
in *Blastocystis hominis* infection, 2876
in *Campylobacter jejuni* infection, 1655
in *Clostridium difficile* infection, 636
coccidia stains for, 617
in *Cryptosporidium* spp. infection, 2901-2902, 2902f, 2902t
in *Dientamoeba fragilis* infection, 2885-2886
in *Diphyllobothrium* spp. infection, 3041
in *Dipylidium caninum* infection, 3041
in *Entamoeba histolytica* infection, 2871
in *Fasciola buski* infection, 3057
in *Fasciola* spp. infection, 3053
in *Giardia lamblia* infection, 2881t, 2882
in *Hymenolepis nana* infection, 3042
immunologic tests on, 618-619
in infant botulism, 1806-1808
leukocytes in, 617
macroscopic, 616-617
microscopic, 617
in microsporidial infection, 2913
molecular tests on, 619
in *Necator americanus* infection, 3018
in *Opisthorchis* spp. infection, 3052
ova in, 617
parasites in, 617
in *Schistosoma* spp. infection, 3066-3067, 3067f
in *Strongyloides stercoralis* infection, 3021

Stool specimen examination *(Continued)*
in *Taenia saginata* infection, 3031
techniques of, 3622-3624, 3624f
in *Trichuris trichiura* infection, 3016
Strawberry tongue, 1073-1074
Streptobacillus moniliformis, 1716-1717, 1716f
Streptobacillus moniliformis infection (rat-bite fever), **1715-1720**
clinical manifestations of, 1717-1718, 1717f
diagnosis of, 1718-1719
differential diagnosis of, 1719
epidemiology of, 1715-1716
exanthem in, 753t-756t, 765, 1717, 1717f
historical perspective on, 1715
joint, 730
pathophysiology of, 1718
prevention of, 1719
treatment of, 1719
Streptococcal toxic shock syndrome, 779, 861, 1141, 3511
non–group A, 1151
Streptococcosis syndrome of infancy, 179
Streptococcus agalactiae, 1153-1154
adherence properties of, 1158
antibodies to, 1157-1158
antigenic structure of, 1153-1154
antimicrobial susceptibility of, 1154
C protein of, 1153-1154
C5a-ase of, 1154, 1158
extracellular products of, 1154
β-hemolysin of, 1154
identification of, 1153
isolation of, 1153
nontypeable, 1153
pili of, 5-6, 1154
serologic classification of, 1153-1154, 1156f
type-specific capsular polysaccharide of, 1154, 1158
Streptococcus agalactiae infection, **1153-1169**
capsule polysaccharide types in, 1156-1157, 1156f
in cellulitis, 778
in cervical lymphadenitis, 176-177, 187
clinical manifestations of, 1159-1162, 1159t, 1163t
community transmission of, 1155
diagnosis of, 1162-1163
differential diagnosis of, 1162-1163
dystonic extrapyramidal syndrome with, 516
early-onset, 1156-1157, 1156f-1157f, 1159-1160, 1159t, 1160f
incidence of, 1155
epidemiology of, 1154-1157
group B–specific antigen in, 1153, 1162
historical perspective on, 1153
immune complexes in, 1158
immune response in, 1158-1159
incidence of, 1155
infant colonization and, 1155
joint, 1161
laboratory studies in, 1162
late, late-onset, 1156-1157, 1156f, 1159t, 1161
late-onset, 1156-1157, 1156f-1157f, 1159t, 1160-1161
incidence of, 1155
maternal, 1155
maternal antibody levels and, 1157-1158
maternal colonization and, 1154-1155, 1157
meningeal, 1160, 1160f, 1162
neonatal, 881-882
Streptococcus agalactiae infection *(Continued)*
nosocomial, 1155
osseous, 1161, 1161f
pathogenesis of, 1157-1159, 1157f
infant host factors in, 1158-1159
maternal factors in, 1157-1158
organism factors in, 1158
pharyngeal. *See* Pharyngitis, streptococcal
prevention of, 1165-1169, 3243
infant prophylaxis in, 1167-1168, 1168f
maternal immunization in, 1168-1169
maternal prophylaxis in, 1165-1167, 1166t
prognosis for, 1165
recurrent, 1161-1162
treatment of, 1165
risk factors for, 1155-1156
soft tissue, 1161, 1162f
treatment of, 1154, 1163-1165
adjunctive, 1164-1165
empirical, 1163, 1164t
supportive, 1164
vertical transmission of, 1155-1157, 1157f
Streptococcus dysgalactiae subsp. *equisimilis* infection, 1150-1152
Streptococcus milleri infection. *See Streptococcus* viridans infection
Streptococcus mitis infection. *See Streptococcus* viridans infection
Streptococcus mutans infection. *See Streptococcus* viridans infection
Streptococcus parasanguinis, pili of, 5-6
Streptococcus pneumoniae, 1210-1223
N-acetylglucosamine deacetylase A of, 1215
antibiotic tolerance of, 1215, 1220-1221
antibodies to, 1223-1224
autolysin of, 1214
capsule of, 1211-1212
carriage of, 1200-1201, 1201f-1202f, 1242-1243, 1243t
cell wall of, 1210-1211, 1211f
choline-binding proteins of, 1213
clonality of, 1216
colonization with, 92, 431-432, 1200-1201
free oxygen radical release by, 1214
genome of, 1211-1212
histidine triad proteins of, 1213
hyaluronidase of, 1214
identification of, 1215-1216
IgA protease of, 1214
invasive capacity of, 1206-1209, 1208f
iron transport in, 1214
isolation of, 1215
lipoteichoic acid of, 1210, 1211f
lysogeny of, 1215
mucosal colonization by, 431-432
NADH oxidase of, 1214
nasopharyngeal colonization by, 92
neuraminidases of, 1212
peptidoglycan of, 1210
phase variation of, 1213
phosphoglucomutase of, 1214
phosphorylcholine esterase of, 1213
plasminogen binding of, 1214
pneumolysin of, 1212-1213
pyruvate oxidase of, 1214
serotypes of, 1205-1209, 1206t, 1207f-1208f, 1216
stem peptides of, 1214
structure of, 1210-1212, 1211f
surface proteins of, 1210-1211, 1211f, 1213
teichoic acid of, 1210, 1211f
virulence factors of, 1212-1215
Streptococcus pneumoniae infection, **1198-1246**
age and, 1199-1200, 1199f-1200f, 1202-1203, 1203f
antibiotic resistance in, 1202, 1219-1223, 1220t-1221t, 3143-3145, 3155
evolution of, 1202, 1202f, 1221-1223, 1222f
in mastoiditis, 234
in meningitis, 427
asplenia and, 1204, 1224, 1232
blood-brain barrier in, 432-433, 433t
bloodstream, 432, 432t, 832-833, 1226-1228, 1227t, 1235
bone, 711, 712t, 718, 1231
breast-feeding and, 1204
bronchial, 269, 269t
in cellulitis, 778
in cervical lymphadenitis, 176-177
conjunctival, 802, 1231
crowding and, 1201, 1204
in daycare facilities, 3561-3562
defective antibody formation and, 1204-1205
diagnosis of, 1217-1218
endocardial, 361, 1232
epidemiology of, 1198-1209, 1199f-1202f
in epiglottitis, 245
ethnicity and, 1203
exanthem in, 750, 753t-756t
gender and, 1203
after heart transplantation, 954
after hematopoietic stem cell transplantation, 935, 1232-1233
historical perspective on, 1198-1199
host defenses against, 1223-1226
alveolar macrophages in, 1225
anticapsular serum IgA in, 1223
anticapsular serum IgG in, 1223
basic fibroblast growth factor in, 1225
C-reactive protein in, 1224
complement in, 1225
cytokines in, 1224
granulocyte colony-stimulating factor in, 1225-1226
intracellular killing in, 1225
intracellular signaling pathways in, 1226
lactoferrin in, 1226
leukocyte IgG receptors in, 1223
leukotrienes in, 1224-1225
mannose-binding lectin in, 1226
phagocytosis in, 1223
platelet-activating factor receptors in, 1224
pneumolysin antibody in, 1223-1224
polymeric immunoglobulin receptor in, 1225
spleen in, 1224
surface protein antibody in, 1223-1224
vitamin A in, 1224
vitamin C in, 1224
immunization against, 285, 292, 315, 1237-1244, 3461-3463
adverse events with, 3463
conjugate vaccine for, 1238-1244, 1238f, 1238t, 1240f-1241f, 1240t, 1243t, 3462
efficacy of, 3462-3463
indications for, 3463
unconjugate vaccine for, 1237
in immunocompromised host, 1204-1205, 1232-1233
joint, 728-730, 729t, 1231
after kidney transplantation, 1014

Streptococcus pneumoniae infection *(Continued)*
mannose-binding lectin and, 1205
mastoid, 234, 235t, 1231
medical conditions and, 1205, 1206t, 1244
meningeal, 426-427, 426t, 450t-451t, 458-459, 1228-1229, 1235. *See also* Meningitis, bacterial
animal model of, 1214
mortality with, 1198, 1199f, 1210
neutropenia and, 1205
in otitis media, 212-215, 212t-213t, 1231, 1233-1235
pathogenesis of, 1209-1210
pericardial, 371-373, 379, 1232
peritoneal, 1232
in preseptal cellulitis, 798-799
prevention of, 1236-1244
immunoprophylactic, 1237-1244
nonimmunologic, 1236
pulmonary, 284-286, 285t, 1229-1231, 1230f, 1230t. *See also* Pneumonia, community-acquired
treatment of, 1233
race and, 1203
in rhinosinusitis, 195, 196t, 1231, 1233
risk factors for, 1202-1204, 1203f
seasonality and, 1203-1204, 1204f
septic, 1228, 1228f
serotype and, 1201, 1201f, 1205-1209, 1206t, 1207f-1208f
serum cholesterol and, 74
in sickle-cell disease, 1203-1204, 1205f, 1232
soft tissue, 778, 1231-1232
splenic dysfunction and, 1204
treatment of, 1233-1235, 1234t-1235t
antibiotic resistance in, 1219-1223, 1220t
antibiotic tolerance in, 1215, 1220-1221
susceptibility testing in, 1218-1219, 1220t-1221t
viral infection and, 1215
vulvovaginal, 568
Streptococcus pyogenes, 1140-1141
adhesins of, 10
C5a peptidase of, 18
capsule of, 1140
carriage of, 1143
enzymes of, 1141
exotoxins of, 1141
hemolysins of, 1141
hyaluronidase of, 17-18
L forms of, 1140
M protein of, 10, 18, 1140
protein F of, 10
Sic protein of, 18
transmission of, 1141-1142
vaccine against, 1150
Streptococcus pyogenes infection, **1140-1152**
vs. *Arcanobacterium haemolyticum* infection, 1326
burn wound, 1051-1052, 1051t
in cervical lymphadenitis, 177, 179, 183t, 184-186
clinical manifestations of, 1142-1143
cutaneous, 753t-756t, 772-773, **776**, 1141, 1143. *See also* Erysipelas; Impetigo
in daycare facilities, 3555
deep neck, 173-174
diagnosis of, 1146-1147
differential diagnosis of, 1146-1147
epidemiology of, 1141-1142
in erysipelas, **776**
Streptococcus pyogenes infection *(Continued)*
finger, **775**
mastoid, 234, 235t
in myositis, **738-739**
osteoarticular, 711. *See also* Osteomyelitis
in otitis media, 212t, 214
PANDAS with, 410, **1143-1146**, 1144f. *See also* PANDAS (pediatric autoimmune neuropsychiatric disorder with streptococcal infection)
pathogenesis of, 1142
perianal, **775**
pharyngeal, 156, 158, 159t-160t
clinical manifestations of, 161-162, 1142-1143
diagnosis of, 163, 1146-1147
epidemiology of, 1141
pathogenesis of, 1142
prevention of, 1141-1142, 1150
tonsillectomy in, 1150
transmission of, 1141
treatment of, 163, 1147-1149, 1148t
prevention of, 1141-1142, 1150
rheumatogenic, 405-406, 405t. *See also* Rheumatic fever
soft tissue, **778-780.** *See also* Necrotizing fasciitis
toxic shock syndrome with, 779, 861, 1141, 1151
treatment of, 1147-1149, 1148t
uvular, 165
vulvovaginal, 568
Streptococcus salivarius infection. *See Streptococcus* viridans infection
Streptococcus sanguinis, 354
Streptococcus sanguinis infection, in aphthous stomatitis, 161
Streptococcus sanguis infection. *See Streptococcus* viridans infection
Streptococcus spp. infection. *See also Streptococcus pneumoniae* infection; *Streptococcus* viridans infection
group A. *See Streptococcus pyogenes* infection
group B. *See Streptococcus agalactiae* infection
group C, 1150-1152
group G, 1150-1152
Streptococcus viridans, 1187-1189, 1188f, 1189t
Streptococcus viridans infection, **1187-1197**
bone, 1193
clinical manifestations of, 1191-1193
dental, 1193, 1195
diagnosis of, 1194
endocardial, 360-361, 361t, 365-366, 365t-366t, 1191-1192, 1195-1196
epidemiology of, 1189-1190
in immunocompromised host, 1191-1192
joint, 1193
neonatal, 1192
pathogenesis of, 1190-1191
pharyngeal, 1193
prevention of, 1195-1196
pulmonary, 1193
treatment of, 1194-1195
antibiotic resistance in, 1194
Streptogramins, resistance to, 3145-3147
Streptokinase, in parapneumonic pleural effusion, 314-315
Streptolysin O, 1141
Streptolysin S, 1141
Streptomycin, 3208-3211, 3208t, 3231t-3233t
in tuberculosis, 1367t, 1369
Streptozyme agglutination test, 1147
Stress
infection and, 109
otitis media and, 222
rhinovirus infection and, 2120-2121
Strongyloides fuelleborni kellyi infection, **3019-3021**
Strongyloides stercoralis infection (strongyloidiasis), **3019-3021**
adult T-cell leukemia and, 2575
biliary, 660
clinical manifestations of, 607t, 615-616, 757t-758t, 3020
CNS, 3020
diagnosis of, 3020-3021
disseminated, 3020
epidemiology of, 3020
after hematopoietic stem cell transplantation, 943
hyperinfection with, 3020
after kidney transplantation, 1014
pathophysiology of, 3020
prevention of, 3021
prognosis for, 3021
treatment of, 625, 3021, 3327t-3346t
Stye, **796**
Subacute necrotizing lymphadenitis, 181
Subacute sclerosing panencephalitis (SSPE), **2386-2390**
clinical manifestations of, 2389
epidemiology of, 2386-2387
laboratory findings in, 2389-2390
ocular manifestations of, 811
pathogenesis of, 2387-2389
pathology of, 2387-2389
treatment of, 2390
Subarachnoid neurocysticercosis, 3035, 3036f, 3038
Subdural effusion, in bacterial meningitis, 438, 440, 454-455
Subdural empyema, **469-470**
clinical manifestations of, 469
CSF examination in, 443t, 469
diagnosis of, 469, 470f
treatment of, 470
Sublingual space, infection of (Ludwig angina), 149-150, 150f
Submandibular space, infection of, 142, 149-150, 150f
Sudden infant death syndrome (SIDS)
Bordetella pertussis and, 1627, 1634
enterovirus and, 2091
Sulfadiazine
in heart transplant–related toxoplasmosis, 953
in *Paracoccidioides* spp. infection, 2792
in *Toxoplasma gondii* infection, 3000-3001, 3001t
Sulfamerazine, in *Toxoplasma gondii* infection, 3000-3001
Sulfamethazine, in *Toxoplasma gondii* infection, 3000-3001
Sulfonamides, 3227-3230, 3231t-3233t, 3351
Sulfur granules, in *Actinomyces* spp. infection, 1821, 1821f, 1823
Superantigens
Kawasaki disease and, 1069-1070
T-cell activation by, 40
in toxic shock syndrome, 849, 865-866, 1141
Supraglottitis. *See* Epiglottitis (supraglottitis)
Suprapubic aspiration, 541, 541f, 541t
Suramin, 3351
in *Trypanosoma brucei* infection, 2972

Surfactant
in ARDS, 872-873
in bronchiolitis, 280
in *Pneumocystis* infection, 3008
Surgery
antibiotic prophylaxis in, 3252-3256, 3253t-3256t
infection after, 3395-3398, 3396t
Sushi, 602-604
Sweat test, in cystic fibrosis, 326
Swimmer's ear. *See* Otitis externa
Swimmer's itch, 3062. *See also Schistosoma* spp. infection (schistosomiasis)
Swimming, rhinosinusitis and, 195, 197
Swollen belly syndrome, in *Strongyloides fulleborni kellyi* infection, 3021
Sydenham, Thomas, 103
Sydenham chorea, 410, 413-414, 3511
Sympathetic nervous system, tetanospasmin effects on, 1813
Synbiotics, 3370
Synovitis, toxic (transient, reactive), 732
Syphilis, 572t-573t, 582, 583t, **1761-1782**, 1783t
acquired, 1762
clinical manifestations of, 1767-1768
treatment of, 1777-1778
cardiovascular, 1768
chancre of, 1765, 1768
clinical manifestations of, 1767-1772
CNS, 494t-498t, 1768
congenital, 812, 819-820, 1762
case definition of, 1763, 1764t
clinical manifestations of, 1769-1772, 1769t, 1771t
CNS involvement in, 1770, 1772
CSF examination in, 1777
cutaneous lesions in, 1772
dental changes in, 1771
diagnosis of, 1775-1777, 1775t
early, 1769-1771, 1769t
ectodermal changes in, 1765, 1771
epidemiology of, 1763-1764
exanthem in, 1770
fissures in, 1770
follow-up for, 1780-1781, 1781t
gastrointestinal involvement in, 1771
hearing loss in, 1772
hematologic findings in, 1770
hepatosplenomegaly in, 1771
interstitial keratitis in, 1771
late, 1771-1772, 1771t
mucous patches in, 1765, 1770
ocular, 1771
pathology of, 1766
pneumonia in, 1770-1771
prevention of, 1764-1765, 1765f, 1765t
rhinitis in, 1770
skeletal involvement in, 1769-1770, 1772
subtherapeutic maternal therapy and, 1763, 1764t
treatment of, 1776t-1777t, 1779-1780, 1780f
diagnosis of, 819-820, 1772-1777
CSF examination in, 443t, 1770, 1777
darkfield examination in, 1772
false-positive, 1774
IgM tests in, 1774
nontreponemal tests in, 1772-1773, 1773t
PCR in, 1774
rabbit infectivity test in, 1772
treponemal tests in, 1773-1774, 1773t
endemic, 1783t, **1787-1788**
Syphilis *(Continued)*
epidemiology of, 1763-1765
exanthem in, 753t-756t, 1770
glomerulonephritis in, 1767
gonococcal infection and, 1297
granuloma inguinale and, 1641
gummas in, 1765-1766, 1768
hepatitis in, 656
historical perspective on, 1761
HIV infection and, 1762, 1778
immune response to, 1767
in international adoptees and refugees, 3126
latent, 1766f, 1768
nephrotic syndrome in, 1767
ocular, 807, 812
pathogenesis of, 1766-1767
pathology of, 1765-1766
in pregnancy, 1769, 1778
prevention of, 1781-1782
primary, 583t, 1765, 1766f, 1768
reporting requirement for, 1782
screening for, 1768
secondary, 1765, 1766f, 1768
stages of, 819, 1765-1766, 1766f
tertiary, 1765-1766, 1766f, 1768
transmission of, 1762
of unknown duration, 1768
Syringomyelia, 524
Systemic inflammatory response syndrome (SIRS), 825t. *See also* Acute-phase reaction
Systemic lupus erythematosus (SLE), pulmonary manifestations of, 297

T

T lymphocytes, 38-40
activation of, 39-40
in acute disseminated encephalomyelitis, 517-518
in adenovirus infection, 1895, 1903-1904, 1907-1908
antigen specificity of, 39
in asthma, 277-278
in *Blastomyces dermatitidis* infection, 2726-2727
in burns, 1050
$CD4^+$, 39
in HIV-1 infection, 2598
in HIV-2 infection, 2585-2586, 2592-2595
$CD8^+$, 39
in bronchiolitis, 275-276
$CD4^+/CD8^+$ ratio
in HIV infection, 2596-2597
in hypersensitivity pneumonitis, 304
CMV-specific, in hematopoietic stem cell transplantation–related CMV infection, 940
in *Cryptococcus neoformans* infection, 2799
in *Cryptosporidium* spp. infection, 2900
development of, 38-39
in DiGeorge syndrome, 48
in EBV infection, 1995
evaluation of, 63, 908
fetal, 42
in *Francisella tularensis* infection, 1661
in granuloma inguinale, 1641
in *Haemophilus influenzae* type b infection, 1672
in hantavirus infection, 2509
in HAV infection, 2136-2137
in HBV infection, 1915
helper, 39
T lymphocytes *(Continued)*
in *Histoplasma capsulatum* infection, 2810
in infectious mononucleosis, 2007, 2007f
in influenza virus infection, 2338
in Kawasaki disease, 1077
in measles, 2379
memory, 40
MHC interaction with, 36, 37f
in *Mycobacterium leprae* infection, 1396-1398
in myocarditis, 386-387
ocular, 810
in parainfluenza virus infection, 2365-2366
in *Pneumocystis* infection, 3007-3008
receptors of, 39
regulatory (suppressor), 39-40
in rhinovirus infection, 135, 2119
in RSV infection, 2416
in rubella virus infection, 2206
superantigen activation of, 40
in syphilis, 1767
in toxic shock syndrome, 865-866
in tuberculosis, 1342
in VZV infection, 2024-2025
in Whipple disease, 641
in Wiskott-Aldrich syndrome, 50
Tache noire, 2655
Tachypnea
in community-acquired pneumonia, 287-288, 287t
in parapneumonic effusion, 310
Tacrolimus
in heart transplantation, 955, 955t
in lung transplantation, 968
Taenia asiatica (pork tapeworm) infection, **3032**
Taenia crassiceps infection (cysticercosis), **3039-3040**
Taenia multiceps infection (coenurosis), 815, **3039-3040**
Taenia saginata, 3030
Taenia saginata (beef tapeworm) infection, **3030-3032**
clinical manifestations of, 3031
diagnosis of, 3031-3032
epidemiology of, 3031
pathogenesis of, 3031
prevention of, 3032
prognosis for, 3032
transmission of, 3030-3031
treatment of, 3032, 3327t-3346t
Taenia solium, 3033
Taenia solium infection (cysticercosis), 739, **3032-3039.** *See also* Neurocysticercosis
clinical manifestations of, 3034-3035
diagnosis of, 3035-3037
epidemiology of, 3033
ocular, 815
pathogenesis of, 3033-3034
prevention of, 3039
prognosis for, 3039
transmission of, 3033
treatment of, 3037-3039, 3327t-3346t
Tafenoquine, in malaria, 2945
Tahyna virus, 2514, 2517
Tampon, in toxic shock syndrome, 852-853, 861
Tanapox, 742t-748t, 749
Tanapox virus infection, **2046-2047**
Tapeworm infection
Diphyllobothrium spp. (fish tapeworm), **3040-3041**
Dipylidium caninum (dog tapeworm), **3041**

Tapeworm infection *(Continued)*
Hymenolepis diminuta (rat tapeworm), **3042**
Hymenolepis nana (dwarf tapeworm), **3042**
Taenia saginata (beef tapeworm), **3030-3032**
TBX1 gene, 48
TC-83 vaccine, 2240-2241
Teeth. *See* Tooth (teeth)
Tefibazumab, in septic shock, 835
Teichoic acid, of *Streptococcus pneumoniae*, 1210, 1211f
Teicoplanin, resistance to, 3151-3154, 3152t
Telaprevir, in HCV infection, 2324
Telavancin, 3206-3207
Telogen effluvium, in toxic shock syndrome, 858
Temperature, body, 83-84. *See also* Fever
Temporal artery thermometer, 86
Tenofovir disoproxil fumarate, in HBV infection, 1922
Terbinafine, 3320-3324, 3321t, 3322f
adverse effects of, 3322-3323
drug interactions with, 3323
indications for, 3323-3324
pharmacokinetics of, 3322, 3323t
spectrum of activity of, 3322
in tinea unguium, 787
Tetanolysin, 1812
Tetanospasmin, 1814
Tetanospasmin, 1812-1813
Tetanus, **1809-1819**
cephalic, 1813
cerebral effects in, 1813
clinical manifestations of, 1813-1815
diagnosis of, 1815
differential diagnosis of, 1815
epidemiology of, 1810, 1811f
generalized, 1813-1815
historical perspective on, 1809-1810
immunization against, 1818, 3470-3472, 3472t
in injection drug users, 1814
local, 1813
mortality with, 1817-1818
motor end-plate effects in, 1812-1813
neonatal, 1814-1815, 1819
pathogenesis of, 1812
pathophysiology of, 1812-1813
prevention of, 1818-1819
prognosis for, 1817
seizures in, 1814, 1816-1817
spinal cord effects in, 1813
sympathetic nervous system effects in, 1813
tetanus toxoid in, 1818-1819, 1818t, 3470-3472
treatment of, 1815-1817, 3511-3512
trismus in, 1814
Tetanus toxoid, 1818-1819, 1818t, 3470-3472
for delayed hypersensitivity skin test, 907
Tetracycline(s), 3217-3220, 3231t-3233t
adverse effects of, 3219-3220, 3351
in *Balantidium coli* infection, 2896
in chronic bronchitis, 269-270
contraindications to, 3219-3220
drug interactions with, 3220
indications for, 3219
in malaria, 2942
mechanisms of action of, 3217
in odontogenic infection, 145
pancreatitis with, 692
pharmacokinetics of, 3218-3219, 3218t
resistance to, 3217-3218
Tetracycline(s) *(Continued)*
topical
in inclusion conjunctivitis, 804
in neonatal conjunctivitis, 806, 1281
in trachoma, 805
in vitro activity of, 3218
Thermogenesis, nonshivering, 84
Thermometers, 85-86
Thermoregulation, 84
Thermostable direct hemolysin, 1557-1558
Thigh, dermatophyte infection of, **786**, 786f
Thimerosal, 1927, 3429
Thoracentesis
in parapneumonic effusion, 310-313
in tuberculosis, 1347-1348
Thoracostomy, in lung abscess, 321
Three-day fever. *See* Roseola infantum
Thrombocytopenia
in *Brucella* spp. infection, 1613-1614
in human erythrovirus B19 (human parvovirus B19) infection, 1850
in infectious mononucleosis, 2002-2003
in measles virus infection, 2380
in rubella virus infection, 2212, 2219
Thrombocytopenic purpura
in measles, 2386
in roseola infantum, 771
in rubella virus infection, 2212
Thrombocytosis, in Kawasaki disease, 1076
Thrombophlebitis
CSF examination in, 443t
venous sinus, 236, 239-240
Thrombosis
in bacterial meningitis, 438
in CMV infection, 1980
IVIG-related, 3496
in *Neisseria meningitidis* infection, 1260
Thrush, **787-788**, 787f
in HIV infection, 148-149
in neonate, 892-893, 896
treatment of, 788
Thumb sign, in epiglottitis, 254f
Thymus, evaluation of, 906, 906f
Thyroglossal duct cysts, 182
Thyroid gland, hormones of, 82
Thyroid-stimulating hormone (TSH), 81
Thyroiditis, adenovirus, 1904
Thyroxine (T_4), 82
Ticarcillin, 3183t-3184t, 3187, 3231t-3233t
in meningitis, 450t
Ticarcillin–potassium clavulanate, 3188, 3231t-3233t
Tick bite, 3071-3072, 3071t, 3549
Aeromonas spp. recovery from, 1543
in *Babesia* spp. infection, 2915-2916, 2916f
in Colorado tick fever virus infection, 2173, 2173f
cutaneous manifestations of, 758t
in *Francisella tularensis* infection, 1659-1660
in Kemerovo-related virus infection, 2175
in Lyme borreliosis, 1730, 1730t, 1737-1738
prevention of, 1737, 2175, 2654, 2920, 3549
in relapsing fever, 1740
in Rocky Mountain spotted fever, 2651
tick removal after, 1737, 2654
Tick-borne encephalitis complex viruses, 2303-2304, 2304f
epidemiology of, 2305-2307, 2306f
Tick-borne encephalitis virus infection, 494t-498t, 500, **2303-2313**
clinical manifestations of, 2307-2309, 2307f, 2308t
Tick-borne encephalitis virus infection *(Continued)*
diagnosis of, 2310
differential diagnosis of, 2310-2311
ecology of, 2304-2305
historical perspective on, 2303
immunization against, 2311-2312, 2312f
meningeal, 487
milk-borne, 2306-2307
MRI in, 2308f, 2309
neurologic sequelae of, 2311
paralysis in, 2308-2309
passive immunization against, 2312
pathogenesis of, 2310
pathology of, 2309
prevention of, 2311-2312, 3534
prognosis for, 2311
seizures in, 2309
in traveler, 3116
treatment of, 2311
Tick-borne relapsing fever, 1731f, **1739-1742**, 1741t
Tick paralysis, **3071-3072**
clinical manifestations of, 3072
diagnosis of, 3072
pathogenesis of, 3071-3072
treatment of, 3072
Tigecycline, 3238
in *Acinetobacter* spp. infection, 1572
in coagulase-negative staphylococcal infection, 1139
in vancomycin-resistant *Enterococcus* spp. infection, 1184
Tilt-table testing, in chronic fatigue syndrome, 1088-1089
Tinea capitis, **784-785**, 785f, 787, 3321t
Tinea corporis, **785**, 787, 3321t
Tinea cruris, **786-787**, 786f
Tinea faciei, **785-787**, 785f, 3321t
Tinea incognita, 785
Tinea nigra, **790**, 790f
Tinea pedis, **786-787**, 3321t
Tinea unguium, **786-787**, 786f, 3321t
Tinea versicolor, 789, 789f
Tinidazole, 3351
Tinnitus, in otitis media, 217
Tipranavir, 2615
Tissue expander, infection with, 1017
TMP-SMX. *See* Trimethoprim-sulfamethoxazole (TMP-SMX)
Tobramycin, 3208-3211, 3208t, 3231t-3234t
Toe(s), desquamation of, in Kawasaki disease, 1073
Toenails, dermatophyte infection of, **786**, 786f
Tokyo guidelines, in cholangitis, 660, 660t
Tolerance
antibiotic, in *Streptococcus pneumoniae* infection, 1215, 1220-1221
immunologic, newborn immunization and, 44
Tonsillectomy
lung abscess and, 316
in peritonsillar abscess, 169
prophylactic, 164
in streptococcal pharyngitis, 1150
Tonsillitis, **158-164.** *See also* Pharyngitis
adenovirus, 1895-1896
Francisella tularensis, 1662
tuberculous, 1357
Tonsillopharyngitis, **158-164.** *See also* Pharyngitis
Tooth (teeth)
caries of. *See* Caries
in congenital syphilis, 1771

Tooth (teeth) *(Continued)*
conical, 916, 916f
extraction of, 146
infection of. *See* Odontogenic infection
Torovirus, 2488
Torulopsis glabrata infection, 569-570
Toscana virus, 2545, 2545f
genetic diversity of, 2550
isolation of, 2549-2550
Toscana virus infection, **2544-2551**
clinical manifestations of, 2548
CNS, 2548
diagnosis of, 2549-2550
ecology of, 2547-2548
epidemiology of, 2545-2547
historical perspective on, 2544
Total parenteral nutrition (TPN), gallstones and, 668
Toxic epidermal necrolysis, 753t-756t
Toxic shock syndrome (TSS), **848-866**
acute phase of, 854-857, 854f-855f
in AIDS, 860
antibody titer in, 852, 856, 863-864
in burn patient, 1055
cervicovaginal ulcerations in, 853
clinical presentation of, 753t-756t, 831, 854-861, 854f
definition of, 848, 849t
dermatologic features of, 854, 855f, 858, 858f
diagnosis of, 849t, 856
differential diagnosis of, 861, 862t
Enterobacteriaceae in, 853
enterotoxins in, 862-863
epidemiology of, 849-853, 850f
foreign body in, 852-853
hematologic features of, 858-859
hepatic findings in, 657, 853
histopathology of, 853-854
hypocalcemia in, 829-830
incidence of, 849-850
vs. Kawasaki disease, 1069-1070
laboratory tests in, 855-856
laryngotracheobronchopneumonitis and, 252
mild episodes of, 859-860
mortality with, 859
MRSA strains in, 856-857
mucosal disruption in, 852
murine, 853
myocardial features of, 853, 858
neonatal, 860-861
nonmenstrual, 851-852, 851t, 855f, 860
oral contraceptives and, 853
physical examination in, 854-855, 855f
postoperative, 860-862
prevention of, 861-862
pulmonary features of, 858
recurrence of, 859-861
renal features of, 853, 858
risk factors for, 850-851, 850f
sequelae of, 859
skin disruption in, 852
Staphylococcus aureus in, 852-853, 862-864. *See also* Toxic shock syndrome toxin–I (TSST-I)
subacute phase of, 854f, 857-859, 858f
surveillance for, 849-850, 850f
tampon in, 852-853, 861
treatment of, 856-857, 856t
antimicrobial agents in, 856-857
corticosteroids in, 857
drainage in, 856
fluid therapy in, 857-858
IVIG in, 835, 857, 3509
Toxic shock syndrome (TSS) *(Continued)*
TSST-I in, 852, 862. *See also* Toxic shock syndrome toxin–I (TSST-I)
Toxic shock syndrome toxin–I (TSST-I), 1115f, 1117
antibody to, 852, 857, 863-864
biologic functions of, 865-866
cellular interactions of, 865
detection of, 856
distributional kinetics of, 865
physiochemical properties of, 864
prevalence of, 863
regulation of, 864-865
in toxic shock syndrome, 852, 862
Toxic (transient) synovitis, 732
Toxin(s), 16-18
Aeromonas hydrophilia, 1540
in animals, 826-827, 827t
Arcanobacterium haemolyticum, 1322
Bacillus anthracis, 1303
Bacillus cereus, 1318-1319
Bordetella pertussis, 17, 1618t, 1619-1620, 1623-1625
Campylobacter spp., 1650
Citrobacter freundii, 17, 1430-1431
Clostridium botulinum, 1791-1792, 1801-1802
Clostridium perfringens, 1796
Clostridium tetani, 1812
Corynebacterium diphtheriae, 1302, 1304
enteroaggregative *Escherichia coli*, 1460
Enterobacter, 1435-1436
enterohemorrhagic *Escherichia coli*, 1457-1459
enteroinvasive *Escherichia coli*, 1459-1460
enterotoxigenic *Escherichia coli*, 1455-1457
epidermolytic, 1117
Haemophilus ducreyi, 1686
Helicobacter pylori, 1693
hemolytic, 1115-1117
in hepatic failure, 94
in humans, 827-831. *See also* Septic shock
Kingella kingae, 1701-1702
leukocidin, 1115-1117
marine, 604, 605t
mushroom, 604, 604t
Mycobacterium ulcerans, 1386-1387
Neisseria meningitidis, 1258
Plesiomonas shigelloides, 1565
Pseudomonas aeruginosa, 1587
Shiga, 17, 1457-1459
Shigella spp., 1479
Staphylococcus aureus, 862-863, 1114-1115, 1117-1118. *See also* Toxic shock syndrome toxin–I (TSST-I)
Staphylococcus epidermidis, 1133
Streptococcus pyogenes, 1141
T-cell activation by, 40
tick, 3071
tissue-degrading, 17-18
Vibrio cholerae, 1550
Vibrio parahaemolyticus, 1557-1558
Yersinia enterocolitica, 1525
Yersinia pestis, 1511-1512
Toxocara canis infection (toxocariasis), **3021-3023**
clinical manifestations of, 3022-3023
covert, 3022
diagnosis of, 3023
epidemiology of, 3022
hepatic, 673
myocardial, 400
ocular, 814, 3022-3023
pathophysiology of, 3022
prevention of, 3023
Toxocara canis infection (toxocariasis) *(Continued)*
prognosis for, 3023
treatment of, 3023
Toxocara cati infection, **3021-3023**
Toxoplasma gondii, 2986-2989, 2987f-2988f
isolation of, 2996
Toxoplasma gondii infection (toxoplasmosis), **2986-3005**
acquired, 757t-758t, 2991
diagnosis of, 2997
epidemiology of, 2989-2990
treatment of, 3002
acute, 2991, 2997
cervical lymphadenitis in, 181
CNS, 494t-498t, 2990-2991, 3327t-3346t
vs. EBV-associated lymphoma, 2012
congenital, 757t-758t, **900**, 900t, 2991-2993, 2992t
diagnosis of, 2997-2999, 2999t
differential diagnosis of, 2993
epidemiology of, 2990
ocular, 816-817, 2994, 2994f
prenatal diagnosis of, 2997-2999
treatment of, 3002-3004
diagnosis of, 2994-3000, 2995t
epidemiology of, 2989-2990
fever of unknown origin in, 844
after heart transplantation, 953
after hematopoietic stem cell transplantation, 943
HIV infection and, 2993, 3003-3004
immune response in, 2991
in immunocompromised host, 2991, 2997
after kidney transplantation, 1014
muscle, 739
myocardial, 400
ocular, 816-817, 2991, 2994, 2994f
diagnosis of, 2999-3000
treatment of, 3000-3004, 3001t
pancreatic, 694-695
pathology of, 2990-2991
in pregnancy, 2990, 2992-2993, 2992t, 2997, 3002, 3004, 3004t
prevention of, 3004, 3004t
Trace elements, metabolism of, 77-79, 77t
Tracheal cytotoxin, 17
of *Bordetella pertussis*, 1618t, 1620, 1624
Tracheal intubation
in epiglottitis, 257-258
otitis media and, 211
Tracheitis
bacterial, 242t, 250t-251t, **252**
treatment of, 257
measles virus, 2385, 2392
Moraxella catarrhalis, 1252
Tracheobronchitis, HSV, after kidney transplantation, 1006
Tracheoesophageal fistula, vs. chronic bronchitis, 268
Tracheostomy, in epiglottitis, 257-258
Trachoma, 804-805, **2636**
Transferrin
CSF, 441
serum, 78
Transglycosylases, of *Neisseria gonorrhoeae*, 1278-1279
Transient hypogammaglobulinemia of infancy, **47**, **912**
Translocated intimin receptor (Tir), 7-8, 9f, 1457
Transmissible spongiform encephalopathies, 503, **2619-2629**, 2620t
clinical manifestations of, 2627-2628, 2627t

Transmissible spongiform encephalopathies *(Continued)*
diagnosis of, 2628
epidemiology of, 2621-2624
pathogenesis of, 2624-2627
pathology of, 2624-2627
treatment of, 2628
Transmission-Based Precautions, 3379, 3381t, 3412
Transplantation. *See* Heart transplantation; Hematopoietic stem cell transplantation (HSCT); Intestinal transplantation; Kidney transplantation; Liver transplantation; Lung transplantation
Transposons, in antibiotic resistance, 3138, 3138t
Transverse myelitis. *See* Acute transverse myelitis
Trauma
HSV infection with, 1944, 1944f, 1953
IVIG in, 3499-3500, 3509
liver abscess with, 673
pancreatitis with, 691, 691t
pyomyositis with, 735-736, 736t
Travel. *See* International travel
Traveler's diarrhea, 600-601, 1451, **3110-3115.** *See also* Enterotoxigenic *Escherichia coli* (ETEC) infection
epidemiology of, 3110-3112
management of, 3113-3115
prevention of, 1463, 3112-3113
Treacher Collins syndrome, 1044
Tremacamra, in common cold, 138
Trematodes, 2865-2866. *See also* Flukes
Trench fever, 363, 753t-756t, **1726**, 3075
diagnosis of, 1727
treatment of, 1728
Trench mouth (acute necrotizing ulcerative gingivitis), 147, 147f
Treponema carateum infection (pinta), 753t-756t, 1783t, **1784-1785**
diagnosis of, 1789
treatment of, 1789-1790
Treponema pallidum, 1761-1762. *See also* Syphilis
identification of, 1772
virulence factors of, 1766-1767
Treponema pallidum subsp. *endemicum* infection (endemic syphilis), 1783t, **1787-1788**
diagnosis of, 1789
treatment of, 1789-1790
Treponema pallidum subsp. *pertenue* infection (yaws), 753t-756t, 1783t, **1785-1787**
diagnosis of, 1789
treatment of, 1789-1790
Treponemataceae, 1097t-1112t
Treponematoses
nonvenereal, **1782-1790**, 1783t
diagnosis of, 1789
treatment of, 1789-1790
venereal. *See* Syphilis
Triatoma infestans, 2959
Triatoma sanguisuga, 758t
Tribec virus, 2175
Trichinella spiralis (trichinosis), 3327t-3346t
cutaneous, 757t-758t
gastrointestinal, 602t-603t
muscle, 739
myocardial, 399
ocular, 815
Trichloroacetic acid, in genital HPV infection, 581
Trichobilharzia ocellata infection, 757t-758t
Trichobilharzia physellae infection, 757t-758t
Trichobilharzia stagnicolae infection, 757t-758t
Trichoderma longibachiatum infection, **2857**
Trichodysplasia spinulosa, 1867-1868
Trichomonas vaginalis, 2887
Trichomonas vaginalis infection (trichomoniasis), 572t-573t, 575-577, **2887-2894**
bacterial infection with, 2889-2891
clinical manifestations of, 2890-2891
cutaneous manifestations of, 757t-758t
diagnosis of, 576-577, 2891-2892, 3626-3627
epidemiology of, 2887-2888
gender and, 2888
immune response to, 2890
partner treatment in, 2893
pathogenesis of, 2889-2890
prepubertal, 575-576, 2888-2889
prognosis for, 2893
risk factors for, 2888
severity of, 2890
transmission of, 2888-2889
treatment of, 2892-2893, 2893t, 3327t-3346t
wet-mount preparation in, 575f, 2891-2892
Trichophyton tonsurans infection (tinea capitis), **784-785**, 785f
Trichosporon spp. infection (trichosporonosis), **790-791**, **2853-2854**
neonatal, 898
Trichostrongylus spp. infection, 3024, 3327t-3346t
Trichuris dysentery syndrome, 3016
Trichuris trichiura infection (trichuriasis), **3015-3016**
clinical manifestations of, 3016
diagnosis of, 3016
epidemiology of, 3015
pathophysiology of, 3015-3016
prevention of, 3016
treatment of, 3016, 3327t-3346t
Triiodothyronine (T_3), 82
Trimethoprim-sulfamethoxazole (TMP-SMX), 3227-3229, 3231t-3233t
adverse effects of, 3229, 3351
in cervical lymphadenitis, 186
in cholangitis, 665
in *Cyclospora cayetanensis* infection, 2907
in *Cystoisospora belli infection*, 2911
drug interactions with, 3229
in heart transplantation, 954
in hematopoietic stem cell transplantation, 938-939
indications for, 3228-3229
mechanisms of action of, 3228
in meningitis, 450t
in methicillin-resistant *Staphylococcus aureus* infection, 3148
in *Nocardia* spp. infection, 1422
pancreatitis with, 692
in *Paracoccidioides* spp. infection, 2792
pharmacokinetics of, 3228
in *Pneumocystis* infection, 3010, 3011t
in *Pneumocystis jiroveci* infection, 973, 2599-2600
resistance to, 3147-3149, 3148f, 3228
in *Staphylococcus aureus* infection, 1125
in *Toxoplasma gondii* infection, 3002
in vitro activity of, 3228
in Whipple disease, 646, 646t
Trismus
in masticator space infection, 149
in odontogenic infection, 144
Trismus *(Continued)*
in pericoronitis, 148
in peritonsillar abscess, 168
in tetanus, 1814
Trivittatus virus, 2514, 2517
Trombicula irritans, 758t
Tropheryma whipplei. *See* Whipple disease
Tropical ear. *See* Otitis externa
Tropical pulmonary eosinophilia, 3327t-3346t
Tropical pyomyositis. *See* Pyomyositis
Tropical splenomegaly syndrome, 2929
Troponin T, in myocarditis, 394
Trypanosoma brucei gambiense, 2968
variable surface glycoproteins of, 19
Trypanosoma brucei gambiense infection (African sleeping sickness), **2967-2973**
clinical manifestations of, 757t-758t, 2969-2970
diagnosis of, 2970-2971
differential diagnosis of, 2971
epidemiology of, 2968-2969
ocular manifestations of, 814
pathogenesis of, 19, 2969
prevention of, 2973
prognosis for, 2971-2973
second stage of, 2970
in traveler, 3116
treatment of, 2971-2972, 3327t-3346t
Trypanosoma brucei rhodesiense, 2968
Trypanosoma brucei rhodesiense infection (African sleeping sickness), **2967-2973**
clinical manifestations of, 2969-2970
diagnosis of, 2970-2971
differential diagnosis of, 2971
epidemiology of, 2968-2969
pathogenesis of, 2969
prevention of, 2973
prognosis for, 2971
in traveler, 3116
treatment of, 2971-2973, 3327t-3346t
Trypanosoma cruzi, 2959-2960, 2960f
Trypanosoma cruzi infection (trypanosomiasis), 398-399, **2959-2967**
acute stage of, 2963-2964
chagoma in, 2961, 2962f
chronic stage of, 2964-2965
clinical manifestations of, 757t-758t, 2962f, 2963-2965
CNS manifestations of, 2963-2964
colonic manifestations of, 387
congenital, 2965
diagnosis of, 2965-2966
early, 2963
epidemiology of, 2960-2961
esophageal manifestations of, 387
immune response to, 2962
in immunocompromised host, 2965
indeterminate stage of, 2964
in international adoptees and refugees, 3129
myocardial manifestations of, 398-399
acute stage, 2963-2964
chronic stage, 2964-2965
pathology of, 2961-2963, 2962f
ocular manifestations of, 814
pathogenesis of, 2961-2963, 2961f
pathology of, 2961-2963, 2962f
prevention of, 2967
in transplantation patient, 388
treatment of, 2966-2967, 3327t-3346t
Tryptophan, 71-72
TSPyV, 1864, 1864t, 1867-1868
TSS. *See* Toxic shock syndrome (TSS)
TSST-I. *See* Toxic shock syndrome toxin–I (TSST-I)

Tuberculids, papulonecrotic, 1354
Tuberculin skin test, 180, 184-185, 313, 1361-1363, 1362f, 1363t
  in fever of unknown origin, 842
  in kidney transplantation, 1004, 1012
  in nontuberculous mycobacterial infection, 1384
Tuberculoma, 1354
Tuberculosis, **1335-1380**, 1382t. *See also Mycobacterium tuberculosis*
  abdominal, 707, 709, 1357-1358
  in adolescents, 1359
  age and, 1342, 1343t, 1347t
  antiapoptotic mechanisms in, 15
  appendiceal, 684
  asymptomatic, 1344
  biliary, 660
  bronchial obstruction in, 1345-1347, 1346f
  care reporting of, 1376
  CNS, 443t, 1352-1354
  congenital, 1358-1359
  cutaneous, 753t-756t, 1354-1355
  in daycare facilities, 3556
  diagnosis of, 1361-1366, 3593-3594
    culture in, 1364-1365
    interferon-γ release assay in, 1363-1364
    nucleic acid amplification in, 1365-1366, 3593-3594
    tuberculin skin test in, 1361-1363, 1362f, 1363t
  dialysis-associated, 1358
  encephalitic, 494t-498t, 504
  epidemiology of, 1336-1341, 1337f-1338f, 1338t, 1340f
  exposure to, 1336
  extrathoracic, 1339, 1340f, 1352-1361
    treatment of, 1373
  fever of unknown origin in, 844-845
  gastrointestinal, 1357-1358
    vs. amebic colitis, 2872
  genetic factors in, 1341-1342
  genital, 1358
  after hematopoietic stem cell transplantation, 935-936
  historical perspective on, 1335-1336
  HIV infection and, 1336, 1339, 1360, 2601
  immune reconstitution inflammatory syndrome and, 1360-1361
  immune response in, 1342
  immunization against, 1376-1378, 1377t
  incidence of, 1336-1339
  incubation period in, 1342
  inoculation, 1358
  in international adoptees and refugees, 3126-3128, 3127f-3128f
  after intestinal transplantation, 1000
  isocitrate lyase in, 15
  after kidney transplantation, 1012, 1358
  before kidney transplantation, 1004
  latent, 1336
  after liver transplantation, 1000
  after lung transplantation, 962-964
  lymphadenitis in, 177, 177t, 179-180, 180f, 187, 1344-1347, 1345f-1346f, 1345t
  lymphohematogenous spread of, 1351-1352, 1356
  mastoid, 234, 237, 239
  meningeal, 488-489, 1352-1354, 1373
  miliary, 1352, 1352f, 1357
    pancreatitis with, 695
    pericarditis with, 373
  murine, 70
Tuberculosis *(Continued)*
  myocardial, 1351
  nitrogen balance in, 68
  nosocomial, 3384-3385
  obstructive hyperaeration in, 1345, 1346f
  ocular, 813, 1357
  otic, 214, 1357
  pancreatitis with, 695
  parotid, 191
  pathogenesis of, 15, 1342-1343, 1342t-1343t, 1343f
  pericardial, 379, 1351
  perinatal, 1358-1359
  peritoneal, 700-701, 1357-1358
  pleural effusion in, 309, 1343f, 1347-1348, 1348f-1349f
  portal of entry in, 1342, 1342t
  postnatal, 1358-1359
  in pregnancy, 1360
  prevalence of, 1336-1339
  prevention of, 1376, 3250-3251
  primary complex of, 1343f, 1344-1347, 1344t-1345t, 1346f, 1347t, 1349f
  pulmonary, 1344-1352
    asymptomatic, 1344
    atelectasis in, 1345
    calcification in, 1347, 1348f-1349f
    chronic, 1350-1351
    collapse-consolidation lesion in, 1345-1346, 1346f, 1347t
    primary complex in, 1343f, 1344-1347, 1344t-1345t, 1346f, 1347t, 1349f
    progressive, 1348-1350, 1350f
  radiography in, 1345, 1346f, 1348f-1350f, 1352f
  renal, 553, 1358
  resistance to, 1341-1342
  retroperitoneal, 707, 709
  skeletal, 1355-1356
  superficial lymph node, 1356
  syringe-transmitted, 1358
  timetable of, 1342-1343, 1343f
  transmission of, 1339-1341
  in traveler, 3116
  treatment of, 1366-1378, 1369t
    activity level and, 1375
    adherence to, 1374
    in adults, 1372
    in children, 1372-1373
    corticosteroids in, 1374-1375
    directly observed, 1374
    drug resistance in, 1337, 1367t, 1369, 1373-1374
    exposure stage, 1370-1371
    in extrapulmonary disease, 1373
    follow-up in, 1375-1376
    infection stage, 1371-1372
    isolation in, 1375
    after lung transplantation, 964
    microbiologic basis for, 1369-1370, 1369t
    pharmacologic, 1366-1369, 1367t
    summary of, 1374-1378
    susceptibility testing for, 1365
  vs. tularemia, 1661
  untreated, 1336
  urinary tract, 540
Tuberculosis verrucosa cutis, 1354
Tubo-ovarian abscess, **590**
Tularemia. *See Francisella tularensis* infection (tularemia)
Tumor. *See also* Cancer; Lymphoma
  brain, 467
  liver, 657
  spinal cord, 524
Tumor necrosis factor (TNF)
  in acute-phase reaction, 80
  in ARDS, 870
  hypertriglyceridemia and, 74
  in malaria, 2926
  in myocarditis, 386
  in septic shock, 826-829, 828f
Tumor necrosis factor-α (TNF-α), 26-27, 26t, 3358-3360, 3359t
  in bacterial meningitis, 434-435
  in burns, 1049
  in *Streptococcus agalactiae* infection, 1158
  in *Streptococcus pneumoniae* infection, 1224
Tumor necrosis factor receptor–associated periodic syndrome, 847-848
Tympanic membrane
  compliance of, 218, 220f
  examination of, 217-218, 217f, 219f
  perforation of, 215-216, 223
  scarring of, 223
  in upper respiratory tract infection, 220
Tympanic neurectomy, 192
Tympanic thermometer, 85-86
Tympanocentesis, 218-220
Tympanometry, 218, 220f
Tympanosclerosis, 223
Tympanostomy tubes
  in mastoiditis, 239
  in otitis media, 229, 232
  in otitis media with effusion, 229
Typhlitis, 679
Typhoid fever. *See Salmonella* ser. Typhi infection (typhoid fever)
Tzanck test, 1956

U

UGT1A1 gene, 668
Ulcer(s)
  Buruli. *See* Buruli ulcer; *Mycobacterium ulcerans* infection
  colonic
    in *Entamoeba histolytic* infection, 2869, 2872
    in *Yersinia enterocolitica* infection, 1527, 1527f
  cutaneous, 764, 764t
    in CMV infection, 1981
    in *Francisella tularensis* infection, 1662
    in *Leishmania* spp. infection, 2950-2951, 2951f
  genital, 582-584, 583t
    in granuloma inguinale, 583t, 1641-1642, 1642f
    in *Haemophilus ducreyi* infection (chancroid), 583t, 1687
    in HSV infection, 561f, 1941, 1942f
    in lymphogranuloma venereum, 583t, 584, 584f
    in syphilis, 583t, 1765, 1768
    in toxic shock syndrome, 853
  oral/labial, in HISV infection, 1940-1941, 1940f, 1950, 1950f
  peptic, 1694. *See also Helicobacter pylori* infection
Ulcerative colitis
  vs. *Clostridium difficile* infection, 637
  fever of unknown origin in, 847
  vs. *Shigella* spp. infection, 1483
Ultrasonography
  in acute lobar nephropathy, 555f
  in appendicitis, 682
  in cervical lymphadenitis, 184
  in cholangitis, 661
  in cholecystitis, 669

Ultrasonography *(Continued)*
in *Clonorchis sinensis* infection, 3052
in echinococcosis, 3045, 3046f
in *Fasciola* spp. infection, 3053
in HAV infection, 2137-2138
in intra-abdominal abscess, 705
in neonatal urinary tract infection, 889
in pancreatitis, 690
in parapneumonic pleural effusion, 310, 311f
in pelvic inflammatory disease, 588
in prostatitis, 560
in pyogenic liver abscess, 674
in pyomyositis, 737, 737f
in renal abscess, 554-556, 556f-557f
in retroperitoneal infection, 708
in rhinosinusitis, 199
in *Schistosoma* spp. infection, 3067
in sickle-cell disease, 670
in urinary tract infection, 544
Umbilical cord
infection of, **890**
in syphilis, 1766
Umbilicus, infection of, **890**
Uncinaria stenocephala infection, 3024
UNG, 909
Upper respiratory tract infection (URI). *See also* Common cold; Epiglottitis (supraglottitis); Laryngitis; Laryngotracheitis; Rhinosinusitis; Tracheitis *and specific infectious agents*
bronchitis and, 263
in cancer patient, 922-923, 923t
fever of unknown origin in, 845-846
Kawasaki disease and, 1068
septic arthritis and, 728
tympanic membrane in, 220
vulvovaginitis and, 568
Urea breath test, in *Helicobacter pylori* infection, 1695, 1696t
Ureaplasma spp., 2668-2669, 2669t, 2688
Ureaplasma spp. infection, **2688-2692**
clinical manifestations of, 2688-2690
differential diagnosis of, 2692
epidemiology of, 2688
in immunocompromised host, 2696
neonatal, 901, 2689-2690
chronic, 2691
clinical manifestations of, 2690-2692
CNS, 2691-2692
pulmonary, 2690
in pregnancy, 2689
prevention of, 2697-2698
pulmonary, 296, 2690
urethral, 532-533, 2689
Urease
Cryptococcus neoformans, 2797
Helicobacter pylori, 1693, 1695
Ureter
bacterial colonization of, 538
ectopic, 559, 563
Urethra
flora of, 91t, 93
inflammation of. *See* Urethritis
noninfectious irritants of, 530-531, 532t, 540
Urethritis, **530-535**
Chlamydia trachomatis, 531
diagnosis of, 533
pathophysiology of, 530, 531f
prognosis for, 534-535
screening for, 535
treatment of, 533-534, 534t
chronic prostatitis with, 559
clinical presentation of, 530-531
Urethritis *(Continued)*
culture in, 532
diagnosis of, 532-533, 532f
epidemiology of, 530
idiopathic, 530-531
infectious, 532, 532t
sexually transmitted, 579
treatment of, 533-534, 534t
Moraxella catarrhalis, 1252
Neisseria gonorrhoeae, 1286-1287, 1293f
carriage and, 534
clinical presentation of, 530-531, 1286, 1287f
complications of, 1287
diagnosis of, 532-533, 532f
female, 1284
pathophysiology of, 530
prevention of, 535
prognosis for, 534
sexual abuse and, 530, 534
treatment of, 533-534, 534t
nongonococcal, 2689, 2692, 2698
noninfectious, 531, 532t
clinical presentation of, 531
pathophysiology of, 530
nonspecific, 531, 559
pathophysiology of, 530, 531f
prevention of, 535
prognosis for, 534-535
treatment of, 533-534, 534t
vs. urinary tract infection, 540
Uric acid, urinary, 70
Urinalysis. *See also* Urine
in fever of unknown origin, 842
in gonococcal infection, 1285, 1291
in renal abscess, 554
in urinary tract infection, 542-543
Urinary catheter, infection with, 537, 540-541, 3393-3395
Urinary incontinence, in St. Louis encephalitis virus infection, 2258
Urinary tract infection (UTI), **535-553**
Achromobacter spp., 1574
Acinetobacter spp., 1571
Aerococcus urinae, 1249
Aeromonas spp., 1543
asymptomatic bacteriuria and, 538-539
bacteriology of, 537, 539
BK virus, 1867, 1867f
in burn patient, 1055-1056
C-reactive protein in, 543
in cancer patient, 925
Candida, 2741-2742, 2748-2749, 2754, 2757
catheter-related, 537, 540-541, 3393-3395
Citrobacter spp., 1431
clinical presentation of, 538-539
CMV, 1981
constipation and, 536
diagnosis of, 540-546
bacteriuria definition in, 542, 542t
CT in, 546
culture in, 542-543
dipsticks in, 543
imaging in, 544-546, 544t
microscopy in, 542-543
MRI in, 545-546
scintigraphy in, 544-545, 545f
site of infection in, 543
ultrasonography in, 544
urine collection for, 540-542, 541f, 541t
voiding cystourethrography in, 546, 546f
differential diagnosis of, 539-540
dysfunctional voiding and, 536
Enterococcus spp., 1173-1174
Urinary tract infection (UTI) *(Continued)*
epidemiology of, 535-537
Escherichia coli, **537-538**
clinical manifestations of, 1444
diagnosis of, 1445-1446
epidemiology of, 1440-1441, 1440t
pathogenesis of, 1441-1443, 1442f, 1443t
prognosis for, 1447
treatment of, 1444t, 1446
fever in, 536
fungal, 540
after heart transplantation, 948
after kidney transplantation, 1005-1006, 1014
Klebsiella spp., 1467-1468
leukocyte esterase in, 543
Morganella morganii, 1470-1471
neonatal, 888-889
nitrite in, 543
nosocomial, 3393-3395
pathogenesis of, **537-538**
physical examination in, 538, 539t
prevention of, 552, 3248
procalcitonin in, 543
prognosis for, 551-552
Proteus mirabilis, 1472-1474
Providencia spp., 1475
Pseudomonas aeruginosa, 1591, 1599
recurrence of, 551-552, 3248
risk factors for, 535-536
Schistosoma spp., 3064-3065
Serratia marcescens, 1490
sexual activity and, 536-537
Shigella spp., 1481
Staphylococcus saprophyticus, 1133, 1137-1138
Stenotrophomonas maltophilia, 1607
treatment of, 546-551
antibiotic resistance in, 3148
antibiotics in, 546-548, 547t
behavioral modification therapy in, 548
corticosteroids in, 548
prophylactic antibiotics in, 548
in uncircumcised boys, 536
vesicoureteral reflux and, 546, 546f, **548-551**
viral, 540
virulence factors in, 537-538
Urine
albumin in, in yellow fever, 2276
bacteria in, 538-539, 542, 542t. *See also* Urinary tract infection (UTI)
Candida in, 2741-2742
cattle, 1744
collection of, 540-542
bag technique for, 541-542
clean-catch, 540
in neonatal infection, 888
suprapubic aspiration for, 541, 541f
urethral catheterization for, 540-541
culture of, 542-543
in chemotherapy-associated infection, 925
in *Escherichia coli* UTI, 1445-1446
in *Leptospira* spp. infection, 1754
in neonatal *Candida* spp. infection, 896
in neonatal infection, 888-889
dipsticks for, 543
immunochromatographic membrane test of, 1217
Leptospira in, 1745, 1752
osmolality of, in septic shock, 832
Schistosoma spp. eggs in, 3066-3067, 3067f
white blood cells in, 542-543

Urine culture
in *Candida* spp. infection, 896
in chemotherapy-associated infection, 925
in *Escherichia coli* UTI, 1445-1446
in *Leptospira* spp. infection, 1754
in neonatal infection, 888-889, 896
in urinary tract infection, 542-543
Uropathogenic *Escherichia coli* (UPEC)
Fim H of, 13, 14f
immunization against, 552
P pilus of, 3-4, 4f, 10, 537
PapG adhesin of, 537
pili of, 537
toxins of, 17, 538
Uropathogenic *Escherichia coli* (UPEC) infection, **537-538.** *See also* Urinary tract infection (UTI)
clinical manifestations of, 1444
diagnosis of, 1445-1446
epidemiology of, 1440-1441, 1440t
outcomes of, 1447
pathogenesis of, 1441-1443, 1442f
treatment of, 1444t, 1446-1447
Urticaria, 763-764, 763t
Usutu virus infection, 2316t
Uterus, *Clostridium perfringens* infection of, 1797-1800
UTI. *See* Urinary tract infection (UTI)
Uveitis, **809-816**
anterior, 809-810
Ascaris lumbricoides, 815
Aspergillus spp., 813
bacterial, 812-813
Bartonella henselae, 813
Baylisascaris procyonis, 815
Borrelia burgdorferi, 812
Brucella spp., 813, 1613
Candida spp., 813
in neonate, 895, 897
CMV, 811
Coccidioides spp., 813-814
Coxiella burnetii, 815
in Creutzfeldt-Jakob disease, 811
Cryptococcus neoformans, 814
EBV, 810
Echinococcus granulosus, 815
echovirus, 2074-2075
Entamoeba histolytica, 814
enterovirus, **810**
epidemiology of, 810
fungal, 813-814
Giardia lamblia, 814
Gnathostoma spinigerum, 815
helminthic, 814-815
herpes B virus, 812
herpes zoster, 804
Histoplasma capsulatum, 813
in HIV infection, 811
HSV, 810
HTLV-1, 2573-2574
human T-cell lymphotropic virus, 811
hypopyon in, 809-810
influenza A virus, 812
insect-induced, 816
intermediate, 809
in Kawasaki disease, 1073
keratic precipitates in, 809-810
LCMV, 811-812
Leishmania, 814
Leptospira spp., 812-813, 1748-1749, 1751-1752
Loa loa, 815
measles virus, 811
Multiceps multiceps, 815
mumps virus, 811
Uveitis *(Continued)*
Mycobacterium leprae, 813
Mycobacterium tuberculosis, 813
Onchocerca volvulus, 815
parvovirus, 811
Plasmodium falciparum, 814
posterior, 809-810
posterior synechiae in, 809-810
postinfectious, 816
protozoal, 814
Rickettsia, 815
Rift Valley fever virus, 812
Rochalimaea quintana, 815
rubella virus, 811, 2213
Schistosoma spp., 815
slit-lamp examination in, 809-810
Sporothrix schenckii, 814
in subacute sclerosing panencephalitis, 811
Taenia solium, 815
Toxocara canis, 814
Treponema pallidum, 812
Trypanosoma spp., 814
viral, 810-812
VZV, 810
Wuchereria bancrofti, 815
Uvulitis, **165-167**
clinical manifestations of, 166, 166f
diagnosis of, 166
differential diagnosis of, 166
epidemiology of, 165-166
etiology of, 165
noninfectious, 166
pathogenesis of, 166
treatment of, 166-167

V

Vaccine(s), 3420-3430, 3421t. *See also* Immunization
anthrax, 1315-1316
arenavirus, 2476-2477
Burkholderia cepacia, 1604
Clostridium botulinum, 1795
Clostridium difficile, 638
dengue virus, 2289
DTaP/DTP, 1630-1636, 1631t-1633t, 1635t
Ebola virus, 2485
EBV, 2015
enterotoxigenic *Escherichia coli*, 628, 1463-1464
Haemophilus ducreyi, 1688
hantavirus, 2511
Helicobacter pylori, 1698
HIV, 2616-2617
hMPV, 2440
HSV, 1966
investigational, 3487
IVIG interactions with, 3496-3497
La Crosse virus, 2526
Norovirus, 2156
Plasmodium spp., 2946
Pseudomonas aeruginosa, 1604
reference sources on, 3429-3430
Rift Valley fever, 2530
Rift Valley fever virus, 2530
RSV, 277, 280, 2430-2431
Schistosoma, 3069
Shigella, 628, 1486
Streptococcus pyogenes, 1150
TC-83, 2240-2241
thimerosal in, 1927, 3429
vaccinia virus, 69, 69f, 749
Yersinia pestis, 1514
Vaccinia immune globulin (VIG), 2036, 3532-3533
Vaccinia necrosum, 2038-2039, 3532
Vaccinia virus, 2045-2046
recombinant, 2045-2046
Vaccinia virus infection, 494t-498t, 742t-748t, 749, 2036, 2038-2039, 2039t, 3532-3533. *See also* Smallpox
transmission of, 2039
Vagina
cancer of, HPV and, 1873t, 1874-1875, 1878
Enterobius vermicularis infection of, 3019
examination of, 561
flora of, 91t, 93, 561-562, 562t
foreign body in, 567, 567f
infection of. *See* Vulvovaginitis
specimen collection from, 561, 561f
Trichomonas vaginalis infection of. *See Trichomonas vaginalis* infection (trichomoniasis)
Vaginal discharge, 561
infectious, 563-564
neonatal, 563, 563f
premenarcheal, 563, 563f
in trichomoniasis, 576, 2891-2892
Vaginitis. *See also* Vulvovaginitis
gonococcal
postmenarcheal, 1284-1285
prepubertal, 1283-1284, 1284f
Shigella spp., 1481
Vaginosis, bacterial, 572t-573t, 577-578, 577f
Valacyclovir, 3278t, 3280-3281
in HSV infection, 1959, 1960t, 1961
in VZV infection, 2029
Valganciclovir, 3278t, 3285-3286
in CMV infection, 1984-1986, 1988
in kidney transplantation, 1008
in lung transplantation, 975-976
Validity, study, 95
Valproic acid
in chorea, 414
pancreatitis with, 691
Vancomycin, 3203-3204, 3231t-3234t
adverse effects of, 3206
in bacterial meningitis, 447-449, 450t, 452
in cervical lymphadenitis, 186
in *Clostridium difficile* infection, 637
in coagulase-negative staphylococcal infection, 1138
guidelines for, 1185, 1185t
in heart transplant–related bacteremia, 947
indications for, 3205-3206
mechanisms of action of, 3204
in methicillin-resistant *Staphylococcus aureus* infection, 1127-1128
monitoring of, 3206
pharmacokinetics of, 3205
resistance to, 3151-3154, 3152t, 3204
in *Arcanobacterium haemolyticum* infection, 1323
in *Enterococcus* spp. infection, 1170, 1179t, 1180-1181, 1183-1186, 1185t, 3399-3400
in methicillin-resistant *Staphylococcus aureus* infection, 1128
in rhinosinusitis, 200
in septic shock, 833-834, 833t
tolerance to, in *Streptococcus pneumoniae* infection, 1220-1221
in vitro activity of, 3204-3205

Variable(s). *See also* Biostatistics
  categorical, 101-102
  confounding, 123-124, 124t
  continuous, 100-101, 122
Variable surface glycoproteins, of trypanosomes, 19
Varicella (chickenpox), **2021-2033**
  acetaminophen in, 85
  in burn patient, 1054
  clinical manifestations of, 2025-2027, 2025f
  complications of, 2026, 2026f
  congenital, 819, 2026-2027
    ocular manifestations of, 819
  conjunctival, 804
  in daycare facilities, 3562
  diagnosis of, 1009, 2028
  differential diagnosis of, 2028
  epidemiology of, 2023-2024
  exanthem in, 742t-748t, 762, 2024-2025, 2025f
  external auditory canal, 208, 208f
  after heart transplantation, 954-955
  after hematopoietic stem cell transplantation, 941
  hepatic, 654
  immune response in, 2024-2025
  immunization against, 2030-2032, 3472-3476
  in immunocompromised host, 2026, 2026f
  after kidney transplantation, 1009-1010
  after lung transplantation, 978-979
  myositis with, 739
  ocular, 810
  pathogenesis of, 2024-2025
  prevention of, 2030-2032, 3522-3525
  prognosis for, 2029-2030
  prophylactic acyclovir in, 2032
  secondary, 2023
  subclinical, 2023
  transmission of, 2022-2023
  treatment of, 2028-2029
Varicella-zoster virus (VZV), 2022
  isolation of, 2028
Varicella-zoster virus (VZV) infection, **2021-2033.** *See also* Herpes zoster; Varicella (chickenpox)
  acute disseminated encephalomyelitis with, 516
  breakthrough, 2031-2032
  clinical manifestations of, 2025-2028
  diagnosis of, 2028
  encephalitic, 494t-498t, 499, 505
  epidemiology of, 2023-2024
  immunization against, 2030-2032, 3472-3476
    active, 2031-2032
    adverse events with, 3473-3474
    contraindications to, 3475-3476
    efficacy of, 2031-2032, 3473
    after hematopoietic stem cell transplantation, 941
    indications for, 3474-3475
    before kidney transplantation, 1009
    before lung transplantation, 978-979
    passive, 2030-2031
    safety of, 2031
  meningeal, 487
  nosocomial, 2025, 2030, 3383
  pathogenesis of, 2024-2025
  postexposure prophylaxis for, 3475
  prevention of, 2030-2032, 3522-3525
  prognosis for, 2029-2030
  prophylactic acyclovir in, 2032
  vs. smallpox, 2036
Varicella-zoster virus (VZV) infection *(Continued)*
  transmission of, 2022-2023
  treatment of, 2028-2029, 3279-3280
Variola virus, 2034. *See also* Smallpox
VariZIG, 2030-2031, 3523-3525, 3524t
Vas deferens, absence of, in cystic fibrosis, 325
Vasculitis
  CNS, vs. acute disseminated encephalomyelitis, 519
  coronary artery. *See* Kawasaki disease
  pancreatitis with, 691t, 692
  pulmonary, 297
  small-vessel (leukocytoclastic), 750. *See also* Exanthem
VDRL (Venereal Disease Research Laboratory) test, 819-820, 1770, 1772-1773, 1773t
Venezuelan equine encephalitis, 494t-498t, **2237-2241**
  clinical manifestations of, 2239-2240
  diagnosis of, 2240
  differential diagnosis of, 2240
  ecology of, 2238-2239
  epidemiology of, 2238-2239, 2238f
  pathogenesis of, 2240
  pathology of, 2240
  prevention of, 2240-2241
  treatment of, 2240
Venezuelan equine encephalitis virus, 2237-2238, 2238t
Venezuelan hemorrhagic fever. *See* Arenavirus infection
Venom, spider, 3077-3078
Venous sinus thrombophlebitis, mastoiditis and, 236, 239-240
Ventilator-associated pneumonia, 3391-3393
  in neonate, 892
Ventricle, brain abscess rupture into, 463, 466
Ventricular neurocysticercosis, 3034-3035, 3036f, 3037-3038
Ventriculitis, in meningitis, 437-438, 886-887
Ventriculoatrial shunt. *See* Cerebrospinal fluid (CSF) shunt
Ventriculography, in myocarditis, 387-389, 388f
Ventriculoperitoneal shunt. *See* Cerebrospinal fluid (CSF) shunt
Verruga peruana, **1725-1726**
  treatment of, 1728
Vertigo, in otitis media, 217, 222
Vesicoureteral reflux, **548-551**
  endoscopic bulking agent injection therapy in, 551
  epidemiology of, 548
  management of, 549
  natural history of, 548-549
  procalcitonin in, 543
  prognosis for, 551-552
  prophylactic antibiotics in, 549-551, 549t, 550f-551f
  surgical treatment of, 551
  voiding cystourethrography in, 546, 546f
Vesicular exanthema of swine virus infection, 2147-2148
Vesivirus, 2149, 2149f, 2149t, 2151
Vesivirus infection, **2154**
Vestibular neuritis, enteroviral infection and, 2090
Vibrio cholerae, 1549-1550. *See also* Cholera
  pili of, 5
  toxins of, 1550
Vibrio parahaemolyticus, 1556
Vibrio parahaemolyticus infection, 602t-603t, 607t, 612, 616t, **1556-1559**
  clinical manifestations of, 1558
  complications of, 1558
  diagnosis of, 618, 1558-1559
  epidemiology of, 1556-1557, 1557t
  extraintestinal, 1558
  pathogenesis of, 1557-1558
  prevention of, 1559
  treatment of, 1559
Vibrio vulnificus, 1560
  virulence factors of, 1561
Vibrio vulnificus infection, **1560-1562**
  clinical manifestations of, 1561
  diagnosis of, 1561
  epidemiology of, 1560
  pathophysiology of, 1560-1561
  prevention of, 1562
  treatment of, 1562
Vicks VapoRub, 138
Video-assisted thoracoscopic surgery (VATS), in parapneumonic pleural effusion, 314
Vincent infection (trench mouth, acute necrotizing ulcerative gingivitis), 147, 147f
Viral infection. *See also specific infections*
  cell invasion in, 16
  fever of unknown origin in, 845
  after hematopoietic stem cell transplantation, 939-943
  host response to, 22-23. *See also* Complement; Immunity
  immune suppression with, 20
  after intestinal transplantation, 995-1000, 997t
  after kidney transplantation, 1006-1007
  laboratory diagnosis of, **3600-3620**
    antigen detection in, 3606t-3607t, 3609
    autopsy specimen for, 3604
    blood specimen for, 3602t-3603t, 3604
    bone marrow specimen for, 3602t-3603t, 3604
    culture in, 3605-3609, 3606t-3607t
    cytology in, 3609
    direct fluorescent antibody test in, 3609-3610
    enzyme-linked immunosorbent assay in, 3610-3612, 3611t
    fecal specimen for, 3602t-3603t, 3604
    highly multiplexed assays in, 3614-3616, 3615t
    multiplexed assays in, 3614
    nasal specimen for, 3601-3604, 3602t-3603t
    nasopharyngeal specimen for, 3601-3604, 3602t-3603t
    nucleic acid amplification test in, 3606t-3607t, 3608, 3612, 3613t
    ocular specimen for, 3602t-3603t, 3604
    quantitative nucleic acid amplification test in, 3616-3617, 3616t
    serology in, 3606t-3607t, 3608
    shell vial assay in, 3608-3609
    single-analyte nucleic acid amplification test in, 3612-3614, 3613t
    specimen collection for, 3601-3604, 3602t-3603t
    specimen transport for, 3604-3605
    throat specimen for, 3601, 3602t-3603t
  latency in, 20
  after liver transplantation, 995-1000, 997t
  after lung transplantation, 957, 974-982

Viral infection *(Continued)*
neonatal, **877-881**, 878t. *See also* Newborn, viral infection in
normal flora and, 89-90
pulmonary. *See* Influenza virus infection; Pneumonia, community-acquired
serum cholesterol and, 73-74
Viridans streptococcal infection. *See* *Streptococcus* viridans infection
Virucidal nasal tissues, in cold prevention, 139, 2117, 2117t
Virulence, 105
Virulence-associated genes, 3
Viruses. *See also* Viral infection *ans specific viral infections*
classification of, 1836-1843, 1837f, 1838t-1840t, 1841f-1842f
DNA-containing, 1841f
RNA-containing, 1842f
Visceral larva migrans, 3022-3023. *See also* *Toxocara canis* infection (toxocariasis)
Visceral leishmaniasis. *See Leishmania* spp. infection (leishmaniasis), visceral
Vision loss
in *Chlamydia trachomatis* infection, **2636**
in Creutzfeldt-Jakob disease, 811
in histoplasmosis, 813
in LCMV infection, 811-812
in onchocerosis, 757t-758t, 809, 815
in toxocariasis, 814
Visitor policy, in infection prevention, 3412
Vitamin A
antioxidant activity of, 77
deficiency of
bronchiolitis and, 280
keratitis and, 807
measles and, 2391-2392
metabolism of, 76
Streptococcus pneumoniae infection and, 1224
Vitamin B_{12}, metabolism of, 77
Vitamin C
antioxidant activity of, 77
in common cold, 137-138
metabolism of, 77
in *Streptococcus pneumoniae* infection, 1224
Vitamin D
deficiency of, bronchiolitis and, 280
metabolism of, 77
Vitamin E
antioxidant activity of, 77
in glutathione synthetase deficiency, 59
metabolism of, 77
Vitritis, 809
Candida, 2743
Loa loa, 815
Voiding cystography, in vesicourethral reflux, 555f
Voiding cystourethrography, in vesicoureteral reflux, 546, 546f
Voiding dysfunction, urinary tract infection and, 536, 548
Vomiting
in appendicitis, 680-681
in *Bacillus cereus* infection, 1319
in enteroviral infection, 2068, 2069t
induced, in botulism, 1794
in pancreatitis, 689
in Reye syndrome, 676
in rotavirus infection, 2185
Voriconazole, 3301f, 3310-3312
in *Aspergillus* spp. infection, 2717-2719
in *Blastomyces dermatitidis* infection, 478
in cancer patient, 928
in *Candida* meningitis, 475
Voriconazole *(Continued)*
in *Coccidioides* spp. infection, 478
dosing for, 3311-3312, 3312t
drug interactions with, 3311
efficacy of, 3311
in *Fusarium* spp. infection, 2848
in heart transplant–related *Aspergillus* infection, 953
in hematopoietic stem cell transplantation–related *Aspergillus* infection, 937
in *Histoplasma capsulatum* infection, 2826
in lung transplant–related *Aspergillus* infection, 967-968
in neonatal *Candida* spp. infection, 2758
pharmacodynamics of, 3310
pharmacokinetics of, 3308t, 3310
prophylactic, 3311
safety of, 3310-3311
in *Scedosporium* spp. infection, 2850
Vulvar cancer, HPV and, 1873t, 1874-1875, 1877
Vulvovaginitis, **563-570**
Candida, 569-570, 570f, 788, 2739-2740
in pregnancy, 2765
treatment of, 2747
Chlamydia trachomatis, 572t-573t, 574-575
Corynebacterium diphtheriae, 569, 1305
diphtheritic, 569
Entamoeba histolytica, 569
fungal, 569-570, 570f
group A streptococcal, 568
HSV, 1941-1943, 1942f. *See also* Herpes simplex virus (HSV) infection, genital
Neisseria gonorrhoeae, 571-574, 572t-573t, 1283-1284, 1284f
nongonococcal *Neisseria* spp., 567
nonspecific, 564-567. *See also* Vaginosis, bacterial
foreign bodies and, 567, 567f
intestinal pinworms and, 566-567, 566f
perineal hygiene deficit and, 564-566
prepubertal, 564, 565t
prepubertal, 563-564, 563f, 566f-567f
sexually transmitted, 570-579
Shigella, 568-569
skin infection and, 568
Trichomonas vaginalis, 572t-573t, 575-577, 575f
upper respiratory tract infection and, 568
Yersinia enterocolitica, 569
VZV. *See* Varicella-zoster virus (VZV)

W

Warthin-Finkeldey giant cells, 2378, 2378f
Warts, 742t-748t. *See also* Human papillomavirus (HPV) infection
anogenital, 572t-573t, **580-581**, 580f-581f, 1873t, 1876-1877
chemical ablation of, 1882-1883
conjunctival, 1879-1880
cutaneous, 1873t, 1876
historical perspective on, 1871
immunomodulatory therapy in, 1883
nasal, 1879
surgical treatment of, 1882-1883
WAS, 913
Wasting syndrome, 79-80
Water-contaminated wound, 781, 781t
Waterborne disease, 606, 627, 3547. *See also* *specific infections*
prevention of, 1486
Waterhouse-Friderichsen syndrome, 1264
Wegener granulomatosis, pulmonary vasculitis in, 297
Wegner sign, 1769
Weight loss, in Whipple disease, 643
Weil syndrome, 1749f, 1752-1753
Wesselsbron virus infection, 2316t
West Nile virus, 2261-2262, 2262f
West Nile virus infection, **2261-2273**
breast-feeding and, 2264
clinical manifestations of, 494t-498t, 500, 510, 2267-2269
culture in, 2270
diagnosis of, 2271
electrodiagnostic studies in, 2270
epidemiology of, 2262-2266, 2265t-2266t, 2266f
exanthem in, 742t-748t, 749, 2268
flaccid paralysis in, 2268-2272
historical perspective on, 2261
immune response to, 2267
immunization against, 2272-2273
after kidney transplantation, 1011
laboratory findings in, 2270
after lung transplantation, 983
meningeal, 487, 489
neuroimaging in, 2270
neuropathology of, 2271-2272
ocular manifestations of, 2268-2269, 2272
pathogenesis of, 2266-2267
in pregnancy, 2264
prevention of, 2272-2273
prognosis for, 2269-2270
vs. Sindbis fever, 2252
vs. St. Louis encephalitis virus infection, 2259
transfusion-associated, 2264
transplantation-associated, 2264
treatment of, 2272
vertical transmission of, 2264
Western equine encephalitis, **2230-2237**
clinical manifestations of, 494t-498t, 2233-2234
diagnosis of, 2235
differential diagnosis of, 2235-2236
ecology of, 2230-2231
epidemiology of, 2231-2233, 2231f-2233f
pathogenesis of, 2234
pathology of, 2234
prevention of, 2236-2237, 2236t
prognosis for, 2234-2235
risk factors for, 2232-2233, 2233t
treatment of, 2236
Western equine encephalitis virus, 2230
Wet-mount preparation
in *Cyclospora cayetanensis* infection, 2907, 2907f
in genital tract infection, 561
in *Naegleria fowleri* infection, 2982
in *Trichomonas vaginalis* infection, 575f, 2891-2892
Wheezing. *See also* Asthma
bronchiolitis and, 272, 273t, 274, 281-282
rhinovirus infection and, 2123
Whipple disease, **639-646**
bacteremia in, 642-643
blood involvement in, 644
cardiac involvement in, 644
clinical manifestations of, 642-644, 642t-644t
CNS, 494t-498t, 643, 644t, 646
cutaneous involvement in, 644
diagnosis of, 645
electron microscopy in, 641-642, 641f-642f
epidemiology of, 640-642

Whipple disease *(Continued)*
gastrointestinal tract involvement in, 642-643, 643t
granulomas in, 642
historical perspective on, 639-640
joint involvement in, 643
light microscopy in, 641, 642f
lymph node involvement in, 644
muscle involvement in, 644
ocular involvement in, 644
PAS-positive macrophage in, 641-642, 642f, 645
pathogenesis of, 640-642, 641f-642f
pneumonia in, 643
pulmonary involvement in, 643-644
renal involvement in, 644
spleen involvement in, 644
transmission of, 641
treatment of, 645-646, 646t
White blood cell (WBC) count
in acute bronchitis, 264
in acute laryngotracheitis, 249
in adenovirus infection, 1895
in arthritides, 731, 732t
in bacteremia, 832-833
in bronchiolitis, 275
in *Clostridium difficile* infection, 636
in Colorado tick fever virus infection, 2174
in croup syndromes, 250t-251t
CSF, 441
in HSV encephalitis, 1946-1947
in intracranial pressure monitor–related infection, 1039-1040
in neonatal meningitis, 885, 1135
in shunt-related infection, 1032-1033
in viral meningitis, 489
in Eastern equine encephalitis, 2228
in fever of unknown origin, 842
in fever without source, 839-840
in hepatitis, 649-650
in infectious mononucleosis, 2007
in infective endocarditis, 358
in Kawasaki disease, 1075-1076
in *Kingella kingae* bacteremia, 1702-1703
in *Leptospira* spp. infection, 1752
in *Mycoplasma pneumoniae* infection, 2678
in neonatal *Bordetella pertussis* infection, 1626, 1629-1630
in occult bacteremia, 838
in parapneumonic pleural effusion, 312-313
in *Pasteurella multocida* meningitis, 1547
in pelvic inflammatory disease, 588
in peritonitis, 702
in pneumococcal meningitis, 1228
in prosthetic joint infection, 1028
in rheumatic fever, 412
in roseola infantum, 770-771
in rubella virus infection, 2211
in sandfly fever, 2536
in sepsis neonatorum, 883
in *Streptobacillus moniliformis* infection, 1718
in *Streptococcus agalactiae* infection, 1162
in tick-borne encephalitis virus infection, 2309
in type 1 leukocyte adhesion deficiency, 52-53
urinary, 542-543
in Venezuelan equine encephalitis, 2240
in visceral leishmaniasis, 2956
in *Yersinia enterocolitica* infection, 1526-1527
White piedra, 790-791
Whitewater Arroyo virus infection, 2467, 2469-2471, 2470t, 2473. *See also* Arenavirus infection
Whitlow, herpetic, 155, 1944-1945, 1944f-1945f
Whooping cough. *See Bordetella pertussis* infection (pertussis, whooping cough)
Widal test, 1503
Wilson disease, 657
Wimberger sign, 1769-1770
Wiskott-Aldrich syndrome, 50, 905-906, 907t, 913
Wolbachia, in *Aedes aegypti* control, 2278, 2289
Wound infection
Aeromonas hydrophila, 1542
Bacillus cereus, 1319
bite-related, 780-781, 780t
Clostridium botulinum, 1792-1794
Clostridium perfringens, 1796
Edwardsiella tarda, 1533
after intestinal transplantation, 994
after kidney transplantation, 1004-1005
Pseudomonas aeruginosa, 1591
soil-related, 781, 781t
Staphylococcus epidermidis, 1137
Stenotrophomonas maltophilia, 1607
Streptococcus aureus, 1121
surgical site, 3395-3398, 3396t
tetanus, 3471, 3472t
Vibrio parahaemolyticus, 1558
Vibrio vulnificus, 1560-1562
water-related, 781, 781t
Wuchereria bancrofti infection (filariasis), 757t-758t, 815, **3025-3026**, 3327t-3346t
WUPyV, 1864, 1864t, 1868

X

X-linked agammaglobulinemia, 46-47, **908-909**
vs. chronic bronchitis, 267
diarrhea in, 600
X-linked lymphoproliferative syndrome, 915, 2000-2001
treatment of, 2013-2014, 3521
X protein, 1913
Xanthogranulomatous pyelonephritis, 539-540
Xanthomonas maltophilia. *See Stenotrophomonas maltophilia*
Xenopsylla cheopis (Oriental rat flea), 1510-1511
Xylitol
in otitis media prevention, 231
in *Streptococcus pneumoniae* infection prevention, 1236
Xylohypha bantiana infection, 479

Y

Yaba-like disease virus infection, **2046-2047**
Yaba monkey tumor virus infection, **2046-2047**
YadA protein, 13
Yale Observation Scale, in occult bacteremia, 839
Yatapoxvirus infection, **2046-2047**
Yaws, 753t-756t, 1783t, **1785-1787**
diagnosis of, 1789
treatment of, 1789-1790
Yellow fever, 656, **2274-2278**
clinical manifestations of, 2275-2276
differential diagnosis of, 2276
Yellow fever *(Continued)*
epidemiology of, 2274f, 2275
historical perspective on, 2274
immunization against, 2277-2278, 3485-3487
laboratory diagnosis of, 2276-2277
pathology of, 2276
prognosis for, 2277
treatment of, 2277
Yellow fever virus, 2274-2275
Yersinia aldovae, 1531
Yersinia alecksiciae, 1531
Yersinia bercovieri, 1531
Yersinia enterocolitica, 1515-1516
adhesin A of, 1524
Ail protein of, 13, 1523-1524
animal reservoirs of, 1516-1517
antibodies against, 1529-1530
carriage of, 1518
enterotoxin of, 1525
injectisomes of, 1524
invasin of, 13, 1523-1524
iron metabolism of, 1525
plasmid-encoded factors of, 1524
type III secretion system of, 1524
virulence factors of, 1521-1526, 1522t-1523t
water contamination with, 1518
yersiniabactin of, 1525
Yops of, 1522t-1523t, 1524-1525
Yersinia enterocolitica infection (yersiniosis), **1514-1532**
adhesin A in, 1524
Ail protein in, 13, 1523-1524
asymptomatic, 1527-1528
bloodstream, 1528
clinical manifestations of, 607t, 612, 616t, 753t-756t, 1526-1528, 1526t, 1527f
community outbreaks of, 1518-1519
diagnosis of, 618, 1530
differential diagnosis of, 1529-1530
enterocolitis in, 1526-1527
epidemiology of, 1516-1519, 1517f, 1517t
extraintestinal, 1528
family epidemics of, 1519
food contamination and, 1517-1518
gastric acidity and, 1526
hepatic, 673
immune response to, 1521
incubation period in, 1518
invasin in, 13, 1523-1524
iron metabolism and, 1525
mesenteric adenitis in, 1520-1521, 1527
nosocomial, 1519
outbreaks of, 1518-1519
pathogenesis of, 13, 18, 1521-1526, 1522t-1523t
pathology of, 1519-1521
plasmid pYV in, 1524
postinfectious syndromes in, 1528-1529
prevention of, 1519
pseudoappendicitis in, 1527
reactive arthritis after, 1528
transfusion-related, 1518
transmission of, 1518
treatment of, 1530-1531
vulvovaginal, 569
water contamination and, 1517-1518
YadA protein in, 13
Yops proteins in, 18
Yersinia frederiksenii, 1531
Yersinia intermedia, 1531
Yersinia kristensenii, 1531
Yersinia massiliensis, 1531
Yersinia mollaretii, 1531

Yersinia pestis, 1509-1510
toxin of, 1511-1512
Yersinia pestis infection (plague), **1509-1514**
bioterrorist, 3583-3584
Caf1 in, 5
cervical lymphadenopathy in, 177, 180
clinical manifestations of, 753t-756t, 1512-1513
diagnosis of, 1513
differential diagnosis of, 1513
epidemiology of, 1509, 1511
historical perspective on, 1509
localized (pestis minor), 1511
nosocomial, 3401
pathogenesis of, 1511-1512
prevention of, 1513-1514
prognosis for, 1513
septicemic, 1512
transmission of, 1510-1511
treatment of, 1513
Yersinia pseudotuberculosis, 1515-1516
invasins of, 13
type III secretion system of, 1524
virulence factors of, 1521, 1522t-1523t
YadA of, 13
Yersinia pseudotuberculosis infection (yersiniosis), 1068, **1514-1532**
clinical manifestations of, 753t-756t, 1529
diagnosis of, 1530
differential diagnosis of, 1529-1530
Yersinia pseudotuberculosis infection (yersiniosis) *(Continued)*
epidemiology of, 1519, 1520f
immune response to, 1521
Kawasaki disease–like illness in, 1529
mesenteric adenitis in, 1521
pathogenesis of, 1521-1526, 1522t-1523t
pathology of, 1521
septicemic, 1529
treatment of, 1530-1531
Yersinia rohdei, 1531
Yersinia ruckeri, 1531
Yersinia spp., 1515-1516
identification of, 1530
typing of, 1515-1516
virulence factors of, 1521-1526, 1522t-1523t
Yersinia spp. infection, **1514-1532.** *See also specific species infections*
epidemiology of, 1516-1519
historical perspective on, 1514-1515
uveitis after, 816
Yops proteins, 18, 1522t-1523t, 1524-1525

Z

Zanamivir, 3268t, 3270-3273, 3270f
in community-acquired pneumonia, 290
in influenza virus infection, 2348-2351, 2349t
in laryngotracheitis, 257
Zeaxanthin, 77
Zidovudine, 2612
Zika fever, 749, **2316-2317**
Zinc
deficiency of, 77t, 79
in gastrointestinal tract infection, 625-626
metabolism of, 77t, 78-79
Zinc lozenges, in common cold, 138
Zonisamide, pancreatitis with, 691
Zoster, **2021-2033**
clinical manifestations of, 2027-2028, 2027f
complications of, 2027-2028
diagnosis of, 2028
epidemiology of, 2023-2024
immunization against, 2023-2024
pathogenesis of, 2024-2025
prevention of, 2030-2032
prognosis for, 2029-2030
risk factors for, 2023-2024
after solid-organ transplantation, 979
transmission of, 2023
treatment of, 2028-2029
Zygomycetes, 2837, 2838f. *See also* Entomophthorales infection (entomophthoromycosis); *Mucor* spp. infection (mucormycosis); Rhizopus spp. infection (mucormycosis)
epidemiology of, 2837-2839, 2839f
neonatal infection with, 899

This page intentionally left blank

CLINICAL KEY™

Smarter search. Faster answers.

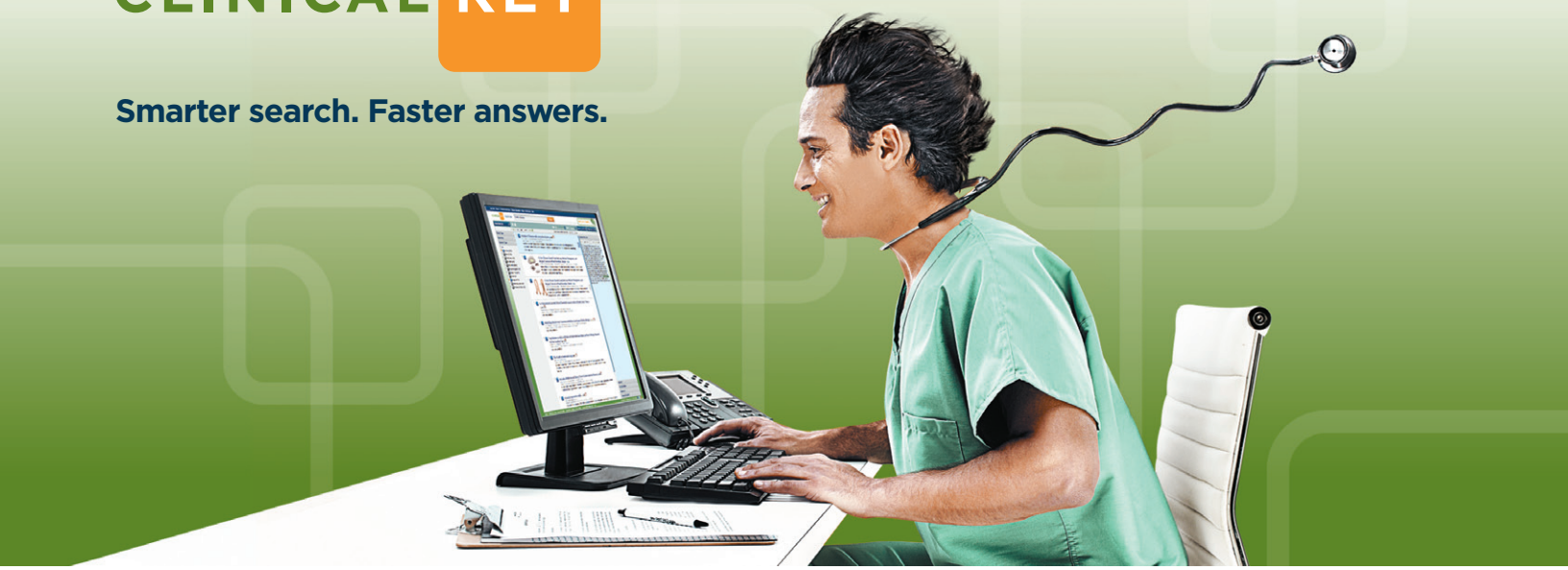

Smarter, Faster Search for Better Patient Care

Unlike a conventional search engine, ClinicalKey is specifically designed to serve doctors by providing three core components:

1 Comprehensive Content

The most current, evidence-based answers available for every medical and surgical specialty.

2 Trusted Answers

Content supplied by Elsevier, the world's leading provider of health and science information.

3 Unrivaled Speed to Answer

Faster, more relevant clinical answers, so you can spend less time searching and more time caring for patients.

Start searching with ClinicalKey today!

Visit ***ClinicalKey.com*** for more information and subscription options.

ELSEVIER

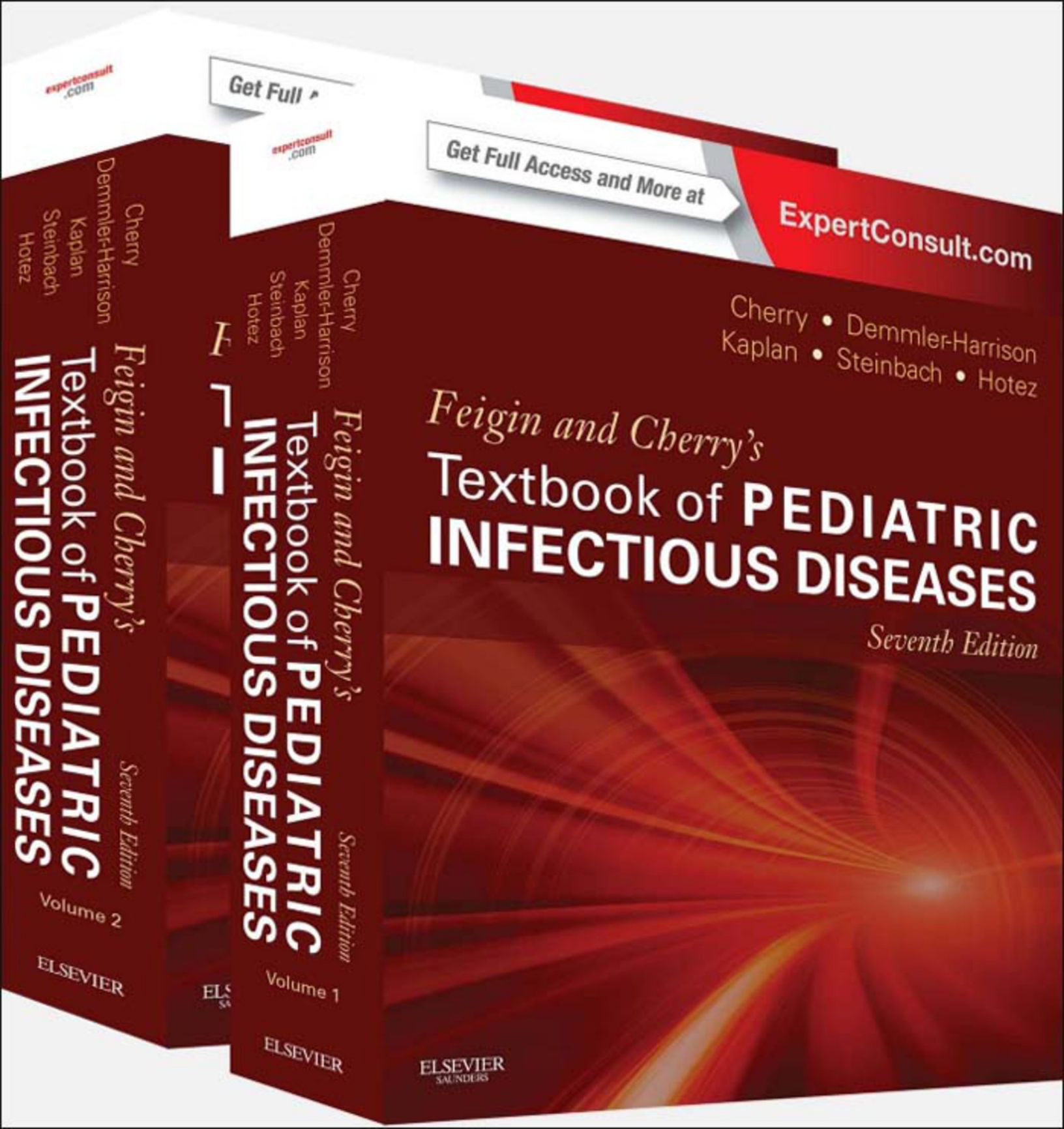
Get Full Access and More at
ExpertConsult.com
Cherry • Demmler-Harrison
Kaplan • Steinbach • Hotez
Feigin and Cherry's
Textbook of PEDIATRIC INFECTIOUS DISEASES
Seventh Edition
Volume 1
Volume 2
ELSEVIER
SAUNDERS

Don't Forget Your Online Access to **ExpertConsult.com**

Mobile. Searchable. Expandable.

ACCESS it on any Internet-ready device

SEARCH all Expert Consult titles you own

LINK to PubMed abstracts

ALREADY REGISTERED?

1. Log in at expertconsult.com
2. Scratch off your Activation Code below
3. Enter it into the "Add a Title" box
4. Click "Activate Now"
5. Click the title under "My Titles"

FIRST-TIME USER?

1. ***REGISTER***
   - Click "Register Now" at expertconsult.com
   - Fill in your user information and click "Continue"
2. ***ACTIVATE YOUR BOOK***
   - Scratch off your Activation Code below
   - Enter it into the "Enter Activation Code" box
   - Click "Activate Now"
   - Click the title under "My Titles"

For technical assistance:
email online.help@elsevier.com
call 800-401-9962 (inside the US)
call +1-314-995-3200 (outside the US)

Activation Code

ExpertConsult.com

FEIGIN AND CHERRY'S TEXTBOOK OF PEDIATRIC INFECTIOUS DISEASES

SEVENTH EDITION

James D. Cherry, MD, MSc
Distinguished Professor of Pediatrics
David Geffen School of Medicine at UCLA
Attending Physician
Pediatric Infectious Diseases
Mattel Children's Hospital UCLA
Los Angeles, California

Gail J. Harrison, MD
Professor
Department of Pediatrics
Section of Infectious Diseases
Baylor College of Medicine
Attending Physician
Infectious Diseases Service
Texas Children's Hospital
Houston, Texas

Sheldon L. Kaplan, MD
Professor and Vice-Chairman for Clinical Affairs
Head, Section of Infectious Diseases
Department of Pediatrics
Baylor College of Medicine
Chief, Infectious Disease Service
Texas Children's Hospital
Houston, Texas

William J. Steinbach, MD
Associate Professor of Pediatrics, Molecular Genetics and Microbiology
Department of Pediatrics
Department of Molecular Genetics and Microbiology
Attending Physician
Division of Pediatric Infectious Diseases
Duke University Medical Center
Durham, North Carolina

Peter J. Hotez, MD, PhD
Dean, National School of Tropical Medicine
Professor, Pediatrics and Molecular & Virology and Microbiology
Head, Section of Pediatric Tropical Medicine
Baylor College of Medicine
Texas Children's Hospital Endowed Chair of Tropical Pediatrics
Director, Sabin Vaccine Institute & Texas Children's Hospital Center for Vaccine Development
President, Sabin Vaccine Institute
Houston, Texas

ELSEVIER
SAUNDERS

ELSEVIER
SAUNDERS

1600 John F. Kennedy Blvd.
Ste 1800
Philadelphia, PA 19103-2899

Feigin & Cherry's Textbook of Pediatric Infectious Diseases

Volume 1: Part no. 9996092720
Volume 2: Part no. 9996092844
Two-volume set ISBN: 978-1-4557-1177-2

Copyright © 2014, 2009, 2004, 1998, 1992, 1987, 1981 by Saunders, an imprint of Elsevier Inc. Chapter 146—Infant Botulism—Stephen S. Arnon—Government employee. This chapter will be in the public domain.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

With respect to any drug or pharmaceutical products identified, readers are advised to check the most current information provided (i) on procedures featured or (ii) by the manufacturer of each product to be administered, to verify the recommended dose or formula, the method and duration of administration, and contraindications. It is the responsibility of practitioners, relying on their own experience and knowledge of their patients, to make diagnoses, to determine dosages and the best treatment for each individual patient, and to take all appropriate safety precautions.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
Feigin & Cherry's textbook of pediatric infectious diseases.
Feigin and Cherry's textbook of pediatric infectious diseases / [edited by] James D. Cherry, Gail J. Demmler-Harrison, Sheldon L. Kaplan, Peter Hotez, William J. Steinbach.—Seventh edition.
p. ; cm.
Textbook of pediatric infectious diseases
Preceded by: Feigin & Cherry's textbook of pediatric infectious diseases / [edited by] Ralph D. Feigin—[et al.]. 6th ed. c2009.
Includes bibliographical references and index.
ISBN 978-1-4557-1177-2 (two-volume set : alk. paper)
I. Cherry, James D. (James Donald), 1930- editor of compilation. II. Demmler-Harrison, Gail J., editor of compilation. III. Kaplan, Sheldon L., editor of compilation. IV. Hotez, Peter J., editor of compilation. V. Steinbach, William J., editor of compilation. VI. Title. VII. Title: Textbook of pediatric infectious diseases.
[DNLM: 1. Communicable Diseases. 2. Child. 3. Pediatrics–methods. WC 100]
RJ401
618.92′98–dc23

2013033020

Senior Content Specialist: Stefanie Jewell-Thomas
Content Development Specialist: Lisa Barnes
Publishing Services Manager: Patricia Tannian
Senior Project Manager: Claire Kramer
Design Direction: Steven Stave

Printed in the United States of America

Last digit is the print number: 9 8 7 6 5 4 3 2 1

Working together to grow libraries in developing countries
www.elsevier.com • www.bookaid.org

To my wife, Jeanne M. Cherry
To my children, James Cherry, Jeffrey Cherry and Kass Hogan, Susan Cherry, and Kenneth and Jennifer Cherry
To my grandchildren, Ferguson, Dennis, and Siena Rose Cherry
James Cherry

To my husband, Neil Harrison
To our children, Emily Demmler Wolfe, Joshua Wolfe, Matthew Demmler, Amy Demmler, Anna Rose Demmler, Kelly Harrison Green, Daniel Green, and Haley Harrison
To our grandchild, Jensen Wolfe
To my in-laws, Elizabeth and Garth Harrison
Gail Harrison

To my wife, Marsha Kaplan
To my children, Lauren Kaplan, Mindy Kaplan Langland, and son-in-law Lance Langland
To my grandchildren, Reece and Macy Langland
Sheldon Kaplan

To my wife, Sandy Steinbach
To my children, Amelia Steinbach, Aidan Steinbach, and Conner Franzen
To my parents, Charles and Kathy Steinbach
William Steinbach

To my wife, Ann Hotez
To my children, Matthew, Emily, Rachel, and Daniel Hotez
To my parents, Ed and Jean Hotez
To my in-laws, Don and Marcia Frifield
Peter Hotez

Ralph D. Feigin, M.D.: April 3, 1938–August 14, 2008

This seventh edition of the *Textbook of Pediatric Infectious Diseases* is dedicated to Ralph D. Feigin. As everyone in pediatrics and, in particular, pediatric infectious diseases, knows, Ralph was an extraordinary individual, and his untimely death in 2008 leaves a void that will never be filled.

Ralph Feigin was born in New York City on April 3, 1938. He graduated from Columbia College in New York City in 1958 and received his M.D. from Boston University School of Medicine in 1962. He married Judith S. Zobel, a childhood friend, in 1960 while in medical school. Ralph completed his first two years of pediatric residency at Boston City Hospital and his third year at the Massachusetts General Hospital. He then fulfilled his military service requirement at the United States Army Research Institute of Infectious Diseases, Ft. Detrick, Frederick, Maryland. While at the United States Army Research Institute, he participated in significant studies relating to circadian periodicity and susceptibility to infections, as well as other studies that resulted in eight publications for which he was the first author. After completing his service commitment, he was Chief Resident at Massachusetts General Hospital during the 1967-68 academic year.

Ralph was recruited to Washington University in St. Louis by Phil Dodge in 1968, and soon thereafter he and one of us (JDC), who was then at St. Louis University, got together and forged an academic and personal friendship that continued until the time of his death. Over 40 years ago, Ralph and Jim recognized the need for a comprehensive book on pediatric infectious diseases, but because of their busy schedules the plan was put on hold, and in 1973 Jim moved to California. In 1976, the pediatric research meetings were held in St. Louis, and at this time Jim and Ralph met with W. B. Saunders representatives, and the book was conceived. The first edition of the textbook was published 5 years later in the fall of 1981. In comparison with this 7th edition, it was a modest effort, with 44 chapters and 124 contributors.

At Washington University and St. Louis Children's Hospital, Ralph developed one of the finest infectious diseases divisions in the country. His "Feigin Rounds" were an unparalleled learning experience and were legendary among medical students and residents. In 1977, Ralph moved to Houston, Texas, to accept the challenge of being the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital. During the ensuing 30 years, the Department grew from 43 faculty members to almost 500. One of us (SLK) came under Ralph's spell in St. Louis and moved to Houston with him. Another one of us (GJH), an intern in Houston in 1977, was waiting for Dr. Feigin when he arrived.

In Houston, Ralph served as the Chair of Pediatrics for Baylor College of Medicine and the Physician-in-Chief at Texas Children's Hospital for 31 years. For 7 years of his tenure, he also served as President and CEO of Baylor College of Medicine. In addition to his commitments in Houston, Ralph served in leadership roles on more than 100 local, regional, and national committees and professional societies. His efforts in persuading government officials of all ranks helped children in Texas, the United States, and in all parts of the world. Many consider him to have been the foremost pediatrician in the world.

Not only was Dr. Feigin a powerhouse of energy, speed, and unsurpassed accomplishments, but he also was a gentleman, full of compassion, warmth, and kindness, and a man who kept people and patients first in his heart and mind. He was a loving husband to his wife, Judy, and a proud father to his three children, Susan, Debra, and Michael; doting grandfather to his six grandchildren, Rebecca, Matthew, Sarah, Rachel, Jacob, and Eli; and a mentor to so many in the field of pediatrics and pediatric infectious diseases. Ralph Feigin is missed by everyone who knew him, particularly by Judy Feigin and the family as well as by the present editors of this seventh edition of Feigin and Cherry.

A Tribute To: Caroline Breese Hall
February 4, 1939–December 10, 2012

It is with great sadness that I present this tribute to Caren Hall. She was a personal friend of mine for over 35 years, and she contributed to the chapters "Respiratory Syncytial Virus" and "Parainfluenza Viruses" in all seven editions of *Feigin and Cherry*.

Caren was a leader in the study of respiratory viral illnesses and also, more recently, in the manifestations of primary HHV-6 infections in young children.

A hallmark of all her chapters in *Feigin and Cherry* was the presentation of original poems at key points in the text. I have taken the liberty to reproduce those here:

As by one bow on varied strings, the tune is played,
By both the microbe and the host, the disease is made

A tiny thistle—
  Of coiled spine
  And outer quill …

Each breath's toll is virus spread and shed. …
Through the newly born the mother flows yet,
  Tiny proteins seeded to protect—
    though imperfect …

We view their chests ballooning, with the fears
That they're "pink puffers" of more tender years.[1]

Too minuscule for us to perceive,
But distinctive fingerprints it leaves.

Thus, if infected at this tender stage
Will they become "blue bloaters" as they age?[1]

With perseverance
And the dreamer's scanning scope,
Reality is made of distant hope …

What occult power pries loose the lid to give you water flight,
But with the lengthening light of spring gives cloak and leaden wing?

The newly born for mother shorn
With seeds unsown and shield unknown …

Though minuscule of micron measure,
It leaves its scent and prints precise;
A fleeing hare for hunt and snare …

In the future, we all will miss Caren's further contributions to the understanding and treatment of respiratory viral infections in children.

J.D.C.

[1]Reprinted with permission from Hall CB. A comment for the future. *Pediatr Res* 11:March, 1977 (Part 2).

James D. Cherry, M.D., M.Sc
Distinguished Professor of Pediatrics
David Geffen School of Medicine at UCLA
Attending Physician
Pediatric Infectious Diseases
Mattel Children's Hospital UCLA
Los Angeles, California

Gail J. Harrison, M.D.
Professor
Department of Pediatrics
Section of Infectious Diseases
Baylor College of Medicine
Attending Physician
Infectious Diseases Service
Texas Children's Hospital
Houston, Texas

Sheldon L. Kaplan, M.D.
Professor and Vice-Chairman for Clinical Affairs
Head, Section of Infectious Diseases
Department of Pediatrics
Baylor College of Medicine
Chief, Infectious Disease Service
Texas Children's Hospital
Houston, Texas

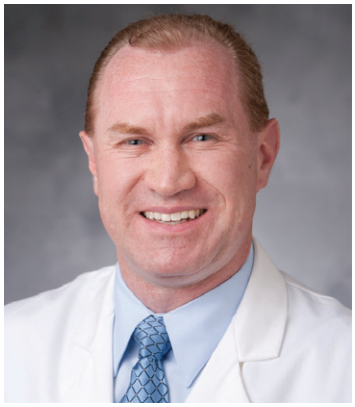

William J. Steinbach, M.D.
Associate Professor of Pediatrics, Molecular Genetics and Microbiology
Department of Pediatrics
Department of Molecular Genetics and Microbiology
Attending Physician
Division of Pediatric Infectious Diseases
Duke University Medical Center
Durham, North Carolina

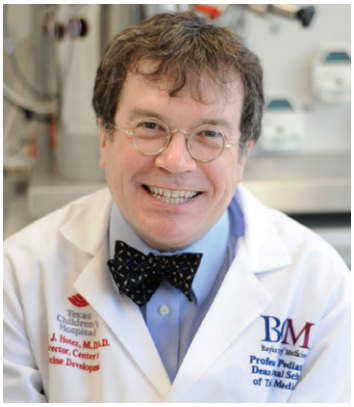

Peter J. Hotez, M.D., Ph.D.
Dean, National School of Tropical Medicine
Professor, Pediatrics and Molecular & Virology and Microbiology
Head, Section of Pediatric Tropical Medicine
Baylor College of Medicine
Texas Children's Hospital Endowed Chair of Tropical Pediatrics
Director, Sabin Vaccine Institute & Texas Children's Hospital Center for Vaccine Development
President, Sabin Vaccine Institute
Houston, Texas

Contributors

John G. Aaskov, B.Sc., Ph.D., F.R.C.Path.
W.H.O. Collaborating Centre for Arbovirus Reference and Research, Institute of Health and Biomedical Innovation. Queensland University of Technology, Brisbane, Australia
Ross River Virus Arthritis; *Murray Valley Encephalitis*

Susan M. Abdel-Rahman, Pharm.D.
Pediatric Clinical Pharmacologist, Clinical Pharmacology and Medical Toxicology, Children's Mercy Hospital and Clinics; Professor, Department of Pediatrics, University of Missouri-Kansas City School of Medicine, Kansas City, Missouri
The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics

Kristina Adachi, M.D., M.A.
Research Fellow in Pediatric Infectious Diseases, Mattel Children's Hosptial, UCLA, Los Angeles, California
Rubella Virus

Christoph Aebi, M.D.
Professor of Pediatrics and Infectious Diseases, Chairman, Department of Pediatrics, University of Bern, Bern, Switzerland
Tick-Borne Encephalitis

Kenneth A. Alexander, M.D., Ph.D.
Professor of Pediatrics, Department of Pediatrics, The University of Chicago; Chief, Section of Pediatric Infectious Diseases, Department of Pediatrics, Comer Children's Hospital, Chicago, Illinois
Human Papillomaviruses

Nicole E. Alexander-Scott, M.D., M.P.H.
Adult-Pediatric Infectious Diseases, Brown University; Assistant Professor of Internal Medicine and Pediatrics, Rhode Island Department of Health; Medical Consultant, Division of Infectious Disease and Epidemiology, Providence, Rhode Island
Active Immunizing Agents

Margot Anderson, M.D.
Pediatric Infectious Disease Fellow, Tulane University, New Orleans, Louisiana
Bacillus cereus

Marsha S. Anderson, M.D.
Associate Professor of Pediatrics, Assistant Dean for Longitudinal Curriculum, University of Colorado School of Medicine, Aurora, Colorado
Meningococcal Disease

Stephen S. Arnon, M.D.
Founder and Chief, Infant Botulism Treatment and Prevention Program, California Department of Health Services, Richmond, California
Infant Botulism

Ann M. Arvin, M.D.
Lucile Salter Packard Professor of Pediatrics and Professor of Microbiology and Immunology, Vice Provost and Dean of Research, Stanford University School of Medicine, Stanford, California
Herpes Simplex Viruses 1 and 2

Robert L. Atmar, M.D.
Professor of Medicine, Baylor College of Medicine, Houston,Texas
Rhinoviruses

Carol J. Baker, M.D.
Professor, Departments of Pediatrics and Molecular Virology and Microbiology, Baylor College of Medicine, Houston, Texas
Cervical Lymphadenitis; *Group B Streptococcal Infections*

Robert S. Baltimore, M.D.
Professor, Pediatrics and Epidemiology, Yale University School of Medicine; Associate Director, Infection Control, Yale-New Haven Hospital, New Haven, Connecticut
Epidemiology and Biostatistics of Infectious Diseases

Stephen J. Barenkamp, M.D.
Professor of Pediatrics, Department of Pediatrics, St. Louis University School of Medicine; Director, Division of Pediatric Infectious Diseases, Cardinal Glennon Children's Medical Center, St. Louis, Missouri
Haemophilus influenzae; Other Haemophilus Species (ducreyi, haemolyticus, influenzae Biogroup aegyptius, parahaemolyticus, and parainfluenzae) and Aggregatibacter (Haemophilus) aphrophilus

Elizabeth D. Barnett, M.D.
Professor of Pediatrics, Department of Pediatrics, Boston University School of Medicine; Director, International Clinic, Department of Pediatrics, Boston Medical Center, Boston, Massachusetts
Malaria

William R. Beisel, M.D.
Adjunct Professor (Retired), W. Harry Feinstone Department of Molecular Microbiology and Immunology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Maryland
Metabolic Response of the Host to Infections

Elizabeth C. Bellino, M.D., M.P.H.
Department of Pediatric Infectious Disease, Tulane University School of Medicine, New Orleans, Louisiana; Staff Pediatrician, Department of Pediatrics, Mutolere Hospital, Kisoro, Uganda
Metabolic Response of the Host to Infections

Gil Benard, M.D., Ph.D.
Medical Researcher, Dermatology, University of São Paulo School of Medicine; Medical Researcher, Laboratory of Medical Mycology, Tropical Medicine Institute of the University of São Paulo, São Paulo, Brazil
Paracoccidioidomycosis

David I. Bernstein, M.D., M.A.
Professor of Pediatrics, Director of the Gamble Program for Clinical Studies, Cincinnati Children's Hospital Medical Center, University of Cincinnati Department of Pediatrics, Cincinnati, Ohio
Rotavirus

Andrea A. Berry, M.D.
Assistant Professor, Department of Pediatrics and Medicine, University of Maryland School of Medicine, Baltimore, Maryland
Diarrhea-Causing and Dysentery-Causing Escherichia coli

Laurence Bichaud, Ph.D.
Emergence des Pathologies Virales, Aix-Marseille University; IRD French Institute of Research for Development; EHESP French School of Public Health; Laboratory of Virology, IHU Mediterranee Infection, AP-HM Public Hospitals of Marseille, Marseille, France
Toscana Virus

Charles D. Bluestone, M.D.
Distinguished Professor of Otolaryngology, Department of Otolaryngology, University of Pittsburgh School of Medicine; Former Director, Department of Pediatric Otolaryngology, Children's Hospital of Pittsburgh of University of Pittsburgh Medical College, Pittsburgh, Pennsylvania
Otitis Media

Jeffrey L. Blumer, Ph.D., M.D.
Professor and Chairman, Department of Pediatrics, The University of Toledo, Toledo, Ohio
Antibiotic Resistance

Robert Bortolussi, M.D., F.R.C.P.C.
Professor, Department of Pediatrics; Professor of Pediatrics and Microbiology and Immunology; Professor, Microbiology and Immunology, Dalhousie University, Halifax, Nova Scotia, Canada
Listeriosis

Kenneth M. Boyer, M.D.
Woman's Board Professor of Pediatrics, Rush Medical College; Chairman, Department of Pediatrics, Rush Children's Hospital of Rush University Medical Center, Chicago, Illinois
Toxoplasmosis

John S. Bradley, M.D.
Professor of Pediatrics, Department of Pediatrics, Division of Infectious Diseases, University of California San Diego School of Medicine; Director, Infectious Diseases, Rady Children's Hospital San Diego, San Diego, California
Pediatric Community-Acquired Pneumonia; *Retroperitoneal Infections*; *Outpatient Intravenous Antimicrobial Therapy for Serious Infections*

Michael T. Brady, M.D.
Chair, Department of Pediatrics, The Ohio State University; Physician-in-Chief, Department of Pediatrics, Nationwide Children's Hospital, Columbus, Ohio
Pseudomonas and Related Genera

William J. Britt, M.D.
Professor, Department of Pediatrics, University of Alabama, Birmingham; Department of Pediatrics, Children's Hospital of Alabama, Birmingham, Alabama
Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinkeer Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia)

David E. Bronstein, M.D., M.S.
Department of Pediatrics, Southern California Permanente Medical Group, Palmdale, California
Aseptic Meningitis and Viral Meningitis; *Encephalitis and Meningoencephalitis*

David A. Bruckner, Sc.D.
Professor Emeritus, Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California
Classification of Fungi; *Nomenclature for Aerobic and Anaerobic Bacteria*; *Fungal Laboratory Analysis: Specimen Collection, Direct Detection, and Culture*; *Parasitic Laboratory Diagnosis*

Kristina Bryant, M.D.
Associate Professor, Department of Pediatrics, University of Louisville, Louisville, Kentucky
Antibiotic-Associated Colitis

Steven C. Buckingham, M.D.
Associate Professor, Department of Pediatrics, University of Tennessee College of Medicine; Attending Physician, Department of Pediatrics, Le Bonheur Children's Hospital, Memphis, Tennessee
Bacteroides, *Fusobacterium*, *and Prevotella*

Carrie L. Byington, M.D.
HA and Edna Benning Presidential Professor of Pediatrics; Vice Chair Research, Department of Pediatrics; Vice Dean, Academic Affairs & Faculty Development, School of Medicine, General Pediatrics and Infectious Diseases, University of Utah School of Medicine, Salt Lake City, Utah
Pediatric Community-Acquired Pneumonia; *Spirillum minus (Rat-Bite Fever)*

Miguel M. Cabada, M.D., M.Sc.
Instructor, Internal Medicine–Infectious Disease, University of Texas Medical Branch, Galveston, Texas; Research Associate, Internal Medicine–Tropical Medicine Institute, Universidad Peruana Cayetano Heredia, Lima, Peru
Foodborne Trematodes

Audrey P. Calzada, M.D.
Chief Resident, Head and Neck Surgery, University of California, Los Angeles, Los Angeles, California
Mastoiditis

Judith R. Campbell, M.D.
Associate Professor, Department of Pediatrics, Director of Fellowship Program in Pediatric Infectious Diseases, Baylor College of Medicine; Attending Physician, Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Parotitis; *Peritonitis and Intra-Abdominal Abscess*

Maria Carrillo-Marquez, M.D.
Assistant Professor, Department of Pediatrics, University of South Dakota, Sioux Falls, South Dakota
Otitis Externa

Mariam R. Chacko, M.B.B.S.
Professor, Department of Pediatrics, Baylor College of Medicine; Medical Director, Baylor College of Medicine Teen Health Clinics; Attending Physician, Adolescent Medicine Service, Texas Children's Hospital, Houston, Texas
Genital Infections; *Calymmatobacterium granulomatis;* *Trichomonas Infections*

Lakshmi Chandramohan, Ph.D.
Research Associate, Molecular Microbiology Laboratory, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Bacterial Laboratory Diagnosis

Louisa E. Chapman, M.D., M.S.P.H.
Medical Epidemiologist, Captain US Public Health Service, Division of Notifiable Health Care Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Hantaviruses

Rémi N. Charrel, M.D., Ph.D.
Professor of Virology, Emergence des Pathologies Virales, Aix-Marseille University; IRD French Institute of Research for Development; EHESP French School of Public Health; Laboratory of Virology, IHU Mediterranee Infection, AP-HM Public Hospitals of Marseille, Marseille, France
Lymphocytic Choriomeningitis Virus; *Arenaviral Hemorrhagic Fevers*; *Toscana Virus*

Elsa Chea-Woo, M.D.
Professor, Medical Clinical Department, Faculty of Medicine, Universidad Peruana Cayetano Heredia; Chief, Department of Pediatrics, Hospital Nacional Cayetano Heredia, Lima, Peru
Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

Ira M. Cheifetz, M.D., F.C.C.M., F.A.A.R.C.
Professor of Pediatrics, Division Chief, Department of Pediatrics, Pediatric Critical Care Medicine, Duke Children's Hospital; Director, Pediatric Critical Care Services, Duke University Health System; Medical Director, Pediatric Intensive Care Unit and Pediatric Respiratory Care Services, Duke University Medical Center, Durham, North Carolina
Acute Respiratory Distress Syndrome in Children

Leon L. Chen, M.D.
Fellow, Pediatric Gastroenterology, Department of Pediatrics, Baylor College of Medicine; Fellow, Pediatric Gastroenterology, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Hepatitis

James D. Cherry, M.D., M.Sc.
Distinguished Professor of Pediatrics, David Geffen School of Medicine at UCLA; Attending Physician, Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
The Common Cold; *Pharyngitis (Pharyngitis*, *Tonsillitis*, *Tonsillopharyngitis*, *and Nasopharyngitis)*; *Rhinosinusitis*; *Mastoiditis*; *Croup (Laryngitis*, *Laryngotracheitis*, *Spasmodic Croup*, *Laryngotracheobronchitis*, *Bacterial Tracheitis*, *and Laryngotracheobronchopneumonitis) and Epiglottitis (Supraglottitis)*; *Acute Bronchitis*; *Cutaneous Manifestations of Systemic Infections*; *Roseola Infantum (Exanthem Subitum)*; *Aeromonas; Pertussis and Other Bordetella Infections*; *Tetanus*; *Adenoviruses*; *Smallpox (Variola Virus)*; *Enteroviruses, Parechoviruses, and Saffold Viruses*; *Reoviruses*; *Rubella Virus*; *Measles Virus*; *Mumps Virus*; *Mycoplasma and Ureaplasma Infections*

Javier Chinen, M.D., Ph.D.
Private Practice Physician, Lake Houston Asthma Allergy and Immunology, Humble, Texas
Primary Immunodeficiencies

Natascha Ching, M.D.
Physician, Department of Pediatric Infectious Diseases, 'Specially for Children, Austin, Texas
Arcanobacterium haemolyticum

Susan E. Coffin, M.D., M.P.H.
Associate Professor of Pediatrics, Division of Infectious Diseases, Perelman School of Medicine at the University of Pennsylvania; Hospital Epidemiologist and Medical Director, Department of Infection Prevention and Control, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Health Care–Associated Infections

Armando G. Correa, M.D.
Assistant Professor, Department of Pediatrics, Baylor College of Medicine; Attending Physician, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Acinetobacter

Holly Corwin, M.P.H.
Research Assistant, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
Global Health

Andrea T. Cruz, M.D., M.P.H.
Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
Tuberculosis; *Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses*

Zev Davidovics, M.D.
Fellow, Department of Pediatric Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas
Whipple Disease

Eszter Deak, M.D., M.P.H.
Postdoctoral Fellow, Clinical Microbiology, Department of Pathology and Laboratory Medicine, University of California, Los Angeles, Los Angeles, California
Classification of Fungi

Xavier de Lamballerie, M.D.
Professor, Emergence des Pathologies Virales, Aix-Marseille University; IRD French Institute of Research for Development; EHESP French School of Public Health; Laboratory of Virology, IHU Mediterranee Infection, AP-HM Public Hospitals of Marseille, Marseille, France
Lymphocytic Choriomeningitis Virus; *Arenaviral Hemorrhagic Fevers*;*Toscana Virus*

Penelope H. Dennehy, M.D.
Director, Division of Pediatric Infectious Diseases, Hasbro Children's Hospital; Professor, Department of Pediatrics, The Warren Alpert Medical School of Brown University, Providence, Rhode Island
Active Immunizing Agents

Minh L. Doan, M.D.
Chief, Pediatric Pulmonology, Department of Pediatrics, Tripler Army Medical Center, Honolulu, Hawaii
Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis

Simon R. Dobson, M.D., F.R.C.P.C.
Clinical Associate Professor, Department of Pediatric Infectious and Immunological Diseases, British Columbia Children's Hospital, Vancouver, Canada
Syphilis

Jan E. Drutz, M.D.
Professor of Pediatrics, Department of Pediatrics, Baylor College of Medicine; Professor of Pediatrics, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Arthropods

Kara Dubray, M.D.
Fellow, Department of Pediatric Infectious Diseases, Children's Hospital & Research Center, Oakland, California; Research Fellow, Communicable Diseases and Emergency Response Branch, California Department of Public Health, Richmond, California
Streptobacillus moniliformis (Rat-Bite Fever); *Spirillum minus (Rat-Bite Fever)*

Andrea Duppenthaler, M.D.
Head of Unit, Department of Pediatric Infectious Diseases, University Children's Hospital Bern, Bern, Switzerland
Tick-Borne Encephalitis

Christopher C. Dvorak, M.D.
Assistant Professor, Department of Pediatric Blood and Marrow Transplant, University of California, San Francisco, San Francisco, California
Opportunistic Infections in Hematopoietic Stem Cell Transplantation

Paul H. Edelstein, M.D.
Professor of Pathology and Laboratory Medicine, Department of Pathology and Laboratory Medicine, Perelman School of Medicine at the University of Pennsylvania; Director of Clinical Microbiology, Department of Pathology and Laboratory Medicine, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania
Legionnaires' Disease, Pontiac Fever, and Related Illnesses

Kathryn M. Edwards, M.D.
Sarah H. Sell Professor of Pediatrics, Department of Pediatrics, Vanderbilt University Medical Center, Nashville, Tennessee
Bartonella Infections

Morven S. Edwards, M.D.
Professor of Pediatrics, Baylor College of Medicine; Attending Physician, Department of Pediatrics, Section of Infectious Diseases, Texas Children's Hospital, Houston, Texas
Mediastinitis; *Anthrax*; *Animal and Human Bites*

B. Keith English, M.D.
Professor and Chair, Department of Pediatrics and Human Development, College of Human Medicine, Michigan State University, East Lansing, Michigan
Enterococcal and Viridans Streptococcal Infections

Janet A. Englund, M.D.
Professor, Department of Pediatrics, University of Washington/Seattle Children's Hospital, Seattle, Washington
Rhinoviruses; *Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome*

Leland L. Fan, M.D.
Professor, Department of Pediatrics, University of Colorado School of Medicine, Aurora, Colorado
Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis

[†]Ralph D. Feigin, M.D.
Formerly J.S. Abercrombie Professor, Chairman, Department of Pediatrics, Distinguished Service Professor, Baylor College of Medicine; Physician-in-Chief, Texas Children's Hospital, Ben Taub General Hospital; Chief, Pediatric Service, The Methodist Hospital, Houston, Texas
Metabolic Response of the Host to Infections

Philip R. Fischer, M.D.
Professor of Pediatrics, Mayo Clinic, Rochester, Minnesota
Cestodes; *Foodborne Trematodes*; *Schistosomiasis*

Brian T. Fisher, D.O., M.P.H., M.S.C.E.
Assistant Professor of Pediatrics, Department of Pediatrics, Division of Infectious Diseases, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania
The Febrile Neutropenic Patient

Randall G. Fisher, M.D.
Associate Professor, Pediatrics, Eastern Virginia Medical School; Medical Director, Division of Pediatric Infectious Diseases, Children's Hospital of The King's Daughters, Norfolk, Virginia
Miscellaneous Gram-Positive Cocci; *Erysipelothrix rhusiopathiae; Corynebacterium and Rhodococcus; Citrobacter; Enterobacter; Klebsiella; Morganella morganii; Proteus; Providencia; Serratia; Miscellaneous Enterobacteriaceae; Vibrio vulnificus; Miscellaneous Non-Enterobacteriaceae Fermentative Bacilli*; *Achromobacter (Alcaligenes); Eikenella corrodens; Elizabethkingia and Chryseobacterium Species*

Douglas S. Fishman, M.D., F.A.S.G.E.
Associate Professor, Department of Pediatrics, Baylor College of Medicine; Director, Gastrointestinal Endoscopy, Department of Pediatric Gastroenterology, Hepatology, and Nutrition, Texas Children's Hospital, Houston, Texas
Cholangitis and Cholecystitis

Anthony R. Flores, M.D., M.P.H., Ph.D.
Assistant Professor, Infectious Diseases, Department of Pediatrics, Baylor College of Medicine; Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Group A, Group C, Group G Beta Hemolytic Streptococcal Infections

Ellen M. Friedman, M.D., F.A.A.P., F.A.C.S.
Professor, Dr. Bobby R. Alford Chair in Pediatric Otolaryngology, Baylor College of Medicine; Chief of Service, Department of Otolaryngology, Texas Children's Hospital, Houston, Texas
Otitis Externa

Carla Garcia, M.D.
Assistant Professor, Department of Pediatrics, University of Texas Southwestern Medical Center, Dallas, Texas
Antibacterial Therapeutic Agents

Lynne S. Garcia, M.S., C.L.S., M.T. (A.S.C.P.), F.A.A.M.
Director, LSG & Associates, Santa Monica, California
Classification and Nomenclature of Human Parasites; *Leishmaniasis*; *Trypanosomiasis*

Gregory M. Gauthier, M.D., M.S.
Assistant Professor (CHS), Department of Medicine, Division of Infectious Diseases, University of Wisconsin, Madison, Wisconsin
Blastomycosis

Patrick J. Gavin, M.D.
Department of Pathology and Laboratory Medicine, Evanston Northwestern Heathcare, Evanston, Illinois
Naegleria, Acanthamoeba, and Balamuthia Infections

[†]Deceased.

Anne A. Gershon, M.D.
Professor, Department of Pediatrics, Columbia University College of Physicians and Surgeons, New York, New York
Varicella-Zoster Virus

Francis Gigliotti, M.D.
Professor and Chief of Infectious Diseases, Associate Chair for Academic Affairs, Department of Pediatrics, University of Rochester School of Medicine and Dentistry, Rochester, New York
Pneumocystis Pneumonia

Mark A. Gilger, M.D.
Professor of Pediatrics, Section of Pediatric Gastroenterology, Hepatology, and Nutrition, Department of Pediatrics, Baylor College of Medicine, Houston, Texas; Physician-in-Chief, Children's Hospital of San Antonio, San Antonio, Texas
Whipple Disease; *Helicobacter pylori*

Susan L. Gillespie, M.D.
Associate Professor, Section of Retrovirology and Global Health, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Oncoviruses (Human T-Cell Lymphotropic Viruses) and Lentiviruses (Human Immunodeficiency Virus Type 2)

Carol A. Glaser, D.V.M., M.P.V.M., M.D.
Chief of Encephalitis and Special Investigations Section, Communicable Disease and Emergency Response Branch, Division of Communicable Disease Control, California Department of Public Health, Richmond, California
Aseptic Meningitis and Viral Meningitis; *Encephalitis and Meningoencephalitis*; *Streptobacillus moniliformis (Rat-Bite Fever)*

Mary P. Glodé, M.D.
Professor of Pediatrics, Department of Pediatrics, University of Colorado School of Medicine; Chief, Pediatric Infectious Disease, Children's Hospital Colorado, Aurora, Colorado
Meningococcal Disease

David Goldman, M.D.
Associate Professor, Department of Pediatrics, Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, New York
Cryptococcosis

Jennifer L. Goldman, M.D.
Department of Pediatrics, Children's Mercy Hospitals and Clinics, Kansas City, Missouri
The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics

Nira A. Goldstein, M.D., M.P.H.
Associate Professor, Attending Physician, Interim Director, Department of Pediatric Otolaryngology, Department of Otolaryngology, State University of New York Downstate Medical Center, Brooklyn, New York
Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses

Edmond T. Gonzales, Jr., M.D.
Professor of Urology, Scott Department of Urology, Baylor College of Medicine; Pediatric Urology Service, Texas Children's Hospital, Houston, Texas
Renal Abscess

Blanca E. Gonzalez, M.D.
Center for Pediatric Infectious Diseases, Associate Staff, Cleveland Clinic, Cleveland, Ohio
Infections of the Oral Cavity

Michael Green, M.D., M.P.H.
Professor, Department of Pediatrics, Surgery and Clinical and Translational Research, University of Pittsburgh School of Medicine; Attending Physician, Division of Infectious Diseases, Children's Hospital of Pittsburgh of University of Pittsburgh Medical College, Pittsburgh, Pennsylvania
Opportunistic Infections in Liver and Intestinal Transplantation

Andreas H. Groll, M.D.
Associate Professor of Pediatrics, Department of Pediatric Hematology and Oncology, Westphalian Wilhelm University; Attending Physician and Head, Infectious Disease Research Program, Center for Bone Marrow Transplantation and Department of Pediatric Hematology/Oncology, Children's University Hospital, Münster, Germany
Antifungal Agents

Charles Grose, M.D.
Professor, Department of Pediatrics, University of Iowa; Director of Infectious Diseases, Children's Hospital, Iowa City, Iowa
Bacterial Myositis and Pyomyositis; *Human Herpesviruses 6, 7, and 8*

Duane J. Gubler, Sc. D., F.A.A.A.S., F.I.D.S.A.
Professor, Signature Research Program in Emerging Infectious Diseases, Duke–NUS Graduate Medical School, Singapore
Yellow Fever

Javier Nieto Guevara, M.D., M.P.H.
Deputy Director, Dirección General, Instituto Conmemorativo Gorgas de Estudios de la Salud, Panamá, Republic of Panama
Parameningeal Infections

Kathleen Gutierrez, M.D.
Associate Professor, Department of Pediatrics, Division of Pediatric Infectious Disease, Stanford University School of Medicine, Stanford, California
Herpes Simplex Viruses 1 and 2

†Caroline Breese Hall, M.D.
Formerly Professor, Department of Pediatrics and Medicine, University of Rochester School of Medicine and Dentistry, Rochester, New York
Parainfluenza Viruses; *Respiratory Syncytial Virus*

Scott B. Halstead, M.D.
Senior Advisor, Dengue Vaccine Initiative, International Vaccine Institute, Seoul, Korea; Adjunct Professor, Department of Preventive Medicine and Biometrics, Uniformed Services University of the Health Sciences, Bethesda, Maryland
Chikungunya; *Dengue, Dengue Hemorrhagic Fever, and Severe Dengue*

Shinjiro Hamano, M.D., Ph.D.
Professor, Institute of Tropical Medicine, Nagasaki University, Japan
Amebiasis

Margaret R. Hammerschlag, M.D.
Professor, Department of Pediatrics and Medicine, State University of New York Downstate Medical Center College of Medicine; Director, Division of Pediatric Infectious Diseases, State University of New York Downstate Medical Center, Brooklyn, New York
Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses; *Chlamydia Infections*

I. Celine Hanson, M.D.
Professor, Department of Pediatrics, Section of Allergy, Immunology and Rheumatology, Baylor College of Medicine; Clinic Chief, Allergy and Immunology Service, Texas Children's Hospital, Houston, Texas
Chronic Bronchitis

Nada Harik, M.D.
Assistant Professor, Department of Pediatrics, Division of Pediatric Infectious Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Nocardia

Kathleen H. Harriman, Ph.D., M.P.H., R.N.
Chief, Vaccine Preventable Diseases Epidemiology Section, Immunization Branch, California Department of Public Health, Richmond, California
Public Health Aspects of Infectious Disease Control

Gail J. Harrison, M.D.
Professor, Department of Pediatrics, Section of Infectious Diseases, Baylor College of Medicine; Attending Physician, Infectious Diseases Service, Texas Children's Hospital, Houston, Texas
Approach to Infections in the Fetus and Newborn; *Opportunistic Infections in Kidney Transplantation*; *Human Papillomaviruses*; *Cytomegalovirus*; *Epstein-Barr Virus*; *Mimiviruses*; *Eastern Equine Encephalitis*; *Western Equine Encephalitis*; *Venezuelan Equine Encephalitis*; *Yellow Fever*; *Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers*; *Rift Valley Fever*; *Crimean-Congo Hemorrhagic Fever*; *Phlebotomus Fever (Sandfly Fever)*; *Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinker Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia)*; *Global Health*; *Antiviral Agents*; *Probiotics*

Rick E. Harrison, M.D.
Professor of Clinical Pediatrics, Pediatrics, David Geffen School of Medicine at UCLA; Medical Director, Mattel Children's Hospital UCLA, Los Angeles, California
Tetanus

C. Mary Healy, M.B., B.Ch., B.A.O., M.D.
Assistant Professor, Department of Pediatrics, Infectious Diseases Section, Baylor College of Medicine; Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Cervical Lymphadenitis

Ulrich Heininger, M.D.
Professor and Chair, Pediatric Infectious Diseases, University Children's Hospital; Member, Medical Faculty, University of Basel, Basel, Switzerland
Pertussis and Other Bordetella Infections

Sheryl L. Henderson, M.D., Ph.D.
Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, American Family Children's Hospital, Madison, Wisconsin
Infections in Child Care Environments

Gloria P. Heresi, M.D.
Professor of Pediatrics, Division Director, Infectious Diseases, Division Director, Fellowship Program, Medical Director, Donald M. Gross Pediatric Group Research, Department of Pediatrics, University of Texas–Houston Medical School, Houston, Texas
Campylobacter jejuni

Peter W. Hiatt, M.D.
Associate Professor and Section Head, Pulmonary Medicine, Department of Pediatrics , Baylor College of Medicine; Chief of Pulmonary Medicine, Department of Pediatrics, Texas Children's Hospital, Houston, Texas
Cystic Fibrosis

†Deceased.

Harry R. Hill, M.D.
Professor, Department of Pathology, Pediatrics, and Internal Medicine, University of Utah, Salt Lake City, Utah
Immunomodulating Agents

David C. Hilmers, M.D., E.E., M.P.H.
Associate Professor, Departments of Internal Medicine and Pediatrics, Center for Space Medicine, Baylor College of Medicine, Houston, Texas
Nonvenereal Treponematoses

Jill A. Hoffman, M.D.
Associate Professor, Department of Pediatrics, Keck School of Medicine of the University of Southern California; Attending Physcian, Division of Infectious Diseases, Children's Hospital Los Angeles, Los Angeles, California
Infections in Pediatric Lung Transplantation

J. Lloyd Holder, Jr., M.D., Ph.D.
Assistant Professor, Department of Pediatrics, Division of Neurology and Developmental Neuroscience, Baylor College of Medicine; Assistant Professor, Department of Pediatrics, Division of Neurology and Developmental Neuroscience, Texas Children's Hospital, Houston, Texas
Parainfectious and Postinfectious Demyelinating Disorders of the Central Nervous System; *Infection-Associated Myelitis and Myelopathies of the Spinal Cord*

Peter J. Hotez, M.D., Ph.D.
Dean, National School of Tropical Medicine, Professor, Pediatrics and Molecular & Virology and Microbiology, Head, Section of Pediatric Tropical Medicine, Baylor College of Medicine; Texas Children's Hospital Endowed Chair of Tropical Pediatrics; Director, Sabin Vaccine Institute & Texas Children's Hospital Center for Vaccine Development; President, Sabin Vaccine Institute; Houston, Texas
Blastocystis hominis and Blastocystis spp. Infection; *Entamoeba coli Infection*; *Balantidium coli Infection*; *Parasitic Nematode Infections*

Leigh M. Howard, M.D., M.P.H.
Fellow, Pediatric Infectious Diseases, Vanderbilt University Medical Center, Nashville, Tennessee
Bartonella Infections

Kristina G. Hulten, Ph.D.
Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

Romney M. Humphries, Ph.D.
Assistant Professor, Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California
Viral Laboratory Diagnosis

David A. Hunstad, M.D.
Associate Professor of Pediatrics and Molecular Microbiology, Washington University; Attending Physician, Pediatric Infectious Diseases, St. Louis Children's Hospital, St. Louis, Missouri
Molecular Determinants of Microbial Pathogenesis

W. Garrett Hunt, M.D.
Associate Professor, Section of Infectious Diseases, Department of Pediatrics, College of Medicine, The Ohio State University, Nationwide Children's Hospital, Columbus, Ohio
La Crosse Virus and Other California Serogroup Viruses

Eugene S. Hurwitz, M.D.
Associate Clinical Professor, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia
Reye Syndrome

W. Charles Huskins, M.D., M.Sc.
Assistant Professor of Pediatrics, Mayo Clinic Medical School; Consultant, Pediatric Infectious Diseases, Mayo Clinic; Chair, Quality and Safety, Department of Pediatric and Adolescent Medicine, Mayo Clinic Children's Center, Rochester, Minnesota
Health Care–Associated Infections

Mohamed Hussein, M.D.
Assistant Professor, Department of Ophthalmology, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas
Ocular Infections

David Y. Hyun, M.D.
Associate Professor, Department of Pediatrics, George Washington University School of Medicine and Health Sciences; Children's National Medical Center, Center for Cancer and Immunology Research, Washington, District of Columbia
Coagulase-Negative Staphylococcal Infections; *Vibrio parahaemolyticus*

Mary Anne Jackson, M.D.
Professor, Department of Pediatrics, University of Missouri, Kansas City School of Medicine; Division Chief, Infectious Diseases, Department of Pediatrics, The Children's Mercy Hospital, Kansas City, Missouri
Bacterial Skin Infections

Michael R. Jacobs, M.B., B.Ch., Ph.D.
Professor, Department of Pathology, Case Western Reserve University; Director, Clinical Microbiology, University Hospitals Case Medical Center, Cleveland, Ohio
Pneumococcal Infections

Richard F. Jacobs, M.D., F.A.A.P.
Robert H. Fiser, Jr. MD Endowed Chair in Pediatrics, Department of Pediatrics, Arkansas Children's Hospital; Professor, Department of Pediatrics, University of Arkansas for Medical Sciences; President, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas
Complications of Pneumonia; *Fungal Meningitis*; *Other Mycobacteria*; *Nocardia; Aggregatibacter actinomycetemcomitans; Actinomycosis*

Ravi Jhaveri, M.D.
Clinical Associate Professor of Pediatrics, Division of Allergy, Immunology, Rheumatology, and Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, North Carolina
Molecular Determinants of Microbial Pathogenesis; *Hepatitis E Virus*; *Hepatitis C Virus*

Samantha H. Johnston, M.D.
Associate Physician, Division of Pediatric Infectious Diseases, Children's Hospital & Research Center Oakland, Oakland, California
Monkeypox and Other Poxviruses

Maureen Jonas, M.D.
Professor, Department of Pediatrics, Harvard Medical School; Senior Associate in Medicine, Gastroenterology, Hepatology, and Nutrition, Boston Children's Hospital, Boston, Massachusetts
Hepatitis B and D Viruses

Meena R. Julapalli, M.D.
Clinical Assistant Professor, Department of Dermatology, University of Texas, Southwestern, Dallas, Texas; Dell Children's Hospital, Department of Pediatric Dermatology, Department of Pediatric and Adolescent Dermatology, 'Specially for Children, Austin, Texas
Viral and Fungal Skin Infections

Sheldon L. Kaplan, M.D.
Professor and Vice-Chairman for Clinical Affairs, Head, Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine; Chief, Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Infectious Pericarditis; *Renal Abscess*; *Prostatitis*; *Pyogenic Liver Abscess*; *Bacteremia and Septic Shock*; *Infections in Pediatric Heart Transplantation*; *Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)*

David A. Kaufman, M.D.
Associate Professor, Department of Pediatrics, University of Virginia School of Medicine, Charlottesville, Virginia
Candidiasis

Gregory L. Kearns, Pharm.D., Ph.D.
Professor and Associate Chairman, Department of Pediatrics, Children's Mercy Hospital, Kansas City, Missouri
The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics

Kwang Sik Kim, M.D.
Professor and Director, Division of Pediatric Infectious Diseases, Johns Hopkins University School of Medicine, Baltimore, Maryland
Bacterial Meningitis Beyond the Neonatal Period

Martin B. Kleiman, M.D.
Ryan White Professor of Pediatrics, Department of Pediatrics, Indiana University School of Medicine, Indianapolis, Indiana
Histoplasmosis

Bruce S. Klein, M.D.
Gerard B. Odell Professor, Department of Pediatrics; Professor, Internal Medicine; Professor, Medical Microbiology and Immunology, University of Wisconsin-Madison, Madison, Wisconsin
Blastomycosis

Jerome O. Klein, M.D.
Professor, Department of Pediatrics, Boston University School of Medicine; Former Director, Division of Pediatric Infectious Diseases, Boston Medical Center, Boston, Massachusetts
Otitis Media

Mark W. Kline, M.D.
J.S. Abercrombie Professor and Chairman, Ralph D. Feigin Chair, Department of Pediatrics, Baylor College of Medicine; Physician-in-Chief, Texas Children's Hospital; President, Baylor International Pediatric AIDS Initiative, Houston, Texas
Primary Immunodeficiencies

Stephan A. Kohlhoff, M.D.
Assistant Professor, Department of Pediatrics and Medicine, State University of New York Downstate Medical Center, Brooklyn, New York
Chlamydia Infections

Tobias Kollmann, M.D., Ph.D.
Assistant Professor, Department of Pediatrics, Division of Infectious Diseases, University of British Columbia, Vancouver, British Columbia, Canada
Listeriosis

Margaret Kosek, M.D.
Assistant Professor, Department of International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland; Principal Investigator, Department of Biomedical Investigation, Asociacion Benefica PRISMA, Iquitos, Loreto, Peru
Shigella

Michael P. Koster, M.D., F.A.A.P.
Clinical Assistant Professor of Pediatrics, Alpert Medical School of Brown University; Hasbro Children's Hospital, Providence, Rhode Island
Active Immunizing Agents

Peter J. Krause, M.D.
Senior Research Scientist, Yale School of Public Health, Yale School of Medicine, New Haven, Connecticut
Lyme Disease; Relapsing Fever; Babesiosis

Leonard R. Krilov, M.D.
Chief, Pediatric Infectious Diseases; Vice Chairman, Department of Pediatrics, Children's Medical Center, Winthrop University Hospital, Mineola, New York; Professor of Pediatrics, State University of New York, Stony Brook School of Medicine, Stony Brook, New York
Chronic Fatigue Syndrome

Paul Krogstad, M.D.
Professor, Departments of Pediatrics and Molecular and Medical Pharmacology, David Geffen School of Medicine at UCLA; Attending Physician, Mattel Children's Hospital UCLA, Los Angeles, California
Esophagitis; *Osteomyelitis*; *Septic Arthritis*; *Enteroviruses, Parechoviruses, and Saffold Viruses*

Damian J. Krysan, M.D., Ph.D.
Associate Professor, Department of Pediatrics and Microbiology/Immunology, University of Rochester, Rochester, New York
Fusariosis and Scedosporiosis

Thomas L. Kuhls, M.D.
Private Practice Pediatrician, Norman Pediatric Associates, Norman, Oklahoma
Appendicitis and Pelvic Abscess; *Pancreatitis*

Paul M. Lantos, M.D.
Medical Instructor, Division of Pediatric Infectious Diseases, Duke University School of Medicine, Durham, North Carolina
Rickettsial and Ehrlichial Diseases

Timothy R. La Pine, M.D.
Professor, Departments of Pediatrics and Pathology, University of Utah; Director of Neonatology, St. Mark's Hospital, Salt Lake City, Utah
Immunomodulating Agents

Matthew B. Laurens, M.D., M.P.H.
Assistant Professor, Howard Hughes Medical Institute, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland
Cholera

Charles T. Leach, M.D.
Professor, Department of Pediatrics, Division of Infectious Diseases & Immunology, University of Texas Health Sciences Center, San Antonio, Texas
Epstein-Barr Virus

Grace E. Lee, M.D.
Fellow, Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Candidiasis

Jan Hau Lee, M.B.B.S., M.R.C.P.C.H.
Pediatric Critical Care Medicine, Duke Children's Hospital, Durham, North Carolina; Children's Intensive Care Unit, KK Women's and Children's Hospital, Singapore
Acute Respiratory Distress Syndrome in Children

Robert J. Leggiadro, M.D.
Adjunct Clinical Professor, Department of Pediatrics, Hofstra North Shore–LIJ School of Medicine, Hempstead, New York; Medical Director, Vaccines, Medicines Development Group, Pfizer Incorporated, Collegeville, Pennsylvania
Bioterrorism

Pedro Legua, M.D., M.Sc. (C.T.M.)
Professor, Department of Medicine, Universidad Peruana Cayetano Heredia; Attending Physician, Departamento de Enfermedades Infecciosas, Tropicales y Dermatológicas, Hospital Nacional Cayetano Heredia, Lima, Peru
Cestodes

Deborah Lehman, M.D.
Professor, Department of Pediatrics, David Geffen School of Medicine at UCLA; Associate Director, Pediatric Infectious Diseases, Cedars-Sinai Medical Center, Los Angeles, California
Passive Immunization

Diana R. Lennon, M.B., Ch.B., F.R.A.C.P.
Professor of Population Health, Child, and Youth, Department of Paediatrics, University of Auckland; Paediatrician, Department of Paediatric Infectious Diseases, Starship Children's Hospital, Auckland, New Zealand
Acute Rheumatic Fever

Eric Leroy, M.D., D.V.M., Ph.D.
Director General, Center for International Medical Research in Franceville, Franceville, Gabon
Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers

Daniel H. Leung, M.D.
Assistant Professor, Department of Pediatrics, Section of Gastroenterology, Hepatology and Nutrition, Baylor College of Medicine; Medical Director, Viral Hepatitis Clinic, Texas Children's Hospital, Houston, Texas
Hepatitis

Moise L. Levy, M.D.
Clinical Professor, Dermatology, University of Texas Southwestern Medical School, Dallas, Texas; Clinical Professor, Dermatology and Pediatrics, Baylor College of Medicine, Houston, Texas; Chief, Pediatric Dermatology, Physician-in-Chief, Dell Children's Medical Center, Austin, Texas
Viral and Fungal Skin Infections

W. Matthew Linam, M.D., M.S.
Assistant Professor, Division of Pediatric Infectious Diseases, University of Arkansas for Medical Sciences and Arkansas Children's Hospital, Little Rock, Arkansas
Other Mycobacteria

Timothy E. Lotze, M.D.
Associate Professor, Departments of Pediatrics and Neurology, Baylor College of Medicine; Medical Director, Blue Bird Circle Multiple Sclerosis, Muscular Dystrophy, and Peripheral Nerve Clinics; Texas Children's Hospital, Houston, Texas
Parainfectious and Postinfectious Demyelinating Disorders of the Central Nervous System; *Infection-Associated Myelitis and Myelopathies of the Spinal Cord*

Yalda C. Lucerno, M.D., Ph.D.
Assistant Professor, Department of Pediatrics, Faculty of Medicine, University of Chile; Pediatrician, Department of Pediatrics, Dr. Luis Calvo Mackenna Hospital, Santiago Metropolitan Region, Chile
Caliciviruses

Ruth Ann Luna, Ph.D.
Director, DNA Sequencing Core, Texas Children's Microbiome Center, Department of Pathology, Texas Children's Hospital, Houston, Texas
Cystic Fibrosis

Timothy Mailman, M.D., F.R.C.P.C.
Associate Professor, Departments of Pathology and Pediatrics, Dalhousie University; Staff, Division of Infectious Diseases, Department of Pediatrics, IWK Health Centre, Halifax, Nova Scotia, Canada
Listeriosis

Susan A. Maloney, M.D., M.H.Sc.
Global TB Coordinator, Center for Disease Control and Prevention, U.S. Centers for Disease Control and Prevention, Atlanta, Georgia
International Travel Issues for Children

Mario J. Marcon, Ph.D.
Clinical Professor of Pathology and Pediatrics, Ohio State University College of Medicine; Director, Clinical/Molecular Microbiology and Virology, Laboratory Medicine, Nationwide Children's Hospital, Columbus, Ohio
Pseudomonas and Related Genera

Kimberly C. Martin, D.O.
Pediatric Infectious Disease Fellow, Infectious Disease, Children's National Medical Center, Washington, District of Columbia; Medical Officer, Division of Anti-Infective Products, U.S. Food and Drug Administration, Silver Spring, Maryland
St. Louis Encephalitis

Laurene Mascola, M.D., M.P.H., F.A.A.P.
Chief, Acute Communicable Disease Program, Los Angeles County Department of Public Health, Los Angeles, California
Public Health Aspects of Infectious Disease Control

Edward O. Mason, Ph.D.
Professor, Department of Pediatrics, Baylor College of Medicine; Infectious Disease Service, Texas Children's Hospital, Houston, Texas
Staphylococcus aureus Infections (Coagulase-Positive Staphylococci)

Wilbert H. Mason, M.D., M.P.H.
Children's Hospital Los Angeles; Department of Pediatrics, The Keck School of Medicine at the University of Southern California, Los Angeles, California
Legionnaires' Disease, *Pontiac Fever*, *and Related Illnesses*

Aldo Maspons, M.D.
Fellow, Pediatric Gastroenterology, Gastroenterology, Hepatology, and Nutrition, Baylor College of Medicine, Houston, Texas
Helicobacter pylori

David O. Matson, M.D., Ph.D.
Professor of Health Professions and Pediatrics, Director, Graduate Program in Public Health, Eastern Virginia Medical School, Old Dominion University; Attending Physician in Pediatric Infectious Diseases, Children's Hospital of The King's Daughters, Sentara Norfolk General Hospital, Norfolk, Virginia
Caliciviruses

Marc A. Mazade, M.D.
Consultant, Pediatric Infectious Diseases, Medical Director of Infection Control, Division of Pediatric Infectious Diseases, Cook Children's Medical Center, Fort Worth, Texas
Infections Related to Craniofacial Surgical Procedures

James B. McAuley, M.D., M.P.H.
Professor of Pediatrics and Internal Medicine, Department of Pediatrics, Rush University Medical Center, Chicago, Illinois; Medical Epidemiologist, Center for Global Health, Centers for Disease Control and Prevention, Lusaka, Zambia
Toxoplasmosis

George H. McCracken, Jr., M.D.
The GlaxoSmthKline Distinguished Professor of Pediatric Infectious Disease, Department of Pediatrics, University of Texas Southwestern Medical Center at Dallas; Attending Physician, Children's Medical Center Dallas, Dallas, Texas
Antibacterial Therapeutic Agents

Kenneth McIntosh, M.D.
Professor, Department of Pediatrics, Harvard Medical School; Senior Physician, Medicine, Children's Hospital, Boston, Massachusetts
Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome

James E. McJunkin, M.D.
Professor of Pediatrics, West Virginia University Health Sciences Center, Charleston South Carolina
La Crosse Virus and Other California Serogroup Viruses

Kelly T. McKee, Jr., M.D., M.P.H.
Vice President and Managing Director, Public Health and Government Services, Quintiles Transnational Corporation, Durham, North Carolina
Hantaviruses

Ross McKinney, Jr., M.D.
Professor, Department of Pediatrics, Duke University School of Medicine, Durham, North Carolina
Rickettsial and Ehrlichial Diseases

Rima L. McLeod, M.D.
Departments of Pediatrics (Infectious Diseases) and Ophthalmology and Visual Sciences, Committees on Molecular Medicine and Immunology and Fellow, Institute of Systems Biology, The University of Chicago; Attending Physician, The University of Chicago Hospitals, Chicago, Illinois
Toxoplasmosis

Monica Malone McNeal, M.S.
Associate Director, Laboratory for Specialized Clinical Studies, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
Rotavirus

H. Cody Meissner, M.D.
Professor of Pediatrics, Tufts University School of Medicine; Chief, Pediatric Infectious Disease, Department of Pediatrics, Tufts Medical Center, Boston, Massachusetts
Respiratory Syncytial Virus

Maria José Soares Mendes-Giannini, Ph.D.
Full Professor, Análises Clínicas, University Estadual Paulista-UNESP; Research Vice-Rector, UNESP, Araraquara, São Paulo, Brazil
Paracoccidioidomycosis

Marian G. Michaels, M.D., M.P.H.
Professor of Pediatrics and Surgery, Department of Pediatrics, University of Pittsburgh School of Medicine; Division of Pediatric Infectious Diseases, Children's Hospital of Pittsburgh of University of Pittsburgh Medical College, Pittsburgh, Pennsylvania
Opportunistic Infections in Liver and Intestinal Transplantation

Ian C. Michelow, M.D.
Assistant Professor of Pediatrics, Warren Alpert Medical School of Brown University, Center for International Health Research, Hasbro Children's Hospital, Division of Pediatric Infectious Diseases, Providence, Rhode Island
Active Immunizing Agents

Aaron M. Miller, M.D., M.B.A.
Clinical Faculty Instructor, Ophthalmology, Baylor College of Medicine; Pediatric Ophthalmologist, Houston Eye Associates, Houston, Texas
Ocular Infections

Marjorie J. Miller, Dr.P.H.
Senior Specialist, Virology, Department of Pathology and Laboratory Medicine, University of California, Los Angeles Medical Center, Los Angeles, California
Classification and Nomenclature of Viruses; *Viral Laboratory Diagnosis*

James N. Mills, M.D.
Adjunct Faculty, Population Biology, Ecology, and Evolution, Emory University, Atlanta, Georgia; Editor-in-Chief, Journal of Wildlife Diseases, Lawrence, Kansas
Hantaviruses

Kathryn S. Moffett, M.D.
Professor of Pediatrics, Section Chief, Division of Pediatric Infectious Diseases, West Virginia University, Morgantown, West Virginia
Cystic Fibrosis

Martin Montes, M.D.
Assistant Professor, Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru; Assistant Professor, Internal Medicine—Division of Infectious Diseases, University of Texas Medical Branch, Galveston, Texas
Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis

Jagmeet Mundi, M.D.
Head and Neck Surgery, University of California, Los Angeles, Los Angeles, California
Rhinosinusitis

James R. Murphy, Ph.D.
Professor, Department of Pediatrics, Pediatric Infectious Diseases, University of Texas Medical School at Houston, Houston, Texas
Campylobacter jejuni

Santhosh Nadipuram, M.D., M. S.
Research Fellow in Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Aeromonas; *Adenoviruses*

Colleen B. Nash, M.D., M.P.H.
Fellow, Pediatric Infectious Diseases, Department of Pediatrics, Section of Pediatric Infectious Diseases, University of Chicago, Chicago, Illinois
Human Papillomaviruses

James P. Nataro, M.D., Ph.D., M.B.A.
Benjamin Armistead Shepherd Professor and Chair, Department of Pediatrics, University of Virginia School of Medicine; Pediatrician-in-Chief, University of Virginia Children's Hospital, Charlottesville, Virginia
Diarrhea-Causing and Dysentery-Causing Escherichia coli

Delma J. Nieves, M.D.
Clinical Instructor, Division of Pediatric Infectious Diseases, University of California Irvine School of Medicine, Children's Hospital of Orange County, Orange, California
Leptospirosis

Richard A. Oberhelman, M.D.
Professor, Tropical Medicine, Tulane School of Public Health and Tropical Medicine; Professor, Department of Pediatrics, Tulane School of Medicine, New Orleans, Louisiana
Bacillus cereus

Theresa J. Ochoa, M.D.
Assistant Professor, Department of Pediatrics/Instituto de Medicina Tropical, Universidad Peruana Cayetano Heredia, Lima, Peru; Assistant Professor, Epidemiology, Center for Infectious Diseases, University of Texas School of Public Health, Houston, Texas
Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning; *Shigella; Salmonella; Cryptosporidiosis*

Rosemary M. Olivero, M.D.
Fellow, Division of Pediatric Infectious Diseases, Boston Medical Center, Boston, Massachusetts
Malaria

Alina Olteanu, M.D., Ph.D.
Pediatrician, MyChildren's Medical Center, Plano, Texas
Metabolic Response of the Host to Infections

Miguel L. O'Ryan, M.D.
Professor and Research Director, Faculty of Medicine, University of Chile; Chair, Chilean National Academy of Medicine, Santiago, Chile
Caliciviruses

Gary D. Overturf, M.D.
Professor Emeritus, Pediatrics and Pathology, Department of Pediatrics, University of New Mexico School of Medicine; Medical Director, Infectious Diseases, Pathology, TriCore Reference Laboratories, Albuquerque, New Mexico
Plague (Yersinia pestis); Clostridial Intoxication and Infection; *Antimicrobial Prophylaxis*

Debra L. Palazzi, M.D.
Associate Professor of Pediatrics, Division of Infectious Diseases, Baylor College of Medicine; Chief, Infectious Diseases Clinic, Texas Children's Hospital, Houston, Texas
Fever Without Source and Fever of Unknown Origin

Pia S. Pannaraj, M.D., M.P.H.
Assistant Professor, Pediatric Infectious Diseases, University of Southern California, Children's Hospital Los Angeles, Los Angeles, California
Cholangitis and Cholecystitis; *Group B Streptococcal Infections*

Janak A. Patel, M.D.
Professor and Division Director, Division of Pediatric Infectious Disease and Immunology, Department of Pediatrics, University of Texas Medical Branch, Galveston, Texas
Infections in Burn Patients

Mary E. Paul, M.D.
Associate Professor, Department of Pediatrics, Baylor College of Medicine; Chief of Service, Retrovirology and Global Health, Texas Children's Hospital, Houston, Texas
Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

Stephen I. Pelton, M.D.
Professor of Pediatrics and Epidemiology, Department of Pediatrics, Boston University Schools of Medicine and Public Health; Chief, Division of Pediatric Infectious Diseases, Department of Pediatrics, Boston Medical Center, Boston, Massachusetts
Pneumococcal Infections

John Perfect, M.D.
Professor of Medicine, Chief, Infectious Diseases, Department of Medicine, Duke University Medical Center, Durham, North Carolina
Cryptococcosis

C. J. Peters, M.D.
John Sealy Distinguished University Chair in Tropical and Emerging Virology; Professor, Departments of Microbiology & Immunology and Pathology; Director for Biodefense, Center for Biodefense & Emerging Infectious Diseases, University of Texas Medical Branch, Galveston, Texas
Hantaviruses; *Rift Valley Fever*

William A. Petri, Jr., M.D., Ph.D.
Chief, Division of Infectious Diseases and International Health, University of Virginia Health System, Charlottesville, Virginia
Amebiasis

Francisco P. Pinheiro, M.D.
Department of Arbovirus, Instituto Evandro Chagas, FNS, Ministry of Health, Belem, Para, Brazil
Oropouche Fever

Kathy Pinkerman, M.D.
Clinical Research Specialist, Baystate Mary Lane Hospital, Ware, Massachusetts
Pasteurella multocida

Benjamin Pinsky, M.D.
Assistant Professor, Department of Pathology, Stanford University School of Medicine; Director, Clinical Virology Laboratory, Stanford Hospital and Clinics and Lucile Packard Children's Hospital; Assistant Professor, Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California
Herpes Simplex Viruses 1 and 2

Stanley A. Plotkin, M.D.
Emeritus Professor of Pediatrics, University of Pennsylvania, Emeritus Professor, Wistar Institute of Research, Emeritus Staff, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania; Adjunct Professor, Johns Hopkins University, Baltimore, Maryland
Rabies Virus

Alice Pong, M.D.
Division of Pediatric Infectious Diseases, University of California San Diego, Rady Children's Hospital San Diego, San Diego, California
Retroperitoneal Infections

Eric A. Porsch, B.S.
Graduate Student, Molecular Genetics and Microbiology, Department of Pediatrics, Duke University, Durham, North Carolina
Kingella kingae

Joan S. Purcell, M.D.
Adolescent Medicine and Pediatrics, STEP Pediatrics, Woodlands, Texas
Trichomonas Infections

Natalie M. Quanquin, M.D., Ph.D.
Research Fellow in Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Mycoplasma and Ureaplasma Infections

Kevin K. Quinn, M.D.
Research Fellow, in Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Mumps Virus

Ramya Ramraj, M.D., M.B.B.S.
Affiliate Assistant Professor, Oregon Health and Sciences University; Attending Pediatrician and Pediatric Gastroenterologist, Doernbecher Children's Hospital and Kaiser NW Permanente, Portland, Oregon
Mimiviruses

Sergio E. Recuenco, M.D., M.P.H., Dr.P.H.
Surveillance, Epidemiology, and Ecology Unit Team Lead, Rabies Program, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia
Rabies Virus

Jack S. Remington, M.D.
Professor Emeritus, Department of Medicine, Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California; Consultant, Research Institute of the Palo Alto Medical Foundation, Palo Alto, California
Toxoplasmosis

Paula A. Revell, Ph.D.
Assistant Professor, Pathology and Immunology and Department of Pediatrics, Baylor College of Medicine; Director of Microbiology and Virology, Pathology, Texas Children's Hospital, Houston, Texas
Bacterial Laboratory Diagnosis

Anne W. Rimoin, Ph.D., M.P.H.
Associate Professor, Epidemiology, University of California, Los Angeles School of Public Health, Los Angeles, California
Monkeypox and Other Poxviruses

Ana Maria Ríos, M.D.
Pediatric Infectious Disease Specialist, Pediatric Infectious Disease, Cook Children's Medical Center, Fort Worth, Texas
Hepatitis A Virus

José R. Romero, M.D.
Horace C. Cabe Professor of Infectious Diseases, Department of Pediatrics, University of Arkansas for Medical Sciences; Director, Pediatric Infectious Diseases Section, Department of Pediatrics, Arkansas Children's Hospital; Director, Clinical Trials Research, Arkansas Children's Hospital Research Institute, Little Rock, Arkansas
Fungal Meningitis; *St. Louis Encephalitis*; *West Nile Virus*

Lawrence A. Ross, M.D.
Professor of Pediatrics, Division of Pediatric Infectious Diseases, Children's Hospital of Los Angeles; Professor of Pediatrics, Division of Pediatric Infectious Diseases, Keck School of Medicine at the University of Southern California, Los Angeles, California
Trypanosomiasis

Charles E. Rupprecht, V.M.D., M.S., PhD.
Director of Research, The Global Alliance for Rabies Research, Manhattan, Kansas
Rabies Virus

Xavier Sáez-Llorens, M.D.
Professor of Pediatrics, Head of Infectious Diseases, Hospital del Niño, Panama City, Panama
Parameningeal Infections

Julia Shaklee Sammons, M.D., M.S.C.E.
Assistant Professor of Pediatrics, University of Pennsylvania School of Medicine; Associate Hospital Epidemiologist and Medical Director, Department of Infection Prevention and Control, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Health Care–Associated Infections

Pablo J. Sánchez, M.D.
Professor of Pediatrics, Department of Pediatrics, University of Texas Southwestern Medical Center; Attending Physician, Parkland Health & Hospital System; Attending Physician, Children's Medical Center, Dallas, Texas
Syphilis

Linette Sande, M.D.
Assistant Professor, Department of Pediatrics, Loma Linda University; Attending Physician, Division of Pediatric Infectious Diseases, Loma Linda University Children's Hospital, Loma Linda, California
Group A, Group C, Group G Beta Hemolytic Streptococcal Infections

Javier Santisteban-Ponce, M.D.
Professor of Pediatrics, Department of Pediatrics, Universidad Peruana Cayetano Heredia; Attending Physician, Department of Pediatrics, Hospital Nacional Edgardo Rebagliati–EsSalud, Lima, Peru
Salmonella

Laura A. Sass, M.D.
Assistant Professor, Department of Pediatrics, Eastern Virginia Medical School; Attending Physician, Division of Pediatric Infectious Diseases, Children's Hospital of The King's Daughters, Norfolk, Virginia
Enterobacter

Carlos A. Sattler, M.D.
Senior Director, Medical Affairs, Merck Vaccines, West Point, Pennsylvania
Stenotrophomonas (Xanthomonas) maltophilia

Robin Schmucker, M.D.
Fellow, Pediatric Infectious Diseases, University of Louisville, Louisville, Kentucky
Antibiotic-Associated Colitis

Danica J. Schulte, M.D.
Assistant Professor in Residence, Department of Pediatrics, University of California, Los Angeles; Attending Physician, Department of Pediatrics, Section of Pediatric Infectious Diseases, Immunology and Allergy, Cedars-Sinai Medical Center, Los Angeles, California
Human Parvovirus B19; *Passive Immunization*

Jennifer E. Schuster, M.D.
Fellow, Pediatric Infectious Diseases, Monroe Carell Jr. Children's Hospital at Vanderbilt, Nashville, Tennessee
Human Bocaviruses; *Human Metapneumovirus*

Gordon E. Schutze, M.D.
Vice Chairman for Educational Affairs, Department of Pediatrics, Baylor College of Medicine; Vice President for International Medical Services, Baylor International Pediatric AIDS Initiative at Texas Children's Hospital, Houston, Texas
Tularemia

Carlos Seas, M.D., M.Sc.
Associate Professor, Department of Medicine, Universidad Peruana Cayetano Heredia; Vice Director, Instituto de Medicina Tropical Alexander von Humboldt; Attending Physician, Infectious and Tropical Medicine, Hospital Nacional Cayetano Heredia, Lima, Peru
Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis

Filiz O. Seeborg, M.D., M.P.H.
Assistant Professor, Department of Pediatrics, Section of Allergy, Immunology, and Rheumatology, Baylor College of Medicine; Allergy and Immunology Service, Texas Children's Hospital, Houston, Texas
Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome

Patrick C. Seed, M.D., Ph.D.
Associate Professor, Pediatrics/Pediatric Infectious Diseases; Assistant Professor, Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina
Extraintestinal Pathogenic Escherichia coli

Eugene D. Shapiro, M.D.
Professor of Pediatrics, Epidemiology, and Investigative Medicine, Yale University, New Haven, Connecticut
Epidemiology and Biostatistics of Infectious Diseases

Nina L. Shapiro, M.D.
Associate Professor, Department of Head and Neck Surgery, UCLA School of Medicine; Director, Pediatric Otolaryngology, University of California, Los Angeles School of Medicine, Los Angeles, California
Rhinosinusitis; *Mastoiditis*

William T. Shearer, M.D., Ph.D.
Professor, Departments of Pediatrics and Immunology, Baylor College of Medicine; Former Chief, Allergy and Immunology and Rheumatology Service, Texas Children's Hospital, Houston, Texas
Chronic Bronchitis; *Primary Immunodeficiencies*; *Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome*

Kareem W. Shehab, M.D.
Fellow, Division of Pediatric Infectious Diseases, Stanford University, Stanford, California
Coccidioidomycosis

Ziad M. Shehab, M.D.
Professor, Departments of Pediatrics and Pathology, University of Arizona, Tucson, Arizona
Coccidioidomycosis

[†]Jerry L. Shenep, M.D.
Formerly Member, Department of Infectious Diseases, St. Jude Children's Research Hospital; Professor, Department of Pediatrics, University of Tennessee Health Science Center, Memphis, Tennessee
Enterococcal and Viridans Streptococcal Infections

W. Donald Shields, M.D.
Professor, Department of Pediatric Neurology, David Geffen School of Medicine at UCLA; Mattel Children's Hospital UCLA, Los Angeles, California
Encephalitis and Meningoencephalitis

Robyn Shimizu-Cohen
Clinical Laboratory Scientist, MT (ASCP), Department of Pathology and Laboratory Medicine, UCLA Health System, Los Angeles, California
Parasitic Laboratory Diagnosis

Debbie-Ann T. Shirley, M.B.B.S.
Fellow, Division of Infectious Diseases and Tropical Pediatrics, Department of Pediatrics, Center for Vaccine Development, University of Maryland School of Medicine, Baltimore, Maryland
Cholera

Stanford T. Shulman, M.D.
Virginia H. Rogers Professor of Pediatric Infectious Diseases, Northwestern University Feinberg School of Medicine; Chief, Division of Infectious Diseases, Department of Pediatrics, Children's Memorial Hospital, Chicago, Illinois
Kawasaki Disease

[†]Deceased.

Constantine Simos, D.M.D.
Assistant Clinical Professor, Oral and Maxillofacial Surgery, Columbia University College of Dental Medicine, New York, New York; Attending Physician, Oral and Maxillofacial Surgery, Robert Wood Johnson University Hospital; Attending Physician Oral and Maxillofacial Surgery, Saint Peter's University Hospital, New Brunswick, New Jersey
Infections of the Oral Cavity

Arnold L. Smith, M.D.
Professor of Pathobiology, University of Washington School of Public Health; Director, Microbial Pathogens Program, Seattle Biomedical Research Institute, Seattle, Washington
Meningococcal Disease

Joseph W. St. Geme III, M.D.
Chairman of Pediatrics and Physician-in-Chief, The Children's Hospital of Philadelphia; Chairman and Professor of Pediatrics and Professor of Microbiology, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, Pennsylvania
Molecular Determinants of Microbial Pathogenesis; *Kingella kingae*

Mary Allen Staat, M.D., M.P.H.
Professor, Department of Pediatrics, University of Cincinnati, College of Medicine; Director, International Adoption Center, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio
Genital Infections; *Rotavirus*; *Infectious Disease Considerations in International Adoptees and Refugees*

Damien Stark, B.Sc., M.Sc., Ph.D., F.A.S.M, F.A.C.T.M.
Associate, Department of Medical and Molecular Biosciences, University of Technology Sydney, Broadway, New South Wales, Australia; Senior Hospital Scientist, Microbiology, St. Vincent's Hospital, Darlinghurst, New South Wales, Australia
Dientamoeba fragilis Infections

Jeffrey R. Starke, M.D.
Professor, Department of Pediatrics, Baylor College of Medicine; Medical Director, Department of Infection Control, Texas Children's Hospital, Houston, Texas
Infective Endocarditis; *Tuberculosis*

Kimberly F. Starr, B.S., M.S.
Graduate Student, Molecular Genetics and Microbiology, Pediatrics, Duke University, Durham, North Carolina
Kingella kingae

Barbara W. Stechenberg, M.D.
Pediatric Infectious Diseases Specialist, Department of Pediatrics, Baystate Children's Hospital, Springfield, Massachusetts; Professor of Pediatrics, Department of Pediatrics, Tufts University School of Medicine, Boston, Massachusetts
Eosinophilic Meningitis; *Moraxella catarrhalis; Diphtheria*; *Pasteurella multocida; Bartonella Infections*

William J. Steinbach, M.D.
Associate Professor of Pediatrics, Molecular Genetics and Micobiology, Department of Pediatrics, Department of Molecular Genetics and Microbiology; Attending Physician, Division of Pediatric Infectious Diseases, Duke University Medical Center, Durham, North Carolina
Opportunistic Infections in Hematopoietic Stem Cell Transplantation; *Aspergillosis*; *Mucormycosis and Entomopthoramycosis (formerly Zygomycosis)*, *Fusariosis and Scedosporiosis*; *Miscellaneous Mycoses*

Paul G. Steinkuller, M.D.
Associate Professor, Departments of Ophthalmology and Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Ocular Infections

Janis Stoll, M.D.
Instructor, Department of Pediatrics, Division of Pediatric Gastroenterology, Washington University School of Medicine; Pediatric Gastroenterology and Liver Transplantation Services, Saint Louis Children's Hospital, Saint Louis, Missouri
Hepatitis B and D Viruses

Jeffrey Suen, M.D.
Clinical Physician, Division of Pediatric Infectious Diseases, David Geffen School of Medicine at UCLA; Attending Physician, Division of Pediatric Infectious Diseases, Mattel Children's Hospital UCLA, Los Angeles, California
Toxic Shock Syndrome

Ciro V. Sumaya, M.D.
Professor, Health Policy and Management, Cox Endowed Chair in Medicine; Founding Dean, School of Rural Public Health, Texas A&M Health Sciences Center, College Station, Texas
Epstein-Barr Virus

Lillian Sung, M.D., Ph.D.
Associate Professor, Division of Haematology/Oncology, The Hospital for Sick Children, Toronto, Ontario, Canada
The Febrile Neutropenic Patient

Douglas S. Swanson, M.D.
Associate Professor of Pediatrics, Pediatric Infectious Diseases, Children's Mercy Hospitals and Clinics; Associate Professor of Pediatrics, University of Missouri–Kansas City, Kansas City, Missouri
The Human Microbiome

Tina Q. Tan, M.D.
Professor of Pediatrics, Department of Pediatrics, Feinberg School of Medicine, Northwestern University; Attending Physician, Division of Infectious Diseases, Co-Director, Travel Medicine Clinic, Director, International Adoptee Clinic, Division of Pediatric Infectious Diseases, Ann and Robert H. Lurie Children's Hospital, Chicago, Illinois
Infections Related to Prosthetic or Artificial Devices; *Giardiasis*; *Naegleria, Acanthamoeba, and Balamuthia Infections*

Herbert B. Tanowitz, M.D.
Medical Director of Diagnostic Parasitology Laboratory, Albert Einstein College of Medicine, Department of Pathology, Bronx, New York
Trypanosomiasis

Robert B. Tesh, M.D.
Professor, Department of Pathology, University of Texas Medical Branch, Galveston, Texas
Crimean-Congo Hemorrhagic Fever; *Phlebotomus Fever (Sandfly Fever)*

Michael A. Tolle, M.D.
Assistant Professor, Department of Pediatrics, Retrovirology and Global Health Section, International Pediatric AIDS Initiative (BIPAI), Baylor College of Medicine Children's Foundation, Texas Children's Hospital, Houston, Texas; Tanzania Bugando Medical Centre, Mwanza, Tanzania
Oncoviruses (Human T-Cell Lymphotropic Viruses) and Lentiviruses (Human Immunodeficiency Virus Type 2)

Philip Toltzis, M.D.
Professor, Department of Pediatrics, Rainbow Babies and Children's Hospital, Cleveland, Ohio
Antibiotic Resistance

Michael F. Tosi, M.D.
Professor of Pediatrics, Division of Pediatric Infectious Diseases, Mount Sinai School of Medicine, New York, New York
Normal and Impaired Immunologic Responses to Infection

Amelia P. A. Travassos da Rosa, B.Sc.
Research Associate, Center for Tropical Diseases, Department of Pathology, University of Texas Medical Branch, Galveston, Texas
Oropouche Fever

Theodore F. Tsai, M.D., M.P.H.
Head, Scientific Affairs, Novartis Vaccines, Cambridge, Massachusetts
Orbiviruses, Coltiviruses, and Seadornaviruses; *Eastern Equine Encephalitis*; *Western Equine Encephalitis*; *Venezuelan Equine Encephalitis*; *Other Alphaviral Infections*; *St. Louis Encephalitis*; *Japanese Encephalitis*; *Tick-Borne Encephalitis*; *Other Flaviviral Infections*

David A. Turner, M.D.
Director, Pediatric Critical Care Fellowship Program, Assistant Professor, Department of Pediatrics, Division of Pediatric Critical Care, Duke Children's Hospital, Duke University Medical Center, Durham, North Carolina
Acute Respiratory Distress Syndrome in Children

Timothy M. Uyeki, M.D., M.P.H., M.P.P.
Deputy Chief for Science, Epidemiology and Prevention Branch, Influenza Division, Centers for Disease Control and Prevention, Atlanta, Georgia; Associate Clinical Professor, Department of Pediatrics, University of California, San Francisco, San Francisco, California
Influenza Viruses

Jesus G. Vallejo, M.D.
Associate Professor, Section of Infectious Diseases, Department of Pediatrics, Baylor College of Medicine; Medical Director, International Patient Services, Texas Children's Hospital, Houston, Texas
Myocarditis; *Bacteremia and Septic Shock*

John A. Vanchiere, M.D., Ph.D.
Associate Professor, Department of Pediatrics, Chief, Pediatric Infectious Diseases, Lousiana State University Health Sciences Center, Shreveport, Louisiana
Human Polyomaviruses

Pedro Fernando da C. Vasconcelos, M.D., Ph.D.
Chief, Department of Arbovirology and Hemorrhagic Fevers, Coordinator, National Reference Laboratory of Arboviruses; Director, National Institute of Science and Technology for Viral Hemorrhagic Fevers; Director, PAHO-WHO CC for Research and Diagnostic Reference on Arbovirus, Instituto Evandro Chagas, SVS/Ministry of Health, Ananindeua, Brazil
Oropouche Fever

Jorge J. Velarde, M.D., Ph.D.
Fellow, Division of Infectious Diseases, Children's Hospital Boston, Boston, Massachusetts
Diarrhea-Causing and Dysentery-Causing Escherichia coli

Robert S. Venick, M.D.
Assistant Professor, Department of Pediatrics and Surgery, David Geffen School of Medicine at UCLA; Attending Physician, Mattel Children's Hospital UCLA, Los Angeles, California
Esophagitis

Ellen R. Wald, M.D.
Professor and Chair, Department of Pediatrics, University of Wisconsin School of Medicine and Public Health; Pediatrician-in-Chief, Department of Pediatrics, American Family Children's Hospital, Madison, Wisconsin
Uvulitis; *Urethritis*; *Cystitis and Pyelonephritis*; *Infections in Child Care Environments*

Thomas J. Walsh, M.D.
Director, Transplantation-Oncology Infectious Diseases Program, Weill Cornell Medical Center of Cornell University, New York, New York
Antifungal Agents

Mark A. Ward, M.D.
Associate Professor, Department of Pediatrics; Director of Pediatric Housestaff Education, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas
Fever: Pathogenesis and Treatment

Michelle Weinberg, M.D., M.P.H.
Medical Epidemiologist, Immigrant, Refugee and Migrant Health Branch, Division of Global Migration and Quarantine, Centers for Disease Control and Prevention, Atlanta, Georgia
International Travel Issues for Children

Nicholas Weinberg, M.D.
Emergency Physican, CVPH Medical Center, Plattsburgh, New York
International Travel Issues for Children

Melanie Wellington, M.D.
Assistant Professor, Pediatric Infectious Diseases, University of Rochester Medical School Center Rochester, New York
Fusariosis and Scedosporiosis

Robert C. Welliver, Sr., M.D.
Professor of Pediatrics, CMRI Hobbs-Recknagel Endowed Chair in Pediatrics, Chief, Division of Pediatric Infectious Diseases, Oklahoma University Health Sciences Center, Oklahoma City, Oklahoma
Bronchiolitis and Infectious Asthma

J. Gary Wheeler, M.D., M.P.S.
Professor of Pediatrics, Division of Infectious Diseases, University of Arkansas for Medical Sciences, Little Rock, Arkansas
Complications of Pneumonia

A. Clinton White, Jr., M.D.
Paul R. Stalnaker Distinguished Professor, Department of Internal Medicine; Director, Division of Infectious Diseases, Internal Medicine, University of Texas Medical Branch, Galveston, Texas
Cryptosporidiosis; *Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis*; *Cestodes*; *Foodborne Trematodes*; *Schistosomiasis*

Suzanne Whitworth, M.D.
Division of Pediatric Infectious Diseases, Cook Children's Healthcare System, Fort Worth, Texas
Aggregatibacter actinomycetemcomitans; *Actinomycosis*

Bernhard L. Wiedermann, M.D., M.A.
Professor, Department of Pediatrics, George Washington University School of Medicine and Health Sciences; Attending Physician, Division of Infectious Diseases, Children's National Medical Center, Washington, District of Columbia
Sporotrichosis

John V. Williams, M.D.
Associate Professor, Department of Pediatrics; Associate Professor, Departments of Pathology, Microbiology, and Immunology, Vanderbilt University School of Medicine, Nashville, Tennesee
Human Bocaviruses; *Human Metapneumovirus*

Natalie Williams-Bouyer, M.D.
Assistant Professor, Department of Pathology; Assistant Director, Division of Clinical Microbiology, University of Texas Medical Branch, Galveston, Texas
Infections in Burn Patients

Murray Wittner, M.D., Ph.D.
Department of Pathology, Albert Einstein College of Medicine, Bronx, New York
Trypanosomiasis

Charles R. Woods, Jr., M.D., M.S.
Professor of Pediatrics (Infectious Diseases), Vice Chair for Faculty Development; Director, Child Health Services Research Unit, Director, Pediatric Infectious Diseases Laboratory, Department of Pediatrics, University of Louisville School of Medicine, Louisville, Kentucky
Genital Infections; *Gonococcal Infections*; *Other Yersinia Species*

Terry W. Wright, Ph.D.
Associate Professor, Department of Pediatrics, University of Rochester School of Medicine, Rochester, New York
Pneumocystis Pneumonia

Ram Yogev, M.D.
Director, Section of Pediatric, Adolescent, and Maternal HIV, Department of Pediatrics, Children's Memorial Hospital; Professor, Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, Illinois
Infections Related to Prosthetic or Artificial Devices; *Parainfluenza Viruses*

Edward Young, A.B., M.S., M.D.
Professor, Department of Medicine, Baylor College of Medicine, Michael E DeBakey Veterans Affairs Medical Center, Houston, Texas
Brucellosis

Ramia Zakhour, M.D.
Fellow, Department of Pediatrics, Pediatric Infectious Diseases, University of Texas–Houston Medical School, Houston, Texas
Campylobacter jejuni

Mara Zambruni, M.D., M.P.H.
Fellow, Pediatric Infectious Diseases, Department of Pediatrics, University of Texas, Houston, Texas
Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning

Theoklis E. Zaoutis, M.D., M.S.C.E.
Thomas Frederick McNair Scott Professor of Pediatrics and Professor of Epidemiology, Perelman School of Medicine, University of Pennsylvania; Associate Chief, Division of Infectious Diseases, The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania
Candidiasis; *Mucormycosis and Entomopthoramycosis (formerly Zygomycosis)*

This page intentionally left blank

Preface

Morbidity and mortality rates related to infectious diseases decreased dramatically during the first half of the 20th century in the developed world because of major improvements in public health (e.g., clean water, adequate sanitation, and vector control) and personal health. Further major reduction in morbidity and mortality rates occurred in the second half of that century following the introduction of antimicrobial therapy, as well as active and passive immunization efforts. Despite these advances in the 20th century, infectious diseases in the developed world remain the leading cause of morbidity in infants and children in the 21st century. Children continue to experience three to nine respiratory infections and one to three gastrointestinal illnesses annually, requiring visits to physicians that outnumber the visits made for the purpose of well-child care. Infectious diseases are also the most common cause of school absenteeism. In more recent years, the emergence of resistance to multiple antibiotics by a large number of bacterial microorganisms (e.g., community-associated methicillin-resistant *Staphylococcus aureus*) has contributed to the morbidity and mortality related to infectious diseases processes, as have new infectious agents (e.g., swine influenza virus, SARS coronavirus) and changes in the clinical manifestations and severity of established infectious agents (e.g., enterovirus 71, swine influenza). The developing world is confronted with the same present-day problems seen in the developed world (antimicrobial resistance and new infectious agents), and these challenges are compounded by malnutrition and lack of adequate public health services and antimicrobial agents.

The first edition of *Feigin and Cherry* was written because Drs. Feigin and Cherry and many of their colleagues were concerned that no single reference text existed that comprehensively covered infectious diseases in children and adolescents. With each subsequent edition, including this one, the goal has been to provide comprehensive coverage of all subjects pertinent to the study of infectious diseases in children. Any attempt to summarize our present understanding of infectious diseases for serious students of the subject is a formidable task. In many areas, new information continues to accrue so rapidly that material becomes dated before it can appear in a text of this magnitude. Nevertheless, in this edition the editors and their author colleagues have endeavored to provide the most comprehensive and up-to-date discussion of pediatric infectious diseases ever compiled. This new edition is available online as well as in print. Purchasers can access the online version by registering their personal identification number (PIN) (found on the inside front cover of the book) at www.expertconsult.com. Online access includes not only fully searchable text, photos, illustrations, and tables, but also references linked to PubMed.

To provide a text as comprehensive and authoritative as possible, we, the editors, have enlisted contributions from a large number of individuals whose collective expertise is responsible for whatever success we may have had in meeting our objective. We offer our most profound appreciation to the 301 fellow contributors from 80 universities or institutions in 17 countries for their professional expertise and devoted scholarship. Their cooperation and willingness to work with us leave us deeply in their debt. Of note is the fact that 18 authors (Carol Baker, Ken Boyer, James Cherry, Morven Edwards, Mary Glode, Charles Grose, Caroline Hall, Scott Halstead, Margaret Hammerschlag, Shelly Kaplan, Jerry Klein, Edward Mason, George McCracken, Stanley Plotkin, Arnold Smith, Barbara Stechenberg, Robert Tesh, and Murray Wittner) have contributed to all seven editions of *Feigin and Cherry*.

Once again, infectious diseases are discussed according to organ systems that may be affected, as well as individually by microorganisms. In all sections in which diseases related to specific agents are discussed, emphasis has been placed, to the greatest extent possible, on the specificity of clinical manifestations that may be related to the organism causing the disease. Detailed information regarding the best means to establish a diagnosis and explicit recommendations for therapy is provided. In the present era of instant information, we have noted that historical perspectives relating to disease categories, as well as specific agents, are ignored. Because history is an important teacher, we have retained relevant historical details in this seventh edition.

Throughout the 32 years and seven-edition history of the *Textbook of Pediatric Infectious Diseases*, a number of classic chapters exist (e.g., measles, rubella, enteroviruses, and mycoplasma infections). The data in these chapters are unavailable in any other single source publication.

The entire text of this seventh edition has been revised extensively. The sixth edition contained almost 4000 pages, which is close to the maximum that can be included in a two-volume book. Therefore, with this seventh edition, we were faced with a major dilemma: specifically, how to include new important material that had become available since the sixth edition but not to substantially increase the size of the book. We approached this dilemma in two ways. One problem in previous editions was redundancy, which we have addressed by reducing the number of chapters from 266 to 253. This reduction has been accomplished by combining information in some previous separate chapters into more concise single presentations. The second way to reduce pages in the printed

version of this textbook was to print only new references. The electronic version, though, contains all references.

This edition continues the format that was initiated in the fourth edition in that infections with specific microorganisms have been organized to provide appropriate emphasis on the common features that may relate specific microorganisms to one another. Thus, all gram-positive coccal organisms are presented sequentially and are followed by gram-negative cocci, gram-positive bacilli, enterobacteria, gram-negative coccobacilli, Treponemataceae, anaerobic bacteria, and so forth. In addition, special sections of the text have been devoted to discussions of each of the following: molecular determinants of microbial pathogenesis; immunologic and phagocytic responses to infection; metabolic response of the host to infections; interaction of infection and nutrition; pathogenesis and treatment of fever; the human microbiome; epidemiology and biostatistics of infectious diseases; infections of the compromised host; Kawasaki disease; chronic fatigue syndrome; international travel issues for children; infectious disease problems of international adoptees and refugees; nosocomial infections; prevention and control of infections in hospitalized children; pharmacology and pharmacokinetics of antibacterial, antiviral, antifungal, and antiparasitic agents; immunomodulating agents; active and passive immunizing agents; public health considerations; infections in day care environments; and use of the bacteriology, mycology, parasitology, virology, and serology laboratories. The section on infections in the compromised host, which includes 16 chapters, has again been expanded. This expansion has been necessitated by the large number of children, particularly posttransplantation recipients, who now serve as the source of many infectious disease problems and constitute a large part of the consulting practice of many pediatric infectious disease physicians.

With some sadness, we have retained a section on bioterrorism, which is necessitated by the current state of world affairs. The section on immunomodulating agents and their potential use in the treatment of infectious diseases has been expanded because information on this subject has become more extensive since the publication of the last edition. Human and animal bites are now combined in one chapter, and a chapter on probiotics has been added.

This project could not have been brought to fruition without the help and assistance of many people whose names do not appear in the text. No words are sufficient to adequately convey our gratitude appropriately; we hope that they know they have our heartfelt thanks.

We would like to single out certain individuals for specific mention. First and foremost, we convey our appreciation to Laura Wennstrom Sheehan (Administrative Analyst, Department of Pediatrics, David Geffen School of Medicine at UCLA, and the Division of Infectious Diseases, Mattel Children's Hospital UCLA) for the thousands of hours she devoted to this seventh edition. From our UCLA office she coordinated the overall process of moving the book forward. She tended to numerous details relating to copyediting, transcribing, references, timing, and communication between the editors and Elsevier. Her expertise in EndNote was invaluable to the authors, editors, and publishing team for the organization of countless references throughout this edition. We are extremely grateful to have her as a part of our team. We would also like to acknowledge the hard work of Jordan Mann, Matthew Sheehan, and Gayla Wennstrom, who assisted Laura throughout this process.

The following students at UCLA played a key role in processing chapters and in particular helping with references: Amabelle Aguelo, Elham Eftekhari, Abe Harn, Jewel Powe, and Anum Qureshi. Tiki Valentino (Administrative Assistant III, Department of Pediatrics, David Geffen School of Medicine at UCLA, and the Division of Infectious Diseases, Mattel Children's Hospital UCLA) coordinated the work of all the students, and her efforts deserve much praise.

We would also like to thank Nathaniel Wilder Wolf at Baylor who coordinated all the parasite chapters.

Of course this seventh edition of *Feigin and Cherry* would not have been possible without Elsevier. In planning this edition we suffered an initial setback when there was a change in key personnel at Elsevier. The continuing efforts, though, of Stefanie Jewell-Thomas (Senior Content Strategist, Global Clinical Reference), Lisa Barnes (Content Development Specialist), and Claire Kramer (Senior Project Manager) allowed the book publishing to proceed in an efficient manner. Their efforts are much appreciated.

Finally, we thank the Baylor College of Medicine and Texas Children's Hospital in Houston, Texas, the David Geffen School of Medicine at UCLA and the Mattel Children's Hospital UCLA in Los Angeles, California, and Duke University School of Medicine in Durham, North Carolina for providing an environment that is supportive of intellectual pursuits.

James D. Cherry, M.D.
Sheldon L. Kaplan, M.D.
Gail J. Harrison, M.D.
William J. Steinbach, M.D.
Peter J. Hotez, M.D

Contents

VOLUME ONE

PART I
HOST-PARASITE RELATIONSHIPS AND THE PATHOGENESIS OF INFECTIOUS DISEASES

1 Molecular Determinants of Microbial Pathogenesis, 3
David A. Hunstad • Ravi Jhaveri • Joseph W. St. Geme III

2 Normal and Impaired Immunologic Responses to Infection, 22
Michael F. Tosi

3 Metabolic Response of the Host to Infections, 65
Elizabeth C. Bellino • Alina Olteanu • Ralph D. Feigin • William R. Beisel

4 Fever: Pathogenesis and Treatment, 83
Mark A. Ward

5 The Human Microbiome, 88
Douglas S. Swanson

6 Epidemiology and Biostatistics of Infectious Diseases, 95
Eugene D. Shapiro and Robert S. Baltimore

PART II
INFECTION OF SPECIFIC ORGAN SYSTEMS

SECTION I
UPPER RESPIRATORY TRACT INFECTIONS, 131

7 The Common Cold, 132
James D. Cherry

8 Infections of the Oral Cavity, 140
Constantine Simos and Blanca E. Gonzalez

9 Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis), 156
James D. Cherry

10 Uvulitis, 165
Ellen R. Wald

11 Peritonsillar, Retropharyngeal, and Parapharyngeal Abscesses, 167
Nira A. Goldstein and Margaret R. Hammerschlag

12 Cervical Lymphadenitis, 175
C. Mary Healy and Carol J. Baker

13 Parotitis, 189
Judith R. Campbell

14 Rhinosinusitis, 193
James D. Cherry • Jagmeet Mundi • Nina L. Shapiro

15 Otitis Externa, 203
Ellen M. Friedman and Maria Carrillo-Marquez

16 Otitis Media, 209
Jerome O. Klein and Charles D. Bluestone

17 Mastoiditis, 233
James D. Cherry • Audrey P. Calzada • Nina L. Shapiro

18 Croup (Laryngitis, Laryngotracheitis, Spasmodic Croup, Laryngotracheobronchitis, Bacterial Tracheitis, and Laryngotracheobronchopneumonitis) and Epiglottitis (Supraglottitis), 241
James D. Cherry

SECTION II
LOWER RESPIRATORY TRACT INFECTIONS, 261

19 Acute Bronchitis, 262
James D. Cherry

20 Chronic Bronchitis, 265
I. Celine Hanson and William T. Shearer

21 Bronchiolitis and Infectious Asthma, 271
Robert C. Welliver, Sr.

22 Pediatric Community-Acquired Pneumonia, 283
Carrie L. Byington and John S. Bradley

23 Children's Interstitial Lung Disease and Hypersensitivity Pneumonitis, 295
Minh L. Doan and Leland L. Fan

24 Complications of Pneumonia, 306
J. Gary Wheeler and Richard F. Jacobs

25 Cystic Fibrosis, 323
Peter W. Hiatt • Ruth Ann Luna • Kathryn S. Moffett

SECTION **III**
INFECTIONS OF THE HEART, 349

26 Infective Endocarditis, 350
Jeffrey R. Starke

27 Infectious Pericarditis, 371
Sheldon L. Kaplan

28 Myocarditis, 381
Jesus G. Vallejo

29 Acute Rheumatic Fever, 401
Diana R. Lennon

30 Mediastinitis, 419
Morven S. Edwards

SECTION **IV**
CENTRAL NERVOUS SYSTEM INFECTIONS, 424

31 Bacterial Meningitis Beyond the Neonatal Period, 425
Kwang Sik Kim

32 Parameningeal Infections, 462
Xavier Sáez-Llorens and Javier Nieto Guevara

33 Fungal Meningitis, 473
José R. Romero and Richard F. Jacobs

34 Eosinophilic Meningitis, 481
Barbara W. Stechenberg

35 Aseptic Meningitis and Viral Meningitis, 484
David E. Bronstein and Carol A. Glaser

36 Encephalitis and Meningoencephalitis, 492
David E. Bronstein • W. Donald Shields • Carol A. Glaser

37 Parainfectious and Postinfectious Disorders of the Nervous System, 513

37A ■ PARAINFECTIOUS AND POSTINFECTIOUS DEMYELINATING DISORDERS OF THE CENTRAL NERVOUS SYSTEM, 513
J. Lloyd Holder, Jr. and Timothy E. Lotze

37B ■ INFECTION-ASSOCIATED MYELITIS AND MYELOPATHIES OF THE SPINAL CORD, 520
J. Lloyd Holder, Jr. and Timothy E. Lotze

SECTION **V**
GENITOURINARY TRACT INFECTIONS, 529

38 Urethritis, 530
Ellen R. Wald

39 Cystitis and Pyelonephritis, 535
Ellen R. Wald

40 Renal Abscess, 553
Edmond T. Gonzales, Jr. and Sheldon L. Kaplan

41 Prostatitis, 558
Sheldon L. Kaplan

42 Genital Infections, 560
Mariam R. Chacko • Mary Allen Staat • Charles R. Woods, Jr.

SECTION **VI**
GASTROINTESTINAL TRACT INFECTIONS, 592

43 Esophagitis, 593
Paul Krogstad and Robert S. Venick

44 Approach to Patients with Gastrointestinal Tract Infections and Food Poisoning, 598
Theresa J. Ochoa • Mara Zambruni • Elsa Chea-Woo

45 Antibiotic-Associated Colitis, 632
Robin Schmucker and Kristina Bryant

46 Whipple Disease, 639
Zev Davidovics and Mark A. Gilger

SECTION **VII**
LIVER DISEASES, 647

47 Hepatitis, 648
Daniel H. Leung and Leon L. Chen

48 Cholangitis and Cholecystitis, 658
Pia S. Pannaraj and Douglas S. Fishman

49 Pyogenic Liver Abscess, 672
Sheldon L. Kaplan

50 Reye Syndrome, 676
Eugene S. Hurwitz

SECTION **VIII**
OTHER INTRA-ABDOMINAL INFECTIONS, 678

51 Appendicitis and Pelvic Abscess, 679
Thomas L. Kuhls

52 Pancreatitis, 689
Thomas L. Kuhls

53 Peritonitis and Intra-Abdominal Abscess, 698
Judith R. Campbell

54 Retroperitoneal Infections, 706
Alice Pong and John S. Bradley

SECTION **IX**
MUSCULOSKELETAL INFECTIONS, 710

55 Osteomyelitis, 711
Paul Krogstad

56 Septic Arthritis, 727
Paul Krogstad

57 Bacterial Myositis and Pyomyositis, 735
Charles Grose

SECTION **X**
SKIN INFECTIONS, 740

58 Cutaneous Manifestatiions of Systemic Infections, 741
James D. Cherry

59 Roseola Infantum (Exanthem Subitum), 768
James D. Cherry

60 Skin Infections, 772

60A ■ BACTERIAL SKIN INFECTIONS, 772
Mary Anne Jackson

60B ■ VIRAL AND FUNGAL SKIN INFECTIONS, 782
Meena R. Julapalli and Moise L. Levy

SECTION **XI**
OCULAR INFECTIOUS DISEASES, 793

61 Ocular Infections, 794
Mohamed Hussein • Aaron M. Miller • Paul G. Steinkuller

SECTION **XII**
SYSTEMIC INFECTIOUS DISEASES, 823

62 Bacteremia and Septic Shock, 824
Sheldon L. Kaplan and Jesus G. Vallejo

63 Fever Without Source and Fever of Unknown Origin, 837
Debra L. Palazzi

64 Toxic Shock Syndrome, 848
Jeffrey Suen

65 Acute Respiratory Distress Syndrome in Children, 866
Jan Hau Lee • Ira M. Cheifetz • David A. Turner

SECTION **XIII**
INFECTIONS OF THE FETUS AND NEWBORN, 876

66 Approach to Infections in the Fetus and Newborn, 877
Gail J. Harrison

SECTION **XIV**
INFECTIONS OF THE COMPROMISED HOST, 902

67 Primary Immunodeficiencies, 903
Javier Chinen • Mark W. Kline • William T. Shearer

68 The Febrile Neutropenic Patient, 921
Brian T. Fisher and Lillian Sung

69 Opportunistic Infections in Hematopoietic Stem Cell Transplantation, 933
Christopher C. Dvorak and William J. Steinbach

70 Infections in Pediatric Heart Transplantation, 945
Sheldon L. Kaplan

71 Infections in Pediatric Lung Transplantation, 957
Jill A. Hoffman

72 Opportunistic Infections in Liver and Intestinal Transplantation, 989
Michael Green and Marian G. Michaels

73 Opportunistic Infections in Kidney Transplantation, 1002
Gail J. Harrison

74 Infections Related to Prosthetic or Artificial Devices, 1015
Ram Yogev and Tina Q. Tan

75 Infections Related to Craniofacial Surgical Procedures, 1043
Marc A. Mazade

76 Infections in Burn Patients, 1047
Janak A. Patel and Natalie Williams-Bouyer

SECTION **XV**
UNCLASSIFIED INFECTIOUS DISEASES, 1063

77 Kawasaki Disease, 1064
Stanford T. Shulman

78 Chronic Fatigue Syndrome, 1085
Leonard R. Krilov

PART III
INFECTIONS WITH SPECIFIC MICROORGANISMS

SECTION **XVI**
BACTERIAL INFECTIONS, 1095

79 Nomenclature for Aerobic and Anaerobic Bacteria, 1096
David A. Bruckner

SUBSECTION **1**
GRAM-POSITIVE COCCI, 1113

80 *Staphylococcus aureus* Infections (Coagulase-Positive Staphylococci), 1113
Sheldon L. Kaplan • Kristina G. Hulten • Edward O. Mason

81 Coagulase-Negative Staphylococcal Infections, 1131
David Y. Hyun

82 Group A, Group C, Group G Beta Hemolytic Streptococcal Infections, 1140
Linette Sande and Anthony R. Flores

83 Group B Streptococcal Infections, 1153
Pia S. Pannaraj and Carol J. Baker

84 Enterococcal and Viridans Streptococcal Infections, 1170
B. Keith English and Jerry L. Shenep

85 Pneumococcal Infections, 1198
Stephen I. Pelton and Michael R. Jacobs

86 Miscellaneous Gram-Positive Cocci, 1246
Randall G. Fisher

SUBSECTION **2**
GRAM NEGATIVE COCCI, 1250

87 *Moraxella catarrhalis*, 1250
Barbara W. Stechenberg

88 Meningococcal Disease, 1253
Marsha S. Anderson • Mary P. Glodé • Arnold L. Smith

89 Gonococcal Infections, 1271
Charles R. Woods, Jr.

SUBSECTION **3**
GRAM-POSITIVE BACILLI, 1301

90 Diphtheria, 1301
Barbara W. Stechenberg

91 Anthrax, 1311
Morven S. Edwards

92 *Bacillus cereus*, 1316
Margot Anderson and Richard A. Oberhelman

93 *Arcanobacterium haemolyticum*, 1322
Natascha Ching

94 Erysipelothrix rhusiopathiae, 1327
Randall G. Fisher

95 Listeriosis, 1329
Tobias Kollmann • Robert Bortolussi • Timothy Mailman

96 Tuberculosis, 1335
Andrea T. Cruz and Jeffrey R. Starke

97 Other Mycobacteria, 1381
W. Matthew Linam and Richard F. Jacobs

98 Leprosy and Buruli Ulcer: The Major Cutaneous Mycobacterioses, 1392
Andrea T. Cruz

99 *Nocardia*, 1418
Nada Harik and Richard F. Jacobs

100 Corynebacterium and Rhodococcus, 1423
Randall G. Fisher

SUBSECTION **4**
GRAM-NEGATIVE BACILLI, 1429

101 Citrobacter, 1429
Randall G. Fisher

102 *Enterobacter*, 1433
Laura A. Sass and Randall G. Fisher

103 Extraintestinal Pathogenic *Escherichia coli*, 1439
Patrick C. Seed

104 Diarrhea-Causing and Dysentery-Causing *Escherichia coli*, 1449
Jorge J. Velarde • Andrea A. Berry • James P. Nataro

105 Klebsiella, 1465
Randall G. Fisher

106 *Morganella morganii*, 1469
Randall G. Fisher

107 *Proteus*, 1471
Randall G. Fisher

108 *Providencia*, 1474
Randall G. Fisher

109 *Shigella*, 1476
Theresa J. Ochoa and Margaret Kosek

110 *Serratia*, 1488
Randall G. Fisher

111 Salmonella, 1491
Theresa J. Ochoa and Javier Santisteban-Ponce

112 Plague *(Yersinia pestis)*, 1509
Gary D. Overturf

113 Other *Yersinia* Species, 1514
Charles R. Woods, Jr.

114 Miscellaneous Enterobacteriaceae, 1532
Randall G. Fisher

115 Aeromonas, 1538
Santhosh Nadipuram and James D. Cherry

116 *Pasteurella multocida*, 1545
Barbara W. Stechenberg and Kathy Pinkerman

117 Cholera, 1548
Debbie-Ann T. Shirley and Matthew B. Laurens

118 Vibrio parahaemolyticus, 1556
David Y. Hyun

119 *Vibrio vulnificus*, 1560
Randall G. Fisher

120 Miscellaneous Non-Enterobacteriaceae Fermentative Bacilli, 1563
Randall G. Fisher

121 *Acinetobacter*, 1568
Armando G. Correa

122 *Achromobacter (Alcaligenes)*, 1573
Randall G. Fisher

123 *Eikenella corrodens*, 1576
Randall G. Fisher

124 *Elizabethkingia* and *Chryseobacterium* Species, 1578
Randall G. Fisher

125 *Pseudomonas* and Related Genera, 1582
Michael T. Brady and Mario J. Marcon

126 *Stenotrophomonas (Xanthomonas) maltophilia*, 1605
Carlos A. Sattler

SUBSECTION **5**
GRAM-NEGATIVE COCCOBACILLI, 1610

127 *Aggregatibacter actinomycetemcomitans*, 1610
Suzanne Whitworth and Richard F. Jacobs

128 Brucellosis, 1611
Edward Young

129 Pertussis and Other *Bordetella* Infections, 1616
James D. Cherry and Ulrich Heininger

130 *Calymmatobacterium granulomatis*, 1640
Mariam R. Chacko

131 Campylobacter jejuni, 1644
Ramia Zakhour • Gloria P. Heresi • James R. Murphy

132 Tularemia, 1657
Gordon E. Schutze

133 *Haemophilus influenzae*, 1665
Stephen J. Barenkamp

134 Other *Haemophilus* Species (*ducreyi, haemolyticus, influenzae* Biogroup aegyptius, *parahaemolyticus,* and *parainfluenzae*) and *Aggregatibacter (Haemophilus) aphrophilus*, 1685
Stephen J. Barenkamp

135 *Helicobacter pylori*, 1691
Aldo Maspons and Mark A. Gilger

136 *Kingella kingae*, 1699
Eric A. Porsch • Kimberly F. Starr • Joseph W. St. Geme III

137 Legionnaires' Disease, Pontiac Fever, and Related Illnesses, 1705
Paul H. Edelstein and Wilbert H. Mason

138 *Streptobacillus moniliformis* (Rat-Bite Fever), 1715
Kara Dubray and Carol A. Glaser

139 *Bartonella* Infections, 1720
Leigh M. Howard • Kathryn M. Edwards • Barbara Stechenberg

SUBSECTION **6**
TREPONEMATACEAE, 1729

140 Borrelia, 1729

140A ■ LYME DISEASE, 1729
Sunil K. Sood and Peter J. Krause

140B ■ RELAPSING FEVER, 1739
Peter J. Krause

141 Leptospirosis, 1743
Delma J. Nieves

142 *Spirillum minus* (Rat-Bite Fever), 1759
Kara Dubray and Carrie L. Byington

143 Syphilis, 1761
Simon R. Dobson and Pablo J. Sánchez

144 Nonvenereal Treponematoses, 1782
David C. Hilmers

SUBSECTION **7**
ANAEROBIC BACTERIA, 1791

145 Clostridial Intoxication and Infection, 1791
Gary D. Overturf

146 Infant Botulism, 1801
Stephen S. Arnon

147 Tetanus, 1809
James D. Cherry and Rick E. Harrison

148 Actinomycosis, 1820
Suzanne Whitworth and Richard F. Jacobs

149 *Bacteroides, Fusobacterium,* and *Prevotella*, 1825
Steven C. Buckingham

VOLUME TWO

SECTION **XVII**
VIRAL INFECTIONS, 1835

150 Classification and Nomenclature of Viruses, 1836
Marjorie J. Miller

DNA VIRUSES—SUBSECTION **1**
PARVOVIRIDAE, 1843

151 Human Parvovirus B19, 1843
Danica J. Schulte

152 Human Bocaviruses, 1858
Jennifer E. Schuster and John V. Williams

DNA VIRUSES—SUBSECTION **2**
POLYOMAVIRIDAE, 1863

153 Human Polyomaviruses, 1863
John A. Vanchiere

154 Human Papillomaviruses, 1871
Colleen B. Nash • Gail J. Harrison • Kenneth A. Alexander

DNA VIRUSES—SUBSECTION **3**
ADENOVIRIDAE, 1888

155 Adenoviruses, 1888
James D. Cherry and Santhosh Nadipuram

DNA VIRUSES—SUBSECTION **4**
HEPATOVIRIDAE, 1911

156 Hepatitis B and D Viruses, 1911
Maureen Jonas and Janis Stoll

DNA VIRUSES—SUBSECTION **5**
HERPESVIRIDAE, 1933

157 Herpes Simplex Viruses 1 and 2, 1933
Kathleen Gutierrez • Benjamin Pinsky • Ann M. Arvin

158 Cytomegalovirus, 1968
Gail J. Harrison

159 Epstein-Barr Virus, 1992
Charles T. Leach • Ciro V. Sumaya • Gail J. Harrison

160 Human Herpesviruses 6, 7, and 8, 2016
Charles Grose

161 Varicella-Zoster Virus, 2021
Anne A. Gershon

DNA VIRUSES—SUBSECTION **6**
POXVIRIDAE, 2033

162 Smallpox (Variola Virus), 2033
James D. Cherry

163 Monkeypox and Other Poxviruses, 2040
Samantha H. Johnston and Anne W. Rimoin

164 Mimiviruses, 2048
Ramya Ramraj and Gail J. Harrison

RNA VIRUSES—SUBSECTION **1**
PICORNAVIRIDAE, 2051

165 Enteroviruses, Parechoviruses, and Saffold Viruses, 2051
James D. Cherry and Paul Krogstad

166 Rhinoviruses, 2109
Robert L. Atmar and Janet A. Englund

167 Hepatitis A Virus, 2128
Ana María Ríos

RNA VIRUSES—SUBSECTION **2**
CALICIVIRIDAE, 2147

168 Caliciviruses, 2147
Yalda C. Lucero • Miguel L. O'Ryan • David O. Matson

169 Hepatitis E Virus, 2158
Ravi Jhaveri

RNA VIRUSES—SUBSECTION **3**
REOVIRIDAE, 2168

170 Reoviruses, 2168
James D. Cherry

171 Orbiviruses, Coltiviruses, and Seadornaviruses, 2172
Theodore F. Tsai

172 Rotavirus, 2176
Mary Allen Staat • Monica Malone McNeal • David I. Bernstein

RNA VIRUSES—SUBSECTION **4**
TOGAVIRIDAE, 2195

173 Rubella Virus, 2195
James D. Cherry and Kristina Adachi

174 Alphaviruses, 2226

174A ■ EASTERN EQUINE ENCEPHALITIS, 2226
Theodore F. Tsai and Gail J. Harrison

174B ■ WESTERN EQUINE ENCEPHALITIS, 2230
Theodore F. Tsai and Gail J. Harrison

174C ■ VENEZUELAN EQUINE ENCEPHALITIS, 2237
Theodore F. Tsai and Gail J. Harrison

174D ■ CHIKUNGUNYA, 2241
Scott B. Halstead

174E ■ ROSS RIVER VIRUS ARTHRITIS, 2248
John G. Aaskov

174F ■ OTHER ALPHAVIRAL INFECTIONS, 2251
Theodore F. Tsai

SUBSECTION **5**
FLAVIVIRIDAE, 2253

175 Flaviviruses, 2253

175A ■ ST. LOUIS ENCEPHALITIS, 2253
Kimberly C. Martin • José R. Romero • Theodore F. Tsai

175B ■ WEST NILE VIRUS, 2261
José R. Romero

175C ■ YELLOW FEVER, 2274
Duane J. Gubler and Gail J. Harrison

175D ■ DENGUE, DENGUE HEMORRHAGIC FEVER, AND SEVERE DENGUE, 2278
Scott B. Halstead

175E ■ JAPANESE ENCEPHALITIS, 2291
Theodore F. Tsai

175F ■ MURRAY VALLEY ENCEPHALITIS, 2301
John G. Aaskov

175G ■ TICK-BORNE ENCEPHALITIS, 2303
Andrea Duppenthaler • Christoph Aebi • Theodore F. Tsai

175H ■ OTHER FLAVIVIRAL INFECTIONS, 2313
Theodore F. Tsai

176 Hepatitis C Virus, 2317
Ravi Jhaveri

SUBSECTION **6**
ORTHOMYXOVIRIDAE, 2326

177 Influenza Viruses, 2326
Timothy M. Uyeki

SUBSECTION **7**
PARAMYXOVIRIDAE, 2360

178 Parainfluenza Viruses, 2360
Ram Yogev and Caroline Breese Hall

179 Measles Virus, 2373
James D. Cherry

180 Mumps Virus, 2395
James D. Cherry and Kevin K. Quinn

181 Respiratory Syncytial Virus, 2407
H. Cody Meissner and Caroline Breese Hall

182 Human Metapneumovirus, 2434
Jennifer E. Schuster and John V. Williams

SUBSECTION **8**
RHABDOVIRIDAE, 2444

183 Rabies Virus, 2444
Sergio E. Recuenco • Stanley A. Plotkin • Charles E. Rupprecht

SUBSECTION **9**
ARENAVIRIDAE AND *FILOVIRIDAE*, 2463

184 Lymphocytic Choriomeningitis Virus, 2463
Rémi N. Charrel and Xavier de Lamballerie

185 Arenaviral Hemorrhagic Fevers, 2466
Rémi N. Charrel and Xavier de Lamballerie

186 Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers, 2478
Eric Leroy and Gail J. Harrison

SUBSECTION **10**
CORONAVIRIDAE AND *TOROVIRIDAE*, 2486

187 Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome, 2486
Kenneth McIntosh and Janet A. Englund

SUBSECTION **11**
BUNYAVIRIDAE, 2496

188 Hantaviruses, 2496
Louisa E. Chapman • C. J. Peters • James N. Mills • Kelly T. McKee, Jr.

189 La Crosse Encephalitis and Other California Serogroup Viruses, 2513
W. Garrett Hunt and James E. McJunkin

190 Other Bunyaviridae, 2528

190A ■ RIFT VALLEY FEVER, 2528
C.J. Peters and Gail J. Harrison

190B ■ CRIMEAN-CONGO HEMORRHAGIC FEVER, 2531
Robert B. Tesh and Gail J. Harrison

190C ■ PHLEBOTOMUS FEVER (SANDFLY FEVER), 2535
Robert B. Tesh and Gail J. Harrison

190D ■ OROPOUCHE FEVER, 2537
Francisco P. Pinheiro • Amelia P. A. Travassos da Rosa • Pedro Fernando da C. Vasconcelos

190E ■ TOSCANA VIRUS, 2544
Rémi N. Charrel • Xavier de Lamballerie • Laurence Bichaud

SUBSECTION **12**
RETROVIRIDAE, 2552

191 Human Retroviruses, 2552

191A ■ ONCOVIRUSES (HUMAN T-CELL LYMPHOTROPIC VIRUSES) AND LENTIVIRUSES (HUMAN IMMUNODEFICIENCY VIRUS TYPE 2), 2552
Michael A. Tolle and Susan L. Gillespie

191B ■ HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME, 2591
Filiz O. Seeborg • Mary E. Paul • William T. Shearer

SUBSECTION **13**
PRION-RELATED DISEASES, 2619

192 Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinker Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia), 2619
William J. Britt and Gail J. Harrison

SECTION **XVIII**
CHLAMYDIA, 2630

193 Chlamydia Infections, 2631
Margaret R. Hammerschlag and Stephan A. Kohlhoff

SECTION **XIX**
RICKETTSIAL DISEASES, 2646

194 Rickettsial and Ehrlichial Diseases, 2647
Paul M. Lantos and Ross McKinney, Jr.

SECTION **XX**
MYCOPLASMA, 2667

195 Mycoplasma and Ureaplasma Infections, 2668
James D. Cherry and Natalie M. Quanquin

SECTION **XXI**
FUNGAL DISEASES, 2701

196 Classification of Fungi, 2702
David A. Bruckner and Eszter Deak

197 Aspergillosis, 2704
William J. Steinbach

198 Blastomycosis, 2723
Gregory M. Gauthier and Bruce S. Klein

199 Candidiasis, 2735
Grace E. Lee • David A. Kaufman • Theoklis E. Zaoutis

200 Coccidioidomycosis, 2769
Ziad M. Shehab and Kareem W. Shehab

201 Paracoccidioidomycosis, 2780
Gil Benard and Maria José Soares Mendes-Giannini

202 Cryptococcosis, 2795
David Goldman and John Perfect

203 Histoplasmosis, 2807
Martin B. Kleiman

204 Sporotrichosis, 2832
Bernhard L. Wiedermann

205 Mucormycosis and Entomopthoramycosis (Formerly Zygomycosis), 2837
Theoklis E. Zaoutis and William J. Steinbach

206 Fusariosis and Scedosporiosis, 2846
Damian J. Krysan • Melanie Wellington • William J. Steinbach

207 Miscellaneous Mycoses, 2852
William J. Steinbach

SECTION **XXII**
PARASITIC DISEASES, 2860

208 Classification and Nomenclature of Human Parasites, 2861
Lynne S. Garcia

SUBSECTION **1**
PROTOZOA 2866

209 Amebiasis, 2866
Shinjiro Hamano and William A. Petri, Jr.

210 *Blastocystis hominis* and *Blastocystis* spp. Infection, 2875
Peter J. Hotez

211 *Entamoeba coli* Infection, 2877
Peter J. Hotez

212 Giardiasis, 2878
Tina Q. Tan

213 *Dientamoeba fragilis* Infections, 2884
Damien Stark

214 Trichomonas Infections, 2887
Joan S. Purcell and Mariam R. Chacko

215 *Balantidium coli* Infection, 2894
Peter J. Hotez

216 Cryptosporidiosis, 2896
Theresa J. Ochoa and A. Clinton White, Jr.

217 Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis, 2905
A. Clinton White, Jr. • Carlos Seas • Martin Montes

218 Babesiosis, 2915
Peter J. Krause

219 Malaria, 2921
Rosemary M. Olivero and Elizabeth D. Barnett

220 Leishmaniasis, 2947
Lynne S. Garcia

221 Trypanosomiasis, 2959
Lawrence A. Ross • Lynne S. Garcia • Murray Wittner • Herbert B. Tanowitz

222 *Naegleria, Acanthamoeba,* and *Balamuthia* Infections, 2975
Patrick J. Gavin and Tina Q. Tan

223 Toxoplasmosis, 2986
James B. McAuley • Kenneth M. Boyer • Jack S. Remington • Rima L. McLeod

224 *Pneumocystis* Pneumonia, 3005
Francis Gigliotti and Terry W. Wright

SUBSECTION **2**
NEMATODES, 3012

225 Parasitic Nematode Infections, 3012
Peter J. Hotez

SUBSECTION **3**
CESTODES, 3030

226 Cestodes, 3030
A. Clinton White, Jr. • Philip R. Fischer • Pedro Legua

SUBSECTION **4**
TREMATODES, 3049

227 Foodborne Trematodes, 3049
Miguel M. Cabada • Philip R. Fischer • A. Clinton White, Jr.

228 Schistosomiasis, 3060
Philip R. Fischer and A. Clinton White, Jr.

SUBSECTION **5**
ARTHROPODS, 3070

229 Arthropods, 3070
Jan E. Drutz

SECTION **XXIII**
GLOBAL HEALTH, 3080

230 Global Health, 3081
Holly Corwin and Gail J. Harrison

231 International Travel Issues for Children, 3091
Michelle Weinberg • Nicholas Weinberg • Susan A. Maloney

232 Infectious Disease Considerations in International Adoptees and Refugees, 3121
Mary Allen Staat

PART IV
THERAPEUTICS

233 Antibiotic Resistance, 3137
Philip Toltzis and Jeffrey L. Blumer

234 The Pharmacokinetic-Pharmacodynamic Interface: Determinants of Anti-Infective Drug Action and Efficacy in Pediatrics, 3159
Susan M. Abdel-Rahman • Jennifer L. Goldman • Gregory L. Kearns

235 Antibacterial Therapeutic Agents, 3182
Carla Garcia and George H. McCracken, Jr.

236 Antimicrobial Prophylaxis, 3242
Gary D. Overturf

237 Outpatient Intravenous Antimicrobial Therapy for Serious Infections, 3257
John S. Bradley

238 Antiviral Agents, 3265
Gail J. Harrison

239 Antifungal Agents, 3291
Andreas H. Groll and Thomas J. Walsh

240 Drugs for Parasitic Infections, 3327

241 Immunomodulating Agents, 3355
Timothy R. La Pine and Harry R. Hill

242 Probiotics, 3369
Gail J. Harrison

PART V
PREVENTION OF INFECTIOUS DISEASES

243 Health Care–Associated Infections, 3377
W. Charles Huskins • Julia Shaklee Sammons • Susan E. Coffin

244 Active Immunizing Agents, 3420
Penelope H. Dennehy • Ian C. Michelow • Michael P. Koster • Nicole E. Alexander-Scott

245 Passive Immunization, 3491
Deborah Lehman and Danica J. Schulte

SECTION **XXIV**
OTHER PREVENTIVE CONSIDERATIONS, 3537

246 Public Health Aspects of Infectious Disease Control, 3538
Kathleen H. Harriman and Laurene Mascola

247 Infections in Child Care Environments, 3550
Sheryl L. Henderson and Ellen R. Wald

248 Animal and Human Bites, 3570
Morven S. Edwards

249 Bioterrorism, 3579
Robert J. Leggiadro

PART VI
APPROACH TO THE LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES

250 Bacterial Laboratory Diagnosis, 3589
Paula A. Revell and Lakshmi Chandramohan

251 Fungal Laboratory Analysis: Specimen Collection, Direct Detection, and Culture, 3596
David A. Bruckner

252 Viral Laboratory Diagnosis, 3600
Romney M. Humphries and Marjorie J. Miller

253 Parasitic Laboratory Diagnosis, 3620
David A. Bruckner and Robyn Shimizu-Cohen

This page intentionally left blank

SECTION **XVII**

VIRAL INFECTIONS

CHAPTER 150

Classification and Nomenclature of Viruses

Marjorie J. Miller

Viruses originally were differentiated from other microorganisms by their small size and their filterability. Initial efforts to classify viruses were based on disease, pathogenesis, organ tropisms, and epidemiologic characteristics rather than on physicochemical properties of the virus particle. During the 1950s and 1960s, many new viruses were being discovered while evidence of virus structure and composition also was emerging, thereby prompting proposals that viruses be grouped on the basis of shared virion properties. The herpesvirus,[1] myxovirus,[2] and poxvirus[6] groups were among the first taxonomic groups delineated. As more information concerning the physicochemical characteristics of viruses accumulated, the need for a universal system of classification and nomenclature became apparent.

The International Committee on Nomenclature of Viruses (ICNV) was established in 1966 at the International Congress of Microbiology in Moscow, some 20 years after a bacterial taxonomy first was published and 75 years after the discovery of viruses. In 1973, the ICNV became the International Committee on Taxonomy of Viruses (ICTV), which operates under the auspices of the Virology Division of the International Union of Microbiological Societies. The ICTV classifies viruses isolated from vertebrates, invertebrates, plants, fungi, protozoa, bacteria, and mycoplasmas and publishes periodic reports summarizing the most recent developments in viral taxonomy.* Interim updates were published in *Intervirology* and more recently in *Archives of Virology*. The most updated information on virus taxonomy can be accessed through the Internet.†

The hierarchic levels of virus taxonomy consist of order, family, subfamily, genus, and species and are based on the structural, physicochemical, biologic, and replicative properties of viruses.[13] Structural characteristics include size, shape, presence or absence of an envelope, and capsid symmetry. Physicochemical characterization is based on type, strandedness, and the number of segments of nucleic acid, as well as the number and size of proteins and their functional activities. Biologic properties include host range, serologic relationships, pathogenicity, and transmission; replicative properties include nucleic acid replication, transcription, and translation.

Virus orders comprise groups of families that share common characteristics (e.g., biochemical composition, viral replication strategy, particle structure, and general genomic organization) distinguishable from those of other orders and families. Virus orders are designated with the suffix *-virales*. Currently, the ICTV has approved six orders, four comprising viruses affecting humans: *Herpesvirales*, family *Herpesviridae; Mononegavirales*, which includes the families *Paramyxoviridae*, *Rhabdoviridae*, and *Filoviridae; Nidovirales*, which comprises the families *Coronaviridae* and *Arteriviridae;* and *Picornavirales*, family *Picornaviridae*.

Virus families represent groups of genera that share common characteristics distinct from those of other families. Virus families are designated with the suffix *-viridae*. Most families have unique virion morphology, genome structure, or strategies of replication (Fig. 150-1). Virus subfamilies are designated with the suffix *-virinae* and have been introduced in seven families: *Coronaviridae (Coronovirinae*, *Torovirinae)*, *Herpesviridae (Alphaherpesvirinae*, *Betaherpesvirinae*, *Gammaherpesvirinae)*, *Paramyxoviridae (Paramyxovirinae*, *Pneumovirinae)*, *Parvoviridae (Parvovirinae)*, *Poxviridae (Chorodopoxvirinae)*, *Reoviridae (Sedoreovirinae*, *Spinareovirinae)* and *Retroviridae (Orthoretrovirinae*, *Spumaretrovirinae)* (Table 150-1).

Virus genera consist of groups of species that share common characteristics different from those of members of other genera. Common properties within a genus include viral replication strategy, genome size, organization, numbers of segments, sequence homologies, and vector transmission. Virus genera are designated with the suffix *-virus*.

The species taxon is the most fundamental unit in biologic classification, but the viruses have proved to be the most difficult with which to deal, and years elapsed before a definition of virus species was accepted internationally. In 1991, the ICTV accepted the definition of a virus species proposed by van Regenmortel[14]: "A virus species is defined as a polythetic class of viruses that constitutes a replicating lineage and occupies a particular ecological niche." Members of a polythetic class have several properties in common, although no single common attribute is present in all members, thereby accommodating the inherent variability of viruses. Common species properties include genome rearrangement, sequence homologies, serologic relationships,

*References 5, 7, 8, 10, 11, 13, 17-19.
†www.ICTVonline.org and www.ncbi.nlm.nih.gov

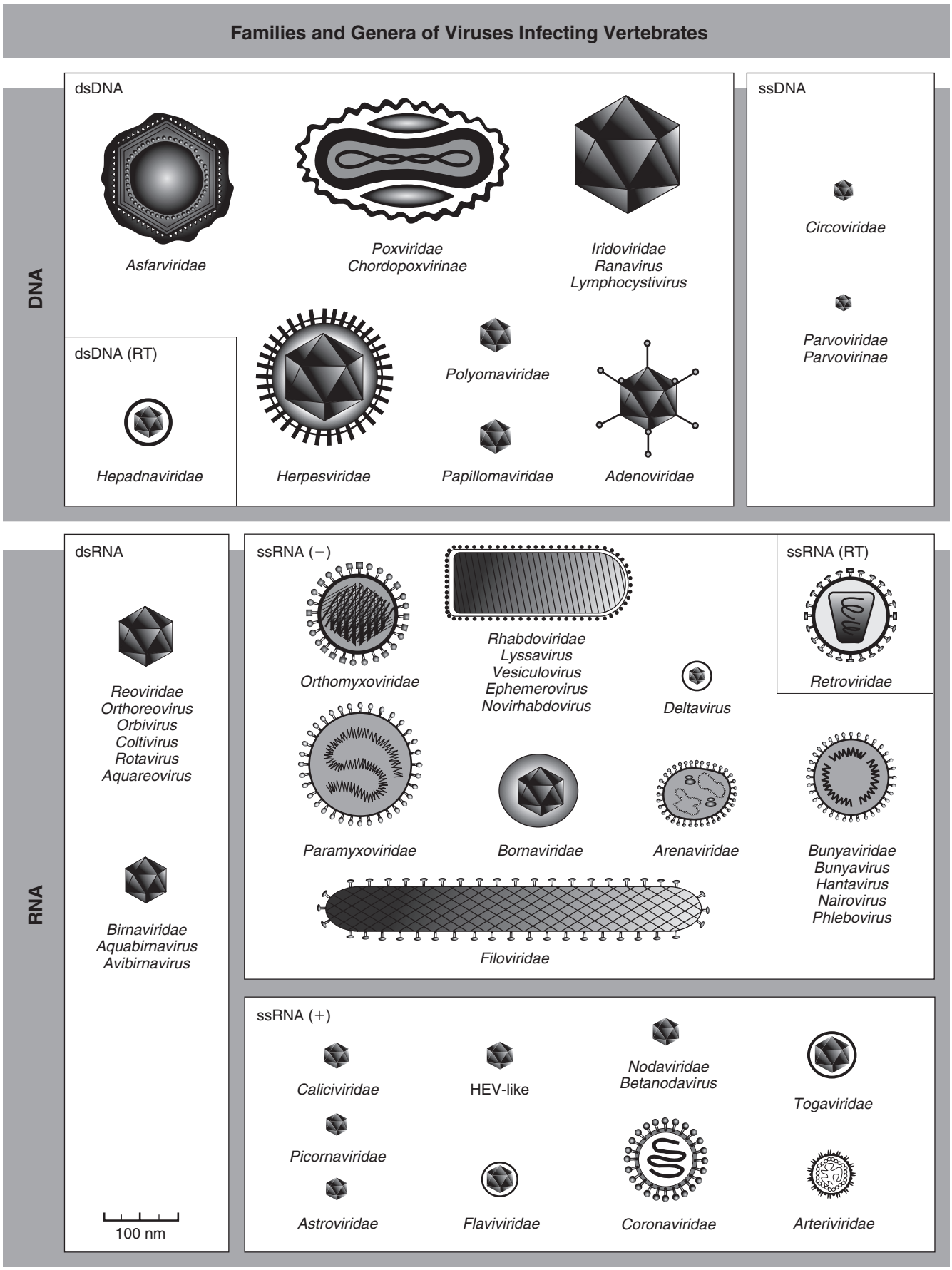

FIGURE 150-1 ■ **Diagram illustrating the shapes and relative sizes of animal viruses of the major families.** (From Van Regenmortel MHV, Fauquet CM, Bishop DHL, et al., editors. *Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses.* San Diego, Calif.: Academic Press; 2000, p. 30.)

TABLE 150-1 **Classification and Nomenclature of Representative Viruses Affecting Humans**

Family	Subfamily	Genus	Representative Species Pathogenic for Humans (Abbreviation, Common Examples, Disease, Synonym, or No. of Members)
DNA Viruses			
Poxviridae	*Chordopoxvirinae*	*Orthopoxvirus*	*Vaccinia virus* (VACV)
			Variola virus (VARV)
		Parapoxvirus	*Orf virus* (ORFH, contagious pustular dermatitis)
			Pseudocowpox virus (PCPV, milkers' nodule)
		Molluscipoxvirus	*Molluscum contagiosum virus* (MOCV)
		Yatapoxvirus	*Yaba monkey tumor virus, tanapox virus* (YMTV, TANV)
Herpesviridae	*Alphaherpesvirinae*	*Simplexvirus*	*Human herpesvirus 1* (HHV-1, herpes simplex virus type 1)
			Human herpesvirus 2 (HHV-2, herpes simplex virus type 2)
			Macacine herpesvirus 1 (McHV-1, monkey B virus)
		Varicellovirus	*Human herpesvirus 3* (HHV-3, varicella-zoster virus)
	Betaherpesvirinae	*Cytomegalovirus*	*Human herpesvirus 5* (HHV-5, cytomegalovirus)
		Roseolovirus	*Human herpesvirus 6A & 6B* (HHV-6, human B lymphotropic virus)
			Human herpesvirus 7 (HHV-7)
	Gammaherpesvirinae	*Lymphocryptovirus*	*Human herpesvirus 4* (HHV-4, Epstein-Barr virus)
		Rhadinovirus	*Human herpesvirus 8* (HHV-8, Kaposi sarcoma–associated herpesvirus)
Adenoviridae		*Mastadenovirus*	*Human adenovirus A* (HAdV-A; 12, 18, 31)
			Human adenovirus B (HAdV-B; 3, 7, 11, 14, 16, 21, 34, 35, 50, 55)
			Human adenovirus C (HAdV-C; 1, 2, 5, 6, 57)
			Human adenovirus D (HAdV-D; 8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, 53, 54, 56)
			Human adenovirus E (HAdV-E; 4)
			Human adenovirus F (HAdV-F; 40, 41)
			Human adenovirus G (HAdV-G; 52)
Papillomaviridae		*Alphapapillomavirus*	*Alphapapillomavirus 7* (HPV 18, 39, 45, 59, 68, 70, 85)
			Alphapapillomavirus 9 (HPV 16, 31, 33, 35, 52, 58, 67)
		Betapapillomavirus	*Betapapillomavirus 1* (HPV 5, 8, 12, 14, 19-21, 25, 36, 47, 93)
		Gammapapillomavirus	*Gammapapillomavirus 1* (HPV 4, 65, 95)
Polyomaviridae		*Polyomavirus*	*BK polyomavirus* (BKPyV, polyomavirus hominis 1)
			JC polyomavirus (JCPyV, polyomavirus hominis 2)
Parvoviridae	*Parvovirinae*	*Bocavirus*	*Human bocavirus,* not classified
		Erythrovirus	*Human parvovirus B19* (erythema infectiosum or fifth disease)
Hepadnaviridae		*Orthohepadnavirus*	*Hepatitis B virus* (HBV)
Hepeviridae		*Hepevirus*	*Hepatitis E virus* (HEV, enterically transmitted non-A, non-B)
RNA Viruses			
Reoviridae	*Sedoreovirinae*	*Orbivirus*	*Changuinola virus* (CGLV); *Great Island virus* (GIV)
		Rotavirus	*Rotavirus A, B, C, D, F, G* (RV)
		Sedovirus	
	Spinarevirinae	*Coltivirus*	*Banna virus* (BAV)
		Orthoreovirus	*Colorado tick fever virus* (CTFV)
			Mammalian orthoreovirus (MRV)
Bornaviridae		*Bornavirus*	*Borna disease virus* (BDV)
Paramyxoviridae	*Paramyxovirinae*	*Henipavirus*	*Hendravirus, Nipahvirus*
		Morbillivirus	*Measles virus* (MeV)
		Respirovirus	*Human parainfluenza virus 1 and 3* (HPIV-1, HPIV-3)
		Rubulavirus	*Mumps virus* (MUV), *Human parainfluenza virus 2* (HPIV-2) *and 4* (HPIV-4a and b)
	Pneumovirinae	*Pneumovirus*	*Human respiratory syncytial virus* (HRSV)
		Metapneumovirus	*Human metapneumovirus* (HMPV)

TABLE 150-1 **Classification and Nomenclature of Representative Viruses Affecting Humans** (Continued)

Family	Subfamily	Genus	Representative Species Pathogenic for Humans (Abbreviation, Common Examples, Disease, Synonym, or No. of Members)
Rhabdoviridae		*Vesiculovirus*	*Vesicular stomatitis Indiana virus* (VSIV)
		Lyssavirus	*Rabies virus* (RABV)
Filoviridae		*Marburgvirus*	*Marburg marburgvirus* (LVMARV, Lake Victoria)
		Ebolavirus	*Zaire ebolavirus* (ZEBOV), *Reston ebolavirus* (REBOV)
Orthomyxoviridae		*Influenzavirus A*	*Influenza A virus* (FLUAV)
		Influenzavirus B	*Influenza B virus* (FLUBV)
		Influenzavirus C	*Influenza C virus* (FLUCV)
Bunyaviridae		*Orthobunyavirus*	*Bunyamwera virus* (BUNV, Bunyamwera serogroup), *California encephalitis virus* (CEV, California serogroup) (mosquito and culicid fly transmitted)
		Hantavirus	*Hantaan virus* (HTNV, Korean hemorrhagic fever or hemorrhagic fever with renal syndrome), *Sin Nombre virus* (SNV, Four Corners hantavirus, hantavirus pulmonary syndrome), and others (rodent associated)
		Nairovirus	*Crimean-Congo hemorrhagic fever virus* (CCHFV) (tick transmitted)
		Phlebovirus	*Sandfly fever Naples virus* (SFNV), *Rift Valley fever virus* (RVFV) (sandfly-borne primarily); *Uukuniemi virus* (UUKV) (phlebotomine, tick, and mosquito transmitted)
Arenaviridae		*Arenavirus*	*Lymphocytic choriomeningitis virus* (LCMV)
			Lassa virus (LASV)
			Junin virus (JUNV, Argentine hemorrhagic fever virus)
			Machupo virus (MAVC, Bolivian hemorrhagic fever virus)
Picornaviridae		*Aphthovirus*	*Foot and mouth disease virus*
		Enterovirus	*Human enterovirus A* (HEV-A; human coxsackieviruses A2-A8, A10, A12, A14, A16; human enterovirus 71,76, 89-92)
			Human enterovirus B (HEV-B; human coxsackieviruses B1-B6; human coxsackievirus A9, A23; human echovirus 1-21, 26-27, 29-33; human enterovirus, 23 serotypes)
			Human enterovirus C (HEV-C; human coxsackieviruses A1, A11, A13, A17, A19-A22, A24; PV, polioviruses 1-3; human enterovirus, 8 serotypes)
			Human enterovirus D (HEV-D; human enteroviruses 68, 70, and 94)
			Human rhinoviruses A , B, and C (HRV-A, B, C; 152 serotypes)
		Parechovirus	*Human parechovirus* (HPeV; human parechovirus 1, formerly echovirus 22; human parechovirus 2, formerly echovirus 23)
		Hepatovirus	*Hepatitis A virus* (HAV, enterovirus 72)
Caliciviridae		*Norovirus*	*Norwalk virus* (NoV, genogroups I-V, 35 genotypes)
		Sapovirus	*Sapporo virus* (SoV, genogroups I-VII)
Astroviridae		*Mamastrovirus*	*Mamastrovirus 1* (MAstV)
Coronaviridae	*Coronavirinae*	*Alphacoronavirus*	*Human coronavirus 229E* (HCoV-229E); *Human coronavirus NL63* (HCoV-NL63)
		Betacoronavirus	*Human coronavirus HKU1* (HCoV-HKU1); *Human coronavirus OC43* (HCoV-OC43); *Severe acute respiratory syndrome–related virus* (SARS)
	Torovirinae	*Torovirus*	*Human torovirus* (HuTV)
Flaviviridae		*Flavivirus*	*St. Louis encephalitis virus* (SLEV), *yellow fever virus* (YFV), *dengue virus* (DENV), a*nd West Nile virus* (WNV) (group B arboviruses, mosquito-borne)

Continued on following page

TABLE 150-1 **Classification and Nomenclature of Representative Viruses Affecting Humans** (Continued)

Family	Subfamily	Genus	Representative Species Pathogenic for Humans (Abbreviation, Common Examples, Disease, Synonym, or No. of Members)
			Kyasanur Forest disease virus (KFDV), *tick-borne encephalitis virus* (TBEV) (tick-borne)
			Vector-unassociated viruses
		Hepacivirus	*Hepatitis C virus* (HCV, parenterally transmitted non-A, non-B hepatitis)
Togaviridae		*Alphavirus*	*Western, eastern, and Venezuelan equine encephalitis viruses* (WEEV, EEEV, VEEV); *Ross River and Sindbis viruses* (RRV, SINV) (group A arboviruses, mosquito-borne)
		Rubivirus	*Rubella virus* (RUBV)
Retroviridae	*Orthoretrovirinae*	*Deltaretrovirus*	*Primate T-cell lymphotropic viruses 1-3* (PTLV-1 to 3, human T-lymphotropic viruses 1-3)
		Lentivirus	*Human immunodeficiency viruses 1 and 2*
Subviral Agents (Satellites, Viroids, and Agents of Spongiform Encephalopathies)			
Prions			Kuru, Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI)
Unclassified Viruses			
		Delta virus	*Hepatitis delta virus* (HDV)

TABLE 150-2 **Representative Properties Used In Virus Taxonomy**

Virion Properties

Morphology
- Size
- Shape
- Presence, absence of envelope and peplomers
- Capsid structure, symmetry

Physical properties
- Molecular mass
- Buoyant density
- Sedimentation coefficient
- pH stability
- Thermal stability
- Solvent, detergent stability
- Cation (Mg^{2+}, Mn^{2+}) stability
- Radiation stability

Genome
- Type of nucleic acid, DNA or RNA
- Strandedness, single- or double-stranded
- Linear or circular
- Sense—positive, negative, or ambisense
- Number of segments
- Size
- Presence or absence and type of 5′ terminal cap
- Nucleotide sequence comparisons

Protein properties
- Number
- Size
- Functional activities
- Amino acid sequence comparisons

Lipids
- Presence or absence
- Nature

Carbohydrates
- Presence or absence
- Nature

Genome Organization and Replication

Organization

Strategy of replication

Characteristics of transcription

Characteristics of translation and post-translational processing

Site of accumulation of assembly, maturation, and release

Antigenic Properties

Serologic relationships

Mapping epitopes

Biologic Properties

Host range

Pathogenesis

Tissue tropisms, pathology

Mode of transmission

Vector relationships

Geographic distribution

vector transmission, host range, pathogenicity, tissue tropism, and geographic distribution.[8,17] Some properties of viruses used in taxonomy are listed in Table 150-2.[13]

The ICTV also has established criteria for formal virus nomenclature[8,9,17] and recommended abbreviations for virus names.[4,8,9,17] In formal taxonomic usage, the names of orders, families, subfamilies, genera, and species are printed in italics, and the first letter of the name is capitalized. This form applies when using a species name to refer to a taxonomic category (e.g., in the Materials and Methods section of a paper when describing the particular virus used in the study). Some examples of

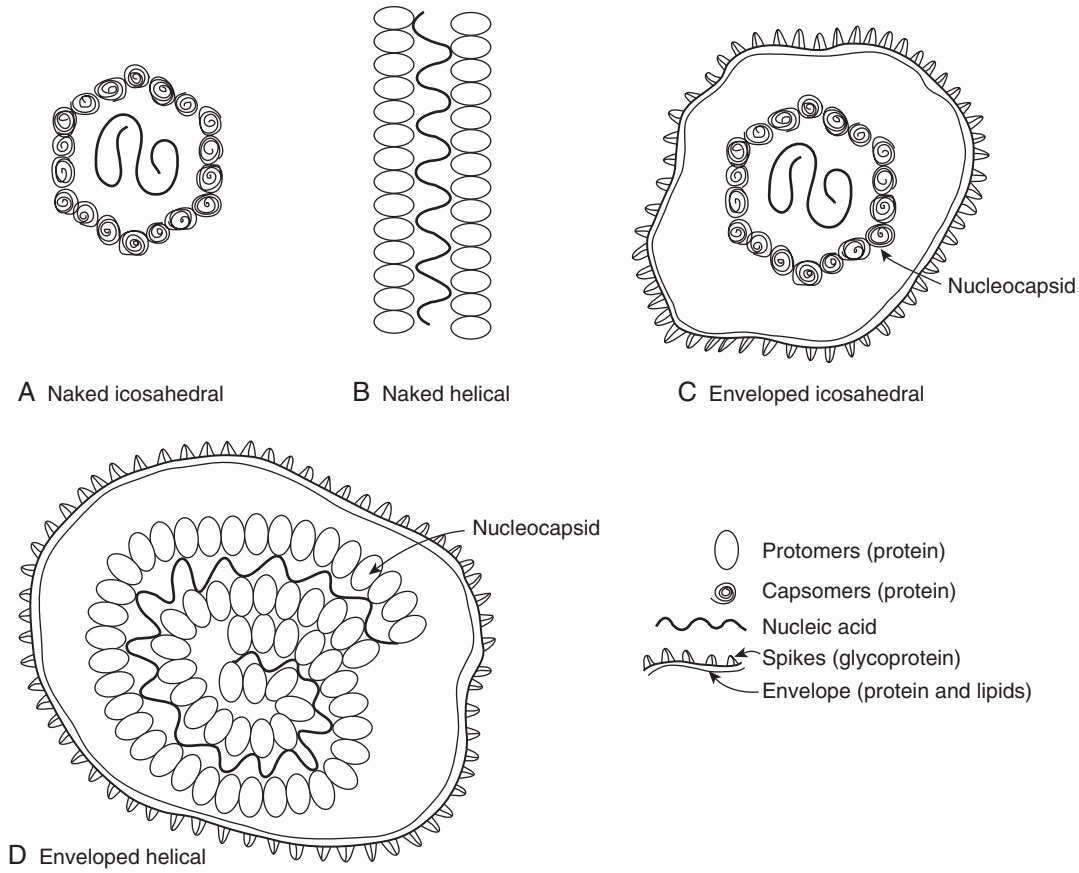

FIGURE 150-2 ■ Schematic diagram of simple forms of virions and their components. The naked icosahedral virions resemble small crystals; the naked helical virions resemble rods with a fine regular helical pattern in their surface. The enveloped icosahedral virions are composed of icosahedral nucleocapsids surrounded by the envelope; the enveloped helical virions are helical nucleocapsids bent to form a coarse, often irregular, coil within the envelope. (From Davis BD, Dulbecco R, Eisen HN, et al. *Microbiology.* 4th ed. Philadelphia: JB Lippincott; 1990, p. 772.)

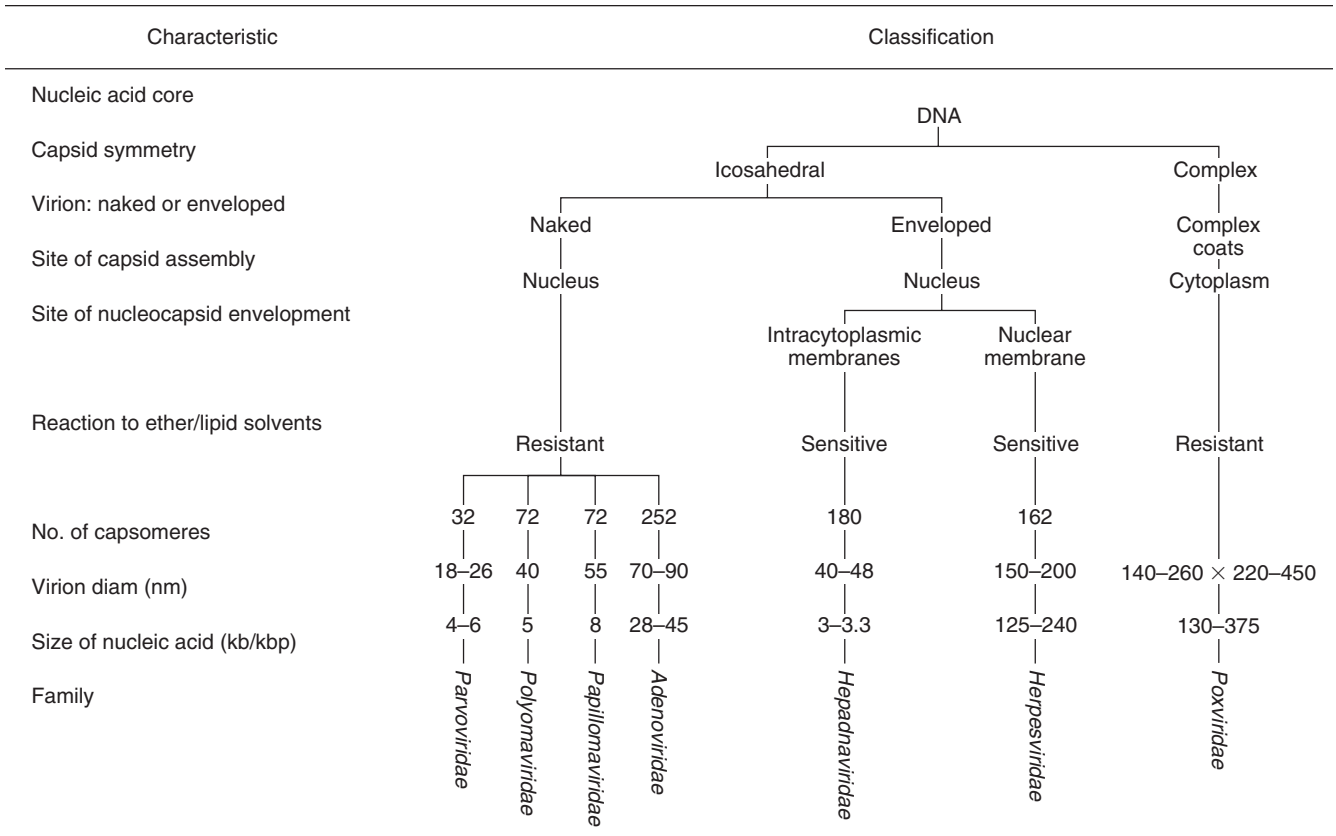

FIGURE 150-3 ■ Classification of major DNA-containing viruses affecting humans. (Modified from Melnick JL. Nomenclature and classification of viruses. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases.* 4th ed. Philadelphia: WB Saunders; 1998, p. 1603-7.)

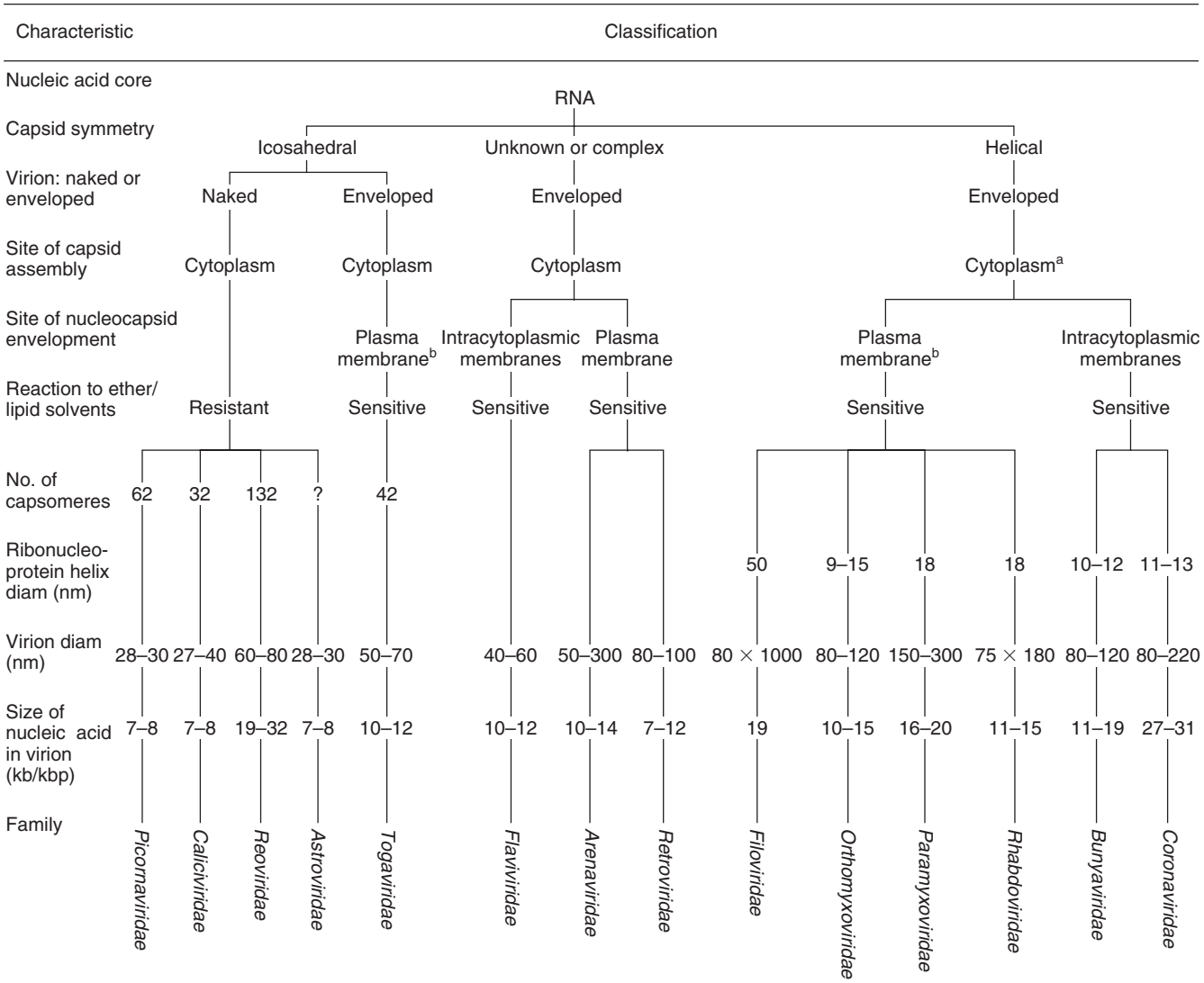

FIGURE 150-4 ■ **Classification of major RNA-containing viruses affecting humans.** (Modified from Melnick JL. Nomenclature and classification of viruses. In Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases.* 4th ed. Philadelphia: WB Saunders; 1998, p. 1603-7.)

formal taxonomic terminology are *Poliovirus*, genus *Enterovirus*, family *Picornaviridae* or *Human herpesvirus 1* (herpes simplex virus type 1) and genus *Simplexvirus*, subfamily *Alphaherpesvirinae*, family *Herpesviridae*. Thereafter, vernacular names can be used throughout the publication. In informal vernacular usage when referring to virions or virus particles or if used as an adjective, italics and capital initial letters are not required and the names are written in lowercase Roman script (e.g., "poliovirus cytopathic effect" or "picornaviruses/polioviruses were inoculated into cell culture").[8,9,15,16] Viruses with uncertain taxonomic status are considered "tentative" species, and names are not italicized, although the initial letter is capitalized. Although not part of the formal international code, abbreviations also have been recommended by the ICTV for every virus name to reduce the possibility of duplication when new abbreviations are proposed[4,8,17] (see Table 150-1).

Figure 150-2 is a schematic diagram showing the basic forms and composition of viruses.[3] The type of nucleic acid, capsid symmetry, presence or absence of an envelope, and peplomers (spikes) all are characteristics used in classification. Figure 150-1 is a more detailed diagram not only illustrating the relative shapes and sizes of families and genera of vertebrate viruses but also indicating the type and nature (single-stranded [ss], double-stranded [ds], sense [+/–]) of the nucleic acid, presence of reverse transcriptase (RT), and presence or absence of an envelope.

Figures 150-3 and 150-4 show classification of DNA and RNA virus families,[12] respectively, by some of the taxonomic characteristics listed in Table 150-2. Representative DNA and RNA viruses affecting humans are listed in Table 150-1, which uses the most recent nomenclature reported by the ICTV (for a complete listing, see King and colleagues[8]).

NEW REFERENCES SINCE THE SIXTH EDITION

8. King AMQ, Adams MJ, Carstens EB, et al, editors. *Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on the Taxonomy of Viruses*. San Diego, Calif.: Elsevier Academic Press; 2011.

9. Kuhn JH, Jahrling PB. Clarification and guidance on the proper usage of virus and virus species names. *Arch Virol* 2010;**55**:445–53.

The full reference list for this chapter is available at expertconsult.com.

DNA VIRUSES

SUBSECTION 1 *PARVOVIRIDAE*

CHAPTER 151

HUMAN PARVOVIRUS B19

Danica J. Schulte

The family *Parvoviridae* includes two subfamilies: *Parvovirinae* and *Densovirinae*.[341,468] The subfamily *Parvovirinae* has six genera: *Erythrovirus*, *Parvovirus*, *Dependovirus*, *Bocavirus*, *Amdovirus*, and *Parv4;* the subfamily *Densovirinae* has five genera: *Brevidensovirus*, *Pefudensovirus*, *Densovirus*, *Iteravirus*, and *Centravirus*. Densonucleosis viruses (*Densovirus* spp.) infect insect hosts and have not been found in humans or mammals,[35,379] whereas the *Parvovirinae* are viruses of vertebrates.[52]

Currently, human parvovirus B19 is the only member of the genus *Erythrovirus* that infects humans; however, two other genera of the subfamily *Parvovirinae* (*Parv4* and *Bocavirus*) also cause human infection.[432] Originally, B19 virus was classified in the genus *Parvovirus*. Despite the reclassification, the contemporary literature still refers to this *Erythrovirus* as parvovirus B19. This virus is autonomous and does not require the presence of a helper virus.

Three human parvovirus B19 genotypes have been defined based on isolates with greater than 10 percent divergence in overall DNA sequence, all causing human disease.[52,404] In 1999, *Erythrovirus* V9, a member of genotype 3, was found to be clinically significant.[189,193,195,370,431] V9 virus was found in the serum and bone marrow of a 6-year-old boy with transient aplastic anemia[348]; however, routine testing for B19 virus failed to identify the V9 virus,[189] because it has more than 11 percent variation from the published B19 genome, with nucleotide discrepancies encompassing the entire genome.[193,195,432]

B19 typically is well conserved, with less than 2 percent sequence variability between isolates.[193,195,348,470] The prevalence of each genotype varies with geographic origin, population, and sample type. Genotype 1 is present worldwide, whereas genotype 3 is prevalent in Africa and genotype 2[135,432,528] primarily circulates in Europe and North America and is very limited.[166,432]

More recently, two additional members of the *Parvovirinae* subfamily have been discovered. One of these, human bocavirus, is discussed in Chapter 152. The other virus, *Parv4*, was found in plasma samples from pooled plasma collections,[155,290,460] placentally,[78] and in persons screened for acute human immunodeficiency virus (HIV) infection.[225,301]

Erythema infectiosum is the most common clinical manifestation of infection with parvovirus B19. Other clinical findings include arthralgia and arthritis, aplastic crisis in patients with red blood cell defects, chronic anemia in immunocompromised patients, fetal hydrops, cardiac disease, neurologic disease, and a variety of other significant illnesses.

HISTORY

The first parvoviruses to be discovered infecting humans were found in stool specimens by electron microscopy.[380] These fecal parvoviruses have been linked with gastrointestinal symptoms, often in outbreaks associated with the consumption of shellfish.[119,477] Their precise pathogenic role remains unclear because these viruses also may be found in the feces of asymptomatic individuals.[380]

In 1974, a second type of parvovirus, human parvovirus B19, was found in the serum of asymptomatic blood donors.[52,100,189] Discovered by chance as an agent responsible for false-positive results in the counterimmunoelectrophoresis tests then in use for detection of hepatitis B surface antigen, it was revealed by electron

REFERENCES

1. Andrewes CH. Nomenclature of viruses. *Nature* 1954;**173**:260–1.
2. Andrewes CH, Bang FB, Burnet FM. A short description of the *Myxovirus* group (influenza and related viruses). *Virology* 1955;**1**: 176–80.
3. Davis BD, Dulbecco R, Eisen HN, et al. *Microbiology*. 4th ed. Philadelphia: JB Lippincott; 1990.
4. Fauquet CM, Pringle CR. Abbreviations for vertebrate virus species names. *Arch Virol* 1999;**144**:1865–80.
5. Fenner F. Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. *Intervirology* 1976;**7**:1–116.
6. Fenner F, Burnet FM. A short description of the poxvirus group (vaccinia and related viruses). *Virology* 1957;**4**:305–10.
7. Francki RIB, Fauquet CM, Knudson DL, et al, editors. Classification and nomenclature of viruses. Fifth Report of the International Committee on Taxonomy of Viruses. *Arch Virol* 1991;**2**(Suppl.): 1–450.
8. King AMQ, Adams MJ, Carstens EB, et al, editors. *Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on the Taxonomy of Viruses*. San Diego, Calif.: Elsevier Academic Press; 2011.
9. Kuhn JH, Jahrling PB. Clarification and guidance on the proper usage of virus and virus species names. *Arch Virol* 2010;**55**: 445–53.
10. Matthews REF. Classification and nomenclature of viruses. Third report of the International Committee on Taxonomy of Viruses. *Intervirology* 1979;**12**:129–296.
11. Matthews REF. Classification and nomenclature of viruses. Fourth report of the International Committee on Taxonomy of Viruses. *Intervirology* 1982;**17**:1–199.
12. Melnick JL. Nomenclature and classification of viruses. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 4th ed. Philadelphia: WB Saunders; 1998.
13. Murphy FA, Fauquet CM, Bishop DHL, et al, editors. Virus taxonomy. Sixth report of the International Committee on Taxonomy of Viruses. *Arch Virol* 1995;**10**(Suppl.):1–503.
14. Van Regenmortel MHV. Virus species, a much overlooked but essential concept in virus classification. *Intervirology* 1990;**31**: 241–54.
15. Van Regenmortel MHV. How to write the names of virus species. *Arch Virol* 1999;**144**:1041–2.
16. Van Regenmortel MHV. On the relative merits of italics, Latin and binomial nomenclature in virus taxonomy. *Arch Virol* 2000;**145**: 433–41.
17. Van Regenmortel MHV, Fauquet CM, Bishop DHL, et al, editors. *Virus taxonomy: classification and nomenclature of viruses. Seventh report of the International Committee on Taxonomy of Viruses*. San Diego, Calif.: Academic Press; 2000.
18. Van Regenmortel MHV, Mahy BWJ. Emerging issues in virus taxonomy. *Emerg Infect Dis* 2004;**10**:8–13.
19. Wildy P. Classification and nomenclature of viruses: first report of the International Committee on Nomenclature of Viruses. In: *Monographs in virology*, vol. 5. Basel: S. Karger; 1971.

microscopy to be uniform icosahedral particles with a mean diameter of 23 nm.

Preliminary studies of the physiochemical nature of this agent suggested its probable identity as a member of the family *Parvoviridae*.[100] In the following years, reports concerning this virus named it variously as human parvovirus–like agent, serum parvovirus–like virus,[93] and B19[438] after one of the original isolates. After its chemical properties were elucidated in 1983,[88,457] the virus was referred to as human parvovirus—or human serum parvovirus—to denote that it is distinct from the fecal viral particles. Today, the virus most commonly is called human parvovirus B19 or simply B19 virus.[9,94,240]

For many years after its discovery, infection with B19 virus appeared to be either asymptomatic or associated with a nonspecific febrile illness.[438] However, in the early 1980s, the central role of B19 virus in the etiology of aplastic crisis in patients with chronic hemolytic anemia was identified.[84,118,229,399,430] Soon thereafter came the appreciation that erythema infectiosum (fifth disease) is the common manifestation of infection with B19 virus.[16] Over 20 years ago, the association between B19 virus infection in pregnancy and fetal death was observed; and during the past few decades, the clinical spectrum of B19 virus infection has broadened considerably.*

Although human parvovirus B19 was not discovered until 1974, its most common clinical disease (erythema infectiosum) was described more than 200 years ago.[46] Tschamer's report in 1889 was considered by most reviewers to be the first description of erythema infectiosum.[473] However, Boysen[46] noted that the English dermatologist Robert Willan had reported the illness first in 1799 and then more completely in 1840.

After Tschamer's report came a succession of Austrian and German reports of the illness.[46] Tschamer thought that erythema infectiosum was a manifestation of rubella, but a few years later Escherich[137] suggested that it was a specific disease. Stricker,[455] in 1899, gave the name erythema infectiosum to the clinical entity.

In 1905, Shaw,[437] after having observed cases of erythema infectiosum in Austria, published the first account in the American literature. Herrick[201] was the first to document in detail an outbreak in the United States; however, cases of erythema infectiosum had been observed previously in St. Louis[533] and in Hamburg, New York.[201] Over the past century, numerous outbreaks and epidemics have been described in North America.† Erythema infectiosum frequently is referred to as fifth disease and in the past also was called ring rubella, large-spotted disease, and epidemic megaloerythema.

PROPERTIES OF THE VIRUS

Human parvovirus B19 is a naked icosahedral virus with a mean diameter of 23 nm and a mean buoyant density in cesium chloride of 1.43 g/dL.[100] The capsid is formed from two major structural proteins (VP1 and VP2) discernible by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.[88] VP2 is the major structural protein, with a molecular weight of 58 kDa; VP1 has a molecular weight of 84 kDa. Ninety-six percent of the capsid is VP2, and 4 percent is VP1. The structure of VP2 has been determined by x-ray crystallography.[189,228] The major nonstructural protein (NS1) has a molecular weight of 77 kDa and acts as a strong transcription activator by recruiting cellular transcription factors and participating in viral replication.[432]

The genome consists of a single molecule of single-stranded DNA approximately 5.6 kb in length.[108] The DNA in B19 virus occurs as both plus and minus strands in approximately equal numbers.[231] When virions are disrupted with protease, the two complementary strands anneal to form a stable duplex.[457] At each end of the molecule are palindromic sequences forming "hairpin" loops. The hairpin at the 3′ end of the genome serves as a primer for DNA polymerases. The hairpin duplex at the 5′ end of the molecule consists of sequences that are neither complementary to those at the 3′ end, as is found in dependoviruses, nor as highly complementary within this 5′ end as are those at the 3′ end.[70] Folding of the VP1 and VP2 proteins creates α-helical loops that appear on the surface of the capsid and are available to the host immune system. The unique region of VP1 is external to the capsid and it contains linear epitopes recognized by neutralizing antibodies and elicits a more efficient immune response than the VP2 region,[528] but antibodies against the VP2 capsid protein are maintained.[95] NS1 can activate tumor necrosis factor-α, interleukin-6, nuclear factor κB, and TP53 and influences signaling pathways involved in the antiviral response.[95] Moreover, B19 DNA can activate Toll-like receptor 9 into erythroid cells, leading to inhibition of cell growth.[432]

Parvovirus infectivity is relatively heat stable, tolerant of a wide range of pH, and resistant to ether.[18] Successful replication of parvoviruses can be accomplished only in a dividing cell because of the absolute requirement for the host-cell function or functions found in late S phase.[185] Human parvovirus B19 can be propagated only in human erythropoietic cells from bone marrow, erythroid cells from a patient with erythroleukemia, human umbilical cord, and peripheral blood and in primary fetal liver culture.* It grows only in dividing cells; therefore, all the aforementioned tissue culture systems require the addition of erythropoietin and are regulated by STAT5A and MEK signaling.[76]

The cellular receptor for B19 virus infection is the blood group P antigen, which is a globoside and a neutral glycolipid.[53] The P antigen also requires a cellular coreceptor, $\alpha_5\beta_1$ integrin, for sufficient entry of parvovirus B19 into human cells.[501] In cooperation with P antigen, B19 virus can also bind to Ku80 autoantigen, expressed on B and T lymphocytes, to mediate efficient cell entry.[95] P antigen is found on erythroblasts, megakaryoblasts, endothelial cells, and fetal myocytes.[53] People who lack

*References 7, 40, 80, 189, 192, 205, 231, 232, 340, 385, 422, 427, 510, 528.

†References 3, 19, 23, 24, 28, 48, 68, 75, 86, 90, 96, 121, 149, 154, 162, 169, 189, 242, 273, 274, 286, 322, 342, 386, 387, 401, 441, 490, 491, 505, 507, 528, 529.

*References 56, 189, 231, 374, 379, 450, 451, 463.

P antigen are resistant to infection with B19 virus, and in vitro studies demonstrate that the erythroid precursors from people lacking P antigen cannot be infected, even in the presence of high concentrations of virus.[55,189,284]

Antigenically, this human erythrovirus is distinct from the parvovirus-like particles found in feces,[381] as well as from the human dependoviruses and autonomous animal parvoviruses.[100] However, the nucleotide sequence and hybridization results reported by Turton and associates[477] suggest that the viruses from gastroenteritis cases in 1977 and 1986 are similar to B19 virus. In contrast to animal parvoviruses, no hemagglutinin has been demonstrated.

The degree to which B19 virus is related to other mammalian parvovirus types has been investigated by DNA-DNA hybridization. Although no relationship is discernible with the human dependoviruses, a distant evolutionary relationship to the genomes of the autonomous parvoviruses of rodents is apparent. Interestingly, this relationship is closer than that between B19 virus and the parvoviruses infecting domestic animals (bovine, feline, and canine parvoviruses).[101] Shackelton and Holmes[435] studied the evolutionary dynamics and phylogenetic history of the *Parvoviridae* and observed a high rate of evolutionary change, with 10^{-4} nucleotide substitutions per site per year.

Many recent studies have examined genetic variability in parvovirus B19 isolates.* Hemauer and associates[199] suggested that greater genome variability occurs in isolates from patients with persistent infection than in isolates from patients with acute infection. Erdman and colleagues[134,470] noted that geographically defined genetic lineages of parvovirus B19 existed but that no particular genotype was associated with a specific clinical manifestation.

EPIDEMIOLOGY

Outbreaks of erythema infectiosum have been observed throughout the world, but most reports have come from nontropical regions. The epidemic pattern of erythema infectiosum is surprisingly similar to that of rubella. Community epidemics are most prevalent in the winter and spring, and they usually last for 3 to 6 months. In a review of 30 well-described epidemics,† 26 had their onset in the period from December through May and 23 of the 30 peaked in March, April, or May. When the North American literature for a 50-year period is examined, a cyclic pattern is evident, with peaks in disease activity occurring approximately every 6 years. The peak periods last for an average of 3 years. A longitudinal study of aplastic crisis in persons with sickle-cell anemia in Jamaica suggested that peaks in incidence occur every 2 to 3 years in that island population.[430]

The case-to-case interval of erythema infectiosum is reported to be between 4 and 14 days.‡ In an epidemic in an elementary school, Greenwald and Bashe[147] noted that the mean case-to-case interval was 8.7 days. Ager and associates[3] in their studies noted clustering of intervals between cases of 7 to 11 days. The data of Wilcox and Evans[507] on multiple cases in households suggest that the case-to-case interval usually is closer to 12 to 14 days. In a volunteer study in which adults were inoculated intranasally, the incubation time until onset of the rash was 17 to 18 days.[13] From this study, the case-to-case interval in the community would be expected to be between 6 and 12 days because shedding of virus occurs between days 5 and 12 of infection. This prediction accords well with intervals in the studies noted earlier, as well as intervals observed in patients with hematologic diseases and aplastic crisis.[328]

In epidemics, the attack rate is high. Lauer and colleagues[273] noted an overall attack rate of 24.3 percent in schoolchildren in grades kindergarten through the eighth grade. Similar attack rates were noted in three other school-related outbreaks.[121,164,169] In the community as a whole, the attack rate is highest in children 5 to 14 years of age[2,3] but secondary cases occur in preschool children, teachers, and parents. In the home, secondary cases are reported more commonly in mothers than in fathers.[3] In school epidemics, the attack rate is considerably higher in girls than in boys.[121,273] Prevalence studies of serum IgG antibody to B19 virus have noted that 40 to 60 percent of adults older than the age of 20 years, up to 85 percent of the elderly, and only 2 to 21 percent of children younger than age 11 years are seropositive.[9,10,15,432] The rate of transmission among household contacts is nearly 50 percent.[481]

Nosocomial infections do occur; most often, the index case is an unrecognized, chronically infected patient.[2,260,389] During community outbreaks, the risk for acquisition by hospital workers is no greater than that in other community residents.

Although erythema infectiosum long had been postulated to be transmitted by droplet via the respiratory tract,[3] proof of this route was not obtained until a group of volunteers were infected successfully with B19 virus after intranasal inoculation. One week after inoculation, virus was excreted from the respiratory tract for 6 days.[13]

The role of human leukocyte antigen (HLA) class I and II alleles was investigated by Kerr and associates[235] in northwestern England. Thirty-six patients with symptomatic B19 infection and 900 controls were studied. The frequency of HLA alleles DRB1*01, DRB1*04, and DRB1*07 was significantly higher in patients with B19 infection than in control subjects.

In a cohort study of 169 children with severe anemia in Papua New Guinea, a strong association between severe anemia and B19 infection was observed; however, the authors were unable to show a combined deleterious effect of malaria and B19 infection.[505] They and others demonstrated that 60 percent of the children were infected with B19 before reaching 2 years of age and that more than 90 percent were infected by 6 years of age.[174,378,418,508]

Parvovirus B19 infection also can be transmitted by blood transfusion and clotting factor concentrates.* One

*References 40, 134, 156, 199, 223, 240.
†References 3, 11, 19, 24, 46, 48, 75, 86, 90, 96, 121, 149, 157, 201, 242, 273, 274, 286, 342, 387, 401, 441, 461, 490, 504, 507, 537.
‡References 3, 16, 121, 169, 392, 490, 507.

*References 17, 116, 189, 311, 330, 391, 417, 449, 513, 519, 531.

case of transmission of B19 DNA via peripheral blood progenitor cell transplantation that resolved after treatment with intravenous immune globulin (IVIG) has been reported.[17] Hayakawa and colleagues[186] reported a case of parvovirus B19 infection that they thought was transmitted by IVIG. Researchers have described donor-transmitted parvovirus infection in transplant patients.[517]

PATHOGENESIS AND PATHOLOGY

The pathogenesis of erythroviral disease involves two quite separate components. The first is caused by the lytic infection of susceptible dividing cells and the second by interaction with products of the immune response.

As stated earlier, parvoviruses replicate only in dividing cells. Thus, infection of an organ or tissue in which a significant proportion of the cells are dividing may give rise to organ-specific disease. This condition is seen clearly in canine and feline parvovirus infections, in which replication of virus in the crypt cells of the intestine gives rise to a severe and often fatal enteritis.[179,296]

Parvovirus B19 is thought not to infect cells of the gastrointestinal tract; virus could not be detected in the feces of volunteers,[13] nor have viruses of this type been found in stool specimens.

After gaining entry via the respiratory tract, the virus sets up a systemic infection with copious viremia (Fig. 151-1) in which 10^{10} or 10^{11} virus particles per milliliter of blood is not an uncommon finding.[13,394]

Parvovirus infection results in profound reticulocytopenia for some 7 to 10 days, commencing during viremia.[13,528] In vitro studies on cultured bone marrow and peripheral blood have shown that B19 virus inhibits the formation of blast-forming erythroid colonies, thus suggesting that an early erythrocyte precursor cell is susceptible to infection with the virus. B19-associated damage to cells of the erythroid lineage is due to the cytotoxicity mediated by viral proteins, whereas the apoptotic features mediated by caspase 3 are induced by NS1.[77,83] Overexpression of NS1 has been found to trigger signaling cascades that help promote proapoptotic and apoptotic processes, resulting in anemia, acute fulminant liver failure, placental insufficiency, and myocarditis.[514] In addition to profound erythroid hypoplasia, giant pronormoblasts typically are seen in the bone marrow at the height of transient aplastic crises and normoblasts have characteristic intranuclear eosinophilic inclusion bodies termed *Lampion* or *lantern cells*.[61]

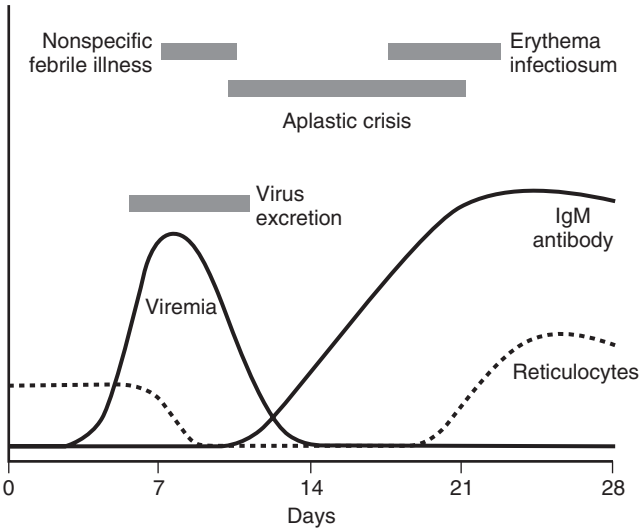

FIGURE 151-1 ■ Selected virologic, immunologic, hematologic, and clinical events in parvovirus B19 virus infection.

Granulocytes and megakaryocytes are unaffected by virus in these in vitro systems. However, during B19 virus infection in a normal host, clinically insignificant lymphopenia and neutropenia occur, as does a drop in platelet number.[13,392] The mechanisms of loss of these cells from peripheral blood remain to be determined. Srivastava and associates[450] noted that B19 virus infection of bone marrow cells in culture results in suppression of megakaryocyte colony formation. In this study, tropism of B19 virus for cells other than erythroid progenitor cells appeared to take place, although viral DNA replication did not occur in the megakaryocyte-enriched fractions. They suggested that the virus might be toxic to cell populations that are nonpermissive of viral DNA replication.

As noted in Figure 151-1, viremia ends at the time of appearance of specific IgM antibody. In addition, researchers have found that the appearance of antibody neutralizes the inhibitory effect of B19 virus on formation of erythrocyte colonies in vitro.[529] The early IgM response is almost entirely VP2 specific. IgG antibody first appears approximately 2 weeks after exposure and later becomes the major antibody subclass; in contrast to the initial IgM response, the IgG response is directed at VP1 rather than VP2. The role of cellular immunity in recovery from infection is unclear. Most patients with persistent infection have T-cell as well as other immune defects, thus suggesting a cellular component in the host response.[40] Von Poblotzki and associates[488] showed that the T cells of persons with parvovirus B19 antibody had HLA class II–restricted responses against B19 structural proteins. Franssila and colleagues[152] demonstrated virus-specific helper T-cell proliferation in recently and remotely infected patients. Their data suggested that B cells that recognize the viral capsid (VP1 and VP2) receive class II–restricted help from $CD4^+$ T lymphocytes. While investigating a parvovirus B19 vaccine, this same group demonstrated that VP1 contains important B-cell epitopes required for protective B-cell immunity, and they concluded that VP2, the major structural protein of B19 virus, appears to provide the major target for B19-specific helper T cells years or decades after initial infection occurs.[150,151] A persistent, activated B19-specific $CD8^+$ T-cell repertoire with maintained effector function occurs in healthy patients who successfully clear the B19 virus. Researchers have hypothesized that these activated $CD8^+$ T cells may play a prominent role in controlling acute B19 infection.[217,227,287,358] Additionally, Kerr and colleagues[233,238] reported that circulating cytokine and chemokine levels in acute B19 disease may correlate with clinical disease.

The most common result of infection with B19 virus is erythema infectiosum. Figure 151-1 shows that the

symptoms of this disease begin 17 or 18 days after inoculation and that approximately 1 week after inoculation virus can be detected in either throat swabs or blood. Skin biopsy results have been reported in three studies.[28,170,207] In three skin biopsy specimens from regions of reticulated eruption, Bard and Perry[28] noted either normal skin or very mild inflammatory changes. Hoffman[207] described edema in the epidermis and perivascular infiltration with mononuclear cells; he also noted swelling of the endothelium of superficial vessels and the presence of cleavage spaces between the epidermis and dermis. The histopathology in 10 cases was investigated by Grimmer and Joseph.[170] They reported dilation of blood vessels with lymphocytic and occasional plasma cell perivascular infiltration. However, the clinical manifestations of many of the cases in the epidemic that Grimmer and Joseph[170] studied were quite atypical.

Virus-specific IgM is present at the time of onset of the rash in erythema infectiosum, thus, although the mechanisms of production of the rash (and arthralgia) of erythema infectiosum remain to be elucidated, postulating an immune-mediated pathogenesis is not unreasonable.[528] The perivascular infiltrations noted by Hoffman[207] would support this suggestion. However, Schwarz and colleagues[426] have found both viral capsid proteins and viral DNA in a skin biopsy specimen of the rash from a patient with erythema infectiosum. They, therefore, suggest that the rash may be a direct effect of the virus rather than being immune complex mediated.

Intrauterine infection results in infection of the fetus and, frequently, abortion.* The main finding in infected fetuses is hydrops fetalis, which results from the anemia caused by infection of the erythrocyte precursor cells. Intranuclear inclusions are seen in nucleated red blood cells, and viral particles are identified in the same cells by electron microscopy.[73] The mechanism responsible for hydrops fetalis stems from the severe anemia, and cardiogenic failure results in generalized ascites, edema, pericardial and pleural effusions, and a thick hydropic placenta.[153] Concomitant myocarditis also occurs, which complicates the clinical picture because the blood group P antigen is expressed on fetal cardiac myocytes.[53,83] The shorter half-life of fetal erythrocytes also exacerbates the disease.[83]

In immunocompromised patients, persistent infection with B19 virus often occurs[267] as a result of failure to produce effective neutralizing antibodies.[83,432] Patients taking immunosuppressive calcineurin inhibitors may be unable to clear B19 virus because these medications inhibit interleukin-2 production and the patients are unable to mount an effective T-helper cell type 1 response.[83,323]

CLINICAL MANIFESTATIONS

Infection with human parvovirus B19 results in a spectrum of clinical manifestations; classic cases of erythema infectiosum occupy a central position in this spectrum. Other major manifestations include arthritis and arthralgia, intrauterine infection and hydrops fetalis, transient aplastic crisis in patients with a variety of underlying hemolytic illnesses, and persistent infection with chronic anemia in patients with immunodeficiencies. In addition, subclinical, nonexanthematous infection occurs, especially in children.[13,392]

Other less common illnesses include myocarditis, vasculitis, glomerulonephritis, and neurologic disease. In addition to the established parvovirus B19 disease associations is an ever-expanding list of categories of illness in which evidence of parvovirus B19 infection has been found (Table 151-1).

Erythema Infectiosum

Although a search of the literature related to outbreaks of erythema infectiosum reveals a conspicuous absence of prodromal symptoms in patients, infected volunteers have had febrile episodes with nonspecific symptoms of headache, chills, myalgia, and malaise accompanying the viremic phase of infection with B19 virus (see Fig. 151-1).[13] These symptoms last for 2 to 3 days and coincide with excretion of virus from the pharynx. This phase is followed by a period of 7 days during which individuals are free of symptoms before onset of the second, or exanthematous, phase of illness. Most likely, the relatively long period between symptoms in this biphasic illness prevented recognition of the link between these nonspecific prodromal symptoms and erythema infectiosum.

The exanthem in classic cases of erythema infectiosum occurs in three stages.* The first stage begins 18 days after the acquisition of infection and is characterized by a fiery red rash on the cheeks (Fig. 151-2) ("slapped-cheek" appearance). The edges of the involved areas may be raised slightly, and a relatively circumoral pallor is present. At this stage, the appearance may be suggestive

FIGURE 151-2 ■ Slapped-cheek appearance with a relative circumoral maculopapular rash in erythema infectiosum. (See companion Expert Consult website for color version.)

*References 7, 9, 68, 73, 106, 162, 189, 245, 397, 406, 424, 510, 527, 528.

*References 3, 23, 48, 75, 81, 86, 169, 437, 481, 490-492.

TABLE 151-1 **Reported Unusual Clinical Findings in Patients with Laboratory Evidence of Parvovirus B19 Virus Infection**

Persistent Arthritis
Juvenile rheumatoid arthritis[81,247,365]
Rheumatoid arthritis[95,352,452,464]
Adult Still disease[288,395]

Neurologic Disease
Encephalitis[25,115,145,194,235,453]
Meningitis[70,258,371,458,461]
Stroke[171,300]
Postinfectious neuralgic amyotropy[27,382]
Guillain-Barré syndrome[27,318]
Transverse myelitis[27]
Facial palsy[302]
Carpal tunnel syndrome[27,414]
Numbness and tingling of fingers[4,27,139]
Myofasciitis[212]

Myocarditis[31,41,42,60,109,112,130,226,261,262,270,337,349,377,411,532]

Cutaneous Manifestations
Papular-purpuric gloves and socks syndrome[6,8,21,37,127,178,209,314,372,442,471,482]
Vesiculopustular exanthema[345]
Erythema multiforme[160]
Henoch-Schönlein syndrome[85,486]
Petechial and purpuric rashes[1,97,117,211,277,310]
Pruritus without rash[295]

Hematologic Manifestations
Thrombocytopenic purpura[5,38,146,216,279,525]
Pancytopenia[180,259,334]
Hemophagocytic syndrome[45,334,448,476,480,497,530]
Neutropenia[307,428]
Diamond-Blackfan syndrome[307,467]
Splenic sequestration[298]

Other Manifestations
Sepsis[22]
Glomerulonephritis[214,220,258,325,506]
Nephrotic syndrome[367]
Kawasaki disease[208,351]
Behçet disease[246]
Polyarteritis nodosa[487]
Wegener granulomatosis[353]
Leukocytoclastic vasculitis[98]
Giant-cell arteritis[157]
Systemic sclerosis[142,143,370]
Systemic vasculitis[105]
Systemic lupus erythematosus[110,202,213,350]
Hepatitis[117,120,184,271,355,447,524]
Raynaud phenomenon[181]
Pseudoappendicitis or mesenteric lymphadenitis[326]
Necrotizing lymphadenitis[224]
Cervical lymphadenopathy[248,475]
Parotitis[302]
Pneumonia[327,453,496]
Pulmonary emboli[18]
Acute respiratory distress syndrome[141]
Mixed cryoglobulinemia[82]
Chronic infection without immunosuppression but with chronic hemolytic anemia[239]
Persistent arthralgia[240]
Chronic fatigue syndrome[221,236,237,240,248,304,308]
Recurrent paresthesias[138]
Fibromyalgia[34]

of scarlet fever, drug sensitivity or other allergic reactions, or collagen vascular disease. The facial exanthem is aggravated by the transition from outdoors to a warm room.

The second stage of the exanthem occurs 1 to 4 days after the onset of facial involvement and consists of the appearance of an erythematous, maculopapular rash on the trunk and limbs. This rash is discrete initially but spreads to involve large areas. Toward the end of this stage, central clearing of the rash from these areas gives the characteristic lacy or reticular pattern (Fig. 151-3).

The third stage of the exanthem is highly variable in duration (i.e., lasting from 1 to 3 or more weeks) and is characterized by marked changes in the intensity of the rash, with periodic complete evanescence and recrudescence. These fluctuations are related to environmental factors such as exposure to sunlight and temperature (a hot bath may result in recrudescence in an apparently recovered child).

The rash often is pruritic, especially in adults, and it generally is more prominent on the extensor surfaces; the palms and soles rarely are affected. Slight desquamation has been noted in a small number of patients in most reviews.

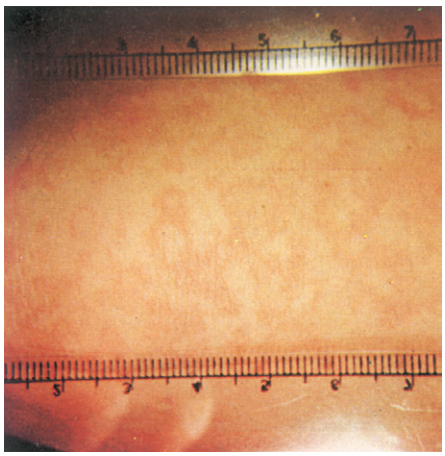

FIGURE 151-3 ■ Rash with a lacelike, or reticular, pattern in erythema infectiosum. (See companion Expert Consult website for color version.)

Although classic cases of erythema infectiosum are easy to recognize clinically, especially during outbreaks, wide variation in the form of the exanthem can be noted—from a very faint, fleeting rash to a florid exanthem that is confluent over large areas (Fig. 151-4). In

FIGURE 151-4 ■ Confluent exanthem in a patient with human parvovirus infection. (See companion Expert Consult website for color version.)

TABLE 151-2 **Frequency of Symptoms and Signs in Erythema Infectiosum**

	Percent Occurrence	
Sign or Symptom	CHILDREN	ADULTS
Rash	100	100
Pruritus	15	50
Headache	20	50
Fever	15	25
Sore throat	15	15
Coryza	10	15
Cough	8	8
Sore eyes	10	10
Anorexia	15	22
Nausea	7	26
Vomiting	4	7
Diarrhea	4	12
Abdominal pain	10	15
Joint pain	10	70
Joint swelling	5	60
Myalgia	4	50

Data from references 3, 121, 169, 491.

many cases the illness may be indistinguishable from rubella. Yermalovich and colleagues published the experience in Belarus following the institution of a national screening program in 2005 for B19 virus in patients with fever and rash who were measles and rubella negative and reported that more than 35 percent of patients were B19 positive.[521]

The overwhelming majority of cases of erythema infectiosum have no enanthem. Kerr and Marsh[242] and Condon[96] noted a few children with pharyngitis.

Other symptoms and signs occur uncommonly in erythema infectiosum (Table 151-2). In general, complaints are expressed more frequently by adults than by children. Headache is reported in approximately a fifth of childhood cases and about half of afflicted adults. Joint pain and swelling and myalgia are particularly troublesome in adults.

Routine laboratory studies are of little use in erythema infectiosum. The leukocyte count usually is normal, although mild eosinophilia is noted occasionally.[23,46,491,492]

The most common complication of erythema infectiosum is joint involvement; it is relatively rare in children (<10% of cases) but is the norm in adults and occurs in 80 percent or more of cases.* A range of severity from mild arthralgia to frank arthritis is observed. The joints most commonly involved are the knees, ankles, and proximal interphalangeal joints; symptoms generally are bilateral. The joint involvement usually is transient and lasts only a few days. In some individuals, symptoms may persist for some weeks or, rarely, months. When joint involvement occurs after an exanthem, the diagnosis may be inferred, but such is not invariably the case. As with rubella, the frequency of arthralgia and arthritis is higher in women than in men.[95]

Other Exanthems

Grimmer and Joseph[170] described cases in which the rashes were morbilliform, hemorrhagic, urticarial, vesicular, and resembled erythema multiforme. Their studies were conducted during an epidemic of erythema infectiosum in Berlin, Germany, in which an estimated 50,000 persons were affected. The cases with markedly unusual manifestations quite possibly were not of the same etiology as the typical cases. Other authors also have noted papular, purpuric, petechial, vesicular, urticarial, and morbilliform eruptions.†

García-Tapia and associates[160] described a 5-year-old girl with erythema multiforme bullosum but without other systemic manifestations. Her serum had B19 virus–specific IgM antibody. Additionally, a papular-purpuric or petechial "glove and sock" syndrome has been reported with acute B19 infection.‡ It is more commonly seen in adults but has been noted in children. B19 virus has been identified in the endothelium of cutaneous biopsy specimens from these patients.[123,415] This unique rash is characterized by petechiae and purpura, with painful or pruritic edema of the hands and feet extending proximally with less severity, and it is self-limited.[6,183,209,215,314] Henoch-Schönlein syndrome and other vasculitic rashes also have been reported.§

Grimmer and Joseph[170] stated that most of their patients in the Berlin epidemic had dark red spots on the pharynx, gums, soft palate, and uvula. These cases, again, must be regarded with some suspicion because the investigators also noted genital lesions and conjunctivitis in a few patients.

Aplastic Crisis

In individuals with chronic hemolytic anemia, the profound reticulocytopenia of human parvovirus B19 infection results in depression of hemoglobin concentrations

*References 9, 15, 16, 23, 187, 192, 205, 343, 402, 405, 493, 505.
†References 75, 96, 97, 147, 211, 277, 298, 310, 345, 387, 516.
‡References 4, 6, 8, 21, 37, 127, 178, 209, 211, 440, 442, 471, 481, 482.
§References 1, 85, 98, 117, 197, 370, 381, 422, 486.

to critical levels.* With resolution of the infection, reticulocytes reappear in the peripheral blood and hemoglobin concentrations return to the normal steady-state values for these patients. This transient arrest in production of erythrocytes is termed *aplastic crisis* and may occur in any individual whose erythrocytes have a short life span. Examples of such conditions include sickle-cell anemia,[165,430,443,536] hereditary spherocytosis,† thalassemia,[277,278,399] glucose-6-phosphate dehydrogenase deficiency,[163] and pyruvate kinase deficiency.[118] However, there are case reports of aplastic anemia secondary to B19 virus developing in immunocompetent patients without underlying diseases.[293,319,398]

Serjeant and coworkers[429] studied the epidemiology of B19 virus infection over time in 308 children with homozygous sickle-cell disease and in 239 controls with normal hemoglobin. B19 virus infection accounted for all 91 episodes of aplastic crisis that occurred. Twenty-three additional patients with sickle-cell disease had B19 virus infections; of these, 10 had mild hematologic changes and 13 had no changes. By 15 years of age, approximately 40 percent of the sickle-cell group and the control group had IgG antibody to B19 virus, thus indicating equal infection rates in the two groups. No patient or control subject had two infections caused by B19 virus.

Smith-Whitley and associates[443] studied a cohort of 633 patients with sickle-cell disease (including patients with various sickle-cell disease genotypes—SS-, SC-, Sβ-thalassemia—as well as those receiving chronic transfusions and hydroxyurea) for complications attributable to acute B19 infection. At study entry, 29.8 percent of the cohort had IgG antibody to B19 virus. The secondary attack rate in the group of children who had siblings with sickle-cell disease was 56 percent. Thirty-five percent of the total patient population with B19 infection did not have worsening anemia; however, transient red cell aplasia developed in 50 percent of the children, with those with SC- or Sβ-thalassemia genotypes characterized by less severe disease. Neutropenia developed in 18 percent of patients and thrombocytopenia in 27 percent. In only one of seven patients receiving chronic transfusions did transient red cell aplasia develop, potentially because of the lower red cell turnover in these patients.

Rao and colleagues[400] found that 70 percent of patients with transient aplastic crisis admitted to their hospitals during a 7-year period had B19 viral infections. No patients had chronic or recurrent infections. In a study of 48 patients with aplastic crises, Mallouh and Qudah[299] found 91 percent to be infected with B19 virus. In addition to the anemia, 21 percent of the patients had leukopenia, 27 percent had neutropenia, and 42 percent had thrombocytopenia. The same investigators noted acute splenic sequestration together with aplastic crisis in three patients with sickle-cell disease and B19 virus infection.[298] Lowenthal and associates[291] noted three young adults with sickle-cell acute chest syndrome associated with B19 virus infection. Yetgin and colleagues[523] reported a case of a 10-year-old child with severe, refractory aplastic anemia ultimately requiring bone marrow transplantation, which resolved the symptoms. Seo reported 13 patients, over a 5-year period, with pure red cell aplasia with associated neutropenia diagnosed with B19 virus on bone marrow biopsy.[428]

Interestingly, reports of exanthematous illness occurring after aplastic crisis are rare.[230,503] However, patients with aplastic crisis require transfusion with packed cells, and rashes occurring after such treatment possibly would be regarded as transfusion reactions. Joint symptoms occur fairly frequently in patients with conditions such as sickle-cell anemia, so although the symptoms may occur as a result of B19 infection, they may be diagnosed as "painful crises."

As noted earlier, human parvovirus B19 infection does not result in aplastic crisis invariably in a chronic hemolytic anemic patient. Some individuals escape this complication if they have undergone transfusion recently,[12] possibly because of a protective effect of transfused antibody (>60% of donors are immune), the substitution of longer-lived, donated erythrocytes for the patients' own fragile ones, or a combination of these two mechanisms.

In populations in which aplastic crisis does occur, the severity of the episode varies among individuals, perhaps reflecting the variation in erythrocyte life span among these patients.[12]

Other Hematologic Manifestations

In addition to pure red blood cell aplasia, numerous other hematologic manifestations, including thrombocytopenic purpura, pancytopenia, hemophagocytic syndrome, neutropenia, and Diamond-Blackfan syndrome, have been observed in association with B19 virus infection.* Thrombocytopenia has been noted as an isolated event; however, idiopathic thrombocytopenic purpura may be associated with aplastic crisis.† Thrombocytopenia is most commonly related to a decrease in megakaryocytes in bone marrow as a result of the inhibitory effect of the virus on granulocyte-megakaryocyte colony-forming-units,[5,393,503] although acquired pure amegakaryocytic thrombocytopenic purpura with resultant absence of bone marrow megakaryocytes secondary to B19 virus also has been described.[38]

Pancytopenia has been observed in association with aplastic crisis, and hemophagocytosis often is demonstrated.‡ Yufu and coauthors[530] described a 15-year-old girl with hemophagocytic syndrome and lymphadenopathy resembling histiocytic necrotizing lymphadenitis (Kikuchi disease).

McClain and colleagues[307] studied 19 children with immune-mediated neutropenia and found by polymerase chain reaction (PCR) assay of bone marrow specimens that 15 had evidence of B19 virus infection.

*References 9, 12, 68, 118, 163, 165, 180, 229, 230, 277, 328, 361, 366, 399, 409, 430, 443, 457, 503, 527, 529, 536.
†References 136, 180, 229, 361, 366, 503.

*References 5, 45, 139, 146, 180, 189, 190, 192, 216, 232, 259, 276, 279, 282, 307, 334, 340, 396, 467, 476, 480, 497, 525.
†References 5, 146, 216, 279, 340, 525.
‡References 45, 180, 192, 232, 259, 272, 334, 340, 473, 480, 497, 530.

Arthritis and Arthralgia

As noted earlier, acute arthritis and arthralgia occur commonly in patients with erythema infectiosum. In most instances, the joint symptoms subside within a few weeks, but the arthralgia and arthritis persist occasionally. Researchers also have observed that the joint manifestations caused by B19 virus infection can occur without any exanthem or with an exanthem not typical of erythema infectiosum, and autoantibodies may be produced.[452,464,485] These findings have led to investigations related to the role of B19 virus in rheumatoid arthritis (RA).*

Nocton and associates[356] described the clinical characteristics of 22 children seen in their rheumatology clinic with joint complaints and evidence of recent B19 virus infection. Of this group, 16 were girls and 6 were boys. The youngest patient was 2 years old, and the oldest was 19 years of age. Seven of the patients had associated rashes, but none had typical erythema infectiosum. One of these children had a petechial rash on the lower extremities, and another child had a past history of urticaria. Fever, anorexia, malaise, or fatigue occurred in 11 of the patients.

Of the 22 patients, 20 had arthritis and 2 had only arthralgia. The knee joints were most commonly involved. The following frequency of specific joint involvement was reported: knees, 82 percent; ankles and wrist, 41 percent; elbows, 32 percent; neck, small joints of the hands and feet, and hips, 27 percent; shoulders, 23 percent; and sternoclavicular joints, 9 percent. Ten of the patients had involvement of five or more joints, and in 12 patients the pattern was pauciarticular, with four or fewer joints involved. Two patients had only a single joint involved, and two patients had migratory illness.

The duration of the arthritis or arthralgia was less than 6 weeks in 50 percent of the children and less than 4 months in 64 percent. Of the 11 children with illness lasting longer than 2 months, 6 had manifestations that fulfilled the criteria for the diagnosis of juvenile rheumatoid arthritis (JRA). Selected laboratory results were as follows: the erythrocyte sedimentation rate was greater than 25 mm/hr in 32 percent of the children, antinuclear antibody titers were greater than 1:40 in 7 of 21 of the patients, and 3 of 6 children had a low total hemolytic complement (CH_{50}) value.

The relationship between B19 virus infection and JRA, RA, and adult Still disease has been studied extensively during the past 15 years, but the findings are difficult to interpret.† Clearly, B19 virus can cause illnesses that fulfill the criteria for a diagnosis of JRA[57,356,365,505] and adult Still disease.[232,288] However, three possible scenarios need to be considered: (1) B19 virus causes a similar illness, (2) B19 virus infection triggers the onset of an illness caused by another condition, and (3) B19 virus directly causes JRA, adult Still disease, and RA.

In one study involving patients with refractory RA and refractory polyarticular JRA, researchers found that both groups had a significantly higher frequency of IgG B19 virus antibody as determined by enzyme-linked immunosorbent assay (ELISA) than did aged-matched controls.[317] However, when the data in this study were examined, the prevalence of antibody in the control population clearly was less than expected. In another study, Kishore and colleagues[247] studied IgM antibody to B19 virus by ELISA and found that 28 percent of patients with JRA, 8 percent of controls, and none of the patients with RA had positive values. They interpreted their JRA findings to be either triggering B19 virus infections or perhaps concurrent infections in patients with established JRA. In a provocative study, Takahashi and coworkers[464] found B19 virus DNA in the synovial tissue of 30 of 39 patients with RA. The data suggested that the virus identified in synovial tissue was active and led to their conclusion that B19 virus is involved in the initiation and perpetuation of RA synovitis. In contrast, Nikkari and associates[352,354] in Finland performed extensive studies and noted that chronic rheumatoid-like arthropathies triggered by B19 virus occasionally occur. However, their data do not support a general role for B19 virus in the etiology or pathogenesis of RA. Varache and colleagues investigated the utility of routine viral screening in patients with early rheumatoid arthritis and determined that the seroprevalence of B19 was not increased compared with the general population.[485] B19 infection can lead to increased circulation of immune complexes, endothelial injury, inflammation, and exacerbation of underlying vasculitic disease.[61]

Tyndall and colleagues[478] described an adult woman with erosive polyarthritis associated with B19 virus infection, and Samii and coworkers[414] reported two adults with bilateral carpal tunnel syndrome associated with B19 virus infection. Berg and colleagues[34] could find no association with either IgG or IgM antibody to B19 virus and fibromyalgia in a study of 26 adult women and matched controls. Guillaume and colleagues[172] reported a case of chronic arthropathy in the lumbar facet joints after B19 infection.

Other rheumatic and vasculitic syndromes have been noted in association with parvovirus B19 infection; however, the strength of these associations is unclear. These associated syndromes include fibromyalgia,[346] systematic lupus erythematosus,[110,202,213,433] Kawasaki disease, leukocytoclastic vasculitis, polyarteritis nodosa, Wegener granulomatosis,[122] chronic fatigue syndrome,[236,237,308] systemic sclerosis,[142,368,369] giant-cell arteritis, transient anticardiolipin syndrome and transient antiphospholipid syndrome,[18] hemophagocytic syndrome,[45,448] mixed cryoglobulinemia,[82] Henoch-Schönlein purpura,[85,197,370] uveitis with and without associated systemic disease,[198] myofasciitis,[212] and Raynaud phenomenon.*

Infection in Immunocompromised Patients

Immunocompromised patients may suffer chronic B19 virus infections.† These patients have persistent anemia caused by continuous lysis of red cell precursors. Lackner

*References 71, 95, 148, 177, 192, 205, 231, 232, 247, 317, 321, 352, 356, 395, 446, 452, 464.
†References 40, 57, 62, 105, 192, 231, 280, 281, 288, 294, 315, 317, 335, 336, 343, 356, 365, 384, 395, 446, 464, 479, 505.

*References 88, 143, 157, 181, 208, 221, 232, 350, 351, 403, 530.
†References 9, 36, 44, 58, 79, 102, 176, 192, 204, 231, 254, 256, 266, 297, 332, 339, 353, 360, 388, 421, 434, 436, 526, 528.

and colleagues reviewed 1059 consecutive patients with various hematologic and oncologic diseases and characterized 35 patients diagnosed with B19 infection.[268] B19 infection has been commonly seen in children with acute lymphocytic leukemia.*

Other immune deficiency states in which chronic B19 virus was documented include Nezelof syndrome, acute myeloid leukemia, chronic myeloid leukemia, Burkitt lymphoma, lymphoblastic lymphoma, myelodysplastic syndrome, astrocytoma, Wilms tumor, HIV infection,[87,344] severe combined immunodeficiency, bone marrow transplantation,[188,243,390,456] other organ transplants,† systemic lupus erythematosus,[459] and patients receiving chemotherapy.[122,140,196,264] The immune deficiency in the aforementioned conditions can be a primary event or a secondary event related to treatment.

Eid and colleagues[125] reviewed 98 transplant recipients in whom acute parvovirus B19 infection developed after transplantation. The median time to B19 disease was 7 weeks after transplantation. Anemia developed in 99 percent, leukopenia in 38 percent, thrombocytopenia in 21 percent, and allograft loss or dysfunction in 10 percent. Three patients died of myocarditis and cardiogenic shock as a result of B19 infection. B19 virus was associated with chronic renal allograft injury.[30]

Patients with chronic anemia have high persistent concentrations of B19 virus in their serum, although the values (10^8 particles/mL) are less than those seen in primary infections (10^{10} to 10^{11} particles/mL) in healthy individuals and in patients with hemoglobinopathies.

Viremia and anemia may, if untreated, persist for years. Fatigue and pallor are the most common clinical symptoms, and other findings of B19 virus infection, such as exanthem and arthralgia, occur rarely. Because of immunodeficiency, IgM antibody studies generally are not useful in making the diagnosis. Therefore, the diagnosis of persistent B19 virus infection depends on demonstration of specific B19 antigen or DNA in blood.[61]

In immunocompromised patients, persistent infection with B19 virus often occurs as a result of failure to produce effective neutralizing antibodies.[61,83,259] IVIG preparations have been used successfully for the treatment of B19 virus in immunocompromised patients.‡ Patients taking immunosuppressive agents, especially calcineurin inhibitors, may be unable to clear B19 virus because these medications inhibit IL-2 production and the patients are unable to mount an effective T-helper cell type 1 response.[83,323] It may be necessary to reduce the dose of immunosuppressive agents in addition to IVIG treatment to successfully eradicate B19 virus.[29,126,285] LaMonte and associates[269] reported five children (three HIV-infected children and two otherwise normal children) with chronic infections without B19-associated anemia.

Intrauterine Infection

Although a large epidemiologic study of an outbreak of erythema infectiosum over 40 years ago failed to reveal evidence of teratogenicity, virologic and serologic studies conducted during the past 25 years indicate that B19 virus crosses the placenta and causes infection in the fetus.* Infection in pregnancy results in fetal hydrops because of the severe anemia and leads to cardiac failure, fetal death, and miscarriage.[83,111,303,363]

Bonvicini and colleagues followed 72 pregnancies complicated by B19 infection. Vertical transmission was documented in 39 percent of the pregnancies, B19 fetal hydrops in 11.9 percent, and overall fetal demise in 10.2 percent, with the highest rate of congenital infection and fatal outcome with maternal infection prior to 20 weeks of gestation.[43]

To determine the rate and outcome of fetal involvement after infection in pregnant women, two prospective studies were performed in England during a 3½-year period from January 1985 to June 1988[397] and from 1992 to 1995.[316] These two studies included 427 pregnant women infected with B19 virus; 367 infants survived, and 129 of them had follow-up examinations at 7 to 10 years of age.[316] The excess rate of fetal loss was 9 percent, and such loss occurred only during the first 20 weeks of gestation. Seven cases of fetal hydrops occurred, and three of these fetuses survived; two of the three received intrauterine transfusions. None of the surviving infants in the two studies had abnormalities attributable to B19 virus infection, and no later effects were found at 7 to 10 years of age. Of the infants exposed in utero after 20 weeks of gestation, more than half were infected, but no clinical effects were identified. In a study by Nyman and colleagues,[364] the frequency of first-trimester fetal loss associated with B19 virus was 3 percent, with 12 percent occurring in the second trimester. Maternal B19 seroconversion during pregnancy was not associated with lower birth weight or reduced length of gestation in live-born children in a study of 35,940 pregnant women in Norway.[416]

In a study in Connecticut related to a large outbreak of erythema infectiosum in which 39 infected pregnant women were monitored, two miscarriages occurred.[407] The frequency of fetal loss caused by B19 virus infection was estimated to be 5 percent. In another study in Spain, fetal loss occurred in only 1 of 60 women infected with B19 virus during pregnancy.[168] Enders and colleagues[132] studied 1018 pregnant women with acute parvovirus infection. The overall rate of fetal loss was 6.3 percent, with a rate of 11 percent if the B19 infection occurred in the first 20 weeks of gestation. The overall rate of hydrops fetalis was 3.9 percent, and the peak incidence occurred between 17 and 24 weeks of gestation.

De Jong and colleagues investigated the neurodevelopmental impairment in children treated with intrauterine transfusion for fetal anemia due to B19 infection. Twenty-eight children were evaluated at a median age of 5, and the study showed increased impairment when compared with the general population.[107]

*References 67, 102, 129, 140, 158, 175, 196, 234, 254, 266, 522.
†References 32, 74, 124, 263, 323, 373.
‡References 29, 40, 57, 113, 192, 200, 218, 231, 254, 434.

*References 3, 7, 9, 47, 54, 58, 68, 106, 114, 128, 161, 162, 168, 191, 192, 222, 231, 245, 249, 250, 252, 253, 303, 306, 309, 316, 320, 359, 362, 371, 385, 397, 406-408, 413, 444, 469, 483, 484, 509-513, 515, 527, 535.

Koch and colleagues[252] performed follow-up examinations on 19 infants born to mothers who had serologically confirmed B19 virus infection between the 4th and 38th weeks of gestation. In none of these infants did hydrops develop during pregnancy, and all were normal after birth. One child whose mother had erythema infectiosum at approximately the 20th week of gestation had a persistent asymptomatic infection for at least the first 7 months of life. Donders and coworkers[114] noted a child in whom a sonogram demonstrated fetal hydrops at 30 weeks of gestation. Cordocentesis revealed a hemoglobin concentration of 2.4 g/dL. One week later, a hydropic 1550-g infant was delivered by cesarean section. The neonate's bone marrow showed an arrest in erythropoiesis with giant pronormoblasts. The infant received multiple transfusions for the first 4 months of life, and B19 viral DNA was identified in the infant's blood at 19 weeks of age. At 2 years of age, this child was found to be clinically, hematologically, and immunologically normal. In contrast to the case just noted, Brown and associates[54] reported an infant with a similar exposure and delivery history who had persistent anemia and, despite therapy, was dependent on transfusions at 4 years of age. In addition, Brown and associates reported two other infants who had received intrauterine transfusions for fetal hydrops and had persistent anemia after birth.

Candotti and colleagues[63] studied plasma samples from 885 pregnant women from Ghana and found that, contrary to the high rate of B19 transmission during primary viremia in pregnancy, pregnant women with B19-specific IgG and low levels of B19 DNA by PCR did not transmit virus to the fetus. Weiffenbach and colleagues determined B19 viral DNA and anti-B19 viral antibody titers in maternal and fetal blood samples obtained from 41 pregnancies complicated by prenatal B19 viral infection and determined that anemia or hydrops due to B19 virus is most likely due to a limited maternofetal transfer of IgG and a poor fetal antibody response.[500]

At the present time, a large number of infants who were exposed to B19 virus in utero have been studied, with no convincing evidence of specific congenital malformations.* However, van Elsacker-Niele and associates[484] noted B19 virus infection in cells other than those of the erythroid series in two aborted fetuses. Ocular malformation was found in one, and evidence of extensive inflammatory reactions in all fetal and placental tissues was noted in both. Fishman and associates report a case of fetal cardiomyopathy with atrioventricular nodal disease.[144] Carlsen and colleagues suggest a higher mortality rate in B19-infected fetuses with fetal trisomy.[64]

Tiesson and colleagues[469] reported an aborted fetus with a bilateral cleft lip, alveolus, and palate; micrognathia; and webbed joints. In another aborted fetus, ocular abnormalities similar to those seen in congenital rubella were observed.[502] In a neonate with anemia, blueberry muffin rash, and hepatomegaly, parvovirus B19 viral gene sequences were found in the liver and placenta.[439] Gulen and associates[173] described a premature infant born at 29 weeks of gestation with leukoerythroblastosis associated with B19-specific IgM and IgG antibody in both the mother and infant.

Yeh and colleagues[520] described severe, transient dyserythropoietic anemia in puerperal woman with preeclampsia and eclampsia with concomitant B19 infection.

Neurologic Illness

Douvoyiannis and colleagues reviewed the literature of 81 cases of neurologic disease associated with B19 virus from 1966 to 2008. The median patient age was 9 years, the majority were immunocompetent patients, and there were no differences in the prevalence of sequelae noted between immunocompetent patients and patients with altered immunity. There were also no difference in sequelae between patients with central nervous system manifestations who received intravenous immunoglobulin with or without corticosteroids and those patients with central nervous system manifestations who did not.[115]

Two children with encephalitis in association with erythema infectiosum were noted in the era before discovery of the causative role of B19 virus in erythema infectiosum.[25,49] In the present era, a number of cases of aseptic meningitis and encephalitis have been reported in association with B19 virus infection in both children and adults.* These cases have been documented by the demonstration of specific B19 virus IgM antibody or the presence of B19 viral DNA in cerebrospinal fluid. Bilge and colleagues[39] reported a 12-year-old boy with a history of renal transplantation who had recurrent episodes of encephalopathy and focal neurologic defects associated with B19 DNA in blood and bone marrow and on skin biopsy specimens. He was treated successfully with IVIG. Nolan and colleagues[357] reported an HIV-positive patient in whom parvovirus B19 encephalitis developed during immune reconstitution while receiving highly active antiretroviral therapy. Chorea-encephalopathy, with parvovirus B19 DNA in both serum and cerebrospinal fluid, developed in a previously healthy 8-year-old girl.[145]

Other neurologic illnesses associated with B19 virus infection[27] include postinfectious neuralgic amyotrophy,[382] Guillain-Barré syndrome,[318] parainfectious myelitis,[419] acute bilateral carpal tunnel syndrome,[292,414] mononeuritis multiplex,[4,283] stroke,[171,300] and numbness and tingling of the fingers.[139]

Myocarditis

Myocarditis in association with parvovirus B19 infection has been observed in immunocompetent children and adults.† B19-associated myocarditis can progress to chronic dilated cardiomyopathy.‡ In a study of 3345 patients who underwent cardiac biopsy for suspected myocarditis or dilated cardiomyopathy, Pankuweit and colleagues[376] found B19 DNA by PCR in 17.6 percent of

*References 245, 329, 397, 406, 407, 510.

*References 27, 39, 70, 145, 182, 192, 194, 231, 241, 257, 357, 371, 453, 458, 461, 499, 518.

†References 31, 42, 60, 109, 112, 130, 261, 270, 289, 337, 349, 375-377, 383, 411.

‡References 261, 262, 270, 289, 375, 376.

patients with dilated cardiomyopathy without significant inflammation, and inflammatory heart disease, ventricular dilation, and reduced ejection fraction were noted in 33.3 percent of patients. Kühl and associates[262] showed that viral persistence in the setting of myocarditis or dilated cardiomyopathy was associated with progressive deterioration of the left ventricular ejection fraction whereas elimination of virus resulted in significant improvement in left ventricular function.

In a study by Wang and colleagues,[495] B19 virus was detected by nested PCR in cardiac tissues in 7 of 42 patients with congenital heart disease and in none of the 38 controls, thus suggesting a correlation between B19 virus and congenital heart disease.

Enders and associates[130] described two children with life-threatening myocarditis. One of these children died, and the second received a cardiac transplant. The illnesses in both these children occurred in the spring, and one had joint pain and urticarial lesions on the flexor surfaces of both arms, erythematous macules on the abdomen and upper part of the chest, and purpuric lesions on the back. Both children had B19 viral DNA identified in the heart and B19-specific IgM antibody.

Nigro and colleagues[349] noted three young children, aged 7, 12, and 18 months, with acute lymphocytic myocarditis and parvovirus B19 infection. Two of these children exhibited full cardiac recovery, but chronic persistent myocarditis developed in the other child. Papadogiannakis and coworkers[377] reported a healthy 11-month-old child who had severe respiratory distress and died of sudden cardiac arrest within 3 hours of admission because of parvovirus-associated lymphocytic myocarditis. Zack and associates[532] reported a 5-year-old girl who suddenly collapsed and died of acute myocarditis secondary to parvovirus B19 infection. Significant lymphocytic infiltration of the sinoatrial node and perivascular infiltration around the atrioventricular node probably resulted in conduction disturbances and death. Jonetzko and colleagues[226] reported a case of fatal parvovirus B19–associated myocarditis in a liver transplant recipient.

Parvovirus B19 infection also can mimic acute myocardial infarction[60,261] and isolated diastolic dysfunction in adults.[474]

It is hypothesized that the blood group P antigen on the endothelial cells of coronary vessels is a portal for infection and that such infection results in endothelial dysfunction, impairment of the coronary microcirculation, and inflammation of the myocardium.[27,261,270,514] The blood group P antigen is not present on the myocytes of children and adults; however, it is present on fetal myocytes.[53,259,261,270]

Acute Hepatitis

Acute hepatitis has been noted as a manifestation of B19 virus infection.[184,447,524] Yoto and coworkers[524] described seven children with acute hepatitis in association with B19 virus infection. They carefully studied and ruled out other common causes of acute hepatitis. Sokal and associates[447] reported fulminant hepatitis in four children younger than 5 years of age in association with parvovirus B19 infection. Their patients had a distinct clinical pattern consisting of low serum bilirubin concentrations and rapid recovery of liver function without transplantation. Nobili and colleagues[355] reported a case of autoimmune hepatitis followed by severe autoimmune hemolytic anemia in a 1-month-old boy with parvovirus B19 infection. Treatment with cyclosporine and prednisone resulted in resolution of his symptoms.

Hsu and associates[210] studied 54 patients with B19 virus infection in addition to hepatitis B virus (HBV) infection and 51 patients with hepatitis C virus (HCV) infection who were not taking immunosuppressive drugs. The study found that the prevalence of parvovirus B19 DNA was high in patients with HBV (85.2%) or HCV (70.6%). Coinfection with B19 and HBV or HCV did not increase the frequency of liver dysfunction in these patients with chronic hepatitis.[210,275] Additionally, in a study by Wong and associates,[512] there was no significant difference in the prevalence of parvovirus B19 DNA in liver biopsy specimens from patients with fulminant hepatitis or hepatitis-associated aplastic anemia and those with HBV or HCV infection. However, Vaillant and colleagues showed that pediatric patients with fulminant hepatic failure who had high prevalence of B19 associated with coinfection of other hepatitis viruses had relatively severe disease and poor outcome.[120]

Other Illnesses

One adult patient had chronic red cell aplasia for a 10-year period that was treated with regular blood transfusions.[265] After a diagnosis of persistent B19 virus infection, the patient was treated with IVIG, which resulted in an apparent cure. A presumably immunologically normal woman had recurrent episodes of paresthesia over the course of a 4-year period in conjunction with persistent B19 viral DNA in her blood.[138] Evidence of persistent infection in immunologically normal patients has been noted in other studies.[239,338]

Kerr and associates[240] studied 53 patients who had contracted acute B19 virus illnesses. Seven of those studied had B19 viral DNA demonstrated in serum specimens 26 to 65 months after their acute illnesses. All seven were women, and four were asymptomatic. Of the other three, one had chronic hemolytic anemia, one had persistent arthralgia of the knees, and the last had arthralgia and chronic fatigue syndrome.

Multiple case reports have described patients with chronic fatigue syndrome and persistent parvovirus B19 DNA in their serum.[221,236,237,304,308] In 2002, Kerr and colleagues[235] described three patients with chronic fatigue syndrome and chronic B19 virus infection treated with IVIG. Such treatment resulted in the clearance of B19 viremia, improvement of symptoms, and resolution of cytokine dysregulation.

Faden and associates[138] described a nurse with recurrent episodes of paresthesia who had B19 viral DNA demonstrated in her serum for almost a 4-year period. This woman also had persistent serum IgM antibody to B19 virus.

Lymphadenitis associated with parvovirus B19 infection has been described by many authors. Tsuda and coworkers[475] reported five young adults with cervical

lymphadenopathy associated with B19 virus infection. All had fever, one had arthralgia, and all had leukopenia. Morinet and colleagues[326] described a 27-year-old man with pseudoappendicitis or acute mesenteric lymphadenitis and serologic evidence of B19 virus infection. A 16-month-old boy had peripheral facial palsy, parotitis, and intraparotid lymphadenitis with evidence of parvovirus B19 IgM antibody and IgG antibody seroconversion.[302] Johnson and associates[224] described an 18-year-old woman with parvovirus B19 resulting in necrotizing lymphadenitis, and Knösel and colleagues[248] reported unilateral cervical lymphadenopathy in a 16-year-old girl. In both cases, parvovirus B19 DNA was detected by PCR on lymph node biopsy specimens.

Parvovirus B19 infection also has been shown to induce proliferative glomerulonephritis, usually endocapillary or mesangioproliferative (or both), with hypocomplementemia and spontaneous recovery.* Ieiri and colleagues[214] studied 10 patients with acute glomerulonephritis associated with human parvovirus B19 infection. These authors found that this specific renal disease characterized by endocapillary hypercellularity was associated with hypocomplementemia, leukopenia, positive antinuclear antibody, and a female preponderance (9 of 10 patients). Ohtomo and associates[367] reported a healthy 8-year-old boy with erythema infectiosum and glomerulonephritis with immune complex deposits associated with B19 virus diagnosed by both monoclonal antibody against human parvovirus B19 antigen and nested PCR on renal biopsy. The association between parvovirus B19 and sepsis also has been reported by multiple authors. These patients exhibited shock, severe respiratory distress, and parvovirus B19 infection.[22,141,327,377,496]

DIFFERENTIAL DIAGNOSIS

Because the exanthem of erythema infectiosum is unique, its diagnosis should be easy. During epidemics, no difficulties should arise, but sporadic cases can be a problem. In the differential diagnosis, rubella and scarlet fever are of most concern. Because rubella virus has been recovered from some patients with illness thought to be erythema infectiosum[25] and because an erythema infectiosum–like illness was observed in volunteers who underwent intranasal administration of a rubella virus strain recovered from a patient with erythema infectiosum–like illness,[420] this diagnostic possibility always should be considered. When the risk for development of congenital rubella is a possibility, rubella-specific diagnostic tests should be performed (see Chapter 173).

Erythema infectiosum can be differentiated from scarlet fever by the usual lack of pharyngitis in the former and a positive culture for *Streptococcus pyogenes* in the latter. Other differential diagnostic considerations are other infectious exanthems (see Chapter 58), collagen vascular diseases, drug reactions, and allergic responses to environmental substances.

Although a presumptive diagnosis of B19 virus infection may be made by exclusion of other etiologic possibilities, a definitive diagnosis can be made only by specific serologic tests or identification of B19 antigens or DNA in blood or tissue specimens.

Aplastic crisis in a patient with chronic hemolytic anemia can be diagnosed by finding a hemoglobin concentration that is 2 g/dL or more below the steady-state value for that patient, together with a reticulocyte count either less than 0.2 percent of the steady-state value or elevated above the steady-state value (indicative of hyperplasia of erythrocyte precursors in the recovery phase). Although B19 virus infection is the most common cause of aplastic crisis, moderate to severe degrees of hypoplasia may be associated with systemic bacterial infections (e.g., *Salmonella*, *Streptococcus pneumoniae*) or marrow-suppressive drugs.[312,430]

DIAGNOSIS

Several tests have been developed and refined that allow a reliable serologic diagnosis of acute and past B19 virus infection to be established and B19 virus in blood and tissues to be demonstrated.* IgM and IgG antibody can be detected by enzyme immunoassay, hemadherence, radioimmunoassay, or immunofluorescence; antigen can be detected by DNA hybridization, PCR, or electron microscopy.

In a normal host, acute or recent infection is determined best by demonstration of specific IgM antibody. In immunocompromised patients with suspected acute or chronic infection, the diagnosis is made by detection of antigen in blood. Similarly, detection of antigen also can be performed early in aplastic crisis and to study aborted fetal tissues. Gallinella and colleagues[159] recommend both specific IgM and B19 DNA PCR to establish a diagnosis of B19 infection. Past infection and immunity to B19 virus are determined by demonstration of specific serum IgG antibody.

Some caution should be observed in accepting the results of IgM serology and antigen detection in unusual clinical circumstances.[51] The sensitivity and specificity of the various serologic tests vary, and additional false-positive and false-negative results can be expected, depending on the skill of workers in individual laboratories.[59,91,472,481] Antigen-detection systems can be contaminated, and such contamination may not be discernible by conventional controls. When the results of tests on specimens from patients with unusual illnesses are positive, repeating the tests in a different laboratory is worthwhile.

Söderlund and associates[445] reported an IgG avidity assay that is highly sensitive and specific for the identification of recent primary infection with parvovirus B19. Enders and coworkers[131] also recommended VP1 IgG avidity or VP2 epitope–type specificity assays in the presence of low-level IgM or B19 DNA to confirm or refute the diagnosis of B19 infection during pregnancy. Because

*References 214, 220, 325, 367, 465, 466, 498.

*References 9, 14, 20, 33, 50, 59, 65, 69, 72, 89, 91, 92, 103, 104, 133, 153, 159, 203, 206, 244, 251, 255, 313, 324, 347, 412, 423, 425, 462, 494, 511, 534.

there is a window of 7 days after initial infection occurs until the development of IgM-specific B19 antibody and because IgM can be negative or low in the presence of overt hydrops fetalis, both serology and B19 DNA PCR are recommended on both maternal and fetal sera (or cord blood).[124]

Beersma and colleagues[33] demonstrated the superiority of B19 VP2 IgM ELISA by showing high correlation with elevated levels of B19 DNA in sera. Persistent infection may be determined by the presence of IgG antibody to NS1.[489]

If parvovirus B19 infection is suspected in pregnancy, ultrasound should be performed to assess for fetal anemia and hydrops (excess of fluid in at least two body compartments of the fetus).[124] The use of middle cerebral artery peak systolic velocity for the detection of fetal anemia may be a useful adjunct to ultrasound scanning.[99,331]

TREATMENT AND PROGNOSIS

No specific treatment of B19 virus infection exists. Symptomatic therapy for erythema infectiosum rarely is necessary, especially in children. Starch baths may be helpful in reducing pruritus. Arthralgia or arthritis may be troublesome and may be treated with analgesics.

The outlook in virtually all cases of erythema infectiosum is excellent. If patients with aplastic crisis receive transfusions with packed erythrocytes when necessary, the prognosis for these patients also is excellent. If B19 virus infection occurs during pregnancy, the pregnancy should be monitored carefully. At delivery, examination of cord blood or blood from the neonate for detection of virus and IgM antibody will reveal whether the virus has crossed the placenta and infected the fetus. When infection has occurred, the child should be examined carefully for any defect and monitored for some years to exclude the possibility of delayed sequelae.

Some researchers have suggested that pregnant women with symptomatic B19 virus infection be observed for fetal aplastic crisis by monitoring maternal serum for elevated levels of α-fetoprotein.[66] If levels are found to be elevated, serial ultrasonography can be performed to detect hydrops fetalis. Fetal hydrops can be treated by in utero transfusions[135,167,410]; however, there is a risk for infection and the potential for congenital malformations with cordocentesis and fetal transfusion. Enders and colleagues[132] reported that 84.6 percent of those with severe hydrops fetalis survived to delivery. In 2005, Matsuda and colleagues[305] successfully treated a B19-infected fetus with hydrops at 21 weeks of gestation with two injections of B19 IgG–rich immunoglobulin injected into the fetal peritoneal cavity. The fetal ascites and pericardial effusion resolved completely 8 days after the initial injection, and a healthy infant was born at 38 weeks of gestation without evidence of cardiac dysfunction or B19 DNA detected in serum. Therapeutic abortion is not indicated for pregnant women with documented B19 virus infection.

Patients with chronic myocarditis caused by B19 virus have been treated successfully with IVIG.[454] Chronic myocarditis secondary to B19 also has been treated with immunosuppressive agents, including corticosteroids and cyclosporine.[270,337,411] Ito and associates[219] described a patient with baseline refractory autoimmune hemolytic anemia who was taking immunosuppressive medications while infected with B19 virus. The patient had a complicated course of illness; persistent pure red cell aplasia subsequently developed but resolved after treatment with cyclosporine and IVIG.

Immunocompromised patients with chronic B19 virus infection can be treated successfully with IVIG preparations,* as can other patients without demonstrated immune deficiencies who have chronic infections.[138,219,221,333,454] Although the amount of specific B19 virus antibody varies among different IVIG products, all contain significant concentrations.[113] No formal treatment studies have been performed, and several different treatment programs have been used. Even though some cures have been achieved with single-dose IVIG therapy, we favor an initial 4-day course with 500 mg/kg/day. After the patient has received treatment, the viremia should cease and clinical improvement should occur. Some immunocompromised patients have required repeated treatment courses.

PREVENTION

B19 virus is spread by the oral and respiratory routes, and virus shedding during routine infection occurs when patients are not aware of their illness. Because B19 virus infections occur in outbreaks, the virus can be widespread in a community, with many infections going unrecognized.

Patients with erythema infectiosum do not need to be isolated because they have passed their period of infectivity. Although patients with aplastic crisis also usually are past the period of virus shedding, they should be isolated because some will be shedding virus at the time of initial evaluation. All patients with chronic infection should be considered contagious until treated with IVIG and demonstrated to be nonviremic.

Pregnant women are of particular concern during an outbreak.[68,162] The rate of parvovirus seropositivity is approximately 50 percent in women of childbearing age, so no risk exists in approximately half of those who might become exposed. Determining an IgG antibody titer can allay the fear in those who are antibody positive. Cartter and associates[68] examined occupational risk factors for B19 virus infection in pregnant women. They found the following rates of infection: school teachers, 16 percent; daycare workers, 9 percent; homemakers, 9 percent; and women working outside the home, 4 percent. In another study, Gillespie and colleagues[162] found the greatest risk for infection occurred in school and daycare personnel. These results suggest that in certain circumstances (in older women and those with past fertility problems), having women in high-risk occupations avoid the workplace during the outbreak period might be reasonable.

Technologic advances have led to the development of experimental recombinant vaccines that show

*References 40, 57, 113, 192, 198, 218, 231, 254, 434.

considerable promise for effective prevention.* These vaccines could be useful in selected populations, such as patients with hemoglobinopathies and seronegative women of child-bearing age.

NEW REFERENCES SINCE SIXTH EDITION

30. Barzon L, Murer L, Pacenti M, et al. Investigation of intrarenal viral infections in kidney transplant recipients unveils an association between parvovirus B19 and chronic allograft injury. *J Infect Dis* 2009;**199**:372–80.
31. Basic D, Gupta S, Kwong RY. Parvovirus B19–induced myocarditis mimicking acute myocardial infarction: clarification of diagnosis by cardiac magnetic resonance imaging. *Circulation* 2010; **121**:e40–2.
42. Bock CT, Klingel K, Kandolf R. Human parvovirus B19–associated myocarditis. *N Engl J Med* 2010;**362**:1248–9.
43. Bonvicini F, Puccetti C, Salfi NC, et al. Gestational and fetal outcomes in B19 maternal infection: a problem of diagnosis. *J Clin Microbiol* 2011;**49**:3514–18.
52. Brown KE. The expanding range of parvoviruses which infect humans. *Rev Med Virol* 2010;**20**:231–44.
64. Carlsen K, Beck BL, Bagger PV, et al. Pregnancy loss ascribable to parvovirus B19/erythrovirus is associated with a high prevalence of trisomy. *Gynecol Obstet Invest* 2010;**70**:328–34.
76. Chen AY, Kleiboeker S, Qiu J. Productive parvovirus B19 infection of primary human erythroid progenitor cells at hypoxia is regulated by STAT5A and MEK signaling but not HIFalpha. *PLoS Pathog* 2011;**7**:e1002088.
77. Chen AY, Zhang EY, Guan W, et al. The small 11 kDa nonstructural protein of human parvovirus B19 plays a key role in inducing apoptosis during B19 virus infection of primary erythroid progenitor cells. *Blood* 2010;**115**:1070–80.
78. Chen MY, Yang SJ, Hung CC. Placental transmission of human parvovirus 4 in newborns with hydrops, Taiwan. *Emerg Infect Dis* 2011;**17**:1954–6.
82. Chiche L, Grados A, Harle JR, et al. Mixed cryoglobulinemia: a role for parvovirus b19 infection. *Clin Infect Dis* 2010;**50**:1074–5.
95. Colmegna I, Alberts-Grill N. Parvovirus B19: its role in chronic arthritis. *Rheum Dis Clin North Am* 2009;**35**:95–110.
107. De Jong EP, Lindenburg IT, van Klink JM, et al. Intrauterine transfusion for parvovirus B19 infection: long-term neurodevelopmental outcome. *Am J Obstet Gynecol* 2012;**206**:204.e1–5.
111. Dijkmans AC, de Jong EP, Dijkmans BA, et al. Parvovirus B19 in pregnancy: prenatal diagnosis and management of fetal complications. *Curr Opin Obstet Gynecol* 2012;**24**:95–101.
112. Dina J, Vabret A, Rambaud C, et al. Fulminant myocarditis associated with parvovirus B19 infection in a child. *J Clin Virol* 2008; **42**:70–1.
115. Douvoyiannis M, Litman N, Goldman DL. Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis* 2009;**48**:1713–23.
120. Dwivedi M, Manocha H, Tiwari S, et al. Coinfection of parvovirus B19 with other hepatitis viruses leading to fulminant hepatitis of unfavorable outcome in children. *Pediatr Infect Dis J* 2009; **28**:649–50.
123. Edmonson MB, Riedesel EL, Williams GP, et al. Generalized petechial rashes in children during a parvovirus B19 outbreak. *Pediatrics* 2010;**125**:e787–92.
126. Eid AJ, Posfay-Barbe KM. Parvovirus B19 in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S147–50.
144. Fishman SG, Pelaez LM, Baergen RN, et al. Parvovirus-mediated fetal cardiomyopathy with atrioventricular nodal disease. *Pediatr Cardiol* 2011;**32**:84–6.
155. Fryer JF, Kapoor A, Minor PD, et al. Novel parvovirus and related variant in human plasma. *Emerg Infect Dis* 2006;**12**:151–4.
158. Gadage VS, Viswanathan S, Kunal S, et al. Parvovirus B19 presenting with persistent pancytopenia in a patient of T-ALL post induction chemotherapy diagnosed on bone marrow examination. *Indian J Pathol Microbiol* 2011;**54**:603–5.
166. Grabarczyk P, Kalinska A, Kara M, et al. Identification and characterization of acute infection with parvovirus B19 genotype 2 in immunocompromised patients in Poland. *J Med Virol* 2011;**83**:142–9.
175. Gustafsson I, Kaldensjo T, Lindblom A, et al. Evaluation of parvovirus B19 infection in children with malignant or hematological disorders. *Clin Infect Dis* 2010;**50**:1426–7.
183. Hashimoto H, Yuno T. Parvovirus B19–associated purpuric-petechial eruption. *J Clin Virol* 2011;**52**:269–71.
184. Hatakka A, Klein J, He R, et al. Acute hepatitis as a manifestation of parvovirus B19 infection. *J Clin Microbiol* 2011;**49**:3422–4.
202. Hession MT, Au SC, Gottlieb AB. Parvovirus B19–associated systemic lupus erythematosus: clinical mimicry or autoimmune induction? *J Rheumatol* 2010;**37**:2430–2.
215. Ilknur T, Fetil E, Akarsu S, et al. A case of papular-purpuric gloves-and-socks syndrome receiving unnecessary treatment. *Clin Exp Dermatol* 2011;**36**:110–11.
268. Lackner H, Sovinz P, Benesch M, et al. The spectrum of parvovirus b19 infection in a pediatric hemato-oncologic ward. *Pediatr Infect Dis J* 2011;**30**:440–2.
283. Lenglet T, Haroche J, Schnuriger A, et al. Mononeuropathy multiplex associated with acute parvovirus B19 infection: characteristics, treatment and outcome. *J Neurol* 2011;**258**:1321–6.
290. Lou S, Xu B, Huang Q, et al. Molecular characterization of the newly identified human parvovirus 4 in the family *Parvoviridae*. *Virology* 2012;**422**:59–69.
404. Rinckel LA, Buno BR, Gierman TM, et al. Discovery and analysis of a novel parvovirus B19 genotype 3 isolate in the United States. *Transfusion* 2009;**49**:1488–92.
415. Santonja C, Nieto-Gonzalez G, Santos-Briz A, et al. Immunohistochemical detection of parvovirus B19 in "gloves and socks" papular purpuric syndrome: direct evidence for viral endothelial involvement: report of three cases and review of the literature. *Am J Dermatopathol* 2011;**33**:790–5.
416. Sarfraz AA, Samuelsen SO, Bruu AL, et al. Maternal human parvovirus B19 infection and the risk of fetal death and low birthweight: a case-control study within 35,940 pregnant women. *BJOG* 2009;**116**:1492–8.
417. Satake M, Hoshi Y, Taira R, et al. Symptomatic parvovirus B19 infection caused by blood component transfusion. *Transfusion* 2011;**51**:1887–95.
419. Scheibe F, Hofmann J, Ruprecht K. Parainfectious myelitis associated with parvovirus B19 infection. *J Neurol* 2010;**257**:1557–8.
428. Seo JY, Kim HJ, Kim SH. Neutropenia in parvovirus B19-associated pure red cell aplasia. *Ann Hematol* 2011;**90**:975–8.
432. Servant-Delmas A, Lefrere JJ, Morinet F, et al. Advances in human B19 erythrovirus biology. *J Virol* 2010;**84**:9658–65.
448. Sood N, Yadav P. Hemophagocytic syndrome associated with concomitant *Klebsiella* and Parvovirus B-19 infection. *Indian J Pathol Microbiol* 2012;**55**:124–5.
460. Szelei J, Liu K, Li Y, et al. Parvovirus 4–like virus in blood products. *Emerg Infect Dis* 2010;**16**:561–4.
485. Varache S, Narbonne V, Jousse-Joulin S, et al. Is routine viral screening useful in patients with recent-onset polyarthritis of a duration of at least 6 weeks? Results from a nationwide longitudinal prospective cohort study. *Arthritis Care Res (Hoboken)* 2011;**63**:1565–70.
500. Weiffenbach J, Bald R, Gloning KP, et al. Serological and virological analysis of maternal and fetal blood samples in prenatal human parvovirus B19 infection. *J Infect Dis* 2012;**205**:782–8.
514. Wurster T, Polzelbauer C, Schonberger T, et al. Green fluorescent protein (GFP) color reporter gene visualizes parvovirus B19 non-structural segment 1 (NS1) transfected endothelial modification. *PLoS One* 2012;**7**:e33602.
521. Yermalovich MA, Hubschen JM, Semeiko GV, et al. Human parvovirus B19 surveillance in patients with rash and fever from Belarus. *J Med Virol* 2012;**84**:973–8.

*References 26, 94, 135, 151, 189, 528.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abuhammour W, Abdel-Haq N, Asmar B. Picture of the month. Petechial eruption with parvovirus B19 infection. *Arch Pediatr Adolesc Med* 1999;**153**:87–8.
2. Adler SP, Manganello AM, Koch WC, et al. Risk of human parvovirus B19 infections among school and hospital employees during endemic periods. *J Infect Dis* 1993;**168**:361–8.
3. Ager EA, Chin TD, Poland JD. Epidemic erythema infectiosum. *N Engl J Med* 1966;**275**:1326–31.
4. Aguilar-Bernier M, Bassas-Vila J, Torne-Gutierrez JI, et al. Presence of perineuritis in a case of papular purpuric gloves and socks syndrome associated with mononeuritis multiplex attributable to B19 parvovirus. *J Am Acad Dermatol* 2006;**54**:896–9.
5. Aktepe OC, Yetgin S, Olcay L, et al. Human parvovirus B19 associated with idiopathic thrombocytopenic purpura. *Pediatr Hematol Oncol* 2004;**21**:421–6.
6. Alfadley A, Aljubran A, Hainau B, et al. Papular-purpuric "gloves and socks" syndrome in a mother and daughter. *J Am Acad Dermatol* 2003;**48**:941–4.
7. Anand A, Gray ES, Brown T, et al. Human parvovirus infection in pregnancy and hydrops fetalis. *N Engl J Med* 1987;**316**:183–6.
8. Anderson DJ, Fangman W, Fowler VG, et al. A returning traveler with fever and rash. *Clin Infect Dis* 2005;**41**:1453–4, 1529–30.
9. Anderson LJ. Human parvoviruses. *J Infect Dis* 1990;**161**:603–8.
10. Anderson LJ, Tsou C, Parker RA, et al. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1986;**24**:522–6.
11. Anderson MJ, Cohen BJ, Schwarz T, et al. Human parvovirus B19 infections in United Kingdom 1984-86. *Lancet* 1987;**1**:738–9.
12. Anderson MJ, Davis LR, Hodgson J, et al. Occurrence of infection with a parvovirus-like agent in children with sickle cell anaemia during a two-year period. *J Clin Pathol* 1982;**35**:744–9.
13. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. *J Infect Dis* 1985;**152**:257–65.
14. Anderson MJ, Jones SE, Minson AC. Diagnosis of human parvovirus infection by dot-blot hybridization using cloned viral DNA. *J Med Virol* 1985;**15**:163–72.
15. Anderson MJ, Kidd IM, Morgan-Capner P. Human parvovirus and rubella-like illness. *Lancet* 1985;**2**:663.
16. Anderson MJ, Lewis E, Kidd IM, et al. An outbreak of erythema infectiosum associated with human parvovirus infection. *J Hyg (Lond)* 1984;**93**:85–93.
17. Arnold DM, Neame PB, Meyer RM, et al. Autologous peripheral blood progenitor cells are a potential source of parvovirus B19 infection. *Transfusion* 2005;**45**:394–8.
18. Asano Y, Sarukawa M, Idezuki T, et al. Multiple small pulmonary emboli associated with transient antiphospholipid syndrome in human parvovirus B19 infection. *Clin Rheumatol* 2006;**25**:585–7.
19. Auriemma PR. Erythema infectiosum: report on a familial outbreak. *Am J Public Health Nations Health* 1954;**44**:1450–4.
20. Azzi A, Zakrzewska K, Gentilomi G, et al. Detection of B19 parvovirus infections by a dot-blot hybridization assay using a digoxigenin-labelled probe. *J Virol Methods* 1990;**27**:125–33.
21. Bagot M, Revuz J. Papular-purpuric "gloves and socks" syndrome: primary infection with parvovirus B19? *J Am Acad Dermatol* 1991;**25**:341–2.
22. Bailey JM. Parvovirus B19 presenting with severe sepsis in a previously healthy 25-year-old female. *J Am Board Fam Med* 2006; **19**:317–19.
23. Balfour HH Jr. Erythema infectiosum (fifth disease): clinical review and description of 91 cases seen in an epidemic. *Clin Pediatr (Phila)* 1969;**8**:721–7.
24. Balfour HH Jr, May DB, Rotte TC, et al. A study of erythema infectiosum: recovery of rubella virus and echovirus-12. *Pediatrics* 1972;**50**:285–90.
25. Balfour HH Jr, Schiff GM, Bloom JE. Encephalitis associated with erythema infectiosum. *J Pediatr* 1970;**77**:133–6.
26. Bansal GP, Hatfield JA, Dunn FE, et al. Candidate recombinant vaccine for human B19 parvovirus. *J Infect Dis* 1993;**167**: 1034–44.
27. Barah F, Vallely PJ, Cleator GM, et al. Neurological manifestations of human parvovirus B19 infection. *Rev Med Virol* 2003; **13**:185–99.
28. Bard JW, Perry HO. Erythema infectiosum. *Arch Dermatol* 1966;**93**:49–53.
29. Barsoum NR, Bunnapradist S, Mougdil A, et al. Treatment of parvovirus B-19 (PV B-19) infection allows for successful kidney transplantation without disease recurrence. *Am J Transplant* 2002;**2**:425–8.
30. Barzon L, Murer L, Pacenti M, et al. Investigation of intrarenal viral infections in kidney transplant recipients unveils an association between parvovirus B19 and chronic allograft injury. *J Infect Dis* 2009;**199**:372–80.
31. Basic D, Gupta S, Kwong RY. Parvovirus B19–induced myocarditis mimicking acute myocardial infarction: clarification of diagnosis by cardiac magnetic resonance imaging. *Circulation* 2010;**121**:e40–2.
32. Becker MR, Schneider B, Reber U, et al. Renal anemia aggravated by long-term parvovirus B19 and cytomegalovirus infection in a renal transplant patient: case report and evaluation of B19 seroprevalence in dialysis patients. *Transplant Proc* 2005;**37**:4306–8.
33. Beersma MF, Claas EC, Sopaheluakan T, et al. Parvovirus B19 viral loads in relation to VP1 and VP2 antibody responses in diagnostic blood samples. *J Clin Virol* 2005;**34**:71–5.
34. Berg AM, Naides SJ, Simms RW. Established fibromyalgia syndrome and parvovirus B19 infection. *J Rheumatol* 1993;**20**: 1941–3.
35. Berns KI. *Parvoviridae and their replication: virology*. 2nd ed. New York: Raven Press; 1990. vol. 2, p. 1743–64.
36. Bertoni E, Rosati A, Zanazzi M, et al. Unusual incidence of aplastic anaemia due to B-19 parvovirus infection in renal transplant recipients. *Transplant Proc* 1997;**29**:818–19.
37. Bessis D. Human parvovirus B19 induced papular-purpuric "gloves and socks" syndrome. *Eur J Dermatol* 1994;**4**:133–4.
38. Bhattacharyya J, Kumar R, Tyagi S, et al. Human parvovirus B19–induced acquired pure amegakaryocytic thrombocytopenia. *Br J Haematol* 2005;**128**:128–9.
39. Bilge I, Sadikoglu B, Emre S, et al. Central nervous system vasculitis secondary to parvovirus B19 infection in a pediatric renal transplant patient. *Pediatr Nephrol* 2005;**20**:529–33.
40. Bloom ME. *Parvoviruses: virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. vol. 2, p. 2361–79.
41. Bock CT, Klingel K, Aberle S, et al. Human parvovirus B19: a new emerging pathogen of inflammatory cardiomyopathy. *J Vet Med B Infect Dis Vet Public Health* 2005;**52**:340–3.
42. Bock CT, Klingel K, Kandolf R. Human parvovirus B19–associated myocarditis. *N Engl J Med* 2010;**362**:1248–9.
43. Bonvicini F, Puccetti C, Salfi NC, et al. Gestational and fetal outcomes in B19 maternal infection: a problem of diagnosis. *J Clin Microbiol* 2011;**49**:3514–18.
44. Borkowski J, Amrikachi M, Hudnall SD. Fulminant parvovirus infection following erythropoietin treatment in a patient with acquired immunodeficiency syndrome. *Arch Pathol Lab Med* 2000;**124**:441–5.
45. Boruchoff SE, Woda BA, Pihan GA, et al. Parvovirus B19–associated hemophagocytic syndrome. *Arch Intern Med* 1990;**150**:897–9.
46. Boysen G. Erythema infectiosum. *Acta Paediatr* 1944;**31**:211–24.
47. Brandenburg H, Los FJ, Cohen-Overbeek TE. A case of early intrauterine parvovirus B19 infection. *Prenat Diagn* 1996;**16**:75–7.
48. Brass C, Elliott LM, Stevens DA. Academy rash: a probable epidemic of erythema infectiosum ("fifth disease"). *JAMA* 1982;**248**: 568–72.
49. Breese C, Horner FA. Encephalopathy with erythema infectiosum. *Am J Dis Child* 1977;**131**:65–7.
50. Brown CS, van Bussel MJ, Wassenaar AL, et al. An immunofluorescence assay for the detection of parvovirus B19 IgG and IgM antibodies based on recombinant viral antigen. *J Virol Methods* 1990;**29**:53–62.
51. Brown KE. Detection and quantitation of parvovirus B19. *J Clin Virol* 2004;**31**:1–4.
52. Brown KE. The expanding range of parvoviruses which infect humans. *Rev Med Virol* 2010;**20**:231–44.
53. Brown KE, Anderson SM, Young NS. Erythrocyte P antigen: cellular receptor for B19 parvovirus. *Science* 1993;**262**:114–17.
54. Brown KE, Green SW, Antunez de Mayolo J, et al. Congenital anaemia after transplacental B19 parvovirus infection. *Lancet* 1994;**343**:895–6.
55. Brown KE, Hibbs JR, Gallinella G, et al. Resistance to parvovirus B19 infection due to lack of virus receptor (erythrocyte P antigen). *N Engl J Med* 1994;**330**:1192–6.

56. Brown KE, Mori J, Cohen BJ, et al. In vitro propagation of parvovirus B19 in primary foetal liver culture. *J Gen Virol* 1991;**72**(Pt 3):741–5.
57. Brown KE, Young NS, Liu JM. Molecular, cellular and clinical aspects of parvovirus B19 infection. *Crit Rev Oncol Hematol* 1994;**16**:1–31.
58. Brown T. Intrauterine human parvovirus infection and hydrops fetalis. *Lancet* 1984;**2**:1033–4.
59. Bruu AL, Nordbo SA. Evaluation of five commercial tests for detection of immunoglobulin M antibodies to human parvovirus B19. *J Clin Microbiol* 1995;**33**:1363–5.
60. Bultmann BD, Klingel K, Sotlar K, et al. Fatal parvovirus B19–associated myocarditis clinically mimicking ischemic heart disease: an endothelial cell–mediated disease. *Hum Pathol* 2003;**34**:92–5.
61. Bultmann BD, Klingel K, Sotlar K, et al. Parvovirus B19: a pathogen responsible for more than hematologic disorders. *Virchows Arch* 2003;**442**:8–17.
62. Caliskan R, Masatlioglu S, Aslan M, et al. The relationship between arthritis and human parvovirus B19 infection. *Rheumatol Int* 2005;**26**:7–11.
63. Candotti D, Danso K, Parsyan A, et al. Maternal-fetal transmission of human parvovirus B19 genotype 3. *J Infect Dis* 2006;**194**: 608–11.
64. Carlsen K, Beck BL, Bagger PV, et al. Pregnancy loss ascribable to parvovirus B19/erythrovirus is associated with a high prevalence of trisomy. *Gynecol Obstet Invest* 2010;**70**:328–34.
65. Carriere C, Boulanger P, Delsert C. Rapid and sensitive method for the detection of B19 virus DNA using the polymerase chain reaction with nested primers. *J Virol Methods* 1993;**44**:221–34.
66. Carrington D, Gilmore DH, Whittle MJ, et al. Maternal serum alpha-fetoprotein—a marker of fetal aplastic crisis during intrauterine human parvovirus infection. *Lancet* 1987;**1**:433–5.
67. Carstensen H, Ornvold K, Cohen BJ. Human parvovirus B19 infection associated with prolonged erythroblastopenia in a leukemic child. *Pediatr Infect Dis J* 1989;**8**:56.
68. Cartter ML, Farley TA, Rosengren S, et al. Occupational risk factors for infection with parvovirus B19 among pregnant women. *J Infect Dis* 1991;**163**:282–5.
69. Carvalho M. Detection of human parvovirus B19 infection: a study of 212 suspected cases in the state of Rio de Janeiro, Brazil. *J Clin Virol* 2002;**25**:223–30.
70. Cassinotti P, Schultze D, Schlageter P, et al. Persistent human parvovirus B19 infection following an acute infection with meningitis in an immunocompetent patient. *Eur J Clin Microbiol Infect Dis* 1993;**12**:701–4.
71. Cassinotti P, Siegl G, Michel BA, et al. Presence and significance of human parvovirus B19 DNA in synovial membranes and bone marrow from patients with arthritis of unknown origin. *J Med Virol* 1998;**56**:199–204.
72. Cassinotti P, Weitz M, Siegl G. Human parvovirus B19 infections: routine diagnosis by a new nested polymerase chain reaction assay. *J Med Virol* 1993;**40**:228–34.
73. Caul EO, Usher MJ, Burton PA. Intrauterine infection with human parvovirus B19: a light and electron microscopy study. *J Med Virol* 1988;**24**:55–66.
74. Cavallo R, Merlino C, Re D, et al. B19 virus infection in renal transplant recipients. *J Clin Virol* 2003;**26**:361–8.
75. Chargin L. Erythema infectiosum: report of an extensive epidemic. *Arch Dermatol Syph* 1942;**47**:467–77.
76. Chen AY, Kleiboeker S, Qiu J. Productive parvovirus B19 infection of primary human erythroid progenitor cells at hypoxia is regulated by STAT5A and MEK signaling but not HIFalpha. *PLoS Pathog* 2011;**7**:e1002088.
77. Chen AY, Zhang EY, Guan W, et al. The small 11 kDa nonstructural protein of human parvovirus B19 plays a key role in inducing apoptosis during B19 virus infection of primary erythroid progenitor cells. *Blood* 2010;**115**:1070–80.
78. Chen MY, Yang SJ, Hung CC. Placental transmission of human parvovirus 4 in newborns with hydrops, Taiwan. *Emerg Infect Dis* 2011;**17**:1954–6.
79. Chernak E, Dubin G, Henry D, et al. Infection due to parvovirus B19 in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1995;**20**:170–3.
80. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.
81. Cherry JD. Parvovirus infections in children and adults. *Adv Pediatr* 1999;**46**:245–69.
82. Chiche L, Grados A, Harle JR, et al. Mixed cryoglobulinemia: a role for parvovirus b19 infection. *Clin Infect Dis* 2010;**50**:1074–5.
83. Chisaka H, Morita E, Yaegashi N, et al. Parvovirus B19 and the pathogenesis of anaemia. *Rev Med Virol* 2003;**13**:347–59.
84. Chorba T, Coccia P, Holman RC, et al. The role of parvovirus B19 in aplastic crisis and erythema infectiosum (fifth disease). *J Infect Dis* 1986;**154**:383–93.
85. Cioc AM, Sedmak DD, Nuovo GJ, et al. Parvovirus B19 associated adult Henoch-Schönlein purpura. *J Cutan Pathol* 2002;**29**:602–7.
86. Clarke HC. Erythema infectiosum: an epidemic with a probable posterythema phase. *Can Med Assoc J* 1984;**130**:603–4.
87. Clarke J, Lee JD. Primary human parvovirus B19 infection in an HIV infected patient on highly active antiretroviral therapy. *Sex Transm Infect* 2003;**79**:336.
88. Clewley JP. Biochemical characterization of a human parvovirus. *J Gen Virol* 1984;**65**(Pt 1):241–5.
89. Clewley JP, Cohen BJ, Field AM. Detection of parvovirus B19 DNA, antigen, and particles in the human fetus. *J Med Virol* 1987;**23**:367–76.
90. Coe HC, Kelly FL. Erythema Infectiosum. *Cal West Med* 1932;**36**:39–40.
91. Cohen BJ, Bates CM. Evaluation of 4 commercial test kits for parvovirus B19–specific IgM. *J Virol Methods* 1995;**55**:11–25.
92. Cohen BJ, Gandhi J, Clewley JP. Genetic variants of parvovirus B19 identified in the United Kingdom: implications for diagnostic testing. *J Clin Virol* 2006;**36**:152–5.
93. Cohen BJ, Mortimer PP, Pereira MS. Diagnostic assays with monoclonal antibodies for the human serum parvovirus-like virus (SPLV). *J Hyg (Lond)* 1983;**91**:113–30.
94. Collett MS. Prospects for a human B19 parvovirus vaccine. *Rev Med Virol* 1994;**4**:91–103.
95. Colmegna I, Alberts-Grill N. Parvovirus B19: its role in chronic arthritis. *Rheum Dis Clin North Am* 2009;**35**:95–110.
96. Condon FJ. Erythema infectiosum: report of an area-wide outbreak. *Am J Public Health Nations Health* 1959;**49**:528–35.
97. Conway SP, Cohen BJ, Field AM, et al. A family outbreak of parvovirus B19 infection with petechial rash in a 7-year-old boy. *J Infect* 1987;**15**:110–12.
98. Cooper CL, Choudhri SH. Photo quiz II. Leukocytoclastic vasculitis secondary to parvovirus B19 infection. *Clin Infect Dis* 1998; **26**:849, 989.
99. Cosmi E, Mari G, Delle Chiaie L, et al. Noninvasive diagnosis by Doppler ultrasonography of fetal anemia resulting from parvovirus infection. *Am J Obstet Gynecol* 2002;**187**:1290–3.
100. Cossart YE, Field AM, Cant B, et al. Parvovirus-like particles in human sera. *Lancet* 1975;**1**:72–3.
101. Cotmore SF, Tattersall P. Characterization and molecular cloning of a human parvovirus genome. *Science* 1984;**226**:1161–5.
102. Coulombel L. Parvovirus infection, leukemia, and immunodeficiency. *Lancet* 1989;**1**:101–2.
103. Cubel RC, Oliveira SA, Brown DW, et al. Diagnosis of parvovirus B19 infection by detection of specific immunoglobulin M antibody in saliva. *J Clin Microbiol* 1996;**34**:205–7.
104. Daly P, Corcoran A, Mahon BP, et al. High-sensitivity PCR detection of parvovirus B19 in plasma. *J Clin Microbiol* 2002;**40**: 1958–62.
105. Dass R, Ramesh P, Ratho RK, et al. Parvovirus B19–induced multisystem disease simulating systemic vasculitis in a young child. *Rheumatol Int* 2005;**25**:125–9.
106. De Jong EP, de Haan TR, Kroes AC, et al. Parvovirus B19 infection in pregnancy. *J Clin Virol* 2006;**36**:1–7.
107. De Jong EP, Lindenburg IT, van Klink JM, et al. Intrauterine transfusion for parvovirus B19 infection: long-term neurodevelopmental outcome. *Am J Obstet Gynecol* 2012;**206**:204.e1–5.
108. Deiss V, Tratschin JD, Weitz M, et al. Cloning of the human parvovirus B19 genome and structural analysis of its palindromic termini. *Virology* 1990;**175**:247–54.
109. Dettmeyer R, Kandolf R, Baasner A, et al. Fatal parvovirus B19 myocarditis in an 8-year-old boy. *J Forensic Sci* 2003;**48**:183–6.
110. Diaz F, Collazos J, Mendoza F, et al. Systemic lupus erythematosus associated with acute parvovirus B19 infection. *Clin Microbiol Infect* 2002;**8**:115–17.
111. Dijkmans AC, de Jong EP, Dijkmans BA, et al. Parvovirus B19 in pregnancy: prenatal diagnosis and management of fetal complications. *Curr Opin Obstet Gynecol* 2012;**24**:95–101.

112. Dina J, Vabret A, Rambaud C, et al. Fulminant myocarditis associated with parvovirus B19 infection in a child. *J Clin Virol* 2008; **42**:70–1.
113. Dockrell DH, Poland GA, Jones MF, et al. Variability in parvovirus B19 IgG levels in intravenous immunoglobulin samples. *Diagn Microbiol Infect Dis* 1996;**26**:133–5.
114. Donders GG, Van Lierde S, Van Elsacker-Niele AM, et al. Survival after intrauterine parvovirus B19 infection with persistence in early infancy: a two-year follow-up. *Pediatr Infect Dis J* 1994;**13**:234–6.
115. Douvoyiannis M, Litman N, Goldman DL. Neurologic manifestations associated with parvovirus B19 infection. *Clin Infect Dis* 2009;**48**:1713–23.
116. Doyle S, Corcoran A. The immune response to parvovirus B19 exposure in previously seronegative and seropositive individuals. *J Infect Dis* 2006;**194**:154–8.
117. Drago F, Semino M, Rampini P, et al. Parvovirus B19 infection associated with acute hepatitis and a purpuric exanthem. *Br J Dermatol* 1999;**141**:160–1.
118. Duncan JR, Potter CB, Cappellini MD, et al. Aplastic crisis due to parvovirus infection in pyruvate kinase deficiency. *Lancet* 1983;**2**:14–16.
119. Dunnet WN. Food poisoning from oysters. *Communicable Disease Report, United Kingdom.* 1984;**36**:3.
120. Dwivedi M, Manocha H, Tiwari S, et al. Coinfection of parvovirus b19 with other hepatitis viruses leading to fulminant hepatitis of unfavorable outcome in children. *Pediatr Infect Dis J* 2009;**28**: 649–50.
121. Edelson RN, Altman R. Erythema infectiosum: a statewide outbreak. *J Med Soc N J* 1970;**67**:805–9.
122. Eden A, Mahr A, Servant A, et al. Lack of association between B19 or V9 erythrovirus infection and ANCA-positive vasculitides: a case-control study. *Rheumatology (Oxford)* 2003;**42**:660–4.
123. Edmonson MB, Riedesel EL, Williams GP, et al. Generalized petechial rashes in children during a parvovirus B19 outbreak. *Pediatrics* 2010;**125**:e787–92.
124. Egbuna O, Zand MS, Arbini A, et al. A cluster of parvovirus B19 infections in renal transplant recipients: a prospective case series and review of the literature. *Am J Transplant* 2006;**6**:225–31.
125. Eid AJ, Brown RA, Patel R, et al. Parvovirus B19 infection after transplantation: a review of 98 cases. *Clin Infect Dis* 2006;**43**: 40–8.
126. Eid AJ, Posfay-Barbe KM. Parvovirus B19 in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S147–50.
127. Eijnden SV, Carlier F, Van Beers D, et al. Gloves and socks lymphangitis associated with acute parvovirus B19 infection. *Pediatr Dermatol* 2003;**20**:184–6.
128. Eis-Hubinger AM, Dieck D, Schild R, et al. Parvovirus B19 infection in pregnancy. *Intervirology* 1998;**41**:178–84.
129. El-Mahallawy HA, Mansour T, El-Din SE, et al. Parvovirus B19 infection as a cause of anemia in pediatric acute lymphoblastic leukemia patients during maintenance chemotherapy. *J Pediatr Hematol Oncol* 2004;**26**:403–6.
130. Enders G, Dotsch J, Bauer J, et al. Life-threatening parvovirus B19–associated myocarditis and cardiac transplantation as possible therapy: two case reports. *Clin Infect Dis* 1998;**26**:355–8.
131. Enders M, Schalasta G, Baisch C, et al. Human parvovirus B19 infection during pregnancy—value of modern molecular and serological diagnostics. *J Clin Virol* 2006;**35**:400–6.
132. Enders M, Weidner A, Zoellner I, et al. Fetal morbidity and mortality after acute human parvovirus B19 infection in pregnancy: prospective evaluation of 1018 cases. *Prenat Diagn* 2004;**24**:513–18.
133. Erdman DD, Durigon EL, Holloway BP. Detection of human parvovirus B19 DNA PCR products by RNA probe hybridization enzyme immunoassay. *J Clin Microbiol* 1994;**32**:2295–8.
134. Erdman DD, Durigon EL, Wang QY, et al. Genetic diversity of human parvovirus B19: sequence analysis of the *VP1/VP2* gene from multiple isolates. *J Gen Virol* 1996;**77**(Pt 11):2767–74.
135. Ergaz Z, Ornoy A. Parvovirus B19 in pregnancy. *Reprod Toxicol* 2006;**21**:421–35.
136. Eriksson BM, Stromberg A, Kreuger A. Human parvovirus B19 infection with severe anemia affecting mother and son. *Scand J Infect Dis* 1988;**20**:335–7.
137. Escherich T. Comptes-rendus du XII Congres International de Medecin. Moscow SP. *Yakovlev* 189;**3**:133.
138. Faden H, Gary GW Jr, Anderson LJ. Chronic parvovirus infection in a presumably immunologically healthy woman. *Clin Infect Dis* 1992;**15**:595–7.
139. Faden H, Gary GW Jr, Korman M. Numbness and tingling of fingers associated with parvovirus B19 infection. *J Infect Dis* 1990; **161**:354–5.
140. Fattet S, Cassinotti P, Popovic MB. Persistent human parvovirus B19 infection in children under maintenance chemotherapy for acute lymphocytic leukemia. *J Pediatr Hematol Oncol* 2004;**26**: 497–503.
141. Ferraz C, Cunha F, Mota TC, et al. Acute respiratory distress syndrome in a child with human parvovirus B19 infection. *Pediatr Infect Dis J* 2005;**24**:1009–10.
142. Ferri C, Azzi A, Magro CM. Parvovirus B19 and systemic sclerosis. *Br J Dermatol* 2005;**152**:819–20.
143. Ferri C, Zakrzewska K, Longombardo G, et al. Parvovirus B19 infection of bone marrow in systemic sclerosis patients. *Clin Exp Rheumatol* 1999;**17**:718–20.
144. Fishman SG, Pelaez LM, Baergen RN, et al. Parvovirus-mediated fetal cardiomyopathy with atrioventricular nodal disease. *Pediatr Cardiol* 2011;**32**:84–6.
145. Fong CY, de Sousa C. Childhood chorea-encephalopathy associated with human parvovirus B19 infection. *Dev Med Child Neurol* 2006;**48**:526–8.
146. Foreman NK, Oakhill A, Caul EO. Parvovirus-associated thrombocytopenic purpura. *Lancet* 1988;**2**:1426–7.
147. Foti C, Bonamonte D, Conserva A, et al. Erythema infectiosum following generalized petechial eruption induced by human parvovirus B19. *New Microbiol* 2006;**29**:45–8.
148. Foto F, Saag KG, Scharosch LL, et al. Parvovirus B19–specific DNA in bone marrow from B19 arthropathy patients: evidence for B19 virus persistence. *J Infect Dis* 1993;**167**:744–8.
149. Fox MJ, Clark JM. Erythema infectiosum. *Am J Dis Child* 1947;**73**:453–7.
150. Franssila R, Auramo J, Modrow S, et al. T helper cell–mediated interferon-gamma expression after human parvovirus B19 infection: persisting VP2-specific and transient VP1u-specific activity. *Clin Exp Immunol* 2005;**142**:53–61.
151. Franssila R, Hedman K. T-helper cell–mediated interferon-gamma, interleukin-10 and proliferation responses to a candidate recombinant vaccine for human parvovirus B19. *Vaccine* 2004; **22**:3809–15.
152. Franssila R, Hokynar K, Hedman K. T helper cell–mediated in vitro responses of recently and remotely infected subjects to a candidate recombinant vaccine for human parvovirus B19. *J Infect Dis* 2001;**183**:805–9.
153. Fridell E, Trojnar J, Wahren B. A new peptide for human parvovirus B19 antibody detection. *Scand J Infect Dis* 1989;**21**: 597–603.
154. Fried RI. Erythema infectiosum: fifth disease; a clinical note. *Ohio Med* 1951;**47**:1027–8.
155. Fryer JF, Kapoor A, Minor PD, et al. Novel parvovirus and related variant in human plasma. *Emerg Infect Dis* 2006;**12**:151–4.
156. Fukada K, Matumoto K, Takakura F, et al. Four putative subtypes of human parvovirus B19 based on amino acid polymorphism in the C-terminal region of non-structural protein. *J Med Virol* 2000;**62**:60–9.
157. Gabriel SE, Espy M, Erdman DD, et al. The role of parvovirus B19 in the pathogenesis of giant cell arteritis: a preliminary evaluation. *Arthritis Rheum* 1999;**42**:1255–8.
158. Gadage VS, Viswanathan S, Kunal S, et al. Parvovirus B19 presenting with persistent pancytopenia in a patient of T-ALL post induction chemotherapy diagnosed on bone marrow examination. *Indian J Pathol Microbiol* 2011;**54**:603–5.
159. Gallinella G, Zuffi E, Gentilomi G, et al. Relevance of B19 markers in serum samples for a diagnosis of parvovirus B19-correlated diseases. *J Med Virol* 2003;**71**:135–9.
160. Garcia-Tapia AM, Fernandez-Gutierrez del Alamo C, Giron JA, et al. Spectrum of parvovirus B19 infection: analysis of an outbreak of 43 cases in Cadiz, Spain. *Clin Infect Dis* 1995;**21**:1424–30.
161. Gentilomi G, Zerbini M, Gallinella G, et al. B19 parvovirus induced fetal hydrops: rapid and simple diagnosis by detection of B19 antigens in amniotic fluids. *Prenat Diagn* 1998;**18**:363–8.
162. Gillespie SM, Cartter ML, Asch S, et al. Occupational risk of human parvovirus B19 infection for school and day-care personnel

during an outbreak of erythema infectiosum. *JAMA* 1990;**263**: 2061–5.
163. Goldman F, Rotbart H, Gutierrez K, et al. Parvovirus-associated aplastic crisis in a patient with red blood cell glucose-6-phosphate dehydrogenase deficiency. *Pediatr Infect Dis J* 1990;**9**:593–4.
164. Goncalves G, Correia AM, Palminha P, et al. Outbreaks caused by parvovirus B19 in three Portuguese schools. *Euro Surveill* 2005;**10**:121–4.
165. Gowda N, Rao SP, Cohen B, et al. Human parvovirus infection in patients with sickle cell disease with and without hypoplastic crisis. *J Pediatr* 1987;**110**:81–4.
166. Grabarczyk P, Kalinska A, Kara M, et al. Identification and characterization of acute infection with parvovirus B19 genotype 2 in immunocompromised patients in Poland. *J Med Virol* 2011;**83**: 142–9.
167. Graesslin O, Andreoletti L, Dedecker F, et al. Successful in utero treatment of parvovirus B19–induced fetal hydrops in a case of twin pregnancy. *Prenat Diagn* 2005;**25**:336–7.
168. Gratacos E, Torres PJ, Vidal J, et al. The incidence of human parvovirus B19 infection during pregnancy and its impact on perinatal outcome. *J Infect Dis* 1995;**171**:1360–3.
169. Greenwald P, Bashe WJ Jr. An Epidemic of erythema infectiosum. *Am J Dis Child* 1964;**107**:30–4.
170. Grimmer H, Joseph A. An epidemic of infectious erythema in Germany. *Arch Dermatol* 1959;**80**:283–5.
171. Guidi B, Bergonzini P, Crisi G, et al. Case of stroke in a 7-year-old male after parvovirus B19 infection. *Pediatr Neurol* 2003;**28**: 69–71.
172. Guillaume MP, Hermanus N, Peretz A. Unusual localisation of chronic arthropathy in lumbar facet joints after parvovirus B19 infection. *Clin Rheumatol* 2002;**21**:306–8.
173. Gulen H, Basarir F, Hakan N, et al. Premature labor and leukoerythroblastosis in a newborn with parvovirus B19 infection. *Haematologica* 2005;**90**(Suppl):ECR38.
174. Gupta R, Singh T. Parvovirus B19 co-infection with falciparum malaria: a cause of severe anemia. *Haematologica* 2005;**90**:ECR41.
175. Gustafsson I, Kaldensjo T, Lindblom A, et al. Evaluation of parvovirus B19 infection in children with malignant or hematological disorders. *Clin Infect Dis* 2010;**50**:1426–7.
176. Gyllensten K, Sonnerborg A, Jorup-Ronstrom C, et al. Parvovirus B19 infection in HIV-1 infected patients with anemia. *Infection* 1994;**22**:356–8.
177. Hajeer AH, MacGregor AJ, Rigby AS, et al. Influence of previous exposure to human parvovirus B19 infection in explaining susceptibility to rheumatoid arthritis: an analysis of disease discordant twin pairs. *Ann Rheum Dis* 1994;**53**:137–9.
178. Halasz CL, Cormier D, Den M. Petechial glove and sock syndrome caused by parvovirus B19. *J Am Acad Dermatol* 1992;**27**: 835–8.
179. Hammon WD, Enders JF. A virus disease of cats, principally characterized by aleucocytosis, enteric lesions and the presence of intranuclear inclusion bodies. *J Exp Med* 1939;**69**:327–52.
180. Hanada T, Koike K, Takeya T, et al. Human parvovirus B19–induced transient pancytopenia in a child with hereditary spherocytosis. *Br J Haematol* 1988;**70**:113–15.
181. Harel L, Straussberg R, Rudich H, et al. Raynaud's phenomenon as a manifestation of parvovirus B19 infection: case reports and review of parvovirus B19 rheumatic and vasculitic syndromes. *Clin Infect Dis* 2000;**30**:500–3.
182. Haseyama K, Kudoh T, Yoto Y, et al. Detection of human parvovirus B19 DNA in cerebrospinal fluid. *Pediatr Infect Dis J* 1997; **16**:324–6.
183. Hashimoto H, Yuno T. Parvovirus B19–associated purpuric-petechial eruption. *J Clin Virol* 2011;**52**:269–71.
184. Hatakka A, Klein J, He R, et al. Acute hepatitis as a manifestation of parvovirus B19 infection. *J Clin Microbiol* 2011;**49**:3422–4.
185. Hauswirth WW. Autonomous parvovirus DNA structure and replication. In: Berns KI, editor. *The parvoviruses*. London: Plenum Press; 1983. p. 129–52.
186. Hayakawa F, Imada K, Towatari M, et al. Life-threatening human parvovirus B19 infection transmitted by intravenous immune globulin. *Br J Haematol* 2002;**118**:1187–9.
187. Hayakawa H, Tara M, Niina K, et al. A clinical study of adult human parvovirus B19 infection. *Intern Med* 2002;**41**:295–9.
188. Hayes-Lattin B, Seipel TJ, Gatter K, et al. Pure red cell aplasia associated with parvovirus B19 infection occurring late after allogeneic bone marrow transplantation. *Am J Hematol* 2004;**75**: 142–5.
189. Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev* 2002;**15**:485–505.
190. Heegaard ED, Hasle H, Clausen N, et al. Parvovirus B19 infection and Diamond-Blackfan anaemia. *Acta Paediatr* 1996;**85**: 299–302.
191. Heegaard ED, Hasle H, Skibsted L, et al. Congenital anemia caused by parvovirus B19 infection. *Pediatr Infect Dis J* 2000;**19**: 1216–18.
192. Heegaard ED, Hornsleth A. Parvovirus: the expanding spectrum of disease. *Acta Paediatr* 1995;**84**:109–17.
193. Heegaard ED, Panum Jensen I, Christensen J. Novel PCR assay for differential detection and screening of erythrovirus B19 and erythrovirus V9. *J Med Virol* 2001;**65**:362–7.
194. Heegaard ED, Peterslund NA, Hornsleth A. Parvovirus B19 infection associated with encephalitis in a patient suffering from malignant lymphoma. *Scand J Infect Dis* 1995;**27**:631–3.
195. Heegaard ED, Qvortrup K, Christensen J. Baculovirus expression of erythrovirus V9 capsids and screening by ELISA: serologic cross-reactivity with erythrovirus B19. *J Med Virol* 2002;**66**: 246–52.
196. Heegaard ED, Schmiegelow K. Serologic study on parvovirus b19 infection in childhood acute lymphoblastic leukemia during chemotherapy: clinical and hematologic implications. *J Pediatr Hematol Oncol* 2002;**24**:368–73.
197. Heegaard ED, Taaning EB. Parvovirus B19 and parvovirus V9 are not associated with Henoch-Schönlein purpura in children. *Pediatr Infect Dis J* 2002;**21**:31–4.
198. Heinz C, Plentz A, Bauer D, et al. Prevalence of parvovirus B19–specific antibodies and of viral DNA in patients with endogenous uveitis. *Graefes Arch Clin Exp Ophthalmol* 2005;**243**:999–1004.
199. Hemauer A, von Poblotzki A, Gigler A, et al. Sequence variability among different parvovirus B19 isolates. *J Gen Virol* 1996;**77**(Pt 8):1781–5.
200. Herbert KE, Prince HM, Westerman DA. Pure red-cell aplasia due to parvovirus B19 infection in a patient treated with alemtuzumab. *Blood* 2003;**101**:1654.
201. Herrick TP. Erythema infectiosum: a clinical report of 74 cases. *Am J Dis Child* 1926;**31**:486–95.
202. Hession MT, Au SC, Gottlieb AB. Parvovirus B19–associated systemic lupus erythematosus: clinical mimicry or autoimmune induction? *J Rheumatol* 2010;**37**:2430–2.
203. Hicks KE, Beard S, Cohen BJ, et al. A simple and sensitive DNA hybridization assay used for the routine diagnosis of human parvovirus B19 infection. *J Clin Microbiol* 1995;**33**:2473–5.
204. Higashida K, Kobayashi K, Sugita K, et al. Pure red blood cell aplasia during azathioprine therapy associated with parvovirus B19 infection. *Pediatr Infect Dis J* 1997;**16**:1093–5.
205. Higgins CS and Anderson MJ. Acute parvovirus infection associated with chronic arthritis. Unpublished data, 1985.
206. Hoebe CJ, Claas EC, Steenbergen JE, et al. Confirmation of an outbreak of parvovirus B19 in a primary school using IgM ELISA and PCR on thumb prick blood samples. *J Clin Virol* 2002;**25**: 303–7.
207. Hoffman E. Erythema infectiosum (groszflecken oder ringelroteln). *Dtsch Med Wochenschr* 1916;**1**:777–9.
208. Holm JM, Hansen LK, Oxhoj H. Kawasaki disease associated with parvovirus B19 infection. *Eur J Pediatr* 1995;**154**:633–4.
209. Hsieh MY, Huang PH. The juvenile variant of papular-purpuric gloves and socks syndrome and its association with viral infections. *Br J Dermatol* 2004;**151**:201–6.
210. Hsu TC, Chen TY, Lin MC, et al. Human parvovirus B19 infection in patients with chronic hepatitis B or hepatitis C infection. *J Gastroenterol Hepatol* 2005;**20**:733–8.
211. Huerta-Brogeras M, Aviles Izquierdo JA, Hernanz Hermosa JM, et al. Petechial exanthem in "bathing trunk" distribution caused by parvovirus B19 infection. *Pediatr Dermatol* 2005;**22**:430–3.
212. Ichinose K, Migita K, Sekita T, et al. Parvovirus B19 infection and myofasciitis. *Clin Rheumatol* 2004;**23**:184–5.
213. Ideguchi H, Ohno S, Ishigatsubo Y. A case of pure red cell aplasia and systemic lupus erythematosus caused by human parvovirus B19 infection. *Rheumatol Int* 2007;**27**:411–14.
214. Ieiri N, Hotta O, Taguma Y. Characteristics of acute glomerulonephritis associated with human parvovirus B19 infection. *Clin Nephrol* 2005;**64**:249–57.

215. Ilknur T, Fetil E, Akarsu S, et al. A case of papular-purpuric gloves-and-socks syndrome receiving unnecessary treatment. *Clin Exp Dermatol* 2011;**36**:110–11.
216. Inoue S, Kinra NK, Mukkamala SR, et al. Parvovirus B-19 infection: aplastic crisis, erythema infectiosum and idiopathic thrombocytopenic purpura. *Pediatr Infect Dis J* 1991;**10**:251–3.
217. Isa A, Kasprowicz V, Norbeck O, et al. Prolonged activation of virus-specific $CD8^+$ T cells after acute B19 infection. *PLoS Med* 2005;**2**:e343.
218. Isobe Y, Sugimoto K, Shiraki Y, et al. Successful high-titer immunoglobulin therapy for persistent parvovirus B19 infection in a lymphoma patient treated with rituximab-combined chemotherapy. *Am J Hematol* 2004;**77**:370–3.
219. Ito S, Oyake T, Uchiyama T, et al. Successful treatment with cyclosporine and high-dose gamma immunoglobulin for persistent parvovirus B19 infection in a patient with refractory autoimmune hemolytic anemia. *Int J Hematol* 2004;**80**:250–3.
220. Iwafuchi Y, Morita T, Kamimura A, et al. Acute endocapillary proliferative glomerulonephritis associated with human parvovirus B19 infection. *Clin Nephrol* 2002;**57**:246–50.
221. Jacobson SK, Daly JS, Thorne GM, et al. Chronic parvovirus B19 infection resulting in chronic fatigue syndrome: case history and review. *Clin Infect Dis* 1997;**24**:1048–51.
222. Jensen IP, Schou O, Vestergaard BF. The 1994 human parvovirus B19 epidemic in Denmark: diagnostic and epidemiological experience. *APMIS* 1998;**106**:843–8.
223. Johansen JN, Christensen LS, Zakrzewska K, et al. Typing of European strains of parvovirus B19 by restriction endonuclease analyses and sequencing: identification of evolutionary lineages and evidence of recombination of markers from different lineages. *Virus Res* 1998;**53**:215–23.
224. Johnson LB, Pasumarthy A, Saravolatz LD. Parvovirus B19 infection presenting with necrotizing lymphadenitis. *Am J Med* 2003;**114**:340–1.
225. Jones MS, Kapoor A, Lukashov VV, et al. New DNA viruses identified in patients with acute viral infection syndrome. *J Virol* 2005;**79**:8230–6.
226. Jonetzko P, Graziadei I, Nachbaur K, et al. Fatal course of parvovirus B19–associated myocarditis in a female liver transplant recipient. *Liver Transpl* 2005;**11**:463–6.
227. Kasprowicz V, Isa A, Jeffery K, et al. A highly restricted T-cell receptor dominates the $CD8^+$ T-cell response to parvovirus B19 infection in HLA-A*2402-positive individuals. *J Virol* 2006;**80**:6697–701.
228. Kaufmann B, Simpson AA, Rossmann MG. The structure of human parvovirus B19. *Proc Natl Acad Sci U S A* 2004;**101**:11628–33.
229. Kelleher JF, Luban NL, Mortimer PP, et al. Human serum "parvovirus": a specific cause of aplastic crisis in children with hereditary spherocytosis. *J Pediatr* 1983;**102**:720–2.
230. Kellermayer R, Faden H, Grossi M. Clinical presentation of parvovirus B19 infection in children with aplastic crisis. *Pediatr Infect Dis J* 2003;**22**:1100–1.
231. Kerr JR. Parvovirus B19 infection. *Eur J Clin Microbiol Infect Dis* 1996;**15**:10–29.
232. Kerr JR. Pathogenesis of human parvovirus B19 in rheumatic disease. *Ann Rheum Dis* 2000;**59**:672–83.
233. Kerr JR. Pathogenesis of parvovirus B19 infection: host gene variability, and possible means and effects of virus persistence. *J Vet Med B Infect Dis Vet Public Health* 2005;**52**:335–9.
234. Kerr JR, Barah F, Chiswick ML, et al. Evidence for the role of demyelination, HLA-DR alleles, and cytokines in the pathogenesis of parvovirus B19 meningoencephalitis and its sequelae. *J Neurol Neurosurg Psychiatry* 2002;**73**:739–46.
235. Kerr JR, Barah F, Cunniffe VS, et al. Association of acute parvovirus B19 infection with new onset of acute lymphoblastic and myeloblastic leukaemia. *J Clin Pathol* 2003;**56**:873–5.
236. Kerr JR, Bracewell J, Laing I, et al. Chronic fatigue syndrome and arthralgia following parvovirus B19 infection. *J Rheumatol* 2002;**29**:595–602.
237. Kerr JR, Cunniffe VS, Kelleher P, et al. Successful intravenous immunoglobulin therapy in 3 cases of parvovirus B19-associated chronic fatigue syndrome. *Clin Infect Dis* 2003;**36**:e100–6.
238. Kerr JR, Cunniffe VS, Kelleher P, et al. Circulating cytokines and chemokines in acute symptomatic parvovirus B19 infection: negative association between levels of pro-inflammatory cytokines and development of B19-associated arthritis. *J Med Virol* 2004;**74**:147–55.
239. Kerr JR, Curran MD, Moore JE, et al. Persistent parvovirus B19 infection. *Lancet* 1995;**345**:1118.
240. Kerr JR, Curran MD, Moore JE, et al. Parvovirus B19 infection—persistence and genetic variation. *Scand J Infect Dis* 1995;**27**:551–7.
241. Kerr JR, Mattey DL, Thomson W, et al. Association of symptomatic acute human parvovirus B19 infection with human leukocyte antigen class I and II alleles. *J Infect Dis* 2002;**186**:447–52.
242. Kerr PS, Marsh EH. Outbreak of erythema infectiosum in Elmsford, N. Y. *Am J Public Health Nations Health* 1933;**23**:1271–4.
243. Ki CS, Kim IS, Kim JW, et al. Incidence and clinical significance of human parvovirus B19 infection in kidney transplant recipients. *Clin Transplant* 2005;**19**:751–5.
244. Kim EC, Durigon EL, Erdman DD, et al. Chemiluminescent microwell hybridization assay for direct detection of human parvovirus B19 DNA. *J Virol Methods* 1994;**50**:349–54.
245. Kinney JS, Anderson LJ, Farrar J, et al. Risk of adverse outcomes of pregnancy after human parvovirus B19 infection. *J Infect Dis* 1988;**157**:663–7.
246. Kiraz S, Ertenli I, Benekli M, et al. Parvovirus B19 infection in Behcet's disease. *Clin Exp Rheumatol* 1996;**14**:71–3.
247. Kishore J, Misra R, Gupta D, et al. Raised IgM antibodies to parvovirus B19 in juvenile rheumatoid arthritis. *Indian J Med Res* 1998;**107**:15–18.
248. Knosel T, Meisel H, Borgmann A, et al. Parvovirus B19 infection associated with unilateral cervical lymphadenopathy, apoptotic sinus histiocytosis, and prolonged fatigue. *J Clin Pathol* 2005;**58**:872–5.
249. Knott PD, Welply GA, Anderson MJ. Serologically proved intrauterine infection with parvovirus. *BMJ (Clin Res Ed)* 1984;**289**:1660.
250. Koch WC, Adler SP. Human parvovirus B19 infections in women of childbearing age and within families. *Pediatr Infect Dis J* 1989;**8**:83–7.
251. Koch WC, Adler SP. Detection of human parvovirus B19 DNA by using the polymerase chain reaction. *J Clin Microbiol* 1990;**28**:65–9.
252. Koch WC, Adler SP, Harger J. Intrauterine parvovirus B19 infection may cause an asymptomatic or recurrent postnatal infection. *Pediatr Infect Dis J* 1993;**12**:747–50.
253. Koch WC, Harger JH, Barnstein B, et al. Serologic and virologic evidence for frequent intrauterine transmission of human parvovirus B19 with a primary maternal infection during pregnancy. *Pediatr Infect Dis J* 1998;**17**:489–94.
254. Koch WC, Massey G, Russell CE, et al. Manifestations and treatment of human parvovirus B19 infection in immunocompromised patients. *J Pediatr* 1990;**116**:355–9.
255. Kock WC. A synthetic parvovirus B19 capsid protein can replace viral antigen in antibody-capture enzyme immunoassays. *J Virol Methods* 1995;**55**:67–82.
256. Koduri PR, Kumapley R, Khokha ND, et al. Red cell aplasia caused by parvovirus B19 in AIDS: use of i.v. immunoglobulin. *Ann Hematol* 1997;**75**:67–8.
257. Koduri PR, Naides SJ. Aseptic meningitis caused by parvovirus B19. *Clin Infect Dis* 1995;**21**:1053.
258. Komatsuda A, Ohtani H, Nimura T, et al. Endocapillary proliferative glomerulonephritis in a patient with parvovirus B19 infection. *Am J Kidney Dis* 2000;**36**:851–4.
259. Kondo Y, Kakami M, Kawaguchi H, et al. Transient pancytopenia associated with parvovirus infection in a healthy child. *Pediatr Int* 2002;**44**:695–7.
260. Koziol DE, Kurtzman G, Ayub J, et al. Nosocomial human parvovirus B19 infection: lack of transmission from a chronically infected patient to hospital staff. *Infect Control Hosp Epidemiol* 1992;**13**:343–8.
261. Kuhl U, Pauschinger M, Bock T, et al. Parvovirus B19 infection mimicking acute myocardial infarction. *Circulation* 2003;**108**:945–50.
262. Kuhl U, Pauschinger M, Seeberg B, et al. Viral persistence in the myocardium is associated with progressive cardiac dysfunction. *Circulation* 2005;**112**:1965–70.
263. Kumar J, Shaver MJ, Abul-Ezz S. Long-term remission of recurrent parvovirus-B associated anemia in a renal transplant recipient induced by treatment with immunoglobulin and positive seroconversion. *Transpl Infect Dis* 2005;**7**:30–3.

264. Kuo SH, Lin LI, Chang CJ, et al. Increased risk of parvovirus B19 infection in young adult cancer patients receiving multiple courses of chemotherapy. *J Clin Microbiol* 2002;**40**:3909–12.
265. Kurtzman G, Frickhofen N, Kimball J, et al. Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. *N Engl J Med* 1989; **321**:519–23.
266. Kurtzman GJ, Cohen B, Meyers P, et al. Persistent B19 parvovirus infection as a cause of severe chronic anaemia in children with acute lymphocytic leukaemia. *Lancet* 1988;**2**:1159–62.
267. Kurtzman GJ, Cohen BJ, Field AM, et al. Immune response to B19 parvovirus and an antibody defect in persistent viral infection. *J Clin Invest* 1989;**84**:1114–23.
268. Lackner H, Sovinz P, Benesch M, et al. The spectrum of parvovirus B19 infection in a pediatric hemato-oncologic ward. *Pediatr Infect Dis J* 2011;**30**:440–2.
269. LaMonte AC, Paul ME, Read JS, et al. Persistent parvovirus B19 infection without the development of chronic anemia in HIV-infected and -uninfected children: the Women and Infants Transmission Study. *J Infect Dis* 2004;**189**:847–51.
270. Lamparter S, Schoppet M, Pankuweit S, et al. Acute parvovirus B19 infection associated with myocarditis in an immunocompetent adult. *Hum Pathol* 2003;**34**:725–8.
271. Langnas AN, Markin RS, Cattral MS, et al. Parvovirus B19 as a possible causative agent of fulminant liver failure and associated aplastic anemia. *Hepatology* 1995;**22**:1661–5.
272. Larroche C, Scieux C, Honderlick P, et al. Spontaneous resolution of hemophagocytic syndrome associated with acute parvovirus B19 infection and concomitant Epstein-Barr virus reactivation in an otherwise healthy adult. *Eur J Clin Microbiol Infect Dis* 2002; **21**:739–42.
273. Lauer BA, MacCormack JN, Wilfert C. Erythema infectiosum: an elementary school outbreak. *Am J Dis Child* 1976;**130**:252–4.
274. Lawton AL. Erythema infectiosum: a clinical study of an epidemic in Branford. *Conn Arch Intern Med* 1931;**47**:28–41.
275. Lee PC, Hung CJ, Lin YJ, et al. A role for chronic parvovirus B19 infection in liver dysfunction in renal transplant recipients? *Transplantation* 2002;**73**:1635–9.
276. Lee YM, Tsai WH, You JY, et al. Parvovirus B19 infection in Taiwanese patients with hematological disorders. *J Med Virol* 2003;**71**:605–9.
277. Lefrère JJ. Human parvovirus and purpura. *Lancet* 1985;**2**:730–1.
278. Lefrere JJ, Courouce AM, Girot R, et al. Human parvovirus and thalassaemia. *J Infect* 1986;**13**:45–9.
279. Lefrere JJ, Courouce AM, Kaplan C. Parvovirus and idiopathic thrombocytopenic purpura. *Lancet* 1989;**1**:279.
280. Lehmann HW, Knoll A, Kuster RM, et al. Frequent infection with a viral pathogen, parvovirus B19, in rheumatic diseases of childhood. *Arthritis Rheum* 2003;**48**:1631–8.
281. Lehmann HW, Kuhner L, Beckenlehner K, et al. Chronic human parvovirus B19 infection in rheumatic disease of childhood and adolescence. *J Clin Virol* 2002;**25**:135–43.
282. Lehmann HW, von Landenberg P, Modrow S. Parvovirus B19 infection and autoimmune disease. *Autoimmun Rev* 2003;**2**:218–23.
283. Lenglet T, Haroche J, Schnuriger A, et al. Mononeuropathy multiplex associated with acute parvovirus B19 infection: characteristics, treatment and outcome. *J Neurol* 2011;**258**:1321–6.
284. Lewin Altschuler E. Molecular method to test if parvovirus B19 is a new virus to the Old World. *Med Hypotheses* 2004;**62**:795–6.
285. Liefeldt L, Buhl M, Schweickert B, et al. Eradication of parvovirus B19 infection after renal transplantation requires reduction of immunosuppression and high-dose immunoglobulin therapy. *Nephrol Dial Transplant* 2002;**17**:1840–2.
286. Lies W, Morgan SK. Erythema infectiosum (fifth disease): report of an outbreak. *J Med Assoc State Ala* 1963;**32**:331–2.
287. Lindblom A, Isa A, Norbeck O, et al. Slow clearance of human parvovirus B19 viremia following acute infection. *Clin Infect Dis* 2005;**41**:1201–3.
288. Longo G, Luppi M, Bertesi M, et al. Still's disease, severe thrombocytopenia, and acute hepatitis associated with acute parvovirus B19 infection. *Clin Infect Dis* 1998;**26**:994–5.
289. Lotze U, Egerer R, Tresselt C, et al. Frequent detection of parvovirus B19 genome in the myocardium of adult patients with idiopathic dilated cardiomyopathy. *Med Microbiol Immunol* 2004;**193**:75–82.
290. Lou S, Xu B, Huang Q, et al. Molecular characterization of the newly identified human parvovirus 4 in the family *Parvoviridae*. *Virology* 2012;**422**:59–69.
291. Lowenthal EA, Wells A, Emanuel PD, et al. Sickle cell acute chest syndrome associated with parvovirus B19 infection: case series and review. *Am J Hematol* 1996;**51**:207–13.
292. Lowry SM, Brent LH, Menaldino S, et al. A case of persistent parvovirus B19 infection with bilateral cartilaginous and ligamentous damage to the wrists. *Clin Infect Dis* 2005;**41**:e42–4.
293. Lugassy G. Chronic pure red cell aplasia associated with parvovirus B19 infection in an immunocompetent patient. *Am J Hematol* 2002;**71**:238–9.
294. Lundqvist A, Isa A, Tolfvenstam T, et al. High frequency of parvovirus B19 DNA in bone marrow samples from rheumatic patients. *J Clin Virol* 2005;**33**:71–4.
295. Lyon CC. Severe acral pruritus associated with parvovirus B19 infection. *Br J Dermatol* 1998;**139**:153–4.
296. Macartney L, McCandlish IA, Thompson H, et al. Canine parvovirus enteritis 2: pathogenesis. *Vet Rec* 1984;**115**:453–60.
297. Malarme M, Vandervelde D, Brasseur M. Parvovirus infection, leukaemia, and immunodeficiency. *Lancet* 1989;**1**:1457.
298. Mallouh AA, Qudah A. Acute splenic sequestration together with aplastic crisis caused by human parvovirus B19 in patients with sickle cell disease. *J Pediatr* 1993;**122**:593–5.
299. Mallouh AA, Qudah A. An epidemic of aplastic crisis caused by human parvovirus B19. *Pediatr Infect Dis J* 1995;**14**:31–4.
300. Mandrioli J, Portolani M, Cortelli P, et al. Middle cerebral artery thrombosis in course of parvovirus B19 infection in a young adult: a new risk factor for stroke? *J Neurovirol* 2004;**10**:71–4.
301. Manning A, Willey SJ, Bell JE, et al. Comparison of tissue distribution, persistence, and molecular epidemiology of parvovirus B19 and novel human parvoviruses PARV4 and human bocavirus. *J Infect Dis* 2007;**195**:1345–52.
302. Martinon-Torres F, Seara MJ, Del Rio Pastoriza I, et al. Parvovirus B19 infection complicated by peripheral facial palsy and parotitis with intraparotid lymphadenitis. *Pediatr Infect Dis J* 1999; **18**:307–8.
303. Marton T, Martin WL, Whittle MJ. Hydrops fetalis and neonatal death from human parvovirus B19: an unusual complication. *Prenat Diagn* 2005;**25**:543–5.
304. Matano S, Kinoshita H, Tanigawa K, et al. Acute parvovirus B19 infection mimicking chronic fatigue syndrome. *Intern Med* 2003; **42**:903–5.
305. Matsuda H, Sakaguchi K, Shibasaki T, et al. Intrauterine therapy for parvovirus B19 infected symptomatic fetus using B19 IgG-rich high titer gammaglobulin. *J Perinat Med* 2005;**33**:561–3.
306. Matsumoto M, Sugitani M, Taguchi N, et al. Neonate with fetomaternal transfusion differentiated from parvo virus B19 infection. *Pediatr Int* 2004;**46**:356–9.
307. McClain K, Estrov Z, Chen H, et al. Chronic neutropenia of childhood: frequent association with parvovirus infection and correlations with bone marrow culture studies. *Br J Haematol* 1993; **85**:57–62.
308. McGhee SA, Kaska B, Liebhaber M, et al. Persistent parvovirus-associated chronic fatigue treated with high dose intravenous immunoglobulin. *Pediatr Infect Dis J* 2005;**24**:272–4.
309. McNamara PJ, Ramanan R. Survival of a preterm neonate with late onset hydrops fetalis due to parvovirus B19 infection. *Acta Paediatr* 1998;**87**:1088–9.
310. McNeely M, Friedman J, Pope E. Generalized petechial eruption induced by parvovirus B19 infection. *J Am Acad Dermatol* 2005;**52**:S109–13.
311. McOmish F, Yap PL, Jordan A, et al. Detection of parvovirus B19 in donated blood: a model system for screening by polymerase chain reaction. *J Clin Microbiol* 1993;**31**:323–8.
312. Megas H, Papadaki E, Constantinides B. *Salmonella* septicemia and aplastic crisis in a patient with sickle-cell anemia. *Acta Paediatr* 1961;**50**:517–21.
313. Mendonca MC, Ribeiro SB, Couceiro JN, et al. Parvovirus B19 infections in state of Rio de Janeiro, Brazil: 526 sera analyzed by IgM-enzyme-linked immunosorbent assay and polymerase chain reaction. *Mem Inst Oswaldo Cruz* 2005;**100**:847–52.
314. Messina MF, Ruggeri C, Rosano M, et al. Purpuric gloves and socks syndrome caused by parvovirus B19 infection. *Pediatr Infect Dis J* 2003;**22**:755–6.

315. Meyer O. Parvovirus B19 and autoimmune diseases. *Joint Bone Spine* 2003;**70**:6–11.
316. Miller E, Fairley CK, Cohen BJ, et al. Immediate and long term outcome of human parvovirus B19 infection in pregnancy. *Br J Obstet Gynaecol* 1998;**105**:174–8.
317. Mimori A, Misaki Y, Hachiya T, et al. Prevalence of antihuman parvovirus B19 IgG antibodies in patients with refractory rheumatoid arthritis and polyarticular juvenile rheumatoid arthritis. *Rheumatol Int* 1994;**14**:87–90.
318. Minohara Y, Koitabashi Y, Kato T, et al. A case of Guillain-Barré syndrome associated with human parvovirus B19 infection. *J Infect* 1998;**36**:327–8.
319. Mishra B, Malhotra P, Ratho RK, et al. Human parvovirus B19 in patients with aplastic anemia. *Am J Hematol* 2005;**79**:166–7.
320. Miyagawa S, Takahashi Y, Nagai A, et al. Angio-oedema in a neonate with IgG antibodies to parvovirus B19 following intrauterine parvovirus B19 infection. *Br J Dermatol* 2000;**143**: 428–30.
321. Moore TL. Parvovirus-associated arthritis. *Curr Opin Rheumatol* 2000;**12**:289–94.
322. Moore WF. Erythema infectiosum review, and report of two cases. *Hawaii Med J* 1956;**16**:35–6.
323. Moreux N, Ranchin B, Calvet A, et al. Chronic parvovirus B19 infection in a pediatric lung transplanted patient. *Transplantation* 2002;**73**:565–8.
324. Mori J, Field AM, Clewley JP, et al. Dot blot hybridization assay of B19 virus DNA in clinical specimens. *J Clin Microbiol* 1989; **27**:459–64.
325. Mori Y, Yamashita H, Umeda Y, et al. Association of parvovirus B19 infection with acute glomerulonephritis in healthy adults: case report and review of the literature. *Clin Nephrol* 2002;**57**: 69–73.
326. Morinet F, Monsuez JJ, Roger P, et al. Parvovirus B19 associated with pseudoappendicitis. *Lancet* 1987;**2**:1466.
327. Morris CN, Smilack JD. Parvovirus B19 infection associated with respiratory distress. *Clin Infect Dis* 1998;**27**:900–1.
328. Mortimer PP. Hypothesis: the aplastic crisis of hereditary spherocytosis is due to a single transmissible agent. *J Clin Pathol* 1983;**36**: 445–8.
329. Mortimer PP, Cohen BJ, Buckley MM, et al. Human parvovirus and the fetus. *Lancet* 1985;**2**:1012.
330. Mortimer PP, Luban NL, Kelleher JF, et al. Transmission of serum parvovirus-like virus by clotting-factor concentrates. *Lancet* 1983;**2**:482–4.
331. Mostello D, Holcomb WL Jr, Talsky JM, et al. Fetal parvovirus B19 infection: Doppler studies allow noninvasive treatment of ascites. *J Ultrasound Med* 2004;**23**:557–60.
332. Moudgil A, Shidban H, Nast CC, et al. Parvovirus B19 infection-related complications in renal transplant recipients: treatment with intravenous immunoglobulin. *Transplantation* 1997;**64**: 1847–50.
333. Mouthon L, Guillevin L, Tellier Z. Intravenous immunoglobulins in autoimmune- or parvovirus B19-mediated pure red-cell aplasia. *Autoimmun Rev* 2005;**4**:264–9.
334. Muir K, Todd WT, Watson WH, et al. Viral-associated haemophagocytosis with parvovirus-B19–related pancytopenia. *Lancet* 1992;**339**:1139–40.
335. Munakata Y, Kodera T, Saito T, et al. Rheumatoid arthritis, type 1 diabetes, and Graves' disease after acute parvovirus B19 infection. *Lancet* 2005;**366**:780.
336. Munakata Y, Saito T. Human parvovirus B19 viraemia is associated with neither IgM positivity nor anaemia in patients with rheumatic diseases. *Ann Rheum Dis* 2006;**65**:272–3.
337. Munro K, Croxson MC, Thomas S, et al. Three cases of myocarditis in childhood associated with human parvovirus (B19 virus). *Pediatr Cardiol* 2003;**24**:473–5.
338. Musiani M, Zerbini M, Gentilomi G, et al. Parvovirus B19 clearance from peripheral blood after acute infection. *J Infect Dis* 1995;**172**:1360–3.
339. Musiani M, Zerbini M, Gentilomi G, et al. Persistent B19 parvovirus infections in haemophilic HIV-1 infected patients. *J Med Virol* 1995;**46**:103–8.
340. Mustafa MM, McClain KL. Diverse hematologic effects of parvovirus B19 infection. *Pediatr Clin North Am* 1996;**43**:809–21.
341. Muzyczka N. *Parvoviridae:* the viruses and their replication. In: Knipe DM, editor. *Virology*. vol. 2. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2327–59.
342. Naides SJ. Erythema infectiosum (fifth disease) occurrence in Iowa. *Am J Public Health* 1988;**78**:1230–1.
343. Naides SJ, Field EH. Transient rheumatoid factor positivity in acute human parvovirus B19 infection. *Arch Intern Med* 1988; **148**:2587–9.
344. Naides SJ, Howard EJ, Swack NS, et al. Parvovirus B19 infection in human immunodeficiency virus type 1-infected persons failing or intolerant to zidovudine therapy. *J Infect Dis* 1993;**168**:101–5.
345. Naides SJ, Piette W, Veach LA, et al. Human parvovirus B19-induced vesiculopustular skin eruption. *Am J Med* 1988;**84**: 968–72.
346. Narvaez J, Nolla JM, Valverde J. No serological evidence that fibromyalgia is linked with exposure to human parvovirus B19. *Joint Bone Spine* 2005;**72**:592–4.
347. Nascimento JP, Hallam NF, Mori J, et al. Detection of B19 parvovirus in human fetal tissues by in situ hybridisation. *J Med Virol* 1991;**33**:77–82.
348. Nguyen QT, Sifer C, Schneider V, et al. Novel human erythrovirus associated with transient aplastic anemia. *J Clin Microbiol* 1999;**37**:2483–7.
349. Nigro G, Bastianon V, Colloridi V, et al. Human parvovirus B19 infection in infancy associated with acute and chronic lymphocytic myocarditis and high cytokine levels: report of 3 cases and review. *Clin Infect Dis* 2000;**31**:65–9.
350. Nigro G, Piazze J, Taliani G, et al. Postpartum lupus erythematosus associated with parvovirus B19 infection. *J Rheumatol* 1997;**24**:968–70.
351. Nigro G, Zerbini M, Krzysztofiak A, et al. Active or recent parvovirus B19 infection in children with Kawasaki disease. *Lancet* 1994;**343**:1260–1.
352. Nikkari S, Luukkainen R, Mottonen T, et al. Does parvovirus B19 have a role in rheumatoid arthritis? *Ann Rheum Dis* 1994;**53**: 106–11.
353. Nikkari S, Mertsola J, Korvenranta H, et al. Wegener's granulomatosis and parvovirus B19 infection. *Arthritis Rheum* 1994;**37**: 1707–8.
354. Nikkari S, Roivainen A, Hannonen P, et al. Persistence of parvovirus B19 in synovial fluid and bone marrow. *Ann Rheum Dis* 1995;**54**:597–600.
355. Nobili V, Vento S, Comparcola D, et al. Autoimmune hemolytic anemia and autoimmune hepatitis associated with parvovirus B19 infection. *Pediatr Infect Dis J* 2004;**23**:184–5.
356. Nocton JJ, Miller LC, Tucker LB, et al. Human parvovirus B19–associated arthritis in children. *J Pediatr* 1993;**122**:186–90.
357. Nolan RC, Chidlow G, French MA. Parvovirus B19 encephalitis presenting as immune restoration disease after highly active antiretroviral therapy for human immunodeficiency virus infection. *Clin Infect Dis* 2003;**36**:1191–4.
358. Norbeck O, Isa A, Pohlmann C, et al. Sustained $CD8^+$ T-cell responses induced after acute parvovirus B19 infection in humans. *J Virol* 2005;**79**:12117–21.
359. Norbeck O, Papadogiannakis N, Petersson K, et al. Revised clinical presentation of parvovirus B19–associated intrauterine fetal death. *Clin Infect Dis* 2002;**35**:1032–8.
360. Nour B, Green M, Michaels M, et al. Parvovirus B19 infection in pediatric transplant patients. *Transplantation* 1993;**56**:835–8.
361. Nunoue T, Koike T, Koike R, et al. Infection with human parvovirus (B19), aplasia of the bone marrow and a rash in hereditary spherocytosis. *J Infect* 1987;**14**:67–70.
362. Nunoue T, Kusuhara K, Hara T. Human fetal infection with parvovirus B19: maternal infection time in gestation, viral persistence and fetal prognosis. *Pediatr Infect Dis J* 2002;**21**:1133–6.
363. Nyman M, Skjoldebrand-Sparre L, Broliden K. Non-hydropic intrauterine fetal death more than 5 months after primary parvovirus B19 infection. *J Perinat Med* 2005;**33**:176–8.
364. Nyman M, Tolfvenstam T, Petersson K, et al. Detection of human parvovirus B19 infection in first-trimester fetal loss. *Obstet Gynecol* 2002;**99**:795–8.
365. Oguz F, Akdeniz C, Unuvar E, et al. Parvovirus B19 in the acute arthropathies and juvenile rheumatoid arthritis. *J Paediatr Child Health* 2002;**38**:358–62.

366. Oh SE, Kim JH, Choi CH, et al. An adult with aplastic crisis induced by human parvovirus B19 as an initial presentation of hereditary spherocytosis. *Korean J Intern Med* 2005;**20**:96–9.
367. Ohtomo Y, Kawamura R, Kaneko K, et al. Nephrotic syndrome associated with human parvovirus B19 infection. *Pediatr Nephrol* 2003;**18**:280–2.
368. Ohtsuka T, Yamazaki S. Increased prevalence of human parvovirus B19 DNA in systemic sclerosis skin. *Br J Dermatol* 2004; **150**:1091–5.
369. Ohtsuka T, Yamazaki S. Altered prevalence of human parvovirus B19 component genes in systemic sclerosis skin tissue. *Br J Dermatol* 2005;**152**:1078–80.
370. Ohtsuka T, Yamazaki S. Prevalence of human parvovirus B19 component *NS1* gene in patients with Henoch-Schönlein purpura and hypersensitivity vasculitis. *Br J Dermatol* 2005;**152**:1080–1.
371. Okumura A, Ichikawa T. Aseptic meningitis caused by human parvovirus B19. *Arch Dis Child* 1993;**68**:784–5.
372. Ongradi J, Becker K, Horvath A, et al. Simultaneous infection by human herpesvirus 7 and human parvovirus B19 in papular-purpuric gloves-and-socks syndrome. *Arch Dermatol* 2000;**136**:672.
373. Onitilo AA, Shaw GR. Parvovirus B19 infection in an isolated pancreas transplant recipient. *Transplant Proc* 2005;**37**:4433–5.
374. Ozawa K, Kurtzman G, Young N. Replication of the B19 parvovirus in human bone marrow cell cultures. *Science* 1986; **233**:883–6.
375. Pankuweit S, Moll R, Baandrup U, et al. Prevalence of the parvovirus B19 genome in endomyocardial biopsy specimens. *Hum Pathol* 2003;**34**:497–503.
376. Pankuweit S, Ruppert V, Eckhardt H, et al. Pathophysiology and aetiological diagnosis of inflammatory myocardial diseases with a special focus on parvovirus B19. *J Vet Med B Infect Dis Vet Public Health* 2005;**52**:344–7.
377. Papadogiannakis N, Tolfvenstam T, Fischler B, et al. Active, fulminant, lethal myocarditis associated with parvovirus B19 infection in an infant. *Clin Infect Dis* 2002;**35**:1027–31.
378. Pasvol G. Parvovirus infection, malaria, and anemia in the tropics—a new hidden enemy? *J Infect Dis* 2006;**194**:141–2.
379. Pattison JR. Parvoviruses: medical and biological aspects. In: Fields BN, editor. *Virology*. vol. 2. 2nd ed. New York: Raven Press; 1990. p. 1765–82.
380. Paver WK, Ashley CR, Caul EO, et al. A small virus in human faeces. *Lancet* 1973;**1**:237–40.
381. Paver WK, Clarke SK. Comparison of human fecal and serum parvo-like viruses. *J Clin Microbiol* 1976;**4**:67–70.
382. Pellas F, Olivares JP, Zandotti C, et al. Neuralgic amyotrophy after parvovirus B19 infection. *Lancet* 1993;**342**:503–4.
383. Peschgens T. Parvovirus B19–assoziierte Myokarditis bei einem 7-Monate-alten kind. *Padiatr Grenzgeb* 1994;**32**:527–30.
384. Peterlana D, Puccetti A, Beri R, et al. The presence of parvovirus B19 VP and NS1 genes in the synovium is not correlated with rheumatoid arthritis. *J Rheumatol* 2003;**30**:1907–10.
385. Petrikovsky BM, Baker D, Schneider E. Fetal hydrops secondary to human parvovirus infection in early pregnancy. *Prenat Diagn* 1996;**16**:342–4.
386. Phillips IE. Erythema infectiosum: outbreak in Bristol, Tennessee-Virginia area. *AMA Arch Derm Syphilol* 1953;**67**:628–9.
387. Phillips IE. Erythema infectiosum; clinical and epidemiological observations. *South Med J* 1954;**47**:253–6.
388. Pillay D, Patou G, Griffiths PD, et al. Secondary parvovirus B19 infection in an immunocompromised child. *Pediatr Infect Dis J* 1991;**10**:623–4.
389. Pillay D, Patou G, Hurt S, et al. Parvovirus B19 outbreak in a children's ward. *Lancet* 1992;**339**:107–9.
390. Plentz A, Hahn J, Holler E, et al. Long-term parvovirus B19 viraemia associated with pure red cell aplasia after allogeneic bone marrow transplantation. *J Clin Virol* 2004;**31**:16–19.
391. Plentz A, Hahn J, Knoll A, et al. Exposure of hematologic patients to parvovirus B19 as a contaminant of blood cell preparations and blood products. *Transfusion* 2005;**45**:1811–15.
392. Plummer FA, Hammond GW, Forward K, et al. An erythema infectiosum–like illness caused by human parvovirus infection. *N Engl J Med* 1985;**313**:74–9.
393. Pont J, Puchhammer-Stockl E, Chott A, et al. Recurrent granulocytic aplasia as clinical presentation of a persistent parvovirus B19 infection. *Br J Haematol* 1992;**80**:160–5.
394. Potter CG, Potter AC, Hatton CS, et al. Variation of erythroid and myeloid precursors in the marrow and peripheral blood of volunteer subjects infected with human parvovirus (B19). *J Clin Invest* 1987;**79**:1486–92.
395. Pouchot J, Ouakil H, Debin ML, et al. Adult Still's disease associated with acute human parvovirus B19 infection. *Lancet* 1993;**341**: 1280–1.
396. Prassouli A, Papadakis V, Tsakris A, et al. Classic transient erythroblastopenia of childhood with human parvovirus B19 genome detection in the blood and bone marrow. *J Pediatr Hematol Oncol* 2005;**27**:333–6.
397. Public Health Laboratory Service Working Party on Fifth Disease. Prospective study of human parvovirus (B19) infection in pregnancy. *BMJ* 1990;**300**:1166–70.
398. Qian XH, Zhang GC, Jiao XY, et al. Aplastic anaemia associated with parvovirus B19 infection. *Arch Dis Child* 2002;**87**:436–7.
399. Rao KR, Patel AR, Anderson MJ, et al. Infection with parvovirus-like virus and aplastic crisis in chronic hemolytic anemia. *Ann Intern Med* 1983;**98**:930–2.
400. Rao SP, Miller ST, Cohen BJ. Transient aplastic crisis in patients with sickle cell disease: B19 parvovirus studies during a 7-year period. *Am J Dis Child* 1992;**146**:1328–30.
401. Rector JM. Erythema infectiosum: clinical observations during an epidemic. *J Pediatr* 1939;**15**:540–5.
402. Reid DM, Reid TM, Brown T, et al. Human parvovirus–associated arthritis: a clinical and laboratory description. *Lancet* 1985;**1**: 422–5.
403. Reitblat T, Drogenikov T, Sigalov I, et al. Transient anticardiolipin antibody syndrome in a patient with parvovirus B19 infection. *Am J Med* 2000;**109**:512–13.
404. Rinckel LA, Buno BR, Gierman TM, et al. Discovery and analysis of a novel parvovirus B19 Genotype 3 isolate in the United States. *Transfusion* 2009;**49**:1488–92.
405. Rivier G, Gerster JC, Terrier P, et al. Parvovirus B19 associated monoarthritis in a 5-year-old boy. *J Rheumatol* 1995;**22**:766–7.
406. Rodis JF, Hovick TJ Jr, Quinn DL, et al. Human parvovirus infection in pregnancy. *Obstet Gynecol* 1988;**72**:733–8.
407. Rodis JF, Quinn DL, Gary GW Jr, et al. Management and outcomes of pregnancies complicated by human B19 parvovirus infection: a prospective study. *Am J Obstet Gynecol* 1990;**163**:1168–71.
408. Rogers BB, Singer DB, Mak SK, et al. Detection of human parvovirus B19 in early spontaneous abortuses using serology, histology, electron microscopy, in situ hybridization, and the polymerase chain reaction. *Obstet Gynecol* 1993;**81**:402–8.
409. Saarinen UM, Chorba TL, Tattersall P, et al. Human parvovirus B19–induced epidemic acute red cell aplasia in patients with hereditary hemolytic anemia. *Blood* 1986;**67**:1411–17.
410. Sahakian V, Weiner CP, Naides SJ, et al. Intrauterine transfusion treatment of nonimmune hydrops fetalis secondary to human parvovirus B19 infection. *Am J Obstet Gynecol* 1991;**164**:1090–1.
411. Saint-Martin J, Choulot JJ, Bonnaud E, et al. Myocarditis caused by parvovirus. *J Pediatr* 1990;**116**:1007–8.
412. Salimans MM, Holsappel S, van de Rijke FM, et al. Rapid detection of human parvovirus B19 DNA by dot-hybridization and the polymerase chain reaction. *J Virol Methods* 1989;**23**:19–28.
413. Saller DN Jr, Rogers BB, Canick JA. Maternal serum biochemical markers in pregnancies with fetal parvovirus B19 infection. *Prenat Diagn* 1993;**13**:467–71.
414. Samii K, Cassinotti P, de Freudenreich J, et al. Acute bilateral carpal tunnel syndrome associated with human parvovirus B19 infection. *Clin Infect Dis* 1996;**22**:162–4.
415. Santonja C, Nieto-Gonzalez G, Santos-Briz A, et al. Immunohistochemical detection of parvovirus B19 in "gloves and socks" papular purpuric syndrome: direct evidence for viral endothelial involvement. Report of three cases and review of the literature. *Am J Dermatopathol* 2011;**33**:790–5.
416. Sarfraz AA, Samuelsen SO, Bruu AL, et al. Maternal human parvovirus B19 infection and the risk of fetal death and low birthweight: a case-control study within 35,940 pregnant women. *BJOG* 2009;**116**:1492–8.
417. Satake M, Hoshi Y, Taira R, et al. Symptomatic parvovirus B19 infection caused by blood component transfusion. *Transfusion* 2011;**51**:1887–95.
418. Scarlata F, Gianelli E, Miceli S, et al. Acute parvovirus B19 infection and anemia during *Plasmodium falciparum* malaria. *Clin Infect Dis* 2002;**35**:1449–51.

419. Scheibe F, Hofmann J, Ruprecht K. Parainfectious myelitis associated with parvovirus B19 infection. *J Neurol* 2010;**257**:1557–8.
420. Schiff G. Challenge study with rubella virus isolated from a patient with erythema infectiosum. *Clin Res* 1971;**19**:675.
421. Schleuning M, Jager G, Holler E, et al. Human parvovirus B19–associated disease in bone marrow transplantation. *Infection* 1999;**27**:114–17.
422. Schwarz TF, Bruns R, Schroder C, et al. Human parvovirus B19 infection associated with vascular purpura and vasculitis. *Infection* 1989;**17**:170–1.
423. Schwarz TF, Jager G, Holzgreve W, et al. Diagnosis of human parvovirus B19 infections by polymerase chain reaction. *Scand J Infect Dis* 1992;**24**:691–6.
424. Schwarz TF, Nerlich A, Hottentrager B, et al. Parvovirus B19 infection of the fetus: histology and in situ hybridization. *Am J Clin Pathol* 1991;**96**:121–6.
425. Schwarz TF, Roggendorf M, Deinhardt F. Human parvovirus B19: ELISA and immunoblot assays. *J Virol Methods* 1988;**20**:155–68.
426. Schwarz TF, Wiersbitzky S, Pambor M. Case report: detection of parvovirus B19 in a skin biopsy of a patient with erythema infectiosum. *J Med Virol* 1994;**43**:171–4.
427. Semble EL, Agudelo CA, Pegram PS. Human parvovirus B19 arthropathy in two adults after contact with childhood erythema infectiosum. *Am J Med* 1987;**83**:560–2.
428. Seo JY, Kim HJ, Kim SH. Neutropenia in parvovirus B19-associated pure red cell aplasia. *Ann Hematol* 2011;**90**:975–8.
429. Serjeant GR, Serjeant BE, Thomas PW, et al. Human parvovirus infection in homozygous sickle cell disease. *Lancet* 1993;**341**: 1237–40.
430. Serjeant GR, Topley JM, Mason K, et al. Outbreak of aplastic crises in sickle cell anaemia associated with parvovirus-like agent. *Lancet* 1981;**2**:595–7.
431. Servant A, Laperche S, Lallemand F, et al. Genetic diversity within human erythroviruses: identification of three genotypes. *J Virol* 2002;**76**:9124–34.
432. Servant-Delmas A, Lefrere JJ, Morinet F, et al. Advances in human B19 erythrovirus biology. *J Virol* 2010;**84**:9658–65.
433. Severin MC, Levy Y, Shoenfeld Y. Systemic lupus erythematosus and parvovirus B-19: casual coincidence or causative culprit? *Clin Rev Allergy Immunol* 2003;**25**:41–8.
434. Seyama K, Kobayashi R, Hasle H, et al. Parvovirus B19–induced anemia as the presenting manifestation of X-linked hyper-IgM syndrome. *J Infect Dis* 1998;**178**:318–24.
435. Shackelton LA, Holmes EC. Phylogenetic evidence for the rapid evolution of human B19 erythrovirus. *J Virol* 2006;**80**:3666–9.
436. Sharma VR, Fleming DR, Slone SP. Pure red cell aplasia due to parvovirus B19 in a patient treated with rituximab. *Blood* 2000;**96**: 1184–6.
437. Shaw HLK. Erythema infectiosum. *Am J Med Sci* 1905;**129**: 16–22.
438. Shneerson JM, Mortimer PP, Vandervelde EM. Febrile illness due to a parvovirus. *BMJ* 1980;**280**:1580.
439. Silver MM, Hellmann J, Zielenska M, et al. Anemia, blueberry-muffin rash, and hepatomegaly in a newborn infant. *J Pediatr* 1996;**128**:579–86.
440. Sklavounou-Andrikopoulou A, Iakovou M, Paikos S, et al. Oral manifestations of papular-purpuric "gloves and socks" syndrome due to parvovirus B19 infection: the first case presented in Greece and review of the literature. *Oral Dis* 2004;**10**:118–22.
441. Smith EH. An epidemic of erythema infectiosum, "the fifth disease." *Arch Pediatr* 1929;**46**:456–8.
442. Smith PT, Landry ML, Carey H, et al. Papular-purpuric gloves and socks syndrome associated with acute parvovirus B19 infection: case report and review. *Clin Infect Dis* 1998;**27**:164–8.
443. Smith-Whitley K, Zhao H, Hodinka RL, et al. Epidemiology of human parvovirus B19 in children with sickle cell disease. *Blood* 2004;**103**:422–7.
444. Smoleniec JS, Pillai M, Caul EO, et al. Subclinical transplacental parvovirus B19 infection: an increased fetal risk? *Lancet* 1994;**343**:1100–1.
445. Soderlund M, Brown CS, Cohen BJ, et al. Accurate serodiagnosis of B19 parvovirus infections by measurement of IgG avidity. *J Infect Dis* 1995;**171**:710–13.
446. Soderlund M, von Essen R, Haapasaari J, et al. Persistence of parvovirus B19 DNA in synovial membranes of young patients with and without chronic arthropathy. *Lancet* 1997;**349**: 1063–5.
447. Sokal EM, Melchior M, Cornu C, et al. Acute parvovirus B19 infection associated with fulminant hepatitis of favourable prognosis in young children. *Lancet* 1998;**352**:1739–41.
448. Sood N, Yadav P. Hemophagocytic syndrome associated with concomitant *Klebsiella* and parvovirus B-19 infection. *Indian J Pathol Microbiol* 2012;**55**:124–5.
449. Soucie JM, Siwak EB, Hooper WC, et al. Human parvovirus B19 in young male patients with hemophilia A: associations with treatment product exposure and joint range-of-motion limitation. *Transfusion* 2004;**44**:1179–85.
450. Srivastava A, Bruno E, Briddell R, et al. Parvovirus B19–induced perturbation of human megakaryocytopoiesis in vitro. *Blood* 1990;**76**:1997–2004.
451. Srivastava A, Lu L. Replication of B19 parvovirus in highly enriched hematopoietic progenitor cells from normal human bone marrow. *J Virol* 1988;**62**:3059–63.
452. Stahl HD, Seidl B, Hubner B, et al. High incidence of parvovirus B19 DNA in synovial tissue of patients with undifferentiated mono- and oligoarthritis. *Clin Rheumatol* 2000;**19**:281–6.
453. Steinfort DP, Dixon B. Parvovirus encephalitis and pneumonia in an immunocompetent adult. *Intern Med J* 2006;**36**:209–10.
454. Stouffer GA, Sheahan RG, Lenihan DJ, et al. The current status of immune modulating therapy for myocarditis: a case of acute parvovirus myocarditis treated with intravenous immunoglobulin. *Am J Med Sci* 2003;**326**:369–74.
455. Stricker G. Die neue Kindersenche in der Umgebung von Giessen (erythema infectiosum). *Z Prakt Aerzte* 1899;**40**:121.
456. Subtirelu MM, Flynn JT, Schechner RS, et al. Acute renal failure in a pediatric kidney allograft recipient treated with intravenous immunoglobulin for parvovirus B19 induced pure red cell aplasia. *Pediatr Transplant* 2005;**9**:801–4.
457. Summers J, Jones SE, Anderson MJ. Characterization of the genome of the agent of erythrocyte aplasia permits its classification as a human parvovirus. *J Gen Virol* 1983;**64**(Pt 11):2527–32.
458. Suzuki N, Terada S, Inoue M. Neonatal meningitis with human parvovirus B19 infection. *Arch Dis Child Fetal Neonatal Ed* 1995;**73**:F196–7.
459. Suzuki T, Saito S, Hirabayashi Y, et al. Human parvovirus B19 infection during the inactive stage of systemic lupus erythematosus. *Intern Med* 2003;**42**:538–40.
460. Szelei J, Liu K, Li Y, et al. Parvovirus 4–like virus in blood products. *Emerg Infect Dis* 2010;**16**:561–4.
461. Tabak F, Mert A, Ozturk R, et al. Prolonged fever caused by parvovirus B19–induced meningitis: case report and review. *Clin Infect Dis* 1999;**29**:446–7.
462. Tabrizi SN, Chen S, Borg AJ, et al. Use of polymerase chain reaction for detection of human parvovirus B19. *J Infect Dis* 1994; **170**:1047–8.
463. Takahashi T, Ozawa K, Takahashi K, et al. Susceptibility of human erythropoietic cells to B19 parvovirus in vitro increases with differentiation. *Blood* 1990;**75**:603–10.
464. Takahashi Y, Murai C, Shibata S, et al. Human parvovirus B19 as a causative agent for rheumatoid arthritis. *Proc Natl Acad Sci U S A* 1998;**95**:8227–32.
465. Takeda S, Takaeda C, Takazakura E, et al. Renal involvement induced by human parvovirus B19 infection. *Nephron* 2001;**89**:280–5.
466. Taylor G, Drachenberg C, Faris-Young S. Renal involvement of human parvovirus B19 in an immunocompetent host. *Clin Infect Dis* 2001;**32**:167–9.
467. Tchernia G, Morinet F, Congard B, et al. Diamond Blackfan anaemia: apparent relapse due to B19 parvovirus. *Eur J Pediatr* 1993;**152**:209–10.
468. The International Committee on Taxonomy of Viruses. Virus taxonomy update. *Arch Virol* 1993;**133**:491–5.
469. Tiessen RG, van Elsacker-Niele AM, Vermeij-Keers C, et al. A fetus with a parvovirus B19 infection and congenital anomalies. *Prenat Diagn* 1994;**14**:173–6.
470. Toan NL, Duechting A, Kremsner PG, et al. Phylogenetic analysis of human parvovirus B19, indicating two subgroups of genotype 1 in Vietnamese patients. *J Gen Virol* 2006;**87**:2941–9.
471. Toyoshima MT, Keller LW, Barbosa ML, et al. Papular-purpuric "gloves and socks" syndrome caused by parvovirus B19 infection in Brazil: a case report. *Braz J Infect Dis* 2006;**10**:62–4.

472. Trotta M, Azzi A, Meli M, et al. Intrauterine parvovirus B19 infection: early prenatal diagnosis is possible. *Int J Infect Dis* 2004; **8**:130–1.
473. Tschamer A. Ueber ortliche Rotheln. *Jahrb Kinderheilkd* 1889;**29**:372–4.
474. Tschope C, Bock CT, Kasner M, et al. High prevalence of cardiac parvovirus B19 infection in patients with isolated left ventricular diastolic dysfunction. *Circulation* 2005;**111**:879–86.
475. Tsuda H, Maeda Y, Nakagawa K. Parvovirus B19–related lymphadenopathy. *Br J Haematol* 1993;**85**:631–2.
476. Tsuda H, Maeda Y, Nakagawa K, et al. Parvovirus B19–associated haemophagocytic syndrome with prominent neutrophilia. *Br J Haematol* 1994;**86**:413–14.
477. Turton J, Appleton H, Clewley JP. Similarities in nucleotide sequence between serum and faecal human parvovirus DNA. *Epidemiol Infect* 1990;**105**:197–201.
478. Tyndall A, Jelk W, Hirsch HH. Parvovirus B19 and erosive polyarthritis. *Lancet* 1994;**343**:480–1.
479. Ueno Y, Umadome H, Shimodera M, et al. Human parvovirus B19 and arthritis. *Lancet* 1993;**341**:1280.
480. Uike N, Miyamura T, Obama K, et al. Parvovirus B19–associated haemophagocytosis in Evans syndrome: aplastic crisis accompanied by severe thrombocytopenia. *Br J Haematol* 1993;**84**:530–2.
481. Vafaie J, Schwartz RA. Parvovirus B19 infections. *Int J Dermatol* 2004;**43**:747–9.
482. Vag T, Sonkoly E, Kemeny B, et al. Familiar occurrence of papular-purpuric 'gloves and socks' syndrome with human herpes virus-7 and human parvovirus B19 infection. *J Eur Acad Dermatol Venereol* 2004;**18**:639–41.
483. Valeur-Jensen AK, Pedersen CB, Westergaard T, et al. Risk factors for parvovirus B19 infection in pregnancy. *JAMA* 1999;**281**: 1099–105.
484. Van Elsacker-Niele AM, Salimans MM, Weiland HT, et al. Fetal pathology in human parvovirus B19 infection. *Br J Obstet Gynaecol* 1989;**96**:768–75.
485. Varache S, Narbonne V, Jousse-Joulin S, et al. Is routine viral screening useful in patients with recent-onset polyarthritis of a duration of at least 6 weeks? Results from a nationwide longitudinal prospective cohort study. *Arthritis Care Res (Hoboken)* 2011;**63**:1565–70.
486. Veraldi S, Mancuso R, Rizzitelli G, et al. Henoch-Schönlein syndrome associated with human parvovirus B19 primary infection. *Eur J Dermatol* 1999;**9**:232–3.
487. Viguier M, Guillevin L, Laroche L. Treatment of parvovirus B19–associated polyarteritis nodosa with intravenous immune globulin. *N Engl J Med* 2001;**344**:1481–2.
488. von Poblotzki A, Gerdes C, Reischl U, et al. Lymphoproliferative responses after infection with human parvovirus B19. *J Virol* 1996;**70**:7327–30.
489. von Poblotzki A, Hemauer A, Gigler A, et al. Antibodies to the nonstructural protein of parvovirus B19 in persistently infected patients: implications for pathogenesis. *J Infect Dis* 1995;**172**: 1356–9.
490. Wadlington WB. Erythema infectiosum: report of an epidemic. *J Tn State Med Assoc* 1957;**50**:1–5.
491. Wadlington WB. Erythema infectiosum. *JAMA* 1965;**192**: 58–60.
492. Wadlington WB. Erythema infectiosum (fifth disease). *Am J Dis Child* 1965;**110**:443–4.
493. Wadlington WB, Riley HD Jr. Arthritis and hemolytic anemia following erythema infectiosum. *JAMA* 1968;**203**:473–5.
494. Wang QY, Erdman DD. Development and evaluation of capture immunoglobulin G and M hemadherence assays by using human type O erythrocytes and recombinant parvovirus B19 antigen. *J Clin Microbiol* 1995;**33**:2466–7.
495. Wang X, Zhang G, Liu F, et al. Prevalence of human parvovirus B19 DNA in cardiac tissues of patients with congenital heart diseases indicated by nested PCR and in situ hybridization. *J Clin Virol* 2004;**31**:20–4.
496. Wardeh A, Marik P. Acute lung injury due to parvovirus pneumonia. *J Intern Med* 1998;**244**:257–60.
497. Watanabe M, Shimamoto Y, Yamaguchi M, et al. Viral-associated haemophagocytosis and elevated serum TNF-alpha with parvovirus-B19–related pancytopenia in patients with hereditary spherocytosis. *Clin Lab Haematol* 1994;**16**:179–82.
498. Watanabe T. Renal involvement in human parvovirus B19 infection. *Pediatr Nephrol* 2003;**18**:966–7.
499. Watanabe T. Ulcerative colitis that developed 8 years after human parvovirus B19 encephalopathy. *Eur J Pediatr* 2004;**163**:341–2.
500. Weiffenbach J, Bald R, Gloning KP, et al. Serological and virological analysis of maternal and fetal blood samples in prenatal human parvovirus B19 infection. *J Infect Dis* 2012;**205**:782–8.
501. Weigel-Kelley KA, Yoder MC, Srivastava A. Alpha5beta1 integrin as a cellular coreceptor for human parvovirus B19: requirement of functional activation of beta1 integrin for viral entry. *Blood* 2003;**102**:3927–33.
502. Weiland HT, Vermey-Keers C, Salimans MM, et al. Parvovirus B19 associated with fetal abnormality. *Lancet* 1987;**1**:682–3.
503. Weinstein M. Atypical presentation of parvovirus infection. *Pediatr Infect Dis J* 2005;**24**:283–4.
504. Werner GH, Brachman PS, Ketler A, et al. A new viral agent associated with erythema infectiosum. *Ann N Y Acad Sci* 1957;**67**:338–45.
505. White DG, Woolf AD, Mortimer PP, et al. Human parvovirus arthropathy. *Lancet* 1985;**1**:419–21.
506. Wierenga KJ, Pattison JR, Brink N, et al. Glomerulonephritis after human parvovirus infection in homozygous sickle-cell disease. *Lancet* 1995;**346**:475–6.
507. Wilcox KR, Evans AS. Erythema infectiosum; report of an outbreak in Marshfield, Wisconsin. *Wis Med J* 1958;**57**:107–14.
508. Wildig J, Michon P, Siba P, et al. Parvovirus B19 infection contributes to severe anemia in young children in Papua New Guinea. *J Infect Dis* 2006;**194**:146–53.
509. Willekes C, Roumen FJ, van Elsacker-Niele AM, et al. Human parvovirus B19 infection and unbalanced translocation in a case of hydrops fetalis. *Prenat Diagn* 1994;**14**:181–5.
510. Woernle CH, Anderson LJ, Tattersall P, et al. Human parvovirus B19 infection during pregnancy. *J Infect Dis* 1987;**156**:17–20.
511. Wong S, Brown KE. Development of an improved method of detection of infectious parvovirus B19. *J Clin Virol* 2006;**35**: 407–13.
512. Wong S, Young NS, Brown KE. Prevalence of parvovirus B19 in liver tissue: no association with fulminant hepatitis or hepatitis-associated aplastic anemia. *J Infect Dis* 2003;**187**:1581–6.
513. Wu CG, Mason B, Jong J, et al. Parvovirus B19 transmission by a high-purity factor VIII concentrate. *Transfusion* 2005;**45**:1003–10.
514. Wurster T, Polzelbauer C, Schonberger T, et al. Green fluorescent protein (GFP) color reporter gene visualizes parvovirus B19 non-structural segment 1 (NS1) transfected endothelial modification. *PLoS One* 2012;7:e33602.
515. Yaegashi N, Okamura K, Tsunoda A, et al. A study by means of a new assay of the relationship between an outbreak of erythema infectiosum and non-immune hydrops fetalis caused by human parvovirus B19. *J Infect* 1995;**31**:195–200.
516. Yamada Y, Iwasa A, Kuroki M, et al. Human parvovirus B19 infection showing follicular purpuric papules with a baboon syndrome-like distribution. *Br J Dermatol* 2004;**150**:788–9.
517. Yango A Jr, Morrissey P, Gohh R, et al. Donor-transmitted parvovirus infection in a kidney transplant recipient presenting as pancytopenia and allograft dysfunction. *Transpl Infect Dis* 2002;**4**: 163–6.
518. Yazawa S, Kawasaki S, Fujimoto C, et al. Case report of meningoencephalitis during a concomitant mumps and parvovirus B19 infection. *Clin Neurol Neurosurg* 2002;**104**:380–2.
519. Yee TT, Cohen BJ, Pasi KJ, et al. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. *Br J Haematol* 1996;**93**:457–9.
520. Yeh SP, Chiu CF, Lee CC, et al. Evidence of parvovirus B19 infection in patients of pre-eclampsia and eclampsia with dyserythropoietic anaemia. *Br J Haematol* 2004;**126**:428–33.
521. Yermalovich MA, Hubschen JM, Semeiko GV, et al. Human parvovirus B19 surveillance in patients with rash and fever from Belarus. *J Med Virol* 2012;**84**:973–8.
522. Yetgin S, Cetin M, Aslan D, et al. Parvovirus B19 infection presenting as pre-B-cell acute lymphoblastic leukemia: a transient and progressive course in two children. *J Pediatr Hematol Oncol* 2004;**26**:689–92.
523. Yetgin S, Cetin M, Ozyurek E, et al. Parvovirus B19 infection associated with severe aplastic anemia in an immunocompetent patient. *Pediatr Hematol Oncol* 2004;**21**:223–6.

524. Yoto Y, Kudoh T, Haseyama K, et al. Human parvovirus B19 infection associated with acute hepatitis. *Lancet* 1996;**347**:868–9.
525. Yoto Y, Kudoh T, Suzuki N, et al. Thrombocytopenia induced by human parvovirus B19 infections. *Eur J Haematol* 1993;**50**: 255–7.
526. Yoto Y, Kudoh T, Suzuki N, et al. Retrospective study on the influence of human parvovirus B19 infection among children with malignant diseases. *Acta Haematol* 1993;**90**:8–12.
527. Young N. Hematologic and hematopoietic consequences of B19 parvovirus infection. *Semin Hematol* 1988;**25**:159–72.
528. Young NS, Brown KE. Parvovirus B19. *N Engl J Med* 2004;**350**: 586–97.
529. Young NS, Mortimer PP, Moore JG, et al. Characterization of a virus that causes transient aplastic crisis. *J Clin Invest* 1984;**73**: 224–30.
530. Yufu Y, Matsumoto M, Miyamura T, et al. Parvovirus B19-associated haemophagocytic syndrome with lymphadenopathy resembling histiocytic necrotizing lymphadenitis (Kikuchi's disease). *Br J Haematol* 1997;**96**:868–71.
531. Zaaijer HL, Koppelman MH, Farrington CP. Parvovirus B19 viraemia in Dutch blood donors. *Epidemiol Infect* 2004;**132**: 1161–6.
532. Zack F, Klingel K, Kandolf R, et al. Sudden cardiac death in a 5-year-old girl associated with parvovirus B19 infection. *Forensic Sci Int* 2005;**155**:13–17.
533. Zahorsky J. An epidemic of erythema infectiosum. *Am J Dis Child* 1924;**28**:261–2.
534. Zerbini M, Gibellini D, Musiani M, et al. Automated detection of digoxigenin-labelled B19 parvovirus amplicons by a capture hybridization assay. *J Virol Methods* 1995;**55**:1–9.
535. Zerbini M, Musiani M, Gentilomi G, et al. Symptomatic parvovirus B19 infection of one fetus in a twin pregnancy. *Clin Infect Dis* 1993;**17**:262–3.
536. Zimmerman SA, Davis JS, Schultz WH, et al. Subclinical parvovirus B19 infection in children with sickle cell anemia. *J Pediatr Hematol Oncol* 2003;**25**:387–9.
537. Zuckerman SN. Erythema infectiosum, with report of an epidemic in San Francisco. *Arch Pediatr* 1940;**57**:168–76.

CHAPTER 152

Human Bocaviruses

Jennifer E. Schuster • John V. Williams

Human bocavirus (HBoV) was discovered in 2005 by Allander and colleagues.[4] Many studies have shown that HBoV infection is ubiquitous worldwide and that nearly all adults are seropositive. HBoV has been detected in respiratory tract specimens from persons with acute respiratory tract illness and in stool specimens from patients with gastroenteritis. However, controlled studies have not shown a consistent association between HBoV and acute disease, and prolonged shedding of HBoV further clouds the issue of causality. Much remains to be elucidated about the epidemiology and biology of this virus.

HISTORY

Allander and coworkers[2] developed an elegant molecular screening approach for virus discovery. The method consisted of pooling clinical specimens, concentrating virus particles by ultracentrifugation, depleting host DNA by filtration and DNase treatment, amplifying the DNA by random polymerase chain reaction (PCR), sequencing the PCR products, and searching published databases for matches to the sequences. The group tested two pools of nasopharyngeal aspirates collected from patients with acute respiratory tract illness during the winter and early spring of 2003 to 2004. Novel parvovirus-like sequences were identified that most closely aligned with *bo*vine parvovirus (BPV) and minute virus of *ca*nines (MVC); thus, the new virus was provisionally designated HBoV. Full-length genomes of two strains were described in this report.

PROPERTIES

HBoV is a member of the Parvoviridae family, subfamily Parvovirinae, and genus *Bocavirus*. Like other parvoviruses, HBoV is a small, nonenveloped virus with a single-stranded, linear DNA genome of approximately 5 kb. The capsid size is approximately 22 to 28 nm.[46] The genomic organization of HBoV closely resembles that of the other known bocaviruses, BPV and MVC. Like all members of the Parvovirinae subfamily, it has two major open reading frames (ORFs) encoding a nonstructural protein (NS1) and two capsid proteins (VP1 and VP2), respectively. However, like MVC and BPV, HBoV also has a third ORF. In MVC and BPV, this ORF encodes a nonstructural protein with unknown function, NP1.[22,96] HBoV has an approximately 42 percent amino acid identity of both major ORFs to MVC and BPV and thus represents a distinct virus. Other HBoV genotypes have been identified and tentatively designated species HBoV2, HBoV3, and HBoV4.[8,59,60] Nucleotide identity between these different HBoVs ranges from 73 to 88 percent.[25,59] Evidence exists of some antigenic cross reactivity between these viruses[56] and the biologic significance of the different types is not clear.

HBoV has been cultivated by one group.[34] Three HBoV PCR-positive specimens from patients were inoculated into well-differentiated primary airway epithelial cell cultures. No cytopathic effect was observed, but the investigators measured increasing amounts of HBoV-specific DNA and RNA in one of the specimens, suggesting active viral replication. HBoV exhibits a complex transcriptional strategy, with multiple monocistronic, polycistronic, and variably spliced mRNAs.[21,34] The overall transcription profile of HBoV is similar to that of BPV and MVC.[87,101] The cellular site of HBoV replication is not known, although viral proteins NS1 and NP1 localize to the nucleus.[21] NP1 has been shown to interfere with innate immune responses by blocking interferon (IFN)-β production via IFN regulatory factor 3 signaling.[109] No animal model of infection has been identified.

EPIDEMIOLOGY

HBoV infections have been identified in humans worldwide. After the discovery of the virus in pooled respiratory tract specimens, some groups tested convenience sample sets collected from patients with acute respiratory tract illness. HBoV was detected by PCR in these specimens at rates between 5 and 20 percent.* The virus is detected most frequently in children younger than 2 years of age. Australian investigators detected HBoV in 18/324 (6%) specimens collected during autumn and winter in a single season from children with acute respiratory tract infection.[98] HBoV was present in 18/318 (6%) Japanese children with lower respiratory tract infection.[75] Investigators in Connecticut tested 425 hospital laboratory specimens collected from children younger than 2 years and detected HBoV in 22 (5%), all between October and April.[62]

The majority of published studies include only patients with symptomatic respiratory tract illness, although those including asymptomatic controls offer conflicting findings. Several studies reported lower rates of HBoV detection in controls in contrast to cases with respiratory tract illness.[3,43,62,76] However, some of the controls were not well age or season matched or they comprised different

*References 5, 9, 40, 41, 62, 68, 75-77, 99.

specimen types. Large prospective studies in Canada, Denmark, and the Netherlands with age- and season-matched controls have found similar rates of HBoV in asymptomatic subjects and children with ARI.[73,103,106] A prospective study in Thailand reported fourfold higher HBoV detection in all respiratory tract illness cases in contrast to controls; however, when considering only HBoV-positive patients without co-detection of other viruses and controlling for age and month, HBoV was not detected significantly more often in either hospitalized pneumonia cases or outpatients with influenza-like illness than in controls.[41] A prospective 1-year cohort study of 119 daycare attendees detected HBoV in 20/45 (44%) asymptomatic subjects at enrollment.[79] In a prospective pneumococcal vaccine trial cohort of 221 children in Papua New Guinea, HBoV was detected in 53/273 (19%) asymptomatic children and 18/80 (22%) children with acute respiratory tract illness.[24]

HBoV is detected year-round, although it is more common during the winter months.* It should be noted that this period is when most respiratory tract specimens are collected, because of the seasonality of acute respiratory tract illnesses. Isolated reports of HBoV infections in immunocompromised hosts exist.[33,54,67,93,94] HBoV was uncommon in adults with exacerbations of chronic obstructive pulmonary disease.[91]

Seroprevalence studies using diverse methods have reported seroprevalence of 74 to 99 percent among adults.[36,52,55,56,72] A prospective serosurvey of 109 Finnish children found that all seroconverted by 6 years of age.[80] One factor that complicates the interpretation of prior seroepidemiology is the recent discovery of serologic cross reactivity among HBoV1, 2, 3, and 4.[56] This study used HBoV virus–like particles to deplete cross-reactive antibodies, and the investigators found that the seroprevalence of HBoV2-4 in adults was 34, 15, and 2 percent and in children aged 1 to 2 years 25, 10, and 5 percent, respectively. The HBoV1 seroprevalence among adults decreased from 96 to 59 percent after depletion of HBoV2-4–reactive antibodies. A similar Chinese study reported that the seroprevalence of HBoV1-4 in adults was 67, 49, 39 and 1 percent, respectively, and in children, the seroprevalence of HBoV1-4 was 50, 37, 29, and 1 percent, respectively.[45] Thus, serologic data suggest that HBoV1 is the most frequent bocavirus type infecting humans.

PATHOGENESIS

The method of transmission of HBoV is not known. However, because the virus is present in the nasopharynx in high concentrations, a reasonable conclusion is that its spread is similar to that of other respiratory viruses.[51] This spread could involve contact transmission with droplet nuclei or by fomites. Parvovirus B19 can be transmitted via respiratory tract secretions.[4,32,44] A few nosocomial infections have been reported.[16,27,62] Parvoviruses are resistant to disinfectants, which could contribute to environmental persistence.[38]

A unique feature of HBoV respiratory tract infections is the high frequency of mixed infections with other established pathogenic respiratory tract viruses, leading to concern that bocavirus is a passenger agent and not the cause of the respiratory tract illness.[95] Studies have identified high rates of 20 to 90 percent coinfection with HBoV and other viruses, including influenza, human metapneumovirus (MPV), parainfluenza viruses (PIV), and respiratory syncytial virus (RSV).* Manning and colleagues[77] detected HBoV in 47/574 (8.2%) specimens from subjects with acute respiratory tract illness, 43 percent of which were coinfected with another virus. Coinfection with another virus, including influenza, PIV, or RSV, was detected in 90 percent of outpatients with influenza-like illness in Thailand.[41] A prospective study of daycare attendees found another established respiratory tract virus in 72 percent of HBoV-positive acute respiratory tract illness episodes during the study.[79] This high rate of co-detection of other viruses with HBoV is similar to other reports of 60 to 90 percent co-detection.† The presence of established respiratory tract pathogens makes it difficult to assign a causal role to HBoV in these reports.

Several studies have shown an association between HBoV single infection and respiratory tract symptoms, and some suggest that a higher HBoV viral load in the nasopharynx correlates with symptomatic disease.‡

Kantola and associates[55] demonstrated the occurrence of immunoglobulin (Ig)M antibody responses and titer increases of IgG antibody in children with respiratory tract illnesses. Concurrent detection of HBoV in nasopharyngeal samples and blood has also been suggested to correlate with acute respiratory tract illness.[28,100] However, HBoV also has been detected in the serum of healthy blood donors.[12]

Another unusual feature of HBoV is prolonged shedding and intermittent detection of HBoV over weeks to months.[11,15,71,106] Martin and colleagues[79] documented prolonged shedding (up to 75 days) in 20 subjects, with intermittent detection several months apart in 18 other children. Three groups detected HBoV in tonsil and adenoid tissues from surgical specimens, suggesting long-term persistence[31,74,86]; interestingly, the rates of detection were higher in adenoids (24% to 56%) than tonsils (5% to 16%). One study detected HBoV in 18/102 (18%) sinus mucosal biopsy specimens from patients with histories of chronic sinusitis but without acute illnesses; 17 of these had HBoV-negative nasopharyngeal swabs and blood.[39] Prolonged detection of HBoV in stool has been reported,[61,93] and HBoV can persist as episomal DNA within gastrointestinal tissue.[58]

HBoV is rarely detected in other tissues. HBoV DNA has been detected in heart tissue from patients undergoing cardiac surgery without evidence of virus-associated cardiomyopathy.[66] HBoV has not been associated with systemic illness or anemia. HBoV has not been detected in amniotic fluid or fetal tissues,[35,90] in contrast to BPV, MVC, and B19, which can cause intrauterine infections.[48,78]

*References 3, 6, 9, 26, 41-43, 62.

*References 3, 41, 64, 76, 77, 89.
†References 3, 13, 17, 30, 41, 50, 73, 84, 103.
‡References 15, 28, 43, 53, 62, 71, 85.

CLINICAL MANIFESTATIONS

Respiratory Illness

HBoV has been detected in nasopharyngeal specimens from children with both upper and lower respiratory tract illnesses. Most studies focus on hospitalized cohorts with predominantly lower tract disease. Many investigations have compared the signs and symptoms associated with HBoV infection with those caused by other respiratory viruses.* These comparative studies show that the clinical findings associated with HBoV infections are similar to those of viral infections caused by RSV, MPV, adenoviruses, influenza viruses, PIV, and rhinoviruses. Laboratory findings associated with HBoV infection are nonspecific. White blood cell counts ranged from 3000 to 31,000 cells/mm^3 in several studies, with means of 11 to 14,000, and C-reactive protein levels ranged from 0.2 to 11.4 mg/dL with median values of 0.4 to 1.2 mg/dL.[6,75,105]

Upper Respiratory Tract Infections

In one study, 86 percent of 49 HBoV infections without coinfection with other agents were classified as upper respiratory tract illnesses.[37] In contrast, only 42 percent of 50 children in this study who had HBoV and a coinfecting virus were diagnosed with an upper respiratory tract illness. Pharyngitis was observed in 55 percent of the cases in which HBoV was the single agent identified. Of the children with HBoV infections without coinfections, 18 percent had acute otitis media and 12 percent had sinusitis. In another study, otitis media was reported in 61 percent of all HBoV infections.[73]

In a large study, Arnold and associates[6] noted the following findings at the time of presentation: fever, 68 percent; rhinorrhea, 67 percent; cough, 85 percent; conjunctivitis, 7 percent; vomiting, 30 percent; diarrhea, 21 percent; and rash, 7 percent. Of interest was that the cough was paroxysmal in 19 percent of the HBoV-infected children, whereas paroxysmal cough was much less common in adenovirus infections (7%) and MPV infections (5%).

In a Korean study, 3/36 (8.3%) children infected with HBoV had croup.[26] In a study in Canada, 2/58 (3%) HBoV-infected children had croup,[10] and in a study in Japan, 1/18 (6%) had laryngotracheitis.[75]

Lower Respiratory Tract Infections

Longtin and colleagues[73] noted that 42 percent of children with HBoV infections had pneumonia and 42 percent had bronchiolitis. In a large study in San Diego, California involving 82 children with HBoV infections, 76 children with adenoviral infections, and 87 children with MPV infections, the investigators were able to compare the rates of various clinical findings.[7] In this study, the following rates of events were noted in HBoV-infected children: hospitalization, 69 percent; need for oxygen administration, 44 percent; admission to intensive care unit, 11 percent; intubation, 4 percent; clinical lower respiratory tract disease, 61 percent; hypoxia, 41 percent; increased work of breathing, 59 percent; abnormal lung findings, 51 percent; "atelectasis versus infiltrate," 11 percent; "infiltrate" or "pneumonia," 9 percent; and bronchiolitis, 46 percent. Comparatively, adenovirus-infected children had less lower respiratory tract disease, less hypoxia, and less increased work of breathing, and they were more likely to have normal chest radiographic findings than were children with HBoV and MPV infections.

In a study in Korea, 53 percent of HBoV-infected children had rales noted on chest auscultation and 42 percent had wheezing.[26] The clinical diagnoses in this study were bronchiolitis (25%), pneumonia (56%), exacerbation of asthma (11%), and croup (8%). In Germany, 32 children with lower respiratory tract disease were studied.[107] The diagnoses were bronchitis (16%), wheezing bronchitis (14%), bronchiolitis (3%), and pneumonia (18%). Of the 11 patients in this study, 10 with pneumonia had coinfections with other respiratory tract viruses. In a study in Canada involving 58 bocavirus infections, 40 percent had bronchiolitis and 22 percent had pneumonia.[10] In a study of 18 children in Japan, the lower respiratory tract diagnoses were wheezy bronchitis (33%), pneumonia (33%), bronchiolitis (11%), bronchitis (11%), and asthmatic attack (6%).[75]

Gastrointestinal Illness

Gastroenteritis has been associated with HBoV infections, and HBoV has been detected in stool[68,69,102,104,108] by PCR, but the biologic significance of this is not clear. Similar to the data on acute respiratory tract illness, established gastrointestinal pathogens are co-detected frequently with HBoV, and HBoV is detected in stool specimens from asymptomatic subjects. In one study in Hong Kong, diarrhea was noted in 11 percent of 79 patients with respiratory tract symptoms.[68] In the same study, HBoV was identified in fecal samples of 25 children with gastroenteritis. Of these children, 16 percent had blood in the stool, 8 percent had mucus in the stool, 32 percent had vomiting, and 68 percent had fever. The following respiratory tract findings were noted in these children with gastroenteritis: coryza (56%), acute bronchitis (16%), and pneumonia (12%). Co-pathogens were identified in 56 percent of the children: rotavirus (36%), *Salmonella* spp. (8%), *Campylobacter* spp. (4%), *Staphylococcus aureus* (4%), and *Clostridium difficile* (4%).

In a study of 962 stool samples from children with acute gastroenteritis in Seoul, Korea, a viral agent was found in 44.4 percent of the specimens.[69] The viruses detected included rotavirus (25.7%), norovirus (13.7%), adenovirus (3.0%), astrovirus (1.1%), and HBoV (0.8%). In another study in Brazil, 2 percent of 705 diarrhea stool samples were PCR positive for HBoV.[1] In studies of respiratory tract illness associated with HBoV infection, diarrhea occurs in 9 to 38 percent.[6,7,37,62,88]

Several studies suggest that the newer bocaviruses HBoV2, 3, and 4, which were discovered in stool specimens, may be more likely to be associated with diarrhea than HBoV1.*[2] HBoV2 is the most frequent type

*References 3, 5, 6, 9, 26, 40, 41, 43, 62, 75-77, 99, 106.

*References 8, 9, 18, 47, 49, 60, 63, 83, 92.

detected in stool from gastroenteritis, at 3 to 5 percent, but in some studies, it is detected at similar rates in asymptomatic controls.[23,60,83,92,108]

Infections in Immunocompromised Patients

Koskenvuo and associates[65] described three children with acute lymphoblastic leukemia who had acute febrile episodes in whom HBoV was detected in respiratory tract secretions. In addition to fever, one of these children had cough, rhinitis, and otitis media, and another child had vomiting and diarrhea. The third child had five consecutive febrile episodes during a 6-month period, and with each of these episodes, HBoV was found in nasal swab samples. Another report described prolonged detection of HBoV in children with various immunodeficiencies.[93] Other cases of HBoV infection in immunosuppressed children and adults have been reported, none fatal.*

Other Clinical Findings

In addition to respiratory and gastrointestinal tracts signs and symptoms, numerous other clinical findings and diagnoses have been observed in children with HBoV infections. Arnold and colleagues[6] noted four children with maculopapular erythematous rashes and exanthems have been noted in other studies.[29,68,82] In three studies, the rate of exanthema varied between 5 and 9 percent in the children studied.

Three studies detected HBoV in respiratory or fecal samples from children clinically thought to have Kawasaki disease.[7,20,68] However, a case-control study of 12 children with Kawasaki disease and 33 age- and gender-matched controls found equal serum levels of HBoV-specific IgA and IgG in both groups, although none in either group were HBoV IgM-positive.[70] None of the Kawasaki disease cases and only one control had HBoV DNA in the serum. Another study performed HBoV PCR on endothelial cells from 22 cases of Kawasaki disease, including 18 with coronary artery lesions; none were positive for HBoV.[14]

Other events noted in temporal association with HBoV infection include intussusception,[19,68] aseptic meningitis,[68] and nephritic syndrome.[68] One case of HBoV DNA detection in the cerebrospinal fluid of four patients with encephalitis has been reported.[81]

DIAGNOSIS

Differential Diagnosis

The findings in HBoV respiratory infections are similar to those in RSV and MPV infections, as well as adenovirus, influenza virus, PIV, and rhinovirus infections. Similarly, gastroenteritis associated with HBoV infection cannot be differentiated from rotavirus infections and illness associated with other viral and bacterial agents.

*References 7, 33, 54, 67, 76, 77, 94, 99.

Specific Diagnosis

Presently, the only diagnostic method for the demonstration of HBoV infection is PCR. Specimens for PCR may be obtained from respiratory tract secretions, stool, and blood. Primer sets target the NP1 region 4 and the NS1 region of the genome.[2,98] A real-time PCR assay targeting the *NS1* gene is capable of detecting all four known HBoV types.[57] Some commercially available multiplex molecular assays include HBoV.

Some authors argue that serodiagnosis or the measurement of HBoV viral load provides a more accurate diagnosis of acute infection versus asymptomatic shedding.[28,55,80,97,100] No commercial reagents for serodiagnosis of HBoV are available at present.

TREATMENT

No specific antiviral treatment for HBoV infections is known. General care should be similar to that employed for other respiratory or gastrointestinal tract viral infections.

PROGNOSIS

Although the full spectrum of HBoV infections and possible sequelae are not known presently, most infections appear to be self-limited.

NEW REFERENCES SINCE THE SIXTH EDITION

8. Arthur JL, Higgins GD, Davidson GP, et al. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathog* 2009;**5**:e1000391.
11. Blessing K, Neske F, Herre U, et al. Prolonged detection of human bocavirus DNA in nasopharyngeal aspirates of children with respiratory tract disease. *Pediatr Infect Dis J* 2009;**28**:1018–19.
12. Bonvicini F, Manaresi E, Gentilomi GA, et al. Evidence of human bocavirus viremia in healthy blood donors. *Diagn Microbiol Infect Dis* 2011;**71**:460–2.
13. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
14. Bowles NE, Hirono K, Yu X, et al. Absence of parvoviral genomes in endothelial cells of Kawasaki disease patients with coronary artery lesions. *Pediatr Infect Dis J* 2009;**28**:345.
15. Brieu N, Guyon G, Rodiere M, et al. Human bocavirus infection in children with respiratory tract disease. *Pediatr Infect Dis J* 2008;**27**:969–73.
16. Calvo C, Garcia-Garcia ML, Blanco C, et al. Human bocavirus infection in a neonatal intensive care unit. *J Infect* 2008;**57**:269–71.
17. Calvo C, Garcia-Garcia ML, Pozo F, et al. Clinical characteristics of human bocavirus infections compared with other respiratory viruses in Spanish children. *Pediatr Infect Dis J* 2008;**27**:677–80.
18. Cashman O, O'Shea H. Detection of human bocaviruses 1, 2 and 3 in Irish children presenting with gastroenteritis. *Arch Virol* 2012;**157**:1767–73.
21. Chen AY, Cheng F, Lou S, et al. Characterization of the gene expression profile of human bocavirus. *Virology* 2010;**403**:145–54.
23. Cheng WX, Jin Y, Duan ZJ, et al. Human bocavirus in children hospitalized for acute gastroenteritis: a case-control study. *Clin Infect Dis* 2008;**47**:161–7.
24. Chidlow GR, Laing IA, Harnett GB, et al. Respiratory viral pathogens associated with lower respiratory tract disease among young children in the highlands of Papua New Guinea. *J Clin Virol* 2012;**54**:235–9.

25. Chieochansin T, Simmonds P, Poovorawan Y. Determination and analysis of complete coding sequence regions of new discovered human bocavirus types 2 and 3. *Arch Virol* 2010;**155**:2023–8.
27. Chow BD, Huang YT, Esper FP. Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio. *J Clin Virol* 2008;**43**:302–6.
28. Christensen A, Nordbo SA, Krokstad S, et al. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *J Clin Virol* 2010;**49**: 158–62.
30. Cilla G, Onate E, Perez-Yarza EG, et al. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. *J Med Virol* 2008;**80**:1843–9.
31. Clement N, Battaglioli G, Jensen RL, et al. Prevalence of human bocavirus in human tonsils and adenoids. *Emerg Infect Dis* 2009;**15**:1149–50.
32. Costa C, Terlizzi ME, Solidoro P, et al. Detection of parvovirus B19 in the lower respiratory tract. *J Clin Virol* 2009;**46**:150–3.
33. de Vries JJ, Bredius RG, van Rheenen PF, et al. Human bocavirus in an immunocompromised child presenting with severe diarrhea. *J Clin Microbiol* 2009;**47**:1241–3.
34. Dijkman R, Koekkoek SM, Molenkamp R, et al. Human bocavirus can be cultured in differentiated human airway epithelial cells. *J Virol* 2009;**83**:7739–48.
35. Enders M, Lindner J, Wenzel JJ, et al. No detection of human bocavirus in amniotic fluid samples from fetuses with hydrops or isolated effusions. *J Clin Virol* 2009;**45**:300–3.
38. Eterpi M, McDonnell G, Thomas V. Disinfection efficacy against parvoviruses compared with reference viruses. *J Hosp Infect* 2009;**73**:64–70.
39. Falcone V, Ridder GJ, Panning M, et al. Human bocavirus DNA in paranasal sinus mucosa. *Emerg Infect Dis* 2011;**17**:1564–5.
42. Garcia ML, Calvo C, Pozo F, et al. Detection of human bocavirus in ill and healthy Spanish children: a 2-year study. *Arch Dis Child* 2009;**94**:249.
43. Garcia-Garcia ML, Calvo C, Pozo F, et al. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. *Pediatr Infect Dis J* 2008;**27**: 358–60.
45. Guo L, Wang Y, Zhou H, et al. Differential seroprevalence of human bocavirus species 1-4 in Beijing, China. *PLoS One* 2012;**7**:e39644.
46. Gurda BL, Parent KN, Bladek H, et al. Human bocavirus capsid structure: insights into the structural repertoire of the Parvoviridae. *J Virol* 2010;**84**:5880–9.
47. Han TH, Kim CH, Park SH, et al. Detection of human bocavirus-2 in children with acute gastroenteritis in South Korea. *Arch Virol* 2009;**154**:1923–7.
49. Huang Y, Mao P, Wang H. Detection of, and frequent co-infection with, human bocavirus in faecal specimens from children in Wuhan, China. *Clin Microbiol Infect* 2009;**16**:490–2.
50. Jacques J, Moret H, Renois F, et al. Human bocavirus quantitative DNA detection in French children hospitalized for acute bronchiolitis. *J Clin Virol* 2008;**43**:142–7.
51. Jartti T, Hedman K, Jartti L, et al. Human bocavirus-the first 5 years. *Rev Med Virol* 2012;**22**:46–64.
52. Kahn JS, Kesebir D, Cotmore SF, et al. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. *J Infect Dis* 2008;**198**:41–50.
53. Kaida A, Kubo H, Takakura K, et al. Detection and quantitative analysis of human bocavirus associated with respiratory tract infection in Osaka City, Japan. *Microbiol Immunol* 2010;**54**: 276–81.
54. Kainulainen L, Waris M, Soderlund-Venermo M, et al. Hepatitis and human bocavirus primary infection in a child with T-cell deficiency. *J Clin Microbiol* 2008;**46**:4104–5.
56. Kantola K, Hedman L, Arthur J, et al. Seroepidemiology of human bocaviruses 1-4. *J Infect Dis* 2011;**204**:1403–12.
57. Kantola K, Sadeghi M, Antikainen J, et al. Real-time quantitative PCR detection of four human bocaviruses. *J Clin Microbiol* 2010;**48**:4044–50.
58. Kapoor A, Hornig M, Asokan A, et al. Bocavirus episome in infected human tissue contains non-identical termini. *PLoS One* 2011;**6**:e21362.
59. Kapoor A, Simmonds P, Slikas E, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J Infect Dis* 2010;**201**:1633–43.
60. Kapoor A, Slikas E, Simmonds P, et al. A newly identified bocavirus species in human stool. *J Infect Dis* 2009;**199**:196–200.
61. Kapusinszky B, Minor P, Delwart E. Nearly constant shedding of diverse enteric viruses by two healthy infants. *J Clin Microbiol* 2012;**50**:3427–34.
63. Khamrin P, Thongprachum A, Shimizu H, et al. Detection of human bocavirus 1 and 2 from children with acute gastroenteritis in Japan. *J Med Virol* 2012;**84**:901–5.
65. Koskenvuo M, Mottonen M, Waris M, et al. Human bocavirus in children with acute lymphoblastic leukemia. *Eur J Pediatr* 2008;**167**:1011–15.
66. Kuethe F, Lindner J, Matschke K, et al. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. *Clin Infect Dis* 2009;**49**:1660–6.
70. Lehmann C, Klar R, Lindner J, et al. Kawasaki disease lacks association with human coronavirus NL63 and human bocavirus. *Pediatr Infect Dis J* 2009;**28**:553–4.
71. Lehtoranta L, Soderlund-Venermo M, Nokso-Koivisto J, et al. Human bocavirus in the nasopharynx of otitis-prone children. *Int J Pediatr Otorhinolaryngol* 2012;**76**:206–11.
72. Lin F, Guan W, Cheng F, et al. ELISAs using human bocavirus VP2 virus-like particles for detection of antibodies against HBoV. *J Virol Methods* 2008;**149**:110–17.
73. Longtin J, Bastien M, Gilca R, et al. Human bocavirus infections in hospitalized children and adults. *Emerg Infect Dis* 2008;**14**: 217–21.
74. Lu X, Gooding LR, Erdman DD. Human bocavirus in tonsillar lymphocytes. *Emerg Infect Dis* 2008;**14**:1332–4.
78. Manteufel J, Truyen U. Animal bocaviruses: a brief review. *Intervirology* 2008;**51**:328–34.
79. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis* 2010;**201**:1625–32.
80. Meriluoto M, Hedman L, Tanner L, et al. Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. *Emerg Infect Dis* 2012;**18**:264–71.
81. Mitui MT, Tabib SM, Matsumoto T, et al. Detection of human bocavirus in the cerebrospinal fluid of children with encephalitis. *Clin Infect Dis* 2012;**54**:964–7.
83. Nawaz S, Allen DJ, Aladin F, et al. Human bocaviruses are not significantly associated with gastroenteritis: results of retesting archive DNA from a case control study in the UK. *PLoS One* 2012;**7**:e41346.
84. Pierangeli A, Scagnolari C, Trombetti S, et al. Human bocavirus infection in hospitalized children in Italy. *Influenza Other Respir Viruses* 2008;**2**:175–9.
85. Proenca-Modena JL, Gagliardi TB, Escremim de Paula F, et al. Detection of human bocavirus mRNA in respiratory secretions correlates with high viral load and concurrent diarrhea. *PLoS One* 2011;**6**:e21083.
86. Proenca-Modena JL, Pereira Valera FC, Jacob MG, et al. High rates of detection of respiratory viruses in tonsillar tissues from children with chronic adenotonsillar disease. *PLoS One* 2012;**7**: e42136.
90. Riipinen A, Vaisanen E, Lahtinen A, et al. Absence of human bocavirus from deceased fetuses and their mothers. *J Clin Virol* 2010;**47**:186–8.
91. Ringshausen FC, Tan AY, Allander T, et al. Frequency and clinical relevance of human bocavirus infection in acute exacerbations of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2009;**4**:111–17.
92. Risku M, Katka M, Lappalainen S, et al. Human bocavirus types 1, 2 and 3 in acute gastroenteritis of childhood. *Acta Paediatr* 2012;**101**:e405–10.
93. Schenk T, Maier B, Hufnagel M, et al. Persistence of human bocavirus DNA in immunocompromised children. *Pediatr Infect Dis J* 2011;**30**:82–4.
100. Soderlund-Venermo M, Lahtinen A, Jartti T, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis* 2009;**15**:1423–30.

101. Sun Y, Chen AY, Cheng F, et al. Molecular characterization of infectious clones of the minute virus of canines reveals unique features of bocaviruses. *J Virol* 2009;**83**:3956–67.
102. Szomor KN, Kapusinszky B, Rigo Z, et al. Detection of human bocavirus from fecal samples of Hungarian children with acute gastroenteritis. *Intervirology* 2009;**52**:17–21.
103. van de Pol AC, Wolfs TF, Jansen NJ, et al. Human bocavirus and KI/WU polyomaviruses in pediatric intensive care patients. *Emerg Infect Dis* 2009;**15**:454–7.
106. von Linstow ML, Hogh M, Hogh B. Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study. *Pediatr Infect Dis J* 2008;**27**: 897–902.
108. Yu JM, Li DD, Xu ZQ, et al. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *J Clin Virol* 2008;**42**:280–5.
109. Zhang Z, Zheng Z, Luo H, et al. Human bocavirus NP1 inhibits IFN-beta production by blocking association of IFN regulatory factor 3 with IFNB promoter. *J Immunol* 2012;**189**:1144–53.

The full reference list for this chapter is available at expertconsult.com.

DNA VIRUSES

SUBSECTION 2 *POLYOMAVIRIDAE*

CHAPTER 153

HUMAN POLYOMAVIRUSES

John A. Vanchiere

The human polyomaviruses, JC virus (JCV), and BK virus (BKV), are believed to have existed in the human population for thousands of years, but their ability to cause disease in modern humans is largely limited to opportunistic diseases in severely immunocompromised patients. The increasing use of immunosuppressive therapies for the treatment of oncologic, neurologic, autoimmune, and rheumatologic disorders has brought the family of *Polyomaviridae* to the foreground of biomedical science in the past decade. With the increasing use of deep-sequencing methods, human polyomaviruses with unique tissue tropisms are being discovered at a rapid pace, highlighting the need to understand the natural history and disease potential of these diverse agents. Although the polyomaviruses derive their name from *poly*, meaning "many," and *oma*, which refers to their ability to induce tumors in laboratory animals, the story of their emergence as opportunistic causes of both malignant and nonmalignant diseases is continuing to unfold. Their colorful history and critical role in the elucidation of fundamental cellular and molecular pathways has immortalized them as research tools.

HISTORY

The recognition of progressive multifocal leukoencephalopathy (PML) as a clinical entity in 1958 marks the beginning of our knowledge of the human polyomaviruses.[13] Edward P. Richardson, Jr. and colleagues described three cases of progressive neurologic disease in patients receiving chemotherapy for leukemia. At autopsy, they found many foci of demyelination, including oligodendrocytes with intranuclear inclusions and astrocytes that were enlarged with bizarre nuclear changes.[13] Richardson subsequently reported a larger series of PML cases and proposed a viral etiology associated with immune suppression.[138] In 1965, papovavirus-like particles were observed by electron microscopy of brain tissue from patients with PML, and in 1971, JCV, the etiologic agent of PML, was isolated from primary human fetal glial cell cultures that had been inoculated with a brain extract from a patient with PML.[126,190] In the same year, another human polyomavirus, BKV, was cultivated from the urine of a renal transplant patient.[56] Each virus was named with the initials of the patient from whom it was isolated.

The respiratory polyomaviruses, KI polyomavirus (KIPyV) and WU polyomavirus (WUPyV), were discovered in 2007 by interrogation of respiratory secretions using deep sequencing methods.[5,57,70,123] The visualization of the trichodysplasia spinulosa polyomavirus (TSPyV) by electron microscopy marked the beginning of the characterization of cutaneous polyomaviruses in the human population, an area of intense study because of the more recent discovery of the Merkel cell polyomavirus (MCPyV) and several additional cutaneous polyomaviruses in the human population.[48,144,156,173]

SV40, the prototype polyomavirus, was isolated in 1960 by Sweet and Hilleman as a contaminant of secondary rhesus monkey kidney cell cultures that were used to produce early polio vaccines.[163] Additionally, several early adenovirus vaccines and a respiratory syncytial virus

REFERENCES

1. Albuquerque MC, Rocha LN, Benati FJ, et al. Human bocavirus infection in children with gastroenteritis, Brazil. *Emerg Infect Dis* 2007;**13**:1756–8.
2. Allander T, Jartti T, Gupta S, et al. Human bocavirus and acute wheezing in children. *Clin Infect Dis* 2007;**44**:904–10.
3. Allander T, Tammi MT, Eriksson M, et al. Cloning of a human parvovirus by molecular screening of respiratory tract samples. *Proc Natl Acad Sci U S A* 2005;**102**:12891–6.
4. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. *J Infect Dis* 1985;**152**:257–65.
5. Arden KE, McErlean P, Nissen MD, et al. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol* 2006;**78**:1232–40.
6. Arnold JC, Singh KK, Spector SA, et al. Human bocavirus: prevalence and clinical spectrum at a children's hospital. *Clin Infect Dis* 2006;**43**:283–8.
7. Arnold JC, Singh KK, Spector SA, et al. Undiagnosed respiratory viruses in children. *Pediatrics* 2008;**121**:e631–7.
8. Arthur JL, Higgins GD, Davidson GP, et al. A novel bocavirus associated with acute gastroenteritis in Australian children. *PLoS Pathog* 2009;**5**:e1000391.
9. Bastien N, Brandt K, Dust K, et al. Human bocavirus infection, Canada. *Emerg Infect Dis* 2006;**12**:848–50.
10. Bastien N, Chui N, Robinson JL, et al. Detection of human bocavirus in Canadian children in a 1-year study. *J Clin Microbiol* 2007;**45**:610–13.
11. Blessing K, Neske F, Herre U, et al. Prolonged detection of human bocavirus DNA in nasopharyngeal aspirates of children with respiratory tract disease. *Pediatr Infect Dis J* 2009;**28**: 1018–19.
12. Bonvicini F, Manaresi E, Gentilomi GA, et al. Evidence of human bocavirus viremia in healthy blood donors. *Diagn Microbiol Infect Dis* 2011;**71**:460–2.
13. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
14. Bowles NE, Hirono K, Yu X, et al. Absence of parvoviral genomes in endothelial cells of Kawasaki disease patients with coronary artery lesions. *Pediatr Infect Dis J* 2009;**28**:345.
15. Brieu N, Guyon G, Rodiere M, et al. Human bocavirus infection in children with respiratory tract disease. *Pediatr Infect Dis J* 2008;**27**:969–73.
16. Calvo C, Garcia-Garcia ML, Blanco C, et al. Human bocavirus infection in a neonatal intensive care unit. *J Infect* 2008; **57**:269–71.
17. Calvo C, Garcia-Garcia ML, Pozo F, et al. Clinical characteristics of human bocavirus infections compared with other respiratory viruses in Spanish children. *Pediatr Infect Dis J* 2008;**27**:677–80.
18. Cashman O, O'Shea H. Detection of human bocaviruses 1, 2 and 3 in Irish children presenting with gastroenteritis. *Arch Virol* 2012;**157**:1767–73.
19. Catalano-Pons C, Bue M, Laude H, et al. Human bocavirus infection in hospitalized children during winter. *Pediatr Infect Dis J* 2007;**26**:959–60.
20. Catalano-Pons C, Giraud C, Rozenberg F, et al. Detection of human bocavirus in children with Kawasaki disease. *Clin Microbiol Infect* 2007;**13**:1220–2.
21. Chen AY, Cheng F, Lou S, et al. Characterization of the gene expression profile of human bocavirus. *Virology* 2010;**403**:145–54.
22. Chen KC, Shull BC, Moses EA, et al. Complete nucleotide sequence and genome organization of bovine parvovirus. *J Virol* 1986;**60**:1085–97.
23. Cheng WX, Jin Y, Duan ZJ, et al. Human bocavirus in children hospitalized for acute gastroenteritis: a case-control study. *Clin Infect Dis* 2008;**47**:161–7.
24. Chidlow GR, Laing IA, Harnett GB, et al. Respiratory viral pathogens associated with lower respiratory tract disease among young children in the highlands of Papua New Guinea. *J Clin Virol* 2012;**54**:235–9.
25. Chieochansin T, Simmonds P, Poovorawan Y. Determination and analysis of complete coding sequence regions of new discovered human bocavirus types 2 and 3. *Arch Virol* 2010;**155**:2023–8.
26. Choi EH, Lee HJ, Kim SJ, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. *Clin Infect Dis* 2006;**43**:585–92.
27. Chow BD, Huang YT, Esper FP. Evidence of human bocavirus circulating in children and adults, Cleveland, Ohio. *J Clin Virol* 2008;**43**:302–6.
28. Christensen A, Nordbo SA, Krokstad S, et al. Human bocavirus in children: mono-detection, high viral load and viraemia are associated with respiratory tract infection. *J Clin Virol* 2010;**49**: 158–62.
29. Chung JY, Han TH, Kim CK, et al. Bocavirus infection in hospitalized children, South Korea. *Emerg Infect Dis* 2006;**12**:1254–6.
30. Cilla G, Onate E, Perez-Yarza EG, et al. Viruses in community-acquired pneumonia in children aged less than 3 years old: High rate of viral coinfection. *J Med Virol* 2008;**80**:1843–9.
31. Clement N, Battaglioli G, Jensen RL, et al. Prevalence of human bocavirus in human tonsils and adenoids. *Emerg Infect Dis* 2009;**15**:1149–50.
32. Costa C, Terlizzi ME, Solidoro P, et al. Detection of parvovirus B19 in the lower respiratory tract. *J Clin Virol* 2009;**46**:150–3.
33. de Vries JJ, Bredius RG, van Rheenen PF, et al. Human bocavirus in an immunocompromised child presenting with severe diarrhea. *J Clin Microbiol* 2009;**47**:1241–3.
34. Dijkman R, Koekkoek SM, Molenkamp R, et al. Human bocavirus can be cultured in differentiated human airway epithelial cells. *J Virol* 2009;**83**:7739–48.
35. Enders M, Lindner J, Wenzel JJ, et al. No detection of human bocavirus in amniotic fluid samples from fetuses with hydrops or isolated effusions. *J Clin Virol* 2009;**45**:300–3.
36. Endo R, Ishiguro N, Kikuta H, et al. Seroepidemiology of human bocavirus in Hokkaido prefecture, Japan. *J Clin Microbiol* 2007;**45**:3218–23.
37. Esposito S, Bosis S, Niesters HG, et al. Impact of human bocavirus on children and their families. *J Clin Microbiol* 2008;**46**:1337–42.
38. Eterpi M, McDonnell G, Thomas V. Disinfection efficacy against parvoviruses compared with reference viruses. *J Hosp Infect* 2009;**73**:64–70.
39. Falcone V, Ridder GJ, Panning M, et al. Human bocavirus DNA in paranasal sinus mucosa. *Emerg Infect Dis* 2011;**17**:1564–5.
40. Foulongne V, Rodiere M, Segondy M. Human bocavirus in children. *Emerg Infect Dis* 2006;**12**:862–3.
41. Fry AM, Lu X, Chittaganpitch M, et al. Human bocavirus: a novel parvovirus epidemiologically associated with pneumonia requiring hospitalization in Thailand. *J Infect Dis* 2007;**195**:1038–45.
42. Garcia ML, Calvo C, Pozo F, et al. Detection of human bocavirus in ill and healthy Spanish children: a 2-year study. *Arch Dis Child* 2009;**94**:249.
43. Garcia-Garcia ML, Calvo C, Pozo F, et al. Human bocavirus detection in nasopharyngeal aspirates of children without clinical symptoms of respiratory infection. *Pediatr Infect Dis J* 2008;**27**:358–60.
44. Grilli EA, Anderson MJ, Hoskins TW. Concurrent outbreaks of influenza and parvovirus B19 in a boys' boarding school. *Epidemiol Infect* 1989;**103**:359–69.
45. Guo L, Wang Y, Zhou H, et al. Differential seroprevalence of human bocavirus species 1-4 in Beijing, China. *PLoS One* 2012;**7**:e39644.
46. Gurda BL, Parent KN, Bladek H, et al. Human bocavirus capsid structure: insights into the structural repertoire of the Parvoviridae. *J Virol* 2010;**84**:5880–9.
47. Han TH, Kim CH, Park SH, et al. Detection of human bocavirus-2 in children with acute gastroenteritis in South Korea. *Arch Virol* 2009;**154**:1923–7.
48. Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev* 2002;**15**:485–505.
49. Huang Y, Mao P, Wang H. Detection of, and frequent co-infection with, human bocavirus in faecal specimens from children in Wuhan, China. *Clin Microbiol Infect* 2010;**16**:490–2.
50. Jacques J, Moret H, Renois F, et al. Human bocavirus quantitative DNA detection in French children hospitalized for acute bronchiolitis. *J Clin Virol* 2008;**43**:142–7.
51. Jartti T, Hedman K, Jartti L, et al. Human bocavirus: the first 5 years. *Rev Med Virol* 2012;**22**:46–64.
52. Kahn JS, Kesebir D, Cotmore SF, et al. Seroepidemiology of human bocavirus defined using recombinant virus-like particles. *J Infect Dis* 2008;**198**:41–50.

53. Kaida A, Kubo H, Takakura K, et al. Detection and quantitative analysis of human bocavirus associated with respiratory tract infection in Osaka City, Japan. *Microbiol Immunol* 2010;**54**:276–81.
54. Kainulainen L, Waris M, Soderlund-Venermo M, et al. Hepatitis and human bocavirus primary infection in a child with T-cell deficiency. *J Clin Microbiol* 2008;**46**:4104–5.
55. Kantola K, Hedman L, Allander T, et al. Serodiagnosis of human bocavirus infection. *Clin Infect Dis* 2008;**46**:540–6.
56. Kantola K, Hedman L, Arthur J, et al. Seroepidemiology of human bocaviruses 1-4. *J Infect Dis* 2011;**204**:1403–12.
57. Kantola K, Sadeghi M, Antikainen J, et al. Real-time quantitative PCR detection of four human bocaviruses. *J Clin Microbiol* 2010;**48**:4044–50.
58. Kapoor A, Hornig M, Asokan A, et al. Bocavirus episome in infected human tissue contains non-identical termini. *PLoS One* 2011;**6**:e21362.
59. Kapoor A, Simmonds P, Slikas E, et al. Human bocaviruses are highly diverse, dispersed, recombination prone, and prevalent in enteric infections. *J Infect Dis* 2010;**201**:1633–43.
60. Kapoor A, Slikas E, Simmonds P, et al. A newly identified bocavirus species in human stool. *J Infect Dis* 2009;**199**:196–200.
61. Kapusinszky B, Minor P, Delwart E. Nearly constant shedding of diverse enteric viruses by two healthy infants. *J Clin Microbiol* 2012;**50**:3427–34.
62. Kesebir D, Vazquez M, Weibel C, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis* 2006;**194**:1276–82.
63. Khamrin P, Thongprachum A, Shimizu H, et al. Detection of human bocavirus 1 and 2 from children with acute gastroenteritis in Japan. *J Med Virol* 2012;**84**:901–5.
64. Kleines M, Scheithauer S, Rackowitz A, et al. High prevalence of human bocavirus detected in young children with severe acute lower respiratory tract disease by use of a standard PCR protocol and a novel real-time PCR protocol. *J Clin Microbiol* 2007;**45**: 1032–4.
65. Koskenvuo M, Mottonen M, Waris M, et al. Human bocavirus in children with acute lymphoblastic leukemia. *Eur J Pediatr* 2008;**167**:1011–15.
66. Kuethe F, Lindner J, Matschke K, et al. Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. *Clin Infect Dis* 2009;**49**:1660–6.
67. Kupfer B, Vehreschild J, Cornely O, et al. Severe pneumonia and human bocavirus in adult. *Emerg Infect Dis* 2006;**12**:1614–16.
68. Lau SK, Yip CC, Que TL, et al. Clinical and molecular epidemiology of human bocavirus in respiratory and fecal samples from children in Hong Kong. *J Infect Dis* 2007;**196**:986–93.
69. Lee JI, Chung JY, Han TH, et al. Detection of human bocavirus in children hospitalized because of acute gastroenteritis. *J Infect Dis* 2007;**196**:994–7.
70. Lehmann C, Klar R, Lindner J, et al. Kawasaki disease lacks association with human coronavirus NL63 and human bocavirus. *Pediatr Infect Dis J* 2009;**28**:553–4.
71. Lehtoranta L, Soderlund-Venermo M, Nokso-Koivisto J, et al. Human bocavirus in the nasopharynx of otitis-prone children. *Int J Pediatr Otorhinolaryngol* 2012;**76**:206–11.
72. Lin F, Guan W, Cheng F, et al. ELISAs using human bocavirus VP2 virus-like particles for detection of antibodies against HBoV. *J Virol Methods* 2008;**149**:110–17.
73. Longtin J, Bastien M, Gilca R, et al. Human bocavirus infections in hospitalized children and adults. *Emerg Infect Dis* 2008;**14**: 217–21.
74. Lu X, Gooding LR, Erdman DD. Human bocavirus in tonsillar lymphocytes. *Emerg Infect Dis* 2008;**14**:1332–4.
75. Ma X, Endo R, Ishiguro N, et al. Detection of human bocavirus in Japanese children with lower respiratory tract infections. *J Clin Microbiol* 2006;**44**:1132–4.
76. Maggi F, Andreoli E, Pifferi M, et al. Human bocavirus in Italian patients with respiratory diseases. *J Clin Virol* 2007;**38**:321–5.
77. Manning A, Russell V, Eastick K, et al. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *J Infect Dis* 2006;**194**:1283–90.
78. Manteufel J, Truyen U. Animal bocaviruses: a brief review. *Intervirology* 2008;**51**:328–34.
79. Martin ET, Fairchok MP, Kuypers J, et al. Frequent and prolonged shedding of bocavirus in young children attending daycare. *J Infect Dis* 2010;**201**:1625–32.
80. Meriluoto M, Hedman L, Tanner L, et al. Association of human bocavirus 1 infection with respiratory disease in childhood follow-up study, Finland. *Emerg Infect Dis* 2012;**18**:264–71.
81. Mitui MT, Tabib SM, Matsumoto T, et al. Detection of human bocavirus in the cerebrospinal fluid of children with encephalitis. *Clin Infect Dis* 2012;**54**:964–7.
82. Naghipour M, Cuevas LE, Bakhshinejad T, et al. Human bocavirus in Iranian children with acute respiratory infections. *J Med Virol* 2007;**79**:539–43.
83. Nawaz S, Allen DJ, Aladin F, et al. Human bocaviruses are not significantly associated with gastroenteritis: results of retesting archive DNA from a case control study in the UK. *PLoS One* 2012;**7**:e41346.
84. Pierangeli A, Scagnolari C, Trombetti S, et al. Human bocavirus infection in hospitalized children in Italy. *Influenza Other Respir Viruses* 2008;**2**:175–9.
85. Proenca-Modena JL, Gagliardi TB, Escremim de Paula F, et al. Detection of human bocavirus mRNA in respiratory secretions correlates with high viral load and concurrent diarrhea. *PLoS One* 2011;**6**:e21083.
86. Proenca-Modena JL, Pereira Valera FC, Jacob MG, et al. High rates of detection of respiratory viruses in tonsillar tissues from children with chronic adenotonsillar disease. *PLoS One* 2012;**7**: e42136.
87. Qiu J, Cheng F, Johnson FB, et al. The transcription profile of the bocavirus bovine parvovirus is unlike those of previously characterized parvoviruses. *J Virol* 2007;**81**:12080–5.
88. Redshaw N, Wood C, Rich F, et al. Human bocavirus in infants, New Zealand. *Emerg Infect Dis* 2007;**13**:1797–9.
89. Regamey N, Frey U, Deffernez C, et al. Isolation of human bocavirus from Swiss infants with respiratory infections. *Pediatr Infect Dis J* 2007;**26**:177–9.
90. Riipinen A, Vaisanen E, Lahtinen A, et al. Absence of human bocavirus from deceased fetuses and their mothers. *J Clin Virol* 2010;**47**:186–8.
91. Ringshausen FC, Tan AY, Allander T, et al. Frequency and clinical relevance of human bocavirus infection in acute exacerbations of chronic obstructive pulmonary disease. *Int J Chron Obstruct Pulmon Dis* 2009;**4**:111–17.
92. Risku M, Katka M, Lappalainen S, et al. Human bocavirus types 1, 2 and 3 in acute gastroenteritis of childhood. *Acta Paediatr* 2012;**101**:e405–10.
93. Schenk T, Maier B, Hufnagel M, et al. Persistence of human bocavirus DNA in immunocompromised children. *Pediatr Infect Dis J* 2011;**30**:82–4.
94. Schenk T, Strahm B, Kontny U, et al. Disseminated bocavirus infection after stem cell transplant. *Emerg Infect Dis* 2007;**13**: 1425–7.
95. Schildgen O, Muller A, Allander T, et al. Human bocavirus: passenger or pathogen in acute respiratory tract infections? *Clin Microbiol Rev* 2008;**21**:291–304, table of contents.
96. Schwartz D, Green B, Carmichael LE, et al. The canine minute virus (minute virus of canines) is a distinct parvovirus that is most similar to bovine parvovirus. *Virology* 2002;**302**:219–23.
97. Simmonds P. Steps towards serological diagnosis of human bocavirus infections. *Clin Infect Dis* 2008;**46**:547–9.
98. Sloots TP, McErlean P, Speicher DJ, et al. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol* 2006;**35**:99–102.
99. Smuts H, Hardie D. Human bocavirus in hospitalized children, South Africa. *Emerg Infect Dis* 2006;**12**:1457–8.
100. Soderlund-Venermo M, Lahtinen A, Jartti T, et al. Clinical assessment and improved diagnosis of bocavirus-induced wheezing in children, Finland. *Emerg Infect Dis* 2009;**15**:1423–30.
101. Sun Y, Chen AY, Cheng F, et al. Molecular characterization of infectious clones of the minute virus of canines reveals unique features of bocaviruses. *J Virol* 2009;**83**:3956–67.
102. Szomor KN, Kapusinszky B, Rigo Z, et al. Detection of human bocavirus from fecal samples of Hungarian children with acute gastroenteritis. *Intervirology* 2009;**52**:17–21.
103. van de Pol AC, Wolfs TF, Jansen NJ, et al. Human bocavirus and KI/WU polyomaviruses in pediatric intensive care patients. *Emerg Infect Dis* 2009;**15**:454–7.

104. Vicente D, Cilla G, Montes M, et al. Human bocavirus, a respiratory and enteric virus. *Emerg Infect Dis* 2007;**13**:636–7.
105. Volz S, Schildgen O, Klinkenberg D, et al. Prospective study of human bocavirus (HBoV) infection in a pediatric university hospital in Germany 2005/2006. *J Clin Virol* 2007;**40**:229–35.
106. von Linstow ML, Hogh M, Hogh B. Clinical and epidemiologic characteristics of human bocavirus in Danish infants: results from a prospective birth cohort study. *Pediatr Infect Dis J* 2008;**27**: 897–902.
107. Weissbrich B, Neske F, Schubert J, et al. Frequent detection of bocavirus DNA in German children with respiratory tract infections. *BMC Infect Dis* 2006;**6**:109.
108. Yu JM, Li DD, Xu ZQ, et al. Human bocavirus infection in children hospitalized with acute gastroenteritis in China. *J Clin Virol* 2008;**42**:280–5.
109. Zhang Z, Zheng Z, Luo H, et al. Human Bocavirus NP1 inhibits IFN-beta production by blocking association of IFN regulatory factor 3 with IFNB promoter. *J Immunol* 2012;**189**:1144–53.

vaccine were contaminated with SV40.[107,117] Although rhesus monkey kidney cells showed no cytopathic effect, when supernatants from vaccine cultures were used to inoculate green monkey kidney cells, a pronounced cytopathic effect was observed, thus giving rise to the original name of SV40, vacuolating virus. By that time, millions of doses of both live and killed preparations of polio vaccine containing SV40 had been given to humans in the United States and Europe. Soon after its identification, the demonstration that SV40 induced tumors in neonatal rodents prompted great concern about its potential effects in humans.[62] Serologic studies showed that the formaldehyde-inactivated Salk poliovirus vaccine, but not the live-attenuated Sabin poliovirus vaccine, induced high-titer, SV40-specific antibody responses.[59] Although the live-attenuated poliovirus vaccine preparations contained higher titers of infectious SV40 virus, they failed to induce virus-specific antibodies in vaccines despite prolonged shedding in stool.[114] A 30-year follow-up of infants possibly exposed to SV40 between 1955 and 1962 via inactivated poliovirus vaccine preparations showed no excess risk for cancer, but such epidemiologic studies have significant limitations.[29,159] Despite numerous reports of the detection of SV40 in human tumors, tissues, and excretions, whether SV40 is the cause of human diseases, including malignancies, remains controversial.[25] Since the 1960s, the mechanism of SV40-induced tumorigenesis has been intensely studied, and as a result of SV40-related research, many facets of cellular and molecular biology have been elucidated, including transcriptional regulation, alternative splicing, eukaryotic DNA replication, tumor suppressor proteins, nuclear localization signals, and viral effects on cell cycle control.[34]

VIROLOGY

Polyomaviruses are classified in the family *Polyomaviridae*. More than 20 polyomaviruses are now known, each with a limited host range in which one or several closely related species are infected.[49a,174] Based on genetic comparisons, members of the family *Polyomaviridae* can be subdivided into avian, primate, and rodent subgroups, but polyomaviruses of ruminants and marine mammals also have been described. Based on their similar genomic structure and replication strategies, it is likely that the polyomaviruses and papillomaviruses evolved from a common progenitor virus. Human polyomaviruses are 45 nm in diameter with a genome approximately 5200 base pairs in length; papillomaviruses are somewhat larger, 55 nm in diameter with a genome approximately 8000 base pairs in length. The genomic organization of the polyomaviruses and papillomaviruses differs in that polyomaviruses use both DNA strands to encode proteins, whereas papillomaviruses use just one DNA strand. Viruses in both families cause infection in immunocompetent and immunodeficient hosts and also have been associated with malignancies.

Based on their tissue tropisms, the human polyomaviruses can be divided into three groups: a nephrotropic group including JCV and BKV, a respiratory group including WUPyV and KIPyV, and a cutaneous group including TdSPyV, MCPyV, and other cutaneous PyVs (Table 153-1). Among the nephrotropic human polyomaviruses, the nucleotide sequence of JCV has approximately 75 percent overall homology with BKV and 69 percent homology with SV40.[34] WU and KI are distant outliers among the primate polyomaviruses, sharing only 30 to 40 percent nucleotide identity with BKV and JCV in the late region. In contrast, the MCPyV and several other cutaneous polyomaviruses are more closely related to nonhuman primate and rodent polyomaviruses.[48,175] Based on the high degree of divergence of the respiratory polyomaviruses from the nephrotropic polyomaviruses, a division of the mammalian polyomaviruses into two genera has been proposed.[85]

TABLE 153-1 **Human Polyomaviruses**

Nephrotropic Group	Respiratory Group	Cutaneous Group
BK virus	WU polyomavirus	Merkel cell polyomavirus
JC virus	KI polyomavirus	Trichodysplasia spinulosa polyomavirus
? SV40		Human polyomavirus 6
		Human polyomavirus 7
		Human polyomavirus 9

Several decades of studies of the biology of BKV, JCV, and SV40 have contributed to the current understanding of the biology of human polyomaviruses, principles of which may be challenged by ongoing studies of the more-recently identified respiratory and cutaneous polyomaviruses that apparently lack a regulatory agnoprotein gene.[175] All polyomaviruses share a characteristic circular genome that can be divided into structural, nonstructural, and regulatory regions. The structural (late) region of the viral genome encodes VP1, VP2, and VP3, the capsid proteins that envelop the viral genome. VP1 is the major capsid protein and accounts for more than 70 percent of the virion mass; it participates in host-cell recognition and stimulation of the host immune response. VP2 and VP3 are smaller, less abundant capsid proteins. The nonstructural (early) region of the polyomavirus genome encodes the large T antigen, the best studied of the polyomavirus proteins; this protein initiates viral DNA replication, stimulates cellular entry into S phase, and influences cellular and viral transcription. The small t antigen is produced by alternative splicing of the early viral mRNA and promotes G_1 cell cycle progression; it is not required for viral growth in cultured cells. The large T antigen of SV40 is responsible for the transformation of cells in vivo and in vitro. Large T antigen is a multifunctional protein that contains binding sites for the cellular pRb and p53 proteins. By binding to pRb, the large T antigen allows E2F-mediated progression of the cell cycle, thus providing the right environment for replication of viral DNA. Through its association with p53, a cellular tumor suppressor protein, the large T antigen blocks p53-induced cellular gene expression, thereby leading to genomic instability.[24] In addition to its

interaction with host proteins, the large T antigen has enzymatic activities that may be useful sites for targeting antiviral compounds.[149] The large T antigens of JCV and BKV, similarly, can interact with a variety of cellular proteins, including the p53 and pRb family proteins.[81] The regulatory region of the polyomavirus genome contains both the origin of replication and the viral promoter. The origin is recognized by the large T antigen, in concert with host-cell factors, including cellular DNA polymerase, and viral DNA replication is initiated. Both early and late viral transcription are mediated by cellular RNA polymerase. T-antigen binding to viral DNA autoregulates the production of early mRNA and, by interaction with cellular factors, stimulates late transcription. Polyomaviruses have either an archetypal (single enhancer) or rearranged (complex) regulatory region that can alter the efficiency of virus replication in vitro.[24,81]

Variants of BKV and JCV with complex regulatory regions are common in immunocompromised patients and, at least for JCV in PML, these variants are considered the pathogenic virus.[49] Although regulatory region variants of BKV may have an impact on viral replication in vitro, no clear evidence exists that such changes are necessary or sufficient for the pathogenesis of BKV-associated nephropathy (BKVAN) in at-risk patients.[155] Recent data on the virology of PML-associated strains of JCV suggests that mutations in the viral coat proteins may alter the tissue tropism of JCV and facilitate its dissemination into the central nervous system.[63] Another important advance in the current understanding of polyomavirus replication has been the discovery of virus-produced micro-RNAs that regulate gene expression and may play a role in the immunologic control of polyomavirus persistence.[160] The clinical importance of this novel regulatory mechanism in the pathogenesis of polyomavirus-associated diseases is an area of intense investigation that may soon provide new targets for therapeutic intervention.

EPIDEMIOLOGY

Polyomaviruses are ubiquitous in the human population and the prevalence of virus-specific antibodies increases with age, beginning in early childhood (Fig. 153-1).[104] BKV seroconversion occurs in 50 percent of children by the time they reach 3 to 4 years of age.[125,152] Population-based seroprevalence data suggest that more than 80 percent of adults have been infected with BKV.[89,184] In contrast to earlier data, recent studies suggest that only about 50 to 70 percent of the adult population has been infected with JCV.* BKV seroconversion has been associated with mild upper respiratory tract illnesses in a small number of children, and BKV viruria has been reported in a child with acute tonsillitis.[65,66,162] No distinct clinical syndrome has been associated with BKV infection of healthy children. The prevalence of antibodies in humans directed against SV40 has not been well established, primarily because of the variability in methods used by different research laboratories. Several studies have reported seroprevalences of 2 to 11 percent for SV40 using a plaque neutralization assay.[27,28,141] However, other studies suggest that much SV40 reactivity may be due to cross reactivity with BKV.[30,95,134,185] Whether BKV-reactive antibodies provide significant cross protection against SV40 infection (or vice versa) has not been determined. As with the nephrotropic human polyomaviruses, the seroprevalence data for the respiratory and cutaneous polyomaviruses suggest that these viruses are ubiquitous in the human population, with infections occurring throughout life and more than 70 percent of humans having detectable specific antibodies to MCPyV, WUPyV, TsPyV, and KIPyV.[32,89,174,184]

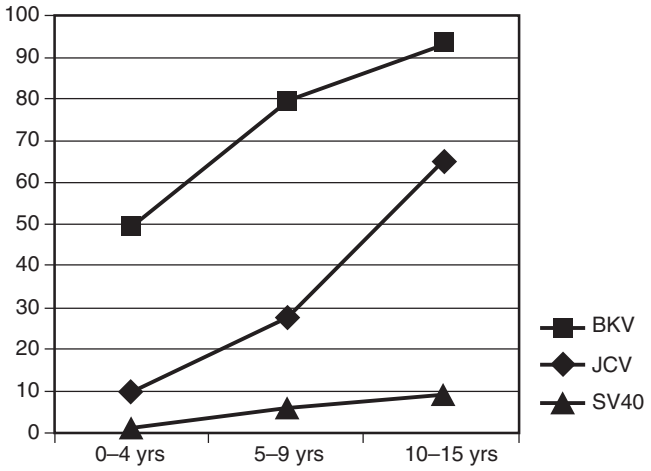

FIGURE 153-1 ■ **Seroprevalence estimates for JC virus *(u),* BK virus *(n),* and SV40 *(s)* in childhood.** (Data from Lednicky JA, Butel JS. Polyomaviruses and human tumors: a brief review of current concepts and interpretations. Front Biosci 1999;**4**:D153-D164.)

Humans are the only known reservoir for JCV and BKV, whereas SV40 primarily is a simian virus that has crossed species from monkeys to humans. Nephrotropic polyomaviruses persist in the kidney and, to a lesser degree, in B lymphocytes and oligodendrocytes in both humans (JCV and BKV) and monkeys (SV40).[42,45,100,120] The exact mechanism of transmission of JCV and BKV is not known, but several studies have shown that fecal excretion of BKV may be common in infancy and adulthood, suggesting that fecal-oral transmission could explain the ubiquity of early childhood infection.[176,177] As described earlier, SV40 was introduced inadvertently into humans as a contaminant of early polio vaccines; however, such direct exposure cannot account for the SV40 seropositivity in children born more recently than 1962, suggesting continued human-to-human spread of the virus, albeit at low levels.[25] In the pediatric population, one SV40 seroprevalence survey found nearly 6 percent of hospitalized children with antibodies to SV40.[27] Among kidney transplant patients in the cohort, 40 percent had evidence of SV40 infection, but whether such infection was related to immunosuppressive medications or the underlying kidney disease was not clear.[27] SV40 DNA sequences have been detected in kidney tissue from

*References 20, 51, 89, 95, 125, 130, 152, 171, 183.

several pediatric renal transplant recipients and in tonsil tissue from immunocompetent children.[26,129]

In immunocompetent individuals, persistence of JCV and BKV in the kidney is asymptomatic and point prevalence studies suggest that intermittent shedding of JCV in urine is more common than shedding of BKV.[2,153] Viral DNA (JCV or BKV) can be found in 30 to 50 percent of kidney samples from immunocompetent adults taken at surgery or autopsy.[33,72,170] The frequency of JCV excretion in urine increases with age, and the prevalence of BKV excretion is approximately 2 to 5 percent in healthy adults.[41,94] Data from a longitudinal study suggest that BKV excretion may be more common than previously thought, having been detected in more than 19 percent of specimens collected daily from 7 of 20 women over a 2-month period.[92]

JCV is lymphotropic, and the finding of JCV in plasma and peripheral blood mononuclear cells generally correlates with immune status in that it is noted less frequently in immunocompetent patients and increases in frequency in human immunodeficiency virus (HIV)-infected patients with lower CD4 counts.[40,55,96] Special clinical conditions, including pregnancy and autoimmune disorders, are associated with excretion of BKV or JCV in urine.[110,113,161,164,165] The immunologic and virologic factors regulating initiation and maintenance of persistence remain largely unknown.

CLINICAL MANIFESTATIONS

Central Nervous System Manifestations

PML is a clinical syndrome characterized by progressive neurologic impairment in severely immunocompromised patients. In PML, the host immune system fails to maintain viral latency and acquired mutations in the VP1 and regulatory region of JCV facilitate neurotropism that leads to CNS invasion.[49,63] JCV replicates in and lyses oligodendroglia and causes destruction of cerebral white matter in a multifocal or patchy distribution (Fig. 153-2). PML is the sentinel acquired immunodeficiency syndrome (AIDS)-defining illness in approximately 5 percent of adults, and its cumulative incidence in HIV-infected adults may be greater than 25 percent.[16,67] PML occurs less commonly in HIV-infected children than in adults. Other immunosuppressed individuals who are at high risk for PML include patients receiving chemotherapy, solid organ or hematopoietic stem cell transplant (HSCT) recipients, and those with primary T-cell defects. Numerous immunosuppressive agents used in the treatment of malignancies, autoimmune diseases, and inflammatory disorders have been associated with an increased risk for PML, including rituximab, natalizumab, efalizumab, and others.[15] For JCV-seropositive patients receiving natalizumab, the risk for PML is approximately 1 percent after 24 to 59 months of natalizumab treatment.[18] At present, serologic testing for JCV-specific immunoglobulin (Ig)G antibodies is the only clinical test that may be useful for risk-stratification of patients receiving prolonged immunosuppression. In addition to JCV seropositivity, both the duration of natalizumab therapy and a history of prior immunosuppressant therapy were associated with increased risk for PML among patients with multiple sclerosis.[18]

Clinical manifestations of PML can include behavior changes; blindness, deafness, and other cranial nerve dysfunctions; motor and cognitive deficits; and incoordination as a result of cerebellar involvement.[17] The onset of symptoms usually is insidious, and associated systemic signs such as fever and headache are uncommon. Progression of symptoms occurs over a period of several

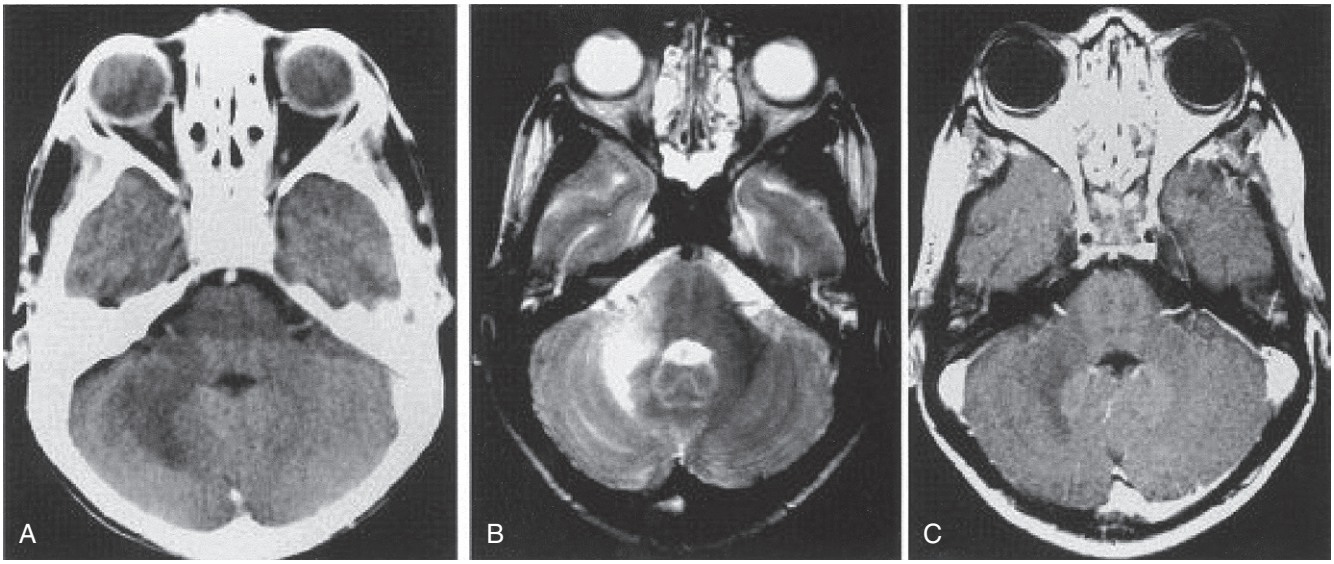

FIGURE 153-2 ■ A, Contrast-enhanced brain computed tomography of a child with progressive multifocal leukoencephalopathy showing low-density, nonenhancing right-sided cerebellar white matter lesion without a mass effect. **B,** On T2-weighted magnetic resonance imaging (MRI), the lesion produces high signal without a mass effect or edema. **C,** The lesion gives low signal on T1-weighted MRI and fails to enhance with intravenous contrast. (From Morriss MC, Rutstein RM, Rudy B, et al.: Progressive multifocal leukoencephalopathy in an HIV-infected child. Neuroradiology 1997,**39**:142-144.)

weeks to months, and the condition is inevitably fatal. The diagnosis of PML is largely clinical and based on the history, physical examination, and characteristic findings on neuroimaging studies. Computed tomography and magnetic resonance imaging (MRI) of the brain show focal demyelination and white matter edema with relative sparing of the gray matter. MRI of a child with AIDS and PML is shown in Figure 153-2.[118] Cerebrospinal fluid (CSF) analysis usually is normal in patients with PML, likely a result of impairment, depletion, or both of T lymphocytes.[17] Neurologic disease associated with BKV has been reported in several AIDS patients, and BKV encephalitis has been reported in a patient without known immunodeficiency in association with polyomavirus seroconversion.[21,172,186]

Urinary Tract Manifestations

Polyomaviruria is a common occurrence in immunocompetent adults and may be so in children as well.[31,83,94,143,178] Hemorrhagic cystitis temporally associated with BKV viruria has been reported rarely in immunocompetent children, but no other nonmalignant diseases have been linked to polyomavirus infection in these children.[146] Urinary excretion of BKV occurs in about 20 to 40 percent of adult and pediatric renal transplant recipients, approximately one third to half of whom also may have BK viremia.[73,76-78,80] Posttransplant polyomaviruria is due predominantly to reactivation of latent infection, and limited data suggest that the reactivated virus is often donor-derived.[181] Excretion of BKV usually begins in the first 2 to 3 months after transplantation. In renal transplant recipients, BKV viruria may be associated with ureteral ulceration and stenosis, sometimes requiring surgical intervention to prevent or relieve obstructive nephropathy. The prognostic significance of polyomavirus reactivation in renal transplant patients has become an important topic, especially as managing immunosuppression has become more sophisticated.

BKV has emerged as the most common infectious cause of renal allograft failure and is increasingly identified as a cause of native-kidney dysfunction in non–renal transplant recipients (Fig. 153-3).[14] BKVAN affects 1 to 10 percent of renal transplant recipients, resulting in allograft loss for approximately 75 percent of those affected. Initially, case reports linking polyomavirus infection and allograft failure identified BKV as a possible pathogen.[121,128,135] Larger studies have since revealed the pathogenesis of BKVAN, as well as the appropriate diagnostic and therapeutic strategies. Retrospective analyses have found that BKVAN is more commonly associated with the use of tacrolimus and mycophenolate, but BKVAN has been reported in patients receiving other immunosuppressive agents.[1,22] Molecular characterization of BKV isolates from patients with BKVAN has not identified particular strains of BKV that are more likely to cause BKVAN.[155] The role of host factors in the pathogenesis of BKVAN remains an area of intense investigation. Importantly, repeat renal transplantation after allograft loss as a result of BKVAN has been successful without increased risk for BKVAN in the second allograft.[58]

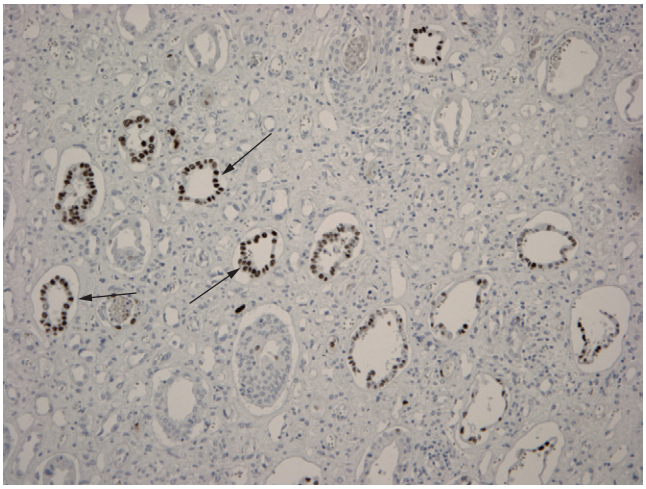

FIGURE 153-3 ■ Immunohistochemical detection of the BKV T antigen in kidney tissue obtained at autopsy from a pediatric patient with disseminated BKV infection after hematopoietic stem cell transplantation. *Arrows* indicate positively stained renal tubular epithelial cells. (See companion Expert Consult website for color version.) (Magnification, ×40). (Courtesy J.A. Vanchiere, MD, PhD.)

Polyomaviruria, especially that involving BKV, is a common finding after hematopoietic stem cell transplantation (HSCT), occurring in 50 to 80 percent of patients within 2 to 8 weeks after transplantation.[7,12,150] BKV viruria is associated with hemorrhagic cystitis (BKV-HC) in 15 to 25 percent of adult and pediatric HSCT recipients.[11,50,104-106] BKV-HC is both painful and distressing for HSCT patients, occasionally necessitating erythrocyte transfusions and, rarely, surgical intervention. For the majority of patients with BKV-HC, supportive care, including intravenous hydration and erythrocyte transfusions, is adequate management, because T-cell engraftment eventually controls BKV replication and leads to resolution of symptoms. Adenovirus is an important but less common cause of hemorrhagic cystitis after HSCT.[4,50,106]

Interstitial nephritis caused by BKV has been described in children with various immunodeficiencies, including hyper-IgM immunodeficiency and AIDS.[36,142] Renal dysfunction is a common finding in AIDS patients, but its etiology has only rarely been linked to polyomaviruses; one study found SV40 in kidney tissue from several AIDS patients with a collapsing variant of focal segmental glomerulosclerosis, but the pathogenesis of this disease remains unclear.[108] Several studies of Balkan endemic nephropathy have associated this enigmatic disease in Eastern Europe with polyomaviruses, but further study is needed to establish a causal link.[119,158] One pediatric study observed an association between SV40 seropositivity and renal transplantation and detected SV40 sequences in renal biopsy specimens, thus suggesting a possible role for SV40 in pediatric renal disease.[26,27]

Cutaneous Manifestations

The clinical manifestations of cutaneous polyomaviruses are evident almost exclusively in immunocompromised

patients, especially among those with marked T-lymphocyte suppression. The association of Merkel cell carcinoma and HIV infection was first described in 2002, but it was not until deep-sequencing approaches were used that the causative agent, MCPyV, was discovered.[48] Numerous case series have now established that MCPyV can be detected in more than 75 percent (range, 43% to 100%) of Merkel cell carcinomas.[9] Mutations in the helicase domain of the large T antigen are commonly found in MCPyV strains from Merkel cell carcinomas and are believed to be necessary for the oncogenesis of MCPyV.[156] As with Merkel cell carcinoma, trichodysplasia spinulosa caused by TSPyV is predominantly a disease of severely immunocompromised patients, especially solid organ transplant recipients and patients with hematologic malignancies.[112] This striking disease is characterized by the eruption of erythematous papules with 1- to 3-mm keratotic spicules on the nose and chin, with occasional lesions on the trunk and back. In addition, patients often have eyelash and eyebrow hair loss.[166] To date, approximately 20 cases of virus-associated trichodysplasia spinulosa have been reported in the medical literature.[112] Other cutaneous polyomaviruses remain enigmatic. Although they appear to be very common in the human population, they have not yet been associated with malignancy or acute clinical diseases.

Pulmonary Manifestations

Although some contend that the nephrotropic polyomaviruses can be transmitted in respiratory secretions, little evidence exists to indicate that such transmission, if it does occur, is accompanied by clinical disease of the respiratory tract. Several case reports of BKV-associated pulmonary disease have been published, uniformly in the setting of severe immunosuppression and with poor outcomes.[36,147,172,189] Although interstitial pneumonitis in a patient with severe immunocompromise might raise the suspicion for polyomavirus-associated disease, other viral pathogens such as cytomegalovirus (CMV) and adenovirus should be considered more likely.

WUPyV and KIPyV can be found in upper and lower respiratory tract secretions from immunocompetent and immunocompromised patients, most often in association with other common respiratory tract pathogens.[5,57,99] The clinical consequences of WUPyV and KIPyV are minimal, even in the most severely compromised patients.[79,99]

Other Manifestations

BKV-associated retinal necrosis in an AIDS-infected patient has been described, as have elevated hepatic transaminase levels in association with BKV viruria and detection of BKV in normal liver specimens.[21,71,93,124] The clinical significance of these manifestations is unknown.

Malignancies

The polyomaviruses are named for their ability to induce tumors in laboratory animals. Their association with human tumors was noted first in 1974 when Soriano and colleagues[157] isolated SV40 from a metastatic malignant melanoma in a patient with SV40 neutralizing antibodies. SV40 T antigen and capsid antigen were detected in lung, liver, and muscle metastases of the patient, but not in normal tissue.[157] Since that time, polyomaviruses have been linked to a variety of neoplasms in humans, frequently analogous to the tumor types produced by SV40 in rodents.[103] SV40 has been detected in association with human cancers much more frequently than JCV and BKV. SV40 T antigen or DNA has been detected in a significant number of meningiomas, ependymomas, choroid plexus tumors, astrocytomas, pleural mesotheliomas, and osteosarcomas.[10,82] Several recent studies reported the presence of SV40 in non-Hodgkin lymphomas.[111,140,182] In the case of pleural mesotheliomas, which also have been associated with asbestos exposure, the presence of SV40 may be a negative prognostic indicator.[132] A possible synergistic effect of SV40 and asbestos has been observed in vitro and in case-control studies of human mesothelioma patients, suggesting that the two agents may work in concert as co-carcinogens.[19,35,97] In a study of pediatric patients, JCV DNA was detected in 11/23 medulloblastomas, and JCV T antigen expression was detected by immunohistochemistry in 4/16 samples tested.[98] Other investigators have not detected polyomavirus DNA or protein in medulloblastoma tissue.[91,188]

JCV also has been detected in colorectal and gastric cancers and in normal tissue samples from the human gastrointestinal tract.[101,136,137] BKV has been detected in both normal and neoplastic prostate tissue, suggesting a potential role for BKV in the pathogenesis of this common cancer of adulthood.[37,38] Recent studies of prostate cancer in different ethnic groups have produced contradictory results and suggest that host and environmental factors also may contribute to the complexity of viral oncogenesis.[3,68] Further studies, including evaluation of host immunogenetic factors, will be required to establish whether polyomaviruses are commonly oncogenic in humans.

Laboratory Diagnosis

Although the suspicion of polyomavirus disease is primarily a clinical one, laboratory techniques are important for making the diagnosis in the proper clinical circumstances. Serologic tests and viral culture are of little use clinically because these tests are laborious and rarely available. Polyomaviruses in urine could be considered normal flora among immunocompetent patients, much like CMV detected in urine after the newborn period. Histologic study of urinary sediment can reveal "decoy cells," which are uroepithelial cells with characteristic polyomavirus inclusions. The presence of decoy cells is a specific and moderately sensitive marker of polyomavirus-associated nephritis in renal transplant patients.[44,102] Detection of specific polyomavirus DNA from urine samples is of little known significance, whereas polymerase chain reaction (PCR) detection from blood samples, especially viremia of greater than 10,000 genomes per milliliter, may correlate with the risk for BKVAN after solid organ transplantation.[102,122,133]

Consensus guidelines suggest routine monitoring of kidney transplant recipients for BKV replication every 1 to 3 months for 1 to 2 years after transplantation, using either urine or plasma quantitative PCR.[75,90] Immunohistochemical detection of BKV T antigen in renal biopsy material is the gold standard for diagnosis of BKVAN, although electron microscopic identification of polyomavirus-like particles in renal biopsy material also may be diagnostic.[77,109] The diagnosis of BKV-HC is based on clinical symptoms and the detection of BKV in urine, usually by PCR. Urinary viral loads in patients with BKV-HC and BKVAN can be extremely high, even 10^{12} or 10^{13} genome copies per milliliter.

Detection of JCV DNA in CSF by PCR is the diagnostic test of choice for suspected PML.[69,96,148,168] In adult patients with AIDS and neurologic symptoms, PCR amplification of JCV DNA from CSF is 99 percent predictive of PML, whereas brain biopsy has a slightly lower sensitivity.[39] On gross examination, tissue typical of PML has been described as "worm-eaten," and microscopic examination reveals multiple foci of demyelination with enlarged oligodendroglia, giant astrocytes, and an intense phagocytic infiltration.[180] Immunohistochemical techniques and other nucleic acid detection methods also can be used to confirm the diagnosis of PML.[84] JC viremia is a general indicator of immunosuppression and a common finding in HIV-infected patients without PML, so its utility in the diagnosis of suspected PML is questionable.[96]

Detection of the cutaneous and respiratory polyomaviruses relies on PCR testing that is performed in research laboratories. With the exception of MCPyV and TdSPyV in immunocompromised patients, there are no clear clinical indications for pursuit of these viruses.[99]

TREATMENT AND PREVENTION

No specific antiviral agents have been studied in randomized trials for the treatment of polyomavirus disease or polyomavirus-associated tumors. Studies in both adult and pediatric patients have demonstrated that routine monitoring of plasma BKV viral loads and preemptive reduction of immunosuppression are useful for the prevention of BKVAN without significant risk for allograft rejection, and routine screening for BKV has been recommended by international panels.[75,90] However, BKV reactivation may persist and lead to chronic renal dysfunction in some patients.[179] BKVAN should be suspected in any renal transplant patient with otherwise unexplained allograft dysfunction. For renal transplant recipients with biopsy-proved BKVAN, reduction of immunosuppression may be helpful for preservation of allograft function, especially among patients with low-grade disease.[43,54] The additional benefit of antiviral agents beyond that of reducing immunosuppression has not been established. Case series have reported variable results with the use of cidofovir and leflunomide in patients with BKVAN.[8,47,86-88] Despite its potential nephrotoxicity, low-dose cidofovir has been suggested by an international consensus group as a potential investigational agent for treatment of BKVAN.[77] Intravenous immunoglobulin has been used to treat several patients with BKVAN, with some authors reporting success and some not.[6,150,187,154] Both leflunomide and cidofovir have been used to treat HSCT recipients with particularly severe hemorrhagic cystitis caused by BKV, but immune restoration by T-cell engraftment is likely the most important factor in resolution of BKV-related cystitis. Recent studies of fluoroquinolone antibiotics, which have some antipolyomavirus activity, for the prevention of BKV disease in immunocompromised patients suggest that these agents may be useful in both renal transplant and HSCT recipients.[53,116] In addition, a lipid derivative of cidofovir called CMX001 has shown in vitro activity against both BKV and JCV.[64,139] Furthermore, this orally bioavailable agent is approximately 100 times more potent than cidofovir and has an improved safety profile in vivo.[52,127]

Initiation of highly active antiretroviral therapy (HAART) can restore immune function and alleviate the symptoms of PML for AIDS patients.[46,131,169] Despite administration of HAART, PML may develop in some HIV-infected patients as a result of incomplete immune reconstitution.[167] Cidofovir has been used successfully in conjunction with HAART in patients with AIDS and PML, but its benefit above that of HAART and in non-AIDS patients with PML has not been determined.[115,145] Adoptive transfer of in vitro–stimulated, virus-specific, allogeneic cytotoxic T-lymphocytes is a potentially promising prophylaxis and treatment option for patients after HSCT. This novel approach has been used successfully to prevent and treat Epstein-Barr virus–related disease after HSCT and may be useful for other viruses as well.[60,61,74] Given the ubiquity and generally benign nature of polyomavirus infections, as well as the paucity of information about the nature of their transmission and clinical significance in immunocompetent patients, specific efforts to prevent polyomavirus transmission are not indicated at this time. Universal precautions are appropriate for care of patients with polyomavirus disease.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Akgul B, Pfister D, Knuchel R, et al. No evidence for a role of xenotropic murine leukaemia virus-related virus and BK virus in prostate cancer of German patients. *Med Microbiol Immunol* 2012;**201**:245–8.
6. Anyaegbu EI, Almond PS, Milligan T, et al. Intravenous immunoglobulin therapy in the treatment of BK viremia and nephropathy in pediatric renal transplant recipients. *Pediatr Transplant* 2012;**16**:E19–24.
9. Arora R, Chang Y, Moore PS. MCV and Merkel cell carcinoma: a molecular success story. *Curr Opin Virol* 2012;**2**:489–98.
14. Babel N, Volk HD, Reinke P. BK polyomavirus infection and nephropathy: the virus-immune system interplay. *Nat Rev Nephrol* 2011;**7**:399–406.
15. Bellizzi A, Nardis C, Anzivino E, et al. Human polyomavirus JC reactivation and pathogenetic mechanisms of progressive multifocal leukoencephalopathy and cancer in the era of monoclonal antibody therapies. *J Neurovirol* 2012;**18**:1–11.
18. Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med* 2012;**366**:1870–80.
20. Bozic C, Richman S, Plavina T, et al. Anti-John Cunningham virus antibody prevalence in multiple sclerosis patients: baseline results of STRATIFY-1. *Ann Neurol* 2011;**70**:742–50.

23. Broekema NM, Abend JR, Bennett SM, et al. A system for the analysis of BKV non-coding control regions: application to clinical isolates from an HIV/AIDS patient. *Virology* 2010;**407**: 368–73.
25. Butel J. Patterns of polyomavirus SV40 infections and associated cancers in humans: a model. *Curr Opin Virol* 2012;**2**:508–14.
32. Chen T, Hedman L, Mattila PS, et al. Serological evidence of Merkel cell polyomavirus primary infections in childhood. *J Clin Virol* 2011;**50**:125–9.
48. Feng H, Shuda M, Chang Y, et al. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008;**319**: 1096–100.
49. Ferenczy MW, Marshall LJ, Nelson CD, et al. Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clin Microbiol Rev* 2012;**25**:471–506.
49a. Fields BN, Knipe DM, Howley PM, et al, editors. *Fields Virology*, 3rd ed. Philadelphia: Lippincott-Raven; 1996. p. 2027–43.
52. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012;**18**:731–8.
53. Gabardi S, Waikar SS, Martin S, et al. Evaluation of fluoroquinolones for the prevention of BK viremia after renal transplantation. *Clin J Am Soc Nephrol* 2010;**5**:1298–304.
58. Geetha D, Sozio SM, Ghanta M, et al. Results of repeat renal transplantation after graft loss from BK virus nephropathy. *Transplantation* 2011;**92**:781–6.
60. Gerdemann U, Christin AS, Vera JF, et al. Nucleofection of DCs to generate multivirus-specific T cells for prevention or treatment of viral infections in the immunocompromised host. *Mol Ther* 2009;**17**:1616–25.
61. Gerdemann U, Keirnan JM, Katari UL, et al. Rapidly generated multivirus-specific cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. *Mol Ther* 2012;**20**:1622–32.
63. Gorelik L, Reid C, Testa M, et al. Progressive multifocal leukoencephalopathy (PML) development is associated with mutations in JC virus capsid protein VP1 that change its receptor specificity. *J Infect Dis* 2011;**204**:103–14.
64. Gosert R, Rinaldo CH, Wernli M, et al. CMX001 (1-O-hexadecyloxypropyl-cidofovir) inhibits polyomavirus JC replication in human brain progenitor-derived astrocytes. *Antimicrob Agents Chemother* 2011;**55**:2129–36.
68. Groom HC, Warren AY, Neal DE, et al. No evidence for infection of UK prostate cancer patients with XMRV, BK virus, *Trichomonas vaginalis* or human papilloma viruses. *PLoS One* 2012;**7**:e34221.
74. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood* 2010;**115**: 925–35.
79. Hormozdi DJ, Arens MQ, Le BM, et al. KI polyomavirus detected in respiratory tract specimens from patients in St. Louis, Missouri. *Pediatr Infect Dis J* 2010;**29**:329–33.
80. Huang G, Chen LZ, Qiu J, et al. Prospective study of polyomavirus BK replication and nephropathy in renal transplant recipients in China: a single-center analysis of incidence, reduction in immunosuppression and clinical course. *Clin Transplant* 2010;**24**: 599–609.
85. Johne R, Buck CB, Allander T, et al. Taxonomical developments in the family Polyomaviridae. *Arch Virol* 2011;**156**:1627–34.
89. Kean JM, Rao S, Wang M, et al. Seroepidemiology of human polyomaviruses. *PLoS Pathog* 2009;**5**:e1000363.
90. Kidney Disease. Improving Global Outcomes. KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 3):S1–155.
92. Kling CL, Wright AT, Katz SE, et al. Dynamics of urinary polyomavirus shedding in healthy adult women. *J Med Virol* 2012; **84**:1459–63.
99. Kuypers J, Campbell AP, Guthrie KA, et al. WU and KI Polyomaviruses in respiratory samples from allogeneic hematopoietic cell transplant recipients. *Emerg Infect Dis* 2012;**18**:1580–8.
112. Matthews MR, Wang RC, Reddick RL, et al. Viral-associated trichodysplasia spinulosa: a case with electron microscopic and molecular detection of the trichodysplasia spinulosa-associated human polyomavirus. *J Cutan Pathol* 2011;**38**:420–31.
113. McClure GB, Gardner JS, Williams JT, et al. Dynamics of pregnancy-associated polyomavirus urinary excretion: a prospective longitudinal study. *J Med Virol* 2012;**84**:1312–22.
116. Miller AN, Glode A, Hogan KR, et al. Efficacy and safety of ciprofloxacin for prophylaxis of polyomavirus BK virus-associated hemorrhagic cystitis in allogeneic hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2011;**17**: 1176–81.
127. Painter W, Robertson A, Trost LC, et al. First pharmacokinetic and safety study in humans of the novel lipid antiviral conjugate CMX001, a broad-spectrum oral drug active against double-stranded DNA viruses. *Antimicrob Agents Chemother* 2012;**56**: 2726–34.
134. Randhawa P, Viscidi R, Carter JJ, et al. Identification of species-specific and cross-reactive epitopes in human polyomavirus capsids using monoclonal antibodies. *J Gen Virol* 2009;**90**: 634–9.
139. Rinaldo CH, Gosert R, Bernhoff E, et al. 1-O-Hexadecyloxypropyl cidofovir (CMX001) effectively inhibits polyomavirus BK replication in primary human renal tubular epithelial cells. *Antimicrob Agents Chemother* 2010;**54**:4714–22.
149. Seguin SP, Ireland AW, Gupta T, et al. A screen for modulators of large T antigen's ATPase activity uncovers novel inhibitors of Simian Virus 40 and BK virus replication. *Antiviral Res* 2012;**96**:70–81.
154. Sharma AP, Moussa M, Casier S, et al. Intravenous immunoglobulin as rescue therapy for BK virus nephropathy. *Pediatr Transplant* 2009;**13**:123–9.
156. Shuda M, Feng H, Kwun HJ, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci U S A* 2008;**105**:16272–7.
166. Tan BH, Busam KJ. Virus-associated trichodysplasia spinulosa. *Adv Anat Pathol* 2011;**18**:450–3.
171. Trampe AK, Hemmelmann C, Stroet A, et al. Anti-JC virus antibodies in a large German natalizumab-treated multiple sclerosis cohort. *Neurology* 2012;**78**:1736–42.
173. van der Meijden E, Janssens RW, Lauber C, et al. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. *PLoS Pathog* 2010;**6**: e1001024.
174. van der Meijden E, Kazem S, Burgers MM, et al. Seroprevalence of trichodysplasia spinulosa-associated polyomavirus. *Emerg Infect Dis* 2011;**17**:1355–63.
175. Van Ghelue M, Khan MT, Ehlers B, et al. Genome analysis of the new human polyomaviruses. *Rev Med Virol* 2012;doi:10.1002/rmv.1711.
176. Vanchiere JA, Abudayyeh S, Copeland CM, et al. Polyomavirus shedding in the stool of healthy adults. *J Clin Microbiol* 2009;**47**: 2388–91.
181. Vera-Sempere FJ, Rubio L, Felipe-Ponce V, et al. Renal donor implication in the origin of BK infection: analysis of genomic viral subtypes. *Transplant Proc* 2006;**38**:2378–81.
183. Viscidi RP, Khanna N, Tan CS, et al. John Cunningham virus antibody and viremia as predictors of progressive multifocal leukoencephalopathy in human immunodeficiency virus-1-infected individuals. *Clin Infect Dis* 2011;**53**:711–15.
184. Viscidi RP, Rollison DE, Sondak VK, et al. Age-specific seroprevalence of Merkel cell polyomavirus, BK virus, and JC virus. *Clin Vaccine Immunol* 2011;**18**:1737–43.
189. Yapa HM, McLornan DP, Raj K, et al. Pneumonitis post-haematopoeitic stem cell transplant: cytopathology clinches diagnosis. *J Clin Virol* 2012;**55**:278–81.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agha I, Brennan DC. BK virus and immunosuppressive agents. *Adv Exp Med Biol* 2006;**577**:174–84.
2. Agostini HT, Ryschkewitsch CF, Stoner GL. Genotype profile of human polyomavirus JC excreted in urine of immunocompetent individuals. *J Clin Microbiol* 1996;**34**:159–64.
3. Akgul B, Pfister D, Knuchel R, et al. No evidence for a role of xenotropic murine leukaemia virus-related virus and BK virus in prostate cancer of German patients. *Med Microbiol Immunol* 2012;**201**:245–8.
4. Akiyama H, Kurosu T, Sakashita C, et al. Adenovirus is a key pathogen in hemorrhagic cystitis associated with bone marrow transplantation. *Clin Infect Dis* 2001;**32**:1325–30.
5. Allander T, Andreasson K, Gupta S, et al. Identification of a third human polyomavirus. *J Virol* 2007;**81**:4130–6.
6. Anyaegbu EI, Almond PS, Milligan T, et al. Intravenous immunoglobulin therapy in the treatment of BK viremia and nephropathy in pediatric renal transplant recipients. *Pediatr Transplant* 2012;**16**:E19–24.
7. Apperley JF, Rice SJ, Bishop JA, et al. Late-onset hemorrhagic cystitis associated with urinary excretion of polyomaviruses after bone marrow transplantation. *Transplantation* 1987;**43**:108–12.
8. Araya CE, Lew JF, Fennell RS III, et al. Intermediate-dose cidofovir without probenecid in the treatment of BK virus allograft nephropathy. *Pediatr Transplant* 2006;**10**:32–7.
9. Arora R, Chang Y, Moore PS. MCV and Merkel cell carcinoma: a molecular success story. *Curr Opin Virol* 2012;**2**:489–98.
10. Arrington AS, Butel JS. SV40 and human tumors. In Khalili K, Stoner GL, editors. *The Human Polyomaviruses JC, BK, and SV40: Molecular and Clinical Perspectives*. New York: John Wiley & Sons; 2011. p. 461–89.
11. Arthur RR, Shah KV, Baust SJ, et al. Association of BK viruria with hemorrhagic cystitis in recipients of bone marrow transplants. *N Engl J Med* 1986;**315**:230–4.
12. Arthur RR, Shah KV, Charache P, et al. BK and JC virus infections in recipients of bone marrow transplants. *J Infect Dis* 1988; **158**:563–9.
13. Astrom KE, Mancall EL, Richardson EPJ. Progressive multifocal leuko-encephalopathy: a hitherto unrecognized complication of chronic lymphatic leukaemia and Hodgkin's disease. *Brain* 1958;**81**:93–111.
14. Babel N, Volk HD, Reinke P. BK polyomavirus infection and nephropathy: the virus-immune system interplay. *Nat Rev Nephrol* 2011;**7**:399–406.
15. Bellizzi A, Nardis C, Anzivino E, et al. Human polyomavirus JC reactivation and pathogenetic mechanisms of progressive multifocal leukoencephalopathy and cancer in the era of monoclonal antibody therapies. *J Neurovirol* 2012;**18**:1–11.
16. Berger JR, Levy RM. The neurologic complications of human immunodeficiency virus infection. *Med Clin North Am* 1993;**77**:1–23.
17. Berger JR, Pall L, Lanska D, et al. Progressive multifocal leukoencephalopathy in patients with HIV infection. *J Neurovirol* 1998;**4**:59–68.
18. Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med* 2012;**366**:1870–80.
19. Bocchetta M, Di RI, Powers A, et al. Human mesothelial cells are unusually susceptible to simian virus 40-mediated transformation and asbestos cocarcinogenicity. *Proc Natl Acad Sci U S A* 2000;**97**:10214–19.
20. Bozic C, Richman S, Plavina T, et al. Anti-John Cunningham virus antibody prevalence in multiple sclerosis patients: baseline results of STRATIFY-1. *Ann Neurol* 2011;**70**:742–50.
21. Bratt G, Hammarin AL, Grandien M, et al. BK virus as the cause of meningoencephalitis, retinitis and nephritis in a patient with AIDS. *AIDS* 1999;**13**:1071–5.
22. Brennan DC, Agha I, Bohl DL, et al. Incidence of BK with tacrolimus versus cyclosporine and impact of preemptive immunosuppression reduction. *Am J Transplant* 2005;**5**:582–94.
23. Broekema NM, Abend JR, Bennett SM, et al. A system for the analysis of BKV non-coding control regions: application to clinical isolates from an HIV/AIDS patient. *Virology* 2010;**407**: 368–73.
24. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. *Carcinogenesis* 2000;**21**:405–26.
25. Butel JS. Patterns of polyomavirus SV40 infections and associated cancers in humans: a model. *Curr Opin Virol* 2012;**2**:508–14.
26. Butel JS, Arrington AS, Wong C, et al. Molecular evidence of simian virus 40 infections in children. *J Infect Dis* 1999;**180**:884–7.
27. Butel JS, Jafar S, Wong C, et al. Evidence of SV40 infections in hospitalized children. *Hum Pathol* 1999;**30**:1496–502.
28. Butel JS, Wong C, Vilchez RA, et al. Detection of antibodies to polyomavirus SV40 in two central European countries. *Centr Eur J Publ Hlth* 2003;**11**:3–8.
29. Carbone M, Pass HI, Miele L, et al. New developments about the association of SV40 with human mesothelioma. *Oncogene* 2003;**22**:5173–80.
30. Carter JJ, Madeleine MM, Wipf GC, et al. Lack of serologic evidence for prevalent simian virus 40 infection in humans. *J Natl Cancer Inst* 2003;**95**:1522–30.
31. Chang H, Wang ML, Tsai RT, et al. High incidence of JC viruria in JC-seropositive older individuals. *J Neurovirol* 2002;**8**:447–51.
32. Chen T, Hedman L, Mattila PS, et al. Serological evidence of Merkel cell polyomavirus primary infections in childhood. *J Clin Virol* 2011;**50**:125–9.
33. Chesters PM, Heritage J, McCance DJ. Persistence of DNA sequences of BK virus and JC virus in normal human tissues and in diseased tissues. *J Infect Dis* 1983;**147**:676–84.
34. Cole CN, Conzen SD. *Polyomaviridae:* the viruses and their replication. In: Knipe DM, Howley PM, editors. *Fields virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2141–74.
35. Cristaudo A, Foddis R, Vivaldi A, et al. SV40 enhances the risk of malignant mesothelioma among people exposed to asbestos: a molecular epidemiologic case-control study. *Cancer Res* 2005;**65**:3049–52.
36. Cubukcu-Dimopulo O, Greco A, Kumar A, et al. BK virus infection in AIDS. *Am J Surg Pathol* 2000;**24**:145–9.
37. Das D, Shah RB, Imperiale MJ. Detection and expression of human BK virus sequences in neoplastic prostate tissues. *Oncogene* 2004;**23**:7031–46.
38. Das D, Wojno K, Imperiale MJ. BKV as a cofactor in the etiology of prostate cancer in its early stages. *J Virol* 2008;**82**:2705–14.
39. De Luca A, Cingolani A, Linzalone A, et al. Improved detection of JC virus DNA in cerebrospinal fluid for diagnosis of AIDS-related progressive multifocal leukoencephalopathy. *J Clin Microbiol* 1996;**34**:1343–6.
40. Degener AM, Pietropaolo V, Di Taranto C, et al. Detection of JC and BK viral genome in specimens of HIV-1 infected subjects. *New Microbiol* 1997;**20**:115–22.
41. Dorries K. Molecular biology and pathogenesis of human polyomavirus infections. *Dev Biol Stand* 1998;**94**:71–9.
42. Dorries K, Vogel E, Gunther S, et al. Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. *Virology* 1994;**198**:59–70.
43. Drachenberg CB, Papadimitriou JC. Polyomavirus-associated nephropathy: update in diagnosis. *Transpl Infect Dis* 2006;**8**:68–75.
44. Drachenberg CB, Papadimitriou JC, Hirsch HH, et al. Histological patterns of polyomavirus nephropathy: correlation with graft outcome and viral load. *Am J Transplant* 2004;**4**:2082–92.
45. Dubois V, Dutronc H, Lafon ME, et al. Latency and reactivation of JC virus in peripheral blood of human immunodeficiency virus type 1-infected patients. *J Clin Microbiol* 1997;**35**:2288–92.
46. Elliot B, Aromin I, Gold R, et al. 2.5 year remission of AIDS-associated progressive multifocal leukoencephalopathy with combined antiretroviral therapy. *Lancet* 1997;**349**:850.
47. Faguer S, Hirsch HH, Kamar N, et al. Leflunomide treatment for polyomavirus BK-associated nephropathy after kidney transplantation. *Transpl Int* 2007;**20**:962–9.
48. Feng H, Shuda M, Chang Y, et al. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008;**319**: 1096–100.
49. Ferenczy MW, Marshall LJ, Nelson CD, et al. Molecular biology, epidemiology, and pathogenesis of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clin Microbiol Rev* 2012;**25**:471–506.

49a. Fields BN, Knipe DM, Howley PM, et al, editors. *Fields Virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996, p. 2027–43.

50. Fioriti D, Degener AM, Mischitelli M, et al. BKV infection and hemorrhagic cystitis after allogeneic bone marrow transplant. *Int J Immunopathol Pharmacol* 2005;**18**:309–16.
51. Flaegstad T, Ronne K, Filipe AR, et al. Prevalence of anti BK virus antibody in Portugal and Norway. *Scand J Infect Dis* 1989;**21**:145–7.

52. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012;**18**:731–8.
53. Gabardi S, Waikar SS, Martin S, et al. Evaluation of fluoroquinolones for the prevention of BK viremia after renal transplantation. *Clin J Am Soc Nephrol* 2010;**5**:1298–304.
54. Gaber LW, Egidi MF, Stratta RJ, et al. Clinical utility of histological features of polyomavirus allograft nephropathy. *Transplantation* 2006;**82**:196–204.
55. Gallia GL, Houff SA, Major EO, et al. JC virus infection of lymphocytes: revisited [Review]. *J Infect Dis* 1997;**176**:1603–9.
56. Gardner SD, Field AM, Coleman DV, et al. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 1971;**19**(1):1253–7.
57. Gaynor AM, Nissen MD, Whiley DM, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog* 2007;**3**:e64.
58. Geetha D, Sozio SM, Ghanta M, et al. Results of repeat renal transplantation after graft loss from BK virus nephropathy. *Transplantation* 2011;**92**:781–6.
59. Gerber P. Patterns of antibodies to SV40 in children following the last booster with inactivated poliomyelitis vaccines. Proc Soc Exp Biol Med 1967;125:1284–7.
60. Gerdemann U, Christin AS, Vera JF, et al. Nucleofection of DCs to generate multivirus-specific T cells for prevention or treatment of viral infections in the immunocompromised host. *Mol Ther* 2009;**17**:1616–25.
61. Gerdemann U, Keirnan JM, Katari UL, et al. Rapidly generated multivirus-specific cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. *Mol Ther* 2012;**20**:1622–32.
62. Girardi AJ, Sweet BH, Slotnick VB, et al. Development of tumors in hamsters inoculated in the neonatal period with vacuolating virus, SV40. *Proc Soc Exp Biol Med* 1962;**109**:649–60.
63. Gorelik L, Reid C, Testa M, et al. Progressive multifocal leukoencephalopathy (PML) development is associated with mutations in JC virus capsid protein VP1 that change its receptor specificity. *J Infect Dis* 2011;**204**:103–14.
64. Gosert R, Rinaldo CH, Wernli M, et al. CMX001 (1-O-hexadecyloxypropyl-cidofovir) inhibits polyomavirus JC replication in human brain progenitor-derived astrocytes. *Antimicrob Agents Chemother* 2011;**55**:2129–36.
65. Goudsmit J, Baak ML, Sleterus KW, et al. Human papovavirus isolated from urine of a child with acute tonsillitis. *Br Med J (Clin Res Ed)* 1981;**283**:1363–4.
66. Goudsmit J, Wertheim-van DP, van Strien A, et al. The role of BK virus in acute respiratory tract disease and the presence of BKV DNA in tonsils. *J Med Virol* 1982;**10**:91–9.
67. Gray F, Chretien F, Vallat-Decouvelaere AV, et al. The changing pattern of HIV neuropathology in the HAART era. *J Neuropathol Exp Neurol* 2003;**62**:429–40.
68. Groom HC, Warren AY, Neal DE, et al. No evidence for infection of UK prostate cancer patients with XMRV, BK virus, Trichomonas vaginalis or human papilloma viruses. *PLoS One* 2012;**7**:e34221.
69. Hammarin AL, Bogdanovic G, Svedhem V, et al. Analysis of PCR as a tool for detection of JC virus DNA in cerebrospinal fluid for diagnosis of progressive multifocal leukoencephalopathy. *J Clin Microbiol* 1996;**34**:2929–32.
70. Han TH, Chung JY, Koo JW, et al. WU polyomavirus in children with acute lower respiratory tract infections, South Korea. *Emerg Infect Dis* 2007;**13**:1766–8.
71. Hedquist BG, Bratt G, Hammarin AL, et al. Identification of BK virus in a patient with acquired immune deficiency syndrome and bilateral atypical retinitis. *Ophthalmology* 1999;**106**:129–32.
72. Heritage J, Chesters PM, McCance DJ. The persistence of papovavirus BK DNA sequences in normal human renal tissue. *J Med Virol* 1981;**8**:143–50.
73. Herman J, Van RM, Snoeck R, et al. Polyomavirus infection in pediatric renal transplant recipients: evaluation using a quantitative real-time PCR technique. *Pediatr Transplant* 2004;**8**:485–92.
74. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. *Blood* 2010;**115**:925–35.
75. Hirsch HH, Randhawa P. BK virus in solid organ transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 4):S136–46.
76. Hirsch HH, Steiger J. Polyomavirus BK. *Lancet Infect Dis* 2003;**3**:611–23.
77. Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. *Transplantation* 2005;**79**:1277–86.
78. Hirsch HH, Knowles W, Dickenmann M, et al. Prospective study of polyomavirus type BK replication and nephropathy in renal-transplant recipients. *New Engl J Med* 2002;**347**:488–96.
79. Hormozdi DJ, Arens MQ, Le BM, et al. KI polyomavirus detected in respiratory tract specimens from patients in St. Louis, Missouri. *Pediatr Infect Dis J* 2010;**29**:329–33.
80. Huang G, Chen LZ, Qiu J, et al. Prospective study of polyomavirus BK replication and nephropathy in renal transplant recipients in China: a single-center analysis of incidence, reduction in immunosuppression and clinical course. *Clin Transplant* 2010;**24**:599–609.
81. Imperiale MJ. The human polyomaviruses, BKV and JCV: Molecular pathogenesis of acute disease and potential role in cancer. *Virology* 2000;**267**:1–7.
82. Jasani B, Cristaudo A, Emri SA, et al. Association of SV40 with human tumours. *Semin Cancer Biol* 2001;**11**:49–61.
83. Jeong BH, Lee KH, Choi EK, et al. Genotyping of the JC virus in urine samples of healthy Korean individuals. *J Med Virol* 2004;**72**:281–9.
84. Jochum W, Weber T, Frye S, et al. Detection of JC virus by anti-VP1 immunohistochemistry in brains with progressive multifocal leukoencephalopathy. *Acta Neuropathol (Berl)* 1997;**94**:226–31.
85. Johne R, Buck CB, Allander T, et al. Taxonomical developments in the family Polyomaviridae. *Arch Virol* 2011;**156**:1627–34.
86. Josephson MA, Gillen D, Javaid B, et al. Treatment of renal allograft polyoma BK virus infection with leflunomide. *Transplantation* 2006;**81**:704–10.
87. Josephson MA, Williams JW, Chandraker A, et al. Polyomavirus-associated nephropathy: update on antiviral strategies. *Transpl Infect Dis* 2006;**8**:95–101.
88. Kadambi PV, Josephson MA, Williams J, et al. Treatment of refractory BK virus-associated nephropathy with cidofovir. *Am J Transplant* 2003;**3**:186–91.
89. Kean JM, Rao S, Wang M, et al. Seroepidemiology of human polyomaviruses. *PLoS Pathog* 2009;**5**:e1000363.
90. Kidney Disease. Improving Global Outcomes: KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009;**9**(Suppl. 3):S1–155.
91. Kim JY, Koralnik IJ, LeFave M, et al. Medulloblastomas and primitive neuroectodermal tumors rarely contain polyomavirus DNA sequences. *Neuro Oncol* 2002;**4**:165–70.
92. Kling CL, Wright AT, Katz SE, et al. Dynamics of urinary polyomavirus shedding in healthy adult women. *J Med Virol* 2012;**84**:1459–63.
93. Knepper JE, diMayorca G. Cloning and characterization of BK virus-related DNA sequences from normal and neoplastic human tissues. *J Med Virol* 1987;**21**:289–99.
94. Knowles WA. Discovery and epidemiology of the human polyomaviruses BK virus (BKV) and JC virus (JCV). *Adv Exp Med Biol* 2006;**577**:19–45.
95. Knowles WA, Pipkin P, Andrews N, et al. Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. *J Med Virol* 2003;**71**:115–23.
96. Koralnik IJ, Boden D, Mai VX, et al. JC virus DNA load in patients with and without progressive multifocal leukoencephalopathy. *Neurology* 1999;**52**:253–60.
97. Kroczynska B, Cutrone R, Bocchetta M, et al. Crocidolite asbestos and SV40 are cocarcinogens in human mesothelial cells and in causing mesothelioma in hamsters. *Proc Natl Acad Sci U S A* 2006;**19**(103):14128–33.
98. Krynska B, Del Valle L, Croul S, et al. Detection of human neurotropic JC virus DNA sequence and expression of the viral oncogenic protein in pediatric medulloblastomas. *Proc Natl Acad Sci USA* 1999;**96**:11519–24.
99. Kuypers J, Campbell AP, Guthrie KA, et al. WU and KI Polyomaviruses in Respiratory Samples from Allogeneic Hematopoietic

Cell Transplant Recipients. *Emerg Infect Dis* 2012;**18**: 1580–8.
100. Lafon ME, Dutronc H, Dubois V, et al. JC virus remains latent in peripheral blood B lymphocytes but replicates actively in urine from AIDS patients. *J Infect Dis* 1998;**177**:1502–5.
101. Laghi L, Randolph AE, Chauhan DP, et al. JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. *Proc Natl Acad Sci USA* 1999;**96**:7484–9.
102. Lee KW, Lee HH, Lee DS, et al. Polymerase chain reaction for the diagnosis of human polyomavirus-associated nephropathy in renal transplant recipients. *Transplant Proc* 2004;**36**:2116–17.
103. Lednicky JA, Butel JS. Polyomaviruses and human tumors: a brief review of current concepts and interpretations. *Front Biosci* 1999;**4**:D153–64.
104. Leung AY, Chan M, Cheng VC, et al. Polyoma BK viruria in patients undergoing autologous hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2005;**35**:1029–30.
105. Leung AY, Mak R, Lie AK, et al. Clinicopathological features and risk factors of clinically overt haemorrhagic cystitis complicating bone marrow transplantation. *Bone Marrow Transplant* 2002;**29**: 509–13.
106. Leung AY, Wong AS, Chan GC, et al. Urinary reactivation of polyoma BK virus but not adenovirus in paediatric and adolescent patients after haematopoietic stem cell transplantation. *Bone Marrow Transplant* 2006;**37**(6):621–3.
107. Lewis AMJ, Levine AS, Crumpacker CS, et al. Studies of nondefective adenovirus 2-simian virus 40 hybrid viruses. V. Isolation of additional hybrids which differ in their simian virus 40-specific biological properties. *J Virol* 1973;**11**:655–64.
108. Li RM, Branton MH, Tanawattanacharoen S, et al. Molecular identification of SV40 infection in human subjects and possible association with kidney disease. *J Am Soc Nephrol* 2002;**13**:2320–30.
109. Liptak P, Kemeny E, Ivanyi B. Primer: histopathology of polyomavirus-associated nephropathy in renal allografts. *Nat Clin Pract Nephrol* 2006;**2**:631–6.
110. Markowitz RB, Eaton BA, Kubik MF, et al. BK virus and JC virus shed during pregnancy have predominantly archetypal regulatory regions. *J Virol* 1991;**65**:4515–19.
111. Martini F, Dolcetti R, Gloghini A, et al. Simian-virus-40 footprints in human lymphoproliferative disorders of HIV^- and HIV^+ patients. *Int J Cancer* 1998;**78**:669–74.
112. Matthews MR, Wang RC, Reddick RL, et al. Viral-associated trichodysplasia spinulosa: a case with electron microscopic and molecular detection of the trichodysplasia spinulosa-associated human polyomavirus. *J Cutan Pathol* 2011;**38**:420–31.
113. McClure GB, Gardner JS, Williams JT, et al. Dynamics of pregnancy-associated polyomavirus urinary excretion: a prospective longitudinal study. *J Med Virol* 2012;**84**:1312–22.
114. Melnick JL, Stinebaugh S. Excretion of vacuolating SV-40 virus (papova virus group) after ingestion as a contaminant of oral poliovaccine. *Proc Soc Exp Biol Med* 1962;**109**:965–8.
115. Meylan PRA, Vuadens P, Maeder P, et al. Monitoring the response of AIDS-related progressive multifocal leukoencephalopathy to HAART and cidofovir by PCR for JC virus DNA in the CSF. *Eur Neurol* 1999;**41**:172–4.
116. Miller AN, Glode A, Hogan KR, et al. Efficacy and safety of ciprofloxacin for prophylaxis of polyomavirus BK virus-associated hemorrhagic cystitis in allogeneic hematopoietic stem cell transplantation recipients. *Biol Blood Marrow Transplant* 2011;**17**: 1176–81.
117. Morris JA, Johnson KM, Aulisio CG, et al. Clinical and serologic responses in volunteers given vacuolating virus (SV_{40}) by respiratory route. *Proc Soc Exp Biol Med* 1961;**108**:56–9.
118. Morriss MC, Rutstein RM, Rudy B, et al. Progressive multifocal leukoencephalopathy in an HIV-infected child. *Neuroradiology* 1997;**39**:142–4.
119. Nastac E, Stoian M, Hozoc M, et al. Prevalence of hemagglutination-inhibiting antibodies to BK virus in patients with endemic Balkan nephropathy. *Virologie* 1982;**33**:169–70.
120. Newman JS, Baskin GB, Frisque RJ. Identification of SV40 in brain, kidney and urine of healthy and SIV-infected rhesus monkeys. *J Neurovirol* 1998;**4**:394–406.
121. Nickeleit V, Hirsch HH, Binet IF, et al. Polyomavirus infection of renal allograft recipients: From latent infection to manifest disease. *J Am Soc Nephrol* 1999;**10**:1080–9.
122. Nickeleit V, Klimkait T, Binet IF, et al. Testing for polyomavirus type BK DNA in plasma to identify renal-allograft recipients with viral nephropathy. *N Engl J Med* 2000;**342**:1309–15.
123. Norja P, Ubillos I, Templeton K, et al. No evidence for an association between infections with WU and KI polyomaviruses and respiratory disease. *J Clin Virol* 2007;**40**:307–11.
124. O'Reilly RJ, Lee FK, Grossbard E, et al. Papovavirus excretion following marrow transplantation: incidence and association with hepatic dysfunction. *Transplant Proc* 1981;**13**:262–6.
125. Padgett BL, Walker DL. Prevalence of antibodies in human sera against JC virus, an isolate from a case of progressive multifocal leukoencephalopathy. *J Infect Dis* 1973;**127**:467–70.
126. Padgett BL, Walker DL, ZuRhein GM, et al. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet* 1971;**1**:1257–60.
127. Painter W, Robertson A, Trost LC, et al. First pharmacokinetic and safety study in humans of the novel lipid antiviral conjugate CMX001, a broad-spectrum oral drug active against double-stranded DNA viruses. *Antimicrob Agents Chemother* 2012;**56**: 2726–34.
128. Pappo O, Demetris AJ, Raikow RB, et al. Human polyoma virus infection of renal allografts: histopathologic diagnosis, clinical significance, and literature review. *Mod Pathol* 1996;**9**:105–9.
129. Patel NC, Vilchez RA, Killen DE, et al. Detection of polyomavirus SV40 in tonsils from immunocompetent children. *J Clin Virol* 2008;**43**:66–72.
130. Portolani M, Marzocchi A, Barbanti-Brodano G, et al. Prevalence in Italy of antibodies to a new human papovavirus (BK virus). *J Med Microbiol* 1974;**7**:543–6.
131. Power C, Nath A, Aoki FY, et al. Remission of progressive multifocal leukoencephalopathy following splenectomy and antiretroviral therapy in a patient with HIV infection. *N Engl J Med* 1997;**336**:661–2.
132. Procopio A, Strizzi L, Vianale G, et al. Simian virus-40 sequences are a negative prognostic cofactor in patients with malignant pleural mesothelioma. *Genes Chrom Cancer* 2000;**29**:173–9.
133. Ramos E, Drachenberg CB, Portocarrero M, et al. BK virus nephropathy diagnosis and treatment: experience at the University of Maryland Renal Transplant Program. *Clin Transpl* 2002; 143–53.
134. Randhawa P, Viscidi R, Carter JJ, et al. Identification of species-specific and cross-reactive epitopes in human polyomavirus capsids using monoclonal antibodies. *J Gen Virol* 2009;**90**:634–9.
135. Randhawa PS, Finkelstein S, Scantlebury V, et al. Human polyoma virus-associated interstitial nephritis in the allograft kidney. *Transplantation* 1999;**67**:103–9.
136. Ricciardiello L, Chang DK, Laghi L, et al. Mad-1 is the exclusive JC virus strain present in the human colon, and its transcriptional control region has a deleted 98-base-Pair sequence in colon cancer tissues. *J Virol* 2001;**75**:1996–2001.
137. Ricciardiello L, Laghi L, Ramamirtham P, et al. JC virus DNA sequences are frequently present in the human upper and lower gastrointestinal tract. *Gastroenterology* 2000;**119**:1228–35.
138. Richardson EPJ. Progressive multifocal leukoencephalopathy. *N Engl J Med* 1961;**265**:815–23.
139. Rinaldo CH, Gosert R, Bernhoff E, et al. 1-O-hexadecyloxypropyl cidofovir (CMX001) effectively inhibits polyomavirus BK replication in primary human renal tubular epithelial cells. *Antimicrob Agents Chemother* 2010;**54**:4714–22.
140. Rizzo P, Carbone M, Fisher SG, et al. Simian virus 40 is present in most United States human mesotheliomas, but it is rarely present in non-Hodgkin's lymphoma. *Chest* 1999;**116**:470S–3S.
141. Rollison DE, Helzlsouer KJ, Alberg AJ, et al. Serum antibodies to JC virus, BK virus, simian virus 40, and the risk of incident adult astrocytic brain tumors. *Cancer Epidemiol Biomarkers Prev* 2003;**12**:460–3.
142. Rosen S, Harmon W, Krensky AM, et al. Tubulo-interstitial nephritis associated with polyomavirus (BK type) infection. *N Engl J Med* 1983;**308**:1192–6.
143. Rossi A, Delbue S, Mazziotti R, et al. Presence, quantitation and characterization of JC virus in the urine of Italian immunocompetent subjects. *J Med Virol* 2007;**79**:408–12.
144. Sadler GM, Halbert AR, Smith N, et al. Trichodysplasia spinulosa associated with chemotherapy for acute lymphocytic leukaemia. *Australas J Dermatol* 2007;**48**:110–14.

145. Sadler M, Chinn R, Healy J, et al. New treatments for progressive multifocal leukoencephalopathy in HIV-1-infected patients. *AIDS* 1998;**12**:533–5.
146. Saitoh K, Sugae N, Koike N, et al. Diagnosis of childhood BK virus cystitis by electron microscopy and PCR. *J Clin Pathol* 1993;**46**:773–5.
147. Sandler ES, Aquino VM, Goss-Shohet E, et al. BK papova virus pneumonia following hematopoietic stem cell transplantation. *Bone Marrow Transplant* 1997;**20**:163–5.
148. Schatzl HM, Sieger E, Jager G, et al. Detection by PCR of human polyomaviruses BK and JC in immunocompromised individuals and partial sequencing of control regions. *J Med Virol* 1994;**42**:138–45.
149. Seguin SP, Ireland AW, Gupta T, et al. A screen for modulators of large T antigen's ATPase activity uncovers novel inhibitors of Simian Virus 40 and BK virus replication. *Antiviral Res* 2012;**96**:70–81.
150. Sener A, House AA, Jevnikar AM, et al. Intravenous immunoglobulin as a treatment for BK virus associated nephropathy: one-year follow-up of renal allograft recipients. *Transplantation* 2006;**81**:117–20.
151. Reference deleted in proofs.
152. Shah KV, Daniel RW, Warszawski RM. High prevalence of antibodies to BK virus, an SV40-related papovavirus, in residents *of Maryland J Infect Dis* 1973;**128**:784–7.
153. Shah KV, Daniel RW, Strickler HD, et al. Investigation of human urine for genomic sequences of the primate polyomaviruses simian virus 40, BK virus, and JC virus. *J Infect Dis* 1997;**176**:1618–21.
154. Sharma AP, Moussa M, Casier S, et al. Intravenous immunoglobulin as rescue therapy for BK virus nephropathy. *Pediatr Transplant* 2009;**13**:123–9.
155. Sharma PM, Gupta G, Vats A, et al. Phylogenetic analysis of polyomavirus BK sequences. *J Virol* 2006;**80**:8869–79.
156. Shuda M, Feng H, Kwun HJ, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci U S A* 2008;**105**:16272–7.
157. Soriano F, Shelburne CE, Gokcen M. Simian virus 40 in a human cancer. *Nature* 1974;**249**:421–4.
158. Stoian M, Hozoc M, Iosipenco M, et al. Serum antibodies to papova viruses (BK and SV 40) in subjects from the area with Balkan endemic nephropathy. *Virologie* 1983;**34**:113–17.
159. Strickler HD, Rosenberg PS, Devesa SS, et al. Contamination of poliovirus vaccines with simian virus 40 (1955-1963) and subsequent cancer rates. *JAMA* 1998;**279**:292–5.
160. Sullivan CS, Grundhoff AT, Tevethia S, et al. SV40-encoded microRNAs regulate viral gene expression and reduce susceptibility to cytotoxic T cells. *Nature* 2005;**435**:682–6.
161. Sundsfjord A, Osei A, Rosenqvist H, et al. BK and JC viruses in patients with systemic lupus erythematosus: prevalent and persistent BK viruria, sequence stability of the viral regulatory regions, and nondetectable viremia. *J Infect Dis* 1999;**180**:1–9.
162. Sundsfjord A, Spein AR, Lucht E, et al. Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. *J Clin Microbiol* 1994;**32**:1390–4.
163. Sweet BH, Hilleman MR. The vacuolating virus, $S.V._{40}$. *Proc Soc Exp Biol Med* 1960;**105**:420–7.
164. Taguchi F, Hara K, Kajioka J, et al. Isolation of BK virus from a patient with systemic lupus erythematosus (SLE). *Microbiol Immunol* 1979;**23**:1131–2.
165. Taguchi F, Nagaki D, Iwasaki K, et al. Prevalence of BK papovavirus antibody in patients with antinuclear antibodies. *Kitasato Arch Exp Med* 1980;**53**:29–35.
166. Tan BH, Busam KJ. Virus-associated trichodysplasia spinulosa. *Adv Anat Pathol* 2011;**18**:450–3.
167. Tantisiriwat W, Tebas P, Clifford DB, et al. Progressive multifocal leukoencephalopathy in patients with AIDS receiving highly active antiretroviral therapy. *Clin Infect Dis* 1999;**28**:1152–4.
168. Telenti A, Aksamit AJ Jr, Proper J, et al. Detection of JC virus DNA by polymerase chain reaction in patients with progressive multifocal leukoencephalopathy. *J Infect Dis* 1990;**162**:858–61.
169. Teofilo E, Gouveia J, Brotas V, et al. Progressive multifocal leukoencephalopathy regression with highly active antiretroviral therapy. *AIDS* 1998;**12**:449.
170. Tominaga T, Yogo Y, Kitamura T, et al. Persistence of archetypal JC virus DNA in normal renal tissue derived from tumor-bearing patients. *Virology* 1992;**186**:736–41.
171. Trampe AK, Hemmelmann C, Stroet A, et al. Anti-JC virus antibodies in a large German natalizumab-treated multiple sclerosis cohort. *Neurology* 2012;**78**:1736–42.
172. Vallbracht A, Lohler J, Gossmann J, et al. Disseminated BK type polyomavirus infection in an AIDS patient associated with central nervous system disease. *Am J Pathol* 1993;**143**:29–39.
173. van der Meijden E, Janssens RW, Lauber C, et al. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromised patient. *PLoS Pathog* 2010;**6**: e1001024.
174. van der Meijden E, Kazem S, Burgers MM, et al. Seroprevalence of trichodysplasia spinulosa-associated polyomavirus. *Emerg Infect Dis* 2011;**17**:1355–63.
175. Van Ghelue M, Khan MT, Ehlers B, et al. Genome analysis of the new human polyomaviruses. *Rev Med Virol* 2012;doi:10.1002/rmv.1711.
176. Vanchiere JA, Abudayyeh S, Copeland CM, et al. Polyomavirus shedding in the stool of healthy adults. *J Clin Microbiol* 2009;**47**:2388–91.
177. Vanchiere JA, Nicome RK, Greer JM, et al. Frequent detection of polyomaviruses in stool samples from hospitalized children. *J Infect Dis* 2005;**192**:658–64.
178. Vanchiere JA, White ZS, Butel JS. Detection of BK virus and simian virus 40 in the urine of healthy children. *J Med Virol* 2005;**75**:447–54.
179. Vats A, Randhawa PS, Shapiro R. Diagnosis and treatment of BK virus-associated transplant nephropathy. *Adv Exp Med Biol* 2006;**577**:213–27.
180. Vazeux R, Cumont M, Girard PM, et al. Severe encephalitis resulting from coinfections with HIV and JC virus. *Neurology* 1990;**40**:944–8.
181. Vera-Sempere FJ, Rubio L, Felipe-Ponce V, et al. Renal donor implication in the origin of BK infection: analysis of genomic viral subtypes. *Transplant Proc* 2006;**38**:2378–81.
182. Vilchez RA, Madden CR, Kozinetz CA, et al. Association between simian virus 40 and non-Hodgkin lymphoma. *Lancet* 2002;**359**:817–23.
183. Viscidi RP, Khanna N, Tan CS, et al. John Cunningham virus antibody and viremia as predictors of progressive multifocal leukoencephalopathy in human immunodeficiency virus-1-infected individuals. *Clin Infect Dis* 2011;**53**:711–15.
184. Viscidi RP, Rollison DE, Sondak VK, et al. Age-specific seroprevalence of Merkel cell polyomavirus, BK virus, and JC virus. *Clin Vaccine Immunol* 2011;**18**:1737–43.
185. Viscidi RP, Rollison DE, Viscidi E, et al. Serological cross-reactivities between antibodies to simian virus 40, BK virus, and JC virus assessed by virus-like-particle-based enzyme immunoassays. *Clin Diagn Lab Immunol* 2003;**10**:278–85.
186. Voltz R, Jager G, Seelos K, et al. BK virus encephalitis in an immunocompetent patient. *Arch Neurol* 1996;**53**:101–3.
187. Wadei HM, Rule AD, Lewin M, et al. Kidney transplant function and histological clearance of virus following diagnosis of polyomavirus-associated nephropathy (PVAN). *Am J Transplant* 2006;**6**:1025–32.
188. Weggen S, Bayer TA, Von Deimling A, et al. Low frequency of SV40, JC and BK polyomavirus sequences in human medulloblastomas, meningiomas and ependymomas. *Brain Pathol* 2000;**10**: 85–92.
189. Yapa HM, McLornan DP, Raj K, et al. Pneumonitis post-haematopoeitic stem cell transplant: cytopathology clinches diagnosis. *J Clin Virol* 2012;**55**:278–81.
190. Zurhein G, Chou SM. Particles resembling papova viruses in human cerebral demyelinating disease. *Science* 1965;**148**:1477–9.

CHAPTER 154

Human Papillomaviruses

Colleen B. Nash • Gail J. Harrison • Kenneth A. Alexander

The human papillomaviruses (HPVs) are members of the virus family *Papillomaviridae*. More than 200 HPV genotypes have been identified. The double-stranded DNA genome of the HPV genome is approximately 8000 base pairs; intact virions are approximately 55 nm in diameter. Although most HPV infections are benign, HPVs can cause malignant transformation of squamous epithelia in both immunocompetent and immunodeficient hosts. HPVs also can cause a variety of benign cutaneous proliferations, including common skin warts. HPVs also have been associated with respiratory papillomatosis, which is a histologically benign but potentially life-threatening process. Our understanding of the complex role HPVs play in these diverse clinical conditions remains incomplete, and management of these diseases remains an ongoing challenge. Recently, the licensure of two prophylactic vaccines against HPVs provides hope that the public health impact of these viruses will decrease.

HISTORY

Warts, or papillomas, have been recognized for centuries to occur at a variety of different body sites, in particular, the skin, genital tract, oral cavity, conjunctiva, and respiratory tract. The viral etiology of warts was demonstrated in 1907, when human volunteers were inoculated experimentally with a cell-free extract prepared from wart tissue.[64] These early experiments also suggested that warts were transmissible from person to person. When electron microscopy became available in the 1940s, virus particles were visualized, initially in skin warts and subsequently in genital warts, thereby confirming the viral etiology of these lesions. However, despite the abundance of virus particles seen in some lesions, virologic investigation of HPV-associated disease processes was hampered by the inability to propagate papillomaviruses in either cell culture or in laboratory animals. The limited amount of information available from the study of virus particles obtained directly from wart tissue led to speculation, in the 1960s, that HPVs were composed of a single virus type. Scientists further theorized that the specific body site and epithelium involved, rather than the virus type, were responsible for the characteristic morphology and disease process.[259] With the advent of molecular biologic techniques in the 1970s, however, more than 70 different types of HPV were recognized, and researchers quickly recognized that specific clinical diseases were associated with infection with specific HPV types. Most recently, HPV infection of the genital tract has been recognized as one of the most prevalent sexually transmitted infections, and a causal link between HPV infection and squamous carcinomas of the cervix is now well accepted. Challenges for the future revolve around developing effective treatment strategies because current measures are palliative, at best, and most lesions recur. New antiviral chemotherapeutic agents are being developed, and immunomodulators such as interferon provide promise for the treatment of serious disease caused by HPVs.

VIROLOGY

The family *Papillomaviridae* are small, nonenveloped viruses with a diameter of about 55 nm and include the HPVs. *Papillomaviridae* have a capsid composed of 72 capsomeres arranged in icosahedral symmetry. The papillomavirus genome is composed of double-stranded circular DNA that is approximately 8 kb in length. The complete nucleotide sequence is available for all clinically relevant HPVs, and partial sequence information is available for more than 120 HPV types.[82] Sequence conservation between the many HPV types is substantial. The papillomavirus genome is divided into three regions: an early region that encodes the proteins required for viral replication (E1, E4), transcription control (E2), and cell transformation (E5, E6, E7) (Table 154-1); a late region that encodes the viral major and minor capsid proteins (L1 and L2); and the viral long control region, which contains the origin of replication and many control elements for viral transcription and replication.[97,114,164,232-234] The virus appears to replicate solely in the nucleus of the cell in association with low-molecular-weight histones.[167,333]

Papillomaviruses display a high degree of species, tissue, and cellular specificity. HPVs appear to infect only humans; most animal papillomaviruses also do not infect other species. Although all HPVs infect epithelial surfaces, each HPV type has a tropism for specific epithelia.[107,306,328,329] The more than 200 HPV types that have been recognized may be classified according to the type of epithelia they infect (cutaneous or mucosal).[236] The mucosal HPV types are further subclassified according to the oncogenic potential of each virus (Table 154-2). The common cutaneous HPV types are found in a variety of skin warts (e.g., palmar warts, plantar warts, flat warts) and are uniformly benign.[32,33,241,304,322] The mucosal HPVs occur mainly in the anogenital tract but also infect and produce disease at mucosal sites in the respiratory tract,

TABLE 154-1 **Major Human Papillomavirus Genes**

Gene	Protein Products and Function
Early (E) Region	
E1	Viral regulatory protein that initiates viral DNA replication
E2	Viral regulatory protein that controls replication and inhibits or activates early transcription of the viral genome
E3	Unknown
E4	A late viral protein that controls viral maturation; expressed in terminally differentiated keratinocytes
E5	Major transforming protein; causes cellular proliferation
E6	Major transforming oncoprotein; associates with the cellular target, TP53, a tumor suppressor protein, and promotes its proteolytic degradation; causes cellular proliferation and perturbation of keratinocyte differentiation
E7	Major transforming protein; associates with the cellular target pRB and inactivates its cell cycle restriction function; causes cellular proliferation and perturbation of keratinocyte differentiation
E8	Unknown; ? regulates viral DNA replication
Late (L) Region	
L1	Major structural viral capsid protein
L2	Minor structural viral capsid protein

the oropharyngeal cavity, and the conjunctiva. The mucosal HPVs also are subgrouped into low-, high-, and intermediate- or moderate-risk types, depending on the frequency with which they are found in invasive cancers.[26,172,264,295,317] A third group of HPVs cause cutaneous diseases in a small group of patients with epidermodysplasia verruciformis (EV, also known as Lewandowsky-Lutz dysplasia or Lutz-Lewandowsky epidermodysplasia verruciformis), which is an extremely rare autosomal recessive disorder marked by extreme warty growths, particularly on the skin of the extremities.[244] These EV-associated viruses (most commonly HPV types 5 and 8, but there are others) have variable oncogenic activity and can underlie the development of squamous tumors in sun-exposed areas.[38,178,220,291,333]

The life cycle of HPVs is intimately entwined with the cell cycle and differentiation of epithelial cells.[92] HPV infection occurs after damage to the mucosa, allowing introduction of the virus into basal keratinocytes. Early infection is marked by E2 protein–regulated transcription of the viral early proteins. HPV infections are maintained in basal keratinocytes. Within basal keratinocytes, two forms of HPV DNA replication occur: maintenance replication and vegetative replication. Maintenance replication occurs in nondifferentiating basal keratinocytes at low levels sufficient only to ensure that HPV DNA is propagated to daughter keratinocytes; viral protein expression is minimal and limited to the viral early proteins. Vegetative replication occurs in differentiating keratinocytes as they enter the stratum spinosum. In the stratum spinosum, high-level viral replication occurs, with expression of the late proteins and production of complete virions. Progeny viruses are not released until fully differentiated keratinocytes are sloughed from the epithelial surface.[205,326]

Interestingly, the HPV genome does not encode any viral DNA or RNA polymerase, rendering the virus wholly dependent on host enzymes for replication. In view of this absolute dependence on host cell DNA and RNA polymerases, replication of papillomaviruses must occur at a time in the host cell cycle during which cellular polymerases, replication factors, and nucleoside triphosphates are in abundance (i.e., S phase). To drive host cells from G0 into S phase, HPVs express the E6 and E7 oncoproteins. Although the E6 and E7 oncoproteins have many identified biochemical activities, the dominant activities of E6 and E7 are as follows: The E7 oncoprotein activates the cell cycle by displacing the E2F regulatory factor from the retinoblastoma tumor suppressor (pRB). This displacement of E2F from pRB allows E2F entry into the nucleus, where E2F activates genes that trigger cell cycle progression from G0 into S phase, with consequent activation of cellular (and viral) DNA replication. To attenuate the normal cellular protective response to unscheduled DNA replication (TP53-induced cellular apoptosis), papillomaviruses express the E6 oncoprotein. The E6 oncoprotein forms a stable complex with the TP53 tumor suppressor. This E6:TP53 complex is then bound by the E6-associated protein (E6-AP), a ubiquitin ligase that, by ubiquitinating TP53, earmarks TP53 for destruction on the proteasome. By displacing E2F from pRB, and by then degrading TP53, papillomaviruses, in effect, "activate the engine" of cell proliferation and then "take away the brakes." The oncogenic activity of HPVs lies largely with the E6 and E7 oncoproteins. Although E6 and E7 expression appear to be tightly regulated in HPV infection, HPVs can integrate, apparently at random, into the host cell genome, leaded to unregulated E6 and E7 expression. Most, but not all, HPV-induced malignancies contain chromosomally integrated copies of the HPV genome.[28,204,326]

Because full epithelial cell differentiation and keratinization of squamous epithelial cells are required for the HPVs to replicate completely, and because such differentiation is not achieved in conventional cell culture, HPVs cannot be propagated using standard tissue culture methods. Limited propagation of HPVs in keratinocyte raft cultures that recapitulate the structure of a

TABLE 154-2 **Human Papillomavirus (HPV) Types and Associated Clinical Conditions**

Condition	Usual Location	Morphology	HPV Type
Cutaneous (Skin) Warts			
Common (verruca vulgaris)	Hands, lips, extremities	Multiple, dome shaped	2, 4
Plantar (verruca plantaris)	Bottom of feet	Single, painful	1
Flat (verruca plana)	Arms, face, knees	Multiple	3, 10, 28, 41
Filiform	Face, neck	Multiple, threadlike	2, 4
Mosaic	Feet, hands	Multiple, superficial	7
Butcher's	Hands	Multiple, dome shaped	7
Epidermodysplasia verruciformis	Face, trunk, extremities	Multiple flat warts or reddish-brown plaques	5, 8, 9, 12, 14, 15, 17, 19-25, 36-38, 47, 49
Immunosuppressed patients	Face, trunk, extremities	Dome-shaped or epidermodysplasia verruciformis–like plaques; may be persistent or progressive	1-5, 8, 10, 20, 23, 28, 49
Mucosal (Anogenital) Warts			
Subclinical	Cervix	Asymptomatic	6, 11, 13, 16, 18, 30-35, 39, 40, 42-45, 51-59
Condylomata acuminata	Cervix, vulva, urethra, anus, penis, scrotum	Multiple exophytic, pink, gray	6, 11, 16
Flat condylomata	Cervix	Asymptomatic, flat plaques	6, 11, 16, 18, 31
Giant condylomata acuminata (Buschke-Löwenstein tumors)	Perirectal area	Large, tumor-like	6, 11
Bowenoid papulosis	Penis, vulva, perirectal area	Multiple, large	16
Cervical cancer	Cervix	Asymptomatic; pigmented papillomas; erythematous or white plaques; ulcerations; mass lesion	Strong association: 16, 18, 31, 45 Moderate association: 30, 33, 35, 39, 51, 52, 56, 58, 59, 68 Weak association: 6, 11, 26, 34, 40, 43, 44, 53-55, 57, 62, 66, 74
Vaginal cancer	Vagina	Asymptomatic; ulcerative mass lesions; vascular pattern with punctation and mosaicism	16
Vulvar cancer	Vulva	Asymptomatic; pigmented papillomas; erythematous or white plaques; ulcerations; mass lesion	16
Penile cancer	Penis	Painless, ulcerative mass lesion	16
Anal cancer	Perianal area, anal canal	Asymptomatic; pigmented papillomas; erythematous or white plaques; ulcerations; mass lesion	16, 18, 31
Ovarian cancer	Ovaries	Unknown	6?, 16?, 18?
Mucosal (Other) Warts			
Respiratory papillomatosis	Larynx, trachea, bronchi, lungs	Multiple papillomas	6, 11
Nasal and paranasal papillomas	Nose, paranasal sinuses	Single or multiple papillomas	6, 11, 57, 57b
Focal epithelial hyperplasia (Heck disease)	Oral cavity	Discrete, multiple nodules	13, 32
Oral cavity papillomas	Gums, buccal mucosa, soft palate, tonsils	Single or multiple papillomas	6, 11, 16
Conjunctival papillomas	Conjunctivae	Single or multiple papillomas	6, 11, 16
Giant cell hepatitis	Liver	Unknown	6?

differentiating epithelium is now performed routinely in research laboratories but has no clinical application. Research laboratories have also propagated papillomaviruses successfully by inoculating virion extracts into susceptible tissue (typically human foreskins) and transplanting this tissue into athymic nude mice.[143,158] Nonetheless, the laboratory and clinical study of HPVs and HPV-associated diseases has been and remains heavily dependent on the use of molecular biology techniques, in particular, polymerase chain reaction (PCR) assay.

EPIDEMIOLOGY

Among humans, HPVs are ubiquitous and, as a group, are the cause of the most common sexually transmitted infection. Although HPVs infect nearly all individuals of both sexes at some point in their lives, most infections are asymptomatic; only the minority of HPV infections come to medical attention. Even though a large proportion of HPV infection is subclinical and does not cause significant disease, the importance of HPV infection lies in the ability of HPVs to cause malignant transformation of squamous epithelial mucosal surfaces, potentially leading to significant cancer morbidity and mortality. Recently developed HPV vaccines have made important strides toward preventing HPV-associated neoplasias and malignancies.[91,115]

HPVs are transmitted by direct skin-to-skin contact, with most sexually transmitted infection resulting from penetrative vaginal and/or anal intercourse. Other types of sexual contact, such as oral sex, and even indirect genital contact, can also transmit the virus.[115,332] Common body warts are transmitted by direct contact, often after minor skin trauma.[170] There is also evidence of latent epithelial disease activation in immunocompromised individuals, including solid-organ transplant recipients and individuals with human immunodeficiency virus (HIV) infection.[171,262,263] After vaginal births, HPV DNA can be detected in the mouths of up to 30 percent of neonates.[50,253,305] Associations between the types of HPV DNA found in maternal and paternal oral and genital mucosa and that found in infant's oral mucosa have been found, although there is a high rate of HPV genotype discordance.[62,150,206,223] Perinatal infection after vertical transmission during childbirth is implicated in recurrent RRP as well as hepatitis in some cases.[85,227,276,337] There have been strong associations found between RRP in children and genital warts present in the mother at the time of delivery.[1,50,303]

The incubation period for HPV infection varies greatly but is approximately 3 months.[173] Nonetheless, disease can appear weeks to years after exposure, with invasive cancers typically developing after a decade or more.

It is estimated that, at any given time, nearly 20 million Americans are infected with HPV, with approximately 6 million new cases occurring each year, and about the same number of individuals clearing their infection each year. Most individuals with HPV infection do not show any signs or symptoms of infection; most infections are transient and cleared uneventfully with median time to clearance of 8 months.[137] Seventy percent of infections are cleared by 12 months; more than 80 percent of genital HPV infections clear spontaneously by 24 months. HPV 16 is associated with increased persistence but still with a high rate (70%) of clearance within 24 months.* For men and women, the lifetime risk for developing a genital HPV infection exceeds 50 percent; estimates are that 80 percent of individuals will have had a genital HPV infection by age 50 years. The highest rates of HPV infection are seen in females younger than 25 years old.[47,142] Higher prevalence of HPV 6 and 11 is found in girls and young women 14 to 19 years old, whereas a higher prevalence of HPV 16 and 18 is found in those 20 to 24 years old.* Most HPV infections are acquired within the first few years after sexual debut. Not surprisingly, the risk for HPV infection is proportional to the number of sexual contacts. Other important risk factors for HPV infection include age older than 30 years, parity, smoking status, use of oral contraceptives, single marital status, and immunosuppression.[52,139,258,260]

The clinical presentation of HPV infection depends on the anatomic location and the type of HPV with which an individual is infected. Males and females infected with HPV experience similar clinical disease processes with related cutaneous warts while experiencing differing manifestations of anogenital condyloma and cancers based on anatomy. In both genders, rates of HPV-associated oropharyngeal and anogenital cancers are increasing.[41]

The Centers for Disease Control and Prevention (CDC) estimates that, in the United States, nearly 30,000 cases of HPV-associated cancers occur annually, with two thirds of those cases affecting females. Cervical cancer is the most common HPV-associated cancer overall; the second most common group of HPV-associated cancers comprises those of the oral cavity and the oropharynx. HPV-associated cancer rates vary greatly by state in both males and females, with higher rates (11 to 16/100,000) seen in southern, southeastern, and mideastern seaboard cities and states (e.g., Louisiana, Kentucky, Tennessee, and the District of Columbia). Rates of HPV-associated cancers in other parts of the country (e.g., Colorado, Utah, and Maryland) are lower (4 to 8/100,000).[54]

Female-Specific HPV-Associated Malignancies

In females, HPV infections can progress to vulvar, vaginal, and cervical cancers. Persistent HPV infection is associated with increased risk for progressive epithelial pathologic process and cancer.

Although the incidence of cervical cancer has declined significantly in the United States in the past 50 years (largely through the development of universal cervical cytology screening programs), cervical cancer remains the second leading cause of cancer death in women worldwide, with more than 80 percent of cases occurring in the developing world.[37] Most cervical cancers are diagnosed in females older than 30 years old. In contrast to vulvar and vaginal cancer, essentially all cervical cancer is HPV associated.[54,312] In the United States, Hispanic (12/100,000) and black (10/100,000) females have higher rates of cervical cancer than females of all other races (7/100,000). Approximately 4,000 females die annually of cervical cancer.[21,54,312] Worldwide, the mortality to incidence ratio of cervical cancer is 52 percent. Globally, nearly 300,000 women die of cervical cancer each year, the majority of whom are in developing countries.[224,283,327]

*References 14, 61, 137, 151 200, 255.

*References 16, 17, 19, 87, 192, 197, 198, 252.

The most studied relationship between HPV and cancer risk is cervical cancer and its preceding grades of dysplasia.[205,207,210,268,311] It is estimated that approximately 10 percent of all women in the world have a cervical HPV infection at any given time, with prevalence of infection being higher in the developing world. Some of the highest rates of infection are seen in Eastern Africa, whereas some of the lowest rates are seen in Southeast Asia. In all developed and developing countries, the prevalence of HPV infection is highest among young women; infection rates decline in younger middle-aged women, with a second resurgence of HPV infection in the perimenopausal age range. The cause for this perimenopausal resurgence of HPV infection phenomenon is not known but may be due to changes in sexual behaviors and acquisition of new HPV infection in middle age, reactivation of latent infection, or a high-exposure population promoting a cohort effect.*

HPV-16 is the most common HPV type in cervical dysplasias and malignancies. HPV-18 is the second most prevalent, with some intercontinental variation seen between Europe, Asia, and the Americas. HPV-16 is the most oncogenic, responsible for nearly half of all cervical cancer. In women with invasive cervical cancer, HPV-16 and HPV-18 together contribute to approximately 70 percent of all cases.[67]

Similar to cervical cancer, nearly all new cases (90%) of anal cancer are HPV associated. In U.S. women there are 3,000 new cases (and 500 deaths) of HPV-associated anal cancers annually. HPV-associated anal cancers affect white females (2/100,000) disproportionately more than females of all other races (0.4 to 1.4/100,000). The incidence of anal cancers in females in the general population is increasing at a rate of nearly 2 percent per year.[54,115] Among HPV-positive females, the prevalence of HPV-related anal cancers is even higher than that for cervical cancer.[224,225]

Since 1986, in the United States the rate of vulvar cancers has increased. In the United States, the overall incidence rate of vulvar cancers is 2.3/100,000 females. Each year, there are approximately 3,000 new cases of vulvar cancer. Half of these cases are HPV associated.[115,140] Worldwide, vulvar cancer contributes to approximately 4 percent of all gynecologic cancers.[8,38]

Vaginal cancer is much rarer than vulvar cancer and occurs most often in females older than 80 years old. In the United States, HPV-associated vaginal cancer cases account for over 700 (65%) new cases annually. Vaginal cancers affect black females (0.7/100,000) disproportionately more than females of all other races (~0.4/100,000).[54,115]

Male-Specific HPV-Associated Malignancies

There are nearly 1,700 new cases (and 250 deaths) of HPV-associated anal cancers diagnosed in males annually. Black males (1.6/100,000) are disproportionately more affected than males of all other races (0.2 to 1.0/100,000).[54,115] Men who have sex with men are a unique population at high risk for persistent low- and high-grade anal lesions that can progress to invasive cancer.[121,321]

Penile cancer is very rare and occurs most commonly in males older than 60 years of age. Approximately 1000 cases of penile cancer occur in the United States each year, of which 35 percent are believed to be HPV associated. Penile cancers affect Hispanic males (1.3/100,000) disproportionately more than males of all other ethnicities (0.7/100,000).[54,115] The incidence of penile cancer is much lower than that of cervical cancer. Penile circumcision affords some degree of protection against penile cancers.

In addition to gender-specific HPV-associated invasive disease, other common HPV-associated disease processes, including cutaneous warts, anogenital warts, and oropharyngeal cancers, affect both sexes similarly.

A progressively higher prevalence of mucosal disease occurs in adolescence and young adulthood. In both males and females, anogenital warts (condylomata acuminata) occur in and around the genitalia and anus.[124] All anogenital warts are caused by HPV, with more than 90 percent caused by HPV types 6 and 11.[121] Every year in the United States there are approximately 1 million new cases of anogenital condylomata diagnosed, often first noticed within weeks or months of exposure. The majority of infections last longer than 4 months.[162] Individuals will have varying clinical courses, some with very few lesions lasting only a few months and some with progressive lesions increasing in size and number and causing significant morbidity.[37,213]

Cancer of the oral and oropharyngeal cavities has gained increasing attention because its relationship to HPV infection has more recently been elucidated. HPV-associated oropharyngeal cancers are increasing in incidence in both males and females. In the United States, HPV-associated oropharyngeal cancers account for nearly 12,000 (60%) new cases annually. HPV-associated oral cancers affect males (9,300 cases) more than females (2,300 cases), with much lower rates found in Asian/Pacific Islander males (3/100,000) and females (0.5/100,000) compared with males and females of all other races (6/100,000 and 0.5/100,000, respectively).[312] In both males and females, the incidence of HPV-associated oral and oropharyngeal cancers increases dramatically after 50 years of age.[43,115,177] Traditionally, oropharyngeal cancer risk has been associated with advanced age and with alcohol and tobacco use. In contrast, HPV-associated oropharyngeal cancers occur in younger adults without strong histories of alcohol or tobacco use. Although the incidence of non–HPV-associated oral and oropharyngeal cancers is declining, the incidence of HPV-associated cancers is increasing at a rate of approximately 5 percent per year.[55,147] Among all oropharyngeal cancers, the prevalence rates of HPV-associated oropharyngeal cancers have risen from 33 percent of new cases in the 1980s, to 70 percent in the 1990s, and over 80 percent in the 2000s, particularly among white males.[42] Those at increased risk for developing HPV-associated head and neck cancer compared with those with HPV-negative disease are generally white, younger, and of a higher socioeconomic status and have a higher education and more lifetime oral sex partners.[22,116]

*References 135, 154, 161, 210, 245, 246, 298-300.

The prevalence of oral HPV infection is estimated between 2 and 3 percent of young adults (18 to 30 years old).[238] Those with concurrent HIV infection, and those who are smokers, have a higher prevalence of oral HPV.[55,57,65,66] Even though white males experience the majority of HPV-associated oropharyngeal disease burden, they also show improved survival over females.[219] Rates of HPV-associated oropharyngeal disease have increased so markedly within the past 20 years in the United States that by 2020 it is anticipated that the number of HPV-associated oropharyngeal cancers will exceed the number of incident cervical cancers.[57,195]

CLINICAL MANIFESTATIONS

Cutaneous Warts

Common cutaneous skin warts, or verrucae, have variable morphology and may appear at any location on the skin.[56,278] Common skin warts, or verrucae vulgaris, present as well-demarcated, dome-shaped papules with multiple conical projections (papillomatosis) that give the surface of the wart a rough appearance and texture. Flat warts, or verrucae planae, in contrast, do not have papillomatosis or hyperkeratosis and often occur in clusters. Warts occasionally have a mosaic appearance; such warts occur most commonly on the plantar surface of the feet and spread superficially over the skin. Warts may also be filiform and appear as threadlike warts on the face and neck.[48]

Common warts generally occur on the hands, especially the dorsum of the fingers, but they also are seen frequently between the fingers. Warts on the hands and feet may occur periungually and on the palms.[145,261] Plantar warts, also called verrucae plantares, occur most commonly in adolescents and young adults.[77,144] Plantar warts generally are single lesions and have a highly thickened corneal layer, or hyperkeratosis, with areas of punctate bleeding. Plantar warts may be painful because they typically are found on pressure-bearing points on the plantar surface of the foot. Flat warts are more common findings in young children and occur most frequently on the arms, face, and knees.[77] Butcher's warts are a form of cutaneous wart seen in meat and poultry handlers who suffer repeated minor trauma to the hands.[80] Although the clinical appearance of cutaneous warts almost always is diagnostic, the differential diagnosis of warty lesions includes other skin disorders, such as molluscum contagiosum, actinomycosis, blastomycosis, sporotrichosis, leishmaniasis, chronic vegetating pyoderma, atypical mycobacterial infection (e.g., swimming pool granuloma), and tuberculosis verrucosa cutis (warty tuberculosis). Giant verrucae or warts also must be differentiated from squamous cell carcinoma.[217]

Epidermodysplasia Verruciformis

Epidermodysplasia verruciformis (EV) is a very rare skin disorder initially manifested in infancy or early childhood. It is characterized by the inability to resolve HPV-induced, cutaneous wartlike lesions.[179] Patients with EV have both nonspecific and HPV-specific defects in cell-mediated immunity, especially T-cell defects; and some patients will have developmental disabilities.* Although the lesions seen with EV are polymorphic, two clinical types of warts are seen primarily in these patients: flat warts and red or reddish-brown macular plaques. Both the flat warts and plaques appear first on the face, trunk, and extremities. They slowly become confluent and then appear to disseminate. Occasionally, the plaques may be achromatic with pigmented borders and resemble pityriasis versicolor. Histopathologically, EV lesions may appear as an in situ or invasive carcinoma.[141] These EV-related carcinomas generally do not metastasize unless exposed to co-carcinogens such as radiation.[221]

Patients with EV may be infected with multiple types of HPV, including HPV-3 and HPV-10, that are associated with flat warts in healthy individuals.[6,179,215,278] The HPV types most commonly associated with lesions in patients with EV (HPV types 5, 8, 17, and 20) do not usually cause lesions in immunocompetent individuals; however, HPV-8 antibodies have been found in healthy individuals, and HPV-5 DNA and HPV-8 DNA have been detected in refractory warts and skin carcinomas in immunocompromised patients such as renal allograft recipients.[78,122,174,235,293] EV-like skin lesions also may be seen in individuals who are immunocompromised as a result of HIV infection, as well as in transplant recipients, those receiving cancer chemotherapy, and other immunosuppressed patients.[25,27,65]

Infections of Male and Female Genital Tract

Genital tract HPV is the most common clinical manifestation of mucosal HPV disease and is, in both sexes, the most common viral sexually transmitted disease. Genital tract HPV infection carries high rates of morbidity and mortality to both males and females by its oncogenic potential. However, condylomata acuminata, or genital warts, are the most common genital tract clinical manifestation and rarely develop into dysplastic lesions or invasive carcinoma.[247,324] Persistent HPV infection is requisite to the development of neoplasia. Oncogenic HPV types establish persistent infection that disrupts the apoptotic machinery of epithelial cells. This, in turn, leads to disorganized and uninhibited cell proliferation and loss of maturation. HPV persistence is seen before the appearance and even after the regression of cytologic pathology.[51,269] Early HPV infection manifests as mucosal dysplasia. Grading systems for intraepithelial neoplasias (INs) are similar for the different anatomic areas, increasing in severity going from grade 1 through grade 3, with carcinoma in situ being the most severe. Dysplasia can be further classified into mild (one-third thickness), moderate (two-thirds thickness), or severe (full thickness) based on the extent to which the cervical epithelium is involved with abnormal cells. The majority of mild dysplastic lesions will regress. Moderate and severe dysplasias are considered premalignant lesions. Invasive carcinomas are preceded by a precursor lesion, a carcinoma in situ that

*References 45, 72, 118, 141, 178, 180, 181, 286, 307, 318.

morphologically resembles the adjacent invasive squamous carcinoma.[209,271]

More common than dysplasia and invasive disease in both males and females are condylomata acuminata, or genital warts. Ninety percent of genital warts are caused by HPV types 6 and 11; a small fraction are caused by HPV-16. Generally, genital warts occur in multiples as flesh-colored, pink, and/or purple-gray papules. Warts, which can also be pedunculated, are covered by an acanthotic squamous epithelium. Giant condylomata acuminata (also known as Buschke-Lowenstein tumors) occur in similar areas as other condylomata but are large, tumor-like lesions that may cause, albeit rarely, inguinal lymphadenopathy, fistulous tracts, inflammation, hemorrhage, and fibrosis of the surrounding tissue. Buschke-Lowenstein tumors can be progress to dysplasia and carcinoma.[240]

In females, genital warts are found on the vulva and in and around the vagina, the perineum, and anus. Although physically visible and palpable, most lesions are asymptomatic. However, lesions can also cause burning, itching, and pain. Intravaginal and cervicovaginal condylomata, usually in the form of less easily visualized flat warts, can also be found and have a higher rate of progression to low- or high-grade dysplastic intraepithelial lesions that external lesions.[98] These flat warts are most commonly caused by HPV types 6, 11, 16, 18, and 31.[12] During pregnancy, genital warts can increase in size and quantity but often regress after delivery. In males, genital warts can be found on the penis, scrotum, groin or thigh area, perineum, and anus. Similar to in females, lesions are often asymptomatic but can cause burning, itching, and dyspareunia. Bowenoid papulosis is another clinical manifestation HPV infection of both the male and female genital tracts, typically occurring in individuals younger than 40 years old. Bowenoid papulosis is characterized by multiple, large erythematous, maculopapular lesions that can take on a red, purple, or brown color with a smooth, velvety surface. Lesions may regress spontaneously or may persist as high-grade intraepithelial lesions and are most commonly associated with HPV 16.[35]

Adolescents can develop genital warts within 1 to 2 months of sexual exposure. Children can have genital and anal warts as a consequence of sexual abuse or from perinatal transmission.[124,126] If a child is older than 2 years, the etiology of genital warts is more likely sexual abuse than perinatal transmission, because beyond 2 years is outside the plausible incubation period for perinatal HPV manifestations.[125,153]

The differential diagnosis of anogenital warts includes condylomata lata associated with secondary syphilis, molluscum contagiosum, epithelial papillae, enlarged sebaceous cysts, seborrheic keratosis, lentigo, pigmented nevi, skin tag, hemorrhoids, Crohn disease, and carcinoma.

Female-Specific Disease

Beyond condylomata acuminata, female-specific genital tract disease involves the cervix, vulva, and vagina. Early infection manifests as dysplasia of varying degrees that most often regresses spontaneously but can progress to invasive cancer.

The site at which the majority of cervical neoplasia takes place (90%) is the squamocolumnar junction, which is on the face of the cervix in adolescents and recedes into the cervical os in adult women.[35] Cervical epithelial lesions identified by Papanicolaou screening are described using the Bethesda system, which classifies precursor lesions into low-grade squamous intraepithelial lesion (LSIL, often correlating with the presence of cervical intraepithelial neoplasia [CIN] grade 1 lesions) or moderate/high-grade squamous intraepithelial lesion (HSIL, which often correlates with the presence of CIN grade 2 or 3 lesions).[209] The majority (approximately 60%) of CIN grade 1 lesions regress spontaneously; only 10 percent progress to CIN grade 3, and only about 1 percent progress to cervical cancer. In contrast, higher-grade lesions have a higher rate of cancer progression. Only one third of CIN grade 3 lesions are likely to regress, and up to 10 to 12 percent of untreated cases progress to cervical cancer.[222] The risk for progression of CIN grade 3 lesions to cervical cancer increases with age, with 0.5 percent per year of CIN grade 3 lesions in young women 20 to 24 years old progressing to cervical cancer; this rate of progression increases to up to 10 percent per year in women 80 years old.[196,201] HPV types 16, 18, 31, and 45 are the most strongly associated with cervical cancer. There is moderate association to cervical cancer and HPV types 33, 35, 39, 51, 52, 56, 58, 59, and 68 and weak association with others, including HPV types 6 and 11.

Cervical dysplasia and carcinoma may be visualized as pigmented papular or plaquelike lesions and as leukoplakic or, if advanced, larger and/or ulcerated lesions. Cervical dysplasia may or may not be amenable to visualization with the naked eye. Common clinical characteristics of cervical carcinoma include atypical nonbranching vessels (e.g., corkscrew, comma shape), abnormal vaginal bleeding or discharge, dysmenorrhea, ulcerations, raised, irregular surface, yellow hue to epithelium, and firmness to palpation.

Vulvar intraepithelial neoplasias (VINs) are named to distinguish lesion morphologies. VIN lesions include VIN, usual type; VIN, warty type; VIN, basaloid type; VIN, mixed (warty/basaloid) type; and VIN, differentiated type. VIN is often asymptomatic and is discovered at the time of gynecologic examination and/or during evaluation for another lesion, such as anogenital warts. VIN lesions are flat or rough elevated papules, are often multicentric, and can occur anywhere on the vulva, including the periurethral and perianal areas. VIN warty type is most associated with high-risk HPV, most often HPV type 16. Regression of VIN is less likely than progression, which usually involves evolution to the basaloid type. Because of the high frequency of multicentric disease, patients with suspected VIN should receive a full examination looking for disease of the cervix and vagina. Up to 50 percent of women with VIN will have preceding or concomitant lower genital tract dysplasia, including cervical or vaginal intraepithelial neoplasia.[316] As VIN progresses, a more distinct vulvar tumor evolves. The most common characteristics of vulvar carcinoma include red, pink, or white bumps, often with rough or eroded surfaces; persistent, nonhealing ulcer with bleeding; intense vulvar pruritus; burning; and dysuria.[38,277]

For vaginal disease, the IN classification is employed, using the term *vaginal intraepithelial neoplasia* (VaIN) divided into grades 1, 2, and 3 to describe the severity of a lesion. Also similar to cervical and vulvar dysplasia, VaIN is usually asymptomatic, but it can present as abnormal vaginal discharge and/or bleeding, most notably postcoital. VaIN usually affects the posterior upper third of the vagina and presents as single or multicentric lesions.[36] Often lesions cannot be seen unless investigated using colposcopy. VaIN lesions often have a vascular pattern with punctation and mosaicism. The vascular pattern can become more unusual as lesions become more invasive. Vaginal cancer is rare. HPV-negative vaginal cancers are seen most often in elderly women. In contrast, VaIN is a disease of younger women and is associated with HPV infection. Progression of VaIN to vaginal cancer is more unpredictable than other female genital intraepithelial lesions. Fortunately, less than 10 percent of VaIN lesions progress to invasive cancer.[231]

Male-Specific Disease

Male-specific genital tract dysplasia and invasive cancer affect the penis. As in all other genital tract dysplasias, the IN system is used to describe the histologic stage of each lesion (penile intraepithelial lesion [PIN] grades 1, 2, 3 and penile carcinoma in situ). Penile dysplasia and invasive disease presents most often as a lump or nodule and sometimes as an ulcerative or erythematous lesion. Penile dysplasia can also present as phimosis, penile pain, and bleeding and with a foul-smelling discharge. Because of social stigma and psychological stress surrounding penile pathology, clinical presentation is often late.[46,79] Squamous cell carcinoma (SCC) is the most common type of neoplasia of the penis, with several different types—classic, basaloid, verrucous, sarcomatoid, and adenosquamous—described.[288] About half of penile carcinomas are HPV associated, most often the warty-basaloid type. HPV-16 and less so HPV-18 are most commonly associated with HPV-associated PIN and invasive penile cancer. Invasive cancer is localized to the glans most commonly then the prepuce, with the least amount of disease seen on the coronal sulcus and shaft. If penile carcinoma in situ involves the glans, it is also known as erythroplasia of Queyrat; if disease involves the shaft, it is also known as Bowen disease. In Bowen disease, inguinal lymph node involvement is common.

Recurrent Respiratory Papillomatosis

Recurrent respiratory papillomatosis (RRP) is a rare, potentially life-threatening condition caused by HPV types 6 and 11.[82,104,133,157,320] Worldwide, RRP probably is the most common tumor of the larynx in children, with an incidence of 0.1 to 2.8 per 100,000 observed.[296,314] In the United States, the estimated incidence of RRP in children is 0.6 to 4.3 per 100,000 and up to 1.8 per 100,000 in adults.[83] RRP is newly diagnosed in an estimated 1500 patients each year.[83,202,314] The disease may be seen in both children and adults. Approximately two thirds of cases of RRP are seen in children; the disease is then referred to as juvenile-onset recurrent respiratory papillomatosis (JORRP).[69] In children, approximately a fourth of the cases will be present before age 1 year; half are present by age 5 years, and the other half by age 11 years. Adult-onset RRP is most often first seen in patients between 20 and 40 years of age, but occasionally RRP may be seen in adolescents.[104,203,278]

Infants and children with JORRP most likely acquire HPV infection perinatally during passage through an HPV-infected birth canal. Thirty to 60 percent of mothers of children with JORRP have a history of genital warts, compared with less than 5 percent of mothers of children who do not have JORRP; children with JORRP rarely are born by cesarean section.[24,228,242,279] Risk factors for JORRP include vaginal delivery, being firstborn, and maternal age younger than 20 years.[280] HPV types 6 and 11, the HPV types most commonly associated with genital warts seen in women, also underlie most cases of JORRP.[34,93,278] Although HPV DNA has been detected in the oropharynx of infants born to mothers with genital HPV infection,[278] RRP develops in only a small proportion of infants born to mothers with genital warts or subclinical HPV infection.[254] The presence of HPV DNA in human breast milk renders this mode of transmission also a possibility for some infants.[265] The mode of transmission of HPV types 6 and 11 in adult-onset RRP is unknown.

The most common initial symptom of RRP and JORRP is hoarseness or a change in voice. Infants and toddlers may have a hoarse cry, stridor, airway obstruction, respiratory distress, or difficulty in phonation. They also may have a crouplike illness. The most common site for RRP and JORRP is the true vocal cord of the larynx. Supraglottic and subglottic extension of the lesions also may occur. In addition, the disease may involve the trachea, bronchi, palate, nasopharynx, paranasal sinuses, and lungs. When the lungs are involved, pulmonary nodules, atelectasis, and secondary bacterial pneumonia may occur (Fig. 154-1). The disease also may produce permanent lung damage with bronchiectasis and

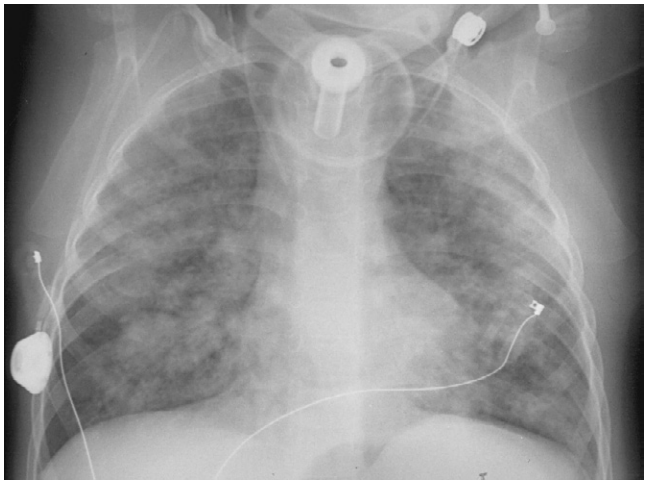

FIGURE 154-1 ■ Chest radiograph of a 6-year-old girl with severe, recurrent, and progressive respiratory papillomatosis since 1 year of age.

cavitations (Fig. 154-2). In 2 to 3 percent of patients, progression to invasive squamous papillomatosis and even malignant transformation to squamous cell carcinoma will occur, with invasion of the soft tissues of the neck, esophagus, and lung parenchyma; such an exceptionally aggressive disease course may occur in association with infection by HPV type 16 (Fig. 154-3).[287,330] The incidence of progression to squamous carcinoma increases to 14 percent in patients who received the radiation therapy that was commonly used previously to treat RRP.[71,113,182,272] Smoking and severe, recurrent disease are also risk factors for development of malignant transformation in adolescents and adults. Clinical courses of RRP and JORRP are highly variable and characterized by common and unpredictable remissions and exacerbations, even despite apparently successful removal of the lesions. Longitudinal studies of JORRP show that infection with HPV type 11 and age younger than 3 years at initial diagnosis are associated with more aggressive tracheal disease requiring frequent surgical debulking.[71,243,330] Sudden and unexpected death may also occur from airway obstruction if the lesions obstruct the laryngeal lumen.[289]

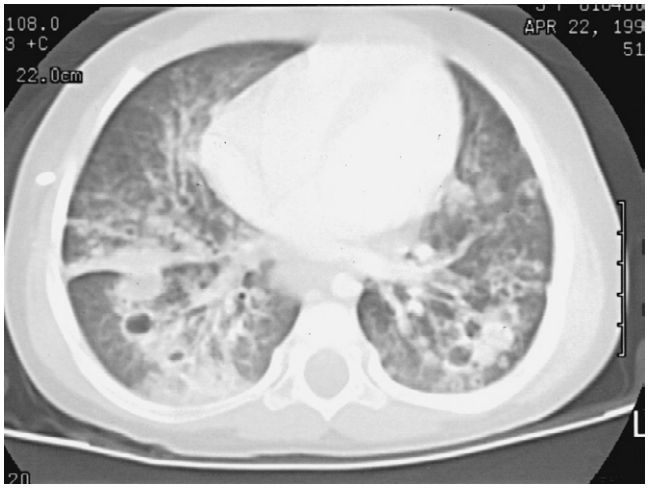

FIGURE 154-2 ■ Chest CT scan of a 6-year-old girl with severe, recurrent, and progressive respiratory papillomatosis shows pulmonary nodules, bronchiectasis, and cavitations.

Upper Respiratory Tract Papillomas

Nasal papillomas or warts are rare tumors that may develop at any age, including childhood, and may occur as solitary lesions or in combination with papillomas elsewhere in the respiratory tract.[38,251] Histologically, they usually resemble the laryngeal papillomas seen in RRP. They most commonly are caused by HPV types 6 and 11, although HPV types 16, 57, and 57b also have been detected in nasal papillomas. Cocaine snorting is one risk factor for the development of nasal papillomas.[275,294] Papillomas also may involve the paranasal sinuses.[39,251]

Conjunctival papillomas occur in all age groups but are exceedingly rare (Fig 154-4). They may be asymptomatic initially and then cause a constant foreign body sensation or chronic conjunctivitis. When large, they appear as pink mulberry- or cauliflower-like growths that may cause pain or interfere with lid closure. In most children and some adults, conjunctival papillomas appear to be caused by HPV types 6 and 11, which characteristically infect the genital tract and, therefore, may be

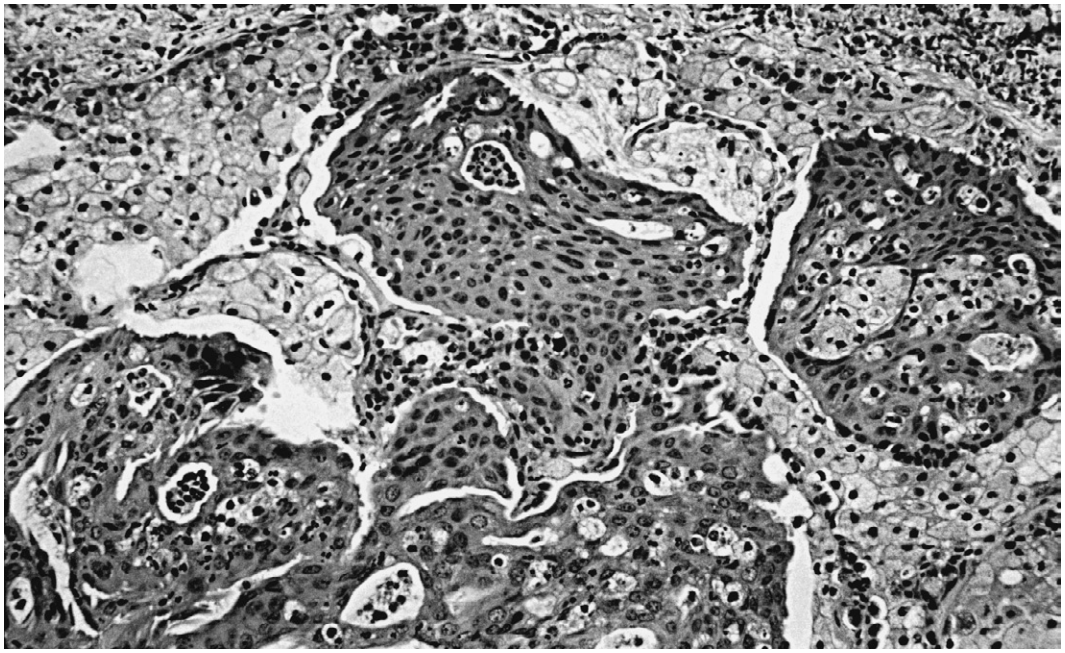

FIGURE 154-3 ■ Lung biopsy tissue from a 6-year-old girl with severe, progressive, recurrent respiratory papillomatosis. This specimen demonstrates invasive squamous papillomatosis. The squamous cells are growing along preformed pulmonary structures, but no evidence of malignancy is apparent at this time because of the absence of invasion of lung tissue (hematoxylin and eosin stain, ×50 original magnification, light microscopy). (Courtesy Dr. Claire Langston, Department of Pathology, Baylor College of Medicine and Texas Children's Hospital, Houston.)

FIGURE 154-4 ■ Focal epithelial hyperplasia of the oral gingival and mucosa (Heck disease). Patient was a 3-year-old Hispanic boy with a history of orthotopic liver transplantation and mild acute organ rejection who had an extensive eruption of painless, rounded, soft nodules present on the tongue, buccal mucosa, and gingivae. (Courtesy Dr. Federico Laham, Department of Pediatrics, Baylor College of Medicine. Photograph taken by Texas Children's Hospital.)

transmitted during birth, similar to RRP.[163,190] HPV type 16, another HPV that commonly infects the genital tract, also has been associated with conjunctival and lacrimal sac carcinoma in adults.[176,189]

Gastrointestinal Disease

Oropharyngeal Cancer

HPV-associated oropharyngeal cancers typically occur within the base of the tongue, tonsils, soft palate, and walls of the pharynx. As previously mentioned, although head and neck cancer risk has been closely linked to increasing age and alcohol and tobacco use, persistent oral HPV infection is now increasingly recognized as a leading cause of oral and oropharyngeal cancers. An association with having a higher number of oral sexual partners (more than five) has been demonstrated in individuals with HPV-positive exfoliative cells within their oropharynx; thus, oral sexual behaviors translate into risk for oropharyngeal disease and cancer. Individuals at risk for persistent oral HPV infection include smokers, individuals of older age, those with low CD4 count, and those on highly active antiretroviral therapy for a long period of time.[43,68]

HPV-associated oral and oropharyngeal cancers are most commonly associated with HPV type 16, which accounts for over 90 percent of HPV-associated disease.[40] This rising HPV-associated disease prevalence is most striking in the United States and less so in Europe and other areas.[56] Not only have HPV-associated oropharyngeal cancers been increasing in incidence overall, but, anatomically, this increase is seen particularly within the palatine and lingual tonsils.[73,131] HPV targets the specialized reticulated epithelium lining the tonsillar crypts.[188] HPV-associated cancer next most commonly occurs within the soft palate and Waldeyer's ring (the uninterrupted ring of lymphoid tissue including the tubal, pharyngeal, palatine, and lingual tonsil). HPV-associated malignancy can also occur in other head and neck regions.[159,211,301,309,319]

Cancer of the oral cavity and oropharynx often presents as delayed clinical signs. Clinical signs and symptoms can include persistent odynophagia, dysphagia, palpable or visible lump in throat or mouth, changes in voice, increased cough, and otalgia.[81] Most often individuals have a nonhealing ulcer or sore.[11,256,310] Given the increasing prevalence of HPV-associated head and neck cancers, an adult with a later neck mass must be assumed to have an HPV-associated malignancy until proven otherwise.

Interestingly, individuals with HPV-associated head and neck cancer have better clinical outcomes after therapy compared with those with non–HPV-associated head and neck cancers. This is likely due to a host of factors, including greater local-regional control of HPV-positive lesions and a higher intrinsic sensitivity of HPV-positive lesions to radiation and cisplatin therapies.[7] HPV-associated cancers are also found to have less cancer recurrence.[219] It is possible that identification of HPV in the oral cavity could serve as a biomarker for monitoring of early disease, disease progression and regression, tumor staging, and continued posttherapeutic surveillance.[188]

Anal Cancer

Even though anal cancer remains a relatively rare occurrence, there are certain at-risk groups that have an increased incidence of anal disease and cancer, particularly men who have sex with men, individuals with a history of solid organ transplantation, and individuals with concurrent HIV infection.[66,99,226]

Similar to all other intraepithelial lesions and cancers, the IN grading system is employed, using the term *anal intraepithelial lesion* (AIN) and a number (i.e., AIN 1 to 3) for classifying the severity of anal disease. Similar to other grade 1 lesions at other sites, AIN 1 is not considered a precancerous lesion. Higher grade lesions (AIN 2 or 3) can progress to invasive cancer.[59] AIN is often warty and white but can be gray and/or pigmented. At the anal verge, lesions are often associated with epithelial stalks, each containing a single-looped capillary. Anal tags can have AIN and are often mistaken as hemorrhoids. Like the cervix, the anus has a squamocolumnar junction, and, like the cervix, it is this squamocolumnar junction that is most susceptible to HPV infection and is most prone to the development of dysplasia. HPV types 16, 18 (and 31 less so) are most commonly associated with anal cancer. Even though anal warts are largely associated with HPV types 6 and 11, anal warts are still associated with invasive anal disease, perhaps because of their association with other sexual risk behaviors and predisposition to other, more oncogenic HPV types.[101,121,160,212]

The age of anal cancer diagnosis in females is most often in the mid to late 60s. Women with related anogenital disease and intraepithelial neoplasia have a higher risk for developing anal cancer. Approximately 30 percent of women with VIN have involvement of the anal canal with AIN.[224]

The average age at anal cancer diagnosis in males is mid to late 50s.[224] In men who have sex with men in particular, there is a high incidence and prevalence of anal disease and cancer found in both HIV-positive and HIV-negative individuals, with age-related prevalence approximately 60 percent across all age groups.[121] HIV-positive men who have sex with men are at highest risk for HPV infection and its sequelae.[128,313] Interestingly, despite the introduction of highly active antiretroviral therapy, the incidence of HPV-associated anal disease and cancer in the HIV-positive men who have sex with men has continued to increase. It is postulated that because individuals are living longer on highly active antiretroviral therapy there is more time for slow-progressing AIN to develop into invasive disease, all the while with unchanged levels of surveillance for anal disease in this surviving population.[86,229,239]

LABORATORY DIAGNOSIS

Although most HPV infections are asymptomatic, the typical appearance of verrucae vulgaris on the skin and condylomata acuminata in the anogenital area in otherwise healthy individuals usually is sufficient to establish the clinical diagnosis of HPV infection. However, laboratory confirmation of HPV-associated lesions may be necessary for unusual manifestations in healthy individuals, for immunocompromised patients, and for patients with suspected malignant lesions. Methods routinely used in foreign diagnosis of many viral infections have limited clinical utility in detecting HPV infection. Instead, a variety of molecular methods, used alone or in combination, are used in research and clinical settings.[337] Cytologic and histologic approaches also are helpful in diagnosing HPV-associated cancers.

Electron Microscopy

Although not used clinically, electron microscopy can be used to detect virions with typical papillomavirus morphology in cutaneous warts. Such virions are difficult to detect in tissue from patients with RRP, JORRP, genital warts, or histologically diagnosed cancers.

Cell Culture

HPVs have not been propagated in cell culture monolayer. Therefore, the routine viral cultures available in most diagnostic virology laboratories will not detect the presence of HPVs.

Serology

HPV serologies are not used clinically and are not available in most diagnostic laboratories. Serologic methods have been used extensively in recent clinical trials of HPV vaccines; however, in natural infection, only 60 percent of women with proven genital HPV infections mount a detectable type-specific IgG response.*

*References 10, 32, 49, 102, 103, 122, 134, 138, 165, 181, 257, 292, 334.

Cytology

Since the 1960s, the Papanicolaou technique for obtaining and staining exfoliated cervical cells (i.e., the Pap smear) has been a procedure used widely as a screening method for detection of dysplastic and malignant cervical epithelial cells. Despite its relative insensitivity, the widespread and consistent use of the Pap smear has reduced dramatically the incidence of invasive squamous cell carcinoma of the cervix in nations with robust screening programs. Although screening guidelines evolve constantly, cervical cytology screening is generally recommended at 1- to 3-year intervals for sexually active women. Updated cervical cytology screening recommendations from the American College of Obstetrics and Gynecology suggest the following (Level A evidence):

- Begin screening at age 21
- Screening should occur every 2 years between ages 21 and 29
- Screening interval can be lengthened to every 3 years after age 30 if there are three negative examinations; no history of CIN grades 2 or 3; no HIV infection or immunocompromise; and no history of diethylstilbestrol (DES) exposure
- Sexually active adolescents should be counseled and tested for sexually transmitted infections and counseled about sex and contraception.[2]

The sensitivity of cervical Pap smear screening is dependent on the expertise of the physician who obtains the specimen and of the cytologist who performs the cytologic analysis.[199] Because most cervical neoplasias arise at the junction of the squamous and columnar epithelium of the cervix (transformation zone), care must be taken to obtain cells from this region. Interobserver variability also exists among cytologists who read Pap smears.[18]

The cytologic abnormalities identified on Pap smear reflected the cytopathic effects of HPV infection in cervical epithelial cells. The cytologic abnormality characteristic of HPV infection is koilocytosis (derived from the Greek word *koilos*, which means "hollow" or "cavity"). Koilocytotic cells display fat, swollen, wrinkled, or raisinoid nuclei surrounded by a halo resulting in an owl's eye–like appearance. Other cytologic abnormalities, including dyskeratosis, parakeratosis, and hyperkeratosis, may occur but are considered secondary or nonspecific. The prevalence of cytologic abnormalities in women screened by Pap smears consistently has been estimated at 2 to 3 percent.[136,273] Reflecting the spontaneous resolution rate of HPV infections, most cytologic abnormalities detected on Pap smears resolve spontaneously in 3 to 6 months. Nonetheless, cytologic abnormalities evolve in some patients, reflecting progression from infection through dysplasia to cervical squamous cell carcinoma. As HPV infection progresses to disease, the koilocytosis typically diminishes and the cervical epithelial cells begin to display dysplastic changes and nuclear abnormalities. Although several Pap smear grading systems exist, the Bethesda system is used in the United States.[29] In the Bethesda system, the cytologic abnormalities seen on Pap smears are graded into four categories: (1) Atypical squamous cells (this category is subdivided into atypical squamous cells of uncertain significance, ASC-US, and

atypical squamous cells–cannot exclude high-grade squamous intraepithelial lesion, ASC-H); (2) low-grade squamous intraepithelial lesion (LSIL); (3) high-grade squamous intraepithelial lesion (HSIL); and (4) squamous cell carcinoma.[209,271]

Colposcopy

Evaluation by cervical colposcopy typically follows for women with abnormal Pap smears or external anogenital warts. Gynecologists, urologists, and family practitioners, as well as pediatricians who are specialists in adolescent medicine, usually perform the procedure. Briefly, the cervix and vulva in females or the urethral meatus, penis, scrotum, and anus in males are visualized under magnification with a colposcope to identify lesions, with special attention paid to their topography, the presence of abnormal whitening, and their vascular architecture. The area then is soaked in dilute acetic acid (3% to 5%) and reexamined for the presence of previously undetected lesions that appear as whitened plaques. Although whitening with acetyl white treatment is useful for detection of lesions, whitening after application of acetic acid is not specific for HPV infection or disease. Therefore, biopsy is required for definitive diagnosis. Tissue biopsy specimens are sent for histologic examination and for detection of HPV DNA by molecular techniques. Anal colposcopy, referred to as anoscopy, is similar to cervical colposcopy and is used to screen men and women at high risk for anal HPV infection.

Histology

HPV infection most often occurs in histologically normal tissue and is detectable only by molecular methods that detect viral DNA. Benign and asymptomatic HPV infection also may cause koilocytosis. Condylomata acuminata lesions display koilocytosis and other histopathologic characteristics of active HPV infection, such as hyperkeratosis, parakeratosis, acanthosis, and lengthening of the rete pegs. Atypical features, including mitotic figures above the basal layer, dysplastic cells, and single-cell keratinization, indicate higher grades of dysplasia. Precancerous and cancerous lesions of the cervix, vulva, vagina, penis, and anus also may be graded as low-grade or high-grade squamous intraepithelial lesions and invasive squamous cell carcinoma. In like manner, HPV-associated oral and oropharyngeal lesions are graded according to cellular atypia and invasion.

Molecular Methods That Detect Human Papillomavirus DNA

A nonradioactive, chemiluminescent liquid hybridization assay (hybrid capture assay) is commercially available and detects as many as 14 HPV types divided into high-risk (types 16, 18, 31, 33, 35, 45, 51, 52, 56) and low-risk (types 6, 11, 42, 43, 44) groups on the basis of association with cervical cancer.[177,178,270] This DNA hybrid capture assay is relatively rapid and simple to perform and is used typically as a reflex test to detect and classify (high-risk or low-risk) HPV DNA in abnormal cervical cytology specimens. Although this DNA hybridization/amplification method is in widespread clinical use, the laboratory and clinical use of other HPV DNA detection methods, including dot-blot, slot-blot, Southern blot, and in situ hybridization, have largely been supplanted by PCR.* Commercially available PCR-based assays capable of identifying and distinguishing 37 high- and low-risk mucosal HPV types are used in clinical trials but are not yet approved for diagnostic use in the United States.

In Situ Hybridization

In situ tissue DNA hybridization assays are performed directly on fresh or fixed tissue sections or on cytologic specimens. In situ assays offer the unique advantage of allowing the examiner to correlate histopathologic abnormalities with the location of HPV DNA. In situ assays may be performed with radioactive or enzyme-labeled probes. Commercial reagents and kits are available. Given the greater sensitivity and ease of detection, immunohistochemical detection of p16 antigen appears to be a sensitive and specific surrogate for tumor HPV positivity.[32,75,284]

TREATMENT

In healthy individuals, most HPV-associated lesions will regress spontaneously in 1 to 2 years. Treatment may be desirable if the lesions are large, multiple, or recurrent; if they cause pain or discomfort; or if they are undesirable cosmetically. Treatment is required if the lesions are life threatening, such as laryngeal papillomas that obstruct the airway, or lead to cervical cancer. A variety of treatment strategies are available, but none produces a universally effective or permanent cure. Rather, current-available approaches seek reduction of the clinically apparent lesion and most require repetitive application. Clinically significant HPV-associated lesions also usually require care by a specialist. For example, a dermatologist should be consulted to assist in the management of a patient with severe recalcitrant cutaneous warts, a gynecologist for patients with cervical dysplasia, an oncologist for patients with cervical cancer, a colorectal surgeon for patients with anal dysplasia or carcinoma, an ophthalmologist for patients with conjunctival papillomas, and an otolaryngologist and pulmonologist for children and adults with severe RRP. The role of the infectious diseases specialist in the management of HPV-associated disease is evolving and is principally to ensure that high-risk patients under their care (i.e., patients who are immunocompromised, post transplant, HIV infected, or have had multiple sexually transmitted infections) are referred regularly for screening and treatment. Infectious diseases physicians will become more directly involved in the care of HPV-associated diseases when HPV-specific antiviral chemotherapy becomes available for clinical trial and eventually for routine clinical use in patients.

Surgical techniques used to treat papillomas include traditional local excision by knife, cryotherapy with liquid

*References 53, 123, 130, 184, 214, 216, 230, 302, 323, 325, 335, 336.

nitrogen or dry ice, electrocautery and curettage, and ultrasonication.* Newer ablative surgical techniques that use carbon dioxide laser vaporization and flash-lamp pulsed-dye laser therapy allow more precise and complete removal of visible papillomas and are becoming widely used to treat genital and laryngeal papillomas.[94,249,297] Surgical excision by knife remains, however, the mainstay of HPV treatment, because excision provides tissue for a histopathologic diagnosis, as well as removal or debulking of large lesions. Ablation by cryotherapy with liquid nitrogen, electrocautery, or laser vaporization may be used to remove small, single, or multiple lesions or may be performed in combination with surgery for large or difficult lesions.[193,267,268] These surgical therapies also may release viral antigens, evoking a local and systemic immunologic response that may assist in eradicating the lesions. Disadvantages of these physical methods of wart and papilloma removal include pain, scarring, and disfigurement. These methods also are relatively invasive and impractical for patients with disseminated disease. Furthermore, recurrent treatments usually are necessary. In addition, laser vapors contain HPV DNA and may be a vehicle for spreading the infection in the patient or to the treating physician or surgeon, and, thus, the vapors or smoke plume should be contained.[110,266] Recurrence of lesions after treatment occurs commonly and most likely is due to the presence of HPV DNA sequences in clinically and histologically normal epithelium adjacent to and beyond the treatment area.[96] The mainstay of treatment for RRP and JORRP is repeated surgical debulking of obstructing papillomas, augmented by immunomodulators and antiviral therapies.[120,133] Surgical rates tend to decrease over the course of time in many patients; however, those patients infected with HPV type 11 may require more frequent, more extensive, and more prolonged surgical procedures over the course of time.[243,331]

Warts and papillomas also may be disrupted physically and removed by using chemical ablatives applied topically to the lesion. Simple organic acids such as bichloracetic and trichloracetic acid or salicylic acid applied twice daily for several days have shown some success in the localized treatment of skin and genital warts.[105] They are caustic substances that produce a white slough that peels off, and they can be applied weekly until the lesion is destroyed.[105] Antimitotic agents such as the traditional podophyllin or the newer preparation podophyllotoxin can be applied twice daily for several weeks. Antimetabolites such as bleomycin, cantharidin, and 5-fluorouracil also have shown efficacy when administered locally once or twice a week because they inhibit the cellular proliferation induced by HPV infection.[156,237] These topical chemicals usually are easy to apply to skin and genital warts, but they also may cause local pain, redness, swelling, irritation, blisters, and scarring.[156] Moreover, they are not virus specific, and recurrences occur commonly. They also are impractical for extensive lesions and may be toxic if used in certain circumstances. For example, they may damage the cornea if used to treat conjunctival papillomas, and they should not be used on pregnant women.[193]

The systemically administered antitumor agent methotrexate also has been administered to individual patients with disseminated HPV disease, with variable success.

Immunomodulation is another treatment strategy for HPV-associated disease. A systemic immunologic response probably is responsible for the spontaneous regression frequently observed in cutaneous and mucosal warts. Therefore, stimulation of the immune system with immunomodulators also may produce remission in patients with HPV-associated disease. Interferons have antiviral, antiproliferative, and immunomodulating properties. Decades of clinical experience have accrued in treating HPV-associated disease with interferon administered topically, intralesionally, or systemically by subcutaneous injection. Both lymphoblastoid and fibroblast interferon have been used with some success to treat patients with genital warts and respiratory papillomatosis topically, locally, and systemically.* However, interferon-γ has not been shown to be beneficial. Both recombinant and natural-source interferon-α preparations have been demonstrated in placebo-controlled trials to be effective and are approved by the U.S. Food and Drug Administration (FDA) for the intralesional treatment of genital warts.[90,91] A 25- to 30-gauge needle is used to inject approximately 0.1 mL (1×10^6 units) at the base of up to five warts at a time for a total dose of 5×10^6 units at each visit. This dose is repeated two to three times weekly for 3 weeks. Maximal effect usually is seen within 4 to 8 weeks. Repeat injections may be given if the warts are persistent or recurrent. Interferon therapy also may be effective if used alone or in combination with laser surgery to treat patients with RRP.[130,134,166,175] Fever, headache, chills, and myalgia frequently occur with local and systemic interferon treatment. Severe and persistent fatigue, nausea, and leukopenia are other fairly common adverse reactions, especially with systemically administered interferon. Systemic natural leukocyte interferon also causes regression of warts and reduction of the virus load in tissues in patients with EV.[5] Cimetidine, an immunomodulator that alters lymphocyte function, has been used in an attempt to treat children with recalcitrant cutaneous warts.[218]

Topical immunotherapeutic agents provide alternative treatment options for HPV-associated disease in some patients.[111,112,152,194,278] Imiquimod, also known as imidazoquinoline, is a first-generation member of a class of immune response modifiers approved for topical treatment of genital and perianal warts.[111,112,152,194] It is a synthetic compound that is a topically active Toll-like receptor–7 agonist. It activates innate immune cells to produce interferon-α and other cytokines to enhance antigen presentation and promote antigen-specific T-helper type 1 cell-mediated immune responses. Imiquimod is a 5 percent cream that is applied to lesions and appears to be effective in some patients. Resiquimod and other related, second-generation imidazoquinoline analogs also are in clinical development.

Retinoids and retinoic acid, which are analogs of vitamin A, can regulate the growth and differentiation of

*References 13, 30, 31, 95, 146, 186, 285.

*References 90, 100, 106, 133, 248, 274, 278.

malignant, premalignant, and even normal cells.[4,250] Clinically, they have a documented effect against squamous cell carcinoma.[168,169] For these reasons, anecdotal reports and small series of patients have emerged in which retinoic acid was used to treat HPV-associated disease, especially RRP. In these reports, retinoids have been used with varying success, primarily as adjuvant agents with surgical therapy, to treat adult patients with severe, refractory RRP.[15,88] The combination of interferon and retinoic acid has been shown to be synergistic against breast cancer cells in vitro and potentially may be useful in refractory cases of RRP.[187]

Unique aspects of HPV infection and disease render designing specific antiviral therapies a challenge. For example, HPV disease usually is focal, the virus is involved intricately in the cell's life cycle, and HPV has great diversity with more than 70 genotypes. The inability to grow HPVs readily in cell culture also hampers our ability to study the antiviral properties and cellular toxicities of candidate compounds. Molecular assays that isolate individual viral functions, therefore, have been used to evaluate the ability of antiviral compounds to inhibit each individual step in the virus life cycle. Furthermore, animal models for HPV-associated disease are lacking. Despite these challenges, the in vitro antiviral activity and clinical efficacy of a variety of compounds have been studied. For example, ribavirin, a nucleoside analog with a broad antiviral spectrum, has been used in clinical trials for the treatment of laryngeal papillomatosis.[191] Cidofovir, an antiviral compound effective against serious disease caused by cytomegalovirus, also appears to have activity against HPVs, and possible clinical benefit for certain HPV-associated disease has been shown. For example, repeated intralesional administration of cidofovir directly into the site of papillomas and also submucosally at the sites of resected papillomas is associated with partial or complete regression of lesions, improvement of voice quality and airway status, and decreased need for repeat surgery. Published studies report a wide variety of intralesional doses (2 to 57 mg of a 5 mg/mL cidofovir concentration solution), frequency of injections (every 2 to 8 weeks), and duration of treatment (4 months to 4 years). Rarely, cidofovir has been administered intravenously in patients with refractory, disseminated papillomatosis.[133] The greatest benefits have most often been documented after the fourth series of injections, and benefit appears to plateau after the eighth series of injections in most patients.* However, because most published reports are single-center, uncontrolled case series with relatively small numbers of patients, controlled, multicenter trials with larger numbers of patients are necessary to accurately determine the benefits of cidofovir therapy, as well as the optimal dose, frequency, and duration of therapy for RRP and JORRP.[308]

PREVENTION

Two vaccines have been developed and marketed for prevention of HPV-associated dysplasias and malignancies. Both are highly effective. Despite the advent of effective vaccines, secondary prevention by cytologic screening remains an essential component of cancer prevention programs.

Historically, screening practices for HPV-associated diseases have focused on cervical pathology. Today, most cases of invasive cervical cancer are preventable with regular screening for precancerous lesions (e.g., by Pap smear) and close follow-up of abnormal results. As mentioned earlier, cervical cytology screening is an ever-evolving process, with the largest changes to the most recent recommendations advocating for screening starting at the older age of 21.[2,3,9]

Data analyses from the National Health Interview Survey find overall cervical cancer screening rates of just over 80 percent, with lower rates among Asian, American Indian/Alaska Native, Hispanic, and foreign-born women. Higher rates of cervical cancer seen among black and Hispanic women are likely multifactorial but are, in large part, related to reduced access to screening and/or follow-up care. In these same disproportionately affected ethnic groups, the disparity of HPV and cancer rates will likely continue to grow if these groups are undervaccinated.[86,229] Two commercially available HPV vaccines are discussed in detail here. A third vaccine is under phase III clinical trial.

Quadrivalent HPV Vaccine

In June 2006, the FDA approved the first vaccine to prevent HPV-associated dysplasias and malignancies. This quadrivalent, recombinant vaccine against serotypes 6, 11, 16, and 18, given as a series of three 0.5-mL intramuscular injections at 0, 2, and 6 months, is now approved for females and males ages 9 through 26 years old. The vaccine, prepared using purified virus-like particles (VLPs) of the capsid (L1) protein of HPV types 6, 11, 16, and 18, stimulates humoral and cellular immune responses to HPV L1 capsid proteins.

The quadrivalent HPV vaccine is indicated for prevention of the following HPV-associated diseases *due to vaccine types* in females:

- Cervical, vulvar, vaginal, and anal cancer
- Genital warts
- Cervical intraepithelial neoplasia (CIN) grades 1/2/3 and cervical adenocarcinoma in situ (AIS)
- Vulvar intraepithelial neoplasia (VIN) grades 2/3
- Vaginal intraepithelial neoplasia (VaIN) grades 2/3
- Anal intraepithelial neoplasia (AIN) grades 1/2/3

The quadrivalent HPV vaccine is indicated for the prevention of the following HPV-associated diseases *due to vaccine types* in males:

- Anal cancer
- Genital warts
- AIN grades 1/2/3

Quadrivalent HPV Vaccine Efficacy

Quadrivalent HPV vaccine efficacy has been assessed in six double-blinded, randomized controlled trials, together evaluating over 28,000 males and females ages 16 through 26 years. Among these trials, the FUTURE I/II trials

*References 60, 63, 84, 108, 129, 183, 281, 282.

of vaccine efficacy and safety provided most of the efficacy and safety data submitted to support vaccine licensure.[10,102,103,148,185]

Females. Primary analysis of efficacy regarding HPV types 6, 11, 16, and 18 were conducted in the per protocol efficacy (PPE) population, which consisted of women ages 16 through 26 years, who were HPV naïve to the relevant strains 1 month before the first dose, remained negative until 1 month after the third dose, received all three doses within 1 year, and had no protocol violations. Detection of CIN grades 2/3, AIS, VIN grades 2/3, and VaIN grades 2/3 were the efficacy end points to assess prevention of cervical, vulvar, and vaginal cancer. Presence of external genital lesions was the efficacy end point to assess prevention of genital warts. Anal disease and anal cancer were not end points in the female studies.

In combined analysis from all clinical trials, efficacy was 98 percent, 100 percent, and 100 percent against HPV type 16 or type 18–related CIN grades 2/3 + AIS, VIN grades 2/3, and VaIN grades 2/3, respectively. By HPV type, vaccine prevented 98 percent of HPV type 16–related lesions and 100 percent of HPV type 18–related lesions. The vaccine prevented 99 percent of anogenital condyloma infections related to HPV types 6, 11, 16, and 18.[42,44]

Secondary analysis approximated the impact on the general population of young American females. This intention-to-treat analysis looked at vaccine efficacy in those receiving at least one dose of the vaccine in both HPV-naïve females and females regardless of HPV status. Females who were HPV naïve at day 1 were found to have reduction of genital warts, CIN grades 2/3 + AIS, and VIN + VaIN grades 2/3 by 83 percent, 43 percent, and 77 percent, respectively. Females regardless of HPV status were found to have reduction of genital warts, CIN grades 2/3 + AIS, and VIN + VaIN grades 2/3 by 63 percent, 18 percent, and 51 percent, respectively.*

Males. To assess the efficacy of the quadrivalent vaccine for prevention of genital warts in males, primary analysis of efficacy against disease due to HPV types 6, 11, 16, and 18 was conducted in the per protocol efficacy (PPE) population, which consisted of males ages 16 through 26 years who were HPV naïve to the relevant strains 1 month before the first dose, remained negative until 1 month after the third dose, received all three doses within 1 year, and had no protocol violations. Presence of external genital warts and penile/perineal/perianal intraepithelial neoplasia (PIN) grades 1/2/3 were the efficacy end points to assess prevention of penile/perineal/perianal cancers. Vaccine efficacy for prevention of anal disease was assessed in a substudy population of men who have sex with men derived from the larger PPE male population.

In combined analyses from all clinical trials, quadrivalent vaccine efficacy was 100 percent and 78 percent for prevention of HPV types 6, 11, 16, and 18-related PIN grades 1/2/3 and AIN grades 1/2/3, respectively. The quadrivalent vaccine prevented 90 percent of anogenital condylomas related to HPV types 6, 11, 16, and 18.[117]

Secondary analysis approximated the impact on the general population of young American males. This intention-to-treat analysis assessed vaccine efficacy in men receiving at least dose of the vaccine in both HPV-naïve males and males regardless of HPV status. Males who were HPV naïve at day 1 were found to have reduction of external genital lesions, genital warts, PIN grades 1/2/3, and AIN grades 1/2/3 by 76 percent, 80 percent, 21 percent, and 77 percent, respectively. Males, regardless of HPV status, were found to have reduction of external genital lesions, genital warts, PIN grades 1/2/3, and AIN grades 1/2/3 by 67 percent, 68 percent, 0.3 percent, and 50 percent, respectively.

Bivalent HPV Vaccine

In 2009, three years after FDA approval of the quadrivalent HPV vaccine, the FDA licensed a second vaccine to prevent HPV-associated diseases due to HPV types 16 and 18. The bivalent vaccine is an HPV-16/18 VLP-based, AS04-adjuvanted vaccine given as a series of three 0.5-mL intramuscular injections given at 0, 1, and 6 months. The bivalent vaccine is FDA approved for use in girls and young women aged 9 through 25 years. The vaccine is prepared by combining adsorbed VLPs (L1 proteins) of HPV types 16 and 18 together with a novel AS04 adjuvant system (3-*O*-decasyl-4′-monophosphoryl lipid A [MPL] adsorbed onto aluminum) in sodium chloride, sodium dihydrogen phosphate dihydrate, and water to stimulate a humoral immune response (IgGs against HPV-L1 capsid proteins).

The vaccine is used for prevention of the following HPV-associated diseases *due to vaccine type* HPVs in females:

- Cervical cancer
- Cervical intraepithelial neoplasia (CIN) grades 1/2/3 and cervical adenocarcinoma in situ (AIS)

Bivalent Vaccine Efficacy

The efficacy of the bivalent vaccine was assessed in two double-blinded, randomized controlled trials, together evaluating over 19,000 young women aged 15 through 25 years, most notably the PATRICIA (PApilloma TRIal against Cancer In young Adults) trial of vaccine efficacy for both according to protocol (ATP) and total vaccinated cohort (TVC) populations.[132,165] Presence of CIN grades 1/2/3 or AIS served as efficacy end points to assess prevention of cervical cancer. Persistence of infection past 12 months served as the virologic end point.

Primary analysis included an ATP population, or HPV-naive population, consisting of women ages 15 through 25 years who received all three vaccine doses, who were HPV naïve to HPV 16 and 18 at baseline and at 6 months, and for whom all efficacy measures were available. Vaccine efficacy for the prevention of HPV type 16 or 18–related CIN grades 2/3 or AIS and CIN grades 1/2/3 or AIS was 93 percent and 92 percent, respectively. Also within the ATP cohort, the vaccine

*References 10, 23, 102, 103, 109, 313.

reduced persistence of infection at 12 months by 91 percent.[132,165]

Further analysis included HPV-naïve and non-naïve females (those with current infection and/or previous exposure) and the total vaccinated cohort (TVC), including females who received at least one vaccine dose, for whom all efficacy measures were available, regardless of HPV status. Females who were HPV naïve (negative for 14 oncogenic HPV types 6, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) at day 1 were found to have vaccine efficacy for CIN grades 1/2/3 or AIS, CIN grades 2/3 or AIS, and CIN grade 3 or AIS of 97 percent, 98 percent, 100 percent, respectively. Females regardless of vaccine-type HPV status were found to have vaccine efficacy for CIN grades 1/2/3 or AIS, CIN grades 2/3 or AIS, and CIN grade 3 or AIS of 56 percent, 53 percent and 34 percent, respectively. Females regardless of all-type (vaccine + nonvaccine) HPV status were found to have vaccine efficacy for CIN grades 1/2/3 or AIS, CIN grades 2/3 or AIS, and CIN grade 3 or AIS of 22 percent, 30 percent, and 33 percent, respectively.[132,165]

Higher Valency Vaccines

A nine-valent L1 virus-like particle-based vaccine is now in clinical trials. If efficacy is demonstrated in clinical trials, this vaccine will extend vaccine protective coverage to nine HPV types (6, 11, 16, 18, 31, 33, 45, 52, and 58), which together account for 90 percent of genital warts and cervical cancers.[208] Six phase III clinical trials are completed or are ongoing. The largest and most significant phase III trial is evaluating vaccine tolerability and efficacy in females between ages 12 and 26 who have already received a three-dose course of quadrivalent vaccine.

Vaccine Limitations

With widespread use, the quadrivalent and bivalent HPV vaccines offer the exciting prospects of substantial reductions in HPV-associated morbidity and mortality. However, as with other vaccines, quadrivalent and bivalent HPV vaccines have specific limitations. It is important to consider the following limitations when giving these vaccines:

- The vaccines do not eliminate the need for continued routine cervical and anal cancer screening.
- The vaccines do not provide protection against nor change the natural history of established infections due to vaccine and nonvaccine HPV types.
- The vaccines are not intended to treat active external genital lesions; any intraepithelial lesion type; or cervical, vulvar, vaginal, and anal cancers.

Although most HPV-associated head and neck malignancies are attributable to HPV type 16 and 18 infections, there are currently no data demonstrating that HPV immunization impacts the incidence or course of HPV-associated head and neck malignancies. Similarly, although it is anticipated that HPV immunization with the quadrivalent vaccine will reduce the incidence of HPV types 6 and 11–associated laryngeal papillomatosis, there are no data indicating that the quadrivalent vaccine will prevent or alter the clinical course of laryngeal papillomatosis at this time.

There is limited evidence that pseudovirion-based HPV vaccines protect against disease due to nonvaccine HPV types. The duration of such heterotypic protection is unknown and may be short.

Given that immunocompromised individuals face increased incidences of HPV-associated morbidity and mortality, it is hoped that both vaccines will provide protection to immunocompromised patients. Although neither vaccine contains live virus and although it is anticipated that the use of these vaccines in immunocompromised patients will be safe, there are few data supporting the use of HPV vaccines in immunocompromised individuals. Several clinical trials of HPV vaccines in immunocompromised individuals are ongoing.

The overall personal and public health, and economic benefits of vaccination have been well demonstrated in both males and females.[58,89,155] The importance of both male and female prevention efforts resides in the fact that not only is there disease burden in women and men alone but there is also significant opportunity for transmission and infection of the other, potentially leading to significant morbidity and mortality (for example, as evidenced by the increased frequency of CIN in females with partners with penile lesions).*

Vaccine benefits vary according to the population immunized. In populations that are provided regular cervical cytology screening, HPV immunization serves largely to prevent high-grade dysplasia and to reduce the economic and emotional costs associated with treatment of premalignant disease. In resource-poor populations that are unscreened, HPV vaccines benefit by preventing cervical cancer and its associated economic and emotional expenses.[70,76]

Based on trends in sexual behaviors, age of sexual debut, knowledge that acquisition of HPV infection occurs within first few years of sexual debut, and the fact that vaccination is most efficacious in HPV-naïve individuals, initiating vaccination prior to any sexual contact (vaginal, anal, oral, digital) will prevent the most infections and their sequelae.[20] It becomes the responsibility of any primary care provider of children, adolescents, and young adults to discuss HPV infection with both patients and their caregivers and to offer vaccination to patients as early as possible to prevent as much disease as possible.[74]

NEW REFERENCES SINCE THE SIXTH EDITION

2. ACOG Committee on Practice Bulletins—Gynecology. ACOG practice bulletin No. 109: cervical cytology screening. *Obstet Gynecol* 2009;**114**:1409–20.
7. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;**363**:24–35.
8. Arbyn M, de Sanjose S, Saraiya M, et al. EUROGIN 2011 roadmap on prevention and treatment of HPV-associated disease. *Int J Cancer* 2012;**131**:1969–82.
22. Benard VB, Johnson CJ, Thompson TD, et al. Examining the association between socioeconomic status and potential human

*References 70, 119, 127, 140, 149, 290, 315.

papillomavirus-associated cancers. *Cancer* 2008;**113**(Suppl 10): 2910–18.
35. Bornstein J, Bentley J, Bösze P, et al. Colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. *Obstet Gynecol* 2012;**120**:166–72.
36. Boonlikit S, Noinual N. Vaginal intraepithelial neoplasia: a retrospective analysis of clinical features and colpohistology. *J Obstet Gynecol Res* 2010;**36**:94–100.
37. Braaten KP, Laufer MR. Human papillomavirus (HPV), HPV-associated disease, and the HPV vaccine. *Rev Obstet Gynecol* 2008;**1**: 2–10.
41. Broomell EM, Reynolds SM, Jacobson RM. Epidemiology, clinical manifestations and recent advances in vaccination against human papillomavirus. *Postgrad Med* 2010;**122**:121–9.
43. Brown LM, David PC, Devesa SS. Oral cavity and pharynx cancer incidence trends by subsite in the United States: changing gender patterns. *J Oncol* 2012;**2012**:1–10.
54. Centers for Disease Control and Prevention (CDC). Human papillomavirus–associated cancers—United States, 2004–2008. *MMWR Morb Mortal Wkly Rep* 2012;**61**:258–61.
57. Chaturvedi AK, Engels EA, Anderson WF, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011;**29**:4294–301.
58. Chesson HW, Ekwueme DU, Saraiya M, et al. The cost-effectiveness of male HPV vaccination in the United States. *Vaccine* 2011;**29**:8443–50.
79. Dee S, Keane T, Bhavsar R. Contemporary diagnosis and management of squamous cell carcinoma (SCC) of the penis. *Br J Urol Int* 2011;**109**(9):1378–92.
86. D'Souza G, Wiley DJ, Li X, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. *J Acquired Immune Defic Syndr* 2008;**48**:491–9.
87. Dunne EF, Sternberg M, Markowitz LE, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 prevalence among females in the United States—National Health and Nutrition examination survey, 2003-2006: opportunity to measure HPV vaccine impact? *J Infect Dis* 2011;**204**:562–5.
89. Elbasha EH, Dasbach EJ. Impact of vaccinating boys and men against HPV in the United States. *Vaccine* 2010;**28**:6858–67.
103. FUTURE I/II Study Group. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. *BMJ* 2010;**341**:c3493.
109. *Gardasil [package insert]*. Whitehouse Station, N.J.: Merck & Co, Inc; 2006.
115. Gillison ML, Chaturvedi AK, Lowy DR. HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. *Cancer* 2008;**113**(10 Suppl):3036–46.
116. Gillison ML, D'Souza G, Westra WH, et al. Distinct risk factor profiles for human papillomavirus type 16–positive and human papillomavirus type 16–negative head and neck cancers. *J Natl Cancer Inst* 2008;**100**:407–20.
117. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. *N Engl J Med* 2011;**364**(5):401–11.
120. Graupp M, Gugatschka M, Kiesler K, et al. Experience of 11 years use of cidofovir in recurrent respiratory papillomatosis. *Eur Arch Otorhinolaryngol* 2013;**270**:641–6.
140. Howlader N, Noone AM, Krapcho M, et al, editors. *SEER Cancer statistics review, 1975-2009 (vintage 2009 populations)*. Bethesda, MD: National Cancer Institute. Based on November 2011 SEER data submission, posted to the SEER web site.2012. Available at http://seer.cancer.gov/csr/1975_2009_pops09/.
147. Joseph AW, D'Souza G. Epidemiology of human papillomavirus–related head and neck cancer. *Otolaryngol Clin North Am* 2012;**45**:739–64.
165. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year-end-of-study analysis of the randomized, double-blind PATRICIA trial. *Lancet* 2012;**13**: 89–99.
188. Marur S, D'Souza G, Westra WH, et al. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet* 2010;**11**: 781–9.
208. National Cancer Institute Clinical Trials. *A study of V503 vaccine in females 12-26 years of age who have previously received Gardasil (V503-006 AM1). 2012*. Available at http://www.cancer.gov/clinicaltrials/search/view/print?cdrid=663767&version=HealthProf. Accessed September 16, 2012.
219. O'Rourke MA, Ellison MV, Murray LJ, et al. Human papillomavirus–related head and neck cancer: a systematic review and meta-analysis. *Oral Oncol* **48**:1–11.2012.
224. Palefsky JM. Human papillomavirus–related disease in men: not just a women's issue. *J Adolesc Health* 2010;**46**:S12–19.
225. Palefsky JM, Giuliano AR, Goldstone S, et al. HPV vaccine against anal infection and anal intraepithelial neoplasia. *N Engl J Med* 2011;**365**:1576–85.
229. Patel P, Hanson DL, Sullivan PS, et al. for the Adult and Adolescent Spectrum of Disease Project and HIV Outpatient Study investigators. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. *Ann Intern Med* 2008;**48**:728–36.
231. Pearson JM, Feltman RS, Twiggs LB. Association of human papillomavirus with vulvar and vaginal intraepithelial disease: opportunities for prevention. *Women's Health* 2008;**4**:143–50.
238. Pickard RKL, Xiao W, Broutain TR, et al. The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18-30 years. *Sex Transm Dis* 2012;**39**:559–66.
239. Picketty C, Selinger-Leneman H, Grabar S, et al. Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. *AIDS* 2008;**22**:1203–11.
256. Rogers SN, Vedpathak LV, Lowe D. Reasons for delayed presentation in oral and oropharyngeal cancer: the patient's perspective. *Br J Oral Maxillofac Surg* 2011;**49**(5):349–53.
284. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus–associated head and neck cancer based on a prospective clinical experience. *Cancer* 2010;**116**:2166–73.
308. Tjon Pian Gi RE, Dietz A, Djukic V, et al. Treatment of recurrent respiratory papillomatosis and adverse reactions following off-label use of cidofovir (Vistide®). *Eur Arch Otorhinolaryngol* 2012;**269**:361–2.
309. Torrente MC, Rodrigo JP, Haigentz M, et al. Human papillomavirus infections in laryngeal cancer. *Head Neck* 2011;**33**:581–6.
312. U.S. Cancer Statistics Working Group. *United States cancer statistics: 1999–2008 incidence and mortality web-based report*. Atlanta, GA: Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute; 2012. Available at http://www.cdc.gov/uscs. Accessed September 16, 2012.
313. U.S. Preventive Services Task Force. *Screening for cervical cancer*. Rockville, MD: U.S. Preventive Services Task Force; 2012. Available at http://www.uspreventiveservicestaskforce.org/uspstf/uspscerv.htm. Accessed September 16, 2012.
315. Vamos C, McDermott R, Daley E. The HPV vaccine: Framing the arguments for and against mandatory vaccination of all middle school girls. *J Sch Health* 2008;**78**:302.
316. van de Nieuwenhof HP, van der Avoort IA, de Hullu JA. Review of squamous premalignant vulvar lesions. *Crit Rev Oncol Hematol* 2008;**68**(2):131–56.
320. Venkatesan NN, Pine HS, Underbrink MP. Recurrent respiratory papillomatosis. *Otolaryngol Clin North Am* 2012;**45**:671–94.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abramson A, DiLorenzo T, Steinberg B. Is papillomavirus detectable in the plume of laser-treated laryngeal papilloma? *Arch Otolaryngol Head Neck Surg* 1990;**116**:604.
2. ACOG Committee on Practice Bulletins—Gynecology. ACOG practice bulletin No. 109: cervical cytology screening. *Obstet Gynecol* 2009;**114**:1409–20.
3. Adams M, Jasani B, Fiander A. Human papillomavirus (HPV) prophylactic vaccination: challenges for public health and implications for screening. *Vaccine* 2008;**25**:3007.
4. Alberts D, Coulthard S, Meyskens F Jr. Regression of aggressive laryngeal papillomatosis with 13-*cis*-retinoic acid (Accutane). *J Biol Response Mod* 1986;**5**:124.
5. Androphy E. Papillomaviruses and interferon. *Ciba Found Symp* 1986;**120**:221.
6. Androphy E, Dvoretzky I, Lowy D. X-linked inheritance of epidermodysplasia verruciformis: genetic and virologic studies of a kindred. *Arch Dermatol* 1985;**121**:864.
7. Ang KK, Harris J, Wheeler R, et al. Human papillomavirus and survival of patients with oropharyngeal cancer. *N Engl J Med* 2010;**363**(10):24–35.
8. Arbyn M, de Sanjose S, Saraiya M, et al. EUROGIN 2011 roadmap on prevention and treatment of HPV-associated disease. *Int J Cancer* 2012;**131**:1969–82.
9. Arbyn M, Dillner J. Review of current knowledge on HPV vaccination: an appendix to the European Guidelines for Quality Assurance in Cervical Cancer Screening. *J Clin Virol* 2007;**38**:189.
10. Ault KA, for the Future II Study Group. Effect of prophylactic human papillomavirus L1 virus-like-particle vaccine on risk of cervical intraepithelial neoplasia grade 2, grade 3 and adenocarcinoma in situ: a combined analysis of four randomized clinical trials. *Lancet* 2007;**369**:1861–8.
11. Baehr P, McDonald G. Infections of the esophagus. In: Surawicz C, Owen R, editors. *Gastrointestinal and hepatic infections*. Philadelphia: WB Saunders; 1995. p. 3–33.
12. Baron EJ, Pfhaller MA. Human papillomaviruses. In: Murray PR, editor. *Textbook of clinical microbiology*. 7th ed. Portland, OR: ASM Press; 1999. p. 1613.
13. Bashi S. Cryotherapy versus podophyllin in the treatment of genital warts. *Int J Dermatol* 1985;**24**:535.
14. Bauer H, Ting Y, Greer CE, et al. Genital human papillomavirus infection in female university students as determined by a PCR-based method. *JAMA* 1991;**265**:472.
15. Bell R, Hong W, Itri LM, et al. The use of *cis*-retinoic acid in recurrent respiratory papillomatosis of the larynx: a randomized pilot study. *Am J Otolaryngol* 1988;**9**:161.
16. Benevolo M, Mottolese M, Marandino F, et al. HPB genotypes concordance between sex partners. *J Exp Clin Cancer Res* 2007;**26**:609.
17. Benevolo M, Mottolese M, Marandino F, et al. HPV prevalence among healthy Italian male sexual partners of women with cervical HPV infection. *J Med Virol* 2008;**80**:1275.
18. Benevolo M, Mottolese M, Piperno G, et al. HLA-A,-B,-C expression in colon carcinoma mimics that of the normal colonic mucosa and is prognostically relevant. *Am J Surg Pathol* 2007;**31**:76.
19. Benevolo M, Vocaturo A, Mottolese M, et al. Clinical role of p161NK4a expression in liquid-based cervical cytology: correlation with HPV testing and histologic diagnosis. *Am J Clin Pathol* 2008;**129**:606.
20. Benevolo M, Vocaturo A, Novelli F, et al. Prognostic value of HER2 and progesterone receptor expression in endometrial carcinoma with positive peritoneal washing. *Anticancer Res* 2007;**27**:2839.
21. Becker T, Wheeler N, McGough C, et al. Sexually transmitted diseases and other risk factors for cervical dysplasia among southwestern Hispanic and non-Hispanic white women. *JAMA* 1994;**271**:1181.
22. Benard VB, Johnson CJ, Thompson TD, et al. Examining the association between socioeconomic status and potential human papillomavius-associated cancers. *Cancer* 2008;**113**(Suppl 10): 2910–18.
23. Benard VB, Lawson HW, Eheman CR, et al. Adherence to guidelines for follow-up of low-grade cytologic abnormalities among medically underserved women. *Obstet Gynecol* 2005;**105**:1323–8.
24. Bennett R, Powell K. Human papillomaviruses: associations between laryngeal papillomas and genital warts. *Pediatr Infect Dis J* 1987;**6**:229.
25. Berger T, Sawchuk W, Leonardi C, et al. Epidermodysplasia verruciformis–associated papillomavirus infection complicating human immunodeficiency virus disease. *Br J Dermatol* 1991;**124**:79.
26. Bergeron C, Barrasso R, Beaudenon S, et al. Human papillomaviruses associated with cervical intraepithelial neoplasia: great diversity and distinct distribution in low- and high-grade lesions. *Am J Surg Pathol* 1992;**16**:641.
27. Berkhout R, Tieben L, Smits H, et al. Nested PCR approach for detection and typing of epidermodysplasia verruciformis–associated human papillomavirus types in cutaneous cancers from renal transplant recipients. *J Clin Microbiol* 1995;**33**:690.
28. Bernard H, Chan S, Manos M, et al. Identification and assessment of known and novel human papillomaviruses by polymerase chain reaction amplification, restriction fragment length polymorphisms, nucleotide sequence, and phylogenetic algorithms. *J Infect Dis* 1994;**170**:1077.
29. 1988 Bethesda System for reporting cervical/vaginal cytologic diagnoses: developed and approved at the National Cancer Institute workshop in Bethesda, MD, December 12-13, 1989. *Diagn Cytopathol* 1989;**5**:331–4.
30. Billingham R, Lewis F. Laser versus electrical cautery in the treatment of condylomata acuminata of the anus. *Surg Gynecol Obstet* 1982;**155**:865.
31. Birch H, Mankart H. Ultrasound for juvenile laryngeal papillomas. *Arch Otolaryngol* 1963;**77**:603.
32. Bonnez W, Da Rin C, Rose R, et al. Use of human papillomavirus type 11 virions in an ELISA to detect specific antibodies in humans with condylomata acuminata. *J Gen Virol* 1991;**72**:1343.
33. Bonnez W, Da Rin C, Rose R, et al. Evolution of the antibody response to human papillomavirus type 11 (HPV-11) in patients with condyloma acuminatum according to treatment response. *J Med Virol* 1993;**39**:340.
34. Bonnez W, Kashima H, Leventhal B, et al. Antibody response to human papillomavirus (HPV) type 11 in children with juvenile-onset recurrent respiratory papillomatosis (RRP). *Virology* 1992;**188**:384.
35. Bornstein J, Bentley J, Bösze P, et al. Colposcopic terminology of the International Federation for Cervical Pathology and Colposcopy. *Obstet Gynecol* 2012;**120**:166–72.
36. Boonlikit S, Noinual N. Vaginal intraepithelial neoplasia: a retrospective analysis of clinical features and colpohistology. *J Obstet Gynecol Res* 2010;**36**:94–100.
37. Braaten KP, Laufer MR. Human papillomavirus (HPV), HPV-associated disease, and the HPV vaccine. *Rev Obstet Gynecol* 2008;**1**(1): 2–10.
38. Brandenberger AW, Rüdlinger R, Hänggi W, et al. Detection of human papillomavirus in vulvar carcinoma: a study by in situ hybridization. *Arch Gynecol Obstet* 1992;**252**:31–5.
39. Brandsma J, Abramson A, Sciubba J. Papillomavirus infections of the nose. In: Steinberg B, Brandsma J, Taichman L, editors. *Papillomaviruses and cancer cells*. New York: Cold Spring Harbor Laboratory; 1987. p. 301–8.
40. Brandsma J, Steinberg B, Abramson A, et al. Presence of human papillomavirus type 16 related sequences in verrucous carcinoma of the larynx. *Cancer Res* 1986;**46**:2185.
41. Broomell EM, Reynolds SM, Jacobson RM. Epidemiology, clinical manifestations and recent advances in vaccination against human papillomavirus. *Postgrad Med* 2010;**122**(2):121–9.
42. Brown D, for the Future II Study Group. HPV type 6/11/16/18 vaccine: first analysis of cross-protection against persistent infection, cervical intraepithelial neoplasia (CIN), and adenocarcinoma in situ (AIS) caused by oncogenic HPV types in addition to 16/18. Poster presented at Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago. September 2007.
43. Brown LM, David PC, Devesa SS. Oral cavity and pharynx cancer incidence trends by subsite in the United States: changing gender patterns. *J Oncol* 2012;**2012**:1–10.
44. Bryan J. Developing an HPV vaccine to prevent cervical cancer and genital warts. *Vaccine* 2007;**25**:3001.
45. Bunney M, Barr B, McLaren K, et al. Human papillomavirus type 5 and skin cancer in renal allograft recipients. *Lancet* 1987;**2**:151.

46. Burgers JK, Badalment RA, Drago JR. Penile cancer: clinical presentation, diagnosis, and staging. *Urol Clin North Am* 1992;**19**(2): 247–56.
47. Burk R, Ho G, Beardsley L, et al. Sexual behavior and partner characteristics are the predominant risk factors for genital human papillomavirus infection in young women. *J Infect Dis* 1996;**174**:679.
48. Carter J, Hagensee M, Taflin M, et al. HPV-1 capsids expressed in vitro detect human serum antibodies associated with foot warts. *Virology* 1993;**195**:456.
49. Carter JJ, Koutsky LA, Wipf GC, et al. The natural history of human papillomavirus type 16 capsid antibodies among a cohort of university women. *J Infect Dis* 1996;**174**:927–36.
50. Cason J, Kaye J, Jewers R, et al. Perinatal infection and persistence of human papillomavirus types 16 and 18 in infants. *J Med Virol* 1995;**47**:209.
51. Castle PE, Wacholder S, Sherman ME, et al. Absolute risk of a subsequent abnormal pap among oncogenic human papillomavirus DNA-positive, cytologically negative women. *Cancer* 2002;**95**: 2145–51.
52. Castellsague X, Munoz N. Chapter 3: Cofactors in human papillomavirus carcinogenesis—role of parity, oral contraceptives an tobacco smoking. *J Natl Cancer Inst Monogr* 2003;**31**:20–8.
53. Caussy D, Orr W, Daya A, et al. Evaluation of methods for detecting human papillomavirus deoxyribonucleotide sequences in clinical specimens. *J Clin Microbiol* 1988;**26**:236.
54. Centers for Disease Control and Prevention (CDC). Human papillomavirus–associated cancers—United States, 2004–2008. *MMWR Morb Mortal Wkly Rep* 2012;**61**:258–61.
55. Chang F, Syrjanen S, Wang L, et al. Infectious agents in the etiology of esophageal cancer. *Gastroenterology* 1992;**103**:1336.
56. Chen S, Tsao Y, Lee J, et al. Characterization and analysis of human papillomaviruses of skin warts. *Arch Dermatol Res* 1993;**285**:460.
57. Chaturvedi AK, Engels EA, Anderson WF, et al. Human papillomavirus and rising oropharyngeal cancer incidence in the United States. *J Clin Oncol* 2011;**29**(32):4294–301.
58. Chesson HW, Ekwueme DU, Saraiya M, et al. The cost-effectiveness of male HPV vaccination in the United States. *Vaccine* 2011;**29**:8443–50.
59. Chin-Hong PV, Vittinghoff E, Cranston RD, et al. Age-specific prevalence of anal human papillomavirus infection in HIV-negative sexually active men who have sex with men: the EXPLORE study. *J Infect Dis* 2004;**190**:2070–6.
60. Christensen N, Hopfl R, DiAngelo S, et al. Assembled baculovirus-expressed human papillomavirus type 11 L1 capsid protein virus-like particles are recognized by neutralizing monoclonal antibodies and induce high titres of neutralizing antibodies. *J Gen Virol* 1994;**75**:2271.
61. Christensen N, Kreider J, Shah K, et al. Detection of human serum antibodies that neutralize infectious human papillomavirus type 11 virions. *J Gen Virol* 1992;**73**:1261.
62. Christian C, Reddel R, Gerwin B. Infection of cultured human cells of respiratory tract origin with human papillomavirus type 1. In: Steinberg B, Brandsma J, Taichman L, editors. *Papillomaviruses and cancer cells*. New York: Cold Spring Harbor Laboratory; 1987. p. 301–8.
63. Chung B, Akst L, Koltai P. 3.5-Year follow-up of intralesional cidofovir protocol for pediatric recurrent respiratory papillomatosis. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1911.
64. Ciuffo G. Innesto positivo con filtrato di verruca vulgare. *G Ital Mal Venereol* 1907;**48**:12.
65. Clark R, Brandon W, Dumestre J, et al. Clinical manifestations of infection with the human immunodeficiency virus in women in Louisiana. *Clin Infect Dis* 1993;**17**:165.
66. Clifford G, Goncalves M, Franceschi S, et al. Human papillomavirus types among women infected with HIV: a meta-analysis. *AIDS* 2006;**20**:2337.
67. Clifford GM, Rana RK, Franceschi S, et al. Comparison of HPV type distribution in high-grade cervical lesions and cervical cancer: a meta-analysis. *Breast Cancer J* 2003;**89**:101–5.
68. Cobo F, Garcia C, Talavera P, et al. Human papillomavirus associated with papillary squamous cell carcinoma of the oropharynx in a renal transplant recipient. *Infection* 2006;**34**:176.
69. Cohen S, Geller K, Seltzer S, et al. Papilloma of the larynx and tracheobronchial tree in children: a retrospective study. *Ann Otol Rhinol Laryngol* 1980;**89**:497.
70. Colgrove J. The ethics and politics of compulsory HPV vaccination. *N Engl J Med* 2006;**355**:2389.
71. Cook T, Brunschwig J, Butel J, et al. Laryngeal papilloma: etiologic and therapeutic considerations. *Ann Otol Rhinol Laryngol* 1973;**82**:649.
72. Cooper K, Androphy E, Lowy D, et al. Antigen presentation and T-cell activation in epidermodysplasia verruciformis. *J Invest Dermatol* 1990;**94**:769.
73. Dahlstrand H, Nasman A, Romanitan M, et al. Human papillomavirus accounts both for increased incidence and better prognosis in tonsillar cancer. *Anticancer Res* 2008;**28**:1133.
74. Daley M, Liddon N, Crane L, et al. A national survey of pediatrician knowledge and attitudes regarding human papillomavirus vaccination. *Pediatrics* 2006;**118**:2280.
75. Daponte A, Pournaras S, Mademtzis I, et al. Evaluation of HPV 16 PCR detection in self-compared with clinician-collected samples in women referred for colposcopy. *Gynecol Oncol* 2006;**103**:463.
76. Das B, Hussain S, Nasare V, et al. Prospects and prejudices of human papillomavirus vaccines in India. *Vaccine* 2008;**26**:2669.
77. Davis A, Emans S. Human papilloma virus infection in the pediatric and adolescent patient. *J Pediatr* 1989;**115**:1.
78. Deau M, Favre M, Orth G. Genetic heterogeneity among human papillomaviruses (HPV) associated with epidermodysplasia verruciformis: evidence for multiple allelic forms of HPV5 and HPV8 E6 genes. *Virology* 1991;**184**:492.
79. Dee S, Keane T, Bhavsar R. Contemporary diagnosis and management of squamous cell carcinoma (SCC) of the penis. *Br J Urol Int* 2011;**109**:1378–92.
80. De Peuter M, De Clercq B, Minette A, et al. An epidemiological survey of virus warts of the hands among butchers. *Br J Dermatol* 1977;**96**:427.
81. de Villiers E, Weidauer H, Otto H, et al. Papillomavirus DNA in human tongue carcinomas. *Int J Cancer* 1985;**36**:575.
82. de Villiers E, Fauquet C, Broker TR, et al. Classification of papillomaviruses. *Virology* 2004;**324**:17–27.
83. Derkay C, Wiatrak B. Recurrent respiratory papillomatosis: a review. *Laryngoscope* 2008;**118**:1236–47.
84. Donnelly J, Martinez D, Jansen K, et al. Protection against papillomavirus with a polynucleotide vaccine. *J Infect Dis* 1996;**173**:314.
85. Drut R, Gomez M, Drut R, et al. Human papillomavirus (HPV)–associated neonatal giant cell hepatitis (NGCH). *Pediatr Pathol Lab Med* 1996;**16**:403.
86. D'Souza G, Wiley DJ, Li X, et al. Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. *J Acquir Immune Defic Syndr* 2008;**48**:491–9.
87. Dunne EF, Sternberg M, Markowitz LE, et al. Human papillomavirus (HPV) 6, 11, 16, and 18 prevalence among females in the United States—National Health and Nutrition examination survey, 2003-2006: opportunity to measure HPV vaccine impact? *J Infect Dis* 2011;**204**:562–5.
88. Eicher S, Taylor-Cooley L, Donovan D. Isotretinoin therapy for recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 1994;**120**:405.
89. Elbasha EH, Dasbach EJ. Impact of vaccinating boys and men against HPV in the United States. *Vaccine* 2010;**28**:6858–67.
90. Eron L, Judson F, Tucker S, et al. Interferon therapy for condylomata acuminata. *N Engl J Med* 1986;**315**:1059.
91. Euvrard S, Chardonnet Y, Pouteil-Noble C, et al. Association of skin malignancies with various and multiple carcinogenic and non-carcinogenic human papillomaviruses in renal transplant recipients. *Cancer* 1993;**72**:2198.
92. Eversole L, Laipis P, Green T. Human papillomavirus type 2 DNA in oral and labial verruca vulgaris. *J Cutan Pathol* 1987;**14**:319.
93. Ferenczy A. HPV-associated lesions in pregnancy and their clinical implications. *Clin Obstet Gynecol* 1989;**32**:191.
94. Ferenczy A, Bergeron C, Richart R. Human papillomavirus DNA in fomites on objects used for the management of patients with genital human papillomavirus infections. *Obstet Gynecol* 1989; **74**:950.
95. Ferenczy A, Bergeron C, Richart R. Human papillomavirus DNA in CO_2 laser-generated plume of smoke and its consequences to the surgeon. *Obstet Gynecol* 1990;**75**:114.
96. Ferenczy A, Mitao M, Nagai N, et al. Latent papillomavirus and recurring genital warts. *N Engl J Med* 1985;**313**:784.

97. Firzlaff J, Kiviat N, Beckmann A, et al. Detection of human papillomavirus capsid antigens in various squamous epithelial lesions using antibodies directed against the L1 and L2 open reading frames. *Virology* 1988;**164**:467.
98. Franceschi S, Doll R, Gallwey J, et al. Genital warts and cervical neoplasia: an epidemiological study. *Br J Cancer* 1983;**48**:621.
99. Friedman H, Saah A, Sherman M, et al. Human papillomavirus, anal squamous intraepithelial lesions, and human immunodeficiency virus in a cohort of gay men. *J Infect Dis* 1998;**178**:45.
100. Friedman-Kien A, Eron L, Conant M, et al. Natural interferon alfa for treatment of condylomata acuminata. *JAMA* 1988;**259**:533.
101. Frisch M, Glimelius B, van den Brule A, et al. Sexually transmitted infection as a cause of anal cancer. *N Engl J Med* 1997;**337**:1350.
102. FUTURE II Study Group. Prophylactic efficacy of a quadrivalent human papillomavirus (HPV) vaccine in women with virological evidence of HPV infection. *J Infect Dis* 2007;**196**:1438.
103. FUTURE I/II Study Group. Four year efficacy of prophylactic human papillomavirus quadrivalent vaccine against low grade cervical, vulvar, and vaginal intraepithelial neoplasia and anogenital warts: randomised controlled trial. *BMJ* 2010;**341**:c3493.
104. Gabbott M, Cossart Y, Kan A, et al. Human papillomavirus and host variables as predictors of clinical course in patients with juvenile-onset recurrent respiratory papillomatosis. *J Clin Microbiol* 1997;**35**:3098.
105. Gabriel G, Thin R. Treatment of anogenital warts: comparison of trichloracetic acid and podophyllin versus podophyllin alone. *Br J Vener Dis* 1983;**59**:124.
106. Gall S, Hughes C, Trofatter K. Interferon for the therapy of condyloma acuminatum. *Am J Obstet Gynecol* 1985;**153**:157.
107. Gallia GL, Houff SA, Major EO, et al. Review: JC virus infection of lymphocytes—revisited. *J Infect Dis* 1997;**176**:1603–9.
108. Galloway D. Human papillomavirus vaccines: a warty problem. *Infect Agents Dis* 1994;**3**:187.
109. *Gardasil [package insert]*. Whitehouse Station. N.J.: Merck & Co, Inc; 2006.
110. Garden J, O'Banion M, Shelnitz L, et al. Papillomavirus in the vapor of carbon dioxide laser-treated verrucae. *JAMA* 1988;**259**:1199.
111. Garland S. Imiquimod. *Curr Opin Infect Dis* 2003;**16**:85.
112. Gaspari A. Mechanism of action and other potential roles of an immune response modifier. *Cutis* 2007;**79**(4 Suppl):36.
113. Gaylis B, Hayden R. Recurrent respiratory papillomatosis: progression to invasion and malignancy. *Am J Otolaryngol* 1991;**12**:104.
114. Ghim S, Jenson A, Schlegel R. HPV-1 L1 protein expressed in cos cells displays conformational epitopes found on intact virions. *Virology* 1992;**190**:548.
115. Gillison ML, Chaturvedi AK, Lowy DR. HPV prophylactic vaccines and the potential prevention of noncervical cancers in both men and women. *Cancer* 2008;**113**(10 Suppl):3036–46.
116. Gillison ML, D'Souza G, Westra WH, et al. Distinct risk factor profiles for human papillomavirus type 16-positive and human papillomavirus type 16-negative head and neck cancers. *J Natl Cancer Inst* 2008;**100**(6):407–20.
117. Giuliano AR, Palefsky JM, Goldstone S, et al. Efficacy of quadrivalent HPV vaccine against HPV infection and disease in males. *N Engl J Med* 2011;**364**:401–11.
118. Glinski W, Obalek S, Jablonska S, et al. T cell defect in patients with epidermodysplasia verruciformis due to human papillomavirus type 3 and 5. *Dermatologica* 1981;**162**:141.
119. Graham S, Priore R, Graham M, et al. Genital cancer in wives of penile cancer patients. *Cancer* 1979;**44**:1870.
120. Graupp M, Gugatschka M, Kiesler K, et al. Experience of 11 years use of cidofovir in recurrent respiratory papillomatosis. *Eur Arch Otorhinolaryngol* 2013;**270**:641–6.
121. Greer C, Wheeler C, Ladner M, et al. Human papillomavirus (HPV) type distribution and serological response to HPV type 6 virus-like particles in patients with genital warts. *J Clin Microbiol* 1995;**33**:2058.
122. Gross G, Ellinger K, Roussaki A, et al. Epidermodysplasia verruciformis in a patient with Hodgkin's disease: characterization of a new papillomavirus type and interferon treatment. *J Invest Dermatol* 1988;**91**:43.
123. Guerrero E, Daniel R, Bosch F, et al. Comparison of ViraPap, Southern hybridization, and polymerase chain reaction methods for human papillomavirus identification in an epidemiological investigation of cervical cancer. *J Clin Microbiol* 1992;**30**:2951.
124. Guido R. Human papillomavirus and cervical disease in adolescents. *Clin Obstet Gynecol* 2008;**51**:290.
125. Gutman L, St. Claire KK, Everett VD, et al. Cervical-vaginal and intraanal human papillomavirus infection of young girls with external genital warts. *J Infect Dis* 1994;**170**:339.
126. Gutman L, St. Claire KK, Herman-Giddens M, et al. Evaluation of sexually abused and nonabused young girls for intravaginal human papillomavirus infection. *Am J Dis Child* 1992;**146**:694.
127. Haber G, Malow R, Zimet G. The HPV vaccine mandate controversy. *J Pediatr Adolesc Gynecol* 2007;**20**:325.
128. Hagensee M, Kiviat N, Critchlow C, et al. Seroprevalence of human papillomavirus types 6 and 16 capsid antibodies in homosexual men. *J Infect Dis* 1997;**176**:625.
129. Hagensee M, Olson N, Baker T, et al. Three-dimensional structure of vaccinia virus-produced human papillomavirus type 1 capsids. *J Virol* 1994;**68**:4503.
130. Haglund S, Lundquist P, Cantell K, et al. Interferon therapy in juvenile laryngeal papillomatosis. *Arch Otolaryngol* 1981;**107**:327.
131. Hammarstedt L, Linquist D, Dahlstrand H, et al. Human papillomavirus as a risk factor for the increase in incidence of tonsillar cancer. *Int J Cancer* 2006;**119**:2620.
132. Harper D, Franco E, Wheeler C, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomized control trial. *Lancet* 2006;**367**:1247.
133. Hawkes M, Campisi P, Zafar R, et al. Time course of juvenile onset recurrent respiratory papillomatosis caused by human papillomavirus. *Pediatr Infect Dis J* 2008;**27**:149.
134. Healy G, Gelber R, Trowbridge A, et al. Treatment of recurrent respiratory papillomatosis with human leukocyte interferon: results of a multicenter randomized clinical trial. *N Engl J Med* 1988;**319**:401.
135. Herrero R, Hildesheim A, Bratti C, et al. Population-based study of human papillomavirus infection and cervical neoplasia in rural Costa Rica. *J Natl Cancer Inst* 2000;**92**:464–74.
136. Hildesheim A, Schiffman M, Gravitt P, et al. Persistence of type-specific human papillomavirus infection among cytologically normal women. *J Infect Dis* 1994;**169**:235.
137. Ho G, Bierman R, Beardsley L, et al. Natural history of cervicovaginal papillomavirus infection in young women. *N Engl J Med* 1998;**338**:423.
138. Ho GY, Studentsov YY, Bierman R, et al. Natural history of human papillomavirus type 16 virus-like particle antibodies in young women. *Cancer Epidemiol Biomarkers Prev* 2004;**13**:110–16.
139. Horn J, McQuillan G, Shah K, et al. Genital human papillomavirus infections in patients attending an inner-city STD clinic. *Sex Transm Dis* 1991;**18**:183.
140. Howlader N, Noone AM, Krapcho M, et al, editors. *SEER Cancer statistics review, 1975-2009 (vintage 2009 populations)*. Bethesda, MD: National Cancer Institute. Based on November 2011 SEER data submission, posted to the SEER web site, 2012. Available at http://seer.cancer.gov/csr/1975_2009_pops09/.
141. Jablonska S, Biczysko W, Jakubowicz K, et al. The ultrastructure of transitional states to Bowen's disease and invasive Bowen's carcinoma in epidermodysplasia verruciformis. *Dermatologica* 1970;**140**:186.
142. Jamison J, Kaplan D, Hamman R, et al. Spectrum of genital human papillomavirus infection in a female adolescent population. *Sex Transm Dis* 1995;**22**:236.
143. Jenison S, Yu X, Valentine J, et al. Human antibodies react with an epitope of the human papillomavirus type 6b L1 open reading frame which is distinct from the type-common epitope. *J Virol* 1989;**63**:809.
144. Jenison S, Yu X, Valentine J, et al. Evidence of prevalent genital-type human papillomavirus infections in adults and children. *J Infect Dis* 1990;**162**:60.
145. Jennings L, Ross A, Faoagali J. The prevalence of warts on the hands of workers in a New Zealand slaughterhouse. *N Z Med J* 1984;**97**:473.
146. Jensen SL. Comparison of podophyllin application with simple surgical excision in clearance and recurrence of perianal condylomata acuminata. *Lancet* 1985;**2**:1146–8.
147. Joseph AW, D'Souza G. Epidemiology of human papillomavirus-related head and neck cancer. *Otolaryngol Clin North Am* 2012;**45**:739–64.

148. Joura E, Leodolter S, Hernandez-Avila M, et al. Efficacy of a quadrivalent prophylactic human papillomavirus (types 6, 11, 16, and 18) L1 virus-like-particle vaccine against high-grade vulval and vaginal lesions: a combined analysis of three randomized clinical trials. *Lancet* 2007;**369**:1693.
149. Kahn J, Rosenthal S, Jin Y, et al. Rates of human papillomavirus vaccination, attitudes about vaccination, and human papillomavirus prevalence in young women. *Obstet Gynecol* 2008;**111**:1103.
150. Kaye J, Cason J, Pakarian F, et al. Viral load as a determinant for transmission of human papillomavirus type 16 from mother to child. *J Med Virol* 1994;**44**:415.
151. Kellogg N, Parra J. The progression of human papillomavirus lesions in sexual assault victims. *Pediatrics* 1995;**96**:1163.
152. Khanna N, Dalby R, Connor A, et al. Phase I clinical trial of repeat dose teramecrocol vaginal ointment in health female volunteers. *Sex Transm Dis* 2008;**35**:577–82.
153. Kiviat N, Koutsky L, Paavonen J, et al. Prevalence of genital papillomavirus infection among women attending a college student health clinic or a sexually transmitted disease clinic. *J Infect Dis* 1989;**159**:293.
154. Kjaer S, Dahl C, Engholm G, et al. Case-control study of risk factors for cervical neoplasia in Denmark, part II: role of sexual activity, reproductive factors, and venereal infections. *Cancer Causes Control* 1992;**3**:339.
155. Koutsky L, Ault K, Wheeler C, et al. A controlled trial of a human papillomavirus type 16 vaccine. *N Engl J Med* 2002;**347**:1645.
156. Krebs H. Treatment of genital condylomata with topical 5-fluorouracil. *Dermatol Clin* 1991;**9**:333.
157. Kreider J, Howett M, Leure-Dupree A, et al. Laboratory production in vivo of infectious human papillomavirus type 11. *J Virol* 1987;**61**:590.
158. Kreider J, Howett M, Lill N, et al. In vivo transformation of human skin with human papillomavirus type 11 from condylomata acuminata. *J Virol* 1986;**59**:369.
159. Kreimer AR, Clifford GM, Boyle P, et al. Human papillomavirus types in head and neck squamous cell carcinomas worldwide: a systematic review. *Cancer Epidemiol Biomarkers Prev* 2004;**14**:467–75.
160. Kuypers J, Kiviat N. Anal papillomavirus infections. In: Surawicz C, Owen R, editors. *Gastrointestinal and hepatic infections*. Philadelphia: W. B. Saunders; 1995. p. 279–85.
161. Labropoulou V, Diakomanolis E, Dailianas S, et al. Genital papillomavirus in Greek women with high-grade cervical intraepithelial neoplasia and cervical carcinoma. *J Med Virol* 1996;**48**:80.
162. Lacey CJN. Therapy for genital human papillomavirus-related disease. *J Clin Virol* 2005;**32**(Suppl):S82–90.
163. Lass J, Grove A, Papale J, et al. Detection of human papillomavirus DNA sequences in conjunctival papilloma. *Am J Ophthalmol* 1983;**96**:670.
164. Le Cann P, Touze A, Enogat N, et al. Detection of antibodies against human papillomavirus (HPV) type 16 virions by enzyme-linked immunosorbent assay using recombinant HPV 16 L1 capsids produced by recombinant baculovirus. *J Clin Microbiol* 1995;**33**:1380.
165. Lehtinen M, Paavonen J, Wheeler CM, et al. Overall efficacy of HPV-16/18 AS04-adjuvanted vaccine against grade 3 or greater cervical intraepithelial neoplasia: 4-year-end-of-study analysis of the randomized, double-blind PATRICIA trial. *Lancet* 2012;**13**: 89–99.
166. Leventhal B, Kashima H, Mounts P, et al. Long-term response of recurrent respiratory papillomatosis to treatment with lymphoblastoid interferon alfa-N1. Papilloma Study Group. *N Engl J Med* 1991;**325**:613.
167. Lin Y, Borenstein L, Ahmed R, et al. Cottontail rabbit papillomavirus L1 protein-based vaccines: protection is achieved only with a full-length, nondenatured product. *J Virol* 1993;**67**:4154.
168. Lippman S, Kessler J, Al Sarraf M, et al. Treatment of advanced squamous cell carcinoma of the head and neck with isotretinoin: a phase II randomized trial. *Invest New Drugs* 1988;**6**:51.
169. Lippman S, Meyskens F Jr. Treatment of advanced squamous cell carcinoma of the skin with isotretinoin. *Ann Intern Med* 1987;**07**:499.
170. Long G, Rickman L. Infectious complications of tattoos. *Clin Infect Dis* 1994;**18**:610.
171. Longuet M, Cassonnet P, Orth G. A novel genital human papillomavirus (HPV), HPV type 74, found in immunosuppressed patients. *J Clin Microbiol* 1996;**34**:1859.
172. Lorincz A, Reid R, Jenson A, et al. Human papillomavirus infection of the cervix: relative risk associations of 15 common anogenital types. *Obstet Gynecol* 1992;**79**:328.
173. Lorincz A, Schiffman M, Jaffurs W, et al. Temporal associations of human papillomavirus infection with cervical cytologic abnormalities. *Am J Obstet Gynecol* 1990;**162**:645.
174. Lutzner M, Orth G, Dutronquay V, et al. Detection of human papillomavirus type 5 DNA in skin cancers of an immunosuppressed renal allograft recipient. *Lancet* 1983;**20**:422.
175. Lyons G, Schlosser J, Lousteau R, et al. Laser surgery and immunotherapy in the management of laryngeal papilloma. *Laryngoscope* 1978;**88**:1586.
176. Madreperla S, Green W, Daniel R, et al. Human papillomavirus in primary epithelial tumors of the lacrimal sac. *Ophthalmology* 1993;**100**:569.
177. Maitland N, Cox M, Lynas C, et al. Detection of human papillomavirus DNA in biopsies of human oral tissue. *Br J Cancer* 1987;**56**:245.
178. Majewski S, Jablonska S. Epidermodysplasia verruciformis: Immunological and clinical aspects. *Curr Top Microbiol Immunol* 1994;**186**:157.
179. Majewski S, Jablonska S. Epidermodysplasia verruciformis as a model of human papillomavirus-induced genetic cancer of the skin. *Arch Dermatol* 1995;**131**:1312–18.
180. Majewski S, Malejczyk J, Jablonska S, et al. Natural cell–mediated cytotoxicity against various target cells in patients with epidermodysplasia verruciformis. *J Am Acad Dermatol* 1990;**22**:423.
181. Majewski S, Skopinska-Rozewska E, Jablonska S, et al. Partial defects of cell-mediated immunity in patients with epidermodysplasia verruciformis. *J Am Acad Dermatol* 1986;**15**:966.
182. Majoros M, Parkhill E, Devine K. Papillomas of the larynx in children: a clinicopathologic study. *Am J Surg* 1964;**108**:470.
183. Mandell D, Arjmand E, Kay D, et al. Intralesional cidofovir for pediatric recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 2004;**130**:1319.
184. Manos M, Ting Y, Wright D. The use of polymerase chain reaction amplification for the detection of genital human papillomaviruses. *Cancer Cells* 1989;**7**:209.
185. Markowitz L, Dunne E, Saraiya M, et al. Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007;**56**(RR-2):1–24.
186. Marres E, Wentges R, Brinkman W. Cryosurgical treatment of juvenile laryngeal papillomatosis. *Laryngoscope* 1966;**76**:1979.
187. Marth C, Daxenbichler G, Dapunt O. Synergistic antiproliferative effect of human recombinant interferons and retinoic acid in cultured breast cancer cells. *J Natl Cancer Inst* 1986;**77**:1197.
188. Marur S, D'Souza G, Westra WH, et al. HPV-associated head and neck cancer: a virus-related cancer epidemic. *Lancet* 2010;**11**: 781–9.
189. McDonnell J, Mayr A, Martin W. DNA of human papillomavirus type 16 in dysplastic and malignant lesions of the conjunctiva and cornea. *N Engl J Med* 1989;**320**:1442.
190. McDonnell P, McDonnell J, Kessis T, et al. Detection of human papillomavirus type 6/11 DNA in conjunctival papillomas by in situ hybridization with radioactive probes. *Hum Pathol* 1987; **18**:1115.
191. McGlennen R, Adams G, Lewis C, et al. Pilot trial of ribavirin for the treatment of laryngeal papillomatosis. *Head Neck* 1993;**15**:504.
192. Melkert P, Hopman E, van den Brule A, et al. Prevalence of HPV in cytomorphologically normal cervical smears, as determined by the polymerase chain reaction, is age dependent. *Int J Cancer* 1993;**53**:919.
193. Miller D, Brodell R, Levine M. The conjunctival wart: report of a case and review of treatment options. *Ophthalmic Surg* 1994;**25**:545.
194. Miller R, Meng T, Tomai M. The antiviral activity of Toll-like receptor 7 and 7/8 agonists. *Drug News Perspect* 2008;**21**:69.
195. Mork J, Lie A, Glattre E, et al. Human papillomavirus infection as a risk factor for squamous-cell carcinoma of the head and neck. *N Engl J Med* 2001;**344**:1125.
196. Morrison E. Natural history of cervical infection with human papillomaviruses. *Clin Infect Dis* 1994;**18**:172.
197. Moscicki A. Impact of HPV infection in adolescent populations. *J Adolesc Health* 2005;**37**(6 Suppl):S3–9.
198. Moscicki A, Palefsky J, Gonzales J, et al. The association between human papillomavirus deoxyribonucleic acid status and the results

of cytologic rescreening tests in young, sexually active women. *Am J Obstet Gynecol* 1991;**165**:67.
199. Moscicki A, Palefsky J, Gonzales J, et al. Colposcopic and histologic findings and human papillomavirus (HPV) DNA test variability in young women positive for HPV DNA. *J Infect Dis* 1992;**166**:951.
200. Moscicki A, Shiboski S, Broering J, et al. The natural history of human papillomavirus infection as measured by repeated DNA testing in adolescent and young women. *J Pediatr* 1998;**132**:277.
201. Moscicki AB, Shiboski S, Hills NK, et al. Regression of low-grade squamous intraepithelial lesions in young women. *Lancet* 2004;**364**:1678–83.
202. Mounts P, Kashima H. Association of human papillomavirus subtype and clinical course in respiratory papillomatosis. *Laryngoscope* 1984;**94**:28.
203. Mounts P, Shah K, Kashima H. Viral etiology of juvenile- and adult-onset squamous papilloma of the larynx. *Proc Natl Acad Sci U S A* 1982;**79**:5425.
204. Münger K, Howley PM, DiMaio D. Human papillomavirus E6 and E7 oncogenes. In: Garcea R, DiMaio D, editors. *The papillomaviruses*. New York: Springer, 2007. p. 195–251.
205. Myers ER, McCrory DC, Nanda K, et al. Mathematical model for the natural history of human papillomavirus infection and cervical carcinogenesis. *Am J Epidemiol* 2000;**151**(12):1158–71.
206. Naghashfar Z, Sawada E, Kutcher M, et al. Identification of genital tract papillomaviruses HPV-6 and HPV-16 in warts of the oral cavity. *J Med Virol* 1985;**17**:313.
207. Nahhas W, Marshall M, Ponziani J, et al. Evaluation of urinary cytology of male sexual partners of women with cervical intraepithelial neoplasia and human papilloma virus infection. *Gynecol Oncol* 1986;**24**:279.
208. National Cancer Institute Clinical Trials. *A study of V503 vaccine in females 12-26 years of age who have previously received Gardasil (V503-006 AM1), 2012*. Available at http://www.cancer.gov/clinicaltrials/search/view/print?cdrid=663767&version=HealthProf. Accessed September 16, 2012.
209. National Cancer Institute Workshop. The 1988 Bethesda system for reporting cervical/vaginal cytologic diagnoses. *JAMA* 1998; **262**:931.
210. Newall A, Brotherton J, Quinn H, et al. Population seroprevalence of human papillomavirus types 6, 11, 16, and 18 in men, women, and children in Australia. *Clin Infect Dis* 2008;**46**:1647.
211. Nishioka S, Fukushima K, Nishizaki K, et al. Human papillomavirus as a risk factor for head and neck cancers—a case control study. *Acta Otolaryngol Suppl* 1999;**527**:66–9.
212. Noffsinger A, Witte D, Fenoglio-Preiser C. The relationship of human papillomaviruses to anorectal neoplasia. *Cancer* 1992; **70**:1276.
213. Nuovo G, Pedemonte B. Human papillomavirus types and recurrent cervical warts. *JAMA* 1990;**263**:1223.
214. Nuovo G, Richart R. A comparison of slot blot, Southern blot, and in situ hybridization analyses for human papillomavirus DNA in genital tract lesions. *Obstet Gynecol* 1989;**74**:673.
215. Obalek S, Favre M, Szymanczyk J, et al. Human papillomavirus (HPV) types specific of epidermodysplasia verruciformis detected in warts induced by HPV3 or HPV3-related types in immunosuppressed patients. *J Invest Dermatol* 1992;**98**:936.
216. Ong C, Bernard H, Villa L. Identification of genomic sequences of three novel human papillomavirus sequences in cervical smears of Amazonian Indians. *J Infect Dis* 1994;**170**:1086.
217. Oraetiruys-Clausen F. Rare oral viral disorder (molluscum contagiosum, localized keratoacanthoma, verrucae, condyloma acuminatum, and focal epithelial hyperplasia). *Oral Surg Oral Med Oral Pathol* 1972;**34**:604.
218. Orlow S, Paller A. Cimetidine therapy for multiple viral warts in children. *J Am Acad Dermatol* 1993;**28**:794.
219. O'Rourke MA, Ellison MV, Murray LJ, et al. Human papillomavirus related head and neck cancer: a systematic review and meta-analysis. *Oral Oncol* 2012;**48**:1–11.
220. Orth G. Epidermodysplasia verruciformis: a model for understanding the oncogenicity of human papillomaviruses. *Ciba Found Symp* 1986;**120**:157–74.
221. Östrow R, Bender M, Niimura M, et al. Human papillomavirus DNA in cutaneous primary and metastasized squamous cell carcinomas from patients with epidermodysplasia verruciformis. *Proc Natl Acad Sci U S A* 1982;**79**:1634.
222. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* 1993;**12**:186–92.
223. Pakarian F, Kaye J, Cason J, et al. Cancer associated human papillomaviruses: perinatal transmission and persistence. *Br J Obstet Gynaecol* 1994;**101**:514.
224. Palefsky JM. Human papillomavirus-related disease in men: not just a women's issue. *J Adolesc Health* 2010;**46**:S12–19.
225. Palefsky JM, Giuliano AR, Goldstone S, et al. HPV vaccine against anal infection and anal intraepithelial neoplasia. *N Engl J Med* 2011;**365**:1576–85.
226. Palefsky J, Holly E, Ralston M, et al. Prevalence and risk factors for human papillomavirus infection of the anal canal in human immunodeficiency virus (HIV)-positive and HIV-negative homosexual men. *J Infect Dis* 1998;**177**:361.
227. Pappo O, Yunis E, Jordan J, et al. Recurrent and de novo giant cell hepatitis after orthotopic liver transplantation. *Am J Surg Pathol* 1994;**18**:804.
228. Pastner B, Baker D, Jackman E. Human papillomaviruses. In: Gonik B, editor. *Viral diseases in pregnancy*. New York: Springer-Verlag; 1994. p. 185–95.
229. Patel P, Hanson DL, Sullivan PS, et al. for the Adult and Adolescent Spectrum of Disease Project and HIV Outpatient Study investigators. Incidence of types of cancer among HIV-infected persons compared with the general population in the United States, 1992-2003. *Ann Intern Med* 2008;**48**:728–36.
230. Payan C, Ducancelle A, Aboubaker M, et al. Human papillomavirus quantification in urine and cervical samples by using the Mx4000 and LightCycler general real-time PCR systems. *J Clin Microbiol* 2007;**45**:897.
231. Pearson JM, Feltman RS, Twiggs LB. Association of human papillomavirus with vulvar and vaginal intraepithelial disease: opportunities for prevention. *Womens Health* 2008;**4**:143–50.
232. Peyton C, Jansen A, Wheeler C, et al. A novel human papillomavirus sequence from an international cervical cancer study. *J Infect Dis* 1994;**170**:1093.
233. Peyton C, Wheeler C. Identification of five novel human papillomavirus sequences in the New Mexico triethnic population. *J Infect Dis* 1994;**170**:1089.
234. Pfister H. Biology and biochemistry of papillomaviruses. *Rev Physiol Biochem Pharmacol* 1984;**99**:111.
235. Pfister H, Iftner T, Fuchs P. Papillomaviruses from epidermodysplasia verruciformis patients and renal allograft recipients. In: Howley P, Broker T, editors. *Papillomaviruses: molecular and clinical aspects*. New York: Liss; 1985. p. 85–100.
236. Pfister H, zur Hausen H. Seroepidemiological studies of human papilloma virus (HPV-1) infections. *Int J Cancer* 1978;**21**:161.
237. Phelps W, Alexander K. Antiviral therapy for human papillomaviruses: rationale and prospects. *Ann Intern Med* 1995;**123**:368.
238. Pickard RKL, Xiao W, Broutain TR, et al. The prevalence and incidence of oral human papillomavirus infection among young men and women, aged 18-30 years. *Sex Transm Dis* 2012;**39**:559–66.
239. Picketty C, Selinger-Leneman H, Grabar S, et al. On behalf of the FHDH-ANRS CO 4. Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. *AIDS* 2008;**22**: 1203–11.
240. Pollanen R, Vuopala S, Lheto VP. Detection of human papillomavirus infection by non-isotopic in situ hybridisation in condylomatous and CIN lesions. *J Clin Pathol* 1993;**46**(10):936–9.
241. Prose N, Knebel-Doeberitz C, Miller S, et al. Widespread flat warts associated with human papillomavirus type 5: a cutaneous manifestation of human immunodeficiency virus infection. *J Am Acad Dermatol* 1990;**23**:978.
242. Quick C, Watts S, Krzyzek R, et al. Relationship between condylomata and laryngeal papillomata: clinical and molecular virological evidence. *Ann Otol Rhinol Laryngol* 1980;**89**:467.
243. Rabah R, Lancaster W, Thomas R, et al. Human papillomavirus–11–associated recurrent respiratory papillomatosis is more aggressive than human papillomavirus–6–associated disease. *Pediatr Dev Pathol* 2001;**4**:68.
244. Ramoz N, Rueda LA, Bouadjar B, et al. Mutations in two adjacent novel genes are associated with epidermodysplasia verruciformis. *Nat Genet* 2002;**32**:579–81.
245. Reeves W, Arosemena J, Garcia M, et al. Genital human papillomavirus infection in Panama City prostitutes. *J Infect Dis* 1989;**160**:599.

246. Reeves W, Brinton L, Garcia M, et al. Human papillomavirus infection and cervical cancer in Latin America. *N Engl J Med* 1989;**320**:1437.
247. Reeves W, Rawls W, Brinton L. Epidemiology of genital papillomaviruses and cervical cancer. *Rev Infect Dis* 1989;**11**:426.
248. Reichman R, Oakes D, Bonnez W, et al. Treatment of condyloma acuminatum with three different interferon-alpha preparations administered parenterally: a double-blind, placebo-controlled trial. *J Infect Dis* 1990;**162**:1270.
249. Reid R. Superficial laser vulvectomy, part I: the efficacy of extended superficial ablation for refractory and very extensive condylomas. *Am J Obstet Gynecol* 1985;**151**:1047.
250. Repucci A, DiLorenzo T, Abramson A. In vitro modulation of human laryngeal papilloma cell differentiation by retinoic acid. *Otolaryngol Head Neck Surg* 1991;**105**:528.
251. Respler D, Jahn A, Pater A, et al. Isolation and characterization of papillomavirus DNA from nasal inverting (schneiderian) papillomas. *Ann Otol Rhinol Laryngol* 1987;**96**:170.
252. Revzina NV, Diclemente RJ. Prevalence and incidence of human papillomavirus in women in the USA: a systematic review. *Int J STD/AIDS* 2005;**16**:528–37.
253. Rintala MA. Transmission of high-risk human papillomavirus (HPV) between parents and infant: a prospective study of HPV in families in Finland. *J Clin Microbiol* 2005;**43**:376–81.
254. Rintala MA, Grénman SE, Järvenkylä ME, et al. High-risk types of human papillomavirus (HPV) DNA in oral and genital mucosa of infants during their first 3 years of life: experience from the Finnish HPV Family Study. *Clin Infect Dis* 2005;**41**:1728–33.
255. Rodriquez A, Schiffman M, Herrero R, et al. Rapid clearance of human papillomavirus and implications for clinical focus on persistent infections. *J Natl Cancer Inst* 2008;**100**:513.
256. Rogers SN, Vedpathak LV, Lowe D. Reasons for delayed presentation in oral and oropharyngeal cancer: the patient's perspective. *Br J Oral Maxillofac Surg* 2011;**49**:349–53.
257. Rose R, Bonnez W, Reichman R, et al. Expression of human papillomavirus type 11 L1 protein in insect cells: in vivo and in vitro assembly of virus-like particles. *J Virol* 1993;**67**:1936.
258. Rosenfeld W, Rose E, Vermund S, et al. Follow-up evaluation of cervicovaginal human papillomavirus infection in adolescents. *J Pediatr* 1992;**121**:307.
259. Rowson K, Mahy B. Human papova (wart) virus. *Bacteriol Rev* 1967;**31**:110.
260. Roye C. Abnormal cervical cytology in adolescents: a literature review. *J Adolesc Health* 1992;**13**:643.
261. Rudlinger R, Bunney M, Grob R, et al. Warts in fish handlers. *Br J Dermatol* 1989;**120**:375.
262. Rudlinger R, Grob R. Papillomavirus infection and skin cancer in renal allograft recipients. *Lancet* 1989;**1**:1132.
263. Rudlinger R, Smith I, Bunney M, et al. Human papillomavirus infections in a group of renal transplant recipients. *Br J Dermatol* 1986;**115**:681.
264. Sabol I, Salakova M, Smahelova J, et al. Evaluation of different techniques for identification of human papillomavirus types of low prevalence. *J Clin Microbiol* 2008;**46**:1606.
265. Sarkola M, Rintala M, Grenman S, et al. Human papillomavirus DNA detected in breast milk. *Pediatr Infect Dis J* 2008;**27**:557–8.
266. Sawchuk W, Felten R. Infectious potential of aerosolized particles. *Arch Dermatol* 1989;**125**:1689.
267. Sawchuk W, Weber P, Lowy D, et al. Infectious papillomavirus in the vapor of warts treated with carbon dioxide laser or electrocoagulation: detection and protection. *J Am Acad Dermatol* 1989;**21**:41.
268. Schiffman M. Recent progress in defining the epidemiology of human papillomavirus infection and cervical neoplasia. *J Natl Cancer Inst* 1992;**84**:394.
269. Schiffman M. Integration of human papillomavirus vaccination, cytology, and human papillomavirus testing. *Cancer* 2007;**111**:145.
270. Schiffman M, Kiviat N, Burk R, et al. Accuracy and interlaboratory reliability of human papillomavirus DNA testing by hybrid capture. *J Clin Microbiol* 1995;**33**:545.
271. Schiffman M, Wheeler CM, Castle PE; Atypical Squamous Cells of Undetermined Significance/Low-Grade Squamous Intraepithelial Lesion Triage Study Group. Human papillomavirus DNA remains detectable longer than related cervical cytologic abnormalities. *J Infect Dis* 2002;**186**:1169–72.
272. Schnadig V, Clark W, Clegg T, et al. Invasive papillomatosis and squamous carcinoma complicating juvenile laryngeal papillomatosis. *Arch Otolaryngol Head Neck Surg* 1986;**112**:966.
273. Schneider A, Kirchhoff T, Meinhardt G, et al. Repeated evaluation of human papillomavirus 16 status in cervical swabs of young women with a history of normal Papanicolaou smears. *Obstet Gynecol* 1992;**79**:683.
274. Schonfeld A, Nitke S, Schattner A, et al. Intramuscular human interferon-beta injections in treatment of condylomata acuminata. *Lancet* 1984;**1**:1038.
275. Schuster D. Snorters' warts. *Arch Dermatol* 1987;**123**:571.
276. Scinicariello F, Sato T, Lee C, et al. Detection of human papillomavirus in primary hepatocellular carcinoma. *Anticancer Res* 1992;**12**:763.
277. Scurry J, Wilkinson EJ. Review of terminology of precursors of vulvar squamous cell carcinoma. *J Lower Genital Tract Dis* 2006;**10**:161–9.
278. Shah K, Howley P. Papillomaviruses. In: Fields B, Knipe D, Howley P, editors. *Fields virology*. Philadelphia: Lippincott-Raven; 2006. p. 2077–109.
279. Shah K, Kashima H, Polk B, et al. Rarity of cesarean delivery in cases of juvenile-onset respiratory papillomatosis. *Obstet Gynecol* 1986;**68**:795.
280. Shah K, Stern W, Shah F, et al. Risk factors for juvenile onset recurrent respiratory papillomatosis. *Pediatr Infect Dis J* 1998;**17**:372.
281. Sheehan P, Sexton S, Russell J. Is intralesional cidofovir worthwhile in juvenile recurrent respiratory papillomatosis? *J Laryngol Otol* 2006;**120**:561.
282. Shehab N, Sweet B, Hogikyan N. Cidofovir for the treatment of recurrent respiratory papillomatosis: a review of the literature. *Pharmacotherapy* 2005;**25**:977.
283. Shen C, Ho M, Chang S, et al. High rate of concurrent genital infections with human cytomegalovirus and human papillomaviruses in cervical cancer patients. *J Infect Dis* 1993;**168**:449.
284. Singhi AD, Westra WH. Comparison of human papillomavirus in situ hybridization and p16 immunohistochemistry in the detection of human papillomavirus-associated head and neck cancer based on a prospective clinical experience. *Cancer* 2010;**116**(9):2166–73.
285. Singleton G, Adkins W. Cryosurgical treatment of juvenile laryngeal papillomatosis: an eight year experience. *Ann Otol Rhinol Laryngol* 1972;**81**:784.
286. Slawsky L, Gilson R, Hockley A, et al. Epidermodysplasia verruciformis associated with severe immunodeficiency, lymphoma, and disseminated molluscum contagiosum. *J Am Acad Dermatol* 1992;**27**:448.
287. Solomon D, Smith R, Kashima H, et al. Malignant transformation in non-irradiated recurrent respiratory papillomatosis. *Laryngoscope* 1985;**95**:900.
288. Solsona E, Algaba F, Horenblas S, et al. EAU guidelines on penile cancer. *Eur Urol* 2004;**46**:1–8.
289. Sperry K. Lethal asphyxiating juvenile laryngeal papillomatosis: a case report with human papillomavirus in situ hybridization analysis. *Am J Forensic Med Pathol* 1994;**15**:146.
290. Stanley M. Prophylactic HPV vaccines: prospects for eliminating anogenital cancer. *Br J Cancer* 2007;**96**:1320.
291. Stark L, Arends M, McLaren KM, et al. Prevalence of human papillomavirus DNA in cutaneous neoplasms from renal allograft recipients supports a possible viral role in tumour promotion. *Br J Cancer* 1994;**69**:222.
292. Steele J, Gallimore P. Humoral assays of human sera to disrupted and nondisrupted epitopes of human papillomavirus type 1. *Virology* 1990;**174**:388.
293. Steger G, Olszewsky M, Stockfleth E, et al. Prevalence of antibodies to human papillomavirus type 8 in human sera. *J Virol* 1990;**64**:4399.
294. Steinberg B, Topp W, Schneider P, et al. Laryngeal papillomavirus infection during clinical remission. *N Engl J Med* 1983;**308**:1261.
295. Stellato G, Nieminen P, Aho H, et al. Human papillomavirus infection of the female genital tract: correlation of HPV DNA with cytologic, colposcopic, and natural history findings. *Eur J Gynaecol Oncol* 1992;**13**:262.
296. St. Louis M, Icenogle J, Manzila T, et al. Genital types of papillomavirus in children of women with HIV-1 infection in Kinshasa, Zaire. *Int J Cancer* 1993;**54**:181.

297. Strong M, Vaughan C, Cooperband S, et al. Recurrent respiratory papillomatosis: management with the CO_2 laser. *Ann Otol Rhinol Laryngol* 1976;**85**:508.
298. Sun X, Kuhn L, Ellerbrock T, et al. Human papillomavirus infection in women infected with the human immunodeficiency virus. *N Engl J Med* 1997;**337**:1343.
299. Sun Y, Eluf-Neto J, Bosch F, et al. Human papillomavirus–related serological markers of invasive cervical carcinoma in Brazil. *Cancer Epidemiol Biomarkers Prev* 1994;**3**:341.
300. Syrjanen K, Hakama M, Saarikoski S, et al. Prevalence, incidence, and estimated life-time risk of cervical human papillomavirus infections in a nonselected Finnish female population. *Sex Transm Dis* 1990;**17**:15.
301. Syrjanen S. Human papillomavirus infections in the oral cavity. In: Syrjanen K, Gissmann L, Koss L, editors. *Papillomaviruses and human disease*. New York: Springer-Verlag; 1987. p. 104–37.
302. Syrjanen S, Partanen P, Mantyjarvi R, et al. Sensitivity of in situ hybridization techniques using biotin- and 35S-labeled human papillomavirus (HPV) DNA probes. *J Virol Methods* 1988;**19**:225.
303. Tasca RA, Clarke RA. Recurrent respiratory papillomatosis. *Arch Dis Child* 2006;**91**:689–91.
304. Taylor S. A prevalence study of virus warts on the hands in a poultry processing and packing station. *J Soc Occup Med* 1980;**30**:20.
305. Tenti P, Zappatore R, Migliora P, et al. Perinatal transmission of human papillomavirus form gravidas with latent infections. *Obstet Gynecol* 1999;**93**:475–9.
306. Thompson D, Douglas J, Foster M, et al. Seroepidemiology of infection with human papillomavirus 16, in men and women attending sexually transmitted disease clinics in the United States. *J Infect Dis* 2004;**190**:1563.
307. Tieben L, Berkhout R, Smits H, et al. Detection of epidermodysplasia verruciformis–like human papillomavirus types in malignant and premalignant skin lesions of renal transplant recipients. *Br J Dermatol* 1994;**131**:226.
308. Tjon Pian Gi RE, Dietz A, Djukic V, et al. Treatment of recurrent respiratory papillomatosis and adverse reactions following off-label use of cidofovir (Vistide®). *Eur Arch Otorhinolaryngol* 2012;**269**:361–2.
309. Torrente MC, Rodrigo JP, Haigentz M, et al. Human papillomavirus infections in laryngeal cancer. *Head Neck* 2011;**33**:581–6.
310. Trottier A, Coutlee F, Leduc R, et al. Human immunodeficiency virus infection is a major risk factor for detection of human papillomavirus DNA in esophageal brushings. *Clin Infect Dis* 1997;**24**:565.
311. Trottier A, Provencher D, Mes-Masson A, et al. Absence of human papillomavirus sequences in ovarian pathologies. *J Clin Microbiol* 1995;**33**:1011.
312. U.S. Cancer Statistics Working Group. *United States cancer statistics: 1999-2008 incidence and mortality web-based report*. Atlanta, GA: Department of Health and Human Services, Centers for Disease Control and Prevention, and National Cancer Institute; 2012. Available at http://www.cdc.gov/uscs. Accessed September 16, 2012.
313. U.S. Preventive Services Task Force. *Screening for cervical cancer*. Rockville, MD: U.S. Preventive Services Task Force; 2012. Available at http://www.uspreventiveservicestaskforce.org/uspstf/uspscerv.htm. Accessed September 16, 2012.
314. Ushikai M, Fujiyoshi T, Kono M, et al. Detection and cloning of human papillomavirus DNA associated with recurrent respiratory papillomatosis in Thailand. *Jpn J Cancer Res* 1994;**85**:699.
315. Vamos C, McDermott R, Daley E. The HPV vaccine: framing the arguments for and against mandatory vaccination of all middle school girls. *J Sch Health* 2008;**78**:302.
316. van de Nieuwenhof HP, van der Avoort IA, de Hullu JA. Review of squamous premalignant vulvar lesions. *Crit Rev Oncol Hematol* 2008;**68**:131–56.
317. van den Brule A, Walboomers J, Du M, et al. Difference in prevalence of human papillomavirus genotypes in cytomorphologically normal cervical smears is associated with a history of cervical intraepithelial neoplasia. *Int J Cancer* 1991;**48**:404.
318. van der Leest R, Zachow K, Ostrow R, et al. Human papillomavirus heterogeneity in 36 renal transplant recipients. *Arch Dermatol* 1987;**123**:354.
319. van Doornum GJ, Korse CM, Buning-Kager JC, et al. Reactivity to human papillomavirus type 16 L1 virus-like particles in sera from patients with genital cancer and patients with carcinomas at the five extragenital sites. *Breast Cancer J* 2003;**88**:1095–100.
320. Venkatesan NN, Pine HS, Underbrink MP. Recurrent respiratory papillomatosis. *Otolaryngol Clin North Am* 2012;**45**:671–94.
321. Vernon S, Holmes K, Reeves W. Human papillomavirus infection and associated disease in persons infected with human immunodeficiency virus. *Clin Infect Dis* 1995;**21**(Suppl. 1):121.
322. Viac J, Chomel J, Chardonnet Y, et al. Incidence of antibodies to human papillomavirus type 1 in patients with cutaneous and mucosal papillomas. *J Med Virol* 1990;**32**:18.
323. ViraPap and ViraType HPV DNA typing kit manuals and product information, 1989.
324. von Krogh G. Clinical relevance and evaluation of genitoanal papillomavirus infection in the male. *Semin Dermatol* 1992; **11**:229–40.
325. Vossler J, Forbes B, Adelson M. Evaluation of the polymerase chain reaction for the detection of human papillomavirus from urine. *J Med Virol* 1995;**45**:354.
326. Vousden K. Mechanisms of transformation by HPV. In: Stern P, Stanley M, editors. *Human papillomaviruses and cervical cancer: biology and immunology*. Oxford: Oxford University Press; 1994. p. 92–116.
327. Walboomers J, Husman A, van den Brule A. Detection of genital human papillomavirus infections: critical review of methods and prevalence studies in relation to cervical cancer. In: Stern P, Stanley M, editors. *Human papillomaviruses and cervical cancer: biology and immunology*. Oxford: Oxford University Press; 1994. p. 41–71.
328. Wallin K, Wiklund F, Angstrom T, et al. Type-specific persistence of human papillomavirus DNA before the development of invasive cervical cancer. *N Engl J Med* 1999;**341**:1633.
329. Ward P, Parry G, Yule R, et al. Human papillomavirus subtype 16a. *Lancet* 1989;**2**:170.
330. Wiatrak B, Wiatrak D, Broker T, et al. Recurrent respiratory papillomatosis: a longitudinal study comparing severity associated with human papilloma viral types 6 and 11 and other risk factors in a large pediatric population. *Laryngoscope* 2004;**114**:1–23.
331. Wilbur D, Stoler M. Testing for human papillomavirus: basic pathobiology of infection, methodologies, and implications for clinical use. *Yale J Biol Med* 1991;**64**:113.
332. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol* 2003;**157**:218–26.
333. Yaegashi N, Jenison S, Batra M, et al. Human antibodies recognize multiple distinct type-specific and cross-reactive regions of the minor capsid proteins of human papillomavirus types 6 and 11. *J Virol* 1992;**66**:2008.
334. Yaegashi N, Jenison S, Valentine J, et al. Characterization of murine polyclonal antisera and monoclonal antibodies generated against intact and denatured human papillomavirus type 1 virions. *J Virol* 1991;**65**:1578.
335. Ylitalo N, Bergstrom T, Gyllensten U. Detection of genital human papillomavirus by single-tube nested PCR and type-specific oligonucleotide hybridization. *J Clin Microbiol* 1995; **33**:1822.
336. Young L, Bevan I, Johnson M, et al. The polymerase chain reaction: a new epidemiological tool for investigating cervical human papillomavirus infection. *BMJ* 1989;**298**:14.
337. Zu R, Chou S. Particles resembling papova viruses in human cerebral demyelination disease. *Science* 1965;**148**:1477.

DNA VIRUSES

SUBSECTION 3 *ADENOVIRIDAE*

CHAPTER 155

Adenoviruses

James D. Cherry • Santhosh Nadipuram

Adenoviruses, which are responsible for a varied array of illnesses in children,* are associated most commonly with respiratory illness and gastroenteritis, but cardiac, neurologic, cutaneous, urinary, and lymphatic manifestations also occur frequently. Although many of the clinical manifestations of adenoviral infections are distinctive, the specific viral etiologic agent rarely is recognized by physicians.

Adenoviruses were noted first in explant cultures of human adenoid tissue; this finding, plus the observation of their apparent general affinity for lymphatic tissue, led to the designation of their name.[165,174,199,526]

HISTORY

The first adenoviral strains were not isolated until 1953, when tissue culture techniques became available, although epidemic disease caused by adenoviruses had been observed throughout the first half of the 20th century. Epidemic keratoconjunctivitis first was noted and reported in Austria by several physicians in 1889.[4,205,508,538,564] Major outbreaks of epidemic keratoconjunctivitis were reported in Bombay in 1901, in Madras in 1920 and 1928, in Hawaii in 1941, and on the West Coast of the United States in 1942.[267,288,289,345,653]

Epidemics of illness such as pharyngoconjunctival fever were observed throughout the 20th century. Béal,[33] in 1907, was the first to report the syndrome; in the 1920s, epidemics associated with swimming in public pools and in lakes were noted in Germany and the United States.[26,473] Initial studies by Rowe and colleagues[526] revealed cytopathic changes in explant tissue cultures of human adenoids that had been removed surgically. Fluid from these cultures caused distinctive cytopathologic changes in other tissue cultures, and antiserum prepared in hyperimmunized rabbits neutralized the effect. In 1954, Hilleman and Werner[282] isolated similar cytopathic agents from the throat washings of military recruits with febrile acute respiratory disease. Shortly thereafter, epidemic keratoconjunctivitis and pharyngoconjunctival fever were seen to be illnesses of adenoviral etiology.[127,130,132,309]

Initially, adenoviruses were known by the following names: *adenoid degeneration agent* because of its recovery in human adenoid tissue explants[526]; *respiratory illness patient number 67 (RI-67) agent*, which was recovered from a military recruit with primary atypical pneumonia during an epidemic of acute respiratory disease[281,282]; *adenoidal-pharyngeal-conjunctival agent*[297]; and *acute respiratory disease agent.*[110] In 1956, investigators in the field selected the term *adenovirus* for the new group of viruses. The name suggested the characteristic involvement of lymphadenoid tissue, as well as the tissue from which the organism was first isolated.[165]

Because of considerable morbidity and major economic considerations related to adenoviral epidemic respiratory disease in military recruits, the development of vaccines received attention early. Initially, inactivated vaccines were produced and they achieved some degree of success.[245,280,371] Later, live viral preparations grown in diploid human fibroblast tissue culture became available and proved quite successful in controlling specific adenoviral infections in military service personnel.*

In 1975, enteric adenoviruses (adenovirus types 40 and 41) first were reported.[432,540,639] They were demonstrated by electron microscopy and subsequently were shown to be a significant cause of diarrhea in children.† Especially important today are adenoviral infections in immunocompromised hosts, particularly transplant recipients.[350]

PROPERTIES OF THE VIRUS

Classification

Adenoviruses that infect humans are placed in the family *Adenoviridae* and the genus *Mastadenovirus.*[183,290,416,543] At present, 57 immunologically distinct adenoviral

*References 2, 3, 63, 65, 97, 163, 199, 285, 292, 357, 516, 530, 557, 610.

*References 153, 154, 234, 371, 521, 556, 585, 588.
†References 63, 104, 356, 424, 474, 584, 598, 610.

types have been recovered from humans.* Additional adenoviral types have been isolated from monkeys, cattle, dogs, mice, and chickens.[270,272,274] Mammalian adenoviruses have a common generic antigen that can be identified by complement fixation and enzyme-linked immunosorbent assay (ELISA).[315,486,518,641] Individual serotypes are identified by neutralization.[251,256,342]

Adenoviruses originally were subclassified on the basis of four hemagglutination patterns with rat and rhesus monkey red blood cells.[522] This subclassification is updated in Table 155-1. In 1962, researchers found that adenovirus type 12 could cause tumors in hamsters, and soon thereafter investigators realized that adenoviruses also could be classified by their oncogenic potential in rodents.[295,591] Grouping by oncogenic potential was similar, with few exceptions, to grouping by hemagglutination properties: organisms in hemagglutination group I had moderate oncogenic potential, group II and group III organisms had low or no potential, and group IV organisms had high oncogenic potential.[225,291,303]

Adenoviruses also have been subclassified (A to F) on the basis of the percentage of guanine plus cytosine in their DNA and other biochemical and biophysical criteria* (Table 155-2). In general, subgroup A organisms are the same as hemagglutination group IV; subgroup B, the same as hemagglutination group I; subgroups C, E, and F, the same as hemagglutination group III; and subgroup D, the same as hemagglutination group II. Restriction enzyme analysis has resulted in genomic typing of specific adenoviral serologic types.[380] In particular, numerous genotypes of adenovirus type 7 have been identified and the severity of disease may relate to the genotype.

TABLE 155-1 **Separation of Human Adenoviruses into Subgroups by Ability to Agglutinate Rhesus Monkey and Rat Erythrocytes**

Subgroup	Characteristic	Type
I	Complete agglutination of monkey erythrocytes	3, 7, 11, 14, 16, 21, 34, 35, 50
II	Complete agglutination of rat erythrocytes	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51
III	Partial agglutination of rat erythrocytes	1, 2, 4-6, 40, 41
IV	No or little agglutination of monkey or rat erythrocytes	12, 18, 31

Data from references 135, 270, 276, 284, 287, 327, 340, 371, 457, 458, 539, 643.

Physical Properties

Adenoviruses are nonenveloped DNA viruses 65 to 80 nm in diameter.† The virion consists of a protein capsid composed of 252 capsomeres and a nucleoprotein core that contains the DNA viral genome and two to four internal proteins.[500] The virion is roughly spherical in the form of an icosahedron; each of the 20 sides is an equilateral triangle, with the vertices of each converging in groups of five and resulting in 12 pentagonal vertices.[63,199,290] Each vertex capsomere contacts five other capsomeres and is designated a *penton*. Each penton contains a base plate and a rodlike projection, the fiber. The virion has 240 nonvertex capsomeres that occur in groups of six and are known as *hexons*.[349,458,600] Hexons contain the generic complement-fixing antigen.[199] Each capsomere has a diameter of 8 nm and a central hole that is 2 to 3 nm in diameter.[648] The capsomeres constitute 87 percent of the dry weight of the virion.

The central core of the virion, which accounts for 12 to 14 percent of its dry weight, is composed of linear

TABLE 155-2 **Grouping of Human Adenoviral Serotypes Based on Biochemical and Biophysical Criteria**

Subgroup	Adenovirus Type(s)	Location and Manifestations of Infection
A	12, 18, 31	Gastrointestinal; may cause disease in children
B:1	3, 7, 16, 21, 50, 55	Respiratory, eye, and gastrointestinal symptomatic infections
B:2	11, 14, 34, 35	Urinary and respiratory; symptomatic urinary tract infections (particularly in immunosuppressed patients) and symptomatic respiratory infections
C	1, 2, 5, 6, 57	Respiratory and gastrointestinal; symptomatic respiratory infections and hepatitis in immunosuppressed patients
D	8-10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, 42-49, 51, 53, 54, 56	Eye and gastrointestinal; symptomatic eye infections, gastrointestinal infections in HIV-infected patients
E	4	Symptomatic eye and respiratory infections
F	40, 41	Symptomatic gastrointestinal infections
G	52	Symptomatic gastrointestinal infections

Data from references 135, 276, 291, 340, 371, 386, 493, 515, 539, 623, 630.

*References 135, 137, 168, 183, 271-274, 276, 277, 292, 340, 514, 539, 623, 625, 642.

*References 276, 340, 493, 539, 622-624.
†References 183, 225, 290, 291, 303, 349, 416.

double-stranded DNA (molecular mass, 23.85 × 10^6 daltons for adenovirus type 2) and two to four basic proteins.[207,291,648]

Adenoviruses are highly stable in general.[142,199,223,303,373] They are resistant to lipid solvents and retain activity at pH values ranging from 2 to 10.

At 24° C, maximal infectivity is maintained between pH 6.0 and 9.5. Adenoviruses are stable at room temperature for 2 weeks, at 36° C for 7 days, and at 4° C for at least 70 days. Infectivity is destroyed by heating to 56° C for 30 minutes. Sodium dodecyl sulfate (0.25%) inactivates virus by disruption of the capsid.[349] Hydrogen peroxide vapor has been shown to inactivate adenoviruses,[47] and free chlorine is effective in controlling adenoviruses in drinking water.[475] Adenovirus serotypes 2, 10, 40, and 41 are highly resistant to ultraviolet radiation, and, therefore, they can be used as markers of the quality of water treatment practices.[279]

Antigenic Composition

The antigenic determinants of adenoviruses are contained on the protein structural subunits (hexons, pentons, and fibers).[600] The hexon antigen (alpha component) carries the generic antigenic component that is common to all mammalian adenoviruses and is measured by complement fixation or ELISA. Another hexon antigen (epsilon component) reacts with neutralizing antibodies lacking hemagglutination-inhibiting activity. The antigen related to viral fiber also induces the production of type-specific neutralizing antibody for some adenoviral types. Minor antigens are related to the pentons. One of them (the cell-detaching factor) causes rounding and clumping of tissue culture cells.

Gerna and associates,[221] using the immunoperoxidase antibody technique, found that the early antigens of all serotypes belonging to one group (see Table 155-1) react strongly with all type-specific immune sera of the same group.

Tissue Culture Growth

Although adenoviruses can be grown in a wide variety of cells of human epithelial origin, primary or diploid cultures of human embryonic kidney are preferable for recovery of agents from clinical specimens.[373] Continuous cell lines such as HeLa and HEp-2 also are quite sensitive.[266,303,514,527] Adenoviruses will grow in monkey kidney tissue culture, but evolution of the cytopathic effect is considerably slower than that occurring in cells of human origin. In addition, the cytopathic effect is more variable in monkey kidney tissue culture and many isolates will be missed.

Enteric adenoviruses have been detected in human feces by electron microscopy.* These enteric adenoviruses, identified as types 40 and 41, usually do not grow in standard tissue culture systems. Both viral types will grow in Graham 293 cells (a human embryonic kidney cell line transformed by adenovirus type 5); type 40 also will grow in tertiary monkey kidney cells, and some strains of type 41 will grow in HEp-2, Chang conjunctiva cells, and tertiary cynomolgus cells.[576,625] Grabow and associates[237] noted that the PLC/PRG/5 cell line was 100 times more sensitive to a laboratory strain of adenovirus type 41 and 10 times more sensitive to a laboratory strain of adenovirus type 40 than were Graham 293 cells.

Specimens for viral culture may be obtained from the eye, pharynx, blood, lungs, pleural and pericardial fluid, liver, stool, intestinal epithelium, lymph nodes, cerebrospinal fluid (CSF), brain, urine, and renal tissue. For best isolation rates, clinical specimens should be inoculated in cell culture within 6 hours of being collected. Tissue culture tubes are incubated best at 37° C, and rolling offers no advantage over stationary incubation.[373] Although the cytopathic effect has considerable variability, the most consistent finding is marked rounding and clumping of cells, often in grapelike clusters.[500,504] A cytopathic effect may be noted as early as 1 to 2 days after inoculation, but it may be delayed as long as 4 weeks. Use of the shell viral technique, in which infected cells in a specimen are centrifuged onto HEp-2 tissue culture cells, yields positive cultures in 1 to 2 days.[171] The cytopathic effect characteristically is followed by detachment of the entire cell sheet from the glass of the tissue culture tube within 2 to 4 days.[242,267,487,524,527]

Virus Multiplication

Initial attachment of adenoviruses to cells is relatively slow, with up to 6 hours being required.[128,224,303] The fiber protein of the virus mediates attachment to the cellular receptor.[544] An important adenovirus receptor is the coxsackievirus and adenovirus receptor (CAR) protein. It is a high-affinity receptor for all adenoviral subgroups except subgroup B. Penetration into the host cell occurs rapidly, either by phagocytosis or by direct entry through the plasma membrane.[303,391] The eclipse period with adenoviruses varies from 11 to 21 hours, depending on the serotype.[224,303] During eclipse, viral DNA uncoating requires approximately 2 hours when determined biochemically.[303] After uncoating, the viral DNA rapidly enters the nucleus of the cell and disassociates from its internal protein.[128,225,391] After the eclipse period, viral DNA accumulates, often doubling the content of the infected cell and becoming part of the typical inclusion bodies of the infected cells.[225]

Characteristic cytopathic changes in tissue culture, as demonstrated by light microscopy of hematoxylin and eosin–stained material or by electron microscopy, appear as early as 8 to 24 hours after infection occurs.[59,61,251] Two types of intranuclear alterations may be seen. In the first, early, small, discrete eosinophilic inclusions are observed. They gradually enlarge, become more prominent, and then form a large crystalline central mass surrounded by a clear zone or halo.[59,231] This type of early cytopathic effect appears to take place in cells that contain only small amounts of infectious virus.[60] The second type of intranuclear alteration, which generally occurs later, consists of large basophilic intranuclear inclusions.[59] Occasionally, giant forms measuring greater than 14 mm across are noted.[59] These mature inclusions may expand the nucleus of the cell so greatly that the cell's DNA content

*References 63, 120, 136, 315, 340, 507, 517, 597, 623, 641.

is 10 times larger than that of an uninfected cell. They contain a large amount of viral antigen and infective virus. Cytopathic changes vary considerably with the different adenoviral serotypes.[61,349]

In general, virus remains within the nucleus of intact, infected cells, with less than 1 percent of the total viral content of a culture being free within the extracellular fluid at any one time.[349]

Animal Susceptibility

Human adenoviruses usually do not produce clinical illness in laboratory animals, although infections with several different serotypes have occurred in selected animals.[303] When adenoviral strains were administered intranasally to young, "pathogen-free," colostrum-deprived pigs or 1-month-old cotton rats, pneumonia developed in the animals and intranuclear inclusions were observed microscopically in their lungs.[303,472] An adenovirus type 5 strain was injected intravenously into a rabbit, and the virus was recovered from the animal's spleen when removed 2 months later. Many adenoviral strains will propagate in a variety of different animal tissue cultures. In addition, in some cultures, marked cytopathic effects are observed but little infectious virus is produced.

Adeno-associated Viruses

Adeno-associated viruses are a group of small, defective, single-stranded, DNA-containing, virus-like particles that replicate in tissue culture only in the presence of adenoviruses.* Four serologic types have been identified; however, only types 2 and 3 have been recovered from humans.[199,349] These agents have been isolated from throat and anal swab specimens of children from whom adenoviral strains also were isolated.[53] Approximately 30 percent of children have complement-fixing antibody to adeno-associated virus types 2 and 3; most of these children have had evidence of adenoviral infection as well.[54] Neutralizing antibody to adeno-associated virus types 2 and 3 is noted in the sera of 60 to 80 percent of children 8 to 14 years of age.[481] The specific relevance of adeno-associated viruses is unknown.

EPIDEMIOLOGY

General Prevalence

Adenoviral infections account for 2 to 5 percent of all respiratory illnesses.† In children, adenoviruses are estimated to be responsible for 2 to 35 percent of respiratory viral illnesses,‡ and they are implicated in 5 to 11 percent of upper respiratory tract infections, 4 to 10 percent of cases of pharyngitis, 3 to 9 percent of cases of croup, 5 to 11 percent of cases of bronchitis, 2 to 16 percent of cases of bronchiolitis, and 4 to 10 percent of cases of pneumonia.* Enteric adenoviral infections are the cause of 2 to 15 percent of acute diarrheal illnesses in children.†

Specific categories of illness caused by adenoviruses vary with respect to viral serotype, patient age, socioeconomic status, and environmental conditions.‡ Adenoviruses have a predilection for infants and children younger than 5 years of age who spend portions of their days in closed environments, such as daycare centers, orphanages, and other institutions.§ Epidemics of disease caused by adenoviral infection have been associated with swimming pools, daycare centers, resident schools, certain industries, hospitals, physicians' offices, and early basic military training centers in young recruits.¶ Adenoviruses are recovered commonly from children in tropical countries and in situations characterized by crowding, such as lower socioeconomic settings.[85,90,107,316,662]

Age Incidence and Prevalence

Although adenoviral infections occur in all age groups, the incidence of infection generally is related inversely to age.[114,349] More than 90 percent of neonates have transplacentally acquired complement-fixing adenoviral serum antibody.[449,565] Most infants have neutralizing antibody to one or more of the common adenoviral types, which appears to be protective during the first 6 months of life. When adenoviral infection does occur in a neonate, it often is severe and occasionally fatal.[16,177,670] By the sixth month of life, only 14 percent of infants have demonstrable adenoviral complement-fixing antibody. By the time children reach 1 year of age, complement-fixing adenoviral antibody is observed in 44 to 50 percent of sera tested.[297,453]

The incidence of adenoviral infection peaks in infants and children between 6 months and 5 years of age.[102,422,488] By the time that they are 5 years of age, 70 to 80 percent of children have neutralizing antibody to adenovirus types 1 and 2 and 50 percent have antibody to adenovirus type 5.**

Adenoviral infections also occur commonly in grade school– and junior high school–aged children, but the incidence diminishes in high school–aged adolescents. Adenoviral infections occur in only 1 to 2 percent of college students, and they are noted infrequently in civilian adults.[172,173,175,241,567] Approximately 1 percent of adults with a respiratory infectious illness will have adenoviral infection; in hospitalized adults, the incidence is approximately 4 percent.[241] The most common adenoviral respiratory tract infections in children are caused by types 1, 2, 3, and 5.†† Types 6 and 7 are the next most frequent isolates associated with childhood respiratory tract infection. Adenovirus types 40 and 41 are the most common causes of diarrhea.[104,354,377]

*References 53, 128, 199, 303, 336, 557.
†References 81, 105, 106, 113, 176, 197, 198, 338, 488.
‡References 62, 81, 88, 90, 290, 389, 399, 448, 503, 506, 542, 619.

*References 62, 88-90, 201, 215, 437, 483.
†References 104, 120, 298, 314, 377, 424, 474, 507, 512, 597, 610, 646.
‡References 85, 114, 249, 265, 316, 662.
§References 40, 43, 85, 257, 390, 621.
¶References 27, 98, 123, 177, 239, 309, 310, 348, 351, 375, 404, 471, 484, 495, 511, 529, 531, 554, 655, 665.
**References 141, 197, 297, 306, 482, 488, 603.
††References 1, 12, 40, 370, 603, 615, 621.

Military Recruits

Epidemic adenoviral disease occurs commonly in military recruits, with virtually all illness developing during the first 8 weeks of basic training.* The attack rate has varied between 40 and 90 percent.[195,242,398,604] Adenoviruses account for 30 to 70 percent of acute respiratory disease, 67 percent of common cold-like illnesses, 62 to 77 percent of cases of acute febrile pharyngitis and tonsillitis, 67 percent of cases of bronchitis, and 24 percent of cases of pneumonia.[46,196] Some adenoviral infections are associated with minimal or no symptoms.[196,242]

A vaccine against adenovirus serotypes 4 and 7 was developed for use in military basic training centers and effectively prevented outbreaks of adenovirus. Routine vaccination began in 1971, but the sole manufacturer of the vaccine ceased its production in 1995. The first large post–vaccine era outbreak of adenovirus serotypes 3 and 7 occurred in naval recruits in Great Lakes, Illinois, in 1997.[531] Subsequent adenoviral epidemics occurred in military recruits in Fort Jackson, South Carolina, in 1998 with type 4 and in Lackland Air Force Base in San Antonio, Texas in 2007 with serotype 14.[68,580,620]

The last remaining stocks of vaccine were depleted or expired by 1999. Because of continued outbreaks, vaccine development was reinitiated, leading to the development of a live oral vaccine against adenovirus 4 and 7. The vaccine was licensed in 2011. It is currently manufactured by Barr Labs.[598] A full review of 8 deaths caused by adenovirus infection in military recruits from 1999 through 2010 was published by Potter and colleagues. All 8 deaths occurred after the cessation of the immunization program in 1999. In contrast, there were no deaths due to adenovirus infection in U.S. military personnel from 1975 through 1998 while this program was in place.[499]

Geographic Distribution

Adenoviral infections have been noted throughout the world.† Epidemic, endemic, and sporadic infections all occur.

Seasonal Patterns

Sporadic infections with adenoviruses occur throughout the year.‡ Epidemic adenoviral respiratory disease usually occurs in the winter, spring, and early summer.§ Seasonal patterns depend on serotypes, population groups, and exposure. Epidemics of disease in military recruits commonly associated with adenovirus types 4 and 7 occur most frequently in the winter and spring.[55,195,196,280] Epidemics of pharyngoconjunctival fever have been noted most commonly in the summer months in school-aged children in association with summer camps or swimming pools.[404,607,648] No seasonal pattern has been identified for adenoviral gastroenteritis.[120,339,517,597]

Host and Social Factors

The overall incidence of adenoviral illnesses is higher in males.[114,267] In a study involving 3313 adenovirus isolates, the male-to-female ratio was 1.3 : 1.[114]

Susceptibility to adenoviral infection apparently does not vary by race. More severe disease has been noted in infants of native and Indian populations in New Zealand and Canada.[9,36,232] However, the relationship of these findings to socioeconomic conditions was not identified initially.

The incidence of adenoviral infection is greatest in lower socioeconomic population groups.[316] Spread of infection has been observed in daycare centers, schools, children's homes, hospitals, clinics, physicians' offices, and certain industrial settings.* Acute, overwhelming illness, including bronchiolitis and pneumonia with severe pulmonary residua, has been reported in neonates, small infants, and occasionally, adults.[16,265,376] Adenoviral infections are a significant problem in immunocompromised hosts; when they occur, they frequently are severe and occasionally are fatal.†

Spread of Infection

Adenoviruses frequently are isolated from the conjunctiva, throat, and stool. Despite the ease of isolating the virus, the effectiveness of spread of infection in the general population varies considerably.[90] Adenovirus types 1, 2, 3, 5, and 7 are effective spreaders; however, they are not as highly contagious as varicella, measles, and influenza viruses.[89,90] Close contact appears to be necessary for infection to spread from one person to another.[565] Illness does not spread rapidly in the usual school setting but does spread dramatically in closed environments.[43,89,257,296] Although adenovirus type 4 spreads less effectively in the general population, spread is rapid in nonimmune military recruits who live in close contact with each other.[100] In an outbreak of adenovirus type 7 in a children's home, 84 percent of the residents were shown to be infected.

Transmission of virus occurs by droplets reaching the conjunctiva, nose, or throat or, alternatively, by the fecal-oral route. In volunteer studies, adenoviruses have been shown to spread by small-droplet aerosols and, to a lesser degree, by large droplets.[19,115,116] Adenovirus type 4 has been recovered from room air and cough samples of patients with the virus in their throats.[20] A study by Russell and colleagues[529] of 341 military recruits and support personnel at the Marine Corps training center in San Diego, California, demonstrated that more than 79 percent of incoming recruits were susceptible to adenoviral infection in the postvaccine era. They identified incoming recruits with prolonged asymptomatic shedding of adenovirus and

*References 195, 351, 398, 414, 529, 531, 605, 606.
†References 27, 88, 92, 122, 131, 180, 193, 199, 204, 207, 232, 265, 275, 284, 301, 306, 308, 316, 329, 336, 338, 346, 348, 349, 358, 362, 428, 429, 480, 545, 551, 560, 564, 565, 571, 577, 602-605, 607, 648, 655, 659, 662.
‡References 197, 202, 230, 284, 296, 389, 399, 428, 437.
§References 12, 55, 62, 199, 257, 289, 306, 358, 480, 498, 503, 603, 606.

*References 40, 43, 46, 87, 98, 177, 249, 251, 310, 471, 474, 484, 554, 610, 665.
†References 79, 82, 124, 129, 189, 243, 350, 392, 420, 463, 558, 670.

the presence of adenoviruses on multiple living quarter surfaces as potential sources of transmission. They also found evidence of extended pharyngeal shedding of adenovirus over the course of several days among recruits who had febrile respiratory illnesses.

In epidemics of pharyngoconjunctival fever, the virus appears to spread from contaminated swimming pool water to the eyes of recipients. In non–swimming-associated outbreaks of adenoviral respiratory illness with appropriate serotypes, conjunctivitis occurs only rarely.* This fact, in conjunction with the finding that pharyngoconjunctival fever occurred after conjunctival administration of virus, but not after nasopharyngeal application in volunteers,[39,557,634] suggests that the conjunctiva must be inoculated directly for the syndrome to occur. Epidemics of keratoconjunctivitis have occurred as a result of contact with contaminated ophthalmic instruments and fingers.† In daycare centers, orphanages, and the military, transmission probably occurs most commonly through small-droplet aerosols in crowded quarters.[495] An alternative in children is the fecal-oral route. Enteric adenoviruses have been identified in fecal specimens for approximately 8 days after the onset of gastroenteritis.[339]

Family members may excrete adenoviruses in their feces intermittently for prolonged periods after initial infection.[65,197] In one study, 20 percent of persons excreted adenoviruses in stool for longer than 3 months. Intrahousehold spread appears to continue as long as susceptible family members are present.[197] Infants born into households in which members are adenoviral fecal excreters often become infected, presumably through the fecal-oral route. In general, 50 percent of susceptible household members will experience infection, although the rate varies inversely with age and also depends on the serotype of adenovirus. Reinfection with specific adenoviral serotypes occurs, but most are asymptomatic or associated with only minimal illness.

Nosocomial spread of both respiratory and enteric adenoviruses is a common occurrence and has been the cause of fatal illnesses in infants and immunocompromised patients.‡

PATHOGENESIS AND PATHOLOGY

Adenoviral infections usually are acute and self-limited, and, therefore, opportunities to study the pathogenesis and pathologic process have been infrequent. Study of the pathologic mechanisms has been performed in human volunteers, tissue and organ culture systems, and recent murine model systems and by examination of specimens from persons dying of adenoviral disease.

Viral Infection

In general, the characteristics of adenoviral infection depend on the host and the serotype of the agent.§ The route of infection with adenoviruses that are capable of causing pharyngoconjunctival fever determines the pathologic manifestations. In most respiratory illnesses, initial viral infection occurs in the respiratory tract and involves the mucous membranes of the nose, oropharynx, and conjunctiva. Adenoviral agents have been isolated from sputum and oral secretions from 2 days before the onset of clinical illness to up to 8 days after the onset of symptoms. Deeper respiratory tract involvement of the trachea, pleurae, and lungs may result from initial small-particle aerosols, from progression of local infection, or perhaps as a result of viremia. Gastrointestinal infection in conjunction with respiratory tract infection also occurs early and probably is the result of swallowed virus. Stool isolates of respiratory viral types frequently are noted concomitantly with respiratory tract infection. However, in contrast to the upper respiratory tract infection, the virus may persist for a long time in the lower gastrointestinal tract. Infection with enteric adenoviral types is presumed to involve intestinal epithelial cells. In one fatal infection, adenoviral antigen was demonstrated in jejunal cells.[507,640]

In experimental studies with aerosolized adenovirus type 4, recovery of virus from throat specimens occurred on day 5 or 6, progressed to a maximum concentration on day 11 or 12, and was seen uncommonly after day 20. Maximal recovery of adenovirus type 4 from anal specimens occurred on day 13, and shedding continued for longer than 3 weeks. Adenovirus types 26 and 27 inoculated into the conjunctiva resulted in short-term isolation of virus from the eyes on days 3 to 7 and, less frequently, from the throat on day 4.[328] Isolation of virus from the rectum was common beginning at the end of the first week. Maximal fecal shedding occurred during the second and third weeks.

Viremia is a common finding in uncomplicated adenoviral respiratory tract infections in children. In a study involving 68 children, adenoviral DNA was noted in acute-phase serum samples from 28 (41%) of these children.[2] In the children with primary infection, 72 percent (21 of 29) had adenoviral DNA in their acute-phase serum samples. Other evidence of the presence of adenoviruses in the bloodstream early in disease is the occurrence of maculopapular, morbilliform, or petechial exanthems (or any combination of such rashes), as well as recovery of virus from multiple organs such as the brain, kidney, urinary bladder, lymphoid tissue, and liver and at postmortem examination.* The virus has been cultured from the mononuclear cells of heparinized blood.[15]

Coinfections

With the expanded use of direct fluorescent assays and PCR, investigators are discovering a common prevalence of coinfections of adenoviruses with multiple serotypes,[32] with other viruses[139,148,278,367,631] and with bacteria.[50] As technology is improving and made more available, the

*References 27, 43, 193, 257, 318, 346.
†References 29, 130, 281, 309, 310, 329, 348, 428, 559.
‡References 5, 140, 188, 353, 554, 670.
§References 45, 65, 71, 109, 227-229, 449, 496, 561, 639, 652.

*References 15, 16, 45, 56, 71, 91, 232, 244, 248, 393, 406, 546.

epidemiology of coinfection, as well as its impact on disease, is becoming clearer.

PATHOLOGY

Early pathologic changes are observed in the epithelium of the respiratory tract. The severity of adenoviral involvement varies with the different serotypes. In tracheal organ cultures, growth of adenovirus type 7 was characterized by an initial focal cytopathologic effect at 100 hours that quickly progressed to involve the whole epithelium.[118] Frequently, the cilia of inclusion-bearing cells were noted to be intact. In contrast to the findings with adenovirus type 7, a type 12 strain resulted in only a mild cytopathologic effect on the organ culture system.[118]

Microscopic examination of autopsy material from patients dying of adenoviral pneumonia revealed a loss of cilia in the tracheal epithelium, proliferation of other respiratory epithelial cells, and the presence of intranuclear inclusions.* In severe pneumonia, total destruction along with necrotizing bronchitis, bronchiolitis, and pneumonia is observed (Fig. 155-1). Mononuclear cellular infiltration is seen, and hyaline membranes and necrosis are present. Cilia and goblet cells are absent, and muscle fiber bundles and elastic fibers are dispersed. Frequently, epithelial cells have a characteristic appearance with adenoviral infection. These infected cells are enlarged grossly and lose their nuclear membranes; the nuclear material has migrated into the cytoplasm.[496] Blood vessels show edema, separation of filaments in their walls, and, occasionally, thrombosis.[672]

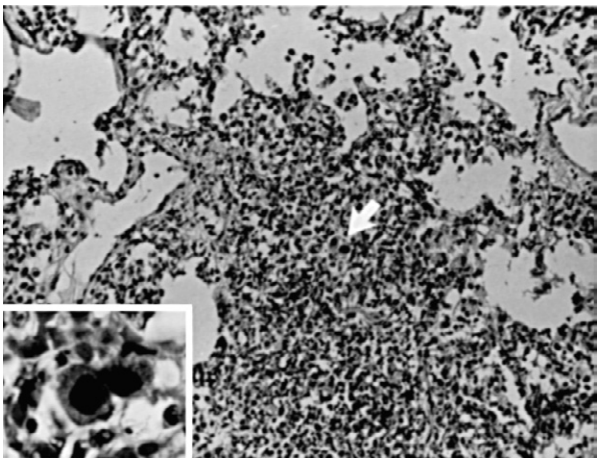

FIGURE 155-1 ■ The lung of a 17-month-old infant with adenoviral pneumonia. The infant died 10 days after the onset of illness, with no other contributing disease. The adenovirus isolated was not typed. A patchy pneumonia is present, and the *arrow* indicates two cells with nuclear inclusions. *Inset,* Two similar cells with typical adenoviral nuclear inclusions at higher magnification (hematoxylin and eosin stain, ×190; *inset,* ×770). (Courtesy Dr. David D. Porter, Department of Pathology, David Geffen School of Medicine at UCLA.)

*References 35, 45, 71, 91, 204, 644, 649, 651, 672.

In cases of pharyngoconjunctival fever, conjunctival biopsy specimens in volunteer studies revealed an inflammatory response with lymphocytic infiltration of the submucosal layer.[38,39] Biopsy material from palatine tonsils of infected volunteers revealed hypertrophy and hyperplasia of the lymphoid tissue, with congestion and edema of the surrounding connective tissue.

On histologic examination, adenoviral inclusions are characterized by small eosinophilic and larger basophilic intranuclear bodies.[35,204,449,561]

Hepatic involvement in adenoviral infection has been reported frequently.* In addition to isolation of virus from the liver, focal areas of liver necrosis accompanied by characteristic hepatic intranuclear inclusions have been demonstrated.[4,82,670] Electron microscopic examination has revealed adenoviral particles within the intranuclear inclusions.[4,45,496,644,670] Hematogenous spread to the central nervous system (CNS) has been observed.[551] Most patients with adenoviral CNS infection have had an associated pneumonia.[119,550] In CNS disease, the brain is edematous and congested. On microscopic examination, a perivascular accumulation of lymphocytes is noted, along with gigantic nuclear inclusions in the cortical neurons. Viral particles have been observed within these nuclear inclusions by electron microscopy.[103]

In epidemic keratoconjunctivitis caused by adenovirus type 8, the walls of the conjunctival vessels are damaged, and aneurysms are present. The surrounding conjunctival connective tissue is edematous. The virus has been suggested to penetrate the cornea along nerves deep into the epithelial layers in a manner similar to that occurring in herpes simplex virus (HSV) infection.[617]

Adenoviral strains have been isolated from, and intranuclear inclusions have been demonstrated in, renal tubular epithelium, lymph nodes, muscle, and gastrointestinal epithelium.†

Immunologic Events

The local response in adenoviral infection depends on the site of viral inoculation, the method of transmission, the viral serotype, the concentration of inoculum, and the antibody status of the host. Three days after infection develops, when virus can be recovered from the nasopharynx, marked transudation of proteins from serum into the respiratory tract occurs, along with the production of secretory IgA antibody.[411] Approximately 7 days after the onset of illness, serum-neutralizing, hemagglutination-inhibiting, and complement-fixing antibodies appear.[128,372,497] At the same time, the nasal secretions contain specific IgA and IgG antibody.[44] In general, neutralizing antibody is the most sensitive indicator of adenoviral infection, hemagglutination-inhibiting antibody is less sensitive, and complement-fixing antibody is the least sensitive.[348,474] Antibody titers peak in 2 to 3 weeks; complement-fixing antibody declines in 2 to 3 months but may persist for up to a year.[128,497] Neutralizing antibody persists for a longer period and is

*References 4, 20, 45, 82, 204 ,496, 558.
†References 42, 82, 640, 667, 668, 670.

measurable in many instances for periods as long as 10 years. Reinfection with the same adenoviral serotype is rare because of type-specific immunity.[39,525]

Although alterations in leukocytes are not common findings, a decrease in the number of lymphocytes has been observed before or at the onset of clinical illness and an increase in neutrophils occurs early in the disease, followed by a decrease later. Neutropenia may develop in severe disseminated illness and is attributed to a direct toxic effect of virus on leukocytes, marrow reserves, or both.[150] The erythrocyte sedimentation rate (ESR) of children has been normal or elevated to 55 mm/hr.[564,565]

Studies by Ginsberg and associates[227-229] in murine adenoviral pneumonia model systems noted that tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6 were elaborated during the first 2 to 3 days of infection. However, only TNF-α played an early role in the initial phase of pathogenesis. The second phase of the inflammatory response is caused by the infiltration of cytotoxic T cells. In an extensive study, Kawasaki and colleagues[331] compared numerous clinical and laboratory test results in children with adenoviral infections with those in children with influenza and respiratory syncytial virus (RSV) infections. They found that the children with adenoviral infections had higher white blood cell (WBC) counts with a greater number of neutrophils than did children with influenza or RSV infections (12,322, 7,638, and 8,460 WBCs/mm,[3] respectively). The children with adenoviral infections also had more atypical lymphocytes and higher C-reactive protein (CRP) and ESR values. The mean serum IL-6 concentration was significantly higher in the children with adenoviral infections than it was in those with influenza and RSV infections (131.0, 26.7, and 15.0 pg/mL, respectively).

Mistchenko and coworkers[423] studied cytokines and adenovirus-specific circulating immune complexes in 38 children with adenoviral infections. They placed the patients into three groups based on the severity of their respiratory illnesses: moderate illness, severe illness, and fatal illness. Serum IL-1 was not detected in children with moderate illness but was found in 7 of 12 with severe illness and in 13 of the 16 children who died. TNF-α frequently was present in the sera of fatal cases, it was not present in the sera of moderately ill children, and it was detected in the sera of only 2 of 12 patients with severe but nonfatal illness. IL-8 was noted in all sera, but values were highest in children with fatal illness. Serum immune complexes (containing IgG) were found in 7 of the 16 children who died. Finally, patients with increased concentrations of IL-6, IL-8, and TNF-α were those with hypoperfusion, febrile peaks, seizures, and a manifestation of septic shock. Five of 10 children with severe or fatal illness had serum autoantibodies specific for smooth muscle.

Matsubara and associates[405] noted the participation of peripheral blood $CD8^+$ T cells and HLA-DR^+, $CD8^+$ T cells in children with adenoviral infection. One child with severe adenoviral type 7 pneumonia had a marked increase in HLA-DR^+ $CD8^+$ T cells, serum levels of interferon-γ, and peripheral blood interferon-γ–producing T cells.

CLINICAL MANIFESTATIONS

Adenoviral infections are exceedingly common events, and the spectrum of disease is quite broad. As noted in Table 155-3, some specific adenoviral diseases exist; however, the overall majority of infections involve a variety of anatomically associated illnesses. In many instances, similar illnesses are caused by other respiratory viruses and the clinical spectra of the various adenoviral serotypes frequently overlap. Certain specific adenoviral types do have clinical characteristics that facilitate etiologic diagnosis, however.

Respiratory Tract

Common Cold

Although adenoviruses frequently receive etiologic consideration in colds, they are, in fact, associated only rarely with this illness.[318] In one report, 3 percent of common colds in children were attributed to adenovirus,[488] and in another study, 6.4 percent of children with coryza had adenoviral isolates.[163] Respiratory tract infections with adenoviruses usually are associated with fever and, frequently, with some degree of pharyngitis; therefore, when strict clinical criteria are applied, they do not qualify as colds.[296,567] Occasionally, adenoviruses, particularly types 1, 2, 3, 5, and 7, have been recovered from patients with typical colds.

Nasopharyngitis, Pharyngitis, and Tonsillitis

Adenoviral pharyngitis is an acute illness characterized by fever, sore throat, extensive exudative tonsillitis, and, frequently, cervical adenopathy.[149,163,318,429,557] In two studies involving 74 children with adenoviral pharyngitis, Ruuskanen and associates[530] described the pharyngeal findings. Most commonly, only mild inflammation and redness were observed. When exudates were present, they found them to be thick and membranous, thin and follicular, or thin and spotty; most typical were follicular and spotty exudates. Associated symptoms include malaise, headache, myalgia, chills, and cough. In infants and preschool-aged children, nasal congestion and discharge are noticeable and abdominal pain is a common complaint.[603] Children with adenoviral acute febrile pharyngitis also frequently have laryngotracheitis, bronchitis, or pneumonia.[659] The usual duration of illness varies from 5 to 7 days, although occasionally, symptoms persist well into the second week.[530]

Acute febrile pharyngitis is the most common adenoviral illness in children and is particularly important as an epidemic illness in closed environments.[43,557,565,659] For example, in an epidemic in a children's home, 63 percent of the residents were infected with adenovirus type 7a.[257] Moffet and colleagues[429] noted that adenoviruses were the etiologic agents recovered most commonly from hospitalized preschool-aged children with febrile exudative pharyngitis; 23 percent of the children studied had adenoviral infections. In a university student health service study, 2.4 percent of illnesses with acute febrile pharyngitis were caused by adenoviruses.[442] Adenoviruses

TABLE 155-3 **Clinical Spectrum of Adenoviral Infections**

System/Organ	Illness Category	Epidemic Occurrence	Frequency	Adenoviral Type(s)
Respiratory	Common cold	No	Rare	1, 2, 3, 5, 7
	Nasopharyngitis, pharyngitis, and tonsillitis	Yes	Common	1,* 2,* 3,* 4, 5,* 7,* 7a, 14, 15, (21/H21+35)†
	Acute respiratory disease	Yes (in military recruits)	Very common	2, 3, 4,* 5, 7,* 8, 11, 14, 21
	Acute laryngotracheitis	No	Occasional	1, 2, 3, 5, 6, 7
	Acute bronchiolitis	No	Occasional	3, 7, 21
	Pneumonia (civilian population)	Yes	Common	1, 2, 3,* 4, 5, 7,* 7a,* 8, 11, 21,* (21/H21 + 35),† 35, 56
	Atypical pneumonia in military recruits	Yes	Common	4,* 7,* 21
	Pertussis-like syndrome	No	Rare	1, 2, 3, 5, 12, 19
	Bronchiolitis obliterans	No	Rare	2, 3, 7, 21
	Unilateral hyperlucent lung	No	Rare	7, 21
Eye	Acute follicular conjunctivitis	Yes	Common	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 15, 16, 17, 19, 20, 22, 31, 34, 37
	Pharyngoconjunctival fever	Yes	Common	1, 2,* 3,* 4,* 5, 6, 7,* 7a,* 8, 14,* 37
	Epidemic keratoconjunctivitis	Yes	Occasional	2, 3, 4, 5, 7, 8,* 10, 11, 13, 14, 15, 16, 17, 19, 23, 29, 37, 54, 56
Skin	Morbilliform and rubelliform exanthem	Rare	Occasional	3, 4, 7, 7a
	Roseola-like	No	Occasional	1, 2
	Stevens-Johnson syndrome	No	Rare	7
	Petechial exanthem	No	Rare	7
Genitourinary	Acute hemorrhagic cystitis	No	Rare	7, 11, 21
	Nephritis	No	Rare	3, 4, 7a
	Orchitis	No	Rare	Unknown
	Oculogenital syndrome	Yes	Rare	19, 37
Gastrointestinal	Gastroenteritis	Yes	Common	1, 2, 3,* 5, 7,* 11, 12, 15, 17, 31,* 32, 33, 40,* 41*
	Mesenteric lymphadenitis	No	Rare	1, 2, 3, 5, 7
	Intussusception	No	Rare	1, 2, 3, 5, 6, 7
	Appendicitis	No	Rare	1, 2, 7
	Hepatitis	No	Rare	1, 2, 3, 5,* 7, (11+35/H11+35)†
Heart	Myocarditis	No	Rare	3, 7, 7a, 21
	Pericarditis	No	Rare	7
Neurologic	Encephalitis and meningitis	No	Rare	1, 2, 3, 5, 6, 7, 11, 12, 26, 32
	Transient encephalopathy	No	Rare	3
	Acute flaccid paralysis	Yes	Rare	21
Joint	Arthritis	No	Rare	7
Auditory	Deafness	No	Rare	3
Endocrine	Thyroiditis	No	Rare	Unknown

*Most common.
†Intermediate strain.

account for 37 to 75 percent of cases of nonstreptococcal pharyngitis in military recruits.

In children, 86 percent of cases of febrile pharyngitis are associated with adenovirus types 1, 2, 3, 5, and 7.[257,296,429]

On occasion, adenovirus types 7a, 9, 14, 15, and the intermediate strain 21/H21+35[277,557,565] also have been noted in association with pharyngitis. In military recruits, adenovirus types 4 and 7 are the main etiologic agents.

Acute Respiratory Disease

Acute respiratory disease is an epidemic disease that occurs predominantly in military recruit populations. This illness was studied extensively during World War II by the Commission on Acute Respiratory Diseases, and although individual cases were quite undifferentiated, the epidemiologic aspects of the outbreaks clearly indicated a specific etiologic agent.* The disease is an acute, febrile respiratory illness of short duration with constitutional and localized respiratory symptoms.[180] After an incubation period of 5 to 7 days, fever (mean temperature of 39.5° C [103.1° F]), pharyngitis, laryngitis, tracheitis, and a nonproductive cough develop.[52,126,226,482] The initial dry, hacking cough may progress to paroxysms. Malaise,

*References 27, 110, 226, 557, 570, 602.

myalgia, chills, headache, dizziness, rhinitis, conjunctivitis, abdominal pain, and local cervical lymphadenopathy are common complaints.[126] The inflammatory process may spread to the bronchi, the bronchioles, and the parenchyma of the lungs. In epidemics, as many as 67 percent of those affected wheeze and have other evidence of small airway obstruction; pneumonia occurs in 10 to 20 percent.[280,376,434] The illness gradually resolves over the course of an 8- to 36-day period.[202] Early in the illness, the total WBC count may be slightly elevated, with a small increase in the percentage of polymorphonuclear leukocytes.

In experimental, aerosol-induced acute respiratory disease the incubation period ranged between 6 and 13 days.[116] Typical illness included fever to 39° C (102.2° F), rhinitis, prostration, malaise, myalgia, and headache. Pneumonia occurred occasionally. The virus was recovered from throat culture specimens 5 days after inoculation, from the nose at 6 days, and after 9 days in fecal specimens. Serum-neutralizing antibody responses occurred between the third and fourth weeks. All ill volunteers had leukopenia and an elevated ESR during the illness.

The syndrome occurs most commonly in military recruits early in basic training; illness has been documented in as many as 90 percent of new trainees within the first 8 weeks of arrival at a training site.[280] The usual etiologic agents are adenovirus types 4 and 7. Occasionally, epidemics have been associated with adenovirus types 3, 11, 14, and 21,[68,199,580,620] and sporadic cases have been noted in connection with adenovirus types 2, 5, and 8.[280,482] The peak seasons of illness are winter and spring.[280]

Because of the magnitude of the problem of acute respiratory disease in military recruits, live attenuated adenoviral vaccines against types 4 and 7 were developed and used in all branches of the U.S. military beginning in the early 1970s.[31,240] These vaccines were used successfully until the early 1990s, when production delays occurred; subsequently, in 1995, the vaccine manufacturer stopped production. From 1999 through 2011, no vaccine was available, and epidemic disease returned to military training bases.* Although illness may occur in civilian adults, it is an uncommon event and not epidemic. In volunteer studies, subjects with serum antibody to a particular adenoviral type are protected against disease with that type of adenovirus induced by intranasal inoculation.[39,116]

Epidemic acute respiratory disease has not been described in children. However, a sporadic comparative illness, usually clinically identified as acute bronchitis, is commonly the result of adenoviral infection.[662] Adenovirus type 7 is the etiologic agent most frequently found in these cases.

Acute Laryngotracheitis

On occasion, adenoviruses have been implicated as a cause of acute laryngotracheitis. In general, the croup caused by adenoviruses is not severe and often is manifested only as a barking, brassy cough. Laryngotracheitis frequently is seen in association with febrile pharyngitis, bronchiolitis, and pneumonia.[597] Epidemics have not been observed. Adenovirus types 1, 2, 3, 5, 6, and 7 have been implicated as etiologic agents.

Acute Bronchiolitis

Adenoviruses account for 5 to 18 percent of cases of bronchiolitis in infants.[215,649] In a recent study of 143 children with adenoviral infections, the admitting diagnosis was bronchiolitis in 24 percent.[516] The bronchiolitis caused by adenoviral infection is sporadic and usually similar to illness associated with other viral agents. Occasionally, adenoviral bronchiolitis occurring early in infancy has been fatal or has resulted in serious residual lung damage and chronic disease.[637] This severe illness has been associated with serotypes 3, 7, and 21.

Pneumonia

Young Children. Adenoviruses are common isolates in children with pneumonia. The overall frequency of adenoviruses as a cause of nonbacterial pneumonia in children is less than that of RSV and parainfluenza virus type 3, but an alarming number of fatal illnesses have been noted. Severe and fatal illnesses in infants and young children have occurred in association with adenovirus types 1, 2, 3, 4, 5, 7, 7a, 7h, 7i, 8, 19, 21, 35, and the intermediate strain 21/H21+35.* The more severe cases of pneumonia have been linked to types 3, 7, and 21.[78,222,552,579,651] Adenoviral pneumonia has been epidemic and sporadic.[84,91,100,170,379]

Severe pneumonia occurs most commonly in neonates and young children aged 3 to 18 months.† The onset of illness is acute, with persistent cough and fever (>39° C). On physical examination, moderate to severe dyspnea is apparent, as is the associated tachypnea. Inspiratory and expiratory wheezes and rales are heard on auscultation. Other signs and symptoms include lethargy, diarrhea and vomiting, pharyngitis, and, occasionally, conjunctivitis. Extrapulmonary complications that occur commonly are meningitis, encephalitis, seizures, splenomegaly, hepatomegaly and hepatitis, myocarditis, nephritis, bleeding tendency, and exanthems.‡ Chest radiographs reveal diffuse infiltrates, which usually are bilateral and may be bronchial, peribronchial, or interstitial.[35,362,516,645] Hyperinflation and lobar collapse occur frequently.[232] Rarely, pleural effusions or mediastinal lymphadenopathy has been described.[362] In surviving infants, symptoms persist for 2 to 4 weeks and radiographic changes resolve slowly, frequently being present at the 3-week follow-up examination.[91,100,552] Recovery often is gradual, and exacerbations occur commonly.[305,480]

Serious sequelae often result from adenoviral lower respiratory disease, particularly in association with

*References 68, 351, 528, 531, 580, 620.

*References 15, 35, 36, 45, 70, 71, 91, 100, 134, 140, 214, 232, 282, 305, 322, 323, 341, 342, 344, 358, 366, 393, 406, 426, 427, 439, 462, 480, 491, 492, 509, 550, 552, 561, 564, 651.

†References 35, 36, 70, 71, 83, 112, 140, 177, 232, 322, 344, 362, 406, 492, 509.

‡References 45, 71, 91, 112, 362, 550, 552, 561, 564.

adenovirus types 3, 7, 7a, and 21.* Such sequelae include bronchiectasis, bronchiolitis obliterans, and unilateral hyperlucent lung.† An estimated 14 to 60 percent of children with documented adenoviral lower respiratory tract disease have some degree of permanent pulmonary sequelae.[232,362,551,552] In a study of 27 children conducted 10 years after they had documented adenoviral type 7 pneumonia, 12 had radiographic evidence of bronchiectasis or residual pulmonary changes; 16 children had abnormal results on pulmonary function studies.[551]

Macek and associates[395] have suggested that persistent adenoviral infection may be the cause of chronic obstructive airway disease in children. They noted adenoviral antigen in bronchoalveolar lavage specimens from 31 of 34 patients with chronic disease but in no bronchoalveolar specimens from a control group.

On occasion, severe and fatal adenoviral pneumonia has been related to malnutrition, environmental crowding, or a preceding severe viral disease such as measles.‡

Severe adenoviral pneumonia associated with adenovirus types 3 and 7 also has been reported occasionally in previously healthy adults.[485,495,555] One adult had severe pneumonia caused by adenovirus type 21 that was associated with myalgia, rhabdomyolysis, and myoglobinuria.[652]

Atypical Pneumonia in Military Recruits. Seven to 20 percent of cases of pneumonia in military recruits are associated with adenoviral infection.[73,196,219] Primary atypical adenoviral pneumonia commonly occurs in the winter months and generally is caused by adenovirus types 4, 7, and 21.[196] The illness is associated with fever, cough, sore throat, rhinorrhea, and chest pain. Other common symptoms include nausea, vomiting, myalgia, headache, and diarrhea. On physical examination, rales and pharyngitis are present in almost all cases. Rhinitis and generalized lymphadenopathy are observed in approximately half of those afflicted, and, occasionally, conjunctivitis is noted. Chest radiographs reveal a bilaterally mottled appearance, most prominent in the lower lobes; they remain abnormal for 4 to 36 days.[73] Although serum cold agglutinins are observed, titers of 1:32 or higher are detected in only 18 percent of patients.[73,219]

Fatal pneumonia, absolute leukopenia, and disseminated disease have been reported in four previously healthy military trainees. Adenovirus type 7 was the etiologic agent in three of these cases, and the fourth illness was caused by adenovirus type 4.[156,376]

Pertussis-like Syndrome. A pertussis-like illness has been noted in association with several adenoviruses, including types 1, 2, 3, 5, 12, and 19.[109,111,257,452,466] The illness occurs commonly in children younger than 36 months. The onset of illness is insidious and initially suggestive of a cold. The cough becomes progressively worse and, by 1 to 2 weeks, is paroxysmal. Severe recurrent episodes of paroxysms result in the production of mucus, posttussive fatigue, and vomiting.[111] Approximately 50 percent of children have a typical whoop, and cyanosis occurs with the paroxysms. Peripheral leukocytosis with WBC counts ranging from 25,000 to 125,000 cells/mm^3 along with lymphocytosis and thrombocytosis is the usual finding.[109,466] The recovery time ranges from 4 to 10 weeks from the onset of illness.[111] Radiologic evidence of bronchiolitis is present in most children; interstitial pneumonia occurs occasionally. In our opinion, most and probably all of these pertussis-like illnesses are indeed *Bordetella pertussis* infections in which adenovirus is a coinfecting agent (see Chapter 129). In young infants, coinfection (adenovirus plus *B. pertussis*) may lead to more severe disease.[37,378,407]

Bronchiolitis Obliterans. Bronchiolitis obliterans is a chronic bronchiolitis that initially was described in 1901 by Lange.[363] It has been noted to occur after measles, influenza, and pertussis, as well as after the inhalation of toxic substances.[25,36,649] Adenovirus types 2, 3, 7, and 21 have resulted in a bronchiolitis obliterans–type chronic illness.* These adenoviruses cause a severe necrotizing bronchiolitis that heals with fibrosis and predominantly obliterates the small airways.[31] The onset of disease is characterized by an acute febrile illness, cough, and respiratory distress. Disease may wax and wane for several weeks or months and is associated with recurrent episodes of atelectasis, pneumonia, and wheezing. Although some children recover from these episodes, the remainder have chronic pulmonary disease, including irreversible atelectasis, bronchiectasis, or hyperlucent lung syndrome.[36,637,649]

Castro-Rodriguez and colleagues[83] conducted a 5-year follow-up study of 38 children hospitalized with adenoviral pneumonia during an outbreak in Santiago, Chile, in 1998. Bronchiolitis obliterans developed in almost 50 percent of these children. Nosocomial acquisition of adenoviral infection was found to be associated more significantly with the development of bronchiolitis obliterans than community-acquired infection was. Murtagh and colleagues conducted a retrospective review of 415 children with lower respiratory tract infection caused by adenoviruses. The found that risk factors for the development of bronchiolitis obliterans included prolonged hospitalization (>30 days), multifocal pneumonia, and hypercapnia.[441]

Unilateral Hyperlucent Lung. Unilateral hyperlucent lung is a well-defined syndrome characterized by increased translucency of all or part of one lung, along with a reduction in lung size.[121,396] The unilateral hyperlucency is associated with a decrease in the size and number of pulmonary vessels, as observed on pulmonary angiograms, and an absence of peripheral filling at bronchography.[396] Although the disease may have a number of causes, including pneumonia secondary to other viruses, it has been noted to occur after severe necrotizing bronchiolitis and pneumonia caused by adenovirus types 7 and 21.[396,649]

Eye

Acute Follicular Conjunctivitis

Acute follicular conjunctivitis is the most common and benign of the adenoviral infections of the eye. The

*References 25, 36, 83, 177, 232, 305, 362, 552.
†References 25, 36, 83, 177, 362, 396, 426, 491, 552, 649.
‡References 25, 35, 36, 232, 358, 449, 552.

*References 6, 25, 36, 83, 426, 491, 637, 649.

infection in this disease is confined to the eye, generally is unilateral, and is manifested by follicular lesions on the palpebral conjunctival surface. Symptoms occur after an incubation period of 5 to 7 days and include lacrimation, itching, burning, a foreign body sensation, and conjunctival erythema.[141] Although the preceding symptoms increase the likelihood of viral infection, rather than a bacterial infection, the diagnosis by clinical symptoms alone can be difficult to make.[461] Examination shows erythema and lymphoid follicular hyperplasia in the conjunctiva in association with serous drainage and increased lacrimation. Occasionally, adenopathy of the preauricular lymph nodes is seen. Symptoms resolve in 10 days to 3 weeks, with recovery usually complete.[115,141] Adenovirus types 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 15, 16, 17, 19, 20, 22, 30, 31, 34, and 37 have been isolated from the eyes of afflicted patients.*

Pharyngoconjunctival Fever

By definition, pharyngoconjunctival fever is a syndrome characterized by fever, pharyngitis, and conjunctivitis. During epidemics, not all patients have the complete syndrome triad. For purposes of this discussion, all descriptions of frequency of signs and symptoms are calculated from the starting point of 100 percent fever, pharyngitis, and conjunctivitis.

Tables 155-4 and 155-5 summarize the frequencies of specific symptoms and signs.† The usual onset of illness is abrupt, with sore throat, generalized aches and pains, eye irritation or pain, and fever. Throat complaints vary from mild to severe. On examination, the tonsils and pharyngeal lymphoid tissue are hypertrophied with pharyngeal redness varying considerably from patient to patient. Approximately one third of affected patients have follicular exudative lesions that cannot be differentiated from streptococcal disease on clinical grounds. Hypertrophy of the adenoids occurs, which may result in nasal blockage. Coryza and posterior nasal discharge are common, leading to cough in many instances.

With respect to conjunctivitis, most patients note some aching or soreness; photophobia and lacrimation are unusual. The appearance of the palpebral conjunctiva usually is granular. The lesions may be almost microscopic or 2 to 3 mm in diameter. Hemorrhages occasionally are noted on the bulbar surface. Frequently, involvement starts in one eye and does not involve the other eye until 2 or 3 days later. Occasionally, the involvement is restricted to one eye.

Compared with other viral respiratory tract infections, the duration of illness with pharyngoconjunctival fever is long. In most patients, the fever is sustained or remittent for 3 to 4 days; however, approximately 10 percent of patients have fever that lasts longer than 7 days. Throat and eye findings usually are improved considerably by the seventh day of illness, but these findings and nasal complaints, fatigue, and headache may persist for 14 days.

*References 57, 112, 141, 177, 401, 536, 543, 596.
†References 11, 14, 18, 27, 30, 38, 39, 43, 108, 159, 167, 193, 194, 207-210, 296, 308, 317-320, 337, 346, 347, 417, 450, 465, 468, 564-566, 603, 607, 626, 634.

TABLE 155-4 **Frequency of Symptoms in Epidemic Pharyngoconjunctival Fever**

Symptoms	Frequency
Throat Complaints	++++
Soreness	++++
Cough	++
Foreign body sensation	++
Dry feeling	+
Eye Complaints	+++
Aching or soreness	+++
Burning sensation	++
Lacrimation	+
Photophobia	+
Nasal Complaints	+++
Coryza	++
Stuffiness or blockage or both	++
Sneezing	+
Epistaxis	+
Other Complaints	++++
Headache	++++
Anorexia	+++
Malaise	++
Generalized aches and pains	++
Nausea	++
Vomiting	+
Diarrhea	+
Abdominal pain	+

++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; +, 1 to 25 percent.
Data from references 18, 38, 77, 194, 208, 319, 337, 343, 404, 484, 557, 607.

In epidemic pharyngoconjunctival fever, the most likely etiologic agent is adenovirus type 3.* The next most prevalent adenovirus associated with epidemic disease is type 7.[77,159,194,658] One or more epidemics also have been noted with adenoviruses 2, 4, 7a, 11, and 14.† Sporadic occurrences of pharyngoconjunctival fever have been observed in association with infections with adenoviruses 1, 2, 3, 4, 5, 6, 7, 7a, 8, 14, 19, and 13/30 (an intermediate type).‡

Epidemic Keratoconjunctivitis

Epidemic keratoconjunctivitis is caused most commonly by adenovirus type 8, but it also has resulted from infection with adenovirus types 2, 3, 4, 5, 7, 10, 11, 13, 14, 15, 16, 17, 19, 22, 23, 29, and 37.§ Currently, adenovirus type

*References 30, 39, 41, 43, 108, 200, 208, 210, 308, 319, 320, 330, 347, 404, 413, 465, 484, 564, 607, 657, 662.
†References 11, 14, 39, 123, 164, 167, 450, 607, 662.
‡References 27, 38, 39, 94, 199, 210, 257, 267, 296, 318, 337, 346, 417, 536, 541, 565, 566, 571, 601, 603, 618, 633.
§References 29, 57, 76, 87, 116, 125, 127, 130, 132, 133, 169, 180, 249, 253, 258, 309, 310, 329, 332, 335, 415, 428, 430, 454, 456, 536, 559, 563, 578, 659.

TABLE 155-5 **Frequency of Signs in Epidemic Pharyngoconjunctival Fever**

Signs	Frequency
Throat Findings	++++
Erythema and infection	++++
Hypertrophied lymphatic tissue	++++
Particulate exudate	++
Eye Findings	++++
Erythema and infection of palpebral and bulbar conjunctiva	++++
Edema	+++
Granular and follicular involvement	++
Eyes unequally affected	++
Superficial punctate keratitis	+
Lymph Node Enlargement	++++
Cervical	++++
Preauricular	+
Generalized	+
Fever	++++
≥39°C (≥102.2°F)	+++
Other	
Flushed face	+++
Enlarged liver or spleen or both	+

++++, 76 to 100 percent; +++, 51 to 75 percent; ++, 26 to 50 percent; +, 1 to 25 percent.
Data from references 18, 38, 77, 194, 208, 255, 343, 484, 557, 607.

37 is the virus most commonly recovered from patients with epidemic keratoconjunctivitis in the United States and Europe. The most severe disease is associated with adenovirus types 5, 8, and 19.[125] More recently in Japan, adenovirus types 54 and 56 have been recovered from patients with epidemic keratoconjunctivitis.[10,302]

The illness occurs most commonly in adults, but a few cases have been reported in children.[258,428,577] It has no seasonal pattern. Although transmission of the viral agent from the respiratory tract to the eye occurs in sporadic cases, the usual method of viral spread is by contaminated ophthalmic instruments and eye solutions, hand-to-eye contact by medical personnel and others, swimming pools, or hands or fomites in close-contact situations, such as in families and in industry.* The incubation period typically is 5 to 10 days but ranges from as short a period as 2 days to as long as 2 weeks.[127,348] The initial symptom generally is unilateral, acute, follicular conjunctivitis that suggests the presence of a foreign body. Photophobia, lacrimation, discharge, hyperemia, and edema of the conjunctiva are notable. Preauricular adenopathy occurs in as many as 90 percent of patients, and 50 percent of those afflicted have pharyngitis and rhinitis. Spread to the other eye may occur in 2 to 7 days. Seven to 10 days after onset of the disease, the conjunctivitis resolves, and painful, superficial, punctate epithelial opacities appear in the center of the cornea. These lesions frequently extend subepithelially and then heal, with subepithelial infiltrates left behind that may persist for months. In severe cases, hazy vision may continue for several years.[29]

An infantile form of epidemic keratoconjunctivitis has been described that usually affects children younger than 2 years old.[169] This pseudomembranous or membranous conjunctivitis generally is accompanied by high fever, pharyngitis, otitis media, diarrhea, and vomiting. Preauricular lymphadenopathy typically is absent.[577]

Virus can be recovered from the eye for a usual period of approximately 2 weeks but has been detected for 2 to 3 years in patients with chronic papillary conjunctivitis.[125,490] In acute illness, conjunctival scrapings obtained during the first 10 days of infection reveal characteristic inclusion bodies when stained with Giemsa.[268] Virus-specific fluorescent antibody staining is diagnostic in epidemic keratoconjunctivitis.[541] Preparations of corneal and conjunctival epithelia reveal adenoviral particles when examined with the electron microscope.[132]

Skin

Adenovirus types 1, 2, 3, 4, 7, and 7a, plus several unknown types, have been noted in connection with exanthematous disease.[96] The cutaneous manifestation most commonly associated with adenoviral infection is an erythematous maculopapular rash that appears while the child is febrile. In many instances, children with this illness have been thought to have either measles or rubella.[358] In most adenoviral infections with exanthems, other clinical findings more characteristic of adenoviruses, such as conjunctivitis, rhinitis, pharyngitis, and lymphadenopathy, also are present. In some instances, the exanthem truly is morbilliform with a characteristic confluence, but Koplik spots do not occur.

A widespread erythematous rash often is present early in the course of severe pneumonia in infants.[232] Chany and colleagues[91] noted a measles-like rash in five patients who died of adenovirus type 7a pneumonia. One report describes a child with an adenovirus type 7 infection and illness suggestive of meningococcemia.[532] This patient had fever, vomiting, diarrhea, and a petechial exanthem. Rocholl and associates[516] noted that rash occurred in 10 percent of 143 adenoviral illnesses in children.

On several occasions, illness characterized by fever and defervescence and then the appearance of a maculopapular rash suggesting the diagnosis of roseola infantum has been observed.[193,207,307,308,453] Adenoviral infections also are confused with rubella. However, the respiratory symptoms and the degree of fever associated with adenoviral infection should clarify the diagnosis. With rubella, respiratory complaints and fever are minimal. On occasion, severe disease with Stevens-Johnson syndrome has been noted. Such patients frequently have pneumonia, and the illness is quite similar to that caused by *Mycoplasma pneumoniae*.

Lähdeaho and associates[361] have noted that serum antibodies to the E1b protein–derived peptides of the

*References 29, 76, 87, 125, 127, 130, 192, 309, 348, 404, 428, 430, 577, 635.

enteric adenovirus type 40 are associated with dermatitis herpetiformis.

Genitourinary Tract

Acute Hemorrhagic Cystitis

Acute hemorrhagic cystitis is an uncommon manifestation of adenoviral infection in immunocompetent children and is characterized by a sudden onset of dysuria and frequency, with hematuria developing 12 to 24 hours later.* Occasionally, fever, suprapubic pain, and enuresis occur. Antecedent upper respiratory tract infection is noted in some children. Symptoms persist for a few days to 2 weeks, with the usual duration being approximately 5 days. Acute hemorrhagic cystitis has been reported in both the United States and Japan. It occurs primarily in children, most often boys, and usually is associated with adenovirus type 11. Occasionally, adenovirus types 7 and 21 have been implicated. Adenoviral antigen has been identified by immunofluorescence in exfoliated bladder cells. Although no sequelae have been reported, the long-term prognosis is unknown.[435]

Nephritis

Hematuria occasionally has been reported in infants with severe pneumonia and disseminated adenoviral infection. Red blood cells and, at times, red blood cell casts also have been noted in the urine of some children with upper respiratory tract infections caused by adenoviruses and specifically in patients with pharyngoconjunctival fever associated with adenovirus types 3, 4, and 7a.[557,562,603,607] In one instance, a young boy had a maculopapular and petechial exanthem and thrombocytopenia associated with adenovirus type 7 infection. Hematuria also was present.

Orchitis

Orchitis developed in one child who had a 5-day history of pain and fever, erythema, and swelling of the right testis.[451] The testicular involvement resolved in several days, and the illness was associated with a 16-fold rise in adenoviral complement-fixation antibody titer.

Oculogenital Syndrome

In 1977, Laverty and associates[368] reported a woman who in addition to pharyngoconjunctival fever had cervicitis and paresthesia of the legs; a type 19 adenovirus was recovered from this woman's cervix. In Perth, Australia, adenovirus type 19 was recovered from the genital tracts of 59 men and women being examined for genital HSV infection in a clinic for sexually transmitted diseases.[301] Several of the patients also had conjunctivitis, and in two patients adenovirus type 19 was isolated from conjunctival specimens. Similar oculogenital illnesses have been noted in association with adenoviral types 2, 8, and 37.[86,536,573]

*References 368, 369, 400, 435, 436, 459.

Hemolytic-Uremic Syndrome

Hemolytic-uremic syndrome in association with adenoviral infection has been described in two 2-year-old children.[51]

Hemorrhagic Fever with Renal Syndrome

An adenovirus type 11 infection associated with hemorrhagic fever with renal syndrome has been reported in a 22-year-old woman.[599]

Gastrointestinal Tract

Gastroenteritis

Infantile diarrhea has been associated with epidemic and sporadic adenoviral diseases such as acute upper respiratory tract infection, severe pneumonia, and pharyngoconjunctival fever.[193,362,557,566,640] Outbreaks of diarrhea characterized by acute abdominal pain followed by diarrhea, nausea and vomiting, fever, headache, and pharyngitis have been associated with adenovirus type 3 and 7 infections.[217,565] Other symptoms occurring in patients with diarrhea include conjunctivitis, rhinitis, pharyngotonsillitis, hepatomegaly, and cervical adenitis.[566] In two patients who had diarrhea and upper respiratory tract illness in association with adenovirus type 15 infection, viral particles were visualized within the nuclei of mucosal cells at autopsy by electron microscopy.[157]

The widespread use of electron microscopy for the study of rotaviral diarrhea led to the finding of previously unrecognized adenoviruses that were fastidious and could not be grown in routine cell cultures.[432,540] These adenoviruses, now identified as types 40 and 41, subsequently were shown to be important causes of gastroenteritis in children.* In enteric adenoviral infection, diarrhea is the most prominent symptom.[596] In children with adenovirus type 40 infection, Uhnoo and colleagues[597] found that the mean duration was 8.6 days, as opposed to 12.2 days in those infected with adenovirus type 41. Most illnesses occur in children younger than 3 years old: the mean age for adenovirus type 40 diarrhea was 15.2 months, whereas it was 28.3 months in type 41 illnesses. Most patients had mild vomiting that lasted approximately 2 days. When compared with illnesses caused by established respiratory adenoviruses, fever occurred less commonly, was less severe, and had a shorter duration in enteric adenoviral infections. Upper respiratory tract symptoms and signs, such as pharyngitis, coryza, cough, and otitis media, were observed in 21 percent of children with enteric adenoviral infection. Brandt and colleagues[64] noted that dual infections with respiratory viruses (such as RSV and enteric adenoviruses) are common occurrences, so caution should be observed in attributing respiratory symptoms to enteric adenoviruses.

Yolken and Franklin[664] found that enteric adenoviruses were an important cause of nosocomial diarrhea in infants

*References 65, 69, 104, 143, 144, 233, 354, 356, 424, 464, 474, 517, 584, 597, 610.

who had previously undergone gastrointestinal surgery for necrotizing enterocolitis.

Mesenteric Lymphadenitis

Adenoviral serotypes 1, 2, 3, 5, and 7 have been recovered from lymph nodes and the appendix in cases of mesenteric lymphadenitis.[42,56,346] Patients with mesenteric lymphadenitis often have abdominal pain and other symptoms similar to those of acute appendicitis. Pharyngitis is a frequently related finding. Mesenteric adenitis often is associated with concurrent or recent adenoviral illness.[112,501] Frequently, the peak incidence of mesenteric lymphadenitis occurs when adenoviral illness is common in the community.

Intussusception

The suggestion that adenoviruses could be an etiologic factor in intussusception arose because these agents frequently can be recovered from throat, stool, and mesenteric lymph node specimens obtained from children who undergo surgery for intussusception.* Most children with intussusception were younger than 2 years old; some had preceding respiratory symptoms.[523,667] Adenovirus serotypes 1, 2, 3, 5, 6, and 7 have been implicated. Typical adenoviral intranuclear inclusions have been demonstrated in cells in stool, intestinal epithelia (ileum), and the appendix by electron microscopy.[460,667,668] Mesenteric lymph nodes often are enlarged at surgery.[668] Increases in antibody titer to adenoviruses have been observed in children after the development of intussusception. Investigators have suggested that bowel wall hypermotility caused by direct viral involvement or by hypertrophy of lymphatic tissue is the lead point for the intussusception.†

Appendicitis

Adenoviruses have been associated with both acute and chronic appendicitis.[56] Right iliac fossa abdominal pain in conjunction with sore throat is a common finding. The virus has been isolated from the appendix and mesenteric lymph nodes at surgery. During acute infection, lymphoid follicles of the ileum, appendix, and mesenteric lymph nodes are infected with virus. In chronic infection, adenovirus remains in cells; on microscopic examination, slight inflammation is seen in the appendix.

Hepatitis

Hepatitis in association with adenoviral infection has been reported many times in small infants, in children with overwhelming disseminated disease, and in immunocompromised patients.‡ In one study, 27 of 30 persons thought to have sporadic infectious hepatitis were found to be infected with adenovirus type 5.[259] Adenovirus types 1, 2, and 3 were isolated from the stool specimens of 12 children younger than 3 years old in an outbreak of infectious hepatitis on an American Indian reservation in Arizona.[261]

In one report, three children with severe, fatal adenovirus type 7 pneumonia had associated findings that simulated Reye syndrome: lethargy, diarrhea, seizures, elevated CSF pressure, myocarditis, hepatitis, and disseminated intravascular coagulation.[360] Edwards and colleagues[162] reported three children with Reye syndrome and adenoviral infection. They suggested that adenoviruses might be an important agent in initiating the syndrome.

Heart

Myocarditis

In children, myocarditis has been noted in association with severe pneumonia and disseminated disease caused by adenovirus types 7, 7a, and 21.[264,269] Similar cardiac involvement has been seen in military recruits with severe acute respiratory disease.[91,100,264,557] One case of sudden death in an 11-year-old boy due to myocarditis with adenovirus type 3 has been documented.[590]

The use of polymerase chain reaction (PCR) assays has led to recognition of adenovirus as the cause of many cases of acute myocarditis.[58,66,246,394,403] In an extensive study of myocarditis and dilated cardiomyopathy, Bowles and coworkers[58] analyzed 773 endomyocardial biopsy samples obtained from patients of all ages. In this large group, 624 patients had myocarditis and adenoviral DNA was identified by PCR in 142 (23%) of the endomyocardial biopsy samples. There were 26 neonates, 37 infants, 20 toddlers, 25 older children, 11 adolescents, and 23 adults. The results of this study suggest that adenoviruses are the leading viral cause of myocarditis, with enteroviruses being the second most common association (14%). On histopathologic examination, only 57 (40%) of the enterovirus-positive samples were found to be acute myocarditis.

Dilated Cardiomyopathy

In the study by Bowles and associates,[58] specimens from 149 patients exhibited dilated cardiomyopathy. Of this subgroup, 18 (12%) had adenoviral DNA demonstrated by PCR in the endomyocardial biopsy specimen. Of these 18 samples, 2 were from neonates, 2 from infants, 2 from toddlers, 4 from older children, 2 from adolescents, and 6 from adults. In a study in adults, 28 explanted hearts from patient undergoing transplant for dilated cardiomyopathy yielded adenovirus type 3 in 25 percent of cases by nested PCR.[581]

Pericarditis

Pericarditis has been associated with severe adenoviral pneumonia. In a patient with adenovirus type 7 pneumonia, electrocardiographic changes consistent with pericarditis were demonstrated and the virus was isolated in high titer from pericardial fluid at postmortem examination.[449] In 1995, Mistchenko and coworkers[425] reported a 10-month-old boy with fatal pericarditis caused by adenovirus type 7. In serum and pericardial fluid from this

*References 42, 48, 107, 216, 294, 460, 495, 523, 667, 668.
†References 42, 107, 216, 460, 498, 523, 667, 668.
‡References 4, 20, 45, 71, 82, 342 ,670.

child, IL-6, TNF-α, and adenovirus-specific immune complexes were identified.

Nervous System

Although CNS disease in adenoviral infection is an uncommon finding, a variety of clinical manifestations have been observed. Both meningitis and encephalitis have been noted as the major manifestations of adenoviral infection or in association with severe disease at other body sites.[457,462] Adenovirus types 1, 2, 3, 5, 6, 7, 12, 26, and 32, in isolated instances, have been recovered from both brain and CSF.* Meningoencephalitis often occurs as part of disseminated adenoviral disease in immunocompromised patients, with the most commonly associated serotypes being 2, 3, 5, and 7. Adenovirus serotype 26 DNA was identified in peripheral blood, CSF, urine, and postmortem brain tissue of a 28-year-old woman undergoing radiation therapy for medulloblastoma; progressive neurologic deterioration developed, followed by her eventual death as a result of disseminated adenoviral infection.[152] In two children with respiratory and CNS symptoms, adenoviruses were recovered from CSF.[181] One child was convalescing from herpes zoster and the other from varicella. Adenovirus type 7 was recovered from brain tissue cultures from an elderly patient with chronic schizophrenia.[393] In another instance, adenovirus type 32 was recovered from the brain of a man with lymphosarcoma and subacute encephalitis.[103,520] In an epidemic of adenoviral infection caused by type 7, 25 percent of the hospitalized patients had symptoms referable to the CNS.[550,552] Many of the patients died; those who survived had little residual effect. Too few cases are reported in the literature to predict the prognosis of CNS disease in children accurately. Adenovirus type 2 was isolated from a muscle biopsy specimen from a patient with inclusion body myositis.[421] Ooi and colleagues[467] reported eight children with adenovirus serotype 21–associated acute flaccid paralysis in the setting of an enterovirus 71–mediated hand-foot-and-mouth disease outbreak in Sarawak, Malaysia, in 1997. Of these eight children, four had upper limb monoparesis, two had lower limb monoparesis, and two had paraparesis. Four of the children experienced complete recovery, one was improving at hospital discharge, and three had residual flaccid weakness and wasting. Seven children with initially appearing typical adenoviral infections had transient encephalopathy.[568] All these children were infected with adenovirus type 3. On initial evaluation, five had pneumonia, one had follicular tonsillitis, and one suffered from diarrhea. Three of the children were lethargic, two were stuporous, and two were obtunded. All had elevated serum aspartate aminotransferase values (range, 39 to 401 U/L), and CSF evaluations were within normal limits. The neurologic findings resolved in all patients in about 1 week.

Infection in Immunocompromised Hosts

Adenovirus types 1, 2, 3, 4, 5, 6, 7, 7a, 11, 29, 31, 32, 34, and 35 have been recovered from children and adults who were immunocompromised by immunodeficiency diseases, malignancies, corticosteroid therapy, immunosuppressive therapy, radiation therapy, and transplantation procedures.* Adenoviral infections occur more frequently in pediatric than in adult hematopoietic stem cell and solid-organ transplant recipients.[28,285,293] Infection is thought to occur as a result of transmission from the donor, reactivation of latent infection, or newly acquired infection.[357] McLaughlin and colleagues[412] examined the incidence of adenovirus infection in 146 children who underwent liver and intestinal transplants. They found that adenoviral infection occurred in 4.1 percent of liver transplant recipients and 20.8 percent of intestinal transplant recipients.

In a study of eight children with liver and small bowel transplants and six with just small bowel transplants, Pinchoff and coworkers[494] noted that all had evidence of adenoviral infection of the graft. In eight of the children, histologic evidence of infection (adenoviral intranuclear inclusions) developed, and these eight had received intensive corticosteroid therapy, had virus isolated from multiple sites, and had persistent positive viral cultures. In a retrospective review of 110 small bowel and liver (combined) transplants in 98 children by Florescu and colleagues, adenovirus infection was found in 38 transplants. Twenty-three of these developed invasive disease, and over 80 percent occurred within the first year of transplant. In this series, the presence of adenovirus did not have an effect on all-cause mortality.[190] For most solid-organ transplants, the source of invasive adenoviral disease often is thought to be the transplanted organ.

Kampmann and colleagues[325] conducted a prospective study on 155 pediatric stem cell transplant recipients and identified 17 percent with adenoviremia (26/155). Their study showed that early detection of adenoviremia, withdrawal of immunosuppression, and early antiviral therapy led to resolution of adenoviremia in 81 percent of the patients. de Mezerville and collaborators[138] retrospectively identified 28 solid-organ transplant recipients and 27 hematopoietic stem cell transplant recipients with adenovirus infection at the Hospital for Sick Children in Toronto, Canada. Their study found a similar rate of posttransplant adenoviral infection in solid-organ transplant recipients (50.9%) and hematopoietic stem cell transplant recipients (49.1%). de Mezerville's group found a much higher mortality rate in the group of hematopoietic stem cell transplant recipients (30% mortality rate vs. 0% in the solid-organ transplant group). Using PCR, Bil-Lula and colleagues reviewed adenovirus infection in 116 patients (98 children, 18 adults) who underwent stem cell transplant. Infection was found in 52 patients (44.8%). Although overall mortality in infected patients was 19 percent, this did not differ significantly from that in uninfected patients.[49,166]

Control of adenovirus infection is thought to be T cell mediated, and patients receiving T cell–suppressive regimens are thought to be at greater risk for adenovirus

*References 93, 112, 119, 152, 181, 213, 334, 478, 489, 520, 564, 571, 606.

*References 4, 28, 34, 79, 82, 103, 124, 129, 179, 184, 189, 218, 243, 250, 262, 271, 321, 333, 384, 392, 442, 455, 463, 479, 494, 520, 545-548, 553, 558, 592, 616, 644, 663, 670.

disease.[636] Myers and colleagues[443] demonstrated an increased risk for development of adenoviral infection in bone marrow transplant recipients receiving alemtuzumab (Campath 1H), a monoclonal antibody used for T-cell depletion, in comparison with patients receiving antithymocyte globulin, which was composed of polyclonal antibodies directed toward T cells. Avivi and colleagues[24] demonstrated that the most important risk factors for progression of adenovirus disease included severe lymphocytopenia (absolute lymphocyte count < 200/mm^3) and continued immunosuppression. They also found a correlation between lack of prophylaxis with ganciclovir for cytomegalovirus infection and risk for progression of adenovirus disease.

Ahmad and coworkers[8] reported a case of severe adenovirus pneumonia developing after infliximab (Remicade) was used to treat a 35-year-old man with Crohn disease. Infliximab is a monoclonal antibody directed against TNF-α that is used frequently in the treatment of autoimmune and rheumatologic diseases. Ahmad's group emphasized the fact that TNF-α antagonists can impair cell-mediated immunity, an essential component of recovery from adenoviral infection in immunocompromised hosts, and, therefore, can predispose patients to the development of viral pneumonia.

Other groups have demonstrated that having low lymphocyte counts at the time of development of adenovirus infection was predictive of adenovirus viremia and that clearance of infection and host survival were dependent on recovery of adenovirus-specific CD4$^+$ T-cell responses.[262,612,636] Lankester and colleagues[365] showed that isolation of adenovirus from multiple sites was associated with the development of adenovirus disease. Lins' group[383] demonstrated an association between isolation of adenovirus in peripheral blood and progression or dissemination (or both) of adenovirus disease. High levels of adenovirus viremia (i.e., detection of PCR product at 100-fold or greater dilution of template DNA) have been correlated with fatal outcomes in children receiving allogeneic stem cell transplants.[537,611]

Baldwin and colleagues[28] studied the outcome and clinical course of 105 adenoviral infections in 100 patients after they received bone marrow transplants. The incidence was higher in unrelated donor transplants than in matched sibling donor transplants. Diarrhea and fever were the most common initial findings. Six deaths were attributed to the adenoviral infections; five of the six patients had pneumonia, and four had associated graft-versus-host disease. Three additional patients had severe disease.

In a study involving 532 recipients of hematopoietic stem cell transplants, a 12 percent incidence of adenoviral infection was noted.[293] Forty-one patients had infections classified as "invasive," and mortality was 76 percent in this group. Recipients of allogeneic transplants were more likely than autologous stem cell recipients to have adenoviral infections.

The most common manifestation of adenoviral infection in pediatric patients with bone marrow transplants at St. Jude Children's Research Hospital was hemorrhagic cystitis, followed by gastroenteritis, pneumonitis, and liver failure.[190] Adenovirus-induced acute hemorrhagic cystitis is a complication encountered after hematopoietic stem cell transplantation and, more rarely, after renal transplantation.[185,286] Gorczynska and colleagues[236] examined the incidence of virus-induced hemorrhagic cystitis in 102 children and adolescents who underwent allogeneic stem cell transplantation. Hemorrhagic cystitis occurred in 25.5 percent of these children, with 3.9 percent of the cases attributed to adenoviruses. Another retrospective review of 266 adults undergoing stem cell transplant showed the incidence of adenoviral hemorrhagic cystitis to be 9.8 percent. Increased risk for cystitis was linked with underlying disease (cancer other than acute leukemia) and with T-cell purging with antithymocyte globulin or alemtuzumab. Patients with acute graft-versus-host disease had a decreased incidence of hemorrhagic cystitis.[431]

Shirali and coworkers[547] noted that demonstration of the adenoviral genome in endomyocardial biopsy specimens from heart transplant recipients was a predictor of graft loss in children. Graft loss with concomitant adenovirus infection has been described in renal transplant patients.[352,355] Scattered reports have found an association between adenoviral infection and viruria and hemorrhagic cystitis in patients with renal transplants.[187,363] Fatal and severe cases of subacute meningoencephalitis caused by an adenovirus have been described in bone marrow transplant recipients.[129,203] Severe necrotizing pneumonia with pulmonary hemorrhage due to adenovirus in a lung transplant patient has been described.[408]

In many instances, a fulminant, bacterial, sepsis-like picture with high fever, cough, and lethargy is associated with adenoviral infection in compromised patients.[670] Severe pneumonia often is demonstrated, both clinically and radiologically, and hepatic involvement with disseminated intravascular coagulation also is a frequent occurrence. Fatalities are reported, and recovery often is slow.

Other Manifestations

Arthritis

Arthritis has been noted in association with adenovirus type 7 infection.[476] The illness was characterized by fever, acute respiratory symptoms, erythematous macular rash, aseptic meningitis, and inflammatory arthritis of both knees.

Thyroiditis

In 1964, Swann[572] reported five patients with acute thyroiditis and thyroid enlargement in whom serologic study revealed greater than fourfold rises in titer of adenoviral complement-fixing antibody.

Deafness

Deafness of sudden onset was reported in an adult with a 2-day history of sore throat, low-grade fever, rhinorrhea, and cough. Adenovirus type 3 was isolated from the patient's throat, and a greater than fourfold rise in neutralizing antibody titer to this virus was observed.[304]

Obesity

Studies have shown that exposure to human adenovirus serotype 36 is associated with increased adiposity in chickens, mice, and nonhuman primates.[145-147] Continuing work in mouse models showed that adenovirus 36 infection increased macrophage infiltration into adipocytes[447] and impairs insulin signaling, thus altering glucose metabolism.[313] Paradoxically, reduced serum cholesterol and serum triglycerides are seen in the same animal models. Atkinson and colleagues[22,613] proposed that adenovirus serotype 36 directly affects adipocytes by increasing the number and size of preadipocytes. Vangipuram and associates[614] explored the possible mechanisms by which adenovirus types 36 and 37 increase adiposity in animal models. They performed ex vivo experiments in human adipocytes and a murine preadipocyte cell line (3T3-L1) and in vivo experiments in the rat model, which demonstrated suppression of leptin mRNA expression, decreased leptin secretion, and increased glucose uptake. In vitro studies have shown that adenovirus 36 infection causes upregulation of adipogenesis-related genes, along with inflammation which lead to lipid accumulation in cells.[446] Fatty acid oxidation was also shown to be impaired in in-vitro models.[632]

Based on studies that adenoviruses increase adiposity in animal models, Atkinson and coworkers[22] screened 502 subjects for neutralizing antibodies to adenovirus 36. They detected antibodies to adenovirus 36 in as many as 30 percent of obese study subjects as opposed to 11 percent of nonobese subjects. Similarly, they found paradoxically decreased serum cholesterol and triglycerides in patients with antibodies to adenovirus 36. Subsequent studies have shown mixed results. Several studies have shown a correlation between seropositivity to adenovirus 36 and obesity.[21,23,212,593] In a study of adults, Na and associates[445] showed that although seropositivity to adenovirus 36 did correlate with overweight body mass index (BMI) (23 to 25), this relationship did not hold when examining obese individuals (BMI >25). A study completed in Holland and Belgium by Goossens and colleagues[235] in active-duty U.S. military personnel[67] failed to show correlations between adenovirus 36 seropositivity and BMI. Interestingly, Trovato and colleagues showed that adenovirus 36 seropositivity was correlated with greater decrease in BMI and reduction of insulin resistance when subjects were placed on diet and exercise programs.[594] One study has shown increased serum cholesterol and triglycerides in children with antibodies against adenovirus 36.[444] Additional studies by Whigham and colleagues[638] demonstrated that human adenovirus serotype 37 also is associated with an increase in adipocyte differentiation and reduced serum triglyceride levels but increased serum cholesterol levels.

Congenital and Neonatal Infections

Neonatal and congenital adenoviral infections reflect disseminated infection with the involvement of multiple organs.* Major manifestations include hepatosplenomegaly, progressive pneumonia, hepatitis, and thrombocytopenia. Towbin and colleagues[589] reported intrauterine adenoviral myocarditis manifested as nonimmune hydrops fetalis. Illnesses frequently appear initially as an early-onset sepsis syndrome. An infant has been reported with a congenital pleural effusion from which type 3 adenovirus was recovered.[419]

Couroucli and associates[117] noted an association between adenoviral infection and bronchopulmonary dysplasia. A significant increase was found in the frequency of adenovirus genome in tracheal aspirates from patients with bronchopulmonary dysplasia versus control subjects.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of adenoviral infection must be subcategorized on the basis of the major clinical manifestations. In many instances, specific clinical symptoms such as pharyngoconjunctival fever or epidemic keratoconjunctivitis can lead to strong suspicion of the adenoviral etiology. With other, more general respiratory diseases such as pharyngitis, bronchitis, croup, bronchiolitis, and pneumonia, the adenoviral etiology cannot be established on clinical grounds. Fever in adenoviral respiratory tract infections tends to be higher than that occurring in parainfluenza and RSV infections but is similar to that in influenza A and B viral infections.[502] High and prolonged fever occurs as commonly in adenoviral infections as it does in bacterial respiratory tract infections. Because the symptom triad of fever, pharyngitis, and conjunctivitis is virtually unique to pharyngoconjunctival fever, establishing the diagnosis of this disease should be easy. Generalized diseases that occasionally might be confused with pharyngoconjunctival fever include leptospirosis, psittacosis, *Mycoplasma pneumoniae* infection, Q fever, Newcastle disease virus infection, and prodromal measles.

In pharyngitis, adenoviral infection must be differentiated both from other viral diseases, such as those caused by Epstein-Barr virus, parainfluenza and influenza viruses, and enteroviruses, and from streptococcal disease. In a young child, follicular pharyngitis is more likely to be caused by adenoviral than by streptococcal infection.

Adenoviral pneumonia in young children frequently is not distinguishable on clinical grounds from that caused by bacteria. In older children and adolescents, in particular, adenoviral pneumonia often can be differentiated from bacterial disease by its bilateral nature. Differentiating adenoviral illness from disease caused by *M. pneumoniae* is more difficult; however, cold agglutinin titers generally are higher and more persistently positive in mycoplasmal disease.

Adenoviral eye disease must be differentiated from that caused by viruses of the herpes group, enteroviral epidemic conjunctivitis (acute hemorrhagic conjunctivitis),[382,660,661] and *Chlamydia* spp., and bacteria, including *Neisseria gonorrhoeae*.

Diarrhea caused by enteric adenoviruses can be differentiated from other viral diarrheas by electron microscopy, by identification with ELISA, and by specific culture of the enteric agents in Graham 293 cell culture. Enteric bacterial and parasitic agents also must be considered in the differential diagnosis.

*References 3, 70, 101, 177, 344, 469, 564.

The high, relatively prolonged fever occurring in association with lymphadenopathy, exanthem, and enanthem noted with adenoviral infection often causes confusion with Kawasaki disease.[95,516] Because Kawasaki disease must be treated early with intravenous immunoglobulin, adenoviral infections should be considered early and appropriate studies performed.

DIAGNOSIS

Adenoviral infection can be diagnosed specifically by isolation of virus in an appropriate tissue culture system, direct antigen detection assay, or by DNA PCR. Most adenoviral types can be recovered from clinical specimens in primary or diploid cultures of human embryonic kidney.[373] The enteric viral types 40 and 41 can be grown in Graham 293 cells.[576,625] The rapidity of detection of adenoviruses by culture is enhanced by centrifugation of specimens in shell vials or plastic-welled plates.[158,171,397]

Direct identification of adenoviruses in respiratory secretions by radioimmunoassay, immunofluorescence techniques, and ELISA now is performed widely.* However, in general, the sensitivity, when compared with that of virus isolation, is relatively low; on the other hand, the specificity is high (>95%). Rocholl and colleagues[516] demonstrated the impact of rapid diagnosis of adenoviral respiratory disease with an adenoviral direct fluorescent antibody technique by noting discontinuation of empirical antibiotic use in a retrospective analysis of hospitalized patients with respiratory cultures positive for adenovirus. Rapid techniques for the identification of enteric adenoviruses in general have been sensitive and specific.† However, in newborns, false-positive results with a latex agglutination test have been reported.[300] Several techniques using DNA probes have been studied for the rapid identification of both respiratory and enteric adenoviral infection.[75,299,575] PCR assay also is useful for the rapid detection of adenoviral infection and is rapidly becoming the gold standard of diagnosis alongside tissue culture isolation.‡ PCR assay is particularly useful for identifying adenoviral DNA in formalin-fixed, paraffin-embedded tissues and other biopsy and postmortem specimens.§

Several groups have demonstrated the utility of PCR detection of adenoviral DNA in acute respiratory tract infections in immunocompetent patients.[2,178,516,546,656] Kuypers and colleagues[359] demonstrated significantly increased sensitivity of quantitative real-time PCR assay over fluorescent antibody detection of adenoviruses. They showed that 77.7 percent of respiratory specimens that were negative for adenovirus by fluorescent antibody detection were positive by PCR detection of adenovirus DNA. This increased sensitivity was replicated by Stroparo and colleagues.[569] Multiple PCR assays have been developed to detect multiple serotypes of adenovirus.[383,656] Buckwalter and colleagues developed real-time PCR for qualitative detection of all 57 serotypes of adenovirus with high sensitivity and specificity in a variety of clinical samples.[74] Others have demonstrated similar multiplex assays for adenovirus, although they were narrower in scope.[470] Aberle and colleagues[2] were able to detect adenoviral DNA with a nested PCR assay in acute-phase sera collected within the first week after the onset of symptoms in 72 percent of immunocompetent children experiencing their first adenoviral infection and in 25 percent of those experiencing recurrent infection.

Other groups have demonstrated the utility of using PCR assays to diagnose and monitor adenoviral infection and disease in immunocompromised hosts.[365,537] Lion's group[385] developed a species-specific, real-time, quantitative PCR assay that allows detection and quantification of 51 human adenovirus subtypes. They showed that detection of adenovirus DNA in the peripheral blood of allogeneic bone marrow transplant recipients preceded the onset of clinical symptoms of disseminated adenoviral disease by a median of 3 weeks. Two other groups[374,628] have used real-time, quantitative PCR assays to detect adenoviral DNA load to aid in both the diagnosis and management of disseminated adenoviral infections in immunocompromised patients. Ebner and colleagues[160] recently have presented a two-stage PCR algorithm for diagnosis in immunosuppressed patients. Although algorithms for detection and typing of adenoviruses have been used in the past,[160,410] the use of type-specific PCR and other techniques such as reverse-line-blot has made serotyping more efficient.[74,80]

Multiplex PCR to detect multiple different viruses in a clinical sample (e.g., respiratory secretions, stool, blood) are now becoming widely available. Commercial, as well as "home-made" assays can now test for up to 13 different viruses with fast turnaround (1 to 4 hours) and excellent sensitivity and specificity.* The availability of inexpensive, reliable, and fast PCR kits has raised the question of whether the detection of viral genetic material can be attributed to true infection or shedding of dead virus from a past event. Kalu and colleagues[324] showed in a cohort of 76 children with 581 episodes of upper respiratory tract infection that adenoviruses of the same type and strain would persistently be detected by PCR in nasopharyngeal washes of the same host for over 200 days. The authors caution that clinical interpretation of PCR test results is necessary, because most practitioners do not have access to phylogenetic analysis. At present, most hospitals rely on fluorescent antibody staining, shell vial culture, and viral culture to identify adenovirus infection. Multiplex PCR, especially for the diagnosis of respiratory viral illness, as well as singleplex adenovirus PCR is quickly taking the place of these methods.

Acute infection also can be diagnosed serologically by the expression of specific serum IgM antibody.[418] Infection can be confirmed serologically by the demonstration of a rise in antibody titer in two sequential serum samples. Antibody response to the adenovirus group antigen can be detected by complement fixation or ELISA.

*References 72, 397, 418, 505, 516, 530, 533, 583.
†References 7, 238, 254, 268, 534, 599, 650.
‡References 2, 13, 58, 160, 161, 178, 184, 263, 311, 312, 359, 365, 374, 385, 412, 433, 479, 516, 537, 546, 628, 656.
§References 58, 246, 394, 403, 547, 595.

*References 182, 211, 247, 252, 278, 370, 402, 609.

Type-specific antibodies can be determined by neutralization, ELISA, or hemagglutination inhibition.

TREATMENT

During the febrile period of illness, adequate hydration should be maintained and excessive activity discouraged. The fever may be controlled with acetaminophen. In children with eye involvement, careful attention should be paid to the possibility of secondary bacterial infection. If local purulence develops, specimens should be procured for culture and topical antimicrobial therapy started. The use of corticosteroid-containing ophthalmic ointments should be avoided.

A consistent, safe, and effective therapy to treat adenovirus infection has yet to be established. Although vidarabine, ribavirin, and cidofovir demonstrate good activity against adenoviruses in vitro, several studies have shown only limited success with the use of vidarabine, ribavirin, ganciclovir, and cidofovir in treating adenovirus disease in humans.[387,438] The potential beneficial effects must be weighed carefully against the adverse side effects of these antiviral agents, including myelosuppression, pancytopenia, and nephrotoxicity. Walls and coworkers[629] conducted a retrospective study of 26 patients undergoing hematopoietic stem cell transplantation. They were unable to demonstrate a direct correlation among adenoviral DNA load, clinical features, and severity of disease. They also identified two children who experienced low-level adenoviral viremia after undergoing hematopoietic stem cell transplantation; in these children the viral infection resolved without treatment. Williams and colleagues created an algorithm to risk-stratify pediatric oncology and stem cell transplant patients with adenovirus infection to determine who would benefit from cidofovir therapy without uneccesary risk for nephrotoxicity. They determined high-risk patients to be those with recent transplant (<60 days allogeneic or <180 days autologous), those with graft-versus-host disease, and those on immunosuppressive therapy. These patients would benefit from cidofovir, whereas those without these risk factors would do well without treatment. Their algorithm was validated in a small prospective cohort of 11 patients.[647]

Ribavirin is a guanosine nucleoside analog used for the treatment of severe RSV infection in its inhaled form and for the treatment of chronic hepatitis C infection. In a number of instances, immunocompromised patients with disseminated adenoviral infection have been treated with ribavirin administered intravenously and successful outcomes have been reported.* In one instance, a loading dose of 30 mg/kg/day divided into three doses was followed by maintenance therapy with 15 mg/kg/day.[409] Gavin and Katz[218] reported complete clinical recovery in two of five patients with severe adenovirus disease. A later study by Lankester and associates[364] demonstrated no effect on plasma adenoviral DNA load despite initiation of treatment with ribavirin at the first onset of clinical symptoms in four pediatric patients receiving allogeneic stem cell transplants. Intravenous ribavirin is available in the United States only as an emergency investigational drug because it is not approved by the U.S. Food and Drug Administration. Data on its use, efficacy, and safety are still very limited.[513]

The antiviral agent with the most promise to date is cidofovir, a cytosine nucleoside analog with efficacy against several DNA viruses, including cytomegalovirus, HSV, polyomaviruses, and adenoviruses. Cidofovir was used successfully in treating a 17-year-old boy who had received a stem cell transplant and had severe adenoviral gastroenteritis.[510] Ljungman and colleagues[388] conducted a study of cidofovir for the Infectious Diseases Working Party for Blood and Marrow Transplantation (EMBT). They demonstrated successful treatment of adenovirus infections in 69 percent of allogeneic hematopoietic stem cell transplant recipients, primarily with a 5-mg/kg dose of cidofovir given intravenously one time per week for the first 3 weeks and every other week after that, along with concomitant use of probenecid and intravenous fluid hydration. Other studies have shown a similar effect with reduced dosing of cidofovir at 1 mg/kg per dose given three times a week.[260,387,438] A case report by Fanourgiakis and colleagues[179] demonstrated successful treatment of severe (grade IV) hemorrhagic cystitis in a 34-year-old man by intravesical instillation of 5 mg/kg of cidofovir in 100 mL saline twice daily. This method avoided the known nephrotoxicity associated with cidofovir. Saquib and colleagues reported on two adult renal transplant patients with adenovirus viremia successfully treated with cidofovir along with intravenous immune globulin, and concurrent reduction in immune suppressive therapy. [535] Yusuf and coworkers[669] reported complete resolution of clinical symptoms in 57 of 58 pediatric hematopoietic stem cell transplant recipients treated with cidofovir. Wallot and colleagues[627] had some success using reduced-dose cidofovir, inhaled nitric oxide, and decreased immunosuppression to treat two liver transplant recipients with disseminated adenoviral disease and acute respiratory distress syndrome. CMX001 (hexadecyloxypropyl-cidofovir) is a new antiviral drug made by Chimerex and is a lipid conjugate of cidofovir. It has broad activity against double-stranded DNA viruses in vitro and is currently undergoing clinical trials to treat adenovirus infections as well as BK polyoma virus, vaccinia, and molluscum contagiosum.[99] Evaluation of treatment of poxviruses in mouse models is also ongoing.[151]

Paolino and colleagues were the first to report successful recovery from disseminated adenovirus infection in a pediatric stem cell transplant patient using CMX001 after treatment failure with cidofovir.[477]

Florescu and colleagues retrospectively reviewed salvage treatment of adenovirus infection with CMX001 (after failure with cidofovir) in 13 solid-organ and stem cell transplant patients: 9 patients had a significant lowering of viral load, whereas 4 patients did not. The former group did have a statistically longer survival time, and no patients discontinued treatment owing to drug side effects.[191]

Further studies into possible immunotherapies for the treatment of adenovirus infections also have been investigated. Feuchtinger and associates[186] were able to perform successful and safe adoptive transfer of

*References 17, 326, 381, 409, 440, 513, 654.

adenovirus-specific, T-cell immunity in five of six patients with documented adenovirus infection after allogeneic hematopoietic stem cell transplantation. This effect was found to be independent of the T-cell dose. Furthermore, the adenovirus-specific, T-cell expansion was sustainable and proliferation of the adenovirus-specific T cells was unimpaired by adenovirus viremia, in contrast to the myelosuppressive effects seen with cytomegaloviral viremia. Currently, emphasis of study is on the efficient and effective in vitro manufacturing of cytotoxic T lymphocytes for use in immunocompromised individuals.[9,206,220,549,671]

Teuchner and colleagues[582] studied *N*-chlorotaurine (NCT), a compound produced by monocytes and granulocytes during the oxidative burst, in a double-blind investigation of adult patients with epidemic keratoconjunctivitis. Among the group of 60 patients, 33 were treated with a 1 percent aqueous solution of NCT and 27 were treated with gentamicin. These researchers analyzed both subjective and objective scores. In the total study group, the NCT-treated subjects were subjectively better than the gentamicin-treated patients at day 8, but there were no objective differences. However, in severe infections, both the subjective and objective scores were better on days 4 and 8 in the NCT-treated patients. They concluded that NCT treatment was well tolerated and that it shortened the duration of illness. A study of the use of 1 percent cidofovir eyedrops with and without 1 percent cyclosporine eyedrops to treat adenovirus-associated keratoconjunctivitis was conducted by Hillenkamp and colleagues.[283] This study demonstrated significant local toxicity in the skin of the eyelids and conjunctiva but a decreased frequency of severe corneal opacities associated with the use of cidofovir. A novel drug, NVC-422 has shown good in vitro activity against adenovirus. This compound is an analog of NCT that has been modified to gain better stability in solid-state solution. A phase 2 clinical trial for adenoviral conjunctivitis is ongoing.[666]

Human fibroblast–derived interferon, applied topically, was used to treat epidemic adenoviral keratoconjunctivitis in a comparative trial.[519] The duration of illness was reduced in the patients treated with interferon, but many of the control patients received dexamethasone. A child with combined immunodeficiency and severe, diffuse adenovirus type 7a pneumonia improved dramatically after receiving a large dose of high-titer adenovirus type 7a immune serum globulin.[124]

PREVENTION

Serious incapacitating epidemics caused by adenoviruses in military recruits in basic training led to the development of adenoviral vaccines.[16,371] The initial vaccines were formalin-inactivated preparations of virus grown in monkey kidney tissue culture and were administered parenterally.[15,245,280] These vaccines achieved only limited success because of variable degrees of potency in different vaccine lots.[36] An inactivated adenoviral vaccine that contained types 3, 4, and 7 was prepared in monkey tissue culture for trial in children.[608] Three doses of this vaccine resulted in high levels of neutralizing antibody to the three viral types, and this antibody persisted in most infants for at least 1 year. Many of the initial lots of inactivated adenoviral vaccine were found to contain live simian adenoviruses that were capable of producing neoplasms in suckling hamsters. Because of this finding, inactivated vaccine trials were discontinued. Next, a live, attenuated adenovirus type 4 vaccine that was cultivated in human diploid tissue culture was developed and administered orally by enteric-coated capsule to volunteers.[586,587] Asymptomatic gastrointestinal infection occurred, and a good serum-neutralizing antibody response was elicited.[245,556,560] Most recipients of live oral vaccine excrete virus in stool for several days to a month after being vaccinated. With military use of the vaccine, shedding in stool was not associated with transmission of the virus to nonimmune contacts.[434] In other studies of married couples and families with children, virus was transmitted to nonimmune contacts.[434,561] Transmission usually occurred without illness.[434]

The administration of live, enteric-coated, type 4 and 7 adenovirus vaccine resulted in a significant decrease in the incidence of acute respiratory disease in military recruits.[155,234,574] In 1995, this vaccination program was terminated, and the last stocks of vaccine were depleted or expired by 1999. However, after a subsequent rise in the incidence of acute respiratory disease and deaths in military servicemen,[29,240,351,531] vaccination was resumed. A new live oral vaccine for type 4 and 7 human adenoviruses is now licensed and in use since 2011.[598] Few attempts have been made to protect children with live adenoviral vaccines. In one study, live enteric-coated adenovirus type 4 vaccine was given to children aged 5 to 11 years. Asymptomatic gastrointestinal infection resulted from administration of the vaccine. Because the major cause of pharyngoconjunctival fever caused by adenoviruses is swimming in contaminated water, discretion in bathing locations is advised. Swimming pool water should be chlorinated adequately, and pool filtration systems should be inspected daily. Ill individuals should be excluded from swimming pools during their illness and for at least 2 weeks after recovery.

PROGNOSIS

The overall prognosis of adenoviral infection generally is excellent. Secondary bacterial complications, if untreated, can result in prolongation of illness and permanent sequelae in some instances. The prognosis of adenoviral infection in the very young and in immunocompromised patients must be guarded.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abd-Jamil J, Teoh BT, Hassan EH, et al. Molecular identification of adenovirus causing respiratory tract infection in pediatric patients at the University of Malaya Medical Center. *BMC Pediatr* 2010;**10**:46.
6. Aguerre V, Castanos C, Pena HG, et al. Postinfectious bronchiolitis obliterans in children: clinical and pulmonary function findings. *Pediatr Pulmonol* 2010;**45**:1180–5.

9. Aissi-Rothe L, Decot V, Venard V, et al. Rapid generation of full clinical-grade human antiadenovirus cytotoxic T cells for adoptive immunotherapy. *J Immunother* 2010;**33**:414–24.
10. Akiyoshi K, Suga T, Fukui K, et al. Outbreak of epidemic keratoconjunctivitis caused by adenovirus type 54 in a nursery school in Kobe City, Japan in 2008. *Jpn J Infect Dis* 2011;**64**:353–5.
12. Alharbi S, Van Caeseele P, Consunji-Araneta R, et al. Epidemiology of severe pediatric adenovirus lower respiratory tract infections in Manitoba, Canada, 1991-2005. *BMC Infect Dis* 2012;**12**:55.
21. Atkinson RL. Human adenovirus-36 and childhood obesity. *Int J Pediatr Obes* 2011;**6**(Suppl. 1):2–6.
23. Atkinson RL, Lee I, Shin HJ, et al. Human adenovirus-36 antibody status is associated with obesity in children. *Int J Pediatr Obes* 2010;**5**:157–60.
32. Barrero PR, Valinotto LE, Tittarelli E, et al. Molecular typing of adenoviruses in pediatric respiratory infections in Buenos Aires, Argentina (1999-2010). *J Clin Virol* 2012;**53**:145–50.
47. Berrie E, Andrews L, Yezli S, et al. Hydrogen peroxide vapour (HPV) inactivation of adenovirus. *Lett Appl Microbiol* 2011;**52**: 555–8.
49. Bil-Lula I, Ussowicz M, Rybka B, et al. PCR diagnostics and monitoring of adenoviral infections in hematopoietic stem cell transplantation recipients. *Arch Virol* 2010;**155**:2007–15.
50. Binks MJ, Cheng AC, Smith-Vaughan H, et al. Viral-bacterial co-infection in Australian indigenous children with acute otitis media. *BMC Infect Dis* 2011;**11**:161.
67. Broderick MP, Hansen CJ, Irvine M, et al. Adenovirus 36 seropositivity is strongly associated with race and gender, but not obesity, among US military personnel. *Int J Obes (Lond)* 2010; **34**:302–8.
68. Brosch L, Tchandja J, Marconi V, et al. Adenovirus serotype 14 pneumonia at a basic military training site in the United States, spring 2007: a case series. *Mil Med* 2009;**174**:1295–9.
74. Buckwalter SP, Teo R, Espy MJ, et al. Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. *J Clin Microbiol* 2012;**50**:766–71.
78. Callaway Z, Kim SH, Kim JY, et al. Adenovirus infection with serious pulmonary sequelae in Korean children. *Clin Respir J* 2011;**5**:92–8.
80. Cao Y, Kong F, Zhou F, et al. Genotyping of human adenoviruses using a PCR-based reverse line blot hybridisation assay. *Pathology* 2011;**43**:488–94.
84. Centers for Disease Control and Prevention. Outbreak of adenovirus 14 respiratory illness—Prince of Wales Island, Alaska, 2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:6–10.
102. Chiu SS, Chan KH, Chen H, et al. Virologically confirmed population-based burden of hospitalization caused by respiratory syncytial virus, adenovirus, and parainfluenza viruses in children in Hong Kong. *Pediatr Infect Dis J* 2010;**29**:1088–92.
105. Chun JK, Lee JH, Kim HS, et al. Establishing a surveillance network for severe lower respiratory tract infections in Korean infants and young children. *Eur J Clin Microbiol Infect Dis* 2009;**28**:841–4.
139. De Paulis M, Gilio AE, Ferraro AA, et al. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011;**87**:307–13.
143. Dey RS, Ghosh S, Chawla-Sarkar M, et al. Circulation of a novel pattern of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. *J Clin Microbiol* 2011;**49**:500–5.
144. Dey SK, Shimizu H, Phan TG, et al. Molecular epidemiology of adenovirus infection among infants and children with acute gastroenteritis in Dhaka City, Bangladesh. *Infect Genet Evol* 2009;**9**:518–22.
148. do Carmo Debur M, Raboni SM, Flizikowski FB, et al. Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal non-pandemic seasonal respiratory infections. *J Clin Pathol* 2010;**63**:930–4.
151. Dropulic LK, Cohen JI. Update on new antivirals under development for the treatment of double-stranded DNA virus infections. *Clin Pharmacol Ther* 2010;**88**:610–19.
166. Ephros M, Friedman BC, Elhasid R, et al. Incidence and clinical manifestations of adenoviral infection among children undergoing allogeneic stem cell transplantation. *Isr Med Assoc J* 2009;**11**: 744–8.
169. Ersoy Y, Otlu B, Turkcuoglu P, et al. Outbreak of adenovirus serotype 8 conjunctivitis in preterm infants in a neonatal intensive care unit. *J Hosp Infect* 2012;**80**:144–9.
170. Esposito DH, Gardner TJ, Schneider E, et al. Outbreak of pneumonia associated with emergent human adenovirus serotype 14—Southeast Alaska, 2008. *J Infect Dis* 2010;**202**:214–22.
176. Fabbiani M, Terrosi C, Martorelli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol* 2009;**81**:750–6.
182. Feeney SA, Armstrong VJ, Mitchell SJ, et al. Development and clinical validation of multiplex TaqMan(R) assays for rapid diagnosis of viral gastroenteritis. *J Med Virol* 2011;**83**:1650–6.
185. Ferreira GF, Oliveira RA, Lucon M, et al. Hemorrhagic cystitis secondary to adenovirus or herpes simplex virus infection following renal transplantation: four case reports. *Transplant Proc* 2009;**41**:4416–19.
190. Florescu DF, Islam MK, Mercer DF, et al. Adenovirus infections in pediatric small bowel transplant recipients. *Transplantation* 2010;**90**:198–204.
191. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012;**18**:731–8.
203. Frange P, Peffault de Latour R, Arnaud C, et al. Adenoviral infection presenting as an isolated central nervous system disease without detectable viremia in two children after stem cell transplantation. *J Clin Microbiol* 2011;**49**:2361–4.
206. Fuji S, Kapp M, Grigoleit GU, et al. Adoptive immunotherapy with virus-specific T cells. *Best Pract Res Clin Haematol* 2011;**24**:413–19.
211. Funahashi Y, Iwata S, Ito Y, et al. Multiplex real-time PCR assay for simultaneous quantification of BK polyomavirus, JC polyomavirus, and adenovirus DNA. *J Clin Microbiol* 2010;**48**:825–30.
212. Gabbert C, Donohue M, Arnold J, et al. Adenovirus 36 and obesity in children and adolescents. *Pediatrics* 2010;**126**:721–6.
214. Garcia-Garcia ML, Calvo C, Falcon A, et al. Role of emerging respiratory viruses in children with severe acute wheezing. *Pediatr Pulmonol* 2010;**45**:585–91.
220. Gerdemann U, Vera JF, Rooney CM, et al. Generation of multivirus-specific T cells to prevent/treat viral infections after allogeneic hematopoietic stem cell transplant. *J Vis Exp* 2011:(51):pii: 2736.
222. Ghanaiem H, Averbuch D, Koplewitz BZ, et al. An outbreak of adenovirus type 7 in a residential facility for severely disabled children. *Pediatr Infect Dis J* 2011;**30**:948–52.
233. Goncalves G, Gouveia E, Mesquita JR, et al. Outbreak of acute gastroenteritis caused by adenovirus type 41 in a kindergarten. *Epidemiol Infect* 2011;**139**:1672–5.
235. Goossens VJ, deJager SA, Grauls GE, et al. Lack of evidence for the role of human adenovirus-36 in obesity in a European cohort. *Obesity (Silver Spring)* 2011;**19**:220–1.
247. Gunson RN, Maclean AR, Shepherd SJ, et al. Simultaneous detection and quantitation of cytomegalovirus, Epstein-Barr virus, and adenovirus by use of real-time PCR and pooled standards. *J Clin Microbiol* 2009;**47**:765–70.
252. Hammitt LL, Kazungu S, Welch S, et al. Added value of an oropharyngeal swab in detection of viruses in children hospitalized with lower respiratory tract infection. *J Clin Microbiol* 2011;**49**:2318–20.
254. Hara M, Takao S, Fukuda S, et al. Rapid diagnosis of adenovirus respiratory tract infections using the chromatographic immunoassay test in the pediatric outpatient setting. *Pediatr Infect Dis J* 2010;**29**:267–9.
269. Heydari H, Mamishi S, Khotaei GT, et al. Fatal type 7 adenovirus associated with human bocavirus infection in a healthy child. *J Med Virol* 2011;**83**:1762–3.
278. Higgins RR, Beniprashad M, Cardona M, et al. Evaluation and verification of the Seeplex Diarrhea-V ACE assay for simultaneous detection of adenovirus, rotavirus, and norovirus genogroups I and II in clinical stool specimens. *J Clin Microbiol* 2011;**49**:3154–62.
298. Huhulescu S, Kiss R, Brettlecker M, et al. Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. *Infection* 2009;**37**:103–8.
302. Ishiko H, Aoki K. Spread of epidemic keratoconjunctivitis due to a novel serotype of human adenovirus in Japan. *J Clin Microbiol* 2009;**47**:2678–9.

311. Jeulin H, Salmon A, Bordigoni P, et al. Comparison of in-house real-time quantitative PCR to the Adenovirus R-Gene kit for determination of adenovirus load in clinical samples. *J Clin Microbiol* 2010;**48**:3132–7.
312. Jeulin H, Salmon A, Bordigoni P, et al. Diagnostic value of quantitative PCR for adenovirus detection in stool samples as compared with antigen detection and cell culture in haematopoietic stem cell transplant recipients. *Clin Microbiol Infect* 2011;**17**:1674–80.
313. Jiang S, Gavrikova TA, Pereboev A, et al. Adenovirus infection results in alterations of insulin signaling and glucose homeostasis. *Am J Physiol Endocrinol Metab* 2010;**298**:E1295–1304.
314. Jin Y, Cheng WX, Yang XM, et al. Viral agents associated with acute gastroenteritis in children hospitalized with diarrhea in Lanzhou, China. *J Clin Virol* 2009;**44**:238–41.
321. Kajon AE, Dickson LM, Fisher BT, et al. Fatal disseminated adenovirus infection in a young adult with systemic lupus erythematosus. *J Clin Virol* 2011;**50**:80–3.
324. Kalu SU, Loeffelholz M, Beck E, et al. Persistence of adenovirus nucleic acids in nasopharyngeal secretions: a diagnostic conundrum. *Pediatr Infect Dis J* 2010;**29**:746–50.
338. Khor CS, Sam IC, Hooi PS, et al. Epidemiology and seasonality of respiratory viral infections in hospitalized children in Kuala Lumpur, Malaysia: a retrospective study of 27 years. *BMC Pediatr* 2012;**12**:32.
352. Komiya T, Goto N, Takeda A, et al. A case of acute rejection with adenovirus infection after ABO-incompatible kidney transplantation. *Clin Transplant* 2009;**23**(Suppl. 20):27–30.
355. Kozlowski T, Nickeleit V, Andreoni K. Donor-transmitted adenovirus infection causing kidney allograft nephritis and graft loss. *Transpl Infect Dis* 2011;**13**:168–73.
367. Lassauniere R, Kresfelder T, Venter M. A novel multiplex real-time RT-PCR assay with FRET hybridization probes for the detection and quantitation of 13 respiratory viruses. *J Virol Methods* 2010;**165**:254–60.
370. Lee JH, Chun JK, Kim DS, et al. Identification of adenovirus, influenza virus, parainfluenza virus, and respiratory syncytial virus by two kinds of multiplex polymerase chain reaction (PCR) and a shell vial culture in pediatric patients with viral pneumonia. *Yonsei Med J* 2010;**51**:761–7.
379. Lewis PF, Schmidt MA, Lu X, et al. A community-based outbreak of severe respiratory illness caused by human adenovirus serotype 14. *J Infect Dis* 2009;**199**:1427–34.
384. Lindblom A, Bhadri V, Soderhall S, et al. Respiratory viruses, a common microbiological finding in neutropenic children with fever. *J Clin Virol* 2010;**47**:234–7.
386. Liu EB, Wadford DA, Seto J, et al. Computational and serologic analysis of novel and known viruses in species human adenovirus D in which serology and genomics do not correlate. *PLoS One* 2012;**7**:e33212.
401. Maranhao AG, Soares CC, Albuquerque MC, et al. Molecular epidemiology of adenovirus conjunctivitis in Rio de Janeiro, Brazil, between 2004 and 2007. *Rev Inst Med Trop Sao Paulo* 2009;**51**:227–9.
402. Marinheiro JC, Sanalios RB, Santos DC, et al. Duplex-PCR assay for the detection of adenovirus and respiratory syncytial virus in nasopharyngeal samples. *Mem Inst Oswaldo Cruz* 2009;**104**:118–20.
408. Mayeur N, Srairi M, Tetu L, et al. Lethal hemorrhagic alveolitis after adenovirus pneumonia in a lung transplant recipient. *Heart Lung* 2012;**41**:401–3.
410. McCarthy T, Lebeck MG, Capuano AW, et al. Molecular typing of clinical adenovirus specimens by an algorithm which permits detection of adenovirus coinfections and intermediate adenovirus strains. *J Clin Virol* 2009;**46**:80–4.
415. Melendez CP, Florentino MM, Martinez IL, et al. Outbreak of epidemic keratoconjunctivitis caused by adenovirus in medical residents. *Mol Vis* 2009;**15**:557–62.
431. Mori Y, Miyamoto T, Kato K, et al. Different risk factors related to adenovirus- or BK virus-associated hemorrhagic cystitis following allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2012;**18**:458–65.
441. Murtagh P, Giubergia V, Viale D, et al. Lower respiratory infections by adenovirus in children: clinical features and risk factors for bronchiolitis obliterans and mortality. *Pediatr Pulmonol* 2009;**44**:450–6.
444. Na HN, Hong YM, Kim J, et al. Association between human adenovirus-36 and lipid disorders in Korean schoolchildren. *Int J Obes (Lond)* 2010;**34**:89–93.
445. Na HN, Kim H, Nam JH. Novel genes and cellular pathways related to infection with adenovirus-36 as an obesity agent in human mesenchymal stem cells. *Int J Obes (Lond)* 2012;**36**:195–200.
446. Na HN, Kim J, Lee HS, et al. Association of human adenovirus-36 in overweight Korean adults. *Int J Obes (Lond)* 2012;**36**:281–5.
447. Na HN, Nam JH. Adenovirus 36 as an obesity agent maintains the obesity state by increasing MCP-1 and inducing inflammation. *J Infect Dis* 2012;**205**:914–22.
448. Naghipour M, Hart CA, Dove W, et al. Adenovirus infections within a family cohort in Iran. *Pediatr Pulmonol* 2009;**44**:749–53.
461. O'Brien TP, Jeng BH, McDonald M, et al. Acute conjunctivitis: truth and misconceptions. *Curr Med Res Opin* 2009;**25**:1953–61.
475. Page MA, Shisler JL, Marinas BJ. Mechanistic aspects of adenovirus serotype 2 inactivation with free chlorine. *Appl Environ Microbiol* 2010;**76**:2946–54.
477. Paolino K, Sande J, Perez E, et al. Eradication of disseminated adenovirus infection in a pediatric hematopoietic stem cell transplantation recipient using the novel antiviral agent CMX001. *J Clin Virol* 2011;**50**:167–70.
499. Potter RN, Cantrell JA, Mallak CT, et al. Adenovirus-associated deaths in US military during postvaccination period, 1999-2010. *Emerg Infect Dis* 2012;**18**:507–9.
503. Puzelli S, Valdarchi C, Ciotti M, et al. Viral causes of influenza-like illness: insight from a study during the winters 2004-2007. *J Med Virol* 2009;**81**:2066–71.
506. Razanajatovo NH, Richard V, Hoffmann J, et al. Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. *PLoS One* 2011;**6**:e17579.
512. Rimoldi SG, Stefani F, Pagani C, et al. Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents. *Arch Virol* 2011;**156**:1583–9.
513. Riner A, Chan-Tack KM, Murray JS. Original research: Intravenous ribavirin—review of the FDA's Emergency Investigational New Drug Database (1997-2008) and literature review. *Postgrad Med* 2009;**121**:139–46.
514. Robinson C, Echavarria M. Adenoviruses. In: Versalovic J, editor. *Manual of clinical microbiology*. vol. 2. 10th ed. Washington, D.C.: ASM Press; 2011. p. 1600–11.
515. Robinson CM, Singh G, Henquell C, et al. Computational analysis and identification of an emergent human adenovirus pathogen implicated in a respiratory fatality. *Virology* 2011;**409**:141–7.
535. Saquib R, Melton LB, Chandrakantan A, et al. Disseminated adenovirus infection in renal transplant recipients: the role of cidofovir and intravenous immunoglobulin. *Transpl Infect Dis* 2010;**12**:77–83.
542. Selvaraju SB, Kovac M, Dickson LM, et al. Molecular epidemiology and clinical presentation of human adenovirus infections in Kansas City children. *J Clin Virol* 2011;**51**:126–31.
549. Sili U, Leen AM, Vera JF, et al. Production of good manufacturing practice-grade cytotoxic T lymphocytes specific for Epstein-Barr virus, cytomegalovirus and adenovirus to prevent or treat viral infections post-allogeneic hematopoietic stem cell transplant. *Cytotherapy* 2012;**14**:7–11.
569. Stroparo E, Cruz CR, Debur Mdo C, et al. Adenovirus respiratory infection: significant increase in diagnosis using PCR comparing with antigen detection and culture methods. *Rev Inst Med Trop Sao Paulo* 2010;**52**:317–21.
579. Tang L, Wang L, Tan X, et al. Adenovirus serotype 7 associated with a severe lower respiratory tract disease outbreak in infants in Shaanxi Province, China. *Virol J* 2011;**8**:23.
580. Tate JE, Bunning ML, Lott L, et al. Outbreak of severe respiratory disease associated with emergent human adenovirus serotype 14 at a US air force training facility in 2007. *J Infect Dis* 2009;**199**:1419–26.
581. Tatrai E, Hartyanszky I Jr, Laszik A, et al. The role of viral infections in the development of dilated cardiomyopathy. *Pathol Oncol Res* 2011;**17**:229–35.
590. Treacy A, Carr MJ, Dunford L, et al. First report of sudden death due to myocarditis caused by adenovirus serotype 3. *J Clin Microbiol* 2010;**48**:642–5.

593. Trovato GM, Castro A, Tonzuso A, et al. Human obesity relationship with Ad36 adenovirus and insulin resistance. *Int J Obes (Lond)* 2009;**33**:1402–9.
594. Trovato GM, Martines GF, Trovato FM, et al. Adenovirus-36 seropositivity enhances effects of nutritional intervention on obesity, bright liver, and insulin resistance. *Dig Dis Sci* 2012;**57**:535–44.
596. U.S. Food and Drug Administration. March 16, 2011 Approval Letter—*Adenovirus type 4 and type 7 vaccine, live, oral. 2011.* Accessed October 10, 2012. Available at: http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm247511.htm.
609. van Maarseveen NM, Wessels E, de Brouwer CS, et al. Diagnosis of viral gastroenteritis by simultaneous detection of Adenovirus group F, Astrovirus, Rotavirus group A, Norovirus genogroups I and II, and Sapovirus in two internally controlled multiplex real-time PCR assays. *J Clin Virol* 2010;**49**:205–10.
616. Varma MC, Kushner YB, Ko DS, et al. Early onset adenovirus infection after simultaneous kidney-pancreas transplant. *Am J Transplant* 2011;**11**:623–7.
619. Venter M, Lassauniere R, Kresfelder TL, et al. Contribution of common and recently described respiratory viruses to annual hospitalizations in children in South Africa. *J Med Virol* 2011;**83**:1458–68.
620. Vento TJ, Prakash V, Murray CK, et al. Pneumonia in military trainees: a comparison study based on adenovirus serotype 14 infection. *J Infect Dis* 2011;**203**:1388–95.
631. Wang X, Yang K, Wei C, et al. Coinfection with EBV/CMV and other respiratory agents in children with suspected infectious mononucleosis. *Virol J* 2010;**7**:247.
632. Wang ZQ, Yu Y, Zhang XH, et al. Human adenovirus 36 decreases fatty acid oxidation and increases de novo lipogenesis in primary cultured human skeletal muscle cells by promoting Cidec/FSP27 expression. *Int J Obes (Lond)* 2010;**34**:1355–64.
636. Watcharananan SP, Kiertiburanakul S, Piyatuctsanawong W, et al. Cytomegalovirus, adenovirus, and polyomavirus co-infection among pediatric recipients of allogeneic stem cell transplantation: characteristics and outcome. *Pediatr Transplant* 2010;**14**:675–81.
646. Wilhelm CM, Hanna SL, Welch CA, et al. Viral gastroenteritis in Charleston, West Virginia, in 2007: from birth to 99 years of age. *Infect Control Hosp Epidemiol* 2010;**31**:816–21.
647. Williams KM, Agwu AL, Dabb AA, et al. A clinical algorithm identifies high risk pediatric oncology and bone marrow transplant patients likely to benefit from treatment of adenoviral infection. *J Pediatr Hematol Oncol* 2009;**31**:825–31.
655. Xie L, Yu XF, Sun Z, et al. Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. *J Clin Microbiol* 2012;**50**:1879–88.
663. Yokose N, Hirakawa T, Inokuchi K. Adenovirus-associated hemorrhagic cystitis in a patient with plasma cell myeloma treated with bortezomib. *Leuk Res* 2009;**33**:e106.
666. Yoon J, Jekle A, Najafi R, et al. Virucidal mechanism of action of NVC-422, a novel antimicrobial drug for the treatment of adenoviral conjunctivitis. *Antiviral Res* 2011;**92**:470–8.
671. Zandvliet ML, Falkenburg JH, van Liempt E, et al. Combined $CD8^+$ and $CD4^+$ adenovirus hexon-specific T cells associated with viral clearance after stem cell transplantation as treatment for adenovirus infection. *Haematologica* 2010;**95**:1943–51.

The full reference list for this chapter is available at expertconsult.com.

DNA VIRUSES

SUBSECTION 4 *HEPATOVIRIDAE*

CHAPTER 156

Hepatitis B and D Viruses

Maureen Jonas • Janis Stoll

HEPATITIS B VIRUS

Hepatitis B virus (HBV) infection occurs throughout the world, although marked geographic differences in rates of both acute and chronic infection exist. An estimated 360 to 400 million individuals (approximately 5% of the world's population) are infected.[68,87,119] In the United States the total number of persons with chronic hepatitis B is estimated at 2 million.[44,78] People chronically infected with HBV are at risk for developing cirrhosis and hepatocellular carcinoma (HCC). Worldwide mortality is estimated at 0.5 to 1.2 million deaths per year.[123] Most of those with chronic HBV infection acquired the virus through vertical transmission, which can now be prevented in the majority of cases by active and passive immunization.

BIOLOGY

HBV is the prototype of a family of DNA viruses called *hepadnaviruses* (hepatotropic DNA viruses). This group consists of enveloped, coated, double-stranded DNA viruses with similar structure and genome organization.

REFERENCES

1. Abd-Jamil J, Teoh BT, Hassan EH, et al. Molecular identification of adenovirus causing respiratory tract infection in pediatric patients at the University of Malaya Medical Center. *BMC Pediatr* 2010;**10**:46.
2. Aberle SW, Aberle JH, Steininger C, et al. Adenovirus DNA in serum of children hospitalized due to an acute respiratory adenovirus infection. *J Infect Dis* 2003;**187**:311–14.
3. Abzug MJ, Levin MJ. Neonatal adenovirus infection: four patients and review of the literature. *Pediatrics* 1991;**87**:890–6.
4. Adler H. Keratitis sub-epithelialis. *Centralbl Prakt Augenheilkd* 1889;**13**:289–95.
5. Adrian T, Wigand R, Richter J. Gastroenteritis in infants, associated with a genome type of adenovirus 31 and with combined rotavirus and adenovirus 31 infection. *Eur J Pediatr* 1987;**146**: 38–40.
6. Aguerre V, Castanos C, Pena HG, et al. Postinfectious bronchiolitis obliterans in children: clinical and pulmonary function findings. *Pediatr Pulmonol* 2010;**45**:1180–5.
7. Ahluwalia GS, Scott-Taylor TH, Klisko B, et al. Comparison of detection methods for adenovirus from enteric clinical specimens. *Diagn Microbiol Infect Dis* 1994;**18**:161–6.
8. Ahmad NM, Ahmad KM, Younus F. Severe adenovirus pneumonia (AVP) following infliximab infusion for the treatment of Crohn's disease. *J Infect* 2007;**54**:e29–32.
9. Aissi-Rothe L, Decot V, Venard V, et al. Rapid generation of full clinical-grade human antiadenovirus cytotoxic T cells for adoptive immunotherapy. *J Immunother* 2010;**33**:414–24.
10. Akiyoshi K, Suga T, Fukui K, et al. Outbreak of epidemic keratoconjunctivitis caused by adenovirus type 54 in a nursery school in Kobe City, Japan in 2008. *Jpn J Infect Dis* 2011;**64**:353–5.
11. Albano A, Salvaggio L, Morrone G. [Epidemic episode of pharyngoconjunctival fever caused by type 2 adenovirus]. *Boll Ist Sieroter Milan* 1961;**40**:580–4 [In Italian].
12. Alharbi S, Van Caeseele P, Consunji-Araneta R, et al. Epidemiology of severe pediatric adenovirus lower respiratory tract infections in Manitoba, Canada, 1991-2005. *BMC Infect Dis* 2012;**12**:55.
13. Allard A, Girones R, Juto P, et al. Polymerase chain reaction for detection of adenoviruses in stool samples. *J Clin Microbiol* 1990;**28**:2659–67.
14. Anderson GR, Carr RD, Flatley FJ, et al. An intrafamilial epidemic of pharyngoconjunctival fever. *AMA Arch Intern Med* 1957;**99**:70–3.
15. Andiman WA, Jacobson RI, Tucker G. Leukocyte-associated viremia with adenovirus type 2 in an infant with lower-respiratory-tract disease. *N Engl J Med* 1977;**297**:100–1.
16. Angella JJ, Connor JD. Neonatal infection caused by adenovirus type 7. *J Pediatr* 1968;**72**:474–8.
17. Arav-Boger R, Echavarria M, Forman M, et al. Clearance of adenoviral hepatitis with ribavirin therapy in a pediatric liver transplant recipient. *Pediatr Infect Dis J* 2000;**19**:1097–100.
18. Aronson B, Aronson S, Sobel G, et al. Pharyngoconjunctival fever; report of an epidemic outbreak. *AMA J Dis Child* 1956;**92**: 596–612.
19. Artenstein MS, Miller WS, Lamson TH, et al. Large-volume air sampling for meningococci and adenoviruses. *Am J Epidemiol* 1968;**87**:567–77.
20. Aterman K, Embil J, Easterbrook KB, et al. Liver necrosis, adenovirus type 2 and thymic dysplasia. *Virchows Arch A Pathol Pathol Anat* 1973;**360**:155–71.
21. Atkinson RL. Human adenovirus-36 and childhood obesity. *Int J Pediatr Obes* 2011;**6**(Suppl. 1):2–6.
22. Atkinson RL, Dhurandhar NV, Allison DB, et al. Human adenovirus-36 is associated with increased body weight and paradoxical reduction of serum lipids. *Int J Obes (Lond)* 2005;**29**: 281–6.
23. Atkinson RL, Lee I, Shin HJ, et al. Human adenovirus-36 antibody status is associated with obesity in children. *Int J Pediatr Obes* 2010;**5**:157–60.
24. Avivi I, Chakrabarti S, Milligan DW, et al. Incidence and outcome of adenovirus disease in transplant recipients after reduced-intensity conditioning with alemtuzumab. *Biol Blood Marrow Transplant* 2004;**10**:186–94.
25. Azizirad H, Polgar G, Borns PF, et al. Bronchiolitis obliterans. *Clin Pediatr (Phila)* 1975;**14**:572–7, 582–4.
26. Bahn CA. Swimming bath conjunctivitis. *New Orleans Med Surg J* 1927;**79**:586–90.
27. Balducci D, Tyrrell DA, Zaiman TE. Acute infections of the respiratory tract and the adenoviruses. *Lancet* 1956;**271**:1326–30.
28. Baldwin A, Kingman H, Darville M, et al. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. *Bone Marrow Transplant* 2000;**26**: 1333–8.
29. Barnard DL, Hart JC, Marmion VJ, et al. Outbreak in Bristol of conjunctivitis caused by adenovirus type 8, and its epidemiology and control. *BMJ* 1973;**2**:165–9.
30. Barr J, Kjellen L, Svedmyr A. Hospital outbreak of adenovirus type 3 infections: a clinical and virologic study on 38 patients partly involved in a nosocomial outbreak. *Acta Paediatr* 1958;**47**:365–82.
31. Barraza EM, Ludwig SL, Gaydos JC, et al. Reemergence of adenovirus type 4 acute respiratory disease in military trainees: report of an outbreak during a lapse in vaccination. *J Infect Dis* 1999;**179**:1531–3.
32. Barrero PR, Valinotto LE, Tittarelli E, et al. Molecular typing of adenoviruses in pediatric respiratory infections in Buenos Aires, Argentina (1999-2010). *J Clin Virol* 2012;**53**:145–50.
33. Béal R. Sur une forme particulaire de conjonctivité aigue avec follicules. *Ann Ocul* 1907;1–33.
34. Beby-Defaux A, Maille L, Chabot S, et al. Fatal adenovirus type 7b infection in a child with Smith-Lemli-Opitz syndrome. *J Med Virol* 2001;**65**:66–9.
35. Becroft DM. Histopathology of fatal adenovirus infection of the respiratory tract in young children. *J Clin Pathol* 1967;**20**:561–9.
36. Becroft DM. Bronchiolitis obliterans, bronchiectasis, and other sequelae of adenovirus type 21 infection in young children. *J Clin Pathol* 1971;**24**:72–82.
37. Beiter A, Lewis K, Pineda EF, et al. Unrecognized maternal peripartum pertussis with subsequent fatal neonatal pertussis. *Obstet Gynecol* 1993;**82**:691–3.
38. Bell JA. Clinical manifestations of pharyngoconjunctival fever. *Am J Ophthalmol* 1957;**43**:11–14.
39. Bell JA, Huebner RJ, Paffenbarger RS Jr, et al. Studies of adenoviruses (APC) in volunteers. *Am J Public Health Nations Health* 1956;**46**:1130–46.
40. Bell JA, Huebner RJ, Rosen L, et al. Illness and microbial experiences of nursery children at junior village. *Am J Hyg* 1961;**74**:267–92.
41. Bell JA, Rowe WP, Engler JI, et al. Pharyngoconjunctival fever; epidemiological studies of a recently recognized disease entity. *JAMA* 1955;**157**:1083–92.
42. Bell TM, Steyn JH. Viruses in lymph nodes of children with mesenteric adenitis and intussusception. *BMJ* 1962;**2**:700–2.
43. Bell TM, Turner G, Macdonald A, et al. Type-3 adenovirus infection. *Lancet* 1960;**2**:1327–9.
44. Bellanti JA, Artenstein MS, Brandt BL, et al. Immunoglobulin responses in serum and nasal secretions after natural adenovirus infections. *J Immunol* 1969;**103**:891–8.
45. Benyesh-Melnick M, Rosenberg HS. The isolation of adenovirus type 7 from a fatal case of pneumonia and disseminated disease. *J Pediatr* 1964;**64**:83–7.
46. Berge TO, England B, Mauris C, et al. Etiology of acute respiratory disease among service personnel at Fort Ord, California. *Am J Hyg* 1955;**62**:283–94.
47. Berrie E, Andrews L, Yezli S, et al. Hydrogen peroxide vapour (HPV) inactivation of adenovirus. *Lett Appl Microbiol* 2011;**52**: 555–8.
48. Bhisitkul DM, Todd KM, Listernick R. Adenovirus infection and childhood intussusception. *Am J Dis Child* 1992;**146**:1331–3.
49. Bil-Lula I, Ussowicz M, Rybka B, et al. PCR diagnostics and monitoring of adenoviral infections in hematopoietic stem cell transplantation recipients. *Arch Virol* 2010;**155**:2007–15.
50. Binks MJ, Cheng AC, Smith-Vaughan H, et al. Viral-bacterial co-infection in Australian indigenous children with acute otitis media. *BMC Infect Dis* 2011;**11**:161.
51. Blachar Y, Leibovitz E, Levin S. The interferon system in two patients with hemolytic uremic syndrome associated with adenovirus infection. *Acta Paediatr Scand* 1990;**79**:108–9.
52. Blacklock JWS. Section of pathology with section of epidemiology and preventive medicine: discussion on adenovirus infections. *Proc R Soc Med* 1957;**50**:753–5.

53. Blacklow NR, Hoggan MD, Kapikian AZ, et al. Epidemiology of adenovirus-associated virus infection in a nursery population. *Am J Epidemiol* 1968;**88**:368–78.
54. Blacklow NR, Hoggan MD, Sereno MS, et al. A seroepidemiologic study of adenovirus-associated virus infection in infants and children. *Am J Epidemiol* 1971;**94**:359–66.
55. Bloom HH, Forsyth BR, Johnson KM, et al. Patterns of adenovirus infections in Marine Corps personnel, part. I: a 42-month survey in recruit and nonrecruit populations. *Am J Hyg* 1964;**80**:328–42.
56. Bonard EC, Paccaud MF. Abdominal adenovirosis and appendicitis. *Helv Med Acta* 1966;**33**:164–71.
57. Boniuk M, Phillips CA, Friedman JB. Chronic adenovirus type 2 keratitis in man. *N Engl J Med* 1965;**273**:924–5.
58. Bowles NE, Ni J, Kearney DL, et al. Detection of viruses in myocardial tissues by polymerase chain reaction. evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol* 2003;**42**:466–72.
59. Boyer GS, Denny FW Jr, Ginsberg HS. Sequential cellular changes produced by types 5 and 7 adenoviruses in HeLa cells and in human amniotic cells; cytological studies aided by fluorescein-labelled antibody. *J Exp Med* 1959;**110**:827–44.
60. Boyer GS, Denny FW, Miller I, et al. Correlation of production of infectious virus with sequential stages of cytologic alteration in HeLa cells infected with adenoviruses types 5 and 7. *J Exp Med* 1960;**112**:865–82.
61. Boyer GS, Leuchtenberger C, Ginsberg HS. Cytological and cytochemical studies of HeLa cells infected with adenoviruses. *J Exp Med* 1957;**105**:195–216.
62. Brandt CD, Kim HW, Jeffries BC, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease, part II: variation in adenovirus infections by year and season. *Am J Epidemiol* 1972;**95**:218–27.
63. Brandt CD, Kim HW, Rodriguez WJ, et al. Adenoviruses and pediatric gastroenteritis. *J Infect Dis* 1985;**151**:437–43.
64. Brandt CD, Kim HW, Rodriguez WJ, et al. Simultaneous infections with different enteric and respiratory tract viruses. *J Clin Microbiol* 1986;**23**:177–9.
65. Brandt CD, Kim HW, Vargosko AJ, et al. Infections in 18,000 infants and children in a controlled study of respiratory tract disease, part I: adenovirus pathogenicity in relation to serologic type and illness syndrome. *Am J Epidemiol* 1969;**90**:484–500.
66. Briassoulis G, Papadopoulos G, Zavras N, et al. Cardiac troponin I in fulminant adenovirus myocarditis treated with a 24-hour infusion of high-dose intravenous immunoglobulin. *Pediatr Cardiol* 2000;**21**:391–4.
67. Broderick MP, Hansen CJ, Irvine M, et al. Adenovirus 36 seropositivity is strongly associated with race and gender, but not obesity, among US military personnel. *Int J Obes (Lond)* 2010;**34**: 302–8.
68. Brosch L, Tchandja J, Marconi V, et al. Adenovirus serotype 14 pneumonia at a basic military training site in the United States, spring 2007: a case series. *Mil Med* 2009;**174**:1295–9.
69. Brown M. Laboratory identification of adenoviruses associated with gastroenteritis in Canada from 1983 to 1986. *J Clin Microbiol* 1990;**28**:1525–9.
70. Brown M, Rossier E, Carpenter B, et al. Fatal adenovirus type 35 infection in newborns. *Pediatr Infect Dis J* 1991;**10**:955–6.
71. Brown RS, Nogrady MB, Spence L, et al. An outbreak of adenovirus type 7 infection in children in Montreal. *Can Med Assoc J* 1973;**108**:434–9.
72. Bruckova M, Grandien M, Pettersson CA, et al. Use of nasal and pharyngeal swabs for rapid detection of respiratory syncytial virus and adenovirus antigens by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1989;**27**:1867–9.
73. Bryant RE, Rhoades ER. Clinical features of adenoviral pneumonia in Air Force recruits. *Am Rev Respir Dis* 1967;**96**:717–23.
74. Buckwalter SP, Teo R, Espy MJ, et al. Real-time qualitative PCR for 57 human adenovirus types from multiple specimen sources. *J Clin Microbiol* 2012;**50**:766–71.
75. Buitenwerf J, Louwerens JJ, De Jong JC. A simple and rapid method for typing adenoviruses 40 and 41 without cultivation. *J Virol Methods* 1985;**10**:39–44.
76. Burns RP, Potter MH. Epidemic keratoconjunctivitis due to adenovirus type 19. *Am J Ophthalmol* 1976;**81**:27–9.
77. Caldwell GG, Lindsey NJ, Wulff H, et al. Epidemic of adenovirus type 7 acute conjunctivitis in swimmers. *Am J Epidemiol* 1974;**99**:230–4.
78. Callaway Z, Kim SH, Kim JY, et al. Adenovirus infection with serious pulmonary sequelae in Korean children. *Clin Respir J* 2011;**5**:92–8.
79. Cames B, Rahier J, Burtomboy G, et al. Acute adenovirus hepatitis in liver transplant recipients. *J Pediatr* 1992;**120**:33–7.
80. Cao Y, Kong F, Zhou F, et al. Genotyping of human adenoviruses using a PCR-based reverse line blot hybridisation assay. *Pathology* 2011;**43**:488–94.
81. Carballal G, Videla CM, Espinosa MA, et al. Multicentered study of viral acute lower respiratory infections in children from four cities of Argentina, 1993-1994. *J Med Virol* 2001;**64**:167–74.
82. Carmichael GP Jr, Zahradnik JM, Moyer GH, et al. Adenovirus hepatitis in an immunosuppressed adult patient. *Am J Clin Pathol* 1979;**71**:352–5.
83. Castro-Rodriguez JA, Daszenies C, Garcia M, et al. Adenovirus pneumonia in infants and factors for developing bronchiolitis obliterans: a 5-year follow-up. *Pediatr Pulmonol* 2006;**41**:947–53.
84. Centers for Disease Control and Prevention. Outbreak of adenovirus 14 respiratory illness—Prince of Wales Island, Alaska, 2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:6–10.
85. Cesario TC, Kreil RL, Caldwell GC, et al. Epidemiologic observations of virus infections in a closed population of young children. *Am J Epidemiol* 1971;**94**:457–66.
86. Cevenini R, Donati M, Landini MP, et al. Adenovirus associated with an oculo-genital infection. *Microbiologica* 1979;**2**:425–7.
87. Chaberny IE, Schnitzler P, Geiss HK, et al. An outbreak of epidemic keratoconjunctivtis in a pediatric unit due to adenovirus type 8. *Infect Control Hosp Epidemiol* 2003;**24**:514–19.
88. Chanock R, Chambon L, Chang W, et al. WHO respiratory disease survey in children: a serological study. *Bull World Health Organ* 1967;**37**:363–9.
89. Chanock RM, Mufson MA, Johnson KM. Comparative biology and ecology of human virus and *Mycoplasma* respiratory pathogens. *Prog Med Virol* 1965;**7**:208–52.
90. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. *Pediatrics* 1965;**36**:21–39.
91. Chany C, Lepine P, Lelong M, et al. Severe and fatal pneumonia in infants and young children associated with adenovirus infections. *Am J Hyg* 1958;**67**:367–78.
92. Chapple PJ. A survey of antibodies to adenovirus 8 and coxsackievirus A21 in human sera. *Bull World Health Organ* 1966;**34**:243–8.
93. Chatterjee NK, Samsonoff WA, Balasubramaniam N, et al. Isolation and characterization of adenovirus 5 from the brain of an infant with fatal cerebral edema. *Clin Infect Dis* 2000;**31**:830–3.
94. Chen HL, Chiou SS, Hsiao HP, et al. Respiratory adenoviral infections in children: a study of hospitalized cases in southern Taiwan in 2001-2002. *J Trop Pediatr* 2004;**50**:279–84.
95. Cherry JD. Unpublished observation.
96. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.
97. Cherry JD. Newer respiratory viruses: their role in respiratory illness of children. *Adv Pediatr* 1973;**20**:225–90.
98. Chiba S, Nakata S, Nakamura I, et al. Outbreak of infantile gastroenteritis due to type 40 adenovirus. *Lancet* 1983;**2**:954–7.
99. Chimerix. *A multicenter, open-label study of CMX001 treatment of serious diseases or conditions caused by dsDNA viruses: 2012*. Accessed October 10, 2012. Available at http://clinicaltrials.gov/ct2/show/NCT01143181.
100. Chin-Hsien T. Adenovirus pneumonia epidemic among Peking infants and preschool children in 1958. *Chin Med J* 1960;**80**: 331–9.
101. Chiou CC, Soong WJ, Hwang B, et al. Congenital adenoviral infection. *Pediatr Infect Dis J* 1994;**13**:664–5.
102. Chiu SS, Chan KH, Chen H, et al. Virologically confirmed population-based burden of hospitalization caused by respiratory syncytial virus, adenovirus, and parainfluenza viruses in children in Hong Kong. *Pediatr Infect Dis J* 2010;**29**:1088–92.
103. Chou SM, Roos R, Burrell R, et al. Subacute focal adenovirus encephalitis. *J Neuropathol Exp Neurol* 1973;**32**:34–50.
104. Christensen ML. Human viral gastroenteritis. *Clin Microbiol Rev* 1989;**2**:51–89.

105. Chun JK, Lee JH, Kim HS, et al. Establishing a surveillance network for severe lower respiratory tract infections in Korean infants and young children. *Eur J Clin Microbiol Infect Dis* 2009;**28**:841–4.
106. Claesson BA, Trollfors B, Brolin I, et al. Etiology of community-acquired pneumonia in children based on antibody responses to bacterial and viral antigens. *Pediatr Infect Dis J* 1989;**8**:856–62.
107. Clarke EJ Jr, Phillips IA, Alexander ER. Adenovirus infection in intussusception in children in Taiwan. *JAMA* 1969;**208**:1671–4.
108. Cockburn TA, Rowe WP, Huebner RJ. Relationship of the 1951 Greeley, Colorado, outbreak of conjunctivitis and pharyngitis to type 3 APC virus infection. *Am J Hyg* 1956;**63**:250–3.
109. Collier AM, Connor JD, Irving WR Jr. Generalized type 5 adenovirus infection associated with the pertussis syndrome. *J Pediatr* 1966;**69**:1073–8.
110. Commission on Acute Respiratory Diseases. Acute respiratory disease among new recruits. *Am J Public Health* 1946;**36**:439–50.
111. Connor JD. Evidence for an etiologic role of adenoviral infection in pertussis syndrome. *N Engl J Med* 1970;**283**:390–4.
112. Connor JD, Buchta RM, DeGenaro F Jr, et al. Specialty conference: potpourri of adenoviral infections. *West J Med* 1974;**120**: 55–61.
113. Cooney MK, Hall CE, Fox JP. The Seattle virus watch, part 3: evaluation of isolation methods and summary of infections detected by virus isolations. *Am J Epidemiol* 1972;**96**:286–305.
114. Cooper RJ, Hallett R, Tullo AB, et al. The epidemiology of adenovirus infections in Greater Manchester, UK 1982-96. *Epidemiol Infect* 2000;**125**:333–45.
115. Couch RB, Cate TR, Douglas RG Jr, et al. Effect of route of inoculation on experimental respiratory viral disease in volunteers and evidence for airborne transmission. *Bacteriol Rev* 1966;**30**:517–29.
116. Couch RB, Cate TR, Fleet WF, et al. Aerosol-induced adenoviral illness resembling the naturally occurring illness in military recruits. *Am Rev Respir Dis* 1965;**93**:529–35.
117. Couroucli XI, Welty SE, Ramsay PL, et al. Detection of microorganisms in the tracheal aspirates of preterm infants by polymerase chain reaction: association of adenovirus infection with bronchopulmonary dysplasia. *Pediatr Res* 2000;**47**:225–32.
118. Craighead JE. Cytopathology of adenoviruses types 7 and 12 in human respiratory epithelium. *Lab Invest* 1970;**22**:553–7.
119. Crandell RA, Dowdle WR, Holcomb TM, et al. A fatal illness associated with two viruses: an intermediate adenovirus type (21-16) and influenza A2. *J Pediatr* 1968;**72**:467–73.
120. Cukor G, Blacklow NR. Human viral gastroenteritis. *Microbiol Rev* 1984;**48**:157–79.
121. Cumming GR, Macpherson RI, Chernick V. Unilateral hyperlucent lung syndrome in children. *J Pediatr* 1971;**78**:250–60.
122. D'Ambrosio E, Del Grosso N, Chicca A, et al. Neutralizing antibodies against 33 human adenoviruses in normal children in Rome. *J Hyg (Lond)* 1982;**89**:155–61.
123. D'Angelo LJ, Hierholzer JC, Keenlyside RA, et al. Pharyngoconjunctival fever caused by adenovirus type 4: report of a swimming pool–related outbreak with recovery of virus from pool water. *J Infect Dis* 1979;**140**:42–7.
124. Dagan R, Schwartz RH, Insel RA, et al. Severe diffuse adenovirus 7a pneumonia in a child with combined immunodeficiency: possible therapeutic effect of human immune serum globulin containing specific neutralizing antibody. *Pediatr Infect Dis* 1984;**3**: 246–51.
125. Darougar S, Quinlan MP, Gibson JA, et al. Epidemic keratoconjunctivitis and chronic papillary conjunctivitis in London due to adenovirus type 19. *Br J Ophthalmol* 1977;**61**:76–85.
126. Dascomb HE, Hilleman MR. Clinical and laboratory studies in patients with respiratory disease caused by adenoviruses (RI-APC-ARD agents). *Am J Med* 1956;**21**:161–74.
127. Davidson SI. Epidemic kerato-conjunctivitis: report of an outbreak which resulted in ward cross-infection. *Br J Ophthalmol* 1964;**48**:573–80.
128. Davis BD, Dulbecco R, Eisen HN, et al. *Microbiology*. Hagerstown, Md.: Harper & Row; 1973. p. 1222–36.
129. Davis D, Henslee PJ, Markesbery WR. Fatal adenovirus meningoencephalitis in a bone marrow transplant patient. *Ann Neurol* 1988;**23**:385–9.
130. Dawson C, Darrell R. Infections due to adenovirus type 8 in the United States, part I: an outbreak of epidemic keratoconjunctivitis originating in a physician's office. *N Engl J Med* 1963;**268**: 1031–4.
131. Dawson C, Darrell R, Hanna L, et al. Infections due to adenovirus type 8 in the United States, part II: community-wide infection with adenovirus type 8. *N Engl J Med* 1963;**268**:1034–7.
132. Dawson CR, Hanna L, Togni B. Adenovirus type 8 infections in the United States, part IV: observations on the pathogenesis of lesions in severe eye disease. *Arch Ophthalmol* 1972;**87**:258–68.
133. Dawson CR, O'Day D, Vastine D. Letter: Adenovirus 19, a cause of epidemic keratoconjunctivitis, not acute hemorrhagic conjunctivitis. *N Engl J Med* 1975;**293**:45–6.
134. Day AS, McGregor DO, Henderson SJ, et al. Fatal adenoviral disease in siblings. *Pediatr Infect Dis J* 1998;**17**:83–5.
135. De Jong JC, Wermenbol AG, Verweij-Uijterwaal MW, et al. Adenoviruses from human immunodeficiency virus–infected individuals, including two strains that represent new candidate serotypes Ad50 and Ad51 of species B1 and D, respectively. *J Clin Microbiol* 1999;**37**:3940–5.
136. De Jong JC, Wigand R, Kidd AH, et al. Candidate adenoviruses 40 and 41: fastidious adenoviruses from human infant stool. *J Med Virol* 1983;**11**:215–31.
137. De Jong JC, Wigand R, Wadell G, et al. Adenovirus 37: identification and characterization of a medically important new adenovirus type of subgroup D. *J Med Virol* 1981;**7**:105–18.
138. de Mezerville MH, Tellier R, Richardson S, et al. Adenoviral infections in pediatric transplant recipients: a hospital-based study. *Pediatr Infect Dis J* 2006;**25**:815–18.
139. De Paulis M, Gilio AE, Ferraro AA, et al. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011;**87**:307–13.
140. de Silva LM, Colditz P, Wadell G. Adenovirus type 7 infections in children in New South Wales, Australia. *J Med Virol* 1989;**29**:28–32.
141. Denny FW Jr. Viruses newly isolated from the upper respiratory tract. *Pediatr Clin North Am* 1960;**7**:295–314.
142. Denny FW Jr, Ginsberg HS. Certain biological characteristics of adenovirus types 5, 6, 7 and 14. *J Immunol* 1961;**86**:567–74.
143. Dey RS, Ghosh S, Chawla-Sarkar M, et al. Circulation of a novel pattern of infections by enteric adenovirus serotype 41 among children below 5 years of age in Kolkata, India. *J Clin Microbiol* 2011;**49**:500–5.
144. Dey SK, Shimizu H, Phan TG, et al. Molecular epidemiology of adenovirus infection among infants and children with acute gastroenteritis in Dhaka City, Bangladesh. *Infect Genet Evol* 2009;**9**: 518–22.
145. Dhurandhar NV, Israel BA, Kolesar JM, et al. Increased adiposity in animals due to a human virus. *Int J Obes Relat Metab Disord* 2000;**24**:989–96.
146. Dhurandhar NV, Israel BA, Kolesar JM, et al. Transmissibility of adenovirus-induced adiposity in a chicken model. *Int J Obes Relat Metab Disord* 2001;**25**:990–6.
147. Dhurandhar NV, Whigham LD, Abbott DH, et al. Human adenovirus Ad-36 promotes weight gain in male rhesus and marmoset monkeys. *J Nutr* 2002;**132**:3155–60.
148. do Carmo Debur M, Raboni SM, Flizikowski FB, et al. Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal non-pandemic seasonal respiratory infections. *J Clin Pathol* 2010;**63**:930–4.
149. Dominguez O, Rojo P, de Las Heras S, et al. Clinical presentation and characteristics of pharyngeal adenovirus infections. *Pediatr Infect Dis J* 2005;**24**:733–4.
150. Douglas RG Jr, Alford RH, Cate TR, et al. The leukocyte response during viral respiratory illness in man. *Ann Intern Med* 1966;**64**:521–30.
151. Dropulic LK, Cohen JI. Update on new antivirals under development for the treatment of double-stranded DNA virus infections. *Clin Pharmacol Ther* 2010;**88**:610–19.
152. Dubberke ER, Tu B, Rivet DJ, et al. Acute meningoencephalitis caused by adenovirus serotype 26. *J Neurovirol* 2006;**12**:235–40.
153. Dudding BA, Bartelloni PJ, Scott RM, et al. Enteric immunization with live adenovirus type 21 vaccine, part I: tests for safety, infectivity, immunogenicity, and potency in volunteers. *Infect Immun* 1972;**5**:295–9.
154. Dudding BA, Top FH Jr, Scott RM, et al. An analysis of hospitalizations for acute respiratory disease in recruits immunized with

adenovirus type 4 and type 7 vaccines. *Am J Epidemiol* 1972;**95**:141–7.
155. Dudding BA, Top FH Jr, Winter PE, et al. Acute respiratory disease in military trainees: the adenovirus surveillance program, 1966-1971. *Am J Epidemiol* 1973;**97**:187–98.
156. Dudding BA, Wagner SC, Zeller JA, et al. Fatal pneumonia associated with adenovirus type 7 in three military trainees. *N Engl J Med* 1972;**286**:1289–92.
157. Duncan IBR. Adenovirus type 15 and human thyroid tissue-culture. *Lancet* 1960;**1**:829–30.
158. Durepaire N, Ranger-Rogez S, Denis F. Evaluation of rapid culture centrifugation method for adenovirus detection in stools. *Diagn Microbiol Infect Dis* 1996;**24**:25–9.
159. Duxbury AE, Mc CR, White J, et al. Epidemic adenovirus infection in a Victorian migrant centre presenting as pharyngo-conjunctival fever. *Med J Aust* 1960;**47**(2):413–17.
160. Ebner K, Rauch M, Preuner S, et al. Typing of human adenoviruses in specimens from immunosuppressed patients by PCR-fragment length analysis and real-time quantitative PCR. *J Clin Microbiol* 2006;**44**:2808–15.
161. Echavarria MS, Ray SC, Ambinder R, et al. PCR detection of adenovirus in a bone marrow transplant recipient: hemorrhagic cystitis as a presenting manifestation of disseminated disease. *J Clin Microbiol* 1999;**37**:686–9.
162. Edwards KM, Bennett SR, Garner WL, et al. Reye's syndrome associated with adenovirus infections in infants. *Am J Dis Child* 1985;**139**:343–6.
163. Edwards KM, Thompson J, Paolini J, et al. Adenovirus infections in young children. *Pediatrics* 1985;**76**:420–4.
164. Ellis AW, McKinnon GT, Lewis FA, et al. Adenovirus type 4 in Melbourne, 1969-1971. *Med J Aust* 1974;**1**:209–11.
165. Enders JF, Bell JA, Dingle JH, et al. Adenoviruses: group name proposed for new respiratory-tract viruses. *Science* 1956;**124**: 119–20.
166. Ephros M, Friedman BC, Elhasid R, et al. Incidence and clinical manifestations of adenoviral infection among children undergoing allogeneic stem cell transplantation. *Isr Med Assoc J* 2009;**11**:744–8.
167. Epshtein FG, Agarkova LG, Areizin RS, et al. [Acute respiratory diseases in children by the 7a type of adenovirus]. *Sov Med* 1962;**25**:81–5 [In Russian].
168. Erdman DD, Xu W, Gerber SI, et al. Molecular epidemiology of adenovirus type 7 in the United States, 1966-2000. *Emerg Infect Dis* 2002;**8**:269–77.
169. Ersoy Y, Otlu B, Turkcuoglu P, et al. Outbreak of adenovirus serotype 8 conjunctivitis in preterm infants in a neonatal intensive care unit. *J Hosp Infect* 2012;**80**:144–9.
170. Esposito DH, Gardner TJ, Schneider E, et al. Outbreak of pneumonia associated with emergent human adenovirus serotype 14—Southeast Alaska, 2008. *J Infect Dis* 2010;**202**:214–22.
171. Espy MJ, Hierholzer JC, Smith TF. The effect of centrifugation on the rapid detection of adenovirus in shell vials. *Am J Clin Pathol* 1987;**88**:358–60.
172. Evans AS. Acute respiratory disease in University of Wisconsin students. *N Engl J Med* 1957;**256**:377–84.
173. Evans AS. Adenovirus infections in children and young adults; with comments on vaccination. *N Engl J Med* 1958;**259**:464–8.
174. Evans AS. Latent adenovirus infections of the human respiratory tract. *Am J Hyg* 1958;**67**:256–66.
175. Evans AS, Dick EC. Acute pharyngitis and tonsillitis in University of Wisconsin Students. *JAMA* 1964;**190**:699–708.
176. Fabbiani M, Terrosi C, Martorelli B, et al. Epidemiological and clinical study of viral respiratory tract infections in children from Italy. *J Med Virol* 2009;**81**:750–6.
177. Faden H, Wynn RJ, Campagna L, et al. Outbreak of adenovirus type 30 in a neonatal intensive care unit. *J Pediatr* 2005;**146**: 523–7.
178. Faix DJ, Houng HS, Gaydos JC, et al. Evaluation of a rapid quantitative diagnostic test for adenovirus type 4. *Clin Infect Dis* 2004;**38**:391–7.
179. Fanourgiakis P, Georgala A, Vekemans M, et al. Intravesical instillation of cidofovir in the treatment of hemorrhagic cystitis caused by adenovirus type 11 in a bone marrow transplant recipient. *Clin Infect Dis* 2005;**40**:199–201.
180. Farkas E, Jancso A, Radnot M. Clinical and virologic studies on the first widespread outbreak of epidemic keratoconjunctivitis in Hungary. *Am J Ophthalmol* 1965;**60**:78–82.
181. Faulkner R, Van Rooyen CE. Adenoviruses types 3 and 5 isolated from the cerebrospinal fluid of children. *Can Med Assoc J* 1962;**87**:1123–5.
182. Feeney SA, Armstrong VJ, Mitchell SJ, et al. Development and clinical validation of multiplex TaqMan(R) assays for rapid diagnosis of viral gastroenteritis. *J Med Virol* 2011;**83**:1650–6.
183. Fenner F. Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. *Intervirology* 1976;**7**:1–115.
184. Ferdman RM, Ross L, Inderlied C, et al. Adenovirus viremia in human immunodeficiency virus-infected children. *Pediatr Infect Dis J* 1997;**16**:413–15.
185. Ferreira GF, Oliveira RA, Lucon M, et al. Hemorrhagic cystitis secondary to adenovirus or herpes simplex virus infection following renal transplantation: four case reports. *Transplant Proc* 2009;**41**:4416–19.
186. Feuchtinger T, Matthes-Martin S, Richard C, et al. Safe adoptive transfer of virus-specific T-cell immunity for the treatment of systemic adenovirus infection after allogeneic stem cell transplantation. *Br J Haematol* 2006;**134**:64–76.
187. Fiala M, Payne JE, Berne TV, et al. Role of adenovirus type 11 in hemorrhagic cystitis secondary to immunosuppression. *J Urol* 1974;**112**:595–7.
188. Finn A, Anday E, Talbot GH. An epidemic of adenovirus 7a infection in a neonatal nursery: course, morbidity, and management. *Infect Control Hosp Epidemiol* 1988;**9**:398–404.
189. Flomenberg P, Babbitt J, Drobyski WR, et al. Increasing incidence of adenovirus disease in bone marrow transplant recipients. *J Infect Dis* 1994;**169**:775–81.
190. Florescu DF, Islam MK, Mercer DF, et al. Adenovirus infections in pediatric small bowel transplant recipients. *Transplantation* 2010;**90**:198–204.
191. Florescu DF, Pergam SA, Neely MN, et al. Safety and efficacy of CMX001 as salvage therapy for severe adenovirus infections in immunocompromised patients. *Biol Blood Marrow Transplant* 2012;**18**:731–8.
192. Ford E, Nelson KE, Warren D. Epidemiology of epidemic keratoconjunctivitis. *Epidemiol Rev* 1987;**9**:244–61.
193. Forssell P, Halonen H, Stenstrom R, et al. An adenovirus epidemic due to types 1 and 2. *Ann Paediatr Fenn* 1962;**8**:35–44.
194. Forssell P, Lapinleimu K, Oker-Blom N, et al. Febrile pharyngitis and conjunctivitis; an epidemic associated with APC virus infection. *Ann Med Exp Biol Fenn* 1956;**34**:287–92.
195. Forsyth BR, Bloom HH, Johnson KM, et al. Patterns of adenovirus infections in Marine Corps personnel, part II: longitudinal study of successive advanced recruit training companies. *Am J Hyg* 1964;**80**:343–55.
196. Forsyth BR, Bloom HH, Johnson KM, et al. Etiology of primary atypical pneumonia in a military population. *JAMA* 1965;**191**: 92–6.
197. Fox JP, Brandt CD, Wassermann FE, et al. The virus watch program: a continuing surveillance of viral infections in metropolitan New York families, part VI: observations of adenovirus infections: virus excretion patterns, antibody response, efficiency of surveillance, patterns of infections, and relation to illness. *Am J Epidemiol* 1969;**89**:25–50.
198. Fox JP, Hall CE, Cooney MK. The Seattle Virus Watch, part VII: observations of adenovirus infections. *Am J Epidemiol* 1977; **105**:362–86.
199. Foy HM. Adenoviruses. In: Evans AS, editor. *Viral infections of humans: epidemiology and control*. 3rd ed. New York: Plenum; 1989. p. 77–94.
200. Foy HM, Cooney MK, Hatlen JB. Adenovirus type 3 epidemic associated with intermittent chlorination of a swimming pool. *Arch Environ Health* 1968;**17**:795–802.
201. Foy HM, Cooney MK, Maletzky AJ, et al. Incidence and etiology of pneumonia, croup and bronchiolitis in preschool children belonging to a prepaid medical care group over a four-year period. *Am J Epidemiol* 1973;**97**:80–92.
202. Foy HM, Cooney MK, McMahan R, et al. Viral and mycoplasmal pneumonia in a prepaid medical care group during an eight-year period. *Am J Epidemiol* 1973;**97**:93–102.
203. Frange P, Peffault de Latour R, Arnaud C, et al. Adenoviral infection presenting as an isolated central nervous system disease without detectable viremia in two children after stem cell transplantation. *J Clin Microbiol* 2011;**49**:2361–4.

204. Freiman I, Super M, Joosting AC, et al. An epidemic of adenovirus type-7 bronchopneumonia in Bantu children. *S Afr Med J* 1971;**45**:107–11.
205. Fuchs E. Keratitis punctata superficialis. *Wien Klin Wochenschr* 1889;**2**:837–41.
206. Fuji S, Kapp M, Grigoleit GU, et al. Adoptive immunotherapy with virus-specific T cells. *Best Pract Res Clin Haematol* 2011;**24**:413–19.
207. Fukumi H, Nishikawa F, Kokubu Y, et al. Isolation of adenovirus from an exanthematous infection resembling roseola infantum. *Jpn J Med Sci Biol* 1957;**10**:87–91.
208. Fukumi H, Nishikawa F, Mizutani H. An epidemic of adenovirus type 3 infections among school children in an elementary school in Tokyo. *Jpn J Med Sci Biol* 1958;**11**:129–40.
209. Fukumi H, Nishikawa F, Nakamura K, et al. Further studies of the cases associated with adenoviruses. *Jpn J Med Sci Biol* 1957;**10**:407–18.
210. Fukumi H, Nishikawa F, Nakamura K, et al. Studies on the adenovirus as an etiological agent of pharyngoconjunctival fever. *Jpn J Med Sci Biol* 1957;**10**:79–85.
211. Funahashi Y, Iwata S, Ito Y, et al. Multiplex real-time PCR assay for simultaneous quantification of BK polyomavirus, JC polyomavirus, and adenovirus DNA. *J Clin Microbiol* 2010;**48**:825–30.
212. Gabbert C, Donohue M, Arnold J, et al. Adenovirus 36 and obesity in children and adolescents. *Pediatrics* 2010;**126**:721–6.
213. Gabrielson MO, Joseph C, Hsiung GD. Encephalitis associated with adenovirus type 7 occurring in a family outbreak. *J Pediatr* 1966;**68**:142–4.
214. Garcia AG, Fonseca ME, de Bonis M, et al. Morphological and virological studies in six autopsies of children with adenovirus pneumonia. *Mem Inst Oswaldo Cruz* 1993;**88**:141–7.
215. Garcia-Garcia ML, Calvo C, Falcon A, et al. Role of emerging respiratory viruses in children with severe acute wheezing. *Pediatr Pulmonol* 2010;**45**:585–91.
216. Gardner PS, Knox EG, Court SD, et al. Virus infection and intussusception in childhood. *BMJ* 1962;**2**:697–700.
217. Gardner PS, McGregor CB, Dick K. Association between diarrhoea and adenovirus type 7. *BMJ* 1960;**1**:91–3.
218. Gavin PJ, Katz BZ. Intravenous ribavirin treatment for severe adenovirus disease in immunocompromised children. *Pediatrics* 2002;**110**:e9.
219. George RB, Ziskind MM, Rasch JR, et al. *Mycoplasma* and adenovirus pneumonias: comparison with other atypical pneumonias in a military population. *Ann Intern Med* 1966;**65**:931–42.
220. Gerdemann U, Vera JF, Rooney CM, et al. Generation of multivirus-specific T cells to prevent/treat viral infections after allogeneic hematopoietic stem cell transplant. *J Vis Exp* 2011:(51):pii: 2736.
221. Gerna G, Cattaneo E, Revello MG, et al. Grouping of human adenoviruses by early antigen reactivity. *J Infect Dis* 1982;**145**:678–82.
222. Ghanaiem H, Averbuch D, Koplewitz BZ, et al. An outbreak of adenovirus type 7 in a residential facility for severely disabled children. *Pediatr Infect Dis J* 2011;**30**:948–52.
223. Ginsberg HS. Characteristics of the new respiratory viruses (adenoviruses), part II: stability to temperature and pH alterations. *Proc Soc Exp Biol Med* 1956;**93**:48–52.
224. Ginsberg HS. Characteristics on the adenoviruses, part III: reproductive cycle of types 1 to 4. *J Exp Med* 1958;**107**:133–52.
225. Ginsberg HS. Adenoviruses. *Am J Clin Pathol* 1972;**57**:771–6.
226. Ginsberg HS, Gold E, Jordan WS Jr, et al. Relation of the new respiratory agents to acute respiratory diseases. *Am J Public Health Nations Health* 1955;**45**:915–22.
227. Ginsberg HS, Horswood RL, Chanock RM, et al. Role of early genes in pathogenesis of adenovirus pneumonia. *Proc Natl Acad Sci U S A* 1990;**87**:6191–5.
228. Ginsberg HS, Moldawer LL, Sehgal PB, et al. A mouse model for investigating the molecular pathogenesis of adenovirus pneumonia. *Proc Natl Acad Sci U S A* 1991;**88**:1651–5.
229. Ginsberg HS, Prince GA. The molecular basis of adenovirus pathogenesis. *Infect Agents Dis* 1994;**3**:1–8.
230. Glezen WP, Loda FA, Clyde WA Jr, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
231. Godman GC, Morgan C, Breitenfeld PM, et al. A correlative study by electron and light microscopy of the development of type 5 adenovirus, part II: light microscopy. *J Exp Med* 1960;**112**:383–402.
232. Gold R, Wilt JC, Adhikari PK, et al. Adenoviral pneumonia and its complications in infancy and childhood. *J Can Assoc Radiol* 1969;**20**:218–24.
233. Goncalves G, Gouveia E, Mesquita JR, et al. Outbreak of acute gastroenteritis caused by adenovirus type 41 in a kindergarten. *Epidemiol Infect* 2011;**139**:1672–5.
234. Gooch WM, 3rd, Mogabgab WJ. Simultaneous oral administration of live adenovirus types 4 and 7 vaccines: protection and lack of emergence of other types. *Arch Environ Health* 1972;**25**:388–94.
235. Goossens VJ, deJager SA, Grauls GE, et al. Lack of evidence for the role of human adenovirus-36 in obesity in a European cohort. *Obesity (Silver Spring)* 2011;**19**:220–1.
236. Gorczynska E, Turkiewicz D, Rybka K, et al. Incidence, clinical outcome, and management of virus-induced hemorrhagic cystitis in children and adolescents after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2005;**11**:797–804.
237. Grabow WO, Puttergill DL, Bosch A. Propagation of adenovirus types 40 and 41 in the PLC/PRF/5 primary liver carcinoma cell line. *J Virol Methods* 1992;**37**:201–7.
238. Grandien M, Pettersson CA, Svensson L, et al. Latex agglutination test for adenovirus diagnosis in diarrheal disease. *J Med Virol* 1987;**23**:311–16.
239. Gray GC. Adenovirus transmission—worthy of our attention. *J Infect Dis* 2006;**194**:871–3.
240. Gray GC, Goswami PR, Malasig MD, et al. Adult adenovirus infections: loss of orphaned vaccines precipitates military respiratory disease epidemics. For the Adenovirus Surveillance Group. *Clin Infect Dis* 2000;**31**:663–70.
241. Grayston JT, Lashof JC, Loosli CG, et al. Adenoviruses, part III: their etiological role in acute respiratory diseases in civilian adults. *J Infect Dis* 1958;**103**:93–101.
242. Grayston JT, Woolridge RL, Loosli CG, et al. Adenovirus infections in naval recruits. *J Infect Dis* 1957;**104**:61–70.
243. Green WR, Greaves WL, Frederick WR, et al. Renal infection due to adenovirus in a patient with human immunodeficiency virus infection. *Clin Infect Dis* 1994;**18**:989–91.
244. Gresser I, Kibrick S. Isolation of vaccinia virus and type 1 adenovirus from urine. *N Engl J Med* 1961;**265**:743–4.
245. Griffin JP, Greenberg BH. Live and inactivated adenovirus vaccines: clinical evaluation of efficacy in prevention of acute respiratory disease. *Arch Intern Med* 1970;**125**:981–6.
246. Griffin LD, Kearney D, Ni J, et al. Analysis of formalin-fixed and frozen myocardial autopsy samples for viral genome in childhood myocarditis and dilated cardiomyopathy with endocardial fibroelastosis using polymerase chain reaction (PCR). *Cardiovasc Pathol* 1995;**4**:3–11.
247. Gunson RN, Maclean AR, Shepherd SJ, et al. Simultaneous detection and quantitation of cytomegalovirus, Epstein-Barr virus, and adenovirus by use of real-time PCR and pooled standards. *J Clin Microbiol* 2009;**47**:765–70.
248. Gutekunst RR, Heggie AD. Viremia and viruria in adenovirus infections: detection in patients with rubella or rubelliform illness. *N Engl J Med* 1961;**264**:374–8.
249. Guyer B, O'Day DM, Hierholzer JC, et al. Epidemic keratoconjunctivitis: a community outbreak of mixed adenovirus type 8 and type 19 infection. *J Infect Dis* 1975;**132**:142–50.
250. Hale GA, Heslop HE, Krance RA, et al. Adenovirus infection after pediatric bone marrow transplantation. *Bone Marrow Transplant* 1999;**23**:277–82.
251. Hamlin A, Harford CG, Parker E, et al. Electron microscopy of HeLa cells infected with adenoviruses. *J Exp Med* 1956;**104**:443–54.
252. Hammitt LL, Kazungu S, Welch S, et al. Added value of an oropharyngeal swab in detection of viruses in children hospitalized with lower respiratory tract infection. *J Clin Microbiol* 2011;**49**:2318–20.
253. Hara J, Ishibashi T, Fujimoto F, et al. Adenovirus type 10 keratoconjunctivitis with increased intraocular pressure. *Am J Ophthalmol* 1980;**90**:481–4.
254. Hara M, Takao S, Fukuda S, et al. Rapid diagnosis of adenovirus respiratory tract infections using the chromatographic immunoassay test in the pediatric outpatient setting. *Pediatr Infect Dis J* 2010;**29**:267–9.
255. Harley D, Harrower B, Lyon M, et al. A primary school outbreak of pharyngoconjunctival fever caused by adenovirus type 3. *Commun Dis Intell* 2001;**25**:9–12.

256. Harnett GB, Newnham WA. Isolation of adenovirus type 19 from the male and female genital tracts. *Br J Vener Dis* 1981;**57**:55–7.
257. Harris DJ, Wulff H, Ray CG, et al. Viruses and disease, part 3: an outbreak of adenovirus type 7A in a children's home. *Am J Epidemiol* 1971;**93**:399–402.
258. Harrison HR, Howe P, Minnich L, et al. A cluster of adenovirus 19 infection with multiple clinical manifestations. *J Pediatr* 1979;**94**:917–19.
259. Hartwell WV, Love GJ, Eidenbock MP. Adenovirus in blood clots from cases of infectious hepatitis. *Science* 1966;**152**:1390.
260. Hatakeyama N, Suzuki N, Kudoh T, et al. Successful cidofovir treatment of adenovirus-associated hemorrhagic cystitis and renal dysfunction after allogenic bone marrow transplant. *Pediatr Infect Dis J* 2003;**22**:928–9.
261. Hatch MH, Siem RA. Viruses isolated from children with infectious hepatitis. *Am J Epidemiol* 1966;**84**:495–509.
262. Heemskerk B, Lankester AC, van Vreeswijk T, et al. Immune reconstitution and clearance of human adenovirus viremia in pediatric stem-cell recipients. *J Infect Dis* 2005;**191**:520–30.
263. Henderson YC, Liu TJ, Clayman GL. A simple and sensitive method for detecting adenovirus in serum and urine. *J Virol Methods* 1998;**71**:51–6.
264. Henson D, Mufson MA. Myocarditis and pneumonitis with type 21 adenovirus infection: association with fatal myocarditis and pneumonitis. *Am J Dis Child* 1971;**121**:334–6.
265. Herbert FA, Wilkinson D, Burchak E, et al. Adenovirus type 3 pneumonia causing lung damage in childhood. *Can Med Assoc J* 1977;**116**:274–6.
266. Herbert H. Superficial punctate keratitis associated with an encapsulated bacillus. *Ophthalmol Rev* 1901;**20**:339–45.
267. Herrmann EC Jr. Experiences in laboratory diagnosis of adenovirus infections in routine medical practice. *Mayo Clin Proc* 1968;**43**:635–44.
268. Herrmann JE, Perron-Henry DM, Blacklow NR. Antigen detection with monoclonal antibodies for the diagnosis of adenovirus gastroenteritis. *J Infect Dis* 1987;**155**:1167–71.
269. Heydari H, Mamishi S, Khotaei GT, et al. Fatal type 7 adenovirus associated with human bocavirus infection in a healthy child. *J Med Virol* 2011;**83**:1762–3.
270. Hierholzer JC. Further subgrouping of the human adenoviruses by differential hemagglutination. *J Infect Dis* 1973;**128**:541–50.
271. Hierholzer JC, Atuk NO, Gwaltney JM Jr. New human adenovirus isolated from a renal transplant recipient: description and characterization of candidate adenovirus type 34. *J Clin Microbiol* 1975;**1**:366–76.
272. Hierholzer JC, Bingham PG. Vero microcultures for adenovirus neutralization tests. *J Clin Microbiol* 1978;**7**:499–506.
273. Hierholzer JC, Gamble WC, Dowdle WR. Reference equine antisera to 33 human adenovirus types: homologous and heterologous titers. *J Clin Microbiol* 1975;**1**:65–74.
274. Hierholzer JC, Kemp MC, Gary GW Jr, et al. New human adenovirus associated with respiratory illness: candidate adenovirus type 39. *J Clin Microbiol* 1982;**16**:15–21.
275. Hierholzer JC, Pumarola A, Rodriguez-Torres A, et al. Occurrence of respiratory illness due to an atypical strain of adenovirus type 11 during a large outbreak in Spanish military recruits. *Am J Epidemiol* 1974;**99**:434–42.
276. Hierholzer JC, Stone YO, Broderson JR. Antigenic relationships among the 47 human adenoviruses determined in reference horse antisera. *Arch Virol* 1991;**121**:179–97.
277. Hierholzer JC, Torrence AE, Wright PF. Generalized viral illness caused by an intermediate strain of adenovirus (21/H21 + 35). *J Infect Dis* 1980;**141**:281–8.
278. Higgins RR, Beniprashad M, Cardona M, et al. Evaluation and verification of the Seeplex Diarrhea-V ACE assay for simultaneous detection of adenovirus, rotavirus, and norovirus genogroups I and II in clinical stool specimens. *J Clin Microbiol* 2011;**49**:3154–62.
279. Hijnen WA, Beerendonk EF, Medema GJ. Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res* 2006;**40**:3–22.
280. Hilleman MR. Epidemiology of adenovirus respiratory infections in military recruit populations. *Ann N Y Acad Sci* 1957;**67**: 262–72.
281. Hilleman MR, Tousimis AJ, Werner JH. Biophysical characterization of the RI (RI-67) viruses. *Proc Soc Exp Biol Med* 1955;**89**:587–93.
282. Hilleman MR, Werner JH. Recovery of new agent from patients with acute respiratory illness. *Proc Soc Exp Biol Med* 1954;**85**:183–8.
283. Hillenkamp J, Reinhard T, Ross RS, et al. The effects of cidofovir 1% with and without cyclosporin A 1% as a topical treatment of acute adenoviral keratoconjunctivitis: a controlled clinical pilot study. *Ophthalmology* 2002;**109**:845–50.
284. Hillis WD, Cooper MR, Bang FB. Adenovirus infections in West Bengal, part I: persistence of viruses in infants and young children. *Indian J Med Res* 1973;**61**:1–9.
285. Hoffman JA. Adenoviral disease in pediatric solid organ transplant recipients. *Pediatr Transplant* 2006;**10**:17–25.
286. Hofland CA, Eron LJ, Washecka RM. Hemorrhagic adenovirus cystitis after renal transplantation. *Transplant Proc* 2004;**36**: 3025–7.
287. Hogan MJ, Crawford JW. Epidemic keratoconjunctivitis (superficial punctate keratitis, keratitis subepithelialis, keratitis maculosa, keratitis nummularis): with a review of the literature and a report of 125 cases. *Am J Ophthalmol* 1942;**25**:1059–78.
288. Holmes WJ. Epidemic infectious conjunctivitis. *Hawaii Med J* 1941;**1**:11–12.
289. Holzel A, Parker L, Patterson WH, et al. Virus isolations from throats of children admitted to hospital with respiratory and other diseases, Manchester 1962-4. *BMJ* 1965;**1**:614–19.
290. Horne RW, Brenner S, Waterson AP, et al. The icosahedral form of an adenovirus. *J Mol Biol* 1959;**1**:84–6.
291. Horwitz MS. Adenoviridae and their replication. In: Fields BN, Knipe DM, Chanock RM, et al, editors. *Virology*. 2nd ed. New York: Raven Press; 1990. p. 1679–721.
292. Horwitz MS. Adenoviruses. In: Knipe DM, Howley PM, editors. *Virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2301–26.
293. Howard DS, Phillips IG, Reece DE, et al. Adenovirus infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 1999;**29**:1494–501.
294. Hsu HY, Kao CL, Huang LM, et al. Viral etiology of intussusception in Taiwanese childhood. *Pediatr Infect Dis J* 1998;**17**: 893–8.
295. Huebner RJ, Casey MJ, Chanock RM, et al. Tumors induced in hamsters by a strain of adenovirus type 3: sharing of tumor antigens and "neoantigens" with those produced by adenovirus type 7 tumors. *Proc Natl Acad Sci U S A* 1965;**54**:381–8.
296. Huebner RJ, Rowe WP, Chanock RM. Newly recognized respiratory tract viruses. *Annu Rev Microbiol* 1958;**12**:49–76.
297. Huebner RJ, Rowe WP, Ward TG, et al. Adenoidal-pharyngeal-conjunctival agents: a newly recognized group of common viruses of the respiratory system. *N Engl J Med* 1954;**251**:1077–86.
298. Huhulescu S, Kiss R, Brettlecker M, et al. Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. *Infection* 2009;**37**:103–8.
299. Hypia T. Detection of adenovirus in nasopharyngeal specimens by radioactive and nonradioactive DNA probes. *J Clin Microbiol* 1985;**21**:730–3.
300. Ieven M, Van Reempts P, Van Overmeire B, et al. A pseudoepidemic of adenoviruses in a neonatal care unit. *Diagn Microbiol Infect Dis* 1994;**18**:157–9.
301. Imre G, Korchmaros I, Geck P, et al. Antigenic specificity of inclusion bodies in epidemic keratoconjunctivitis. *Ophthalmologica* 1964;**148**:7–12.
302. Ishiko H, Aoki K. Spread of epidemic keratoconjunctivitis due to a novel serotype of human adenovirus in Japan. *J Clin Microbiol* 2009;**47**:2678–9.
303. Jackson GG, Muldoon RL. Viruses causing common respiratory infection in man, part IV: reoviruses and adenoviruses. *J Infect Dis* 1973;**128**:811–66.
304. Jaffe BF, Maassab HF. Sudden deafness associated with adenovirus infection. *N Engl J Med* 1967;**276**:1406–9.
305. James AG, Lang WR, Liang AY, et al. Adenovirus type 21 bronchopneumonia in infants and young children. *J Pediatr* 1979;**95**: 530–3.
306. Jansson E, Wager O. Adenovirus antibodies in patients with respiratory infection. *Ann Med Intern Fenn* 1961;**50**:221–7.
307. Jansson E, Wager O, Forssell P, et al. An exanthema subitum–like rash in patients with adenovirus infection. *Ann Paediatr Fenn* 1961;**7**:3–11.

308. Jansson E, Wager O, Forssell P, et al. Epidemic occurrence of adenovirus type 7 infection in Helsinki. *Ann Paediatr Fenn* 1962;**8**:24–34.
309. Jawetz E. The story of shipyard eye. *BMJ* 1959;**1**:873–6.
310. Jernigan JA, Lowry BS, Hayden FG, et al. Adenovirus type 8 epidemic keratoconjunctivitis in an eye clinic: risk factors and control. *J Infect Dis* 1993;**167**:307–13.
311. Jeulin H, Salmon A, Bordigoni P, et al. Comparison of in-house real-time quantitative PCR to the Adenovirus R-Gene kit for determination of adenovirus load in clinical samples. *J Clin Microbiol* 2010;**48**:3132–7.
312. Jeulin H, Salmon A, Bordigoni P, et al. Diagnostic value of quantitative PCR for adenovirus detection in stool samples as compared with antigen detection and cell culture in haematopoietic stem cell transplant recipients. *Clin Microbiol Infect* 2011;**17**:1674–80.
313. Jiang S, Gavrikova TA, Pereboev A, et al. Adenovirus infection results in alterations of insulin signaling and glucose homeostasis. *Am J Physiol Endocrinol Metab* 2010;**298**:E1295–1304.
314. Jin Y, Cheng WX, Yang XM, et al. Viral agents associated with acute gastroenteritis in children hospitalized with diarrhea in Lanzhou, China. *J Clin Virol* 2009;**44**:238–41.
315. Johansson ME, Uhnoo I, Kidd AH, et al. Direct identification of enteric adenovirus, a candidate new serotype, associated with infantile gastroenteritis. *J Clin Microbiol* 1980;**12**:95–100.
316. Joncas J, Moisan A, Avilanis V. Incidence of adenovirus infection: a family study. *Can Med Assoc J* 1962;**87**:52–8.
317. Jones BR. Sporadic ocular disease associated with adenovirus infection in London. *Proc R Soc Med* 1957;**50**:758–60.
318. Jordan WS, Badger GF, Curtiss C, et al. A study of illness in a group of Cleveland families, part X: the occurrence of adenovirus infections. *Am J Hyg* 1956;**64**:336–48.
319. Kaji M, Kamiya S, Tatewaki E. An epidemic of pharyngoconjunctival fever in Moji, Kyushu. *Kyushu J Med Sci* 1961;**12**:241–9.
320. Kaji M, Kimura M, Kamiya S. An epidemic of pharyngoconjunctival fever among school children in an elementary school in Fukuoka prefecture. *Kyushu J Med Sci* 1960;**12**:1–8.
321. Kajon AE, Dickson LM, Fisher BT, et al. Fatal disseminated adenovirus infection in a young adult with systemic lupus erythematosus. *J Clin Virol* 2011;**50**:80–3.
322. Kajon AE, Murtagh P, Garcia Franco S, et al. A new genome type of adenovirus 3 associated with severe lower acute respiratory infection in children. *J Med Virol* 1990;**30**:73–6.
323. Kajon AE, Wadell G. Molecular epidemiology of adenoviruses associated with acute lower respiratory disease of children in Buenos Aires, Argentina (1984-1988). *J Med Virol* 1992;**36**:292–7.
324. Kalu SU, Loeffelholz M, Beck E, et al. Persistence of adenovirus nucleic acids in nasopharyngeal secretions: a diagnostic conundrum. *Pediatr Infect Dis J* 2010;**29**:746–50.
325. Kampmann B, Cubitt D, Walls T, et al. Improved outcome for children with disseminated adenoviral infection following allogeneic stem cell transplantation. *Br J Haematol* 2005;**130**:595–603.
326. Kapelushnik J, Or R, Delukina M, et al. Intravenous ribavirin therapy for adenovirus gastroenteritis after bone marrow transplantation. *J Pediatr Gastroenterol Nutr* 1995;**21**:110–12.
327. Kasel JA, Banks PA, Wigand R, et al. An immunologic classification of heterotypic antibody responses to adenoviruses in man. *Proc Soc Exp Biol Med* 1965;**119**:1162–5.
328. Kasel JA, Evans HE, Spickard A, et al. Conjunctivitis and enteric infection with adenovirus types 26 and 27: responses to primary, secondary and reciprocal cross-challenges. *Am J Hyg* 1963;**77**:265–82.
329. Kasova V, Bruckova M, Kotelensky F, et al. Isolation of adenovirus type 29 from an outbreak of epidemic keratoconjunctivitis. *Acta Virol* 1977;**21**:173.
330. Kawana R, Kaneko M, Matsumoto I, et al. An outbreak of pharyngoconjunctival fever due to adenovirus type 3. *Jpn J Microbiol* 1966;**10**:149–57.
331. Kawasaki Y, Hosoya M, Katayose M, et al. Correlation between serum interleukin 6 and C-reactive protein concentrations in patients with adenoviral respiratory infection. *Pediatr Infect Dis J* 2002;**21**:370–4.
332. Keenlyside RA, Hierholzer JC, D'Angelo LJ. Keratoconjunctivitis associated with adenovirus type 37: an extended outbreak in an ophthalmologist's office. *J Infect Dis* 1983;**147**:191–8.
333. Keller EW, Rubin RH, Black PH, et al. Isolation of adenovirus type 34 from a renal transplant recipient with interstitial pneumonia. *Transplantation* 1977;**23**:188–91.
334. Kelsey DS. Adenovirus meningoencephalitis. *Pediatrics* 1978;**61**:291–3.
335. Kemp MC, Hierholzer JC, Cabradilla CP, et al. The changing etiology of epidemic keratoconjunctivitis: antigenic and restriction enzyme analyses of adenovirus types 19 and 37 isolated over a 10-year period. *J Infect Dis* 1983;**148**:24–33.
336. Kendall EJ, Cook GT, Stone DM. Acute respiratory infections in children: isolation of Coxsackie B virus and adenovirus during a survey in a general practice. *BMJ* 1960;**2**:1180–4.
337. Kendall EJ, Riddle RW, Tuck HA, et al. Pharyngo-conjunctival fever; school outbreaks in England during the summer of 1955 associated with adenovirus types 3, 7, and 14. *BMJ* 1957;**2**:131–6.
338. Khor CS, Sam IC, Hooi PS, et al. Epidemiology and seasonality of respiratory viral infections in hospitalized children in Kuala Lumpur, Malaysia: a retrospective study of 27 years. *BMC Pediatr* 2012;**12**:32.
339. Kidd AH, Cosgrove BP, Brown RA, et al. Faecal adenoviruses from Glasgow babies: studies on culture and identity. *J Hyg (Lond)* 1982;**88**:463–74.
340. Kidd AH, Jonsson M, Garwicz D, et al. Rapid subgenus identification of human adenovirus isolates by a general PCR. *J Clin Microbiol* 1996;**34**:622–7.
341. Kim KS, Gohd RS. Fatal pneumonia caused by adenovirus type 35. *Am J Dis Child* 1981;**135**:473–5.
342. Kim YJ, Schmidt NJ, Mirkovic RR. Isolation of an intermediate type of adenovirus from a child with fulminant hepatitis. *J Infect Dis* 1985;**152**:844.
343. Kimura SJ, Hanna L, Nicholas A, et al. Sporadic cases of pharyngoconjunctival fever in Northern California, 1955-1956. *Am J Ophthalmol* 1957;**43**:14–16.
344. Kinney JS, Hierholzer JC, Thibeault DW. Neonatal pulmonary insufficiency caused by adenovirus infection successfully treated with extracorporeal membrane oxygenation. *J Pediatr* 1994;**125**:110–12.
345. Kirkpatrick H. An epidemic of macular keratitis. *Br J Ophthalmol* 1920;**4**:16–20.
346. Kjellen L, Sterner G, Svedmyr A. On the occurrence of adenoviruses in Sweden. *Acta Paediatr* 1957;**46**:164–76.
347. Kjellen L, Zetterberg B, Svedmyr A. An epidemic among Swedish children caused by adenovirus type 3. *Acta Paediatr* 1957;**46**:561–8.
348. Kjer P, Mordhorst CH. Studies on an epidemic of keratoconjunctivitis caused by adenovirus type 8, part II: clinical and epidemiological aspects. *Acta Ophthalmol (Copenh)* 1961;**39**:984–92.
349. Knight V, Kasel JA. Adenoviruses. In: Knight V, editor. *Viral and mycoplasmal infections of the respiratory tract*. Philadelphia: Lea & Febiger; 1973. p. 65–86.
350. Kojaoghlanian T, Flomenberg P, Horwitz MS. The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 2003;**13**:155–71.
351. Kolavic-Gray SA, Binn LN, Sanchez JL, et al. Large epidemic of adenovirus type 4 infection among military trainees: epidemiological, clinical, and laboratory studies. *Clin Infect Dis* 2002;**35**:808–18.
352. Komiya T, Goto N, Takeda A, et al. A case of acute rejection with adenovirus infection after ABO-incompatible kidney transplantation. *Clin Transplant* 2009;**23**(Suppl. 20):27–30.
353. Koneru B, Jaffe R, Esquivel CO, et al. Adenoviral infections in pediatric liver transplant recipients. *JAMA* 1987;**258**:489–92.
354. Kotloff KL, Losonsky GA, Morris JG Jr, et al. Enteric adenovirus infection and childhood diarrhea: an epidemiologic study in three clinical settings. *Pediatrics* 1989;**84**:219–25.
355. Kozlowski T, Nickeleit V, Andreoni K. Donor-transmitted adenovirus infection causing kidney allograft nephritis and graft loss. *Transpl Infect Dis* 2011;**13**:168–73.
356. Krajden M, Brown M, Petrasek A, et al. Clinical features of adenovirus enteritis: a review of 127 cases. *Pediatr Infect Dis J* 1990;**9**:636–41.
357. Krilov LR. Adenovirus infections in the immunocompromised host. *Pediatr Infect Dis J* 2005;**24**:555–6.
358. Kuei-Fang J, Ying T, Yu-Ch'un L, et al. The role of adenovirus in the etiology of infantile pneumonia and pneumonia complicating measles. *Chin Med J* 1962;**81**:141–6.

359. Kuypers J, Wright N, Ferrenberg J, et al. Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children. *J Clin Microbiol* 2006;**44**:2382–8.
360. Ladisch S, Lovejoy FH, Hierholzer JC, et al. Extrapulmonary manifestations of adenovirus type 7 pneumonia simulating Reye syndrome and the possible role of an adenovirus toxin. *J Pediatr* 1979;**95**:348–55.
361. Lahdeaho ML, Parkkonen P, Reunala T, et al. Antibodies to E1b protein-derived peptides of enteric adenovirus type 40 are associated with celiac disease and dermatitis herpetiformis. *Clin Immunol Immunopathol* 1993;**69**:300–5.
362. Lang WR, Howden CW, Laws J, et al. Bronchopneumonia with serious sequelae in children with evidence of adenovirus type 21 infection. *BMJ* 1969;**1**:73–9.
363. Lange W. Über eine eigentumliche Erkrankung der kleinen Bronchien und Bronchiolen. *Dtsch Arch Klin Med* 1901;**70**:342.
364. Lankester AC, Heemskerk B, Claas EC, et al. Effect of ribavirin on the plasma viral DNA load in patients with disseminating adenovirus infection. *Clin Infect Dis* 2004;**38**:1521–5.
365. Lankester AC, van Tol MJ, Claas EC, et al. Quantification of adenovirus DNA in plasma for management of infection in stem cell graft recipients. *Clin Infect Dis* 2002;**34**:864–7.
366. Larranaga C, Kajon A, Villagra E, et al. Adenovirus surveillance on children hospitalized for acute lower respiratory infections in Chile (1988-1996). *J Med Virol* 2000;**60**:342–6.
367. Lassauniere R, Kresfelder T, Venter M. A novel multiplex real-time RT-PCR assay with FRET hybridization probes for the detection and quantitation of 13 respiratory viruses. *J Virol Methods* 2010;**165**:254–60.
368. Laverty CR, Russell P, Black J, et al. Adenovirus infection of the cervix. *Acta Cytol* 1977;**21**:114–17.
369. Lee HJ, Pyo JW, Choi EH, et al. Isolation of adenovirus type 7 from the urine of children with acute hemorrhagic cystitis. *Pediatr Infect Dis J* 1996;**15**:633–4.
370. Lee JH, Chun JK, Kim DS, et al. Identification of adenovirus, influenza virus, parainfluenza virus, and respiratory syncytial virus by two kinds of multiplex polymerase chain reaction (PCR) and a shell vial culture in pediatric patients with viral pneumonia. *Yonsei Med J* 2010;**51**:761–7.
371. Lee SG, Hung PP. Vaccines for control of respiratory disease caused by adenoviruses. *Rev Med Virol* 1993;**3**:209–16.
372. Lehrich JR, Kasel JA, Rossen RD. Immunoglobulin classes of neutralizing antibody formed after human inoculation with soluble adenoviral antigens. *J Immunol* 1966;**97**:654–62.
373. Lennette EH, Schmidt NJE. *Diagnostic procedures for viral and rickettsial infections*. 4th ed. New York: American Public Health Association; 1969. p. 205–25.
374. Leruez-Ville M, Minard V, Lacaille F, et al. Real-time blood plasma polymerase chain reaction for management of disseminated adenovirus infection. *Clin Infect Dis* 2004;**38**:45–52.
375. Levandowski RA, Rubenis M. Nosocomial conjunctivitis caused by adenovirus type 4. *J Infect Dis* 1981;**143**:28–31.
376. Levin S, Dietrich J, Guillory J. Fatal nonbacterial pneumonia associated with adenovirus type 4: occurrence in an adult. *JAMA* 1967;**201**:155–7.
377. Lew JF, Glass RI, Petric M, et al. Six-year retrospective surveillance of gastroenteritis viruses identified at ten electron microscopy centers in the United States and Canada. *Pediatr Infect Dis J* 1990;**9**:709–14.
378. Lewis K, Saubolle MA, Tenover FC, et al. Pertussis caused by an erythromycin-resistant strain of *Bordetella pertussis*. *Pediatr Infect Dis J* 1995;**14**:388–91.
379. Lewis PF, Schmidt MA, Lu X, et al. A community-based outbreak of severe respiratory illness caused by human adenovirus serotype 14. *J Infect Dis* 2009;**199**:1427–34.
380. Li QG, Wadell G. Analysis of 15 different genome types of adenovirus type 7 isolated on five continents. *J Virol* 1986;**60**:331–5.
381. Liles WC, Cushing H, Holt S, et al. Severe adenoviral nephritis following bone marrow transplantation: successful treatment with intravenous ribavirin. *Bone Marrow Transplant* 1993;**12**:409–12.
382. Lim KH, Yin-Murphy M. An epidemic of conjunctivitis in Singapore in 1970. *Singapore Med J* 1971;**12**:247–9.
383. Lin B, Vora GJ, Thach D, et al. Use of oligonucleotide microarrays for rapid detection and serotyping of acute respiratory disease–associated adenoviruses. *J Clin Microbiol* 2004;**42**:3232–9.
384. Lindblom A, Bhadri V, Soderhall S, et al. Respiratory viruses, a common microbiological finding in neutropenic children with fever. *J Clin Virol* 2010;**47**:234–7.
385. Lion T, Baumgartinger R, Watzinger F, et al. Molecular monitoring of adenovirus in peripheral blood after allogeneic bone marrow transplantation permits early diagnosis of disseminated disease. *Blood* 2003;**102**:1114–20.
386. Liu EB, Wadford DA, Seto J, et al. Computational and serologic analysis of novel and known viruses in species human adenovirus D in which serology and genomics do not correlate. *PLoS One* 2012;**7**:e33212.
387. Ljungman P. Treatment of adenovirus infections in the immunocompromised host. *Eur J Clin Microbiol Infect Dis* 2004;**23**:583–8.
388. Ljungman P, Ribaud P, Eyrich M, et al. Cidofovir for adenovirus infections after allogeneic hematopoietic stem cell transplantation: a survey by the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2003;**31**:481–6.
389. Loda FA, Clyde WA Jr, Glezen WP, et al. Studies on the role of viruses, bacteria, and M. pneumoniae as causes of lower respiratory tract infections in children. *J Pediatr* 1968;**72**:161–76.
390. Loda FA, Glezen WP, Clyde WA Jr. Respiratory disease in group day care. *Pediatrics* 1972;**49**:428–37.
391. Lonberg-Holm K, Philipson L. Early events of virus-cell interaction in an adenovirus system. *J Virol* 1969;**4**:323–38.
392. Londergan TA, Walzak MP. Hemorrhagic cystitis due to adenovirus infection following bone marrow transplantation. *J Urol* 1994;**151**:1013–14.
393. Lord A, Sutton RN, Corsellis JA. Recovery of adenovirus type 7 from human brain cell cultures. *J Neurol Neurosurg Psychiatry* 1975;**38**:710–12.
394. Lozinski GM, Davis GG, Krous HF, et al. Adenovirus myocarditis: retrospective diagnosis by gene amplification from formalin-fixed, paraffin-embedded tissues. *Hum Pathol* 1994;**25**:831–4.
395. Macek V, Sorli J, Kopriva S, et al. Persistent adenoviral infection and chronic airway obstruction in children. *Am J Respir Crit Care Med* 1994;**150**:7–10.
396. Macpherson RI, Cumming GR, Chernick V. Unilateral hyperlucent lung: a complication of viral pneumonia. *J Can Assoc Radiol* 1969;**20**:225–31.
397. Mahafzah AM, Landry ML. Evaluation of immunofluorescent reagents, centrifugation, and conventional cultures for the diagnosis of adenovirus infection. *Diagn Microbiol Infect Dis* 1989;**12**:407–11.
398. Maisel JC, Pierce WE, Crawford YE, et al. Virus pneumonia and adenovirus infection: a reappraisal. *Am J Hyg* 1962;**75**:56–68.
399. Maletzky AJ, Cooney MK, Luce R, et al. Epidemiology of viral and mycoplasmal agents associated with childhood lower respiratory illness in a civilian population. *J Pediatr* 1971;**78**:407–14.
400. Manalo D, Mufson MA, Zollar LM, et al. Adenovirus infection in acute hemorrhagic cystitis: a study in 25 children. *Am J Dis Child* 1971;**121**:281–5.
401. Maranhao AG, Soares CC, Albuquerque MC, et al. Molecular epidemiology of adenovirus conjunctivitis in Rio de Janeiro, Brazil, between 2004 and 2007. *Rev Inst Med Trop Sao Paulo* 2009;**51**:227–9.
402. Marinheiro JC, Sanalios RB, Santos DC, et al. Duplex-PCR assay for the detection of adenovirus and respiratory syncytial virus in nasopharyngeal samples. *Mem Inst Oswaldo Cruz* 2009;**104**:118–20.
403. Martin AB, Webber S, Fricker FJ, et al. Acute myocarditis: rapid diagnosis by PCR in children. *Circulation* 1994;**90**:330–9.
404. Martone WJ, Hierholzer JC, Keenlyside RA, et al. An outbreak of adenovirus type 3 disease at a private recreation center swimming pool. *Am J Epidemiol* 1980;**111**:229–37.
405. Matsubara T, Inoue T, Tashiro N, et al. Activation of peripheral blood $CD8^+$ T cells in adenovirus infection. *Pediatr Infect Dis J* 2000;**19**:766–8.
406. Matsuoka T, Naito T, Kubota Y, et al. Disseminated adenovirus (type 19) infection in a neonate: rapid detection of the infection by immunofluorescence. *Acta Paediatr Scand* 1990;**79**:568–71.
407. Mattoo S, Cherry JD. Molecular pathogenesis, epidemiology, and clinical manifestations of respiratory infections due to *Bordetella*

pertussis and other *Bordetella* subspecies. *Clin Microbiol Rev* 2005;**18**:326–82.
408. Mayeur N, Srairi M, Tetu L, et al. Lethal hemorrhagic alveolitis after adenovirus pneumonia in a lung transplant recipient. *Heart Lung* 2012;**41**:401–3.
409. McCarthy AJ, Bergin M, De Silva LM, et al. Intravenous ribavirin therapy for disseminated adenovirus infection. *Pediatr Infect Dis J* 1995;**14**:1003–4.
410. McCarthy T, Lebeck MG, Capuano AW, et al. Molecular typing of clinical adenovirus specimens by an algorithm which permits detection of adenovirus coinfections and intermediate adenovirus strains. *J Clin Virol* 2009;**46**:80–4.
411. McCormick DP, Wenzel RP, Davies JA, et al. Nasal secretion protein responses in patients with wild-type adenovirus disease. *Infect Immun* 1972;**6**:282–8.
412. McLaughlin GE, Delis S, Kashimawo L, et al. Adenovirus infection in pediatric liver and intestinal transplant recipients: utility of DNA detection by PCR. *Am J Transplant* 2003;**3**:224–8.
413. McMillan NS, Martin SA, Sobsey MD. Outbreak of pharyngoconjunctival fever at a summer camp: North Carolina, 1991. *MMWR Morb Mortal Wkly Rep* 1992;**41**:342–3.
414. McNamara MJ, Pierce WE, Crawford YE, et al. Patterns of adenovirus infection in the respiratory diseases of naval recruits: a longitudinal study of two companies of naval recruits. *Am Rev Respir Dis* 1962;**86**:485–97.
415. Melendez CP, Florentino MM, Martinez IL, et al. Outbreak of epidemic keratoconjunctivitis caused by adenovirus in medical residents. *Mol Vis* 2009;**15**:557–62.
416. Melnick JL. Taxonomy of viruses, 1976. *Prog Med Virol* 1976;**22**:211–21.
417. Merchant RK, Rowe WP, Kasel JA, et al. Pharyngoconjunctival fever due to type 1 adenovirus; report of three cases. *N Engl J Med* 1958;**258**:131–3.
418. Meurman O, Ruuskanen O, Sarkkinen H. Immunoassay diagnosis of adenovirus infections in children. *J Clin Microbiol* 1983;**18**: 1190–5.
419. Meyer K, Girgis N, McGravey V. Adenovirus associated with congenital pleural effusion. *J Pediatr* 1985;**107**:433–5.
420. Michaels MG, Green M, Wald ER, et al. Adenovirus infection in pediatric liver transplant recipients. *J Infect Dis* 1992;**165**:170–4.
421. Mikol J, Felten-Papaiconomou A, Ferchal F, et al. Inclusion-body myositis: clinicopathological studies and isolation of an adenovirus type 2 from muscle biopsy specimen. *Ann Neurol* 1982;**11**: 576–81.
422. Miller DG, Gabrielson MO, Horstmann DM. Clinical virology and viral surveillance in a pediatric group practice: the use of double-seeded tissue culture tubes for primary virus isolation. *Am J Epidemiol* 1968;**88**:245–56.
423. Mistchenko AS, Diez RA, Mariani AL, et al. Cytokines in adenoviral disease in children: association of interleukin-6, interleukin-8, and tumor necrosis factor alpha levels with clinical outcome. *J Pediatr* 1994;**124**:714–20.
424. Mistchenko AS, Huberman KH, Gomez JA, et al. Epidemiology of enteric adenovirus infection in prospectively monitored Argentine families. *Epidemiol Infect* 1992;**109**:539–46.
425. Mistchenko AS, Maffey AF, Casal G, et al. Adenoviral pericarditis: high levels of interleukin 6 in pericardial fluid. *Pediatr Infect Dis J* 1995;**14**:1007–9.
426. Mistchenko AS, Robaldo JF, Rosman FC, et al. Fatal adenovirus infection associated with new genome type. *J Med Virol* 1998;**54**:233–6.
427. Mitchell LS, Taylor B, Reimels W, et al. Adenovirus 7a: a community-acquired outbreak in a children's hospital. *Pediatr Infect Dis J* 2000;**19**:996–1000.
428. Mitsui Y, Hanna L, Hanabusa J, et al. Association of adenovirus type 8 with epidemic keratoconjunctivitis. *AMA Arch Ophthalmol* 1959;**61**:891–8.
429. Moffet HL, Siegel AC, Doyle HK. Nonstreptococcal pharyngitis. *J Pediatr* 1968;**73**:51–60.
430. Mordhorst CH, Kjer P. Studies on an epidemic of keratoconjunctivitis caused by adenovirus type 8, part I: virus isolation in human amniotic cells and serological observations. *Acta Ophthalmol (Copenh)* 1961;**39**:974–83.
431. Mori Y, Miyamoto T, Kato K, et al. Different risk factors related to adenovirus- or BK virus-associated hemorrhagic cystitis following allogeneic stem cell transplantation. *Biol Blood Marrow Transplant* 2012;**18**:458–65.
432. Morris CA, Flewett TH, Bryden AS, et al. Epidemic viral enteritis in a long-stay children's ward. *Lancet* 1975;**1**:4–5.
433. Morris DJ, Cooper RJ, Barr T, et al. Polymerase chain reaction for rapid diagnosis of respiratory adenovirus infection. *J Infect* 1996;**32**:113–17.
434. Mueller RE, Muldoon RL, Jackson GG. Communicability of enteric live adenovirus type 4 vaccine in families. *J Infect Dis* 1969;**119**:60–6.
435. Mufson MA, Belshe RB. A review of adenoviruses in the etiology of acute hemorrhagic cystitis. *J Urol* 1976;**115**:191–4.
436. Mufson MA, Belshe RB, Horrigan TJ, et al. Cause of acute hemorrhagic cystitis in children. *Am J Dis Child* 1973;**126**:605–9.
437. Mufson MA, Krause HE, Mocega HE, et al. Viruses, *Mycoplasma pneumoniae* and bacteria associated with lower respiratory tract disease among infants. *Am J Epidemiol* 1970;**91**:192–202.
438. Muller WJ, Levin MJ, Shin YK, et al. Clinical and in vitro evaluation of cidofovir for treatment of adenovirus infection in pediatric hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2005;**41**:1812–16.
439. Munoz FM, Piedra PA, Demmler GJ. Disseminated adenovirus disease in immunocompromised and immunocompetent children. *Clin Infect Dis* 1998;**27**:1194–200.
440. Murphy GF, Wood DP Jr, McRoberts JW, et al. Adenovirus-associated hemorrhagic cystitis treated with intravenous ribavirin. *J Urol* 1993;**149**:565–6.
441. Murtagh P, Giubergia V, Viale D, et al. Lower respiratory infections by adenovirus in children: clinical features and risk factors for bronchiolitis obliterans and mortality. *Pediatr Pulmonol* 2009;**44**:450–6.
442. Myerowitz RL, Stalder H, Oxman MN, et al. Fatal disseminated adenovirus infection in a renal transplant recipient. *Am J Med* 1975;**59**:591–8.
443. Myers GD, Krance RA, Weiss H, et al. Adenovirus infection rates in pediatric recipients of alternate donor allogeneic bone marrow transplants receiving either antithymocyte globulin (ATG) or alemtuzumab (Campath). *Bone Marrow Transplant* 2005;**36**: 1001–8.
444. Na HN, Hong YM, Kim J, et al. Association between human adenovirus-36 and lipid disorders in Korean schoolchildren. *Int J Obes (Lond)* 2010;**34**:89–93.
445. Na HN, Kim J, Lee HS, et al. Association of human adenovirus-36 in overweight Korean adults. *Int J Obes (Lond)* 2012;**36**:281–5.
446. Na HN, Kim H, Nam JH. Novel genes and cellular pathways related to infection with adenovirus-36 as an obesity agent in human mesenchymal stem cells. *Int J Obes (Lond)* 2012;**36**:195–200.
447. Na HN, Nam JH. Adenovirus 36 as an obesity agent maintains the obesity state by increasing MCP-1 and inducing inflammation. *J Infect Dis* 2012;**205**:914–22.
448. Naghipour M, Hart CA, Dove W, et al. Adenovirus infections within a family cohort in Iran. *Pediatr Pulmonol* 2009;**44**:749–53.
449. Nahmias AJ, Griffith D, Snitzer J. Fatal pneumonia associated with adenovirus type 7. *Am J Dis Child* 1967;**114**:36–41.
450. Nakayama M, Miyazaki C, Ueda K, et al. Pharyngoconjunctival fever caused by adenovirus type 11. *Pediatr Infect Dis J* 1992;**11**:6–9.
451. Naveh Y, Friedman A. Letter: Clinical memorandum: orchitis associated with adenoviral infection. *Am J Dis Child* 1975;**129**:257–8.
452. Nelson KE, Gavitt F, Batt MD, et al. The role of adenoviruses in the pertussis syndrome. *J Pediatr* 1975;**86**:335–41.
453. Neva FA, Enders JF. Isolation of a cytopathogenic agent from an infant with a disease in certain respects resembling roseola infantum. *J Immunol* 1954;**72**:315–21.
454. Newland JC, Cooney MK. Characteristics of an adenovirus type 19 conjunctivitis isolate and evidence for a subgroup associated with epidemic conjunctivitis. *Infect Immun* 1978;**21**:303–9.
455. Niemann TH, Trigg ME, Winick N, et al. Disseminated adenoviral infection presenting as acute pancreatitis. *Hum Pathol* 1993;**24**:1145–8.
456. Noda M, Miyamoto Y, Ikeda Y, et al. Intermediate human adenovirus type 22/H10,19,37 as a new etiological agent of conjunctivitis. *J Clin Microbiol* 1991;**29**:1286–9.

457. Norrby E. Biological significance of structural adenovirus components. *Curr Top Microbiol Immunol* 1968;**43**:1–43.
458. Norrby E. The structural and functional diversity of Adenovirus capsid components. *J Gen Virol* 1969;**5**:221–36.
459. Numazaki Y, Kumasaka T, Yano N, et al. Further study on acute hemorrhagic cystitis due to adenovirus type 11. *N Engl J Med* 1973;**289**:344–7.
460. Numazaki Y, Yano N, Ikeda M, et al. Adenovirus infection in intussusception of Japanese infants. *Jpn J Microbiol* 1973;**17**:87–9.
461. O'Brien TP, Jeng BH, McDonald M, et al. Acute conjunctivitis: truth and misconceptions. *Curr Med Res Opin* 2009;**25**:1953–61.
462. Odio C, McCracken GH Jr, Nelson JD. Disseminated adenovirus infection: a case report and review of the literature. *Pediatr Infect Dis* 1984;**3**:46–9.
463. Ohori NP, Michaels MG, Jaffe R, et al. Adenovirus pneumonia in lung transplant recipients. *Hum Pathol* 1995;**26**:1073–9.
464. Oishi I, Yamazaki K, Minekawa Y, et al. Three-year survey of the epidemiology of rotavirus, enteric adenovirus, and some small spherical viruses including "Osaka-agent" associated with infantile diarrhea. *Biken J* 1985;**28**:9–19.
465. Oker-Blom N, Wager O, Strandstrom H, et al. Adenoviruses associated with pharyngoconjunctival fever; isolation of adenovirus type 7 & serological studies suggesting its etiological role in an epidemic in Helsinki. *Ann Med Exp Biol Fenn* 1957;**35**: 342–51.
466. Olson LC, Miller G, Hanshaw JB. Acute infectious lymphocytosis presenting as a pertussis-like illness: its association with adenovirus type 12. *Lancet* 1964;**1**:200–1.
467. Ooi MH, Wong SC, Clear D, et al. Adenovirus type 21-associated acute flaccid paralysis during an outbreak of hand-foot-and-mouth disease in Sarawak, Malaysia. *Clin Infect Dis* 2003;**36**:550–9.
468. Ormsby HL, Aitchison WS. The role of the swimming pool in the transmission of pharyngeal-conjunctival fever. *Can Med Assoc J* 1955;**73**:864–6.
469. Osamura T, Mizuta R, Yoshioka H, et al. Isolation of adenovirus type 11 from the brain of a neonate with pneumonia and encephalitis. *Eur J Pediatr* 1993;**152**:496–9.
470. Pabbaraju K, Wong S, Fox JD. Detection of adenoviruses. *Methods Mol Biol* 2011;**665**:1–15.
471. Pacini DL, Collier AM, Henderson FW. Adenovirus infections and respiratory illnesses in children in group day care. *J Infect Dis* 1987;**156**:920–7.
472. Pacini DL, Dubovi EJ, Clyde WA Jr. A new animal model for human respiratory tract disease due to adenovirus. *J Infect Dis* 1984;**150**:92–7.
473. Paderstein R. Was ist schwimmbad-konjunktivitis? *Klin Monatsbl Augenheilkd* 1925;**74**:634–42.
474. Paerregaard A, Hjelt K, Genner J, et al. Role of enteric adenoviruses in acute gastroenteritis in children attending day-care centres. *Acta Paediatr Scand* 1990;**79**:370–1.
475. Page MA, Shisler JL, Marinas BJ. Mechanistic aspects of adenovirus serotype 2 inactivation with free chlorine. *Appl Environ Microbiol* 2010;**76**:2946–54.
476. Panush RS. Adenovirus arthritis. *Arthritis Rheum* 1974;**17**:534–6.
477. Paolino K, Sande J, Perez E, et al. Eradication of disseminated adenovirus infection in a pediatric hematopoietic stem cell transplantation recipient using the novel antiviral agent CMX001. *J Clin Virol* 2011;**50**:167–70.
478. Papapetropoulou M, Vantarakis AC. Detection of adenovirus outbreak at a municipal swimming pool by nested PCR amplification. *J Infect* 1998;**36**:101–3.
479. Parizhskaya M, Walpusk J, Mazariegos G, et al. Enteric adenovirus infection in pediatric small bowel transplant recipients. *Pediatr Dev Pathol* 2001;**4**:122–8.
480. Parker WL, Wilt JC, Stackiw W. Adenovirus infections. *Can J Public Health* 1961;**52**:246–51.
481. Parks WP, Boucher DW, Melnick JL, et al. Seroepidemiological and ecological studies of the adenovirus-associated satellite viruses. *Infect Immun* 1970;**2**:716–22.
482. Parrott RH. Newly isolated viruses in respiratory disease. *Pediatrics* 1957;**20**:1066–83.
483. Parrott RH. Viral respiratory tract illnesses in children. *Bull N Y Acad Med* 1963;**39**:629–48.
484. Parrott RH, Rowe WP, Huebner RJ, et al. Outbreak of febrile pharyngitis and conjunctivitis associated with type 3 adenoidal-pharyngeal-conjunctival virus infection. *N Engl J Med* 1954;**251**:1087–90.
485. Pearson RD, Hall WJ, Menegus MA, et al. Diffuse pneumonitis due to adenovirus type 21 in a civilian. *Chest* 1980;**78**:107–9.
486. Pereira HG. Typing of adenoidal-pharyngeal-conjunctival (APC) viruses by complement-fixation. *J Pathol Bacteriol* 1956;**72**: 105–9.
487. Pereira HG. A protein factor responsible for the early cytopathic effect of adenoviruses. *Virology* 1958;**6**:601–11.
488. Pereira MS. Adenovirus infections. *Postgrad Med J* 1973;**49**: 798–801.
489. Pereira MS, Maccallum FO. Infection with adenovirus type 12. *Lancet* 1964;**1**:198–9.
490. Pettit TH, Holland GN. Chronic keratoconjunctivitis associated with ocular adenovirus infection. *Am J Ophthalmol* 1979;**88**: 748–51.
491. Pichler MN, Reichenbach J, Schmidt H, et al. Severe adenovirus bronchiolitis in children. *Acta Paediatr* 2000;**89**:1387–9.
492. Piedra PA, Kasel JA, Norton HJ, et al. Description of an adenovirus type 8 outbreak in hospitalized neonates born prematurely. *Pediatr Infect Dis J* 1992;**11**:460–5.
493. Pina M, Green M. Biochemical studies on adenovirus multiplication, part IX: chemical and base composition analysis of 28 human adenoviruses. *Proc Natl Acad Sci U S A* 1965;**54**:547–51.
494. Pinchoff RJ, Kaufman SS, Magid MS, et al. Adenovirus infection in pediatric small bowel transplantation recipients. *Transplantation* 2003;**76**:183–9.
495. Pingleton SK, Pingleton WW, Hill RH, et al. Type 3 adenoviral pneumonia occurring in a respiratory intensive care unit. *Chest* 1978;**73**:554–5.
496. Pinkerton H, Carroll S. Fatal adenovirus pneumonia in infants: correlation of histologic and electron microscopic observations. *Am J Pathol* 1971;**65**:543–8.
497. Portnoy B, Salvatore MA, Hanes B, et al. The sensitivity of the complement fixation test for the detection of adenovirus infections in infants and children with lower respiratory disease. *Am J Epidemiol* 1967;**86**:362–71.
498. Potter CW. Adenovirus infection as an aetiological factor in intussusception of infants and young children. *J Pathol Bacteriol* 1964;**88**:263–74.
499. Potter RN, Cantrell JA, Mallak CT, et al. Adenovirus-associated deaths in US military during postvaccination period, 1999-2010. *Emerg Infect Dis* 2012;**18**:507–9.
500. Prage L, Pettersson U, Philipson L. Internal basic proteins in adenovirus. *Virology* 1968;**36**:508–11.
501. Prince RL. Evidence for an aetiological role for adenovirus type 7 in the mesenteric adenitis syndrome. *Med J Aust* 1979;**2**:56–7.
502. Putto A, Ruuskanen O, Meurman O. Fever in respiratory virus infections. *Am J Dis Child* 1986;**140**:1159–63.
503. Puzelli S, Valdarchi C, Ciotti M, et al. Viral causes of influenza-like illness: insight from a study during the winters 2004-2007. *J Med Virol* 2009;**81**:2066–71.
504. Rafajko RR. Differences in cytopathic effects of adenovirus in monkey kidney tissue culture. *Proc Soc Exp Biol Med* 1965;**119**:975–82.
505. Ray CG, Minnich LL. Efficiency of immunofluorescence for rapid detection of common respiratory viruses. *J Clin Microbiol* 1987;**25**:355–7.
506. Razanajatovo NH, Richard V, Hoffmann J, et al. Viral etiology of influenza-like illnesses in Antananarivo, Madagascar, July 2008 to June 2009. *PLoS One* 2011;**6**:e17579.
507. Retter M, Middleton PJ, Tam JS, et al. Enteric adenoviruses: detection, replication, and significance. *J Clin Microbiol* 1979;**10**:574–8.
508. Reuss VA. Keratitis maculosa. *Wien Klin Wochenschr* 1889;**2**: 665–6.
509. Reynolds MA, Hart CA, Sills JA, et al. Two cases of adenovirus type I pneumonia: diagnosis by direct electron microscopy and culture. *Pediatr Infect Dis* 1986;**5**:105–7.
510. Ribaud P, Scieux C, Freymuth F, et al. Successful treatment of adenovirus disease with intravenous cidofovir in an unrelated stem-cell transplant recipient. *Clin Infect Dis* 1999;**28**:690–1.
511. Richmond SJ, Caul EO, Dunn SM, et al. An outbreak of gastroenteritis in young children caused by adenoviruses. *Lancet* 1979;**1**:1178–81.

512. Rimoldi SG, Stefani F, Pagani C, et al. Epidemiological and clinical characteristics of pediatric gastroenteritis associated with new viral agents. *Arch Virol* 2011;**156**:1583–9.
513. Riner A, Chan-Tack KM, Murray JS. Original research: Intravenous ribavirin—review of the FDA's Emergency Investigational New Drug Database (1997-2008) and literature review. *Postgrad Med* 2009;**121**:139–46.
514. Robinson C, Echavarria M. Adenoviruses. In: Versalovic J, editor. *Manual of clinical microbiology*. vol. 2. 10th ed. Washington, D.C.: ASM Press; 2011. p. 1600–11.
515. Robinson CM, Singh G, Henquell C, et al. Computational analysis and identification of an emergent human adenovirus pathogen implicated in a respiratory fatality. *Virology* 2011;**409**:141–7.
516. Rocholl C, Gerber K, Daly J, et al. Adenoviral infections in children: the impact of rapid diagnosis. *Pediatrics* 2004;**113**:e51–6.
517. Rodriguez WJ, Kim HW, Brandt CD, et al. Fecal adenoviruses from a longitudinal study of families in metropolitan Washington, D.C. laboratory, clinical, and epidemiologic observations. *J Pediatr* 1985;**107**:514–20.
518. Roggendorf M, Wigand R, Deinhardt F, et al. Enzyme-linked immunosorbent assay for acute adenovirus infection. *J Virol Methods* 1982;**4**:27–35.
519. Romano A, Revel M, Guarari-Rotman D, et al. Use of human fibroblast-derived (beta) interferon in the treatment of epidemic adenovirus keratoconjunctivitis. *J Interferon Res* 1980;**1**:95–100.
520. Roos R, Chou SM, Rogers NG, et al. Isolation of an adenovirus 32 strain from human brain in a case of subacute encephalitis. *Proc Soc Exp Biol Med* 1972;**139**:636–40.
521. Rose HM, Lamson TH, Buescher EL. Adenoviral infection in military recruits. *Arch Environ Health* 1970;**21**:356–61.
522. Rosen L. A hemagglutination-inhibition technique for typing adenoviruses. *Am J Hyg* 1959;**71**:120–8.
523. Ross JG, Potter CW, Zachary RB. Adenovirus infection in association with intussusception in infancy. *Lancet* 1962;**2**:221–3.
524. Rowe WP, Hartley JW, Roizman B, et al. Characterization of a factor formed in the course of adenovirus infection of tissue cultures causing detachment of cells from glass. *J Exp Med* 1958;**108**:713–29.
525. Rowe WP, Huebner RJ. Present knowledge of the clinical significance of the adenoidal-pharyngeal-conjunctival group of viruses. *Am J Trop Med Hyg* 1956;**5**:453–60.
526. Rowe WP, Huebner RJ, Gilmore LK, et al. Isolation of a cytopathogenic agent from human adenoids undergoing spontaneous degeneration in tissue culture. *Proc Soc Exp Biol Med* 1955; **84**:570–3.
527. Rowe WP, Huebner RJ, Hartley JW, et al. Studies of the adenoidal-pharyngeal-conjunctival (APC) group of viruses. *Am J Hyg* 1955;**61**:197–218.
528. Russell KL, Broderick MP, Franklin SE, et al. Transmission dynamics and prospective environmental sampling of adenovirus in a military recruit setting. *J Infect Dis* 2006;**194**:877–85.
529. Ruuskanen O, Meurman O, Sarkkinen H. Adenoviral diseases in children: a study of 105 hospital cases. *Pediatrics* 1985;**76**:79–83.
530. Ruuskanen O, Sarkkinen H, Meurman O, et al. Rapid diagnosis of adenoviral tonsillitis: a prospective clinical study. *J Pediatr* 1984;**104**:725–8.
531. Ryan MA, Gray GC, Smith B, et al. Large epidemic of respiratory illness due to adenovirus types 7 and 3 in healthy young adults. *Clin Infect Dis* 2002;**34**:577–82.
532. Sahler OJ, Wilfert CM. Fever and petechiae with adenovirus type 7 infection. *Pediatrics* 1974;**53**:233–5.
533. Salomon HE, Grandien M, Avila MM, et al. Comparison of three techniques for detection of respiratory viruses in nasopharyngeal aspirates from children with lower acute respiratory infections. *J Med Virol* 1989;**28**:159–62.
534. Sanekata T, Taniguchi K, Demura M, et al. Detection of adenovirus type 41 in stool samples by a latex agglutination method. *J Immunol Methods* 1990;**127**:235–9.
535. Saquib R, Melton LB, Chandrakantan A, et al. Disseminated adenovirus infection in renal transplant recipients: the role of cidofovir and intravenous immunoglobulin. *Transpl Infect Dis* 2010;**12**:77–83.
536. Schaap GJ, de Jong JC, van Bijsterveld OP, et al. A new intermediate adenovirus type causing conjunctivitis. *Arch Ophthalmol* 1979;**97**:2336–8.
537. Schilham MW, Claas EC, van Zaane W, et al. High levels of adenovirus DNA in serum correlate with fatal outcome of adenovirus infection in children after allogeneic stem-cell transplantation. *Clin Infect Dis* 2002;**35**:526–32.
538. Schloesser C. Comments on keratitis punctata. *Zentralbl Prakt Augenheilkd* 1889;**13**:360.
539. Schnurr D, Dondero ME. Two new candidate adenovirus serotypes. *Intervirology* 1993;**36**:79–83.
540. Schoub BD, Koornhof HJ, Lecatsas G, et al. Letter: Viruses in acute summer gastroenteritis in black infants. *Lancet* 1975;**1**: 1093–4.
541. Schwartz HS, Vastine DW, Yamashiroya H, et al. Immunofluorescent detection of adenovirus antigen in epidemic keratoconjunctivitis. *Invest Ophthalmol* 1976;**15**:199–207.
542. Selvaraju SB, Kovac M, Dickson LM, et al. Molecular epidemiology and clinical presentation of human adenovirus infections in Kansas City children. *J Clin Virol* 2011;**51**:126–31.
543. Severe JL, Traub RG. Conjunctivitis with follicles associated with adenovirus type 22. *N Engl J Med* 1962;**266**:1375–6.
544. Shenk TE. Adenoviruses. In: Knipe DM, Howley PM, editors. *Virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2265–300.
545. Shields AF, Hackman RC, Fife KH, et al. Adenovirus infections in patients undergoing bone-marrow transplantation. *N Engl J Med* 1985;**312**:529–33.
546. Shike H, Shimizu C, Kanegaye J, et al. Quantitation of adenovirus genome during acute infection in normal children. *Pediatr Infect Dis J* 2005;**24**:29–33.
547. Shirali GS, Ni J, Chinnock RE, et al. Association of viral genome with graft loss in children after cardiac transplantation. *N Engl J Med* 2001;**344**:1498–503.
548. Siegal FP, Dikman SH, Arayata RB, et al. Fatal disseminated adenovirus 11 pneumonia in an agammaglobulinemic patient. *Am J Med* 1981;**71**:1062–7.
549. Sili U, Leen AM, Vera JF, et al. Production of good manufacturing practice-grade cytotoxic T lymphocytes specific for Epstein-Barr virus, cytomegalovirus and adenovirus to prevent or treat viral infections post-allogeneic hematopoietic stem cell transplant. *Cytotherapy* 2012;**14**:7–11.
550. Simila S, Jouppila R, Salmi A, et al. Encephaloningitis in children associated with an adenovirus type 7 epidemic. *Acta Paediatr Scand* 1970;**59**:310–16.
551. Simila S, Linna O, Lanning P, et al. Chronic lung damage caused by adenovirus type 7: a ten-year follow-up study. *Chest* 1981;**80**:127–31.
552. Simila S, Ylikorkala O, Wasz-Hockert O. Type 7 adenovirus pneumonia. *J Pediatr* 1971;**79**:605–11.
553. Simsir A, Greenebaum E, Nuovo G, et al. Late fatal adenovirus pneumonitis in a lung transplant recipient. *Transplantation* 1998;**65**:592–4.
554. Singh-Naz N, Brown M, Ganeshananthan M. Nosocomial adenovirus infection: molecular epidemiology of an outbreak. *Pediatr Infect Dis J* 1993;**12**:922–5.
555. Smith RH. Fatal adenovirus infection with misleading positive serology for infectious mononucleosis. *Lancet* 1979;**1**:299–302.
556. Smith TJ, Buescher EL, Top FH Jr, et al. Experimental respiratory infection with type 4 adenovirus vaccine in volunteers: clinical and immunological responses. *J Infect Dis* 1970;**122**:239–48.
557. Sohier R, Chardonnet Y, Prunieras M. Adenoviruses: status of current knowledge. *Prog Med Virol* 1965;**7**:253–325.
558. South MA, Dolen J, Beach DK, et al. Fatal adenovirus hepatic necrosis in severe combined immune deficiency. *Pediatr Infect Dis* 1982;**1**:416–19.
559. Sprague JB, Hierholzer JC, Currier RW, 2nd, et al. Epidemic keratoconjunctivitis: a severe industrial outbreak due to adenovirus type 8. *N Engl J Med* 1973;**289**:1341–6.
560. Stanley ED, Jackson GG. Spread of enteric live adenovirus type 4 vaccine in married couples. *J Infect Dis* 1969;**119**:51–9.
561. Steen-Johnsen J, Orstavik I, Attramadal A. Severe illnesses due to adenovirus type 7 in children. *Acta Paediatr Scand* 1969;**58**: 157–63.
562. Steigbigel RT, LaScolea LJ, Marx G. Renal hematuria associated with adenovirus 7a infection. *Am J Dis Child* 1978;**132**:208–10.
563. Stellwag VK. A peculiar form of corneal inflammation. *Wien Klin Wochenschr* 1889;**August**:613–14.

564. Sterner G. Infections with adenovirus type 7 in children and their relationship to acute respiratory disease. *Acta Paediatr* 1959;**48**: 287–98.
565. Sterner G. Adenovirus infection in childhood: an epidemiological and clinical survey among Swedish children. *Acta Paediatr* 1962;**142**:5–30.
566. Sterner G, Gerzen P, Ohlson M, et al. Acute respiratory illness and gastroenteritis in association with adenovirus type 7 infections. *Acta Paediatr* 1961;**50**:457–68.
567. Stovin S. Sporadic acute respiratory infections in adults with special reference to adenovirus infections. *J Hyg (Lond)* 1958;**56**:404–14.
568. Straussberg R, Harel L, Levy Y, et al. A syndrome of transient encephalopathy associated with adenovirus infection. *Pediatrics* 2001;**107**:E69.
569. Stroparo E, Cruz CR, Debur Mdo C, et al. Adenovirus respiratory infection: significant increase in diagnosis using PCR comparing with antigen detection and culture methods. *Rev Inst Med Trop Sao Paulo* 2010;**52**:317–21.
570. Stuart-Harris CH. The adenoviruses and respiratory disease in man. *Lect Sci Basis Med* 1958–1959;**8**:148–64.
571. Sutton RN, Pullen HJ, Blackledge P, et al. Adenovirus type 7; 1971-74. *Lancet* 1976;**2**:987–91.
572. Swann NH. Acute thyroiditis: five cases associated with adenovirus infection. *Metabolism* 1964;**13**:908–10.
573. Swenson PD, Lowens MS, Celum CL, et al. Adenovirus types 2, 8, and 37 associated with genital infections in patients attending a sexually transmitted disease clinic. *J Clin Microbiol* 1995;**33**: 2728–31.
574. Takafuji ET, Gaydos JC, Allen RG, et al. Simultaneous administration of live, enteric-coated adenovirus types 4, 7 and 21 vaccines: safety and immunogenicity. *J Infect Dis* 1979;**140**:48–53.
575. Takiff HE, Seidlin M, Krause P, et al. Detection of enteric adenoviruses by dot-blot hybridization using a molecularly cloned viral DNA probe. *J Med Virol* 1985;**16**:107–18.
576. Takiff HE, Straus SE, Garon CF. Propagation and in vitro studies of previously non-cultivable enteral adenoviruses in 293 cells. *Lancet* 1981;**2**:832–4.
577. Tanaka C. Epidemic keratoconjunctivitis in Japan and the Orient. *Am J Ophthalmol* 1957;**43**:46–50.
578. Tanaka C. A study of the relationship between adenovirus and epidemic keratoconjunctivitis. *AMA Arch Ophthalmol* 1958;**59**: 49–54.
579. Tang L, Wang L, Tan X, et al. Adenovirus serotype 7 associated with a severe lower respiratory tract disease outbreak in infants in Shaanxi Province, China. *Virol J* 2011;**8**:23.
580. Tate JE, Bunning ML, Lott L, et al. Outbreak of severe respiratory disease associated with emergent human adenovirus serotype 14 at a US air force training facility in 2007. *J Infect Dis* 2009;**199**:1419–26.
581. Tatrai E, Hartyanszky I Jr, Laszik A, et al. The role of viral infections in the development of dilated cardiomyopathy. *Pathol Oncol Res* 2011;**17**:229–35.
582. Teuchner B, Nagl M, Schidlbauer A, et al. Tolerability and efficacy of N-chlorotaurine in epidemic keratoconjunctivitis—a double-blind, randomized, phase-2 clinical trial. *J Ocul Pharmacol Ther* 2005;**21**:157–65.
583. Thiele GM, Okano M, Purtilo DT. Enzyme-linked immunosorbent assay (ELISA) for detecting antibodies in sera of patients with adenovirus infection. *J Virol Methods* 1989;**23**:321–32.
584. Tiemessen CT, Wegerhoff FO, Erasmus MJ, et al. Infection by enteric adenoviruses, rotaviruses, and other agents in a rural African environment. *J Med Virol* 1989;**28**:176–82.
585. Top FH Jr. Control of adenovirus acute respiratory disease in U.S. Army trainees. *Yale J Biol Med* 1975;**48**:185–95.
586. Top FH Jr, Buescher EL, Bancroft WH, et al. Immunization with live types 7 and 4 adenovirus vaccines, part II: antibody response and protective effect against acute respiratory disease due to adenovirus type 7. *J Infect Dis* 1971;**124**:155–60.
587. Top FH Jr, Dudding BA, Russell PK, et al. Control of respiratory disease in recruits with types 4 and 7 adenovirus vaccines. *Am J Epidemiol* 1971;**94**:142–6.
588. Top FH Jr, Grossman RA, Bartelloni PJ, et al. Immunization with live types 7 and 4 adenovirus vaccines, part I: safety, infectivity, antigenicity, and potency of adenovirus type 7 vaccine in humans. *J Infect Dis* 1971;**124**:148–54.
589. Towbin JA, Griffin LD, Martin AB, et al. Intrauterine adenoviral myocarditis presenting as nonimmune hydrops fetalis: diagnosis by polymerase chain reaction. *Pediatr Infect Dis J* 1994;**13**: 144–50.
590. Treacy A, Carr MJ, Dunford L, et al. First report of sudden death due to myocarditis caused by adenovirus serotype 3. *J Clin Microbiol* 2010;**48**:642–5.
591. Trentin JJ, Yabe Y, Taylor G. The quest for human cancer viruses. *Science* 1962;**137**:835–41.
592. Trifajova J, Bruckova M, Ryc M, et al. Type 5 adenovirus isolated from urine of patient with Hodgkin's disease. *J Hyg Epidemiol Microbiol Immunol* 1981;**25**:321–3.
593. Trovato GM, Castro A, Tonzuso A, et al. Human obesity relationship with Ad36 adenovirus and insulin resistance. *Int J Obes (Lond)* 2009;**33**:1402–9.
594. Trovato GM, Martines GF, Trovato FM, et al. Adenovirus-36 seropositivity enhances effects of nutritional intervention on obesity, bright liver, and insulin resistance. *Dig Dis Sci* 2012;**57**: 535–44.
595. Turner PC, Bailey AS, Cooper RJ, et al. The polymerase chain reaction for detecting adenovirus DNA in formalin-fixed, paraffin-embedded tissue obtained post mortem. *J Infect* 1993;**27**: 43–6.
596. Uchio E, Matsuura N, Takeuchi S, et al. Acute follicular conjunctivitis caused by adenovirus type 34. *Am J Ophthalmol* 1999;**128**: 680–6.
597. Uhnoo I, Wadell G, Svensson L, et al. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. *J Clin Microbiol* 1984;**20**:365–72.
598. U.S. Food and Drug Administration. *March 16, 2011 Approval Letter—Adenovirus type 4 and type 7 vaccine, live, oral: 2011*. Accessed October 10, 2012. Available at http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm247511.htm.
599. Usami T, Mugiya S, Ushiyama T, et al. Systemic infection resembling hemorrhagic fever with the renal syndrome caused by adenovirus type 11. *J Urol* 1997;**157**:617–18.
600. Valentine RC, Pereira HG. Antigens and structure of the adenovirus. *J Mol Biol* 1965;**13**:13–20.
601. van Bijsterveld OP, de Jong JC, Muzerie CJ, et al. Pharyngoconjunctival fever caused by adenovirus type 19. *Ophthalmologica* 1978;**177**:134–9.
602. Van Der Veen J. Infections with adenovirus in Europe. *Soc Belge Med Trop Ann* 1958;**38**:891–904.
603. Van Der Veen J. The role of adenoviruses in respiratory disease. *Am Rev Respir Dis* 1963;**88**(Suppl.):167–80.
604. Van Der Veen J, Dijkman JH. Association of type 21 adenovirus with acute respiratory illness in military recruits. *Am J Hyg* 1962;**76**:149–59.
605. Van Der Veen J, Kok G. Isolation and typing of adenoviruses recovered from military recruits with acute respiratory disease in The Netherlands. *Am J Hyg* 1957;**65**:119–29.
606. van der Veen J, Oei KG, Abarbanel MF. Patterns of infections with adenovirus types 4, 7 and 21 in military recruits during a 9-year survey. *J Hyg (Lond)* 1969;**67**:255–68.
607. Van Der Veen J, Van Der Ploeg G. An outbreak of pharyngoconjunctival fever caused by types 3 and 4 adenovirus at Waalwijk, The Netherlands. *Am J Hyg* 1958;**68**:95–105.
608. van der Veen J, van Zaane DJ, Sprangers MA, et al. Homotypic and heterotypic antibody response in infants to adenovirus vaccine. *Arch Gesamte Virusforsch* 1967;**21**:320–33.
609. van Maarseveen NM, Wessels E, de Brouwer CS, et al. Diagnosis of viral gastroenteritis by simultaneous detection of Adenovirus group F, Astrovirus, Rotavirus group A, Norovirus genogroups I and II, and Sapovirus in two internally controlled multiplex real-time PCR assays. *J Clin Virol* 2010;**49**:205–10.
610. Van R, Wun CC, O'Ryan ML, et al. Outbreaks of human enteric adenovirus types 40 and 41 in Houston day care centers. *J Pediatr* 1992;**120**:516–21.
611. van Tol MJ, Claas EC, Heemskerk B, et al. Adenovirus infection in children after allogeneic stem cell transplantation: diagnosis, treatment and immunity. *Bone Marrow Transplant* 2005;**35**(Suppl. 1):S73–6.
612. van Tol MJ, Kroes AC, Schinkel J, et al. Adenovirus infection in paediatric stem cell transplant recipients: increased risk in young children with a delayed immune recovery. *Bone Marrow Transplant* 2005;**36**:39–50.

613. Vangipuram SD, Sheele J, Atkinson RL, et al. A human adenovirus enhances preadipocyte differentiation. *Obes Res* 2004;**12**:770–7.
614. Vangipuram SD, Yu M, Tian J, et al. Adipogenic human adenovirus-36 reduces leptin expression and secretion and increases glucose uptake by fat cells. *Int J Obes (Lond)* 2007;**31**:87–96.
615. Vargosko AJ, Kim HW, Parrott RH, et al. Recovery and identification of adenovirus in infections of infants and children. *Bacteriol Rev* 1965;**29**:487–95.
616. Varma MC, Kushner YB, Ko DS, et al. Early onset adenovirus infection after simultaneous kidney-pancreas transplant. *Am J Transplant* 2011;**11**:623–7.
617. Vass Z. Histological findings in epidemic keratoconjunctivitis. *Acta Ophthalmol (Copenh)* 1964;**42**:119–21.
618. Vastine DW, Schwartz HS, Yamashiroya HM, et al. Cytologic diagnosis of adenoviral epidemic keratoconjunctivitis by direct immunofluorescence. *Invest Ophthalmol Vis Sci* 1977;**16**:195–200.
619. Venter M, Lassauniere R, Kresfelder TL, et al. Contribution of common and recently described respiratory viruses to annual hospitalizations in children in South Africa. *J Med Virol* 2011;**83**:1458–68.
620. Vento TJ, Prakash V, Murray CK, et al. Pneumonia in military trainees: a comparison study based on adenovirus serotype 14 infection. *J Infect Dis* 2011;**203**:1388–95.
621. Vihma L. Surveillance of acute viral respiratory diseases in children. *Acta Paediatr* 1969;**192**:27–41.
622. Wadell G. Classification of human adenoviruses by SDS-polyacrylamide gel electrophoresis of structural polypeptides. *Intervirology* 1979;**11**:47–57.
623. Wadell G. Molecular epidemiology of human adenoviruses. *Curr Top Microbiol Immunol* 1984;**110**:191–220.
624. Wadell G, Hammarskjold ML, Winberg G, et al. Genetic variability of adenoviruses. *Ann N Y Acad Sci* 1980;**354**:16–42.
625. Wadell G, Sundell G, de Jong JC. Characterization of candidate adenovirus 37 by SDS-polyacrylamide gel electrophoresis of virion polypeptides and DNA restriction site mapping. *J Med Virol* 1981;**7**:119–25.
626. Wallis AL. An unusual epidemic. *Lancet* 1955;**269**:290.
627. Wallot MA, Dohna-Schwake C, Auth M, et al. Disseminated adenovirus infection with respiratory failure in pediatric liver transplant recipients: impact of intravenous cidofovir and inhaled nitric oxide. *Pediatr Transplant* 2006;**10**:121–7.
628. Walls T, Hawrami K, Shingadia D, et al. Polymerase chain reaction in the management of disseminated adenovirus infection after stem cell transplantation. *Pediatr Infect Dis J* 2004;**23**:1167–9.
629. Walls T, Hawrami K, Ushiro-Lumb I, et al. Adenovirus infection after pediatric bone marrow transplantation: is treatment always necessary? *Clin Infect Dis* 2005;**40**:1244–9.
630. Walsh MP, Seto J, Liu EB, et al. Computational analysis of two species C human adenoviruses provides evidence of a novel virus. *J Clin Microbiol* 2011;**49**:3482–90.
631. Wang X, Yang K, Wei C, et al. Coinfection with EBV/CMV and other respiratory agents in children with suspected infectious mononucleosis. *Virol J* 2010;**7**:247.
632. Wang ZQ, Yu Y, Zhang XH, et al. Human adenovirus 36 decreases fatty acid oxidation and increases de novo lipogenesis in primary cultured human skeletal muscle cells by promoting Cidec/FSP27 expression. *Int J Obes (Lond)* 2010;**34**:1355–64.
633. Ward TG. Viruses of the respiratory tract. *Prog Med Virol* 1973;**15**:126–58.
634. Ward TG, Huebner RJ, Rowe WP, et al. Production of pharyngoconjunctival fever in human volunteers inoculated with APC viruses. *Science* 1955;**122**:1086–7.
635. Warren D, Nelson KE, Farrar JA, et al. A large outbreak of epidemic keratoconjunctivitis: problems in controlling nosocomial spread. *J Infect Dis* 1989;**160**:938–43.
636. Watcharananan SP, Kiertiburanakul S, Piyatuctsanawong W, et al. Cytomegalovirus, adenovirus, and polyomavirus co-infection among pediatric recipients of allogeneic stem cell transplantation: characteristics and outcome. *Pediatr Transplant* 2010;**14**:675–81.
637. Wenman WM, Pagtakhan RD, Reed MH, et al. Adenovirus bronchiolitis in Manitoba: epidemiologic, clinical, and radiologic features. *Chest* 1982;**81**:605–9.
638. Whigham LD, Israel BA, Atkinson RL. Adipogenic potential of multiple human adenoviruses in vivo and in vitro in animals. *Am J Physiol Regul Integr Comp Physiol* 2006;**290**:R190–4.
639. White GB, Stancliffe D. Letter: Viruses and gastroenteritis. *Lancet* 1975;**2**:703.
640. Whitelaw A, Davies H, Parry J. Electron microscopy of fatal adenovirus gastroenteritis. *Lancet* 1977;**1**:361.
641. Wigand R, Baumeister HG, Maass G, et al. Isolation and identification of enteric adenoviruses. *J Med Virol* 1983;**11**:233–40.
642. Wigand R, Gelderblom H, Wadell G. New human adenovirus (candidate adenovirus 36), a novel member of subgroup D. *Arch Virol* 1980;**64**:225–33.
643. Wigand R, Keller D. Relationship of human adenoviruses 12, 18, and 31 as determined by hemagglutination inhibition. *J Med Virol* 1978;**2**:137–42.
644. Wigger HJ, Blanc WA. Fatal hepatic and bronchial necrosis in adenovirus infection with thymic alymphoplasia. *N Engl J Med* 1966;**275**:870–4.
645. Wildin SR, Chonmaitree T, Swischuk LE. Roentgenographic features of common pediatric viral respiratory tract infections. *Am J Dis Child* 1988;**142**:43–6.
646. Wilhelm CM, Hanna SL, Welch CA, et al. Viral gastroenteritis in Charleston, West Virginia, in 2007: from birth to 99 years of age. *Infect Control Hosp Epidemiol* 2010;**31**:816–21.
647. Williams KM, Agwu AL, Dabb AA, et al. A clinical algorithm identifies high risk pediatric oncology and bone marrow transplant patients likely to benefit from treatment of adenoviral infection. *J Pediatr Hematol Oncol* 2009;**31**:825–31.
648. Wilt JC, Stackiw W. Adenovirus infections in Manitoba. *Can Med Assoc J* 1970;**102**:269–72.
649. Wohl ME, Chernick V. State of the art: bronchiolitis. *Am Rev Respir Dis* 1978;**118**:759–81.
650. Wood DJ, Bijlsma K, de Jong JC, et al. Evaluation of a commercial monoclonal antibody-based enzyme immunoassay for detection of adenovirus types 40 and 41 in stool specimens. *J Clin Microbiol* 1989;**27**:1155–8.
651. Wright HT Jr, Beckwith JB, Gwinn JL. A fatal case of inclusion body pneumonia in an infant infected with adenovirus type 3. *J Pediatr* 1964;**64**:528–33.
652. Wright J, Couchonnal G, Hodges GR. Adenovirus type 21 infection: occurrence with pneumonia, rhabdomyolysis, and myoglobinuria in an adult. *JAMA* 1979;**241**:2420–1.
653. Wright RE. Superficial punctate keratitis: a record of an epidemic of superficial punctate keratitis which appeared in Madras about May, 1928, and continued during the year that followed, embodying observations relating to the clinical appearances, biomicroscopy, epidemiology, aetiology and histopathology, of the disease, based on the investigation of over 900 cases. *Br J Ophthalmol* 1930;**14**:257–91.
654. Wulffraat NM, Geelen SP, van Dijken PJ, et al. Recovery from adenovirus pneumonia in a severe combined immunodeficiency patient treated with intravenous ribavirin. *Transplantation* 1995;**59**:927.
655. Xie L, Yu XF, Sun Z, et al. Two adenovirus serotype 3 outbreaks associated with febrile respiratory disease and pharyngoconjunctival fever in children under 15 years of age in Hangzhou, China, during 2011. *J Clin Microbiol* 2012;**50**:1879–88.
656. Xu W, Erdman DD. Type-specific identification of human adenovirus 3, 7, and 21 by a multiplex PCR assay. *J Med Virol* 2001;**64**:537–42.
657. Yamadera S, Yamashita K, Akatsuka M, et al. Adenovirus surveillance, 1982-1993, Japan: a report of the National Epidemiological Surveillance of Infectious Agents in Japan. *Jpn J Med Sci Biol* 1995;**48**:199–210.
658. Yamadera S, Yamashita K, Akatsuka M, et al. Trend of adenovirus type 7 infection, an emerging disease in Japan: a report of the National Epidemiological Surveillance of Infectious Agents in Japan. *Jpn J Med Sci Biol* 1998;**51**:43–51.
659. Yin-Coggrave M. Isolation of adenoviruses from cases of epidemic keratoconjunctivitis in Singapore. *Am J Ophthalmol* 1963;**55**: 575–83.
660. Yin-Murphy M. Simple tests for the diagnosis of picornavirus epidemic conjunctivitis (acute haemorrhagic conjunctivitis). *Bull World Health Organ* 1976;**54**:675–9.
661. Yin-Murphy M, Lim KH. Picornavirus epidemic conjunctivitis in Singapore. *Lancet* 1972;**2**:857–8.
662. Yodfat Y, Nishmi M. Successive overlapping outbreaks of febrile pharyngitis and pharyngoconjunctival fever associated with adenovirus types 2 and 7, in a kibbutz. *Isr J Med Sci* 1974;**10**:1505–9.

663. Yokose N, Hirakawa T, Inokuchi K. Adenovirus-associated hemorrhagic cystitis in a patient with plasma cell myeloma treated with bortezomib. *Leuk Res* 2009;**33**:e106.
664. Yolken RH, Franklin CC. Gastrointestinal adenovirus: an important cause of morbidity in patients with necrotizing enterocolitis and gastrointestinal surgery. *Pediatr Infect Dis* 1985;**4**:42–7.
665. Yolken RH, Lawrence F, Leister F, et al. Gastroenteritis associated with enteric type adenovirus in hospitalized infants. *J Pediatr* 1982;**101**:21–6.
666. Yoon J, Jekle A, Najafi R, et al. Virucidal mechanism of action of NVC-422, a novel antimicrobial drug for the treatment of adenoviral conjunctivitis. *Antiviral Res* 2011;**92**:470–8.
667. Yunis EJ, Atchison RW, Michaels RH, et al. Adenovirus and ileocecal intussusception. *Lab Invest* 1975;**33**:347–51.
668. Yunis EJ, Hashida Y. Electron microscopic demonstration of adenovirus in appendix vermiformis in a case of ileocecal intussusception. *Pediatrics* 1973;**51**:566–70.
669. Yusuf U, Hale GA, Carr J, et al. Cidofovir for the treatment of adenoviral infection in pediatric hematopoietic stem cell transplant patients. *Transplantation* 2006;**81**:1398–404.
670. Zahradnik JM, Spencer MJ, Porter DD. Adenovirus infection in the immunocompromised patient. *Am J Med* 1980;**68**:725–32.
671. Zandvliet ML, Falkenburg JH, van Liempt E, et al. Combined $CD8^+$ and $CD4^+$ adenovirus hexon-specific T cells associated with viral clearance after stem cell transplantation as treatment for adenovirus infection. *Haematologica* 2010;**95**:1943–51.
672. Zinserling A. Pecularities of lesions in viral and *Mycoplasma* infections of the respiratory tract. *Virchows Arch A Pathol Pathol Anat* 1972;**356**:259–73.

All primarily infect the livers of their respective hosts and can cause acute and chronic infections. HBV is the most studied member of the family, which also includes the woodchuck hepatitis virus (WHV), the ground squirrel hepatitis virus, the duck hepatitis virus (DHV), and hepatitis viruses found in herons, Ross's geese, and arctic squirrels.[178] The WHV and DHV share approximately 70 percent sequence homology with HBV but do not infect humans.[182] The use of these animal models has facilitated the study of hepadnavirus structure and replication, supplementing information obtained from infected humans and cloning studies.

Three types of particles are found in the sera of HBV-infected people and are shown in Figure 156-1: the Dane particle and two subviral particles, one spherical and the other filament shaped. All three types share a common surface antigen, hepatitis B surface antigen (HBsAg), but only one, the Dane particle, contains viral DNA and is capable of replication. Neither the filament, 20 nm in width and ranging from 50 to 1000 nm in length, nor the sphere, also of 20 nm diameter, contains nucleic acid. The Dane particle or whole virion is 42 nm in diameter and contains a core, or nucleocapsid, enclosing the DNA. The outer shell is composed of large amounts of hepatitis B surface proteins in a lipoprotein envelope, which is derived from host cells. Inside this shell is found the nucleocapsid, an icosahedral structure composed of 240 core protein subunits with regular penetrating channels. The nucleocapsid proteins are detected serologically as hepatitis B core antigen (HBcAg). This nucleocapsid is 25 to 27 nm in diameter, and within it are contained the viral genome and polymerase. HBV e antigen (HBeAg) is a soluble antigen produced from the same open reading frame as is HBcAg, but, unlike core antigen, e antigen is secreted into serum, where it is thought to have a role in induction of tolerance to HBV. The viral genome is composed of double-stranded circular DNA that can be relaxed or closed depending on the stage in the reproduction cycle. This 3.2-kb DNA encodes four overlapping open reading frames (ORFs) that overlap with other regulatory *cis*-acting sequences such as promoters, enhancers, and polyadenylation and genome packaging signals.

The subviral particles (spheres and filaments) are composed of envelope proteins and host-derived lipid components.[178] These particles are abundant in the serum of infected individuals (10^6 to 10^{14} particles/mL), and they are highly immunogenic, stimulating the production of neutralizing antibodies. One hypothesis is that they absorb neutralizing antibodies, thus shielding the intact virion from the host immune response. Subviral particles were used to produce the first effective HBV vaccine.[203]

Molecular Virology

The viral genome, as noted previously, contains four ORFs in its circular DNA. The DNA strands are not completely symmetric; the minus strand is shorter, leading to a region of single-stranded DNA. This gap can be filled by the virus's own polymerase when required. The four genes are *S* for the surface or envelope gene; *P* for the viral polymerase gene; *C* for the core gene, which encodes nucleocapsid protein; and *X* for the X gene. The surface proteins synthesized by HBV are termed the

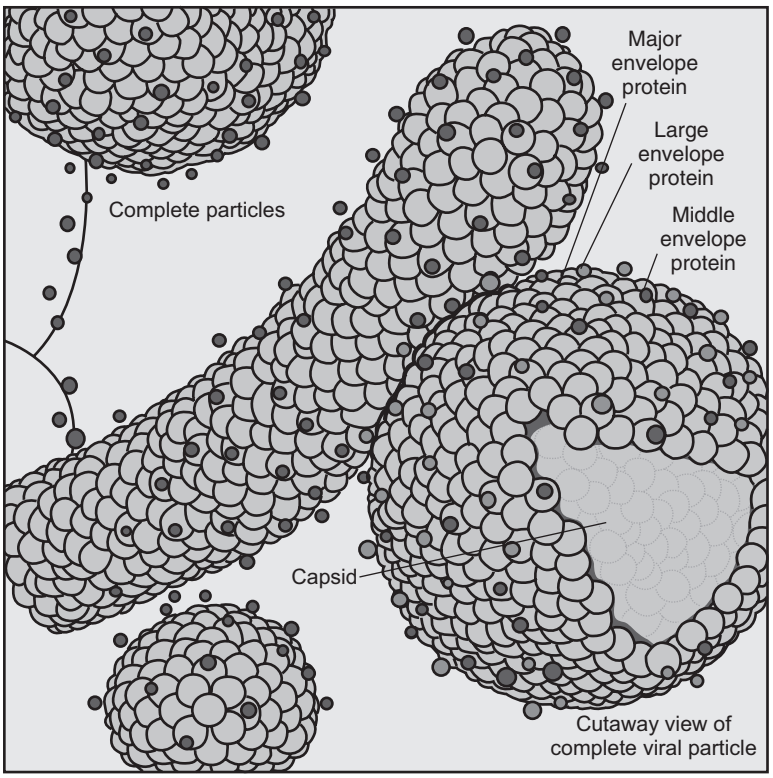

FIGURE 156-1 ■ The three forms of HBV surface antigen are whole virions, rods, and proteins. A cutaway view of the whole virion shows the three envelope proteins: major, middle, and large. (From Mutchnik MG. Acute and chronic hepatitis B. In: Feldman M, Maddrey WC, editors. *The liver.* Philadelphia: Current Medicine; 1996.)

large, *middle*, *and major proteins;* they are type II transmembrane proteins that can form multimers. Transcription initiated at the *pre-S1*, *pre-S2*, or *S* epitopes leads to formation of these three proteins. Although antibodies to S protein alone will provide protection against HBV infection, epitopes of *pre-S1* also can stimulate neutralizing antibody production.[120] A group of antigens used for subtyping HBV strains in epidemiologic studies is found in association with S proteins. Analysis of subdeterminants of HBsAg has revealed four major serotypes, *adw*, *adr*, *ayw*, and *ayr*, thus allowing differentiation of HBV infections from different sources. The "a" determinant is found in all isolates. Antibody to hepatitis B surface antigen (anti-HBs) is directed against this determinant and provides protection against all serotypes.

Eight HBV genotypes and multiple subgenotypes have been identified by use of polymerase chain reaction (PCR)–restriction fragment length polymorphism analysis of the complete viral genome.[105,130] They have been designated A through H. Genotypes B, C, and D have been further categorized into five subgenotypes each (HBV B1 to B5, HBV C1 to C5, and HBV D1 to D5).[140,176] Genotype and subgenotype predominance varies by geographic location: A study of 187 patients with chronic HBV infection revealed the following marked regional variations in genotype: North Europeans, 63 percent A; South Europeans and Middle Easterners, 96 percent D; Africans, 53 percent A, 27 percent D, 20 percent E; East Asians, 14 percent A, 43 percent B, and 43 percent C.[130] The clinical differences among HBV genotypes are being elucidated. Infection with genotype C was found in Taiwanese adults to be associated with more severe liver disease and HCC in those older than 50 years of age, whereas genotype B is associated with HCC in younger persons.[104] In a study of 487 Chinese HBV-infected children, genotype C was more common (76.4%) than genotype B (22.3%) or genotype D (1.3%). Children with chronic HBV B2 had less severe inflammation and fibrosis than those with HBV C2.[228] An association of genotype with response to interferon-α (IFN-α) treatment also has been demonstrated. A study from Taiwan analyzed the responses of 58 patients with chronic HBV infection genotypes B and C to treatment with IFN-α. The patients with genotype C had higher aminotransferase levels at baseline. After 48 weeks of follow-up, only 15 percent of those with genotype C were HBeAg negative and HBV DNA negative, as compared with 42 percent of those with genotype B.[106]

HBV polymerase consists of two major domains tethered by an intervening spacer region. Elucidating the structure and biochemistry of hepadnavirus polymerases has proved difficult because they require cellular factors, which are lost during extraction from cells, for enzymatic activity.[156] The ORF that encodes the *P* gene overlaps to a large extent with the core protein ORF; indeed, both proteins are translated from the same mRNA.[32]

The core gene encodes the viral capsid proteins that combine to form the core particles. The core proteins combine first into dimers; 120 dimers unite to form a shell 36 nm in diameter.[47] HBcAg appears to be phosphorylated within cells and becomes dephosphorylated upon export.[196] The main role of core proteins is enclosure of viral DNA, but they also play a role in the cytolysis of infected cells when expressed on the cell surface.[127] Mutations in the core gene, the pre-core region, and the promoter for *C* have become clinically important.

Mutations affecting all ORFs of the HBV genome have been described. Most viral genomes carry more than one mutation, and most individuals are infected with more than one variant. Some of the mutations are thought to contribute to viral latency, level of infection, severity of liver disease, and vaccine escape. The group best studied is the pre-core mutants, which result in lack of HBeAg production, even in the presence of active viral replication, the so-called HBe antigen–negative infection. In contrast to the situation in persons with wild-type virus, in whom absence of HBeAg usually signifies absent HBV replication and mild liver disease (see later discussion), in HBeAg-negative hepatitis, HBV DNA levels are high, antibody to hepatitis B e antigen (anti-HBe) is detected, and liver disease may be severe. Sporadic cases and outbreaks of fulminant HBV infection have been attributed to pre-core mutants. Mutations in the *S* or *pre-S* gene, which encode envelope protein, have been reported in patients infected after vaccination and in individuals who received monoclonal antibody to HBV after liver transplantation. This variant contains a missense mutation in the *a* determinant of the surface antigen. Subsequently, these mutants were demonstrated in chronic HBV carriers even without these immune pressures. This mutation causes an infection in which HBsAg is undetectable but in which HBeAg and HBV-DNA are found, in contrast to the typical serologic pattern (see later discussion).

The role of X protein is not fully understood, but it is required for establishment of infection in vivo although not for transfection of cells in vitro.[16,42] X protein is a regulatory protein involved in transcriptional activation during viral replication[127]; it has also has been shown to transactivate the promoters of other viruses such as human immunodeficiency virus (HIV) and human T-lymphotropic virus type 1.[172] In addition, hepatitis B X protein appears to be involved in the development of HCC.[13]

Viral Life Cycle Overview

Like retroviruses, hepadnaviruses replicate by means of reverse transcription of an RNA intermediate[200] (i.e., the flow of genetic information is from DNA to RNA to DNA). As shown in Figure 156-2, after the virus binds to receptors on the cell surface, virion nucleocapsids are delivered to the cytoplasm, where they translocate to the nucleus. There the genomic DNA is converted to the covalently closed circular DNA (cccDNA) form that serves as a template for the reverse transcription. This cccDNA is transcribed by host RNA polymerase II, and the resulting RNAs are translated to originate the *P*, *C*, *S/pre-S*, and *X* gene products. The viral pol initiates encapsidation of viral pregenomic RNAs into the cytoplasm by binding to the 5′ encapsidation signal, epsilon, on the pgRNA.[137] Through a series of complex host-viral interactions, negative strand DNA synthesis is

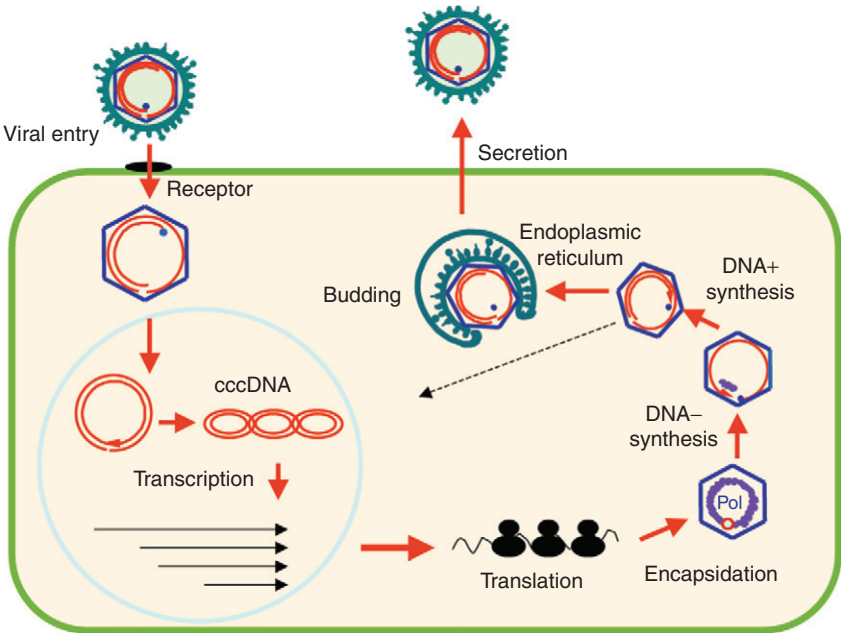

FIGURE 156-2 ■ The virus enters the cell through a receptor or receptors, and the viral genome is imported into the nucleus, where transcription occurs. Pregenomic RNA encapsidation occurs in the cytoplasm. Reverse transcription leads to negative-strand and then positive-strand synthesis. The nucleocapsid undergoes assembly with hepatitis B surface antigen, followed by budding and secretion in blood endoplasmic reticulum. (See companion Expert Consult website for color version.) (From Benhenda S, Cougot D, Buendia MA, Neuveut C: Hepatitis B virus X protein: molecular functions and its role in virus life cycle. *Adv Cancer Res* 2009;**103**:75-109.)

accomplished, followed by plus-strand synthesis and circularization of the genome. The progeny cores then bud into intracellular membranes and acquire glycoprotein envelopes to form nucleocapsids. Nucleocapsids are exported from the hepatocyte cytoplasm toward the cell membrane, where they become invested with surface proteins bound in the membrane bilayer, resulting in budding of mature virions into the bloodstream.

Although HBV can infect numerous tissues, including spleen, kidney, peripheral blood mononuclear cells, and pancreas, the virus replicates exclusively in the liver, because the tissue-specific viral promoter and enhancer are in liver cells. There is no in vitro HBV infection system that can support the entire life cycle of HBV. In the cell culture systems presently available, such as primary duck hepatocytes infected with DHV and immortalized human hepatoma cell lines such as HepG2 and HuH7, the viral replicative cycle is not cytopathic. The infected hepatocytes are normal morphologically and display growth rates identical to those of uninfected controls.[15,174] Human primary hepatocyte cultures are only susceptible to HBV infection for a limited time after explantation,[67] so usefulness has been limited.

Viral Binding and Cell Entry

Studies of the early phases of the viral life cycle have primarily used DHV. The binding reaction has two components: one is of low affinity and is nonsaturable, and the second is of high affinity and saturable.[110] At 37° C, cell entry and viral infection occur. After binding, the virus must fuse with the host cell membrane. This phenomenon appears to occur by a pH-independent mechanism. The binding and entry processes are slow, with maximal infection occurring after 16 hours of cell exposure to the inoculum.[161] The pre-S proteins appear to be involved primarily in the cell fusion interactions, because monoclonal antibodies to these proteins prevent infection.[110] The pre-S proteins also appear to control the narrow spectrum of host determination (i.e., if the virus entry process is bypassed by transfection into heterologous cells, heron HBV is able to replicate in duck cells, which normally are not susceptible to this virus).

The cellular receptors for human HBV are unknown and remain difficult to detect owing to the challenge of maintaining an HBV-susceptible cell line. The hepatotropism of HBV is presumed to be due to the presence of a specific receptor, although none has been identified.[182] Many proteins, such as apolipoprotein H[27] and endonexin 2 (a phospholipid-binding protein), bind to the HBV envelope glycoproteins, particularly S protein.[84] Receptors for pre-S proteins still have not been identified, and the biologic significance of these findings is yet to be determined. The mechanisms by which viral DNA is transported through the hepatocyte is not well understood but likely involve alteration of the nucleocapsid core protein after viral entry.[110]

In the nucleus, viral DNA is transformed to the cccDNA, a process that entails repair of the single-stranded gap, removal of the 5′-terminal structures, and covalent ligation of the strands.[2] Host mechanisms appear to contribute greatly to this process.[113]

Genomic Replication

The first step in HBV genomic replication is the encapsidation of the genomic RNA template.[201] The pregenomic RNA is encapsidated into the core particle.[59] Both C protein and *P* gene products are required to package this small RNA portion into the capsid.[85] Encapsidation of polymerase occurs by binding of the P protein to the RNA that will be packaged.[9] Single-stranded pregenomic

RNA is converted to partially duplex virion DNA through reverse transcription and elongations of the plus and minus strands.

Viral Assembly and Release

The 20-nm subviral particles are assembled between the endoplasmic reticulum and the Golgi apparatus.[92] These 20-nm particles contain predominantly S proteins, which are synthesized in the endoplasmic reticulum.[76] In addition, these particles may contain M subunits but generally do not have L proteins.[81] The assembly process is encoded totally in the S domain.[122] Assembled particles are transported through the secretory pathway and traverse the Golgi complex.[107,198] The S protein carries out the entire assembly process without the involvement of other viral proteins.[2] Therefore, subviral particles containing only envelope proteins are released. In vitro studies have shown that overexpression of L proteins gives rise to filamentous viral particles in the endoplasmic reticulum.[43] The overabundance of L, M, and S aggregates apparently is cytopathic to hepatocytes in vitro. Similar cytopathic features have been seen in human infections with HBV, yet the role of envelope protein expression in hepatocyte injury remains to be determined.[2]

The assembly of the Dane particle differs from the assembly of the 20-nm particles in that all three proteins, L, M, and S, are present.[81] Studies of HepG2 cell lines transfected with HBV mutants have shown that no virus budding occurs if the envelope proteins are not present (mutations in *S*) and that for virion formation and release, both L and S proteins are needed.[25] M proteins apparently are not necessary for viral assembly.

Viral Persistence

Nuclear cccDNA appears to be crucial to viral persistence. Hepatocytes that harbor this form of viral genome are the only ones that produce virus.[2] As mentioned earlier, the replicative cycle of HBV is not cytopathic to hepatocytes, and, therefore, infected cells multiply normally. The cytoplasmic mechanism of reverse transcription, which produces the cccDNA and consequently delivers this product to the nucleus, must therefore be passed on to progeny cells. When HBV DNA is integrated into the cell chromosome, viral DNA may be rearranged, although whether this happens before or after integration is unclear. This rearrangement may lead to disruption of viral genes, especially the core and polymerase genes, whereas the coding regions and promoters for the envelope proteins remain intact.[171]

IMMUNOPATHOGENESIS

HBV is not directly cytopathic, and the variability in liver damage is due to host immune responses. Although HBV preferentially infects hepatocytes and replicates therein, it can infect other liver cells, such as cholangiocytes, as well as cells in extrahepatic tissue such as peripheral blood lymphocytes, pancreatic acinar cells, cornea, spleen, thyroid gland, kidney, adrenal gland, and smooth muscle.[143]

Responses to HBV infection and the likelihood of developing chronic infection are dependent on both age at the time of acquisition and immune competence. Ninety percent of infants born to mothers who are HBeAg positive become chronically infected with HBV, compared with only 5 percent of those infected as adults. The production of antibodies to pre-S and S antigens by B cells, as well as cytotoxic T-lymphocyte (CTL) responses, mediates recovery from acute HBV infection. Individuals who clear HBV have a strong polyclonal human leukocyte antigen (HLA) class I–restricted CTL response to multiple epitopes in the HBV envelope, nucleocapsid, and polymerase regions. This CTL response can be reactivated many years after clearance of all detectable evidence of HBV infection.[131]

Infants infected perinatally are at the highest risk for development of chronic HBV infection. During childhood, those infected as neonates characteristically have high circulating levels of HBV DNA but low disease activity, with very low rates of HBeAg seroconversion, either spontaneously or after treatment with IFN.[131] Researchers have offered numerous hypotheses as to why immune tolerance develops in neonates. One theory proposes that transplacental passage of HBeAg induces $CD4^+$ anergy and, therefore, no CTL responses to HBeAg develop. However, HBeAg-specific T-cell proliferation eventually is detected in those who subsequently seroconvert, rendering clonal deletion an unlikely mechanism.[131] Defective interleukin-2 production has been demonstrated in children who have chronic HBV, although whether it is a cause or consequence of chronic infection is not clear.[75]

The development of chronic infection is either a failure of host immune responses or a testament to the ability of HBV to evade that response. As the virus replicates within hepatocytes, viral antigens appear on the cell surface. Subsequently, cytotoxic T lymphocytes directed against HBcAg infiltrate the liver and cause hepatocyte necrosis.[51] In addition, HBV interferes with the production of cytokines of the Th1 response, especially IFNs that would otherwise elicit class II major histocompatibility complex (MHC) antigen expression and enhance viral clearance. For reasons not fully understood, both the HBV-specific CTL response and the $CD4^+$ helper T-cell response are weak in chronic HBV infection. The high HBV viral load may lead to depletion of peripheral T-cell responses.[63] However, HBeAg, produced in large amounts in chronic HBV infection, and HBsAg share T-cell epitopes. HBeAg may enter the thymus and induce immune tolerance to both HBsAg and HBeAg by decreasing the production of HBV-specific cytotoxic T lymphocytes. However, these cells can be reactivated in patients who undergo a spontaneous or IFN-induced HBeAg seroconversion.[166] Soluble HBeAg may itself contribute to persistence of HBV; there is some evidence that HBeAg favors production of T-helper cell type 2 cytokines rather than T-helper cell type 1 cytokines, which have a greater role in noncytolytic control of HBV.[63] Persistence of HBV infection may also be due to infection of extrahepatic tissues such as the kidney.[63]

Fulminant hepatitis is seen in less than 1 percent of infected individuals and has a high mortality rate. It is characterized by a brisk immune response and viral clearance. Those who survive clear HBV and do not develop chronic HBV infection. A higher incidence of fulminant hepatitis occurs in those patients infected with HBV mutants that do not produce the HBeAg, suggesting either an increased rate of replication or lack of immune detection of the mutant HBV.[116]

Carcinogenesis

Hepatocellular carcinoma is a recognized sequela of chronic infection with HBV; worldwide, most cases of HCC are linked to HBV infection. In Taiwan, the incidence of HCC in chronically infected individuals is 200 to 812 cases per 100,000 person-years, compared with 10 to 30 cases per 100,000 person-years in the general population. In those with liver cirrhosis secondary to chronic HBV infection, the incidence increases to 1,000 to 5,000 cases per 100,000 person-years (1% to 5% per year).[40] Prior to effective therapies, the lifetime risk for development of HCC in a chronically infected person was estimated at 40 to 50 percent.[12] The incidence of HCC is associated with serum HBV DNA levels in a dose-response relationship.[38] The risk for HCC after treatment is not clear; most treated individuals remain HBsAg positive and remain at some risk. The age at which surveillance for HCC should commence and the optimal frequency has not been determined.[135]

The detection of integrated HBV sequences in both human HCC and animal models has led to numerous theories regarding the mechanisms leading to HCC. Current hypotheses center on the integration of viral DNA into host genome, which may be associated with activation of oncogenes, deactivation of tumor suppressor genes, or other genetic instability. The finding that HCC cells in an infected individual contain HBV DNA in discrete rather than random sites, indicating clonal expansion of tumor cells, supports this theory. In general terms, the integrated HBV may affect genes locally by replacing or activating genes by insertional mutagenesis, or HBV may affect more distant genes by means of gene products that can be diffused.[162] Integration of HBV occurs at random sites on host hepatocyte chromosomes rather than at one fixed site. HCC tissue can itself contain a variety of chromosomal abnormalities, such as polyploidy, allelic imbalance, and translocations.[158] More than a dozen genes have been implicated in HCC, not only suggesting heterogeneity of genetic etiologic factors but also indicating that an accumulation of genetic mutations is required for HCC to develop.

Although many mutations are found in HCC, they can be clustered into three main pathways.[202] The first is that of tumor suppressor or DNA damage repair genes typified by *TP53*. A mutation in *TP53* is detected in approximately 30 percent of HCC, although this figure varies by geographic region, being more common in China and less in Europe. The rate of *TP53* mutation is increased by exposure to aflatoxin.[158] A second pathway is that of cell cycle control, the *RB1* pathway. An example is the retinoblastoma gene, which is mutated in about 15 percent of HCC.[227] The final pathway associated with transformation of a normal hepatocyte to a malignant one is the β-catenin and adenomatosis polyposis coli *(APC)* gene system, which is involved in signal transduction. The E-adherin gene, which produces a protein that forms complexes with β-catenin, frequently is mutated in HCC.[186]

Abnormalities in hepatocyte regulatory genes may be activated by the integration of HBV X protein (HBx). HBx can function as a transcription activator, protease inhibitor, and effector of cell signaling pathways. Two other HBV proteins, middle hepatitis B virus surface protein and the large hepatitis B virus protein, have similar properties.[162]

The frequency and significance of integration of viral DNA into the host genome are unresolved issues in childhood HBV infection; some studies have shown early integration, whereas others describe it as a rare event.* Although HCC is detected most often after at least 20 years of chronic HBV infection, cases in children as young as 8 months old have been reported.[72,205,220] Childhood HCC associated with HBV infection has been described in both Asian[37,205] and Western[72,160] populations. Because most cases are reported retrospectively, data regarding incidence are not available. No guidelines have been developed for prospective monitoring of children with chronic HBV infection for development of HCC, although periodic measurement of serum α-fetoprotein levels and hepatic ultrasound evaluations are recommended in adults.

Genotype of HBV may be an indirect marker of HCC risk. Genotype C with TCC at codon 15 of the precore region is associated with an increased risk for HCC in a Hong Kong population with cirrhosis.[30] Prevention of HBV infection and eradication of existing infection remain the most effective measures to prevent HCC. After a nationwide hepatitis B vaccination program implemented in Taiwan in 1984, the incidence of HCC in children between 6 and 14 years old declined from 0.7 per 100,000 children between 1981 and 1986 to 0.36 per 100,000 children between 1990 and 1994.[35] The incidence of HCC was lower in vaccinated versus unvaccinated Taiwanese children, with an age- and sex-adjusted relative risk reduction of 0.31.[33] The 5-year survival rate in children with HCC is less than 30 percent, despite treatment.[223]

EPIDEMIOLOGY

HBV infection occurs throughout the world, although there are marked geographic differences.

Areas of high, intermediate, and low endemicity, as reflected by the number of carriers, have been identified. Highly endemic areas are those in which more than 8 percent of the population is infected. Examples include Southeast Asia, Africa, Commonwealth of Independent States, and China. More than 70 percent of the world's population lives in these high-endemicity areas. The Middle East, Central and South America, and parts of Eastern Europe are examples of intermediate-endemicity regions, where infection is found in 2 to 7 percent of the

*References 10, 36, 74, 181, 217, 221.

population. The United States, Australia, and Western Europe are areas of low endemicity, with chronic infection rates of less than 2 percent of the total population.[211] In areas of low endemicity, acute infections occur more commonly in adults, which, although causing significant morbidity, are less likely to result in chronic carriage than are asymptomatic infections in children.

In the United States, the prevalence of chronic HBV infection (HBsAg positive) among the general population, from 1999 to 2006, was 0.27 percent. This study revealed that 4.7 percent of the U.S. population had evidence of either past or chronic infection with HBV,[215] not statistically different from that reported in the National Health and Nutrition Examination Surveys (NHANES III) survey from 1988 to 1994.[147] The rates among ethnic groups varied, with non-Hispanic blacks having the highest rates (12.2%) compared with non-Hispanic whites (2.8%) and Mexican Americans (2.9%). The prevalence rate of chronic HBV infection was higher among foreign-born children (0.89%) than U.S.-born children (0.16%).[215]

HBV may be transmitted vertically, horizontally, parenterally, or sexually. Vertical transmission of HBV occurs in the perinatal setting when an infected mother transmits the infection to her child. Horizontal transmission occurs among children in groups with moderate to high endemicity[58] who were not infected at birth. This form of transmission occurs in households of an infected family member, in which other members acquire HBV without sexual or overt contact with contaminated bodily fluid. In adolescents and adults, infection is transmitted by contaminated blood or other bodily fluids through percutaneous or mucous membrane routes. Examples are intravenous drug use; occupational exposure to blood; sexual contact, especially in men who have sex with men; and nosocomial infections from contaminated shared equipment.[142]

In countries where HBV infection is highly endemic, perinatal or vertical transmission is very common, whereas in regions of low endemicity, sexual transmission is more common. If a mother is seropositive for both HBeAg and HBsAg, the risk for transmission of HBV to her child is 85 to 90 percent without immunoprophylaxis.[8] The most common risk factors for acquisition of acute HBV in adults in the United States from 1982 to 1998 were heterosexual exposure to a person with hepatitis or having multiple sexual partners (27%), intravenous drug use (18%), men who have sex with men (13%), household contact (3.6%), and occupational exposure (16%).[73] Approximately one third of people reported no risk factors during the study period.[73]

Within the United States, the number of reported cases of acute hepatitis B decreased 81 percent between 1990 and 2006 to the lowest rate ever recorded (1.6 per 100,000 population).[214] The largest decline was in children younger than 15 years old. Hepatitis B elimination strategies such as screening of pregnant women, universal immunization of infants, and catch-up immunization of older children are credited with the reduced incidence in children. Strategies to eliminate hepatitis B are outlined in Table 156-1.

The experience from Taiwan, the first country to implement mass vaccination against HBV, is encouraging. Since 1986, all neonates have been vaccinated against HBV. Seroprevalence of HBsAg declined from 9.8 percent before this strategy to 0.6 percent 20 years later.[150] There was a significant decrease in the incidence of HCC, from 0.54 to 0.20 per 100,000 children, between 1986 and 2000. The proportion of infants and children with hepatitis B as a cause of fulminant hepatic failure in Taiwan decreased from 82 percent before mass vaccination to 57 percent.[39]

Researchers have estimated that only 45 percent of the expected 20,000 HBsAg-positive women giving birth in the United States between 1993 and 1996 were identified.[187] This low figure may be due to numerous factors; women likely to be infected often do not receive prenatal care,[185] or women may be tested but the information not conveyed to the place of birth or to the pediatrician caring for the child.[8] Attention to this aspect of care is extremely important because the reduction in rate of transmission from mother to child with use of hepatitis B immunoglobulin (HBIG) and hepatitis B vaccine is as high as 90 percent.[197] HBsAg can be found in the breast milk of HBV-infected mothers. However, in studies performed in Taiwan and England, breast feeding by HBsAg-seropositive mothers did not significantly increase the risk for neonatal acquisition of HBV infection.[159] A study of 230 babies in China demonstrated that breast-fed babies had similar response rates to HBV vaccination and failure rates to HBV immunoprophylaxis as bottle-fed babies.[213] Effective immunoprophylaxis should allow for safe breast feeding.

TABLE 156-1 Strategies to Eliminate Transmission of Hepatitis B Virus Infection in the United States

- Universal vaccination of infants beginning at birth
- Prevention of perinatal HBV infection through:
  - Routine screening of all pregnant women for hepatitis B surface antigen (HBsAg)
  - Immunoprophylaxis of infants born to HBsAg-positive women and infants born to women with unknown HBsAg status
- Routine vaccination of all previously unvaccinated children and adolescents
- Vaccination of previously unvaccinated adults at increased risk for infection

From Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005;**54**(RR-16):1-31.

NATURAL HISTORY

Acute HBV Infection

Acute hepatitis B infection is most common in young men older than 19 years and women older than 40 years,[54,73] but most do not develop chronic disease. The clinical and serologic findings in acute hepatitis B infection are shown in Figure 156-3. Children infected

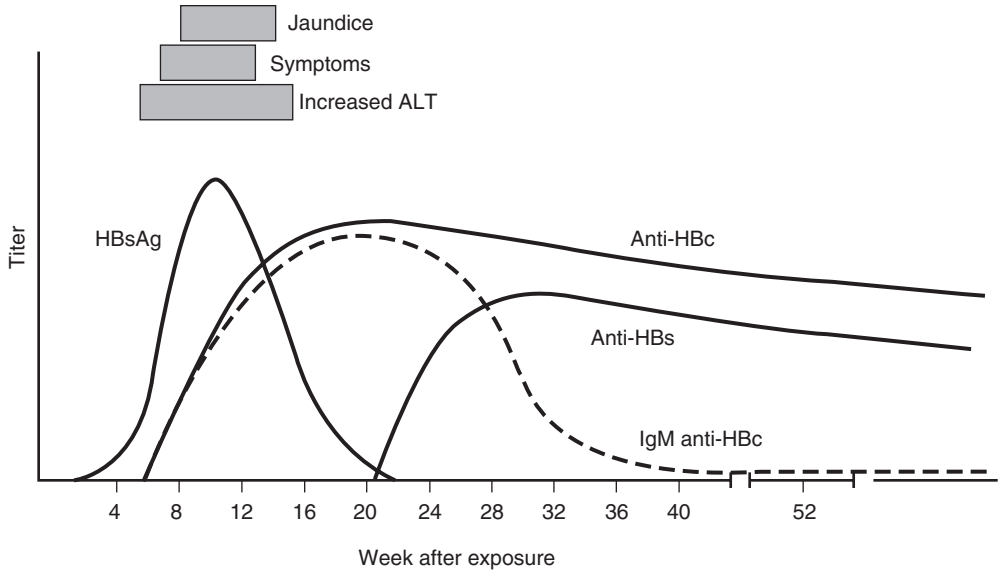

FIGURE 156-3 ■ Clinical and serologic features of acute infection with hepatitis B virus. Hepatitis B surface antigen (HBsAg) is detectable within 4 weeks and reaches a peak value at 12 weeks. This coincides with the onset of jaundice. Hepatitis B e antigen becomes detectable at the same time. Anti–hepatitis B core (Anti-HBc) IgM is detected from 6 to 8 weeks and peaks at 20 weeks. It then declines, but anti-HBc IgG persists. On resolution of acute infection, the serum contains anti-HBc, IgG type, and anti-HBs. ALT, alanine aminotransferase.

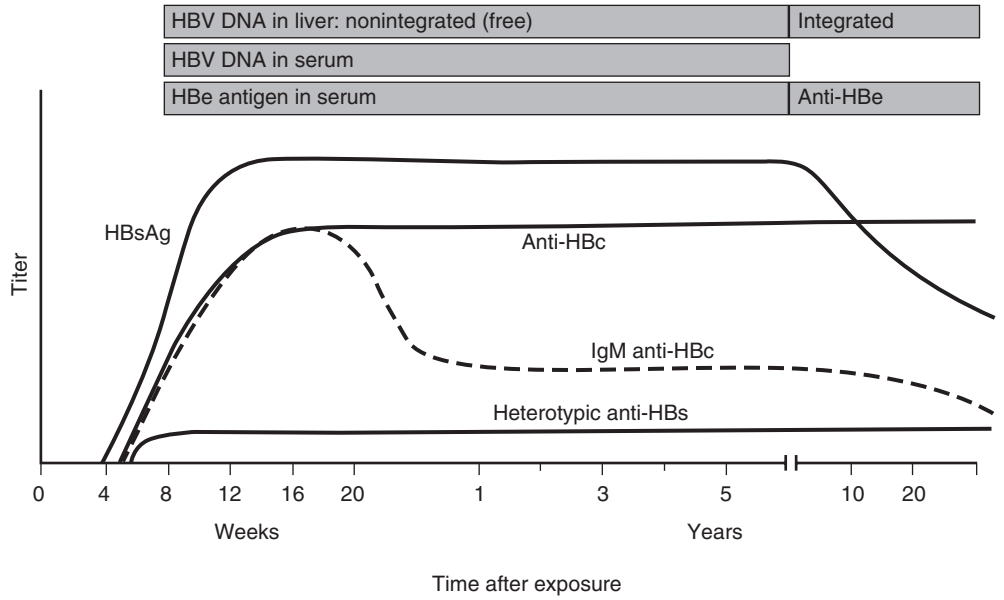

FIGURE 156-4 ■ Clinical and serologic features of chronic hepatitis B virus (HBV) infection. Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and anti–hepatitis B core (HBc) antibody are detectable and may persist for years. Seroconversion from HBeAg to HBe antibody is associated with very low levels of HBV DNA in serum. The diagnosis of chronic HBV infection is dependent on finding HBsAg for at least 6 months or the combination of HBsAg and anti-HBc of the IgG class.

perinatally rarely develop acute hepatitis, but fulminant hepatic failure can develop, particularly in infants born to HBeAg-negative mothers.[39]

Chronic HBV Infection

The natural history of chronic HBV infection in children has been examined in several populations. The clinical and serologic findings of chronic hepatitis B are shown in Figure 156-4. Most Chinese children, in whom chronic HBV usually is perinatally acquired, remain HBeAg positive, with very high levels of viral replication yet only minimal clinical liver disease.[133,134] In contrast, children in the West with chronic HBV infection frequently clear HBeAg and HBV DNA from serum during the first 2 decades.[22] In extended follow-up of 14.5 ± 6.1 years of 89

white children in Italy after seroclearance of HBeAg, 4 experienced reactivation, 3 of whom had HBeAg-negative hepatitis.[23] Those who lose HBeAg tend to have higher alanine aminotransferase (ALT) levels early in life, indicating more active liver disease. Some of the children (15.4%) become seronegative for HBsAg as well.[23] Reactivation of viral replication is observed only rarely. Children born to Asian and Afro-Caribbean mothers in the United Kingdom who acquired HBV infection perinatally had a much lower (29%) rate of HBeAg seroconversion than white children in this U.K. study (80%)[24] or Italian children (98%).[23] These differences are ascribed to the older age at acquisition of HBV in the Western children, associated with a more efficient immune response. These observations influence the management and counseling of children with chronic HBV infection as well as the design and interpretation of therapeutic trials.

The risk for acquiring chronic hepatitis B is related to a number of factors, such as very young age, other medical conditions, and other infections. If an infected person has other medical problems, such as end-stage renal disease requiring hemodialysis, or is coinfected with HIV, the risk for development of chronic HBV infection increases. The risk for development of HCC is increased in all HBV chronic carriers but especially those with active viral replication.[12] Four of 99 Italian children had HBeAg-positive cirrhosis at the start of the study, and after 14.5 ± 6.1 years follow-up 2 developed HCC and 2 had lost histopathologic features of cirrhosis.[21,23]

Extrahepatic manifestations of both acute and chronic HBV infection occur more commonly in adults. Up to 25 percent of adults with acute HBV infection have extrahepatic manifestations such as arthralgia and even serum-like sickness. Vasculitides can develop in both acute and chronic HBV infection, and examples include polyarteritis nodosa, renal disease, and mononeuritis.[146] Membranoproliferative glomerulonephritis is an extrahepatic manifestation of chronic HBV infection in some children.[109,194] There is a case report of epididymitis related to acute hepatitis B in a 12-year-old boy.[206]

HISTOPATHOLOGIC FEATURES

The purposes of obtaining a liver biopsy in chronic HBV infection are to quantify the severity of changes, to rule out other causes of liver disease, and to allow comparison of changes after treatment. In typical acute viral hepatitis, a liver biopsy usually is not required or indicated. If performed, the findings are liver cell degeneration and necrosis, with lobular disarray. The acute injury to hepatocytes is manifested as both ballooning and acidophilic degeneration, either of which can resolve or progress to cell death. Inflammatory cell infiltration and liver cell regeneration are also seen.[89]

The histopathologic changes of chronic HBV infection are characterized by portal tract inflammation, interface hepatitis, and fibrosis, which may progress to architectural distortion of lobules and eventual cirrhosis.[89] Chronic HBV infection is characterized by "ground-glass" cells (Fig. 156-5A), which are hepatocytes containing HBsAg and most easily identified by staining with orcein, Victoria blue, or aldehyde fuchsin. However, these cells also are found in other liver diseases, and the pattern of staining is important. In hepatitis B, ground-glass cells are found either singly or in clusters arranged in a haphazard pattern. "Sanded" liver nuclei are caused by accumulation of HBcAg and generally are identified by electron microscopy[94] or immunoperoxidase techniques (see Fig. 156-5B).

Histopathologic staging and grading systems are used to allow standardization and meaningful comparisons in chronic hepatitis. One such system is the Knodell-Ishak score, which evaluates four histopathologic features—each scored using specific criteria.[111] This system has proved reproducible, is now is in common use, and is also known as the modified histologic activity index (HAI). Other systems are the Batts-Ludwig[11] and METAVIR systems.[173] The systematic classifications are useful in estimating progression of disease and response to therapy and for comparing outcomes of therapeutic trials. A study from Poland demonstrated that these scores were useful

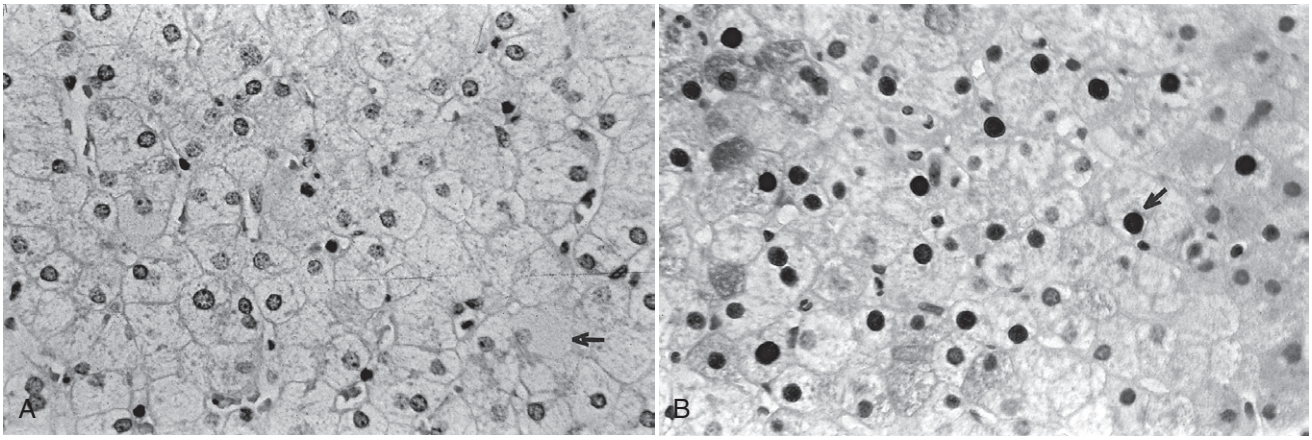

FIGURE 156-5 ■ A, Photomicrograph of a liver biopsy specimen from a child with chronic hepatitis B virus (HBV) infection. Ground-glass hepatocytes *(arrow)* are demonstrated (hematoxylin and eosin stain; original magnification ×20). **B,** Photomicrograph of the liver of a child with chronic HBV infection. The darkly stained nuclei *(arrow)* contain hepatitis B core antigen (HBcAg) (immunoperoxidase stain for HBcAg; original magnification ×20).

in comparing liver biopsy specimens in children before and after IFN-α treatment and that inflammatory but not fibrosis scores improved after treatment.[188] However, another study demonstrated interobserver variability in grading and staging of pediatric liver biopsy specimens.[218]

Serum markers of hepatic fibrosis, such as apolipoprotein A1, haptoglobin, and α2-macroglobulin have been investigated in children. They present many theoretical advantages for noninvasive monitoring of patients, but no single marker has yet proven useful in children with chronic HBV infection.[125] Serum noninvasive models using a combination of serum markers and enzyme immunoassays now more commonly used in chronic hepatitis C were applied to 179 adult patients with chronic hepatitis B.[164] The authors suggest that these values may be helpful in determining which patients with normal ALT values would benefit from early therapy.

Other noninvasive techniques studied in adults include transient elastography and acoustic radiation force impulse. These modalities appear promising in identifying patients with normal or near-normal ALT but significant fibrosis who might benefit from therapy.[108]

TREATMENT

Treatment of acute hepatitis B is purely supportive, and most patients recover fully and with permanent immunity (anti-HBs). The role of antiviral therapy in fulminant HBV infection has not been studied systematically. Treatment, when indicated, is directed at those with chronic HBV infection. In this setting, the long-term goals of therapy are eradication of HBV and improvement or regression of liver disease. Other goals for treatment include cessation or decrease in viral replication as indicated by loss of HBeAg and loss of HBV DNA from serum, clearance of HBsAg, normalization of liver histopathology, normalization of aminotransferase values, and reduction of the risk for HCC.

To date, no agents fulfill all of these goals in either adults or children. Children and adults have differences in immune tolerance and rate of progression of liver disease. Therefore, studies of treatments of chronic HBV in adults cannot be extrapolated directly to children. In addition, the timing and choice of therapeutic agent are crucial because of differences in age, maturity, stage in natural history, and comorbidities.

A child being considered for treatment should have serologic evidence of HBV infection, such as HBsAg for at least 6 months, as well as evidence of active HBV replication, HBeAg, or HBV DNA (in case of eAg-negative HBV infection). In addition, children with consistent elevation of ALT to at least twice the upper limit of normal are most likely to benefit from treatment. A liver biopsy should be obtained before the start of therapy to provide evidence of chronic hepatitis, to stage the disease, and to rule out other processes. In most studies performed before the high prevalence of eAg-negative infections, seroconversion from HBeAg to anti-HBe in serum was used as the primary outcome variable for response to therapy. Therapeutic trial results must be compared with the "background rate" of spontaneous conversion from HBeAg positive to anti-HBe, which in children younger than 3 years of age are very low (<2% per year) but increase with age so that in children older than 6 years of age, the yearly clearance rates vary from 14.3 to 35.3 percent.[34]

Quantitative HBsAg and quantitative HBeAg are being investigated as predictors for viral loads and treatment responses. Varying levels of quantitative HBsAg have been documented over the natural course of HBV infection and also between different genotypes.[96] Levels of HBsAg decrease with increasing age and progression of disease with increased fibrosis.[95] These values may be helpful in determining treatment strategies and algorithms for stopping therapy.[31,126]

Two medications that are approved in the United States for treatment of chronic HBV in young children younger than the age of 12 are IFN-α and lamivudine. Neither of these is still used in adults, because of the availability of drugs with better tolerance and better resistance profiles. Adefovir dipivoxil is licensed for adolescents age 12 years and older, and entecavir is licensed for those 16 years of age and older.

Interferon

IFNs are a family of cytokines with immunomodulatory, antiproliferative, and direct antiviral actions. There are three types: α, β, and γ. IFN-α has been the primary form used in treatment of chronic HBV infection. It induces the display of HLA class I molecules on hepatocyte membranes, which in turn promotes lysis by $CD8^+$ cytotoxic lymphocytes. At the same time, it directly inhibits viral protein synthesis.[127] IFN-α is available in several forms, including IFN-α2a, IFN-α2b, and lymphoblastoid IFN-α. Only IFN-α2a and IFN-α2b have been licensed in the United States for use in children. Peginterferon-α, in which the IFN molecule is attached to polyethylene glycol to allow for once weekly versus three times weekly injections, is the preferred form in adults but is not currently approved in the United States for children with chronic HBV.

IFN-α treatment led to loss of HBV DNA or HBeAg seroconversion in 20 to 58 percent of European as compared with 8 to 17 percent of control children.[210] Success rates were not as high for treatment of children in Asian countries, most of whom were perinatally infected; only 3 to 17 percent cleared HBV DNA or had HBeAg seroconversion in early studies.[118] However, in a large multinational, randomized control trial of IFN-α2b, no differences were found in loss of HBsAg between children born in Asian countries (22%) and those from Europe and North America (26%) if aminotransferase values were elevated.[190] If only children with elevated aminotransferase levels are selected for treatment with IFN-α, then children younger than 13 years of age with intermediate HBV DNA levels are most likely to have a good response, regardless of ethnicity.[190]

Reported studies of IFN-α included only children older than 18 months of age without hepatitis C and HIV coinfections because these factors may alter treatment algorithms. Children with evidence of active

immunologic responses to HBV (i.e., low levels of viral replication [HBV-DNA]) and high ALT values (greater than twice normal) are more likely to have a response to therapy with IFN-α.[208] Children who acquired disease by vertical transmission are more likely to have established immune tolerance and may be less likely to respond to treatment. Children with very low HBV DNA levels may be in the process of spontaneous seroconversion; therefore, an expectant policy may be prudent.

Ten percent of treated adults and children lose HBsAg, compared with 1 percent of untreated patients.[115,191] Long-term (1.1 to 11.5 years) follow-up of adult responders to IFN-α showed a significantly reduced incidence of HCC compared with either treated nonresponders or untreated control patients.[129] The long-term outlook for treated children is not yet clear. In a Turkish study, none of 23 treatment nonresponder developed cirrhosis or HCC at a mean follow-up of 4.5 years.[112] A second study compared long-term (5.6 ± 3.1 years) outcome in two groups of 37 children: one group was treated with IFN-α2β and the second control group of children age matched for age, sex, and baseline ALT level received no treatment. At time of follow-up, the rates of HBeAg and HBsAg loss were 54.1 percent (20/37) and 8.1 percent (3/37) in treated children compared with 35.1 percent (13/37) and 2.7 percent (1/37), respectively, in untreated children (NS). Children with ALT levels elevated more than twice normal who were treated had higher rates of HBeAg seroconversion at follow-up compared with children with elevated ALT who were not treated.[212] A study from Sweden demonstrated that pretreatment HBV DNA levels were associated with sustained virologic response.[189] Histologic outcomes in children with chronic HBV treated with IFN-α for 20 weeks have been reported in 93 children, average age 7.1 years, in Poland. Liver biopsy samples were obtained before treatment and 12 months after the end of the treatment and graded for fibrosis and inflammation using three different scoring systems. Thirty-five children underwent HBeAg/anti-HBe seroconversion. Liver fibrosis did not improve regardless of responder status, but inflammatory activity was decreased after treatment in both responders and nonresponders.[218]

Side effects of IFN-α include a transient influenza-like syndrome (fever, myalgia, headache, arthralgia, and anorexia) that occurs in virtually all patients at the start of therapy. Bone marrow suppression, especially neutropenia, is a common side effect, seen in 39 percent of children in one series.[93] Changes in personality, irritability, and temper tantrums are reported more frequently in children than in adults.[93,191] These problems resolve once treatment is withdrawn. Febrile seizures and marked elevation of aminotransferase levels have also been reported. Quality of life is impaired in children during IFN therapy compared with their pretreatment levels because of medication side effects and fear of injections. However, within 3 months of cessation of treatment, these effects are reversed.[93] Two meta-analyses of treatment of chronic HBV in children with IFN-α have been conducted.[208,209] Each concludes that treatment leads to a higher rate of HBeAg seroconversion than would occur spontaneously. Approximately two thirds of children treated with IFN-α show no sustained response to therapy; more effective and better- tolerated treatments are required.

HBV Polymerase Inhibitors

This class of agents includes the nucleoside and nucleotide analogs that inhibit HBV polymerase by incorporation into viral DNA, leading to termination. They are administered orally and generally well tolerated.[137]

Lamivudine

Lamivudine (2′,3′-dideoxycytosine), also known as 3TC, is an oral nucleoside analog. It is triphosphorylated intracellularly to an active intermediate that is incorporated into the growing DNA chain, thereby terminating the chain and inhibiting viral replication. Two large, randomized, controlled studies of lamivudine as initial treatment for chronic HBV infection in adults, showed serologic, biochemical, and histologic evidence of benefit.[55,117]

There are a number of studies demonstrating benefit of lamivudine in children. The first was a double-blind, placebo-controlled, randomized, multicenter study of lamivudine for 52 weeks in 191 children aged 2 to 17 years at a dose of 3 mg/kg/day (maximum dose, 100 mg) compared with placebo in 97 children.[101] All children in the study were HBeAg positive and had detectable HBV DNA and elevated ALT values. Twenty-three percent (44/191) of those treated with lamivudine cleared HBV DNA and HBeAg compared with 13 percent (12/97) in the placebo group (p <.05). Nineteen percent (31/166) developed the primary mutation associated with lamivudine resistance (i.e., tyrosine-methionine-aspartate-aspartate [YMDD]) in the first 48 weeks of therapy. Higher response rates were observed in those with higher ALT levels, as had been noted with IFN. In an open-label study of lamivudine in 29 adolescents and young adults with maternally transmitted chronic HBV infection in Taiwan, only those with ALT more than five times the upper limit of normal demonstrated a response (undetectable HBV DNA, normal ALT, and HBeAg/anti-HBe seroconversion) than control patients after 52 weeks of treatment.[151]

The optimum duration of lamivudine therapy was investigated in a multicenter, open-label study of prolonged therapy in children who had participated in the previous 1-year study.[192] Two hundred and 13 children were HBeAg positive; 133 had originally received lamivudine and 77 placebo, and all were offered lamivudine for a further 2 years. At the end of the study, 51 more children had achieved virologic response. However, 100/173 had developed the YMDD variant of HBV. The virologic response to those with YMDD variant was only 5 percent (5/100) compared with 49 percent (34/70) in those with wild-type HBV. Sixty-three children who were HBeAg negative (49 previously treated with lamivudine and 14 with placebo) at the start of the study were observed for 2 more years. At the end of the study period, 48/54 (89%) had a durable virologic response. No serious side effects of lamivudine were reported.

Based on the observation that both therapeutic response and development of resistant mutants increase in frequency with treatment duration, the optimal duration of lamivudine therapy has not been defined. In fact, this agent has largely been replaced in adult HBV treatment by newer drugs with more favorable resistance profiles.

Selecting the children most likely to benefit from lamivudine remains a challenge. Those most likely to have a sustained virologic response to lamivudine are those with HBeAg-positive chronic hepatitis and aminotransferase levels twice the upper limit of normal. If the YMDD variant emerges, lamivudine should be discontinued and liver enzyme levels monitored. In patients with advanced liver disease, alternate therapy may need to be provided. Lamivudine is excreted by the kidneys; thus, the dose should be reduced in patients with renal failure.

On cessation of therapy in adults, 25 percent of treated patients had elevation of serum ALT of up to three times baseline, as compared with 8 percent of placebo-treated patients. This "lamivudine withdrawal flare" may progress to jaundice and incipient liver failure, as noted in 2 of 41 patients in a Dutch study.[86] Therefore, monitoring patients after a course of lamivudine is important.

Combination of IFN-α and Lamivudine

Studies of combination therapy with IFN-α and lamivudine have been done in both children and adults. In adults with HBeAg-positive chronic HBV, peginterferon-α2a either alone or in combination with lamivudine for 48 weeks was superior to lamivudine alone.[121] At the end of week 72, the endpoint defined as HBeAg seroconversion, normalization of ALT and HBV DNA less than 100,000 copies/mL was achieved by 56 of 271 (21%) persons with peginterferon plus lamivudine compared with 62 of 271 (23%) persons who received peginterferon plus placebo and 28 of 272 (10%) who received lamivudine alone. An open-label, single-treatment arm, single-center study of combined IFN-α and lamivudine in children with perinatally acquired chronic hepatitis B was reported from the United Kingdom.[49] The treated children were traditionally regarded as poor responders, that is, infected in the first year of life, were HBeAg positive, and had normal aminotransferases. Only 23 children were treated with lamivudine alone for 8 weeks followed by lamivudine plus IFN-α 5 MU/m^2 three times weekly for 10 months. Five seroconverted to anti-HBe and four of these five became HBsAg negative and HBsAb positive. No child developed a YMDD mutant, and most (78%) became HBV DNA negative. These results have not yet been duplicated in larger studies.

Adefovir Dipivoxil

Adefovir dipivoxil is a synthetic nucleotide analog of adenosine monophosphate.[226] It suppresses HBV in both HBeAg-positive and HBeAg-negative adults and children, and drug resistance is less frequent and emerges later compared with lamivudine. Adefovir dipivoxil has been used to decrease HBV DNA and aminotransferase levels with prolonged treatment (144 weeks) in adults with HBeAg-negative chronic hepatitis B infection.[79] When treatment was withdrawn in one subgroup after 48 weeks, the virologic and biochemical benefits were lost, suggesting long-term treatment is required for viral suppression in those with HBeAg-negative chronic hepatitis B. The rate of development of resistance was somewhat lower (5.9% after 144 weeks) than that observed with lamivudine. Elevation of serum creatinine level was the most serious adverse event, and on-and-off treatment ALT elevations were common. In a double-blind randomized trial of 480 HBeAg-positive adults in China, suppression of HBV DNA was observed in those receiving adefovir 10 mg daily for 52 weeks whether they had wild-type or YMDD mutant infection.[226] A study of adefovir dipivoxil in 173 children with HBeAg-positive chronic hepatitis B showed that 23 percent of children aged 12 to 18 years of age were able to achieve a normal ALT and HBV DNA less than 1000 copies/mL compared with no subjects treated with placebo. No subjects developed mutations from the adefovir.[99] No statistical difference in this primary outcome was seen in patients younger than 12 years of age, but some HBeAg seroconversions were noted in the younger group of treated children. A follow-up of longer-term treatment in these patients noted continued safety, and some cumulative virologic responses with prolonged therapy, but treatment was discontinued in subjects with virologic failure (persistence of HBV DNA >1000 copies/mL) to minimize resistance risk.[100]

Entecavir

Entecavir (ETV) is a cyclopentyl guanosine analog that inhibits HBV polymerase; it was approved in the United States for use in patients 16 years and older in 2005.[137] Entecavir for 48 weeks suppresses HBV DNA levels in both HBeAg-positive and HBeAg-negative adults at higher rates compared with lamivudine.[132] Entecavir is considered one of the first-line agents for patients with HBeAg-positive or HBeAg-negative chronic HBV infection given its low risk for resistance. Entecavir is able to suppress both lamivudine-resistant and adefovir-resistant viruses, although a higher dose of the medication is indicated in lamivudine-experienced patients, owing to some cross resistance. Entecavir resistance is rare, except in individuals who harbor lamivudine-resistant HBV. Most adverse events are mild, although there is a theoretical risk for hepatotoxicity with lactic acidosis. A phase III randomized, controlled trial with entecavir in subjects 2 to 16 years of age is underway.

Tenofovir disoproxil fumarate (TDF) is a nucleotide analog reverse-transcriptase inhibitor (NRTI) that was licensed in the United States for use in adults with HBV infection in 2008. It is also used in combination with other antiretroviral agents for the treatment of HIV infection in adults. A randomized, placebo-controlled trial using tenofovir in adolescents demonstrated potent antiviral effects with acceptable safety. One hundred six patients were enrolled to take either tenofovir 300 mg or placebo once daily for 72 weeks. Normalization of the ALT value occurred in 74 percent of patients taking tenofovir and 31 percent of patients taking placebo. Adverse events were not common in either group, but the bone mineral density increased less in the tenofovir group than the placebo group over the course of therapy group.[149]

Table 156-2 summarizes published trials to date in children with chronic HBV.

HEPATITIS B IN SPECIAL POPULATIONS

Hepatitis B and Human Immunodeficiency Virus Coinfection

The prevalence and course of HBV infection in adults who are coinfected with HIV has been examined in numerous studies. Of 181 HIV-infected adults in Greece, 71.8 percent of the men who have sex with men and 91.7 percent of intravenous drug users had evidence of HBV infection.[57] In a study from Australia conducted between 1985 and 1989, men with HIV infection were more likely to have chronic HBV infection than were HIV-negative men.[17] In Western Europe and the United States among HIV-infected persons, 6 to 14 percent have chronic hepatitis B, including 4 to 6 percent of heterosexuals, 9 to 17 percent of men who have sex with men, and 7 to 10 percent of injection drug users.[3] A recent study in Thailand found the prevalence of HBV coinfection in perinatally HIV-infected adolescents to be 3.3 percent.[6] No evidence has been found for direct interaction between HBV and HIV. HIV and HBV coinfected individuals have higher circulating levels of HBV DNA but not worse hepatic necroinflammation.[45] In patients with

TABLE 156-2 **Summary of Therapeutic Trials in Children**

Author, Year	Treatment Regimens	HBeAg-Negative 6 Months after Therapy	HBV DNA–Negative 6 Months After Therapy
Barbera, 1994[7]	Group 1 (n = 21): IFN-α2a, 7.5 MU/m^2	Group 1: 5/21	Group 1: 5/20
	Group 2 (n = 19): IFN-α2a, 3 MU/m^2	Group 2: 4/19	Group 2: 4/19
	Controls (n = 37): No treatment	Controls: 3/37	Controls: 7/37
		p <.05, group 1 vs. 3	NS
Sokal, 1998[190]	Treatment (n = 72): IFN-α2b	Treatment: 25/72	Treatment: 27/72
	Control (n = 77): No treatment	Controls: 8/74	Controls: 8/74
		p <.05	p <.05
Gurakan, 2000[77]	Group 1 (n = 15): IFN-α2b, 5 MU/m^2	Group 1: 5/15	Group 1: 6/15
	Group 2 (n = 15): IFN-α2b, 10 MU/m^2	Group 2: 9/15	Group 2: 9/15
		p <.05	p <.05
Giacchino, 1995[71]	Group 1 (n = 16): Human lymphoblastoid IFN-α preceded by 4 wk of prednisolone, 0.6 mg/kg/day	Group 1: 7/16	Group 1: 10/11
	Group 2 (n = 19): IFN-α preceded by placebo	Group 2: 0/19	Group 2: 11/19
		NS	NS
Jonas, 2002[101]	Treatment (n = 191): Lamivudine daily × 52 wk	Treatment: 50/191	Treatment: 117/191
	Controls (n = 95): Placebo daily × 52 wk	Placebo: 14/95	Placebo: 15/191
			p <.001
Sokal, 2006[192]	Treatment (n = 213): Lamivudine daily × 52 wk, all HBeAg-positive (133 previously treated with lamivudine × 52 wk)	Treatment: 51/213	As before
	Observation (n = 63): Previously treated with lamivudine × 52 wk, all HBeAg negative	Control: 48/54 (sustained response 52 wk after end of treatment)	
Dikici, 2001[56]	Group 1 (n = 30): IFN-α2b and lamivudine	Group 1: 11/30	Group 1: 29/30
	Group 2 (n = 27): Same medications but for 12 months	Group 2: 15/27	Group 2: 26/29
		NS	NS
D'Antiga, 2006[49]	Pilot study (n = 23)		
	Lamivudine, 3 mg/kg daily alone × 8 wk, then lamivudine, 3 mg/kg daily × 48 wk, and IFN-α, 5 MU/m^2 3/wk × 40 wk	5/23 anti-HBe	18/23
Jonas, 2008[99]	Group 1 (n = 113): adefovir	Group 1: 12/113	Group 1: 18/113
	Group 2 (n = 57): placebo	Group 2: 3/57	Group 2: 0/57
Sokucu, 2006[193]	Group 1 (n = 35): IFN-α (5 MU/m^2)	Group 1: 8/35	Group 1: 7/35
	Group 2 (n = 30): IFN-α with lamivudine	Group 2: 12/30	Group 2: 19/30
		NS	p = .01
Murray, 2012[149]	Group 1 (n = 52): tenofovir 300 mg	Group 1: 10/48	Group 1: 46/52
	Group 2 (n = 54): placebo	Group 2: 7/48	Group 2: 0/54
		NS	p <.0001

HBeAg, hepatitis B e antigen; IFN, interferon; NS, not significant.

HBV and HIV coinfection there are higher rates of HBV persistence, HBV relapse (reemergence of HBsAg), and more severe and rapid progression of liver disease. Spontaneous HBeAg seroconversion rarely occurs in this group. Special considerations for treatment and immunization are discussed in the appropriate subsections.

Hepatitis B in Solid-Organ Transplant Recipients

Chronic HBV infection became a relative contraindication to liver transplantation after initial poor results. In the 1970s and 1980s, more than 80 percent of patients became reinfected quickly and 55 percent died within 60 days of surgery. Patients who had recurrence of HBV infection after liver transplantation developed a characteristic histologic lesion, termed *fibrosing cholestatic hepatitis*, which eventually led to loss of the allograft.[14] The term *de novo HBV infection* is defined as HBV infection occurring in HBsAg-negative liver recipients who become HBsAg positive after transplant. In a retrospective study of 71 HBsAg-negative Taiwanese children who received orthotopic liver transplantation, 15.3 percent of patients developed de novo HBV infection. In this study, higher HBsAb titers (>200 mIU/mL) were thought to be sufficient to possibly prevent de novo HBV infection in liver transplant patients.[199] The demonstration in the 1990s that HBIG is efficacious in decreasing infection of the allograft has allowed successful transplantation. The use of long-term HBIG decreases the risk for recurrence to 30 percent at 12 months and increases patient survival to greater than 90 percent at 12 months after transplantation.[177] Lamivudine has been added to HBIG after the transplant procedure. In a study from China of 69 patients with chronic HBV infection who received liver transplants and treatment to prevent reinfection, 10 died fewer than 3 months after transplant.[222] Of the remaining 59 patients, 8 were not included in follow-up because they did not receive treatment with HBIG and lamivudine, leaving 51 patients in the final analysis. The mean follow-up was 14.1 months, and 2 of 51 had recurrent disease; 1 died and the infection in the other resolved.[222] The allograft reinfection rate is even lower if the patient does not have active HBV replication at the time of transplantation, which is why lamivudine has been added in the pretransplant period. The cost of HBIG is a major factor, and duration of therapy appears to be lifelong to maintain protective HBs titer.

HBV infection is the fourth most common cause of acute liver failure in adults in the United States.[157] Rarely, however, does a child in the United States with HBV need a liver transplant for either acute or chronic liver failure.[195] Of 215 children who underwent transplantation between 1986 and 1992 at a major French center, only 4 had HBV-associated disease.[141]

The course of HBV infection in heart transplant recipients was examined in a French study in which 69 of 874 heart transplant patients were infected with HBV, most through nosocomial infection during endomyocardial biopsies. HBV infection had little impact on 5-year survival.[139] In a group of 120 lung transplant recipients in Israel, 11 patients with either chronic hepatitis B or receipt of an organ from an HBcAb-positive donor were given lamivudine prophylaxis. Only 1 patient developed resistance, which responded to treatment with adefovir. Two patients had reactivation of HBV infection with high aminotransferase levels and high HBV-DNA levels.[184] In a study from Taiwan, overall patient and allograft survival after kidney transplantation were not affected by HBV infection, despite increased hepatic morbidity.[91] Of the 113 patients who received kidney transplants between 1986 and 1998, 20 patients were HBsAg positive and 9 were positive for both HBsAg and HCV antibody. Of the 20 who were infected with HBV alone, 4 developed fulminant hepatic failure and died within 2 years of renal transplantation, 2 developed cirrhosis, and 2 others developed HCC.[91] Overall, however, the 5-year survival was not dissimilar in those infected with HBV and those not infected.

The child most likely to be treated successfully with today's agents is one who acquired HBV beyond infancy, has evidence of immune responsiveness to HBV, and has mild to moderate inflammatory changes on liver biopsy. The child ideally has no other medical problems, and child and family can comply with the regimen and monitoring. A perinatally infected infant may still respond to therapy, as long as there is evidence of immune activation, indicated primarily by ALT values consistently at least one and one-half times the upper limit of normal.[98]

Even if the child has some success with treatment but remains HBsAg positive, the child is still at risk for HCC or reactivation to HBeAg and HBV. Yearly follow-up of virologic status, determination of α-fetoprotein level, and ultrasonography are recommended by most experts, even though clear evidence to support this practice is lacking.

IMMUNOPROPHYLAXIS

A comprehensive strategy to prevent HBV infection, both acute hepatitis B and the sequelae of chronic HBV infection, must eliminate transmission that occurs during infancy and childhood as well as during adolescence and adulthood. It has been clearly demonstrated that HBV transmission cannot be prevented through vaccination strategies targeting only the groups considered at highest risk for developing infection. A comprehensive immunization strategy is currently recommended by the Centers for Disease Control and Prevention (CDC). There are a number of parts to this strategy, as detailed in Table 156-3.

Two types of products are available for prophylaxis against HBV infection. HBIG provides antibody to hepatitis B surface antigen, which gives temporary (3 to 6 months) protection by means of passive immunity; it is indicated only in certain postexposure settings. Hepatitis B vaccine evokes active immunity and is recommended for both preexposure and postexposure prophylaxis.

Hepatitis B Immunoglobulin

HBIG is prepared from plasma known to contain a high titer of anti-HBs. The human plasma from which HBIG is prepared is screened for antibodies to HIV and HCV, and the process used to prepare HBIG inactivates and

TABLE 156-3 **Prevention of Horizontal Transmission Within the Home and School**

Home
Hepatitis B virus immunization of all household members
Universal precautions for all blood and body fluids
Coverage of all abrasions with dressings
Ban on sharing potentially contaminated items such as razors or toothbrushes
School
Hepatitis B virus universal immunization
Universal precautions for all blood and body fluids
Coverage of all abrasions with dressings
Full participation by child in normal school life

eliminates live viruses from the final product. HBIG is used in the postexposure prophylaxis of neonates of HBV-infected women and for susceptible individuals with sexual, needle-stick, or mucosal exposures. It is administered intramuscularly. The dose for infants with perinatal HBV exposure is 0.5 mL. The dose for adults is 0.06 mL/kg. Efficacy ranges from 70 to 95 percent.[8] HBIG in large doses is used after liver transplantation in HBV-infected patients to prevent infection of the allograft. HBIG is typically administered together with the HBV vaccine.

Hepatitis B Vaccine

Hepatitis B vaccines are available as single-antigen formulations or are combined with other vaccines. The two licensed single recombinant vaccines are produced by using *Saccharomyces cerevisiae* (common baker's yeast), into which a plasmid containing the gene for HBsAg has been inserted. Purified HBsAg is obtained by lysis of the yeast cells and separation of HBsAg by biochemical and biophysical techniques. Hepatitis B vaccines are packaged to contain 10 to 40 μg of HBsAg protein/mL after adsorption to aluminum hydroxide (0.5 mg/mL). Vaccines available in the United States are Recombivax HB and Engerix-B. Recombinant HBsAg is also used in the three combination vaccines available in the United States: Comvax, Pediarix, and Twinrix. The immunogenicity is similar for all preparations.

The recommended series of three intramuscular doses of hepatitis B vaccine induces a protective antibody response (anti-HBs) in more than 90 percent of healthy adults and in more than 95 percent of infants, children, and adolescents. Hepatitis B vaccine should be administered into the deltoid muscles of adults and children or the anterolateral thigh muscles of neonates and infants; the immunogenicity is substantially lower when injections are administered into the buttocks. When hepatitis B vaccine is administered to infants at the same time as other vaccines, separate sites in the anterolateral thigh may be used for the multiple injections.

The vaccination schedule used most often for adults and children has been three intramuscular injections, the second and third administered 1 and 6 months, respectively, after the first. Each of the two available vaccines has been evaluated to determine the age-specific dose at which an optimal antibody response is achieved.

Perinatal transmission of HBV can be prevented effectively if the HBsAg-positive mother is identified and if her infant receives appropriate immunoprophylaxis. Administration of HBIG and the first dose of hepatitis B vaccine within 12 hours of birth prevents 90 percent of perinatal infections.[102] Serologic testing of infants who receive immunoprophylaxis to prevent perinatal infection should be performed to identify the 5 percent of infants who become HBV carriers despite these measures. Testing for anti-HBs and HBsAg in children 12 to 15 months of age determines the success of vaccination and, in the case of failure, identifies HBV-infected infants or those who may require treatment or revaccination. The following are the current recommendations as to timing of immunizations for infants in the United States.[144]

1. *Infants born to HBsAg-negative mothers* should receive the first single antigen vaccine dose at birth or before hospital discharge. The plan thereafter depends on whether a single antigen or combination vaccine is used. For single antigen vaccine, the second dose should be administered at age 1 to 2 months and the third at age 6 to 18 months. If combination vaccines are used, the first dose should be of a single antigen vaccine at birth or before hospital discharge. The second dose should be at 2 months, the third at 4 months, and a fourth dose at either 6 months or 12 to 15 months depending on the combination of vaccines used. Testing for serologic response is not necessary and is not recommended.
2. *Infants born to HBsAg-positive mothers* should receive 0.5 mL of HBIG and the first dose of single-antigen hepatitis B vaccine within 12 hours of birth, administered intramuscularly at separate sites. The infant can then complete the immunization series with either single antigen or combination vaccine. If using single antigen vaccine, the infant should receive the second dose at 1 to 2 months and the third when the child is at least 164 days (6 months) of age. If using combination vaccine, the child should receive single antigen vaccine and HBIG at birth and then combination vaccine at 2, 4, and either 6 months or 12 to 15 months depending on the combination. Premature infants of HBV-infected women should receive vaccine in the same schedule, but those weighing less than 2000 g at birth have a decreased response to hepatitis B vaccination below 1 month of age. For such infants the dose given at birth should not be counted as the start of the vaccine series and three additional doses of vaccine (for a total of four doses) should be administered beginning when the infant reaches 1 month of age.[144] Testing for serologic response (HBsAg and anti-HBs) should be done when the child is 12 to 15 months of age. If anti-HBs response is inadequate (<10 mEq/L) and the child is HBsAg negative, he or she should be revaccinated. If the child's anti-HBs titers are greater than 10 mU/L and the child is HBsAg negative, that child is immune and not infected. If the child is HBsAg

positive at age 12 to 18 months, he or she should be considered as potentially chronically infected and followed appropriately.

3. *Infants born to mothers whose HBsAg status is unknown* should receive the first dose of vaccine within 12 hours of birth. Meanwhile, a maternal blood sample should be sent for HBsAg testing. If positive, HBIG should be given as soon as possible (not later than 1 week of age). The infant then should complete the routine vaccination schedule. If the infant is preterm and weighs less than 2000 g and the mother's HBsAg is not established within 12 hours of birth, the infant should receive HBIG and single-antigen hepatitis B vaccine and then complete four doses of hepatitis B vaccinations.

Others Who Should Receive Hepatitis B Vaccine

All nonimmunized children and young adults younger than 19 years of age should begin HBV vaccination at any health care provider visit. For older children and adolescents aged 11 to 15 years, the routine three-dose schedule may be used, but a two-dose schedule of vaccination also has been approved, using the Recombivax HB vaccine. The adult dose (1.0 mL/10 μg) is administered to the adolescent, and the second dose is given 4 to 6 months later. Short-term (2-year) follow-up in groups immunized by either schedule showed similar rates of anti-HBs decline. Of emphasis is that a lower dose of vaccine is used in the three-dose schedule; if the original dose is not known, the full three-dose schedule should be used.[29] Immunization also should be offered to high-risk groups, although universal childhood immunization has created an immune adult population for the most part, except immigrants, who comprise the largest reservoir of adult HBV infection in the United States.

In a three-dose schedule, increasing the interval between administrations of the first and second doses of hepatitis B vaccine has little effect on immunogenicity or final antibody titer. The third dose confers optimal protection, acting as a booster dose. Longer intervals between the last two doses (4 to 12 months) result in higher final titers of anti-HBs antibodies. Larger vaccine doses (40 μg) or an increased number of doses is required to induce protective antibody in many hemodialysis patients and other immunocompromised persons (e.g., those who take immunosuppressive drugs or who are infected with HIV). Children with end-stage renal disease respond better than do adults to HBV immunization. Ninety percent of pediatric chronic dialysis patients and 100 percent of children vaccinated before beginning hemodialysis became anti-HBs positive in one study.[65] An immunocompromised person who has previously shown an adequate anti-HBs antibody titer that then falls to less than 10 mIU/mL should receive a booster dose.

Testing for immunity (anti-HBs titer) is advised only for patients whose subsequent clinical management depends on knowledge of their immune status (e.g., infants born to HBsAg-positive mothers, hemodialysis patients and staff, patients with HIV infection). Postvaccination testing also should be considered for those at occupational risk who may have exposures from injuries with sharp instruments because knowledge of their antibody response helps determine appropriate postexposure prophylaxis.

When revaccinated, 15 to 25 percent of people who do not respond to the primary vaccine series produce an adequate antibody response after one additional dose and 30 to 50 percent do so after three additional doses.[97] Therefore, revaccination should be considered for people who do not respond to the initial series.

The duration of vaccine-induced immunity has been evaluated in long-term follow-up studies of both adults and children. The duration of response from neonatal or later immunization is still not clear, but studies in both Asian and European children vaccinated in infancy demonstrate immune memory persisted at least 10 years in Italy,[225] was 12 years in Hong Kong,[224] but started to wane by 15 years post immunization in Taiwanese children.[138] Booster doses were administered to the children in the latter study with anti-HBs titers less than 100 mIU/mL, and 4 of 128 demonstrated no response.[138] A previous study from the same group demonstrated excellent response in all subjects to a booster dose at age 10 years in those who were anti-HBs seronegative despite neonatal vaccination after being born to HBsAg- and HBeAg-positive mothers.[88] The timing and necessity of a booster dose is still unclear and is not recommended on a population basis.[103]

Therapeutic vaccination, that is, immunization of those already infected in an effort to boost their HBV-specific T-cell responses, has shown limited clinical success in adults. However, a trial of combination immunization (pre-S2/S vaccine) with IFN-α2b compared with IFN-α2b alone in children with chronic HBV infection did not show any benefit compared with IFN-α2b alone.[82]

Vaccine Side Effects and Adverse Reactions

Hepatitis B vaccines have been shown to be safe for both adults and children. Anaphylaxis is the only serious adverse event in children and adolescents; the observed risk was reported in 2003 as 1.1 per million vaccine doses administered.[18] Pain at the injection site (3% to 29%) and a temperature greater than 37.7° C (99.9° F) (1% to 6%) have been the side effects most frequently reported, but they were reported no more frequently among vaccinees than among subjects receiving a placebo.[28,204] In the United States, surveillance from the CDC of adverse reactions has shown a possible association between Guillain-Barré syndrome (GBS) and receipt of the first dose of plasma-derived hepatitis B vaccine in adults; plasma-derived HBV vaccine is no longer in use. An expert review did not accept or reject a causal association between HBV vaccine and Guillain-Barré syndrome.[28]

Reports of multiple sclerosis developing after vaccination for HBV led to concern that the vaccine might cause multiple sclerosis in previously healthy subjects. This theory was refuted in a nested control study of two large cohorts of nurses in the United States that compared the HBV immunization histories of each of 192 women who developed multiple sclerosis with five healthy controls. No association was found.[5] In a case crossover study using the European multiple sclerosis database, recent vaccination against HBV, tetanus, or influenza did not appear to increase the short-term risk for relapse in multiple sclerosis.[46]

Until 1999, hepatitis B vaccines were prepared with thimerosal (sodium ethylmercuric thiosalicylate) to prevent bacterial and fungal contamination. This preservative aroused public concern regarding mercury toxicity and the amount of mercury exposure relative to body weight. No adverse outcomes were associated clearly with thimerosal use, but HBV vaccination of neonates was suspended temporarily in 1999 until thimerosal-free vaccines became available, unless the mother was infected with HBV. Two thimerosal-free hepatitis B vaccines now are available in the United States for use in infants. Hence, parents can be reassured about the lack of exposure to mercury in hepatitis B vaccines.

Contraindications

There are only a few clinical situations in which hepatitis B vaccine is not recommended; those with a history of anaphylaxis to the vaccine and those with history of hypersensitivity to yeast. Vaccination should be deferred in those with an intercurrent moderate to severe illness. Pregnancy is not a contraindication to immunization.[144]

Recommendations to Prevent Household Transmission

When a person is found to have acute or chronic infection with HBV, immunization of household contacts is recommended. Guidance on universal precautions should be provided. As outlined in Table 156-4, parents of infected children and siblings should learn to treat bodily fluids, especially blood, as potentially infectious. All bloody emissions and blood-soiled items should be handled with gloves, and any blood-stained items should be either cleaned with bleach or disposed of carefully. Both the infected person and others in the house should cover skin abrasions with waterproof bandages. Razors and toothbrushes never should be shared. If needle sharps are in use, they should be secured and disposed of according to local guidelines. Schools, workplaces, and health care providers should practice universal precautions for all individuals regardless of whether HBV status is known.

TABLE 156-4 **Recommended Physician Visits for Children with Chronic Hepatitis B Virus Infections**

Every 6 Months
History taking
Physical examination, including growth
Blood work: AST/ALT, CBC, HBsAg, anti-HBs, HBeAg, anti-HBe
Review of risk factors for transmission
6 Months to Annual
Liver ultrasound evaluation
α-Fetoprotein level (optimum frequency not yet clear)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBC, complete blood cell count; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen.

FUTURE STRATEGIES/TARGETS FOR TREATMENT

As new technologies are developed, potential treatments for HBV infection continue to emerge. These can be classified as (1) disruption of HBV life cycle other than HBV polymerase-based replication, (2) gene therapy, and (3) immunomodulators other than IFN-α.

Disruption of the formation of the nucleocapsid by members of the heteroaryl dihydropyrimidine class or phenylpropanamides have shown some efficacy in cell and mouse models.[137] Direct manipulation of the HBV gene offers the possibility of viral eradication using technology such as small interfering RNAs (siRNAs), once technical problems such as introduction of siRNAs into hepatocytes and prevention of enzymatic degradation have been overcome. However, proof of principle has already been demonstrated in mice. Short hairpin RNAs, a form of RNA interference, homologous to HBV RNAs were introduced into a cell culture model and into immunocompetent and immunodeficient mice. There were substantial reductions in mouse liver HBV RNAs and replicated genome as well as a reduction of 84.5 percent in secreted HBsAg in mouse serum.[145] siRNA molecules against different regions of the HBV gene have been shown to decrease HBsAg secretion in a human hepatoblastoma cell line.[114] Another group has shown that this effect can persist through multiple cell passages, suggesting integration of siRNA into the HBV genome.[167] Recently, altritol-containing synthetic siRNAs were tested in vivo in an HBV transgenic mouse model, and these were capable of silencing HBV replication without evidence of off-target toxicity.[80] Other members of the IFN class (e.g., IFN-γ, IFN-λ) as well as cytokines have shown some efficacy in either cell culture or animal models but have not yet been tried in humans.[137]

Despite advances in treatment, prevention of new infections must remain at the forefront of all therapeutic endeavors. Vertical transmission of HBV to children can be prevented by proper antenatal testing, careful follow-up of results, and availability of immunization programs to infants.

HEPATITIS D

Hepatitis D virus (HDV) is a subviral particle that requires a helper virus, HBV, to establish infection. As a subviral particle, HDV, which also is known as the delta agent or virus, does not encode its own envelope protein and requires HBV envelope production to become an infectious virion. HDV first was described in 1977 when a new antigen was described in the hepatocytes of patients infected with HBV. Transmission experiments

demonstrated that the antigen was indeed a new virus.[168] Infection with HDV and HBV can be contracted at the same time (coinfection), or HDV can be contracted as a new infection in a person previously infected with HBV (superinfection). There are currently eight major genotypes (clades) of HDV.[163,170] There is geographic variation in both incidence and genotype distribution.[183] HDV genotype I is the most frequent genotype found worldwide, but HDV genotypes 2 and 4 are found in the Far East and are associated with milder disease.[124] HDV genotype 1 infection appears to be associated with a more fulminant hepatitis in acute infection and increased rates of cirrhosis and HCC compared with genotype 2.[219]

VIROLOGY

HDV is a spherical particle with a diameter of 36 nm.[148] When the envelope is degraded, a nucleocapsid containing hepatitis D antigen (HDAg) and the HDV RNA genome is released. The two forms of HDAg are the small s-HDAg, which is 195 amino acids in length and is required for replication, and the larger l-HDAg, which is 214 amino acids long, is required for virion assembly, and inhibits HDV replication.[148] s-HDAg is a nuclear protein that undergoes phosphorylation by protein kinase C, and inhibitors of protein kinase C inhibit HDV replication in cell culture systems.[41] The ratio of the two forms of HDAg found in patients may vary.

The HDV RNA genome is single stranded and circular, with a length of 1679 nucleotides. During viral replication, the genomic RNA serves as a template for complementary RNA, which in turn is a template for more genomic RNA. A host-derived RNA polymerase is required.[207] Each copy of the genome and antigenome contains ribozyme activity that cleaves RNA and is required for HDV replication.[148] Posttranscriptional modification of the RNA leads to the production of the l-HDAg in addition to the s-HDAg already transcribed.[41,207]

To leave the host cell, HDV RNA and HDAg must be packaged into virions. The l-HDAg undergoes isoprenylation of a cysteine residue near its C terminus to allow virion assembly. Inhibition of this isoprenylation interferes with viral production by preventing interaction with HBsAg.[41] Once this interaction occurs, HBsAg and host cell membrane lipids enclose HDV RNA, l-HDAg, and s-HDAg to form virions. Noninfectious HDV particles, which contain only l-HDAg and HBsAg, can be found in serum.[148]

EPIDEMIOLOGY

The epidemiology of HDV infection parallels that of HBV infection, for obvious reasons. Modes of transmission of HDV also are similar to those for HBV and include direct or indirect parenteral exposure to blood or body fluids and sexual and perinatal transmission. Sexual transmission is less efficient than that of HBV, as evidenced by the relatively low frequency of HDV infection in homosexual men.[179] Perinatal transmission can occur but is rare because HBV carrier mothers also infected with HDV usually are anti-HBe positive and thus less infectious. Intrafamilial transmission of HDV has been demonstrated in endemic areas, such as southern Italy, by means of a combined epidemiologic and molecular study, which demonstrated that within family units the HDV strains were nearly identical.[152] Transmission of HDV infection has also been documented among residents of institutions for the developmentally disabled.

The geographic differences in HDV endemicity in the world are great, and four levels have been characterized. High endemicity (i.e., HDV is found in more than 20% of asymptomatic HBV carriers and more than 60% of HBV carriers with chronic liver disease) is seen in northern South America and Africa as well as in Romania.[35] In these populations, HDV superinfection of HBV carriers is a significant cause of chronic liver disease and causes outbreaks of fulminant hepatitis. In these areas, HDV infection is seen commonly in both children and adults and household transmission has been implicated. Parts of the Middle East, Africa, some Pacific Islands, and parts of Asia report intermediate HDV endemicity (in 10% to 20% of asymptomatic HBV carriers and 30% to 60% of HBV carriers with chronic liver disease). Infection in these regions occurs predominantly in adults, and outbreaks are uncommon, but HDV is an important cause of chronic liver disease. Low endemicity (in 3% to 9% of asymptomatic HBV carriers and 10% to 25% of HBV carriers with chronic liver disease) is observed in most developed countries, including the United States, but subpopulations in these countries, such as parenteral drug abusers and prostitutes, have a high infection rate. In areas of low HBV endemicity, transmission of HDV appears to be mainly by the percutaneous route, with higher rates of HDV infection found in intravenous drug users and hemophiliacs than in other HBV high-risk groups. HDV is considered only a moderately important cause of chronic liver disease in developed countries.[217] Finally, for as yet unexplained reasons, certain subpopulations have a high carriage rate of HBV but virtually no HDV infection; they include American Indians, Alaska Natives, and residents of some Asian countries.

Within the U.S. blood donor population, the prevalence of HDV infection is low; only 1.4 to 8 percent of donors are infected or have evidence of prior infection.[4] Estimates from the CDC for 1990 indicate that among the approximately 250,000 cases of acute HBV infection each year, 7500 simultaneously acquire HDV infection. There are about 70,000 carriers of HDV in the United States, where about 1000 deaths per year are caused by chronic HDV and 35 by fulminant HDV. In certain regions, the prevalence of HDV infection appears to be decreasing. In Italy, the prevalence of HDV infection in HBsAg carriers decreased from 23 percent in 1987 to 14 percent in 1992 and 8.3 percent in 1997.[66,175] Corresponding data for the United States are not available.

IMMUNOPATHOGENESIS

Unlike HBV, HDAg is found only in the liver or serum.[69] The mechanism by which HDV infection leads to hepatic injury has not been elucidated. Despite some evidence that HDV can be directly cytopathic, cytopathic viruses

seldom result in chronic infections. In addition, some HDV carriers have no evidence of hepatic cell damage. Both cellular and humoral components of the host immune system are involved in the response to HDV, although the relative contribution of each is controversial. Investigations into the humoral response to HDV demonstrated that HDAg contains epitopes or immunogenic domains to which sera from acutely infected humans and woodchucks react. The predominantly recognized epitopes are not exposed on the virion surface; thus, antibodies that recognize these epitopes cannot neutralize virus particles, which may explain why no association exists between the humoral response and clinical outcome.[148] Autoantibodies, especially liver-kidney microsomal type 3 antibodies and anti–basal cell layer antibody, are found occasionally in people with HDV infection. The significance of these antibodies is unclear, but they may play a role in the immunopathogenesis of HDV.

Cellular immune responses to HDV also have been demonstrated. Both $CD4^+$ and $CD8^+$ lymphocytes have been found in liver of patients infected with HDV; it is not clear whether these cells contribute to hepatitis or aid in control of HDV infection.[148] Patients with HDV superinfection of chronic HBV who had peripheral $CD4^+$ cells that reacted to four epitopes on HDAg were seronegative for HDV IgM, implying inactive disease.[152] Such $CD4^+$ T-cell clones produce IFN-γ and may be directly cytopathic. Individuals without these specific $CD4^+$ cells had active HDV disease.[155] These epitopes are in highly conserved regions of HDV, which is encouraging for the prospect of vaccine development.

An unusual mechanism of HDAg presentation by hepatocytes has been reported. The processing of HDAg peptide appears to occur outside the cell, where the peptide is cleaved into smaller fragments that bind to class II major histocompatibility complex molecules of all three human leukocyte antigen types found on hepatocytes and mononuclear cells. This form of antigen presentation could have many possible effects on T-cell responses: amplification of T-cell response to HDAg, stimulation of cytotoxic T cells to kill uninfected hepatocytes, or exhaustion of T-cell responses to HDAg, allowing infected cells to escape detection.[148]

The relationship of HBV infection status to pathogenesis of HDV is also unclear. Patients with active HBV replication (i.e., HBV DNA and HBeAg seropositive) have high levels of HDV viremia.[168,169] Conversely, in HBV and HDV carriers without liver disease or with minimal inflammatory hepatic lesions, markers of HBV replication are absent and HDV viremia is low.[19,169] In coinfected liver transplant recipients, HDV reinfection can occur without evidence of active HBV replication but necroinflammatory activity does not develop until HBV infection is reactivated.[50]

DIAGNOSIS

Establishing the diagnosis of either acute or chronic HBV infection is a prerequisite for the diagnosis of HDV infection, which is made by detection of antibody to HDV (anti-HDV). HDV antigen can be measured in serum, but it is usually present only transiently during acute infection and in immunosuppressed individuals.[124] Assays for both IgM and IgG anti-HDV are available. HDV RNA can be detected by reverse-transcriptase and real-time polymerase chain reaction assays, but these tests are not available commercially.

HDV infection may occur at the same time as HBV infection, a pattern that is termed *coinfection*. If an individual has acute, resolving hepatitis B, the HDV infection also will resolve.[52] In this instance, anti-HDV is found in the IgM form during the acute illness and persists for 2 to 6 weeks. Subsequently, IgG anti-HDV is found in serum, but it also diminishes to undetectable levels when the HBV infection resolves (Fig. 156-6). In HDV superinfection, both IgM and IgG anti-HDV are detected. Most often, superinfection with HDV leads to chronic HDV infection, diagnosed by persistence of both IgM and IgG anti-HDV (Fig. 156-7). Anti-HDV becomes predominantly IgG after about 6 weeks and persists if infection does not resolve; titers correlate well with ongoing viral replication.

Histopathologic features of HDV infection are very similar to those of isolated HBV infection (see earlier discussion). HDAg can be identified in nuclei and, to a lesser extent, in the cytoplasm of infected hepatocytes.

CLINICAL MANIFESTATIONS

In general, coinfection with HDV infection does not have clinical features that distinguish it from HBV infection alone. Symptoms develop 2 months after exposure, are similar to those of acute HBV infection, and usually resolve by 4 months after exposure. However, two peaks in aminotransferase levels often are noted, a pattern that is unusual in HBV infection alone. The infections resolve together.[53] Coinfection with both viruses does not increase the risk for development of chronic HBV infection.[136]

HDV superinfection in individuals with chronic HBV infection have different manifestations. HDV infection becomes persistent in most individuals (Fig. 156-8), and the predominant clinical pattern of chronic HDV infection varies by geographic location. In the United States and Europe, HDV superinfection leads to chronic liver disease in more than 90 percent of cases, whereas in some Pacific Island and African populations, most individuals with chronic HDV are asymptomatic.[169] When chronic liver disease does occur, the progression to cirrhosis is rapid; in studies in both adults and children, cirrhosis has been noted to occur in as few as 2 to 10 years. The cirrhosis associated with HDV infection has two patterns: in a minority of patients, especially the high-risk drug users, it progresses rapidly to hepatic failure and portal hypertension. In others, more stable cirrhosis occurs, with little inflammatory component, compatible with prolonged survival.[22] In a small minority of those who are superinfected, a self-limited hepatitis develops and subsequently both HBV and HDV infections are cleared. HDV infection has been demonstrated to lead to HCC at a younger age and a higher rate than does HBV

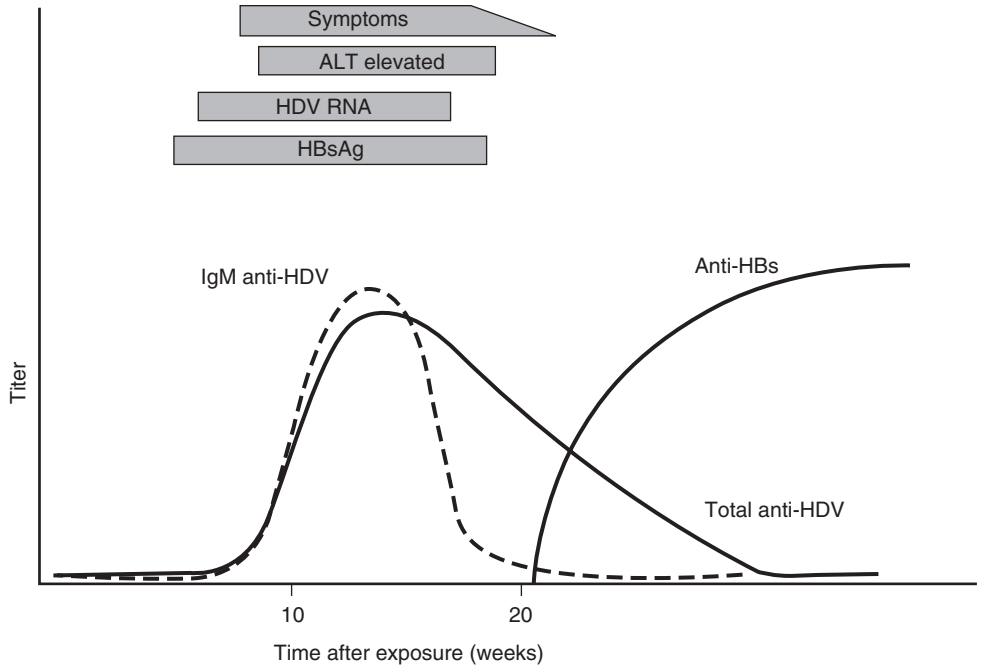

FIGURE 156-6 ■ Serologic events during hepatitis B virus (HBV) and hepatitis D virus (HDV) coinfection that resolves. After exposure to both viruses, hepatitis B surface antigen (HBsAg) and HDV RNA can be detected shortly before the onset of symptoms. Serum alanine aminotransferase (ALT) is elevated for the duration of detectable serum HDV RNA and HBsAg. Serum IgM anti-HDV level rises when symptoms are present and falls as HDV infection resolves and the anti-HBs titer becomes elevated. When both infections have resolved, anti-HBs is detectable in serum but total anti-HDV is very low.

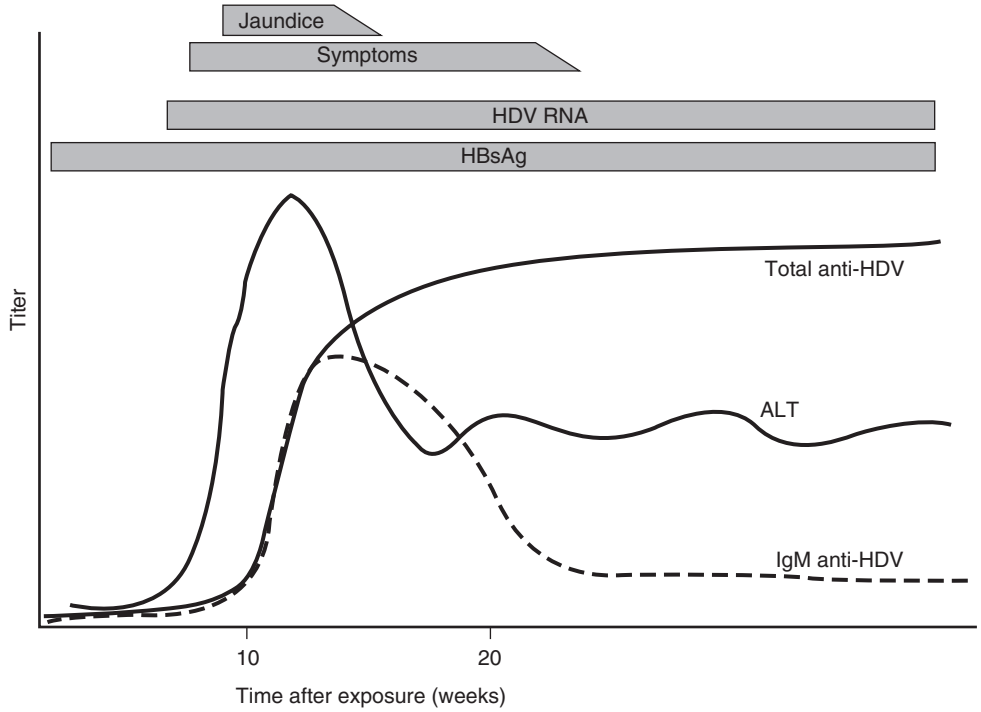

FIGURE 156-7 ■ Serologic events during hepatitis B virus (HBV) and hepatitis D virus (HDV) superinfection. If an individual already infected with HBV is infected with HDV, there is a marked elevation of alanine aminotransferase (ALT) coincident with the onset of symptoms and the onset of jaundice. HDV RNA also can be detected at the onset of symptoms. ALT levels fluctuate over the next few months as IgM anti-HDV decreases and total anti-HDV rises. Throughout the course of HDV superinfection, hepatitis B surface antigen (HBsAg) remains detectable.

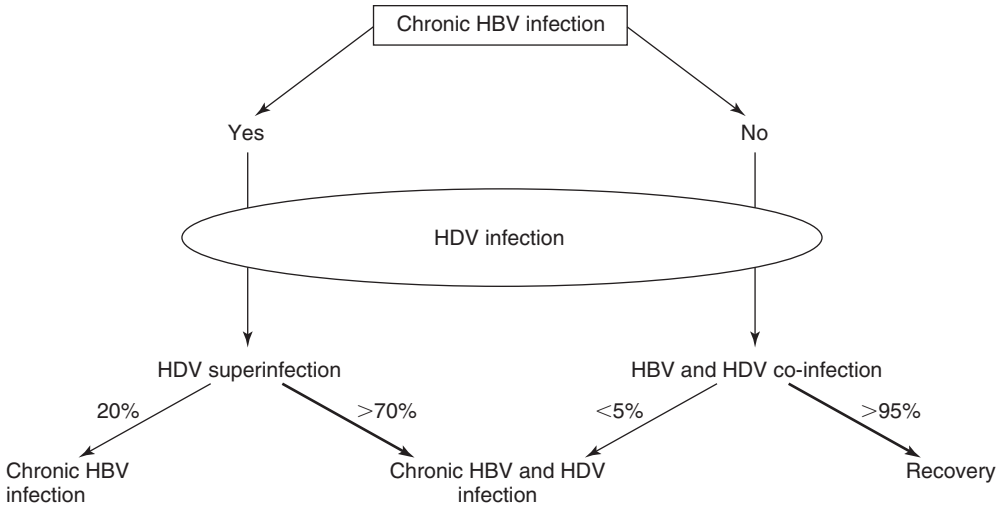

FIGURE 156-8 ■ Outcomes of hepatitis B virus (HBV) and hepatitis D virus (HDV) superinfection and coinfection. When a person already infected with HBV becomes infected with HDV there is a greater than 70 percent chance of becoming chronically infected with both HDV and HBV. In contrast, if a person acquires HBV and HDV infection de novo, there is less than a 5 percent chance of chronic HBV and HDV infection.

infection alone.[169] In a study of 166 patients with HBV and HDV from Romania, the median survival after the diagnosis of HDV-compensated cirrhosis was 58.3 months.[70] A multicenter European study of 200 HBsAg-positive patients with compensated cirrhosis found that 39 (20%) were anti-HDV positive. Those with HDV, HBV, and cirrhosis were younger than those with only HBV and cirrhosis; indeed, HDV infection may lead to the development of cirrhosis 15 years earlier.[62] In this study, HDV infection increased the risk for HCC three times and risk for mortality twofold compared with those with HBV and cirrhosis.[62] The natural history in those without cirrhosis is difficult to establish. In a study of 13 institutions for those with development disabilities in Illinois, 67 of 238 (28%) of HBsAg carriers also had anti-HDV antibodies.[83] Long-term (20 years) follow-up information was available on 65 of 66 anti-HDV– and HBsAg–positive individuals and 166 persons who were HBsAg positive. Liver-related deaths occurred in 7 of 65 (11%) anti-HDV–positive persons but only 1 in 166 anti-HDV–negative person.[1] Hepatitis D was associated with both a higher rate of chronic liver disease and a higher liver-related mortality rate.[1]

A retrospective study of 188 chronically HDV-infected individuals in Italy was performed over an 8-year period. At presentation 44 percent of patients had chronic hepatitis and 56 percent had cirrhosis. A fourth of the patients with chronic hepatitis progressed to cirrhosis during the study period. In 40 patients the viremia resolved either while on therapy or spontaneously. Nine percent of patients were diagnosed with HCC during the 8-year follow-up period.[154]

Children with HDV infection follow a course similar to that of adults. In one European series, the subset of children chronically infected with HBV who also were infected with HDV had more advanced liver disease and the prevalence of HDV infection increased in parallel with the activity of hepatitis. During the study period, disease progressed more rapidly in children infected with both viruses.[61] Testing for HDV infection is recommended in any child with chronic hepatitis B and unusually severe liver disease or an acute exacerbation of stable liver disease.

HDV infection after liver transplantation can appear without apparent HBV reinfection of either the graft or serum. However, use of PCR assay has demonstrated that low levels of HBV infection do, in fact, occur in patients with recurrent HDV infection, and this replication of HBV is sufficient to permit replication of HDV. The HDV virion found in such infections shares the characteristics of typical HDV infection.[20]

TREATMENT

Although several antiviral agents have been studied, the only treatment that has had any beneficial effect in HDV infection is IFN-α. Trials in adults, in both Europe and the United States, have shown that treatment with either 9 million units three times weekly or 5 million units daily of IFN-α results in normalization of serum aminotransferase levels, decrease or disappearance of HDV-RNA from serum, and improvement in hepatic inflammation in about 50 percent of cases.[61] However, most patients experienced a relapse shortly after treatment was discontinued. Response maintained after treatment was noted primarily in individuals who became HBsAg negative.

Pegylated INF-α2b has shown promise as a treatment for chronic HDV in two small trials. Fourteen patients were treated with pegylated IFN-α2b 1.5 μg/kg weekly for 12 months and 6 (43%) had a sustained virologic response (undetectable HDV RNA) 6 months after the end of treatment.[26] Combination ribavirin and pegylated IFN-α2b did not improve virologic response compared

with pegylated IFN-α2b alone.[153] No factors predictive of response have been identified. Prognosis in nonresponders to IFN-α and in those who relapse has been poor. The author of an editorial in *Hepatology* recommended continuation of IFN-α or pegylated IFN-α2b in patients with a decline in HDV RNA as long as possible, until HDV RNA and HBsAg disappear.[60] A recent randomized trial compared a 48-week treatment regimen of pegylated IFN-α2a plus adefovir dipivoxil, pegylated IFN-α2a alone, and adefovir dipivoxil alone. Twenty-three percent of patients treated with combination therapy and 24 percent of those treated with pegylated IFN-α2a monotherapy cleared HDV RNA. No patients treated with adefovir dipivoxil alone cleared HDV RNA at 48 weeks.[216]

Currently, no recommendations have been established regarding the use of IFN-α or pegylated IFN-α2b for the treatment of chronic HDV infection in children. A trial in seven children in Greece treated with IFN-α in a dosage of 3 million units/m^2 of body surface area three times a week for 1 year yielded disappointing results. All remained anti-HDV IgM positive, and four of the seven had persistent HDV RNA in serum. A significant reduction in serum aminotransferase values was noted after 1 year of treatment, but there was no significant improvement in liver histology.[48] A study of eight children from Germany also found no effect of IFN-α on replication of HDV. However, serum aminotransferase values improved and children underwent earlier anti-HBe seroconversion compared with historical controls.[180] Lamivudine has been shown to have no effect on either HDV viremia or liver disease activity in chronic HDV infection.[165]

Some children and adults require liver transplantation for management of end-stage HDV-associated liver disease. Patients coinfected with HDV and HBV have lower rates of HBV infection of the allograft than do those in patients with isolated HBV infection. These data were reported in a series of 58 patients with HBV infection, of whom 25 also had HDV infection and who underwent liver transplantation between 1984 and 1996 in Belgium. Fifty-two patients survived longer than 3 months, and median follow-up was 74 months. HDV infection improved survival at 5 years: 96 ± 4 percent compared with 63 ± 10 percent for those infected with HBV alone.[128] As yet, no explanation has been given for the better outcome of transplantation in individuals who have both HBV and HDV infections. Administration of HBIG is required before and after transplantation, as in those patients with HBV infection alone.

IMMUNOPROPHYLAXIS

Passive or active immunization specifically against HDV infection is not available. HDAg itself is highly immunogenic, but antibodies are not neutralizing. Description of a T-cell response to several HDV epitopes in individuals with less severe disease activity has stimulated speculation about possible vaccine development, but studies are preliminary. A DNA-based HDV vaccine successfully induced cellular responses in an animal model and also was successful when used with a HBV vaccine.[90] A trial of vaccine derived from B-cell epitopes of HDAg and HDAg p24 expressed in *Escherichia coli*, yeast, or baculovirus in woodchucks provoked a specific antibody response but did not protect against HDV superinfection.[64]

At the present time, individuals with chronic HBV infection, who are the same people at risk for acquiring HDV, cannot be protected from HDV infection except by avoidance of high-risk behaviors and exposures. However, prevention of HBV infection in susceptible individuals will prevent HDV coinfection and will be the most important mechanism for decreasing the prevalence of HDV infection in a population. The current strategy aimed at decreasing susceptibility to HBV infection by universal immunization of neonates and catch-up immunization of older children and adolescents should reduce substantially the risk for subsequent development of HDV infection during young adulthood. In Italy, the decrease in HDV prevalence in those with chronic HBV infection from 23 percent in 1987 to 8.3 percent in 1997 has led to speculation that chronic hepatitis D may be a "vanishing disease."[66]

NEW REFERENCES SINCE THE SIXTH EDITION

6. Aurpibul L, Lumbiganon P, Kolasaraksa P, et al. HIV and hepatitis B coinfection among perinatally HIV-infected Thai adolescents. *Pediatr Infect Dis J* 2012;**31**:943–7.
13. Benhenda S, Cougot D, Buendia MA, Neuveut C. Hepatitis B VIRUs X protein: molecular functions and its role in virus life cycle and pathogenesis. *Adv Cancer Res* 2009;**103**:75–109.
38. Chen CJ, Yang HI. Natural history of chronic hepatitis B REVEALed. *J Gastroenterol Hepatol* 2011;**26**:628–38.
44. Cohen C, Evans AA, London WT, et al. Underestimation of chronic hepatitis B virus infection in the United States of America *J Viral Hepatitis* 2008;**15**:12–13.
78. Haber BA, Block JM, Jonas MM, et al. Recommendations for screening, monitoring, and referral of pediatric chronic hepatitis B. *Pediatrics* 2009;**124**:e1007–13.
80. Hean J, Crowther C, Ely A, et al. Inhibition of hepatitis B virus replication in vivo using lipoplexes containing altritol-modified antiviral siRNAs. *Artif DNA PNA XNA* 2010;**1**:17–26.
95. Jang JW, Yoo SH, Kwon JH, et al. Serum hepatitis B surface antigen levels in the natural history of chronic hepatitis B infection. *Aliment Pharmacol Ther* 2011;**34**:1337–46.
96. Jaroszewicz J, Calle Serrano B, Wursthorn K, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV) infection: a European perspective. *J Hepatol* 2010;**52**:514–22.
98. Jonas MM, Block JM, Haber BA, et al. Treatment of children with chronic hepatitis B virus infection in the United States: patient selection and therapeutic options. *Hepatology* 2010;**52**:2192–205.
99. Jonas MM, Kelly D, Pollack H, et al. Safety, efficacy, and pharmacokinetics of adefovir dipivoxil in children and adolescents (age 2 to <18 years) with chronic hepatitis B. *Hepatology* 2008;**47**:1863–71.
100. Jonas MM, Kelly D, Pollack H, et al. Efficacy and safety of long-term adefovir dipivoxil therapy in children with chronic hepatitis B infection. *Pediatr Infect Dis J* 2012;**31**:578–82.
108. Kim SU, Han KH, Ahn SH. Transient elastography in chronic hepatitis B: an Asian perspective. *World J Gastroenterol* 2010;**16**:5173–80.
126. Lee JMAS, Kim HS, Park H, et al. Quantitative hepatitis B surface antigen and hepatitis B e antigen titers in prediction of treatment response to entecavir. *Hepatology* 2011;**2011**:1486–93.
140. Lusida M, Nugrahaputra VE, Handajani SR, et al. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. *J Clin Microbiol* 2008;**46**:2160–6.
149. Murray K, Szenborn L, Wysocki J, et al. Randomized, placebo-controlled trial of tenofovir disoproxil fumarate in adolescents with chronic hepatitis B. *Hepatology* 2012;**56**(6):2018–26.

154. Niro GA, Smedile A, Ippolito AM, et al. Outcome of chronic delta hepatitis in Italy: a long-term cohort study. *J Hepatol* 2010;**53**: 834–40.
164. Raftopoulos SC, George J, Bourliere M, et al. Comparison of noninvasive models of fibrosis in chronic hepatitis B. *Hepatol Int* 2011 Jul 12. [Epub ahead of print].
170. Rizzetto M. Hepatitis D: thirty years after. *J Hepatol* 2009;**50**: 1043–50.
199. Su WJ, Ho MC, Ni YH, et al. Clinical course of de novo hepatitis B infection after pediatric liver transplantation. *Liver Transpl* 2010;**16**:215–21.
214. Wasley A, Grytdal S, Gallagher K, Centers for Disease Control and Prevention (CDC). Surveillance for acute viral hepatitis—United States, 2006. *MMWR Surveill Summ* 2008;**57**(2):1–24.
215. Wasley A, Kruszon-Moran D, Kuhnert W, et al. The prevalence of hepatitis B virus infection in the United States in the era of vaccination. *J Infect Dis* 2010;**202**:192–201.
216. Wedemeyer H, Yurdaydin C, Dalekos GN, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;**364**:322–31.
228. Zhong YW, Li J, Song HB, et al. Virologic and clinical characteristics of HBV genotypes/subgenotypes in 487 Chinese pediatric patients with CHB. *BMC Infect Dis* 2011;**11**:262.

The full reference list for this chapter is available at expertconsult.com.

DNA VIRUSES

SUBSECTION 5 *HERPESVIRIDAE*

CHAPTER 157

Herpes Simplex Viruses 1 and 2

Kathleen Gutierrez • Benjamin Pinsky • Ann M. Arvin

Herpes simplex virus (HSV) types 1 and 2 are in the family of nine human herpesviruses, which also includes cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), and human herpesviruses (HHV) types 6A, 6B, 7, and 8.

HSV-1 is the prototype of the α-herpesvirus subfamily, which includes HSV-2 and VZV.[317] These viruses are ubiquitous and neurotropic and establish latency in sensory ganglia; persistence is associated with periodic reactivation and reappearance of infectious virus at mucocutaneous sites. Most individuals infected with HSV-1 and HSV-2 do not have clinical manifestations, either at the time of initial acquisition or during episodes of reactivation. When HSV-1 or HSV-2 causes disease, the illness may range from minor, such as "fever blisters," to life threatening, as exemplified by HSV encephalitis or neonatal disease. Proper management of HSV-1 and HSV-2 infection depends on clinical recognition of the common and atypical syndromes caused by these viruses, knowledge of laboratory methods that are useful for proving the diagnosis, and early and appropriate antiviral therapy.

THE VIRUSES

HSV-1 and HSV-2 virions consist of an icosahedral protein capsid enclosing a core of double-stranded DNA, surrounded by a protein tegument, and enclosed in a lipid-containing envelope.[317] The genomic DNA of HSV-1 and HSV-2 has substantial sequence homology (approximately 50%), but the viruses also have unique sequences that encode variant proteins, and they differ biologically in their patterns of replication in vitro and in vivo. Tegument proteins are located between the capsid and the viral envelope. The envelope has glycoproteins that are important targets of the humoral and cellular immune responses (gB, gC, gD, and gG). The glycoproteins gE and gI function as immunoglobulin (Ig) Fc receptors. Other glycoproteins include gH and gL, which form a complex with gB that plays a role in cell entry and spread; gK, which is required for viral exocytosis; and gM, which has a role in capsid envelopment and exocytosis. These glycoproteins are found in both HSV-1 and HSV-2 and exhibit a high degree of amino acid similarity. The gG of HSV-2 is larger than its HSV-1 homologue and has unique sites that are recognized by virus-specific host responses. Both forms of HSV can infect either oral or genital sites; however, HSV-1 usually causes infections of oral mucocutaneous sites and most genital mucocutaneous infections are caused by HSV-2.[118,317] HSV-1 is more likely to recur at oral sites, and HSV-2 reactivates more frequently in the genital area, even in persons who were infected initially at both oral and genital sites with HSV-1 or HSV-2.[220]

HSV attaches to and penetrates cells via gB and gD through specific cell surface herpes family receptors. This

REFERENCES

1. Abiad H, Ramani R, Currie JB, et al. The natural history of hepatitis D virus infection in Illinois state facilities for the developmentally disabled. *Am J Gastroenterol* 2001;**96**:534–40.
2. Acs G, Sells MA, Purcell RH, et al. Hepatitis B virus produced by transfected Hep G2 cells causes hepatitis in chimpanzees. *Proc Natl Acad Sci U S A* 1987;**84**:4641–4.
3. Alter MJ. Epidemiology of viral hepatitis and HIV co-infection. *J Hepatol* 2006;**44**:S6–9.
4. Alter MJ, Mast EE. The epidemiology of viral hepatitis in the United States. *Gastroenterol Clin North Am* 1994;**23**(3):437–55.
5. Ascherio A, Zhang SM, Hernan MA, et al. Hepatitis B vaccination and the risk of multiple sclerosis. *N Engl J Med* 2001;**344**: 327–32.
6. Aurpibul L, Lumbiganon P, Kolasaraksa P, et al. HIV and hepatitis B coinfection among perinatally HIV-infected Thai adolescents. *Pediatr Infect Dis J* 2012;**31**:943–7.
7. Barbera C, Bortoletti F, Crivellaro C, et al. Recombinant interferon-alpha 2a hastens the rate of HBeAg clearance in children with chronic hepatitis B. *Hepatology* 1994;**20**:287–90.
8. Barr D, Hershow R, Furner S, et al. Assessing prenatal hepatitis B screening in Illinois with an inexpensive study design adaptable to other jurisdictions. *Am J Public Health* 1999;**89**:19–24.
9. Bartenschlager R, Schaller H. Hepadnaviral assembly is initiated by polymerase binding to the encapsidation signal in the viral RNA genome. *Embo J* 1992;**11**:3413–20.
10. Bartolome J, Moraleda G, Ruiz-Moreno M, et al. Hepatitis B virus DNA patterns in the liver of children with chronic hepatitis B. *J Med Virol* 1990;**31**:195–9.
11. Batts K, Ludwig J. Chronic hepatitis: an update on terminology and reporting. *Am J Surg Pathol* 1995;**19**:1409–17.
12. Beasley RP. Hepatitis B virus: the major etiology of hepatocellular carcinoma. *Cancer* 1988;**61**:1942–56.
13. Benhenda S, Cougot D, Buendia MA, Neuveut C. Hepatitis B VIRUs X protein: molecular functions and its role in virus life cycle and pathogenesis. *Adv Cancer Res* 2009;**103**:75–109.
14. Benner KG, Lee RG, Keeffe EB, et al. Fibrosing cytolytic liver failure secondary to recurrent hepatitis B after liver transplantation. *Gastroenterology* 1992;**103**:1307–12.
15. Bishop N, Civitico G, Wang YY, et al. Antiviral strategies in chronic hepatitis B virus infection, part I: establishment of an in vitro system using the duck hepatitis B virus model. *J Med Virol* 1990;**31**:82–9.
16. Blum HE, Zhang ZS, Galun E, et al. Hepatitis B virus X protein is not central to the viral life cycle in vitro. *J Virol* 1992;**66**:1223–7.
17. Bodsworth NJ, Cooper DA, Donovan B. The influence of human immunodeficiency virus type 1 infection on the development of the hepatitis B virus carrier state. *J Infect Dis* 1991;**163**:1138–40.
18. Bohlke K, Davis R, Marcy M, et al. Risk of anaphylaxis after vaccination of children and adolescents. *Pediatrics* 2003;**112**:815–20.
19. Bonino F, Brunetto MR, Negro F. Factors influencing the natural course of HDV hepatitis. *Prog Clin Biol Res* 1991;**364**:137–46.
20. Borghesio E, Rosina F, Smedile A, et al. Serum immunoglobulin M antibody to hepatitis D as a surrogate marker of hepatitis D in interferon-treated patients and in patients who underwent liver transplantation. *Hepatology* 1998;**27**:873–6.
21. Bortoletti F, Guido M, Cadrobbi P, et al. Spontaneous regression of hepatitis B virus–associated cirrhosis developed in childhood. *Dig Liver Dis* 2005;**37**:964–7.
22. Bortolotti F, Cadrobbi P, Crivellaro C, et al. Long-term outcome of chronic type B hepatitis in patients who acquire hepatitis B virus infection in childhood. *Gastroenterology* 1990;**99**:805–10.
23. Bortolotti F, Guido M, Bartolacci S, et al. Chronic hepatitis B in children after e antigen seroclearance: final report of a 29 year longitudinal study. *Hepatology* 2006;**43**:556–62.
24. Boxall EH, Sira J, Standish RA, et al. Natural history of hepatitis B in perinatally infected carriers. *Arch Dis Child Fetal Neonatal Ed* 2004;**89**:F456–60.
25. Bruss V, Ganem D. The role of envelope proteins in hepatitis B virus assembly. *Proc Natl Acad Sci U S A* 1991;**88**:1059–63.
26. Castlenau C, Le Gal F, Ripault M-P, et al. Efficacy of peginterferon alpha-2b in chronic hepatitis delta: relevance of quantitative RT-PCR for follow-up. *Hepatology* 2006;**44**:728–35.
27. Centers for Disease Control. Hepatitis B virus: a comprehensive strategy for the eliminating transmission in the United States through universal childhood vaccination. Recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991;**40**(RR-13):1–25.
28. Centers for Disease Control and Prevention. Update: vaccine side effects, adverse reactions, contraindications, and precautions. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1996;**45**:1–35.
29. Centers for Disease Control and Prevention. Alternate two-dose hepatitis B vaccination schedule for adolescents aged 11-15 years. *JAMA* 2000;**283**(16):2100.
30. Chan HL, Tse CH, Ng EY, et al. Phylogenetic, virological, and clinical characteristics of genotype C hepatitis B virus with TCC at codon 15 of the precore region. *J Clin Microbiol* 2006;**44**: 681–7.
31. Chan HL, Wong VW, Tse AM, et al. Serum hepatitis B surface antigen quantitation can reflect hepatitis B virus in the liver and predict treatment response. *Clin Gastroenterol Hepatol* 2007;**5**: 1462–8.
32. Chang LJ, Pryciak P, Ganem D, et al. Biosynthesis of the reverse transcriptase of hepatitis B viruses involves de novo translational initiation not ribosomal frameshifting. *Nature* 1989;**337**:364–8.
33. Chang MH. Hepatitis B: long term outcome and benefit from mass vaccination in children. *Acta Gastroenterol Belg* 1998;**61**:210–13.
34. Chang MH, Chen PJ, Chen JY, et al. Hepatitis B virus integration in hepatitis B virus–related hepatocellular carcinoma in childhood. *Hepatology* 1991;**13**:316–20.
35. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997;**336**:1906–7.
36. Chang MH, Sung JL, Lee CY, et al. Factors affecting clearance of hepatitis B e antigen in hepatitis B surface antigen carrier children. *J Pediatr* 1989;**115**:385–90.
37. Cheah PL, Looi LM, Lin HP, et al. Childhood primary hepatocellular carcinoma and hepatitis B virus infection. *Cancer* 1990;**65**:174–6.
38. Chen CJ, Yang HI. Natural history of chronic hepatitis B REVEALed. *J Gastroenterol Hepatol* 2011;**26**:628–38.
39. Chen HL, Chang C-J, Kong MS, et al. Pediatric fulminant hepatic failure in endemic areas of hepatitis B infection: 15 years after universal hepatitis B vaccination. *Hepatology* 2004;**39**:58–63.
40. Chen PJ, Chen DS. Hepatitis B virus infection and hepatocellular carcinoma: molecular genetics and clinical perspectives. *Semin Liver Dis* 1999;**19**:253–62.
41. Chen PJ, Wu HL, Wang CJ, et al. Molecular biology of hepatitis D virus: research and potential for application. *J Gastroenterol Hepatol* 1997;**12**:S188–92.
42. Chen ST, La Porte P, Yee JK. Mutational analysis of hepatitis B virus enhancer 2. *Virology* 1993;**196**:652–9.
43. Chisari FV, Filippi P, Buras J, et al. Structural and pathological effects of synthesis of hepatitis B virus large envelope polypeptide in transgenic mice. *Proc Natl Acad Sci U S A* 1987;**84**:6909–13.
44. Cohen C, Evans AA, London WT, et al. Underestimation of chronic hepatitis B virus infection in the United States of America *J Viral Hepatitis* 2008;**15**:12–13.
45. Colin JF, Cazals-Hatem D, Loriot MA, et al. Influence of human immunodeficiency virus infection on chronic hepatitis B in homosexual men. *Hepatology* 1999;**29**:1306–10.
46. Confavreux C, Suissa S, Saddier P, et al. Vaccinations and the risk of relapse in multiple sclerosis. Vaccines in Multiple Sclerosis Study Group. *N Engl J Med* 2001;**344**:319–26.
47. Crowther RA, Kiselev NA, Bottcher B, et al. Three-dimensional structure of hepatitis B virus core particles determined by electron cryomicroscopy. *Cell* 1994;**77**:943–50.
48. Dalekos GN, Galanakis E, Zervou E, et al. Interferon-alpha treatment of children with chronic hepatitis D virus infection: the Greek experience. *Hepatogastroenterology* 2000;**47**:1072–6.
49. D'Antiga L, Aw M, Atkins M, et al. Combined lamivudine/interferon-alpha treatment in "immunotolerant" children perinatally infected with hepatitis B: a pilot study. *J Pediatr* 2006;**148**:228–33.
50. David E, Rahier J, Pucci A, et al. Recurrence of hepatitis D (delta) in liver transplants: histopathological aspects. *Gastroenterology* 1993;**104**:1122–8.

51. Desmet VJ. Immunopathology of chronic viral hepatitis. *Hepatogastroenterology* 1991;**38**:14–21.
52. Di Bisceglie AM, Hoofnagle JH, Krawczynski K. Changes in hepatitis C virus antigen in liver with antiviral therapy. *Gastroenterology* 1993;**105**:858–62.
53. Di Bisceglie AM, Negro F. Diagnosis of hepatitis delta virus infection. *Hepatology* 1989;**10**:1014–16.
54. Diel R, Helle J, Gottschalk R. Transmission of hepatitis B in Hamburg, Germany, 1998-2003: a prospective, population study. *Med Microbiol Immunol* 2005;**194**:193–9.
55. Dienstag JL, Schiff ER, Wright TL, et al. Lamivudine as initial treatment for chronic hepatitis B in the United States. *N Engl J Med* 1999;**341**:1256–63.
56. Dikici B, Bosnak M, Kara IH, et al. Lamivudine and interferon-alpha combination treatment of childhood patients with chronic hepatitis B infection. *Pediatr Infect Dis J* 2001;**20**:988–92.
57. Dimitrakopoulos A, Takou A, Haida A, et al. The prevalence of hepatitis B and C in HIV-positive Greek patients: relationship to survival of deceased AIDS patients. *J Infect* 2000;**40**:127–31.
58. Doganci T, Uysal G, Kir T, et al. Horizontal transmission of hepatitis B in children with chronic hepatitis B. *World J Gastroenterol* 2005;**11**:418–20.
59. Enders GH, Ganem D, Varmus HE. 5′-Terminal sequences influence the segregation of ground squirrel hepatitis virus RNAs into polyribosomes and viral core particles. *J Virol* 1987;**61**:35–41.
60. Farci P. Treatment of chronic hepatitis D: New advances, old challenges. *Hepatology* 2006;**44**:536–39.
61. Farci P, Mandas A, Coiana A, et al. Treatment of chronic hepatitis D with interferon alfa-2a. *N Engl J Med* 1994;**330**:88–94.
62. Fattovich G, Giustina G, Christensen E, et al. Influence of hepatitis delta virus infection on morbidity and mortality in compensated cirrhosis type B. The European Concerted Action on Viral Hepatitis (Eurohep). *Gut* 2000;**46**:420–6.
63. Ferrari C, Missale G, Boni C, et al. Immunopathogenesis of hepatitis B. *J Hepatol* 2003;**39**:S36–42.
64. Fiedler M, Roggendorf M. Vaccination against hepatitis delta virus infection: studies in the woodchuck *(Marmota monax)* model. *Intervirology* 2001;**44**:154–61.
65. Fivush BA, Neu AM. Immunization guidelines for pediatric renal disease. *Semin Nephrol* 1998;**18**:256–63.
66. Gaeta G, Stroffolini T, Chiaramonte M, et al. Chronic hepatitis D; a vanishing disease? An Italian multicenter study. *Hepatology* 2000;**32**:824–7.
67. Galle PR, Hagelstein J, Kommerell B, et al. In vitro experimental infection of primary human hepatocytes with hepatitis B virus. *Gastroenterology* 1994;**106**:664–73.
68. Ganem D, Prince AM. Hepatitis B virus infection—natural history and clinical consequences. *N Engl J Med* 2004;**350**:1118–29.
69. Gerken G, Meyer zum Buschenfelde KH. Chronic hepatitis delta virus (HDV) infection. *Hepatogastroenterology* 1991;**38**:29–32.
70. Gheorghe L, Iacob S, Simionov I, et al. Natural history of compensated viral B and D cirrhosis. *Rom J Gastroenterol* 2005;**14**: 329–35.
71. Giacchino R, Main J, Timitilli A, et al. Dual-centre, double-blind, randomised trial of lymphoblastoid interferon alpha with or without steroid pretreatment in children with chronic hepatitis B. *Liver* 1995;**15**:143–8.
72. Giacchino R, Navone C, Facco F, et al. HBV-DNA-related hepatocellular carcinoma occurring in childhood: report of three cases. *Dig Dis Sci* 1991;**36**:1143–6.
73. Goldstein ST, Alter MJ, Williams IT, et al. Incidence and risk factors for acute hepatitis B in the United States, 1982-1998: implications for vaccination programs. *J Infect Dis* 2002;**185**: 713–19.
74. Goto Y, Yoshida J, Kuzushima K, et al. Patterns of hepatitis B virus DNA integration in liver tissue of children with chronic infections. *J Pediatr Gastroenterol Nutr* 1993;**16**:70–4.
75. Guida S, Fiore M, Scotese I, et al. Defective interleukin-2 production in children with chronic hepatitis B: role of adherent cells. *J Pediatr Gastroenterol Nutr* 1997;**24**:312–16.
76. Gunther S, Meisel H, Reip A, et al. Frequent and rapid emergence of mutated pre-C sequences in HBV from e-antigen positive carriers who seroconvert to anti-HBe during interferon treatment. *Virology* 1992;**187**:271–9.
77. Gurakan F, Kocak N, Ozen H, et al. Comparison of standard and high dosage recombinant interferon alpha 2b for treatment of children with chronic hepatitis B infection. *Pediatr Infect Dis J* 2000;**19**:52–6.
78. Haber BA, Block JM, Jonas MM, et al. Recommendations for screening, monitoring, and referral of pediatric chronic hepatitis B. *Pediatrics* 2009;**124**:e1007–13.
79. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005;**352**:2673–81.
80. Hean J, Crowther C, Ely A, et al. Inhibition of hepatitis B virus replication in vivo using lipoplexes containing altritol-modified antiviral siRNAs. *Artif DNA PNA XNA* 2010;**1**:17–26.
81. Heermann KH, Goldmann U, Schwartz W, et al. Large surface proteins of hepatitis B virus containing the pre-s sequence. *J Virol* 1984;**52**:396–402.
82. Helvaci M, Kizilqunesler A, Kasirga E, et al. Efficacy of hepatitis B vaccination and interferon-alpha-2b combination therapy versus inferferon-alpha-2b monotherapy in children with chronic hepatitis B. *J Gastroenterol Hepatol* 2004;**19**:785–91.
83. Hershow RC, Chomel BB, Graham DR, et al. Hepatitis D virus infection in Illinois state facilities for the developmentally disabled: epidemiology and clinical manifestations. *Ann Intern Med* 1989;**110**:779–85.
84. Hertogs K, Leenders WP, Depla E, et al. Endonexin II, present on human liver plasma membranes, is a specific binding protein of small hepatitis B virus (HBV) envelope protein. *Virology* 1993;**197**:549–57.
85. Hirsch RC, Lavine JE, Chang LJ, et al. Polymerase gene products of hepatitis B viruses are required for genomic RNA packaging as wel as for reverse transcription. *Nature* 1990;**344**:552–5.
86. Honkoop P, De Man RA, Niesters HG, et al. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. *Hepatology* 2000;**32**:635–9.
87. Hou J. Epidemiology and prevention of hepatitis B virus infection. *Int J Med Sci* 2005;**2**:50–7.
88. Huang LM, Chiang BL, Lee CY, et al. Long-term response to hepatitis B vaccination and response to booster in children born to mothers with hepatitis B e antigen. *Hepatology* 1999;**29**:954–9.
89. Huang SN, Chen TC, Tsai SL, Liaw YP. Histopathology and pathobiology of hepatropic virus-induced liver injury. *J Gastroenterol Hepatol* 1997;**12**:S195–217.
90. Huang YH, Wu JC, Tao MH, et al. DNA-based immunization produces Th1 immune responses to hepatitis delta virus in a mouse model. *Hepatology* 2000;**32**:104–10.
91. Huo TI, Yang WC, Wu JC, et al. Impact of hepatitis B and C virus infection on the outcome of kidney transplantation in Chinese patients. *Chung Hua I Hsueh Tsa Chih (Taipei)* 2000;**63**:93–100.
92. Huovila AP, Eder AM, Fuller SD. Hepatitis B surface antigen assembles in a post-ER, pre-Golgi compartment. *J Cell Biol* 1992;**118**:1305–20.
93. Iorio R, Pensati P, Botta S, et al. Side effects of alpha-interferon therapy and impact on health-related quality of life in children with chronic viral hepatitis. *Pediatr Infect Dis J* 1997;**16**:984–90.
94. Ishak K. Chronic hepatitis; morphology and nomenclature. *Mod Pathol* 1994;**7**:690–713.
95. Jang JW, Yoo SH, Kwon JH, et al. Serum hepatitis B surface antigen levels in the natural history of chronic hepatitis B infection. *Aliment Pharmacol Ther* 2011;**34**:1337–46.
96. Jaroszewicz J, Calle Serrano B, Wursthorn K, et al. Hepatitis B surface antigen (HBsAg) levels in the natural history of hepatitis B virus (HBV) infection: a European perspective. *J Hepatol* 2010;**52**:514–22.
97. Jilg W, Schmidt M, Deinhardt F. Immune response to hepatitis B revaccination. *J Med Virol* 1988;**24**:377–84.
98. Jonas MM, Block JM, Haber BA, et al. Treatment of children with chronic hepatitis B virus infection in the United States: patient selection and therapeutic options. *Hepatology* 2010;**52**:2192–205.
99. Jonas MM, Kelly D, Pollack H, et al. Safety, efficacy, and pharmacokinetics of adefovir dipivoxil in children and adolescents (age 2 to <18 years) with chronic hepatitis B. *Hepatology* 2008;**47**: 1863–71.
100. Jonas MM, Kelly D, Pollack H, et al. Efficacy and safety of long-term adefovir dipivoxil therapy in children with chronic hepatitis B infection. *Pediatr Infect Dis J* 2012;**31**:578–82.
101. Jonas MM, Mizerski J, Badia IB, et al. Clinical trial of lamivudine in children with chronic hepatitis B. *N Engl J Med* 2002;**346**: 1706–13.

102. Jonas MM, Reddy RK, DeMedina M, et al. Hepatitis B infection in a large municipal obstetrical population: characterization and prevention of perinatal transmission. *Am J Gastroenterol* 1990;**85**: 277–80.
103. Kao JH, Chen DS. Hepatitis B vaccination: to boost or not too boost. *Lancet* 2005;**366**:1337–8.
104. Kao JH, Chen PJ, Lai MY, et al. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000;**118**:554–9.
105. Kao JH, Chen PJ, Lai MY, et al. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. *J Clin Microbiol* 2002;**40**:1207–9.
106. Kao JH, Wu NH, Chen PJ, et al. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000;**33**:998–1002.
107. Kelly RB. Pathways of protein secretion in eukaryotes. *Science* 1985;**230**:25–32.
108. Kim SU, Han KH, Ahn SH. Transient elastography in chronic hepatitis B: an Asian perspective. *World J Gastroenterol* 2010;**16**:5173–80.
109. Kleinknecht C, Levy M, Peix A, et al. Membranous glomerulonephritis and hepatitis B surface antigen in children. *J Pediatr* 1979;**95**:946–52.
110. Klingmuller U, Schaller H. Hepadnavirus infection requires interaction between the viral pre-S domain and a specific hepatocellular receptor. *J Virol* 1993;**67**:7414–22.
111. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;**1**:431–5.
112. Koçak N, Gürakan F, Saltik IN, et al. Long-term prognosis of interferon nonresponder children with hepatitis B. *Am J Gastroenterol* 2000;**95**:1841.
113. Kock J, Schlicht HJ. Analysis of the earliest steps of hepadnavirus replication: genome repair after infectious entry into hepatocytes does not depend on viral polymerase activity. *J Virol* 1993;**67**:4867–74.
114. Konishi M, Wu C, Wu G. Inhibition of HBV replication bt siRNA in a stable HBV-producing cell line. *Hepatology* 2003;**38**:842–50.
115. Korenman J, Baker B, Waggoner J, et al. Long-term remission of chronic hepatitis B after alpha-interferon therapy. *Ann Intern Med* 1991;**114**:629–34.
116. Kosake Y, Takase K, Kojima M, et al. Fulminant hepatitis B: induction by the hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. *Gastroenterology* 1991;**100**:1087–94.
117. Lai CL, Chien R-N, Leung NWY, et al. A one-year trial of lamivudine for chronic hepatitis B. *N Engl J Med* 1998;**339**:61–8.
118. Lai CL, Lok AS, Lin HJ, et al. Placebo-controlled trial of recombinant alpha2-interferon in Chinese HBsAg-carrier children. *Lancet* 1987;**2**:877–80.
119. Lai CL, Ratziu V, Yuen MF, et al. Viral hepatitis B. *Lancet* 2003;**362**:2089–94.
120. Lambert V, Fernholz D, Sprengel R, et al. Virus-neutralizing monoclonal antibody to a conserved epitope on the duck hepatitis B virus pre-S protein. *J Virol* 1990;**64**:1290–7.
121. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005;**352**:2682–95.
122. Laub O, Rall LB, Truett M, et al. Synthesis of hepatitis B surface antigen in mammalian cells: expression of the entire gene and the coding region. *J Virol* 1983;**48**:271–80.
123. Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004;**11**:97–107.
124. Le Gal F, Gordien E, Affolabi D, et al. Quantification of hepatitis delta virus RNA in serum by consensus real-time PCR indicates different patterns of virological response to interferon therapy in chronically infected patients. *J Clin Microbiol* 2005;**43**:2363–69.
125. Lebensztejn D, Skiba E, Tobolczyk J, et al. Diagnostic accuracy of serum biochemical fibrosis markers in children with chronic hepatitis B evaluated but receiver operating characteristics analysis. *World J Gastroenterol* 2005;**11**:7192–96.
126. Lee JM, Ahn SH, Kim HS, et al. Quantitative hepatitis B surface antigen and hepatitis B e antigen titers in prediction of treatment response to entecavir. *Hepatology* 2011;**53**:1486–93.
127. Lee WM. Hepatitis B virus infection. *N Engl J Med* 1997; **337**:1733–45.
128. Lerut JP, Donataccio M, Ciccarelli O, et al. Liver transplantation and HBsAg-positive postnecrotic cirrhosis: adequate immunoprophylaxis and delta virus co-infection as the significant determinants of long-term prognosis. *J Hepatol* 1999;**30**:706–14.
129. Lin SM, Sheen IS, Chien RN, et al. Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999;**29**:971–5.
130. Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus—large-scale analysis using a new genotyping method. *J Infect Dis* 1997;**175**:1285–93.
131. Lok AS. Hepatitis B infection: pathogenesis and management. *J Hepatol* 2000;**32**:89–97.
132. Lok AS. The maze of treatments for hepatitis B. *N Engl J Med* 2005;**352**:2743–6.
133. Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen–positive Chinese children. *Hepatology* 1988;**8**:1130–3.
134. Lok AS, Lai CL, Su PC, et al. Treatment of chronic hepatitis B with interferon: experience in Asian patients. *Semin Liver Dis* 1989;**9**:249–53.
135. Lok AS, McMahon BJ. [AASLD Practice Guidelines. Chronic hepatitis B: update of therapeutic guidelines]. *Rom J Gastroenterol* 2004;**13**:150–4.
136. London W, Evan A. The epidemiology of hepatitis viruses B, C and D. *Clin Lab Med* 1996;**16**:251–71.
137. Loomba R, Liang TJ. Novel approaches to new therapies for hepatitis B virus infection. *Antivir Ther* 2006;**11**:1–15.
138. Lu CY, Chiang BL, Chi WK, et al. Waning immunity to plasma-derived hepatitis B vaccine and the need for boosters 15 years after neonatal vaccination. *Hepatology* 2004;**40**:1415–20.
139. Lunel F, Cadranel JF, Rosenheim M, et al. Hepatitis virus infections in heart transplant recipients: epidemiology, natural history, characteristics, and impact on survival. *Gastroenterology* 2000; **119**:1064–74.
140. Lusida M, Nugrahaputra VE, Handajani SR, et al. Novel subgenotypes of hepatitis B virus genotypes C and D in Papua, Indonesia. *J Clin Microbiol* 2008;**46**:2160–6.
141. Lykavieris P, Fabre M, Yvart J, et al. HBV infection in pediatric liver transplantation. *J Pediatr Gastroenterol Nutr* 1993;**16**:321–7.
142. Maddrey WC. Hepatitis B: an important public health issue. *J Med Virol* 2000;**61**:362–6.
143. Mason A, Wick M, White H, et al. Hepatitis B virus replication in diverse cell types during chronic hepatitis B virus infection. *Hepatology* 1993;**18**:781–9.
144. Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), part 1: immunization of infants, children, and adolescents. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1–31.
145. McCaffrey A, Nakai H, Pandey K, et al. Inhibition of hepatitis B virus in mice by RNA interference. *Nat Biotechnol* 2003; **21**:639–44.
146. McMahon BJ, Heyward WL, Templin DW, et al. Hepatitis B–associated polyarteritis nodosa in Alaskan Eskimos: clinical and epidemiologic features and long-term follow-up. *Hepatology* 1989;**9**:97–101.
147. McQuillan GM, Coleman PJ, Kruszon-Moran D, et al. Prevalence of hepatitis B virus infection in the United States: the National Health and Nutrition Examination Surveys, 1976 through 1994 [see comments]. *Am J Public Health* 1999;**89**:14–18.
148. Modahl LE, Lai MM. Hepatitis delta virus: the molecular basis of laboratory diagnosis. *Crit Rev Clin Lab Sci* 2000;**37**:45–92.
149. Murray K, Szenborn L, Wysocki J, et al. Randomized, placebo-controlled trial of tenofovir disoproxil fumarate in adolescents with chronic hepatitis B. *Hepatology* 2012;**56**(6):2018–26.
150. Ni YH, Chen DS. Hepatitis B vaccination in children: the Taiwan experience. *Pathol Biol (Paris)* 2010;**58**:296–300.
151. Ni YH, Huang F-C, Wu TC, et al. Lamivudine treatment in maternally transmitted chronic hepatitis B virus infection patients. *Pediatr Int* 2005;**47**:372–7.
152. Niro GA, Casey JL, Gravinese E, et al. Intrafamilial transmission of hepatitis delta virus: molecular evidence. *J Hepatol* 1999; **30**:564–9.

153. Niro GA, Ciancio A, Gaeta G, et al. Pegylated interferon alpha-2b as monotherapy or in combination with ribavarin in chronic hepatitis delta. *Hepatology* 2006;**44**:713–20.
154. Niro GA, Smedile A, Ippolito AM, et al. Outcome of chronic delta hepatitis in Italy: a long-term cohort study. *J Hepatol* 2010;**53**:834–40.
155. Nisini R, Paroli M, Accapezzato D, et al. Human $CD4^+$ T-cell response to hepatitis delta virus: identification of multiple epitopes and characterization of T-helper cytokine profiles. *J Virol* 1997;**71**:2241–51.
156. Oberhaus SM, Newbold JE. Detection of DNA polymerase activities associated with purified duck hepatitis B virus core particles by using an activity gel assay. *J Virol* 1993;**67**:6558–66.
157. Ostapowicz G, Fontana R, Scholdt F, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* 2002;**137**:947–54.
158. Ozturk M. Genetic aspects of hepatocellular carcinogenesis. *Semin Liver Dis* 1999;**19**:235–42.
159. Pickering L. *2000 Red book: report of the Committee on Infectious Disease*. 25th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2000. p. 98–101.
160. Pontisso P, Basso G, Perilongo G, et al. Does hepatitis B virus play a role in primary liver cancer in children of Western countries? *Cancer Detect Prevent* 1991;**15**:363–8.
161. Pugh JC, Summers JW. Infection and uptake of duck hepatitis B virus by duck hepatocytes maintained in the presence of dimethyl sulfoxide. *Virology* 1989;**172**:564–72.
162. Rabe C, Caselmann WH. Interaction of hepatitis B virus with cellular processes in liver carcinogenesis. *Crit Rev Clin Lab Sci* 2000;**37**:407–29.
163. Radjef N, Gordien E, Ivaniushina V, et al. Molecular phylogenetic analyses indicate a wide and ancient radiation of African hepatitis delta virus, suggesting a *Deltavirus* genus of at least seven major clades. *J Virol* 2004;**78**:2537–44.
164. Raftopoulos SC, George J, Bourliere M, et al. Comparison of noninvasive models of fibrosis in chronic hepatitis B. *Hepatol Int* 2011 Jul 12. [Epub ahead of print].
165. Rayes N, Seehofer D, Hopf U, et al. Comparison of famciclovir and lamivudine in the long-term treatment of hepatitis B infection after liver transplantation. *Transplantation* 2001;**71**:96–101.
166. Rehermann B, Lau D, Hoofnagle JH, et al. Cytotoxic T lymphocyte responsiveness after resolution of chronic hepatitis B virus infection. *J Clin Invest* 1996;**97**:1655–65.
167. Ren X, Luo G, Xie Z, et al. Inhibition of multiple gene expression and virus replication of HBV by stable RNA interference in 2.2.15 cells. *J Hepatol* 2006;**44**:663–70.
168. Rizzetto M. The delta agent. *Hepatology* 1983;**3**:729–37.
169. Rizzetto M. Hepatitis delta virus disease: an overview. *Prog Clin Biol Res* 1993;**382**:425–30.
170. Rizzetto M. Hepatitis D: thirty years after. *J Hepatol* 2009;**50**:1043–50.
171. Rogler CE, Summers J. Novel forms of woodchuck hepatitis virus DNA isolated from chronically infected woodchuck liver nuclei. *J Virol* 1982;**44**:852–63.
172. Rossner MT. Review: hepatitis B virus X-gene product: a promiscuous transcriptional activator. *J Med Virol* 1992;**36**:101–17.
173. Rozario R, Ramakrishna B. Histopathological study of chronic hepatitis B and C: a comparison of two scoring systems. *J Hepatol* 2003;**38**:223–9.
174. Rumin S, Gripon O, Le Seyec, et al. Long-term productive episomal hepatitis B virus replication in promary cultures. *J Viral Hepat* 1996;**3**:227–38.
175. Sagnelli E, Stroffolini T, Ascione A, et al. Decrease in HDV endemicity in Italy. *J Hepatol* 1997;**26**:20–4.
176. Sakamoto T, Tanaka Y, Simonetti J, et al. Classification of hepatitis B virus genotype B into 2 major types based on characterization of a novel subgenotype in arctic indigenous populations. *J Infect Dis* 2007;**196**:1487–92.
177. Samuel D, Muller R, Alexander G, et al. Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med* 1993;**329**:1842–7.
178. Scaglioni PP, Melegari M, Wands JR. Recent advances in the molecular biology of hepatitis B virus. *Baillieres Clin Gastroenterol* 1996;**10**:207–25.
179. Scharsschmidt B, Hed M, Hollander H. Hepatitis B in patients with HIV infection: relationship to AIDS and patient survival. *Ann Intern Med* 1992;**117**:837.
180. Schneider A, Habermehl P, Gerner P, et al. Alpha-interferon treatment in HBeAg positive children with chronic hepatitis B and associated hepatitis D. *Klin Padiatr* 1998;**210**:363–5.
181. Scotto J, Hadchouel M, Hery C, et al. Hepatitis B virus DNA in children's liver diseases: detection by blot hybridisation in liver and serum. *Gut* 1983;**24**:618–24.
182. Seeger C, Mason WS. Hepatitis B virus biology. *Microbiol Mol Biol Rev* 2000;**64**:51–68.
183. Shakil AO, Hadziyannis S, Hoofnagle JH, et al. Geographic distribution and genetic variability of hepatitis delta virus genotype I. *Virology* 1997;**234**:160–7.
184. Shitrit A, Kramer M, Bakal I, et al. Lamivudine prophylaxis for hepatits B virus infection after lung transplantation. *Ann Thorac Surg* 2006;**81**:1851–2.
185. Silverman N, Darby M, Ronkin S, et al. Hepatitis B prevalence in an unregistered prenatal population: implications for neonatal therapy,. *JAMA* 1991;**266**:2852–5.
186. Slagle BL, Zhou YZ, Birchmeier W, et al. Deletion of the E-cadherin gene in hepatitis B virus-positive Chinese hepatocellular carcinomas. *Hepatology* 1993;**18**:757–62.
187. Smith N, Yusuf H, Averhoff F. Surveillance and prevention of hepatitis B virus transmission. *Am J Public Health* 1999;**89**: 11–13.
188. Sobaniec-Lotowska ME, Lebensztejn D. Histological outcome of chronic hepatitis B in children treated with interferon alpha. *World J Gastroenterol* 2006;**11**:7179–82.
189. Soderstrom A, Lindh M, Ekholm K, et al. Predictive factors and virological response to interferon treatment in children with chronic hepatitis B. *Scand J Infect Dis* 2005;**37**:40–7.
190. Sokal EM, Conjeevaram HS, Roberts EA, et al. Interferon alfa therapy for chronic hepatitis B in children: a multinational randomized controlled trial. *Gastroenterology* 1998;**114**:988–95.
191. Sokal EM, Conjeevaram HS, Roberts EA, et al. Interferon alfa therapy for chronic hepatitis B in children: a multinational randomized controlled trial. *Gastroenterology* 1998;**114**:988–95.
192. Sokal EM, Kelly DA, Mizerski J, et al. Long-term lamivudine therapy for children with HBeAg-positive chronic hepatitis B. *Hepatology* 2006;**43**:225–32.
193. Sokucu S, Gokçe S, Suoglu OD, et al. Comparison of interferon monotherapy with interferon-lamivudine combination treatment in children with chronic hepatitis B. *Indian J Gastroenterol* 2006;**25**:136–9.
194. Southwest Pediatric Nephrology Study Group. Hepatitis B surface antigenemia in North American children with membranous glomerulonephropathy. *J Pediatr* 1985;**106**:571–8.
195. Squires R, Shneider B, Bucuvalas J, et al. Acute liver failure in children: the first 349 patients in the pediatrics acute liver failure study group. *J Pediatr* 2006;**148**:652–8.
196. Standring DN, Ou JH, Masiarz FR, et al. A signal peptide encoded within the precore region of hepatitis B virus directs the secretion of a heterogeneous population of e antigens in Xenopus oocytes. *Proc Natl Acad Sci U S A* 1988;**85**:8405–9.
197. Stevens CE, Taylor PE, Tong MJ, et al. Yeast recombinant hepatitis B vaccine: efficacy with hepatitis B immune globulin in prevention of perinatal hepatitis B virus transmission. *JAMA* 1987;**257**:2612–16.
198. Stibbe W, Gerlich WH. Structural relationships between minor and major proteins of hepatitis B surface antigen. *J Virol* 1983;**46**:626–8.
199. Su WJ, Ho MC, Ni YH, et al. Clinical course of de novo hepatitis B infection after pediatric liver transplantation. *Liver Transpl* 2010;**16**:215–21.
200. Summers J, Mason WS. Replication of the genome of a hepatitis B–like virus by reverse transcription of an RNA intermediate. *Cell* 1982;**29**:403–15.
201. Summers J, O'Connell A, Millman I. Genome of hepatitis B virus: restriction enzyme cleavage and structure of DNA extracted from Dane particles. *Proc Natl Acad Sci U S A* 1975;**72**:4597–601.
202. Szabo E, Paska C, Kaposi Novak P, et al. Similarities and differences in hepatitis B and C induced hepatocarcinogenesis. *Pathol Oncol Res* 2004;**10**:5–11.

203. Szmuness W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980;**303**:833–41.
204. Szmuness W, Stevens CE, Harley EJ, et al. Hepatitis B vaccine: demonstration of efficacy in a controlled clinical trial in a high-risk population in the United States. *N Engl J Med* 1980;**303**:833–41.
205. Tanaka T, Miyamoto H, Hino O, et al. Primary hepatocellular carcinoma with hepatitis B virus-DNA-integration in a 4-year-old boy. *Hum Pathol* 1986;**17**:202.
206. Tasar M, Bostanci I, Karabulut B, et al. A rare extrahepatic syndrome related to acute hepatitis type B: epididymitis in an adolescent. *Acta Gastroenterol Belg* 2005;**68**:270–1.
207. Taylor JM. Replication of human hepatitis delta virus: influence of studies on subviral plant pathogens. *Adv Virus Res* 1999;**54**:45–60.
208. Torre D, Tambini R. Interferon-alpha therapy for chronic hepatitis B in children: a meta-analysis. *Clin Infect Dis* 1996;**23**:131–7.
209. Vajro P, Migliaro F, Fontanella A, et al. Interferon: a meta-analysis of published studies in pediatric chronic hepatitis B. *Acta Gastroenterol Belg* 1998;**61**:219–23.
210. Vajro P, Tedesco M, Fontanella A, et al. Prolonged and high dose recombinant interferon alpha-2b alone or after prednisone priming accelerates termination of active viral replication in children with chronic hepatitis B infection. *Pediatr Infect Dis J* 1996;**15**:223–31.
211. Van Damme P, Vellinga A. Epidemiology of hepatitis B and C in Europe. *Acta Gastroenterol Belg* 1998;**61**:175–82.
212. Vo Thi Diem H, Bourgois A, Bontems P, et al. Chronic hepatitis B infection: long term comparison of children receiving interferon alpha and untreated controls. *J Pediatr Gastroenterol Nutr* 2005;**40**:141–5.
213. Wang J, Zhu Q, Wang X. Breastfeeding does not pose any additional risk of immunoprophylaxis failure on infants of HBV carrier mothers. *Int J Clin Pract* 2003;**57**:100–2.
214. Wasley A, Grytdal S, Gallagher K, Centers for Disease Control and Prevention (CDC). Surveillance for acute viral hepatitis—United States, 2006. *MMWR Surveill Summ* 2008;**57**(2):1–24.
215. Wasley A, Kruszon-Moran D, Kuhnert W, et al. The prevalence of hepatitis B virus infection in the United States in the era of vaccination. *J Infect Dis* 2010;**202**:192–201.
216. Wedemeyer H, Yurdaydin C, Dalekos GN, et al. Peginterferon plus adefovir versus either drug alone for hepatitis delta. *N Engl J Med* 2011;**364**:322–31.
217. Wirth S, Keller K-M, Schaefer E, et al. Hepatitis B virus DNA in liver tissue of chronic HBsAg carriers in childhood and its relationship to other viral markers. *J Pediatr Gastroenterol Nutr* 1992;**14**:431–5.
218. Woynarowski M, Cielecka-Kuszyk J, Kaluzynski A, et al. Interobserver variability in histopathological assessment of liver biopsies taken in a pediatric open label therapeutic program for chronic HBV infection treatment. *World J Gastroenterol* 2006;**12**:1713–17.
219. Wu JC, Choo KB, Chen CM, et al. Genotyping of hepatitis D virus by restriction-fragment length polymorphism and relation to outcome of hepatitis D. *Lancet* 1995;**346**:939–41.
220. Wu TC, Tong MJ, Hwang B, et al. Primary hepatocellular carcinoma and hepatitis B infection during childhood. *Hepatology* 1987;**7**:46–8.
221. Yaginuma K, Kobayashi H, Kobayashi M, et al. Multiple integration site of hepatitis B virus DNA in hepatocellular carcinoma and chronic active hepatitis tissues from children. *J Virol* 1987;**61**:1808–13.
222. Yan M-L, Yan L-N, Li B, et al. Intramuscular hepatitis B immune globulin combined with lamivudine in prevention of hepatitis B recurrence after liver transplantation. *Hepatobiliary Pancreat Dis Int* 2006;**5**:360–63.
223. Yu SB, Kim HY, Eo H, et al. Clinical characteristics and prognosis of pediatric hepatocellular carcinoma. *World J Surg* 2006;**30**:43–50.
224. Yuen MF, Lim WL, Cheng CC, et al. Twelve-year follow-up of a prospective randomized trial of hepatitis B recombinant DNA yeast vaccine versus plasma-derived vaccine without booster doses in children. *Hepatology* 1999;**29**:924–7.
225. Zanetti AR, Mariano A, Romano L, et al. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* 2005;**366**:1379–84.
226. Zeng M, Mao Y, Yao G, et al. A double-blind randomized trial of adefovir dipivoxil in Chinese subjects with HBeAg-positive chronic hepatitis B. *Hepatology* 2006;**44**:108–16.
227. Zhang X, Xu HJ, Murakami Y, et al. Deletions of chromosome 13q, mutations in Retinoblastoma 1, and retinoblastoma protein state in human hepatocellular carcinoma. *Cancer Res* 1994;**54**:4177–82.
228. Zhong YW, Li J, Song HB, et al. Virologic and clinical characteristics of HBV genotypes/subgenotypes in 487 Chinese pediatric patients with CHB. *BMC Infect Dis* 2011;**11**:262.

process also involves gH and gL. The herpes family coreceptors belong to the tumor necrosis factor family (Hve-A), the immunoglobulin superfamily of receptors (nectins), and the 3-*O*-sulfated heparin sulfates.[317] After the virion enters the cell, an orderly expression of immediate early, or α, genes occurs that triggers viral gene transcription and inhibits host cell function; early, or β, viral genes that encode regulatory proteins and DNA replication enzymes, including thymidine kinase and viral DNA polymerase; and finally, late, or γ, genes that encode structural proteins. Replication of viral DNA and accumulation of structural proteins allow assembly of the virion capsid in the cell nucleus and envelopment by modified cellular membranes that are incorporated into the virion envelope during virion egress. Infectious virions are released from the infected cell or can spread to adjacent cells via membrane fusion.

Human HSV replicates in tissue culture cells derived from many mammalian species and various laboratory animals, including rodents, rabbits, and primates. Lack of restriction of the host range distinguishes HSV-1 and HSV-2 from the other human herpesviruses. HSV-1 and HSV-2 replication progresses rapidly in cell culture and causes characteristic focal cytopathologic effects. Confirmation that these effects are caused by HSV can be accomplished by staining the infected cells with specific antisera or monoclonal antibodies that differentiate HSV-1 from HSV-2 and from other viral pathogens.

Like other herpesviruses, HSV-1 and HSV-2 have gene products that inhibit intrinsic cellular responses that interfere with replication and facilitate the avoidance of immune surveillance during primary infection, latency, and reactivation. Among others, these mechanisms include elaboration of virally encoded IgG Fc receptors formed by gE/gI and complement receptors that bind host immune components; downregulation of major histocompatibility complex (MHC) class I antigen, which prevents recognition of infected cells by $CD8^+$ T cells; interference with upregulation of MHC class II molecules by interferon-γ, which inhibits $CD4^+$ T-cell–mediated adaptive immune responses; and inhibition of the lytic activity of natural killer cells.[317]

TRANSMISSION

Infectious HSV-1 and HSV-2 virions are released into the oral or genital secretions of infected but asymptomatic persons who are experiencing primary or recurrent infection. This phenomenon is referred to as viral excretion or *viral shedding* and results in periodic opportunities for the virus to be transferred to susceptible contacts. Most HSV-1 and HSV-2 transmission is caused by silent infections, and transmission occurs with equivalent frequency regardless of whether the individual who is shedding the virus has ever had symptoms of oral or genital herpes.

Although HSV-1 and HSV-2 are highly infectious after inoculation of mucocutaneous sites, they are not transmitted casually from person to person. The enveloped virions are unstable outside mammalian cells, and close interpersonal contact is required for transfer of infectious virus particles. In most circumstances, transmission requires direct apposition of infected with uninfected mucous membranes or skin during intimate contact such as kissing or sexual contact. Outbreaks associated with gingivostomatitis have occurred at daycare centers.[344] If uninfected exposed skin or mucous membranes are damaged, the risk for transmission appears to be enhanced. Although skin is less susceptible to direct inoculation than the mucous membranes are, transmission via inoculation of skin may be increased by local trauma. For example, affected areas are more susceptible in burned patients[135] and infants with diaper rash. Children with eczema are at risk for contracting serious disseminated HSV infections (Kaposi varicelliform eruption).[13,430] Transfer of HSV-1 between wrestlers (herpes gladiatorum)[351] and between rugby players (herpes rugbeiorum or "scrum-pox")[353] probably takes place after the abrasion of saliva-contaminated skin. One outbreak at a wrestling camp involved 60 of 175 participants.[36,76] Health care workers may acquire HSV infections of the paronychial region (herpetic whitlow), presumably from direct contact of ungloved hands with oropharyngeal secretions.[142,319] Medical personnel can transfer HSV to their patients. Children acquire HSV whitlow in the course of primary HSV-1 gingivostomatitis through nail biting or thumb sucking. When herpetic whitlow develops in persons who are not health care workers, HSV-2 is a common cause and often is associated with primary genital HSV infection.[144] The usual route of transmission to neonates is by exposure during passage through an HSV-infected birth canal. Genital and anal HSV infections are transmitted through direct contact with infected genitalia or from orogenital or oroanal contact.

Newly acquired HSV-1 and HSV-2 infections are associated with shedding of high titers of infectious virus, regardless of whether the individual is symptomatic. In addition, active recurrent HSV-1 or HSV-2 lesions contain substantial titers of virus, which may increase the likelihood of transmission occurring during close contact with a susceptible individual.

The incidence of acquisition of HSV-2 is 5 to 15 percent per year in individuals who are HSV-1 and HSV-2 seronegative or HSV-1 seropositive but HSV-2 seronegative and whose sexual partners have recurrent genital herpes.[257,258] In studies of discordant couples, transmission occurred in 10 percent of partners, with higher transmission from men (17%) than from women (4%). In women lacking antibodies to HSV-1 and HSV-2, the rate of transmission was 32 percent; if they had antibodies to type 1, the rate was 9 percent.[257] Studies of patients by HSV-2–specific serology show that many individuals remain uninfected despite having long-term contact with a person who is infected with HSV-2.[215] The extent to which HSV-1 immunity protects against HSV-2 infection is not clear, but the evidence is that symptoms of new HSV-2 infection may be prevented if the exposed individual has HSV-1 immunity, whereas subclinical infection frequently is not blocked.[46,60] Information about whether HSV-1 or HSV-2 acquired earlier interferes with the acquisition of infection by unrelated HSV-1 or HSV-2 strains is limited, but a few individuals with multiple sexual partners have been found to shed HSV-2 viruses that are genetically distinct.

HSV has been isolated from the hands of patients with oral lesions and has been shown to persist for several hours on inanimate objects or in distilled water. Nonetheless, inanimate sources have not been implicated as important reservoirs of HSV persistence and spread.[275,402] HSV transmission from transplanted organs[119] and inseminated donor sperm has been reported.[262]

When HSV-1 and HSV-2 shedding has been evaluated prospectively in seropositive individuals with or without a history of symptoms, the usual frequency of detection of virus in oral or genital secretions is 1 to 2 percent.[15,317] In patients with known genital herpes, silent excretion has been documented as often as 10 percent of the time when they appear to be lesion free. Twelve percent of women with primary HSV-1 genital infection and 18 percent with primary genital HSV-2 infection subsequently shed virus asymptomatically, especially in the first 3 months after resolution of the primary infection.[204] Two percent of women attending a sexually transmitted disease clinic shed HSV-2 asymptomatically.[213] In partner studies, 70 percent of HSV-2 transmissions were associated with sexual contact during periods of asymptomatic viral shedding.[258]

Polymerase chain reaction (PCR) is considerably more sensitive than is culture for detection of HSV on mucosal surfaces, and the use of this technology has improved understanding of the extent of HSV viral shedding. However, it is important to recognize that the detection of segments of the viral genome by PCR does not necessarily mean that infectious virus is present. PCR detects subclinical shedding of HSV-2 on 20 to 25 percent of days. Factors that influence the magnitude and frequency of shedding include the site of infection, immune status of the patient, and time of acquisition of the virus.[324] Previous studies using viral cultures rarely reported isolation of HSV-2 from the mouth, but recent quantitative PCR studies detected asymptomatic oral HSV-2 reactivation from 40 percent of men with HSV-2 genital herpes. Oral HSV-2 shedding frequently is concurrent with genital HSV-2 shedding.[197]

Investigation of HSV-1 transmission is less extensive, but the high prevalence of asymptomatic HSV-1 infection in the population and its acquisition in early childhood suggest a similar pattern of spread by exposure to virus present in oral secretions from asymptomatic individuals.

Both HSV-1 and HSV-2 can be transmitted to a neonate. Approximately 4 percent of neonatal herpes cases are acquired in utero, 86 percent around the time of birth, and 10 percent postnatally. Three fourths of natal/postnatal cases of neonatal herpes infection are caused by HSV-2, and the remainder are caused by HSV-1 strains.[283] Virtually all neonatal HSV-2 infections probably are acquired from mothers having an active genital herpes infection by contact with infected genital secretions at or during delivery from an asymptomatic mother who acquired her first episode of genital herpes infection in the third trimester or near the time of delivery.[283,384,441,451] The majority of neonates with HSV appear to acquire the infection by contact with infected genital secretions at or during delivery from an asymptomatic mother who acquired her first episode of genital herpes infection in the third trimester or near the time of delivery.[51,52,55-57] Because 60 to 80 percent of mothers who have newborns with HSV have no signs or symptoms of genital herpes at the time of labor and delivery and have a negative past history of genital herpes or contact with a partner who had a genital vesicular rash, some experts recommend routine serologic screening of pregnant women and their partners to identify potential at-risk mothers.* Neonates with HSV-1 infection can acquire the virus from several sources: maternal genital tract, oral contact with mother, breast lesions,† oral herpes in the father or other family members,[117,384,452,453] or health care–associated transmission from other infected infants or health care workers.[161,241] Although HSV-1 cold sores and asymptomatic oral shedding may be common findings in nursery personnel,[170,171] transmission to a neonate from this source appears to be rare.[316,405] Male neonates may also contract HSV infection after out-of-hospital ritual circumcision.[80] In a recent report, 11 neonates were reported infected with HSV after orthodox Jewish ritual circumcision that included metzitzah B'peh, a practice in which the circumciser uses his mouth to apply direct orogenital suction to the newly circumcised penis to clean the blood away from the wound.[80]

EPIDEMIOLOGY

Understanding HSV epidemiology requires distinguishing between the prevalence of infection, which is high, and the frequency of HSV-related disease, which is low. These viruses maintain their persistence in the human population through their capacity to persist and reactivate in the individual host. The prevalence of HSV-1 and HSV-2 is a consequence of efficient transmission to susceptible close contacts and the continuous presence of a large pool of infected persons in the population. Because HSV-1 and HSV-2 are not cleared after primary infection but instead establish latency and reactivate frequently in an infected host, opportunities for viral spread to new susceptible persons are common. Seroepidemiologic studies have shown that HSV infections are found in all human populations, even in the most remote and isolated communities. HSV-1 and HSV-2 have no seasonal pattern because the mechanism of transmission is by intimate contact, which occurs year round. Individuals who have been infected with HSV-1 or HSV-2 remain a reservoir for infectious virus throughout their lifetimes and, as intermittent shedders, are sources of virus that spreads to susceptible contacts. Most transmission is from asymptomatic infection, and most acquisition is asymptomatic. For example, the rate of subclinical shedding of HSV-2 in individuals with no reported history of genital herpes was similar to that in subjects with such a history (3.0% vs. 2.7%).[420] Shedding of HSV-1 in oral secretions is equally prevalent.

When symptoms of a new HSV-1 infection do occur, the source of the infection rarely is identified because the susceptible individual usually has many infected contacts.

*References 51, 52, 54, 249, 434, 437, 438.
†References 52, 54, 56, 121, 240, 383, 452, 453.

The first symptomatic episodes of HSV-1 or HSV-2 often represent reactivations of earlier infections.

The average age at acquisition and the prevalence of HSV-1 and HSV-2 infection are influenced by the virus type. Although neonatal HSV infections are usually acquired from maternal genital infection and usually are caused by HSV-2, HSV-1 acquisition predominates in childhood. Depending on social and economic factors, 20 to 40 percent of young children are seropositive by the time they reach 5 years of age, with higher rates of acquisition in lower socioeconomic groups, non-Hispanic blacks, and children born outside the United States.[450] Earlier acquisition of HSV-1 may be influenced by whether the mother, or other major caregivers, has HSV-1 infection. Attendance at child care centers, which brings children together in close contact, may increase the likelihood of acquiring HSV-1 at an earlier age.[218,344] Acquisition of HSV-1 continues with age at an average rate of 1 to 2 percent per year throughout the childhood and adult years; by later ages, 70 to 90 percent of individuals have been infected by HSV-1.[360] Seroepidemiologic studies of higher socioeconomic groups have reported HSV-1 infection in 30 to 46 percent of university students.[141]

The prevalence of HSV-2 is highly variable and depends on the country, region, sex, age, population subgroup, and whether a population is "high risk."[357] Because transmission of HSV-2 is associated with sexual activity, the prevalence of HSV-2 begins to increase with adolescence. Now that serologic methods can be used to differentiate HSV-1 and HSV-2 infection, the seroprevalence of HSV-2 in adults has been shown to range from 20 to 60 percent. Continuing comprehensive investigation of HSV-2 epidemiology in the United States by the National Health and Nutrition Examination Survey (NHANES) documented a 30 percent increase in prevalence from the 1970s to the 1990s, which resulted in HSV-2 infection in approximately 20 percent of adults in the period between 1988 and 1994.[75,134,186] Since that time, HSV-2 seroprevalence has decreased to approximately 16% in individuals 14 to 49 years of age.[78] High HSV-2 infection rates are documented in higher as well as lower socioeconomic groups.[46,213,215,382] Rates are consistently higher in women, non-Hispanic blacks, and individuals with multiple sexual partners.

Changes in the epidemiology of symptomatic HSV-2 infection have been suggested by rising numbers of medical visits for genital herpes. The extent to which the increase in symptomatic cases of HSV-2 infection reflects a true increase in the prevalence of HSV-2 or a higher likelihood that HSV-2 infection will result in symptoms is not certain. The decrease in the number of young adults with HSV-1 infection acquired in childhood could be associated with loss of cross-reactive HSV immunity and an increased risk for the development of symptomatic genital herpes. A higher risk for contracting genital HSV-2 infection is associated with lower socioeconomic status, early age at first intercourse, increased numbers of sexual partners, female sex, previous marriage, urban living, black race, and incidents of trichomoniasis, bacterial vaginosis, and other sexually transmitted diseases.[134,149,187]

A retrospective analysis of genital HSV cultures obtained from a group of college students over the course of a 9-year period showed that HSV-1 is an increasingly common cause of genital herpes in some populations in the United States. In this particular population, HSV-1 accounted for more than 70 percent of positive genital cultures by the end of the study period, as compared with approximately 30 percent at the beginning.[314]

Symptomatic disease occurs after newly acquired HSV infection at an estimated incidence of 10 to 20 percent or less. However, silent infections can give rise to symptoms that appear months or years later as a result of viral reactivation. The epidemiology of HSV-1 or HSV-2 reactivation as a cause of symptomatic illness depends on the prevalence of these infections in the population as a whole, but it also is influenced by host factors that perturb the balance between the virus and the infected individual. For example, HSV-related symptoms may be triggered by exposure to sunlight (ultraviolet), febrile illnesses, immunosuppression from illness or necessary therapies, and other variables. These variables are cofactors that affect the frequency of HSV-related disease within the infected cohort but not the epidemiologic pattern of infection.

HSV infections may be transmitted from the mother to her fetus and neonate. Fulminant or disseminated primary HSV disease may occur in pregnancy, but whether it develops more often than in nonpregnant women is not clear.[221] A review[292] of seven such cases revealed (1) infection during the third trimester in all; (2) four beginning as a genital HSV infection and three as oral disease; (3) hepatitis in six, encephalitis in two, and pancreatitis in two; (4) maternal death in three (43%)—two from hepatitis and one from encephalitis; and (5) three instances of fetal death—each secondary to severe maternal systemic illness rather than direct infection with HSV. Postpartum HSV endometritis may also occur and result in disseminated infection in both mother and neonate.[130,253]

An increased rate of spontaneous abortion occurs in women with primary genital herpes during early pregnancy, regardless of socioeconomic status.[55,164,165,269] Premature delivery does not occur more commonly in prospectively monitored women with recurrent genital herpes,[164,283,409,447] but most reports of neonatal herpes cases find a greater preponderance of premature infants than in the general population.[283,441,452] This finding, however, may reflect a higher susceptibility of premature infants to HSV infection secondary to a lack of transplacentally acquired IgG neutralizing antibodies rather than being a cause of the prematurity. Recurrent genital herpes in middle-class pregnant women is no more severe or frequent than in nonpregnant ones,[164,409,447] but older studies demonstrated more frequent and longer episodes in women of lower socioeconomic groups.[269,276] Between 74 and 88 percent of middle-class pregnant women with a history of genital herpes had at least one clinical recurrence during an observed pregnancy.[164,409,447] A mean of 2.7 to 3.0 episodes occurred during gestation, and HSV was isolated from lesions in 56 to 75 percent of the recurrences and in 0.6 to 12 percent of concomitant cervical cultures obtained during the recurrence. Asymptomatic

shedding of HSV from the cervix, either between clinical recurrences or in a history-positive woman with no episodes during the observed pregnancy, was detected in 0.5 to 2.3 percent of the cultures obtained. Importantly, the presence of HSV shedding during the latter weeks of pregnancy was not predictive of shedding at the time of delivery.[15,447]

The number of cases of neonatal herpes occurring in the United States each year is far less than one would expect from the probable number of pregnancies complicated by genital herpes. Of the 3.5 to 4 million annual pregnancies, 7,000 to 200,000 of these women have genital herpes at some time during the pregnancy and 3,500 to 14,000 have positive HSV cultures at the time of delivery.* The actual number of cases of neonatal herpes per year in Seattle and Atlanta (0.1 and 0.3 per 1000 live births, respectively) would project to a national estimate of 350 to 1,050 per year in the United States.[384] An estimate of the incidence of neonatal HSV is between 1 in 3,000 and 1 in 20,000 and may vary with the demographics and geographic location of the population. Because asymptomatic HSV infection of the neonate rarely, if ever, occurs, subclinical cases or missed diagnoses probably do not account for the differences between the estimated number of maternal and neonatal infections. More likely, neonatal infection rates in the neonates of mothers with genital herpes are far lower than currently assumed, possibly because of the preventive measures used. The estimated infection rates quoted most often currently are (1) a 33 to 50 percent rate for infants vaginally delivered by mothers with primary genital herpes, (2) a 3 to 5 percent rate for infants vaginally delivered by mothers with recurrent lesions, and (3) less than 3 percent for those delivered by mothers with recurrent asymptomatic shedding at the time of delivery.† In the early 1970s,[269] the following neonatal infection rates in neonates born to mothers with genital herpes during pregnancy were reported: (1) 33 percent in mothers with primary disease after the 32nd week of gestation, (2) 3 percent in mothers with recurrent episodes after 32 weeks, and (3) 42 percent in mothers with virus-positive lesions at the time of delivery (primary vs. recurrent episodes not specified). More recent studies have determined a significant risk for neonatal herpes occurring in neonates born to mothers with asymptomatic shedding at the time of vaginal delivery: 33 percent with subclinical first episodes and 0 to 3 percent with recurrent shedding.‡ Accurate knowledge of the actual neonatal infection risk is important for making decisions about management (e.g., cesarean section, prophylactic antiviral therapy). Researchers and experts have estimated that as many as 75 percent of neonates with HSV disease are born to mothers with no history or clinical findings suggestive of active HSV infection during pregnancy, labor, or delivery.[5,52,69,70,71]

Several factors influence the risk for acquiring neonatal herpes and the severity of neonatal disease once the infection develops. The greater risk for transmitting neonatal infection by mothers with primary, as opposed to recurrent, vesicular or ulcerative lesions at the time of delivery is well established. Also, a woman with virus-positive recurrent lesions at the time of delivery is likely to be at greater risk than is a woman who is asymptomatic and shedding virus identified by HSV surveillance cultures. Other aspects of the anatomic site and the severity of maternal genital herpes also may be associated with a greater risk for development of neonatal disease: (1) maternal cervical as opposed to vulvar or buttock skin involvement—resulting in more virus being shed into vaginal secretions; (2) multiple as opposed to single vesicles or ulcers—again, more virus from multiple lesions; (3) higher titer of virus in vaginal secretions—cultures positive sooner or more intensely positive in the diagnostic virology laboratory; and (4) longer duration of fetal exposure to infected vaginal secretions because of prolonged rupture of membranes or of fetal scalp monitors.[92,281-283]

Investigations by Yeager and associates[452,453] indicate that no anti-HSV antibody or a low titer of such antibody in maternal and neonatal serum is associated with a greater risk for acquisition of neonatal infection and more serious disease and that high-titer antibody is associated with a lower risk. Studies of HSV antibody titers in neonates do not show an association between antibody titer and outcome of disease. More investigations are required, therefore, to determine the potential protective role of maternal transplacental anti-HSV antibody.

Premature infants have accounted for 40 to 50 percent of cases of neonatal herpes,[394,434,444] in contrast to the usual prematurity rates of 6 to 18 percent reported in the general maternal population. Whether the increased frequency of prematurity among neonates with herpes indicates a greater propensity of mothers with genital herpes to deliver prematurely or a greater susceptibility of premature infants to HSV infection is not known. Premature infants appear more likely to have a fatal outcome.[71,434,435]

Instrumentation of the neonate, particularly scalp electrodes for fetal monitoring, is known to increase the risk for acquiring neonatal HSV infection.[122,195,287] Mortality rates appear to be higher in neonates with HSV-2 than in those with HSV-1 infection, probably because of the greater proportion of HSV-2–infected infants with dissemination.[435] In neonatal herpes survivors, neurologic damage occurs much more frequently in infants with HSV-2 infection than in those with HSV-1 infection.[96,435] Infants with disseminated infection (liver, lungs, adrenals), with or without central nervous system (CNS) involvement, have the highest mortality rates (70% to 80%); those with encephalitis have only intermediate rates (30% to 40%); and those with infection limited to the skin, eye, or mouth have the lowest rates (0%).[444] Rarely, neonatal HSV has been implicated as a cause of sudden unexpected death in infancy.[426]

The prevalence of HSV infection in febrile neonates presenting to an acute pediatric emergency treatment center appears similar to that of bacterial meningitis, suggesting that HSV infection should be in the differential of neonatal fever and evaluated accordingly.[69-71] Factors associated with neonatal HSV infection in febrile or hypothermic neonates presenting to acute care centers

*References 44, 151, 165, 390, 409, 437, 447.
†References 44, 52, 92, 283, 447.
‡References 52, 69, 70, 71, 284, 301.

include history of peripartum maternal fever, history of maternal primary HSV infection, prematurity, vaginal delivery, neonatal seizures, vesicular rash, respiratory distress, thrombocytopenia, elevated hepatic enzymes, and CSF pleocytosis.[69-71] Because the presentation of neonatal HSV is nonspecific, and delay in diagnosis and treatment is associated with significant morbidity and mortality, some experts recommend all neonates younger than 21 days of age receive evaluation for HSV infection and early empirical treatment with acyclovir.[69-71,246]

PATHOGENESIS AND PATHOLOGY

HSV-1 and HSV-2 exhibit particular tropism for cells of ectodermal origin, including skin and neuronal cells. Initial viral replication is thought to occur at the portal of entry, usually in mucous membranes or skin. In symptomatic cases, the incubation period for primary infection appears to vary from 2 to 20 days. In contrast to varicella, HSV viremia is difficult to detect by culture in a normal host, although it may be found in some immunocompromised patients.[158,370] HSV DNA in plasma and peripheral blood mononuclear cells in HSV-infected neonates has been detected with PCR techniques.[111] HSV DNA has also been detected in approximately 25% of patients with confirmed primary genital HSV infection. Women are more likely to be viremic than men, and viremia is detected more frequently early after infection.[188]

If mucocutaneous lesions are induced during primary or recurrent infection, the pathologic cell changes caused by HSV-1 or HSV-2 replication include cytoplasmic enlargement and nuclear alterations. Cell fusion may lead to the formation of multinucleated giant cells. The nuclei of infected cells often have eosinophilic intranuclear inclusions and marginated nuclear chromatin. As the cells manifest injury, a local inflammatory response ensues, intercellular edema develops, and vesicles form. The vesicles become visible as they enlarge and usually are surrounded by an erythematous margin. At later stages, the vesicles become pustular and then dry and crust. HSV lesions typically are superficial and do not scar. Vesicles that form on mucous membranes are transient, with rapid sloughing of the superficial layer, and are seen first as shallow ulcers.

HSV-1 and HSV-2 establish latency in neurons of the sensory ganglia by mechanisms that are as yet unidentified. The viruses persist in this latent state for various intervals, although frequent asymptomatic reactivation is common; reactivation induces viral replication with the production of infectious virus at mucosal or other sites. Persons who have recurrent HSV infections frequently describe tingling sensations, itching, and burning at the site of recurrence beginning several hours before the appearance of clusters of vesicles. Persons who have recurrent genital herpes may experience severe shooting pain in their legs and even urinary retention in connection with recurrence. Occasionally, recurrent HSV skin eruptions may occur in a zosteriform pattern in a distribution reflecting the sensory innervation of a particular dermatome.

Replication at the portal of entry appears to result in infection of sensory nerve endings, followed by transport of the virus to the cranial and dorsal root ganglia along neuronal axons. Latency is established regardless of whether the primary infection is symptomatic, and it appears to be an invariable consequence of HSV infection with either virus type.[27,315,317] HSV-1 and HSV-2 persist in neuronal cells of the sensory ganglia, with substantial numbers of these cells harboring latent virus, as demonstrated by the detection of latency-associated transcripts. The latency-associated transcripts overlap the viral genes *ICPO* and *ICP34.5* in an antisense direction.[97,317,372] The role of these transcripts in the establishment or maintenance of latency remains to be clarified. The presence of latency-associated transcripts appears to be necessary for establishment of latency and efficient reactivation of viral production in some animal models in vivo.[294] Viral particles are not produced in latently infected cells. HSV-1 persists most predominantly in the cranial nerve ganglia, whereas HSV-2 latency occurs in the lumbosacral ganglia. Cocultivation techniques have been used to recover HSV from the dorsal root ganglia innervating the areas of skin in which persons have experienced recurrent herpes lesions. HSV-1 has been found in trigeminal ganglia, and HSV-2 has been recovered from sacral ganglia.[29] Once latency is established in the sensory ganglia, antiviral drugs cannot eradicate the latent virus from infected neurons. Because the latent virus does not multiply, it is not susceptible to drugs that affect viral DNA synthesis, such as acyclovir. When reactivation is triggered, HSV-1 or HSV-2 is transported back down axons to mucocutaneous sites, where they replicate and release infectious virus into secretions.[27,99,315,317] Individuals with symptomatic recurrent HSV infections almost always have HSV lesions in the identical or a directly adjacent site. Reactivation is not prevented by adaptive immunity, although whether symptoms occur and whether the virus disseminates may be influenced by the host response.

The stimulus for reactivation of latent virus may be provided by iatrogenic or naturally occurring episodes of immunosuppression and by endocrine (e.g., menstruation) or exogenous (e.g., trauma, sun, acupuncture, emotional stress) factors. Ultraviolet irradiation of the usual site of recurrence of persons who have a history of recurrent oral herpes reliably induces recurrence either quickly (within 48 hours) or 2 to 7 days later.[361] Skin subject to recurrent HSV infection has been transplanted elsewhere on the body and exchanged with skin taken from the site of graft placement and previously not involved in HSV infections. Subsequent recurrence of HSV infection was found to be localized to the original site, not to the original skin. These studies and others strongly suggest that latent virus does not reside in skin cells.

Primary HSV infection elicits humoral and cellular immunity, which can be detected shortly after the appearance of lesions in individuals in whom symptoms develop. Based on animal models and some human studies, the initial host response is mediated by innate, nonspecific mechanisms, followed by the acquisition of virus-specific adaptive immunity.[205] The nonspecific response consists of mobilization of polymorphonuclear leukocytes and monocytes to the site of infection, release of interferon-γ and other cytokines, and activation of macrophages and natural killer cells. The innate response is followed by

the production of antiviral antibodies that can be detected 2 to 12 weeks after infection occurs. HSV-specific antibodies mediate neutralization of virus, complement fixation, and cellular or complement cytotoxicity. Induction of specific cellular immunity is detected by measuring T-cell recognition of HSV proteins in proliferation and cytotoxicity assays and production of interleukin-2 and interferon-γ. Failure of virus-specific, cell-mediated immunity to develop, as may occur in neonates and children with genetic immunodeficiencies, immunocompromised children, and other high-risk populations, can be associated with life-threatening dissemination of HSV-1 or HSV-2. Disseminated HSV can infect and destroy cells in many organs (e.g., the lungs, liver, adrenals, and brain), with multiple- organ hyperinflammation leading to massive apoptosis, with catastrophic effects. It is likely disseminated HSV in the neonate and in the older child and adult causes a systemic inflammatory response syndrome that often causes a fatal outcome. One case report reported the sequential changes of hyperinflammation in a neonate with disseminated HSV disease. As the HSV DNA copies rose in the patient's serum, an increase in the biomarker concentrations of first high-mobility group box 1 and then cytochrome c was observed. Anti-inflammatory interventions with prednisolone, high-dose immunoglobulin, blood exchange, and high dose acyclovir were used for treatment, and the patient survived.[267] A small group of immune-seronegative individuals with HSV-specific T-cell responses has been described. None of these individuals had clinical symptoms of oral or genital HSV. All had exposure to HSV-2–positive sexual partners, but HSV was never isolated by culture or PCR assay from these exposed individuals, even after extensive sampling over a prolonged period.[300] Studies to determine whether genetic immunologic determinants of disease or mutations in HSV entry receptors alter susceptibility to HSV infection are in progress.[379]

Symptomatic HSV-1 and HSV-2 recurrences are less severe than are primary lesions in immunologically intact persons. In persons previously infected with one type of virus (e.g., HSV-1), new infections with the second type (e.g., HSV-2) more often are silent, or the symptoms are less severe than in a host who has never been infected with either virus. When reinfection with unrelated strains has been identified, symptoms also have been mild and, without molecular analysis of viral DNA, would be attributed to reactivation of endogenous virus.[62] The immune response to symptomatic reactivation is not associated with a significant increase in the production of antibody, although fourfold rises and reemergence of IgA and IgM antiviral antibody may occur. Natural killer cell activity and cytokine production increase. Relative defects in these and HSV-specific T-cell responses may predispose the individual to frequent or severe symptomatic recurrences. A cohort of patients with severe eczema, sinopulmonary infections, elevated serum IgE levels, eosinophilia, and cutaneous viral infection (including severe HSV) caused by DOCK8 deficiency has been described.[455] Patients who have diseases or are being treated with agents that reduce cell-mediated immunity, such as those undergoing antitumor chemotherapy or individuals with acquired immunodeficiency syndrome (AIDS), often have frequent HSV recurrences that are longer in duration and more severe, but dissemination rarely occurs.

Viral encephalitis is the most severe consequence of HSV infection in an otherwise healthy host. HSV-1 is the pathogen in almost all these cases, and the pathologic mechanism is thought to be ascending infection along neuronal pathways from the cranial nerve ganglia to the brain.[104] Encephalitis may accompany or develop after primary HSV infection, but it also occurs as a consequence of reactivation of latent virus. When the virus reaches brain parenchymal cells, it replicates efficiently and induces widespread hemorrhagic necrosis and vascular compromise. The neuroimmune response to infection contributes to brain inflammation and subsequent sequelae. Activated glial cells (astrocytes and microglia) respond to HSV infection with the production of cytokines and chemokines.[245] HSV encephalitis has been associated with an acute-phase elevation in β_2-microglobulin, neopterin, interleukin-6, and interferon-γ in cerebrospinal fluid (CSF).[21,22] During convalescence, increased levels of soluble CD8, β_2-microglobulin, neopterin, and specific anti-HSV IgG have been detected. How these markers relate to the pathogenesis of disease has not been established.

Genetic heterogeneity involving a collection of inborn errors of immunity to HSV may predispose to herpes encephalitis. To date, signal transducer and activator of transcription–1 (STAT1), UNC93B, Toll-like receptor–3 (TLR3), and TNF receptor-associated factor–3 (TRAF3) deficiencies and nuclear factor-κB (NF-κB) essential modulator mutations have been found in a few children with herpes encephalitis.[20,67,456] Most recently, TIR-domain–containing adapter-inducing interferon-β (TRIF) deficiency has been reported in children with herpes encephalitis, demonstrating the importance of TRIF for the TLR3-dependent production of antiviral interferons in the CNS during primary infection with HSV-1 in childhood.[334] A recent genetic epidemiology survey suggests that childhood HSV encephalitis, although sporadic, may result from mendelian predisposition (from autosomal recessive susceptibility in particular), at least in some children.[1]

HSV may be transmitted to the neonate in utero either by a transplacental (congenital infection) or ascending route, at the time of birth (natal infection), or after birth (postnatal infection).[394] Congenital infection probably results from transplacental transmission of virus secondary to leukocyte-associated viremia in a mother with genital herpes, but no direct evidence supports this hypothesis. HSV-2 viremia has been documented in two women with primary genital herpes.[189] A report of 13 neonates with intrauterine HSV infection indicated that primary genital herpes was present during pregnancy in four mothers, recurrent disease was present in one, and no history of genital herpes was elicited in the remaining eight.[356] In the fetus, HSV appears to be transmitted directly from the placenta through the bloodstream to target organs. Because of the known tropism of HSV for the CNS, one is not surprised that most infants with congenital infection have evidence of brain involvement at birth.[356] Intrauterine infection also may occur by an ascending route from an infected maternal genital tract,

and such transmission is supported by the development of clinical signs of HSV infection in the first 5 days of life.

The natal infection presumably is acquired secondary to aspiration of infected vaginal secretions into the nares, oropharyngeal cavity, conjunctivae, and upper respiratory tract of the infant. Other portals of entry for natal infection include the scalp, skin, and umbilical cord. In postnatal HSV infection, no evidence supports the genital tract as a source of virus. Most postnatally acquired infections appear to result from contact with saliva from persons with oral herpes or with virus carried on the hands of personnel. After natal or postnatal acquisition of HSV occurs, initial replication of the virus occurs at the portal of entry, which most often may be the conjunctivae or oral mucosa, with subsequent viremic dissemination to viscera. Involvement of the CNS occurs either from hematogenous spread to the brain in infants with disseminated disease, which results in meningoencephalitis and multiple areas of cortical hemorrhagic necrosis, or from retrograde axonal transport of the virus to the CNS from localized, superficial replication in the skin, eye, or mouth.

CLINICAL MANIFESTATIONS

Most HSV-1 and HSV-2 infections do not cause symptoms, but when infection is symptomatic the clinical manifestations usually are self-limited and not severe. If symptoms occur, the disease associated with primary infection tends to be much more extensive than are the minor, localized lesions at mucocutaneous junctions caused by viral reactivation. Nonetheless, prospective studies document that new HSV infections can be as mild as symptomatic recurrences, and, as a result, definitive differentiation of primary from recurrent infection is not possible with clinical criteria.

Gingivostomatitis

Gingivostomatitis is the most common form of HSV-induced primary illness in children. A history of such symptoms has been reported in as few as 1 percent to as many as 31 percent of seropositive children, the higher percentage being from a study involving the Navajo Indians. It usually is seen in young children between 10 months and 3 years of age. In those younger than 10 months old, residual maternal antibody probably modifies or prevents the appearance of recognizable symptoms in association with primary HSV-1 infection. Although acute gingivostomatitis caused by HSV is a relatively infrequent occurrence, it is sufficiently common that most pediatricians are familiar with the condition and learn to distinguish this infection from herpangina.[286,317]

The illness begins with irritability and fever. Despite these systemic symptoms, HSV is not cultured from blood during this period[158]; however, investigators have detected HSV-1 DNA by PCR assay in whole blood or plasma early in the course of illness in approximately a third of young children with primary HSV-1 gingivostomatitis.[163] The infant usually refuses to eat and may refuse

FIGURE 157-1 ■ Primary HSV gingivostomatitis in a normal toddler: ulcerative-vesicular stage. (Courtesy Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)

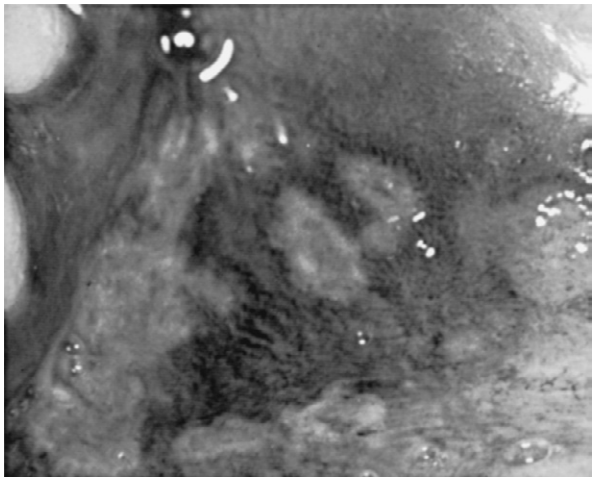

FIGURE 157-2 ■ Primary HSV gingivostomatitis in a 6-year-old child, demonstrating involvement of the oral mucosa with ulcers.

fluids. Thereafter, vesicular lesions appear around and on the lips, along the gingiva, on the anterior tongue, and on the anterior (hard) portion of the palate (Figs. 157-1 and 157-2). The vesicles break down rapidly, and, when seen, lesions usually appear as 1- to 3-mm shallow gray ulcers on an erythematous base. The gums are erythematous, mildly swollen, and ulcerated. They may appear friable and bleed on contact. The child experiences extreme discomfort, will not eat, and, if fluids are refused as well, may require hospitalization to ensure that adequate hydration is maintained. The risk for dehydration is compounded by the fever that generally accompanies this syndrome. Vesicles often extend around the lips and chin or down the neck in an immunologically intact child. The child frequently has foul-smelling breath (fetor oris). The lesions bleed easily and may become covered with a black crust. The cervical and submental nodes usually are swollen and tender. The clinical signs continue to evolve

for 4 to 5 days, and the process of resolution requires at least an additional week. In an analysis of the natural history of HSV gingivostomatitis in 36 children, oral lesions persisted for an average of 12 days, most children had extraoral lesions, fever lasted for 2 to 6 days, and difficulty taking liquids was noted for 4 to 10 days. The duration of viral shedding was 7 days (range, 2 to 12 days).[7] Herpetic epiglottitis[45] and acute otitis media[84] are unusual complications.

HSV gingivostomatitis is differentiated from herpangina, a manifestation of enteroviral infection, by the location of ulcers in the anterior portion of the oropharynx. Herpangina generally causes posterior pharyngeal ulcers. In addition, unlike HSV infection, herpangina often has a more acute onset, a shorter duration, and seasonal occurrence.[286] Whereas enterovirus-associated hand, foot, and mouth disease can manifest as oral ulcers and a vesicular eruption on the distal portions of extremities, its bilateral distribution should differentiate it from HSV gingivostomatitis and concurrent HSV autoinoculation of a digit. Severe Stevens-Johnson syndrome (erythema multiforme) may mimic HSV infection, but the generalized macular rash accompanied by "bull's-eye" lesions is characteristic of erythema multiforme. Rarely, recurrent HSV infection can be associated with erythema multiforme (see later discussion). Impetigo may be confused with the lesions of HSV infection, and misdiagnosis is reinforced because colonization of skin by *Staphylococcus aureus* may be identified in bacterial cultures and be considered causative.

Parents and caregivers are familiar with "cold sores" or "fever blisters" but may not know that these lesions are caused by HSV. Because HSV infection may be thought of as a sexually transmitted disease, the physician is advised to anticipate confusion and anxiety when making the diagnosis of HSV oral infection and to address these concerns by explaining the normal mode of acquisition of oral HSV infection by young children.

In adolescents, primary HSV-1 infection can be seen initially as exudative pharyngitis.[255] Characteristic findings are shallow tonsillar ulcers with a gray exudate. HSV infection must be differentiated from streptococcal and Epstein-Barr virus infection and rarely from diphtheria, acute human immunodeficiency virus (HIV) infection, and tularemia-induced pharyngitis. In one study of college students of a higher socioeconomic level, HSV was the most common etiologic agent of acute pharyngitis (24%).[143] Another study of 613 college students with upper respiratory tract complaints documented an incidence of 5.7 percent with positive HSV cultures. Twelve of the 35 students with positive cultures had vesicular lesions on their lips, throat, or gums, and 29 of the 35 had a primary diagnosis of pharyngitis that was indistinguishable from other causes of pharyngitis.[255] In some cases, acute pharyngitis is caused by HSV-2; the symptoms and clinical course appear to be similar, and viral cultures are required to identify the type of infecting virus.

Vulvovaginitis

Primary herpetic vulvovaginitis occurs rarely in infants and children. HSV-1 may cause this clinical syndrome, perhaps if inadvertently introduced when the genital area is touched by a caregiver with HSV on the hands. Progression of the infection may be limited to a few lesions, or it may resemble symptomatic primary genital herpes caused by HSV-2. Obtaining material for culture to identify the type of infecting virus is important because HSV-1 infection is less likely to recur and HSV-2 infection may reflect sexual abuse of the child. Genital HSV infection in young children warrants a careful and sensitive appraisal of the circumstances that possibly led to the infection. Children infected with HSV-2 in the neonatal period have had genital lesions later in childhood.

Pediatricians are likely to encounter genital herpes in their adolescent patients. Reports on the incidence of genital herpes in which data are provided for subgroups of children and adolescents are limited, but a rate of 3 per 100,000 in the 10- to 14-year-old age group and 76 per 100,000 in the 15- to 19-year-old age group was reported in a Minnesota study of a predominantly white, middle-class population of northern European ethnicity.[85] Older NHANES studies reported the prevalence in 12- to 19-year-old subjects to be 5.6 percent.[134] Recent NHANES data (2005 to 2008) show a decrease in HSV-2 seroprevalence in this age group, most recently to 1.4 percent in 14- to 19-year olds.[451] In one report of 379 adolescents aged 14 to 19 years who were treated at a sexually transmitted disease or urban community clinic, 12 percent had HSV-2 antibodies and only 22 percent of these patients had a history of genital herpes. HSV-2 seropositivity correlated with black race or female gender but not with condom use, the number of sexual partners in the previous 2 months, or a previous history of a sexually transmitted disease, thus indicating that prevalence was related to demographic rather than behavioral variables.[380]

Prospective studies have documented that many infections are subclinical and that new genital HSV-2 infections have a wide range of manifestations.[228,258] Variation is common with respect to the extent and severity of lesions; whether the lesions are bilateral or localized; the presence or absence of systemic symptoms such as malaise, myalgia, headache, and fever; dysuria; and regional lymphadenopathy. Symptoms can be mild enough to suggest recurrent infection or may be those of severe, "classic" primary genital herpes. In such cases in adults, local genital symptoms include severe pain (in 95% of men and 99% of women), itching, dysuria (44% of men and 83% of women), vaginal or urethral discharge, and tender inguinal adenopathy (80%). The lesions begin as vesicles or pustules and progress to wet ulcers and then to healing ulcers with or without crusts (Figs. 157-3 to 157-5). Crusting usually occurs only on squamous epithelium. Lesions tend to last for 2 to 3 weeks (mean, 19 days) before complete healing occurs. They may spread in a wavelike fashion from the initial site to the thighs, buttocks, and urethra. Virus shedding generally persists for 1 to 2 weeks or longer. Some primary infections are associated with atypical lesions (e.g., fissures, furuncles, excoriations), and extragenital lesions, typically on the buttocks, may be observed.[38,213]

When evaluating potential primary genital herpes in a sexually active adolescent, the history may reveal recent contact with a partner who is known to have recurrent

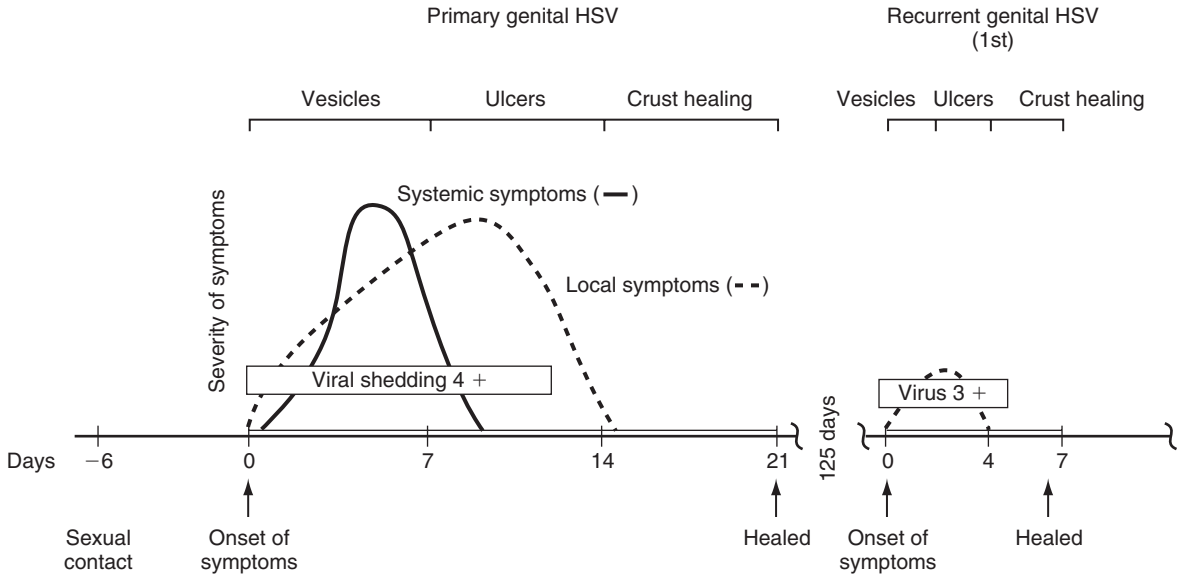

FIGURE 157-3 ■ Graphic representation of the course of primary and recurrent HSV genital infection. The days illustrated are for average cases.

FIGURE 157-4 ■ **Primary genital herpes in a female.** (Courtesy Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)

FIGURE 157-5 ■ **Primary genital HSV infection in a male.** (Courtesy Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)

genital herpes. When exposure can be documented, the incubation period is estimated to be 2 to 14 days. However, most episodes will not be associated with a known exposure. It is important to recognize that as many as two thirds of first-reported episodes of symptomatic genital herpes actually are caused by reactivation. The exposure may have occurred months or years before the signs of HSV-2 infection appear. In some adult series, 50 to 60 percent of persons without a history of HSV infection can identify symptoms consistent with previous infection after receiving education about the typical signs, but many have not had any previous episodes.[226] HSV-1 accounts for 5 to 25 percent of primary genital HSV infections in the United States. Up to 50 percent of cases diagnosed in other geographic areas, such as England and Japan, are caused by HSV-1. Indications are that HSV-1 is becoming a more common primary genital isolate in the United States. In recent years, HSV-1 has been isolated from genital cultures more often than HSV-2 has in some populations.[314] Patients with primary HSV-1 genital infection occasionally exhibit lesions elsewhere (25%), typically on the hands and face.[38]

Primary genital HSV-2 infections may be associated with viral meningitis. This complication is manifested as fever, stiff neck, headache, and photophobia with a CSF lymphocytic cellular response and usually a normal glucose level.[173,392] HSV-2 can be isolated from CSF in the early phase of the illness. HSV-2–associated meningitis differs from HSV-1 encephalitis in that it is almost always mild, self-limited, and not associated with neurologic residua.

Other complications of primary HSV genital infection include sacral autonomic nervous dysfunction, which is manifested as poor rectal sphincter tone, constipation, sacral anesthesia, urinary retention, or impotence. Extragenital lesions may be present. These are more frequent in women and occur on the buttocks, groin, thighs, or, less commonly, the fingers or conjunctiva, generally in the second week of disease. In addition, secondary yeast infections in women, and pharyngitis, which usually is associated with fever, malaise, myalgia, headache, tender anterior cervical adenopathy, and a mildly erythematous to diffusely ulcerative or exudative posterior pharyngitis may be seen. Most patients have throat cultures positive for HSV. HSV has been associated uncommonly with acute salpingitis[235] and inguinal lymphadenitis.[388] Transmission to the fetus or neonate is an important and very serious complication of primary or recurrent genital herpes in women of child-bearing age.

Of critical importance is the association of previous HSV infection with an increased incidence of HIV infection.[368] Investigators have considered the possibility that the risk for infection with HIV is enhanced by mucosal ulcers or that the increase in local $CD4^+$ lymphocytes resulting from HSV infection offers a greater number of target cells for HIV attachment and entry. Conversely, individuals with HIV infection reactivate HSV frequently and also have HIV present in mucosal ulcers.[417] A study from Kenya showed that HSV shedding was associated with significantly higher vaginal and cervical HIV shedding, even after controlling for plasma HIV load and $CD4^+$ count.[26] Although all patients with HSV-2 genital infection appear to be at higher risk for acquiring HIV infection, those with recent HSV-2 seroconversion appear to be more susceptible.[312,396] This finding has been confirmed in other populations.[194]

Genital herpes creates significant psychological difficulties for many patients. Whereas many people cope well with the illness and the likelihood of recurrent disease, a syndrome of profound depression, poor self-esteem, complete abstention from sexual activities, and general withdrawal develops in some. This reaction, or "leper syndrome," must be anticipated and discussed in a sensitive and caring manner. Information about the high prevalence of these infections in the population may be helpful, with emphasis placed on the fact that most infected people do not realize that they are infected and shed the virus intermittently, just like those who have had symptoms, as well as effective treatment options.

Other Primary Skin Infections

Mucocutaneous junction areas are common sites of HSV infection, and damaged skin often provides a portal of entry for HSV. Vesicular lesions spread throughout the affected skin and usually crust and resolve in approximately 1 week. The illness accompanying eczema herpeticum can be severe (Fig. 157-6) and even fatal, although in most cases it resolves without specific therapy and leaves no sequelae. Increased use of topical calcineurin inhibitors has raised concern regarding possible immune

FIGURE 157-6 ■ Extensive HSV infection in an infant with atopic eczema (Kaposi varicelliform eruption). (Courtesy Dr. Theodore Rosen, Department of Dermatology, Baylor College of Medicine, Houston.)

suppression and an increase in cases of eczema herpeticum. One study showed the incidence density of herpesvirus skin infections to be similar in infants who received 1 percent pimecrolimus cream and those who received the vehicle without active drug.[288] However, of those children characterized as having eczema herpeticum (more extensive skin involvement), all infants were in the group receiving 1 percent pimecrolimus cream. Further studies are needed to determine the magnitude of risk, if any, of the use of topical calcineurin inhibitors in the development of eczema herpeticum. Current recommendations include discontinuing topical calcineurin inhibitor therapy and beginning appropriate antiviral therapy expeditiously if eczema herpeticum develops.[288]

Widespread herpetic lesions may occur in skin altered by thermal or chemical burns. In this situation, a secondary fever may occur, usually 1 to several weeks after the initial insult. Careful inspection of the burn site or adjacent normal tissue may reveal vesicles (Fig. 157-7) or nonspecific ulcerative lesions. Without therapy, several of these patients have died of disseminated HSV infection.[135] A similar syndrome occurs rarely in children after they have incurred simple skin abrasions (Fig. 157-8). Herpetic recurrences may follow in these cases, but recurrences are localized to the area of skin that was affected initially. HSV can cause severe infection at the site of common diaper rash.[184]

Herpetic whitlow is a painful, erythematous, swollen lesion that occurs on the terminal phalanx, sometimes associated with a damaged cuticle.[34,142,319] The fingers (69%) and thumb (21%) are involved most frequently.[142] Less commonly, the palm may be the site of inoculation and major involvement.[340] The digit is swollen, and the painful white swelling appears to be filled with pus but, when opened for drainage, is found to contain little fluid and no purulent material. The white appearance is caused by the presence of necrotic epithelial cells. Occasionally, the whitlow, which may persist for 7 to 10 days, is accompanied initially by a few vesicles that may give a clue to the etiologic agent of the primary infection. Less commonly, the whitlow is associated with fever, lymphadenopathy, and lymphangitis.[335]

Whitlow is seen in four typical situations. Most commonly, infants with primary herpetic gingivostomatitis autoinoculate their fingers (Fig. 157-9). At times, whitlow is encountered in infants without obvious oral disease, and sometimes it may be caused by adults kissing their children's fingers. In these settings, the viral isolate almost always is HSV-1.[34,142] In sexually active patients, the whitlow more commonly is a manifestation of concurrent genital disease, which should be sought by appropriate history and physical examination.[144] These infections are caused most frequently by HSV-2. In the fourth setting, persons such as dentists, respiratory therapists, nurses, and pediatricians who examine oral cavities or handle

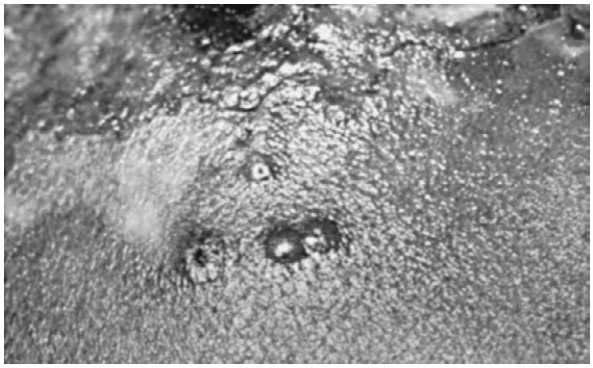

FIGURE 157-7 ■ Secondary HSV infection at a burn site in a 2-year-old child. Note the vesicles at the border of the burn.

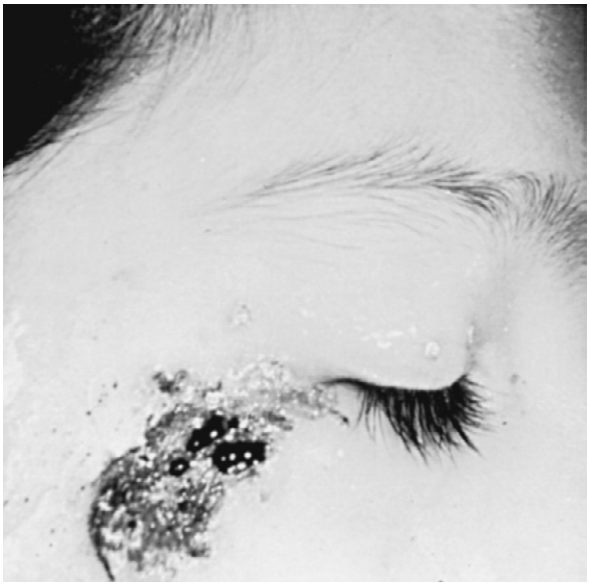

FIGURE 157-8 ■ Facial HSV infection in a young girl after a mild abrasion. (Courtesy Dr. Johnie Frazier, Department of Pediatrics, University of Texas Medical School, Houston.)

FIGURE 157-9 ■ Extensive herpetic whitlow in a toddler with oral HSV infection.

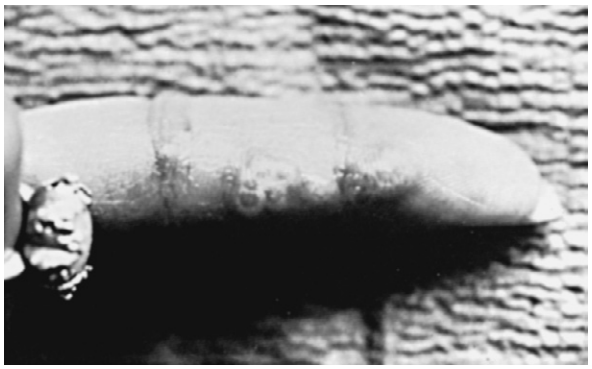

FIGURE 157-10 ■ Recurrent herpetic whitlow in a pediatrician.

secretion-contaminated material without wearing gloves are at risk for acquiring herpetic whitlow (Fig. 157-10).[129] In addition, health care–associated transmission of HSV to other patients, especially in intensive care settings, may occur if careful attention to use of gloves and other personal protective equipment is not practiced.[3]

Herpetic whitlow frequently is confused with bacterial felon, paronychia, or osteomyelitis of the fingertip. Such confusion may lead to the incorrect intervention of incision and drainage. This procedure is not indicated and is not beneficial in the management of herpetic whitlow, which should be treated with oral acyclovir. Needle aspiration and bacterial and viral cultures provide the diagnosis of whitlow, and initiation of antiviral therapy can be based on clinical signs.

In several sports, cutaneous (especially facial) HSV infection is a hazard, particularly in sports with close physical contact such as wrestling (herpes gladiatorum) and rugby (scrum pox, a name derived from the lineup of rugby players, the "scrum" or "scrummage").[36,76,351,353,431] Frequently, the initial lesions are misdiagnosed as impetigo. HSV infection develops in approximately 3 percent of wrestlers in high school, typically on the head and neck but also on the extremities and trunk and in the eyes.[76]

Infection of the Eye

Ocular herpes occurs as a result of primary infection or reactivation of virus in the trigeminal ganglion. Primary infection may manifest as blepharitis or follicular conjunctivitis. The symptoms often are accompanied by preauricular lymphadenopathy, corneal injection, watering, discharge, itching, lid swelling, and, in a third of cases, malaise and fever. If the infection is restricted to the conjunctivae (which can be accompanied by vesicular herpetic lesions elsewhere on the face or in the nose or mouth), it usually resolves without sequelae or specific features. Herpetic infection of the eye may progress to involve the cornea and progress to keratoconjunctivitis, with far more serious potential consequences. Neonatal HSV infection may also present as keratoconjunctivitis and has been reported after cesarean section delivery over intact chorioamniotic membranes and in twin deliveries.[139,421] For this reason, an ophthalmologic consultant always should examine and evaluate these cases.

Corneal involvement by HSV may be manifested initially as minute vesicles at the corneal margin. Progression of the corneal infection (best seen with the use of topical fluorescein dye) is marked by the appearance of branching lesions (a dendritic pattern) or the less diagnostic irregular (ameboid or geographic) ulcer.[35,101] The child complains of severe photophobia often accompanied by blurred vision, chemosis, and lacrimation. Primary eye infection may include stromal involvement, uveitis, and, rarely, retinitis.[289] Retinitis is manifested as multiple whitish yellow, punctate retinal lesions. Spontaneous healing, which generally requires 2 to 3 weeks, can be speeded by the application of topical antiviral therapy. Corticosteroids are contraindicated. The risk for visual impairment caused by direct viral damage, immunopathologic reactions, or both is enhanced greatly with recurrences. With each bout of infection, the corneal ulcers become more extensive and can result in scarring and impairment of sight. Herpetic infection of the eye recurs in approximately 32 percent of patients who have primary symptomatic infection and occurs more commonly in younger patients.[446] Recurrences may be manifested as blepharitis, follicular conjunctivitis with or without lid lesions, or ulcers and keratitis, which more often are accompanied by ulcerations deeper into the corneal stroma (diskiform or necrotizing keratitis) with resultant extensive scarring, irregular astigmatism, and even corneal perforation.[446] Children with dendritic ulcers have a better prognosis for good vision than do those with geographic or diskiform keratitis.[35]

Rarely, HSV eye infection results in acute retinal necrosis. HSV-2 is the most common cause of acute retinal necrosis identified in childhood. Most affected children have a history of congenital or perinatal herpes infection.[225] Acute retinal necrosis may also be a late sequelae of HSV-1 encephalitis.[100] Many patients have preexisting chorioretinal scars. Reactivation is triggered by periocular trauma or occurs with immunosuppression, such as during treatment with systemic corticosteroids.[398] Symptoms include eye irritation and photophobia.

Very infrequently, an oculoglandular syndrome of conjunctivitis and preauricular adenopathy may be caused by HSV infection.[66]

Infections of the Central Nervous System

HSV is the most common identifiable cause of serious or life-threatening sporadic, endemic encephalitis.[238] It accounts for 2 to 5 percent of all cases of encephalitis in the United States and for as many as 20 percent of all cases with an etiologic diagnosis (60% to 70% of cases of encephalitis have no established cause).[308] With the advent of immunization for measles, mumps, rubella, and varicella, the relative incidence of HSV in cases of encephalitis has increased, although absolute numbers remain stable in children.[210,238,308] The case-fatality rate associated with untreated HSV encephalitis is approximately 70 percent,[443] and survivors generally have severe and permanent neurologic disability. Both HSV-1 and HSV-2 have been implicated in the etiology of CNS

infection by HSV, but typical HSV encephalitis in patients outside the neonatal age range is caused by HSV-1.[1,97,317,339] Spread of HSV-1 to the CNS seems to proceed via either neurogenic pathways or by hematogenous dissemination or perhaps through the cribriform plate from infected nasopharyngeal mucosa during primary infection. Recurrent infection probably results from spread via sensory neurons. HSV-2 meningitis is not associated with brain parenchymal infection; whether the infection is a consequence of hematogenous delivery or spread along neuronal pathways is not known.

Although HSV encephalitis may involve virtually any area of the brain, this infection has a striking tendency to involve the orbital region of the frontal lobes and, with particular frequency, portions of the temporal lobes. The predilection of HSV to involve regions of the brain governing olfaction suggests that a pathogenetic pathway proceeds from the nasal-respiratory mucosa via the olfactory bulbs and along the subsequent tracts into the brain.[187] Other researchers have suggested that reactivated virus travels from the trigeminal ganglia via fifth nerve fibers to the meninges of the anterior and middle fossae.[104,105] HSV brain stem encephalitis also occurs and may be associated with neuro-ophthalmologic findings and cranial nerve deficits.[293] Studies in children involving diagnosis by PCR assay and magnetic resonance imaging (MRI) have defined cases with more diffuse cerebral involvement.[317,341,410]

HSV encephalitis must be differentiated from HSV meningitis, which usually is caused by HSV-2 and is a complication of primary genital or oropharyngeal infection. In HSV meningitis, symptoms and signs of meningitis, including headache, photophobia, and stiff neck, appear before or shortly after genital or oropharyngeal lesions are noted. The signs are similar to those of other acute viral meningitides such as enteroviral meningitis. This syndrome may occur in children as well as adults.[114] HSV-2 meningitis is reported in the absence of lesions[343] and rarely in neonates.[342] Seizures and focal CNS findings generally are absent. Examination of CSF reveals lymphocytosis (with 300 to 2600 white blood cells) and may demonstrate low glucose levels. In cases of HSV meningitis, in contrast to HSV encephalitis, virus is often cultured from CSF,[15,17,97,173] and HSV PCR assay also is diagnostic.[343] Recovery usually is complete without specific therapy, but with the availability of effective antiviral agents, HSV meningitis should be treated with acyclovir. HSV meningitis may reappear with or without obvious genital recurrences. Studies using PCR DNA detection analysis have shown that HSV is the major agent responsible for benign recurrent lymphocytic meningitis, also known as *Mollaret meningitis*.[389] These adult patients had three to nine attacks of recurrent lymphocytic meningitis, with 48 to 1600 cells per liter, normal glucose, and protein concentrations of 41 to 240 mg/dL in CSF. PCR analysis detected HSV-2 and, less commonly, HSV-1. Acute viral meningitis caused by reactivation of HSV-1 also has been described in a preadolescent child.[91]

HSV encephalitis is a highly lethal disease caused by HSV-1 in 93 to 96 percent of cases.[24,270,317,432,436] It may be a result of primary (30%) or recurrent (70%) infection.[270,317] Although no specific data exist, researchers have suggested that HSV encephalitis is more likely to be associated with primary infection in younger persons because new infections occur more commonly in this age group. One report suggests that primary infection is more likely to be associated with fatal encephalitis. Of 113 cases of biopsy-documented HSV encephalitis, 31 percent occurred in patients younger than 20 years and 6 to 10 percent of patients were between 6 months and 10 years of age.[355,356] Unlike most other common forms of viral meningoencephalitis such as enterovirus or arbovirus infection, HSV encephalitis is not seasonal. It is an acute illness characterized by fever, headache, malaise, irritability, and nonspecific symptoms lasting 1 to 7 days, with progression to the signs and symptoms of CNS involvement in 3 to 7 days and, finally, to coma and death (Table 157-1). A biphasic illness consisting of initial improvement followed by worsening may occur. The signs of HSV encephalitis resemble those of other viral encephalitides, with initial fever and altered behavior.[166] Meningeal signs are uncommon findings. No correlation exists between isolation of HSV from sites extrinsic to the CNS (e.g., the oropharynx or genital tract) and the diagnosis of HSV encephalitis.[270,433] Thus, the presence of oral or genital lesions is of no help in the establishing or excluding the diagnosis of HSV encephalitis. Nonetheless, if a patient has HSV encephalitis, identical viruses have been isolated from the brain and oral secretions.[439]

The CSF generally reveals pleocytosis, with as many as 2000 white blood cells/mm^3 but usually (80% of the time) more than 50 white blood cells/mm^3. In 90 percent of cases, more than 60 percent of the cells are lymphocytes. Early in the course of infection, neutrophils may predominate. In 75 to 85 percent of cases, red blood cells, reflecting the hemorrhagic necrosis, are seen in the CSF. Between 5 and 25 percent of patients have

TABLE 157-1 **Historical and Clinical Findings in HSV Encephalitis**

Historical Finding	Initial Clinical Finding (%)
Alteration of consciousness	97
Memory loss	92
Personality changes	85
Fever	81
Dysphasia	76
Persistent seizures	71
Headache	67
Autonomic dysfunction	60
Personality change	46
Ataxia	40
Seizures	38
Focal	28
Generalized	10
Vomiting	33
Cranial nerve defects	32
Hemiparesis	24
Visual field loss	14
Papilledema	14

hypoglycorrhachia, and 80 to 88 percent have elevated protein levels in CSF (median, 80 mg/dL), which rise to striking levels as the disease progresses. Two to 3 percent of patients with early HSV encephalitis have normal CSF.[211,270] Repeat analysis of CSF usually reveals abnormalities consistent with encephalitis. HSV almost never is cultured from lumbar CSF in patients outside the neonatal age range and rarely has been grown from ventricular fluid.[136] Thus, whereas CSF examination is helpful, it is not at all diagnostic of HSV encephalitis. When the CSF of patients with HSV encephalitis is compared with that of patients undergoing biopsy for suspected HSV but with another resultant diagnosis, no differentiating characteristics of CSF are found that could allow one to predict HSV infection accurately. HSV DNA can be detected by the use of PCR assay of the CSF of patients who have HSV encephalitis.*

Neurodiagnostic tests have been of limited assistance. Probably one of the most useful is the electroencephalogram (EEG) (Fig. 157-11).[50] A "typical" pattern of unilateral or bilateral (poor prognosis) periodic focal spikes against a background of slow (flattened) activity (paroxysmal lateral epileptiform discharges [PLEDs]) has been associated with HSV encephalitis. These findings are suggestive but not pathognomonic. Other findings include large-amplitude, irregular slow activity, sharp waves, and variable spikes. In 80 to 90 percent of patients, the EEG is not only abnormal but also localizing. In many cases in the pediatric and adult age groups, the EEG may be one of the earliest localizing laboratory tests.[112,150,209] Less commonly, the results of a radionuclide or computed tomographic (CT) scan are abnormal and localizing (50% to 60% of cases).[193,236] CT results may be characteristic late in the illness and consist of low-density, contrast-enhanced lesions in the temporal area, mass effect, edema, and hemorrhage (Fig. 157-12); early in the illness, when establishing the diagnosis is critical, CT results more often are unremarkable.[150,209,264] Abnormal CT results are a poor prognostic factor.[264] MRI findings are more likely to be abnormal at initial evaluation for HSV encephalitis because of its high sensitivity to changes in brain water content[348] (Fig. 157-13), and MRI is more sensitive than is CT for detection of HSV encephalitis.[109,302] Findings include hyperintensity of the temporal and brain stem areas on T2-weighted MR images, and imaging results may be normal if performed in the early stages of the disease.[4]

Focal abnormalities in HSV encephalitis are significantly more likely to be observed on EEG, CT, MRI, or radionuclide brain scan than in other illnesses confused with it. All these findings are biased by the current concept of HSV encephalitis as a focal encephalitis, with very few biopsy data available on the etiology of nonfocal encephalitis. Whether a significant number of cases of HSV encephalitis are milder and nonfocal is unknown, because few of them ever come to brain biopsy or even careful retrospective serologic diagnosis. Studies using MRI and PCR technology have identified cases with multifocal brain involvement.[236,341]

Clinical and laboratory evaluations of patients with suspected HSV encephalitis are valuable only for increasing the index of suspicion, not for confirming the diagnosis. In one series of 24 children with HSV encephalitis diagnosed by PCR assay and compared with 38 children in whom HSV encephalitis was excluded by PCR, no significant differences were found in clinical manifestations at onset or in CSF cell counts, protein, or glucose.[181] However, more children with HSV encephalitis had localizing findings detected by CT (75% vs. 31%), whereas 36 percent of those without HSV encephalitis had EEG abnormalities as opposed to no EEG abnormalities detected in those with HSV encephalitis.

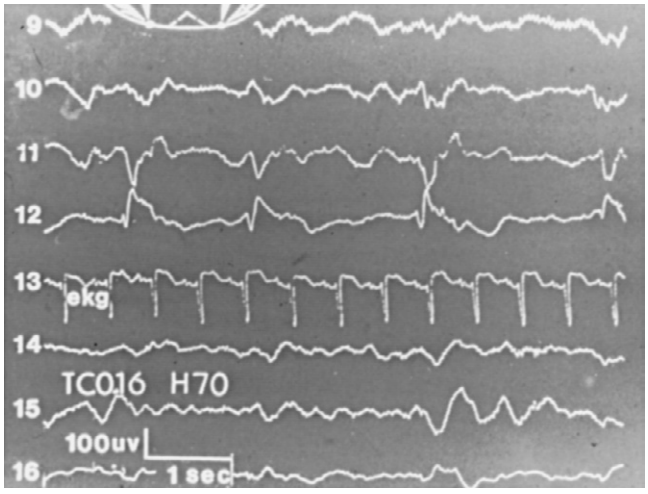

FIGURE 157-11 ■ Electroencephalogram in a 9-month-old infant with HSV encephalitis. Note the paroxysmal discharges, especially in lead 12.

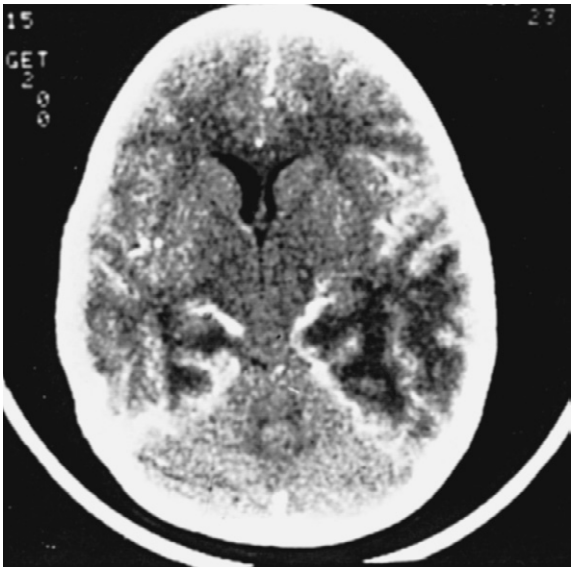

FIGURE 157-12 ■ CT scan 1 week after the onset of HSV encephalitis in a 6-year-old child. Note the bilateral temporal low-density areas with dye enhancement and the greater mass effect on the patient's left side than on the right side.

*References 9, 23, 110, 115, 222, 321, 399.

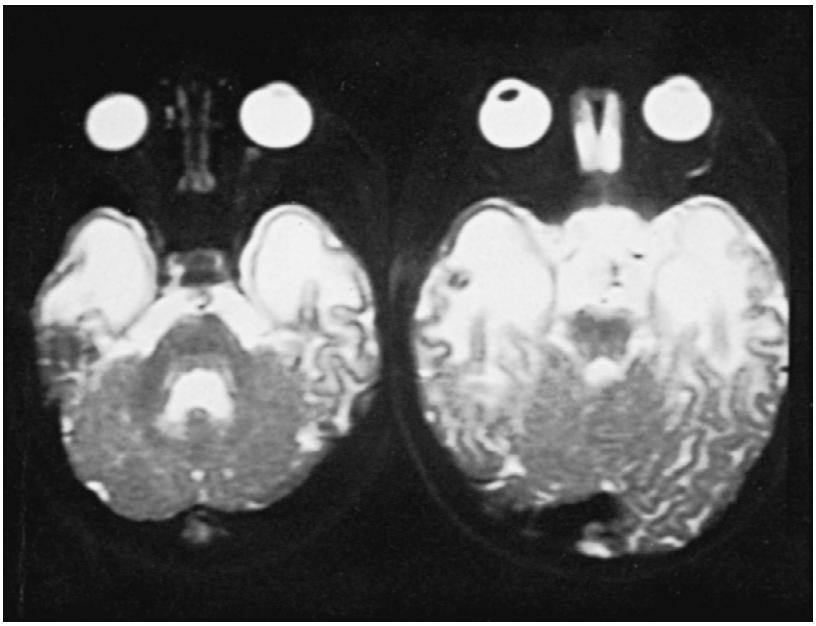

FIGURE 157-13 ■ MR image in a patient with HSV encephalitis. Note the increased signal intensity bilaterally in the temporal lobes. (From Kohl S. Herpes simplex virus encephalitis in children. *Pediatr Clin North Am* 1988;**35**:465-83.)

The differential diagnosis of HSV encephalitis is large (Table 157-2). Especially in the pediatric age range, the ability to discriminate HSV encephalitis from other etiologic agents mimicking it is poor (50% in the national collaborative series in 71 patients younger than 20 years and 42% in a smaller series of 12 patients younger than 12 years).[209] Confirmation of the specific diagnosis of HSV encephalitis remains essential, both to provide optimal aggressive therapy for that condition and, of equal importance, to achieve a diagnosis for the 50 to 60 percent of patients without HSV infection, roughly 16 percent of whom would benefit from other specific therapies.[112,209,264,433] In most cases, the diagnosis can be established by PCR analysis of CSF.* Large studies show PCR analysis to be 98 percent sensitive and 94 percent specific when compared with brain biopsy.[222] Some of the 6 percent "false-positive" results probably were due to poor handling of the biopsy tissue for culture. PCR analysis may yield positive results by 1 day after the onset of symptoms.[153] However, a negative PCR result obtained soon after the onset of symptoms does not exclude the diagnosis of HSV encephalitis. In two small series of patients, repeat testing of CSF 4 to 7 days after an initial negative HSV PCR assay showed positive PCR results.[427] HSV DNA persists in most specimens for a week or more, even after antiviral therapy.[399,432] PCR primers must be chosen to detect HSV-2, as well as HSV-1, because 4 to 6 percent of HSV encephalitis cases may be caused by HSV-2.[24]

If PCR results are negative in a patient who has symptoms and signs of HSV encephalitis, repeat CSF HSV PCR analysis should be performed, and if still negative, and the patient's condition is progressively worsening, brain biopsy may be contemplated. The risk associated with brain biopsy is low. In the national collaborative study of 432 biopsies, six complications occurred (hemorrhage in three patients and poorly controlled brain edema in three, for a 1.4% complication rate).[436] Roughly 2 to 3 percent of brain biopsies yielded false-negative results, usually because of biopsy of the wrong site.[436] Decision analysis suggests that performing a biopsy is especially critical in a patient with low CSF glucose levels.[337]

Quantitative PCR analysis may have value as a prognostic tool and as a method of assessing the response to antiviral therapy in patients with HSV encephalitis.[14,432] In 16 patients, those with high copy numbers of HSV DNA in CSF tended to have abnormal CT scans, be older, and exhibit more severe sequelae. HSV DNA levels were found to decrease during acyclovir therapy in seven patients; the exception was a patient whose copy numbers increased and who subsequently had a fatal infection.

A less acute form of HSV encephalitis in immunocompromised patients has been reported. Also less commonly, HSV has been implicated in brain stem encephalitis.[124]

Relapse occurs in approximately 5 percent of treated patients.[209] Choreoathetosis may be an initial sign of relapse.[216,422] Reports of a postherpetic encephalomyelitis caused by a probable autoimmune or demyelinating etiologic factor have appeared[2,206]; moreover, virus-positive recurrence of HSV encephalitis has been described months after patients have undergone apparently successful therapy.[113] These consequences can be differentiated and documented only by PCR analysis or, if necessary, brain biopsy and appropriate tissue histologic examination and culture.

Infection of the Gastrointestinal Tract in Normal Hosts

Whereas infection of visceral organs is well recognized in immunocompromised hosts, case reports of such infection in apparently healthy persons are less common.

*References 9, 23, 153, 222, 321, 341, 343, 404.

TABLE 157-2 Differential Diagnosis of HSV Encephalitis

Infections
Fungal
Cryptococcus spp.
Coccidioides immitis
Histoplasma spp.
Bacterial
Abscess, cerebritis
Listeria monocytogenes meningitis
Borrelia burgdorferi meningitis
Subdural, epidural empyema
Mycobacterium tuberculosis meningitis
Bacterial endocarditis
Bacterial meningitis (e.g., *Neisseria meningitidis, Haemophilus influenzae, Streptococcus pneumoniae*)
Mycoplasma pneumoniae encephalitis
Protozoal
Toxoplasmosis
Amebic meningoencephalitis
Rickettsial
Viral
Mumps virus
Coxsackievirus, echovirus
Arbovirus (St. Louis, California, eastern and western equine encephalitis, West Nile virus)
Influenza
Reye syndrome
Lymphocytic choriomeningitis virus
Rabies virus
Epstein-Barr virus
Human herpesvirus–6
Rubella virus
Cytomegalovirus
Adenovirus
Tick-borne encephalitis virus
Powassan virus
Subacute sclerosing encephalitis (measles virus)
Progressive multifocal leukoencephalopathy
Acute disseminated encephalomyelitis (ADEM)
Noninfectious Disorders
Tumor
Vascular disease
Arteriovenous malformations
Toxins
Alcoholic encephalopathy
Leukemia
Cerebral infarction
Subdural or epidural hematoma
Adrenal leukodystrophy

Nonetheless, HSV esophagitis has been described in patients, including several children who were 11 months to 17 years of age.* Esophagitis has recently been reported as complication of wrestling.[196] HSV-1 is the usual pathogen, and the syndrome is associated with primary infection.[117] Initial symptoms include fever, severe odynophagia, retrosternal and subxiphoid pain, and an inability to eat. Oral lesions may be present, but skin lesions generally are absent. Esophagoscopy reveals ulcerations and fibrinous and, at times, hemorrhagic exudate. Distal involvement may be more extensive than the proximal esophageal findings suggest. Double-contrast esophagography may be diagnostic, although endoscopy and biopsy usually are necessary for establishing a definitive diagnosis. Symptoms generally remit in 5 to 7 days after the administration of acyclovir and nonspecific therapy such as antacids, histamine-2 blockers, and hydration.[18,64,263] Perforation of the esophagus is a rare complication.

HSV infection can manifest as an anorectal infection in males who have sex with males.[147,304] In most series of HSV proctitis, cases are in younger adolescent males involved in passive anal intercourse. This syndrome occasionally affects women practicing passive anal intercourse. Initial symptoms include severe anorectal pain, discharge, tenesmus, hematochezia, and, in particular, fever, difficulty urinating, sacral paresthesias, constipation, and (in 50% to 70% of patients) ulcers or vesicles in the perianal or distal rectal area.[147] The duration of symptoms is 2 to 3 weeks. Primary HSV infection accounts for proctitis in this group in 25 to 30 percent of cases. Syphilis and infection with *Giardia lamblia*, *Entamoeba histolytica*, *Campylobacter fetus*, *Shigella* spp., other enteric bacteria, and *Neisseria gonorrhoeae* also must be considered in the differential diagnosis of this entity. Appropriate cultures and histologic analysis are crucial for establishing a specific diagnosis.

HSV is a rare cause of hepatitis and represents a broad spectrum of disease from mild elevation of aminotransferases to fulminant liver failure and death. HSV-1 and HSV-2 should always be considered in the differential diagnosis of acute liver failure.[407] HSV hepatitis occurs in all ages, from neonate to adult, and occurs in both immune-competent and special hosts.[28,254,260] In one review of the literature describing 35 patients who had HSV-associated hepatitis, 14 percent had no underlying condition. The remainder had various immunocompromising conditions such as transplantation, corticosteroid administration, pregnancy, burns, primary immunodeficiency, or cancer. These patients tended to have fulminant hepatic necrosis, extremely elevated serum aminotransferase levels, disseminated intravascular coagulation, and a mortality rate of 86 percent.[82] In a prospective study of healthy young adults with genital herpes (primary or recurrent), 14 percent had mild elevations in liver enzyme tests.

Recurrent Infections

HSV-1 and HSV-2 have the characteristic capacity of herpesviruses to establish latency and undergo episodes of reactivation despite the presence of an apparently adequate immune response. Persistence is facilitated because latent infection of neurons occurs in an immunologically "privileged" site. Reactivation of HSV infection with viral shedding generally causes no lesions. HSV can be recovered from the pharynx and genital sites of asymptomatic persons. It has been found in the tears of persons with a history of recurrent ophthalmic disease,

*References 18, 30, 64, 138, 263, 285.

even in the absence of eye lesions or symptoms. All HSV-2–seropositive women from whom samples of vaginal secretions were obtained for more than 100 days had documented asymptomatic viral shedding, and shedding occurred on 1 percent of the days on which cultures were performed.[420]

Patterns of HSV reactivation include asymptomatic reactivation after silent primary infection, asymptomatic reactivation after symptomatic primary infection, and symptomatic recurrence after either silent or symptomatic primary infection. The risk for having symptomatic recurrences may be higher in individuals who have symptomatic primary infections. When reactivation causes disease, the clinical manifestations of recurrent HSV infection depend on the area involved. Most HSV recurrences are milder than the primary illness, although patients may have symptomatic recurrences without having had clinically apparent primary disease. Recurrent infection in a normal host also may be more severe than is the primary infection. This pattern is particularly evident in HSV infection of the eye, in which recurrent illness is associated with deep stromal damage and scarring.[299,454] This enhanced severity may be related to a greater extent to a more exuberant immune response than to viral damage. HSV encephalitis, which may be a manifestation of viral reactivation, is devastating.[270,317]

The most common manifestation of recurrent HSV infection is herpes labialis ("cold sores," "fever blisters"). Recurrences are observed in 25 to 50 percent of persons who have had symptomatic primary HSV-1 oral infection but in only 24 percent of those who had primary HSV-2 oral infection. The mean rate of recurrence after symptomatic primary HSV-1 infection in adults is approximately 0.1 per month. These recurrences often are associated with a variety of febrile illnesses, local trauma, sun exposure, or menstruation.[25,363,366] Whether acquisition of HSV-1 in childhood is less likely to be associated with recurrent herpes labialis is not known.

Most persons with herpes labialis experience a prodrome (pain, burning, tingling, or itching) at the site that lasts a few hours to several days. Subsequently, an orderly progression ensues from papules (lasting 12 to 36 hours) to vesicles (usually gone by 48 hours) and finally to ulcers and crusting (lasting 2 to 4 days) (Fig. 157-14). The typical lesion measures 35 to 80 mm. The majority of outbreaks are healed by 5 to 10 days (mean, 200 hours). Most pain occurs during the vesicular stage. Virus is isolated readily from vesicles (80% to 90% of the time) and less commonly from ulcers and crusts (34% of the time). Maximal virus titers (10^7 to 10^8) in lesions are detected in the first 1 or 2 days, and virus generally is not isolated after 120 hours.[25,366] Virus is detected in the saliva and on the hands of persons with herpes labialis.

Recurrences tend to affect the same location or closely related areas. In general, they occur on the lips, mucocutaneous junction, or other parts of the face. Recurrent lesions found inside the mouths of normal hosts rarely are caused by HSV and more likely are aphthous lesions. When HSV recurrences are within the mouth, they tend to be on tissue adjacent to bone, such as the gums or palate, and not on the lips or buccal mucosa.[361] A differential diagnosis of the condition also includes pemphigus, lichen planus, ulcers caused by cyclic neutropenia, and ulcers associated with celiac disease, ulcerative colitis, Crohn disease, pernicious anemia, and Behçet syndrome.

FIGURE 157-14 ■ Recurrent herpes labialis (cold sore) in an adolescent.

Recurrent genital herpes is the second most common manifestation of HSV. Studies have elucidated several factors that increase the risk for recurrent genital disease after symptomatic primary genital infection. Recurrence rates are much higher after primary HSV-2 (90%) than after primary HSV-1 (25% to 55%) infection.[220,309] The mean rate of recurrence is 0.02 to 0.1 per month after primary genital HSV-1 infection and 0.3 per month after primary HSV-2 genital infection.[220,309] Recurrences are seen more commonly in men than in women and after a recurrent lesion than after a first attack.[309]

Only 5 to 12 percent of persons with recurrent genital herpes have constitutional symptoms. Local symptoms include pain (average, 4 to 6 days), itching, dysuria (10% to 30%), adenopathy (20% to 30%), and lesions (average, 50 to 60 mm) lasting 4 to 5 days until crusting, with healing taking place by 9 to 11 days (range, 4 to 29 days) (see Fig. 157-3). Symptoms in females tend to be more severe than those in males. Virus is shed for an average of 3 to 4 days (but in some cases as long as 20 days). Virus generally is shed with titers of 10^2 to 10^4 per lesion. Vesicles are seen in dry areas, but in wet areas the vesicles rapidly break down into ulcers. Symptoms generally are milder and of shorter duration than those in primary genital disease.[53] New crops of lesions commonly occur during the course of recurrence. The severity of

recurrence is quite variable; in some cases, several discrete recurrences blend into a single, prolonged recurrence, and in rare cases, patients have almost continuous recurrences.[154] In one study of patients who were HSV-2 seropositive with no history of genital herpes, 62 percent had recognized herpetic lesions at later evaluation.[420] Recurrences in the previously asymptomatic group were shorter (3 vs. 5 days) and less frequent (3.0 vs. 8.2 per year) when the pattern of recurrence in this cohort was compared with that of subjects with a known history of genital herpes.

With endonuclease restriction analysis, researchers have clear evidence that, whereas most recurrences represent endogenous reactivation of the same latent virus, reinfection with a new homologous virus (i.e., HSV-2 and new HSV-2), as well as heterologous virus (i.e., HSV-2 and then HSV-1 or vice versa), is possible.[62] How common this occurrence is remains to be ascertained and must depend to some degree on the sexual activity and number of partners of the persons studied.[346]

Other cutaneous recurrences may develop at each anatomic site of primary infection. HSV infection may recur on the face or trunk in a typical dermatome distribution, such as that associated with VZV. Indeed, frequent repeated attacks of zosteriform-like lesions on any part of the body in a normal host suggest HSV and not VZV infection.

Erythema Multiforme

Erythema multiforme is thought to be an immune-mediated, "allergic" response to recurrent HSV infection.[133,178,261,280,429] It has been associated with the presence of human leukocyte DQw3 antigen.[192] In several series, approximately 15 to nearly 100 percent of patients who have erythema multiforme, especially those with recurrent erythema multiforme, gave a history of recurrent HSV infection before the skin eruption, which may be macular or urticarial.[178] In one series, 5 of 80 patients who had recurrent oral HSV infection experienced a rash (presumably erythema multiforme) that manifested 8 to 14 days after the onset of a cold sore.[366] Studies in adults and children have documented HSV antigen-antibody immune complexes and HSV DNA (detected by PCR analysis and in situ hybridization) in the skin of patients who had erythema multiforme after having HSV infection.[47,48,280] In a series of 20 children with erythema multiforme (10 who had antecedent herpes), 16 were documented to have HSV DNA at the site of the rash.[429] The mechanism of transport of viral DNA fragments is not clearly understood. Recent studies suggest that $CD34^+$ cells play a role in transport of HSV DNA fragments to lesional skin.[279]

The skin manifestations may last 14 to 21 days, and therapy generally is directed toward the allergic and not the viral component of the illness. Suppression of HSV recurrences prevents the associated episodes of erythema multiforme. Indeed, suppressive treatment of erythema multiforme with acyclovir, even in the absence of recurrent HSV infection, completely suppresses clinical manifestations.[347,387] In one series, the syndrome developed in 12 children at a mean age of 8 years within 4 days after the appearance of herpes labialis lesions and symptoms lasted for an average of 10 days; 9 children had recurrent erythema multiforme, with an average of 2.6 episodes per year.[429] Detection of HSV by PCR assay of skin biopsy tissue was described in all cases. None of three children given acyclovir suppressive therapy had recurrences during treatment for at least 6 months, but erythema multiforme with recurrent HSV developed in one child when use of the drug was discontinued, thus supporting a causative role of HSV in the syndrome.

HSV Infection in Immunocompromised Hosts

As the practice of pediatrics continues to include more patients with severe acquired immunodeficiency states brought about by increasingly intensive therapy for malignancies and primary immune deficiency disorders, expanding application of bone marrow and organ transplantation, and HIV infection, the prevalence of severe HSV infection in immunocompromised hosts is increasing. Table 157-3 lists conditions associated with unusually severe HSV infections. Aside from several cases of HSV encephalitis in patients with agammaglobulinemia

TABLE 157-3 **Conditions Contributing to Unusually Severe HSV Infections**

Neonatal period
Malnutrition
Malignancy
Immunosuppressive therapy
Antineoplastic
Transplantation
Corticosteroids or adrenocorticotropic hormone
Primary immunodeficiency
Agammaglobulinemia
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
Severe combined immunodeficiency syndrome
Nucleoside phosphorylase deficiency
Thymoma and hypogammaglobulinemia
Common variable agammaglobulinemia
Chronic mucocutaneous candidiasis
Natural killer cell defect
Acquired immunodeficiency syndrome
DOCK8 deficiency
Pregnancy
Burns
Trauma
Skin abnormalities
Atopic eczema
Bullous impetigo
Burns
Darier disease
Ichthyosiform erythroderma
Pemphigus
Viral infection
Measles
Bordetella pertussis
Tuberculosis
Severe bacterial infection
Haemophilus influenzae meningitis
Sarcoidosis

(who also had concomitant infections with enterovirus),[242] common links in these varied groups are either skin abnormalities (eczema, burns) or immunologic defects, primarily in the cell-mediated components of the immune system.* The critical defects have not been defined and may involve one or a combination of inadequate functions (e.g., of $CD4^+$ T cells, $CD8^+$ T cells, natural killer cells, macrophage antigen processing, or other factors).

The incidence of severe HSV infection in children with diseases that predispose them to these complications (see Table 157-3) is defined poorly but, in limited series, was similar to that seen in adults.[137,145,306,317] Most infections are caused by reactivation, as would be expected given the relative frequency of primary and recurrent infections.

In series of pediatric or adult patients who have received renal, bone marrow, or cardiac transplants, 70 to 90 percent of seropositive persons excreted HSV, usually from the oropharynx and generally at the time of peak immunosuppression (in the first month after transplantation).[271] HSV disease usually occurs within 2 to 3 weeks of solid organ or hematopoietic transplantation in patients who do not receive antiviral prophylaxis.[145] Of 68 children who underwent renal transplantation, a herpesvirus was isolated in 43 percent, with 28 percent of the isolates being HSV.[397] HSV in cardiac transplant cases causes symptomatic illness in 45 to 85 percent of seropositive patients, depending on the intensity of immunosuppression. HSV was the virus most commonly isolated in children who underwent bone marrow transplantation (23%).[424] HSV has been suggested to be one of the etiologic agents of the interstitial pneumonitis that develops after bone marrow transplantation.[274,307] In children with leukemia, HSV infection occurs more commonly in those with myelocytic leukemia than in those with lymphocytic leukemia and the risk for the development of infection increases with neutropenia and chemotherapy. HSV infection was the most common serious viral infection in children with leukemia. Whereas most infections occurred during periods of remission, on a per-day basis, the risk for development of infection was seven times higher during induction.[448]

In Africa, patients suffering from underlying malnutrition and concomitant measles infection may contract fatal disseminated HSV infection; such events rarely occur in industrialized nations.[33,201,391]

In children with HIV infection, HSV causing chronic mucocutaneous ulcers (persisting for more than 1 month), bronchitis, pneumonitis, and esophagitis is an AIDS-defining condition.[77] Chronic ulcerative HSV lesions often develop in children with HIV infection.[333] Chronic HSV mucocutaneous disease or widespread organ involvement was reported as the AIDS-defining condition in 7 of 789 (0.9%) HIV-infected children in one analysis.[401] In another series of children with AIDS, 5 to 29 percent contracted HSV opportunistic infection.[137] Many of these children have perianal ulcers from which HSV-1 is isolated. In HIV-infected patients, dissemination is a rare occurrence.[19] Multiple recurrences of infection are common occurrences as the immunodeficiency worsens. In patients with AIDS, HSV can cause a typical[159,408] or more indolent encephalitis. HSV usually does not result in mortality in HIV-infected patients, but it causes significant morbidity.[203] HSV genital ulcer disease increases the risk for acquisition of HIV.[175] Studies show that suppression of clinical and subclinical reactivation of HSV is associated with a decrease in HIV viral load.[338] In one recent study, daily acyclovir therapy did not reduce the risk for transmission of HIV-1, despite a reduction in plasma HIV-1 RNA and a 73% reduction in the occurrence of genital ulcers due to HSV-2, suggesting that a more substantial reduction in plasma HIV-1 RNA is necessary to reduce transmission.[74]

Several major syndromes are attributable to HSV in immunocompromised patients, with some overlap and occasionally progression from one to another. The first and most common manifestation is a local, chronic, often extensive cutaneous or mucocutaneous infection. The second form is infection involving a single organ (e.g., esophagitis or pneumonitis). The most serious illness is characterized by more widespread dissemination involving distant areas of the skin or visceral organs (e.g., the lungs, liver, adrenal glands) and the CNS. Although data are limited, disseminated disease probably most often represents primary infection except in the most severely immunocompromised patients. More localized syndromes may be a manifestation of either primary infection or recurrent illness.

The typical localized HSV infection begins in the mouth or about the lips, often appearing innocuously as recurrence of ordinary herpes labialis. Over the course of several days, the papules and vesicles progress to bullae, frequently with hemorrhagic fluid. The bullae or vesicles evolve into huge, chronic, bloody, coalescing, ulcerated, oozing lesions eroding into subcutaneous tissue and occasionally destroying underlying structures. The tissue is malodorous, and the lesions are painful (Fig. 157-15). The lip and palate are the sites most commonly affected. Oral lesions account for approximately 60 percent of

FIGURE 157-15 ■ Chronic, hemorrhagic HSV infection in a girl with leukemia and a bone marrow transplant.

*References 41, 135, 172, 296, 373, 391.

FIGURE 157-16 ■ HSV proctitis and rectal infection in a homosexual male with AIDS. (Courtesy Dr. Victor Fainstein, Department of Internal Medicine, M. D. Anderson Cancer Center, Houston.)

HSV infections in children undergoing transplantation.[397] A similar syndrome, usually caused by HSV-2 infection, may be seen in the perianal or vaginal area and is one of the characteristic syndromes that occur in males who have sex with HIV-infected males (Fig. 157-16). If untreated, the lesions may lead to death because of local destruction and hemorrhage, or they may regress as the immune status of the host improves or as antiviral chemotherapy is administered. A syndrome of herpetic geometric glossitis has been reported in HIV-infected patients.[152] Affected patients have a tender tongue accompanied by dorsal longitudinal crossed and branching fissures.

Extensive HSV skin infections may occur in patients with burns, eczema, pemphigus, or abrasions, often with conversion of second-degree tissue damage to third-degree damage (see Figs. 157-6 and 157-7). Of 179 children with eczema, herpes skin infections developed in 10, 7 of whom required hospitalization.[103] Although rare, local infection may progress to dissemination in some cases, possibly as a result of the more severe immunodeficiency occurring with several of these conditions. The widespread necrotizing lesions are known commonly as *Kaposi varicelliform eruption* or *eczema herpeticum* (see Fig. 157-6). The predisposition to severe HSV-1 infection in children with atopic eczema appears to be due to the cutaneous abnormalities because these children have T-cell responses to HSV, as well as high HSV IgG antibody titers.[148] Studies have looked at the role of the cathelicidin family of antimicrobial peptides in protecting children with atopic dermatitis against disseminated skin infection with HSV. Variable skin expression of cathelicidin peptide LL-37 may explain why eczema herpeticum develops in some children with atopic dermatitis and others appear to be at lower risk.[148,176] One study evaluating phenotypic risks for eczema herpeticum demonstrated that children with severe atopy and greater T-helper type 2 (Th2) polarity (as demonstrated by an increase in Th2 chemokines) were at increased risk for HSV and *Staphylococcus aureus* skin infections.[32]

Herpes skin lesions must be differentiated from bacterial infections caused by gram-positive or gram-negative organisms, chronic fungal infections (as seen with *Mucor* or *Blastomyces*), other viral infections (vaccinia, varicella), mycobacterial infection, and various noninfectious lesions such as pyoderma gangrenosum, chemotherapy-induced ulcers, or Sweet syndrome.

HSV esophagitis rarely has been reported in normal children,[30,64,196] but it is a relatively common finding in immunocompromised patients. Pathologic studies have suggested that approximately 25 percent of cases of autopsy-proven esophagitis (1% to 6% of all autopsies) are secondary to HSV infection (14 of 55 cases).[273] Underlying conditions included burns, aplastic anemia, malignancies, transplantation, a variety of other serious medical problems, and postoperative trauma induced by nasogastric tubes.[64,248,273] Twenty to 50 percent of these patients have HSV involvement elsewhere (the lungs, trachea, and, less commonly, the skin). The esophagitis may be asymptomatic or associated with dysphagia, odynophagia, epigastric discomfort, and retrosternal pain. Characteristic findings in the esophagus are ulcers with raised granular margins. The ulcers often are covered with fibrinous exudate and, in advanced cases, are confluent, with progression to complete mucosal loss in large segments of the esophagus. Typically, visceral herpes infection is not suspected before the patient's death, but, uncommonly, involvement of adjacent gastric tissue can be documented. Diagnostic evaluation should include a barium swallow, which may demonstrate edema, nodules, and ulceration of the esophagus; however, barium studies cannot differentiate HSV from other etiologic agents of esophagitis. Esophagoscopy accompanied by biopsy and viral culture is diagnostic and helps exclude other common causes of this syndrome, including *Candida*, CMV, and possibly other fungi and bacteria or chemotherapy-induced changes.[63,64,273] Although the oral cavity, esophagus, and rectum are the usual sites of HSV infection in the gastrointestinal tract of immunocompromised patients, HSV-2 colitis has been reported in a young child after combined liver and small bowel transplantation.[108]

HSV pneumonia is an unusual condition, but lung involvement has been described in neonates[39] and immunocompromised hosts. This diagnosis frequently is made post mortem almost exclusively in immunocompromised patients. In one series of 1000 consecutive autopsies,[272] HSV pneumonia was identified in 10 cases. HSV

pulmonary infection occurred in 3 percent of children who had undergone renal transplantation.[397] In most adult cases, the process is a result of endogenous viral mucocutaneous reactivation and involvement of lung tissue by contiguous spread, which causes focal pneumonia (60% of the time), or involvement by hematogenous spread from an oral or genital site, which results in diffuse pneumonitis.[307] Although the largest series consisted primarily of adult patients, three of the patients were aged 7 years or younger (with Down syndrome and congestive heart failure, rhabdomyosarcoma, and pneumococcal pneumonia and a seizure disorder).[179] Patients had cough, dyspnea, fever, and hypoxia; and 50 percent had rales. Most had other concomitant pulmonary infections with bacteria, *Candida*, *Aspergillus*, and CMV. HSV pneumonia cannot be diagnosed by the association of upper airway cultures and radiographic abnormalities. It must rest on an aggressive approach involving culture and histopathologic examination of involved lung tissue obtained by either biopsy or bronchoscopy.

HSV meningoencephalitis is not a common occurrence in immunocompromised patients. It may occur as a component of widely disseminated disease, or it may be a localized condition. Several cases have been reported in patients who had agammaglobulinemia in association with concomitant enteroviral infection of the brain.[242] The meningoencephalitis may be fulminant, such as occurs in a normal host. An interesting case that followed an atypical subacute course accompanied by bilateral brain involvement has been reported in an anergic patient who had Hodgkin disease. HSV encephalitis may also involve the brain stem.[243] Although HSV does not appear to have a predilection for CNS infection in immunocompromised hosts, patients with AIDS are an exception and may have ascending myelitis, acute transverse myelitis, and encephalitis.[137,140,159,408]

Hepatitis caused by HSV has been reported most commonly after solid organ or bone marrow transplantation[185,217] and during pregnancy.[202] Signs include fever, abdominal pain, and elevated liver enzyme levels. Hepatitis usually occurs during the first 3 weeks after transplantation, unless prophylactic acyclovir is given. The mortality rate is very high (67% to 100%). In at least one case, orthotopic liver transplantation resulted in survival.[352] HSV is reported as the most frequently identified viral etiology of hepatitis on one series of patients with systemic lupus erythematosus.[306]

The most severe form of HSV in an immunocompromised host is widely disseminated disease that can involve the liver, adrenals, lungs, spleen, kidney, and often the brain. In studies from South Africa and Kenya,[33,201,391] measles and severe malnutrition were frequent cofactors in children who had widely disseminated HSV infection. These illnesses represented fatal primary infections. Similar syndromes have been described; they and other underlying conditions are listed in Table 157-3. Dissemination has been reported coincident with *Bordetella pertussis*, *Haemophilus influenzae* meningitis,[183] and other bacterial infections.[33] Disseminated HSV infection occurred in 10 percent and 25 percent of children who had HSV infection and underwent bone marrow and renal transplantation, respectively.[271,424]

Disseminated HSV infection initially usually manifests as initial fever and skin or mucocutaneous involvement in 80 percent of cases, but instead of healing as expected, the infection disseminates. The cutaneous dissemination may involve a widespread vesicular eruption that looks much like varicella, or it may involve more local, large hemorrhagic vesicles and bullae. Involvement of the major target organs, as noted previously, gives rise to syndromes of hepatitis, pneumonia, shock, bleeding, disseminated intravascular coagulopathy, seizures, coma, renal failure, hypothermia, and death in days to weeks. Laboratory examination may reveal leukopenia, thrombocytopenia, elevated liver function test values, hyponatremia, azotemia, pneumonitis, hypoglycemia, CSF pleocytosis, abnormal EEG results, and electrocardiographic abnormalities. Death occurs commonly in this syndrome (90%), even after the institution of antiviral chemotherapy. Because the liver frequently is involved and biopsy may be precluded by the tenuous condition of the patient, HSV infection should be considered in all high-risk groups (see Table 157-3) with fulminant hepatitis.

Fetus and Newborn

HSV may infect the fetus before delivery (congenital or in utero infection), infect the newborn during delivery (perinatal or natal infection), or infect the infant after birth (postnatal infection).

The clinical spectrum in infants with congenital HSV infection is different from that observed in infants with natal or postnatal disease. The classic triad of cutaneous findings, CNS disease, and ophthalmologic disease is seen in approximately one third of neonates with congenital HSV.[250] The most prominent and most common features of congenital infection are vesicular rash, bullae, or cutaneous scars present at birth or within a few days of birth. In some cases, the extent and severity of these lesions may result in an initial diagnosis of epidermolysis bullosa. At least one case report of limb hypoplasia, similar to congenital varicella syndrome, has been shown to be caused by HSV-2.[185a] Two thirds of infants demonstrate extensive involvement of the CNS at birth, either clinically or from autopsy findings: diffuse brain damage, microcephaly, or intracranial calcifications. In some infants, eye findings, including chorioretinitis, microphthalmos, retinal scars and retinal dysplasia, and cataracts, have been noted.

The three general patterns of natal or postnatal neonatal HSV infection are categorized by the extent of disease: (1) disseminated infection with or without CNS involvement, present in 25 percent of cases; (2) infection localized to the CNS in 30 percent of cases; and (3) infection localized to the skin, eye, or mouth in 45 percent of cases.

Infants with natal or postnatal HSV infection commonly have a clinical picture resembling that of bacterial sepsis: alterations in temperature, lethargy, respiratory distress, anorexia or vomiting, and cyanosis. In febrile neonates presenting to an acute care treatment center, HSV infection should be considered in the differential diagnosis of neonatal fever, especially in the presence of

mononuclear CSF pleocytosis, hypothermia, or sepsis-like syndrome.[69-71] If skin involvement is present, then skin vesicles or mucous membrane ulcers may be apparent. If the conjunctivae or cornea is involved, then nonexudative conjunctivitis, tearing, and pain will be present. If the CNS is involved, then seizures may occur.[421] The frequency of disseminated disease has decreased after a high of 51 percent reported in the years 1973 to 1981; this decrease probably represents earlier diagnosis and treatment of localized infection before dissemination occurs.[436a] The disseminated form of infection in the earlier years of recognition presented in infants between 9 and 11 days of age and usually involved the liver, where it caused fulminant hepatitis with disseminated intravascular coagulation, and the adrenal glands, and it most closely resembled the picture of bacterial sepsis.[407] Current reports show disseminated neonatal HSV may present as early as the first week of life, without cutaneous or localizing signs, similar to bacterial sepsis, and confirm that HSV remains an important cause of neonatal morbidity and mortality. A disseminated infection can affect multiple organs, including the brain, larynx, trachea, lungs, esophagus, stomach, lower gastrointestinal tract, spleen, kidneys, pancreas, and heart. Pneumonia occurs in 37 percent of infants. Markedly elevated aminotransferase levels, direct hyperbilirubinemia, coagulopathy, and thrombocytopenia are common findings late in the disease process. Sixty to 75 percent of infants with disseminated disease have hematogenously acquired CNS involvement and meningoencephalitis, as manifested by irritability, a bulging fontanelle, localized or generalized seizures, flaccid or spastic paralysis, opisthotonos, decerebrate rigidity, or coma. Importantly, 39 percent of infants with disseminated disease do not have skin vesicles or eye lesions at initial evaluation and vesicles do not develop during the acute HSV disease; only 56 percent initially have fever, and many are hypothermic on presentation.

CNS disease from retrograde axonal transmission of virus presents later than does disseminated disease, usually when the infant is 11 to 17 days old but occasionally when the infant is as old as 4 to 6 weeks of age. Typically, these infants have focal seizures that subsequently generalize. Examination of CSF demonstrates mononuclear pleocytosis with a predominance of lymphocytes, a moderately low glucose concentration, and elevated protein levels. A normal cell count, glucose, and protein concentration, however, may be found on the initial lumbar puncture, if performed early in the disease process. An elevated red blood cell count secondary to hemorrhagic brain involvement may be present but by itself is an unreliable sign. Fever is present at initial evaluation in only 44 percent of infants with HSV encephalitis, and 32 percent of infants do not have skin vesicles either initially or during the course of their illness. Neonatal HSV encephalitis can be multifocal or limited to only the temporal lobes, brain stem, or cerebellum; and watershed distribution ischemia may be seen in some cases.[293,410]

Localized skin, eye, or mouth disease usually presents within the infant's first 7 to 10 days of life. Approximately 83 percent of these neonates have skin lesions: usually single vesicles, occasionally vesicle clusters, and rarely a zoster-like rash. As many as 46 to 80 percent of neonates with skin lesions have 1 to 12 recurrences during the first 6 to 12 months of life. Thirteen to 25 percent of infants have eye involvement; disease is limited to only the eye in one third of these cases, but the risk for disseminated disease is significant.[421] Common manifestations include eye pain and eyelid swelling, fussiness, tearing, nonexudative conjunctivitis, keratitis, and chorioretinitis. As many as 83 percent of infants are afebrile initially. One third of all infants have evidence of herpetic mouth lesions.

DIAGNOSIS

Clinical findings may suggest a probable diagnosis of HSV infection, but obtaining a definitive laboratory diagnosis is necessary or useful in many circumstances and should always be sought in neonates and patients with severe disease.[16,72] Accurate laboratory diagnosis requires giving attention to obtaining the correct specimens necessary for identifying the etiology of the clinical syndrome. Appropriate interpretation of the laboratory results is the second critical factor. Evaluation of mucocutaneous lesions in high-risk patients guides the use of antiviral therapy if the lesion is herpetic. However, because HSV often is shed in immunosuppressed and otherwise healthy individuals, finding HSV in oropharyngeal secretions does not mean that the clinical condition is caused by HSV. In general, the virus must be isolated from the relevant tissue for confirmation of the diagnosis. The test selected also is important (e.g., HSV is rarely found in CSF cultures of patients outside the neonatal age group with HSV encephalitis but often is present in aseptic meningitis caused by HSV). Detection of HSV DNA in CSF by PCR assay or by brain biopsy is indicated to prove the diagnosis and exclude other treatable causes of encephalitis. Laboratory diagnosis also is valuable when HSV infections are not life threatening.[72] For example, establishing that a genital lesion is caused by HSV-1 or HSV-2 facilitates decisions about antiviral therapy and allows initiation of appropriate counseling regarding the risk for recurrences and measures that may reduce transmission of HSV to contacts and during pregnancy to the neonate.

Viral Culture

The original gold standard for HSV diagnosis was recovery of infectious virus in cell culture. HSV replicates rapidly in cell culture, typically in 1 to 2 days, and produces a characteristic cytopathic effect (CPE) (Fig. 157-17).[72,224,332] A variety of cell lines (human fibroblasts, A549, and many others) support HSV replication and are utilized by diagnostic laboratories to isolate HSV. Although the presence of HSV is suggested by the cell types infected as well as the character and timing of the CPE, definitive identification of the infecting virus is accomplished via microscopy of fixed cells with immunofluorescence assay with HSV-1– and HSV-2–specific antibodies.

Several culture methods that do not rely on the evaluation of CPE have been devised that provide more rapid

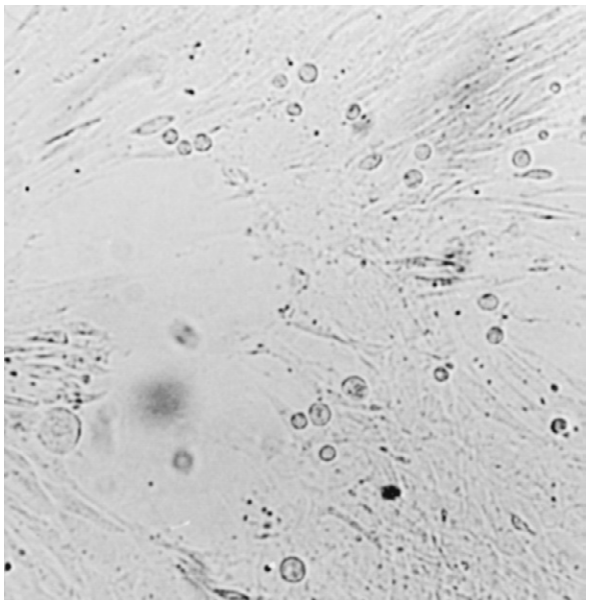

FIGURE 157-17 ■ Culture of HSV on human fibroblasts demonstrating disruption of the cell monolayer and rounding and enlargement of cells to show the cytopathogenic effect.

diagnosis of HSV than traditional culture. One approach is the use of a genetically modified baby hamster kidney cell line containing a reporter gene encoding β-galactosidase under control of an HSV-specific promoter (Enzyme-Linked Viral Inducible System [ELVIS]).[98,231] Addition of a colorimetric reporter substrate after centrifugation-enhanced infection and overnight incubation allows identification of cells infected with HSV. Similarly, the shell vial method utilizes centrifugation-enhanced infection of cell monolayers and overnight incubation, followed by immunofluorescence assay staining with HSV-specific antibodies.[128,180,295,400] Although these methods may reduce the time to result, they are typically less sensitive than traditional cell culture.[81,189]

HSV culture methods, both traditional and rapid, are being phased out by many diagnostic laboratories and replaced by type-specific nucleic acid amplification tests for HSV-1 and HSV-2 DNA. These tests do not show definitively that infectious virus is present in the specimen but suggest that it is or may have been shortly before the specimen was obtained.

Direct Detection of HSV-Infected Cells

Direct detection methods allow a diagnosis of HSV infection to be established more rapidly than any available HSV culture method.[16,102,231,345] Direct immunofluorescent assay of cells taken from mucocutaneous lesions is the procedure used most commonly. Specimens are obtained by unroofing the lesion, swabbing the exposed base to remove infected cells, and transferring the swab to viral transport media. The collected cells are centrifuged and washed, applied to a glass slide, then fixed and stained with fluorescently labeled, HSV-specific antibodies. This method, the direct viral examination or direct fluorescent antibody (DFA) testing, yields sensitivities, when compared with traditional viral culture, in the range of 61 to 80 percent with few false-positive reactions.[72,81] To optimize DFA performance, diagnostic laboratories set criteria for specimen adequacy (e.g., requiring a certain number of basal, parabasal, and intermediate cells from lesion sites), thereby emphasizing the importance of proper specimen collection. Despite these precautions, direct detection of HSV by immunofluorescence remains less sensitive than culture. Because of this, it is recommended that DFA-negative lesional samples be tested by a more sensitive method to rule out false-negative results.[72]

HSV antigens can be detected directly in clinical specimens by a variety of additional methods including enzyme-linked immunosorbent assays (ELISAs) and immunoperoxidase assays.* However, these assays have been replaced in the diagnostic laboratory by nucleic acid amplification tests.

Nonspecific methods such as Papanicolaou (Pap) staining or the Tzanck test have been used in the past to suggest possible HSV infection. Even though these methods may show cytologic changes in specimens obtained from suspected HSV lesions, the diagnosis of HSV should not rely on these methods in current practice because rapid, specific methods are available. Although cytologic examination of HSV lesions reveals typical giant cells and, less commonly, Cowdry type A intranuclear inclusions, these changes also are seen with CMV and VZV. Cytologic changes are identified in approximately 70 percent of culture-positive specimens, but sensitivity is dependent on the experience of the examiner and may be low.[268,359] Electron microscopy of vesicular fluid or tissue preparations may reveal the characteristic virus of the herpes family (Fig. 157-18). However, the microscopist cannot differentiate HSV from other herpesviruses, and this diagnostic approach rarely is available as a rapid method. Because selection of antiviral therapy for herpesviruses requires specific identification of the infecting virus, nonspecific methods have limited clinical value in the current era of specific antiviral therapy.

Polymerase Chain Reaction Assays

Type-specific HSV nucleic acid amplification assays, particularly PCR assay, have become the gold standard for HSV diagnosis and have replaced virus culture and direct examination in many diagnostic laboratories. The detection of HSV DNA by PCR is more sensitive than the isolation of virus by culture.[9,24,174,321,329] In one study of mucosal specimens, the ratio of PCR positivity to virus culture ranged from 3.8:1 in the winter to 8.8:1 in the summer. PCR does not require the presence of infectious virus for detection, and this is one of the great advantages and disadvantages of HSV DNA testing. Given the high sensitivity of HSV PCR, false-positive results may occur, particularly if original specimen contamination occurs at time of collection or if cross contamination between specimens occurs during performance of PCR assay. However, the implementation of careful molecular diagnostic workflows and the development of

*References 8, 49, 90, 231, 278, 345, 406.

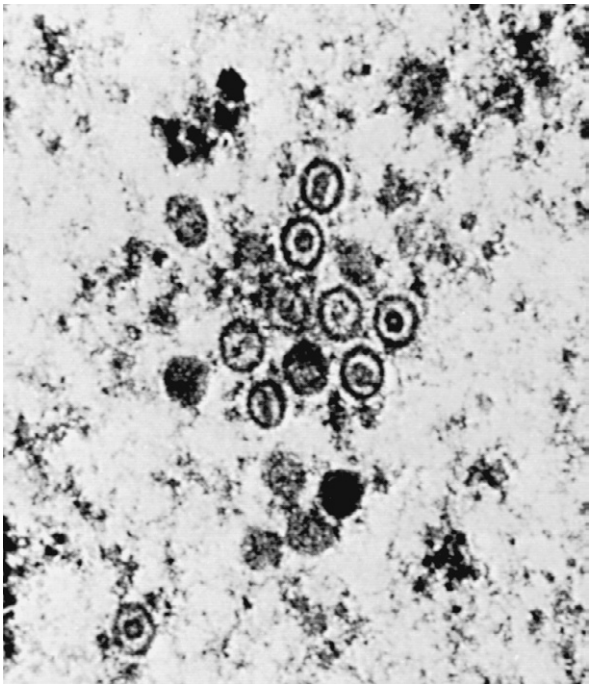

FIGURE 157-18 ■ Electron-microscopic demonstration of HSV particles in a brain biopsy specimen from a 9-month-old infant with HSV encephalitis.

the real-time PCR methodology have considerably reduced the risk for false-positive reactions.

The clinical utility of HSV PCR was first demonstrated for the diagnosis of HSV encephalitis.* Given its high negative predictive value, HSV PCR testing of CSF is often used to rule out HSV infection in patients with suspected herpes encephalitis. However, it is important to recognize that HSV DNA may not be detectable in CSF early in the course of disease.[427] A single negative test should not exclude the diagnosis of HSV when there is high clinical suspicion, and sequential testing of CSF by PCR has rendered the use of brain biopsy unnecessary in most situations. In one study, HSV DNA was detected by PCR in the CSF of 53 (98%) of 54 patients with biopsy-proven herpes simplex encephalitis and was detected in all 18 CSF specimens obtained before brain biopsy was performed on patients with proven herpes encephalitis.[222] Testing febrile neonates with CSF pleocytosis using CSF HSV PCR assay and empirically treating with acyclovir until PCR test results are known has also been shown to save lives and be cost effective.[71]

PCR assay also has demonstrated the presence of HSV DNA in the plasma or whole blood and used to diagnose HSV viremia in neonates, immune-competent patients with eczema herpeticum and primary herpetic gingivostomatitis, as well as in immunocompromised patients with disseminated disease. PCR analysis also may demonstrate prolonged presence of HSV DNA in genital lesions.[89] The sensitivity of the PCR method is adequate for detecting asymptomatic shedding of HSV.[65,88,162] HSV PCR also has utility when the clinical diagnosis is difficult to make, as illustrated by detection of HSV in vitreous fluid[167] and in unusual mucocutaneous lesions in patients with AIDS.[230] HSV DNA may also be quantitated in clinical specimens using PCR-based methods and used to predict severity of disease, especially in hepatitis and encephalitis, and to follow virologic response in these patients.[31,65]

Serologic Diagnosis

Serologic methods are used to assess HSV immune status and document whether an individual has been infected with HSV. Because these viruses cause persistent infections, the presence of HSV IgG antibodies may only signify that the individual has latent HSV-1 or HSV-2 (or both) in sensory ganglia and that the virus can be expected to reactivate and intermittently shed. Clinical laboratories utilize ELISA or immunoblot methods to detect HSV-1 and HSV-2 type-specific IgG antibodies in patient sera. These assays use specific HSV gG antigens and can be used to show whether an individual is infected with HSV-1, HSV-2, or both, but not when the infection was acquired.* If paired sera are available, these assays are capable of demonstrating type-specific seroconversion, even if a patient is already exposed to a single type. The sensitivities of commercial gG type-specific HSV serology tests range from 90 to 98 percent, with specificities greater than 94 percent when compared with Western blot analysis.[414] As with any serologic test, false-negative results are more likely early in the course of infection, and false-positive results may occur in patients with a low pretest probability of exposure.

If a patient is seronegative at the onset of symptoms or at initial evaluation, primary HSV infection can be documented by demonstrating seroconversion with paired sera.[191,208] However, serologic tests are not useful for evaluating HSV recurrence, because IgG antibody titers often do not rise during viral reactivation. On the other hand, increases in IgG antibody titer may occur in the absence of recurrent disease. In addition, testing for IgM antibodies does not improve the serologic diagnosis. HSV IgM assays cannot be used to distinguish primary from recurrent infection because reactivation can also induce the production of IgM antibody.[168]

Local production of HSV IgG in CSF samples can be used to document HSV encephalitis in some patients, but a 2- to 4-week interval is required to demonstrate this change and it cannot be used to guide antiviral therapy.[270,399,404] Testing CSF for HSV IgM antibodies has no demonstrated diagnostic value.

Genetic Analysis for Molecular Epidemiology

HSV DNA from epidemiologically unrelated isolates has a specific cleavage pattern when digested by endonuclease restriction enzymes and subjected to gel electrophoresis (Fig. 157-19).[61] This restriction fragment length polymorphism (RFLP) method is primarily used for

*References 24, 68, 153, 199, 303, 311, 321, 355, 399.

*References 123, 169, 232, 382, 360, 414.

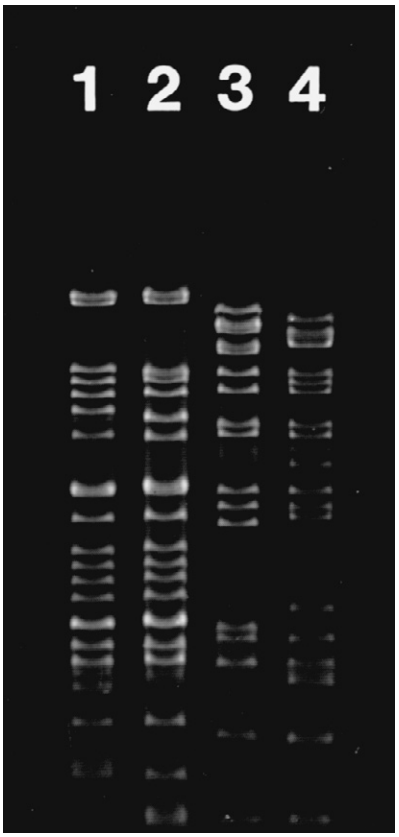

FIGURE 157-19 ■ Electrophoretic separation of the DNA fragments of four HSV isolates by size, produced by restriction endonuclease enzyme digestion using the BamHI enzyme. Lanes 1 and 2 are two different HSV-2 clinical isolates, whereas lanes 3 and 4 are two different HSV-1 clinical isolates. The origin of the gel is at the top. (Courtesy Dr. Saul Kit, Division of Biochemical Virology, Baylor College of Medicine, Houston.)

epidemiologic purposes to demonstrate relatedness among isolates during the evaluation of apparent outbreaks or nosocomial transmission events.[61,62,160,241,439] In addition, RFLP analysis has contributed to our understanding of exogenous reinfection versus recurrence and the possibility that individuals may harbor more than one latent virus strain. This method is laborious and not typically performed in diagnostic laboratories. Precise differentiation of HSV strains requires genomic sequencing, which is performed on a research basis.

Antiviral Drug Susceptibility Testing

The persistence of lesions, emergence of new lesions, or increasing plasma viral load despite appropriate antiviral therapy is suggestive of treatment failure and should prompt laboratory evaluation of antiviral resistance. This testing may be useful to guide selection of an alternative therapy, but, unfortunately, testing is not readily available in clinical practice. Although resistance is uncommon in immunocompetent patients (prevalence 0.1% to 0.7%), immunocompromised patients, many of whom receive long-term HSV prophylaxis or treatment, are at relatively high risk for the development of resistance (prevalence 2.5% to 11%).[298] The gold standard method for HSV antiviral susceptibility testing is the plaque reduction assay. This phenotypic assay utilizes Vero cells (African green monkey kidney cells) inoculated with a standardized viral inoculum and incubated for 48 to 72 hours in the presence of serial drug concentrations and no drug controls.[305,385,428] After incubation, the cells are fixed and stained, the number of plaques or focal areas of cytopathic effect is counted, and the concentration of drug that reduces the plaque number by 50 percent (IC_{50}) is calculated. The plaque reduction assay has well-described breakpoint values of 2 μg/mL or greater for acyclovir and 100 μg/mL or greater for foscarnet. Other phenotypic susceptibility methods utilize a variety of techniques to measure HSV replication in culture, including dye uptake, ELISA, DNA hybridization, and PCR assay.[229,298,393,428] Specific breakpoint values will vary based on the assay. Although laborious, phenotypic evaluation remains the primary approach to HSV susceptibility testing in clinical laboratories. Sequence analysis of the genes encoding thymidine kinase *(UL23)* and DNA polymerase *(UL30)*, the targets of HSV antiviral agents acyclovir and foscarnet, have revealed a large number of resistance mutations as well as polymorphisms that do not contribute to the resistance phenotype.[298,237,331] Genotypic susceptibility testing is primarily utilized as a research tool to understand the mechanism of antiviral resistance and is not generally performed for clinical purposes.

PROGNOSIS, COMPLICATIONS, AND SEQUELAE

Most HSV infections occurring beyond the fetal and neonatal periods cause minor morbidity but very rarely are life threatening. The introduction of antiviral drugs that inhibit HSV replication has changed the management and outcome of these infections. Even before these drugs were available, eczema herpeticum resolved without sequelae in most cases. Despite administration of antiviral therapy, HSV encephalitis can be fatal or be associated with serious consequences ranging from moderate to extensive and permanent neurologic disability.[423] The case-fatality rate for untreated encephalitis may be as high as 75 percent,[443] and progressive neurologic damage may occur.[113,157,207] In a series of PCR-diagnosed cases, a Glasgow Coma Scale score lower than 11 was associated with a poor outcome, and the youngest cohort, those younger than 3 years, had higher morbidity and mortality rates.[181] Genital herpes causes significant physical and psychological morbidity. In immunocompromised patients, disseminated HSV infection can result in fatal or serious illness. HSV is one of the most common infectious causes of blindness in developed countries.

TREATMENT

HSV-1 and HSV-2 infections are treated with acyclovir or related drugs. Acyclovir is a nucleoside analog that is a competitive inhibitor of HSV DNA polymerase, and it terminates DNA chain elongation. Acyclovir is an inactive agent. Inhibition of viral DNA synthesis by blocking viral DNA polymerase and DNA chain elongation

requires phosphorylation of acyclovir. HSV-1 and HSV-2 viral thymidine kinases are much more active than are mammalian cell kinases, and acyclovir becomes a specific antiviral agent in the presence of viral thymidine kinase.

Because the oral bioavailability of acyclovir is only approximately 20 percent, efficacy with oral administration requires high and frequent dosing. Concentrations of acyclovir achieved in CSF are approximately 50 percent of plasma levels. Acyclovir is excreted by glomerular filtration, so doses must be reduced in patients with impaired renal function. Acyclovir has been an exceptionally safe drug in clinical practice, but adverse reactions, including rash, nausea, vomiting, diarrhea, abdominal pain, headache, and neutropenia, can occur. Neurotoxicity may occur with an overdose, usually when the dose is not adjusted for poor renal clearance. Acyclovir also can cause acute renal insufficiency by precipitation in the renal tubules in dehydrated patients with high plasma concentrations or by rapid infusion of the drug. Valacyclovir, the L-valine ester of acyclovir, is converted to acyclovir[403] and has much higher bioavailability after oral administration, with plasma concentrations similar to those obtained after intravenous administration of acyclovir. Famciclovir is an oral prodrug of penciclovir[107]; it also is well absorbed orally and is metabolized to the active form on absorption. The major advantage of these agents is that less frequent dosing is required than with acyclovir. HSV-1 and HSV-2 seldom are resistant to acyclovir or related drugs in the immunocompetent host (<1%). Resistance can occur in up to 10% of isolates from immunocompromised patients who receive prolonged antiviral therapy. HSV isolates resistant to acyclovir usually have mutations in the gene encoding viral thymidine kinase *(UL23)* and less often to *UL30*, which encodes for the viral target DNA polymerase enzyme. Foscarnet or, in some cases, cidofovir is an alternative for the treatment of acyclovir-resistant HSV.[42] Resistance to foscarnet and cidofovir has been reported.[298,449]

Oral HSV Infection

Although published experience is limited, administration of oral acyclovir to children with gingivostomatitis is appropriate and can be expected to modify the duration of symptoms if it is initiated early in the clinical course.[177] Several small placebo-controlled trials of primary herpes gingivostomatitis in young children have been reported.[6,11] In one study, the pain and hypersalivation resolved more quickly in acyclovir-treated patients.[120] In a larger study, symptoms resolved more rapidly with a 10-day course of oral acyclovir 600 mg/m^2 four times a day; differences were observed in drooling (4 vs. 8 days with placebo), gum swelling (5 vs. 7 days), speed of healing of intraoral lesions (6 vs. 8 days), appearance of new lesions (57% vs. 94%), and viral shedding in saliva (4 vs. 10 days). Therapy had no effect on the subsequent development of cold sores.[11] A comparison of oral acyclovir suspension, 15 mg/kg five times a day for 7 days, and placebo started within 72 hours documented that drug recipients had a shorter duration of oral lesions (median of 4 vs. 10 days [difference, 6 days; 95% confidence interval (CI), 4.0 to 8.0]) and earlier resolution of other symptoms, including fever (1 vs. 3 days [difference, 2 days; CI, 0.8 to 3.2]), extraoral lesions (0 vs. 5.5 days [difference, 5.5 days; CI, 1.3 to 4.7]), difficulty eating (4 vs. 7 days [difference, 3 days; CI, 1.31 to 4.69]), and difficulty drinking (3 vs. 6 days [difference, 3 days; CI, 1.1 to 4.9]).[6] Treatment also decreased the period of viral shedding (1 vs. 5 days [difference, 4 days; CI, 2.9 to 5.1]). Symptomatic therapy includes antipyretics and oral hydration. The use of oral anesthetics is not recommended and has resulted in self-injury as a result of children chewing on anesthetized oral mucosa or lips.

Although the duration of symptoms is shorter with recurrences, children with frequent or severe recurrences may benefit from treatment with oral acyclovir as soon as new lesions appear. Topical acyclovir has been licensed for the treatment of recurrent oral herpes labialis based on experience in adults. However, its benefits on clinical symptoms are minimal. If treatment in children is indicated, oral acyclovir is the best option. Studies in adult patients with herpes labialis show that patient-initiated, high-dose, short-duration treatment with valacyclovir or famciclovir initiated at the onset of symptoms significantly reduces the time to healing and hastens resolution of pain and tenderness when compared with placebo.[362,364]

Several studies have produced conflicting results regarding the use of topical acyclovir for the treatment of oral herpes recurrences. Topical acyclovir can decrease the duration of HSV shedding from 2 to 3 days with placebo treatment to 1 to 2 days,[367] and the effect on symptoms is either none or subtle (1 less day with vesicles and a 2- to 3-day acceleration of healing). Although no controlled studies have been conducted, acyclovir therapy should be used for the treatment of rare cases of HSV esophagitis in normal hosts.[138]

HSV Keratitis

Children with HSV keratitis should be referred for immediate evaluation by an ophthalmologist who is familiar with this illness and its progressive, sight-threatening forms and with optimal antiviral therapy.[421] The use of cycloplegic and anti-inflammatory agents, which may be necessary, requires specialized experience. Topical preparations have been shown to have a beneficial effect on HSV keratitis and probably other superficial ocular HSV infections such as conjunctivitis and blepharitis.[239] These preparations include idoxuridine (Stoxil), vidarabine (Vira-A), trifluridine (Viroptic), and acyclovir. Neonates with ocular involvement with HSV should also receive both a topical ophthalmic drug and parenteral acyclovir or other antiviral treatment. Orally administered acyclovir also has been used to treat dendritic keratitis, with effects that were similar to those achieved with topical acyclovir in the treatment of dendritic corneal ulceration. Several series suggest that oral acyclovir is therapeutic in patients who have stromal keratitis or keratouveitis.[233,349] When given in conjunction with topical antiviral therapy, the response to oral acyclovir is good, even in those who had failed topical therapy.[350] Oral therapy also is useful for prophylaxis against viral reactivation in those with stromal keratitis,[445] and prophylaxis is associated with decreased recurrence of corneal disease.[454]

HSV Encephalitis

Intravenous acyclovir is the drug of choice for HSV encephalitis.[432] For children 3 months to 12 years of age, 10 to 15 mg/kg/dose every 8 hours is recommended. For children older than 12 years of age and for adults, 10 mg/kg/dose every 8 hours is recommended.[182] Renal function and hydration status should both be monitored closely while the patient is receiving intravenous acyclovir. The recommended duration of therapy ranges from 14 to 21 days or longer and should be determined by clinical response and HSV DNA PCR levels in CSF. Neonates with any form of HSV infection, including encephalitis, should receive parenteral acyclovir until HSV DNA PCR assay of the CSF is negative. Thereafter, oral acyclovir suppressive therapy should be administered at a dose of 300 mg/m^2/dose every 8 hours for 6 months, to improve neurodevelopmental outcomes and prevent skin recurrences.[200]

Two well-controlled clinical studies in Sweden and the United States that compared vidarabine with acyclovir 30 mg/kg/day in three divided doses for 10 to 12 days provided evidence that acyclovir is significantly more effective than vidarabine in treating HSV encephalitis.[356,433] In the Swedish study, the early mortality rate was 19 percent in the acyclovir-treated group and 50 percent in the vidarabine-treated group.[356] The National Institutes of Health collaborative study[433] generated similar data. As was observed in the earlier vidarabine studies,[443] younger age and less severe neurologic signs at initiation of therapy markedly influence the outcome of HSV encephalitis treated with acyclovir. Lethargic patients have a 15 percent mortality rate, whereas comatose patients have a 40 percent mortality rate.

In addition to antiviral therapy, intensive care is required for optimizing the outcome of these patients. Fluid management for the prevention of overhydration or underhydration is critical. Frequently, direct measurement of intracranial pressure is necessary for effective monitoring of increased pressure and treatment with diuretic agents. Anticonvulsant therapy for management of the often severe and prolonged seizures, as well as ventilatory support, usually is necessary at some time during the illness.

Genital HSV Infection

Recommended antiviral therapies for genital herpes simplex infection include acyclovir, famciclovir, and valacyclovir (Table 157-4), but acyclovir remains the best studied drug in children.[79,107] Although topical acyclovir was the first antiviral therapy used to treat genital herpes, it has been replaced by the oral agent. Some benefit was observed with the topical drug, but it is difficult to use, and its effectiveness is limited when compared with oral acyclovir; treatment decreased the mean duration of viral shedding (4.1 vs. 7.0 days with placebo) and time to crusting of lesions (7.1 vs. 10.5 days).[94] In the treatment of recurrent genital disease, topical acyclovir generally tended to have a minor effect on the duration of viral shedding (from 1 to 2 days in treated patients vs. 2 to 3 days in placebo recipients), with little or no effect on symptoms.[247,310] These results were not improved markedly with the immediate use of topical ointment. Early studies in which intravenous acyclovir was given to patients with primary genital HSV infection also were performed.[93,259] When used in a placebo-controlled, double-blind study at a dose of 5 mg/kg every 8 hours for 5 days, acyclovir decreased the duration of viral shedding (2 vs. 8 to 13 days in placebo-treated patients), shortened the duration of local and systemic symptoms by 2 to 5 days, and decreased the time to healing by 7 to 12 days. Complications such as extragenital lesions or urinary retention were reduced significantly.[291]

Oral acyclovir has therapeutic effects on both primary and recurrent HSV infection in adults and should be given to adolescents with these infections. The major effect of intravenous acyclovir therapy on the first episode of genital HSV infection is seen in those who have true primary disease, as opposed to those who have HSV-1 immunity and are experiencing new HSV-2 infection.[291] The intravenous route of administration has been replaced by oral therapy with acyclovir and related agents. In either case, optimal benefit requires early intervention. In a double-blind, placebo-controlled study of patients with primary genital infection, acyclovir at a dose of 200 mg five times per day for 5 to 10 days significantly reduced viral shedding (1 to 6 days vs. 13 to 15 days with placebo), lesion formation after 48 hours (0% to 4% vs. 43% to 44%), the duration of lesions (10 to 12 vs. 16 to 21 days), and the duration and severity of symptoms.[59] In

TABLE 157-4 **Recommended Treatment Regimens for Adults and Adolescents with Genital HSV Infection**

First Clinical Episode
Acyclovir 400 mg orally three times a day for 7 to 10 days
or
Acyclovir 200 mg orally 5 times a day for 7 to 10 days
or
Famciclovir 250 mg orally three times a day for 7 to 10 days
or
Valacyclovir 1 g orally twice a day for 7 to 10 days
Suppressive Therapy
Acyclovir 400 mg orally twice a day
or
Famciclovir 250 mg orally twice a day
or
Valacyclovir 500 mg orally once a day
or
Valacyclovir 1 g orally once a day
Episodic Therapy*
Acyclovir 400 mg orally three times a day for 5 days
or
Famciclovir 125 mg orally twice daily for 5 days
or
Valacyclovir 500 mg orally twice a day for 3 days

*Additional dosing recommendations can be found in the guidelines referenced.

From Centers for Disease Control and Prevention: Sexually transmitted disease treatment guidelines 2010. MMWR Recomm Rep 2010;**59**(RR-12);1-109.

similar studies of adult patients with recurrent genital HSV infection, acyclovir 200 mg five times per day for 5 days decreased the duration of virus shedding (1 to 2 vs. 2 to 4 days), time to healing (5 to 6 vs. 6 to 7 days), and development of new lesions (2% to 10% vs. 19% to 25%), especially when administered early in the recurrence.[277]

In these short-term studies in which patients were cautioned regarding hydration, no significant side effects were observed. Furthermore, no cytogenetic effects were noted.[86] Studies of virus from either placebo or acyclovir recipients showed that 4 to 15 percent of viruses isolated after therapy were more resistant to acyclovir, regardless of therapy.[87] Neither oral nor intravenous acyclovir reduces the rate of recurrence when used to treat either primary or recurrent genital infection. Oral antiviral agents are appropriate for patients who have primary genital HSV infection, HSV proctitis,[79,318] and frequent recurrent disease. Intravenous acyclovir should be reserved for patients who have severe local or disseminated disease with pneumonitis, hepatitis, or meningoencephalitis or complications such as urinary retention or aseptic meningitis syndrome.[79,291]

In adults, valacyclovir administered two times a day was as effective as acyclovir administered five times a day in the treatment of first-episode genital herpes.[131] When compared with placebo, valacyclovir is as effective as acyclovir in suppressing HSV viral replication as measured by culture and HSV PCR assay.[156] Similarly, famciclovir is efficacious in the treatment of first-episode and recurrent genital HSV infection.[12,323]

Symptomatic therapy for HSV lesions should be directed toward reduction of local discomfort, promotion of healing, and prevention of autoinoculation and superinfection. Nonspecific creams and ointments probably delay healing and increase risk for the development of maceration and infection. Keeping lesions clean and dry probably is the most important local measure. Urinating sometimes is painful and can be made less so if done in a bathtub or sitz bath. Some experts advise Burow solution sitz baths or short compress treatments. Prolonged soaking delays healing.

Mucocutaneous HSV Infection in Immunocompromised Hosts

Acyclovir and related drugs given orally or intravenously are the agents of choice for serious mucocutaneous HSV-1 or HSV-2 infection in high-risk hosts. Early initiation of treatment provides the best outcome. Given the limited oral bioavailability of acyclovir, higher plasma concentrations can be achieved with valacyclovir or famciclovir, but clinical experience with these drugs remains limited in children. If the infection is considered potentially life threatening, intravenous acyclovir is the treatment of choice. Therapeutic responses to HSV infection in immunocompromised hosts have been difficult to study because of the variable nature of the disease. The historical experience with vidarabine demonstrated the benefits of antiviral therapy initially. In a randomized, controlled, crossover study of mucocutaneous HSV infection, vidarabine 10 mg/kg/day intravenously in a 12-hour infusion was shown to decrease pain and induce defervescence in patients older than 40 years. In none of the 85 patients in this study did visceral dissemination of HSV develop. However, these observations are of historical interest now because the drug is not available and has been replaced by acyclovir. Acyclovir ointment appears to have some minor effects on pain, viral shedding, and time to complete healing of lesions in immunocompromised persons with mild, non–life-threatening mucocutaneous HSV infection,[440] but oral acyclovir is more effective for mild illness.

Patients with more severe illness should receive care in a hospital setting with the intravenous preparation. Intravenous acyclovir has been analyzed extensively in immunocompromised patients with mucocutaneous HSV infection.[137,317] When used early, acyclovir arrests the progression of infection. In several double-blind, placebo-controlled studies, acyclovir has been shown to be highly effective. It decreased the time to cessation of new lesions (1 vs. 3 days with placebo), time to lesion crusting (3 to 7 vs. 9 to 14 days), time to lesion healing (12 to 14 vs. 18 to 28 days), cessation of pain (4 to 10 vs. 7 to 16 days), and termination of viral shedding (3 vs. 14 to 17 days).[413] Acyclovir administered intravenously is the most effective agent and the drug of choice for treating HSV in an immunocompromised host. The dosage is 250 mg/m^2 or 5 to 10 mg/kg/dose every 8 hours infused over a 1-hour period. In patients with severe disease, the dose may be doubled. The major toxic effect has been renal, with reversible obstructive nephropathy and transient rises in serum creatinine levels (5% to 10% of patients). Adequate hydration, keeping urine specific gravity measurements less than 1.010, usually prevents this problem. Dosage guidelines have been established in patients who have impaired renal function (Table 157-5). One to 5 percent of patients may experience nausea and vomiting. Less commonly (1%), reversible neurologic symptoms (lethargy, agitation, tremor, disorientation, coma, transient hemiparesthesia) and laboratory abnormalities (abnormal EEG, increased CSF myelin basic protein) have developed in bone marrow transplant recipients. These patients usually had received interferon and CNS chemoprophylaxis for leukemia.[412] Other less serious problems include phlebitis (14%) and hives (5%).

Several studies have focused on the use of oral acyclovir in this patient population.[377] The IC_{50} of acyclovir generally is 0.1 to 0.5 μg/mL for HSV-1 and 0.5 to 2 μg/mL for HSV-2. Many studies use molar concentrations; in the case of acyclovir, the dose in micromoles divided by 4 equals the dose in micrograms per milliliter.

TABLE 157-5 **Guidelines for Dosing Intervals of Acyclovir in Patients with Renal Impairment***

Creatinine Clearance (mL/min/1.7 m^2)	Dose	Dosing Interval (hr)
≥50	Normal	8
25-50	Normal	12
10-25	Normal	24
0-10	50% of normal	24

*A dose should be administered after each dialysis session in patients undergoing hemodialysis.

Whereas peak serum levels with intravenous acyclovir vary from 8 to 15 μg/mL, the levels achieved with oral therapy are considerably lower (1 to 2 μg/mL). In preliminary studies of relatively small populations of immunocompromised adults, oral acyclovir at doses of 200 mg five times per day effectively promoted the healing of lesions and inhibited viral shedding.[377] Pediatric studies have used oral doses of 600 mg/m^2 given four times per day.[381] The relative efficacies of oral and intravenous acyclovir have not been compared in this setting. The choice of route of administration requires clinical judgment about the degree of immunosuppression, assessment for evidence of dissemination, and frequent reevaluation if oral drug is given.

Preliminary pharmacokinetic information regarding valacyclovir in immunocompromised children is available.[266] Valacyclovir was administered to 28 immunocompromised children aged 5 to 12 years in an open-label, randomized, dose-ranging study. Doses of valacyclovir administered were 250 or 500 mg twice a day or 500 mg three times a day. Pharmacokinetic evaluation has estimated that the bioavailability of oral valacyclovir is twofold to fourfold greater than that of oral acyclovir. The information provided in this study provides preliminary guidance for dosing of oral valacyclovir in children; however, more studies are needed to determine efficacy and safety in children. Recently published data regarding the pharmacokinetics of valacyclovir suspension in children suggest that valacyclovir oral suspension at doses of 10 and 20 mg/kg administered two to three times daily in children 3 months to 12 years of age produces drug levels similar to doses of 500-mg and 1000-mg caplets, respectively in adults.[198] Pharmacokinetics of famciclovir in children are also being evaluated with a recently proposed dosing scheme that awaits formal clinical evaluation.[43]

All studies of acyclovir in immunosuppressed patients have documented the propensity of recurrent lesions to reappear when therapy is withdrawn. Whether acyclovir reduces the humoral and cellular immune responses to HSV is not certain.[411]

Neonatal Herpes Simplex Virus Infection

Antiviral therapy for neonatal HSV disease significantly improves mortality rates and long-term outcomes of infected neonates. Historically, vidarabine was the first commercially available drug for the treatment of neonatal HSV disease. Placebo-controlled trials demonstrated that vidarabine at doses of 15 or 30 mg/kg/day given as a 12-hour infusion significantly reduced the mortality rate from 62 percent in historic cases and placebo controls to 35 percent in treated cases and increased the percentage of normal survivors from 19 to 43 percent. Results were best in infants with disseminated infection and disease localized to the CNS, with the mortality rate being reduced from 70 to 40 percent and the percentage of normal survivors increased from 10 to 30 percent. No difference was found between the 15-mg and the 30-mg dose in side effects, but the higher dose appeared to inhibit more effectively the progression of disease from skin-eye-mouth involvement to disseminated or CNS disease. None of the 49 treated patients had significant adverse clinical reactions or laboratory abnormalities attributable to the drug. On the negative side, 21 percent of infants progressed to more serious disease while receiving therapy and serious neurologic sequelae developed in 27 percent of the total. Fifteen percent of infants with disseminated or CNS disease continued to excrete HSV in the throat after 10 days of therapy.

The results of a vidarabine-acyclovir comparison trial in the treatment of neonatal herpes did not show any difference in efficacy and safety between the two drugs.[434] When the results with vidarabine and vidarabine-acyclovir were combined, the mortality rate was reduced from 49 percent in untreated historical controls to 17 percent in treated patients. Normal survivors increased from 26 to 67 percent. Although the results in the total group of patients were encouraging, the outcome in the group of patients with disseminated disease was not: a mortality rate of 54 percent in treated patients versus 76 percent in untreated historical controls and normal development in 59 percent of treated survivors versus 54 percent of controls. Factors that predicted mortality and their relative risk were as follows: disseminated disease, 33; CNS disease, 5.8; semicoma or coma, 5.2; disseminated intravascular coagulation, 3.8; prematurity, 3.7; and pneumonitis, 3.6.[435] Factors significantly associated with neurologic sequelae in survivors and their relative risk in these studies were as follows: skin-eye-mouth disease with three or more recurrent skin lesions after completion of acute therapy, 21; skin-eye-mouth disease caused by HSV-2, 14; HSV-2 infection, regardless of disease category, 4.9; CNS disease, 4.4; and seizures, 3.0. In infants with HSV disease limited to the skin, eye, or mouth, neonates with HSV-1 infection were all normal developmentally at 1 year of age as compared with 86 percent of those with HSV-2 infection. Among the latter infants, those with impaired neurologic outcome were significantly more likely to have had three or more skin recurrences in the first 6 months of life.

Because of greater ease of administration, intravenous acyclovir has supplanted vidarabine as the drug of choice for the treatment of neonatal HSV infection. Vidarabine no longer is available commercially. Because mortality and morbidity in neonates with HSV infection remained high using standard (30 mg/kg/day) dosing of acyclovir for 10 days, intermediate-dose (45 mg/kg/day) and high-dose (60 mg/kg/day) intravenous acyclovir for 21 days in 79 neonates was evaluated to treat neonatal HSV infection. Data were compared with those of a previous study in which all infants received standard doses of acyclovir (30 mg/kg/day) for 10 days. Overall, after stratification for disease category, the survival rate for patients treated with high-dose acyclovir was significantly higher than that for patients treated with the standard dose. Specifically, in neonates who received high-dose acyclovir, those with disseminated disease but not encephalitis had a significantly higher survival rate. The mortality rate at 24 months for patients with disseminated disease who received 21 days of high-dose acyclovir was 31 percent, compared with 57 percent for patients who received an intermediate dose for 21 days and 61 percent for those

who received the standard dose for 10 days in the earlier study. For encephalitis, the mortality rate of infants at 24 months was 6 percent with high-dose acyclovir, 20 percent with the intermediate dose, and 19 percent with the standard dose. In addition, recipients of high-dose acyclovir had less morbidity than did historical control infants who received the standard dose of acyclovir. Patients treated with high-dose acyclovir were 6.6 times as likely to have normal development at 12 months of age. Among infants with encephalitis, however, 31 percent of high-dose acyclovir recipients were developing normally at 12 months as compared with 29 percent of patients treated with the standard dose. With respect to toxicity in the high-dose acyclovir recipients, 21 percent had a transient neutropenia that resolved either during continuation of high-dose acyclovir or after its cessation. Nephrotoxicity occurred in four (6%) infants who had disseminated disease. Because acyclovir is eliminated by the kidneys, the dose should be adjusted for renal disease. High-dose acyclovir did not impede the development of an adequate antibody response to HSV in infected infants.

These studies have led to the current recommendation for the use of intravenous acyclovir at a dose of 60 mg/kg/day for a minimum of 21 days to treat neonatal CNS and disseminated HSV disease and a minimum for 14 days for skin-eye-mouth disease.[5] In addition, infants with ocular involvement should receive a topical ophthalmic drug (1% to 2% trifluridine, 1% idoxuridine, or 3% vidarabine), in addition to systemic acyclovir. Consultation with an ophthalmologist is also recommended to assist in the management of ocular HSV in the neonate, because corticosteroids and other medications may be indicated in some patients to preserve vision. All patients with HSV CNS infection should have a repeat lumbar puncture performed at the completion of acyclovir therapy to determine resolution of abnormal CSF parameters and to document whether the CSF specimen is HSV culture and/or HSV DNA PCR negative. A positive result from a CSF HSV PCR assay at the end of 21 days is an indication for continuing intravenous acyclovir therapy and for performing repeat neuroimaging studies to evaluate the extent of CNS damage. Repeat samples of blood and CSF should be obtained weekly until HSV studies are negative and provide a guideline for duration of antiviral therapy.

Concern has been raised that some infants may have progressive neurologic injury after acquiring neonatal herpes encephalitis. In addition, infants who have three or more skin recurrences with HSV-2 in the first 6 months of life have been shown to be at increased risk for having neurodevelopmental abnormalities at follow-up, whether as a consequence of previously undetected CNS infection or because of CNS dissemination during skin reactivation. Suppression of HSV skin reactivation by the administration of acyclovir (300 mg/m^2 per dose given orally three times daily) for 6 months is recommended.[5] Oral suppression for 6 months also improves neurodevelopmental outcome in neonates with all forms of HSV infection. The infant's blood cell counts should be monitored while receiving long-term oral acyclovir therapy because documented suppressive therapy resulted in neutropenia in 50 percent of treated infants. In addition, emergence of acyclovir-resistant HSV isolates has been documented. Acyclovir suppression also has been used after development of HSV eye infection in which reactivation might imperil vision.

General supportive measures, such as maintenance of fluid and electrolyte balance, correction of hypoglycemia, management of disseminated intravascular coagulation and shock, control of seizures with anticonvulsants, mechanical support of the respiratory system, nutritional support, and antimicrobial therapy for complicating bacterial infections, also are critical in improving the outcome for neonates with HSV infection. Because neonates with a disseminated form of HSV with hepatitis and hepatic necrosis may have secondary peritonitis and gram-negative bacteremia or sepsis, administration of antibiotics is also recommended. In severe cases of hepatic failure associated with neonatal disseminated HSV infection, liver transplantation has been effective treatment for some infants.[260,313]

Acyclovir-Resistant HSV Infection

Despite its widespread use, acyclovir remains an effective antiviral compound. In immunocompetent hosts, acyclovir resistance has been an unusual problem, even with prolonged courses of suppressive therapy. Mutant, thymidine kinase–negative virus strains have been recovered in 3 to 10 percent of patients treated with acyclovir, but acyclovir-resistant virus also can be recovered in placebo-treated patients, thus indicating that subpopulations of HSV are present in the lesions.[87] Thymidine kinase–negative HSV recurrences in immunocompromised patients have been documented well, with demonstration of the ability of these viruses to establish latency and recur. These viral mutants are less virulent in animal models. Patients who were culture positive for such mutants often have thymidine kinase–positive HSV isolates recovered from lesions during their next recurrence.[354]

Acyclovir-resistant virus occasionally has been shed by immunocompetent patients before, during, or after receiving therapy, yet it usually has not been associated with treatment failure.[155,212,234,378] In pretreatment patients, 3.6 percent of isolates (31 of 870) were resistant to acyclovir (not inhibited by 3 μg/mL). A similar percentage (3.1%) of resistant isolates was recovered from 663 immunocompetent patients after administration of acyclovir therapy.[87] An immunocompetent patient was reported who had an acyclovir-resistant virus containing an altered thymidine kinase that caused multiple recurrences of genital herpes unresponsive to acyclovir therapy.[212] In another immunocompetent patient receiving prophylactic acyclovir for recurrent genital herpes and long-term prednisone therapy for chronic urticaria, recurrent genital lesions with acyclovir- and penciclovir-resistant HSV-2 developed.[214] To date, these occurrences remain rare findings in the immunocompetent patient population. Acyclovir-resistant HSV has also been documented in neonates treated for HSV infection.[238]

Drug resistance occasionally is a problem in the acyclovir-treated immunocompromised population. Cases of acyclovir-resistant virus causing local inva-

sive disease and, less commonly, dissemination and acyclovir-unresponsive meningoencephalitis have been reported.[137,140,244,326] Recurrences with acyclovir-resistant HSV-1 may be encountered, as described in a child with Wiskott-Aldrich syndrome.[330,331] Of the three mechanisms of altered sensitivity of HSV to acyclovir (absent thymidine kinase [TK^-], altered thymidine kinase [TK^A], and altered viral DNA polymerase), the TK^- type is encountered in the vast majority of cases.[87,137,140] These viruses cannot phosphorylate acyclovir and convert it to the active triphosphate. Among bone marrow transplant recipients receiving multiple courses of acyclovir, acyclovir-resistant virus was recovered from 2 percent of patients during initial therapy and from 9 percent after treatment of a second recurrence. In a tertiary care center, acyclovir-resistant, clinically significant virus was recovered from 5 percent of immunocompromised patients (usually after receiving acyclovir) but not from any immunocompetent hosts.[125] Illness caused by acyclovir-resistant viruses was more severe in pediatric patients and occurred more commonly in very immunocompromised patients, such as those with AIDS or those who had undergone bone marrow transplantation.[126] Among bone marrow transplant recipients and patients with AIDS who were tested after receiving acyclovir therapy, 18 percent (105 of 582) had isolates that were acyclovir resistant.[87] In vitro viral susceptibility of HSV has been highly associated with clinical response to acyclovir in HIV-infected patients.[327] Acyclovir-resistant isolates are resistant to penciclovir and ganciclovir, which also require phosphorylation. Foscarnet has been used and is associated with successful clinical response and cessation of viral shedding. The most common side effects of foscarnet are nephrotoxicity (azotemia), alterations in serum calcium and phosphorus, and neutropenia, which has been observed in 10 to 25 percent of patients.[190,326] Foscarnet therapy is indicated for an immunocompromised patient who has HSV infection unresponsive to acyclovir. Viral susceptibility testing may aid in clinical management.[298,328]

In patients receiving chronic or multiple courses of foscarnet, foscarnet-resistant HSV isolates (IC_{50} >100 μg/mL) have been recovered from lesions failing to respond to foscarnet.[327,328] Of note, these lesions often responded to acyclovir, alone or in combination with foscarnet.[328] Perhaps the mutation in DNA polymerase responsible for foscarnet resistance was in an area not related to acyclovir activity. Other experimental strategies used to treat acyclovir-resistant viruses have included high-dose, continuous-infusion acyclovir (1.5 to 2.0 mg/kg/hr) or intravenous cidofovir,[58,125,223,358] although recent cases of cidofovir-resistant isolates have been reported.[449] In the case of acyclovir- and foscarnet-resistant viruses, topical 3-hydroxy-2-phosphonomethoxypropyl cytosine (HPMPC or cidofovir) or a combination of topical trifluorothymidine and interferon-α[40,325,330] has been used successfully in patients with chronic mucocutaneous lesions.

PREVENTION AND INFECTION CONTROL

Because HSV infection is ubiquitous in the human population and intermittent asymptomatic shedding is extremely common, preventing transmission of HSV is difficult. By diminishing the passage of infectious HSV-2, condoms offer moderate protection and should be recommended routinely.[251,416] Circumcised males are also less likely to acquire HSV-2 infection compared with uncircumcised controls.[395]

HSV is sensitive to heat and lipid solvents, so the use of antiseptics, soap and hot water, or chlorine decreases the risk for transfer of virus in settings such as the home, spas, pools, wrestling meets, and hospitals. In addition, appropriate use of gloves and other personal protective wear, by all health care personnel who come in contact with potentially infected body secretions or rashes should decrease the acquisition and health care–associated transmission of HSV. Health care workers with an active herpetic whitlow should wear gloves and not have direct patient care responsibilities for neonates and immunocompromised patients.[5] Health care workers with active oral herpes lesions should practice good hygiene and precautions, and reassignment to non–patient care areas should be made on an individual basis. Health care providers should wear gloves and wash carefully before and after contact with respiratory or genital tract secretions. Parents and caretakers of infants with eczema or severe diaper rash should be especially careful to avoid making direct or indirect contact of this altered skin with an active HSV lesion. Burn patients should be protected against exposure to or direct contact with personnel or visitors who have active HSV lesions. Immunosuppressed patients with evidence of HSV infection usually are experiencing reactivation of latent virus. Primary HSV infections in immunosuppressed persons, as in neonates, may be especially severe, and protecting these susceptible patients against exposure to HSV lesions is important. Wrestlers and rugby players who have exposed skin lesions caused by HSV should be excluded from competition.

The major approaches to the prevention of neonatal herpes involve interruption of transmission from sites of HSV infection and disease at the time of delivery and postnatally from a variety of potential sources. Because in utero transplacental congenital infection occurs so infrequently and predicting the occurrence of congenital infection during pregnancy is not possible, no standard recommendations for prophylaxis are available.

Cesarean delivery within 4 hours of rupture of fetal membranes in a mother with active genital herpes at the time of delivery is recommended to reduce the risk for natal infection. Because serial vaginal cultures taken during the latter stages of pregnancy have failed to predict shedding at the time of delivery, such monitoring is not recommended. Cesarean delivery is recommended only for women with active vesicular or ulcerative lesions at the time of delivery.[5] Cesarean delivery is not always 100 percent protective, even if the membranes are intact. Between 1.2 and 3 percent and, in one study, up to 33 percent of neonates with HSV infection are delivered by cesarean delivery. The cost-effectiveness of performing a cesarean delivery in women with recurrent lesions at delivery has been questioned by some experts, but it remains the current recommendation in the United States. Multiple clinical trials have shown that administration of prophylactic acyclovir and valacyclovir beginning at 36 weeks' gestation reduces the risk for clinically apparent HSV

genital vesicles and ulcers at delivery, reduces the risk for HSV viral shedding at delivery, and also reduces the incidence of cesarean delivery. However, although the hope is that the risk for HSV disease in the neonate also would be reduced, in none of these studies was sufficient power or evidence available to determine the effect of peripartum prophylaxis on the incidence of neonatal HSV. No adverse effects have been seen in infants exposed to maternal acyclovir during pregnancy. Because fetal scalp electrode monitor, forceps, and maneuvers that might cause a break in the infant's skin during delivery appear to increase the risk for acquisition of neonatal HSV, they should be used only with clear indication and a careful assessment of the risk-to-benefit ratio.

The results of antenatal genital HSV cultures from pregnant women with a history of genital herpes do not predict the infant's risk for exposure to HSV at delivery. Because 60 to 80 percent of mothers of infants with neonatal herpes are asymptomatic or have unrecognized infection, the optimal approach may be to perform a screening test to identify women shedding or likely to shed HSV at the time of delivery. Although HSV rapid antigen-detection tests continue to be developed commercially, their sensitivity in *asymptomatic* women still is only 60 to 75 percent. Screening of pregnant women for non–type-specific HSV antibody would be confounded by the presence of HSV-1 antibody, which is present in 60 to 80 percent of the population. The presence of HSV-1 antibody probably represents oral herpes, which poses little risk to the neonate. Although type-specific antibody assays are available to identify pregnant women infected with HSV-2, such an approach would miss women with genital HSV-1 infection or those with asymptomatic primary HSV-2 infection near the time of delivery before antibody is detectable. Its use has been recommended in identifying discordant couples, that is, women with no history of having genital HSV infection but whose sexual partner has had previous infection, so barrier precautions may be used before delivery to decrease the chance of development of a maternal primary infection that poses a greater risk to her infant. Culturing the genital tract of women at the time of vaginal delivery would identify infants exposed to HSV and allow anticipatory management. However, because genital HSV infection rates are only 0.1 to 0.4 percent, this approach likely would not be practical or cost effective for *all* women. Culturing of *selected* women at delivery could focus on (1) positive or partner-discordant HSV-2 serologic tests, (2) those women with a history of genital herpes in themselves or their sexual partners, (3) those women with a history of another sexually transmitted disease or multiple sexual partners, and (4) mothers with a history of maternal fever in labor. However, routine or selective serologic screening, although recommended by some experts, is not currently a standard recommendation, and further investigation is required to determine whether screening of pregnant women to prevent neonatal herpes is beneficial, practical, and cost effective.

No studies have been conducted on the optimal management of asymptomatic infants exposed to maternal HSV infection at delivery, although guidelines on maternal and neonatal evaluation and prophylactic or anticipatory antiviral therapy are available.[197a] A recently published algorithm for the evaluation of asymptomatic neonates following vaginal or cesarean delivery to women with active genital herpes lesions recommends the following approach:

Obstetricians who note a visible lesion should obtain a swab for HSV culture and HSV PCR and determine maternal history of genital HSV preceding pregnancy.

If there is a positive maternal history of prior genital HSV, HSV surface cultures and PCR, and blood PCR should be obtained from the asymptomatic infant at 24 hours of age. If the mother has recurrent infection, the risk of neonatal disease is probably less than 3 to 5 percent. Acyclovir does not need to be initiated if the infant remains asymptomatic. If the laboratory studies from the infant are negative, then the family is educated regarding signs and symptoms of neonatal HSV disease and the infant is followed closely. If any of the infant testing is positive, then further evaluation is performed and intravenous acyclovir is initiated.

If there is no maternal history of prior genital HSV, then it is recommended that type-specific serology for HSV-1 and HSV-2 antibodies be sent from the mother. At 24 hours of age, HSV surface cultures and HSV PCR, HSV blood PCR, CSF studies, and serum ALT should be obtained from the infant. Intravenous acyclovir at 60 mg/kg/dose in three divided doses is then initiated pending results of maternal serology. The duration of treatment is dependent on whether maternal lesion culture and PCR are positive and whether maternal serology reflects first episode primary, first episode nonprimary, or recurrent infection. If maternal serology is consistent with primary or first episode nonprimary infection at delivery, the neonatal infection rate may be as high as 30 to 50 percent.

It is recommended that this management algorithm be used only in facilities where access to PCR and type-specific serologic testing is readily available and turnaround time for test results is reasonably short. In addition, evaluation and treatment is indicated in any infant before 24 hours of age if signs and symptoms of HSV disease are present. Immediate evaluation and treatment may be considered if infant is preterm (≤37 weeks' gestation) or there was prolonged rupture of membranes (>4-6 hours). Situations that increase the risk for neonatal transmission despite maternal recurrent disease include prematurity, the use of a scalp electrode monitor, or skin lacerations. All infants should be monitored closely for any clinical evidence of HSV infection. Any positive culture or the occurrence of signs or symptoms suggesting HSV infection in the neonate suggests that a full virologic evaluation, including blood and CSF analysis for HSV by PCR, should be performed and antiviral therapy initiated. Antiviral therapy also should be administered to neonates with positive HSV cultures of mucosal sites, and these neonates should not be assessed as being merely colonized, because all require intravenous acyclovir therapy.[5] Breast-feeding is contraindicated only if the mother has a vesicular lesion on her breast. Physicians also have recommended that circumcision be delayed for 1 month in infants at highest risk for transmission.

Prevention of postnatally acquired neonatal infection needs to take into account the potential sources of HSV: (1) maternal oral herpes or breast lesions; (2) household

member, such as father or sibling, or other close contact, such as grandparent or caretaker, with oral herpes or herpetic gingivostomatitis; and (3) health care–associated transmission from other infected infants or health care workers. Transmission to the neonate from family members with oral herpes lesions can be interrupted by education, avoidance of kissing and nuzzling of the neonate if they have oral herpes ulcers or history of "cold sores," and avoidance of touching the neonate if they have a herpetic skin lesion or whitlow, as well as common-sense personal hygiene, including hand washing. Health care–associated transmission can be reduced by contact isolation and hospital infection control measures, as well as universal precautions and good hand washing techniques. Personnel with herpetic whitlow should not have direct patient care responsibilities until the lesion has healed. Infants with HSV infection should be placed in contact isolation for the duration of the illness.[5] The median duration of viral shedding from skin vesicles and mucosal sites in infants receiving acyclovir is 5 to 8 days. Asymptomatic but high-risk infants born to mothers with herpes at delivery also should be in contact isolation; alternatively, they may room in with the mother in a private room.

Immunoprophylaxis and Chemoprophylaxis

The development of vaccines is a long-term objective that has significant potential to ameliorate the disease burden associated with HSV-1 and HSV-2.[14] However, to date, attempts to create effective HSV vaccines have met with limited success.[37,257,320,369] In one early report, a vaccine containing the recombinant glycoprotein D of HSV-2 (gD_2) was used in patients who had established recurrent genital herpes infections, and vaccine recipients had a third fewer genital herpes recurrences than those experienced by placebo recipients during the study year.[374] However, experience with the use of recombinant vaccines to prevent primary HSV infections has yielded mixed results. Recombinant HSV-2 gD_2 and gB_2 have been demonstrated to induce high humoral and cell-mediated immune responses in seronegative and seropositive recipients.[227] Some vaccines may protect women who have no HSV immunity at baseline.[369] In a study of discordant couples, an HSV-2 gD_2 subunit vaccine with alum and monophosphoryl lipid adjuvant was approximately 73 percent effective in preventing symptomatic genital HSV-2 disease in women who were seronegative for both HSV-1 and HSV-2.[371] However, when the same vaccine was given to 8323 women who were negative for antibodies to HSV-1 and HSV-2 in a randomized double-blind efficacy field trial, the vaccine was not efficacious, presumably owing to differences in the populations studied.[37] These studies illustrate that high levels of neutralizing antibody do not guarantee protection against infection. Other vaccine types in early stages of development include disabled infectious single-cycle virus vaccines, live genetically attenuated vaccines, and naked DNA.[369,442] No adequate controlled trials of high-dose intravenous immune globulin as an intervention to suppress frequently recurring genital herpes have been reported, and this method is not likely to be effective because infected patients have high titers of HSV-specific antibodies.[252]

Intramuscular human interferon-α administered before and after surgery on the trigeminal nerve root significantly decreased the incidence of HSV shedding and clinical reactivation.[290] In similar studies, interferon had no significant effect on HSV shedding or reactivation in renal transplant patients.[83]

Intravenous acyclovir has been shown to prevent reactivation of HSV almost completely in immunosuppressed patients receiving a bone marrow transplant or antileukemic chemotherapy.[336] In patients with bone marrow transplants, intravenous therapy is begun at the onset of immunosuppression and continued through the immediate transplant period, and then therapy is switched to oral acyclovir for several months.[145] Oral acyclovir administered chronically markedly suppressed recurrence in immunodeficient patients.[386] The use of prophylactic acyclovir is indicated in HSV-seropositive, immunosuppressed pediatric patients. Long-term use of suppressive prophylactic acyclovir appears to prevent the emergence of drug-resistant HSV disease in hematopoietic stem cell transplant recipients.[107,127]

Oral acyclovir 200 mg two to five times per day administered chronically can prevent reactivation of genital HSV nearly completely in patients with frequent recurrences. Recurrences decreased by 50 to 70 percent, and the time to recurrence changed from 14 to 25 days in patients receiving placebo to 100 to 125 days in those receiving acyclovir.[116,378] Breakthrough recurrences tended to be mild and to have less viral shedding and rarely were caused by acyclovir-resistant virus. When acyclovir was discontinued (after 12 to 15 weeks of administration), HSV recurrences reverted to the pretreatment frequency. Higher doses of acyclovir suppressed recurrences in patients experiencing a "breakthrough" with more conventional doses. In patients receiving therapy for 5 years, the number of recurrences declined from the first year (1.7) to the fifth year (0.8). Twenty to 25 percent of patients are recurrence free for 4 to 5 years.[146] After prophylactic therapy ended, suppressive therapy no longer was warranted in some patients because of longer periods between recurrences.[132,375] Patients who had resistant virus exhibited re-isolation of acyclovir-sensitive virus. Thus, acyclovir suppressed recurrences without eliminating latent virus. One study has reported that acyclovir does not seem to change the rate of asymptomatic viral shedding.[376] A subsequent study demonstrated that acyclovir 400 mg twice a day resulted in a 95 percent reduction in subclinical viral shedding when compared with placebo in women with genital herpes.[419] Side effects of chronic acyclovir therapy include an increase in mean corpuscular volume and hemoglobin concentration without anemia or megaloblastic changes, neutropenia and asthenia, and mild gastrointestinal upset.[378] Valacyclovir and famciclovir also can be used to suppress frequently recurring genital herpes effectively, and they suppress both symptomatic and asymptomatic HSV shedding.* Both drugs are more expensive than is acyclovir. The decision of

*References 106, 156, 322, 418.

whether to use episodic or suppressive therapy for individuals with recurrent genital HSV infection depends on the frequency of recurrences. Those with a recent primary infection and frequent recurrences may prefer long-term suppressive therapy. If episodic therapy is used, it should be initiated as soon as symptoms begin. Oral herpetic recurrences were prevented in skiers given a brief course of acyclovir during their ski trip.[365]

Chronic antiviral suppressive therapy with valacyclovir reduced genital HSV transmission in heterosexual HSV-2–discordant couples in one study.[95] Once-daily suppressive therapy with valacyclovir or placebo was given to the HSV-2–seropositive partner. Acquisition of HSV-2 occurred in 4 of 743 susceptible partners given valacyclovir and 16 of 741 susceptible partners given placebo (hazard ratio, 0.52). HSV was identified in genital secretions 2.9 percent of days in source partners receiving valacyclovir and 10.8 percent of days in placebo recipients. Use of condoms and abstinence during symptomatic outbreaks still are recommended. Use of HSV type-specific serologic testing to identify HSV-seropositive individuals and disclosure of HSV status, in combination with condom use, risk reduction counseling, and long-term suppressive antiviral therapy have the potential to control the spread of HSV-2 infection.[149,415] The potential utility of antiviral therapy against herpes simplex is being evaluated as a strategy for prevention of HIV infection.[73,74]

The role of antiviral therapy during pregnancy to prevent neonatal HSV infection is undergoing evaluation for safety and efficacy. In one randomized, blinded trial, women with recurrent genital HSV received either acyclovir, 400 mg three times a day, or placebo from 36 weeks' gestation until delivery. Genital lesions were present at delivery in 14 percent of women receiving placebo and 5 percent of women taking acyclovir ($p = .08$). A significant decrease in HSV culture and PCR positivity was noted in the acyclovir group near the time of delivery. No significant difference in delivery by cesarean section was found between the groups. Suppressive acyclovir treatment decreased but did not eliminate genital lesions or HSV viral detection at delivery.[425] Another randomized, placebo-controlled study evaluated the effect of daily valacyclovir suppression initiated at 36 weeks' gestation. Women receiving valacyclovir had fewer recurrences of genital HSV; however, valacyclovir suppression did not decrease the number of women who had viral shedding or lesions near delivery. A limited sample size may have contributed to failure to observe a significant difference in groups treated with drug versus placebo.[10] Neonatal HSV infection may still occur after maternal acyclovir suppression, as illustrated by a recently published case series.[297]

Experience with acyclovir prophylaxis for reactivation of HSV in children is limited. Prophylactic oral acyclovir 30 to 60 mg/kg/day in three to five doses for 1 week has been shown to decrease seroconversion effectively and to eradicate symptomatic cases of HSV gingivostomatitis in a nursery setting in which outbreaks of primary HSV infection were occurring.[219] Suppression, at doses of 300 mg/m^2 three times daily, has been effective in infants with frequent recurrences of HSV after neonatal HSV infection and has been safe, although prolonged use has been associated with reversible neutropenia. To date, prolonged suppressive therapy in adults has not resulted in serious side effects* or an increase in the problem of drug-resistant HSV in a healthy host.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abel L, Plancoulaine S, Jouanguy E, et al. Age-dependent Mendelian predisposition to herpes simplex virus type 1 encephalitis in childhood. *J Pediatr* 2010;**157**:623–9.
13. Aronson PL, Yan AC, Mittal MK, et al. Delayed acyclovir and outcomes of children hospitalized with eczema herpeticum. *Pediatrics* 2011;**128**:1161–7.
20. Audry M, Ciancanelli M, Yang K, et al. NEMO is a key component of NF-κβ- and IRF-3-dependent TLR3-mediated immunity to herpes simplex virus. *J Allergy Clin Immunol* 2011;**128**:610–17.
31. Beersma M, Verjans G, Metselaar H, et al. Quantification of viral DNA and liver enzymes in plasma improves early diagnosis and management of herpes simplex virus hepatitis. *J Viral Hepat* 2011;**18**:e160–6.
32. Beck LA, Boguniewicz M, Hata T, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. *J Allergy Clin Immunol* 2009;**124**:260–9.
43. Blumer J, Rodriguez A, Sánchez PJ, et al. Single-dose pharmacokinetics of famciclovir in infants and population pharmacokinetic analysis in infants and children. *Antimicrob Agents Chemother* 2010;**54**:2032–41.
64. Canalejo CE, Garcia DF, Cabello N, et al. Herpes esophagitis in healthy adults and adolescents: report of 3 cases and a review of the literature. *Medicine (Baltimore)* 2010;**89**:204–10.
65. Cantey J, Mejias A, Wallihan R, et al. Use of blood polymerase chain reaction testing for diagnosis of herpes simplex virus infection. *J Pediatr* 2012;**161**:357–61.
69. Caviness AC, Demmler G, Almendarez Y, et al. The prevalence of neonatal herpes simplex virus infection compared with serious bacterial illness in hospitalized neonates. *J Pediatr* 2008;**143**:164–9.
70. Caviness AC, Demmler G, Selwyn B. Clinical and laboratory features of neonatal herpes simplex virus infection: a case-control study. *Pediatr Infect Dis J* 2008;**27**:425–30.
71. Caviness AC, Demmler G, Swint J, et al. Cost-effectiveness analysis of herpes simplex virus testing and treatment strategies in febrile neonates. *Arch Pediatr Adolesc Med* 2008;**162**:665–74.
72. Caviness AC, Oelze LL, Saz UE, et al. Direct immunofluorescence assay compared to cell culture for the diagnosis of mucocutaneous herpes simplex virus infections in children. *J Clin Virol* 2010;**49**:58–60.
74. Celum C, Wald A, Lingappa JR, et al. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *N Engl J Med* 2010;**362**:427–39.
77. Centers for Disease Control and Prevention. Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 months and for HIV infection and AIDS among children aged 18 months to <13 years—United States, 2008. *MMWR Recomm Rep* 2008;**57**(No. RR-10):[Appendix A].
78. Centers for Disease Control and Prevention. Seroprevalence of herpes simplex virus type 2 among persons Aged 14–49 Years—United States, 2005–2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:456–9. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5915a3.htm.
79. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(No. RR-12):20–5.
80. Centers for Disease Control and Prevention. Neonatal herpes simplex virus infection following Jewish ritual circumcisions that included direct orogenital suction—New York City, 2000-2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:405–9.
100. Curtis T, Mandava N. Acute retinal necrosis as a late sequel of herpes simplex type 1 encephalitis in a child. *J AAPOS* 2007;**11**:509–10.
177. Hudson B, Powell C. Towards evidence-based medicine for pediatricians: does oral aciclovir improve clinical outcome in immunocompetent children with primary herpes simplex gingivostomatitis? *Arch Dis Child* 2009;**94**:165–7.

*References 132, 146, 375.

182. James SH, Whitley RJ. Treatment of herpes simplex virus infections in pediatric patients: current status and future needs. *Clin Pharmacol Ther* 2010;**88**:720–4.
188. Johnston C, Magaret A, Selke S, et al. Herpes simplex virus viremia during primary genital infection. *J Infect Dis* 2008;**198**:31–4.
196. Khlevner J, Beneri C, Morganstern JA. Wrestling and herpetic esophagitis. *Pediatr Infect Dis J* 2011;**30**:911–42.
197a. Kimberlin DW, Baley J. Committee on Infectious Diseases, and Committee on Fetus and Newborn: Guidance on management of asymptomatic neonates born to women with active genital herpes lesions. *Pediatrics* 2013:**131**;383-6.
198. Kimberlin DW, Jacobs RF, Weller S, et al. Pharmacokinetics and safety of extemporaneously compounded valacyclovir oral suspension in pediatric patients from 1 month through 11 years of age. *Clin Infect Dis* 2010;**15**:221–8.
200. Kimberlin DW, Whitley R, Wan W, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N Engl J Med* 2011;**365**:1284–92.
216. Kullnat MW, Morse RP. Choreoathetosis after herpes simplex encephalitis with basal ganglia involvement on MRI. *Pediatrics* 2008;**121**:e1003–7.
243. Livorsi D, Anderson E, Qureshi S, et al. Brainstem encephalitis: an unusual presentation of herpes simplex virus infection. *J Neurol* 2010;**257**:1432–7.
246. Long S, Pool T, Vodzak J, et al. Herpes simplex virus infection in young infants during 2 decades of empiric acyclovir therapy. *Pediatr Infect Dis J* 2011;**30**:556–61.
251. Martin ET, Krantz E, Gottlieb SL, et al. A pooled analysis of the effect of condoms in preventing HSV-2 acquisition. *Arch Intern Med* 2009;**169**:1233–40.
250. Marquez L, Levy M, Munoz F, et al. A report of three cases and review of intrauterine herpes simplex virus infection. *Pediatr Infect Dis J* 2011;**30**:153–7.
253. McGill A, Bavaro M, You W. Postpartum herpes simplex virus endometritis and disseminated infection in both mother and neonate. *Obstet Gynecol* 2012;**120**:471–3.
254. McGoogan K, Haafiz A, Gonzalez-Peralta R. Herpes simplex virus hepatitis in infants: clinical outcomes and correlates of disease severity. *J Pediatr* 2011;**159**:608–11.
260. Moldovan B, Mentha G, Majno P, et al. Demographics and outcomes of severe herpes simplex virus hepatitis: a registry-based study. *J Hepatol* 2011;**55**:1222–6.
267. Nagamori T, Koyano S, Asai Y, et al. Sequential changes in pathophysiology of systemic inflammatory response in a disseminated neonatal herpes simplex virus (HSV) infection. *J Clin Virol* 2012;**53**:265–7.
297. Pinninti SG, Angara R, Feja KN, et al. Neonatal herpes disease following maternal antenatal antiviral suppressive therapy: a multicenter case series. *J Pediatr* 2012;**161**:134-8.e1–3.
298. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrob Agents Chemother* 2011;**55**:459–72.
306. Ramos-Casals M, Cuadrado MJ, Alba P, et al. Acute viral infections in patients with systemic lupus erythematosus: description of 23 cases and review of the literature. *Medicine (Baltimore)* 2008;**87**:311–18.
313. Riediger C, Sauer P, Matevossian E, et al. Herpes simplex virus sepsis and acute liver failure. *Clin Transplant* 2009;**23**(Suppl 21):37–41.
334. Sancho-Shimizu V, Perez do Diego R, Lorenzo L, et al. Herpes simplex encephalitis in children with autosomal recessive and dominant TRIF deficiency. *J Clin Invest* 2011;**121**:4889–902.
335. Thompson C, Whitley R. Neonatal herpes simplex virus infections: where are we now? *Adv Exp Med Biol* 2011;**697**:221–30.
395. Tobian AA, Serwadda D, Quinn TC, et al. Male circumcision for the prevention of HSV-2 and HPV infections and syphilis. *N Engl J Med* 2009;**360**:1298–309.
410. Vossough A, Zimmerman R, Vilaniuk L, et al. Imaging findings of neonatal herpes simplex virus type 2 encephalitis. *Neuroradiology* 2008;**50**:355–66.
412. Wang E, Schnall B, Rotschild T, et al. Unilateral keratitis following death of a twin as the presenting sign of herpetic infection in a neonate. *J AAPOS* 2011;**15**:489–90.
423. Ward KN, Ohrling A, Bryant NJ, et al. Herpes simplex serious neurological disease in young children: incidence and long-term outcome. *Arch Dis Child* 2012;**97**:162–5.
426. Weber M, Hartley J, Ashworth M, et al. Virological investigations in sudden unexpected deaths in infancy (SUDI). *Forensic Sci Med Pathol* 2010;**6**:261–7.
454. Young RC, Hodge DO, Liesegang TJ, et al. Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976-2007: the effect of oral antiviral prophylaxis. *Arch Ophthalmol* 2010;**128**:1178–83.
455. Zhang Q, Jeremiah CD, Lamborn IT, et al. Combined immunodeficiency associated with DOCK8 mutations. *N Engl J Med* 2009;**361**:2046–55.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abel L, Plancoulaine S, Jouanguy E, et al. Age-dependent Mendelian predisposition to herpes simplex virus type 1 encephalitis in childhood. *J Pediatr* 2010;**157**:623–9.
2. Abramson JS, Roach ES, Levy HB. Postinfectious encephalopathy after treatment of herpes simplex encephalitis with acyclovir. *Pediatr Infect Dis* 1984;**3**:146–7.
3. Adams G, Stover BH, Keenlyside RA, et al. Nosocomial herpetic infections in a pediatric intensive care unit. *Am J Epidemiol* 1981;**113**:126–32.
4. Al-Shekhlee A, Kocharian N, Suarez JJ. Re-evaluating the diagnostic methods in herpes simplex encephalitis. *Herpes* 2006;**13**:17–19.
5. American Academy of Pediatrics. Herpes simplex virus. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 398–408.
6. Amir J, Harel L, Smetana Z, Varsano I. Treatment of herpes simplex gingivostomatitis with acyclovir in children: a randomised double blind placebo controlled study. *BMJ* 1997;**314**:1800–3.
7. Amir J, Harel L, Smetana Z, Varsano I. The natural history of primary herpes simplex type 1 gingivostomatitis in children. *Pediatr Dermatol* 1999;**16**:259–63.
8. Amir JR, Straussberg L, Harel L, et al. Evaluation of a rapid enzyme immunoassay for the detection of herpes simplex virus antigen in children with herpetic gingivostomatitis. *Pediatr Infect Dis J* 1996;**15**:627–9.
9. Ando Y, Kimura H, Miwata H, et al. Quantitative analysis of herpes simplex virus DNA in cerebrospinal fluid of children with herpes simplex encephalitis. *J Med Virol* 1993;**41**:170–3.
10. Andrews WW, Kimberlin DF, Whitley R, et al. Valacyclovir therapy to reduce recurrent genital herpes in pregnant women. *Am J Obstet Gynecol* 2006;**194**:774–81.
11. Aoki FY, Law BJ, Hammond GW, et al. Acyclovir suspension for treatment of acute herpes simplex virus gingivostomatitis in children: a placebo-controlled, double-blind trial. Abstract. Presented at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy, 1993, New Orleans, p. 399.
12. Aoki FY, Tyring S, Diaz-Mitoma F, et al. Single-day, patient-initiated famciclovir therapy for recurrent genital herpes: a randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2006;**42**:8–13.
13. Aronson PL, Yan AC, Mittal MK, et al. Delayed acyclovir and outcomes of children hospitalized with eczema herpeticum. *Pediatrics* 2011;**128**:1161–7.
14. Arvin AM. Genital herpesvirus infections: rationale for a vaccine strategy. In: Hitchcock PJ, MacKay HT, Wasserheit JN, et al, editors. *Sexually transmitted diseases and adverse outcomes of pregnancy*. Washington, D.C.: American Society for Microbiology; 1999. p. 259–67.
15. Arvin AM, Hensleigh PA, Au DS, et al. Failure of antepartum maternal cultures to predict the infant's risk for exposure to herpes simplex virus at delivery. *N Engl J Med* 1986;**315**:796–800.
16. Arvin AM, Prober CG. Herpes simplex virus type 2, a persistent problem. *N Engl J Med* 1997;**337**:1158–9.
17. Arvin AM, Prober CG. Herpes simplex viruses. In: Murphy P, editor. *Manual of clinical microbiology*. 7th ed. Washington, D.C.: American Society for Microbiology; 1998. p. 878–87.
18. Ashenburg C, Rothstein FC, Dahms BB. Herpes esophagitis in the immunocompetent child. *J Pediatr* 1986;**108**:584–7.
19. Ashkenazi S, Kohl S. Nervous system abnormalities in pediatric HIV infection and AIDS. *Semin Pediatr Infect Dis* 1990; **1**:94–106.
20. Audry M, Ciancanelli M, Yang K, et al. NEMO is a key component of NF-κβ- and IRF-3-dependent TLR3-mediated immunity to herpes simplex virus. *J Allergy Clin Immunol* 2011;**128**:610–17.
21. Aurelius E, Andersson B, Forsgren M, et al. Cytokines and other markers of intrathecal immune response in patients with herpes simplex encephalitis. *J Infect Dis* 1994;**170**:678–80.
22. Aurelius E, Forsgren M, Skoldenberg B, et al. Persistent intrathecal immune activation in patients with herpes simplex encephalitis. *J Infect Dis* 1993;**168**:1248–52.
23. Aurelius E, Johansson B, Skoldenberg B, et al. Rapid diagnosis of herpes simplex encephalitis by nested polymerase chain reaction assay of cerebrospinal fluid. *Lancet* 1991;**337**:189–92.
24. Aurelius E, Johansson B, Skoldenberg B, et al. Encephalitis in immunocompetent patients due to herpes simplex virus type 1 or 2 as determined by type-specific polymerase chain reaction and antibody assays of cerebrospinal fluid. *J Med Virol* 1993; **39**:179–86.
25. Bader C, Crumpacker CS, Schnipper LE, et al. The natural history of recurrent facial-oral infection with herpes simplex virus. *J Infect Dis* 1978;**138**:897–905.
26. Baeten JM, McClelland RS, Corey L, et al. Vitamin A supplementation and genital shedding of herpes simplex virus among HIV-1–infected women: a randomized clinical trial. *J Infect Dis* 2004;**189**:1466–71.
27. Baringer JR. Recovery of herpes simplex virus from human trigeminal ganglions. *N Engl J Med* 1974;**291**:828.
28. Barton LL, Weaver-Woodard S, Gutierrez JA, Lee DM. Herpes simplex virus hepatitis in a child: case report and review. *Pediatr Infect Dis J* 1999;**18**:1026–8.
29. Bastian FO, Rabson AS, Yee CL, et al. Herpes-virus hominis: Isolation from human trigeminal ganglion. *Science* 1972;**178**: 306–7.
30. Bastian JF, Kaufman IA. Herpes simplex esophagitis in a healthy 10-year-old boy. *J Pediatr* 1982;**100**:426–7.
31. Beersma M, Verjans G, Metselaar H, et al. Quantification of viral DNA and liver enzymes in plasma improves early diagnosis and management of herpes simplex virus hepatitis. *J Viral Hepat* 2011;**18**:e160–6.
32. Beck LA, Boguniewicz M, Hata T, et al. Phenotype of atopic dermatitis subjects with a history of eczema herpeticum. *J Allergy Clin Immunol* 2009;**124**:260–9.
33. Becker WB, Kipps A, McKenzie D. Disseminated herpes simplex virus infection: its pathogenesis based on virological and pathological studies in 33 cases. *Am J Dis Child* 1968;**115**:1–8.
34. Behr JT, Daluga DJ, Light TR, et al. Herpetic infections in the fingers of infants: report of five cases. *J Bone Joint Surg Am* 1987;**69**:137–9.
35. Beigi B, Algawi K, Foley-Nolan A, et al. Herpes simplex keratitis in children. *Br J Ophthalmol* 1994;**78**:458–60.
36. Belongia EA, Goodman JL, Holland EJ, et al. An outbreak of herpes gladiatorum at a high-school wrestling camp. *N Engl J Med* 1991;**325**:906–10.
37. Belshe RB, Leone PA, Bernstein DI, et al. Efficacy results of a trial of a herpes simplex vaccine. *N Engl J Med* 2012;**366**:34–43.
38. Benedetti JK, Zeh J, Selke S, et al. Frequency and reactivation of nongenital lesions among patients with genital herpes simplex virus. *Am J Med* 1995;**98**:237–42.
39. Bennett RE Jr, Barnett DW. Perinatal herpes simplex infection presenting with pneumonia and pleural effusions. *Pediatr Infect Dis J* 2001;**20**:228–30.
40. Birch CJ, Tyssen DP, Tachedjian G, et al. Clinical effects and in vitro studies of trifluorothymidine combined with interferon-α for treatment of drug-resistant and sensitive herpes simplex virus infections. *J Infect Dis* 1992;**166**:108–12.
41. Biron CA, Byron KS, Sullivan JL. Severe herpes virus infections in an adolescent without natural killer cells. *N Engl J Med* 1989;**320**:1731–5.
42. Blot N, Schneider P, Young P, et al. Treatment of an acyclovir and foscarnet-resistant herpes simplex virus infection with cidofovir in a child after an unrelated bone marrow transplant. *Bone Marrow Transplant* 2000;**26**:903–5.
43. Blumer J, Rodriguez A, Sánchez PJ, et al. Single-dose pharmacokinetics of famciclovir in infants and population pharmacokinetic analysis in infants and children. *Antimicrob Agents Chemother* 2010;**54**:2032–41.
44. Boehm F, Estes W, Wright P, et al. Management of genital herpes simplex virus infection occurring during pregnancy. *Am J Obstet Gynecol* 1981;**141**:735.
45. Bogger-Goren S. Acute epiglottitis caused by herpes simplex virus. *Pediatr Infect Dis J* 1987;**6**:1133–4.
46. Breinig MK, Kingsley LA, Armstrong JA, et al. Epidemiology of genital herpes in Pittsburgh: serologic, sexual and racial correlates of apparent and inapparent herpes simplex infections. *J Infect Dis* 1990;**162**:299–305.
47. Brice SL, Krzemien D, Weston WL, et al. Detection of herpes simplex virus DNA in cutaneous lesions of erythema multiforme. *J Invest Dermatol* 1989;**93**:183–7.

48. Brice SL, Stockert SS, Jester JD, et al. Herpes simplex virus–associated erythema multiforme in children. *Abstract Clin Res* 1990;**38**:182.
49. Brinker JP, Herrmann JE. Comparison of three monoclonal antibody–based enzyme immunoassays for detection of herpes simplex virus in clinical specimens. *Eur J Clin Microbiol Infect Dis* 1995;**14**:314–17.
50. Brodtkorb E, Lindqvist M, Johnson M, et al. Diagnosis of herpes simplex encephalitis: a comparison between electroencephalography and computed tomography findings. *Acta Neurol Scand* 1982;**66**:462–71.
51. Brown E, Gardella C, Maim G, et al. Effect of maternal HSV serostatus and HSV type on risk for neonatal herpes. *Acta Obstet Gynecol Scand* 2007;**86**:523.
52. Brown Z, Benedetti JD, Ashley R. Neonatal herpes simplex virus infection in relation to asymptomatic maternal infection at the time of labor. *N Engl J Med* 1991;**324**:1247.
53. Brown Z, Benedetti JD, Watts DH. A comparison between detailed and simple histories in the diagnosis of genital herpes complicating pregnancy. *Am J Obstet Gynecol* 1995;**172**: 1299–303.
54. Brown Z, Gardella C, Maim G, et al. Effect of maternal HSV serostatus and HSV type on risk for neonatal herpes. *Acta Obstet Gynecol Scand* 2007;**86**:523.
55. Brown Z, Selke A, Zeh J, et al. The acquisition of HSV during pregnancy. *N Engl J Med* 1997;**337**:509.
56. Brown Z, Vontver L, Benetdetti J, et al. Effects on infants of a first episode of genital herpes during pregnancy. *N Engl J Med* 1987;**317**:1246.
57. Brown Z, Wald A, Morrow R, et al. Effect of serologic status and cesarean delivery on the rates of HSV from mother to infant. *JAMA* 2003;**289**:203.
58. Bryant P, Sasadeusz J, Carapetis J, et al. Successful treatment of foscarnet-resistant herpes simplex stomatitis with intravenous cidofovir in a child. *Pediatr Infect Dis J* 2001;**20**:1083–6.
59. Bryson Y, Dillon M, Lovett M, et al. Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir: a randomized double-blind controlled trial in normal subjects. *N Engl J Med* 1983;**308**:916–21.
60. Bryson Y, Dillon M, Bernstein DI, et al. Risks of acquisition of genital herpes simplex virus type 2 in sex partners of persons with genital herpes: a prospective couple study. *J Infect Dis* 1993;**167**:942–6.
61. Buchman TG, Roizman B, Adams G, et al. Restriction endonuclease fingerprinting of herpes simplex virus DNA: a novel epidemiological tool applied to a nosocomial outbreak. *J Infect Dis* 1978;**138**:488–98.
62. Buchman TG, Roizman B, Nahmias AJ. Demonstration of exogenous genital reinfection with herpes simplex virus type 2 by restriction endonuclease fingerprinting of viral DNA. *J Infect Dis* 1979;**140**:295–304.
63. Buss DH, Scharyj M. Herpesvirus infection of the esophagus and other visceral organs in adults: Incidence and clinical significance. *Am J Med* 1979;**66**:457–62.
64. Canalejo CE, Garcia DF, Cabello N, et al. Herpes esophagitis in healthy adults and adolescents: report of 3 cases and a review of the literature. *Medicine (Baltimore)* 2010;**89**:204–10.
65. Cantey J, Mejias A, Wallihan R, et al. Use of blood polymerase chain reaction testing for diagnosis of herpes simplex virus infection. *J Pediatr* 2012;**161**:357–61.
66. Caputo GM, Byck H. Concomitant oculoglandular and ulceroglandular fever due to herpes simplex type 1. *Am J Med* 1992;**93**:577–80.
67. Casrouge A, Zhang SY, Eidenschenk C, et al. Herpes simplex virus encephalitis in human UNC-93B deficiency. *Science* 2006;**314**: 308–12.
68. Cassinotti P, Mietz H, Siegl G. Suitability and clinical application of a multiplex nested PCR assay for the diagnosis of herpes simplex virus infections. *J Med Virol* 1996;**50**:75–81.
69. Caviness AC, Demmler G, Almendarez Y, et al. The prevalence of neonatal herpes simplex virus infection compared with serious bacterial illness in hospitalized neonates. *J Pediatr* 2008;**143**:164–9.
70. Caviness AC, Demmler G, Selwyn B. Clinical and laboratory features of neonatal herpes simplex virus infection: a case-control study. *Pediatr Infect Dis J* 2008;**27**:425–30.
71. Caviness AC, Demmler G, Swint J, et al. Cost-effectiveness analysis of herpes simplex virus testing and treatment strategies in febrile neonates. *Arch Pediatr Adolesc Med* 2008;**162**:665–74.
72. Caviness AC, Oelze LL, Saz UE, et al. Direct immunofluorescence assay compared to cell culture for the diagnosis of mucocutaneous herpes simplex virus infections in children. *J Clin Virol* 2010;**49**:58–60.
73. Celum CL, Robinson NJ, Cohen MS. Potential effect of HIV type 1 antiretroviral and herpes simplex virus type 2 antiviral therapy on transmission and acquisition of HIV type 1 infection. *J Infect Dis* 2005;**191**(Suppl. 1):S107–14.
74. Celum CL, Wald A, Lingappa JR, et al. Acyclovir and transmission of HIV-1 from persons infected with HIV-1 and HSV-2. *N Engl J Med* 2010;**362**:427–39.
75. Centers for Disease Control and Prevention. Genital herpes infections, United States, 1966-1984. *MMWR Morb Mortal Wkly Rep* 1986;**35**(24):402–4.
76. Centers for Disease Control and Prevention. Herpes gladiatorum at a high school wrestling camp—Minnesota. *MMWR Morb Mortal Wkly Rep* 1990;**39**:69–71.
77. Centers for Disease Control and Prevention. Revised surveillance case definitions for HIV infection among adults, adolescents, and children aged <18 months and for HIV infection and AIDS among children aged 18 months to <13 years—United States, 2008. *MMWR Recomm Rep* 2008;**57**(No. RR-10):[Appendix A].
78. Centers for Disease Control and Prevention. Seroprevalence of herpes simplex virus type 2 among persons Aged 14–49 Years—United States, 2005–2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:456–9. Also available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5915a3.htm.
79. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(No. RR-12):20–5.
80. Centers for Disease Control and Prevention (CDC). Neonatal herpes simplex virus infection following Jewish ritual circumcisions that included direct orogenital suction—New York City, 2000-2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 405–9.
81. Chan EL, Brandt K, Horsman GB. Comparison of Chemicon SimulFluor direct fluorescent antibody staining with cell culture and shell vial direct immunoperoxidase staining for detection of herpes simplex virus and with cytospin direct immunofluorescence staining for detection of varicella-zoster virus. *Clin Diagn Lab Immunol* 2001;**8**:909–12.
82. Chase RA, Pottage JC Jr, Haber MH, et al. Herpes simplex viral hepatitis in adults: two case reports and review of the literature. *Rev Infect Dis* 1987;**9**:329–33.
83. Cheeseman SH, Rubin RH, Stewart JA, et al. Controlled clinical trial of prophylactic human-leukocyte interferon in renal transplantation. effect on cytomegalovirus and herpes simplex virus infections. *N Engl J Med* 1979;**300**:1345–9.
84. Chonmaitree T, Owen M, Patel J, et al. Presence of cytomegalovirus and herpes simplex virus in middle ear fluids from children with acute otitis media. *Clin Infect Dis* 1992;**15**:650–3.
85. Chuang T-Y, Su WPD, Perry HO, et al. Incidence and trend of herpes progenitalis. a 15-year population study. *Mayo Clin Proc* 1983;**58**:436–41.
86. Clive D, Corey L, Reichman RC, et al. A double-blind, placebo-controlled cytogenetic study of oral acyclovir in patients with recurrent genital herpes. *J Infect Dis* 1991;**164**:753–7.
87. Collins P, Ellis MN. Sensitivity monitoring of clinical isolates of herpes simplex virus to acyclovir. *J Med Virol* 1993;**1**(Suppl.): 58–66.
88. Cone RW, Hobson AC, Brown Z, et al. Frequent detection of genital herpes simplex virus DNA by polymerase chain reaction among pregnant women. *JAMA* 1994;**272**:792–6.
89. Cone RW, Hobson AC, Palmer J, et al. Extended duration of herpes simplex virus DNA in genital lesions detected by the polymerase chain reaction. *J Infect Dis* 1991;**164**:757–60.
90. Cone RW, Swenson PD, Hobson AC, et al. Herpes simplex virus detection from genital lesions: a comparative study using antigen detection (HerpChek) and culture. *J Clin Microbiol* 1993;**31**: 1774–6.
91. Conway JH, Weinberg KA, Ashley RL, et al. Viral meningitis in a preadolescent child caused by reactivation of latent herpes simplex (type 1). *Pediatr Infect Dis J* 1997;**16**:627–9.

92. Corey L, Adams H, Grown Z, et al. Genital herpes simplex virus infections: clinical manifestations, course, and complications. *Ann Intern Med* 1983;**98**:958–72.
93. Corey L, Fife KH, Benedetti JK, et al. Intravenous acyclovir for the treatment of primary genital herpes. *Ann Intern Med* 1983;**98**:914–21.
94. Corey L, Nahmias AJ, Guinan ME. A trial of topical acyclovir in genital herpes simplex virus infection. *N Engl J Med* 1982;**306**:1313–19.
95. Corey L, Wald A, Patel R, et al. Once-daily valacyclovir to reduce the risk for transmission of genital herpes. *N Engl J Med* 2004;**350**:11–20.
96. Corey L, Whitley R, Stone E, et al. Difference between herpes simplex virus type 1 and type 2 neonatal encephalitis in neurological outcome. *Lancet* 1988;**1**:1–10.
97. Craig C, Nahmias AJ. Different patterns of neurologic involvement with herpes simplex virus types 1 and 2: isolation of herpes simplex virus type 2 from the buffy coat of two adults with meningitis. *J Infect Dis* 1973;**127**:365–72.
98. Crist GA, Langer JM, Woods GL, et al. Evaluation of the ELVIS plate method for the detection and typing of herpes simplex virus in clinical specimens. *Diagn Microbiol Infect Dis* 2004;**49**:173–7.
99. Croen KD, Ostrove SM, Dragovic L, et al. Characterization of herpes simplex virus type 2 latency-associated transcription in human sacral ganglia and in cell culture. *J Infect Dis* 1991;**163**: 23–8.
100. Curtis T, Mandava N. Acute retinal necrosis as a late sequel of herpes simplex type 1 encephalitis in a child. *J AAPOS* 2007;**11**: 509–10.
101. Darougar S, Wishart MS, Viswalingam ND. Epidemiological and clinical features of primary herpes simplex virus ocular infection. *Br J Ophthalmol* 1985;**69**:2–6.
102. Dascal A, Chan-Thim J, Morahan M, et al. Diagnosis of herpes simplex virus infection in a clinical setting by a direct enzyme immunoassay kit. *J Clin Microbiol* 1989;**27**:700–4.
103. David TJ, Langson M. Herpes simplex infection in atopic eczema. *Arch Dis Child* 1985;**60**:338–43.
104. Davis LE, Johnson RT. An explanation for the localization of herpes simplex encephalitis? *Ann Neurol* 1979;**5**:2–5.
105. Davis LE, McLaren LC. Relapsing herpes simplex encephalitis following antiviral therapy? *Ann Neurol* 1983;**13**:192–5.
106. DeJesus E, Wald A, Warren T, et al. Valacyclovir for the suppression of recurrent genital herpes in human immunodeficiency virus–infected subjects. *J Infect Dis* 2003;**188**:1009–16.
107. Dekker CL, Prober CG. Pediatric uses of valacyclovir, penciclovir and famciclovir. *Pediatr Infect Dis J* 2001;**20**:1079–81.
108. Delis S, Kato T, Ruiz P, et al. Herpes simplex colitis in a child with combined liver and small bowel transplant. *Pediatr Transplant* 2001;**5**:374–7.
109. Demaeral PH, Wilms G, Robberecht W, et al. MRI of herpes simplex encephalitis. *Neuroradiology* 1992;**34**:490–3.
110. DeVincenzo JP, Thorne G. Mild herpes simplex encephalitis diagnosed by polymerase chain reaction: a case report and review. *Pediatr Infect Dis J* 1994;**13**:662–4.
111. Diamond C, Mohan K, Hobson A, et al. Viremia in neonatal herpes simplex virus infections. *Pediatr Infect Dis J* 1999;**18**:487–9.
112. DiSclafani A, Kohl S, Ostrow PT. The importance of brain biopsy in suspected herpes simplex encephalitis. *Surg Neurol* 1982;**17**: 101–6.
113. Dix RD, Baringer JR, Panitch HS, et al. Recurrent herpes simplex encephalitis: recovery of virus after Ara-A treatment. *Ann Neurol* 1983;**13**:196–200.
114. Do AN, Green PA, Demmler GJ. Herpes simplex type 2 meningitis and associated genital lesions in a three-year-old child. *Pediatr Infect Dis J* 1994;**13**:1014–16.
115. Domingues RB, Lakeman FD, Mayo MS, Whitley RJ. Application of competitive PCR to cerebrospinal fluid samples from patients with herpes simplex encephalitis. *J Clin Microbiol* 1998;**36**:2229–34.
116. Douglas JM, Critchlow C, Benedetti J. A double-blind study of oral acyclovir for suppression of recurrences of genital herpes simplex virus infection. *N Engl J Med* 1984;**310**:1551–6.
117. Douglas JM, Schmidt O, Corey L. Acquisition of neonatal HSV-1 infection from a paternal source contact. *J Pediatr* 1983;**103**:908.
118. Dowdle WR, Nahmias AJ, Harwell RW, et al. Association of antigenic type of herpesvirus hominis with site of viral recovery. *J Immunol* 1967;**99**:774–80.
119. Drummer JS, Armstrong J, Somers J, et al. Transmission of infection with herpes simplex virus by renal transplantation. *J Infect Dis* 1987;**155**:202–6.
120. Ducoulombier H, Cousin J, Dewilde A, et al. [A controlled clinical trial versus placebo of acyclovir in the treatment of herpetic gingivostomatitis in children.] *Ann Pediatr (Paris)* 1988;**35**:212–16. In French.
121. Dunkle L, Schmidt R, O'Connor D. Neonatal herpes simplex infection possibly acquired via maternal breast milk. *Pediatrics* 1979;**63**:250.
122. Echeverria P, Miller G, Campbell A, et al. Scalp vesicles within the first week of life: a clue to early diagnosis of herpes neonatorum. *J Pediatr* 1973;**83**:1062.
123. Eis-Hübinger AM, Däumer M, Matz B, Schneweis KE. Evaluation of three glycoprotein G2–based enzyme immunoassays for detection of antibodies to herpes simplex virus type 2 in human sera. *J Clin Microbiol* 1999;**37**:1242–6.
124. Ellison PH, Hanson PA. Herpes simplex: a possible cause of brainstem encephalitis. *Pediatrics* 1977;**59**:240–3.
125. Engel JP, England JA, Fletcher CV, Hill EL. Treatment of resistant herpes simplex virus with continuous-infusion aceclovir. *JAMA* 1990;**263**:1662–4.
126. Englund JA, Zimmerman ME, Swierhosz EM, et al. Herpes simplex virus resistant to acyclovir: a study in a tertiary care center. *Ann Intern Med* 1990;**112**:416–22.
127. Erard V, Wald A, Corey L, et al. Use of long-term suppressive acyclovir after hematopoietic stem-cell transplantation: impact on herpes simplex virus (HSV) disease and drug-resistant HSV disease. *J Infect Dis* 2007;**196**:266–70.
128. Espy M, Smith TF. Detection of herpes simplex virus in conventional tube cell cultures and in shell vials with a DNA probe kit and monoclonal antibodies. *J Clin Microbiol* 1988;**26**:22–4.
129. Fedler HM, Long SS. Herpetic whitlow: epidemiology, clinical characteristics, diagnosis and treatment. *Am J Dis Child* 1983;**137**:861–3.
130. Frederick D, Bland D, Gollin Y. Fatal disseminated herpes simplex virus infection in a previously healthy pregnant woman: a case report. *J Reprod Med* 2002;**47**:591–6.
131. Fife KH, Barbarash RA, Rudolph T, et al. Valaciclovir versus acyclovir in the treatment of first-episode genital herpes infection: results of an international, multicenter, double-blind, randomized clinical trial. The Valaciclovir International Herpes Simplex Virus Study Group. *Sex Transm Dis* 1997;**24**:481–6.
132. Fife KH, Crumpacker CS, Mertz GJ. Recurrence and resistance patterns of herpes simplex virus following cessation of greater than or equal to 6 years of chronic suppression with acyclovir. *J Infect Dis* 1994;**169**:1338–41.
133. Fiumara NJ, Solomon J. Recurrent herpes simplex virus infections and erythema multiforme: a report of three patients. *Sex Transm Dis* 1983;**10**:144–7.
134. Fleming DT, McQuillan GM, Johnson RE, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* 1997;**337**:1105–11.
135. Foley FD, Greenwald KA, Nash G, et al. Herpesvirus infection in burned patients. *N Engl J Med* 1970;**282**:652–6.
136. Frank AL, Tucker G. Isolation of herpes simplex type 1 from ventricular fluid of an infant with encephalitis. *J Pediatr* 1978;**92**:601–2.
137. Frenck RW, Kohl S. Herpes simplex virus in the immunocompromised child. In: Patrick CC, editor. *Infections in immunocompromised infants and children*. New York: Churchill Livingstone; 1992. p. 603–24.
138. Galbraith JCT, Shafran S. Herpes simplex esophagitis in the immunocompetent patient: report of four cases and review. *Clin Infect Dis* 1992;**14**:894–901.
139. Gallardo M, Johnson D, Gaviria J, et al. Isolated herpes simplex keratoconjunctivitis in a neonate born by cesarean delivery. *J AAPOS* 2005;**9**:285–7.
140. Gateley A, Gander RM, Johnson PC, et al. Herpes simplex virus type 2 meningoencephalitis resistant to acyclovir in a patient with AIDS. *J Infect Dis* 1990;**116**:711–20.
141. Gibson JJ, Hornung CA, Alexander GR, et al. A cross-sectional study of herpes simplex virus types 1 and 2 in college students: occurrence and determinants of infection. *J Infect Dis* 1990;**162**:306–12.
142. Gill MJ, Arlette J, Buchan K. Herpes simplex virus infection of the hand: a profile of 79 cases. *Am J Med* 1988;**84**:89–93.

143. Glezen WP, Fernald GW, Lohr JA. Acute respiratory disease of university students with special reference to the etiologic role of herpesvirus hominis. *Am J Epidemiol* 1975;**101**:111–21.
144. Glogau R, Hanna L, Jawetz E. Herpetic whitlow as part of genital virus infection. *J Infect Dis* 1977;**136**:689–92.
145. Gnann JW Jr. Herpes simplex and varicella zoster virus infection after hemopoietic stem cell or solid organ transplantation. In: Bowden R, Lungman P, Paya CV, editors. *Transplant infections*. Philadelphia: Lippincott Williams & Wilkins; 2004. p. 350–65.
146. Goldberg LH, Kaufman R, Kurtz TO, et al. Long-term suppression of recurrent genital herpes with acyclovir: a 5-year benchmark. *Arch Dermatol* 1993;**129**:582–7.
147. Goodell SE, Quinn TC, Mkrtichian E, et al. Herpes simplex virus proctitis in homosexual men: clinical, sigmoidoscopic, and histopathological features. *N Engl J Med* 1983;**308**:868–71.
148. Goodyear HM, McLeish P, Randall S, et al. Immunological studies of herpes simplex virus infection in children with atopic eczema. *Br J Dermatol* 1996;**134**:85–93.
149. Gottlieb SL, Douglas JM Jr, Foster M, et al. Incidence of herpes simplex virus type 2 infection in 5 sexually transmitted disease (STD) clinics and the effect of HIV/STD risk-reduction counseling. *J Infect Dis* 2004;**190**:1059–67.
150. Greenberg SB, Taber L, Septimus E, et al. Computerized tomography in brain-biopsy–proven herpes simplex encephalitis: early normal results. *Arch Neurol* 1981;**38**:58–9.
151. Grossman J. Herpes simplex virus (HSV) infection. *Clin Obstet Gynecol* 1982;**25**:555.
152. Grossman ME, Stevens AW, Cohen PR. Brief report: herpetic geometric glossitis. *N Engl J Med* 1993;**329**:1859–60.
153. Guffond T, Dewilde A, Lobert PE, et al. Significance and clinical relevance of the detection of herpes simplex virus DNA by polymerase chain reaction in cerebrospinal fluid from patients with presumed encephalitis. *Clin Infect Dis* 1994;**18**:744–9.
154. Guinan ME, MacCalman J, Kern ER, et al. The course of untreated recurrent genital herpes simplex infection in 27 women. *N Engl J Med* 1983;**304**:759–63.
155. Gupta R, Hill EL, McClernon D, et al. Acyclovir sensitivity of sequential herpes simplex virus type 2 isolates from the genital mucosa of immunocompetent women. *J Infect Dis* 2005;**192**:1102–7.
156. Gupta R, Wald A, Krantz E, et al. Valacyclovir and acyclovir for suppression of shedding of herpes simplex virus in the genital tract. *J Infect Dis* 2004;**190**:1374–81.
157. Gutman LT, Wilfert CM, Eppes S. Herpes simplex virus infection in children: analysis of cerebrospinal fluid and progressive neurodevelopmental deterioration. *J Infect Dis* 1986;**154**:415–21.
158. Halperin SA, Shehab Z, Thacker D, et al. Absence of viremia in primary herpetic gingivostomatitis. *Pediatr Infect Dis* 1983;**2**:452–3.
159. Hamilton RL, Achim C, Grafe MR, et al. Herpes simplex virus brainstem encephalitis in an AIDS patient. *Clin Neuropathol* 1995;**14**:45–50.
160. Hammer SM, Buchman TG, D'Angelo LJ, et al. Temporal cluster of herpes simplex encephalitis: investigation by restriction endonuclease cleavage of viral DNA. *J Infect Dis* 1980;**141**:436–40.
161. Hammerburg O, Watts J, Chenesky M, et al. An outbreak of herpes simplex virus type 1 in an intensive care nursery. *Pediatr Infect Dis* 1983;**2**:290.
162. Hardy DA, Arvin AM, Yasukawa LL, et al. The successful identification of asymptomatic genital herpes simplex infection at delivery using the polymerase chain reaction. *J Infect Dis* 1990;**162**:1031–5.
163. Harel L, Smetana Z, Prais D, et al. Presence of viremia in patients with primary herpetic gingivostomatitis. *Clin Infect Dis* 2004;**39**:636–40.
164. Harger JB. Indications for antepartum HSV screening cultures. *Infect Surg* 1989;**8**:24.
165. Harger JB, Pazin G, Armstrong J, et al. Characteristics and management of pregnancy in women with genital herpes simplex virus infection. *Am J Obstet Gynecol* 1983;**145**:784.
166. Hargrave DR, Webb DW. Movement disorders in association with herpes simplex virus encephalitis in children: a review. *Dev Med Child Neurol* 1998;**40**:640–2.
167. Harper TW, Miller D , Schiffman JC, Davis JL. Polymerase chain reaction analysis of aqueous and vitreous specimens in the diagnosis of posterior segment infectious uveitis. *Am J Ophthalmol* 2009;**147**:140–7.
168. Hashido M, Kawana T. Herpes simplex virus–specific IgM, IgA and IgG subclass antibody responses in primary and nonprimary genital herpes patients. *Microbiol Immunol* 1997;**41**(5):415–20.
169. Hashido M, Lee FK, Inouye S. Detection of herpes simplex virus type-specific antibodies by an enzyme-linked immunosorbent assay based on glycoprotein G. *J Med Virol* 1997;**53**:319–23.
170. Hatherley L, Hayes K, Hennessy E, Jack I. Herpesvirus in an obstetric hospital, part I: herpetic eruptions. *Med J Aust* 1980;**2**:206.
171. Hatherley L, Hayes K, Jack I. Herpesvirus in an obstetric hospital, part II: asymptomatic virus excretion in staff members. *Med J Aust* 1980;**2**:273.
172. Herrod HG. Chronic mucocutaneous candidiasis in childhood and complications of non-*Candida* infection: a report of the pediatric immunodeficiency study group. *J Pediatr* 1990;**116**:377–82.
173. Hevron JE. Herpes simplex virus type 2 meningitis. *Obstet Gynecol* 1977;**49**:622–4.
174. Hobson A, Wald A, Wright N, et al. Evaluation of a quantitative competitive PCR assay for measuring herpes simplex virus DNA content in genital tract secretions. *J Clin Microbiol* 1997;**35**:548–52.
175. Hook EWI, Cannon RO, Nahmras AJ, et al. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. *J Infect Dis* 1992;**165**:251–5.
176. Howell MD, Wollenberg A, Gallo RL, et al. Cathelicidin deficiency predisposes to eczema herpeticum. *J Allergy Clin Immunol* 2006;**117**:836–41.
177. Hudson B, Powell C. Towards evidence-based medicine for pediatricians: does oral aciclovir improve clinical outcome in immunocompetent children with primary herpes simplex gingivostomatitis? *Arch Dis Child* 2009;**94**:165–7.
178. Huff JC, Weston WL. Recurrent erythema multiforme. *Medicine (Baltimore)* 1989;**68**:133–40.
179. Hull HF, Blumhagen JD, Benjamin D, et al. Herpes simplex viral pneumonitis in childhood. *J Pediatr* 1984;**104**:211–15.
180. Hursh DA, Wendt SF, Lee CF, et al. Detection of herpes simplex virus by using A549 cells in centrifugation culture with a rapid membrane enzyme immunoassay. *J Clin Microbiol* 1989;**27**:1695–6.
181. Ito Y, Ando Y, Kimura H, et al. Polymerase chain reaction–proved herpes simplex encephalitis in children. *Pediatr Infect Dis J* 1998;**17**:29–32.
182. James SH, Whitley RJ. Treatment of herpes simplex virus infections in pediatric patients: current status and future needs. *Clin Pharmacol Ther* 2010;**88**:720–4.
183. Jaworski MA, Moffatt MEK, Ahronheim GA. Disseminated herpes simplex associated with *H. influenzae* infection in a previously healthy child. *J Pediatr* 1980;**96**:426–9.
184. Jenson HB, Shapiro ED. Primary herpes simplex virus infection of a diaper rash. *Pediatr Infect Dis J* 1987;**6**:1136–8.
185. Johnson JR, Egaas S, Gleaves CA, et al. Hepatitis due to herpes simplex virus in marrow-transplant recipients. *Clin Infect Dis* 1992;**14**:38–45.
185a. Johansson AB, Rassart A, Blum D, et al. Lower-limb hypoplasia due to intra-uterine infection with herpes simplex virus type 2: possible confusion with intrauterine varicella-zoster syndrome. *Clin Infect Dis* 2004;**38**:e57–62.
186. Johnson RE, Nahmias AJ, Magder LS, et al. A seroepidemiologic survey of the prevalence of herpes simplex virus type 2 infection in the United States. *N Engl J Med* 1989;**321**:7–12.
187. Johnson RT, Mims CA. Pathogenesis of viral infections of the nervous system. *N Engl J Med* 1968;**278**:23–30.
188. Johnston C, Magaret A, Selke S, et al. Herpes simplex virus viremia during primary genital infection. *J Infect Dis* 2008;**198**:31–4.
189. Johnston SL, Siegel CS. Comparison of enzyme immunoassay, shell vial culture, and conventional cell culture for the rapid detection of herpes simplex virus. *Diagn Microbiol Infect Dis* 1990;**13**:241–4.
190. Jones TJ, Paul R. Disseminated acyclovir-resistant herpes simplex virus type 2 treated successfully with foscarnet. *J Infect Dis* 1995;**171**:508–9.
191. Kahlon J, Lakeman FD, Ackermann M, et al. Human antibody response to herpes simplex virus–specific polypeptides after

primary and recurrent infection. *J Clin Microbiol* 1986;**23**: 725–30.
192. Kampgen E, Bung G, Wank R. Association of herpes simplex virus–induced erythema multiforme with the human leukocyte antigen DQw3. *Arch Dermatol* 1988;**124**:1372–5.
193. Kao CH, Wang SJ, Mak SC, et al. Viral encephalitis in children: detection with technetium-99 mm HMPAO brain single-photon emission CT and its value in prediction of outcome. *AJNR Am J Neuroradiol* 1994;**15**:1369–73.
194. Kapiga SH, Sam NE, Bang H, et al. The role of herpes simplex virus type 2 and other genital infections in the acquisition of HIV-1 about high risk women in northern Tanzania. *J Infect Dis* 2007;**195**:1260–9.
195. Kaye E, Dooling E. Neonatal herpes simplex meningoencephalitis associated with fetal monitor scalp electrodes. *Neurology* 1981; **31**:1045.
196. Khlevner J, Beneri C, Morganstern JA. Wrestling and herpetic esophagitis. *Pediatr Infect Dis J* 2011;**30**:911–42.
197. Kim HN, Meier A, Huang ML, et al. Oral herpes simplex virus type 2 reactivation in HIV-positive and -negative men. *J Infect Dis* 2006;**194**:420–7.
197a. Kimberlin DW, Baley J. Committee on Infectious Diseases, and Committee on Fetus and Newborn: Guidance on management of asymptomatic neonates born to women with active genital herpes lesions. *Pediatrics* 2013:**131**;383–6.
198. Kimberlin DW, Jacobs RF, Weller S, et al. Pharmacokinetics and safety of extemporaneously compounded valacyclovir oral suspension in pediatric patients from 1 month through 11 years of age. *Clin Infect Dis* 2010;**15**:221–8.
199. Kimberlin DW, Lakeman FD, Arvin AM, et al. Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J Infect Dis* 1996;**174**:1162–7.
200. Kimberlin DW, Whitley R, Wan W, et al. Oral acyclovir suppression and neurodevelopment after neonatal herpes. *N Engl J Med* 2011;**365**:1284–92.
201. Kipps A, Becker W, Wainwright J, et al. Fatal disseminated primary herpes virus infection in children: epidemiology based on 93 non-neonatal cases. *S Afr Med J* 1967;**41**:6 47–51.
202. Klein NA, Mabie WC, Shaver DC, et al. Herpes simplex virus hepatitis in pregnancy: two patients successfully treated with acyclovir. *Gastroenterology* 1991;**100**:239–44.
203. Kline MW, Bohannon B, Kozinetz CA, et al. Characteristics of human immunodeficiency virus–associated mortality in pediatric patients with vertically transmitted infection. *Pediatr Infect Dis J* 1992;**11**:676–7.
204. Koelle DM, Benedetti J, Langenberg A, et al. Asymptomatic reactivation of herpes simplex virus in women after the first episode of genital herpes. *Ann Intern Med* 1992;**116**:433–7.
205. Koelle DM, Corey L. Recent progress in herpes simplex virus immunobiology and vaccine research. *Clin Microbiol Rev* 2003;**16**:96–113.
206. Koenig H, Rabinowitz SG, Day E, et al. Postinfectious encephalomyelitis after successful treatment of herpes simplex encephalitis with adenine arabinoside: ultrastructural observations. *N Engl J Med* 1979;**300**:1089–93.
207. Kohl S. Herpes simplex virus encephalitis in children. *Pediatr Clin North Am* 1988;**35**:465–83.
208. Kohl S, Adam E, Matson DO, et al. Kinetics of human antibody responses to primary genital herpes simplex virus infection. *Intervirology* 1982;**18**:164–8.
209. Kohl S, James AR. Herpes simplex virus encephalitis during childhood: the importance of brain biopsy diagnosis. *J Pediatr* 1985;**107**:212–15.
210. Koskiniemi M, Vaheri A. Effect of measles, mumps, rubella vaccination on patterns of encephalitis in children. *Lancet* 1989;**1**:31–4.
211. Koskiniemi M, Vaheri A, Taskinen E. Cerebrospinal fluid alterations in herpes simplex virus encephalitis. *Rev Infect Dis* 1984;**6**: 608–19.
212. Kost RG, Hill EL, Tigges M, et al. Brief report: recurrent acyclovir-resistant genital herpes in an immunocompetent patient. *N Engl J Med* 1993;**329**:1777–82.
213. Koutsky LA, Stevens CE, Holmes KK, et al. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. *N Engl J Med* 1992;**326**:1533–9.
214. Kriesel JD, Spruance SL, Prichard M, et al. Recurrent antiviral-resistant genital herpes in an immunocompetent patient. *J Infect Dis* 2005;**192**:156–61.
215. Kulhanjian JA, Soroush V, Au DS, et al. Identification of women at unsuspected risk for contracting primary herpes simplex virus type 2 infections during pregnancy. *N Engl J Med* 1992;**326**:916–20.
216. Kullnat MW, Morse RP. Choreoathetosis after herpes simplex encephalitis with basal ganglia involvement on MRI. *Pediatrics* 2008;**121**:e1003–7.
217. Kusne S, Schwartz M, Breinig MK, et al. Herpes simplex virus hepatitis after solid organ transplantation in adults. *J Infect Dis* 1991;**163**:1001–7.
218. Kuzushima K, Kimura H, Kino Y, et al. Clinical manifestations of primary herpes simplex virus type 1 infection in a closed community. *Pediatrics* 1991;**87**:152–8.
219. Kuzushima K, Kudo T, Kimura H, et al. Prophylactic oral acyclovir in outbreaks of primary herpes simplex virus type 1 infection in a closed community. *Pediatrics* 1992;**89**:379–83.
220. Lafferty WE, Coombs RW, Benedetti J, et al. Recurrences after oral and genital herpes simplex infection: influence of site of infection and viral type. *N Engl J Med* 1987;**316**:1444–9.
221. Lagew D Jr, Furlow T, Harper W, et al. Disseminated herpes simplex virus infection in pregnancy: Successful treatment with acyclovir. *JAMA* 1984;**252**:2058.
222. Lakeman FD, Whitley RJ. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. *J Infect Dis* 1995;**171**:857–63.
223. Lalezari JP, Drew HL, Glutzer E, et al. Treatment with intravenous (S)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl] cytosine of acyclovir-resistant mucocutaneous infection with herpes simplex virus in a patient with AIDS. *J Infect Dis* 1994;**170**:570–2.
224. Landry ML, Mayo DR, Hsiung GD. Comparison of guinea pig embryo cells, rabbit kidney cells, and human embryonic lung fibroblast cell strains for isolation of herpes simplex virus. *J Clin Microbiol* 1982;**15**:842–7.
225. Landry ML, Mullangi P, Nee P, Klein BR. Herpes simplex virus type 2 acute retinal necrosis 9 years after neonatal herpes. *J Pediatr* 2005;**146**:836–8.
226. Langenberg A, Benedetti J, Jenkins J, et al. Development of clinically recognizable genital lesions among women previously identified as having "asymptomatic" herpes simplex virus type 2 infection. *Ann Intern Med* 1989;**110**:882–7.
227. Langenberg AG, Burke RL, Adair SF, et al. A recombinant glycoprotein vaccine for herpes simplex type 2: safety and efficacy. *Ann Intern Med* 1995;**122**:889–98.
228. Langenberg AG, Corey L, Ashley RL, et al. A prospective study of new infections with herpes simplex virus type 1 and type 2. *N Engl J Med* 1999;**341**:1432–8.
229. Langlois M, Allard JP, Nuglar F, et al, A rapid and automated colorimetric assay for evaluating the sensitivity of herpes simplex strains to antiviral drugs. *J Biol Stand* 1986;**14**:201–11.
230. Langtry JA, Ostlere LS, Hawkins DA, et al. The difficulty in diagnosis of cutaneous herpes simplex virus infection in patients with AIDS. *Clin Exp Dermatol* 1994;**19**:224–6.
231. LaRocco MT. Evaluation of an enzyme-linked viral inducible system for the rapid detection of herpes simplex virus. *Eur J Clin Microbiol Infect Dis* 2000;**19**:233–5.
232. Lee FK, Coleman RM, Pereira L, et al, Detection of herpes simplex virus type 2-specific antibody with glycoprotein G. *J Clin Microbiol* 1985;**22**:641–4.
233. Lee SY, Pavan-Langston D. Role of acyclovir in the treatment of herpes simplex virus keratitis. *Int Ophthalmol Clin* 1994;**34**:9–18.
234. Lehrman SN, Douglas JM, Corey L, et al. Recurrent genital herpes and suppressive oral acyclovir therapy: relation between clinical outcome and in vitro drug sensitivity. *Ann Intern Med* 1986;**104**:786–90.
235. Lehtinen M, Runtala I, Teisala K, et al. Detection of herpes simplex virus in women with acute pelvic inflammatory disease. *J Infect Dis* 1985;**152**:78–82.

236. Leonard JR, Moran CJ, Cross DT 3rd, et al. MR imaging of herpes simplex type 1 encephalitis in infants and young children: a separate pattern of findings. *AJR Am J Roentgenol* 2000;**174**:1651–5.
237. Levin J, Weinberg A, Leary J, Sarisky R. Development of acyclovir resistant herpes simplex virus early during treatment of herpes neonatorum. *Pediatr Infect Dis J* 2001;**20**:1094–7.
238. Lewis P, Glaser CA. Encephalitis. *Pediatr Rev* 2005;**26**:353–63.
239. Liesegang TJ. Classification of herpes simplex virus keratitis and anterior uveitis. *Cornea* 1999;**18**:127–43.
240. Light IJ. Postnatal acquisition of herpes simplex by the newborn infant: a review of the literature. *Pediatrics* 1979;**63**:480.
241. Linnemann CC, Buchman TG, Light IJ, et al. Transmission of herpes simplex virus type 1 in a nursery for the newborn: identification of viral isolates by DNA "fingerprinting." *Lancet* 1978;**1**:964–6.
242. Linnemann CC, May DB, Schubert WK, et al. Fatal viral encephalitis in children with X-linked hypogammaglobulinemia. *Am J Dis Child* 1973;**126**:100–3.
243. Livorsi D, Anderson E, Qureshi S, et al. Brainstem encephalitis: an unusual presentation of herpes simplex virus infection. *J Neurol* 2010;**257**:1432–7.
244. Ljungman P, Ellis MN, Hackman RC, et al. Acyclovir-resistant herpes simplex virus causing pneumonia after marrow transplantation. *J Infect Dis* 1990;**162**:244–8.
245. Lokensgard JR, Cheeran MC, Hu S, et al. Glial cell responses to herpesvirus infections: role in defense and immunopathogenesis. *J Infect Dis* 2002;**186**(Suppl. 2):S171–9.
246. Long S, Pool T, Vodzak J, et al. Herpes simplex virus infection in young infants during 2 decades of empiric acyclovir therapy. *Pediatr Infect Dis J* 2011;**30**:556–61.
247. Luby JP, Gnann JW, Alexander WJ, et al. A collaborative study of patient-initiated treatment of recurrent genital herpes with topical acyclovir or placebo. *J Infect Dis* 1984;**150**:1–6.
248. Lumbreras C, Fernandez I, Velosa J, et al. Infectious complications following pancreatic transplantation: incidence, microbiological and clinical characteristics, and outcome. *Clin Infect Dis* 1995;**20**:514–20.
249. Mark K, Kim H, Wald A, et al. Targeted prenatal HSV testing: can we identify women at risk for transmission to the neonate? *Am J Obstet Gynecol* 2006;**194**:408.
250. Marquez L, Levy M, Munoz F, et al. A report of three cases and review of intrauterine herpes simplex virus infection. *Pediatr Infect Dis J* 2011;**30**:153–7.
251. Martin ET, Krantz E, Gottlieb SL, et al. A pooled analysis of the effect of condoms in preventing HSV-2 acquisition. *Arch Intern Med* 2009;**169**:1233–40.
252. Masci S, DeSimone C, Famularo G, et al. Intravenous immunoglobulins suppress the recurrences of genital herpes simplex virus: a clinical and immunological study. *Immunopharmacol Immunotoxicol* 1995;**17**:33–47.
253. McGill A, Bavaro M, You W. Postpartum herpes simplex virus endometritis and disseminated infection in both mother and neonate. *Obstet Gynecol* 2012;**120**:471–3.
254. McGoogan K, Haafiz A, Gonzalez-Peralta R. Herpes simplex virus hepatitis in infants: clinical outcomes and correlates of disease severity. *J Pediatr* 2011;**159**:608–11.
255. McMillan JA, Weiner LB, Higgins AM, et al. Pharyngitis associated with herpes simplex virus in college students. *Pediatr Infect Dis J* 1993;**12**:280–4.
256. Reference deleted in proofs.
257. Mertz GJ, Ashley R, Burke RL, et al. Double-blind, placebo-controlled trial of a herpes simplex virus type 2 glycoprotein vaccine in persons at high risk for genital herpes infection. *J Infect Dis* 1990;**116**:653–60.
258. Mertz GJ, Benedetti J, Ashley R, et al. Risk factors for the sexual transmission of genital herpes. *Ann Intern Med* 1992;**116**: 197–202.
259. Mindel A, Adler MW, Sutherland S, et al. Intravenous acyclovir treatment for primary genital herpes. *Lancet* 1982;**1**:697–700.
260. Moldovan B, Mentha G, Majno P, et al. Demographics and outcomes of severe herpes simplex virus hepatitis: a registry-based study. *J Hepatol* 2011;**55**:1222–6.
261. Molin L. Oral acyclovir prevents herpes simplex virus–associated erythema multiforme. *Br J Dermatol* 1987;**116**:109–11.
262. Moore DE, Ashley RL, Zarutskie PW, et al. Transmission of genital herpes by donor insemination. *JAMA* 1989;**261**:3441–3.
263. Moore DJ, Davidson GP, Binns GF. Herpes simplex oesophagitis in young children. *Med J Aust* 1986;**144**:716–17.
264. Morawetz RB, Whitley RJ, Murphy DM. Experience with brain biopsy for suspected herpes encephalitis: a review of forty consecutive cases. *Neurosurgery* 1983;**12**:654–7.
265. Moseley RD, Corey L, Benjamin D, et al. Comparison of viral isolation, direct immunofluorescence, and indirect immunoperoxidase techniques for detection of genital herpes simplex virus infection. *J Clin Microbiol* 1981;**13**:913–18.
266. Nadal D, Leverger G, Sokal EM, et al. An investigation of the steady-state pharmacokinetics of oral valacyclovir in immunocompromised children. *J Infect Dis* 2002;**186**(Suppl. 1):S123–30.
267. Nagamori T, Koyano S, Asai Y, et al. Sequential changes in pathophysiology of systemic inflammatory response in a disseminated neonatal herpes simplex virus (HSV) infection. *J Clin Virol* 2012;**53**(3):265–7.
268. Nahass GT, Goldstein BA, Zhu WY, et al. Comparison of Tzanck smear, viral culture, and DNA diagnostic methods in detection of herpes simplex and varicella-zoster infection. *JAMA* 1992;**268**: 2541–4.
269. Nahmias A, Josey W, Nib Z, et al. Perinatal risk associated with maternal genital herpes simplex virus infection. *Am J Obstet Gynecol* 1971;**110**:825.
270. Nahmias AJ, Whitley RJ, Visintine AN, et al. Herpes simplex virus encephalitis: laboratory evaluations and their diagnostic significance. *J Infect Dis* 1982;**145**:829–36.
271. Naraqi S, Jackson GG, Jonasson O, et al. Prospective study of prevalence, incidence, and source of herpesvirus infections in patients with renal allografts. *J Infect Dis* 1977;**136**:531–40.
272. Nash G. Necrotizing tracheobronchitis and bronchopneumonia consistent with herpetic infection. *Hum Pathol* 1972;**3**:283–91.
273. Nash G, Ross JS. Herpetic esophagitis, a common cause of esophageal ulceration. *Hum Pathol* 1974;**5**:339–45.
274. Neiman PE, Reeves W, Ray G, et al. A prospective analysis of interstitial pneumonia and opportunistic viral infection among recipients of allogeneic bone marrow grafts. *J Infect Dis* 1977;**136**:754–67.
275. Nerurkar LS, West F, May M, et al. Survival of herpes simplex virus in water specimens collected from hot tubs in spa facilities and on plastic surfaces. *JAMA* 1983;**250**:3081–3.
276. Ng A, Reagan J, Yen S. Herpes genitalis. *Obstet Gynecol* 1970;**36**:645.
277. Nilsen A, Aasen T, Halsos T, et al. Efficacy of oral acyclovir in the treatment of initial and recurrent genital herper. *Lancet* 1982;**2**:571–3.
278. Ogburn JR, Hoffpauir JT, Cole E, et al. Evaluation of new transport medium for detection of herpes simplex virus by culture and direct enzyme-linked immunosorbent assay. *J Clin Microbiol* 1994;**32**:3082–4.
279. Ono F, Sharma BK, Smith CC, et al. $CD34^+$ cells in the peripheral blood transport herpes simplex virus DNA fragments to the skin of patients with erythema multiforme (HAEM). *J Invest Dermatol* 2005;**124**:1215–24.
280. Orton PW, Huff JC, Tonnesen MG, et al. Detection of a herpes simplex viral antigen in skin lesions of erythema multiforme. *Ann Intern Med* 1984;**101**:48–50.
281. Overall J. Genital and perinatal herpes simplex virus infections. In: de la Maza L, Peterson E, editors. *Medical virology*. IV. Hillsdale, NJ: L. Erlbaum Associates; 1985. p. 253–304.
282. Overall J. Empiric therapy with acyclovir for suspected neonatal herpes simplex infections. *Pediatric Infect Dis J* 1989;**8**:808.
283. Overall J. Herpes simplex virus infection of the fetus and newborn. *Pediatr Ann* 1994;**23**:131.
284. Overall J, Whitley R, Yeager A, et al. Prophylactic or anticipatory antiviral therapy for newborns exposed to herpes simplex infection. *Pediatr Infect Dis* 1984;**3**:193.
285. Owensby LC, Stammer JL. Esophagitis associated with herpes simplex infection in an immunocompetent host. *Gastroenterology* 1978;**74**:1305–6.
286. Parrott RH, Wolf SI, Nudelman J, et al. Clinical and laboratory differentiation between herpangina and infectious (herpetic) gingivostomatitis. *Pediatrics* 1954;**14**:122–9.
287. Parvey L, Chien L. Neonatal herpes simplex virus infection introduced by fetal monitor scalp electrodes. *Pediatrics* 1980;**65**:1150.
288. Paul C, Cork M, Rossi AB, et al. Safety and tolerability of 1% pimecrolimus cream among infants: experience with 1133 patients treated for up to 2 years. *Pediatrics* 2006;**117**:e118–28.

289. Pavan-Langston D, Brockhurst RJ. Herpes simplex panuveitis: a clinical report. *Arch Ophthalmol* 1969;**81**:783–7.
290. Pazin GJ, Armstrong JA, Lam MT, et al. Prevention of reactivated herpes simplex infection by human leukocyte interferon after operation on the trigeminal root. *N Engl J Med* 1979;**301**: 225–30.
291. Peacock JE, Kaplowitz LG, Sparling PF, et al. Intravenous acyclovir therapy of first episodes of genital herpes: a multicenter double-blind, placebo controlled trial. *Am J Med* 1988;**85**: 301–6.
292. Peacock J Jr, Sarrubi F. Disseminated herpes simplex virus infection during pregnancy. *Obstet Gynecol* 1983;**6**(Suppl)13:13S–8S.
293. Pelligra G, Lynch N, Miller S, et al. Brainstem involvement in neonatal herpes simplex virus type 2 encephalitis. *Pediatrics* 2007;**120**(2):e442–6.
294. Perng GC, Dunkel EC, Geary PA, et al. The latency-associated gene of herpes simplex virus type 1 (HSV-1) is required for efficient in vivo spontaneous reactivation of HSV-1 from latency. *J Virol* 1994;**68**:8045–55.
295. Peterson EM, Hughes BL, Aarnaes SL, et al. Comparison of primary rabbit kidney and MRC-5 cells and two stain procedures for herpes simplex virus detection by a shell vial centrifugation method. *J Clin Microbiol* 1988;**26**:222–4.
296. Pien FD, Smith TF, Anderson CF, et al. Herpesviruses in renal transplant patients. *Transplantation* 1973;**16**:489–95.
297. Pinninti SG, Angara R, Feja KN, et al. Neonatal herpes disease following maternal antenatal antiviral suppressive therapy: a multicenter case series. *J Pediatr* 2012;**161**:134-8.e1–3.
298. Piret J, Boivin G. Resistance of herpes simplex viruses to nucleoside analogues: mechanisms, prevalence, and management. *Antimicrob Agents Chemother* 2011;**55**:459–72.
299. Poirier RH. Herpetic ocular infections of childhood. *Arch Ophthalmol* 1980;**98**:704–6.
300. Posavad CM, Wald A, Hosken N, et al. T cell immunity to herpes simplex viruses in seronegative subjects: silent infection or acquired immunity? *J Immunol* 2003;**170**:4380–8.
301. Prober C, Corey L, Brow Z, et al. The management of pregnancies complicated by genital infection with herpes simplex virus. *Clin Infect Dis* 1992;**15**:1031.
302. Prober CG, Enzmann DR. Early diagnosis and management of herpes simplex encephalitis. *Pediatr Infect Dis J* 1996;**15**: 387–8.
303. Puchhammer-Stoeckl E, Heinz FX, Kundi M, et al. Evaluation of the polymerase chain reaction for diagnosis of herpes simplex virus encephalitis. *J Clin Microbiol* 1993;**31**:146–8.
304. Quinn TC, Corey L, Chaffee RG, et al. The etiology of anorectal infections in homosexual men. *Am J Med* 1981;**71**:395–406.
305. Rabella N, Otegui M, Labeaga R, et al. Antiviral susceptibility of herpes simplex viruses and its clinical correlates: a single center's experience. *Clin Infect Dis* 2002;**34**:1055–60.
306. Ramos-Casals M, Cuadrado MJ, Alba P, et al. Acute viral infections in patients with systemic lupus erythematosus: description of 23 cases and review of the literature. *Medicine (Baltimore)* 2008;**87**:311–18.
307. Ramsey PG, Fife KH, Hackman RC, et al. Herpes simplex virus pneumonia: clinical, virologic, and pathologic features in 20 patients. *Ann Intern Med* 1982;**97**:813–20.
308. Rantala H, Uhari M. Occurrence of childhood encephalitis: a population-based study. *Pediatr Infect Dis J* 1989;**8**:426–30.
309. Reeves WC, Corey L, Adams HG, et al. Risk for recurrence after first episodes of genital herpes: relation to HSV type antibody response. *N Engl J Med* 1981;**305**:315–19.
310. Reichman RC, Badger GJ, Guinan ME, et al. Topically administered acyclovir in the treatment of recurrent herpes simplex genitalis: a controlled trial. *J Infect Dis* 1983;**147**:336–40.
311. Revello MGF, Baldanti A, Sarasini D, et al. Quantitation of herpes simplex virus DNA in cerebrospinal fluid of patients with herpes simplex encephalitis by the polymerase chain reaction. *Clin Diagn Virol* 1997;**7**:183–91.
312. Reynolds SJ, Risbud AR, Shepherd ME, et al. Recent herpes simplex virus type 2 infection and the risk for human immunodeficiency virus type 1 acquisition in India. *J Infect Dis* 2003;**187**:1513–21.
313. Riediger C, Sauer P, Matevossian E, et al. Herpes simplex virus sepsis and acute liver failure. *Clin Transplant* 2009;**23**(Suppl 21):37–41.
314. Roberts CM, Pfister JR, Spear SJ. Increasing proportion of herpes simplex virus type 1 as a cause of genital herpes infection in college students. *Sex Transm Dis* 2003;**30**:797–800.
315. Rock DL, Fraser NW. Detection of HSV-1 genome in central nervous system of latently infected mice. *Nature* 1983;**302**:523.
316. Roizman B, Buchman T. The molecular epidemiology of herpes simplex virus. *Hosp Pract* 1979;**14**:95.
317. Roizman B, Knipe DM, Whitley. RJ. Herpes simplex viruses. In: Knipe D, Howley P, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 2502.
318. Rompalo AM, Mertz GJ, Davis LG. Oral acyclovir for treatment of first episode herpes simplex virus proctitis. *JAMA* 1988;**19**:2879–81.
319. Rosato FE, Rosato EF, Plotkin SA. Herpetic paronychia: an occupational hazard of medical personnel. *N Engl J Med* 1970;**283**:804–5.
320. Rouse BT, Kaistha SD. A tale of 2 alpha-herpesviruses: lessons for vaccinologists. *Clin Infect Dis* 2006;**42**:810–17.
321. Rowley AH, Whitley RJ, Lakeman FO, et al. Rapid detection of herpes-simplex virus DNA in cerebrospinal fluid of patients with herpes simplex encephalitis. *Lancet* 1990;**335**:440–1.
322. Sacks SL. Famciclovir suppression of asymptomatic and symptomatic recurrent anogenital herpes simplex virus shedding in women: a randomized, double-blind, double-dummy, placebo-controlled, parallel-group, single-center trial. *J Infect Dis* 2004;**189**:1341–7.
323. Sacks SL, Aoki FY, Diaz-Mitoma F, et al. Patient-initiated, twice-daily oral famciclovir for early recurrent genital herpes: a randomized, double-blind multicenter trial. Canadian Famciclovir Study Group. *JAMA* 1996;**276**:44–9.
324. Sacks SL, Griffiths PD, Corey L, et al. HSV shedding. *Antiviral Res* 2004;**63**(Suppl. 1):S19–26.
325. Sacks SL, Shafran SD, Diaz-Mitoma F, et al. A multicenter phase I/II dose escalation study of single-dose cidofovir gel for treatment of recurrent genital herpes. *Antimicrob Agents Chemother* 1998;**42**:2996–9.
326. Safrin S, Crumpaker C, Chatis P, et al. A controlled trial comparing foscarnet with vidarabine for acyclovir-resistant, mucocutaneous herpes simplex in the acquired immunodeficiency syndrome. *N Engl J Med* 1991;**325**:551–5.
327. Safrin S, Elbeik T, Phan L, et al. Correlation between response to acyclovir and foscarnet therapy and in vitro susceptibility results from isolates of herpes simplex virus from human immunodeficiency virus–infected patients. *Antimicrob Agents Chemother* 1994;**38**:1246–50.
328. Safrin S, Kemmerly S, Plotkin B, et al. Foscarnet-resistant herpes simplex virus in patients with AIDS. *J Infect Dis* 1994;**169**: 193–6.
329. Safrin S, Shaw H, Bolan G, et al. Comparison of virus culture and the polymerase chain reaction for diagnosis of mucocutaneous herpes simplex virus infection. *Sex Transm Dis* 1997;**24**: 176–80.
330. Saijo M, Suzutani T, Murono K, et al. Recurrent aciclovir-resistant herpes simplex in a child with Wiskott-Aldrich syndrome. *Br J Dermatol* 1998;**139**:311–14.
331. Saijo M, Yasuda Y, Yabe H, et al. Bone marrow transplantation in a child with Wiskott-Aldrich syndrome latently infected with acyclovir-resistant (ACVr) herpes simplex virus type 1: emergence of foscarnet-resistant virus originating from the ACVr virus. *J Med Virol* 2002;**68**:99–104.
332. Salmon VC, Stanberry LR, Overall JC Jr. More rapid isolation of herpes simplex virus in a continuous line of mink lung cells than in Vero or human fibroblast cells. *Diagn Microbiol Infect Dis* 1984;**2**:317–24.
333. Salvini F, Carminati G, Pinzani R, et al. Chronic ulcerative herpes simplex virus infection in HIV-infected children. *AIDS Patient Care STDS* 1997;**11**:421–8.
334. Sancho-Shimizu V, Perez do Diego R, Lorenzo L, et al. Herpes simplex encephalitis in children with autosomal recessive and dominant TRIF deficiency. *J Clin Invest* 2011;**121**:4889–902.
335. Sands M, Brown R. Herpes simplex lymphangitis: two cases and a review of the literature. *Arch Intern Med* 1988;**148**:2066–7.
336. Saral R, Ambinder RF, Burns WH, et al. Acyclovir prophylaxis against herpes simplex virus infection in patients with leukemia: a randomized, double-blind, placebo-controlled study. *Ann Intern Med* 1983;**99**:773–6.

337. Sawyer J, Ellner J, Ransohoff DF. To biopsy or not to biopsy in suspected herpes simplex encephalitis: a quantitative analysis. *Med Decis Making* 1988;**8**:95–101.
338. Schacker T, Zeh J, Hu H, et al. Changes in plasma human immunodeficiency virus type 1 RNA associated with herpes simplex virus reactivation and suppression. *J Infect Dis* 2002;**186**: 1718–25.
339. Schauseil-Zipf U, Harden A, Hoare RD, et al. Early diagnosis of herpes simplex encephalitis in childhood: clinical, neurophysiological and neuroradiological studies. *Eur J Pediatr* 1982;**138**:154–61.
340. Schleiss MR, Fong W. Primary palmar herpes simplex virus 1 infection in a ten-year-old girl. *Pediatr Infect Dis J* 1992;**11**:338–9.
341. Schlesinger Y, Buller RS, Brunstrom JE, et al. Expanded spectrum of herpes simplex encephalitis in childhood. *J Pediatr* 1995;**126**:234–41.
342. Schlesinger Y, Storch GA. Herpes simplex meningitis in infancy. *Pediatr Infect Dis J* 1994;**13**:141–4.
343. Schlesinger Y, Tebas P, Gaudreault-Keener M, et al. Herpes simplex virus type 2 meningitis in the absence of genital lesions: improved recognition with use of the polymerase chain reaction. *Clin Infect Dis* 1995;**20**:842–8.
344. Schmitt DL, Johnson DW, Henderson FW. Herpes simplex type 1 infections in group day care. *Pediatr Infect Dis J* 1991;**10**:729–34.
345. Schmidt NJ, Dennis J, Devlin V, et al. Comparison of direct immunofluorescence and direct immunoperoxidase procedures for detection of herpes simplex virus antigen in lesion specimens. *J Clin Microbiol* 1983;**18**:445–8.
346. Schmidt OW, Fife KH, Corey L. Reinfection is an uncommon occurrence in patients with symptomatic recurrent genital herpes. *J Infect Dis* 1984;**149**:645–6.
347. Schofield JK, Tatnall FM, Leigh IM. Recurrent erythema multiforme: clinical features and treatment in a large series of patients. *Br J Dermatol* 1993;**128**:542–5.
348. Schroth G, Gawehn J, Thron A, et al. Early diagnosis of herpes simplex encephalitis by MRI. *Neurology* 1987;**37**:179–83.
349. Schwab IR. Oral acyclovir in the management of herpes simplex ocular infection. *Ophthalmology* 1988;**95**:423–30.
350. Schwartz GS, Holland EJ. Oral acyclovir for the management of herpes simplex virus keratitis in children. *Ophthalmology* 2000;**107**:278–82.
351. Selling B, Kibrick S. An outbreak of herpes simplex among wrestlers (herpes gladiatorum). *N Engl J Med* 1964;**270**: 979–82.
352. Shanley CJ, Braun DK, Brown K, et al. Fulminant hepatic failure secondary to herpes simplex virus hepatitis: successful outcome after orthotopic liver transplantation. *Transplantation* 1995;**59**: 145–9.
353. Shute P, Jeffries DJ, Maddocks AC. Scrumpox caused by herpes simplex virus. *BMJ* 1979;**2**:1629.
354. Sibrack CD, Gutman LT, Wilfert CM, et al. Pathogenicity of acyclovir-resistant herpes simplex virus type 1 from an immunodeficient child. *J Infect Dis* 1982;**146**:673–82.
355. Skoldenberg B. Herpes simplex encephalitis. *Scand J Infect Dis Suppl* 1996;**100**:8–13.
356. Skoldenberg B, Alestig K, Burman L, et al. Acyclovir versus vidarabine in herpes encephalitis: randomized, multicentre study in consecutive Swedish patients. *Lancet* 1984;**2**:707–11.
357. Smith JS, Robinson NJ. Age-specific prevalence of infection with herpes simplex virus types 2 and 1: a global review. *J Infect Dis* 2002;**186**(Suppl 1):S3–28.
358. Snoeck R, Andrei G, Gerard M, et al. Successful treatment of progressive mucocutaneous infection due to acyclovir- and foscarnet-resistant herpes simplex virus with (S)-1-(3-hydroxy-2-phosphonylmethyloxypropyl) cytosine (HPMPC). *Clin Infect Dis* 1994;**18**:570–8.
359. Solomon AR, Rasmussen JE, Varani J, et al. The Tzanck smear in the diagnosis of cutaneous herpes simplex. *JAMA* 1984;**251**(5): 633–5.
360. Spicher VM, Bouvier P, Schlegel-Haueter SE, et al. Epidemiology of herpes simplex virus in children by detection of specific antibodies in saliva. *Pediatr Infect Dis J* 2001;**20**:265–72.
361. Spruance SL. Pathogenesis of herpes simplex labialis: excretion of virus in the oral cavity. *J Clin Microbiol* 1984;**19**:675–9.
362. Spruance SL, Bodsworth N, Resnick H, et al. Single-dose, patient-initiated famciclovir: a randomized, double-blind, placebo-controlled trial for episodic treatment of herpes labialis. *J Am Acad Dermatol* 2006;**55**:47–53.
363. Spruance SL, Freeman DJ, Stewart JCB. The natural history of ultraviolet radiation–induced herpes simplex labialis and response to therapy with peroral and topical formulations of acyclovir. *J Infect Dis* 1991;**163**:728–34.
364. Spruance SL, Jones TM, Blatter MM, et al. High-dose, short-duration, early valacyclovir therapy for episodic treatment of cold sores: results of two randomized, placebo-controlled, multicenter studies. *Antimicrob Agents Chemother* 2003;**47**:1072–80.
365. Spruance SL, Hamill ML, Hoge WS, et al. Acyclovir prevents reactivation of herpes simplex labialis in skiers. *JAMA* 1988;**260**:1597–9.
366. Spruance SL, Overall JC, Kern ER, et al. The natural history of recurrent herpes simplex labialis: implication for antiviral therapy. *N Engl J Med* 1977;**297**:69–75.
367. Spruance SL, Schnipper LE, Overall JC Jr, et al. Treatment of herpes simplex labialis with topical acyclovir in polyethylene glycol. *J Infect Dis* 1982;**146**:85–90.
368. Stamm WE, Handsfield HH, Rompalo AM, et al. The association between genital ulcer disease and acquisition of HIV infection in homosexual men. *J A M A* 1988;**260**:1429–33.
369. Stanberry LR. Clinical trials of prophylactic and therapeutic herpes simplex virus vaccines. *Herpes* 2004;**11**(Suppl 3):161A–9A.
370. Stanberry LR, Floyd-Reising SA, Connelly BL, et al. Herpes simplex viremia: report of eight pediatric cases and review of the literature. *Clin Infect Dis* 1994;**18**:401–7.
371. Stanberry LR, Spruance SL, Cunningham AL, et al. Glycoprotein-D-adjuvant vaccine to prevent genital herpes. *N Engl J Med* 2002;**347**:1652–61.
372. Stevens JG, Cook ML. Latent herpes simplex virus in spinal ganglia of mice. *Science* 1971;**173**:843.
373. St. Geme JW, Prince JT, Burke BA, et al. Impaired cellular resistance to herpes-simplex virus in Wiskott-Aldrich syndrome. *N Engl J Med* 1965;**273**:229–34.
374. Straus SE, Corey L, Burke RL, et al. Placebo-controlled trial of vaccination with recombinant glycoprotein D of herpes simplex virus type 2 for immunotherapy of genital herpes. *Lancet* 1994;**343**:1460–3.
375. Straus SE, Croen KD, Sawyer MH, et al. Acyclovir suppression of frequently recurring genital herpes: efficacy and diminished need during successive years of treatment. *J A M A* 1988;**260**:2227–30.
376. Straus SE, Seidlin M, Takiff HE, et al. Effect of oral acyclovir treatment on symptomatic and asymptomatic virus shedding in recurrent genital herpes. *Sex Transm Dis* 1989;**16**:107–13.
377. Straus SE, Smith HA, Brickman C, et al. Acyclovir for chronic mucocutaneous herpes simplex virus infection in immunosuppressed patients. *Ann Intern Med* 1982;**96**:270–7.
378. Straus SE, Takiff HE, Seidlin M, et al. Suppression of frequently recurring genital herpes: a placebo-controlled double-blind trial of oral acyclovir. *N Engl J Med* 1984;**310**:1545–50.
379. Struyf F, Posavad CM, Keyaerts E, et al. Search for polymorphisms in the genes for herpesvirus entry mediator, nectin-1, and nectin-2 in immune seronegative individuals. *J Infect Dis* 2002;**185**:36–44.
380. Sucato G, Celum C, Dithmer D, et al. Demographic rather than behavioral risk factors predict herpes simplex virus type 2 infection in sexually active adolescents. *Pediatr Infect Dis J* 2001;**20**:422–6.
381. Sullender WM, Arvin AM, Diaz PS, et al. Pharmacokinetics of acyclovir suspension in infants and children. *Antimicrob Agents Chemother* 1987;**31**:1722–6.
382. Sullender WM, Yasukawa LL, Schwartz M, et al. Type-specific antibodies to herpes simplex virus type 2 (HSV-2) glycoprotein G in pregnant women, infants exposed to maternal HSV 2 infections at delivery, and infants with neonatal herpes. *J Infect Dis* 1988;**157**:164–71.
383. Sullivan-Bolyai J, Fife K, Jacobs R, et al. Disseminated neonatal herpes simplex virus type 1 from a maternal breast lesion. *Pediatrics* 1983;**71**:455.
384. Sullivan-Bolyai J, Hull H, Wilson C, et al. Neonatal herpes simplex virus infection in King County, Washington: Increasing incidence and epidemiologic correlates. *JAMA* 1983;**250**:3059.

385. Swierkosz EM, Hodinka RL, Moore BM. *Antiviral susceptibility testing: herpes simplex virus by plaque reduction assay*. Approved standard, vol. 24. Wayne, PA: Clinical and Laboratory Standards Institute; 2004.
386. Tang IYS, Maddax MS, Veremis SA, et al. Low-dose oral acyclovir for prevention of herpes simplex virus infection during OKT3 therapy. *Transplant Proc* 1989;**21**:1758–60.
387. Tatnall FM, Schofield JK, Leigh IM. A double-blind, placebo-controlled trial of continuous acyclovir therapy in recurrent erythema multiforme. *Br J Dermatol* 1995;**132**:267–70.
388. Taxy JB, Tillaw I, Goldman PM. Herpes simplex lymphadenitis. *Arch Pathol Lab Med* 1985;**109**:1043–4.
389. Tedder DG, Ashley R, Tyler KL, et al. Herpes simplex virus infection as a cause of benign recurrent lymphocytic meningitis. *Ann Intern Med* 1994;**121**:334–8.
390. Tejani N, Lei S, Kaplan M. Subclinical herpes simplex genitalis infections in the perinatal period. *Am J Obstet Gynecol* 1979;**135**:547.
391. Templeton AC. Generalized herpes simplex in malnourished children. *J Clin Pathol* 1970;**23**:24–30.
392. Terni M, Caccialanza P, Cassai E, et al. Aseptic meningitis in association with herpes progenitalis. *N Engl J Med* 1971;**285**:503–5.
393. Thi TN, Deback C, Malet I, et al. Rapid determination of antiviral drug susceptibility of herpes simplex virus types 1 and 2 by real time PCR. *Antiviral Res* 2006;**69**:152–7.
394. Thompson C, Whitley R. Neonatal herpes simplex virus infections: Where are we now? *Adv Exp Med Biol* 2011;**697**:221–30.
395. Tobian AA, Serwadda D, Quinn TC, et al. Male circumcision for the prevention of HSV-2 and HPV infections and syphilis. *N Engl J Med* 2009;**360**:1298–309.
396. Todd J, Grosskurth H, Changalucha J, et al. Risk factors influencing HIV infection incidence in a rural African population: a nested case-control study. *J Infect Dis* 2006;**193**:458–66.
397. Trachtman H, Weiss RA, Spigland I, et al. Clinical manifestations of herpesvirus infections in pediatric renal transplant recipients. *Pediatr Infect Dis J* 1985;**4**:480–6.
398. Tran TH, Stanescu D, Caspers-Velu L, et al. Clinical characteristics of acute HSV-2 retinal necrosis. *Am J Ophthalmol* 2004;**137**:872–9.
399. Troendle-Atkins J, Demmler GJ, Buffone GJ. Rapid diagnosis of herpes simplex virus encephalitis by using the polymerase chain reaction. *J Pediatr* 1993;**123**:376–80.
400. Tse P, Aarnaes SL, de la Meza LM, Peterson EM. Detection of herpes simplex virus by 8 h in shell vial cultures with primary rabbit kidney cells. *J Clin Microbiol* 1989;**27**:199–200.
401. Turner BJ, Denison M, Eppes SC, et al. Survival experience of 789 children with the acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 1993;**12**:310–20.
402. Turner R, Shehab Z, Osborne K, et al. Shedding and survival of herpes simplex virus from "fever blisters." *Pediatrics* 1982;**70**: 547–9.
403. Tyring SK, Douglas JM Jr, Corey L, et al. A randomized, placebo-controlled comparison of oral valacyclovir and acyclovir in immunocompetent patients with recurrent genital herpes infections. The Valaciclovir International Study Group. *Arch Dermatol* 1998;**134**:185–91.
404. Uren EC, Johnson PD, Montanaro J, et al. Herpes simplex virus encephalitis in pediatrics: diagnosis by detecting antibodies and DNA in cerebrospinal fluid. *Pediatr Infect Dis J* 1993;**12**:1001–6.
405. VanDyke R, Spector S. Transmission of herpes simplex virus type 1 to a newborn infant during endotracheal suctioning for meconium aspiration. *Pediatr Infect Dis* 1984;**3**:153.
406. Verano L, Michalski FJ. Comparison of a direct antigen enzyme immunoassay, Herpchek, with cell culture for detection of herpes simplex virus from clinical specimens. *J Clin Microbiol* 1995;**33**:1378–9.
407. Verma A, Dhawan A, Zuckerman M, et al. Neonatal herpes simplex virus infection presenting as acute liver failure: prevalent role of herpes simplex virus type 1. *J Pediatr Gastroenterol Nutr* 2006;**42**:282–6.
408. Vital C, Monlun E, Vital A, et al. Concurrent herpes simplex virus type 1 necrotizing encephalitis, cytomegalovirus ventriculoencephalitis and cerebral lymphoma in an AIDS patient. *Acta Neuropathol* 1995;**89**:105–8.
409. Vontver L, Hickok D, Brown Z, et al. Recurrent genital herpes simplex virus infection in pregnancy: infant outcome and frequency of asymptomatic recurrences. *Am J Obstet Gynecol* 1982;**143**:75.
410. Vossough A, Zimmerman R, Vilaniuk L, et al. Imaging findings of neonatal herpes simplex virus type 2 encephalitis. *Neuroradiology* 2008;**50**:355–66.
411. Wade JC, Day LM, Crowley JJ, et al. Recurrent infection with herpes simplex virus after marrow transplantation: role of the specific immune response and acyclovir treatment. *J Infect Dis* 1984;**149**:750–6.
412. Wade JC, Meyers JD. Neurologic symptoms associated with parenteral acyclovir treatment after marrow transplantation. *Ann Intern Med* 1983;**98**:921–5.
413. Wade JC, Newton B, McLaren C, et al. Intravenous acyclovir to treat mucocutaneous herpes simplex virus infection after marrow transplantation: a double-blind trial. *Ann Intern Med* 1982;**96**:265–9.
414. Wald A, Ashley-Morrow R. Serological testing for herpes simplex virus (HSV)-1 and HSV-2 infection. *Clin Infect Dis* 2002;**35**(Suppl. 2):S173–82.
415. Wald A, Krantz E, Selke S, et al. Knowledge of partners' genital herpes protects against herpes simplex virus type 2 acquisition. *J Infect Dis* 2006;**194**:42–52.
416. Wald A, Langenberg AG, Krantz E, et al. The relationship between condom use and herpes simplex virus acquisition. *Ann Intern Med* 2005;**143**:707–13.
417. Wald A, Link K. Risk for human immunodeficiency virus infection in herpes simplex virus type 2–seropositive persons: a meta-analysis. *J Infect Dis* 2002;**185**:45–52.
418. Wald A, Selke S, Warren T, et al. Comparative efficacy of famciclovir and valacyclovir for suppression of recurrent genital herpes and viral shedding. *Sex Transm Dis* 2006;**33**:529–33.
419. Wald A, Zeh J, Barnum G, et al. Suppression of subclinical shedding of herpes simplex virus type 2 with acyclovir. *Ann Intern Med* 1996;**124**:8–15.
420. Wald A, Zeh J, Selke S, et al. Reactivation of genital herpes simplex virus type 2 infection in asymptomatic seropositive persons. *N Engl J Med* 2000;**342**:844–50.
421. Wang E, Schnall B, Rotschild T, et al. Unilateral keratitis following death of a twin as the presenting sign of herpetic infection in a neonate. *J AAPOS* 2011;**15**:489–90.
422. Wang HS, Kuo MF, Huang SC, et al. Choreoathetosis as an initial sign of relapsing of herpes simplex encephalitis. *Pediatr Neurol* 1994;**11**:341–5.
423. Ward KN, Ohrling A, Bryant NJ, et al. Herpes simplex serious neurological disease in young children: incidence and long-term outcome. *Arch Dis Child* 2012;**97**:162–5.
424. Wasserman R, August CS, Plotkin SA. Viral infections in pediatric bone marrow transplant patients. *Pediatr Infect Dis J* 1988;**7**:109–15.
425. Watts DH, Brown ZA, Money D, et al. A double-blind, randomized, placebo-controlled trial of acyclovir in late pregnancy for the reduction of herpes simplex virus shedding and cesarean delivery. *Am J Obstet Gynecol* 2003;**188**:836–43.
426. Weber M, Hartley J, Ashworth M, et al. Virological investigations in sudden unexpected deaths in infancy (SUDI). *Forensic Sci Med Pathol* 2010;**6**:261–7.
427. Weil AA, Glaser CA, Amad Z, et al. Patients with suspected herpes simplex encephalitis: rethinking an initial negative polymerase chain reaction result. *Clin Infect Dis* 2002;**34**:1154–7.
428. Weinberg A, Leary JJ, Sarisky RT, Levin MJ. Factors that affect in vitro measurement of the susceptibility of herpes simplex virus to nucleoside analogues. *J Clin Virol* 2007;**38**:139–45.
429. Weston WL, Brice SL, Jester JD, et al. Herpes simplex virus in childhood erythema multiforme. *Pediatrics* 1992;**89**:32–4.
430. Wheeler CE Jr, Abele DC. Eczema herpeticum, primary and recurrent. *Arch Dermatol* 1966;**93**:162–73.
431. White WB, Grant-Kels JM. Transmission of herpes simplex virus type 1 infection in rugby players. *JAMA* 1984;**252**:533–5.
432. Whitley RJ. Herpes simplex encephalitis: adolescents and adults. *Antiviral Res* 2006;**71**:141–8.
433. Whitley RJ, Alford CA, Hirsch MS, et al. Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *N Engl J Med* 1986;**314**:144–9.
434. Whitley RJ, Arvin A, Prober C, et al. A controlled trial comparing vidarabine with acyclovir in neonatal herpes simplex virus infection. *N Engl J Med* 1991;**324**:444.

435. Whitley RJ, Arvin A, Prober C, et al. Predictors of morbidity and mortality in neonates with herpes simplex virus infections. *N Engl J Med* 1991;**324**:450.
436. Whitley RJ, Cobbs CG, Alford CA Jr, et al. Diseases that mimic herpes simplex encephalitis: diagnosis, presentation, and outcome. *JAMA* 1989;**262**:234–9.
436a. Whitley RJ, Corey L, Arvin A, et al. Changing presentation of herpes simplex infection in neonates. *J Infect Dis* 1988: **158**;109.
437. Whitley RJ, Davis E, Suppapanya N. Incidence of neonatal HSV infection in a managed care population. *Sex Trans Dis* 2007;**34**:704.
438. Whitley RJ, Hutto C. Neonatal herpes simplex virus infections. *Pediatr Rev* 1985;**7**:119.
439. Whitley RJ, Lakeman AD, Nahmias A, et al. DNA restriction-enzyme analysis of herpes simplex virus isolates obtained from patients with encephalitis. *N Engl J Med* 1982;**307**:1060–2.
440. Whitley RJ, Levin M, Marton N, et al. Infections caused by herpes simplex virus in the immunocompromised host: natural history and topical acyclovir therapy. *J Infect Dis* 1984;**150**:323–9.
441. Whitley RJ, Nahmias A, Visintine A, et al. The natural history of herpes simplex virus infection of mother and newborn. *Pediatrics* 1980;**66**:489.
442. Whitley RJ, Roizman B. Herpes simplex viruses: is a vaccine tenable? *J Clin Invest* 2002;**110**:145–51.
443. Whitley RJ, Soong SJ, Dolin R, et al. Adenine arabinoside therapy of biopsy-proved herpes simplex encephalitis. *N Engl J Med* 1977;**297**:289–94.
444. Whitley RJ, Yeager A, Kartus P, et al. Neonatal herpes simplex virus infection: follow-up evaluation of vidarabine therapy. *Pediatrics* 1983;**72**:778–85.
445. Wilhelmus KR, Beck RW, Moke PS, et al. Acyclovir for the prevention of recurrent herpes simplex virus eye disease. The Herpetic Eye Disease Study Group. *N Engl J Med* 1998;**339**: 300–6.
446. Wishart MS, Darougar S, Viswalingam ND. Recurrent herpes simplex virus ocular infection: epidemiological and clinical features. *Br J Ophthalmol* 1987;**71**:669–72.
447. Wittek A, Yeager A, Au D, et al. Asymptomatic shedding of herpes simplex from the cervix and lesion site during pregnancy: correation of antepartum shedding with shedding at delivery. *Am J Dis Child* 1984;**138**:439.
448. Wood DJ, Corbitt G. Viral infections in childhood leukemia. *J Infect Dis* 1985;**152**:266–73.
449. Wyles DL, Patel A, Madinger N, et al. Development of herpes simplex virus disease in patients who are receiving cidofovir. *Clin Infect Dis* 2005;**41**:676–80.
450. Xu F, Lee FK, Morrow RA, et al. Seroprevalence of herpes simplex virus type 1 in children in the United States. *J Pediatr* 2007;**151**:374–7.
451. Xu F, Sternberg M, Kottiri B, et al. Trends in herpes simplex virus type 1 and type 2 seroprevalence in the United States. *JAMA* 2006;**296**:964–73.
452. Yeager A, Arvin A. Reasons for the absence of a history of recurrent genital infections in mothers of neonates infected with herpes simplex virus. *Pediatrics* 1984;**73**:188.
453. Yeager A, Arvin A, Urbani L, et al. Relationship of antibody to outcome in neonatal herpes simplex virus infections. *Infect Immun* 1980;**29**:532.
454. Young RC, Hodge DO, Liesegang TJ, et al. Incidence, recurrence, and outcomes of herpes simplex virus eye disease in Olmsted County, Minnesota, 1976-2007: the effect of oral antiviral prophylaxis. *Arch Ophthalmol* 2010;**128**:1178–83.
455. Zhang Q, Jeremiah CD, Lamborn IT, et al. Combined immunodeficiency associated with DOCK8 mutations. *N Engl J Med* 2009;**361**:2046–55.
456. Zhang SY, Jouanguy E, Ugolini S, et al. TLR3 deficiency in patients with herpes simplex encephalitis. *Science* 2007;**317**: 1522–7.

HISTORY

The recorded history of CMV probably began when Ribbert[360] described in 1881 and then reported in 1904 "protozoan-like cells" in the organs of an infant who died of presumed congenital syphilis. In 1921, Goodpasture and Talbot[175] hypothesized that these swollen cells, or "cytomegalia," were host cells that had been injured by a virus. During the first half of the 20th century, CMV was recognizable only by the pathologic changes that it produced in infected cells; and because these cells frequently were seen in the salivary glands of animals and humans, CMV originally was called the "salivary gland virus."

In 1956, human CMV was isolated in tissue culture by independent investigators Rowe and colleagues,[372] Smith,[396] and Weller and associates.[454] Because several human and animal viruses subsequently were found to replicate in salivary glands, the descriptive name *cytomegalovirus* was proposed by Weller in 1960. The ability to cultivate CMV in tissue culture led to the development of serologic techniques, which in the 1960s and 1970s resulted in many important clinical and epidemiologic observations. For example, CMV was found to infect people of all ages throughout the world. By 1962, CMV was established as a significant pathogen of the fetus and neonate that was capable of producing a spectrum of clinical manifestations and neurologic sequelae.[453] In 1966, Kaariainen and colleagues[224] presented evidence supporting CMV as a cause of posttransfusion mononucleosis syndrome.

The molecular biology of the virus was explored during the 1970s and 1980s and continues to be studied by many investigators. In addition, during the 1970s and 1980s, CMV emerged as a major cause of morbidity and mortality in immunosuppressed patients, especially those who underwent organ or marrow transplantation or who had acquired immunodeficiency syndrome (AIDS). In 1976, the first clinical trial of the live attenuated CMV vaccine Towne 125 was reported; since then, more than 500 renal allograft recipients as well as healthy young men and women have received the vaccine under investigational protocols.[156,334] In addition, research on a glycoprotein B subunit vaccine based on one of the glycoproteins of the virus began in the 1980s; and in the 1990s and the early part of the 21st century, other recombinant vaccines were developed and tested in human volunteers. Clinical trials with other vaccines, such as alphavirus replicon particles, live attenuated viral vaccines, and DNA vaccines began in 2005.[424] However, after decades of research, a successful CMV vaccine remains elusive. In 1999, the Institute of Medicine of the National Academy of Sciences assigned a high priority for a vaccine to prevent CMV infection, an action that was quickly endorsed in 2000 by the National Vaccine Advisory Committee, based on the lifetime costs to society and the human suffering associated with CMV disease in neonates and immune-compromised individuals.[24,231] A resurgence of awareness of CMV from public health officials and the lay community began in 2004, providing hope that prevention and treatment of CMV disease may become a priority for research, education, and public health policy.[75] Attempts by clinicians to treat CMV disease began in the late 1960s and early 1970s,[147] and now CMV infection is a treatable disease; several specific and effective antiviral agents, including ganciclovir (licensed in 1989), foscarnet (licensed in 1991), and cidofovir (licensed in 1996), are available to clinicians to treat seriously ill patients.[70] Most recently, in 2000 to 2012, immunomodulating agents, such as CMV hyperimmune globulin and adoptive immunotherapies using CMV-specific cytotoxic T lymphocytes, are being evaluated in clinical trials as adjuncts to available antiviral therapies.

VIROLOGY

CMV is a member of the *Herpesviridae* family of DNA viruses. This family contains many important human pathogens and is subdivided into three subfamilies: (1) *Alphaherpesvirinae*, fast-growing, cytolytic viruses that are latent in neurons and include herpes simplex virus (HSV) types 1 and 2 and varicella-zoster virus (VZV); (2) *Betaherpesvirinae*, slow-growing, cytomegalic viruses that contain the CMVs; and (3) *Gammaherpesvirinae*, herpesviruses that preferentially grow in lymphocytes and sometimes transform them into malignant states; this subfamily includes Epstein-Barr virus and human herpesviruses 6, 7, and 8.

CMV is the largest member of *Herpesviridae*. The genome is double-stranded DNA 240 kb in size (150×10^6 daltons; guanine + cytosine [G + C] content, 58%), and it has a unique long sequence and a unique short sequence, both of which are bounded by repetitive sequences that are inverted relative to each other. The genome, therefore, can assume four isomeric forms. The viral particle has a 110-nm icosahedral capsid composed of 162 capsomeres. The entire virion is enclosed by a lipid envelope, which yields a final diameter of approximately 200 nm.[418]

Replication of CMV is slow compared with that of HSV. Whereas it takes herpes simplex only 4 to 8 hours to produce infectious progeny virus, CMV requires at least 24 hours. The CMV genome is transcribed slowly in a regulated sequence; on the basis of the appearance of different classes of CMV-specific proteins, the replicative cycle can be divided into three periods: immediate-early, early, and late. The immediate-early period is defined as the first 4 hours after infection occurs. During this period, specific segments of the DNA genome undergo restricted transcription and certain regulatory proteins are produced that allow the virus to take control of host-cell macromolecular synthesis. The early period of replication begins after the immediate-early phase and persists for almost 20 hours. This period is characterized by replication of viral DNA, synthesis of infected cell proteins, and production of progeny virus. The late period usually is considered to occur 24 hours after infection develops. During this period, the structural components of the virus are produced and infectious virus is released from the cell. Monoclonal antibodies against the various immediate-early, early, and late proteins produced by CMV have been used as rapid viral diagnostic tools.

CMV has no distinct serotypes. However, strain relatedness or differences can be determined by molecular analysis of viral DNA. Restriction enzyme analysis of DNA extracted from CMV isolates that are linked epidemiologically (e.g., serial isolates from the same person, mother-infant pairs, or family members experiencing temporally related CMV infections) shows identical or similar DNA fragment mapping patterns. This technique or modifications of this technique have been applied to the epidemiology of CMV in a variety of patients, such as transplant recipients, congenitally infected infants, and patients whose CMV was transmitted from person to person in hospitals, daycare centers, and residences. Polymerase chain reaction (PCR) methodology has determined CMV genotypes exist, by showing differences in the a sequence, major immediate-early, glycoprotein B (gB or UL55), H (gH or UL75), N (gN or UL73), and UL144 regions of the viral genome.* Four gB genotypes have been characterized, and although all genotypes cause congenital and acquired infections, studies suggest that adverse outcomes after CMV infection may be linked to infection with a specific gB genotype, but none has been conclusive. Infection with the CMV gB3 genotype appears to be associated more commonly with fatal infections in bone marrow transplant recipients and hearing loss in congenitally infected neonates, and CMV gB2 and gB4 genotypes appear to be more often fatal in patients with AIDS, in some studies.[35,165,348,469,473] Some studies have suggested neonates with gN-4 genotype may have higher risk for sequelae, whereas others have not confirmed this association.[209,330,331] Pregnant women, neonates with congenital CMV infection, healthy toddlers, and immune-compromised patients may harbor more than one CMV genotype and may have different genotypes in different compartments of the body.[123,186,302,371] Mixed gB genotype infections in transplant recipients may be associated with higher viral loads and delayed viral clearance.[270]

EPIDEMIOLOGY

Seroepidemiologic studies have shown that infection with CMV occurs commonly and usually is inapparent.[482] However, the burden of CMV infection and severe disease may be increasing in certain high-risk groups, such as neonates and premature infants and older children and adults with immune deficiencies as well as transplant recipients.[332,387,426] The incidence of CMV infection does not appear to be seasonal. However, the prevalence of CMV IgG antibody is influenced by many factors, including the age, geographic location, cultural and socioeconomic status, race or ethnicity, and child-rearing practices of the group. For example, in developed countries such as the United Kingdom and the United States, the prevalence of CMV antibody is 40 to 60 percent in adult populations of middle-to-upper socioeconomic status and more than 80 percent in lower socioeconomic status groups.[182,412,481] CMV seroprevalence in the United States also may differ by race and ethnicity, with reports of 51 percent of non-Hispanic white individuals being CMV seropositive compared with 76 percent of non-Hispanic black individuals and 82 percent of Hispanic or Mexican Americans.[102,129,415] In contrast, in developing countries, 80 percent of children acquire CMV by the time they reach 3 years of age and almost all persons have been infected by adulthood.[17,25,449] Studies on the age-related prevalence of infection with CMV in the United States suggest three periods of increased incidence of acquisition: early childhood, adolescence, and the childbearing years.[17,455,480]

Pregnancy

Between 45 to 100 percent of women of childbearing age will be CMV seropositive, depending on age, race, ethnicity, and geographic location.[78] Annual seroconversion rates among CMV-seronegative pregnant women vary between 1 to 7 percent, with an average of 2.3 percent.[210] The risk for intrauterine infection during pregnancy appears to vary with type of maternal infection.[260,269] Maternal CMV infection that is either primary or recurrent during pregnancy can result in an infant who is infected congenitally with CMV. However, the rate of intrauterine infection with recurrent infection in the mother is less than 1 percent, whereas transmission to the fetus occurs in 32 to 50 percent of mothers who are infected primarily with CMV during pregnancy.[230,412] Naturally occurring immunity to CMV infection reduces the risk for congenital CMV infection in future pregnancies by at least 69 percent.[161] Symptoms and sequelae are also much more likely to occur in infants congenitally infected as a result of the mother's primary infection during pregnancy than in infants congenitally infected from a recurrent maternal infection. However, even though symptoms are more likely in neonates congenitally infected as a result of a maternal primary infection, congenitally infected neonates from maternal recurrent CMV infections, resulting from reactivation of endogenous strain or reinfection with a new strain, may also experience long-term sequelae, including hearing loss and neurologic disease.[90]

Congenital Infection

Approximately 1 percent of all neonates are born congenitally infected with CMV; ranges of 0.18 to 2.5 percent are reported, varying between countries and the social demographics of the population.[230,269] There are an estimated 40,000 neonates with congenital CMV infection born annually in the United States.[41,84,374] Less than 20 percent (estimated 5% to 15% in most studies) of children with congenital CMV infection will show obvious clinical signs at birth, resulting in an estimated 8,000 to 10,000 children annually in the United States with long-term neurologic and sensory sequelae from congenital CMV disease. Although most neonates congenitally infected with CMV are asymptomatic, between 10 and 15 percent of apparently asymptomatic neonates have congenital hearing loss or may develop progressive sensorineural hearing loss.

*References 35, 121, 186, 209, 210, 302, 330, 331.

Perinatal Infection

CMV can be transmitted perinatally from a mother to her infant.[332] The virus can be shed in the CMV-seropositive mother's cervicovaginal secretions, urine, saliva, and breast milk. The most common and most efficient routes of perinatal transmission are ingestion or aspiration of cervicovaginal secretions at the time of delivery or ingestion of fresh breast milk after delivery. CMV-seropositive mothers, especially mothers with high levels of CMV IgG antibody, frequently shed CMV in their breast milk; and as many as 53 percent of children who are breast-fed with milk that contains infectious virus or CMV DNA can become infected with CMV.[138,220,312,446] These infections usually are benign in normal term infants but may be extremely serious in preterm, very low-birth-weight neonates.[274,445] In addition, as many as 57 percent of neonates whose mothers shed CMV at or around the time of delivery become infected with CMV.[359]

Postnatal Infection in Childhood

Children not congenitally or perinatally infected with CMV may be infected during the toddler or preschool years.[332] The acquisition of CMV by children between 1 and 3 years of age is influenced by home exposure to the virus, by the socioeconomic status or country of origin of the family, and by group daycare exposure.[77] Weller and Hanshaw[453] suggested that the child-rearing practices in Sweden, where group daycare was common practice, accounted for the relatively high prevalence of CMV infection in Swedish children compared with children in the United States and Great Britain, where daycare centers were not common at that time.

Pass and colleagues[318] first reported the prevalence of CMV in daycare centers in the United States in 1982. They found that 51 percent of children who attended a daycare center in Alabama excreted CMV in their saliva or urine. In this study, the prevalence of CMV excretion varied with age; 83 percent of children aged 13 to 24 months shed virus as opposed to only 9 percent of children younger than 1 year old. Pass and colleagues concluded that the high prevalence of CMV infection probably was due to horizontal spread between the children in daycare. Subsequent studies have confirmed a high prevalence of CMV excretion in children in daycare centers across the United States. Overall prevalence rates of 11 to 57 percent have been observed in many other studies in the United States and other countries, with the highest prevalence of active CMV infection (29% to 78%) found in children 1 to 3 years of age.[4,221,293,306,307] Child care homes may have lower prevalence of CMV excretion in children (8% to 15%); however, CMV seroconversion in home child care providers appears similar to that of child care providers in larger daycare centers.[36,318,320,321] Children who attend daycare centers also shed high titers of virus (up to 10^5 median tissue culture infective dose per milliliter), with a mean duration of viral shedding of 13 months in urine and 7 months in saliva.[292] This prolonged, generally asymptomatic shedding of large quantities of virus, coupled with mobility and the less than hygienic daily habits notorious in toddlers, no doubt facilitates the transmission of CMV in daycare centers.

Several studies support the idea that the high prevalence of CMV infection in young children who attend daycare centers is due to horizontal transmission; these studies used molecular fingerprinting techniques to show that infected children in contact with each other shed strains of CMV with similar or identical restriction enzyme banding patterns and that predominant strains of CMV appeared to circulate during a given period in a given daycare center.[4,5,208] Reinfection with a genetically different strain of CMV also has been observed and may be important in child-to-child transmission of CMV in the daycare center environment.[8] The importance of horizontal spread of CMV has been shown in special care centers for mentally retarded children as well.[390] CMV also has been isolated from plastic toys and from the hands of daycare center workers.[145,208,318]

CMV-infected children may transmit the virus to the daycare center workers who care for them.[5,7] In addition, children who attend daycare centers may transmit CMV to their CMV-seronegative parents. In one study of parents of children who attended one of three daycare centers in Alabama, 14 of 67 parents (21%) whose children attended daycare seroconverted, compared with none of 31 parents whose children were cared for at home.[322] Excretion of CMV by the child clearly was a key risk factor for parental seroconversion because none of the seroconversions occurred in parents whose children attended the daycare center but did not shed CMV. Moreover, this study revealed a strong trend toward a greater risk for acquiring CMV infection (seroconversion rate, 45%) in the parents of children 18 months or younger. Additional evidence implicating young children who attend daycare centers as a source of CMV infection for their parents was provided by a study in Virginia daycare centers, in which the parents of children in daycare shed CMV strains identical to the strains shed by their children.[6] Furthermore, CMV strains from daycare settings, when transmitted from toddlers to their pregnant mothers, may be the source of congenital infection.[293]

Whereas early childhood probably is the most rapid period of acquisition of CMV, annual seroconversion rates of only 3 to 6.2 percent have been observed in older children aged 3 to 10 years in the United States and other countries, thus reflecting an age-related plateau in the acquisition of CMV. One identified risk factor for the acquisition of CMV infection in a school-aged child has been recent or active CMV infection in a family member.*

Adolescents

In support of these early findings, a seroepidemiologic study of CMV infection in sexually active adolescent girls found a strong association between indicators of sexual activity and serologic evidence of CMV infection and concluded that sexual activity is an important risk factor

*References 102, 113, 129, 415, 416, 480.

for the acquisition of CMV infection in adolescent girls.[88,402] Early cross-sectional epidemiologic studies of CMV implied that a gradual increase in CMV antibody prevalence occurs during the teenage years, and this period of rapid acquisition was attributed to the intimate physical contact that commonly occurs during the teenage years.[17,367] However, these studies were conducted primarily in lower socioeconomic groups. In a seroepidemiologic study of several groups in Houston, Texas, on the acquisition of CMV infection in late childhood and adolescence, the prevalence of antibody increased with age in subjects of nonwhite races, as it did according to previous studies, but the prevalence of antibody in white subjects did not increase with age.[455]

Vertical transmission of CMV acquired during the teenage years may result in congenital infection with CMV in infants born to teenage mothers. In 1984, Kumar and colleagues[242] studied primary CMV infection in more than 3000 pregnant adolescents in Ohio. They found that 57 percent were CMV seropositive, that 1 percent of susceptible pregnant adolescents acquired CMV, and that the risk for intrauterine transmission was 50 percent if primary CMV infection occurred during pregnancy. Information provided by the National Congenital Cytomegalovirus Disease Registry and the National Center for Health Statistics suggests that adolescents who are pregnant actually may be at higher risk than are older mothers for giving birth to an infant with congenital CMV disease because 34 percent of mothers who give birth to an infant with congenital CMV disease are younger than 20 years old; however, this age group represents only 16 percent of the mothers giving birth in the United States.[115,212]

Intrafamilial Transmission

CMV also can be transmitted within the family setting. Evidence for intrafamilial transmission of CMV has been provided in the form of case reports, seroepidemiologic studies, and accounts in which molecular analysis of CMV strains was used to trace the transmission of CMV in family members or extended family members with temporally related CMV infections. In most studies, the index case was a child; when a CMV-infected child entered a household, attack rates were 47 to 53 percent.[427,433,474]

Three patterns of intrafamilial transmission have been observed: transmission between siblings, transmission between parents, and transmission between children and parents. Additional support for the intrafamilial transmission of CMV has been provided by published studies in which molecular analysis was performed on CMV isolates from family members or extended family members who experienced temporally related CMV infections. In each of these studies, restriction enzyme analysis of viral DNA from CMV isolates in family members showed that the strain of CMV was the same within each family.[136,323,410,474] New molecular techniques based on PCR amplification of a hypervariable region of the CMV genome also have corroborated the observation that genetically similar strains of CMV can be transmitted between family members over the course of time.[403]

Sexual Transmission

CMV also appears to be transmitted by heterosexual and homosexual contact. The evidence to support sexual transmission is anecdotal, virologic, serologic, and molecular and, taken together, suggests that sexual transmission of CMV is important in certain groups. However, because the virus is shed in saliva, cervicovaginal secretions, and semen, which form of intimate contact results in transmission between sex partners is unclear.[118,252,359]

Several observations support the idea that CMV can be transmitted sexually. For example, CMV antibody prevalence increases with age; CMV antibody is more prevalent in sexually active women than in celibate women; CMV antibody is associated with indices of sexual activity, such as recent infection with *Chlamydia trachomatis* or *Neisseria gonorrhoeae;* and a strikingly high annual incidence (37%) of primary CMV infection has been observed in young women with a recent first sexual experience.* In addition, in a longitudinal study of the site-specific shedding of CMV in human immunodeficiency virus (HIV)–seropositive homosexual and bisexual men, CMV was cultured from semen more frequently than from other body sites or fluids.[362] Moreover, molecular analysis of viral DNA has shown strains of CMV isolated from sex partners to be identical in most cases analyzed.[118,194]

Infection of the genital tract can recur by reactivation of an endogenous strain or by reinfection with an exogenous or different strain of CMV.[402] The consequences of such reinfections are unknown but may have important implications for transmission, especially if a CMV vaccine is used to control congenital CMV disease in the future. Sexual debut and recent sexual activity significantly influence CMV seroprevalence among women of childbearing age. In addition, engaging in sexual activity within 2 years of delivery of an infant adds increased risk for delivering a congenitally infected infant. Because sexual activity appears to influence CMV seroprevalence and congenital CMV infection rates, strategies to reduce sexual transmission of CMV may be important.[160,416]

Nosocomial Transmission

CMV can be transmitted in the hospital setting by transfusion of blood products, by bone marrow and organ transplantation, and, rarely, by person-to-person transmission. Despite numerous studies that have used serologic, virologic, and molecular epidemiologic techniques, transmission of CMV from a CMV-infected patient to a health care worker has not been documented.† Therefore, even though health care workers are exposed to CMV-infected patients, their risk for acquiring CMV appears to be no greater than that of the general population. In addition, unlike homes and daycare centers, hospitals routinely perform rigorous infection control procedures, including universal precautions, which probably accounts for the relatively low risk for acquiring CMV as well as other infections in the hospital setting.

*References 89, 101, 112, 160, 222, 402, 416, 461.
†References 9, 31, 37, 62, 120, 137, 206.

However, infant-to-infant transmission has been shown to occur, albeit infrequently, in crowded hospital units with a high prevalence of CMV excretion in patients.[120,406] CMV also can survive on plastic surfaces and has been cultured from inanimate objects in contact with CMV-infected patients in the hospital setting.[120,406]

Blood products are a well-established source of CMV infection, and donor-to-recipient transmission of CMV has been documented by restriction enzyme analysis of viral DNA.[432] Post-transfusion CMV mononucleosis can be seen in adults who receive large volumes of fresh whole blood.[224] In addition, 15 to 17 percent of CMV-seronegative neonates who receive blood products from CMV-seropositive donors acquire CMV.[474,475] Post-transfusion CMV infection in neonates, especially premature infants, can cause a syndrome of shock, lymphocytosis, and pneumonitis; CMV infection also appears to hasten the progression to bronchopulmonary dysplasia in these patients.[378,475]

CMV apparently is transmitted in the residual leukocytes found in whole blood, packed red blood cells, and platelet fractions as well as by pure leukocyte transfusions. The risk for development of post-transfusion CMV infection is approximately 3 percent per unit transfused, and the risk for symptomatic infection is much higher in CMV-seronegative recipients than in CMV-seropositive recipients.

Immunosuppressed Patients

Primary and reactivation infections as well as reinfection with CMV occur commonly in organ and marrow transplant recipients, as well as infants with primary immune deficiency syndromes that involve T-cell or natural killer (NK)-cell dysfunction or acquired immune deficiency from human immunodeficiency virus (HIV) infection or AIDS. The health care burden of severe CMV disease appears to be increasing in both these special patient groups.[387] Active infection with CMV occurs in almost all organ and marrow transplant recipients and usually is manifested clinically and virologically 30 to 90 days after transplantation. Rarely, CMV retinitis can occur years after a patient has undergone transplantation and generally is associated with chronic CMV viremia.[152] CMV can be transmitted to the recipient by the transplanted organ, by transfused blood products, and, theoretically, also by intimate contact with persons actively infected with CMV.

Infections with CMV are primary when they occur in CMV-seronegative recipients who receive transplants or blood products from CMV-seropositive donors. Recipients who are CMV seropositive before undergoing transplantation can experience reactivation of their own endogenous strain of CMV. In addition, CMV-seropositive transplant recipients may be superinfected by strains of CMV present in the donor organ.[94] In fact, in one study of renal transplant recipients in the United Kingdom, proven superinfection with the donor's strain of CMV occurred more frequently than did proven reactivation of the recipient's strain of virus.[188] Although reactivation, reinfection, and primary CMV infection all can produce symptoms in immunosuppressed transplant recipients, primary CMV infections are much more likely to be severe and even fatal.[340]

The type of transplant also appears to influence the type of CMV disease expressed. For example, the most severe and lethal form of CMV interstitial pneumonitis is seen in bone marrow transplant recipients, especially those experiencing a graft-versus-host reaction. CMV pneumonia also is a common occurrence after heart, lung, or renal transplantation, and severe CMV hepatitis is a special problem after liver transplantation.[16]

The iatrogenic immunosuppression essential for graft maintenance probably is responsible for the common occurrence of reactivation infection after transplantation, as well as the increased incidence of severe and symptomatic infection seen after primary CMV infection in transplant recipients. Cytotoxic drugs such as cyclophosphamide and azathioprine, in addition to corticosteroids, have been associated with reactivation of latent CMV infection, and the addition of antilymphocyte globulin to an immunosuppressive regimen can increase the morbidity of CMV infections.[324] The use of cyclosporine as the primary immunosuppressive agent, even in conjunction with corticosteroids, does not appear to reduce the risk for acquiring CMV infection compared with a regimen of azathioprine and corticosteroids; however, it may decrease the incidence of severe, symptomatic CMV disease in some transplant recipients.[135] The use of OKT3 to treat rejection in certain transplant patients increases the risk for dissemination in those with primary CMV infection.[393]

The frequency and morbidity of CMV infection in patients with malignant neoplasms are not as high as in patients who have undergone marrow and organ transplantation; however, the use of chemotherapy, especially for leukemia, is associated with significant CMV disease, particularly pneumonitis and persistent fever with viral dissemination. CMV-seropositive patients receiving immunosuppressive therapy for connective tissue disease also can have significant reactivation infection with CMV.[130]

The progressive and profound immunosuppression in adults and children with AIDS also is associated with CMV infection and disease. Although the source of CMV infection in adults with AIDS most likely is heterosexual or homosexual contact, sources of CMV infection in young children with AIDS have not been determined. One can hypothesize, however, that many of these infections are acquired from the mother, either congenitally or perinatally. Infants infected perinatally with HIV who also acquire CMV congenitally appear to be at significant risk for experiencing rapid progression of disease in the first 18 months of life, as well as debilitating neurologic disease.[131,241,386] Patients with AIDS also may be infected with multiple strains of CMV.[134,407]

Infants with primary immune deficiency syndromes involving T-cell or NK-cell function may have persistent CMV infection and progressive, life-threatening CMV disease. They often will have very high levels of CMV DNA levels by quantitative PCR in their blood or plasma, hepatosplenomegaly, pneumonitis, colitis, retinitis, thrombocytopenia, or elevated aminotransferase levels. Prompt diagnosis and management of possible

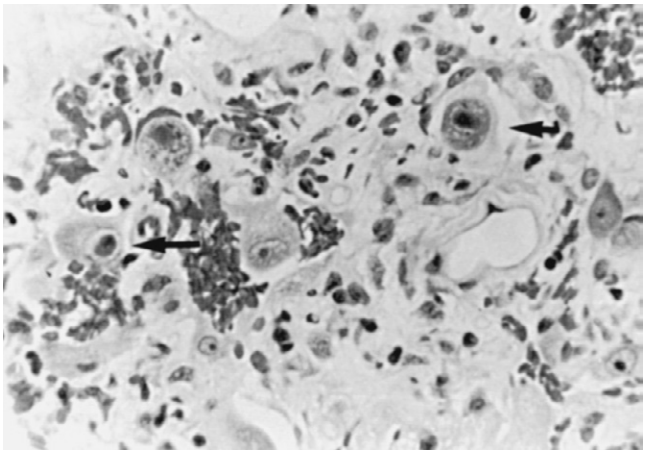

FIGURE 158-1 ■ Typical cytomegalic inclusion cells seen in lung tissue obtained by open-lung biopsy from a bone marrow transplant recipient with fatal cytomegalovirus pneumonitis (×640). (Courtesy Dr. Milton J. Finegold, Department of Pathology, Texas Children's Hospital, Houston.)

underlying immune disorder and specific antiviral therapy against CMV are important in these patients.

PATHOLOGY, PATHOGENESIS, AND IMMUNITY

CMV infection causes characteristic type A Cowdry intranuclear inclusions and massive enlargement of the affected cells (Fig. 158-1). It is this property of "cytomegaly" from which CMV acquired its name. The cytomegalic cells (25 to 40 μm in diameter) are two to four times larger than normal cells, and the nucleus usually is more than 10 μm in diameter. The intranuclear inclusion also is large (up to 10 μm in diameter) and is surrounded by an intranuclear halo and then the nuclear membrane, which gives a characteristic "owl's eye" appearance. Basophilic, granular, intracytoplasmic inclusions (2 to 4 μm in diameter) also may be present in cells that have intranuclear inclusions. These large cells represent productive virus infection, and both the nuclear and cytoplasmic inclusions contain viral nucleocapsids and express virus-specific antigens.[201] These cytomegalic cells frequently are associated with epithelial cells, and their presence generally indicates a productive and symptomatic infection with CMV. Cells also may be infected latently with CMV. These cells may express virus-specific antigen and contain viral nucleic acid without producing typical cytomegaly or a cytopathic effect. The significance of these cells must be considered carefully within the clinical context of the patient.

With severe, disseminated CMV disease, involvement can be seen in virtually all organ systems. The salivary glands frequently are infected in both symptomatic and asymptomatic infections, and the ductal epithelium usually is the site of pathologic involvement. The kidneys also are infected frequently in both symptomatic and asymptomatic CMV infection. In the kidneys, cytomegalic cells are most pronounced in the proximal tubules and interstitial cellular infiltrates, and even immune complex deposits can be seen in the glomeruli.

CMV infection often causes prolonged viruria, but it rarely results in significant renal dysfunction. In the lungs, cytomegalic cells are seen in alveolar and bronchial epithelium and are associated with mononuclear cell inflammation. Pulmonary alveolar macrophages also may express viral antigen and contain CMV DNA. The brain parenchyma can be involved, and a variety of pathologic processes can be observed and include sensorineural hearing loss. Other organs that can be infected or diseased with CMV include the liver, pancreas, adrenals, eyes, lymph nodes, heart, skin, bone, male and female genital tracts, esophagus, stomach, intestine, and placenta.

Infections with CMV can be latent and nonproductive, productive yet asymptomatic, or productive and symptomatic. Therefore, determination of whether a patient is "sick with CMV" or "sick from CMV" sometimes is difficult. Viral strain differences have not been shown to date to influence pathogenicity. However, immune responses, including maturity of the immune response, appear to be a major factor in virulence of the infection because CMV disease occurs more frequently in fetuses, premature neonates, transplant recipients, and patients with AIDS than in older healthy infants, children, and adults with acquired CMV infection.

The cell-mediated immune response, both specific and nonspecific, is thought to be important in host defense against CMV. The nonspecific immune mechanisms of NK cells and interferon production occur soon after the development of CMV infection, when early antigens are being produced and before infectious virus is released from the cell. The generation of cytotoxic T cells against CMV early antigens probably is the most important specific host immune response to CMV, and patients who are defective in this T-cell response are at high risk for acquiring serious CMV disease.[183]

Clinical and laboratory evidence also supports the concept of CMV as an immunosuppressive agent. The proliferative response of T cells to stimulation with mitogens and CMV antigen is suppressed in patients with CMV mononucleosis, and immunosuppressed patients with active CMV disease frequently have other opportunistic infections.[361] In addition, CMV pneumonitis in bone marrow transplant recipients has been hypothesized to result from host cell–mediated events produced in response to chronic viral replication.[483] Homology between certain CMV proteins and class I and class II major histocompatibility complex products has been observed. This "molecular mimicry" implies that the severe tissue destruction seen in CMV pneumonitis may be partly an autoimmune phenomenon in these patients.

Humoral immunity, on the other hand, does not appear to be a key factor in the host's defense against CMV infection. For example, the fetus can be infected by intrauterine transmission as a result of reactivation of CMV infection in a woman who is CMV seropositive before pregnancy, and infants frequently are infected perinatally from infected cervicovaginal secretions or breast milk in the presence of passive maternal antibody.[412] In addition, CMV-seropositive transplant

recipients can be reinfected with a new strain of CMV from the donor organ, and viruria and viremia occur in transplant recipients despite high titers of neutralizing antibody against the specific strain of CMV.[94,95] The presence of CMV antibody, therefore, should be considered a marker of previous or current infection with the virus rather than a measure of immunity per se.

Although humoral immunity does not appear to prevent infection with CMV, it does appear to lessen the severity of associated symptoms. Infants congenitally infected with CMV as a result of reactivation of infection in their mother almost always are asymptomatic, whereas perinatally infected infants rarely have significant symptoms. Primary infections in transplant recipients are more likely to be symptomatic than is reinfection or reactivation. One hypothesis regarding how CMV eludes host humoral defenses is that the virus binds to the host protein β_2-microglobulin and masks the antigenic determinants that are important for neutralization by antibody.[277]

CLINICAL MANIFESTATIONS

Fetal and Congenital Infections

The fetus with in utero CMV infection may show ultrasonographic abnormalities, including signs of abnormal somatic or head growth, enlarged liver or spleen, echogenic bowel patterns, and hepatic calcifications, as well as biologic findings such as fetal thrombocytopenia and lymphopenia and elevated liver enzymes and elevated CMV-specific IgM antibody levels. In addition, cerebral fetal ultrasonography may show enlarged ventricles in the fetal brain, intracranial calcifications, or microcephaly or mimic a Dandy-Walker malformation in severe cases.[29,190,263] Annually, 30,000 to 40,000 infants are born with congenital CMV infection. Of these infants, as many as 10 percent have severe, classic "cytomegalic inclusion disease," characterized by intrauterine growth retardation, jaundice, hepatosplenomegaly, thrombocytopenia with petechiae and purpura, pneumonia, and severe central nervous system (CNS) damage with microcephaly, intracerebral calcifications, chorioretinitis, and sensorineural hearing loss. Another 5 percent have atypical involvement, such as ventriculomegaly, periventricular leukomalacia, periventricular cystic malformations with or without calcifications, polymicrogyria, cerebral thrombosis, strabismus, optic atrophy, long bone osteitis characterized by fine vertical metaphyseal striations, isolated and transient thrombocytopenia and petechiae, cutaneous vasculitis, hemolytic anemia, ascites, chronic hepatitis, and intrahepatic cholestatic disease.* As many as 90 percent of these infants who are symptomatic at birth later have neurologic sequelae, vision loss, or progressive deafness. However, the range of severity of these sequelae appears to be broad, and severity may be predicted by head circumference and computed tomographic (CT) findings at birth.[57,304] In addition, CMV viremia and high levels of CMV DNA by PCR analysis in the blood or plasma in congenitally infected neonates is associated with symptomatic disease and hearing loss at birth, suggesting that the level of virus contributes to disease severity.[56,61]

The differential diagnosis of symptomatic congenital CMV disease includes congenital toxoplasmosis, congenital HSV infection, congenital syphilis, congenital rubella syndrome, congenital infection with lymphocytic choriomeningitis virus, and congenital HIV infection. In addition, noninfectious causes, such as genetic disorders, metabolic disease, and maternal exposure to drugs and toxins, should be considered.

Most infants who are infected congenitally with CMV are asymptomatic at birth. However, 10 to 17 percent of these infants later may have unilateral or bilateral deafness or differences in higher-level auditory function.[103,412,460] Progressive or late-onset hearing loss also may occur in these infants, thus rendering it likely that universal neonatal hearing screening programs will miss rather than detect many of these children.[158,305] Small retinal lesions may occur as well.[99] Earlier studies suggested that developmental problems may occur in asymptomatically infected children, but more recent studies suggest that cognitive outcome appears to be normal compared with uninfected children.[228]

Very low-birth-weight infants who are infected congenitally with CMV may experience pulmonary and systemic deterioration temporally associated with corticosteroid therapy.[436]

Neonates and young infants younger than 6 months of age with persistently high levels of CMV viremia or high levels of CMV DNA by PCR analysis in the blood or plasma, or infants who have significant rebound of their CMV DNA levels after antiviral treatment, may have a primary immune disorder of T-cell function, such as severe combined immune deficiency, or a disorder of NK-cell function. Prompt diagnosis and management of their underlying immune disorder, as well as specific antiviral therapy against CMV, are important in these patients.

Perinatal Infections

Perinatally acquired infections in healthy infants usually manifest when the child is between 4 and 16 weeks of age, but most such infections are asymptomatic. However, as many as one third of infants exposed to CMV perinatally may have signs and symptoms of disease associated with CMV infection, most often self-limited lymphadenopathy, hepatosplenomegaly, hepatitis, or pneumonitis.[244] Perinatally acquired infection with CMV also can cause a viral, sepsis-like syndrome or severe, protracted pneumonitis that has been associated with the development of bronchopulmonary dysplasia in premature infants.[274,378] A limited number of studies suggests that these infections, however, do not appear to cause neurodevelopmental sequelae or deafness.[245] Infants with primary immune deficiency of T-cell or NK-cell function, or AIDS from perinatally acquired HIV infection, who acquire CMV perinatally may have CMV viremia, high levels of CMV DNA by PCR analysis in blood or plasma, and associated hepatosplenomegaly, thrombocytopenia, viral sepsis, pneumonia, retinitis, or colitis.

*References 18, 27, 99, 155, 218, 306.

Prompt diagnosis of an underlying primary immune disorder, as well as antiviral therapy, is lifesaving management in these patients. The differential diagnosis includes other perinatally acquired infections, such as *Chlamydia* pneumonitis, hepatitis B virus infection, and infection with HIV, as well as postnatally acquired infections with enteroviruses, adenovirus, and a variety of bacterial pathogens.

Mononucleosis Syndrome

CMV-induced mononucleosis occurs as a primary infection in both immunocompetent and immunosuppressed persons, in pregnant women, and, occasionally, as a reactivation infection in immunosuppressed patients.[260] It can result from person-to-person transmission of the virus as well as from transmission by blood products or by organ or marrow transplantation. Although originally described in adults and most often occurring in patients between 20 and 40 years of age, it also can be seen in adolescents, children, and even infants.[268,316] Typical CMV-induced mononucleosis is characterized by fever and strikingly severe malaise of 1 to 4 weeks' duration, peripheral lymphocytosis with atypical lymphocytes, and mildly elevated liver enzymes. In some patients, headache, myalgias, and abdominal pain with diarrhea are prominent symptoms. In premature infants with transfusion-acquired CMV mononucleosis, prominent manifestations include shock, hepatosplenomegaly, pneumonitis, thrombocytopenia, and renal failure.[406] In contrast to Epstein-Barr virus–induced mononucleosis, CMV-induced mononucleosis rarely causes pharyngitis, tonsillitis, or significant splenomegaly, and it does not result in production of heterophil antibodies.[100,207] However, like Epstein-Barr virus–induced mononucleosis, it can be associated with morbilliform rash after administration of ampicillin, elevated erythrocyte sedimentation rate, polyclonal hypergammaglobulinemia, and production of other antibodies such as rheumatoid factor, cold agglutinins, and antinuclear antibodies. Complications rarely occur but include interstitial pneumonitis, myocarditis, pericarditis, hemolytic anemia, thrombocytopenia with or without petechiae or purpura, hemophagocytic syndrome, arthralgias and arthritis, maculopapular rashes, adrenal insufficiency, splenic infarction, ulcerative colitis and proctitis, Guillain-Barré syndrome, and meningoencephalitis.* Severe, icteric hepatitis as well as granulomatous hepatitis also can occur, but hepatic necrosis and liver failure caused by CMV have not been documented convincingly in normal hosts.[54,100,207,240,356]

The differential diagnosis of CMV-induced mononucleosis includes mononucleosis induced by other viruses such as Epstein-Barr virus, hepatitis A or B virus, and HIV. In addition, acquired toxoplasmosis can produce a mononucleosis syndrome in healthy persons.

Interstitial Pneumonitis

CMV is a major cause of interstitial pneumonia in both adults and children who are immunosuppressed because of congenital primary immunodeficiency of T-cell or NK-cell function, AIDS, organ or marrow transplantation, or malignant disease. In recipients of bone marrow transplants, CMV accounts for 17 to 70 percent of cases of interstitial pneumonitis in adult patients but only 10 percent of cases in patients younger than 21 years.[452,453] Pneumonia also can occur in apparently immunocompetent young infants with perinatally acquired CMV infection and in healthy adults with CMV-induced mononucleosis.[233,244,411,457] Whereas the pneumonia in immunocompetent hosts almost always is benign and self-limited, CMV pneumonia in immunosuppressed patients is a serious, often fatal illness, especially in bone marrow transplant recipients, who have a mortality rate of up to 90 percent. It also can be particularly troublesome after pediatric heart and lung transplantation.[303] CMV pneumonitis usually occurs 1 to 3 months after the patient has undergone transplantation and begins with symptoms of fever and a dry, nonproductive cough. It then can progress during the course of 1 to 2 weeks to dyspnea, retractions, wheezing, and hypoxia, which require ventilatory support. It may occur as the only disease manifestation or be part of a disseminated CMV infection. The radiographic appearance of CMV pneumonia usually is diffuse, interstitial infiltrates, but peribronchial infiltrates with hyperinflation and nodular pulmonary infiltrates also have been described (Fig. 158-2).[233,429] Coinfection with other pathogens, especially gram-negative enteric bacteria and fungal pathogens in transplant recipients and in patients with *Pneumocystis jiroveci* infection and AIDS, can occur.[448]

Congenitally infected infants also may be born with CMV pneumonitis, which often is sufficiently severe to require ventilatory support and usually is part of a multisystemic CMV disease process. Very low-birth-weight

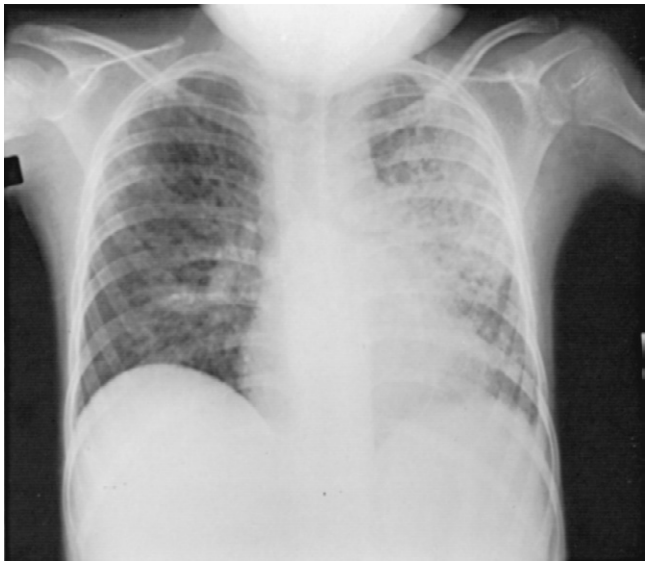

FIGURE 158-2 ■ Chest radiograph of a bone marrow transplant recipient with rapidly fatal cytomegalovirus pneumonitis. An open-lung biopsy specimen showed numerous cytomegalic inclusion cells, exhibited cytomegalovirus early antigens by immunoperoxidase staining, and grew cytomegalovirus after inoculation into tissue culture.

*References 87, 100, 109, 151, 207, 239, 240, 259.

infants who are infected congenitally with CMV may experience CMV pneumonitis in temporal association with corticosteroid therapy.[436]

The differential diagnosis of CMV pneumonitis in immunocompromised patients, including neonates, is extensive. It includes infection with other viruses, such as HSV, VZV, measles virus, respiratory syncytial virus, influenza A and B viruses, parainfluenza viruses, and adenoviruses; bacterial pneumonia caused by a variety of gram-positive and gram-negative organisms; infection with protozoa, such as *P. jiroveci* and *Toxoplasma gondii;* infection with *Chlamydia* and *Mycoplasma;* and fungal pneumonia, caused especially by *Candida* and *Aspergillus.* Noninfectious causes of pneumonitis, such as pulmonary hemorrhage, aspiration pneumonia, rejection, and pulmonary damage from chemotherapeutic agents, also should be considered.

Retinitis and Other Eye Abnormalities

Chorioretinitis as well as optic atrophy, cortical blindness, and strabismus occur in 17 to 41 percent of neonates with symptomatic congenital CMV infection and rarely in children born with asymptomatic congenital CMV infection.[55,99,374] Although most retinal lesions in congenitally infected infants appear to be inactive at birth, some observations suggest that progression of preexisting lesions and late-onset new lesions resulting in vision loss may occur rarely in both symptomatic and asymptomatic congenitally infected infants.[55,104] Retinitis does not seem to be a prominent part of perinatally or early postnatally acquired infection in immunologically normal infants but has been rarely reported to occur.[245,329] Infants with primary immune disorders involving T-cell or NK-cell dysfunction, on the other hand, often have CMV viremia, usually at persistently high titers, and CMV retinitis. CMV retinitis once was a rare manifestation of CMV disease in solid-organ transplant recipients undergoing chronic immunosuppression for more than a year and in patients receiving chemotherapy for malignant disease.[152] In the 1980s, CMV retinitis emerged as a frequent manifestation of CMV disease in patients with severe immunosuppression, especially bone marrow transplant recipients and patients with AIDS. It probably is a result of hematogenous spread of the virus to the retina, with continued local viral replication. Despite the common occurrence of CMV retinitis in adults with AIDS, however, it rarely has been reported in children with AIDS.[261]

CMV produces characteristic white, perivascular infiltrates and hemorrhage, with a necrotic, rapidly progressive retinitis. It descriptively has been called "cottage cheese" retinitis and "ketchup" or "brushfire" retinitis.[52] Early, peripheral retinitis can be asymptomatic, or the complaints may be minimal and nonspecific; it is especially difficult to ascertain in infants and young children. It does not cause eye pain, photophobia, or conjunctivitis. Once the retinitis has progressed, it can cause blurred vision, decreased visual acuity, visual field defects, and blindness. Young children and infants who have suffered visual loss as a result of CMV retinitis may exhibit strabismus or failure to fix and follow objects within their visual field. CMV retinitis also can progress rapidly to total blindness if the macula is involved. Immunosuppressed children from any cause who have CMV disease or persistently high levels of CMV DNA in their blood or plasma should receive regular expert ophthalmologic examinations to monitor for the development of sight-threatening retinitis. Establishing the diagnosis early may allow prompt institution of antiviral therapy, which may be sight saving.[261]

The ophthalmoscopic appearance of CMV retinitis usually is characteristic. However, in patients in whom the appearance of the retina is not typical or in whom the retinitis has progressed despite specific antiviral treatment, other causes of retinal lesions that should be considered include cotton-wool spots associated with hypertension, diabetes, connective tissue disease, anemia and leukemia, ocular toxoplasmosis, and candidal infection of the retina as well as syphilis, HSV infection, lymphocytic choriomeningitis virus infection, and VZV infection. Detection of the virus by culture or its DNA by a PCR-based method in vitreous fluid may help establish the diagnosis in difficult or atypical patients.[149] CMV antibodies also have been detected in the tears of patients with active ocular infections, including retinitis.[373]

CMV also has been associated in some studies with other unusual eye abnormalities. In coincidentally congenitally infected infants, microphthalmos, anophthalmia, optic nerve hypoplasia and coloboma, optic nerve atrophy, Peter anomaly, and irregular retinal pigment have been observed.[164] However, the role that CMV is playing in these anomalies is unclear and unsubstantiated by prospective studies.[99] CMV also does not appear to cause congenital cataracts.[392] CMV also has been isolated from tears and has been associated with conjunctivitis in patients with CMV mononucleosis and AIDS as well as with corneal epithelial keratitis and disk neovascularization.[256]

Hepatitis

CMV hepatitis in neonates with congenital CMV disease, in children who are bone marrow, heart, and lung transplant recipients, in patients with cancer or AIDS, in infants with primary immune deficiency of T-cell or NK-cell function, and even in healthy persons experiencing a primary CMV infection usually is manifested as mild to moderate hepatomegaly and mildly to moderately elevated serum hepatic enzyme levels. Infants with primary immune disorders of T-cell and NK-cell function may have progressive hepatomegaly and increasing aminotransferase levels and the presence of CMV evident on histopathology in the liver. CMV hepatitis commonly occurs in conjunction with fever, thrombocytopenia, and lymphopenia or lymphocytosis. Jaundice and hyperbilirubinemia may occur, severe hepatitis or cirrhosis is exceedingly rare, and hepatic necrosis and liver failure caused by CMV hepatitis have not been documented convincingly in these patients. CMV infection also has been associated with granulomatous hepatitis.[54,356] In addition, CMV hepatitis is a unique and prominent problem in children who have undergone liver transplantation.[64] Most CMV hepatitis occurs 1 to 2 months after

the patient has undergone transplantation, but it may be noted as early as 2 weeks or as long as 4 months afterward.[66] It is more common and more severe after a primary CMV infection and is associated with liver transplantation from a CMV-seropositive donor and the use of OKT3 antibodies for severe rejection.[394] CMV hepatitis in liver transplant recipients is characterized by prolonged fever, leukopenia, thrombocytopenia, elevated levels of liver enzymes, hyperbilirubinemia, and liver failure. Distinguishing between CMV hepatitis and acute rejection often is difficult, even with a liver biopsy, and the two commonly coexist. CMV infection also has been associated with ascending cholangitis, chronic rejection, and the vanishing bile duct syndrome in liver transplant recipients.[66,308]

Infants with congenital CMV disease also may have hepatitis. The liver usually is smooth and nontender and commonly extends 3 to 5 cm below the right costal margin. Ascites may be present prenatally and may persist postnatally for 1 to 2 weeks. The hepatomegaly usually resolves by the time the infant is 3 months of age, and persistence beyond 1 year is highly unusual. Mild hepatitis generally is present, and aminotransferase levels in neonatal hepatitis caused by CMV rarely exceed 500 IU. Hyperbilirubinemia is present at birth in approximately a third of neonates with congenital CMV and may be striking, with conjugated (direct) bilirubin levels up to 30 mg/dL.[212] The abnormal results of liver function tests gradually resolve during the course of the first few weeks of life, and chronic hepatitis as a result of congenital infection with CMV is an unusual occurrence. Congenital CMV disease also has been associated with intrahepatic and extrahepatic biliary atresia in some studies, but the direct role of CMV in neonatal cholestatic disease is unclear.[18,155,218] A neonate or infant with congenital CMV disease and recurrent, persistent or progressive hepatomegaly and hepatitis, despite antiviral treatment, may have an underlying primary immune deficiency of NK-cell or T-cell function.

The differential diagnosis of CMV hepatitis includes other causes of viral hepatitis, such as hepatitis A virus, hepatitis B virus, hepatitis C virus, Epstein-Barr virus, HSV, enterovirus, and adenovirus as well as toxoplasmosis; other infections, such as bacterial ascending cholangitis; and noninfectious causes, such as ischemic injury, vascular thrombosis, hemolysis, rejection, and hepatitis induced by drugs or toxins. In neonates with a significant and persistent direct hyperbilirubinemia, the diagnosis of biliary atresia should be considered as well.

Gastrointestinal Disease

Serious gastrointestinal disease causing esophagitis, gastritis, gastric outlet obstruction, gastroenteritis, pyloric and small bowel obstruction, duodenitis, colitis, proctitis, pancreatitis, hemorrhage, and acalculous cholecystitis has been associated with CMV infection in immunocompromised persons, especially patients with AIDS or neoplastic disorders and those who have undergone bone marrow, kidney, intestinal, or liver transplantation.* Rarely, self-limited CMV gastroenteritis, colitis, and proctitis have been associated with CMV mononucleosis syndrome in apparently normal individuals.* CMV enterocolitis has also been reported as a cause of intractable diarrhea, that often is bloody, in infants with congenital and postnatal CMV disease.† Characteristic signs and symptoms in infants and children include nausea, vomiting, dysphagia, epigastric pain and tenderness, delayed gastric emptying, watery guaiac-positive stools or gastrointestinal hemorrhage, and disaccharide and monosaccharide intolerance.[275,286,440] Severe disease may cause dehydration and failure to thrive. Endoscopy with biopsy is required to establish a definitive diagnosis and usually shows linear, localized, or punctate ulcers. Hemorrhagic lesions or diffuse erosion can occur in severe disease. Characteristic cytomegalic inclusion cells can be seen in the gastrointestinal endothelium, epithelium, and glandular tissue; CMV may be cultured from stool or biopsy specimens; or CMV DNA may be detected by PCR-based methods.[174] In addition, these patients often are viremic with CMV DNA by PCR detectable in their blood or plasma and occasionally have evidence of disseminated CMV infection with involvement of the lungs and retina. Infants with severe end organ disease caused by CMV should receive an immunologic workup to exclude primary immune deficiency of T-cell or NK-cell function.

The differential diagnosis of CMV colitis includes infection with other viruses, especially HSV and adenovirus, and infection with bacteria, particularly *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia*, as well as *Clostridium difficile* and *Mycobacterium avium-intracellulare*. Parasitic infection with *Cryptosporidium*, *Giardia*, and amebae also should be excluded. The differential diagnosis of CMV esophagitis and gastritis includes HSV infection, *Candida* esophagitis, reflux esophagitis, and peptic ulcer disease.

Meningoencephalitis and Other Neurologic Disorders

CNS involvement is well described and occurs relatively frequently in infants with symptomatic congenital CMV infection. Although the severity of damage to the CNS during congenital infection varies greatly, postmortem examination of severely affected infants has demonstrated necrotizing encephalitis, especially in the deep periventricular structures, and scattered areas of necrosis and inclusion-bearing cells. Although direct viral infection of neural structures probably plays a major role in CNS disease in congenital CMV infection, infectious vasculitis also may occur. In addition, because congenital CMV disease can be associated with marked thrombocytopenia, intracranial hemorrhage can contribute to CMV-related CNS injury.[34,73,196,443] Clinical manifestations of this disease process include microcephaly, cerebral palsy, intracerebral calcifications, seizures, hemiparesis, developmental delay, ventriculomegaly, paraventricular cysts, intraventricular strands, periventricular leukomalacia, lissencephaly-pachygyria, porencephaly, schizencephaly, cortical malformations such as

*References 126, 275, 286, 342, 440.

*References 16, 100, 126, 207, 286, 344, 345.

†References 125, 126, 162, 211, 275, 342, 343, 366.

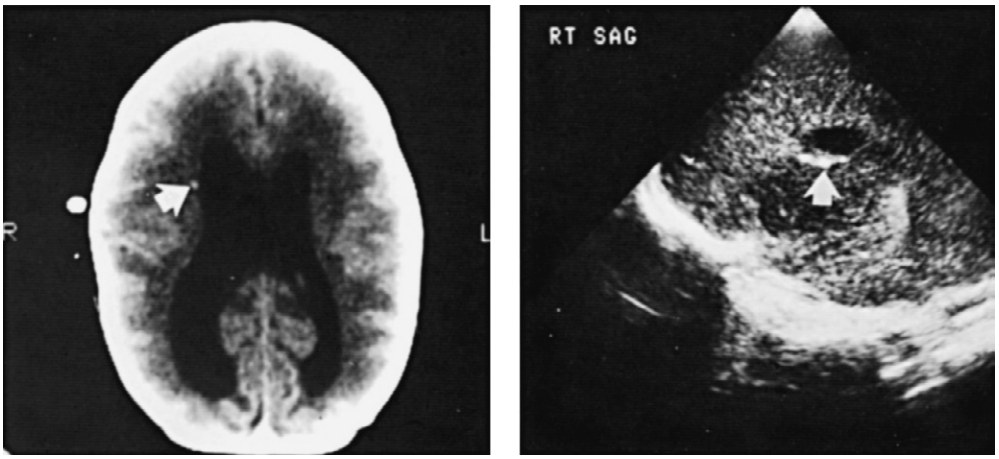

FIGURE 158-3 ■ CT scan *(left)* and ultrasound examination *(right)* of the head of an infant with congenital cytomegalovirus disease. Both tests showed moderate asymmetric enlargement of the lateral ventricles with punctate periventricular calcifications.

polymicrogyria, multifocal deep white matter lesions and gliosis, atrophy from cerebral volume loss, parenchymal and ependymal cysts, periventricular cysts, cerebellar lesions, meningoencephalitis, and sensorineural deafness (Fig. 158-3).[90,247,306,425,438] Remarkably, despite the well-documented neuropathologic process in congenital disease, isolation of CMV from the cerebrospinal fluid (CSF) of a congenitally infected child is an extremely unusual finding.[216] CMV DNA has been detected in the CSF of congenitally infected infants, and its presence at birth appeared to identify infants at risk for a poor neurodevelopmental outcome.[26]

The differential diagnosis of symptomatic congenital CMV infection with neurologic disease includes congenital toxoplasmosis, congenital HSV infection, congenital rubella syndrome, congenital infection with lymphocytic choriomeningitis virus, brain tumors such as craniopharyngioma, and calcified hematoma. In addition, congenital CMV disease involving the CNS also may be mimicked by genetic disorders such as tuberous sclerosis, Sturge-Weber syndrome, and Aicardi syndrome; metabolic conditions such as hyperthyroidism; α_1-antitrypsin deficiency; galactosemia; peroxisome disorders such as Zellweger syndrome, neonatal adrenoleukodystrophy, and infantile Refsum disease; urea cycle deficiencies; organic acidemias; and liposomal storage disorders.[33] Maternal exposure to drugs and toxins, especially isotretinoin, cocaine, and alcohol, also is included in the differential diagnosis. The presence or absence of intracerebral calcification as well as the pattern of calcifications when they are present may be helpful in distinguishing among these disorders. In addition, the appropriate microbiologic studies, chromosome analysis, metabolic studies, and drug screens should be performed.

In postnatal life, CMV meningoencephalitis appears to be rare yet well documented.[32] It may occur as a complication of CMV mononucleosis, as an isolated manifestation of primary CMV infection in a normal host, or as a primary or recurrent infection in an immunocompromised patient.[345] In infants, it may be the presenting condition of a primary immune deficiency of T-cell or NK-cell function. Symptoms include headache, photophobia, nuchal rigidity, memory deficits, and inability to concentrate. CSF findings include mild mononuclear pleocytosis and slightly elevated protein. Although the virus is isolated from the CSF and brain parenchyma exceedingly rarely, neuropathologic findings of intranuclear inclusions and microglial nodules are characteristic.

CMV encephalitis may complicate adult and pediatric immunocompromised transplant recipients and patients with AIDS.[39] As many as 50 percent of patients with AIDS may have evidence of CMV infection of the CNS at postmortem examination.[296] CMV has been reported to cause a subacute, occasionally progressive encephalitis in patients with AIDS and has been implicated as a cofactor in the pathogenesis of the AIDS dementia complex seen in both adults and children.[108,200,287,397,459] In this disease, CMV may be isolated from CSF or CMV DNA may be detected in the fluid or brain by PCR-based methods.[177] In children, this syndrome is characterized by weakness, confusion, and loss of developmental milestones.

The differential diagnosis of CMV encephalitis and meningoencephalitis in a normal host includes primarily infection with other neurotropic viruses, such as HSV, Epstein-Barr virus, VZV, enterovirus, and arboviruses. Neurosyphilis and tuberculous meningitis also should be considered. In immunocompromised patients, especially those with AIDS, the following should be added to the differential diagnosis of CNS infection: progressive multifocal leukoencephalopathy caused by papovavirus; HIV encephalitis; fungal CNS infection caused by *Cryptococcus neoformans*, *Candida* spp., *Aspergillus*, or *Histoplasma;* protozoal infections with *T. gondii* and, rarely, *P. jiroveci* and *Strongyloides stercoralis;* bacterial infections with *M. avium-intracellulare* and *Nocardia asteroides;* noninfectious diseases such as primary cerebral lymphoma and lymphomatoid granulomatosis; and vascular complications such as hemorrhage and infarction.[20] In immunosuppressed patients, more than one of these conditions can coexist in the CNS.

CMV also can invade the peripheral nervous system and cause a painful peripheral neuropathy in patients with AIDS.[166,365,442] Ascending paralysis caused by myelitis, with or without vasculitis or necrosis, also can occur

and may appear similar to Guillain-Barré syndrome.[442] In addition, CMV polyradiculopathy has been described in adult patients with AIDS and may occur in older children. This disease usually begins as leg pain and sacral paresthesias and may progress to weakness and flaccid paralysis. The CSF characteristically has a polymorphonuclear pleocytosis and moderately elevated protein level.[234] The association of CMV infection with infantile spasms, Guillain-Barré syndrome, Charcot-Marie-Tooth disease, Huntington disease, Alzheimer disease, myasthenia gravis, and neuropsychiatric diseases (e.g., schizophrenia) has been reported, but a definite causal relationship between CMV and these diseases remains to be proved.[32,238]

Deafness and Other Ear Disorders

Hearing loss is present at birth in 25 to 50 percent of infants with symptomatic congenital CMV infection and in approximately 15 percent of infants with asymptomatic congenital CMV infection.[212] Given that congenital CMV infection affects 30,000 to 40,000 infants annually in the United States, this congenital infection probably is the most common cause of nonhereditary sensorineural deafness. Investigators have estimated that congenital CMV infection may account for as many as 40 percent of cases of congenital hearing loss.[40,127,187] Progression or fluctuation of the hearing loss occurs in at least two thirds of these children through the preschool years, and continued progression may occur through the school-age and adolescent years.[159,460] Although CMV has been detected in the endolabyrinth of infants who died of congenital CMV disease, whether this progressive deafness is caused by continued viral replication in the inner ear, reinfection with a new strain of virus, or a complex cascade of immunopathologic events remains unclear.[111] Mondini dysplasia of the temporal bones has been seen in infants with congenital CMV infection, but the importance of this observation relative to the pathogenesis of the progressive hearing loss commonly seen in congenitally infected infants is unknown at this time.[47] Some studies suggest that higher levels of CMV in urine and blood are present in neonates who develop hearing loss from congenital CMV infection,[22,251,364,447] whereas other studies speculate that genetic mutations or inflammatory genes may play a role in CMV-associated hearing loss.[305,309,370,385]

CMV has been isolated from the middle ear effusions of healthy and immunocompromised children with otitis media.[93,141] In addition, CMV infection, defined serologically, has been associated with sudden-onset deafness, acute labyrinthitis, and Meniere syndrome.[420,462,463] Older children who were born with asymptomatic congenital CMV infection also may exhibit differences in higher auditory function, even though they do not have sensorineural or conductive hearing loss.[103]

Myocarditis and Other Cardiovascular Disorders

Myocarditis has been described as a rare complication of severe congenital CMV disease and CMV mononucleosis in presumably otherwise healthy adults and children.[431,450,463] CMV myocarditis also has been seen in renal and heart transplant recipients, usually as part of a disseminated CMV infection and associated with graft rejection treated with high-dose immunosuppressive therapy.[173,341,388] Myocarditis caused by CMV may be present in infants with primary immune deficiency disorders of T-cell and NK-cell function. Patients can have heart failure, cardiomegaly, electrocardiographic abnormalities, and poor left ventricular function on echocardiography; cytomegalic inclusion cells and the presence of CMV DNA can be documented by myocardial biopsy.

The association of CMV infection with other cardiac disorders such as congenital heart block, structural cardiac anomalies, and pericarditis is anecdotal, and a cause-and-effect relationship is not well documented.[227]

Thrombotic phenomena, often serious and life threatening, have been associated with primary and active CMV infection in neonates, infants, adolescents, and adults, as well as transplant recipients and patients with HIV infection/AIDS.[2,114,266] The virus may cause damage to endothelial cells, activate coagulation factors, and induce production of antiphospholipid antibodies.[405]

CMV coronary endotheliitis with superimposed thrombosis and myocardial infarction has been described in adult heart transplant recipients and was reported in an infant who died of disseminated CMV disease with an apical ventricular aneurysm.[285,376] CMV primary or acute infection also has been associated with venous and arterial thrombosis in previously healthy individuals, in patients undergoing solid-organ transplant (especially renal and heart transplant recipients), and in infants with congenital and early postnatal CMV infection.[1,27,266] Primary CMV infection has been associated with pulmonary embolism and portal vein thrombosis in an adolescent with CMV mononucleosis.[1-3,250] CMV infection has been postulated to play a role in the pathogenesis of atherosclerosis and coronary artery disease in animal models, as well as in both normal persons and heart transplant recipients.[179,219,276,279,429] High levels of CMV antibody have also been epidemiologically associated with coronary artery disease in studies in performed in many countries and continents, including India, Finland, the United States, and China.*

CMV also has been associated with hypertension in animal (murine) models, but studies definitely linking CMV to hypertension in humans have not been definitive.[92]

Endocrine System

Histopathologic evidence of involvement of the organs of the endocrine system is described well in both congenital and postnatally acquired disseminated CMV infections.[201] Endocrine disorders such as Graves disease and diabetes insipidus have been associated with congenital CMV infection, but these reports may represent coincidental findings.[280,375] Longitudinal studies are required to determine whether the autoimmune endocrinopathies in children with congenital rubella parallel the findings

*References 1-3, 219, 290, 291, 405.

in children with congenital CMV infection. CMV infection also has been associated with autoimmune type 1 diabetes, although a specific cause-and-effect relationship has not been established.[315] In addition, immunosuppressed patients, especially persons with AIDS, may manifest clinical endocrinopathies caused by CMV infection, such as adrenal insufficiency and adrenal necrosis.[180] CMV inclusions also have been found in the pituitary gland of patients with AIDS, all of whom showed evidence of CMV encephalitis or disseminated infection elsewhere in the body.[150] Moreover, involvement of the thyroid and parathyroid glands with CMV has been reported in adults with AIDS.[163]

Genitourinary System

Disease of the male and female genitourinary system as a result of CMV has been reported. Patients with AIDS may have symptomatic epididymitis and cystitis caused by CMV.[49,347,422] Lower urinary tract infections and hemorrhagic cystitis caused by CMV have been diagnosed in patients after they have undergone stem cell and solid-organ transplantation.[314] CMV also commonly yet asymptomatically infects the cervicovaginal secretions and semen of both healthy and immunosuppressed adults.[222,252]

Skin

Cutaneous manifestations of CMV infection are described well and can occur with both congenital and acquired CMV infection.[259] Infants with symptomatic congenital infection may have nonpalpable petechiae, purpura, or bruises, usually as a result of thrombocytopenia. Violaceous or dark, magenta infiltrative papules or nodules, called "blueberry muffin" lesions, also may occur, but these lesions are more characteristic of congenital rubella syndrome. The skin lesions associated with acquired CMV infection usually are localized cutaneous ulcers or a widespread, exanthematous, maculopapular eruption, although vesiculobullous lesions also have been described.[326] CMV mononucleosis syndrome in adults and children may be accompanied by a maculopapular, rubelliform rash that may be pruritic. In addition, ampicillin-associated rashes may occur with CMV mononucleosis. CMV also may cause a cutaneous, leukoblastic vasculitis.[377] Well-demarcated, ulcerated lesions that show histopathologic evidence of CMV infection may be seen in immunocompromised patients who have undergone transplantation or in those who suffer from AIDS. Finally, CMV may play a cofactor role with human herpesvirus type 8 in the neoplastic process in Kaposi sarcoma, but definitive proof of a causal relationship remains to be shown.

Unusual Associations

CMV infection, either congenital or acquired, has been detected in association with a wide variety of conditions, including defects in tooth structure and the formation of enamel, arterial or venous thrombosis, atherosclerosis, portal vein thrombosis associated with protein S and protein C deficiency, unexplained fevers in burn patients, bacterial sepsis in burn patients, congenital eventration of the diaphragm, inguinal hernia, and fatal *Staphylococcus epidermidis* infection in very low-birth-weight infants.* CMV infection also may be involved in the genesis and maintenance of immunopathologic phenomena, such as allograft rejection in solid-organ transplant recipients and graft-versus-host disease in bone marrow and stem cell transplant recipients, as well as autoimmune diseases.[246,438] Case reports have linked primary CMV infection with the onset of autoimmune disorders or recurrent CMV infection with worsening of autoimmune disease.[192,328,439] CMV also has been associated with hemophagocytic syndrome in adults and children.[246] However, given the common occurrence of CMV infection, it is difficult to determine if the relationships are coincidental or comorbid, rather than part of a cause-and-effect relationship.

LABORATORY DIAGNOSIS

Detection of the Infectious Agent

CMV can be isolated in cell culture with fibroblast cell lines such as human foreskin fibroblasts and human embryonic lung fibroblasts.[384] Specimens that contain a high titer of virus, such as those from congenitally infected infants, may show growth in 24 hours. Some specimens, such as those from persons with acquired asymptomatic infection, may require as long as 6 weeks for detectable growth, but most cultures grow in 1 to 2 weeks. CMV has been isolated from a variety of specimens, including urine, saliva, nasopharyngeal and sinus washings, conjunctiva, tears, middle ear fluid, breast milk, semen, cervicovaginal secretions, stool, CSF, peripheral white blood cells and plasma, amniotic fluid, bronchial lavage samples, and biopsy or autopsy specimens. All samples for isolation of viruses (except blood, which should be at room temperature) should be held at 4° C (on wet ice or in a refrigerator) until processed in the virology laboratory. Specimens for isolation of virus should be inoculated within hours of collection for an optimal isolation rate. Although isolation of CMV proves that a productive infection is present, it does not confirm an etiologic relationship with the disease process and requires careful interpretation within the patient's clinical context.

An adaptation of tissue culture is a low-speed centrifugation enhancement, monoclonal antibody culture technique, also called a shell vial assay. In this test, inoculated tissue culture cells in small vials are stained with a fluorescein-conjugated monoclonal antibody to either an early or a late CMV antigen (or both). Cells infected with CMV exhibit nuclear and membrane fluorescence 18 to 72 hours after inoculation. This rapid viral diagnostic technique is especially reliable with urine and bronchoalveolar lavage specimens and has been applied with variable results to blood and tissue specimens.[171] However,

*References 1, 3, 23, 27, 48, 191, 225, 243, 250, 264, 267, 413, 429.

maximal sensitivity and specificity are obtained when shell vials are used as an adjunct to and not in place of routine tissue culture. CMV-infected cells also can be detected by direct immunofluorescence assay on exfoliated cells in bronchoalveolar lavage specimens or in frozen tissue specimens.[193] These procedures, however, require a laboratory experienced in direct immunofluorescence assay technique.

Real-time PCR-based methods are available in many laboratories for detection and quantitation of CMV DNA. These very sensitive tests may be used to detect and to quantify CMV DNA in a variety of samples and are useful for diagnosis or prediction of CMV disease, as well as for monitoring response to antiviral treatment.*

Detection of CMV DNA in the CSF of immunecompromised patients with AIDS, of patients with primary immune disorders, and of transplant recipients seems to be a reliable diagnostic method for detection of CMV infection of the CNS, and detection of CMV DNA in the CSF of neonates with congenital CMV disease correlates with poor neurodevelopmental outcome.[26,98,177,467] Similarly, detection of CMV DNA by PCR analysis in vitreous fluid provides persuasive evidence that a patient's retinitis is CMV related.[135] CMV DNA also may be detected in the peripheral white blood cells and plasma of patients with CMV infection, and detection of CMV DNA in the plasma or serum of neonates and immunocompromised patients appears to correlate with severity of disease and viral dissemination.[295,391,408,409] Detection of CMV DNA in amniotic fluid provides prenatal diagnosis of congenital CMV infection and, when correlated with fetal ultrasonography and other clinical evaluations of the fetus, supports the presence of CMV associated in utero disease.[263] PCR assays also have detected CMV DNA in dried blood spots or Guthrie cards collected, as well as in urine and saliva, as part of neonatal screening programs and used to diagnose congenital CMV infection and disease.[40,44,58,59,447]

Another use for PCR-based diagnosis of CMV infection is the very early diagnosis of CMV infection in high-risk patients, such as transplant recipients, before the development of potentially fatal CMV disease, such as pneumonitis. This approach may allow preemptive antiviral therapy to be initiated when CMV infection appears active but before overt disease is detected.[68,468] CMV quantitative DNA detection by PCR assay is now standard for monitoring a patient's response to antiviral therapy and should be correlated with the resolution of CMV-related clinical symptoms.[131,168]

CMV pp65 antigen also may be detected in the white blood cells of patients with CMV infection and disease, and this CMV antigenemia test is still used by clinical laboratories as a rapid screen for CMV viremia, providing results the same day. It is relatively easy to perform, and the degree of antigenemia may be quantitated to monitor response to antiviral therapy.[131]

Exfoliated cells in urine or bronchoalveolar lavage specimens or cells in tissue obtained by biopsy can be examined for histologic evidence of CMV infection. Cells that are infected productively with CMV are enlarged, have type A Cowdry intranuclear inclusions, and occasionally have perinuclear inclusions. The appearance of these cells is characteristic and has been likened to owl's eyes. Immunohistochemical staining with immunoperoxidase can be used to augment the detection of these typical cells. The presence of these cells correlates with the presence of active CMV disease and may be useful clinically.

Serology

Standard serologic techniques also can be applied to the diagnosis of CMV infections. CMV IgG antibody can be determined in serum by several different methods, including complement fixation, hemagglutination inhibition, indirect fluorescent antibody assay, anticomplement immunofluorescence assay, enzyme-linked immunosorbent assay (ELISA), and latex agglutination and neutralization tests. ELISA and the indirect fluorescent antibody assay are used most commonly in clinical virology laboratories. The presence of CMV IgG antibody in a single serum specimen implies that the patient at some time has been infected with CMV. On the other hand, a negative IgG antibody determination is good evidence against current or past CMV infection because CMV antibody usually is present at the time of infection and persists for life. Severely immunocompromised patients, especially bone marrow transplant recipients, or infants and children with a primary immune deficiency, however, can lose their ability to make IgG antibody and become CMV seronegative, even though they are infected actively with CMV. This occurrence has a poor prognosis and requires immediate reconstitution with antibody and aggressive antiviral therapy until immune reconstitution is achieved. Primary infection with CMV is documented best by clear seroconversion from negative to positive CMV IgG antibody. A fourfold rise in CMV IgG antibody titer is not diagnostic of a primary infection because reactivation or reinfection also can cause titers to fluctuate. In addition, the height of the titer or ELISA index in a single serum specimen is not diagnostic of a recent infection or indicative of severity of CMV disease.

CMV IgM antibody can be determined in serum by radioimmunoassay, indirect fluorescent antibody assay, or ELISA. Both the indirect fluorescent antibody assay and ELISA are used commonly in clinical laboratories, although some indirect fluorescent antibody assays have a considerable false-positive rate. Accurate interpretation of CMV IgM antibody results requires knowledge of the methods used and careful consideration of the clinical context to exclude diseases that produce cross-reacting antibody or polyclonal responses. Test methods also should remove rheumatoid factor from the test serum, a common cause of false-positive IgM reactions. If the test is performed properly, the presence of CMV IgM antibody implies a current or recent primary CMV infection. In healthy adults, CMV IgM antibody usually persists for 6 weeks and may be present for as long as 3 to 12 months, rarely even longer, after the primary infection.[119] Commercial immunoblot and avidity assays may be used to supplement conventional CMV IgG and IgM serology.[253]

*References 69, 83, 110, 117, 170, 397, 402, 409.

The CMV IgG avidity index and IgM immunoblot assay is now available in clinical reference laboratories in many countries and continents, including Europe, Asia, and the United States. This testing may be used to help time a suspected primary infection with CMV.[143,254,346,357] A low avidity index (30%) suggests a recent primary infection, usually within 3 months, because low-to-moderate CMV avidity anti-CMV antibody may persist for 18 to 20 weeks after primary CMV infection.[254] A high avidity index (above 60%) suggests a past or recurrent CMV infection.[178] There are several manufacturers of these supplemental CMV assays, and results may vary between manufacturers, so interpretation should be within clinical context and in concert with other CMV testing, if available.[346] In immunocompromised adults experiencing clinically significant reactivation infection with CMV, CMV IgM antibody may be detected for prolonged periods.[297] The sensitivity and specificity of CMV IgM antibody determination in the diagnosis of acquired, primary CMV infection or clinically significant reactivation infection in infants and children have not been studied systematically, although clinicians frequently use this test for this purpose.

Western blot assays using viral structural proteins separated from purified viral particles or from recombinant viral proteins appear to be sensitive and specific for detection of glycoprotein-specific CMV IgG and IgM. Currently, these tests are available only in research or reference laboratories.[255]

Laboratory Diagnosis of Specific Clinical Syndromes

Pregnancy

Diagnosis of primary maternal CMV infection is best made by de novo seroconversion of virus-specific CMV IgG antibody or by the presence of both CMV IgG antibody and CMV IgM antibody associated with a low IgG avidity index.[477] For timing of the onset of CMV infection (before or in early pregnancy), CMV avidity testing is most helpful if performed during the first or early second trimester.[143] In primary maternal CMV infection, the risk for transmission of CMV to the fetus is approximately 40 percent, and it is up to 25 percent overall to the fetus for development of sequelae of some type. A maternal recurrent infection may result from reactivation of the woman's own endogenous strain(s) of CMV or by reinfection with a new strain of CMV. Recurrent CMV infection is difficult to diagnosis prenatally and may be considered if there is a significant rise of CMV IgG antibody, with the presence of IgM and a high avidity IgG index. The diagnosis of recurrent CMV infection in the mother can also be considered if she is CMV IgG antibody positive and CMV IgM antibody negative at the beginning of pregnancy yet gives birth to a congenitally infected but usually asymptomatic neonate. Routine serologic screening of pregnant women for CMV infection is controversial; and although recommended by some experts, it is not currently performed in the United States or Canada.[260] However, selective serologic screening for diagnosis should be performed for women who request it, and for those women in high-risk circumstances, including those with clinical evidence of CMV infection, such as mononucleosis during pregnancy or detection of fetal abnormalities on screening ultrasonography suggestive of in utero CMV disease.[128] Serologic screening and counseling may also be considered for child care workers and for women with a young child or toddler in the household. Prenatal diagnosis of in utero CMV infection of the fetus is best accomplished by amniocentesis, which should be done at least 7 weeks after the presumed time of maternal primary CMV infection and after 21 weeks' gestation.[205,368] This interval is important because it may take 5 to 7 weeks (at least 2 weeks) after maternal infection to produce fetal infection and subsequent replication in the kidney for a detectable quantity of CMV to be detected in the amniotic fluid.[205] Amniotic fluid may be sent for CMV culture or detection of CMV DNA by qualitative or quantitative PCR. Quantitative determination of CMV DNA in amniotic fluid that shows very high levels of DNA may predict more serious disease in the fetus and poorer outcome in the neonate. After diagnosis of maternal primary or recurrent CMV infection, monitoring of the fetus by serial ultrasound examinations should be performed every 2 to 4 weeks to detect abnormalities, which may aid in management.

Congenital Infection

Viral culture or CMV culture is the traditional diagnostic test for congenital infection with CMV.[115] The diagnosis is established by isolation of the virus from urine or saliva in the infant's first 1 to 3 weeks of life. Cell culture of urine or saliva has been used successfully to screen large numbers of neonates for congenital CMV, as well as diagnose individual patients clinically.[115,121,311,412,481] Urine cultures obtained after 3 weeks of life must be interpreted cautiously because perinatally acquired and transfusion-acquired infections with CMV may be manifested as early as 3 weeks of age.[378] Detection of nuclear inclusion-bearing renal epithelial cells in urinary sediment collected in the first 2 weeks of life also implies the presence of congenital infection. This technique is insensitive, however, compared with cell culture and is important only historically. Detection of CMV DNA in the urine, saliva, blood, or CSF of neonates by DNA PCR amplification techniques correlates with congenital infection and disease, and this method is currently used by many clinicians and laboratories for diagnosis of congenital CMV.[26,67,117,295] Detection of CMV DNA in dried blood spots (DBS) or Guthrie cards collected for neonatal metabolic screening also has been used for the retrospective and prospective diagnosis of congenital CMV infection and disease.[42,43,45,470] However, this method may not detect all congenitally infected neonates, especially those with silent or asymptomatic infection, because the level of CMV in blood is lower in asymptomatic congenital CMV infection. Studies evaluating real-time PCR assays performed on DNA extracted from DBS samples used to screen neonates for other congenital conditions have mostly shown DBS relatively insensitive for neonatal screening programs when compared with traditional

methods but possibly are useful for identification of neonates with symptomatic disease or at high risk for sequelae.[58,258,470] Genotyping may be performed by PCR assays on DNA extracted from DBS samples, providing a tool to study the possible association of CMV gB or gH genotype with disease in neonates.[121,123] Real-time PCR assays performed on saliva specimens (both liquid and dried) have shown high sensitivity compared with traditional saliva rapid culture and may be a potential screening tool for diagnosis of congenital CMV in neonates.* Neonates with high CMV DNA levels by quantitative PCR methods appear to be at highest risk for severe illness in the neonatal period and for sequelae such as progressive or late-onset hearing loss and, therefore, are candidates for CMV antiviral therapy.[251,447]

The diagnosis of fetal intrauterine CMV disease in single, twin, and multiple births also may be established by isolating the virus from amniotic fluid or detection of CMV DNA by PCR amplification in amniotic fluid obtained at least 2 weeks after the suspected time of maternal primary infection and after 21 weeks' gestation.[196,368,478] Fetal condition also can be determined by serial fetal ultrasound examinations or fetal MRI in special circumstances.[98,159,269] Fetal blood samples that show the presence of CMV DNA by PCR assay, CMV IgM antibody, thrombocytopenia, or leukopenia or elevated liver function test results also provide supportive evidence of CMV-associated fetal disease and may predict severe disease. Prenatal screening of pregnant women and prenatal diagnosis of congenital CMV infection should be accompanied by consultation with a maternal fetal medicine specialist as well as a pediatric infectious diseases specialist and a prenatal counselor knowledgeable about all the possible outcomes that can occur and all the options that are available to the parents and physician.[142,299,357]

Standard serologic tests also can be applied to diagnose congenital infection with CMV, but this approach is cumbersome, retrospective, and rarely used clinically. The absence of CMV IgG antibody in cord blood rules out congenital infection, whereas its presence may imply passive transfer from the mother or indicate a congenital infection. Serial serologic specimens also can be obtained when the infant is 1, 3, and 6 months of age. If CMV IgG antibody levels disappear during the infant's first months of life, congenital infection is ruled out. However, if CMV IgG antibody persists, the infant either was infected congenitally or acquired CMV infection perinatally or postnatally. The presence of CMV IgM antibody in cord or infant blood collected in the first 3 weeks of the infant's life suggests the diagnosis of congenital CMV infection.[184,414] However, CMV IgM antibody may be insensitive (22%) compared with urine or saliva CMV culture or CMV DNA detection for the diagnosis of congenital infection.[295]

Perinatal and Postnatal Infection

The diagnosis of perinatal infection with CMV is difficult to establish but is documented best by a negative CMV viral culture or negative CMV DNA PCR analysis of urine or saliva and negative CMV IgM antibody level at birth, followed by a positive viral culture and positive CMV IgM antibody at 8 to 16 weeks of age and persistence of CMV IgG antibody beyond 4 to 6 months of age. Postnatal primary CMV infection is diagnosed by CMV IgG seroconversion, the presence of CMV IgM antibody, and CMV excretion or "shedding" detected by culture or PCR in saliva, urine, and other body fluids.

Cytomegalovirus Syndromes in Immunocompromised Hosts

In immunocompromised patients, determination of whether serologic or virologic evidence of active CMV infection correlates with disease is difficult because CMV commonly is shed from saliva, urine, and respiratory tract secretions in these patients without clear evidence of a disease process. Therefore, detection of a productive virus infection in the organ system suspected to be involved usually is often necessary to establish the diagnosis of CMV disease in an immunocompromised patient. For example, interstitial pneumonitis caused by CMV is documented best by an open-lung biopsy specimen that shows characteristic CMV histopathologic features and positive viral culture.[107] Detection of CMV in bronchoalveolar lavage specimens also correlates with lung biopsy results.[107,170] Similarly, the diagnosis of CMV hepatitis or colitis is documented best by the presence of cytomegalic inclusion cells, isolation of CMV, or detection of CMV by DNA PCR or immunohistochemical stains from biopsy specimens. If biopsy of suspected organ is not feasible or practical, then detection of CMV viremia or rising titers of CMV DNA by quantitative PCR can support the diagnosis, because it often precedes or accompanies serious disease, such as CMV pneumonitis in bone marrow or solid-organ transplant recipients, CMV retinitis or colitis in patients with AIDS, or disseminated disease in infants with primary immune deficiency syndromes.[168,409,441]

Prospective monitoring of high-risk patients by serial samples of blood or plasma for quantitative CMV DNA PCR analysis or, alternatively, by CMV cultures of urine or blood or bronchial lavage samples and collection of serum to detect seroconversion by serologic testing is indicated and often performed because positive tests indicate the need for preemptive therapy with a CMV-specific antiviral agent, such as ganciclovir or valganciclovir, when CMV infection is active but before overt disease develops.[168,349,383,430]

TREATMENT

Antiviral agents with activity against CMV include derivatives of acyclovir, such as ganciclovir and valganciclovir, as well as foscarnet and cidofovir (Fig. 158-4).* Investigational agents, such as maribavir, are in clinical trials to determine safety, antiviral effects, and efficacy for treatment and prevention of CMV infection in transplant

*References 42, 43, 45, 59, 401, 438, 471, 472.

*References 15, 72, 75, 226, 257, 363, 404, 421, 451.

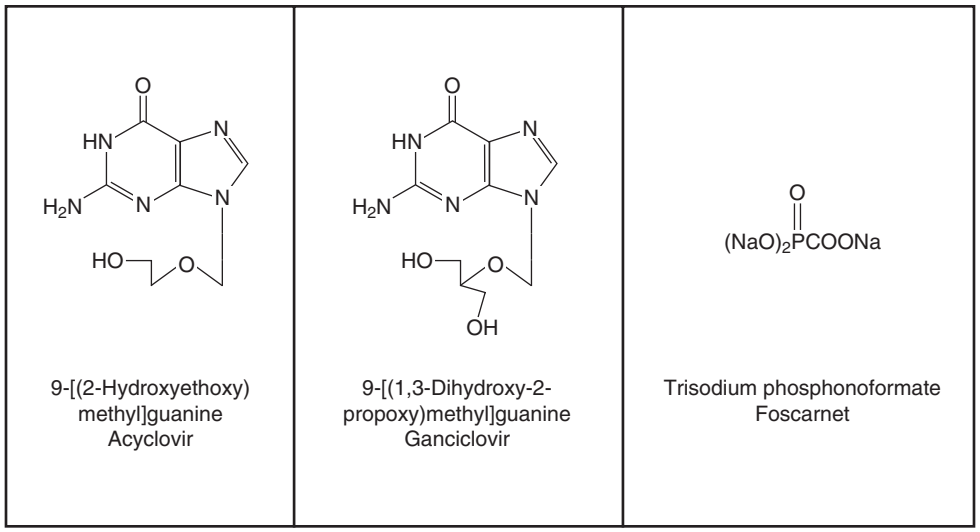

FIGURE 158-4 ■ Structures of acyclovir, ganciclovir, foscarnet.

recipients and possibly, in the near future, for congenital CMV disease.[28,273,274,464,465] Acyclovir and valacyclovir also may have limited activity against CMV under certain conditions. In addition, biological agents such as immune globulin and CMV hyperimmune globulin may benefit selected patients and novel approaches such as cell-based, cytotoxic T-cell adoptive immunotherapy also are being investigated in clinical trials.* Treatment is beneficial for adult and pediatric immunocompromised hosts as well as for neonates with congenital CMV disease.† Normal hosts with severe CMV disease also may benefit from antiviral therapy.[345]

Treatment of CMV-associated disease, such as retinitis, pneumonitis, hepatitis, colitis, esophagitis, or encephalitis, in immunocompromised hosts, such as those patients with primary immune deficiency disorders, AIDS, or transplant recipients usually involves a 2- to 3-week period of induction therapy with an intravenous antiviral medication, usually ganciclovir, as first-line treatment.‡ In special circumstances, such as suspected drug resistance or marrow suppression, foscarnet or cidofovir may be indicated.[144,423] The administration of ganciclovir plus foscarnet or cidofovir is advocated by some experts for treatment of CMV disease in infants with primary immune deficiency of T-cell or NK-cell function, to avoid emergence of resistant strains, until immune reconstitution is achieved. The induction period should be accompanied by clinical improvement and a virologic response, with reduction in the blood quantitative CMV DNA PCR titers. If the host is expected to remain severely immunocompromised after receiving successful induction therapy, or is awaiting immune reconstitution, maintenance therapy at the same dosage as treatment levels or, alternatively, a reduced dosage schedule, three to five times a week, administered intravenously or orally is indicated through the expected period of immune suppression. In patients with AIDS, primary immune disorders of T-cell or NK-cell function, solid-organ transplant recipients receiving high levels of immunosuppression, and bone marrow/stem cell transplant recipients who have not yet engrafted and shown T-cell function may receive full doses of antiviral medication as maintenance therapy. Such therapy is usually continued indefinitely, or until immune reconstitution or resolution of immune suppression.[132] In patients with AIDS or other immune deficiency conditions who have refractory CMV retinitis, treatment with a ganciclovir implant by an ophthalmologist may augment systemic therapy, but published experience evaluating this approach in infants and children is limited.[80,435] Valganciclovir, an orally bioavailable prodrug of ganciclovir, appears to be as effective as is intravenous ganciclovir for induction therapy for CMV retinitis and for mild to moderate CMV disease in solid-organ transplant recipients.[139,271] Clinical experience has shown valganciclovir is also useful for other CMV-associated illnesses in mild to moderately ill patients. If, despite administration of antiviral therapy, CMV disease persists or progresses or if a virologic response does not occur, drug resistance should be considered. Resistance to ganciclovir has been documented most commonly in patients with AIDS, but it also can occur in patients with primary immune deficiency disorders of T-cell and NK-cell function or malignant neoplasms and in solid-organ and stem cell transplant recipients.[96,213] Most ganciclovir-resistant CMV strains have a mutation in the UL97 phosphotransferase gene, but specific mutations in the UL54 DNA polymerase gene also may occur alone or in combination with UL97 mutations.[85] Most ganciclovir-resistant CMV strains are susceptible to foscarnet but may exhibit cross resistance to cidofovir, and strains simultaneously resistant to ganciclovir, cidofovir, and foscarnet also rarely may occur.[97,423] Reports of repopulation of previously susceptible wild-type strain of CMV may occur after discontinuation of ganciclovir in patients in whom a ganciclovir strain has emerged.[133] Resistant strains of CMV may

*References 21, 51, 195, 298, 339, 434.
†References 70, 107, 124, 148, 157, 189, 198, 203, 229, 235-239, 262, 301, 351, 354.
‡References 70, 107, 124, 157, 189, 214, 229, 451.

compartmentalize in the eye or brain and cause progressive disease, even if plasma and peripheral sites appear to harbor susceptible strains.

The association of CMV immune globulin administration and acute and long-term clinical outcomes in transplantation has not been well defined.[46,140,154] The administration of ganciclovir along with intravenous immune globulin or CMV hyperimmune globulin to bone marrow transplant recipients with CMV pneumonitis has been shown to increase survival over that of historical controls who were treated with a variety of other antiviral regimens, including ganciclovir alone, immune globulin alone, acyclovir, vidarabine, and interferon.[140] However, differences in the method used to diagnose CMV pneumonitis, duration of illness, and treatment regimens between study subjects and historical controls obscure interpretation of these studies.[108,263] Nonetheless, CMV-associated interstitial pneumonitis in bone marrow transplant recipients, and other types of CMV disease in immune compromised hosts, may be an immunopathologic process, and combination therapy may prevent active virus replication (ganciclovir, foscarnet, cidofovir) while enhancing immune responsiveness of cellular immunity in immune globulin–deficient patients or blunting the immune response to viral antigens already expressed on CMV-infected cells.[215,389] CMV hyperimmune globulin administered to liver transplant recipients has been shown to increase the risk for organ rejection but results in greater long-term graft and patient survival.[154] In pediatric and adult heart transplant recipients, administration of CMV hyperimmune globulin with or without antiviral prophylaxis is also associated with decreased mortality and graft loss.[398,399]

Oral maribavir has been reported in clinical trials to be possibly effective for CMV prophylaxis for prevention of CMV infection in stem cell transplant recipients and treatment of refractory or drug-resistant CMV in transplant recipients.[434,466] Clinical trials for treatment of congenital CMV infection may also be conducted. Standard immune globulin (IVIG) or CMV hyperimmune globulin, although possibly beneficial in selected patients when combined with specific antiviral therapy, should not be used alone for the treatment of established CMV infections in immunocompromised patients.[154,278,398] The combination of high-dose corticosteroids with ganciclovir does not appear to improve survival in bone marrow transplant recipients with biopsy-proven CMV pneumonitis.[352] On the other hand, a regimen combining ganciclovir with hematopoietic growth factor may allow ganciclovir to be continued in selected patients with mild marrow toxicity.

CMV hyperimmune globulin or CMV-enriched immune globulin has been shown to be of possible benefit during pregnancy in women experiencing a primary CMV infection. Case series show it appears to reduce the risk for transmission of CMV to the fetus when administered during a primary maternal infection during pregnancy, may reverse fetal abnormalities, and may improve outcome in the CMV-infected fetus with in utero disease.[74,248,298,444] Randomized clinical trials are now in progress. Treatment of infants with symptomatic congenital CMV infection with the antiviral agent floxuridine was reported first by Feigin and colleagues in 1969 and 1971.[147] More recent reports suggest that antiviral agents, such as ganciclovir and valganciclovir, benefit congenitally infected infants.* Anecdotal reports, case series, and randomized clinical trials have shown clinical and virologic improvement in congenitally infected neonates treated with ganciclovir and valganciclovir.† A multicenter phase I/II study of the pharmacokinetics, antiviral effects, and safety of intravenous ganciclovir was conducted in 47 neonates with symptomatic congenital CMV disease and neurologic involvement.[433,458,484] In this study, a 6-mg/kg dose of ganciclovir administered by a 1-hour infusion every 12 hours for a period of 6 weeks produced a significant reduction in the quantity of urinary virus excretion; however, viral shedding recurred when ganciclovir treatment was discontinued. Neutropenia, thrombocytopenia, and elevated levels of liver enzymes also were observed. Limited clinical follow-up in 30 of the original 47 infants showed that hearing loss improved or stabilized in 16 percent of the treated infants. Subsequently, a phase III, multicenter, randomized clinical trial enrolled 100 infants and was completed in 1999.[237] In this study, ganciclovir treatment in the neonatal period appeared to have an impact on hearing loss by slightly improving hearing, maintaining normal hearing, or preventing hearing deterioration, as measured when the infants reached 6 and 12 months of age. Because only 44 percent of the enrolled infants were assessed in follow-up at 1 year of age, a long-term follow-up study of treated infants is needed to determine whether the early beneficial effects last through childhood and adolescence. In addition to an effect on hearing loss, treatment provided more rapid resolution of hepatitis, when present, and improved short-term growth in weight and head circumference and developmental milestones.[310,311] Currently, most experts agree antiviral treatment is indicated in neonates with congenital CMV disease with neurologic involvement and/or eye disease and/or sensorineural hearing loss. Some experts recommend treatment of all symptomatic neonates to reduce the risk for late-onset hearing loss and improve neurodevelopmental outcome. For maximum benefit, treatment should begin within the first month of life.

The decision to administer antiviral therapy to a neonate with congenital CMV disease remains at the discretion of the clinician and should be based on disease severity and risk factors for sequelae.[395,428,456] Short-term antiviral therapy is likely to benefit infants who have multisystem disease with viremia detected by positive CMV quantitative DNA PCR assay, pneumonia, severe or persistent thrombocytopenia, hepatitis, enterocolitis, or active sight-threatening retinitis.[211] In these clinical circumstances, a reduction in viral load should improve the infant's clinical condition and resolve end-organ disease. However, the sustained effect beyond 1 year of life of short-term treatment on long-term sequelae, such as hearing loss and neurodevelopmental disabilities, is not clear at this time and requires long-term follow-up studies to confirm this benefit.[71] A longer duration of therapy for 6 months or longer, with an oral antiviral agent such as valganciclovir, may improve outcomes and

*References 71, 249, 283, 284, 294, 310, 311, 380, 476.
†References 202, 300, 311, 355, 428, 436.

reduce the development of long-term sequelae, including hearing loss, in these infants.[19,428,456] Anecdotal reports and phase I pharmacokinetic and pharmacodynamic studies have demonstrated that valganciclovir oral solution provides plasma concentrations and antiviral effects similar to those levels achievable with use of intravenously administered ganciclovir in neonates.[236,289] Clinical trials evaluating the long-term benefits of prolonged treatment with oral valganciclovir in congenitally infected infants are needed. Case series of children with asymptomatic congenital CMV infection and hearing loss treated with ganciclovir and valganciclovir have reported improvement in hearing loss with treatment.[198,476] In addition to specific antiviral therapy, management of congenitally infected infants includes supportive care, nutritional and feeding support, and control of seizures. If thrombocytopenia is severe and persistent, platelet transfusions and immune globulin also is of benefit. Long-term management includes careful attention to nutrition to ensure adequate growth, serial hearing tests to detect progressive or late-onset hearing loss, developmental assessments to evaluate for cognitive and motor disabilities, physical therapy, occupational therapy, and ophthalmologic evaluations to follow up abnormalities present in the neonatal period, such as retinitis, and to monitor for vision-threatening, late-onset sequelae.[456,460]

PREVENTION

Prevention of CMV disease is important because it causes significant morbidity and mortality in a variety of patients. In immunocompromised hosts, it also is associated with the development of other complications, such as opportunistic coinfections and graft rejection in transplant recipients, and it increases resource use in transplant programs. In neonates and infants with primary immune deficiency disorders of T-cell and NK-cell function, congenital or acquired CMV infection is sight and life threatening. In premature neonates, potentially fatal viral sepsis syndromes can occur. Furthermore, congenital infection is a leading cause of deafness, vision loss, and developmental disabilities in children. Approaches to the prevention of CMV disease include the use of CMV-seronegative blood products, selection of CMV-seronegative donors for transplant recipients, passive immunoprophylaxis with immune globulin, prophylactic or preemptive use of antiviral agents, active immunization with a CMV vaccine, and behavioral strategies to reduce exposures to CMV-infected secretions.

Blood Product, Human Milk, and Transplant Donor Selection

Transplant recipients who are CMV seronegative and receive solid-organ or bone marrow or stem cell transplants from CMV-seropositive donors are at significant risk for acquiring symptomatic primary CMV infection. Therefore, whenever possible, CMV-seronegative recipients should receive transplants from CMV-seronegative donors and all blood product transfusions should be from CMV-seronegative donors. CMV-seropositive transplant recipients may also experience CMV infection and disease after transplant.

Infection with CMV in seriously ill CMV-seronegative neonates can be prevented by use of blood products from CMV-seronegative donors or by use of frozen deglycerolized red blood cells.[63,475] Saline-washed red blood cells also reduce but do not always prevent the acquisition of CMV infection in neonates, even though as many as 90 percent of the leukocytes can be removed by this method.[116]An alternative method of preparing leukocyte-depleted blood, filtration through a cotton-wool filter, appears to prevent posttransfusion acquisition of CMV infection in neonates.[169] Many institutions now provide CMV-seronegative or leukocyte-depleted blood products routinely to all neonates or to all low-birth-weight neonates, as well as other immune compromised infants and children, regardless of the CMV serostatus of the mother.

Ingestion of CMV-positive breast milk by premature infants, especially those of less than 28 weeks' gestation, has been associated with illness. Therefore, donor selection for CMV seronegative donors or pretreatment through pasteurization or freeze-thaw cycles of human milk administered to extremely premature infants may reduce the risk for acquisition of CMV-associated disease in these infants.* Healthy term infants may receive breast milk from CMV-seropositive mothers and donors.[327]

Passive Immunoprophylaxis

Although immune globulin, CMV hyperimmune globulin, or CMV-enriched immune globulin should not be used alone for the treatment of established CMV disease in immunocompromised patients, these preparations may be used with antiviral agents to treat CMV disease in selected patients and to prevent the acquisition of serious CMV disease in selected immunocompromised patients. The association of CMV immune globulin administration and acute and long-term clinical outcomes in transplantation has not been well defined, but some studies suggest potential benefit.[46,154]

Passive immunization remains controversial, however, partly because studies or protocols have used different dosages (100 to 400 mg/kg) administered at varying intervals (1 week before transplantation and every 1 to 3 weeks after transplantation) for varying times (60 to 120 days). CMV immune globulin has been shown to decrease the incidence of symptomatic CMV disease from 60 to 21 percent in CMV-seronegative renal transplant recipients who received a kidney from a CMV-seropositive donor.[400] CMV immunoprophylaxis also has decreased the incidence of CMV pneumonitis in CMV-seronegative bone marrow transplant recipients who did not receive granulocyte transfusions.[60,281,350,464] CMV hyperimmune globulin administered to liver transplant recipients has been shown to increase the risk for organ rejection but improves long-term graft and patient survival.[154] In pediatric and adult heart transplant recipients, administration of CMV hyperimmune globulin with or without antiviral prophylaxis is also associated with decreased mortality and graft loss.[398,399] A clinical trial with a CMV-specific monoclonal antibody

*References 81, 220, 274, 312, 445, 446.

specific to CMV gH (MSL-109) did not show benefit in a randomized, placebo-controlled, double-blind study in allogeneic stem cell transplant recipients, nor in infants with congenital CMV infection.[53]

The use of immunoglobulin preparations (CMV hyperimmune globulin, CMV-enriched immune globulin, and standard human intravenous immunoglobulin that may contain high titers of CMV IgG) in pregnant women with primary CMV infections to prevent or to ameliorate CMV infection in the fetus has been reported, and results were favorable.* CMV immunoglobulin reduces placental inflammation and thickening, neutralizes virus with high avidity antibodies, and reduces cytokine-mediated cellular immune responses.[13,14,248] In one case-control study, the severity of neurodevelopmental and hearing disabilities in the infants and children of mothers with primary CMV infection who received CMV immunoglobulin was less when compared with those who did not receive CMV immunoglobulin.[298] However, despite these promising results, definitive recommendations for the routine clinical use of CMV immunoglobulin immunoprophylaxis in women experiencing primary CMV infection during pregnancy, or to treat or ameliorate in utero CMV disease in the fetus, await results of randomized clinical trials.[288,299,301,339]

Prophylaxis and Early Preemptive Therapy with Antiviral Agents

The prophylactic use of antiviral agents in transplant recipients reduces the incidence of serious CMV disease.[139] However, no regimen has been shown to completely prevent the acquisition of CMV infection or disease in all types of transplant patients.[327]

Acyclovir is used by some clinicians as prophylaxis for CMV disease in organ transplant recipients, despite evidence that acyclovir is inactive against most strains of CMV and CMV disease occurs despite such prophylaxis.[394] In one study, intravenous administration of acyclovir 500 mg/m^2 of body surface area per dose every 8 hours for 5 days before and 30 days after transplantation to CMV-seropositive bone marrow transplant recipients appeared to reduce the incidence of CMV disease.[282] High-dose oral acyclovir administered 1 day before and for 12 weeks after transplantation also has been shown to reduce the incidence of CMV disease and infection in renal transplant recipients.[38]

The prophylactic administration of human leukocyte interferon-α has been shown to reduce the incidence of severe CMV disease in renal transplant recipients, but it has no apparent benefit in bone marrow transplant recipients, and it is not used routinely in any patient population.[91,199,204,353]

The prophylactic use of intravenous ganciclovir and oral valganciclovir in solid-organ and bone marrow and stem cell transplant recipients at high risk for acquiring serious CMV disease is now routine and favored over preemptive treatment.[139,232,272] Prophylaxis treatment regimens vary, however, and it is difficult to make recommendations about which regimen is best. Most solid-organ transplant recipients appear to benefit from intravenous ganciclovir 5 to 10 mg/kg/day administered once or twice daily for 2 to 6 weeks after transplantation, usually followed by continuing antiviral prophylaxis with a reduced dose of ganciclovir or with orally administered valganciclovir.[30,437] Administration of a short 2-week course of ganciclovir near the time of transplantation, without continuing antiviral prophylaxis, does not appear to reduce significantly the incidence of CMV disease in most solid-organ transplant recipients studied.[30,437] Antiviral prophylaxis not only reduces the risk for serious CMV disease but also improves solid-organ graft survival and overall patient survival.[139] A duration of prophylaxis with oral valganciclovir extended to 200 days compared with 100 days after transplantation appears to significantly reduce the incidence of CMV disease. However delayed-onset CMV disease may still occur and antiviral resistance may emerge in some patients.[232,272]

Intravenous immune globulin or CMV hyperimmune or CMV-enriched immune globulin when it is given with a prophylactic antiviral agent in solid-organ recipients improves allograft and patient survival. In bone marrow transplant recipients, the administration of ganciclovir prophylaxis after marrow engraftment reduces CMV disease. In addition, some ganciclovir-treated bone marrow transplant recipients may experience prolonged neutropenia.[176] Even though most transplant centers have adopted prophylaxis strategies with antiviral agents and CMV hyperimmune globulin to reduce the risk for CMV infection and disease, some experts believe the strategy of preemptive antiviral therapy has certain advantages over strategies that either treat only patients with overt clinical disease or administer prophylactic antiviral agents to many patients at risk, only a few of whom appear to benefit.[167] In addition, preemptive or very early antiviral treatment strategies include viral surveillance of blood, urine, and respiratory samples. Viral surveillance of these samples can be by standard viral culture, viral antigen detection, viral nucleic acid detection by qualitative or quantitative PCR assay, or a combination of these tests. Detection of CMV viremia, or rising quantitative CMV DNA PCR levels, and culture-positive or PCR-positive bronchoalveolar lavage samples have correlated with the development of serious CMV disease in transplant recipients.[68] In bone marrow, stem cell, and solid-organ transplant recipients, early intervention with intravenous ganciclovir therapy at the time of positive CMV surveillance cultures is beneficial.[176,383,396] Another approach to preemptive therapy, that is currently not practiced routinely and that does not entail viral surveillance techniques, is to administer an antiviral agent, such as ganciclovir, only during times of rejection, when CMV reactivation is likely to occur. This approach has been modestly successful in high-risk renal transplant recipients.[197] Simultaneous virologic and immunologic follow-up is the best approach for monitoring CMV infection in transplant recipients. Lack of immune reconstitution or presence of graft rejection or graft-versus-host disease usually heralds repeated episodes of recurrent or persistent CMV infection or disease, requiring multiple or prolonged courses of antiviral and immunoglobulin therapy.[167] Antiviral resistance to single or multiple agents

*References 13, 14, 74, 106, 299, 301, 444.

may occur in these high-risk patients and has been increasingly recognized as a significant problem.[85]

Active Immunization

Prevention of acquisition of CMV disease through active immunization is a priority for the 21st century.[379,419] Universal vaccination of adolescent females to protect their future children against congenital CMV infection has been determined to be cost effective, if a CMV vaccine with at least 61 percent efficacy is available.[122] In addition to pregnant women and their fetuses, transplant recipients would benefit greatly if a safe, effective CMV vaccine became widely available.[24,223,379,381] The ideal CMV vaccine should be safe, effective, immunogenic, and cost effective. It should prevent the development of primary CMV infection without causing chronic persistent infection. The vaccine also should not be capable of infecting the fetus, and it should not be oncogenic. However, because the optimal composition and strategy for an ideal CMV vaccine that would be licensed for clinical use remains to be discovered, research continues in animal models and human clinical trials.[50,172,185,379,382]

In 1975, Plotkin and associates[336] characterized and reported a candidate CMV vaccine strain, Towne 125, that was isolated originally from the urine of a congenitally infected infant named Towne. Since then, more than 500 subjects, including renal transplant recipients and healthy adult male and female volunteers, received the investigational Towne 125 vaccine.* Studies of these subjects showed that Towne 125 is attenuated and relatively safe and that it induces humoral and cellular immunity in both healthy and immunosuppressed subjects.

The Towne 125 vaccine also appeared to be protective in a randomized, placebo-controlled study of 91 immunosuppressed renal transplant recipients.[335] In this study, 30 CMV-seronegative vaccine recipients received a kidney from a CMV-seropositive donor, and the incidence of severe CMV disease was significantly lower in the vaccine group than in the placebo group. However, the CMV infection rate did not differ significantly between the groups, and members of both groups experienced mild to moderate CMV disease. Subsequent studies have confirmed that CMV-seronegative renal transplant recipients who receive a live attenuated CMV vaccine are more resistant to serious CMV disease.[337] Another vaccine strategy investigated was the development of mutant hybrid strains of CMV that combine the safety of the Towne strain with the more virulent and potentially more immunogenic Toledo strain. Early clinical trials demonstrated, enhanced immunogenicity and possibly, however, also enhanced virulence.[12,333,424] Other investigators have evaluated subunit vaccines for CMV that contain purified glycoprotein B (gB) complexed with a powerful adjuvant—alpha replicon particle vaccines and live-attenuated CMV vaccines.[65,146,319,424] A CMV gB vaccine with MF59 adjuvant recent showed vaccine efficacy of 50 percent and provided proof that vaccination has the potential to decrease maternal and congenital CMV infection.[317,325] Most recently, the cellular immune system has been targeted by "DNA vaccines" that encode gB and appear to induce protective cellular immunity against CMV. Finally, adoptive immunotherapy with sensitized T cells has shown promising results for prevention of CMV infection and disease in transplant recipients and is being evaluated in clinical trials.[195]

*References 82, 156, 223, 334-336, 338, 417.

Behavioral Strategies to Prevent Primary Cytomegalovirus Infection

With the current complexities associated with the development of a CMV vaccine and the challenges of administering effective antiviral therapy, an alternative practical option for prevention of CMV infection during pregnancy and other high-risk times of life is education to increase public awareness about CMV and its modes of transmission.* Surveys have documented that knowledge about cytomegalovirus around the world is low.[265] Most studies have shown less than 20 percent of responders to CMV knowledge surveys have heard of CMV. For example, approximately 7 percent of men and 13 percent of women in one study from the 2010 HealthStyles survey had even heard of CMV, even though it is considered a common viral infection.[79,369] Educational materials are available from clinicians, from public health and university web sites,† as well as nonprofit organizations and social media web sites. These sites provide factual information to promote CMV awareness and guidelines for reducing the risk for CMV transmission to pregnant women, women of child-bearing age, and other susceptible individuals.[368] Reliable, relatively inexpensive serologic evaluation is available, so all women contemplating pregnancy should know their CMV serologic status. In addition, because epidemiologic studies have shown a major source of CMV infection to be close contact with young children, women who are pregnant, especially women who are known to be CMV seronegative, should be aware that a high percentage of young children are infected actively with CMV and that while pregnant they should exercise good hygienic practices when in close contact with young children, especially those who attend daycare centers or are known to have an active CMV infection.[76,79,105,320] Studies from the United States and Europe have shown that the incidence of child-to-parent transmission of CMV may be reduced by interventions that identify susceptible pregnant women and educate them about increasing protective behaviors, such as handwashing after diaper changes and wiping tears and nasopharyngeal mucus and decreasing risky behavior for acquiring CMV, such as kissing on the mouth and sharing eating utensils.[10,11,105,153] Many experts recommend women of childbearing age should be aware about CMV and practice recommended hygienic precautions during pregnancy.[76,105,121,181] In addition, CMV can be transmitted from husband to wife, and if the spouse or partner experiences a CMV mononucleosis syndrome, a CMV-seronegative woman may wish to consider avoiding pregnancy for an individualized period of time.[118]

*References 86, 115, 121, 181, 217, 358, 368, 479.

†Centers for Disease Control and Prevention: www.cdc.gov/cmv; and Baylor College of Medicine: www.bcm.edu/pedi/infect/cmv.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abueguen P, Delbos V, Ducancelle A, et al. Venous thrombosis in immunocompetent patients with acute cytomegalovirus infection: a complication that may be underestimated. *Clin Microbiol Infect* 2010;**16**:851–4.
13. Adler S, Nigro G. The importance of cytomegalovirus-specific antibodies for the prevention of fetal cytomegalovirus infection or disease. *Herpes* 2008;**15**:24–7.
14. Adler S, Nigro G. Findings and conclusions from CMV hyperimmune globulin treatment trials. *J Clin Virol* 2009;**46**(Suppl. 4):S54–7.
27. Atzmony L, Halutz O, Avidor B, et al. Incidence of cytomegalovirus-associated thrombosis and its risk factors: a case control study. *Thromb Res* 2010;**126**:e439–43.
28. Avery R, Marty F, Strasfeld L. et al. Oral maribavir for treatment of refractor or resistant cytomegalovirus infections in transplant recipients. *Transpl Infect Dis* 2010;**12**:489–96.
46. Baron C, Forconi C, Lebranchu Y. Revisiting the effects of CMV on long-term transplant outcome. *Curr Opin Organ Transplant* 2010;**15**:492–8.
50. Bernstein D. Vaccines for cytomegalovirus. *Infect Disord Drug Targets* 2011;**11**:514–25.
58. Boppana S, Ross S, Novak Z, et al. Dried blood spot real-time polymerase reaction assays to screen newborns for congenital cytomegalovirus infection. *JAMA* 2010;**303**:1375–82.
59. Boppana S, Ross S, Shimamura M, et al. Saliva polymerase chain reaction assay for cytomegalovirus screening in newborns. *N Engl J Med* 2011;**364**:2111–18.
71. Buonesenso D, Serranti D, Gariguillo L, et al. Congenital cytomegalovirus infection: current strategies and future perspectives. *Eur Rev Med Pharmacol Sci* 2012;**16**:919–35.
74. Buxmann H, Stackelberg O, Schlosser R, et al. Use of cytomegalovirus hyperimmunoglobulin for prevention of congenital cytomegalovirus disease: a retrospective analysis. *J Perinat Med* 2012;**49**:439–46.
77. Cannon M, Hyde T, Schmid D. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. *Rev Med Virol* 2011;**21**:240–55.
78. Cannon M, Schmid D, Hyde T. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol* 2010;**29**:202–13.
79. Cannon M, Westbrook K, Lewis D, et al. Awareness of and behaviors related to child-to-mother transmission of cytomegalovirus. *Prev Med* 2012;**54**:351–7.
81. Capretti M, Lanari M, Lazzarotto T, et al. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study. *J Pediatr* 2009;**154**:842–8.
90. Cheeran M, Lokensgard J, Schleiss M. Neuropathogensis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev* 2009;**22**:99–126.
92. Cheng J, Ke Q, Jin Z, et al. Cytomegalovirus infection causes an increase of arterial blood pressure. *PLoS Pathog* 2009;**5**:e1000427.
104. Coors L, Spencer R. Delayed presentation of cytomegalovirus retinitis in an infant with severe congenital cytomegalovirus infection. *Retina* 2010;**30**:S59–62.
105. Cordier A, Guitton A, Vauloup-Fellous C. et al. Awareness of cytomegalovirus infection among pregnant women in France. *J Clin Virol* 2012;**53**:332–7.
122. Dempsey A, Pangborn H, Prosser L. Cost-effectiveness of routine vaccination of adolescent females against cytomegalovirus. *Vaccine* 2012;**30**:4060–6.
123. deVries J, Wessels E, Korver A, et al. Rapid genotyping of cytomegalovirus in dried blood spots by multiplex real-time PCR assays targeting the envelope glycoprotein gB and gH genes. *J Clin Microbiol* 2012;**59**:232–7.
133. Drew W, Liu C. Repopulation of ganciclovir-resistant cytomegalovirus by wild-type virus. *Clin Transplant* 2012;**26**:949–52.
139. Eid A, Razonable R. New developments in the management of cytomegalovirus infection after solid organ transplantation. *Drugs* 2010;**70**:965–81.
143. Enders G, Daiminger A, Bader U, et al. The value of CMV IgG avidity and immunoblot for timing the onset of primary CMV infection in pregnancy. *J Clin Virol* 2013;**56**:102–7.
154. Fischer R, Kristler K, Ulsh P, et al. The association between cytomegalovirus immune globulin and long-term recipient and graft survival following liver transplantation. *Transpl Infect Dis* 2012;**14**:121–31.
185. Griffiths PD. CMV vaccine trial endpoints. *J Clin Virol* 2009;**46**(Suppl. 4):S64–7.
186. Grojean J, Hantz S, Cotin S, et al. Direct genotyping of cytomegalovirus envelope glycoproteins from toddler's saliva samples. *J Clin Virol* 2009;**46**(Suppl. 4):S43–8.
191. Haarala A, Kahonen M, Lehtimaki T, et al. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: The Cardiovascular Risk in Young Finns Study. *Clin Exp Immunol* 2012;**167**:309–16.
192. Hachfi W, Laurichesse J, Chauveheid M. et al. Acute cytomegalovirus infection revealing systemic lupus erythematosus. *Rev Med Interne* 2011;**32**:e6–8.
195. Hanley P, Cruz C, Savoldo B, et al. Functionally active virus-specific T cells that target CMV, adenovirus, and EBV can be expanded from naive T-cell populations in cord blood and will targe a range of viral epitopes. *Blood* 2009;**114**:1958–67.
198. Hilgendorff A, Daiminger A, Dangel V, et al. Oral valganciclovir treatment in a CMV congenital infected infant with sensorineural hearing loss (SNHL) first detected at 4 months of age. *Klin Padiar* 2009;**221**:448–9.
203. Hodson E, Craig J, Strippoli G, et al. Antiviral medications for preventing cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev* 2008;(2):CD003774.
209. Hyde T, Schmid D, Cannon M. Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. *Rev Med Virol* 2010;**20**:311–26.
210. Ikuta K, Ischioka K, Satao Y, et al. A novel real-time PCR method for determination and quantification of each cytomegalovirus glycoprotein H subtype in clinical samples. *J Clin Microbiol* 2012;**50**:499–501.
211. Irizarry K, Honigbaum S, Demmler-Harrison G, et al. Successful treatment with oral valganciclovir of primary CMV enterocolitis in a congenitally infected infant. *Fetal Pediatr Pathol* 2011;**30**:437–41.
219. Ji Y, An L, Zhan P, et al. Cytomegalovirus infection and coronary heart disease risk: a meta-analysis. *Mol Biol Rep* 2012;**39**:6537–46.
232. Kim Y, Boeckh M, Cook L, et al. Cytomegalovirus infection and ganciclovir resistance caused by UL97 mutations in pediatric transplant recipients. *Transpl Infect Dis* 2012;**10**:1399.
238. Kishi M, Sakakibara R, Ogawa E, et al. Bilateral abducens palsy in a case of cytomegalovirus-associated Guillain-Barré syndrome. *Neurol Sci* 2011;**32**:1219–22.
246. Kwan C, Jung Y, Yun D, et al. A case of acute pericarditis with hemophagocytic syndrome, cytomegalovirus infection and systemic lupus erythematosus. *Rheumatol Int* 2008;**28**: 271–3.
249. Lackner A, Acham A, Alborno T, et al. Effect on hearing of ganciclovir therapy for asymptomatic congenital cytomegalovirus infection: four to 10 year follow up. *J Laryngol Otol* 2009;**123**:391–6.
250. Ladd A, Goyal R, Rosainz L, et al. Pulmonary embolism and portal vein thrombosis in an immunocompetent adolescent with acute cytomegalovirus hepatitis. *J Thromb Thrombolysis* 2009;**28**:496–9.
253. Lazarotto T, Guerra B, Gabrielli L, et al. Update on the prevention, diagnosis, and management of cytomegalovirus infection during pregnancy. *Clin Microbiol Infect* 2011;**17**:1285–93.
254. Lazzarotto T, Guerra B, Lanari M, et al. New advances in the diagnosis of congenital cytomegalovirus infection. *J Clin Virol* 2008;**41**:192–7.
258. Leruez-Ville M, Vauloup-Fellous C, Coulderc S, et al. Prospective identification of congenital cytomegalovirus infectioin in newborns using real-time polymerase chain reaction assays in dried blood spots. *Clin Infect Dis* 2011;**52**:575–81.
260. Leung J, Cannon M, Grosse S, et al. Laboratory testing for cytomegalovirus among pregnant women in the United States: a retrospective study using administrative claims data. *BMC Infect Dis* 2012;**12**:334–40.
264. Lifering W, de Vries A, Veeger N, et al. Possible contribution of cytomegalovirus infection to the high risk of (recurrent) venous thrombosis after renal transplantation. *Thromb Haemost* 2008;**99**:127–32.

265. Lim S, Tan W, Tan L. Awareness of and attitudes toward congenital cytomegalovirus infection among pregnant women in Singapore. *Int J Gynaecol Obstet* 2012;**117**:268–72.
270. Manuel O, Asberg A, Pang X, et al. Impact of genetic polymorphisms in cytomegalovirus glycoprotein B on outcomes in solid-organ transplant recipients with cytomegalovirus disease. *Clin Infect Dis* 2009;**49**:1160–6.
272. Martin M, Goyette N, Ives J, Boivin G. Incidence and characterization of cytomegalovirus resistance mutations (UL97) among pediatric solid organ transplant patients who received valganciclovir prophylaxis. *J Clin Virol* 2010;**47**:321–4.
273. Marty F, Ljungman P, Papanicolaou G, et al. Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplants: a phase 3, double-blind, placebo-controlled randomized trial. *Lancet Infect Dis* 2011;**11**:284–92.
290. Mundkur L, Rao V, Hebbagudi S, et al. Pathogen burden, cytomegalovirus infection and inflammatory markers in the risk of premature coronary artery disease in individuals of Indian origin. *Exp Clin Cardiol* 2012;**17**:63–8.
291. Mundkur L, Shivananandan H, Hebbagudi S, et al. Human cytomegalovirus neutralizing antibodies and increased risk of coronary artery disease in Indian population. *Heart* 2012;**98**:982–7.
294. Nassetta L, Kimberlin D, Whitley R. Treatment of congenital cytomegalovirus infection: implications for future therapeutic strategies. *J Antimicrob Chemother* 2009;**63**:862–7.
298. Nigro G, Adler S, Parruti G, et al. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy-a case-control study of the outcome in children. *J Infect Dis* 2012;**205**:215–27.
301. Nigro G, Torre R, Pentimalli H. et al. Regression of fetal cerebral abnormalities by primary cytomegalovirus infection following hyperimmunoglobulin therapy. *Prenat Diagn* 2008;**28**:512–17.
302. Novak Z, Ross S, Patro P, et al. Cytomegalovirus strain diversity in seropositive women. *J Clin Microbiol* 2008;**46**:882–6.
306. Noyola D, Jimenez-Capdeville M, Demmler-Harrison G. Central nervous system disorders in infants with congenital cytomegalovirus infection. *Neurol Res* 2010;**32**:278–84.
311. Oliver S, Cloud G, Sanchez P, et al. Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system. *J Clin Virol* 2009;**46**(Suppl. 4):S22–6.
317. Pass R. Development and evidence for efficacy of CMV glycoprotein B vaccine wit MF59 adjuvant. *J Clin Virol* 2009;**46**(Suppl. 4):S73–6.
325. Pass R, Zhang C, Evans A, et al. Vaccine prevetion of maternal cytomegalovirus infection. *N Engl J Med* 2009;**360**:1191–9.
328. Perez-Mercado A, Vila-Perez S. Cytomegalovirus as a trigger for systemic lupus erythematosus. *J Clin Rheumatol* 2010;**16**:335–7.
329. Piersigilli F, Catena G, De Gasperis M, et al. Active retinitis in an infant with postnatally acquired cytomegalovirus infection. *J Perinatol* 2012;**32**:559–62.
330. Pignatelli S, DalMonte P. Epidemiology of human cytomegalovirus strains through comparison of methodological approaches to explore gN variants. *New Microbol* 2009;**32**:1–10.
331. Pignatelli S, Maurizio D, Ladini M, Dal Monte P. Development of a multiplex PCR for simultaneous amplification and genotyping of gN among human cytomegalovirus strains. *New Microbiol* 2010;**33**:257–62.
332. Plosa E, Esbenshade J, Fuller M, Weitkamp J. Cytomegalovirus infection. *Pediatr Rev* 2012;**33**:156–63.
339. Polilli E, Parruti G, D'Arcangelo F. et al. Preliminary evaluation of the safety and efficacy of standard intravenous immunoglobulins in pregnant women with primary cytomegalovirus infection. *Clin Vaccine Immunol* 2012;**12**:1991–3.
346. Rajasekariah H, Scott G, Robertson P, et al. Improving diagnosis of primary cytomegalovirus infection in pregnant women using immunoblots. *J Med Virol* 2013;**85**:315–19.
349. Razonable R. Cytomegalovirus infection after liver transplantation: current concepts and challenges. *World J Gastroenterol* 2008;**14**:4849–60.
369. Ross D, Victor M, Sumaratojo E, et al. Women's knowledge of congenital cytomegalovirus: results from the 2005 HealthStyles survey. *J Womens Health (Larchmt)* 2008;**17**:849–58.
371. Ross S, Novak Z, Pati S, et al. Mixed infection and strain diversity in congenital cytomegalovirus infection. *J Infect Dis* 2011;**204**:1003–7.
382. Schleiss M. Comparison of vaccine strategies against congenital CMV infection in the guinea pig model. *J Clin Virol* 2008;**41**:224–30.
398. Snydman D, Kristler K, Ulsh P, et al. The impact of CMV prevention on long-term recipient and graft survival in heart transplant recipients: analysis of the Scientific Registry of Transplant Recipients (SRTR) database. *Clin Transplant* 2011;**25**:e55–62.
399. Snydham D, Kristler K, Ulsh P, et al. Cytomegalovirus prevention and long-term recipient and graft survival in pediatric heart transplant recipients. *Transplantation* 2010;**90**:1432–8.
401. Soetens A, Vauloup-Fellous C, Foulon I, et al. Evaluation of different cytomegalovirus (CMV) DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates wit congenital CMV infections. *J Clin Microbiol* 2008;**46**:943–6.
424. Sung H, Schleiss M. Update on the current status of cytomegalovirus vaccines. *Expert Rev Vaccines* 2010;**9**:1303–14.
429. Tang-Feldman Y, Lochhead S, Lochhead G, et al. Murine cytomegalovirus (MCMV) infection upregulates P38 MAP kinase in aortas of apoEKO mice: a molecular mechanism for MCMV-induced acceleration of atherosclerosis. *J Cardiovasc Transl Res* 2013;**6**:54–64.
434. Trofe J, Pote L, Wade E, et al. Maribavir: a novel antiviral agent with activity against cytomegalovirus. *Ann Pharmacother* 2008;**42**:144–7.
439. Varani S, Landini M. Cytomegalovirus-induced immunopathology and its clinical consequences. *Herpesviridae* 2011;**2**:6–10.
444. Visentin S, Manara R, Milanese L, et al. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. *Clin Infect Dis* 2012;**55**:497–503.
465. Winston D, Saliva F, Blumberg E, et al. Efficacy and safety of maribavir dosed at 100 mg orally twice daily for the prevention of cytomegalovirus disase in liver transplantation recipients: a randomized, double-blind, multicenter controlled trial. *Am J Transplant* 2012;**12**:3021–30.
466. Winston D, Young J, Pullarkat V, et al. Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem cell transplant recipients: a multicenter, randomized, double-blind, placebo-controlled, dose ranging study. *Blood* 2008;**111**:5403–10.
469. Wu X, Wang Y, Xu Y. Cytomegalovirus glycoprotein B genotype in hematopoietic stem cell transplant patients from China. *Biol Blood Marrow Transplant* 2010;**16**:647–52.
476. Yilmaz-Cliftdogan D, Vardar F. Effect on hearing of oral valganciclovir for asymptomatic congenital cytomegalovirus infection. *J Trop Pediatr* 2011;**57**:132–4.
477. Yinon Y, Farine D, Yudin M, et al. Cytomegalovirus infection during pregnancy. *J Obstet Gynaecol Can* 2010;**332**:348–54.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abgueguen P, Delbos V, Chennebault J, et al. Vascular thrombosis and acute cytomegalovirus infection in immunocompetent patients: report of 2 cases and literature review. *Clin Infect Dis* 2003;**36**:E134–9.
2. Abgueguen P, Delbos V, Ducancelle A, et al. Venous thrombosis in immunocompetent patients with acute cytomegalovirus infection: a complication that may be underestimated. *Clin Microbiol Infect* 2010;**16**:851–4.
3. Adam E, Melnick J, DeBakey M. Cytomegalovirus infection and atherosclerosis. *Cent Eur J Pub Health* 1997;**5**:99–106.
4. Adler S. The molecular epidemiology of cytomegalovirus transmission among children attending a daycare center. *J Infect Dis* 1985;**152**:760.
5. Adler S. Cytomegalovirus transmission among children in daycare, their mothers and caretakers. *Pediatr Infect Dis* 1988;**7**:279.
6. Adler S. Molecular epidemiology of cytomegalovirus: viral transmission among children attending a daycare center, their parents, and caretakers. *J Pediatr* 1988;**116**:366.
7. Adler S. Cytomegalovirus and child daycare: evidence for an increased infection rate among day-care workers. *N Engl J Med* 1989;**321**:1290.
8. Adler S. Molecular epidemiology of cytomegalovirus: a study of factors affecting transmission among children at three day-care centers. *Pediatr Infect Dis J* 1991;**10**:584.
9. Adler S, Baggett J, Wilson M, et al. Molecular epidemiology of cytomegalovirus in a nursery: lack of evidence for nosocomial transmission. *J Pediatr* 1986;**108**:117.
10. Adler S, Finney J, Manganello A, et al. Prevention of child-to-mother transmission of cytomegalovirus by changing behaviors: a randomized controlled trial. *Pediatr Infect Dis J* 1996;**15**:240.
11. Adler S, Finney J, Manganello A, et al. Prevention of child to mother transmission of CMV among pregnant women. *J Pediatr* 2004;**145**:485.
12. Adler S, Hempfling S, Starr S, et al. Safety and immunogenicity of the Towne strain cytomegalovirus vaccine. *Pediatr Infect Dis J* 1998;**17**:200.
13. Adler S, Nigro G. The importance of cytomegalovirus-specific antibodies for the prevention of fetal cytomegalovirus infection or disease. *Herpes* 2008;**15**:24–7.
14. Adler S, Nigro G. Findings and conclusions from CMV hyperimmune globulin treatment trials. *J Clin Virol* 2009;**46**(Suppl. 6):S54–7.
15. Akeeson-Johansson A, Lernestedt J, Rigden O, et al. Sensitivity of cytomegalovirus to intravenous foscarnet treatment. *Bone Marrow Transplant* 1986;**1**:215.
16. Alexander J, Cuellar R, Fadden R, et al. Cytomegalovirus infection of the upper gastrointestinal tract before and after liver transplantation. *Transplantation* 1988;**46**:378.
17. Alford C, Stagno S, Pass R, et al. Epidemiology of cytomegalovirus infections. In: Nahmias A, Dowdle W, Schinazi R, editors. *The human herpesviruses: an interdisciplinary perspective*. New York, Elsevier North Holland; 1987. p. 159–71.
18. Amer O, Abd-el Rahma H, Sherief L, et al. Role of some viral infections in neonatal cholestasis. *Egypt J Immunol* 2004; **11**:149.
19. Amir J, Wolf D, Levy I. Treatment of symptomatic congenital cytomegalovirus infection with intravenous ganciclovir followed by long-term oral valganciclovir. *Eur J Pediatr* 2010;**169**:1061–7.
20. Anders K, Steinsapir K, Iverson D, et al. Neuropathologic findings in the acquired immunodeficiency syndrome (AIDS). *Clin Neuropathol* 1986;**5**:1.
21. Andrei G, DeClercq E, Snoeck R. Novel inhibitors of hCMV. *Curr Opin Investig Drugs* 2008;**9**:132.
22. Arav-Boger R, Pass R. Viral load in congenital CMV infection. *Herpes* 2007;**14**:17.
23. Arav-Boger R, Reif S, Bujanover Y. Portal vein thrombosis caused by protein C and protein S deficiency associated with cytomegalovirus infection. *J Pediatr* 1995;**126**:586.
24. Arvin A, Fast P, Myers M, et al. Vaccine development to prevent CMV disease: recommendations from the National Vaccine Advisory Committee. *Clin Infect Dis* 2004;**39**:233.
25. Ashraf S, Parande C, Arya S. Cytomegalovirus antibodies of patients in the Gizen area of Saudi Arabia. *J Infect Dis* 1985; **152**:1351.
26. Atkins J, Demmler G, Williamson W, et al. Polymerase chain reaction to detect cytomegalovirus DNA in the cerebrospinal fluid of neonates with congenital infection. *J Infect Dis* 1994;**169**:1334.
27. Atzmony L, Halutz O, Avidor B, et al. Incidence of cytomegalovirus-associated thrombosis and its risk factors: a case control study. *Thromb Res* 2010;**126**:e439–43.
28. Avery R, Marty F, Strasfeld L., et al. Oral maribavir for treatment of refractor or resistant cytomegalovirus infections in transplant recipients. *Transpl Infect Dis* 2010;**12**:489–96.
29. Azam A, Vial Y, Fawer C, et al. Prenatal diagnosis of congenital CMV infection. *Obstet Gynecol* 2001;**97**:443.
30. Bailey T, Trulock E, Ettinger N, et al. Failure of prophylactic ganciclovir to prevent cytomegalovirus disease in recipients of lung transplants. *J Infect Dis* 1992;**165**:548.
31. Balcarek K, Bagley R, Cloud G, et al. Cytomegalovirus infection among employees in a children's hospital: no evidence for increased risk associated with patient care. *JAMA* 1990;**263**:840.
32. Bale J. Human cytomegalovirus infection and disorders of the nervous system. *Arch Neurol* 1984;**41**:310.
33. Bale J. Conditions mimicking congenital infections. *Semin Pediatr Neurol* 1994;**1**:63–7.
34. Bale J, Bray P, Bell W. Neuroradiographic abnormalities in congenital cytomegalovirus infection. *Pediatr Neurol* 1985;**1**:42.
35. Bale J, Murph J, Demmler G, et al. Intrauterine cytomegalovirus infection and glycoprotein B genotypes. *J Infect Dis* 2000;**182**:933.
36. Bale J, Zimmerman B, Dawson J, et al. Cytomegalovirus transmission in child care homes. *Arch Pediatr Adolesce Med* 1999; **153**:75–9.
37. Balfour C, Balfour H. Cytomegalovirus is not an occupational risk for nurses in renal transplant and neonatal units: results of a prospective surveillance study. *JAMA* 1986;**256**:1909.
38. Balfour H, Chace B, Stapleton J, et al. A randomized, placebo-controlled trial of oral acyclovir for the prevention of cytomegalovirus disease in recipients of renal allografts. *N Engl J Med* 1989;**320**:1381.
39. Bamborschke S, Wullen T, Huber M, et al. Early diagnosis and successful treatment of acute cytomegalovirus encephalitis in a renal transplant recipient. *J Neurol* 1992;**239**:205.
40. Barbi M, Binda S, Caroppo S, et al. Multicity Italian study of congenital CMV infection. *Pediatr Infect Dis J* 2006;**25**:156.
41. Barbi M, Binda S, Caroppo S, et al. A wider role for congenital CMV infection in sensorineural hearing loss. *Pediatr Infect Dis J* 2003;**22**:39.
42. Barbi M, Binda S, Caroppo S. Diagnosis of congenital CMV infection via dried blood spots. *Rev Med Virol* 2006;**16**:385–92.
43. Barbi M, Binda S, Caroppo S, et al. Neonatal screening for congenital CMV infection and hearing loss. *J Clin Virol* 2006;**35**:206.
44. Barbi M, Binda S, Primache V, et al. CMV DNA detection in Guthrie cards: A powerful tool for diagnosis of congenital infection. *J Clin Virol* 2000;**17**:159.
45. Barbi M, McKay W, Binda S, et al. External quality assessment of CMV DNA determination on dried blood spots. *B M C Microbiol* 2008;**8**:2.
46. Baron C, Forconi C, Lebranchu Y. Revisiting the effects of CMV on long-term transplant outcome. *Curr Opin Organ Transplant* 2010;**15**:492–8.
47. Bauman N, Kirby-Keyser J, Dolan K, et al. Mondini dysplasia and congenital cytomegalovirus infection. *J Pediatr* 1994;**124**:71.
48. Becraft D. Prenatal cytomegalovirus infection and muscular deficiency (eventration) of the diaphragm. *J Pediatr* 1979;**94**:74.
49. Benson M, Kaplan M, O'Toole K, et al. A report of cytomegalovirus cystitis and a review of other genitourinary manifestations of the acquired immune deficiency syndrome. *J Urol* 1988;**140**:153.
50. Bernstein D. Vaccines for cytomegalovirus. *Infect Disord Drug Targets* 2011;**11**:514–25.
51. Biron K. Antiviral drugs for CMV disease. *Antiviral Res* 2006;**71**:154.
52. Bloom J, Palestine A. The diagnosis of cytomegalovirus retinitis. *Ann Intern Med* 1988;**109**:963.
53. Boeckh M, Bowden R, Storer B, et al. Randomized, placebo-controlled, double-blind study of a cytomegalovirus-specific monoclonal antibody (MSL-109) for prevention of cytomegalovirus infection after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001;**7**:343–61.
54. Bonkowsky H, Lee R, Klatskin G. Acute granulomatous hepatitis: occurrence in cytomegalovirus mononucleosis. *JAMA* 1975;**233**:1284.

55. Boppana S, Amos C, Britt W, et al. Late onset and reactivation of chorioretinitis in children with congenital cytomegalovirus infection. *Pediatr Infect Dis J* 1994;**13**:1139.
56. Boppana S, Fowler K, Pass R, et al. Congenital CMV infection: association between virus burden in infancy and hearing loss. *J Pediatr* 2005;**146**:817.
57. Boppana S, Fowler K, Vaid Y, et al. Neuroradiographic findings in the newborn period and long-term outcome in children with symptomatic congenital cytomegalovirus infection. *Pediatrics* 1997;**99**:409.
58. Boppana S, Ross S, Novak Z, et al. Dried blood spot real-time polymerase reaction assays to screen newborns for congenital cytomegalovirus infection. *JAMA* 2010;**303**:1375–82.
59. Boppana S, Ross S, Shimamura M, et al. Saliva polymerase chain reaction assay for cytomegalovirus screening in newborns. *N Engl J Med* 2011;**364**:2111–18.
60. Bowden R, Sayers S, Flournoy N, et al. Cytomegalovirus immune globulin and seronegative blood products to prevent primary cytomegalovirus infection after marrow transplantation. *N Engl J Med* 1986;**16**:1006.
61. Bradford R, Cloud G, Lakeman A, et al. Detection of cytomegalovirus (CMV) DNA by PCR is associated with hearing loss in newborns with symptomatic congenital CMV infection involving the central nervous system. *J Infect Dis* 2005;**191**:227.
62. Brady M. Cytomegalovirus infections: occupational risk for health professionals. *Am J Infect Control* 1986;**14**:197.
63. Brady M, Milam J, Anderson D, et al. Use of deglycerolized red blood cells to prevent post transfusion infection with cytomegalovirus in neonates. *J Infect Dis* 1984;**150**:334.
64. Breinig M, Zitelli B, Starzl T, et al. Epstein-Barr virus, cytomegalovirus, and other viral infections in children after liver transplantation. *J Infect Dis* 1987;**156**:273.
65. Britt W, Vugler L, Butfiloski E, et al. Cell surface expression of human cytomegalovirus (HCMV) gp55-116 (gB): use of HCMV-recombinant vaccinia virus–infected cells in analysis of the human neutralizing antibody response. *J Virol* 1990;**64**:1079.
66. Bronsther O, Makowka L, Jaffe R, et al. Occurrence of cytomegalovirus hepatitis in liver transplant patients. *J Med Virol* 1988; **24**:423.
67. Buffone G, Demmler G, Schimbor C, et al. DNA hybridization assay for congenital cytomegalovirus infection. *J Clin Microbiol* 1988;**26**:2184.
68. Buffone G, Frost A, Samo T, et al. The diagnosis of CMV pneumonitis in lung and heart/lung transplant patients by PCR compared with traditional laboratory criteria. *Transplantation* 1993;**56**:342.
69. Buffone G, Schimbor C, Demmler G, et al. Detection of cytomegalovirus in urine by nonisotopic DNA hybridization. *J Infect Dis* 1986;**154**:163.
70. Buhles W, Mastre J, Tinker A, et al. Ganciclovir treatment of life- or sight-threatening cytomegalovirus infection: experience in 314 immunocompromised patients. *Rev Infect Dis* 1988;**10**(Suppl.):495.
71. Buonesenso D, Serranti D, Gariguillo L, et al. Congenital cytomegalovirus infection: current strategies and future perspectives.. *Eur Rev Med Pharmacol Sci* 2012;**16**:919–35.
72. Butler K, DeSmet M, Husson R, et al. Treatment of aggressive cytomegalovirus retinitis with ganciclovir in combination with foscarnet in a child infected with human immunodeficiency virus. *J Pediatr* 1992;**120**:483.
73. Butt W, Mackey R, deCrespigny L, et al. Intracranial lesions of congenital cytomegalovirus infection detected by ultrasound scanning. *Pediatrics* 1984;**73**:611.
74. Buxmann H, Stackelberg O, Schlosser R, et al. Use of cytomegalovirus hyperimmunoglobulin for prevention of congenital cytomegalovirus disease: a retrospective analysis. *J Perinat Med* 2012;**49**:439–46.
75. Cacoub P, Deray G, Baumelou A, et al. Acute renal failure induced by foscarnet: 4 cases. *Clin Nephrol* 1988;**29**:315.
76. Cannon M, Davis K. Washing our hands of the congenital CMV disease epidemic. *BMC Public Health* 2005;**5**:70.
77. Cannon M, Hyde T, Schmid D. Review of cytomegalovirus shedding in bodily fluids and relevance to congenital cytomegalovirus infection. *Rev Med Virol* 2011;**21**:240–55.
78. Cannon M, Schmid D, Hyde T. Review of cytomegalovirus seroprevalence and demographic characteristics associated with infection. *Rev Med Virol* 2010;**29**:202–13.
79. Cannon M, Westbrook K, Lewis D, et al. Awareness of and behaviors related to child-to-mother transmission of cytomegalovirus. *Prev Med* 2012;**54**:351–7.
80. Cantrill H, Henry K, Melroe N, et al. Treatment of cytomegalovirus retinitis with intravitreal ganciclovir. *Ophthalmology* 1989;**96**:367.
81. Capretti M, Lanari M, Lazzarotto T, et al. Very low birth weight infants born to cytomegalovirus-seropositive mothers fed with their mother's milk: a prospective study. *J Pediatr* 2009;**154**:842–8.
82. Carney W, Hirsch M, Iacoviello V, et al. T-lymphocyte subsets and proliferative responses following immunization with cytomegalovirus vaccine. *J Infect Dis* 1983;**147**:958.
83. Cassol S, Poon M, Pal R, et al. Primer-mediated enzymatic amplification of cytomegalovirus (CMV) DNA: application to the early diagnosis of CMV infection in marrow transplant recipients. *J Clin Invest* 1989;**83**:1109.
84. Casteels A, Naessens A, Gordts F, et al. Neonatal screening for congenital CMV infection. *J Perinat Med* 1999;**27**:116.
85. Castor J, Cook L, Corey L, et al. Rapid determination directly from patient serum samples of hCMV UL97 mutations conferring ganciclovir resistance. *J Clin Microbiol* 2007;**45**:2681.
86. Centers for Disease Control and Prevention. Knowledge and practice of obstetricians and gynecologists regarding CMV infection during pregnancy—United States 2007. *M M W R Morb Mortal Wkly Rep* 2008;**57**:65.
87. Chanarin I, Walford D. Thrombocytopenic purpura in cytomegalovirus mononucleosis. *Lancet* 1973;**2**:238.
88. Chandler S, Handsfield H, McDougall J. Isolation of multiple strains of cytomegalovirus from women attending a clinic for sexually transmitted diseases. *J Infect Dis* 1987;**155**:655.
89. Chandler S, Holmes K, Wentworth B, et al. The epidemiology of cytomegaloviral infection in women attending a sexually transmitted disease clinic. *J Infect Dis* 1985;**152**:597.
90. Cheeran M, Lokensgard J, Schleiss M. Neuropathogenesis of congenital cytomegalovirus infection: disease mechanisms and prospects for intervention. *Clin Microbiol Rev* 2009;**22**:99–126.
91. Cheeseman S, Rubin R, Stewart J, et al. Controlled clinical trial of prophylactic human leukocyte interferon in renal transplantation. *N Engl J Med* 1979;**300**:1345.
92. Cheng J, Ke Q, Jin Z, et al. Cytomegalovirus infection causes an increase of arterial blood pressure. *PLoS Pathog* 2009;**5**:e1000427.
93. Chonmaitree T, Owen M, Pater J, et al. Presence of cytomegalovirus and herpes simplex virus in middle ear fluids from children with acute otitis media. *Clin Infect Dis* 1992;**15**:650.
94. Chou S. Acquisition of donor strains of cytomegalovirus by renal transplant recipients. *N Engl J Med* 1986;**314**:1418.
95. Chou S. Neutralizing antibody responses to reinfecting strains of cytomegalovirus in transplant recipients. *J Infect Dis* 1989;**160**:16.
96. Chou S, Guentzel S, Michels K, et al. Frequency of UL97 phosphotransferase mutations related to ganciclovir resistance in clinical cytomegalovirus isolates. *J Infect Dis* 1995;**172**:238.
97. Chou S, Marousek G, Guentzel S, et al. Evolution of mutations conferring multidrug resistance during prophylaxis and therapy for cytomegalovirus disease. *J Infect Dis* 1997;**176**:786.
98. Cinque P, Vago L, Brytling M. Cytomegalovirus infection of the central nervous system in patients with AIDS: diagnosis by DNA amplification from cerebrospinal fluid. *J Infect Dis* 1992;**166**:1408.
99. Coats D, Demmler G, Paysse E, et al. Ophthalmologic findings in children with congenital cytomegalovirus infection. *J AAPOS* 2000;**4**:110.
100. Cohen J, Corey G. Cytomegalovirus infection in the normal host. *Medicine (Baltimore)* 1985;**64**:100.
101. Collier A, Handsfield H, Roberts P, et al. Cytomegalovirus infection in women attending a sexually transmitted disease clinic. *J Infect Dis* 1990;**162**:46.
102. Colugnati F, Staras S, Dollard S, et al. Incidence of CMV infection among the general population and pregnant women in the United States. *B M C Infect Dis* 2007;**7**:71.
103. Connolly P, Jerger S, Williamson W, et al. Evaluation of higher-level auditory function in children with asymptomatic congenital cytomegalovirus infection. *Am J Otol* 1992;**13**:185.
104. Coors L, Spencer R. Delayed presentation of cytomegalovirus retinitis in an infant with severe congenital cytomegalovirus infection. *Retina* 2010;**30**:S59–62.
105. Cordier A, Guitton A, Vauloup-Fellous C. et al. Awareness of cytomegalovirus infection among pregnant women in France. *J Clin Virol* 2012;**53**:332–7.

106. Cosmi E, Mazzocco M, La Torre R, et al. Therapy or prevention of fetal infection by cytomegalovirus immunoglobulin infusion in pregnant women with primary infection. *Acta Biomed Ateneo Parmense* 2000;**71**(Suppl. 1):547–51.
107. Crumpacker C, Marlowe S, Zhang J, et al. Treatment of cytomegalovirus pneumonia. *Rev Infect Dis* 1988;**10**(Suppl.):538.
108. Curless R, Scott G, Post M, et al. Progressive cytomegalovirus encephalopathy following congenital infection in an infant with acquired immunodeficiency syndrome. *Childs Nerv Syst* 1987;**3**: 255.
109. Danish E, Dahms B, Kumar M. Cytomegalovirus-associated hemophagocytic syndrome. *Pediatrics* 1985;**75**:280.
110. Dankner W, McCutchan J, Richman D, et al. Localization of human cytomegalovirus in peripheral blood leukocytes by in situ hybridization. *J Infect Dis* 1990;**161**:31.
111. Davis G, Spector G, Strauss M, et al. Cytomegalovirus endolabyrinthitis. *Arch Pathol Lab Med* 1977;**101**:118.
112. Davis L, Stewart J, Garvin S. Cytomegalovirus infection: a seroepidemiologic comparison of nuns and women from a venereal disease clinic. *Am J Epidemiol* 1975;**102**:327.
113. deOry F, Ramirez R, Garcia-Comas L, et al. Is there a change in CMV seroepidemiology in Spain? *Eur J Epidemiol* 2004;**19**: 85.
114. Delbos V, Abueguen P, Chennebault J, et al. Acute cytomegealovirus infection and venous thrombosis: role of antiphospholipid antibiodies. *J Infect* 2007;**54**:e47–50.
115. Demmler G. Summary of a workshop on surveillance for congenital CMV disease. *Rev Infect Dis* 1991;**13**:315.
116. Demmler G, Brady M, Bijou H, et al. Post-transfusion cytomegalovirus infection in neonates: role of saline-washed red blood cells. *J Pediatr* 1986;**108**:762.
117. Demmler G, Buffone G, Schimbor C, et al. Detection of cytomegalovirus in urine from newborns by using polymerase chain reaction DNA amplification. *J Infect Dis* 1988;**158**:1177.
118. Demmler G, O'Neil G, O'Neil J, et al. Transmission of cytomegalovirus from husband to wife. *J Infect Dis* 1986;**154**:545.
119. Demmler G, Six H, Hurst S, et al. Enzyme-linked immunosorbent assay for the detection of IgM-class antibodies to cytomegalovirus. *J Infect Dis* 1986;**153**:1152.
120. Demmler G, Yow M, Spector S, et al. Nosocomial cytomegalovirus infections in two hospitals caring for infants and children. *J Infect Dis* 1987;**156**:9.
121. Demmler-Harrison G. Congenital cytomegalovirus: public health action towards awareness, prevention, and treatment. *J Clin Virol* 2009;**46**(Suppl. 4):S1–5.
122. Dempsey A, Pangborn H, Prosser L. Cost-effectiveness of routine vaccination of adolescent females against cytomegalovirus. *Vaccine* 2012;**30**:4060–6.
123. deVries J, Wessels E, Korver A, et al. Rapid genotyping of cytomegalovirus in dried blood spots by multiplex real-time PCR assays targeting the envelope glycoprotein gB and gH genes. *J Clin Microbiol* 2012;**59**:232–7.
124. Dietrich D, Chachoua A, François L, et al. Ganciclovir treatment of gastrointestinal infections caused by cytomegalovirus in patients with AIDS. *Rev Infect Dis* 1988;**10**(Suppl.):532.
125. Dimmick J, Bove K. Cytomegalovirus infection of the bowel in infancy: pathogenetic and diagnostic significance. *Pediatr Pathol* 1984;**2**:95.
126. Dolgin S, Larsen J, Shah K, et al. CMV enteritis causing hemorrhage and obstruction in an infant with AIDS. *J Pediatr Surg* 1990;**25**:696.
127. Dollard S, Grosse S, Ross D. New estimates on the prevention of neurologic and sensory sequelae and mortality. *Rev Med Virol* 2007;**17**:355.
128. Donner C, Liesnard C, Congent J, et al. Prenatal diagnosis of 52 pregnancies at risk for congenital cytomegalovirus infection. *Obstet Gynecol* 1993;**82**:481.
129. Dowd J, Aiello A, Alley D. Socioeconomic disparities in the seroprevalence of CMV infection in the US population: NHANES III. *Epidemiol Infect* 2009;**137**:58–65.
130. Dowling J, Saslow A, Armstrong J, et al. Cytomegalovirus infection in patients receiving immunosuppressive therapy for rheumatologic disorders. *J Infect Dis* 1976;**133**:399.
131. Doyle M, Atkins J-T, Rivera-Matos IR. Congenital cytomegalovirus infection in infants infected with human immunodeficiency virus type 1. *Pediatr Infect Dis J* 1996;**15**:1102.
132. Drew W, Ives D, Lalezari J, et al. Oral ganciclovir as maintenance treatment for cytomegalovirus retinitis in patients with AIDS. *N Engl J Med* 1995;**333**:615.
133. Drew W, Liu C. Repopulation of ganciclovir-resistant cytomegalovirus by wild-type virus. *Clin Transplant* 2012;**26**:949–52.
134. Drew W, Sweet E, Miner R, et al. Multiple infections by cytomegalovirus in patients with acquired immunodeficiency syndrome: documentation by Southern blot hybridization. *J Infect Dis* 1984;**150**:952.
135. Dummer J, White L, Ho M, et al. Morbidity of cytomegalovirus infection in recipients of heart-lung transplants who received cyclosporine. *J Infect Dis* 1985;**152**:1182.
136. Dworsky M, Lakeman A, Stagno S. Cytomegalovirus transmission within a family. *Pediatr Infect Dis* 1984;**3**:236.
137. Dworsky M, Weoch K, Cassady G, et al. Occupational risk for primary cytomegalovirus infection among pediatric health-care workers. *N Engl J Med* 1983;**309**:950.
138. Dworsky M, Yow M, Stagno S, et al. Cytomegalovirus infection of breast milk and transmission in infancy. *Pediatrics* 1983; **72**:295.
139. Eid A, Razonable R. New developments in the management of cytomegalovirus infection after solid organ transplantation. *Drugs* 2010;**70**:965–81.
140. Emanuel D, Cunningham I, Jules-Elysee K, et al. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous immune globulin. *Ann Intern Med* 1988;**109**:777.
141. Embil J, Goldbloom A, McFarlane E. Isolation of cytomegalovirus from middle ear effusion. *J Pediatr* 1985;**107**:435.
142. Enders G, Bader V, Lindemann L, et al. Prenatal diagnosis of congenital cytomegalovirus infection in 189 pregnancies with known outcome. *Prenat Diagn* 2001;**21**:326.
143. Enders G, Daiminger A, Bader U, et al. The value of CMV IgG avidity and immunoblot for timing the onset of primary CMV infection in pregnancy. *J Clin Virol* 2013;**56**:102–7.
144. Erice A, Chou S, Biron K, et al. Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. *N Engl J Med* 1989;**320**:289.
145. Faix R. Survival of cytomegalovirus on environmental surfaces. *J Pediatr* 1985;**106**:649.
146. Farrar G, Bull J, Greenaway P. Prospects for the clinical management of human cytomegalovirus infections. *Vaccine* 1986; **4**:217.
147. Feigin R, Shackleford P, Haymond M, et al. Floxuridine treatment of congenital cytomegalovirus inclusion disease. *Pediatrics* 1971;**48**:318.
148. Felsenstein E, D'Amico D, Hirsch M, et al. Treatment of cytomegalovirus retinitis with 9-[2-hydroxy-1-(hydroxymethyl)ethoxymethyl]guanine. *Ann Intern Med* 1985;**103**:377.
149. Fenner T, Garweg J, Hufert F, et al. Diagnoses of human cytomegalovirus-induced retinitis in human immunodeficiency virus type-1–infected subjects by using the polymerase chain reaction. *J Clin Microbiol* 1991;**29**:2621.
150. Ferreiro J, Vinters H. Pathology of the pituitary gland in patients with the acquired immune deficiency syndrome (AIDS). *Pathology* 1988;**20**:211.
151. Fiala M, Kattlove H. Cytomegalovirus mononucleosis with severe thrombocytopenia. *Ann Intern Med* 1973;**79**:450.
152. Fiala M, Payne J, Berne T, et al. Epidemiology of cytomegalovirus infection after transplantation and immunosuppression. *J Infect Dis* 1975;**132**:421.
153. Finney J, Miller K, Adler S. Changing protective and risky behaviors to prevent child-to-parent transmission of cytomegalovirus. *J Appl Behav Anal* 1993;**26**:471.
154. Fischer R, Kristler K, Ulsh P, et al. The association between cytomegalovirus immune globulin and long-term recipient and graft survival following liver transplantation. *Transpl Infect Dis* 2012;**14**:121–31
155. Fjaer R, Bruu A, Nordbo S. Extrahepatic bile duct atresia and viral involvement. *Pediatr Transplant* 2005;**9**:68.
156. Fleisher G, Starr S, Friedman H, et al. Vaccination of pediatric nurses with live attenuated cytomegalovirus. *Am J Dis Child* 1982;**136**:294.
157. Flores-Aguilar J, Huang J, Wiley C, et al. Long-acting therapy of viral retinitis with (S)-1-(3-hydroxy-2-phosphorylmethoxypropyl) cytosine. *J Infect Dis* 1994;**169**:642.

158. Fowler K, Dahle A, Boppana S, et al. Newborn hearing screening: will children with hearing loss caused by congenital cytomegalovirus infection be missed? *J Pediatr* 1999;**135**:60.
159. Fowler K, McCollister F, Dahle A, et al. Progressive and fluctuating sensorineural hearing loss in children with asymptomatic congenital cytomegalovirus infection. *J Pediatr* 1997;**130**:626.
160. Fowler K, Pass R. Risk factors for congenital CMV infection in the offspring of young women: exposure to young children and recent onset of sexual activity. *Pediatrics* 2006;**118**:e286.
161. Fowler K, Stagno S, Pass R. Maternal immunity and prevention of congenital cytomegalovirus infection. *JAMA* 2003;**289**: 1008–11.
162. Fox L, Gerber M, Penix L, et al. Intractable diarrhea from cytomegalovirus enterocolitis in an immunocompetent infant. *Pediatrics* 1999;**103**:E10.
163. Frank T, LiVolsi V, Connor A. Cytomegalovirus infection of the thyroid in immunocompromised adults. *Yale J Biol Med* 1987;**60**:1.
164. Frenkel L, Keys M, Hefferen S, et al. Unusual eye abnormalities associated with congenital cytomegalovirus infection. *Pediatrics* 1980;**5**:763.
165. Fries B, Chou S, Boeckh M, et al. Frequency distribution of cytomegalovirus envelope glycoprotein genotypes in bone marrow transplant recipients. *J Infect Dis* 1994;**169**:769.
166. Fuller G, Jacobs J, Guiloff R. Association of painful peripheral neuropathy in AIDS with cytomegalovirus infection. *Lancet* 1989;**2**:937.
167. Gerna G, Lillerli D. Monitoring transplant patients for human CMV: diagnostic update. *Herpes* 2006;**13**:4.
168. Gerna G, Zipeto D, Parea M, et al. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia and DNAemia. *J Infect Dis* 1991;**164**:488.
169. Gilbert G, Hudson I, Hayes K, et al. Prevention of transfusion-acquired cytomegalovirus infection in infants by blood filtration to remove leukocytes. *Lancet* 1989;**1**:1228.
170. Gleaves C, Myerson D, Bowden R, et al. Direct detection of cytomegalovirus from bronchoalveolar lavage samples by using a rapid in situ DNA hybridization assay. *J Clin Microbiol* 1989;**27**:2429.
171. Gleaves C, Smith T, Shuster E, et al. Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J Clin Microbiol* 1985;**21**:217.
172. Gonczol E, Plotkin S. Development of a cytomegalovirus vaccine: lessons from recent clinical trials. *Expert Opin Biol Ther* 2001;**1**:401–12.
173. Gonwa T, Capehart J, Pilcher J, et al. Cytomegalovirus myocarditis as a cause of cardiac dysfunction in a heart transplant. *Transplantation* 1989;**47**:197.
174. Goodgame R, Genta R, Estrada R, et al. Frequency of positive tests for cytomegalovirus in AIDS patients: endoscopic lesions compared with normal mucosa. *Am J Gastroenterol* 1993;**88**:338.
175. Goodpasture E, Talbot F. Concerning the nature of "protozoan-like" cells in certain lesions of infancy. *Am J Dis Child* 1921;**21**:415.
176. Goodrich J, Mori M, Gleaves C, et al. Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplant. *N Engl J Med* 1991;**325**:1601.
177. Gozlan J, Salord J, Roullet E, et al. Rapid detection of cytomegalovirus DNA in cerebrospinal fluid of AIDS patients with neurologic disorders. *J Infect Dis* 1992;**166**:1416.
178. Grangeot-Keros L, Mayaux M, Lebon P, et al. Value of cytomegalovirus (CMV) IgG avidity index for the diagnosis of primary CMV infection in pregnant women. *J Infect Dis* 1997;**175**:944.
179. Grattan M, Moreno-Cabral C, Starnes V, et al. Cytomegalovirus infection is associated with cardiac allograft rejection and atherosclerosis. *JAMA* 1989;**261**:3561.
180. Greene L, Cole W, Greene J, et al. Adrenal insufficiency as a complication of the acquired immunodeficiency syndrome. *Ann Intern Med* 1984;**101**:497.
181. Griffiths P. Strategies to prevent CMV infection in the neonate. *Semin Neonatol* 2001;**7**:293–9.
182. Griffiths P, Baboonian C. A prospective study of primary cytomegalovirus infection during pregnancy: Final report. *Br J Obstet Gynaecol* 1984;**92**:307.
183. Griffiths P, Grundy J. Molecular biology and immunology of cytomegalovirus. *Biochem J* 1987;**241**:313.
184. Griffiths P, Stagno S, Pass R, et al. Congenital cytomegalovirus infection: diagnostic and prognostic significance of the detection of specific immunoglobulin M antibodies in cord serum. *Pediatrics* 1982;**69**:544.
185. Griffiths PD. CMV vaccine trial endpoints. *J Clin Virol* 2009;**46**(Suppl. 4):S64–7.
186. Grojean J, Hantz S, Cotin S, et al. Direct genotyping of cytomegalovirus envelope glycoproteins from toddler's saliva samples. *J Clin Virol* 2009;**46**(Suppl. 4):S43–48.
187. Grosse S, Ross D, Dollard S. Congenital cytomegalovirus infection as a cause of permanent bilateral hearing loss: a quantitative assessment. *J Clin Virol* 2008;**41**:57.
188. Grundy JE, Super M, Sweny P, et al. Symptomatic cytomegalovirus infection in seropositive kidney recipients: reinfection with donor virus rather than reactivation of recipient virus. *Lancet* 1988;**1**:132–5.
189. Gudnason T, Belani K, Balfour H. Ganciclovir treatment of cytomegalovirus disease in immunocompromised children. *Pediatr Infect Dis* 1989;**8**:436.
190. Guerra B, Simonazzi G, Puccetti C, et al. Ultrasound prediction of symptomatic congenital cytomegalovirus infection. *Am J Obstet Gynecol* 2008;**198**:380.
191. Haarala A, Kahonen M, Lehtimaki T, et al. Relation of high cytomegalovirus antibody titres to blood pressure and brachial artery flow-mediated dilation in young men: The Cardiovascular Risk in Young Finns Study. *Clin Exp Immunol* 2012;**167**:309–16.
192. Hachfi W, Laurichesse J, Chauveheid M, et al. Acute cytomegalovirus infection revealing systemic lupus erythematosus. *Rev Med Interne* 2011;**32**:e6–8.
193. Hackman R, Myerson D, Meyers J, et al. Rapid diagnosis of cytomegalovirus pneumonia by tissue immunofluorescence with a murine monoclonal antibody. *J Infect Dis* 1985;**151**:325.
194. Handsfield H, Chandler S, Caine V, et al. Cytomegalovirus infection in sex partners: evidence for sexual transmission. *J Infect Dis* 1985;**151**:344.
195. Hanley P, Cruz C, Savoldo B, et al. Functionally active virus-specific T cells that target CMV, adenovirus, and EBV can be expanded from anaive T-cell populations in cord blood and will target a range of viral epitopes. *Blood* 2009;**114**:1958–67.
196. Hayward J, Titelbaum D, Clancy R, et al. Lissencephalypachygyria associated with congenital cytomegalovirus infection. *J Child Neurol* 1991;**6**:109.
197. Hibberd P, Tokoff-Rubin N, Conti D, et al. Preemptive ganciclovir therapy to prevent cytomegalovirus disease in cytomegalovirus antibody–positive renal transplant recipients. *Ann Intern Med* 1995;**123**:18.
198. Hilgendorff A, Daiminger A, Dangel V, et al. Oral valganciclovir treatment in a CMV congenital infected infant with sensorineural hearing loss (SNHL) first detected at 4 months of age. *Klin Padiar* 2009;**221**:448–9.
199. Hirsch M, Schooley R, Cosimi A, et al. Effects of interferon-alpha on cytomegalovirus reactive syndromes in renal transplant recipients. *N Engl J Med* 1983;**308**:1489.
200. Ho D, Bredesen D, Vinters H, et al. The acquired immunodeficiency sydrome (AIDS) dementia complex. *Ann Intern Med* 1989;**111**:400.
201. Ho M. Characteristics of cytomegalovirus. In: Greenough W, Merigan T, editors. *Cytomegalovirus biology and infection: current topics in infectious disease*. New York: Plenum; 1982. p. 9–32.
202. Hocker J, Cook L, Addams G, et al. Ganciclovir therapy of congenital cytomegalovirus pneumonia. *Pediatr Infect Dis J* 1990;**9**:743.
203. Hodson E, Craig J, Strippoli G, et al. Antiviral medications for preventing cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev* 2008;(2):CD003774.
204. Hodson E, Jones C, Strippoli G, et al. Immunoglobulins, vaccines or interferon for preventing cytomegalovirus disease in solid organ transplant recipients. *Cochrane Database Syst Rev* 2007;(2):CD005129.
205. Hohlfeld P, Vial Y, Maillard-Grignon C, et al. Cytomegalovirus fetal infections. *Obstet Gynecol* 1991;**78**:615.
206. Hokeberg I, Olding-Stenkvist E, Grillner L, et al. No evidence of hospital-acquired cytomegalovirus infection in a pregnant pediatric nurse using restriction endonuclease analysis. *Pediatr Infect Dis J* 1988;**7**:812.
207. Horwitz C, Henle W, Henle G, et al. Clinical and laboratory evaluation of cytomegalovirus-induced mononucleosis in previously healthy individuals. *Medicine (Baltimore)* 1986;**65**:124.
208. Hutto C, Little E, Ricks R, et al. Isolation of cytomegalovirus from toys and hands in a daycare center. *J Infect Dis* 1986;**154**:527.

209. Hyde T, Schmid D, Cannon M. Cytomegalovirus seroconversion rates and risk factors: implications for congenital CMV. *Rev Med Virol* 2010;**20**:311–26.
210. Ikuta K, Ischioka K, Satao Y, et al. A novel real-time PCR method for determination and quantification of each cytomegalovirus glycoprotein H subtype in clinical samples. *J Clin, Microbiol* 2012;**50**:499–501.
211. Irizarry K, Honigbaum S, Demmler-Harrison G, et al. Successful treatment with oral valganciclovir of primary CMV enterocolitis in a congenitally infected infant. *Fetal Pediatr Pathol* 2011;**30**: 437–41.
212. Istas A, Demmler G, Dobbins J, et al. Surveillance for congenital cytomegalovirus disease: a report from the National Congenital Cytomegalovirus Disease Registry. *Clin Infect Dis* 1995;**20**:665.
213. Jacobs D, Enger C, Dunn J, et al. Cytomegalovirus retinitis and viral resistance: ganciclovir resistance. *J Infect Dis* 1998;**177**: 770.
214. Jacobson M, Mills J. Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1988;**108**:585.
215. Jacobson M, O'Donnel J, Rousell R, et al. Failure of adjunctive cytomegalovirus immune globulin to improve efficacy of ganciclovir in patients with acquired immunodeficiency syndrome and cytomegalovirus retinitis: a phase 1 study. *Antimicrob Agents Chemother* 1990;**34**:176.
216. Jamison R, Hathorn A. Isolation of cytomegalovirus from cerebrospinal fluid of a congenitally infected infant. *Am J Dis Child* 1978;**132**:63.
217. Jeon J, Victor M, Adler S, et al. Knowledge and awareness of congenital CMV among women. *Infect Dis Obstet Gynecol* 2006; **2006**:80383.
218. Jevon G, Dimmick J. Biliary atresia and CMV infection. *Pediatr Dev Pathol* 1999;**2**:11.
219. Ji Y, An L, Zhan P, et al. Cytomegalovirus infection and coronary heart disease risk: a meta-analysis. *Mol Biol Rep* 2012; **39**:6537–46.
220. Jim W, Shu C, Chiu N, et al. Transmission of CMV from mothers to preterm infants by breast milk. *Pediatr Infect Dis J* 2004;**23**:848.
221. Jones L, Duke-Duncan P, Yeager A. Cytomegaloviral infections in infant-toddler centers: centers for the developmentally delayed versus regular daycare. *J Infect Dis* 1985;**151**:953.
222. Jordan M, Rousseau W, Noble G, et al. Association of cervical cytomegalovirus with venereal disease. *N Engl J Med* 1973;**288**:932.
223. Just M, Buergin-Wolff A, Emoedi G, et al. Immunization trials with live attenuated cytomegalovirus Towne 125. *Infection* 1975;**3**:111.
224. Kaariainen L, Klemola E, Paloheimo J. Rise of cytomegalovirus antibodies in an infectious-mononucleosis-like syndrome after transfusion. *BMJ* 1966;**1**:1270.
225. Kagan R, Naragi S, Matsuda T, et al. Herpes simplex virus and cytomegalovirus infections in burned patients. *J Trauma* 1985;**25**:40.
226. Kamar N, Mengelle C, Yahyaoui S, et al. Followup of 28 HCMV seropositive renal transplant recipients: comparison of clinical, biologic and virologic parameters in the groups of treated versus untreated infected patients. *J Clin Virol* 2005;**33**:35.
227. Karn K, Julian T, Ogburn P. Fetal heart block associated with congenital cytomegalovirus infection: a case report. *J Reprod Med* 1984;**29**:278.
228. Kashden J, Frison S, Fowler K, et al. Intellectual assessment of children with asymptomatic congenital cytomegalovirus infection. *J Dev Behav Pediatr* 1998;**19**:254.
229. Keay S, Petersen E, Icenogle T, et al. Ganciclovir treatment of serious cytomegalovirus infection in heart and heart-lung transplant recipients. *Rev Infect Dis* 1988;**10**(Suppl.):563.
230. Kenneson A, Cannon M. Review and meta-analysis of the epidemiology of congenital cytomegalovirus infection. *Rev Med Virol* 2007;**17**:253.
231. Khanna R, Diamond D. Human cytomegalovirus vaccine: time to look for alternative options. *Trends Mol Med* 2006;**12**:26.
232. Kim Y, Boeckh M, Cook L, et al. Cytomegalovirus infection and ganciclovir resistance caused by UL97 mutations in pediatric transplant recipients. *Transpl Infect Dis* 2012;**10**:1399.
233. Kim Y, Gururaj V, Mirkovic R. Concomitant diffuse nodular pulmonary infiltration in an infant with cytomegalovirus infection. *Pediatr Infect Dis* 1982;**1**:173.
234. Kim Y, Hollander H. Polyradiculopathy due to cytomegalovirus: report of two cases in which improvement occurred after prolonged therapy and review of the literature. *Clin Infect Dis* 1993;**17**:32.
235. Kimberlin D. Antiviral therapy for CMV infection in pediatric patients. *Semin Pediatr Infect Dis* 2002;**13**:22.
236. Kimberlin D, Acosta E, Sanchez P, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital CMV disease. *J Infect Dis* 2008;**197**:836.
237. Kimberlin D, Lin C-Y, Sanchez P, et al. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus disease involving the central nervous system: a randomized, controlled trial. *J Pediatr* 2003;**143**:17.
238. Kishi M, Sakakibara R, Ogawa E, et al. Bilateral abducens palsy in a case of cytomegalovirus-associated Guillain-Barré syndrome. *Neurol Sci* 2011;**32**:1219–22.
239. Klemola E, Stenstrom R, von Essen R. Pneumonia as a clinical manifestation of cytomegalovirus infection in previously healthy adults. *Scand J Infect Dis* 1972;**4**:7.
240. Klemola E, von Essen R, Henle G, et al. Infectious mononucleosis–like disease with negative heterophil agglutination test: clinical features in relation to Epstein-Barr virus and cytomegalovirus and antibodies. *J Infect Dis* 1970;**121**:608.
241. Kovacs A, Schluchter M, Easley K, et al. Cytomegalovirus infection and HIV-1 disease progression in infants born to HIV-1 infected mothers. *N Engl J Med* 1999;**341**:77.
242. Kumar M, Gold E, Jacobs I, et al. Primary cytomegalovirus infection in adolescent pregnancy. *Pediatrics* 1984;**74**:493.
243. Kumar M, Jenson H, Dahms B. Fatal *Staphylococcal epidermidis* infections in very low-birth-weight infants with cytomegalovirus infection. *Pediatrics* 1985;**76**:110.
244. Kumar M, Nankervis G, Cooper A, et al. Postnatally acquired cytomegalovirus infections in infants of CMV-excreting mothers. *J Pediatr* 1984;**104**:669.
245. Kumar M, Nankervis G, Jacobs I, et al. Congenital and postnatally acquired cytomegalovirus infections: long-term follow-up. *J Pediatr* 1984;**104**:674.
246. Kwan C, Jung Y, Yun D, et al. A case of acute pericarditis with hemophagocytic syndrome, cytomegalovirus infection and systemic lupus erythematosus. *Rheumatol Int* 2008;**28**:271–3.
247. Kylat R, Kelly E, Ford-Hones E. Clinical findings and adverse outcome in neonates with symptomatic congenital cytomegalovirus infection. *Eur J Pediatr* 2006;**165**:773.
248. La Torre R, Nigro G, Mazzocco M, et al. Placental enlargement in women with primary maternal cytomegalovirus infection is associated with fetal and neonatal disease. *Clin Infect Dis* 2006;**43**:994–1000.
249. Lackner A, Acham A, Alborno T, et al. Effect on hearing of ganciclovir therapy for asymptomatic congenital cytomegalovirus infection: four to 10 year follow up. *J Laryngol Otol* 2009; **123**:391–6.
250. Ladd A, Goyal R, Rosainz L, et al. Pulmonary embolism and portal vein thrombosis in an immunocompetent adolescent with acute cytomegalovirus hepatitis. *J Thromb Thrombolysis* 2009;**28**:496–9.
251. Lanari M, Lazzarotto T, Venturi V, et al. Neonatal CMV blood load and risk of sequelae in symptomatic and asymptomatic congenitally infected newborns. *Pediatrics* 2006;**117**:376.
252. Lang D, Kummer J. Cytomegalovirus in semen: observations in selected populations. *J Infect Dis* 1975;**132**:472.
253. Lazzarotto T, Guerra B, Gabrielli L, et al. Update on the prevention, diagnosis, and management of cytomegalovirus infection during pregnancy. *Clin Microbiol Infect* 2011;**17**:1285–93.
254. Lazzarotto T, Guerra B, Lanari M, et al. New advances in the diagnosis of congenital CMV infection. *J Clin Virol* 2008; **41**:192.
255. Lazzarotto T, Maine G, Dalmonte P, et al. A novel Western blot test containing both viral and recombinant proteins for anticytomegalovirus immunoglobulin MN detection. *J Clin Microbiol* 1997;**35**:393.
256. Lee R, Ai E. Disc neovascularization in patients with AIDS and cytomegalovirus retinitis. *Retina* 1991;**11**:305.
257. Lehoang P, Giarard B, Robinet M, et al. Foscarnet in the treatment of cytomegalovirus retinitis in acquired immunodeficiency syndrome. *Ophthalmology* 1989;**96**:865.

258. Leruez-Ville M, Vauloup-Fellous C, Coulderc S, et al. Prospective identification of congenital cytomegalovirus infectioin in newborns using real-time polymerase chain reaction assays in dried blood spots. *Clin Infect Dis* 2011;**52**:575–81.
259. Lesher JL. Cytomegalovirus infections and the skin. *J Am Acad Dermatol* 1988;**18**:1333.
260. Leung J, Cannon M, Grosse S, et al. Laboratory testing for cytomegalovirus among pregnant women in the United States: a retrospective study using administrative claims data. *BMC Infect Dis* 2012;**12**:334–40.
261. Levin A, Zeichner S, Duker J, et al. Cytomegalovirus retinitis in an infant with acquired immunodeficiency syndrome. *Pediatrics* 1989;**84**:683.
262. Levin M. Current approaches to the prevention and treatment of cytomegalovirus disease after bone marrow transplantation: an overview. *Semin Hematol* 1990;**27**:1.
263. Liegenard C, Donner C, Brancart F, et al. Prenatal diagnosis of congenital CMV infection: prospective study of 237 pregnancies at risk. *Obstet Gynecol* 2000;**95**:881.
264. Lifering W, de Vries A, Veeger N, et al. Possible contribution of cytomegalovirus infection to the high risk of (recurrent) venous thrombosis after renal transplantation. *Thromb Haemost* 2008;**99**:127–32.
265. Lim S, Tan W, Tan L. Awareness of and attitudes toward congenital cytomegalovirus infection among pregnant women in Singapore. *Int J Gynaecol Obstet* 2012;**117**:268–72.
266. Lim W, Kahn E, Gupta A, et al. Treatment of cytomegalovirus enterocolitis with ganciclovir in an infant with acquired immunodeficiency syndrome. *Pediatr Infect Dis* 1988;**7**:354.
267. Linneman C, MacMillan B. Viral infections in pediatric burn patients. *Am J Dis Child* 1981;**135**:750.
268. Lui W, Chang W. Cytomegalovirus mononucleosis in Chinese infants. *Arch Dis Child* 1972;**47**:643.
269. Malm G, Engman M. Congenital cytomegalovirus infections. *Semin Fetal Neonatal Med* 2007;**12**:154–9.
270. Manuel O, Asberg A, Pang X, et al. Impact of genetic polymorphisms in cytomegalovirus glycoprotein B on outcomes in solid-organ transplant recipients with cytomegalovirus disease. *Clin Infect Dis* 2009;**49**:1160–6.
271. Martin D, Sierra-Madero J, Walmsley S, et al. A controlled trial of valganciclovir as induction therapy for cytomegalovirus retinitis. *N Engl J Med* 2002;**346**:1119.
272. Martin M, Goyette N, Ives J, et al. Incidence and characterization of cytomegalovirus resistance mutations (UL97) among pediatric solid organ transplant patients who received valganciclovir prophylaxis. *J Clin Virol* 2010;**47**:321–4.
273. Marty F, Ljungman P, Papanicolaou G, et al. Maribavir prophylaxis for prevention of cytomegalovirus disease in recipients of allogeneic stem-cell transplants: a phase 3, double-blind, placebo-controlled randomized trial. *Lancet Infect Dis* 2011;**11**:284–92.
274. Maschmann J, Hamprecht K, Dietz K, et al. Cytomegalovirus infection of extremely low–birth weight infants via breast milk. *Clin Infect Dis* 2000;**33**:1998.
275. Matthews T, Boehme R. Antiviral activity and mechanism of action of ganciclovir. *Rev Infect Dis* 1988;**10**(Suppl.):490.
276. McDonald K, Rector T, Braunlin E, et al. Association of coronary artery disease in cardiac transplant recipients with cytomegalovirus infection. *Am J Cardiol* 1989;**64**:359.
277. McKeating J, Griffiths P, Grundy J. Cytomegalovirus in urine specimens has host beta 2 microglobulin bound to the viral envelope: a mechanism of evading the host immune response. *J Gen Virol* 1987;**68**:785.
278. Meine-Jansen C, Toet M, Rademaker C, et al. Treatment of symptomatic congenital CMV infection with valganciclovir. *J Perinat Med* 2005;**33**:364.
279. Melnick J, Adam E, DeBakey M. Possible role of cytomegalovirus in atherogenesis. *JAMA* 1990;**263**:2204.
280. Mena W, Royal S, Pass R, et al. Diabetes insipidus associated with symptomatic congenital cytomegalovirus infection. *J Pediatr* 1993;**122**:911.
281. Meyers J, Leszcynski J, Zaia J, et al. Prevention of cytomegalovirus infection by cytomegalovirus immune globulin after marrow transplantation. *Ann Intern Med* 1983;**98**:442.
282. Meyers J, Reed E, Shepp D, et al. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N Engl J Med* 1988;**318**:70.
283. Michaels M. Treatment of congenital CMV: where are we now? *Expert Rev Anti Infect Ther* 2007;**5**:441.
284. Michaels M, Greenberg D, Sabo D, et al. Treatment of children with congenital cytomegalovirus infection with ganciclovir. *Pediatric Infect Dis J* 2003;**22**:504–9.
285. Millett R, Tomita T, Marshall H, et al. Cytomegalovirus endomyocarditis in a transplanted heart. *Arch Pathol Lab Med* 1991;**115**:511.
286. Mohammed A, Benmousa A, Almeghaiseeb I, et al. Gastric outlet obstruction. *Hepatogastroenterology* 2007;**54**:2415.
287. Morgello S, Cho E, Nielsen S, et al. Cytomegalovirus encephalitis in patients with acquired immunodeficiency syndrome: an autopsy study of 30 cases and a review of the literature. *Hum Pathol* 1987;**18**:289.
288. Moxley K, Knudtson E. Resolution of hydrops secondary to CMV after maternal and fetal treatment with human CMV hyperimmune globulin. *Obstet Gynecol* 2008;**111**:524.
289. Muller A, Eis-Hubinger A, Brandhorst G, et al. Oral valganciclovir for symptomatic congenital CMV infection in an extremely low birth weight infant. *J Perinatol* 2008;**28**:74.
290. Mundkur L., Rao V, Hebbagudi S, et al. Pathogen burden, cytomegalovirus infection and inflammatory markers in the risk of premature coronary artery disease in individuals of Indian origin. *Exp Clin Cardiol* 2012;**17**:63–8.
291. Mundkur L, Shivananandan H, Hebbagudi S, et al. Human cytomegalovirus neutralizing antibodies and increased risk of coronary artery disease in Indian population. *Heart* 2012;**98**:982–7.
292. Murph J, Bale J. The natural history of acquired cytomegalovirus infection among children in group daycare. *Am J Dis Child* 1988;**142**:843.
293. Murph J, Souze I, Dawson J, et al. Epidemiology of congenital cytomegalovirus infection: maternal risk factors and molecular analysis of cytomegalovirus strains. *Am J Epidemiol* 1998;**47**:940.
294. Nassetta L, Kimberlin D, Whitley R. Treatment of congenital cytomegalovirus infection: implications for future therapeutic strategies. *J Antimicrob Chemother* 2009;**63**:862–7.
295. Nelson C, Istas A, Wilkerson M, et al. Polymerase chain reaction detection of cytomegalovirus DNA in serum as a diagnostic test for congenital cytomegalovirus infection. *J Clin Microbiol* 1995;**33**:3317.
296. Nielsen S, Petito C, Urmacher C, et al. Subacute encephalitis in acquired immune deficiency syndrome: a postmortem study. *Am J Clin Pathol* 1984;**82**:678.
297. Nielsen S, Sorensen I, Anderson H. Kinetics of specific immunoglobulins M, E, A and G in congenital, primary and secondary cytomegalovirus infection studied by antibody-capture enzyme-linked immunosorbent assay. *J Clin Microbiol* 1988;**26**:654.
298. Nigro G, Adler S, Parruti G, et al. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy—a case-control study of the outcome in children. *J Infect Dis* 2012;**205**:215–27.
299. Nigro G, Adler S, LaTorre R, et al. Passive immunization during pregnancy for congenital CMV infection. *N Engl J Med* 2005;**353**:1350–62.
300. Nigro G, Scholz H, Bartman U. Ganciclovir therapy for symptomatic congenital cytomegalovirus infection in infants: a two-regimen experience. *J Pediatr* 1994;**124**:318.
301. Nigro G, Torre R, Pentimalli H. et al. Regression of fetal cerebral abnormalities by primary cytomegalovirus infection following hyperimmunoglobulin therapy. *Prenat Diagn* 2008;**28**:512–17.
302. Novak Z, Ross S, Patro P, et al. Cytomegalovirus strain diversity in seropositive women. *J Clin Microbiol* 2008;**46**:882–6.
303. Noyes B, Kurland G, Orenstein D, et al. Experience with pediatric lung transplantation. *J Pediatr* 1994;**124**:261.
304. Noyola D, Demmler G, Nelson C, et al. Early predictors of neurodevelopmental outcome in symptomatic congenital cytomegalovirus infection. *J Pediatr* 2001;**138**:325.
305. Noyola D, Demmler G, Williamson W, et al. Cytomegalovirus urinary excretion and long term outcome in children with congenital cytomegalovirus infection. Congenital CMV Longitudinal Study Group. *Pediatr Infect Dis J* 2000;**19**:505.
306. Noyola D, Jimenez-Capdeville M, Demmler-Harrison G. Central nervous system disorders in infants with congenital cytomegalovirus infection. *Neurol Res* 2010;**32**:278–84.
307. Noyola D, Valdez-Lopez B, Hernandez-Salinas A, et al. Cytomegalovirus excretion in children attending day-care centers. *Arch Med Res* 2005;**36**:590–3.

308. O'Grady J, Sutherland S, Harvey R, et al. Cytomegalovirus infection and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bile duct syndrome after liver transplantation. *Lancet* 1988;**2**:302.
309. Ogawa H, Suzutani T, Baba Y, et al. Etiology of severe sensorineural hearing loss in children: independent impact of congenital CMV infection and GJB2 mutation. *J Infect Dis* 2007;**195**: 782.
310. Oliver S, Cloud G, Sanchez P, et al. Effect of ganciclovir therapy on neurodevelopmental outcomes in symptomatic congenital CMV infection involving the central nervous system: a randomized, controlled study. Presented at Pediatric Academic Societies Annual Meeting, San Francisco, Calif, May 2, 2006.
311. Oliver S, Cloud G, Sanchez P, et al. Neurodevelopmental outcomes following ganciclovir therapy in symptomatic congenital cytomegalovirus infections involving the central nervous system. *J Clin Virol* 2009;**46**(Suppl. 4):S22–6.
312. Omarsdottir S, Casper C, Zweygberg-Wirgart B, et al. Transmission of cytomegalovirus to extremely preterm infants through breast milk. *Acta Pediatr* 2006;**96**:492.
313. Ong E, Ellis M, Tweele D, et al. Cytomegalovirus cholecystitis and colitis associated with the acquired immunodeficiency syndrome. *J Infect Dis* 1989;**18**:73.
314. Paduch D. Viral lower urinary tract infections. *Ann Urol Rev* 2007;**8**:324.
315. Pak C, McArthur R, Eun H, et al. Association of cytomegalovirus infection with autoimmune type 1 diabetes. *Lancet* 1988;**2**:1.
316. Pannuti C, Vilasboas L, Angelo M, et al. Cytomegalovirus mononucleosis in children and adults: differences in clinical presentation. *Scand J Infect Dis* 1985;**17**:153.
317. Pass R. Development and evidence for efficacy of CMV glycoprotein B vaccine with MF59 adjuvant. *J Clin Virol* 2009;**46**(Suppl. 4):S73–6.
318. Pass R, August A, Dworsky M, et al. Cytomegalovirus infection in a day-care center. *N Engl J Med* 1982;**307**:477.
319. Pass R, Duliege A-M, Boppana S, et al. A subunit cytomegalovirus vaccine based on recombinant envelope glycoprotein B and a new adjuvant. *J Infect Dis* 1999;**180**:990.
320. Pass R, Hutto S. Group daycare and cytomegaloviral infections of mothers and children. *Rev Infect Dis* 1986;**8**:599.
321. Pass R, Hutto S, Reynolds D, et al. Increased frequency of cytomegalovirus infection in children in group daycare. *Pediatrics* 1984;**74**:121.
322. Pass R, Hutto S, Ricks R, et al. Increased rate of cytomegalovirus infection among parents of children attending day-care centers. *N Engl J Med* 1986;**314**:1414.
323. Pass R, Little E, Stagno S, et al. Young children as a probable source of maternal congenital cytomegalovirus infection. *N Engl J Med* 1987;**316**:1366.
324. Pass R, Whitley R, Diethelm A, et al. Cytomegalovirus infection in patients with renal transplants: potentiation by antithymocyte globulin and an incompatible graft. *J Infect Dis* 1980;**142**:9.
325. Pass R, Zhang C, Evans A, et al. Vaccine prevetion of maternal cytomegalovirus infection. *N Engl J Med* 2009;**360**:1191–9.
326. Patterson J, Broecker A, Kornstein M, et al. Cutaneous cytomegalovirus infection in a liver transplant patient: diagnosis by in situ DNA hybridization. *Am J Dermatopathol* 1988;**10**:524.
327. Paya C. Prevention of cytomegalovirus disease in recipients of solid organ transplants. *Clin Infect Dis* 2001;**32**:596.
328. Perez-Mercado A, Vila-Perez S. Cytomegalovirus as a trigger for systemic lupus erythematosus. *J Clin Rheumatol* 2010;**16**:335–7.
329. Piersigilli F, Catena G, De Gasperis M, et al. Active retinitis in an infant with postnatally acquired cytomegalovirus infection. *J Perinatol* 2012;**32**:559–62.
330. Pignatelli S, DalMonte P. Epidemiology of human cytomegalovirus strains through comparison of methodological approaches to explore gN variants. *New Microbiol* 2009;**32**:1–10.
331. Pignatelli S, Maurizio D, Ladini M, et al. Development of a multiplex PCR for simultaneous amplification and genotyping of gN among human cytomegalovirus strains. *New Microbiol* 2010; **33**:257–62.
332. Plosa E, Esbenshade J, Fuller M, et al. Cytomegalovirus infection. *Pediatr Rev* 2012;**33**:156–63.
333. Plotkin S. Vaccination against cytomegalovirus, the challenging demon. *Pediatr Infect Dis J* 1999;**18**:313.
334. Plotkin S, Farquhar J, Hornberger E. Clinical trials of immunization with the Towne 125 strain of human cytomegalovirus. *J Infect Dis* 1976;**134**:470.
335. Plotkin S, Friedman H, Fleisher G, et al. Towne-vaccine-induced prevention of cytomegalovirus disease after renal transplants. *Lancet* 1984;**1**:528.
336. Plotkin S, Furukawa T, Zygraich N, et al. Candidate cytomegalovirus strain for human vaccination. *Infect Immun* 1975;**12**:521.
337. Plotkin S, Higgins R, Kurtz J, et al. Multicenter trial of Towne strain attenuated virus vaccine in seronegative renal transplant recipients. *Transplantation* 1994;**58**:1176.
338. Plotkin S, Smiley M, Friedman H, et al. Prevention of cytomegalovirus disease by Towne strain live attenuated vaccine. *Birth Defects* 1984;**20**:271.
339. Polilli E, Parruti G, D'Arcangelo F, et al. Preliminary evaluation of the safety and efficacy of standard intravenous immunoglobulins in pregnant women with primary cytomegalovirus infection. *Clin Vaccine Immunol* 2012;**12**:1991–3.
340. Pollard R. Cytomegalovirus infections in renal, heart, heart-lung and liver transplantation. *Pediatr Infect Dis* 1988;**7**(Suppl.):97.
341. Powell K, Bellamy A, Catton M, et al. Cytomegalovirus myocarditis in a heart transplant recipient: sensitive monitoring of viral DNA by the polymerase chain reaction. *J Heart Transplant* 1989;**8**:465.
342. Proujansky R, Orenstein S, Kocoshis S, et al. Cytomegalovirus gastroenteritis after liver transplantation. *J Pediatr* 1988;**113**:700.
343. Quiros-Tejeira R, Ament M. et al. Cytomegalovirus enterocolitis in an immunocompetent infant host: another cause of treatable intractable diarrhea in infancy. *J Pediatr Gastroenterol Nutr* 1999;**29**:86–90.
344. Rabinowitz M, Bassan I, Robinson M. Sexually transmitted cytomegalovirus proctitis in a woman. *Am J Gastroenterol* 1988;**83**:885.
345. Rafailidis P, Mourtzoukou E, Varbobitis I, et al. Severe CMV infection in apparently immunocompetent patients: a systematic review. *Virol J* 2008;**5**:47.
346. Rajasekariah H, Scott G, Robertson P, et al. Improving diagnosis of primary cytomegalovirus infection in pregnant women using immunoblots. *J Med Virol* 2013;**85**:315–19.
347. Randazzo R, Hulette C, Gottlieb M, et al. Cytomegaloviral epididymitis in a patient with the acquired immune deficiency syndrome. *J Urol* 1986;**136**:1095.
348. Rasmussen L, Hong C, Zipeto D, et al. Cytomegalovirus gB genotype distribution differs in human immunodeficiency virus–infected patients and immunocompromised allograft recipients. *J Infect Dis* 1997;**175**:179.
349. Razonable R. Cytomegalovirus infection after liver transplantation: current concepts and challenges. *World J Gastroenterol* 2008;**14**:4849–60.
350. Reed E, Bowden R, Dandliker P, et al. Efficacy of cytomegalovirus immunoglobulin in marrow transplant recipients with cytomegalovirus pneumonia. *J Infect Dis* 1987;**156**:641.
351. Reed E, Bowden R, Dandliker P, et al. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immune globulin in patients with bone marrow transplants. *Ann Intern Med* 1988;**109**:783.
352. Reed E, Dandliker P, Meyers J, et al. Treatment of cytomegalovirus pneumonia with 9-[2-hydroxy-1-(hydroxymethyl)-ethoxymethyl]guanine and high dose corticosteroids. *Ann Intern Med* 1986;**105**:214.
353. Reed E, Meyers J. Treatment of cytomegalovirus infection: diagnosis and treatment of viral infections. *Clin Lab Med* 1987;**7**:831.
354. Reed E, Wolford J, Kopecky K, et al. Ganciclovir for the treatment of cytomegalovirus gastroenteritis in bone marrow transplant patients. *Ann Intern Med* 1990;**112**:505.
355. Reigstad H, Bjerknes R, Markestad T, et al. Ganciclovir therapy of congenital cytomegalovirus disease. *Acta Paediatr* 1992;**81**:707.
356. Reller L. Granulomatous hepatitis associated with acute cytomegalovirus infection. *Lancet* 1973;**1**:20.
357. Revello M, Baldanti F, Furione M, et al. Polymerase chain reaction for prenatal diagnosis of congenital human cytomegalovirus infection. *J Med Virol* 1995;**47**:462.
358. Revello M, Gerna G. Diagnosis and management of hCMV infection in the mother, fetus and newborn infant. *Clin Microbiol Rev* 2002;**15**:680.
359. Reynolds D, Stagno S, Hosty T, et al. Maternal CMV excretion and perinatal infection. *N Engl J Med* 1973;**289**:4.

360. Ribbert H. Über protozoanartige Zellen in der Niere eines syphilitischen Neugeborenen und in der Parotis von Kindern. *Zentralbl Allg Pathol* 1904;**15**:945.
361. Rinaldo C, Carney W, Richter B, et al. Mechanisms of immunosuppression of cytomegalovirus mononucleosis. *J Infect Dis* 1980;**141**:488.
362. Rinaldo C, Kingsley L, Ho M, et al. Enhanced shedding of cytomegalovirus in semen of human immunodeficiency virus–seropositive homosexual men. *J Clin Microbiol* 1992;**30**:1148.
363. Ringden O, Lonnqvist B, Paulin T, et al. Pharmacokinetics, safety and preliminary clinical experiences using foscarnet in the treatment of cytomegalovirus infections in bone marrow and renal transplant recipients. *J Antimicrob Chemother* 1986;**17**:378.
364. Rivera L, Boppana S, Fowler K, et al. Predictors of hearing loss in children with symptomatic congenital CMV infection. *Pediatrics* 2002;**110**:762.
365. Robert M, Geraghty J, Miles S, et al. Severe neuropathy in a patient with acquired immune deficiency syndrome (AIDS): evidence for widespread cytomegalovirus infection of peripheral nerve and human immunodeficiency virus–like immunoreactivity of anterior horn cells. *Acta Neuropathol* 1989;**79**:255.
366. Rongkavilit C, Bedard M, Ang J, et al. Severe cytomegalovirus enterocolitis in an immunocompetent infant. *Pediatr Infect Dis J* 2004;**23**:579–81.
367. Rosenthal S, Stanberry L, Biro F, et al. Seroprevalence of herpes simplex virus types 1 and 2 and cytomegalovirus in adolescents. *Clin Infect Dis* 1997;**24**:135.
368. Ross D, Dollard S, Victor M, et al. The epidemiology and prevention of congenital CMV infection and disease: activities of the Centers for Disease Control and Prevention Workgroup. *J Womens Health (Larchmt)* 2006;**15**:224.
369. Ross D, Victor M, Sumaratojo E, et al. Women's knowledge of congenital cytomegalovirus: results from the 2005 HealthStyles survey. *J Womens Health (Larchmt)* 2008;**17**:849–58.
370. Ross S, Novak Z, Kumbla R, et al. *GJB2* and *GJB6* mutations in children with congenital CMV infection. *Pediatr Res* 2007;**61**:687.
371. Ross S, Novak Z, Pati S, et al. Mixed infection and strain diversity in congenital cytomegalovirus infection. *J Infect Dis* 2011;**204**:1003–7.
372. Rowe W, Hartley J, Waterman S, et al. Cytopathogenic agent resembling human salivary gland virus recovered from tissue cultures of human adenoids. *Proc Soc Exp Biol Med* 1956;**92**:418.
373. Rozanova E, Teplinskaia L, Kaliberdina A, et al. Cytomegalovirus antibodies in tear fluid of patients with retinitis. *Arch Virol* 2006;**151**:2407.
374. Saigal S, Luny K, Larke R, et al. The outcome in children with congenital cytomegalovirus infection. *Am J Dis Child* 1982;**136**:896.
375. Salisbury S, Embil J. Graves disease following congenital cytomegalovirus infection. *J Pediatr* 1978;**92**:954.
376. Sanchez G, Neches W, Jaffe R. Myocardial aneurysm in association with disseminated cytomegalovirus infection. *Pediatr Cardiol* 1982;**2**:63.
377. Sandler A, Snedeker J. Cytomegalovirus infection in an infant presenting with cutaneous vasculitis. *Pediatr Infect Dis* 1987;**6**:422.
378. Sawyer M, Edwards D, Spector S. Cytomegalovirus infection and bronchopulmonary dysplasia in premature infants. *Am J Dis Child* 1987;**141**:303.
379. Schleiss M, Heineman T. Progress toward an elusive goal: current status of CMV vaccines. *Expert Rev Vaccines* 2005;**4**:481.
380. Schleiss M. Antiviral therapy for congenital CMV infection. *Semin Pediatr Infect Dis* 2005;**16**:50.
381. Schleiss M. Prospects for development and potential impact of a vaccine against congenital CMV infection. *J Pediatr* 2007;**151**:564.
382. Schleiss M. Comparison of vaccine strategies against congenital CMV infection in the guinea pig model. *J Clin Virol* 2008;**41**:224–30.
383. Schmidt G, Horak D, Niland J. A randomized, controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants. *N Engl J Med* 1991;**324**:1005.
384. Schmidt N, Emmons R. *Diagnostic procedures for viral, rickettsial and chlamydial infections*. 6th ed. Washington, DC: American Public Health Association; 1989. p. 321–78.
385. Schraff S, Brown D, Schleiss M, et al. The role of inflammatory genes in hearing loss. *Otol Neurotol* 2007;**28**:964.
386. Scott G, Buck B, Letterman J, et al. Acquired immunodeficiency syndrome in infants. *N Engl J Med* 1984;**310**:76.
387. Seale H, McIntyre C, Dwyer D, et al. The changing epidemiology of severe CMV disease in Australia. *Hum Vaccine* 2007;**3**:239.
388. Shabtai M, Luft B, Waltzer W, et al. Massive cytomegalovirus pneumonia and myocarditis in a renal transplant recipient: successful treatment with DHPG. *Transplant Proc* 1988;**20**:562.
389. Shanley J, Via C, Sharrow S, et al. Interstitial pneumonitis during murine cytomegalovirus infection and graft-versus-host reaction. *Transplantation* 1987;**44**:658.
390. Shen C, Chang W, Chang S, et al. Cytomegalovirus transmission in special-care centers for mentally retarded children. *Pediatrics* 1993;**91**:79.
391. Shibata D, Martin W, Appleman M, et al. Detection of cytomegalovirus DNA in peripheral blood of patients infected with human immunodeficiency virus. *J Infect Dis* 1988;**158**:1185.
392. Shyamala G, Sowmya P, Mahavan H, et al. Relative efficiency of PCR and EIA in determining of viral etiology in congenital cataracts. *J Postgrad Med* 2008;**54**:17.
393. Singh N, Dummer J, Kusne S, et al. Infections with cytomegalovirus and other herpesviruses in 121 liver transplant recipients: transmission by donated organ and the effect of OKT3 antibodies. *J Infect Dis* 1988;**158**:124.
394. Singh N, Yu V, Mieles L, et al. High-dose acyclovir compared with short-course preemptive ganciclovir therapy to prevent cytomegalovirus disease in liver transplant recipients. *Ann Intern Med* 1994;**120**:375.
395. Smets K, DeCoen K, Dhoge I, et al. Selecting neonates with congenital CMV infection for ganciclovir therapy. *Eur J Pediatr* 2006;**165**:885.
396. Smith M. Propagation in tissue cultures of a cytopathogenic virus from human salivary gland virus (SGV) disease. *Proc Soc Exp Biol Med* 1956;**92**:424.
397. Snider W, Simpson D, Nielsen S, et al. Neurological complications of acquired immune deficiency syndrome: analysis of 50 patients. *Ann Neurol* 1984;**14**:403.
398. Snydman D, Kristler K, Ulsh P, et al. The impact of CMV prevention on long-term recipient and graft survival in heart transplant recipients: analysis of the Scientific Registry of Transplant Recipients (SRTR) database. *Clin Transplant* 2011;**25**:e55–62.
399. Snydham D, Kristler K, Ulsh P, et al. Cytomegalovirus prevention and long-term recipient and graft survival in pediatric heart transplant recipients. *Transplantation* 2010;**90**:1432–8.
400. Snydman D, Werner B, Heinze-Lacey B, et al. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal transplant recipients. *N Engl J Med* 1987;**317**:1049.
401. Soetens O, Vaulous-Fellous C, Foulon I, et al. Evaluation of different CMV DNA PCR protocols for analysis of dried blood spots from consecutive cases of neonates with congenital CMV infection. *J Clin Microbiol* 2008;**46**:943.
402. Sohn Y, Oh M, Balcarek K, et al. Cytomegalovirus infection in sexually active adolescents. *J Infect Dis* 1991;**163**:460.
403. Sokol D, Demmler G, Buffone G. Rapid epidemiologic analysis of cytomegalovirus by using polymerase chain reaction amplification of the L-S junction region. *J Clin Microbiol* 1992;**30**:839.
404. Sommadossi J, Bevan R, Ling T, et al. Clinical pharmacokinetics of ganciclovir in patients with normal and impaired renal function. *Rev Infect Dis* 1988;**10**(Suppl.):507.
405. Sorlie P, Nieto F, Adam E, et al. A prospective study of cytomegalovirus, herpes simplex virus 1, and coronary heart disease: the atherosclerosis risk in communities (ARIC) study. *Arch Intern Med* 2000;**160**:2027–32.
406. Spector S. Transmission of cytomegalovirus among infants in hospital documented by restriction-endonuclease-digestion analyses. *Lancet* 1983;**1**:378.
407. Spector S, Hirata K, Neuman T. Identification of multiple cytomegalovirus strains in homosexual men with acquired immunodeficiency syndrome. *J Infect Dis* 1984;**150**:953.
408. Spector S, Merrill R, Wolf D, et al. Detection of human cytomegalovirus in plasma of AIDS patients during acute visceral disease by DNA amplification. *J Clin Microbiol* 1992;**30**:2359.
409. Spector S, Rua J, Spector D, et al. Detection of human cytomegalovirus in clinical specimens by DNA-DNA hybridization. *J Infect Dis* 1984;**150**:121.
410. Spector S, Spector D. Molecular epidemiology of cytomegalovirus infections in premature twin infants and their mother. *Pediatr Infect Dis* 1982;**1**:405.

411. Stagno S, Brasfield D, Brown M, et al. Infant pneumonitis associated with cytomegalovirus, *Chlamydia*, *Pneumocystis*, and *Ureaplasma:* a prospective study. *Pediatrics* 1981;**68**:322.
412. Stagno S, Pass R, Dworsky M, et al. Congenital cytomegalovirus infection: the relative importance of primary and recurrent maternal infection. *N Engl J Med* 1982;**306**:945.
413. Stagno S, Pass R, Thomas J, et al. Defects of tooth structure in congenital cytomegalovirus infection. *Pediatrics* 1982;**69**:646.
414. Stagno S, Tinker M, Elrod C, et al. Immunoglobulin M antibodies detected by enzyme-linked immunosorbent assay and radioimmunoassay in the diagnosis of cytomegalovirus infections in pregnant women and newborn infants. *J Clin Microbiol* 1985;**21**:930.
415. Staras S, Dollard S, Radford K, et al. Seroprevalence of CMV infection in the United States, 1988-1994. *Clin Infect Dis* 2006;**43**:1143.
416. Staras S, Flanders W, Dollard S, et al. Influence of sexual activity of CMV seroprevalence in the United States: 1988–94. *Sex Transm Dis* 2008;**35**:472.
417. Starr S, Glazer J, Friedman H, et al. Specific cellular and humoral immunity after immunization with live Towne strain cytomegalovirus vaccine. *J Infect Dis* 1981;**143**:585.
418. Stinski M. Cytomegalovirus and its replication. In: Fields BN, editor. *Virology*. 2nd ed. New York: Raven Press; 1990. p. 1959–80.
419. Stratton K, Durch J, Lawrence R. *Vaccines for the 21st century: a tool for decision making.* Report of the Committee to Study Priorities for Vaccine Development; Division of Health Promotion and Disease Prevention. Washington, DC, Institute of Medicine, National Academy of Sciences, National Academy Press, 1999.
420. Strauss M. Human cytomegalovirus labyrinthitis. *Am J Otolaryngol* 1990;**11**:292.
421. Studies of Ocular Complications of AIDS Research Group, in collaboration with the AIDS Clinical Trials Group. Mortality in patients with the acquired immunodeficiency syndrome treated with either foscarnet or ganciclovir for cytomegalovirus retinitis. *N Engl J Med* 1992;**326**:213.
422. Subietas A, Deppisch L, Astarloa J. Cytomegalovirus oophoritis: ovarian cortical necrosis. *Hum Pathol* 1977;**8**:285.
423. Sullivan V, Coen D. Isolation of foscarnet-resistant human cytomegalovirus patterns of resistance and sensitivity to other antiviral drugs. *J Infect Dis* 1991;**164**:781.
424. Sung H, Schleiss M. Update on the current status of cytomegalovirus vaccines. *Expert Rev Vaccines* 2010;**9**:1303–14.
425. Suzuki Y, Toribe Y, Mogami Y, et al. Epilepsy in patients with congenital CMV infection. *Brain Dev* 2008;**30**:420.
426. Svahn A, Berggren J, Parke A, et al. Changes in seroprevalence to four herpesviruses over 30 years in Swedish children aged 9 to 12 years. *J Clin Virol* 2006;**37**:118.
427. Taber L, Frank A, Yow M, et al. Acquisition of cytomegaloviral infections in families with young children: a serologic study. *J Infect Dis* 1985;**151**:948.
428. Tanak-Kitajma N, Sugaya N, Futatani T, et al. Ganciclovir therapy for congenital CMV infection in six infants. *Pediatr Infect Dis J* 2005;**24**:782.
429. Tang-Feldman Y, Lochhead S, Lochhead G, et al. Murine cytomegalovirus (MCMV) infection upregulates P38 MAP kinase in aortas of apoEKO Mice: a molecular mechanism for MCMV-induced acceleration of atherosclerosis. *J Cardiovasc Transl Res* 2013;**6**:54–64.
430. Tanner D, Buckley P, Hong R, et al. Fatal cytomegalovirus bronchiolitis in a patient with Nezelof's syndrome. *Pediatrics* 1980;**65**:98.
431. Tiula E, Leinikki P. Fatal cytomegalovirus infection in a previously healthy boy with myocarditis and consumption coagulopathy as presenting signs. *Scand J Infect Dis* 1972;**4**:57.
432. Tolpin M, Stewart J, Warren D, et al. Transfusion transmission confirmed by restriction endonuclease analysis. *J Pediatr* 1985;**107**:953.
433. Trang J, Kidd L, Gruber W, et al. Linear single-dose pharmacokinetics of ganciclovir in newborns with congenital cytomegalovirus infections. *Clin Pharmacol Ther* 1993;**53**:15.
434. Trofe J, Pote L, Wade E, et al. Maribavir: a novel antiviral agent with activity against cytomegalovirus. *Ann Pharmacother* 2008;**42**:144–7.
435. Ussery F, Gibson S, Conklin R, et al. Intravitreal ganciclovir in the treatment of AIDS-associated cytomegalovirus retinitis. *Ophthalmology* 1988;**95**:640.
436. Vallejo J, Englund J, Garcia-Prats J, et al. Ganciclovir treatment of steroid-associated cytomegalovirus disease in a congenitally infected neonate. *Pediatr Infect Dis J* 1994;**13**:239.
437. Van der Bij W, Speich R. Management of cytomegalovirus infection and disease after solid organ transplantation. *Clin Infect Dis* 2001;**33**(Suppl. 1):533.
438. Vander Knaap A, Vermeulen G, Bankhof F, et al. Pattern of white matter abnormalities on MRI: use of PCR testing of Guthrie cards to link pattern with congenital CMV infection. *Radiology* 2004;**230**:529.
439. Varani S, Landini M. Cytomegalovirus-induced immunopathology and its clinical consequences. *Herpesviridae* 2011;**2**:6–10.
440. Victoria M, Nangia B, Jindrak K. Cytomegalovirus pyloric obstruction in a child with acquired immunodeficiency syndrome. *Pediatr Infect Dis* 1985;**4**:550.
441. Vilmer E, Mazeron M, Rabian C, et al. Clinical significance of cytomegalovirus viremia in bone marrow transplantation. *Transplantation* 1985;**40**:30.
442. Vinters H, Kwok M, Ho H, et al. Cytomegalovirus in the nervous system of patients with the acquired immune deficiency syndrome. *Brain* 1989;**112**:245.
443. Virnig N, Balfour H. Hemiatrophy and hemiparesis in a patient with congenital cytomegalovirus infection. *Am J Dis Child* 1975;**129**:1359.
444. Visentin S, Manara R, Milanese L, et al. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. *Clin Infect Dis* 2012;**55**:497–503.
445. Vochem M, Hamprecht K, Jahn G, et al. Transmission of cytomegalovirus to preterm infants through breast milk. *Pediatr Infect Dis J* 1998;**17**:53.
446. Vollmer B, Seibold-Weiger K, Schmitz-Salue C, et al. Postnatally acquired CMV infection via breast milk: effects on hearing and development in preterm infants. *Pediatr Infect Dis J* 2004;**23**:322.
447. Walter S, Atkinson C, Sharland M, et al. Congenital CMV: association between dried blood spot viral load and hearing loss. *Arch Dis Child Fetal Neonatal Ed* 2008;**93**:F280.
448. Wang N, Huang S, Thurkbeck W. Combined *Pneumocystis carinii* and cytomegalovirus infection. *Arch Pathol* 1970;**90**:529.
449. Wang P, Evans A. Prevalence of antibodies to Epstein-Barr virus and cytomegalovirus in sera from a group of children in the People's Republic of China. *J Infect Dis* 1986;**153**:150.
450. Waris E, Rasanen P, Kreus K, et al. Fatal cytomegalovirus disease in a previously healthy adult. *Scand J Infect Dis* 1972;**4**:61.
451. Watson F, O'Connell J, Amber I, et al. Treatment of cytomegalovirus pneumonia in heart transplant recipients with 9(1,3-dihydroxy-2-propoxymethyl)-guanine (DHPG). *J Heart Transplant* 1988;**7**:102.
452. Weiner R, Bortin M, Gale R, et al. Interstitial pneumonitis after bone marrow transplantation. *Ann Intern Med* 1986;**104**:168.
453. Weller T, Hanshaw J. Virological and clinical observation of cytomegalic inclusion disease. *N Engl J Med* 1962;**266**:1233.
454. Weller T, Macauley J, Craig J, et al. Isolation of intranuclear inclusion-producing agents from infants with illnesses resembling cytomegalic inclusion disease. *Proc Soc Exp Biol Med* 1957; **94**:4.
455. White N, Yow M, Demmler G, et al. Prevalence of cytomegalovirus antibody in subjects between the ages of 6 and 22 years. *J Infect Dis* 1989;**159**:1013.
456. Whitley R. Congenital CMV infection: epidemiology and treatment. *Adv Exp Med Biol* 2004;**549**:115.
457. Whitley R, Brasfield D, Reynolds D, et al. Protracted pneumonitis in young infants associated with perinatally acquired cytomegaloviral infection. *J Pediatr* 1976;**89**:16.
458. Whitley R, Cloud G, Gruber W, et al. Ganciclovir treatment of symptomatic congenital cytomegalovirus infection: results of a phase II study. *J Infect Dis* 1997;**175**:1080.
459. Wiley C, Schrier R, Denaro F, et al. Localization of cytomegalovirus proteins and genome during fulminant central nervous system infection in an AIDS patient. *J Neuropathol Exp Neurol* 1986;**45**:127.
460. Williamson W, Demmler G, Percy A, et al. Progressive hearing loss in infants with asymptomatic congenital cytomegalovirus infection. *Pediatrics* 1992;**90**:862.
461. Wilmott F. Cytomegalovirus in female patients attending a VD clinic. *Br J Vener Dis* 1975;**51**:278.

462. Wilson W. The relationship of the herpesvirus family to sudden hearing loss: a prospective clinical study and literature review. *Laryngoscope* 1986;**96**:870.
463. Wink K, Schmitz H. Cytomegalovirus myocarditis. *Am Heart J* 1980;**100**:667.
464. Winston D, Pollard R, Ho W, et al. Cytomegalovirus immune plasma in bone marrow transplant recipients. *Ann Intern Med* 1982;**97**:11.
465. Winston D, Saliva F, Blumberg E, et al. Efficacy and safety of maribavir dosed at 100 mg orally twice daily for the prevention of cytomegalovirus disase in liver transplantation recipients: a randomized, double-blind, multicenter controlled trial. *Am J Transplant* 2012;**12**:3021–30.
466. Winston D, Young J, Pullarkat V, et al. Maribavir prophylaxis for prevention of cytomegalovirus infection in allogeneic stem cell transplant recipients: a multicenter, randomized, double-blind, placebo-controlled, dose ranging study. *Blood* 2008;**111**:5403–10.
467. Wolf D, Spector S. Diagnosis of human cytomegalovirus central nervous system disease in AIDS patients by DNA amplification from cerebrospinal fluid. *J Infect Dis* 1992;**166**:1412.
468. Wolf D, Spector S. Early diagnosis of human cytomegalovirus disease in transplant recipients by DNA amplification in plasma. *Transplantation* 1993;**56**:330.
469. Wu X, Wang Y, Xu Y. Cytomegalovirus glycoprotein B genotype in hematopoietic stem cell transplant patients from China. *Biol Blood Marrow Transplant* 2010;**16**:647–52.
470. Yamagashi Y, Miyagawa H, Wada K, et al. CMV DNA detection in dried blood spots for diagnosing congenital CMV infection in Japan. *J Med Virol* 2006;**78**:923–5.
471. Yamamoto A, Mussi-Pinhata M, Marin L, et al. Is saliva as reliable as urine for detection of cytomegalovirus DNA for neonatal screening of congenital CMV infection? *J Clin Virol* 2006;**36**:228.
472. Yamamoto A, Mussi-Pinhata M, Pinto P, et al. Usefulness of blood and urine samples collected on filter paper in detecting CMV by PCR technique. *J Virol Methods* 2001;**97**:159.
473. Yan H, Koyano S, Inami Y, et al. Genetic variations in the gB, UL144, and UL149 genes of hCMV strains collected from congenitally and postnatally infected Japanese children. *Arch Virol* 2008;**153**:667.
474. Yeager A. Transmission of cytomegalovirus to mothers by infected infants: another reason to prevent transfusion-acquired infections. *Pediatr Infect Dis* 1983;**2**:295.
475. Yeager A, Grumet F, Hafleigh E, et al. Prevention of transfusion-acquired cytomegalovirus infections in newborn infants. *J Pediatr* 1981;**98**:281.
476. Yilmaz-Cliftdogan D, Vardar F. Effect on hearing of oral valganciclovir for asymptomatic congenital cytomegalovirus infection. *J Trop Pediatr* 2011;**57**:132–4.
477. Yinon Y, Farine D, Yudin M, et al. Cytomegalovirus infection during pregnancy. *J Obstet Gynaecol Can* 2010;**332**:348–54.
478. Yinon Y, Yagel S, Tepperberg-Dikawa M, et al. Prenatal diagnosis and outcome of congenital CMV infection in twin pregnancies. *BJOG* 1996;**113**:295.
479. Yow M. Congenital cytomegalovirus disease: a NOW problem. *J Infect Dis* 1989;**159**:163.
480. Yow M, White N, Taber L, et al. Acquisition of cytomegalovirus infection from birth to 10 years: a longitudinal serologic study. *J Pediatr* 1987;**110**:37.
481. Yow M, Williamson D, Leeds L, et al. Epidemiologic characteristics of cytomegalovirus infection in mothers and their infants. *Am J Obstet Gynecol* 1988;**158**:1189.
482. Zaia J. Epidemiology and pathogenesis of cytomegalovirus disease. *Semin Hematol* 1990;**27**:1.
483. Zaloga G, Chernow B, Eil C. Hypercalcemia and disseminated cytomegalovirus infection in the acquired immunodeficiency syndrome. *Ann Intern Med* 1985;**102**:331.
484. Zhou X, Gruber W, Demmler G, et al. Population pharmacokinetics of ganciclovir in newborns with congenital cytomegalovirus infections. *Antimicrob Agents Chemother* 1996;**40**:2202.

CHAPTER 159

Epstein-Barr Virus

Charles T. Leach • Ciro V. Sumaya • Gail J. Harrison

Epstein-Barr virus (EBV) is an extremely common herpesvirus that is the etiologic agent of classic infectious mononucleosis (IM).[492] Evidence that the virus also plays a principal role in the development of certain lymphoproliferative diseases and epithelial malignant neoplasms continues to mount. Its ability to remain in a dormant (latent) state after infection allows reactivation and recurrence of disease, especially under immunosuppressive conditions. Unfortunately, antiviral agents have little effect on EBV-associated diseases, but progress is being made toward improved treatment of EBV-associated malignant diseases. Eventually, vaccination may provide protection for those persons at highest risk for development of severe disease manifestations.

This chapter reviews progress in understanding of the immunobiology of EBV and its disease associations. Enhanced understanding of EBV is leading to newer diagnostic methods and improved management of EBV-associated diseases.

HISTORY

One can identify four distinct periods of history related to IM and its etiologic agent, EBV[127,457]: (1) early descriptions of IM, (2) identification of the heterophil antibody test associated with IM, (3) discovery of EBV in Burkitt lymphoma (BL) cells, and (4) association of acute EBV infection with heterophil-positive IM.

Credit for the original description of a disease consistent with what is now called infectious mononucleosis commonly is given to a German physician named Pfeiffer,[403] who in 1889 described relatively mild illness characterized by fever and lymphadenopathy; later, his report was translated into English.[336] The illness came to be known as Drüsenfieber, or glandular fever. Although some cases of glandular fever were typical of what is now recognized as IM, certain characteristics of this illness, including its predominance in children and familial clustering, did not fit. In England, glandular fever and IM have become synonymous, yet many experts consider glandular fever to be a different illness.

In the early 1900s, numerous case descriptions of illnesses epidemiologically and clinically compatible with IM appeared.[56,190,241] However, because the illness now recognized as IM had not yet been described and these illnesses were suggestive of leukemia, most of them were reported erroneously as acute leukemia with spontaneous cure. Although the term *infectious mononucleosis* was used first in one of these case reports,[241] the illness was not distinguished from leukemia until Sprunt and Evans published their landmark paper in 1920.[482] These authors described a series of patients with clinical syndromes consistent with typical IM, including classic hematologic findings. A subsequent report by Downey and McKinlay provided a more detailed description of the hematologic manifestations.[110]

Although most cases of IM could be identified by hematologic and clinical findings, not until a specific serologic test was identified in 1932 could classic IM be better defined. In the course of investigating heterophil antibodies in serum from patients with rheumatic fever and other diseases, Paul and Bunnell[395] noted high titers of heterophil antibodies in serum from a control patient. Further investigation revealed that the control patient was a medical student with IM. Evaluation of sera from three other patients confirmed the presence of high titers of heterophil antibodies during acute IM.

In 1958, Dennis Burkitt, an English surgeon, described 38 cases of "round-cell sarcoma" developing in children and adolescents living in one region of Uganda, Africa.[54] Eventually, the tumors were determined to represent lymphomas, not sarcomas. Although a new mosquito-borne infectious disease was suspected as the culprit initially, Epstein and Achong[118] eventually were able to cultivate and evaluate tumor cells by electron microscopy; they noted particles with ultrastructural characteristics similar to those of herpes simplex virus (HSV), which had been described a few years earlier. Further work established this virus as the fourth human herpesvirus (after HSV-1, HSV-2, and cytomegalovirus [CMV]).

The association between this new herpesvirus and IM was noted serendipitously in 1968 when an IM-like illness developed in a technician working in the laboratory of the Henles.[205] Stored, pre-illness serum from this technician contained neither heterophil nor EBV antibodies, but both heterophil and EBV antibodies appeared 6 days after the onset of symptoms. In addition, virus was cultivated from the technician's peripheral blood cells. Subsequent testing of serial specimens from other patients with IM also demonstrated seroconversion to EBV in all cases. This work as well as studies from Yale University,[126,374] among others, firmly established EBV as the cause of IM. Investigators subsequently showed that EBV has the capacity to transform (immortalize) human B lymphocytes in vitro, which allows the derivation of EBV-transformed B-lymphoblastoid cell lines capable of being propagated indefinitely in tissue culture.[207,407]

VIROLOGY

EBV is in the family Herpesviridae, subfamily Gammaherpesvirinae, genus *Lymphocryptovirus*.[98] Its closest relative, human herpesvirus 8 (also known as Kaposi sarcoma–associated herpesvirus), is also a gammaherpesvirus. Although its taxonomic name (human herpesvirus type 4) is used by most bibliographic search services, most scientists and physicians use its historic name.

Structure and Genome

The structure of the EBV is typical for a member of the herpesvirus family; it displays an inner core of DNA surrounded by a nucleocapsid, tegument, and envelope.[119] Within the inner core, which measures approximately 45 nm, exist approximately 184 kilobase pairs (kbp) of double-stranded DNA coding for nearly 100 proteins. The surrounding nucleocapsid displays icosahedral symmetry (162 capsomeres) and measures approximately 100 to 110 nm in diameter. Outside the capsid lies the tegument, which consists of loosely packed, amorphous proteinaceous material, and the tegument in turn is surrounded by the viral envelope containing glycoprotein spikes. The complete virion measures approximately 150 to 220 nm.

The entire EBV genome has been sequenced.[28] The structure of EBV is characteristic of all the herpesviruses, with short and long sections of "unique" sequences (U_S and U_L, respectively) separated by a major internal repeat region (IR_1) generally consisting of 6 to 12 direct repeats of a 3-kbp sequence.[102] The virus also has numerous other regions of repeated nucleotide sequences, including direct repeat of a 0.5-kbp sequence at its termini.

MOLECULAR BIOLOGY

Much of the early information about the molecular biology of EBV comes from laboratory strains derived from BL tumor cell lines (e.g., HR-1)[216] and marmoset lymphoid cell lines transformed by viral strains infecting human beings (e.g., B95-8).[355]

Replication

EBV infection of cells is initiated by attachment of EBV glycoprotein gp350 to a cell surface receptor (CR2, CD21) found primarily on B lymphocytes and nasopharyngeal epithelial cells[472,563] and less commonly on other cells.[262,345,558] This receptor is the same as the surface moiety that functions as the receptor for the third component of serum complement (the C3d receptor).[136] Penetration of the cell requires a complex consisting of viral glycoproteins gH, gL, and gp42 (for B-cell infection), whereas only gH and gL are necessary for infection of epithelial cells[534]; gp42 binds with human leukocyte antigen (HLA) class protein found in B cells but not present in epithelial cells. After entering the B lymphocyte, the virus is transported through the cytoplasm and is stripped of its envelope in endocytic vesicles.[371] Subsequently, the viral genome circularizes and is maintained in the cell nucleus as a multicopy plasmid (or episome).[327] The initial and predominantly replicative (lytic) EBV infection probably occurs in epithelial cells of the oropharynx and adjacent structures,[470,551] with virus then transmitted to circulating B cells passing through these lymphoepithelial tissues. Although lytic genes may be found in EBV-associated tumors (e.g., posttransplant lymphoproliferative disease [PTLD]),[358] the overall proportion of replicating cells is far surpassed by latently infected B cells. In contrast, after infection of epithelial cells occurs, active replication occurs and leads to lysis and death of the cell. Alternating replication of viral glycoproteins in B cells and epithelial cells appears to modify the viral tropism, allowing virus from replicating B cells to infect epithelial cells more efficiently, and vice versa.[46] Recent data also suggest that monocytes may play an important role as a reservoir for EBV and could provide an important link for the transfer of virus from B cells to the oral epithelium.[521] Proteins synthesized during viral replication are important for viral gene regulation, replication of the genome, production of mature particles, and modulation of the immune system.

Latency

Latently infected B cells are the primary reservoir of EBV in the body. In a typical healthy EBV-seropositive adult, 1 to 50 per million B cells are infected with EBV, and this condition remains relatively constant during many years.[25] Although more than 80 gene products may be expressed during active viral replication, only a limited number are expressed during viral latency. This is one method by which the virus limits cytotoxic T-cell recognition of EBV-infected cells. The EBV gene products that may be expressed during latency include six nuclear proteins (EBV nuclear antigens [EBNAs] 1, 2, 3A, 3B, and 3C and a leader protein [EBNA-LP]); three membrane proteins (latent membrane proteins [LMPs] 1, 2A, and 2B); two small, nonpolyadenylated (noncoding) RNA molecules (EBV-encoded RNAs [EBERs] 1 and 2); and the Bam H1A rightward transcripts (BARTs).

On the basis of in vitro genetic and somatic cell hybrid experiments, three different patterns of latent gene expression can exist in EBV-infected cells from patients with EBV-associated disease.* Type I latency, exemplified by cells derived from BL, exhibits a limited repertoire of genes consisting of EBNA-1, EBERs, and BARTs. Type II latency typically is observed in cells derived from Hodgkin disease (HD) and nasopharyngeal carcinoma (NPC). Besides EBNA-1, EBERs, and BARTs, these cells also express latent membrane proteins (LMP-1, LMP-2A, LMP-2B). Lymphoblastoid cell lines as well as tissues from patients with IM, PTLD, and acquired immunodeficiency syndrome (AIDS)–associated lymphomas display the broadest pattern of latent gene expression (type III), wherein all genes associated with latency are expressed.[291,453,511,562] A "type 0" latency also may exist within circulating EBV-infected memory B cells from

*References 102, 291, 428, 499, 513, 559, 565.

healthy persons, wherein only EBERs and BARTs are expressed.[428] Although these latency expression pattern categories may be useful for histopathologic characterization, significant heterogeneity among histopathologically similar tumors can occur.

A brief description of latency-associated EBV genes follows.

EBNA. EBNA-1 is required for replication and maintenance of EBV episomal DNA in latently infected cells.[291] It also interacts with various viral promoters in regulating transcription of the EBNAs (including itself) as well as LMP-1. As mentioned previously, amino acid repeat structures in the EBNA-1 protein protect it from proteosomal degradation, thereby stabilizing protein levels and possibly preventing presentation by major histocompatibility complex class I molecules.[316] A role for EBNA-1 in B-cell transformation and oncogenesis remains somewhat debatable.[499] EBNA-2 clearly is a viral oncogene that is essential for cellular transformation. It transcriptionally activates several viral genes (e.g., LMP-1, LMP-2A) and cellular genes (e.g., CD21 and CD23) by its interaction with the DNA-binding protein RBP-Jκ (Jκ recombination-binding protein).[203] All three EBNA-3 proteins (A, B, and C) are transcriptional regulators of key cellular and viral promoters, but only 3A and 3C have proved to be indispensable for in vitro B-cell transformation.[518] The primary regulatory functions of EBNA-3 proteins, like those of EBNA-2, occur through interactions with RBP-Jκ.[432] Moreover, EBNA-3C is able to overcome the arrest of the retinoblastoma (Rb) gene cell cycle and increases expression of LMP-1.[325] The exact mechanistic contribution of EBNA-LP (also called EBNA-5) to B-cell immortalization is not well understood. Although EBNA-LP is not required for transformation of B cells, the absence of EBNA-LP drastically reduces the efficiency of transformation.[340] EBNA-LP possibly contributes to immortalization by enhancing EBNA-2–mediated transcriptional activation of the LMP-1 gene.[399] Also, previous studies have demonstrated that EBNA-LP cooperates with EBNA-2 in driving cells into the G_1 cell cycle phase by binding and inactivating the tumor suppressors Rb and p53.[501]

Latent Membrane Proteins. Like EBNA-2, LMP-1 (a viral oncogene) is essential for EBV transformation of B cells and appears to be the main transforming protein of EBV. LMP-1 displays pleiotropic effects when it is expressed in EBV-infected cells. Most prominently, LMP-1 mimics the CD40 B-cell receptor, which plays a key role in the activation and differentiation of B cells.[280] The protein also induces expression of antiapoptosis genes (e.g., BCL-2 and A20) as well as activation and adhesion of B cells.[457] LMP-2 consists of two transmembrane proteins: LMP-2A contains two intracytoplasmic domains (at the amino and carboxy termini) and 12 integral membrane domains; LMP-2B differs by the lack of the amino-terminal cytoplasmic domain. LMP-2 generally is recognized as a modifier of normal B-cell development, promoting maintenance of viral latency.[565] LMP-2A mimics the B-cell receptor and alters normal B-cell signal transduction by inducing a range of genes involved in cell cycle induction, inhibition of apoptosis, and suppression of cell-mediated immunity. Functions of LMP-2B are less well described, but a report indicates a key role in regulating LMP-2A activity.[443]

EBERs. These two small nonpolyadenylated (noncoding) RNAs are the most abundant viral transcripts found in latently infected EBV cells (approximately 10^7 copies per cell) and, indeed, serve as important diagnostic tools through the use of in situ hybridization (see later section on diagnosis, EBV nucleic acid). Studies indicate that EBERs may play important roles in viral persistence and oncogenesis for some tumors. For example, EBERs bind to double-stranded, RNA-activated protein kinase R, which serves to inhibit interferon-inducible apoptosis.[369] Moreover, EBERs play a key role in the maintenance of malignant phenotypes of BL cells.[503]

BARTs. These consist of a family of differentially spliced RNAs, first identified in NPC and later found in other EBV-associated malignant diseases.[475] Although noted abundantly in all forms of latency, protein products of their open reading frames have not been clearly identified.

Transformation

In vitro, resting human B cells infected with EBV develop into lymphoblastoid cell lines. These cells are described as immortalized or transformed because instead of following one of two normal pathways (differentiation or apoptosis [programmed cell death]), they proliferate continuously.[269] Current evidence suggests that EBV proteins coded by six latency-associated genes (described previously) are important for this cell transformation process: EBNA-2 and LMP-1 are absolutely essential; EBNA-1, EBNA-3A, EBNA-3C, and EBNA-LP also play critical roles.[428] The remaining latency-associated viral gene products, although not essential for transformation, are involved in the initiation and maintenance of cell transformation.

EBV generally transforms relatively mature B lymphocytes, secreting a complete immunoglobulin product (heavy chain plus light chain).[50,379] EBV also is capable of infecting and transforming B cells in earlier stages of development (e.g., pre-B cells [producing only μ chains] and lymphoid precursors lacking immunoglobulin gene rearrangement)[146,147,269,276]; mature plasma cells cannot be infected by EBV. Tumor cells and cell lines from BLs are B cells that generally express small amounts of immunoglobulin, which may be detected as surface immunoglobulin molecules, cytoplasmic/secreted molecules, or both. The major heavy-chain isotype expressed by Burkitt tumors appears to be μ chain (IgM), although other isotypes can be observed.[379] Similarly, the EBV-infected cells observed in the blood of persons with mononucleosis and the tumor cells found in immunodeficient hosts with EBV-associated lymphoproliferative diseases and lymphoma are B lymphocytes; these EBV-infected cell populations may include cells producing any of the major classes of human immunoglobulin (IgG, IgA, or IgM).[50,379,434,435] EBV-related malignant lymphoproliferations in immunosuppressed patients may be polyclonal, oligoclonal, or monoclonal; the lymphoproliferations probably are polyclonal at first but eventually progress to monoclonality.[101] BLs, on the other hand, always appear to be monoclonal at initial evaluation.[34,379]

EBV Genotypes

Two genotypes of EBV (EBV-1 and EBV-2) were identified in the 1980s on the basis of marked genomic differences in EBNA LP, 2, 3A, 3B, and 3C genes.[444,451] By use of this methodology, seroepidemiologic and virologic studies suggested geographic differences in the prevalence of these two genotypes, with both types found widely in central Africa and elsewhere but type 1 predominating in Western countries. Most EBV-infected persons appear to harbor one genotype, yet immunocompromised persons may be coinfected with both type 1 and type 2 genotypes. More detailed genetic analyses have been conducted, revealing more subtle differences in the genome and the presence of multiple EBV "strains" in healthy patients.[468,516,532] Multiple strains probably are acquired at the time that the initial EBV infection occurs,[516] yet it is possible that superinfections with other EBV strains occur after primary infection with one strain. No substantial evidence supports the view that any one genotype (or strain) is responsible for specific lymphoproliferative diseases.[414]

IMMUNOPATHOGENESIS

Infectious Mononucleosis

EBV elicits a wide range of immunologic responses in humans, partly because of its propensity to infect B lymphocytes. In a normal host, the immunologic effects probably are responsible for most EBV-associated disease manifestations. Both cellular and humoral immunity develop in response to EBV infection. Detection of humoral antibodies directed against viral capsid and nuclear proteins is important for establishment of the diagnosis of acute infection. However, the responsibility for effective control of EBV infection lies primarily with the cellular immune elements.[168] Two to 7 weeks after exposure, up to 20 percent of circulating B lymphocytes become infected during primary EBV infection, although 1 percent is typical.[433] A brisk cellular immune response ensues. In the acute stage, proliferating EBV-infected B cells are controlled principally by natural killer (NK) T cells, helper ($CD4^+$) T cells, and cytotoxic-suppressor ($CD8^+$) T cells.[427] In some rare EBV syndromes, these T cells can themselves become infected and directly cause disease.[250,283] In adults the proliferation of $CD8^+$ T cells causes a temporary inversion of the normal $CD4^+/CD8^+$ T-cell ratio; however, $CD8^+$ T-lymphocyte expansion is less marked in children.[537] After this T-cell response, the number of EBV-infected B cells falls dramatically during the next 4 to 6 weeks to concentrations of approximately 1 per million circulating B cells.[436] During convalescence, HLA-restricted cytotoxic T lymphocytes keep EBV in check, primarily by targeting the latently expressed EBNA-3 protein.[505]

During the acute stages of IM, despite a brisk immune system response to EBV, widespread and extensive impairment in general cell-mediated and humoral immunity also exists. For example, low or absent delayed-type hypersensitivity reactions develop in response to tuberculin and other antigens.[187,339] In addition, T cells from patients with IM display unusually weak proliferative responses when they are exposed to recall antigens (e.g., *Candida albicans*, tetanus toxoid).[359,510] Although EBV-specific cytotoxic and suppressor T cells appear during acute infection, the T-cell response is primarily nonspecific and HLA unrestricted.[284,285,392,423] Furthermore, a polyclonal humoral response occurs during acute infection with EBV.[442] Each B cell committed to one isotype continues to produce that isotype after transformation of EBV, thereby leading to secretion of all classes of immunoglobulins, although primarily IgM.[498,552]

Once primary infection occurs, EBV, like other herpesviruses, is able to persist in a latent state in a human host throughout that person's lifetime. This ability indicates that EBV exerts some influence on the immune response to prevent its complete eradication. With its large genome, this process of immune system evasion is carried out primarily through the coding of functional homologues of many cellular factors that are involved in cell cycle regulation, signal transduction, and inhibition of programmed cell death (apoptosis). For example, the EBNA-1 protein contains a spacer region consisting of multiple Gly-Ala repeats that inhibit protein processing and major histocompatibility complex class I recognition of cells expressing EBNA-1.[317] Another viral protein (BARF1) blocks the receptor for colony-stimulating factor type 1, which normally stimulates secretion of antiviral factors such as interferon-α (IFN-α) through interaction with monocytes.[81] In addition, one crucial EBV protein, LMP-1, upregulates certain antiapoptosis factors, allowing prolonged survival of cells.[564]

Epstein-Barr Virus–Associated Tumors

In normal hosts, cellular immune responses are adequate for control and sequestration of EBV-infected cells. However, cellular immune deficiency may allow uncontrolled proliferation of EBV-infected B cells to occur during primary or reactivated (recrudescent) EBV infection. Such an excessive EBV-associated production of B cells may be histologically pleomorphic (such as B-cell lymphoproliferative diseases) or relatively uniform (monomorphic, such as B-cell lymphomas). In organ transplant recipients, no consistent chromosomal translocations are associated with the lymphomas. Many of these lesions regress once immunosuppressive therapy initiated after transplantation is withdrawn.[562] In rare, severe cases, B cells harboring EBV DNA and expressing EBNA may become disseminated throughout the body as plasmacytoid cells visible on peripheral blood smears and as B-lymphoid cells potentially invading all organs of the body. Life-threatening EBV infections also may be correlated with an overly strong virus-induced T-cell proliferation that might cause autoaggressive activity, producing hypogammaglobulinemia or other major organ dysfunctions.[33]

The pathogenesis of EBV-associated malignant disorders in some settings may be multifactorial and involve a mixture of virologic, genetic, and environmental factors. NPC, which occurs predominantly in a specific geographic locale, has been linked etiologically with

EBV.[209,217] The identification of epithelial cells as potential targets for EBV infection, thereby allowing a lytic (productive) infection, has strengthened this relationship.[470] Further studies have revealed that NPC is a monoclonal proliferation that develops subsequent to EBV infection, with EBV gene products required for tumor growth.[393] However, NPC also clearly has a genetic association, inasmuch as strong linkage to a specific HLA type exists.[310] Furthermore, early EBV gene expression can affect profoundly the growth of infected cells, with full malignant potential reached when mutations occur on chromosome 3 or 9 (presumably affecting tumor suppressor genes).[231,232] These mutations possibly result from adverse environmental conditions (e.g., exposure to chemical carcinogens, such as the volatile nitrosamines and polycyclic hydrocarbons in salted fish).[255]

The EBV genome and expression of EBNA-1 are present in more than 95 percent of BL cells derived from African patients with BL (endemic BL), with the genome existing as circular episomes. In contrast, only 15 to 30 percent of cases of nonendemic (or sporadic) BL occurring in Europe and the United States contain EBV DNA.[235,573] On the other hand, EBV is present in more than 95 percent of primary lymphomas of the central nervous system (CNS) in patients with AIDS.[333] BL cells, but not the lymphoid cells found in IM, exhibit characteristic chromosomal alterations associated with enhanced malignant potential. Approximately 90 percent of BL tumors exhibit a reciprocal translocation involving the long arms of chromosomes 8 and 14, t(8;14); most of the remainder have t(8;2) and t(8;22).[287,445] These translocations place the c-*myc* oncogene under the control of an immunoglobulin promoter,[95,509] which results in transcriptional deregulation and overexpression of the c-*myc* oncogene.[90,306] Malaria is well known to cause chronic B-cell stimulation, providing enhanced opportunities for c-*myc* translocations within germinal centers of lymphatic tissue. Healthy children living in areas with holoendemic malaria may have high viral loads of EBV in peripheral blood, with acute malaria further augmenting the viral load[561] and antimalaria treatment subsequently reducing the EBV load in the peripheral blood.[108] Therefore, the evolution of endemic BL appears to be a multistep process involving EBV infection, malaria-induced B-cell proliferation, c-*myc* gene rearrangement and activation, and probably other elements.[99,100,286,378,463]

PTLD may develop in organ and bone marrow transplant recipients during the intense immunosuppression that occurs after placement of the graft. PTLDs span a spectrum of lymphoproliferation from polyclonal disease to monoclonal, monomorphic disease that often is difficult to distinguish from typical malignant lymphomas developing in patients with intact immune systems. No proof exists that an orderly progression leading to malignant lymphoma occurs; however, one model for the pathogenesis of PTLD proposes that defective immune surveillance after transplantation allows polyclonal proliferation of EBV-infected cells displaying a type III latency pattern (similar to that observed in IM).[82] Subsequently, genetic mutations at various loci (e.g., c-*myc*, p53) may allow progression to full malignant lymphoma to occur.[101]

HISTOPATHOLOGY

Infectious Mononucleosis[90,162,500]

Because IM typically is benign and the diagnosis is based primarily on clinical and serologic findings, histopathologic examination of tissues seldom is required.[92] However, histopathologic information is available on some tissues (e.g., lymph nodes, tonsils, spleen) from unusual cases and from patients requiring surgery.[517] For patients in whom lymphoproliferative disease and other serious manifestations of EBV infection develop, a larger amount of information is available from a broader range of tissues.[359]

Lymph nodes are enlarged diffusely during acute IM. On histologic examination, active lymphoid follicles are noted, with lymphoid proliferation extending to the sinuses, blood vessels, trabecula, and capsule.[359] The capsule remains intact despite substantial hyperplasia. The lymphoid response consists primarily of T and B immunoblasts, typically with a pleomorphic pattern and frequent mitoses indicative of rapid cell turnover.[517] Other cells identifiable in lymph node tissues include a substantial number of small lymphocytes, large atypical lymphocytes, plasma cells, histiocytes, and eosinophils. Micronecrosis may be present, although it is less extensive than that observed with herpes lymphadenitis. Tonsillar tissue also contains an active lymphoproliferative response but with more prominent follicles and extensive necrosis.

The spleen is enlarged two to three times its normal weight during acute IM, primarily as a result of hyperplasia of the red pulp. Like in the lymph nodes, the cellularity is principally from immunoblasts with substantial pleomorphism. Hemorrhage, primarily subcapsular in location, is identified commonly. The liver typically shows minimal disease; infiltration by lymphocytes and monocytes occurs principally in the portal areas, and possibly minor degenerative changes take place in hepatocytes. Bone marrow may appear relatively normal during IM, but some series have reported hypercellularity and small granulomas.[92] From the relatively few cases of CNS disease associated with EBV infection, histopathology reveals lymphocytic infiltration of the meninges.[9] Less common findings include demyelination, degeneration, focal hemorrhage, congestion, and edema.

Epstein-Barr Virus–Associated Malignant Diseases

The most common form of non-Hodgkin lymphoma (NHL) is BL, which consists of sheets of small noncleaved cells that are histologically uniform. Most arise from a single infected cell (monoclonality). The majority of CNS NHL tumors display large-cell morphology, with virtually all containing monoclonal EBV DNA. In PTLD, a wide range of histologic findings may be noted. At one extreme, one finds benign lymphoid hyperplasia with normal tissue architecture and a pleomorphic response arising from numerous infected cells (polyclonality). At the other end is observed frank malignant lymphoma.[88]

HD is unique among cancers as less than 1 percent of cells within the tumor are actually neoplastic.[272] HD tissues are characterized by the presence of diffuse inflammatory cell infiltrates admixed with a low level of neoplastic cells: classic Reed-Sternberg cells and mononuclear Hodgkin cells (referred to collectively as H-RS cells). Reed-Sternberg cells are large (15 to 45 μm), multinucleated cells demonstrated to derive from germinal center–derived B cells.[291] These cells exhibit strongly stained nucleoli surrounded by a characteristic clear area resembling a halo. The World Health Organization classification system for HD has been revised,[246] and two main subgroups now are defined: nodular lymphocytic-predominant HD and classic HD. Within classic HD are four distinctive histologic categories based on the predominant cell type and characteristics of the background cellularity: (1) lymphocyte rich, (2) nodular sclerosis, (3) mixed cellularity, and (4) lymphocyte depleted. In Western countries, EBV can be identified in approximately 40 percent of HD tissue specimens; mixed cellularity and lymphocyte-depleted types are associated most strongly with EBV. Children most commonly develop the nodular sclerosing variety.

Of the three histopathologic categories of epithelial tumors in the nasopharynx, the most common form in children is the undifferentiated variety, which is associated most strongly with EBV.[24] Examination of these tumors reveals undifferentiated squamous cells, with substantial infiltration by lymphocytes. Malignant cells consistently contain multiple copies of monoclonal EBV DNA within episomes, and several EBV proteins are expressed.

Other Epstein-Barr Virus–Associated Diseases

The tissues involved in hemophagocytic lymphohistiocytosis typically include the bone marrow, spleen, liver, and lymph nodes, but the skin or brain may be affected as well.[137] In the bone marrow, hypocellularity often is noted. Activated macrophages (or histiocytes) appearing "stuffed" are observed engulfing all bone marrow cellular elements or their precursors or fragments, including erythrocytes, leukocytes, and platelets.[137,431,548]

Tissues affected by oral hairy leukoplakia (predominantly in adult patients with AIDS)[117] reveal keratin or parakeratin projections, mild hyperparakeratosis and acanthosis, ballooning and hyperplasia of the prickle cell layer, and only a sparse inflammatory cell infiltrate in the subepithelial connective tissue.[172] The principal histologic findings in pulmonary tissue from children infected with human immunodeficiency virus (HIV) and having lymphocytic interstitial pneumonitis are lymphoid nodules and diffuse infiltration of the alveolar septa and peribronchiolar regions by lymphocytic cells, including lymphocytes, plasma cells, plasmacytoid lymphocytes, and immunoblasts.[17] Some lymphoid nodules may be observed as well. The infiltrating lymphocytes consist of B cells and T cells.[264] Necrosis is not observed, and blood vessels are not affected.

EPIDEMIOLOGY

Seroprevalence

Epidemiologic studies of EBV were stimulated by development of reagents for detection of specific anti-EBV antibodies in serum in the mid-1960s. Specifically, tests detecting antibodies to VCA (long-lasting, early in infection) and EA (short duration, early in infection) were used. Henle and Henle,[204] using an immunofluorescence method, first noted a high prevalence of EBV VCA antibodies (100%) in patients with BL, but they also discovered a relatively high prevalence (85%) in normal adults. Subsequent studies in American[156,206,409,489,490] and British[401] populations have established that 80 to 95 percent of adults have serologic evidence of past EBV infection, with most infections occurring during infancy and childhood.

The age at initial (primary) infection varies markedly in different cultural and socioeconomic settings, a fact that has great pertinence to manifestations of disease associated with primary infection. In developing countries, EBV infection generally occurs at an early age; 80 to 100 percent of children become infected by the time they reach 3 to 6 years of age. In these settings, most children with primary EBV infection have clinically silent or mild disease.[38,138,472,490] In privileged communities and in industrialized countries, primary infection with EBV commonly occurs later in life. In these settings and for reasons that are unclear, primary infection in older age groups, for example, individuals between 10 and 30 years of age, is more likely to induce clinical symptoms, most often a mononucleosis syndrome.[68] An IM case rate of 50 to 75 percent associated with primary EBV infection was documented in U.S. college students.[375] An unexpectedly high rate of IM also has been noted with eventual development of primary EBV infection in siblings of a pediatric case of IM.[496]

Incidence

The incidence of IM from population-based studies ranges between 50 and 100 cases per 100,000 population.[126,200,202] These studies indicate that the highest incidence rates for IM occur in the age group of 15 to 19 years. Although two reports have described a modest seasonal change for IM,[180,200] most studies have indicated no seasonal predilection. IM consistently has developed at a higher rate in persons of white race than in other ethnic groups.[200] In one study, IM developed in males at a rate slightly higher than that in females.[202]

Viral Shedding

The initial indication that EBV is shed in oropharyngeal secretions came from Chang and Golden[65,69] in 1971 when they identified a "leukocyte transforming agent" in throat washings from four of eight adults with IM. Although EBV was suspected, specific laboratory tests could not confirm it.[166] Other investigators using more specific identification methods clearly established EBV as the leukocyte transforming agent.[158,328,356,376] Subsequent

studies in healthy populations have indicated the following: (1) most children and adults with acute IM shed EBV in their oropharynx, (2) between 6 and 20 percent of the general population is shedding EBV in the oropharynx at any given point of time, (3) oropharyngeal shedding may be intermittent or continuous, and (4) high concentrations of EBV in oropharyngeal secretions are associated with high concentrations of EBV in B lymphocytes in the peripheral blood but not with concentrations of EBV-specific serum antibodies.

In addition to the oropharynx, EBV DNA may be found in the urine of persons during and for several months after an episode of IM.[297] Shedding of EBV also has been reported in the uterine cervix,[471,512] the male reproductive tract,[243,512] and breast milk.[265]

Transmission

Common Modes of Transmission

Reports in the first half of the 20th century, before widespread acceptance and use of the heterophil antibody test, suggested the occurrence of epidemics of IM.[189,380,515] However, researchers generally thought that the criteria used in most of those reports to establish the diagnosis of IM were inadequate to convince one that the outbreaks were indeed associated with EBV.[220] Although some success was reported for transmission of human EBV infections to certain primate species,[258] attempts to experimentally infect humans with EBV collected from the oropharyngeal secretions, blood, urine, and feces of patients with IM generally failed.[123,124,373,478] These experiments, conducted before development of specific EBV antibody tests, presumably were unsuccessful because most experimental subjects were immune as a result of having had a previous EBV infection.

Intimate sharing of oral secretions is thought to play a major role in the transmission of EBV.[125,220] The fact that EBV infection is extremely common worldwide, especially in underdeveloped regions, suggests that the virus is spread relatively efficiently in the general population. Surprisingly, outbreaks of EBV infection are uncommon events. However, transmission of EBV, even among close contacts of a person with acute EBV infection, may occur slowly. Studies of families of index cases of IM have indicated that EBV is not transmitted efficiently.[67,141,202] Furthermore, Chang[67] reported a lack of EBV transmission from a man with IM to his EBV-seronegative wife, even after 16 months of follow-up. In addition, data from another family study indicated that after the index IM episode, EBV antibodies (seroconversion) developed in only 35 percent of the nonimmune sibling contacts after an average observation period of 5.6 contact months.[496] Nonetheless, this same study also noted that the eventual seroconversion event in siblings was more likely to be associated with an IM episode than would be expected in the general population.[496] The complexity of EBV transmission and induction of IM is suggested further by the finding of multiple strains and variability of strains in distinct body compartments of individuals with primary EBV infections.[468]

Evidence has shown that EBV replicates in oropharyngeal epithelial cells[470]; subsequent infection of B cells possibly occurs as a result of contact with these epithelial cells.[4] Further studies indicate that latent and productive EBV infection occurs in lymphoid cells on the surface of the tonsillar epithelium and within the crypt, but not in epithelial cells.[10] Thus, transmission of EBV from person to person may be due to transfer of virus from persistently infected EBV lymphoid cells; EBV-infected epithelial cells may not be required for this phenomenon.[377,557] The incubation period for typical EBV-associated IM is imprecise but is estimated at 30 to 50 days.[141,220]

Transmission via Blood Products or Transplanted Organs

Transmission of EBV via blood transfusion has been reported,* but it occurs much less commonly than does transmission of CMV. High levels of immunity, transfer of neutralizing antibodies during transfusion, and limited survival of B cells may account for the rarity of this occurrence.[211] In transplant recipients, EBV-positive donor organs may transmit EBV to the recipient.[63,69,197]

Intrauterine and Perinatal Transmission

Because most females reaching child-bearing age already have been infected with EBV, primary infections during pregnancy occur infrequently.[140,237,303] Therefore, the risk of fetal transmission is difficult to study. One prospective study of primary infections occurring during pregnancy (silent or symptomatic) indicated no adverse fetal outcomes and, when studied, no viral transmission to the fetus.[139] Moreover, Chang and colleagues[66,70] could find only one EBV-infected specimen among 2696 samples of cord blood lymphocytes. On the other hand, case reports suggest that rare instances of intrauterine transmission may occur.[51,165,261] The significance of one report noting EBV DNA in breast milk of approximately 50 percent of healthy women is unclear.[265]

Sexual Transmission

Detection of EBV in genital ulcers,[193,411] the uterine cervix,[471] and the male reproductive tract[243] raises the possibility of venereal transmission. Additional epidemiologic findings suggest that EBV transmission and onset of IM can be correlated with sexual intercourse or closely associated behaviors.[89]

NONMALIGNANT CLINICAL SYNDROMES ASSOCIATED WITH EBV INFECTION

Silent, Nonspecific Infections

Similar to other lymphotropic human herpesvirus infections, primary EBV infection in children typically is silent but may result in mild clinical symptoms. Nonspecific clinical symptoms may include prolonged low-grade

*References 2, 157, 208, 220, 351, 507, 557.

fever with or without lymphadenopathy, cough, rhinorrhea, and pharyngitis.

Infectious Mononucleosis

The signs and symptoms of classic IM* develop in most adolescents and adults, as well as in some young children, when they experience a primary (initial) EBV infection. Factors determining an asymptomatic versus symptomatic response to EBV infection are largely unknown. Adults developing IM may have an exaggerated, pathologic T-cell response to primary EBV infection. The degree of viremia does not appear to be related to symptoms, with EBV viral loads in blood from symptomatic subjects being comparable to those undergoing silent EBV infection.[465]

Prodrome

In most persons with IM, a prodrome of 2 to 5 days develops during the end of the incubation period. Prodromal symptoms typically are mild and may consist of malaise, fatigue, and possibly fever. At this stage, differentiation of IM from other viral infections is difficult.

Acute Phase

The prodrome is followed by the classic clinical features of IM (i.e., fever, sore throat, malaise, and fatigue), and physical examination reveals lymphadenopathy and tonsillopharyngitis (Table 159-1). The temperature ranges from 38° C (100.4° F) to 40.5° C (104.9° F) (usually <39° C [102.2° F]); the fever usually begins abruptly, is more prominent in the afternoon and evening, and persists for 1 to 2 weeks. On occasion, the fever may last 4 to 5 weeks. Lymphadenopathy occurs in more than 90 percent of children and adults and typically involves the anterior and posterior cervical lymph nodes (Fig. 159-1). Affected nodes are moderately enlarged in a symmetric manner. However, generalized lymphadenopathy also is characteristic and involves the occipital, supraclavicular, axillary, epitrochlear, and inguinal chains. Like with other causes of viral lymphadenitis, the lymph nodes in patients with IM may be slightly tender, but they generally are nontender, nonerythematous, and discrete. Lymphadenopathy is most prominent during weeks 2 to 4 of the illness. Tonsillopharyngitis develops during the first week of illness and usually resolves abruptly the next week. Although mild symptoms occur in some patients, most have substantial symptoms. Exudative pharyngitis, similar to that found with *Streptococcus pyogenes* pharyngitis, occurs in approximately half of patients (Fig. 159-2). *S. pyogenes* is present in the posterior pharyngeal region of 5 percent of patients with IM and probably represents carriage rather than simultaneous bacterial tonsillopharyngitis.

Splenomegaly occurs in approximately 50 percent of patients with IM but more frequently in younger children. Usually only the tip of the spleen is palpable, with maximal size developing by the end of the second week

FIGURE 159-1 ■ Anterior cervical lymphadenopathy in an 8-year-old child with infectious mononucleosis. (Courtesy Dr. James Brien, Scott and White Hospital, Temple, Texas.)

*References 71, 113, 135, 221, 492, 494.

TABLE 159-1 **Clinical Manifestations of Infectious Mononucleosis in Children and Adults**

	Frequency (%)		
Sign or Symptom	**Age < 4 yr***	**Age 4-16 yr***	**Adults (Range)***
Lymphadenopathy	94	95	93-100
Fever	92	100	63-100
Sore throat or tonsillopharyngitis	67	75	70-91
Exudative tonsillopharyngitis	45	59	40-74
Splenomegaly	82	53	32-51
Hepatomegaly	63	30	6-24
Cough or rhinitis	51	15	5-31
Rash	34	17	0-15
Abdominal pain or discomfort	17	0	2-14
Eyelid edema	14	14	5-34

*From references 71, 113, 135, 221, 492, 494.

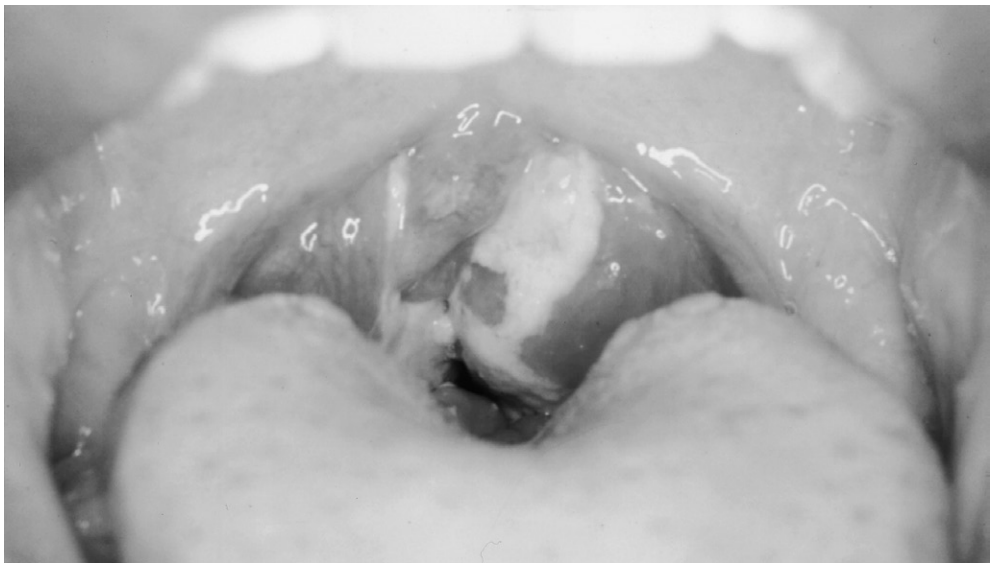

FIGURE 159-2 ■ Exudative pharyngitis in an 8-year-old child with infectious mononucleosis. (Courtesy Dr. James Brien, Scott and White Hospital, Temple, Texas.)

of illness. The splenomegaly typically resolves by the third or fourth week. Hepatomegaly occurs in approximately 60 percent of young children with IM and less commonly in older age groups. However, silent inflammation of the liver is a common finding.[229] Cough and rhinitis are noted frequently in young children with IM, perhaps as a result of other concurrent viral infections. Rash (unassociated with antibiotic administration) develops in less than 15 percent of adults with IM but occurs more commonly in children and adolescents (18% to 34%).[494] The strong correlation in young adults between the administration of ampicillin and the subsequent development of a rash is not observed in children with IM, possibly overshadowed by the overall increased incidence of cutaneous manifestations in children with IM (see Complications of Infectious Mononucleosis for a detailed discussion of rashes associated with IM). Children are more likely than are adults to have abdominal pain, failure to thrive, otitis media, and recurrent tonsillopharyngitis preceding or following IM.[494] A palatal enanthem is present in approximately a third of adults, but it is a nonspecific finding. Eyelid edema also is described in up to a third of adults but occurs less commonly (15%) in children.[494]

Resolution Phase

The acute phase of IM lasts several days to 3 to 4 weeks, with gradual and uneventful resolution. On occasion, biphasic illness is observed, with recrudescence of acute symptoms after significant improvement. Organomegaly may persist for 1 to 3 months. The severe fatigue usually resolves within 3 to 4 weeks, but several months may be required for persons with IM to fully resume their preillness activity levels. There is evidence, albeit minimal, suggesting that subsequent fatigue-like syndromes in patients with IM may be related to (or predicted by) the presence of preexisting lower physical fitness or prolonged bed rest during convalescence, whereas mood disorders are more related to premorbid psychiatric history.[543]

Infectious Mononucleosis in Young Children

Increasing numbers of EBV-IM or IM-like illness are being reported in very young children, usually accompanied by compatible EBV-specific laboratory testing.* Young children may have less intense EBV-specific serologic responses or not yield these immune responses until later in the disease course. EBV genomic testing by quantitative polymerase chain reaction (PCR) of peripheral blood mononuclear cells and plasma has been suggested as a laboratory diagnostic aid in some cases.[73]

Disseminated EBV Infection in X-Linked Lymphoproliferative Disease

Overwhelming primary EBV infection, seen rarely in immunocompetent patients, occurs more commonly in children with certain primary immunodeficiency disorders, most notably boys with X-linked lymphoproliferative disease (XLP).[417,488] These patients typically first present with features consistent with IM, but fulminant disease leading to death subsequently develops. Widespread, uncontrolled lymphoproliferation is found at autopsy, and causes of death include acute hemorrhage, meningoencephalitis, secondary bacterial infection, and liver failure.[364] Survivors usually have hypogammaglobulinemia, and B-cell lymphomas may develop later. XLP is associated with the deletion or mutation of a specific gene (SLAM-associated protein) located in the chromosomal Xq25 region, which is involved in key cellular

*References 73, 109, 154, 493, 494, 533.

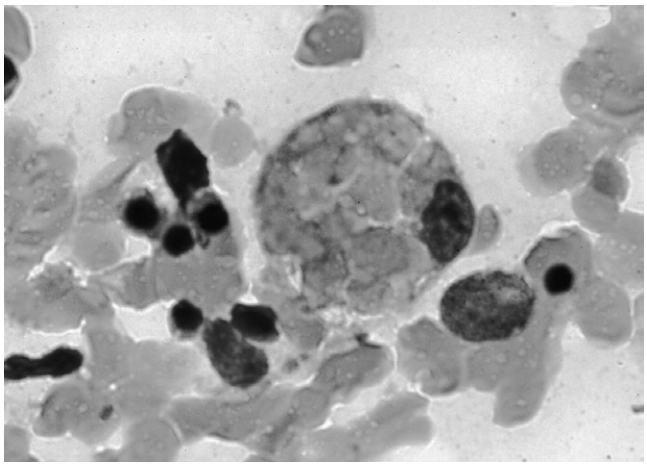

FIGURE 159-3 ■ Bone marrow from an 18-year-old woman with Epstein-Barr virus—associated hemophagocytic lymphohistiocytosis revealing a macrophage (center) filled with phagocytosed red blood cells. (From Blood 93:1991, 1999. Photo courtesy Lindsey Baden, M.D., and Frank Evangelista, M.D., Beth Israel Deaconess Medical Center, Departments of Medicine and Pathology, 330 Brookline Ave., SL-435, Boston.)

signaling pathways for immune responses[80,372,455]; absence of this functional protein permits overwhelming and usually fatal infection from a primary EBV infection.

Hemophagocytic Lymphohistiocytosis

Hemophagocytosis, first described in 1939, is a pathologic condition demonstrating engulfment of bone marrow cellular elements (erythrocytes, leukocytes, platelets, and their precursors) by activated macrophages (Fig. 159-3). Hemophagocytic lymphohistiocytosis (HLH, also called hemophagocytic syndrome) is characterized primarily by fever, pancytopenia, splenomegaly, and hemophagocytosis in lymphoreticular tissue (bone marrow, spleen, and lymph nodes). Since the initial description of its association with viral infections in 1979,[431] numerous viral infections, including EBV, have been linked with HLH.[388,424] Hereditary forms of HLH exist, most prominently those occurring in children with XLP (see preceding section).[364] A disseminated form of EBV infection with features of HLH may develop in these patients; most other cases are sporadic. In contrast to IM, which involves infection of B cells, primary infection in patients with sporadic HLH results in an EBV-driven T-cell proliferation with proinflammatory cytokines playing a central pathogenetic role.[250] Untreated EBV-associated HLH carries a high mortality rate.

Chronic Active Disease

Although first described in 1978,[529] chronic active Epstein-Barr virus (CAEBV) infection is not as well characterized as are other EBV-associated diseases.[149] It shares with HLH the central theme of infection and proliferation of non-B cells. CAEBV infection occurs more commonly in Japan,[282] with children affected more frequently than adults. Two forms of the disease have been described, one involving EBV-infected T cells and the other affecting NK cells.[283] Patients with the more severe form can be considered to have a chronic version of HLH, with similar symptoms and a uniformly poor prognosis. After having a typical IM-like illness, these patients develop persistent infection of their peripheral T cells ($CD4^+$, $CD8^+$, or both) and do not recover fully. Continuous or intermittent signs and symptoms, persisting for 3 months or longer, typically include fever, hepatosplenomegaly, lymphadenopathy, headache, malaise, and fatigue.[462,545,546] More severe cases display neurologic disease (e.g., encephalitis), malignant disease (e.g., lymphoma or leukemia), hepatic failure, hematologic disease (e.g., hemophagocytosis), and disease localized to other organs (e.g., myocarditis, pneumonitis).[282] One distinctive characteristic of these patients is the extremely high antibody titers against EBV replicative antigens, including VCA and EA, and low or absent responses to EBNA. Poor prognostic factors include older age and high EBV viral loads.[282] Some cases may be related to mutations in the perforin gene.[262,270] Clonal expansion of EBV-infected T cells probably plays a central role in disease pathogenesis.[282] A second form of the disease, described principally in Japanese patients, is characterized by milder IM symptoms, less extreme EBV serologic abnormalities, granular lymphocytosis, and high IgE levels.[283] Interestingly, these patients also typically develop pronounced skin reactions in response to mosquito bites. Large expansions of EBV-infected, CD56-positive NK cells are observed in peripheral blood and in the skin reactions. CAEBV infection should not be confused with chronic fatigue disorder, which has no clear association with EBV.

Other Diseases

EBV has been linked with various autoimmune disorders in children and adults, primarily on the basis of seroepidemiologic information. These disorders include rheumatoid arthritis,[57] systemic lupus erythematosus,[248,349] and multiple sclerosis.[5] Further careful studies must be conducted to substantiate these early reports of an etiologic relationship with EBV.

COMPLICATIONS OF INFECTIOUS MONONUCLEOSIS

Acute EBV infection in a healthy child or adolescent, regardless of whether it is associated with typical IM, rarely is complicated by serious illness.[492] The most frequent complications, exanthems and mild hepatitis, more appropriately are considered part of the normal spectrum of disease.[290] More serious complications involving major organs have been reported regularly since IM first was described as a distinct clinical entity, but they remain rare (for review, see elsewhere[6,253,400,494]). Most complications, including the more severe ones, are transient.

In one prospective study,[494] significant complications involving mainly the pulmonary, neurologic, and hematologic systems were noted in approximately 20 percent of children with IM. Thrombocytopenia with

hemorrhagic manifestations, severe airway obstruction, and neurologic complications developed in children with IM more frequently than in adults; jaundice occurred less frequently.

Exanthems

Ampicillin Rash. Historically, patients with IM were treated frequently with antibiotics, primarily for suspected streptococcal pharyngitis or bacterial lymphadenitis. Soon after the release of ampicillin, reports linked the antibiotic with the occurrence of exanthems in patients with IM. Two large case series have indicated that administration of ampicillin (or amoxicillin) precipitates an exanthem in most (95% to 100%) adolescents and adults with EBV-associated IM[391,415]; it is less common in children with IM.[494] This phenomenon has become known as the ampicillin rash associated with IM. The rash usually develops 5 to 10 days after treatment is initiated and resolves within a few days of discontinuation. Characteristically, the cutaneous lesions are maculopapular and pruritic and involve the trunk, face, and extremities (Fig. 159-4).[391] A rash also may develop in patients with IM treated with other β-lactam antibiotics, but such rashes do not represent hypersensitivity reactions.

Other Exanthems. An exanthem will develop in approximately 3 to 15 percent of persons with IM not treated with β-lactam antibiotics.* These rashes are maculopapular but can be urticarial, scarlatiniform, or erythema multiforme–like. Erythema nodosum also has been noted.[43] Although most commonly associated with hepatitis B, some cases of Gianotti-Crosti syndrome (papular acrodermatitis of childhood) also have been related to primary EBV infection.[225]

Cardiac

Mild electrocardiographic abnormalities (mostly nonspecific ST and T wave changes) have been observed in 6 to 16 percent of adults with IM.[113,222] Abnormalities usually are noted during the second or third week of illness and disappear by the fourth week, but they may persist longer. A case of myocarditis heralding typical IM has been noted in a young adult.[531] Serious cardiac complications occur extremely rarely.[145]

Hematologic

Hemolytic Anemia. Hemolytic anemia occurs in 1 to 3 percent of patients with IM,[223,494] and many cases have been reported in children with previously unrecognized hemoglobinopathies. The anemia typically develops during the second or third week of illness and resolves in 1 to 2 weeks. Corticosteroid therapy may hasten resolution.[155]

Aplastic Anemia. This anemia is a rare complication of IM, with only scattered case reports in the literature.[175,302,524] Steroids have been beneficial,[169] but bone marrow transplantation has been necessary in some cases.[318] Fatalities have been recorded, usually in association with pancytopenia.[1,524]

Thrombocytopenia. Thrombocytopenia occurs in 25 to 50 percent of patients with IM[59,60] and probably is immune mediated. Platelet counts usually return to normal levels 4 to 6 weeks after the onset of illness. Severe thrombocytopenia (platelet counts $<20 \times 10^3/mm^3$) occurs rarely, with fewer than 40 cases identified in one review.[405] These authors noted a complication rate of 27 percent and a mortality rate of 5 percent associated

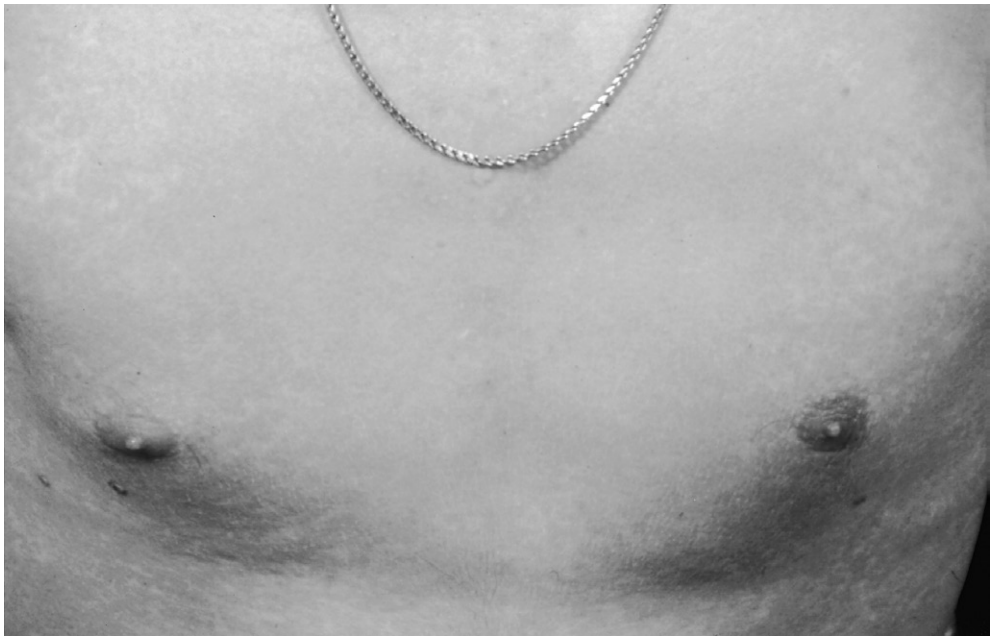

FIGURE 159-4 ■ Ampicillin rash occurring in an adolescent with infectious mononucleosis after the administration of amoxicillin. (Courtesy Dr. James Brien, Scott and White Hospital, Temple, Texas.)

*References 27, 221, 259, 343, 421, 494.

with severe thrombocytopenia. Case reports have suggested beneficial effects of corticosteroids[322,504] or intravenous immune globulin[112] for severe, life-threatening thrombocytopenia.

Neutropenia. Mild to moderate neutropenia occurs frequently during IM.[58,61] Neutrophil counts typically reach their lowest values during the third and fourth weeks of illness but may persist for another month or more. Although severe neutropenia (absolute neutrophil count <200) and agranulocytosis are rare events, with patients recovering uneventfully, death from overwhelming bacterial infection has been reported.[370] Antineutrophil antibodies elicited during EBV-induced polyclonal antibody stimulation probably are the source.[456]

Pancytopenia. Pancytopenia has been reported to develop in a small number of children and adults with IM.[293] Fatalities attributable to overwhelming bacterial infection have occurred.[247,524]

Spleen

Detectable splenomegaly develops in up to 50 percent of patients with IM; one study demonstrated splenic enlargement by ultrasound examination in 100 percent of patients hospitalized with IM.[107] Patients with a palpable spleen are at risk for having spontaneous rupture. In the United States, spontaneous rupture of the spleen occurs in an estimated 1 in every 1000 adults with IM[130,337]; the rate of splenic rupture in children is unknown but probably is lower. Rupture typically occurs during the first to third week of illness and is heralded by abdominal pain. Signs of hypovolemia occur frequently and include orthostasis, tachycardia, and syncope. Pain in the left shoulder (Kehr sign),[331] caused by diaphragmatic irritation from blood, is observed in approximately 50 percent of patients with splenic rupture; right shoulder and subscapular pain may develop as well.[452] Splenic abscess has been described in a child with IM.[75]

To reduce the risk of splenic rupture, physicians customarily caution patients against engaging in strenuous physical activity and contact sports during recovery from the acute illness and for the period of significant organomegaly.[448,535] Recommendations for reduced activity vary between 3 weeks and 6 months. In one review, the lack of evidence-based information about the timing of return to activity for athletes is acknowledged, and physicians are discouraged from making decisions on the basis of a single parameter.[535] Instead, individualized decisions must be made. In general, if the patient is asymptomatic without a palpable liver or spleen, a gradual return to pre-IM levels of activity probably is safe. Imaging procedures (e.g., computed tomography or ultrasonography) generally are not cost-effective or accurate in determining the timing for normal activity, but they may be considered for athletes desiring to return to play early and for those with ambivalent examination findings.

Most cases of splenic rupture require emergency splenectomy.[130] However, in an effort to maintain the patient's hematologic and immunologic competence, practical management of splenic rupture is evolving. At some institutions, selected patients with splenic rupture who meet certain requirements, including hemodynamic stability with low transfusion requirements and a normal level of consciousness, may be managed with medical therapy[22] or endovascular interventional methods[48] instead of traditional splenectomy.

Gastrointestinal Tract

Liver. Mild to moderate, subclinical inflammation of the liver occurs in more than 90 percent of patients with IM.[229] Rarely, more serious cases of hepatic disease, including fulminant hepatic failure in children, have been described.* Chronic hepatitis also has been reported.[506]

Other. Other gastrointestinal diseases purportedly associated with IM include pancreatitis, gastritis, typhlitis, and cholecystitis.†

Neurologic

Acute neurologic disorders[85,466] develop in 1 to 5 percent of persons with IM.[36,221,466] Neurologic complications usually occur at the height of the typical manifestations of IM, but they also may develop during the resolution phase. In addition, some reports document neurologic disease as a heralding event, before the development of typical IM, or even as the sole manifestation of EBV infection, especially in children.[45,104,106,121,363] The most common neurologic complications include those occurring in the CNS, such as aseptic meningitis, meningoencephalitis, and encephalitis; however, other central and peripheral neurologic complications have been described. The pathophysiologic process of CNS manifestations is not well established. Possible mechanisms include direct viral invasion, immunologic mechanisms, and inflammation.

Encephalitis and Aseptic Meningitis. EBV frequently affects the CNS, causing cerebrospinal fluid (CSF) abnormalities and electroencephalographic abnormalities, yet most patients exhibit no symptoms.[398] In two large series of patients with encephalitis, EBV was responsible for 2 to 5 percent of cases,[275,288] and EBV caused 8 percent of cases of focal encephalitis.[544] Clinical findings in EBV encephalitis include fever, headache, vomiting, seizures, alteration in mental status, irritability, disorientation, lethargy, and, occasionally, a comatose state.[104] Symptoms suggestive of schizophrenia, such as delusions, hallucinations, and extreme agitation, are seen occasionally. Patients with aseptic meningitis typically complain of a stiff neck, headache, fever, and vomiting. Symptoms of IM occur uncommonly in children developing EBV encephalitis. Typical CSF findings in children with EBV encephalitis include mild to moderate pleocytosis (lymphocytic predominance) in 50 percent, mildly elevated protein levels in half of cases, and normal glucose concentration.[106] EBV antibodies, EBV DNA, and even EBV itself have been identified in the CSF or brain tissue of persons with EBV-associated neurologic complications.[192,238,260,396] Some reports suggest that the immune dysregulation caused by primary EBV infection may act as a triggering mechanism for the development of measles-associated subacute sclerosing panencephalitis.[132,224] Because typical IM symptoms seldom

*References 103, 148, 199, 290, 330, 334, 341.
†References 201, 290, 321, 390, 464, 568.

occur in children with EBV encephalitis and clinical findings are not specific, EBV should be considered a possible etiology for any child with acute encephalitis.

Other CNS Manifestations. EBV-associated cerebellitis is a rare occurrence that develops primarily in children and young adults.[78,85,121,163] Full recovery occurs within weeks to months. Cranial nerve palsies, singly or in combination, also occur rarely; the facial nerve is involved most commonly.[353,566] Other manifestations of cranial nerve involvement include optic neuritis,[143] deafness,[121,547] and ophthalmoplegia.[450] Some cases of brain stem encephalitis[381,461] and recurrent aseptic (Mollaret) meningitis[169] have been reported.

Non-CNS Neurologic Complications. Outside the CNS, Guillain-Barré syndrome (ascending polyradiculoneuritis) is the most common complication of EBV infection.[176,177,426] Other non-CNS neurologic complications of IM include peripheral neuropathy,[115] autonomic dysfunction,[556] hemiplegia,[29] and transverse myelitis.[520]

Renal

Silent abnormalities of the urine sediment, including proteinuria and microscopic hematuria, occur commonly in patients with IM.[221,237,308] However, clinically significant renal disease is an extremely rare event. Tubulointerstitial disease and nephrotic syndrome have been observed most commonly.[42,527] Acute renal failure with and without rhabdomyolysis has been reported.[144,311,527] Some children and adolescents have developed hemolytic-uremic syndrome related to acute EBV infection.[459,467]

Respiratory Tract

Airway Obstruction. Severe airway obstruction occurs in 1 to 5 percent of children and adults with IM. Alpert and Fleisher[6] observed severe airway obstruction in a fourth of children admitted for complications of IM. Patients typically have severe tonsillopharyngitis with progressive symptoms of airway obstruction, usually in association with dysphagia and odynophagia. Airway obstruction may occur at any of several levels, including inflammation and hypertrophy in the Waldeyer ring, edema of the epiglottis and pharynx, and development of pseudomembranes in large airways.[162,185] Management of airway obstruction has included systemic corticosteroids, tonsillectomy, tracheostomy, and nasopharyngeal airway placement as well as general supportive measures, such as humidification and elevation of the head of the bed.[477,549] Nonsurgical management, including the systemic administration of steroids, usually is beneficial, although tonsillectomy may be necessary in refractory cases.[477,485,549] With the widespread availability of intensive care and mechanical ventilation, mortality from this complication is much lower than previously observed.

Neck Abscesses. Cervical and peritonsillar abscesses have been described in association with IM.[55,195,257,541] This uncommon complication follows severe tonsillopharyngitis in patients with IM and typically is caused by bacteria found in the oral cavity, including alpha-hemolytic streptococci and anaerobes. Appropriate management includes administration of antibiotics and surgical drainage. Some concern exists that corticosteroid therapy of incipient airway obstruction may predispose to the development of an abscess.[55,195] On the other hand, abscesses may develop in the absence of previous corticosteroid therapy.[152,410]

Pulmonary Disease. Radiographic evidence of pulmonary infiltrates occurs in 0 to 5 percent of patients with IM,[113,298] yet significant symptoms rarely are present.[113,221,298] Some cases of IM with pulmonary disease may represent coinfection with other etiologic agents.[15,134] However, several immunocompetent adults (but few children) with acute EBV infection have been described with pulmonary disease seemingly unrelated to other organisms.[79,191,382] Typically, unilateral or bilateral interstitial infiltrates are observed, and pleural effusions may be present.[116,289] In some cases, EBV has been detected in lung tissues.[483,525] Recovery is the rule, although mechanical ventilation may be necessary.

Psychiatric

Chronic Fatigue. In the early 1980s, a chronic debilitating disorder (now designated chronic fatigue syndrome) began receiving increased publicity and purportedly was linked etiologically to EBV.[263,487] The syndrome occurs more commonly in middle-aged women and is characterized primarily by chronic debilitating fatigue as well as by low-grade fever, mild lymphadenopathy, pharyngitis, neuropsychological problems, and other symptoms. The association with EBV was drawn mainly from epidemiologic features and some abnormal EBV-specific immune responses. Variable general immunologic abnormalities have been described in some cases. However, no consistency exists in these findings, and a plausible pathogenetic association has not been demonstrated. Most experts today do not regard EBV as a major etiologic agent in the development of this syndrome, although it may play a contributory role along with other viruses (e.g., human herpesvirus 6, CMV). Variably prolonged episodes of fatigue symptoms after IM continue to be reported, particularly among young adult women and those with premorbid mood disorders.[273,402]

"Alice in Wonderland" Syndrome. This unusual syndrome, technically termed metamorphopsia, is a visual illusion manifested as a distortion in size, form, movement, or color, and it is associated most commonly with migraine headaches, epilepsy, and hallucinogenic drugs. Copperman[87] first described its association with IM in 1977, and other similar cases also have been described.[76,122,295,296] Onset may occur during or soon after resolution of the clinical IM symptoms, and it may resolve within 4 to 6 weeks. Visual evoked potentials suggest diminished cerebral perfusion in affected cases.[296]

Miscellaneous Complications

Other complications purportedly associated with IM or primary EBV infection include recurrent tonsillitis,[526] sinusitis,[491] periorbital cellulitis,[491] ocular disease,[344] acute dacryocystitis,[160] rhabdomyolysis,[386] genital ulcers,[192,193,290,411] and orchitis.[420] EBV has been implicated in the pathogenesis of advanced types of periodontal disease.[473]

MALIGNANT DISEASES ASSOCIATED WITH EPSTEIN-BARR VIRUS

Burkitt Lymphoma

Endemic BL is a rapidly progressive and fatal tumor that predominantly affects young children in central Africa and Papua New Guinea (Fig. 159-5).[54] Approximately 60 percent of African patients with BL present with jaw masses; abdominal masses are second most common.[570] Less common sites include the CNS and the eye. Non-African patients with BL are most likely to have abdominal masses initially. In some patients with BL and some with congenital or acquired immunodeficiencies (including AIDS), CNS involvement is present or eventually develops and is characterized by intracranial mass lesions of lymphomatous cells or a CSF pleocytosis consisting of malignant cells, or both.[14,570,571]

Hodgkin Disease

The association of EBV with HD is based primarily on serologic studies[315,365] and identification of EBV DNA in H-RS cells (the malignant cells characteristic of HD).[276,539] The differential incidence distribution of HD in children throughout the world corresponds with the age at first EBV infection: it increases in adolescence in developed countries and increases in early childhood in developing countries.[184,256] Virtually all cases of HD in developing countries are associated with the presence of EBV, whereas the virus is present in approximately 40 percent of patients with HD in developed countries.[277] However, in developed countries, EBV is strongly associated with HD in children younger than 10 years and in tumors displaying the mixed cellularity variant.[20,213,389] HD rarely occurs in children younger than 5 years in Western countries, but it is seen more commonly at younger ages in developing countries. The precise pathogenetic mechanisms for EBV-associated HD are being unraveled, but evidence indicates that HD is primarily a result of chronic inflammation, with EBV contributing to the overproduction of various cytokines and chemokines by H-RS cells and the surrounding inflammatory cells.[277] Patients typically have cervical or supraclavicular lymphadenopathy as well as mediastinal adenopathy (occasionally with obstructive symptoms).[233] Constitutional symptoms such as fever, night sweats, and weight loss develop in approximately a fourth of patients with HD, and these symptoms worsen the prognosis.

FIGURE 159-5 ■ African boy with Burkitt lymphoma involving the jaw. (Courtesy Dr. George Miller, Yale University.)

Nasopharyngeal Carcinoma

NPC occurs primarily among adults in southern China, but it may be seen in Greenland, Alaska, and the Mediterranean region as well; the tumor is extremely rare in Western countries.[128] In endemic regions, children younger than 16 years account for only 1 to 2 percent of all cases of NPC, whereas this proportion is higher (10%) in the United States.[240,319] Children with NPC most commonly present with a painless unilateral neck mass, nasal symptoms (congestion, epistaxis, discharge), auditory symptoms (otalgia, otitis media, hearing loss), and cranial nerve abnormalities.[24,255] At initial evaluation, children with NPC are more likely than are adults to have locoregional disease.[24,240]

Posttransplant Lymphoproliferative Disease

Children immunosuppressed for organ transplantation have an increased risk for development of EBV-related lymphoproliferative syndromes and B-cell lymphomas (collectively termed PTLD).[219,292,479] Ho and colleagues[219] reported a greater frequency of lymphoproliferative lesions in pediatric transplant recipients (4%) than in adult transplant recipients (0.8%). Pediatric organ transplant recipients are thought to have an increased risk for development of EBV-related PTLDs because, compared with adult transplant recipients, they are more likely to be seronegative for EBV at transplantation, and primary infection is more likely to progress to PTLD and other severe EBV-associated disorders.[218,219] Furthermore, among pediatric renal transplant recipients with primary EBV infection, adolescents are at a higher risk for development of PTLD and have poorer outcomes compared with younger recipients.[474] Some variability exists in the incidence of PTLD according to the specific organ transplanted. The incidence is highest in children receiving intestinal transplants (31%),[486] probably attributable to more profound immunosuppressive regimens and greater loads of lymphatic tissue in the grafts; generally lower PTLD incidences are noted in other pediatric organ transplant populations (liver: 7% to 13%; thoracic organs: 4% to 12%; kidney: 1% to 9%).[486] After human stem cell transplantation, the 10-year cumulative incidence rate for PTLD is approximately 1 percent overall.[91] Rates of PTLD are substantially higher if benign, hyperplastic cases of PTLD are included.[567]

The clinical history, physical examination findings, and confirmatory virologic laboratory testing are required to establish a diagnosis of PTLD.[230] Symptoms may be nonspecific, especially early in the course, and include fever, malaise, weight loss, abdominal symptoms (bleeding, nausea, vomiting), sore throat, swollen lymph nodes, headache, focal neurologic symptoms, and indications of graft dysfunction.[171,230] On examination, signs may include lymphadenopathy, large tonsils, hepatosplenomegaly,

pallor, subcutaneous nodules, and focal neurologic findings.[171]

Subsequent EBV-associated smooth muscle tumors (e.g., leiomyosarcomas) have been found to develop in some adolescent and young adult patients undergoing organ transplantation.[307,437]

Lymphoproliferative Disease in Other Immunodeficient Patients

Patients with a wide variety of congenital immunodeficiencies (e.g., severe combined immunodeficiency syndrome, Wiskott-Aldrich syndrome, ataxia-telangiectasia, Chédiak-Higashi syndrome, common variable immunodeficiency) as well as iatrogenically immunosuppressed patients (e.g., patients receiving cancer chemotherapy or anti–tumor necrosis factor-α monoclonal antibodies) also are at risk for development of various EBV-associated lymphoproliferative diseases and severe atypical EBV infections. One should keep in mind, however, that IM and other forms of EBV infections in immunocompromised children and adults usually run a course similar to that in immunocompetent persons; actually, even in immunosuppressed patients (and posttransplantation subjects), the majority of EBV infections are clinically silent.[210,219]

Other Malignant Diseases

Other rare lymphoproliferative diseases (B-cell and T-cell) as well as carcinomas have putative associations with EBV. The strength of these associations is variable. Pyothorax-associated lymphomas, occurring at sites of long-standing inflammation (e.g., tuberculosis), are strongly associated with EBV, with EBV identified in tumor cells in 70 to 100 percent of cases.[150] Lymphomatoid granulomatosis, seen predominantly in immunosuppressed patients, has the distinctive histopathologic finding of malignant cells surrounding blood vessels (angiocentricity). This malignant disease is considered to have a pathogenesis similar to that of PTLD, with diminished T-cell surveillance probably allowing proliferation of EBV-transformed B cells.[425] Lymphomatoid granulomatosis occurs primarily in the lung but may be found at other sites, including lymph nodes of children.[111] Plasmablastic lymphomas (extraordinarily rare in children) are unusual tumors typically found in the oral cavity of adult patients with AIDS, but they also sometimes are found in immunocompetent patients.[62] They have a strong association with EBV.

Several T-cell lymphomas are associated with EBV.[425] They include (1) angioblastic lymphadenopathy[540]; (2) enteropathy-type T-cell lymphoma (often associated with celiac disease)[555]; (3) extranodal nasal T/NK-cell lymphoma, an aggressive tumor involving the nasal or midline facial area or other non-nasal, extranodal sites[245]; and (4) aggressive NK-cell leukemia, a rapidly fatal disease heralded by an acute symptom complex that includes fever and multiorgan system failure.[245]

Gastric carcinomas are more common in Asia, but they are extremely rare in children. In adults, two types of gastric tumors are noted to be associated with EBV. Gastric adenocarcinoma is a more common tumor, but EBV is found in only approximately 10 percent of tissues. In contrast, EBV can be found in the majority of tissues from patients with gastric tumors displaying an undifferentiated (lymphoepithelioma-like) histopathologic pattern.[279] Also in Asian populations, EBV is detectable within most lymphoepithelioma-like salivary gland tumors[460] and could play an etiologic role. Considerable ongoing debate continues about the etiologic relationship of EBV with breast cancer.[301]

EPSTEIN-BARR VIRUS AND HUMAN IMMUNODEFICIENCY VIRUS

Several studies have indicated that underlying HIV infection is responsible for abnormal responses to EBV infections. These findings probably are attributable to the relative ineffectiveness of T cells from patients with AIDS in controlling EBV-infected lymphocytes.[41] In HIV-infected adults, these unusual reactions include exaggerated antibody responses to certain EBV antigens, including VCA and EA,[419] and enhanced oropharyngeal shedding of EBV.[7,133,251,376] Oropharyngeal shedding of EBV occurs more commonly in younger HIV-infected children, and children whose HIV disease progressed more rapidly had a substantially higher rate of excretion.[133,251] In another study, EBV infection developed in HIV-infected children at an earlier age and was more likely to involve hepatosplenomegaly.[397] Whether EBV contributes directly to the progression of immune deficiency in these patients continues to be a matter of conjecture, although in vitro studies do indicate that some EBV proteins (e.g., LMP, BZLF1) are able to transactivate HIV-1.[194,338]

Studies clearly have revealed that certain lymphoproliferative and other diseases develop in HIV-infected patients at rates higher than those in healthy persons. These diseases include NHL, HD, body cavity lymphoma, smooth muscle tumors, lymphocytic interstitial pneumonitis (LIP), and oral hairy leukoplakia.

NHL has remained the most common malignant disease occurring in children with AIDS[39] and is an AIDS-defining condition.[86] In the present era of highly active antiretroviral therapy (HAART), NHL also has surpassed Kaposi sarcoma as the most common malignant disease in adults.[117] In one pediatric study, approximately one third of NHL tumors contained EBV DNA.[348] Primary CNS lymphomas are extremely rare occurrences in healthy children, but they have accounted for 8 percent[348] to 23 percent[39] of NHLs in children with AIDS. Primary CNS lymphomas are linked strongly to EBV, with the virus present in virtually every case.[333] NHL invariably occurs in patients with AIDS who have profound immune suppression (e.g., $CD4^+$ concentration <200 cells/μL). Pediatric patients with AIDS and NHL typically have systemic symptoms of fever and weight loss. Signs of extranodal disease include hepatosplenomegaly, abdominal distention, jaundice, bone marrow involvement, and, rarely, CNS symptoms.[347] Although it is not an AIDS-defining condition, HD in adults with AIDS historically has occurred at rates 5 to 15 times higher than in the general

population. HD is an uncommon occurrence in children with AIDS, but it has been described.[348,413] HIV-infected patients, including children,[413] appear more likely than non–HIV-infected patients to present with disseminated disease, sometimes without obvious nodal involvement on physical examination.[406] In adults with AIDS, the incidence of HD has increased significantly in the HAART era.[117] Interestingly, HD incidence rates are higher in patients with AIDS and moderate compared with severe immune suppression, and the increased incidence is postulated possibly to be related to the inability of EBV-infected H-RS cells to recruit sufficient lymphocytes and histiocytes for their survival.[40]

Body cavity lymphoma (or primary effusion lymphoma) is a rare malignant disease that occurs almost exclusively in adult patients with AIDS, but it has been reported in children.[305] Patients typically have immunoblastic lymphomatous effusions without solid tumors in body cavities, such as the peritoneum or pleura.[367] More than 75 percent of these nonsolid tumors are associated with human herpesvirus 8; however, EBV also is identified frequently in tumor cells. EBV may play a cofactorial role in the pathogenesis of these unusual tumors.[425]

Leiomyosarcoma is an extremely rare occurrence in healthy patients,[560] but it is the second most common malignant disease in children with AIDS[346] and is an AIDS-defining condition (Category B).[86] It also has developed in solid-organ transplant recipients[307] and in patients with other types of immunodeficiency.[354] EBV may be responsible for the unusually high incidence of these smooth muscle tumors found in children with AIDS.[252,348] Leiomyosarcoma tissues from patients with AIDS have high concentrations of EBV (CD21) receptors, contain clonal (or oligoclonal) EBV DNA, and express EBERs and EBNA-1.[252,348] Leiomyosarcoma may develop in any smooth muscle tissue in patients with AIDS, but more typically it is noted first in the gastrointestinal tract and lungs.

LIP is a lymphoproliferative disease that occurs primarily in children with AIDS.[16] In the era before HAART, the incidence of LIP was 12 percent among children enrolled in a large longitudinal natural history study[167]; in the HAART era, this incidence has dropped to less than one case of LIP per 1000 person-years. Previous serologic and virologic evidence pointed to an association with EBV,[16,272,422,446] but the exact role that EBV plays in this lymphoid proliferation is unclear at present. LIP is associated with an improved prognosis for survival.[446] On radiographic examination, diffuse interstitial infiltrates are noted. The course of disease is variable. Many infections in infants are asymptomatic, and whereas some children remain stable for long periods, others may exhibit a slow but relentless progression to chronic lung disease typified by tachypnea, cough, wheezing, and hypoxemia. Advanced disease is characterized by clubbing, wasting, cor pulmonale, and bronchiectasis.

Oral hairy leukoplakia is a nonmalignant squamous cell proliferation that develops in the oropharynx of patients with AIDS. Although a common occurrence in adults with AIDS, it occurs in less than 5 percent of HIV-infected children.[335] EBV DNA and virus replication can be detected within epithelial cells,[173,476] with defective variants possible.[151] Oral hairy leukoplakia lesions are manifested as nonremovable gray or white plaques on the lateral surface of the tongue; they often have vertical corrugations and a shaggy or "hairy" appearance when dry.[458]

DIAGNOSIS OF INFECTIOUS MONONUCLEOSIS

General Laboratory Findings

Patients with IM typically have an absolute lymphocytosis (>50% lymphocytes, with total leukocytes >5000/μL), prominent atypical lymphocytes (usually >10% of total leukocytes), and a positive test result for Paul-Bunnell heterophil antibodies (positive differential heterophil). The atypical lymphocytes observed in the blood of patients with mononucleosis (Fig. 159-6) predominantly consist of activated T lymphocytes responding to the B-cell infection.[394] Although often considered a hallmark of IM, atypical lymphocytes also may be observed in association with CMV infection, toxoplasmosis, measles, mumps, roseola, rubella, drug reactions, and other conditions.[74,553]

For a typical uncomplicated case of IM, establishing a specific diagnosis of EBV infection is unnecessary; a complete blood count, differential, and heterophil antibody test are sufficient for diagnostic testing. The results of these tests are uncommonly normal early in the course of disease, and thus the tests may need to be repeated during the first 3 to 4 weeks before diagnostic results are achieved. The clinician should be aware that very young (<4 years) patients with primary EBV infection frequently have a negative heterophil test result.[493] Some studies suggest that a higher ratio (>0.35) of lymphocytes to white blood cell counts[550] or the detection of lymphatic tissue with fibrinous membranes observed by nasopharyngeal endoscopy[536] may distinguish between IM and acute bacterial tonsillitis.

Heterophil antibodies classically were measured as sheep erythrocyte agglutinins.[395] Beef, ox, and horse red blood cells also are agglutinated by the heterophil

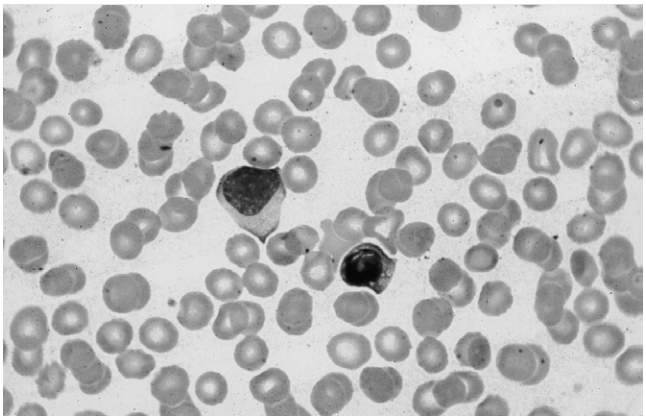

FIGURE 159-6 ■ Atypical lymphocytes in the peripheral blood of a patient with infectious mononucleosis. (Courtesy Dr. Margaret Gulley, University of North Carolina, Chapel Hill, NC.)

antibodies found in the serum of patients with IM, but these heterophil antibodies do not bind to guinea pig kidney antigen extracts. These properties of mononucleosis heterophil antibodies distinguish them from the naturally occurring Forssman heterophil antibodies and from the heterophil antibodies found in serum sickness and other conditions. Thus, traditional tests for Paul-Bunnell heterophil antibodies involve absorption of the test serum with beef or ox red blood cells (which remove Paul-Bunnell heterophil antibodies) and guinea pig extract (which does not remove them). Tests in current use (e.g., mono test, Mono-Diff, Monospot) typically use horse red blood cells, which provide a more sensitive assay than do sheep erythrocytes. However, when horse erythrocyte agglutination tests are performed, the specificity of a positive result always should be confirmed by absorption of the serum with at least guinea pig kidney extract or, preferably, with both guinea pig kidney extract and beef (or ox) red blood cells. Materials for the absorption steps are included in many but not all of the kits available commercially. Another heterophil antibody test, the beef (or ox) erythrocyte hemolysin assay, does not require absorption of the test sera but is somewhat less sensitive than is horse erythrocyte agglutination testing. Other tests[142,188,312,439] using purified forms of heterophil antigen do not seem to offer any significant advantage over the rapid slide test using horse erythrocyte agglutination.

Other laboratory tests in patients with severe mononucleosis syndromes or in those with atypical clinical manifestations may indicate involvement of major organs, but such involvement rarely is associated with severe complications (see the previous section, Complications of Infectious Mononucleosis). These laboratory abnormalities include elevated transaminase levels, mild hemolytic anemia, and neutropenia.

EBV-specific laboratory testing should be reserved for patients with an EBV-suspected disease (1) lacking a heterophil antibody response, (2) exhibiting atypical, severe manifestations, or (3) associated with significant lymphoproliferative, oncogenic, or chronic findings.[495] In most cases of EBV-associated IM episodes in immunocompetent patients, EBV-specific serology (i.e., antibody determination) is sufficient to affirm the viral-specific diagnosis. Isolation of virus from body secretions, fluids, and tissues (see later section, Virus Isolation) is labor-intensive and not widely available. Quantitation or semiquantitation of EBV-specific viral load provides some measure of activity or putative influence of the virus in a variety of EBV-associated diseases. Other laboratory methods demonstrating high concentrations of actively replicating virus or latent antigens (such as immunohistochemical methods for antigen testing or quantitative PCR) may be more useful in associating EBV infection with disease, particularly in immunocompromised patients (Table 159-2).

Epstein-Barr Virus Antibodies

The viral-specific diagnosis of EBV-associated primary infections as IM in immunocompetent individuals (and commonly in immunocompromised patients) usually requires three types of antibody analysis on acute serum: IgG to EB VCA (VCA IgG), IgM to EB VCA (VCA IgM), and IgG to EBNA (EBNA IgG).[495] (The EBNA complex is composed of six nuclear antigens or proteins, principally EBNA-1; the antibody response to the complex will herein be referred to as IgG to EBNA, or EBNA IgG.) The three EBV antibodies can be detected by commercially available immunofluorescent techniques or by various enzyme immunoassays. The need to add the determination of IgG to EB early antigen, a serologic response commonly found early in the primary infection, usually is not necessary for IM-like episodes.

Evolution of the serologic response to EBV antigens after a prototypic EBV-associated IM episode is depicted

TABLE 159-2 **Summary of Laboratory Tests for Epstein-Barr Virus (EBV)–Associated Diseases**

Detection of	Test	Purpose
Antibodies	Heterophil antibody	Detect heterophil antibodies indicating infectious mononucleosis (more reliable in patients older than 4 years)
	EBV antibodies	Measure antibody response to viral proteins in serum samples; distinguish acute from remote infection
EBV DNA and RNA	Southern blot	Assess clonality of lesions with respect to EBV DNA structure; distinguish latent from replicative infection
	In situ hybridization	
	RNA (EBERs)	Identify EBER transcripts in specific cell types within histologic lesions
	DNA	Identify EBV DNA in specific cell types within histologic lesions
	PCR	Detect and quantitate EBV DNA in blood or CSF to diagnose and monitor disease
EBV proteins	Immunohistochemistry	Identify EBV protein expression in specific cell types within histologic lesions; distinguish latent from replicative infection on the basis of expression profiles
EBV	Virus culture	Detect infectious virions or latently infected B cells; impractical for routine clinical use
	Electron microscopy	Identify whole virions representing replicative infection; impractical for routine clinical use

CSF, cerebrospinal fluid; EBER, EBV-encoded RNA; PCR, polymerase chain reaction.
Modified from Gulley ML: Molecular diagnosis of Epstein-Barr virus–related diseases. *J Mol Diagn* 2001;**3**:1-10.

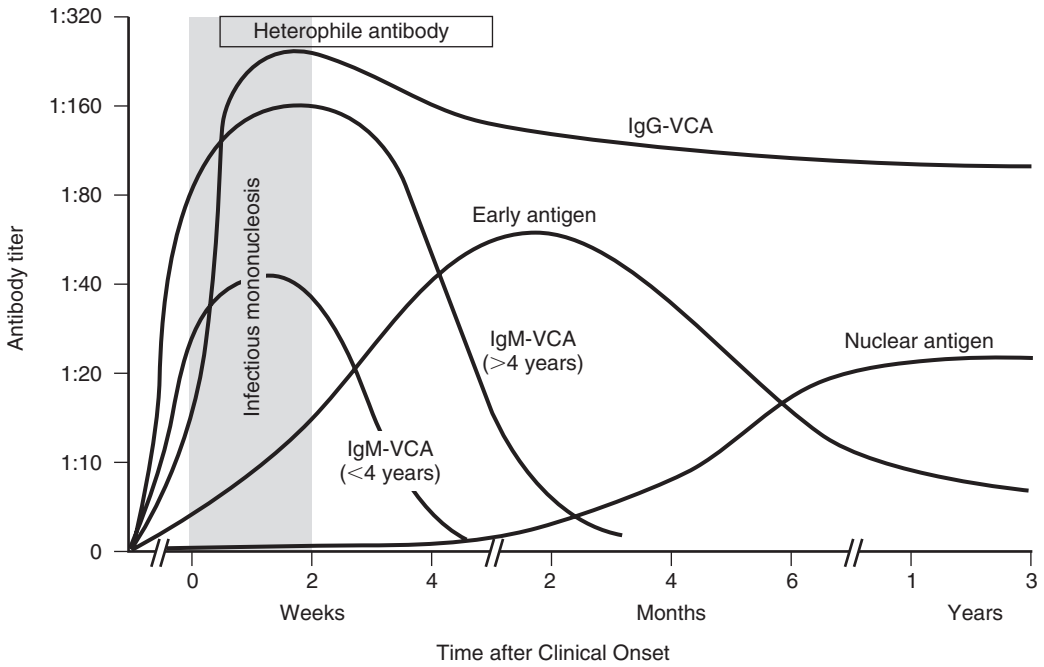

FIGURE 159-7 ■ Schematic representation of the evolution of antibodies to various Epstein-Barr virus antigens in patients with infectious mononucleosis. The titers are geometric mean values expressed as reciprocals of the serum dilution. The minimal titer tested for viral capsid antigen (VCA) and early antigen (EA) antibodies was 1:10; for Epstein-Barr nuclear antigen (EBNA), it was 1:2.5. The IgM response to capsid antigen was divided because of the significant differences noted according to age. (From Jenson HB, Ench Y, Sumaya CV. Epstein-Barr virus. In: Rose NR, de Macario EC, Folds JD, et al, editors. *Manual of clinical laboratory immunology*. 5th ed. Washington, DC: American Society for Microbiology; 1997. p. 634-43.)

in Figure 159-7. By the time clinical evaluation for IM and probably other forms of primary EBV infection is performed, an appreciable antibody response to EBV VCA has developed in most persons.[209,493] In the case of primary infections (e.g., IM), the initial serum sample often contains both IgG and IgM antibodies to VCA. Most children also have (or they will develop shortly) antibodies to the EA complex, which can be measured separately as antibodies to the restricted (R) or diffuse (D) components.[138,493] During the early phase of primary infection, most persons do not have detectable IgG antibodies to EBNA; a few have marginally detectable anti-EBNA titers. Thus, the serologic diagnosis of a recent or current primary EBV infection typically consists of a positive IgG and IgM anti-VCA response and negative anti-EBNA, with positive results in the anti-EA assay, if it is performed.

After the acute primary EBV infection (reflected in IM), IgM antibodies to VCA typically disappear within 2 to 3 months, and IgG antibodies to EA usually disappear within 6 to 12 months after infection (see Fig. 159-7).[493,495] IgG anti-VCA and anti-EBNA antibodies persist for life and are indicative of a typical, chronic virus-carrying state.[493] Some studies also have shown the persistence of anti-EA antibodies for several years after an acute EBV infection and the occasional appearance of anti-EA antibodies in healthy seropositive persons; however, the clinical or virologic importance of this finding is not clear. Heterophil antibodies may persist for months and sometimes for a year or more in persons who have recovered from IM; they remain detectable longer (usually more than a year) when the more sensitive horse erythrocyte agglutination and a quantitative method to determine the antibody response are used.

The antibody profile for an acute primary EBV infection, particularly that associated with an IM episode, is relatively clear in comparison with the variability of serologic or antibody profiles found in persons with other EBV-related disorders (Table 159-3). When a disease is not a consequence of primary infection but may be related to persistent or reactivated EBV infection (e.g., BL, NPC, some lymphomas of immunocompromised hosts, some chronic or atypical illnesses), the following serologic profile may be observed: positive IgG anti-VCA, often in high titer; usually negative IgM anti-VCA; often elevated anti-EA titer; and positive anti-EBNA assay (although sometimes at low titer and occasionally undetectable).* Of note is that males with XLP frequently have no or low levels of EBV antibodies in response to EBV infection.[449]

Some studies[342,429,440] suggest that monitoring for decreases in anti-EBNA antibodies or identification of increased immunologic mediators such as interleukin-4 and soluble CD23 and increased circulating EBV may be useful in providing early serologic signs of EBV-associated tumor development in organ and bone marrow transplant recipients. Further, testing for IgA antibody responses to EBV antigens may be helpful in establishing a diagnosis and evaluating the response to therapy for NPC.[266]

Molecular cloning of subregions of the EBV genome into high-expression plasmid vectors can generate large

*References 195, 210, 211, 254, 417, 488.

TABLE 159-3 **Correlation of Clinical Status and Characteristic Serologic Responses to Epstein-Barr Virus (EBV) Infection***

Clinical Status	Heterophil Antibodies (Quantitative Test)	Response EBV-Specific Antibodies				
		IgM-VCA	IgG-VCA	EA-D	EA-R	EBNA
Susceptible	—	—	—	—	—	—
Infectious mononucleosis						
Acute primary infection	++[†]	++/+++	+/+++	+/+++	–[‡]	–/+
Recent primary infection	+/–	–/+	++++	+++	–[‡]	—
Remote infection	—	—	++	–[§]	–/+	+/++
Reactivation immunosuppressed/ immunocompromised	—	—	+++/++++	–[§]	++/+++	–/+++
Burkitt lymphoma	—	—	+++/++++	–[§]	++/+++	+/++
Nasopharyngeal carcinoma	—	—	+++/++++	++/+++	–[¶]	++/+++

*Antibody response scale is none or negative (–), low (+), average (++), above average (+++), and very high (++++). A scale is used for application to various antibody tests and techniques. This table is modified from numerous studies. Individual responses outside the characteristic range may occur.
†Children younger than 4 years may have weak or undetectable heterophil antibody responses as determined by qualitative rapid slide tests.
‡In some young children and in adults with asymptomatic seroconversion, the anti-EA response may be mainly to the EA-R component.
§A minority of individuals will have the anti-EA response mainly to the EA-D component.
¶A minority of individuals will have the anti-EA response mainly to the EA-R component.

amounts of pure proteins containing EBV-encoded polypeptide sequences for use in immunoblot and enzyme-linked immunosorbent assay (ELISA)–based tests.* These methods have extended the availability and use of EBV laboratory diagnosis and follow-up. There is a growing number of commercially available EBV serologic tests, particularly enzyme immunoassays.[52,214] Yet after several decades of use, immunofluorescent techniques still remain the "gold standard" in EBV-specific serologic testing because of their overall excellent specificity and sensitivity. However, they require more expensive equipment, time, effort, and experience in interpretation. ELISAs are important alternatives for serologic testing because of their ease in performance and enhanced sensitivity. However, their specificity may be inferior to that of immunofluorescent techniques.[314] It is noteworthy that there is insufficient standardization of antigen preparation, antigen and substrate selection, and cross-comparability of serologic techniques and manufacturers involved with commercially available EBV serologic tests. Therefore, interpretations should be drawn carefully, particularly with serum of young children, an age group that has been less well evaluated.[313]

Improvements in standardization as well as in specificity and sensitivity of commercially available serologic testing would benefit the laboratory diagnosis of primary EBV infections. Current EBV-specific serologic testing for nonprimary infections, particularly in immunocompromised patients, provides more limited diagnostic interpretations. The expanding capacity in the detection and quantitation of EBV viral load in body compartments and lesions (see next section) is of growing diagnostic importance in these circumstances.

*References 53, 64, 129, 215, 342, 429, 440.

Epstein-Barr Virus Nucleic Acid (DNA or RNA)

In young patients, EBV serologic testing may reveal false-positive results owing to retained maternal antibodies from the placenta, excessive blood product infusions, or asymptomatic seroconversion. In patients treated with immunosuppressants, a false-negative serologic response to EBV antigens may occur. In such situations, demonstrating EBV nucleic acid in tissue sections or in blood may be necessary for diagnostic purposes.[8] Methods include Southern blotting, in situ hybridization, and detection of EBV DNA by PCR.[49] Besides their utility as diagnostic aids, these tests also are useful for epidemiologic and pathogenetic purposes relevant to EBV infections.

Southern blot hybridization has been useful in demonstrating the association of EBV with disease.[14,49,538] Although it is not as sensitive as PCR, Southern blotting has the advantages of providing additional information about the monoclonality of EBV-associated lesions, detecting lytic EBV processes, and recognizing viral integration into the genome.[172,182,186,418]

Because of their ubiquity in EBV-infected cells (up to 10^7 copies per cell), detection of EBERs in tissues by in situ RNA hybridization has become a standard method for detecting and localizing latent EBV in tissues.[32,183,333] Probes for detection of EBERs (riboprobes) are labeled conveniently by immunoperoxidase methods. Detection of EBV DNA by in situ hybridization also can be performed,[48] although DNA detection is less sensitive because of the lower copy numbers per cell in comparison with EBERs.

Amplification of EBV DNA by PCR has emerged as an important test for establishing the diagnosis and treatment of several EBV-associated diseases. In adult patients

with AIDS and primary CNS lymphomas, detection of EBV DNA in CSF has high sensitivity and specificity in most studies.[18,77,93,333] PCR for EBV in CSF, possibly in combination with certain imaging studies,[18] may be sufficient to establish the diagnosis of CNS lymphoma and could preclude performance of brain biopsy, which can be associated with complications. On the other hand, lumbar puncture in patients with cerebral lesions also must be considered carefully because of the risk for cerebral herniation.

In stem cell and solid-organ transplant recipients, quantitative PCR for EBV DNA in peripheral blood serves as a useful tool for the diagnosis of PTLD and as a marker during follow-up.[171,309,530,559] Levels of EBV DNA in blood typically are low in healthy control subjects, high in those with PTLD or other EBV-associated diseases, and intermediate in asymptomatic organ transplant patients. A high EBV load in the blood has a clear relationship with development of PTLD,[170] and with successful management, EBV DNA concentrations typically diminish to baseline levels.[170,228,230,438] Although negative predictive values for these tests are higher than 90 percent, positive predictive values are substantially lower. Healthy subjects with IM, asymptomatic seroconverters, and transplant recipients with PTLD may have comparably high levels of EBV detected in the blood, thus making it difficult to separate out true PTLD from increases unassociated with PTLD. Detection of EBV within cellular specimens, such as whole blood or peripheral blood mononuclear cells, appears to be more reproducible and accurate than are tests measuring cell-free EBV in plasma or serum. Further studies clearly are necessary to establish quantitative laboratory standards for diagnosis and monitoring of EBV-associated PTLD. Histopathologic evaluation of a suspicious tumor site is much more informative and less controversial regarding diagnostic utility.

Quantitative PCR also may be useful for diagnosis and monitoring of other EBV-associated malignant diseases, including NPC,[304] HD,[310] and NHL.[484] Larger studies are necessary to define better the role of EBV viral load testing in these diseases. PCR testing may also be of assistance in the laboratory testing for EBV-induced disease (as IM) in young children and infants.[73,109]

Epstein-Barr Virus Proteins

Immunofluorescent techniques may be used to detect the presence of cells carrying EBV proteins in touch imprints of biopsy material or in frozen sections of cryopreserved tissue.[172,333,408,562] Most commonly, tissues are examined for VCA, EBNA-1, and LMP-1 proteins with the use of mouse monoclonal antibodies.

Virus Isolation

EBV from blood or oropharyngeal specimens (rarely, other specimens) can be isolated in tissue culture with a transformation assay.[254] For oropharyngeal specimens, the test requires incubation with fresh cord blood mononuclear cells for as long as 6 weeks. Transforming virus can be identified in peripheral blood by incubating mononuclear cells in the presence of cyclosporine (for inhibition of cytotoxic T cells, which can suppress the growth of EBV-positive B cells). An end-point dilution assay may be used to quantitate the amount of EBV within blood specimens.[436] Virus isolation tests are used primarily for research purposes and are not widely available.

Electron Microscopy

EBV virions also may be detected by electron microscopy, which has been used in patients with oral hairy leukoplakia,[244] NPC,[21] and other EBV-associated lesions. The ultrastructural features of EBV virions, however, are indistinguishable from those of other herpesviruses, and confirmatory tests usually are necessary. Electron microscopy generally is available only at larger research centers and therefore is impractical for routine diagnostic testing.

Imaging Studies

Imaging studies seldom are necessary in typical patients with IM. In one study of patients hospitalized with IM, the most common findings on chest radiography were splenomegaly (47%), hilar adenopathy (13%), and interstitial infiltrates (5%).[298] Imaging studies are used more commonly for evaluation of EBV-associated neurologic disease, especially in immunocompromised patients. Children with EBV-associated encephalitis usually have normal neuroimaging results.[106] However, some patients may display diffuse swelling or patchy low-density lesions by computed tomography, and magnetic resonance imaging may reveal increased signal on T2-weighted images as well as atrophy.[106,461] Contrast-enhanced computed tomography scans of primary cerebral lymphoma in patients with AIDS typically reveal large (2 to 6 mm) lesions occurring anywhere in the cerebrum with diffuse and homogeneous enhancement[502]; however, some overlap exists with findings observed in other diseases, such as cerebral toxoplasmosis. Thallium 201 single-photon emission computed tomography, in conjunction with magnetic resonance imaging and PCR testing of CSF for EBV DNA, may be useful in distinguishing CNS lymphomas from toxoplasmosis in adult patients with AIDS.[18]

DIFFERENTIAL DIAGNOSIS

Infectious Mononucleosis. EBV causes an estimated 80 to 95 percent of IM syndromes.[497] Numerous agents have been associated with heterophil-negative IM. CMV is responsible for most cases of heterophil-negative IM with typical clinical and hematologic findings.[228] Certain features of CMV mononucleosis may help distinguish it from EBV (e.g., less common sore throat and lymphadenopathy, less intense atypical lymphocytosis, and prominent splenomegaly), but these characteristics have considerable overlap with EBV-associated IM. Serologic testing consistent with acute CMV infection in the

absence of characteristic EBV serologic findings confirms the diagnosis. The remainder of cases may be attributable to infection with *Toxoplasma gondii*, adenoviruses, rubella virus, and hepatitis A virus. Acute HIV syndrome also may mimic IM. However, in children with non-EBV IM, the etiology frequently remains elusive. In heterophil-negative children with mononucleosis and both the clinical and hematologic characteristics of the syndrome, the most likely known agents are EBV and CMV.

Other EBV-Associated Disorders. EBV-associated HLH may be confused with IM, systemic connective tissue disorders, septicemia, and certain hematologic malignant neoplasms.[239] EBV-associated CNS lymphomas in adults with AIDS are difficult to distinguish from cerebral toxoplasmosis on the basis of clinical findings and imaging studies. Historically, a negative *T. gondii* serum antibody test response eliminates toxoplasmosis, whereas a positive test response may necessitate a trial of antitoxoplasmic therapy. Response within a week suggests toxoplasmosis, but a nonresponse indicates the need for stereotactic brain biopsy to establish a diagnosis. Identification of EBV DNA in CSF by PCR may help confirm the diagnosis of lymphoma. Because PTLDs may affect the graft, a high EBV viral load in conjunction with graft abnormalities (e.g., elevated liver enzyme values in a liver transplant recipient) may be confused with graft rejection; histologic evaluation of biopsy tissue from the graft should differentiate PTLD from acute graft rejection.

TREATMENT

Infectious Mononucleosis

Supportive Care. Support, including rest, fluids, and antipyretics, is all that is required for uncomplicated IM. Although clinical trials and case reports dating to the 1950s generally have demonstrated a beneficial effect of corticosteroids in reducing the symptoms associated with IM (e.g., fever, pharyngitis, lymphadenitis), current guidelines suggest that physicians use corticosteroids cautiously if at all.[350] This decision is based partly on the unknown long-term consequences of such therapy, including a potential deleterious effect on the immune system. Nonetheless, small series and anecdotal reports suggest that some severely symptomatic patients (e.g., those with high fever, severe pharyngitis) may benefit from short-term courses of corticosteroids.[13,35,47] Although little controversy exists about the use of corticosteroids for patients with complicated illnesses (e.g., with stridor from massively enlarged tonsils or paratracheal adenopathy, or for those with hematologic or neurologic complications; see Complications of Infectious Mononucleosis earlier), personal experience and clinical judgment largely dictate the use of corticosteroids in practice.[105,514] For example, in a report of patients with IM evaluated as outpatients and inpatients, 45 percent received systemic corticosteroids, with only a small proportion (<10%) of these patients having airway concerns.[514]

Antiviral Treatment. Numerous antiviral agents inhibit replication of EBV in vitro. Such agents include acyclovir, famciclovir, penciclovir, ganciclovir, and other nucleoside analogues.* None of these agents is capable of eliminating latent EBV present as episomes in infected cells. Five randomized, placebo-controlled trials of acyclovir (with or without corticosteroids) in adolescents and adults with IM demonstrated no beneficial effect in resolving the clinical signs and symptoms associated with illness as well as no reduced rate of complications.[11,12,387,522,523] Moreover, a meta-analysis substantiated these findings.[519] Acyclovir did significantly reduce the rate of EBV shedding in the oropharynx, albeit temporarily.[519] A study reported a decrease in the quantity of EBV DNA in oral washings and blood as well as in illness severity during treatment with valacyclovir.[528]

Epstein-Barr Virus–Associated Malignant Diseases

Providing details about treatment of EBV-associated lymphoproliferative diseases, including lymphomas, is beyond the scope of this chapter. Pediatric oncologists must be involved in the care of children with these malignant diseases. A brief overview of treatment of the principal diseases is provided.

Posttransplant Lymphoproliferative Disease

The most effective therapy for PTLD in transplant recipients is reduction (or complete elimination)[236] of immunosuppression to allow more effective control of rampant EBV infection by the cell-mediated immune system. This measure halts progression of PTLD in approximately two thirds of pediatric solid-organ transplant recipients developing polymorphic PTLD in the first year after undergoing transplantation.[171] Decisions concerning reduction of immunosuppression must be weighed against the risk of graft rejection, and careful follow-up with frequent EBV viral load measurements must be ensured. If no improvement occurs after a trial of reduced immune suppression, other therapeutic modalities must be considered singly or in combination.

Although antiviral agents (acyclovir, ganciclovir, valganciclovir) are used frequently for PTLD, largely on the basis of an anecdotal report published in 1982,[196] no clinical trials have confirmed their efficacy. Because these agents treat lytic EBV infections, yet PTLD primarily involves latent virus, the consensus has been that these agents should have minimal impact on PTLD. However, this view has been challenged by reports in experimental animals suggesting that the small proportion of lytically infected cells within these tumors may contribute to the growth of EBV-associated malignant lesions by enhancing angiogenesis and inducing paracrine B-cell growth factors.[226,227]

There is now considerable experience with the use of rituximab, a chimeric monoclonal antibody directed at CD20, against PTLD.[161] Although no randomized controlled clinical trials have been conducted to date in

*References 26, 83, 96, 164, 326, 481.

children, data from cohort studies, case reports, and a phase II trial indicate that rituximab is safe and effective.[161,179] Most adverse events are mild or moderate and usually are related to the first infusion of the antibody. Despite a profound reduction of circulating B cells in peripheral blood, hypogammaglobulinemia is an uncommon occurrence with rituximab treatment (although more likely in young children), and patients are not at higher risk for development of infectious complications. Most centers now use rituximab as a primary therapeutic agent for PTLD if reduction of immune suppression is unsuccessful.

IFN-α has induced complete remissions in some studies,[97,320] but relapses and graft rejections have occurred. Conventional NHL chemotherapy (CHOP regimen) consisting of cyclophosphamide, daunorubicin, vincristine, and prednisone may have increased toxicity and mortality associated with infection when standard doses are used,[368,481,500] thus leading to investigation of low-dose CHOP regimens consisting of cyclophosphamide and prednisone.[30,178]

Experimental therapy using EBV-specific cytotoxic T lymphocytes has been successful for PTLD in human stem cell transplant recipients,[441] but it is more complicated for solid-organ transplant recipients with PTLD. In contrast to stem cell transplant recipients, whose EBV-associated PTLD tumors are derived from the donor, tumors in solid-organ transplant recipients are invariably derived from the recipient. Therefore, EBV-specific cytotoxic T lymphocytes must be prepared by ex vivo EBV transformation of either the recipient's B cells[44,159,278] or at least partially HLA-matched banked B cells.[198] Surgery and radiation therapy generally are reserved for local complications of tumors, including compression of an airway or gastrointestinal bleeding,[171] but they have been used more widely at some institutions.[84] A multifaceted approach, such as that used by Comoli and colleagues,[84] ultimately may yield the highest response rates against the most aggressive forms of PTLD. Clinical trials of cytotoxic T lymphocytes as prophylaxis and treatment of PTLD continue to show promising results.[44,159]

B-Cell Lymphoma

Endemic (African) BLs, which are highly associated with EBV,[293] are treated primarily with cyclophosphamide-based chemotherapy.[294,569] Favorable responses were observed by Labrecque and coworkers[294] in patients with tumors expressing lytic genes. Treatment of sporadic BL also is based primarily on multiagent chemotherapy that includes cyclophosphamide.[323] Case reports have indicated positive responses to rituximab, and Children's Oncology Group protocols now include rituximab as first-line treatment of BL.[161]

B-cell lymphomas in patients with AIDS are extremely difficult to treat, primarily because of the underlying severe host immune deficiency. Early regimens using standard chemotherapeutic regimens were largely failures, with survival rates lower than those of untreated patients; most patients died of opportunistic infections, drug toxicity, and resistant tumors. Alternative therapeutic strategies have improved outcomes for this malignant disease, yet mortality rates remain substantial.[329] These strategies have included lower doses of standard drugs,[267] continuous infusion of chemotherapy,[465] hematopoietic growth factors,[300] novel immunotherapies (e.g., interleukin-2),[37] and adoptive immunotherapy[542] as well as improved management of opportunistic infections and HIV infection. In vitro data indicate the effectiveness of anti–NF-κB treatments, including bortezomib[572] and certain statin drugs,[271] and the hope is that human studies will follow. An exciting emerging area of therapy is termed lytic induction and involves treatment of EBV-positive lymphoma cells with certain agents that induce a transition from latent to lytic infection, thus rendering them susceptible to antiviral therapy (e.g., ganciclovir).[94]

AIDS-related primary CNS lymphomas are extremely difficult to treat. Historically, palliative whole brain radiation therapy in conjunction with corticosteroids was used for treatment but with disappointing survival rates of only a few months. Combined modality therapy with whole brain radiation and chemotherapy has been investigated but generally also produces poor outcomes.[268] However, most studies were conducted before wide availability of HAART. A minority of healthier patients with AIDS may respond better to combined modality regimens.[268] For patients not already receiving HAART, initiation of an appropriate regimen is recommended because case reports have described improvement.

Hodgkin Disease

Historically, children with HD were treated with high-dose radiation. However, high rates of skeletal growth inhibition and secondary malignant neoplasms were noted in survivors, so new trials using lower involved-field radiation in conjunction with multiagent chemotherapy were conducted. These trials form the basis for combination treatment regimens used currently in growing children.[234,412] Although the presence of EBV in tumor (more likely in mixed-cellularity types) currently plays no role in pretherapy staging, some studies suggest improved outcomes for patients with EBV-positive tumors.[360,366]

Nasopharyngeal Carcinoma

NPCs are sensitive to radiotherapy as well as to chemotherapy. Surgery usually is not an option because of the location of the primary tumor, although it may be necessary for persistent or recurrent lymph node disease. For early localized disease, radiotherapy generally is indicated, whereas combined modality treatment (radiation and chemotherapy) may be considered for more advanced disease.[3] Children whose treatment is failing have a high rate of distant metastases, principally to bone.[23,240]

Nonmalignant Epstein-Barr Virus–Associated Diseases

XLP. In an effort to prevent fulminant IM, intravenous immune globulin is recommended for patients with XLP who display hypogammaglobulinemia, although such

therapy is not fully effective.[153] Traditional therapy with antivirals and IFN-α has been disappointing for fulminant IM, but reported success with rituximab (in combination with corticosteroids and other agents) has provided some enthusiasm.[367] Males with XLP also can develop lymphomas, which may respond to traditional chemotherapy regimens. Presently, the only definitive cure for XLP is allogeneic hematopoietic stem cell transplantation. Fifteen such transplants were reported between 1986 and 2005, with 10 survivors.[299] Younger age appears to be associated with improved survival in this limited population. Administration of rituximab may be considered a bridge between the diagnosis of XLP and a transplantation.[299] Now that the gene defect in XLP has been identified, consideration may be given to a gene therapeutic approach.[357]

HLH. Early studies using non–etoposide-containing regimens were largely unsuccessful in significantly reducing the high mortality rate associated with HLH.[19] Chemotherapeutic regimens including etoposide (toxic to macrophages) have reduced the mortality rate significantly. Results of a standardized protocol (HLH-94) developed by the Histiocyte Society have been published.[212] Agents in this protocol included etoposide and corticosteroids for initial therapy, followed by etoposide, corticosteroids, and cyclosporine (continuation therapy), leading to bone marrow transplantation. A similar standardized protocol (HLH-2004), but including cyclosporine at initiation, currently is being evaluated.[250] Few data are available on salvage therapy for those children unresponsive to the HLH-94 protocol, but Janka[250] has summarized anecdotal successes with standard NHL or HD treatment protocols, some monoclonal antibodies (e.g., anti-CD25 and anti-CD52), and treatment with fludarabine (an antiviral agent) of one child who survived until bone marrow transplantation could be performed.

CAEBV Infection. Treatment modalities for CAEBV infection have varied widely because the rarity of this disease precludes performing proper clinical trials. Resolution of symptoms has been reported in case reports with use of antiviral drugs (e.g., acyclovir, ganciclovir),[242] etoposide,[384] immunomodulatory agents (e.g., corticosteroids, intravenous immune globulin, cytokines),[142,149,274] adoptive transfer of cytotoxic T cells,[454] or combinations thereof. For the most severe forms of disease, stem cell transplantation may be considered.[383]

HIV-Associated Diseases. Oral hairy leukoplakia generally does not require therapy because patients typically are asymptomatic and the lesions have no malignant potential. However, therapy may be required for troubling symptoms or cosmetic or psychological reasons. Small lesions may be treated topically,[361] but large lesions require systemic oral antiherpesviral agents.[390] Treatment strategies for LIP are not defined well. Supportive care, including supplemental oxygen, may be necessary. Suppression of HIV with effective antiretroviral agents also is logical to improve immune function and to minimize the potential direct effect of HIV infection. Although no controlled studies have documented the value of antiretrovirals, benefit is suggested by the declining prevalence of LIP in children during the HAART era.[167] Corticosteroids also may be of benefit.[17,352] No controlled studies have evaluated antiherpesviral drugs (e.g., acyclovir) for LIP.

PROGNOSIS

The prognosis for healthy persons with IM is extremely good, with mortality considered a rare occurrence. In a series of 30 fatal cases of IM from the 1950s, Lukes and Cox[332] found splenic rupture, Guillain-Barré syndrome, hemorrhage, and secondary infection to be the most common causes of death. In Penman's[400] later study of 20 documented deaths, predominantly of young adults, the causes of death (in order of frequency) were neurologic complications, splenic rupture, secondary infection, hepatic failure, and myocarditis. EBV-induced aplastic anemia, a rare but dreaded hematologic complication, now is thought to have a better prognosis for eventual recovery if the patient can be supported successfully through the acute stages.[31,302] It has been suggested that older patients are at higher risk for severe EBV-related IM.[508]

The prognosis for patients with EBV-associated lymphoproliferative diseases is variable and depends on the immunologic status of the patient and the type of lymphoproliferation. A registry of 161 males with XLP indicated a mortality rate of 70 percent by the age of 10 years and 100 percent by the age of 40 years.[174] HLH has a greater than 90 percent fatality rate without treatment; most deaths are caused by hemorrhage, overwhelming infection, disseminated intravascular coagulation, or multiorgan failure.[72,249] Etoposide-based treatment has improved outcomes considerably.[250] The largest case series of CAEBV infection (from Japan), which included 21 children younger than 18 years, reported a mortality rate of approximately 30 percent despite various forms of therapy.[281] Historically, PTLD that develops after solid-organ transplantation has carried a relatively high mortality rate (50% to 80%), with higher mortality rates in bone marrow transplant recipients.[131] Presently, the mortality rate is probably much lower, but more time is necessary to assess the impact of newer therapeutic modalities. The prognosis for healthy children with NHL generally is good, with 5-year relative survival rates of 80 percent.[430] However, the prognosis for patients with AIDS and NHL is poor. In adults with AIDS, median survival is 2 to 4 months for CNS lymphomas (virtually all of which are EBV associated), with only slightly longer survival noted in patients receiving HAART.[268] Childhood HD generally has a very good prognosis, with 5-year survival rates of approximately 95 percent.[430] With use of combined modality therapy, children with NPC have long-term survival rates in the range of 70 to 90 percent.[24]

PREVENTION

EBV is shed regularly in saliva, and therefore infection control policies are difficult to develop. In addition, mechanisms for EBV transmission are not well understood, and EBV outbreaks occur rarely if at all. For these reasons, no more than standard infection control precautions are recommended for patients hospitalized with EBV infections, including IM.[404] Although transfusion-associated EBV infection occurs rarely, persons with recent EBV infection of mononucleosis-like illnesses are urged to refrain from donating blood or organs.[404] Because EBV is spread

through salivary contact, individuals should avoid sharing food and drinks, especially with someone known to have had a recent EBV infection, if they wish to reduce the risk of acquiring EBV.

Vaccine

Efforts continue in the development of an EBV vaccine. Because of concern about the administration of a vaccine with unknown long-term consequences, including malignant transformation, most efforts have been focused on development of subunit EBV vaccines. A subunit vaccine containing the EBV gp350 protein has proved effective in preventing EBV infection in an experimental animal model.[120,469] A vaccinia virus–based gp350 vaccine has been evaluated in a small number of Chinese patients, eliciting good antibody responses and providing some protection from EBV infection[181]; however, these findings were not corroborated, and this vaccine was not developed further. A recombinant gp350 vaccine with adjuvant has been evaluated in Belgium, with phase I and phase II data published.[362,480] The vaccine was immunogenic, safe, and well tolerated. Although vaccine recipients were not protected from EBV infection, symptoms of IM were reduced compared with controls.[428,480] The EBV vaccine may soon enter phase III trials.[416] An effective vaccine could be considered for persons at high risk for development of EBV-associated lymphoproliferative disease and cancer, such as patients with AIDS, those with XLP, and organ or stem cell transplant recipients. Before such a vaccine is approved, further research must be conducted to address issues such as protection against exogenous superinfection from wild-type EBV strains.[532] Therapeutic vaccines, aimed primarily at latent antigens expressed in EBV-associated malignant diseases such as NPC, also are under investigation.[114,324,468] Virus-like particle–based EBV vaccine candidates are also in preclinical development.[447]

Prevention of Posttransplant Lymphoproliferative Disease

Strategies for prevention of PTLD reflect many of those used for treatment of established PTLD (see earlier section on treatment of PTLD).[357] Children at high risk for development of PTLD (e.g., EBV-seronegative children and infants younger than 12 months) typically are given prophylactic anti-CMV immune globulin and an antiviral after transplantation. A retrospective analysis of a large renal transplant database covering Europe and North America (96% adults) has suggested a beneficial effect of anti-CMV immune globulin (e.g., CytoGam) in the prevention of B-cell lymphomas.[385]

After undergoing transplantation, these children should have frequent EBV viral load testing. If EBV viral loads persist above a significant cutoff value established by the laboratory, preemptive measures should be considered. Although real-time PCR tests are sensitive in predicting development of PTLD, they are not highly specific and lack standardization. Cutoff values for "significant" concentrations of EBV DNA vary from laboratory to laboratory. Once a decision is made that preemptive therapy is warranted, reduction or elimination of immunosuppressive medications should be performed. In addition, rituximab treatment administered preemptively in patients who have significant viremia (>40,000 copies/mL in blood) may respond with resolution of viremia, without development of PTLD.[554]

Adoptive transfer of cytotoxic T lymphocytes specific for EBV, produced from donor peripheral blood mononuclear cells stimulated with EBV target antigens, is also under clinical development as an antiviral prophylactic agent as well as an antiviral treatment agent for EBV-expressing lymphoproliferative diseases in allogeneic hematopoietic stem cell transplant recipients.[44,159]

NEW REFERENCES SINCE THE SIXTH EDITION

44. Bollard C, Cooper L, Heslop H. Immunotherapy targeting EBV-expressing lymphoproliferative diseases. *Best Pract Res Clin Haematol* 2008;**21**:405–20.
73. Cheng C, Chang L, Shao P, et al. Clinical manifestations and quantitative analysis of virus load in Taiwanese children with Epstein-Barr virus–associated infectious mononucleosis. *J Microbiol Immunol Infect* 2007;**40**: 216–21.
109. Donho S, Maeda A, Ishiura Y, et al. Diagnosis of infectious mononucleosis caused by Epstein-Barr virus in infants. *Pediatr Int* 2010;**52**:536–40.
154. Gao L. Xie Z, Liu Y, et al. Epidemiology and clinical characteristics of infectious mononucleosis associated with Epstein-Barr virus infection in children in Beijing, China. *World J Pediatr* 2011;**7**:45–9.
159. Gerdemann U, Keirnan J, Katri U, et al. Rapidly generated multivirus cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. *Mol Ther* 2012;**20**:1622–32.
160. Ghauri A, Keane P, Scotcher SM, et al. Acute dacrocystitis associated with Epstein-Barr virus infection. *Orbit* 2011;**30**:245–8.
179. Gross T, Orjuela M, Perkins S, et al. Low-dose chemotherapy and rituximab for posttransplant lymphoproliferative disease (PTLD): a Children's Oncology Group report. *Am J Transplant* 2012;**12**:3069–75.
193. Halversen J, Brevig T, Aas T, et al. Genital ulcers as initial manifestation of Epstein-Barr virus infection: two new cases and a review of the literature. *Acta Derm Venereol* 2006;**86**:439–42.
447. Ruiss R, Jochum S, Wanner G, et al. A virus-like particle–based Epstein-Barr virus vaccine. *J Virol* 2011;**85**:13105–13.
469. Sitompul L, Widodo N, Djati M, Utomo D. Epitope mapping of gp350/220 conserved domain of Epstein-Barr virus to develop nasopharyngeal carcinoma (npc) vaccine. *Bioinformation* 2012;**8**:479–82.
473. Slots J, Saygun I, Sabeti M, Kubar A. Epstein-Barr virus oral disease. *J Periodontal Res* 2006;**41**:235–44.
474. Smith J, Corey L, Healey P, et al. Adolescents are more likely to develop posttransplant lymphoproliferative disorder after primary Epstein-Barr virus infection than younger renal transplant recipients. *Transplantation* 2007;**83**:1423–8.
508. Tattevin P, Le Tulzo Y, Minjolle S, et al. Increasing incidence of severe Epstein-Barr virus–related infectious mononucleosis: surveillance study. *J Clin Microbiol* 2006;**44**:1873–4.
528. Vezena H, Balfour H, Weller D, et al. Valacycovir pharmacokinetics and exploratory pharmacodynamics in young adults with EBV infectious mononucleosis. *J Clin Pharmcol* 2010;**50**:734–42.
533. Wan K, Yu I, Wu W. Primary Epstein-Barr virus infection in 2-year old children: report of 3 cases. *Turk Pediatr* 2010;**52**:655–8.
554. Worth A, Conyers R, Cohen J, et al. Pre-emptive rituximab based on virameia and T cell reconstitution: a highly effective strategy for the prevention of Epstein-Barr–associated lymphoproliferative disease following stem cell transplantation. *Br J Haematol* 2011;**155**:377–85.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ahronheim GA, Auger F, Joncas JH, et al. Primary infection by Epstein-Barr virus presenting as aplastic anemia. *N Engl J Med* 1983;**309**:313–14.
2. Alfieri C, Tanner J, Carpentier L, et al. Epstein-Barr virus transmission from a blood donor to an organ transplant recipient with recovery of the same virus strain from the recipient's blood and oropharynx. *Blood* 1996;**87**:812–17.
3. Ali H, al-Sarraf M. Nasopharyngeal cancer. *Hematol Oncol Clin North Am* 1999;**13**:837–47.
4. Allday MJ, Crawford DH. Role of epithelium in EBV persistence and pathogenesis of B-cell tumours. *Lancet* 1988;**1**:855–7.
5. Alotaibi S, Kennedy J, Tellier R, et al. Epstein-Barr virus in pediatric multiple sclerosis. *JAMA* 2004;**291**:1875–9.
6. Alpert G, Fleisher GR. Complications of infection with Epstein-Barr virus during childhood: a study of children admitted to the hospital. *Pediatr Infect Dis* 1984;**3**:304–7.
7. Alsip GR, Ench Y, Sumaya CV, et al. Increased Epstein-Barr virus DNA in oropharyngeal secretions from patients with AIDS, AIDS-related complex, or asymptomatic human immunodeficiency virus infections. *J Infect Dis* 1988;**157**:1072–6.
8. Ambinder RF, Mann RB. Detection and characterization of Epstein-Barr virus in clinical specimens. *Am J Pathol* 1994;**145**:239–52.
9. Ambler M, Stoll J, Tzamaloukas A, et al. Focal encephalomyelitis in infectious mononucleosis. A report with pathological description. *Ann Intern Med* 1971;**75**:579–83.
10. Anagnostopoulos I, Hummel M, Kreschel C, et al. Morphology, immunophenotype, and distribution of latently and/or productively Epstein-Barr virus–infected cells in acute infectious mononucleosis: implications for the interindividual infection route of Epstein-Barr virus. *Blood* 1995;**85**:744–50.
11. Andersson J, Britton S, Ernberg I, et al. Effect of acyclovir on infectious mononucleosis: a double-blind, placebo-controlled study. *J Infect Dis* 1986;**153**:283–90.
12. Andersson J, Skoldenberg B, Henle W, et al. Acyclovir treatment in infectious mononucleosis: a clinical and virological study. *Infection* 1987;**15**:S14–20.
13. Andersson J, Ernberg I. Management of Epstein-Barr virus infections. *Am J Med* 1988;**85**:107–15.
14. Andiman W, Gradoville L, Heston L, et al. Use of cloned probes to detect Epstein-Barr viral DNA in tissues of patients with neoplastic and lymphoproliferative diseases. *J Infect Dis* 1983;**148**: 967–77.
15. Andiman WA, McCarthy P, Markowitz RI, et al. Clinical, virologic, and serologic evidence of Epstein-Barr virus infection in association with childhood pneumonia. *J Pediatr* 1981;**99**:880–6.
16. Andiman WA, Eastman R, Martin K, et al. Opportunistic lymphoproliferations associated with Epstein-Barr viral DNA in infants and children with AIDS. *Lancet* 1985;**2**:1390–3.
17. Andiman WA, Shearer WT. Lymphoid interstitial pneumonitis. In: Pizzo PA, Wilfert CM, editors. *Pediatric AIDS: the challenge of HIV infection in infants, children, and adolescents*. Baltimore: Williams & Wilkins; 1998. p. 323–34.
18. Antinori A, De Rossi G, Ammassari A, et al. Value of combined approach with thallium-201 single-photon emission computed tomography and Epstein-Barr virus DNA polymerase chain reaction in CSF for the diagnosis of AIDS-related primary CNS lymphoma. *J Clin Oncol* 1999;**17**:554–60.
19. Arico M, Janka G, Fischer A, et al. Hemophagocytic lymphohistiocytosis. Report of 122 children from the International Registry. FHL Study Group of the Histiocyte Society. *Leukemia* 1996;**10**:197–203.
20. Armstrong AA, Alexander FE, Paes RP, et al. Association of Epstein-Barr virus with pediatric Hodgkin's disease. *Am J Pathol* 1993;**142**:1683–8.
21. Arnold W, Huth F. Light and electron microscopic investigations of nasopharyngeal carcinomas with regard to the viral etiology of these tumors. *J Cancer Res Clin Oncol* 1979;**94**:87–109.
22. Asgari MM, Begos DG. Spontaneous splenic rupture in infectious mononucleosis: a review. *Yale J Biol Med* 1997;**70**:175–82.
23. Ayan I, Altun M. Nasopharyngeal carcinoma in children: retrospective review of 50 patients. *Int J Radiat Oncol Biol Phys* 1996;**35**:485–92.
24. Ayan I, Kaytan E, Ayan N. Childhood nasopharyngeal carcinoma: from biology to treatment. *Lancet Oncol* 2003;**4**:13–21.
25. Babcock GJ, Decker LL, Volk M, et al. EBV persistence in memory B cells in vivo. *Immunity* 1998;**9**:395–404.
26. Bacon TH, Boyd MR. Activity of penciclovir against Epstein-Barr virus. *Antimicrob Agents Chemother* 1995;**39**:1599–602.
27. Baehner RL, Shuler SE. Infectious mononucleosis in childhood: clinical expressions, serologic findings, complications, prognosis. *Clin Pediatr (Phila)* 1967;**6**:393–9.
28. Baer R, Bankier AT, Biggin MD, et al. DNA sequence and expression of the B95-8 Epstein-Barr virus genome. *Nature* 1984; **310**:207–11.
29. Baker FJ, Kotchmar GS Jr, Foshee WS, et al. Acute hemiplegia of childhood associated with Epstein-Barr virus infection. *Pediatr Infect Dis* 1983;**2**:136–8.
30. Balfour IC, Wall D, Luisiri A, et al. Cyclosphosphamide/prednisone for combination immunosuppression and therapy of lymphoproliferative disease. *J Heart Lung Transplant* 1999;**18**:492–5.
31. Baranski B, Armstrong G, Truman JT, et al. Epstein-Barr virus in the bone marrow of patients with aplastic anemia. *Ann Intern Med* 1988;**109**:695–704.
32. Barletta JM, Kingma DW, Ling Y, et al. Rapid in situ hybridization for the diagnosis of latent Epstein-Barr virus infection. *Mol Cell Probes* 1993;**7**:105–9.
33. Baumgarten E, Herbst H, Schmitt M, et al. Life-threatening infectious mononucleosis: is it correlated with virus-induced T cell proliferation? *Clin Infect Dis* 1994;**19**:152–6.
34. Bechet JM, Fialkow PJ, Nilsson K, et al. Immunoglobulin synthesis and glucose-6-phosphate dehydrogenase as cell markers in human lymphoblastoid cell lines. *Exp Cell Res* 1974;**89**:275–82.
35. Bender CE. The value of corticosteroids in the treatment of infectious mononucleosis. *JAMA* 1967;**199**:529–31.
36. Bernstein TC, Wolff HG. Involvement of the nervous system in infectious mononucleosis. *Ann Intern Med* 1950;**33**:1120–38.
37. Bernstein ZP, Porter MM, Gould M, et al. Prolonged administration of low-dose interleukin-2 in human immunodeficiency virus–associated malignancy results in selective expansion of innate immune effectors without significant clinical toxicity. *Blood* 1995;**86**:3287–94.
38. Biggar RJ, Henle W, Fleisher G, et al. Primary Epstein-Barr virus infections in African infants. I. Decline of maternal antibodies and time of infection. *Int J Cancer* 1978;**22**:239–43.
39. Biggar RJ, Frisch M, Goedert JJ. Risk of cancer in children with AIDS. AIDS-Cancer Match Registry Study Group. *JAMA* 2000;**284**:205–9.
40. Biggar RJ, Jaffe ES, Goedert JJ, et al. Hodgkin lymphoma and immunodeficiency in persons with HIV/AIDS. *Blood* 2006;**108**:3786–91.
41. Birx DL, Redfield RR, Tosato G. Defective regulation of Epstein-Barr virus infection in patients with acquired immunodeficiency syndrome (AIDS) or AIDS-related disorders. *N Engl J Med* 1986;**314**:874–9.
42. Blowey DL. Nephrotic syndrome associated with an Epstein-Barr virus infection. *Pediatr Nephrol* 1996;**10**:507–8.
43. Bodansky HJ. Erythema nodosum and infectious mononucleosis. *Br Med J* 1979;**2**:17.
44. Bollard C, Cooper L, Heslop H. Immunotherapy targeting EBV-expressing lymphoproliferative diseases. *Best Pract Res Clin Haematol* 2008;**21**:405–20.
45. Bonforte RJ. Convulsion as a presenting sign of infectious mononucleosis. *Am J Dis Child* 1967;**114**:429–32.
46. Borza CM, Hutt-Fletcher LM. Alternate replication in B cells and epithelial cells switches tropism of Epstein-Barr virus. *Nat Med* 2002;**8**:594–9.
47. Brandfonbrener A, Epstein A, Wu S, et al. Corticosteroid therapy in Epstein-Barr virus infection. Effect on lymphocyte class, subset, and response to early antigen. *Arch Intern Med* 1986;**146**:337–9.
48. Brichkov I, Cummings L, Fazylov R, et al. Nonoperative management of spontaneous splenic rupture in infectious mononucleosis: the role for emerging diagnostic and treatment modalities. *Am Surg* 2006;**72**:401–4.
49. Brousset P, Butet V, Chittal S, et al. Comparison of in situ hybridization using different nonisotopic probes for detection of Epstein-Barr virus in nasopharyngeal carcinoma and immunohistochemical correlation with anti–latent membrane protein antibody. *Lab Invest* 1992;**67**:457–64.
50. Brown NA, Miller G. Immunoglobulin expression by human B lymphocytes clonally transformed by Epstein Barr virus. *J Immunol* 1982;**128**:24–9.

51. Brown ZA, Stenchever MA. Infectious mononucleosis and congenital anomalies. *Am J Obstet Gynecol* 1978;**131**:108–9.
52. Bruu AL, Hjetland R, Holter E, et al. Evaluation of 12 commercial tests for detection of Epstein-Barr virus–specific and heterophile antibodies. *Clin Diagn Lab Immunol* 2000;**7**:451–6.
53. Buisson M, Fleurent B, Mak M, et al. Novel immunoblot assay using four recombinant antigens for diagnosis of Epstein-Barr virus primary infection and reactivation. *J Clin Microbiol* 1999;**37**:2709–14.
54. Burkitt DP. A sarcoma involving the jaws in African children. *Br J Surg* 1958;**46**:218–23.
55. Burstin PP, Marshall CL. Infectious mononucleosis and bilateral peritonsillar abscesses resulting in airway obstruction. *J Laryngol Otol* 1998;**112**:1186–8.
56. Cabot RC. The lymphocytosis of infection. *Am J Med Sci* 1913;**145**:335–9.
57. Callan MF. Epstein-Barr virus, arthritis, and the development of lymphoma in arthritis patients. *Curr Opin Rheumatol* 2004;**16**: 399–405.
58. Cantow EF, Kostinas JE. Studies on infectious mononucleosis. IV. Changes in the granulocytic series. *Am J Clin Pathol* 1966;**46**:43–7.
59. Cantow EF, Kostinas JE. Studies on infectious mononucleosis. 3. *Platelets Am J Med Sci* 1966;**251**:664–7.
60. Carter RL. Platelet levels in infectious mononucleosis. *Blood* 1965;**25**:817–21.
61. Carter RL. Granulocyte changes in infectious mononucleosis. *J Clin Pathol* 1966;**19**:279–83.
62. Cattaneo C, Facchetti F, Re A, et al. Oral cavity lymphomas in immunocompetent and human immunodeficiency virus infected patients. *Leuk Lymphoma* 2005;**46**:77–81.
63. Cen H, Breinig MC, Atchison RW, et al. Epstein-Barr virus transmission via the donor organs in solid organ transplantation: polymerase chain reaction and restriction fragment length polymorphism analysis of IR2, IR3, and IR4. *J Virol* 1991;**65**:976–80.
64. Chan KH, Luo RX, Chen HL, et al. Development and evaluation of an Epstein-Barr virus (EBV) immunoglobulin M enzyme-linked immunosorbent assay based on the 18-kilodalton matrix protein for diagnosis of primary EBV infection. *J Clin Microbiol* 1998;**36**:3359–61.
65. Chang RS, Golden HD. Transformation of human leucocytes by throat washing from infectious mononucleosis patients. *Nature* 1971;**234**:359–60.
66. Chang RS, Blankenship W. Spontaneous in vitro transformation of leukocytes from a neonate. *Proc Soc Exp Biol Med* 1973;**144**:337–9.
67. Chang RS. Interpersonal transmission of EB-virus infection [letter]. *N Engl J Med* 1975;**293**:454–5.
68. Chang RS. What is the determinant for susceptibility to infectious mononucleosis? [letter] *N Engl J Med* 1975;**292**:12.
69. Chang RS, Lewis JP, Reynolds RD, et al. Oropharyngeal excretion of Epstein-Barr virus by patients with lymphoproliferative disorders and by recipients of renal homografts. *Ann Intern Med* 1978;**88**:34–40.
70. Chang RS, Seto DS. Perinatal infection by Epstein-Barr virus. *Lancet* 1979;**2**:28.
71. Chang RS. *Infectious mononucleosis*. Boston: G. K. Hall Medical Publishers; 1980.
72. Chen RL, Lin KH, Lin DT, et al. Immunomodulation treatment for childhood virus-associated haemophagocytic lymphohistiocytosis. *Br J Haematol* 1995;**89**:282–90.
73. Cheng C, Chang L, Shao P, et al. Clinical manifestations and quantitative analysis of virus load in Taiwanese children with Epstein-Barr virus–associated infectious mononucleosis. *J Microbiol Immunol Infect* 2007;**40**:216–21.
74. Chin TD. Diagnosis of infectious mononucleosis. *South Med J* 1976;**69**:654–8.
75. Chulay JD, Lankerani MR. Splenic abscess. Report of 10 cases and review of the literature. *Am J Med* 1976;**61**:513–22.
76. Cinbis M, Aysun S. Alice in Wonderland syndrome as an initial manifestation of Epstein-Barr virus infection. *Br J Ophthalmol* 1992;**76**:316.
77. Cinque P, Brytting M, Vago L, et al. Epstein-Barr virus DNA in cerebrospinal fluid from patients with AIDS-related primary lymphoma of the central nervous system. *Lancet* 1993;**342**: 398–401.
78. Cleary TG, Henle W, Pickering LK. Acute cerebellar ataxia associated with Epstein-Barr virus infection. *JAMA* 1980;**243**:148–9.
79. Cloney DL, Kugler JA, Donowitz LG, et al. Infectious mononucleosis with pleural effusion. *South Med J* 1988;**81**:1441–2.
80. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* 1998;**20**:129–35.
81. Cohen JI, Lekstrom K. Epstein-Barr virus BARF1 protein is dispensable for B-cell transformation and inhibits alpha interferon secretion from mononuclear cells. *J Virol* 1999;**73**:7627–32.
82. Cohen JI. Epstein-Barr virus infection. *N Engl J Med* 2000;**343**:481–92.
83. Colby BM, Shaw JE, Elion GB, et al. Effect of acyclovir [9-(2-hydroxyethoxymethyl)guanine] on Epstein-Barr virus DNA replication. *J Virol* 1980;**34**:560–8.
84. Comoli P, Maccario R, Locatelli F, et al. Treatment of EBV-related post–renal transplant lymphoproliferative disease with a tailored regimen including EBV-specific T cells. *Am J Transplant* 2005;**5**:1415–22.
85. Connelly KP, DeWitt LD. Neurologic complications of infectious mononucleosis. *Pediatr Neurol* 1994;**10**:181–4.
86. Centers for Disease Control. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Recomm Rep* 1994;**43**(RR-12):1–10.
87. Copperman SM. "Alice in Wonderland" syndrome as a presenting symptom of infectious mononucleosis in children: a description of three affected young people. *Clin Pediatr (Phila)* 1977;**16**: 143–6.
88. Craig FE, Gulley ML, Banks PM. Posttransplantation lymphoproliferative disorders. *Am J Clin Pathol* 1993;**99**:265–76.
89. Crawford DH, Swerdlow AJ, Higgins C, et al. Sexual history and Epstein-Barr virus infection. *J Infect Dis* 2002;**186**:731–6.
90. Croce CM, Tsujimoto Y, Erikson J, et al. Chromosome translocations and B cell neoplasia. *Lab Invest* 1984;**51**:258–67.
91. Curtis RE, Travis LB, Rowlings PA, et al. Risk of lymphoproliferative disorders after bone marrow transplantation: a multi-institutional study. *Blood* 1999;**94**:2208–16.
92. Custer RP. The pathology of infectious mononucleosis. *Blood* 1948;**3**:830–57.
93. d'Arminio Monforte A, Cinque P, Vago L, et al. A comparison of brain biopsy and CSF-PCR in the diagnosis of CNS lesions in AIDS patients. *J Neurol* 1997;**244**:35–9.
94. Daibata M, Bandobashi K, Kuroda M, et al. Induction of lytic Epstein-Barr virus (EBV) infection by synergistic action of rituximab and dexamethasone renders EBV-positive lymphoma cells more susceptible to ganciclovir cytotoxicity in vitro and in vivo. *J Virol* 2005;**79**:5875–9.
95. Dalla-Favera R, Bregni M, Erikson J, et al. Human c-myc onc gene is located on the region of chromosome 8 that is translocated in Burkitt lymphoma cells. *Proc Natl Acad Sci U S A* 1982; **79**:7824–7.
96. Datta AK, Hood RE. Mechanism of inhibition of Epstein-Barr virus replication by phosphonoformic acid. *Virology* 1981;**114**: 52–9.
97. Davis CL, Wood BL, Sabath DE, et al. Interferon-alpha treatment of posttransplant lymphoproliferative disorder in recipients of solid organ transplants. *Transplantation* 1998;**66**:1770–9.
98. Davison AJ, Eberle R, Hayward GS, et al. Herpesviridae. In: Fauquet C, Mayo MA, Maniloff J, et al, editors. *Virus taxonomy: classification and nomenclature of viruses: 8th report of the International Committee on Taxonomy of Viruses*. Oxford: Elsevier/Academic Press; 2005. p. 193–212.
99. de-The G. Is Burkitt's lymphoma related to perinatal infection by Epstein-Barr virus? *Lancet* 1977;**1**:335–8.
100. de-The G, Geser A, Day NE, et al. Epidemiological evidence for causal relationship between Epstein-Barr virus and Burkitt's lymphoma from Ugandan prospective study. *Nature* 1978;**274**: 756–61.
101. Delecluse HJ, Kremmer E, Rouault JP, et al. The expression of Epstein-Barr virus latent proteins is related to the pathological features of post-transplant lymphoproliferative disorders. *Am J Pathol* 1995;**146**:1113–20.
102. Delecluse HJ, Hammerschmidt W. The genetic approach to the Epstein-Barr virus: from basic virology to gene therapy. *Mol Pathol* 2000;**53**:270–9.

103. Deutsch J, Wolf H, Becker H, et al. Demonstration of Epstein-Barr virus DNA in a previously healthy boy with fulminant hepatic failure. *Eur J Pediatr* 1986;**145**:94–8.
104. Diaz-Mitoma F, Vanast WJ, Tyrrell DL. Increased frequency of Epstein-Barr virus excretion in patients with new daily persistent headaches. *Lancet* 1987;**1**:411–15.
105. Disney FA. Corticosteroids for infectious mononucleosis. *Pediatr Infect Dis J* 1988;**7**:820–1.
106. Domachowske JB, Cunningham CK, Cummings DL, et al. Acute manifestations and neurologic sequelae of Epstein-Barr virus encephalitis in children. *Pediatr Infect Dis J* 1996;**15**:871–5.
107. Dommerby H, Stangerup SE, Stangerup M, et al. Hepatosplenomegaly in infectious mononucleosis, assessed by ultrasonic scanning. *J Laryngol Otol* 1986;**100**:573–9.
108. Donati D, Espmark E, Kironde F, et al. Clearance of circulating Epstein-Barr virus DNA in children with acute malaria after antimalaria treatment. *J Infect Dis* 2006;**193**:971–7.
109. Donho S, Maeda A, Ishiura Y, et al. Diagnosis of infectious mononucleosis caused by Epstein-Barr virus in infants. *Pediatr Int* 2010;**52**:536–40.
110. Downey H, McKinlay CA. Acute lymphadenosis compared with acute lymphatic leukemia. *Arch Intern Med* 1923;**32**:82–112.
111. Drut R, Drut RM. Angiocentric immunoproliferative lesion and angiocentric lymphoma of lymph node in children. A report of two cases. *J Clin Pathol* 2005;**58**:550–2.
112. Duncombe AS, Amos RJ, Metcalfe P, et al. Intravenous immunoglobulin therapy in thrombocytopenic infectious mononucleosis. *Clin Lab Haematol* 1989;**11**:11–15.
113. Dunnet WN. Infectious mononucleosis. *Br Med J* 1963;**1**:1187–91.
114. Duraiswamy J, Sherritt M, Thomson S, et al. Therapeutic LMP1 polyepitope vaccine for EBV-associated Hodgkin disease and nasopharyngeal carcinoma. *Blood* 2003;**101**:3150–6.
115. Dussaix E, Le Touze P, Tardieu M. Neuropathy of the brachial plexus complicating infectious mononucleosis in an 18-month-old child. *Arch Fr Pediatr* 1986;**43**:129–30.
116. Eaton OM, Little PF, Silver HM. Infectious mononucleosis with pleural effusion. Review of the literature. *Arch Intern Med* 1965;**115**:87–9.
117. Engels EA, Pfeiffer RM, Goedert JJ, et al. Trends in cancer risk among people with AIDS in the United States 1980-2002. *AIDS* 2006;**20**:1645–54.
118. Epstein MA, Achong BG. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1964;**1**:702–3.
119. Epstein MA, Achong BG. Morphology of the virus and of virus-induced cytopathologic changes. In: Epstein MA, Achong BG, editors. *The Epstein-Barr virus*. New York: Springer-Verlag; 1979. p. 23–37.
120. Epstein MA, Morgan AJ, Finerty S, et al. Protection of cottontop tamarins against Epstein-Barr virus–induced malignant lymphoma by a prototype subunit vaccine. *Nature* 1985;**318**:287–9.
121. Erzurum S, Kalavsky SM, Watanakunakorn C. Acute cerebellar ataxia and hearing loss as initial symptoms of infectious mononucleosis. *Arch Neurol* 1983;**40**:760–2.
122. Eshel GM, Evov A, Lahat E, et al. Alice in Wonderland syndrome, a manifestation of acute Epstein-Barr virus infection. *Pediatr Infect Dis J* 1987;**6**:68.
123. Evans AS. Experimental attempts to transmit infectious mononucleosis to man. *Yale J Biol Med* 1947;**20**:19–26.
124. Evans AS. Further experimental attempts to transmit infectious mononucleosis to man. *J Clin Invest* 1950;**29**:508–12.
125. Evans AS. Infectious mononucleosis in University of Wisconsin students: report of a five-year investigation. *Am J Hyg* 1960;**71**: 342–62.
126. Evans AS, Niederman JC, McCollum RW. Seroepidemiologic studies of infectious mononucleosis with EB virus. *N Engl J Med* 1968;**279**:1121–7.
127. Evans AS. The history of infectious mononucleosis. *Am J Med Sci* 1974;**267**:189–95.
128. Fandi A, Altun M, Azli N, et al. Nasopharyngeal cancer: epidemiology, staging, and treatment. *Semin Oncol* 1994;**21**:382–97.
129. Farber I, Hinderer W, Rothe M, et al. Serological diagnosis of Epstein-Barr virus infection by novel ELISAs based on recombinant capsid antigens p23 and p18. *J Med Virol* 2001;**63**:271–6.
130. Farley DR, Zietlow SP, Bannon MP, et al. Spontaneous rupture of the spleen due to infectious mononucleosis. *Mayo Clin Proc* 1992;**67**:846–53.
131. Faye A, Vilmer E. Post-transplant lymphoproliferative disorder in children: incidence, prognosis, and treatment options. *Paediatr Drugs* 2005;**7**:55–65.
132. Feorino PM, Humphrey D. Mononucleosis-associated subacute sclerosing panencephalitis. *Lancet* 1975;**2**:530–2.
133. Ferbas J, Rahman MA, Kingsley LA, et al. Frequent oropharyngeal shedding of Epstein-Barr virus in homosexual men during early HIV infection. *AIDS* 1992;**6**:1273–8.
134. Fermaglich DR. Pulmonary involvement in infectious mononucleosis. *J Pediatr* 1975;**86**:93–5.
135. Finch SC. Clinical symptoms and signs of infectious mononucleosis. In: Carter RL, Penman HG, editors. *Infectious mononucleosis*. Oxford, England: Blackwell Scientific Publications; 1969. p. 19–46.
136. Fingeroth JD, Weis JJ, Tedder TF, et al. Epstein-Barr virus receptor of human B lymphocytes is the C3d receptor CR2. *Proc Natl Acad Sci U S A* 1984;**81**:4510–14.
137. Fisman DN. Hemophagocytic syndromes and infection. *Emerg Infect Dis* 2000;**6**:601–8.
138. Fleisher G, Henle W, Henle G, et al. Primary infection with Epstein-Barr virus in infants in the United States: clinical and serologic observations. *J Infect Dis* 1979;**139**:553–8.
139. Fleisher G, Bologonese R. Infectious mononucleosis during gestation: report of three women and their infants studied prospectively. *Pediatr Infect Dis* 1984;**3**:308–11.
140. Fleisher G, Bologonese R. Epstein-Barr virus infections in pregnancy: a prospective study. *J Pediatr* 1984;**104**:374–9.
141. Fleisher GR, Pasquariello PS, Warren WS, et al. Intrafamilial transmission of Epstein-Barr virus infections. *J Pediatr* 1981;**98**:16–19.
142. Fletcher MA, Lo TM, Levey BA, et al. Immunochemical studies of infectious mononucleosis. VI. A radioimmunoassay for the detection of infectious mononucleosis heterophile antibody and antigen. *J Immunol Methods* 1977;**14**:51–8.
143. Frey T. Optic neuritis in children: infectious mononucleosis as an etiology. *Doc Ophthalmol* 1973;**34**:183–8.
144. Friedman BI, Libby R. Epstein-Barr virus infection associated with rhabdomyolysis and acute renal failure. *Clin Pediatr (Phila)* 1986;**25**:228–9.
145. Frishman W, Kraus ME, Zabkar J, et al. Infectious mononucleosis and fatal myocarditis. *Chest* 1977;**72**:535–8.
146. Fu SM, Hurley JN. Human cell lines containing Epstein-Barr virus but distinct from the common B cell lymphoblastoid lines. *Proc Natl Acad Sci U S A* 1979;**76**:6637–40.
147. Fu SM, Hurley JN, McCune JM, et al. Pre-B cells and other possible precursor lymphoid cell lines derived from patients with X-linked agammaglobulinemia. *J Exp Med* 1980;**152**:1519–26.
148. Fuhrman SA, Gill R, Horwitz CA, et al. Marked hyperbilirubinemia in infectious mononucleosis. Analysis of laboratory data in seven patients. *Arch Intern Med* 1987;**147**:850–3.
149. Fujisaki T, Nagafuchi S, Okamura T. Gamma-interferon for severe chronic active Epstein-Barr virus. *Ann Intern Med* 1993;**118**:474–5.
150. Fukayama M, Hayashi Y, Ooba T, et al. Pyothorax-associated lymphoma: development of Epstein-Barr virus–associated lymphoma within the inflammatory cavity. *Pathol Int* 1995;**45**:825–31.
151. Gan YJ, Shirley P, Zeng Y, et al. Human oropharyngeal lesions with a defective Epstein-Barr virus that disrupts viral latency. *J Infect Dis* 1993;**168**:1349–55.
152. Ganzel TM, Goldman JL, Padhya TA. Otolaryngologic clinical patterns in pediatric infectious mononucleosis. *Am J Otolaryngol* 1996;**17**:397–400.
153. Gaspar HB, Sharifi R, Gilmour KC, et al. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. *Br J Haematol* 2002;**119**:585–95.
154. Gao L, Xie Z, Liu Y, et al. Epidemiology and clinical characteristics of infectious mononucleosis associated with Epstein-Barr virus infection in children in Beijing, China. *World J Pediatr* 2011;**7**:45–9.
155. Gelati G, Verucchi G, Chiodo F, et al. Hemolytic anemia as a complication of Epstein-Barr virus infection: a report of two cases. *J Exp Pathol* 1987;**3**:485–9.
156. Gerber P, Birch SM. Complement-fixing antibodies in sera of human and nonhuman primates to viral antigens derived from Burkitt's lymphoma cells. Proc Natl Acad Sci U S A 1967;58:478–84.

157. Gerber P, Walsh JH, Rosenblum EN, et al. Association of EB-virus infection with the post-perfusion syndrome. *Lancet* 1969;**1**:593–5.
158. Gerber P, Lucas S, Nonoyama M, et al. Oral excretion of Epstein-Barr virus by healthy subjects and patients with infectious mononucleosis. *Lancet* 1972;**2**:988–9.
159. Gerdemann U, Keirnan J, Katri U, et al. Rapidly generated multivirus cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. *Mol Ther* 2012;**20**:1622–32.
160. Ghauri A, Keane P, Scotcher SM, et al. Acute dacrocystitis associated with Epstein-Barr virus infection. *Orbit* 2011;**30**:245–8.
161. Giulino LB, Bussel JB, Neufeld EJ, et al. Treatment with rituximab in benign and malignant hematologic disorders in children. *J Pediatr* 2007;**150**:338–44.
162. Glynn FJ, Mackle T, Kinsella J. Upper airway obstruction in infectious mononucleosis. *Eur J Emerg Med* 2007;**14**:41–2.
163. Gohlich-Ratmann G, Wallot M, Baethmann M, et al. Acute cerebellitis with near-fatal cerebellar swelling and benign outcome under conservative treatment with high dose steroids. *Eur J Paediatr Neurol* 1998;**2**:157–62.
164. Goldani LZ. Treatment of severe infectious mononucleosis with famciclovir. *J Infect* 2002;**44**:92–3.
165. Goldberg GN, Fulginiti VA, Ray CG, et al. In utero Epstein-Barr virus (infectious mononucleosis) infection. *JAMA* 1981;**246**: 1579–81.
166. Golden HD, Chang RS, Prescott W, et al. Leukocyte-transforming agent: prolonged excretion by patients with mononucleosis and excretion by normal individuals. *J Infect Dis* 1973;**127**:471–3.
167. Gona P, Van Dyke RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *JAMA* 2006;**296**:292–300.
168. Gowing NF. Infectious mononucleosis: histopathologic aspects. *Pathol Annu* 1975;**10**:1–20.
169. Graman PS. Mollaret's meningitis associated with acute Epstein-Barr virus mononucleosis. *Arch Neurol* 1987;**44**:1204–5.
170. Green M, Cacciarelli TV, Mazariegos GV, et al. Serial measurement of Epstein-Barr viral load in peripheral blood in pediatric liver transplant recipients during treatment for posttransplant lymphoproliferative disease. *Transplantation* 1998;**66**:1641–4.
171. Green M, Webber S. Posttransplantation lymphoproliferative disorders. *Pediatr Clin North Am* 2003;**50**:1471–91.
172. Greenspan D, Greenspan JS, Conant M, et al. Oral "hairy" leucoplakia in male homosexuals: evidence of association with both papillomavirus and a herpes-group virus. *Lancet* 1984;**2**:831–4.
173. Greenspan JS, Greenspan D, Lennette ET, et al. Replication of Epstein-Barr virus within the epithelial cells of oral "hairy" leukoplakia, an AIDS-associated lesion. *N Engl J Med* 1985; **313**:1564–71.
174. Grierson H, Purtilo DT. Epstein-Barr virus infections in males with the X-linked lymphoproliferative syndrome. *Ann Intern Med* 1987;**106**:538–45.
175. Grishaber JE, McClain KL, Mahoney DH Jr, et al. Successful outcome of severe aplastic anemia following Epstein-Barr virus infection. *Am J Hematol* 1988;**28**:273–5.
176. Grose C, Feorino PM. Epstein-Barr virus and Guillain-Barré syndrome. *Lancet* 1972;**2**:1285–7.
177. Grose C, Henle W, Henle G, et al. Primary Epstein-Barr-virus infections in acute neurologic diseases. *N Engl J Med* 1975;**292**: 392–5.
178. Gross TG, Hinrichs SH, Winner J, et al. Treatment of posttransplant lymphoproliferative disease (PTLD) following solid organ transplantation with low-dose chemotherapy. *Ann Oncol* 1998;**9**:339–40.
179. Gross T, Orjuela M, Perkins S, et al. Low-dose chemotherapy and rituximab for posttransplant lymphoproliferative disease (PTLD): a Children's Oncology Group report. *Am J Transplant* 2012; **12**:3069–75.
180. Grotto I, Mimouni D, Huerta M, et al. Clinical and laboratory presentation of EBV positive infectious mononucleosis in young adults. *Epidemiol Infect* 2003;**131**:683–9.
181. Gu SY, Huang TM, Ruan L, et al. First EBV vaccine trial in humans using recombinant vaccinia virus expressing the major membrane antigen. *Dev Biol Stand* 1995;**84**:171–7.
182. Gulley ML, Raphael M, Lutz CT, et al. Epstein-Barr virus integration in human lymphomas and lymphoid cell lines. *Cancer* 1992;**70**:185–91.
183. Gulley ML, Eagan PA, Quintanilla-Martinez L, et al. Epstein-Barr virus DNA is abundant and monoclonal in the Reed-Sternberg cells of Hodgkin's disease: association with mixed cellularity subtype and Hispanic American ethnicity. *Blood* 1994;**83**:1595–602.
184. Gutensohn N, Cole P. Epidemiology of Hodgkin's disease in the young. *Int J Cancer* 1977;**19**:595–604.
185. Gutgesell HP Jr. Acute airway obstruction in infectious mononucleosis. *Pediatrics* 1971;**47**:141–3.
186. Gutierrez MI, Bhatia K, Magrath I. Replicative viral DNA in Epstein-Barr virus associated Burkitt's lymphoma biopsies. *Leuk Res* 1993;**17**:285–9.
187. Haider S, Coutinho M de L, Emond RT, et al. Tuberculin anergy and infectious mononucleosis. *Lancet* 1973;**2**:14.
188. Halbert SP, Anken M, Henle W, et al. Detection of infectious mononucleosis heterophil antibody by a rapid, standardized enzyme-linked immunosorbent assay procedure. *J Clin Microbiol* 1982;**15**:610–16.
189. Halcrow JP, Owen LM, Rodger NO. Infectious mononucleosis, with an account of an epidemic in an E.M.S. hospital. *Br Med J* 1943;**2**:443–7.
190. Hall AJ. A case resembling acute lymphocytic leukemia ending in complete recovery. *Proc R Soc Med* 1915;**8**:15–19.
191. Haller A, von Segesser L, Baumann PC, et al. Severe respiratory insufficiency complicating Epstein-Barr virus infection: case report and review. *Clin Infect Dis* 1995;**21**:206–9.
192. Halsted CC, Chang RS. Infectious mononucleosis and encephalitis: recovery of EB virus from spinal fluid. *Pediatrics* 1979; **64**:257–8.
193. Halversen J, Brevig T, Aas T, et al. Genital ulcers as initial manifestation of Epstein-Barr virus infection: two new cases and a review of the literature. *Acta Derm Venereol* 2006;**86**:439–42.
194. Hammarskjold ML, Simurda MC. Epstein-Barr virus latent membrane protein transactivates the human immunodeficiency virus type 1 long terminal repeat through induction of NF-kappa B activity. *J Virol* 1992;**66**:6496–501.
195. Handler SD, Warren WS. Peritonsillar abscess: a complication of corticosteroid treatment in infectious mononucleosis. *Int J Pediatr Otorhinolaryngol* 1979;**1**:265–8.
196. Hanto DW, Frizzera G, Gajl-Peczalska KJ, et al. Epstein-Barr virus–induced B-cell lymphoma after renal transplantation: acyclovir therapy and transition from polyclonal to monoclonal B-cell proliferation. *N Engl J Med* 1982;**306**:913–18.
197. Haque T, Thomas JA, Falk KI, et al. Transmission of donor Epstein-Barr virus (EBV) in transplanted organs causes lymphoproliferative disease in EBV-seronegative recipients. *J Gen Virol* 1996;**77**:1169–72.
198. Haque T, Wilkie GM, Taylor C, et al. Treatment of Epstein-Barr-virus–positive post-transplantation lymphoproliferative disease with partly HLA-matched allogeneic cytotoxic T cells. *Lancet* 2002;**360**:436–42.
199. Hart GK, Thompson WR, Schneider J, et al. Fulminant hepatic failure and fatal encephalopathy associated with Epstein-Barr virus infection. *Med J Aust* 1984;**141**:112–13.
200. Heath CW Jr, Brodsky AL, Potolsky AI. Infectious mononucleosis in a general population. *Am J Epidemiol* 1972;**95**:46–52.
201. Hedstrom SA, Belfrage I. Acute pancreatitis in two cases of infectious mononucleosis. *Scand J Infect Dis* 1976;**8**:124–6.
202. Henke CE, Kurland LT, Elveback LR. Infectious mononucleosis in Rochester, Minnesota, 1950 through 1969. *Am J Epidemiol* 1973;**98**:483–90.
203. Henkel T, Ling PD, Hayward SD, et al. Mediation of Epstein-Barr virus EBNA2 transactivation by recombination signal-binding protein J kappa. *Science* 1994;**265**:92–5.
204. Henle G, Henle W. Immunofluorescence in cells derived from Burkitt's lymphoma. *J Bacteriol* 1966;**91**:1248–56.
205. Henle G, Henle W, Diehl V. Relation of Burkitt's tumor–associated herpes-type virus to infectious mononucleosis. *Proc Natl Acad Sci U S A* 1968;**59**:94–101.
206. Henle G, Henle W, Clifford P, et al. Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. *J Natl Cancer Inst* 1969;**43**:1147–57.
207. Henle W, Diehl V, Kohn G, et al. Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. *Science* 1967;**157**:1064–5.

208. Henle W, Henle G, Scriba M, et al. Antibody responses to the Epstein-Barr virus and cytomegaloviruses after open-heart and other surgery. *N Engl J Med* 1970;**282**:1068–74.
209. Henle W, Henle G. Evidence for an oncogenic potential of the Epstein-Barr virus. *Cancer Res* 1973;**33**:1419–23.
210. Henle W, Henle G. Immunology of Epstein-Barr virus. In: Roizman B, editor. *The herpesviruses*. New York: Plenum Press; 1982. p. 209–52.
211. Henle W, Henle G. Epstein-Barr virus and blood transfusions. *Prog Clin Biol Res* 1985;**182**:201–9.
212. Henter JI, Samuelsson-Horne A, Arico M, et al. Treatment of hemophagocytic lymphohistiocytosis with HLH-94 immunochemotherapy and bone marrow transplantation. *Blood* 2002;**100**: 2367–73.
213. Herbst H, Stein H, Niedobitek G. Epstein-Barr virus and CD30$^+$ malignant lymphomas. *Crit Rev Oncog* 1993;**4**:191–239.
214. Hess RD. Routine Epstein-Barr virus diagnostics from the laboratory perspective: still challenging after 35 years. *J Clin Microbiol* 2004;**42**:3381–7.
215. Hinderer W, Lang D, Rothe M, et al. Serodiagnosis of Epstein-Barr virus infection by using recombinant viral capsid antigen fragments and autologous gene fusion. *J Clin Microbiol* 1999;**37**:3239–44.
216. Hinuma Y, Grace JT Jr. Cloning of immunoglobulin-producing human leukemic and lymphoma cells in long-term cultures. *Proc Soc Exp Biol Med* 1967;**124**:107–11.
217. Ho HC, Ng MH, Kwan HC, et al. Epstein-Barr-virus–specific IgA and IgG serum antibodies in nasopharyngeal carcinoma. *Br J Cancer* 1976;**34**:655–60.
218. Ho M, Miller G, Atchison RW, et al. Epstein-Barr virus infections and DNA hybridization studies in posttransplantation lymphoma and lymphoproliferative lesions: the role of primary infection. *J Infect Dis* 1985;**152**:876–86.
219. Ho M, Jaffe R, Miller G, et al. The frequency of Epstein-Barr virus infection and associated lymphoproliferative syndrome after transplantation and its manifestations in children. *Transplantation* 1988;**45**:719–27.
220. Hoagland RJ. The transmission of infectious mononucleosis. *Am J Med Sci* 1955;**229**:262–72.
221. Hoagland RJ. The clinical manifestations of infectious mononucleosis: a report of two hundred cases. *Am J Med Sci* 1960;**240**: 21–9.
222. Hoagland RJ. Mononucleosis and heart disease. *Am J Med Sci* 1964;**248**:1–6.
223. Hoagland RJ. *Infectious mononucleosis*. New York: Grune & Stratton; 1967.
224. Hochberg FH, Lehrich JR, Richardson EP Jr, et al. Mononucleosis-associated subacute sclerosing panencephalitis. *Acta Neuropathol (Berl)* 1976;**34**:33–40.
225. Hofmann B, Schuppe HC, Adams O, et al. Gianotti-Crosti syndrome associated with Epstein-Barr virus infection. *Pediatr Dermatol* 1997;**14**:273–7.
226. Hong GK, Gulley ML, Feng WH, et al. Epstein-Barr virus lytic infection contributes to lymphoproliferative disease in a SCID mouse model. *J Virol* 2005;**79**:13993–4003.
227. Hong GK, Kumar P, Wang L, et al. Epstein-Barr virus lytic infection is required for efficient production of the angiogenesis factor vascular endothelial growth factor in lymphoblastoid cell lines. *J Virol* 2005;**79**:13984–92.
228. Horwitz CA, Henle W, Henle G, et al. Heterophil-negative infectious mononucleosis and mononucleosis-like illnesses. Laboratory confirmation of 43 cases. *Am J Med* 1977;**63**:947–57.
229. Horwitz CA, Burke MD, Grimes P, et al. Hepatic function in mononucleosis induced by Epstein-Barr virus and cytomegalovirus. *Clin Chem* 1980;**26**:243–6.
230. Hoshino Y, Kimura H, Kuzushima K, et al. Early intervention in post-transplant lymphoproliferative disorders based on Epstein-Barr viral load. *Bone Marrow Transplant* 2000;**26**:199–201.
231. Huang DP, Lo KW, Choi PH, et al. Loss of heterozygosity on the short arm of chromosome 3 in nasopharyngeal carcinoma. *Cancer Genet Cytogenet* 1991;**54**:91–9.
232. Huang DP, Lo KW, van Hasselt CA, et al. A region of homozygous deletion on chromosome 9p21-22 in primary nasopharyngeal carcinoma. *Cancer Res* 1994;**54**:4003–6.
233. Hudson MM, Donaldson SS. Hodgkin's disease. *Pediatr Clin North Am* 1997;**44**:891–906.
234. Hudson MM, Donaldson SS. Treatment of pediatric Hodgkin's lymphoma. *Semin Hematol* 1999;**36**:313–23.
235. Hummel M, Anagnostopoulos I, Korbjuhn P, et al. Epstein-Barr virus in B-cell non-Hodgkin's lymphomas: unexpected infection patterns and different infection incidence in low- and high-grade types. *J Pathol* 1995;**175**:263–71.
236. Hurwitz M, Desai DM, Cox KL, et al. Complete immunosuppressive withdrawal as a uniform approach to post-transplant lymphoproliferative disease in pediatric liver transplantation. *Pediatr Transplant* 2004;**8**:267–72.
237. Icart J, Didier J, Dalens M, et al. Prospective study of Epstein Barr virus (EBV) infection during pregnancy. *Biomedicine* 1981;**34**: 160–3.
238. Imai S, Usui N, Sugiura M, et al. Epstein-Barr virus genomic sequences and specific antibodies in cerebrospinal fluid in children with neurologic complications of acute and reactivated EBV infections. *J Med Virol* 1993;**40**:278–84.
239. Imashuku S, Hibi S, Ohara T, et al. Effective control of Epstein-Barr virus–related hemophagocytic lymphohistiocytosis with immunochemotherapy. *Histiocyte Society Blood* 1999;**93**: 1869–74.
240. Ingersoll L, Woo SY, Donaldson S, et al. Nasopharyngeal carcinoma in the young: a combined M.D. Anderson and Stanford experience. *Int J Radiat Oncol Biol Phys* 1990;**19**:881–7.
241. Ireland RA, Baetjer WA, Ruhrah J. A case of lymphatic leukemia with apparent cure. *JAMA* 1915;**65**:948–9.
242. Ishida Y, Yokota Y, Tauchi H, et al. Ganciclovir for chronic active Epstein-Barr virus infection. *Lancet* 1993;**341**:560–1.
243. Israele V, Shirley P, Sixbey JW. Excretion of the Epstein-Barr virus from the genital tract of men. *J Infect Dis* 1991;**163**:1341–3.
244. Itin PH, Bircher AJ, Litzisdorf Y, et al. Oral hairy leukoplakia in a child: confirmation of the clinical diagnosis by ultrastructural examination of exfoliative cytologic specimens. *Dermatology* 1994;**189**:167–9.
245. Jaffe ES. Mature T-cell and NK-cell lymphomas in the pediatric age group. *Am J Clin Pathol* 2004;**122**(Suppl.):s110–21.
246. Jaffe ES, Banks PM, Nathwani B, et al. Recommendations for the reporting of lymphoid neoplasms: a report from the Association of Directors of Anatomic and Surgical Pathology. *Mod Pathol* 2004;**17**:131–5.
247. Jain S, Sherlock S. Infectious mononucleosis with jaundice, anaemia, and encephalopathy. *Br Med J* 1975;**3**:138–9.
248. James JA, Kaufman KM, Farris AD, et al. An increased prevalence of Epstein-Barr virus infection in young patients suggests a possible etiology for systemic lupus erythematosus. *J Clin Invest* 1997;**100**:3019–26.
249. Janka G, Imashuku S, Elinder G, et al. Infection- and malignancy-associated hemophagocytic syndromes. Secondary hemophagocytic lymphohistiocytosis. *Hematol Oncol Clin North Am* 1998;**12**: 435–44.
250. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. *Eur J Pediatr* 2007;**166**:95–109.
251. Jenson H, McIntosh K, Pitt J, et al. Natural history of primary Epstein-Barr virus infection in children of mothers infected with human immunodeficiency virus type 1. *J Infect Dis* 1999;**179**: 1395–404.
252. Jenson HB, Leach CT, McClain KL, et al. Benign and malignant smooth muscle tumors containing Epstein-Barr virus in children with AIDS. *Leuk Lymphoma* 1997;**27**:303–14.
253. Jenson HB. Acute complications of Epstein-Barr virus infectious mononucleosis. *Curr Opin Pediatr* 2000;**12**:263–8.
254. Jenson HB, Ench Y, Sumaya CV. Epstein-Barr virus. In: Detrick B, Hamilton RG, Folds JD, editors. *Manual of molecular and clinical laboratory immunology*. Herndon, Va: ASM Press; 2006. p. 637–47.
255. Jeyakumar A, Brickman TM, Jeyakumar A, et al. Review of nasopharyngeal carcinoma. *Ear Nose Throat J* 2006;**85**:168–70.
256. Johansson B, Klein G, Henle W, et al. Epstein-Barr virus (EBV)–associated antibody patterns in malignant lymphoma and leukemia. I. Hodgkin's disease. *Int J Cancer* 1970;**6**:450–62.
257. Johnsen T, Katholm M, Stangerup SE. Otolaryngological complications in infectious mononucleosis. *J Laryngol Otol* 1984;**98**: 999–1001.
258. Joncas J, Lussier G, Pavilanis V. Studies on infectious mononucleosis. II. Attempts at experimental transmission to monkeys. *Can Med Assoc J* 1966;**95**:151–4.

259. Joncas J, Chiasson JP, Turcotte J, et al. Studies on infectious mononucleosis. 3. Clinical data, serologic and epidemiologic findings. *Can Med Assoc J* 1968;**98**:848–54.
260. Joncas JH, Chicoine L, Thivierge F, et al. Epstein-Barr virus antibodies in the cerebrospinal fluid. A case of infectious mononucleosis with encephalitis. *Am J Dis Child* 1974;**127**:282–5.
261. Joncas JH, Alfieri C, Leyritz-Wills M, et al. Simultaneous congenital infection with Epstein-Barr virus and cytomegalovirus. *N Engl J Med* 1981;**304**:1399–403.
262. Jondal M, Klein G. Surface markers on human B and T lymphocytes. II. Presence of Epstein-Barr virus receptors on B lymphocytes. *J Exp Med* 1973;**138**:1365–78.
263. Jones JF, Ray CG, Minnich LL, et al. Evidence for active Epstein-Barr virus infection in patients with persistent, unexplained illnesses: elevated anti-early antigen antibodies. *Ann Intern Med* 1985;**102**:1–7.
264. Joshi VV, Oleske JM, Minnefor AB, et al. Pathologic pulmonary findings in children with the acquired immunodeficiency syndrome: a study of ten cases. *Hum Pathol* 1985;**16**:241–6.
265. Junker AK, Thomas EE, Radcliffe A, et al. Epstein-Barr virus shedding in breast milk. *Am J Med Sci* 1991;**302**:220–3.
266. Kantakamalakul W, Chongkolwatana C, Naksawat P, et al. Specific IgA antibody to Epstein-Barr viral capsid antigen: a better marker for screening nasopharyngeal carcinoma than EBV-DNA detection by polymerase chain reaction. *Asian Pac J Allergy Immunol* 2000;**18**:221–6.
267. Kaplan LD, Straus DJ, Testa MA, et al. Low-dose compared with standard-dose m-BACOD chemotherapy for non-Hodgkin's lymphoma associated with human immunodeficiency virus infection. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group. *N Engl J Med* 1997;**336**:1641–8.
268. Kasamon YL, Ambinder RF. AIDS-related primary central nervous system lymphoma. *Hematol Oncol Clin North Am* 2005;**19**:665–87.
269. Katamine S, Otsu M, Tada K, et al. Epstein-Barr virus transforms precursor B cells even before immunoglobulin gene rearrangements. *Nature* 1984;**309**:369–72.
270. Katano H, Ali MA, Patera AC, et al. Chronic active Epstein-Barr virus infection associated with mutations in perforin that impair its maturation. *Blood* 2004;**103**:1244–52.
271. Katano H, Pesnicak L, Cohen JI. Simvastatin induces apoptosis of Epstein-Barr virus (EBV)–transformed lymphoblastoid cell lines and delays development of EBV lymphomas. *Proc Natl Acad Sci U S A* 2004;**101**:4960–5.
272. Katz BZ, Berkman AB, Shapiro ED. Serologic evidence of active Epstein-Barr virus infection in Epstein-Barr virus–associated lymphoproliferative disorders of children with acquired immunodeficiency syndrome. *J Pediatr* 1992;**120**:228–32.
273. Katz B, Shirashi Y, Mears C, et al. Chronic fatigue syndrome after infectious mononucleosis in adolescents. *Pediatrics* 2009;**124**: 189–93.
274. Kawa-Ha K, Franco E, Doi S, et al. Successful treatment of chronic active Epstein-Barr virus infection with recombinant interleukin-2. *Lancet* 1987;**1**:17.
275. Kennard C, Swash M. Acute viral encephalitis: its diagnosis and outcome. *Brain* 1981;**104**:129–48.
276. Khan G, Coates PJ, Gupta RK, et al. Presence of Epstein-Barr virus in Hodgkin's disease is not exclusive to Reed-Sternberg cells. *Am J Pathol* 1992;**140**:757–62.
277. Khan G. Epstein-Barr virus, cytokines, and inflammation: a cocktail for the pathogenesis of Hodgkin's lymphoma? *Exp Hematol* 2006;**34**:399–406.
278. Khanna R, Bell S, Sherritt M, et al. Activation and adoptive transfer of Epstein-Barr virus–specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. *Proc Natl Acad Sci U S A* 1999;**96**:10391–6.
279. Kijima Y, Hokita S, Takao S, et al. Epstein-Barr virus involvement is mainly restricted to lymphoepithelial type of gastric carcinoma among various epithelial neoplasms. *J Med Virol* 2001;**64**: 513–18.
280. Kilger E, Kieser A, Baumann M, et al. Epstein-Barr virus–mediated B-cell proliferation is dependent upon latent membrane protein 1, which simulates an activated CD40 receptor. *EMBO J* 1998;**17**:1700–9.
281. Kimura H, Hoshino Y, Kanegane H, et al. Clinical and virologic characteristics of chronic active Epstein-Barr virus infection. *Blood* 2001;**98**:280–6.
282. Kimura H, Morishima T, Kanegane H, et al. Prognostic factors for chronic active Epstein-Barr virus infection. *J Infect Dis* 2003;**187**:527–33.
283. Kimura H. Pathogenesis of chronic active Epstein-Barr virus infection: is this an infectious disease, lymphoproliferative disorder, or immunodeficiency? *Rev Med Virol* 2006;**16**:251–61.
284. Klein E, Masucci MG, Berthold W, et al. Lymphocyte-mediated cytotoxicity toward virus-induced tumor cells; natural and activated killer lymphocytes in man. In: Essex M, Todaro G, Zur Hausen H, editors. *Viruses in naturally occurring cancers*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory; 1980. p. 1187–97.
285. Klein E, Ernberg I, Masucci MG, et al. T-cell response to B-cells and Epstein-Barr virus antigens in infectious mononucleosis. *Cancer Res* 1981;**41**:4210–15.
286. Klein G. Lymphoma development in mice and humans: diversity of initiation is followed by convergent cytogenetic evolution. *Proc Natl Acad Sci U S A* 1979;**76**:2442–6.
287. Klein G. The role of gene dosage and genetic transpositions in carcinogenesis. *Nature* 1981;**294**:313–18.
288. Kolski H, Ford-Jones EL, Richardson S, et al. Etiology of acute childhood encephalitis at The Hospital for Sick Children, Toronto, 1994-1995. *Clin Infect Dis* 1998;**26**:398–409.
289. Kopec SE, Irwin RS, Mello CJ, et al. Bilateral anaerobic empyemas complicating infectious mononucleosis. *Chest* 1997;**112**: 833–5.
290. Koutras A. Epstein-Barr virus infection with pancreatitis, hepatitis and proctitis. *Pediatr Infect Dis* 1983;**2**:312–13.
291. Kuppers R. B cells under influence: transformation of B cells by Epstein-Barr virus. *Nat Rev Immunol* 2003;**3**:801–12.
292. Kurland G, Orenstein DM. Complications of pediatric lung and heart-lung transplantation. *Curr Opin Pediatr* 1994;**6**:262–71.
293. Labrecque LG, Lampert I, Kazembe P, et al. Correlation between cytopathological results and in situ hybridisation on needle aspiration biopsies of suspected African Burkitt's lymphomas. *Int J Cancer* 1994;**59**:591–6.
294. Labrecque LG, Xue SA, Kazembe P, et al. Expression of Epstein-Barr virus lytically related genes in African Burkitt's lymphoma: correlation with patient response to therapy. *Int J Cancer* 1999;**81**:6–11.
295. Lahat E, Eshel G, Arlazoroff A. "Alice in Wonderland" syndrome and infectious mononucleosis in children. *J Neurol Neurosurg Psychiatry* 1990;**53**:1104.
296. Lahat E, Berkovitch M, Barr J, et al. Abnormal visual evoked potentials in children with "Alice in Wonderland" syndrome due to infectious mononucleosis. *J Child Neurol* 1999;**14**:732–5.
297. Landau Z, Gross R, Sanilevich A, et al. Presence of infective Epstein-Barr virus in the urine of patients with infectious mononucleosis. *J Med Virol* 1994;**44**:229–33.
298. Lander P, Palayew MJ. Infectious mononucleosis—a review of chest roentgenographic manifestations. *J Can Assoc Radiol* 1974; **25**:303–6.
299. Lankester AC, Visser LFA, Hartwig NG, et al. Allogeneic stem cell transplantation in X-linked lymphoproliferative disease: two cases in one family and review of the literature. *Bone Marrow Transplant* 2005;**36**:99–105.
300. Laporte JP, Lesage S, Woler M, et al. Administration of three cytokines instead of bone marrow transplantation in an HIV^+ patient with high-grade lymphoma. *Eur J Haematol* 1994; **53**:123–5.
301. Lawson JS, Gunzburg WH, Whitaker NJ. Viruses and human breast cancer. *Future Microbiol* 2006;**1**:33–51.
302. Lazarus KH, Baehner RL. Aplastic anemia complicating infectious mononucleosis: a case report and review of the literature. *Pediatrics* 1981;**67**:907–10.
303. Le CT, Chang RS, Lipson MH. Epstein-Barr virus infections during pregnancy. A prospective study and review of the literature. *Am J Dis Child* 1983;**137**:466–8.
304. Le QT, Jones CD, Yau TK, et al. A comparison study of different PCR assays in measuring circulating plasma Epstein-Barr virus DNA levels in patients with nasopharyngeal carcinoma. *Clin Cancer Res* 2005;**11**:5700–7.

305. Leach CT, Frantz C, Head DR, et al. Human herpesvirus-8 (HHV-8) associated with small non–cleaved cell lymphoma in a child with AIDS. *Am J Hematol* 1999;**60**:215–21.
306. Leder P, Battey J, Lenoir G, et al. Translocations among antibody genes in human cancer. *Science* 1983;**222**:765–71.
307. Lee ES, Locker J, Nalesnik M, et al. The association of Epstein-Barr virus with smooth-muscle tumors occurring after organ transplantation. *N Engl J Med* 1995;**332**:19–25.
308. Lee S, Kjellstrand CM. Renal disease in infectious mononucleosis. *Clin Nephrol* 1978;**9**:236–40.
309. Lee TC, Savoldo B, Rooney CM, et al. Quantitative EBV viral loads and immunosuppression alterations can decrease PTLD incidence in pediatric liver transplant recipients. *Am J Transplant* 2005;**5**:2222–8.
310. Lei KI, Chan LY, Chan WY, et al. Quantitative analysis of circulating cell-free Epstein-Barr virus (EBV) DNA levels in patients with EBV-associated lymphoid malignancies. *Br J Haematol* 2000;**111**:239–46.
311. Lei PS, Lowichik A, Allen W, et al. Acute renal failure: unusual complication of Epstein-Barr virus–induced infectious mononucleosis. *Clin Infect Dis* 2000;**31**:1519–24.
312. Levey BA, Lo TM, Caldwell KE, et al. Latex test for serodiagnosis of infectious mononucleosis. *J Clin Microbiol* 1980;**11**:256–62.
313. Levine D, Tilton RC, Parry MF, et al. False positive EBNA IgM and IgG antibody tests for infectious mononucleosis in children. *Pediatrics* 1994;**94**:892–4.
314. Levine D, Klenk R, Morelli A, et al. Let the user beware: specificity and sensitivity limits for in vitro diagnostic devices. *Pediatrics* 1997;**100**:267–9.
315. Levine PH, Ablashi DV, Berard CW, et al. Elevated antibody titers to Epstein-Barr virus in Hodgkin's disease. *Cancer* 1971; **27**:416–21.
316. Levitskaya J, Coram M, Levitsky V, et al. Inhibition of antigen processing by the internal repeat region of the Epstein-Barr virus nuclear antigen-1. *Nature* 1995;**375**:685–8.
317. Levitskaya J, Sharipo A, Leonchiks A, et al. Inhibition of ubiquitin/proteasome-dependent protein degradation by the Gly-Ala repeat domain of the Epstein-Barr virus nuclear antigen 1. *Proc Natl Acad Sci U S A* 1997;**94**:12616–21.
318. Levy M, Kelly JP, Kaufman DW, et al. Risk of agranulocytosis and aplastic anemia in relation to history of infectious mononucleosis: a report from the international agranulocytosis and aplastic anemia study. *Ann Hematol* 1993;**67**:187–90.
319. Liebowitz D. Nasopharyngeal carcinoma: the Epstein-Barr virus association. *Semin Oncol* 1994;**21**:376–81.
320. Liebowitz D, Anastasi J, Hagos F, et al. Post-transplant lymphoproliferative disorders (PTLD): clinicopathologic characterization and response to immunomodulatory therapy with interferon-alpha. *Ann Oncol* 1996;**7**(Suppl. 3):28.
321. Lifschitz C, LaSala S. Pancreatitis, cholecystitis, and choledocholithiasis associated with infectious mononucleosis. *Clin Pediatr (Phila)* 1981;**20**:131.
322. Likic R, Kuzmanic D. Severe thrombocytopenia as a complication of acute Epstein-Barr virus infection. *Wien Klin Wochenschr* 2004;**116**:47–50.
323. Lilleyman JS, Pinkerton CR. Lymphoblastic leukaemia and non-Hodgkin's lymphoma. *Br Med Bull* 1996;**52**:742–63.
324. Lin CL, Lo WF, Lee TH, et al. Immunization with Epstein-Barr virus (EBV) peptide-pulsed dendritic cells induces functional $CD8^+$ T-cell immunity and may lead to tumor regression in patients with EBV-positive nasopharyngeal carcinoma. *Cancer Res* 2002;**62**:6952–8.
325. Lin J, Johannsen E, Robertson E, et al. Epstein-Barr virus nuclear antigen 3C putative repression domain mediates coactivation of the LMP1 promoter with EBNA-2. *J Virol* 2002;**76**:232–42.
326. Lin JC, Smith MC, Pagano JS. Comparative efficacy and selectivity of some nucleoside analogs against Epstein-Barr virus. *Antimicrob Agents Chemother* 1985;**27**:971–3.
327. Lindahl T, Adams A, Bjursell G, et al. Covalently closed circular duplex DNA of Epstein-Barr virus in a human lymphoid cell line. *J Mol Biol* 1976;**102**:511–30.
328. Lipman M, Andrews L, Niederman J, et al. Direct visualization of enveloped Epstein-Barr herpesvirus in throat washing with leukocyte-transforming activity. *J Infect Dis* 1975;**132**:520–3.
329. Little RF, Yarchoan R, Wilson WH. Systemic chemotherapy for HIV-associated lymphoma in the era of highly active antiretroviral therapy. *Curr Opin Oncol* 2000;**12**:438–44.
330. Lloyd-Still JD, Scott JP, Crussi F. The spectrum of Epstein-Barr virus hepatitis in children. *Pediatr Pathol* 1986;**5**:337–51.
331. Lowenfels AB. Kehr's sign—a neglected aid in rupture of the spleen. *N Engl J Med* 1966;**274**:5.
332. Lukes RJ, Cox FH. Clinical and morphologic findings in 30 fatal cases of infectious mononucleosis. *Am J Pathol* 1958;**34**:586.
333. MacMahon EM, Glass JD, Hayward SD, et al. Epstein-Barr virus in AIDS-related primary central nervous system lymphoma. *Lancet* 1991;**338**:969–73.
334. Madigan NP, Newcomer AD, Campbell DC, et al. Intense jaundice in infectious mononucleosis. *Mayo Clin Proc* 1973;**48**: 857–62.
335. Magalhaes MG, Bueno DF, Serra E, et al. Oral manifestations of HIV positive children. *J Clin Pediatr Dent* 2001;**25**:103–6.
336. Major RH. *Classic descriptions of disease, with biographical sketches of the authors*. Springfield, Ill: Charles C Thomas; 1945.
337. Maki DG, Reich RM. Infectious mononucleosis in the athlete. Diagnosis, complications, and management. *Am J Sports Med* 1982;**10**:162–73.
338. Mallon R, Borkowski J, Albin R, et al. The Epstein-Barr virus BZLF1 gene product activates the human immunodeficiency virus type 1 5′ long terminal repeat. *J Virol* 1990;**64**:6282–5.
339. Mangi RJ, Niederman JC, Kelleher JE Jr, et al. Depression of cell-mediated immunity during acute infectious mononucleosis. *N Engl J Med* 1974;**291**:1149–53.
340. Mannick JB, Cohen JI, Birkenbach M, et al. The Epstein-Barr virus nuclear protein encoded by the leader of the EBNA RNAs is important in B-lymphocyte transformation. *J Virol* 1991;**65**:6826–37.
341. Markin RS, Linder J, Zuerlein K, et al. Hepatitis in fatal infectious mononucleosis. *Gastroenterology* 1987;**93**:1210–17.
342. Martinez OM, Villanueva JC, Lawrence-Miyasaki L, et al. Viral and immunologic aspects of Epstein-Barr virus infection in pediatric liver transplant recipients. *Transplantation* 1995;**59**:519–24.
343. Mason WR Jr, Adams EK. Infectious mononucleosis; an analysis of 100 cases with particular attention to diagnosis, liver function tests and treatment of selected cases with prednisone. *Am J Med Sci* 1958;**236**:447–59.
344. Matoba AY. Ocular disease associated with Epstein-Barr virus infection. *Surv Ophthalmol* 1990;**35**:145–50.
345. McClain KL, Leach CT, Jenson HB, et al. Association of Epstein-Barr virus with leiomyosarcomas in children with AIDS. *N Engl J Med* 1995;**332**:12–18.
346. McClain KL. Epstein-Barr virus lymphoproliferative diseases. *Semin Pediatr Infect Dis* 1996;**7**:1–8.
347. McClain KL, Joshi VV, Murphy SB. Cancers in children with HIV infection. *Hematol Oncol Clin North Am* 1996;**10**:1189–201.
348. McClain KL, Leach CT, Jenson HB, et al. Molecular and virologic characteristics of lymphoid malignancies in children with AIDS. *J Acquir Immune Defic Syndr* 2000;**23**:152–9.
349. McClain MT, Heinlen LD, Dennis GJ, et al. Early events in lupus humoral autoimmunity suggest initiation through molecular mimicry. *Nat Med* 2005;**11**:85–9.
350. McGowan JE Jr, Chesney PJ, Crossley KB, et al. Guidelines for the use of systemic glucocorticosteroids in the management of selected infections. Working Group on Steroid Use, Antimicrobial Agents Committee, Infectious Diseases Society of America. *J Infect Dis* 1992;**165**:1–13.
351. McMonigal K, Horwitz CA, Henle W, et al. Post-perfusion syndrome due to Epstein-Barr virus. Report of two cases and review of the literature. *Transfusion* 1983;**23**:331–5.
352. McSherry GD. Human immunodeficiency-virus-related pulmonary infections in children. *Semin Respir Infect* 1996;**11**:173–83.
353. Mendonca D. A case of infectious mononucleosis presenting with a bilateral facial palsy. *J Laryngol Otol* 1971;**85**:981–2.
354. Mierau GW, Greffe BS, Weeks DA. Primary leiomyosarcoma of brain in an adolescent with common variable immunodeficiency syndrome. *Ultrastruct Pathol* 1997;**21**:301–5.
355. Miller G, Shope T, Lisco H, et al. Epstein-Barr virus: transformation, cytopathic changes, and viral antigens in squirrel monkey and marmoset leukocytes. *Proc Natl Acad Sci U S A* 1972;**69**:383–7.

356. Miller G, Niederman JC, Andrews LL. Prolonged oropharyngeal excretion of Epstein-Barr virus after infectious mononucleosis. *N Engl J Med* 1973;**288**:229–32.
357. Milone MC, Tsai DE, Hodinka RL, et al. Treatment of primary Epstein-Barr virus infection in patients with X-linked lymphoproliferative disease using B-cell-directed therapy. *Blood* 2005; **105**:994–6.
358. Montone KT, Hodinka RL, Salhany KE, et al. Identification of Epstein-Barr virus lytic activity in post-transplantation lymphoproliferative disease. *Mod Pathol* 1996;**9**:621–30.
359. Moody CE, Casazza BA, Christenson WN, et al. Lymphocyte transformation induced by autologous cells. VIII. Impaired autologous mixed lymphocyte reactivity in patients with acute infectious mononucleosis. *J Exp Med* 1979;**150**:1448–55.
360. Morente MM, Piris MA, Abraira V, et al. Adverse clinical outcome in Hodgkin's disease is associated with loss of retinoblastoma protein expression, high Ki67 proliferation index, and absence of Epstein-Barr virus–latent membrane protein 1 expression. *Blood* 1997;**90**:2429–36.
361. Moura MD, Guimaraes TR, Fonseca LM, et al. A random clinical trial study to assess the efficiency of topical applications of podophyllin resin (25%) versus podophyllin resin (25%) together with acyclovir cream (5%) in the treatment of oral hairy leukoplakia. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103**:64–71.
362. Moutschen M, Leonard P, Sokal EM, et al. Phase I/II studies to evaluate safety and immunogenicity of a recombinant gp350 Epstein-Barr virus vaccine in healthy adults. *Vaccine* 2007;**25**:4697–705.
363. Mozes B, Pines A, Werner D, et al. Grand-mal as the major presenting symptom of infectious mononucleosis. *J Neurol Neurosurg Psychiatry* 1984;**47**:569–70.
364. Mroczek EC, Weisenburger DD, Grierson HL, et al. Fatal infectious mononucleosis and virus-associated hemophagocytic syndrome. *Arch Pathol Lab Med* 1987;**111**:530–5.
365. Mueller N, Evans A, Harris NL, et al. Hodgkin's disease and Epstein-Barr virus. Altered antibody pattern before diagnosis. *N Engl J Med* 1989;**320**:689–95.
366. Murray PG, Billingham LJ, Hassan HT, et al. Effect of Epstein-Barr virus infection on response to chemotherapy and survival in Hodgkin's disease. *Blood* 1999;**94**:442–7.
367. Nador RG, Cesarman E, Chadburn A, et al. Primary effusion lymphoma: a distinct clinicopathologic entity associated with the Kaposi's sarcoma–associated herpes virus. *Blood* 1996;**88**: 645–56.
368. Nalesnik MA, Makowka L, Starzl TE. The diagnosis and treatment of posttransplant lymphoproliferative disorders. *Curr Probl Surg* 1988;**25**:367–472.
369. Nanbo A, Yoshiyama H, Takada K. Epstein-Barr virus–encoded poly(A)–RNA confers resistance to apoptosis mediated through Fas by blocking the PKR pathway in human epithelial intestine 407 cells. *J Virol* 2005;**79**:12280–5.
370. Neel EU. Infectious mononucleosis. Death due to agranulocytosis and pneumonia. *JAMA* 1976;**236**:1493–4.
371. Nemerow GR, Cooper NR. Early events in the infection of human B lymphocytes by Epstein-Barr virus: the internalization process. *Virology* 1984;**132**:186–98.
372. Nichols KE, Harkin DP, Levitz S, et al. Inactivating mutations in an SH2 domain–encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* 1998;**95**:13765–70.
373. Niederman JC, Scott RB. Studies on infectious mononucleosis: attempts to transmit the disease to human volunteers. *Yale J Biol Med* 1965;**38**:1–10.
374. Niederman JC, McCollum RW, Henle G, et al. Infectious mononucleosis. Clinical manifestations in relation to EB virus antibodies. *JAMA* 1968;**203**:205–9.
375. Niederman JC, Evans AS, Subrahmanyan L, et al. Prevalence, incidence and persistence of EB virus antibody in young adults. *N Engl J Med* 1970;**282**:361–5.
376. Niederman JC, Miller G, Pearson HA, et al. Infectious mononucleosis. Epstein-Barr-virus shedding in saliva and the oropharynx. *N Engl J Med* 1976;**294**:1355–9.
377. Niedobitek G, Agathanggelou A, Herbst H, et al. Epstein-Barr virus (EBV) infection in infectious mononucleosis: virus latency, replication and phenotype of EBV-infected cells. *J Pathol* 1997;**182**:151–9.
378. Niedobitek G, Meru N, Delecluse HJ. Epstein-Barr virus infection and human malignancies. *Int J Exp Pathol* 2001;**82**:149–70.
379. Nilsson K. The nature of lymphoid cell lines and their relationship to the virus. In: Epstein MA, Achong BG, editors. *The Epstein-Barr virus*. New York: Springer-Verlag; 1979. p. 225–95.
380. Nolan RA. Report of a so-called epidemic of glandular fever (infectious mononucleosis). *U S Navy Med Bull* 1935;**33**: 479–83.
381. North K, de Silva L, Procopis P. Brain-stem encephalitis caused by Epstein-Barr virus. *J Child Neurol* 1993;**8**:40–2.
382. Offit PA, Fleisher GR, Koven NL, et al. Severe Epstein-Barr virus pulmonary involvement. *J Adolesc Health Care* 1981;**2**:121–5.
383. Okamura T, Hatsukawa Y, Arai H, et al. Blood stem-cell transplantation for chronic active Epstein-Barr virus with lymphoproliferation. *Lancet* 2000;**356**:223–4.
384. Okano M. Therapeutic approaches for severe Epstein-Barr virus infection. *Pediatr Hematol Oncol* 1997;**14**:109–19.
385. Opelz G, Daniel V, Naujokat C, et al. Effect of cytomegalovirus prophylaxis with immunoglobulin or with antiviral drugs on post-transplant non-Hodgkin lymphoma: a multicentre retrospective analysis. *Lancet Oncol* 2007;**8**:212–18.
386. Osamah H, Finkelstein R, Brook JG. Rhabdomyolysis complicating acute Epstein-Barr virus infection. *Infection* 1995;**23**:119–20.
387. Pagano JS, Sixbey JW, Lin JC. Acyclovir and Epstein-Barr virus infection. *J Antimicrob Chemother* 1983;**12**:113–21.
388. Palazzi DL, McClain KL, Kaplan SL. Hemophagocytic syndrome in children: an important diagnostic consideration in fever of unknown origin. *Clin Infect Dis* 2003;**36**:306–12.
389. Pallesen G, Hamilton-Dutoit SJ, Rowe M, et al. Expression of Epstein-Barr virus latent gene products in tumour cells of Hodgkin's disease. *Lancet* 1991;**337**:320–2.
390. Pastore L, De Benedittis M, Petruzzi M, et al. Efficacy of famciclovir in the treatment of oral hairy leukoplakia. *Br J Dermatol* 2006;**154**:378–9.
391. Patel BM. Skin rash with infectious mononucleosis and ampicillin. *Pediatrics* 1967;**40**:910–11.
392. Patel PC, Menezes J. Epstein-Barr virus (EBV)–lymphoid cell interactions. II. The influence of the EBV replication cycle on natural killing and antibody-dependent cellular cytotoxicity against EBV-infected cells. *Clin Exp Immunol* 1982;**48**:589–601.
393. Pathmanathan R, Prasad U, Sadler R, et al. Clonal proliferations of cells infected with Epstein-Barr virus in preinvasive lesions related to nasopharyngeal carcinoma. *N Engl J Med* 1995;**333**:693–8.
394. Pattengale PK, Smith RW, Perlin E. Atypical lymphocytes in acute infectious mononucleosis. Identification by multiple T and B lymphocyte markers. *N Engl J Med* 1974;**291**:1145–8.
395. Paul JR, Bunnell WW. The presence of heterophile antibodies in infectious mononucleosis. *Am J Med Sci* 1932;**183**:90–104.
396. Pedneault L, Katz BZ, Miller G. Detection of Epstein-Barr virus in the brain by the polymerase chain reaction. Ann. *Neurol* 1992;**32**:184–92.
397. Pedneault L, Lapointe N, Alfieri C, et al. Natural history of Epstein-Barr virus infection in a prospective pediatric cohort born to human immunodeficiency virus–infected mothers. *J Infect Dis* 1998;**177**:1087–90.
398. Pejme J. Infectious mononucleosis: a clinical and haematological study of patients and contacts, and a comparison with healthy subjects. *Acta Med Scand Suppl* 1964;**413**:1–83.
399. Peng R, Moses SC, Tan J, et al. The Epstein-Barr virus EBNA-LP protein preferentially coactivates EBNA2-mediated stimulation of latent membrane proteins expressed from the viral divergent promoter. *J Virol* 2005;**79**:4492–505.
400. Penman HG. Fatal infectious mononucleosis: a critical review. *J Clin Pathol* 1970;**23**:765–71.
401. Pereira MS, Blake JM, Macrae AD. EB virus antibody at different ages. *Br Med J* 1969;**4**:526–7.
402. Peterson I, Thomas J, Hamilton W, White P. Risk and predictors of fatigue after infectious mononucleosis in a large primary-care cohort. *QJM* 2006;**99**:49–55.
403. Pfeiffer E. Drüsenfieber. *Jahrb Kinderheilkd* 1889;**29**:257–64.
404. Pickering LK, Baker CJ, Kimberlin D, Long SS, editors. *Epstein-Barr virus infections (infectious mononucleosis). Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 318–24.
405. Pipp ML, Means ND, Sixbey JW, et al. Acute Epstein-Barr virus infection complicated by severe thrombocytopenia. *Clin Infect Dis* 1997;**25**:1237–9.

406. Ponzoni M, Fumagalli L, Rossi G, et al. Isolated bone marrow manifestation of HIV-associated Hodgkin lymphoma. *Mod Pathol* 2002;**15**:1273–8.
407. Pope JH, Horne MK, Scott W. Transformation of foetal human leukocytes in vitro by filtrates of a human leukaemic cell line containing herpes-like virus. *Int J Cancer* 1968;**3**:857–66.
408. Poppema S, van Imhoff G, Torensma R, et al. Lymphadenopathy morphologically consistent with Hodgkin's disease associated with Epstein-Barr virus infection. *Am J Clin Pathol* 1985;**84**:385–90.
409. Porter DD, Wimberly I, Benyesh-Melnick M. Prevalence of antibodies to EB virus and other herpesviruses. *JAMA* 1969; **208**:1675–9.
410. Portman M, Ingall D, Westenfelder G, et al. Peritonsillar abscess complicating infectious mononucleosis. *J Pediatr* 1984;**104**: 742–4.
411. Portnoy J, Ahronheim G, Ghibu F, et al. Recovery of Epstein-Barr virus from genital ulcers. *N Engl J Med* 1984;**311**:966–8.
412. Potter R. Paediatric Hodgkin's disease. *Eur J Cancer* 1999; **35**:1466–74.
413. Preciado MV, De Matteo E, Fallo A, et al. EBV-associated Hodgkin's disease in an HIV-infected child presenting with a hemophagocytic syndrome. *Leuk Lymphoma* 2001;**42**:231–4.
414. Puchhammer-Stockl E, Gorzer I. Cytomegalovirus and Epstein-Barr virus subtypes—the search for clinical significance. *J Clin Virol* 2006;**36**:239–48.
415. Pullen H, Wright N, Murdoch JM. Hypersensitivity reactions to antibacterial drugs in infectious mononucleosis. *Lancet* 1967;**2**:1176–8.
416. Pullen L. Epstein-Barr vaccine may soon enter phase 3 trials [abstract]. *Sci Transl Med* 2011;**3**:107fs7.
417. Purtilo DT, Tatsumi E, Manolov G, et al. Epstein-Barr virus as an etiological agent in the pathogenesis of lymphoproliferative and aproliferative diseases in immune deficient patients. *Int Rev Exp Pathol* 1985;**27**:113–83.
418. Raab-Traub N, Flynn K. The structure of the termini of the Epstein-Barr virus as a marker of clonal cellular proliferation. *Cell* 1986;**47**:883–9.
419. Rahman MA, Kingsley LA, Breinig MK, et al. Enhanced antibody responses to Epstein-Barr virus in HIV-infected homosexual men. *J Infect Dis* 1989;**159**:472–9.
420. Ralston LS, Saiki AK, Powers WT. Orchitis as a complication of infectious mononucleosis. *JAMA* 1960;**173**:1348–9.
421. Rea TD, Russo JE, Katon W, et al. Prospective study of the natural history of infectious mononucleosis caused by Epstein-Barr virus. *J Am Board Fam Pract* 2001;**14**:234–42.
422. Reddy A, Lyall EG, Crawford DH. Epstein-Barr virus and lymphoid interstitial pneumonitis: an association revisited. *Pediatr Infect Dis J* 1998;**17**:82–3.
423. Reinherz EL, O'Brien C, Rosenthal P, et al. The cellular basis for viral-induced immunodeficiency: analysis by monoclonal antibodies. *J Immunol* 1980;**125**:1269–74.
424. Reisman RP, Greco MA. Virus-associated hemophagocytic syndrome due to Epstein-Barr virus. *Hum Pathol* 1984;**15**:290–3.
425. Rezk SA, Weiss LM. Epstein-Barr virus–associated lymphoproliferative disorders. *Hum Pathol* 2007;**38**:1293–304.
426. Ricker W, Blumberg A, Peters CH, et al. The association of Guillain-Barré syndrome with infectious mononucleosis with a report of two fatal cases. *Blood* 1947;**2**:217–26.
427. Rickinson AB, Moss DJ. Human cytotoxic T lymphocyte responses to Epstein-Barr virus infection. *Annu Rev Immunol* 1997;**15**: 405–31.
428. Rickinson AB, Kieff E. Epstein-Barr virus. In: Knipe DM, Howley PM, editors. *Fields' virology*. Philadelphia: Wolters Kluwer Health/ Lippincott Williams & Wilkins; 2007. p. 2655–700.
429. Riddler SA, Breinig MC, McKnight JL. Increased levels of circulating Epstein-Barr virus (EBV)–infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. *Blood* 1994;**84**: 972–84.
430. Ries LAG, Melbert D, Krapcho M, et al. *SEER cancer statistics review, 1975-2004*. Bethesda, Md: National Cancer Institute; 2007.
431. Risdall RJ, McKenna RW, Nesbit ME, et al. Virus-associated hemophagocytic syndrome: a benign histiocytic proliferation distinct from malignant histiocytosis. *Cancer* 1979;**44**:993–1002.
432. Robertson ES, Lin J, Kieff E. The amino-terminal domains of Epstein-Barr virus nuclear proteins 3A, 3B, and 3C interact with RBPJ(kappa). *J Virol* 1996;**70**:3068–74.
433. Robinson J, Smith D, Niederman J. Mitotic EBNA-positive lymphocytes in peripheral blood during infectious mononucleosis. *Nature* 1980;**287**:334–5.
434. Robinson JE, Brown N, Andiman W, et al. Diffuse polyclonal B-cell lymphoma during primary infection with Epstein-Barr virus. *N Engl J Med* 1980;**302**:1293–7.
435. Robinson JE, Smith D, Niederman J. Plasmacytic differentiation of circulating Epstein-Barr virus–infected B lymphocytes during acute infectious mononucleosis. *J Exp Med* 1981;**153**:235–44.
436. Rocchi G, Felici A, Ragona G, et al. Quantitative evaluation of Epstein-Barr-virus–infected mononuclear peripheral blood leukocytes in infectious mononucleosis. *N Engl J Med* 1977;**296**: 132–4.
437. Rogatsch H, Bonatti H, Menet A, et al. Epstein-Barr virus–associated multicentric leiomyosarcoma in an adult patient after heart transplantation: case report and review of the literature. *Am J Surg Pathol* 2000;**24**:614–21.
438. Rogers BB, Sommerauer J, Quan A, et al. Epstein-Barr virus polymerase chain reaction and serology in pediatric post-transplant lymphoproliferative disorder: three-year experience. *Pediatr Dev Pathol* 1998;**1**:480–6.
439. Rogers R, Windust A, Gregory J. Evaluation of a novel dry latex preparation for demonstration of infectious mononucleosis heterophile antibody in comparison with three established tests. *J Clin Microbiol* 1999;**37**:95–8.
440. Rooney CM, Loftin SK, Holladay MS, et al. Early identification of Epstein-Barr virus–associated post-transplantation lymphoproliferative disease. *Br J Haematol* 1995;**89**:98–103.
441. Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus–induced lymphoma in allogeneic transplant recipients. *Blood* 1998;**92**: 1549–55.
442. Rosen A, Gergely P, Jondal M, et al. Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes in vitro. *Nature* 1977;**267**:52–4.
443. Rovedo M, Longnecker R. Epstein-Barr virus latent membrane protein 2B (LMP2B) modulates LMP2A activity. *J Virol* 2007;**81**:84–94.
444. Rowe M, Young LS, Cadwallader K, et al. Distinction between Epstein-Barr virus type A (EBNA 2A) and type B (EBNA 2B) isolates extends to the EBNA 3 family of nuclear proteins. *J Virol* 1989;**63**:1031–9.
445. Rowley JD. Identification of the constant chromosome regions involved in human hematologic malignant disease. *Science* 1982;**216**:749–51.
446. Rubinstein A, Morecki R, Silverman B, et al. Pulmonary disease in children with acquired immune deficiency syndrome and AIDS-related complex. *J Pediatr* 1986;**108**:498–503.
447. Ruiss R, Jochum S, Wanner G, et al. A virus-like particle–based Epstein-Barr virus vaccine. *J Virol* 2011;**85**:13105–13.
448. Rutkow IM. Rupture of the spleen in infectious mononucleosis: a critical review. *Arch Surg* 1978;**113**:718–20.
449. Sakamoto K, Freed HJ, Purtilo DT. Antibody responses to Epstein-Barr virus in families with the X-linked lymphoproliferative syndrome. *J Immunol* 1980;**125**:921–5.
450. Salazar A, Martinez H, Sotelo J. Ophthalmoplegic polyneuropathy associated with infectious mononucleosis. *Ann Neurol* 1983;**13**:219–20.
451. Sample J, Young L, Martin B, et al. Epstein-Barr virus types 1 and 2 differ in their EBNA-3A, EBNA-3B, and EBNA-3C genes. *J Virol* 1990;**64**:4084–92.
452. Sargison KD, Cole TP, Kyle J. Traumatic rupture of the spleen. *Br J Surg* 1968;**55**:506–8.
453. Sauce D, Larsen M, Leese AM, et al. IL-7R alpha versus CCR7 and CD45 as markers of virus-specific $CD8^+$ T cell differentiation: contrasting pictures in blood and tonsillar lymphoid tissue. *J Infect Dis* 2007;**195**:268–78.
454. Savoldo B, Huls MH, Liu Z, et al. Autologous Epstein-Barr virus (EBV)–specific cytotoxic T cells for the treatment of persistent active EBV infection. *Blood* 2002;**100**:4059–66.
455. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 1998;**395**:462–9.

456. Schooley RT, Densen P, Harmon D, et al. Antineutrophil antibodies in infectious mononucleosis. *Am J Med* 1984;**76**:85–90.
457. Schooley RT. Etiology. In: Schlossberg D, editor. *Infectious mononucleosis*. New York: Springer-Verlag; 1989. p. 1–7.
458. Scully C, Laskaris G, Pindborg J, et al. Oral manifestations of HIV infection and their management. I. More common lesions. *Oral Surg Oral Med Oral Pathol* 1991;**71**:158–66.
459. Shashaty GG, Atamer MA. Hemolytic uremic syndrome associated with infectious mononucleosis. *Am J Dis Child* 1974;**127**: 720–2.
460. Sheen TS, Tsai CC, Ko JY, et al. Undifferentiated carcinoma of the major salivary glands. *Cancer* 1997;**80**:357–63.
461. Shian WJ, Chi CS. Epstein-Barr virus encephalitis and encephalomyelitis: MR findings. *Pediatr Radiol* 1996;**26**:690–3.
462. Shibata Y, Hoshino Y, Hara S, et al. Clonality analysis by sequence variation of the latent membrane protein 1 gene in patients with chronic active Epstein-Barr virus infection. *J Med Virol* 2006;**78**:770–9.
463. Shiramizu B, Barriga F, Neequaye J, et al. Patterns of chromosomal breakpoint locations in Burkitt's lymphoma: relevance to geography and Epstein-Barr virus association. *Blood* 1991;**77**: 1516–26.
464. Siirci A, Akinci A, Ozgen U, et al. Neutropenic enterocolitis (typhlitis) associated with infectious mononucleosis. *Pediatr Radiol* 2006;**36**:155–7.
465. Silins SL, Sherritt MA, Silleri JM, et al. Asymptomatic primary Epstein-Barr virus infection occurs in the absence of blood T-cell repertoire perturbations despite high levels of systemic viral load. *Blood* 2001;**98**:3739–44.
466. Silverstein A, Steinberg G, Nathanson M. Nervous system involvement in infectious mononucleosis. The heralding and-or major manifestation. *Arch Neurol* 1972;**26**:353–8.
467. Simonetti GD, Dumont–Dos Santos K, Pachlopnik JM, et al. Hemolytic uremic syndrome linked to infectious mononucleosis. *Pediatr Nephrol* 2003;**18**:1193–4.
468. Sitki-Green DL, Edwards RH, Covington MM, et al. Biology of Epstein-Barr virus during infectious mononucleosis. *J Infect Dis* 2004;**189**:483–92.
469. Sitompul L, Widodo N, Djati M, Utomo D. Epitope mapping of gp350/220 conserved domain of Epstein-Barr virus to develop nasopharyngeal carcinoma (npc) vaccine. *Bioinformation* 2012;**8**: 479–82.
470. Sixbey JW, Nedrud JG, Raab-Traub N, et al. Epstein-Barr virus replication in oropharyngeal epithelial cells. *N Engl J Med* 1984;**310**:1225–30.
471. Sixbey JW, Lemon SM, Pagano JS. A second site for Epstein-Barr virus shedding: the uterine cervix. *Lancet* 1986;**2**:1122–4.
472. Sixbey JW, Davis DS, Young LS, et al. Human epithelial cell expression of an Epstein-Barr virus receptor. *J Gen Virol* 1987;**68**:805–11.
473. Slots J, Saygun I, Sabeti M, Kubar A. Epstein-Barr virus oral disease. *J Periodontal Res* 2006;**41**:235–44.
474. Smith J, Corey L, Healey P, et al. Adolescents are more likely to develop posttransplant lymphoproliferative disorder after primary Epstein-Barr virus infection than younger renal transplant recipients. *Transplantation* 2007;**83**:1423–8.
475. Smith PR, de Jesus O, Turner D, et al. Structure and coding content of CST (BART) family RNAs of Epstein-Barr virus. *J Virol* 2000;**74**:3082–92.
476. Snijders PJ, Schulten EA, Mullink H, et al. Detection of human papillomavirus and Epstein-Barr virus DNA sequences in oral mucosa of HIV-infected patients by the polymerase chain reaction. *Am J Pathol* 1990;**137**:659–66.
477. Snyderman NL, Stool SE. Management of airway obstruction in children with infectious mononucleosis. *Otolaryngol Head Neck Surg* 1982;**90**:168–70.
478. Sohier R, Lepine P, Sautter V. Recherces sur de la transmission experimentale de la mononucleose infectieuse au singe et a l'homme. *Ann Inst Pasteur (Paris)* 1940;**65**:50–62.
479. Sokal EM, Caragiozoglou T, Lamy M, et al. Epstein-Barr virus serology and Epstein-Barr virus–associated lymphoproliferative disorders in pediatric liver transplant recipients. *Transplantation* 1993;**56**:1394–8.
480. Sokal EM, Hoppenbrouwers K, Vardermuelen C, et al. Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an Epstein-Barr virus vaccine in healthy young adults. *J Infect Dis* 2007;**196**:1749–53.
481. Sparano JA, Wiernik PH, Leaf A, et al. Infusional cyclophosphamide, doxorubicin, and etoposide in relapsed and resistant non-Hodgkin's lymphoma: evidence for a schedule-dependent effect favoring infusional administration of chemotherapy. *J Clin Oncol* 1993;**11**:1071–9.
482. Sprunt TP, Evans FA. Mononuclear leukocytosis in reaction to acute infections (infectious mononucleosis). *Bull Johns Hopkins Hosp* 1920;**31**:410–17.
483. Sriskandan S, Labrecque LG, Schofield J. Diffuse pneumonia associated with infectious mononucleosis: detection of Epstein-Barr virus in lung tissue by in situ hybridization. *Clin Infect Dis* 1996;**22**:578–9.
484. Stevens SJ, Pronk I, Middeldorp JM. Toward standardization of Epstein-Barr virus DNA load monitoring: unfractionated whole blood as preferred clinical specimen. *J Clin Microbiol* 2001;**39**: 1211–16.
485. Stevenson DS, Webster G, Stewart IA. Acute tonsillectomy in the management of infectious mononucleosis. *J Laryngol Otol* 1992;**106**:989–91.
486. Straathof KC, Bollard CM, Rooney CM, et al. Immunotherapy for Epstein-Barr virus–associated cancers in children. *Oncologist* 2003;**8**:83–98.
487. Straus SE, Tosato G, Armstrong G, et al. Persisting illness and fatigue in adults with evidence of Epstein-Barr virus infection. *Ann Intern Med* 1985;**102**:7–16.
488. Sullivan JL. Epstein-Barr virus and the X-linked lymphoproliferative syndrome. *Adv Pediatr* 1983;**30**:365–99.
489. Sumaya CV. Epidemiologic study of a leukocyte-transforming agent in a general population. *J Clin Microbiol* 1975;**2**:520–3.
490. Sumaya CV, Henle W, Henle G, et al. Seroepidemiologic study of Epstein-Barr virus infections in a rural community. *J Infect Dis* 1975;**131**:403–8.
491. Sumaya CV, Neerhout RC. Sinusitis and periorbital infections complicating infectious mononucleosis. *Am J Dis Child* 1976; **130**:777.
492. Sumaya CV. Primary Epstein-Barr virus infections in children. *Pediatrics* 1977;**59**:16–21.
493. Sumaya CV, Ench Y. Epstein-Barr virus infectious mononucleosis in children. II. Heterophil antibody and viral-specific responses. *Pediatrics* 1985;**75**:1011–19.
494. Sumaya CV, Ench Y. Epstein-Barr virus infectious mononucleosis in children. I. Clinical and general laboratory findings. *Pediatrics* 1985;**75**:1003–10.
495. Sumaya CV. Epstein-Barr virus serologic testing: diagnostic indications and interpretations. *Pediatr Infect Dis* 1986;**5**:337–42.
496. Sumaya CV, Ench Y. Epstein-Barr virus infections in families: the role of children with infectious mononucleosis. *J Infect Dis* 1986;**154**:842–50.
497. Sumaya CV, Ench Y. Childhood infectious mononucleosis–like illness not associated with Epstein-Barr virus. *Pediatr Res* 1987;**21**:336A.
498. Sutton RN, Reynolds K, Almond JP, et al. Immunoglobulins and EB virus antibodies in infectious mononucleosis. *Clin Exp Immunol* 1973;**13**:359–66.
499. Swaminathan S. Molecular biology of Epstein-Barr virus and Kaposi's sarcoma–associated herpesvirus. *Semin Hematol* 2003;**40**: 107–15.
500. Swinnen LJ, Mullen GM, Carr TJ, et al. Aggressive treatment for postcardiac transplant lymphoproliferation. *Blood* 1995;**86**: 3333–40.
501. Szekely L, Selivanova G, Magnusson KP, et al. EBNA-5, an Epstein-Barr virus–encoded nuclear antigen, binds to the retinoblastoma and p53 proteins. *Proc Natl Acad Sci U S A* 1993;**90**:5455–9.
502. Taiwo BO. AIDS-related primary CNS lymphoma: a brief review. *AIDS Read* 2000;**10**:486–91.
503. Takada K, Nanbo A. The role of EBERs in oncogenesis. *Semin Cancer Biol* 2001;**11**:461–7.
504. Takahashi T, Hamasaka N, Harada S, et al. Methylprednisolone pulse therapy for severe immune thrombocytopenia associated with infectious mononucleosis. *Am J Hematol* 2007;**82**:416–17.
505. Tan LC, Gudgeon N, Annels NE, et al. A re-evaluation of the frequency of $CD8^+$ T cells specific for EBV in healthy virus carriers. *J Immunol* 1999;**162**:1827–35.

506. Tanaka K, Shimada M, Sasahara A, et al. Chronic hepatitis associated with Epstein-Barr virus infection in an infant. *J Pediatr Gastroenterol Nutr* 1986;**5**:467–71.
507. Tattevin P, Cremieux AC, Descamps D, et al. Transfusion-related infectious mononucleosis. *Scand J Infect Dis* 2002;**34**:777–8.
508. Tattevin P, Le Tulzo Y, Minjolle S, et al. Increasing incidence of severe Epstein-Barr virus–related infectious mononucleosis: surveillance study. *J Clin Microbiol* 2006;**44**:1873–4.
509. Taub R, Kirsch I, Morton C, et al. Translocation of the c-myc gene into the immunoglobulin heavy chain locus in human Burkitt lymphoma and murine plasmacytoma cells. *Proc Natl Acad Sci U S A* 1982;**79**:7837–41.
510. ten Napel CH, The TH. Lymphocyte reactivity in infectious mononucleosis. *J Infect Dis* 1980;**141**:716–23.
511. Thomas JA, Hotchin NA, Allday MJ, et al. Immunohistology of Epstein-Barr virus–associated antigens in B cell disorders from immunocompromised individuals. *Transplantation* 1990;**49**:944–53.
512. Thomas R, Macsween KF, McAulay K, et al. Evidence of shared Epstein-Barr viral isolates between sexual partners, and low level EBV in genital secretions. *J Med Virol* 2006;**78**:1204–9.
513. Thompson MP, Kurzrock R. Epstein-Barr virus and cancer. *Clin Cancer Res* 2004;**10**:803–21.
514. Thompson SK, Doerr TD, Hengerer AS. Infectious mononucleosis and corticosteroids: management practices and outcomes. *Arch Otolaryngol Head Neck Surg* 2005;**131**:900–4.
515. Tidy HL, Daniel EC. Glandular fever and infective mononucleosis, with an account of an epidemic. *Lancet* 1923;**2**:9–13.
516. Tierney RJ, Edwards RH, Sitki-Green D, et al. Multiple Epstein-Barr virus strains in patients with infectious mononucleosis: comparison of ex vivo samples with in vitro isolates by use of heteroduplex tracking assays. *J Infect Dis* 2006;**193**:287–97.
517. Tindle BH. Pathology of infectious mononucleosis. In: Schlossberg D, editor. *Infectious mononucleosis*. New York: Springer-Verlag; 1989. p. 126–41.
518. Tomkinson B, Robertson E, Kieff E. Epstein-Barr virus nuclear proteins EBNA-3A and EBNA-3C are essential for B-lymphocyte growth transformation. *J Virol* 1993;**67**:2014–25.
519. Torre D, Tambini R. Acyclovir for treatment of infectious mononucleosis: a meta-analysis. *Scand J Infect Dis* 1999;**31**:543–7.
520. Tsutsumi H, Kamazaki H, Nakata S, et al. Sequential development of acute meningoencephalitis and transverse myelitis caused by Epstein-Barr virus during infectious mononucleosis. *Pediatr Infect Dis J* 1994;**13**:665–7.
521. Tugizov S, Herrera R, Veluppillai P, et al. Epstein-Barr virus (EBV)–infected monocytes facilitate dissemination of EBV within the oral mucosal epithelium. *J Virol* 2007;**81**:5484–96.
522. Tynell E, Aurelius E, Brandell A, et al. Acyclovir and prednisolone treatment of acute infectious mononucleosis: a multicenter, double-blind, placebo-controlled study. *J Infect Dis* 1996;**174**:324–31.
523. van der Horst C, Joncas J, Ahronheim G, et al. Lack of effect of peroral acyclovir for the treatment of acute infectious mononucleosis. *J Infect Dis* 1991;**164**:788–92.
524. van Doornik MC, van't Veer-Korthof ET, Wierenga H. Fatal aplastic anaemia complicating infectious mononucleosis. *Scand J Haematol* 1978;**20**:52–6.
525. Veal CF Jr, Carr MB, Briggs DD Jr. Diffuse pneumonia and acute respiratory failure due to infectious mononucleosis in a middle-aged adult. *Am Rev Respir Dis* 1990;**141**:502–4.
526. Veltri RW, Sprinkle PM, McClung JE. Epstein-Barr virus associated with episodes of recurrent tonsillitis. *Arch Otolaryngol* 1975;**101**:552–6.
527. Verma N, Arunabh S, Brady TM, et al. Acute interstitial nephritis secondary to infectious mononucleosis. *Clin Nephrol* 2002;**58**:151–4.
528. Vezena H, Balfour H, Weller D, et al. Valacyclovir pharmacokinetics and exploratory pharmacodynamics in young adults with EBV infectious mononucleosis. *J Clin Pharmacol* 2010;**50**:734–42.
529. Virelizier JL, Lenoir G, Griscelli C. Persistent Epstein-Barr virus infection in a child with hypergammaglobulinaemia and immunoblastic proliferation associated with a selective defect in immune interferon secretion. *Lancet* 1978;**2**:231–4.
530. Wagner HJ, Cheng YC, Huls MH, et al. Prompt versus preemptive intervention for EBV lymphoproliferative disease. *Blood* 2004;**103**:3979–81.
531. Walenta K, Kindermann I, Gartner B, et al. Dangerous kisses: Epstein-Barr virus myocarditis mimicking myocardial infarction. *Am J Med* 2006;**119**:e3–6.
532. Walling DM, Brown AL, Etienne W, et al. Multiple Epstein-Barr virus infections in healthy individuals. *J Virol* 2003;**77**:6546–50.
533. Wan K, Yu I, Wu W. Primary Epstein-Barr virus infection in 2-year old children: report of 3 cases. *Turk Pediatr* 2010;**52**:655–8.
534. Wang X, Kenyon WJ, Li Q, et al. Epstein-Barr virus uses different complexes of glycoproteins gH and gL to infect B lymphocytes and epithelial cells. *J Virol* 1998;**72**:5552–8.
535. Waninger KN, Harcke HT. Determination of safe return to play for athletes recovering from infectious mononucleosis: a review of the literature. *Clin J Sport Med* 2005;**15**:410–16.
536. Weber R, Hegenbarth V, Kaftan H, et al. Nasopharyngeal endoscopy adds to reliability of clinical diagnosis of infectious mononucleosis. *J Laryngol Otol* 2001;**115**:792–5.
537. Weigle KA, Sumaya CV, Montiel MM. Changes in T-lymphocyte subsets during childhood Epstein-Barr virus infectious mononucleosis. *J Clin Immunol* 1983;**3**:151–5.
538. Weiss LM, Strickler JG, Warnke RA, et al. Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol* 1987;**129**:86–91.
539. Weiss LM, Movahed LA, Warnke RA, et al. Detection of Epstein-Barr viral genomes in Reed-Sternberg cells of Hodgkin's disease. *N Engl J Med* 1989;**320**:502–6.
540. Weiss LM, Jaffe ES, Liu XF, et al. Detection and localization of Epstein-Barr viral genomes in angioimmunoblastic lymphadenopathy and angioimmunoblastic lymphadenopathy–like lymphoma. *Blood* 1992;**79**:1789–95.
541. Westmore GA. Cervical abscess: a life-threatening complication of infectious mononucleosis. *J Laryngol Otol* 1990;**104**:358–9.
542. Wheatley GH III, McKinnon KP, Iacobucci M, et al. Dendritic cells improve the generation of Epstein-Barr virus–specific cytotoxic T lymphocytes for the treatment of posttransplantation lymphoma. *Surgery* 1998;**124**:171–6.
543. White PD, Thomas JM, Kangro HO, et al. Predictions and associations of fatigue syndromes and mood disorders that occur after infectious mononucleosis. *Lancet* 2001;**358**:1946–54.
544. Whitley RJ, Cobbs CG, Alford CA Jr, et al. Diseases that mimic herpes simplex encephalitis. Diagnosis, presentation, and outcome. NIAID Collaborative Antiviral Study Group. *JAMA* 1989;**262**:234–9.
545. Williams H, McAulay K, Macsween KF, et al. The immune response to primary EBV infection: a role for natural killer cells. *Br J Haematol* 2005;**129**:266–74.
546. Williams H, Crawford DH. Epstein-Barr virus: the impact of scientific advances on clinical practice. *Blood* 2006;**107**:862–9.
547. Williams LL, Lowery HW, Glaser R. Sudden hearing loss following infectious mononucleosis: possible effect of altered immunoregulation. *Pediatrics* 1985;**75**:1020–7.
548. Woda BA, Sullivan JL. Reactive histiocytic disorders. *Am J Clin Pathol* 1993;**99**:459–63.
549. Wohl DL, Isaacson JE. Airway obstruction in children with infectious mononucleosis. *Ear Nose Throat J* 1995;**74**:630–8.
550. Wolf DM, Friedrichs I, Toma AG. Lymphocyte–white blood cell count ratio: a quickly available screening tool to differentiate acute purulent tonsillitis from glandular fever. *Arch Otolaryngol Head Neck Surg* 2007;**133**:61–4.
551. Wolf H, Haus M, Wilmes E. Persistence of Epstein-Barr virus in the parotid gland. *J Virol* 1984;**51**:795–8.
552. Wollheim FA, Williams RC Jr. Studies on the macroglobulins of human serum. I. Polyclonal immunoglobulin class M (IgM) increase in infectious mononucleosis. *N Engl J Med* 1966;**274**:61–7.
553. Wood TA, Frenkel EP. The atypical lymphocyte. *Am J Med* 1967;**42**:923–36.
554. Worth A, Conyers R, Cohen J, et al. Pre-emptive rituximab based on virameia and T cell reconstitution: a highly effective strategy for the prevention of Epstein-Barr–associated lymphoproliferative disease following stem cell transplantation. *Br J Haematol* 2011;**155**:377–85.
555. Wright DH. Enteropathy associated T cell lymphoma. *Cancer Surv* 1997;**30**:249–61.
556. Yahr MD, Frontera AT. Acute autonomic neuropathy. Its occurrence in infectious mononucleosis. *Arch Neurol* 1975;**32**:132–3.

557. Yao QY, Rickinson AB, Epstein MA. A re-examination of the Epstein-Barr virus carrier state in healthy seropositive individuals. *Int J Cancer* 1985;**35**:35–42.
558. Yefenof E, Bakacs T, Einhorn L, et al. Epstein-Barr virus (EBV) receptors, complement receptors, and EBV infectibility of different lymphocyte fractions of human peripheral blood. I. Complement receptor distribution and complement binding by separated lymphocyte subpopulations. *Cell Immunol* 1978;**35**:34–42.
559. Yin CC, Jones D. Molecular approaches towards characterization, monitoring and targeting of viral-associated hematological malignancies. *Exp Rev Mol Diagn* 2006;**6**:831–41.
560. Yokoi S, Iizasa T, Hiroshima K, et al. Pulmonary artery leiomyosarcoma expressing Epstein-Barr virus in an immunocompetent individual. *Ann Thorac Surg* 2006;**81**:1897–9.
561. Yone CL, Kube D, Kremsner PG, et al. Persistent Epstein-Barr viral reactivation in young African children with a history of severe *Plasmodium falciparum* malaria. *Trans R Soc Trop Med Hyg* 2006;**100**:669–76.
562. Young L, Alfieri C, Hennessy K, et al. Expression of Epstein-Barr virus transformation-associated genes in tissues of patients with EBV lymphoproliferative disease. *N Engl J Med* 1989;**321**:1080–5.
563. Young LS, Clark D, Sixbey JW, et al. Epstein-Barr virus receptors on human pharyngeal epithelia. *Lancet* 1986;**1**:240–2.
564. Young LS, Dawson CW, Eliopoulos AG. Epstein-Barr virus and apoptosis: viral mimicry of cellular pathways. *Biochem Soc Trans* 1999;**27**:807–12.
565. Young LS, Rickinson AB. Epstein-Barr virus: 40 years on. *Nat Rev Cancer* 2004;**4**:757–68.
566. Zafeiriou DI, Pavlou E. Images in clinical medicine. Hypoglossal-nerve palsy. *N Engl J Med* 2004;**350**:29.
567. Zangwill SD, Hsu DT, Kichuk MR, et al. Incidence and outcome of primary Epstein-Barr virus infection and lymphoproliferative disease in pediatric heart transplant recipients. *J Heart Lung Transplant* 1998;**17**:1161–6.
568. Zhang Y, Molot R. Severe gastritis secondary to Epstein-Barr viral infection. Unusual presentation of infectious mononucleosis and associated diffuse lymphoid hyperplasia in gastric mucosa. *Arch Pathol Lab Med* 2003;**127**:478–80.
569. Ziegler JL. Chemotherapy of Burkitt's lymphoma. *Cancer* 1972;**30**:1534–40.
570. Ziegler JL. Burkitt's lymphoma. *N Engl J Med* 1981;**305**:735–45.
571. Ziegler JL, Beckstead JA, Volberding PA, et al. Non-Hodgkin's lymphoma in 90 homosexual men. Relation to generalized lymphadenopathy and the acquired immunodeficiency syndrome. *N Engl J Med* 1984;**311**:565–70.
572. Zou P, Kawada J, Pesnicak L, et al. Bortezomib induces apoptosis of Epstein-Barr virus (EBV)–transformed B cells and prolongs survival of mice inoculated with EBV-transformed B cells. *J Virol* 2007;**81**:10029–36.
573. zur Hausen H, Schulte-Holthausen H, Klein G, et al. EBV DNA in biopsies of Burkitt tumours and anaplastic carcinomas of the nasopharynx. *Nature* 1970;**228**:1056–8.

CHAPTER 160

Human Herpesviruses 6, 7, and 8

Charles Grose

As of the year 2012, there are nine human herpesviruses. Before 1986, five human herpesviruses were known. They were herpes simplex virus (HSV) type 1 (oral) and type 2 (genital), cytomegalovirus (CMV), varicella-zoster virus (VZV), and Epstein-Barr virus (EBV). The herpesviruses are important pathogens causing a variety of childhood diseases that are described in other chapters of this textbook. An inherent characteristic of herpesviruses is their ability to form a latent infection, in which the viral genome continues to reside within the host. When the virus reactivates, it often causes further symptoms and signs. Thus, herpesviruses cause a spectrum of illnesses during the lifetime of the infected human host.

During the last decades of the 20th century, four novel human herpesviruses (HHVs) were discovered. Two were originally designated HHV-6 and HHV-7, whereas the newest member of the family has been called either Kaposi sarcoma–associated herpesvirus or HHV-8. In 2012, the decision was made to separate HHV-6 into two distinct species called HHV-6A and HHV-6B. These viruses and the diseases they cause are described in this chapter. The HHV-6 strains are unique among human herpesviruses in that their genomes can occasionally integrate into the human chromosome. Because many studies of HHV-6 cited here were carried out before the formal separation into two species, the term HHV-6 often will not distinguish HHV-6A from HHV-6B species.

HUMAN HERPESVIRUSES 6A, 6B, AND 7

One unexpected consequence of the epidemic of acquired immunodeficiency syndrome (AIDS) was the discovery of a new human DNA virus. The virus was isolated first from the white blood cells of six patients with lymphoproliferative disorders, two of whom had AIDS.[27] Further electron microscopic characterization of the virus demonstrated properties of a herpesvirus, including (1) an icosahedral capsid composed of 162 capsomers covered by a lipid membrane and (2) an enveloped particle with a diameter of 200 nm (Fig. 160-1). Because the virus was isolated originally from B lymphocytes, the agent was designated human B-lymphotropic virus. However, this apparent tropism for B lymphocytes was not confirmed by other investigators, who found that the virus preferentially infected $CD4^+$ T lymphocytes and not B lymphocytes.[20,30] Thus, the initial designation of human B-lymphotropic virus was changed to HHV-6.

The DNA sequence and the deduced amino acid sequence of the HHV-6 genome have been published.[6] Calculation of the percentage of amino acid identity shared by HHV-6 proteins with those in other herpesviruses revealed that HHV-6 proteins most closely resembled those of human CMV. The strains of HHV-6 have been divided into group A and group B. The HHV-6 isolates related to prototype strain U1102 have been called group A, whereas those related to strain Z29 have been designated group B. The genomes of the group A and B viruses exhibit a 90 percent nucleotide sequence identity. In 1990, while searching for additional strains of HHV-6, Frenkel and coworkers[14] isolated a new T-lymphotropic herpesvirus that was designated HHV-7. HHV-7 is related closely at a genetic level to HHV-6 and to a lesser degree to CMV.[6] In 2012, the International Committee on Taxonomy of Viruses decided to designate HHV-6A and HHV-6B as separate species.

Most persons contract their primary HHV-6 infection before reaching the age of 5 years.[3] In the United States, primary HHV-6 infection occurred in 40 percent of children by the time they reached 12 months of age and in 77 percent by the age of 24 months.[41] In these young children, HHV-6B strains appear to be a major cause of the disease roseola (exanthem subitum). Roseola is discussed at greater length in Chapter 59. Children also may contract a primary HHV-6 infection without manifesting a rash. After acute infection, HHV-6 forms a latent infection, probably in T lymphocytes. In a small number of infants, acute HHV-7 infection appears to cause a roseola illness much like that of acute HHV-6 infection.[13a]

Diseases Caused by HHV-6 and HHV-7

Studies in Japan first delineated the nature of the illness associated with acute HHV-6 infection in young children.[37] HHV-6 was cultured from the peripheral blood leukocytes of four infants with roseola (exanthem subitum); each subject was 6 months old and had an acute febrile illness followed by a concurrent fall in temperature and onset of a rash. The blood samples were collected during the febrile stage of the disease. In a subsequent paper by the same group, two infants (6 and 7 months of age) were described who developed HHV-6 infection *without* a rash.[29] Both infants had been seen by physicians because of a 2- to 3-day history of high temperature (38.5°C to 39.5°C [101.3°F to 103.1°F]). The only abnormal clinical finding was congestion of the throat. In both cases, the temperature rapidly returned to normal around day 3 of illness, but no rash was ever observed. Cultures of peripheral blood cells from both infants were positive for HHV-6.[29] Further seroprevalence studies performed in Japan showed that most Japanese children (86%) contracted HHV-6 infection by

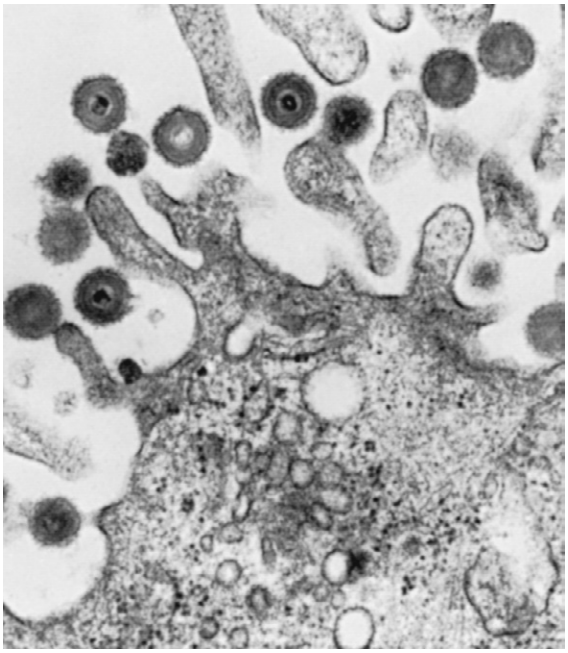

FIGURE 160-1 ■ Electron micrograph of cultured mononuclear cells infected with HHV-6. Several enveloped viral particles are visible in the extracellular area. (Courtesy Dr. Y. Asano.)

TABLE 160-1 **Selected Features in Roseola Associated with Infection in Children**

Clinical Findings	%
Temperature above 37.5°C (99.5°F)	98
Rash	98
Diarrhea	68
Nagayama spots	65
Cervical adenopathy	31
Edematous eyelids	30
Bulging fontanelle	26
Prodromal symptoms	14
Convulsions	8
Cough	0

Data modified from Asano Y, Grose C. Human herpesvirus type 6 infections. In: Glaser R, Jones J, editors. *Herpesvirus infections*. New York: Marcel Dekker; 1994, p. 227–4.

the time they were 24 months of age.[40] Thereafter, the increase in seropositivity among the childhood populations was small. These statistics suggest that HHV-6 infection goes unrecognized in most children.

Primary HHV-6 infection has been implicated in a few cases of severe hepatitis. The first case was that of a 21-year-old patient with cystic fibrosis who received a liver transplant.[36] The recipient lacked antibodies to HHV-6, and the organ donor was seropositive. Two weeks after undergoing transplantation, the recipient developed fever and grand mal seizures; her hepatic function deteriorated. HHV-6 was cultured from her peripheral blood cells. The apparent source of infection was the donor liver, from which HHV-6 presumably was reactivated after transplantation. She gradually recovered her hepatic function during the course of several weeks.

A fatal case of fulminant HHV-6 hepatitis has been reported in a 3-month-old boy.[4] The infant was admitted to a hospital because of fever, jaundice, convulsions, and loss of consciousness. His serum bilirubin concentration, liver transaminases, and blood ammonia level were elevated markedly. Within 7 days, he became comatose and died. As part of the diagnostic evaluation, HHV-6 was cultured from his mononuclear cells. Furthermore, HHV-6 DNA was detected in biopsy samples of liver and brain, which were obtained immediately after death. A serum sample drawn before death showed reactivity to HHV-6 antigens.

In a large retrospective review of roseola in association with acute HHV-6 infection, the signs and symptoms were tabulated.[3] The study population included 94 boys and 82 girls who ranged in age from 3 weeks to 18 months (Table 160-1). As would be predicted, the two most common clinical findings were fever and rash. The temperature often rose to 39°C (102.2°F), and fever persisted for 2 to 4 days. The rash usually appeared when the fever lessened; the rash was papular in 54 percent, macular in 40 percent, and maculopapular in the remainder of the children. The exanthem typically persisted for 3 to 4 days and was not followed by desquamation. Diarrhea was a surprisingly frequent occurrence, but it was not severe. An enanthem called Nagayama spots in Japan consisted of papules on the mucosa of the soft palate and uvula. Of the total study group, 8 percent developed febrile seizures. More severe central nervous system (CNS) complications have been documented in children not enrolled in this study. A few children with HHV-6 encephalitis have had abnormal electroencephalographic recordings. Some children with encephalitis have had permanent neurologic sequelae. In the United States in particular, acute HHV-6 infection also appears to be associated with concurrent acute otitis media.[24]

Reactivation of previous HHV-6 infection has been demonstrated in some healthy children and adults who contracted a second herpes-type infection, such as primary EBV infection (infectious mononucleosis) or primary CMV infection.[3] A similar serologic survey was performed in 10 renal transplant recipients who initially were HHV-6 seropositive.[3] After transplantation, all 10 showed greater than fourfold rises in antibody to HHV-6. Only 2 of the 10 developed a febrile illness, and both of them also had primary CMV infection. Among the human herpesviruses, HHV-6 infections are unique in that HHV-6 DNA can be integrated into a human chromosome. In a recent survey of 21 cases of integrated HHV-6 infection after transplantation, HHV-6B DNA was found more often among solid-organ transplant recipients, whereas integrated HHV-6A was more common among allogeneic hematopoietic stem cell recipients. None of the 21 transplant patients developed clinical symptoms related to the integrated HHV-6 infections.[18] Furthermore, HHV-6 does not appear to be related causally to Kawasaki disease,[22] nor does HHV-6 infection appear to alter the course of human immunodeficiency virus (HIV) infection.[28]

Whether HHV-7 causes a distinct illness has been the subject of many medical investigations since 1990. In general, HHV-7 antibody is acquired by mid-childhood, but there appears to be no corresponding sentinel illness. However, in two infants with roseola, both isolation of HHV-7 and seroconversion to HHV-7 were documented; in addition, another five children with roseola were found to have undergone seroconversion to HHV-7.[32] HHV-7 also has been isolated from one infant with an acute febrile illness.[25] Therefore, in a small number of young children, acute HHV-7 infection may be associated with fever and sometimes a roseola-like rash.

Finally, congenital infections also can occur in pregnant women already infected with HHV-6. In a survey of 5638 cord blood samples, HHV-6 DNA was detected in 57 samples (1%), but the congenital infections did not cause an identifiable disease in the newborn.[16] Of great interest, congenital HHV-6 infections appear to be caused mainly by chromosomally integrated HHV-6 DNA. Therefore, the HHV-6 genome will be present in all cell types, including tissues in the CNS of the newborn. Chromosomal integration during congenital infection appears to occur more commonly with HHV-6A than with HHV-6B species.

Neurologic Complications

In the preceding paragraphs, CNS symptoms were mentioned briefly. In a large series of clinical studies performed in Japan, seizures were a common feature of acute HHV-6 infection.[3,38] In one series of 105 young children with acute febrile convulsions, 21 had evidence of primary HHV-6 infection, as assayed by either isolation of virus from blood or seroconversion of HHV-6 antibodies. When the age of the patient was assessed, HHV-6 infection was found in 13 of 23 seizure patients younger than 1 year; thus, the seizure group with HHV-6 infection was significantly younger than the seizure group without HHV-6 infection. In addition, the frequency of clustering seizures, long-lasting seizures, partial seizures, and postictal paralysis in children having their first febrile convulsion episode was significantly higher in those with primary HHV-6 infection than in those without HHV-6 infection. One child with fatal acute necrotizing encephalitis and concurrent HHV-6 infection also has been described.[21]

In studies from Japan, assays for HHV-6 and HHV-7 DNA were performed on cerebrospinal fluid samples from 43 children with CNS symptoms.[39] All children had symptoms compatible with aseptic meningitis. HHV-6 DNA was detected in the peripheral blood cells of 15 and in the cerebrospinal fluid of 7 children; all were HHV-6 variant B. HHV-7 DNA was detected in the peripheral blood cells of 28 and in the cerebrospinal fluid of 6 patients. Thus, the clinical study demonstrated that both HHV-6 and HHV-7 can invade the CNS or, alternatively, frequently reactivate within the CNS during childhood neurologic disease. The results from Japan have been confirmed in a similar large analysis of young children with seizures in Britain and Ireland.[35] Among 156 children hospitalized with febrile seizures, 26 (17%) were associated with acute HHV-6 or HHV-7 infection. The median age for hospitalization in the HHV-6 group was 53 weeks, and the median age for the HHV-7 group was 60 weeks.

HHV-6 infection is an important cause of posttransplantation acute limbic encephalitis (PALE) after hematopoietic stem cell transplantation. Among six children who underwent cord blood transplantation for hematologic malignant disease at one hospital in Australia, six developed PALE in association with HHV-6 infection.[13a] Three patients developed confusion, general impairment of memory, and sporadic seizures. The other three had more severe symptoms, including generalized epilepsy that presented during the third week after the transplantation. The diagnosis of HHV-6 infection was made by detection of viral DNA in blood and spinal fluid. The seizures were initially controlled and the HHV-6 infection was treated. However, after another 11 to 18 months, all three patients developed generalized seizures that were much more difficult to control. All three patients experienced a severe regression in their motor and intellectual functioning.

Pathogenesis of HHV-6 and HHV-7 Infection

The pathogenesis of HHV-6 infection certainly includes a viremia while the child is asymptomatic; the total duration of the incubation period is not well defined but may be approximately 10 days. The prodrome, which signals the end of the incubation period, includes 2 to 4 days of fever, which precedes the onset of the rash.[3,26] During this period, virtually 100 percent of peripheral blood cell cultures are positive for HHV-6. By day 3 or 4, when the rash first appears, the viremia is abating. Between 5 and 7 days after the onset of the fever (or 2 to 4 days after appearance of exanthem), viremia persists in less than 20 percent of the children. Viremia is absent later in convalescence, when the antibody response is detectable. HHV-6 occasionally reactivates from latency throughout the lifetime of an infected human, but usually no apparent illness is present.[7]

The mothers of infants with HHV-6 infection have been studied to determine whether they are the source of the infectious agent.[16] Because most mothers are seropositive, the possibility exists that HHV-6 infection could reactivate in the mother, who would then transmit the infection to her child, possibly through exchange of saliva. Obviously, mothers with an integrated HHV-6 DNA infection can pass the infection to their fetus during gestation.[16] However, for mothers without an integrated infection but with HHV-6 seropositivity, HHV-6 DNA was detected in the vaginal secretions, so perinatal HHV-6 transmission remains a possibility. Older siblings are another possible source of infection within a household.[41]

In contrast with HHV-6, HHV-7 seropositivity occurs in children but often after the first 2 years of life. HHV-7 transmission has been investigated in large multigenerational Japanese families living in the same household.[31] The results indicated that HHV-7 is transmitted gradually from older generation to younger generation (Fig. 160-2). Transmission often occurs in children before the child enters a traditional primary school at the age of 6 years. Either parent or presumably an older sibling can

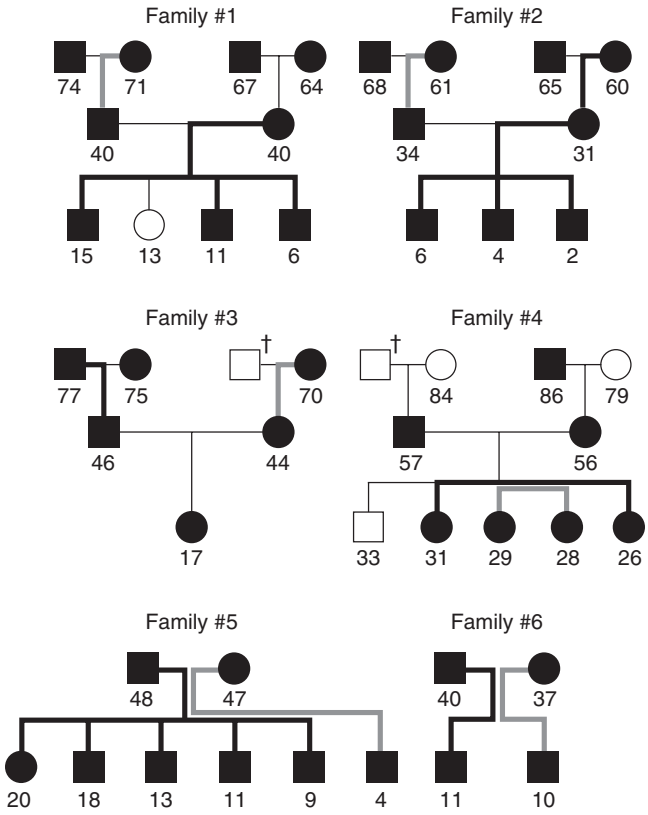

FIGURE 160-2 ■ Pedigrees of six families with HHV-7 infection. All families resided in Okayama, Japan. Males and females are indicated as boxes and circles, respectively. Persons from whom HHV-7 was isolated are shaded. A person who was already deceased is marked †. Members who have similar HHV-7 DNA restriction patterns are connected with bold lines. Numbers under the boxes and circles indicate ages. (From Takahashi Y, Yamada M, Nakamura J, et al. Transmission of human herpesvirus 7 through multigenerational families in the same household. *Pediatr Infect Dis J* 1997;**16**:975–8.)

transmit the virus. A reasonable explanation for this pattern of transmission is repeated exchange of infectious saliva among family members living in the same household during the course of many years.

Diagnosis

Infection with HHV-6 can be diagnosed by several means, including (1) measurement of antibody, (2) isolation of virus, (3) detection of viral antigen, and (4) detection of viral DNA. The first method (i.e., the traditional approach for diagnosis of virus infection) usually requires acute and convalescent serum samples for determination of a fourfold or greater rise in titer of virus-specific IgG antibody. The finding of IgM specific for HHV-6 in a single serum sample also would indicate an acute infection in a young child. The titer of IgG antibody rises during reactivation of the virus in adults, but whether IgM antibody to HHV-6 appears regularly at the same time is not known. HHV-6 antibodies usually have been measured by an indirect immunofluorescence method, although neutralization tests also have been performed.[3,4,34]

Isolation of HHV-6 was the original technique for identification of this herpesvirus.[27] The method is more difficult to perform than are those methods commonly used for most herpesviruses (e.g., HSV, CMV, VZV) but is similar to that required for isolation of another herpesvirus, EBV. HHV-6 usually is isolated in a cell substrate consisting of mononuclear cells obtained from cord blood of a newborn infant. The source of virus in the patient also is the peripheral blood mononuclear cell population.[34]

Viral genome can be detected in infected tissues by DNA hybridization techniques.[34] Viral DNA also can be detected in tissue samples by polymerase chain reaction (PCR). In one fatal case of HHV-6 infection,[4] DNA was extracted from postmortem liver and brain samples and amplified by the PCR test. On direct gel electrophoresis, HHV-6–specific DNA was detected in both the liver and the brain specimens. These studies indicate that HHV-6 infection can be diagnosed by both traditional and newer molecular techniques. Likewise, HHV-7 infection usually is diagnosed by isolation of virus or detection of viral DNA by PCR testing in samples from patients.

Treatment of HHV-6 Infection

In most instances of HHV-6 infection in healthy children, treatment with an antiviral medication is not indicated. Recovery is complete within a few days after onset of the rash, and sequelae rarely occur. However, HHV-6 disease in immunocompromised persons may be more persistent or severe (e.g., encephalitis). Several groups have analyzed the in vitro sensitivity of HHV-6 to four antiviral drugs: acyclovir, ganciclovir, phosphonoformic acid, and cidofovir.[1,3] All four compounds have been used in treatment of other herpesvirus infections. Multiplication of HHV-6 in cell culture is inhibited readily by ganciclovir at a concentration of 2 to 10 μg/mL, easily achievable levels in humans. This effect is of interest because ganciclovir inhibits human CMV to a similar degree; as mentioned earlier, CMV and HHV-6 are related closely at a genomic level. Phosphonoformic acid (foscarnet) at a concentration of 6 μg/mL also is effective against HHV-6 infection. On the other hand, HHV-6 is affected by acyclovir only at concentrations of 50 μg/mL, considerably higher levels than those required for treatment of HSV-1, HSV-2, and VZV infections and probably toxic in humans. Likewise, cidofovir is more toxic than is ganciclovir and would not be recommended for HHV-6 treatment. At present, none of these antiviral drugs has been approved specifically for treatment of HHV-6 infections. One caveat for antiviral therapy for HHV-6 infection is the consideration whether the HHV-6 infection is related to chromosomally integrated HHV-6 DNA; these patients often have high copy numbers of HHV-6 DNA in the blood. Under these circumstances, however, antiviral therapy may exert no demonstrable effect.[18]

KAPOSI SARCOMA–ASSOCIATED HERPESVIRUS (HUMAN HERPESVIRUS 8)

Yet another consequence of the HIV epidemic in the 1980s was the appearance of Kaposi sarcoma in many people with AIDS. The increase in Kaposi sarcoma was

especially puzzling because the tumor occurred more frequently in people who acquired HIV by sexual transmission rather than by infusion of infected blood products, such as factor VIII in hemophiliac patients. The question often arose whether a second infectious agent was involved in the etiology of Kaposi sarcoma. The answer to that question was provided by a report published in late 1994. The authors announced the identification of a previously unknown herpesvirus-like DNA sequence collected from patients with AIDS in New York.[9] The viral DNA was called Kaposi sarcoma–associated herpesvirus (KSHV). Other virologists prefer that the agent be called human herpesvirus 8 (HHV-8).

Diseases Caused by Kaposi Sarcoma–Associated Herpesvirus

The authors of the original KSHV report were investigating patients with Kaposi sarcoma. They discovered the herpesvirus-like DNA sequences by using a combination of genetic techniques, including amplification of DNA by PCR and subsequent representational difference analysis.[9] Thereby, they were able to identify and to characterize unique DNA sequences in Kaposi sarcoma that were absent from nonmalignant tissue from the same patient. One such sequence showed close homology to regions of the genome of *Herpesvirus saimiri*, a simian herpesvirus, and to a lesser degree to EBV, the agent that causes infectious mononucleosis. A second DNA fragment was homologous to another region in the *H. saimiri* genome. In their study, the authors located one or both of these herpesvirus-like DNA sequences in 20 of 27 different samples of Kaposi sarcoma tissue. The investigators concluded that they had discovered DNA of a previously unknown herpesvirus within Kaposi sarcoma tissues.

Their results were confirmed quickly by other groups. In one study from California, investigators detected identical viral sequences in 13 of 13 biopsy specimens of Kaposi sarcoma from patients with AIDS.[2] This study also found the same sequence in the peripheral blood cells collected from 10 of the 13 patients but not in the blood samples of 20 patients with no history of Kaposi sarcoma. A third study from France found the herpesvirus-like DNA in biopsy specimens from 5 patients with Mediterranean-type Kaposi sarcoma; all 5 patients were HIV seronegative.[11] In subsequent studies, HHV-8 DNA has been detected in most Kaposi sarcomas (classic) in HIV-seronegative individuals, often men residing in the Mediterranean area.

The authors of the original KSHV report subsequently found the herpesvirus-like DNA sequences in body cavity–based lymphomas from patients with AIDS.[8] HHV-8 DNA also has been found in the B-cell lymphoproliferative disorder known as multicentric Castleman disease.[5] Of equal importance, KSHV sequences were not found in several other lymphomas or leukemias (e.g., small-lymphocyte lymphoma, monocytoid B-cell lymphoma, follicular lymphoma, diffuse large-cell lymphoma, Burkitt lymphoma, lymphoblastic lymphoma, anaplastic large-cell lymphoma, multiple myeloma, hairy cell leukemia, acute lymphoblastic leukemia, or cutaneous T-cell lymphoma). Recently, multicentric Castleman disease has been diagnosed in an HHV-8–infected child, who may have had errors in innate immunity secondary to consanguineous parents.[19]

Transmission of HHV-8 Among Children

One of the most perplexing aspects of HHV-8 epidemiology is the mode of transmission. That the seroprevalence is high among a population of adult men who have sex with men is well known.[23] Yet, the question remains whether larger segments of the population are infected on a worldwide basis and, in particular, whether children are infected. To answer this question, several HHV-8 seroepidemiology studies were surveyed. Two studies provide partial answers to the question of childhood infection.

The first study was performed in a village in French Guiana, South America, among 1337 individuals of African origin.[24] They ranged in age from 2 to 91 years. The serologic data indicated that HHV-8 seropositivity is strongly age dependent: at the ages of 2 to 4 years, 3 of 146 were positive; at the ages of 5 to 9 years, 14 of 278 were positive; at the ages of 10 to 14 years, 31 of 232 were positive; at the ages of 15 to 19 years, 24 of 149 were positive; at the ages of 20 to 29 years, 38 of 236 were positive; at the ages of 30 to 39 years, 16 of 120 were positive; at the ages of 40 to 49 years, 19 of 70 were positive; and older than 50 years, 32 of 106 were positive. These seroprevalence data clearly show a gradual acquisition of infection during early childhood, with a stepwise increment from approximately 5 to 12 percent at the age of 10 years.

HIV infection did not play a role in the likelihood of transmission because HIV infection is not a common occurrence in this village. Instead, extensive analyses of intrafamilial relationships demonstrated a highly significant familial correlation in HHV-8 seropositivity between mother and child (especially when children were younger than 10 years) and between siblings. The correlation was highest when the siblings had an age difference of less than 5 years. Of interest, a similarity in transmission appears to exist between HHV-8 and HHV-7, when HHV-7 was studied in multigenerational Japanese households (Fig. 160-2).

A second large HHV-8 seroepidemiology analysis was performed in Israel.[10] The incidence of classic Kaposi sarcoma in Israel is among the highest in the developed world. Because of this statistic, the investigators undertook a study to ascertain the HHV-8 seroprevalence in Israel and also to investigate HHV-8 intrafamilial transmission. The study population included 1648 healthy blood donors determined to be positive for hepatitis B antigen and 2403 family members.

The seroprevalence data showed that 9 percent of children aged 2 to 14 years and 9 percent of adolescents and young adults aged 15 to 24 years were HHV-8 seropositive. Seroprevalence in older adults ranged from 12 to 18 percent. HHV-8 positivity was more likely to occur in children when at least one of the parents was positive compared with children with neither parent positive. The most important predictor of a child's HHV-8 seropositivity status was maternal seropositivity.

These two large seroepidemiologic studies demonstrate that HHV-8 is spread by nonsexual means in children in many countries around the world. In these same countries, HHV-8 seropositivity rises throughout adolescence and adulthood, regardless of HIV infection status.[33] However, in North America and western Europe, the seropositivity rate among HIV-seronegative adults is considered to be low, approximately 3 to 5 percent. The reasons for these differences between continents are not resolved. Nonetheless, the most reasonable hypothesis for transmission is exchange of saliva within family groups. Studies have proved that the virus is present in saliva, in particular in epithelial cells contained within saliva.[12] An alternative mode of infection, described next, is transmission by blood transfusion.

Hemophagocytic Lymphohistiocytosis

Hemophagocytic lymphohistiocytosis (HLH) is a rare and sometimes fatal disorder of immune regulation, often associated with mutations in the perforin gene. Even carriers of the mutated gene may require an acute infection to trigger HLH. HHV-8 is one of the triggers. HLH in association with HHV-8 infection has occurred in adults with severe HIV infection or with a recent kidney transplant.[13,19]

HLH has been diagnosed in two identical siblings from Iowa during the first 6 months of life.[15] The siblings were born prematurely and spent several months in intensive care. Both siblings had mutations in the perforin gene. Both siblings had HHV-8 infection. Because the mother of the two was negative for HHV-8 infection, one possible route of transmission was a blood transfusion given while they were in the nursery. In fact, in a large study in East Africa, blood transfusions were shown to transmit HHV-8 infection.[18] Therefore, children who have had multiple blood transfusions may be at risk for acquiring HHV-8 infection.[11]

Diagnosis and Treatment of HHV-8 Infection

Diagnosis of prior HHV-8 infection can be made by serology. Diagnosis of acute HHV-8 infection requires a scraping of cells from the mouth for testing by PCR amplification of HHV-8–specific DNA.[9,11,12] Mononuclear blood cells also may be tested by HHV-8–specific PCR. These PCR protocols are becoming more widely available.

Treatment is not necessary in most healthy hosts because the virus quickly enters latency after the primary infection. No disease has been associated with a latent infection in children, except those with defects in the perforin gene. However, acute HHV-8 infection in recent transplant recipients or HIV-seropositive children could be an indication for treatment.[13,19] Some of the same antiviral drugs described for HHV-6 treatment also inhibit HHV-8 replication (e.g., ganciclovir), but currently there are too few clinical data on which to base a standard regimen, so treatment would need to be individualized for each infected patient.

NEW REFERENCES SINCE THE SIXTH EDITION

13a. Epstein LG, Shinnar S, Hesdorffer DC, et al. Human herpesvirus 6 and 7 in febrile status epilepticus. *Epilepsia* 2012;**53**:1481–8.

16. Hall CB, Caserta MT, Schnabel KC, et al. Transplacental human herpesvirus 6 congenital infection caused by maternal chromosomally integrated virus. *J Infect Dis* 2010;**201**:505–7.

18. Lee SO, Brown RA, Razonable RR. Chromosomally integrated human herpesvirus-6 in transplant recipients. *Transpl Infect Dis* 2012;**14**:359–65.

19. Leroy S, Moshous D, Cassar O, et al. Multicentric Castleman disease in an HHV-8 infected child born to consanguineous parents. *Pediatrics* 2012;**129**:199–203.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agut H, Collandre H, Aubin JT, et al. In vitro sensitivity of human herpesvirus-6 to antiviral drugs. *Res Virol* 1989;**140**:219–28.
2. Ambroziak JA, Blackbourn DJ, Herndier BG, et al. Herpes-like sequences in HIV-infected and uninfected Kaposi's sarcoma patients. *Science* 1995;**268**:582–3.
3. Asano Y, Grose C. Human herpesvirus type 6 infections. In: Glaser R, Jones J, editors. *Herpesvirus infections*. New York: Marcel Dekker; 1994. p. 227–44.
4. Asano Y, Yoshikawa T, Suga S, et al. Fatal fulminant hepatitis in an infant with herpesvirus-6 infection. *Lancet* 1990;**1**:862–3.
5. Ascoli V, Signoretti S, Onetti-Muda A, et al. Primary effusion lymphoma in HIV-infected patients with multicentric Castleman's disease. *J Pathol* 2001;**193**:200–9.
6. Berneman ZN, Dharam VA, Ge L, et al. Human herpesvirus 7 is a T-lymphotropic virus and is related to, but significantly different from, human herpesvirus 6 and human cytomegalovirus. *Proc Natl Acad Sci U S A* 1992;**89**:10552–6.
7. Caserta MT, McDermott MP, Dewhurst S, et al. Human herpesvirus 6 (HHV6) DNA persistence and reactivation in healthy children. *J Pediatr* 2004;**145**:478–84.
8. Cesarman E, Chang Y, Moore PS, et al. Kaposi's sarcoma–associated herpesvirus-like DNA sequences in AIDS-related body-cavity-based lymphomas. *N Engl J Med* 1995;**332**:1186–91.
9. Chang Y, Cesarman E, Pessin MS, et al. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 1994;**266**:1865–9.
10. Davidovici B, Karakis I, Bourboulia D, et al. Seroepidemiology and molecular epidemiology of Kaposi's sarcoma–associated herpesvirus among Jewish population groups in *Israel J Natl Cancer Inst* 2001;**93**:194–202.
11. Duus KM, Grose C. Human herpesvirus 8, blood transfusions, and hemophagocytic syndrome. *Pediatr Infect Dis J* 2007;**26**:278–9.
12. Duus KM, Lentchitsky V, Wagenaar T, et al. Wild-type Kaposi's sarcoma–associated herpesvirus isolated from the oropharynx of immune-competent individuals has tropism for cultured oral epithelial cells. *J Virol* 2004;**78**:4074–84.
13. Fardet L, Blum L, Kerob D, et al. Human herpesvirus 8–associated hemophagocytic lymphohistiocytosis in human immunodeficiency virus–infected patients. *Clin Infect Dis* 2003;**37**:285–2.

13a. Epstein LG, Shinnar S, Hesdorffer DC, et al. Human herpesvirus 6 and 7 in febrile status epilepticus. *Epilepsia* 2012;**53**:1481–8.

14. Frenkel N, Schirmer EC, Wyatt LS, et al. Isolation of a new herpesvirus from $CD4^+$ T cells. *Proc Natl Acad Sci U S A* 1990;**87**:748–52.
15. Grossman WJ, Radhi M, Schauer D, et al. Development of hemophagocytic lymphohistiocytosis in triplets infected with HHV-8. *Blood* 2005;**106**:1203–6.
16. Hall CB, Caserta MT, Schnabel KC, et al. Transplacental human herpesvirus 6 congenital infection caused by maternal chromosomally integrated virus. *J Infect Dis* 2010;**201**:505–7.
17. Reference deleted in proofs.
18. Lee SO, Brown RA, Razonable RR. Chromosomally integrated human herpesvirus-6 in transplant recipients. *Transpl Infect Dis* 2012;**14**:359–65.
19. Leroy S, Moshous D, Cassar O, et al. Multicentric Castleman disease in an HHV-8 infected child born to consanguineous parents. *Pediatrics* 2012;**129**:199–203.
20. Lusso P, Markham PD, Tschachler E, et al. In vitro cellular tropism of human B-lymphotropic virus (human herpesvirus-6). *J Exp Med* 1988;**167**:1659–70.
21. Ohsaka M, Houkin K, Takigami M, Koyanagi I. Acute necrotizing encephalopathy associated with human herpesvirus-6 infection. *Pediatr Neurol* 2006;**34**:160–3.
22. Okano M, Luka J, Thiele GM, et al. Human herpesvirus 6 infection and Kawasaki disease. *J Clin Microbiol* 1989;**27**:2379–80.
23. Pauk J, Huang ML, Brodie SJ, et al. Mucosal shedding of human herpesvirus 8 in men. *N Engl J Med* 2000;**343**:1369–77.
24. Plancoulaine S, Abel L, van Beveren M, et al. Human herpesvirus 8 transmission from mother to child between siblings in an endemic population. *Lancet* 2000;**356**:1062–5.
25. Portolani M, Cermelli C, Mirandola P, et al. Isolation of human herpesvirus 7 from an infant with febrile syndrome. *J Med Virol* 1995;**45**:282–3.
26. Pruksananonda P, Hall CB, Insel RA, et al. Primary human herpesvirus 6 infection in young children. *N Engl J Med* 1992;**326**:1445–50.
27. Salahuddin SZ, Ablashi DV, Markham PD, et al. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. *Science* 1986;**234**:596–601.
28. Spira TJ, Bozemann LH, Sanderlin KC, et al. Lack of correlation between human herpesvirus-6 infection and the course of human immunodeficiency virus infection. *J Infect Dis* 1990;**161**:567–70.
29. Suga S, Yoshikawa T, Asano Y, et al. Human herpesvirus-6 infection (exanthem subitum) without rash. *Pediatrics* 1989;**83**:1003–6.
30. Takahashi K, Sonoda S, Higashi K, et al. Predominant CD4 T-lymphocyte tropism of human herpesvirus 6–related virus. *J Virol* 1989;**63**:3161–3.
31. Takahashi Y, Yamada M, Nakamura J, et al. Transmission of human herpesvirus 7 through multigenerational families in the same household. *Pediatr Infect Dis J* 1997;**16**:975–8.
32. Tanaka K, Kondo T, Torigoe S, et al. Human herpesvirus 7: another causal agent for roseola (exanthem subitum). *J Pediatr* 1994;**125**:1–5.
33. Vitale F, Viviano E, Perna AM, et al. Serological and virological evidence of non-sexual transmission of human herpesvirus type 8 (HHV8). *Epidemiol Infect* 2000;**125**:671–5.
34. Ward KN. The natural history and laboratory diagnosis of human herpesviruses-6 and -7 infections in the immunocompetent. *J Clin Virol* 2005;**32**:183–93.
35. Ward KN, Andrews NJ, Verity CM, et al. Human herpesviruses-6 and -7 each cause significant neurological morbidity in Britain and *Ireland Arch Dis Child* 2005;**90**:619–23.
36. Ward KN, Gray JJ, Efstathious S. Brief report: primary human herpesvirus 6 infection in a patient following liver transplantation from a seropositive donor. *J Med Virol* 1989;**28**:69–72.
37. Yamanishi K, Okuno T, Shiraki K, et al. Identification of human herpesvirus-6 as a causal agent for exanthem subitum. *Lancet* 1988;**1**:1065–7.
38. Yoshikawa T, Asano Y. Central nervous system complications in human herpesvirus-6 infection. *Brain Dev* 2000;**22**:307–14.
39. Yoshikawa T, Ihira M, Suzuki K, et al. Invasion by human herpesvirus 6 and human herpesvirus 7 of the central nervous system in patients with neurological signs and symptoms. *Arch Dis Child* 2000;**83**:170–1.
40. Yoshikawa T, Suga S, Asano Y, et al. Distribution of antibodies to a causative agent of exanthem subitum (human herpesvirus-6) in healthy individuals. *Pediatrics* 1989;**84**:675–7.
41. Zerr DM, Meier AS, Selke SS, et al. A population-based study of primary human herpesvirus 6 infection. *N Engl J Med* 2005;**352**:768–76.

chemotherapy, organ transplantation, and irradiation. During primary infection, VZV establishes a latency in sensory nerve ganglia; zoster results when latent VZV reactivates in the ganglion. Usually, reactivation results in skin infection and produces a unilateral dermatomal eruption that may be painful or pruritic. On occasion, however, symptoms other than rash are the result of reactivation of VZV. Reactivation may also occur in the absence of symptoms.

The origin of the name "chickenpox" is uncertain, but it may have come from the French *pois chiche* (chickpea) or from the domestic fowl (in Old English, *cicen*, and in Middle High German, *kuchen*).[46] *Herpes* is the Greek word meaning "to creep," and *zoster* comes from the Greek and Latin word meaning "girdle" or "belt."

THE ORGANISM

VZV is an alpha herpesvirus. It has a DNA core surrounded by a nucleocapsid composed of 162 hexagonal capsomers that form an icosahedron with a diameter of approximately 100 nm. A tegument surrounds this structure, which in turn is surrounded by a lipid-containing envelope. Enveloped virions have a diameter of approximately 200 nm.[55] The genome of VZV has been sequenced; it contains 71 open-reading frames.[57] The linear, double-stranded DNA consists of a long unique segment, U_L, and a short unique sequence, U_S, flanked by internal and terminal repeats.[58,61,202] The genome of the Oka vaccine strain also has been sequenced; the molecular basis for attenuation of the strain is still under investigation.[4,93,139,224]

At least six genes of VZV, 4, 21, 29, 62, 63, and 66 are expressed in human sensory ganglia during latent VZV infection. VZV is synthesized by an orderly cascade of gene expression consisting of immediate-early (IE), early (E), and, finally, late (L) lytic genes that encode structural proteins. In *latent* infection there is a block in the cascade that permits synthesis of only roughly one tenth of the genes that are synthesized in productive or *lytic* infection. The genes expressed during latent infection are IE and E genes; L genes are not expressed. One hypothesis is that cellular immunity to VZV normally can control early replication of the virus so that productive or lytic infection does not occur.*

During productive or lytic infection, VZV synthesizes more than 30 polypeptides; the function of most of these polypeptides remains unknown, but some are structural and others are nonstructural and presumably have regulatory action. VZV synthesizes at least eight glycoproteins (g): gB, gC, gE, gH, gI, gK, gL and gM.[49,56,98,124] These glycoproteins correspond somewhat to those of herpes simplex virus (HSV), but, unlike HSV, VZV has no equivalent of gD, which is the major glycoprotein of HSV. The major glycoprotein of VZV is gE. The VZV glycoproteins not only provide structures for the virion but also play roles in infectivity, such as mediating adhesion and promoting entry of VZV from an infected cell into another uninfected cell. The glycoproteins also are antigenic, as is the tegument, and immune responses are directed toward these structures.

Just one antigenic type of VZV has been identified. Three major classifications of wild-type VZV were originally described, referred to as European (E), Japanese (J, for strains homologous to the Oka parental vaccine strain), and Mosaic (M, for strains displaying a combination of European and Japanese genotype-like mutations).[17,143,187,212] Worldwide, seven VZV clades have now been identified, which have differing geographic origins, that have been influenced by worldwide immigration patterns. All seven clades have been observed in the United States, although clades 1 and 3 (both E) predominate.[24]

Wild-type VZV DNA can be distinguished from that of vaccine-type (Oka) virus by restriction enzyme analysis of DNA from cultured virus and/or by polymerase chain reaction (PCR) assay and by direct sequencing, particularly of segments in open-reading frame 62, where the major mutations in Oka are present.*

VZV replicates in the nuclei of infected cells, where the DNA core and capsid are synthesized. The capsid is enveloped by a complex process in the Golgi apparatus, after which it traverses the cell in cytoplasmic vesicles.[82] In vitro, and in patients with VZV infections, the virus grows rather slowly and is highly cell associated. Its synthetic pathway entraps it in endosomic vesicles, where it is inactivated before it can be released from cells in infectious form. In vivo, however, infectious VZV is released from cells of the superficial epidermis and is highly contagious; VZV avoids the endosomal route in this particular tissue because of the loss of its receptor (the large calcium-independent mannose-6-phosphate receptor) and thereby exits cells by the secretory pathway.[43]

VZV has an extremely limited host range and infects mostly primates. Various strains of guinea pigs, including hairless ones, may be infected with VZV, but either no symptoms are produced or the illness produced is extremely mild, but specific immune responses can be demonstrated.[159] Animal models of latent VZV infection have been described in rodents, including guinea pigs.[41,155,179,223] An in vitro model of latency and reactivation of VZV in intestinal neurons from guinea pigs also has been developed.[42]

TRANSMISSION

VZV is spread by the airborne route[133] and mostly requires contact with an infected individual for transmission to occur. In varicella, VZV is transmitted mainly from the skin[43,207]; the respiratory tract cannot be excluded but is not likely to be a significant mode of spread. VZV is isolated readily from skin lesions, but isolation of the virus from the respiratory tract just before or during rash is extremely rare.[92,163,205] Spread of VZV from varicella patients before the onset of rash in closed communities has been reported, however, thus implicating respiratory

*References 42, 50-52, 106, 120-122, 145, 146.

*References 4, 93, 131, 142, 151, 158, 174, 185.

spread, although early skin lesions could have been overlooked.[25,94] VZV DNA has been detected by PCR assay in the nasopharynx of children during the preeruptive and early stages of varicella.[123,164,183] Investigations of leukemic recipients of live attenuated varicella vaccine have implicated spread of VZV from skin. No transmission to siblings from vaccinees who did not have a rash was found, but a 14 percent transmission rate occurred when siblings were exposed to a recently immunized child who had a vaccine-associated rash.[207] The chance of transmission occurring was directly proportional to the number of skin lesions present.[207] Observations in children with wild-type varicella despite their having been immunized indicated that higher transmission rates are associated with greater numbers of skin lesions.[189] Interestingly, an attack rate of more than 20 percent occurred after medical students were exposed to VZV during the autopsy of a patient with disseminated varicella; clearly, this transmission could not have occurred by the respiratory route.[166] Taken together, these observations suggest that VZV spreads mainly from skin lesions rather than from the respiratory tract. Epidemiologic studies suggest that transmission is most likely to take place in the early stages of varicella.[33,157]

Zoster is not transmissible per se, but the vesicular lesions of zoster contain infectious VZV and can transmit varicella to other individuals who have not yet had primary infection.[26,128] Zoster is perhaps 75 percent less contagious than is varicella,[177,186] and whether VZV is spread from the respiratory tract of patients with zoster is unknown. Presumably, however, the major source of contagion, as for varicella, is the skin lesions.[186]

EPIDEMIOLOGY

VZV infections occur worldwide, and both sexes are affected equally. The virus spreads less efficiently in countries with tropical climates than in those with temperate climates,[110] thereby resulting in a high rate of susceptibility in adults reared in tropical countries. In the pre-vaccine era, approximately 3.8 million cases of chickenpox, an entire birth cohort, occurred annually in the United States. At that time, 8 to 9 percent of children between the ages of 1 and 9 years contracted the illness annually.[171] These data were collected before many children attended daycare facilities; exposure to VZV in the daycare setting might have led to earlier acquisition of disease. Today there is little varicella disease in the United States owing to widespread routine vaccination. (See the later discussion of the health effects of widespread immunization in the United States.)

The link between varicella and zoster was recognized first about 100 years ago when Bokay[22] appreciated that cases of varicella often occurred after exposure to a patient with zoster. Early in the 20th century, medical investigators inoculated vesicular fluid from zoster patients into varicella-susceptible children, who then contracted chickenpox.[26,128] Weller first successfully isolated VZV in vitro, gave the virus its name, and demonstrated that the viruses isolated from patients with varicella and zoster are identical.[216] Garland proposed that zoster is the result of reactivation of latent VZV.[70] Hope-Simpson presciently recognized the importance of the immune system in preventing reactivation of VZV; he postulated that zoster results when immunity to VZV wanes with age.[109] Declining cell-mediated immunity to VZV has been identified as contributing to the development of zoster.[9,18,28,104] With the use of molecular techniques for DNA analysis, researchers have established that the DNA of the viruses causing varicella and zoster is the same, and autopsies have revealed that latent VZV is detectable in neurons of the sensory ganglia of individuals with a past history of varicella or vaccination.[53,77,121,144,147] Zoster is not acquired by contact with patients who have zoster or chickenpox.[74] Zoster develops in approximately 30 percent of the varicella-immune population during their lifetimes, resulting in an annual rate of 1 million cases.[162]

Varicella is highly contagious; clinical infection develops in 80 to 90 percent of susceptible individuals exposed in a household.[178] Secondary varicella cases in a family usually are more severe than primary cases, probably because of intensity of exposure.[170,178] Approximately 75 percent of American adults with no past history of varicella have detectable antibodies to VZV,[132] thus indicating that subclinical varicella can occur. One epidemiologic study suggests that its incidence is approximately 5 percent.[178]

Subclinical zoster also may occur, and zoster with dermatomal pain and no rash has been described (zoster sine herpete).[89] Increases in VZV immune responses and episodes of viremia shown by PCR assay in asymptomatic individuals suggest that silent reactivation occurs.[85,99,221] Asymptomatic shedding of infectious VZV does not seem to exist.

Second attacks of varicella are uncommon but may happen more frequently in persons who are immunocompromised than in immunologically normal individuals.[86,116] Immunologic boosting to VZV occurs commonly on reexposure to the virus.[8,79,86] Whether exogenous boosting is necessary for long-term maintenance of immunity to VZV is not known for certain. A recent study suggests this is unlikely; the incidence of herpes zoster was similar in isolated communities of elderly people and in those with contact with the general public.[54,67]

Adults and children older than age 2 years with zoster usually have a history of a previous attack of varicella or vaccination.[109,191] Zoster may be due to vaccine type or wild-type VZV. In vaccinated children with zoster, approximately one third of cases are caused by wild-type VZV.[39,40,68,191] Zoster is an unusual event in children, but the incidence is increased in young children who had varicella either in utero or before reaching their second birthday.[60] Chickenpox in the first year of life increases the risk for development of childhood zoster by a relative factor between roughly 3 and 21,[100] possibly because of immaturity of the immune response to VZV in young infants. Infants with the congenital varicella syndrome are at greatly increased risk, as high as 18 percent in the first few years of life, for the development of zoster.[72]

The incidence of zoster in a population begins to increase sharply at approximately 50 years of age.[109] The loss of cell-mediated immunity to VZV that occurs

naturally with normal aging contributes to the increased incidence of zoster,[18,28] and it is now known that zoster can be prevented by therapeutic immunization. In a double-blind, placebo-controlled study involving more than 38,000 healthy individuals older than 60 years, immunization with a live attenuated zoster vaccine (Oka strain) reduced the burden of illness of zoster by 61 percent.[162] Zoster vaccine in the United States contains approximately 20,000 plaque-forming units of VZV, and it is approximately 14 times as potent as monovalent varicella vaccine.[162] Recently, the efficacy and safety of this vaccine in persons between ages 50 and 59 years was demonstrated, so that this vaccine is now recommended for healthy persons older than the age of 50 years.[184] Zoster also develops with increased frequency in patients with neoplasms and organ transplants[141]; in severely immunocompromised patients with zoster, disseminated infection with viremia also may occur.[63] Spinal trauma, irradiation, and corticosteroid therapy may be additional precipitating factors. Children who are infected with human immunodeficiency virus (HIV) and have $CD4^+$ levels less than 15 percent when varicella develops are at great risk for the development of zoster; in one published series, the incidence of herpes zoster was 70 percent.[80] HIV-infected children who have received varicella vaccine, however, rarely experience zoster.[195]

On occasion, zoster may develop in healthy children or young adults who are not immunocompromised, presumably the result of a transient fall in cell-mediated immunity to VZV caused by a stimulus such as another viral infection or stress. Zoster does not develop in all immunocompromised persons; the deficiency of cell-mediated immunity to VZV is a necessary but not sufficient requirement for this illness. The distribution of lesions in chickenpox, which primarily involves the trunk and head, is reflected in a proportionately greater representation of these regions in the dermatomal lesions of zoster.[200] Zoster may recur in either the same or a different dermatome; the chance of developing recurrent zoster is similar to the chance of having a first attack for a particular age group and is reported as occurring in 2 to 5 percent of adults.[109,175]

Varicella occurs most commonly in the winter and early spring. In contrast, zoster occurs at equal rates during all seasons of the year. VZV may seed ganglia and establish neuronal latency either directly from skin lesions or by viremia.[42]

PATHOGENESIS

The incubation period of varicella ranges from 10 to 23 days (average, 14 days).[94] During the incubation period, VZV is thought to spread to regional lymph nodes, undergo multiplication, and cause a primary low-grade viremic phase after approximately 5 days that spreads the virus to the viscera, where further viral multiplication occurs. This process results in the second and greater viremic phase, which delivers the virus to the skin, where it causes the characteristic rash.[97] VZV can be isolated from blood cultures either a few days before the onset of rash or within 1 to 2 days after onset in immunocompetent children.[11] An alternative, recently proposed pathogenic mechanism is that VZV reaches keratinocytes soon after infection occurs, by virus-infected $CD4^+$ memory T cells. These memory cells, when uninfected, normally circulate through the skin. In this model, overcoming of innate immunity in skin accounts for the 2- to 3-week incubation period that follows infection.[126,127]

The skin lesions of VZV infection begin as macules and progress rapidly to papules, vesicles, pustules, and scabs. Histologic changes in the skin lesions are similar for chickenpox and zoster. The hallmarks of each disease are multinucleated giant cells and intranuclear inclusions. In varicella, they are localized primarily in the dermis and epidermis, where ballooning degeneration of cells in the deeper layers is accompanied by intercellular edema. As the edema progresses, the cornified layers and basal layers separate to form a thin-roofed vesicle. An exudate of mononuclear cells is seen in the dermis.[2,216] In zoster, in addition to skin lesions that resemble those of varicella, a mononuclear inflammatory infiltrate is present in the dorsal root ganglion of the affected dermatome. Hemorrhagic necrosis of ganglion cells and demyelination of the corresponding axon also may be present.[62,107,144]

Both humoral and cell-mediated immune responses to VZV develop within a few days after the onset of varicella. Peak antibody levels are attained 4 to 8 weeks after onset, remain high for approximately 6 months, and then decline. IgG VZV antibody titers are detectable in healthy adults for decades after recovery from varicella.[84,199,220] After active immunization against varicella, antibody titers are lower than after natural infection occurs but persist for as long as 20 years in healthy vaccinees immunized as children.[217] Serum IgG, IgA, and IgM are detectable after both varicella and zoster. Zoster occurs despite high levels of specific antibodies, but significantly higher titers develop during convalescence and are indicative of an anamnestic response to VZV in this secondary infection.[209]

Cell-mediated immune responses are thought to play the major role in host defense against the virus. Cell-mediated immunity to VZV can be demonstrated in vitro by stimulation of lymphocytes with VZV antigens,[104,225] an intradermal skin test,[132] specific lysis of histocompatible target cells by cytotoxic T cells,[6] and an enzyme-linked immunosorbent spot assay.[138] Natural killer cell and antibody-dependent cellular toxicity to VZV also has been described.[112] Immune responses of normal subjects with remote clinical evidence of varicella are characterized by occasional high activity in the absence of symptoms, thus suggesting either exposure to VZV with boosting of the immune response or subclinical reactivation of the virus. Cell-mediated immune reactions remain positive for years after a case of varicella, although this response wanes in many individuals older than 50 years.[18,28] The predominant cell in VZV vesicular lesions is the polymorphonuclear leukocyte. These leukocytes may play a role in generating interferon in vesicular lesions, which may be a factor in recovery.[201]

Exactly how immunity to varicella and zoster is mediated is unclear and also complex. Because patients with isolated agammaglobulinemia do not experience either

severe or recurrent varicella, researchers long presumed that cell-mediated immunity is more important in host defense than humoral immunity; we now recognize that T-cell cytotoxicity is crucial in recovery from VZV infection.[7] The response or responses that prevent clinical illness after reinfection with VZV are presumed to be those of cytotoxic T lymphocytes, but antibodies also may play a role. Elderly individuals with poor cell-mediated immune responses to VZV are not subject to recurrent chickenpox. In addition, specific antibodies must have some importance because passive immunization is used successfully to prevent or to modify varicella in exposed susceptible persons, perhaps by neutralization of cell-free VZV early in the infection. These issues, however, are not straightforward. Varicella may develop in young infants after exposure despite detectable transplacental antibody titers,[14] and modified cases of breakthrough varicella have developed in vaccinated leukemic children despite the presence of humoral or cell-mediated immune responses at exposure to VZV.[83,87] Still, clinical illness is far less likely to develop in individuals with detectable antibody titers or positive cell-mediated immune responses (or both) at exposure to VZV than in those without positive immune responses. Thus, some forms of cell-mediated immunity and specific antibodies each probably play roles in host defense against VZV, and there may be some redundancy in the system.

NOSOCOMIAL VARICELLA

Although it is probably less of a problem since licensure of varicella vaccine, nosocomial varicella is a potentially serious and expensive problem in hospitals, where both patients and employees may be susceptible to chickenpox.[96,102,214] Because varicella-susceptible hospital employees may serve as vectors for the spread of VZV to susceptible patients, serologic testing of employees for immunity to chickenpox if they have no past history of having had clinical varicella and offering vaccine to those who are susceptible are now standard measures in many hospitals.

The risk for horizontal transmission in maternity wards or the neonatal nursery after hospital exposure to an adult or child is surprisingly low.[72] A few episodes of nursery transmission of varicella have been reported,[66,81,101] but the low incidence of such transmission may be, at least partly, a result of the fact that many infants are in isolettes. Furthermore, most hospital employees and most mothers and their neonates have antibodies to VZV and are at low risk for the development of clinical illness. Even in low-birth-weight infants, antibodies to VZV may be detectable.[154,176]

CLINICAL MANIFESTATIONS

Varicella

Varicella is a highly contagious, usually self-limited systemic infection characterized by fever and a generalized pruritic rash lasting approximately 5 days. A prodromal phase in children is unusual, but malaise and fever for 1 to 2 days before the onset of rash is a common manifestation in adults.[46] The rash is more intense on the trunk and head than on the extremities, and it typically evolves as a series of "crops" during the course of 1 to 2 days in normal hosts. Most children with varicella have 250 to 500 superficial skin lesions, many of which are vesicular (Fig. 161-1).[178] Not uncommonly, a few lesions may develop in the mouth, conjunctiva, or other mucosal sites. Residual scarring is exceptional but can occur, and depigmented areas of skin may occur in dark-skinned patients. A self-limited increase in hepatic transaminase levels without jaundice is not an uncommon occurrence during varicella.[169] Rarely, thrombocytopenia and neutropenia may transiently occur. Severe infections are more likely to develop in adults than in children, presumably because of less robust cell-mediated immune responses to VZV in adults than in children.[75,160] Neonates who acquire varicella from their mothers in the few days before delivery also are at risk for acquisition of severe varicella because of immaturity of the cell-mediated immune response.[72]

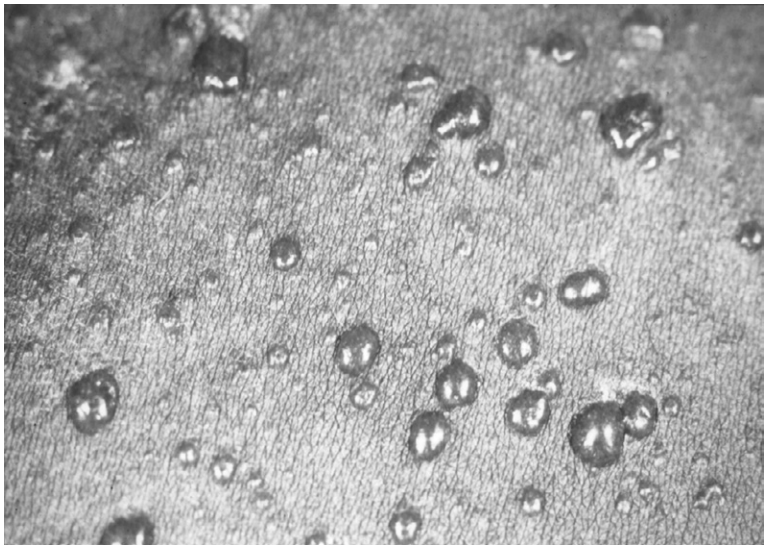

FIGURE 161-1 ■ Typical skin lesions of varicella.

Complications of Varicella

The most frequent complication of varicella in normal hosts is bacterial superinfection of the skin, lungs, or bones, most often caused by *Staphylococcus aureus* or group A β-hemolytic streptococci.[23,222] Central nervous system involvement, which may precede or follow varicella, includes transient cerebellar ataxia, severe cerebral encephalitis, aseptic meningitis, and transverse myelitis.[114,115] Encephalopathy as a sequela of Reye syndrome has become a rare complication because aspirin no longer is recommended for children with varicella. Other complications of chickenpox encountered less commonly include arthritis, glomerulonephritis, myocarditis, and purpura fulminans.[216]

Varicella may be severe and even fatal in immunocompromised patients, including those with an underlying malignant neoplasm or congenital or acquired deficits in cell-mediated immunity, such as patients who have undergone organ transplantation or have underlying HIV infection, and in children receiving high doses of corticosteroids for any reason (Fig. 161-2).[65] These patients may be susceptible to progressive varicella, with continuing fever and the development of new vesicular lesions for 2 weeks or longer. Their skin lesions characteristically are large, umbilicated, and hemorrhagic, and primary varicella pneumonia is a frequent complication. Alternatively, in some immunocompromised patients, an acute form of varicella with disseminated intravascular coagulation develops and is rapidly fatal, at times before antiviral therapy can be instituted. A 30 percent rate of dissemination with a 7 percent fatality rate was reported in leukemic children in whom chickenpox developed in the pre–antiviral drug era.[64] Severe varicella has been observed in children with underlying HIV infection, especially those classified as having acquired immunodeficiency syndrome (AIDS).[117] In most HIV-infected children, however, mild to moderate forms of varicella develop, although the illness generally is more severe than that in otherwise healthy children.[80] Varicella does not seem to be a cofactor for clinical progression of HIV infection to AIDS, but in approximately 5 percent of these children, chronic wartlike hyperkeratotic VZV lesions may develop, presumably being a chronic form of zoster in which the infection is low grade but persistent.[5]

Primary varicella pneumonia accounts for many of the fatalities ascribed to varicella, particularly in immunocompromised patients, and adults.[167] In male military recruits with varicella, radiographic evidence of pneumonia was found in 16 percent.[215] Symptoms include fever, cough, and dyspnea. Other common symptoms and signs are cyanosis, rales, hemoptysis, and chest pain. The chest radiograph typically reveals a diffuse nodular or miliary pattern that is most pronounced in the perihilar region.[204] Blood gas analyses and pulmonary function tests indicate a diffusion defect that may persist in some cases for months after recovery.[21] The availability of antiviral chemotherapy has greatly improved the outcome of this complication.

Congenital Varicella Syndrome

LaForet and Lynch, who were medical students in 1947, were the first to describe an infant with multiple congenital anomalies after contraction of maternal chickenpox.[130]

FIGURE 161-2 ■ Progressive varicella in a 9-year-old child with underlying leukemia.

The infant had hypoplasia of the right lower extremity, clubfoot, and absent right deep tendon reflexes, along with cerebral cortical atrophy, cerebellar aplasia, chorioretinitis, torticollis, insufficiency of the anal and vesical sphincters, and cicatricial cutaneous lesions on the left lower extremity. These manifestations are now recognized to be typical of this syndrome. In 1974, Srabstein and colleagues rediscovered this syndrome, which appeared to have been forgotten.[197] Their report of another case and review of the literature concluded that although the virus could not be isolated from affected infants as with congenital rubella or cytomegalovirus infection, the congenital syndrome consisted of a typical constellation of birth defects as originally described by LaForet and Lynch. This syndrome occurs after maternal VZV infection develops in the first or second trimester of pregnancy; approximately 2 percent of the offspring of pregnancies complicated by varicella are affected. Approximately 100 affected infants have been described in the literature; 95 percent occurred after maternal varicella and 5 percent after maternal zoster. In the prevaccine era, approximately 40 affected infants were thought to be born annually in the United States.[72] Today in the vaccine era, this syndrome may once more be forgotten.

Cicatricial scars of the skin, the most prominent stigmata, are reported in more than 60 percent of cases.[72] Other frequent abnormalities include chorioretinitis, microphthalmos, Horner syndrome, cataract, nystagmus, hypoplastic limbs, cortical atrophy or mental retardation (or both), and early death. Many of these children develop zoster (in which VZV can be identified) in the early months of life. There is a high correlation between limb abnormalities and a poor outcome in these infants. The Oka strain is not known to cause the congenital syndrome.[72]

Zoster

Zoster usually begins as a localized unilateral vesicular skin eruption involving one to three dermatomal segments (Fig. 161-3). The skin lesions resemble those of varicella but tend more toward confluence and are likely to be painful or pruritic, especially in adults. Zoster generally is a milder disease in children than in adults. Zoster may follow natural varicella or vaccination; however, the incidence of zoster is clearly lower after vaccination than after varicella disease, even in immunocompromised vaccinees.[103,104,206] Zoster is a considerable cause of morbidity, but mortality is unusual or rare.[148] Visceral zoster, involving the gastrointestinal tract, may produce severe abdominal symptomatology, at times in the absence of rash.[41,72]

Complications of Zoster

Two kinds of pain are associated with herpes zoster: (1) acute pain that is related to reactivation of VZV in sensory

FIGURE 161-3 ■ Zoster in an otherwise healthy 10-year-old boy.

neurons and (2) pain that develops after the acute phase of the illness, often in the area of healed rash. Between 25 and 50 percent of persons older than 50 years in whom zoster develops may experience late protracted pain, or postherpetic neuralgia, after the rash has healed. Postherpetic neuralgia also occurs frequently in immunocompromised patients. Pain may persist for months to years and is described as aching, jabbing, or boring. The cause of postherpetic neuralgia is unknown but may be related to healing of injured nerves. Otherwise healthy children rarely experience postherpetic neuralgia; both the incidence and duration are related directly to increasing age.[90,91] Zoster may be particularly severe and common after bone marrow transplantation; in the first year after undergoing transplantation, 20 to 70 percent of recipients develop zoster.[71]

DIAGNOSIS

Clinical Diagnosis of Varicella and Zoster

It usually is not difficult to establish a clinical diagnosis of VZV infection because the vesicular rash is so characteristic. In questionable cases of varicella, epidemiologic information, such as a history of recent exposure to varicella or zoster and subsequent transmission of varicella to another person, may be useful. The differential diagnosis of varicella includes generalized HSV infection, enterovirus infections, rickettsialpox, impetigo, allergic reactions, including Stevens-Johnson syndrome and poison ivy, and insect bites.

In one study of zoster, 13 percent of clinically diagnosed cases were proved by culture to be caused by HSV infection.[118] The unilateral rash of zoster most frequently appears on the trunk or face. The trigeminal nerve, most commonly the ophthalmic branch, is an especially significant site because the eye may be involved.

Laboratory Diagnosis

Laboratory diagnosis of VZV infection is facilitated by the presence of VZV in superficial skin lesions, where it is easily accessible for testing. The diagnosis is made best by the demonstration of specific viral antigens or VZV DNA in skin vesicles or scrapings. VZV antigens are demonstrable by immunofluorescence with a commercial monoclonal antibody to VZV that is conjugated to fluorescein.[73] Immunofluorescence testing is highly sensitive and can be completed within approximately an hour. PCR assay is used to demonstrate VZV in skin lesions and also in saliva and cerebrospinal fluid (CSF) when there is central nervous system involvement with VZV. PCR is more sensitive than immunofluorescence and is becoming the preferred method for diagnosis of VZV.[73,210] PCR also may be used to distinguish between vaccine (Oka strain) and wild-type VZV infections[73,174]

The diagnosis also may be made by isolation of virus from skin lesions very early in the course of illness. Within several days after the onset, vesicular fluid no longer is likely to be infectious, although viable VZV may be present in zoster lesions for a longer period, especially in immunocompromised patients. VZV cannot be isolated from skin lesions that have become pustular or dry. Isolation of VZV is a slow method because approximately 48 hours is required before the first signs of a viral cytopathic effect are seen. It is also less sensitive than is immunofluorescence or PCR. VZV rarely is isolated from respiratory secretions or from infected CSF during VZV meningitis.[92] Demonstration of VZV DNA, antigens, or isolation of virus from samples obtained from skin, other sites, or autopsy tissue is diagnostic of a current infection with VZV because, unlike other herpesviruses, no known carrier state or shedding of VZV by asymptomatic individuals exists.[73] VZV infections can also be diagnosed by demonstration of viral DNA in saliva.[136,152,153]

Numerous serologic tests, including the fluorescent antibody to membrane antigen (FAMA) method and enzyme-linked immunosorbent assay (ELISA), are useful for measuring antibodies to VZV.[73] Antibody to VZV develops within a few days after the onset of varicella, persists for many years, and is present before the onset of zoster. VZV infections may be documented by a fourfold or greater rise in VZV antibody titer in acute and convalescent serum specimens. Specific IgM in one serum specimen suggests recent VZV infection. Persistence of VZV antibody beyond 8 months of age is highly suggestive of intrauterine varicella.[81] Immunity to varicella is present if a positive FAMA titer to VZV is demonstrated on a single serum sample after varicella or immunization.[156] Serologic methods, however, particularly commercial ELISAs, may fail to identify individuals who have had natural infection or have been immunized.[73,199]

The value of serologic procedures for the diagnosis of zoster is limited. Heterologous increases in antibody titer against VZV in patients with HSV infection who previously had varicella may occur and have been ascribed to antigens common to the two viruses.[73] Laboratory diagnosis of zoster is best accomplished by PCR assay on skin lesions or in saliva.

TREATMENT

Traditionally, nonspecific measures, such as frequent bathing to discourage bacterial skin infection, antihistamines given orally, calamine lotion applied locally, oatmeal baths to decrease itching, and cutting fingernails short to discourage scratching, have been used to treat varicella. Fever is controlled best with acetaminophen rather than aspirin, which may predispose to Reye syndrome.[105] The issue of whether treatment with ibuprofen is associated with group A streptococcal superinfection in varicella has not been resolved, and, therefore, avoidance of its use for symptomatic treatment of varicella seems to be the best approach.[29,45,135,227]

Useful specific antiviral therapy for VZV became available in the mid 1980s with the introduction of the nucleoside analog acyclovir, an inhibitor of DNA polymerase and a DNA chain terminator. The antiviral effect of acyclovir depends on its being phosphorylated in the

body by virus-induced thymidine kinases, which accounts for its relative lack of toxicity.[219] Antiviral therapy for varicella and zoster is similar.

Patients with severe or potentially severe VZV infections should be treated with intravenous acyclovir 30 mg/kg/day for adults and adolescents and 1500 mg/m^2/day for children, both given in three divided doses. Orally administered acyclovir is less reliable for immunocompromised patients because only approximately 20 percent of this formulation is absorbed from the gastrointestinal tract, and no data on its efficacy in high-risk patients have been published. Because acyclovir is excreted by the kidneys, patients with a creatinine clearance of less than 50 mL/min/1.73 m^2 should receive one half to one third of this dosage. Intravenous acyclovir is infused for at least 1 hour, with maintenance fluids given both before and during the infusion. Providing adequate hydration is important to prevent renal damage from precipitation of the drug in the renal tubules. Other adverse effects of acyclovir include phlebitis, rash, nausea, and neurologic manifestations such as headache and tremor. In general, however, acyclovir is extremely well tolerated.[219]

Early intravenous therapy should be instituted for patients at high risk for development of severe VZV infection, such as leukemic children and those who have undergone organ or bone marrow transplantation, to prevent the dissemination of VZV.[192,218] Not only may this therapy be potentially lifesaving in immunocompromised patients, but it also prevents considerable morbidity from VZV infection. Children thought to be somewhat less immunocompromised, such as those with HIV infection but without AIDS, may be given a treatment trial with oral acyclovir under close medical supervision. For such patients who are not doing well, intravenous therapy should be administered. In zoster patients, the use of intravenous acyclovir is associated with more rapid healing of skin lesions and resolution of acute pain than if no specific treatment is given.[219]

Considerable controversy has ensued about the role of orally administered acyclovir for the treatment of varicella and zoster in otherwise healthy children because most of these infections are self-limited. Customarily, however, adults, who are at greater risk for development of severe infection, are treated. Oral dosages used are 80 mg/kg/day (in four divided doses) for children and 4 g/day (in five divided doses) for adults. Double-blind, placebo-controlled studies in healthy children given oral acyclovir at 80 mg/kg/day for 5 days or placebo beginning within 24 hours of the onset of varicella rash have revealed that the number of chickenpox skin lesions is reduced significantly by acyclovir. A modest benefit was derived from acyclovir; children who received it had fever for approximately 1 day less, but they did not return to school any more rapidly, nor did they have fewer complications of chickenpox.[16,59]

Some evidence indicates that early administration of oral acyclovir may decrease the acute pain associated with zoster.[111] However, the need to administer specific therapy for zoster in otherwise healthy children for whom pain is not a particular problem rarely occurs. Usually however, children with herpes zoster are treated in order to speed recovery.

The newer drug, penciclovir, has an action similar to that of acyclovir.[108] It is administered as famciclovir, a prodrug that when given orally is converted rapidly to penciclovir in the body. A major advantage of famciclovir is that it is administered only three times a day (1500 mg/day for an adult), whereas acyclovir is given four or five times daily. Penciclovir has an antiviral action similar to that of acyclovir. One study suggests that famciclovir given to elderly patients with zoster early in the course of infection decreases the duration of postherpetic neuralgia, although not its incidence.[208] No data regarding whether varicella may be treated successfully with famciclovir have been published, nor have any data on the use of famciclovir in immunocompromised patients or in children.

The prodrug of acyclovir, valacyclovir, also is given orally, reaches blood levels that are approximately three to four times higher than those following oral acyclovir, and has been shown in one study to be superior to acyclovir for treatment of zoster.[19] Valacyclovir may be used in children older than age 2 with chickenpox. The dose of valacyclovir is 20 mg/kg per dose TID for 5 days, not to exceed 1 g per dose 3 times daily.

Of concern about the potential widespread use of acyclovir is that drug resistance may develop. At present, resistance is less a problem with VZV than with HSV, but VZV resistant to acyclovir has been reported in a few patients with underlying AIDS.[113] Two vaccinated children with neuroblastoma who developed zoster caused by the Oka strain that became resistant to acyclovir after prolonged treatment have been described.[27,137] Foscarnet was licensed by the Food and Drug Administration for the treatment of VZV infections that are resistant to acyclovir and famciclovir. Foscarnet inhibits the synthesis of VZV DNA polymerase.[180,181,194] Intravenous foscarnet is given at a dosage of 180 mg/kg/day in two divided doses, adjusted according to renal function. The main toxicity of foscarnet is renal damage and electrolyte imbalance.[71]

PROGNOSIS

The prognosis of varicella and zoster is excellent in children without underlying health problems. The outlook for immunocompromised patients receiving antiviral chemotherapy also is good, especially if treatment is begun at an early stage of the illness. In general, zoster carries a better prognosis than varicella does, possibly because it is a secondary infection. If the disease is diagnosed promptly, complications such as bacterial superinfections usually can be treated successfully with antimicrobial agents. In the pre-vaccine era, despite the availability of antiviral therapy and passive immunization, the Centers for Disease Control and Prevention (CDC) has estimated that approximately 100 deaths from varicella occurred annually in the United States, mostly in children with no previous health problems. An epidemiologic study of more than 250,000 health maintenance organization records indicated that the rates of varicella in adolescents and young adults and the complication and

hospitalization rates had increased between 1992 and 1995 by a factor of approximately 5.[44]

The widespread use of varicella vaccine in the United States decreased the morbidity and mortality rates from chickenpox substantially. By 2000, in the three sentinel communities in the United States, vaccine coverage among children 19 to 35 months was more than 80 percent, and reported varicella cases had declined roughly from 70 to 80 percent, compared with the pre-vaccine era. Hospitalizations for varicella also declined by approximately 80 percent. The greatest declines occurred in young children.[161] Deaths from varicella have become rare.[150] At present, more than 90 percent of children in the United States are routinely vaccinated against varicella.[35]

PREVENTION

VZV is such an infectious agent that general measures are not useful for prevention of varicella in susceptible individuals. Some protection, however, can be achieved by isolation of hospitalized patients. Hospitalized patients with active VZV infection should be admitted to a private room, preferably with negative-pressure ventilation, and hospital personnel and visitors should wash their hands before and after entering the room and wear masks, gowns, and gloves while in it. The CDC now recommends postexposure vaccination for healthy varicella-susceptible exposed individuals.[31]

Passive Immunization Against Varicella

Varicella-susceptible children at high risk for development of severe chickenpox should be passively immunized if they are closely exposed to VZV. Passive immunization may be lifesaving; it may modify varicella or prevent it.

Passive immunization is indicated for varicella-susceptible individuals who have been closely exposed to varicella or zoster and are at high risk for development of severe chickenpox. This population includes immunocompromised children and adults, pregnant women, neonates whose mothers have active varicella at the time of delivery, and premature infants of less than 28 weeks' gestation or who weighed less than 1000 g at birth. Children at high risk should be considered to be susceptible to varicella if they have no history of having had chickenpox. False-positive antibody test results may occur in immunocompromised individuals, and, therefore, susceptible children may be identified serologically as immune; hence, a history of illness is a preferred indication of these patients' immune status. Passive immunization usually is reserved for VZV-exposed adults only if they have been proved serologically to be susceptible to chickenpox, because most adults with a negative history of varicella raised in the continental United States are immune.

Patients with HIV infection, especially those with AIDS, have some increased risk for development of severe varicella, and their management should be similar to that of immunocompromised children with regard to passive immunization. Even children who are receiving intravenous globulin for treatment of HIV infection should receive passive immunization if they have no past history of varicella and close exposure has occurred.

Infants whose mothers have an onset of chickenpox 5 days or less before delivery or within 48 hours after delivery should be given passive immunization as soon as possible after birth.[72] The transplacental route of infection and the immaturity of the immune system probably account for the severity of varicella in these infants.[72] Attack rates for varicella as high as 50 percent in infants exposed to mothers who have varicella have been reported, despite their having passive immunization.[72,173] Passively immunized infants should be observed closely, but usually they can be managed as outpatients. Intravenous acyclovir should be reserved for the rare, passively immunized infant with varicella in whom an extensive rash (more than 200 vesicles) or possible pneumonia develops.[15]

Passive immunization need not be given to full-term infants who are exposed to VZV after they are 48 hours old. Passive immunization is optional for neonates (<1 week old) if their siblings at home have active varicella. Infants exposed to VZV after birth almost always have mild varicella. Although the reported mortality rate from varicella in children younger than 1 year is four times that in older children, both rates are exceedingly low—8 per 100,000 cases and 2 per 100,000 cases, respectively.[172] The mortality rate for adults and for leukemic children receiving chemotherapy, in contrast, is 20 and 1000 times higher, respectively.[171] Passive immunization is not useful to treat or to prevent zoster. Whether it is useful for pregnant varicella-susceptible women who have been exposed to VZV to protect the fetus from developing the congenital varicella syndrome is a good possibility.[48]

Until recently, varicella-zoster immune globulin (VZIG), distributed by the Massachusetts Public Health Biological Laboratory and the American Red Cross, was used for passive immunization. Because of limited requests for VZIG since licensure of varicella vaccine in the United States, however, VZIG no longer is being produced. Another product, varicella immune globulin (human), manufactured in Canada, is now available under an investigational new drug application expanded-access protocol.[34] VariZIG can be obtained in the United States,* but central institutional review board approval is required, and local institutional review board approval also may be necessary. An alternative to VariZIG is intravenous immune globulin 400 mg/kg.

Although passive immunization has been shown to be effective when it is given within 4 days and perhaps as long as 5 days, it should be administered as soon as possible after an exposure to VZV.[3,34] In 2012, the CDC has recommended passive immunization for as long as 10 days after close exposure to VZV (based on limited data) with the possibility that it will have a salutary effect.[37] The dose is 1 vial or 125 units for every 10 kg of body weight, with a maximal dosage of 5 vials or 625 units, given intramuscularly. VariZIG should be readministered

*The sole distributor of VariZIG is FFF Enterprises in Temecula, California (800-843-7477).

to high-risk susceptible individuals who are closely reexposed 3 weeks after a first dose.[3]

Active Immunization Against Varicella

A live attenuated varicella vaccine, the Oka strain, was developed in Japan more than 35 years ago.[148] It was licensed in the United States in 1995 for universal immunization of healthy children and adults who are susceptible to varicella[30,35] and has proved to be extremely safe and well tolerated.[20,35,39,68,191] Varicella vaccine is labile. The lyophilized vaccine must be stored frozen at an average temperature of 5° F or colder. The freezer must have its own door; frost-free freezers are acceptable. Prior to use, the vaccine must be diluted with its supplied diluent and administered subcutaneously to the patient within 30 minutes to preserve its potency.[35]

Originally a monovalent formulation of varicella vaccine was licensed in the United States. Currently two licensed varicella vaccine formulations are available in the United States, both produced by Merck. Monovalent varicella vaccine, which contains 1,350 plaque-forming units (PFU) at expiry, is recommended for healthy varicella-susceptible persons older than the age of 12 months. A combination vaccine, MMRV, which was licensed in 2005, contains a larger amount of VZV than Varivax, approximately 10,000 PFU at expiry. MMRV is recommended for healthy children between 1 and 12 years of age. Varicella vaccines worldwide are the Oka strain and appear to be similar to the vaccine used in the United States.

Safety

The most frequent adverse reaction to vaccination is a mild rash that develops several weeks later in approximately 5 percent of healthy children, particularly after the first dose.[84,88,191,217] These rashes can be serious in immunocompromised children who are vaccinated inadvertently[76,78,191] but usually respond to antiviral therapy. When vaccinees develop rash from the Oka strain, the potential for transmission of the vaccine virus exists, but transmission is extremely rare from healthy individuals, with fewer than 10 reported instances from healthy vaccinated individuals after more than 15 years of routine immunization of as many as 60 million American children.[35,68,95,191] In contrast, the rate of transmission of the Oka strain was 14 percent after household exposure from vaccinees with underlying leukemia who had a rash and exposed their susceptible healthy siblings.[207]

It was observed that the incidence of febrile seizures was double that in children from 1 to 2 years of age in the 5 to 12 days after MMRV compared with monovalent varicella vaccine (one extra febrile seizure for every 2500 vaccinees).[125] After careful consideration the CDC recommended that for the first dose of measles, mumps, rubella, and varicella at age 12 to 47 months, either MMR vaccine and monovalent varicella vaccine or MMRV may be used. Providers are advised to discuss the risks and benefits of each of the two choices with the infant's parent or caregiver, particularly if it is anticipated to give MMRV; the CDC favors giving MMR and varicella vaccines in separate sites. For the second doses, at any age, and if the first dose is given at age 4 years or older, MMRV is preferred.[36]

Few serious neurologic events have been causally demonstrated to be connected to varicella vaccine. There have been nine reports of herpes zoster due to the Oka strain with accompanying meningitis (VZV DNA found in CSF by PCR assay) in a group of healthy and immunocompromised patients; all recovered with antiviral therapy.[165] It is well known that meningitis may also accompany herpes zoster caused by wild-type VZV,[38,89,168,196,198] so it is unclear whether these nine patients should be classified as having a vaccine-associated adverse event or not. Because there is no known denominator, the incidence of these complications after is not known. Transient cerebellar ataxia has been reported after vaccination, but its connection to VZV has not been proven.[68,191]

Effectiveness

Live attenuated varicella vaccine is highly effective in healthy children and adults, but not all vaccinees are completely protected. Ten to 20 percent of children given one dose may develop a modified breakthrough illness after intimate exposure to VZV. Varicella vaccine has, however, been 97 percent effective in preventing severe varicella.[210,211] The vaccine is also highly effective in adults; after two doses, more than 75 percent are completely protected from varicella after household exposure to the virus.[1,84,182] Severe wild-type varicella in vaccinated adults is very rare.

Originally, it was observed that approximately 85 percent of healthy children are protected after only one dose of vaccine,[129,210,211,217] and there was little concern about breakthrough varicella because it had been found to be almost always a modified illness. There was no significant waning of immunity in a case-control study with laboratory verification of varicella.[211] Loss of VZV antibodies, moreover, occurs rarely in healthy vaccinated children, some of whom have been monitored for as long as 20 years after immunization.*

Beginning in approximately 2000, however, breakthrough varicella became a growing concern in the United States. The incidence of varicella in sentinel geographic areas where active surveillance was being performed revealed that although the incidence of disease fell after 1995, it leveled off at a low rate in about 2000.[188,189] The number of reports of outbreaks of varicella among vaccinated children was, moreover, increasing; some of these studies indicated vaccine effectiveness as low as 44 and 56 percent, although most showed 80 to 85 percent effectiveness.[69,134] Also recognized was that children with breakthrough infections could spread wild-type VZV to contacts almost as efficiently as unvaccinated children with varicella.[189] Attempts at outbreak control with second doses of varicella vaccine proved to be expensive and complicated.[149] Finally, one study suggested that the seroconversion rate could be as low as 76 percent, indicating an unexpectedly high rate of primary

*References 10, 12, 47, 129, 193, 213, 226.

vaccine failure after one dose of vaccine.[156] The fear was that without a second routine dose, a gradual accumulation of susceptible young adults would develop as a result of primary vaccine failure and would be at risk to develop severe chickenpox.

In the light of all these problems, a second dose of varicella vaccine was recommended for all children, not just those older than 12 years, by the CDC in June 2006. Early studies exploring immune responses to two doses had indicated the safety of a second dose and a marked boost in immunity and the seroconversion rate afterward. An interval of 4 to 6 years between the first and second doses was recommended in 2007, but there was flexibility in that the second dose could be administered at any time 1 month after the first dose.[35] Catch-up immunization was also recommended for children who received only one dose of varicella vaccine.[35] With the institution of two routine doses, protection achieved 98 percent in a case-control study,[190] and the incidence of varicella was reported to have decreased significantly in Connecticut.[119]

Because the risk from wild-type VZV is greater than is the risk from vaccine-type VZV, immunization is recommended for health care workers and persons whose varicella-susceptible family members are immunocompromised or pregnant.[35] Varicella vaccine is recommended only for healthy persons. Vaccinees in whom an extensive VZV rash develops within 2 to 3 weeks after immunization are likely to have wild-type infection.[191] In such situations, PCR and restriction fragment length polymorphism analysis can be used to differentiate between wild-type and vaccine-type VZV. Passive immunization could be considered for immunocompromised individuals inadvertently exposed to a vaccinee with rash resembling chickenpox that might be caused by wild-type VZV.

Most states now require varicella vaccination for children to attend daycare or school. In geographic areas where vaccine use in children younger than 3 years is 70 percent or more, the incidence of varicella has decreased sharply, not only in the vaccinated group but also in all age groups, a process indicative of herd immunity.[188,195] As has been mentioned, the incidence of zoster appears to be less of a problem after immunization than after natural infection in vaccinated immunocompromised individuals and in healthy vaccinees.

HIV-infected children may be immunized with monovalent varicella vaccine if they are free of symptoms and have 15 percent or more CD4 T lymphocytes (for children > 8 years old, a CD4 T lymphocyte level of ≥200 cells/μL is used). For these children, varicella vaccine has been shown to be safe, immunogenic, and effective.[35,195]

Drug Prophylaxis

Prophylaxis of varicella in exposed persons may be achieved by the administration of acyclovir.[13,110,140,203] This approach is not recommended often, however, because the optimal dosage and timing of administration have not been studied in large groups of children, nor is it known whether long-term maintenance of immunity exists in all children. In the United States, prevention of varicella by vaccination is a preferable approach. Long-term acyclovir therapy may be used to prevent the development of zoster in patients who have undergone bone marrow transplantation; however, its value is questionable because zoster commonly develops after administration of acyclovir is stopped.[32]

NEW REFERENCES SINCE THE SIXTH EDITION

24. Breuer J, Grose C, Norberg P, et al. A proposal for a common nomenclature for viral clades that form the species varicella-zoster virus: summary of VZV Nomenclature Meeting 2008, Barts and the London School of Medicine and Dentistry, 24-25 July 2008. *J Gen Virol* 2010;**91**:821–8.
36. Centers for Disease Control and Prevention. Update: Recommendations from the Advisory Committee on Immunization Practices (ACIP) regarding administration of combination MMRV vaccine. *MMWR Morb Mortal Wkly Rep* 2008;**57**:258–60.
37. Centers for Disease Control and Prevention. FDA approval of an extended period for administering VariZIG for postexposure prophylaxis of varicella. *MMWR Morb Mortal Wkly Rep* 2012; **61**:212.
38. Chang CC, McLean C, Vujovic O, et al. Fatal acute varicella-zoster virus hemorrhagic meningomyelitis with necrotizing vasculitis in an HIV-infected patient. *Clin Infect Dis* 2009;**48**:372–3.
39. Chaves SS, Haber P, Walton K, et al. Safety of varicella vaccine after licensure in the United States: experience from reports to the vaccine adverse event reporting system, 1995-2005. *J Infect Dis* 2008;**197**(Suppl 2):S170–7.
40. Chaves SS, Lopez AS, Watson TL, et al. Varicella in infants after implementation of the US varicella vaccination program. *Pediatrics* 2011;**128**:1071–7.
41. Chen J, Gershon AA, Li Z, et al. Varicella zoster virus (VZV) infects and establishes latency in enteric neurons. *J Neurovirol* 2011;**17**:578–89.
48. Cohen A, Moschopoulos P, Stiehm RE, et al. Congenital varicella syndrome: the evidence for secondary prevention with varicella-zoster immune globulin. *CMAJ* 2011;**183**:204–8.
54. Crumpacker C. Absence of exposure to varicella does not increase the risk of zoster. *Clin Infect Dis* 2011;**53**:412–13.
67. Gaillat J, Gajdos V, Launay O, et al. Does monastic life predispose to the risk of Saint Anthony's fire (herpes zoster)? *Clin Infect Dis* 2011;**53**:405.
68. Galea SA, Sweet A, Beninger P, et al. The safety profile of varicella vaccine: a 10-year review. *J Infect Dis* 2008;**197**(Suppl 2): S165–169.
72. Gershon A. Chickenpox, measles, and mumps. In: Remington J, Klein J, Wilson C, et al, editors. *Infections of the fetus and newborn infant*. 7th ed. Philadelphia: Elsevier Saunders; 2010.
77. Gershon AA, Chen J, Davis L, et al. Latency of varicella-zoster virus in dorsal root, cranial nerve, and enteric ganglia in vaccinated children. *Trans Am Clin Climatol Assoc* 2012;**123**:17–33; discussion 33-5.
78. Gershon AA, Gershon MD. Perspectives on vaccines against varicella-zoster virus infections. *Curr Top Microbiol Immunol* 2010;**342**:359–72.
89. Gilden D, Cohrs RJ, Mahalingam R, et al. Neurological disease produced by varicella zoster virus reactivation without rash. *Curr Top Microbiol Immunol* 2010;**342**:243–53.
95. Goulleret N, Mauvisseau E, Essevaz-Roulet M, et al. Safety profile of live varicella virus vaccine (Oka/Merck): five-year results of the European Varicella Zoster Virus Identification Program (EU VZVIP). *Vaccine* 2010;**28**:5878–82.
103. Hambleton S, Steinberg SP, Larussa PS, et al. Risk of herpes zoster in adults immunized with varicella vaccine. *J Infect Dis* 2008;**197**(Suppl 2):S196–9.
119. Kattan JA, Sosa LE, Bohnwagner HD, et al. Impact of 2-dose vaccination on varicella epidemiology: Connecticut—2005-2008. *J Infect Dis* 2011;**203**:509–12.
121. Kennedy PG, Cohrs RJ. Varicella-zoster virus human ganglionic latency: a current summary. *J Neurovirol* 2011;**16**:411–18.
125. Klein NP, Fireman B, Yih WK, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;**126**:e1–8.

136. Leung J, Harpaz R, Baughman AL, et al. Evaluation of laboratory methods for diagnosis of varicella. *Clin Infect Dis* 2010;**51**:23–32.
148. Mahamud A, Marin M, Nickell SP, et al. Herpes zoster–related deaths in the United States: validity of death certificates and mortality rates 1979-2007. *Clin Infect Dis* 2012;**55**:960–6.
150. Marin M, Zhang JX, Seward JF. Near elimination of varicella deaths in the US after implementation of the vaccination program. *Pediatrics* 2011;**128**:214–20.
153. Mehta SK, Tyring SK, Gilden DH, et al. Varicella-zoster virus in the saliva of patients with herpes zoster. *J Infect Dis* 2008;**197**:654–7.
156. Michalik DE, Steinberg SP, LaRussa PS, et al. Primary vaccine failure after 1 dose of varicella vaccine in healthy children. *J Infect Dis* 2008;**197**:944–9.
165. Pahud BA, Glaser CA, Dekker CL, et al. Varicella zoster disease of the central nervous system: epidemiological, clinical, and laboratory features 10 years after the introduction of the varicella vaccine. *J Infect Dis* 2011;**203**:316–23.
168. Pena JA, Pirics ML, DiCaprio HS, et al. Varicella reactivation presenting as shingles and aseptic meningitis in an immunocompetent 11-year-old boy. *Clin Pediatr (Phila)* 2009;**48**:435–7.
174. Quinlivan ML, Jensen NJ, Radford KW, et al. Novel genetic variation identified at fixed loci in ORF62 of the Oka varicella vaccine and in a case of vaccine-associated herpes zoster. *J Clin Microbiol* 2012;**50**:1533–8.
184. Schmader KE, Levin MJ, Gnann JW Jr, et al. Efficacy, safety, and tolerability of herpes zoster vaccine in persons aged 50-59 years. *Clin Infect Dis* 2012;**54**:922–8.
185. Schmid DS. Varicella-zoster virus vaccine: molecular genetics. *Curr Top Microbiol Immunol* 2010;**342**:323–40.
190. Shapiro ED, Vazquez M, Esposito D, et al. Effectiveness of 2 doses of varicella vaccine in children. *J Infect Dis* 2011;**203**:312–15.
195. Son M, Shapiro ED, LaRussa P, et al. Effectiveness of varicella vaccine in children infected with HIV. *J Infect Dis* 2010;**201**:1806–10.
196. Spiegel R, Miron D, Lumelsky D, et al. Severe meningoencephalitis due to late reactivation of varicella-zoster virus in an immunocompetent child. *J Child Neurol* 2010;**25**:87–90.
198. Srinivasan S, Ahn G, Anderson A. Meningoencephalitis-complicating herpes zoster ophthalmicus infection. *J Hosp Med* 2009;**4**:E19–22.
206. Tseng HF, Smith N, Marcy SM, et al. Risk factors of herpes zoster among children immunized with varicella vaccine: results from a nested case-control study. *Pediatr Infect Dis J* 2010;**29**:205–8.
224. Yamanishi K. Molecular analysis of the Oka vaccine strain of varicella-zoster virus. *J Infect Dis* 2008;**197**(Suppl 2):S45–8.

The full reference list for this chapter is available at expertconsult.com.

DNA VIRUSES

SUBSECTION 6 *POXVIRIDAE*

CHAPTER 162

SMALLPOX (VARIOLA VIRUS)

James D. Cherry

Smallpox was a dreaded febrile exanthematous disease caused by the orthopoxvirus variola virus.* After an extensive decade-long World Health Organization (WHO) program to eradicate smallpox, the world was certified free of smallpox in 1979.[14,78] Increased concern relating to biologic terrorism occurred during the 1990s, and the events of September 11, 2001 and immediately thereafter brought home the potential reality of this threat for the present and future.†

HISTORY*

Evidence suggests that endemic smallpox was occurring before 1500 BCE. Three mummies dating from the 18th to the 20th dynasties in Egypt had pustular lesions all over their bodies.[30] Reliable written accounts of smallpox first appeared during the 4th century. Smallpox was differentiated from measles in 340 CE by Ko Hung in China. At that time in China, smallpox was an established

*References 3, 4, 7, 19, 22, 31.
†References 2, 20, 42, 43, 46, 58, 60, 72, 78.

*References 22, 28, 30, 39, 48, 67, 68, 75.

REFERENCES

1. Ampofo K, Saiman L, LaRussa P, et al. Persistence of immunity to live attenuated varicella vaccine in healthy adults. *Clin Infect Dis* 2002;**34**:774–9.
2. Annunziato PW, Lungu O, Panagiotidis C, et al. Varicella-zoster virus proteins in skin lesions: implications for a novel role of ORF29p in chickenpox. *J Virol* 2000;**74**:2005–10.
3. Anonymous. VariZIG for prophylaxis after exposure to varicella. *Med Lett* 2006;**48**:69–70.
4. Argaw T, Cohen JI, Klutch M, et al. Nucleotide sequences that distinguish Oka vaccine from parental Oka and other varicella-zoster virus isolates. *J Infect Dis* 2000;**181**:1153–7.
5. Aronson JE, McSherry G, Hoyt L, et al. Varicella does not appear to be a cofactor for human immunodeficiency virus infection in children. *Pediatr Infect Dis J* 1992;**11**:1004–8.
6. Arvin AM. Cell-mediated immunity to varicella-zoster virus. *J Infect Dis* 1992;**166**:S35–41.
7. Arvin AM, Koropchak CM, Sharp M, et al. The T-lymphocyte response to varicella-zoster viral proteins. *Adv Exp Med Biol* 1990;**278**:71–81.
8. Arvin AM, Koropchak CM, Wittek AE. Immunologic evidence of reinfection with varicella-zoster virus. *J Infect Dis* 1983;**148**: 200–5.
9. Arvin AM, Pollard RB, Rasmussen LE, et al. Selective impairment of lymphocyte reactivity to varicella-zoster virus antigen among untreated patients with lymphoma. *J Infect Dis* 1978;**137**: 531–40.
10. Asano Y. Varicella vaccine: the Japanese experience. *J Infect Dis* 1996;**174**(Suppl 3):S310–13.
11. Asano Y, Itakura N, Hiroishi Y. Viremia is present in incubation period in nonimmunocompromised children with varicella. *J Pediatr* 1985;**106**:69–71.
12. Asano Y, Suga S, Yoshikawa T, et al. Experience and reason: twenty-year follow-up of protective immunity of the Oka strain live varicella vaccine. *Pediatrics* 1994;**94**:524–6.
13. Asano Y, Yoshikawa T, Suga S, et al. Postexposure prophylaxis of varicella in family contact by oral acyclovir. *Pediatrics* 1993;**92**: 219–22.
14. Baba K, Yabuuchi H, Takahashi M, et al. Immunologic and epidemiologic aspects of varicella infection acquired during infancy and early childhood. *J Pediatr* 1982;**100**:881–5.
15. Bakshi SS, Miller TC, Kaplan M, et al. Failure of varicella-zoster immunoglobulin in modification of severe congenital varicella. *Pediatr Infect Dis* 1986;**5**:699–702.
16. Balfour HH Jr, Kelly JM, Suarez CS, et al. Acyclovir treatment of varicella in otherwise healthy children. *J Pediatr* 1990;**116**: 633–9.
17. Barrett-Muir W, Scott FT, Aaby P, et al. Genetic variation of varicella-zoster virus: evidence for geographical separation of strains. *J Med Virol* 2003;**70**(Suppl 1):S42–7.
18. Berger R, Florent G, Just M. Decrease of the lymphoproliferative response to varicella-zoster virus antigen in the aged. *Infect Immun* 1981;**32**:24–7.
19. Beutner KR, Friedman DJ, Forszpaniak C, et al. Valacyclovir compared with acyclovir for improved therapy for herpes zoster in immunocompetent adults. *Antimicrob Agents Chemother* 1995;**39**: 1546–53.
20. Black S, Shinefield H, Ray P, et al. Postmarketing evaluation of the safety and effectiveness of varicella vaccine. *Pediatr Infect Dis J* 1999;**18**:1041–6.
21. Bocles JS, Ehrenkranz NJ, Marks A. Abnormalities of respiratory function in varicella pneumonia. *Ann Intern Med* 1964;**60**:183–95.
22. Bokay JV. Über den aetiologischen Zusammenhang der Varizellen mit gewissen Fallen von Herpes Zoster. *Wien Klin Wochenschr* 1909;**22**:1323–6.
23. Bradley JS, Schlievert PM, Sample TG Jr. Streptococcal toxic shock-like syndrome as a complication of varicella. *Pediatr Infect Dis J* 1991;**10**:77–8.
24. Breuer J, Grose C, Norberg P, et al. A proposal for a common nomenclature for viral clades that form the species varicella-zoster virus: summary of VZV Nomenclature Meeting 2008, Barts and the London School of Medicine and Dentistry, 24-25 July 2008. *J Gen Virol* 2010;**91**:821–8.
25. Brunell PA. Transmission of chickenpox in a school setting prior to the observed exanthem. *Am J Dis Child* 1989;**143**:1451–2.
26. Bruusgaard E. The mutual relation between zoster and varicella. *Br J Dermatol* 1932;**44**:1–24.
27. Bryan CJ, Prichard MN, Daily S, et al. Acyclovir-resistant chronic verrucous vaccine strain varicella in a patient with neuroblastoma. *Pediatr Infect Dis J* 2008;**27**:946–8.
28. Burke BL, Steele RW, Beard OW, et al. Immune responses to varicella-zoster in the aged. *Arch Intern Med* 1982;**142**:291–3.
29. Byington CL, Spencer LY, Johnson TA, et al. An epidemiological investigation of a sustained high rate of pediatric parapneumonic empyema: risk factors and microbiological associations. *Clin Infect Dis* 2002;**34**:434–40.
30. Centers for Disease Control and Prevention. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1996;**45**:1–36.
31. Centers for Disease Control and Prevention. Prevention of varicella: update. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1–6.
32. Centers for Disease Control and Prevention. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *MMWR Recomm Rep* 2000;**49**:1–125.
33. Centers for Disease Control and Prevention. Varicella outbreak among primary school students—Beijing, China, 2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:39–43.
34. Centers for Disease Control and Prevention. A new product (VariZIG) for postexposure prophylaxis of varicella available under an investigational new drug application expanded access protocol. *MMWR Morb Mortal Wkly Rep* 2006;**55**:209–10.
35. Centers for Disease Control and Prevention. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007;**56**: 1–40.
36. Centers for Disease Control and Prevention. Update: Recommendations from the Advisory Committee on Immunization Practices (ACIP) regarding administration of combination MMRV vaccine. *MMWR Morb Mortal Wkly Rep* 2008;**57**:258–60.
37. Centers for Disease Control and Prevention. FDA approval of an extended period for administering VariZIG for postexposure prophylaxis of varicella. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 212.
38. Chang CC, McLean C, Vujovic O, et al. Fatal acute varicella-zoster virus hemorrhagic meningomyelitis with necrotizing vasculitis in an HIV-infected patient. *Clin Infect Dis* 2009;**48**:372–3.
39. Chaves SS, Haber P, Walton K, et al. Safety of varicella vaccine after licensure in the United States: experience from reports to the vaccine adverse event reporting system, 1995-2005. *J Infect Dis* 2008;**197**(Suppl 2):S170–7.
40. Chaves SS, Lopez AS, Watson TL, et al. Varicella in infants after implementation of the US varicella vaccination program. *Pediatrics* 2011;**128**:1071–7.
41. Chen J, Gershon AA, Li Z, et al. Varicella zoster virus (VZV) infects and establishes latency in enteric neurons. *J Neurovirol* 2011;**17**:578–89.
42. Chen J, Gershon A, Silverstein SJ, et al. Latent and lytic infection of isolated guinea pig enteric and dorsal root ganglia by varicella zoster virus. *J Med Virol* 2003;**70**:S71–78.
43. Chen JJ, Zhu Z, Gershon AA, et al. Mannose 6-phosphate receptor dependence of varicella zoster virus infection in vitro and in the epidermis during varicella and zoster. *Cell* 2004;**119**:915–26.
44. Choo PW, Donahue JG, Manson JE, et al. The epidemiology of varicella and its complications. *J Infect Dis* 1995;**172**:706–12.
45. Choo PW, Donahue JG, Platt R. Ibuprofen and skin and soft tissue superinfections in children with varicella. *Ann Epidemiol* 1997;**7**:440–5.
46. Christie AB. *Chickenpox*. Edinburgh: E. & S. Livingstone, Ltd.; 1969.
47. Clements DA, Armstrong CB, Ursano AM, et al. Over five-year follow-up of Oka/Merck varicella vaccine recipients in 465 infants and adolescents. *Pediatr Infect Dis J* 1995;**14**:874–9.
48. Cohen A, Moschopoulos P, Stiehm RE, et al. Congenital varicella syndrome: the evidence for secondary prevention with varicella-zoster immune globulin. *CMAJ* 2011;**183**:204–8.
49. Cohen JI, Straus SE, Arvin A. Varicella-zoster virus: replication, pathogenesis, and management. In: Knipe DM, Howley PM, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 2773–818.
50. Cohrs RJ, Barbour M, Gilden DH. Varicella-zoster virus (VZV) transcription during latency in human ganglia: detection of

transcripts mapping to genes 21, 29, 62, and 63 in a cDNA library enriched for VZV RNA. *J Virol* 1996;**70**:2789–96.
51. Cohrs RJ, Gilden DH. Varicella zoster virus transcription in latently-infected human ganglia. *Anticancer Res* 2003;**23**:2063–9.
52. Cohrs RJ, Gilden DH, Kinchington PR, et al. Varicella-zoster virus gene 66 transcription and translation in latently infected human ganglia. *J Virol* 2003;**77**:6660–5.
53. Croen KD, Ostrove JM, Dragovic LJ, et al. Patterns of gene expression and sites of latency in human nerve ganglia are different for varicella-zoster and herpes simplex viruses. *Proc Natl Acad Sci U S A* 1988;**85**:9773–7.
54. Crumpacker C. Absence of exposure to varicella does not increase the risk of zoster. *Clin Infect Dis* 2011;**53**:412–13.
55. Davison AJ. Varicella-zoster virus. The Fourteenth Fleming lecture. *J Gen Virol* 1991;**72**(Pt 3):475–86.
56. Davison AJ, Edson CM, Ellis RW. New common nomenclature for glycoprotein genes of varicella-zoster virus and their glycosylated products. *J Virol* 1986;**57**:1195–7.
57. Davison AJ, Scott JE. The complete DNA sequence of varicella-zoster virus. *J Gen Virol* 1986;**67**(Pt 9):1759–816.
58. Dumas AM, Geelen JL, Weststrate MW, et al. XbaI, PstI, and BglII restriction enzyme maps of the two orientations of the varicella-zoster virus genome. *J Virol* 1981;**39**:390–400.
59. Dunkel L, Arvin A, Whitley R, et al. A controlled trial of oral acyclovir for chickenpox in normal children. *N Engl J Med* 1991;**325**: 1539–44.
60. Dworsky M, Whitley R, Alford C. Herpes zoster in early infancy. *Am J Dis Child* 1980;**134**:618–19.
61. Ecker JR, Hyman RW. Varicella zoster virus DNA exists as two isomers. *Proc Natl Acad Sci U S A* 1982;**79**:156–60.
62. Esiri MM, Tomlinson AH. Herpes zoster: demonstration of virus in trigeminal nerve and ganglion by immunofluorescence and electron microscopy. *J Neurol Sci* 1972;**15**:35–48.
63. Feldman S, Chaudary S, Ossi M, et al. A viremic phase for herpes zoster in children with cancer. *J Pediatr* 1977;**91**:597–600.
64. Feldman S, Hughes WT, Daniel CB. Varicella in children with cancer: seventy seven cases. *Pediatrics* 1975;**56**:388–97.
65. Feldman S, Lott L. Varicella in children with cancer: impact of antiviral therapy and prophylaxis. *Pediatrics* 1987;**80**:465–72.
66. Friedman CA, Temple DM, Robbins KK, et al. Outbreak and control of varicella in a neonatal intensive care unit. *Pediatr Infect Dis J* 1994;**13**:152–4.
67. Gaillat J, Gajdos V, Launay O, et al. Does monastic life predispose to the risk of Saint Anthony's fire (herpes zoster)? *Clin Infect Dis* 2011;**53**:405.
68. Galea SA, Sweet A, Beninger P, et al. The safety profile of varicella vaccine: a 10-year review. *J Infect Dis* 2008;**197**(Suppl 2): S165–169.
69. Galil K, Lee B, Strine T, et al. Outbreak of varicella at a day-care center despite vaccination. *N Engl J Med* 2002;**347**:1909–15.
70. Garland J. Varicella following exposure to herpes zoster. *N Engl J Med* 1943;**228**:336–7.
71. Gershon A. Zoster in immunosuppressed patients. In: Watson CPN, Gershon A, editors. *Herpes zoster and postherpetic neuralgia*. Amsterdam: Elsevier; 2001. p.107–17.
72. Gershon A. Chickenpox, measles, and mumps. In: Remington J, Klein J, Wilson C, et al, editors. *Infections of the fetus and newborn infant*. 7th ed. Philadelphia: Elsevier Saunders; 2010.
73. Gershon A, Chen J, LaRussa P, et al. Varicella-zoster virus. In: Murray PR, editor. *Manual of clinical microbiology*. 9th ed. Washington, D.C.: ASM Press; 2007. p. 1537–48.
74. Gershon A, LaRussa P, Steinberg S, et al. The protective effect of immunologic boosting against zoster: an analysis in leukemic children who were vaccinated against chickenpox. *J Infect Dis* 1996;**173**:450–3.
75. Gershon AA. Varicella-zoster virus: prospects for control. *Adv Pediatr Infect Dis* 1995;**10**:93–124.
76. Gershon AA. Varicella vaccine: rare serious problems—but the benefits still outweigh the risks. *J Infect Dis* 2003;**188**:945–7.
77. Gershon AA, Chen J, Davis L, et al. Latency of varicella-zoster virus in dorsal root, cranial nerve, and enteric ganglia in vaccinated children. *Trans Am Clin Climatol Assoc* 2012;**123**:17–33; discussion 33-5.
78. Gershon AA, Gershon MD. Perspectives on vaccines against varicella-zoster virus infections. *Curr Top Microbiol Immunol* 2010; **342**:359–72.
79. Gershon AA, Larussa P, Steinberg S, et al. The protective effect of immunologic boosting against zoster: an analysis in leukemic children who were vaccinated against chickenpox. *J Infect Dis* 1996;**173**:450–3.
80. Gershon AA, Mervish N, LaRussa P, et al. Varicella-zoster virus infection in children with underlying human immunodeficiency virus infection. *J Infect Dis* 1997;**176**:1496–500.
81. Gershon AA, Raker R, Steinberg S. Antibody to varicella zoster virus in parturient women and their offspring during the first year of life. *Pediatrics* 1976;**58**:692–6.
82. Gershon AA, Sherman DL, Zhu Z, et al. Intracellular transport of newly synthesized varicella-zoster virus: final envelopment in the trans-Golgi network. *J Virol* 1994;**68**:6372–90.
83. Gershon AA, Steinberg SP. Persistence of immunity to varicella in children with leukemia immunized with live attenuated varicella vaccine. *N Engl J Med* 1989;**320**:892–7.
84. Gershon AA, Steinberg SP. Live attenuated varicella vaccine: protection in healthy adults compared with leukemic children. National Institute of Allergy and Infectious Diseases Varicella Vaccine Collaborative Study Group. *J Infect Dis* 1990;**161**: 661–6.
85. Gershon AA, Steinberg SP, Borkowsky W, et al. IgM to varicella-zoster virus: demonstration in patients with and without clinical zoster. *Pediatr Infect Dis* 1982;**1**:164–7.
86. Gershon AA, Steinberg SP, Gelb L. Clinical reinfection with varicella-zoster virus. *J Infect Dis* 1984;**149**:137–42.
87. Gershon AA, Steinberg SP, Gelb L, et al. Live attenuated varicella vaccine: efficacy for children with leukemia in remission. *JAMA* 1984;**252**:355–62.
88. Gershon AA, Steinberg SP, LaRussa P, et al. Immunization of healthy adults with live attenuated varicella vaccine. *J Infect Dis* 1988;**158**:132–7.
89. Gilden D, Cohrs RJ, Mahalingam R, et al. Neurological disease produced by varicella zoster virus reactivation without rash. *Curr Top Microbiol Immunol* 2010;**342**:243–53.
90. Gilden DH, Dueland AN, Cohrs R, et al. Preherpetic neuralgia. *Neurology* 1991;**41**:1215–18.
91. Gilden DH, Kleinschmidt-DeMasters BK, LaGuardia JJ, et al. Neurologic complications of the reactivation of varicella-zoster virus. *N Engl J Med* 2000;**342**:635–45.
92. Gold E. Serologic and virus-isolation studies of patients with varicella or herpes-zoster infection. *N Engl J Med* 1966;**274**: 181–5.
93. Gomi Y, Imagawa T, Takahashi M, et al. Oka varicella vaccine is distinguishable from its parental virus in DNA sequence of open reading frame 62 and its transactivation activity. *J Med Virol* 2000;**61**:497–503.
94. Gordon JE. Chickenpox: an epidemiological review. *Am J Med Sci* 1962;**244**:362–89.
95. Goulleret N, Mauvisseau E, Essevaz-Roulet M, et al. Safety profile of live varicella virus vaccine (Oka/Merck): five-year results of the European Varicella Zoster Virus Identification Program (EU VZVIP). *Vaccine* 2010;**28**:5878–82.
96. Gray GC, Palinkas LA, Kelley PW. Increasing incidence of varicella hospitalizations in United States Army and Navy personnel: are today's teenagers more susceptible? Should recruits be vaccinated? *Pediatrics* 1990;**86**:867–73.
97. Grose C. Variation on a theme by Fenner: the pathogenesis of chickenpox. *Pediatrics* 1981;**68**:735–7.
98. Grose C. Glycoproteins encoded by varicella-zoster virus: biosynthesis, phosphorylation, and intracellular trafficking. *Annu Rev Microbiol* 1990;**44**:59–80.
99. Grose C, Litwin V. Immunology of the varicella-zoster virus glycoproteins. *J Infect Dis* 1988;**157**:877–81.
100. Guess HA, Broughton DD, Melton LJ 3rd, et al. Epidemiology of herpes zoster in children and adolescents: a population-based study. *Pediatrics* 1985;**76**:512–17.
101. Gustafson TL, Shehab Z, Brunell PA. Outbreak of varicella in a newborn intensive care nursery. *Am J Dis Child* 1984;**138**: 548–50.
102. Haiduven-Griffiths D, Fecko H. Varicella in hospital personnel: a challenge for the infection control practitioner. *AJIC Am J Infect Control* 1987;**15**:207–11.
103. Hambleton S, Steinberg SP, Larussa PS, et al. Risk of herpes zoster in adults immunized with varicella vaccine. *J Infect Dis* 2008;**197**(Suppl 2):S196–9.

104. Hardy I, Gershon AA, Steinberg SP, et al. The incidence of zoster after immunization with live attenuated varicella vaccine: a study in children with leukemia. Varicella Vaccine Collaborative Study Group. *N Engl J Med* 1991;**325**:1545–50.
105. Haverkos HW, Amsel Z, Drotman DP. Adverse virus-drug interactions. *Rev Infect Dis* 1991;**13**:697–704.
106. Hay J, Ruyechan W. Varicella zoster virus—a different kind of herpesvirus latency? *Semin Virol* 1994;**5**:241–7.
107. Head H, Campbell AW. The pathology of herpes zoster and its bearing on sensory localisation. *Brain* 1900;**23**:353–62.
108. Hodge RAV. Antiviral portrait series: 3. Famciclovir and penciclovir: the mode of action of famciclovir including its conversion to penciclovir [Review]. *Antiviral Chem Chemother* 1993;**4**:67–84.
109. Hope-Simpson RE. The Nature of herpes zoster: a long-term study and a new hypothesis. *Proc R Soc Med* 1965;**58**:9–20.
110. Huang YC, Lin TY, Chiu CH. Acyclovir prophylaxis of varicella after household exposure. *Pediatr Infect Dis J* 1995;**14**:152–4.
111. Huff JC, Bean B, Balfour HH Jr, et al. Therapy of herpes zoster with oral acyclovir. *Am J Med* 1988;**85**:84–9.
112. Ihara T, Starr S, Ito M, et al. Human polymorphonuclear leukocyte–mediated cytotoxicity against varicella-zoster virus–infected fibroblasts. *J Virol* 1984;**51**:110–16.
113. Jacobson MA, Berger TG, Fikrig S, et al. Acyclovir-resistant varicella zoster virus infection after chronic oral acyclovir therapy in patients with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1990;**112**:187–91.
114. Jenkins RB. Severe chickenpox encephalopathy; treatment with intravenous urea, hypothermia, and dexamethasone. *Am J Dis Child* 1965;**110**:137–9.
115. Johnson R, Milbourn PE. Central nervous system manifestations of chickenpox. *Can Med Assoc J* 1970;**102**:831–4.
116. Junker AK, Angus E, Thomas EE. Recurrent varicella-zoster virus infections in apparently immunocompetent children. *Pediatr Infect Dis J* 1991;**10**:569–75.
117. Jura E, Chadwick EG, Josephs SH, et al. Varicella-zoster virus infections in children infected with human immunodeficiency virus. *Pediatr Infect Dis J* 1989;**8**:586–90.
118. Kalman CM, Laskin OL. Herpes zoster and zosteriform herpes simplex virus infections in immunocompetent adults. *Am J Med* 1986;**81**:775–8.
119. Kattan JA, Sosa LE, Bohnwagner HD, et al. Impact of 2-dose vaccination on varicella epidemiology: Connecticut—2005-2008. *J Infect Dis* 2011;**203**:509–12.
120. Kennedy PG. Varicella-zoster virus latency in human ganglia. *Rev Med Virol* 2002;**12**:327–34.
121. Kennedy PG, Cohrs RJ. Varicella-zoster virus human ganglionic latency: a current summary. *J Neurovirol* 2011;**16**:411–18.
122. Kennedy PG, Grinfeld E, Bontems S, et al. Varicella-zoster virus gene expression in latently infected rat dorsal root ganglia. *Virology* 2001;**289**:218–23.
123. Kido S, Ozaki T, Asada H, et al. Detection of varicella-zoster virus (VZV) DNA in clinical samples from patients with VZV by the polymerase chain reaction. *J Clin Microbiol* 1991;**29**:76–9.
124. Kinchington PR, Cohen JI. Viral proteins. In: Arvin AM, Gershon AA, editors. *Varicella-zoster virus: virology and clinical management*. Cambridge: Cambridge University Press; 2000. p. 74–104.
125. Klein NP, Fireman B, Yih WK, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;**126**:e1–8.
126. Ku CC, Besser J, Abendroth A, et al. Varicella-zoster virus pathogenesis and immunobiology: new concepts emerging from investigations with the SCIDhu mouse model. *J Virol* 2005;**79**:2651–8.
127. Ku CC, Zerboni L, Ito H, et al. Varicella-zoster virus transfer to skin by T cells and modulation of viral replication by epidermal cell interferon-alpha. *J Exp Med* 2004;**200**:917–25.
128. Kundratitz K. Experimentelle Übertragung von Herpes Zoster auf den Menschen und die Beziehungen von Herpes Zoster zu *Varicellen Monatsschr Kinderheilkd* 1925;**29**:516–23.
129. Kuter BJ, Weibel RE, Guess HA, et al. Oka/Merck varicella vaccine in healthy children: final report of a 2-year efficacy study and 7-year follow-up studies. *Vaccine* 1991;**9**:643–7.
130. Laforet EG, Lynch CL Jr. Multiple congenital defects following maternal varicella; report of a case. *N Engl J Med* 1947;**236**:534–7.
131. LaRussa P, Lungu O, Hardy I, et al. Restriction fragment length polymorphism of polymerase chain reaction products from vaccine and wild-type varicella-zoster virus isolates. *J Virol* 1992;**66**:1016–20.
132. LaRussa P, Steinberg SP, Seeman MD, et al. Determination of immunity to varicella-zoster virus by means of an intradermal skin test. *J Infect Dis* 1985;**152**:869–75.
133. Leclair JM, Zaia JA, Levin MJ, et al. Airborne transmission of chickenpox in a hospital. *N Engl J Med* 1980;**302**:450–3.
134. Lee BR, Feaver SL, Miller CA, et al. An elementary school outbreak of varicella attributed to vaccine failure: policy implications. *J Infect Dis* 2004;**190**:477–83.
135. Lesko SM. The safety of ibuprofen suspension in children. *Int J Clin Pract Suppl*:50–3. 2003;
136. Leung J, Harpaz R, Baughman AL, et al. Evaluation of laboratory methods for diagnosis of varicella. *Clin Infect Dis* 2010;**51**:23–32.
137. Levin MJ, Dahl KM, Weinberg A, et al. Development of resistance to acyclovir during chronic infection with the Oka vaccine strain of varicella-zoster virus, in an immunosuppressed child. *J Infect Dis* 2003;**188**:954–9.
138. Levin MJ, Smith JG, Kaufhold RM, et al. Decline in varicella-zoster virus (VZV)-specific cell-mediated immunity with increasing age and boosting with a high-hose VZV vaccine. *J Infect Dis* 2003;**188**:1336–44.
139. Lim SM, Song SW, Kim SL, et al. Comparison between of the attenuated BR-Oka and the wild type strain of varicella zoster virus (VZV) on the DNA level. *Arch Pharm Res* 2000;**23**:418–23.
140. Lin TY, Huang YC, Ning HC, et al. Oral acyclovir prophylaxis of varicella after intimate contact. *Pediatr Infect Dis J* 1997;**16**:1162–5.
141. Locksley RM, Flournoy N, Sullivan KM, et al. Infection with varicella-zoster virus after marrow transplantation. *J Infect Dis* 1985;**152**:1172–81.
142. Loparev VN, Argaw T, Krause PR, et al. Improved identification and differentiation of varicella-zoster virus (VZV) wild-type strains and an attenuated varicella vaccine strain using a VZV open reading frame 62-based PCR. *J Clin Microbiol* 2000;**38**:3156–60.
143. Loparev VN, Gonzalez A, Deleon-Carnes M, et al. Global identification of three major genotypes of varicella-zoster virus: longitudinal clustering and strategies for genotyping. *J Virol* 2004;**78**:8349–58.
144. Lungu O, Annunziato PW, Gershon A, et al. Reactivated and latent varicella-zoster virus in human dorsal root ganglia. *Proc Natl Acad Sci U S A* 1995;**92**:10980–4.
145. Lungu O, Panagiotidis CA, Annunziato PW, et al. Aberrant intracellular localization of Varicella-zoster virus regulatory proteins during latency. *Proc Natl Acad Sci U S A* 1998;**95**:7080–5.
146. Mahalingham R, Wellish M, Cohrs R, et al. Expression of protein encoded by varicella-zoster virus open reading frame 63 in latently infected human ganglionic neurons. *Proc Nat Acad Sci U S A* 1996;**93**:2122–4.
147. Mahalingam R, Wellish MC, Dueland AN, et al. Localization of herpes simplex virus and varicella zoster virus DNA in human ganglia. *Ann Neurol* 1992;**31**:444–8.
148. Mahamud A, Marin M, Nickell SP, et al. Herpes zoster–related deaths in the United States: validity of death certificates and mortality rates 1979-2007. *Clin Infect Dis* 2012;**55**:960–6.
149. Marin M, Nguyen HQ, Keen J, et al. Importance of catch-up vaccination: experience from a varicella outbreak, Maine, 2002-2003. *Pediatrics* 2005;**115**:900–5.
150. Marin M, Zhang JX, Seward JF. Near elimination of varicella deaths in the US after implementation of the vaccination program. *Pediatrics* 2011;**128**:214–20.
151. Martin JH, Dohner DE, Wellinghoff WJ, et al. Restriction endonuclease analysis of varicella-zoster vaccine virus and wild-type DNAs. *J Med Virol* 1982;**9**:69–76.
152. Mehta SK, Cohrs RJ, Forghani B, et al. Stress-induced subclinical reactivation of varicella zoster virus in astronauts. *J Med Virol* 2004;**72**:174–9.
153. Mehta SK, Tyring SK, Gilden DH, et al. Varicella-zoster virus in the saliva of patients with herpes zoster. *J Infect Dis* 2008;**197**:654–7.
154. Mendez DB, Sinclair MB, Garcia S, et al. Transplacental immunity to varicella-zoster virus in extremely low birthweight infants. *Am J Perinatol* 1992;**9**:236–8.

155. Merville-Louis MP, Sadzot-Delvaux C, Delree P, et al. Varicella-zoster virus infection of adult rat sensory neurons in vitro. *J Virol* 1989;**63**:3155–60.
156. Michalik DE, Steinberg SP, LaRussa PS, et al. Primary vaccine failure after 1 dose of varicella vaccine in healthy children. *J Infect Dis* 2008;**197**:944–9.
157. Moore DA, Hopkins RS. Assessment of a school exclusion policy during a chickenpox outbreak. *Am J Epidemiol* 1991;**133**: 1161–7.
158. Mori C, Takahara R, Toriyama T, et al. Identification of the Oka strain of the live attenuated varicella vaccine from other clinical isolates by molecular epidemiologic analysis. *J Infect Dis* 1998; **178**:35–8.
159. Myers MG, Connelly BL. Animal models of varicella. *J Infect Dis* 1992;**166**(Suppl 1):S48–50.
160. Nader S, Bergen R, Sharp M, et al. Comparison of cell-mediated immunity (CMI) to varicella-zoster virus (VZV) in children and adults immunized with live attenuated varicella vaccine. *J Infect Dis* 1995;**171**:13–17.
161. Nguyen HQ, Jumaan AO, Seward JF. Decline in mortality due to varicella after implementation of varicella vaccination in the United States. *N Engl J Med* 2005;**352**:450–8.
162. Oxman MN, Levin MJ, Johnson GR, et al. A vaccine to prevent herpes zoster and postherpetic neuralgia in older adults. *N Engl J Med* 2005;**352**:2271–84.
163. Ozaki T, Matsui Y, Asano Y, et al. Study of virus isolation from pharyngeal swabs in children with varicella. *Am J Dis Child* 1989; **143**:1448–50.
164. Ozaki T, Miwata H, Asano Y, et al. Varicella zoster virus DNA in throat swabs of vaccinees. *Arch Dis Child* 1992;**67**:328–9.
165. Pahud BA, Glaser CA, Dekker CL, et al. Varicella zoster disease of the central nervous system: epidemiological, clinical, and laboratory features 10 years after the introduction of the varicella vaccine. *J Infect Dis* 2011;**203**:316–23.
166. Paul N, Jacob ME. An outbreak of cadaver-acquired chickenpox in a health care setting. *Clin Infect Dis* 2006;**43**:599–601.
167. Pearson HE. Parturition varicella-zoster. *Obstet Gynecol* 1964;**23**: 21–7.
168. Pena JA, Pirics ML, DiCaprio HS, et al. Varicella reactivation presenting as shingles and aseptic meningitis in an immunocompetent 11-year-old boy. *Clin Pediatr (Phila)* 2009;**48**:435–7.
169. Pitel PA, McCormick KL, Fitzgerald E, et al. Subclinical hepatic changes in varicella infection. *Pediatrics* 1980;**65**:631–3.
170. Poulsen A, Cabral F, Nielsen J, et al. Varicella zoster in Guinea-Bissau: intensity of exposure and severity of infection. *Pediatr Infect Dis J* 2005;**24**:102–7.
171. Preblud SR. Varicella: complications and costs. *Pediatrics* 1986; **78**:728–35.
172. Preblud SR, Bregman DJ, Vernon LL. Deaths from varicella in infants. *Pediatr Infect Dis* 1985;**4**:503–7.
173. Preblud SR, Nelson WL, Levin M, et al. Modification of congenital varicella infection with VZIG. Presented before the Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, 1986. p. 317.
174. Quinlivan ML, Jensen NJ, Radford KW, et al. Novel genetic variation identified at fixed loci in ORF62 of the Oka varicella vaccine and in a case of vaccine-associated herpes zoster. *Clin Microbiol* 2012;**50**:1533–8.
175. Ragozzino MW, Melton LJ 3rd, Kurland LT, et al. Population-based study of herpes zoster and its sequelae. *Medicine (Baltimore)* 1982;**61**:310–16.
176. Raker RK, Steinberg S, Drusin LM, et al. Antibody to varicella zoster virus in low-birth-weight newborn infants. *J Pediatr* 1978; **93**:505–6.
177. Riegle L, Cooperstock M. Contagiousness of zoster in a day care setting. *Pediatr Infect Dis* 1985;**4**:413.
178. Ross AH. Modification of chicken pox in family contacts by administration of gamma globulin. *N Engl J Med* 1962;**267**: 369–76.
179. Sadzot-Delvaux C, Debrus S, Nikkels A, et al. Varicella-zoster virus latency in the adult rat is a useful model for human latent infection. *Neurology* 1995;**45**:S18–20.
180. Safrin S, Berger TG, Gilson I, et al. Foscarnet therapy in five patients with AIDS and acyclovir-resistant varicella-zoster virus infection. *Ann Intern Med* 1991;**115**:19–21.
181. Safrin S, Crumpacker C, Chatis P, et al. A controlled trial comparing foscarnet with vidarabine for acyclovir-resistant mucocutaneous herpes simplex in the acquired immunodeficiency syndrome. The AIDS Clinical Trials Group. *N Engl J Med* 1991;**325**: 551–5.
182. Saiman L, Larussa P, Steinberg SP, et al. Persistence of immunity to varicella-zoster virus after vaccination of healthcare workers. *Infect Control Hosp Epidemiol* 2001;**22**:279–83.
183. Sawyer MH, Wu YN, Chamberlin CJ, et al. Detection of varicella-zoster virus DNA in the oropharynx and blood of patients with varicella. *J Infect Dis* 1992;**166**:885–8.
184. Schmader KE, Levin MJ, Gnann JW Jr, et al. Efficacy, safety, and tolerability of herpes zoster vaccine in persons aged 50-59 years. *Clin Infect Dis* 2012;**54**:922–8.
185. Schmid DS. Varicella-zoster virus vaccine: molecular genetics. *Curr Top Microbiol Immunol*:323–40. 2010;
186. Seiler HE. A study of herpes zoster particularly in its relationship to chickenpox. *J Hyg (Lond)* 1949;**47**:253–62.
187. Sergeev N, Rubtcova E, Chizikov V, et al. New mosaic subgenotype of varicella-zoster virus in the USA: VZV detection and genotyping by oligonucleotide-microarray. *J Virol Methods* 2006; **136**:8–16.
188. Seward JF, Watson BM, Peterson CL, et al. Varicella disease after introduction of varicella vaccine in the United States, 1995-2000. *JAMA* 2002;**287**:606–11.
189. Seward JF, Zhang JX, Maupin TJ, et al. Contagiousness of varicella in vaccinated cases: a household contact study. *JAMA* 2004;**292**:704–8.
190. Shapiro ED, Vazquez M, Esposito D, et al. Effectiveness of 2 doses of varicella vaccine in children. *J Infect Dis* 2011;**203**: 312–15.
191. Sharrar RG, LaRussa P, Galea SA, et al. The postmarketing safety profile of varicella vaccine. *Vaccine* 2000;**19**:916–23.
192. Shepp DH, Dandliker PS, Meyers JD. Treatment of varicella-zoster virus infection in severely immunocompromised patients: a randomized comparison of acyclovir and vidarabine. *N Engl J Med* 1986;**314**:208–12.
193. Shinefield HR, Black SB, Staehle BO, et al. Vaccination with measles, mumps and rubella vaccine and varicella vaccine: safety, tolerability, immunogenicity, persistence of antibody and duration of protection against varicella in healthy children. *Pediatr Infect Dis J* 2002;**21**:555–61.
194. Smith KJ, Kahlter DC, Davis C, et al. Acyclovir-resistant varicella zoster responsive to foscarnet. *Arch Dermatol* 1991;**127**:1069–71.
195. Son M, Shapiro ED, LaRussa P, et al. Effectiveness of varicella vaccine in children infected with HIV. *J Infect Dis* 2010;**201**: 1806–10.
196. Spiegel R, Miron D, Lumelsky D, et al. Severe meningoencephalitis due to late reactivation of varicella-zoster virus in an immunocompetent child. *J Child Neurol* 2010;**25**:87–90.
197. Srabstein JC, Morris N, Larke RP, et al. Is there a congenital varicella syndrome? *J Pediatr* 1974;**84**:239–43.
198. Srinivasan S, Ahn G, Anderson A. Meningoencephalitis-complicating herpes zoster ophthalmicus infection. *J Hosp Med* 2009;**4**:E19–22.
199. Steinberg SP, Gershon AA. Measurement of antibodies to varicella-zoster virus by using a latex agglutination test. *J Clin Microbiol* 1991;**29**:1527–9.
200. Stern ES. The mechanism of herpes zoster and its relation to chickenpox. *Br J Dermatol Syph* 1937;**49**:264–71.
201. Stevens DA, Ferrington RA, Jordan GW, et al. Cellular events in zoster vesicles: relation to clinical course and immune parameters. *J Infect Dis* 1975;**131**:509–15.
202. Straus SE, Aulakh HS, Ruyechan WT, et al. Structure of varicella-zoster *virus DNA J Virol* 1981;**40**:516–25.
203. Suga S, Yoshikawa T, Ozaki T, et al. Effect of oral acyclovir against primary and secondary viraemia in incubation period of varicella. *Arch Dis Child* 1993;**69**:639–42; discussion 642-633.
204. Triebwasser JH, Harris RE, Bryant RE, et al. Varicella pneumonia in adults: report of seven cases and a review of literature. *Medicine (Baltimore)* 1967;**46**:409–23.
205. Trlifajova J, Bryndova D, Ryc M. Isolation of varicella-zoster virus from pharyngeal and nasal swabs in varicella patients. *J Hyg Epidemiol Microbiol Immunol* 1984;**28**:201–6.

206. Tseng HF, Smith N, Marcy SM, et al. Risk factors of herpes zoster among children immunized with varicella vaccine: results from a nested case-control study. *Pediatr Infect Dis J* 2010;**29**: 205–8.
207. Tsolia M, Gershon AA, Steinberg SP, et al. Live attenuated varicella vaccine: evidence that the virus is attenuated and the importance of skin lesions in transmission of varicella-zoster virus. *J Pediatr* 1990;**116**:184–9.
208. Tyring S, Barbarash RA, Nahlik JE, et al. Famciclovir for the treatment of acute herpes zoster: effects on acute disease and postherpetic neuralgia: a randomized, double-blind, placebo-controlled trial. Collaborative Famciclovir Herpes Zoster Study Group. *Ann Intern Med* 1995;**123**:89–96.
209. Uduman SA, Gershon AA, Brunell PA. Should patients with zoster receive zoster immune globulin? *JAMA* 1975;**234**:1049–51.
210. Vazquez M, LaRussa PS, Gershon AA, et al. The effectiveness of the varicella vaccine in clinical practice. *N Engl J Med* 2001; **344**:955–60.
211. Vazquez M, LaRussa PS, Gershon AA, et al. Effectiveness over time of varicella vaccine. *JAMA* 2004;**291**:851–5.
212. Wagenaar TR, Chow VT, Buranathai C, et al. The out of Africa model of varicella-zoster virus evolution: single nucleotide polymorphisms and private alleles distinguish Asian clades from European/North American clades. *Vaccine* 2003;**21**:1072–81.
213. Watson B, Gupta R, Randall T, et al. Persistence of cell-mediated and humoral immune responses in healthy children immunized with live attenuated varicella vaccine. *J Infect Dis* 1994;**169**: 197–9.
214. Weber DJ, Rutala WA, Hamilton H. Prevention and control of varicella-zoster infections in healthcare facilities. *Infect Control Hosp Epidemiol* 1996;**17**:694–705.
215. Weber DM, Pellecchia JA. Varicella pneumonia: study of prevalence in adult men. *JAMA* 1965;**192**:572–3.
216. Weller TH. Varicella and herpes zoster: changing concepts of the natural history, control, and importance of a not-so-benign virus. *N Engl J Med* 1983;**309**:1434–40.
217. White CJ, Kuter BJ, Hildebrand CS, et al. Varicella vaccine (VARIVAX) in healthy children and adolescents: results from clinical trials, 1987 to 1989. *Pediatrics* 1991;**87**:604–10.
218. Whitley RJ. Therapeutic approaches to varicella-zoster virus infections. *J Infect Dis* 1992;**166**(Suppl 1):S51–57.
219. Whitley RJ, Middlebrooks M, Gnann JW Jr. Acyclovir: the past ten years. *Adv Exp Med Biol* 1990;**278**:243–53.
220. Williams V, Gershon A, Brunell PA. Serologic response to varicella zoster membrane antigens measured by indirect immunofluorescence. *J Infect Dis* 1974;**130**:669–72.
221. Wilson A, Sharp M, Koropchak CM, et al. Subclinical varicella-zoster virus viremia, herpes zoster, and T lymphocyte immunity to varicella-zoster viral antigens after bone marrow transplantation. *J Infect Dis* 1992;**165**:119–26.
222. Wilson GJ, Talkington DF, Gruber W, et al. Group A streptococcal necrotizing fasciitis following varicella in children: case reports and review. *Clin Infect Dis* 1995;**20**:1333–8.
223. Wroblewska Z, Valyi-Nagy T, Otte J, et al. A mouse model for varicella-zoster virus latency. *Microb Pathog* 1993;**15**:141–51.
224. Yamanishi K. Molecular analysis of the Oka vaccine strain of varicella-zoster virus. *J Infect Dis* 2008;**197**(Suppl 2):S45–8.
225. Zaia JA, Leary PL, Levin MJ. Specificity of the blastogenic response of human mononuclear cells to herpes antigens. *Infect Immun* 1978;**20**:646–51.
226. Zerboni L, Nader S, Aoki K, et al. Analysis of the persistence of humoral and cellular immunity in children and adults immunized with varicella vaccine. *J Infect Dis* 1998;**177**:1701–4.
227. Zerr DM, Alexander ER, Duchin JS, et al. A case-control study of necrotizing fasciitis during primary varicella. *Pediatrics* 1999; **103**:783–90.

endemic disease that had come from the west 300 years earlier. During the period from 340 to 1000 CE, the disease was described in Egypt, India, Korea, Japan, southern Europe, and North Africa. A major contributor to the spread of smallpox was the great Islamic expansion across North Africa and into Spain in the 7th and 8th centuries.

By the year 1000 CE, smallpox probably was endemic in populated areas of Europe, Asia, and the African Mediterranean countries. Smallpox was established further in northern Europe by the population movements related to the Crusades. By the 16th century, smallpox was a serious disease in Europe, as indicated by death statistics in Geneva, London, and Sweden. In London during the 17th century, approximately 10 percent of the yearly deaths were attributed to smallpox.

The disease was introduced into the American colonies in 1507 and into Mexico soon thereafter. Epidemics of smallpox posed serious problems for the colonists, as well as for the Native Americans. These epidemics also may have been important in certain stages of the American Revolution.[4,32] Smallpox persisted in the United States and Mexico until the 1940s and 1950s, respectively, despite concerted efforts to eliminate the problem.

Soon after this disease was recognized, the Chinese are reported to have made efforts to prevent it. The technique used presumably was variolation, the intentional intranasal or intracutaneous inoculation of susceptible persons with vesicular fluid or crusts from patients with smallpox. This technique apparently originated in China and subsequently was used in many countries, including the United States. As recently as 1968 and 1969, isolated instances of the use of variolation were noted in remote areas of Africa and Asia.[32,47]

After making his observations in 1796 on the immunity against smallpox that was conferred by inoculation with material obtained from cowpox lesions, Jenner extended his research to include intentional and deliberate exposure of some of those persons immunized with cowpox. This strategy provided convincing evidence of solid protection against smallpox.[50] Encouraged by the results, Jenner predicted in 1801 the ultimate eradication of smallpox, a remarkable prediction indeed.[32,42]

The Intensified Smallpox Eradication Program of the WHO, established in 1967, initiated remarkable progress toward total global eradication of this disease.[41,45] Several factors responsible for this rapid progress included emphasis on surveillance of disease, with containment rather than routine vaccination; improved vaccines and vaccination technology; and sound administrative and fiscal support.[32,33,44,45,54] Of the more than 30 countries in 1967 in which smallpox was endemic, the disease persisted in only 5 in 1975 and in 2 in 1977. The world's last case of endemic smallpox occurred on October 31, 1977, in Merca, Somalia.[26] A year later, in 1978, a photographer working at the University of Birmingham in England was infected with a laboratory strain of smallpox virus and died.[74]

After the worldwide eradication of smallpox was achieved, the World Health Assembly (WHA) in 1980 recommended that all countries cease vaccination.[78] Subsequently, a WHO expert committee recommended that all laboratories throughout the world destroy their stocks of variola virus or transfer them into either of two WHO reference laboratories.[46] The laboratories were the Russian State Research Center on Virology and Biotechnology, Koltsovo, Novosibirsk Region, and the Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia.[6] Reportedly, all countries complied with this recommendation.

The WHO committee at a later date recommended that all variola virus stocks be destroyed by 1999, and the WHA concurred with this recommendation.[8] In 1998, a committee of the Institute of Medicine reviewed the possible importance of retaining variola virus for research purposes.[49] In May 1999, the WHA agreed to postpone the destruction of all variola stocks until 2002.[34] In 2005, the WHA again voted not to destroy smallpox stocks.[70] Most recently, in May 2011, the WHA agreed to postpone setting a date for destruction of smallpox stocks.[71,77] It is to be considered again at the 67th WHA meeting in 2014. At the present time, research using the virus is being conducted in the United States and Russia. This research is related to the development of safer vaccines and to antiviral agents.

In the early 1990s, evidence revealed that during the previous 10 years, the Soviet government apparently had developed a successful program to produce variola virus for use as a biologic weapon.[46,69] In the late 1990s, concern increased with regard to bioterrorism and, in particular, with the possibility that Russian expertise and equipment could have fallen into non-Russian hands because financial support for laboratories and workers had declined.

Smallpox was used as a biologic weapon almost 250 years ago.[24] In the French and Indian Wars (1754 to 1767), British forces sent smallpox-contaminated blankets and handkerchiefs to the Native Americans surrounding Fort Pitt in the summer of 1763.

ETIOLOGY

Smallpox is caused by a variola virus, an orthopoxvirus.[31,45,62] The virus particles are brick shaped and measure 250 to 300 × 200 to 250 × 100 nm; the size and shape are similar for all orthopoxviruses. The distinctive appearance is helpful in rapidly identifying members of this virus group by electron microscopy of vesicular fluid or crusts. The virus is stable when dried and may remain viable for long periods in dried crusts and indefinitely under freeze-drying techniques in the laboratory. It is propagated readily on the chorioallantoic membrane of embryonated eggs and in a variety of mammalian cell cultures.

EPIDEMIOLOGY

In infected patients, variola virus was found in respiratory secretions and in vesicular fluid from skin lesions. Transmission usually occurred after close personal contact, but airborne spread also could occur.[5,45,46,74] Patients with

smallpox were most infectious from the onset through the first 7 to 10 days of rash.

All persons were susceptible to smallpox unless they previously had been infected with variola virus itself or with cowpox or vaccinia virus. Thus, the occurrence of smallpox depended entirely on the presence of a human source of infection and effective exposure of susceptible persons. The age distribution of cases varied in individual countries, depending largely on vaccination practices. Preschool-aged children often were not vaccinated and thus had the greatest prevalence of disease.

The seasonal influence was substantial and perhaps is shown best by the spread of disease after the winter and spring introduction of smallpox into Europe; spread of infection was more than 30 times as great after the introduction of infection into European nations between December and May, in contrast to substantially less frequent spread after introduction between June and November.[79]

Substantial contrast in case-fatality rates was recognized in different geographic areas; these differences did not seem to depend on the supportive care available and did not appear to be related to ethnic or specific resistance factors. The only logical explanation for these different rates was substantial differences in the virulence of the virus prevalent in the regions. The mildest form of smallpox, described as *variola minor*, or *alastrim*, was prevalent in Brazil, Ethiopia, and adjacent countries. Case-fatality rates of 1 percent or less were customary, and permanent residual scarring and blindness were most unusual occurrences. In other areas of Africa and Indonesia, the disease appeared to be of intermediate severity, whereas on the Indian subcontinent, case-fatality rates were as high as 30 percent, with residual permanent scarring, blindness, and other sequelae occurring frequently in those who survived the acute illness.[32]

PATHOLOGY

The characteristic pathologic lesions affected the skin and mucous membranes. They involved the deeper layers of the skin and progressed through macular, papular, vesicular, and pustular stages, with the subsequent formation of crusts. Lesions similar to those on the skin also were found in the lower respiratory and gastrointestinal tracts.

Secondary bacterial infection occurred frequently and often affected the skin and lungs. Hemorrhagic complications (hemorrhagic smallpox) were not uncommon events.

CLINICAL MANIFESTATIONS[28,45,53,61]

After an incubation period of 7 to 17 days, the disease began abruptly with fever (i.e., temperatures between 38.9° and 40.5°C [102° and 105°F]), headache, and marked malaise.[8] Backache and muscle pain were prominent symptoms. Nausea, vomiting, and abdominal pain also were present.

After these prodromal signs and symptoms were present for 2 to 4 days, the fever usually decreased, and the characteristic cutaneous eruption appeared. The rash was most extensive on the face and extremities, and the individual lesions passed through the stages of macules and papules; by the third or fourth day, they clearly were vesicular. Lesions in a single area of the body were characteristically at the same stage of development.

By the sixth day, the vesicular fluid usually was cloudy and the individual pustules frequently were umbilicated. Pustules often converged and become confluent. By the 10th day, the individual lesions began to dry and formed characteristic crusts that remained intact for several days before they were shed. The patient's temperature customarily fell during the early appearance of the rash, although the fever usually returned. Significant fever that persisted after the 10th or 12th day of disease suggested the presence of bacterial superinfection.

Although most cases were similar to this description, which was characteristic of the ordinary type of smallpox, other clinical variations were described. The hemorrhagic type was the most severe form, with a case-fatality rate that approached 100 percent. In this form, hemorrhagic manifestations appeared during the prodromal stage, with extensive cutaneous extravasation of blood and bleeding from the various body orifices. Death usually occurred within the first week of illness, and, frequently few typical diagnostic lesions appeared on the skin surfaces before death ensued. Fortunately, this form of disease occurred in only 2 or 3 percent of the total cases of variola major in Asia, and it rarely was seen elsewhere.

A flat variety had been reported in approximately 6 percent of cases observed in India. In this variety, the cutaneous lesions remained flat and soft to the touch, in contrast to the ordinary variety; these lesions characteristically resolved without pustulation. This form was associated with case-fatality rates of 75 to 96 percent.[32]

A modified form of disease occurred almost exclusively in previously vaccinated persons. Although the prodromal illness often was severe, skin lesions were few, evolved rapidly, and were more superficial. The prognosis was excellent.

The mildest form of the disease, alastrim, or variola minor, was caused by a specific variola virus having less pathogenicity in humans. Serious forms of the illness, as with variola major, were unusual occurrences. The skin lesions tended to be superficial, and the clinical course resembled that of varicella, with the exception of the distribution of cutaneous lesions. These lesions involved the face and extremities, in contrast to the characteristic central body distribution of varicella. Residual scarring, if it occurred at all, usually reflected secondary bacterial infection of individual lesions.

In recently vaccinated persons, asymptomatic infection with variola virus after exposure had been demonstrated by increases in antibody titer against the virus when acute and convalescent sera were tested. This uncommon event was of neither clinical nor epidemiologic significance.

Complications included hemorrhagic events and various secondary bacterial infections, including impetigo,

pneumonia, empyema, and otitis media. Nephritis and arthritis with permanent joint changes were described.

DIFFERENTIAL DIAGNOSIS

The typical course, the characteristic cutaneous lesions, and the presence of other cases after contact 7 to 17 days earlier left little doubt about the etiologic agent. Varicella presented the greatest problem in differential diagnosis, particularly from the variola minor, or alastrim, form of the disease, but the distribution of cutaneous lesions for each condition was characteristic. Generalized vaccinia or eczema vaccinatum was distinguished by a history of exposure to vaccinia virus, previous skin lesions, and the distribution of the rash. Impetigo (especially the bullous variety caused by staphylococcal infection), scabies, secondary syphilis, and yaws were other considerations. Usually, little difficulty was encountered clinically in distinguishing among these infections.

Monkeypox can be clinically similar to smallpox, and, therefore, specific laboratory procedures are required for diagnosis (see Chapter 163). Other conditions included in the differential diagnosis were erythema multiforme, pityriasis rosea, measles, rickettsialpox, disseminated herpes simplex infection, syphilis, enteroviral exanthems, and bacterial, viral, and rickettsial petechial and purpuric rashes.

SPECIFIC DIAGNOSIS

Several laboratory procedures are available to provide specific and accurate diagnosis. Vesicular fluid, crusts, or scrapings from skin lesions reveal the characteristic brick-shaped viral particles of the variola-vaccinia virus group when examined by electron microscopy. These orthopoxviruses differ in appearance from the herpesviruses, which in the past were the agents most frequently confused with smallpox. Electron microscopy is precise and rapid and is preferred when facilities for this examination are available.[23] Smallpox virus DNA also can be identified rapidly by polymerase chain reaction (PCR).[28,29,52,66] PCR has an advantage over electron microscopy in that it can specifically distinguish smallpox virus from the orthopoxviruses.

Orthopoxviruses may be recovered and propagated on the chorioallantoic membrane of embryonated eggs or in tissue culture.[28] Virus recovery requires more time (3 to 7 days) than the direct antigen-detection tests, but it provides the specific active virus required for differentiation among the various orthopoxvirus types.[63,65] Infection also can be determined serologically by using acute-phase and convalescent-phase sera in enzyme-linked immunosorbent assay (ELISA), Western blotting, or virus neutralization assays.[28]

The specific diagnosis of a disease that does not currently exist anywhere in the world presents unique challenges. Recognizing these challenges, the CDC has a diagnosis and evaluation plan with laboratory testing recommendations (http://emergency.cdc.gov/agent/smallpox/diagnosis).

TREATMENT

Therapy primarily was supportive and symptomatic. The skin was kept clean, the bed linen was changed at regular intervals, and local or systemic therapy was provided for the frequent bacterial complications. Attention given to appropriate fluid and nutritional support was required.

Methisazone, convalescent smallpox serum, and vaccinia immune globulin (VIG) were effective in preventing the disease after exposure, but no evidence showed that these agents altered the course of the disease once symptoms occurred.[51] Idoxuridine was used for corneal lesions.[4]

At the present time the United States has a stockpile of VIG, which is produced by Cangene Corporation in Canada (http://www.cdc.gov/laboratory/drugservice/formulary.html).[76] This product is presently available for the treatment of smallpox vaccination complications (i.e., eczema vaccinatum, vaccinia necrosum, or severe generalized vaccinia). In the event of a biological terrorism smallpox release, it is assumed that VIG would be available for treatment.

Studies with the antivirals cidofovir and ST-246 show broad antiviral activity against orthopoxviruses.[1,20,61] Through the *Project Bioshield Act* of 2004, 1.7 million doses of ST-246 have been stockpiled.

PREVENTION

Active Immunization

In the 20th century, many strains of vaccinia virus were used in the effective prophylaxis of smallpox, which led to the control and then eradication of smallpox from the world. After smallpox was eliminated from the United States and Western Europe, general concern shifted to morbidity and mortality resulting from smallpox vaccines.[38,56,57] It was realized that the risk for smallpox was remote and that vaccine reactions were significant (Table 162-1). Because of this, routine smallpox vaccination was discontinued in the United States in the 1970s.

In the late 1990s, concerns arose that smallpox could be used as a weapon of bioterrorism. The events of September 11, 2001 and immediately thereafter highlighted the potential reality of this threat.* Estimates were that probably less than 20 percent of the U.S. population had any immunity.[43] These concerns led to recommendations by the Advisory Committee on Immunization Practices (ACIP) for the use of smallpox vaccine to protect persons who may need to work with orthopoxviruses and to prepare for a possible bioterrorism attack.[13] The government took steps to prepare the country in the event of a bioterrorist attack involving smallpox. These measures included building an adequate supply of vaccine, enhancing the laboratory capacity for diagnosis of variola and vaccinia virus infection, and training health care and public health personnel.[37]

*References 2, 20, 42, 43, 46, 60, 72.

TABLE 162-1 **Rates of Reported Complications Associated with Vaccinia Vaccination (Cases/Million Vaccinations)**

Age (yr) and Status Total*	Inadvertent Inoculation	Generalized Vaccinia	Eczema Vaccinatum	Progressive Vaccinia	Postvaccinial Encephalitis	Total
Primary						
<1	507.0	394.4	14.1	—†	42.3	1549.3
1-4	577.3	233.4	44.2	3.2	9.5	1261.8
5-19	371.2	139.7	34.9	—	8.7	855.9
≥20	606.1	212.1	30.3	—	—	1515.2
Overall rates	529.2	241.5	38.5	1.5	12.3	1253.8
Revaccination						
<1	—	—	—	—	—	—
1-4	109.1	—	—	—	—	200.0
5-19	47.7	9.9	2.0	—	—	85.5
≥20	25.0	9.1	4.5	6.8	4.5	113.6
Overall rates	42.1	9.0	3.0	3.0	2.0	108.2

From Lane JM, Ruben FL, Neff JM., et al. Complications of smallpox vaccination, 1968: results of 10 statewide surveys. *J Infect Dis* 1970;**122**:303–9.

*Rates of overall complications by age group include complications not provided in this table, including severe local reactions, bacterial superinfection of the vaccination site, and erythema multiforme.

†No instances of this complication were identified during the 1968 10-state survey.

Through the *Project BioShield Act*, the United States has an ample supply of smallpox vaccine.[64] This is presently available for immunization of selected people (e.g., first responders and laboratory workers) and would be available if a bioterrorist attack were to occur. This stockpile includes ACAM 2000, which is prepared in cell culture, and a modified vaccinia, Ankara (MVA), a new vaccine. This vaccine is thought to be safer than conventional smallpox vaccines and it was purchased by the United States to be used in immunocompromised persons if a terrorist smallpox release occurred.[42,44]

The primary strategy used in the past to control an outbreak of smallpox and to interdict the transmission of virus was known as a *surveillance and containment (ring vaccination)* strategy. This strategy identified infected persons through intensive surveillance and subsequently both isolated the individuals with the disease and vaccinated household and close contacts of the infected patient. Vaccination also was recommended for contacts of the primary contact with the patient (secondary contacts). This strategy was instrumental in the eradication of smallpox as a naturally occurring disease. Depending on the size of any smallpox outbreak and the availability of resources for rapid and thorough contact tracing, ring vaccination was supplemented previously in areas with identified smallpox cases to include voluntary vaccination of all individuals without vaccine contraindications. This strategy was pursued to expand the ring of immune individuals within an outbreak area and to reduce further the chance of any secondary transmission of smallpox to patients before they could be identified and isolated.

The CDC developed protocols to permit rapid, simultaneous delivery of smallpox vaccine to every state in U.S. territory within 12 to 24 hours. Smallpox response planning at the federal level has been conveyed to state and local health departments to address the rapid distribution of the vaccine if vaccination programs are suggested.

The CDC currently recommends that cases of febrile rash illnesses for which smallpox is considered in the differential diagnosis be reported immediately to local or state health departments or both. After evaluation is performed by local or state health departments, if smallpox laboratory diagnostics are considered necessary, the CDC Rash Illness Evaluation Team should be consulted. At this time, laboratory confirmation for smallpox is available routinely only at the CDC. Both a clinical consultation and a preliminary laboratory diagnosis can be completed within a period of 8 to 24 hours.

In the present bioterrorism era the CDC and the ACIP have devoted considerable effort into the diagnosis of smallpox and have looked at risks and benefits of smallpox immunization programs. To assist public health and other medical personnel in evaluating the possibility of smallpox in patients with febrile rash illnesses, the CDC developed a rash illness assessment algorithm (http://emergency.cdc.gov/agent/smallpox/diagnosis/riskalgorithm/).

Medical evaluation of the risks for acquiring smallpox under current conditions and in the absence of a case of smallpox or a confirmed smallpox bioterrorism threat in contrast to the potential risks for developing vaccine complications was such that the ACIP concluded that vaccination of the general population was not recommended because the potential benefits of vaccination did not outweigh the risks for vaccine complications. In 2001, the ACIP recommended smallpox vaccine for laboratory workers who directly handle (1) cultures or (2) animals contaminated or infected with vaccinia, recombinant vaccinia viruses, or other orthopoxviruses that infect humans (e.g., monkeypox and cowpox).[13] Other health care workers (e.g., physicians and nurses) whose contact with these viruses is limited to contaminated material (e.g., dressings) but who adhere to appropriate infection control measures are at lower risk for inadvertently acquiring infection than are laboratory workers. However,

because a theoretical risk for developing infection does exist, vaccination may be considered for this group. Because of the low risk for developing infection, vaccination was not recommended for persons who do not handle virus cultures or materials directly or who do not work with animals contaminated or infected with these viruses. According to available data on the persistence of neutralizing antibody after vaccination, persons working with vaccinia, recombinant vaccinia viruses, or other nonvariola orthopoxviruses should be revaccinated every 10 years.

In 2003, the ACIP and the Healthcare Infection Control Practices Advisory Committee (HICPAC) published supplemental recommendations for using smallpox vaccine in a pre-event vaccination program.[15] A summary of these recommendations is presented here:

To facilitate preparedness and response, smallpox vaccination is recommended for persons designated by public health authorities to conduct investigation and follow-up of initial smallpox cases that could necessitate direct patient contact.[15] The ACIP recommended that each state and territory establish and maintain more than one smallpox response team. The ACIP and the HICPAC recommended that each acute-care hospital identify health care workers who can be vaccinated and trained to provide direct medical care for the first smallpox patients requiring hospital admission and to evaluate and manage patients who are suspected as having smallpox. When feasible, the first-stage vaccination program was to include previously vaccinated health care personnel to decrease the potential for adverse events. Additionally, persons administering smallpox vaccine in this pre-event vaccination program were to be vaccinated.

Side Effects and Adverse Events of Smallpox Vaccines

The risk for adverse events resulting from smallpox vaccine today is presumed potentially to be at least as great, in terms of severity and frequency of events, as during the era when universal vaccinia vaccination was performed.[16,38] One can suspect that the number of adverse reactions may be increased in frequency in the current era in contrast to the 1960s and 1970s because a larger proportion of the population of the United States and the world may have an inherited or acquired a form of immunodeficiency or may be receiving a variety of agents that suppress the immune system. Factors thought to predispose to development of adverse events include pregnancy or breast-feeding, extensive skin eruptions, atopic dermatitis, T-cell immune defect, immunosuppressive therapy, inflammatory or disruptive diseases of the cornea, and age younger than 1 year.[16]

Fever occurs commonly after smallpox vaccination. As many as 70 percent of children have 1 or more days with a temperature of 37.8° C (100° F) or higher 4 to 14 days after receiving primary vaccination,[18,59] and 15 to 20 percent have temperatures of 38.9° C (102° F) or higher. After receiving revaccination, 35 percent of children have temperatures of 37.8° C (100° F) or higher, and 5 percent have temperatures of 38.9° C (102° F) or higher.[59] Fever after vaccination or revaccination occurs less commonly in adults than in children.

An erythematous or urticarial rash may occur approximately 10 days after receipt of primary vaccination. The vaccinee usually is afebrile, and the rash resolves spontaneously within 2 to 4 days. Rarely, bullous erythema multiforme (Stevens-Johnson syndrome) occurs.[53]

Inadvertent inoculation at other sites, the most frequent complication of vaccinia vaccination, accounts for approximately half of all complications of primary vaccination and revaccination.[17] Inadvertent inoculation usually results from autoinoculation of vaccine virus transferred from the site of vaccination. The most common sites involved are the face, eyelid, nose, mouth, genitalia, and rectum. Most lesions heal without specific therapy, but VIG may be useful for cases of ocular implantation.

Generalized vaccinia in persons who have no underlying illness is characterized by a vesicular rash of varying extent. The rash generally is self-limited and requires little or no therapy, except in patients whose conditions appear to be toxic or in those who have a serious underlying illness.

Other expected systemic symptoms associated with smallpox vaccination include malaise, myalgia, headache, chills, nausea, and fatigue (0.3% to 37%).[35,36] Soreness at the site of the vaccination is a very common development, as are local lymphadenopathy and erythema surrounding the vaccination site.[37]

Expected local events occurring relatively infrequently require symptomatic treatment. They include satellite lesions within 2.5 cm of the primary lesion, viral lymphangitis, local edema, and intense inflammation surrounding the papule.[35-37]

More severe complications of vaccinia vaccination include eczema vaccinatum, progressive vaccinia, myopericarditis, and postvaccinial encephalitis. These complications, with the exception of myopericarditis, occur at least 10 times more often among primary vaccinees than among revaccinees and more frequently among infants than among older children and adults.[55-57]

The number of adverse events per million doses of primary smallpox vaccination and revaccination is reported in Table 162-1. The rates described are based on surveys of vaccination-associated complications that occurred in the late 1960s in the United States. The high prevalence of immunosuppression in the United States today in contrast to the late 1960s may render the rates of vaccinia necrosum higher than in 1968. The rate of postvaccinial encephalitis in adults was difficult to document in the 1960s because very few adults were primary vaccinees. Studies performed in Europe showed higher rates of postvaccinial encephalitis in adults than in young children. Studies performed since 2002, when efforts were made to vaccinate members of the military and civilians, have shown similar rates of adverse reactions, although cardiac side effects have been more notable (Table 162-2).

Eczema vaccinatum is a localized or systemic dissemination of vaccinia virus in persons who have eczema or a history of eczema and other chronic or exfoliative skin conditions (e.g., atopic dermatitis). The illness often is

TABLE 162-2 Number of Reported Events Associated with Smallpox Vaccination in the Military: 2002 to 2007

Event Type	Department of Defense Experience*	Grabenstein and Winkenwerder†
Inadvertent inoculation	61	Self: 48 Transfer to contact: 21
Generalized vaccinia	43	36
Eczema vaccinatum	1	0
Progressive vaccinia	0	0
Myopericarditis	140	37
Ischemic heart disease	16	N/A
Encephalitis	N/A	1
Treated with VIG	6	N/A
Death‡	8	0

*Department of Defense experience: December, 2002 to May, 2007, 1,200,000 million vaccinees.[28]
†Experience of U.S. military: December, 2002 to May, 2003, 450,293 vaccinees.[50]
‡After review, one death was the result of an acute lupus-like illness that may have been caused by the vaccine, and others were thought to result from other causes.[28]
NA, Not applicable; *VIG,* vaccinia immune globulin.

mild and self-limited but may be severe and occasionally fatal. The most serious cases in vaccine recipients occur in primary vaccinees and appear to be independent of the activity of the underlying eczema.[73] Severe cases also have been observed after contact infection has occurred.

Progressive vaccinia (vaccinia necrosum) is a severe, potentially fatal illness characterized by progressive necrosis in the area of vaccination, often with metastatic lesions. It occurs almost exclusively in persons with cellular immunodeficiency.

In the 1960s, cardiac complications were reported but were not considered significant. Myopericarditis now is thought to be a true adverse event related to smallpox vaccine, although most cases resolve without further complications.[9,27] Cases of ischemic heart disease occurring after receipt of smallpox vaccination also have been reported. Although this association has not been shown to be causal, the ACIP recommended excluding persons with cardiac disease from participation in current smallpox vaccination programs.[15] The incidence of cardiac events in children is not known because the recent vaccination program has targeted adults.

The most serious complication is postvaccinial encephalitis. Usually, it affected primary vaccinees younger than 1 year of age. Of affected vaccinees with this complication, 15 to 25 percent died and 25 percent had permanent neurologic sequelae.[40,56,57]

Death rarely occurred after receipt of vaccinia vaccination; reports note approximately one to two deaths per million primary vaccinations and 0.1 death per million revaccinations. Death most often was the result of postvaccinial encephalitis or progressive vaccinia.

Vaccinia may be transmitted when a recently vaccinated person has contact with a susceptible person. In the CDC's 10-state survey of complications of smallpox vaccination, the risk for transmission to contacts was 27 infections per million total vaccinations; 44 percent of these contact cases occurred in children 5 years of age or younger.[57] Since 1980, several cases of contact transmission of vaccinia from vaccinated military recruits have been reported and include six cases transmitted by a single vaccine recipient.[10-12]

Since 2001, among 1,200,000 vaccinated military personnel, 61 suspected instances of transfer of vaccinia occurred, 36 of which were laboratory confirmed.[25] This number included two cases of tertiary transfer, one from a service member to his wife and then to their breastfeeding baby and another among male sports partners.[15,37] Fetal vaccinia occurs rarely (<40 reported cases), but usually it is fatal.[38]

More than 60 percent of cases of contact transmission result in uncomplicated inadvertent inoculation. Approximately 30 percent of these cases result in eczema vaccinatum, which may be fatal.[57] Eczema vaccinatum may be more severe in contacts than in vaccinated persons, possibly because of simultaneous multiple inoculation at several sites.[21,56] Contact transmission rarely results in postvaccinial encephalitis or vaccinia necrosum.

Smallpox Vaccine Availability

The CDC is the only source of smallpox vaccine and VIG for civilians. In the advent of need to carry out smallpox vaccination, procedural information will be available from the CDC.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Andrei G, Snoeck R. Cidofovir activity against poxvirus infections. *Viruses* 2010;**2**:2803–30.
64. Nalca A, Zumbrun EE. ACAM2000: the new smallpox vaccine for United States Strategic National Stockpile. *Drug Des Devel Ther* 2010;**4**:71–9.
77. World Health Assembly. *Decision on smallpox virus destruction delayed for three years*. May 27, 2011. Accessed. Available at: http://www.homelandsecuritynewswire.com/decision-smallpox-virus-destruction-delayed-three-years.
79. World Health Organization. human monkeypox: the past five years. *WHO Chron* 1984;**38**:227–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Andrei G, Snoeck R. Cidofovir activity against poxvirus infections. *Viruses* 2010;**2**:2803–30.
2. Barbera J, Macintyre A, Gostin L, et al. Large-scale quarantine following biological terrorism in the United States: scientific examination, logistic and legal limits, and possible consequences. *JAMA* 2001;**286**:2711–17.
3. Behbehani AM. The smallpox story: historical perspective—globally eradicated today, smallpox once killed scores of people over thousands of years. *ASM News* 1991;**57**:571–6.
4. Benenson AS. Smallpox. In: Wehrle PF, Top FH, editors. *Communicable and infectious diseases*. 9th ed. St. Louis: C. V. Mosby; 1982. p. 577–88.
5. Bhatnagar V, Stoto MA, Morton SC, et al. Transmission patterns of smallpox: systematic review of natural outbreaks in Europe and North America since World War II. *BMC Public Health* 2006;**6**:126.
6. Breman JG, Arita I. The confirmation and maintenance of smallpox eradication. *N Engl J Med* 1980;**303**:1263–73.
7. Breman JG, Henderson DA. Poxvirus dilemmas: monkeypox, smallpox, and biologic terrorism. *N Engl J Med* 1998;**339**:556–9.
8. Breman JG, Henderson DA. Diagnosis and management of smallpox. *N Engl J Med* 2002;**346**:1300–8.
9. Cassimatis DC, Atwood JE, Engler RM, et al. Smallpox vaccination and myopericarditis: a clinical review. *J Am Coll Cardiol* 2004;**43**:1503–10.
10. Centers for Disease Control. Vaccinia necrosum after smallpox vaccination: Michigan. *MMWR Morb Mortal Wkly Rep* 1982;**31**:501–2.
11. Centers for Disease Control. Contact spread of vaccinia from a recently vaccinated Marine: Louisiana. *MMWR Morb Mortal Wkly Rep* 1984;**33**:37–8.
12. Centers for Disease Control. Contact spread of vaccinia from a National Guard vaccinee: Wisconsin. *MMWR Morb Mortal Wkly Rep* 1985;**34**:182–3.
13. Centers for Disease Control and Prevention. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:1–26.
14. Centers for Disease Control and Prevention. 25th Anniversary of the last case of naturally acquired smallpox. *MMWR Morb Mortal Wkly Rep* 2002;**51**:952.
15. Centers for Disease Control and Prevention. Recommendations for using smallpox vaccine in a pre-event vaccination program: supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Healthcare Infection Control Practices Advisory Committee (HICPAC). *MMWR Morb Mortal Wkly Rep* 2003;**52**:1–16.
16. Centers for Disease Control and Prevention. Smallpox vaccination and adverse reactions: guidance for clinicians. *MMWR Morb Mortal Wkly Rep* 2003;**52**:1–28.
17. Centers for Disease Control and Prevention. Secondary and tertiary transfer of vaccinia virus among U.S. military personnel: United States and worldwide, 2002-2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:103–5.
18. Cherry JD, McIntosh K, Connor JD, et al. Primary percutaneous vaccination. *J Infect Dis* 1977;**135**:145–54.
19. Cohen J. Smallpox vaccinations: how much protection remains? *Science* 2001;**294**:985.
20. Cohen J, Marshall E. Vaccines for biodefense: a system in distress. *Science* 2001;**294**:498–501.
21. Coperman PWM, Wallace HJ. Eczema vaccinatum. *Br Med J* 1964;**2**:906.
22. Crosby AW. Smallpox. In: Kiple KF, editor. *The Cambridge world history of human diseases*. New York: Cambridge University Press; 1993. p. 1008–13.
23. Cruickshank JG, Bedson HS, Watson DH. Electron microscopy in the rapid diagnosis of smallpox. *Lancet* 1966;**2**:527–30.
24. d'Errico P. *Jeffrey Amherst and smallpox blankets: Lord Jeffrey Amherst's letters discussing germ warfare against American Indians*. Available at http://www.nativeweb.org/pages/legal/amherst/lord_jeff.html.
25. Department of Defense. *Smallpox vaccination program*. Available at http://www.smallpox.army.mil/event.SPSafetySum/asp.
26. Deria A, Jezek Z, Markvart K, et al. The world's last endemic case of smallpox: surveillance and containment measures. *Bull World Health Organ* 1980;**58**:279–83.
27. Eckart RE, Love SS, Atwood JE, et al. Incidence and follow-up of inflammatory cardiac complications after smallpox vaccination. *J Am Coll Cardiol* 2004;**44**:201–5.
28. Esposito JJ, Fenner F. Poxviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2885–920.
29. Esposito JJ, Massung RF. Poxvirus infections in humans. In: Murray PR, Tenover F, Baron EJ, editors: *Clinical microbiology*. Washington, D.C.: American Society of Microbiology; 1995. p. 1131–8.
30. Fenner F. Smallpox, "the most dreadful scourge of the human species:" its global spread and recent eradication (2). *Med J Aust* 1984;**141**:728–35.
31. Fenner F. Poxviruses. In: Fields BN, Knipe DM, Howley PM, et al., editors. *Fields virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996. p. 2673–702.
32. Fenner F, Henderson DA, Arita I, et al. *Smallpox and its eradication*. Geneva: World Health Organization; 1988.
33. Foege WH, Millar JD, Lane JM. Selective epidemiologic control in smallpox eradication. *Am J Epidemiol* 1971;**94**:311–15.
34. Fox JL. Current topics: World Health Assembly delaying smallpox destruction. *ASM News* 1999;**65**:464–5.
35. Frey SE, Couch RB, Tacket CO, et al. Clinical responses to undiluted and diluted smallpox vaccine. *N Engl J Med* 2002;**346**:1265–74.
36. Frey SE, Newman FK, Cruz J, et al. Dose-related effects of smallpox vaccine. *N Engl J Med* 2002;**346**:1275–80.
37. Fulginiti VA, Papier A, Lane JM, et al. Smallpox vaccination: a review. I. Background, vaccination technique, normal vaccination and revaccination, and expected normal reactions. *Clin Infect Dis* 2003;**37**:241–50.
38. Fulginiti VA, Papier A, Lane JM, et al. Smallpox vaccination: a review. II. Adverse events. *Clin Infect Dis* 2003;**37**:251–71.
39. Geddes AM. The history of smallpox. *Clin Dermatol* 2006;**24**:152–7.
40. Goldstein JA, Neff JM, Lane JM, et al. Smallpox vaccination reactions, prophylaxis, and therapy of complications. *Pediatrics* 1975;**55**:342–7.
41. Henderson DA. Eradication of smallpox: the critical year ahead. *Proc R Soc Med* 1973;**66**:493–500.
42. Henderson DA. The looming threat of bioterrorism. *Science* 1996;**283**:1279–82.
43. Henderson DA. Countering the posteradication threat of smallpox and polio. *Clin Infect Dis* 2002;**34**:79–83.
44. Henderson DA, Arita I, Shafa E. *Studies of the bifurcated needle and recommendations for its use*. Document SE/72.5. Geneva: World Health Organization Smallpox Eradication Unit; 1972.
45. Henderson DA, Borio LL, Lane JM. Smallpox and vaccinia. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 4th ed. Philadelphia: WB Saunders; 2004. p. 123–53.
46. Henderson DA, Inglesby TV, Bartlett JG, et al. Smallpox as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 1999;**281**:2127–37.
47. Hopkins DR. *Princes and peasants: smallpox in history*. Chicago: University of Chicago Press; 1983.
48. Huth E. Quantitative evidence for judgments on the efficacy of inoculation for the prevention of smallpox: England and New England in the 1700s. *J R Soc Med* 2006;**99**:262–6.
49. Institute of Medicine. *Assessment of future scientific need for live variola virus*. Washington, D.C.: National Academies Press; 1999.
50. Jenner E. *The origin of the vaccine inoculation*. London: D. N. Shury; 1801. Cited in Crookshank EM. *History and pathology of vaccination*. Vol. II. London: HK Lewis; 1889. p. 276.
51. Kempe CH, Berge TO, England B. Hyperimmune vaccinal gamma globulin; source, evaluation, and use in prophylaxis and therapy. *Pediatrics* 1956;**18**:177–88.
52. Knight JC, Massung RF, Esposito JJ. Polymerase chain reaction identification of smallpox virus. In: Becker Y, Darai G, editors. *PCR: Protocols for diagnosis of human and animal viral disease*. Heidelberg: Springer-Verlag; 1995. p. 297–302.
53. Krugman S, Ward R. Smallpox and vaccinia. In: Krugman S, Ward, R, editors. *Infectious disease of children*. 4th ed. St. Louis: CV Mosby; 1968. p. 314–34.
54. Ladnyj ID, Ziegler P, Kima E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bull World Health Organ* 1972;**46**:593–7.

55. Lane JM, Millar JD, Neff JM. Smallpox and smallpox vaccination policy. *Annu Rev Med* 1971;**22**:251–72.
56. Lane JM, Ruben FL, Neff JM, et al. Complications of smallpox vaccination, 1968. *N Engl J Med* 1969;**281**:1201–8.
57. Lane JM, Ruben FL, Neff JM, et al. Complications of smallpox vaccination, 1968: results of ten statewide surveys. *J Infect Dis* 1970; **122**:303–9.
58. Longini IM Jr, Halloran ME, Nizam A, et al. Containing a large bioterrorist smallpox attack: a computer simulation approach. *Int J Infect Dis* 2007;**11**:98–108.
59. McIntosh K, Cherry JD, Benenson AS, et al. Standard percutaneous revaccination of children who receive primary percutaneous vaccination. *J Infect Dis* 1977;**135**:155–66.
60. Meltzer MI, Damon I, LeDuc JW, et al. Modeling potential responses to smallpox as a bioterrorist weapon. *Emerg Infect Dis* 2001;**7**:595–969.
61. Moore ZS, Seward JF, Lane JM. Smallpox. *Lancet* 2006;**367**: 425–35.
62. Moss B. Poxviridae: The viruses and their replication. In: Knipe DM, Howley PM, editors. Fields virology, 4th ed. **vol. 2**. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2849–83.
63. Nakano JH, Bingham PG. Smallpox, vaccinia, and human infections with monkeypox virus. In: Lennette EH, Spaulding EH, Truant JP, editors. *Manual of clinical microbiology*. 2nd ed. Washington, D.C.: American Society for Microbiology; 1974. p. 782–94.
64. Nalca A, Zumbrun EE. ACAM2000: the new smallpox vaccine for United States Strategic National Stockpile. *Drug Des Devel Ther* 2010;**4**:71–9.
65. Nizamuddin M, Dumbell KR. A simple laboratory test to distinguish the virus of smallpox from that of alastrim. *Lancet* 1961;**1**: 68–9.
66. Ropp SL, Jin Q, Knight JC, et al. PCR strategy for identification and differentiation of small pox and other orthopoxviruses. *J Clin Microbiol* 1995;**33**:2069–76.
67. Spiegel AD, Kavaler F, Kucinski KM. Smallpox and New York City's smallpox hospital. *J Community Health* 2005;**30**:391–413.
68. Stewart AJ, Devlin PM. The history of the smallpox vaccine. *J Infect* 2006;**52**:329–34.
69. Stone R. Down to the wire on bioweapons talks. *Science* 2001; **293**:414–16.
70. Tanne JH. World Health Assembly votes not to destroy smallpox virus. *BMJ* 2005;**330**:1230.
71. Texas Tech University Center for Biodefense Law and Public Policy. *64th World Health Assembly gives smallpox a temporary stay of execution*. 2011. Accessed. Available at: http://www.ttu.edu/biodefense/images/Smallpox_Decision_at_the_64th_World_Health_Assembly.pdf.
72. Tucker JB. Historical trends related to bioterrorism: an empirical analysis. *Emerg Infect Dis* 1999;**5**:498–504.
73. Waddington E, Bray PT, Evans AD, et al. Cutaneous complications of mass vaccination against smallpox in South Wales 1962. *Trans St Johns Hosp Dermatol Soc* 1964;**50**:22–42.
74. Wehrle PF. Smallpox eradication: a global appraisal. *JAMA* 1978; **240**:1977–9.
75. White PJ, Shackelford PG. Edward Jenner, MD, and the scourge that was. *Am J Dis Child* 1983;**137**:864–9.
76. Wittek R. Vaccinia immune globulin: current policies, preparedness, and product safety and efficacy. *Int J Infect Dis* 2006;**10**: 193–201.
77. World Health Assembly. *Decision on smallpox virus destruction delayed for three years*. May 27, 2011. Accessed. Available at: http://www.homelandsecuritynewswire.com/decision-smallpox-virus-destruction-delayed-three-years.
78. World Health Organization. *The Global eradication of smallpox: final report of the Global Commission for the Certification of Smallpox Eradication. History of International Public Health No. 4*. Geneva: World Health Organization; 1980.
79. World Health Organization. Human monkeypox: the past five years. *WHO Chron* 1984;**38**:227–9.

CHAPTER 163

Monkeypox and Other Poxviruses

Samantha H. Johnston • Anne W. Rimoin

Smallpox, caused by variola virus (see Chapter 162), at one time was the most important human poxvirus disease, but this disease has been eradicated from the world. Today, only one human poxvirus, molluscum contagiosum virus, causes specific human illness. In addition, at least nine other poxviruses can cause human infections. Eight of these viruses are acquired zoonotically, and the other, vaccinia, is acquired by active immunization against smallpox, inadvertently by laboratory accident, or from a vaccinated person to an unvaccinated person.

PROPERTIES OF THE VIRUSES

Classification

The family *Poxviridae* has two subfamilies: *Chordopoxvirinae* (vertebrate poxviruses) and *Entomopoxvirinae* (insect poxviruses).[45,48,93] Eight genera are included in the *Chordopoxvirinae* subfamily, and four of these genera contain species that infect humans (Table 163-1).

Structure

Poxviruses are the largest animal viruses and are discernible by light microscopy.[11,95] In general, by electron microscopy, orthopoxviruses appear brick shaped, with a length of 350 nm and a width of 270 nm. They contain double-stranded DNA genomes that vary from 130 to 300 kbp, depending on the particular species. A 30-nm lipoprotein bilayer (envelope) surrounds the virus core. The envelope contains seven or more distinct glycoproteins. *Parapoxvirus* organisms have a different structure than do *Orthopoxvirus*, *Yatapoxvirus*, and *Molluscipoxvirus* organisms. They are ovoid and vary from 260 to 160 nm for orf virus to 300 to 190 nm for pseudocowpox virus.

SPECIFIC VIRUSES AND THEIR ILLNESSES

Monkeypox Virus

Monkeypox is a zoonotic orthopoxvirus that causes a serious smallpox-like illness in humans.* Monkeypox virus was first discovered in 1958, when it was isolated from the lesions of a generalized smallpox-like vesiculopustular disease among captive monkeys at the State Serum Institute in Copenhagen, Denmark.[130] The disease was not recognized as being distinct from smallpox until 1970, when, after smallpox had been eliminated from many countries, a similar illness continued to circulate in rural regions of the Democratic Republic of Congo (DRC).[39,61,68,95] Between 1970 and 1979, 55 cases of monkeypox were confirmed by the World Health Organization (WHO) in forested areas of Western and Central Africa, of which 44 cases (80%) occurred in the DRC.[10,69,82]

In 1980, smallpox was declared eradicated by the WHO Advisory Committee on Orthopoxvirus Infections. Despite sporadic reports of human monkeypox in Africa, the WHO Advisory Committee also recommended that smallpox vaccination cease globally because of the cost and complexity of sustaining a special vaccination program for what was then a comparatively rare disease.[134] To address the concern that in the absence of smallpox vaccination campaigns, monkeypox might have the potential to emerge from central Africa and occupy the niche vacated by smallpox, a WHO-sponsored active surveillance program was carried out in selected districts in the DRC from 1981 to 1986.[108] This surveillance program initiated modeling of monkeypox human-to-human transmission patterns. Models predicted that the reproductive number (R_o) was less than 1 and that human-to-human transmission was at low enough levels not to present a significant public health threat. Thus, the WHO recommended against sustained vaccinia vaccination for protection against monkeypox.[58,134]

During this period 338 cases were identified. Most cases were found in children (mean age, 4.4 years), leading to the hypothesis that smallpox vaccine may confer a degree of immunity in adults.[68,92,95] Since the WHO's initial efforts in monkeypox surveillance were initiated, outbreaks, mostly in the DRC, have been reported in the literature. Monkeypox has since supplanted smallpox as the most important orthopoxvirus affecting humans, and more attention has been directed to understanding the transmission patterns, epidemiology and control strategies of this illness.

Epidemiology

Epidemiologic studies beginning in 1970 identified several outbreaks of human monkeypox, with most cases arising in the DRC. Jezek and associates[72,74] reported disease in 338 patients and found that among patients with primary infection (i.e., cases associated with an animal source), 50 percent were younger than 4 years of age and 93 percent were younger than 14 years of age. Ninety-six percent of these children were not vaccinated against smallpox.[10] Animal sources were suspected in 245

*References 9, 10, 14, 22-28, 39, 61, 62, 68, 73, 74, 78, 92, 94, 95, 103.

TABLE 163-1 **Poxviruses That Can Cause Human Illness**

Genus	Species
Orthopoxvirus	Variola virus
	Monkeypox virus
	Vaccinia virus
	Cowpox virus
Parapoxvirus	Orf virus
	Bovine papular stomatitis virus
	Pseudocowpox virus
	Parapox of seals
	Parapoxvirus of reindeer
Yatapoxvirus	Tanapoxvirus
	Yabapoxvirus
Molluscipoxvirus	Molluscum contagiosum virus

of the 338 cases, and human-to-human transmission occurred in the remaining 93 cases. Analysis of unvaccinated contacts of the patients showed a secondary attack rate of 9.3 percent.[10] The longest chain of infection was four generations.[72] Index cases of monkeypox were usually women or children who had frequent contact with animals.[26,35] Since initial pox lesions on the hands or arms indicative of direct inoculation were not described, infection was presumably acquired by inhaling material aerosolized during handling or butchering of an animal. Person-to-person transmission was generally limited to family members and other close contacts, suggesting monkeypox was less transmissible than smallpox, but the small size and low population density of rural villages also may have helped limit the size of outbreaks.[35]

At the end of the active WHO surveillance program in 1986, only 13 cases of human monkeypox were reported until 1995, likely as a result of political instability in the region. In 1996 to 1997, the largest outbreak ever recorded occurred in the Kasai Oriental region of the DRC. During an initial investigation by the WHO and the Centers for Disease Control and Prevention (CDC), 92 cases were identified and a follow-up investigation suggested an additional 419 suspected cases. However, confusion with a concurrent varicella outbreak made precise estimates of the true outbreak size difficult to ascertain.[21,58] Analysis of 320 possible and probable cases showed attack rates of 18 percent in susceptible individuals, 3 percent if vaccinated, and 26 percent if unvaccinated.[58] Eighty-five percent of cases occurred in patients younger than 16 years of age. The case-fatality rate was 1.5 percent, lower than the previously reported rate of 10 percent.[21] The broad case definition used for monkeypox case identification of fever and vesiculopustular rash possibly could include infections with varicella as well, thus artificially increasing the rates of reported secondary transmission as well as artificially decreasing the case-fatality rates.[39]

Meyer and colleagues[92] used viral isolation and amplification by polymerase chain reaction (PCR) to detect monkeypox virus after a 2001 outbreak in the DRC. The group found that coinfection and cotransmission of varicella could have misrepresented actual cases of monkeypox. Of the seven small outbreaks, two were proved to be the result of monkeypox (16 cases, 4 deaths); two showed evidence of co-circulating monkeypox and varicella (7 cases, 1 death); two were the result of varicella-zoster virus (6 cases, 0 deaths); and neither virus was isolated in the final outbreak. Children were the principal victims in all outbreaks.

Rimoin and colleagues[108] conducted active surveillance for human monkeypox in the DRC between 2005 and 2007 to assess the current burden of infection and noted a dramatic increase in monkeypox cases since the 1980s. The most heavily affected health zones showed a 20-fold increase in monkeypox activity in contrast to the 1980s, with an increase in cases from 0.72 to 14.42/10,000. Ninety-two percent of cases occurred in those born after smallpox vaccination ended in 1980. The study concluded that risk for monkeypox is "inversely associated with smallpox vaccination."

These surveillance activities have supported the three main factors determining monkeypox burden in enzootic areas: smallpox vaccination, exposure to animal reservoir species, and human-to-human transmission of disease. Smallpox vaccination officially ceased in the DRC in 1982; thus, vaccine-induced herd immunity has waned, partially contributing to increased incidence.[78] Another contribution has been the change in animal contact during this time period. Monkeypox generally occurs in villages in or near tropical rainforest. Clearing of these forests as well as increased reliance on reservoir species for nutrition likely has brought humans into closer contact with reservoir species.[108] The effect of increased human-to-human transmission has not been formally investigated, but waning immunity to smallpox is thought to allow more sustained transmission of monkeypox in a generally naïve population.

Although monkeypox outbreaks in humans had been reported during the 1970s, few cases have been noted outside of DRC.[6,104] However, serologic evidence shows that orthopoxvirus, presumably monkeypox, may be circulating in West Africa among multiple species, including humans.[89,105]

Only one known outbreak has occurred outside of Africa. In May of 2003, the CDC reported the first outbreak of monkeypox in humans in the Western Hemisphere among 47 patients with laboratory-confirmed or probable cases.[13,22,23-25,106] No case fatalities and no cases of secondary transmission occurred. Fourteen cases resulted in hospitalization, two of these were severe cases in children. One girl developed encephalitis that resolved over the course of a 14-day hospitalization; a second girl developed diffuse oral and pharyngeal lesions that impaired swallowing, and she also recovered.[23] The outbreak was linked to the distribution of North American prairie dogs as pets that had become ill after being housed with an infected rope squirrel and an infected giant pouched rat shipped from Ghana. Patients were identified in Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin.[12,23-25,63,103] PCR analysis of tissue obtained from the prairie dogs revealed 100 percent similarity with the monkeypox virus strain Zaire 96-I-16 that had been isolated previously from the DRC.[79]

The virus is thought to spread to humans by handling of infected animals or by direct contact with an infected animal's body fluids or lesions.[79] Person-to-person spread occurs by large respiratory droplets, although monkeypox transmission is much less efficient than smallpox.[26,95,114] A risk factor for secondary transmission is close contact with a primary case. Evidence of previous vaccination is thought to be protective, with surveillance programs estimating vaccine efficacy between 80 and 85 percent.[34,49,108] Mortality rates have varied between 0 and 17 percent during outbreaks.*

Serologic evidence suggests that many animals, including both arboreal and terrestrial rodents and nonhuman primates, can be infected with monkeypox virus, however, rope squirrels have been targeted as the primary host.[39,52,63] Epidemiologic studies from the DRC have implicated squirrels living in agricultural areas as primary candidates to sustain transmission among people in nearby settlements, although no definitive host has been identified. Concerns that monkeypox virus could spread through North American rodent populations and serve as a vector for continued zoonotic exposure have not played out.[39]

Clinical Features

The clinical features of human monkeypox virus infection are similar to those of smallpox, but the overall illness tends to be less severe. The characterization of symptoms during outbreaks in Africa has been limited by poor access to medical care, retrospective identification, and civil unrest in areas of outbreaks. The outbreak reported in the midwestern United States allowed researchers to describe clinical and laboratory parameters of disease.[63]

After a 10- to 14-day period of incubation (range, 1 to 31 days), a 2-day prodrome of fever, malaise, and lymphadenopathy precedes the manifestation of rash.[10,39,68] Lymphadenopathy occurs in 90 percent of unvaccinated individuals and is a key feature distinguishing monkeypox from smallpox because lymphadenopathy generally does not occur in smallpox. Affected lymph nodes may be unilateral or bilateral and include submandibular, cervical, postauricular, axillary, inguinal, or any combination of these locations.[9] Other signs and symptoms include chills, sweats, headache, backache, sore throat, cough, and shortness of breath.

The rash usually appears first on the face. As in smallpox, lesions develop and progress together in the same body region through the stages of macules, papules, vesicles, and pustules. Over the course of a 2- to 4-week period, the lesions progress until they finally scab over and desquamate. Most patients have discrete lesions, and it is estimated that 23 percent have semiconfluent lesions and 7 percent have confluent lesions. Mucous membrane involvement and conjunctivitis are common manifestations.[74,92] Lesions usually are found on the extremities, head, and trunk, as well as on the palms and soles.[61] During the first week of the rash, patients are considered to be infectious.[73] Cases without rash have been described.[84] The presence of rash on the palms and soles and the stages of progression of the rash distinguish this from varicella.

Complications include secondary bacterial infection of the skin; pneumonia; vomiting, diarrhea, and dehydration; keratitis and corneal ulceration; septicemia; and encephalitis. The death rate in nonvaccinated patients was 11 percent in the study reported by Jezek and associates,[74] and all deaths occurred in children aged 8 years or younger, possibly reflecting protective effect in older individuals who may have received vaccinia immunization.

One case of congenital monkeypox infection has been reported.[67] The child's mother presented with clinical characteristics of monkeypox on August 12, 1983, and the child was born prematurely on September 23, 1983. At birth, the child had generalized skin lesions and died 6 weeks later of malnutrition. No data are available on the clinical course of monkeypox in patients with human immunodeficiency virus and acquired immunodeficiency syndrome (HIV/AIDS) or other immunocompromised states.

Unlike African patients, in whom more severe illness is noted, most of the patients in the United States had what was described as a mild, self-limited febrile rash illness.[25] The majority of patients had localized lesions associated with direct contact with infected animals, and only one patient, a child, had a generalized rash. Nevertheless, the outbreak resulted in 14 hospitalizations among the 47 cases. Differences in the severity of disease may result from differences in virulence among genetically distinct clades of monkeypox viruses: the West African monkeypox virus group and the Congo basin isolates.[88] Congo basin isolates have been shown to be more virulent in nonhuman primates and based on epidemiologic and genetic sequence analyses are thought to be more virulent in humans as well.[30,45,68,90,113]

Laboratory alterations noted in patients of the midwestern U.S. outbreak included elevated transaminases in 50 percent of patients, low blood urea nitrogen (61%), hypoalbuminemia (50%), leukocytosis (45%), and thrombocytopenia (35%).[63]

Diagnosis

All suspect cases should be reported immediately to the local health department. Laboratory confirmation is required for establishing a definitive diagnosis. The CDC case definitions from 2004 can help guide clinicians (Table 163-2).[26] Samples used for diagnosis include blood and cutaneous tissue from at least two scabs that have been unroofed and collected in a sterile fashion. Once lesions have been unroofed, the base of the vesicle should be swabbed with sterile cotton or polyester swab and the material applied to a clean microscope slide and air-dried. The material should be sent to the CDC or a national reference laboratory in the country of origin for diagnostic testing. Other specimens used for analysis include skin biopsy specimens, which may be indistinguishable from those of smallpox lesions, with necrosis of the stratum basale, adjacent dermal papillae, and stratum spinosum.

*References 1, 7, 37, 50, 62, 68, 71, 73, 92.

TABLE 163-2 **Centers for Disease Control and Prevention Case Definition for Human Cases of Monkeypox (January 2004)**

Human Monkeypox Case Classification	Human Monkeypox Case Classification
Suspect Case The patient meets one of the epidemiologic criteria and has fever or unexplained rash and two or more other signs or symptoms with onset of first sign or symptom within 21 days after the last exposure. **Probable Case** The patient meets one of the epidemiologic criteria and has fever and vesicular-pustular rash with onset of the first sign or symptom less than 21 days after the last exposure meeting epidemiologic criteria; or if rash is present but type is not described, the patient demonstrates elevated levels of IgM antibodies reactive with orthopoxvirus between at least 7 and 56 days after the rash onset. **Confirmed Case** The patient meets one of the laboratory criteria. ***Clinical Criteria*** Rash (macular, papular, vesicular, pustular, generalized or localized, discrete or confluent) Fever (subjective or measured, ≥37.4°C) Other signs and symptoms (chills and/or sweats, headache, backache, lymphadenopathy, sore throat, cough, shortness of breath) ***Epidemiologic Criteria*** Exposure (includes living in a household, petting or handling, or visiting a pet-holding facility such as a pet store or veterinary clinic) to an exotic or wild mammalian pet (including prairie dogs, Gambian giant rats, and rope squirrels, among others to be considered on a case-by-case basis) obtained on or after April 15, 2003, with clinical signs of illness (e.g., conjunctivitis, respiratory symptoms, and/or rash)	Exposure (as described earlier) to an exotic or wild mammalian pet (as described earlier) with or without clinical signs of illness that has been in contact with either a mammalian pet (living in a household or originating from the same pet holding facility as another animal with monkeypox) or a human Exposure (skin-to-skin or face-to-face contact) to a suspect, probable, or confirmed human case ***Laboratory Criteria*** Isolation of monkeypox virus in culture Demonstration of monkeypox virus DNA by PCR testing of a clinical specimen Demonstration of virus morphologically consistent with an orthopoxvirus by electron microscopy in the absence of exposure to another orthopoxvirus Demonstration of presence of orthopoxvirus in tissue using immunohistochemical testing methods in the absence of exposure to another orthopoxvirus ***Exclusion Criteria*** An alternative diagnosis can explain the illness. *OR* The case was reported on the basis of primary or secondary exposure to an exotic or wild mammalian pet (as described earlier) or a human subsequently determined not to have monkeypox, provided other possible epidemiologic exposure criteria are not present. *OR* A patient without a rash does not develop a rash within 10 days of onset of clinical symptoms consistent with monkeypox. The case is determined to be negative for nonvariola generic orthopoxvirus by PCR testing of a well-sampled rash lesion by the approved protocol. The case is determined to have undetectable levels of IgM antibody 7 to 56 days after rash onset.

Modified from Centers for Disease Control and Prevention: Updated interim case definition for human monkeypox, Atlanta, 2004, CDC. Available at http://www.cdc.gov/ncidod/monkeypox/casedefinition.htm; and Centers for Disease Control and Prevention: *Update: multistate outbreak of monkeypox—Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003.* MMWR Morb Mortal Wkly Rep 2003;**52**:561–4, 2003.
IgM, Immunoglobulin M; *PCR,* polymerase chain reaction.

Electron microscopy of the lesions shows abundant large, brick-shaped orthopoxvirus particles in the cytoplasm of infected epidermal cells.[122]

Blood samples may be analyzed for paired acute-phase and convalescent-phase serum samples; however, serologic testing is limited because of the close antigenic relationship that exists among orthopoxviruses. More specific methods used by the CDC include cell culture or chick chorioallantoic membrane isolation, along with PCR and sequencing to differentiate monkeypox virus from other orthopoxviruses.[35,81,86] PCR also can differentiate between Congo Basin and West African monkeypox strains.[87,112]

Treatment and Prevention

In 1968, investigators reported that monkeys could be immunized against monkeypox virus using smallpox vaccine.[90] In outbreaks in the DRC described by Jezek and colleagues, as well as by more recent surveillance by Rimoin and colleagues,[74,108] prior smallpox inoculation was found to be protective and result in a milder form of disease, often difficult to distinguish from chickenpox. Hammarlund and associates[56] also described protective immunity to monkeypox virus in persons previously vaccinated against smallpox. Vaccine efficacy was estimated to be 85 percent during surveillance trials in the 1980s and more than 80 percent for more than 30 years during more recent surveillance.[50,108] However, the smallpox vaccination status did not alter either the severity of illness or hospitalization rates in the midwestern U.S. outbreak.[61] The CDC recommended vaccination with smallpox vaccine for those without contraindications to vaccination, including health care workers and household contacts of patients with suspected or confirmed monkeypox cases. Preexposure vaccination is preferred, but vaccination can be administered after laboratory confirmation of an infection. Vaccination should be given within 4 days but may be considered protective up to 14 days after exposure.[26]

Because of the high frequency of complications associated with administering vaccinia, Stittelaar and colleagues[124] described the potential use of a modified vaccinia virus strain, Ankara, as safer vaccine against orthopox infections. Laboratory studies demonstrated that macaques given Ankara were protected against lethal respiratory challenges with monkeypox virus. This vaccine candidate, IMVAMUNE, was shown to be safe and provide immunity in humans; as of 2010 it had been granted fast track status by the U.S. Food and Drug Administration (FDA).[128] Another candidate, LC16m8, also underwent phase I and II clinical trials; it achieved antivaccinia, antivariola, and antimonkeypox neutralizing antibodies without any observed cardiac toxicities.[77] A variety of other vaccines have been studied in various primate models, and these have also shown promise.[37,43,59]

Important questions have been raised as to the risks and benefits of testing these vaccines for efficacy and vaccinating humans in monkeypox endemic areas.[107] These issues may become more relevant global public health issues should monkeypox become established in an animal reservoir outside of Africa.

Roess and colleagues[110] evaluated education-based prevention efforts in areas endemic for monkeypox; after a community intervention trial, they found improved disease recognition and transmission and risk-limitation methods in place. Until an effective and inexpensive monkeypox vaccine becomes available, this type of intervention may be the best option to provide some degree of disease prevention.[110]

As a potential preventive measure, 28 residents (26 adults and 2 children) affected in the U.S. outbreak area received a smallpox vaccine to try to prevent further transmission of monkeypox.[24] No data exist on the effectiveness of vaccinia immunoglobulin; however, it may be considered as a treatment for severe cases of monkeypox infection and as a prophylaxis in exposed persons who are severely immunocompromised.[39]

Additionally, cidofovir has been shown to be active in vitro and in vivo in animal models as a potential treatment,[2,36] but no reported data on its effectiveness for treating infected humans exists to date. However, the CDC recommends that it be considered as a treatment in severe monkeypox virus infections and in immunocompromised hosts, but should not be used as a prophylaxis because of its significant toxicities.[14] Tecovirimat, or ST-246, a compound with activity against orthopoxviruses, also has shown promise in treating severe monkeypox virus infection in rodent and nonhuman primate models and may one day be approved for use in humans.[76,115,121] Mitoxantrone, a drug already FDA approved for multiple sclerosis and cancer, recently demonstrated the ability to carry some antiviral effects against monkeypox and cowpox viruses, although less effectively than cidofovir.[1]

Among hospitalized patients, isolation precautions should be taken because the virus may be transmitted by direct contact or large respiratory droplets that may be airborne.[26] Isolation can be discontinued for patients with vesiculopustular rash after all lesions have crusted; however, contact with immunocompromised persons should be limited until all crusts have separated. For patients without rash, isolation should be continued for 7 days after the onset of fever. Asymptomatic contacts should remain under symptom surveillance for 21 days after the last exposure, and temperature should be monitored twice daily. Affected individuals should not donate any body fluids or organs while ill.[26]

Bioterrorism Concerns

Concern over monkeypox virus as an agent of bioterrorism has been raised, but there has been no evidence to suggest that the virus becomes more severe, virulent, or easily transmissible after passages through human hosts.[72] Furthermore, evidence that monkeypox evolved independently from smallpox alleviated some fears that the virus could "evolve back" into smallpox. Further, low rates of primary infection, limited transmissibility, and low case fatality make monkeypox an unlikely bioterrorist threat.[8] However, some concerns remain about the potential of molecular biologic techniques to manipulate the virus genetically into a more lethal form.[65,116]

Cowpox Virus

Cowpox disease has been recognized for hundreds of years in Europe. The infection causes ulcers on the teats of cows.[45] Those working with infected cows, including milkmaids, often developed similar ulcers on their hands and were known to be immune to smallpox. In May of 1796, this led Edward Jenner to inoculate James Phipps, an 8-year-old boy, with cowpox material obtained from a lesion on a local dairy maid.[6,66-68] No infection occurred after exposing him to material from a smallpox lesion.

Recent studies have found that the reservoir hosts are not cows, but instead wild rodents. Further, it is a rare illness with a geographic distribution throughout Western Europe.[4,6,11] Baxby[4] reported 12 human patients with illnesses caused by cowpox virus; only 5 were exposed to infected cows and the other 7 reported no direct contact with cows. All patients, however, lived in or had visited rural areas before the onset of illness. Of the 10 patients with lesions, 6 had lesions on the hand only, 3 had lesions on the chin or face only, and a single patient had lesions on both the face and hand. Most patients had local edema, lymphadenitis, and fever. Initially, lesions were confused with anthrax in two patients.

An additional 12 cases of cowpox causing cutaneous infection were reported from various European countries. Most identified cases resolved between 3 weeks to 6 months, but one fatality was reported. The authors reported a 7-year-old girl with persistent lymph node enlargement 2 years after initial presentation of infection. Patients developed infection after they had exposure to ill animals, including cats (9), dogs (2), and wild rats (1).[97] One immunocompromised 18-year-old man died; he was thought to have acquired the infection from a cat with a skin lesion on the anterior paw.[33] Schupp and associates[116] reported a single case of cowpox virus potentially from a cat, causing necrotic ulcers on a 12-year-old boy in Germany. The virus was isolated and detected by PCR.

Similar to monkeypox virus, several compounds have shown antiviral effects for cowpox virus in vitro or

in animal models. These include cidofovir, ST-246, terameprocol, and mitoxantrone.[3,100,102]

Vaccinia Virus

Vaccinia virus is the live immunizing antigen successfully used in the global program to eradicate smallpox. This orthopoxvirus is different from cowpox virus, the agent that Jenner and others used for vaccination in the early 19th century. Vaccinia virus has been used for vaccination for more than 100 years. Restriction endonuclease studies indicated that various strains of vaccinia virus from different geographic regions are similar to each other but distinctly different from cowpox virus.[45] The origin of vaccinia virus is unknown, but various hypotheses exist regarding its origin,[11] including (1) evolving from variola virus through continual passage in the skin of cows or humans, (2) evolving from cowpox virus through continual passage in the skin of animals, (3) hybridization between cowpox virus and variola virus, and (4) evolving in a an extinct natural animal host.

Vaccinia virus has caused outbreaks in several countries in milk herds; most commonly it has been reported in India and Brazil, with reports published since 1999. An outbreak in India was described in which infected buffaloes were thought to have had contact with vaccinated humans during smallpox eradication programs, rather than the virus being a primary buffalo pathogen.[11,45] In several Brazilian provinces, skin lesions on dairy cows and their milkers resembling cowpox were found to be caused by a variety of vaccinia virus strains belonging to two distinct groups. During the 18th and 19th centuries, cases with vaccinia virus were thought to have evolved from repeated introduction of vaccinia.[126] In addition to cutaneous symptoms, patients reported fever, malaise, myalgia, headache, cough, and adenopathy.[118] Secondary bacterial infection of cutaneous lesions has been reported.

Because of the success of the smallpox eradication program in the United States, routine vaccinia vaccination was discontinued in 1971,[19,20] and by 1976, the recommendation of vaccinating health care workers also was discontinued.[15] However, in 1980, the Advisory Committee on Immunization Practices (ACIP) recommended vaccinating laboratory workers to protect them from possible infection while working with nonvariola orthopoxviruses (e.g., vaccinia, monkeypox, and recombinant vaccinia).[16,51,57]

In 1982, the only active licensed producer of vaccinia vaccine in the United States ceased production for general use, and distribution to civilian populations was discontinued in 1983.[18] However, the CDC has continued to provided vaccinia vaccine for laboratory workers.[17] In 1984, the laboratory recommendations were formally included in guidelines for biosafety in microbiologic and biomedical laboratories. This was expanded to include persons working in animal care areas where studies with orthopoxviruses were performed.[127]

Since January of 1982, smallpox vaccination has not been required for international travelers, and International Certificates of Vaccination no longer include smallpox vaccination.[135] Military personnel continued to be vaccinated routinely for several years after discontinuation. More recently, only selected groups of military personnel are vaccinated against smallpox; groups include those designated for military action in areas where the threat of biological warfare exists.

Recombinant Vaccinia Virus

Vaccinia virus is the prototype of the genus *Orthopoxvirus*.[45] It is a double-stranded DNA virus that has a broad host range under experimental conditions and is rarely isolated from animals outside the laboratory.* Many strains of vaccinia virus exist with various levels of virulence in humans and animals. Some examples include the Temple of Heaven and Copenhagen vaccinia strains, which are highly pathogenic in animals, and the NYCBOH strain, from which the Wyeth vaccine was derived, which has relatively low pathogenicity in animals and humans.[49]

Vaccinia virus can be engineered genetically to contain and express foreign DNA without impairing the ability of the virus to replicate. Such foreign DNA can encode protein antigens that induce protection against one or more infectious agents. These recombinant vaccinia viruses created from several different strains have been engineered to express the immunizing antigens of many viruses, bacteria, parasites, and tumors of veterinary and medical importance.† In the United States, recombinant viruses have been made from the NYCBOH strain or a mouse neuroadapted derivative, the WR strain. Some recombinant viruses have been made from the Copenhagen and Lister vaccinia strains, which are more pathogenic in animals than the NYCBOH strain. More recently, studies using recombinant vaccinia to vaccinate against prostate cancer, malaria, tuberculosis, and HIV have used the Ankara strain.[5,64,101] Animal studies generally suggest that recombinant viruses may be no more pathogenic than the parent strain of vaccinia virus. However, no consistently reliable laboratory marker or animal test has been able to predict the attenuation of vaccinia virus or a particular recombinant virus for humans.[136] Laboratory-acquired infections with vaccinia or recombinant viruses have been reported[75,99,117]; however, no surveillance system exists to monitor laboratory workers, thus the risk for acquiring infection in persons who handle virus cultures or contaminated materials is not known.

With the initiation of human and veterinary trials using recombinant vaccines, physicians, nurses, veterinarians, and other personnel may be susceptible to both vaccinia and recombinant agents. This exposure could occur from direct contact with the recombinant virus or contaminated dressings, but the risk for transmission is unknown. To date, no reports of transmission to health care personnel from vaccine recipients have been published. When appropriate infection control precautions are observed, health care workers exposed to smaller volumes and lower titers of virus may be at less risk for acquiring infection in contrast to their laboratory

*References 9, 32, 43, 46, 53, 137.
†References 32, 51, 55, 62, 80, 119, 120, 134.

counterparts.[54,133] However, because the risk for transmission exists in such persons, the ACIP suggests that personnel with direct exposure to volunteers in clinical studies be considered for vaccination and observe appropriate biosafety guidelines and infection control procedures.[54,127,133]

Camelpox

Camelpox virus is an orthopoxvirus that is genetically the closest known virus to variola virus (smallpox virus).[42] Its disease is restricted to camels, and it is enzootic in all regions (except Australia) where camel breeding is practiced. The illness in camels is severe, with smallpox-like lesions. Bera and associates[7] noted three human cases in animal handlers in India. The illness in all three was mild and limited to a few lesions on the hands.

Orf Virus

Orf virus is a member of the genus *Parapoxvirus* (see Table 58-1 in Chapter 58). Orf disease, also called *ecthyma contagiosum*,[45] is an occupationally acquired disease, with most cases occurring in adults.[53,83] The reservoir hosts of orf virus are sheep and goats, and the virus has worldwide distribution. Human disease is characterized by single or multiple lesions that last approximately 35 to 40 days, progress through six stages, and most often are located on the hands.[53,83] The stages include maculopapular, 1 to 7 days; target, 7 to 14 days; acute, 14 to 21 days; regenerative, 21 to 28 days; papillomatous, 28 to 35 days; and regressive, 35 to 40 or more days. Patients may have low-grade fever, but regional lymphadenitis is an uncommon finding. However, two patients with a widespread papulovesicular eruption, fever, malaise, and lymphadenopathy were described.[132] In addition, a giant orf granuloma developed at the site of a rope burn in a 12-year-old boy who lived on a farm.[98] These atypically proliferating giant orf lesions have occurred in both immunosuppressed and immunocompetent individuals. Complications after infection can occur, including erythema multiforme, bullous pemphigoid, swan-neck deformity of a finger, paresthesia, and autoimmune blistering disorders. Mortality among flocks of goats and sheep infected with orf virus can be very high, which could be economically damaging.[60]

The CDC reported four unrelated cases of cutaneous, self-resolving orf virus infections in people either exposed to sheep or goats with oral lesions or who had been vaccinated recently with live orf virus vaccine. These cases occurred in New York, Illinois, California, and Tennessee, and patients' ages ranged from 11 to 51 years.[27] Uzel and colleagues[129] reported nine cases of orf virus infection in Turkey related to feasts of sacrifice; adults involved in the preparation of the animals for consumption were found to be at risk. Four additional cases reported in the United States between 2009 and 2011 highlighted the potential for household exposures to the virus, including how food was prepared from infected animals.[29]

Until recently, diagnosis of orf virus depended on electron microscopy or serology. In 2001, Torfason and Gunadóttir[125] described the PCR method to detect orf virus that helped make identification of the virus more accessible.[96]

Orf virus has been shown to have antiviral activity against recurrent genital herpes disease in guinea pigs and hepatitis B and herpes simplex virus 1 in mice. The protection in mice happens by modulating the host innate immune system, inducing phagocytosis, natural killer cell activity, and release of interferon-α, tumor necrosis factor-α, interleukin-2, and granulocyte-macrophage colony-stimulating factor. This finding may have implications for immune therapy for patients with serious infection from these viruses.[131] Treatment with imiquimod of complicated cases in immunocompetent hosts[44] and in immunocompromised patients has been reported.[84]

Other Parapoxviruses

Human infections with pseudocowpox virus and bovine papular stomatitis virus are considered occupational diseases. Pseudocowpox infections occur on the hands of milkers, and infections with bovine papular stomatitis virus have also been reported on the hands of veterinarians and others with close contact with cows.[12,45,91] The initially red lesions of the pseudocowpox nodule are relatively painless, but they may be pruritic and will eventually become purple. They are firm, do not ulcerate, and typically last 4 to 6 weeks. In comparison, the lesions caused by bovine papular stomatitis virus are wartlike and typically last 3 to 4 weeks.

Additionally, reports on humans infected with other parapoxviruses from reindeer and seals are available.[46,47,109] One report noted that in a human infection with sealpox virus, the person had orflike lesions that resolved after biopsy specimens were obtained.[31]

Yatapoxviruses

Tanapox virus, yaba-like disease virus (YLDV), and yaba monkey tumor virus (YMTV) are three viruses found in monkeys that can cause human infection.[35,40,41,45,70] DNA maps of tanapox virus and YLDV are extremely similar, indicating they may be the same. Tanapox in humans first was observed along the Tana River in Kenya in 1957, and outbreaks in Africa, including the DRC, have involved both children and adults.[45,70]

The illness starts with fever, headache, backache, and mild prostration and skin lesions occur 2 to 4 days after onset. Individual lesions start as an itchy path, followed by the development of a pocklike lesion. At 7 days, lesions are approximately 10 mm in diameter with surrounding erythema. Lymphadenitis is both local and regional. The lesions ulcerate during the second week of illness and last approximately 6 weeks. Most patients have a single lesion, but some have 2 to 10 lesions. The prognosis usually is good, although secondary bacterial infection can occur.

Two cases of tanapox were reported in the United States. The first case was a 51-year-old man with papular lesions with general malaise after visiting Tanzania and being exposed to a cat.[123] A second case occurred in a 21-year-old female student from New Hampshire, who developed fever, headaches, malaise, and several papules after being exposed to chimpanzees in the DRC.[38] The

symptoms of both patients were diagnosed using PCR techniques and resolved spontaneously. As international travel increases, more case reports of such poxvirus illnesses may surface. PCR techniques to diagnose such infections quickly have been developed,[38,123,138] and these methods will assist in rapid diagnosis of poxvirus illnesses that may be confused with more serious illnesses such as smallpox and monkeypox. Of interest, tanapox virus has been recently been studied in human cancer cell lines in hope of developing an oncolytic viral vaccine to be used therapeutically in a variety of human cancers with minimal side effects.[85]

YMTV was isolated from tumors in monkeys in Nigeria, and animal handlers in primate centers in the United States have become ill as a result of this virus. Human infections have not been identified in the field in Africa. YMTV produces epidermal histiocytomas, tumor-like masses of histiocytic polygonal mononuclear cell infiltrates that may advance to suppurative inflammation.[35]

Molluscum Contagiosum Virus

In contrast to the other zoonotic poxviruses discussed in this chapter, *molluscum contagiosum* virus is a human virus that is a common cause of human skin lesions[45] (see Chapter 60B and Table 58-1 in Chapter 58). The virus has not been grown in cell culture as yet, but has been extracted from human lesions, and it does not cause infection in experimental animals. Analysis of viral DNA from lesions in geographically different patients indicates existence of two major subtypes and a third, rare subtype. The clinical aspects of molluscum contagiosum virus infection are presented in Chapter 60B.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Because all but two viruses presented in this chapter are zoonotic agents, careful attention must be given to geographic location and animal exposure. All diseases described here are characterized by local lesions, which easily can be collected for direct identification. All poxviruses can be identified by examination of material from lesions by electron microscopy and by PCR. All viruses except molluscum contagiosum virus grow in one or more tissue culture systems, the chorioallantoic membrane of embryonated eggs, or both. Unusual agents should be referred to specific reference laboratories for species identification.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Altmann SE, Smith AL, Dyall J, et al. Inhibition of cowpox virus and monkeypox virus infection by mitoxantrone. *Antiviral Res* 2012;**93**:305–8.
2. Andrei G, Snoeck R. Cidofovir activity against poxvirus infections. *Viruses* 2010;**2**:2803–30.
7. Bera BC, Shanmugasundaram K, Barua S, et al. Zoonotic cases of camelpox infection in India. *Vet Microbiol* 2011;**152**:29–38.
29. Centers for Disease Control and Prevention. Human Orf virus infection from household exposures: United States, 2009-2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:245–8.
37. Denzler KL, Babas T, Rippeon A, et al. Attenuated NYCBH vaccinia virus deleted for the *E3L* gene confers partial protection against lethal monkeypox virus disease in cynomolgus macaques. *Vaccine* 2011;**29**:9684–90.
42. Duraffour S, Meyer H, Andrei G, et al. Camelpox virus. *Antiviral Res* 2011;**92**:167–86.
43. Earl PL, Americo JL, Wyatt LS, et al. Rapid protection in a monkeypox model by a single injection of a replication-deficient vaccinia virus. *Proc Natl Acad Sci U S A* 2008;**105**:10889–94.
46. Essbauer S, Pfeffer M, Meyer H. Zoonotic poxviruses. *Vet Microbiol* 2010;**140**:229–36.
52. Fuller T, Thomassen HA, Mulembakani PM, et al. Using remote sensing to map the risk of human monkeypox virus in the Congo Basin. *Ecohealth* 2011;**8**:14–25.
59. Hirao LA, Draghia-Akli R, Prigge JT, et al. Multivalent smallpox DNA vaccine delivered by intradermal electroporation drives protective immunity in nonhuman primates against lethal monkeypox challenge. *J Infect Dis* 2011;**203**:95–102.
60. Hosamani M, Scagliarini A, Bhanuprakash V, et al. Orf: an update on current research and future perspectives. *Expert Rev Anti Infect Ther* 2009;**7**:879–93.
76. Jordan R, Goff A, Frimm A, et al. ST-246 antiviral efficacy in a nonhuman primate monkeypox model: determination of the minimal effective dose and human dose justification. *Antimicrob Agents Chemother* 2009;**53**:1817–22.
77. Kennedy JS, Gurwith M, Dekker CL, et al. Safety and immunogenicity of LC16m8, an attenuated smallpox vaccine in vaccinia-naive adults. *J Infect Dis* 2011;**204**:1395–402.
79. Khodakevich L, Szczeniowski M, Manbu-ma-Disu, et al. The role of squirrels in sustaining monkeypox virus transmission. *Trop Geogr Med* 1987;**39**:115–22.
85. Lee HL, Essani K. Differential susceptibility of human cancer cell lines to wild-type tanapoxvirus infection. *Open Virol J* 2010;**4**:1–6.
87. Li Y, Zhao H, Wilkins K, et al. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J Virol Methods* 2010;**169**:223–7.
89. Macneil A, Abel J, Reynolds MG, et al. Serologic evidence of human orthopoxvirus infections in Sierra Leone. *BMC Res Notes* 2011;**4**:465.
100. Pollara JJ, Laster SM, Petty IT. Inhibition of poxvirus growth by terameprocol, a methylated derivative of nordihydroguaiaretic acid. *Antiviral Res* 2010;**88**:287–95.
102. Quenelle DC, Kern ER. Treatment of vaccinia and cowpox virus infections in mice with CMX001 and ST-246. *Viruses* 2010;**2**:2681–95.
105. Reynolds MG, Carroll DS, Olson VA, et al. A silent enzootic of an orthopoxvirus in Ghana, West Africa: evidence for multi-species involvement in the absence of widespread human disease. *Am J Trop Med Hyg* 2010;**82**:746–54.
104. Reynolds MG, Damon IK. Outbreaks of human monkeypox after cessation of smallpox vaccination. *Trends Microbiol* 2012;**20**:80–7.
107. Rimoin AW, Graham BS. Whither monkeypox vaccination. *Vaccine* 2011;**29**(Suppl. 4):D60–4.
108. Rimoin AW, Mulembakani PM, Johnston SC, et al. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. *Proc Natl Acad Sci U S A* 2010;**107**:16262–7.
110. Roess AA, Monroe BP, Kinzoni EA, et al. Assessing the effectiveness of a community intervention for monkeypox prevention in the Congo basin. *PLoS Negl Trop Dis* 2011;**5**:e1356.
113. Saijo M, Ami Y, Suzaki Y, et al. Virulence and pathophysiology of the Congo Basin and West African strains of monkeypox virus in non-human primates. *J Gen Virol* 2009;**90**:2266–71.
112. Saijo M, Ami Y, Suzaki Y, et al. Diagnosis and assessment of monkeypox virus (MPXV) infection by quantitative PCR assay: differentiation of Congo Basin and West African MPXV strains. *Jpn J Infect Dis* 2008;**61**:140–2.
118. Silva-Fernandes AT, Travassos CE, Ferreira JM, et al. Natural human infections with Vaccinia virus during bovine vaccinia outbreaks. *J Clin Virol* 2009;**44**:308–13.
121. Smith SK, Self J, Weiss S, et al. Effective antiviral treatment of systemic orthopoxvirus disease: ST-246 treatment of prairie dogs infected with monkeypox virus. *J Virol* 2011;**85**:9176–87.
128. U.S. Food and Drug Administration. Fast Track status for Imvamune. *Hum Vaccin* 2010;**6**:368–72.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Altmann SE, Smith AL, Dyall J, et al. Inhibition of cowpox virus and monkeypox virus infection by mitoxantrone. *Antiviral Res* 2012;**93**:305–8.
2. Andrei G, Snoeck R. Cidofovir Activity against Poxvirus Infections. *Viruses* 2010;**2**:2803–30.
3. Arita I, Jezek Z, Khodakevich L, et al. Human monkeypox: a newly emerged orthopoxvirus zoonosis in the tropical rain forests of Africa. *Am J Trop Med Hyg* 1985;**34**:781–9.
4. Baxby D. Is cowpox misnamed? A review of 10 human cases. *Br Med J* 1977;**1**:1379–81.
5. Bejon P, Kai OK, Mwacharo J, et al. Alternating vector immunizations encoding pre-erythrocytic malaria antigens enhance memory responses in a malaria endemic area. *Eur J Immunol* 2006;**36**: 2264–72.
6. Bennett M, Baxby D. Cowpox. *J Med Microbiol* 1996;**45**:157–8.
7. Bera BC, Shanmugasundaram K, Barua S, et al. Zoonotic cases of camelpox infection in India. *Vet Microbiol* 2011;**152**:29–38.
8. Breman JG, Henderson DA. Poxvirus dilemmas: monkeypox, smallpox, and biologic terrorism. *N Engl J Med* 1998;**339**:556–9.
9. Breman JG, Henderson DA. Monkeypox: An emerging infection in humans. In: Scheld WM, Craig WA, Hughes JM, editors. *Emerging infections*. 4th ed. Washington, D.C.: ASM Press; 2000.
10. Breman JG, Kalisa R, Steniowski MV, et al. Human monkeypox, 1970-79. *Bull World Health Organ* 1980;**58**:165–82.
11. Buller RM, Palumbo GJ. Poxvirus pathogenesis. *Microbiol Rev* 1991;**55**:80–122.
12. Carson CA, Kerr KM. Bovine papular stomatitis with apparent transmission to man. *J Am Vet Med Assoc* 1967;**151**:183–7.
13. Centers for Disease Control and Prevention. *Monkeypox: report of cases in the United States*. Atlanta: CDC; n.d.
14. Centers for Disease Control and Prevention. *Updated interim cdc guidance for use of smallpox vaccine, cidofovir, and vaccinia immune globulin (vig) for prevention and treatment in the setting of an outbreak of monkeypox infections*. Atlanta: CDC; n.d.
15. Centers for Disease Control and Prevention. Recommendation of the Immunization Practices Advisory Committee (ACIP): smallpox vaccination of hospital and health personnel. *MMWR Morb Mortal Wkly Rep* 1976;**25**:9.
16. Centers for Disease Control and Prevention. Smallpox vaccine: recommendation of the Immunization Practices Advisory Committee. *MMWR Morb Mortal Wkly Rep* 1980;**29**:417–20.
17. Centers for Disease Control and Prevention. Smallpox vaccine available for protection of at-risk laboratory workers. *MMWR Morb Mortal Wkly Rep* 1983;**32**:543.
18. Centers for Disease Control and Prevention. Smallpox vaccine no longer available for civilians: United States. *MMW R Morb Mortal Wkly Rep* 1983;**32**:387.
19. Centers for Disease Control and Prevention. Public Health Service recommendations on smallpox vaccination. *MMWR Morb Mortal Wkly Rep* 1987;**20**:339.
20. Centers for Disease Control and Prevention. Vaccinia (smallpox) vaccine: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991;**40**:1–10.
21. Centers for Disease Control and Prevention. Human monkeypox: Kasai Oriental, Democratic Republic of Congo, February 1996-October 1997. *MMWR Morb Mortal Wkly Rep* 1997;**46**:1168–71.
22. Centers for Disease Control and Prevention. Multistate outbreak of monkeypox: Illinois, Indiana, and Wisconsin, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**:537–40.
23. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox: Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**: 561–4.
24. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox: Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**: 616–18.
25. Centers for Disease Control and Prevention. Update: multistate outbreak of monkeypox: Illinois, Indiana, Kansas, Missouri, Ohio, and Wisconsin, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**: 642–6.
26. Centers for Disease Control and Prevention. *Updated interim case definition for human monkeypox*. Atlanta: CDC; 2004. Available at http://www.cdc.gov/ncidod/monkeypox/casedefinition.htm.
27. Centers for Disease Control and Prevention. Orf virus infection in humans: New York, Illinois, California and Tennessee, 2004-2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:65–8.
28. Centers for Disease Control and Prevention. *Updated interim infection control and exposure management guidance in the health-care and community setting for patients with possible monkeypox virus infection*. Atlanta: CDC; 2012. Available at http://www.vidyya.com/vol5/v5i161_2.htm.
29. Centers for Disease Control and Prevention. Human Orf virus infection from household exposures: United States, 2009-2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:245–8.
30. Chen N, Li G, Liszewski MK, et al. Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. *Virology* 2005;**340**:46–63.
31. Clark C, McIntyre PG, Evans A, et al. Human sealpox resulting from a seal bite: confirmation that sealpox virus is zoonotic. *Br J Dermatol* 2005;**152**:791–3.
32. Cooney EL, Collier AC, Greenberg PD, et al. Safety of and immunological response to a recombinant vaccinia virus vaccine expressing HIV envelope glycoprotein. *Lancet* 1991;**337**:567–72.
33. Czerny CP, Zeller-Lue C, Eis-Hubinger AM, et al. Characterization of a cowpox-like orthopox virus which had caused a lethal infection in man. *Arch Virol Suppl* 1997;**13**:13–24.
34. Damaso CR, Esposito JJ, Condit RC, et al. An emergent poxvirus from humans and cattle in Rio de Janeiro State: Cantagalo virus may derive from Brazilian smallpox vaccine. *Virology* 2000;**277**: 439–49.
35. Damon IK. Poxviruses that infect people. In: Murray PR, Baron EJ, Jorgensen JH, et al, editors. *Manual of clinical microbiology*. 9th ed. Washington, D.C.: ASM Press; 2007. p. 1631–40.
36. De Clercq E. Potential of acyclic nucleoside phosphonates in the treatment of DNA virus and retrovirus infections. *Expert Rev Anti Infect Ther* 2003;**1**:21–43.
37. Denzler KL, Babas T, Rippeon A, et al. Attenuated NYCBH vaccinia virus deleted for the E3L gene confers partial protection against lethal monkeypox virus disease in cynomolgus macaques. *Vaccine* 2011;**29**:9684–90.
38. Dhar AD, Werchniak AE, Li Y, et al. Tanapox infection in a college student. *N Engl J Med* 2004;**350**:361–6.
39. Di Giulio DB, Eckburg PB. Human monkeypox: an emerging zoonosis. *Lancet Infect Dis* 2004;**4**:15–25.
40. Downie AW, Espana C. A comparative study of Tanapox and Yaba viruses. *J Gen Virol* 1973;**19**:37–49.
41. Downie AW, Taylor-Robinson CH, Caunt AE, et al. Tanapox: a new disease caused by a pox virus. *Br Med J* 1971;**1**:363–8.
42. Duraffour S, Meyer H, Andrei G, et al. Camelpox virus: *antiviral Res* 2011;**92**:167–86.
43. Earl PL, Americo JL, Wyatt LS, et al. Rapid protection in a monkeypox model by a single injection of a replication-deficient vaccinia virus. *Proc Natl Acad Sci U S A* 2008;**105**:10889–94.
44. Erbagci Z, Erbagci I, Almila Tuncel A. Rapid improvement of human orf (ecthyma contagiosum) with topical imiquimod cream: report of four complicated cases. *J Dermatolog Treat* 2005;**16**: 353–6.
45. Esposito JJ, Fenner F. Poxviruses. In: Knipe DM, Howley PM, editors: *Fields virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 2885–920.
46. Essbauer S, Pfeffer M, Meyer H. Zoonotic poxviruses. *Vet Microbiol* 2010;**140**:229–36.
47. Falk ES. Parapoxvirus infections of reindeer and musk ox associated with unusual human infections. *Br J Dermatol* 1978;**99**:647–54.
48. Fenner F, Wittek R, Dumbell KR. *The orthopoxviruses*. San Diego: Academic Press; 1989. p. 10–13.
49. Fenner F, Henderson DA, Arita I, et al. *Smallpox and its eradication*. Geneva: World Health Organization; 1988. p. 581–3.
50. Fine PE, Jezek Z, Grab B, et al. The transmission potential of monkeypox virus in human populations. *Int J Epidemiol* 1988;**17**: 643–50.
51. Flexner C, Moss B. Vaccinia virus as a live vector for expression of immunogens. In: Levine MM, Woodrow GC, Kaper JB, et al, editors. *New generation vaccines*. 2nd ed. New York: Marcel Dekker; 1997. p. 297–314.
52. Fuller T, Thomassen HA, Mulembakani PM, et al. Using remote sensing to map the risk of human monkeypox virus in the Congo Basin. *Ecohealth* 2011;**8**:14–25.

53. Ganske JG, Miller SH, Demuth RJ. Ecthyma contagiosum (orf). *Plast Reconstr Surg* 1981;**68**:779–80.
54. Garner JS, Simmons BP. Guideline for isolation precautions in hospitals. *Infect Control* 1983;**4**:245–325.
55. Graham BS, Belshe R, Clements ML, et al. HIV-GP-160 recombinant vaccinia vaccination of vaccinia-naive adults followed by RGP160 booster. Sixth International Conference on AIDS. Florence, Italy, 1991.
56. Hammarlund E, Lewis MW, Carter SV, et al. Multiple diagnostic techniques identify previously vaccinated individuals with protective immunity against monkeypox. *Nat Med* 2005;**11**:1005–11.
57. Henderson DA, Moss B. Smallpox and vaccinia. In: Plotkin SA, Orenstein WA, editors. *Vaccines*. 3rd ed. Philadelphia: W. B. Saunders; 1999. p. 74–97.
58. Heymann DL, Szczeniowski M, Esteves K. Re-emergence of monkeypox in Africa: a review of the past six years. *Br Med Bull* 1998;**54**:693–702.
59. Hirao LA, Draghia-Akli R, Prigge JT, et al. Multivalent smallpox DNA vaccine delivered by intradermal electroporation drives protective immunity in nonhuman primates against lethal monkeypox challenge. *J Infect Dis* 2011;**203**:95–102.
60. Hosamani M, Scagliarini A, Bhanuprakash V, et al. Orf: an update on current research and future perspectives. *Expert Rev Anti Infect Ther* 2009;**7**:879–93.
61. Huhn GD, Bauer AM, Yorita K, et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin Infect Dis* 2005;**41**:1742–51.
62. Hutin YJ, Williams RJ, Malfait P, et al. Outbreak of human monkeypox, Democratic Republic of Congo, 1996 to 1997. *Emerg Infect Dis* 2001;**7**:434–8.
63. Hutson CL, Lee KN, Abel J, et al. Monkeypox zoonotic associations: insights from laboratory evaluation of animals associated with the multi-state US outbreak. *Am J Trop Med Hyg* 2007;**76**: 757–68.
64. Ibanga HB, Brookes RH, Hill PC, et al. Early clinical trials with a new tuberculosis vaccine, MVA85A, in tuberculosis-endemic countries: issues in study design. *Lancet Infect Dis* 2006;**6**:522–8.
65. Jackson RJ, Ramsay AJ, Christensen CD, et al. Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic lymphocyte responses and overcomes genetic resistance to mousepox. *J Virol* 2001;**75**:1205–10.
66. Jenner E. *An inquiry into the causes and effects of the variolae vaccinae, a disease discovered in some of the western counties of England, particularly Gloucestershire, and known by the name of the cowpox*. London: Sampson Low; 1798.
67. Jenner E. *Further observations on the variolae vacciniae or cowpox*. London: Sampson Low; 1799.
68. Jezek Z, Fenner F. Human monkeypox. *Monogr Virol* 1988;**17**: 1–140.
69. Jezek Z, Fenner F. *Monographs in virology*, vol. 17. Basel, Switzerland: Karger; 1988.
70. Jezek Z, Arita I, Szczeniowski M, et al. Human tanapox in Zaire: clinical and epidemiological observations on cases confirmed by laboratory studies. *Bull World Health Organ* 1985;**63**: 1027–35.
71. Jezek Z, Grab B, Paluku KM, et al. Human monkeypox: disease pattern, incidence and attack rates in a rural area of northern Zaire. *Trop Geogr Med* 1988;**40**:73–83.
72. Jezek Z, Grab B, Szczeniowski MV, et al. Human monkeypox: secondary attack rates. *Bull World Health Organ* 1988;**66**:465–70.
73. Jezek Z, Marennikova SS, Mutumbo M, et al. Human monkeypox: a study of 2,510 contacts of 214 patients. *J Infect Dis* 1986;**154**: 551–5.
74. Jezek Z, Szczeniowski M, Paluku KM, et al. Human monkeypox: clinical features of 282 patients. *J Infect Dis* 1987;**156**:293–8.
75. Jones L, Ristow S, Yilma T, et al. Accidental human vaccination with vaccinia virus expressing nucleoprotein gene. *Nature* 1986; **319**:543.
76. Jordan R, Goff A, Frimm A, et al. ST-246 antiviral efficacy in a nonhuman primate monkeypox model: determination of the minimal effective dose and human dose justification. *Antimicrob Agents Chemother* 2009;**53**:1817–22.
77. Kennedy JS, Gurwith M, Dekker CL, et al. Safety and immunogenicity of LC16m8, an attenuated smallpox vaccine in vaccinia-naive adults. *J Infect Dis* 2011;**204**:1395–402.
78. Khodakevich L, Jezek Z, Messinger D. Monkeypox virus: ecology and public health significance. *Bull World Health Organ* 1988;**66**: 747–52.
79. Khodakevich L, Szczeniowski M, Manbu ma D, et al. The role of squirrels in sustaining monkeypox virus transmission. *Trop Geogr Med* 1987;**39**:115–22.
80. Kieny MP, Lathe R, Drillien R, et al. Expression of rabies virus glycoprotein from a recombinant vaccinia virus. *Nature* 1984;**312**: 163–6.
81. Kulesh DA, Loveless BM, Norwood D, et al. Monkeypox virus detection in rodents using real-time 3′-minor groove binder TaqMan assays on the Roche LightCycler. *Lab Invest* 2004;**84**: 1200–8.
82. Ladnyj ID, Ziegler P, Kima E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bull World Health Organ* 1972;**46**:593–7.
83. Leavell UW Jr, McNamara MJ, Muelling R, et al. Orf. Report of 19 human cases with clinical and pathological observations. *JAMA* 1968;**204**:657–64.
84. Lederman ER, Austin C, Trevino I, et al. ORF virus infection in children: clinical characteristics, transmission, diagnostic methods, and future therapeutics. *Pediatr Infect Dis J* 2007;**26**:740–4.
85. Lee HL, Essani K. Differential susceptibility of human cancer cell lines to wild-type tanapoxvirus infection. *Open Virol J* 2010;**4**:1–6.
86. Li Y, Olson VA, Laue T, et al. Detection of monkeypox virus with real-time PCR assays. *J Clin Virol* 2006;**36**:194–203.
87. Li Y, Zhao H, Wilkins K, et al. Real-time PCR assays for the specific detection of monkeypox virus West African and Congo Basin strain DNA. *J Virol Methods* 2010;**169**:223–7.
88. Likos AM, Sammons SA, Olson VA, et al. A tale of two clades: monkeypox viruses. *J Gen Virol* 2005;**86**:2661–72.
89. Macneil A, Abel J, Reynolds MG, et al. Serologic evidence of human orthopoxvirus infections in Sierra Leone. *BMC Res Notes* 2011;**4**:465.
90. McConnell S, Hickman RL, Wooding WL, Jr, et al. Monkeypox: experimental infection in chimpanzee (Pan satyrus) and immunization with vaccinia virus. *Am J Vet Res* 1968;**29**:1675–80.
91. McEvoy JD, Allan BC. Isolation of bovine papular stomatitis virus from humans. *Med J Aust* 1972;**1**:1254–6.
92. Meyer H, Perrichot M, Stemmler M, et al. Outbreaks of disease suspected of being due to human monkeypox virus infection in the Democratic Republic of Congo in 2001. *J Clin Microbiol* 2002;**40**:2919–21.
93. Moss B. Poxviridae: The viruses and their replication. In: Fields BN, Knipe DM, Howley PM, et al. editors: *Fields virology*. 4th ed. Philadelphia: Lippincott-Raven; 1996. p. 2637–71.
94. Mutombo M, Arita I, Jezek Z. Human monkeypox transmitted by a chimpanzee in a tropical rain-forest area of Zaire. *Lancet* 1983; **1**:735–7.
95. Nalca A, Rimoin AW, Bavari S, et al. Reemergence of monkeypox: prevalence, diagnostics, and countermeasures. *Clin Infect Dis* 2005; **41**:1765–71.
96. Nitsche A, Buttner M, Wilhelm S, et al. Real-time PCR detection of parapoxvirus DNA. *Clin Chem* 2006;**52**:316–19.
97. Pahlitzsch R, Hammarin AL, Widell A. A case of facial cellulitis and necrotizing lymphadenitis due to cowpox virus infection. *Clin Infect Dis* 2006;**43**:737–42.
98. Pether JV, Guerrier CJ, Jones SM, et al. Giant orf in a normal individual. *Br J Dermatol* 1986;**115**:497–9.
99. Pike RM. Laboratory-associated infections: summary and analysis of 3921 cases. *Health Lab Sci* 1976;**13**:105–14.
100. Pollara JJ, Laster SM, Petty IT. Inhibition of poxvirus growth by Terameprocol, a methylated derivative of nordihydroguaiaretic acid. *Antiviral Res* 2010;**88**:287–95.
101. Qiu JT, Chang TC, Lin CT, et al. Novel codon-optimized GM-CSF gene as an adjuvant to enhance the immunity of a DNA vaccine against HIV-1 Gag. *Vaccine* 2007;**25**:253–63.
102. Quenelle DC, Kern ER. Treatment of vaccinia and cowpox virus infections in mice with CMX001 and ST-246. *Viruses* 2010;**2**: 2681–95.
103. Reed KD, Melski JW, Graham MB, et al. The detection of monkeypox in humans in the Western Hemisphere. *N Engl J Med* 2004;**350**:342–50.
104. Reynolds MG, Damon IK. Outbreaks of human monkeypox after cessation of smallpox vaccination. *Trends Microbiol* 2012;**20**:80–7.

105. Reynolds MG, Carroll DS, Olson VA, et al. A silent enzootic of an orthopoxvirus in Ghana, West Africa: evidence for multi-species involvement in the absence of widespread human disease. *Am J Trop Med Hyg* 2010;**82**:746–54.
106. Reynolds MG, Yorita KL, Kuehnert MJ, et al. Clinical manifestations of human monkeypox influenced by route of infection. *J Infect Dis* 2006;**194**:773–80.
107. Rimoin AW, Graham BS. Whither monkeypox vaccination. *Vaccine* 2011;**29**(Suppl. 4):D60–4.
108. Rimoin AW, Mulembakani PM, Johnston SC, et al. Major increase in human monkeypox incidence 30 years after smallpox vaccination campaigns cease in the Democratic Republic of Congo. *Proc Natl Acad Sci U S A* 2010;**107**:16262–7.
109. Robinson AJ, Lyttle DJ. Parapoxviruses: Their biology and potential as recombinant vaccines. In: Binns MM, Smith GL, editors. *Recombinant poxviruses*. Boca Raton, FL: CRC Press; 1992. p. 285–327.
110. Roess AA, Monroe BP, Kinzoni EA, et al. Assessing the effectiveness of a community intervention for monkeypox prevention in the Congo basin. *PLoS Negl Trop Dis* 2011;**5**:e1356.
111. Rotz LD, Dotson DA, Damon IK, et al. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. *MMWR Recomm Rep* 2011;**50**:1–25; quiz CE21–7.
112. Saijo M, Ami Y, Suzaki Y, et al. Diagnosis and assessment of monkeypox virus (MPXV) infection by quantitative PCR assay: differentiation of Congo Basin and West African MPXV strains. *Jpn J Infect Dis* 2008;**61**:140–2.
113. Saijo M, Ami Y, Suzaki Y, et al. Virulence and pathophysiology of the Congo Basin and West African strains of monkeypox virus in non-human primates. *J Gen Virol* 2009;**90**:2266–71.
114. Sale TA, Melski JW, Stratman EJ. Monkeypox: an epidemiologic and clinical comparison of African and US disease. *J Am Acad Dermatol* 2006;**55**:478–81.
115. Sbrana E, Jordan R, Hruby DE, et al. Efficacy of the antipoxvirus compound ST-246 for treatment of severe orthopoxvirus infection. *Am J Trop Med Hyg* 2007;**76**:768–73.
116. Schupp P, Pfeffer M, Meyer H, et al. Cowpox virus in a 12-year-old boy: rapid identification by an orthopoxvirus-specific polymerase chain reaction. *Br J Dermatol* 2001;**145**:146–50.
117. Shimojo H. Virus infections in laboratories in Japan. *Bibl Haematol* 1975;771–3.
118. Silva-Fernandes AT, Travassos CE, Ferreira JM, et al. Natural human infections with Vaccinia virus during bovine vaccinia outbreaks. *J Clin Virol* 2009;**44**:308–13.
119. Smith GL, Mackett M, Moss B. Infectious vaccinia virus recombinants that express hepatitis B virus surface antigen. *Nature* 1983;**302**:490–5.
120. Smith GL, Murphy BR, Moss B. Construction and characterization of an infectious vaccinia virus recombinant that expresses the influenza hemagglutinin gene and induces resistance to influenza virus infection in hamsters. *Proc Natl Acad Sci U S A* 1983;**80**:7155–9.
121. Smith SK, Self J, Weiss S, et al. Effective antiviral treatment of systemic orthopoxvirus disease: ST-246 treatment of prairie dogs infected with monkeypox virus. *J Virol* 2011;**85**:9176–87.
122. Stagles MJ, Watson AA, Boyd JF, et al. The histopathology and electron microscopy of a human monkeypox lesion. *Trans R Soc Trop Med Hyg* 1985;**79**:192–202.
123. Stich A, Meyer H, Kohler B, et al. Tanapox: first report in a European traveller and identification by PCR. *Trans R Soc Trop Med Hyg* 2002;**96**:178–9.
124. Stittelaar KJ, van Amerongen G, Kondova I, et al. Modified vaccinia virus Ankara protects macaques against respiratory challenge with monkeypox virus. *J Virol* 2005;**79**:7845–51.
125. Torfason EG, Gunadottir S. Polymerase chain reaction for laboratory diagnosis of orf virus infections. *J Clin Virol* 2002;**24**:79–84.
126. Trindade GS, Emerson GL, Carroll DS, et al. Brazilian vaccinia viruses and their origins. *Emerg Infect Dis* 2007;**13**:965–72.
127. U.S. Department of Health and Human Services. *Biosafety in microbiological and biomedical laboratories*. Washington, D.C.: U. S. Government Printing Office; 1984. HHS Publication No. (NIH) 88-8395, p. 66.
128. U.S. Food and Drug Administration. Fast Track status for Imvamune. *Hum Vaccin* 2010;**6**:368–72.
129. Uzel M, Sasmaz S, Bakaris S, et al. A viral infection of the hand commonly seen after the feast of sacrifice: human orf (orf of the hand). *Epidemiol Infect* 2005;**133**:653–7.
130. von Magnus P, Andersen EK, Petersen KB, et al. A pox-like disease in cynomolgus monkeys. *Acta Pathol Microbiol Scand* 1959;**46**:156–76.
131. Weber O, Siegling A, Friebe A, et al. Inactivated parapoxvirus ovis (Orf virus) has antiviral activity against hepatitis B virus and herpes simplex virus. *J Gen Virol* 2003;**84**:1843–52.
132. Wilkinson JD. Orf: a family with unusual complications. *Br J Dermatol* 1977;**97**:447–50.
133. Williams WW. Guideline for infection control in hospital personnel. *Infect Control* 1983;**4**:326–49.
134. World Health Organization. *The global eradication of smallpox: final report of the Global Commission for the Certification of Smallpox Eradication—history of international public health*, vol. 4. Geneva: World Health Organization; 1980.
135. World Health Organization. Smallpox vaccination certificates. *Wkly Epidemiol Rec* 1981;**39**:305.
136. World Health Organization. Recombinant vaccinia viruses as live virus vectors for vaccine antigens: memorandum from a WHO/USPHS/NIBSC meeting. *World Health Organ* 1985;**63**:471–7.
137. Zagury D, Leonard R, Fouchard M, et al. Immunization against AIDS in humans. *Nature* 1987;**326**:249–50.
138. Zimmermann P, Thordsen I, Frangoulidis D, et al. Real-time PCR assay for the detection of tanapox virus and yaba-like disease virus. *J Virol Methods* 2005;**130**:149–53.

CHAPTER 164

MIMIVIRUSES

Ramya Ramraj • Gail J. Harrison

In 1992, while investigating the source of an outbreak of community-acquired pneumonia in northern England, investigators discovered a bizarre microorganism.[10,11] The microorganism, resembling a small gram-positive coccus, was found inside a free-living ameba in a water cooling tower. Researchers initially thought it was a bacterium, but subsequent analysis demonstrated a double-stranded DNA virus. Originally named mimivirus because it resembled bacteria in Gram-stained smears, it now is designated officially as a quasi-autonomous virus, *Acanthamoeba polyphaga mimivirus.* There is a proposed new order, Megaviridae, defining a new fourth domain of life, under consideration, which may include mimivirus in the future.[4,5,12]

STRUCTURE AND PROPERTIES

A. polyphaga mimivirus is a giant, double-stranded, icosahedral DNA virus.[26] With a diameter of approximately 650 nm, mimivirus is the largest virus known to date. Mimivirus morphologically resembles nucleocytoplasmic large DNA viruses (NCLDVs), such as the iridovirus, asfarvirus, and phycodnavirus.[7,23] However, evolutionary dissimilarities define a new family, the Mimiviridae.[10] The mimivirus comprises a central dense core that is surrounded by two lipid membrane layers inside a capsid protein shell covered by fibrils. The 1.2-Mb genome sequence[15,18,21] is complex and possesses genes that are shared by the NCLDV families as well as several that have not been identified previously in viruses. The origins and the various processes[1] involved in shaping gene content of the mimivirus are not entirely clear at this time.[10,15]

The virus can survive on inanimate surfaces for at least 30 days, is resistant to alcohol-based biocidal agents, but is inactivated by free chlorine and glutaraldeyde.[3,7]

VIRAL REPLICATION

In *A. polyphaga*, the replication cycle of the mimivirus starts with entry of the virus by phagocytosis, followed by a 4-hour eclipse phase inside the nucleus,[10] and then the formation of the "virus factory" in the cytoplasmic space,[22] ending with cell lysis and virus release 24 hours after infection (Fig. 164-1). The virus factory,[16] where virus replication and assembly occur, is the major site of production of mimivirus DNA and induces profound alteration of the infected cell structure, such as by recruitment of organelles and organization of cellular compartments. Very specific mechanisms and complex interactions between viral and cellular factors that are not entirely clear at this time must be involved to build this remarkably large and efficient virus factory, which can generate this sophisticated microorganism rapidly.[22]

ANIMAL SUSCEPTIBILITY

An experimental model of infection[8] was developed to establish a possible role of mimivirus as a human pathogen. Autopsy specimens of laboratory mice that were inoculated intracardially with infecting units of mimivirus revealed histopathologic evidence of pneumonia, and mimiviruses were reisolated from samples from the lung. Inoculation of human macrophages with mimiviruses showed evidence of infection, although no evidence of a lytic cycle was observed.[19] Efforts to induce infection in other cell lines were futile, and the target cell in mice remains to be identified.

MIMIVIRUS INFECTION IN HUMANS

In December 2004, a laboratory technician in France[20] developed subacute pneumonia with dry cough, fever, and chest pain. This technician was in charge of performing mimivirus serologic testing and Western blot analysis and thus had handled relatively large amounts of mimivirus antigens. Radiographic examination of his chest revealed bilateral basilar infiltrates (Fig. 164-2). The illness did not respond to amoxicillin-clavulanate treatment prescribed on day 15 after the onset of symptoms, but after an additional 2 weeks, the illness resolved, and the technician made a full recovery. Serum samples screened against pneumonia agents showed seroconversion (<1:50 to 1:3200) only for mimivirus, which was confirmed by testing with two-dimensional Western blotting against 23 identified proteins of mimivirus.

Prevalence of Antibodies to Mimivirus in Patients with Pneumonia

Three early studies investigated the prevalence of antibodies to mimivirus in specific human populations.[19] The first study tested serum samples obtained from 376 Canadian patients with community-acquired pneumonia and from 511 healthy subjects. A total of 9.66 percent of patients with pneumonia had antibodies to mimivirus,

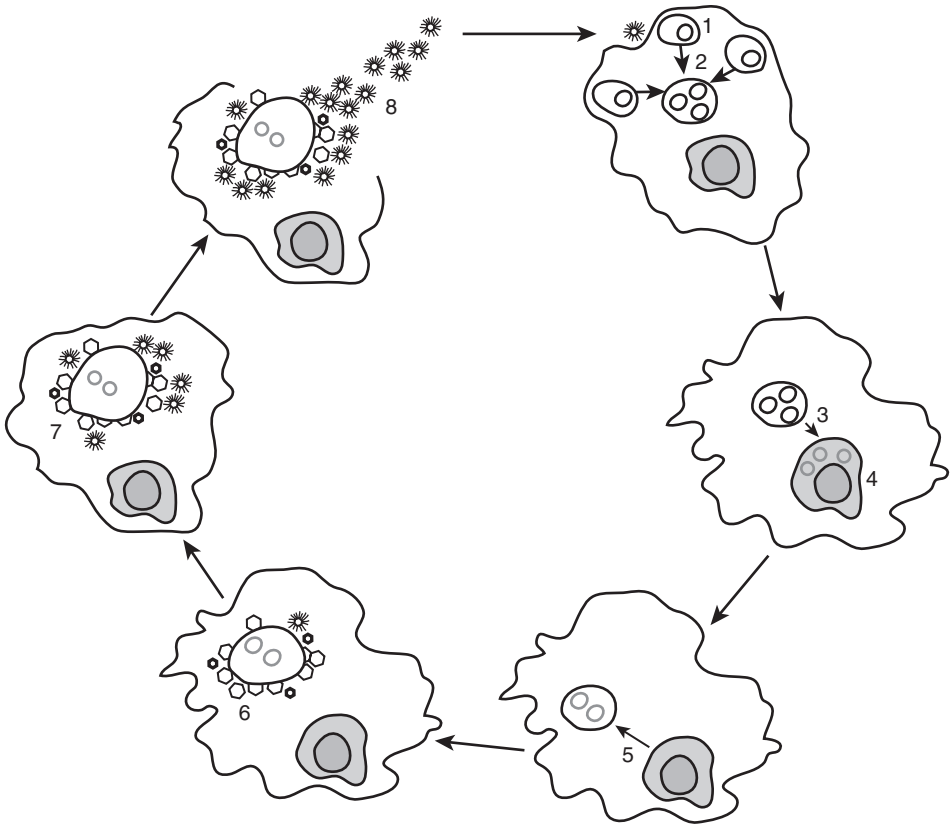

FIGURE 164-1 ■ Schematic representation of the *Acanthamoeba polyphaga mimivirus* replication cycle. *1,* Mimivirus entry through a phagocytic vacuole. *2,* Fusion of phagocytic vacuoles and delivery of mimivirus genetic material into the cell cytoplasm *(3). 3,* Mimivirus DNA entry into the host nucleus, where the first round of DNA replication could begin *(4). 5,* At 3 hours after infection, mimivirus DNA came from the host nucleus to form the virus factory (VF) replication center. *6,* At 5 hours after infection, the VF size showed a 50 percent increase, and viral proteins began to be detected; proviral capsid assembly and viral capsids budding from the VF central core could be observed. *7,* Empty or DNA-filled capsids accumulated near the central core, resulting in a growing VF with viral particles free in the cytoplasm. *8,* Complete viral capsids surrounded by fibrils may be released through cell lysis. (From Suzan-Monti M, La Scola B, Barrassi L, et al. Ultrastructural characterization of the giant volcano-like virus factory of *Acanthamoeba polyphaga Mimivirus. PLoS One* 2007;**2**:e328.)

compared with 2.3 percent of control subjects.[14] Patients with pneumonia who had antibodies to mimivirus were more likely to be hospitalized from a nursing home and to be rehospitalized after discharge.[13] The second study included 26 patients from Marseille, France, who acquired pneumonia while in an intensive care unit and 50 control serum samples (from blood donors). Antibodies to mimivirus were detected in samples obtained from 5 patients but in none of the control samples.[13] The third serosurvey involved 157 patients in intensive care units who had pneumonia. Serum specimens obtained from these patients were tested against a panel of antigens from "conventional" pneumonia agents as well as ameba-associated microorganisms, including mimivirus.[2] Evidence of infection with conventional pathogens was found in 28 cases and with ameba-associated pathogens in 18 cases. In the group infected by ameba-associated pathogens, more patients had seroconversion to mimivirus than to any other pathogen (5 cases), and seroconversion was more common among patients with ventilator-associated pneumonia than among those with community-acquired pneumonia (31.6% and 10.5% of patients, respectively). Additional serologic studies have shown an association of mimivirus seropositivity and increased duration of mechanical ventilation and prolonged stay in the intensive care unit in adult patients.[24] However, studies that have employed molecular detection techniques of reverse transcription polymerase chain reaction (RT-PCR) have not detected mimivirus DNA in respiratory secretions of hospitalized adult and pediatric patients with pneumonia.[6,9,24,25] Taken together, these studies reveal a significant rate of seroconversion and seropositivity in hospitalized patients with either community-acquired pneumonia or hospital-acquired pneumonia, but direct detection of the pathogen in clinical respiratory secretions has not been confirmed.[19]

DIAGNOSTIC METHODS

Diagnostic strategies include collection of bronchoalveolar lavage (BAL) fluid and serologic samples. Serologic testing includes immunofluorescence assay of samples of acute-phase and convalescent-phase serum for antibodies to proteins specific for mimivirus (Becton Dickinson, Rutherford, NJ).[2] However, one study employing

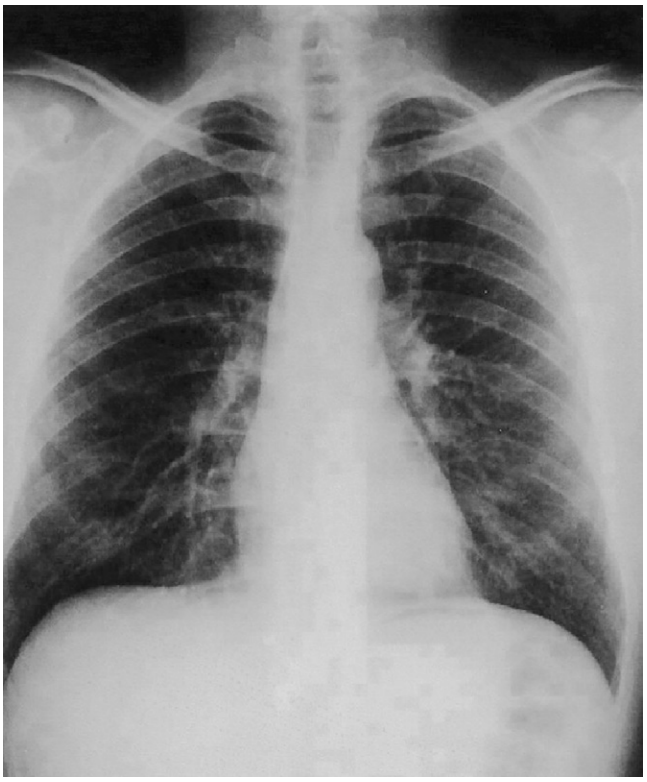

FIGURE 164-2 ■ Chest radiograph of a laboratory technician who was infected with mimivirus, with bilateral basilar infiltrates evident. (From Raoult D, La Scola B, Birtles R, et al. The discovery and characterization of Mimivirus, the largest known virus and putative pneumonia agent. *Clin Infect Dis* 2007;**45**:95–102.)

immunoproteomics showed cross reaction between mimivirus and *Francisella tularensis*, suggesting caution in interpretation of results when only serologic methods are employed to diagnose mimivirus infection or to determine associations of mimivirus and pneumonia.[17] Mimivirus also can be cultivated by co-culture with ameba. It also can be detected in DNA extracted (QIAamp tissue kit, Qiagen, Hilden, Germany) from BAL samples amplified by PCR assay.[2] However, real-time PCR methods have failed to consistently detect mimivirus in clinical respiratory secretions obtained from patients with pneumonia. The strongest evidence of infection includes a combination of positive BAL culture, positive PCR assays, and fourfold increase in antibody titer between acute-phase and convalescent-phase serum samples or significant seroconversion for mimivirus.[2]

MIMIVIRUS AS AN EMERGING PATHOGEN

Evidence indicates that mimivirus is a new human pathogen that causes pneumonia.[3] However, mimivirus does not replicate efficiently in co-culture with any of the mammalian cells tested to date, and serologic cross reactions among pathogens are observed commonly. Isolation of the virus or DNA detection by RT-PCR from respiratory secretions of patients with pneumonia has not confirmed the presence of the pathogen directly in clinical samples.[13] Mimivirus infection in hospitalized children with pneumonia has not been confirmed, and seroprevalence data in children have not been published.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Campos R. Andrade K, Ferreira P, et al. Virucidal activity of chemical biocides against mimivirus, a putative pneumonia agent. *J Clin Virol* 2012;**55**:323–8.
4. Claverie J, Abergel C. Mimiviruses: the emerging paradox of quasi-autonomous viruses. *Trends Genet* 2010;**26**:431, 437.
5. Colson P, de Lamballerie Y, Fournous G, Baoult D. Reclassification of giant viruses composing a fourth domain of life in new order Megaviridae. *Intervirology* 2012;**55**:321–2.
6. Dare RK, Chittaganpitch M, Erdman D. Screening pneumonia patients for mimivirus. *Emerg Infect Dis* 2008;**14**:465–7.
17. Pelleteir N, Taoult D, La Scola B. Specific recognition of the major capsid protein of *Acanthamoeba polyphaga mimivirus* by sera of patients infected with *Francisella tularensis*. *FEMS Microbiol Lett* 2009;**297**:117–23.
24. Vincent A, La Scola B, Forel J, et al. Clinical significance of a positive serology for mimivirus in patients presenting with a suspicion of ventilator-associated pneumonia. *Crit Care Med* 2009;**37**:111–18.
25. Vincent A, La Scola B, Papazian L. Advances in Mimivirus pathogenicity. *Intervirology* 2010;**53**:304–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Benarroch D, Claverie JM, Raoult D, Shuman S. Characterization of mimivirus DNA topoisomerase IB suggests horizontal gene transfer between eukaryal viruses and bacteria. *J Virol* 2006;**80**:314–21.
2. Berger P, Papazian L, Drancourt M, et al. Ameba-associated microorganisms and diagnosis of nosocomial pneumonia. *Emerg Infect Dis* 2006;**12**:248–55.
3. Campos R. Andrade K, Ferreira P, et al. Virucidal activity of chemical biocides against mimivirus, a putative pneumonia agent. *J Clin Virol* 2012;**55**:323–8.
4. Claverie J, Abergel C. Mimiviruses: the emerging paradox of quasi-autonomous viruses. *Trends Genet* 2010;**26**:431, 437.
5. Colson P, de Lamballerie Y, Fournous G, Baoult D. Reclassification of giant viruses composing a fourth domain of life in new order Megaviridae. *Intervirology* 2012;**55**:321–2.
6. Dare RK, Chittaganpitch M, Erdman D. Screening pneumonia patients for mimivirus. *Emerg Infect Dis* 2008;**14**:465–7.
7. Iyer LM, Aravind L, Koonin E. Common origin of four diverse families of large eukaryotic DNA viruses. *J Virol* 2001;**75**:11720–34.
8. Khan M, Lepidi H, Raoult D, et al. Pneumonia in mice inoculated experimentally with *Acanthamoeba polyphaga mimivirus*. *Microb Pathog* 2007;**42**:56–61.
9. Larcher C, Jeller V, Fischer H, Huemer H. Prevalence of respiratory viruses, including newly identified viruses, in hospitalized children in Austria. *Eur J Clin Microbiol Infect Dis* 2006;**25**:61–3.
10. La Scola B, Audic S, Robert C, et al. A giant virus in amoebae. *Science* 2003;**299**:2033.
11. La Scola B, Birtles RJ, Greub G, et al. *Legionella drancourtii* sp. nov., a strictly intracellular amoebal pathogen. *Int J Syst Evol Microbiol* 2004;**54**:699–703.
12. La Scola B, De Lamballerie X, Claverie JM, et al. Genus *Mimivirus*. In: Fauquet CM, Mayo MA, Maniloff J, et al, editors. *Virus taxonomy: VIIIth report of the International Committee on Taxonomy of Viruses*. 2nd ed. New York: Elsevier Academic Press; 2005. p. 275–6.
13. La Scola B, Marrie TJ, Auffray JP, et al. Mimivirus in pneumonia patients. *Emerg Infect Dis* 2005;**11**:449–52.
14. Marrie TJ, Raoult D, La Scola B, et al. *Legionella*-like and other amoebal pathogens as agents of community-acquired pneumonia. *Emerg Infect Dis* 2001;**7**:1026–9.
15. Moreira D, Lopez-Garcia P. Comment on "The 1.2-megabase genome sequence of Mimivirus." *Science* 2005;**308**:1114.
16. Novoa RR, Calderita G, Arranz R, et al. Virus factories: associations of cell organelles for viral replication and morphogenesis. *Biol Cell* 2005;**97**:147–72.
17. Pelleteir N, Taoult D, La Scola B. Specific recognition of the major capsid protein of *Acanthamoeba polyphaga mimivirus* by sera of patients infected with *Francisella tularensis*. *FEMS Microbiol Lett* 2009;**297**:117–23.
18. Raoult D, Audic S, Robert C, et al. The 1.2-megabase genome sequence of Mimivirus. *Science* 2004;**306**:1344–50.
19. Raoult D, La Scola B, Birtles R, et al. The discovery and characterization of Mimivirus, the largest known virus and putative pneumonia agent. *Clin Infect Dis* 2007;**45**:95–102.
20. Raoult D, Renesto P, Brouqui P. Laboratory infection of a technician by mimivirus. *Ann Intern Med* 2006;**144**:702–3.
21. Renesto-Audiffren P, Abergel C, Decloquement P, et al. Mimivirus giant particles incorporate a large fraction of anonymous and unique gene products. *J Virol* 2006;**80**:11678–85.
22. Suzan-Monti M, La Scola B, Barrassi L, et al. Ultrastructural characterization of the giant volcano-like virus factory of *Acanthamoeba polyphaga Mimivirus*. *PLoS One* 2007;**2**:e328.
23. Suzan-Monti M, La Scola B, Raoult D. Genomic and evolutionary aspects of *Mimivirus*. *Virus Res* 2006;**117**:145–55.
24. Vincent A, La Scola B, Forel J, et al. Clinical significance of a positive serology for mimivirus in patients presenting with a suspicion of ventilator-associated pneumonia. *Crit Care Med* 2009;**37**:111–18.
25. Vincent A, La Scola B, Papazian L. Advances in Mimivirus pathogenicity. *Intervirology* 2010;**53**:304–9.
26. Xiao C, Chipman PR, Battisti AJ, et al. Cryo-electron microscopy of the giant mimivirus. *J Mol Biol* 2005;**353**:493–6.

RNA VIRUSES

SUBSECTION 1
PICORNAVIRIDAE

CHAPTER 165

Enteroviruses, Parechoviruses, and Saffold Viruses

James D. Cherry • Paul Krogstad

Enteroviruses (EVs) and parechoviruses (PeVs) make up two genera of the family *Picornaviridae*. Saffold viruses (SAFVs) are genetically related to Theiler marine encephalomyelitis virus.[1114] They are classified as a new species in the genus *Cardiovirus*. These small RNA viruses are responsible for frequent and often significant human illnesses with protean manifestations.* To bring order to the growing list of poliovirus-like agents, coxsackieviruses, echoviruses, and polioviruses first were categorized together and named in 1957 by a committee sponsored by the National Foundation for Infantile Paralysis.[711] These viruses were grouped together because of similarities in their biophysical properties, shared features in their epidemiology and pathogenesis, and the many disease syndromes they caused. The human alimentary tract was thought to be the natural habitat of these agents, and the term *enterovirus* was coined. Newer agents later assigned to the genus *Enterovirus* were simply numbered sequentially, and more than 110 distinct human EV (HEV) types now are recognized. The two viruses originally classified as echoviruses 22 and 23 were reclassified into a new genus *(Parechovirus)* within the *Picornaviridae* family because of features of their structure and replication that distinguish them from the EVs.[954]

HISTORY

Poliomyelitis, the first enteroviral disease to be recognized and the most important one, has a long history.[812] The earliest record is an Egyptian stele of the 18th dynasty (1403 BCE to 1365 CE) that shows a young man with a withered, shortened leg, the characteristic deformity of paralytic poliomyelitis.[461,704] Michael Underwood,[1015] a London pediatrician, published the first medical description in 1789 in *A Treatise on Diseases of Children*. During the 19th century, many reports appeared in Europe and the United States describing small clusters of cases of "infantile paralysis." The authors were puzzled about the nature of the affliction; not until the 1860s and 1870s was the spinal cord firmly established as the seat of the pathologic process. The contagious nature of poliomyelitis was not appreciated until the late 19th century. Medin, a Swedish pediatrician, was the first to describe the epidemic nature of poliomyelitis (1890), and his pupil Wickman worked out the basic principles of the epidemiology.[1066]

The virus was isolated first in monkeys by Landsteiner and Popper[613] in 1908. The availability of a laboratory animal assay system opened many avenues of research that in the ensuing decades led to the demonstration that an unrecognized intestinal infection was the usual manifestation and the paralytic disease was a relatively uncommon event.

Coxsackieviruses and echoviruses have had a shorter history. Epidemic pleurodynia was described clinically in 1735 by Hannaeus[419] more than 200 years before the coxsackieviral origin of this disease was discovered. In 1948, Dalldorf and Sickles[254] first reported the isolation of a coxsackievirus after inoculation of suckling mice.

In 1949, Enders and associates[302] described the growth of poliovirus 2 in tissue culture, and their techniques paved the way for recovery of a large number of other cytopathic viruses. Most of these "new" viruses failed to produce illness in laboratory animals. Because the relationships of many of these newly recovered agents with human disease were unknown, these agents were called *orphan viruses*.[703] Later, several agents were grouped together and were called *e*nteric *c*ytopathogenic *h*uman *o*rphan viruses, or echoviruses. More recently, using molecular detection methods, SAFVs were identified in human infections.[531,1114]

*References 180, 182,183, 253, 404, 569, 650, 703, 707, 923, 1059-1062, 1063, 1114.

TABLE 165-1 **Original Classification of Human Enteroviruses: Animal and Tissue Culture Spectrum***

		Cytopathic Effect		*Illness and Pathology*	
Virus	**Antigenic Types†**	**Monkey Kidney Tissue Culture**	**Human Tissue Culture**	**Suckling Mouse**	**Monkey**
Polioviruses	1-3	+	+	–	+
Coxsackieviruses A	1-24‡	–	–	+	–
Coxsackieviruses B	1-6	+	+	+	–
Echoviruses	1-34§	+	±	–	–

Modified from Cherry J D. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* 3rd ed. Philadelphia, W. B. Saunders, 1990, p. 325-66.

*Many enterovirus strains have been isolated that do not conform to these categories, leading to the revised classification scheme shown in Table 165-2.

†New types, beginning with type 68, were initially assigned enterovirus type numbers instead of coxsackievirus or echovirus numbers. Types 68 through 71 were identified before the strategy for taxonomy was changed, as described in the text.

‡Coxsackievirus A23 was found to be the same as echovirus 9.

§Echovirus 10 was reclassified as a reovirus; echoviruses 22 and 23 were made the first members of the parechovirus genus within the picornavirus family and echovirus 28 was reclassified as a rhinovirus.

+, Present; –, absent; ±, variably present.

Inactivated and live-attenuated poliovirus vaccines became available in the late 1950s and early 1960s, and the most notable advance since the late 1980s has been a dramatic reduction in worldwide poliomyelitis as a result of the global immunization initiative.[227,483,802,988] The last case of confirmed paralytic poliomyelitis in the Western Hemisphere caused by a wild-type virus strain occurred in 1991.[868]

THE VIRUSES

Classification

EVs, PeVs, and SAFVs are RNA viruses belonging to the family *Picornaviridae* (*pico*, "small").* These viruses are grouped together because they share certain physical, biochemical, and molecular properties. In electron micrographs, the viruses are seen as 30-nm particles that consist of naked (nonenveloped) protein capsids, constituting approximately 70 to 75 percent of the mass of particles that contain the genomic RNA.

The original classification of HEVs is shown in Table 165-1. The EVs originally were distributed into four groups based on their different effects in tissue culture and pattern of disease in experimentally infected animals: polioviruses (causal agents of poliomyelitis in humans and nonhuman primates), coxsackieviruses A (associated with herpangina, human central nervous system [CNS] disease, and flaccid paralysis in suckling mice), coxsackieviruses B (human CNS and cardiac disease, spastic paralysis in mice), and the echoviruses (nonpathogenic in mice and not linked to human disease at first).

Although this scheme was useful initially, many strains were isolated subsequently that do not conform to such rigid specificities. For example, several coxsackievirus A strains replicate and have a cytopathic effect in monkey kidney tissue cultures, and some echovirus strains cause paralysis in mice. For this reason, and to simplify the nomenclature, EVs subsequently were assigned sequential numbers. Following this convention, the prototype EV strains Fermon, Toluca-1, J 670/71, and BrCr (identified between 1959 and 1973) were designated EV 68 through EV 71, respectively. Additional EVs continued to be identified that could not be using antisera specific for the classic serotypes. More than 40 distinct EVs have been identified by culture and molecular detection methods, although many have not been linked to human disease.

Since the late 1990s, genetic, biologic, and molecular properties have been used to revise picornavirus taxonomy. Determining the nucleotide sequence encoding the viral VP1 capsid protein plays a major role in this approach and predictably identifies viruses originally classified by serologic means, thus leading to the term *molecular serotyping*.[787,788,833,839] This phylogenetic technique formed the basis of a reorganization of the HEVs into four alphabetically designated species (HEV-A, B, C, and D) (Table 165-2). The three human polioviruses (PV1 to 3) have been classified as members of HEV-C, in view of their close molecular relationship to other members of the species.[108]

Two of the original echoviruses (E22 and E23) have long been known to exhibit genomic and proteomic differences from the other early "enteroviruses" and were reclassified as first members of the new genus *Parechovirus* as *Parechovirus* types 1 and 2.[843,954,955] More than a dozen additional types of human PeV have now been identified. Similarly, hepatitis A virus initially was assigned the designation EV 72, but it was reclassified as the sole member of the *Hepatovirus* genus within the picornavirus family because of marked genetic and biologic distinctions from the EVs.

The *Cardiovirus* genus of the Picornaviridae has recently been augmented by identification of the SAFVs. Beginning with a strain isolated in 2007, eight genetically distinct SAFVs have been identified since 2007 and added to the *Cardiovirus* genus as members of the *theilovirus* spp.[531]

*References 319, 491, 707, 708, 709, 710, 801, 890, 956.

TABLE 165-2 **Genomic Classification of Enteroviruses**

Species Designation	Types
Human enterovirus A (HEV-A)	Coxsackievirus A2-8, 10, 12, 14, 16
	Enterovirus A71, A76, A89, A90, A91, A114, A119
Human enterovirus B (HEV-B)	Coxsackievirus A9
	Coxsackievirus B 1-6
	Echovirus 1-9, 11-21, 24-27, 29-33
	Enterovirus B69, B73-75, B77-88, B93, B97, B98, B100, B101, B106, B107
Human enterovirus C (HEV-C)	Poliovirus 1-3
	Coxsackievirus A 1, 11, 13, 17, 19-22, 24
	Enterovirus C95, C96, C99, C102, C104, C105, C109, C113, C116-C118
Human enterovirus D (HEV-D)	Enterovirus D68, D70, D94, D111

King AMQ, Adams MJ, Carstens EB, et al, editors. Virus taxonomy: classification and nomenclature of viruses: ninth report of the International Committee on Taxonomy of Viruses. San Diego: Elsevier Academic Press, 2011.
Coxsackievirus (CV) A15 has been reclassified as a strain of CVA11, and CVA18 as a strain of CVA13. Echovirus 34 has been recognized as a variant of coxsackievirus A24.

TABLE 165-3 **Cellular Receptors and Cofactors for Infection of Representative Enteroviruses**

Virus	HEV Species	Receptor	Cofactor for Infection*
Enterovirus 71	HEV-A	PSGL-1, SCARB2	
Coxsackieviruses B1-6	HEV-B	CAR	Some group B coxsackieviruses may use CD55 (DAF) or heparan for attachment
Echovirus 9	HEV-B	$\alpha_v\beta_3$ integrin (vitronectin receptor)	MAP-70
Echoviruses 1, 8	HEV-B	VLA-2 ($\alpha_2\beta_1$ integrin)	Heparan sulfate
Coxsackieviruses A13, 17, 20, 21, 24	HEV-C	ICAM-1	
Polioviruses 1-3	HEV-C	CD155(PVR)	
Enterovirus 70	HEV-D	CD55 (DAF)	

*The cofactors generally facilitate adhesion to cells, but their sole expression is insufficient to permit infection to occur.
CAR, Coxsackie and adenovirus receptor; *DAF,* decay accelerating factor; *HEV,* human enterovirus; *ICAM 1,* intercellular adhesion molecule 1; *MAP-70,* microtubule-associated protein 70; *PSGL-1,* P-selectin glycoprotein ligand 1; *Pvr,* poliovirus receptor; *SCARB2,* scavenger receptor class B member 2; *VLA-2,* a human integrin.

The application of molecular phylogenetic approaches also revealed that recombination between circulating picornaviruses is a frequent event and is likely to increase their genetic diversity of EVs, cardioviruses, and PeVs.* This propensity for recombination has played a role in more recent outbreaks of paralytic diseases involving vaccine-derived strains of poliovirus.[563]

Morphology and Replication

The genome of EVs and PeVs is a single-stranded, positive-sense RNA molecule approximately 7.4 kb in length.[834] It consists of a 5′ noncoding region followed by a single long open-reading frame, a short 3′ noncoding region, and a polyA tail. The 5′ noncoding region contains an internal ribosome entry site that recruits the translational machinery to an initiation site approximately 740 nucleotides downstream from the 5′ terminus of the viral RNA . The 3′ noncoding region folds into highly conserved secondary and tertiary structures that are thought to play a role in the initiation of the replication of the viral genome. This genome is packaged into capsids that exhibit icosahedral symmetry with 20 triangular faces and 12 vertices.

Replication of EVs begins with the adsorption of virions to cell surface receptors, which, for the most part, are integrins or immunoglobulin-like proteins (Table 165-3). The virions penetrate the surface of cell and undergo conformational changes that release the viral genome, which functions as messenger RNA for the viral polyprotein.[636,927] This polypeptide contains three domains, P1 to P3, which are cleaved into three or four proteins each. The P1 region is liberated from the polyprotein by the viral 2A protein, a chymotrypsin-like protease. P1 initially is split into three proteins, VP0, VP1, and VP3, by the viral 3C protease. VP0 then is processed further into two smaller proteins, VP4 and VP2. Portions of VP1, VP2, and VP3 are exposed at the surface of the virion, whereas VP4 is entirely internal. VP1, VP2, and VP3 have no sequence homology but share the same topology.[843] Specifically, they form an eight-stranded antiparallel β-barrel that is wedge shaped and comprises two antiparallel β-sheets. The amino acid sequences in

*References 74, 160, 197, 281, 657-659, 785, 796, 942, 970.

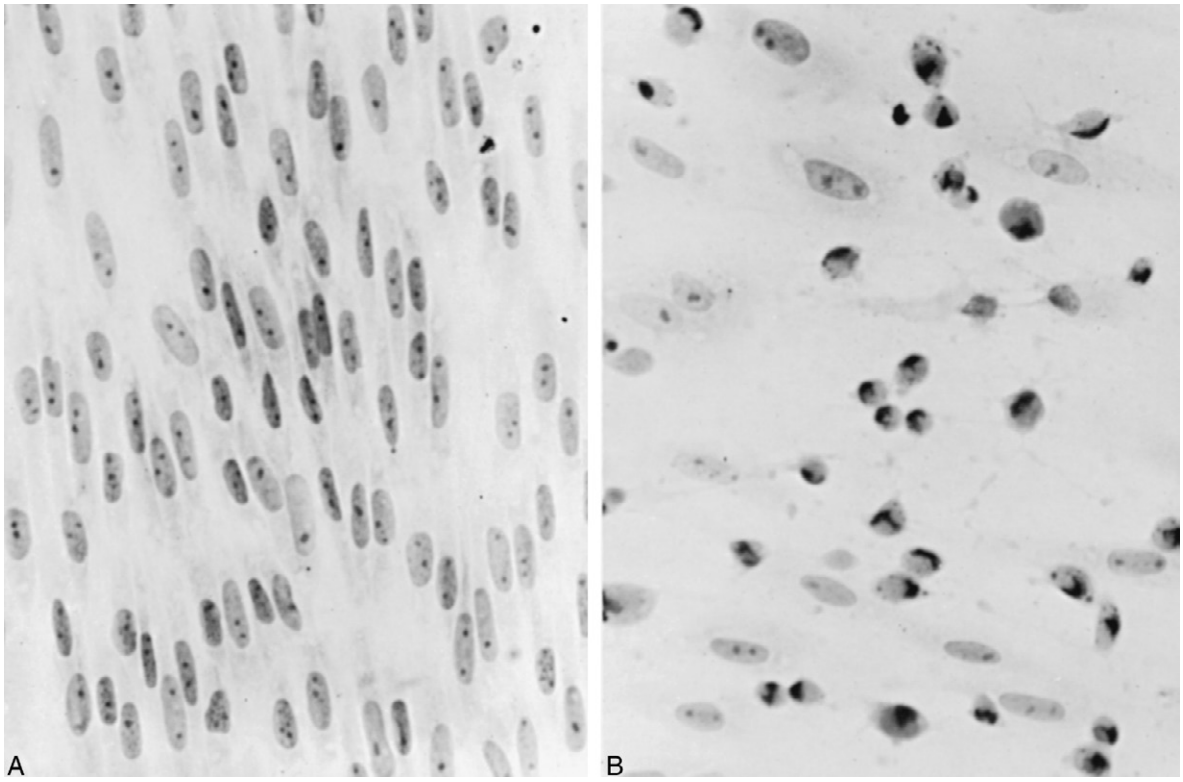

FIGURE 165-1 ■ Fetal rhesus monkey kidney tissue culture (HL-8). **A,** Uninoculated tissue culture. **B,** Echovirus 11 cytopathic effect. (From Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* Philadelphia, W.B. Saunders, 1976.)

the loops that connect the β-strands and the N- and C-terminal sequences that extend from the β-barrel domain of VP1, VP2, and VP3 give each EV its distinct antigenicity. The replication of EVs occurs in the cytoplasm in membrane-associated replication complexes and is completed rapidly (5 to 10 hours). Studies of polioviruses and coxsackieviruses have shown that enteroviral replication is associated with disruption of cellular protein secretion, and host-cell protein synthesis is suppressed because of cleavage of human eukaryotic translation initiation factor 4G (eIF4G) by enteroviral 2A proteases, which abrogates cap-dependent translation. The coxsackievirus 2A protein also cleaves dystrophin, a cytoskeletal protein; this activity has been hypothesized to play a role in damage to the myocardium.[43,272]

The PeVs replicate in a similar fashion.[597] Integrins ($\alpha_v\beta_3$ and perhaps $\alpha_v\beta_1$ and $\alpha_v\beta_6$) are used as receptors, and replication occurs in cytoplasmic structures.[926] However, the P1 portion of the viral polyprotein is processed into only three capsid proteins, and only one protease has been identified in the PeVs. In addition, PeV replication occurs in small, discrete foci in the cytoplasm, rather than in large accumulations of membranous vesicles like the EVs. Moreover, transcription and translation do not appear to be disrupted by the PeVs, thus perhaps explaining their relatively mild and delayed cytopathic effect when they are grown in tissue culture.[957]

The replication of the Saffold cardioviruses has not been specifically examined, but genetic similarities to Theilers murine encephalomyelitis virus (TMEV) and other murine cardioviruses suggest that they possess a single protease related to the 3C protease of other picornaviruses.[451] The SAFV genome also appears to encode a leader (L) protein at the amino terminus of the viral polyprotein. The L proteins of TMEV and encephalomyocarditis viruses are thought to modulate viral translation, innate immune responses, and cellular apoptosis.[450]

Replication Characteristics and Host Systems

EVs and PeVs are relatively stable viruses in that they retain activity for several days at room temperature and can be stored indefinitely at ordinary freezer temperatures (–20°C [–4°F]). They are inactivated quickly by heat (>56°C [132.8°]), formaldehyde, chlorination, and ultraviolet light, but they are refractory to ether, ethanol, and isopropanol.[708,711,803,878]

Enteroviral strains grow rapidly when adapted to susceptible host systems and cause cytopathologic features in 2 to 7 days. The typical tissue culture cytopathic effect is shown in Figure 165-1; characteristic pathologic findings in mice are shown in Figures 165-2 and 165-3. Final titers of virus recovered in the laboratory vary markedly among different viral strains and the host system used; typically, concentrations of 10^4 to 10^8 infectious units per milliliter of tissue culture fluid or tissue homogenate are obtained. Unadapted viral strains frequently require long periods of incubation. In both tissue culture and suckling mice, evidence of growth usually is visible. Blind passage

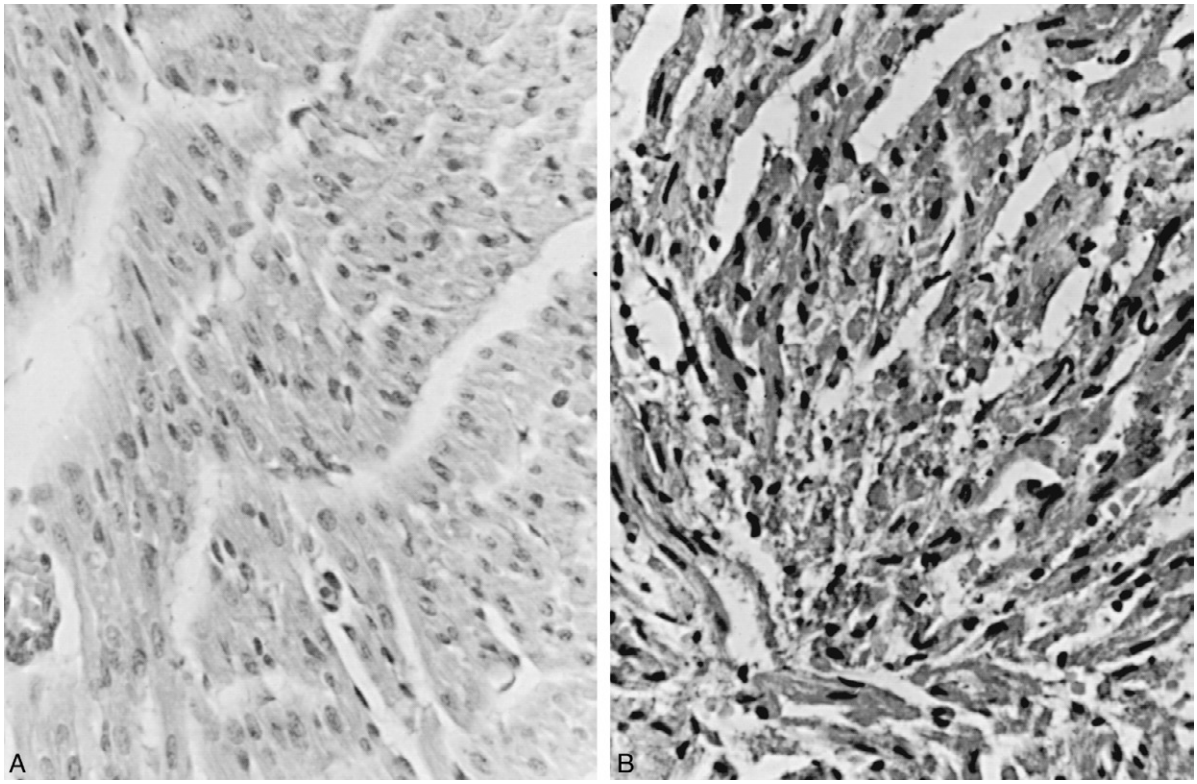

FIGURE 165-2 ■ Suckling mouse myocardium. **A,** Normal suckling mouse myocardium. **B,** Myocardium of a suckling mouse infected with coxsackievirus B1. (From Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* Philadelphia, W.B. Saunders, 1976.)

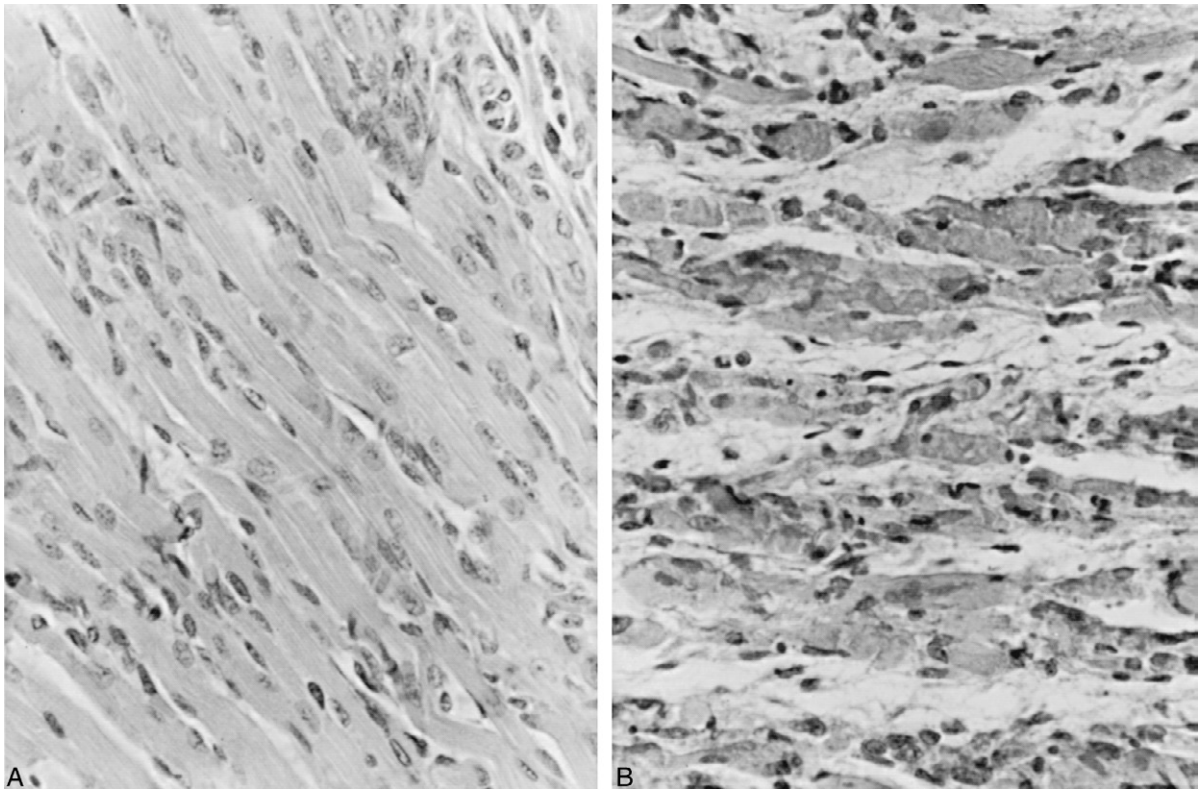

FIGURE 165-3 ■ Suckling mouse skeletal muscle. **A,** Normal suckling mouse skeletal muscle. **B,** Skeletal muscle of a mouse infected with coxsackievirus A16. (From Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* Philadelphia, W.B. Saunders, 1976.)

occasionally is necessary for the cytopathologic features to become apparent.

Although many different primary and secondary tissue culture systems support the growth of various EVs, primary rhesus monkey kidney cultures generally are accepted to have the most inclusive spectrum.[113] Other simian kidney tissue cultures, however, also have the same broad spectrum. Tissue cultures of human origin have a more limited spectrum, but several echovirus types have shown more consistent primary isolation in human than in monkey kidney culture. A satisfactory system for the primary recovery of EVs from clinical specimens would include primary rhesus, cynomolgus, or African green monkey kidney; a diploid, human embryonic lung fibroblast cell strain; rhabdomyosarcoma cell line tissue cultures; and intraperitoneal and intracerebral inoculation of suckling mice younger than 24 hours.*

Antigenic Characteristics

Although some minor cross reactions exist among several coxsackievirus and echovirus types, common group antigens of diagnostic importance have not been defined well.[704,706,707,711,801] Heat treatment of virions and the use of synthetic peptides have produced antigens with broad enteroviral reactivity.[899,990] These antigens have been used in enzyme-linked immunosorbent assay (ELISA) and complement fixation tests to determine immunoglobulin (Ig)G and IgM enteroviral antibodies and for antigen detection. In one study, Terletskaia-Ladwig and colleagues[990] reported the identification of patients infected with EVs by the use of an IgM enzyme immunoassay (EIA). This test used heat-treated coxsackievirus B5 and echovirus 9 as antigens, and it identified patients infected with echoviruses 4, 11, and 30. The sensitivity of the test was 35 percent. In another study involving heat-treated virus or synthetic peptides, the respective sensitivities were 67 and 62 percent.[899] However, both tests lacked specificity. Intratypic strain differences are common findings, and some strains (prime strains) are neutralized poorly by antisera to prototype viruses. In animals, however, these prime strains induce antibodies that neutralize the specific prototype viruses.

The identification of polioviral, coxsackieviral, and echoviral types by neutralization in suckling mice or tissue culture with antiserum pools is relatively well defined. Neutralization is induced by the epitopes on structural proteins VP1, VP2, and VP3; in particular, several epitopes are clustered on VP1. Prime strains do cause diagnostic difficulty because frequently they are not neutralized by the reference antisera, a particular problem with echoviruses 4, 9, and 11 and EV 71. If these types are suspected, in some instances this problem can be overcome by using antisera in less diluted concentrations or antisera prepared against several different strains of problem viruses. Kubo and associates[600] were able to type enteroviral isolates not identified through neutralization by nucleotide sequence analysis of the VP4 gene. They specifically identified prime strains of echovirus 18 and EV 71. Sequence analysis of the VP1 gene also is useful for typing enteroviral prime strains not identified by neutralization.[775]

Host Range

Humans are the only natural hosts of polioviruses, coxsackieviruses, and echoviruses.* However, EVs have been recovered in nature from sewage, flies, swine, dogs, a calf, a budgerigar, a fox, mussels, monkeys, and oysters.[182] In addition, serologic evidence of infection with EVs similar to human strains has been noted in chimpanzees, cattle, rabbits, a fox, a chipmunk, and a marmot.[182] A variety of EVs that are distinct from the human EVs have been isolated from nonhuman primates.[784] Contamination of shellfish also is interesting because in addition to their possible role in human infection, shellfish offer a source of enteroviral storage during periods of cold weather.[182] Contaminated food is another possible source of human infection.[182]

EPIDEMIOLOGY

Transmission

The spread of HEVs, PeVs, and also presumably SAVFs, is from person to person by the fecal-oral and possibly the oral-oral (respiratory spread) route. Swimming and wading pools may serve as a means of spread of EVs during the summer.[559] Oral-oral transmission by way of the contaminated hands of health care personnel and transmission by fomites were documented in a chronic care pediatric ward.[520] EVs have been recovered from trapped flies, and such carriage probably contributes to the spread of human infection, particularly in lower socioeconomic populations with poor sanitation facilities.[182] Between 1972 and 2004, Sinclair and colleagues[944] noted 13 outbreaks of EV disease related to swimming in lakes, pools, or the ocean. One noteworthy point source outbreak occurred in a group of travelers to Mexico who swam in contaminated seawater.[944]

Children are the main susceptible cohort; they are immunologically susceptible, and their unhygienic habits facilitate the spread of infection. Spread is from child to child (from feces to skin to mouth) and then within family groups. Recovery of EVs is related inversely to age, but the prevalence of specific antibodies is related directly to age. The incidence of infection and the prevalence of antibodies do not differ between boys and girls.

Geographic Distribution and Season

EVs, PeVs, and SAFVs have worldwide distribution.[182,330,332,347,365,1114] Neutralizing antibodies for specific viral types have been noted in serologic surveys throughout the world, and most strains have been recovered in global isolation studies. In any given area, frequent fluctuations occur in predominant types. Epidemics probably depend on newly susceptible individuals in the population rather than on reinfection. Epidemics may be

*References 64, 427, 440, 707, 709, 876.

*References 253, 365, 569, 703, 761, 923, 1060, 1062.

localized and sporadic, and they may vary in origin from place to place in the same year. Pandemic waves of infection also occur.

In temperate climates, enteroviral infections occur primarily in the summer and fall, but in the tropics, they are prevalent all year.[182,365,706] A basic concept in understanding the epidemiology of these illnesses concerns the far greater frequency of unrecognized infection than clinical disease, as illustrated by poliomyelitis, which remained an epidemiologic mystery until researchers appreciated that unrecognized infections were the main source of contagion. Serologic surveys were instrumental in elucidating the problem; in populations living in conditions of poor sanitation and hygiene, epidemics did not occur, but wide dissemination of polioviruses was confirmed by demonstrating the presence of specific antibodies to all three types in almost 100 percent of children by the time they reached 5 years of age.

Epidemics of poliomyelitis first began to appear in Europe and the United States during the latter part of the 19th century; they continued with increasing frequency in economically advanced countries until the introduction of effective vaccines in the 1950s and 1960s.[94,217,461,812] The evolution from endemic to epidemic follows a characteristic pattern beginning with collections of a few cases, then endemic rates that are higher than usual, followed by severe epidemics with high attack rates. The age group attacked in endemic areas and in early epidemics is the youngest; more than 90 percent of paralytic cases begin in children younger than 5 years of age. Once this pattern of epidemicity begins, it is irreversible unless preventive vaccination is performed.

As epidemics recur over a period of years, a shift in age incidence occurs whereby relatively fewer cases are seen in the youngest children; the peak often is in the 5- to 14-year-old group, and increasing percentages of cases develop in young adults. These changes are correlated with socioeconomic factors and improved standards of hygiene—when children are protected from immunizing infections in the first few years of life, the pool of susceptible persons builds up, and introduction of a virulent strain often is followed by an epidemic.[461] The extensive use of vaccines since the late 1950s resulted in the elimination of paralytic poliomyelitis from large geographic areas, but the disease remains endemic in various parts of the world. Although seasonal periodicity is distinct in temperate climates, some viral activity does take place during the winter months.[182] Infection and the acquisition of postinfection immunity occur with greater intensity and at earlier ages in crowded, economically deprived populations with less efficient sanitation.

Molecular techniques have allowed researchers to study the genotypes of specific viral types in populations over the course of time.[278,498,644,741] For example, Mulders and colleagues[741] studied the molecular epidemiology of wild poliovirus 1 in Europe, the Middle East, and the Indian subcontinent. These investigators found that four major genotypes were circulating. Two genotypes were found predominantly in eastern Europe, a third genotype was circulating mainly in Egypt, and the fourth genotype was dispersed widely. All four genotypes were present in Pakistan.

Prevalence of Different Types

The epidemiologic behavior of coxsackieviruses and echoviruses parallels that of polioviruses, in which unrecognized infections far outnumber infections with distinctive symptoms. The agents are disseminated widely throughout the world, and outbreaks caused by one or another type occur regularly. These outbreaks tend to be localized, with different agents prevalent in different years. In the late 1950s, however, echovirus 9 had a far wider circulation; it swept through a large part of the world and infected not only children but also young adults.

Coxsackievirus A16 viral infection with hand-foot-and-mouth disease first was noted in Toronto and New Zealand in 1957.[188] Subsequently, illness caused by this virus was noted throughout the world. Antibody studies and viral isolation studies in 1957 to 1963 in California and Wisconsin indicated that coxsackievirus A16 was a newly circulating virus.[186,188] Since the 1960s, coxsackievirus A16 outbreaks of hand-foot-and-mouth disease continued to occur in the United States. However, since 1968, this virus has not been one of the five most prevalent yearly isolates (Table 165-4).

EV 71 was first isolated in California from patients with aseptic meningitis from 1969 to 1972.[795,917] Subsequently, this virus has been noted in the United States in association with aseptic meningitis and occasionally with encephalitis. The clinical manifestations of EV 71 changed, and the chronology of this is presented by Oii and colleagues.[795] In addition to aseptic meningitis, hand-foot-and-mouth disease and a broad range of encephalitis presentations (e.g., paralytic disease and brain stem encephalitis) occurred. In Japan, in 1973,1977, and 1978, 1031 cases occurred; most patients had hand-foot-and-mouth disease. Since 1997, severe hand-foot-and-mouth disease with paralysis, cardiorespiratory dysfunction, and brain stem encephalitis has had epidemic occurrence in Malaysia, Taiwan, Singapore, Brunei, Japan, China, and other Asian-Pacific countries.

Although severe EV 71 disease has occurred occasionally in North America and Western Europe, epidemics similar to those occurring in the Asian-Pacific region have not been noted.[44,717,795] There are three genotypes of EV 71: A, B, and C. B and C have subtypes B 1 to 5, and C 1 to 5. However, there does not appear to be a clear association with specific sub-types and the occurrence of more severe disease. Chang and colleagues[162] found that the human leukocyte antigen (HLA)-A33 phenotype was associated with EV 71 infections. This HLA-A33 phenotype is common in Asian populations but is rare in white populations. This suggests a possible reason for the difference in epidemic occurrence in the Asian-Pacific region and the United States and Western Europe.

After a long absence, a particular agent may return and circulate among susceptible persons of different ages who have been born since the last epidemic. Other agents remain endemic in a given area and surface as sporadic cases and occasionally in small outbreaks. Multiple types frequently are active at the same time, although one agent commonly is predominant in a given locality.

TABLE 165-4 **Predominant Types of Nonpolio Enteroviral Isolations in the United States: 1961 to 2008***

	Five Most Common Viral Types Per Year				
	First	**Second**	**Third**	**Fourth**	**Fifth**
1961	Coxsackievirus B5	Coxsackievirus B2	Coxsackievirus B4	Echovirus 11	Echovirus 9
1962	Coxsackievirus B3	Echovirus 9	Coxsackievirus B2	Echovirus 4	Coxsackievirus B5
1963	Coxsackievirus B1	Coxsackievirus A9	Echovirus 9	Echovirus 4	Coxsackievirus B4
1964	Coxsackievirus B4	Coxsackievirus B2	Coxsackievirus A9	Echovirus 4	Echovirus 6, coxsackievirus B1
1965	Echovirus 9	Echovirus 6	Coxsackievirus B2	Coxsackievirus B5	Coxsackievirus B4
1966	Echovirus 9	Coxsackievirus B2	Echovirus 6	Coxsackievirus B5	Coxsackievirus A9, A16
1967	Coxsackievirus B5	Echovirus 9	Coxsackievirus A9	Echovirus 6	Coxsackievirus B2
1968	Echovirus 9	Echovirus 30	Coxsackievirus A16	Coxsackievirus B3	Coxsackievirus B4
1969	Echovirus 30	Echovirus 9	Echovirus 18	Echovirus 6	Coxsackievirus B4
1970	Echovirus 3	Echovirus 9	Echovirus 6	Echovirus 4	Coxsackievirus B4
1971	Echovirus 4	Echovirus 9	Echovirus 6	Coxsackievirus B4	Coxsackievirus B2
1972	Coxsackievirus B5	Echovirus 4	Echovirus 6	Echovirus 9	Coxsackievirus B3
1973	Coxsackievirus A9	Echovirus 9	Echovirus 6	Coxsackievirus B2	Coxsackievirus B5, echovirus 5
1974	Echovirus 11	Echovirus 4	Echovirus 6	Echovirus 9	Echovirus 18
1975	Echovirus 9	Echovirus 4	Echovirus 6	Coxsackievirus A9	Coxsackievirus B4
1976	Coxsackievirus B2	Echovirus 4	Coxsackievirus B4	Coxsackievirus A9	Coxsackievirus B3, echovirus 6
1977	Echovirus 6	Coxsackievirus B1	Coxsackievirus B3	Echovirus 9	Coxsackievirus A9
1978	Echovirus 9	Echovirus 4	Coxsackievirus A9	Echovirus 30	Coxsackievirus B4
1979	Echovirus 11	Echovirus 7	Echovirus 30	Coxsackievirus B2	Coxsackievirus B4
1980	Echovirus 11	Coxsackievirus B3	Echovirus 30	Coxsackievirus B2	Coxsackievirus A9
1981	Echovirus 30	Echovirus 9	Echovirus 11	Echovirus 3	Coxsackievirus A9, echovirus 5
1982	Echovirus 11	Echovirus 30	Echovirus 5	Echovirus 9	Coxsackievirus B5
1983	Coxsackievirus B5	Echovirus 30	Echovirus 20	Echovirus 11	Echovirus 24
1984	Echovirus 9	Echovirus 11	Coxsackievirus B5	Echovirus 30	Coxsackievirus B2, A9
1985	Echovirus 11	Echovirus 21	Echovirus 6, 7†		Coxsackievirus B2
1986	Echovirus 11	Echovirus 4	Echovirus 7	Echovirus 18	Coxsackievirus B5
1987	Echovirus 6	Echovirus 18	Echovirus 11	Coxsackievirus A9	Coxsackievirus B2
1988	Echovirus 11	Echovirus 9	Coxsackievirus B4	Coxsackievirus B2	Echovirus 6
1989	Coxsackievirus B5	Echovirus 9	Echovirus 11	Coxsackievirus B2	Echovirus 6
1990	Echovirus 30	Echovirus 6	Coxsackievirus B2	Coxsackievirus A9	Echovirus 11
1991	Echovirus 30	Echovirus 11	Coxsackievirus B1	Coxsackievirus B2	Echovirus 7
1992	Echovirus 11	Echovirus 30	Echovirus 9	Coxsackievirus B1	Coxsackievirus A9
1993	Echovirus 30	Coxsackievirus B5	Coxsackievirus A9	Echovirus 7	Coxsackievirus B3
1994	Coxsackievirus B2	Coxsackievirus B3	Echovirus 6	Echovirus 30	Enterovirus 71
1995	Echovirus 9	Echovirus 11	Coxsackievirus A9	Coxsackievirus B2	Echovirus 30, coxsackievirus B5
1996	Coxsackievirus B5	Echovirus 17	Echovirus 6	Coxsackievirus A9	Coxsackievirus B4
1997	Echovirus 30	Echovirus 6	Echovirus 7	Echovirus 11	Echovirus 18
1998	Echovirus 30	Echovirus 9	Echovirus 11	Coxsackievirus B3	Echovirus 6
1999	Echovirus 11	Echovirus 16	Echovirus 9	Echovirus 14	Echovirus 25
2000	Coxsackievirus B5	Echovirus 6	Coxsackievirus A9	Coxsackievirus B4	Echovirus 11
2001	Echovirus 18	Echovirus 13	Coxsackievirus B2	Echovirus 6	Echovirus 4
2002	Echovirus 7	Echovirus 9	Coxsackievirus B1	Echovirus 11	Coxsackievirus B5
2003	Echovirus 9	Echovirus 30	Coxsackievirus B1	Coxsackievirus B4	Coxsackievirus A9
2004	Echovirus 30	Echovirus 9	Coxsackievirus A9	Coxsackievirus B5	Coxsackievirus B4
2005	Coxsackievirus B5	Echovirus 6	Echovirus 30	Echovirus 18	Coxsackievirus B3
2006	Echovirus 6	Echovirus 9	Coxsackievirus A9	Coxsackievirus B5	Coxsackievirus B3
2007	Coxsackievirus B1	Echovirus 18	Echovirus 9	Coxsackievirus B4	Echovirus 11
2008	Coxsackievirus B1	Echovirus 30	Echovirus 6	Echovirus 9	Echovirus 11

Data from references 13, 138, 142-145, 150, 182, 356, 967 and personal communication from LaMonte-Fowlkes A, Epidemiology Branch, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, for the 2005 data.

*The majority of patients from whom viruses were isolated had neurologic illnesses.

†Third and fourth place tie.

Listed in Table 165-4 are the five most prevalent nonpolio enteroviral isolations per year in the United States from 1961 through 2008* (also, Personal communication, JP Alexander, LJ Anderson, Respiratory and Enterovirus Branch, Centers for Disease Control and Prevention [CDC], 1990; H. Gary, Respiratory and Enteric Viruses Branch, CDC, 1996; A. LaMonte-Fowlkes, Epidemiology Branch, Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, CDC).

Most patients from whom viruses were isolated had neurologic illnesses. Other EVs also possibly were prevalent but did not cause clinical disease sufficiently severe enough to induce physicians to submit specimens for study. In addition, probably many coxsackievirus A infections, even in epidemic situations, have gone undiagnosed because inoculation of suckling mice was not performed. Although more than 62 nonpolio EV types and 4 PeV types have been identified, of interest is that in the 48 years covered in Table 165-4, only 24 different virus types are noted. Khetsuriani and associates[153] at the CDC presented an extensive report on enteroviral surveillance in the United States for the period from 1970 to 2005. During this period, the most common enteroviral isolates, in order, were echoviruses 9, 11, and 30; coxsackievirus B5; and echovirus 6. During the period from 2000 to 2005, the most common isolates, in order, were echoviruses 9, 30, 18, and 13 and coxsackievirus B5. The extensive EV surveillance report (between 1970 and 2005) contained data on both serotype and genomic species designation.[153] Of 49,637 viral isolates, 58 different EV serotypes and 2 PeV serotypes were identified. Of these isolates, 94.6, 3.1, 0.3, and 0.1 percent were genomic species HEV-B, HEV-A, HEV-C, and HEV-D, respectively, and 1.9 percent were PeVs. Similar serotype and genomic species data are also available for 2006 to 2008.[150] During this period, 15 serotypes were identified, and 13 of which were genomic species HEV-B.

Similar data for the most common enteroviral isolates in Spain from 1988 to 1997 and in Belgium from 1980 to 1994 are available.[284,1005] The EV isolated most frequently in both countries was echovirus 30. In 1997 and 1998, major epidemic disease caused by EV 71 occurred in Taiwan, Malaysia, Australia, and Japan.[109,159,453,591,694]

Even though the use of live polioviral vaccine has eliminated epidemic poliomyelitis in the United States, determining the effect polio vaccine viruses has had on EV ecology has been difficult. In 1970, polioviruses accounted for only 6 percent of the total number of enteroviral isolations from patients with neurologic illnesses.[182] Although the numbers are not directly comparable, more than one third of the enteroviral isolations in 1962 from similar patients were polioviruses.[182] However, Horstmann and colleagues[463] studied specimens from sewage and from asymptomatic children during the early vaccine era and noted that the number of yearly polioviral isolations (presumably vaccine strains) was greater than the number of nonpolio EVs. The prevalence of vaccine viruses apparently did not affect the seasonal epidemiology of other EVs.

*References 138, 145, 147, 150, 172, 173, 182, 355, 966.

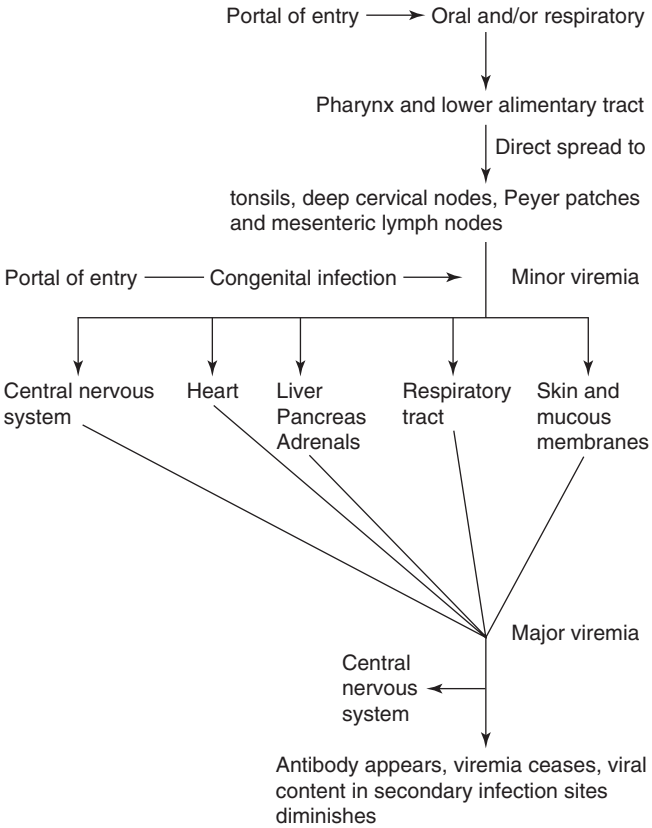

FIGURE 165-4 ■ Pathogenesis of enteroviral infections. (From Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* Philadelphia, W.B. Saunders, 1976.)

PATHOGENESIS AND PATHOLOGY

Events During Pathogenesis

Figure 165-4 diagrams the events in pathogenesis. After initial acquisition of virus by the oral or respiratory route, implantation occurs in the pharynx and the lower alimentary tract.* Within 1 day, the infection extends to the regional lymph nodes. On approximately the third day, minor viremia develops and results in the involvement of many secondary infection sites. In congenital infections, the infection is initiated during the minor viremia phase. Multiplication of virus in secondary infection sites coincides with the onset of clinical symptoms. Illness can vary from minor infection to fatal disease. Major viremia occurs during the period of multiplication of virus in secondary infection sites; this period usually lasts from the third to the seventh day of infection. In many echovirus and coxsackievirus infections, involvement of the CNS apparently occurs at the same time as other secondary organ involvement. Occasionally, the CNS symptoms of enteroviral infections are delayed, thus suggesting that seeding occurred later in association with the major viremic phase.

*References 94, 181, 182, 455, 707, 801, 895.

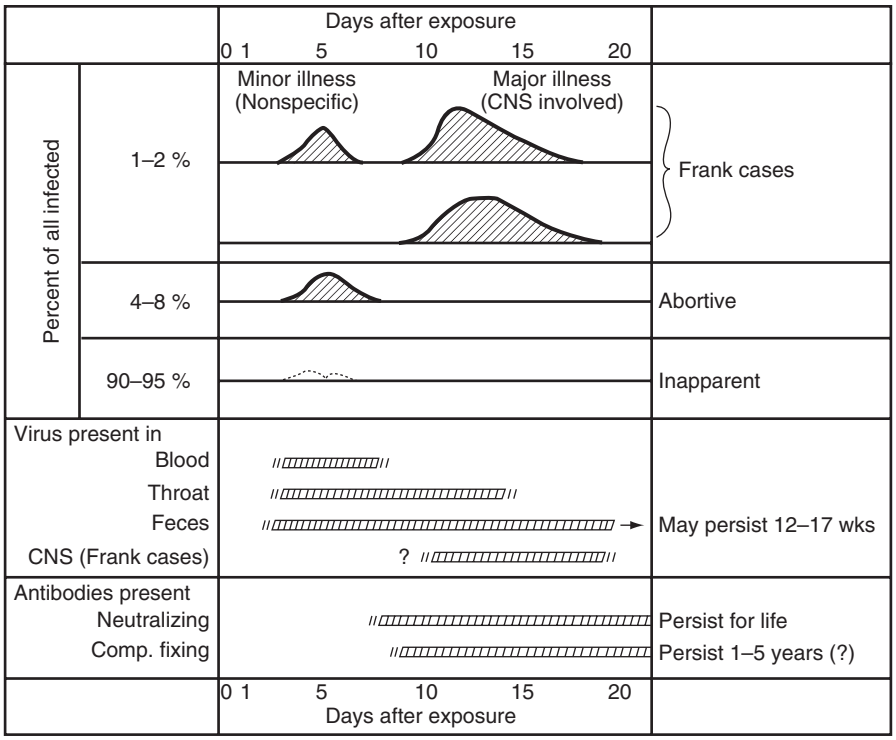

FIGURE 165-5 ■ The course of clinical and subclinical forms of poliovirus infection in relation to the presence of virus and the development of antibodies. *CNS,* Central nervous system. (From Bodian D, Horstmann DM. Poliomyelitis. In: Horsfall FL, Tamm I, editors. *Viral and rickettsial infections of man.* 4th ed. Philadelphia, J. B. Lippincott, 1965, p. 430-73.)

Cessation of viremia correlates with the appearance of serum antibody. The viral concentration in secondary infection sites begins to diminish on approximately the seventh day. However, infection continues in the lower intestinal tract for prolonged periods.

In Figure 165-5, clinical and subclinical events in polioviral infections are presented. By 3 to 5 days after exposure, virus can be recovered from blood, the throat, and feces. This finding may be accompanied by symptoms of the "minor illness," or the infection may be unrecognized clinically. The end of the period of viremia coincides with the appearance of antibodies and the onset of clinical signs of CNS involvement. The available evidence favors blood as the main pathway of CNS invasion in natural disease, but experimental infections in monkeys indicated that the virus can travel along the axons of peripheral nerves. Possibly, when tonsillectomy is performed on a child with an inapparent poliovirus infection, the virus enters the nerve fibers exposed during surgery and spreads to the cranial nerve nuclei in the brain, thereby resulting in bulbar paralysis.

Factors That Affect Pathogenesis

The pathogenesis and pathology of enteroviral and parechoviral infections depend on the virulence, tropism, and inoculum concentration of virus, as well as on many specific host factors. EVs obviously have marked differences in both tropism and virulence. Although some generalizations can be made with regard to tropism, marked differences occur even among strains of specific viral types. Differences in the virulence of specific enteroviral types may be the result of recombination among EVs or point mutations.[849,866,908]

Van Eden and associates[1021] studied 17 families during an outbreak of poliomyelitis caused by type 1 virus in the Netherlands. The findings suggested that HLA-related genetic factors were important in the occurrence of paralytic disease.

Enteroviral infections in the fetus and neonate generally are thought to be more severe than similar infections in older persons. This situation undoubtedly is the case with coxsackievirus B infection and probably also with coxsackievirus A and echovirus infections. Although the reasons for this increased severity are unknown, several aspects of neonatal immune mechanisms have been suggested. In addition, the similarity of coxsackievirus B infections in suckling mice to those in human neonates provided a useful animal model system. Heineberg and associates[434] compared coxsackievirus B1 infections in 24-hour-old suckling mice with similar infections in older mice. These investigators noted that adult mice produced interferon in all infected tissues, whereas suckling mice produced only small amounts of interferon in the liver. These researchers thought that the difference in outcome of coxsackievirus B1 infection in suckling and older mice could be explained by the inability of cells of the immature animals to elaborate interferon. Additional studies of abnormalities of innate immunity in neonates may enhance our understanding of the severity of enteroviral infections in newborns.[637]

Other researchers suggested that the increased susceptibility of suckling mice to severe coxsackievirus infections is related to the transplacentally acquired, increased

concentrations of adrenocortical hormones.[63,96] Kunin[603] suggested that the difference in age-specific susceptibility could be explained at the cellular level. He showed that various tissues of newborn mice bind coxsackievirus B3, whereas tissues of adult mice are virtually inactive in this regard.[602,603] The progressive loss of receptor-containing cells with increasing age may be the mechanism that accounts for less severe infections in older animals. Supporting this suggestion, Ito and colleagues[505] showed that expression of coxsackievirus and adenovirus receptor (CAR) decreases with increasing age in rats. In the past, researchers assumed that specific disease in various organs and tissues in enteroviral infections was caused by the direct cytopathic effect and tropism of a particular virus. In more recent years, however, large numbers of studies using murine model systems suggested that host immune responses contribute to the pathologic process.* These studies suggested that T-cell–mediated processes and virus-induced autoimmunity cause both acute and chronic tissue damage. In contrast, other studies suggested that the primary viral cytopathic effect is responsible for the tissue damage and that the various T-cell responses are a reaction to the damage and not its cause.[693]

Since the early 1960s, the clinical manifestations caused by several enteroviral serotypes have changed. For example, echovirus 11 infection initially was noted in association with an outbreak of upper respiratory tract infection in a daycare center in 1958.[822] Then, in the 1960s, this infection was found to be related to exanthem and aseptic meningitis.[187,194] Following this event and occurring at present is the association of echovirus 11 infection and severe sepsis-like illness with hepatitis in neonates.†

Another example relates to EV 71 infections. Initially, this virus was noted in association with aseptic meningitis, and only a few patients also had exanthem.[557,917] Since the late 1990s, severe epidemic disease with EV 71 has occurred in Taiwan, Singapore, Australia, Malaysia, and Japan. In these epidemics, hand-foot-and-mouth disease is a major finding, and the neurologic disease is more severe than in the past.‡

These phenotypic changes could be the result either of point mutations or of recombination among EVs.§ Chan and AbuBakar[160] presented evidence indicating that a recombination event occurred between EV 71 and coxsackievirus A16.

Pathology

The clinical signs of enteroviral infection vary widely, so great variations in pathology also exist. Because pathologic material generally is available only from patients with fatal illness, this section discusses only the more severe manifestations. Worth emphasizing, however, is that these fatal infections account for just a small portion of all enteroviral infections. The pathologic findings in children with milder infections, such as nonspecific febrile illness, have not been described.

Coxsackieviruses A

Records of severe illness associated with coxsackieviruses A are rare. Gold and colleagues,[382] in a study of sudden unexpected death in infants, recovered coxsackievirus A4 from the brains of three children. In none of these patients were histologic abnormalities noted in the brain or spinal cord. An adult with a fatal coxsackievirus A7 infection had diffuse pancarditis and organized pneumonitis.[46]

Coxsackieviruses B

Of the nonpolio EVs, coxsackieviruses B have been associated most frequently with severe and catastrophic disease. The most common findings in these cases have been myocarditis, meningoencephalitis, or both. Involvement of the adrenals, pancreas, liver, and lungs also has been noted.

Heart

Grossly, the heart usually is enlarged, with dilation of the chambers and flabby musculature.[180,314,364,1081]

Microscopically, the pericardium frequently contains some inflammatory cells along with thickening and edema, and the endocardium may have focal infiltrations of inflammatory cells. The myocardium (Fig. 165-6) is congested and contains infiltrations of inflammatory cells (i.e., lymphocytes, mononuclear cells, reticulum cells, histiocytes, plasma cells, and polymorphonuclear and eosinophil leukocytes). Involvement of the myocardium often is patchy and focal but occasionally diffuse. The muscle shows loss of striation, as well as edema and eosinophilic degeneration. Muscle necrosis without extensive cellular infiltration is a common finding.

Brain and Spinal Cord

The meninges are congested, edematous, and occasionally mildly infiltrated with inflammatory cells.[182,314,727,1081] Lesions in the brain and spinal cord are focal rather than diffuse, but they frequently involve many different areas. The lesions consist of areas of eosinophilic degeneration of cortical cells, clusters of mononuclear and glial cells (Fig. 165-7), and perivascular cuffing. Two children with fatal coxsackieviral B infection (types 2 and 4) had, in addition to typical inflammatory encephalitic lesions, widespread multifocal areas of liquefaction necrosis without inflammation.[305]

Other Organs

The lungs frequently have areas of mild focal pneumonitis with peribronchiolar mononuclear cellular infiltration.[12,108,182,362,726] The liver often is engorged and occasionally contains isolated foci of liver cell necrosis

*References 27, 106, 173, 357, 360, 437, 440, 454, 533, 613, 786, 800, 803, 840, 849, 850, 874, 928.
†References 97, 182,183, 413, 530, 720, 736.
‡References 127, 158, 159, 163, 164, 168, 169, 178, 208, 343, 453, 467, 474, 476, 477, 499, 540, 591, 639, 645-648, 656, 679, 695, 773, 794, 835, 1040-1042, 1096.
§References 160, 197, 561, 657, 658, 785, 908, 942.

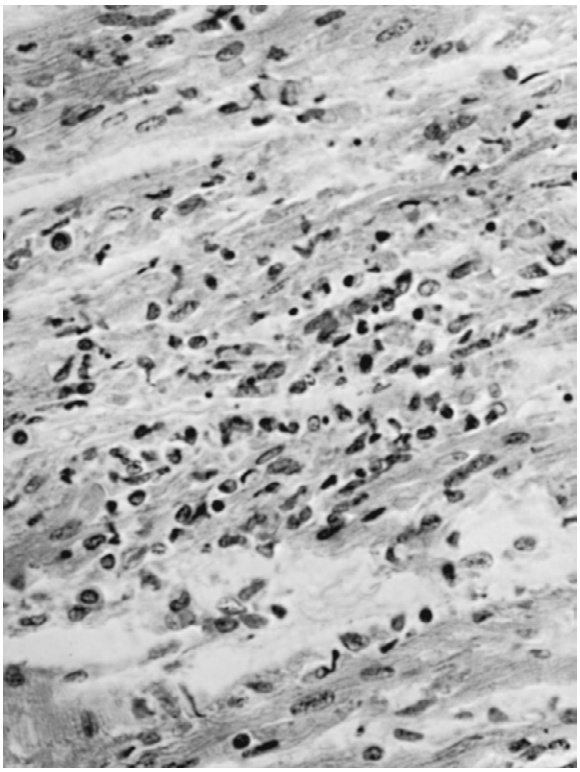

FIGURE 165-6 ■ Coxsackievirus B4 myocarditis in a 9-day-old infant. Note the myocardial necrosis and mononuclear cellular infiltration. (From Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* Philadelphia, W.B. Saunders, 1976.)

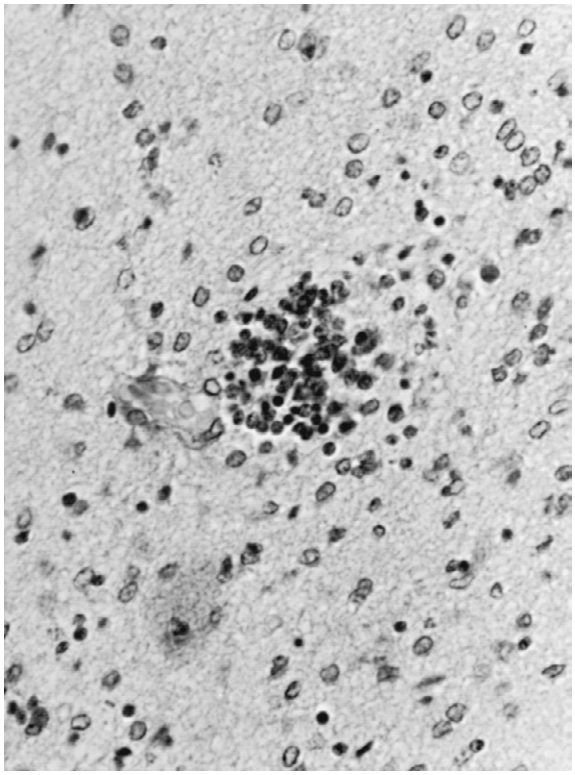

FIGURE 165-7 ■ Coxsackievirus B4 encephalitis in a 9-day-old infant with focal infiltration of mononuclear and glial cells. (From Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* Philadelphia, W.B. Saunders, 1976.)

and mononuclear infiltration. In the pancreas, occasional focal degeneration of islet cells occurs. Congestion has been observed in the adrenal glands, along with mild to severe cortical necrosis; inflammatory cells are present.

Echoviruses

Although frequently responsible for illness, echoviruses rarely were associated with fatal infection until relatively recently. In several different reports with eight different echovirus types, hepatic necrosis was a major pathologic finding.* Massive hepatic necrosis has been seen with echoviruses 3, 6, 7, 9, 11, 14, 19, and 21. At autopsy, one infant with echovirus 6 infection had cloudy and thickened leptomeninges, liver necrosis, adrenal and renal hemorrhage, and mild interstitial pneumonitis.[182] One infant with echovirus 9 infection had an enlarged and congested liver with marked central necrosis,[182] and another with this virus had interstitial pneumonitis without liver involvement.[183] Three infants with echovirus 11 infection had renal and adrenal hemorrhage and small vessel thrombi in the renal medulla and in both the medulla and inner cortex of the adrenal glands.[745] These patients' livers were normal. Two infants, one with echovirus 6 and the other with echovirus 31 infection, had only extensive pneumonia.[104,182]

*References 89, 182, 367, 413, 720, 736, 1089.

Enteroviruses

The brain of a child who died of acute encephalitis caused by EV 71 infection was examined.[1094] Grossly, the brain appeared normal, but microscopically, it revealed typical features of acute encephalitis consisting of perivascular cuffing with mixed inflammatory cells, neuronophagia, and inflammatory nodules.

Polioviruses

The neuropathy of poliomyelitis usually is pathognomonic; only certain cells and areas of the neuraxis are susceptible to the virus.[94,217] Neuronal damage is caused directly by virus multiplication, but not all affected neurons are killed. The injury may be reversible, and function may be restored within 3 to 4 weeks after onset. Little histologic evidence of meningeal reaction exists. Perivascular cuffing and some interstitial glial infiltration are present. Histologic sections generally reveal more widespread lesions than would be estimated from the clinical findings. Scattered neurons may undergo considerable destruction without causing clinical disability.

Regions in which neuronal lesions occur are (1) the spinal cord (anterior horn cells chiefly and, to a lesser degree, the intermediate and dorsal horn and dorsal root ganglia), (2) the medulla (vestibular nuclei, cranial nerve nuclei, and the reticular formation that contains the vital centers), (3) the cerebellum (nuclei in the roof and vermis

only), (4) the midbrain (chiefly the gray matter but also the substantia nigra and occasionally the red nucleus), (5) the thalamus and hypothalamus, (6) the pallidum, and (7) the cerebral cortex (motor cortex). The viruses spare the following areas: (1) the entire cerebral cortex except the motor area, (2) the cerebellum except the vermis and deep midline nuclei, and (3) the white matter of the spinal cord. This distribution of lesions permits the physician to establish a histologic diagnosis of poliomyelitis.

Extraneural disease usually is a secondary phenomenon. Bronchopulmonary changes such as aspiration pneumonia, atelectasis, and purulent bronchitis may occur because of impaired coughing and decreased thoracic movement. Cardiovascular changes may result in hypertension, cardiac failure, and pulmonary edema. Prolonged immobilization leads to negative nitrogen and calcium balance, along with urinary lithiasis, renal failure, hypertension with encephalopathy, and seizures. Treatment itself may cause untoward complications, such as urinary tract infection (after catheterization), decubitus ulcers, and psychotic disturbances. Ulcerations in the alimentary tract may result in serious bleeding and occasional perforation. Respiratory failure culminates in respiratory acidosis and anoxic changes.

Parechoviruses

Although parechoviral infections have a broad spectrum of clinical manifestations, including encephalitis and sepsis-like neonatal disease, pathologic data are not available.[1,76,95,620,1049]

CLINICAL MANIFESTATIONS: NONPOLIO ENTEROVIRUSES, PARECHOVIRUSES, AND SAFFOLD VIRUSES

Nonpolio enteroviral, parechoviral and Saffold viral infections are exceedingly common, and the spectrum of disease is protean. Many of the clinical-virologic associations listed in Tables 165-5 through 165-19 are based on a limited number of cases. Because EVs, PeVs, and SAFVs frequently are carried asymptomatically in the gastrointestinal tract for relatively long periods, some of the observed illnesses and the viruses concomitantly recovered may not have a cause-and-effect relationship. However, repeated observations since the 1960s have supported many virus–illness associations, even though their occurrence has been sporadic.

Few specific EV, PeV, or SAFV diseases exist, but, rather, various interrelated syndromes and anatomically associated illnesses are reported. Many illnesses and syndromes can be caused by different viral types, and most types are capable of inducing a variety of clinical syndromes. Conversely, certain specific coxsackieviral and echoviral types have clinical characteristics that facilitate an etiologic diagnosis.

Since the late 1980s, few careful studies have been conducted in which specific clinical findings were correlated with individual enteroviral types. This paucity was fostered by the expense of serotyping and the lack of using inoculation of suckling mice for isolation of virus. The increased use of polymerase chain reaction (PCR) also has contributed to the lack of identification of manifestations of disease by specific viral type. This situation is unfortunate because the clinical manifestations caused by specific viral types are not constant, and disease severity varies by specific enteroviral type.

Asymptomatic Infection

Because researchers have known that 90 to 95 percent of poliovirus infections are not recognized clinically, they have assumed that most infections with EVs, PeVs, and SAFVs are asymptomatic. This opinion is strengthened by the finding that these viruses can be recovered frequently from the stools of healthy children. However, relatively few data are available on the rate of asymptomatic infection with nonpolio EVs, PeVs, and SAFVs. All too frequently, isolation of EVs and PeVs from stool is equated with asymptomatic infection. This assumption is an error because illness, if it takes place, occurs shortly after the acquisition of virus and is short-lived; a particular infection may have been associated with a nonspecific illness 2 or 3 months before a stool specimen is obtained in a surveillance program. Unfortunately, in several studies involving controlled population groups in which accurate clinical expression rates could have been determined, clinical observations apparently were of secondary importance to the investigators.[182,365,366,368,590] The data available suggest differences in clinical expression among coxsackieviral and echoviral types.

Table 165-5 lists the approximate frequency of asymptomatic infections with selected coxsackieviruses and echoviruses. As can be seen, the rates vary among the group as a whole and even within specific types. Overall, approximately half, and perhaps more, of all nonpolio enteroviral infections appear to be associated with clinical

TABLE 165-5 Approximate Frequencies of Asymptomatic Infection with Selected Coxsackieviruses and Echoviruses

	Asymptomatic Infection Frequency (%)
Coxsackieviruses	
A	50
A16	50
B	20
B2	11-50
B3	25-96
B4	30-70
B5	5-40
Echoviruses	50
4	Uncommon-60
6	Rare
9	15-60
18	Rare-20
20	33
25	30
30	50

TABLE 165-6 **Enteroviruses and Parechoviruses Noted in Association with Nonspecific Febrile Illness and Respiratory Disease**

	Virus Types		
Clinical Categories	**Coxsackieviruses A**	**Coxsackieviruses B**	**Enteroviruses and Parechoviruses**
Nonspecific febrile illness	All types	All types	All types
Common cold	Mainly 21, 24; rarely other types	Mainly 1-5; rarely 6	Mainly 2, 20; rarely other types
Pharyngitis (pharyngitis, tonsillitis, tonsillopharyngitis, and nasopharyngitis)	Probably all types; mainly 9	Probably all types; mainly 1-5	Probably all types; mainly 2, 4, 6, 9, 11, 16, 19, 25, 30, 71, PeV 3, 6
Herpangina	1-10, 16, 22	1-5	6, 9, 11, 16, 17, 25, PeV 1
Lymphonodular pharyngitis	10		
Stomatitis and other lesions in the anterior of the mouth	5, 9, 10, 16	2, 5	9, 11, 20, 71
Parotitis	Coxsackievirus A not typed	3, 4	70
Croup	9	4, 5	4, 11, 21
Bronchitis		1,4	8, 12-14, PeV 1
Bronchiolitis and infectious asthma	Many types	Many types	Many types
Pneumonia	9, 16	1-6	6, 7, 9, 11, 12, 19, 20, 30, 68, 74, 78, PeV 1
Pleurodynia	1, 2, 4, 6, 9, 16	1-6	1-3, 6-9, 11, 12, 14, 16-19, 24, 25, 30, PeV 2

PeV, Parechoviruses.

manifestations. In general, the more carefully clinical symptoms are examined, the smaller is the percentage of truly asymptomatic infections. Clinical expression also is related inversely to age. With coxsackievirus A16, asymptomatic infection occurs in only approximately 10 percent of children younger than 5 years of age, whereas rates are higher in older children and adults.[10,182] Sabin[894] reported no asymptomatic infections in infants with echovirus 18, and Nishmi and Yodfat[770] noted fewer than 20 percent of infections without illness in children younger than 8 years of age during an echovirus 4 epidemic. At the other extreme, Clemmer and associates[225] reported that 96 percent of infections with coxsackievirus B3 were asymptomatic. In other studies, the illness rate of coxsackievirus B3 was 60 to 75 percent. Extensive seroepidemiologic studies by Zoll and colleagues[1114] and Galama and associates[348] of SAFV types 2 and 3 indicate that infection is very common in young children with these viruses. Because other studies have only occasionally noted clinical associations with these viruses, it appears that Saffold viral infections are most often asymptotic.*

Nonspecific Febrile Illness

Nonspecific febrile illness is the most common manifestation of coxsackieviral and echoviral infection (Table 165-6). All viral types cause this clinical finding, but its frequency varies considerably among the individual viruses.

The onset of illness usually is abrupt, without a prodrome. In young children, the initial finding is fever and associated malaise. In older children, headache generally is noted. The temperature ranges between 38.3° and 40°C (101° and 104°F) and has a mean duration of 3 days. In some instances, the fever is biphasic; it occurs for 1 day, is absent for 2 to 3 days, and then recurs for an additional 2 to 4 days. In many young children, the only manifestation of illness is fever, and its presence is discovered by chance by a parent.

Malaise and anorexia often are related to the degree of temperature elevation, as is headache in older patients. Sore throat is a common complaint, but an inflamed pharynx is not seen on examination. Nausea and vomiting occasionally occur at the onset of illness, as does mild abdominal discomfort. One or two loose stools may be noted. Generalized myalgia also is observed, and children complain of a scratchy feeling in the throat.

Physical examination usually yields benign findings. Minimal conjunctivitis, infection of the pharynx, and cervical lymphadenitis may be present. The duration of illness varies from 24 hours to approximately 6 days, with an average of 3 to 4 days. The white blood cell count (WBC) count is normal. EVs, particularly coxsackieviruses A, are significant causes of febrile seizures in young children.[468] An SAFV was noted in a stool sample from a child with a fever of unknown origin.[531]

Respiratory Manifestations

Common Cold

Although numerous coxsackieviruses and echoviruses have been recovered from children with mild upper respiratory tract infections, only rarely do the illnesses qualify as common colds (see Table 165-6). (The common

*References 2, 90, 205, 282, 450, 501, 502, 531, 564, 763, 856, 857, 1009, 1010, 1093.

cold is an acute illness with nasal stuffiness, rhinitis, no objective evidence of pharyngitis, and no or minimal fever.) In most instances, significant fever (temperature >38.3°C [>101°F]) is associated with enteroviral infections and usually some degree of pharyngitis.

Coxsackievirus A21 is the only EV that clearly qualifies as a common cold virus.[183,522] This agent has produced epidemics of mild respiratory illness in military populations. In adult volunteers, instillation of this virus in the nose has resulted in the common cold syndrome. Epidemic disease has not been observed in children. Other viruses that have been associated with the common cold syndrome include echoviruses 2 and 20 and coxsackieviruses B1, B2, B3, B4, B5, and A24.[182]

Pharyngitis (Pharyngitis, Tonsillitis, Tonsillopharyngitis, and Nasopharyngitis)

Pharyngitis is a common clinical manifestation of coxsackieviral and echoviral infection. Probably all EVs on occasion cause mild pharyngitis. The most common coxsackieviruses and echoviruses associated with pharyngitis are as follows: coxsackieviruses A9, B1, B2, B3, B4, and B5; echoviruses 2, 4, 6, 9, 11, 16, 19, 25, and 30; and EV 71.*

Pharyngitis in coxsackievirus and echovirus infections frequently is associated with other clinical findings, such as meningitis, pleurodynia, and exanthem. These other manifestations become more important than pharyngitis in individual cases in the minds of parents as well as clinicians.

Pharyngitis caused by coxsackieviruses and echoviruses usually is abrupt in onset, without a prodrome. Although pharyngeal involvement is present at the onset of disease, the initial complaint usually is fever. The temperature usually ranges between 38.3° and 40°C (101° and 104°F), but higher temperatures are not unusual. In general, fever tends to be more pronounced in younger patients. Young children have malaise and anorexia. School-age children complain of headache and myalgia. Sore throat, coryza and vomiting, diarrhea, or a combination thereof also may be noted.

Examination of the tonsils and pharynx shows varying degrees of erythema. In some cases, only infection is noted, whereas in others, severe pharyngitis with patches of exudate is seen. The usual duration of uncomplicated coxsackieviral or echoviral pharyngitis is 3 to 6 days. Routine laboratory study is of minimal value in enteroviral pharyngitis; the total WBC count may be normal or slightly elevated with a normal differential determination. Throat culture rules out disease caused by group A streptococci.

SAFV type 2 was noted in 13 children with pharyngitis and 12 children with tonsillitis in a Japanese study.[501]

Other Intraoral Manifestations

Herpangina. The onset of herpangina is typical of most EV infections and is characterized by the sudden awareness of fever.[809,1046,1108,1109] No characteristic prodrome usually exists, but young children may be irritable and occasionally listless and anorexic for a few hours before the febrile state is recognized. The initial temperature can be variable, with a range from normal to 41°C (106°F). In general, the temperature tends to be higher in younger patients. Breese noted that the most common temperature in young children was between 39.5° and 40°C (103° and 104°F). Older children frequently complain of headache and backache. Vomiting occurs in approximately 25 percent of children younger than 5 years. In one outbreak of illness caused by coxsackievirus A4,[328] initial symptoms were anorexia and drooling (100%); sore throat (50%); coryza (45%); headache (18%); and vomiting, diarrhea, or both (36%).

In most instances of herpangina, the oropharyngeal lesions are present on the first examination at the time of fever or shortly after fever is noted. In the coxsackievirus A4 outbreak described by Forman and Cherry,[328] the enanthem was not observed until 24 to 48 hours after the onset of initial nonspecific symptoms. The characteristic lesions in herpangina are small (1 to 2 mm) vesicles and ulcers. These lesions apparently start as papules, become vesicular, and then ulcerate in a short but variable period. In my experience (J. D. C.), the lesions most commonly observed are ulcers. Breese[105] noted in some children seen early in the course of the illness that a petechial appearance preceded the appearance of typical vesicular-ulcerative enanthem.

The lesions usually are discrete, with an average of 5 per patient; some patients will have only 1 or 2 lesions, whereas others may have 14 or more. When seen early, the vesicular lesions are observed to enlarge from 1 to 2 mm to 3 to 4 mm during a 2- to 3-day period.[809] Each vesicular and ulcerative lesion is surrounded by an erythematous ring that varies in size up to 10 mm in diameter. The most common site of the lesions is the anterior tonsillar pillars. Lesions also occur on the soft palate, uvula, tonsils, and pharyngeal wall and occasionally on the posterior buccal surfaces. In some cases, additional lesions have been noted on the dorsum or tip of the tongue. However, by definition, cases in which the primary involvement is on the tongue or anterior mouth and in which the lesions are of a general size greater than 5 mm are not considered to be herpangina.

Aside from the specific lesions, the remainder of the throat appears normal, minimally injected, or mildly erythematous. The usual duration of signs and symptoms is 3 to 6 days. Most cases of herpangina are mild and without complications, but aseptic meningitis and other more severe enteroviral manifestations also occur occasionally. Severe neurologic manifestations have been observed in children with herpangina caused by EV 71.[162,165,453,591] During epidemic disease in Taiwan in 1998, children with herpangina and aseptic meningitis, acute flaccid paralysis, and rhombencephalitis were described.[453,473] In one study, a biphasic course was described, with herpangina occurring first and neurologic manifestations 2 to 5 days later.[450]

EVs, PeVs, and SAFVs found in association with sporadic or epidemic herpangina occurrence are presented in Table 165-7.

*References 182, 192-194, 238, 308, 414, 430, 545, 546, 556, 569, 629-631, 734, 892, 897, 940.

TABLE 165-7 **Etiologic Agents Found in Association with Sporadic or Epidemic Herpangina Occurrence**[184]

Virus	Occurrence	
	Epidemic	Sporadic
Coxsackievirus A		
1	+	
2	+	
3	+	
4	+	
5	+	
6	+	
7		+
8	+	
9		+
10	+	
12		+
16		+
22	+	
Coxsackievirus B		
1	+	+
2		+
3		+
4		+
5		+
Echovirus		
6		+
9		+
11		+
16	+	+
17		+
25	+	
Parechovirus 1 and 6		+
Enterovirus 71	+	
Saffold virus types 2, 3		+

Acute Lymphonodular Pharyngitis. In 1962, Steigman and colleagues[959] reported a unique enanthem associated with coxsackievirus A10 infection. The lesions had the typical distribution of herpangina; they were papular, discrete, 3 mm in diameter, and surrounded by a zone of erythema. The lesions were white to yellow and persisted for 6 to 10 days. This entity has not been reported again, although coxsackievirus A10 has been noted in association with hand-foot-and-mouth disease.[182,926]

Stomatitis and Other Lesions in the Anterior of the Mouth. The main enteroviral cause of stomatitis and ulcerative lesions in the anterior of the mouth is coxsackievirus A16, and the clinical entity is hand-foot-and-mouth disease. This condition is presented in detail later in this chapter. Worth mentioning here, however, is that occasionally enanthem occurs without the exanthem and this enanthem also has been associated with other coxsackieviruses (A5, A9, A10, B2, B5), echovirus 33, and EV 71.*

In echovirus 9 infection, Tyrrell and colleagues[1014] reported six children with a unique enanthem: painless whitish dots, ulcers, or vesicles on the buccal surfaces near the Stensen duct. These investigators also noted the occasional occurrence of similar lesions under the tongue.

Deseda-Tous and colleagues[267] reported an adolescent with hemorrhagic vesicular lesions on the pharynx, mucosal surfaces, and tongue in association with an echovirus 11 infection. Clarke and Stott[220] observed reddish macules on the buccal mucosa of a patient with echovirus 20 infection, and Cherry and Jahn[187] noted a child with hand-foot-and-mouth disease in whom the buccal lesions suggested Koplik spots, although the lesions were larger and yellower in this patient.

Parotitis

Parotitis in association with herpangina and coxsackievirus A infection was reported in 1957 by Howlett and associates.[471] In 1960, Kraus[596] described two additional cases, and Bertaggia and associates[88] and Winsser and Altieri[1078] noted parotitis in association with coxsackieviruses B3 and B4, respectively. Three patients with acute hemorrhagic conjunctivitis and parotitis caused by EV 70 also have been reported.[913]

Croup

In large studies of respiratory illness in young children, croup is associated sporadically with coxsackieviral and echoviral infection.[170,182,334,373,1064] In general, these illnesses are mild in contrast to croup caused by parainfluenza and influenza viruses.

An outbreak of croup associated with echovirus 11 in a daycare center was reported.[822] In this instance, 17/53 ill children were found to be infected with the U strain of echovirus 11. A subsequent study noted the same virus in 4 children with croup.[823] Croup also was reported in outbreaks of coxsackievirus B5 infection.[42,236] Other specific agents associated with croup include coxsackieviruses A9 and B4 and echoviruses 4 and 21.[182]

Bronchitis

An acute febrile illness with cough, rhonchi, and referred breath sounds occasionally is a sporadic manifestation of enteroviral infection. A specific association of coxsackieviruses B1 and B4, echoviruses 8, 12, 13, and 14, and PeV 1 has been found.[170,182,236,292,294] A SAFV type 2 was noted in one child with bronchitis.[501]

Bronchiolitis and Infectious Asthma

Coxsackieviruses and echoviruses have been associated sporadically with bronchiolitis, infectious asthma, and the precipitation of asthmatic attacks in atopic children.* Epidemic disease has not been observed, and the illnesses usually have been mild.

*References 182, 263, 285, 324, 649, 924.

*References 170, 182, 292, 334, 373, 395, 458, 1064.

Pneumonia

In general, studies of respiratory tract infections in children, sporadic coxsackieviruses, and echoviruses have been noted in 1 to 7 percent of patients with pneumonia and positive viral cultures.[170,182,334,373] Specific virus types include coxsackieviruses A9, A16, B1, B2, B3, B4, B5, and B6; echoviruses 6, 7, 9, 11, 12, 19, 20, and 30; and EV 71.*

In only three instances can an outbreak of pneumonia caused by specific viruses be suggested to have occurred. During the summer of 1959, Lerner and colleagues[632] noted that 3/15 children infected with coxsackievirus A9 had pneumonia. The patients with pneumonia had a vesicular rash, and one of these children died.

Eckert and associates[292] observed 6 children with coxsackievirus B1 infection and pneumonia during the summer of 1963. These illnesses were not described further, although the mean WBC count in 12 children who had lower respiratory tract infections with coxsackieviruses B was reported as 11,383 with a normal differential. All children were hospitalized.

Goldwater[384] reported a coxsackievirus B6 outbreak in south Australia during the summer of 1992 to 1993. Twenty-seven patients had pneumonia associated with high fever and severe cough that lasted several weeks. In addition to the fatal case of coxsackievirus A9 infection described by Lerner and colleagues,[632] deaths have resulted from infection with coxsackieviruses B1, B5, and A16.[323,509,1088]

In the Philippines in 2009, 21 children with EV 68, severe respiratory tract infections, and presumed pneumonia were noted[493] All children had cough and chest in-drawing. Two deaths occurred.

Pleurodynia (Bornholm Disease)

Epidemic pleurodynia† is an illness that was noted first in 1735 by Hannaeus, a Danish physician.[419] Not until the late 19th century, however, did the illness receive further attention in the medical literature. At this time, several epidemics in Scandinavian countries were described.[182,245,322] Although Finsen referred to the disease as *pleurodynia*, others who designated outbreaks used geographic names, such as Skien disease, Bamle disease, Drangedal disease, and Bornholm disease, or descriptive names such as epidemic myalgia, devil's grippe, epidemic diaphragmatic spasm, or epidemic benign pleurisy.[388] In 1933, Sylvest[975] published the classic monograph on the subject, and from this publication the name Bornholm disease (from Bornholm Island, a Danish island in the Baltic Sea) came to be associated with the illness. The enteroviral origin of epidemic pleurodynia was established in 1949.[244,1057]

Historically, pleurodynia is an epidemic disease, but sporadic cases do occur. A characteristic incubation period of approximately 4 days is followed by the sudden onset of fever and pain. The pain typically is located in the chest or upper part of the abdomen and is muscular in origin and of variable intensity. Occasionally, the pain occurs in other areas of the body. Frequently, the pain is excruciatingly severe and sudden and is associated with profuse sweating, so the patient may appear pale and as though in shock. The pain is spasmodic, with durations varying from a few minutes to several hours. Most commonly, the spasmodic periods last approximately 15 to 30 minutes. During spasms, respirations usually are rapid, shallow, and grunting, suggestive of pneumonia or pleural inflammation. Coughing, sneezing, or deep breathing makes the pain worse. Older children and adults describe the pain as stabbing or knifelike. An older person often fears that a heart attack is occurring.

When pain localizes in the abdomen, it frequently is crampy and suggests colic in a younger child. The child may double over and refuse to walk or move. Occasionally, the abdominal pain in association with a pale, sweaty, shocklike appearance suggests acute intestinal obstruction. Splinting and guarding of the abdomen also lead to consideration of appendicitis and peritonitis.

The fever and pain usually last 1 to 2 days. Frequently, however, the illness is biphasic; after the initial febrile period, the patient is asymptomatic for several days, and then the pain and fever recur. Rarely, patients have several recurrent episodes over the course of several weeks. In these patients, fever is less prominent.

Some degree of tenderness is present in the areas of pain, but frank myositis with muscle swelling is not observed. Pleural friction rubs may be noted on auscultation, and they may appear and disappear with the coming and going of episodes of pain.

In epidemics, both children and adults are afflicted, but most cases occur in persons younger than 30 years. Most children have other symptoms of enteroviral infection, such as anorexia, nausea, vomiting, headache, and sore throat. Routine laboratory study is not very helpful. The WBC count varies considerably, but increased percentages of polymorphonuclear neutrophils and band forms are frequent findings. The erythrocyte sedimentation rate also is inconsistent; normal to extremely high values may be observed. The chest radiograph usually is normal.

Complications in pleurodynia seldom develop. Aseptic meningitis has been noted in some patients, and men have experienced orchitis. Cardiac involvement, in the form of myocarditis and pericarditis, also may complicate pleurodynia.

The major etiologic agents in epidemic pleurodynia are coxsackieviruses B3 and B5.* Other viruses associated with epidemic disease include coxsackieviruses B1 and B2 and echoviruses 1 and 6.[32,182,287] Agents associated with sporadic occurrences of pleurodynia include coxsackieviruses A1, A2, A4, A6, A9, A16, B1, B2, B3, B4, B5, and B6; echoviruses 1, 2, 3, 6, 7, 8, 9, 11, 12, 14, 16, 17, 18, 19, 24, 25, and 30; and PeV 2.†

Pleurodynia rarely is reported today. Because the EVs that cause pleurodynia still circulate, cases probably are overlooked or misdiagnosed. An outbreak caused by coxsackievirus B1 in football players at a public high school

*References 182, 238, 292, 323, 369, 509, 546, 632, 726, 914, 940, 1061.
†References 45, 182, 253, 387, 404, 569, 725, 975, 1048.

*References 45,182, 480, 892, 920, 940.
†References 66,182, 236, 287, 415, 539, 574, 692, 725, 973.

TABLE 165-8 **Enteroviruses and Parechoviruses Noted in Association with Gastrointestinal Complaints**

	Virus Types		
Clinical Categories	**Coxsackieviruses A**	**Coxsackieviruses B**	**Enteroviruses and Parechoviruses (PE)**
Gastrointestinal, not specified	2, 4, 5, 6, 7, 9, 10, 14, 16	1-5	1-9, 11, 12, 14, 16-21, 24, 25, 30, 71, PeV 1-6, 8, 10, 11
Nausea and vomiting	9, 16	2-5	2, 4, 6, 9, 11, 16, 18-20, 30, PeV 1
Diarrhea	1, 9, 16	2-5	3, 4, 6, 7, 9, 11-14, 16-21, 25, 30, PeV 1
Constipation	9	3-5	4, 6, 9, 11
Abdominal pain	9, 16	2-5	4, 6, 9, 11, 18, 19, 30
Pseudoappendicitis			1, 8, 14
Peritonitis		1	
Mesenteric adenitis		5	7, 9, 11
Appendicitis		2, 5	
Intussusception		3	7, 9
Hepatitis	4, 9, 10, 20, 24	1-5	1, 3, 4, 6, 7, 9, 11, 14, 20, 21, 30
Reye syndrome	2	4	14, PeV 1
Pancreatitis	9	3-5	
Diabetes mellitus		1-5	

PeV, Parechoviruses.

was reported.[492] Unfortunately, no clinical data were presented. In 2005, 28 pediatric patients with pleurodynia were observed in a medical center in Northern Taiwan.[472] This outbreak was due to coxsackievirus B3.

Gastrointestinal Manifestations

Gastrointestinal manifestations occur commonly in enteroviral and parechoviral infections.* Clinical manifestations in addition to vomiting and diarrhea are varied (Table 165-8).

Early studies paid particular attention to diarrheal disease in children. However, when specific studies of infantile diarrhea were undertaken, the enteroviral association was far from clear.[62,182,388,809,1104] The correlation of infection and disease in these studies was compromised by the problem that the main source of culture in study patients and controls was from stool; persistent infection in the bowel rendered separation of controls and ill patients impossible.

Infections with all coxsackieviruses and echoviruses frequently have one or more gastrointestinal symptoms as part of the general illness. The intensity and spectrum do vary among the specific agents, however, and also among strains of particular viral types. In a review of World Health Organization (WHO) virus reports covering a 4-year period, Assaad and Cockburn[34] found that the main clinical sign or symptom was gastrointestinal in 12 percent of coxsackieviral infections and in 6.8 percent of echoviral infections. In another analysis, Assaad and Borecka[33] noted 16 deaths during the period 1967 to 1975 in patients with coxsackievirus and echovirus infections in whom the principal clinical association was gastrointestinal.

In a 20-year survey in Wisconsin, Nelson and colleagues[755] noted that gastrointestinal symptoms occurred in approximately one third of all patients from whom these investigators recovered nonpolio EVs. Morens and colleagues[729] reported that in 4 percent of patients from whom nonpolio EVs were recovered during the 1971 to 1975 period, gastrointestinal disease was the major diagnosis. Horn and coworkers,[458] studying respiratory viral infections, noted that 21.2 percent of subjects from whom EVs were recovered had gastrointestinal complaints.

Vomiting

Vomiting is a common manifestation of infection with many coxsackieviral and echoviral types, but it rarely is the major complaint of the patient or the parent.* The frequency of vomiting during outbreaks of illness caused by coxsackieviruses and echoviruses depends on the specific types of virus and on the major manifestation during a particular outbreak. Table 165-9 presents the frequency of vomiting by viral type. In 14 different enteroviral types, vomiting has been noted as a significant aspect of the illness during disease outbreaks. Except for coxsackievirus A16 infections (hand-foot-and-mouth disease), in which it is an uncommon complaint, vomiting occurs in approximately 50 percent of all patients with epidemic enteroviral disease. Vomiting is noted most commonly in meningitis and least commonly in pleurodynia and uncomplicated exanthematous disease.

*References 33, 34, 104, 182, 211, 253, 404, 458, 504, 569, 616, 681, 684, 689, 694, 703, 725, 728, 729, 761, 807, 818-820, 846, 925, 1020, 1059-1060, 1062, 1080.

*References 10, 32, 182, 192, 193, 204, 238, 239, 265, 318, 317, 388, 408, 414, 415, 421, 542, 545, 546, 554, 573, 582, 629, 672, 674, 770, 827, 832, 853, 871, 889, 894, 897, 940, 960, 999, 1048, 1054, 1065, 1074, 1075.

TABLE 165-9 **Frequency of Vomiting in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses**

Virus Type	Age Group (yr)	Vomiting (%)	Main Characteristics of Outbreak	References
Coxsackievirus A9	Mainly children	60-73	Meningitis	182
	Children	14-20	Rash	182, 632
Coxsackievirus A16	Children and adults	3-15	Hand-foot-and mouth disease	10, 871
Coxsackievirus B2	Mainly children	50	Meningitis	182
	Mainly children	18-66	Febrile illness, respiratory illness	183
Coxsackievirus B3	Adults and children	33	Pleurodynia	182
	Children	9	Febrile illness, respiratory illness	182
	Mainly children	25	Fever, diarrhea	316
Coxsackievirus B4	Mainly children	27	Nonspecific fever	318
Coxsackievirus B5	Adults and children	31-37	Pleurodynia	389, 940
	Adults and children	50-95	Meningitis	182, 204, 408, 889
	Mainly children	25-33	Nonspecific febrile illness	204, 889, 1075
	Children	45	Febrile illness, respiratory illness	907
	Mainly children	100	Hepatosplenic syndrome	940
	Children	40	Rash	193
Echovirus 2	Children	12	Respiratory illness, rash	182
Echovirus 4	Mainly children	70-90	Meningitis	545, 853
	Mainly children	9-50	Epidemic disease, including meningitis, minor illness	182, 770
Echovirus 6	Mainly children	55-98	Meningitis	421, 546, 572, 608, 1054
	Mainly children	50-75	Epidemic disease, including meningitis	182, 1075
Echovirus 9	0-4	64-71	Rash, meningitis	182, 897
	5-9	81-83		
	10-19	61-92		
	>20	20-38		
	Mainly children	39-95	Rash, meningitis	182, 832, 1054, 1065
	Mainly children	26	Pharyngitis	182
	Mainly children	71	Epidemic disease, including meningitis	182
Echovirus 11	Not specified	100	Febrile illness, respiratory illness	182
Echovirus 16	Children	30	Rash	414
Echovirus 19	<0.5	25	Meningitis, upper respiratory tract illness, nonspecific febrile illness	182
	0.5-2	31		
	3-5	37		
	6-12	68		
	13-18	61		
	19-25	53		
	26+	43		
Echovirus 20	Children	67	Febrile illness, respiratory illness	238
Echovirus 30	Mainly children	15-91	Meningitis	392, 542, 827, 999
	<5	50	Febrile illness, respiratory illness	182
	>5	70		

Diarrhea

Diarrhea occurs commonly in enteroviral and parechoviral infections, but it usually is just one of many manifestations of systemic illness.* Specific studies of diarrheal disease in infants and children have had varied results; some studies indicate an enteroviral origin, whereas others reveal that coxsackieviruses and echoviruses were recovered from well children at the same prevalence.

Ramos-Alvarez and Olarte[847] carried out an extensive study of diarrheal disease in Mexico City and noted that echoviruses were recovered eight times more frequently from children with diarrhea than from control children without diarrhea. These investigators found that echoviruses 6 and 19 predominated, but types 3, 7, 9, 12, 14, 18, and 21 also were recovered. Pelon and colleagues[813] noted an association between coxsackieviruses B4 and B5

*References 10, 31, 32, 82, 111, 114, 182, 185, 236-239, 265, 317, 318, 383, 388, 404, 414, 415, 421, 545, 546, 556, 569, 582, 609, 622, 629, 672, 681, 684, 689, 747, 807, 808, 827, 831, 836, 846-848, 871, 892, 894, 923, 952, 953, 986, 1062, 1075, 1104, 1105.

and acute diarrhea; they observed a 42 percent coxsackievirus B isolation rate in children with acute diarrhea and a corresponding rate of only 12 percent in those without diarrhea. Goodwin and associates[387] noted echoviruses in the stools of children with diarrhea at twice the rate observed in healthy children. In a study by Yow and colleagues,[1104,1105] no association was found between EVs and infantile diarrhea. Echoviruses were recovered twice as frequently from children ill with diarrhea as from children without gastrointestinal illness.[1105] In Canada, McLean and colleagues[689] could find no association between enteroviral infection and gastroenteritis. In a large study of diarrhea in India, coxsackieviruses A9, B3, and B6 and echoviruses 12 and 21 were recovered more commonly from ill patients than from children without diarrhea.[747]

In several studies of specific diarrhea outbreaks, afflicted persons were noted to be actively infected with a particular virus type. Goldwater and Laws[383] observed three children younger than 6 months who were infected with echovirus 19 and who had gastroenteritis without other signs or symptoms. Klein and colleagues[582] recovered echovirus 11 from the blood of two laboratory workers with acute gastroenteritis. Diarrhea occurred in three of nine volunteers given echovirus 11.[114] Eichenwald and associates[296] and Cramblett and colleagues[236] reported epidemic diarrhea caused by echovirus 18 in neonates. An outbreak of diarrhea associated with coxsackievirus A1 was noted in 7/14 bone marrow transplant recipients during a 3-week period.[1001]

Table 165-10 presents the frequency of diarrhea in outbreaks of specific coxsackieviral and echoviral illnesses. Diarrhea varied with regard to the viruses represented and in different studies with the same agents. Diarrhea in enteroviral disease rarely is severe. In most instances, loose stools occur for a 2- to 4-day period. The stools rarely are watery, never are bloody, and at most number six to eight per day. In two childcare centers, diarrhea associated with a parechoviral infection occurred in 21 percent of the children during a one half year period.[104] The following PeV genotypes have been identified in children with diarrhea: 1, 3, 4, 5, 6, 8, 10, 11.[818-820,1111] SAFVs have been identified in stool samples from children with diarrhea, but a cause-and-effect relationship has not been established.[90,422,564,856,1091]

Constipation

Some degree of constipation is a rather frequent occurrence in many acute infectious illnesses, but evaluation is rendered difficult by the subjective nature of the complaint. In coxsackieviral and echoviral diseases, a short period of constipation may be associated with fever, vomiting, and anorexia early in the course of the illness. This period frequently is followed by mild diarrhea.[114] As noted in Table 165-8, constipation has been reported specifically as a symptom with four coxsackieviral and four echoviral types. Constipation is a particularly common event in children with enteroviral meningitis; it occurs in 10 to 40 percent of cases.*

*References 182, 317, 318, 545, 546, 607.

Abdominal Pain

Abdominal pain is a common symptom in many coxsackieviral and echoviral infections. Table 165-11 lists the frequency of occurrence of abdominal pain and the main characteristic of the illness by virus type. Approximately 10 percent of patients with coxsackievirus A16 hand-foot-and-mouth disease report abdominal pain. In approximately one fourth of patients, many coxsackieviruses and echoviruses associated with meningitis cause abdominal pain.

The magnitude of abdominal pain as a clinical symptom in coxsackieviral and echoviral infections varies considerably. For example, in aseptic meningitis, headache and other neurologic reports overshadow the abdominal symptoms. In other situations, fever and abdominal pain are diagnostically troublesome because of the possible presence of a surgical abdomen (discussed further in the next paragraph). The pain most frequently is periumbilical; it may be either constant or colicky. The fever is most often higher than 38.3°C (101°F).

Peritonitis, Pseudoperitonitis, Appendicitis, Pseudo-obstruction, Mesenteric Adenitis, and Intussusception

Occasionally, coxsackieviruses and echoviruses are associated with illnesses that suggest severe abdominal involvement. Liebman and St. Geme[641] described two children with abdominal findings suggestive of acute appendicitis (i.e., semirigid, tender abdomen, rebound tenderness, and rectal tenderness) who had associated infections with echoviruses 1 and 14. McLean[688] described a surgical abdomen in one child with coxsackievirus B1 infection. In this case, virus was recovered from the peritoneal exudate; at surgery, this boy was found to have peritonitis, but his appendix was normal. Thomas[992] reported a 5-year-old boy with coxsackievirus B5 infection who at surgery was found to have excessive clear peritoneal fluid and markedly enlarged mesenteric lymph nodes. A pregnant woman with acute abdominal pain and rebound tenderness associated with echovirus 8 infection was described.[811]

In immunofluorescence studies, Tobe[997] showed the presence of coxsackievirus B2 and B5 antigens in the mucous membranes and mesenteric lymph nodes of patients with appendicitis more often than in similar studies in control subjects. He suggested that the viral infection acts as a trigger for appendicitis. Bell and Steyn[70] recovered echoviruses 7 and 9 from the mesenteric lymph nodes of children with intussusception.

Hepatitis

Marked liver involvement is not a rare finding in disseminated coxsackieviral and echoviral infections in neonates.* The association of hepatitis and enteroviral infection in older children is defined less clearly but

*References 11, 155, 169, 180, 253, 466, 569, 616, 621, 674, 728, 729, 734, 783, 914, 920, 921, 940, 971, 1030, 1045.

TABLE 165-10 **Frequency of Diarrhea in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses**

Virus Type	Age Group (yr)	Diarrhea (%)	Main Characteristics of Outbreak	References
Coxsackievirus A9	Adults and children	7-100	Fever, rash	182, 634
Coxsackievirus A16	Mainly children	4-33	Hand-foot-and-mouth disease	10, 185, 629, 874
Coxsackievirus B2	Mainly children	9-56	Febrile illness, respiratory illness	182
	Mainly children	12	Meningitis	671
Coxsackievirus B3	Mainly children	2-5	Pleurodynia	182
	Children	11	Meningitis	236
	Mainly children	54	Nonspecific febrile illness	316
Coxsackievirus B4	Children	9	Meningitis	237
	Mainly children	8	Febrile illness, respiratory illness	317
Coxsackievirus B5	<1	21	Meningitis and fever, respiratory illness	831
	1-9	15		
	10-29	3		
	Mainly children	5-9	Meningitis	182, 236, 1075
Echovirus 4	Mainly children	5-75	Meningitis	545, 622, 894
	Mainly children	65	Nonspecific febrile illness	622
Echovirus 6	Mainly children	6-12	Meningitis	
Echovirus 9	0-4	14	Meningitis, rash	182, 420, 546, 894
	5-9	10		
	10-19	8		
	<20	0		
	Mainly children	3-40	Meningitis	182, 239, 894
	Children	15	Rash, febrile illness	283
	Mainly children	5-15	Respiratory illness	182
Echovirus 11	Adults	33-100	Gastrointestinal illness	114, 582
	Mainly infants	40	Nonspecific febrile illness	82
Echovirus 13	Children	11	Respiratory illness	182
Echovirus 16	Children	20	Rash	414
Echovirus 17	<1-4	100	Fever, diarrhea	182
Echovirus 18	Infants	100	Diarrhea	296
Echovirus 19	<2	30	Meningitis	182
	2-4	7		
	5-11	7		
	12-17	0		
	>18	9		
	Mainly children	3	Meningitis	383
	Mainly children	10	Respiratory illness	235, 383
	Mainly children	9	Diarrhea	395
Echovirus 20	Children	100	Respiratory, enteric illness	238
Echovirus 30	<5	12	Meningitis	182
	>5	5		
	Adults and children	7-10	Meningitis	415, 827, 999

probably occurs more commonly than generally realized, based on the number of individual cases reported. Caution must be exercised in accepting EVs as the exclusive etiologic agent of hepatitis, however, because hepatitis A virus infection has been ruled out in only a few of the available studies.

Morris and associates[734] described an illness suggestive of a coxsackieviral or echoviral infection in an 18-month-old child who had hyperbilirubinemia and abnormal liver function test results. From this child, coxsackievirus A4 was recovered from blood during the acute illness, and a rise in neutralizing antibody titer to the isolated virus was demonstrated. Chang and Weinstein[169] reported a 3-year-old boy with pharyngitis, urinary abnormalities, and neurologic symptoms who had elevated liver enzyme values (i.e., aspartate transaminase value of 1600 and alanine transaminase value of 1180). Coxsackievirus A9 was recovered from this child's cerebrospinal fluid (CSF), and the child's antibody response to this agent was significant. Coxsackievirus A20 has been associated with clinical hepatitis, and simultaneous infections with coxsackievirus A24 and hepatitis A virus have been demonstrated.[921]

An adult with a coxsackievirus B1 infection had both myocarditis and liver involvement.[11] A similar illness caused by coxsackievirus B3 in a 19-year-old woman was described.[971] An 11-month-old boy had a Reye-like syndrome in conjunction with coxsackievirus B2 infection.[550]

TABLE 165-11 **Frequency of Abdominal Pain in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses**

Virus Type	Age Group (yr)	Abdominal Pain (%)	Main Characteristics of Outbreak	References
Coxsackievirus A9	Mainly children	7	Meningitis	182
	Children	20	Rash	192
Coxsackievirus A16	Mainly children	12	Hand-foot-and-mouth disease	632, 871
Coxsackievirus B2	Mainly children	22	Meningitis	672
Coxsackievirus B3	Children and adults	25-90	Pleurodynia	182
Coxsackievirus B4	Children and adults	10	Meningitis	182
	Mainly children	36	Febrile illness, respiratory illness	319
Coxsackievirus B5	Mainly children	10	Meningitis	182
	Children	13	Febrile illness, respiratory illness	907
	Children and adults	23-67	Pleurodynia	388, 892, 940
Echovirus 4	Mainly children	28-50	Meningitis	182, 545
	Mainly children	13	Febrile illness, respiratory illness	770
Echovirus 6	Mainly children	17-43	Meningitis	182, 421, 546, 572
Echovirus 9	0-4	28	Meningitis, rash	182, 204, 629, 897
	5-9	30		
	10-19	25		
	≥20	0		
Echovirus 11	Adults	38	Gastrointestinal illness	114
Echovirus 16	Children	20	Rash	414
Echovirus 19	<0.5	3	Meningitis	182
	0.5-2	3		
	3-5	27		
	6-12	38		
	13-18	13		
	19-25	9		
	26+	3		
Echovirus 30	Mainly children	17	Meningitis	542, 999

Reye syndrome has been associated with echovirus 3 infection.[429] Siegel and colleagues[940] described a hepatosplenic syndrome in which 15 patients had hepatomegaly and coxsackievirus B5 infection. These patients, most of whom were children, had otherwise typical enteroviral illnesses. Liver function studies were performed in only three instances, and the results were normal.

Echoviruses 1, 3, 4, 6, 7, 9, 11, 14, 20, 21, and 30 have been associated with hepatitis.* Hepatomegaly occurs commonly in enteroviral infections.[185,187,316,609,632] During the period from 1971 through 1975, more than 7000 cases of nonpolio enteroviral infection were reported to the Viral Diseases Division at the CDC.[728] In this group were 13 cases of hepatitis and 6 of Reye syndrome; coxsackieviruses A2 and B4 and echoviruses 14 and 22 were associated with Reye syndrome.

Pancreatitis

One of the effects of coxsackievirus B in suckling mice is extensive infection in the pancreas. As with other similarities of infection between suckling mice and human neonates, generalized coxsackievirus B infection in neonates also is accompanied frequently by extensive pancreatic damage. In contrast, pancreatic involvement in older children and adults is not a common occurrence.[126,743,748,1016] Coxsackieviruses B3, B4, B5, and A9 have been noted. Groeneweg and coworkers[405] described a 3-year-old girl with chronic hereditary pancreatitis associated with a serine protease inhibitor Kazal type 1 gene mutation and a high antibody titer to coxsackievirus type 5.

Diabetes Mellitus

A possible relationship between juvenile diabetes mellitus and the seasonal occurrence of coxsackievirus B4 was suggested in 1969 by Gamble and Taylor.[354] In a second study, Gamble and associates[353] noted that titers of coxsackievirus B4 antibodies in patients with type 1 diabetes mellitus (DM) within 3 months of the onset of disease were higher than those in physiologically normal subjects or in patients with chronic diabetes.

Since the 1960s, many studies in animal models and children have looked at the relationship between juvenile-onset, type 1 DM and coxsackieviruses B.* Between 1973

*References 182, 481, 569, 670, 725, 914.

*References 22, 35, 48, 49, 156, 174, 223, 242, 271, 339, 340, 393, 424, 435, 444, 449, 451, 465, 534, 535, 538, 577, 653-655, 798, 875, 919, 925, 995, 1018, 1024, 1073.

and 1984, 10 case-control studies examined the prevalence of antibody to various coxsackieviruses B in patients with type 1 DM and controls. In 8 of these studies, the prevalence was greater in the patients with type 1 DM. In 3 of these studies, IgM antibody to the coxsackieviruses B was examined, and the prevalence in each was greater in the patients with type 1 DM than in controls. A Finnish study noted that patients with type 1 DM were more likely to have IgA antibodies to coxsackievirus B4 than were controls.[488]

Helfand and associates[435] performed a well-done case-control study of type 1 DM. These investigators found that new-onset cases of type 1 DM in patients 13 to 18 years of age were more likely than were controls to be IgM antibody–positive for 9/14 EV serotypes. The serotypes were coxsackieviruses B2, B3, B4, B5, and B6; coxsackievirus A9; and echoviruses 9, 30, and 34. Green and associates[393] performed a systematic review of published case-control studies relating to coxsackievirus B serology and type 1 DM. In 13 studies that looked at antibody to all coxsackievirus B types, 7 (54%) had significantly more positive antibody values in the cases than in the controls. The findings were less impressive when rates of antibody by specific coxsackievirus B serotypes were compared. However, with coxsackievirus B4, the odds ratio was higher than 1/9 of 17 (53%) comparisons, and this difference was significant in 6 (35%) of the studies.

In an extensive study, Skarsvik and colleagues[947] noted that children with type 1 DM had an impaired type 1 T-cell response against coxsackievirus B4 in contrast to the response in healthy children. The findings led these investigators to suggest that the defect in T-cell function delayed clearance of coxsackievirus B4 and made damage to β cells more likely. In another study involving adults, Varela-Calvino and coworkers[1024] noted that T-cell proliferative responses against the VP2 protein of coxsackievirus B4 was significantly reduced in patients with type 1 DM in contrast to the responses in control subjects.

Cudworth and colleagues[242] noted a correlation between HLA type BW15 and coxsackieviruses B1, B2, B3, and B4 antibodies in patients with type 1 DM. In 1979, Yoon and colleagues[1101] reported the recovery of coxsackievirus B4 from the pancreas of a previously healthy 10-year-old boy who died after being in a diabetic coma. Hindersson and coworkers[451] noted the simultaneous onset of type 1 DM in a mother and her 10-year-old son coincident with a coxsackievirus B5 infection.

Clements and coworkers[223] noted that 9/14 serum samples from children with new-onset type 1 DM were positive for EV RNA by PCR. In contrast, only 4 percent of serum samples from control children had evidence of EV RNA. Recently, Oikarinen and associates[793] conducted a nested case control study involving children in Finland who took part in a type 1 diabetes prediction and prevention study. All case children (N = 38) had progressed to clinical type 1 diabetes. Nondiabetic control children (N = 140) were pairwised matched for sex, birth date, hospital district, and HLA-DP-conferred genetic susceptibility. Serum samples (at 3- to 12-month intervals) were examined for EV RNA. EV RNA-positive samples (5.1%) were significantly more frequent among cases than among controls (1.9%). In another Finnish study involving adults, 50 percent of the patients with type 1 diabetes had RNA evidence of ongoing enteroviral infection in the gut, whereas none of the control subjects had similar infections.[792]

In a murine model, See and Tilles[925] found that a diabetogenic strain of coxsackievirus B4 infection resulted in persistent detection of viral RNA in the pancreases of most infected mice. This persistence of antigen was associated with chronic islet cell inflammation and elevated islet cell antibody levels. Stimulated peritoneal macrophages caused lysis of islet cells either directly or by an antibody-dependent mechanism. A study in prediabetic children found that the presence of EV RNA in serum was a risk factor for development of β-cell autoimmunity and type 1 DM.[655] Glutamic acid decarboxylase (GAD_{65}) is one of the major β-cell target antigens in the autoimmune β-cell damaging process that leads to the development of type 1 DM.[653] Antibody and cellular immunity cross reactivity exists between GAD_{65} and the 2C protein of coxsackievirus B4.[35,653] The results of several studies suggest that enteroviral infections are associated with the development of β-cell autoimmunity and the eventual destruction of islet cells.[121,449,654] Time periods from enteroviral infection to the development of β-cell autoimmunity and the eventual development of type 1 DM from β-cell destruction have a great range that perhaps explains the lack of a more specific seasonally related onset of type 1 DM. A case of neonatal type 1 DM associated with a maternal echovirus 6 infection has been reported.[798]

Eye Findings

Acute Hemorrhagic Conjunctivitis

Although conjunctivitis has been a frequent finding in nonpolio enteroviral illnesses since the 1950s, its occurrence as a dominant symptom has been observed only since the 1960s.[134,182,404,592]

In June 1969, Chatterjee and associates[171] noted an epidemic of acute hemorrhagic conjunctivitis in Accra, Ghana. This disease was nicknamed Apollo 11 disease because it coincided with the time of the Apollo 11 moon landing. Since 1969, many epidemics of acute hemorrhagic conjunctivitis have been described. In most epidemics, EV 70 has been the etiologic agent, but similar epidemics have been caused by a variant of coxsackievirus A24.[218] More recently, this virus has been the cause of more epidemics than has EV 70.* Most epidemics have occurred in tropical and semitropical countries; however, outbreaks have been observed in Minnesota, as well as in Moscow, London, and other European cities.[606,1099] In the continental United States, epidemic disease has occurred in Florida and North Carolina.[136] During epidemics, all age groups are affected, but the highest attack rate is in school-age children.[1052]

*References 122, 139, 146, 224, 379, 565, 604, 806, 853, 987, 1050, 1090.

TABLE 165-12 **Frequency of Conjunctivitis in Outbreaks of Illness Caused by Coxsackieviruses and Echoviruses**

Virus Type	Age Group	Conjunctivitis (%)	Main Characteristics of Illness	References
Coxsackievirus A9	Children	20	Pharyngitis, rash	632
Coxsackievirus A16	Mainly children	0-18	Hand-foot-and-mouth disease	171, 666, 871
Coxsackievirus B2	Mainly children	2	Nonspecific febrile illness, respiratory illness	182
Coxsackievirus B3	Children	2-42	Upper respiratory illness	182
Coxsackievirus B4	Children	25	Upper respiratory illness	182
Coxsackievirus B5	Mainly children	7-50	Rash, meningitis, hepatosplenic illness, respiratory illness, pleurodynia	194, 236, 892, 907
Echovirus 2	Children	25	Rhinorrhea	182
Echovirus 4	Children	Rare	Meningitis	670
Echovirus 9	Mainly children	13-30	Rash, meningitis	140, 182, 899
Echovirus 11	Children	15	Rash	192
Echovirus 16	Children	6-10	Rash	414, 759
Echovirus 30	Mainly children	10-60	Meningitis, nonspecific febrile illness	182, 415, 999

Acute hemorrhagic conjunctivitis has a sudden onset, with severe eye pain and associated photophobia, blurred vision, lacrimation, erythema, and congestion of the eye, as well as edema and chemosis of the lids.[445] Subconjunctival hemorrhages of varying size, frequently transient punctate epithelial keratitis, conjunctival follicles, and preauricular lymphadenopathy are present. The eye discharge initially is serous but becomes mucopurulent with secondary bacterial infection. Systemic symptoms, including fever, are rare manifestations. Within 2 to 3 days after the onset of illness, patients note some improvement, and recovery is usually complete in 7 to 12 days. In a study in American Samoa, researchers found that illness caused by coxsackievirus A24 was somewhat different from that caused by EV 70.[911] In cases caused by coxsackievirus A24, conjunctival hemorrhage was less severe, and upper respiratory and systemic symptoms occurred more frequently. In a study in American Samoa of disease caused by EV 70, researchers found that children 2 to 10 years of age had the highest attack rate, and antibody from previous infection gave only partial protection against symptomatic reinfection.[86]

Occasionally, findings suggestive of pharyngoconjunctival fever have been noted. A few patients have had a poliomyelitis-like illness or polyradiculomyeloneuropathy after EV 70 acute hemorrhagic conjunctivitis.[485,1037] Epidemics are explosive, with spread mainly by the eye-hand-fomite-eye route.

Conjunctivitis Associated with Other Enteroviral Illness

Conjunctivitis is a common minor manifestation of enteroviral illness with several specific agents. Table 165-12 presents the frequency of occurrence of conjunctivitis in selected outbreaks of disease caused by coxsackieviruses and echoviruses. Conjunctivitis was most prevalent in coxsackievirus B3 and B5 and echovirus 9 and 30 infections. In addition to the outbreaks reported in Table 165-12, conjunctivitis has been noted in isolated illnesses associated with echoviruses 1, 6, and 20.[569]

Photophobia

As would be expected, photophobia occurs commonly in aseptic meningitis caused by coxsackieviruses and echoviruses. During epidemic meningitis, researchers have noted it with the following viruses: coxsackievirus A9 and echoviruses 3, 4, 6, 7, 9, 19, and 30.* It is a most common occurrence with echovirus 9 and 30 infections, but the incidence of this complaint varies greatly in different reports. In one echovirus 30 outbreak, 80 percent of the patients studied had photophobia.[1000] On average, 20 percent of patients with meningitis have remarkable photophobia. Photophobia also is associated with pleurodynia caused by coxsackievirus B infections.[892,1048]

Other Eye Findings

Nodular lesions on the palpebral conjunctiva were observed in some patients with coxsackievirus A10 infection and lymphonodular pharyngitis.[959] A corneal ulcer occurred in one patient with hand-foot-and-mouth disease.[667] Optic neuritis was described in a boy with pleurodynia, although the enteroviral origin of the illness was not confirmed in the laboratory.[1012] Keratoconjuctivitis was noted in two boys with echovirus 13 infections.[576] Periorbital edema was observed in one patient with echovirus 9 meningitis. A woman with panuveitis associated with coxsackievirus B3 infection was described.[329] One report detailed monofocal outer retinitis in a 36-year-old man with hand-foot-and-mouth disease.[409] Five extensive outbreaks of uveitis caused by echoviruses 11 and 19 in hospitalized young children were noted in three Siberian cities.[1025] These cases occurred predominately in infants hospitalized for other illnesses such as bronchitis, pneumonia, gastroenteritis, sepsis, dystrophy, and premature birth. Three normal adult patients with anterior uveitis in whom parechovirus

*References 69, 182, 388, 395, 541, 542, 545, 546, 572, 629, 670, 827, 999.

was identified in ocular fluid have been noted.[259] In the same study an adult positive for human immunodeficiency virus (HIV) had panuveitis. Sore eyes and other unspecified eye complaints also have been noted frequently in patients with coxsackieviral and echoviral infections.*

Cardiovascular Manifestations

Pericarditis, Myocarditis, and Dilated Cardiomyopathy

Pericarditis or myocarditis or both have been noted in association with 27 different nonpolio EVs. The relative importance of the different serotypes is presented in Table 165-13. The coxsackieviruses B have been implicated most frequently in heart disease. Coxsackievirus B5 has been the most common causative agent, but types 2, 3, and 4 also have been reported frequently. Of the echoviruses, type 6 has been associated most often with cardiac involvement, but the clinical findings with this agent have been described in only a few cases.

In patients with coxsackievirus B cardiac disease, hepatitis, pneumonia, nephritis, meningitis, and orchitis have been occasional associated findings. In 2007 to 2008, detection of severe neonatal disease (often with myocarditis) associated with coxsackievirus B1 infection was detected.[149,800,1067] These severe cases were due to a single genetic lineage. Sometimes, arrhythmias are the only clinical manifestations of myocarditis.[189,1011] Constrictive pericarditis occurs occasionally.[680] The mortality rate for acute coxsackieviral and echoviral heart disease is unknown, but it is significant. Unfortunately, proper virologic study rarely is performed in nonfatal disease. In the only published follow-up study, researchers found that patients who survived acute coxsackievirus myocarditis usually recovered completely, without any residual disability.[966]

The early descriptions of coxsackievirus B infection with myocarditis in the 1950s and early 1960s indicated acute, usually fulminant illnesses in which a coxsackievirus B serotype could be recovered from multiple sites, such as the throat, stool, and CSF, as well as from the heart and other organs at autopsy.[364,402,568,570,1019] Coxsackieviruses B originally were isolated from suckling mice, and the infection in these mice caused acute, overwhelming fatal infections involving multiple organs, including the heart.[253,527] Coxsackievirus B infections in mice were studied in mouse model systems in the 1950s and 1960s, and researchers found that infections in older mice were affected markedly by the virus strain, mouse genetics, and drugs.[96,470,575] Since the 1970s, studies in mouse model systems have become more sophisticated, but they still depend on specific coxsackievirus B strains and specific mouse strains.†

More recent studies in mouse model systems noted the progression of acute coxsackievirus B infection to chronic infection and cardiomyopathy.[20,454,537,680,841,858] Since the mid-1980s, acute, subacute, and chronic cardiomyopathy has been studied in patients by antigen-detection techniques and newer serologic methods.*

Bowles and colleagues[103] found coxsackievirus B nucleic acid sequences in myocardial biopsy samples from several patients with cardiomyopathy. Muir and colleagues[740] detected EV-specific IgM antibodies in the sera of 9 patients with chronic relapsing pericarditis. Fujioka and coworkers[345] reported positive enteroviral PCR results from endomyocardial biopsy specimens in 6/31 patients (19%) with dilated cardiomyopathy (DCM). Andréoletti and collaborators[23] noted EV RNA in 18/25 samples from the heart tissue of patients with DCM or ischemic cardiomyopathy. However, in two other well-controlled studies, enteroviral RNA could not be identified in endomyocardial biopsy samples or in heart tissue from patients undergoing heart transplantation.[228,642] In 1994, Muir and Archard[737] and Melchers and associates[701] reviewed the evidence for persistent enteroviral infection in chronic medical conditions, including idiopathic DCM. Muir and Archard[737] concluded that persistent enteroviral infection is associated causally with chronic medical conditions, whereas Melchers and colleagues[702] found no clear evidence for enteroviral persistence and the subsequent development of chronic medical conditions.

Since the mid-1990s, additional studies in humans have been performed, with mixed results with regard to the role of coxsackievirus B in the origin of chronic cardiomyopathy. Bowles and associates[101] studied cardiac samples for PCR analysis from 624 patients of all ages with myocarditis and 149 patients with DCM. Enteroviral genome was found in the cardiac samples of 85 patients with myocarditis and in the samples from 12 patients with DCM. The histopathologic findings in 9 of these 12 patients demonstrated borderline or mild findings of inflammatory infiltrates. Another study by investigators in the same group examined 80 explanted hearts from children with end-stage heart disease, and an enteroviral genome was not detected.[335] In another study, Zhang and coworkers[1113] found evidence of enteroviral capsid protein VP1 by immunohistochemical staining in 47/89 patients with DCM. In a study performed in Japan, Fujioka and associates[344] detected EV RNA in 7/30 American patients (23%) and in 15/47 Japanese patients (32%) with DCM. In our opinion, the data in these studies suggest that EVs have a role in some patients with DCM.

The general theme in most contemporary mouse model studies is that damage to the myocardium is not caused by the direct cytopathic effect of the virus, but rather is caused by the cellular immune response of the host. Unfortunately, the mouse model data and the less than definitive data from human studies have led cardiologists and others to think that the model systems are representative of human disease.[315,651] No adequate follow-up studies of survivors of acute enteroviral myocarditis or pericarditis have been conducted. Levi and colleagues[634] compared follow-up cardiac data on 10

*References 182, 312, 327, 408, 622, 664, 757, 909, 915.

†References 20, 120, 125, 214, 357-359, 364, 375, 438, 441, 454, 478, 479, 503, 537, 566, 573, 581, 614, 663, 680, 693, 756, 780, 804, 841, 859, 861, 862, 872, 928, 929,1003, 1006, 1079.

*References 19, 21, 25, 29, 101, 228, 260, 335, 344, 345, 376, 381, 433, 640, 642, 682, 702, 737, 738, 740, 1112.

TABLE 165-13 **Nonpolio Enteroviruses and Parechoviruses Associated with Pericarditis and Myocarditis**

Virus Type		Age Group or Age of Individual Patients	Heart Involvement: Etiologic Importance*	Heart Involvement: Pericarditis	Heart Involvement: Myocarditis	Heart Involvement: Unspecified	Other Aspects of Illness	References
Coxsackievirus	A1	Infants and adults	±	+	+			182, 399
	A2	Adults	±			+		34, 182
	A4	Mainly children	+	+	+		Sudden infant death	34, 383, 400, 1051
	A5	—	±	+				1022
	A7	Adults	±	+	+		Hand-foot-and-mouth disease	46
	A8	—	±			+		399
	A9	Mainly infants	+		+		High fatality rate	400
	A10	—	±			+		34
	A16	Infants and child	±		+		High fatality rate	182, 1045, 1088
Coxsackievirus	B1	Infants, children, and adults	++	+	+		Rare hepatitis	11, 31, 34, 117, 182, 265, 399, 569, 692, 755, 874, 918, 1060
	B2	Infants, children, and adults	+++	+	+		Occasional pneumonia	31, 34, 116, 117, 182, 265, 319, 404, 466, 569, 593, 692, 755, 874, 918, 923, 1051, 1060
	B3	Infants, children, and adults	+++	+	+		Rare hepatitis	34, 40, 68, 116, 117, 182, 265, 404, 467, 569, 593, 638, 755, 874, 923, 971, 1046, 1047, 1051, 1060
	B4	Infants, children, and adults	+++	+	+		Occasional pneumonia; myocardial calcifications; rare hepatitis, orchitis, nephritis, hemolytic uremic syndrome	31, 34, 40, 54, 107, 115-117, 182, 265, 320, 404, 467, 569, 593, 755, 854, 874, 973, 1011, 1014, 1051
	B5	Infants, children, and adults	+++	+	+		Occasional pneumonia, pleurodynia, and meningitis; rare orchitis	31, 33, 45, 98, 117, 182, 370, 388, 431, 432, 593, 652, 692, 732, 755, 779, 829, 831, 837, 918, 923, 1051, 1056, 1060, 1075
Echovirus	1	Adolescents	±	+	+			34, 915, 1060
	4	—	±			+		34, 700
	6	Children and adults	++	+				34, 66, 182, 1060
	7	Children and adults	±			+		34, 182, 725
	8	Adolescents	±	+				182, 526, 539
	9	Children and adults	+	+	+			34, 182, 189, 638, 700, 723, 1051
	11	Children and adults	+			+		34, 66, 182, 279, 874
	14	—	±			+		725, 1022
	17	—	±			+		34
	19	Children and adults	±	+	+			66
	25	Children	±		+			65, 66
	30	—	±			+		34, 638
Enterovirus	71			±		+	Encephalitis	453
Parechovirus	1	Children	±		+			671, 891

*++++, Most common; ±, rare.

adults who had acute myocarditis associated with coxsackieviral infections 42 to 68 months previously with similar cardiac data from normal age- and sex-matched subjects. No statistically significant differences in the two groups were found in the seven tests evaluated.

Orinius[797] performed cardiac examinations on 53 adults who had coxsackievirus B infections 2 to 11 years previously. The initial diagnoses in these 53 patients were meningitis in 33 cases, encephalitis in 3 cases, pleurodynia in 6 cases, pericarditis in 2 cases, and nonspecific complaints in 9 cases. Of this group, the "possibility of cardiomyopathy was established" in 2 (4%) cases. Two patients who previously had pericarditis were normal at follow-up.

Sainani and associates[898] performed follow-up studies on 20 adults and 2 teenagers who had coxsackievirus B myocarditis or pericarditis and found that 5 (23%) patients had chronic heart failure. Unfortunately, the duration of time since the primary illness of the patients was not reported.

Bergström and colleagues[81] performed follow-up examinations on five patients (two teenagers and three adults) who had had enteroviral myopericarditis and found that none had significant symptoms or physical signs. No follow-up data are available for neonates, infants, and children who have had enteroviral myocarditis.

In 1954, Stürup[968] reported a follow-up study of patients admitted to the hospital in 1930 to 1932 with pleurodynia. Of nine patients evaluated, including four who had a history of acute pericarditis, none had constructive pericarditis.

Other Cardiac Manifestations

Several investigators have studied the possible association of coxsackievirus B infection and myocardial infarction.* In many instances, patients with infarction have been demonstrated to have concomitant coxsackievirus B infection.† In controlled studies, the results have varied. Nikoskelainen and associates[769] noted that 9/59 patients with acute myocardial infarction had coxsackievirus B infection, whereas in the control group of 38 patients without infarction, only 1 patient had evidence of infection. Similarly, Nicholls and Thomas[762] found that 26 percent of patients had infections, but no infections were found in the control subjects.

In contrast, five other studies failed to show an increased rate of infection in patients with myocardial infarction in contrast to controls.[396,402,781,830,1083] However, two of these studies were performed during a nonenteroviral season,[830,1083] and in a third study, little coxsackievirus B activity occurred in the community during the study period.[396] Analysis of the available studies indicates that coxsackievirus B infection would appear to have a role in myocardial infarction. In one coxsackievirus B5 infection, inferolateral wall myocardial necrosis occurred, but coronary arteriography did not demonstrate obstruction.[266]

Chandy and associates[161] presented data in which antibody to group B coxsackieviruses was related to rheumatic-like valvular heart disease, and Burch and associates[117] demonstrated coxsackievirus B antigens in rheumatic lesions of the heart. Limson and colleagues[643] could find no association between group B coxsackieviruses and rheumatic fever. Soboleva and associates[949] noted an association between coxsackievirus A13 and rheumatic fever; they reported seven children with concomitant streptococcal and coxsackievirus A13 infection. Children with fulminant EV 71 infection have been noted to have acute left ventricular dysfunction.[159,166,474] A 27-month-old girl who died suddenly was found to have an SAFV identified in the CSF, blood, and myocardium.[763]

A 4-month-old boy who had repair of an atrioventricular septal defect was found to have a coxsackievirus B-2 endocarditis.[92] This virus was recovered from the excised prosthetic patch.

Genitourinary Manifestations

Orchitis and Epididymitis

Group B coxsackieviruses are second only to mumps as causative agents of orchitis.* Coxsackievirus B5 is the virus associated most commonly with this disease, although coxsackieviruses B2 and B4 also have been implicated on many occasions. In almost all instances, orchitis is a secondary event in enteroviral infections. The most common association is with pleurodynia. The illness frequently is biphasic: fever and pleurodynia develop initially, and then apparent recovery is followed by orchitis approximately 2 weeks after onset. Many patients also have epididymitis. In epidemics of disease caused by group B coxsackieviruses, the occurrence of testicular involvement varies considerably. Generally, orchitis occurs infrequently, but in one outbreak of coxsackievirus B2, 17 percent of postpubertal male patients had orchitis, and 7 percent also had epididymitis.[182] Orchitis also is associated frequently with pericarditis and myocarditis. In one instance, coxsackievirus B5 was recovered from a testicular biopsy specimen.[234]

In virtually all instances, testicular involvement has occurred in postpubertal patients, mostly in young adults. In addition to coxsackieviruses B2, B4, and B5, other EVs have been implicated: coxsackieviruses B1 and B3[182,569] and echoviruses 6, 9, and 11.[569,1058] Meningitis and exanthem have been associated with orchitis.

Nephritis

Scattered cases of nephritis associated with nonpolio enteroviral infections have been reported. Bayatpour and colleagues[60] noted acute glomerulonephritis in a 9-year-old boy with an extensive coxsackievirus B4 infection. This child had a concomitant rise in antistreptolysin O titer. Yuceoglu and associates[1106] reported twins with echovirus 9 infection and acute glomerulonephritis; Burch and Colcolough[115] observed a patient with progressive

*References 298, 396, 402, 618, 619, 762, 769, 781, 830, 1083, 1085.
†References 298, 396, 402, 618, 619, 676, 762, 769, 781, 1083, 1085.

*References 31, 182, 234, 337, 404, 569, 729, 923, 973, 1060.

fatal pancarditis and nephritis in whom coxsackievirus B4 antigen was found in the kidneys. Mesangiolytic glomerulonephritis associated with an echovirus 6 infection was described in an infant with immune deficiency.[476]

Other Genitourinary Findings

Hemolytic-uremic syndrome has been associated with virologic or serologic evidence of infection with coxsackieviruses A2, A4, A9, A10, A16, A21 and B1 to B6; echoviruses 4, 6, and 7; and PeV 1.* De Petris and colleagues[261] demonstrated titer rises to various EVs in patients with hemolytic-uremic syndrome who were positive or negative for infections with verocytotoxin-producing *Escherichia coli*. Other abnormal renal or urinary findings in nonpolio enteroviral infections include acute oliguric renal failure with coxsackievirus B5[30]; pyuria, hematuria, or proteinuria with echoviruses 1, 6, and 9 and coxsackievirus B5[182,546,629,898,915]; and hemorrhagic cystitis.[729] A 7-year-old girl with coxsackievirus A10 infection had vaginal ulcerative lesions,[718] and Wassermann test results have been falsely positive in patients with echovirus 9 and coxsackievirus B5 infections.[840]

Hematologic Findings

In 1968, Horwitz and Moore[464] described an outbreak of infectious lymphocytosis during which 27 mentally handicapped children were studied. The mean WBC count of the study group patients was 57,200; lymphocytes accounted for at least 50 percent of the total in all cases. Approximately half of the patients had low-grade fever, and 15/27 had moderate diarrhea. A nontypeable EV suggestive of coxsackievirus group A was recovered from the ill patients, and neutralizing antibody to a strain (EVU-16) of the isolated virus developed in 31 percent of the patients in whom serologic studies were performed.[406,465]

Letsas and colleagues[633] reported a 17-year-old-boy with fulminant myocarditis and hemophagocytic syndrome. Over the course of a 3-day period, his hematocrit decreased from 40.9 to 31.7 percent, and his platelet count went from 90,000 to 38,000/mm^3. Examination of the bone marrow revealed many mature histiocytes with active hemophagocytosis. The patient improved dramatically with inotropic and intravenous immunoglobulin (IVIG) treatment. Katsibardi and coworkers[548] reported the occurrence of hemophagocytic syndrome associated with enteroviral infections in three children with malignancies; in spite of IVIG and pleconaril treatment, all cases had a fatal outcome.

Muscle and Joint Manifestations

Arthritis

During the period from 1971 to 1975, during which EVs were recovered from 7075 persons in the United States, 9 instances of rheumatic disease were reported[729]; although not clearly specified, 3 patients apparently had arthritis. Blotzer and Myers[91] noted an adult with echovirus 9 infection and the concomitant onset of arthritis that persisted for 3 months. Echovirus 9 was recovered from the synovial fluid of a man with acute monocytic arthritis,[601] and one adult and one adolescent had serologic evidence of coxsackievirus B4 infection in association with the onset of rheumatoid arthritis–like illness.[487]

Myositis

Because group A coxsackieviruses routinely cause myositis in suckling mice, a reasonable approach is to suspect a similar clinical manifestation in people. Myalgia also is a common complaint in illnesses caused by many coxsackieviruses and echoviruses.[31,182,337,415,608,692] However, almost no direct (i.e., demonstration of virus in muscle) or indirect (i.e., elevations in muscle enzymes) evidence of muscle involvement in routine enteroviral illnesses exists. Of the nine patients with rheumatic disease described by Morens and colleagues,[729] six apparently had myositis; in one instance, coxsackievirus A2 was implicated, and in another patient with polymyositis, coxsackievirus A9 was the related agent. Three patients with echovirus 18 infection and polymyositis have been reported.[558] Acute rhabdomyolysis in one adult and myositis, myoglobinemia, and myoglobinuria in another adult have been associated with echovirus 9 infection.[515,532] Christensen and associates[216] noted that children with dermatomyositis were more likely to have serum antibody to one or more coxsackieviruses B than were control children. Chou and Gutmann[212] noted EV-like particles in the muscles of two patients with fatal dermatomyositis; Tang and colleagues[984] demonstrated coxsackievirus A9 in the muscles of an 11-year-old girl with chronic myopathy. Using both PCR and dot-blot hybridization assays, Fox and colleagues[333] could not find evidence of persistent enteroviral infection in 32 adults with inflammatory muscle disease (1 patient with dermatomyositis and 31 patients with polymyositis).

Yousef and coworkers[1102] reported EV RNA in biopsy specimens from 6/13 patients (46%) with idiopathic polymyositis or dermatomyositis but in no samples from 13 patients with other muscle disorders. Behan and associates[61] looked for picornavirus RNA in biopsy specimens from 41 patients with inflammatory myopathy, but all results were negative.

Dekel and associates[264] reported a 4-year-old girl with localized thigh swelling caused by coxsackievirus A21. On muscle biopsy, perivascular infiltrates were noted, but the muscle fibers were not affected. Widespread edema was found in the muscle.

Skin Manifestations

Nonpolio EVs as a group are a common cause of a variety of skin manifestations.[180,1061,1065] In the summer and fall, these viruses are the leading cause of exanthems. Variation in the clinical expression rate of exanthem and in the age of the host is marked among the various viral types. In general, dermatologic expression is related inversely to the age of the infected patient. The frequency of

*References 36, 261, 372, 782, 854, 855.

exanthem and common associated illnesses by viral type are presented in Table 165-14.

Coxsackievirus A2

Rash associated with coxsackievirus A2 has occurred rarely. Febrile illness with exanthem in conjunction with coxsackievirus A2 infection was observed in Cincinnati in 1957; no details of this outbreak were presented.[894] Assaad and Cockburn[34] reviewed approximately 15,000 enteroviral illnesses for the period from 1967 to 1970 that were reported to the WHO and noted that in 45 instances, skin or mucous membrane lesions associated with coxsackievirus A2 infection were the main clinical manifestations.

Coxsackievirus A3

One case was reported, but no details are available.[725]

Coxsackievirus A4

Exanthem has been noted infrequently with coxsackievirus A4 infection.[34,187,328,734,1110] However, many cases may be missed because this virus grows poorly in tissue culture and suckling mouse inoculation rarely is used in diagnostic laboratories. Six children were observed in one outbreak.[328] All patients initially had herpangina, and then the exanthem appeared concurrently with or after defervescence. It initially was erythematous, maculopapular, and discrete. In some children, the rash resolved within 4 days, but in others it progressed and became vesicular. The vesicular lesions occurred in crops, spread to the extremities, but not the palms and soles, and were yellowish, opaque, and 5 to 10 mm in size. They persisted for 1 to 2 weeks and regressed, with a brownish discoloration. The lesions easily were confused with resolving bug bites. Other manifestations of coxsackievirus A4 infection include an exanthem suggesting combined scarlet fever and rubella,[363] a maculopapular rash that clears with desquamation,[734] and an anaphylactoid, purpura-like rash (Fig. 165-8).[187]

FIGURE 165-8 ■ Erythematous, papular, papulovesicular, and petechial lesions suggestive of anaphylactic purpura in a child with coxsackievirus A4 infection. (See companion Expert Consult website for color version.)

Coxsackievirus A5

During the 4-year period studied by Assaad and Cockburn,[34] coxsackievirus A5 was second only to coxsackievirus A16 in the number of instances for which exanthem or mucous membrane involvement was the main clinical manifestation. Even with this apparent frequency, most instances of exanthem have been sporadic rather than part of an outbreak of exanthematous disease. The most common finding is hand-foot-and-mouth disease, which is not clinically discernible from that caused by coxsackievirus A16.[241,524,328] In one patient, the virus was recovered from vesicular fluid.[324]

Coxsackievirus A6

Recently, severe hand-foot-and-mouth disease has been found in coxsackievirus A6 outbreaks in the United States, Taiwan, Finland, and Japan.[151] In the United States, there were 63 cases noted between November 7, 2011 and February 29, 2012. In California, cases continued to occur in the spring and summer of 2012 (Carol Glaser, California, 2013, Department of Public Health, personal communication). Lesions in this coxsackievirus A6 infection have often been bullous and involved extensive regions of the body. Shedding of nails occurred in two patients.

Coxsackievirus A7

Coxsackievirus A7 was recovered from one child with a morbilliform rash and aseptic meningitis.[398] An adult with a fatal coxsackievirus 7 infection had pancarditis, pneumonia, and hand-foot-and-mouth disease.[46]

Coxsackievirus A9

Coxsackievirus A9 is a common cause of exanthem (Figs. 165-9 and 165-10).* In contrast to coxsackievirus A16 and echovirus 9, in which rates of clinical exanthem expression are high, the rate of skin manifestations in coxsackievirus A9 infection is low. A review of 259 coxsackievirus A9 infections in 1970 revealed an exanthem rate of 4 percent.[182] Most cases of coxsackievirus A9 exanthem occur sporadically, although a few outbreaks have been described.[192,631,778]

Skin manifestations in coxsackievirus A9 infection have been interesting and varied. The most common rash illness is characterized by fever and an erythematous, maculopapular rash that starts on the face and neck and spreads to the trunk and extremities. Aseptic meningitis is a common associated finding.

In 1960, Lerner and colleagues[632] reported on 11 children with exanthem associated with coxsackievirus A9 infection. Of the patients, 6 had vesicular lesions and 2 patients had associated viral pneumonia. Since 1960, vesicular exanthem has been noted on several occasions.[182,192,482] Most commonly, illnesses have been described as hand-foot-and-mouth disease, but when the reports were analyzed, the rashes were vesicular but not always in a peripheral pattern.

*References 34, 182, 187, 192, 460, 482, 525, 529, 574, 632, 729, 778, 1072.

TABLE 165-14 **Frequency of Exanthem in Outbreaks of Illness Caused by Enteroviruses and Parechoviruses**

Virus Type		Age Group	Occurrence of Rash	Characteristics of Rash	Associated Manifestations	References
Coxsackievirus	A2	Children	Rare	Maculopapular	Fever	34, 894
	A4	Children	Rare	Maculopapular, vesicular	Fever, herpangina, hepatitis	34, 187, 328, 734, 1110
	A5	Mainly children	Occasional	Hand-foot-and-mouth disease	Fever	34, 241, 324, 328
	A6	Children and adults	Common	Hand-foot-and-mouth disease	Fever	151
	A7	Children and adults	Rare	Morbilliform; hand-foot-and-mouth disease, pancarditis	Meningitis, pneumonia	46, 400
	A9	Mainly children	4%	Maculopapular, vesicular, urticarial, petechial; Hand-foot-and-mouth disease	Fever, meningitis, pneumonia	34, 182, 187, 192, 460, 482, 524, 529, 574, 632, 729, 778, 1070
	A10	Mainly children	Occasional	Hand-foot-and-mouth disease	Fever	34, 118, 182, 222, 285, 635
	A16	Children and adults	88%, <5 yr 38%, 5-12 yr 11%, adults	Hand-foot-and-mouth disease	Fever	10, 15, 22, 31, 34, 110, 182, 185, 187, 307, 328, 341, 362, 381, 381, 446, 447, 635, 665, 666, 697, 732, 746, 865, 871, 996
	B1	Children	Occasional	Maculopapular, vesicular	Fever, meningitis	34, 182, 187, 191, 263, 662
	B2	Children	Rare	Maculopapular, vesicular, petechial	Fever, herpangina, meningitis	31, 34, 328
	B3	Mainly children	Occasional	Maculopapular, vesicular, petechial	Fever, hepatosplenomegaly	31, 34, 124, 183, 232, 316, 421, 480, 631
	B4	Mainly children	Occasional	Maculopapular, vesicular, urticarial	Fever, respiratory illness	182, 317, 545, 571
	B5	Mainly children	10%	Maculopapular, petechial, urticarial	Fever, meningitis	34, 98, 182, 193, 232, 265, 408, 431, 755, 772, 829, 831, 907, 940, 963, 967, 974, 1075, 1092
	B6	Children and adults	20%	Morbilliform	Pneumonia	384
Echovirus	1	Children	Rare	Maculopapular	Conjunctivitis	683
	2	Children	Rare	Macular, maculopapular	Fever, pharyngitis	182, 897, 957
	3	Children	Rare	Petechial	Fever, meningitis	34, 429, 462
	4	Mainly children	10%-20%	Macular, maculopapular, petechial	Fever, meningitis	182, 191, 293, 351, 545, 622, 670, 770, 853, 954

	5	Infants and adults	Occasional	Macular	Fever	368, 932
	6	Mainly children	Rare	Maculopapular, macular, papulopustular, vesicular	Fever, meningitis	34, 546, 677, 691, 696, 1031, 1034, 1075
	7	Children	Occasional	Maculopapular	Fever, meningitis	34, 513, 683
	9	Children and adults 41%, 5-9 yr 6%, >10 yr	57%, <5 yr	Maculopapular, petechial, vesicular	Fever, meningitis	34, 78, 182, 221, 230, 239, 257, 312, 325, 352, 355, 428, 460, 511, 524, 536, 569, 571, 584, 607, 626, 664, 690, 729, 755, 768, 833, 839, 852, 889, 898, 951, 965, 979, 1065
	11	Mainly children	Occasional	Maculopapular, vesicular, urticarial	Fever, meningitis	34, 114, 182, 194, 267, 923
	13	Children	Occasional	Maculopapular		457, 951
	14	Mainly children	Rare	Maculopapular, scarlatiniform	Fever, meningitis	34, 897
	16	Children	Occasional	Roseola-like	Fever, herpangina	180, 233, 414, 757-760
	17	Children	Occasional	Macular, maculopapular, papulovesicular	Fever, diarrhea, herpangina, meningitis	34, 111, 191, 190
	18	Children and adults	Occasional, epidemic	Rubelliform	Fever, meningitis	558, 701, 731, 755
	19	Children and adults	Occasional	Maculopapular	Fever, meningitis, upper respiratory illness	229, 237, 383
	25	Children	Occasional	Maculopapular, hemangioma-like	Fever, pharyngitis	65, 733, 751
	30	Children and adults	Occasional	Macular, maculopapular	Fever, meningitis	34, 182, 542, 999, 1093
	32	Children	Rare	Hemangioma-like	Fever	191
	33	—	Rare	—	Meningitis	544, 555
Enterovirus	71	Children	Occasional	Macular, maculopapular, vesicular; Hand-foot-and-mouth disease	Fever, meningitis, encephalitis, paralytic disease	13, 44, 369, 499, 557, 705, 717, 719, 795, 893, 1113
Parechovirus	1	Infants	Rare	Morbilliform	Respiratory illness	83
	3	Infants	Rare	—	—	1049
	6	Infants	Rare	—	—	1049

FIGURE 165-9 ■ Papular-urticarial lesions in coxsackievirus A9 infection. (See companion Expert Consult web site for color version.) (From Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.)

FIGURE 165-10 ■ Petechial and purpuric rash in a child with coxsackievirus A9 infection. (See companion Expert Consult website for color version.)

Papular urticaria and large urticarial lesions have been an occasional manifestation of coxsackievirus A9 infection (see Fig. 165-9).[187,192] Other manifestations include Stevens-Johnson syndrome and a severe illness simulating meningococcemia (see Fig. 165-10).[187,729]

Coxsackievirus A10

The most common exanthematous illness associated with coxsackievirus A10 infection is hand-foot-and-mouth disease.* Stevens-Johnson syndrome was associated with coxsackievirus A10 infection in one instance,[182] and a child with ulcerative genital lesions also was reported.[718]

Coxsackievirus A16

In the WHO review of enteroviral infections for the 4-year period from 1967 through 1970, coxsackievirus A16 was associated with almost half of all skin or mucous membrane diseases. Coxsackievirus A16 is the major cause of hand-foot-and-mouth disease (Figs. 165-11 through 165-14). Of historical interest is that hand-foot-and-mouth disease apparently was a new clinical entity in 1956.[777] It was noted in sporadic outbreaks until approximately

*References 118, 182, 222, 285, 500, 635.

FIGURE 165-11 ■ Vesicular and maculopapular lesions on the foot and lower part of the leg as part of the hand, foot, and mouth syndrome caused by coxsackievirus A16. (From Cherry JD, Jahn CL. Hand, foot, and mouth syndrome: Report of six cases due to coxsackievirus, group A, type 16. *Pediatrics* 1966;**37**:637. Copyright American Academy of Pediatrics 1966.)

FIGURE 165-12 ■ Erythematous maculopapular lesions on the buttocks as part of the hand, foot, and mouth syndrome caused by coxsackievirus A16. (See companion Expert Consult website for color version.) (From Cherry JD, Jahn CL. Hand, foot, and mouth syndrome: Report of six cases due to coxsackievirus, group A, type 16. *Pediatrics* 1966;**37**:637. Copyright American Academy of Pediatrics 1966.)

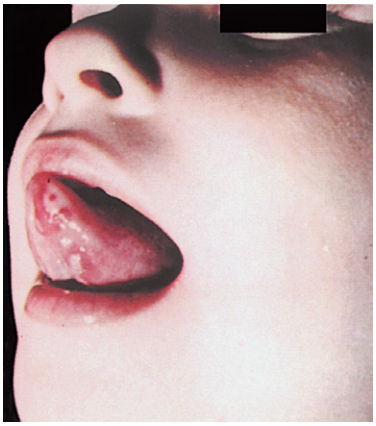

FIGURE 165-13 ■ Two large ulcerative lesions on the underside of the tongue in a patient with hand, foot, and mouth syndrome caused by coxsackievirus A16. (See companion Expert Consult website for color version.)

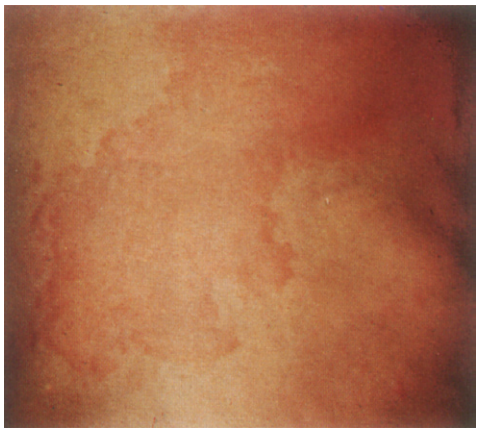

FIGURE 165-14 ■ Acute urticaria in a child with hand, foot, and mouth syndrome caused by coxsackievirus A16 infection. (See companion Expert Consult website for color version.)

1963, and since that time it has been a regularly recurring disease throughout the world.* Serologic data suggest that coxsackievirus A16 was not in wide circulation until approximately 1963.[185]

The signs and symptoms of coxsackievirus A16 hand-foot-and-mouth disease are recorded in Table 165-15. All illnesses have a typically enteroviral pattern with a short incubation period (4 to 6 days) and a summer and fall seasonal pattern. The clinical expression rate of the enanthem-exanthem complex is high—close to 100 percent in young children, 38 percent in schoolchildren, and 11 percent in adults.[730] Exanthem occurs more commonly in children, but in adults, the rash occurs more frequently with coxsackievirus A16 than with any of the other enteroviral agents.

Illness is ushered in with a mild prodromal fever, anorexia, malaise, and frequently a sore mouth. Enanthem occurs 1 to 2 days after the onset of fever, and the exanthem appears shortly thereafter. Of the cutaneous lesions, oral lesions are present more consistently than lesions of the skin. Because of this prevalence, illness, particularly in adults, is identified mistakenly as aphthous stomatitis (canker sores) or herpes simplex virus (HSV) infection. The intraoral lesions are ulcerative and average approximately 4 to 8 mm. The tongue and buccal mucosa are involved most frequently.

As noted in Table 165-15, the hands are involved more commonly than the feet. Buttock lesions also occur frequently, but they usually do not progress to vesiculation. The lesions on the hands and feet generally are vesicular and vary in size from 3 to 7 mm; they typically occur more commonly on the dorsal surfaces but frequently on the palms and soles, as well. The vesicles contain virus, but cytologic examination usually does not reveal diagnostic findings. These lesions clear by absorption of the fluid in approximately 1 week.

Of considerable interest is the frequent association of coxsackievirus A16 with subacute, chronic, and recurring skin lesions.[307,753] Evans and Waddington[307] described an 84-year-old woman with chronic recurring skin lesions of more than 2 years' duration. Nankervis and associates[753] noted both subacute and recurring lesions in children. Higgins and Crow[446] reported a 31-year-old woman with Darier disease who had a Kaposi varicelliform eruption caused by coxsackievirus A16, and a similar illness was noted in a 1-year-old boy with eczema.[746] One child with a Gianotti-Crosti–like eruption (papular acrodermatitis of childhood) associated with coxsackievirus A16 infection was described.[510]

*References 10, 15, 26, 31, 111, 182, 185, 215, 307, 328, 341, 362, 378, 380, 381, 446, 448, 635, 665, 666, 697, 730, 753, 865, 871, 996.

TABLE 165-15 **Symptoms and Signs of Coxsackievirus A16 Illness (Hand-Foot-and-Mouth Disease)***

Symptoms and Signs	Percentage of Cases (%)
Enanthem	90
Buccal	61
Tongue	44
Palate, uvula, anterior pillars	36
Gums	15
Exanthem	64
Hands	52
Feet	31
Buttocks	31
Legs	13
Arms	10
Face	5
Sore mouth or throat	67
Malaise	61
Anorexia	52
Fever	42
Submandibular and/or cervical adenitis	22
Coryza	11
Cough	11
Diarrhea	10
Nausea and vomiting	3

From Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233-86.
*Data from references 16, 186, 328, 341, 667, 698, 866, 872.

Coxsackievirus B1

Exanthem occasionally occurs in coxsackievirus B1 infections.* The most common cutaneous finding is an erythematous maculopapular eruption that is discrete. Two children with illnesses suggestive of hand-foot-and-mouth disease have been observed.[187]

Coxsackievirus B2

Coxsackievirus B2–associated exanthem is a rare occurrence. Maculopapular, vesicular, and petechial lesions have been noted.[31,34,328]

Coxsackievirus B3

Exanthem occasionally is reported as a sporadic event in coxsackievirus B3 infections, and a small outbreak

*References 34, 182, 187, 191, 263, 662.

occurred in one instance.* Erythematous maculopapular eruptions occur most commonly, but petechial rash illnesses suggestive of meningococcemia also have been observed. Hand-foot-and-mouth disease was reported in one child,[631] and another child had a more generalized vesicular eruption. An adult with recurrent Hand-foot-and-mouth disease had a rise in neutralizing antibody titer to coxsackievirus B3.[232]

Coxsackievirus B4

Although cutaneous manifestations with coxsackievirus B4 were noted as frequently as were those associated with coxsackieviruses B1, B2, and B3,[34,182] few clinical descriptions exist.[545,571] Morbilliform, petechial, and urticarial rashes have been described.

Coxsackievirus B5

Of the group B coxsackieviruses, type B5 is noted most frequently to have skin manifestations.† The rash usually is maculopapular; petechial lesions are observed occasionally, and one child with urticaria was reported.[193] Several children studied by Cherry and colleagues[193] had a roseola-like illness pattern. During outbreaks of coxsackievirus B5 illness, approximately 10 percent of young children have an exanthem. Many patients have concomitant aseptic meningitis. One adult patient with recurrent hand-foot-and-mouth disease had serologic evidence of coxsackievirus B5 infection.

Coxsackievirus B6

Goldwater[384] identified 97 children and adults with coxsackievirus B6 infection. The most prominent finding in these cases was cough and pneumonia. Twenty patients had exanthems; the rash was morbilliform and was associated with high fever and cough.

Echovirus 1

Exanthem and conjunctivitis have been associated with echovirus 1 infection in several children.[683]

Echovirus 2

Exanthem occasionally occurs with echovirus 2 infection.[182,897,957] The rash has been erythematous macular in some instances but usually is maculopapular and discrete. Most children have had associated fever and pharyngitis.

Echovirus 3

Exanthem was noted in 2/29 children with echovirus 3 infection.[429] In both instances, the rashes were petechial; most patients in this outbreak had aseptic meningitis. Details of other echovirus 3 infections and exanthems are lacking.[31,462]

*References 31, 34, 124, 232, 318, 421, 480, 631, 1107.
†References 34, 98, 182, 193, 232, 265, 408, 431, 772, 829, 831, 907, 940, 963, 967, 974, 1075.

Echovirus 4

Echovirus 4 is a common cause of epidemic aseptic meningitis, and exanthem is an associated finding in 10 to 20 percent of pediatric cases.* The rash usually is macular or maculopapular, has its onset 1 to 3 days after the initial fever, and lasts 1 to 2 days. A child with a petechial rash was noted.[545]

Echovirus 5

One major outbreak of echovirus 5 infection with exanthem was described.[368] This outbreak, which involved a maternity unit, resulted in a macular rash in 36 percent of the infants and 14 percent of the mothers. The rash was most prominent on the limbs and buttocks, appeared 24 to 36 hours after the onset of fever, and lasted 2 days. Selwyn and Howitt[932] reported a child who had a macular rash with a zoster distribution. A neonate with sepsis-like illness and exanthem was described.[411]

Echovirus 6

Although echovirus 6 has been one of the more prevalent EVs since the late 1970s, it has been associated only sporadically with exanthematous disease. The following manifestations have been observed: morbilliform,[1031] maculopapular,[691,1075] macular,[677] and papulopustular exanthems[546]; Stevens-Johnson syndrome[1034]; and pityriasis rosea in an adult and a child.[546] Meade and Chang[696] reported an interesting zoster-like eruption in a 7-year-old boy in whom echovirus 6 was recovered from several bullae.

Echovirus 7

Echovirus 7 has been associated occasionally with exanthem.[34,246,513,725] In one outbreak of aseptic meningitis, 5/13 patients had erythematous maculopapular rashes that occurred during fever.[513] One child with a discrete maculopapular rash and thrombocytopenia was described.[683] The case of a 37-year-old woman with echovirus 7 infection, leukocytoclastic vasculitis, and palpable purpura was reported.[200]

Echovirus 9

Since the 1960s, echovirus 9 has been the most prevalent nonpolio EV, and exanthem is a common clinical manifestation (Table 165-16, Fig. 165-15).† Nonspecific febrile illness and aseptic meningitis are the usual major manifestations of echovirus 9 infection. Exanthem occurs in approximately one third of cases. The prevalence of exanthem is related inversely to age; 57 percent of children younger than 5 years of age have a rash, whereas only 6 percent of those older than 10 years have similar cutaneous findings.[629] The rash usually is rubelliform, but

*References 187, 326, 351, 545, 622, 670, 770, 853, 952.
†References 34, 79, 182, 221, 230, 239, 257, 312, 325, 342, 352, 355, 385, 428, 460, 511, 525, 536, 569, 571, 584, 607, 626, 664, 690, 729, 767, 832, 838, 851, 888, 897, 950, 964, 979, 1065.

TABLE 165-16 **Signs and Symptoms in Echovirus 9 Disease***

Sign or Symptom	Percentage of Cases (%)
Fever	92
Headache	85
Nuchal rigidity	83
Nausea and vomiting	71
Pain (neck, back, trunk)	44
Exanthem	35
Nonexudative pharyngitis	28
Cough	24
Sore throat	20
Cervical lymphadenopathy	18
Coryza	18
Abdominal pain	17
Photophobia	16

From Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233-86.
*Modified from references 80, 257, 311, 325, 352, 512, 608, 627, 889, 951, 1066.

FIGURE 165-15 ■ Erythematous, discrete, maculopapular, and petechial rash of echovirus 9 infection. (See companion Expert Consult website for color version.) (From Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233-286.)

in addition or as the sole manifestation, petechiae are common findings. Rash and fever generally appear at approximately the same time, and frequently the illness closely mimics meningococcemia. The rash usually lasts approximately 3 to 5 days. In one instance, the rash progressed to a vesicular stage.[626]

Echovirus 11

Exanthem occasionally occurs with echovirus 11 infection, and it varies considerably in appearance.* The most notable lesions have been bug bite–like vesicles and urticaria.[194] A child with subacute recurrent vesicular lesions[194] and a woman with a disseminated vesicular eruption were reported.[267]

*References 34, 114, 182, 194, 267, 923.

Echovirus 13

One child with a maculopapular eruption was described.[457] During an outbreak of echovirus 13 aseptic meningitis in Israel, 15 patients had a concomitant exanthem.[951]

Echovirus 14

Echovirus 14 is a rare cause of exanthem, and few details are available.[34,897] One child with a scarlatiniform eruption and aseptic meningitis was reported.[897]

Echovirus 16

The first of the enteroviral exanthematous diseases to be described was that caused by echovirus 16. It initially was studied by Neva and Enders[758] and, later, Enders and colleagues,[302] and the illness was called *Boston exanthem*. Two outbreaks of echovirus 16 infection with exanthem were documented by Neva[757] in 1951 and by Neva and Zuffante[762] in 1954, and another occurred in Paris in 1960.[234] Since then, Boston exanthem has been reported only once. Seven cases were observed in 1974.[414]

The exanthem associated with echovirus 16 infection is erythematous, maculopapular, and discrete and is similar to that of other enteroviral infections. What has been unique with echovirus 16 infection is the relationship of rash with fever. Frequently, the illness resembles roseola in that the rash occurs at the time of or after defervescence.[180,414] Ulcerative lesions on the soft palate and tonsillar pillars (herpangina) sometimes have been observed.

Echovirus 17

Rash is an occasional occurrence in echovirus 17 infection.[34,111,187,190] In one outbreak, transient erythematous rashes were noted.[111] In cases that one of us (J. D. C.) studied, papular, maculopapular, and papulovesicular lesions were noted; two patients had herpangitic enanthems, and one had aseptic meningitis.[187,190]

Echovirus 18

Kennett and associates[558] described an extensive epidemic of echovirus 18 infection in which aseptic meningitis with or without exanthem was the major finding. In 15 patients, exanthem was the chief complaint. The rash was described as rubelliform. Most patients were children, but adults also had exanthem.

Echovirus 19

In one extensive epidemic of echovirus 19 infection, exanthem was a common occurrence.[229] Fifty percent of children younger than 6 months of age had exanthem, and many of these infants presented a picture of septicemia with peripheral circulatory failure. Rash occurred in adults and older children, but the percentage decreased with age. The rash usually was erythematous, maculopapular, and discrete. It started on the face and upper part of the trunk and spread to the extremities. Fever occurred in all cases, and meningitis and signs of upper respiratory

tract involvement were noted frequently. In another outbreak of echovirus 19 infection, 33 percent of those infected had exanthem,[383] whereas in an outbreak studied by Cramblett and colleagues,[237] exanthem was a rare finding.

Echovirus 21

A 19-day-old infant with aseptic meningitis and rash was reported.[209]

Echovirus 24

A 4-month-old infant had rash and aseptic meningitis as a result of echovirus 24 infection.[209]

Echovirus 25

Echovirus 25 has been associated with an array of different skin manifestations.[67,186,407,733,751] In one epidemic of febrile pharyngitis, approximately one third of the patients had exanthem.[733] The rash usually was maculopapular and discrete, but occasionally it displayed a morbilliform confluence. The rash most frequently occurred during the period of defervescence.

Of considerable interest are two children with acute hemangioma-like lesions.[186] The lesions were erythematous and papular and surrounded by a 1- to 4-mm–wide halo of blanched-appearing skin. The center of each lesion had a bright red dot that suggested a dilated capillary or terminal arteriole. The whole lesion blanched with pinpoint pressure applied to its middle.

Echovirus 30

Echovirus 30 is a common cause of epidemic aseptic meningitis, and exanthem occasionally is noted concomitantly. The rash is macular or maculopapular.*

Echovirus 32

Two children with echovirus 32 infection and hemangioma-like lesions were described by Cherry and colleagues.[191] Some lesions seemed to be composed of many dilated capillaries; they easily blanched with pressure.

Echovirus 33

Four patients with aseptic meningitis and echovirus 33 infection also had exanthem.[544,555] Two adults had vesicular lesions that suggested HSV infection.[263]

Enterovirus 71

Kennett and associates[557] in Australia studied 49 patients with EV 71 infection and noted exanthem in 11. Six patients had aseptic meningitis, and rash was the predominant complaint in 5 patients. The exanthems varied: some were erythematous maculopapular, some vesicular, and some a combination of lesions. Two children had hand-foot-and-mouth disease, and a single child had a florid, diffuse erythematous rash. Since the 1970s, outbreaks and epidemics of hand-foot-and-mouth disease caused by EV 71 have been noted throughout the world.* Most notable have been epidemics in Asian-Pacific countries. In addition to hand-foot-and-mouth disease, these illnesses also have severe encephalitic manifestations.

*References 34, 182, 495, 540, 624, 999, 1093.

Parechoviruses

Exanthem has been noted in association with PeV infections on several occasions. In some reports, genotypes have been reported,[83,504,800,931,1020] whereas in others only PeV is indicated.[50,825,1027,1028,1080] In one study that included 110 PeV infections, 9.1 percent had unspecified exanthems and, in addition, 6.4 percent had hand-foot-and-mouth disease.[504] Genotypes included types 1 and 3. In a study by Selvarargan and associates[931] of CNS infections with PeV type 3, 60.3 percent had associated maculopapular exanthems. A morbilliform rash has been described in three infants with genotype 1 infections.[83] Genotypes 1 to 6 have all had infections associated with rash.

Clinical Exanthematous Manifestations and Syndromes

The major clinical exanthematous manifestations and syndromes of coxsackieviruses and echoviruses are presented in Table 165-17. Unusual findings include hemangioma-like lesions with echoviruses 25 and 32, anaphylactoid purpura with coxsackievirus A4 and echoviruses 9 and 18, zoster-like rash with echoviruses 5 and 6, pityriasis-like rash with echovirus 6, and chronic or recurrent rash with coxsackievirus A16 and echovirus 11.

Neurologic Manifestations

Neurologic illness is a frequent manifestation of infection with most EVs and PeV. The most common illness is aseptic meningitis, but encephalitis and other manifestations also occur. Parechoviral neurologic manifestations are particularly common and significant in neonates and during the first 3 months of life (these will be discussed in the section on neonatal infections). The prevalence of nonpolio EVs and PeVs in the various clinical syndromes is presented in Table 165-18.

Aseptic Meningitis

Aseptic meningitis caused by EVs occurs both in epidemics and as isolated cases. The etiologic agents most often associated with epidemic disease are presented in Table 165-19. Epidemic disease has occurred most commonly with coxsackievirus B5 and echoviruses 4, 6, 9, 13, and 30. In general, illness occurs more frequently in children, but if a specific outbreak is large, adults also are involved. Virtually all patients have fever and pharyngitis; other respiratory manifestations occur commonly. Rash is a frequent occurrence but varies with the specific viral

*References 44, 125, 158, 159, 163, 165-167, 178, 208, 343, 369, 453, 467, 472, 474, 475, 499, 540, 591, 639, 645-648, 656, 679, 694, 705, 717, 773, 783, 794-796, 835, 893, 1040-1042, 1094, 1096, 1113.

TABLE 165-17 **Clinical Exanthematous Manifestations of Enteroviruses**

		Associated Viral Agents and Prevalence of Manifestations		
Clinical Manifestations	**Virus Subgroup**	**Common**	**Occasional**	**Rare**
Macular rash	Coxsackievirus A			
	Coxsackievirus B		1, 2, 5	
	Echovirus and enterovirus		2, 4, 5, 13, 14, 17, 19, 30	18, 71
Maculopapular rash	Coxsackievirus A	9	2, 4, 5, 10, 16	6, 7
	Coxsackievirus B		1-5	
	Echovirus and enterovirus	4, 9	2, 5-7, 11, 16-19, 25, 30, 71	1, 3, 13, 14, 27, 33
Vesicular rash	Coxsackievirus A	5, 16	8-10	4, 7
	Coxsackievirus B			1-3, 5
	Echovirus and enterovirus		11	6, 9, 17, 71
Petechial or purpuric rash	Coxsackievirus A	9	4	
	Coxsackievirus B		2-5	
	Echovirus	9	4, 7	3
Urticarial rash	Coxsackievirus A	9	16	
	Coxsackievirus B		4, 5	
	Echovirus		11	
Erythema multiforme or Stevens-Johnson syndrome	Coxsackievirus A		9	10, 16
	Coxsackievirus B			4, 5
	Echovirus			6, 11
Exanthem and meningitis	Coxsackievirus A		2, 9	7
	Coxsackievirus B		1, 2, 4, 5	
	Echovirus and enterovirus	4, 9	6, 11, 17, 18, 25, 30	3, 14, 33, 71
Exanthem and pneumonia	Coxsackievirus A		9	7
	Coxsackievirus B		6	1
	Echovirus			9, 11
Hand-foot-and-mouth disease	Coxsackievirus A	6, 16	5, 10	7, 9
	Coxsackievirus B			1, 3, 5
	Echovirus and enterovirus	71		
Hemangioma-like lesions	Coxsackievirus A			
	Coxsackievirus B			
	Echovirus			25, 32
Herpangina and exanthem	Coxsackievirus A		4	9
	Coxsackievirus B			2
	Echovirus		16, 17	
Roseola-like illness	Coxsackievirus A			6, 9
	Coxsackievirus B		5	1, 2, 4
	Echovirus		16, 25	9, 11, 27, 30
Anaphylactoid purpura	Coxsackievirus A			4
	Coxsackievirus B			
	Echovirus			9, 18
Zoster-like rash	Coxsackievirus A			
	Coxsackievirus B			
	Echovirus			5, 6
Pityriasis-like rash	Coxsackievirus A			
	Coxsackievirus B			6
	Echovirus			
Chronic or recurrent rash	Coxsackievirus A	16		
	Coxsackievirus B			
	Echovirus			11

agents. Between one third and half of all patients with echovirus 9 meningitis have exanthem. Abdominal pain is a common symptom in patients with epidemic enteroviral aseptic meningitis.

Except for rash, herpangina, pleurodynia, or myocarditis, little else occurs clinically to help identify the origin in a sporadic case of aseptic meningitis. Initial symptoms include fever, headache, malaise, nausea, and vomiting. The headache usually is frontal or generalized; adolescents and adults frequently note retrobulbar pain. Pain in the neck, back, and legs occurs commonly. Abdominal pain is noted in approximately one fifth of patients, but this symptom varies with the specific etiologic viral type. Photophobia is a common occurrence.

TABLE 165-18 **Neurologic Manifestations of Nonpolio Enteroviruses and Parechoviruses**

	Associated Viral Agents and Prevalence of Manifestations				
Clinical Manifestations	**Virus Subgroup**	**Common**	**Occasional**	**Rare**	**References**
Aseptic meningitis	Coxsackievirus A	9	7	1-6, 8, 10, 11-14, 16-18, 21, 22, 24	13, 14, 37, 76, 79, 85, 133, 144, 159, 166, 182, 257, 291, 301, 312, 313, 325, 351, 352, 387, 404, 410, 418, 436, 453, 511, 513, 517, 524, 553, 554, 569, 580, 583, 587, 591, 607, 623, 624, 629, 686, 691, 694, 705, 725, 731, 776, 864, 877, 882, 890, 894, 923, 933, 936, 950, 951, 1004, 1017, 1033, 1049, 1065, 1070, 1093
	Coxsackievirus B	2, 4, 5	1, 3	6	
	Echovirus	4, 6, 9, 13, 30, 33	3, 11, 12, 14, 16, 18, 19, 25, 31	1, 2, 5, 7, 8, 15, 17, 21, 24, 26, 27, 29, 32	
	Enterovirus	71	75	77	
	Parechovirus			1, 2, 3	
Encephalitis	Coxsackievirus A		9	2, 4-7, 10, 16	13, 127, 158, 159, 163, 164, 166, 167, 178, 182, 208, 343, 404, 431, 453, 467, 472, 474, 475, 499, 540, 569, 591, 595, 611, 620, 639, 645-648, 656, 660, 679, 694, 695, 722, 725, 773, 794, 796, 835, 882, 917, 1040-1042, 1096
	Coxsackievirus B	5	1, 2, 4	3	
	Echovirus	4, 6, 9, 11, 30	3, 25	1, 2, 5, 7, 8, 12, 13, 14, 15, 17-21, 24, 27, 31, 33	
	Enterovirus	71			
	Parechovirus			1, 2	
Paralysis (lower motor neuron involvement)	Coxsackievirus A		4, 7, 9	2, 5, 6, 10, 11, 14, 21, 24	4, 5, 7, 12, 13, 15, 21, 34, 159, 166, 172, 182, 211, 255, 288, 321, 326, 369, 377, 397, 401, 403, 404, 430, 453, 499, 569, 591, 594, 664-667, 694, 705, 789, 828, 852, 904, 923, 958, 1035, 1037, 1099, 1107
	Coxsackievirus B		2, 3	1, 4-6	
	Echovirus		9, 11, 30	1-4, 6-8, 12, 14, 16-19, 25, 27, 31, 33	
	Enterovirus	70, 71		74, 75	
	Parechovirus			1, 6	
Guillain-Barré syndrome and transverse myelitis	Coxsackievirus A		9	2, 4, 5, 6, 19	13, 51, 67, 69, 89, 265, 321, 362, 390, 485, 507, 519, 568, 627, 694, 716, 904, 977, 978
	Coxsackievirus B			1, 4	
	Echovirus		6	5, 7, 19	
	Enterovirus		70		
	Parechovirus			1	
Cerebellar ataxia	Coxsackievirus A		9	4, 7	77, 316, 404, 414, 499, 569, 677, 685, 749, 1110
	Coxsackievirus B			3, 4	
	Echovirus		6, 9	16	
	Enterovirus		71		
Peripheral neuritis	Coxsackievirus A				923
	Coxsackievirus B				
	Echovirus		9		
Neurologic sequelae and other neurologic illness	Coxsackievirus A		3	9	154, 159, 166, 212, 453, 569, 591, 694, 817, 873, 923, 930, 1039, 1043, 1086
	Coxsackievirus B		3	2, 4	
	Echovirus		9	19, 25, 30, 33	
	Enterovirus	71			

TABLE 165-19 **Coxsackieviruses and Echoviruses Associated with Epidemic Aseptic Meningitis**

Virus Type		Age Group	Common Non-neurologic Findings	References
Coxsackievirus	A7	Children and adults	Fever	398
	A9	Mainly children	Fever, rash, pharyngitis	182, 632
	B1	Mainly children	Fever, pharyngitis	182
	B2	Children and adults	Fever, pharyngitis, rhinitis, abdominal pain, diarrhea	182, 673
	B3	Children and adults	Fever, pharyngitis, conjunctivitis	182
	B4	Children and adults	Fever, pharyngitis, rash, conjunctivitis	182, 317
	B5	Mainly children	Fever, pharyngitis, rash, pleurodynia, abdominal pain, diarrhea, rhinitis, myocarditis	45, 182, 193, 202, 399, 688, 831, 889, 967, 974, 976
Echovirus	3	Children	Fever, rash	429
	4	Mainly children	Fever, pharyngitis, abdominal pain, rash, conjunctivitis	182, 327, 351, 541, 545
	6	Children and adults	Fever, pharyngitis, abdominal pain, pleurodynia, cardiac involvement	182, 420, 546, 572, 608
		Children	Fever, rash	512
	9	Mainly children	Fever, rash, abdominal pain, pharyngitis	182, 257, 325, 342, 385, 428, 511, 536, 607, 664, 770, 838, 888, 897, 950, 964, 1000, 1054, 1062
	11	Mainly children	Fever, upper respiratory illness, pneumonia	703
	13	Mainly children	Fever	144
	16	Children	Fever, rash	414
	18	Children and adults	Fever, rash	558
	19	Mainly children	Fever, upper respiratory illness, rash	229, 383
	25	Children and adults	Fever, rash	65
	30	Children and adults	Fever, pharyngitis, rhinitis, conjunctivitis, rash, abdominal pain	182, 392, 415, 542, 827, 1093
	31	Children and adults	Fever	623
	33	Children and adults	Fever, rash	544, 555
Enterovirus	71	Mainly children	Fever, rash	159, 166, 453, 557, 591, 694

Physical examination reveals a temperature in the range of 38° to 40° C (100.4° to 104° F). Skin rash occurs often and usually is erythematous, maculopapular, and discrete. Frequently, particularly with echovirus 9 infection, the rash is petechial and suggests meningococcemia. Hand-foot-and-mouth disease is a common event in cases of aseptic meningitis caused by EV 71 infection.[159,166,453,591,694] Pharyngitis occurs frequently. Generalized muscle stiffness or spasm usually is observed, although the degree varies considerably; the Kernig and Brudzinski signs are positive in fewer than half of the cases. Deep tendon reflexes usually are normal. In one study, 9 percent of children with enteroviral meningitis had the syndrome of inappropriate secretion of antidiuretic hormone.[175] The onset of this syndrome was noted 36 hours after admission to the hospital, and it usually lasted less than 2 days.

Examination of CSF reveals considerable variation among patients and in the same patient on repeated examination. CSF leukocyte counts vary from a few cells to a few thousand per cubic millimeter; the median is in the range of 100 to 500 cells/mm^3. The percentage of neutrophils also varies greatly. Initial examinations frequently reveal a predominance of neutrophils, but rarely more than 90 percent, as seen in bacterial disease. Usually, a range between 30 and 60 percent neutrophils is found on initial examination. Repeated examinations of CSF demonstrate an increasing percentage of mononuclear cells. Dagan and colleagues[247] observed that the rate of isolation of virus from the CSF in enteroviral meningitis was directly proportional to the number of leukocytes in the fluid. Dalal and associates[252] found that children with echovirus 4 meningitis had elevated values of interleukin (IL)-6 and interferon in the CSF and that the IL-6 value correlated with the CSF leukocyte values.

CSF protein levels usually are elevated mildly, and glucose concentrations usually are normal; hypoglycorrhachia rarely occurs.* Occasionally, the CSF findings suggest tuberculosis meningitis with mononuclear pleocytosis, hypoglycorrhachia, and elevated protein levels.[669] A child with coxsackievirus B4 meningitis had eosinophils in the CSF.[195] The results of other routine laboratory studies such as the WBC count occasionally are abnormal but are not helpful diagnostically.

The duration of illness varies significantly. In most patients, the temperature returns to normal within 4 to 6 days, and disability as a result of neurologic involvement lasts 1 to 2 weeks. Occasionally, the pattern of illness is biphasic; an initial period with fever, headache, nausea, vomiting, and muscle aches and pains of a few days' duration is followed by general recovery and then

*References 38, 196, 297, 609, 628, 673, 933, 941, 945, 969.

a return to the same symptoms in addition to more pronounced neurologic involvement.

Wilfert and associates[1069] performed a longitudinal assessment of children who had enteroviral meningitis early in life and found that receptive language functioning was significantly worse than that of children in a control group without meningitis. In another study, Sells and colleagues[930] examined the long-term effects of CNS enteroviral infection in 19 children. Of this group, 11 were free of detectable abnormalities, 5 had possible defects, and 3 had definite neurologic sequelae. In more recent studies, patients who have had meningitis have performed as well as controls in follow-up developmental evaluations.[80,817,882]

Encephalitis

In the United States, approximately 2500 cases of encephalitis per year, on average, are reported to the CDC.[137] Of this group, only approximately 2 percent of these patients demonstrate an enteroviral origin. However, the seasonal pattern of disease and the absence of arboviral activity in many geographic locations suggest that 500 to 1000 cases of enteroviral encephalitis actually occur each year in the United States. The prevalence of coxsackieviruses and echoviruses as etiologic agents in encephalitis is presented in Table 165-18. Echovirus 9 is the most frequent cause of enteroviral encephalitis. Other enteroviral types commonly associated are echoviruses 4, 6, 11, and 30 and coxsackievirus B5. Echoviruses 4, 6, and 11 have been noted most frequently over a long period.

In general, the prognosis in encephalitis caused by enteroviral infection is good, but fatalities do occur. The viral types that have been isolated from the brain or CSF in fatal cases are coxsackieviruses B3 and B6; echoviruses 2, 9, 17, and 25; and EV 71.[404,569] First noted in Japan in the 1970s were outbreaks of hand-foot-and-mouth disease associated with a diverse array of encephalitic manifestations.[499] Since the mid-1970s, epidemics of hand-foot-and-mouth disease with encephalitis have been occurring in Asian-Pacific countries.*

In an outbreak in Taiwan in 1998, 30/78 (38%) patients with severe illness had encephalitis and 25 of these patients with encephalitis also had pulmonary edema or hemorrhage, 2 had myocarditis, and 1 had acute flaccid paralysis.[453] In another study, 30/34 children with CNS involvement had brain stem encephalitis and 9 of this group (30%) died.[1044] Children who recovered from EV 71 encephalitis associated with cardiopulmonary failure often had neurologic sequelae with delayed neurodevelopment, reduced cognitive function, and attention-deficit/hyperactivity–related problems.[164,835]

Paralysis

Paralysis caused by anterior horn cell disease occasionally results from infection with nonpolio EVs. In contrast to the prevalence of poliovirus, which in the prevaccine era resulted in epidemic paralytic disease, paralysis caused by nonpolio EVs usually is a sporadic event. Coxsackievirus A7 has been associated with outbreaks of paralytic disease on three occasions.[402,40,1038] Many cases of illness similar to poliomyelitis have occurred during outbreaks and epidemics of illness caused by EV 71.[16,256,371,433,502,707,907] Paralytic disease also has been noted during epidemics of acute hemorrhagic conjunctivitis caused by EV 70.[1040,1102]

GUILLAIN-BARRÉ SYNDROME AND TRANSVERSE MYELITIS

As Table 165–17 shows, many coxsackieviruses and echoviruses apparently have been associated with Guillain-Barré syndrome. In general, no specific viral types appear to cause the disease. Rather, the disease occurs sporadically in association with the prevalent enteroviral types.

Other Neurologic Illnesses

Cerebellar ataxia has been associated with coxsackieviruses A4, A7, A9, B3, and B4 and echoviruses 6, 9, and 16 (see Table 165-18). A 16-month-old boy with ataxia was found to have an SAFV in the CSF and in a fecal sample.[763] Scott[923] specifically commented on peripheral neuritis with echovirus 9 infection. Coxsackievirus A9 has been associated with focal encephalitis and acute hemiplegia on two occasions,[154,182] and echovirus 25 infection was noted in a 5-year-old boy with focal encephalitis and subacute hemichorea.[815] Coxsackievirus B4 was isolated from the CSF of a 22-year-old woman with intracranial hypertension,[1086] and postencephalitis Parkinson syndrome occurring after an episode of coxsackievirus B2 meningoencephalitis was described.[1039] Coxsackievirus B3 and EV 71 infections have been associated with opsoclonus-myoclonus.[599] The case of a 4-year-old boy with "Alice in Wonderland" syndrome (complex symptoms of perceptual distortion) associated with coxsackievirus B1 infection was described.[1043]

Phillips and colleagues[824] and Barrett and associates[53] noted the simultaneous occurrence of enteroviral and St. Louis encephalitis viral infection in the same community. In six instances, dual infections occurred, and the afflicted children tended to have more serious illnesses. Ribai and colleagues[863] noted an 18 month-old boy with transient cerebral arteriopathy and an enteroviral infection. The child had aphasia, right hemiplegia, and seizures, which occurred intermittently over a 3-day period.

Ergul and associates[303] described a 7-year-old boy with vestibular neuritis from whom enteroviral RNA was found in both the CSF and a nasopharyngeal sample. Multiple attacks of enteroviral aseptic meningitis in the same individuals have been noted occasionally.[585,750]

Woodall and associates[1084] found conserved enteroviral sequences in spinal cords from subjects with sporadic motor neuron disease and from one patient with possible familial motor neuron disease. Berger and associates[80] noted EV RNA sequences in spinal cord specimens from 13/17 patients with amyotrophic lateral sclerosis, but Walker and colleagues[1038] could not duplicate these findings in a study of 20 spinal cord specimens from similar

*References 127, 157, 158, 163, 165,166, 178, 208, 343, 453, 467, 472, 474, 475, 499, 540, 591, 639, 645-648, 656, 679, 694, 695, 773, 794, 796, 835, 1040-1042, 1096.

patients. Simonsen and coworkers[943] noted an outbreak of vertigo in Wyoming in August of 1992 in which IgM antibody studies suggested an enteroviral origin.

Chronic Fatigue Syndrome and Fibromyalgia

From molecular techniques and antibody prevalence data, chronic enteroviral infections have been suggested to play a role in the chronic fatigue and postviral fatigue syndromes.[25,123,224,737,1103] However, these findings have not been confirmed by other investigators.[702,715,973] More recently, Douche-Aourik and associates[275] found enteroviral RNA in muscle biopsies from 4/30 (13%) patients with fibromyalgia or chronic fatigue syndrome but in no biopsy samples from 29 healthy subjects. However, in none of the 4 positive samples could the presence of VP1 enteroviral capsid protein be demonstrated using a specific monoclonal antibody. In another study, Lane and colleagues[615] found enteroviral RNA in muscle biopsy samples of 10/48 patients (20.8%) with chronic fatigue syndrome and in no samples from 29 controls. These investigators also found that 9 of the 10 subjects with positive muscle biopsies had abnormal lactate responses to exercise in the subanaerobic threshold exercise test. In an editorial relating to these data, Dalakas[249] warned that similar findings in the past were "epiphenomenal" because EVs are ubiquitous in humans and technical flaws inherently connected to contamination in laboratories working with these viruses are inevitable.

The biggest proponents for a causal role of EV in chronic fatigue syndrome have been Chia and Chia.[199,201] Although they have had extensive experience with chronic fatigue syndrome and have conducted several studies, none of their data had been adequately controlled.[589] More recently, they have presented data suggesting that some cases of chronic fatigue syndrome are the result of chronic enteroviral infections of the stomach.[202,203]

Sudden Infant Death

Balduzzi and Greendyke[47] recovered coxsackievirus A5 from the stool of a 1-month-old child who experienced sudden infant death. In a similar investigation of sudden infant death, Gold and associates[382] recovered coxsackievirus A4 from the brains of three babies. Coxsackievirus A8 also was recovered from the stool of a child in whom anorexia was noted on the day before death. Coxsackievirus B3 was recovered at the autopsy of an infant who died suddenly on the eighth day of life.[47] Morens and colleagues[729] noted sudden infant death eight times in association with enteroviral infection; PeV was found on two occasions. In a subgroup of infants with sudden unexplained death in whom the "clinical, biologic, and histologic" findings suggested viral infection, evidence of enteroviral infection was found more frequently than in infants without findings of viral infection.[391] Specifically, enteroviral RNA was detected in the respiratory tract in 54 percent of the viral infection group and in none of the group without findings suggestive of viral infection. These results were supported by IgM antibodies to coxsackieviruses B in 56 percent of the first group and in none of the second group. Four infants with suspected sudden infant death syndrome were found to have coxsackievirus B3 myocarditis with minimal histopathologic changes.[269] In five instances of crib death in one study, echovirus 11 was isolated from the lungs in two cases, from the myocardium in one case, and from the nose or feces in the other two cases.[88] Drexler and associates[280] noted RNA evidence of genotype 2 SAFV in the CSF of an infant with sudden infant death syndrome.

Chronic Enteroviral Infections in Immunocompromised Patients

Patients with cell-mediated and combined immunodeficiencies are susceptible to chronic and often fatal infections with many viruses.[350] Patients with agammaglobulinemia and normal cell-mediated function generally survive infections with these same viruses. EVs are the exception, however, in that chronic, unusual infections with a variety of EVs have been reported.* The most common illness is meningoencephalitis, but arthritis and polymyositis are other frequent findings. Echovirus 11 has been the most common cause of chronic infection, but the following other EVs also have been causative: echoviruses 2, 3, 5, 6, 7, 9, 13, 14, 15, 17, 18, 19, 21, 24, 25, 26, 29, 30, and 33; coxsackieviruses A11, A15, B1, B2, and B3; and PeV 1.[687] EVs have been recovered from many other body sites, such as the liver, heart, lung, pancreas, lymph nodes, bone marrow, muscle, throat, and stool, in addition to the CSF.

Several patients with X-linked agammaglobulinemia have had polymyositis-like or dermatomyositis-like syndromes caused by echovirus infections; the following echoviruses have been implicated: types 2, 3, 5, 9, 11, 17, 19, 24, 25, 30, and 33.[52,240,698] The case of a 15-year-old boy with X-linked agammaglobulinemia and chronic arthritis caused by echovirus 11 was reported.[9] Three children with X-linked hyper-IgM syndrome and persistent enteroviral meningoencephalitis were described.[243] Persistent enteroviral infections of the CNS were reported in pediatric and adult patients infected with HIV.[290,803] In addition, enteroviral infections caused deaths in bone marrow transplant recipients.[24,348]

Congenital Infections

Abortion

Landsman and associates[612] studied 2631 pregnancies during an epidemic of echovirus 9 and could find no difference in antibody to echovirus 9 in women who aborted and in those who delivered term infants. A similar study in Finland revealed no increase in the abortion rate in women infected in early pregnancy with echovirus 9.[851] Although coxsackieviral infections occur commonly, epidemics with specific viral types involving large populations have not been studied.

Two women with coxsackievirus A16 hand-foot-and-mouth disease had spontaneous abortions.[791] In one instance, coxsackievirus A16 was recovered from the

*References 9, 87, 93, 231, 240, 243, 286, 289, 304, 412, 416, 442, 547, 687, 885, 1023, 1053, 1055, 1068.

products of conception. Frisk and Diderholm[338] found that 33 percent of women with abortions had IgM antibody to coxsackieviruses B, whereas only 8 percent of controls had similar antibody. In a second, larger study, the same research group confirmed their original findings.[39]

Ljungan virus (a PeV) is endemic in some rodent populations, and it can cause fetal death in these animals. Krous and Langlois[598] and Samsioe and colleagues[903] suggest that this virus may be the cause of some intrauterine deaths in humans. In one study, both PCR and immunohistochemical staining identified Ljungan virus in specimens from five human intrauterine fetal deaths.[903]

Congenital Malformations

In a large prospective study, Brown and Karunas[110] made a serologic search for selected maternal enteroviral infections in association with congenital malformations. Sera from 630 mothers of infants with anomalies and from 1164 mothers of children without defects were studied carefully. Specifically, serologic evidence of infection with coxsackieviruses B1, B2, B3, B4, B5, and A9 and with echoviruses 6 and 9 was sought during the first trimester and during the last 6 months of pregnancy. In this study, infants were examined for 113 specific abnormalities; these anomalies were grouped into 12 categories for analysis. The investigators demonstrated a positive correlation between maternal infection and infant anomaly with coxsackieviruses B2, B3, B4, and A9. The overall anomaly rate associated with first-trimester infection with coxsackievirus B4 was significantly higher than that in controls. Maternal coxsackievirus B2 infection throughout pregnancy, coxsackievirus B4 infection during the first trimester of pregnancy, and infection with at least one of the five group B coxsackieviruses during pregnancy were associated with urogenital anomalies in contrast to controls. Coxsackievirus A9 infection was associated with digestive anomalies and coxsackieviruses B3 and B4 with cardiovascular defects. When coxsackieviruses B were analyzed as a group (B1 to B5), an overall association with congenital heart disease was found; the likelihood of having cardiovascular anomalies was increased when maternal infection with two or more coxsackieviruses B occurred.

Gauntt and colleagues[360] found that ventricular fluid from 4/28 babies with severe anatomic defects contained neutralizing antibody to one or more coxsackievirus B types. In one case, specific IgM antibody to coxsackievirus B6 was demonstrated.

In a serologic study in Scotland, Ross and colleagues[878] found no association between maternal coxsackievirus B infection and fetal developmental anomalies. Elizan and associates[299] were unable to find any relationship between maternal infection with coxsackieviruses B and congenital CNS malformations. In three studies, no association between maternal echovirus 9 infection and congenital malformation was noted.[584,612,851]

Niklasson and associates[768] studied CNS malformations in terminated pregnancies. PeV (Ljungan virus) was diagnosed in 9/10 cases of hydrocephalus and in 5/9 cases of anencephaly, whereas this virus was found in only 1/18 trisomy 21 controls.

Prematurity and Stillbirth

Bates[57] reported an 8-month-old stillborn fetus with calcific pancarditis and hydrops fetalis at autopsy. Fluorescent antibody study revealed coxsackievirus B3 antigen in the myocardium. Burch and colleagues[116] reported three stillborn infants who had fluorescent antibody evidence of coxsackievirus B myocarditis, one each with coxsackieviruses B2, B3, and B4. These investigators also described a premature boy who had histologic and immunofluorescent evidence of cardiac infection with coxsackieviruses B2, B3, and B4; he lived only 24 hours.

Freedman[336] reported the occurrence of a full-term stillbirth in a woman infected with echovirus 11. Because the baby had no pathologic or virologic evidence of infection, Freedman attributed the event to a secondary consequence of maternal infection caused by fever and dehydration rather than primary transplacental infection. In another stillbirth in which echovirus 11 was recovered from amniotic fluid, the fetus was found to have evidence of focal encephalitis, massive adrenal hemorrhage, and diffuse subarachnoid hemorrhage.[948] Echovirus 27 was recovered from amniotic fluid in an intrauterine fetal death at 28 weeks of gestation.[762] A 26-week, 1300-g stillborn fetus with hydrocephalus, fibrotic peritonitis, and hepatosplenomegaly was found by PCR and immunohistochemical study to have an EV 71 infection.[213] A baby of 26 weeks' gestation with nonimmune hydrops fetalis and an intrauterine infection with coxsackievirus B3 was reported by Ouellet and coworkers.[799]

Neonatal Infections

Epidemiology and Pathogenesis

Neonatal infection with EVs and PeVs can result from transplacental viral transmission, contact infection during birth, and human-to-human contact after birth. Transplacental passage of coxsackieviruses and echoviruses at term has been noted on many occasions. Benirschke[71] studied the placentas in three cases of congenital coxsackievirus B disease and could find no histologic evidence of infection. In 1956, Kibrick and Benirschke[570] reported the first case of intrauterine infection with coxsackievirus B3. In this instance, the infant was delivered by cesarean section and became symptomatic several hours after birth. Brightman and associates[106] recovered coxsackievirus B5 from the placenta and rectum of a premature infant. No histologic abnormalities of the placenta were noted.

Berkovich and Smithwick[84] noted an asymptomatic neonate who had specific IgM PeV 1 antibody in cord blood, thus suggesting intrauterine infection with this virus. Hughes and colleagues[481] reported a newborn infant with echovirus 14 infection who had markedly elevated IgM (190 mg/dL) on the sixth day of life. This child probably also was infected in utero. In addition, echovirus 19 has been noted in a transplacentally acquired infection.[821] Other evidence of intrauterine infection has

been presented for coxsackieviruses A4, B1, B2, B3, B4, and B5 and echoviruses 9, 11, and 19.*

Little definitive evidence exists for either ascending infection or contact infection with coxsackieviruses or echoviruses during birth. However, transmission of infection during the birth process seems probable.[179,190,543] The fecal carriage rate of EVs in asymptomatic adult patients varies between 0 and 6 percent or higher in different population groups.[182] Cherry and colleagues[190] reported that in 2/55 mothers (4%), EVs were present in feces shortly after delivery.

Coxsackievirus B5 was recovered from the cervices of four women with febrile illnesses during the third trimester of pregnancy.[860] Echovirus 11 was isolated from the cervix of a mother whose baby became ill on the third day of life with fatal echovirus 11 necrotizing hepatitis.[859] Chang and colleagues[168] detected coxsackievirus B3 in breast milk of two symptomatic mothers, and their babies both suffered severe illnesses with hepatic necrosis and meningitis caused by coxsackievirus B3.

Several epidemics with coxsackieviruses and echoviruses in newborn nurseries have been studied.[106,128,712,745] Brightman and associates[106] observed an epidemic of coxsackievirus B5 in a premature nursery. Their data suggested that the virus was introduced into this nursery by an asymptomatic infant who had been infected in utero. Secondary infections occurred in 12 babies and 2 nurses. The timing of the secondary cases suggested that three generations had occurred and that the nurses had been infected during the second generation. The investigators suggested that the infection had spread from infant to infant and from infant to nurse.

Javett and associates[514] reported an acute epidemic of myocarditis associated with coxsackievirus B3 infection in a Johannesburg maternity home. Unfortunately, no epidemiologic investigation or search for asymptomatic infected infants was performed. However, in analyzing the dates of onset of the illnesses, single infections apparently occurred for five generations, and then five children became ill within a 3-day period.

Kipps and colleagues[579] carried out epidemiologic investigations in two coxsackievirus B3 nursery epidemics. In the first epidemic, the initial infection probably was transmitted from a mother to her baby; this baby then was the source of five secondary cases in newborn infants and one illness in a nurse. Four of the five secondary cases were located on one side of the nursery, but only one crib was close to that of the index baby, and this crib did not adjoin the cribs of the three other contact cases. In the second outbreak, a baby who also was infected by his mother probably introduced the virus into the nursery. The three secondary cases were geographically far removed from the primary case.

Cramblett and colleagues[235] reported an outbreak of echovirus 11 disease in four infants in an intensive care nursery. All infants were in enclosed incubators, and three patients became ill within 24 hours; the fourth child became ill 4 days later. Echovirus 11 was recovered from two members of the nursery staff. These data suggest that transmission from personnel to infants occurred because of inadequate washing of hands.

In another outbreak in an intensive care unit, the initial patient was transferred to the nursery because of severe echovirus 11 disease.[745] After transfer, the senior house officer and a psychologist in the unit were infected. The investigators inferred that these infected personnel spread the disease by respiratory droplet to nine other babies. In another nursery outbreak of echovirus 11, Mertens and colleagues[712] found that the infection spread through close contact between infected newborns and the nurses in the unit. Spread of infection was interrupted with the installation of vigorous hygienic and isolation measures. In an outbreak of echovirus 11 in an intermediate care unit, Kinney and colleagues[578] found that gavage feeding, mouth care, and being a twin were risk factors for acquiring illness.

Many other instances of isolated nursery infections and small outbreaks with coxsackieviruses and echoviruses have been reported. The most consistent source of original nursery infection seems to be transmission from a mother to her baby,* but virus can be introduced into the nursery by hospital personnel.[496,617,974]

In a longitudinal study of neonatal enteroviral infections carried out during the summer and fall of 1981, Jenista andassociates[516] found that the nonpolio enteroviral infection rate was 12.8 percent. Lower socioeconomic status and lack of breast-feeding were found to be risk factors for acquiring infection. Nonpolio enteroviral infections were determined to be a significant cause for readmission of the cohort neonates to the hospital. During a community outbreak of echovirus 11 disease, Modlin[721] found that passive transplacental passage of antibody to neonates prevented the development of severe disease but not mucosal infection.

Clinical Manifestations

Enteroviral and parechoviral infections in neonates result in a wide variety of clinical manifestations ranging from asymptomatic infection to fatal encephalitis and myocarditis.[183] An overview by illness category and prevalence is presented in Table 165-20.

Inapparent Infection. Although inapparent infection probably occurs occasionally with many different EVs, little documentation of this assumption exists. Cherry and colleagues[190] studied 590 normal neonates during a 6-month period and noted only a single asymptomatic infection. This child was infected in utero or immediately thereafter with coxsackievirus B2. The mother had an upper respiratory tract illness 10 days before delivery.

During a survey of perinatal viral infections, 44 babies were found to be infected with PeV 1 in the study period May to December of 1966.[506] The prevalence of virus and the incidence of new infections during this period were fairly uniform. No illness was attributed to PeV 1 infection, and the virus disappeared from the nursery in mid-December. Asymptomatic infections with PeV 1 were

*References 57, 72, 97, 182, 190, 306, 421, 530, 543, 668, 799, 821, 992.

*References 28, 55, 173, 177, 183, 196, 229, 256, 297, 309, 414, 497, 609, 650, 672, 721, 745, 842, 974, 983.

TABLE 165-20 **Major Manifestations of Neonatal Nonpolio Enteroviral and Parechoviral Infections**

Specific Involvement	Common	Rare	References
Inapparent infection		Cox A9, B1, B2, B4, B5	84, 182, 190, 296, 310, 368, 505, 737, 754
	Echo 22	Echo 3, 5, 9, 11, 14, 20, 30, 31	
Mild, nonspecific, febrile illness	Cox B5	Cox B1-B4, A9, A16	26, 55, 75, 82, 99, 183, 190, 256, 297, 368, 425, 508, 544, 650, 724, 745, 939, 1013
	Echo 5, 11, 33	Echo 4, 7, 9, 17, 30	
		PeV 4	
Sepsis-like illness	Cox B2-B5	Cox B1, A9	6, 27, 76, 87, 95, 97, 176, 182, 183, 206, 229, 235, 256, 311, 382, 413, 530, 543, 609, 650, 673, 712, 720, 736, 745, 821, 859, 906, 910, 961, 974, 1026, 1088
	Echo 5, 11, 16	Echo 2-4, 6, 9, 14,19, 21	
		PeV 1, 2, 3	
Respiratory illness (general)		Cox B1, B4, B5, A9	190, 439, 481, 505
	Echo 11	Echo 9, 17	
	PeV 1, 3		
Herpangina		Cox A5	173
Coryza		Cox A9	82, 237, 439, 752, 917
		Echo 11, 17, 19	
		PeV 1	
Pharyngitis		Cox B4	82, 439, 699, 906, 939
		Echo 11, 17, 18	
Laryngotracheitis or bronchitis		Cox B1, B4	292, 439, 729
		Echo 11	
Pneumonia		Cox B4, A9	84, 182, 206, 256, 311, 439, 505, 713, 998, 1071, 1078
		Echo 9, 11, 17, 31	
		PeV 1	
Cloud baby		Echo 20	296
Gastrointestinal			
Vomiting or diarrhea		Cox B1, B2, B5	82, 182, 229, 296, 295, 311, 368, 423, 439, 505, 650, 713, 847, 906, 974
	Echo 5, 17, 18	Echo 4, 6, 8, 9, 11, 16, 19, 21	
		PeV 1	
Hepatitis		Cox A9, B1, B3, B4	168, 182, 183, 467, 530, 569, 720, 736, 1088, 1097
	Echo 11, 19	Echo 6, 9, 14, 21	
Pancreatitis		Cox B3, B4, B5	588, 1078
Necrotizing enterocolitis		Cox B2, B3	609
Cardiovascular			
Myocarditis and pericarditis	Cox B1-B4	Cox B5, A9	54, 116, 182, 296, 312, 362, 413, 466, 486, 497, 505, 514, 543, 568-570
		Echo 11, 19	571, 579, 724, 727, 980, 1013, 1088
Skin	Cox B5	Cox B1, 3	84, 99, 182, 187, 193, 235, 246, 367, 414, 543, 650, 699, 713, 772, 906, 991
	Echo 5, 17	Echo 4, 7, 9, 11, 16, 18	
	PeV 1, 3		
Neurologic			
Aseptic meningitis	Cox B2-B5	Cox A9, A14, B1	84, 100, 182, 183, 193, 235, 273, 296, 314, 413, 421, 428-430, 524, 543, 673, 712, 713, 772, 916, 917, 921, 961, 974
	Echo 3, 9, 11, 17	Echo 1, 14, 21, 30	
		EV 71	
Encephalitis	Cox B1-B4	Cox B5	76, 196, 309, 310, 428, 910, 922, 1088
		Echo 9	
		PeV 2, 3	
Paralysis		Cox B2	524
Sudden infant death		Cox B3, A4, A5, A8	47, 382, 729
		PeV 1	

Cox, Coxsackievirus; *echo,* echovirus; *EV,* enterovirus; *PeV,* parechovirus.

noted on two other occasions.[84,753] Infections without evidence of illness also have been noted with coxsackieviruses A9, B1, B4, and B5 and echoviruses 3, 5, 9, 11, 13, 14, 20, 30, and 31.*

Mild, Nonspecific Febrile Illness. In a review of 338 enteroviral infections in early infancy, 9 percent were classified as nonspecific febrile illnesses.[728] Illness may be sporadic or part of an outbreak with a specific viral type. When the illness is part of an outbreak, the clinical manifestations vary, depending on the viral type; some infants have aseptic meningitis and other signs and symptoms, whereas others simply have nonspecific fever. Specific viruses related to nonspecific fever are listed in Table 165-20. Although by definition illness in this category is mild, awareness that viral infection may be extensive is important. When sought, virus may be isolated from the blood, urine, and CSF of infants with mild illnesses.[55,510] In an outbreak of relatively benign illness caused by echovirus 7 in six neonates, all the infants were found to have high C-reactive protein values.[246]

Sepsis-Like Illness. The main diagnostic problem in neonatal enteroviral and parechoviral infections is differentiation of bacterial from viral disease. Even in an infant with mild, nonspecific fever, bacterial disease must be considered strongly. The sepsis-like illness described here always is alarming. Illness is characterized by fever, poor feeding, abdominal distention, irritability, rash, lethargy, and hypotonia.† Other findings include diarrhea, vomiting, seizures, and apnea. In severe, frequently fatal illnesses, most often caused by echovirus 11 infection, jaundice, hepatitis, disseminated intravascular coagulation (DIC), thrombocytopenia, and hypotension occur.[530,720,736,859]

Sepsis-like illness is a common occurrence. Morens[728] noted its presence in one fifth of 338 enteroviral infections in infants. In an attempt to differentiate bacterial from viral disease, Lake and associates[609] studied 27 infants with enteroviral infection. WBC counts were not helpful because the total count, the number of neutrophils, and the number of band-form neutrophils were elevated in most cases. Of most importance were historical data. Most mothers had suffered a recent febrile, viral-like illness. In addition, other factors often associated with bacterial sepsis, such as prolonged rupture of membranes, prematurity, and low Apgar scores, were unusual findings in the enteroviral infection group. Bone marrow failure developed in a newborn baby boy with a sepsis-like illness caused by echovirus 11 infection.[985] The neutropenia resolved spontaneously, and the thrombocytopenia normalized after treatment with IVIG.

Abzug[5] reviewed the prognosis in 16 neonates who had a sepsis-like illness with hepatitis and coagulopathy. The case-fatality rate was 31 percent. In addition to having hepatitis and coagulopathy, the 5 patients with fatal cases had myocarditis and 3 patients had encephalitis. The follow-up of 6 survivors noted normalization of liver function and platelet counts and the absence of subsequent significant medical problems.

Respiratory Illness. Respiratory complaints generally are overshadowed by other manifestations of neonatal enteroviral disease. Only 7 percent of 338 enteroviral infections in early infancy were classified as respiratory illness in one study.[728] Herpangina has been observed and photographed only once; Chawareewong and associates[173] noted several infants with herpangina and coxsackievirus A5 infection.

Hercík and colleagues[439] reported an epidemic of respiratory illness in 22 neonates associated with echovirus 11 infection. All of these infants had rhinitis and pharyngitis, 50 percent had laryngitis, and 32 percent had interstitial pneumonitis. Berkovich and Pangan[83] studied respiratory illnesses in premature infants and reported 64 babies with illness, 18 of whom had virologic or serologic evidence of PeV 1 infection. In addition, many had high but constant levels of serum antibody to PeV 1. Some of the infants with high antibody levels probably also were infected with PeV 1. The children with proved PeV 1 infection could not be differentiated clinically from those without evidence of such infection. Ninety percent of the infants had coryza, and 39 percent had radiographic evidence of pneumonia.

Except for echoviruses 11 and PeV 1, respiratory illness associated with EVs has occurred sporadically. The following other viruses have been noted: coxsackieviruses A5, A9, B1, B4, and B5 and echoviruses 9, 17, 18, 19, 20, 22, and 31. In the review by Morens,[728] only 7/338 enteroviral infections of infancy were classified as pneumonia. A newborn with an echovirus 11 infection developed meningitis, DIC, and persistent pulmonary hypertension.[1071] Postmortem examination revealed pneumonia, hyaline membranes, pulmonary interstitial emphysema, meningitis, and DIC.

Eichenwald and Kostevalov[295] recovered echovirus 20 from four full-term infants younger than 8 days of age. Although these infants were asymptomatic, they were found to be colonized extensively with staphylococci, and they disseminated these organisms into the air around them. Because of this ability to disseminate staphylococci, they were called *cloud babies*. The investigators thought these cloud babies contributed to the epidemic spread of staphylococci in the nursery. Because active staphylococcal dissemination occurred only when echovirus 20 could be recovered from the nasopharynx, viral–bacterial synergistic activity was thought to be present.

Gastrointestinal Manifestations. Significant gastrointestinal illness occurs in approximately 7 percent of enteroviral infections of infancy.[728] Vomiting and diarrhea occur commonly but usually are only part of the overall illness complex and not the major manifestations. In 1958, Eichenwald and associates[296] described epidemic diarrhea associated with echovirus 18 infection. In a nursery unit of premature infants, 12/21 babies were mildly ill. Neither temperature elevation nor hypothermia occurred. Six infants were lethargic and listless, and moderate abdominal distention developed in 2 infants. The diarrhea lasted from 1 to 5 days; these infants had

*References 183, 182, 296, 310, 368, 496, 505, 516, 617, 735, 974.
†References 50, 414, 426, 609, 825, 931, 1019, 1080, 1087.

five or six watery, greenish stools per day, occasionally expelled explosively. Two infants had a small amount of blood, but no mucus or pus cells, noted in their stools. Five babies in another nursery also had similar diarrheal illness. Echovirus 18 was recovered from all ill infants.

In 22 infants with epidemic respiratory disease caused by echovirus 11, all had vomiting as a manifestation of the illness.[439] Linnemann and colleagues[650] reported vomiting in 36 percent and diarrhea in 7 percent of neonates with echoviral infection. In another study, Lake and associates[609] found diarrhea in 81 percent and vomiting in 33 percent of neonates with nonpolio enteroviral infections.

Hepatitis is an important neonatal nonpolio enteroviral illness. Morens[728] reported that 2 percent of neonates with clinically severe enteroviral disease had hepatitis. Lake and colleagues[609] observed that hepatomegaly was present in 37 percent of neonates with enteroviral infection, and hepatosplenomegaly was observed by Hercík and associates[439] in 12/22 newborns with echovirus 11 respiratory illness.

Severe hepatitis, frequently with hepatic necrosis, has been noted with echoviruses 6, 9, 11, 14, 19, 21, and 30.* Echovirus 11 most often has been associated with severe and usually fatal hepatitis; findings include DIC and thrombocytopenia, as well as apnea, lethargy, poor feeding, and jaundice.

Philip and Larson[821] reported three catastrophic neonatal echovirus 19 infections that resulted in hepatic necrosis and massive terminal hemorrhage. One infant, infected in utero, was symptomatic at birth. The Apgar score was 3, and multiple petechiae were observed. Generalized ecchymoses and apneic episodes occurred, and the infant died at 3.5 hours of age. Thrombocytopenia was noted, and echovirus 19 was isolated from the brain, liver, spleen, and lymph nodes. The other two infants who died of echovirus 19 infection were twins. They were normal during the first 3 days of life but then became mildly cyanotic and lethargic. Shortly thereafter, apneic episodes occurred, and jaundice and petechiae developed. Both twins became oliguric, and they died on the eighth and ninth days of life with severe, terminal gastrointestinal bleeding. Both twins were thrombocytopenic, and virus was recovered from systemic sites in both.

Pancreatitis was found in three of four newborns with coxsackievirus B5 meningitis[588] and in a coxsackievirus B4 infection at autopsy.[1079] In other fatal coxsackievirus B infections, pancreatic involvement has been detected, but clinical manifestations rarely have been observed.

Lake and associates[609] reported three infants with necrotizing enterocolitis. Coxsackievirus B3 was recovered from two of these infants, and coxsackievirus B2 was recovered from the third.

Cardiovascular Manifestations. In contrast to enteroviral cardiac disease in children and adults, in which pericarditis is a common finding, neonatal disease almost always involves the heart muscle. Most cases of neonatal myocarditis are caused by coxsackievirus B infection, and nursery outbreaks have occurred on several occasions. In 1961, Kibrick[568] reviewed the clinical findings in 45 cases of neonatal myocarditis; his findings are summarized in Table 165-21. Of interest is that many of the early experiences, particularly in South Africa, involved catastrophic nursery epidemics. Since the observation in 1972 of five newborns with echovirus 11 infection and myocarditis, no other nursery epidemics have been reported.[278]

TABLE 165-21 **Signs and Symptoms of Neonatal Coxsackievirus B Myocarditis**

Category	Frequency (%)
Feeding difficulty	84
Listlessness	81
Cardiac signs	81
Respiratory distress	75
Cyanosis	72
Fever	70
Pharyngitis	64
Hepatosplenomegaly	53
Biphasic course	35
Central nervous system signs	27
Hemorrhage	13
Jaundice	13
Diarrhea	8

Modified from Kibrick S. Viral infections of the fetus and newborn. *Perspect Virol* 1961;**2**:140-159.

The illness caused by coxsackieviruses B most commonly was abrupt in onset, with symptoms of listlessness, anorexia, and fever. A biphasic pattern was noted in approximately one third of the patients. Progression was rapid, and signs of circulatory failure appeared in a 2-day period. If death did not occur, recovery occasionally was rapid but usually took place gradually over an extended period. Most patients had cardiac findings such as tachycardia, cardiomegaly, electrocardiographic changes, and transitory systolic murmurs. Many patients showed signs of respiratory distress and cyanosis. Approximately one third of the infants had signs suggesting neurologic involvement. Of the 45 cases analyzed by Kibrick,[568] only 12 patients survived.

In an echovirus 11 nursery outbreak reported by Drew,[278] 5/10 babies had tachycardia out of proportion to their fever. Three of these babies had electrocardiograms; supraventricular tachycardia was noted in all, and ST-segment depression was observed in 2 patients' records. Supraventricular tachycardia also has occurred with coxsackievirus B infection.[505] Echovirus 19 has been associated with myocarditis, and coxsackievirus A9 was noted in a child with pericarditis.[237,980]

In the 1970s, neonatal myocarditis caused by EVs was seen less commonly than it was in the 1950s and early 1960s. In his review, Morens[728] noted only two instances among 248 severe neonatal enteroviral illnesses. Chan and Lun[456] reported a neonate with coxsackievirus B4 myocarditis who developed a ventricular aneurysm.

*References 82, 97, 183, 182, 413, 481, 530, 720, 736, 821, 826.

Exanthem. Exanthem as a manifestation of neonatal enteroviral infection has been reported with coxsackieviruses B1, B3, and B5; with echoviruses 4, 5, 7, 9, 11, 16, 17, and 18; and with PeV 1, 3, 4, 5, and 6. In most instances, rash is just a minor manifestation of severe neonatal disease. In 27 infants studied by Lake and colleagues,[609] 41 percent had exanthem. Cutaneous manifestations generally commence between the third and fifth days of illness. The rash usually is macular or maculopapular. Petechial lesions are noted occasionally. Surprisingly, vesicular lesions have not been described, nor has any rash illness in neonates been associated with coxsackievirus A16. Hall and associates[414] reported two neonates with echovirus 16 infections in which the illnesses resembled roseola. These patients had fever for 2 and 3 days, defervescence, and then the appearance of a maculopapular rash.

A newborn with a vesiculopapular-crusted rash on the face, trunk, and extremities caused by coxsackievirus B3 was reported by Theodoridou and associates.[991] It was a congenital infection in which new lesions appeared over the course of a 5-day period, and the total duration of the rash was 10 days.

Neurologic Manifestations. As noted in Table 165-20, neurologic illness has been associated with coxsackieviruses B1, B2, B3, B4, and B5, many echoviruses and PeVs. In neonates, differentiating meningitis from meningoencephalitis usually is difficult. Meningoencephalitis occurs commonly in infants with sepsis-like illness, and postmortem studies revealed many infants with disseminated viral disease (heart, liver, adrenal glands) in addition to CNS involvement. In Morens' review,[728] 50 percent of the patients with enteroviral infection who were analyzed had encephalitis or meningitis.

The initial clinical findings in neonatal meningitis or meningoencephalitis are similar to those in nonspecific febrile illness or sepsis-like illness. Most often, the child is normal and then becomes febrile, anorectic, and lethargic. Jaundice frequently is noted in newborns, and vomiting occurs in neonates of all ages. Less common findings include apnea, tremulousness, and general increased tonicity. Seizures occur occasionally. Extensive white matter injury has been noted in neonatal PeV encephalitis.[1027] This finding can be confused with white matter changes associated with hypoxic-ischemic injuries.

Examination of CSF reveals considerable variation in protein, glucose, and cellular values. In seven newborns with meningitis caused by coxsackievirus B5 studied by Swender and colleagues,[974] the mean CSF protein value was 244 mg/dL, and the highest value was 480 mg/dL. The mean CSF glucose value was 57 mg/dL, and one of the seven infants had pronounced hypoglycorrhachia (a value of 12 mg/dL). The mean CSF leukocyte count in the seven babies was 1069 cells/mm^3, with 67 percent polymorphonuclear cells. The highest cell count was 4526 cells/mm^3, with 85 percent polymorphonuclear cells. In another study involving 28 children younger than 2 months of age in which coxsackievirus B5 was the implicated pathogen, 36 percent of the infants had CSF leukocyte counts of 500 cells/mm^3 or greater.[673] In this same study, only 13 percent of the infants had CSF protein values of 120 mg/dL or greater; 12 percent of the infants had glucose values lower than 40 mg/dL.

In summary, the CSF findings in neonatal nonpolio enteroviral and parechoviral infections frequently are similar to those in bacterial disease. In particular, the most consistent finding in bacterial disease, hypoglycorrhachia, is noted in approximately 10 percent of newborns with enteroviral meningitis.[196,297,609,673,974]

Johnson and associates[525] reported a 1-month-old boy with right-sided facial paralysis and loss of abdominal reflexes. The facial paralysis persisted through convalescence; the reflexes returned to normal within 2 weeks. The boy was infected with coxsackievirus B2.

Euscher and associates[306] detected coxsackievirus RNA in placental tissue from six of seven newborn infants with respiratory difficulties and other manifestations at birth. Of these infants, one died shortly after birth, and the other six suffered neurodevelopmental delays.

CLINICAL MANIFESTATIONS: POLIOVIRUSES

When a susceptible person has had effective contact with a poliovirus, one of several responses may occur, in the following order of frequency: (1) inapparent infection, (2) minor illness (abortive poliomyelitis), (3) nonparalytic poliomyelitis (aseptic meningitis), and (4) paralytic poliomyelitis.* Paralytic poliomyelitis is the most dramatic expression of the infection and the only one clinically recognizable as caused by a poliovirus; it accounts for not more than 1 to 2 percent of infections during epidemics and considerably less under endemic conditions (see Fig. 165-5). The aseptic meningitis syndrome is similarly infrequent; nonspecific "minor illness" is estimated to occur in 4 to 8 percent, and 90 to 95 percent of those infected have inapparent infections. Factors that determine the type of clinical response are poorly understood, but the degree of virulence of the virus and certain host characteristics are important.

Age has a significant effect on patterns of infection; older patients are more likely to have severe paralytic disease and a higher mortality rate. Pregnancy increases the risk, probably primarily because of hormonal factors but also because pregnant women may be exposed more to young children, who are the main sources of contagion. Tonsillectomy in the presence of inapparent infection can precipitate bulbar poliomyelitis; evidence also suggests that tonsillectomy at any time in the past results in enhanced susceptibility to the bulbar form of the disease. Recent diphtheria-tetanus-pertussis vaccination increases the likelihood of development of paralysis; the site of injection and the site of paralysis appear to be correlated. Physical exertion and trauma around the time of onset also increase the risk for severe paralysis, especially in adults.

*References 16, 94, 181, 183, 217, 469, 1100.

Minor Illness (Abortive Poliomyelitis)

The minor illness is mild and nonspecific, with low-grade fever, malaise, anorexia, and sore throat. Physical examination reveals no significant abnormalities, CSF is normal, and recovery occurs within 24 to 72 hours. The illness often is so mild that it goes unrecognized, and patients rarely are seen by a physician.

Nonparalytic Poliomyelitis (Aseptic Meningitis)

The onset of nonparalytic poliomyelitis is associated with vague malaise followed by fever, headache, aching of the muscles, and sometimes hyperesthesia and paresthesia. Anorexia, nausea, vomiting, constipation, or diarrhea also may be present. The temperature rises to 37.8° to 39.5° C (101° to 103° F); stiffness of the neck, back, and hamstrings soon appears.

Approximately two thirds of affected children have a short, symptom-free interlude between the first phase (minor illness) and the second phase (CNS or major illness). This two-phase course occurs less commonly in adults, in whom the evolution of symptoms is more insidious. Nuchal and spinal rigidity is necessary for establishing the diagnosis of nonparalytic poliomyelitis during the second phase.

Physical examination reveals nuchal-spinal signs and changes in superficial and deep reflexes. With cooperative patients, the nuchal-spinal signs are sought first by active tests. The child is asked to sit up unassisted. If doing so causes undue effort, if the knees flex upward, and if the patient writhes a bit from side to side while sitting up and uses the hands on the bed for the tripod supporting position, unmistakable spinal rigidity is present. Still sitting, the patient is asked to flex chin to chest and is observed for nuchal rigidity. Alternatively, from the supine position with the knees held down gently, the patient is asked to sit up and kiss the knees. If the knees draw up sharply or if the maneuver cannot be completed adequately, the patient has stiffness of the spine caused by muscle spasm. If the diagnosis still is uncertain, attempts should be made to elicit the Kernig and Brudzinski signs. Gentle forward flexion of the occiput and neck elicits nuchal rigidity, which may precede spinal rigidity. Head drop may be demonstrated by placing the hands under the patient's shoulders and raising the trunk. Normally, the head follows the plane of the trunk, but in poliomyelitis, it often falls backward limply. The frequency of the head-drop sign, even in nonparalytic poliomyelitis, with no subsequent residuals indicates that it is not caused by true paresis of the neck flexors. In struggling infants, distinguishing voluntary resistance from clinically important involuntary nuchal rigidity may be difficult. One may place the infant's shoulders flush with the edge of the table, support the weight of the occiput in the hand, and then flex the head anteriorly. Nuchal rigidity that persists during this maneuver may be interpreted as involuntary. When not closed, the anterior fontanelle also may be tense or bulging.

In the early stages, the reflexes are normally active and remain so unless paralysis supervenes. Changes in reflexes, either increased or depressed, may precede the onset of weakness by 12 to 24 hours; hence, detecting such changes is important, especially in nonparalytic patients managed at home. The superficial reflexes (i.e., cremasteric and abdominal and the reflexes of the spinal and gluteal muscles) are usually the first to be diminished. The spinal and gluteal reflexes are elicited by tapping segmentally downward on each side of the spine and buttocks. These reflexes may disappear before the abdominal and cremasteric ones. Changes in the deep tendon reflexes, whether exaggerated or depressed, generally occur 8 to 24 hours after depression of the superficial reflexes and indicate impending paresis of the extremities.

Laboratory findings consist of a normal or slightly elevated WBC count and the characteristic CSF changes of aseptic meningitis—approximately 20 to 300 cells, predominantly lymphocytes, a normal glucose level, and a normal or slightly elevated protein level. If a spinal tap is performed in the first few hours after onset, a predominance of polymorphonuclear leukocytes may be seen, but it shifts in 6 to 12 hours to more than 90 percent lymphocytes. If no further progression of clinical signs occurs, the disease remains nonparalytic, the temperature falls to normal, and signs of meningeal irritation gradually disappear. Recovery ensues in 3 to 10 days, depending on the severity of the illness.

Paralytic Poliomyelitis

The manifestations of paralytic poliomyelitis are those enumerated earlier for nonparalytic poliomyelitis in addition to weakness in one or more muscle groups, either skeletal or cranial. Patients in whom paralysis is destined to develop often wear an anxious expression; they are extremely alert, restless, and flushed and appear acutely ill. The fever is higher than that in abortive disease, and the patient may have intense muscle pain. Shortly before actual muscle weakness is detected, the superficial and deep reflexes often diminish or disappear on the affected side. Frequently, a symptom-free interlude of several days occurs between the initial illness phase and the recurrence of symptoms that culminate in paralysis.

The onset of paralysis may be extraordinarily sudden and may progress in a few hours to complete loss of motion in one or more extremities. Asymmetric involvement is typical in milder cases. More gradual spread of weakness also occurs and may continue over a period of 3 to 5 days. Bladder paralysis of 1 to 3 days' duration develops in approximately 20 percent of patients, and bowel atony frequently is noted, occasionally to the point of paralytic ileus. In general, when the fever subsides, no further paralysis is likely to occur. The lower limbs are affected more commonly than are the upper, but in severe cases, quadriplegia and loss of function of the intercostal, abdominal, and trunk muscles with resultant respiratory difficulty may ensue. The superficial and deep reflexes in the affected limbs are lost; twitching of the muscles and diffuse fasciculations may be seen transiently. Sensory abnormalities are rare occurrences.

Flaccid paralysis is the most obvious clinical expression of the neuronal changes. The ensuing muscular atrophy is caused by denervation in addition to the atrophy of disuse. The pain, spasticity, nuchal and spinal rigidity, and hypertonia early in the illness probably are caused by lesions in the brain stem, spinal ganglia, and posterior columns. Respiratory and cardiac arrhythmias, blood pressure and vasomotor changes, and similar manifestations reflect damage to vital centers in the medulla.

On physical examination, the distribution of paralysis characteristically is spotty. To detect mild muscular weakness, one often must apply gentle resistance in opposition to the muscle group being tested. The spinal form has weakness of some of the muscles of the neck, abdomen, trunk, diaphragm, thorax, or extremities. The bulbar form is characterized by weakness in the motor distribution of one or more cranial nerves, with or without dysfunction of the vital centers of respiration and circulation. Patients with bulbar disease often are extremely agitated, even delirious, or they may become stuporous. The 10th cranial nerve nuclei are involved most commonly and result in paralysis of the pharynx, soft palate, and vocal cords. Facial paralysis occurs less commonly; it usually is asymmetric and involves only selected muscle groups. Ocular palsies are unusual findings.

Components of both the bulbar and spinal forms occur together in bulbospinal poliomyelitis. In the encephalitic form of the disease, irritability, disorientation, drowsiness, and coarse tremors not explained by inadequate ventilation are noted. Even during epidemics of poliomyelitis, this form can be recognized only if some peripheral or cranial nerve paralysis coexists or ensues. Hypoxia and hypercapnia caused by inadequate ventilation from respiratory insufficiency may produce disorientation without true encephalitis.

Numerous components acting together may result in insufficiency in ventilation (Table 165-22). The most serious consequences are hypoxia and hypercapnia, which may produce profound effects on many other systems. Respiratory insufficiency should be detected early to diminish its widespread effects, and because the situation may shift rapidly, continued clinical evaluation is essential. Despite weakness of the respiratory muscles, the patient may respond with so much respiratory effort that normal alveolar ventilation is maintained. In fact, the increased effort (associated with anxiety and fear) actually may produce overventilation at the outset and may result in respiratory alkalosis. Such effort is fatiguing and soon leads to respiratory failure.

For clarity, certain terms characterizing patterns of disease need definition. First, *pure spinal poliomyelitis with respiratory insufficiency* refers to tightness, weakness, or paralysis of the respiratory muscles (chiefly the diaphragm and intercostals) without discernible clinical involvement of the cranial nerves or vital centers. The cervical and thoracic spinal cord segments chiefly are involved. Second, *pure bulbar poliomyelitis* refers to paralysis of the motor cranial nerve nuclei with or without involvement of the vital centers that control respiration, circulation, and body temperature. Involvement of the 9th, 10th, and 12th cranial nerves is most important because it results in paralysis of the pharynx, tongue, and larynx, with consequent airway obstruction. Third, *bulbospinal poliomyelitis with respiratory insufficiency* refers to involvement of the respiratory muscles with coexisting bulbar paralysis.

TABLE 165-22 Common Sources of Hypoxia and Hypercapnia in Poliomyelitis

1. Cranial nerves IX to XII involved with
   a. Pharyngeal paralysis and pooling of secretions
   b. Laryngeal involvement, either spasm of laryngeal muscles or paralysis of vocal cords
   c. Lingual paralysis
   d. Tracheal accumulation of secretions from inability to cough
   e. Aspiration of vomitus
2. Vital center involvement with
   a. Inefficient, irregular respiration
   b. Cardiovascular disturbance
   c. Hyperpyrexia causing increased oxygen consumption
3. Cervical and spinal cord involvement causing paresis of the primary and accessory muscles of respiration
4. Pulmonary complications (e.g., pneumonia, atelectasis, and edema)
5. Contributory factors
   a. Panic
   b. Gastric dilation
   c. Sedation
   d. Inadequate equipment (e.g., small-bore tracheostomy tubes, unsuitable respirator settings)

From Cherry JD. Enteroviruses. In: Behrman RE, Vaughan VC, editors. *Nelson textbook of pediatrics.* 12th ed. Philadelphia, W.B. Saunders, 1983, p. 791-804.

The clinical findings resulting from involvement of the respiratory muscles are (1) an anxious expression; (2) inability to speak without frequent pauses, a situation resulting in short, jerky, "breathless" sentences that can be demonstrated by asking the child to count numbers serially; (3) increased respiratory rate; (4) movement of the alae nasi and the accessory muscles of respiration; (5) inability to cough or sniff with full depth; (6) paradoxical abdominal movements caused by diaphragmatic immobility from spasm or weakness of one or both leaves; and (7) relative immobility of the intercostal spaces, whether segmental, unilateral, or bilateral. When the arms are weak and especially when deltoid paralysis occurs, one should beware of impending respiratory paralysis because the phrenic nerve nuclei are in adjacent areas of the spinal cord. Observing the patient's capacity for thoracic breathing while the abdominal muscles are splinted manually can be performed to assess minor degrees of paresis. Light manual splinting of the thoracic cage helps in evaluating the effectiveness of diaphragmatic movement.

The clinical findings of bulbar poliomyelitis with respiratory difficulty (other than paralysis of the extraocular, facial, and masticatory muscles) include the following:

1. Nasal twang to the voice or cry as a result of palatal and pharyngeal weakness (hard consonant words such as "cookie" or "candy" bring out this condition best.
2. Inability to swallow smoothly that results in an accumulation of saliva in the pharynx and

indicates partial immobility (holding the larynx lightly and asking the patient to swallow confirms the immobility).
3. Accumulated pharyngeal secretions, which may cause irregular respiration because each inspiration must be "planned" and cannot be "subconscious" in view of the risk for aspirating; the respirations thus may appear interrupted and abnormal even to the point of falsely simulating intercostal or diaphragmatic weakness.
4. The impossibility of effective coughing, with resultant constant and fatiguing efforts to clear the throat.
5. Nasal regurgitation of saliva and fluids caused by palatal paralysis, with an inability to separate the oropharynx from the nasopharynx during swallowing.
6. Deviation of the palate, uvula, or tongue.
7. Involvement of vital centers, as manifested by an irregularity in the rate, depth, and rhythm of respiration; by cardiovascular alterations that include changes in blood pressure (especially increased), alternate flushing and mottling of the skin, and cardiac arrhythmias; and by rapid changes in body temperature.
8. Paralysis of one or both vocal cords that causes hoarseness, aphonia, and, ultimately, asphyxia unless recognized by laryngoscopy and managed by immediate tracheostomy.
9. The "rope sign," an acute angulation between the chin and larynx caused by weakness of the hyoid muscles (the hyoid bone is pulled posteriorly, thus narrowing the hypopharyngeal inlet).

Myocardial failure sometimes develops secondary to pulmonary complications or as a result of acute myocarditis. The initial manifestation of poliovirus infection on occasion can resemble that of Guillain-Barré syndrome.[1103]

Congenital Infections

Abortion. Poliomyelitis is associated with an increased incidence of abortion. Horn[459] noted 43 abortions in 325 pregnancies complicated by maternal poliomyelitis. Abortion was related directly to the severity of the maternal illness, including the degree of fever during the acute phase of illness. However, abortion also has occurred in association with mild nonparalytic poliomyelitis. Siegel and Greenberg[394] noted that fetal death occurred in 14/30 instances (46.7%) of maternal poliomyelitis during the first trimester. Kaye and associates[551] reviewed the literature in 1953 and recorded 19 abortions in 101 cases of poliomyelitis in pregnancy. In a small study in Evanston Hospital in Illinois, the abortion rate in maternal poliomyelitis was little different from the expected rate.[100]

Congenital Malformations. Although isolated instances of congenital malformation and maternal poliomyelitis have been noted, little statistical evidence supports the suggestion that polioviruses are teratogens. In their review of the literature, Kaye and colleagues[551] noted 6 anomalies in 101 infants born to mothers with poliomyelitis during pregnancy. In the reviews of Horn,[459] Bates,[58] and Siegel and Greenberg,[394] no evidence of maternal polioviral infection–induced anomalies was found. Similarly, no evidence suggests that infection with poliovirus vaccine during pregnancy causes congenital malformations.[423]

Prematurity and Stillbirth. In Horn's study[459] of 325 pregnancies, 9 infants died in utero. In each instance, the mother was critically ill with poliomyelitis. Horn[459] also noted that 45 infants weighed less than 6 lb, and 17 of them had a birth weight less than 5 lb. These low-birth-weight infants were born predominantly to mothers who had poliomyelitis early in pregnancy. In New York City, Siegel and Greenberg[394] also noted an increase in prematurity after maternal poliomyelitis infection. It was related specifically to maternal paralytic poliomyelitis.

Neonatal Infections

General. In the excellent review by Bates[58] in 1955, 58 cases of poliomyelitis in infants younger than 1 month were described. Although complete data were not available on many of the cases, 51 had paralysis, died of their disease, or both. Of the total number of infants on whom these investigators had clinical data, only one infant had nonparalytic disease. More than half the cases were secondary to maternal disease. Because other investigators have noted congenital infection without symptomatic maternal infection, infection in the mother probably was the source for an even greater percentage of the neonatal illnesses. The incubation period of neonatal poliomyelitis has not been established, and, therefore, determining how many of the babies were infected in utero is difficult. Probably, most illnesses that occurred within the first 5 days of life were congenital. Most of the neonates had symptoms of fever, anorexia or dysphagia, and listlessness. Almost half of the infants noted in this review died, and of those surviving, 48 percent had residual paralysis.

Infection Acquired In Utero. Elliott and associates[300] described an infant girl in whom "complete flaccidity" was noted at birth. This child's mother had mild paralytic poliomyelitis, with the onset of minor illness occurring 19 days before the infant's birth. Fetal movements had ceased 6 days before delivery, a finding suggesting that paralysis had occurred at this time. On examination, the baby was severely atonic; when supported under the back, she was passively opisthotonic. Respiratory efforts were abortive and were confined to the accessory muscles; laryngoscopy revealed complete flaccidity in the larynx.

Johnson and Stimson[521] reported a case in which the mother's probable abortive infection occurred 6 weeks before the birth of the baby. The baby initially was thought to be normal but apparently underwent no medical examination until the fourth day of life. At this time, the physician noted right hemiplegia. On the following day, a more complete examination revealed lateral bulging of the right side of the abdomen accompanied by crying and maintenance of the lower extremities in a frog leg position. Adduction and flexion at the hips were weak, and the knee and ankle jerks were absent. Laboratory

studies were unremarkable except for examination of CSF. It revealed 20 lymphocytes and a protein level of 169 mg/dL. During a 6-month period, this child's paralysis gradually improved and resulted in only residual weakness of the left lower extremity.

Paresis of the left arm was noted shortly after birth in another child with apparent transplacentally acquired poliomyelitis.[661] At 2 days of age, the baby was quadriplegic, but patellar reflexes were present, and the child had no respiratory or swallowing difficulties. This child had pneumonia at 3 weeks of age, but otherwise, general neurologic improvement occurred. Examination at 8 weeks of age revealed bilateral atrophy of the shoulder girdle muscles. The CSF from this patient had 63 leukocytes/mm^3, 29 percent of them polymorphonuclear cells, and a protein concentration of 128 mg/dL.

All three of the infants just discussed apparently were infected in utero several days before birth. Their symptoms were exclusively neurologic; fever, irritability, and vomiting did not occur.

Postnatally Acquired Infection. In contrast to infections acquired in utero, those acquired postnatally are more typical of classic poliomyelitis. Shelokov and Weinstein[937] described a child who was asymptomatic at birth. The onset of minor symptoms in the mother occurred 3 weeks before delivery, and the onset of major symptoms occurred 1 day before delivery. On the sixth day of life, the infant suddenly became ill with watery diarrhea. He looked grayish and pale. On the following day, he was irritable, lethargic, and limp and had a temperature of 38° C (100.4° F). Mild opisthotonos and weakness of both lower extremities developed. He was responsive to sound, light, and touch. The CSF had an elevated protein level and an increased number of leukocytes. His condition worsened during a total period of 3 days, and then gradual improvement began. At 1 year of age, he had severe residual paralysis of the right leg and moderate weakness in the left leg.

Baskin and associates[56] described two infants with neonatal poliomyelitis. The first child, whose mother had severe poliomyelitis at the time of delivery, was well for 3 days and then had a temperature of 38.3° C (100.9° F). On the fifth day of life, the boy became listless and cyanotic. Examination of CSF revealed a protein level of 300 mg/dL and 108 leukocytes/mm^3. His condition worsened, and extreme flaccidity, irregular respiration, and progressive cyanosis developed; he died on the seventh day of life. The second infant was a boy who was well until he was 8 days old but then became listless, with a temperature of 38.38° C (101.08° F). During the next 5 days, flaccid quadriplegia developed, as did irregular, rapid, and shallow respirations and an inability to swallow. The child died on the 14th day of life. Acute poliomyelitis had developed in his mother 6 days before the onset of his symptoms.

Abramson and colleagues[4] reported four children with neonatal poliomyelitis, two of whom died. In three of the children, the illnesses were typical of acute poliomyelitis in older children. The other child died at 13 days of age with generalized paralysis. The onset of his illness was difficult to define, and he was never febrile. Bates[58] described infants with acute poliomyelitis and clinical illnesses similar to those that occur in older persons.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Clinical Diagnosis

Clinical differentiation of enteroviral disease frequently is thought to be impossible. Although treatable bacterial illnesses always should be considered and treated first, also true is that when all the circumstances of a particular illness are considered, enteroviral diseases can be suspected on clinical grounds. The most important factors in clinical diagnosis are the season of the year, geographic location, exposure, incubation period, and clinical symptoms.

In temperate climates, prevalence of EVs is distinctly seasonal, so disease usually is seen in the summer and fall. Enteroviral disease is less likely to occur in the winter. In the tropics, EVs are prevalent throughout the year, and the season, therefore, is not diagnostically helpful.

As with all infectious illnesses, knowledge of exposure and incubation time is important. A careful history of maternal illness is critical in neonatal disease. For example, nonspecific, mild febrile illness in a mother that occurs in the summer and fall should warn of the possibility of severe neonatal illness. Specific findings (i.e., aseptic meningitis, pleurodynia, herpangina, pericarditis, myocarditis) should alert the clinician to enteroviral illnesses. The short incubation period of enteroviral infections should be considered.

Laboratory Diagnosis

Virus Isolation and Detection Techniques

Most viral diagnostic laboratories have facilities for the recovery of most EVs that cause illness. A three-tissue culture system that includes primary monkey kidney, a diploid, human embryonic lung fibroblast cell strain, and the RD cell line will allow the isolation of virtually all coxsackieviruses B and echoviruses and some coxsackieviruses A (e.g., coxsackieviruses A9 and A16). In a study in which Buffalo green monkey kidney cells and subpassage of primary human embryonic kidney cells were used in addition to primary monkey kidney and human diploid fibroblast (MRC-5) cells, the recovery rate of EVs was increased 11 percent.[210] For a complete diagnostic isolation spectrum, suckling mouse inoculation also should be performed.

Proper selection and handling of specimens are most important in the isolation of viruses. Enteroviral infections tend to be generalized, so collection of material from multiple sites is important; specimens should be collected from any or all of the following: nasopharynx, throat, stool, blood, urine, CSF, and any other body fluids that are available. Swabs from the nose, throat, and rectum should be placed in a carrying medium containing a small amount of protein. Hanks balanced salt solution with 2 percent agamma calf serum and antibiotics is

satisfactory. Fluid specimens should be collected in sterile vials; specimens of postmortem material are collected best in vials that contain carrying medium. In general, specimens should be refrigerated immediately after collection and during transportation to the laboratory. The specimens must not be exposed to sunlight during transportation. If an extended period will elapse before a specimen is processed in the laboratory, shipping and storing it frozen is advisable.

Contrary to popular belief, tissue culture evidence of enteroviral growth takes only a few days in many cases and less than a week in most.[392] The use of spin amplification, the shell vial technique, and monoclonal antibodies has been shown to reduce the time of detection in enteroviral cultures significantly.[586,814,935,1002] After an EV has been isolated, type identification is performed conventionally by neutralization, and this process, unfortunately, frequently takes a long time.

Nucleic acid techniques with cDNA and RNA probes have been shown to be useful for direct identification of EVs.* Most important, however, has been the development of numerous PCR techniques. Since 1990, many reports have described enteroviral, parechoviral, and SAFV PCR methods and their use in identifying viral RNA in clinical specimens.†

PCR has proved most useful for the direct identification of EVs and PeVs in the CSF of patients with meningitis.[887,912,982,993,1098] When compared with culture of CSF specimens, PCR is faster and more sensitive and the specificity is equal.

PCR also has proved useful in the identification of EVs and PeVs in blood, urine, and throat specimens.‡ Particularly impressive are the findings of Byington and associates[119] and Rittichier and associates.[867] Using PCR on specimens of blood and CSF, these investigators found that more than 25 percent of infants admitted to the hospital for suspected sepsis in 1997 had nonpolio enteroviral infections. Based on this study and the work of Andréoletti and coworkers,[18] we consider that the general workup for febrile children hospitalized for possible sepsis should include PCR for EVs and PeVs in both blood and CSF, if available. A shortcoming of PCR is that EV RNA is identified but a specific enteroviral type is not. Because of this shortcoming, we recommend that conventional culture be performed in addition to PCR. However, in the research setting, further molecular study often can identify the genotype of a causative agent from the original sample submitted for PCR assay.[567]

EV RNA also has been identified in numerous tissue specimens from patients with chronic medical conditions such as idiopathic DCM. However, as discussed earlier in the section on cardiovascular Manifestations, the possibility of a lack of specificity (false-positive results) is a concern. Polioviruses can be separated from other EVs, and poliovirus vaccine strains can be identified rapidly by PCR.[3,198,293,1095]

Serology

Except in special circumstances, the use of serologic techniques for establishing the primary diagnosis of suspected enteroviral infection is impractical. Standard serologic study depends on the demonstration of a rise in antibody titer to a specific virus as an indication of infection with that agent. Although ELISA, hemagglutination inhibition, and complement fixation take only a short time to perform, these tests can be carried out only after a second, convalescent-phase blood specimen has been collected. These tests also are impractical in searching for the cause of a specific illness in a child because of the existence of so many antigenically different EVs. As noted previously in the discussion of antigenic characteristics, group antigens can be produced that allow one to establish a serologic diagnosis by IgM EIA (enzyme immunoassay) and complement fixation, but these tests lack specificity.[899,905,990]

In the evaluation of a patient with a suspected enteroviral infection, serum should be collected as soon as possible after the onset of illness and then again 2 to 4 weeks later. This serum should be stored frozen. In most clinical situations, performing serologic tests on the collected serum is not necessary because demonstration of a rise in antibody titer in the serum of an infant from whom a specific virus has been isolated from a body fluid obviously is superfluous. However, collected serum can be useful diagnostically if the prevalence of specific EVs in a community is known. In this situation, looking for antibody titer changes to a selected number of viral types is relatively easy. Faster diagnosis can be made with a single serum sample if a search for specific IgM enteroviral antibody is made.*

Unfortunately, no EV IgM antibody tests are commercially available in the United States. Commercial laboratories offer enteroviral complement fixation antibody panels. However, these tests are expensive, and their results in the clinical setting almost always are meaningless unless acute-phase and convalescent-phase sera are analyzed.

Histology

Enteroviral infections have no specific histologic findings such as those seen in cytomegalovirus or HSV infection. However, tissues can be examined for specific enteroviral antigens by immunofluorescence and for RNA by PCR.[116,155,675]

Differential Diagnosis

The differential diagnosis of enteroviral infection depends on the clinical manifestations. In general, the most important considerations relate to bacterial diseases such as those commonly associated with pharyngitis, pneumonia, pericarditis, meningitis, and septicemia. Other viruses must be considered with upper respiratory tract illnesses,

*References 129, 258, 489, 816, 880, 881, 884.
†References 3, 7, 18, 23, 41, 73, 119, 198, 223, 224, 228, 293, 333, 345, 389, 417, 490, 567, 605, 610, 642, 676, 701, 737, 764, 771, 774, 790, 844, 867, 870, 879, 882, 887, 909, 916, 932, 934, 938, 946, 962, 972, 981, 982, 989, 993, 1008, 1029, 1032, 1036,1082, 1097, 1098.
‡References 7, 18, 119, 764, 765, 934.

*References 157, 207, 356, 374, 375, 386, 939, 1007, 1115.

gastrointestinal infections, rashes, encephalitis, and neonatal illness.

Paralytic poliomyelitis usually presents no diagnostic problem in the presence of an outbreak, but sporadic cases are another matter, especially in countries such as the United States, where the disease (except for the vaccine-associated form) has disappeared and many pediatricians have never seen a case. Rarely, other EVs have been shown to cause paralytic syndromes that are indistinguishable from poliomyelitis.

Several other diseases must be considered in the differential diagnosis of sporadic cases of paralytic illness. Guillain-Barré syndrome is the most common and difficult differential diagnostic problem. Fever, headache, and meningeal signs usually occur less commonly in Guillain-Barré syndrome; the paralysis characteristically is symmetric, and sensory changes are common findings. Also in Guillain-Barré syndrome, the CSF contains few cells but a significant elevation in protein concentration. Other illnesses confused with paralytic poliomyelitis include peripheral neuritis (postinjection, toxic, herpes zoster), arboviral infection, rabies, tetanus, botulism, and tick paralysis.

TREATMENT

Specific Therapy

No specific therapy for any enteroviral infection is approved for use in the United States. In severe, catastrophic, and generalized neonatal infection, in all probability the baby received no specific antibody for the particular virus from the mother. In this situation, administering immunoglobulin to the infant probably is advisable because high titers of neutralizing antibody to many EVs frequently are present in immunoglobulin.[213,248] Little evidence supports the claim that this therapy is beneficial; however, it can be expected to stop further organ seeding secondary to continued viremia. Intravenous or intraventricular injection of immunoglobulin with IVIG and administration of hyperimmune plasma have been useful on some occasions and not others in the treatment of enteroviral infection in patients with agammaglobulinemia.*

Abzug and associates[8] performed a small but controlled study in which nine EV-infected neonates received IVIG and seven similarly infected infants received supportive care. In this study, no significant difference was found in clinical scores, antibody values, or the magnitude of viremia and viruria in those treated versus control infants. However, five infants received IVIG with a high neutralizing antibody titer (>1:800) to their individual viral isolates, and they experienced faster cessation of viremia and viruria. A neonate with disseminated echovirus 11 infection and hepatitis, pneumonitis, meningitis, DIC, decreased renal function, and anemia who survived after receiving a large dose of IVIG and supportive care was described.[528] Administering IVIG to older children with life-threatening enteroviral infection also seems reasonable. A specific recommended dosage is unknown, but 400 mg/kg/day for 4 days or 2 g/kg in one dose has been used.

Jantausch and associates[512] reported an infant with a disseminated echovirus 11 infection who survived after receiving maternal plasma transfusions. A neonate with fulminant echovirus 11 infection survived after undergoing orthotopic liver transplantation.[219]

The antiviral drug pleconaril offers promise for the treatment of enteroviral infections.* This drug is a novel compound that integrates into the capsid of EVs. It prevents virus attachment to cellular receptors and the uncoating and subsequent release of viral RNA into the host cell. In a double-blinded, placebo-controlled study of 39 patients with enteroviral meningitis, a statistically significant shortening of duration of disease was noted—from 9.5 days in controls to 4.0 days in drug recipients.[886] Pleconaril also has been used on a compassionate-release basis for the treatment of patients with life-threatening infection.[59,112,883] The following categories of enteroviral illness have been treated: chronic meningoencephalitis in patients with agammaglobulinemia or hypogammaglobulinemia, neonatal sepsis, myocarditis, poliomyelitis (wild-type or vaccine associated), and encephalitis, as well as in bone marrow transplant recipients. Although these treatment studies have not had control arms, favorable clinical responses have been observed in 26/50 treated patients, including 12/18 patients with chronic meningoencephalitis. Pleconaril is not available in the United States.

In severe illnesses such as neonatal myocarditis or encephalitis, a frequent temptation is to administer corticosteroids. Although some workers have thought that this therapy is beneficial in treating coxsackieviral myocarditis, we recommend that corticosteroids not be given during acute enteroviral infection. The deleterious effects of these agents in coxsackieviral infection of mice are particularly persuasive factors in this opinion.[96,576] Immunosuppressive therapy for myocarditis of unknown origin with prednisone and cyclosporine or azathioprine was evaluated in a controlled trial of 111 adults, but no beneficial effect was observed.[678]

Because the possibility of bacterial sepsis cannot be ruled out in many instances of enteroviral infection, antibiotics frequently should be administered for the most likely potential pathogens. Care in the selection and administration of antibiotics is urged so that drug toxicity is not added to the problems of the patient.

Nonspecific Therapy

Mild, Nonspecific Febrile Illness

In patients in whom fever is the only symptom, careful observation is important. Many patients who eventually become severely ill initially have 2 to 3 days of fever without other localized findings. Care should be taken to administer adequate fluids to febrile infants, and excessive elevation of temperature should be prevented if possible.

*References 9, 93, 240, 289, 304, 349, 412, 442, 687, 698, 702, 1023, 1055.

*References 59, 112, 268, 552, 817, 886, 883, 1052.

Myocarditis

Myocarditis has no specific therapy. However, congestive heart failure and arrhythmias occur, and they should be treated by the usual methods. In administering digitalis to patients with enteroviral myocarditis, paying careful attention to the initial dosage is most important because the heart often is extremely sensitive; frequently, only small amounts of digoxin are necessary.

Meningoencephalitis

In patients with meningoencephalitis, seizures, cerebral edema, and disturbances in fluid and electrolyte balance all occur frequently and respond to treatment. Seizures are treated best with phenobarbital, phenytoin, or lorazepam. Cerebral edema can be treated with urea, mannitol, or large doses of corticosteroids. As noted, the use of corticosteroids in patients with active enteroviral infection seems unwise because the local benefit may be outweighed by the overall deleterious effects. Fluids should be monitored closely, and serum electrolyte levels should be determined frequently because inappropriate antidiuretic hormone secretion is a common occurrence.

Poliomyelitis

The broad principles of management are to allay fear, minimize the ensuing skeletal deformities, anticipate and meet complications in addition to the neuromusculoskeletal ones, and prepare the child and family for the prolonged treatment that may be required and for permanent disability when it seems likely. Patients with the nonparalytic and mildly paralytic forms of the disease may be treated at home.

Most patients with paralytic poliomyelitis require hospitalization. A calm atmosphere is desired. Suitable body alignment is necessary to avoid excessive skeletal deformity. A neutral position with the feet at a right angle, knees slightly flexed, and hips and spine straight is achieved by the use of boards, sandbags, and, occasionally, light splint shells. Active and passive motion is indicated as soon as the pain has disappeared. The orthopedist and physiatrist should see these patients as early in the illness as possible and should assume responsibility before fixed deformities develop.

Management of pure bulbar poliomyelitis consists essentially of maintaining the airway and avoiding all risks for inhalation of saliva, food, or vomitus. Gravity drainage of accumulated secretions is favored by the head-low (foot of the bed elevated 20 to 25 degrees), prone position with the face to one side. Aspirators with rigid or semirigid tips are preferred for direct oral and pharyngeal use, and soft flexible catheters may be used for nasopharyngeal aspiration. Fluid and electrolyte balance is maintained best by intravenous infusion because tube or oral feeding in the first few days may incite vomiting. After the initial few days, sips of sterile water may be given from a spoon, with increments as indicated by the child's ability to swallow. In addition to close observation for respiratory insufficiency, blood pressure should be recorded at least twice daily. Hypertension is not an uncommon occurrence and occasionally leads to hypertensive encephalopathy. Patients with pure bulbar poliomyelitis may require tracheostomy because of paralysis of the vocal cords or constriction of the hypopharynx. Most patients with pure bulbar poliomyelitis who recover have little residual impairment; some patients exhibit mild dysphagia and occasional vocal fatigue with slurring of speech.

Impaired ventilation must be recognized early; mounting anxiety, restlessness, and fatigue are early indications for prompt intervention. Tracheostomy is indicated for some patients with pure bulbar poliomyelitis, spinal respiratory muscle paralysis, and bulbospinal paralysis. Unlike other patients on whom tracheostomy is performed, these patients generally are unable to cough, sometimes for many months. Frequent and swift endotracheal aspiration under aseptic conditions is necessary. Mechanical ventilation often is needed. Patients are fully conscious and aware; terrifying procedures are performed best with an outward atmosphere of calm. Explaining the procedure and having the parents on hand may be helpful. A reduction in thoracic compliance occurs early, and higher than expected pressure gradients may be required to achieve adequate ventilation. Weaning a patient from dependency on respiratory assistance is a torturous process, as is total musculoskeletal rehabilitation. Motivation of the patient and the team of personnel is paramount.

PROGNOSIS

The prognosis for nonpolio enteroviral infections is excellent in most instances. Virtually all morbidity and mortality are related to cardiac and neurologic disease in older children and to these diseases in addition to general disseminated infection with hepatitis in neonates.

The prognosis for poliomyelitis varies with the degree of muscle involvement. In patients with mild muscle weakness, complete recovery is the rule. If paralysis is present, recovery of muscle function continues for a period of approximately 18 months to 2 years. By 3 months, approximately 60 percent of the ultimate improvement has been achieved, and by 6 months, 80 percent. The final result depends on the extent and localization of nerve cell damage.

Respiratory failure is responsible for most of the deaths in paralytic poliomyelitis. With the many improvements in techniques for handling this complication, the overall mortality rate has been reduced to approximately 4 percent; with the bulbar form and in adults, it still may be as high as 10 percent.

Occasionally, new neuromuscular symptoms develop later in life in patients who have had paralytic poliomyelitis.* Although the cause of this late-onset weakness and muscle atrophy (postpolio syndrome) is not understood completely, it most likely is the result of routine attrition of anterior horn cells associated with aging rather than persistent neural infection with polioviruses. However,

*References 130, 250, 251, 523, 845, 994, 1077, 1076.

specific immunopathologic mechanisms possibly play a role in some instances.[371]

Leparc-Goffart and associates[625] presented data suggesting the presence of poliovirus-specific genomic sequences in the CSF of patients with postpolio syndrome. However, Muir and colleagues,[739] who performed similar studies, found no association of chronic neurologic disease with the presence of enteroviral RNA in CSF.

PREVENTION

Nonpolio Enteroviral Infections

Attenuated and inactivated viral vaccines for EVs other than polioviruses are not available. However, if a virulent enteroviral type were to emerge, a specific vaccine for active immunization probably could be developed. It would seem that EV 71 would qualify for vaccine development and use in the Asian-Pacific region. In this light, several inactivated vaccines are in various studies of development (see http://clinicaltrials.gov/ct2/results?term=enterovirus+71).

Passive protection with IVIG may be useful in preventing disease. In practice, however, it would seem worthwhile only for sudden and virulent nursery outbreaks. For example, if several cases of myocarditis occurred in a nursery, administering IVIG to all babies in the nursery would seem reasonable. Pooled human immunoglobulin in most instances can be expected to contain antibodies against coxsackieviruses B1, B2, B3, B4, and B5, as well as several coxsackieviruses A and echoviruses.[248] Therefore, this procedure would offer protection to infants without transplacentally acquired specific antibody who had not yet become infected. Immune serum was useful in the management of three nursery enteroviral outbreaks.[128,744,810]

Polioviral Infections

In the United States, the total annual number of paralytic cases fell from an average of 16,000 in the 5 years before the introduction of vaccine to approximately 10 cases per year between 1980 and 1984. The experience in 1979, however, when 26 paralytic cases were reported, served as a reminder that virulent polioviruses still could surface in susceptible persons.[134,135] Most of the 1979 cases occurred in Pennsylvania and several other states in Amish population groups who had not been immunized. A similar epidemic in Connecticut in 1972 involved a pocket of unimmunized students in a Christian Science school.[131] These outbreaks reflected the reality that poliomyelitis was still occurring in many parts of the world. The possibility of the introduction of virulent strains was ever present, and only through continued and extensive immunization programs could the disease be prevented from reappearing in epidemic form.[461]

The remarkable overall record of the decline in paralytic poliomyelitis in the United States was a result of the development and use of two effective vaccines.[461] Inactivated poliovirus vaccine, the first to be licensed, was used extensively beginning in 1955 and considerably reduced the incidence of the disease, although epidemics continued to occur. Live-attenuated oral poliovirus vaccine, licensed in 1961 and 1962, subsequently was recommended as the method of choice in the United States based on its superiority in terms of immunogenic capacity, ability to induce local IgA antibody in the oropharynx and intestinal tract and thus provide greater resistance to reinfection, and ease of administration. Oral poliovirus vaccine gradually supplanted inactivated poliovirus vaccine, and between 1973 and 1978, it was the only vaccine available. Its extraordinary effectiveness at that time, despite reaching only 65 percent of children younger than 5 years of age with the recommended three doses, suggested that the capacity of the attenuated strains to spread contributed to a much higher immunization rate than was indicated by vaccination statistics. The potential impact of such spread on the immunity of the population was illustrated by the observations of Fox and Hall,[331] who conducted long-term virologic surveillance of middle-income families in Seattle. Polioviruses (vaccine strains) accounted for 50 percent of the 2937 viral isolates from healthy children, their parents, and others in the community. In an analysis of 611 of the poliovirus isolates, researchers found that 75.6 percent were from vaccinees, 10.5 percent were from vaccinee contacts, and 14 percent were from persons without recent known contact with vaccine or a vaccinee. These findings provided a vivid picture of the pervasiveness of the attenuated strains and their continuous circulation in the population. This feature also was supported by the almost invariable recovery of polioviruses from weekly samples of sewage that were collected throughout the year, in the early vaccine era, in urban communities.[463]

Despite its striking success, the oral vaccine had some problems. One was greatly reduced seroconversion rates when the vaccine was given to children living in the tropics: as few as 50 percent had satisfactory responses, in contrast to the more than 95 percent response rate in the United States and similar countries.[274,706] Viral interference from other EVs played some role, and the presence of an inhibitory substance in the oropharynx that prevented significant multiplication of the vaccine strains also was involved. The seroconversion problem was lessened by using pulse immunization programs.[518,896] For example, the strategy of national annual vaccination days twice per year, 2 months apart, has been successful in developing countries.

Another problem with oral poliovirus vaccine—and the major problem in the United States—was the occurrence of a small number of vaccine-associated cases of poliomyelitis.[461,706] The immunogenic effectiveness of the vaccine depends on multiplication of the attenuated strains in the intestinal tract. Because no poliovirus strain is completely stable, the progeny of vaccine strains underwent a certain degree of mutation, which rarely resulted in increased virulence and vaccine-associated cases in recipients and their contacts, most often their parents.

Since 1980, no indigenous cases of wild poliovirus disease have occurred in the United States.[141,965] From 1980 to 1989, 80 cases of vaccine-associated paralytic poliomyelitis and 5 cases of imported disease were

reported. The overall rate of vaccine-associated paralytic poliomyelitis was 1 case per 2.5 million doses of distributed vaccine; the risk for recipients was 1 case per 6.8 million doses, and for household contacts it was 1 case per 6.4 million doses. Of the 80 cases, 30 occurred in vaccinees, 32 occurred in household contacts, 4 were community acquired, and 14 occurred in immunologically abnormal persons.

Further analysis revealed that the risk associated with the first dose of vaccine was 1 case per 700,000 doses, but it was only 1 case per 6.9 million for subsequent doses; for vaccine recipients, the calculated risks were 1 case per 1.4 million initial doses and 1 case per 41.5 million subsequent doses. The calculated risks for contact cases were 1 case per 1.9 million initial doses of vaccine and 1 case per 13.8 million subsequent doses.

Immunodeficient children are at particular risk for acquiring vaccine-associated paralytic poliomyelitis.[132,965] From 1969 through 1976, 11 percent of vaccine-associated cases occurred in immunodeficient patients; 10/11 of these patients were children younger than 1 year of age. From 1980 to 1989, 18 percent of vaccine-associated paralytic poliomyelitis cases occurred in immunodeficient patients.[965]

Although the risks mentioned earlier were considered acceptable in view of the benefits provided, the question raised repeatedly since the 1970s was whether the United States should return to the use of inactivated poliovirus vaccine, which does not carry a risk for acquiring paralytic disease and has been highly successful in several small European countries in which more than 95 percent of the population is immunized.*

The problem was reviewed in detail by a committee of the Institute of Medicine (IOM) of the National Academy of Sciences, which reported its recommendations in April of 1977.[766] The conclusion at the time was that given the situation in the United States, in which not more than 65 percent of susceptible children were vaccinated, oral poliovirus vaccine should continue to be the principal vaccine for routine immunization. Inactivated poliovirus vaccine, conversely, should be provided for two groups: immunodeficient persons, because of their greatly enhanced risk for acquiring vaccine-associated disease after receiving the oral vaccine; and adults receiving primary immunization, because of their greater susceptibility to paralytic disease. Also suggested was that the inactivated vaccine be available as an alternative for those who prefer it. In addition, a single dose of trivalent oral poliovirus vaccine was suggested for all entrants into the seventh grade of school as a means of added protection for later years when they became parents.

In January of 1988, a panel appointed by the IOM again reviewed policy options for vaccination against poliomyelitis in the United States.[494] The IOM panel concluded that no change in policy should be recommended at that time. However, they did recommend that a new enhanced inactivated poliovirus vaccine replace the old vaccine when inactivated vaccine was indicated. They also suggested that when a new enhanced diphtheria-tetanus-pertussis inactivated poliovirus vaccine became available, a regimen of two or more doses of it followed by the oral vaccine be considered.

In 1996, the U.S. poliovirus vaccine immunization program was evaluated extensively by both the Advisory Committee on Immunization Practices (ACIP) and the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP). The ACIP recommended that sequential administration of inactivated poliovirus vaccine followed by oral poliovirus vaccine be the schedule of choice in the United States (Orenstein, WA, personal communication, 1996). The schedule consisted of two doses of the inactivated vaccine at 2 and 4 months of age and two doses of the oral vaccine at 12 to 18 months and 4 to 6 years of age. Both committees indicated that schedules that include all of the doses of each vaccine also were acceptable (Hanneman RE, personal communication, 1996; Orenstein, WA, personal communication, 1996). At the present time, an all-inactivated poliovirus vaccination schedule is recommended in the United States.[17,714] It is a four-dose schedule with doses at 2 and 4 months, 6 to 18 months, and 4 to 6 years. The reader is advised to consult the recommendations of the ACIP and the AAP, as well as the manufacturers' literature, for full consideration of the contraindications and indications for poliovirus vaccines.

Routine primary poliovirus vaccination of adults older than 18 years is not conducted in the United States. However, adults at risk for exposure to wild polioviruses (i.e., laboratory workers, international travelers, health care workers) should be immunized. For the vaccination of adults, inactivated poliovirus vaccine is recommended. Patients with immunodeficiency diseases should not be given oral poliovirus vaccine; live virus also should not be used in households in which an immunodeficient person resides.

Global Eradication of Poliomyelitis

The WHO established the Expanded Program on Immunization (EPI) in 1974.[484,1047,1089] Subsequently, the use of oral poliovirus vaccine in developing countries vastly increased. In 1980 in Brazil, researchers demonstrated that mass administration with the oral vaccine on National Immunization Days (NIDs) led to a dramatic reduction in the incidence of poliomyelitis.[262] This demonstration led in 1985 to the targeted eradication of poliomyelitis from the Western Hemisphere by 1990. This campaign was successful in that the last confirmed case of paralytic poliomyelitis caused by wild poliovirus occurred in 1991 in Peru.[135] In September 1994, an international commission convened by the Pan American Health Organization certified that indigenous transmission of wild poliovirus had been interrupted in the Americas.[140]

In 1988, the World Health Assembly established the objective of global eradication of polio by 2000.[484] This program was based on four strategies recommended by the WHO: (1) maintenance of high vaccination coverage levels among children with at least three doses of oral poliovirus vaccine; (2) development of sensitive systems of epidemiologic and laboratory surveillance, including use of the standard WHO case definition (i.e., a confirmed case of poliomyelitis is defined as acute flaccid

*References 331, 346, 452, 533, 549, 742, 805, 899-902, 965.

paralysis and at least one of the following: laboratory-confirmed wild poliovirus infection, residual paralysis of 60 days, death, or no follow-up investigation at 60 days); (3) administration of supplementary doses of oral poliovirus vaccine to all young children (usually those <5 years of age) during NIDs to rapidly interrupt the transmission of poliovirus; and (4) "mopping up" vaccination campaigns, which are localized campaigns targeted at high-risk areas where wild poliovirus transmission was most likely to persist at low levels. NIDs are mass campaigns conducted over the course of a short period (i.e., days to weeks) during which two doses of oral poliovirus vaccine are administered to all children in the target age group regardless of previous vaccination history, with an interval of 4 to 6 weeks between doses.

From 1985 through 1990, worldwide routine vaccination coverage levels increased from 47 to 85 percent and stabilized at 80 to 81 percent from 1991 to 1994.[141] From 1985 through 1994, the number of cases reported annually decreased 84 percent, from 39,361 to 6241. The number of countries reporting polio cases decreased steadily from 1985 (99/196 [51%]) to 1988 (88/196 [45%]) and 1994 (51/214 [24%]). In addition, the number of countries reporting zero polio cases increased from 1985 (84 [43%]) to 1988 (104 [53%]) and 1994 (145 [68%]). The number of countries with endemic polio that conducted NIDs each year increased from 15 in 1988 to 37 in April of 1995; 24 additional countries scheduled their first NIDs for later in 1995.

At the beginning of the 21st century, NIDs had been conducted in every country in the world with endemic polio. In 1999, 7141 cases of poliomyelitis were reported worldwide. These cases occurred in 30 countries, mainly in south Asia and central Africa. By the end of 2000, fewer than 3500 cases occurred in endemic areas throughout the world, and wild-type 2 poliovirus has not been detected since October 1999.[988] At the close of 2001, the area of endemic poliomyelitis had been reduced to 10 countries, with fewer than 1000 cases reported.[754]

The global eradication program suffered a major setback in 2004 when oral poliovirus vaccine immunizations in northern Nigeria were suspended because of misinformation regarding vaccine safety.[443] After this suspension, epidemic disease caused by type 1 virus occurred throughout Nigeria and also spread directly or indirectly to 21 previously polio-free countries.[148] This epidemic, which had extensive spread, had a major negative impact on the Global Polio Eradication Initiative. During 2005, 1856 cases were reported globally, and 1000 of these cases occurred in countries with outbreaks caused by importation.

In October 2011, the Independent Monitoring Board for the Global Polio Eradication Initiative (GPEI) issued a very critical report of the GPEI.[869] This report reinvestigated the initiative, but a goal of stopping polio transmission in 2012 will not be needed. In 2011, there were four endemic countries (Afghanistan, India, Nigeria, and Pakistan).[152] In addition, reestablished transmission occurred in Angola, Chad, and the Democratic Republic of the Congo.

Much attention is being paid to the end-game strategy for global polio eradication.[226,276,277,560] Of particular concern for the future after world eradication of wild poliovirus strains is vaccine-associated paralytic poliomyelitis.[270,562,563] Other concerns are the long-term excretion of highly evolved vaccine-derived polioviruses in persons with primary immunodeficiencies and polio outbreaks associated with circulating vaccine-derived polioviruses in areas with low rates of oral poliovirus vaccine coverage. Ideally, an end-game strategy would transition to the use of inactivated poliovirus vaccine, but this strategy is not realistic in much of the developing world.

Despite the substantial progress that has been made toward global eradication of polio, several challenges remain, including (1) increasing vaccination levels in unvaccinated subpopulations; (2) preventing the reintroduction of wild poliovirus into polio-free areas by eliminating reservoirs in polio-endemic countries (particularly in the Indian subcontinent); (3) increasing the awareness of donor agencies and governments in industrialized countries of the substantial financial and humanitarian benefits of global eradication of polio, thus engendering support from unaffected countries beyond that already provided by organizations such as Rotary International; (4) encouraging all countries that remain polio endemic to make eradication of polio a high priority, including the implementation of NIDs and the initiation of acute flaccid paralysis surveillance; and (5) providing support to vaccination program managers for training to develop managerial skills for implementing and maintaining effective vaccination and surveillance programs in all countries.[141] The success of the Global Polio Eradication Initiative will depend on finding solutions to these financial, managerial, political, and technical challenges.

World events and new knowledge relating to reversion of poliovirus vaccine strains and recombination of poliovirus vaccine strains with nonpolio EVs led to the realization that decisions related to discontinuation of immunization after world eradication are exceedingly complex.[561,754,988,1082]

NEW REFERENCES SINCE THE SIXTH EDITION

17. American Academy of Pediatrics. Poliovirus infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *2012 red book: report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 588–92.
44. Badran SA, Midgley S, Andersen P, et al. Clinical and virological features of terovirus 71 infections in Denmark, 2005 to 2008. *Scand J Infect Dis* 2011;**43**:642–8.
50. Bangalore H, Ahmed J, Bible J, et al. Abdominal distension: an important feature in human parechovirus infection. *Pediatr Infect Dis J* 2011;**30**:260–2.
74. Benschop KS, de Vries M, Minnaar RP, et al. Comprehensive full-length sequence analyses of human parechoviruses: diversity and recombination. *J Gen Virol* 2010;**91**:145–54.
90. Blinkova O, Kapoor A, Victoria J, et al. Cardioviruses are genetically diverse and cause common enteric infections in South Asian children. *J Virol* 2009;**83**:4631–41.
92. Blumental S, Reynders M, Willems A, et al. Enteroviral infection of a cardiac prosthetic device. *Clin Infect Dis* 2011;**52**:710–16.
104. Braun LE, Renaud C, Fairchok MP, et al. Human parechovirus and other enteric viruses in childcare attendees in the era of rotavirus vaccines. *J Ped Infect Dis* 2012;**1**:136–43.
150. Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance: United States, 2006-2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1577–80.

151. Centers for Disease Control and Prevention. Notes from the field: severe hand, foot, and mouth disease associated with coxsackievirus A6—Alabama, Connecticut, California, and Nevada, November 2011-February 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:213–14.
152. Centers for Disease Control and Prevention. Progress toward interruption of wild poliovirus transmission: worldwide, January 2011-March 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 353–7.
184. Cherry JD. Herpangina. In: Feigin R, Cherry JD, Demmler-Harrison G, et al, editors. *Textbook of pediatric infectious diseases*. 6th ed. Philadelphia: Saunders; 2009. p. 169–71.
203. Chia J, Chia A, Voeller M, et al. Acute enterovirus infection followed by myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and viral persistence. *J Clin Pathol* 2010;**63**:165–8.
226. Cochi SL, Linkins RW. The final phase of polio eradication: new vaccines and complex choices. *J Infect Dis* 2012;**205**:169–71.
259. de Groot-Mijnes JD, de Visser L, Zuurveen S, et al. Identification of new pathogens in the intraocular fluid of patients with uveitis. *Am J Ophthalmol* 2010;**150**:628–36.
270. DeVries AS, Harper J, Murray A, et al. Vaccine-derived poliomyelitis 12 years after infection in Minnesota. *N Engl J Med* 2011;**364**:2316–23.
280. Drexler JF, Baumgarte S, Eschbach-Bludau M, et al. Human cardioviruses, meningitis, and sudden infant death syndrome in children. *Emerg Infect Dis* 2011;**17**:2313–15.
281. Drexler JF, Baumgarte S, Luna LK, et al. Genomic features and evolutionary constraints in Saffold-like cardioviruses. *J Gen Virol* 2010;**91**:1418–27.
347. Galama J, Lanke K, Zoll J, et al. Seroepidemiology of Saffold cardiovirus type 2. *Emerg Infect Dis* 2011;**17**:1572–3.
405. Groeneweg M, Poley JW, Dansen M, et al. Chronic hereditary pancreatitis in a girl with a serine protease inhibitor Kazal type I (SPINK-1) gene mutation and a coxsackie type B5 infection. *Pediatr Infect Dis J* 2009;**28**:169–70.
422. Harada S, Okada M, Yahiro S, et al. Surveillance of pathogens in outpatients with gastroenteritis and characterization of sapovirus strains between 2002 and 2007 in Kumamoto Prefecture, Japan. *J Med Virol* 2009;**81**:1117–27.
426. Harvala H, Robertson I, Chieochansin T, et al. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. *J Infect Dis* 2009;**199**:1753–60.
450. Himeda T, Ohara Y. Saffold virus, a novel human cardiovirus with unknown pathogenicity. *J Virol* 2012;**86**:1292–6.
477. Huang WT, Lee PI, Chang LY, et al. Epidemic pleurodynia caused by coxsackievirus B3 at a medical center in northern Taiwan. *J Microbiol Immunol Infect* 2010;**43**:515–18.
493. Imamura T, Fuji N, Suzuki A, et al. Enterovirus 68 among children with severe acute respiratory infection: the Philippines. *Emerg Infect Dis* 2011;**17**:1430–5.
501. Itagaki T, Abiko C, Aoki Y, et al. Saffold cardiovirus infection in children associated with respiratory disease and its similarity to coxsackievirus infection. *Pediatr Infect Dis J* 2011;**30**:680–3.
502. Itagaki T, Abiko C, Ikeda T, et al. Sequence and phylogenetic analyses of Saffold cardiovirus from children with exudative tonsillitis in Yamagata, Japan. *Scand J Infect Dis* 2010;**42**:950–2.
504. Ito M, Yamashita T, Tsuzuki H, et al. Detection of human parechoviruses from clinical stool samples in Aichi, Japan. *J Clin Microbiol* 2010;**48**:2683–8.
564. Khamrin P, Chaimongkol N, Nantachit N, et al. Saffold cardioviruses in children with diarrhea, Thailand. *Emerg Infect Dis* 2011;**17**:1150–2.
567. Kiang D, Newbower EC, Yeh E, et al. An algorithm for the typing of enteroviruses and correlation to serotyping by viral neutralization. *J Clin Virol* 2009;**45**:334–40.
598. Krous HF, Langlois NE. Ljungan virus: a commentary on its association with fetal and infant morbidity and mortality in animals and humans. *Birth Defects Res A Clin Mol Teratol* 2010; **88**:947–52.
604. Kuo PC, Lin JY, Chen LC, et al. Molecular and immunocytochemical identification of coxsackievirus A-24 variant from the acute haemorrhagic conjunctivitis outbreak in Taiwan in 2007. *Eye (Lond)* 2010;**24**:131–6.
636. Levy HC, Bostina M, Filman DJ, et al. Catching a virus in the act of RNA release: a novel poliovirus uncoating intermediate characterized by cryo-electron microscopy. *J Virol* 2010;**84**: 4426–41.
717. Mirand A, Schuffenecker I, Henquell C, et al. Phylogenetic evidence for a recent spread of two populations of human enterovirus 71 in European countries. *J Gen Virol* 2010;**91**:2263–77.
763. Nielsen AC, Bottiger B, Banner J, et al. Serious invasive Saffold virus infections in children, 2009. *Emerg Infect Dis* 2012;**18**: 7–12.
768. Niklasson B, Samsioe A, Papadogiannakis N, et al. Zoonotic Ljungan virus associated with central nervous system malformations in terminated pregnancy. *Birth Defects Res A Clin Mol Teratol* 2009;**85**:542–5.
774. Nolte FS, Rogers BB, Tang YW, et al. Evaluation of a rapid and completely automated real-time reverse transcriptase PCR assay for diagnosis of enteroviral meningitis. *J Clin Microbiol* 2011;**49**: 528–33.
793. Oikarinen S, Martiskainen M, Tauriainen S, et al. Enterovirus RNA in blood is linked to the development of type 1 diabetes. *Diabetes* 2011;**60**:276–9.
795. Ooi MH, Wong SC, Lewthwaite P, et al. Clinical features, diagnosis, and management of enterovirus 71. *Lancet Neurol* 2010;**9**: 1097–105.
800. Pajkrt D, Benschop KS, Westerhuis B, et al. Clinical characteristics of human parechoviruses 4-6 infections in young children. *Pediatr Infect Dis J* 2009;**28**:1008–10.
818. Pham NT, Chan-It W, Khamrin P, et al. Detection of human parechovirus in stool samples collected from children with acute gastroenteritis in Japan during 2007-2008. *J Med Virol* 2011; **83**:331–6.
819. Pham NT, Takanashi S, Tran DN, et al. Human parechovirus infection in children hospitalized with acute gastroenteritis in Sri Lanka. *J Clin Microbiol* 2011;**49**:364–6.
820. Pham NT, Trinh QD, Khamrin P, et al. Diversity of human parechoviruses isolated from stool samples collected from Thai children with acute gastroenteritis. *J Clin Microbiol* 2010;**48**: 115–19.
825. Pineiro L, Vicente D, Montes M, et al. Human parechoviruses in infants with systemic infection. *J Med Virol* 2010;**82**:1790–6.
856. Ren L, Gonzalez R, Xiao Y, et al. Saffold cardiovirus in children with acute gastroenteritis, Beijing, China. *Emerg Infect Dis* 2009;**15**:1509–11.
857. Ren L, Gonzalez R, Xie Z, et al. Saffold cardioviruses of 3 lineages in children with respiratory tract infections, Beijing, China. *Emerg Infect Dis* 2010;**16**:1158–61.
869. Roberts L. Polio campaign: the polio emergency. *Science* 2012; **337**:514–16.
893. Ryu WS, Kang B, Hong J, et al. Clinical and etiological characteristics of enterovirus 71-related diseases during a recent 2-year period in Korea. *J Clin Microbiol* 2010;**48**:2490–4.
903. Samsioe A, Papadogiannakis N, Hultman T, et al. Ljungan virus present in intrauterine fetal death diagnosed by both immunohistochemistry and PCR. *Birth Defects Res A Clin Mol Teratol* 2009;**85**:227–9.
926. Seitsonen J, Susi P, Heikkila O, et al. Interaction of alphaVbeta3 and alphaVbeta6 integrins with human parechovirus 1. *J Virol* 2010;**84**:8509–19.
927. Seitsonen JJ, Shakeel S, Susi P, et al. Structural analysis of coxsackievirus A7 reveals conformational changes associated with uncoating. *J Virol* 2012;**86**:7207–15.
931. Selvarangan R, Nzabi M, Selvaraju SB, et al. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. *Pediatr Infect Dis J* 2011;**30**:238–42.
944. Sinclair RG, Jones EL, Gerba CP. Viruses in recreational waterborne disease outbreaks: a review. *J Appl Microbiol* 2009;**107**: 1769–80.
970. Sun G, Zhang X, Yi M, et al. Analysis of the genomic homologous recombination in Theilovirus based on complete genomes. *Virol J* 2011;**8**:439.
1009. Tsukagoshi H, Masuda Y, Mizutani T, et al. Sequencing and phylogenetic analyses of Saffold cardiovirus (SAFV) genotype 3 isolates from children with upper respiratory infection in Gunma, Japan. *Jpn J Infect Dis* 2010;**63**:378–80.
1010. Tsukagoshi H, Mizuta K, Abiko C, et al. The impact of Saffold cardiovirus in patients with acute respiratory infections in Yamagata, Japan. *Scand J Infect Dis* 2011;**43**:669–71.

1067. Wikswo ME, Khetsuriani N, Fowlkes AL, et al. Increased activity of coxsackievirus B1 strains associated with severe disease among young infants in the United States, 2007-2008. *Clin Infect Dis* 2009;**49**:e44–51.
1091. Xu ZQ, Cheng WX, Qi HM, et al. New Saffold cardiovirus in children, China. *Emerg Infect Dis* 2009;**15**:993–4.
1111. Zhang DL, Jin Y, Li DD, et al. Prevalence of human parechovirus in Chinese children hospitalized for acute gastroenteritis. *Clin Microbiol Infect* 2011;**17**:1563–9.
1112. Zhang Y, Zhu Z, Yang W, et al. An emerging recombinant human enterovirus 71 responsible for the 2008 outbreak of hand foot and mouth disease in Fuyang city of China. *Virol J* 2010;**7**:94.
1115. Zoll J, Erkens Hulshof S, Lanke K, et al. Saffold virus, a human Theiler's-like cardiovirus, is ubiquitous and causes infection early in life. *PLoS Pathog* 2009;**5**:e1000416.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 166

RHINOVIRUSES

Robert L. Atmar • Janet A. Englund

HISTORY

The first recognized human rhinovirus (HRV) (type 1A) was isolated in monkey kidney cell culture by Pelon and associates[227] in 1954 from a recruit at Great Lakes Naval Training Center in Chicago during an outbreak of afebrile common colds in his training company. Independently, Price and colleagues[237] reported the isolation of an antigenically identical virus from nurses and children with colds. In 1963, the HRVs were so named because of their association with illnesses of the nasal passages.[282]

Before these actual virus isolations, evidence suggested that viruses cause the common cold. Despite indications that some coldlike illnesses could be complicated by bacterial infections,[75,292] bacteria-free nasal filtrates from persons with apparent symptomatic respiratory tract infections clearly were able to initiate these illnesses. Kruse,[179] in 1914, first demonstrated transmission with apparently sterile filtrates, and his results were confirmed in the late 1920s in a series of experiments in humans and chimpanzees by Dochez and associates.[75] In the 1930s, workers from this latter laboratory reported growth of the agent in tissue culture and embryonated eggs, but their results were not confirmed.[5] In the 1940s and 1950s, Andrewes and colleagues[6] at the Common Cold Unit in Salisbury, England, and Dingle and the members of the U.S. Armed Forces Commission on Acute Respiratory Diseases were able to transmit colds from person to person with apparently sterile filtrates.[54] Jackson and colleagues[158] performed similar experiments in Chicago and also demonstrated immunity. In 1953, the Salisbury researchers reported isolation of an agent, DC, in serially passaged filtrates of tubed tissue cultures of human embryonic lungs; the virus was detected by its ability to produce colds in humans. Although these results could not be substantiated at the time, a virus *was* present; in 1968, the DC filtrates were found to contain HRV-9.[5,56]

The next major advance in growing common cold viruses took place in the late 1950s in Salisbury. Tyrrell and Parsons[283] inoculated human embryonic kidney cells with nasal filtrates and incubated the cultures under conditions simulating those of the nasal passages (e.g., 33° C, neutral pH, and in a roller drum for aeration). Six distinct types of HRVs (types 1B to 6)[61] were isolated by observing cytopathic effects.[270] Shortly thereafter, Hamparian and coworkers[61,132,173] isolated 18 HRVs (types 7 to 12 and 18 to 29) by using a semi-continuous diploid cell strain obtained by Hayflick and Moorhead[139] from human fetal lung cultures. The use of Hayflick's cell lines greatly accelerated the isolation and characterization of "new" HRVs, leading to the identification of 100 serotypes.[131] More remain untyped, but the number of additional cultivable serotypes seems limited.[131,170,212]

The use of molecular methods for the sensitive detection of rhinoviruses, beginning in the 1990s, further revolutionized the field by enhanced detection and typing of rhinoviruses in the clinical setting.[126,182,193,228] In 2006, nonculturable rhinoviruses were further expanded by the detection of a wider diversity of recognized rhinoviruses associated with respiratory illness, including a new species (clade C).[9,31,182] At least 49 new types have been identified by sequencing methods.[201] In the past decade, the contribution of HRVs to disease in children, hospitalized patients, and immunocompromised patients has been increasingly appreciated with the widespread application of sensitive and more rapid molecular methods in research and clinical practice. These new detection methods have clearly demonstrated that although the HRVs are

REFERENCES

1. Abed Y, Boivin G. Human parechovirus types 1, 2 and 3 infections in Canada. *Emerg Infect Dis* 2006;**12**:969–75.
2. Abed Y, Boivin G. New Saffold cardioviruses in 3 children: Canada. *Emerg Infect Dis* 2008;**14**:834–6.
3. Abraham R, Chonmaitree T, McCombs J, et al. Rapid detection of poliovirus by reverse transcription and polymerase chain amplification: application for differentiation between poliovirus and nonpoliovirus enteroviruses. *J Clin Microbiol* 1993;**31**: 395–9.
4. Abramson H, Greenberg M, Magee MC. Poliomyelitis in the newborn infant. *J Pediatr* 1953;**43**:167–73.
5. Abzug MJ. Prognosis for neonates with enterovirus hepatitis and coagulopathy. *Pediatr Infect Dis J* 2001;**20**:758–63.
6. Abzug MJ, Levin MJ, Rotbart HA. Profile of enterovirus disease in the first two weeks of life. *Pediatr Infect Dis J* 1993;**12**:820–4.
7. Abzug MJ, Loeffelholz M, Rotbart HA. Diagnosis of neonatal enterovirus infection by polymerase chain reaction. *J Pediatr* 1995;**126**:447–50.
8. Abzug MJ, Keyserling HL, Lee ML, et al. Neonatal enterovirus infection: virology, serology, and effects of intravenous immune globulin. *Clin Infect Dis* 1995;**20**:1201–6.
9. Ackerson BK, Raghunathan R, Keller MA, et al. Echovirus 11 arthritis in a patient with X-linked agammaglobulinemia. *Pediatr Infect Dis J* 1987;**6**:485–8.
10. Adler JL, Mostow SR, Mellin H, et al. Epidemiologic investigation of hand, foot, and mouth disease: infection caused by coxsackievirus A 16 in Baltimore, June through September 1968. *Am J Dis Child* 1970;**120**:309–14.
11. Agranat AL. A near-fatal case of coxsackie B1 myocarditis (with pericarditis) in an adult. *S Afr Med J* 1961;**35**:831–3.
12. Ahmad N, Abraham AA. Pancreatic isleitis with coxsackie virus B5 infection. *Hum Pathol* 1982;**13**:661–2.
13. Alexander JP Jr, Baden L, Pallansch MA, et al. Enterovirus 71 infections and neurologic disease: United States, 1977-1991. *J Infect Dis* 1994;**169**:905–8.
14. Alexander JP Jr, Chapman LE, Pallansch MA, et al. Coxsackievirus B2 infection and aseptic meningitis: a focal outbreak among members of a high school football team. *J Infect Dis* 1993;**167**: 1201–5.
15. Alsop J, Flewett TH, Foster JR. "Hand-foot-and-mouth disease" in Birmingham in 1959. *Br Med J* 1960;**2**:1708–11.
16. American Academy of Pediatrics. Poliovirus infections. In: Peter G, editor. *1994 red book: report of the Committee on Infectious Diseases*. 23th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 1994. p. 379–86.
17. American Academy of Pediatrics. Poliovirus infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *2012 red book: report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 588–92.
18. Andreoletti L, Blassel-Damman N, Dewilde A, et al. Comparison of use of cerebrospinal fluid, serum, and throat swab specimens in diagnosis of enteroviral acute neurological infection by a rapid RNA detection PCR assay. *J Clin Microbiol* 1998;**36**:589–91.
19. Andreoletti L, Bourlet T, Moukassa D, et al. Enteroviruses can persist with or without active viral replication in cardiac tissue of patients with end-stage ischemic or dilated cardiomyopathy. *J Infect Dis* 2000;**182**:1222–7.
20. Andreoletti L, Hober D, Becquart P, et al. Experimental CVB3-induced chronic myocarditis in two murine strains: evidence of interrelationships between virus replication and myocardial damage in persistent cardiac infection. *J Med Virol* 1997;**52**: 206–14.
21. Andreoletti L, Hober D, Decoene C, et al. Detection of enteroviral RNA by polymerase chain reaction in endomyocardial tissue of patients with chronic cardiac diseases. *J Med Virol* 1996;**48**: 53–9.
22. Andreoletti L, Hober D, Hober-Vandenberghe C, et al. Detection of coxsackie B virus RNA sequences in whole blood samples from adult patients at the onset of type I diabetes mellitus. *J Med Virol* 1997;**52**:121–7.
23. Andreoletti L, Wattre P, Decoene C, et al. Detection of enterovirus-specific RNA sequences in explanted myocardium biopsy specimens from patients with dilated or ischemic cardiomyopathy. *Clin Infect Dis* 1995;**21**:1315–17.
24. Aquino VM, Farah RA, Lee MC, et al. Disseminated coxsackie A9 infection complicating bone marrow transplantation. *Pediatr Infect Dis J* 1996;**15**:1053–4.
25. Archard LC, Bowles NE, Behan PO, et al. Postviral fatigue syndrome: persistence of enterovirus RNA in muscle and elevated creatine kinase. *J R Soc Med* 1988;**81**:326–9.
26. Archibald E, Purdham DR. Coxsackievirus type A16 infection in a neonate. *Arch Dis Child* 1979;**54**:649.
27. Arnon R, Naor N, Davidson S, et al. Fatal outcome of neonatal echovirus 19 infection. *Pediatr Infect Dis J* 1991;**10**:788–9.
28. Arola A, Kalimo H, Ruuskanen O, et al. Experimental myocarditis induced by two different coxsackievirus B3 variants: aspects of pathogenesis and comparison of diagnostic methods. *J Med Virol* 1995;**47**:251–9.
29. Arola A, Kallajoki M, Ruuskanen O, et al. Detection of enteroviral RNA in end-stage dilated cardiomyopathy in children and adolescents. *J Med Virol* 1998;**56**:364–71.
30. Aronson MD, Phillips CA. Coxsackievirus B5 infections in acute oliguric renal failure. *J Infect Dis* 1975;**132**:303–6.
31. Artenstein MS, Cadigan FC Jr, Buescher EL. Clinical and epidemiological features of coxsackie group B virus infections. *Ann Intern Med* 1965;**63**:597–603.
32. Ash I. Large epidemic outbreak of vomiting associated with meningism and exanthem: clinical and epidemiological observations. *Br Med J* 1958;**1**:316–18.
33. Assaad F, Borecka I. Nine-year study of WHO virus reports on fatal viral infections. *Bull World Health Organ* 1977;**55**:445–53.
34. Assaad F, Cockburn WC. Four-year study of WHO virus reports on enteroviruses other than poliovirus. *Bull World Health Organ* 1972;**46**:329–36.
35. Atkinson MA, Bowman MA, Campbell L, et al. Cellular immunity to a determinant common to glutamate decarboxylase and coxsackie virus in insulin-dependent diabetes. *J Clin Invest* 1994;**94**:2125–9.
36. Austin TW, Ray CG. Coxsackie virus group B infections and the hemolytic-uremic syndrome. *J Infect Dis* 1973;**127**:698–701.
37. Avellon A, Rubio G, Palacios G, et al. Enterovirus 75 and aseptic meningitis, Spain, 2005. *Emerg Infect Dis* 2006;**12**:1609–11.
38. Avner ED, Satz J, Plotkin SA. Hypoglycorrachia in young infants with viral meningitis [Letter]. *J Pediatr* 1975;**87**:833–4.
39. Axelsson C, Bondestam K, Frisk G, et al. Coxsackie B virus infections in women with miscarriage. *J Med Virol* 1993;**39**:282–5.
40. Ayuthya PSN, Jayavasu J, Pongpanich B. Coxsackie group B virus and primary myocardial disease in infants and children. *Am Heart J* 1974;**88**:311–14.
41. Baasner A, Dettmeyer R, Graebe M, et al. PCR-based diagnosis of enterovirus and parvovirus B19 in paraffin-embedded heart tissue of children with suspected sudden infant death syndrome. *Lab Invest* 2003;**83**:1451–5.
42. Babb JM, Stoneman ME, Stern H. Myocarditis and croup caused by coxsackie virus type B5. *Arch Dis Child* 1961;**36**:551–6.
43. Badorff C, Lee GH, Lamphear BJ, et al. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med* 1999;**5**:320–6.
44. Badran SA, Midgley S, Andersen P, et al. Clinical and virological features of enterovirus 71 infections in Denmark, 2005 to 2008. *Scand J Infect Dis* 2011;**43**:642–8.
45. Bain HW, Mc LD, Walker SJ. Epidemic pleurodynia (Bornholm disease) due to coxsackie B-5 virus: the inter-relationship of pleurodynia, benign pericarditis and aseptic meningitis. *Pediatrics* 1961;**27**:889–903.
46. Baker DA, Phillips CA. Fatal hand-foot-and-mouth disease in an adult caused by coxsackievirus 47. *JAMA* 1979;**242**:1065.
47. Balduzzi PC, Greendyke RM. Sudden unexpected death in infancy and viral infection. *Pediatrics* 1966;**38**:201–6.
48. Banatvala JE. Insulin-dependent (juvenile-onset, type 1) diabetes mellitus coxsackie B viruses revisited. *Prog Med Virol* 1987;**34**: 33–54.
49. Banatvala JE, Bryant J, Schernthaner G, et al. Coxsackie B, mumps, rubella, and cytomegalovirus specific IgM responses in patients with juvenile-onset insulin-dependent diabetes mellitus in Britain, Austria, and Australia. *Lancet* 1985;**1**:1409–12.
50. Bangalore H, Ahmed J, Bible J, et al. Abdominal distension: an important feature in human parechovirus infection. *Pediatr Infect Dis J* 2011;**30**:260–2.

51. Barak Y, Schwartz JF. Acute transverse myelitis associated with ECHO type 5 infection. *Am J Dis Child* 1988;**142**:128.
52. Bardelas JA, Winkelstein JA, Seto DS, et al. Fatal ECHO 24 infection in a patient with hypogammaglobulinemia: relationship to dermatomyositis-like syndrome. *J Pediatr* 1977;**90**:396–9.
53. Barrett FF, Yow MD, Phillips CA. St. Louis encephalitis in children during the 1964 epidemic. *JAMA* 1965;**193**:381–5.
54. Barson WJ, Craenen J, Hosier DM, et al. Survival following myocarditis and myocardial calcification associated with infection by coxsackie virus B-4. *Pediatrics* 1981;**68**:79–81.
55. Barton LL. Febrile neonatal illness associated with echo virus type 5 in the cerebrospinal fluid. *Clin Pediatr (Phila)* 1977;**16**: 383–5.
56. Baskin JL, Soule EH, Mills SD. Poliomyelitis of the newborn; pathologic changes in 2 cases. *Am J Dis Child* 1950;**80**:10–21.
57. Bates HR Jr. Coxsackie virus B3 calcific pancarditis and hydrops fetalis. *Am J Obstet Gynecol* 1970;**106**:629–30.
58. Bates T. Poliomyelitis in pregnancy, fetus, and newborn. *Am J Dis Child* 1955;**90**:189–95.
59. Bauer S, Gottesman G, Sirota L, et al. Severe coxsackie virus B infection in preterm newborns treated with pleconaril. *Eur J Pediatr* 2002;**161**:491–3.
60. Bayatpour M, Zbitnew A, Dempster G, et al. Role of coxsackievirus B4 in the pathogenesis of acute glomerulonephritis. *Can Med Assoc J* 1973;**109**:873 passim.
61. Behan WM, Gow JW, Simpson K, et al. Search for picornaviruses at onset of inflammatory myopathy. *J Clin Pathol* 1996;**49**: 592–4.
62. Behbehani AM, Wenner HA. Infantile diarrhea: a study of the etiologic role of viruses. *Am J Dis Child* 1966;**111**:623–9.
63. Behbehani AM, Sulkin SE, Wallis C. Factors influencing susceptibility of mice to coxsackie virus infection. *J Infect Dis* 1962; **110**:147–54.
64. Bell EJ, Cosgrove BP. Routine enterovirus diagnosis in a human rhabdomyosarcoma cell line. *Bull World Health Organ* 1980;**58**: 423–8.
65. Bell EJ, Grist NR. Echovirus 25 Infections in Scotland, 1961-64. *Lancet* 1965;**2**:464–6.
66. Bell EJ, Grist NR. Echoviruses, carditis, and acute pleurodynia. *Lancet* 1970;**1**:326–8.
67. Bell EJ, Russell SJ. Acute transverse myelopathy and echo-2 virus infection. *Lancet* 1963;**2**:1226–7.
68. Bell JF, Meis A. Pericarditis in infection due to coxsackie virus group B type 3. *N Engl J Med* 1959;**261**:126–8.
69. Bell TM, Clark NS, Chambers W. Outbreak of illness associated with E.C.H.O. Type 7 Virus. *Br Med J* 1963;**2**:292–4.
70. Bell TM, Steyn JH. Viruses in lymph nodes of children with mesenteric adenitis and intussusception. *Br Med J* 1962;**2**: 700–2.
71. Benirschke K. *Viral infection of the placenta: viral etiology of congenital malformations*, Washington, D.C.: U.S. Government Printing Office; 1968.
72. Benirschke K, Pendleton ME. Coxsackie virus infection: an important complication of pregnancy. *Obstet Gynecol* 1958;**12**: 305–9.
73. Benschop K, Molenkamp R, van der Ham A, et al. Rapid detection of human parechoviruses in clinical samples by real-time PCR. *J Clin Virol* 2008;**41**:69–74.
74. Benschop KS, de Vries M, Minnaar RP, et al. Comprehensive full-length sequence analyses of human parechoviruses: diversity and recombination. *J Gen Virol* 2010;**91**:145–54.
75. Benschop KS, Schinkel J, Luken ME, et al. Fourth human parechovirus serotype. *Emerg Infect Dis* 2006;**12**:1572–5.
76. Benschop KS, Schinkel J, Minnaar RP, et al. Human parechovirus infections in Dutch children and the association between serotype and disease severity. *Clin Infect Dis* 2006;**42**:204–10.
77. Berg R, Jelke H. Acute cerebellar ataxia in children associated with coxsackie viruses group B. *Acta Paediatr Scand* 1965;**54**: 497–502.
78. Berger MM, Kopp N, Vital C, et al. Detection and cellular localization of enterovirus RNA sequences in spinal cord of patients with ALS. *Neurology* 2000;**54**:20–5.
79. Berglund A, Bottiger M, Johnsson T, et al. An outbreak of aseptic meningitis with a rubella-like rash probably caused by ECHO virus type 9. *Arch Gesamte Virusforsch* 1958;**8**:294–305.
80. Bergman I, Painter MJ, Wald ER, et al. Outcome in children with enteroviral meningitis during the first year of life. *J Pediatr* 1987;**110**:705–9.
81. Bergström K, Erikson U, Nordbring F, et al. Acute nonrheumatic myopericarditis: a follow-up study. *Scand J Infect Dis* 1970;**2**:7–16.
82. Berkovich S, Kibrick S. Echo II outbreak in newborn infants and mothers. *Pediatrics* 1964;**33**:534–40.
83. Berkovich S, Pangan J. Recoveries of virus from premature infants during outbreaks of respiratory disease: the relation of ECHO virus type 22 to disease of the upper and lower respiratory tract in the premature infant. *Bull N Y Acad Med* 1968; **44**:377–87.
84. Berkovich S, Smithwick EM. Transplacental infection due to ECHO virus type 22. *J Pediatr* 1968;**72**:94–6.
85. Berlin LE, Rorabaugh ML, Heldrich F, et al. Aseptic meningitis in infants < 2 years of age: diagnosis and etiology. *J Infect Dis* 1993;**168**:888–92.
86. Bern C, Pallansch MA, Gary HE Jr, et al. Acute hemorrhagic conjunctivitis due to enterovirus 70 in American Samoa: serum-neutralizing antibodies and sex-specific protection. *Am J Epidemiol* 1992;**136**:1502–6.
87. Berry PJ, Nagington J. Fatal infection with echovirus 11. *Arch Dis Child* 1982;**57**:22–9.
88. Bertaggia A, Meneghetti F, Carretta M. Observations on a case of parotitis due to coxsackie virus B3. *G Mal Infekt* 1976;**28**: 188–9.
89. Biren VP, Meitens C. Nachweis einer Coxsackie B2-Infektion bei Polyradikulitis (Guillain-Barré syndrom). *Ann Paediatr* 1965; **204**:312–22.
90. Blinkova O, Kapoor A, Victoria J, et al. Cardioviruses are genetically diverse and cause common enteric infections in South Asian children. *J Virol* 2009;**83**:4631–41.
91. Blotzer JW, Myers AR. Echovirus-associated polyarthritis: report of a case with synovial fluid and synovial histologic characterization. *Arthritis Rheum* 1978;**21**:978–81.
92. Blumental S, Reynders M, Willems A, et al. Enteroviral infection of a cardiac prosthetic device. *Clin Infect Dis* 2011;**52**:710–16.
93. Bodensteiner JB, Morris HH, Howell JT, et al. Chronic ECHO type 5 virus meningoencephalitis in X-linked hypogammaglobulinemia: treatment with immune plasma. *Neurology* 1979;**29**: 815–19.
94. Bodian D, Horstmann DM. Poliomyelitis. In: Horsfall FL, Tamm I, editors. *Viral and rickettsial infections of man*. 4th ed. Philadelphia: J. B. Lippincott; 1965. p. 430–73.
95. Boivin G, Abed Y, Boucher FD. Human parechovirus 3 and neonatal infections. *Emerg Infect Dis* 2005;**11**:103–5.
96. Boring WD, Angevine DM, Walker DL. Factors influencing host-virus interactions. I. A comparison of viral multiplication and histopathology in infant, adult, and cortisone-treated adult mice infected with the Conn. -5 strain of coxsackie virus. *J Exp Med* 1955;**102**:753–66.
97. Bose CL, Gooch WM 3rd, Sanders GO, et al. Dissimilar manifestations of intrauterine infection with echovirus 11 in premature twins. *Arch Pathol Lab Med* 1983;**107**:361–3.
98. Bottiger M, Johnsson T, Zeipel G. Family infections with acute pericarditis and myocarditis and coxsackie virus B5. *Arch Gesamte Virusforsch* 1963;**13**:153–5.
99. Bowen GS, Fisher MC, DeForest A, et al. Epidemic of meningitis and febrile illness in neonates caused by ECHO type 11 virus in Philadelphia. *Pediatr Infect Dis* 1983;**2**:359–63.
100. Bowers VM Jr, Danforth DN. The significance of poliomyelitis during pregnancy: an analysis of the literature and presentation of twenty-four new cases. *Am J Obstet Gynecol* 1953;**65**:34–9.
101. Bowles NE, Ni J, Kearney DL, et al. Detection of viruses in myocardial tissues by polymerase chain reaction. evidence of adenovirus as a common cause of myocarditis in children and adults. *J Am Coll Cardiol* 2003;**42**:466–72.
102. Bowles NE, Richardson PJ, Olsen EG, et al. Detection of coxsackie-B-virus-specific RNA sequences in myocardial biopsy samples from patients with myocarditis and dilated cardiomyopathy. *Lancet* 1986;**1**:1120–3.
103. Boyd MT, Jordan SW, Davis LE. Fatal pneumonitis from congenital echovirus type 6 infection. *Pediatr Infect Dis J* 1987;**6**: 1138–9.

104. Braun LE, Renaud C, Fairchok MP, et al. Human parechovirus and other enteric viruses in childcare attendees in the era of rotavirus vaccines. *J Ped Infect Dis* 2012;**1**:136–43.
105. Reference deleted in proofs.
106. Brightman VJ, Scott TF, Westphal M, et al. An outbreak of coxsackie B-5 virus infection in a newborn nursery. *J Pediatr* 1966;**69**:179–92.
107. Brodie HR, Marchessault V. Acute benign pericarditis caused by coxsackie virus group B. *N Engl J Med* 1960;**262**:1278–80.
108. Brown B, Oberste MS, Maher K, et al. Complete genomic sequencing shows that polioviruses and members of human enterovirus species C are closely related in the noncapsid coding region. *J Virol* 2003;**77**:8973–84.
109. Brown BA, Oberste MS, Alexander JP Jr, et al. Molecular epidemiology and evolution of enterovirus 71 strains isolated from 1970 to 1998. *J Virol* 1999;**73**:9969–75.
110. Brown GC, Karunas RS. Relationship of congenital anomalies and maternal infection with selected enteroviruses. *Am J Epidemiol* 1972;**95**:207–17.
111. Brown JM, Wright JA, Ogden WS. Hand, foot, and mouth disease. *Br Med J* 1964;**1**:58.
112. Bryant PA, Tingay D, Dargaville PA, et al. Neonatal coxsackie B virus infection-a treatable disease? *Eur J Pediatr* 2004;**163**: 223–8.
113. Bryden AS. Isolation of enteroviruses and adenoviruses in continuous simian cell lines. *Med Lab Sci* 1992;**49**:60–5.
114. Buckland FE, Bynoe ML, Philipson L, et al. Experimental infection of human volunteers with the U-virus-a strain of ECHO virus type 11. *J Hyg (Lond)* 1959;**57**:274–84.
115. Burch GE, Colcolough HL. Progressive Coxsackie viral pancarditis and nephritis. *Ann Intern Med* 1969;**71**:963–70.
116. Burch GE, Sun SC, Chu KC, et al. Interstitial and coxsackievirus B myocarditis in infants and children: a comparative histologic and immunofluorescent study of 50 autopsied hearts. *JAMA* 1968;**203**:1–8.
117. Burch GE, Sun SC, Colcolough HL, et al. Coxsackie B viral myocarditis and valvulitis identified in routine autopsy specimens by immunofluorescent techniques. *Am Heart J* 1967;**74**:13–23.
118. Burry JN, Moore B, Mattner C. Hand, foot and mouth disease in South Australia. *Med J Aust* 1968;**2**:587–9.
119. Byington CL, Taggart EW, Carroll KC, et al. A polymerase chain reaction-based epidemiologic investigation of the incidence of nonpolio enteroviral infections in febrile and afebrile infants 90 days and younger. *Pediatrics* 1999;**103**:E27.
120. Cabinian AE, Kiel RJ, Smith F, et al. Modification of exercise-aggravated coxsackievirus B3 murine myocarditis by T lymphocyte suppression in an inbred model. *J Lab Clin Med* 1990;**115**: 454–62.
121. Cabrera-Rode E, Sarmiento L, Tiberti C, et al. Type 1 diabetes islet associated antibodies in subjects infected by echovirus 16. *Diabetologia* 2003;**46**:1348–53.
122. Cabrerizo M, Echevarria JE, Otero A, et al. Molecular characterization of a coxsackievirus A24 variant that caused an outbreak of acute haemorrhagic conjunctivitis in Spain, 2004. *J Clin Virol* 2008;**43**:323–7.
123. Calder BD, Warnock PJ, McCartney RA, et al. Coxsackie B viruses and the post-viral syndrome: a prospective study in general practice. *J R Coll Gen Pract* 1987;**37**:11–14.
124. Canby JP. Petachiae and fever: infection with coxsackie virus group B, type 3—case report. *Clin Pediatr (Phila)* 1963;**2**:187–8.
125. Cao Y, Schnurr DP, Schmidt NJ. Differing cardiotropic and myocarditic properties of group B type 4 coxsackievirus strains. *Arch Virol* 1984;**80**:119–30.
126. Capner P, Lendrum R, Jeffries DJ, et al. Viral antibody studies in pancreatic disease. *Gut* 1975;**16**:866–70.
127. Cardosa MJ, Perera D, Brown BA, et al. Molecular epidemiology of human enterovirus 71 strains and recent outbreaks in the Asia-Pacific region: comparative analysis of the VP1 and VP4 genes. *Emerg Infect Dis* 2003;**9**:461–8.
128. Carolane DJ, Long AM, McKeever PA, et al. Prevention of spread of echovirus 6 in a special care baby unit. *Arch Dis Child* 1985;**60**:674–6.
129. Carstens JM, Tracy S, Chapman NM, et al. Detection of enteroviruses in cell cultures by using in situ transcription. *J Clin Microbiol* 1992;**30**:25–35.
130. Cashman NR, Maselli R, Wollmann RL, et al. Late denervation in patients with antecedent paralytic poliomyelitis. *N Engl J Med* 1987;**317**:7–12.
131. Centers for Disease Control and Prevention. Follow-up on poliomyelitis, Connecticut, New York, Massachusetts, New Hampshire. *MMWR Morb Mortal Wkly Rep* 1972;**21**:365–6.
132. Centers for Disease Control and Prevention. *Poliomyelitis surveillance summary: 1974-6*. Atlanta: U.S. Department of Health, Education and Welfare; 1977.
133. Centers for Disease Control and Prevention. *Aseptic meningitis surveillance: annual smmary 1976*. Atlanta: U.S. Department of Health, Education and Welfare; 1979.
134. Centers for Disease Control and Prevention. Follow-up on poliomyelitis: United States, Canada, Netherlands. *MMWR Morb Mortal Wkly Rep* 1979;**28**:345–6.
135. Centers for Disease Control and Prevention. Annual summary 1979: Reported morbidity and mortality in the United States. *MMWR Morb Mortal Wkly Rep* 1980;**28**:1–119.
136. Centers for Disease Control and Prevention. Acute hemorrhagic conjunctivitis: Florida, North Carolina. *MMWR Morb Mortal Wkly Rep* 1981;**30**:501–2.
137. Centers for Disease Control and Prevention. *Encephalitis surveillance: annual summary, 1978*. Atlanta: U.S. Department of Health and Human Services; 1981.
138. Centers for Disease Control and Prevention. Enterovirus surveillance: United States, 1985. *MMWR Morb Mortal Wkly Rep* 1985;**34**:494–5.
139. Centers for Disease Control and Prevention. Acute hemorrhagic conjunctivitis: Mexico. *MMWR Morb Mortal Wkly Rep* 1989;**38**: 327–9.
140. Centers for Disease Control and Prevention. Certification of poliomyelitis eradication: the Americas, 1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**:720–2.
141. Centers for Disease Control and Prevention. Progress toward global poliomyelitis eradication, 1985-1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**:273–5, 281.
142. Centers for Disease Control and Prevention. Nonpolio enterovirus surveillance: United States, 1993-1996. *MMWR Morb Mortal Wkly Rep* 1997;**46**:748–857.
143. Centers for Disease Control and Prevention. Enterovirus surveillance: United States, 1997-1999. *MMWR Morb Mortal Wkly Rep* 2000;**49**:913–16.
144. Centers for Disease Control and Prevention. Echovirus type 13: United States, 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**: 777–80.
145. Centers for Disease Control and Prevention. Enterovirus surveillance: United States, 2000-2001. *MMWR Morb Mortal Wkly Rep* 2002;**51**:1047–9.
146. Centers for Disease Control and Prevention. Acute hemorrhagic conjunctivitis outbreak caused by coxsackievirus A24: Puerto Rico, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**: 632–4.
147. Centers for Disease Control and Prevention. Enterovirus surveillance: United States, 2002-2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:153–6.
148. Centers for Disease Control and Prevention. Resurgence of wild poliovirus type 1 transmission and consequences of importation: 21 countries, 2002-2005. *MMWR Morb Mortal Wkly Rep* 2006; **55**:145–50.
149. Centers for Disease Control and Prevention. Increased detections and severe neonatal disease associated with coxsackievirus B1 infection: United States, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:553–6.
150. Centers for Disease Control and Prevention. Nonpolio enterovirus and human parechovirus surveillance: United States, 2006-2008. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1577–80.
151. Centers for Disease Control and Prevention. Notes from the field: severe hand, foot, and mouth disease associated with coxsackievirus A6—Alabama, Connecticut, California, and Nevada, November 2011-February 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:213–14.
152. Centers for Disease Control and Prevention. Progress toward interruption of wild poliovirus transmission: worldwide, January 2011-March 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 353–7.

153. Centers for Disease Control and Prevention. Khetsuriani N, Lamonte-Fowlkes A, et al. Enterovirus surveillance: United States, 1970-2005. *MMWR Surveill Summ* 2006;**55**:1–20.
154. Chalhub EG, Devivo DC, Siegel BA, et al. Coxsackie A9 focal encephalitis associated with acute infantile hemiplegia and porencephaly. *Neurology* 1977;**27**:574–9.
155. Chambon M, Delage C, Bailly JL, et al. Fatal hepatic necrosis in a neonate with echovirus 20 infection: use of the polymerase chain reaction to detect enterovirus in liver tissue. *Clin Infect Dis* 1997;**24**:523–4.
156. Champsaur HF, Bottazzo GF, Bertrams J, et al. Virologic, immunologic, and genetic factors in insulin-dependent diabetes mellitus. *J Pediatr* 1982;**100**:15–20.
157. Chan D, Hammond GW. Comparison of serodiagnosis of group B coxsackievirus infections by an immunoglobulin M capture enzyme immunoassay versus microneutralization. *J Clin Microbiol* 1985;**21**:830–4.
158. Chan KP, Goh KT, Chong CY, et al. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. *Emerg Infect Dis* 2003;**9**:78–85.
159. Chan LG, Parashar UD, Lye MS, et al. Deaths of children during an outbreak of hand, foot, and mouth disease in sarawak, malaysia: clinical and pathological characteristics of the disease. For the Outbreak Study Group. *Clin Infect Dis* 2000;**31**:678–83.
160. Chan YF, AbuBaker S. Recombinant human enterovirus 71 in hand, foot and mouth disease patients. *Emerg Infect Dis* 2004; **10**:1468–70.
161. Chandy KG, John TJ, Mukundan P, et al. Coxsackie B antibodies in "rheumatic" valvular heart-disease. *Lancet* 1979;**1**:381.
162. Chang LY, Hsia SH, Wu CT, et al. Outcome of enterovirus 71 infections with or without stage-based management: 1998 to 2002. *Pediatr Infect Dis J* 2004;**23**:327–32.
163. Chang LY, Hsiung CA, Lu CY, et al. Status of cellular rather than humoral immunity is correlated with clinical outcome of enterovirus 71. *Pediatr Res* 2006;**60**:466–71.
164. Chang LY, Huang LM, Gau SS, et al. Neurodevelopment and cognition in children after enterovirus 71 infection. *N Engl J Med* 2007;**356**:1226–34.
165. Chang LY, King CC, Hsu KH, et al. Risk factors of enterovirus 71 infection and associated hand, foot, and mouth disease/ herpangina in children during an epidemic in Taiwan. *Pediatrics* 2002;**109**:e88.
166. Chang LY, Lin TY, Hsu KH, et al. Clinical features and risk factors of pulmonary oedema after enterovirus-71-related hand, foot, and mouth disease. *Lancet* 1999;**354**:1682–6.
167. Chang LY, Tsao KC, Hsia SH, et al. Transmission and clinical features of enterovirus 71 infections in household contacts in Taiwan. *JAMA* 2004;**291**:222–7.
168. Chang ML, Tsao KC, Huang CC, et al. Coxsackievirus B3 in human milk. *Pediatr Infect Dis J* 2006;**25**:955–7.
169. Chang TW, Weinstein L. Infection of the nervous system by coxsackie A9 virus. *Bull Tufts N E Med Ctr* 1960;**6**:181–93.
170. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. *Pediatrics* 1965;**36**:21–39.
171. Chatterjee S, Quarcoopome CO, Apenteng A. Unusual type of epidemic conjunctivitis in Ghana. *Br J Ophthalmol* 1970;**54**: 628–30.
172. Chaves SS, Lobo S, Kennett M, et al. Coxsackie virus A24 infection presenting as acute flaccid paralysis. *Lancet* 2001; **357**:605.
173. Chawareewong S, Kiangsiri S, Lokaphadhana K, et al. Neonatal herpangina caused by coxsackie A-5 virus. *J Pediatr* 1978;**93**: 492–4.
174. Chehadeh W, Weill J, Vantyghem MC, et al. Increased level of interferon-alpha in blood of patients with insulin-dependent diabetes mellitus: relationship with coxsackievirus B infection. *J Infect Dis* 2000;**181**:1929–39.
175. Chemtob S, Reece ER, Mills EL. Syndrome of inappropriate secretion of antidiuretic hormone in enteroviral meningitis. *Am J Dis Child* 1985;**139**:292–4.
176. Chen CA, Tsao PN, Chou HC, et al. Severe echovirus 30 infection in twin neonates. *J Formos Med Assoc* 2003;**102**:59–61.
177. Chen JH, Chiu NC, Chang JH, et al. A neonatal echovirus 11 outbreak in an obstetric clinic. *J Microbiol Immunol Infect* 2005; **38**:332–7.
178. Chen KT, Chang HL, Wang ST, et al. Epidemiologic features of hand-foot-mouth disease and herpangina caused by enterovirus 71 in Taiwan, 1998-2005. *Pediatrics* 2007;**120**:e244–252.
179. Cheng LL, Ng PC, Chan PK, et al. Probable intrafamilial transmission of coxsackievirus b3 with vertical transmission, severe early-onset neonatal hepatitis, and prolonged viral RNA shedding. *Pediatrics* 2006;**118**:e929–933.
180. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.
181. Cherry JD. Enteroviruses. In: Behrman RE, Vaughan VC, editors. *Nelson textbook of pediatrics*. 12th ed. Philadelphia: W. B. Saunders; 1983. p. 791–804.
182. Cherry JD. Enteroviruses: polioviruses (poliomyelitis), coxsackieviruses, echoviruses, and enteroviruses. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 2nd ed. Philadelphia: W. B. Saunders; 1987. p. 1729–90.
183. Cherry JD. Enteroviruses. In: Remington JS, Klein JO, editors. *Infectious Diseases of the Fetus and Newborn Infant*. 3rd ed. Philadelphia: W. B. Saunders; 1990. p. 325–66.
184. Cherry JD. Herpangina. In: Feigin R, Cherry JD, Demmler-Harrison G, et al, editors. *Textbook of pediatric infectious diseases*. 6th ed. Philadelphia: Saunders; 2009. p. 169–71.
185. Cherry JD, Jahn CL. Concomitant enterovirus infecton, smallpox vaccination, and exanthem. *J Pediatr* 1965;**67**:679–81.
186. Cherry JD, Jahn CL. Hand, foot, and mouth syndrome: report of six cases due to coxsackie virus, group A, type 16. *Pediatrics* 1966;**37**:637–43.
187. Cherry JD, Jahn CL. Virologic studies of exanthems. *J Pediatr* 1966;**68**:204–14.
188. Cherry JD, Nelson DB. Enterovirus infections: their epidemiology and pathogenesis: with particular reference to coxsackie A 16 virus. *Clin Pediatr (Phila)* 1966;**5**:659–64.
189. Cherry JD, Jahn CL, Meyer TC. Paroxysmal atrial tachycardia associated with ECHO 9 virus infection. *Am Heart J* 1967;**73**: 681–6.
190. Cherry JD, Soriano F, Jahn CL. Search for perinatal viral infection: a prospective, clinical, virologic, and serologic study. *Am J Dis Child* 1968;**116**:245–50.
191. Cherry JD, Bobinski JE, Horvath FL, et al. Acute hemangioma-like lesions associated with ECHO viral infections. *Pediatrics* 1969;**44**:498–502.
192. Cherry JD, Lerner AM, Klein JO, et al. Coxsackie A9 infections with exanthems, with particular reference to urticaria. *Pediatrics* 1963;**31**:819–23.
193. Cherry JD, Lerner AM, Klein JO, et al. Coxsackie B5 infections with exanthems. *Pediatrics* 1963;**31**:455–62.
194. Cherry JD, Lerner AM, Klein JO, et al. Echo 11 Virus infections associated with exanthems. *Pediatrics* 1963;**32**:509–16.
195. Chesney JC, Hoganson GE, Wilson MH. CSF eosinophilia during an acute coxsackie B4 viral meningitis. *Am J Dis Child* 1980;**134**:703.
196. Chesney PJ, Quennec P, Clark C. Hypoglycorrhachia and coxsackie B3 meningoencephalitis. *Am J Clin Pathol* 1978;**70**: 947–8.
197. Chevaliez S, Szendroi A, Caro V, et al. Molecular comparison of echovirus 11 strains circulating in Europe during an epidemic of multisystem hemorrhagic disease of infants indicates that evolution generally occurs by recombination. *Virology* 2004;**325**: 56–70.
198. Chezzi C. Rapid diagnosis of poliovirus infection by PCR amplification. *J Clin Microbiol* 1996;**34**:1722–5.
199. Chia JK. The role of enterovirus in chronic fatigue syndrome. *J Clin Pathol* 2005;**58**:1126–32.
200. Chia JK, Bold EJ. Life-threatening leukocytoclastic vasculitis with pulmonary involvement due to echovirus 7. *Clin Infect Dis* 1998;**27**:1326–7.
201. Chia JK, Chia A. Diverse etiologies for chronic fatigue syndrome. *Clin Infect Dis* 2003;**36**:671–2; author reply 672–3.
202. Chia JK, Chia AY. Chronic fatigue syndrome is associated with chronic enterovirus infection of the stomach. *J Clin Pathol* 2008;**61**:43–8.
203. Chia J, Chia A, Voeller M, et al. Acute enterovirus infection followed by myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) and viral persistence. *J Clin Pathol* 2010;**63**:165–8.
204. Chin TD, Lehan PH, Rubin H, et al. Epidemiological studies of aseptic meningitis caused by coxsackie virus B5. *Am J Public Health Nations Health* 1958;**48**:1193–200.

205. Chiu CY, Greninger AL, Kanada K, et al. Identification of cardioviruses related to Theiler's murine encephalomyelitis virus in human infections. *Proc Natl Acad Sci U S A* 2008; **105**:14124–9.
206. Cho CT, Janelle JG, Behbehani A. Severe neonatal illness associated with echo 9 virus infection. *Clin Pediatr (Phila)* 1973;**12**: 304–5.
207. Chomel JJ, Thouvenot D, Fayol V, et al. Rapid diagnosis of echovirus type 33 meningitis by specific IgM detection using an enzyme linked immunosorbent assay (ELISA). *J Virol Methods* 1985;**10**:11–19.
208. Chong CY, Chan KP, Shah VA, et al. Hand, foot and mouth disease in Singapore: a comparison of fatal and non-fatal cases. *Acta Paediatr* 2003;**92**:1163–9.
209. Chonmaitree T, Menegus MA, Powell KR. The clinical relevance of "CSF viral culture": a two-year experience with aseptic meningitis in Rochester, NY. *JAMA* 1982;**247**:1843–7.
210. Chonmaitree T, Ford C, Sanders C, et al. Comparison of cell cultures for rapid isolation of enteroviruses. *J Clin Microbiol* 1988;**26**:2576–80.
211. Chonmaitree T, Menegus MA, Schervish-Swierkosz EM, et al. Enterovirus 71 infection: report of an outbreak with two cases of paralysis and a review of the literature. *Pediatrics* 1981;**67**: 489–93.
212. Chou SM, Gutmann L. Picornavirus-like crystals in subacute polymyositis. *Neurology* 1970;**20**:205–13.
213. Chow KC, Lee CC, Lin TY, et al. Congenital enterovirus 71 infection: a case study with virology and immunohistochemistry. *Clin Infect Dis* 2000;**31**:509–12.
214. Chow LH, Gauntt CJ, McManus BM. Differential effects of myocarditic variants of coxsackievirus B3 in inbred mice: a pathologic characterization of heart tissue damage. *Lab Invest* 1991; **64**:55–64.
215. Christen AG, Crandell RA, Kerstein MH. Hand-foot-and-mouth disease in a father and son. *Oral Surg Oral Med Oral Pathol* 1967;**24**:427–32.
216. Christensen ML, Pachman LM, Schneiderman R, et al. Prevalence of coxsackie B virus antibodies in patients with juvenile dermatomyositis. *Arthritis Rheum* 1986;**29**:1365–70.
217. Christie AB. Acute poliomyelitis. In: Christie AB, editor. *Infectious diseases: epidemiology and clinical practice*. 2nd ed. Edinburgh: Churchill Livingstone; 1974. p. 567–614.
218. Christopher S, Theogaraj S, Godbole S, et al. An epidemic of acute hemorrhagic conjunctivitis due to coxsackievirus A24. *J Infect Dis* 1982;**146**:16–19.
219. Chuang E, Maller ES, Hoffman MA, et al. Successful treatment of fulminant echovirus 11 infection in a neonate by orthotopic liver transplantation. *J Pediatr Gastroenterol Nutr* 1993;**17**: 211–14.
220. Clarke A, Stott J. E.C.H.O. type 20. *Br Med J* 1961;**1**:900–1.
221. Clarke M, Hunter M, Mc NG, et al. Seasonal aseptic meningitis caused by coxsackie and ECHO viruses, Toronto, 1957. *Can Med Assoc J* 1959;**81**:5–8.
222. Clarke SKR, Morley T, Warin RP. Hand, foot, and mouth disease. *Br Med J* 1964;**1**:58.
223. Clements GB, Galbraith DN, Taylor KW. Coxsackie B virus infection and onset of childhood diabetes. *Lancet* 1995;**346**: 221–3.
224. Clements GB, McGarry F, Nairn C, et al. Detection of enterovirus-specific RNA in serum: the relationship to chronic fatigue. *J Med Virol* 1995;**45**:156–61.
225. Clemmer DI, Li F, Le Blanc DR, et al. An outbreak of subclinical infection with coxsackievirus B3 in southern Louisiana. *Am J Epidemiol* 1966;**83**:123–9.
226. Cochi SL, Linkins RW. The final phase of polio eradication: new vaccines and complex choices. *J Infect Dis* 2012;**205**: 169–71.
227. Cochi SL, Hull HF, Sutter RW, et al. Commentary: the unfolding story of global poliomyelitis eradication. *J Infect Dis* 1997;**175** Suppl 1:S1–3.
228. Cochrane HR, May FE, Ashcroft T, et al. Enteroviruses and idiopathic dilated cardiomyopathy. *J Pathol* 1991;**163**:129–31.
229. Codd AA, Hale JH. Epidemic of echovirus 19 in the north-east of England. *J Hyg (Lond)* 1976;**76**:307–17.
230. Constable FL, Howitt LF. Outbreak of E.C.H.O. type 9 infection in a children's home. *Br Med J* 1961;**1**:1483–6.
231. Cooper JB, Pratt WR, English BK, et al. Coxsackievirus B3 producing fatal meningoencephalitis in a patient with X-linked agammaglobulinemia. *Am J Dis Child* 1983;**137**:82–3.
232. Coucke C, Kint A, Gabriel P. Hand, foot and mouth disease in the adult subject. *Dermatologica* 1976;**153**:272–6.
233. Couvreur J, Cook CM, Chany C, et al. [An epidemic of ECHO virus type 16 exanthema in a hospital with the statistics of a longitudinal study]. *Arch Gesamte Virusforsch* 1963;**13**:215–32.
234. Craighead JE, Mahoney EM, Carver DH, et al. Orchitis due to coxsackie virus group B, type 5: report of a case with isolation of virus from the testis. *N Engl J Med* 1962;**267**:498–500.
235. Cramblett HG, Haynes RE, Azimi PH, et al. Nosocomial infection with Echovirus type II in handicapped and premature infants. *Pediatrics* 1973;**51**:603–7.
236. Cramblett HG, Moffet HL, Black JP, et al. Coxsackie virus infections: clinical and laboratory studies. *J Pediatr* 1964;**64**: 406–14.
237. Cramblett HG, Moffet HL, Middleton GK, et al. ECHO 19 virus infections: clinical and laboratory studies. *Arch Intern Med* 1962;**110**:574–9.
238. Cramblett HG, Rosen L, Parrott RH, et al. Respiratory illness in six infants infected with a newly recognized ECHO virus. *Pediatrics* 1958;**21**:168–77.
239. Crawford M, Macrae AD, O'Reilly JN. An unusual illness in young children associated with an enteric virus. *Arch Dis Child* 1956;**31**:182–8.
240. Crennan JM, Van Scoy RE, McKenna CH, et al. Echovirus polymyositis in patients with hypogammaglobulinemia: failure of high-dose intravenous gammaglobulin therapy and review of the literature. *Am J Med* 1986;**81**:35–42.
241. Crow KD, Warin R, Wilkinson DS. Hand, foot, and mouth disease. *Br Med J* 1963;**2**:1267–8.
242. Cudworth AG, White GBB, Woodrow JC, et al. Etiology of juvenile-onset diabetes: a prospective study. *Lancet* 1977;**1**: 385–8.
243. Cunningham CK, Bonville CA, Ochs HD, et al. Enteroviral meningoencephalitis as a complication of X-linked hyper IgM syndrome. *J Pediatr* 1999;**134**:584–8.
244. Curnen EC, Shaw EW, Melnick JL. Disease resembling nonparalytic poliomyelitis associated with a virus pathogenic for infant mice. *J Am Med Assoc* 1949;**141**:894–901.
245. Daae A. Epidemi i drangedal af akut muskelrheumatisme udbredt ved smitte. *Norsk Mag F Laegevidensk* 1872;**2**:409–13, 529–42.
246. Daboval T, Ferretti E, Duperval R. High C-reactive protein levels during a benign neonatal outbreak of echovirus type 7. *Am J Perinatol* 2006;**23**:299–304.
247. Dagan R, Jenista JA, Menegus MA. Association of clinical presentation, laboratory findings, and virus serotypes with the presence of meningitis in hospitalized infants with enterovirus infection. *J Pediatr* 1988;**113**:975–8.
248. Dagan R, Prather SL, Powell KR, et al. Neutralizing antibodies to non-polio enteroviruses in human immune serum globulin. *Pediatr Infect Dis* 1983;**2**:454–6.
249. Dalakas MC. Enteroviruses in chronic fatigue syndrome: "now you see them, now you don't." *J Neurol Neurosurg Psychiatry* 2003;**74**:1361–2.
250. Dalakas MC, Elder G, Hallett M, et al. A long-term follow-up study of patients with post-poliomyelitis neuromuscular symptoms. *N Engl J Med* 1986;**314**:959–63.
251. Dalakas MC, Sever JL, Madden DL, et al. Late postpoliomyelitis muscular atrophy: clinical, virologic, and immunologic studies. *Rev Infect Dis* 1984;**6**(suppl 2):S562–567.
252. Dalal I, Tzhori S, Somekh E, et al. Cytokine profile in cerebrospinal fluid of children with echovirus type 4 meningitis. *Pediatr Neurol* 2003;**29**:312–16.
253. Dalldorf F, Melnick JL. Coxsackie viruses. In: Horsfall FL, Tamm I. editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1965. p. 474–512.
254. Dalldorf G, Sickles GM. An unidentified, filtrable agent isolated from the feces of children with paralysis. *Science* 1948;**108**: 61–2.
255. DaSilva EE, Winkler MT, Pallansch MA. Role of enterovirus 71 in acute flaccid paralysis after the eradication of poliovirus in Brazil. *Emerg Infect Dis* 1996;**2**:231–3.
256. Davies DP, Hughes CA, MacVicar J, et al. Echovirus-11 infection in a special-care baby unit. *Lancet* 1979;**1**:96.

257. Davies JW, Mc DA, Severs D. Epidemic virus meningitis due to ECHO 9 virus in Newfoundland. *Can Med Assoc J* 1958;**79**:162–7.
258. De L, Nottay B, Yang CF, et al. Identification of vaccine-related polioviruses by hybridization with specific RNA probes. *J Clin Microbiol* 1995;**33**:562–71.
259. de Groot-Mijnes JD, de Visser L, Zuurveen S, et al. Identification of new pathogens in the intraocular fluid of patients with uveitis. *Am J Ophthalmol* 2010;**150**:628–36.
260. de Leeuw N, Melchers WJ, Balk AH, et al. No evidence for persistent enterovirus infection in patients with end-stage idiopathic dilated cardiomyopathy. *J Infect Dis* 1998;**178**:256–9.
261. De Petris L, Gianviti A, Caione D, et al. Role of non-polio enterovirus infection in pediatric hemolytic uremic syndrome. *Pediatr Nephrol* 2002;**17**:852–5.
262. De Quadros CA, Andrus JK, Olive JM, et al. Eradication of poliomyelitis: progress in the Americas. *Pediatr Infect Dis J* 1991;**10**:222–9.
263. Dechamps C, Peigue-Lafeuille HH, Laveran H, et al. Four cases of vesicular lesions in adults caused by enterovirus infections. *J Clin Microbiol* 1988;**26**:2182–3.
264. Dekel B, Yoeli R, Shulman L, et al. Localized thigh swelling mimicking a neoplastic process: involvement of coxsackie virus type A21. *Acta Paediatr* 2002;**91**:357–9.
265. Dery P, Marks MI, Shapera R. Clinical manifestations of coxsackievirus infections in children. *Am J Dis Child* 1974;**128**:464–8.
266. DesA'neto A, Bullington JD, Bullington RH, et al. Coxsackie B5 heart disease: demonstration of inferolateral wall myocardial necrosis. *Am J Med* 1980;**68**:295–8.
267. Deseda-Tous J, Byatt PH, Cherry JD. Vesicular lesions in adults due to echovirus 11 infections. *Arch Dermatol* 1977;**113**:1705–6.
268. Desmond RA, Accortt NA, Talley L, et al. Enteroviral meningitis: natural history and outcome of pleconaril therapy. *Antimicrob Agents Chemother* 2006;**50**:2409–14.
269. Dettmeyer R, Baasner A, Schlamann M, et al. Coxsackie B3 myocarditis in 4 cases of suspected sudden infant death syndrome: diagnosis by immunohistochemical and molecular-pathologic investigations. *Pathol Res Pract* 2002;**198**:689–96.
270. DeVries AS, Harper J, Murray A, et al. Vaccine-derived poliomyelitis 12 years after infection in Minnesota. *N Engl J Med* 2011;**364**:2316–23.
271. Dippe SE, Bennett PH, Miller M, et al. Lack of causal association between coxsackie B4 virus infection and diabetes. *Lancet* 1975;**1**:1314–17.
272. Doedens JR, Kirkegaard K. Inhibition of cellular protein secretion by poliovirus proteins 2B and 3A. *EMBO J* 1995;**14**:894–907.
273. Domok I, Molnar E. An outbreak of meningoencephalomyocarditis among newborn infants during the epidemic of Bornholm disease of 1958 in Hungary. II. Aetiological findings. *Ann Paediatr* 1960;**194**:102–14.
274. Domok I, Balayan MS, Fayinka OA, et al. Factors affecting the efficacy of live poliovirus vaccine in warm climates: efficacy of type 1 Sabin vaccine administered together with antihuman gamma-globulin horse serum to breast-fed and artificially fed infants in Uganda. *Bull World Health Organ* 1974;**51**:333–47.
275. Douche-Aourik F, Berlier W, Feasson L, et al. Detection of enterovirus in human skeletal muscle from patients with chronic inflammatory muscle disease or fibromyalgia and healthy subjects. *J Med Virol* 2003;**71**:540–7.
276. Dowdle W, Kew O. Vaccine-derived polioviruses: is it time to stop using the word "rare"? *J Infect Dis* 2006;**194**:539–41.
277. Dowdle WR, De Gourville E, Kew OM, et al. Polio eradication: the OPV paradox. *Rev Med Virol* 2003;**13**:277–91.
278. Drebot MA, Nguan CY, Campbell JJ, et al. Molecular epidemiology of enterovirus outbreaks in Canada during 1991-1992: identification of echovirus 30 and coxsackievirus B1 strains by amplicon sequencing. *J Med Virol* 1994;**44**:340–7.
279. Drew JH. Echo 11 virus outbreak in a nursery associated with myocarditis. *Aust Paediatr J* 1973;**9**:90–5.
280. Drexler JF, Baumgarte S, Eschbach-Bludau M, et al. Human cardioviruses, meningitis, and sudden infant death syndrome in children. *Emerg Infect Dis* 2011;**17**:2313–15.
281. Drexler JF, Baumgarte S, Luna LK, et al. Genomic features and evolutionary constraints in Saffold-like cardioviruses. *J Gen Virol* 2010;**91**:1418–27.
282. Drexler JF, Luna LK, Stocker A, et al. Circulation of 3 lineages of a novel Saffold cardiovirus in humans. *Emerg Infect Dis* 2008;**14**:1398–405.
283. Drouhet V. Enterovirus infection and associated clinical symptoms in children. *Ann Inst Pasteur* 1960;**98**:562–8.
284. Druyts-Voets E. Epidemiological features of entero non-poliovirus isolations in Belgium 1980-94. *Epidemiol Infect* 1997;**119**:71–7.
285. Duff MF. Hand-foot-and-mouth syndrome in humans: coxackie A10 infections in New Zealand. *Br Med J* 1968;**2**:661–4.
286. Dunn JJ, Romero JR, Wasserman R, et al. Stable enterovirus 5′ nontranslated region over a 7-year period in a patient with agammaglobulinemia and chronic infection. *J Infect Dis* 2000;**182**:298–301.
287. Duxbury AE, Warner P. Epidemiological and laboratory investigations on Bornholm disease in Adelaide, 1957. *Med J Aust* 1958;**45**:518–23.
288. Duxbury AE, White J, Lipscomb BM, et al. Illness simulating paralytic poliomyelitis associated with coxsackie group A type 4 virus infection. *Med J Aust* 1961;**48**:708–11.
289. Dwyer JM, Erlendsson K. Intraventricular gamma-globulin for the management of enterovirus encephalitis. *Pediatr Infect Dis J* 1988;**7**:S30–33.
290. Dyer JR, Edis RH, French MA. Enterovirus associated neurological disease in an HIV-1 infected man. *J Neurovirol* 1998;**4**:569–71.
291. Eckert GL, Barron AL, Karzon DT. Aseptic meningitis due to ECHO virus type 18. *AMA J Dis Child* 1960;**99**:1–3.
292. Eckert HL, Portnoy B, Salvatore MA, et al. Group B, coxsackie virus infection in infants with acute lower respiratory disease. *Pediatrics* 1967;**39**:526–31.
293. Egger D, Pasamontes L, Ostermayer M, et al. Reverse transcription multiplex PCR for differentiation between polio- and enteroviruses from clinical and environmental samples. *J Clin Microbiol* 1995;**33**:1442–7.
294. Ehrnst A, Eriksson M. Epidemiological features of type 22 echovirus infection. *Scand J Infect Dis* 1993;**25**:275–81.
295. Eichenwald HF, Kotsevalov O. Immunologic responses of premature and full-term infants to infection with certain viruses. *Pediatrics* 1960;**25**:829–39.
296. Eichenwald HF, Ababio A, Arky AM, et al. Epidemic diarrhea in premature and older infants caused by ECHO virus type 18. *J Am Med Assoc* 1958;**166**:1563–6.
297. Eilard T, Kyllerman M, Wennerblom I, et al. An outbreak of coxsackie virus type B2 among neonates in an obstetrical ward. *Acta Paediatr Scand* 1974;**63**:103–7.
298. El-Hagrassy MM, Banatvala JE, Coltart DJ. Coxsackie-B-virus-specific IgM responses in patients with cardiac and other diseases. *Lancet* 1980;**2**:1160–2.
299. Elizan TS, Ajero-Froehlich L, Fabiyi A, et al. Viral infection in pregnancy and congenital CNS malformations in man. *Arch Neurol* 1969;**20**:115–19.
300. Elliott GB, McAllister JE. Fetal poliomyelitis. *Am J Obstet Gynecol* 1956;**72**:896–902.
301. Elvin-Lewis M, Melnick JL. ECHO 11 virus associated with aseptic meningitis. *Proc Soc Exp Biol Med* 1959;**102**:647–9.
302. Enders JF, Weller TH, Robbins FC. Cultivation of the Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. *Science* 1949;**109**:85–7.
303. Ergul Y, Ekici B, Tastan Y, et al. Vestibular neuritis caused by enteroviral infection. *Pediatr Neurol* 2006;**34**:45–6.
304. Erlendsson K, Swartz T, Dwyer JM. Successful reversal of echovirus encephalitis in X-linked hypogammaglobulinemia by intraventricular administration of immunoglobulin. *N Engl J Med* 1985;**312**:351–3.
305. Estes ML, Rorke LB. Liquefactive necrosis in coxsackie B encephalitis. *Arch Pathol Lab Med* 1986;**110**:1090–2.
306. Euscher E, Davis J, Holzman I, et al. Coxsackie virus infection of the placenta associated with neurodevelopmental delays in the newborn. *Obstet Gynecol* 2001;**98**:1019–26.
307. Evans AD, Waddington E. Hand, foot and mouth disease in south Wales, 1964. *Br J Dermatol* 1967;**79**:309–17.

308. Evans AS, Dick EC. Acute Pharyngitis and tonsillitis in University of Wisconsin students. *JAMA* 1964;**190**:699–708.
309. Farmer K, MacArthur BA, Clay MM. A follow-up study of 15 cases of neonatal meningoencephalitis due to coxsackie virus B5. *J Pediatr* 1975;**87**:568–71.
310. Farmer K, Patten PT. An outbreak of coxsackie B5 infection in a special care unit for newborn infants. *N Z Med J* 1968; **68**:86–9.
311. Faulkner RS, Van Rooyen CE. Echovirus type 17 in the neonate. *Can Med Assoc J* 1973;**108**:878 passim.
312. Faulkner RS, Macleod AJ, Van Rooyen CE. Virus meningitis: seven cases in one family. *Can Med Assoc J* 1957;**77**:439–44.
313. Faustini A, Fano V, Muscillo M, et al. An outbreak of aseptic meningitis due to echovirus 30 associated with attending school and swimming in pools. *Int J Infect Dis* 2006;**10**:291–7.
314. Fechner RE, Smith MG, Middlekamp JN. Coxsackie B virus infection of the newborn. *Am J Pathol* 1963;**42**:493–505.
315. Feldman AM, McNamara D. Myocarditis. *N Engl J Med* 2000;**343**:1388–98.
316. Feldman W, Larke RP. Acute cerebellar ataxia associated with the isolation of coxsackievirus type A9. *Can Med Assoc J* 1972; **106**:1104 passim.
317. Felici A, Gregorig B. Contribution to the study of diseases in Italy caused by the coxsackie B group of viruses. II. Epidemiological, clinical and virological data obtained in the course of a summer outbreak caused by coxsackie B 4 virus. *Arch Gesamte Virusforsch* 1959;**9**:317–28.
318. Felici A, Archetti I, Russi F, et al. Contribution to the study of diseases caused by the Coxsackie B group of viruses in Italy. III. Role of coxsackie B virus type 3 in summer diarrheal infections in infants and children. *Arch Gesamte Virusforsch* 1962;**11**: 592–8.
319. Fenner F. Classification and nomenclature of viruses: second report of the International Committee on Taxonomy of Viruses. *Intervirology* 1976;**7**:1–115.
320. Ferreira Junior AG, Ferreira SM, Gomes ML, et al. Enteroviruses as a possible cause of myocarditis, pericarditis and dilated cardiomyopathy in Belem, Brazil. *Braz J Med Biol Res* 1995;**28**: 869–74.
321. Figueroa JP, Ashley D, King D, et al. An outbreak of acute flaccid paralysis in Jamaica associated with echovirus type 22. *J Med Virol* 1989;**29**:315–19.
322. Finsen J. *Iagttagelser sygdomsforholdeme i Island*. Copenhagen, C. A. Reitzels: University of Copenghagen; 1874.
323. Flewett TH. Histological study of two cases of coxsackie B virus pneumonia in children. *J Clin Pathol* 1965;**18**:743–6.
324. Flewett TH, Warin RP, Clarke SK. "Hand, foot, and mouth disease" associated with coxsackie A5 virus. *J Clin Pathol* 1963; **16**:53–5.
325. Flugsrud L, Abrahamsen AM, Lahelle O. An outbreak of aseptic meningitis associated with a virus related to ECHO type 9. *Acta Med Scand* 1958;**162**:129–35.
326. Foley JF, Chin TD, Gravelle CR. Paralytic disease due to infection with ECHO virus type 9 report of a case with residual paralysis. *N Engl J Med* 1959;**260**:924–6.
327. Forbes JA. Meningitis in Melbourne due to E.C.H.O.. virus. I. Clinical aspects. *Med J Aust* 1958;**45**:246–8.
328. Forman ML, Cherry JD. Enanthems associated with uncommon viral syndromes. *Pediatrics* 1968;**41**:873–82.
329. Forster W, Bialasiewicz AA, Busse H. Coxsackievirus B3-associated panuveitis. *Br J Ophthalmol* 1993;**77**:182–3.
330. Fox JP. Epidemiological aspects of coxsackie and echo virus infections in tropical areas. *Am J Public Health Nations Health* 1964;**54**:1134–42.
331. Fox JP. Eradication of poliomyelitis in the United States: a commentary on the Salk reviews. *Rev Infect Dis* 1980;**2**:277–81.
332. Fox JP, Hall CE. *Viruses in families*. Littleton, Mass: PSG Publishing; 1980.
333. Fox SA, Finklestone E, Robbins PD, et al. Search for persistent enterovirus infection of muscle in inflammatory myopathies. *J Neurol Sci* 1994;**125**:70–6.
334. Foy HM, Cooney MK, Maletzky AJ, et al. Incidence and etiology of pneumonia, croup and bronchiolitis in preschool children belonging to a prepaid medical care group over a four-year period. *Am J Epidemiol* 1973;**97**:80–92.
335. Francalanci P, Chance JL, Vatta M, et al. Cardiotropic viruses in the myocardium of children with end-stage heart disease. *J Heart Lung Transplant* 2004;**23**:1046–52.
336. Freedman PS. Echovirus 11 infection and intrauterine death. *Lancet* 1979;**1**:96–7.
337. Freij L, Norrby R, Olsson B. A small outbreak of coxsackie B5 infection with two cases of cardiac involvement and orchitis followed by testicular atrophy. *Acta Med Scand* 1970;**187**: 177–81.
338. Frisk G, Diderholm H. Increased frequency of coxsackie B virus IgM in women with spontaneous abortion. *J Infect* 1992;**24**: 141–5.
339. Frisk G, Diderholm H. Antibody responses to different strains of coxsackie B4 virus in patients with newly diagnosed type I diabetes mellitus or aseptic meningitis. *J Infect* 1997;**34**:205–10.
340. Frisk G, Tuvemo T. Enterovirus infections with beta-cell tropic strains are frequent in siblings of children diagnosed with type 1 diabetes children and in association with elevated levels of GAD65 antibodies. *J Med Virol* 2004;**73**:450–9.
341. Froeschle JE, Nahmias AJ, Feorino PM, et al. Hand, foot, and mouth disease (coxsackievirus A16) in Atlanta. *Am J Dis Child* 1967;**114**:278–83.
342. Frothingham TE. ECHO virus type 9 associated with three cases simulating meningococcemia. *N Engl J Med* 1958;**259**:484–5.
343. Fu YC, Chi CS, Jan SL, et al. Pulmonary edema of enterovirus 71 encephalomyelitis is associated with left ventricular failure: implications for treatment. *Pediatr Pulmonol* 2003;**35**:263–8.
344. Fujioka S, Kitaura Y, Deguchi H, et al. Evidence of viral infection in the myocardium of American and Japanese patients with idiopathic dilated cardiomyopathy. *Am J Cardiol* 2004;**94**:602–5.
345. Fujioka S, Koide H, Kitaura Y, et al. Molecular detection and differentiation of enteroviruses in endomyocardial biopsies and pericardial effusions from dilated cardiomyopathy and myocarditis. *Am Heart J* 1996;**131**:760–5.
346. Fulginiti VA. The problems of poliovirus immunization. *Hosp Pract* 1980;**15**:61–7.
347. Galama J, Lanke K, Zoll J, et al. Seroepidemiology of Saffold cardiovirus type 2. *Emerg Infect Dis* 2011;**17**:1572–3.
348. Galama JM, de Leeuw N, Wittebol S, et al. Prolonged enteroviral infection in a patient who developed pericarditis and heart failure after bone marrow transplantation. *Clin Infect Dis* 1996; **22**:1004–8.
349. Galama JM, Vogels MT, Jansen GH, et al. Antibodies against enteroviruses in intravenous Ig preparations: great variation in titres and poor correlation with the incidence of circulating serotypes. *J Med Virol* 1997;**53**:273–6.
350. Galama JMD. Enteroviral infections in the immunocompromised host. *Rev Med Microbiol* 1997;**8**:33–40.
351. Gallacher K, Ghosh K, Patel A, et al. An outbreak of echovirus type 4 infections and its implications for diagnosis and management in general practice. *J Infect* 1993;**26**:321–4.
352. Galpine JF, Clayton TM, Ardley J, et al. Outbreak of aseptic meningitis with exanthem. *Br Med J* 1958;**1**:319–21.
353. Gamble DR, Kinsley ML, FitzGerald MG, et al. Viral antibodies in diabetes mellitus. *Br Med J* 1969;**3**:627–30.
354. Gamble DR, Taylor KW. Seasonal incidence of diabetes mellitus. *Br Med J* 1969;**3**:631–3.
355. Garnett DG, Burlingham A, Van Zwanenberg D. An outbreak of aseptic meningitis of virus origin in East Suffolk. *Lancet* 1957;**272**:500–2.
356 Gaudin OG, Pozzetto B, Aouni M, et al. Detection of neutralizing IgM antibodies in the diagnosis of enterovirus infections. *J Med Virol* 1989;**28**:200–5.
357. Gauntt C, Higdon A, Bowers D, et al. What lessons can be learned from animal model studies in viral heart disease? *Scand J Infect Dis Suppl* 1993;**88**:49–65.
358. Gauntt CJ, Arizpe HM, Higdon AL, et al. Molecular mimicry, anti-coxsackievirus B3 neutralizing monoclonal antibodies, and myocarditis. *J Immunol* 1995;**154**:2983–95.
359 Gauntt CJ, Gomez PT, Duffey PS, et al. Characterization and myocarditic capabilities of coxsackievirus B3 variants in selected mouse strains. *J Virol* 1984;**52**:598–605.
360. Gauntt CJ, Gudvangen RJ, Brans YW, et al. Coxsackievirus group B antibodies in the ventricular fluid of infants with severe anatomic defects in the central nervous system. *Pediatrics* 1985; **76**:64–8.

361. Gauntt CJ, Higdon AL, Arizpe HM, et al. Epitopes shared between coxsackievirus B3 (CVB3) and normal heart tissue contribute to CVB3-induced murine myocarditis. *Clin Immunol Immunopathol* 1993;**68**:129–34.
362. Gear J. Coxsackie virus infections in Southern Africa. *Yale J Biol Med* 1961;**34**:289–303.
363. Gear JH. Coxsackie virus infections of the newborn. *Prog Med Virol* 1958;**1**:106–21.
364. Gebhard JR, Perry CM, Harkins S, et al. Coxsackievirus B3-induced myocarditis: perforin exacerbates disease, but plays no detectable role in virus clearance. *Am J Pathol* 1998;**153**: 417–28.
365. Gelfand HM. The occurrence in nature of the coxsackie and ECHO viruses. *Prog Med Virol* 1961;**3**:193–244.
366. Gelfand HM, Holguin AH, Marchetti GE, et al. A continuing surveillance of enterovirus infections in healthy children in Six United States cities. I. Viruses Isolated during 1960 and 1961. *Am J Hyg* 1963;**78**:358–75.
367. Georgieff MK, Johnson DE, Thompson TR, et al. Fulminant hepatic necrosis in an infant with perinatally acquired echovirus 21 infection. *Pediatr Infect Dis J* 1987;**6**:71–3.
368. German LJ, McCracken AW, Wilkie KM. Outbreak of febrile illness associated with E.C.H.O. virus type 5 in maternity unit in Singapore. *Br Med J* 1968;**1**:742–4.
369. Gilbert GL, Dickson KE, Waters MJ, et al. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. *Pediatr Infect Dis J* 1988;**7**: 484–8.
370. Gillett RL. Acute benign pericarditis and the Coxsackie viruses. *N Engl J Med* 1959;**261**:838–43.
371. Ginsberg AH, Gale MJ Jr, Rose LM, et al. T-cell alterations in late postpoliomyelitis. *Arch Neurol* 1989;**46**:497–501.
372. Glasgow LA, Balduzzi P. Isolation of Coxsackie virus group A, type 4, from a patient with hemolytic-uremic syndrome. *N Engl J Med* 1965;**273**:754–6.
373. Glezen WP, Loda FA, Clyde WA Jr, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
374. Glimaker M, Ehrnst A, Magnius L, et al. Early diagnosis of enteroviral meningitis by a solid-phase reverse immunosorbent test and virus isolation. *Scand J Infect Dis* 1990;**22**:519–26.
375. Glimaker M, Samuelson A, Magnius L, et al. Early diagnosis of enteroviral meningitis by detection of specific IgM antibodies with a solid-phase reverse immunosorbent test (SPRIST) and mu-capture EIA. *J Med Virol* 1992;**36**:193–201.
376. Godeny EK, Gauntt CJ. Involvement of natural killer cells in coxsackievirus B3-induced murine myocarditis. *J Immunol* 1986; **137**:1695–702.
377. Godtfredsen A, Hansen B. A case of mild paralytic disease due to Echo virus type 11. *Acta Pathol Microbiol Scand* 1961;**53**: 111–16.
378. Goh KT, Doraisingham S, Tan JL, et al. An outbreak of hand, foot, and mouth disease in Singapore. *Bull World Health Organ* 1982;**60**:965–9.
379. Goh KT, Ooi PL, Miyamura K, et al. Acute haemorrhagic conjunctivitis: seroepidemiology of coxsackievirus A24 variant and enterovirus 70 in Singapore. *J Med Virol* 1990;**31**:245–7.
380. Gohd RS, Faigel HC. Hand-foot-and-mouth-disease resembling measles a life-threatening disease: case report. *Pediatrics* 1966;**37**: 644–8.
381. Gohd RS, Gordon W. Endemic nature of coxsackievirus A-16. *Bacteriol Proc* 1967;**108**:152.
382. Gold E, Carver DH, Heineberg H, et al. Viral infection: a possible cause of sudden, unexpected death in infants. *N Engl J Med* 1961;**264**:53–60.
383. Goldwater PN, Laws J. Echovirus 19 outbreak in Auckland 1975-76. *N Z Med J* 1977;**86**:319–22.
384. Goldwater PN. Immunoglobulin M capture immunoassay in investigation of coxsackievirus B5 and B6 outbreaks in South Australia. *J Clin Microbiol* 1995;**33**:1628–31.
385. Gondo K, Kusuhara K, Take H, et al. Echovirus type 9 epidemic in Kagoshima, southern Japan: seroepidemiology and clinical observation of aseptic meningitis. *Pediatr Infect Dis J* 1995;**14**: 787–91.
386. Gong CM, Ho DW, Field PR, et al. Immunoglobulin responses to echovirus type 11 by enzyme linked immunosorbent assay: single-serum diagnosis of acute infection by specific IgM antibody. *J Virol Methods* 1984;**9**:209–21.
387. Goodwin MH Jr, Love GJ, Mackel DC, et al. Observations on the association of enteric viruses and bacteria with diarrhea. *Am J Trop Med Hyg* 1967;**16**:178–85.
388. Gordon RB, Lennette EH, Sandrock RS. The varied clinical manifestations of coxsackie virus infections: observations and comments on an outbreak in California. *AMA Arch Intern Med* 1959;**103**:63–75.
389. Gorgievski-Hrisoho M, Schumacher JD, Vilimonovic N, et al. Detection by PCR of enteroviruses in cerebrospinal fluid during a summer outbreak of aseptic meningitis in Switzerland. *J Clin Microbiol* 1998;**36**:2408–12.
390. Graber D, Fossoud C, Grouteau E, et al. Acute transverse myelitis and coxsackie A9 virus infection. *Pediatr Infect Dis J* 1994;**13**:77.
391. Grangeot-Keros L, Broyer M, Briand E, et al. Enterovirus in sudden unexpected deaths in infants. *Pediatr Infect Dis J* 1996;**15**: 123–8.
392. Gravelle CR, Noble GR, Feltz ET, et al. An epidemic of echovirus type 30 meningitis in an arctic community. *Am J Epidemiol* 1974;**99**:368–74.
393. Green J, Casabonne D, Newton R. Coxsackie B virus serology and type 1 diabetes mellitus: a systematic review of published case-control studies. *Diabet Med* 2004;**21**:507–14.
394. Greenberg M, Siegel M. Poliomyelitis in pregnancy: effect on fetus and newborn infant. *J Pediatr* 1956;**49**:280–8.
395. Gregg I. The role of viral infection in asthma and bronchitis. In: Proudfoot AT, editor. *Symposium on viral diseases*. Edinburgh: Royal College of Physicians; 1975. p. 82–98.
396. Griffiths PD, Hannington G, Booth JC. Coxsackie B virus infections and myocardial infarction: results from a prospective, epidemiologically controlled study. *Lancet* 1980;**1**:1387–9.
397. Grimwood K, Huang QS, Sadleir LG, et al. Acute flaccid paralysis from echovirus type 33 infection. *J Clin Microbiol* 2003;**41**: 2230–2.
398. Grist NR. Further studies of coxsackie a7 virus infection in the west of Scotland. *Lancet* 1965;**2**:261–3.
399. Grist NR, Bell EJ. Coxsackie virus heart diseases. *Br Med J* 1968;**3**:556.
400. Grist NR, Bell EJ. Coxsackie viruses and the heart. *Am Heart J* 1969;**77**:295–300.
401. Grist NR, Bell EJ. Enteroviral etiology of the paralytic poliomyelitis syndrome. *Arch Environ Health* 1970;**21**:382–7.
402. Grist NR, Bell EJ. A six-year study of coxsackievirus B infections in heart disease. *J Hyg (Lond)* 1974;**73**:165–72.
403. Grist NR, Bell EJ. Paralytic poliomyelitis and nonpolio enteroviruses: studies in Scotland. *Rev Infect Dis* 1984;**6**(Suppl. 2): S385–386.
404. Grist NR, Bell EJ, Assaad F. Enteroviruses in human disease. *Prog Med Virol* 1978;**24**:114–57.
405. Groeneweg M, Poley JW, Dansen M, et al. Chronic hereditary pancreatitis in a girl with a serine protease inhibitor kazal type I (SPINK-1) gene mutation and a coxsackie type B5 infection. *Pediatr Infect Dis J* 2009;**28**:169–70.
406. Grose C, Horwitz MS. Characterization of an enterovirus associated with acute infectious lymphocytosis. *J Gen Virol* 1976;**30**: 347–55.
407. Guidotti MB. An outbreak of skin rash by echovirus 25 in an infant home. *J Infect* 1983;**6**:67–70.
408. Guthrie N. Coxsackie B5 meningitis: report of an outbreak in a high school football squad. *J Tn State Med Assoc* 1962;**55**: 355–6.
409. Haamann P, Kessel L, Larsen M. Monofocal outer retinitis associated with hand, foot, and mouth disease caused by coxsackievirus. *Am J Ophthalmol* 2000;**129**:552–3.
410. Habel K, Silverberg RJ, Shelokov A. Isolation of enteric viruses from case of aseptic meningitis. *Ann N Y Acad Sci* 1957;**67**: 223–9.
411. Haddad J, Gut JP, Wendling MJ, et al. Enterovirus infections in neonates: a retrospective study of 21 cases. *Eur J Med* 1993;**2**: 209–14.
412. Hadfield MG, Seidlin M, Houff SA, et al. Echovirus meningomyeloencephalitis with administration of intrathecal immunoglobulin. *J Neuropathol Exp Neurol* 1985;**44**:520–9.
413. Halfon N, Spector SA. Fatal echovirus type 11 infections. *Am J Dis Child* 1981;**135**:1017–20.

414. Hall CB, Cherry JD, Hatch MH, et al. The return of Boston exanthem: echovirus 16 infections in 1974. *Am J Dis Child* 1977; **131**:323–6.
415. Hall CE, Cooney MK, Fox JP. The Seattle virus watch program. I. Infection and illness experience of virus watch families during a communitywide epidemic of echovirus type 30 aseptic meningitis. *Am J Public Health Nations Health* 1970;**60**: 1456–65.
416. Halliday E, Winkelstein J, Webster AD. Enteroviral infections in primary immunodeficiency (PID): a survey of morbidity and mortality. *J Infect* 2003;**46**:1–8.
417. Halonen P, Rocha E, Hierholzer J, et al. Detection of enteroviruses and rhinoviruses in clinical specimens by PCR and liquid-phase hybridization. *J Clin Microbiol* 1995;**33**:648–53.
418. Hammon WM, Yohn DS, Ludwig EH, et al. A study of certain nonpoliomyelitis and poliomyelitis enterovirus infections: clinical and serologic associations. *J Am Med Assoc* 1958;**167**: 727–35.
419. Hannaeus G. Dissertation. Copenhagen. 1735.
420. Hanninen P, Pohjonen R. Echovirus type 6 meningitis: clinical and virological observations during an epidemic in Turku in 1968. *Scand J Infect Dis* 1971;**3**:121–5.
421. Hanson LA, Lundgren S, Lycke E, et al. Clinical and serological observations in cases of coxsackie B3 infections in early infancy. *Acta Paediatr Scand* 1966;**55**:577–83.
422. Harada S, Okada M, Yahiro S, et al. Surveillance of pathogens in outpatients with gastroenteritis and characterization of sapovirus strains between 2002 and 2007 in Kumamoto Prefecture, Japan. *J Med Virol* 2009;**81**:1117–27.
423. Harjulehto T, Aro T, Hovi T, et al. Congenital malformations and oral poliovirus vaccination during pregnancy. *Lancet* 1989;**1**: 771–2.
424. Harkonen T, Puolakkainen M, Sarvas M, et al. Picornavirus proteins share antigenic determinants with heat shock proteins 60/65. *J Med Virol* 2000;**62**:383–91.
425. Hart EW, Brunton GB, Taylor CED, et al. Infection of newborn babies with ECHO virus type 5. *Lancet* 1962;**2**:402.
426. Harvala H, Robertson I, Chieochansin T, et al. Specific association of human parechovirus type 3 with sepsis and fever in young infants, as identified by direct typing of cerebrospinal fluid samples. *J Infect Dis* 2009;**199**:1753–60.
427. Hatch MH, Marchetti GE. Isolation of echoviruses with human embryonic lung fibroblast cells. *Appl Microbiol* 1971;**22**:736–7.
428. Haynes RE, Cramblett HG, Kronfol HJ. Echovirus 9 meningoencephalitis in infants and children. *JAMA* 1969;**208**: 1657–60.
429. Haynes RE, Cramblett HG, Hilty MD, et al. ECHO virus type 3 infections in children: clinical and laboratory studies. *J Pediatr* 1972;**80**:589–95.
430. Hayward JC, Gillespie SM, Kaplan KM, et al. Outbreak of poliomyelitis-like paralysis associated with enterovirus 71. *Pediatr Infect Dis J* 1989;**8**:611–16.
431. Heathfield KW, Pilsworth R, Wall BJ, et al. Coxsackie B5 infections in Essex, 1965, with particular reference to the nervous system. *Q J Med* 1967;**36**:579–95.
432. Hedlund P, Lycke E, Tibblin G. The association of acute benign pericarditis in adults with coxsackie B5 virus infection. *Arch Gesamte Virusforsch* 1963;**13**:156–9.
433. Heim A, Grumbach I, Hake S, et al. Enterovirus heart disease of adults: a persistent, limited organ infection in the presence of neutralizing antibodies. *J Med Virol* 1997;**53**:196–204.
434. Heineberg H, Gold E, Robbins FC. Differences in interferon content in tissues of mice of various ages infected with coxsackie B1 virus. *Proc Soc Exp Biol Med* 1964;**115**:947–53.
435. Helfand RF, Gary HE Jr, Freeman CY, et al. Serologic evidence of an association between enteroviruses and the onset of type 1 diabetes mellitus. Pittsburgh Diabetes Research Group. *J Infect Dis* 1995;**172**:1206–11.
436. Helfand RF, Khan AS, Pallansch MA, et al. Echovirus 30 infection and aseptic meningitis in parents of children attending a child care center. *J Infect Dis* 1994;**169**:1133–7.
437. Helin M, Savola J, Lapinleimu K. Cardiac manifestations during a coxsackie B5 epidemic. *Br Med J* 1968;**3**:97–9.
438. Henke A, Huber S, Stelzner A, et al. The role of CD8+ T lymphocytes in coxsackievirus B3-induced myocarditis. *J Virol* 1995; **69**:6720–8.
439. Hercík L, Huml M, Mimra J, et al. [Epidemic of respiratory tract diseases in newborn infants due to ECHO 11 virus]. *Zentralbl Bakteriol Orig* 1970;**213**:18–27.
440. Herrmann EC Jr. Experience in providing a viral diagnostic laboratory compatible with medical practice. *Mayo Clin Proc* 1967;**42**:112–23.
441. Herskowitz A, Beisel KW, Wolfgram LJ, et al. Coxsackievirus B3 murine myocarditis: wide pathologic spectrum in genetically defined inbred strains. *Hum Pathol* 1985;**16**:671–3.
442. Hertel NT, Pedersen FK, Heilmann C. Coxsackie B3 virus encephalitis in a patient with agammaglobulinaemia. *Eur J Pediatr* 1989;**148**:642–3.
443. Heymann DL, Aylward RB. Eradicating polio. *N Engl J Med* 2004;**351**:1275–7.
444. Hierholzer JC, Farris WA. Follow-up of children infected in a coxsackievirus B-3 and B-4 outbreak: no evidence of diabetes mellitus. *J Infect Dis* 1974;**129**:741–6.
445. Hierholzer JC, Hilliard KA, Esposito JJ. Serosurvey for "acute hemorrhagic conjunctivitis" virus (Enterovirus 70) antibodies in the Southeastern United States, with review of the literature and some epidemiologic implications. *Am J Epidemiol* 1975;**102**: 533–44.
446. Higgins PG, Crow KD. Recurrent Kaposi's varicelliform eruption in Darier's disease. *Br J Dermatol* 1973;**88**:391–4.
447. Higgins PG, Warin RP. Hand, foot, and mouth disease: a clinically recognizable virus infection seen mainly in children. *Clin Pediatr (Phila)* 1967;**6**:373–6.
448. Higgins PG, Ellis EM, Boston DG. Hand, foot and nouth disease, 1963-64: a study of cases and family contacts. *Mon Bull Minist Health Public Health Lab Serv* 1965;**24**:38–45.
449. Hiltunen M, Hyoty H, Knip M, et al. Islet cell antibody seroconversion in children is temporally associated with enterovirus infections. Childhood Diabetes in Finland (DiMe) Study Group. *J Infect Dis* 1997;**175**:554–60.
450. Himeda T, Ohara Y. Saffold virus, a novel human cardiovirus with unknown pathogenicity. *J Virol* 2012;**86**:1292–6.
451. Hindersson M, Elshebani A, Orn A, et al. Simultaneous type 1 diabetes onset in mother and son coincident with an enteroviral infection. *J Clin Virol* 2005;**33**:158–67.
452. Hinman AR, Koplan JP, Orenstein WA, et al. Live or inactivated poliomyelitis vaccine: an analysis of benefits and risks. *Am J Public Health* 1988;**78**:291–5.
453. Ho M, Chen ER, Hsu KH, et al. An epidemic of enterovirus 71 infection in Taiwan. Taiwan Enterovirus Epidemic Working Group. *N Engl J Med* 1999;**341**:929–35.
454. Hober D, Andreoletti L, Shen L, et al. Coxsackievirus B3-induced chronic myocarditis in mouse: use of whole blood culture to study the activation of TNF alpha-producing cells. *Microbiol Immunol* 1996;**40**:837–45.
455. Hober D, Chehadeh W, Bouzidi A, et al. Antibody-dependent enhancement of coxsackievirus B4 infectivity of human peripheral blood mononuclear cells results in increased interferon-alpha synthesis. *J Infect Dis* 2001;**184**:1098–108.
456. Hoi-shan Chan S, Lun KS. Ventricular aneurysm complicating neonatal coxsackie B4 myocarditis. *Pediatr Cardiol* 2001;**22**: 247–9.
457. Hooft C, Nihoul E, Lambert Y, et al. Clinical findings during an echo virus type 13 endemic infection. *Helv Paediatr Acta* 1963;**18**:230–9.
458. Horn ME, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967-1972. *J Hyg (Lond)* 1975;**74**:157–68.
459. Horn P. Poliomyelitis in pregnancy: a twenty-year report from Los Angeles County, California. *Obstet Gynecol* 1955;**6**:121–37.
460. Horstmann DM. The new ECHO viruses and their role in human disease. *Arch Intern Med* 1958;**102**:155–62.
461. Horstmann DM. The poliomyelitis story: a scientific hegira. *Yale J Biol Med* 1985;**58**:79–90.
462. Horstmann DM. Viral exanthems and enanthems. *Pediatrics* 1968;**41**:867–70.
463. Horstmann DM, Emmons J, Gimpel L, et al. Enterovirus surveillance following a community-wide oral poliovirus vaccination program: a seven-year study. *Am J Epidemiol* 1973;**97**:173–86.
464. Horwitz MS, Moore GT. Acute infectious lymphocytosis: an etiologic and epidemiologic study of an outbreak. *N Engl J Med* 1968;**279**:399–404.

465. Horwitz MS, Ilic A, Fine C, et al. Coxsackieviral-mediated diabetes: induction requires antigen-presenting cells and is accompanied by phagocytosis of beta cells. *Clin Immunol* 2004;**110**: 134–44.
466. Hosier DM, Newton WA Jr. Serious coxsackie infection in infants and children; myocarditis, meningoencephalitis, and hepatitis. *AMA J Dis Child* 1958;**96**:251–67.
467. Hosoya M, Kawasaki Y, Sato M, et al. Genetic diversity of enterovirus 71 associated with hand, foot and mouth disease epidemics in Japan from 1983 to 2003. *Pediatr Infect Dis J* 2006;**25**:691–4.
468. Hosoya M, Sato M, Honzumi K, et al. Association of nonpolio enteroviral infection in the central nervous system of children with febrile seizures. *Pediatrics* 2001;**107**:E12.
469. Howe HA, Wilson JL. Poliomyelitis. In: Rivers TM, Horsfall FL Jr, editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1959. p. 432–518.
470. Howes DW. Studies of coxsackie viruses. I. Comparison of age-susceptibility relationships in mice. *Aust J Exp Biol Med Sci* 1954;**32**:253–64.
471. Howlett JG, Somlo F, Kalz F. A new syndrome of parotitis with herpangina caused by the coxsackie virus. *Can Med Assoc J* 1957;**77**:5–7.
472. Hsia SH, Wu CT, Chang JJ, et al. Predictors of unfavorable outcomes in enterovirus 71-related cardiopulmonary failure in children. *Pediatr Infect Dis J* 2005;**24**:331–4.
473. Huang CC, Liu CC, Chang YC, et al. Neurologic complications in children with enterovirus 71 infection. *N Engl J Med* 1999; **341**:936–42.
474. Huang FL, Jan SL, Chen PY, et al. Left ventricular dysfunction in children with fulminant enterovirus 71 infection: an evaluation of the clinical course. *Clin Infect Dis* 2002;**34**:1020–4.
475. Huang MC, Wang SM, Hsu YW, et al. Long-term cognitive and motor deficits after enterovirus 71 brainstem encephalitis in children. *Pediatrics* 2006;**118**:e1785–1788.
476. Huang TW, Wiegenstein LM. Mesangiolytic glomerulonephritis in an infant with immune deficiency and echovirus infection. *Arch Pathol Lab Med* 1977;**101**:125–8.
477. Huang WT, Lee PI, Chang LY, et al. Epidemic pleurodynia caused by coxsackievirus B3 at a medical center in northern Taiwan. *J Microbiol Immunol Infect* 2010;**43**:515–18.
478. Huber SA, Lodge PA. Coxsackievirus B-3 myocarditis in Balb/c mice: evidence for autoimmunity to myocyte antigens. *Am J Pathol* 1984;**116**:21–9.
479. Huber SA, Mortensen A, Moulton G. Modulation of cytokine expression by CD4+ T cells during coxsackievirus B3 infections of BALB/c mice initiated by cells expressing the gamma delta + T-cell receptor. *J Virol* 1996;**70**:3039–44.
480. Huebner RJ, Risser JA, Bell JA, et al. Epidemic pleurodynia in Texas: a study of 22 cases. *N Engl J Med* 1953;**248**:267–74.
481. Hughes JR, Wilfert CM, Moore M, et al. Echovirus 14 infection associated with fatal neonatal hepatic necrosis. *Am J Dis Child* 1972;**123**:61–7.
482. Hughes RO, Roberts C. Hand, foot, and mouth disease associated with coxsackie A9 virus. *Lancet* 1972;**2**:751–2.
483. Hull HF. Progress towards global polio eradication. *Dev Biol (Basel)* 2001;**105**:3–7.
484. Hull HF, Ward NA, Hull BP, et al. Paralytic poliomyelitis: seasoned strategies, disappearing disease. *Lancet* 1994;**343**: 1331–7.
485. Hung TP, Green IJ, Sung SM. Neurologic complications with elevated antibody titer after acute hemorrhagic conjunctivitis. *Am J Ophthalmol* 1975;**80**:832–4.
486. Hurley R, Norman AP, Pryse-Davies J. Massive pulmonary haemorrhage in the newborn associated with coxsackie B virus infection. *Br Med J* 1969;**3**:636–7.
487. Hurst NP, Martynoga AG, Nuki G, et al. Coxsackie B infection and arthritis. *Br Med J (Clin Res Ed)* 1983;**286**:605.
488. Hyoty H, Huupponen T, Kotola L, et al. Humoral immunity against viral antigens in type 1 diabetes: altered IgA-class immune response against coxsackie B4 virus. *Acta Pathol Microbiol Immunol Scand C* 1986;**94**:83–8.
489. Hyypia T. Identification of human picornaviruses by nucleic acid probes. *Mol Cell Probes* 1989;**3**:329–43.
490. Hyypia T, Auvinen P, Maaronen M. Polymerase chain reaction for human picornaviruses. *J Gen Virol* 1989;**70**(pt 12):3261–8.
491. Hyypia T, Hovi T, Knowles NJ, et al. Classification of enteroviruses based on molecular and biological properties. *J Gen Virol* 1997;**78**(pt 1):1–11.
492. Ikeda RM, Kondracki SF, Drabkin PD, et al. Pleurodynia among football players at a high school: an outbreak associated with coxsackievirus B1. *JAMA* 1993;**270**:2205–6.
493. Imamura T, Fuji N, Suzuki A, et al. Enterovirus 68 among children with severe acute respiratory infection: the Philippines. *Emerg Infect Dis* 2011;**17**:1430–5.
494. Institute of Medicine. *An evaluation of poliomyelitis vaccine policy options*. Institute of Medicine Publication 88-04. Washington, D.C.: National Academy of Sciences; 1988.
495. Irvine DH, Irvine AB, Gardner PS. Outbreak of E.C.H.O. virus type 30 in a general practice. *Br Med J* 1967;**4**:774–6.
496. Isaacs D, Dobson SR, Wilkinson AR, et al. Conservative management of an echovirus 11 outbreak in a neonatal unit. *Lancet* 1989;**1**:543–5.
497. Isacsohn M, Eidelman AI, Kaplan M, et al. Neonatal coxsackievirus group B infections: experience of a single department of neonatology. *Isr J Med Sci* 1994;**30**:371–4.
498. Ishiko H, Takeda N, Miyamura K, et al. Phylogenetic analysis of a coxsackievirus A24 variant: the most recent worldwide pandemic was caused by progenies of a virus prevalent around 1981. *Virology* 1992;**187**:748–59.
499. Ishimaru Y, Nakano S, Yamaoka K, et al. Outbreaks of hand, foot, and mouth disease by enterovirus 71: high incidence of complication disorders of central nervous system. *Arch Dis Child* 1980;**55**: 583–8.
500. Itagaki A, Ishihara J, Mochida K, et al. A clustering outbreak of hand, foot, and mouth disease caused by coxsackie virus A10. *Microbiol Immunol* 1983;**27**:929–35.
501. Itagaki T, Abiko C, Aoki Y, et al. Saffold cardiovirus infection in children associated with respiratory disease and its similarity to coxsackievirus infection. *Pediatr Infect Dis J* 2011;**30**: 680–3.
502. Itagaki T, Abiko C, Ikeda T, et al. Sequence and phylogenetic analyses of Saffold cardiovirus from children with exudative tonsillitis in Yamagata, Japan. *Scand J Infect Dis* 2010;**42**:950–2.
503. Ito M, Kodama M, Masuko M, et al. Expression of coxsackievirus and adenovirus receptor in hearts of rats with experimental autoimmune myocarditis. *Circ Res* 2000;**86**:275–80.
504. Ito M, Yamashita T, Tsuzuki H, et al. Detection of human parechoviruses from clinical stool samples in Aichi, Japan. *J Clin Microbiol* 2010;**48**:2683–8.
505. Jack I, Townley RR. Acute aseptic myocarditis of newborn infants, due to Coxsackie viruses. *Med J Aust* 1961;**48**:25–9.
506. Jack I, Grutzner J, Gray N, et al. A survey of prenatal virus disease in Melbourne. July 21, 1967. Personal communication.
507. Jackson AL. A clinical study of the Landry-Guillain-Barré syndrome with reference to aetiology, including the role of coxsackie virus infections. *S Afr J Lab Clin Med* 1961;**7**:121–37.
508. Jahn CL, Cherry JD. Mild neonatal illness associated with heavy enterovirus infection. *N Engl J Med* 1966;**274**:394–5.
509. Jahn CL, Felton OL, Cherry JD. Coxsackie B1 pneumonia in an adult. *JAMA* 1964;**189**:236–7.
510. James WD, Odom RB, Hatch MH. Gianotti-Crosti-like eruption associated with coxsackievirus A-16 infection. *J Am Acad Dermatol* 1982;**6**:862–6.
511. Jamieson WM, Kerr M, Sommerville RG. ECHO type-9 meningitis in East Scotland. *Lancet* 1958;**1**:581–3.
512. Jantausch BA, Luban NL, Duffy L, et al. Maternal plasma transfusion in the treatment of disseminated neonatal echovirus 11 infection. *Pediatr Infect Dis J* 1995;**14**:154–5.
513. Jarvis WR, Tucker G. Echovirus type 7 meningitis in young children. *Am J Dis Child* 1981;**135**:1009–12.
514. Javett SN, Heymann S, Mundel B, et al. Myocarditis in the new newborn infant: a study of an outbreak associated with coxsackie group B virus infection in a maternity home in Johannesburg. *J Pediatr* 1956;**48**:1–22.
515. Jehn UW, Fink MK. Myositis, myoglobinemia, and myoglobinuria associated with enterovirus echo 9 infection. *Arch Neurol* 1980;**37**:457–8.
516. Jenista JA, Powell KR, Menegus MA. Epidemiology of neonatal enterovirus infection. *J Pediatr* 1984;**104**:685–90.
517. Jhala CI, Draper J, Walcher DN. Aseptic meningitis syndrome due to virus echo type 23. *Am J Dis Child* 1961;**102**:868–70.

518. John TJ, Pandian R, Gadomski A, et al. Control of poliomyelitis by pulse immunisation in Vellore, India. *Br Med J (Clin Res Ed)* 1983;**286**:31–2.
519. Johnson DA, Eger AW. Myelitis associated with an echovirus. *JAMA* 1967;**201**:637–8.
520. Johnson I, Hammond GW, Verma MR. Nosocomial coxsackie B4 virus infections in two chronic-care pediatric neurological wards. *J Infect Dis* 1985;**151**:1153–6.
521. Johnson JF, Stimson PM. Clinical poliomyelitis in the early neonatal period: report of a case. *J Pediatr* 1952;**40**:733–7.
522. Johnson KM, Bloom HH, Forsyth B, et al. Relative role of identifiable agents in respiratory disease. II. The role of enteroviruses in respiratory disease. *Am Rev Respir Dis* 1962;**88**: 240–5.
523. Johnson RT. Late progression of poliomyelitis paralysis: discussion of pathogenesis. *Rev Infect Dis* 1984;**6**(Suppl. 2):S568–570.
524. Johnson RT, Shuey HE, Buescher EL. Epidemic central nervous system disease of mixed enterovirus etiology. I. Clinical and epidemiologic description. *Am J Hyg* 1960;**71**:321–30.
525. Johnson RT, Buescher EL, Rogers NG, et al. Epidemic central nervous system disease of mixed enterovirus etiology. II. Analysis of laboratory investigations. *Am J Hyg* 1960;**71**:331–41.
526. Johnson RT, Portnoy B, Rogers NG, et al. Acute benign pericarditis: virologic study of 34 patients. *Arch Intern Med* 1961; **108**:823–32.
527. Johnsson T, Lundmark C. A histopathological study of Coxsackie virus infections in mice. *Arch Gesamte Virusforsch* 1955;**6**:262–81.
528. Johnston JM, Overall JC Jr. Intravenous immunoglobulin in disseminated neonatal echovirus 11 infection. *Pediatr Infect Dis J* 1989;**8**:254–6.
529. Joncas JH, Podoski MO, Pavilanis V. Rash associated with coxsackie A9 infection. *Can Med Assoc J* 1966;**95**:372–3.
530. Jones MJ, Kolb M, Votava HJ, et al. Intrauterine echovirus type 11 infection. *Mayo Clin Proc* 1980;**55**:509–12.
531. Jones MS, Lukashov VV, Ganac RD, et al. Discovery of a novel human picornavirus in a stool sample from a pediatric patient presenting with fever of unknown origin. *J Clin Microbiol* 2007;**45**:2144–50.
532. Josselson J, Pula T, Sadler JH. Acute rhabdomyolysis associated with an echovirus 9 infection. *Arch Intern Med* 1980;**140**: 1671–2.
533. Judelsohn R. Changing the US polio immunization schedule would be bad public health policy. *Pediatrics* 1996;**98**:115–16.
534. Juhela S, Hyoty H, Hinkkanen A, et al. T cell responses to enterovirus antigens and to beta-cell autoantigens in unaffected children positive for IDDM-associated autoantibodies. *J Autoimmun* 1999;**12**:269–78.
535. Juhela S, Hyoty H, Roivainen M, et al. T-cell responses to enterovirus antigens in children with type 1 diabetes. *Diabetes* 2000;**49**:1308–13.
536. Kahlmeter O. Clinical aspects of echo viruses. *Ciba Found Symp* 1959;**7**:24–36.
537. Kandolf R, Klingel K, Zell R, et al. Molecular mechanisms in the pathogenesis of enteroviral heart disease: acute and persistent infections. *Clin Immunol Immunopathol* 1993;**68**:153–8.
538. Kang Y, Chatterjee NK, Nodwell MJ, et al. Complete nucleotide sequence of a strain of coxsackie B4 virus of human origin that induces diabetes in mice and its comparison with nondiabetogenic coxsackie B4 JBV strain. *J Med Virol* 1994;**44**:353–61.
539. Kantor FS, Hsiung GD. Pleurodynia associated with ECHO virus type 8. *N Engl J Med* 1962;**266**:661–3.
540. Kao SJ, Yang FL, Hsu YH, et al. Mechanism of fulminant pulmonary edema caused by enterovirus 71. *Clin Infect Dis* 2004; **38**:1784–8.
541. Kaplan GJ, Bender TR, Clark PS, et al. Echovirus type 4 meningitis and related febrile illness: epidemiologic study of an outbreak in two Eskimo communities in 1970. *Am J Epidemiol* 1972;**96**:74–85.
542. Kaplan GJ, Clark PS, Bender TR, et al. Echovirus type 30 meningitis and related febrile illness: epidemiologic study of an outbreak in an Eskimo community. *Am J Epidemiol* 1970;**92**:257–65.
543. Kaplan MH, Klein SW, McPhee J, et al. Group B coxsackievirus infections in infants younger than three months of age: a serious childhood illness. *Rev Infect Dis* 1983;**5**:1019–32.
544. Kapsenberg JG. ECHO virus type 33 as a cause of meningitis. *Arch Gesamte Virusforsch* 1968;**23**:144–7.
545. Karzon DT, Eckert GL, Barron AL, et al. Aseptic meningitis epidemic due to ECHO 4 virus. *Am J Dis Child* 1961;**101**: 610–22.
546. Karzon DT, Hayner NS, Winkelstein W Jr, et al. An epidemic of aseptic meningitis syndrome due to ECHO virus type 6. II. A clinical study of ECHO 6 infection. *Pediatrics* 1962;**29**:418–31.
547. Katamura K, Hattori H, Kunishima T, et al. Non-progressive viral myelitis in X-linked agammaglobulinemia. *Brain Dev* 2002;**24**:109–11.
548. Katsibardi K, Moschovi MA, Theodoridou M, et al. Enterovirus-associated hemophagocytic syndrome in children with malignancy: report of three cases and review of the literature. *Eur J Pediatr* 2008;**167**:97–102.
549. Katz SL. Poliovaccine policy: time for a change. *Pediatrics* 1996;**98**:116–17.
550. Kaul A, Cohen ME, Broffman G, et al. Reye-like syndrome associated with coxsackie B2 virus infection. *J Pediatr* 1979;**94**: 67–9.
551. Kaye BM, Rosner DC, Stein IF. Viral diseases in pregnancy and their effect upon the embryo and fetus. *Am J Obstet Gynecol* 1953;**65**:109–19.
552. Kearns GL, Bradley JS, Jacobs RF, et al. Single dose pharmacokinetics of pleconaril in neonates. Pediatric Pharmacology Research Unit Network. *Pediatr Infect Dis J* 2000;**19**:833–9.
553. Kelen AE, Lesiak JM, Labzoffsky NA. An outbreak of aseptic meningitis due to echo 25 virus. *Can Med Assoc J* 1964;**90**:1349–51.
554. Kelen AE, Lesiak JM, Labzoffsky NA. Sporadic occurrence of echo virus types 27 and 31 associated with aseptic meningitis in Ontario. *Can Med Assoc J* 1964;**91**:1266–8.
555. Kelen AE, Lesiak JM, Labzoffsky NA. Occurrence of echovirus 33 infections in Ontario. *Can Med Assoc J* 1968;**98**:985–7.
556. Kendall EJ, Cook GT, Stone DM. Acute respiratory infections in children: isolation of coxsackie B virus and adenovirus during a survey in a general practice. *Br Med J* 1960;**2**:1180–4.
557. Kennett ML, Birch CJ, Lewis FA, et al. Enterovirus type 71 infection in Melbourne. *Bull World Health Organ* 1974;**51**: 609–15.
558. Kennett ML, Ellis AW, Lewis FA, et al. An epidemic associated with echovirus type 18. *J Hyg (Lond)* 1972;**70**:325–34.
559. Keswick BH, Gerba CP, Goyal SM. Occurrence of enteroviruses in community swimming pools. *Am J Public Health* 1981;**71**: 1026–30.
560. Kew O. What role for inactivated poliovirus vaccine in the eradication endgame? *J Infect Dis* 2006;**193**:1341–3.
561. Kew O, Morris-Glasgow V, Landaverde M, et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science* 2002;**296**:356–9.
562. Kew OM, Mulders MN, Lipskaya GY, et al. Molecular epidemiology of wild poliovirus type 1 in Europe, the Middle East, and the Indian subcontinent. *J Infect Dis* 1995;**171**:1399–405.
563. Kew OM, Sutter RW, de Gourville EM, et al. Vaccine-derived polioviruses and the endgame strategy for global polio eradication. *Annu Rev Microbiol* 2005;**59**:587–635.
564. Khamrin P, Chaimongkol N, Nantachit N, et al. Saffold cardioviruses in children with diarrhea, Thailand. *Emerg Infect Dis* 2011;**17**:1150–2.
565. Khan A, Sharif S, Shaukat S, et al. An outbreak of acute hemorrhagic conjunctivitis (AHC) caused by coxsackievirus A24 variant in Pakistan. *Virus Res* 2008;**137**:150–2.
566. Khatib R, Chason JL, Silberberg BK, et al. Age-dependent pathogenicity of group B coxsackieviruses in Swiss-Webster mice: infectivity for myocardium and pancreas. *J Infect Dis* 1980;**141**:394–403.
567. Kiang D, Newbower EC, Yeh E, et al. An algorithm for the typing of enteroviruses and correlation to serotyping by viral neutralization. *J Clin Virol* 2009;**45**:334–40.
568. Kibrick S. Viral infections of the fetus and newborn. *Perspect Virol* 1961;**2**:140–59.
569. Kibrick S. Current Status of Coxsackie and Echo Viruses in Human Disease. *Prog Med Virol* 1964;**6**:27–70.
570. Kibrick S, Benirschke K. Acute aseptic myocarditis and meningoencephalitis in the newborn child infected with coxsackle virus group B, type 3. *N Engl J Med* 1956;**255**:883–9.

571. Kibrick S, Enders JF. Disease due to ECHO virus type 9 in Massachusetts, 1958. *N Engl J Med* 1958;**259**:482–4.
572. Kibrick S, Melendez L, Enders JF. Clinical associations of enteric viruses with particular reference to agents exhibiting properties of the ECHO group. *Ann N Y Acad Sci* 1957;**67**:311–25.
573. Kiel RJ, Smith FE, Chason J, et al. Coxsackievirus B3 myocarditis in C3H/HeJ mice: description of an inbred model and the effect of exercise on virulence. *Eur J Epidemiol* 1989;**5**:348–50.
574. Kilbourne ED, Goldfield M. Coxsackie viruses and virus-like diseases of the adult: a three-year study in a contagious disease hospital. *Am J Med* 1956;**21**:175–83.
575. Kilbourne ED, Wilson CB, Perrier D. The induction of gross myocardial lesions by a Coxsackie (pleurodynia) virus and cortisone. *J Clin Invest* 1956;**35**:362–70.
576. Kimura H, Saitoh M, Miyakubo H, et al. Keratoconjunctivitis caused by echovirus type 13 in Japanese children. *Pediatr Infect Dis J* 2003;**22**:758–9.
577. King ML, Shaikh A, Bidwell D, et al. Coxsackie-B-virus-specific IgM responses in children with insulin-dependent (juvenile-onset; type I) diabetes mellitus. *Lancet* 1983;**1**:1397–9.
578. Kinney JS, McCray E, Kaplan JE, et al. Risk factors associated with echovirus 11′ infection in a hospital nursery. *Pediatr Infect Dis* 1986;**5**:192–7.
579. Kipps A, Naude W, du T, et al. Coxsackie virus myocarditis of the newborn: epidemiological features. *Med Proc* 1958;**4**:401–6.
580. Kirschke DL, Jones TF, Buckingham SC, et al. Outbreak of aseptic meningitis associated with echovirus 13. *Pediatr Infect Dis J* 2002;**21**:1034–8.
581. Kishimoto C, Kitazawa M, Hiraoka Y, et al. Extracellular matrix remodeling in coxsackievirus B3 myocarditis. *Clin Immunol Immunopathol* 1997;**85**:47–55.
582. Klein JO, Lerner AM, Finland M. Acute gastroenteritis associated with ECHO virus, type 11. *Am J Med Sci* 1960;**240**:749–53.
583. Kleinman H, Ramras DG, Cooney MK, et al. Aseptic meningitis due to echo virus type 7. *N Engl J Med* 1962;**267**:1116–21.
584. Kleinman H, Rogers D, Ellwood PM Jr, et al. Epidemic of ECHO 9 aseptic meningitis in Minnesota, 1957. *Univ Minn Med Bull* 1958;**29**:306.
585. Klemola E, Lapinleimu K. Multiple attacks of aseptic meningitis in the same individual. *Br Med J* 1964;**1**:1087–90.
586. Klespies SL, Cebula DE, Kelley CL, et al. Detection of enteroviruses from clinical specimens by spin amplification shell vial culture and monoclonal antibody assay. *J Clin Microbiol* 1996;**34**:1465–7.
587. Kobayashi K, Haruta T, Kubota M, et al. Clinical spectrum in hospitalized children with echovirus type 13 infection. *Pediatr Int* 2005;**47**:185–9.
588. Koch VF, Enders-Ruckle G, Wokittel E. Coxsackie B-5 Infektionen mit signifikanter Antikorperentwicklung bei Neugeborenen. *Arch Kinderheilkd* 1962;**165**:245–58.
589. Koelle DM, Barcy S, Huang M, et al. Reply to: Markers of viral infection in monozygotic twins discordant for chronic fatigue syndrome. *Clin Infect Dis* 2003;**36**:672–3.
590. Kogon A, Spigland I, Frothingham TE, et al. The virus watch program: a continuing surveillance of viral infections in metropolitan New York families. VII. Observations on viral excretion, seroimmunity, intrafamilial spread and illness association in coxsackie and echovirus infections. *Am J Epidemiol* 1969;**89**:51–61.
591. Komatsu H, Shimizu Y, Takeuchi Y, et al. Outbreak of severe neurologic involvement associated with enterovirus 71 infection. *Pediatr Neurol* 1999;**20**:17–23.
592. Kono R. Apollo 11 disease or acute hemorrhagic conjunctivitis: a pandemic of a new enterovirus infection of the eyes. *Am J Epidemiol* 1975;**101**:383–90.
593. Koontz CH, Ray CG. The role of coxsackie group B virus infections in sporadic myopericarditis. *Am Heart J* 1971;**82**:750–8.
594. Kopel FB, Shore B, Hodes HL. Nonfatal bulbospinal paralysis due to ECHO 4 virus. *J Pediatr* 1965;**67**:588–94.
595. Koskiniemi M, Paetau R, Linnavuori K. Severe encephalitis associated with disseminated echovirus 22 infection. *Scand J Infect Dis* 1989;**21**:463–6.
596. Kraus NS. [Parotitis caused by coxsackie virus]. *Minerva Med* 1960;**51**:1370–81.
597. Krogerus C, Egger D, Samuilova O, et al. Replication complex of human parechovirus 1. *J Virol* 2003;**77**:8512–23.
598. Krous HF, Langlois NE. Ljungan virus: a commentary on its association with fetal and infant morbidity and mortality in animals and humans. *Birth Defects Res A Clin Mol Teratol* 2010;**88**:947–52.
599. Kuban KC, Ephros MA, Freeman RL, et al. Syndrome of opsoclonus-myoclonus caused by coxsackie B3 infection. *Ann Neurol* 1983;**13**:69–71.
600. Kubo H, Iritani N, Seto Y. Molecular classification of enteroviruses not identified by neutralization tests. *Emerg Infect Dis* 2002;**8**:298–304.
601. Kujala G, Newman JH. Isolation of echovirus type 11 from synovial fluid in acute monocytic arthritis. *Arthritis Rheum* 1985;**28**:98–9.
602. Kunin CM. Virus-tissue union and the pathogenesis of enterovirus infections. *J Immunol* 1962;**88**:556–69.
603. Kunin CM. Cellular susceptibility to enteroviruses. *Bacteriol Rev* 1964;**28**:382–90.
604. Kuo PC, Lin JY, Chen LC, et al. Molecular and immunocytochemical identification of coxsackievirus A-24 variant from the acute haemorrhagic conjunctivitis outbreak in Taiwan in 2007. *Eye (Lond)* 2010;**24**:131–6.
605. Kupila L, Vuorinen T, Vainionpaa R, et al. Diagnosis of enteroviral meningitis by use of polymerase chain reaction of cerebrospinal fluid, stool, and serum specimens. *Clin Infect Dis* 2005;**40**:982–7.
606. Kuritsky JN, Weaver JH, Bernard KW, et al. An outbreak of acute hemorrhagic conjunctivitis in central Minnesota. *Am J Ophthalmol* 1983;**96**:449–52.
607. Laforest RA, McNaughton GA, Beale AJ, et al. Outbreak of aseptic meningitis (meningoencephalitis) with rubelliform rash: Toronto, 1956. *Can Med Assoc J* 1957;**77**:1–4.
608. Lahelle O. Aseptic meningitis caused by ECHO virus. *J Hyg (Lond)* 1957;**55**:475–84.
609. Lake AM, Lauer BA, Clark JC, et al. Enterovirus infections in neonates. *J Pediatr* 1976;**89**:787–91.
610. Landry ML, Garner R, Ferguson D. Rapid enterovirus RNA detection in clinical specimens by using nucleic acid sequence-based amplification. *J Clin Microbiol* 2003;**41**:346–50.
611. Landry ML, Fonseca SN, Cohen S, et al. Fatal enterovirus type 71 infection: rapid detection and diagnostic pitfalls. *Pediatr Infect Dis J* 1995;**14**:1095–100.
612. Landsman JB, Grist NR, Ross CA. Echo 9 virus infection and congenital malformations. *Br J Prev Soc Med* 1964;**18**:152–6.
613. Landsteiner K, Popper E. Übertragung der Poliomyelitis acuta auf Affen. *Z Immun Forsch* 1909;**2**:377–90.
614. Lane JR, Neumann DA, Lafond-Walker A, et al. Role of IL-1 and tumor necrosis factor in coxsackie virus-induced autoimmune myocarditis. *J Immunol* 1993;**151**:1682–90.
615. Lane RJ, Soteriou BA, Zhang H, et al. Enterovirus related metabolic myopathy: a postviral fatigue syndrome. *J Neurol Neurosurg Psychiatry* 2003;**74**:1382–6.
616. Lansky LL, Krugman S, Hug G. Anicteric coxsackie B hepatitis. *J Pediatr* 1979;**94**:64–5.
617. Lapinleimu K, Kaski U. An outbreak caused by coxsackievirus B5 among newborn infants. *Scand J Infect Dis* 1972;**4**:27–30.
618. Lau RC. Coxsackie B virus infection in acute myocardial infarction and adult heart disease. *Med J Aust* 1982;**2**:520–2.
619. Lau RC. Coxsackie B virus infections in New Zealand patients with cardiac and non-cardiac diseases. *J Med Virol* 1983;**11**:131–7.
620. Legay V, Chomel JJ, Fernandez E, et al. Encephalomyelitis due to human parechovirus type 1. *J Clin Virol* 2002;**25**:193–5.
621. Leggiadro RJ, Chwatsky DN, Zucker SW. Echovirus 3 infection associated with anicteric hepatitis. *Am J Dis Child* 1982;**136**:744–5.
622. Lehan PH, Chick EW, Doto IL, et al. An epidemic illness associated with a recently recognized enteric virus (ECHO virus type 4). I. Epidemiologic and clinical features. *Am J Hyg* 1957;**66**:63–75.
623. Lennette EH, Magoffin RL, Knouf EG. Viral central nervous system disease: an etiologic study conducted at the Los Angeles County General Hospital. *JAMA* 1962;**179**:687–95.
624. Leonardi GP, Greenberg AJ, Costello P, et al. Echovirus type 30 infection associated with aseptic meningitis in Nassau County, New York, USA. *Intervirology* 1993;**36**:53–6.

625. Leparc-Goffart I, Julien J, Fuchs F, et al. Evidence of presence of poliovirus genomic sequences in cerebrospinal fluid from patients with postpolio syndrome. *J Clin Microbiol* 1996;**34**: 2023–6.
626. Lepow ML, Carver DH, Robbins FC. Clinical and epidemiologic observation on enterovirus infection in a circumscribed community during an epidemic of ECHO 9 infection. *Pediatrics* 1960;**26**:12–26.
627. Lepow ML, Carver DH, Wright HT Jr, et al. A clinical, epidemiologic and laboratory investigation of aseptic meningitis during the four-year period, 1955-1958. I. Observations concerning etiology and epidemiology. *N Engl J Med* 1962;**266**: 1181–7.
628. Lepow ML, Coyne N, Thompson LB, et al. A clinical, epidemiologic and laboratory investigation of aseptic meningitis during the four-year period, 1955-1958. II. The clinical disease and its sequelae. *N Engl J Med* 1962;**266**:1188–93.
629. Lerner AM, Klein JO, Cherry JD, et al. New viral exanthems. *N Engl J Med* 1963;**269**:678–85.
630. Lerner AM, Klein JO, Finland M. A laboratory outbreak of infections with coxsackie virus group A, type 9. *N Engl J Med* 1960;**263**:1302–4.
631. Lerner AM, Klein JO, Finland M. Brief recording: Infection with coxsackie virus group B, type 3, with vesicular eruption: report of two cases. *N Engl J Med* 1960;**263**:1305.
632. Lerner AM, Klein JO, Levin HS, et al. Infections due to coxsackie virus group A, type 9, in Boston, 1959, with special reference to exanthems and pneumonia. *N Engl J Med* 1960;**263**: 1265–72.
633. Letsas KP, Filippatos GS, Delimpasi S, et al. Enterovirus-induced fulminant myocarditis and hemophagocytic syndrome. *J Infect* 2007;**54**:e75–7.
634. Levi GF, Proto C, Quadri A, et al. Coxsackie virus heart disease and cardiomyopathy. *Am Heart J* 1977;**93**:419–21.
635. Levin S, Measroch V, Pech W, et al. Hand-foot-and-mouth disease. *S Afr Med J* 1962;**36**:502–4.
636. Levy HC, Bostina M, Filman DJ, et al. Catching a virus in the act of RNA release: a novel poliovirus uncoating intermediate characterized by cryo-electron microscopy. *J Virol* 2010;**84**: 4426–41.
637. Levy O. Innate immunity of the newborn: basic mechanisms and clinical correlates. *Nat Rev Immunol* 2007;**7**:379–90.
638. Lewes D, Rainford DJ, Lane WF. Symptomless myocarditis and myalgia in viral and *Mycoplasma pneumoniae* infections. *Br Heart J* 1974;**36**:924–32.
639. Li CC, Yang MY, Chen RF, et al. Clinical manifestations and laboratory assessment in an enterovirus 71 outbreak in southern Taiwan. *Scand J Infect Dis* 2002;**34**:104–9.
640. Li Y, Bourlet T, Andreoletti L, et al. Enteroviral capsid protein VP1 is present in myocardial tissues from some patients with myocarditis or dilated cardiomyopathy. *Circulation* 2000;**101**: 231–4.
641. Liebman WM, St Geme JW Jr. Enteroviral pseudoappendicitis. *Am J Dis Child* 1970;**120**:77–8.
642. Liljeqvist JA, Bergstrom T, Holmstrom S, et al. Failure to demonstrate enterovirus aetiology in Swedish patients with dilated cardiomyopathy. *J Med Virol* 1993;**39**:6–10.
643. Limson BM, Chan VF, Guzman SV, et al. Occurrence of infection with group B coxsackievirus in rheumatic and nonrheumatic Filipino children. *J Infect Dis* 1979;**140**:415–18.
644. Lin KH, Wang HL, Sheu MM, et al. Molecular epidemiology of a variant of coxsackievirus A24 in Taiwan: two epidemics caused by phylogenetically distinct viruses from 1985 to 1989. *J Clin Microbiol* 1993;**31**:1160–6.
645. Lin TY, Chang LY, Hsia SH, et al. The 1998 enterovirus 71 outbreak in Taiwan: pathogenesis and management. *Clin Infect Dis* 2002;**34**(Suppl. 2):S52–57.
646. Lin TY, Chang LY, Huang YC, et al. Different proinflammatory reactions in fatal 6nd non-fatal enterovirus 71 infections: implications for early recognition and therapy. *Acta Paediatr* 2002; **91**:632–5.
647. Lin TY, Hsia SH, Huang YC, et al. Proinflammatory cytokine reactions in enterovirus 71 infections of the central nervous system. *Clin Infect Dis* 2003;**36**:269–74.
648. Lin TY, Twu SJ, Ho MS, et al. Enterovirus 71 outbreaks, Taiwan: occurrence and recognition. *Emerg Infect Dis* 2003;**9**:291–3.
649. Lindenbaum JE, Van Dyck PC, Allen RG. Hand, foot and mouth disease associated with coxsackievirus group B. *Scand J Infect Dis* 1975;**7**:161–3.
650. Linnemann CC Jr, Steichen J, Sherman WG, et al. Febrile illness in early infancy associated with ECHO virus infection. *J Pediatr* 1974;**84**:49–54.
651. Liu P, Wang EEL, Sole M. Viral, myocarditis: changing concepts in pathogenesis and implications in diagnosis and treatment. *Cardiovasc Pathol* 1993;**22**:47–257.
652. Longson M, Cole FM, Davies D. Isolation of a coxsackie virus group B, type 5, from the heart of a fatal case of myocarditis in an adult. *J Clin Pathol* 1969;**22**:654–8.
653. Lonnrot M, Hyoty H, Knip M, et al. Antibody cross-reactivity induced by the homologous regions in glutamic acid decarboxylase (GAD65) and 2C protein of coxsackievirus B4: Childhood diabetes in Finland Study Group. *Clin Exp Immunol* 1996;**104**: 398–405.
654. Lonnrot M, Korpela K, Knip M, et al. Enterovirus infection as a risk factor for beta-cell autoimmunity in a prospectively observed birth cohort: the Finnish Diabetes Prediction and Prevention Study. *Diabetes* 2000;**49**:1314–18.
655. Lonnrot M, Salminen K, Knip M, et al. Enterovirus RNA in serum is a risk factor for beta-cell autoimmunity and clinical type 1 diabetes: a prospective study. Childhood Diabetes in Finland (DiMe) Study Group. *J Med Virol* 2000;**61**:214–20.
656. Lu CY, Lee CY, Kao CL, et al. Incidence and case-fatality rates resulting from the 1998 enterovirus 71 outbreak in Taiwan. *J Med Virol* 2002;**67**:217–23.
657. Lukashev AN. Role of recombination in evolution of enteroviruses. *Rev Med Virol* 2005;**15**:157–67.
658. Lukashev AN, Lashkevich VA, Ivanova OE, et al. Recombination in circulating enteroviruses. *J Virol* 2003;**77**:10423–31.
659. Lukashev AN, Lashkevich VA, Ivanova OE, et al. Recombination in circulating human enterovirus B: independent evolution of structural and non-structural genome regions. *J Gen Virol* 2005; **86**:3281–90.
660. Lum LC, Chua KB, McMinn PC, et al. Echovirus 7 associated encephalomyelitis. *J Clin Virol* 2002;**23**:153–60.
661. Lycke E, Nilsson LR. Poliomyelitis in a newborn due to intrauterine infection. *Acta Paediatr* 1962;**51**:661–4.
662. Lycke E, Hiltigardh A, Redin B. Coxsackie B1 virus and febrile illness with rash. *Lancet* 1959;**1**:1097.
663. Lyden DC, Huber SA. Aggravation of coxsackievirus, group B, type 3-induced myocarditis and increase in cellular immunity to myocyte antigens in pregnant Balb/c mice and animals treated with progesterone. *Cell Immunol* 1984;**87**:462–72.
664. Macleod AJ, Faulkner RS, Van Rooyen CE. ECHO 9 virus infections in eastern Canada: clinical and laboratory studies. *Can Med Assoc J* 1958;**78**:661–5.
665. Magoffin RL, Lennette EH. Nonpolioviruses and paralytic disease. *Calif Med* 1962;**97**:1–7.
666. Magoffin RL, Jackson EW, Lennette EH. Vesicular stomatitis and exanthem: a syndrome associated with coxsackie virus, type A16. *JAMA* 1961;**175**:441–5.
667. Magoffin RL, Lennette EH, Schmidt NJ. Association of Coxsackie viruses with illnesses resembling mild paralytic polimyelitis. *Pediatrics* 1961;**28**:602–13.
668. Makower H, Skurska Z, Halazinska L. On transplacental infection with coxsackie virus. *Tex Rep Biol Med* 1958;**16**:346–54.
669. Malcom BS, Eiden JJ, Hendley JO. ECHO virus type 9 meningitis simulating tuberculous meningitis. *Pediatrics* 1980;**65**:725–6.
670. Malherbe H, Harwin R, Smith AH. An outbreak of aseptic meningitis associated with ECHO virus type 4. *S Afr Med J* 1957; **31**:1261–4.
671. Maller HM, Powars DF, Horowitz RE, et al. Fatal myocarditis associated with ECHO virus, type 22, infection in a child with apparent immunological deficiency. *J Pediatr* 1967;**71**:204–10.
672. Marchessault V, Pavilanis V, Podoski MO, et al. An epidemic of aseptic meningitis caused by coxsackie B type 2 virus. *Can Med Assoc J* 1961;**85**:123–6.
673. Marier R, Rodriguez W, Chloupek RJ, et al. Coxsackievirus B5 infection and aseptic meningitis in neonates and children. *Am J Dis Child* 1975;**129**:321–5.
674. Marks MI, Joncas JH, Mauer SM. Fatal hepatitis in siblings: isolation of coxsackievirus B5 and herpes simplex virus. *Can Med Assoc J* 1970;**102**:1391–3, passim.

675. Martin AB, Webber S, Fricker FJ, et al. Acute myocarditis: rapid diagnosis by PCR in children. *Circulation* 1994;**90**:330–9.
676. Martino TA, Sole MJ, Penn LZ, et al. Quantitation of enteroviral RNA by competitive polymerase chain reaction. *J Clin Microbiol* 1993;**31**:2634–40.
677. Marzetti G, Midulla M. Acute cerebellar ataxia associated with echo type 6 infection in two children. *Acta Paediatr Scand* 1967; **56**:547–51.
678. Mason JW, O'Connell JB, Herskowitz A, et al. A clinical trial of immunosuppressive therapy for myocarditis. The Myocarditis Treatment Trial Investigators. *N Engl J Med* 1995;**333**:269–75.
679. Matsubayashi T, Miwa Y, Takeda S, et al. Percutaneous cardiopulmonary support in a child with enterovirus 71 encephalitis. *Pediatr Int* 2006;**48**:327–9.
680. Matsumori A, Kawai C. Coxsackie virus B3 perimyocarditis in BALB/c mice: experimental model of chronic perimyocarditis in the right ventricle. *J Pathol* 1980;**131**:97–106.
681. Matsuura K, Hasegawa S, Nakayama T, et al. Epidemiological studies on echovirus type 18 infection in Toyama Prefecture. *Microbiol Immunol* 1983;**27**:359–68.
682. Matthews JD, Cameron SJ, George M. Constrictive pericarditis following coxsackie virus infection. *Thorax* 1970;**25**:624–6.
683. Matumoto M. Newer respiratory disease viruses in Japan and some Far Eastern countries. *Am Rev Respir Dis* 1963;**88**(Suppl.): 46–60.
684. McAllister R. ECHO virus infections. *Pediatr Clin North Am* 1960;**7**:927–45.
685. McAllister RM, Hummeler K, Coriell LL. Report of a case with isolation of type 9 ECHO virus from the cerebrospinal fluid. *N Engl J Med* 1959;**261**:1159–62.
686. McIntyre JP, Keen GA. Laboratory surveillance of viral meningitis by examination of cerebrospinal fluid in Cape Town, 1981-9. *Epidemiol Infect* 1993;**111**:357–71.
687. McKinney RE Jr, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. *Rev Infect Dis* 1987;**9**:334–56.
688. McLean D. Patterns of infection with enteroviruses. *J Pediatr* 1959;**54**:823–8.
689. McLean DM, Chatiyanonda K, Ladyman SR, et al. Gastroenteritis of infants in two Canadian communities. *Can Med Assoc J* 1970;**102**:1247–51.
690. McLean DM, Melnick JL. Association of mouse pathogenic strain of echo virus type 9 with aseptic meningitis. *Proc Soc Exp Biol Med* 1957;**94**:656–60.
691. McLean DM, Larke RP, Cobb C, et al. Mumps and enteroviral meningitis in Toronto, 1966. *Can Med Assoc J* 1967;**96**:1355–61.
692. McLean DM, Larke RP, McNaughton GA, et al. Enteroviral syndromes in Toronto, 1964. *Can Med Assoc J* 1965;**92**:658–61.
693. McManus BM, Chow LH, Wilson JE, et al. Direct myocardial injury by enterovirus: a central role in the evolution of murine myocarditis. *Clin Immunol Immunopathol* 1993;**68**:159–69.
694. McMinn P, Stratov I, Nagarajan L, et al. Neurological manifestations of enterovirus 71 infection in children during an outbreak of hand, foot, and mouth disease in Western Australia. *Clin Infect Dis* 2001;**32**:236–42.
695. McMinn PC. An overview of the evolution of enterovirus 71 and its clinical and public health significance. *FEMS Microbiol Rev* 2002;**26**:91–107.
696. Meade RH 3rd, Chang TW. Zoster-like eruption due to echovirus 6. *Am J Dis Child* 1979;**133**:283–4.
697. Meadow SR. Hand, foot, and mouth diseases. *Arch Dis Child* 1965;**40**:560–4.
698. Mease PJ, Ochs HD, Wedgwood RJ. Successful treatment of echovirus meningoencephalitis and myositis-fasciitis with intravenous immune globulin therapy in a patient with X-linked agammaglobulinemia. *N Engl J Med* 1981;**304**:1278–81.
699. Medearis DN Jr, Kramer RA. Exanthem associated with echo virus type 18 viremia. *J Pediatr* 1959;**55**:367–73.
700. Meehan WF, Bertrand CA. Ventricular tachycardia associated with echovirus infection. *JAMA* 1970;**212**:1701–3.
701. Melchers W, Zoll J, van Kuppeveld F, et al. There is no evidence for persistent enterovirus infections in chronic medical conditions in humans. *Rev Med Virol* 1994;**4**:235–43.
702. Mellouli F, Arrouji Z, Debre M, et al. [Successful treatment of echovirus 27 meningoencephalitis in agammaglobulinaemia with intraventricular injection of gammaglobulin: a case report]. *Arch Pediatr* 2003;**10**:130–3.
703. Melnick JL. Echoviruses. In: Horsfall JR, Tamm I, editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1965. p. 513–45.
704. Melnick JL. Portraits of viruses: the picornaviruses. *Intervirology* 1983;**20**:61–100.
705. Melnick JL. Enterovirus type 71 infections: a varied clinical pattern sometimes mimicking paralytic poliomyelitis. *Rev Infect Dis* 1984;**6**(Suppl. 2):S387–390.
706. Melnick JL. Enteroviruses. In: Evans AS, editor. *Viral infections on humans: epidemiology and control*. 3rd ed. New York: Plenum Medical; 1989. p. 191–263.
707. Melnick JL. Enteroviruses: polioviruses, coxsackieviruses, echoviruses and newer enteroviruses. In: Fields BN, Knipe DM, editors. *Virology*. 2nd ed. New York: Raven Press; 1990. p. 549–605.
708. Melnick JL. My role in the discovery and classification of the enteroviruses. *Annu Rev Microbiol* 1996;**50**:1–24.
709. Melnick JL, Wenner HA. Enteroviruses. In: Lennette EH, Schmidt NJ, editors. *Diagnostic procedures for viral and rickettsial infections*. 4th ed. New York: American Public Health Association; 1969.
710. Melnick JL, Cockburn WC, Dalldorf G, et al. Picornavirus group. *Virology* 1963;**19**:114–16.
711. Melnick JL, Dalldorf G, Enders JF, et al. The enteroviruses. *Am J Public Health* 1957;**47**:1556–66.
712. Mertens T, Hager H, Eggers HJ. Epidemiology of an outbreak in a maternity unit of infections with an antigenic variant of echovirus 11. *J Med Virol* 1982;**9**:81–91.
713. Miller DG, Gabrielson MO, Bart KJ, et al. An epidemic of aseptic meningitis, primarily among infants, caused by echovirus 11-prime. *Pediatrics* 1968;**41**:77–90.
714. Miller MA, Sutter RW, Strebel PM, et al. Cost-effectiveness of incorporating inactivated poliovirus vaccine into the routine childhood immunization schedule. *JAMA* 1996;**276**:967–71.
715. Miller NA, Carmichael HA, Calder BD, et al. Antibody to coxsackie B virus in diagnosing postviral fatigue syndrome. *BMJ* 1991;**302**:140–3.
716. Minami K, Tsuda Y, Maeda H, et al. Acute transverse myelitis caused by coxsackie virus B5 infection. *J Paediatr Child Health* 2004;**40**:66–8.
717. Mirand A, Schuffenecker I, Henquell C, et al. Phylogenetic evidence for a recent spread of two populations of human enterovirus 71 in European countries. *J Gen Virol* 2010;**91**:2263–77.
718. Mitchell SC, Dempster G. The finding of genital lesions in a case of coxsackie virus infection. *Can Med Assoc J* 1955;**72**: 117–19.
719. Miwa C, Ohtani M, Watanabe H, et al. Epidemic of hand, foot and mouth disease in Gifu Prefecture in 1978. *Jpn J Med Sci Biol* 1980;**33**:167–80.
720. Modlin JF. Fatal echovirus 11 disease in premature neonates. *Pediatrics* 1980;**66**:775–80.
721. Modlin JF. Perinatal echovirus infection: insights from a literature review of 61 cases of serious infection and 16 outbreaks in nurseries. *Rev Infect Dis* 1986;**8**:918–26.
722. Modlin JF, Dagan R, Berlin LE, et al. Focal encephalitis with enterovirus infections. *Pediatrics* 1991;**88**:841–5.
723. Monif GR, Lee CW, Hsiung GD. Isolated myocarditis with recovery of ECHO type 9 virus from the myocardium. *N Engl J Med* 1967;**277**:1353–5.
724. Montgomery J, Gear J, Prinsloo FR, et al. Myocarditis of the newborn; an outbreak in a maternity home in Southern Rhodesia associated with coxsackie group-B virus infection. *S Afr Med J* 1955;**29**:608–12.
725. Moore M. Centers for Disease Control. Enteroviral disease in the United States, 1970-1979. *J Infect Dis* 1982;**146**:103–8.
726. Moore ML, Hooser LE, Davis EV, et al. Sudden unexpected death in infancy. isolations of echo type 7 virus. *Proc Soc Exp Biol Med* 1964;**116**:231–4.
727. Moossy J, Geer JC. Encephalomyelitis, myocarditis and adrenal cortical necrosis in coxasackie B3 virus infection: distribution of the central nervous system lesions. *Arch Pathol* 1960;**70**:614–22.
728. Morens DM. Enteroviral disease in early infancy. *J Pediatr* 1978;**92**:374–7.

729. Morens DM, Zweighaft RM, Bryan JM. Non-polio enterovirus disease in the United States, 1971–1975. *Int J Epidemiol* 1979;**8**: 49–54.
730. Morgante O, Wilkinson D, Burchak EC, et al. Outbreak of hand-foot-and-mouth disease among Indian and Eskimo children in a hospital. *J Infect Dis* 1972;**125**:587–94.
731. Mori I, Matsumoto K, Hatano M, et al. An unseasonable winter outbreak of echovirus type 30 meningitis. *J Infect* 1995;**31**: 219–23.
732. Morita H, Kitaura Y, Deguchi H, et al. Coxsackie B5 myopericarditis in a young adult: clinical course and endomyocardial biopsy findings. *Jpn Circ J* 1983;**47**:1077–83.
733. Moritsugu Y, Sawada K, Hinohara M, et al. An outbreak of type 26 Echovirus infection with exanthem in an infant home near Tokyo. *Am J Epidemiol* 1968;**87**:599–608.
734. Morris JA, Elisberg BL, Pond WL, et al. Hepatitis associated with coxsackie virus group A, type 4. *N Engl J Med* 1962;**267**: 1230–3.
735. Moscovici C, Maisel J. Intestinal viruses of newborn and older prematures. *Am J Dis Child* 1961;**101**:771–7.
736. Mostoufizadeh M, Lack EE, Gang DL, et al. Postmortem manifestations of echovirus 11 sepsis in five newborn infants. *Hum Pathol* 1983;**14**:818–23.
737. Muir P, Archard LC. There is evidence for persistent enterovirus infections in chronic medical conditions in humans. *Rev Med Virol* 1994;**4**:245–50.
738. Muir P, Nicholson F, Illavia SJ, et al. Serological and molecular evidence of enterovirus infection in patients with end-stage dilated cardiomyopathy. *Heart* 1996;**76**:243–9.
739. Muir P, Nicholson F, Spencer GT, et al. Enterovirus infection of the central nervous system of humans: lack of association with chronic neurological disease. *J Gen Virol* 1996;**77**(pt 7): 1469–76.
740. Muir P, Nicholson F, Tilzey AJ, et al. Chronic relapsing pericarditis and dilated cardiomyopathy: serological evidence of persistent enterovirus infection. *Lancet* 1989;**1**:804–7.
741. Mulders MN, Lipskaya GY, van der Avoort HG, et al. Molecular epidemiology of wild poliovirus type 1 in Europe, the Middle East, and the Indian subcontinent. *J Infect Dis* 1995;**171**:1399–405.
742. Murdin AD, Barreto L, Plotkin S. Inactivated poliovirus vaccine: past and present experience. *Vaccine* 1996;**14**:735–46.
743. Murphy AM, Simmul R. Coxsackie B4 virus infections in New South Wales during 1962. *Med J Aust* 1964;**2**:443–5.
744. Nagington J, Gandy G, Walker J, et al. Use of normal immunoglobulin in an echovirus 11 outbreak in a special-care baby unit. *Lancet* 1983;**2**:443–6.
745. Nagington J, Wreghittt TG, Gandy G, et al. Fatal echovirus 11 infections in outbreak in special-care baby unit. *Lancet* 1978;**2**: 725–8.
746. Nahmias AJ, Froeschle JE, Feorino PM, et al. Generalized eruption in a child with eczema due to coxsackievirus A16. *Arch Dermatol* 1968;**97**:147–8.
747. Nair E, Kalra SL. Cytopathic enteroviruses in Delhi area. I. From cases of diarrhoea and healthy controls. *Indian J Med Res* 1969;**57**:141–8.
748. Nakao T. Coxsackie viruses and diabetes. *Lancet* 1971;**2**:1423.
749. Nakao T, Horino K. Clinical and virological studies of acute cerebellar ataxia in childhood. *Tohoku J Exp Med* 1970;**101**: 47–53.
750. Nakao T, Miura R. Recurrent virus meningitis. *Pediatrics* 1971;**47**:773–6.
751. Nakao T, Morita M. Exanthem associated with ECHO 25 virus. *Jika Rinsho* 1965;**18**:772–3.
752. Nakao T, Miura R, Sato M. ECHO virus type 22 infection in a premature infant. *Tohoku J Exp Med* 1970;**102**:61–8.
753. Nankervis G, Starr J, Gold E. *Hand, foot and mouth syndrome in a group of families*. Program for the Society for Pediatric Research; 1968. p. 152.
754. Nathanson N, Fine P. Virology. Poliomyelitis eradication: a dangerous endgame. *Science* 2002;**296**:269–70.
755. Nelson D, Hiemstra H, Minor T, et al. Non-polio enterovirus activity in Wisconsin based on a 20-year experience in a diagnostic virology laboratory. *Am J Epidemiol* 1979;**109**:352–61.
756. Neu N, Beisel KW, Traystman MD, et al. Autoantibodies specific for the cardiac myosin isoform are found in mice susceptible to coxsackievirus B3-induced myocarditis. *J Immunol* 1987;**138**: 2488–92.
757. Neva FA. A second outbreak of Boston exanthem disease in Pittsburgh during 1954. *N Engl J Med* 1956;**254**:838–43.
758. Neva FA, Enders JF. Cytopathogenic agents isolated from patients during an unusual epidemic exanthem. *J Immunol* 1954;**72**:307–14.
759. Neva FA, Feemster RF, Gorbach IJ. Clinical and epidemiological features of an usual epidemic exanthem. *J Am Med Assoc* 1954; **155**:544–8.
760. Neva FA, Zuffante SM. Agents isolated from patients with Boston exanthem disease during 1954 in Pittsburgh. *J Lab Clin Med* 1957;**50**:712–19.
761. News and Notes. Epidemiology: echovirus infections. *Br Med J* 1970;**3**:594.
762. Nicholls AC, Thomas M. Coxsackie virus infection in acute myocardial infarction. *Lancet* 1977;**1**:883–4.
763. Nielsen AC, Bottiger B, Banner J, et al. Serious invasive Saffold virus infections in children, 2009. *Emerg Infect Dis* 2012;**18**: 7–12.
764. Nielsen JL, Berryman GK, Hankins GD. Intrauterine fetal death and the isolation of echovirus 27 from amniotic fluid. *J Infect Dis* 1988;**158**:501–2.
765. Nielsen LP, Modlin JF, Rotbart HA. Detection of enteroviruses by polymerase chain reaction in urine samples of patients with aseptic meningitis. *Pediatr Infect Dis J* 1996;**15**:625–7.
766. Nightingale EO. Recommendations for a national policy on poliomyelitis vaccination. *N Engl J Med* 1977;**297**:249–53.
767. Nihoul E, Quersin-Thiry L, Weynants A. ECHO virus type 9 as the agent responsible for an important outbreak of aseptic meningitis in Belgium. *Am J Hyg* 1957;**66**:102–18.
768. Niklasson B, Samsioe A, Papadogiannakis N, et al. Zoonotic Ljungan virus associated with central nervous system malformations in terminated pregnancy. *Birth Defects Res A Clin Mol Teratol* 2009;**85**:542–5.
769. Nikoskelainen J, Kalliomaki JL, Lapinleimu K, et al. Coxsackie B virus antibodies in myocardial infarction. *Acta Med Scand* 1983;**214**:29–32.
770. Nishmi M, Yodfat Y. An outbreak among Kibbutz children of a febrile illness associated with Echo virus type 4. *Isr J Med Sci* 1970;**6**:535–9.
771. Nix WA, Maher K, Johansson ES, et al. Detection of all known parechoviruses by real-time PCR. *J Clin Microbiol* 2008;**46**: 2519–24.
772. Nogen AG, Lepow ML. Enteroviral meningitis in very young infants. *Pediatrics* 1967;**40**:617–26.
773. Nolan MA, Craig ME, Lahra MM, et al. Survival after pulmonary edema due to enterovirus 71 encephalitis. *Neurology* 2003;**60**:1651–6.
774. Nolte FS, Rogers BB, Tang YW, et al. Evaluation of a rapid and completely automated real-time reverse transcriptase PCR assay for diagnosis of enteroviral meningitis. *J Clin Microbiol* 2011;**49**: 528–33.
775. Norder H, Bjerregaard L, Magnius LO. Homotypic echoviruses share aminoterminal VP1 sequence homology applicable for typing. *J Med Virol* 2001;**63**:35–44.
776. Norder H, Bjerregaard L, Magnius L, et al. Sequencing of "untypable" enteroviruses reveals two new types, EV-77 and EV-78, within human enterovirus type B and substitutions in the BC loop of the VP1 protein for known types. *J Gen Virol* 2003;**84**:827–36.
777. Norton H. Report of an outbreak of "hand-foot-and-mouth disease" in Sydney. *Med J Aust* 1961;**2**:570.
778. Novack A, Feldman HA, Wang SS, et al. A community-wide coxsackievirus A9 outbreak. *JAMA* 1967;**202**:862–6.
779. Null FC Jr, Castle CH. Adultpericarditis and myocarditis due to coxsackie virus group B, type 5. *N Engl J Med* 1959;**261**:937–42.
780. O'Connell JB, Reap EA, Robinson JA. The effects of cyclosporine on acute murine coxsackie B3 myocarditis. *Circulation* 1986;**73**:353–9.
781. O'Neill D, McArthur JD, Kennedy JA, et al. Coxsackie B virus infection in coronary care unit patients. *J Clin Pathol* 1983;**36**: 658–61.
782. O'Regan S, Robitaille P, Mongeau JG, et al. The hemolytic uremic syndrome associated with ECHO 22 infection. *Clin Pediatr (Phila)* 1980;**19**:125–7.

783. O'Shaughnessey WJ, Buechner HA. Hepatitis associated with a coxsackie B5 virus infection during late pregnancy. *JAMA* 1962;**179**:71–2.
784. Oberste MS, Maher K, Pallansch MA. Molecular phylogeny and proposed classification of the simian picornaviruses. *J Virol* 2002;**76**:1244–51.
785. Oberste MS, Maher K, Pallansch MA. Evidence for frequent recombination within species human enterovirus B based on complete genomic sequences of all thirty-seven serotypes. *J Virol* 2004;**78**:855–67.
786. Oberste MS, Penaranda S, Pallansch MA. RNA recombination plays a major role in genomic change during circulation of coxsackie B viruses. *J Virol* 2004;**78**:2948–55.
787. Oberste MS, Maher K, Kilpatrick DR, et al. Typing of human enteroviruses by partial sequencing of VP1. *J Clin Microbiol* 1999;**37**:1288–93.
788. Oberste MS, Maher K, Nix WA, et al. Molecular identification of 13 new enterovirus types, EV79-88, EV97, and EV100-101, members of the species human enterovirus B. *Virus Res* 2007; **128**:34–42.
789. Oberste MS, Michele SM, Maher K, et al. Molecular identification and characterization of two proposed new enterovirus serotypes, EV74 and EV75. *J Gen Virol* 2004;**85**:3205–12.
790. Oberste MS, Nix WA, Maher K, et al. Improved molecular identification of enteroviruses by RT-PCR and amplicon sequencing. *J Clin Virol* 2003;**26**:375–7.
791. Ogilvie MM, Tearne CF. Spontaneous abortion after hand-foot-and-mouth disease caused by coxsackie virus A16. *Br Med J* 1980;**281**:1527–8.
792. Oikarinen M, Tauriainen S, Honkanen T, et al. Detection of enteroviruses in the intestine of type 1 diabetic patients. *Clin Exp Immunol* 2008;**151**:71–5.
793. Oikarinen S, Martiskainen M, Tauriainen S, et al. Enterovirus RNA in blood is linked to the development of type 1 diabetes. *Diabetes* 2011;**60**:276–9.
794. Ooi EE, Phoon MC, Ishak B, et al. Seroepidemiology of human enterovirus 71, Singapore. *Emerg Infect Dis* 2002;**8**: 995–7.
795. Ooi MH, Wong SC, Lewthwaite P, et al. Clinical features, diagnosis, and management of enterovirus 71. *Lancet Neurol* 2010;**9**: 1097–105.
796. Ooi MH, Wong SC, Podin Y, et al. Human enterovirus 71 disease in Sarawak, Malaysia: a prospective clinical, virological, and molecular epidemiological study. *Clin Infect Dis* 2007;**44**: 646–56.
797. Orinius E. The late cardiac prognosis after coxsackie-B infection. *Acta Med Scand* 1968;**183**:235–7.
798. Otonkoski T, Roivainen M, Vaarala O, et al. Neonatal Type I diabetes associated with maternal echovirus 6 infection: a case report. *Diabetologia* 2000;**43**:1235–8.
799. Ouellet A, Sherlock R, Toye B, et al. Antenatal diagnosis of intrauterine infection with coxsackievirus B3 associated with live birth. *Infect Dis Obstet Gynecol* 2004;**12**:23–6.
800. Pajkrt D, Benschop KS, Westerhuis B, et al. Clinical characteristics of human parechoviruses 4-6 infections in young children. *Pediatr Infect Dis J* 2009;**28**:1008–10.
801. Pallansch MA, Roos RP. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, editors. Fields virology, **vol. 1**. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 723–75.
802. Pallansch MA, Sandhu HS. The eradication of polio: progress and challenges. *N Engl J Med* 2006;**355**:2508–11.
803. Palomba E, Tovo PA. Persistent fever as the only manifestation of chronic coxsackievirus B4 infection in the brain of a human immunodeficiency virus type 1-infected child. *Clin Infect Dis* 1999;**28**:912–13.
804. Paque RE. Role of anti-idiotypic antibodies in induction, regulation, and expression of coxsackievirus-induced myocarditis. *Prog Med Virol* 1992;**39**:204–27.
805. Paradiso PR. The future of polio immunization in the United States: are we ready for change? *Pediatr Infect Dis J* 1996;**15**: 645–9.
806. Park K, Lee K, Lee J, et al. Acute hemorrhagic conjunctivitis epidemic caused by coxsackievirus A24 variants in Korea during 2002-2003. *J Med Virol* 2006;**78**:91–7.
807. Parks WP, Queiroga LT, Melnick JL. Studies of infantile diarrhea in Karachi, Pakistan. II. Multiple virus isolations from rectal swabs. *Am J Epidemiol* 1967;**85**:469–78.
808. Parrott RH. The clinical importance of group A coxsackie viruses. *Ann N Y Acad Sci* 1957;**67**:230–40.
809. Parrott RH, Ross S, Burke FG, et al. Herpangina; clinical studies of a specific infectious disease. *N Engl J Med* 1951;**245**:275–80.
810. Pasic S, Jankovic B, Abinun M, et al. Intravenous immunoglobulin prophylaxis in an echovirus 6 and echovirus 4 nursery outbreak. *Pediatr Infect Dis J* 1997;**16**:718–20.
811. Paterson WG, Smith IW. An unusual acute abdomen in pregnancy: an ECHO 8 virus infection. *Practitioner* 1969;**203**:337–9.
812. Paul JR. *A history of poliomyelitis*. New Haven, CT: Yale University Press; 1971.
813. Pelon W, Villarejos VM, Rhim JS, et al. Coxsackie group B virus infection and acute diarrhoea occurring among children in Costa Rica. *Arch Dis Child* 1966;**41**:636–41.
814. Perez-Ruiz M, Navarro-Mari JM, Palacios del Valle E, et al. Human rhabdomyosarcoma cells for rapid detection of enteroviruses by shell-vial assay. *J Med Microbiol* 2003;**52**:789–91.
815. Peters AC, Vielvoye GJ, Versteeg J, et al. ECHO 25 focal encephalitis and subacute hemichorea. *Neurology* 1979;**29**:676–81.
816. Petitjean J, Freymuth F, Kopecka H, et al. Detection of enteroviruses in cerebrospinal fluids: enzymatic amplification and hybridization with a biotinylated riboprobe. *Mol Cell Probes* 1994;**8**:15–22.
817. Pevear DC, Tull TM, Seipel ME, et al. Activity of pleconaril against enteroviruses. *Antimicrob Agents Chemother* 1999;**43**: 2109–15.
818. Pham NT, Chan-It W, Khamrin P, et al. Detection of human parechovirus in stool samples collected from children with acute gastroenteritis in Japan during 2007-2008. *J Med Virol* 2011;**83**: 331–6.
819. Pham NT, Takanashi S, Tran DN, et al. Human parechovirus infection in children hospitalized with acute gastroenteritis in Sri Lanka. *J Clin Microbiol* 2011;**49**:364–6.
820. Pham NT, Trinh QD, Khamrin P, et al. Diversity of human parechoviruses isolated from stool samples collected from Thai children with acute gastroenteritis. *J Clin Microbiol* 2010;**48**: 115–19.
821. Philip AG, Larson EJ. Overwhelming neonatal infection with ECHO 19 virus. *J Pediatr* 1973;**82**:391–7.
822. Philipson L. Association between a recently isolated virus and an epidemic of upper respiratory disease in a day nursery. *Arch Gesamte Virusforsch* 1958;**8**:204–15.
823. Philipson L, Wesslen T. Recovery of a cytopathogenic agent from patients with non-diphtheritic croup and from day-nursery children. I. Properties of the agent. *Arch Gesamte Virusforsch* 1958;**8**:76–94.
824. Phillips CA, Melnick JL, Barrett FF, et al. Dual virus infections: simultaneous enteroviral disease and St. Louis encephalitis. *JAMA* 1966;**197**:169–72.
825. Pineiro L, Vicente D, Montes M, et al. Human parechoviruses in infants with systemic infection. *J Med Virol* 2010;**82**:1790–6.
826. Pino-Ramirez RM, Pertierra-Cortada A, Iriondo-Sanz M, et al. Neonatal echovirus 30 infection associated with severe hepatitis in twin neonates. *Pediatr Infect Dis J* 2008;**27**:88.
827. Plager H, Deibel R. Echo 30 virus infections: outbreak in New York State. *N Y State J Med* 1970;**70**:391–3.
828. Plager H, Harrison FF. Paralysis associated with ECHO virus type nine. *N Y State J Med* 1961;**61**:798–800.
829. Plager H, Beebe R, Miller JK. Coxsackie B-5 pericarditis in pregnancy. *Arch Intern Med* 1962;**110**:735–8.
830. Ponka A, Jalanko H, Ponka T, et al. Viral and mycoplasmal antibodies in patients with myocardial infarction. *Ann Clin Res* 1981;**13**:429–32.
831. Pope JG, Pollock TM. Coxsackie B5 virus infections during 1965: a report to the Director of the Public Health Laboratory Service from various laboratories in the United Kingdom. *Br Med J* 1967;**4**:575–7.
832. Portnoy B, Hanes B, Pierce NF, et al. Aseptic meningitis associated with echo virus type 9 infection. with special reference to variability by sex and incidence of paralytic sequelae. *Calif Med* 1965;**102**:261–7.

833. Poyry T, Hyypia T, Horsnell C, et al. Molecular analysis of coxsackievirus A16 reveals a new genetic group of enteroviruses. *Virology* 1994;**202**:982–7.
834. Poyry T, Kinnunen L, Hyypia T, et al. Genetic and phylogenetic clustering of enteroviruses. *J Gen Virol* 1996;**77**(pt 8):1699–717.
835. Prager P, Nolan M, Andrews IP, et al. Neurogenic pulmonary edema in enterovirus 71 encephalitis is not uniformly fatal but causes severe morbidity in survivors. *Pediatr Crit Care Med* 2003;**4**:377–81.
836. Prakash CV. Entero-viruses in infantile diarrhoea in India. I. Investigations carried out in Bombay. *Indian J Med Res* 1962; **50**:343–7.
837. Price RA, Garcia JH, Rightsel WA. Choriomeningitis and myocarditis in an adolescent with isolation of coxsackie B-5 virus. *Am J Clin Pathol* 1970;**53**:825–31.
838. Prince JT, Geme JW Jr, Scherer WF. ECHO-9 virus exanthema. *J Am Med Assoc* 1958;**167**:691–6.
839. Pulli T, Koskimies P, Hyypia T. Molecular comparison of coxsackie A virus serotypes. *Virology* 1995;**212**:30–8.
840. Quaife RA, Gostling JV. False positive Wassermann reaction associated with evidence of enterovirus infection. *J Clin Pathol* 1971;**24**:120–1.
841. Rabausch-Starz I, Schwaiger A, Grunewald K, et al. Persistence of virus and viral genome in myocardium after coxsackievirus B3-induced murine myocarditis. *Clin Exp Immunol* 1994;**96**: 69–74.
842. Rabkin CS, Telzak EE, Ho MS, et al. Outbreak of echovirus 11 infection in hospitalized neonates. *Pediatr Infect Dis J* 1988;**7**: 186–90.
843. Racaniello VR. Picornaviridae: The viruses and their replication. In: Knipe DM, Howley PM , editors. Fields virology, **vol. 1**. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 685–722.
844. Ramers C, Billman G, Hartin M, et al. Impact of a diagnostic cerebrospinal fluid enterovirus polymerase chain reaction test on patient management. *JAMA* 2000;**283**:2680–5.
845. Ramlow J, Alexander M, LaPorte R, et al. Epidemiology of the post-polio syndrome. *Am J Epidemiol* 1992;**136**:769–86.
846. Ramos-Alvarez M. Cytopathogenic enteric viruses associated with undifferentiated diarrheal syndromes in early childhood. *Ann N Y Acad Sci* 1957;**67**:326–31.
847. Ramos-Alvarez M, Olarte J. Diarrheal diseases of children. the occurrence of enteropathogenic viruses and bacteria. *Am J Dis Child* 1964;**107**:218–31.
848. Ramos-Alvarez M, Sabin AB. Enteropathogenic viruses and bacteria; role in summer diarrheal diseases of infancy and early childhood. *J Am Med Assoc* 1958;**167**:147–56.
849. Ramsingh AI, Collins DN. A point mutation in the VP4 coding sequence of coxsackievirus B4 influences virulence. *J Virol* 1995;**69**:7278–81.
850. Ramsingh AI, Lee WT, Collins DN, et al. T cells contribute to disease severity during coxsackievirus B4 infection. *J Virol* 1999;**73**:3080–6.
851. Rantasalo I, Penttinen K, Saxen L, et al. Echo 9 virus antibody status after an epidemic period and the possible teratogenic effect of the infection. *Ann Paediatr Fenn* 1960;**6**:175–84.
852. Ranzenhofer ER, Dizon FC, Lipton MM, et al. Clinical paralytic poliomyelitis due to coxsackie virus group A, type 7. *N Engl J Med* 1958;**259**:182.
853. Ray CG, McCollough RH, Doto IL, et al. Echo 4 illness. Epidemiological, clinical and laboratory studies of an outbreak in a rural community. *Am J Epidemiol* 1966;**84**:253–67.
854. Ray CG, Portman JN, Stamm SJ, et al. Hemolytic-uremic syndrome and myocarditis: association with coxsackievirus B infection. *Am J Dis Child* 1971;**122**:418–20.
855. Ray CG, Tucker VL, Harris DJ, et al. Enteroviruses associated with the hemolytic-uremic syndrome. *Pediatrics* 1970;**46**:378–88.
856. Ren L, Gonzalez R, Xiao Y, et al. Saffold cardiovirus in children with acute gastroenteritis, Beijing, China. *Emerg Infect Dis* 2009; **15**:1509–11.
857. Ren L, Gonzalez R, Xie Z, et al. Saffold cardioviruses of 3 lineages in children with respiratory tract infections, Beijing, China. *Emerg Infect Dis* 2010;**16**:1158–61.
858. Reyes MP, Ho KL, Smith F, et al. A mouse model of dilated-type cardiomyopathy due to coxsackievirus B3. *J Infect Dis* 1981; **144**:232–6.
859. Reyes MP, Ostrea EM Jr, Roskamp J, et al. Disseminated neonatal echovirus 11 disease following antenatal maternal infection with a virus-positive cervix and virus-negative gastrointestinal tract. *J Med Virol* 1983;**12**:155–9.
860. Reyes MP, Zalenski D, Smith F, et al. Coxsackievirus-positive cervices in women with febrile illnesses during the third trimester in pregnancy. *Am J Obstet Gynecol* 1986;**155**:159–61.
861. Rezkalla S, Khatib G, Khatib R. Coxsackievirus B3 murine myocarditis: deleterious effects of nonsteroidal anti-inflammatory agents. *J Lab Clin Med* 1986;**107**:393–5.
862. Rezkalla SH, Raikar S, Kloner RA. Treatment of viral myocarditis with focus on captopril. *Am J Cardiol* 1996;**77**:634–7.
863. Ribai P, Liesnard C, Rodesch G, et al. Transient cerebral arteriopathy in infancy associated with enteroviral infection. *Eur J Paediatr Neurol* 2003;**7**:73–5.
864. Rice SK, Heinl RE, Thornton LL, et al. Clinical characteristics, management strategies, and cost implications of a statewide outbreak of enterovirus meningitis. *Clin Infect Dis* 1995;**20**: 931–7.
865. Richardson HB Jr, Leibovitz A. "Hand, foot, and mouth disease" in children: an epidemic associated with coxsakie virus A-16. *J Pediatr* 1965;**67**:6–12.
866. Rinehart JE, Gomez RM, Roos RP. Molecular determinants for virulence in coxsackievirus B1 infection. *J Virol* 1997;**71**:3986–91.
867. Rittichier KR, Bryan PA, Bassett KE, et al. Diagnosis and outcomes of enterovirus infections in young infants. *Pediatr Infect Dis J* 2005;**24**:546–50.
868. Robbins FC, de Quadros CA. Certification of the eradication of indigenous transmission of wild poliovirus in the Americas. *J Infect Dis* 1997;**175**(Suppl. 1):S281–285.
869. Roberts L. Polio campaign: the polio emergency. *Science* 2012; **337**:514–16.
870. Robinson CC, Willis M, Meagher A, et al. Impact of rapid polymerase chain reaction results on management of pediatric patients with enteroviral meningitis. *Pediatr Infect Dis J* 2002;**21**: 283–6.
871. Robinson CR, Doane FW, Rhodes AJ. Report of an outbreak of febrile illness with pharyngeal lesions and exanthem: Toronto, summer 1957—isolation of group A coxsackie virus. *Can Med Assoc J* 1958;**79**:615–21.
872. Robinson JA, O'Connell JB, Roeges LM, et al. Coxsackie B3 myocarditis in athymic mice. *Proc Soc Exp Biol Med* 1981;**166**: 80–91.
873. Roden VJ, Cantor HE, O'Connor DM, et al. Acute hemiphegia of childhood associated with coxsackie A9 viral infection. *J Pediatr* 1975;**86**:56–8.
874. Rodriguez-Torres R, Lin JS, Berkovich S. A sensitive electrocardiographic sign in myocarditis associated with viral infection. *Pediatrics* 1969;**43**:846–51.
875. Roivainen M, Knip M, Hyoty H, et al. Several different enterovirus serotypes can be associated with prediabetic autoimmune episodes and onset of overt IDDM. Childhood Diabetes in Finland (DiMe) Study Group. *J Med Virol* 1998;**56**:74–8.
876. Romero JR. Enteroviruses and parechoviruses. In: Murray PR, Baron EJ, Jorgensen JH, et al. editors. *Manual of clinical microbiology*. 9th ed. Washington, D.C.: ASM Press; 2007. p. 1392–404.
877. Rorabaugh ML, Berlin LE, Heldrich F, et al. Aseptic meningitis in infants younger than 2 years of age: acute illness and neurologic complications. *Pediatrics* 1993;**92**:206–11.
878. Ross CA, Bell EJ, Kerr MM, et al. Infective agents and embryopathy in the west of Scotland 1966-1970. *Scott Med J* 1972; **17**:252–8.
879. Rotbart HA. Diagnosis of enteroviral meningitis with the polymerase chain reaction. *J Pediatr* 1990;**117**:85–9.
880. Rotbart HA. Enzymatic RNA amplification of the enteroviruses. *J Clin Microbiol* 1990;**28**:438–42.
881. Rotbart HA. Nucleic acid detection systems for enteroviruses. *Clin Microbiol Rev* 1991;**4**:156–68.
882. Rotbart HA. Enteroviral infections of the central nervous system. *Clin Infect Dis* 1995;**20**:971–81.

883. Rotbart HA, Webster AD, Pleconaril Treatment Registry G. treatment of potentially life-threatening enterovirus infections with pleconaril. *Clin Infect Dis* 2001;**32**:228–35.
884. Rotbart HA, Abzug MJ, Levin MJ. Development and application of RNA probes for the study of picornaviruses. *Mol Cell Probes* 1988;**2**:65–73.
885. Rotbart HA, Kinsella JP, Wasserman RL. Persistent enterovirus infection in culture-negative meningoencephalitis: demonstration by enzymatic RNA amplification. *J Infect Dis* 1990;**161**: 787–91.
886. Rotbart HA, O'Connell JF, McKinlay MA. Treatment of human enterovirus infections. *Antiviral Res* 1998;**38**:1–14.
887. Rotbart HA, Sawyer MH, Fast S, et al. Diagnosis of enteroviral meningitis by using PCR with a colorimetric microwell detection assay. *J Clin Microbiol* 1994;**32**:2590–2.
888. Rotem CE. Meningitis of virus origin. *Lancet* 1957;**272**:502–4.
889. Rubin H, Lehan PH, Doto IL, et al. Epidemic infection with coxsackie virus group B, type 5. I. Clinical and epidemiologic aspects. *N Engl J Med* 1958;**258**:255–63.
890. Rueckert RR. Picornaviridae and their replication. In: Fields BN, Knipe DM, editor. *Virology*. 2nd ed. New York: Raven Press; 1990. p. 507–48.
891. Russell SJ, Bell EJ. Echoviruses and carditis. *Lancet* 1970;**1**: 784–5.
892. Ryder DE, Doane FW, Zbitnew A, et al. Report of an outbreak of Bornholm disease, with isolation of coxsackie B5 virus: Toronto, 1958. *Can J Public Health* 1959;**50**:265–9.
893. Ryu WS, Kang B, Hong J, et al. Clinical and etiological characteristics of enterovirus 71-related diseases during a recent 2-year period in Korea. *J Clin Microbiol* 2010;**48**:2490–4.
894. Sabin AB. Role of ECHO viruses in human disease. In: Rose HM, editor. *Viral infections of infancy and childhood*. New York: Hoeber-Harper; 1960. p. 78–100.
895. Sabin AB. Poliomyelitis. In: Braude AI, editor. *Medical microbiology and infectious diseases*. Philadelphia: W. B. Saunders; 1981. p. 1348–65.
896. Sabin AB. Oral poliovirus vaccine: history of its development and use and current challenge to eliminate poliomyelitis from the world. *J Infect Dis* 1985;**151**:420–36.
897. Sabin AB, Krumbiegel ER, Wigand R. ECHO type 9 virus disease. *J Dis Child* 1958;**96**:197–219.
898. Sainani GS, Krompotic E, Slodki SJ. Adult heart disease due to the coxsackie virus B infection. *Medicine (Baltimore)* 1968;**47**: 133–47.
899. Salk D. Eradication of poliomyelitis in the United States. I. Live virus vaccine-associated and wild poliovirus disease. *Rev Infect Dis* 1980;**2**:228–42.
900. Salk D. Eradication of poliomyelitis in the United States. II. Experience with killed poliovirus vaccine. *Rev Infect Dis* 1980;**2**:243–57.
901. Salk D. Eradication of poliomyelitis in the United States. III. Poliovaccines: practical considerations. *Rev Infect Dis* 1980;**2**: 258–73.
902. Salk D. Polio immunization policy in the United States: a new challenge for a new generation. *Am J Public Health* 1988;**78**:296–300.
903. Samsioe A, Papadogiannakis N, Hultman T, et al. Ljungan virus present in intrauterine fetal death diagnosed by both immunohistochemistry and PCR. *Birth Defects Res A Clin Mol Teratol* 2009;**85**:227–9.
904. Samuda GM, Chang WK, Yeung CY, et al. Monoplegia caused by enterovirus 71: an outbreak in Hong Kong. *Pediatr Infect Dis J* 1987;**6**:206–8.
905. Samuelson A, Glimaker M, Skoog E, et al. Diagnosis of enteroviral meningitis with IgG-EIA using heat-treated virions and synthetic peptides as antigens. *J Med Virol* 1993;**40**: 271–7.
906. Sanders DY, Cramblett HG. Viral infections in hospitalized neonates. *Am J Dis Child* 1968;**116**:251–6.
907. Sanders DY, Powell RV, Smith A. Outbreak of coxsackie B5 virus in a children's home. *South Med J* 1969;**62**:474–6.
908. Santti J, Hyypia T, Kinnunen L, et al. Evidence of recombination among enteroviruses. *J Virol* 1999;**73**:8741–9.
909. Saslaw S, Wooley CF, Anderson GR. Aseptic meningitis syndrome: report of eleven cases with cerebrospinal fluid isolation of enteroviruses. *Arch Intern Med* 1960;**105**:69–75.
910. Sato K, Yamashita T, Sakae K, et al. A new-born baby outbreak of echovirus type 33 infection. *J Infect* 1998;**37**:123–6.
911. Sawyer LA, Hershow RC, Pallansch MA, et al. An epidemic of acute hemorrhagic conjunctivitis in American Samoa caused by coxsackievirus A24 variant. *Am J Epidemiol* 1989;**130**:1187–98.
912. Sawyer MH, Holland D, Aintablian N, et al. Diagnosis of enteroviral central nervous system infection by polymerase chain reaction during a large community outbreak. *Pediatr Infect Dis J* 1994;**13**:177–82.
913. Saxena RC, Bhatia M, Chaturvedi UC. Recent epidemic conjunctivitis in Lucknow: a clinical study. *Orient Arch Ophthalmol* 1982;**10**:253–7.
914. Schleissner LA, Portnoy B. Hepatitis and pneumonia associated with ECHO virus, type 9, infection in two adult siblings. *Ann Intern Med* 1968;**68**:1315–19.
915. Schleissner LA, Portnoy B. Spectrum of ECHO virus 1 disease in a young diabetic. *Chest* 1973;**63**:457–9.
916. Schlesinger Y, Sawyer MH, Storch GA. Enteroviral meningitis in infancy: potential role for polymerase chain reaction in patient management. *Pediatrics* 1994;**94**:157–62.
917. Schmidt NJ, Lennette EH, Ho HH. An apparently new enterovirus isolated from patients with disease of the central nervous system. *J Infect Dis* 1974;**129**:304–9.
918. Schmidt NJ, Magoffin RL, Lennette EH. Association of group B coxsackie viruses with cases of pericarditis, myocarditis, or pleurodynia by demonstration of immunoglobulin M antibody. *Infect Immun* 1973;**8**:341–8.
919. Schmidt WA, Brade L, Muntefering H, et al. Course of coxsackie B antibodies during juvenile diabetes. *Med Microbiol Immunol* 1978;**164**:291–8.
920. Schoub BD, Johnson S, McAnerney JM, et al. Epidemic coxsackie B virus infection in Johannesburg, South Africa. *J Hyg (Lond)* 1985;**95**:447–55.
921. Schultz WW, Weiss E. Demonstration of specific antibodies to a coxsackie-like virus in patients of a hepatitis outbreak. *Am J Epidemiol* 1979;**110**:124–31.
922. Schurmann W, Statz A, Mertens T, et al. Two cases of coxsackie B2 infection in neonates: clinical, virological, and epidemiological aspects. *Eur J Pediatr* 1983;**140**:59–63.
923. Scott TF. Clinical syndromes associated with entero virus and REO virus infections. *Adv Virus Res* 1961;**8**:165–97.
924. Seddon JH, Duff MF. Hand-foot-and-mouth disease: coxsackie virus types A 5, A 10, and A 16 infections. *N Z Med J* 1971; **74**:368–73.
925. See DM, Tilles JG. Pathogenesis of virus-induced diabetes in mice. *J Infect Dis* 1995;**171**:1131–8.
926. Seitsonen J, Susi P, Heikkila O, et al. Interaction of alphaVbeta3 and alphaVbeta6 integrins with human parechovirus 1. *J Virol* 2010;**84**:8509–19.
927. Seitsonen JJ, Shakeel S, Susi P, et al. Structural analysis of coxsackievirus A7 reveals conformational changes associated with uncoating. *J Virol* 2012;**86**:7207–15.
928. Seko Y, Matsuda H, Kato K, et al. Expression of intercellular adhesion molecule-1 in murine hearts with acute myocarditis caused by coxsackievirus B3. *J Clin Invest* 1993;**91**:1327–36.
929. Seko Y, Yoshifumi E, Yagita H, et al. Restricted usage of T-cell receptor V alpha genes in infiltrating cells in murine hearts with acute myocarditis caused by coxsackie virus B3. *J Pathol* 1996;**178**:330–4.
930. Sells CJ, Carpenter RL, Ray CG. Sequelae of central-nervous-system enterovirus infections. *N Engl J Med* 1975;**293**:1–4.
931. Selvarangan R, Nzabi M, Selvaraju SB, et al. Human parechovirus 3 causing sepsis-like illness in children from midwestern United States. *Pediatr Infect Dis J* 2011;**30**:238–42.
932. Selwyn S, Howitt LF. A mosaic of enteroviruses poliovirus, coxsackie, and Echo infections in a group of families. *Lancet* 1962; **2**:548–51.
933. Severien C, Jacobs KH, Schoenemann W. Marked pleocytosis and hypoglycorrhachia in coxsackie meningitis. *Pediatr Infect Dis J* 1994;**13**:322–3.
934. Sharland M, Hodgson J, Davies EG, et al. Enteroviral pharyngitis diagnosed by reverse transcriptase-polymerase chain reaction. *Arch Dis Child* 1996;**74**:462–3.
935. She RC, Crist G, Billetdeaux E, et al. Comparison of multiple shell vial cell lines for isolation of enteroviruses: a national perspective. *J Clin Virol* 2006;**37**:151–5.

936. Shelokov A, Habel K. Viremia in coxsackie B meningitis. *Proc Soc Exp Biol Med* 1957;**94**:782–4.
937. Shelokov A, Weinstein L. Poliomyelitis in the early neonatal period: report of a case of possible intrauterine infection. *J Pediatr* 1951;**38**:80–3.
938. Shimizu H, Schnurr DP, Burns JC. Comparison of methods to detect enteroviral genome in frozen and fixed myocardium by polymerase chain reaction. *Lab Invest* 1994;**71**:612–16.
939. Sieber OF Jr, Kilgus AH, Fulginiti VA, et al. Immunological response of the newborn infant to coxsackie B-4 infection. *Pediatrics* 1967;**40**:444–6.
940. Siegel W, Spencer FJ, Smith DJ, et al. Two new variants of infection with coxsackie virus group B, type 5, in young children: A syndrome of lymphadenopathy, pharyngitis and hepatomegaly or splenomegaly, or both, and one of pneumonia. *N Engl J Med* 1963;**268**:1210–16.
941. Silver TS, Todd JK. Hypoglycorrhachia in pediatric patients. *Pediatrics* 1976;**58**:67–71.
942. Simmonds P, Welch J. Frequency and dynamics of recombination within different species of human enteroviruses. *J Virol* 2006;**80**:483–93.
943. Simonsen L, Khan AS, Gary HE Jr, et al. Outbreak of vertigo in Wyoming: possible role of an enterovirus infection. *Epidemiol Infect* 1996;**117**:149–57.
944. Sinclair RG, Jones EL, Gerba CP. Viruses in recreational waterborne disease outbreaks: a review. *J Appl Microbiol* 2009;**107**:1769–80.
945. Singer JI, Maur PR, Riley JP, et al. Management of central nervous system infections during an epidemic of enteroviral aseptic meningitis. *J Pediatr* 1980;**96**:559–63.
946. Singh S, Chow VT, Phoon MC, et al. Direct detection of enterovirus 71 (EV71) in clinical specimens from a hand, foot, and mouth disease outbreak in Singapore by reverse transcription-PCR with universal enterovirus and EV71-specific primers. *J Clin Microbiol* 2002;**40**:2823–7.
947. Skarsvik S, Puranen J, Honkanen J, et al. Decreased in vitro type 1 immune response against coxsackie virus B4 in children with type 1 diabetes. *Diabetes* 2006;**55**:996–1003.
948. Skeels MR. Perinatal echovirus infection. *N Engl J Med* 1981;**305**:1529–30.
949. Soboleva VD, Lozovskaya LS, Alekseveva VB, et al. Coxsackie A 13 virus in the foci of rheumatism. *J Hyg Epidemiol Microbiol Immunol* 1978;**22**:195–202.
950. Solomon P, Weinstein L, Chang TW, et al. Epidemiologic, clinical, and laboratory features of an epidemic of type 9 ECHO virus meningitis. *J Pediatr* 1959;**55**:609–19.
951. Somekh E, Cesar K, Handsher R, et al. An outbreak of echovirus 13 meningitis in central Israel. *Epidemiol Infect* 2003;**130**:257–62.
952. Sommerville RG. Enteroviruses and diarrhoea in young persons. *Lancet* 1958;**2**:1347–9.
953. Spudis EV, Cramblett HG. Echo 4 Meningoencephalitis. *Arch Neurol* 1965;**12**:404–9.
954. Stanway G, Hyypia T. Parechoviruses. *J Virol* 1999;**73**:5249–54.
955. Stanway G, Joki-Korpela P, Hyypia T. Human parechoviruses: biology and clinical significance. *Rev Med Virol* 2000;**10**:57–69.
956. Stanway G, Brown F, Christian P, et al. Family Picornaviridae. In: Fauquet CM, Mayo MA, Maniloff J, et al., editors. *Virus taxonomy: VIIIth Report of the International Committee on Taxonomy of Viruses*. London: Elsevier/Academic Press; 2005. p. 757–78.
957. Steigman AJ. Poliomyelitic properties of certain non-polio viruses: enteroviruses and heine-Medin disease. *J Mt Sinai Hosp N Y* 1958;**25**:391–404.
958. Steigman AJ, Lipton MM. Fatal bulbospinal paralytic poliomyelitis due to ECHO 11 virus. *JAMA* 1960;**174**:178–9.
959. Steigman AJ, Lipton MM, Braspennickx H. Acute lymphonodular pharyngitis: a newly described condition due to coxsackie A virus. *J Pediatr* 1962;**61**:331–6.
960. Steinhoff MC. Viruses and diarrhea: a review. *Am J Dis Child* 1978;**132**:302–7.
961. Steinmann J, Albrecht K. [Echovirus 11 outbreak among premature newborn infants in a neonatal intensive care unit]. *Zentralbl Bakteriol Mikrobiol Hyg A* 1985;**259**:284–93.
962. Stellrecht KA, Harding I, Woron AM, et al. The impact of an enteroviral RT-PCR assay on the diagnosis of aseptic meningitis and patient management. *J Clin Virol* 2002;**25**(Suppl. 1):S19–26.
963. Stern H. Aetiology of central nervous system infections during prevalence of poliovirus and coxsackie virus. *Br Med J* 1961;**1**:1061–6.
964. Stones PB. Isolation of E.C.H.O. virus type 9 during an outbreak of meningo-encephalitis. *Br Med J* 1958;**2**:1514.
965. Strebel PM, Sutter RW, Cochi SL, et al. Epidemiology of poliomyelitis in the United States one decade after the last reported case of indigenous wild virus-associated disease. *Clin Infect Dis* 1992;**14**:568–79.
966. Strikas RA, Anderson LJ, Parker RA. Temporal and geographic patterns of isolates of nonpolio enterovirus in the United States, 1970-1983. *J Infect Dis* 1986;**153**:346–51.
967. Strom J. Coxsackie B5 infection causing serous meningitis with exanthema and benign pericarditis with myocarditis in two siblings. *Acta Paediatr Suppl* 1962;**135**:197–202.
968. Stürup H. The long-term prognosis of acute non-specific pericarditis; a follow-up examination including cases of dry pleurisy from an epidemic of Bornholm disease (myalgia epidemica) in 1930-32. *Dan Med Bull* 1954;**1**:89–91.
969. Sumaya CV, Corman LI. Enteroviral meningitis in early infancy: significance in community outbreaks. *Pediatr Infect Dis* 1982;**1**:151–4.
970. Sun G, Zhang X, Yi M, et al. Analysis of the genomic homologous recombination in Theilovirus based on complete genomes. *Virol J* 2011;**8**:439.
971. Sun NC, Smith VM. Hepatitis associated with myocarditis: unusual manifestation of infection with coxsackie virus group B, type 3. *N Engl J Med* 1966;**274**:190–3.
972. Swanink CM, Melchers WJ, van der Meer JW, et al. Enteroviruses and the chronic fatigue syndrome. *Clin Infect Dis* 1994;**19**:860–4.
973. Swann NH. Epidemic pleurodynia, orchitis, and myocarditis in an adult due to coxsackie virus, group B, type 4. *Ann Intern Med* 1961;**54**:1008–13.
974. Swender PT, Shott RJ, Williams ML. A community and intensive care nursery outbreak of coxsackievirus B5 meningitis. *Am J Dis Child* 1974;**127**:42–5.
975. Sylvest E. *Epidemic myalgia: Bornholm disease*. London: Oxford University Press; 1934.
976. Syverton JT, McLean DM, Martins Da Silva M, et al. Outbreak of aseptic meningitis caused by coxsackie B5 virus; laboratory, clinical, and epidemiologic study. *J Am Med Assoc* 1957;**164**:2015–19.
977. Takahashi S, Miyamoto A, Oki J, et al. Acute transverse myelitis caused by ECHO virus type 18 infection. *Eur J Pediatr* 1995;**154**:378–80.
978. Takeuchi Y, Kikuchi T, Kimura M. Guillain-Barré syndrome associated with ECHO virus type 7 infections. *Pediatrics* 1970;**45**:294–5.
979. Takos MJ, Weil M, Sigel MM. Outbreak of ECHO 9 exanthema traced to a children's party. *Am J Dis Child* 1960;**100**:360–4.
980. Talsma M, Vegting M, Hess J. Generalised coxsackie A9 infection in a neonate presenting with pericarditis. *Br Heart J* 1984;**52**:683–5.
981. Tan EL, Yong LL, Quak SH, et al. Rapid detection of enterovirus 71 by real-time TaqMan RT-PCR. *J Clin Virol* 2008;**42**:203–6.
982. Tanel RE, Kao SY, Niemiec TM, et al. Prospective comparison of culture vs genome detection for diagnosis of enteroviral meningitis in childhood. *Arch Pediatr Adolesc Med* 1996;**150**:919–24.
983. Tang JW, Bendig JW, Ossuetta I. Vertical transmission of human echovirus 11 at the time of Bornholm disease in late pregnancy. *Pediatr Infect Dis J* 2005;**24**:88–9.
984. Tang TT, Sedmak GV, Siegesmund KA, et al. Chronic myopathy associated with coxsackievirus type A9: a combined electron microscopical and viral isolation study. *N Engl J Med* 1975;**292**:608–11.
985. Tarcan A, Ozbek N, Gurakan B. Bone marrow failure with concurrent enteroviral infection in a newborn. *Pediatr Infect Dis J* 2001;**20**:719–21.
986. Tateno I, Suzuki S, Kagawa S, et al. On an ECHO-like agent constantly recoverable from volunteers given Niigata strain of acute epidemic gastroenteritis. *Jpn J Exp Med* 1956;**26**:125–38.

987. Tavares FN, Costa EV, Oliveira SS, et al. Acute hemorrhagic conjunctivitis and coxsackievirus A24v, Rio de Janeiro, Brazil, 2004. *Emerg Infect Dis* 2006;**12**:495–7.
988. Technical Consultative Group to the World Health Organization on the Global Eradication of Poliomyelitis. "Endgame" issues for the global polio eradication initiative. *Clin Infect Dis* 2002; **34**:72–7.
989. Terletskaia-Ladwig E, Metzger C, Schalasta G, et al. A new enzyme immunoassay for the detection of enteroviruses in faecal specimens. *J Med Virol* 2000;**60**:439–45.
990. Terletskaia-Ladwig E, Metzger C, Schalasta G, et al. Evaluation of enterovirus serological tests IgM-EIA and complement fixation in patients with meningitis, confirmed by detection of enteroviral RNA by RT-PCR in cerebrospinal fluid. *J Med Virol* 2000;**61**:221–7.
991. Theodoridou M, Kakourou T, Laina I, et al. Vesiculopapular rash as a single presentation in intrauterine coxsackie virus infection. *Eur J Pediatr* 2002;**161**:412–13.
992. Thomas HM Jr. Acute viral peritonitis due to group B coxsackie viruses. *Md State Med J* 1962;**11**:282–5.
993. Thoren A, Widell A. PCR for the diagnosis of enteroviral meningitis. *Scand J Infect Dis* 1994;**26**:249–54.
994. Thorsteinsson G. Management of postpolio syndrome. *Mayo Clin Proc* 1997;**72**:627–38.
995. Tian J, Lehmann PV, Kaufman DL. T cell cross-reactivity between coxsackievirus and glutamate decarboxylase is associated with a murine diabetes susceptibility allele. *J Exp Med* 1994;**180**: 1979–84.
996. Tindall JP, Callaway JL. Hand-foot-and-mouth disease: it's more common than you think. *Am J Dis Child* 1972;**124**:372–5.
997. Tobe T. Inapparent virus infection as a trigger of appendicitis. *Lancet* 1965;**1**:1343–6.
998. Toce SS, Keenan WJ. Congenital echovirus 11 pneumonia in association with pulmonary hypertension. *Pediatr Infect Dis J* 1988;**7**:360–2.
999. Torphy DE, Ray CG, Thompson RS, et al. An epidemic of aseptic meningitis due to echo-virus type 30: epidemiologic features and clinical and laboratory findings. *Am J Public Health Nations Health* 1970;**60**:1447–55.
1000. Toth M, Osvath P, Galambos M, et al. Kindergarten outbreak of an exanthematous disease caused by echovirus type 9. *Acta Paediatr Acad Sci Hung* 1964;**5**:235–9.
1001. Townsend TR, Bolyard EA, Yolken RH, et al. Outbreak of coxsackie A1 gastroenteritis: a complication of bone-marrow transplantation. *Lancet* 1982;**1**:820–3.
1002. Trabelsi A, Grattard F, Nejmeddine M, et al. Evaluation of an enterovirus group-specific anti-VP1 monoclonal antibody, 5-D8/1, in comparison with neutralization and PCR for rapid identification of enteroviruses in cell culture. *J Clin Microbiol* 1995;**33**:2454–7.
1003. Tracy S, Hofling K, Pirruccello S, et al. Group B coxsackievirus myocarditis and pancreatitis: connection between viral virulence phenotypes in mice. *J Med Virol* 2000;**62**:70–81.
1004. Trallero G, Casas I, Avellon A, et al. First epidemic of aseptic meningitis due to echovirus type 13 among Spanish children. *Epidemiol Infect* 2003;**130**:251–6.
1005. Trallero G, Casas I, Tenorio A, et al. Enteroviruses in Spain: virological and epidemiological studies over 10 years (1988-97). *Epidemiol Infect* 2000;**124**:497–506.
1006. Traystman MD, Chow LH, McManus BM, et al. Susceptibility to coxsackievirus B3-induced chronic myocarditis maps near the murine Tcr alpha and Myhc alpha loci on chromosome 14. *Am J Pathol* 1991;**138**:721–6.
1007. Tsao KC, Chan EC, Chang LY, et al. Responses of IgM for enterovirus 71 infection. *J Med Virol* 2002;**68**:574–80.
1008. Tsao LY, Lin CY, Yu YY, et al. Microchip, reverse transcription-polymerase chain reaction and culture methods to detect enterovirus infection in pediatric patients. *Pediatr Int* 2006;**48**:5–10.
1009. Tsukagoshi H, Masuda Y, Mizutani T, et al. Sequencing and phylogenetic analyses of Saffold cardiovirus (SAFV) genotype 3 isolates from children with upper respiratory infection in Gunma, Japan. *Jpn J Infect Dis* 2010;**63**:378–80.
1010. Tsukagoshi H, Mizuta K, Abiko C, et al. The impact of Saffold cardiovirus in patients with acute respiratory infections in Yamagata, Japan. *Scand J Infect Dis* 2011;**43**:669–71.
1011. Tubman TR, Craig B, Mulholland HC. Ventricular tachycardia associated with coxsackie B4 virus infection. *Acta Paediatr Scand* 1990;**79**:572–5.
1012. Turnbull DC. Optic neuritis associated with Bornholm disease. *Am J Ophthalmol* 1958;**46**:81–3.
1013. Tuuteri L, Lapinleimu K, Meurman L. Fatal myocarditis associated with coxsackie by B3 infection in the newborn. *Ann Paediatr Fenn* 1963;**9**:56–64.
1014. Tyrrell DAJ, Lane RR, Snell B, et al. Clinical and laboratory studies of a syndrome characterized by meningitis and an exanthem, caused by a virus related to ECHO 9. In: Rose HM, editor. *Viral infections of infancy and childhood: a symposium of the Section on Microbiology*. New York: Hoeber-Harper; 1960. p. 101–18.
1015. Underwood M. *A treatise on the diseases of children*. 2nd ed. London: J. Mathews; 1789.
1016. Ursing B. Acute pancreatitis in coxsackie B infection. *Br Med J* 1973;**3**:524–5.
1017. Utz JP, Shelokov AI. Coxsackie B virus infection: presence of virus in blood, urine, and cerebrospinal fluid. *J Am Med Assoc* 1958;**168**:264–7.
1018. Vague P, Vialettes B, Prince MA, et al. Coxsackie B viruses and autoimmune diabetes. *N Engl J Med* 1981;**305**:1157–8.
1019. Van Creveld S. Virus myocarditis in infancy-acute phase and possible late sequels. In: Rose HM, editor. *Viral infections of infancy and childhood: a symposium of the Section on Microbiology*. The New York Academy of Medicine. New York: Paul B. Hoeber; 1960. p. 33–54.
1020. van der Sanden S, de Bruin E, Vennema H, et al. Prevalence of human parechovirus in the Netherlands in 2000 to 2007. *J Clin Microbiol* 2008;**46**:2884–9.
1021. van Eden W, Persijn GG, Bijkerk H, et al. Differential resistance to paralytic poliomyelitis controlled by histocompatibility leukocyte antigens. *J Infect Dis* 1983;**147**:422–6.
1022. Van Loon GR, Masson AM. Viral pericarditis: a report of five cases. *Can Med Assoc J* 1968;**99**:163–8.
1023. Van Maldergem L, Mascart F, Ureel D, et al. Echovirus meningoencephalitis in X-linked hypogammaglobulinemia. *Acta Paediatr Scand* 1989;**78**:325–6.
1024. Varela-Calvino R, Ellis R, Sgarbi G, et al. Characterization of the T-cell response to coxsackievirus B4: evidence that effector memory cells predominate in patients with type 1 diabetes. *Diabetes* 2002;**51**:1745–53.
1025. Vasilii AL, Galina AK, Alexander NL, et al. Enterovirus uveitis. *Rev Med Virol* 2004;**14**:241–54.
1026. Ventura KC, Hawkins H, Smith MB, et al. Fatal neonatal echovirus 6 infection: autopsy case report and review of the literature. *Mod Pathol* 2001;**14**:85–90.
1027. Verboon-Maciolek MA, Groenendaal F, Hahn CD, et al. Human parechovirus causes encephalitis with white matter injury in neonates. *Ann Neurol* 2008;**64**:266–73.
1028. Verboon-Maciolek MA, Krediet TG, Gerards LJ, et al. Severe neonatal parechovirus infection and similarity with enterovirus infection. *Pediatr Infect Dis J* 2008;**27**:241–5.
1029. Verboon-Maciolek MA, Nijhuis M, van Loon AM, et al. Diagnosis of enterovirus infection in the first 2 months of life by real-time polymerase chain reaction. *Clin Infect Dis* 2003;**37**:1–6.
1030. Verboon-Maciolek MA, Swanink CM, Krediet TG, et al. Severe neonatal echovirus 20 infection characterized by hepatic failure. *Pediatr Infect Dis J* 1997;**16**:524–7.
1031. Verlinde JD, van TH, Wilterdink JB, et al. "Biak fever": an epidemic illness associated with ECHO6 virus. *Trop Geogr Med* 1959;**11**:276–80.
1032. Verstrepen WA, Bruynseels P, Mertens AH. Evaluation of a rapid real-time RT-PCR assay for detection of enterovirus RNA in cerebrospinal fluid specimens. *J Clin Virol* 2002;**25**(Suppl. 1): S39–43.
1033. Vieth UC, Kunzelmann M, Diedrich S, et al. An echovirus 30 outbreak with a high meningitis attack rate among children and household members at four day-care centers. *Eur J Epidemiol* 1999;**15**:655–8.
1034. Von Zeipel G, Svedmyr A. A study of the association of ECHO viruses to aseptic meningitis. *Arch Gesamte Virusforsch* 1957;**7**: 355–68.
1035. Voroshilova MK, Chumakov MP. Poliomyelitis-like properties of AB-IV coxsackie A7 group of viruses. *Prog Med Virol* 1959;**2**: 106–70.

1036. Vuorinen T, Vainionpaa R, Hyypia T. Five years' experience of reverse-transcriptase polymerase chain reaction in daily diagnosis of enterovirus and rhinovirus infections. *Clin Infect Dis* 2003; **37**:452–5.
1037. Wadia NH, Katrak SM, Misra VP, et al. Polio-like motor paralysis associated with acute hemorrhagic conjunctivitis in an outbreak in 1981 in Bombay, India: clinical and serologic studies. *J Infect Dis* 1983;**147**:660–8.
1038. Walker MP, Schlaberg R, Hays AP, et al. Absence of echovirus sequences in brain and spinal cord of amyotrophic lateral sclerosis patients. *Ann Neurol* 2001;**49**:249–53.
1039. Walters JH. Postencephalitic Parkinson syndrome after meningoencephalitis due to coxsackie virus group B, type 2. *N Engl J Med* 1960;**263**:744–7.
1040. Wang JN, Yao CT, Yeh CN, et al. Critical management in patients with severe enterovirus 71 infection. *Pediatr Int* 2006; **48**:250–6.
1041. Wang SM, Lei HY, Huang KJ, et al. Pathogenesis of enterovirus 71 brainstem encephalitis in pediatric patients: roles of cytokines and cellular immune activation in patients with pulmonary edema. *J Infect Dis* 2003;**188**:564–70.
1042. Wang SM, Lei HY, Huang MC, et al. Modulation of cytokine production by intravenous immunoglobulin in patients with enterovirus 71-associated brainstem encephalitis. *J Clin Virol* 2006;**37**:47–52.
1043. Wang SM, Liu CC, Chen YJ, et al. Alice in Wonderland syndrome caused by coxsackievirus B1. *Pediatr Infect Dis J* 1996; **15**:470–1.
1044. Wang SM, Liu CC, Tseng HW, et al. Clinical spectrum of enterovirus 71 infection in children in southern Taiwan, with an emphasis on neurological complications. *Clin Infect Dis* 1999; **29**:184–90.
1045. Wang SM, Liu CC, Yang YJ, et al. Fatal coxsackievirus B infection in early infancy characterized by fulminant hepatitis. *J Infect* 1998;**37**:270–3.
1046. Ward C. Severe arrhythmias in coxsackievirus B3 myopericarditis. *Arch Dis Child* 1978;**53**:174–6.
1047. Ward NA, Milstien JB, Hull HF, et al. The WHO-EPI initiative for the global eradication of poliomyelitis. *Biologicals* 1993;**21**: 327–33.
1048. Warin JF, Davies JB, Sanders FK, et al. Oxford epidemic of Bornholm disease, 1951. *Br Med J* 1953;**1**:1345–51.
1049. Watanabe K, Oie M, Higuchi M, et al. Isolation and characterization of novel human parechovirus from clinical samples. *Emerg Infect Dis* 2007;**13**:889–95.
1050. Waterman SH, Casas-Benabe R, Hatch MH, et al. Acute hemorrhagic conjunctivitis in Puerto Rico, 1981-1982. *Am J Epidemiol* 1984;**120**:395–403.
1051. Waterson AP. Virological investigations in congestive cardiomyopathy. *Postgrad Med J* 1978;**54**:505–9.
1052. Webster AD. Pleconaril: an advance in the treatment of enteroviral infection in immuno-compromised patients. *J Clin Virol* 2005;**32**:1–6.
1053. Webster AD, Rotbart HA, Warner T, et al. Diagnosis of enterovirus brain disease in hypogammaglobulinemic patients by polymerase chain reaction. *Clin Infect Dis* 1993;**17**:657–61.
1054. Wehrle PF, Judge ME, Parizeau MC, et al. Disability associated with ECHO virus infections. *N Y State J Med* 1959;**59**:3941–5.
1055. Weiner LS, Howell JT, Langford MP, et al. Effect of specific antibodies on chronic echovirus type 5 encephalitis in a patient with hypogammaglobulinemia. *J Infect Dis* 1979;**140**:858–63.
1056. Weinstein SB. Acute benign pericarditis associated with coxsackie virus group B, type 5. *N Engl J Med* 1957;**257**:265–7.
1057. Weller TH, Enders JF, Buckingham M, et al. The etiology of epidemic pleurodynia: a study of two viruses isolated from a typical outbreak. *J Immunol* 1950;**65**:337–46.
1058. Welliver RC, Cherry JD. Aseptic meningitis and orchitis associated with echovirus 6 infection. *J Pediatr* 1978;**92**:239–40.
1059. Wenner HA. The ECHO viruses. *Ann N Y Acad Sci* 1962;**101**: 398–412.
1060. Wenner HA. The enteroviruses. *Am J Clin Pathol* 1972;**57**: 751–61.
1061. Wenner HA. Virus diseases associated with cutaneous eruptions. *Prog Med Virol* 1973;**16**:269–336.
1062. Wenner HA, Behbehani AM. ECHO viruses. *Monogr Virol* 1968;**1**:1–72.
1063. Wenner HA, Lou TY. Virus diseases associated with cutaneous eruptions. *Prog Med Virol* 1963;**5**:219–94.
1064. Wenner HA, Christodoulopoulou G, Weston J, et al. The etiology of respiratory illnesses occurring in infancy and childhood. *Pediatrics* 1963;**31**:4–17.
1065. Wesslen T, Eriksson S, Ehinger A, et al. Epidemic of aseptic meningitis associated with ECHO virus type 9. *Arch Gesamte Virusforsch* 1958;**8**:183–91.
1066. Wickman I. Classics in infectious diseases. On the epidemiology of Heine-Medin's disease. Reprinted from *Acute Poliomyelitis (Heine-Medin's disease)*, Chapter VIII, by Ivar Wickman, Trans. J. W. J. Maloney. The Journal of Nervous and Mental Disease Publishing Company, New York, 1913. *Rev Infect Dis* 1980;**2**: 319–27.
1067. Wikswo ME, Khetsuriani N, Fowlkes AL, et al. Increased activity of coxsackievirus B1 strains associated with severe disease among young infants in the United State, 2007-2008. *Clin Infect Dis* 2009;**49**:e44–51.
1068. Wilfert CM, Buckley RH, Mohanakumar T, et al. Persistent and fatal central-nervous-system ECHO virus infections in patients with agammaglobulinemia. *N Engl J Med* 1977;**296**: 1485–9.
1069. Wilfert CM, Thompson RJ Jr, Sunder TR, et al. Longitudinal assessment of children with enteroviral meningitis during the first three months of life. *Pediatrics* 1981;**67**:811–15.
1070. Wilkins AJ, Kotze DM, Melvin J. Meningo-encephalitis due to coxsackie B virus in Southern Rhodesia. *S Afr Med J* 1955; **29**:25–8.
1071. Willems A, Benne CA, Timmer A, et al. Fatal illness associated with pulmonary hypertension in a neonate caused by intrauterine echovirus 11 infection. *Am J Perinatol* 2006;**23**:59–61.
1072. Willems WR, Hornig C, Bauer H, et al. A case of coxsackie A9 virus infection with orchitis. *J Med Virol* 1978;**3**:137–40.
1073. Wilson C, Connolly JM, Thomson D. Coxsackie B2 virus infection and acute-onset diabetes in a child. *Br Med J* 1977;**1**: 1008.
1074. Wilt JC, Medovy H, Besant D, et al. Aseptic meningitis in Manitoba, 1957. *Can Med Assoc J* 1958;**78**:839–42.
1075. Wilt JC, Parker WL, Owens AL, et al. Enterovirus infections in Manitoba, 1959. *Can Med Assoc J* 1960;**83**:839–43.
1076. Windebank AJ, Litchy WJ, Daube JR, et al. Late effects of paralytic poliomyelitis in Olmsted County, Minnesota. *Neurology* 1991;**41**:501–7.
1077. Windebank AJ, Litchy WJ, Daube JR, et al. Lack of progression of neurologic deficit in survivors of paralytic polio: a 5-year prospective population-based study. *Neurology* 1996;**46**:80–4.
1078. Winsser J, Altieri RH. A three-year study of coxsackie virus, group B, infection in Nassau County. *Am J Med Sci* 1964;**247**: 269–73.
1079. Wolfgram LJ, Rose NR. Coxsackievirus infection as a trigger of cardiac autoimmunity. *Immunol Res* 1989;**8**:61–80.
1080. Wolthers KC, Benschop KS, Schinkel J, et al. Human parechoviruses as an important viral cause of sepsislike illness and meningitis in young children. *Clin Infect Dis* 2008;**47**:358–63.
1081. Wong SN, Tam AY, Ng TH, et al. Fatal coxsackie B1 virus infection in neonates. *Pediatr Infect Dis J* 1989;**8**:638–41.
1082. Wood DJ, Sutter RW, Dowdle WR. Stopping poliovirus vaccination after eradication: issues and challenges. *Bull World Health Organ* 2000;**78**:347–57.
1083. Wood SF, Rogen AS, Bell EJ, et al. Role of coxsackie B viruses in myocardial infarction. *Br Heart J* 1978;**40**:523–5.
1084. Woodall CJ, Riding MH, Graham DI, et al. Sequences specific for enterovirus detected in spinal cord from patients with motor neurone disease. *BMJ* 1994;**308**:1541–3.
1085. Woods JD, Nimmo MJ, Mackay-Scollay EM. Acute transmural myocardial infarction associated with active coxsackie virus B infection. *Am Heart J* 1975;**89**:283–7.
1086. Wooley CF. Intracranial hypertension associated with recovery of a coxsackie virus from the cerebrospinal fluid. *Neurology* 1960; **10**:572–4.
1087. Wreghitt TG, Sutehall GM, King A, et al. Fatal echovirus 7 infection during an outbreak in a special care baby unit. *J Infect* 1989;**19**:229–36.
1088. Wright HT Jr, Landing BH, Lennette EH, et al. Fatal infection in an infant associated with coxsackie virus group A, type 16. *N Engl J Med* 1963;**268**:1041–4.

1089. Wright PF, Kim-Farley RJ, de Quadros CA, et al. Strategies for the global eradication of poliomyelitis by the year 2000. *N Engl J Med* 1991;**325**:1774–9.
1090. Wu D, Ke CW, Mo YL, et al. Multiple outbreaks of acute hemorrhagic conjunctivitis due to a variant of coxsackievirus A24: Guangdong, China, 2007. *J Med Virol* 2008;**80**:1762–8.
1091. Xu ZQ, Cheng WX, Qi HM, et al. New Saffold cardiovirus in children, China. *Emerg Infect Dis* 2009;**15**:993–4.
1092. Yaffee HS. Erythema multiforme caused by coxsackie B5. A possible association with epidemic pustular stomatitis of children. *Arch Dermatol* 1960;**82**:737–9.
1093. Yamashita K, Miyamura K, Yamadera S, et al. Epidemics of aseptic meningitis due to echovirus 30 in Japan: a report of the National Epidemiological Surveillance of Infectious Agents in Japan. *Jpn J Med Sci Biol* 1994;**47**:221–39.
1094. Yan JJ, Wang JR, Liu CC, et al. An outbreak of enterovirus 71 infection in Taiwan 1998: a comprehensive pathological, virological, and molecular study on a case of fulminant encephalitis. *J Clin Virol* 2000;**17**:13–22.
1095. Yang CF, De L, Holloway BP, et al. Detection and identification of vaccine-related polioviruses by the polymerase chain reaction. *Virus Res* 1991;**20**:159–79.
1096. Yang TT, Huang LM, Lu CY, et al. Clinical features and factors of unfavorable outcomes for non-polio enterovirus infection of the central nervous system in northern Taiwan, 1994-2003. *J Microbiol Immunol Infect* 2005;**38**:417–24.
1097. Yen HR, Lien R, Fu RH, et al. Hepatic failure in a newborn with maternal peripartum exposure to echovirus 6 and enterovirus 71. *Eur J Pediatr* 2003;**162**:648–9.
1098. Yerly S, Gervaix A, Simonet V, et al. Rapid and sensitive detection of enteroviruses in specimens from patients with aseptic meningitis. *J Clin Microbiol* 1996;**34**:199–201.
1099. Yin-Murphy M. Acute hemorrhagic conjunctivitis. *Prog Med Virol* 1984;**29**:23–44.
1100. Yohannan MD, Ramia S, al Frayh AR. Acute paralytic poliomyelitis presenting as Guillain-Barré syndrome. *J Infect* 1991;**22**:129–33.
1101. Yoon J, Notkins AL, Onodera T, et al. Virus-induced diabetes mellitus: infection of mice with variants of encephalomyocarditis virus, coxsackievirus B4, and reovirus type 3. *Adv Exp Med Biol* 1979;**119**:137–46.
1102. Yousef GE, Isenberg DA, Mowbray JF. Detection of enterovirus specific RNA sequences in muscle biopsy specimens from patients with adult onset myositis. *Ann Rheum Dis* 1990;**49**:310–15.
1103. Yousef GE, Bell EJ, Mann GF, et al. Chronic enterovirus infection in patients with postviral fatigue syndrome. *Lancet* 1988;**1**:146–50.
1104. Yow MD, Melnick JL, Blattner RJ, et al. Enteroviruses in infantile diarrhea. *Am J Hyg* 1963;**77**:283–92.
1105. Yow MD, Melnick JL, Phillips CA, et al. An etiologic investigation of infantile diarrhea in Houston during 1962 and 1963. *Am J Epidemiol* 1966;**83**:255–61.
1106. Yuceoglu AM, Berkovich S, Minkowitz S. Acute glomerulonephritis associated with ECHO virus type 9 infection. *J Pediatr* 1966;**69**:603–9.
1107. Yui LA, Gledhill RF. Limb paralysis as a manifestation of coxsackie B virus infection. *Dev Med Child Neurol* 1991;**33**:427–38.
1108. Zahorsky J. Herpetic sore throat. *Med J* 1920;**13**:871–2.
1109. Zahorsky J. Herpangina: a specific infectious disease. *Arch Pediatr* 1924;**41**:181–4.
1110. Zanetti AR. Enterovirus associated sporadic cases and epidemics. *Ann Sclavo* 1977;**19**:187–92.
1111. Zhang DL, Jin Y, Li DD, et al. Prevalence of human parechovirus in Chinese children hospitalized for acute gastroenteritis. *Clin Microbiol Infect* 2011;**17**:1563–9.
1112. Zhang H, Li Y, McClean DR, et al. Detection of enterovirus capsid protein VP1 in myocardium from cases of myocarditis or dilated cardiomyopathy by immunohistochemistry: further evidence of enterovirus persistence in myocytes. *Med Microbiol Immunol* 2004;**193**:109–14.
1113. Zhang Y, Zhu Z, Yang W, et al. An emerging recombinant human enterovirus 71 responsible for the 2008 outbreak of hand foot and mouth disease in Fuyang city of China. *Virol J* 2010;**7**:94.
1114. Zoll J, Erkens Hulshof S, Lanke K, et al. Saffold virus, a human Theiler's-like cardiovirus, is ubiquitous and causes infection early in life. *PLoS Pathog* 2009;**5**:e1000416.
1115. Zuniga MDR, Reichardt J, Braun W, et al. Detection of IgM antibodies against coxsackie B viruses by a western blot technique. *Acta Virol* 1993;**37**:1–10.

associated primarily with mild upper respiratory tract disease, they also may be involved in bronchitis, sinusitis, and, on occasion, pneumonia in all age groups. These viruses seem to be precipitants of "infectious asthma" attacks. HRVs cause 30 to 50 percent of all acute respiratory illnesses.[61,99]

THE ORGANISM

Rhinoviruses are a member of the *Enterovirus* genus in the family *Picornaviridae*.[176] Other members of the *Enterovirus* genus include polioviruses, coxsackieviruses, and echoviruses. Like the other picornaviruses, the HRVs are small (30 nm), nonenveloped (therefore resistant to lipid solvents such as ether and chloroform), and icosahedral (20-sided, hexagonal in cross section), with a genome consisting of single-stranded RNA (molecular weight, 2.5×10^6 daltons) approximately 7200 nucleotides long.[176] A picornavirus can be thought of as an RNA genome surrounded by a 20-sided protein coat (the capsid) (Fig. 166-1). The RNA alone is infectious and can serve as a messenger.

HRVs previously were distinguished from classic enteroviruses by being rendered noninfectious at an acidity lower than pH 5 and by their higher buoyant density in cesium chloride.[122] HRVs are subdivided based on various virus characteristics as follows: (1) into three species (HRV A, HRV B, and HRV C) based on genomic sequence analysis; (2) into three groups based on receptor usage (see later); (3) into two groups based on susceptibility to capsid-binding antiviral agents; and (4) into more than 100 serotypes, as described earlier.[254] Currently, polymerase chain reaction (PCR) assays are typically used to distinguish other enterovirus species from HRVs.[192]

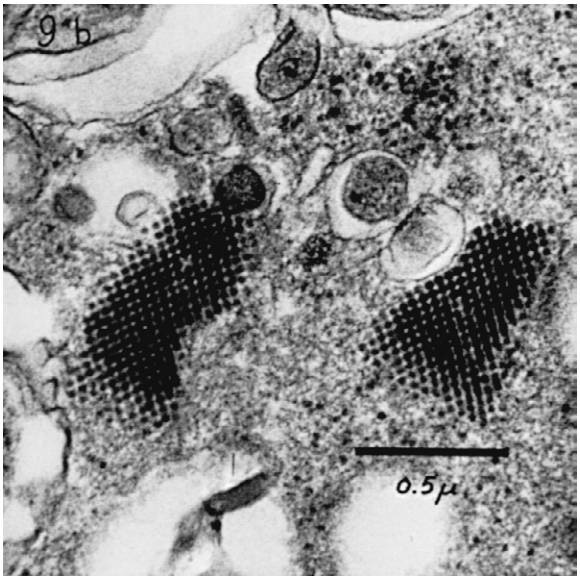

FIGURE 166-1 ■ Rhinovirus type 2 in human fetal lung cells. Note the hexagonal virus crystals closely packed in a rectangular lattice (×40,000). (From Kawana R, Matsumoto I. Electron microscopic study of rhinovirus replication in human fetal lung cells. *Jpn J Microbiol* 1971;**15**:207-17.)

The genomes of most culturable HRV serotypes have been sequenced completely.[223] Within an HRV species, a 61 to 98 percent pairwise nucleotide sequence identity of the VP1 gene exists among the different serotypes, whereas between HRV serotypes the pairwise nucleotide sequence identity for this gene is 46 to 55 percent.[181] HRV-87 has greater sequence homology to the *Enterovirus D* species (EV-68 and EV-70) than to other HRVs and has been reclassified into the *Enterovirus D* species.[28]

Structure of the Virion

The virion is composed of 60 copies of each of four structural proteins: VP1, VP2, VP3, and VP4. Knowledge of the structure of the HRVs is based on the determination of the capsid structures in atomic detail for five serotypes, HRV-1A, HRV-2, HRV-3, HRV-14, and HRV-16.* The thin (5-nm) protein capsid has an undulating exterior marked by 12 vertices (the icosahedral fivefold axis) equally spaced over the surface. Surrounding these vertices is a steep (2.5-nm deep), narrow canyon. VP1, VP2, and VP3 are exposed on the surface of the virion, whereas VP4 is buried within the capsid.

A pocket of unknown function whose walls are lined by 17 amino acids that vary with each HRV type has been found at the base of the canyon.[7] Various organic molecules have been determined to bind in this pocket; when these molecules are bound, either the virus is prevented from uncoating (necessary to release viral RNA for its translation to viral protein) or the cell receptor is prevented from docking in the canyon.[100,231] Numerous drugs made by several different drug companies in the United States, Europe, and Japan have been developed to exploit the anti-picornavirus potentialities of this pocket, and the sensitivity of the HRVs to these drugs has been used as an additional means of classifying the HRVs (into inhibitor groups A and B).[7,8] One group of these drugs, the WIN compounds synthesized by Sterling-Winthrop, has had wide scientific exposure; however, clinical trials have been discouraging.[273,275] One disadvantage of these compounds is that the various HRV serotypes vary widely in sensitivity to these organic molecules.[7] Another problem has been failure to attain effective concentrations of drug at the site of infection.

Virus Life Cycle

The first step in the virus life cycle is binding to its cellular receptor. There are only two different receptor binding sites for the culturable HRV serotypes: 90 serotypes bind to intercellular adhesion molecule-1 (ICAM-1, the major receptor group), and 10 other serotypes bind to members of the low-density lipoprotein receptor family (LDL-R, the minor receptor group.[1,115,285] The receptor(s) of clade C viruses have not been identified for clade C rhinoviruses because members of this species are currently noncultivable.

ICAM-1 binds to the base of the canyon of HRVs in the major receptor group.[247,249,285] Virus-neutralizing

*References 10, 174, 247, 248, 288, 304.

antibody prevents virus attachment to cells by adhering to serospecific sites and blocking the cell receptor from access to its binding site at the canyon base. These antibody sites may directly overlap ICAM-1–binding sites, or they may be separate from the ICAM-1–binding sites but at a point sufficiently close to allow the binding site to be blocked by steric hindrance.[46,261] Binding of the major receptor group HRVs to ICAM-1 leads to destabilization of the virus capsid and uncoating of the viral RNA.[116]

The LDL-R family binds to a different site for the minor receptor group of HRVs than that recognized by ICAM-1. The binding site for these HRVs is the small, star-shaped dome above the canyon on the icosahedral fivefold axis. Attachment of minor receptor group viruses to LDL-R does not lead to virus uncoating, in contrast to what is seen with binding of major receptor group HRVs to ICAM-1. Instead, internalization into acidic endosomal compartments is required for uncoating of minor receptor group HRVs.[144]

Replication of the HRVs is similar to that of the enteroviruses.[243] After attachment to the cellular receptor and endocytosis of the virus, RNA is released into the cytoplasm. Genomic viral RNA functions as messenger RNA and attaches to ribosomes, which stimulates its translation by host enzymes into a single long polyprotein. This polyprotein is cleaved by viral proteases to yield viral RNA polymerase, capsid proteins, proteases, and proteins to halt the synthesis of host proteins and other products. Under laboratory conditions, infectious virus first is formed after approximately 2 hours and reaches a maximum of approximately 1000 infectious particles per cell at approximately 7 hours. Infectious virus, however, comprises a minority of the virus-like particles formed; only approximately 1 in 200 is a complete virus capable of replicating in cell culture. All viral replication occurs in the cytoplasm, and viruses are released by cell lysis in a process involving apoptosis.[66,243]

Host Range

Animals

Rhinoviruses have been isolated from natural infections in only chimpanzees and humans.[176] Chimpanzee infections are subclinical. A natural, subclinical outbreak of HRV-31 in chimpanzees was reported in a primate center.[69,70]

Many attempts have been made to infect a wide variety of animals with HRVs. In addition to the chimpanzee, the gibbon has been susceptible, but it is not reliably so.[70] Mice do not naturally express ICAM-1, the major receptor, and are not infected by these viruses. The block can be overcome by engineering mouse cells to express ICAM-1, a finding that recently led to the development of a transgenic mouse model for HRV infection.[25,134,280] Mice also can be infected by HRVs that express the minor receptor.[25,302] Mutations in viral nonstructural proteins can improve the replication efficiency of mouse-adapted rhinoviruses in both major and minor receptor groups.[134,302] Equine and bovine "rhinoviruses" have been described, but these viruses belong to different picornavirus genera.[176]

Cell and Tissue Cultures

The spectrum of tissue culture cells infected by HRVs also is narrow.[58,183] For initial isolation, human embryonic diploid cells generally are used (Fig. 166-2), although for some HRV types, an especially sensitive strain[55] of a continuous cell line, HeLa, serves as well or better.[58,183] Some HRVs grow only in human organ cultures and, as noted earlier, some only in living humans.[183]

The first HRV (HRV-1A) was propagated in primary cell culture from primate kidneys (rhesus monkey cell cultures are used most commonly); surprisingly, chimpanzee kidney cell cultures do not propagate HRVs.[67] However, most HRVs propagate on original isolation only in cells of human origin, and they are called H strains; others also can be isolated in monkey kidney cell cultures and are called M strains. After laboratory propagation, all HRVs seem adaptable to the HRV-sensitive HeLa cell strain.[55] HeLa-grown HRVs attain much higher titers than those in the primary human diploid cells used for initial isolation.

Antigenic Properties

A prominent characteristic of HRV is its great antigenic diversity; more than 100 cultivable serotypes exist.[131] Evidence has shown, however, that the number of additional serotypes of cultivable strains may be limited.[99,131,170,212] Certain serotypes cross react, but little evidence indicates that this cross reaction could be exploited in vaccination.[57,97,210] Genetic analyses suggest that close to 50 additional serotypes may exist among clade C HRVs.[201,258]

HRVs often are poor antigens. Significant (fourfold or greater) increases in serum antibody may not develop in as many as 50 percent of patients from whom HRVs are isolated, and the levels attained often are low.[97-99]

HRVs may be undergoing continuous antigenic change.[210] One mechanism that may lead to the emergence of new strains is recombination. The sequencing of complete viral genomes has identified a number of probable recombination events between different virus serotypes. The recombinants then diverged further from the parental strains and became new serotypes.[223] Sufficiently marked antigenic variation has been found for HRV-22 and HRV-51 to interfere with their typing.[256,266]

EPIDEMIOLOGY

HRVs cause infection with a worldwide distribution, and large epidemiologic studies have consistently identified HRVs as the most common cause of viral respiratory disease. For example, two population-based studies in Roehampton, England[148] and Tecumseh, Michigan,[213] both found HRVs more frequently than other respiratory viruses, with HRVs causing 26.3 percent and 38.1 percent, respectively, of all respiratory illnesses. More recent studies of acute respiratory illness in children also identified HRVs most commonly.[9,180] Infection is more common in children than in adults. The annual incidence in children is approximately one infection per year, although

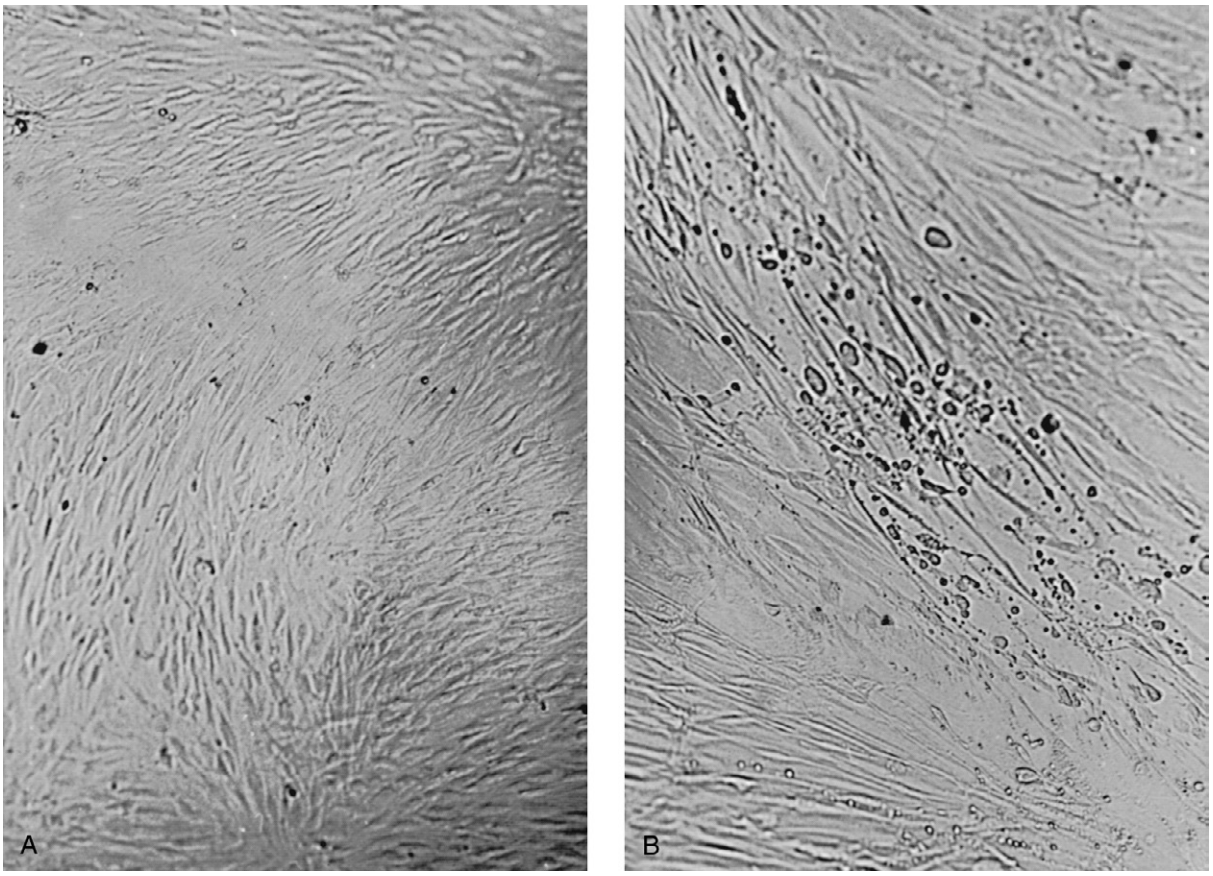

FIGURE 166-2 ■ Rhinovirus type 16 infection. **A,** Normal human diploid (fetal tonsil) cell sheet. **B,** Cell sheet infected for 2 days with rhinovirus type 16. The rounded and misshapen cells are characteristic. (Courtesy Dr. David M. Warshauer, University of Wisconsin, Madison, WI.)

one study reported an incidence as high as six infections per year.[298]

Seasonal Distribution

HRV causes infections year round, although there are seasonal peaks of occurrence.* During the usual September through May "cold season," HRV infections often are predominant at both ends, early fall and middle-to-late spring (Fig. 166-3). They also are important causes of summer colds. This seasonal pattern seems general because similar findings have been reported from families in Charlottesville, Virginia, Seattle, Washington, and elsewhere.† In the Southern Hemisphere, a similar seasonal pattern occurs, but during opposing months.[164]

Cycling and Circulation of Individual Rhinovirus Types

Several HRV serotypes usually circulate simultaneously within a community,[133] frequently coincident with other respiratory viruses.[68,148,213] For example, in a study of 24 neighboring families in Madison, Wisconsin (Eagle Heights Village), 14 different HRV types in addition to several nontypeable HRVs were found during the two academic years 1963 to 1965. Only a single type (HRV-15) was found both years. The other common respiratory viruses also were present. In virus surveillance studies performed in families in Seattle and Tecumseh a few "common" serotypes were found to be more prevalent in a given respiratory season and to be identified in subsequent respiratory seasons.[99,212] However, most individual HRV types do not repeat within a population from year to year.

Although several HRV types usually circulate concurrently, all types present are not equal in prevalence. In a Chicago nursery school study,[26] 14 different serotypes were isolated during the academic year 1962 to 1963, but 10 of the serotypes did not spread at all. Only 3 types disseminated widely, and they infected more than 40 percent of the children. Similarly, in the 1963 to 1965 Eagle Heights Village study, 14 HRV types were isolated, but only 3 types (HRV-43, HRV-51, and HRV-55) were "spreaders." Similar patterns of serologic prevalence were reported from laboratories in Tecumseh,[213] New York City, Seattle,[98,99] and Charlottesville.[142]

Predominating Rhinovirus Types

Although the pattern of predominating HRV types within a circumscribed population and a defined time frame

*References 61, 86, 98, 99, 195, 212, 245.
†References 18, 98, 99, 122, 142, 195.

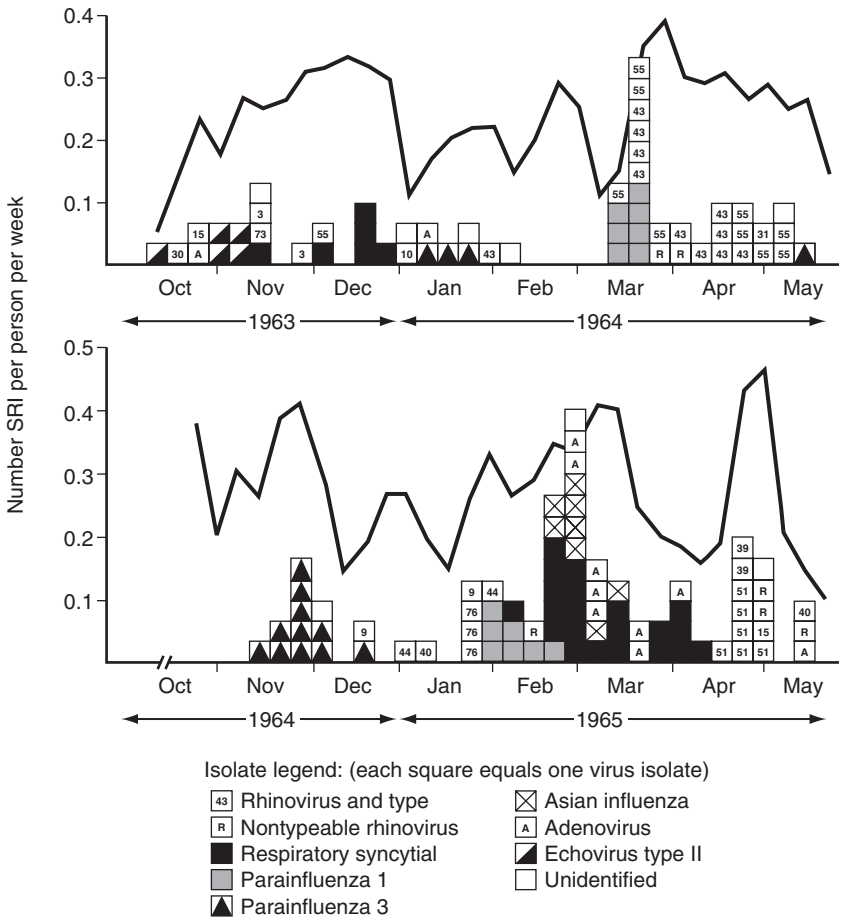

FIGURE 166-3 ■ Rhinoviruses and other respiratory viruses associated with symptomatic respiratory infection. This study included three neighboring apartment buildings in Eagle Heights, a University of Wisconsin housing village in Madison, Wisconsin (24 to 26 families, ≈100 persons), from 1963 to 1965.

seem to be well established, a predominance of serotypes over large geographic areas or over many years does not seem to occur. This phenomenon was studied exhaustively in widely separated locations and over many years: the Gulf South from 1962 to 1970, Tecumseh from 1966 to 1971 and from 1976 to 1981, and Seattle from 1965 to 1969 and from 1975 to 1979.[98,99,210,212,214] Although "common types" (usually the isolation of at least five to eight strains of a serotype during the period studied) occurred during each period and at each place, different types were "common" findings in different places and times. For example, Monto and colleagues,[212,214] during two 6-year study periods in Tecumseh obtained 475 HRV isolates covering 70 serotypes (of a possible 89 at that time),[169] but only HRV-1B, HRV-10, HRV-28, and HRV-58 were isolated in both periods. However, these viruses were not particularly common because only 16, 22, 15, and 18 isolates, respectively, of these four types were found during the 12 years. In the neighboring state of Wisconsin from 1963 to 1965,[68] the common types were HRV-43, HRV-51, and HRV-55, which were completely different from those in Michigan. Finally, the late John Fox sifted data from family populations surveyed by him and others in New York City, Seattle, and Tecumseh.[98,99] He found only 4 common serotypes (HRV-1B, HRV-12, HRV-15, and HRV-38) from among 802 isolates. Dominant HRV serotypes often appear to occur locally over relatively short periods but do not extend for decades or over the nation, at least in the United States.

More recent studies using molecular assays indicate that the number of circulating strains in a given geographic area is even greater than previously appreciated using only cell culture methods. Investigators in the Netherlands identified 27 different genotypes of HRV infection among a cohort of 18 healthy children 7 years of age and younger sampled at least every 2 weeks over a 6-month period.[287] Mackay and colleagues[193] identified 74 distinct HRV genotypes from all 3 HRV species circulating over a 1-year period in 234 children in Melbourne, Australia. Thus, the diversity of HRVs present in a community is greater than previously recognized.

Median Human Infectious Dose for Rhinovirus

As documented in HRV challenge experiments with laboratory-grown virus administered by nose drops or aerosol, less than one median tissue culture infective dose ($TCID_{50}$) can initiate infection.[64,76] Much more (2000 times) is required when placed on the tongue or dried just outside the anterior nares (10,000 times).[64] How accurately these conditions approach the natural state is not known.

PERSON-TO-PERSON TRANSMISSION

Epidemiologic Observations

Individual HRV serotypes often disseminate with surprising difficulty, as noted in studies of Eagle Heights Village,[68] a Madison, Wisconsin, elementary school, and a Chicago nursery school.[26] At least within family populations, the most common finding was that a specific HRV serotype did not spread from the index case. Hendley and associates,[142] in a study of 19 families in Charlottesville, found that HRVs from 10 of 22 index cases did not spread at all, and in only 7 families did dissemination to at least another person take place. In only 4 of the 19 families did further sequential spread occur. Fox and associates,[98,99] in a surveillance of more than 200 Seattle families, found intrafamily secondary attack rates in susceptible individuals (no homotypic antibody) to be 44 percent from 1965 to 1969 and 28 percent from 1975 to 1979. In both periods, children younger than 5 years had the highest secondary attack rate of 60 and 30 percent, respectively. Monto and Johnson and others[211,215] reported similar findings in 48 Panamanian families: with 5 HRV isolates used as antigens, the secondary attack rate varied from 10.5 to 56 percent.

In Madison, interfamily and intrafamily dissemination of the 3 "spreading" HRV types, HRV-43, HRV-51, and HRV-55, was examined in the 24 to 26 neighboring families living in fourplex apartments in Eagle Heights Village (Fig. 166-4). These 3 serotypes were the only ones of 14 to spread beyond the index family. HRV-51 attacked 23 percent of susceptible individuals, and many family members and close neighbors remained uninfected. HRV-43 infected 34 percent of susceptible individuals; in one building, only the families in a single-end fourplex became infected. Conversely, HRV-55 caused a mini-epidemic in two buildings; several families had all members infected. HRV dissemination seemed to focus on the fourplex, probably because in the winter months the children played in the hallways and stairs connecting the apartments. Nonetheless, even the spreading HRVs left many susceptible individuals untouched among next-door neighbors and within many families.

Determination of the reason for such erratic spreading patterns can be sought in well-controlled chain-of-infection experiments with human volunteers. HRV transmission experiments from several laboratories are described next.

Person-to-Person Transmission to Human Volunteers

Early Experiments with Rhinovirus Colds

Early experiments with different HRV serotypes showed that person-to-person transmission occurred infrequently. Couch and colleagues[59,63] attained no transmission after housing 15 donors (persons infected intranasally with laboratory-grown HRV-15) with 12 antibody-negative recipients for 26 days. At approximately the same time, the Madison laboratory performed transmission trials with HRV-55 and HRV-16 in a series of experiments ranging in duration from a 1- to 1.5-minute kiss to a 3-day weekend in a dormitory room. In 26 donors and 33 recipients, only two transmissions occurred, one from a 1.5-minute kiss and the other from a weekend in a dormitory.[64] Only when young married student couples were used was a substantial rate attained; among 24 couples, 9 donor spouses infected their mates, for a transmission rate of 38 percent.[65]

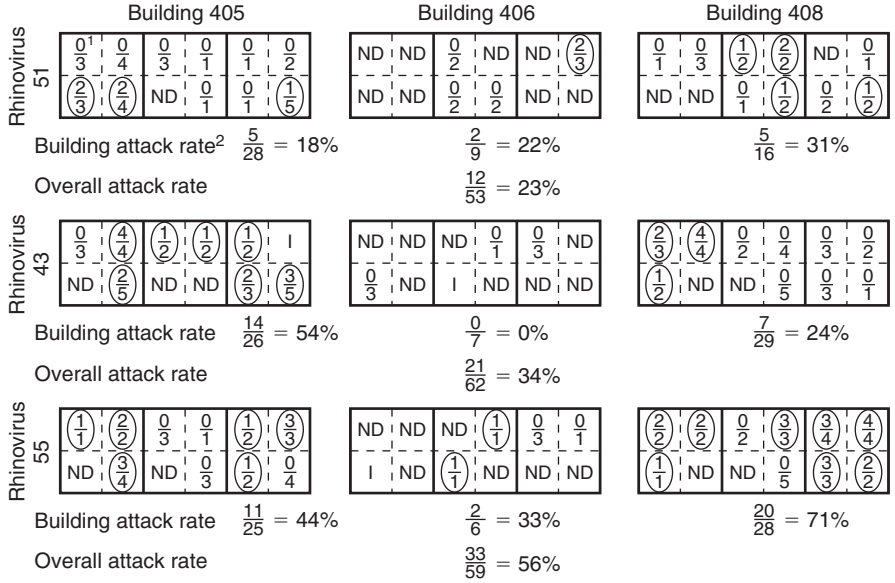

[1]No. diagnosed cases/no. susceptibles (no detectable antibody)
[2]As diagnosed by either virus isolation and/or a fourfold or greater serologic response

FIGURE 166-4 ■ Attack rates of three "spreading" rhinoviruses in three neighboring Eagle Heights apartment buildings in Madison, Wisconsin. I, all members immune; ND, not done; O, family with one or more infected members.

Characteristics of a "Good" Rhinovirus Transmitter

In the experiments with married couples, "successful" donors shed enough virus to contaminate their environment, exhibited signs and symptoms of a moderate to severe cold, and spent many hours at home with their spouses.[65] As an example of the effect of high virus shedding, intracouple transmission rates were 71 percent for donors with nasopharyngeal HRV titers of 5,000 to more than 80,000 $TCID_{50}$, 33 percent for those with 1,000 to 5,000 $TCID_{50}$, and only 18 percent for those with less than 1,000 $TCID_{50}$ ($p = .025$). Illustrative of both shedding and production of sufficient nasal secretions was contamination of the hands with HRV: donors whose hands were assayed for virus and who transmitted infections to their spouses all had HRV on their hands, whereas none of the nontransmitters did ($p = .03$). The amount of time spent together seemed important in transmission, although many infected spouses who spent hours of direct contact with their partners did not transmit the infection.

Some Early Conclusions

The preceding epidemiologic human volunteer studies suggest that attending a concert with persons who have even obvious signs of colds is unlikely to result in infection. Conversely, students who spend day after day in classrooms may have a higher chance of acquiring infection. In addition, a relatively short stay (a few hours) among friends or relatives with HRV-caused colds is not likely to cause infection, even with brief embracing.

Route-of-Transmission Experiments

Because HRVs often seem to spread with relative difficulty under many normal circumstances, the route of transmission becomes important because controlling respiratory virus transmission in habitats such as schoolrooms and families may be feasible by blocking transmission routes. Since the early 1970s, human volunteer experimental infection models have been used to explore HRV transmission routes. These studies were performed primarily at the University of Virginia, England's Common Cold Unit, and the University of Wisconsin.

Gwaltney and Hendley[123] at the University of Virginia focused on the possibility that HRV colds were disseminated by direct or indirect hand contact. Interest in this approach had been provoked by the inadvertent transmission of HRVs from infected to noninfected persons through the vehicle of an ethanol-sterilized nasal speculum. Subsequently, the plausibility of direct hand transmission of HRVs was supported by the observation that nose picking and eye rubbing were common among Department of Medicine Grand Rounds participants and Sunday school attendees,[143] following previous documentation of infection through conjunctival swabs.[37] These investigators then showed that HRV-39 retained infectivity for several hours on various surfaces and could be found on the hands of persons infected with HRV-39. In another study, infectious HRV was isolated from the fingertips of 22 percent of persons who touched objects that had been contaminated with nasal mucus of adults with natural colds one hour earlier.[299] These experiments suggest that transmission of HRV could occur through self-inoculation by environmentally contaminated hands and demonstrated HRV-39 self-inoculation from an environmental source in human volunteers.[143]

The University of Virginia team in Charlottesville subsequently conducted a series of three experiments that identified direct inoculation as a route of HRV transmission.[125] In two of the experiments, groups of recipients were exposed to aerosols generated by donors infected with an untypeable HRV strain, HH, and the exposures occurred in separate closed rooms for large-particle (12 recipients) or small-particle (10 recipients) aerosols produced by donors during periods of 45 minutes (large particles) or 3 days (small particles). One of the large-particle aerosol recipients was infected, whereas no one exposed to small-particle aerosols was. These essentially negative transmission rates by aerosol exposure were not surprising.[123] Previously, Couch[60] had been unsuccessful in transmitting infection through the airborne route over a period of 26 days, and the Madison laboratory attained only a single HRV transmission among 11 recipients after 3 days' exposure in a dormitory.[64] The third group of Charlottesville recipients was exposed to HRV by inoculating themselves with the donor's nasal mucus. The donors blew their noses into their hands. The recipients then stroked the donors' contaminated hands for 10 seconds and then inoculated themselves by deliberately placing their fingers, two or three times, on their nasal and conjunctival mucosa on 3 successive days. Eleven of the 15 hand-contact recipients (73%) were infected with the donors' HRV. The authors concluded that transmission by the hand-contact/self-inoculation route was much more efficient than the aerosol route was and "may be an important natural route of HRV transmission."[125]

One deficiency of this experiment was that donors and recipients shared the same air space over a measured, often brief, period during which fresh nasal mucus was transferred from the donor to the recipient. Nonetheless, Gwaltney did achieve a high rate of HRV transmission with nasal secretions, something that had not been accomplished readily in more "natural" experiments.[59,64] Also in support of hand transmission was the observation in the aforementioned married couple experiments that the ability to transmit HRV was correlated significantly with the presence of the virus on the donors' hands.[65] As a result of these various pieces of evidence and lack of compelling evidence to the contrary, hand-to-hand or fomite transmission became the accepted route for HRV contagion.[40,60] In later experiments, the Charlottesville group demonstrated approximately 50 percent transmission through fomites by using the same hand-contact/self-inoculation method described earlier, except either a coffee cup handle or a plastic tile was interposed between donors' and recipients' hands.[124]

At England's Common Cold Unit, Reed[241] investigated the likelihood of natural hand-contact transmission among residents (roommates) of several housing units, 38 of whom had been infected with HRV-2. Reed[244] found

that 16 of the donors had HRV on their fingers, but none of the virus was transmitted to the fingers of their 18 roommates, even though virus could be recovered from some (6 of 40) objects recently handled by HRV-2–infected donors. Reed also found that none of 29 virus transfers from virus dried on the fingers could pass through a fomite to a recipient; the conclusion was that "spread of colds is unlikely to occur via objects contaminated by the hands of the virus shedder …"

Laboratory workers at the University of Wisconsin continued their attempts to devise a natural transmission model to examine routes of transmission and, potentially, methods for interruption of transmission. Although the results of the transmission experiment with married couples were illuminating, the system itself had one great deficiency as an experimental model: the participants could not be observed during their interaction periods.[65] A model was needed in which donors and recipients could be monitored at all times. Ultimately, the scheme for such a system was suggested by two virologic-epidemiologic events in Antarctica, one from the summer seasons of 1975 to 1980 in which Dick and associates studied respiratory virus dissemination in an isolated Antarctic population and the other from a human volunteer experiment conducted in an Antarctic field party by England's Common Cold Unit.[291] Both experiences suggested that high natural transmission rates after just a few days of exposure could be achieved only in an environment with a high donor-to-recipient ratio and in a population density like that in classrooms and dormitories of a boarding school. Accordingly, a model was devised in which donors (with the qualities of a "good" transmitter as described earlier) and recipients interacted during waking hours by playing cards and board and video games and engaging in communal studying. During the night, donors and recipients bunked together in the same or an adjoining room. With such an arrangement, graduate student monitors recorded activities and all clinical signs 24 hours a day. These artifices were successful; with 8 donors and 12 to 16 recipients, a 50 percent recipient attack rate usually was attained over a 12-hour period and a 100 percent rate over the course of a week.[74,204] Informally, this model, a fully observed monotypic HRV mini-epidemic, was named "Antarctic Hut," from its origins.[141]

Route-of-Transmission "Blocking" Experiments

With the use of the Antarctic Hut model, a series of four HRV-16 route-of-transmission blocking experiments was conducted to determine whether the virus was spread by hand contact, aerosol, or both.[74] The first three experiments lasted 12 hours, with 8 donors and 12 recipients; the donor-recipient interaction was through playing variants of stud and draw poker (Fig. 166-5). This interaction mode facilitated both aerosol and fomite/hand-contact transmission. Half the recipients were blocked completely from any hand contact/self-inoculation by wearing arm restraints (see Fig. 166-5). Little transmission resulted from hand or fomite contact because the HRV-16 infection rate in the restrained recipients was only slightly lower than in the unrestrained recipients, 56 versus 67 percent, an insignificant reduction ($p = .494$).

The fourth experiment, involving aerosol blocking, was begun immediately after the third. The poker game

FIGURE 166-5 ■ A typical "Antarctic Hut" transmission experiment in progress using stud and draw poker as the interaction promoter. At each of four tables are two "donors" (infected with rhinovirus type 16) and three "recipients" (no antibody to rhinovirus type 16) (wearing surgical scrub suits). This particular experiment is one in which hand-contact/self-inoculation transmission is blocked in 6 of the 12 recipients by wearing Thatcher collars. The collars block hand-contact transmission by preventing touching of the face by the hands; therefore, virus can pass from donors to recipients only by aerosol.

continued in the original room with eight donors alone, and all the equipment from the third experiment, including the cards (which literally were gummy from the previous 12-hour game), poker chips, pencils, and so forth, was moved to an identical room across the hall. The continuing eight-donor poker game in the room used for the third experiment was refurnished with poker implements. Subsequently, 12 new recipients entered the second room, began playing poker with the now heavily used cards and other paraphernalia, and made exaggerated hand-to-nose self-inoculation movements. Aerosol transmission was blocked effectively by the two brick walls between the rooms. Once each hour, the cards, poker chips, and other portables were exchanged between the donor and recipient rooms to maintain a freshly contaminated supply of fomites in the recipient room. The result was extremely surprising; not 1 of the 12 "fomite recipients" caught HRV-16. Judging from the results of these Antarctic Hut experiments in which fomite/hand-contact and aerosol transmission were blocked alternately, aerosol transmission clearly seemed the predominant route, at least for HRV-16 among student poker players at the University of Wisconsin. A few months after the 1987 publication of these results, an editorial appeared in *The Lancet* titled "Splints Don't Stop Colds: Surprising!"[74,263] It concluded that "… it looks as though coughs and sneezes *do* spread these diseases more than sticky fingers."

Subsequently, a set of experiments was conducted to determine how HRV-16 had disappeared along the five-step fomite transmission chain from nose to hand to fomite to hand to nose.[163] The same results were noted.[244] The virus seemed to disappear precipitously at each step in the chain; 10^6 $TCID_{50}$ of HRV-16 in the donor's nose was reduced to 0 to 64 $TCID_{50}$ on the card-playing implements, and then the virus nearly disappeared on reaching the recipients' hands.

Additional studies examined other methods to interrupt transmission. The University of Virginia team performed a blocking experiment in the field in which mothers attempted to prevent hand contact/self-inoculation by dipping their fingers in iodine. The results of this 1979 to 1982 study were reported as part of a general review.[141] The illness rate during the 4 years was 40 percent lower in the mothers with iodinated fingers than in the placebo mothers, and the difference was significant ($p = .047$). However, the authors noted the difficulty of conducting and interpreting these investigations.[141] In another set of experimental human infection studies, the application of organic acids present in hand cleansers (e.g., salicylic acid, pyroglutamic acid) to the hands of volunteers decreased the likelihood of rhinovirus infection when volunteers subsequently touched their nasal or conjunctival mucosa 10 minutes after rhinovirus had been placed on their hands.[276]

The Madison group also examined transmission interruption methods to prevent spread from person to person. As part of a general investigation of the epidemiology of respiratory viruses in an isolated (≈200 persons) Antarctic population at McMurdo Station in 1979, use of an iodinated facial tissue developed by the S. C. Johnson Company of Racine, Wisconsin, was successful in interrupting transmission.[71,291] Three days after all personnel began copious use of these tissues, the incidence of respiratory illness dropped rapidly and significantly ($p < .01$); in fact, respiratory illness nearly disappeared from the base.[73] Although this effect was striking, the trial could be controlled only historically. However, the apparent success was sufficient to interest the Kimberly-Clark Corporation in applying virucidal facial tissue technology to interrupt HRV-16 transmission in the Antarctic Hut model. The Kimberly-Clark Corporation soon made available a nontoxic, highly virucidal facial tissue. The tissues completely stopped transmission of HRV-16, in comparison to transmission rates of 42 and 75 percent in control recipients using ordinary cotton handkerchiefs ($p < .001$) (Table 166-1).[72] The corporation test-marketed these tissues under the brand name "Avert," but their sales were lower than expected. Subsequently, two field trials of Avert in families were conducted in Charlottesville and Tecumseh, with reductions in viral transmission of 5 to 39 percent, respectively.[91,189] These results were disappointing but not unexpected. Changing established habits of personal nasal sanitation was not easy in the highly supervised, well-motivated adults in Antarctica; it may be impossible in unsupervised children.

Despite all the experimental studies to identify the principal route of virus transmission, no single route

TABLE 166-1 **Apparent Complete Interruption by Virucidal Tissues of Rhinovirus Type 16 Transmission***

Study Group	Cotton Handkerchiefs Experiment A	Virucidal Tissues Experiment B	Virucidal Tissues Experiment C	Cotton Handkerchiefs Experiment D
Recipients who "caught" HRV-16 colds/total (%)†	5/12 (42)	0/12	0/12	9/12 (75)

*In each of four experiments, 8 volunteers with HRV-16 colds played poker for 12 hours with 12 other volunteers (without HRV-16 antibody) and for nasal sanitation used either ordinary cotton handkerchiefs (experiments A and D) or virucidal tissues (experiments B and C). Three-ply Kleenex tissues were treated, per 100 g, with 9.1 g citric acid, 4.5 g malic acid, and 1.8 g sodium lauryl sulfate. Cotton or paper handkerchiefs were used to clear nasal passages, to smother coughs and sneezes, and to wipe surfaces. Virucidal tissues were used carefully and copiously.

†Diagnosed by at least one isolation of HRV-16 or a fourfold rise in antibody to HRV-16 in the convalescent serum sample, or both.

HRV-16, human rhinovirus type 16.

Modified from Dick EC, Hossain SU, Mink KA, et al. Interruption of transmission of rhinovirus colds among human volunteers using virucidal handkerchiefs. *J Infect Dis* 1986;**153**:352-6.

appears to be predominant. What is clear from these studies is that rhinovirus illnesses may be transmitted by both the hand-contact and aerosol routes.

PATHOGENESIS AND HOST FACTORS

General Course of Infection

Infection usually occurs through the respiratory route, although infection by the conjunctival route has been demonstrated.[37,143] When the virus is given intranasally by pipette, only one $TCID_{50}$ is needed to initiate infection.[64,76] The incubation period for a cold normally is 2 to 3 days, but a period of up to 7 days has been reported.[78,142] Ciliary dysfunction can predispose to respiratory tract infection.[29,39] HRVs replicate well in the upper respiratory tract (see later discussion), and substantial evidence indicates that they replicate in the lower respiratory tract as well.[109,129,224,225]

HRVs may be shed in large amounts (1000 to 1 million infectious particles per milliliter of nasal washing) during the first 2 to 3 days of a cold and may be produced for 2 to 3 weeks thereafter.[74,76,78,204] However, the effect on cells of the nasopharyngeal cavity seems benign despite much local reaction to the virus, which results in the usual signs and symptoms of a cold. Systematic studies of HRV-inoculated human volunteers at the University of Virginia demonstrated that damage to the respiratory epithelium was slight, although a few sloughed ciliated epithelial cells did contain HRV antigen.[277,295] HRV was recovered only at focal sites in the nose and nasopharynx at the time of maximal symptoms, and in situ hybridization studies demonstrated evidence of HRV infection in only a minority of epithelial cells.[15,23,297] Using primary monolayer cultures of ciliated and nonciliated epithelial cells, Winther and associates[296] demonstrated that HRV, coronavirus, influenza A virus, and adenovirus all grew well in these cell cultures; however, HRV and coronavirus produced no discernible cytopathic effect compared with the widespread destruction of the cell monolayer by influenza virus and adenovirus by 96 hours.

Innate Immune Response

Innate immunity is the initial host response to infection with rhinoviruses. Toll-like receptor 3 (TLR3) is activated during virus replication, is upregulated during HRV infection, and is associated with decreased viral replication.[145] Signaling occurs through both retinoic acid inducible gene-I (RIG-I) and melanoma differentiation associate gene-5, and inhibition of these molecules leads to increased viral replication.[264] One mechanism of inhibition of intracellular signaling developed by the virus is cleavage of the signaling molecules (e.g., RIG-I, interferon-β [IFN-β] promoter stimulator 1 [IPS-1]) by the viral protease.[24,84] Toll-like receptor activation leads to the production of type 1 IFNs, which is likely the mechanism by which viral replication is inhibited. Impaired induction of IFN-β leads to increased viral replication and is a defect that has been identified in the respiratory epithelium of asthmatic patients.[290] Numerous other components of innate immunity are also induced by rhinovirus infection, including β-defensins, nitric oxide, and a variety of proinflammatory cytokines and chemokines (e.g., interleukin [IL]-1, IL-6, IL-8, RANTES [regulated on activation, normal T cell expressed and secreted]).[241,253]

Because HRVs seem to cause only mild pathologic changes in cells of the respiratory tract, attention has turned to various immunologic or inflammatory substances as possible causes of symptomatic illness.[272] Researchers have long known that peripheral blood neutrophils increase in the first 2 to 3 days of illness in HRV-infected volunteers.[41,272] Phagocytes (neutrophils and monocytes) also have been demonstrated in large numbers in the nasal secretions of HRV-infected symptomatic human volunteers.[188] Although the microbe-killing ability of phagocytes is well known, these cells in themselves also can cause cold symptoms through the release of various toxic products, such as superoxide and hydrogen peroxide, during the respiratory burst.[35,269] In fact, increasing numbers of leukocytes in the peripheral blood correlate with increased symptoms and in infected human volunteers who are not ill the white blood cell count does not increase.[272] An association has been noted between the nasal levels of two proinflammatory mediators involved in granulocyte regulation, granulocyte colony-stimulating factor and IL-8, and blood and nasal neutrophilia have been identified.[110,221] Nasal IL-8 levels also correlate with the severity of clinical symptoms.[117,279]

Increased nasal levels of other inflammatory mediators (e.g., IL-1β, IL-6, RANTES, kinins) have been associated with more severe clinical symptoms.[218,221,238,239,306] Furthermore, topical nasal administration of IL-6 or bradykinin leads to development of some of the symptoms of the common cold.[105,240] At least some of these inflammatory responses appear to depend on an activation pathway regulated by nuclear factor κB (NF-κB).[305,306] Resolution of clinical symptoms may be caused by the elaboration of inhibitors of inflammation, such as IL-1 receptor antagonist.[303] These inflammatory mediators may serve as a target for the development of drugs to relieve the symptoms of HRV infection, although the use of a specific kinin inhibitor did not lead to clinical improvement.[120,147]

Immunity Associated with Serum Antibody

Serum antibody to the various HRV types develops with age (presumably from repeated infections); and by adulthood, antibody may be detected against approximately 50 percent of HRV types tested.[121] The presence of serum antibody correlates positively with immunity, and resistance to infection and the degree of disease expression are related to the amount of antibody present.[2,3,36] As examples, in the Eagle Heights Village families, 21 of 75 persons (28%) without antibody were infected with either HRV-43 or HRV-55, whereas only 5 of 35 (14%) with homologous antibody to either of these agents were infected.[68] No one was infected who had homologous

serum antibody levels above 1:16. Similar results were observed in the Charlottesville and Seattle families.[98,99,142] However, large doses can overwhelm antibody. At the clinical center of the National Institute of Allergy and Infectious Diseases, where human volunteers were used, 1000 $TCID_{50}$ was found to infect persons with serum antibody titers up to 1:256.[42] Judging from the paucity of reports of natural infections in persons with serum antibody titers greater than 1:16, virus inocula in naturally contracted cases must be low.

Immunity Associated with Antibody in Nasal Secretions

Researchers at the National Institutes of Health found that the presence of antibody in nasal secretion is associated with protection from homologous HRV infection and disease.[43] They found that intranasal administration of inactivated HRV vaccine produced both serum and nasal antibody, whereas two intramuscular injections of this vaccine produced little, if any, nasal antibody.[229] When the volunteers given the vaccine intranasally were challenged with approximately 100 $TCID_{50}$ of homologous HRV, they were protected significantly against both clinical illness and infection, whereas those administered the same vaccine intramuscularly remained susceptible.[229]

Conversely, when the same vaccine preparation was administered subcutaneously in three injections, nasal antibody responses were observed in 21 of 46 (45%) volunteers.[79] Challenge of these volunteers with 3 $TCID_{50}$ of virus produced significantly less virus shedding and reduced the duration and severity of illness than was observed in unimmunized controls who had no HRV-specific antibody. Possibly, this lower infectious dose more closely approximates a natural situation. This study also showed that protection against infection with the low virus challenge correlated with the magnitude of serum antibody and not with the presence of secretory antibody. The relative roles of serum and secretory antibody in HRV infections are still unsettled.

Antibody Appearance over the Course of Infection

A general pattern of the development of humoral (secretory and serum) antibody based on human studies has emerged.[36,81] Approximately 24 hours after infection, a sharp increase in nasal immunoglobulin (Ig) A secretion occurs. At the time symptoms appear (approximately 48 hours after infection), rhinorrhea begins, and the transudate is composed of significant amounts of IgG. After approximately 1 week and after the actual episode of illness, virus-specific antibody, predominantly IgA, appears in the nasal passages, tears, and the parotid saliva. Serum antibody (usually IgG but occasionally IgM) also begins to be formed at 1 week and rises to peak levels at 1 month. Both serum and secretory antibodies appear sooner and rise more rapidly in persons with detectable neutralizing antibody in their preinfection serum specimens. Antibody has been detected in nasal secretions and serum approximately 1 year after infection and, judging from the high proportion of the adult population with antibody to many HRV serotypes, probably lasts much longer.[33,121]

Cell-Mediated Immunity

Substantial evidence indicates that neutrophils increase both in the peripheral blood and in the nasopharynx during the first few days of an HRV cold.[41,294] Peripheral blood lymphocytes actually decrease in the first 2 to 3 days of an HRV cold, and migration of lymphocytes into the nasal secretions increases at this time.[41,188] Specific humoral antibody usually is not present in the serum or nasopharynx this early in the illness, a finding suggesting that these in situ nasopharyngeal lymphocytes, through cell-mediated immunity, play an important role in controlling HRV proliferation. HRV infection of primary epithelial cells cultured in vitro leads to increased expression of the T-cell attractant molecule CXCL10; and activated T cells cocultured with the epithelial cells produce IFN-γ and tumor necrosis factor (TNF) and activate the nitric oxide pathway, which can help inhibit viral replication.[166] Hsia and colleagues[150] examined the ability of peripheral blood lymphocytes to liberate various cytokines (IL-2 and IFN-γ) and participate in other cellular immune processes (cytotoxicity and antigen-stimulated blastogenesis) during the early days of HRV colds in experimentally infected human volunteers. These investigators found that all these cell-mediated immunity activities during infection were increased significantly when compared with preinfection levels. Of special interest, the cellular ability to liberate higher levels of IL-2 correlated inversely and significantly ($p < .02$) with virus shedding and nasal mucus production. Cross-reactive T-cell epitopes are shared by HRVs, and T cells isolated from peripheral blood and from tonsillar tissue proliferate and secrete a variety of cytokines (IFN-γ, IL-2, IL-4, IL-5) after exposure to HRV antigens.[108,135,293] Thus, activation of T cells may contribute not only to virus clearance but also to airway inflammation.[108]

Interference Among Rhinoviruses

Interference in infection with heterotypic HRV serotypes, which lasts between 5 and 16 weeks, has been reported in human volunteers.[92] Complete resistance to infection was observed after inoculation with as much as 2000 $TCID_{50}$ of HRV-16 (administered by nasopharyngeal spray) in a subject with an unsuspected "wild" HRV (not typed) infection present at the time of inoculation.[186] However, epidemiologic studies in a nursery school population,[26] a military population,[246] and a family population[209] showed sequential heterotypic HRV infections occurring frequently, sometimes at intervals as short as 2 days. In the family population, one subject was infected by three different HRVs within a 30-day period. The factor or factors responsible for the interference observed in the experimental human infection model have not been identified. Simultaneous infection with an HRV and other respiratory viruses, including influenza A and B

viruses, coronaviruses, parainfluenza type 1 virus, respiratory syncytial virus (RSV), adenoviruses, various enteroviruses, or other HRV serotypes, also has been commonly reported, particularly in children.[85,99,184]

Influence of a Cold Environment on the Course of Infection

Exposure to cold temperatures has been thought either to initiate or to exacerbate respiratory tract infections. In fact, chilling animals was shown to increase the severity and frequency of viral infection.[289] However, investigation of the effects of chilling on humans infected with "common cold viruses" and with HRV-15 did not demonstrate significant effects.[80] In the HRV-15 experiments, the conditions were realistic. The subjects (men) were cooled sufficiently in air to cause shivering for approximately an hour or were immersed in water long enough to cause a decline in rectal temperature.

Effect of Age and Sex

A much higher prevalence of HRV infection is found in infants and young children than in older persons. In Seattle,[98] the HRV infection rate in children from birth to 5 years of age was nearly twice that of older children and adults (0.77 vs. 0.41 infections per person per year, respectively); in Tecumseh,[214] the isolation rate in the 1- to 4-year-old group was far higher than that in any other age bracket. The high attack rates in these young children were not unexpected because fewer than 10 percent of them had antibody to any of the 56 HRV types,[123] and children ordinarily are subjected continuously to the family-like environment so conducive to transmission of HRVs. In especially crowded populations, attack rates may be high. In an Alaskan Eskimo village, 70 percent of 395 children were infected during a spring outbreak of HRV-16 infection.[300]

The frequency of infection with HRV generally declines throughout life. In a prospective study of children attending daycare where PCR methods were utilized to analyze samples collected weekly in symptomatic children, HRVs were the most common respiratory virus identified (Fig. 166-6), and the incidence/child-year of HRV infection was 1.3/year for children enrolled in daycare at birth to 12 months of age versus 0.8 for children older than 12 to 24 months.[90] Beginning at the age of school attendance, the number of HRV infections declines gradually from one to two per year to 0.25 per year in the age group older than 60 years.[121,216] Results from most laboratories have found that persons 20 to 30 years of age account for an exception to the general decline in incidence with age[98,142,213,216]; HRV infections as well as infections with other respiratory pathogens increase during this period of life. Because the increase is especially marked in mothers,[98,142] it probably is the result of the transfer of respiratory illness in small children to parents.

In Tecumseh, the number of respiratory illnesses per year was consistently higher for female patients than for male patients (3 to >60 years of age).[216] If one assumes that HRVs account for a similar proportion of disease in each gender, this finding would indicate that girls and women generally have more HRV infections. As noted earlier, women with young children clearly seem to have more HRV infections than do others in their age group. In Seattle,[98] mothers had 1.5 times more HRV infections than fathers did, and in Charlottesville, women of childbearing age had approximately 1.2 times more HRV illness than male study subjects did.[121]

The number of moderate to severe rhinovirus illnesses was higher in boys than girls during the first 3 years of life in a longitudinal birth cohort study examining factors associated with developing wheezing illness.[284] However, the number of HRV-associated wheezing illnesses was similar between the two sexes.

Effect of Psychosocial Factors

Numerous psychosocial factors have been shown to influence susceptibility to HRV infection, primarily in the experimental human infection model. Increased psychological stress, as measured by numerous stress indices, and poorer sleep efficiency were associated with an increased susceptibility to infection and an increased likelihood of developing illness.[50,52,53] In contrast, having a more diverse societal network (increased number of social ties) and having a positive emotional style (i.e., sense of well-being, vigor, and calmness) were associated with resistance to infection and illness.[51,83] Antibody-negative (serum antibody levels < 1:2) subjects were equally susceptible to infection regardless of the degree

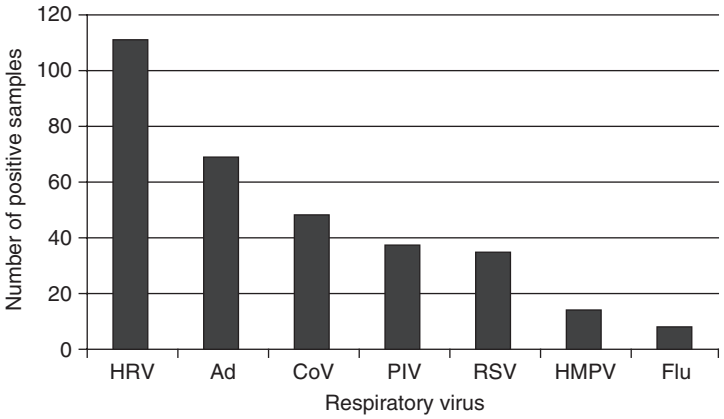

FIGURE 166-6 ■ Number of respiratory viruses identified using PCR from 318 respiratory tract illnesses in children attending a daycare center. HRV, human rhinovirus; Ad, adenovirus; CoV, coronavirus; PIV, parainfluenza virus types 1 to 4; RSV, respiratory syncytial virus; HMPV, human metapneumovirus; Flu, influenza A and B. (Modified from Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16-20.)

of stress or number of social ties, although these factors did influence the likelihood of clinical illness.[51,52] Increased stress in working adults also was associated with an increased risk for having a natural common cold.[268]

CLINICAL MANIFESTATIONS

HRV infections in any age group usually cause only mild upper respiratory tract illness or common colds.[60] Investigators estimate that HRVs cause 30 to 50 percent of common colds, at least in adults.[191] A variety of respiratory symptoms and signs can be present, and the relative frequency varies of such symptoms varies between children and adults (Table 166-2). The development of serious illness is also possible: HRV-associated, radiograph-positive, atypical pneumonia has been described in military trainees[106] and in immunocompromised adults,[112,154] graft dysfunction has occurred in lung transplant recipients,[168] and exacerbation of underlying lung disease has been seen in adults with asthma and chronic obstructive pulmonary disease.[19,114,219,257] Severe respiratory disease, pneumonia, and death also have occurred in outbreaks of rhinovirus infection in geriatric patients residing in nursing homes.[146,190]

Children, particularly the very young, are subject to serious, sometimes fatal HRV-caused illness. These include bronchiolitis and pneumonia. Viremia has been noted to occur in a minority of children with HRV infection and respiratory disease.[301] Deaths of infants with rhinoviremia has also been observed, including one of which that occurred during a cold and six "cot" deaths that were diagnosed as bronchiolitis/pneumonia and from which HRVs were isolated.[245,286] At least in theory, generalized illness after viremia seems possible with HRV infections; the HRVs are a division of the larger picornavirus group, which contains many viruses capable of generalized and fatal infection and whose genomes have considerable homology with the HRVs (see Structure of the Virion, earlier).[222] HRVs have also been isolated from patients with a variety of other respiratory clinical illnesses, including pharyngitis, epiglottitis, and laryngotracheobronchitis (croup) (Table 166-3). However, detection of HRV may also occur in persons who are

TABLE 166-2 **Signs and Symptoms of Respiratory Illness in 20 Adults and 15 Children with Rhinovirus Infections in Philadelphia and New Jersey: 1959 to 1960***

Sign or Symptom	Adults	Children (Age 2 Mo to 8 Yr)
Fever†	4 (20%)	9 (60%)
Eye, ear, nose, and throat		
Rhinorrhea	18 (90%)	10 (67%)
Purulent nasal discharge	4 (20%)	1 (7%)
Pharyngitis	10 (50%)	5 (33%)
Conjunctival infection	2 (10%)	1 (7%)
Anterior cervical lymphadenopathy	1 (5%)	8 (53%)
Hoarseness	4 (20%)	0
Croup	0	1 (7%)
Infection of the tympanic membrane	0	3 (20%)
Chest		
Cough	8 (40%)	15 (100%)
Dyspnea	0	4 (27%)
Refractions	0	3 (20%)
Rhonchi	0	7 (47%)
Rales	0	2 (13%)
Wheezing	0	4 (27%)

*The children were from outpatient clinics and wards of the Children's Hospital of Philadelphia, and the adults were employees of Merck and Company, Inc., Rahway, NJ.
†In adults, 37.2°C (99°F) or higher by mouth: the peak was 37.3°C (99.2°F). In children, 37.7°C (100°F) or higher by rectum; range, 37°C (98.6°F) to 39.1°C (102.4°F); mean, 37.9°C (100.4°F).
Adapted from Reilly CM, Hoch SM, Stokes J Jr, et al: Clinical and laboratory findings in cases of respiratory illness caused by coryzaviruses. *Ann Intern Med* 1962;**57**:515-25.

TABLE 166-3 **Clinical Diagnosis of Respiratory Illnesses of Young Children* at St. Anna Children's Hospital, Vienna, from 1984 to 1986: Comparison Between Rhinovirus and Respiratory Syncytial Virus Infections**

Physical Findings	All Patients† (*n* = 519)	RSV (*n* = 119)	HRV (*n* = 60)
Upper Respiratory Tract Infection			
Rhinitis	65 (12%)	4 (3%)	5 (9%)
Otitis media	10 (2%)	0	2 (3%)
Epiglottitis	6 (1%)	1 (1%)	2 (3%)
Pharyngitis	23 (4%)	3 (2%)	4 (6%)
Laryngitis	8 (2%)	1 (1%)	2 (3%)
Other diseases	25 (5%)	0	1 (2%)
Total	137 (26%)	9 (7%)	16 (26%)†
Lower Respiratory Tract Infection			
Croup	14 (3%)	4 (3%)	4 (6%)
Tracheobronchitis	100 (19%)	46 (39%)	16 (27%)
Bronchitis	24 (5%)	3 (2%)	0
Obstructive bronchitis‡	78 (15%)	28 (24%)	8 (14%)
Pneumonia	126 (24%)	29 (25%)	15 (25%)
Other diseases	40 (8%)	0	1 (2%)
Total	382 (74%)	110 (93%)	44 (74%)

*Ten days to 3 years of age (median age, 6.6 months).
†HRVs were more likely to cause upper respiratory tract infection ($p < .01$).
‡This diagnosis included children with expiratory wheeze and evidence of air trapping. In a later article, this same definition was used for wheezy bronchitis.
HRV, human rhinovirus; RSV, respiratory syncytial virus.
Modified from Kellner G, Popow-Kraupp T, Kundi M, et al. Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. *Acta Paediatr Scand* 1989;**78**:390-4.

totally asymptomatic; up to 25 percent of infections detected by PCR assay in otherwise healthy children and adults are asymptomatic or subclinical,[155,259] and asymptomatic shedding for up to 3 months has been documented even in hematopoietic stem cell transplant recipients.[205]

Spectrum of Respiratory Disease

Rhinovirus Infections in Hospitalized Children

Many investigators have identified HRV infections in children hospitalized with severe respiratory disease, and these studies illustrate the high number of hospitalizations associated with HRV in children overall. In 1971 in Bristol, England, prior to the widespread use of molecular diagnostic tools, 377 infants hospitalized for respiratory disease yielded 199 (53%) viral diagnoses.[160] RSV was predominant and accounted for 79 percent of the diagnoses, but HRVs were second at 12 percent (23 patients). Half of the HRV diagnoses were in infants with bronchiolitis or pneumonia. HRV illnesses were comparable in severity to those caused by RSV.

At St. Anna Children's Hospital in Vienna from 1984 to 1986, Kellner and colleagues[172] compared the clinical features of HRV and RSV infection in 519 children aged 10 days to 3 years (median age, 6.6 months). Viral pathogens were detected in 227 (44%) of the children; and of these, 119 (23%) were RSV and 60 (12%) were HRV. The physical findings (Table 166-4) and the clinical diagnosis (see Table 166-3) of the children with HRV or RSV infection were the same, except HRV infections were more likely than were RSV infections to be associated with upper respiratory tract infection.

Glezen and colleagues[113] performed a prospective study to evaluate the occurrence of respiratory virus infection in subjects with respiratory or cardiac disorders in Houston, Texas, between 1991 and 1995. HRV infections were identified in 51 subjects from a total of 1198 evaluated illnesses; 6 were associated with a second respiratory virus infection.[88] In children younger than 5 years of age, exacerbation of asthma, bronchiolitis, and suspected sepsis were the most common clinical findings, whereas in older children and young adults, almost all subjects had an exacerbation of asthma. In older adults, a complication of an underlying disease (asthma, chronic obstructive lung disease, congestive heart failure) or pneumonia was diagnosed. In this prospective study, HRV infections were an important cause of lower respiratory tract illness in all age groups.[88] When molecular methods were used to identify viral respiratory tract infections from specimens collected during this study, HRVs were present in almost 25 percent of analyzed samples and in approximately half of children younger than 1 year of age.[20]

Bronchiolitis is one of the more common reasons for young children to be hospitalized with respiratory illness. HRVs have been identified in several studies as the second most common virus infection identified in association with bronchiolitis after RSV.[38,161,196,197] In some studies, the bronchiolitis associated with HRV infection was less severe than that caused by RSV,[196-198,265] whereas in others the severity was similar.[38] In a large multicenter study reported by Mansbach and colleagues,[196] children younger than 2 years of age and coinfected with RSV and HRV had significantly longer lengths of stay after adjustment for clinical and demographic parameters whereas HRV coinfections with other respiratory viruses were not

TABLE 166-4 **Physical Findings in Young Children* with Respiratory Illness at the Time of Their Admission to St. Anna Children's Hospital, Vienna, from 1984 to 1986: Comparison Between Rhinovirus and Respiratory Syncytial Virus Infections**

Physical Findings	All Patients† (*n* = 519)	RSV (*n* = 119)	HRV (*n* = 60)
Stridor during expiration	67 (13%)	15 (13%)	10 (16%)
Cyanosis	18 (3%)	4 (3%)	2 (3%)
Swollen cervical glands	87 (17%)	17 (14%)	9 (15%)
Red throat	344 (66%)	75 (63%)	41 (68%)
Nasal flaring	75 (14%)	29 (16%)	11 (18%)
Crepitations	20 (4%)	7 (6%)	2 (3%)
Chest radiograph (positive)	260 (50%)	79 (66%)	35 (58%)
Wheezing	113 (22%)	30 (25%)	13 (22%)
Moist rales	174 (34%)	43 (36%)	22 (36%)
Dry rales	49 (9%)	13 (11%)	5 (8%)
Median respiratory rate/min (range)	43 (10-96)	40 (10-84)	36 (10-64)
Median body temperature (range)	38.4°C (36.4°C-41.8°C)	39.9°C (36.8°C-41.0°C)	38.5°C (36.9°C-41.0°C)
Median duration of illness (range)	11.6 days (2-45)	10.5 days (4-12)	12.8 days (3-31)

*Ten days to 3 years of age (median age, 6.6 months).
†One hundred twelve (21%) children had upper respiratory tract illnesses, and 342 (66%) children had lower respiratory tract illnesses: 471 (91%) were inpatients, and 48 (9%) were outpatients.
HRV, human rhinovirus; RSV, respiratory syncytial virus.
Modified from Kellner G, Popow-Kraupp T, Kundi M, et al. Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. *Acta Paediatr Scand* 1989;**78**:390-4.

associated with prolonged lengths of stay. Decreased severity of illness in hospitalized children who are coinfected with both HRV and RSV or human metapneumovirus has also been documented.[30,198]

Several studies have examined the prevalence of HRV infection in pediatric patients presenting with community-acquired pneumonia. Juven and colleagues[167] examined 254 children hospitalized with community-acquired pneumonia and found HRV infection in 25 percent. A concomitant bacterial co-pathogen was identified in half of the patients. Tsolia and associates[271] reported HRV infection in 45 percent of 75 school-aged children hospitalized with community-acquired pneumonia, with a co-pathogen found in 58 percent of the HRV-infected patients. García-García and colleagues[104] detected HRV infection in approximately 20 percent of children hospitalized with pneumonia; HRV was the most common viral pathogen identified in children older than 18 months of age and the sole virus identified in 55 percent of the HRV-infected patients.

Iwane and colleagues[155] examined the contribution of HRVs in children younger than 5 years of age who were hospitalized with severe acute respiratory illness. Overall, HRV infection was identified in 17.4 percent of the children, and a second viral respiratory pathogen was identified in 18 percent of HRV-infected patients (most commonly RSV). These investigators also included a control group of age-similar children presenting for well-child visits and without active respiratory symptoms, and they found that 12.5 percent of the control subjects were asymptomatically infected with HRV. Infection with HRV-A viruses was significantly more common in the hospitalized children 24 months of age and older compared with controls (8.1% vs. 2.2%), and HRV-C infection was significantly more frequent in hospitalized children 6 months of age and older (8.2% vs. 3.9%). HRV-B infection was uncommon in both groups (<1% each).

In summary, there is substantial evidence that HRVs can cause serious lower respiratory tract illness, especially in young children. In some populations, HRVs may be second to RSV in frequency and be associated with comparably severe signs and symptoms. However, the prevalence of asymptomatic virus shedding and the frequent identification of another viral respiratory tract infection make it difficult to determine the clinical significance of HRV infection in the individual patient.

Asthma

HRV infection is a risk factor for both the development of asthma and for exacerbations of wheezing in children with asthma. Having either an RSV or an HRV infection that results in a wheezing illness during the first year of life is an independent risk factor for wheezing later in childhood.[178,180,187] Children who have a HRV-associated wheezing illness in the first 3 years of life are more likely to be diagnosed with asthma at 6 years of age than are those who had an RSV-associated illness.[156] Sensitization to aeroallergens (atopy) in the first year of life also was an independent risk factor for the subsequent development of asthma, particularly after HRV infection.[156,157,180] It is still not clear at this time whether the infection with HRV (or RSV) that is associated with wheezing in the first several years of life plays an etiologic role in the subsequent development of asthma or whether it is a marker for children already at risk for developing asthma.[175]

Viral respiratory tract infection is a common precipitant of wheezing in children with asthma.[34] HRVs are the most frequent respiratory virus identified in children with acute wheezing exacerbation of asthma; however, not all HRV infections in an asthmatic child will result in wheezing. Two investigative groups serotyped the HRVs isolated from children with wheezy illness and found no types to be particularly "asthmagenic"; and a total of 30 different serotypes were associated with asthma exacerbations.[170,206-208] At the present time it is not clear whether HRV strain differences are important factors in precipitating asthma attacks.

The mechanism of bronchospasm in virus-infected patients has generated much speculation and research. HRVs can replicate in the lower respiratory tract and have been found in the lower airways.[109,129,152,224,225] Whether the virus-associated bronchospasm is caused by viral replication in the lower airways or by induction of inflammatory mediators resulting from their growth in the upper respiratory tract has been a subject of debate, although accumulating evidence favors the former possibility.[107] HRV infection clearly induces the production of numerous proinflammatory cytokines, and an increased number of inflammatory cells has been demonstrated in the lower respiratory tract in association with HRV infection.[94,102,107] The presence of activated cytotoxic T cells in the lower airways has been suggested to contribute to asthma-associated mortality.[220] Host factors also play a role, in that innate immune responses to rhinovirus are impaired. For example, bronchial epithelial cells from asthmatic individuals produce less IFN-β and have less apoptosis and greater viral replication after infection with HRV than do cells from nonasthmatic subjects.[290] Aeroallergen–sensitized asthmatic children have higher expression of the high affinity IgE receptor FcεRI than nonallergic, nonasthmatic children, and this was associated with a decreased production of IFN-α and IFN-γ1 by plasma-derived dendritic cells after HRV stimulation.[87] IFN production was further decreased after cross linking of the FcεRI receptor. Animal models also suggest the possibility that reflex neurogenic pathways may be activated in association with viral respiratory tract infection and may lead to bronchoconstriction. The pathogenesis of respiratory virus–induced bronchospasm is an area of active research, with the hope that preventive or therapeutic intervention strategies can be developed.

Otitis Media

Although acute otitis media is chiefly a bacterial disease, with approximately two thirds of middle ear fluid specimens yielding *Streptococcus pneumoniae*, *Haemophilus influenzae*, or both, and *Moraxella catarrhalis* and other bacteria playing lesser roles, otitic involvement often is preceded or accompanied by a putative viral upper respiratory tract illness.[11,13,177,251] Most bacterial pathogens found in

middle ear fluid are colonizers of the nasopharynx, and bacterial infection of the middle ear is considered to be a direct extension from that area.[177] A history of a preceding viral upper respiratory tract illness is common, and clear and comprehensive evidence of viral extension from the nasopharynx to the middle ear has been established. Many different respiratory viruses have been identified in middle ear effusions from children with otitis media, and HRVs are one of the most commonly identified when molecular methods of detection are used. Pitkaranta and colleagues[234] identified respiratory viruses from the middle ear effusions in 30 percent of 100 children who had otitis media with effusion. HRVs constituted approximately two thirds of the respiratory viruses identified. Bacterial pathogens were identified in 35 percent of the samples, and both bacteria and viruses were found in 11 percent. A criterion for selection was the absence of an upper respiratory tract illness within the preceding week, thus whether the presence of HRV RNA represented evidence of ongoing or past infection is unclear. Blomqvist and colleagues[27] found HRV RNA in middle ear effusions from 41 percent of children with otitis media in the first 2 years of life, and Chantzi and colleagues[45] found a similar prevalence of HRV RNA in otitis media effusions.

The presence of both bacterial and viral pathogens in middle ear fluid does not, in general, interfere with the effectiveness of antibacterial therapy, although in those specific patients whose acute otitis media is refractory to treatment it may.[14] When measured against a comparison group of 66 "normal" acute otitis media cases (controls), 22 patients with refractory cases harbored significantly more viral pathogens than did controls (68% vs. 41%, respectively; $p < .05$). In addition, when only the middle ear fluid of these groups was examined for the presence of viruses, 32 percent of patients with refractory cases had virus versus 15 percent in controls. HRVs were the dominant viruses in both groups. Bacterial pathogens were grown from the middle ear fluid of 4 of the 22 in the poor-responder group, and all harbored concomitant respiratory viruses, two of which were HRVs; only one of the four bacteria was resistant to the antibacterial agent used in therapy. These investigators also examined patients in the 66-patient control group whose illnesses were refractory to treatment and found significantly more viruses ($p < .05$) in the middle ear fluid of this group than in those with a good response to therapy. These authors concluded that the presence of viruses in the middle ear fluid of children with acute otitis media can delay response to antibacterial therapy.

Chonmaitree and associates[47] in Galveston, Texas, also investigated the role of viruses in middle ear fluid as agents that may interfere with antibacterial therapy. In their initial report, viruses were cultured from the middle ear fluid of 11 of 58 children (19%); RSV and HRV were the most frequent viruses isolated, at 3 each. Of the patients in whom therapy failed, significantly more ($p < .05$) harbored viruses as well, and of those whose bacteria were susceptible to the treatment antibiotic, significantly more had combined viral-bacterial infections. In two subsequent reports by these authors, the highest rate of poor bacteriologic outcome occurred when HRV was isolated from middle ear fluid. Seven of nine instances of HRV infection resulted in bacteriologic treatment failure.[267]

These studies suggest that viruses, including HRVs, may play a role in acute otitis media, especially in prolonging the response to antimicrobial treatment. McBride and associates[200] at the University of Pittsburgh and the University of Virginia used HRV-infected human volunteers and found that the eustachian tube became occluded in 50 percent of the volunteers and that abnormal middle ear pressure was present for as long as 10 days. These studies were extended by Buchman and colleagues,[32] who inoculated 60 volunteers with HRV-39. Middle ear pressures of less than –100 mm H_2O were noted in 22 subjects (37%). In 2 of the 3 subjects who had pressures less than –100 mm H_2O, upper respiratory tract illness with middle ear effusion developed. In this study, the otologic manifestations of experimental HRV infection were extended to include otitis media.

Arola and colleagues[12] examined the role of viruses in 61 children (mean age, 3.2 years) with subacute or chronic asymptomatic otitis media with effusion. Five HRVs and one adenovirus were found in the middle ear fluid. In addition, bacterial pathogens were isolated from the patient with adenovirus infection and from two of the patients with HRV infection. None of these patients with otitis media and effusion were ill with an upper respiratory tract infection at the time the specimen was obtained, and the effusion had endured 30 to 60 days before myringotomy was performed.

Sinusitis

HRVs can cause a clinical syndrome of rhinosinusitis similar to that caused by bacterial infection. Gwaltney and colleagues[126] performed computed tomography of the sinuses on adults with acute common colds of less than 4 days' duration in which HRV infection was documented by culture in 27 percent. Radiographic abnormalities included mucosal thickening and air-fluid levels. Associated clinical signs and symptoms resolved without antibiotic therapy, a finding suggesting that the radiographic signs were caused by an acute viral infection. Few attempts have been made to recover viruses directly from the sinuses. Two reports from Virginia noted that 140 aspirates obtained by direct puncture of the maxillary sinuses yielded 86 positive specimens, only 12 of which contained viruses. Seven of these viruses were HRVs, and the remainder were influenza A or parainfluenza viruses.[127,130] Five of the viruses were isolated in conjunction with bacterial pathogens. In a more recent study, Pitkaranta and associates[233,235] used real-time PCR analysis and in situ hybridization to identify HRVs in 50 percent of adults with acute community-acquired sinusitis. Because the signs and symptoms of viral rhinosinusitis and bacterial sinusitis can be difficult to distinguish, the American Academy of Pediatrics developed clinical practice guidelines for the diagnosis and treatment of bacterial sinusitis, and these were updated in 2012 by the Infectious Diseases Society of America.[48,49]

Immunocompromised Patients

Community-acquired respiratory viral infections are important causes of morbidity and mortality in immunocompromised patients.[89] HRV infections in these populations are common, with a cumulative 100-day incidence as high as 22.3 percent in one hematopoietic stem cell transplant population and with infections occurring year round and prolonged virus shedding noted.[205] Clinical manifestations can range from mild upper respiratory tract symptoms to fatal pneumonia.[112,119,205] Many patients with fatal infections have been coinfected with other pathogens, making it difficult to determine the relative contribution of HRV infection to the outcome.[12,154] Overall, higher viral loads in clinical samples correlated with more symptomatic and more severe disease.[111,205]

DIAGNOSIS OF INFECTION

HRV infections cannot be diagnosed solely on the basis of clinical grounds because they cause such a wide spectrum of respiratory illness, particularly in infants and children. However, because of the characteristic spring-summer-fall seasonal pattern of the HRVs, tentatively assigning an HRV origin in patients with mild-to-moderate respiratory illness during these months is not unreasonable.

Obtaining a culture of the etiologic agent and interpreting the meaning of a positive specimen can be difficult with HRVs. These viruses often propagate unpredictably, even in relatively sensitive diploid cell cultures such as WI-38 and FT cells or in Ohio strain HeLa cells, and none of the HRV-C strains can be propagated in vitro,[16,55,58,61,121] Isolation of HRV from children can also be hampered by the common practice of relying on a throat swab. Inocula from many throat swabs contain one tissue culture infectious virus particle or less (see Table 166-5). Hendley and colleagues[142] found nasal specimens to be superior for HRV studies, and diagnostic rhinovirologists from many nations successfully use nasal aspirates.[149,171,203] Specimens should be placed in cell culture as soon as possible and without previous freezing and thawing (however, refrigeration [4° C] overnight results in little loss). If freezing is necessary and dry ice is used, great care must be taken to avoid contamination of the specimen with sublimed carbon dioxide because the resultant carbonic acid rapidly kills the virus. Cell cultures should be incubated at 33° C and slowly rolled. Evidence of virus growth usually is seen between 2 days and 2 weeks. Once a cytopathic effect is evident, standard techniques for identification are used.[62]

As described early in this chapter, HRVs have many base sequences in common among the RNA genomes of the various serotypes, especially in the noncoding region at the 5′ end. Several laboratories have taken advantage of this observation by designing RT-PCR assays for the detection of HRVs. Some assays do not distinguish HRVs from enteroviruses, whereas others are HRV specific.[103,128,153] The superiority of RT-PCR techniques compared with cell culture methods first became evident in a clinical study of children with acute respiratory illnesses. Johnston and colleagues[165] detected HRV by PCR in 146 of 292 samples (50%) that yielded HRV in only 47 (16%) by standard culture. PCR was thus three times more sensitive than was culture for detecting HRV infection. Many subsequent studies confirmed the superiority of RT-PCR assays over culture methods for the detection of HRVs.[4,18,19,151] The development of sensitive real-time RT-PCR assays raises the possibility that clinical virology laboratories would become able to identify HRV infection rapidly.[255] Multiplex assays that identify HRVs as well as other respiratory viruses are also commercially available.[22,232] The increased sensitivity of molecular assays can make interpretation of a positive result problematic, because many asymptomatic infections are identified. Viral quantitation may have a role in identifying clinically significant HRV infections.[111]

TABLE 166-5 **Quantity of Virus Found in 87 Throat Swab Squeezings from Children Aged 3 to 11 Years: Madison, Wisconsin, from 1971 to 1972***

	Per 0.1 mL
≤1 $TCID_{50}$	22
>1 to ≤50 $TCID_{50}$	46
≥50 to <50 $TCID_{50}$	14
>50 to <500 $TCID_{50}$	5

*Fourteen rhinovirus types are represented.
$TCID_{50}$, median tissue culture infective dose.

PREVENTION AND TREATMENT

Prevention and treatment are addressed thoroughly in Chapter 7 and elsewhere, and only measures specific to the HRVs are added here. Although some protection after the use of inactivated vaccines has been demonstrated,[79,230] the existence of more than 100 serotypes limits the prospect of vaccine development. However, based on the observation that cross-reacting antigenic groupings exist among the HRVs, developing a vaccine that targets these epitopes may be possible.[57]

The most effective HRV cold-preventive agent described to date that has been demonstrated to be effective for natural HRV colds is IFN-α, a protein produced as part of the host's natural antiviral defense and now produced for experimentation by genetic recombinant methods.[82,136] Investigators administered this agent by nasal spray to other family members after symptoms appeared in the index case; 80 percent of the secondary HRV colds were prevented. However, IFN given in this fashion does not seem to be effective against other respiratory viruses. For example, in a follow-up study in Seattle, IFN-α did not reduce the incidence of colds when administered in a protocol identical to that used in the previous family studies, chiefly because HRV infections were not the majority of agents causing colds.[77,82,101,136]

HRVs often have a decided seasonality, so rapid diagnosis of the index case by PCR analysis would be helpful to determine when IFN-α could be used effectively to stop intrafamily spread. A combination of rapid diagnosis and family prophylaxis with IFN-α could be helpful in families such as those with asthmatic children, in whom HRV infection may be serious (see Clinical Manifestations). The disadvantage of IFN prophylaxis is that prolonged intranasal administration (>7 days) or repeated treatment produces an inflammatory response consisting of nasal stuffiness, ulceration, and blood-tinged discharge.[252]

Numerous antiviral agents with in vitro activity against HRVs have been developed. The earlier section in this chapter on structure of the virion describes the development of some of these agents, which was made possible by taking advantage of current detailed knowledge of HRV structure and specific cell receptors. Several of these preparations, including capsid-binding agents, soluble receptor (soluble ICAM-1), and antibody to the receptor (ICAM-1), have been tested in clinical trials, but development of most of these agents has not been pursued for a variety of reasons,[21,278] as reviewed by Arruda and Hayden.[17] Another capsid-binding agent, pleconaril, has in vitro antiviral activity against many enteroviruses and HRVs, and phase III clinical trials on naturally acquired colds demonstrated some reduction in the severity and duration of several respiratory symptoms.[137] However, pleconaril induces the cyp3A4 enzyme that can lead to menstrual irregularities in women taking oral contraceptives.[93] This concern, along with the drug's modest efficacy, prevented pleconaril from being approved by the U.S. Food and Drug Administration; an intranasal form is still under development.[226] Another anti-RV drug, rupintrivir (formerly AG7088), is a potent, irreversible inhibitor of viral 3C protease that has reached clinical trial.[138,199] Further studies of both these agents, especially in children, will be needed to determine their potential benefits.

Colds usually are treated symptomatically with mild nonsteroidal anti-inflammatory drugs, sympathomimetics (e.g., phenylpropanolamine), and antihistamines; these agents are discussed in Chapter 7.[262] Anticholinergic agents (e.g., topical ipratropium bromide) and first-generation antihistamines reduce severity of rhinorrhea, whereas second-generation ("nonsedating") antihistamines have no effect. The efficacy of the first-generation antihistamines may result from the ability of these agents to block muscarinic as well as histaminic receptors and to cross the blood-brain barrier.[217] Sympathomimetics decrease nasal obstruction, although topical administration may lead to rebound nasal obstruction. Nonsteroidal anti-inflammatory agents decrease the severity of some of the systemic symptoms associated with colds (e.g., malaise, headache).[273] A combination of antiviral and antimediator preparations may provide effective therapy.[122] Various other treatments, including vitamin C,[44,140] *Echinacea*,[202,274] intranasal humidified air,[96,281] and zinc lozenges,[159,194,236] have been evaluated, and some studies have reported a clinical benefit, although other studies have failed to confirm such findings.[273] Almost all clinical evaluations of symptomatic and alternative therapies have been performed in adults; very few data exist on the utility of such therapy in children.[194,260] In 2007, the U.S. Food and Drug Administration released a warning against using over-the-counter therapies including antihistamines, decongestants, and antitussives for the treatment of the common cold in children younger than 2 years of age because of potentially dangerous side effects linked to several deaths, in the absence of data demonstrating benefit in this age group.[95]

The use of oral or inhaled corticosteroids appears to enhance the shedding of viable virus.[118,242] One potential consequence of corticosteroid use, seen in a study of pediatric patients with a common cold syndrome, is a significant increase in the risk for developing acute otitis media in HRV-infected subjects.[250] However, hospitalized children with a first episode of wheezing associated with HRV infection were less likely to have recurrent wheezing over the following year if treated with prednisolone, and corticosteroid therapy can decrease the duration of symptoms associated with acute asthma exacerbations.[162,185] Thus, corticosteroids do not have a role in the treatment of uncomplicated HRV infections. Similarly, no role exists for the use of antibiotic therapy in uncomplicated HRV infection.

Acknowledgment

The authors would like to acknowledge the contributions of Elliot C. Dick, Stanley L. Inhorn, and W. Paul Glezen, who contributed to this chapter in previous editions of the text.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Arden KE, McErlean P, Nissen MD, et al. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol* 2006;**78**:1232–40.
20. Atmar RL, Piedra PA, Patel SM, et al. Picornavirus, the most common respiratory virus causing infection among patients of all ages hospitalized with acute respiratory illness. *J Clin Microbiol* 2012;**50**:506–8.
22. Barbady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast assay and the Idaho Technology FilmArray RP Assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J Clin Microbiol* 2012;**50**:2282–8.
24. Barral PM, Sarkar D, Fisher PB, et al. RIG-I is cleaved during picornavirus infection. *Virology* 2009;**391**:171–6.
30. Brand JK, de Groot R, Galama JM, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. *Pediatr Pulmonol* 2012;**47**:393–400.
31. Briese T, Renwick N, Venter M, et al. Global distribution of novel rhinovirus genotype. *Emerg Infect Dis* 2008;**14**:944–7.
34. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet* 2010;**276**:826–34.
38. Calvo C, Pozo F, Garcia-Garcia ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Arch Paediatr* 2010;**99**:883–7.
48. Chow AW, Benninger MS, Brook I, et al. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults. *Clin Infect Dis* 2012;**54**:e72–112.
50. Cohen S, Doyle WJ, Alper CM, et al. Sleep habits and susceptibility to the common cold. *Arch Intern Med* 2009;**169**:62–7.
84. Drahos J, Racaniello VR. Cleavage of IPS-1 in cells infected with human rhinovirus. *J Virol* 2009;**83**:11581–7.
86. Du Prel JB, Puppe W, Grondahl B, et al. Are meteorological parameters associated with acute respiratory tract infections? *Clin Infect Dis* 2009;**49**:861–8.

87. Durrani SR, Montville JJ, Pratt AS, et al. Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children. *J Allergy Clin Immunol* 2012;**130**:489–95.
89. Englund JA. Diagnosis and epidemiology of community-acquired respiratory virus infections in the immunocompromised host. *Biol Blood Marrow Transplant* 2001;**7**(Suppl):S2–4.
90. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
94. Fleming HE, Little FF, Schnurr D, et al. Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways. *Am J Respir Crit Care Med* 1999;**160**:100–8.
95. Food and Drug Administration (FDA). *OTC cough and cold products: not for infants and children under 2 years of age*. Available at http://www.fda.gov/forconsumers/consumerupdates/ucm048682. Accessed August 6, 2012.
104. García-García ML, Calvo C, Pozo F, et al. Spectrum of respiratory viruses in children with community acquired pneumonia. *Pediatr Infect Dis J* 2012;**31**:808–13.
111. Gerna G, Piralla A, Rovida F, et al. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. *J Med Virol* 2009;**81**:1498–507.
119. Gutman JA, Peck AJ, Kuypers J, et al. Rhinovirus as a cause of fatal lower respiratory tract infection in adults stem cell transplantation patients: a report of two cases. *Bone Marrow Transplant* 2007;**40**:809–11.
155. Iwane MK, Prill MM, Lu X, et al. Human rhinovirus species associated with hospitalization for acute respiratory illness in young US children. *J Infect Dis* 2011;**204**:1702–10.
156. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;**178**:667–72.
157. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Resp Crit Care Med* 2012;**185**:281–5.
162. Jartti T, Lehtinen P, Vanto T, et al. Efficacy of prednisolone in children hospitalized for recurrent wheezing. *Pediatr Allergy Immunol* 2007;**18**:326–34.
166. Jornot L, Cordey S, Caruso A, et al. T lymphocytes promote the antiviral and inflammatory responses of airway epithelial cells. *PLoS One* 2011;**6**:e26293.
167. Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 2000;**19**:293–8.
175. Kim WK, Gern JE. Updates in the relationship between human rhinovirus and asthma. *Allergy Asthma Immunol Res* 2012;**4**:116–21.
176. King AMQ, Adams MJ, Carstens EB, et al. *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*. Waltham, Mass.: Elsevier Academic Press; 2012.
178. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, et al. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? *J Allergy Clin Immunol* 2003;**111**:66–71.
180. Kusel MM, de Klerk NH, Holt PG, et al. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006;**25**:680–6.
182. Lamson D, Renwick N, Kapor V, et al. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York state during 2004-2005. *J Infect Dis* 2006;**194**:1398–402.
185. Lehtinen P, Ruohola A, Vanto T, et al. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. *J Allergy Clin Immunol* 2007;**119**:570–5.
192. Lu X, Holloway B, Dare RK, et al. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. *J Clin Microbiol* 2008;**46**:533–9.
193. Mackay IM, Lambert SB, Faux CE, et al. Community-wide, contemporaneous circulation of a broad spectrum of human rhinoviruses in healthy Australian preschool-aged children during a 12-month period. *J Infect Dis* 2013;**207**:1433–41
196. Mansbach JM, Piedra PA, Teach SJ, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med* 2012;**166**:700–6.
197. Marguet C, Lubrano M, Gueudin M, et al. In very young infants severity of acute bronchiolitis depends on carried viruses. *PLoS One* 2009;**4**:34596.
198. Martin ET, Kuypers J, Wald A, et al. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respi Viruses* 2012;**6**:71–7.
201. McIntyre CL, McWilliam Leitch EC, Savolainen-Kopra C, et al. Analysis of genetic diversity and sites of recombination in human rhinovirus species C. *J Virol* 2010;**84**:10297–310.
205. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;**115**:2088–94.
223. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science* 2009;**324**:55–9.
228. Peltola V, Waris M, Osterback R, et al. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis* 2008;**197**:382–9.
232. Pierce VM, Elkan M, Leet M, et al. Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. *J Clin Microbiol* 2012;**50**:364–71.
243. Racaniello VR. *Picornaviridae:* the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, et al, editors. *Fields virology*, vol. 1. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 795–838.
258. Simmonds P, McIntyre C, Savolainen-Kopra C, et al. Proposals for the classification of human rhinovirus species C into genotypically assigned types. *J Gen Virol* 2010;**91**:2409–19.
259. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol* 2010;**82**:1282–90.
264. Slater L, Bartlett NW, Haas JJ, et al. Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium. *PLoS Pathog* 2010;**6**:e1001178.
265. Stempel H, Martin ET, Kuypers J, et al. Multiple viral respiratory pathogens in children with bronchiolitis. *Acta Paediatr* 2009;**98**:123–6.
271. Tsolia MN, Psarra S, Bossios A, et al. Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 2004;**39**:681–6.
284. Uekert S, Akan G, Evans MD, et al. Sex-related differences in immune development and the expression of atopy in early childhood. *J Allergy Clin Immunol* 2006;**118**:1375–81.
287. van der Zalm MM, Wilbrink B, van Ewijk BE, et al. Highly frequent infections with human rhinovirus in healthy young children: a longitudinal cohort study. *J Clin Virol* 2011;**52**:317–20.
298. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. *J Med Virol* 2006;**78**:644–50.
299. Winther B, McCue K, Ashe K, et al. Rhinovirus contamination of surfaces in homes of adults with natural colds: transfer of virus to fingertips during normal daily activities. *J Med Virol* 2011;**83**:906–11.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abraham G, Colonno RJ. Many rhinovirus serotypes share the same cellular receptor. *J Virol* 1984;**51**:3–345.
2. Alper CM, Doyle WJ, Skoner DP, et al. Pre-challenge antibodies moderate infection rate, signs, and symptoms in adults experimentally challenged with rhinovirus type 39. *Laryngoscope* 1996;**106**: 1298–305.
3. Alper CM, Doyle WJ, Skoner DP, et al. Pre-challenge antibodies moderate disease expression in adults experimentally exposed to rhinovirus strain Hanks. *Clin Infect Dis* 1998;**27**:119–28.
4. Andeweg AC, Bestebroer TM, Huybreghs M, et al. Improved detection of rhinoviruses in clinical samples by using a newly developed nested reverse transcription-PCR assay. *J Clin Microbiol* 1999;**37**:524–30.
5. Andrewes CH, Chaproniere DM, Gompels AEH, et al. Propagation of common-cold virus in tissue cultures. *Lancet* 1953;**2**: 546–7.
6. Andrewes CH, Lovelock JE, Sommerville T. An experiment on the transmission of colds. *Lancet* 1951;**1**:25–7.
7. Andries K, Dewindt B, Snoeks J, et al. Two groups of rhinoviruses revealed by a panel of antiviral compounds present sequence divergence and differential pathogenicity. *J Virol* 1990;**64**:1117–23.
8. Andries K, Dewindt B, Snoeks J, et al. A comparative test of fifteen compounds against all known human rhinovirus serotypes as a basis for a more rational screening program. *Antiviral Res* 1991;**16**:213–25.
9. Arden KE, McErlean P, Nissen MD, et al. Frequent detection of human rhinoviruses, paramyxoviruses, coronaviruses, and bocavirus during acute respiratory tract infections. *J Med Virol* 2006;**78**: 1232–40.
10. Arnold E, Rossmann MG. Analysis of the structure of a common cold virus, human rhinovirus 14, refined at a resolution of 3.0 Å. *J Mol Biol* 1990;**211**:763–801.
11. Arola M, Ruuskanen O, Ziegler T, et al. Clinical role of respiratory virus infection in acute otitis media. *Pediatrics* 1990;**86**: 848–55.
12. Arola M, Ziegler T, Puhakka H, et al. Rhinovirus in otitis media with effusion. *Ann Otol Rhinol Laryngol* 1990;**99**:451–3.
13. Arola M, Ziegler T, Ruuskanen O, et al. Rhinovirus in acute otitis media. *J Pediatr* 1988;**113**:693–5.
14. Arola M, Ziegler T, Ruuskanen O. Respiratory virus infection as a cause of prolonged symptoms in acute otitis media. *J Pediatr* 1990;**116**:697–701.
15. Arruda E, Boyle TR, Winther B, et al. Localization of human rhinovirus replication in the tract by in situ hybridization. *J Infect Dis* 1995;**171**:1329–33.
16. Arruda E, Crump CE, Rollins BS, et al. Comparative susceptibilities of human embryonic fibroblasts and HeLa cells for isolation of human rhinoviruses. *J Clin Microbiol* 1996;**34**:1277–9.
17. Arruda E, Hayden FG. Clinical studies of antiviral agents for picornaviral infections. In: Jeffries DJ, de Clerq E, editors. *Antiviral chemotherapy*. New York: John Wiley & Sons; 1995. p. 321–55.
18. Arruda E, Pitkaranta A, Witek TJ Jr, et al. Frequency and natural history of rhinovirus infections in adults during autumn. *J Clin Microbiol* 1997;**35**:2864–8.
19. Atmar RL, Guy E, Guntupalli KK, et al. Respiratory tract viral infections in inner-city asthmatic adults. *Arch Intern Med* 1998;**158**:2453–9.
20. Atmar RL, Piedra PA, Patel SM, et al. Picornavirus, the most common respiratory virus causing infection among patients of all ages hospitalized with acute respiratory illness. *J Clin Microbiol* 2012;**50**:506–8.
21. Bangham CR, McMichael AJ. Nosing ahead in the cold war. *Nature* 1990;**344**:16.
22. Barbady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast assay and the Idaho Technology FilmArray RP Assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J Clin Microbiol* 2012;**50**:2282–8.
23. Bardin PG, Johnston SL, Sanderson G, et al. Detection of rhinovirus infection of the nasal mucosa by oligonucleotide in situ hybridization. *Am J Respir Cell Mol Biol* 1994;**10**:207–13.
24. Barral PM, Sarkar D, Fisher PB, et al. RIG-I is cleaved during picornavirus infection. *Virology* 2009;**391**:171–6.
25. Bartlett NW, Walton RP, Edwards MR, et al. Mouse models of rhinovirus-induced disease and exacerbation of allergic airway inflammation. *Nat Med* 2008;**14**:199–204.
26. Beem MO. Acute respiratory illness in nursery school children: a longitudinal study of the occurrence of illness and respiratory viruses. *Am J Epidemiol* 1969;**90**:30–44.
27. Blomqvist S, Roivainen M, Puhakka T, et al. Virological and serological analysis of rhinovirus infections during the first two years of life in a cohort of children. *J Med Virol* 2002;**66**:263–8.
28. Blomqvist S, Savolainen C, Raman L, et al. Human rhinovirus 87 and enterovirus 68 represent a unique serotype with rhinovirus and enterovirus features. *J Clin Microbiol* 2002;**40**:4218–23.
29. Boat TF, Carson JL. Ciliary dysmorphology and dysfunction: primary or acquired? *N Engl J Med* 1990;**323**:1700–2.
30. Brand JK, de Groot R, Galama JM, et al. Infection with multiple viruses is not associated with increased disease severity in children with bronchiolitis. *Pediatr Pulmonol* 2012;**47**:393–400.
31. Briese T, Renwick N, Venter M, et al. Global distribution of novel rhinovirus genotype. *Emerg Infect Dis* 2008;**14**:944–7.
32. Buchman CA, Doyle WJ, Skoner D, et al. Otologic manifestations of experimental rhinovirus infection. *Laryngoscope* 1994;**104**: 1295–9.
33. Buscho RF, Perkins JC, Knopf HL, et al. Further characterization of the local respiratory tract antibody response induced by intranasal instillation of inactivated rhinovirus 13 vaccine. *J Immunol* 1972;**108**:169–77.
34. Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet* 2010;**276**: 826–34.
35. Busse WW, Vrtis RF, Dick EC. The role of viral infections in intrinsic asthma: activation of neutrophil inflammation. *Agents Actions Suppl* 1989;**28**:41–56.
36. Butler WT, Waldmann TA, Rossen RD, et al. Changes in IgA and IgG concentrations in nasal secretions prior to the appearance of antibody during viral respiratory infection in man. *J Immunol* 1970;**105**:584–91.
37. Bynoe ML, Hobson D, Horner J, et al. Inoculation of human volunteers with a strain of virus isolated from a common cold. *Lancet* 1961;**1**:1194–6.
38. Calvo C, Pozo F, García-García ML, et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Arch Paediatr* 2010;**99**:883–7.
39. Carson JL, Collier AM, Hu SS. Acquired ciliary defects in nasal epithelium of children with acute viral upper respiratory infections. *N Engl J Med* 1985;**312**:463–8.
40. Cate TR. Self-control of the common cold? *Ann Intern Med* 1978;**88**:569–70.
41. Cate TR, Couch RB, Fleet WF, et al. Production of tracheobronchitis in volunteers with rhinovirus in a small-particle aerosol. *Am J Epidemiol* 1965;**81**:95–105.
42. Cate TR, Couch RB, Johnson KM. Studies with rhinoviruses in volunteers: production of illness, effect of naturally acquired antibody, and demonstration of a protective effect not associated with serum antibody. *J Clin Invest* 1964;**43**:56–67.
43. Cate TR, Rossen RD, Douglas RG Jr, et al. The role of nasal secretion and serum antibody in the rhinovirus common cold. *Am J Epidemiol* 1966;**84**:352–63.
44. Chalmers TC. Effects of ascorbic acid on the common cold: an evaluation of the evidence. *Am J Med* 1975;**58**:532–6.
45. Chantzi FM, Papadopoulos NG, Bairamis T, et al. Human rhinoviruses in otitis media with effusion. *Pediatr Allergy Immunol* 2006;**17**:514–18.
46. Che Z, Olson NH, Leippe D, et al. Antibody-mediated neutralization of human rhinovirus 14 explored by means of cryoelectron microscopy and X-ray crystallography of virus-Fab complexes. *J Virol* 1998;**72**:4610–22.
47. Chonmaitree T, Owen MJ, Howie VM. Respiratory viruses interfere with bacteriologic response to antibiotic in children with acute otitis media. *J Infect Dis* 1990;**162**:546–9.
48. Chow AW, Benninger MS, Brook I, et al. IDSA clinical practice guideline for acute bacterial rhinosinusitis in children and adults. *Clin Infect Dis* 2012;**54**:e72–e112.
49. Clinical practice guideline: management of sinusitis. *Pediatrics* 2001;**108**:798–808.
50. Cohen S, Doyle WJ, Alper CM, et al. Sleep habits and susceptibility to the common cold. *Arch Intern Med* 2009;**169**:62–7.

51. Cohen S, Doyle WJ, Skoner DP, et al. Social ties and susceptibility to the common cold. *JAMA* 1997;**277**:1940–4.
52. Cohen S, Frank E, Doyle WJ, et al. Types of stressors that increase susceptibility to the common cold in healthy adults. *Health Psychol* 1998;**17**:214–23.
53. Cohen S, Tyrrell DA, Smith AP. Psychological stress and susceptibility to the common cold. *N Engl J Med* 1991;**325**:606–12.
54. Commission on Acute Respiratory Diseases. Experimental transmission of minor respiratory illness to human volunteers by filter-passing agents, part I: Demonstration of two types of illness characterized by long and short incubation periods and different clinical features. *J Clin Invest* 1947;**26**:957–73.
55. Conant RM, Hamparian VV. Rhinoviruses: basis for a numbering system. 1. HeLa cells for propagation and serologic procedures. *J Immunol* 1968;**100**:107–13.
56. Conant RM, Hamparian VV, Stott EJ, et al. Identification of rhinovirus strain D.C. *Nature* 1968;**217**:1264.
57. Cooney MK, Fox JP, Kenny GE. Antigenic groupings of 90 rhinovirus serotypes. *Infect Immun* 1982;**37**:642–7.
58. Cooney MK, Kenny GE. Demonstration of dual rhinovirus infection in humans by isolation of different serotypes in human heteroploid (HeLa) and human diploid fibroblast cell cultures. *J Clin Microbiol* 1977;**5**:202–7.
59. Couch RB. Personal communication. 1978.
60. Couch RB. The common cold: control? *J Infect Dis* 1984;**150**: 167–73.
61. Couch RB. Rhinoviruses. In: Knipe DM, Howley PM, Griffin DE, et al, editors. *Fields virology*, **vol.**. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 777–97.
62. Couch RB, Atmar RL. Rhinoviruses. In: Lennette EH, Smith TF, editors. *Laboratory diagnosis of viral infection*. 3rd ed. New York: Marcel Dekker; 1999. p. 787–802.
63. Couch RB, Douglas RG Jr, Lindgren KM, et al. Airborne transmission of respiratory infection with coxsackievirus A type 21. *Am J Epidemiol* 1970;**91**:78–86.
64. D'Alessio DJ, Meschievitz CK, Peterson JA, et al. Short-duration exposure and the transmission of rhinoviral colds. *J Infect Dis* 1984;**150**:189–94.
65. D'Alessio DJ, Peterson JA, Dick CR, et al. Transmission of experimental rhinovirus colds in volunteer married couples. *J Infect Dis* 1976;**133**:28–36.
66. Deszcz L, Gaudernak E, Kuechler E, et al. Apoptotic events induced by human rhinovirus infection. *J Gen Virol* 2005;**86**: 1379–89.
67. Dick EC. Chimpanzee kidney tissue cultures for growth and isolation of viruses. *J Bacteriol* 1963;**86**:573–6.
68. Dick EC, Blumer CR, Evans AS. Epidemiology of infections with rhinovirus types 43 and 55 in a group of university of Wisconsin student families. *Am J Epidemiol* 1967;**86**:386–400.
69. Dick EC, Dick CR. A subclinical outbreak of human rhinovirus 31 infection in chimpanzees. *Am J Epidemiol* 1968;**88**:267–72.
70. Dick EC, Dick CR. Natural and experimental infections of non-human primates with respiratory viruses. *Lab Anim Sci* 1974;**24**: 177–81.
71. Dick EC, Gavinshi SS, Mahl MC, et al. A virucidal handkerchief for helping prevent transmission of respiratory infection at McMurdo Station and Scott Base during the winter fly-in period. *Antarctic J U S* 1979;**14**:189–90.
72. Dick EC, Hossain SU, Mink KA, et al. Interruption of transmission of rhinovirus colds among human volunteers using virucidal paper handkerchiefs. *J Infect Dis* 1986;**153**:352–6.
73. Dick EC, Jennings LC, Meschievitz CK, et al. Possible modification of the normal winter fly-in respiratory disease outbreak at McMurdo Station. *Antarctic J U S* 1980;**15**:173–4.
74. Dick EC, Jennings LC, Mink KA, et al. Aerosol transmission of rhinovirus colds. *J Infect Dis* 1987;**156**:442–8.
75. Dochez AR, Shibley GS, Mills KC. Studies in the common cold, part IV: Experimental transmission of the common cold to anthropoid apes and human beings by means of a filtrable agent. *J Exp Med* 1930;**52**:701–16.
76. Douglas RG Jr. Pathogenesis of rhinovirus common colds in human volunteers. *Ann Otol Rhinol Laryngol* 1970;**79**:563–71.
77. Douglas RG Jr. The common cold: relief at last? *N Engl J Med* 1986;**314**:114–15.
78. Douglas RG Jr, Cate TR, Gerone PJ, et al. Quantitative rhinovirus shedding patterns in volunteers. *Am Rev Respir Dis* 1966;**94**:159–67.
79. Douglas RG Jr, Couch RB. Parenteral inactivated rhinovirus vaccine: minimal protective effect. *Proc Soc Exp Biol Med* 1972; **139**:899–902.
80. Douglas RG Jr, Lindgren KM, Couch RB. Exposure to cold environment and rhinovirus common cold: failure to demonstrate effect. *N Engl J Med* 1968;**279**:742–7.
81. Douglas RG Jr, Rossen RD, Butler WT, et al. Rhinovirus neutralizing antibody in tears, parotid saliva, nasal secretions and serum. *J Immunol* 1967;**99**:297–303.
82. Douglas RM, Moore BW, Miles HB, et al. Prophylactic efficacy of intranasal alpha 2-interferon against rhinovirus infections in the family setting. *N Engl J Med* 1986;**314**:65–70.
83. Doyle WJ, Gentile DA, Cohen S. Emotional style, nasal cytokines, and illness expression after experimental rhinovirus exposure. *Brain Behav Immun* 2006;**20**:175–81.
84. Drahos J, Racaniello VR. Cleavage of IPS-1 in cells infected with human rhinovirus. *J Virol* 2009;**83**:11581–7.
85. Drews AL, Atmar RL, Glezen WP, et al. Dual respiratory virus infections. *Clin Infect Dis* 1997;**25**:1421–9.
86. Du Prel JB, Puppe W, Grondahl B, et al. Are meteorological parameters associated with acute respiratory tract infections? *Clin Infect Dis* 2009;**49**:861–8.
87. Durrani SR, Montville JJ, Pratt AS, et al. Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children. *J Allergy Clin Immunol* 2012;**130**: 489–95.
88. El Sahly HM, Atmar RL, Glezen WP, et al. Spectrum of clinical illness in hospitalized patients with "common cold" virus infections. *Clin Infect Dis* 2000;**31**:96–100.
89. Englund JA. Diagnosis and epidemiology of community-acquired respiratory virus infections in the immunocompromised host. *Biol Blood Marrow Transplant* 2001;7(Suppl):S2–4.
90. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
91. Farr BM, Hendley JO, Kaiser DL, et al. Two randomized controlled trials of virucidal nasal tissues in the prevention of natural upper respiratory infections. *Am J Epidemiol* 1988;**128**: 1162–72.
92. Fleet WF, Couch RB, Cate TR, et al. Homologous and heterologous resistance to rhinovirus common cold. *Am J Epidemiol* 1965;**82**:185–96.
93. Fleischer R, Laessig K. Safety and efficacy evaluation of pleconaril for treatment of the common cold. *Clin Infect Dis* 2003;**37**:1722.
94. Fleming HE, Little FF, Schnurr D, et al. Rhinovirus-16 colds in healthy and in asthmatic subjects: similar changes in upper and lower airways. *Am J Respir Crit Care Med* 1999;**160**:100–8.
95. Food and Drug Administration (FDA). *OTC cough and cold products: not for infants and children under 2 years of age*. Available at http://www.fda.gov/forconsumers/consumerupdates/ucm048682. Accessed August 6, 2012.
96. Forstall GJ, Macknin ML, Yen-Lieberman BR, et al. Effect of inhaling heated vapor on symptoms of the common cold. *JAMA* 1994;**271**:1109–11.
97. Fox JP. Is a rhinovirus vaccine possible? *Am J Epidemiol* 1976;**103**:345–54.
98. Fox JP, Cooney MK, Hall CE. The Seattle virus watch, part V. Epidemiologic observations of rhinovirus infections, 1965-1969, in families with young children. *Am J Epidemiol* 1975;**101**:122–43.
99. Fox JP, Cooney MK, Hall CE, et al. Rhinoviruses in Seattle families, 1975-1979. *Am J Epidemiol* 1985;**122**:830–46.
100. Fox MP, Otto MJ, McKinlay MA. Prevention of rhinovirus and poliovirus uncoating by WIN 51711, a new antiviral drug. *Antimicrob Agents Chemother* 1986;**30**:110–16.
101. Foy HM, Fox JP, Cooney MK. Efficacy of alpha 2-interferon against the common cold. *N Engl J Med* 1986;**315**:513–14.
102. Fraenkel DJ, Bardin PG, Sanderson G, et al. Lower airways inflammation during rhinovirus colds in normal and in asthmatic subjects. *Am J Respir Crit Care Med* 1995;**151**:879–86.
103. Gama RE, Horsnell PR, Hughes PJ, et al. Amplification of rhinovirus specific nucleic acids from clinical samples using the polymerase chain reaction. *J Med Virol* 1989;**28**:73–7.
104. García-García ML, Calvo C, Pozo F, et al. Spectrum of respiratory viruses in children with community acquired pneumonia. *Pediatr Infect Dis J* 2012;**31**:808–13.

105. Gentile DA, Yokitis J, Angelini BL, et al. Effect of intranasal challenge with interleukin-6 on upper airway symptomatology and physiology in allergic and nonallergic patients. *Ann Allergy Asthma Immunol* 2001;**86**:531–6.
106. George RB, Mogabgab WJ. Atypical pneumonia in young men with rhinovirus infections. *Ann Intern Med* 1969;**71**:1073–8.
107. Gern JE, Busse WW. Association of rhinovirus infections with asthma. *Clin Microbiol Rev* 1999;**12**:9–18.
108. Gern JE, Dick EC, Kelly EA, et al. Rhinovirus-specific T cells recognize both shared and serotype-restricted viral epitopes. *J Infect Dis* 1997;**175**:1108–14.
109. Gern JE, Galagan DM, Jarjour NN, et al. Detection of rhinovirus RNA in lower airway cells during experimentally induced infection. *Am J Respir Crit Care Med* 1997;**155**:1159–61.
110. Gern JE, Vrtis R, Grindle KA, et al. Relationship of upper and lower airway cytokines to outcome of experimental rhinovirus infection. *Am J Respir Crit Care Med* 2000;**162**:2226–31.
111. Gerna G, Piralla A, Rovida F, et al. Correlation of rhinovirus load in the respiratory tract and clinical symptoms in hospitalized immunocompetent and immunocompromised patients. *J Med Virol* 2009;**81**:1498–507.
112. Ghosh S, Champlin R, Couch R, et al. Rhinovirus infections in myelosuppressed adult blood and marrow transplant recipients. *Clin Infect Dis* 1999;**29**:528–32.
113. Glezen WP, Greenberg SB, Atmar RL, et al. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000;**283**:499–505.
114. Greenberg SB. Viral respiratory infections in elderly patients and patients with chronic obstructive pulmonary disease. *Am J Med* 2002;**112**:28S–32S.
115. Greve JM, Davis G, Meyer AM, et al. The major human rhinovirus receptor is ICAM-1. *Cell* 1989;**56**:839–47.
116. Greve JM, Forte CP, Marlor CW, et al. Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1. *J Virol* 1991;**65**:6015–23.
117. Grunberg K, Timmers MC, Smits HH, et al. Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. *Clin Exp Allergy* 1997;**27**:36–45.
118. Gustafson LM, Proud D, Hendley JO, et al. Oral prednisone therapy in experimental rhinovirus infections. *J Allergy Clin Immunol* 1996;**97**:1009–14.
119. Gutman JA, Peck AJ, Kuypers J, et al. Rhinovirus as a cause of fatal lower respiratory tract infection in adults stem cell transplantation patients: a report of two cases. *Bone Marrow Transplant* 2007;**40**:809–11.
120. Gwaltney JM Jr. Combined antiviral and antimediator treatment of rhinovirus colds. *J Infect Dis* 1992;**166**:776–82.
121. Gwaltney JM Jr. Rhinoviruses. In: Evans AS, Kaslow RA, editors. *Viral infections of humans: epidemiology and control*. 4th ed. New York: Plenum; 1997. p. 815–38.
122. Gwaltney JM Jr, Heinz BA. Rhinovirus. In: Richman DD, Whitley RJ, Hayden FG, editors. *Clinical virology*. 2nd ed. Washington, D.C.: ASM Press; 2002. p. 995–1018.
123. Gwaltney JM Jr, Hendley JO. Rhinovirus transmission: one if by air, two if by hand. *Am J Epidemiol* 1978;**107**:357–61.
124. Gwaltney JM Jr, Hendley JO. Transmission of experimental rhinovirus infection by contaminated surfaces. *Am J Epidemiol* 1982; **116**:828–33.
125. Gwaltney JM Jr, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Ann Intern Med* 1978;**88**:463–7.
126. Gwaltney JM Jr, Phillips CD, Miller RD, et al. Computed tomographic study of the common cold. *N Engl J Med* 1994;**330**: 25–30.
127. Gwaltney JM Jr, Sydnor A Jr, Sande MA. Etiology and antimicrobial treatment of acute sinusitis. *Ann Otol Rhinol Laryngol Suppl* 1981;**90**:68–71.
128. Halonen P, Rocha E, Hierholzer J, et al. Detection of enteroviruses and rhinoviruses in clinical specimens by PCR and liquid-phase hybridization. *J Clin Microbiol* 1995;**33**:648–53.
129. Halperin SA, Eggleston PA, Hendley JO, et al. Pathogenesis of lower respiratory tract symptoms in experimental rhinovirus infection. *Am Rev Respir Dis* 1983;**128**:806–10.
130. Hamory BH, Sande MA, Sydnor A Jr, et al. Etiology and antimicrobial therapy of acute maxillary sinusitis. *J Infect Dis* 1979; **139**:197–202.
131. Hamparian VV, Colonno RJ, Cooney MK, et al. A collaborative report: rhinoviruses—extension of the numbering system from 89 to 100. *Virology* 1987;**159**:191–2.
132. Hamparian VV, Ketler A, Hilleman MR. Recovery of new viruses (coryzaviruses) from cases of common cold in human adults. *Proc Soc Exp Biol Med* 1961;**108**:444–53.
133. Hamparian VV, Leagus MB, Hilleman MR, et al. Epidemiologic investigations of rhinovirus infections. *Proc Soc Exp Biol Med* 1964;**117**:469–76.
134. Harris JR, Racaniello VR. Changes in rhinovirus protein 2C allow efficient replication in mouse cells. *J Virol* 2003;**77**:4773–80.
135. Hastings GZ, Rowlands DJ, Francis MJ. Proliferative responses of T cells primed against human rhinovirus to other rhinovirus serotypes. *J Gen Virol* 1991;**72**:2947–52.
136. Hayden FG, Albrecht JK, Kaiser DL, et al. Prevention of natural colds by contact prophylaxis with intranasal alpha 2-interferon. *N Engl J Med* 1986;**314**:71–5.
137. Hayden FG, Herrington DT, Coats TL, et al. Efficacy and safety of oral pleconaril for treatment of colds due to picornaviruses in adults: results of 2 double-blind, randomized, placebo-controlled trials. *Clin Infect Dis* 2003;**36**:1523–32.
138. Hayden FG, Turner RB, Gwaltney JM, et al. Phase II, randomized, double-blind, placebo-controlled studies of rupintrivir nasal spray 2-percent suspension for prevention and treatment of experimentally induced rhinovirus colds in healthy volunteers. *Antimicrob Agents Chemother* 2003;**47**:3907–16.
139. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961;**25**:585–621.
140. Hemila H, Herman ZS. Vitamin C and the common cold: a retrospective analysis of Chalmers' review. *J Am Coll Nutr* 1995;**14**:116–23.
141. Hendley JO, Gwaltney JM Jr. Mechanisms of transmission of rhinovirus infections. *Epidemiol Rev* 1988;**10**:243–58.
142. Hendley JO, Gwaltney JM Jr, Jordan WS Jr. Rhinovirus infections in an industrial population, part IV. Infections within families of employees during two fall peaks of respiratory illness. *Am J Epidemiol* 1969;**89**:184–96.
143. Hendley JO, Wenzel RP, Gwaltney JM Jr. Transmission of rhinovirus colds by self-inoculation. *N Engl J Med* 1973;**288**:1361–4.
144. Hewat EA, Neumann E, Conway JF, et al. The cellular receptor to human rhinovirus 2 binds around the 5-fold axis and not in the canyon: a structural view. *EMBO J* 2000;**19**:6317–25.
145. Hewson CA, Jardine A, Edwards MR, et al. Toll-like receptor 3 is induced by and mediates antiviral activity against rhinovirus infection of human bronchial epithelial cells. *J Virol* 2005;**79**: 12273–9.
146. Hicks LA, Shepard CW, Britz PH, et al. Two outbreaks of severe respiratory disease in nursing homes associated with rhinovirus. *J Am Geriatr Soc* 2006;**54**:284–9.
147. Higgins PG, Barrow GI, Tyrrell DA. A study of the efficacy of the bradykinin antagonist, NPC 567, in rhinovirus infections in human volunteers. *Antiviral Res* 1990;**14**:339–44.
148. Horn ME, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967-1972. *J Hyg (Lond)* 1975;**74**:157–68.
149. Horn ME, Reed SE, Taylor P. Role of viruses and bacteria in acute wheezy bronchitis in childhood: a study of sputum. *Arch Dis Child* 1979;**54**:587–92.
150. Hsia J, Goldstein AL, Simon GL, et al. Peripheral blood mononuclear cell interleukin-2 and interferon-gamma production, cytotoxicity, and antigen-stimulated blastogenesis during experimental rhinovirus infection. *J Infect Dis* 1990;**162**:591–7.
151. Hyypia T, Puhakka T, Ruuskanen O, et al. Molecular diagnosis of human rhinovirus infections: comparison with virus isolation. *J Clin Microbiol* 1998;**36**:2081–3.
152. Imakita M, Shiraki K, Yutani C, et al. Pneumonia caused by rhinovirus. *Clin Infect Dis* 2000;**30**:611–12.
153. Ireland DC, Kent J, Nicholson KG. Improved detection of rhinoviruses in nasal and throat swabs by semi-nested RT-PCR. *J Med Virol* 1993;**40**:96–101.
154. Ison MG, Hayden FG, Kaiser L, et al. Rhinovirus infections in hematopoietic stem cell transplant recipients with pneumonia. *Clin Infect Dis* 2003;**36**:1139–43.
155. Iwane MK, Prill MM, Lu X, et al. Human rhinovirus species associated with hospitalization for acute respiratory illness in young US children. *J Infect Dis* 2011;**204**:1702–10.

156. Jackson DJ, Gangnon RE, Evans MD, et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am J Respir Crit Care Med* 2008;**178**:667–72.
157. Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Resp Crit Care Med* 2012;**185**:281–5.
158. Jackson GG, Dowling HF, Anderson TO. Neutralization of common cold agents in volunteers by pooled human globulin. *Science* 1958;**128**:27–8.
159. Jackson JL, Peterson C, Lesho E. A meta-analysis of zinc salts lozenges and the common cold. *Arch Intern Med* 1997;**157**: 2373–6.
160. Jacobs JW, Peacock DB, Corner BD, et al. Respiratory syncytial and other viruses associated with respiratory disease in infants. *Lancet* 1971;**1**:871–6.
161. Jacques J, Bouscambert-Duchamp M, Moret H, et al. Association of respiratory picornaviruses with acute bronchiolitis in French infants. *J Clin Virol* 2006;**35**:463–6.
162. Jartti T, Lehtinen P, Vanto T, et al. Efficacy of prednisolone in children hospitalized for recurrent wheezing. *Pediatr Allergy Immunol* 2007;**18**:326–34.
163. Jennings LC, Dick EC, Mink KA, et al. Near disappearance of rhinovirus along a fomite transmission chain. *J Infect Dis* 1988;**158**:888–92.
164. Jennings LC, MacDiarmid RD, Miles JA. A study of acute respiratory disease in the community of Port Chalmers, part I. Illnesses within a group of selected families and the relative incidence of respiratory pathogens in the whole community. *J Hyg (Lond)* 1978;**81**:49–66.
165. Johnston SL, Sanderson G, Pattemore PK, et al. Use of polymerase chain reaction for diagnosis of picornavirus infection in subjects with and without respiratory symptoms. *J Clin Microbiol* 1993;**31**:111–17.
166. Jornot L, Cordey S, Caruso A, et al. T lymphocytes promote the antiviral and inflammatory responses of airway epithelial cells. *PLoS One* 2011;**6**:e26293.
167. Juven T, Mertsola J, Waris M, et al. Etiology of community-acquired pneumonia in 254 hospitalized children. *Pediatr Infect Dis J* 2000;**19**:293–8.
168. Kaiser L, Aubert JD, Pache JC, et al. Chronic rhinoviral infection in lung transplant recipients. *Am J Respir Crit Care Med* 2006;**174**: 1392–9.
169. Kapikian AZ, Conant RM, Hamparian VV, et al. Rhinoviruses: extension of the numbering system. *Virology* 1971;**43**:524–6.
170. Kellner G, Popow-Kraupp T, Binder C, et al. Respiratory tract infections due to different rhinovirus serotypes and the influence of maternal antibodies on the clinical expression of the disease in infants. *J Med Virol* 1991;**35**:267–72.
171. Kellner G, Popow-Kraupp T, Kundi M, et al. Contribution of rhinoviruses to respiratory viral infections in childhood: a prospective study in a mainly hospitalized infant population. *J Med Virol* 1988;**25**:455–69.
172. Kellner G, Popow-Kraupp T, Kundi M, et al. Clinical manifestations of respiratory tract infections due to respiratory syncytial virus and rhinoviruses in hospitalized children. *Acta Paediatr Scand* 1989;**78**:390–4.
173. Ketler A, Hamparian VV, Hilleman MR. Characterization and classification of ECHO 28–rhinovirus-coryzavirus agents. *Proc Soc Exp Biol Med* 1962;**110**:821–31.
174. Kim SS, Smith TJ, Chapman MS, et al. Crystal structure of human rhinovirus serotype 1A (HRV1A). *J Mol Biol* 1989;**210**: 91–111.
175. Kim WK, Gern JE. Updates in the relationship between human rhinovirus and asthma. *Allergy Asthma Immunol Res* 2012;**4**:116–21.
176. King AMQ, Adams MJ, Carstens EB, et al. *Virus taxonomy: ninth report of the International Committee on Taxonomy of Viruses*. Waltham, Mass.: Elsevier Academic Press; 2012.
177. Klein BS, Dollete FR, Yolken RH. The role of respiratory syncytial virus and other viral pathogens in acute otitis media. *J Pediatr* 1982;**101**:16–20.
178. Kotaniemi-Syrjanen A, Vainionpaa R, Reijonen TM, et al. Rhinovirus-induced wheezing in infancy—the first sign of childhood asthma? *J Allergy Clin Immunol* 2003;**111**:66–71.
179. Kruse VW. The causative agent of coughs and colds: from the Hygienic Institute of the University of Leipzig. *Munch Med Wochenschr* 1914;**61**:1547.
180. Kusel MM, de Klerk NH, Holt PG, et al. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006; **25**:680–6.
181. Laine P, Savolainen C, Blomqvist S, et al. Phylogenetic analysis of human rhinovirus capsid protein VP1 and 2A protease coding sequences confirms shared genus-like relationships with human enteroviruses. *J Gen Virol* 2005;**86**:697–706.
182. Lamson D, Renwick N, Kapor V, et al. MassTag polymerase-chain-reaction detection of respiratory pathogens, including a new rhinovirus genotype, that caused influenza-like illness in New York state during 2004-2005. *J Infect Dis* 2006;**194**:1398–402.
183. Larson HE, Reed SE, Tyrrell DA. Isolation of rhinoviruses and coronaviruses from 38 colds in adults. *J Med Virol* 1980;**5**:221–9.
184. Lefkowitz LB Jr, Jackson GG. Dual respiratory infection with parainfluenza and rhinovirus: the pathogenesis of transmitted infection in volunteers. *Am Rev Respir Dis* 1966;**93**:519–28.
185. Lehtinen P, Ruohola A, Vanto T, et al. Prednisolone reduces recurrent wheezing after a first wheezing episode associated with rhinovirus infection or eczema. *J Allergy Clin Immunol* 2007;**119**: 570–5.
186. Lemanske RF Jr, Dick EC, Swenson CA, et al. Rhinovirus upper respiratory infection increases airway hyperreactivity and late asthmatic reactions. *J Clin Invest* 1989;**83**:1–10.
187. Lemanske RF Jr, Jackson DJ, Gangnon RE, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing. *J Allergy Clin Immunol* 2005;**116**:571–7.
188. Levandowski RA, Weaver CW, Jackson GG. Nasal-secretion leukocyte populations determined by flow cytometry during acute rhinovirus infection. *J Med Virol* 1988;**25**:423–32.
189. Longini IM Jr, Monto AS. Efficacy of virucidal nasal tissues in interrupting familial transmission of respiratory agents: a field trial in Tecumseh, Michigan. *Am J Epidemiol* 1988;**128**:639–44.
190. Louie JK, Yagi S, Nelson FA, et al. Rhinovirus outbreak in a long term care facility for elderly persons associated with unusually high mortality. *Clin Infect Dis* 2005;**41**:262–5.
191. Lowenstein SR, Parrino TA. Management of the common cold. *Adv Intern Med* 1987;**32**:207–33.
192. Lu X, Holloway B, Dare RK, et al. Real-time reverse transcription-PCR assay for comprehensive detection of human rhinoviruses. *J Clin Microbiol* 2008;**46**:533–9.
193. Mackay IM, Lambert SB, Faux CE, et al. Community-wide, contemporaneous circulation of a broad spectrum of human rhinoviruses in healthy Australian preschool-aged children during a 12-month period. *J Infect Dis* 2013;**207**:1433–41
194. Macknin ML, Piedmonte M, Calendine C, et al. Zinc gluconate lozenges for treating the common cold in children: a randomized controlled trial. *JAMA* 1998;**279**:1962–7.
195. Makela MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 1998;**36**: 539–42.
196. Mansbach JM, Piedra PA, Teach SJ, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med* 2012;**166**:700–6.
197. Marguet C, Lubrano M, Gueudin M, et al. In very young infants severity of acute bronchiolitis depends on carried viruses. *PLoS One* 2009;**4**:34596.
198. Martin ET, Kuypers J, Wald A, et al. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respi Viruses* 2012;**6**:71–7.
199. Matthews DA, Dragovich PS, Webber SE, et al. Structure-assisted design of mechanism-based irreversible inhibitors of human rhinovirus 3C protease with potent antiviral activity against multiple rhinovirus serotypes. *Proc Natl Acad Sci U S A* 1999;**96**:11000–7.
200. McBride TP, Doyle WJ, Hayden FG, et al. Alterations of the eustachian tube, middle ear, and nose in rhinovirus infection. *Arch Otolaryngol Head Neck Surg* 1989;**115**:1054–9.
201. McIntyre CL, McWilliam Leitch EC, Savolainen-Kopra C, et al. Analysis of genetic diversity and sites of recombination in human rhinovirus species C. *J Virol* 2010;**84**:10297–310.
202. Melchart D, Walther E, Linde K, et al. Echinacea root extracts for the prevention of upper respiratory tract infections: a double-blind, placebo-controlled randomized trial. *Arch Fam Med* 1998; **7**:541–5.
203. Mertsola J, Ziegler T, Ruuskanen O, et al. Recurrent wheezy bronchitis and viral respiratory infections. *Arch Dis Child* 1991; **66**:124–9.

204. Meschievitz CK, Schultz SB, Dick EC. A model for obtaining predictable natural transmission of rhinoviruses in human volunteers. *J Infect Dis* 1984;**150**:195–201.
205. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;**115**:2088–94.
206. Minor TE, Baker JW, Dick EC, et al. Greater frequency of viral respiratory infections in asthmatic children as compared with their nonasthmatic siblings. *J Pediatr* 1974;**85**:472–7.
207. Minor TE, Dick EC, Baker JW, et al. Rhinovirus and influenza type A infections as precipitants of asthma. *Am Rev Respir Dis* 1976;**113**:149–53.
208. Minor TE, Dick EC, DeMeo AN, et al. Viruses as precipitants of asthmatic attacks in children. *JAMA* 1974;**227**:292–8.
209. Minor TE, Dick EC, Peterson JA, et al. Failure of naturally acquired rhinovirus infections to produce temporal immunity to heterologous serotypes. *Infect Immun* 1974;**10**:1192–3.
210. Mogabgab WJ, Holmes BJ, Pollock B. Antigenic relationships of common rhinovirus types from disabling upper respiratory illnesses. *Dev Biol Stand* 1975;**28**:400–11.
211. Monto AS. A community study of respiratory infections in the tropics, part 3. Introduction and transmission of infections within families. *Am J Epidemiol* 1968;**88**:69–79.
212. Monto AS, Bryan ER, Ohmit S. Rhinovirus infections in Tecumseh, Michigan: frequency of illness and number of serotypes. *J Infect Dis* 1987;**156**:43–9.
213. Monto AS, Cavallaro JJ. The Tecumseh study of respiratory illness, part II. Patterns of occurrence of infection with respiratory pathogens, 1965-1969. *Am J Epidemiol* 1971;**94**:280–9.
214. Monto AS, Cavallaro JJ. The Tecumseh study of respiratory illness, part IV. Prevalence of rhinovirus serotypes, 1966-1969. *Am J Epidemiol* 1972;**96**:352–60.
215. Monto AS, Johnson KM. A community study of respiratory infections in the tropics, part II. The spread of six rhinovirus isolates within the community. *Am J Epidemiol* 1968;**88**:55–68.
216. Monto AS, Ullman BM. Acute respiratory illness in an American community: the Tecumseh study. *JAMA* 1974;**227**:164–9.
217. Muether PS, Gwaltney JM Jr. Variant effect of first- and second-generation antihistamines as clues to their mechanism of action on the sneeze reflex in the common cold. *Clin Infect Dis* 2001;**33**:1483–8.
218. Naclerio RM, Proud D, Lichtenstein LM, et al. Kinins are generated during experimental rhinovirus colds. *J Infect Dis* 1988;**157**:133–42.
219. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *B M J* 1993;**307**:982–6.
220. O'Sullivan S, Cormican L, Faul JL, et al. Activated, cytotoxic CD8(+) T lymphocytes contribute to the pathology of asthma death. *Am J Respir Crit Care Med* 2001;**164**:560–4.
221. Pacifico L, Iacobini M, Viola F, et al. Chemokine concentrations in nasal washings of infants with rhinovirus illnesses. *Clin Infect Dis* 2000;**31**:834–8.
222. Pallansch MA, Roos RP. Enteroviruses: polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. In: Knipe DM, Howley PM, Griffin DE, et al, editors. *Fields virology*, **vol. 1**. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 723–6.
223. Palmenberg AC, Spiro D, Kuzmickas R, et al. Sequencing and analyses of all known human rhinovirus genomes reveal structure and evolution. *Science* 2009;**324**:55–9.
224. Papadopoulos NG, Bates PJ, Bardin PG, et al. Rhinoviruses infect the lower airways. *J Infect Dis* 2000;**181**:1875–84.
225. Papadopoulos NG, Sanderson G, Hunter J, et al. Rhinoviruses replicate effectively at lower airway temperatures. *J Med Virol* 1999;**58**:100–4.
226. Patick AK. Rhinovirus chemotherapy. *Antiviral Res* 2006;**71**:391–6.
227. Pelon W, Mogabgab WJ, Phillips IA, et al. A cytopathogenic agent isolated from naval recruits with mild respiratory illnesses. *Proc Soc Exp Biol Med* 1957;**94**:262–7.
228. Peltola V, Waris M, Osterback R, et al. Rhinovirus transmission within families with children: incidence of symptomatic and asymptomatic infections. *J Infect Dis* 2008;**197**:382–9.
229. Perkins JC, Tucker DN, Knopf HL, et al. Comparison of protective effect of neutralizing antibody in serum and nasal secretions in experimental rhinovirus type 13 illness. *Am J Epidemiol* 1969;**90**:519–26.
230. Perkins JC, Tucker DN, Knopf HL, et al. Evidence for protective effect of an inactivated rhinovirus vaccine administered by the nasal route. *Am J Epidemiol* 1969;**90**:319–26.
231. Pevear DC, Fancher MJ, Felock PJ, et al. Conformational change in the floor of the human rhinovirus canyon blocks adsorption to HeLa cell receptors. *J Virol* 1989;**63**:2002–7.
232. Pierce VM, Elkan M, Leet M, et al. Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. *J Clin Microbiol* 2012;**50**:364–71.
233. Pitkaranta A, Arruda E, Malmberg H, et al. Detection of rhinovirus in sinus brushings of patients with acute community-acquired sinusitis by reverse transcription-PCR. *J Clin Microbiol* 1997;**35**:1791–3.
234. Pitkaranta A, Jero J, Arruda E, et al. Polymerase chain reaction–based detection of rhinovirus, respiratory syncytial virus, and coronavirus in otitis media with effusion. *J Pediatr* 1998;**133**:390–4.
235. Pitkaranta A, Starck M, Savolainen S, et al. Rhinovirus RNA in the maxillary sinus epithelium of adult patients with acute sinusitis. *Clin Infect Dis* 2001;**33**:909–11.
236. Prasad AS, Fitzgerald JT, Bao B, et al. Duration of symptoms and plasma cytokine levels in patients with the common cold treated with zinc acetate: A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 2000;**133**:245–52.
237. Price WH, Emerson H, Ibler I, et al. Studies of the JH and 2060 viruses and their relationship to mild upper respiratory disease in humans. *Am J Hyg* 1959;**69**:224–49.
238. Proud D, Gwaltney JM Jr, Hendley JO, et al. Increased levels of interleukin-1 are detected in nasal secretions of volunteers during experimental rhinovirus colds. *J Infect Dis* 1994;**169**:1007–13.
239. Proud D, Naclerio RM, Gwaltney JM, et al. Kinins are generated in nasal secretions during natural rhinovirus colds. *J Infect Dis* 1990;**161**:120–3.
240. Proud D, Reynolds CJ, Lacapra S, et al. Nasal provocation with bradykinin induces symptoms of rhinitis and a sore throat. *Am Rev Respir Dis* 1988;**137**:613–16.
241. Proud D, Sanders SP, Wiehler S. Human rhinovirus infection induces airway epithelial cell production of human beta-defensin 2 both in vitro and in vivo. *J Immunol* 2004;**172**:4637–45.
242. Puhakka T, Makela MJ, Malmstrom K, et al. The common cold: effects of intranasal fluticasone propionate treatment. *J Allergy Clin Immunol* 1998;**101**:726–31.
243. Racaniello VR. *Picornaviridae:* the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, et al, editors. *Fields virology*, vol. 1. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 795–838.
244. Reed SE. An investigation of the possible transmission of rhinovirus colds through indirect contact. *J Hyg (Lond)* 1975;**75**:249–58.
245. Roebuck MO. Rhinoviruses in Britain: 1963-1973. *J Hyg (Lond)* 1976;**76**:137–46.
246. Rosenbaum MJ, De Berry P, Sullivan EJ, et al. Epidemiology of the common cold in military recruits with emphasis on infections by rhinovirus types 1A, 2, and two unclassified rhinoviruses. *Am J Epidemiol* 1971;**93**:183–93.
247. Rossmann MG. The canyon hypothesis: hiding the host cell receptor attachment site on a viral surface from immune surveillance. *J Biol Chem* 1989;**264**:14587–90.
248. Rossmann MG, Arnold E, Erickson JW, et al. Structure of a human common cold virus and functional relationship to other picornaviruses. *Nature* 1985;**317**:145–53.
249. Rossmann MG, Palmenberg AC. Conservation of the putative receptor attachment site in picornaviruses. *Virology* 1988;**164**:373–82.
250. Ruohola A, Heikkinen T, Waris M, et al. Intranasal fluticasone propionate does not prevent acute otitis media during viral upper respiratory infection in children. *J Allergy Clin Immunol* 2000;**106**:467–71.
251. Ruuskanen O, Arola M, Putto-Laurila A, et al. Acute otitis media and respiratory virus infections. *Pediatr Infect Dis J* 1989;**8**:94–9.
252. Samo TC, Greenberg SB, Couch RB, et al. Efficacy and tolerance of intranasally applied recombinant leukocyte A interferon in normal volunteers. *J Infect Dis* 1983;**148**:535–42.
253. Sanders SP, Proud D, Permutt S, et al. Role of nasal nitric oxide in the resolution of experimental rhinovirus infection. *J Allergy Clin Immunol* 2004;**113**:697–702.
254. Savolainen C, Blomqvist S, Hovi T. Human rhinoviruses. *Paediatr Respir Rev* 2003;**4**:91–8.

255. Scheltinga SA, Templeton KE, Beersma MF, et al. Diagnosis of human metapneumovirus and rhinovirus in patients with respiratory tract infections by an internally controlled multiplex real-time RNA PCR. *J Clin Virol* 2005;**33**:306–11.
256. Schieble JH, Lennette EH, Fox VL. Antigenic variation of rhinovirus type 22. *Proc Soc Exp Biol Med* 1970;**133**:329–33.
257. Seemungal TA, Harper-Owen R, Bhowmik A, et al. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. *Eur Respir J* 2000;**16**:677–83.
258. Simmonds P, McIntyre C, Savolainen-Kopra C, et al. Proposals for the classification of human rhinovirus species C into genotypically assigned types. *J Gen Virol* 2010;**91**:2409–19.
259. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections inhospitalized and community control children in Alaska. *J Med Virol* 2010;**82**:1282–90.
260. Smith MB, Feldman W. Over-the-counter cold medications: a critical review of clinical trials between 1950 and 1991. *JAMA* 1993;**269**:2258–63.
261. Smith TJ, Chase ES, Schmidt TJ, et al. Neutralizing antibody to human rhinovirus 14 penetrates the receptor-binding canyon. *Nature* 1996;**383**:350–4.
262. Sperber SJ, Hayden FG. Chemotherapy of rhinovirus colds. *Antimicrob Agents Chemother* 1988;**32**:409–19.
263. Splints don't stop colds: surprising! *Lancet* 1988;**1**:277–8.
264. Slater L, Bartlett NW, Haas JJ, et al. Co-ordinated role of TLR3, RIG-I and MDA5 in the innate response to rhinovirus in bronchial epithelium. *PLoS Pathog* 2010;**6**:e1001178.
265. Stempel H, Martin ET, Kuypers J, et al. Multiple viral respiratory pathogens in children with bronchiolitis. *Acta Paediatr* 2009;**98**:123–6.
266. Stott EJ, Walker M. Antigenic variation among strains of rhinovirus type 51. *Nature* 1969;**224**:1311–12.
267. Sung BS, Chonmaitree T, Broemeling LD, et al. Association of rhinovirus infection with poor bacteriologic outcome of bacterial-viral otitis media. *Clin Infect Dis* 1993;**17**:38–42.
268. Takkouche B, Regueira C, Gestal-Otero JJ. A cohort study of stress and the common cold. *Epidemiology* 2001;**12**:345–9.
269. Taussig LM, Busse WW, Lemen RJ, et al. NHLBI workshop summary: models of infectious airway injury in children. *Am Rev Respir Dis* 1988;**137**:979–84.
270. Taylor-Robinson D, Tyrrell DA. Serotypes of viruses (rhinoviruses) isolated from common colds. *Lancet* 1962;**1**:452–4.
271. Tsolia MN, Psarra S, Bossios A, et al. Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* **39**:681–6, 2004
272. Turner RB. The role of neutrophils in the pathogenesis of rhinovirus infections. *Pediatr Infect Dis J* 1990;**9**:832–5.
273. Turner RB. The treatment of rhinovirus infections: progress and potential. *Antiviral Res* 2001;**49**:1–14.
274. Turner RB, Bauer R, Woelkart K, et al. An evaluation of *Echinacea angustifolia* in experimental rhinovirus infections. *N Engl J Med* 2005;**353**:341–8.
275. Turner RB, Dutko FJ, Goldstein NH, et al. Efficacy of oral WIN 54954 for prophylaxis of experimental rhinovirus infection. *Antimicrob Agents Chemother* 1993;**37**:297–300.
276. Turner RB, Hendley JO. Virucidal hand treatments for prevention of rhinovirus infection. *J Antimicrob Chemother* 2005;**56**:805–7.
277. Turner RB, Hendley JO, Gwaltney JM Jr. Shedding of infected ciliated epithelial cells in rhinovirus colds. *J Infect Dis* 1982;**145**:849–53.
278. Turner RB, Wecker MT, Pohl G, et al. Efficacy of tremacamra, a soluble intercellular adhesion molecule 1, for experimental rhinovirus infection: a randomized clinical trial. *J A M A* 1999;**281**:1797–804.
279. Turner RB, Weingand KW, Yeh CH, et al. Association between interleukin-8 concentration in nasal secretions and severity of symptoms of experimental rhinovirus colds. *Clin Infect Dis* 1998;**26**:840–6.
280. Tuthill TJ, Papadopoulos NG, Jourdan P, et al. Mouse respiratory epithelial cells support efficient replication of human rhinovirus. *J Gen Virol* 2003;**84**:2829–36.
281. Tyrrell D, Barrow I, Arthur J. Local hyperthermia benefits natural and experimental common colds. *BMJ* 1989;**298**:1280–3.
282. Tyrrell DA, Chanock RM. Rhinoviruses: a description. *Science* 1963;**141**:152–3.
283. Tyrrell DA, Parsons R. Some virus isolations from common colds, part III. Cytopathic effects in tissue cultures. *Lancet* 1960;**1**:239–42.
284. Uekert S, Akan G, Evans MD, et al. Sex-related differences in immune development and the expression of atopy in early childhood. *J Allergy Clin Immunol* 2006;**118**:1375–81.
285. Uncapher CR, DeWitt CM, Colonno RJ. The major and minor group receptor families contain all but one human rhinovirus serotype. *Virology* 1991;**180**:814–17.
286. Urquhart GE, Stott EJ. Rhinoviraemia. *BMJ* 1970;**4**:28–30.
287. van der Zalm MM, Wilbrink B, van Ewijk BE, et al. Highly frequent infections with human rhinovirus in healthy young children: a longitudinal cohort study. *J Clin Virol* 2011;**52**:317–20.
288. Verdaguer N, Blaas D, Fita I. Structure of human rhinovirus serotype 2 (HRV2). *J Mol Biol* 2000;**300**:1179–94.
289. Walker DL, Boring WD. Factors influencing host-virus interactions, part III: Further studies on the alteration of Coxsackie virus infection in adult mice by environmental temperature. *J Immunol* 1958;**80**:39–44.
290. Wark PA, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med* 2005;**201**:937–47.
291. Warshauer DM, Dick EC, Mandel AD, et al. Rhinovirus infections in an isolated antarctic station: transmission of the viruses and susceptibility of the population. *Am J Epidemiol* 1989;**129**:319–40.
292. Webster LT, Clow AD. The association of pneumococci, *H. influenzae* and *Streptococcus hemolyticus* with coryza, pharyngitis, and sinusitis in man. *J Exp Med* 1932;**55**:445–53.
293. Wimalasundera SS, Katz DR, Chain BM. Characterization of the T cell response to human rhinovirus in children: implications for understanding the immunopathology of the common cold. *J Infect Dis* 1997;**176**:755–9.
294. Winther B, Brofeldt S, Christensen B, et al. Light and scanning electron microscopy of nasal biopsy material from patients with naturally acquired common colds. *Acta Otolaryngol* 1984;**97**:309–18.
295. Winther B, Farr B, Turner RB, et al. Histopathologic examination and enumeration of polymorphonuclear leukocytes in the nasal mucosa during experimental rhinovirus colds. *Acta Otolaryngol Suppl* 1984;**413**:19–24.
296. Winther B, Gwaltney JM, Hendley JO. Respiratory virus infection of monolayer cultures of human nasal epithelial cells. *Am Rev Respir Dis* 1990;**141**:839–45.
297. Winther B, Gwaltney JM Jr, Mygind N, et al. Sites of rhinovirus recovery after point inoculation of the upper airway. *JAMA* 1986;**256**:1763–7.
298. Winther B, Hayden FG, Hendley JO. Picornavirus infections in children diagnosed by RT-PCR during longitudinal surveillance with weekly sampling: association with symptomatic illness and effect of season. *J Med Virol* 2006;**78**:644–50.
299. Winther B, McCue K, Ashe K, et al. Rhinovirus contamination of surfaces in homes of adults with natural colds: transfer of virus to fingertips during normal daily activities. *J Med Virol* 2011;**83**:906–11.
300. Wulff H, Noble GR, Maynard JE, et al. An outbreak of respiratory infection in children associated with rhinovirus types 16 and 29. *Am J Epidemiol* 1969;**90**:304–11.
301. Xatzipsalti M, Kyrana S, Tsolia M, et al. Rhinovirus viremia in children with respiratory infections. *Am J Respir Crit Care Med* 2005;**172**:1037–40.
302. Yin FH, Lomax NB. Establishment of a mouse model for human rhinovirus infection. *J Gen Virol* 1986;**67**:2335–40.
303. Yoon HJ, Zhu Z, Gwaltney JM Jr, et al. Rhinovirus regulation of IL-1 receptor antagonist in vivo and in vitro: a potential mechanism of symptom resolution. *J Immunol* 1999;**162**:7461–9.
304. Zhao R, Pevear DC, Kremer MJ, et al. Human rhinovirus 3 at 3.0 Å resolution. *Structure* 1996;**4**:1205–20.
305. Zhu Z, Tang W, Gwaltney JM Jr, et al. Rhinovirus stimulation of interleukin-8 in vivo and in vitro: role of NF-kappaB. *Am J Physiol* 1997;**273**:L814–24.
306. Zhu Z, Tang W, Ray A, et al. Rhinovirus stimulation of interleukin-6 in vivo and in vitro: evidence for nuclear factor kappa B–dependent transcriptional activation. *J Clin Invest* 1996;**97**:421–30.

CHAPTER 167

Hepatitis A Virus

Ana María Ríos

HISTORY

Hepatitis A has been recognized as a clinical entity for centuries, and large epidemics of jaundice occurred during military campaigns in ancient and modern times. During the past several decades, epidemiologic and clinical studies defined the infectious nature of the disease, which led to the differentiation of "infectious hepatitis," now designated hepatitis A.[204] Before 1970, attempts to isolate a virus associated with hepatitis A were uniformly unsuccessful, and information concerning the clinical course of infection, the fecal-oral route of transmission, and the efficacy of immunoglobulin in preventing disease was obtained from studies conducted in humans.[204,206,412] In 1973, hepatitis A virus (HAV) was identified by immune electron microscopy in stool samples of patients with hepatitis A.[133] This discovery led to the development of serologic tests that differentiate acute and resolved infections, characterization of the virus, definition of pathogenic events during infection, and further definition of the epidemiology of HAV infection. HAV has been propagated in cell culture,[91,295,395] which has led to the development and licensure of vaccines shown to be highly efficacious in preventing infection and disease in immunized individuals.[64,176]

PROPERTIES

Classification

HAV is a 27-nm, nonenveloped, positive-sense RNA virus belonging to the family *Picornaviridae*. Although HAV initially was classified in the genus *Enterovirus*, nucleotide analysis indicates that HAV is distinct from all other picornaviruses.[225,386,404] In contrast to other enteroviruses, HAV has essentially no nucleotide or amino acid homology, does not have an intestinal replication phase, replicates slowly in cell culture and rarely produces a cytopathic effect, and is relatively resistant to inactivation by heating.[87] For these reasons, HAV has been reclassified in a separate genus designated *Hepatovirus*.[255,404]

Genomic Organization and Genetic Variation

Hepatitis A is composed of an icosahedral capsid that contains a positive-sense, single-stranded RNA genome of approximately 7.5 kb in length with a single open reading frame flanked by 5′ and 3′ untranslated regions (UTR). The 5′ UTR is the most conserved region of the genome and contains an internal ribosome entry site. The open reading frame encodes a polyprotein organized into three functional regions: P1, P2, and P3. P1 is secondarily cleaved into four capsid proteins, VP1 to VP4, whereas P2 and P3 proteins encode nonstructural proteins associated with replication.[225,355,396,404]

HAV exists as a single serotype. The neutralization site appears to be conformational and derived from epitopes located on VP1 and VP3, as identified by neutralizing monoclonal antibodies.[262,292] A high degree of nucleotide conservation exists among geographically diverse human HAV isolates. However, enough genetic diversity exists to define several HAV genotypes and subgenotypes. The entire nucleic acid sequences of several HAV strains have been determined by molecular cloning, and a large number of HAV isolates have been characterized by sequencing of short genome segments.[265]

The genomic regions most commonly used to define HAV genotypes include (1) the C terminus of the VP3 region, (2) The N terminus of the VP1 region, (3) the junction of the VP1 and P2A regions, (4) the VP1-P2B regions and the entire VP1 region (Fig. 167-1).[80,265]

Sequence variation of a 168-nucleotide fragment encompassing the VP1/2A junction had initially defined seven genotypes that differ at least by 15 percent and subtypes that differ by 7 to 7.5 percent.

Six genotypes (I to VI) are now defined based on analysis of the 900 nucleotides of the complete VP1 protein. Genotypes I, II, and III, divided into subtypes A and B, infect humans. The former genotype VII was reclassified within the genotype IIB.[81,265] The other three (IV, V, and VI) appear to be restricted in their primary replication to Old World monkeys.[264,367]

The worldwide geographic distribution of HAV serotypes has been described by many reports.[80,265,308] Genotype I is the most prevalent worldwide, particularly genotype IA, which dominates in North, Central, and South America; China; Japan; Thailand; and Europe.[50,177,184,308] Subgenotype IB is reported mainly from the Mediterranean region and South Africa and is the most prevalent in Turkey.[273] Subgenotype IIIA is the most prevalent in India, Nepal, and Korea.[170,341,410] In low-endemicity areas, such as the United States and Western Europe, subgenotype IA dominates, but all genotypes have been reported.[81,265] Of note, subgenotypes IIA, IIB, and IIIB rarely have been reported.[103]

The analysis of the other genomic regions (i.e., the N terminus of the VP1, VP1-2B regions, the entire VP, and the C terminus of VP3) allows better strain discrimination. Growing sequence databases allow for the identification of the geographic origin of a given subgenotype.

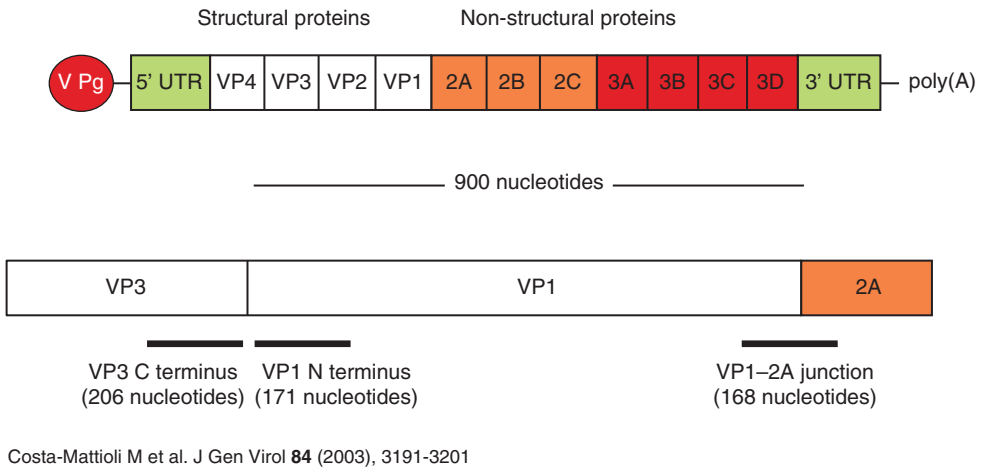

Costa-Mattioli M et al. J Gen Virol **84** (2003), 3191-3201

FIGURE 167-1 ■ Genomic organization of hepatitis A virus (HAV). (See companion Expert Consult website for color version.)

This genetic variation has proved useful in identifying clusters and linking apparently sporadic cases.*

Genotype does not appear to influence the clinical presentation or outcome of infection.[2,144,170] However, two recent publications from Korea proposed that genotypes might account for differences in disease severity. A publication about a nationwide hepatitis A outbreak in Korea from 2006 to 2008 suggested that genotype IIIA infection, in contrast to genotype IA infection, might show more severe clinical manifestations.[410] Similarly, a separate study from Korea compared disease severity related to genotype in adolescents and young adults. Genotype IIIA was found to be associated with higher alanine aminotransferase (ALT) levels and longer hospitalization in a multivariate logistic regression model.[407]

HAV replicates more slowly in cell culture than other picornaviruses; wild-type virus requires many weeks of adaptation before infectious foci or HAV antigen is detected.[34,224,333] HAV produces a high ratio of defective to complete (infectious) virus both in cell culture and during infection.[44,404] Cell culture adaptation is associated with mutations in nonstructural proteins and the 5′ nontranslated region.[120,122] Mutations in the VP1/2A and 2C genes are associated with attenuation, and virulence can be restored if genes from the wild-type virus are reintroduced.[121] Cell culture–adapted HAV rarely produces a cytopathic effect, although cytopathic strains have been isolated and serve as a useful model for laboratory studies.[88,267] Adaptation also results in loss of virulence (attenuation) when it is evaluated in the chimpanzee model of infectivity.[187] Cell culture systems that are more permissive to replication of virulent wild-type virus have been reported.[132,201]

The HAV virion appears to be extremely stable, although the molecular determinants of this characteristic are not known. HAV is stable in the environment, with only a 100-fold decline in infectivity when it is stored for longer than 4 weeks at room temperature.[87,244,245,288] The virus retains infectivity when it is treated with nonionic detergents, organic solvents, and low pH at 38° C (100.4° F) for 90 minutes.[87,254] HAV is more resistant than poliovirus to heat in that it is inactivated only partially at 60° C (140° F) for 1 hour.[87] Temperatures of 85° to 95° C (185° to 203° F) for 1 minute are required for complete inactivation of HAV in foods such as shellfish.[87,255] HAV is inactivated completely by formalin (0.02% at 37° C [98.6° F] for 72 hours) but appears to be relatively resistant to free chlorine, especially when the virus is associated with organic matter.[233,331] For general-purpose disinfection, a 1:100 dilution of 5 percent sodium hypochlorite (i.e., household bleach) in tap water inactivates HAV in most situations.[64,129]

*References 10, 101, 167, 172, 263, 306.

EPIDEMIOLOGY

Routes of Transmission

Routes of HAV transmission are determined by the timing and location of virus replication, circulation, and excretion during infection. HAV replicates in the liver, is excreted in bile, and is found in highest concentration in stool (up to 10^8 infectious particles per milliliter).[355] The highest concentration in stool occurs during the 2-week period before jaundice develops or liver enzymes become elevated, followed by a rapid decline after jaundice appears (Fig. 167-2).[133,338,355] Children and infants may shed HAV for longer periods than do adults. Through the use of polymerase chain reaction (PCR) to amplify viral nucleic acid, HAV RNA has been detected in the stool of infected neonates for as long as 6 months after infection, and some studies have shown excretion in older children and adults 1 to 3 months after the onset of clinical illness.[176,310,409] Chronic shedding of HAV does not occur; however, virus may be present in stool during relapsing illness (see the section on relapsing hepatitis A).[337] Viremia occurs during the prodromal stage of infection and extends through the period of liver enzyme elevation (see Fig. 167-2), with virus concentrations several orders of magnitude lower than those in stool.[43,77,205,225] In experimentally infected animals, HAV can be detected in saliva during periods of peak excretion

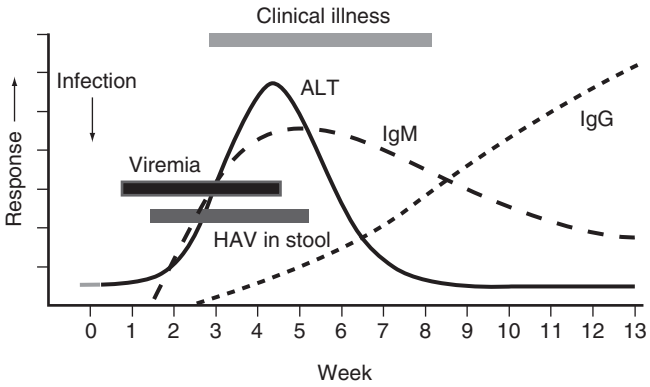

FIGURE 167-2 ■ Immunologic, virologic, and biochemical events during the course of a typical hepatitis A virus *(HAV)* infection. *ALT,* Alanine aminotransferase; *IgG,* immunoglobulin G; *IgM,* immunoglobulin M.

in stool.[77] HAV also has been detected in the saliva of infected patients with concurrent HAV viremia through HAV RNA with identical serum and saliva sequences.[231] The simplicity of obtaining saliva allows the recommendation of the use of saliva for investigation of outbreaks.[6,7] The saliva as an extrahepatic site of early HAV replication could create a potential risk for saliva-transmitted infection.[5]

Detection of HAV antigen in stool by enzyme immunoassay or detection of HAV RNA in serum or stool by PCR does not demonstrate that an infected person is infectious, because these assays may detect defective as well as infectious viral particles. Nucleic acid amplification by immunocapture PCR requires the presence of intact virus,[46,187] and HAV RNA may be detected in stool for months with immunocapture PCR. However, the period of infectivity appears to be shorter than the period when HAV RNA can be detected in stool. Data from epidemiologic studies suggest that peak infectivity occurs during the 2 weeks before the onset of symptoms. For practical purposes, both children and adults with hepatitis A can be assumed to be noninfectious 1 week after jaundice appears.

Because of the high concentration of virus in the stool of infected persons, HAV transmission occurs primarily by the fecal-oral route, usually by person-to-person transmission in households and extended-family settings and between sexual contacts.[344] Person-to-person transmission results in high rates of infection in young children in developing countries and has been the predominant mode of transmission in the United States, particularly during community-wide outbreaks, as well as in outbreaks in child care centers.[25,38] HAV can remain infectious in the environment,[245] and fecal contamination of food or water can result in common-source outbreaks. HAV has been transmitted by transfusion, but such transmission occurs rarely because the blood donation must occur during the early prodromal stage of the disease or from an asymptomatic person who is viremic.[221] In the United States, nucleic acid amplification tests such as PCR now are applied to screening of source plasma used for the manufacture of plasma-derived products. These assays are sufficiently sensitive to remove most units that have HAV, and serosurveillance for HAV infections among clotting factor recipients in the United States indicates that HAV infections are now rare in this risk group.[60]

Two published case reports have described intrauterine transmission of HAV during the first trimester that resulted in fetal meconium peritonitis.[218,246] After delivery, both infants were found to have a perforated ileum. The risk for transmission to newborns by pregnant women in whom hepatitis A develops in the third trimester of pregnancy appears to be low.[259,319,362] Newborns who acquire HAV infection in this manner or from a transfusion usually are asymptomatic, and the infection is detected by the development of hepatitis A in hospital staff or other persons having contact with the infant.[310,389] A couple case reports have described vertical HAV transmission associated with jaundice and raised liver enzymes during the first weeks of life with uneventful improvement.[123,303] HAV RNA was detected in breast milk of two of three women with acute HAV infection in whom anti-HAV immunoglobulin (Ig)M and IgG were detected in serum and breast milk, as well as HAV RNA in serum. Transmission by breast milk has not been demonstrated.[97]

Patterns of Disease Worldwide

Worldwide, the endemicity of HAV infection differs markedly among and within countries (Fig. 167-3).[182]

Patterns of HAV infection can be differentiated, each being characterized by distinct age-specific profiles of prevalence of antibody to HAV (anti-HAV), incidence of hepatitis A, and prevailing environmental (hygienic and sanitary) and socioeconomic conditions (Fig. 167-4).[23,25,157,181,182]

In areas with a high endemic pattern of infection, represented by the least developed countries (i.e., parts of Africa, Asia, and Central and South America), poor socioeconomic conditions allow HAV to spread readily (see Fig. 167-3). Most persons are infected as young children, and essentially the entire population is infected before reaching adolescence, as demonstrated by the age-specific prevalence of anti-HAV (see Fig. 167-4).[22,83,181,325,368] Because virtually all HAV infections occur in age groups in which asymptomatic infection predominates, reported disease rates may be low and outbreaks rare.

In areas of intermediate endemicity, HAV is not transmitted as readily because of better sanitary and living conditions, and the predominant age at infection is older than that in highly endemic areas (see Figs. 167-3 and 167-4).[73,181] Paradoxically, the overall incidence and average age of reported cases often increase because high levels of virus circulate in a population that includes many susceptible older children, adolescents, and young adults, in whom symptoms are likely to develop with HAV infection.[155] Large common-source outbreaks also can occur because of the relatively high rate of virus transmission and the large number of susceptible persons, especially among those of higher socioeconomic levels. Such an outbreak occurred in Shanghai in 1988, with more than 300,000 cases associated with the consumption of

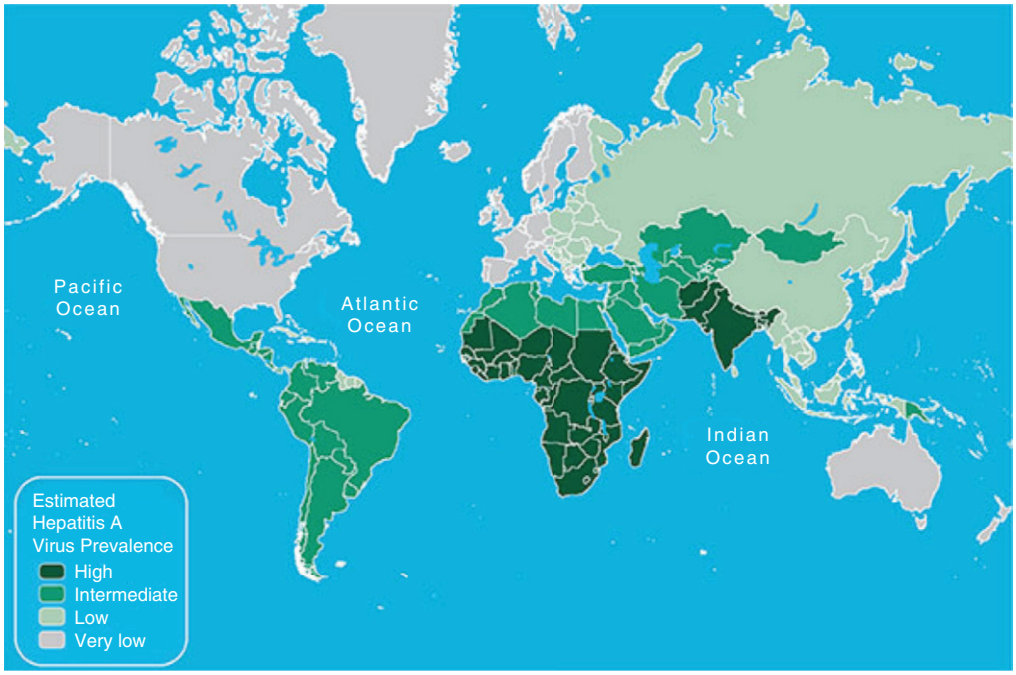

FIGURE 167-3 ■ Estimated geographic distribution of patterns of endemicity of hepatitis A virus (HAV) infection. The distribution was generalized from the best available data. (See companion Expert Consult website for color version.)

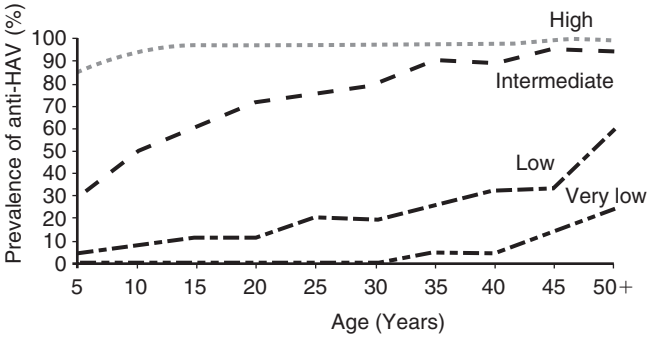

FIGURE 167-4 ■ Patterns of hepatitis A virus *(HAV)* infection worldwide.

contaminated clams.[160] Nonetheless, person-to-person transmission in community-wide epidemics continues to account for much of the disease in these countries.[382]

Considerable hepatitis A–related morbidity and associated costs can occur, even in developing countries. Hepatitis A is reported to account for 50 to 60 percent of all acute viral hepatitis cases among children in Pakistan, and 232 children with fulminant hepatic failure secondary to hepatitis A were admitted to one tertiary care referral hospital in Karachi during a 9-year period.[321] Hepatitis A was the etiology of the fulminant hepatitis of two thirds of the children treated at two hospitals in Argentina during a 15-year period; and in one of these hospitals performing liver transplantation, a third of the liver transplantations in children were required because of fulminant hepatitis A.[74] Another small series from Turkey indicated that HAV was the most common identifiable cause of acute liver failure among children, accounting for 26 percent of cases.[18]

In most areas of North America and Western Europe, sanitary and hygienic conditions are such that the endemicity of HAV infection is low (see Figs. 167-3 and 167-4). Relatively fewer children are infected, and disease often occurs in the context of community-wide and child care center outbreaks and occasionally as common-source outbreaks.[28,149,294,324,358] In some regions (e.g., Scandinavia), the endemicity of HAV infection is very low and disease occurs almost exclusively in defined risk groups, such as travelers returning from areas where HAV infection is endemic or illicit drug users.[41]

Some changes have occurred in the epidemiologic trends and patterns of hepatitis A infection around the world related to vaccine A implementation, socioeconomic progress, and improvement in hygiene measures.*

Shifts in age-specific prevalence patterns indicating a transition from high to intermediate endemicity are occurring in many parts of the world (see Fig. 167-4). As this transition occurs, marked variations in hepatitis A epidemiology are seen among countries and within countries and cities, with some areas displaying a pattern typical of high endemicity and others of intermediate endemicity (see Fig. 167-3).[25,155,182]

Systematic review and meta-analysis of published data using IgG anti-HAV seroprevalence data between 1990 and 2005 from different world regions indicated that high-income regions (i.e., Western Europe, Australia, New Zealand, Canada, the United States, Japan, the Republic of Korea, and Singapore) remain as having a very low HAV endemicity levels and a high proportion of susceptible adults. The low-income regions (i.e., sub-Saharan Africa and parts of South Asia) still present high endemicity levels and almost no susceptible adolescents

*References 182, 192, 209, 215, 216, 257, 369.

and adults, and most middle-income regions (i.e., Asia, Latina America, Eastern Europe, and the Middle East) demonstrated a mix of intermediate and low endemicity levels.[182]

Patterns of Disease in the United States

The epidemiology of hepatitis A in the United States has been altered profoundly by the introduction of hepatitis A vaccines, with a significant reduction as a vaccine-preventable infectious disease (Fig. 167-5A). Rates have declined sharply since the mid-1990s. In 2010, 1670 cases were reported to the Centers for Disease Control and Prevention (CDC), representing a 94 percent decline in contrast to rates from 1990 to 1997 and significantly lower than previous incidence nadirs.[68,70] However, national surveillance systems collect data on symptomatic cases, and incidence models indicate that most infections are not symptomatic. One such analysis estimated that an average of 271,000 infections occurred per year during 1980 to 1999—10.4 times the reported number of symptomatic cases.[12] With use of similar modeling methodology, an estimated 17,000 infections occurred in 2010 (see Figure 167-5B).[69]

Variation by Age and Race or Ethnicity

Historically, the highest hepatitis A rates were reported among children 5 to 14 years of age, with approximately one third of cases occurring among children younger than 15 years.[58] Because many young children have unrecognized or asymptomatic infection, they also are likely to represent a major reservoir for HAV transmission. Incidence models indicated that during the 1980s

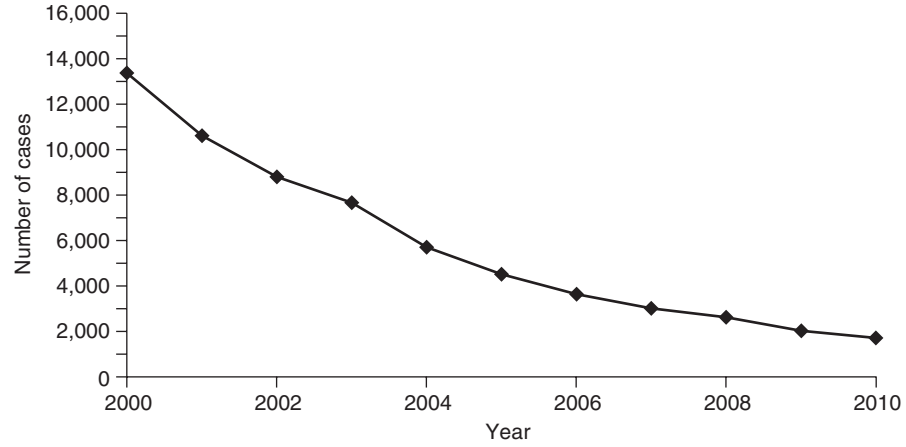

A Source: National Notifiable Diseases Surveillance System (NNDSS)

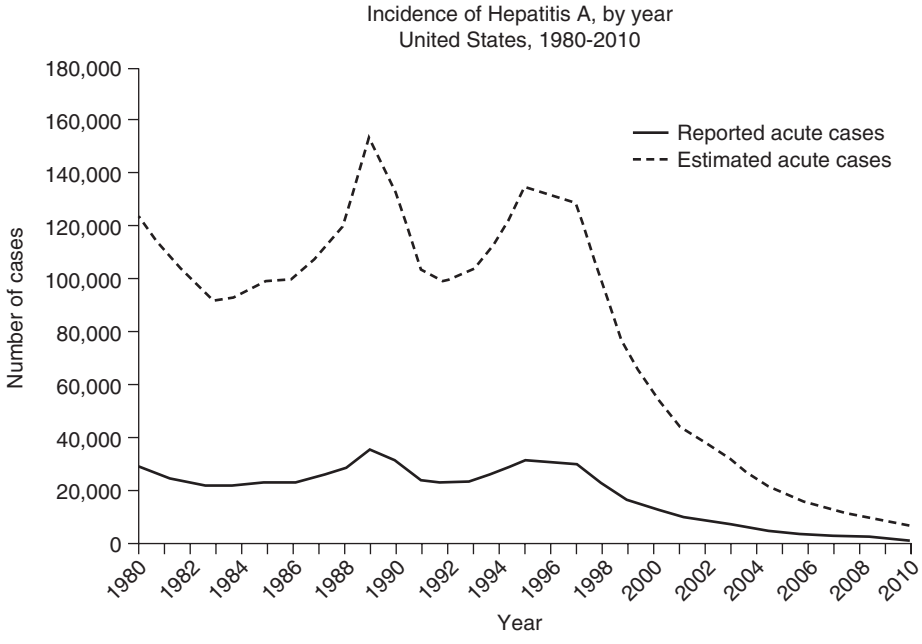

B Source: http://www.cdc.gov/hepatitis/HAV/HAVfaq.htm

FIGURE 167-5 ■ **A,** Reported number of hepatitis A cases, United States, 2000-2010. **B,** Hepatitis A incidence rates (per 100,000 population) based on cases reported to the Centers for Disease Control and Prevention (1980-2010).

and 1990s, more than half of HAV infections occurred among children younger than 10 years of age, most of whom were younger than 4 years.[12] Since the mid-1990s, decreases in rates among children have been greater than among adults, and the proportion of cases among children dropped from 35 percent in the prevaccine era to 19 percent in 2003.[388]

From 2002 to 2010, rates were similar and low among persons in all age groups (<1.0 cases/100,000 population). In 2010, the range was from 0.23 to 0.74 cases/100,000 population.[68,70] The highest rates were for persons 15 to 24 years of age (0.74 cases/100,000 population) and the lowest rates were among children younger than 9 years of age (0.3 cases/100,000 population).[70]

Among racial and ethnic groups, before the use of hepatitis A vaccine, rates among Native Americans and Alaska Natives were more than 10 times the rates in other racial and ethnic groups, and rates among Hispanics were approximately three times higher than among non-Hispanics. These disparities have lessened or disappeared in the era of routine childhood vaccination, with rates among Hispanics in 2003 approximately twice those among non-Hispanics (86% decline in contrast to 1990 to 1997); and rates among Native Americans and Alaska Natives were the same as or lower than those among other ethnic groups.[388] In 2010, the rate for hepatitis A among Hispanics was 0.7 cases/100,000 population, the lowest rate ever reported for this group. Rates among Native Americans and Alaska Natives are now the same as or lower than those among other ethnic groups. The 2010 rate of hepatitis A among Native Americans and Alaskan Natives was the lowest ever recorded (0.2/100,000 population) (Fig. 167-6).[70] Seroprevalence of antibody to HAV (anti-HAV) in the population reflects immunity to either previous infection or vaccination. As determined by the National Health and Nutrition Examination Survey (NHANES); the overall seroprevalence on anti-HAV in United States was 34.9 percent (95% confidence interval [CI], 33.1, 36.7) during 1999 to 2006.[195,197] Seroprevalence among children has increased from 8 percent (6.3% to 10.1%) in 1988 to 1994 to 35.3 percent in 2007 to 2008, which probably reflects increased immunization of children and others.[251] For U.S.-born children 6 to 19 years of age, the strongest factor associated with seroprevalence was residence in vaccinating states. Among U.S.-born adults aged older than 19 years, the overall age-adjusted seroprevalence of anti-HAV was 29.9 percent (95% CI, 28.3, 31.5) during 1999 to 2006, which was not significantly different from the seroprevalence during 1988 to 1994 (32.2%; 95% CI 30.1, 34.4).[195,197] Among U.S.-born adults (40 years of age and older), HAV antibody prevalence decreased from 1988 to 1994 to 2003 to 2006, which may reflect lower rates of infection resulting from reduced exposure to the virus because of higher rates of immunization among younger age groups.[251] The decrease in seroprevalence in the adult population reflects the replacement of adults who were exposed naturally to HAV in the past by an adult population who are neither vaccinated nor previously exposed.[167] For all of the periods, the Mexican-American population had a significantly higher prevalence of HAV antibody in contrast to the non-Hispanic white and non-Hispanic black populations among both children 6 to 19 years of age and adults 20 years of age and older. This is probably a reflection of higher rates of vaccination within this subgroup, but also a continued greater exposure to natural infection from travel to or visitors from Mexico, where the disease is endemic.[251]

Geographic Variation

Analyses of national surveillance data collected during the 1980s and 1990s showed striking regional variation in the incidence of hepatitis A. The national average

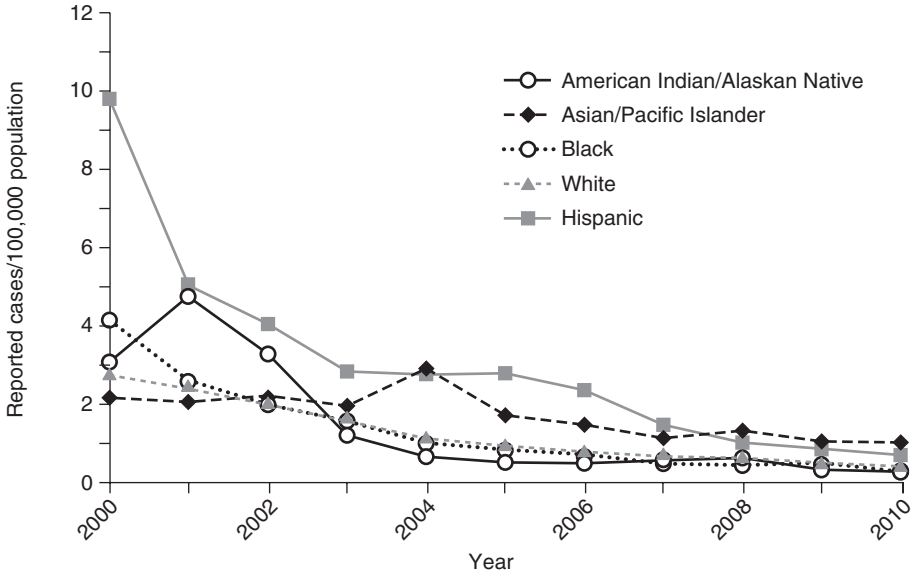

Source: National Notifiable Diseases Surveillance System (NNDSS)

FIGURE 167-6 ■ Hepatitis A incidence, United States, by race and ethnicity, 2000 through 2010. (Data from the Centers for Disease Control and Prevention, National Notifiable Diseases Surveillance System, Atlanta, Ga.)

incidence was approximately 10 cases/100,000 persons per year during 1987 to 1997, with the highest rates and almost all cases consistently occurring in a limited number of states and counties concentrated in the western and southwestern United States.[57] Approximately two thirds of cases were reported from the 17 states with the highest rates, even though only one third of U.S. residents lived in these states. With the implementation of routine vaccination of children in these states in 1999, rates equalized across the country,[388] with significantly greater reductions in incidence in areas where vaccination was recommended.[314,388]

HAV infection rates are now the lowest ever reported and similar in all regions. The rate of acute hepatitis A declined from 1.5 cases/100,000 population to 0.54/100,000 population during 2005 to 2010.[70]

In 2010, the case rate ranged from 0.1 case/100,000 population (Arkansas, Mississippi, and South Dakota) to 1.0 case/100,000 population (Arizona).[68]

Potential Sources of Infection

The distribution of potential HAV exposures among cases for which information regarding exposures is available has changed in the vaccination era. The proportion of cases attributable to household or sexual contact with a person who has hepatitis A, the most commonly reported potential source of infection, has declined as routine hepatitis A vaccination of children has increased, from 15 to 25 percent to 5.6 to 7.3 percent of reported cases in 2009 and 2010.[61,63,67,68] The number of international travel–associated cases has remained approximately the same; but as overall incidence declined, the proportion of cases attributable to this exposure has risen to 14 to 15 percent reported in 2009 to 2010.[67,68] In sites conducting enhanced hepatitis surveillance during 2005 to 2007 as part of the Emerging Infection Program of the CDC; the most frequent (46%) potential source of infection among persons reported with HAV disease was travel outside of the United States and Canada. These cases mostly reflected persons who traveled, but also included some who were exposed to a traveler but did not travel themselves.[196] As in previous years, most travel-related cases (85%) were associated with travel to Mexico, Central America, and South America.[196,325]

Recognized foodborne outbreaks account for a small proportion of cases in most years (3% to 5%), but large outbreaks such as those associated with contaminated green onions in 2003 have increased the proportion of cases associated with food in some years to as high as 16 percent.[10,61,63,393] In 2010, the linkage to an outbreak could be determined in 10 percent of the reported cases for which this information was available.[68]

Cyclic outbreaks occur among men who have sex with men (MSM) and users of injecting and noninjecting drugs; during outbreak years, this exposure can account for 10 to 15 percent of nationally reported cases.*

In 2010, only 5 percent of the case reports with information available about sexual practices indicated sex with another man and only 2 percent reported use of injection drugs.[68] Nearly 50 percent of patients with hepatitis A do not have a recognized source of infection[61,63,67,68] but might be contacts of persons, especially children, with asymptomatic infection.

Community-Wide Epidemic

Historically, most cases of hepatitis A in the United States occurred in the context of community-wide epidemics, during which infection is transmitted from person to person in households and extended-family settings.[28] Once initiated, these epidemics often persisted for several years and proved difficult to control[283,329] even when attempts were made to vaccinate some portion of the population rapidly.[16,85] Children played an important role in HAV transmission during these epidemics. In communities with historically the highest hepatitis A rates, as exemplified by Native American and Alaska Native communities, the pattern of transmission was similar to that found in countries with an intermediate endemicity of hepatitis A infection (see the discussion on patterns of disease worldwide).[49,102,212,328,401] The best explanation for these observations is that asymptomatic transmission was occurring in susceptible young children at low levels during interepidemic periods until a cohort of susceptible children became large enough to sustain a community-wide outbreak.[328,339] Asymptomatic transmission among children also has been important in sustaining outbreaks in areas with consistently elevated rates. During community-wide outbreaks, serologic studies of members of households with an adult case and no identified source found that 25 to 40 percent of contacts younger than 6 years of age had serologic evidence of having had recent HAV infection.[306,344] In one of these studies, 52 percent of households of adults without an identified source of infection included a child younger than 6 years and the presence of a young child was associated with household transmission of HAV.[344] In this study, transmission chains were identified involving as many as six generations and more than 20 cases. With the advent of routine vaccination of children, community-wide outbreaks have largely ceased. Community-wide outbreaks that have been sustained by transmission among adults in high-risk groups continue to be identified, and novel strategies to provide vaccination in settings such as jails have had some success.[61,263,274,359,386]

EPIDEMIOLOGY OF HEPATITIS A IN SPECIFIC SETTINGS

Child Care Centers

The role of children with asymptomatic infection has been recognized in outbreaks in child care centers since the 1970s.[322,323] Because infection in children usually is mild or asymptomatic, these outbreaks often are not recognized until adult contacts (usually parents) become ill.[159] Outbreaks rarely occur in centers that do not have children in diapers and occur more commonly in larger

*References 61, 63, 82, 143, 162, 171, 315.

centers.[159] Both poor hygiene in these children and the need for staff to handle and change diapers contribute to spread. Despite the occurrence of outbreaks when HAV is introduced into a child care center, studies of child care center employees do not show a significantly increased prevalence of HAV infection in contrast to control populations.[140,180] On occasion, outbreaks in child care centers can be the source of more extensive transmission within a community.[104,158,378] However, most disease within child care centers probably reflects transmission from the community. Hepatitis A outbreaks within child care centers have become unusual events in recent years and are likely to be eliminated as routine vaccination of all young children is implemented.

Other Groups and Settings

Hepatitis A cases in school children usually reflect disease that has been acquired in the community. However, multiple cases among children within a school may indicate a common-source outbreak.[172] Historically, HAV infection was endemic in institutions for the developmentally disabled; but with smaller facilities and improved conditions, the incidence and prevalence of infection have decreased, and outbreaks rarely are reported in the United States.[349]

During the past 2 decades, outbreaks have been reported with increasing frequency in illicit drug users in North America, Australia, and Europe.* In the United States, these outbreaks often have occurred in the context of a large community-wide outbreak; in the past decade, they frequently have involved users of injected and noninjected methamphetamine, who may account for as many as 30 percent of reported cases in these communities during outbreaks.[28,171,173,263,386] Cross-sectional serologic surveys have demonstrated that injection drug users have a higher prevalence of anti-HAV than the general U.S. population.[178,384] Transmission among injection drug users probably occurs through both percutaneous and fecal-oral routes.[173]

Hepatitis A outbreaks in MSM have been reported frequently, most recently in urban areas in the United States, Canada, Europe, and Australia.† These outbreaks may occur in the context of an outbreak in the larger community.[28] Some studies conducted during outbreaks and seroprevalence surveys among MSM identified specific sex practices associated with illness, whereas others have not demonstrated such associations.‡

Transfusion-related hepatitis A rarely occurs because HAV does not result in chronic infection and blood donors are screened for elevated aminotransferase levels. Currently, nucleic acid amplification tests also are used to screen source plasma used in the manufacture of plasma-derived products. However, during the mid-1990s, outbreaks were reported in Europe and the United States in patients who received factor VIII and factor IX concentrates prepared by solvent-detergent treatment to inactivate lipid-containing viruses.[236,342] HAV is resistant to solvent-detergent treatment, and contamination presumably occurred from plasma donors with hepatitis A who donated during the incubation period. The risk for acquiring infection in patients with hemophilia is not known, although data from one serologic survey of hemophiliac patients suggested that they might be at increased risk for acquisition of HAV infection.[232] However, no HAV infections attributed to blood products were identified in an analysis of serosurveillance data collected from 140 hemophilia treatment centers in the United States between 1998 and 2002, suggesting that improved viral inactivation procedures, donor screening, and increased hepatitis A vaccine coverage among clotting factor recipients can reduce the risk for transmission of HAV to recipients of clotting factors.[60] Transmission related to blood transfusions also has resulted in nosocomial outbreaks in neonatal intensive care units.[194,272,310] Hepatitis A has been reported in adult cancer patients treated with lymphocytes that apparently were incubated in serum from a donor with HAV infection, although the patient source was not identified.[391]

Nosocomial transmission from adult patients to health care workers usually has been associated with fecal incontinence of the patient.[153,281] Such transmission is a rare event, however, because most patients with hepatitis A are hospitalized after the onset of jaundice, when infectivity is low.[130,338] Health care workers have not been found to have an increased prevalence of anti-HAV in contrast to control populations in serologic surveys conducted in the United States.[148]

Persons from developed countries who travel to developing countries with a high, transitional, or intermediate endemicity of HAV infection are at substantial risk for acquiring hepatitis A (see Fig. 167-3).[182,325,346]

Hepatitis A infection is one of the most common vaccine-preventable infections acquired during travel.[325] A more recent study of Swiss travelers estimated the risk to be 6 to 30 cases/100,000 person-months of stay in developing countries among persons who did not receive immunoglobulin or vaccine before departure.[261] The risk is higher among travelers staying in areas with poor hygienic conditions,[210] it varies according to the region and the length of stay, and it appears to be increased even among travelers who reported staying in urban areas or luxury hotels.[325,346] In some European countries, returning international travelers with hepatitis A account for a substantial proportion of reported cases (16% to 40%).[72,397] In the United States, the proportion of cases attributed to recent international travel has increased from 5 to 15 percent.[67,68]

The potential for new foodborne outbreaks around the world has been enhanced with the world globalization by importation of food products from countries where hepatitis A remains endemic to countries with low endemicity. This has been nicely demonstrated during recent outbreak investigations of hepatitis A infection, including the multistate outbreak associated with semidried tomatoes in Australia during 2009[112] and similar outbreaks in the Netherlands[289,290] and France during 2010.[146]

Foodborne hepatitis A outbreaks are now recognized relatively infrequently in the United States and are

*References 56, 162, 171, 173, 274, 315, 327, 359, 386.
†References 39, 53, 82, 143, 320, 347, 364, 365.
‡References 31, 82, 84,165, 188, 384.

associated most commonly with contamination of food during preparation by a food handler with HAV infection. However, persons who work as food handlers are not at increased risk for acquiring hepatitis A because of their occupation. Food contaminated before retail distribution, such as lettuce or fruits contaminated at the growing or processing stage, has been recognized as the source of hepatitis A outbreaks.* Outbreaks related to contaminated shellfish, commonly noted in the past,[105,107,277,293] have become increasingly uncommon but occasionally are identified.[32] Since the large multistate outbreaks linked to oysters in 2005[32] and the one linked to green onions in 1998 through 2003,[101,393] no large outbreaks have been detected in the United States. No water-borne hepatitis A outbreaks in the United States have been reported since the 1980s; earlier outbreaks were linked to sewage contamination or inadequate treatment of water.†

Control of food outbreaks usually requires intensive public health effort.[94] HAV foodborne disease investigation with direct food testing through HAV RNA molecular techniques has made the recovery of implicated food faster and more efficient, as was demonstrated during the investigation of the large multistate outbreak of hepatitis A caused by consumption of contaminated oysters in the United States in 2005.[32] Molecular epidemiology through sequencing of outbreak strains has been demonstrated to be is an excellent tool for the investigation of HAV infection outbreaks within the United States and European countries.‡

PATHOLOGY AND PATHOGENESIS

Pathology

The light microscopic findings in acute hepatitis A, which include inflammatory cell infiltration, hepatocellular necrosis, and liver cell regeneration, are common to all forms of acute viral hepatitis. These histologic findings vary with the stage and severity of hepatitis. Early biopsy specimens generally show portal infiltration by lymphocytes, plasma cells, and macrophages positive for periodic acid–Schiff.[108,357] Spotty or focal necrosis, as evidenced by balloon degeneration, shrinkage, and fragmentation of hepatocytes, is seen commonly.[108] HAV antigen is found primarily in the cytoplasm of hepatocytes, but it also can be found in liver macrophages. However, because HAV infection is self-limited and does not result in chronic liver disease, liver biopsy rarely is indicated (see the discussion on treatment).

Differences have been noted in light microscopic findings of hepatitis A and other forms of viral hepatitis, particularly hepatitis B. In addition to degeneration of hepatocytes in the perivenular area, periportal inflammation and destruction of hepatocytes adjacent to the portal area may be more pronounced than in hepatitis B.[1,278,357] Findings in some patients, including extension of the inflammatory infiltration from the periportal area into the hepatic parenchyma and disruption of the limiting plate, may be difficult to distinguish from those of chronic hepatitis.[1,108] Cholestasis may be more prominent than in hepatitis B.[316]

The histologic findings in fulminant hepatitis A are indistinguishable from those in other forms of fulminant viral hepatitis. Examination of pathologic specimens shows massive hepatic necrosis, abnormal architecture of surviving hepatocytes, and a diffuse inflammatory response.[199] Viral antigen can be found in pathologic specimens.

Pathogenesis

The mechanism by which HAV crosses gastrointestinal epithelium and infects liver cells has not been completely established. Previous studies have shown that HAV-IgA complex can translocate across epithelial cells and that HAV-IgA complexes are taken up by hepatocytes through the asialoglycoprotein receptor.[113,114] In this model of HAV infection, virus would first infect gastrointestinal cells and then be transported into the enterohepatic circulation as part of an HAV-IgA complex, with subsequent selective uptake by hepatocytes. HAV is able to bind to human cells through a specific cellular receptor inserted into the plasma membrane, and DNA that encodes this receptor (huhavcr-1) has been identified in many human tissues.[131] HAVCR-1 was first discovered as the cellular receptor for HAV (HAVcr-1) in African green monkeys in 1996.[186] This cellular receptor is a member of the T-cell immunoglobulin mucin (TIM, also known as TIM-1) gene family. It appears to regulate the size and effector functions of T cells and also might play a role in the development of atopy.[247] The relationship of TIM1 and atopy with HAV exposure is important, and various epidemiologic studies around the world (in Italy, Denmark, Australia, Korea, and Turkey) have shown lower prevalence of allergy and asthma among the HAV-seropositive individuals in contrast to HAV-seronegative individuals.[242,243] Immunoglobulin (Ig)A is a natural ligand of HAVCR-1, and the association of IgA with HAVCR1 enhances virus receptor activity with synergistic effect in virus-receptor interactions.[353]

HOST INNATE AND ADAPTIVE IMMUNE RESPONSES

Cellular Immune Response

Unlike other picornaviruses, infection of cultured cells with wild-type HAV has no cytopathic effect. The general assumption is that HAV infection also is noncytopathic in vivo and therefore the cytopathic changes in the liver associated with hepatitis A are immune mediated. HAV is able to suppress interferon (INF)-β gene expression in the initial stages of infection, which might facilitate prolonged viral production and infection of neighboring cells.[134] This could be explained in part by a disruption in the signaling through the Toll-like receptor 3 pathway.[240,298] A recent research study demonstrated that

*References 10, 101, 105, 172, 270, 301, 309, 394.
†References 30, 37, 42, 47, 98, 142, 233.
‡References 32, 112, 146, 167, 289, 365.

the interaction between HAV and its receptor HAVCR1 inhibits CD4 T-cell regulatory cell function, resulting in an immune imbalance allowing viral expansion with limited hepatocellular damage during the early phase of infection, which is characteristic of HAV infection.[235] Symptoms and biochemical evidence of liver injury do not occur at the time of maximum virus replication and fecal shedding (during the late incubation period). Rather, liver injury is associated closely with viral clearance. $CD8^+$, class 1–dependent, cytotoxic, and virus-specific T cells that are capable of producing IFN-γ are present in the circulation and the liver.[139,371] Additional inflammatory cells, recruited to the site of infection by IFN-γ and other cytokines secreted by $CD8^+$ cells, may be responsible for much of the liver injury. Complement has been shown to bind to HAV capsid proteins, and serum complement levels drop during infection, but whether complement-mediated cellular injury occurs is unclear.[239] The mechanism by which infection is resolved remains uncertain.

Humoral Immune Response

Unlike the situation with hepatitis C, the adaptive immune response to HAV is robust and extremely effective in eliminating the virus. Neutralizing antibodies to the virus (anti-HAV) generally appear in the serum concurrent with the earliest evidence of serum aminotransferase elevation and hepatocellular injury (see Fig. 167-2).

Antibodies directed against conformational epitopes displayed on intact virions as well as empty viral capsids are produced during the later stages of infection (see the section on genomic organization and genetic variation). Virus-specific IgM and IgG and IgA antibodies are present in serum; IgM anti-HAV generally can be detected at the onset of symptoms (see Fig. 167-2).[240]

CLINICAL MANIFESTATIONS

Similar to other forms of viral hepatitis, the clinical manifestations of HAV infection are variable and range from asymptomatic anicteric infection to symptoms of acute hepatitis, including fever, malaise, anorexia, nausea, vomiting, right upper quadrant pain, and jaundice. The likelihood of having symptomatic HAV infection and the severity of the illness are related to the age of the patient. In early childhood, infection usually is asymptomatic, whereas infection in adulthood generally is accompanied by symptoms. The diagnosis of hepatitis A must be confirmed by serologic testing because no constellation of symptoms is pathognomonic of the disease.

Incubation Period

The average incubation period is 28 to 30 days but can range from 15 to 50 days.[204] The average incubation period has been reported to be shorter in patients who acquired HAV infection by parenteral transmission from contaminated blood products and in chimpanzees infected parenterally than in those infected orogastrically.[239,330]

Spectrum of Illness

HAV infection, confirmed by the detection of IgM anti-HAV in serum, can be inapparent (asymptomatic, with no elevation in serum aminotransferase levels), subclinical (asymptomatic, with elevation of serum aminotransferase levels), or clinically evident (with symptoms). Specific symptoms of liver dysfunction include jaundice and dark urine caused by hyperbilirubinemia. However, symptomatic hepatitis A without jaundice (anicteric) does occur. Nonspecific symptoms of acute hepatitis A can include fever, myalgia, anorexia, nausea, right upper quadrant pain or discomfort, diarrhea, and pruritus.

Many acute HAV infections, particularly inapparent and subclinical infection and anicteric hepatitis A, are not recognized as cases of viral hepatitis.[344,406] The frequency of symptoms with acute infection is influenced strongly by age. Children are less likely than adults to have symptomatic infection, and jaundice rarely occurs in children younger than 6 years of age.[151] In one report describing outbreaks in several day care centers, the proportion of infected children without symptoms was 84 percent in children younger than 3 years, 50 percent in children 3 to 4 years of age, and 20 percent in children 5 years or older.[159] Symptoms develop in most adults with acute infection. In a study of two outbreaks among young adult U.S. military personnel, symptoms developed in 76 to 97 percent of infected persons, and approximately 55 percent were icteric.[213]

Clinical Signs and Symptoms

In an individual patient, the clinical symptoms of acute hepatitis A are indistinguishable from those caused by other forms of viral hepatitis. Particularly in older children and adults, the onset of illness often is quite abrupt and may consist of fever, myalgia, anorexia, malaise, nausea, intermittent dull abdominal pain, and vomiting. Fever (temperature rarely higher than 38.9° C [102° F]) and headache occur more frequently than in other forms of acute viral hepatitis.[350] Pediatric patients may have diarrhea or, less commonly, upper respiratory tract symptoms such as cough, sore throat, and runny nose, and the diagnosis of hepatitis A might not be considered in children with predominantly respiratory or gastrointestinal symptoms and transient fever without the typical malaise, fatigue, and anorexia.[138,219] Dark urine followed by jaundice and light-colored stool, if present, will appear within a few days to a week after onset of the prodromal symptoms.[29,156,213,219] When this icteric phase begins, symptoms often resolve and appetite returns in young children, but older children and adult patients may experience a transient worsening in the prodromal symptoms of anorexia, malaise, and weakness.[199,260]

In addition to jaundice and scleral icterus, physical findings may include mild hepatomegaly and tenderness, but severe tenderness suggests other diagnoses. The spleen may be palpable in 10 to 20 percent of patients, and posterior cervical adenopathy may be present.[76,213,363] Pleural effusions have been reported to occur, do not appear to be associated with more severe disease, and resolve spontaneously.[4] Ascites, peripheral edema, and

findings indicative of hepatic encephalopathy suggest the presence of a more severe form of hepatitis (see the discussion on atypical clinical manifestations and complications of hepatitis A). Ultrasonographic findings in children with uncomplicated hepatitis A have included edema or thickening of the gallbladder wall, abdominal lymphadenopathy, and, less commonly, transient ascites and pancreatic abnormalities.[76,193]

The symptoms of hepatitis A last for several weeks on average and usually not longer than 2 months.[200] Prolonged or relapsing hepatitis A can occur (see the discussion on relapsing hepatitis A) and, in the case of prolonged hepatitis A, may be associated with genetic markers for autoimmune hepatitis.[127]

Laboratory Abnormalities

As in other forms of viral hepatitis, during HAV infection, inflammation of the liver is accompanied by abnormalities in serum hepatic enzymes, with increases in serum, aspartate aminotransferase (AST), alkaline phosphatase, and γ-glutamyltranspeptidase (GGTP) levels. Elevations of serum ALT and AST occur most consistently and may precede the appearance of symptoms by a week or more (see Fig. 167-2). Peak levels generally occur 3 to 10 days after the onset of symptoms and are between 200 and 5000 IU but can reach as high as 20,000 IU. The level of ALT usually is higher than that of AST because the inflammatory response is destructive, particularly to the plasma membrane, in acute viral hepatitis. ALT is found in the cytosol of the plasma membrane, whereas AST is located mainly in cell mitochondria.[156]

Serum bilirubin levels, although frequently elevated, usually remain below 10 mg/dL and peak 1 to 2 weeks after illness begins. Higher levels can be seen in some patients, especially when HAV infection is complicated by cholestasis (see the section on cholestatic hepatitis A) or hemolysis secondary to an underlying glucose-6-phosphate dehydrogenase (G6PD) deficiency state.[71] In patients with G6PD deficiency, indirect bilirubin may account for more than 50 percent of the total bilirubin. Alkaline phosphatase concentration and 5′-nucleotidase activity usually are elevated only mildly, rarely reaching more than 2 or 3 times the normal level. GGTP levels generally are 3 to 10 times the upper limit of normal.

Serum immunoglobulin levels often are elevated, and IgM levels frequently are higher than those in acute hepatitis B and non-A, non-B hepatitis.[411] In patients without underlying liver disease, the prothrombin time usually is normal. A prolonged prothrombin time, generally associated with severe liver damage, is a prognostic indicator for the development of fulminant hepatitis.[402]

Patients with acute HAV infection usually have a mild lymphocytosis with occasional atypical mononuclear cells.[260] Except for patients who have hemolysis associated with G6PD deficiency, the hematocrit generally is normal.[71,260]

Apart from patients who have relapsing or cholestatic hepatitis A (see the section on atypical clinical manifestations and complications of hepatitis A), serum bilirubin and aminotransferase levels usually return to normal by 2 to 3 months after the onset of illness in most patients.[200]

Diagnostic Tests

Because the pattern and magnitude of symptoms and the hepatic enzyme abnormalities of hepatitis A are not distinctive for hepatitis A, the diagnosis requires serologic detection of IgM anti-HAV along with demonstration of an acute onset of signs and symptoms of hepatitis and jaundice or elevated aminotransferase levels. Persons with signs and symptoms of hepatitis and an epidemiologic link to other confirmed cases are likely to have hepatitis A, even if no laboratory confirmation is available. Sensitive and specific radioimmunoassays or enzyme immunoassays show that virtually all patients have detectable IgM anti-HAV during the acute or early convalescent phase of HAV infection (see Fig. 167-2).[228] A small proportion (3%) of patients tested within 3 days of the onset of symptoms may be IgM anti-HAV negative but become IgM anti-HAV positive within the initial 2 weeks of illness.[228] During the first 4 to 8 weeks after the onset of symptoms, the titer of IgM anti-HAV in serum is high.[163] Antibody generally disappears within 6 months, although rarely it can be detected for 2 years or longer.[110,163,198,228]

False-positive IgM anti-HAV test results among persons who have no other evidence of recent infection have been reported, suggesting a low positive predictive value when it is used to test asymptomatic persons with no known recent HAV exposures.[62,65] IgM anti-HAV may be detectable for a longer time in patients with symptomatic illness than in those with asymptomatic infection.[163,185,340]

IgG anti-HAV is present in low titer at or shortly after the onset of acute HAV infection, and the titer rises during the course of several weeks as the IgM anti-HAV titer falls (see Fig. 167-2). IgG anti-HAV remains detectable in serum for the lifetime of the individual and confers lasting protection against disease. Secretory IgA antibodies are detected in a minority of humans or primates with acute HAV infection but are unlikely to provide any significant protection against HAV infection.[345] IgG anti-HAV is transferred passively across the placenta and declines to undetectable levels in most infants by the time they reach 12 to 15 months of age.[27,229,230]

Commercially available enzyme immunoassays either detect total (IgG and IgM) antibody against HAV capsid proteins by using a competitive inhibition (blocking) format or detect IgM antibody to capsid proteins by using an IgM capture format.[220] These assays do not measure the neutralizing antibodies responsible for biologic activity against HAV, but detection of total anti-HAV by conventional assays is correlated with the appearance of neutralizing antibodies.[202,220] Neutralizing antibody can be detected by assays that measure inhibition of HAV in cell culture (i.e., radioimmunofocus inhibition test or plaque assay).[11,88,220,222] Neutralizing antibodies elicited against one strain of HAV have been shown to have biologic activity against other HAV strains.[222]

When tested in parallel with a World Health Organization anti-HAV reference reagent, the lower limit of

detection of most commercially available assays is approximately 100 mIU/mL of anti-HAV.[147,220] Administration of immunoglobulin provides a high level of protection against hepatitis A, although the antibody concentrations achieved by passive immunization with immunoglobulin or active immunization with vaccine are 10- to 100-fold lower than those produced after natural infection.[220] Antibody concentrations achieved by passive or active immunization and known to provide protection against HAV infection both in vivo and in vitro may be below the level of detection of most commercial immunoassays.

The lower limit of antibody necessary to provide protection against HAV infection is unknown. In vitro studies with cell culture–derived virus suggest that low levels of antibody (e.g., <20 mIU/mL) are neutralizing.[222] Clinical trials that evaluated vaccine efficacy have not provided an estimate of the minimum protective antibody level because vaccine-induced levels of antibody have been very high and few infections have occurred in vaccinees. Experimental studies in chimpanzees indicate that very low levels of passively transferred antibody (<10 mIU/mL) obtained from immunized individuals do not protect against infection but prevent clinical hepatitis and shedding of virus.[297]

PCR assays can be used to detect HAV RNA in stools and sera of persons with HAV infection, although these tests are available only in a few research laboratories. PCR techniques using serum specimens have been useful in some clinical, epidemiologic, or environmental studies. Although HAV concentrations in stool decline quickly after illness onset, HAV RNA can be detected in most serum specimens that are collected within 4 to 6 weeks after onset of illness.[43]

ATYPICAL CLINICAL MANIFESTATIONS AND COMPLICATIONS OF HEPATITIS A

Table 167-1 lists the atypical clinical manifestations and complications of hepatitis A.

TABLE 167-1 **Atypical Clinical Manifestations and Complications of Hepatitis A Virus Infection**

Relapsing hepatitis A[152,317,361]
Fulminant hepatitis A[241,275,300,360]
Cholestatic hepatitis A[154,361]
Hepatitis A triggering autoimmune hepatitis[361,379]
Extrahepatic manifestations
Transient rash or arthralgias[150,361]
Papular acrodermatitis of childhood[312]
Cutaneous vasculitis[95,174,175]
Cryoglobulinemia[174,175,317]
Guillain-Barré acute syndrome[351]
Other neurologic syndromes (e.g., myeloradiculopathy, mononeuritis, vertigo, meningoencephalitis)[19,40,351]
Renal syndromes (acute renal failure, nephritic syndrome, acute glomerulonephritis)[17,100,183]
Pancreatitis[256]
Aplastic anemia and thrombocytopenia[124,234]

Relapsing Hepatitis A

Relapsing hepatitis is a relatively common manifestation of hepatitis A that occurs in approximately 10 percent of patients.[317] One to 4 months after having the initial episode of acute hepatitis, these patients have a second episode; more than one relapse rarely occurs.[152,317,361] Patients with relapsing hepatitis A have no distinctive clinical features of their first disease episode. After the first episode, most patients experience a significant improvement in symptoms and biochemical abnormalities. However, the frequency with which serum aminotransferase levels completely normalize during this period has been variable; in one report, normalization occurred in only one of seven patients with relapsing hepatitis A.[361] The relapse episode of hepatitis rarely is more severe than the initial episode and is accompanied by elevated serum aminotransferase levels (typically to >1000 U/L) and persistence of IgM anti-HAV. Molecular studies have demonstrated the presence of HAV in stool and HAV RNA in serum during relapse, but whether patients are infectious is unknown.[152] The illness usually lasts a total of 16 to 40 weeks and results in full recovery.[152,317] Although the pathogenesis of relapsing hepatitis is unknown, it probably is immunologically mediated.[317] Persistent HAV infection with a relapsing clinical course has been reported in patients after they have undergone liver transplantation for fulminant hepatitis A; HAV-specific genomic sequences have been identified in the grafts of these patients.[126,402]

The IgA has been postulated as a carrier supporting hepatotropic transport of HAV, which may contribute to different clinical outcomes.[115] Over a period of several weeks after infection, anti-HAV IgA is able to promote an enterohepatic cycling of HAV, resulting in continuous endogenous reinfection of the liver. A mouse model demonstrated that highly avid IgG antibodies, which are present in later times of the infection, can terminate the reinfection. The endogenous reinfections in the presence of a developing neutralizing immunity might contribute to prolonged, as well as relapsing, courses of HAV infections, and the IgA may act as a protracting factor.[115]

Fulminant Hepatitis A

In the United States, a relatively small proportion of all fulminant hepatitis is caused by hepatitis A.[241,279,300,318,360] A prospective multicenter study conducted among 348 children with acute liver failure in the United States, Canada, and the United Kingdom found that only three cases (1%) could be attributed to hepatitis A.[343] However, 26 to 60 percent of children with acute liver failure had hepatitis A in tertiary care center–based studies conducted in Turkey, India, Argentina, and Pakistan.[15,18,21,321] Among patients hospitalized with hepatitis A, the case-fatality rate has been estimated to be 0.14 percent.[250] In the 1988 Shanghai epidemic that involved primarily adolescents and young adults, 47 deaths (0.015%) were recorded among the 310,746 diagnosed cases.[79] Between 1998 and 2005, 29 adults with HAV IgM–positive acute liver failure were enrolled in the Acute Liver Failure Study Group (ALFSG) in the United States. Acute HAV

accounted for 3.1 percent of the patients enrolled. At 3 weeks follow-up, 16 had spontaneously recovered (55%), 9 underwent transplantation (31%), and 4 had died (14%). The proportion of HAV cases enrolled in the ALFSG significantly decreased from 5 to 0.8 percent between 1988 and 2005 (p <.007).[356]

On the basis of national surveillance data in the United States; death occurs in 0.3 to 0.6 percent of persons with symptomatic hepatitis A, reaching 1.8 percent among those over 50 years of age.[325] In 2008, the mortality rate of hepatitis A was 0.02/100,000 population (n = 83). During 2004 to 2008, the mortality rates were highest among persons older than 75 years in contrast to other age groups, and the overall mortality rate for that population was 0.12/100,000 population in 2008. From 2004 through 2008, the hepatitis A mortality rate was consistently higher among males than among females.[68]

Host factors reported to be associated with an increased risk for development of fulminant hepatitis include older age[45,208,250,385,405]* and underlying chronic liver disease.* Reported findings among persons with fulminant disease include nucleotide or amino acid substitutions in the 5′ untranslated region,[145] P2 region, and P3 region of the HAV genome.[265] Viral factors such as substitution rate in the entire genome were not associated with acute liver failure in the 29 patients identified with HAV liver failure in the adult ALFSG from 1998 to 2004; however, a negative PCR (undetectable or very low viral load) was the single factor that correlated more significantly with more severity and worse outcomes.[2] This finding has been previously described in the literature and suggests that the excessive host response maybe a determining factor in the development of fulminant hepatitis A.[304] An in depth analysis conducted in India for a 5-year period concluded that higher CD8 T cells and coinfection with other viral hepatitis infections were more commonly associated with fulminant hepatitis and contrary to previous findings; higher viral load was also an important contributing factor.[170]

Fulminant hepatitis A has no distinctive clinical features that distinguish it from fulminant hepatic failure of other causes. Within approximately 8 weeks of the onset of illness, symptoms of hepatic encephalopathy and marked prolongation of prothrombin time are noted in patients with no history of previous liver disease.[276] Complications can include cerebral edema, sepsis, gastrointestinal bleeding, and hypoglycemia. The prognosis of fulminant hepatitis A without transplantation is better than that of fulminant disease related to other viral causes, and 40 to 70 percent of patients can be expected to recover.[150,275,318,360] In one hospital series, more rapid onset to liver failure after onset of jaundice was associated with improved outcome, and higher bilirubin concentration and more prolonged prothrombin time were associated with poor outcomes.[99]

Extrahepatic Manifestations

During acute hepatitis A, transient rash and arthralgias occur in as many as 14 and 19 percent of patients, respectively, particularly during the prodromal period.[150,361] Urticaria has been reported but occurs less frequently than in acute hepatitis B.[109] Papular acrodermatitis of childhood, the Gianotti-Crosti syndrome, rarely occurs in the United States but has been reported elsewhere in association with HAV infection.[312] The cutaneous lesions, which consist of nonpruritic, symmetric flat papules on the face, extremities, and buttocks, may persist for several weeks before spontaneously resolving.[116]

Other extrahepatic manifestations that occur chiefly in association with cholestatic or relapsing hepatitis A include cutaneous vasculitis and cryoglobulinemia.[95,174,175] The vasculitis, manifested as erythematous maculopapular lesions often affecting the lower extremities and buttocks and typically associated with purpura, appears as leukocytoclastic vasculitis and granular deposits of IgM anti-HAV and complement in blood vessel walls in skin biopsy specimens. Cryoglobulinemia includes cryoglobulins composed of IgG and IgM and IgM anti-HAV antibodies.[95,174,175,317] These manifestations resolve spontaneously with resolution of the hepatitis.

In the absence of fulminant disease, neurologic syndromes have been observed only rarely in association with hepatitis A. Guillain-Barré syndrome has been reported to occur 3 days to 2 weeks after the onset of hepatitis A, as have myeloradiculopathy, vertigo, mononeuritis (cranial or peripheral nerve), meningoencephalitis, and exacerbation of multiple sclerosis.[19,40,286,351] Renal complications, including acute renal failure, nephrotic syndrome, and acute glomerulonephritis, also rarely have been reported in children who did not have fulminant disease.[17,100,183] Self-limited, mild pancreatitis likewise appears to occur.[256] Reported hematologic complications include aplastic anemia and severe thrombocytopenia.[124,234] Gestational complications, including premature rupture of membranes and placental separation, have been reported among pregnant women with acute hepatitis A, but infants born to these women were healthy otherwise.[118,311]

Cholestatic Hepatitis A

Cholestatic hepatitis occurs in a small percentage of patients with hepatitis A. These patients are deeply icteric and may have pruritus, fatigue, fever, loose stools, anorexia, dark urine, and weight loss. In two reports of 10 patients with cholestatic hepatitis A, peak serum bilirubin levels generally were higher than 10 mg/dL, with some as high as 38 mg/dL, and remained elevated for 12 to 16 weeks.[154,361] Serum aminotransferase levels declined but remained elevated during the period of cholestasis. In one of these reports, five patients had prolonged prothrombin times that normalized with the administration of vitamin K.[154]

Cholestatic hepatitis A can be distinguished from obstructive jaundice by normal abdominal ultrasound findings. Conducting further invasive diagnostic procedures, such as liver biopsy or direct forms of cholangiography, is not necessary in most cases.[317] Although patients will recover completely without therapy, a course of corticosteroids with a gradual taper during a span of at least

*References 3, 96, 189, 223, 380, 385, 403, 405.

4 weeks has been reported to hasten relief of symptoms and resolution of cholestasis.[207,317,408]

Hepatitis A Triggering Autoimmune Hepatitis

Several reports have described patients in whom hepatitis A is followed by type 1 autoimmune chronic hepatitis.[299,361,379] Laboratory studies demonstrated a T-cell defect in these patients, suggesting a genetic predisposition to the development of autoimmune hepatitis that is "triggered" by HAV infection. These patients have required corticosteroid therapy, sometimes for long periods.

TREATMENT

Hepatitis A has no specific therapy, and because HAV infection is self-limited and does not result in chronic infection or chronic liver disease, treatment generally is supportive. Hospitalization may be necessary for patients who are dehydrated from nausea and vomiting or who have fulminant hepatic failure. Because no conclusive data indicate that bed rest or inactivity influences the course of illness, no restriction of activity is necessary. Similarly, no specific diet is indicated, although many patients may have an intolerance to fatty foods during their illness. Medications, particularly those that have the potential to cause hepatic damage and those that are metabolized by the liver, including acetaminophen, should be used with caution. The half-life of these medications may be prolonged.

A specific therapy for fulminant hepatic failure caused by hepatitis A has not been established. Small uncontrolled trials conducted among adults in the 1980s suggested that some patients benefited from prostaglandin E or IFN, but no additional data to indicate that these therapies are efficacious have been published. No evidence exists that exchange transfusions, plasmapheresis, or corticosteroids are effective.[125,156] Adults with fulminant hepatitis A who received prostaglandin E or IFN have been reported to show improvement in small, uncontrolled trials.[227,335] Amantadine and ribavirin have shown activity in vitro against HAV.[86] Liver transplantation is successful in some patients.[164,334,360,384] Persistent HAV infection has been demonstrated in some transplant recipients, but whether it affects survival is unknown.[117,126] One case report from Korea described an adult patient with recurrent hepatitis A with liver failure after a successful transplantation; which prompted a second liver transplant with later evidence of hepatitis A ongoing infection.[282] Because survival rates of adult and pediatric patients are relatively high without transplantation and no single factor is predictive of a poor outcome, establishment of criteria for choosing candidates for transplantation has been difficult.[150,276,360,377] Reported survival rates after transplantation in patients with fulminant hepatitis from all viral causes range from 40 to 89 percent, depending on the severity of liver failure and other factors.[214,360]

PREVENTION

In addition to general measures of good personal hygiene, particularly handwashing; provision of safe drinking water; and proper disposal of sanitary waste, preexposure or postexposure immunization primarily with hepatitis A vaccine or with immunoglobulin can prevent the acquisition of hepatitis A.

Immunoglobulin

Immunoglobulin is a sterile solution of antibodies prepared by a serial cold ethanol precipitation procedure from pooled human plasma that has tested negative for hepatitis B surface antigen, antibody to human immunodeficiency virus (HIV), and antibody to hepatitis C virus.[78] This precipitation procedure has been shown to inactivate hepatitis B virus and HIV.[52] Since 1995, immunoglobulin prepared in the United States has been required to be negative for hepatitis C virus RNA by PCR amplification or to be produced by a method that ensures additional virus inactivation. When it is administered before exposure or within 2 weeks after exposure, immunoglobulin is more than 85 percent effective in preventing hepatitis A by passive transfer of anti-HAV.[198,258,347] Whether immunoglobulin completely prevents infection or leads to asymptomatic infection and the development of persistent anti-HAV (passive-active immunity) probably is related to the amount of time that has elapsed between exposure and administration of immunoglobulin.[219,347] Although in recent years immunoglobulin lots have had slightly lower titers of anti-HAV, probably because of a decreasing prevalence of previous HAV infection in plasma donors, no clinical or epidemiologic evidence of decreased efficacy has been reported.[354]

Immunoglobulin may be given to persons who are traveling to countries with high, transitional, or intermediate endemicity of HAV infection (see Fig. 167-3) instead of or in addition to hepatitis A vaccine.[65] Immunoglobulin should be given to children younger than 12 months who are traveling to such countries because hepatitis A vaccine is not licensed in the United States for children in this age group (see the section on vaccines).[65] Although hepatitis A often is asymptomatic in infants and young children, preexposure prophylaxis is indicated to prevent the rare severe cases and transmission to others after return from abroad.

For preexposure prophylaxis of travelers, the dose of immunoglobulin is 0.02 mL/kg body weight if travel will be for less than 3 months. Because of the decay of passive immunity during the course of time, a dose of 0.06 mL/kg is necessary for persons who will be abroad for 3 to 5 months, and readministration every 5 months is necessary for extended trips. Hepatitis A vaccine, if it is not contraindicated, is a better choice for such persons.

Aggressive use of immunoglobulin or vaccine is indicated to control hepatitis A outbreaks in child care centers in which hepatitis A is diagnosed in a child or employee[65,64,324] and in other settings (e.g., hospitals and facilities for developmentally disabled persons) when outbreaks occur.[58] When a food handler is identified with

hepatitis A, postexposure prophylaxis should be administered to other food handlers at the establishment and under limited circumstances to patrons.[51,65,135] Once cases are identified that are associated with a food service establishment, it generally is too late to administer postexposure prophylaxis to patrons because the 2-week postexposure period during which prophylaxis is effective will have passed.

Previously unvaccinated household or sexual contact of patients with hepatitis A should receive postexposure prophylaxis. In the absence of postexposure prophylaxis, secondary attack rates of 15 to 30 percent have been reported in households, with higher rates of transmission occurring from infected young children than from infected adolescents and adults. Attack rates in food handlers are generally lower.[197]

Immunoglobulin is usually used for postexposure prophylaxis in susceptible persons who are either children younger than 12 months or persons older than 40 years of age, immunocompromised persons, and persons with chronic liver disease.[197]

For postexposure prophylaxis, 0.02 mL/kg body weight of immunoglobulin is administered intramuscularly. For infants and young children, the injection can be administered in the anterolateral aspect of the thigh or the deltoid muscles; for older children and adolescents, the injection should be administered in the deltoid or gluteus muscles, into which a large volume of immunoglobulin can be injected.[59] If the immunoglobulin is administered in the gluteus, the injection should be given in the superolateral aspect to avoid injury to the sciatic nerve.[59]

Vaccine is recommended as postexposure prophylaxis in healthy persons 12 months through 40 years of age as of 2007 because it induces active immunity providing longer protection, has higher acceptability and availability, and is easy to administer.[65,69,197]

Immunoglobulin does not interfere with the immune response to oral poliovirus vaccine, to yellow fever vaccine, or, in general, to inactivated vaccines. However, immunoglobulin can interfere with the immune response to some live attenuated vaccines (e.g., measles-mumps-rubella vaccine [MMR] and varicella vaccine). Administration of MMR and varicella vaccines should be delayed for at least 3 months and at least 5 months, respectively, after the administration of immunoglobulin. Immunoglobulin should not be given within 2 weeks after the administration of MMR or within 3 weeks of the administration of varicella vaccine, unless the benefits of immunoglobulin administration are greater than the benefits of vaccination.[59] For travelers younger than 1 year of age in whom the use of immunoglobulin may interfere with the administration of other needed vaccines (e.g., MMR and varicella), the use of inactivated hepatitis A vaccine could be considered (see the discussion on inactivated vaccines).

Serious adverse events from immunoglobulin rarely occur. Because anaphylaxis has been reported after repeated administration to persons with IgA deficiency, these persons should not receive immunoglobulin.[119] Pregnancy or lactation is not a contraindication to the administration of immunoglobulin. For infants and pregnant women, a preparation that does not include thimerosal is preferable.

TABLE 167-2 Recommended Doses and Schedules for Inactivated Hepatitis A Vaccines Available in the United States

Age (yr)	Vaccine*	Dose	Volume (mL)	No. of Doses	Schedule† (mo)
1-18	HAVRIX	720 ELU	0.5	2	0, 6-12
	VAQTA	25 U	0.5	2	0, 6-18
≥19	HAVRIX	1440 ELU	1.0	2	0, 6-12
	VAQTA	50 U	1.0	2	0, 6-18
>18	TWINRIX	720 ELU	1.0	3	0, 1, 6‡

From Center for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendation from the advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2006;**55**(RR-7):1-23.

*HAVRIX is manufactured from HAV strain HM175 by GlaxoSmithKline; VAQTA is manufactured from HAV strain CR326F′ by Merck & Co, Inc.; TWINRIX is a combined hepatitis A and hepatitis B vaccine that also contains 20 µg per dose recombinant hepatitis B surface antigen protein.

†Zero months indicates initial dose; subsequent numbers represent months after the initial dose.

‡An alternative four-dose schedule, given on days 0, 7, 21-30, followed by a dose at month 12, also is licensed in the United States.

ELU, Enzyme-linked immunosorbent assay units.

Hepatitis A Vaccine

The ability to propagate HAV in cell culture allowed the development of hepatitis A vaccines. Both inactivated and live attenuated hepatitis A vaccines have been developed by use of defined isolates from infected cell lines.*

However, only inactivated vaccines have been evaluated for efficacy in controlled clinical trials and licensed in the United States, and the live attenuated vaccines evaluated to date do not appear to offer any distinct advantage over inactivated vaccines.[176,392] Available data indicate that both inactivated and live attenuated hepatitis vaccine are capable of providing long-term protection.[280]

Vaccine Preparation and Performance

Inactivated hepatitis A vaccines are prepared by a method similar to that used to prepare inactivated poliovirus vaccine, by propagation of cell culture–adapted virus in human fibroblasts, purification by ultrafiltration or other methods, formalin inactivation, and adsorption to an aluminum hydroxide adjuvant.[14,75] Inactivated vaccines using the HM175 strain and the CR326F′ strain have been licensed in pediatric and adult formulations for intramuscular administration and are available in the United States for persons 12 months of age and older (Table 167-2). One of these vaccines (HM175 strain) is formulated with 2-phenoxyethanol as a preservative, whereas

*References 90, 237, 238, 253, 296, 336.

the other is formulated without a preservative (see Table 167-2).[284] The antigen content of one vaccine (CR326F′ strain) is expressed as units of HAV antigen as defined by a standard; the antigen content of the other vaccine (HM175 strain) is determined by reactivity in a quantitative immunoassay for HAV antigen and is expressed as enzyme-linked immunosorbent assay units (ELU; see Table 167-2).

Two other inactivated hepatitis A vaccines are manufactured and available in Europe and other parts of the world.[175,211,287,383] In addition, a combination inactivated hepatitis A and recombinant hepatitis B vaccine is available in the United States for persons 18 years and older[64] and a pediatric formulation is available in Europe, Canada, and other parts of the world.[106]

In extensive studies in children and adults, the inactivated hepatitis A vaccines available in the United States have been found to be highly immunogenic. In general, after one dose of vaccine, 95 to 100 percent of children 1 year or older and adults respond with concentrations of antibody considered to be protective; a second dose given 6 to 18 months later results in a boost in antibody concentration and probably is important for long-term protection.* Persons whose second dose is delayed as long as 3 to 8 years after the first dose have responses to vaccination similar to the responses of those who receive the vaccine on licensed schedules.[24,179,400] Studies conducted among infants and children younger than 18 months have demonstrated that simultaneous administration of hepatitis A vaccine with diphtheria–tetanus–acellular pertussis (DTaP), *Haemophilus influenzae* type b, hepatitis B, MMR, and inactivated poliovirus vaccines does not affect the immunogenicity and reactogenicity of these vaccines.[27,35,92,252,370] IgM anti-HAV occasionally can be detected by standard assays, primarily if it is measured soon (i.e., 2 to 3 weeks) after vaccination.[332,381]

Conditions that may result in reduced immunogenicity include HIV infection, chronic liver disease, and older age. Among adults with HIV infection, 61 to 87 percent had protective antibody concentrations after completing the vaccination series.[166,191,269,387] Higher $CD4^+$ T-lymphocyte count at baseline was associated with response to vaccination.[13,166,191,305,387] However, HIV-infected persons with normal or near-normal $CD4^+$ T-lymphocyte counts have response rates that exceed 95 percent.[390] A retrospective study in adult patients with HIV who received two doses of HAV vaccine demonstrated a durable seropositive response for up to 6 to 10 years after HAV vaccination. Suppressed HIV RNA levels were associated with durable HAV responses.[89] Awareness of hepatitis susceptibility and hepatitis coinfection status in HIV-infected patients is essential for optimal clinical management. Despite recommendations for hepatitis screening and vaccination of HIV-infected MSM, rates for screening and vaccination remained suboptimal in the United States from 2004 to 2007.[168] HIV-infected patients have an elevated risk for HAV coinfection, which is significant considering the elevated prevalence of liver disease in this population. Furthermore, HIV-infected persons may experience prolonged HAV viremia, which has important public health implications for transmission within the community.[89] Among persons with chronic liver disease, seroprotection rates were similar to those observed among healthy adults, but the final antibody concentrations were substantially lower.[190,217]

Studies in children younger than 1 year of age indicate that the vaccine is safe and immunogenic for those who do not have passively transferred antibody from previous maternal HAV infection.[27,226,291,366] In studies of infants who received hepatitis A vaccine according to several different schedules, those with passively transferred maternal antibody at the time of vaccination responded, but final antibody concentrations were approximately one-third to one-tenth those of infants who did not have passively transferred antibody and were vaccinated according to the same schedule.* The clinical significance, if any, of these lower antibody concentrations is unknown. One study found that all infants vaccinated in the presence of passively transferred maternal antibody responded to a booster dose given 6 months later with an anamnestic response, suggesting that they had been primed by the primary series.[93] However, in another small study, two of six children who had lost detectable antibody did not have an anamnestic response to a booster dose administered approximately 6 years after they had received the primary vaccine series in infancy in the presence of passively transferred antibody.[136] Most infants born to anti-HAV–positive mothers have lost detectable antibody by the time they reach 12 to 15 months of age, and both pediatric hepatitis A vaccines are now licensed for use in children as young as 12 months.[141,230]

Inactivated hepatitis A vaccine has been shown to be highly efficacious in preventing clinically apparent disease. In a study of approximately 40,000 Thai children 1 to 16 years of age, the efficacy of inactivated vaccine (HM175 strain) was 94 percent (95% CI, 79%, 99%) after two doses (360 ELU per dose) administered 1 month apart.[176] In a study of another inactivated vaccine (CR326F strain) involving approximately 1000 children 2 to 16 years of age in a New York community with high hepatitis A rates, efficacy was 100 percent (lower bound of the 95% CI, 87%) starting 17 days after the administration of one dose (25 U).[392]

Since hepatitis A vaccines became available in the United States in 1995, studies, demonstration projects, and surveillance data have evaluated their effectiveness in controlling and preventing the development of hepatitis A in communities. In areas with the highest hepatitis A rates, such as Native American and Alaska Native communities, vaccination of the majority of children—and in some cases adolescents and young adults—resulted in a rapid decline in the incidence of disease, and with ongoing routine vaccination of children, the reduction in incidence of disease has been sustained.[33,55,248] In larger, more heterogeneous communities with lower but consistently elevated hepatitis A rates, interruption of ongoing

*References 20, 27, 75, 169, 249, 266.

*References 27, 93, 226, 229, 291, 366.

community-wide epidemics by vaccination of children proved more difficult.[85] In contrast, sustained routine vaccination of children can reduce hepatitis A incidence markedly during the course of time (see discussion on vaccine recommendations and use).[16,92,111]

Hepatitis A vaccine also is effective when it is used to prevent infection after exposure. Hepatitis A vaccine administered soon after exposure prevented infection in a chimpanzee model.[307] Hepatitis A vaccine was found to be 79 percent efficacious in preventing infection in contrast to no treatment in a small randomized trial conducted in Italy.[313] However, the confidence interval was wide (7% to 95%), and the study did not include a comparison group that received passive postexposure prophylaxis with immunoglobulin.[26] In a randomized, controlled trial conducted in Kazakhstan, among persons aged 2 to 40 years who were contacts of hepatitis A cases, the efficacy of hepatitis A vaccine administered within 14 days after exposure to HAV was shown to be similar to that of immunoglobulin in healthy children and adults younger than 40 years of age.[381] Based on their results and because the vaccine offers advantages over immunoglobulin, the Advisory Committee on Immunization Practices (ACIP) of the U.S. Public Health Service currently recommends the hepatitis A vaccine in preference to immunoglobulin for postexposure prophylaxis of healthy persons from the ages of 12 months to 40 years.[65] Advisory groups or expert panels in some other countries also have recommended that hepatitis A vaccine be used as the primary means of preventing hepatitis A after exposure.[268,352]

Experience to date indicates that the incidence of adverse events after vaccination is comparable to that after the administration of other widely used vaccines. In prelicensure clinical studies in children, the side effects reported most frequently included soreness, tenderness, warmth, or induration at the injection site (4% to 19%); feeding problems (8%); and headache (4%).[35,64,252] Through 2005, more than 188 million doses have been sold worldwide, including more than 50 million doses in the United States.[64] No serious adverse events in children or adults have been identified that could be definitively ascribed to hepatitis A vaccine.[36,64] The Vaccine Adverse Events Reporting System (maintained by the U.S. Food and Drug Administration and the CDC) has received approximately 6000 reports of adverse events occurring after receipt of hepatitis A vaccine. The most common events were minor and of brief duration, such as fever, injection-site reactions, rash, and headache. The 871 serious adverse events included Guillain-Barré syndrome, transaminitis, idiopathic thrombocytopenic purpura, and seizures among children.[64,271] Rare adverse events reported after marketing include syncope, jaundice, erythema multiforme, anaphylaxis, brachial plexus neuropathy, transverse myelitis, encephalopathy, and others. For events for which incidence rates are available, such as Guillain-Barré syndrome, reported rates were not higher than reported background rates.[64]

Anti-HAV persists at protective levels in vaccinated adults for at least 10 to 12 years after vaccination,[372,376,375] and more recent publications have demonstrated protective levels up to 15 and 17 years after vaccination[373,374] and in children for at least 5 to 10 years after vaccination.[128,161,372] Available data indicate that both inactivated and live attenuated hepatitis A vaccine are capable of providing long-term protection for up to 15 years in both children and adults.[280]

In one follow-up study, two thirds of infants who did not have passively transferred maternal antibody at the time of vaccination had detectable anti-HAV 6 years later, and all who had lost antibody had an anamnestic response to a booster dose.[136] Estimates based on kinetic models of decline in antibody suggest that the duration of protection could be 15 to 25 years or longer.[372,398,399] A study randomized by maternal anti-HAV status included 197 children younger than 2 years of age who received different schedules of HAV vaccine and demonstrated that the seropositivity induced by hepatitis A vaccine can persists for at least 10 years regardless of presence of maternal anti-HAV.[326]

In some settings, performing prevaccination serologic testing may be considered in an attempt to reduce cost by not vaccinating persons with previous immunity.[48] Testing of children is not indicated because of their expected low prevalence of infection and the lower cost of vaccine for this age group. Testing may be considered for older adolescents and adults in certain population groups with a high prevalence of infection (e.g., Native Americans and Alaska Natives), persons who either were born in or lived for extensive periods in geographic areas that have high endemicity of HAV infection (e.g., Central and South America, Africa, and Asia), and adults 40 years of age and older, but the cost of testing, the vaccine cost, and the likelihood that the person will return for vaccination should be taken into account. Postvaccination testing is not indicated because of the high rate of response to the vaccine. Furthermore, most anti-HAV testing methods licensed for use in the United States cannot reliably detect the low anti-HAV concentrations generated by immunization.[69]

Vaccine Recommendations and Use

Recommendations for the use of hepatitis A vaccine were issued first by the ACIP (Table 167-3), the American Academy of Pediatrics, and other groups in 1996 and updated in 1999 and 2006.[8,54,58,64] As part of an incremental strategy aimed at achieving widespread routine vaccination, the 1999 recommendations called for routine vaccination of children living in areas where rates of hepatitis A consistently had been elevated (see Table 167-3). Various vaccination strategies that were suggested included vaccinating one or more single-age cohorts of children or adolescents, vaccinating children in selected settings (e.g., day care), and vaccinating children and adolescents over a wide range of ages in a variety of settings, such as when they seek health care for other purposes. The final step in this incremental strategy, routine vaccination of children older than 12 months nationwide, was recommended by the ACIP in 2006.[64]

The impact of routine vaccination of children initially was shown in areas that historically have had the highest hepatitis A rates (e.g., Native American communities), where this strategy had been recommended since 1996.

TABLE 167-3 Advisory Committee on Immunization Practices Recommendations for Routine Preexposure Use of Hepatitis A Vaccine

Group	Comments
All children at age 12-23 months*	Integrate into routine childhood vaccination schedule; children who are not vaccinated by age 2 years can be vaccinated at subsequent visits
Children aged 2-18 years	Maintain existing program†; can be considered in areas without existing programs
International travelers	Except persons traveling to Canada, Western Europe, Japan, Australia, or New Zealand, who are at no greater risk than in the United States
Men who have sex with men	Includes adolescents
Illicit drug users	Includes adolescents
Persons with chronic liver disease	Increased risk of fulminant hepatitis A with HAV infection
Persons receiving clotting factor concentrates	
Persons who work with HAV in research settings	
Anyone wishing to obtain immunity	

From Centers for Disease Control and Prevention: Prevention of hepatitis A through active or passive immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 2006;**55**(RR-7):1-23.

*Hepatitis A vaccine is not licensed for children younger than 12 months.

†States covered by 1999 ACIP recommendations (Alabama, Alaska, Arizona, California, Colorado, Idaho, Minnesota, Missouri, Nevada, New Mexico, Oklahoma, Oregon, South Dakota, Texas, Utah, Washington, Wyoming, and selected areas in other states).[58]

HAV, Hepatitis A virus.

Surveys conducted in 1999 to 2000 indicated vaccination coverage of 50 to 80 percent of preschool- and school-age Native American and Alaska Native children, thus suggesting that the recommendation for routine vaccination was being implemented.[33] By 2000, the incidence of hepatitis A in Native Americans and Alaska Natives had declined by 97 percent in contrast to the beginning of the preceding decade and was the same as the overall U.S. rate.[33]

National surveillance data indicate that routine vaccination of children living in areas with consistently elevated rates, which was recommended in 1999, has had an impact on the overall incidence of hepatitis A in the United States. Rates declined most dramatically among children in parts of the country where routine vaccination of children was recommended and where the estimated coverage was highest.[388]

Since 2001, incidence rates in each successive year have been historic lows, with the 2010 rate of 0.54 cases/100,000 population demonstrating a 94 percent decline in contrast to previous lows observed in the early 1990s.[70]

Vaccine coverage data are limited but indicate that declines in incidence have occurred with modest levels of coverage, suggesting a strong herd immunity effect.[9,93,314] Modeling studies suggested that 39 percent of potential cases were averted in 2001 because of the direct effects of immunization and herd immunity.[314] Similar observations have been made in Israel and Spain. The incidence of hepatitis A declined by 95 percent in Israel within 3 years after initiation of an immunization program among children 18 to 24 months old, even though no catch-up vaccination of other age groups was attempted.[93] Routine vaccination of 12-year-old children in Catalonia, Spain was followed by a 58 percent reduction in overall incidence.[111] Hepatitis A incidence is cyclic, and data from additional years are needed to verify that low rates are sustained and attributable to vaccination and to provide a definitive determination of the overall impact of this strategy of routine childhood vaccination. However, the consistency and strength of these data render alternative explanations unlikely.

The data indicating that vaccination of children could have a powerful impact on incidence rates in all ages and the demonstration of vaccine safety and immunogenicity for children as young as 1 year were key factors supporting the decision to expand routine vaccination to all children aged 12 to 23 months in the United States. Economic analyses also were considered. One study estimated that routine vaccination given when children reached 1 year of age in the United States would result in 183,806 fewer infections and 32 fewer deaths in a single U.S. birth cohort, with a cost-effectiveness ratio of $173,000 per life year gained and $24,000 per quality-adjusted life year gained.[302]

Vaccination of successive cohorts of children eventually should result in a sustained reduction in the incidence of disease nationwide and thus provide the opportunity to eliminate HAV transmission. To achieve this goal, implementation of recent ACIP recommendations for vaccination of young children nationwide will be needed. Pediatric combination vaccines that include hepatitis A vaccine would facilitate this effort.

Vaccination of persons at increased risk for acquiring hepatitis A, including travelers to countries where hepatitis A is endemic, adolescent and adult MSM, persons who use illegal drugs, those who work with HAV in research settings, and persons who have clotting factor disorders, also is recommended.[64] Persons with chronic liver disease who acquire hepatitis A have been reported to have a high case-fatality rate and are recommended for vaccination also.[64]

In 2009, ACIP recommended hepatitis A vaccination for all previously unvaccinated persons who anticipate close personal contact (e.g., household contact or regular babysitting) with an international adoptee from a country of high or intermediate endemicity during the first 60 days after arrival of the adoptee in the United States. The first dose of the two-dose hepatitis A vaccine series should

be administered as soon as adoption is planned, ideally within 2 weeks before the arrival of the adoptee.[66]

ACIP considered the likelihood that a child adopted by parents in the United States may be actively infected with hepatitis A and shedding virus at the time of the adoption. During 1998 to 2008, approximately 18.000 children were adopted from foreign countries by families in the United States each year. Of these, 99.8 percent came from countries where hepatitis A is considered of high or intermediate endemicity and approximately 85 percent were younger than 5 years of age.

ACIP also considered reports of HAV infection among persons in close contact with new adoptees from countries of high or intermediate endemicity. In 2007, the CDC was notified of a case of fulminant hepatitis A in a nontraveling household contact of an asymptomatic Ethiopian adoptee confirmed to have acute hepatitis A (IGM HAV positive). This case prompted an investigation that led to an identification of 20 additional cases of acute hepatitis A among persons who had close contact with newly arriving international adopted children and no history of traveling abroad.[137,285] HAV infection in international adoptees and their contacts between 2007 and 2009 was also reported in Minnesota.[348]

HAV infection during pregnancy may be associated with the risk for gestational complications. HAV serology and vaccination for pregnant women should be considered a high-prevalence area of acute hepatitis A.[311]

Hepatitis A is not routinely recommended for health care personnel, persons attending or working in child care centers, or persons who work in liquid or solid waste management (e.g., sewer workers or plumbers). ACIP does not recommend routine hepatitis A vaccination for food service workers, but vaccination may be considered on local epidemiology.[69]

NEW REFERENCES SINCE THE SIXTH EDITION

2. Ajmera V, Xia G, Vaughan G, et al. What factors determine the severity of hepatitis A-related acute liver failure? *J Viral Hepat* 2011;**18**:e167–74.
5. Amado LA, Marchevsky RS, de Paula VS, et al. Experimental hepatitis A virus (HAV) infection in cynomolgus monkeys *(Macaca fascicularis):* evidence of active extrahepatic site of HAV replication. *Int J Exp Pathol* 2010;**91**:87–97.
7. Amado LA, Villar LM, de Paula VS, et al. Exposure to multiple subgenotypes of hepatitis A virus during an outbreak using matched serum and saliva specimens. *J Med Virol* 2011;**83**: 768–75.
13. Armstrong KE, Bush HM, Collins JD, et al. Role of CD4 count in immunity development after hepatitis A and B vaccination among HIV-infected patients: Kentucky, 2002-2007. *J Int Assoc Physicians AIDS Care (Chic)* 2010;**9**:179–86.
21. Bariş Z, Saltik T, Temizel IN, et al. Acute Liver failure in children: 20 year experience. *Turk J Gastroenterol* 2012;**23**:127–34.
31. Bialek SR, Barry V, Bell BP, et al. Seroprevalence and correlates of hepatitis a among HIV-negative American men who have sex with men. *Sex Health* 2011;**8**:343–8.
39. Bordi L, Rozera G, Scognamiglio P, et al. Monophyletic outbreak of hepatitis A involving HIV-infected men who have sex with men, Rome, Italy 2008-2009. *J Clin Virol* 2012;**54**:26–9.
50. Cao J, Bi S, Meng Q, et al. Genotyping of acute hepatitis a virus isolates from China, 2003-2008. *J Med Virol* 2011;**83**:1134–41.
66. Centers for Disease Control and Prevention. Updated recommendations from the Advisory Committee on Immunization practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1006–7.
67. Centers for Disease Control and Prevention. *Viral hepatitis surveillance, United States 2009*. Atlanta: CDC; 2009. Available at: http://www.cdc.gov/hepatitis/Statistics/2009Surveillance/index.htm.
68. Centers for Disease Control and Prevention. *Viral hepatitis surveillance, United States*. Atlanta: CDC; 2010. Available at: http://www.cdc.gov/hepatitis/Statistics/2010Surveillance/index.htm.
69. Centers for Disease Control and Prevention. Hepatitis A. In: *Epidemiology and prevention of vaccine preventable disease*. U. S. Department of Health and Human Services; CDC, ed, 2012. p. 101–14.
70. Centers for Disease Control and Prevention. Summary of notifiable disease, United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–11.
89. Crum-Cianflone NF, Wilkins K, Lee AW, et al. Long-term durability of immune responses after hepatitis A vaccination among HIV-infected adults. *J Infect Dis* 2011;**203**:1815–23.
97. Daudi N, Shouval D, Stein-Zamir C, et al. Breastmilk hepatitis A virus RNA in nursing mothers with acute hepatitis A virus infection. *Breastfeed Med* 2012;**7**:313–15.
103. Desbois D, Couturier E, Mackiewicz V, et al. Epidemiology and genetic characterization of hepatitis A virus genotype IIA. *J Clin Microbiol* 2010;**48**:3306–15.
112. Donnan EJ, Fielding JE, Gregory JE, et al. A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. *Clin Infect Dis* 2012;**54**:775–81.
115. Dotzauer A, Heitmann A, Laue T, et al. The role of immunoglobulin A in prolonged and relapsing hepatitis A virus infections. *J Gen Virol* 2012;**93**(Pt 4):754–60.
146. Gallot C, Grout L, Roque-Afonso AM, et al. Hepatitis A associated with semidried tomatoes, France, 2010. *Emerg Infect Dis* 2011;**17**:566–7.
164. Hayashi MST, Tsunematsu I, Hirokawa F, et al. Hepatitis A virus-related late-onset hepatic failure: a case report. *Exp Clin Transplant* 2011;**9**:150–2.
167. Holmberg SD. Hepatitis a epidemiology goes global. *Clin Infect Dis* 2012;**54**:782–3.
168. Hoover KW, Butler M, Workowski KA, et al. Low rates of hepatitis screening and vaccination of HIV-infected MSM in HIV clinics. *Sex Transm Dis* 2012;**39**:349–53.
170. Hussain Z, Husain SA, Almajhdi FN, et al. Immunological and molecular epidemiological characteristics of acute and fulminant viral hepatitis A. *Virol J* 2011;**8**:254.
177. Ishii K, Kiyohara T, Yoshizaki S, et al. Epidemiological and genetic analyses of a diffuse outbreak of hepatitis A in Japan, 2010. *J Clin Virol* 2012;**53**:219–24.
182. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine* 2010;**28**:6653–7.
192. Kim YJ, Lee HS. Increasing incidence of hepatitis A in Korean adults. *Intervirology* 2010;**53**:10–14.
195. Klevens RM, Kruszon-Moran D, Wasley A, et al. Seroprevalence of hepatitis A virus antibodies in the U.S.: results from the National Health and Nutrition Examination Survey. *Public Health Rep* 2011;**126**:522–32.
196. Klevens RM, Miller JT, Iqbal K, et al. The evolving epidemiology of hepatitis a in the United States: incidence and molecular epidemiology from population-based surveillance, 2005-2007. *Arch Intern Med* 2010;**170**:1811–18.
197. Klevens RM, Sharapov U Hepatitis A. *Manual for the surveillance of vaccine-preventable diseases*. 5th ed. Atlanta: Centers for Disease Control and Prevention; 2011.
207. Kumar P, Bhatia V. Prolonged cholestasis due to hepatitis A virus infection. *Indian Pediatr* 2011;**48**:485–6.
209. Kurugol Z, Aslan A, Turkoglu E, et al. Changing epidemiology of hepatitis A infection in Izmir, Turkey. *Vaccine* 2011;**29**:6259–61.
215. Lee H, Cho HK, Kim JH, et al. Seroepidemiology of hepatitis A in Korea: changes over the past 30 years. *J Korean Med Sci* 2011;**26**:791–6.
216. Lee HC, Ang LW, Chiew PK, et al. Changing epidemiological patterns of hepatitis A infection in Singapore. *Ann Acad Med Singapore* 2011;**40**:439–47.
235. Manangeeswaran M, Jacques J, Tami C, et al. Binding of hepatitis a virus to its cellular receptor 1 inhibits T-regulatory cell functions in humans. *Gastroenterol* 2012;**142**:1516–25 e1513.
251. McQuillan G, Kruszon-Moran D, Enniston M, et al. Viral hepatitis. *NCHS Data Brief* 2010;**27**:1–8.

257. Mor Z, Srur S, Dagan R. Hepatitis A disease following the implementation of universal vaccination: who is at risk? *J Viral Hepat* 2010;**17**:293–7.
259. Motte A, Blanc J, Minodier P. Acute hepatitis A in a pregnant woman at delivery. *Int J Infect Dis* 2009;**13**:e49–51.
280. Ott JJ, Irving G, Wiersma ST. Long-term protective effects of hepatitis A vaccines: a systematic review. *Vaccine* 2012;**31**:3–11.
282. Park GC, Hwang S, Yu YD, et al. Intractable recurrent hepatitis A virus infection requiring repeated liver transplantation: a case report. *Transplant Proc* 2010;**42**:4658–60.
285. Pelletier AR, Mehta PJ, Burgess DR, et al. An outbreak of hepatitis A among primary and secondary contacts of an international adoptee. *Public Health Rep* 2010;**125**:642–6.
289. Petrignani M, Harms M, Verhoef L, et al. Update: a food-borne outbreak of hepatitis A in the Netherlands related to semi-dried tomatoes in oil, January-February 2010. *Euro Surveill* 2010; **15**(20).
290. Petrignani M, Verhoef L, van Hunen R, et al. A possible food-borne outbreak of hepatitis A in the Netherlands, January-February 2010. *Euro Surveill* 2010;**15**(11).
298. Qu L, Feng Z, Yamane D, et al. Disruption of TLR3 signaling due to cleavage of TRIF by the hepatitis A virus protease-polymerase processing intermediate, 3CD. *PLoS Pathog* 2011;**7**: e1002169.
311. Ryu HS, Park SY, Lim SR, et al. Clinical characteristics and gestational complications associated with acute hepatitis a in pregnancy. *Korean J Gastroenterol* 2010;**56**:307–13.
319. Selander B, Blackberg J, Widell A, et al. No evidence of intrauterine transmission of hepatitis A virus from a mother to a premature infant. *Acta Paediatr* 2009;**98**:1603–6.
320. Sfetcu O, Irvine N, Ngui SL, et al. Hepatitis A outbreak predominantly affecting men who have sex with men in Northern Ireland, October 2008 to July 2009. *Euro Surveill* 2011;**16**(9).
326. Sharapov UM, Bulkow LR, Negus SE, et al. Persistence of hepatitis A vaccine induced seropositivity in infants and young children by maternal antibody status: 10-year follow-up. *Hepatology* 2012;**56**:516–22.
334. Simsek Y, Isik B, Karaer A, et al. Fulminant hepatitis A infection in second trimester of pregnancy requiring living-donor liver transplantation. *J Obstet Gynaecol Res* 2012;**38**:745–8.
341. Song HU, Hwang SG, Kwon CI, et al. Molecular epidemiology of hepatitis A virus in the South-East area of Gyeonggi-do in Korea. *Yonsei Med J* 2009;**50**:512–16.
348. Sweet K, Sutherland W, Ehresmann K, et al. Hepatitis A infection in recent international adoptees and their contacts in Minnesota, 2007-2009. *Pediatrics* 2011;**128**:e333–8.
364. Tortajada C, de Olalla PG, Diez E, et al. Hepatitis A among men who have sex with men in Barcelona, 1989-2010: insufficient control and need for new approaches. *BMC Infectious diseases* 2012;**12**:11.
365. Tortajada C, de Olalla PG, Pinto RM, et al. Outbreak of hepatitis A among men who have sex with men in Barcelona, Spain, September 2008-March 2009. *Euro Surveill* 2009;**14**(15).
369. Tsou TP, Liu CC, Huang JJ, et al. Change in hepatitis A epidemiology after vaccinating high risk children in Taiwan, 1995-2008. *Vaccine* 2011;**29**:2956–61.
373. Van Herck K, Crasta PD, Messier M, et al. Seventeen-year antibody persistence in adults primed with two doses of an inactivated hepatitis A vaccine. *Human Vaccin Immunother* 2012;**8**:323–7.
374. Van Damme P, Leroux-Roels G, Crasta P, et al. Antibody persistence and immune memory in adults, 15 years after a three-dose schedule of a combined hepatitis A and B vaccine. *J Med Virol* 2012;**84**:11–17.
407. Yoon YK, Yeon JE, Kim JH, et al. Comparative analysis of disease severity between genotypes IA and IIIA of hepatitis A virus. *J Med Virol* 2011;**83**:1308–14.
408. Yoon EL, Yim HJ, Kim SY, et al. Clinical courses after administration of oral corticosteroids in patients with severely cholestatic acute hepatitis A: three cases. *Korean J Hepatol* 2010;**16**:329–33.
410. Yun H, Lee HJ, Jang JH, et al. Hepatitis A virus genotype and its correlation with the clinical outcome of acute hepatitis A in Korea: 2006-2008. *J Med Virol* 2011;**83**:2073–81.

The full reference list for this chapter is available at expertconsult.com.

RNA VIRUSES

SUBSECTION 2 *CALICIVIRIDAE*

CHAPTER 168

CALICIVIRUSES

Yalda C. Lucero • Miguel L. O'Ryan • David O. Matson

Caliciviridae is a family that currently includes five genera that differ in genomic organization and phylogenetic comparisons of genomic sequence identity, named *Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*, and *Nebovirus*.[38,69]

The first calicivirus-associated disease in animals was described in animals in 1932, when outbreaks of a vesicular exanthema restricted to domestic pigs occurred in California and spread through the United States (vesicular exanthema of swine virus [VESV] infection, prototype of the *Vesivirus* genus).[11] Although diagnostic tools were not available to identify the causative agent, epidemiologic observations suggested an infective origin that was transmitted probably through contaminated food. Extensive programs of sterilization of feed and restriction of feed types finally stopped the outbreaks in the 1950s. In the 1970s, VESV strains were first

REFERENCES

1. Abe H, Beninger PR, Ikejiri N, et al. Light microscopic findings of liver biopsy specimens from patients with hepatitis type A and comparison with type B. *Gastroenterology* 1982;**82**(5 Pt 1): 938–47.
2. Ajmera V, Xia G, Vaughan G, et al. What factors determine the severity of hepatitis A-related acute liver failure? *J Viral Hepatit* 2011;**18**:e167–74.
3. Akriviadis EA, Redeker AG. Fulminant hepatitis A in intravenous drug users with chronic liver disease. *Ann Inter Med* 1989;**110**: 838–9.
4. Alhan E, Yildizdaş D, Yapicioğlu H, et al. Pleural effusion associated with acute hepatitis A infection [Letter]. *Pediatr Infect Dis J* 1999;**18**:1111–12.
5. Amado LA, Marchevsky RS, de Paula VS, et al. Experimental hepatitis A virus (HAV) infection in cynomolgus monkeys *(Macaca fascicularis):* evidence of active extrahepatic site of HAV replication. *Int J Exp Pathol* 2010;**91**:87–97.
6. Amado LA, Villar LM, de Paula VS, et al. Comparison between serum and saliva for the detection of hepatitis A virus RNA. *J Virol Methods* 2008;**148**:74–80.
7. Amado LA, Villar LM, de Paula VS, et al. Exposure to multiple subgenotypes of hepatitis A virus during an outbreak using matched serum and saliva specimens. *J Med Virol* 2011;**83**: 768–75.
8. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, editor. *2006 red book: report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006.
9. Amon JJ, Darling N, Fiore AE, et al. Factors associated with hepatitis a vaccination among children 24 to 35 months of age: United States, 2003. *Pediatrics* 2006;**117**:30–3.
10. Amon JJ, Devasia R, Xia G, et al. Molecular epidemiology of foodborne hepatitis a outbreaks in the United States, 2003. *J Inf Dis* 2005;**192**:1323–30.
11. Anderson DA. Cytopathology, plaque assay, and heat inactivation of hepatitis A virus strain HM175. *J Med Virol* 1987;**22**:35–44.
12. Armstrong GL, Bell BP. Hepatitis A virus infections in the United States: model-based estimates and implications for childhood immunization. *Pediatrics* 2002;**109**:839–45.
13. Armstrong KE, Bush HM, Collins JD, et al. Role of CD4 count in immunity development after hepatitis A and B vaccination among HIV-infected patients: Kentucky, 2002-2007. *J Int Assoc Physicians AIDS Care (Chic)* 2010;**9**:179–86.
14. Armstrong ME, Giesa PA, Davide JP, et al. Development of the formalin-inactivated hepatitis A vaccine, VAQTA from the live attenuated virus strain CR326F. *J Hepatol* 1993;**18**(Suppl. 2): S20–26.
15. Arora NK, Nanda SK, Gulati S, et al. Acute viral hepatitis types E, A, and B singly and in combination in acute liver failure in children in north India. *J Med Virol* 1996;**48**:215–21.
16. Averhoff F, Shapiro CN, Bell BP, et al. Control of hepatitis A through routine vaccination of children. *JAMA* 2001;**286**:2968–73.
17. Aydin A, Mikla S, Ficicioglu C, et al. Nephrotic syndrome associated with hepatitis A virus infection. *Pediatr Infect Dis J* 1999; **18**:391.
18. Aydoğdu S, Ozgenç F, Yurtsever S, et al. Our experience with fulminant hepatic failure in Turkish children: etiology and outcome. *J Trop Pediatr* 2003;**49**:367–70.
19. Azuri J, Lerman-Sagie T, Mizrahi A, et al. Guillain-Barré syndrome following serological evidence of hepatitis A in a child. *Eur J Pediatr* 1999;**158**:341–2.
20. Balcarek KB, Bagley MR, Pass RF, et al. Safety and immunogenicity of an inactivated hepatitis A vaccine in preschool children. *J Infect Dis* 1995;**171**(Suppl. 1):S70–72.
21. Bariş Z, Saltik T, Temizel IN, et al. Acute Liver failure in children: 20 year experience. *Turk J Gastroenterol* 2012;**23**:127–34.
22. Bartoloni A, Aquilini D, Roselli M, et al. Prevalence of antibody to hepatitis A virus in the Santa Cruz region of Bolivia. *J Trop Med Hyg* 1989;**92**:279–81.
23. Batra Y, Bhatkal B, Ojha B, et al. Vaccination against hepatitis A virus may not be required for schoolchildren in northern India: results of a seroepidemiological survey. *Bull World Health Organ* 2002;**80**:728–31.
24. Beck BR, Hatz C, Bronnimann R, Herzog C. Successful booster antibody response up to 54 months after single primary vaccination with virosome-formulated, aluminum-free hepatitis A vaccine. *Clin Infect Dis* 2003;**37**:e126–8.
25. Bell BP. Global epidemiology of hepatitis A: Implications for control strategies. In: Margolis HS, Alter MJ, Liang TJ, et al, editors: *Viral hepatitis and liver disease*. London: International Medical Press; 2002.
26. Bell BP, Margolis HS. Hepatitis A vaccine for secondary hepatitis A infection [Letter]. *Lancet* 1999;(354):341.
27. Bell BP, Negus S, Fiore AE, et al. Immunogenicity of an inactivated hepatitis A vaccine in infants and young children. *Pediatr Infect Dis J* 2007;**26**:116–22.
28. Bell BP, Shapiro CN, Alter MJ, et al. The diverse patterns of hepatitis A epidemiology in the United States: implications for vaccination strategies. *J Infect Dis* 1998;**178**:1579–84.
29. Benenson MW, Takafuji ET, Bancroft WH, et al. A military community outbreak of hepatitis type A related to transmission in a child care facility. *Am J Epidemiol* 1980;**112**:471–81.
30. Bergeisen GH, Hinds MW, Skaggs JW. A waterborne outbreak of hepatitis A in Meade County, Kentucky. *Am J Public Health* 1985;**75**:161–4.
31. Bialek SR, Barry V, Bell BP, et al. Seroprevalence and correlates of hepatitis a among HIV-negative American men who have sex with men. *Sex Health* 2011;**8**:343–8.
32. Bialek SR, George PA, Xia GL, et al. Use of molecular epidemiology to confirm a multistate outbreak of hepatitis A caused by consumption of oysters. *Clin Infect Dis* 2007;**44**:838–40.
33. Bialek SR, Thoroughman DA, Hu D, et al. Hepatitis A incidence and hepatitis A vaccination among American Indians and Alaska Natives, 1990-2001. *Am J Public Health* 2004;**94**:996–1001.
34. Binn LN, Lemon SM, Marchwicki RH, et al. Primary isolation and serial passage of hepatitis A virus strains in primate cell cultures. *J Clin Microbiol* 1984;**20**:28–33.
35. Biologicals GlaxoSmithKline. *HAVRIX [package insert]*. Rixensart, Belgium: GlaxoSmithKline Biologicals; 2005.
36. Black S, Shinefield H, Hansen J, et al. A post-licensure evaluation of the safety of inactivated hepatitis A vaccine (VAQTA, Merck) in children and adults. *Vaccine* 2004;**22**:766–72.
37. Bloch AB, Stramer SL, Smith JD, et al. Recovery of hepatitis A virus from a water supply responsible for a common source outbreak of hepatitis A. *Am J Public Health* 1990;**80**:428–30.
38. Bonanni P, Colombai R, Franchi G, et al. Experience of hepatitis A vaccination during an outbreak in a nursery school of Tuscany, Italy. *Epidemiol Infection* 1998;**121**:377–80.
39. Bordi L, Rozera G, Scognamiglio P, et al. Monophyletic outbreak of hepatitis A involving HIV-infected men who have sex with men, Rome, Italy 2008-2009. *J Clin Virol* 2012;**54**:26–9.
40. Bosch VV, Dowling PC, Cook SD. Hepatitis A virus immunoglobulin M antibody in acute neurological disease. *Ann Neurol* 1983;**14**:685–7.
41. Bottiger M, Christenson B, Grillner L. Hepatitis A immunity in the Swedish population: a study of the prevalence of markers in the Swedish population. *Scand J Infect Dis* 1997;**29**:99–102.
42. Bowen GS, McCarthy MA. Hepatitis A associated with a hardware store water fountain and a contaminated well in Lancaster County, Pennsylvania, 1980. *Am J Epidemiol* 1983;**117**:695–705.
43. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. *J Infect Dis* 2000;**182**: 12–17.
44. Bradley DW, Fields HA, McCaustland KA. Biochemical and biophysical characterization of light and heavy density hepatitis A virus particles: evidence HAV is an RNA virus. *J Med Virol* 1978; **2**:175–87.
45. Brown GR, Persley K. Hepatitis A epidemic in the elderly. *South Med J* 2002;**95**:826–33.
46. Brown VK, Robertson. BH. Immunoselection of clinical specimens containing virus followed by polymerase chain reaction amplification and rapid direct sequencing. *Biotechniques* 1990;**8**: 262–4.
47. Bryan JA, Lehmann JD, Setiady IF, et al. An outbreak of hepatitis-A associated with recreational lake water. *Am J Epidemiol* 1974;**99**: 145–54.
48. Bryan JP, Nelson M. Testing for antibody to hepatitis A to decrease the cost of hepatitis A prophylaxis with immune globulin or hepatitis A vaccines. *Arch Inter Med* 1994;**154**:663–8.

49. Bulkow LR, Wainwright RB, McMahon BJ, et al. Secular trends in hepatitis A virus infection among Alaska Natives. *J Infect Dis* 1993;**168**:1017–20.
50. Cao J, Bi S, Meng Q, et al. Genotyping of acute hepatitis a virus isolates from China, 2003-2008. *J Med Virol* 2011;**83**:1134–41.
51. Carl M, Francis DP, Maynard JE. Food-borne hepatitis A: recommendations for control. *J Infect Dis* 1983;**148**:1133–5.
52. Center for Disease Control and Prevention. Safety of therapeutic immune globulin preparations with respect to transmission of human T-lymphotropic virus type III/lymphadenopathy-associated virus infection. *MMWR Morb Mortal Wkly Rep* 1986; **35**:231–3.
53. Center for Disease Control and Prevention. Hepatitis A among homosexual men: United States, Canada, and Australia. *MMWR Morb Mortal Wkly Rep* 1992;**41**(155):161–4.
54. Center for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1996;**45**(RR-15):1–30.
55. Center for Disease Control and Prevention. Hepatitis A vaccination programs in communities with high rates of hepatitis A. *MMWR Morb Mortal Wkly Rep* 1997;**46**:600–3.
56. Center for Disease Control and Prevention. Hepatitis A among drug abusers. *MMWR Morb Mortal Wkly Rep* 1988;**37**:297–300, 305.
57. Center for Disease Control and Prevention. Summary of notifiable diseases, United States, 1997. *MMWR Morb Mortal Wkly Rep* 1998;**46**:ii–vii, 3–87.
58. Center for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1999;**48**(RR-12):1–36.
59. Center for Disease Control and Prevention. General recommendations on immunizations: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2002;**51**:1–36.
60. Center for Disease Control and Prevention. Blood safety and Monitoring among persons with bleeding disorders-United States, May 1998-June 2002. *MMWR Morb Mortal Wkly Rep* 2003; **51**:1152–4.
61. Center for Disease Control and Prevention. *Hepatitis surveillance, report no. 60*. Atlanta: CDC; 2005. Available at: http://www.cdc.gov/ncidod/diseases/hepatitis/resource/PDFs/hep_surveillance_60.pdf.
62. Center for Disease Control and Prevention. Positive test results for acute hepatitis A virus among persons with no recent history of acute hepatitis: United States, 2002-2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:453–6.
63. Center for Disease Control and Prevention. *Hepatitis surveillance, report no 61*. Atlanta: CDC; 2006. Available at: http://www.cdc.gov/ncidod/diseases/hepatitis/resource/PDFs/hep_surveillance_61.pdf.
64. Center for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendation from the advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2006;**55**(RR-7):1–23.
65. Center for Disease Control and Prevention. Prevention of hepatitis A after exposure to hepatitis A virus in international travelers: updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007;**56**:1080–4.
66. Center for Disease Control and Prevention. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1006–7.
67. Center for Disease Control and Prevention. *Viral hepatitis surveillance, United States 2009*. Atlanta: CDC; 2009. Available at: http://www.cdc.gov/hepatitis/Statistics/2009Surveillance/index.htm.
68. Center for Disease Control and Prevention. *Viral hepatitis surveillance, United States*. Atlanta: CDC; 2010. Available at: http://www.cdc.gov/hepatitis/Statistics/2010Surveillance/index.htm.
69. Center for Disease Control and Prevention. Hepatitis A. In: *Epidemiology and prevention of vaccine preventable disease*. U. S. Department of Health and Human Services. CDC; ed, 2012. p. 101–14.
70. Center for Disease Control and Prevention. Summary of notifiable disease, United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–11.
71. Chan TK, Todd D. Haemolysis complicating viral hepatitis in patients with glucose-6-phosphate dehydrogenase deficiency. *Br Med J* 1975;**1**:131–3.
72. Christenson B. Epidemiological aspects of acute viral hepatitis A in Swedish travellers to endemic areas. *Scand J Infect Dis* 1985;**17**:5–10.
73. Cianciara J. Hepatitis A shifting epidemiology in Poland and Eastern Europe. *Vaccine* 2000;**18**(Suppl. 1):S68–70.
74. Ciocca M. Clinical course and consequences of hepatitis A infection. *Vaccine* 2000;**18**(Suppl. 1):S71–74.
75. Clemens R, Safary A, Hepburn A, et al. Clinical experience with an inactivated hepatitis A vaccine. *J Infect Dis* 1995;**171**(Suppl. 1): S44–49.
76. Cohen HA, Amir J, Frydman M, et al. Infection with the hepatitis A virus associated with ascites in children. *Am J Dis Child* 1992; **146**:1014–16.
77. Cohen JI, Feinstone S, Purcell RH. Hepatitis A virus infection in a chimpanzee: duration of viremia and detection of virus in saliva and throat swabs. *J Infect Dis* 1989;**160**:887–90.
78. Cohn EJ, Oncley JL, Strong LE, et al. Chemical, clinical, and immunological studies on the products of human plasma fractionation. I. The characterization of the protein fractions of human plasma. *J Clin Invest* 1944;**23**:417–32.
79. Cooksley WGE. What did we learn from the Shanghai hepatitis A epidemic? *J Viral Hepat* 2000;**7**(Suppl. 1):1–3.
80. Costa-Mattioli M, Di Napoli A, Ferre V, et al. Genetic variability of hepatitis A virus. *J Gen Virol* 2003;**84**(Pt 12):3191–201.
81. Costa-Mattioli M, Di Napoli A, Ferre V, et al. Genetic variability of hepatitis A virus. *PowerPoint*, *J Gen Virol* 2003.
82. Cotter S, Sansom S, Lee B, et al. Outbreak of hepatitis A among men who have sex with men: implications for hepatitis A vaccination strategies. *J Infect Dis* 2003;**187**:1235–40.
83. Coursaget P, Leboulleux D, Gharbi Y, et al. Etiology of acute sporadic hepatitis in adults in Senegal and Tunisia. *Scand J Infec Dis* 1995;**27**:9–11.
84. Coutinho RA, Albrecht-van Lent P, Lelie N, et al. Prevalence and incidence of hepatitis A among male homosexuals. *Br Med J* 1983;**287**:1743–5.
85. Craig AS, Sockwell DC, Schaffner W, et al. Use of hepatitis A vaccine in a community-wide outbreak of hepatitis A. *Clin Infect Dis* 1998;**27**:531–5.
86. Crance JM, Biziagos E, Passagot J, et al. Inhibition of hepatitis A virus replication in vitro by antiviral compounds. *J Med Virol* 1990;**31**:155–60.
87. Cromeans T, Nainan OV, Fields HA, et al. Hepatitis A and E viruses. In: Hui YH, Gorham JR, Murrel KD, et al, editors: *Foodborne disease handbook*. New York: Marcel Dekker; 1994. p. 1–56.
88. Cromeans T, Sobsey MD, Fields HA. Development of a plaque assay for a cytopathic, rapidly replicating isolate of hepatitis A virus. *J Med Virol* 1987;**22**:45–56.
89. Crum-Cianflone NF, Wilkins K, Lee AW, et al. Long-term durability of immune responses after hepatitis A vaccination among HIV-infected adults. *J Infect Dis* 2011;**203**:1815–23.
90. D'Hondt E. Possible approaches to develop vaccines against hepatitis A. *Vaccine* 1992;**10**(Suppl. 1):S48–52.
91. Daemer RJ, Feinstone SM, Gust ID, et al. Propagation of human hepatitis A virus in African green monkey kidney cell culture: primary isolation and serial passage. *Infect Immun* 1981;**32**:388–93.
92. Dagan R, Amir J, Mijalovsky A, et al. Safary A. and Ashkenazi S. Immunization against hepatitis A in the first year of life: priming despite the presence of maternal antibody. *Pediatr Infect Dis J* 2000;**19**:1045–52.
93. Dagan R, Leventhal A, Anis E, et al. Incidence of hepatitis A in Israel following universal immunization of toddlers. *JAMA* 2005;**294**:202–10.
94. Dalton CB, Haddix A, Hoffman RE, et al. The cost of a foodborne outbreak of hepatitis A in Denver, Colo. *Arch Intern Med* 1996;**156**:1013–16.
95. Dan M, Yaniv R. Cholestatic hepatitis, cutaneous vasculitis, and vascular deposits of immunoglobulin M and complement associated with hepatitis A virus infection. *Am J Med* 1990;**89**: 103–4.

96. Datta D, Williams I, Culver D, et al. Association between deaths due to hepatitis A and chronic liver disease, United States, 1981-1997. *Antiviral Ther* 2000;**5**(Suppl. 1):507–10.
97. Daudi N, Shouval D, Stein-Zamir C, Ackerman Z. Breastmilk hepatitis A virus RNA in nursing mothers with acute hepatitis A virus infection. *Breastfeed Med* 2012;**7**:313–15.
98. De Serres G, Cromeans TL, Levesque B, et al. Molecular confirmation of hepatitis A virus from well water: epidemiology and public health implications. *J Infect Dis* 1999;**179**:37–43.
99. Debray D, Cullufi P, Devictor D, et al. Liver failure in children with hepatitis A. *Hepatol* 1997;**26**:1018–22.
100. Demircin G, Oner A, Tinaztepe K, et al. Acute glomerulonephritis in hepatitis A virus infection. *J Pediatr Gastrogastroenterol Nutr* 1998;**27**:86–9.
101. Dentinger CM, Bower WA, Nainan OV, et al. An outbreak of hepatitis A associated with green onions. *J Infect Dis* 2001;**183**: 1273–6.
102. Dentinger CM, Heinrich NL, Bell BP, et al. A prevalence study of hepatitis A virus infection in a migrant community: is hepatitis A vaccine indicated? *J Pediatr* 2001;**138**:705–9.
103. Desbois D, Couturier E, Mackiewicz V, et al. Epidemiology and genetic characterization of hepatitis A virus genotype IIA. *J Clin Microbiol* 2010;**48**:3306–15.
104. Desenclos JC, MacLafferty L. Community wide outbreak of hepatitis A linked to children in day care centres and with increased transmission in young adult men in Florida 1988-9. *J Epidemiol Community Health* 1993;**47**:269–73.
105. Desenclos JC, Klontz KC, Wilder MH, et al. A. A multistate outbreak of hepatitis A caused by the consumption of raw oysters. *Am J Public Health* 1991;**81**:1268–72.
106. Diaz-Mitoma F, Law B, Parsons J. A combined vaccine against hepatitis A and B in children and adolescents. *Pediatr Infect Dis J* 1999;**18**:109–14.
107. Dienstag JL, Gust ID, Lucas CR, et al. Mussel-associated viral hepatitis, type A: serological confirmation. *Lancet* 1976;**1**:561–3.
108. Dmochowski L. Viral type A and type B hepatitis: morphology, biology, immunology and epidemiology: a review. *Am J Clin Pathol* 1976;**65**(Suppl. 5):741–86.
109. Dollberg S, Berkun Y, Gross-Kieselstein E. Urticaria in patients with hepatitis A virus infection. *Pediatr Infec Dis J* 1991;**10**:702–3.
110. Dollberg S, Kerem E, Klar A, et al. Disappearance of IgM antibodies to hepatitis A virus after an acute infection in children and adolescents. *J Pediatr Gastroenterol Nutr* 1990;**10**:307–9.
111. Dominguez A, Salleras L, Carmona G, et al. Effectiveness of a mass hepatitis A vaccination program in preadolescents. *Vaccine* 2003;**21**:698–701.
112. Donnan EJ, Fielding JE, Gregory JE, et al. A multistate outbreak of hepatitis A associated with semidried tomatoes in Australia, 2009. *Clin Infect Dis* 2012;**54**:775–81.
113. Dotzauer A, Brenner M, Gebhardt U, et al. IgA-coated particles of Hepatitis A virus are translocalized antivectorially from the apical to the basolateral site of polarized epithelial cells via the polymeric immunoglobulin receptor. *J Gen Virol* 2005;**86** (Pt 10):2747–51.
114. Dotzauer A, Gebhardt U, Bieback K, et al. Hepatitis A virus-specific immunoglobulin A mediates infection of hepatocytes with hepatitis A virus via the asialoglycoprotein receptor. *J Virol* 2000; **74**:10950–7.
115. Dotzauer A, Heitmann A, Laue T, et al. The role of immunoglobulin A in prolonged and relapsing hepatitis A virus infections. *J Gen Virol* 2012;**93**(Pt 4):754–60.
116. Draelos ZK, Hansen RC, James WD. Gianotti-Crosti syndrome associated with infections other than hepatitis B. *JAMA* 1986;**256**: 2386–8.
117. Eisenbach C, Longerich T, Fickenscher H, et al. Recurrence of clinically significant hepatitis A following liver transplantation for fulminant hepatitis A. *J Clin Virol* 2006;**35**:109–12.
118. Elinav E, Ben-Dov IZ, Shapira Y, et al. Acute hepatitis A infection in pregnancy is associated with high rates of gestational complications and preterm labor. *Gastroenterol* 2006;**130**:1129–34.
119. Ellis EF, Henney CS. Adverse reactions following administration of human gamma globulin. *J Allergy Clin Immunolol* 1969;**43**:45–54.
120. Emerson SU, Huang YK, McRill C, et al. Mutations in both the 2B and 2C genes of hepatitis A virus are involved in adaptation to growth in cell culture. *J Virol* 1992;**66**:650–4.
121. Emerson SU, Huang YK, Nguyen H, et al. Identification of VP1/2A and 2C as virulence genes of hepatitis A virus and demonstration of genetic instability of 2C. *J Virol* 2002;**76**:8551–9.
122. Emerson SU, McRill C, Rosenblum B, et al. Mutations responsible for adaptation of hepatitis A virus to efficient growth in cell culture. *J Virol* 1991;**65**:4882–6.
123. Erkan T, Kutlu T, Cullu F, et al. A case of vertical transmission of hepatitis A virus infection. *Acta Paediatr* 1998;**87**:1008–9.
124. Ertem D, Acar Y, Arat C, et al. Thrombotic and thrombocytopenic complications secondary to hepatitis A infection in children. *Am J Gastroenterol* 1999;**94**:3653–5.
125. European Association for the study of the liver. Randomised trial of steroid therapy for acute liver failure. *Gut* 1979;**20**: 620–3.
126. Fagan E, Yousef G, Brahm J, et al. Persistence of hepatitis A virus in fulminant hepatitis and after liver transplantation. *J Med Virol* 1990;**30**:131–6.
127. Fainboim L, Canero Velasco MC, Marcos CY, et al. Protracted, but not acute, hepatitis A virus infection is strongly associated with HLA-DRB*1301, a marker for pediatric autoimmune hepatitis. *Hepatol* 2001;**33**:1512–17.
128. Fan PC, Chang MH, Lee PI, et al. Follow-up immunogenicity of an inactivated hepatitis A virus vaccine in healthy children: results after 5 years. *Vaccine* 1998;**16**:232–5.
129. Favero MS. Disinfection and sterilization. In: Zuckerman AJ, Thomas HC, editors: *Viral hepatitis: scientific basis and management*. New York: Churchill Livingstone; 1993. p. 565–75.
130. Favero MS, Maynard JE, Leger RT, et al. Guidelines for the care of patients hospitalized with viral hepatitis. *Ann Intern Med* 1979;**91**:872–6.
131. Feigelstock D, Thompson P, Mattoo P, et al. The human homolog of HAVcr-1 codes for a hepatitis A virus cellular receptor. *J Virol* 1998;**72**:6621–8.
132. Feigelstock DA, Thompson P, Kaplan GG. Growth of hepatitis A virus in a mouse liver cell line. *J Virol* 2005;**79**:2950–5.
133. Feinstone SM, Kapikian AZ, Purcell RH. Hepatitis A: Detection by immune electron microscopy of a virus like antigen association with acute illness. *Science* 1973;**182**:1026–8.
134. Fensterl V, Grotheer D, Berk I, et al. Hepatitis A virus suppresses RIG-I-mediated IRF-3 activation to block induction of beta interferon. *J Virol* 2005;**79**:10968–77.
135. Fiore AE. Hepatitis A transmitted by food. *Clin Infect Dis* 2004;**38**:705–15.
136. Fiore AE, Shapiro CN, Sabin K, et al. Hepatitis A vaccination of infants: effect of maternal antibody status on antibody persistence and response to a booster dose. *Pediatr Infect Dis J* 2003;**22**: 354–9.
137. Fischer GE, Teshale EH, Miller C, et al. Hepatitis A among international adoptees and their contacts. *Clin Infect Dis* 2008;**47**:812–14.
138. Fishman LN, Jonas MM, Lavine JE. Update on viral hepatitis in children. *Pediatr Clin North Am* 1996;**43**:57–74.
139. Fleischer B, Fleischer S, Maier K, et al. Clonal analysis of infiltrating T lymphocytes in liver tissue in viral hepatitis A. *Immunology* 1990;**69**:14–19.
140. Fornasini MA, Morrow AL, Pickering LK. Illness and health-related benefits among child daycare providers. Abstract 835. Paper presented at a meeting of the American Pediatric Society and Society for Pediatric Research, Seattle, 1994.
141. Franzen C, Frosner G. Placental transfer of hepatitis A antibody. *N Engl J Med* 1981;**304**:427.
142. Friedman LS, O'Brien TF, Morse LJ, et al. Revisiting the Holy Cross football team hepatitis outbreak (1969) by serological analysis. *JAMA* 1985;**254**:774–6.
143. Friedman MS, Blake PA, Koehler JE, et al. Factors influencing a communitywide campaign to administer hepatitis A vaccine to men who have sex with men. *Am J Public Health* 2000;**90**: 1942–6.
144. Fujiwara K, Yokosuka O, Ehata T, et al. Association between severity of type A hepatitis and nucleotide variations in the 5′ non-translated region of hepatitis A virus RNA: strains from fulminant hepatitis have fewer nucleotide substitutions. *Gut* 2002;**51**: 82–8.
145. Fujiwara K, Yokosuka O, Fukai K, et al. Analysis of full-length hepatitis A virus genome in sera from patients with fulminant and self-limited acute type A hepatitis. *J Hepatol* 2001;**35**:112–19.

146. Gallot C, Grout L, Roque-Afonso AM, et al. Hepatitis A associated with semidried tomatoes, France, 2010. *Emerg Infect Dis* 2011;**17**:566–7.
147. Gerety RJ, Smallwood LA, Finlayson JS, et al. Standardization of the antibody to hepatitis A virus (anti-HAV) content of immunoglobulin. *Dev Biol Stand* 1983;**54**:411–16.
148. Gibas A, Blewett DR, Schoenfeld DA, et al. Prevalence and incidence of viral hepatitis in health workers in the prehepatitis B vaccination era. *Am J Epidemiol* 1992;**136**:603–10.
149. Gil A, Gonzalez A, Dal-Re R, et al. Prevalence of antibodies against varicella zoster, herpes simplex (types 1 and 2), hepatitis B and hepatitis A viruses among Spanish adolescents. *J Infect* 1998; **36**:53–6.
150. Gimson AE, White YS, Eddleston AL, et al. Clinical and prognostic differences in fulminant hepatitis type A, B and non-A non-B. *Gut* 1983;**24**:1194–8.
151. Gingrich GA, Hadler SC, Elder HA, et al. Serologic investigation of an outbreak of hepatitis A in a rural day-care center. *Am J Public Health* 1983;**73**:1190–3.
152. Glikson M, Galun E, Oren R, et al. Relapsing hepatitis A: review of 14 cases and literature survey. *Medicine (Baltimore)* 1992;**71**:14–23.
153. Goodman RA. Nosocomial hepatitis A. *Ann Intern Med* 1985; **103**:452–4.
154. Gordon SC, Reddy KR, Schiff L, et al. Prolonged intrahepatic cholestasis secondary to acute hepatitis A. *Ann Intern Med* 1984; **101**:635–7.
155. Green MS, Block C, Slater PE. Rise in the incidence of viral hepatitis in Israel despite improved socioeconomic conditions. *Rev Infect Dis* 1989;**11**:464–9.
156. Gust ID, Feinstone SM. History. In: Gust ID, Feinstone SM, editors. *Hepatitis*. Boca Raton, Fla: CRC Press; 1988.
157. Hadler SC. Global impact of hepatitis A virus infection: changing patterns. In: Hollinger FB, Lemon SM, Margolis HS, editors: *Viral hepatitis and liver disease*. Baltimore: Williams & Wilkins; 1991. p. 14–20.
158. Hadler SC, Erben JJ, Matthews D, et al. Effect of immunoglobulin on hepatitis A in day-care centers. *JAMA* 1983;**249**:48–53.
159. Hadler SC, Webster HM, Erben JJ, et al. Hepatitis A in day-care centers: a community-wide assessment. *New Engl J Med* 1980;**302**: 1222–7.
160. Halliday ML, Kang LY, Zhou TK, et al. An epidemic of hepatitis A attributable to the ingestion of raw clams in Shanghai, China. *J Infect Dis* 1991;**164**:852–9.
161. Hammitt LL, Bulkow L, Hennessy TW. Persistence of antibody to hepatitis A virus 10 years after vaccination among children and adults. *J Infect Dis* 2008;**198**:1776–82.
162. Harkess J, Gildon B, Istre GR. Outbreaks of hepatitis A among illicit drug users, Oklahoma, 1984-87. *Am J Public Health* 1989; **79**:463–6.
163. Hatzakis A, Hadziyannis S. Sex-related differences in immunoglobulin M and in total antibody response to hepatitis A virus observed in two epidemics of hepatitis A. *Am J Epidemiol* 1984; **120**:936–42.
164. Hayashi MST, Tsunematsu I, Hirokawa F, et al. Hepatitis A virus-related late-onset hepatic failure: a case report. *Exp Clin Transplant* 2011;**9**:150–2.
165. Henning KJ, Bell E, Braun J, et al. A community-wide outbreak of hepatitis A: risk factors for infection among homosexual and bisexual men. *Am J Med* 1995;**99**:132–6.
166. Hess G, Clemens R, Bienzle U, et al. Immunogenicity and safety of an inactivated hepatitis A vaccine in anti-HIV positive and negative homosexual men. *J Med Virol* 1995;**46**:40–2.
167. Holmberg SD. Hepatitis a epidemiology goes global. *Clin Infect Dis* 2012;**54**:782–3.
168. Hoover KW, Butler M, Workowski KA, et al. Low rates of hepatitis screening and vaccination of HIV-infected MSM in HIV clinics. *Sex Transm Dis* 2012;**39**:349–53.
169. Horng YC, Chang MH, Lee CY, et al. Safety and immunogenicity of hepatitis A vaccine in healthy children. *Pediatr Infect Dis J* 1993;**12**:359–62.
170. Hussain Z, Husain SA, Almajhdi FN, et al. Immunological and molecular epidemiological characteristics of acute and fulminant viral hepatitis A. *Virol J* 2011;**8**:254.
171. Hutin YJ, Bell BP, Marshall KL, et al. Identifying target groups for a potential vaccination program during a hepatitis A communitywide outbreak. *Am J Public Health* 1999;**89**:918–21.
172. Hutin YJ, Pool V, Cramer EH, et al. A multistate, foodborne outbreak of hepatitis A. National Hepatitis A Investigation Team. *N Engl J Med* 1999;**340**:595–602.
173. Hutin YJ, Sabin KM, Hutwagner LC, et al. Multiple modes of hepatitis A virus transmission among methamphetamine users. *Am J Epidemiol* 2000;**152**:186–92.
174. Ilan Y, Hillman M, Oren R, et al. Vasculitis and cryoglobulinemia associated with persisting cholestatic hepatitis A virus infection. *Am J Gastroenterol* 1990;**85**:586–7.
175. Inman RD, Hodge M, Johnston ME, et al. Arthritis, vasculitis, and cryoglobulinemia associated with relapsing hepatitis A virus infection. *Ann Intern Med* 1986;**105**:700–3.
176. Innis BL, Snitbhan R, Kunasol P, et al. Protection against hepatitis A by an inactivated vaccine. *JAMA* 1994;**271**:1328–34.
177. Ishii K, Kiyohara T, Yoshizaki S, et al. Epidemiological and genetic analyses of a diffuse outbreak of hepatitis A in Japan, 2010. *J Clin Virol* 2012;**53**:219–24.
178. Ivie K, Spruill C, Bell BP. Prevalence of hepatitis A virus infection among illicit drug users, 1993-1994. *Antiviral Ther* 2000;**5**(Suppl. 1):A7.
179. Iwarson S, Lindh M, Widerstrom L. Excellent booster response 4 to 8 years after a single primary dose of an inactivated hepatitis A vaccine. *J Travel Med* 2004;**11**:120–1.
180. Jackson LA, Stewart LK, Solomon SL, et al. Risk of infection with hepatitis A, B or C, cytomegalovirus, varicella or measles among child care providers. *Pediatr Infect Dis J* 1996;**15**:584–9.
181. Jacobsen KH, Koopman JS. Declining hepatitis A seroprevalence: a global review and analysis. *Epidemiol Infect* 2004;**132**:1005–22.
182. Jacobsen KH, Wiersma ST. Hepatitis A virus seroprevalence by age and world region, 1990 and 2005. *Vaccine* 2010;**28**:6653–7.
183. Jamil SM, Massry SG. Acute anuric renal failure in nonfulminant hepatitis A infection. *Am J Nephrol* 1998;**18**:329–32.
184. Jansen RW, Siegl G, Lemon SM. Molecular epidemiology of human hepatitis A virus defined by an antigen-capture polymerase chain reaction method. *Proc Natl Acad Sci USA* 1990;**87**:2867–71.
185. Kao HW, Ashcavai M, Redeker AG. The persistence of hepatitis A IgM antibody after acute clinical hepatitis A. *Hepatol* 1984;**4**: 933–6.
186. Kaplan G, Totsuka A, Thompson P, et al. Identification of a surface glycoprotein on African green monkey kidney cells as a receptor for hepatitis A virus. *EMBO J* 1996;**15**:4282–96.
187. Karron RA, Daemer R, Ticehurst J, et al. Studies of prototype live hepatitis A virus vaccines in primate models. *J Infect Dis* 1988;**157**:338–45.
188. Katz MH, Hsu L, Wong E, et al. Seroprevalence of and risk factors for hepatitis A infection among young homosexual and bisexual men. *J Infect Dis* 1997;**175**:1225–9.
189. Keeffe EB. Is hepatitis A more severe in patients with chronic hepatitis B and other chronic liver diseases? *Am J Gastroenterol* 1995;**90**:201–5.
190. Keeffe EB, Iwarson S, McMahon BJ, et al. Safety and immunogenicity of hepatitis A vaccine in patients with chronic liver disease. *Hepatol* 1998;**27**:881–6.
191. Kemper CA, Haubrich R, Frank I, et al. Safety and immunogenicity of hepatitis A vaccine in human immunodeficiency virus-infected patients: a double-blind, randomized, placebo-controlled trial. *J Infect Dis* 2003;**187**:1327–31.
192. Kim YJ, Lee HS. Increasing incidence of hepatitis A in Korean adults. *Intervirology* 2010;**53**:10–14.
193. Klar A, Branski D, Nadjari M, et al. Gallbladder and pancreatic involvement in hepatitis. *J Clin Gastroenterol* 1998;**27**:143–5.
194. Klein BS, Michaels JA, Rytel MW, et al. Nosocomial hepatitis A: a multinursery outbreak in Wisconsin. *JAMA* 1984;**252**:2716–21.
195. Klevens RM, Kruszon-Moran D, Wasley A, et al. Seroprevalence of hepatitis A virus antibodies in the U.S.: results from the National Health and Nutrition Examination Survey. *Public Health Rep* 2011;**126**:522–32.
196. Klevens RM, Miller JT, Iqbal K, et al. The evolving epidemiology of hepatitis a in the United States: incidence and molecular epidemiology from population-based surveillance, 2005-2007. *Arch Intern Med* 2010;**170**:1811–18.
197. Klevens RM, Sharapov U Hepatitis A. *Manual for the surveillance of vaccine-preventable diseases*. 5th ed. Atlanta: Centers for Disease Control and Prevention; 2011.

198. Kluge I. Gamma-globulin in the prevention of viral hepatitis: a study of the effect of medium-size doses. *Acta Med Scand* 1963;**174**:469–77.
199. Koff RS. Viral hepatitis. In: Walker WA, Durie PR, Halmiton JR, et al, editors. *Pediatric gastroenterologic disease: pathophysiology, diagnosis, management*. Philadelphia: B. C. Decker; 1991. p. 857–74.
200. Koff RS. Clinical manifestations and diagnosis of hepatitis A virus infection. *Vaccine* 1992;**10**(Suppl. 1):S15–17.
201. Konduru K, Kaplan GG. Stable growth of wild-type hepatitis A virus in cell culture. *J Virol* 2006;**80**:1352–60.
202. Krah DL. A simplified multiwell plate assay for the measurement of hepatitis A virus infectivity. *Biologicals* 1991;**19**:223–7.
203. Krugman S, Giles JP. Infectious hepatitis; Evidence of two distinctive clinical, epidemiological and immunological types of infection. *JAMA* 1967;**200**:365–73.
204. Krugman S, Giles JP. Viral hepatitis. New light on and old disease. *JAMA* **212**: 1019–29. 1970.
205. Krugman S, Ward R, Giles JP. The natural history of infectious hepatitis. *Am J Med* 1962;**32**:717–28.
206. Krugman S, Ward R, Giles JP, et al. Infectious hepatitis, study on effect of gamma globulin and on the incidence of apparent infection. *JAMA* 1960;**174**:823–30.
207. Kumar P, Bhatia V. Prolonged cholestasis due to hepatitis A virus infection. *Indian pediatrics* 2011;**48**:485–6.
208. Kumashiro R, Sata M, Suzuki H, et al. Clinical study of acute hepatitis type A in patients older than 50 years. *Acta Hepatol Jpn* 1988;**29**:457–62.
209. Kurugol Z, Aslan A, Turkoglu E, Koturoglu G. Changing epidemiology of hepatitis A infection in Izmir, Turkey. *Vaccine* 2011;**29**: 6259–61.
210. Lange WR, Frame JD. High incidence of viral hepatitis among American missionaries in Africa. *Am J Trop Med Hyg* 1990;**43**: 527–33.
211. Lea AP, Balfour LA. Virosomal hepatitis A vaccine (strain RG-SB). *Biodrugs* 1997;**7**:232–48.
212. Leach CT, Koo FC, Hilsenbeck SG, et al. The epidemiology of viral hepatitis in children in South Texas: increased prevalence of hepatitis A along the Texas-Mexico border. *J Infect Dis* 1999; **180**:509–13.
213. Lednar WM, Lemon SM, Kirkpatrick JW, et al. Frequency of illness associated with epidemic hepatitis A virus infections in adults. *Am J Epidemiol* 1985;**122**:226–33.
214. Lee H, Vacanti JP. Liver transplantation and its long term management in children. *Pediatr Clin North Am* 1996;**43**:99–124.
215. Lee H, Cho HK, Kim JH, et al. Seroepidemiology of hepatitis A in Korea: changes over the past 30 years. *J Korean Med Sci* 2011;**26**:791–6.
216. Lee HC, Ang LW, Chiew PK, et al. Changing epidemiological patterns of hepatitis A infection in Singapore. *Ann Acad Med Singapore* 2011;**40**:439–47.
217. Lee SD, Chan CY, Yu MI, et al. Safety and immunogenicity of inactivated hepatitis A vaccine in patients with chronic liver disease. *J Med Virol* 1997;**52**:215–18.
218. Leikin E, Lysidiewicz A, Garry D, et al. Intrauterine transmission of hepatitis A virus. *Obstet Gynecol* 1996;**88**:690–1.
219. Lemon SM. Type A viral hepatitis: new developments in an old disease. *New Engl J Med* 1985;**313**:1059–67.
220. Lemon SM. Immunologic approaches to assessing the response to inactivated hepatitis A vaccine. *J Hepatol* 1993;**18**(Suppl. 2): S15–19.
221. Lemon SM. The natural history of hepatitis A: the potential for transmission by transfusion of blood or blood products. *Vox Sang* 1994;**67**(Suppl. 4):19–23; discussion 24–6.
222. Lemon SM, Binn LN. Serum neutralizing antibody response to hepatitis A virus. *J Infect Dis* 1983;**148**:1033–9.
223. Lemon SM, Shapiro CN. The value of immunization against hepatitis A. *Infect Agents Dis* 1994;**3**:38–49.
224. Lemon SM, Binn LN, Marchwicki RH. Radioimmunofocus assay for quantitation of hepatitis A virus in cell cultures. *J Clin Microbiol* 1983;**17**:834–9.
225. Lemon SM, Whetter LE, Chang KH, et al. Recent advances in understanding the molecular virology of hepatoviruses: contrasts and comparisons with hepatitis C virus. In: Nishioka K, Suzuki H, Mishiro S, et al, editors. *Viral hepatitis and liver disease*. Tokyo: Springer-Verlag; 1994. p. 22–7.
226. Letson GW, Shapiro CN, Kuehn D, et al. Effect of maternal antibody on immunogenicity of hepatitis A vaccine in infants. *J Pediatr* 2004;**144**:327–32.
227. Levin S, Leibowitz E, Torten J, et al. Interferon treatment in acute progressive and fulminant hepatitis. *Isr J Med Sci* 1989;**25**:364–72.
228. Liaw YF, Yang CY, Chu CM, et al. Appearance and persistence of hepatitis A IgM antibody in acute clinical hepatitis A observed in an outbreak. *Infection* 1986;**14**:156–8.
229. Lieberman JM, Marcy M, Partridge S, et al. *Evaluation of hepatitis A vaccine in infants: effect of maternal antibodies on the antibody response*. Alexandria, Va: Infectious Diseases Society of America; 1998.
230. Linder N, Karetnyi Y, Gidony Y, et al. Decline of hepatitis A antibodies during the first 7 months of life in full-term and preterm infants. *Infection* 1999;**2**:128–31.
231. Mackiewicz V, Dussaix E, le Petitcorps MF, et al. Detection of hepatitis A virus RNA in saliva. *J Clin Microbiol* 2004;**42**:4329–31.
232. Mah MW, Royce RA, Rathouz PJ, et al. Prevalence of hepatitis A antibodies in hemophiliacs: preliminary results from the Southeastern Delta Hepatitis Study. *Vox Sang* 1994;**67**(Suppl. 1):21–2; discussion 23.
233. Mahoney FJ, Farley TA, Kelso KY, et al. An outbreak of hepatitis A associated with swimming in a public pool. *J Infect Dis* 1992; **165**:613–18.
234. Maiga MY, Oberti F, Rifflet H, et al. Manifestations hématologiques liées au virus de l'hépatite A. *Gastroenterol Clin Biol* 1997;**21**:327–30.
235. Manangeeswaran M, Jacques J, Tami C, et al. Binding of hepatitis a virus to its cellular receptor 1 inhibits T-regulatory cell functions in humans. *Gastroenterol* 2012;**142**:1516–25 e1513.
236. Mannucci PM, Gdovin S, Gringeri A, et al. Transmission of hepatitis A to patients with hemophilia by factor VIII concentrates treated with organic solvent and detergent to inactivate viruses. The Italian Collaborative Group. *Ann Inter Med* 1994; **120**:1–7.
237. Mao JS, Chai SA, Xie RY, et al. Further evaluation of the safety and protective efficacy of live attenuated hepatitis A vaccine (H2-strain) in humans. *Vaccine* 1997;**15**:944–7.
238. Mao JS, Dong DX, Zhang HY, et al. Primary study of attenuated live hepatitis A vaccine (H2 strain) in humans. *J Infect Dis* 1989; **159**:621–4.
239. Margolis HS, Nainan OV, Krawczynski K, et al. Appearance of immune complexes during experimental hepatitis A infection in chimpanzees. *J Med Virol* 1988;**26**:315–26.
240. Martin A, Lemon SM. Hepatitis A virus: from discovery to vaccines. *Hepatology* 2006;**43**(2 Suppl. 1):S164–72.
241. Mathiesen LR, Skinoj P, Nielsen JO, et al. Hepatitis type A, B, and non-A non-B in fulminant hepatitis. *Gut* 1980;**21**:72–7.
242. Matricardi PM, Rosmini F, Ferrigno L, et al. Cross sectional retrospective study of prevalence of atopy among Italian military students with antibodies against hepatitis A virus. *BMJ* 1997;**314**: 999–1003.
243. Matricardi PM, Rosmini F, Panetta V, et al. Hay fever and asthma in relation to markers of infection in the United States. *J Allergy Clin Immunol* 2002;**110**:381–7.
244. Mbithi JN, Springthorpe VS, Boulet JR, et al. Survival of hepatitis A virus on human hands and its transfer on contact with animate and inanimate surfaces. *J Clin Microbiol* 1992;**30**:757–63.
245. McCaustland KA, Bond WW, Bradley DW, et al. Survival of hepatitis A virus in feces after drying and storage for 1 month. *J Clin Microbiol* 1982;**16**:957–8.
246. McDuffie RS Jr, Bader T. Fetal meconium peritonitis after maternal hepatitis A. *Am J of obstet Gynecol* 1999;**180**:1031–2.
247. McIntire JJ, Umetsu SE, Macaubas C, et al. Immunology: hepatitis A virus link to atopic disease. *Nature* 2003;**425**:576.
248. McMahon BJ, Beller M, Williams J, et al. A program to control an outbreak of hepatitis A in Alaska by using an inactivated hepatitis A vaccine. *Arch Pediatr Adolesc Med* 1996;**150**:733–9.
249. McMahon BJ, Williams J, Bulkow L, et al. Immunogenicity of an inactivated hepatitis A vaccine in Alaska Native children and Native and non-Native adults. *J Infect Dis* 1995;**171**:676–9.
250. McNeil M, Hoy JF, Richards MJ, et al. Aetiology of fatal viral hepatitis in Melbourne: a retrospective study. *Med J Aust* 1984; **141**:637–40.

251. McQuillan G, Kruszon-Moran D, Enniston M, et al. Viral hepatitis. *NCHS Data Brief* 2010;**27**:1–8.
252. Merck & Co. I. VAQTA (package Insert). *Whitehouse Station*. NJ: Merck & Co., Inc; 2001.
253. Midthun K, Ellerbeck E, Gershman K, et al. Safety and immunogenicity of a live attenuated hepatitis A virus vaccine in seronegative volunteers. *J Infect Dis* 1991;**163**:735–9.
254. Millard J, Appleton H, Parry JV. Studies on heat inactivation of hepatitis A virus with special reference to shellfish. I. Procedures for infection and recovery of virus from laboratory-maintained cockles. *Epidemiol Infect* 1987;**98**:397–414.
255. Minor PD. Picornaviridae. In: Francki RIB, Fauquet CM, Knudson DL, et al, editors. *Classification and nomenclature of the viruses*. The fifth report of the international Committee on Taxonomy of Virus. Vienna: Springer Verlag; 1991. p. 320–6.
256. Mishra A, Saigal S, Gupta R, et al. Acute pancreatitis associated with viral hepatitis: a report of six cases with review of literature. *Am J Gastroenterol* 1999;**94**:2292–5.
257. Mor Z, Srur S, Dagan R, et al. Hepatitis A disease following the implementation of universal vaccination: who is at risk? *J Viral Hepat* 2010;**17**:293–7.
258. Mosley JW, Reisler DM, Brachott D, et al. Comparison of two lots of immune serum globulin for prophylaxis of infectious hepatitis. *Am J Epidemiol* 1968;**87**:539–50.
259. Motte A, Blanc J, Minodier P, et al. Acute hepatitis A in a pregnant woman at delivery. *Int J Infect Dis* 2009;**13**:e49–51.
260. Mowat AP. *Liver disorders in childhood*. 3rd ed. Oxford: Butterworth; 1994.
261. Mutsch M, Spicher VM, Gut C, et al. Hepatitis A virus infections in travelers, 1988-2004. *Clin Infect Dis* 2006;**42**:490–7.
262. Nainan OV, Brinton MA, Margolis HS. Identification of amino acids located in the antibody binding sites of human hepatitis A virus. *Virology* 1992;**191**:984–7.
263. Nainan OV, Armstrong GL, Han XH, et al. Hepatitis a molecular epidemiology in the United States, 1996-1997: sources of infection and implications of vaccination policy. *J Infect Dis* 2005;**19**: 957–63.
264. Nainan OV, Margolis HS, Robertson BH, et al. Sequence analysis of a new hepatitis A virus naturally infecting cynomolgus macaques *(Macaca fascicularis)*. *J Gen Virol* 1991;**72** (Pt 7):1685–9.
265. Nainan OV, Xia G, Vaughan G, et al. Diagnosis of hepatitis a virus infection: a molecular approach. *Clin Microbiol Rev* 2006; **19**:63–79.
266. Nalin DR. VAQTA, hepatitis A vaccine, purified inactivated. *Drugs Future* 1995;**20**:24–9.
267. Nasser AM, Metcalf TG. Production of cytopathology in FRhK-4 cells by BS-C-1-passaged hepatitis A virus. *Appl Environ Microbiol* 1987;**53**:2967–71.
268. National Advisory. Committee on immunization. Immunization practices: supplementary statement on hepatitis A vaccine. *Can Commun Dis Rep* 2000;**26**:12–18.
269. Neilsen GA, Bodsworth NJ, et al. Response to hepatitis A vaccination in human immunodeficiency virus-infected and -uninfected homosexual men. *J Infect Dis* 1997;**176**:1064–7.
270. Niu MT, Polish LB, Robertson BH, et al. Multistate outbreak of hepatitis A associated with frozen strawberries. *J Infect Dis* 1992;**166**:518–24.
271. Niu MT, Salive M, Krueger C, et al. Two-year review of hepatitis A vaccine safety: data from the Vaccine Adverse Event Reporting System (VAERS). *Clin Infect Dis* 1998;**26**:1475–6.
272. Noble RC, Kane MA, Reeves SA, et al. Posttransfusion hepatitis A in a neonatal intensive care unit. *JAMA* 1984;**252**:2711–15.
273. Normann A, Badur S, Onel D, et al. Acute hepatitis A virus infection in Turkey. *J Med Virol* 2008;**80**:785–90.
274. O'Donovan D, Cooke RP, Joce R, et al. An outbreak of hepatitis A amongst injecting drug users. *Epidemiol Infect* 2001;**127**:469–73.
275. O'Grady J. Management of acute and fulminant hepatitis A. *Vaccine* 1992;**10**(Suppl. 1):S21–23.
276. O'Grady JG, Alexander GJ, Hayllar KM, et al. Early indicators of prognosis in fulminant hepatic failure. *Gastroenterology* 1989;**97**: 439–45.
277. Ohara H, Naruto H, Watanabe W, et al. An outbreak of hepatitis A caused by consumption of raw oysters. *J Hyg* 1983;**91**:163–5.
278. Okuno T, Sano A, Deguchi T, et al. Pathology of acute hepatitis A in humans: comparison with acute hepatitis B. *Am J Clin Pathol* 1984;**81**:162–9.
279. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. *Ann Intern Med* 2002;**137**:947–54.
280. Ott JJ, Irving G, Wiersma ST. Long-term protective effects of hepatitis A vaccines: a systematic review. *Vaccine* 2012;**31**:3–11.
281. Papaevangelou GJ, Roumeliotou-Karayannis AJ, Contoyannis PC. The risk of hepatitis A and B virus infections from patients under care without isolation precautions. *J Med Virol* 1981;**7**: 143–8.
282. Park GC, Hwang S, Yu YD, et al. Intractable recurrent hepatitis A virus infection requiring repeated liver transplantation: a case report. *Transplant Proc* 2010;**42**:4658–60.
283. Pavia AT, Nielson L, Armington L, et al. A communitywide outbreak of hepatitis A in a religious community: Impact of mass administration of immune globulin. *Am J Epidemiol* 1990;**131**: 1085–93.
284. Peetermans J. Production, quality control and characterization of an inactivated hepatitis A vaccine. *Vaccine* 1992;**10**(Suppl. 1): s99–101.
285. Pelletier AR, Mehta PJ, Burgess DR, et al. An outbreak of hepatitis A among primary and secondary contacts of an international adoptee. *Public Health Rep* 2010;**125**:642–6.
286. Pelletier G, Elghozi D, Trepo C, et al. Mononeuritis in acute viral hepatitis. *Digestion* 1985;**32**:5306.
287. Perez OM, Herzog C, Zellmeyer M, et al. Efficacy of virosome hepatitis A vaccine in young children in Nicaragua: randomized placebo-controlled trial. *J infect Dis* 2003;**188**:671–7.
288. Peterson DA, Wolfe LG, Larkin EP, Deinhardt FW. Thermal treatment and infectivity of hepatitis A virus in human feces. *J Med Virol* 1978;**2**:201–6.
289. Petrignani M, Harms M, Verhoef L, et al. Update: a food-borne outbreak of hepatitis A in the Netherlands related to semi-dried tomatoes in oil, January-February 2010. *Euro Surveill* 2010;**15**(20).
290. Petrignani M, Verhoef L, van Hunen R, et al. A possible food-borne outbreak of hepatitis A in the Netherlands, January-February 2010. *Euro Surveill* 2010;**15**(11).
291. Piazza M, Safary A, Vegnente A, et al. Safety and immunogenicity of hepatitis A vaccine in infants: a candidate for inclusion in the childhood vaccination programme. *Vaccine* 1999;**17**:585–8.
292. Ping LH, Jansen RW, Stapleton JT, et al. Identification of an immunodominant antigenic site involving the capsid protein VP3 of hepatitis A virus. *Proc Natl Acad Sci US A* 1988;**85**:8281–5.
293. Portnoy BL, Mackowiak PA, Caraway CT, et al. Oyster-associated hepatitis: failure of shellfish certification programs to prevent outbreaks. *JAMA* 1975;**233**:1065–8.
294. Prodinger WM, Larcher C, Solder BM, et al. Hepatitis A in Western Austria: the epidemiological situation before the introduction of active immunisation. *Infection* 1994;**22**:53–5.
295. Provost PJ, Hilleman MR. Propagation of human hepatitis A virus in cell culture in vitro. *Proc Soc Exp Biol Med* 1979;**160**:213–21.
296. Provost PJ, Bishop RP, Gerety RJ, et al. New findings in live, attenuated hepatitis A vaccine development. *J Med Virol* 1986; **20**:165–75.
297. Purcell RH, D'Hondt E, Bradbury R, et al. Inactivated hepatitis A vaccine: active and passive immunoprophylaxis in chimpanzees. *Vaccine* 1992;**10**(Suppl. 1):S148–151.
298. Qu L, Feng Z, Yamane D, et al. Disruption of TLR3 signaling due to cleavage of TRIF by the hepatitis A virus protease-polymerase processing intermediate, 3CD. *PLoS Pathog* 2011;**7**: e1002169.
299. Rahaman SM, Chira P, Koff RS. Idiopathic autoimmune chronic hepatitis triggered by hepatitis A. *Am J Gastroenterol* 1994;**89**: 106–8.
300. Rakela J, Redeker AG, Edwards VM, et al. Hepatitis A virus infection in fulminant hepatitis and chronic active hepatitis. *Gastroenterology* 1978;**74**:879–82.
301. Reid TMS, Robinson HG. Frozen raspberries and hepatitis A. *Epidemiol Infect* 1987;**98**:109–12.
302. Rein DB, Hicks KA, Wirth KE, et al. Cost-effectiveness of routine childhood vaccination for hepatitis A in the United States. *Pediatrics* 2007;**119**:e12–21.
303. Renge RL, Dani VS, Chitambar SD, et al. Vertical transmission of hepatitis A. *Indian J Pediatr* 2002;**69**:535–6.
304. Rezende G, Roque-Afonso AM, Samuel D, et al. Viral and clinical factors associated with the fulminant course of hepatitis A infection. *Hepatology* 2003;**38**:613–18.

305. Rimland D, Guest JL. Response to hepatitis A vaccine in HIV patients in the HAART era. *AIDS* 2005;**19**:1702–4.
306. Robertson BH, Averhoff F, Cromeans TL, et al. Genetic relatedness of hepatitis A virus isolates during a community-wide outbreak. *J Med Virol* 2000;**62**:144–50.
307. Robertson BH, D'Hondt EH, Spelbring J, et al. Effect of postexposure vaccination in a chimpanzee model of hepatitis A virus infection. *J Med Virol* 1994;**43**:249–51.
308. Robertson BH, Jansen RW, Khanna B, et al. Genetic relatedness of hepatitis A virus strains recovered from different geographical regions. *J Gen Virol* 1992;**73**(Pt 6):1365–77.
309. Rosenblum LS, Mirkin IR, Allen DT, et al. A multifocal outbreak of hepatitis A traced to commercially distributed lettuce. *Am J Public Health* 1990;**80**:1075–9.
310. Rosenblum LS, Villarino ME, Nainan OV, et al. Hepatitis A outbreak in a neonatal intensive care unit: risk factors for transmission and evidence of prolonged viral excretion among preterm infants. *J Infect, Dis* 1991;**164**:476–82.
311. Ryu HS, Park SY, Lim SR, et al. Clinical characteristics and gestational complications associated with acute hepatitis a in pregnancy. *Korean J Gastroenterol* 2010;**56**:307–13.
312. Sagi EF, Linder N, Shouval D. Papular acrodermatitis of childhood associated with hepatitis A virus infection. *Pediatr Dermatol* 1985;**3**:31–3.
313. Sagliocca L, Amoroso P, Stroffolini T, et al. Efficacy of hepatitis A vaccine in prevention of secondary hepatitis A infection: a randomised trial. *Lancet* 1999;**353**:1136–9.
314. Samandari T, Bell BP, Armstrong GL. Quantifying the impact of hepatitis A immunization in the United States, 1995-2001. *Vaccine* 2004;**22**:4342–50.
315. Schade CP, Komorwska D. Continuing outbreak of hepatitis A linked with intravenous drug abuse in Multnomah County. *Public Health Rep* 1988;**103**:452–9.
316. Scheuer PJ. *Liver biopsy interpretation*. 4th ed. London: Bailliére Tindall; 1988.
317. Schiff ER. Atypical clinical manifestations of hepatitis A. *Vaccine* 1992;**10**(Suppl. 1):S18–20.
318. Schiodt FV, Atillasoy E, Shakil AO, et al. Etiology and outcome for 295 patients with acute liver failure in the United States. *Liver Transpl Surg* 1999;**5**:29–34.
319. Selander B, Blackberg J, Widell A, et al. No evidence of intrauterine transmission of hepatitis A virus from a mother to a premature infant. *Acta Paediatr* 2009;**98**:1603–6.
320. Sfetcu O, Irvine N, Ngui SL, et al. Hepatitis A outbreak predominantly affecting men who have sex with men in Northern Ireland, October 2008 to July 2009. *Euro Surveill* 2011;**16**(9).
321. Shah U, Habib Z, Kleinman RE. Liver failure attributable to hepatitis A virus infection in a developing country. *Pediatrics* 2000;**105**:436–8.
322. Shapiro C, Hadler S. Significance of hepatitis in children in day care. *Semin Pediatr Infect Dis* **1**:270–9, 1990
323. Shapiro CN, Hadler SC. Hepatitis A and hepatitis B virus infections in day care centers. *Pediatr Ann* 1990;**20**:435–41.
324. Shapiro CN, Coleman PJ, McQuillan GM, et al. Epidemiology of hepatitis A: seroepidemiology and risk groups in the USA. *Vaccine* 1992;**10**(Suppl. 1):S59–62.
325. Sharapov IM, Hepatitis A. *The CDC travel health information for international travel 2012: the yellow book*. New York: Oxford University Press; 2011.
326. Sharapov UM, Bulkow LR, Negus SE, et al. Persistence of hepatitis A vaccine induced seropositivity in infants and young children by maternal antibody status: 10-year follow-up. *Hepatology* 2012;**56**:516–22.
327. Shaw DD, Whiteman DC, Merritt AD. Hepatitis A outbreaks among illicit drug users and their contacts in Queensland, 1997. *Med J Aust* 1999;**170**:584–7.
328. Shaw FE Jr, Shapiro CN, Welty TK, et al. Hepatitis transmission among the Sioux Indians of South Dakota. *Am J Public Health* 1990;**80**:1091–4.
329. Shaw FE Jr, Sudman JH, Smith SM, et al. A community-wide epidemic of hepatitis A in Ohio. *Am J Epidemiol* 1986;**123**:1057–65.
330. Sherertz RJ, Russell BA, Reuman PD. Transmission of hepatitis A by transfusion of blood products. *Arch Intern Med* 1984;**144**:1579–80.
331. Shi GR, Li SQ, Qian L. The epidemiological study on a foodborne outbreak of non-A, non-B hepatitis. *J Chin Med Univ* 1987;**16**:150.
332. Shouval D, Ashur Y, Adler R, et al. Single and booster dose responses to an inactivated hepatitis A virus vaccine: comparison with immune serum globulin prophylaxis. *Vaccine* 1993;**11**(Suppl. 1):S9–14.
333. Simmonds RS, Szucs G, Metcalf TG, Melnick JL. Persistently infected cultures as a source of hepatitis A virus. *Appl Environ Microbiol* 1985;**49**:749–55.
334. Simsek Y, Isik B, Karaer A, et al. Fulminant hepatitis A infection in second trimester of pregnancy requiring living-donor liver transplantation. *J Obstet Gynaecol Res* 2012;**38**:745–8.
335. Sinclair SB, Levy GA. Treatment of fulminant viral hepatic failure with prostaglandin E: a preliminary report. *Dig Dis Sci* 1991;**36**: 791–800.
336. Sjogren MH, Purcell RH, McKee K, et al. Clinical and laboratory observations following oral or intramuscular administration of a live attenuated hepatitis A vaccine candidate. *Vaccine* 1992; **10**(Suppl. 1):S135–137.
337. Sjogren MH, Tanno H, Fay O, et al. Hepatitis A virus in stool during clinical relapse. *Ann Intern Med* 1987;**106**:221–6.
338. Skinhoj P, Mathiesen LR, Kryger P, et al. Faecal excretion of hepatitis A virus in patients with symptomatic hepatitis A infection. *Scand J Gastroenterol* 1981;**16**:1057–9.
339. Smith PF, Grabau JC, Werzberger A, et al. The role of young children in a community-wide outbreak of hepatitis A. *Epidemiol Infect* 1997;**118**:243–52.
340. Snydman DR, Dienstag JL, Stedt B, et al. Use of IgM-hepatitis A antibody testing: investigating a common-source, food borne outbreak. *JAMA* 1981;**245**:827–30.
341. Song HU, Hwang SG, Kwon CI, et al. Molecular epidemiology of hepatitis A virus in the South-East area of Gyeonggi-do in Korea. *Yonsei Med J* 2009;**50**:512–16.
342. Soucie JM, Robertson BH, Bell BP, et al. Hepatitis A virus infections associated with clotting factor concentrate in the United States. *Transfusion* 1998;**38**:573–9.
343. Squires RH Jr, Shneider BL, Bucuvalas J, et al. Acute liver failure in children: the first 348 patients in the pediatric acute liver failure study group. *J Pediatr* 2006;**148**:652–8.
344. Staes CJ, Schlenker TL, Risk I, et al. Sources of infection among persons with acute hepatitis A and no identified risk factors during a sustained community-wide outbreak. *Pediatrics* 2000;**106**:E54.
345. Stapleton JT, Lange DK, LeDuc JW, et al. The role of secretory immunity in hepatitis A virus infection. *J Infec Dis* 1991;**163**: 7–11.
346. Steffen R, Kane MA, Shapiro CN, et al. Epidemiology and prevention of hepatitis A in travelers. *JAMA* 1994;**272**:885–9.
347. Stokes J, Neefe JR. The prevention and attenuation of infectious hepatitis by gamma globulin. *JAMA* 1945;**127**:144–5.
348. Sweet K, Sutherland W, Ehresmann K, et al. Hepatitis A infection in recent international adoptees and their contacts in Minnesota, 2007-2009. *Pediatrics* 2011;**128**:e333–8.
349. Szmuness W, Purcell RH, Dienstag JL, et al. Antibody to hepatitis A antigen in institutionalized mentally retarded patients. *JAMA* 1977;**237**:1702–5.
350. Tabor E. Clinical presentation of hepatitis A. In: Gerety RJ, editor. *Hepatitis A*. New York: Academic Press; 1987.
351. Tabor E. Guillain-Barré syndrome and other neurologic syndromes in hepatitis A, B, and non-A, non-B. *J Med Virol* 1987; **21**:207–16.
352. Taliani G, Gaeta GB. Hepatitis A: post-exposure prophylaxis. *Vaccine* 2003;**21**:2234–7.
353. Tami C, Silberstein E, Manangeeswaran M, et al. Immunoglobulin A (IgA) is a natural ligand of hepatitis A virus cellular receptor 1 (HAVCR1), and the association of IgA with HAVCR1 enhances virus-receptor interactions. *J Virol* 2007;**81**:3437–46.
354. Tankersley DL, Preston MS. Quality control of immune globulins. In: Krijnen HW, Strengers PFW, VanAken X, editors. *Immunoglobulins: proceedings of an international symposium*. Amsterdam: Central Laboratory of the Netherlands Red Cross Transfusion Service; 1988. p. 381–99.
355. Tassopoulos NC, Papaevangelou GJ, Ticehurst JR, et al. Fecal excretion of Greek strains of hepatitis A virus in patients with hepatitis A and in experimentally infected chimpanzees. *J Infect Dis* 1986;**154**:231–7.
356. Taylor RM, Davern T, Munoz S, et al. Fulminant hepatitis A virus infection in the United States: incidence, prognosis, and outcomes. *Hepatology* 2006;**44**:1589–97.

357. Teixera MR Jr, Weller IVD, Murray A, et al. The pathology of hepatitis A in man. *Liver* 1982;**2**:53–60.
358. Termorshuizen F, Dorigo-Zetsma JW, de Melker HE, et al. The prevalence of antibodies to hepatitis A virus and its determinants in the Netherlands: a population-based survey. *Epidemiol Infect* 2000;**124**:459–66.
359. Thorburn KM, Bohorques R, Stepak P, et al. Immunization strategies to control a community-wide hepatitis A epidemic. *Epidemiol Infect* 2001;**127**:461–7.
360. Tibbs CJ, Williams R. Liver transplantation for acute and chronic viral hepatitis. *J Viral Hepat* 1995;**2**:65–72.
361. Tong MJ, el-Farra NS, Grew MI. Clinical manifestations of hepatitis A: recent experience in a community teaching hospital. *J Infect Dis* 1995;**171**(Suppl. 1):S15–18.
362. Tong MJ, Thursby M, Rakela J, et al. Studies on the maternal-infant transmission of the viruses which cause acute hepatitis. *Gastroenterology* 1981;**80**(5 pt 1):999–1004.
363. Toppet V, Souayah H, Delplace O, et al. Lymph node enlargement as a sign of acute hepatitis A in children. *Pediatr Radiol* 1990; **20**:249–52.
364. Tortajada C, de Olalla PG, Diez E, et al. Hepatitis A among men who have sex with men in Barcelona, 1989-2010: insufficient control and need for new approaches. *BMC Infect Dis* 2012;**12**:11.
365. Tortajada C, de Olalla PG, Pinto RM, et al. Outbreak of hepatitis A among men who have sex with men in Barcelona, Spain, September 2008-March 2009. *Euro Surveill* 2009;**14**(15).
366. Troisi CL, Hollinger FB, Krause DS, et al. Pickering L.K. Immunization of seronegative infants with hepatitis A vaccine (HAVRIX; SKB): a comparative study of two dosing schedules. *Vaccine* 1997;**15**:1613–17.
367. Tsarev SA, Emerson SU, Balayan MS, et al. Simian hepatitis A virus (HAV) strain AGM-27: comparison of genome structure and growth in cell culture with other HAV strains. *J Gen Virol* 1991;**72**(Pt 7):1677–83.
368. Tsega E, Mengesha B, Hansson BG, et al. Hepatitis A, B, and delta infection in Ethiopia: a serologic survey with demographic data. *Am J Epidemiol* 1986;**123**:344–51.
369. Tsou TP, Liu CC, Huang JJ, et al. Change in hepatitis A epidemiology after vaccinating high risk children in Taiwan, 1995-2008. *Vaccine* 2011;**29**:2956–61.
370. Usonis V, Meriste S, Bakasenas V, et al. Immunogenicity and safety of a combined hepatitis A and B vaccine administered concomitantly with either a measles-mumps-rubella or a diphtheria-tetanus-acellular pertussis-inactivated poliomyelitis vaccine mixed with a *Haemophilus influenzae* type b conjugate vaccine in infants aged 12-18 months. *Vaccine* 2005;**23**:2602–6.
371. Vallbracht A, Maier K, Stierhof YD, et al. Liver-derived cytotoxic T cells in hepatitis A virus infection. *J Infect Dis* 1989;**160**: 209–17.
372. Van Damme P, Banatvala J, Fay O, et al. Hepatitis A booster vaccination: is there a need? *Lancet* 2003;**362**:1065–71.
373. Van Herck K, Crasta PD, Messier M, et al. Seventeen-year antibody persistence in adults primed with two doses of an inactivated hepatitis A vaccine. *Hum Vaccin Immunother* 2012;**8**:323–7.
374. Van Damme P, Leroux-Roels G, Crasta P, et al. Antibody persistence and immune memory in adults, 15 years after a three-dose schedule of a combined hepatitis A and B vaccine. *J Med Virol* 2012;**84**:11–17.
375. Van Herck K, Van Damme P, Dieussaert I, et al. Antibody persistence 10 years after immunization with a two-dose inactivated hepatitis A vaccine. *Int J Infect Dis* 2004;**8**(supp. 1):s225.
376. Van Herck K, Van Damme P, Lievens M, et al. Hepatitis A vaccine: indirect evidence of immune memory 12 years after the primary course. *J Med Virol* 2004;**72**:194–6.
377. Van Thiel D. When should the decision to proceed with transplantation actually be made in cases of subfulminant liver failure: at admission to hospital or when a donor organ is made available? *J Hepatol* 1993;**17**:1–2.
378. Venczel LV, Desai MM, Vertz PD, et al. The role of child care in a community-wide outbreak of hepatitis A. *Pediatrics* 2001;**108**:E78.
379. Vento S, Garofano T, Di Perri G, et al. Identification of hepatitis A virus as a trigger for autoimmune chronic hepatitis type 1 in susceptible individuals. *Lancet* 1991;**337**:1183–7.
380. Vento S, Garofano T, Renzini C, et al. Fulminant hepatitis associated with hepatitis A virus superinfection in patients with chronic hepatitis C. *N Engl J Med* 1998;**338**:286–90.
381. Victor JC, Monto AS, Surdina TY, et al. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. *N Engl J Med* 2007;**357**:1685–94.
382. Victor JC, Surdina TY, Suleimenova SZ, et al. Person-to-person transmission of hepatitis A virus in an urban area of intermediate endemicity: implications for vaccination strategies. *Am J Epidemiol* 2006;**163**:204–10.
383. Vidor E, Fritzell B, Plotkin S. Clinical development of a new inactivated hepatitis A vaccine. *Infection* 1996;**24**:447–58.
384. Villano SA, Nelson KE, Vlahov D, et al. Hepatitis A among homosexual men and injection drug users: more evidence for vaccination. *Clin Infect Dis* 1997;**25**:726–8.
385. Vogt TM, Wise ME, Bell BP, et al. Declining hepatitis A mortality in the United States during the era of hepatitis A vaccination. *J Infect Dis* 2008;**197**:1282–8.
386. Vong S, Fiore AE, Haight DO, et al. Vaccination in the county jail as a strategy to reach high risk adults during a community-based hepatitis A outbreak among methamphetamine drug users. *Vaccine* 2005;**23**:1021–8.
387. Wallace MR, Brandt CJ, Earhart KC, et al. Safety and immunogenicity of an inactivated hepatitis A vaccine among HIV-infected subjects. *Clin Infect Dis* 2004;**39**:1207–13.
388. Wasley A, Samandari T, Bell BP. Incidence of hepatitis A in the United States in the era of vaccination. *JAMA* 2005;**294**: 194–201.
389. Watson JC, Fleming DW, Borella AJ, et al. Vertical transmission of hepatitis A resulting in an outbreak in a neonatal intensive care unit. *J Infect Dis* 1993;**167**:567–71.
390. Weinberg A, Gona P, Nachman SA, et al. Antibody responses to hepatitis A virus vaccine in HIV-infected children with evidence of immunologic reconstitution while receiving highly active antiretroviral therapy. *J Infect Dis* 2006;**193**:302–11.
391. Weisfuse IB, Graham DJ, Will M, et al. An outbreak of hepatitis A among cancer patients treated with interleukin-2 and lymphokine-activated killer cells. *J infect Dis* 1990;**161**:647–52.
392. Werzberger A, Mensch B, Kuter B, et al. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992;**327**:453–7.
393. Wheeler C, Vogt T, Armstrong GL, et al. A foodborne outbreak of hepatitis A in Pennsylvania associated with green onions. *N Engl J Med* 2005;**353**:890–7.
394. Wheeler C, Vogt TM, Armstrong GL, et al. An outbreak of hepatitis A associated with green onions. *N Engl J Med* 2005;**353**:890–7.
395. Wheeler CM, Fields HA, Schable CA, et al. Adsorption, purification, and growth characteristics of hepatitis A virus strain HAS-15 propagated in fetal rhesus monkey kidney cells. *J Clin Microbiol* 1986;**23**:434–40.
396. Wheeler CM, Robertson BH, Van Nest G, et al. Structure of the hepatitis A virion: peptide mapping of the capsid region. *J Virol* 1986;**58**:307–13.
397. Widell A, Hansson BG, Moestrup T, et al. Acute hepatitis A, B and non-A, non-B in a Swedish community studied over a ten-year period. *Scand J Infect Dis* 1982;**14**:253–9.
398. Wiedermann G, Kundi M, Ambrosch F. Estimated persistence of anti-HAV antibodies after single dose and booster hepatitis A vaccination (0-6 schedule). *Acta Trop* 1998;**69**:121–5.
399. Wiens BL, Bohidar NR, Pigeon JG, et al. Duration of protection from clinical hepatitis A disease after vaccination with VAQTA. *J Med Virol* 1996;**49**:235–41. (398)
400. Williams JL, Bruden DA, Cagle HH, et al. Hepatitis A vaccine: immunogenicity following administration of a delayed immunization schedule in infants, children and adults. *Vaccine* 2003;**21**: 3208–11.
401. Williams R. Prevalence of hepatitis A virus antibody among Navajo school children. *Am J Public Health* 1986;**76**:282–3.
402. Williams R, Wendon J. Indications for orthotopic liver transplantation in fulminant liver failure. *Hepatology* 1994;**20**(supppl.):5–10.
403. Willner IR, Uhl MD, Howard SC, et al. Serious hepatitis A: an analysis of patients hospitalized during an urban epidemic in the United States. *Ann Inter Med* 1998;**128**:111–14.
404. Wimmer E, Murdin AD. Hepatitis A virus and the molecular biology of picornaviruses: a case for a new genus of the family Picornaviridae. In: Hollinger FB, Lemon SM, Margolis H, editors. *Viral hepatitis and liver disease*. 11th ed. Baltimore: Williams and Wilkins; 1991. p. 31–41.

405. Wise ME, Sorvillo F. Hepatitis A–related mortality in California, 1989-2000: analysis of multiple cause-coded death data. *Am J Public Health* 2005;**95**:900–5.
406. Yang NY, Yu PH, Mao ZX, et al. Inapparent infection of hepatitis A virus. *Am J Epidemiol* 1988;**127**:599–604.
407. Yoon YK, Yeon JE, Kim JH, et al. Comparative analysis of disease severity between genotypes IA and IIIA of hepatitis A virus. *J Med Virol* 2011;**83**:1308–14.
408. Yoon EL, Yim HJ, Kim SY, et al. Clinical courses after administration of oral corticosteroids in patients with severely cholestatic acute hepatitis A; three cases. *Korean J Hepatol* 2010;**16**:329–33.
409. Yotsuyanagi H, Koike K, Yasuda K, et al. Prolonged fecal excretion of hepatitis A virus in adult patients with hepatitis A as determined by polymerase chain reaction. *Hepatology* 1996;**24**:10–13.
410. Yun H, Lee HJ, Jang JH, et al. Hepatitis A virus genotype and its correlation with the clinical outcome of acute hepatitis A in Korea: 2006-2008. *J Med Virol* 2011;**83**:2073–81.
411. Zhuang H, Kaldor J, Locarnini SA, et al. Serum immunoglobulin levels in acute A, B, and non-A, non-B hepatitis. *Gastroenterology* 1982;**82**:549–53.
412. Zuckerman AJ. Aspects of the ecology of viral hepatitis. *Ecol Dis* 1983;**2**:135–44.

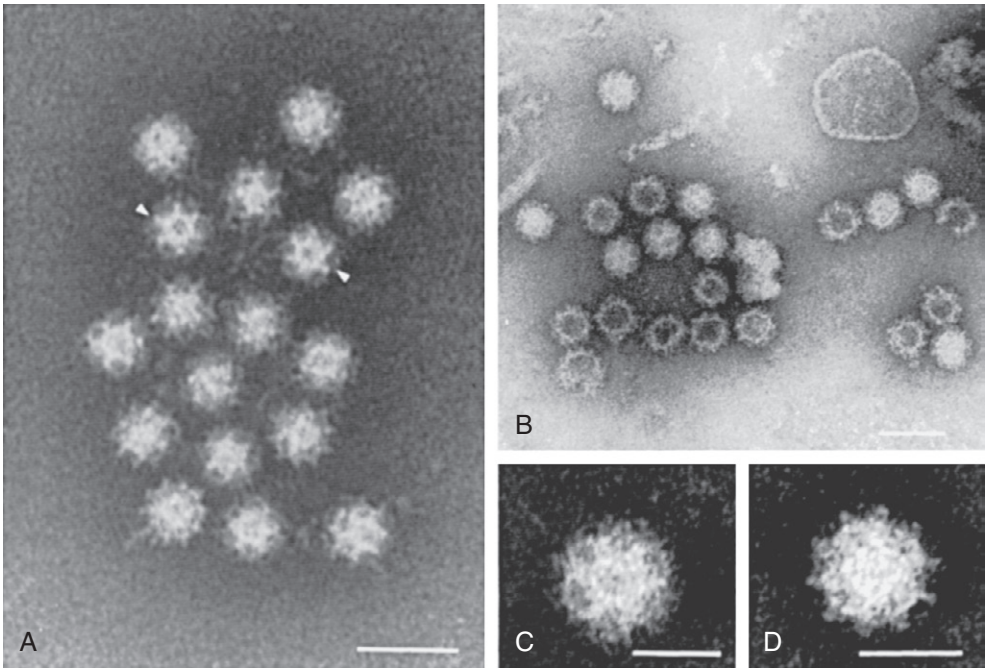

FIGURE 168-1 ■ Human calicivirus particles visualized by electron microscopy. Particles in a preparation of a purified Sapporo/1982/Japan prototype strain show distinct surface cups, and the Star of David pattern is visible on some particles (**a,** *arrowheads*). Particle staining, particle type, and debris in clinical specimens are variable, which frustrates the recognition of distinct structural features. **b,** Specimen from a child from Houston, Texas, infected with an antigenically distinct *Sapovirus.* Two particles at high magnification show the Star of David pattern (**c**) and the 10 surface projections (**d**) characteristic of calicivirus (specimen from a symptomatic child attending a daycare center in Phoenix, Arizona). Bar in **a** and **b** = 50 nm; bar in **c** and **d** = 25 nm.

visualized by electron microscopy; they had distinctive and unique features that led to the name *calicivirus* (from "chalice," for the virion's surface cups).[140]

Caliciviruses in humans were recognized first in 1972, when viral particles were isolated from stool samples of gastroenteritis patients involved in an outbreak, affecting schoolchildren, teachers, and their household contacts in Norwalk, Ohio.[1,101] Volunteer challenge studies proved the causal role of these round and rough-surfaced particles visualized by immune electron microscopy, which were named the Norwalk agent (prototype of the *Norovirus* genus). Many similar small, round-structured viruses (SRSVs) were described subsequently from outbreaks of gastroenteritis around the world. In parallel, investigators using electron microscopy to survey stool specimens from children with sporadic gastroenteritis visualized particles that did not have the appearance of SRSVs but instead were similar to the animal caliciviruses previously identified.[3,129,131] These "typical" caliciviruses occurred in a small percentage of sporadic diarrhea stool specimens and in a few outbreaks of gastroenteritis, including an outbreak in an orphanage in Sapporo, Japan (prototype of the *Sapovirus* genus). Biochemical and genomic sequencing analysis confirmed subsequently that the SRSVs (noroviruses) and human viruses with typical calicivirus morphologic features (sapoviruses) both belong to the *Caliciviridae*.[69,92]

In 1984, a highly fatal, highly contagious hepatitis was observed in European rabbits bred in China.[209] This syndrome spread some 7000 miles across Asia into Europe and reached Spain within 4 years, a dispersal rate of approximately 5 miles (8 kilometers) per day in a sedentary host. European rabbit populations experienced mortality rates exceeding 90 percent within 3 days of exposure. Typical calicivirus particles were visualized in infected rabbit livers, hence the rabbit hemorrhagic disease virus (RHDV; prototype of the *Lagovirus* genus).[146,156]

More recently, calicivirus isolates associated to gastroenteritis in bovines were recognized as a new genus, *Nebovirus.*[82,89,103]

These summaries illustrate the diversity of the ecologic relationships and the broad spectrum of illness (from mild gastroenteritis to fatal hepatitis) associated with members of the *Caliciviridae*. Next, we will discuss the structural, genetic, pathogenic, epidemiologic, and clinical features of caliciviruses, with emphasis on human infection.

PROPERTIES OF CALICIVIRUSES

Structural Features of the Virion

The *Caliciviridae* are a family of nonenveloped, icosahedral viruses with a positive-sense, polyadenylated, single-stranded RNA genome of approximately 7500 nucleotides in length. Cryoelectron microscopy and x-ray crystallography have resolved the three-dimensional structure of representative members of four of the five genera.[14,34,86,160] Typical calicivirus virion particles are 40 nm in diameter and have a T = 3 icosahedral symmetry, conformed by 90 dimers of the main structural protein, VP1 (Fig. 168-1).[160] VP1, is a 60-kDa protein that has two

TABLE 168-1 **Genera of Human and Animal Caliciviruses**

Genus	Known Hosts	Examples
*Norovirus**	Human, bovine, pig	Norwalk, Snow Mountain and Lordsdale viruses; Jena virus (bovine)
*Sapovirus**	Human, pig	Sapporo caliciviruses 82/Japan, 86/Houston, 90/Houston; Manchester and Plymouth viruses; porcine enteric calicivirus
Lagovirus	Rabbit, hare, pig?, dog?, human? fox? mouse? bird?	Rabbit hemorrhagic disease virus of Europe, Asia, Australia, New Zealand; European brown hare syndrome virus
Vesivirus	Pig, cat, chimpanzee, sea lion, dolphin, mussel, sea otter, raccoon, Aruba Island rattlesnake, human	A48; feline caliciviruses F4, CFI, F65, F9; Pan-1, Tur-1; San Miguel sea lion virus types 1 to 17; SMSV-5 Hom-1
Nebovirus	Bovine	Newbury-1 virus

*Previous names of these genera were Norwalk-like and Sapporo-like viruses; synonyms for *Norovirus* and *Sapovirus* were small, round-structured viruses and typical caliciviruses, respectively.
Data from references 12, 69, 176, and 177.

domains (S and P) linked by a hinge. The S domain is responsible for the shell structure of the capsid, and the P domain protrudes from the shell, generating spikes that denote the "cuplike" depressions giving the name to this family.[34,160] The genomic RNA is covered by the capsid and remains covalently attached by its 5′ end to the viral genome–linked protein (VPg).[46]

Taxonomic Relationships Among the Caliciviruses

Caliciviruses were classified as picornaviruses when first identified. However, the International Committee on Taxonomy of Viruses decided to separate them, thus the *Caliciviridae* family was created in 1978.[41] Hepatitis E virus was initially included in the *Caliciviridae* until 1999, when it was separated.[13,130] The *Caliciviridae* currently comprises five genera: *Norovirus*, *Sapovirus*, *Vesivirus*, *Lagovirus*, and *Nebovirus*.[89,127]

According to the 2011 report of the International Committee on Taxonomy of Viruses, the following species are recognized within each genus: Norwalk virus for *Norovirus*, Sapporo virus for *Sapovirus*, feline calicivirus and vesicular exanthema of swine virus for *Vesivirus*, European brown hare syndrome virus and rabbit hemorrhagic disease virus for *Lagovirus*, and Newbury-1 virus for *Nebovirus* (Table 168-1). In addition, according to sequence analysis of whole-genome and capsid regions, multiple genogroups, denoted by "G" plus a roman number (e.g., genogroup two is denoted as GII), and genetic clades, named by arabic numbers following the genogroup name (e.g., GII.4 for genotype 4 of genogroup II) of uncertain biologic significance have been identified within each genus.

Genomic Organization

The genomic organization among the calicivirus genera differ in the number and size of open-reading frames (ORFs), the presence of certain genes, and the need for posttranslational cleavage for gene product function

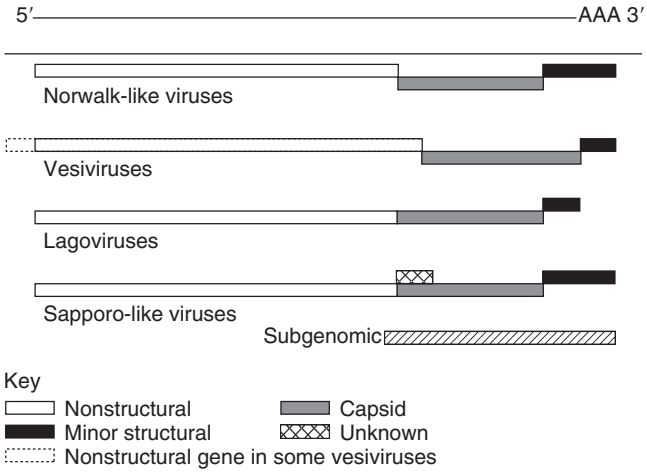

FIGURE 168-2 ■ Genomic organization of calicivirus genera. The genome of *Norovirus* has three open-reading frames (ORFs) that in the 5′ to 3′ direction include a nonstructural polypeptide, the virion capsid gene, and a minor structural protein. The genomes of the other three genera differ from that of noroviruses in the length of the ORFs, including a unique gene at the 5′ end of ORF1 in some vesiviruses and a posttranslationally cleaved N terminus of the capsid protein in vesiviruses and sapoviruses. A subgenomic RNA beginning just 5′ of ORF2 and continuing to the poly-A tail is synthesized during replication. Some sequence comparisons suggest that the longer ORF3 of *Norovirus* (and the comparable gene of *Sapovirus*) arose by intragenic recombination.

(Fig. 168-2).* The genomes of noroviruses and vesiviruses have three ORFs. ORF1 encodes a polyprotein cleaved by a viral protease during replication into a set of nonstructural proteins, including the RNA-dependent RNA polymerase (RdRp), the nucleoside triphosphatase, the protease, and the VPg; ORF2 encodes the capsid protein; and ORF3 encodes a protein that appears to be a minor structural protein.[79,94] Some vesiviruses strains

*References 28, 50, 94, 133, 165, 182.

differ from noroviruses in harboring a longer genome (e.g., Pan-1),[164] although this is not universal (e.g., feline calicivirus).[28] This longer genome includes a longer ORF1 with an additional predicted protein at the N terminus (p6),[148] a longer ORF2 with extra nucleotides at the 5′ end, which are posttranslationally cleaved, and a shorter ORF3 (approximately half the size of that of noroviruses.[148] ORF2 and ORF3 genes of these genera are included in a subgenomic RNA that can be transcribed independently to the genomic RNA.[94,164]

Sapoviruses, lagoviruses, and neboviruses have two ORFs. ORF1 is a fusion of the corresponding ORF1 and ORF2 of noroviruses and vesiviruses, whereas ORF2 encodes for a minor structural protein.[147,152] An additional ORF, in another frame, at the 5′ end of the capsid gene is present in some strains of sapoviruses.[147]

Calicivirus genomes have a highly conserved nontranscribed nucleotide sequence at the 5′ end of the whole genome and at the 5′ end of the capsid gene.[108] In addition, many caliciviruses have an overlap between the 5′ end of ORF1 and the 3′ end of the ORF2. Both features of genomic organization eventually favor recombination (see later discussion).[22] Conserved sequences of some regions of the capsid gene have been appropriate targets for primer design used in reverse-transcriptase polymerase chain reaction (RT-PCR) assays. Sequence analysis of these fragments has allowed accurate discrimination between genus and genogroups, equivalent to whole-genome sequence analysis.[213]

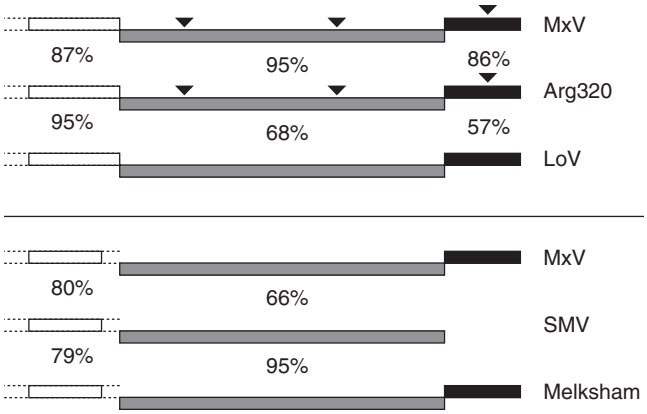

FIGURE 168-3 ■ Sequence comparisons of Argentine calicivirus strain 320 (Arg320; *top*) and Snow Mountain virus (SMV; *bottom*), each with two other noroviruses. Arg320 is significantly closer to Lordsdale virus (LoV) than to Mexico virus (MxV) in the RNA polymerase genome region and significantly closer to MxV than LoV in the capsid and ORF3 genes. Deletions or insertions in the capsid and ORF3 genes of Arg320 are shared with MxV but not with LoV. SMSV is equally close to MxV and Melksham virus in the known polymerase region but much closer to Melksham than MxV in the capsid region. The ORF3 sequence is not available for SMV. Box shadings are as in Figure 168-2. (Data from references 80 and 91.)

Recombinants

Evidence for Recombination Within Caliciviruses

The description of distinct phylogenetic clades within *Caliciviridae* genera and discrepancies of genotyping of some strains when RdRp and capsid sequences were analyzed independently encouraged studies aimed to recognize strains that might be natural recombinants within caliciviruses. Two examples are the well-characterized Argentine strain 320 (Arg320) and the prototype Snow Mountain virus, recognized in the 1990s to be recombinants, at the time when the RdRp and capsid regions of these strains were characterized (Fig. 168-3). In both strains, the change in relative sequence identity took place at the ORF1/ORF2 junction, where sequence conservation at the 5′ end of the genome also occurs (see earlier description), thus indicating that the recombination site occurred in this region. For Arg320, the ORF1 sequence had the highest identity with the Lordsdale virus (prototype of GII.4 genotype) and the capsid and ORF3 sequences had the highest identity with the Mexico virus (prototype of GII.3 genotype), both circulating simultaneously with strain Arg320. A similar change in relative sequence identity also occurred with Snow Mountain virus, which was detected when partial RdRp and capsid sequences were compared with reference Mexico (GII.3) and Melksham (GII.2) viruses respectively, again strains circulating during the same time period.[79,93] After the description of these recombinant strains, multiple naturally recombinant noroviruses and sapoviruses have been identified worldwide.[78,120,126,203] Although this phenomenon has been described also in vesiviruses, lagoviruses, and neboviruses, it seems to be much more infrequent in these genera.[42,49,57]

Other than recognizing a recombinant genomic structure, the recombinants themselves seem unremarkable in terms of clinical consequences. Recombination may be a mechanism by which caliciviruses quickly escape host immunity, analogous to antigenic shifts among influenza viruses but by a different molecular mechanism. The recombination event could be recent or have occurred in the remote past.

Generation of recombinants within caliciviruses requires certain biologic and molecular attributes. First, the infection of single cells simultaneously by two calicivirus strains, in absence of immune or molecular interference. Outbreaks caused by multiple *Norovirus* strains and coinfection with different *Norovirus* strains occur.[11,59,62,89] Second, the genomic structure of caliciviruses favors recombination, owing to overlapping regions between ORFs, conserved regions at 5′ end of the ORFs, and independent transcription of high copy number of the subgenomic region. Finally, the calicivirus RdRp can recognize and process the highly conserved 5′ end sequence of both ORF1 (for whole-genome transcription) and ORF2 (for subgenomic transcription) and can eventually switch from one RNA molecule to another.[22] The recognized recombinants may be a viable subset, among a larger set of recombination events occurring during replication.

Antigenic Properties of the Virion

The presence of a single main capsid protein theoretically ought to limit the antigenic complexity of the virion. However, circulating caliciviruses are highly diverse.[78,84,100,120] This diversity is explained, as in other

RNA viruses, mostly by the high error rate in genomic transcription performed by the viral RdRp, which has no proofreading activity.

Many vesiviruses can be cultivated readily in cell culture, whereas cultivation of viruses in the other genera has been successful for only a few strains.[53,56,181,185] The vesiviruses include a large number of serotypes (>40 neutralization types).[176] Similarly, characterization of lagoviruses suggests the existence of serotypes.[144,207] Determination of whether the other genera have similar diversity has not been possible because of the lack of ability to test strains for their neutralization characteristics. Antigenic characterization of caliciviruses is at a primitive level of development.

Even though no human calicivirus have been successfully cultivated to date, reverse genetic systems and recombinant technology developed to express capsid protein in different cell lines have allowed studies on virus assembly and characterization of antigenic properties of viral capsids.* Enzyme-linked immunosorbent assays to detect viral antigens and antiviral antibodies directed against specific strains have been developed.[74,96] An intense, clear reaction is usually evidenced for the specific strain, but frequent cross reactivity between genotypes and genogroups have also been reported.[77] Neutralization assays have not been performed for human calicivirus because of culture limitations; however, innovative receptor (histoblood group antigen [HBGA]) blocking assays, and hemagglutination inhibition assays have demonstrated a high correlation between blocking/inhibiting antibody titers and protection against a specific strain.[44,163]

NOROVIRUS AND *SAPOVIRUS*, HUMAN CALICIVIRUSES

Strains commonly recovered from human gastroenteritis illness fall into two genera: *Norovirus* (mainly GI and GII) and *Sapovirus*, the called human caliciviruses. Noroviruses and sapoviruses share a number of properties. To avoid repetition, we will discuss features common to both genera followed by distinct features for each genus.

Epidemiology

Human caliciviruses have worldwide distribution, and serologic studies indicate that infections by these agents occur commonly and early in life, with virtually all individuals having antibodies at 5 years of age.[67,145,152]

Human caliciviruses are described as causing acute gastroenteritis mainly in two distinct situations, sporadic endemic cases affecting children younger than 5 years of age and as a cause of outbreaks affecting individuals of all ages. *Norovirus* is the second cause of endemic acute diarrhea in children after rotavirus (before rotavirus vaccine introduction), causing 10 to 20 percent of overall cases and near 20 percent of diarrhea-associated hospitalizations.[5,20,36,154] *Sapovirus* is comparatively less frequent, accounting for 1 to 10 percent of endemic gastroenteritis.[132,192,195]

Noroviruses are the main cause of gastroenteritis outbreaks, associated mostly with contaminated water and food, particularly shellfish.[5,107,187] Noroviruses account for approximately 50 percent of all outbreaks in countries with adequate sanitation and more than 90 percent of outbreaks for which no bacterial or parasitic cause can be found.[5,30,107,187,204] Unlike noroviruses, sapoviruses are known primarily from sporadic cases of diarrhea in children, although outbreaks in closed populations do occur.[113,190] Most of human caliciviruses outbreaks are recognized in close settings where common exposure occurs, such as nurseries, childcare centers, schools, hospitals, camps, hotels, and ships. In these outbreaks, in addition to the primary source (contaminated water or food), person-to person spread also plays a significant role in prolonging outbreak duration. Long persistence of norovirus gastroenteritis outbreaks has been described and has been associated with persistent asymptomatic excretion in food handlers, continual surface contamination, and reexposure to the same strain from contaminated foods.[37,52,105] Primary attack rates in outbreaks frequently are high, near 30 percent, and secondary attack rates range from 5 to 30 percent.[65,206]

Although, high genetic and antigenic diversity have been described for human caliciviruses, mainly for *Norovirus*,[60,77,85] a clear predominance of specific genotypes has been described and large changes in the relative prevalence of these genotypes have occurred during the past 40 years.[5] For example, Norwalk virus, prototype of GI.1 strains, and closely related strains appear to have been the predominant noroviruses in the 1970s in several regions studied.[71] Thereafter, Snow Mountain virus (prototype of GII.2 strains) and related strains predominated, whereas strains within the clade that contains the prototype Norwalk virus (GI) were found infrequently (<5%).[5,100,125] Since the mid 1990s, GII.4 strains emerged and globally spread, representing currently more than 80 percent of noroviruses identified.[114,143,162,174] Distribution of GII.4 lineages differs remarkably from region to region and/or over time, which could be at least partially explained by selective pressure due to population-acquired immunity.[23,114,174]

Seasonal Patterns

Both sporadic and outbreak-associated *Norovirus* infections have winter seasonality (Fig. 168-4).[70,129,138,214] Exceptions to this trend are observed for particular transmission sources. Infections related to certain food will occur when harvested and eaten fresh (fruit, salads, shellfish) or processed, shipped, and consumed later (frozen fruits, ice).[7,30,169] Infections from exposure associated with recreation facilities, such as swimming, tend to have a summer seasonal predominance.[168,188] In addition, contamination of water supplies occurs more frequently in a flooding situation resulting in the mixing of clear water and sewage and in situations associated with shellfish harvesting.[8,119,158,198]

A distinct seasonal prevalence of *Sapovirus*-associated illness is not as apparent, but winter predominance is suggested from several studies.[48,154,214]

*References 5, 67, 81, 96, 161, 211, 212.

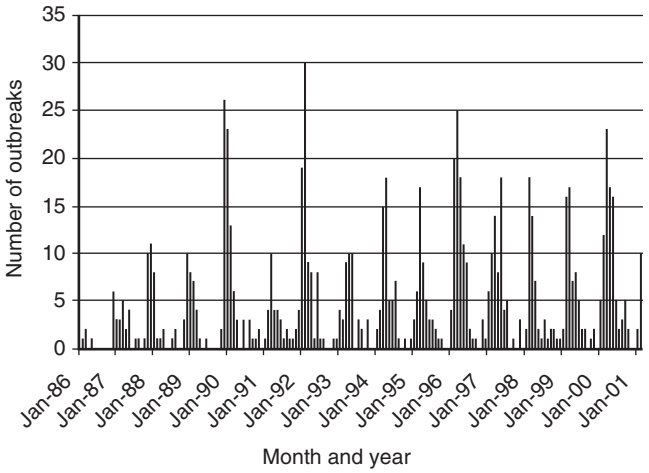

FIGURE 168-4 ■ Seasonality of 840 presumed viral outbreaks in nursing homes for the elderly in Maryland, 1986 to 2000. Detailed analyses of 20 outbreaks found noroviruses in 18. (Data from Figure 1 in Green KY, Belliot G, Taylor JL, et al. A predominant role for Norwalk-like viruses as agents of epidemic gastroenteritis in Maryland nursing homes for the elderly. *J Infect Dis* 2002;**185**:133-46.)

Pathogenesis, Pathology, and Immunology

Pathogenesis

Human calicivirus have not been successfully cultivated in cell lines to date, and animal models are still under development, a situation that has hindered pathogenesis elucidation. Most of the information in this area comes from human volunteer studies, in vitro assays with virus-like proteins (VLPs), and animal models infected with murine norovirus.

Human volunteer studies performed in 1970s showed a group of individuals who seemed resistant to Norwalk virus infection after challenge even with high viral doses.[5,158] The mechanism proposed for this differential risk was the absence or presence of HBGAs on digestive mucosa that could act as a host receptor. HBGAs are oligosaccharides present in human secretions and epithelia and are part of the innate immune system. The expression of HBGAs on mucosa is controlled by the activity of a fucosyl transferase (FUT2), which is responsible for the synthesis of H antigen, the precursor of the A and B antigens. Individuals carrying an inactivating mutation of FUT2 do not express HBGAs on their epithelia, are eventually resistant to *Norovirus*, and are named nonsecretors, whereas subjects with normal active FUT2 express these oligosaccharides and are denominated secretors. More recently, volunteer challenge studies, epidemiologic surveys, and in vitro assays have supported the role of these sugar structures in *Norovirus* virion attachment.* A genogroup-specific HBGA-attachment pattern has been described in vitro that could be related with strain-specific susceptibility.[87,116]

Secretor status does not completely explain the differences seen among infected and uninfected individuals for all *Norovirus* strains. Additional mechanisms are likely involved and remain an ongoing field of research.

*References 19, 87, 109, 116, 121, 200.

Pathology

Knowledge about pathogenic mechanisms of human calicivirus has been limited by the lack of an accurate animal model. Small bowel biopsy specimens obtained from *Norovirus*-infected volunteers in challenge studies mainly evaluating the prototype Norwalk virus have given some pathologic clues.[4] Villous atrophy, epithelia disarrangement, crypt hyperplasia, cytoplasmic vacuolization, and lamina propria inflammatory infiltrate have been described in duodenal/jejunal biopsy specimens obtained from Norwalk virus–infected individuals.[2,5,157,170,171] These morphologic changes have been evidenced both in symptomatic and asymptomatic infected subjects and as early as few hours before starting of clinical manifestations. No viral particle or specific cytopathic effects in enterocytes have been described. Histologic changes resolve after 2 weeks in immunocompetent individuals but could take months in immunosuppressed patients.[5,136,157] Sucrase, trehalase, and alkaline phosphatase activities from the intestinal brush border of symptomatic patients are decreased, inducing a transient malabsorptive state.[2]

The pathogenic features of *Sapovirus* infection in humans have not been determined and are presumed to be the same as those for *Norovirus* caliciviruses.

Immunology

The first barrier against human calicivirus is innate immunity, mainly represented by HBGA on enterocyte surface, which acts as host receptors (see earlier). Nonsecretor individuals lacking HBGA on their epithelia have a lower risk of *Norovirus* infection and *Norovirus*-associated gastroenteritis.[5,19,116,200] HBGAs present in human breast milk can inhibit the binding of noroviral VLPs to saliva and synthetic oligosaccharides in vitro; breast-fed infants from secretor mothers have a lower risk of gastroenteritis caused by *Norovirus* compared with other enteric pathogens.[94,137]

Although human calicivirus infection triggers both humoral and cellular immune acquired responses, infection by these agents can occur many times in the same individual and at any age. Protective acquired immunity to *Norovirus* seems to be complex and remains incompletely understood. Volunteer and clinical studies have demonstrated that 2 to 3 weeks after *Norovirus* infection, serum specific antibodies can be detected and persist for many months.[43,116,157] Adult individuals rechallenged by the same strain seems to be protected shortly after (6 to 14 weeks) the initial exposure but can be infected again when the exposure occurs 24 to 72 months after the first challenge.[5,157] Cross reactivity has been described, but it seems to be restricted to strains of the same genogroups.[115,209] It has not been possible to correlate specific antibody titers with protection, and individuals with high serum and duodenal titers challenged with the same strain of *Norovirus* can even be more susceptible than subjects who have lower titers or are seronegative.[15,97] However, the viral infectious dose given to volunteers in

these challenge studies was severalfold greater than naturally occurring infective doses and thus may not actually represent the phenomenon of natural immunity.

Viral neutralization assays have not been possible, owing to the lack of an adequate cell culture system. HBGA blocking assays and hemagglutination inhibition assays strongly suggest that natural infections are followed by the production of strain-specific protective antibodies.[44,163]

Clinical Manifestations

The incubation period after exposure to *Norovirus* ranges from 12 to 60 hours, with a mean of 24 hours.[6,66,199] Stool excretion of *Norovirus* begins as early as 15 hours after the development of infection, peaks during the second day, and persists at least 7 days and up to 2 months.[6] Noroviruses also have been detected in vomitus, thus suggesting the possibility of spread by aerosol or dispersion of vomitus particles.[31,122] Illness is usually characterized by a self-limited episode of nonbloody diarrhea, nausea, vomiting, and low fever, lasting 1 to 2 days in outbreak setting and 5 to 6 days in endemic cases.[88,165] In children older than 1 year of age, vomiting predominates, whereas in infants and adults, diarrhea tends to predominate.[5,65] This clinical presentation is indistinguishable from those of other enteric pathogens.

A wide disease spectrum ranging from asymptomatic excretion to severe disease with dehydration requiring hospital admission can occur for human caliciviruses as for all enteric viruses; death from *Norovirus* illness rarely occurs.[6,21,65,150,165] In general, human calicivirus gastroenteritis cases are milder than rotavirus cases, even though these agents may represent up to 20 percent of diarrhea-associated hospital admissions in children younger than 5 years of age.[151,214] Chronic symptomatic infections have been described in immunocompromised individuals.[58,142,167] Although the predominant feature of human calicivirus infections is by far gastrointestinal, some researchers have described viral RNA in serum samples, suggesting the possibility of occasional systemic infections; febrile and nonfebrile seizures during norovirus gastroenteritis have been described.[32,35,193] Specific factors associated with clinical severity have not been clearly identified; GII.4 *Norovirus* strains were associated with more severe cases in one study.[21]

Sapovirus illnesses are also indistinguishable from those caused by other gastroenteritis pathogens with a wide spectrum of severity, although most gastroenteritis cases are mild.[48,61,132] A few children in one study intensively evaluated during hospitalization for a *Sapovirus* illness had prolonged malabsorption, frank rectal bleeding, severe dehydration and acidosis, and transient leukopenia.[18]

Differential Diagnosis

In sporadic cases, human caliciviruses are almost indistinguishable by clinical presentation from other viral and toxic causes of gastroenteritis. Persons with calicivirus-associated infections tend to have less fever and more vomiting than if infected with other agents.[5,123,151,214] Characteristic human calicivirus outbreaks are associated with a rapid onset of symptoms after contaminated water or food ingestion (24 to 48 hours of incubation), high primary and secondary attack rate, and a broad age range of affected individuals. The pattern of symptoms is a fairly good marker for calicivirus-associated outbreaks: a brief duration of illness, 2 to 3 days; vomiting as a prominent symptom in more than 50 percent of the outbreak cases; and cultures of freshly processed stool specimens negative for bacterial, parasitic, or fungal pathogens.* When outbreaks have such characteristics, noroviruses will be detected in more than 80 percent.

Diagnosis

Molecular and immunologic based techniques have been developed for human calicivirus detection in both clinical and environmental samples.

Real-time quantitative RT-PCR assay directed against the ORF1-ORF2 junction (a highly conserved region) is currently the most sensitive test for *Norovirus* detection in both stool and environmental samples,[45,99,153,191,201] and can be applied in endemic and outbreak gastroenteritis cases. Cautious interpretation has to be given to detection of very low viral loads, because asymptomatic excretion is common.[151] This assay discriminates between genogroups (GI and GII) but not between subtypes, which may make epidemiologic studies and tracking of a potential infectious source in outbreaks difficult.[45,191] Technical modifications for simultaneous screening of high number of samples (one-step and duplex assays) and simultaneous detection of multiple enteric pathogens have been developed.[55,83,153,201]

Conventional RT-PCR was the main diagnostic technique until the mid 2000s, after which it has been largely replaced by the highly sensitive and faster real-time assay; currently its main application is on nucleic acids amplification for DNA sequencing and genotyping.[30] The gold standard for *Norovirus* genotyping is full capsid sequencing; however, this procedure is very time consuming and technically difficult, making it impractical in clinical and epidemiologic settings.[213] Four conserved genomic regions, specifically from the RdRp and capsid genes (designated regions A to D), have been used successfully for *Norovirus* genotyping.[93,205] Genotyping based on the capsid gene, particularly on region D, allows better discrimination between strains, with only slightly less differentiation when compared with the full capsid sequencing, which is the reason why it is more practical for clinical sample analysis.[30]

Enzyme immunoassays (EIAs) for stool antigen detection have been developed, and commercial kits are currently available.[29] Among the advantages of these methods are their high specificity, lower cost, and lower requirements for skilled personal and complex instruments[30]; the disadvantage is their lower sensitivity due to a high antigenic diversity of human caliciviruses and a low stool viral load that can be under the threshold of detection. Compared with RT-PCR, the sensitivity of new-generation commercial EIA kits ranged from 61 to 80 percent, and

*References 8, 30, 65, 75, 102, 117.

specificity has ranged from 92 to 100 percent.[64,104,106,135] Because of the modest sensitivity of these commercial kits, they are not currently recommended for *Norovirus* detection in sporadic gastroenteritis cases. However, given their ease of use and rapid results, these methods have been recommended for screening of multiple stool samples obtained from gastroenteritis outbreaks; negative results do not exclude *Norovirus* etiology, and molecular tests should be performed in outbreak situations.

Immunochromatographic rapid tests have also been developed. The advantages of these methods are their potential use as point of care tests to provide rapid results. However, their low sensitivity (near 70%) currently limits their clinical application.[64]

VESIVIRUS

Epidemiology

General Prevalence

Vesiviruses include a large number of serotypes isolated from many different animal hosts, including pigs, cows, cats, dogs, sea lions, sea otters, walruses, whales, several snakes, pygmy chimpanzees, and gorillas, as well as a few isolates from humans.* These viruses probably have a worldwide distribution in terrestrial and marine environments, although studies of San Miguel calicivirus–like strains are lacking outside North America. Vesiviruses include at least 40 neutralization types (serotypes). The prevalence of these serotypes in different regions has had little study.

Morbidity and Mortality

Vesiviruses cause a variety of severe illnesses in animals and are not currently considered a relevant human pathogen, with rare cases reported mainly in people with close exposure to animals. Nevertheless, a role in human disease cannot be excluded because anti-*Vesivirus* antibodies were detected in 12 percent of normal blood donors, at higher prevalence (21%) among otherwise normal donors with elevated serum levels of liver aminotransferases and at highest prevalence (47%) among patients with hepatitis associated with blood exposures.[179] Some individuals in the groups with normal and elevated aminotransferase levels also had *Vesivirus* RNA circulating in the blood. The exact clinical significance of this finding from one study is still unclear.

One laboratory worker was infected with SMSV-5 and experienced an influenza-like illness, followed during the next several days by viremia and a vesicular exanthem similar to that observed in the original pinniped host.[177] SMSV-5 was visualized in vesicular fluid by electron microscopy and was cultured from the lesions; the genome was amplified and sequenced. The worker also mounted a greater than fourfold serum neutralizing antibody response to SMSV-5. Biologists working with marine mammals had similar illnesses but were studied less intensively than was the laboratory worker.[11,182]

*References 12, 54, 63, 124, 176, 178, 183, 186.

Host and Social Factors

Although vesiviruses cause a variety of diseases, only a few strains cause gastroenteritis, a feature common to *Norovirus* and *Sapovirus* caliciviruses. Among the vesiviruses are a number that exhibit a broad range of hosts. For example, SMSV-5 has been found to infect sea lions, seals, opal-eye fish, pigs, cattle, and laboratory workers.[176,184]

Pathogenesis and Pathology

Vesiviruses cause infection on mucosal surfaces (pneumonia, aphthous ulcers) and disease after viremia (generalized vesicular eruption, encephalitis, hepatitis, spontaneous abortion). The incubation period after exposure is short: 1 to 4 days.[189] Excretion varies among species. For three cultivable vesiviruses, asymptomatic and persistent excretion of virus was detected for months after the primary infection.[98,181,185]

The pathogenic features of *Vesivirus* infection vary and depend on the syndrome of the affected host.

Clinical Manifestations

The proven clinical manifestations of *Vesivirus* infection in humans are limited to a few cases. Because vesiviruses cause a variety of illnesses in animals and these viruses occur naturally in primates, the potential spectrum of illness in humans is broad. Groups at greatest risk of acquiring infection probably would be zoo workers, indigenous populations handling marine mammals, veterinarians, and laboratory workers, but studies of these groups have been limited.[183] The clinical implications of the finding of anti-*Vesivirus* antibody and RNA in serum are uncertain. The RNA in serum was found in persons judged well enough to donate blood.[179]

Diagnosis

Many vesiviruses can be cultivated in cell culture, and many are found in sufficiently high concentration in clinical samples to permit the use of electron microscopy and classic virologic methods for detection of infecting viruses. Other strains have resisted cultivation, and molecular techniques have been developed for them.[127,141,173] Vesiviruses are heat labile, and clinical samples should be submitted promptly and unfrozen for analysis.

LAGOVIRUS

Epidemiology

General Prevalence

The prototype *Lagovirus* is RHDV. Lagoviruses include the genetically distinct European brown hare syndrome virus and multiple genetically distinct strains recovered from asymptomatic rabbits.* Rabbits also are infected

*References 25, 26, 110-112, 207, 210.

with *Vesivirus* strains, which may lead to confusion.[124] Antigenic diversity occurs in the *Lagovirus* genus, but the number of serotypes in the genus is unknown, because like noroviruses and sapoviruses, lagoviruses cannot be cultivated in the laboratory.[207] RHDV has been epidemic in Asia and Europe since it was first recognized in China in 1984.[210] Rabbits in more than 40 countries have been affected. Disease spreads rapidly and appears to "leap" large distances in a short time. The ability of the virus to spread rapidly across large bodies of water (e.g., the English Channel) suggests that arthropods or other flying vectors have a role in transmission.[33] One outbreak in Mexico was traced to a shipment of rabbit meat from Asia; extensive slaughter of rabbits in Mexico was required to eradicate the agent.[68] Three outbreaks have occurred in North America and Cuba.[24] The source of these outbreaks is unknown, although deliberate importation and release was suspected.

RHDV was released in 1995 in controlled field studies off the coast of mainland Australia but escaped from the study site the same year and spread onto the continent.[90] Subsequently, the virus was introduced illegally into the South Island of New Zealand. Clandestine, deliberate spread in both countries to control exotic European rabbits led to wide dispersal of the agent. These releases occurred despite conflicting evidence of RHDV infection in other animal species and, subsequently, in humans.[27,128]

Morbidity and Mortality

Lagoviruses are not currently considered a human pathogen. RHDV infections are highly fatal (>80%) in wild and domesticated European rabbits. Subclinical infections occur in young rabbits, and immunity from early infection appears to attenuate later exposure. In the food-associated outbreak in Mexico, antibody developed in one human. Zoo workers, rabbit breeders, and foresters would have the largest occupational risk of RHDV exposure. The human infection in the Mexican outbreak apparently was subclinical.[68] Ostensibly, subclinical infection also occurred in some persons exposed to rabbits in Australia after escape of the virus there.[128]

Host and Social Factors

The range of hosts of lagoviruses and the host of RHDV before it was recognized in rabbits in China are unknown. In Australian studies, numerous Australian animal species challenged with a subimmunogenic dose of RHDV had no antibody response. On the other hand, antibodies did develop in kiwis, mice, dogs, and foxes; in the case of foxes, the serum antibody response was attributable to oral feeding.[39] Natural spread in rabbits is enhanced by their colony breeding behavior.

Clinical Manifestations and Pathogenesis

The incubation period after exposure is 1 to 3 days.[202] Lagoviruses cause a fatal hepatitis in rabbits and hares that results in disseminated intravascular coagulation and diffuse hemorrhage. Death is rapid, occurring 6 to 12 hours after the onset of illness. RHDV and European brown hare syndrome virus appear to be sufficiently distinct to preclude cross protection. Because the infections in the Mexican worker exposed during the RHDV outbreak and in the workers in Australia who tested antibody positive apparently were subclinical, the spectrum of potential infection in humans is unknown.

Diagnosis

Samples from persons with suspected infection should be submitted to reference laboratories after consultation. Diagnosis can be established with immunologic and molecular techniques.

NEBOVIRUS

Epidemiology

Nebovirus and its prototype strain Newbury-1 virus are bovine pathogens, causing acute gastroenteritis mainly in calves.[103,149,155,175] Although the first report of these bovine pathogens was in the mid 1970s, they were not recognized as a different *Caliciviridae* genus until recently[103,149,175,208] Neboviruses have been detected in 3 to 10 percent of calves with diarrhea[82,103] and have not already been isolated in humans.

Clinical Manifestations and Pathogenesis

After 1 to 3 days of incubation, a self-limited acute nonbloody diarrhea manifests and lasts less than 7 days.[76,175] The histologic damage predominates in the proximal small bowel and is characterized by stunted villi and a decrease in galactosidase activity, and carbohydrate absorption has been demonstrated in challenge studies with gnotobiotic calves.[76]

Diagnosis

Bovine stool samples are processed by RT-PCR for *Nebovirus* detection.[103]

TREATMENT AND PREVENTION

Nonspecific Measures

Children with diarrhea are more likely than are children without diarrhea to be excreting enteric viruses, including caliciviruses. Infection with noroviruses requires a very small inoculum, on the order of 100 particles, which favors transmission in closed setting and through fomites.[30,51,118,199,209] For children in care settings as well as for hospitalized children, a few infection control measures are likely to reduce the spread of infection. Such measures include personnel trained in hygiene and isolation procedures, cleanliness of surfaces (with sodium

hypochlorite solution or 70% ethanol) and in food preparation, exclusion of ill care providers, adequate hand washing, and exclusion or cohorting of ill children.[30,51,73] Laboratory workers exposed to caliciviruses should be aware of the risk for acquiring infection.

Specific Measures

No specific treatment for calicivirus infection has been established. Feline interferon and ribavirin inhibit the replication of feline calicivirus (a *Vesivirus*) in feline cell lines.[134,159] Morpholino antisense oligomers blocked production of *Vesivirus* and murine norovirus virions cultivated in cell lines and inhibit the protein expression of Norwalk virus in replicon-bearing cells.[17,188] These antisense compounds have potential for prophylaxis and treatment of illness, although their potential future clinical use in humans seems highly unlikely.[180] More recently, a nucleoside analog, 2′-C-MeC, showed significant in vitro activity against a GII norovirus prototype and could be further explored for human use.[40] Use of HBGA receptor blocking molecules has also been postulated as a possible therapeutic strategy for the future.[139]

Vaccines for feline calicivirus and RHDV indicated that this prevention method could work for other caliciviruses. The development of human vaccine candidates has focused on noroviruses with substantial advances during the past decade. *Norovirus* vaccines incorporating an immunogen-expressed capsid protein that self-assembles into VLPs have been the candidates most extensively studied.[4,9,10,72,194] More recently, viral particles formed specifically by the P domain of the capsid protein (P particles) have been studied as potential vaccine candidates.[197]

A safe and effective *Norovirus* vaccine could play a significant role in reducing the impact of large gastroenteritis outbreaks occurring in specific settings in which large groups of individuals are compromised. Army personnel especially those allocated to large ships, foreign aid workers, cruise ship workers, and residents of elder care facilities are examples of populations that could benefit from *Norovirus* disease prevention.[51] More importantly, a vaccine targeted for infants, following the age strategy used for rotavirus, could have a significant impact in further reducing the toll from death, hospitalization, and medical visits associated with childhood endemic gastroenteritis. Current epidemiologic data indicate that an effective vaccine could reduce up to 20 percent of emergency department visits or hospitalizations associated with gastroenteritis.[151]

Barriers to *Norovirus* vaccine development have been related with the perception that immunity is short termed and strain specific and that the significant genetic/antigenic variability may impair the effectiveness of a vaccine including a limited number of antigenic variants. The concept of short-term immunity was obtained from challenge studies in adults[157,209]; the situation in children may be different. A cohort study in infants from Chile indicates that *Norovirus* infections and reinfections are very common and caused largely by GII strains but that symptomatic reinfections are uncommon during the first 3 years of life. Not a single case of a symptomatic GII infection was detected.[150] Thus, similar to rotavirus, it is possible that a GII *Norovirus* vaccine would be protective against symptomatic disease during the first critical 3 years of life and probably less protective against asymptomatic and mild infections. This vaccine outcome would be of unquestionable epidemiologic benefit worldwide.

The most advanced *Norovirus* vaccine candidate is a VLP formulation in a powder form for intranasal use or in a formulation for parenteral use.[4] Intranasal administration in a placebo-controlled trial including 90 adults receiving two doses of a Norwalk virus G1.1 VLP or placebo demonstrated a 70 percent immunoglobulin A antibody response in vaccine recipients. A Norwalk virus challenge caused gastroenteritis in 69 percent of placebo recipients and in 37 percent of vaccinated individuals ($p = .006$), whereas infection occurred in 82 percent and 61 percent, respectively ($p = .05$). These results are encouraging and open a path for the development of GI and, more importantly, GII vaccines for infants.

P-particle–based vaccines may be less immunogenic and thus far seem less likely to succeed as a vaccine. The advantage of this approach is the possibility of combining different antigens such as rotavirus and influenza virus[196,197] in a parenteral vaccination strategy, although antigen combination also seems feasible for VLPs, at least with rotavirus VP6 antigen.[16] In any case, these potential strategies are at least 5 years away from being implemented.

PROGNOSIS

Understanding of the features of calicivirus infection in humans has increased rapidly since 1990, when molecular tools for studying the viruses became available. The availability of refined diagnostic techniques has significantly increased our understanding of calicivirus-associated disease. Gastroenteritis that is mostly mild to moderate, but also severe, is the sole recognized disease associated with these viruses in humans. Severe cases have been reported mostly in infants, elder adults, young healthy men under physical stress (e.g., military personal), and immunocompromised patients.[47,58,150,167] Disease is usually short-lived with the exception of infection in immunocompromised individuals, including children with solid-organ transplants, in whom it can cause prolonged diarrhea and viral shedding for months.[58,166] Severe chronic diarrhea can also occur in children with stem cell transplantation.[167] Deaths associated with *Norovirus* infections have been reported in adults with hematopoietic stem cell transplantation.[172] Nevertheless, these complications are rare, and most infected individuals fully recover within 1 week.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J Med* 2011;**365**:2178–87.
6. Atmar RL, Opekun AR, Gilger MA, et al. Norwalk virus shedding after experimental human infection. *Emerg Infect Dis* 2008;**14**: 1553–7.

7. Baert L, Mattison K, Loisy-Hamon F, et al. Review: norovirus prevalence in Belgian, Canadian and French fresh produce: a threat to human health? *Int J Food Microbiol* 2011;**151**:261–9.
8. Baker K, Morris J, McCarthy N, et al. An outbreak of norovirus infection linked to oyster consumption at a UK restaurant, February 2010. *J Public Health (Oxf)* 2011;**33**:205–11.
16. Blazevic V, Lappalainen S, Nurminen K, et al. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. *Vaccine* 2011;**29**:8126–33.
19. Bucardo F, Nordgren J, Carlsson B, et al. Pediatric norovirus diarrhea in Nicaragua. *J Clin Microbiol* 2008;**46**:2573–80.
20. Bucardo F, Kindberg E, Paniagua M, et al. Genetic susceptibility to symptomatic norovirus infection in Nicaragua. *J Med Virol* 2009;**81**:728–35.
21. Bucardo F, Nordgren J, Carlsson B, et al. Asymptomatic norovirus infections in Nicaraguan children and its association with viral properties and histo-blood group antigens. *Pediatr Infect Dis J* 2010;**29**:934–9.
23. Bull RA, White PA. Mechanisms of GII.4 norovirus evolution. *Trends Microbiol* 2011;**19**:233–40.
30. Centers for Disease Control and Prevention (CDC). Surveillance for foodborne disease outbreaks—United States, 2008. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1197–202.
32. Chan CM, Chan CW, Ma CK, et al. Norovirus as cause of benign convulsion associated with gastroenteritis. *J Paediatr Child Health* 2011;**47**:373–7.
35. Chen SY, Tsai CN, Lai MW, et al. Norovirus infection as a cause of diarrhea-associated benign infantile seizures. *Clin Infect Dis* 2009;**48**:849–55.
36. Cheng W, Ye X, Yang X, et al. Epidemiological study of human calicivirus infection in children with gastroenteritis in Lanzhou from 2001 to 2007. *Arch Virol* 2010;**155**:553–5.
38. Clarke IN, Estes MK, Green KY, et al. Caliciviridae. In: King AMQ, Adams MJ, Carstens EB, et al, editors. *Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier; 2011. p. 977–86.
44. Czakó R, Atmar RL, Opekun AR, et al. Serum hemagglutination inhibition activity correlates with protection from gastroenteritis in persons infected with Norwalk virus. *Clin Vaccine Immunol* 2012;**19**:284–7.
47. Delacour H, Dubrous P, Koeck JL. Noroviruses: a challenge for military forces. *J R Army Med Corps* 2010;**156**:251–4.
49. Di Martino B, Di Profio F, Martella V, et al. Evidence for recombination in neboviruses. *Vet Microbiol* 2011;**153**:367–72.
51. Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recomm Rep* 2011;**60**:1–18.
55. Feeney SA, Armstrong VJ, Mitchell SJ, et al. Development and clinical validation of multiplex TaqMan® assays for rapid diagnosis of viral gastroenteritis. *J Med Virol* 2011;**83**:1650–6.
57. Forrester NL, Moss SR, Turner SL, et al. Recombination in rabbit haemorrhagic disease virus: possible impact on evolution and epidemiology. *Virology* 2008;**376**:390–6.
58. Frange P, Touzot F, Debré M, et al. Prevalence and clinical impact of norovirus fecal shedding in children with inherited immune deficiencies. *J Infect Dis* 2012;**206**:1269–74.
64. Geginat G, Kaiser D, Schrempf S. Evaluation of third-generation ELISA and a rapid immunochromatographic assay for the detection of norovirus infection in fecal samples from inpatients of a German tertiary care hospital. *Eur J Clin Microbiol Infect Dis* 2012;**31**:733–7.
82. Hassine-Zaafrane M, Kaplon J, Sdiri-Loulizi K, et al. Molecular prevalence of bovine noroviruses and neboviruses detected in central-eastern Tunisia. *Arch Virol* 2012;**157**:1599–604.
83. Higgins RR, Beniprashad M, Cardona M, et al. Evaluation and verification of the Seeplex Diarrhea-V ACE assay for simultaneous detection of adenovirus, rotavirus, and norovirus genogroups I and II in clinical stool specimens. *J Clin Microbiol* 2011;**49**:3154–62.
86. Hu Z, Tian X, Zhai Y, et al. Cryo-electron microscopy reconstructions of two types of wild rabbit hemorrhagic disease viruses characterized the structural features of lagovirus. *Protein Cell* 2010;**1**:48–58.
89. International Committee on Taxonomy of Viruses. *Master species list. 2011*. Available at http://www.ictvonline.org/virusTaxonomy.asp?taxnode_id=20113430. Accessed August 10, 2012.
103. Kaplon J, Guenau E, Asdrubal P, et al. Possible novel nebovirus genotype in cattle, France. *Emerg Infect Dis* 2011;**17**:1120–3.
104. Kele B, Lengyel G, Deak J. Comparison of an ELISA and two reverse transcription polymerase chain reaction methods for norovirus detection. *Diagn Microbiol Infect Dis* 2011;**70**:475–8.
105. Kimura H, Nagano K, Kimura N, et al. A norovirus outbreak associated with environmental contamination at a hotel. *Epidemiol Infect* 2011;**139**:317–25.
106. Kirby A, Gurgel RQ, Dove W, et al. An evaluation of the RIDASCREEN and IDEIA enzyme immunoassays and the RIDAQUICK immunochromatographic test for the detection of norovirus in faecal specimens. *J Clin Virol* 2010;**49**:254–7.
107. Koo HL, Ajami N, Atmar RL, et al. Noroviruses: The leading cause of gastroenteritis worldwide. *Discov Med* 2010;**10**:61–70.
113. Lee LE, Cebelinski EA, Fuller C, et al. Sapovirus outbreaks in long-term care facilities, Oregon and Minnesota, USA, 2002-2009. *Emerg Infect Dis* 2012;**18**:873–6.
116. Lindesmith L, Donaldson E, LoBue A, et al. Mechanisms of GII.4 norovirus persistence in human populations. *PLoS Med* 2008;**5**:269–90.
118. Lopman B, Gastañaduy P, Park GW, et al. Environmental transmission of norovirus gastroenteritis. *Curr Opin Virol* 2012;**2**:96–102.
119. Lowther JA, Henshilwood K, Lees DN. Determination of norovirus contamination in oysters from two commercial harvesting areas over an extended period, using semiquantitative real-time reverse transcription PCR. *J Food Prot* 2008;**71**:1427–33.
120. Mahar JE, Kirkwood CD. Characterization of norovirus strains in Australian children from 2006 to 2008: prevalence of recombinant strains. *J Med Virol* 2011;**83**:2213–19.
126. Mathijs E, Denayer S, Palmeira L, et al. Novel norovirus recombinants and of GII.4 sub-lineages associated with outbreaks between 2006 and 2010 in Belgium. *Virol J* 2011;**8**:310.
129. Matthews JE, Dickey BW, Miller RD, et al. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. *Epidemiol Infect* 2012;**140**:1161–72.
132. Medici M, Tummolo F, Albonetti V, et al. Molecular detection and epidemiology of astrovirus, bocavirus, and sapovirus in Italian children admitted to hospital with acute gastroenteritis, 2008-2009. *J Med Virol* 2012;**84**:643–50.
135. Morillo SG, Luchs A, Cilli A, et al. Norovirus 3rd generation kit: an improvement for rapid diagnosis of sporadic gastroenteritis cases and valuable for outbreak detection. *J Virol Methods* 2011;**173**:13–16.
139. Nasir W, Frank M, Koppisetty CA, et al. Lewis histo-blood group α1,3/α1,4 fucose residues may both mediate binding to GII.4 noroviruses. *Glycobiology* 2012;**22**:1163–72.
150. O'Ryan ML, Lucero Y, Prado V, et al. Symptomatic and asymptomatic rotavirus and norovirus infections during infancy in a Chilean birth cohort. *Pediatr Infect Dis J* 2009;**28**:879–84.
151. O'Ryan M, Peña A, Vergara R, et al. Prospective characterization of norovirus compared with rotavirus acute diarrhea episodes in Chilean children. *Pediatr Infect Dis J* 2010;**29**:855–9.
155. Park SI, Jeong C, Park SJ, et al. Molecular detection and characterization of unclassified bovine enteric caliciviruses in South Korea. *Vet Microbiol* 2008;**130**:371–9.
162. Puustinen L, Blazevic V, Huhti L, et al. Norovirus genotypes in endemic acute gastroenteritis of infants and children in Finland between 1994 and 2007. *Epidemiol Infect* 2012;**140**:268–75.
163. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. *J Infect Dis* 2010;**202**:1212–18.
166. Roos-Weil D, Ambert-Balay K, Lanternier F, et al. Impact of norovirus/sapovirus-related diarrhea in renal transplant recipients hospitalized for diarrhea. *Transplantation* 2011;**92**:61–9.
167. Saif MA, Bonney DK, Bigger B, et al. Chronic norovirus infection in pediatric hematopoietic stem cell transplant recipients: a cause of prolonged intestinal failure requiring intensive nutritional support. *Pediatr Transplant* 2011;**15**:505–9.

169. Sarvikivi E, Roivainen M, Maunula L, et al. Multiple norovirus outbreaks linked to imported frozen raspberries. *Epidemiol Infect* 2012;**140**:260–7.
174. Siebenga J, Vennema H, Zheng D, et al. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants; 2001-2007. *J Infect Dis* 2009;**200**:802–12.
187. Soller JA, Bartrand T, Ashbolt NJ, et al. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Res* 2010;**44**:4736–47.
192. Svraka S, Vennema H, van der Veer B, et al. Epidemiology and genotype analysis of emerging sapovirus-associated infections across Europe. *J Clin Microbiol* 2010;**48**:2191–8.
193. Takanashi S, Hashira S, Matsunaga T, et al. Detection, genetic characterization, and quantification of norovirus RNA from sera of children with gastroenteritis. *J Clin Virol* 2009;**44**:161–3.
195. Tam CC, O'Brien SJ, Tompkins DS, et al. Changes in causes of acute gastroenteritis in the United Kingdom over 15 years: microbiologic findings from 2 prospective, population-based studies of infectious intestinal disease. *Clin Infect Dis* 2012;**54**: 1275–86.
196. Tamminen K, Huhti L, Koho T, et al. A comparison of immunogenicity of norovirus GII-4 virus-like particles and P-particles. *Immunology* 2012;**135**:89–99.
197. Tan M, Jiang X. Norovirus P particle: a subviral nanoparticle for vaccine development against norovirus, rotavirus and influenza virus. *Nanomedicine (Lond)* 2012;**7**:889–97.
198. Terio V, Martella V, Moschidou P, et al. Norovirus in retail shellfish. *Food Microbiol* 2010;**27**:29–32.
212. Yunus MA, Chung LM, Chaudhry Y, et al. Development of an optimized RNA-based murine norovirus reverse genetics system. *J Virol Methods* 2010;**169**:112–18.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 169

Hepatitis E Virus

Ravi Jhaveri

Hepatitis E virus (HEV) was only recently characterized but has likely been infecting humans for hundreds of years. New information about the animal reservoirs, epidemiology, and molecular biology is constantly becoming available. Recent natural disasters and regional warfare have highlighted HEV as an agent that causes epidemics in areas where sanitation and public health systems have broken down. New developments offer hope that protection using vaccine will soon become a reality. This chapter is a summary of the discoveries so far with emphasis on the impact of HEV infection in children.

HISTORY

As methods to detect hepatitis A and B virus as the major causes of fecal-oral and blood-borne hepatitis, respectively, became more reliable in the 1970s, more cases were being classified as "non-A, non-B hepatitis." Investigations in regions of South Asia examined epidemic and endemic cases of acute hepatitis.[43,82,172] Case patients all had a clinical course that was very similar to that of hepatitis A virus (HAV), despite a remote history of HAV infection, and testing for other causes of viral hepatitis was negative. Many believed that a distinct enteric viral agent was the cause of these cases.

The first evidence of this came when Balayan and colleagues[18] used stool extracts from infected humans orally administered to a human volunteer known to be HAV immune. Classic hepatitis symptoms ensued, and stool from the infected volunteer was used to reinfect cynomolgus macaques. Electron microscopy of stool samples from the asymptomatic and early symptomatic phase showed virus-like particles (VLPs) approximately 30 nm in diameter that banded at 1.35 g/cm^3 on a cesium chloride gradient.[26,27] VLPs showed no cytopathic effect when passaged in tissue culture and mice inoculated with the VLPs showed no signs of disease.[18]

The field experienced a major advance after the reverse-transcriptase polymerase chain reaction (RT-PCR) identification of hepatitis C virus (HCV) in 1989.[32] Similar methods were used to determine the nature of the non-A, non-B enteric virus.[132] Reyes and colleagues[132] constructed cDNA clones from infected bile that subsequently hybridized with RNA from infected animal and human liver, as well as isolates from five distinct geographic outbreaks. Sequence analysis revealed the presence of an RNA-dependent RNA polymerase, a common feature of plus-strand RNA viruses. Northern blot hybridization identified a 7.4-kilobase (kb) molecule present only in homogenates from infected liver samples. The agent was pronounced "hepatitis E virus" and further efforts by the same group cloned and sequenced HEV during the subsequent year.[149]

REFERENCES

1. Adler JL, Zickl R. Winter vomiting disease. *J Infect Dis* 1969; **119**:668–73.
2. Agus S, Dolin R, Wyatt R, et al. Acute infectious nonbacterial gastroenteritis: intestinal histopathology. Histologic and enzymatic alterations during illness produced by the Norwalk agent in man. *Ann Intern Med* 1973;**79**:18–25.
3. Appleton H, Higgins PG. Viruses and gastroenteritis in infants. *Lancet* 1975;**1**:1297.
4. Atmar RL, Bernstein DI, Harro CD, et al. Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J Med* 2011;**365**:2178–87.
5. Atmar RL, Estes MK. The epidemiologic and clinical importance of norovirus infection. *Gastroenterol Clin North Am* 2006;**35**:275–90.
6. Atmar RL, Opekun AR, Gilger MA, et al. Norwalk virus shedding after experimental human infection. *Emerg Infect Dis* 2008;**14**: 1553–7.
7. Baert L, Mattison K, Loisy-Hamon F, et al. Review: norovirus prevalence in Belgian, Canadian and French fresh produce: a threat to human health? *Int J Food Microbiol* 2011;**151**:261–9.
8. Baker K, Morris J, McCarthy N, et al. An outbreak of norovirus infection linked to oyster consumption at a UK restaurant, February 2010. *J Public Health (Oxf)* 2011;**33**:205–11.
9. Ball JM, Estes MK, Hardy ME, et al. Recombinant Norwalk virus–like particles as an oral vaccine. *Arch Virol Suppl* 1996;**12**: 243–9.
10. Ball JM, Hardy ME, Atmar RL, et al. Oral immunization with recombinant Norwalk virus–like particles induces a systemic and mucosal immune response in mice. *J Virol* 1998;**72**:1345–53.
11. Barlough JE, Berry ES, Skilling D, et al. The marine calicivirus story. *Compend Contin Educ Practicing Veterinarian* 1986;**8**:F5–F14.
12. Barlough JE, Matson DO, Skilling DE, et al. Isolation of reptilian calicivirus Crotalus type 1 from feral pinnipeds. *J Wildl Dis* 1998; **34**:451–6.
13. Berke T, Matson DO. Reclassification of the *Caliciviridae* into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. *Arch Virol* 2000;**145**:1421–36.
14. Bertolotti-Ciarlet A, White L, Chen R, et al. Structural requirements for the assembly of Norwalk virus–like particles. *J Virol* 2002;**76**:4044–55.
15. Blacklow N, Cukor G, Bedigian M, et al. Immune response and prevalence of antibody to Norwalk enteritis virus as determined by radioimmunoassay. *J Clin Microbiol* 1979;**10**:903–9.
16. Blazevic V, Lappalainen S, Nurminen K, et al. Norovirus VLPs and rotavirus VP6 protein as combined vaccine for childhood gastroenteritis. *Vaccine* 2011;**29**:8126–33.
17. Bok K, Cavanaugh VJ, Matson DO, et al. Inhibition of norovirus replication by morpholino oligomers targeting the 5′-end of the genome. *Virology* 2008;**380**:328–37.
18. Booth IW, Chrystie IL, Levinsky RJ, et al. Protracted diarrhoea, immunodeficiency and viruses. *Eur J Pediatr* 1982;**138**:271–2.
19. Bucardo F, Kindberg E, Paniagua M, et al. Genetic susceptibility to symptomatic norovirus infection in Nicaragua. *J Med Virol* 2009;**81**:728–35.
20. Bucardo F, Nordgren J, Carlsson B, et al. Pediatric norovirus diarrhea in Nicaragua. *J Clin Microbiol* 2008;**46**:2573–80.
21. Bucardo F, Nordgren J, Carlsson B, et al. Asymptomatic norovirus infections in Nicaraguan children and its association with viral properties and histo-blood group antigens. *Pediatr Infect Dis J* 2010;**29**:934–9.
22. Bull RA, Hansman GS, Clancy LE, et al. Norovirus recombination in ORF1/ORF2 overlap. *Emerg Infect Dis* 2005;**11**:1079–85.
23. Bull RA, White PA. Mechanisms of GII.4 norovirus evolution. *Trends Microbiol* 2011;**19**:233–40.
24. Campagnolo ER, Ernst MJ, Berninger ML, et al. Outbreak of rabbit hemorrhagic disease in domestic lagomorphs. *J Am Vet Med Assoc* 2003;**223**:1151–5, 1128.
25. Capucci L, Fusi P, Lavazza A, et al. Detection and preliminary characterization of a new rabbit calicivirus related to rabbit hemorrhagic disease virus but nonpathogenic. *J Virol* 1996;**70**: 8614–23.
26. Capucci L, Lavazza A. A brief update on rabbit hemorrhagic disease virus. *Emerg Infect Dis* 1998;**4**:343–4.
27. Carman JA, Garner MG, Catton MG, et al. Viral haemorrhagic disease of rabbits and human health. *Epidemiol Infect* 1998;**121**: 409–18.
28. Carter MJ, Milton ID, Meanger J, et al. The complete nucleotide sequence of a feline calicivirus. *Virology* 1992;**190**:443–8.
29. Castriciano S, Luinstra K, Petrich A, et al. Comparison of the RIDASCREEN norovirus enzyme immunoassay to IDEIA NLV GI/GII by testing stools also assayed by RT-PCR and electron microscopy. *J Virol Methods* 2007;**141**:216–19.
30. Centers for Disease Control and Prevention. Surveillance for foodborne disease outbreaks—United States, 2008. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1197–202.
31. Chadwick PR, Walker M, Rees AE. Airborne transmission of a small round structured virus. *Lancet* 1994;**343**:171.
32. Chan CM, Chan CW, Ma CK, et al. Norovirus as cause of benign convulsion associated with gastroenteritis. *J Paediatr Child Health* 2011;**47**:373–7.
33. Chasey D. Possible origin of rabbit haemorrhagic disease in the United Kingdom. *Vet Rec* 1994;**135**:496–9.
34. Chen R, Neill JD, Estes MK, et al. X-ray structure of a native calicivirus: structural insights into antigenic diversity and host specificity. *Proc Natl Acad Sci U S A* 2006;**103**:8048–53.
35. Chen SY, Tsai CN, Lai MW, et al. Norovirus infection as a cause of diarrhea-associated benign infantile seizures. *Clin Infect Dis* 2009;**48**:849–55.
36. Cheng W, Ye X, Yang X, et al. Epidemiological study of human calicivirus infection in children with gastroenteritis in Lanzhou from 2001 to 2007. *Arch Virol* 2010;**155**:553–5.
37. Cheesbrough JS, Green J, Gallimore CI, et al. Widespread environmental contamination with Norwalk-like viruses (NLV) detected in a prolonged hotel outbreak of gastroenteritis. *Epidemiol Infect* 2000;**125**:93–8.
38. Clarke IN, Estes MK, Green KY, et al. Caliciviridae. In: King AMQ, Adams MJ, Carstens EB, et al, editors. *Virus taxonomy: classification and nomenclature of viruses. Ninth report of the International Committee on Taxonomy of Viruses*. San Diego: Elsevier; 2011. p. 977–86.
39. Coman BJ. *Environmental impact associated with the proposed use of rabbit calicivirus disease for integrated rabbit control in Australia*. Sydney: Australia and New Zealand Rabbit Calicivirus Program; 1996.
40. Costantini VP, Whitaker T, Barclay L, et al. Antiviral activity of nucleoside analogues against norovirus. *Antivir Ther* 2012;**17**: 981–91.
41. Cooper PD, Agolk VI, Bachrach HL, et al. Picornaviridae: second report. *Intervirology* 1978;**10**:165.
42. Coyne KP, Reed FC, Porter CJ, et al. Recombination of feline calicivirus within an endemically infected cat colony. *J Gen Virol* 2006;**87**:921–6.
43. Cukor G, Nowak N, Blacklow N. Immunoglobulin M responses to the Norwalk virus of gastroenteritis. *Infect Immun* 1982;**37**: 463–8.
44. Czakó R, Atmar RL, Opekun AR, et al. Serum hemagglutination inhibition activity correlates with protection from gastroenteritis in persons infected with Norwalk virus. *Clin Vaccine Immunol* 2012;**19**:284–7.
45. da Silva AK, Le Saux JC, Parnaudeau S, et al. Evaluation of removal of noroviruses during wastewater treatment, using real-time reverse transcription-PCR: different behaviors of genogroups I and II. *Appl Environ Microbiol* 2007;**73**:7891–7.
46. Daughenbaugh KF, Fraser CS, Hershey JW, et al. The genome-linked protein VPg of the Norwalk virus binds eIF3, suggesting its role in translation initiation complex recruitment. *EMBO J* 2003;**22**:2852–9.
47. Delacour H, Dubrous P, Koeck JL. Noroviruses: a challenge for military forces. *J R Army Med Corps* 2010;**156**:251–4.
48. Dey SK, Phan TG, Nguyen TA, et al. Prevalence of sapovirus infection among infants and children with acute gastroenteritis in Dhaka City, Bangladesh during 2004-2005. *J Med Virol* 2007;**79**: 633–8.
49. Di Martino B, Di Profio F, Martella V, et al. Evidence for recombination in neboviruses. *Vet Microbiol* 2011;**153**:367–72.
50. Dingle KE, Lambden PR, Caul EO, et al. Human enteric *Caliciviridae:* the complete genome sequence and expression of virus-like particles from a genetic group II small round structured virus. *J Gen Virol* 1995;**76**(pt. 9):2349–55.

51. Division of Viral Diseases, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention. Updated norovirus outbreak management and disease prevention guidelines. *MMWR Recomm Rep* 2011;**60**:1–18.
52. D'Souza DH, Sair A, Williams K, et al. Persistence of caliciviruses on environmental surfaces and their transfer to food. *Int J Food Microbiol* 2006;**108**:84–91.
53. Duizer E, Schwab KJ, Neill FH, et al. Laboratory efforts to cultivate noroviruses. *J Gen Virol* 2004;**85**:79–87.
54. Etherington GJ, Ring SM, Charleston MA, et al. Tracing the origin and co-phylogeny of the caliciviruses. *J Gen Virol* 2006; **87**(pt. 5):1229–35.
55. Feeney SA, Armstrong VJ, Mitchell SJ, et al. Development and clinical validation of multiplex TaqMan® assays for rapid diagnosis of viral gastroenteritis. *J Med Virol* 2011;**83**:1650–6.
56. Flynn WT, Saif LJ. Serial propagation of porcine enteric calicivirus-like virus in primary porcine kidney cell cultures. *J Clin Microbiol* 1988;**26**:206–12.
57. Forrester NL, Moss SR, Turner SL, et al. Recombination in rabbit haemorrhagic disease virus: possible impact on evolution and epidemiology. *Virology* 2008;**376**:390–6.
58. Frange P, Touzot F, Debré M, et al. Prevalence and clinical impact of norovirus fecal shedding in children with inherited immune deficiencies. *J Infect Dis* 2012;**206**:1269–74.
59. Gallimore CI, Cheesbrough JS, Lamden K, et al. Multiple norovirus genotypes characterized from an oyster-associated outbreak of gastroenteritis. *Int J Food Microbiol* 2005;**103**:323–30.
60. Gallimore CI, Green J, Lewis D, et al. Diversity of noroviruses cocirculating in the north of England from 1998 to 2001. *J Clin Microbiol* 2004;**42**:1396–401.
61. Gallimore CI, Iturriza-Gomara M, Lewis D, et al. Characterization of sapoviruses collected in the United Kingdom from 1989 to 2004. *J Med Virol* 2006;**78**:673–82.
62. Gallimore CI, Pipkin C, Shrimpton H, et al. Detection of multiple enteric virus strains within a foodborne outbreak of gastroenteritis: an indication of the source of contamination. *Epidemiol Infect* 2005;**133**:41–7.
63. Ganova-Raeva L, Smith AW, Fields H, Khudyakov Y. New calicivirus isolated from walrus. *Virus Res* 2004;**102**:207–13.
64. Geginat G, Kaiser D, Schrempf S. Evaluation of third-generation ELISA and a rapid immunochromatographic assay for the detection of norovirus infection in fecal samples from inpatients of a German tertiary care hospital. *Eur J Clin Microbiol Infect Dis* 2012;**31**:733–7.
65. Gotz H, Ekdahl K, Lindback J, et al. Clinical spectrum and transmission characteristics of infection with Norwalk-like virus: findings from a large community outbreak in Sweden. *Clin Infect Dis* 2001;**33**:622–8.
66. Graham DY, Jiang X, Tanaka T, et al. Norwalk virus infection of volunteers: New insights based on improved assays. *J Infect Dis* 1994;**170**:34–43.
67. Gray JJ, Jiang X, Morgan-Capner P, et al. Prevalence of antibodies to Norwalk virus in England: detection by enzyme-linked immunosorbent assay using baculovirus-expressed Norwalk virus capsid antigen. *J Clin Microbiol* 1993;**31**:1022–5.
68. Gregg DA, House C, Meyer R, Berninger M. Viral haemorrhagic disease of rabbits in Mexico: epidemiology and viral characterization. *Rev Sci Tech* 1991;**10**:435–51.
69. Green KY, Ando T, Balayan MS, et al. Taxonomy of the caliciviruses. *J Infect Dis* 2000;**181**(Suppl. 2):S322–30.
70. Green KY, Belliot G, Taylor JL, et al. A predominant role for Norwalk-like viruses as agents of epidemic gastroenteritis in Maryland nursing homes for the elderly. *J Infect Dis* 2002;**185**: 133–46.
71. Greenberg HB, Valdesuso J, Yolken RH, et al. Role of Norwalk virus in outbreaks of nonbacterial gastroenteritis. *J Infect Dis* 1979;**139**:564–8.
72. Guerrero RA, Ball JM, Krater SS, et al. Recombinant Norwalk virus–like particles administered intranasally to mice induce systemic and mucosal (fecal and vaginal) immune responses. *J Virol* 2001;**75**:9713–22.
73. Gulati BR, Allwood PB, Hedberg CW, et al. Efficacy of commonly used disinfectants for the inactivation of calicivirus on strawberry, lettuce, and a food-contact surface. *J Food Prot* 2001;**64**:1430–4.
74. Guo M, Qian Y, Chang KO, et al. Expression and self-assembly in baculovirus of porcine enteric calicivirus capsids into virus-like particles and their use in an enzyme-linked immunosorbent assay for antibody detection in swine. *J Clin Microbiol* 2001;**39**:1487–93.
75. Hale A, Mattick K, Lewis D, et al. Distinct epidemiological patterns of Norwalk-like virus infection. *J Med Virol* 2000;**62**:99–103.
76. Hall GA, Bridger JC, Brooker BE, et al. Lesions of gnotobiotic calves experimentally infected with a calicivirus-like (Newbury) agent. *Vet Pathol* 1984;**21**:208–15.
77. Hansman GS, Natori K, Shirato-Horikoshi H, et al. Genetic and antigenic diversity among noroviruses. *J Gen Virol* 2006;**87**:909–19.
78. Hansman GS, Oka T, Katayama K, et al. Human sapoviruses: genetic diversity, recombination, and classification. *Rev Med Virol* 2007;**17**:133–41.
79. Hardy M. Norovirus protein structure and function. *FEMS Microbiol* 2005;**253**:1–8.
80. Hardy ME, Kramer SF, Treanor JJ, et al. Human calicivirus genogroup II capsid sequence diversity revealed by analyses of the prototype Snow Mountain agent. *Arch Virol* 1997;**142**:1469–79.
81. Harrington PR, Yount B, Johnston RE, et al. Systemic, mucosal, and heterotypic immune induction in mice inoculated with Venezuelan equine encephalitis replicons expressing Norwalk virus-like particles. *J Virol* 2002;**76**:730–42.
82. Hassine-Zaafrane M, Kaplon J, Sdiri-Loulizi K, et al. Molecular prevalence of bovine noroviruses and neboviruses detected in central-eastern Tunisia. *Arch Virol* 2012;**157**:1599–604.
83. Higgins RR, Beniprashad M, Cardona M, et al. Evaluation and verification of the Seeplex Diarrhea-V ACE assay for simultaneous detection of adenovirus, rotavirus, and norovirus genogroups I and II in clinical stool specimens. *J Clin Microbiol* 2011;**49**:3154–62.
84. Hohdatsu T, Sato K, Tajima T, et al. Neutralizing feature of commercially available feline calicivirus (FCV) vaccine immune sera against FCV field isolates. *J Vet Med Sci* 1999;**61**:299–301.
85. Honma S, Nakata S, Numata K, et al. Epidemiological study of prevalence of genogroup II human calicivirus (Mexico virus) infections in Japan and Southeast Asia as determined by enzyme-linked immunosorbent assays. *J Clin Microbiol* 1998;**36**:2481–4.
86. Hu Z, Tian X, Zhai Y, et al. Cryo-electron microscopy reconstructions of two types of wild rabbit hemorrhagic disease viruses characterized the structural features of lagovirus. *Protein Cell* 2010;**1**: 48–58.
87. Huang P, Farkas T, Zhong W, et al. Norovirus and histo-blood group antigens: demonstration of a wide spectrum of strain specificities and classification of two major binding groups among multiple binding patterns. *J Virol* 2005;**79**:6714–22.
88. Hutson A, Atmar R, Estes M. Norovirus disease: changing epidemiology and host susceptibility factors. *Trends Microbiol* 2004;**12**: 279–87.
89. International Committee on Taxonomy of Viruses. *Master species list. 2011*. Available at http://www.ictvonline.org/virusTaxonomy.asp?taxnode_id=20113430. Accessed August 10, 2012.
90. Jarvis BDW. *Dilemmas and implications*. Miscellaneous Series of the Royal Society of New Zealand; 1999. p. 55.
91. Jiang X, Espul C, Zhong WM, et al. Characterization of a novel human calicivirus that may be a naturally occurring recombinant. *Arch Virol* 1999;**144**:2377–87.
92. Jiang X, Graham DY, Wang K, et al. Norwalk virus genome cloning and characterization. *Science* 1990;**250**:1580–3.
93. Jiang X, Huang PW, Zhong WM, et al. Design and evaluation of a primer pair that detects both Norwalk- and Sapporo-like caliciviruses byRT-PCR. *J Virol Methods* 1999;**83**:145–54.
94. Jiang X, Huang P, Zhong W, et al. Human milk contains elements that block binding of noroviruses to histo-blood group antigens in saliva. *Adv Exp Med Biol* 2004;**554**:447–50.
95. Jiang X, Wang M, Wang K, et al. Sequence and genomic organization of Norwalk virus. *Virology* 1993;**195**:51–61.
96. Jiang X, Wilton N, Zhong WM, et al. Diagnosis of human caliciviruses by use of enzyme immunoassays. *J Infect Dis* 2000; **181**(Suppl. 2):S349–59.
97. Johnson P, Mathewson J, DuPont H, et al. Multiple-challenge study of host susceptibility to Norwalk gastroenteritis in US adults. *J Infect Dis* 1990;**161**:18–21.
98. Johnson RP, Povey RC. Feline calicivirus infection in kittens borne by cats persistently infected with the virus. *Res Vet Sci* 1984;**37**:114–19.

99. Kageyama T, Kojima S, Shinohara M, et al. Broadly reactive and highly sensitive assay for Norwalk-like viruses based on real-time quantitative reverse transcription-PCR. *J Clin Microbiol* 2003;**41**: 1548–57.
100. Kageyama T, Shinohara M, Uchida K, et al. Coexistence of multiple genotypes, including newly identified genotypes, in outbreaks of gastroenteritis due to norovirus in Japan. *J Clin Microbiol* 2004;**42**:2988–95.
101. Kapikian A, Wyatt RG, Dolin R, et al. Visualization by immune electron microscopy of a 27-nm particle associated with acute infectious non-bacterial gastroenteritis. *J Virol* 1972;**10**:1075–81.
102. Kaplan JE, Feldman R, Campbell DS, et al. The frequency of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. *Am J Public Health* 1982;**72**:1329–32.
103. Kaplon J, Guenau E, Asdrubal P, et al. Possible novel nebovirus genotype in cattle, France. *Emerg Infect Dis* 2011;**17**:1120–3.
104. Kele B, Lengyel G, Deak J. Comparison of an ELISA and two reverse transcription polymerase chain reaction methods for norovirus detection. *Diagn Microbiol Infect Dis* 2011;**70**:475–8.
105. Kimura H, Nagano K, Kimura N, et al. A norovirus outbreak associated with environmental contamination at a hotel. *Epidemiol Infect* 2011;**139**:317–25.
106. Kirby A, Gurgel RQ, Dove W, et al. An evaluation of the RIDASCREEN and IDEIA enzyme immunoassays and the RIDAQUICK immunochromatographic test for the detection of norovirus in faecal specimens. *J Clin Virol* 2010;**49**:254–7.
107. Koo HL, Ajami N, Atmar RL, et al. Noroviruses: the leading cause of gastroenteritis worldwide. *Discov Med* 2010;**10**:61–70.
108. Lambden PR, Liu B, Clarke IN. A conserved sequence motif at the 5′ terminus of the Southampton virus genome is characteristic of the *Caliciviridae*. *Virus Genes* 1995;**10**:149–52.
109. Larsson M, Rydell G, Grahn A, et al. Antibody prevalence and titer to norovirus (genogroup ii) correlate with secretor *(FUT2)* but not with ABO phenotype or Lewis *(FUT3)* genotype. *J Infect Dis* 2006;**194**:1422–7.
110. Laurent S, Vautherot JF, Le Gall G, et al. Structural, antigenic and immunogenic relationships between European brown hare syndrome virus and rabbit haemorrhagic disease virus. *J Gen Virol* 1997;**78**(pt. 11):2803–11.
111. Lavazza A, Scicluna MT, Capucci L. Susceptibility of hares and rabbits to the European brown hare syndrome virus (EBHSV) and rabbit haemorrhagic disease virus (RHDV) under experimental conditions. *Zentralbl Veterinarmed B* 1996;**43**:401–10.
112. Le Gall G, Huguet S, Vende P, et al. European brown hare syndrome virus: Molecular cloning and sequencing of the genome. *J Gen Virol* 1996;**77**(pt. 8):1693–7.
113. Lee LE, Cebelinski EA, Fuller C, et al. Sapovirus outbreaks in long-term care facilities, Oregon and Minnesota, USA, 2002-2009. *Emerg Infect Dis* 2012;**18**:873–6.
114. Lindesmith L, Donaldson E, LoBue A, et al. Mechanisms of GII.4 Norovirus persistence in human populations. *Plos Med* 2008;**5**:269–90.
115. Lindesmith L, Moe C, LePendu J, et al. Cellular and humoral immunity following Snow Mountain virus challenge. *J Virol* 2005; **79**:2900–9.
116. Lindesmith L, Moe C, Marionneau S, et al. Human susceptibility and resistance to Norwalk virus infection. *Nat Med* 2003;**9**:548–53.
117. Lopman BA, Adak GK, Reacher MH, et al. Two epidemiologic patterns of norovirus outbreaks: surveillance in England and Wales, 1992-2000. *Emerg Infect Dis* 2003;**9**:71–7.
118. Lopman B, Gastañaduy P, Park GW, et al. Environmental transmission of norovirus gastroenteritis. *Curr Opin Virol* 2012;**2**:96–102.
119. Lowther JA, Henshilwood K, Lees DN. Determination of norovirus contamination in oysters from two commercial harvesting areas over an extended period, using semiquantitative real-time reverse transcription PCR. *J Food Prot* 2008;**71**:1427–33.
120. Mahar JE, Kirkwood CD. Characterization of norovirus strains in Australian children from 2006 to 2008: prevalence of recombinant strains. *J Med Virol* 2011;**83**:2213–19.
121. Marionneau S, Ruvoen N, Le Moullac-Vaidye B, et al. Norwalk virus binds to histo-blood group antigens present on gastroduodenal epithelial cells of secretor individuals. *Gastroenterology* 2002;**122**:1967–77.
122. Marks PJ, Vipond IB, Regan FM, et al. A school outbreak of Norwalk-like virus: evidence for airborne transmission. *Epidemiol Infect* 2003;**131**:727–36.
123. Marshall JA, Hellard ME, Sinclair MI, et al. Incidence and characteristics of endemic Norwalk-like virus–associated gastroenteritis. *J Med Virol* 2003;**69**:568–78.
124. Martin-Alonso JM, Skilling DE, Gonzalez-Molleda L, et al. Isolation and characterization of a new vesivirus from rabbits. *Virology* 2005;**337**:373–83.
125. Martinez N, Espul C, Cuello H, et al. Sequence diversity of human caliciviruses recovered from children with diarrhea in Mendoza, Argentina, 1995-1998. *J Med Virol* 2002;**67**:289–98.
126. Mathijs E, Denayer S, Palmeira L, et al. Novel norovirus recombinants and of GII.4 sub-lineages associated with outbreaks between 2006 and 2010 in Belgium. *Virol J* 2011;**8**:310.
127. Matson DO. Re-analysis of serologic data from the Australian study of human health risks of infection by rabbit hemorrhagic disease virus. *Royal Society of New Zealand, Wellington: Misc Series* 1999;**55**:62–6.
128. Matson DO, Berke T, Dinulos MB, et al. Partial characterization of the genome of nine animal caliciviruses. *Arch Virol* 1996;**141**: 2443–56.
129. Matthews JE, Dickey BW, Miller RD, et al. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. *Epidemiol Infect* 2012;**140**:1161–72.
130. Mayo MA. Virus taxonomy—Houston 2002. *Arch Virol* 2002; **147**:1071–6.
131. McSwiggan DA, Cubitt D, Moore W. Calicivirus associated with winter vomiting disease. *Lancet* 1978;**1**:1215.
132. Medici M, Tummolo F, Albonetti V, et al. Molecular detection and epidemiology of astrovirus, bocavirus, and sapovirus in Italian children admitted to hospital with acute gastroenteritis, 2008-2009. *J Med Virol* 2012;**84**:643–50.
133. Meyers G, Wirblich C, Thiel HJ. Rabbit hemorrhagic disease virus—molecular cloning and nucleotide sequencing of a calicivirus genome. *Virology* 1991;**184**:664–76.
134. Mochizuki M, Nakatani H, Yoshida M. Inhibitory effects of recombinant feline interferon on the replication of feline enteropathogenic viruses in vitro. *Vet Microbiol* 1994;**39**:145–52.
135. Morillo SG, Luchs A, Cilli A, et al. Norovirus 3rd generation kit: an improvement for rapid diagnosis of sporadic gastroenteritis cases and valuable for outbreak detection. *J Virol Methods* 2011; **173**:13–16.
136. Morotti R, Kaufman S, Fishbein T, et al. Calicivirus infection in pediatric small intestine transplant recipients: pathological considerations. *Hum Pathol* 2004;**35**:1236–40.
137. Morrow A, Ruiz-Palacios G, Jiang X, et al. Human milk oligosaccharides are associated with protection against diarrhea in breastfed infants. *J Pediatr* 2004;**145**:297–303.
138. Mounts AW, Ando T, Koopmans M, et al. Cold weather seasonality of gastroenteritis associated with Norwalk-like viruses. *J Infect Dis* 2000;**181**(Suppl 2):S284–7.
139. Nasir W, Frank M, Koppisetty CA, et al. Lewis histo-blood group α1,3/α1,4 fucose residues may both mediate binding to GII.4 noroviruses. *Glycobiology* 2012;**22**:1163–72.
140. Neill JD, Meyer RF, Seal BS. Genetic relatedness of the caliciviruses: San Miguel sea lion and vesicular exanthema of swine viruses constitute a single genotype within the *Caliciviridae*. *J Virol* 1995;**69**:4484–8.
141. Neill JD, Seal BS. Development of PCR primers for specific amplification of two distinct regions of the genomes of San Miguel sea-lion and vesicular exanthema of swine viruses. *Mol Cell Probes* 1995;**9**:33–7.
142. Nilsson M, Hendlund K, Thorhagen M, et al. Evolution of human calicivirus RNA in vivo: accumulation of mutations in the protruding P2 domain of the capsid leads to structural changes and possibly a new phenotype. *J Virol* 2003;**77**:13117–24.
143. Noel JS, Fankhauser RL, Ando T, et al. Identification of a distinct common strain of "Norwalk-like viruses" having a global distribution. *J Infect Dis* 1999;**179**:1334–44.
144. Nowotny N, Bascunana CR, Ballagi-Pordany A, et al. Phylogenetic analysis of rabbit haemorrhagic disease and European brown hare syndrome viruses by comparison of sequences from the capsid protein gene. *Arch Virol* 1997;**142**:657–73.

145. Numata K, Nakata S, Jiang X, et al. Epidemiological study of Norwalk virus infections in Japan and Southeast Asia by enzyme-linked immunosorbent assays with Norwalk virus capsid protein produced by the baculovirus expression system. *J Clin Microbiol* 1994;**32**:121–6.
146. Ohlinger VF, Thiel HJ. Identification of the viral haemorrhagic disease virus of rabbits as a calicivirus. *Rev Sci Tech* 1991;**10**: 311–23.
147. Oka T, Yamamoto M, Katayama K, et al. Identification of the cleavage sites of sapovirus open reading frame 1 polyprotein. *J Gen Virol* 2006;**87**:3329–38.
148. Oka T, Yamamoto M, Yokoyama M, et al. Highly conserved configuration of catalytic amino acid residues among calicivirus-encoded proteases. *J Virol* 2007;**81**:6798–806.
149. Oliver SL, Asobayire E, Dastjerdi AM, et al. Genomic characterization of the unclassified bovine enteric virus Newbury agent-1 (Newbury1) endorses a new genus in the family of *Caliciviridae*. *Virology* 2006;**350**:240–50.
150. O'Ryan ML, Lucero Y, Prado V, et al. Symptomatic and asymptomatic rotavirus and norovirus infections during infancy in a Chilean birth cohort. *Pediatr Infect Dis J* 2009;**28**:879–84.
151. O'Ryan M, Peña A, Vergara R, et al. Prospective characterization of norovirus compared with rotavirus acute diarrhea episodes in Chilean children. *Pediatr Infect Dis J* 2010;**29**:855–9.
152. O'Ryan ML, Vial PA, Mamani N, et al. Seroprevalence of Norwalk virus and Mexico virus in Chilean individuals: assessment of independent risk factors for antibody acquisition. *Clin Infect Dis* 1998;**27**:789–95.
153. Pang XL, Preiksaitis JK, Lee B. Multiplex real time RT-PCR for the detection and quantitation of norovirus genogroups I and II in patients with acute gastroenteritis. *J Clin Virol* 2005;**33**:168–71.
154. Pang XL, Zeng SQ, Honma S, et al. Effect of rotavirus vaccine on Sapporo virus gastroenteritis in Finnish infants. *Pediatr Infect Dis J* 2001;**20**:295–300.
155. Park SI, Jeong C, Park SJ, et al. Molecular detection and characterization of unclassified bovine enteric caliciviruses in South Korea. *Vet Microbiol* 2008;**130**:371–9.
156. Parra F, Prieto M. Purification and characterization of a calicivirus as the causative agent of a lethal hemorrhagic disease in rabbits. *J Virol* 1990;**64**:4013–15.
157. Parrino T, Schreiber D, Trier J, et al. Clinical immunity in acute gastroenteritis caused by Norwalk agent. *N Engl J Med* 1977; **297**:86–9.
158. Phanuwan C, Takizawa S, Oguma K, et al. Monitoring of human enteric viruses and coliform bacteria in waters after urban flood in Jakarta, Indonesia. *Water Sci Technol* 2006;**54**:203–10.
159. Povey RC. In vitro antiviral efficacy of ribavirin against feline calicivirus, feline viral rhinotracheitis virus, and canine parainfluenza virus. *Am J Vet Res* 1978;**39**:175–8.
160. Prassad BV, Hardy ME, Dokland T, et al. X-ray crystallographic structure of the Norwalk virus capsid. *Science* 1999;**286**:287–90.
161. Prassad BV, Matson DO, Smith AW. Three-dimensional structure of calicivirus. *J Mol Biol* 1994;**240**:256–64.
162. Puustinen L, Blazevic V, Huhti L, et al. Norovirus genotypes in endemic acute gastroenteritis of infants and children in Finland between 1994 and 2007. *Epidemiol Infect* 2012;**140**:268–75.
163. Reeck A, Kavanagh O, Estes MK, et al. Serological correlate of protection against norovirus-induced gastroenteritis. *J Infect Dis* 2010;**202**:1212–18.
164. Rinehart-Kim JE, Zhong WM, Jiang X, et al. Complete nucleotide sequence and genomic organization of a primate calicivirus, Pan-1. *Arch Virol* 1999;**144**:199–208.
165. Rockx B, de Witt M, Vennema H, et al. Natural history of human calicivirus infection: a prospective cohort study. *Clin Infect Dis* 2002;**35**:246–53.
166. Roos-Weil D, Ambert-Balay K, Lanternier F, et al. Impact of norovirus/sapovirus-related diarrhea in renal transplant recipients hospitalized for diarrhea. *Transplantation* 2011;**92**:61–9.
167. Saif MA, Bonney DK, Bigger B, et al. Chronic norovirus infection in pediatric hematopoietic stem cell transplant recipients: a cause of prolonged intestinal failure requiring intensive nutritional support. *Pediatr Transplant* 2011;**15**:505–9.
168. Sartorius B, Andersson Y, Velicko I, et al. Outbreak of norovirus in Västra Götaland associated with recreational activities at two lakes during August 2004. *Scand J Infect Dis* 2007;**39**:323–31.
169. Sarvikivi E, Roivainen M, Maunula L, et al. Multiple norovirus outbreaks linked to imported frozen raspberries. *Epidemiol Infect* 2012;**140**:260–7.
170. Schreiber DS, Blacklow NR, Trier JS. The mucosal lesion of the proximal small intestine in acute infectious nonbacterial gastroenteritis. *N Engl J Med* 1973;**288**:1318–23.
171. Schreiber DS, Blacklow NR, Trier JS. The small intestinal lesion induced by Hawaii agent acute infectious nonbacterial gastroenteritis. *J Infect Dis* 1974;**129**:705–8.
172. Schwartz S, Vergoulidou M, Schreier E, et al. Norovirus gastroenteritis causes severe and lethal complications after chemotherapy and hematopoietic stem cell transplantation. *Blood* 2011;**117**: 5850–6.
173. Seal BS, Lutze-Wallace C, Kreutz LC, et al. Isolation of caliciviruses from skunks that are antigenically and genotypically related to San Miguel sea lion virus. *Virus Res* 1995;**37**:1–12.
174. Siebenga J, Vennema H, Zheng D, et al. Norovirus illness is a global problem: emergence and spread of norovirus GII.4 variants; 2001-2007. *J Infect Dis* 2009;**200**:802–12.
175. Smiley JR, Chang KO, Hayes J, et al. Characterization of anenteropathogenic bovine calicivirus representing a potentially new calicivirus genus. *J Virol* 2002;**76**:10089–98.
176. Smith AW. Virus cycles in aquatic mammals, poikilotherms, and invertebrates. In: Hurst C, editor. *Viral ecology*. San Diego: Academic Press; 2000. p. 447–91.
177. Smith AW, Berry ES, Skilling DE, et al. In vitro isolation and characterization of a calicivirus causing a vesicular disease of the hands and feet. *Clin Infect Dis* 1998;**26**:434–9.
178. Smith AW, Boyt PM. Caliciviruses of ocean origin: a review. *J Zoo Wildlife Med* 1990;**21**:3–23.
179. Smith AW, Iversen PL, Skilling DE, et al. Vesivirus viremia and seroprevalence in humans. *J Med Virol* 2006;**78**:693–701.
180. Smith AW, Matson DO, Stein DA, et al. Antisense treatment of *Caliciviridae:* an emerging disease agent of animals and humans. *Curr Opin Mol Ther* 2002;**4**:177–84.
181. Smith AW, Mattson DE, Skilling DE, et al. Isolation and partial characterization of a calicivirus from calves. *Am J Vet Res* 1983;**44**: 851–5.
182. Smith AW, Prato C, Skilling DE. Caliciviruses infecting monkeys and possibly man. *Am J Vet Res* 1978;**39**:287–9.
183. Smith AW, Skilling DE, Cherry N, et al. Calicivirus emergence from ocean reservoirs: zoonotic and interspecies movements. *Emerg Infect Dis* 1998;**4**:13–20.
184. Smith AW, Skilling DE, Dardiri AH, et al. Calicivirus pathogenic for swine: a new serotype isolated from opal eye *Girella nigricans*, an ocean fish. *Science* 1980;**209**:940–1.
185. Smith AW, Skilling DE, Ensley PK, et al. Calicivirus isolation and persistence in a pygmy chimpanzee *(Pan paniscus)*. *Science* 1983; **221**:79–81.
186. Smith AW, Skilling DE, Matson DO, et al. Detection of vesicular exanthema of swine-like calicivirus in tissues from a naturally infected spontaneously aborted bovine fetus. *J Am Vet Med Assoc* 2002;**220**:455–8.
187. Soller JA, Bartrand T, Ashbolt NJ, et al. Estimating the primary etiologic agents in recreational freshwaters impacted by human sources of faecal contamination. *Water Res* 2010;**44**:4736–47.
188. Stein DA, Skilling DE, Iversen PL, Smith AW. Inhibition of vesivirus infections in mammalian tissue culture with antisense morpholino oligomers. *Antisense Nucleic Acid Drug Dev* 2001;**11**: 317–25.
189. Studdert MJ. Caliciviruses: brief review. *Arch Virol* 1978;**58**: 157–91.
190. Sugieda M, Nakajima K, Nakajima S. Outbreaks of Norwalk-like virus–associated gastroenteritis traced to shellfish: coexistence of two genotypes in one specimen. *Epidemiol Infect* 1996;**116**:339–46.
191. Svraka S, Duizer E, Vennema H. Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005. *J Clin Microbiol* 2007;**45**:1389–94.
192. Svraka S, Vennema H, van der Veer B, et al. Epidemiology and genotype analysis of emerging sapovirus-associated infections across Europe. *J Clin Microbiol* 2010;**48**:2191–8.
193. Takanashi S, Hashira S, Matsunaga T, et al. Detection, genetic characterization, and quantification of norovirus RNA from sera of children with gastroenteritis. *J Clin Virol* 2009;**44**:161–3.

194. Tacket CO, Mason HS, Losonsky G, et al. Human immune responses to a novel Norwalk virus vaccine delivered in transgenic potatoes. *J Infect Dis* 2000;**182**:302–5.
195. Tam CC, O'Brien SJ, Tompkins DS, et al. Changes in causes of acute gastroenteritis in the United Kingdom over 15 years: microbiologic findings from 2 prospective, population-based studies of infectious intestinal disease. *Clin Infect Dis* 2012;**54**:1275–86.
196. Tamminen K, Huhti L, Koho T, et al. A comparison of immunogenicity of norovirus GII-4 virus-like particles and P-particles. *Immunology* 2012;**135**:89–99.
197. Tan M, Jiang X. Norovirus P particle: a subviral nanoparticle for vaccine development against norovirus, rotavirus and influenza virus. *Nanomedicine (Lond)* 2012;**7**:889–97.
198. Terio V, Martella V, Moschidou P, et al. Norovirus in retail shellfish. *Food Microbiol* 2010;**27**:29–32.
199. Thornhill TS, Kalica AR, Wyatt RG, et al. Pattern of shedding of the Norwalk particle in stools during experimentally induced gastroenteritis in volunteers as determined by immune electron microscopy. *J Infect Dis* 1975;**132**:28–34.
200. Thorven M, Grahn A, Hedlund KO, et al. A homozygous nonsense mutation (428G→A) in the human secretor *(FUT2)* gene provides resistance to symptomatic norovirus (GGII) infections. *J Virol* 2005;**79**:15351–5.
201. Trujillo AA, McCaustland KA, Zheng DP, et al. Use of TaqMan real-time reverse transcription-PCR for rapid detection, quantification, and typing of norovirus. *J Clin Microbiol* 2006;**44**:1405–12.
202. Ueda K, Park JH, Ochiai K, et al. Disseminated intravascular coagulation (DIC) in rabbit haemorrhagic disease. *Jpn J Vet Res* 1992;**40**:133–41.
203. Vidal R, Roessler P, Solari V, et al. Novel recombinant norovirus causing outbreaks of gastroenteritis in Santiago, Chile. *J Clin Microbiol* 2006;**44**:2271–5.
204. Vidal R, Solari V, Mamani N, et al. Caliciviruses and foodborne gastroenteritis, Chile. *Emerg Infect Dis* 2005;**11**:1134–6.
205. Vinje J, Hamidjaja RA, Sobsey MD. Development and application of a capsid VP1 (region D) based reverse transcription PCR assay for genotyping of genogroup I and II noroviruses. *J Virol Methods* 2004;**116**:109–17.
206. Widdowson MA, Cramer EH, Hadley L, et al. Outbreaks of acute gastroenteritis on cruise ships and on land: identification of a predominant circulating strain of norovirus—United. States, 2002. *J Infect Dis* 2004;**190**:27–36.
207. Wirblich C, Meyers G, Ohlinger VF, et al. European brown hare syndrome virus: relationship to rabbit hemorrhagic disease virus and other caliciviruses. *J Virol* 1994;**68**:5164–73.
208. Woode GN, Bridger JC. Isolation of small viruses resembling astroviruses and caliciviruses from acute enteritis of calves. *J Med Microbiol* 1978;**11**:441–52.
209. Wyatt RG, Dolin R, Blacklow NR, et al. Comparison of three agents of acute infectious nonbacterial gastroenteritis by cross-challenge in volunteers. *J Infect Dis* 1974;**129**:709–14.
210. Xu ZJ, Chen WX. Viral hemorrhagic disease in rabbits: a review. *Vet Res Commun* 1989;**13**:205–12.
211. Yoda T, Terano Y, Suzuki Y, et al. Characterization of monoclonal antibodies generated against Norwalk virus GII capsid protein expressed in *Escherichia coli*. *Microbiol Immunol* 2000;**44**:905–14.
212. Yunus MA, Chung LM, Chaudhry Y, et al. Development of an optimized RNA-based murine norovirus reverse genetics system. *J Virol Methods* 2010;**169**:112–18.
213. Zheng DP, Ando T, Fankhauser RL, et al. Norovirus classification and proposed strain nomenclature. *Virology* 2006;**346**:312–23.
214. Zintz C, Bok K, Parada E, et al. Prevalence and genetic characterization of caliciviruses among children hospitalized for acute gastroenteritis in the United States. *Infect Genet Evol* 2005;**5**:281–90.

MICROBIOLOGY AND GENOME ORGANIZATION

HEV is a plus sense RNA virus with a genome size of approximately 7200 base pairs (bps).[149,174] Initial cloning of the Burma strain revealed the presence of three overlapping reading frames (ORFs).[149,162] ORF1 is approximately 5000 bps in length and encodes for the nonstructural proteins.[89,149] ORF2 begins 37 bps downstream from the ORF1 stop codon and codes for the viral capsid.[149] ORF3 is only 369 bps and overlaps both ORF1 and ORF2. The viral RNA contains a short 5′ untranslated region (UTR) of approximately 27 bps and a 3′ UTR of approximately 65 bps.[149] The genome has a 7-methyl cap at the 5′ UTR and a poly-A tail of approximately 150 to 200 bases.[73] The organization of the genome is represented in Figure 169-1.

Initial comparative genomic analysis deduced the likely functions of the ORF1 nonstructural regions.[89] They include the methyltransferase "capping" motif (~amino acids 56-240), papain-like cysteine protease (~amino acids 433-592), RNA helicase (~amino acids 960-1204), and the RNA-dependent RNA polymerase (RdRp) (~amino acids 1207-1693). Details of ORF1 processing are limited, but recent data examining ORF1 using a baculovirus system showed extensive cysteine protease processing.[9,121,137] The enzymatic activities of the methyltransferase and RdRp have been demonstrated in independent studies.[7,100]

ORF2 codes for the viral capsid protein.[149] Specific studies of the ORF2 have shown a membrane export signal sequence (amino acids 1-36) at its extreme N-terminal, several glycosylation sites, and multiple smaller processed forms.[69,133,149,179] ORF2 has dimerization sites and can assemble into VLPs when the first 110 amino acids are deleted.[96,95,176] Studies have shown that ORF2 dimers can encapsidate and deliver exogenous HEV RNA to naïve cells.[122]

The role of ORF3 appears to be multifaceted. A key function of the protein appears to be in mediating viral egress from cells.[177] ORF3 deletion mutant viruses can replicate RNA, but this RNA is almost entirely intracellular. Specific studies have shown that a specific proline-containing motif within ORF3 is responsible for interacting with cellular Tsg101, which has also been shown to interact with several other viruses.[45,81,116] Other effects include interaction with the MAPK/ERK pathway, ORF2 binding, and self-association.[79,90,167,168] Recent studies have shown that ORF3 also mediates the evasion of interferon responses in A549 cells.[41]

Viral Structure and Stability

Electron micrographs from fecal samples of patients and animals infected with HEV show the virus to be approximately 27 to 30 nm in diameter.[18,26,27]

Ultrastructural studies of HEV have involved ORF2 expression, and the empty VLPs produced are slightly smaller than infectious HEV.[96,176] The VLPs assemble from 30 dimers to form a spiky hollow sphere.[176] Although the empty VLP shares much similarity with the native virion in appearance, further confirmation is lacking until a tissue culture system is available.

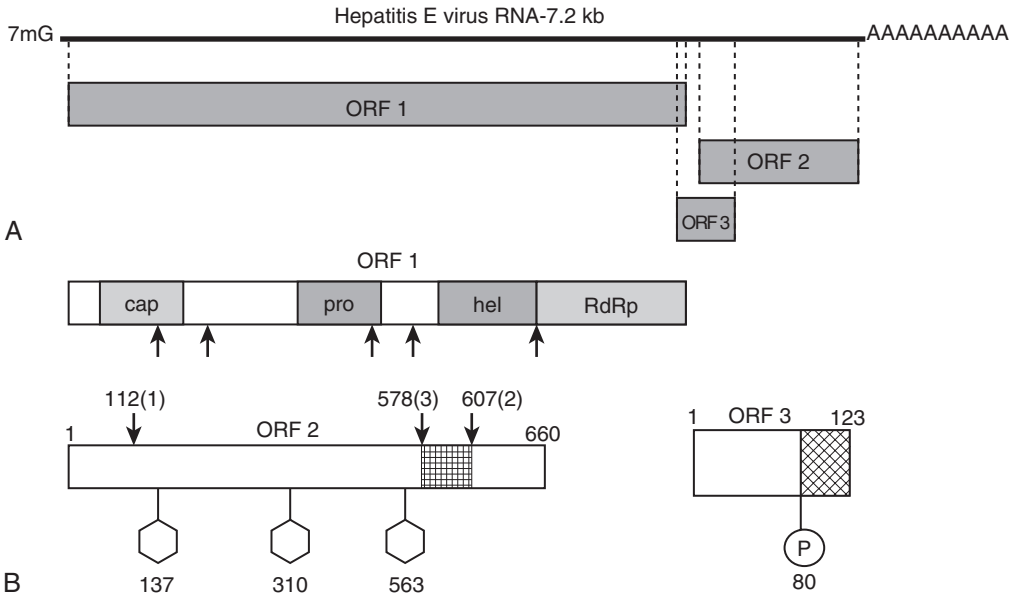

FIGURE 169-1 ■ Representation of HEV genome and open-reading frames. **A,** Hepatitis E virus (HEV) genome overview. The HEV RNA is single stranded and features three overlapping reading frames: ORF1, ORF2, and ORF3. **B,** Individual features of the HEV ORFs. ORF1 contains the nonstructural protein domains *(shaded),* which include the methyltransferase *(cap),* cysteine protease *(pro),* helicase *(hel),* and RNA-dependent RNA polymerase *(RdRp).* Proposed cleavage sites are illustrated with the *bold arrows* at their approximate locations. ORF2 is the structural capsid protein, which is 660 amino acids in length. Cleavage sites are indicated by the *bold arrows* at their determined amino acid positions. Glycosylation sites are represented with *polygons* at their determined amino acid positions. The domain of ORF2 that has been determined to be the neutralization epitope is represented by the *cross-hatched box.* ORF3 is the smallest protein, and its phosphorylation site is represented by the *circle labeled P.* The domain responsible for dimerization is represented by *the diagonal hatched box.*

Several strains of HEV are almost completely heat inactivated by 60°C (140°F), in contrast to 66°C (150.8°F) for HAV[44] with some interstrain variability. Virus-containing stocks have been stable at extreme cold temperatures with several freeze/thaw cycles.[44] Based on persistent HEV infections during an outbreak from water containing chlorine concentrations of 0.3 to 0.6 mg/L, HEV may be resistant to chlorine inactivation.[57]

Viral Life Cycle

For many years, HEV did not grow in tissue culture conditions, so detailed information regarding viral structure and life cycle were limited.[43] Limited studies have shown evidence of HEV replication in primary macaque hepatocyte cultures.[150,151] When in vitro transcribed RNA from a full-length cDNA clone was transfected into liver cell lines, viral proteins could be detected and the culture supernatant could be used to infect a rhesus monkey.[121] However, recent efforts have demonstrated that HEV clinical strains isolated from feces and blood can replicate to high titers in the PLC/PRF/5 liver cell line and the A549 lung cell line.[152] Antibodies from patient serum, be it immunoglobulin (Ig)M, long after convalescence, or against heterotypic stains, were able to neutralize HEV in cell culture, demonstrating the validity and utility of the system.

Currently no information is available regarding the viral receptor for HEV. However, progress has been made to demonstrate that HEV VLPs enter the cell via clathrin-mediated endocytosis.[78] After the virus gains access to the cell, it uncoats and translates its RNA to produce enough nonstructural proteins to begin the transcription and replication process.[121] A genomic and 2-kb subgenomic RNA are produced, as well as a negative strand replication template.[53,151] Studies using real-time PCR coupled with live cell imaging demonstrated a peak of negative-strand RNA at 8 hours after infection, followed by a decline.[170] Positive strand RNA peaked at 14 hours after infection. With the accumulation of transcripts, structural proteins begin to accumulate, as well as more plus sense daughter RNA. ORF2 gets cleaved, dimerizes, and associates with the HEV RNA to form new virions.[146] These accumulated virions are subsequently released by an unknown mechanism.

Viral Genetics

HEV has four main genotypes.[174] Genotype 1 is distributed throughout the world from South Asia to Northern Africa. Genotype 2 is most common in Mexico but has been isolated in certain countries in Africa. Genotypes 3 and 4 are the zoonotic strains of HEV that are found in swine and humans from developed Western countries and China, respectively. Genotypes 1 and 2 are approximately 70 to 80 percent homologous, and genotype 3 strains are more removed from genotype 2 strains (25% to 50% homology).[109] Generally, genotypes 1 and 2 are closer to each other and genotypes 3 and 4 are closer to each other.[99] This makes sense because genotypes 1 and 2 are responsible for endemic and epidemic diseases and genotypes 3 and 4 are responsible for zoonotic disease and sporadic human cases.

There is only one serotype of HEV.[107,110,136,174] There is some evidence that HEV develops quasi-species during epidemics that allow for adaptation within individual hosts.[54]

Taxonomy and Classification

Before the characterization of HEV, it was proposed to be closely aligned with the Calicivirus family based on physical characteristics.[18,149] After the full cloning of the virus and the identification of the three ORFs, the virus was formally classified with the Caliciviruses.

Subsequent work showed that HEV shared several characteristics with rubella virus and other "alpha-like" viruses.[89] An analysis performed comparing the helicase and polymerase regions of HEV with members from the Caliciviridae, Picornaviridae, and Togoviridae families showed significant divergence.[22] The International Committee on Taxonomy of Viruses decided to separate HEV from the Caliciviridae family based on these findings and leave it unclassified, where it still remains.[55]

EPIDEMIOLOGY

Endemic Hepatitis E Virus

HEV is endemic in areas of the developing world where sanitation and clean water are not readily available. South and Southeast Asia and North Africa experience endemic disease, as well as regions of Mexico and South America. Large epidemics of HEV infection occur in these endemic countries related to widespread contamination of water after natural disasters. Cases of HEV infection outside these areas were inevitably traced back to some travel to one of these areas. Global estimates of the burden of HEV infection in endemic countries arrived at 20 million incident infections in 2005, with over 3 million symptomatic cases and 70,000 deaths.[131] Sixty percent of these cases occur in South and East Asia, with another 14 percent in North Africa.

Most of the seroprevalence studies performed in endemic countries have shown that unlike in HAV infection, children are not universally infected with HEV early in life. Children in the first decade have anti-HEV–positive rates of approximately 3 to 4 percent,[12,15] and the peak of HEV seropositivity is the second and third decades of life. However, studies from regions of Egypt and northern regions of India demonstrate a pattern that is similar to that of HAV, with 60 to 70 percent of children under 10 years being anti-HEV–positive.[6,47,106] Egypt has much higher prevalence than neighboring Israel, and southern and central India have anti-HEV rates much lower than those of the northern regions described above.[12,80,113] A study of children from Moscow had HEV seroprevalence rates that between those of most other studies.[1] Explanations for these regional differences are lacking. Table 169-1 is a summary of HEV

TABLE 169-1 **Seroprevalence Studies of Hepatitis E in Children**

Country/Region	Age Group	% Positive	NO. Positive/Tested	Reference
India: South Chennai	0-2 yr	5.3	1/19	Mohanavalli 2003[113]
	2-4 yr	9.0	2/22	
	4-6 yr	7.3	4/55	
	6-8 yr	7.9	3/37	
	8-10 yr	16.7	3/15	
	10-12 yr	9.0	3/33	
India: South Vellore	1-5 yr	0.5	1/200	Daniel 2004[38]
	6-15 yr	1.0	2/200	
	16-40 yr	8.0	8/100	
India: Central Pune	6-10 yr	3.9	50/1302	Arankalle 2001[12]
India: North New Delhi	11-15 yr	6.3	83/1349	
	6-12 mo	11.0	23/210	Mathur 2001[106]
	13-24 mo	9.3	19/203	
	25-48 mo	23.7	96/405	
	49-72 mo	29.5	126/426	
	73-96 mo	31.3	129/412	
	97-120 mo	35.8	152/424	
India: North Lucknow	0-5 yr	64	18/28	Aggarwal 1997[6]
	6-10 yr	59	13/22	
	11-18 yr	64	16/25	
Bangladesh: Southern	1-5 yr	0.7	Not stated	Labrique 2009[91]
	6-10 yr	1.8		
	11-15 yr	11.7		
	16-20 yr	28.2		
Nepal: Kathmandu	12-19 yr	16	10/64	Clayson 1997[34]
Japan: Nagoya	1-11 yr	2.6	8/309	Goto 2006[52a]
Taiwan: Central	3 yr	3.1	6/196	Lin 2004[97]
	4 yr	3.4	22/652	
	5 yr	3.4	42/1247	
	6 yr	3.4	15/443	
Russia: Moscow	0-3 yr	11.8	2/17	Abe 2004[1]
	4-6 yr	7.7	3/39	
	7-9 yr	23.2	19/82	
	10-12 yr	20	18/90	
	12-15 yr	17.7	20/113	
Turkey: Istanbul	0.5-4 yr	3.7	12/321	Sidal 2001[139]
	5-9 yr	2.1	7/318	
	10-16	0.3	1/270	
Turkey: Varied	<19 yr	0	0/105	Thomas 1993[157]
Morocco: Melilla	Mean 9.2 yr (±4.03)	0	0/321	Bernal 1995[23]
Israel: Varied	1-5 yr	1.1	1/93	Karetnyi 1995[80]
	6-10 yr	0	0/30	
	11-15 yr	1.6	2/124	
	16-20 yr	3.8	4/105	
Egypt: Nile delta + upper	0-4 yr	36.2	130/359	Fix 2000[47]
	5-9 yr	64.7	1600/2473	
	10-14 yr	75.6	1944/2571	
	15-19 yr	75.5	1438/1905	
Yemen	0-18	7.7	17/220	Bawazir 2010[20]
Spain: Madrid	2-10 yr	1	7/724	Fogeda 2012[48]
	11-20 yr	1.8	12/685	
United States: Northern California	<20 yr	0.4-0.7	Not stated	Mast 1997[105]
Cuba: Havana	5-15 yr	0	0/22	Quintana 2005[129]
Mexico: National sample	1-4 yr	1.1	1/91	Alvarez-Munoz 1999[8]
	5-14 yr	4.4	27/619	
	15-19 yr	9.6	109/1138	
Chile: Valdivia	Not stated	36	60/166	Ibarra 1994[68]
Bolivia: Chaco region	1-5 yr	3.9	2/51	Bartoloni 1999[19]
	6-10 yr	3.8	4/105	
	11-20 yr	5.5	5/90	
Venezuela: Rural villages	4 yr	1.3	1/75	Pujol 1994[127]
	5-9 yr	2.7	2/75	
	10-15 yr	5.8	4/69	

seroprevalence studies performed in children from different parts of the world.*

One of the Egypt seroprevalence studies performed a follow-up study to examine seroconversion and estimated the incidence of HEV infection.[144] Of the 919 villagers who were anti-HEV–negative at the beginning of the study, 34 seroconverted over 10 months, or an incidence of 41.6/1000 person-years for this group.

Studies of sporadic acute hepatitis in endemic areas indicate the burden of HEV disease in those countries. Studies examining children with acute hepatitis symptoms in Egypt and Sudan show that HEV infection is either the leading cause or second leading cause in children.[42,66,67] A similar study from India showed that although HEV infection was the second leading cause after HAV infection of acute hepatitis requiring admission to a children's hospital, it caused a slightly higher percentage of acute liver failure.[125] One study found that HAV and HEV infections led to worse outcomes in acute liver failure, but others have not substantiated those findings.[16,102]

Several studies from endemic areas were specifically designed to examine the differences between HAV and HEV infections.[29,135] The results of these studies have shown that HAV disease peaks earlier in life than HEV disease and that although the clinical symptoms of acute hepatitis are very similar, the hepatic dysfunction from HEV infection tends to peak earlier in the illness and may take longer to resolve. Because the peaks of HAV and HEV seroprevalence and disease in endemic countries do not overlap, this has led some to think that HAV disease somehow protects against or interferes with HEV infection.[147] No specific proof of this hypothesis is available currently.

Although the reservoir for HEV is not known, the detection of HEV from various sewage samples combined with patients with prolonged viremia make it likely that the virus can exist in these environments to sustain its transmission cycle.[118,124,169]

Epidemic Hepatitis E Virus

HEV epidemics are often related to events that cause disruption of sanitary conditions (e.g., war, earthquakes, and flooding). Recent war-associated epidemics among the refugees from the Darfur region of Sudan and Iraq in 2004 illustrate the nature of HEV epidemic circulation.[24,51,57,173]

In a refugee camp in Western Sudan, more than 2600 cases of HEV infection were reported among the estimated 78,000 residents in the second half of 2004.[24,57] Of the total cases, almost 10 percent required hospitalization, and of the hospitalized patients, 45 (17.8%) died.[24] The bulk of the mortality was in the pregnant women—19/45 (42.2%); in fact, the sentinel cases for the HEV infection epidemic were 2 pregnant women who died in July 2004. The overall mortality rate for the epidemic was 1.7 percent. Of the 1133 pregnant women in the refugee camp, 220 reported jaundice (19.4% attack rate) and the mortality rate was 8.2 percent

The main source of water for the camp was unchlorinated groundwater pumped in through pipelines or surface water with chlorine added that refugees could access.[57] In the analysis of those patients with symptomatic HEV infection and those with asymptomatic infection, patients aged 15 to 45 years had a higher attack rate and more symptomatic infections (4.3% and 71%, respectively) in contrast to the group younger than 15 years of age (2.4% and 22%, respectively). Intrafamilial spread of HEV previously did not appear to play a significant role during epidemic conditions.[4,14] However, a large outbreak in northern Uganda with over 3000 cases demonstrated in households with two or more cases that they were more likely to have had contact with people likely to have been infected with HEV.[156] All tested water and zoonotic sources were negative for HEV, thus suggesting person-to-person spread.

Zoonotic Hepatitis E Virus

As more widespread serologic studies were performed to assess rates of anti-HEV seropositivity, cases with no identifiable travel risks were discovered in developed countries with no known endemic HEV activity.[50,104,158] Many of the cases had heavy animal exposures, most commonly to swine. This led researchers to investigate the possibility that HEV was in fact a zoonosis and that either direct animal contact or ingestion of raw or undercooked meat could produce HEV infection. Meng and associates[111] conducted a multiphase study to determine if swine were infected with HEV and to isolate the virus.[111] They performed enzyme-linked immunosorbent assays (ELISAs) on multiple swine herds and found the majority to be anti-HEV–positive and that the seroprevalence increased with age. They followed newborn piglets, monitored for seroconversion, and demonstrated concurrent histologic hepatitis and viremia by RT-PCR. In a follow-up study, serum from the infected piglets was used to successfully infect pathogen-free experimental pigs.[108] Pigs could not be infected with human strains of HEV. Rhesus monkeys and chimpanzees, a human surrogate, were successfully infected with the swine HEV.[109] An isolate of HEV from the United States that was genetically similar to the swine HEV successfully infected pathogen-free pigs, thus proving that swine HEV could infect both pigs and humans and was the likely cause of sporadic cases of HEV in the United States and other developed countries. It has now been established that HEV genotypes 3 and 4 are the strains most frequently isolated from animals that are found in nonendemic HEV infection cases from industrialized countries.[36]

Many animal species are likely infected with HEV. Among those described are swine, deer, chickens, rodents, and cats.[62-64,72,119] Of note, avian HEV, which has been demonstrated as the cause of hepatosplenomegaly syndrome in chickens, shares only 50 to 60 percent homology with human and swine HEV and has not been shown to be able to infect nonhuman primates.[63,65]

*References 1, 4, 8, 12, 19, 20, 23, 34, 38, 47, 48, 68, 80, 91, 97, 105, 106, 127, 129, 139, 157.

Vertical and Breast Milk Transmission

Studies done on pregnant women with HEV have shown that vertical transmission does occur. The small studies performed show that HEV is likely transmitted at high rates, and the infants can be severely affected or suffer fetal demise before or soon after birth.[85,86,140]

Limited studies have been conducted on breast milk transmission. HEV RNA and anti-HEV antibody have been detected in colostrum components, but at significantly lower titers in contrast to serum.[31] No cases of HEV transmission in the infants were definitively linked to breast milk; however, the mothers with advanced liver disease were not allowed or able to breast-feed in this study. In short, no recommendations exist to contraindicate breast-feeding in this population aside from those mothers with severe disease.

Other Modes of Hepatitis E Virus Transmission

Cases have been reported of HEV linked with transfusion of contaminated blood. Donors were in the presymptomatic viremic phase and recipients of those blood products developed symptoms of acute hepatitis with a shorter incubation period than patients infected via the usual fecal-oral route.[10,11,25,87]

Consumption of undercooked wild game meat has been implicated in HEV infections. Several cases have been reported of acute HEV infection after consumption of uncooked or undercooked deer and wild boar meat.[93,154,155] An outbreak in France was traced to consumption of uncooked pig liver sausage.[35] Comparison of HEV RNA sequences from index cases and sausages revealed 99.4 percent identity, leading to a new package label recommending the sausage be cooked before eating.

PATHOGENESIS AND IMMUNITY

Pathogenesis

The details of pathogenesis in HEV infection are largely unknown. After an individual consumes HEV-contaminated water or food, it is unknown how the virus infects enterocytes and spreads from the intestine to the liver. Studies in swine show replicative HEV RNA present in colon, small intestine, lymph nodes, tonsils, and liver as early at 3 days after infection.[171]

Evidence of HEV replication within the liver as detected by HEV antigen is apparent by 7 days after infection. At its peak, HEV antigens can be detected in 70 to 90 percent of hepatocytes before the onset of symptoms and then drops off rapidly.[3] Viremia is detectable in serum, stool, and bile before the onset of symptoms.[30,98,160] Anti-HEV appears at the same time as the histologic signs of inflammation.[33] Similar to HAV infection, disease is likely caused by the immune response to the virus and not the virus itself.[3]

The majority of the information regarding progression and pathogenesis come from HEV inoculation of animals.[18,98,160,163] These studies usually involved intravenous administration, with an incubation period from administration to viremia of approximately 3 weeks, approximately 1 week less on average than humans. HEV RNA can be detected in serum, stool, and bile 1 week before clinical symptoms become apparent.

In the HEV infection volunteer studies, the patients developed constitutional symptoms at approximately 4 weeks after administration.[18,30] Specific symptoms and the biochemical abnormalities of hepatitis developed 1 week later. Early studies showed viral particles appeared in stool with the onset of symptoms and continued through the peak hepatitis symptoms and antibody was present at the only time they tested, 1 week after the peak hepatitis symptoms.[18] Subsequent studies using RT-PCR showed HEV RNA was present in serum before stool samples, and was present from 1 week before to 2 weeks after symptoms had begun.[30] Peak alanine aminotransferase elevations corresponded with peak symptoms, and symptoms lasted between 30 and 80 days. HEV IgG persisted well beyond the study end point.[30]

The presence of HEV RNA in stool and serum can vary significantly from patient to patient.[5] In 20 patients diagnosed with sporadic HEV infection, HEV RNA appeared in serum at days 4 to 7 after the onset of symptoms in 89 percent of patients tested and persisted beyond day 22 in 21 percent. HEV RNA in stool samples was present in 73 percent of patients at days 8 to 14 after symptoms, peaking slightly later than serum HEV RNA, and persisted beyond day 22 in 13 percent. A subset of patients will have HEV RNA in serum and stool for up to 120 days.[118] It is possible that these patients with prolonged shedding may serve as a reservoir for spread of HEV to the environment.

A summary of the clinical progression of HEV infection is graphically represented in Figure 169-2.

Immunity

HEV antibody appears with the onset of symptoms and after the peak of viremia. IgM appears just ahead of IgG (Fig. 169-3). HEV IgA appears at the same time as IgM and appears to persist slightly about 1 month longer after infection than IgM.[148,159] ORF2 is the dominant immunogen across all types of HEV infection, although antibodies to ORF3 can be detected in patients.[56,66,67,104,178] A more detailed discussion of neutralizing antibodies can be found in the discussion of vaccines.

Some studies of patients after infection have shown HEV IgG responses wane quickly after infection, whereas others show a more durable antibody response.[52] The waning of IgG levels forms the basis for the theory that susceptible adults are the vulnerable population during HEV outbreaks.

Cellular immunity to HEV plays a role in eradiation of infection and persistence of viral immunity.[117] Specific studies comparing patients with resolved acute HEV and transplant patients with resolved and chronic HEV infection have demonstrated a role for broad polyspecific T-cell responses against the virus.[145] Regulatory T cells appear to increase in number during acute HEV infection.[161]

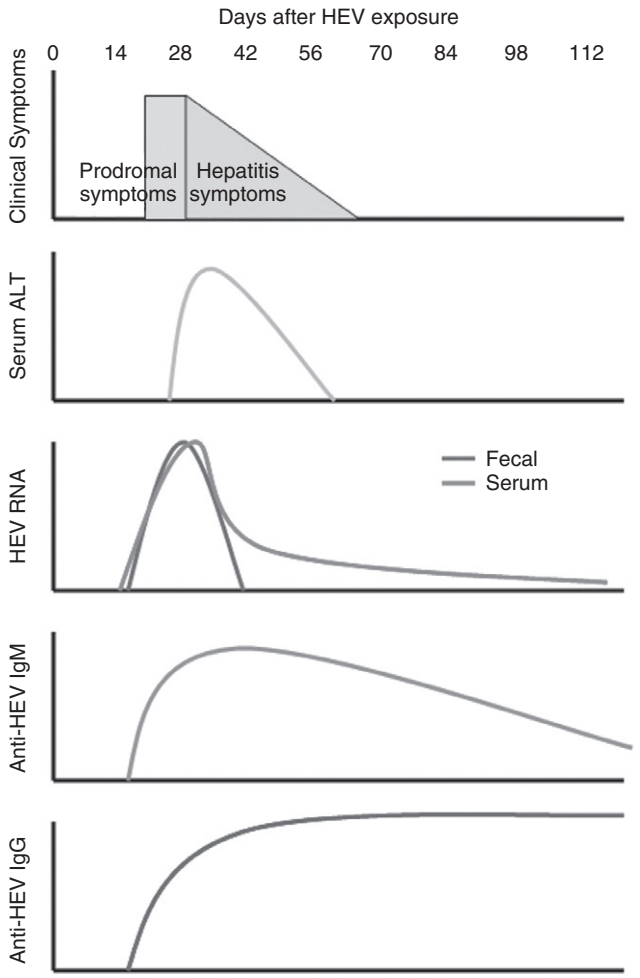

FIGURE 169-2 ■ Representation of the course of hepatitis E virus (HEV) infection with approximate times after HEV exposure. In volunteer and animal experiments, prodromal symptoms will appear approximately 3 weeks after infection, which precedes the symptoms of hepatitis by about 1 week. The hepatitis symptoms can last 4 to 6 weeks on average, with significant variation. Serum alanine aminotransferase peaks with the onset of hepatitis symptoms. HEV RNA can be detected in either stool or serum approximately 3 weeks after infection with the onset of the prodrome and can be detected for 1 to 3 weeks in duration, with some patients experiencing prolonged viremia. HEV immunoglobulin (Ig)M and IgG appear in concert with the onset of symptoms and after the peak of HEV RNA. IgM peaks slightly earlier than IgG and can taper quickly, with some disappearing by 3 months. IgG peaks later and lasts several months after infection.

CLINICAL MANIFESTATIONS

The signs and symptoms of acute HEV infection are indistinguishable from those seen with HAV infection.[3,29,67,135] A prodromal illness consisting of malaise, anorexia, nausea, abdominal pain, fever, myalgia, and headache precedes the findings of jaundice, hepatomegaly, and serum transaminase elevations.

One study examining children after a common source exposure provides insight into the manifestations of HEV infection in this group. Twenty students from a private school in urban North India were exposed to HEV while on a camping trip.[17] The exact source was never identified, but investigators were able to monitor the children for signs and symptoms of HEV infection and serologic and virologic confirmation of infection. The clinical symptoms are summarized in Figure 169-3. Of the 20 children, 17 developed clinical illness (85%) but only 10 developed symptoms of acute hepatitis. The other 7 remained anicteric, with symptoms of malaise, nausea, abdominal pain, and headache. The children with jaundice had a different incubation period (25 to 38 days, median 33 days) in contrast to the children without jaundice (22 to 95 days, median 27 days). HEV IgM was found in all patients. HEV RNA was found in 11 children, including one of the children who never developed symptoms. Because this study is one of the few to have a point source of exposure, it is difficult to know if the very high rate of infection is due to the younger age of the patients, the strain of the virus, or the nature of the exposure.

Rates of fulminant hepatic failure and mortality from HEV infection are estimated to be approximately 1 to 4 percent. Studies from endemic areas suggest that the rate may be higher in children, but those data have not been widely substantiated.[126] The rate is closer to 15 to 50 percent in patients with preexisting liver diseases and compensated cirrhosis, whether due to chronic hepatitis B or C or other metabolic liver disease.[130]

It was previously thought that HEV caused only acute disease; however, recent studies demonstrated that chronic HEV infection can occur in immunocompromised and transplant patients.[59,75] This has been observed in children, with outcomes including cirrhosis and death.[60,61]

Pregnant women are highly vulnerable to HEV infection.[37,84] It was recognized before HEV was fully characterized that infected pregnant women had high rates of fulminant liver failure and mortality rates of 20 percent or higher.[88] One study from India of pregnant women with acute hepatitis demonstrated not only that HEV caused fulminant hepatitis and death more frequently, but it was also associated with many obstetric complications (i.e., antepartum and postpartum hemorrhage, premature rupture of membranes, and intrauterine fetal death) and poor fetal outcomes (i.e., preterm delivery and stillbirths) in contrast to other forms of viral hepatitis.[123] This feature of HEV is not restricted to a particular region or genotype, as illustrated by the Darfur outbreaks. The mechanism for severe disease in these women is unknown, although one study showed an increased Th2 cytokine bias in peripheral blood morphonuclear cells from pregnant HEV-infected patients that could allow for a dysregulated inflammatory response.[24,120] Pregnant women with HEV infection have significantly longer periods of viremia than nonpregnant counterparts, also supporting the idea of a muted or dysfunctional immune response.[21]

Extrahepatic manifestations of HEV infection have been reported, but they are uncommon. Pancreatitis can occur shortly after the onset of acute hepatitis symptoms.[70,101,112] Several reports have described patients with significant hemolysis with glucose-6-phosphate dehydrogenase (G6PD) deficiency associated with acute HEV

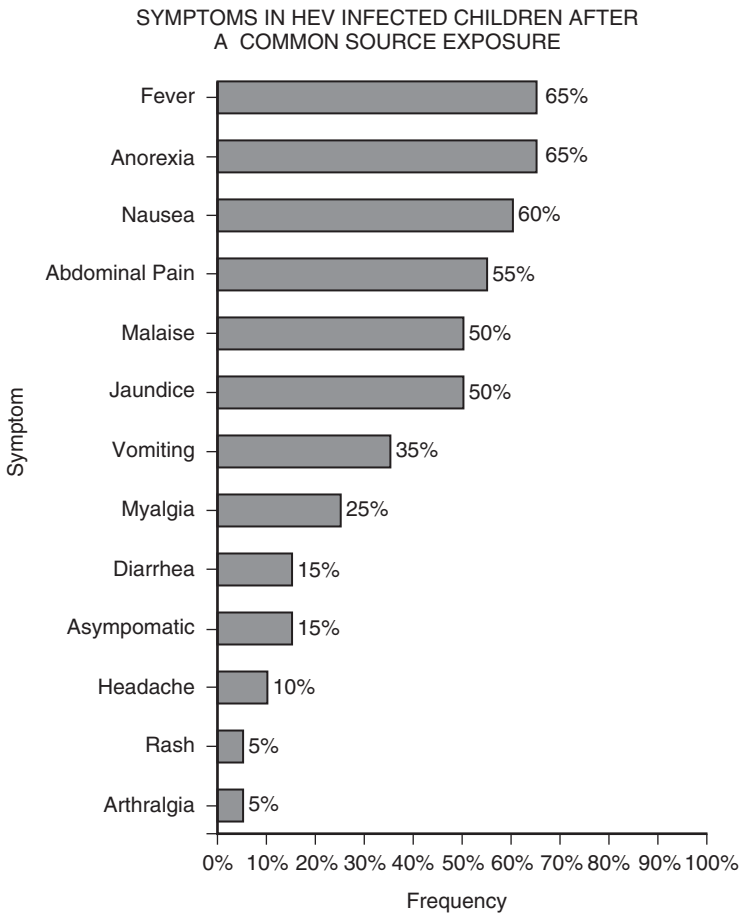

FIGURE 169-3 ■ Symptoms observed in children after a common-source exposure to hepatitis E virus. Jaundice was seen in only 50 percent of patients overall, and 15 percent were completely asymptomatic. (From Arora NK, Panda SK, Nanda SK, et al. Hepatitis E infection in children: study of an outbreak. *J Gastroenterol Hepatol* 1999;**14**: 572-577.)

infection.[2,180] Neurologic manifestations described include transverse myelitis, Bell palsy, and Guillain-Barré syndrome.[39,40,74,103,141] All of these manifestations resolve spontaneously in conjunction with the hepatitis. It is not clear whether these are a result of direct viral effects or an immune-mediated phenomenon. It is interesting that most of the extrahepatic manifestations have been reported in children.

DIAGNOSTIC TESTING

Serology

Antibody confirmation is the most widely available method used to diagnose HEV infection. ELISA for both IgG and IgM can be performed at many reference laboratories. The ORF2 capsid protein is the most common antigen used to detect antibody.[164] Some studies have shown that inclusion of the other ORFs can increase the specificity and positive predictive value of the test. A few studies indicate that HEV IgA may perform better than IgM based testing.[148,159] This needs to be confirmed in larger studies.

A comparison of various HEV ELISA kits during an epidemic in Indonesia found that the sensitivity of the tests was much better in symptomatic patients than in asymptomatic ones (72% to 91% vs. 39% to 51%, respectively).[114] Also, the Walter Reed kits for IgG and IgM performed better than the GeneLabs and Abbott IgG tests. Testing of normal healthy controls that were HEV PCR–negative showed that the serologic testing had high specificity, again with the Walter Reed tests performing best. The caveat of the study is that the GeneLabs IgM kit was an older version of the test, and the limitations of the assay may not apply to the newer kit.

A new rapid immunochromatographic assay showed promising results when it was evaluated for use in resource-poor countries to diagnose acute HEV infection.[115] Using 25 μL of serum or 35 μL of whole blood applied to the test strip and a 15-minute incubation period, the kit had a sensitivity of 93 percent and a specificity of 99.7 percent.

Polymerase Chain Reaction

RT-PCR for HEV RNA can be performed within the first month of illness on either serum or stool.[5] The testing is less commercially available and likely conducted in research or select public health laboratories. Several recent studies have shown that real-time PCR testing may allow more rapid and sensitive detection of HEV RNA, with the additional benefits of detecting multiple HEV genotypes and the ability to test environmental samples in addition to clinical samples.[46,71]

Electron Microscopy

HEV has also been visualized in stool samples from patients using electron microscopy.[18,142] In general, these methods incorporate HEV antibody to adsorb intact virus to increase the likelihood of detection. Again, this testing is not generally available.

PREVENTION AND VACCINE CANDIDATES

Disease Avoidance

Practical measures can be taken to reduce the risk for HEV infection when traveling to endemic regions.[28] Consumption of only boiled or bottled water, avoidance of ice, eating fruits that can be peeled, and eating only well-cooked foods are all recommended measures.

Specific consideration should be made for pregnant women and patients with chronic liver disease to avoid travel to areas endemic for HEV or regions experiencing HEV infection outbreaks.

Antibody Preparations

No antibody preparations are available that can be used as preexposure or postexposure prophylaxis for HEV infection. Small studies have shown successful passive immunization in animals.[165] Some studies have used immune serum globulin in endemic areas with mixed effects.[13,84] These studies are all small, and the antibody preparations were of varying amounts and given at varying times, so definitive conclusions about the role of antibody cannot be made.

Therapies

Most patients do not need therapy for HEV infection. However, patients with less common manifestations might benefit if therapies existed. Ribavirin has been used with success in patients with fulminant infection, although it is not clear whether the drug was critical in the recovery of the patient.[49] For immunocompromised patients with chronic HEV infection, several anecdotal studies have examined using pegylated interferon–α for 3 to 6 months in duration with high rates (60% to 80%) of resolution of HEV viremia and only a few reported relapses.[58,76]

Few studies have examined therapeutic options for acute HEV infection. One uncontrolled study used glycyrrhizin in patients with significant acute hepatitis from HEV infection, but no meaningful conclusions can be drawn from its results.[153] A hammerhead ribozyme targeted to the 3′ UTR have shown decreases in the in vitro activity of an HEV reporter.[143] Development of therapies for HEV infection is not likely to be effective for the simple reason that the peak of viral activity is usually before and along with the onset of symptoms. The patients with prolonged viremia are not necessarily the sickest patients and are often asymptomatic.

Vaccine Candidates and Future Therapies

Early vaccine studies used cynomolgus monkeys actively immunized with a recombinant ORF2 protein. Subjects were protected after challenge with homologous virus in contrast to passively immunized or control animals.[165]

Work to identify neutralizing antibody showed that the epitopes recognized were in the terminal region of ORF2, amino acids 578-607.[136] These antibodies were protective of HEV infection when incubated with HEV before administration to monkeys. This epitope is present within the 56-kDa form of the capsid protein.

A recombinant 56-kDa ORF2 vaccine protected rhesus monkeys challenged with HEV 1 month after a 2-dose series.[128,166] Repeat challenge at 6 months and 1 year showed protection against hepatitis in 75 percent, which increased to 100 percent in animals given a third dose of vaccine 1 month before challenge.[181] Postexposure vaccination did not prevent HEV infection.

Subsequent phase I testing in humans induced anti-HEV titers 40 U/mL and greater in 88 percent of vaccinees after three doses.[134] Only a few minor local reactions occurred and no significant adverse events. Based on these early trials, a large human field trial was completed in Nepal in 2001. A group of 2000 seronegative soldiers were randomized to receive three doses over 6 months of the recombinant HEV vaccine or placebo.[138] The subjects were followed for signs and symptoms of acute hepatitis for 2.5 years. The study included 69 total cases of acute HEV infection, 66 in the placebo group, and 3 in the vaccine group, with an efficacy of 95.5 percent in subjects receiving three doses of vaccine. The intent to treat analysis showed an efficacy of 88.5 percent. The vaccine was well tolerated, with local pain at the injection site being the most frequent complaint.

Another version of recombinant vaccine against hepatitis E produced in bacterial cells has moved through preclinical testing with promising results. A large phase III trial in China with more than 90,000 subjects showed the vaccine was highly protective, with rates of 95 to 100 protection.[182] Only one case of HEV infection was observed in a subject that received only 1 dose of vaccine, in contrast to 22 in the placebo group. A subsequent analysis of women in the study who became pregnant after enrollment demonstrated no associated pregnancy complications or fetal morbidity or mortality.[175] Antibody levels among those women who received study vaccine were comparable to those in nonpregnant counterparts in the study. These findings certainly raise the possibility that an HEV vaccine may become available to protect pregnant women in endemic areas.

Alternative vaccine strategies being investigated include VLPs given orally to induce local and systemic immunity. Mice immunized with HEV VLPs in increasing amounts developed serum IgG and IgM responses at 2 to 4 weeks after immunization.[92] Mice given the higher inoculums (50 and 100 μg) also developed fecal IgA responses. Subsequent challenge experiments in cynomolgus macaques showed that oral immunization with

VLPs not only produced serum and fecal antibody but protected the animals from intravenous challenge with native HEV strains.[94] DNA vaccines have shown encouraging initial results as well. Macaques given a DNA plasmid encoding for the HEV ORF2 using the gene gun technique developed significant anti-HEV IgG within 4 months of vaccination; vaccinated animals were protected against challenge with infectious HEV and control animals developed clinical disease.[77]

SUMMARY

Despite the lack of a tissue culture system for HEV, much progress has been made toward a vaccine that is effective. Fundamental questions of virus entry and pathogenesis still linger, as well as why certain patients are highly susceptible to severe HEV disease, whereas others are not. The nature of the differences in the epidemiology and clinical manifestations of HEV infection in children also needs to be studied further. Recent large-scale trials offer promise that an efficacious vaccine may be available in the near future to protect those most vulnerable to HEV infection.

NEW REFERENCES SINCE THE SIXTH EDITION

20. Bawazir AA, Hart CA, Sallam TA, et al. Seroepidemiology of hepatitis A and hepatitis E viruses in Aden, Yemen. *Trans R Soc Trop Med Hyg* 2010;**104**:801–5.
21. Begum N, Polipalli SK, Husain SA, et al. Duration of hepatitis E viremia in pregnancy. *Int J Gynaecol Obstet* 2010;**108**:207–10.
35. Colson P, Borentain P, Queyriaux B, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis* 2010;**202**:825–34.
41. Dong C, Zafrulah M, Mixson-Hayden T, et al. Suppression of interferon-alpha signaling by hepatitis E virus. *Hepatology* 2012; **55**:1324–32.
45. Emerson SU, Nguyen HT, Torian U, et al. Release of genotype 1 hepatitis E virus from cultured hepatoma and polarized intestinal cells depends on open reading frame 3 protein and requires an intact PXXP motif. *J Virol* 2010;**84**:9059–69.
48. Fogeda M, Avellon A, Echevarria JM. Prevalence of specific antibody to hepatitis E virus in the general population of the community of Madrid, Spain. *J Med Virol* 2012;**84**:71–4.
49. Gerolami R, Borentain P, Raissouni F, et al. Treatment of severe acute hepatitis E by ribavirin. *J Clin Virol* 2011;**52**:60–2.
58. Haagsma EB, Riezebos-Brilman A, van den Berg AP, et al. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl* 2010;**16**: 474–7.
59. Haagsma EB, van den Berg AP, Porte RJ, et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2008;**14**:547–53.
60. Halac U, Béland KK, Lapierre P, et al. Chronic hepatitis E infection in children with liver transplantation. *Gut* 2012;**61**:597–603.
61. Halac U, Béland K, Lapierre P, et al. Cirrhosis due to chronic hepatitis e infection in a child post-bone marrow transplant. *J Pediatr* 2012;**160**:871–4.
75. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008;**358**:811–17.
76. Kamar N, Rostaing L, Abravanel F, et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin Infect Dis* 2010;**50**:e30–3.
78. Kapur N, Thakral D, Durgapal H, et al. Hepatitis E virus enters liver cells through receptor-dependent clathrin-mediated endocytosis. *J Viral Hepat* 2012;**19**:436–48.
81. Kenney SP, Pudupakam RS, Huang YW, et al. The PSAP motif within the ORF3 protein of an avian strain of the hepatitis E virus is not critical for viral infectivity in vivo but plays a role in virus release. *J Virol* 2012;**86**:5637–46.
91. Labrique AB, Zaman K, Hossain Z, et al. Population seroprevalence of hepatitis E virus antibodies in rural Bangladesh. *Am J Trop Med Hyg* 2009;**81**:875–81.
116. Nagashima S, Takahashi M, Jirintai S, et al. A PSAP motif in the ORF3 protein of hepatitis E virus is necessary for virion release from infected cells. *J Gen Virol* 2011;**92**(Pt 2):269–78.
122. Parvez MK, Purcell RH, Emerson SU. Hepatitis E virus ORF2 protein over-expressed by baculovirus in hepatoma cells, efficiently encapsidates and transmits the viral RNA to naive cells. *Virol J* 2011;**8**:159.
123. Patra S, Kumar A, Trivedi SS, et al. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. *Ann Intern Med* 2007;**147**:28–33.
131. Rein DB, Stevens GA, Theaker J, et al. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology* 2012; **55**:988–97.
145. Suneetha PV, Pischke S, Schlaphoff V, et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. *Hepatology* 2012;**55**:695–708.
152. Tanaka T, Takahashi M, Kusano E, et al. Development and evaluation of an efficient cell-culture system for hepatitis E virus. *J Gen Virol* 2007;**88**(Pt 3):903–11.
156. Teshale EH, Grytdal SP, Howard C, et al. Evidence of person-to-person transmission of hepatitis E virus during a large outbreak in Northern Uganda. *Clin Infect Dis* 2010;**50**:1006–10.
161. Tripathy AS, Das R, Rathod SB, et al. Peripheral T regulatory cells and cytokines in hepatitis E infection. *Eur J Clin Microbiol Infect Dis* 2012;**31**:179–84.
170. Varma SP, Kumar A, Kapur N, et al. Hepatitis E virus replication involves alternating negative- and positive-sense RNA synthesis. *J Gen Virol* 2011;**92**(Pt 3):572–81.
175. Wu T, Zhu FC, Huang SJ, et al. Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis. *Hepatology* 2012; **55**:2038.
177. Yamada K, Takahashi M, Hoshino Y, et al. ORF3 protein of hepatitis E virus is essential for virion release from infected cells. *J Gen Virol* 2009;**90**(Pt 8):1880–91.
182. Zhu FC, Zhang J, Zhang XF, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet* 2010;**376**:895–902.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abe K, Hayakawa E, Sminov AV, et al. Molecular epidemiology of hepatitis B, C, D and E viruses among children in Moscow, Russia. *J Clin Virol* 2004;**30**:57–61.
2. Abid S, Khan AH. Severe hemolysis and renal failure in glucose-6-phosphate dehydrogenase deficient patients with hepatitis E. *Am J Gastroenterol* 2002;**97**:1544–7.
3. Aggarwal R, Krawczynski K. Hepatitis E: an overview and recent advances in clinical and laboratory research. *J Gastroenterol Hepatol* 2000;**15**:9–20.
4. Aggarwal R, Naik SR. Hepatitis E: intrafamilial transmission versus waterborne spread. *J Hepatol* 1994;**21**:718–23.
5. Aggarwal R, Kini D, Sofat S, et al. Duration of viraemia and faecal viral excretion in acute hepatitis E. *Lancet* 2000;**356**: 1081–2.
6. Aggarwal R, Shahi H, Naik S, et al. Evidence in favour of high infection rate with hepatitis E virus among young children in India. *J Hepatol* 1997;**26**:1425–6.
7. Agrawal S, Gupta D, Panda SK. The 3′ end of hepatitis E virus (HEV) genome binds specifically to the viral RNA-dependent RNA polymerase (RdRp). *Virology* 2001;**282**:87–101.
8. Alvarez-Munoz MT, Torres J, Damasio L, et al. Seroepidemiology of hepatitis E virus infection in Mexican subjects 1 to 29 years of age. *Arch Med Res* 1999;**30**:251–4.
9. Ansari IH, Nanda SK, Durgapal H, et al. Cloning, sequencing, and expression of the hepatitis E virus (HEV) nonstructural open reading frame 1 (ORF1). *J Med Virol* 2000;**60**:275–83.
10. Arankalle VA, Chobe LP. Hepatitis E virus: can it be transmitted parenterally? *J Viral Hepat* 1999;**6**:161–4.
11. Arankalle VA, Chobe LP. Retrospective analysis of blood transfusion recipients: evidence for post-transfusion hepatitis E. *Vox Sang* 2000;**79**:72–4.
12. Arankalle VA, Chadha MS, Chitambar SD, et al. Changing epidemiology of hepatitis A and hepatitis E in urban and rural India (1982-98). *J Viral Hepat* 2001;**8**:293–303.
13. Arankalle VA, Chadha MS, Dama BM, et al. Role of immune serum globulins in pregnant women during an epidemic of hepatitis E. *J Viral Hepat* 1998;**5**:199–204.
14. Arankalle VA, Chadha MS, Mehendale SM, et al. Epidemic hepatitis E: serological evidence for lack of intrafamilial spread. *Indian J Gastroenterol* 2000;**19**:24–8.
15. Arankalle VA, Tsarev SA, Chadha MS, et al. Age-specific prevalence of antibodies to hepatitis A and E viruses in Pune, India, 1982 and 1992. *J Infect Dis* 1995;**171**:447–50.
16. Arora NK, Nanda SK, Gulati S, et al. Acute viral hepatitis types E, A, and B singly and in combination in acute liver failure in children in north India. *J Med Virol* 1996;**48**:215–21.
17. Arora NK, Panda SK, Nanda SK, et al. Hepatitis E infection in children: study of an outbreak. *J Gastroenterol Hepatol* 1999;**14**: 572–7.
18. Balayan MS, Andjaparidze AG, Savinskaya SS, et al. Evidence for a virus in non-A, non-B hepatitis transmitted via the fecal-oral route. *Intervirology* 1983;**20**:23–31.
19. Bartoloni A, Bartalesi F, Roselli M, et al. Prevalence of antibodies against hepatitis A and E viruses among rural populations of the Chaco region, south-eastern Bolivia. *Trop Med Int Health* 1999;**4**: 596–601.
20. Bawazir AA, Hart CA, Sallam TA, et al. Seroepidemiology of hepatitis A and hepatitis E viruses in Aden, Yemen. *Trans R Soc Trop Med Hyg* 2010;**104**:801–5.
21. Begum N, Polipalli SK, Husain SA, et al. Duration of hepatitis E viremia in pregnancy. *Int J Gynaecol Obstet* 2010;**108**:207–10.
22. Berke T, Matson DO. Reclassification of the Caliciviridae into distinct genera and exclusion of hepatitis E virus from the family on the basis of comparative phylogenetic analysis. *Arch Virol* 2000;**145**:1421–36.
23. Bernal MC, Leyva A, Garcia F, et al. Seroepidemiological study of hepatitis E virus in different population groups. *Eur J Clin Microbiol Infect Dis* 1995;**14**:954–8.
24. Boccia D, Guthmann JP, Klovstad H, et al. High mortality associated with an outbreak of hepatitis E among displaced persons in Darfur, Sudan. *Clin Infect Dis* 2006;**42**:1679–84.
25. Boxall E, Herborn A, Kochethu G, et al. Transfusion-transmitted hepatitis E in a 'nonhyperendemic' country. *Transfus Med* 2006; **16**:79–83.
26. Bradley D, Andjaparidze A, Cook EH Jr, et al. Aetiological agent of enterically transmitted non-A, non-B hepatitis. *J Gen Virol* 1988;**69**(Pt 3):731–8.
27. Bradley DW, Balayan MS. Virus of enterically transmitted non-A, non-B hepatitis. *Lancet* 1988;**1**:819.
28. Centers for Disease Control and Prevention. *Hepatitis E fact sheet*. Atlanta: CDC; 2006.
29. Chau TN, Lai ST, Tse C, et al. Epidemiology and clinical features of sporadic hepatitis E as compared with hepatitis A. *Am J Gastroenterol* 2006;**101**:292–6.
30. Chauhan A, Jameel S, Dilawari JB, et al. Hepatitis E virus transmission to a volunteer. *Lancet* 1993;**341**:149–50.
31. Chibber RM, Usmani MA, Al-Sibai MH. Should HEV infected mothers breast feed? *Arch Gynecol Obstet* 2004;**270**:15–20.
32. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;**244**:359–62.
33. Clayson ET, Myint KS, Snitbhan R, et al. Viremia, fecal shedding, and IgM and IgG responses in patients with hepatitis E. *J Infect Dis* 1995;**172**:927–33.
34. Clayson ET, Shrestha MP, Vaughn DW, et al. Rates of hepatitis E virus infection and disease among adolescents and adults in Kathmandu, Nepal. *J Infect Dis* 1997;**176**:763–6.
35. Colson P, Borentain P, Queyriaux B, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. *J Infect Dis* 2010;**202**:825–34.
36. Cooper K, Huang FF, Batista L, et al. Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. *J Clin Microbiol* 2005;**43**:1684–8.
37. Dahiya M, Kumar A, Kar P, et al. Acute viral hepatitis in third trimester of pregnancy. *Indian J Gastroenterol* 2005;**24**:128–9.
38. Daniel HD, Warier A, Abraham P, et al. Age-wise exposure rates to hepatitis e virus in a southern Indian patient population without liver disease. *Am J Trop Med Hyg* 2004;**71**:675–8.
39. Dixit VK, Abhilash VB, Kate MP, et al. Hepatitis E infection with Bell's palsy. *J Assoc Physicians India* 2006;**54**:418.
40. Dixit VK, Kate MP, Abhilash VB, et al. Association of Guillain-Barré syndrome and hepatitis E infection. *Indian J Gastroenterol* 2006;**25**:48; author reply 48.
41. Dong C, Zafrullah M, Mixson-Hayden T, et al. Suppression of interferon-alpha signaling by hepatitis E virus. *Hepatology* 2012; **55**:1324–32.
42. el-Zimaity DM, Hyams KC, Imam IZ, et al. Acute sporadic hepatitis E in an Egyptian pediatric population. *Am J Trop Med Hyg* 1993;**48**:372–6.
43. Emerson SU, Purcell RH. Hepatitis E virus. *Rev Med Virol* 2003; **13**:145–54.
44. Emerson SU, Arankalle VA, Purcell RH. Thermal stability of hepatitis E virus. *J Infect Dis* 2005;**192**:930–3.
45. Emerson SU, Nguyen HT, Torian U, et al. Release of genotype 1 hepatitis E virus from cultured hepatoma and polarized intestinal cells depends on open reading frame 3 protein and requires an intact PXXP motif. *J Virol* 2010;**84**:9059–69.
46. Enouf V, Dos Reis G, Guthmann JP, et al. Validation of single real-time TaqMan PCR assay for the detection and quantitation of four major genotypes of hepatitis E virus in clinical specimens. *J Med Virol* 2006;**78**:1076–82.
47. Fix AD, Abdel-Hamid M, Purcell RH, et al. Prevalence of antibodies to hepatitis E in two rural Egyptian communities. *Am J Trop Med Hyg* 2000;**62**:519–23.
48. Fogeda M, Avellon A, Echevarria JM. Prevalence of specific antibody to hepatitis E virus in the general population of the community of Madrid, Spain. *J Med Virol* 2012;**84**:71–4.
49. Gerolami R, Borentain P, Raissouni F, et al. Treatment of severe acute hepatitis E by ribavirin. *J Clin Virol* 2011;**52**:60–2.
50. Gessoni G, Manoni F. Hepatitis E virus infection in north-east Italy: serological study in the open population and groups at risk. *J Viral Hepat* 1996;**3**:197–202.
51. Glanz J, Times NY. *Hepatitis Outbreak Laid to Water and Sewage Failures*. New York; 2006. p. 2004. Available at http://query.nytimes.com/gst/fullpage.html?res=D04E4D61E39F936A1575AC0A9629C8B63.
52. Goldsmith R, Yarbough PO, Reyes GR, et al. Enzyme-linked immunosorbent assay for diagnosis of acute sporadic hepatitis E in Egyptian children. *Lancet* 1992;**339**:328–31.

52a. Goto K, Ito K, Sugiura T, et al. Prevalence of hepatitis E virus infection in Japanese children. *J Pediatr Gastroenterol Nutr* 2006;**42**:89–92.
53. Graff J, Torian U, Nguyen H, et al. A bicistronic subgenomic mRNA encodes both the ORF2 and ORF3 proteins of hepatitis E virus. *J Virol* 2006;**80**:5919–26.
54. Grandadam M, Tebbal S, Caron M, et al. Evidence for hepatitis E virus quasispecies. *J Gen Virol* 2004;**85**:3189–94.
55. Green KY, Ando T, Balayan MS, et al. Taxonomy of the caliciviruses. *J Infect Dis* 2000;**181**(Suppl. 2):S322–30.
56. Guo H, Zhou EM, Sun ZF, et al. Identification of B-cell epitopes in the capsid protein of avian hepatitis E virus (avian HEV) that are common to human and swine HEVs or unique to avian HEV. *J Gen Virol* 2006;**87**:217–23.
57. Guthmann JP, Klovstad H, Boccia D, et al. A large outbreak of hepatitis E among a displaced population in Darfur, Sudan, 2004: the role of water treatment methods. *Clin Infect Dis* 2006;**42**: 1685–91.
58. Haagsma EB, Riezebos-Brilman A, van den Berg AP, et al. Treatment of chronic hepatitis E in liver transplant recipients with pegylated interferon alpha-2b. *Liver Transpl* 2010;**16**:474–7.
59. Haagsma EB, van den Berg AP, Porte RJ, et al. Chronic hepatitis E virus infection in liver transplant recipients. *Liver Transpl* 2008; **14**:547–53.
60. Halac U, Beland K, Lapierre P, et al. Chronic hepatitis E infection in children with liver transplantation. *Gut* 2012;**61**:597–603.
61. Halac U, Beland K, Lapierre P, et al. Cirrhosis due to chronic hepatitis e infection in a child post-bone marrow transplant. *J Pediatr* 2012;**160**:871–4.
62. Haqshenas G, Huang FF, Fenaux M, et al. The putative capsid protein of the newly identified avian hepatitis E virus shares antigenic epitopes with that of swine and human hepatitis E viruses and chicken big liver and spleen disease virus. *J Gen Virol* 2002;**83**: 2201–9.
63. Haqshenas G, Shivaprasad HL, Woolcock PR, et al. Genetic identification and characterization of a novel virus related to human hepatitis E virus from chickens with hepatitis-splenomegaly syndrome in the United States. *J Gen Virol* 2001;**82**:2449–62.
64. Hsieh SY, Meng XJ, Wu YH, et al. Identity of a novel swine hepatitis E virus in Taiwan forming a monophyletic group with Taiwan isolates of human hepatitis E virus. *J Clin Microbiol* 1999;**37**:3828–34.
65. Huang FF, Sun ZF, Emerson SU, et al. Determination and analysis of the complete genomic sequence of avian hepatitis E virus (avian HEV) and attempts to infect rhesus monkeys with avian HEV. *J Gen Virol* 2004;**85**:1609–18.
66. Hyams KC, McCarthy MC, Kaur M, et al. Acute sporadic hepatitis E in children living in Cairo, Egypt. *J Med Virol* 1992;**37**: 274–7.
67. Hyams KC, Purdy MA, Kaur M, et al. Acute sporadic hepatitis E in Sudanese children: analysis based on a new western blot assay. *J Infect Dis* 1992;**165**:1001–5.
68. Ibarra HV, Riedemann SG, Siegel FG, et al. Hepatitis E virus in Chile. *Lancet* 1994;**344**:1501.
69. Jameel S, Zafrullah M, Ozdener MH, et al. Expression in animal cells and characterization of the hepatitis E virus structural proteins. *J Virol* 1996;**70**:207–16.
70. Jaroszewicz J, Flisiak R, Kalinowska A, et al. Acute hepatitis E complicated by acute pancreatitis: a case report and literature review. *Pancreas* 2005;**30**:382–4.
71. Jothikumar N, Cromeans TL, Robertson BH, et al. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods* 2006;**131**: 65–71.
72. Kabrane-Lazizi Y, Fine JB, Elm J, et al. Evidence for widespread infection of wild rats with hepatitis E virus in the United States. *Am J Trop Med Hyg* 1999;**61**:331–5.
73. Kabrane-Lazizi Y, Meng XJ, Purcell RH, et al. Evidence that the genomic RNA of hepatitis E virus is capped. *J Virol* 1999;**73**: 8848–50.
74. Kamani P, Baijal R, Amarapurkar D, et al. Guillain-Barré syndrome associated with acute hepatitis E. *Indian J Gastroenterol* 2005;**24**:216.
75. Kamar N, Selves J, Mansuy JM, et al. Hepatitis E virus and chronic hepatitis in organ-transplant recipients. *N Engl J Med* 2008;**358**:811–17.
76. Kamar N, Rostaing L, Abravanel F, et al. Pegylated interferon-alpha for treating chronic hepatitis E virus infection after liver transplantation. *Clin Infect Dis* 2010;**50**:e30–3.
77. Kamili S, Spelbring J, Carson D, et al. Protective efficacy of hepatitis E virus DNA vaccine administered by gene gun in the cynomolgus macaque model of infection. *J Infect Dis* 2004;**189**: 258–64.
78. Kapur N, Thakral D, Durgapal H, et al. Hepatitis E virus enters liver cells through receptor-dependent clathrin-mediated endocytosis. *J Viral Hepat* 2012;**19**:436–48.
79. Kar-Roy A, Korkaya H, Oberoi R, et al. The hepatitis E virus open reading frame 3 protein activates ERK through binding and inhibition of the MAPK phosphatase. *J Biol Chem* 2004;**279**: 28345–57.
80. Karetnyi YV, Favorov MO, Khudyakova NS, et al. Serological evidence for hepatitis E virus infection in Israel. *J Med Virol* 1995;**45**:316–20.
81. Kenney SP, Pudupakam RS, Huang YW, et al. The PSAP motif within the ORF3 protein of an avian strain of the hepatitis e virus is not critical for viral infectivity in vivo but plays a role in virus release. *J Virol* 2012;**86**:5637–46.
82. Khuroo MS. Study of an epidemic of non-A, non-B hepatitis: possibility of another human hepatitis virus distinct from post-transfusion non-A, non-B type. *Am J Med* 1980;**68**:818–24.
83. Khuroo MS, Dar MY. Hepatitis E: evidence for person-to-person transmission and inability of low dose immune serum globulin from an Indian source to prevent it. *Indian J Gastroenterol* 1992; **11**:113–16.
84. Khuroo MS, Kamili S. Aetiology, clinical course and outcome of sporadic acute viral hepatitis in pregnancy. *J Viral Hepat* 2003;**10**:61–9.
85. Khuroo MS, Kamili S. Clinical course and duration of viremia in vertically transmitted hepatitis E virus (HEV) infection in babies born to HEV-infected mothers. *J Viral Hepat* 2009;**16**: 519–23.
86. Khuroo MS, Kamili S, Jameel S. Vertical transmission of hepatitis E virus. *Lancet* 1995;**345**:1025–6.
87. Khuroo MS, Kamili S, Yattoo GN. Hepatitis E virus infection may be transmitted through blood transfusions in an endemic area. *J Gastroenterol Hepatol* 2004;**19**:778–84.
88. Khuroo MS, Teli MR, Skidmore S, et al. Incidence and severity of viral hepatitis in pregnancy. *Am J Med* 1981;**70**:252–5.
89. Koonin EV, Gorbalenya AE, Purdy MA, et al. Computer-assisted assignment of functional domains in the nonstructural polyprotein of hepatitis E virus: delineation of an additional group of positive-strand RNA plant and animal viruses. *Proc Natl Acad Sci U S A* 1992;**89**:8259–63.
90. Korkaya H, Jameel S, Gupta D, et al. The ORF3 protein of hepatitis E virus binds to Src homology 3 domains and activates MAPK. *J Biol Chem* 2001;**276**:42389–400.
91. Labrique AB, Zaman K, Hossain Z, et al. Population seroprevalence of hepatitis E virus antibodies in rural Bangladesh. *Am J Trop Med Hyg* 2009;**81**:875–81.
92. Li T, Takeda N, Miyamura T. Oral administration of hepatitis E virus-like particles induces a systemic and mucosal immune response in mice. *Vaccine* 2001;**19**:3476–84.
93. Li TC, Chijiwa K, Sera N, et al. Hepatitis E virus transmission from wild boar meat. *Emerg Infect Dis* 2005;**11**:1958–60.
94. Li TC, Suzaki Y, Ami Y, et al. Protection of cynomolgus monkeys against HEV infection by oral administration of recombinant hepatitis E virus-like particles. *Vaccine* 2004;**22**:370–7.
95. Li TC, Takeda N, Miyamura T, et al. Essential elements of the capsid protein for self-assembly into empty virus-like particles of hepatitis E virus. *J Virol* 2005;**79**:12999–3006.
96. Li TC, Yamakawa Y, Suzuki K, et al. Expression and self-assembly of empty virus-like particles of hepatitis E virus. *J Virol* 1997;**71**: 7207–13.
97. Lin DB, Lin JB, Chen SC, et al. Seroepidemiology of hepatitis E virus infection among preschool children in Taiwan. *J Med Virol* 2004;**74**:414–18.
98. Longer CF, Denny SL, Caudill JD, et al. Experimental hepatitis E: pathogenesis in cynomolgus macaques *(Macaca fascicularis)*. *J Infect Dis* 1993;**168**:602–9.
99. Lu L, Li C, Hagedorn CH. Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol* 2006;**16**:5–36.

100. Magden J, Takeda N, Li T, et al. Virus-specific mRNA capping enzyme encoded by hepatitis E virus. *J Virol* 2001;**75**:6249–55.
101. Maity SG, Ray G. Severe acute pancreatitis in acute hepatitis E. *Indian J Gastroenterol* 2002;**21**:37–8.
102. Malathi S, Mohanavalli B, Menon T, et al. Clinical and viral marker pattern of acute sporadic hepatitis in children in Madras, South India. *J Trop Pediatr* 1998;**44**:275–8.
103. Mandal K, Chopra N. Acute transverse myelitis following hepatitis E virus infection. *Indian Pediatr* 2006;**43**:365–6.
104. Mast EE, Alter MJ, Holland PV, et al. Evaluation of assays for antibody to hepatitis E virus by a serum panel. Hepatitis E Virus Antibody Serum Panel Evaluation Group. *Hepatology* 1998;**27**: 857–61.
105. Mast EE, Kuramoto IK, Favorov MO, et al. Prevalence of and risk factors for antibody to hepatitis E virus seroreactivity among blood donors in Northern California. *J Infect Dis* 1997;**176**: 34–40.
106. Mathur P, Arora NK, Panda SK, et al. Sero-epidemiology of hepatitis E virus (HEV) in urban and rural children of North India. *Indian Pediatr* 2001;**38**:461–75.
107. Meng J, Dai X, Chang JC, et al. Identification and characterization of the neutralization epitope(s) of the hepatitis E virus. *Virology* 2001;**288**:203–11.
108. Meng XJ, Halbur PG, Haynes JS, et al. Experimental infection of pigs with the newly identified swine hepatitis E virus (swine HEV), but not with human strains of HEV. *Arch Virol* 1998;**143**: 1405–15.
109. Meng XJ, Halbur PG, Shapiro MS, et al. Genetic and experimental evidence for cross-species infection by swine hepatitis E virus. *J Virol* 1998;**72**:9714–21.
110. Meng J, Pillot J, Dai X, et al. Neutralization of different geographic strains of the hepatitis E virus with anti-hepatitis E virus-positive serum samples obtained from different sources. *Virology* 1998;**249**:316–24.
111. Meng XJ, Purcell RH, Halbur PG, et al. A novel virus in swine is closely related to the human hepatitis E virus. *Proc Natl Acad Sci U S A* 1997;**94**:9860–5.
112. Mishra A, Saigal S, Gupta R, et al. Acute pancreatitis associated with viral hepatitis: a report of six cases with review of literature. *Am J Gastroenterol* 1999;**94**:2292–5.
113. Mohanavalli B, Dhevahi E, Menon T, et al. Prevalence of antibodies to hepatitis A and hepatitis E virus in urban school children in Chennai. *Indian Pediatr* 2003;**40**:328–31.
114. Myint KS, Endy TP, Gibbons RV, et al. Evaluation of diagnostic assays for hepatitis E virus in outbreak settings. *J Clin Microbiol* 2006;**44**:1581–3.
115. Myint KS, Guan M, Chen HY, et al. Evaluation of a new rapid immunochromatographic assay for serodiagnosis of acute hepatitis E infection. *Am J Trop Med Hyg* 2005;**73**:942–6.
116. Nagashima S, Takahashi M, Jirintai, et al. A PSAP motif in the ORF3 protein of hepatitis E virus is necessary for virion release from infected cells. *J Gen Virol* 2011;**92**:269–78.
117. Naik S, Aggarwal R, Naik SR, et al. Evidence for activation of cellular immune responses in patients with acute hepatitis E. *Indian J Gastroenterol* 2002;**21**:149–52.
118. Nanda SK, Ansari IH, Acharya SK, et al. Protracted viremia during acute sporadic hepatitis E virus infection. *Gastroenterology* 1995;**108**:225–30.
119. Okamoto H, Takahashi M, Nishizawa T, et al. Presence of antibodies to hepatitis E virus in Japanese pet cats. *Infection* 2004;**32**:57–8.
120. Pal R, Aggarwal R, Naik SR, et al. Immunological alterations in pregnant women with acute hepatitis E. *J Gastroenterol Hepatol* 2005;**20**:1094–101.
121. Panda SK, Ansari IH, Durgapal H, et al. The in vitro-synthesized RNA from a cDNA clone of hepatitis E virus is infectious. *J Virol* 2000;**74**:2430–7.
122. Parvez MK, Purcell RH, Emerson SU. Hepatitis E virus ORF2 protein over-expressed by baculovirus in hepatoma cells, efficiently encapsidates and transmits the viral RNA to naive cells. *Virol J* 2011;**8**:159.
123. Patra S, Kumar A, Trivedi SS, et al. Maternal and fetal outcomes in pregnant women with acute hepatitis E virus infection. *Ann Intern Med* 2007;**147**:28–33.
124. Pina S, Jofre J, Emerson SU, et al. Characterization of a strain of infectious hepatitis E virus isolated from sewage in an area where hepatitis E is not endemic. *Appl Environ Microbiol* 1998;**64**: 4485–8.
125. Poddar U, Thapa BR, Prasad A, et al. Changing spectrum of sporadic acute viral hepatitis in Indian children. *J Trop Pediatr* 2002;**48**:210–13.
126. Poddar U, Thapa BR, Prasad A, et al. Natural history and risk factors in fulminant hepatic failure. *Arch Dis Child* 2002;**87**: 54–6.
127. Pujol FH, Favorov MO, Marcano T, et al. Prevalence of antibodies against hepatitis E virus among urban and rural populations in Venezuela. *J Med Virol* 1994;**42**:234–6.
128. Purcell RH, Nguyen H, Shapiro M, et al. Pre-clinical immunogenicity and efficacy trial of a recombinant hepatitis E vaccine. *Vaccine* 2003;**21**:2607–15.
129. Quintana A, Sanchez L, Larralde O, et al. Prevalence of antibodies to hepatitis E virus in residents of a district in Havana, Cuba. *J Med Virol* 2005;**76**:69–70.
130. Ramachandran J, Eapen CE, Kang G, et al. Hepatitis E superinfection produces severe decompensation in patients with chronic liver disease. *J Gastroenterol Hepatol* 2004;**19**:134–8.
131. Rein DB, Stevens GA, Theaker J, et al. The global burden of hepatitis E virus genotypes 1 and 2 in 2005. *Hepatology* 2012;**55**: 988–97.
132. Reyes GR, Purdy MA, Kim JP, et al. Isolation of a cDNA from the virus responsible for enterically transmitted non-A, non-B hepatitis. *Science* 1990;**247**:1335–9.
133. Robinson RA, Burgess WH, Emerson SU, et al. Structural characterization of recombinant hepatitis E virus ORF2 proteins in baculovirus-infected insect cells. *Protein Expr Purif* 1998;**12**:75–84.
134. Safary A. Perspectives of vaccination against hepatitis E. *Intervirology* 2001;**44**:162–6.
135. Sainokami S, Abe K, Kumagai I, et al. Epidemiological and clinical study of sporadic acute hepatitis E caused by indigenous strains of hepatitis E virus in Japan compared with acute hepatitis A. *J Gastroenterol* 2004;**39**:640–8.
136. Schofield DJ, Glamann J, Emerson SU, et al. Identification by phage display and characterization of two neutralizing chimpanzee monoclonal antibodies to the hepatitis E virus capsid protein. *J Virol* 2000;**74**:5548–55.
137. Sehgal D, Thomas S, Chakraborty M, et al. Expression and processing of the Hepatitis E virus ORF1 nonstructural polyprotein. *Virol J* 2006;**3**:38.
138. Shrestha MP, Scott RM, Joshi DM, et al. Safety and efficacy of a recombinant hepatitis E vaccine. *N Engl J Med* 2007;**356**: 895–903.
139. Sidal M, Unuvar E, Oguz F, et al. Age-specific seroepidemiology of hepatitis A, B, and E infections among children in Istanbul, Turkey. *Eur J Epidemiol* 2001;**17**:141–4.
140. Singh S, Mohanty A, Joshi YK, et al. Mother-to-child transmission of hepatitis E virus infection. *Indian J Pediatr* 2003;**70**:37–9.
141. Sood A, Midha V, Sood N. Guillain-Barré syndrome with acute hepatitis E. *Am J Gastroenterol* 2000;**95**:3667–8.
142. Sreenivasan MA, Arankalle VA, Sehgal A, et al. Non-A, non-B epidemic hepatitis: visualization of virus-like particles in the stool by immune electron microscopy. *J Gen Virol* 1984;**65**(Pt 5): 1005–7.
143. Sriram B, Thakral D, Panda SK. Targeted cleavage of hepatitis E virus 3′ end RNA mediated by hammerhead ribozymes inhibits viral RNA replication. *Virology* 2003;**312**:350–8.
144. Stoszek SK, Engle RE, Abdel-Hamid M, et al. Hepatitis E antibody seroconversion without disease in highly endemic rural Egyptian communities. *Trans R Soc Trop Med Hyg* 2006;**100**: 89–94.
145. Suneetha PV, Pischke S, Schlaphoff V, et al. Hepatitis E virus (HEV)-specific T-cell responses are associated with control of HEV infection. *Hepatology* 2012;**55**:695–708.
146. Surjit M, Jameel S, Lal SK. The ORF2 protein of hepatitis E virus binds the 5′ region of viral RNA. *J Virol* 2004;**78**:320–8.
147. Syed SA, Sarwari AR, Smego RA Jr, et al. Does infection with hepatitis A virus provide protection against hepatitis E virus? *Med Hypotheses* 2003;**60**:337–9.
148. Takahashi M, Kusakai S, Mizuo H, et al. Simultaneous detection of immunoglobulin A (IgA) and IgM antibodies against hepatitis E virus (HEV) Is highly specific for diagnosis of acute HEV infection. *J Clin Microbiol* 2005;**43**:49–56.

149. Tam AW, Smith MM, Guerra ME, et al. Hepatitis E virus (HEV): molecular cloning and sequencing of the full-length viral genome. *Virology* 1991;**185**:120–31.
150. Tam AW, White R, Reed E, et al. In vitro propagation and production of hepatitis E virus from in vivo-infected primary macaque hepatocytes. *Virology* 1996;**215**:1–9.
151. Tam AW, White R, Yarbough PO, et al. In vitro infection and replication of hepatitis E virus in primary cynomolgus macaque hepatocytes. *Virology* 1997;**238**:94–102.
152. Tanaka T, Takahashi M, Kusano E, et al. Development and evaluation of an efficient cell-culture system for hepatitis E virus. *J Gen Virol* 2007;**88**:903–11.
153. Tandon A, Tandon BN, Bhujwala RA. Clinical spectrum of acute sporadic hepatitis E and possible benefit of glycyrrhizin therapy. *Hepatol Res* 2002;**23**:55–61.
154. Tei S, Kitajima N, Ohara S, et al. Consumption of uncooked deer meat as a risk factor for hepatitis E virus infection: an age- and sex-matched case-control study. *J Med Virol* 2004;**74**: 67–70.
155. Tei S, Kitajima N, Takahashi K, et al. Zoonotic transmission of hepatitis E virus from deer to human beings. *Lancet* 2003;**362**: 371–3.
156. Teshale EH, Grytdal SP, Howard C, et al. Evidence of person-to-person transmission of hepatitis E virus during a large outbreak in Northern Uganda. *Clin Infect Dis* 2010;**50**:1006–10.
157. Thomas DL, Mahley RW, Badur S, et al. Epidemiology of hepatitis E virus infection in Turkey. *Lancet* 1993;**341**:1561–2.
158. Thomas DL, Yarbough PO, Vlahov D, et al. Seroreactivity to hepatitis E virus in areas where the disease is not endemic. *J Clin Microbiol* 1997;**35**:1244–7.
159. Tian DY, Chen Y, Xia NS. Significance of serum IgA in patients with acute hepatitis E virus infection. *World J Gastroenterol* 2006;**12**:3919–23.
160. Ticehurst J, Rhodes LL Jr, Krawczynski K, et al. Infection of owl monkeys *(Aotus trivirgatus)* and cynomolgus monkeys *(Macaca fascicularis)* with hepatitis E virus from Mexico. *J Infect Dis* 1992;**165**:835–45.
161. Tripathy AS, Das R, Rathod SB, et al. Peripheral T regulatory cells and cytokines in hepatitis E infection. *Eur J Clin Microbiol Infect Dis* 2012;**31**:179–84.
162. Tsarev SA, Emerson SU, Reyes GR, et al. Characterization of a prototype strain of hepatitis E virus. *Proc Natl Acad Sci U S A* 1992;**89**:559–63.
163. Tsarev SA, Emerson SU, Tsareva TS, et al. Variation in course of hepatitis E in experimentally infected cynomolgus monkeys. *J Infect Dis* 1993;**167**:1302–6.
164. Tsarev SA, Tsareva TS, Emerson SU, et al. ELISA for antibody to hepatitis E virus (HEV) based on complete open-reading frame-2 protein expressed in insect cells: identification of HEV infection in primates. *J Infect Dis* 1993;**168**:369–78.
165. Tsarev SA, Tsareva TS, Emerson SU, et al. Successful passive and active immunization of cynomolgus monkeys against hepatitis E. *Proc Natl Acad Sci U S A* 1994;**91**:10198–202.
166. Tsarev SA, Tsareva TS, Emerson SU, et al. Recombinant vaccine against hepatitis E: dose response and protection against heterologous challenge. *Vaccine* 1997;**15**:1834–8.
167. Tyagi S, Jameel S, Lal SK. Self-association and mapping of the interaction domain of hepatitis E virus ORF3 protein. *J Virol* 2001;**75**:2493–8.
168. Tyagi S, Surjit M, Roy AK, et al. The ORF3 protein of hepatitis E virus interacts with liver-specific alpha1-microglobulin and its precursor alpha1-microglobulin/bikunin precursor (AMBP) and expedites their export from the hepatocyte. *J Biol Chem* 2004; **279**:29308–19.
169. Vaidya SR, Chitambar SD, Arankalle VA. Polymerase chain reaction-based prevalence of hepatitis A, hepatitis E and TT viruses in sewage from an endemic area. *J Hepatol* 2002;**37**: 131–6.
170. Varma SP, Kumar A, Kapur N, et al. Hepatitis E virus replication involves alternating negative- and positive-sense RNA synthesis. *J Gen Virol* 2011;**92**:572–81.
171. Williams TP, Kasorndorkbua C, Halbur PG, et al. Evidence of extrahepatic sites of replication of the hepatitis E virus in a swine model. *J Clin Microbiol* 2001;**39**:3040–6.
172. Wong DC, Purcell RH, Sreenivasan MA, et al. Epidemic and endemic hepatitis in India: evidence for a non-A, non-B hepatitis virus aetiology. *Lancet* 1980;**2**:876–9.
173. World Health Organization, Organization WH. *Hepatitis E in Chad. Geneva* 2006:*disease outbreak news*. 2004.
174. Worm HC, van der Poel WH, Brandstatter G. Hepatitis E: an overview. *Microbes Infect* 2002;**4**:657–66.
175. Wu T, Zhu FC, Huang SJ, et al. Safety of the hepatitis E vaccine for pregnant women: a preliminary analysis. *Hepatology* 2012;**55**: 2038.
176. Xing L, Kato K, Li T, et al. Recombinant hepatitis E capsid protein self-assembles into a dual-domain T = 1 particle presenting native virus epitopes. *Virology* 1999;**265**:35–45.
177. Yamada K, Takahashi M, Hoshino Y, et al. ORF3 protein of hepatitis E virus is essential for virion release from infected cells. *J Gen Virol* 2009;**90**:1880–91.
178. Yarbough PO, Tam AW, Fry KE, et al. Hepatitis E virus: identification of type-common epitopes. *J Virol* 1991;**65**:5790–7.
179. Zafrullah M, Ozdener MH, Kumar R, et al. Mutational analysis of glycosylation, membrane translocation, and cell surface expression of the hepatitis E virus ORF2 protein. *J Virol* 1999;**73**: 4074–82.
180. Zamvar V, McClean P, Odeka E, et al. Hepatitis E virus infection with nonimmune hemolytic anemia. *J Pediatr Gastroenterol Nutr* 2005;**40**:223–5.
181. Zhang M, Emerson SU, Nguyen H, et al. Recombinant vaccine against hepatitis E: duration of protective immunity in rhesus macaques. *Vaccine* 2002;**20**:3285–91.
182. Zhu FC, Zhang J, Zhang XF, et al. Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial. *Lancet* 2010;**376**:895–902.

RNA VIRUSES
SUBSECTION 3 *REOVIRIDAE*

CHAPTER 170

Reoviruses

James D. Cherry

Reoviruses are ubiquitous in nature, but their role in human disease is vague. After reoviruses were classified in 1959, numerous reports noted their association with human disease.[44,75,86] Since that time, these viruses have been evaluated extensively in laboratory animal studies, but few cases of human disease have been reported during the last 4 decades.

HISTORY

On the basis of its cytopathic effect in monkey kidney tissue culture and its recovery from stool specimens at a time of enterovirus surveillance, the first recovered reovirus was designated ECHO virus type 10.[12,13] This virus and four similar strains were recovered in 1954 from the stools of healthy children in Cincinnati and Mexico.[68,69] By 1959, researchers were aware that ECHO 10 viral strains appeared to have many characteristics that were different from those of enteroviruses, such as large size and unique cytopathic effect, and they were withdrawn from this grouping. The term *reovirus* was chosen to stress the association of these agents with both the respiratory *(r)* and enteric *(e)* tracts. The *o* for orphan was retained in the designation. In the early 1960s, reoviral infections were noted in association with human illness.*

PROPERTIES†

Reoviruses are members of the family *Reoviridae*.[20,58,60] This family has numerous double-stranded RNA viruses that infect mammals, birds, reptiles, fish, mollusks, insects, plants, and arthropods.[60] Nine genera are in the family, and they can be subdivided by the presence or absence of a turretlike protein that projects from the innermost capsid layer. Of the turreted *Reoviridae*, only orthoreoviruses infect humans and are addressed in this chapter. Orthoreoviruses commonly are called simply reoviruses. Nonturreted *Reoviridae* include the following viruses that cause disease in humans: rotaviruses (Chapter 172) and orbiviruses and coltiviruses (Chapter 171). Three serotypes of orthoreoviruses cause infection in humans. All mammalian reoviruses are related by a common group-specific, complement-fixing antigen.[83,104] Three distinct human serotypes can be identified by neutralization or hemagglutination inhibition.[73]

Reovirus particles are composed of an inner protein shell (core) with a diameter of 60 nm that is surrounded by an outer protein shell (outer capsid) measuring 81 nm in diameter.[60] The outer capsid has icosahedral symmetry and is composed of 92 to 180 hexagonal and pentagonal subunits (capsomers). The genome of orthoreoviruses has a total size of approximately 23,500 base pairs with 10 gene segments: three large (L1, L2, L3), three medium (M1, M2, M3), and four small (S1, S2, S3, S4). These genes encode 12 proteins. The outer capsid comprises four proteins: σ-1, σ-3, λ-2, and μ-1. The σ-1 protein is the reovirus cell-attachment protein against which serotype-specific neutralizing antibodies are developed. This protein also is the hemagglutinin. The reovirus receptor is the junctional adhesion molecule 1 (JAM1).[21,97] The σ-3 protein binds double-stranded RNA and is sensitive to protease degradation. It is a zinc metalloprotein that has effects on translation. The λ-2 protein is important in particle assembly and μ-1 in cell penetration.

The inner capsid is composed of four proteins: λ-3, λ-1, μ-2, and σ-2. The λ-3 protein is an RNA-dependent RNA polymerase. The λ-1 protein is a zinc metalloprotein that binds RNA. The μ-2 protein binds RNA, and σ-2 binds double-stranded RNA.

The four nonstructural proteins are μ-NS, μ-NSC, σ-1s, and σ-NS. The μ-NS protein is a core-binding protein, and σ-NS binds single-stranded RNA. The roles of μ-NSC and σ-1s are unknown.

Reoviruses are moderately heat-stable. The half-life at 56° C (132.8° F) of a type 3 strain is 1.6 minutes; at 37° C (98.6° F), it is approximately 2.5 hours. Reoviruses are inactivated by visible light in the presence of heterocyclic dyes. They are inactivated by ultraviolet light but are more resistant to this treatment than are RNA viruses with single-stranded nucleic acid. Reoviruses are stable through a wide pH range and also as aerosols, particularly when the relative humidity is high. They are relatively resistant to 3 percent formaldehyde solution, 1 percent hydrogen peroxide, and 1 percent phenol but are inactivated completely by 70 percent ethyl alcohol at room temperature for 1 hour. Brief exposure to 70 percent ethanol is ineffective in disinfecting reoviruses, but brief

*References 18, 31, 35, 44, 79, 80, 112.
†References 11, 59, 75, 76, 103, 105.

exposure to 95 percent ethanol or sodium hypochlorite is effective.

Reoviruses replicate with a cytopathic effect in a large number of tissue culture systems of both primate and other animal origins. For recovery from clinical material, monkey kidney tissue culture is satisfactory. Cytopathic effect is enhanced in rolled in contrast to stationary cultures.[43] Infected cells develop characteristic cytoplasmic inclusions that contain double-stranded RNA, virus-specific proteins, and complete and incomplete viral particles. All three serotypes agglutinate human erythrocytes, and this virus-cell interaction is stable in a wide temperature and pH range.

Of all viruses that naturally infect humans, reoviruses have the broadest host range. They have been recovered from natural infections in cattle, chimpanzees, monkeys, mice, dogs, turkeys, sheep, pigs, chickens, and cats.* In addition, hemagglutination-inhibiting and neutralizing antibodies to one or more of the three reoviral serotypes have been found in the serum of rabbits, horses, trout, guinea pigs, antelopes, zebras, warthogs, bats, wallabies, quokkas, kangaroos, and several genera of New World monkeys.†

Because of their widespread prevalence in nature and the ease of causing infection in laboratory animals, reoviruses have been used widely in pathogenicity studies. The following unique illnesses have been observed: diabetes mellitus in mice; hydrocephalus in hamsters, ferrets, rats, and mice; encephalitis in mice; chronic infection with runting in mice; myocarditis in mice; chronic obstructive jaundice associated with choledochal obliteration in mice; and lymphomas in mice with chronic infection.‡

EPIDEMIOLOGY

The serologic data presented in the preceding section of this chapter demonstrate that reoviruses are prevalent infectious agents in the animal world. Similarly, surveys of sera collected from humans indicate worldwide human infection.§ The occurrence in nature of identical viruses in many different animals and humans leads to consideration of possible transmission from species to species. At present, this transmission has not been demonstrated. However, Chua and associates[8-10] present data suggesting that human reoviruses associated with respiratory infections were probably of bat origin. In addition, Ouattara and colleagues[64] have noted a reovirus type 2 swine reovirus reassortant associated with encephalitis.

Reoviruses are recovered frequently from sewage.¶ In San Diego, reoviruses have been recovered more consistently than has any other virus throughout the year from sewage.[17] This finding suggests that reoviral infection is endemic in humans in the San Diego area.

*References 14, 26, 28, 49, 51, 52, 77, 82, 108, 109.
†References 57, 73, 74, 83, 92, 94.
‡References 32, 38, 47, 48, 62, 63, 67, 88, 93, 95, 104.
§References 4-6, 22, 36, 42, 44, 45, 66, 81, 85, 99, 102.
¶References 19, 34, 40, 46, 50, 61.

TABLE 170-1 **Hemagglutination-Inhibiting (H1) Antibodies to Reovirus Type 2 in 253 Serum Specimens Collected in Boston, Massachusetts, 1959 to 1962**

Age Group	Percentage with H1 Titer ≥1:20
Premature newborn	68
0-6 mo	25
7-12 mo	9
13-24 mo	27
2-5 yr	37
6-10 yr	52
11-20 yr	54
21-40 yr	34
41-60 yr	83
>60 yr	73

From Lerner AM, Cherry JD, Klein JO, et al. Infections with reoviruses. *N Engl J Med* 1962;**267**:947-952. Reprinted with permission from *The New England Journal of Medicine*.

The type 2 hemagglutination-inhibiting antibody prevalence pattern by age in sera collected in Boston from 1959 to 1962 is presented in Table 170-1. Antibody is transmitted transplacentally to the newborn. During the first year of life, this acquired antibody wanes, after which antibody prevalence increases with increasing age. Approximately 50 percent of school-aged children have hemagglutination-inhibiting antibody titers to type 2 reovirus of 1:20 or greater; approximately 80 percent of adults have similar titers. Two more recent studies have shown a similar prevalence of antibody to reovirus type 3.[87,98]

The method of transmission of reoviruses is unknown. However, because they are recovered most frequently from the feces, the primary spread probably is by the fecal-oral route, similar to that of enteroviruses. Because the reoviruses are stable in aerosols and because respiratory illness has been associated with reovirus infections, the respiratory route is an additional possibility.

CLINICAL MANIFESTATIONS

The role of reoviral infections in human disease is far from clear. In most instances, virologic or serologic evidence of reoviral infection has been a sporadic finding in human illness, so that cause and effect are difficult to establish. However, in volunteer studies in young adults, infection resulted in clinical illness.[31,78]

The prevalence of antibody to all three reoviral types in humans and the frequency of reovirus isolation from human sewage indicate that human infection is a common occurrence, but the relative paucity of virus recovered during studies of community disease suggests that most infections are inapparent or associated with trivial illness.

Upper Respiratory Tract Illness

In the winter of 1957, Rosen and colleagues[79] noted an outbreak of infection with reovirus type 1 in nursery children in a welfare institution. Illness was noted in

16/22 infected children and was characterized by low-grade fever (rectal temperatures from 38.1° to 38.6°C [100.6°F to 101.5°F]), rhinorrhea, and pharyngitis. The average duration of fever was 2.2 days; in 9 children, the duration was only 1 day. Three children had diarrhea, and 3 had mild otitis media. In another study conducted at the same institution during the winter of 1955 to 1956, 4 children with reovirus type 3 infection and illness were noted.[80] One child had a temperature of 38.9°C (102°F), coryza, and tonsillitis; another child had fever (temperature of 38.2°C [100.8°F]), cough, and diarrhea; and 2 children had only coryza. During another reovirus type 3 outbreak in the fall of 1957, all 6 infected infants had symptoms. Five children had mild fever, 5 had coryza, and 4 had diarrhea. Pharyngitis was not observed in any of the infected children.

Other sporadic instances of similar mild upper respiratory tract illnesses have been described.[15,27,31,90,91] In volunteer trials in young adults, reovirus type 1 infection was associated with malaise, rhinorrhea, cough, sneezing, pharyngitis, and headache in some subjects in one study,[78] and coldlike illness was observed in 37 percent of subjects in another trial.[31] In both volunteer studies and natural infection, mild diarrhea occurred with the upper respiratory tract illness.

In three separate investigations, Chua and coworkers[8-10] noted acute influenza-like illnesses in four adult patients. The reoviruses in these four cases may have been of bat origin, and in one instance evidence of human-to-human transmission was found.

Pneumonia

Tillotson and Lerner[101] described a 5-year-old girl who had extensive pneumonia and died after 15 days of illness. This child initially had fever, cough, rhinorrhea, and a generalized maculopapular rash. When admitted to the hospital on the 10th day of illness, the child was cyanotic and in marked respiratory distress; rash was no longer present, but mild pharyngitis and conjunctivitis were. A chest radiograph revealed diffuse confluent pneumonia, and reovirus type 3 was recovered from the lungs, adrenals, liver, spleen, kidney, a lymph node, heart, brain, and blood.

Joske and associates[35] described a 10-month-old girl who died after having a respiratory illness of 4 days' duration. A reovirus type 1 was recovered from the stool and brain of this child, and postmortem study revealed interstitial pneumonia, myocarditis, hepatitis, and encephalitis. El-Rai and Evans[18] reported the case of an 18-year-old man who had fever (temperature of 39.4°C [103°F]), nausea, vomiting, cough, and patchy pneumonia. He had serologic evidence of infection with reovirus type 1. Pneumonia has been noted in another child with reovirus type 3 infection.[90]

Gastrointestinal Manifestations

Mild diarrhea has been noted both in association with upper respiratory tract illness and as an isolated event.[70,78-80,82] Because reovirus type 3 consistently produces steatorrhea in mice, this clinical manifestation has been sought in illnesses of children and noted in six.[34,84] Three patients with hepatitis and encephalitis have been described.[35] Zalan and associates[112] have noted two patients in whom abdominal pain and cramps were prominent.

In 1980, Bangaru and associates[1] reported the similarity of induced hepatobiliary injury caused by reovirus type 3 infection in mice and biliary atresia in human infants. Subsequent to this observation, Glaser and associates[23] and Morecki and colleagues[55,56] looked for an association between reovirus type 3 infection and biliary atresia in humans. In their first report, they found that 17/25 patients (68%) with biliary atresia had antibodies (indirect immunofluorescent antibody technique) to reovirus type 3, whereas similar antibodies were recovered in only 3/37 control sera.[55] In a second study, they found that 62 percent of babies with extrahepatic biliary atresia and 52 percent of infants with idiopathic neonatal hepatitis had antibodies to reovirus type 3; only 12 percent of control children had similar antibodies.[23] In an ultrastructural and immunocytochemical study, they found evidence of reovirus type 3 in the porta hepatis of an infant with extrahepatic biliary atresia.[56]

Using similar serologic techniques, Dussaix and associates[16] were unable to find any relationship between reovirus type 3 antibody and either biliary atresia or neonatal hepatitis. They found reovirus type 3 antibody in sera from 45 percent of infants with biliary atresia, 50 percent of infants with neonatal hepatitis, and 50 percent of control infants. Minuk and colleagues[54] found no association between reovirus type 3 infection and idiopathic cholestatic liver disease in adults. Brown and colleagues[7] reported a relatively large study of reovirus type 3 infection and extrahepatic biliary atresia and neonatal hepatitis. They interpreted their data as demonstrating no correlation between the virus and the illnesses studied. However, the geometric mean antibody value in the combined biliary atresia and neonatal hepatitis groups was significantly higher than that of the control group. Richardson and associates[72] reported a study in which they examined the percentage of immunoglobulin (Ig)G, IgA, and IgM serum antibodies to reovirus type 3 in 40 infants with extrahepatic biliary atresia, 59 infants with neonatal hepatitis, 61 infants with cholestatic liver disease with causes other than extrahepatic biliary atresia or neonatal hepatitis, and 138 control infants with no liver disease. They found no difference in the prevalence of IgG and IgA antibodies between the groups with liver disease and the control subjects. They did, however, note a greater prevalence of IgM antibody in each of the groups with liver disease in contrast to the rate in the control group. However, this increased prevalence of IgM antibody could be the result of false-positive titers as a result of the liver disease rather than evidence of recent or ongoing infection. Steele and associates,[96] using a reverse transcriptase–mediated polymerase chain reaction (RT-PCR), found no evidence of reovirus type 3 in preserved tissues from infants with cholestatic liver disease. Saito and associates,[83] using RT-PCR, could find no amplification product in tissue or stool specimens from patients with biliary atresia, infantile obstructive cholangiopathy, or congenital dilation of the bile duct.

In 2009, Rauschenfels and colleagues[71] did an extensive study on the incidence of hepatotropic viruses in

biliary atresia. They obtained liver biopsies from 64 infants with biliary atresia at the time of the Kasai procedure. They identified reovirus type 3 RNA by RT-PCR in 21 (33%) biopsies. They noted that the 21 patients who were reovirus-positive were older (mean age, 60 days) than those who were virus-negative (mean age, 54 days). This finding led them to suggest that the viral infection was a secondary phenomenon and not the cause of the biliary atresia.

Exanthem

Exanthem has been a common manifestation of clinically apparent reoviral infections.[18,35,44,101] Lerner and associates[44] noted exanthem in six of seven children infected with reovirus type 2. Predominant symptoms in these patients included fever, malaise, anorexia, and pharyngitis. Two children had adenopathy, and one child had diarrhea. The rash was maculopapular in five patients and vesicular in one child. One child had a measleslike illness with photophobia, conjunctivitis, cervical lymphadenopathy, and a confluent maculopapular rash that lasted approximately 1 week.

Exanthem has been noted in a 5-year-old girl with pneumonia and type 3 reovirus infection, a 28-month-old girl with encephalitis and type 2 infection, and an 18-year-old adolescent male with pharyngitis and cervical and posterior occipital lymph node enlargement and type 2 infection.[18,35,101]

Neurologic Disease

Joske and colleagues[35] described three cases of hepatitis-encephalitis syndrome with reoviral infections. All of these cases had abnormal liver function test results and clinical and laboratory evidence of meningeal and cerebral involvement. One child died, one had mild neurologic residua at the 6-week follow-up, and one recovered without difficulty. Two patients were infected with reovirus type 2 and one with reovirus type 3.

El-Rai and Evans[18] found serologic evidence of reovirus type 2 infection in two children with aseptic meningitis, and Zalan and colleagues[112] described two children with reovirus type 2 infections associated with leg weakness and pain. Johansson and colleagues[33] noted a 3-month-old girl with meningitis, diarrhea, vomiting, and fever. Reovirus type 1 was isolated from this child's cerebrospinal fluid (CSF), and the child had a fourfold rise in neutralizing antibody to the isolated virus. Krainer and Aronson[40] described a 29-year-old woman who died of disseminated demyelinating encephalomyelitis in which a reovirus was recovered from the CSF and brain. Tyler and associates[106] isolated a type 3 reovirus from the CSF of a 6.5-week-old child with meningitis. This child recovered without obvious neurologic sequelae.

Ouattara and associates[64] reported a 6-year-old boy and his 2-month-old girl cousin with acute encephalitis associated with a reovirus type 2 infection. The boy had a febrile prodrome and then vomiting and impaired consciousness. On examination, he had a palsy of the left facial nerve. His CSF had a mildly elevated protein without pleocytosis. Magnetic resonance imaging (MRI) showed multiple symmetric lesions. He recovered completely in a few weeks. The cousin had fever, asthenia, and rhinorrhea. She also had a mildly elevated CSF protein and MRI findings similar to those of her older cousin. At hospital discharge at 18 days, she had marked hypotonia and the absence of language. At 1 year follow-up, she had uncoordinated movements. A similar virus was isolated from the urine of both children. This virus was thought to be a reassortant between human and swine strains. Krainer and Aronson reported a 29-year-old woman who died of a disseminated demyelinating encephalomyelitis in which a reovirus was recovered from the cerebrospinal fluid and brain.[41]

Other Manifestations

A 25-year-old man with Hodgkin disease had persistent reovirus type 1 viruria for a 5-week period, but no associated clinical illness was demonstrated.[17] Reoviruses have been recovered from biopsy material from patients with Burkitt lymphoma.[2,3] One child with hemorrhagic bullous myringitis was infected with reovirus type 2.[112] Terheggen and colleagues[100] noted a 28-year-old man with mild myocarditis from whom a reovirus was isolated from the stool sample.

REOVIRUSES AS POTENTIAL ANTI-CANCER AGENTS

Studies during the present decade indicate the potential of reoviruses to selectively destroy many different types of neoplastic cells.* Many malignant cells have an activated Ras pathway that results in a deficient ability to mount an antireovirus response that is mediated by the cellular protein PKR. Organ culture and animal model systems have shown promising results with reovirus type 3 in numerous different malignant neoplasms. At present, many phase I and phase II trials are in progress.[25,65,107]

DIAGNOSIS

Because no specific clinical features suggest reoviral infection, virologic and serologic studies are necessary for establishing the diagnosis. Reoviruses can be recovered from clinical material in primary monkey kidney tissue culture.[76] Care must be taken in interpreting results, however, because reoviruses can be contaminants of monkey tissue cultures. Cytopathic effect in tissue culture is enhanced by rolling during incubation.[43] Identification of virus is made by neutralization; the distinctive cytopathic effect should be helpful in selecting strains for study with reovirus antisera. In research laboratories, reoviruses also can be detected by molecular techniques such as in situ and dot-blot hybridization and PCR.[71,104] Paired serum specimens can be examined for antibody titer rise to reoviruses by neutralization, indirect immunofluorescent antibody technique, enzyme-linked immunosorbent assay (ELISA), or hemagglutination inhibition.

*References 10, 17, 19, 23, 24, 28, 29, 36, 38, 52, 53, 89, 106, 110, 111.

Recent infection can also be identified by the demonstration of specific IgM antibody by ELISA.

NEW REFERENCES SINCE THE SIXTH EDITION

8. Chua KB, Crameri G, Hyatt A, et al. A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. *Proc Natl Acad Sci U S A* 2007;**104**:11424–9.
9. Chua KB, Voon K, Crameri G, et al. Identification and characterization of a new orthoreovirus from patients with acute respiratory infections. *PLoS One* 2008;**3**:e3803.
10. Chua KB, Voon K, Yu M, et al. Investigation of a potential zoonotic transmission of orthoreovirus associated with acute influenza-like illness in an adult patient. *PLoS One* 2011;**6**:e25434.
25. Harrington KJ, Vile RG, Melcher A, et al. Clinical trials with oncolytic reovirus: moving beyond phase I into combinations with standard therapeutics. *Cytokine Growth Factor Rev* 2010;**21**:91–8.
30. Ilett EJ, Barcena M, Errington-Mais F, et al. Internalization of oncolytic reovirus by human dendritic cell carriers protects the virus from neutralization. *Clin Cancer Res* 2011;**17**:2767–76.
37. Kapadia R, Coffey MC. The use of immunohistochemistry to determine oncolytic reovirus distribution and replication in-human tumors. *Methods* 2010;**52**:301–6.
39. Kim M, Williamson CT, Prudhomme J, et al. The viral tropism of two distinct oncolytic viruses, reovirus and myxoma virus, is modulated by cellular tumor suppressor gene status. *Oncogene* 2010;**29**:3990–6.
58. Murray PR, Rosenthal KS, Pfaller MA. Reoviruses. In: Schmitt W, DeFrancesco K, editor. *Medical Microbiology*. 6th ed. Mosby Elsevier; 2009. p. 601–8.
64. Ouattara LA, Barin F, Barthez MA, et al. Novel human reovirus isolated from children with acute necrotizing encephalopathy. *Emerg Infect Dis* 2011;**17**:1436–44.
71. Rauschenfels S, Krassmann M, Al-Masri AN, et al. Incidence of hepatotropic viruses in biliary atresia. *Eur J Pediatr* 2009;**168**:469–76.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 171

Orbiviruses, Coltiviruses, and Seadornaviruses

Theodore F. Tsai

Viruses in the genera *Orbivirus*, *Coltivirus*, and *Seadornavirus* differ from others in the family *Reoviridae* structurally, in physicochemical properties, and by their arthropod mode of transmission.[2,3] More than 100 orbiviruses are classified into serogroups by relationships in complement fixation, agar gel precipitation, and immunofluorescent assays. Additional serocomplex relationships are recognized, whereas individual viruses are differentiated by neutralization tests. Orbiviruses are principally animal pathogens (e.g., the bluetongue viruses); only viruses in the Kemerovo, Orungo, Lebombo, and Changuinola serogroups have been shown to cause human illness. Colorado tick fever (CTF) virus is the only *Coltivirus* recognized to cause human illness, and among the seadnornaviruses, only Banna virus is a confirmed human pathogen.[2,3]

The orbiviruses are spherical nonenveloped viruses with two protein shells: an outer shell (capsid) approximately 86 nm in diameter and an inner shell (core) 69 nm in diameter. The inner core VP7 protein, arranged in trimers, contains group-reactive antigens, and the outer capsid VP2 or VP5 proteins bear serotype-specific antigens. The viruses are acid labile, and some exhibit sensitivity to lipid solvents. The double-stranded RNA viral genome comprises 10 segments associated with viral structural and nonstructural proteins. Their complete nucleotide sequences and coding assignments have been determined for a bluetongue virus, and partial information is available for others. In general, reassortments of genomic segments among viruses within serogroups are viable, but not between groups, thus validating the broad serogroup classification. The genetic relationships (e.g., RNA hybridization) among viruses within serogroups diverge from the antigenic relationships in some instances, which provides an additional basis for taxonomic classification.

The coltiviruses and seadornaviruses resemble the orbiviruses in size and in having two capsids but possess genomes organized into 12 RNA segments.[2] Seadornaviruses' morphologic features and structural protein similarities with rotaviruses suggest an evolutionary relationship.[36]

REFERENCES

1. Bangaru B, Morecki R, Glaser JH, et al. Comparative studies of biliary atresia in the human newborn and reovirus-induced cholangitis in weanling mice. *Lab Invest* 1980;**43**:456–62.
2. Bell TM, Massie A, Ross MG, et al. Isolation of a reovirus from a case of Burkitt's Lymphoma. *Br Med J* 1964;**1**:1212–13.
3. Bell TM, Massie A, Ross MG, et al. Further isolations of reovirus type 3 from cases of Burkitt's lymphoma. *Br Med J* 1966;**1**: 1514–17.
4. Berger RH, Brody JA. Reovirus antibody patterns in Alaska. *Am J Epidemiol* 1967;**86**:724–35.
5. Bricout F, Regnard J, Duval J. [Pathogenicity and diffusion of reoviruses]. *Ann Pediatr (Paris)* 1965;**12**:43–8.
6. Brown PK, Taylor-Robinson D. Respiratory virus antibodies in sera of persons living in isolated communities. *Bull World Health Organ* 1966;**34**:895–900.
7. Brown WR, Sokol RJ, Levin MJ, et al. Lack of correlation between infection with reovirus 3 and extrahepatic biliary atresia or neonatal hepatitis. *J Pediatr* 1988;**113**:670–6.
8. Chua KB, Crameri G, Hyatt A, et al. A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans. *Proc Natl Acad Sci U S A* 2007;**104**:11424–9.
9. Chua KB, Voon K, Crameri G, et al. Identification and characterization of a new orthoreovirus from patients with acute respiratory infections. *PLoS One* 2008;**3**:e3803.
10. Chua KB, Voon K, Yu M, et al. Investigation of a potential zoonotic transmission of orthoreovirus associated with acute influenza-like illness in an adult patient. *PLoS One* 2011;**6**:e25434.
11. Cohen JA, Williams WV, Weiner DB, et al. Reoviruses. In: von Regenmortel MHV, Neurath AR, editors. *Immunochemistry of Viruses*. Vol. 2. The Basis for Serodiagnosis and Vaccines. New York: Elsevier Science; 1990. p. 381–401.
12. Committee on the Enteroviruses. Viruses. *Science* 1955;**122**: 1187–8.
13. Committee on the Enteroviruses. The enteroviruses. *Am J Public Health* 1957;**47**:1556–66.
14. Csiza CK. Characterization and serotyping of three feline reovirus isolates. *Infect Immun* 1974;**9**:159–66.
15. De Lavergne E, Olive D, Lemoyne MT. [The reoviruses: difficulties in demonstrating their pathogenic capacity in man]. *Presse Med* 1965;**73**:951–6.
16. Dussaix E, Hadchouel M, Tardieu M, et al. Biliary atresia and reovirus type 3 infection. *N Engl J Med* 1984;**310**:658.
17. Edmonson JH, Millian SJ, Goodenow M, et al. Persistent viruria due to reovirus in a patient treated for Hodgkin's disease in a protected environment. *J Infect Dis* 1970;**121**:438–41.
18. El-Rai FM, Evans AS. Reovirus infections in children and young adults. *Arch Environ Health* 1963;**7**:700–4.
19. England B. Concentration of reovirus and adenovirus from sewage and effluents by protamine sulfate (salmine) treatment. *Appl Microbiol* 1972;**24**:510–12.
20. Fenner F. Classification and nomenclature of viruses: second report of the International Committee on Taxonomy of Viruses. *Intervirology* 1976;**7**:34.
21. Forrest JC, Dermody TS. Reovirus receptors and pathogenesis. *J Virol* 2003;**77**:9109–15.
22. George S, John TJ. Reovirus antibodies in human sera in South India. *Indian J Med Res* 1970;**58**:1680–5.
23. Glaser JH, Balistreri WF, Morecki R. Role of reovirus type 3 in persistent infantile cholestasis. *J Pediatr* 1984;**105**:912–15.
24. Hanel EG, Xiao Z, Wong KK, et al. A novel intravesical therapy for superficial bladder cancer in an orthotopic model: oncolytic reovirus therapy. *J Urol* 2004;**172**:2018–22.
25. Harrington KJ, Vile RG, Melcher A, et al. Clinical trials with oncolytic reovirus: moving beyond phase I into combinations with standard therapeutics. *Cytokine Growth Factor Rev* 2010;**21**: 91–8.
26. Hartley JW, Rowe WP, Huebner RJ. Recovery of reoviruses from wild and laboratory mice. *Proc Soc Exp Biol Med* 1961;**108**: 390–5.
27. Hilleman MR, Hamparian VV, Ketler A, et al. Acute respiratory illnesses among children and adults: field study of contemporary importance of several viruses and appraisal of the literature. *JAMA* 1962;**180**:445–53.
28. Hull RN, Minner JR, Smith JW. New viral agents recovered from tissue cultures of monkey kidney cells. I. Origin and properties of cytopathogenic agents S.V.1, S.V.2, S.V.4, S.V.5, S.V.6, S.V.11, S.V.12 and S.V.15. *Am J Hyg* 1956;**63**:204–15.
29. Ikeda Y, Nishimura G, Yanoma S, et al. Reovirus oncolysis in human head and neck squamous carcinoma cells. *Auris Nasus Larynx* 2005;**31**:407–12.
30. Ilett EJ, Barcena M, Errington-Mais F, et al. Internalization of oncolytic reovirus by human dendritic cell carriers protects the virus from neutralization. *Clin Cancer Res* 2011;**17**:2767–76.
31. Jackson GG, Muldoon RL, Cooper GS. Reovirus type 1 as an etiologic agent of the common cold. *J Clin Invest* 1961;**40**: 1051.
32. Jenson AB, Rabin ER, Phillips CA, et al. Reovirus encephalitis in newborn mice: an electron microscopic and virus assay study. *Am J Pathol* 1965;**47**:223–39.
33. Johansson PJ, Sveger T, Ahlfors K, et al. Reovirus type 1 associated with meningitis. *Scand J Infect Dis* 1996;**28**:117–20.
34. Jopkiewics TK, Krzeminska K, Stachowska Z. Virologic survey of sewage in the city of Bydgoszcz [English summary]. *Przegl Epidemiol* 1968;**22**:521–7.
35. Joske RA, Keall DD, Leak PJ, et al. Hepatitis-encephalitis in humans with reovirus infection. *Arch Intern Med* 1964;**113**: 811–16.
36. Kalra SL, Nair E, Nair CMG. Reovirus antibodies in Delhi. *Indian J Med Res* 1969;**57**:1–4.
37. Kapadia R, Coffey MC. The use of immunohistochemistry to determine oncolytic reovirus distribution and replication in-human tumors. *Methods* 2010;**52**:301–6.
38. Kilham L, Margolis G. Hydrocephalus in hamsters, ferrets, rats, and mice following inoculations with reovirus type I. I. Virologic studies. *Lab Invest* 1969;**21**:183–8.
39. Kim M, Williamson CT, Prudhomme J, et al. The viral tropism of two distinct oncolytic viruses, reovirus and myxoma virus, is modulated by cellular tumor suppressor gene status. *Oncogene* 2010;**29**:3990–6.
40. Knocke KW, Pittler H, Hopken W. [Demonstration of reoviruses in sewage]. *Zentralbl Bakteriol Orig* 1967;**203**:417–21.
41. Krainer L, Aronson BE. Disseminated encephalomyelitis in humans with recovery of hepato-encephalitis virus (HEV). *J Neuropathol Exp Neurol* 1969;**18**:339–42.
42. Leers WD, Rozee KR. A survey of reovirus antibodies in sera of urban children. *Can Med Assoc J* 1966;**94**:1040–2.
43. Lerner AM, Cherry JD, Finland M. Enhancement of cytopathic effects of reoviruses in rolled cultures of rhesus kidney. *Proc Soc Exp Biol Med* 1962;**110**:727–9.
44. Lerner AM, Cherry JD, Klein JO, et al. Infections with reoviruses. *N Engl J Med* 1962;**267**:947–52.
45. Levy JA, Tanabe E, Curnen EC. Occurrence of reovirus antibodies in health African children and in children with Burkitt's lymphoma. *Cancer* 1968;**21**:53–7.
46. Malherbe HH, Strickland-Cholmley M. Quantitative studies on viral survival in sewage purification processes. In: Berg G, editor. *Transmission of Viruses by the Water Route*. New York: John Wiley & Sons; 1967. p. 379–87.
47. Maratos-Flier E. Reovirus and endocrine cells. *Curr Top Microbiol Immunol* 1998;**233**:85–91.
48. Margolis G, Kilham L. Hydrocephalus in hamsters, ferrets, rats, and mice following inoculations with reovirus type I. II. Pathologic studies. *Lab Invest* 1969;**21**:189–98.
49. Massie EL, Shaw ED. Reovirus type 1 in laboratory dogs. *Am J Vet Res* 1966;**27**:783–7.
50. Matsuura K, Ishikura M, Nakayama T, et al. Ecological studies on reovirus pollution of rivers in Toyama Prefecture. II. Molecular epidemiological study of reoviruses isolated from river water. *Microbiol Immunol* 1993;**37**:305–10.
51. McFerran JB, Connor T. A reovirus isolated from a pig. *Res Vet Sci* 1970;**11**:388–90.
52. McFerran JB, Nelson R, Clarke JK. Isolation and characterization of reoviruses isolated from sheep. *Arch Gesamte Virusforsch* 1973; **40**:72–81.
53. Mechor B, Seikaly H, Wong K, et al. Reovirus salvage of positive resection margin: a novel treatment adjunct. *J Otolaryngol* 2006; **35**:97–101.

54. Minuk GY, Paul RW, Lee PW. The prevalence of antibodies to reovirus type 3 in adults with idiopathic cholestatic liver disease. *J Med Virol* 1985;**16**:55–60.
55. Morecki R, Glaser JH, Cho S, et al. Biliary atresia and reovirus type 3 infection. *N Engl J Med* 1982;**307**:481–4.
56. Morecki R, Glaser JH, Johnson AB, et al. Detection of reovirus type 3 in the porta hepatis of an infant with extrahepatic biliary atresia: ultrastructural and immunocytochemical study. *Hepatology* 1984;**4**:1137–42.
57. Munz E, Reimann M, Ackermann E. [Seroepidemiologic studies on reovirus infections of man, domestic and wild animals in Tanzania (author's transl)]. *Acta Trop* 1979;**36**:277–88.
58. Murray PR, Rosenthal KS, Pfaller MA. Reoviruses. In: Schmitt W, DeFrancesco K, editor. *Medical Microbiology*. 6th ed. Mosby Elsevier; 2009. p. 601–8.
59. Nibert ML, Schiff LA. Reoviruses and their replication. *Virology* 2001;**2**:1679–728.
60. Nibert ML, Schiff LA. Reoviruses and their replication. In: Knipe DM, Howley PM, editors. *Fields' Virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1679–728.
61. Nupen EM. Virus studies on the Windhoek waste-water reclamation plant (southwest Africa). *Water Res* 1970;**4**:661–72.
62. Onodera T, Jenson AB, Yoon JW, et al. Virus-induced diabetes mellitus: reovirus infection of pancreatic beta cells in mice. *Science* 1978;**201**:529–31.
63. Organ EL, Rubin DH. Pathogenesis of reovirus gastrointestinal and hepatobiliary disease. *Curr Top Microbiol Immunol* 1998;**233**: 67–83.
64. Ouattara LA, Barin F, Barthez MA, et al. Novel human reovirus isolated from children with acute necrotizing encephalopathy. *Emerg Infect Dis* 2011;**17**:1436–44.
65. Overview R. *Oncolytics Biotech Inc*. http://www.oncolyticsbiotech.com/tech.html. Date accessed May 31, 2007.
66. Pal SR, Agarwal SC. Sero-epidemiological study of reovirus infection amongst the normal population of the Chandigarh area: Northern India. *J Hyg (Lond)* 1968;**66**:519–29.
67. Phillips PA, Keast D, Walters MN, et al. Murine lymphoma induced by reovirus 3. *Pathology* 1971;**3**:133–8.
68. amos-Alvarez M, Sabin AB. Characteristics of poliomyelitis and other enteric viruses recovered in tissue culture from healthy American children. *Proc Soc Exp Biol Med* 1954;**87**:655–61.
69. Ramos-Alvarez M, Sabin AB. Intestinal viral flora of healthy children demonstrable by monkey kidney tissue culture. *Am J Public Health Nations Health* 1956;**46**:295–9.
70. Ramos-Alvarez M, Sabin AB. Enteropathogenic viruses and bacteria; role in summer diarrheal diseases of infancy and early childhood. *JAMA* 1958;**167**:147–56.
71. Rauschenfels S, Krassmann M, Al-Masri AN, et al. Incidence of hepatotropic viruses in biliary atresia. *Eur J Pediatr* 2009;**168**: 469–76.
72. Richardson SC, Bishop RF, Smith AL. Reovirus serotype 3 infection in infants with extrahepatic biliary atresia or neonatal hepatitis. *J Gastroenterol Hepatol* 1994;**9**:264–8.
73. Rosen L. Serologic grouping of reoviruses by hemagglutination inhibition. *Am J Hyg* 1960;**71**:242–9.
74. Rosen L. Reoviruses in animals other than man. *Ann N Y Acad Sci* 1962;**101**:461–5.
75. Rosen L. Reoviruses. In: Wenner HA, Behbehani AM, Rosen L, editors. *Virology Monographs*. New York: Springer-Verlag; 1968. p. 74–107.
76. Rosen L. Reoviruses. In: Lennette EH, Schmidt NJ, editors. *Diagnostic Procedures for Viral and Rickettsial Infections*. 4th ed. New York: American Public Health Association; 1969. p. 354–63.
77. Rosen L, Abinanti FR, Hovis JF. Further observations on the natural infection of cattle with reoviruses. *Am J Hyg* 1963;**77**:38–48.
78. Rosen L, Evans HE, Spickard A. Reovirus infections in human volunteers. *Am J Hyg* 1963;**77**:29–37.
79. Rosen L, Hovis JF, Mastrota FM, et al. An outbreak of infection with a type 1 reovirus among children in an institution. *Am J Hyg* 1960;**71**:266–74.
80. Rosen L, Hovis JF, Mastrota FM, et al. Observations on a newly recognized virus (Abney) of the reovirus family. *Am J Hyg* 1960; **71**:258–65.
81. Ruiz-Gomez J, Faingezicht-Gutman I, Sosa-Martinez J. [Reovirus: investigation of antibodies in persons of various ages]. *Bol Med Hosp Infant Mex* 1965;**22**:359–63.
82. Sabin AB. The significance of viruses recovered from the intestinal tracts of healthy infants and children. *Ann N Y Acad Sci* 1956;**66**: 226–30.
83. Sabin AB. Reoviruses. A new group of respiratory and enteric viruses formerly classified as ECHO type 10 is described. *Science* 1959;**130**:1387–9.
84. Saito T, Shinozaki K, Matsunaga T, et al. Lack of evidence for reovirus infection in tissues from patients with biliary atresia and congenital dilatation of the bile duct. *J Hepatol* 2004;**40**: 203–11.
85. Schmidt J, Tauchnitz C, Kuhn O. [Investigations on the occurrence of hemagglutination inhibition antibodies against the reovirus types 1 and 2 in the population]. *Z Hyg Infektionskr* 1965;**150**: 269–79.
86. Scott TFM. Clinical syndromes associated with entero virus and reo virus infections. *Adv Virus Res* 1962;**8**:165–97.
87. Selb B, Weber B. A study of human reovirus IgG and IgA antibodies by ELISA and western blot. *J Virol Methods* 1994;**47**: 15–25.
88. Sherry B, Blum MA. Multiple viral core proteins are determinants of reovirus-induced acute myocarditis. *J Virol* 1994;**68**:8461–5.
89. Smakman N, van der Bilt JD, van den Wollenberg DJ, et al. Immunosuppression promotes reovirus therapy of colorectal liver metastases. *Cancer Gene Ther* 2006;**13**:815–18.
90. Stanley NF. Reovirus: a ubiquitous orphan. *Med J Aust* 1961; **48**(2):815–18.
91. Stanley NF. Reoviruses. *Br Med Bull* 1967;**23**:150–4.
92. Stanley NF, Leak PJ. The serologic epidemiology of reovirus infection with special reference to the Rottnest Island Quokka (*Setonix brachyurus*). *Am J Hyg* 1963;**78**:82–8.
93. Stanley NF, Dorman DC, Ponsford J. Studies on the pathogenesis of a hitherto undescribed virus (hepato-encephalomyelitis) producing unusual symptoms in suckling mice. *Aust J Exp Biol Med Sci* 1953;**31**:147–59.
94. Stanley NF, Leak PJ, Grieve GM, et al. The ecology and epidemiology of reovirus. *Aust J Exp Biol Med Sci* 1964;**42**:373–84.
95. Stanley NF, Leak PJ, Walters MN, et al. Murine infections with reovirus. II. The chronic disease following reovirus type 3 infection. *Br J Exp Pathol* 1964;**45**:142–9.
96. Steele MI, Marshall CM, Lloyd RE, et al. Reovirus 3 not detected by reverse transcriptase-mediated polymerase chain reaction analysis of preserved tissue from infants with cholestatic liver disease. *Hepatology* 1995;**21**:697–702.
97. Stehle T, Dermody TS. Structural similarities in the cellular receptors used by adenovirus and reovirus. *Viral Immunol* 2004; **17**:129–43.
98. Tai JH, Williams JV, Edwards KM, et al. Prevalence of reovirus-specific antibodies in young children in Nashville, Tennessee. *J Infect Dis* 2005;**191**:1221–4.
99. Taylor-Robinson D. Respiratory virus antibodies in human sera from different regions of the world. *Bull World Health Organ* 1965;**32**:833–47.
100. Terheggen F, Benedikz E, Frissen PH, et al. Myocarditis associated with reovirus infection. *Eur J Clin Microbiol Infect Dis* 2003;**22**:197–8.
101. Tillotson JR, Lerner AM. Reovirus type 3 associated with fatal pneumonia. *N Engl J Med* 1967;**276**:1060–3.
102. Toth M, Honty A. Age-incidence of haemagglutination-inhibiting antibodies to reovirus types 1, 2 and 3. *Acta Microbiol Acad Sci Hung* 1966;**13**:119–25.
103. Tyler KL. Mammalian reoviruses. *Virology* 2001;**2**:1729–45.
104. Tyler KL. Mammalian reoviruses. In: Knipe DM, Howley PM, editors. *Fields' Virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1729–45.
105. Tyler KL, Fields BN. Reoviruses. In: Fields BN, Knipe DM, Chanock RM, et al, editors. *Fields' Virology*. 2nd ed. New York: Raven Press; 1990. p. 1307–28.
106. Tyler KL, Barton ES, Ibach ML, et al. Isolation and molecular characterization of a novel type 3 reovirus from a child with meningitis. *J Infect Dis* 2004;**189**:1664–75.
107. Vidal L, Pandha H, Spicer J, et al. A phase I study of reolysin given intravenously to patients with advanced malignancies. *J Clin Oncol ASCO Ann Meet Proc* 2006;**24**:3064.
108. Walker ER, Friedman MH, Olson NO. Electron microscopic study of an avian reovirus that causes arthritis. *J Ultrastruct Res* 1972;**41**:67–79.

109. Wooley RE, Dees TA, Cromack AS, et al. Infectious enteritis of turkeys: characterization of two reoviruses isolated by sucrose density gradient centrifugation from turkeys with infectious enteritis. *Am J Vet Res* 1972;**33**:157–64.
110. Yang WQ, Lun X, Palmer CA, et al. Efficacy and safety evaluation of human reovirus type 3 in immunocompetent animals: racine and nonhuman primates. *Clin Cancer Res* 2004;**10**:8561–76.
111. Yang WQ, Senger DL, Lun XQ, et al. Reovirus as an experimental therapeutic for brain and leptomeningeal metastases from breast cancer. *Gene Ther* 2004;**11**:1579–89.
112. Zalan E, Leers WD, Labzoffsky NA. Occurrence of reovirus infection in Ontario. *Can Med Assoc J* 1962;**87**:714–15.

COLORADO TICK FEVER VIRUS

CTF is an acute tick-borne febrile illness caused by the eponymous *Coltivirus*.[5,13,18,19] Eyach virus, isolated from *Ixodes* ticks in France and Germany; S6-1403, isolated in California from a gray squirrel; and mosquito strains from Indonesia are related to but distinct from CTF virus and have not been confirmed to cause human illness.[2,28] RNA hybridization studies of CTF viral strains from a 33-year interval found minor heterogeneity, thus suggesting that a single gene pool has been maintained by mixing of viral strains and by constraints on viral replication within tick vectors and vertebrate hosts. A wide variety of small and large mammals are infected naturally with the virus, but clinical illness develops only in humans. Experimental infection of rhesus monkeys, hamsters, and mice produces hematologic changes similar to those occurring in human infections.[21]

Epidemiology

Cases occur sporadically in association with the habitats of the wood tick *Dermacentor andersoni*. Most cases occur in May and June, when adult ticks are most active, but infections from March to November have been reported. Infections are acquired principally in the western part of the United States and Canada in the known geographic distribution of the vector (Fig. 171-1).[8,11-13] In rare cases, CTF has occurred in persons who had not traveled to areas of known risk, such as those exposed to ticks brought home on the clothing of family members and, in one case, by transfusion.[7,35,41,45] The annual incidence of disease in the Rocky Mountain region, approximately 3/million residents, is declining, possibly a result of a larger elk population that has reduced habitats of small vertebrate hosts.[4]

CTF virus is maintained in a 2- to 3-year cycle among small mammals, principally rodents, and *D. andersoni* ticks (Fig. 171-2).[5,13,19] Once infected in the larval stage, ticks remain infected through the nymphal and adult stages (trans-stadial transmission). Larvae are infected by viremic rodents. After molting, they carry the virus through the winter, and as nymphs, they infect other rodents and renew the cycle of transmission the following spring. Humans become infected incidentally by the bite of infected adult ticks.

The least chipmunk *(Eutamias minimus)*, the golden-mantled ground squirrel *(Spermophilus lateralis)*, and the porcupine *(Erethizon dorsatum)* appear to be the primary hosts for larval, nymphal, and adult *D. andersoni;* secondary hosts include rock mice *(Peromyscus maniculatus)*, meadow voles *(Microtus pennsylvanicus)*, and pine squirrels *(Tamiasciurus hudsonicus)*.[6]

D. andersoni is found exclusively in the high plains and in mountainous terrain between 4000 and 10,000 feet in altitude in the geographic distribution previously mentioned. The specific microhabitats where infected ticks are most prevalent are south-facing slopes with open stands of ponderosa pine, moderate shrubs, and rocky surfaces that provide favorable habitats for the intermediate rodent hosts.[6,34] Both male and female adult ticks can transmit infection to humans, and the period of attachment required for transmission of the virus may be very brief.

Clinical Manifestations

A history of a tick bite or tick exposure is given by more than 90 percent of patients.[22] The incubation period usually is 3 to 4 days, with a range of 0 to 14 days after known exposure to a tick.[4,22] The onset typically is abrupt,

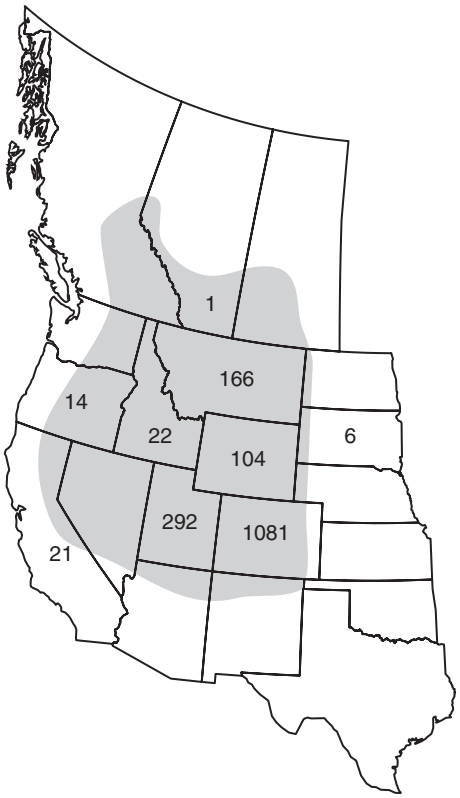

FIGURE 171-1 ■ Geographic distribution of *Dermacentor andersoni* ticks and reported cases of Colorado tick fever, 1980 to 1991.

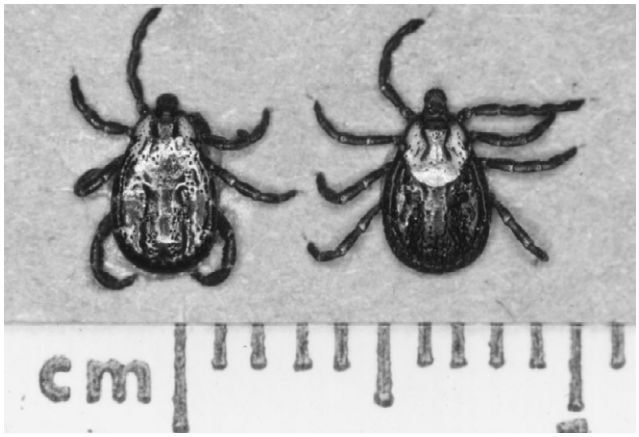

FIGURE 171-2 ■ Adult male *(left)* and female *(right) Dermacentor andersoni* ticks. Both can transmit Colorado tick fever virus.

with fever, chills, malaise, headache, retro-orbital pain, myalgia, lumbar pain, and hyperesthesia. Nausea, vomiting, and abdominal pain occur less frequently. Upper respiratory tract symptoms usually are absent, although conjunctival injection may develop. Lymphadenopathy and hepatosplenomegaly are found in some patients. Maculopapular and petechial eruptions have been observed in 5 to 12 percent of cases.[43]

The disease classically is diphasic; an initial attack lasting 2 to 3 days is followed by an equal interval of defervescence and a second and, rarely, a third recurrence. However, this saddleback pattern is absent in more than 50 percent of cases. Symptoms resolve several days after the second bout of fever, but prolonged asthenia lasting for several weeks is a typical finding, especially in adults.[22]

Leukopenia is a hallmark of the illness. The mean initial leukocyte count is 3900/mm^3, which diminishes to a nadir 5 to 6 days after the onset of illness, often during the period of remission. A left shift with an absolute neutropenia and relative lymphocytosis is a usual finding. Examination of bone marrow aspirates shows maturational arrest in the granulocytic series, with absent mature forms and numerous metamyelocytes and myelocytes. Megakaryocytes are depleted, and a reduced peripheral platelet count (<150,000/m^3) is found in most patients; however, thrombocytopenia rarely reaches a level of clinical significance.[26,33]

Uncomplicated recovery is the rule. Epididymo-orchitis, pneumonitis, hepatitis, and pericarditis have been reported as complications, but the most serious complications are encephalitis and hemorrhage,[15,20,22,24,32] which have been reported nearly exclusively in children younger than 10 years.[11,22,44] Three deaths have been reported, all in children who exhibited signs of generalized bleeding accompanied by a reduced platelet count.[22] Examination of the bone marrow in one child disclosed a generalized depression of myeloid and erythroid elements and a marked reduction in megakaryocytes. Central nervous system signs consistent with meningitis and encephalitis have been reported in both children and adults. Examination of cerebrospinal fluid disclosed elevated protein and mononuclear cell pleocytosis in the few cases that have been described.

D. andersoni is a vector for both CTF and Rocky Mountain spotted fever.[2] Although dual isolation of CTF virus and *Rickettsia rickettsii* from the same tick has not been reported, a concurrent rickettsial infection could not be discounted in all of the previously mentioned fatal cases attributed to CTF. Furthermore, a dual infection could develop in an individual exposed to more than one tick.

CTF virus is teratogenic in experimentally infected mice, but few clinical data are available on the teratogenic potential of the virus in humans.[23] One pregnant woman aborted 2 weeks after having an illness; and in an infant delivered 6 days after the onset of CTF in the mother, a febrile illness with leukopenia developed at 3 days of age, thus indicating a possible vertically acquired infection. In a single reported instance, a second attack of CTF has been documented, which indicates that immunity may not be permanent.[22]

The combined historical elements of tick exposure and outdoor activity in an enzootic area should suggest the diagnosis in patients with a spring-summer grippe-like illness. In a controlled study, abdominal pain, pharyngitis, and rash were less common symptoms in patients with CTF than in patients with other acute illnesses in the same season. Although Rocky Mountain spotted fever occurs rarely in the states in which CTF is prevalent, the possibility of this potentially fatal disease should be considered in the differential diagnosis. Helpful differentiating features include the rarity of a petechial rash in CTF and its biphasic course. Tick-borne relapsing fever also follows a remitting course, but it has a more acute and toxic onset; splenomegaly occurs commonly, and remission is by crisis. The geographic distribution of the diseases overlaps; however, human encounters with the argasid (soft) tick vectors of relapsing fever (*Ornithodoros* ticks) are likely to occur in cabins and other protected areas.

Eyach virus has been implicated in central nervous system infections and polyradiculitis in Europe. However, its tick-borne transmission from *Ixodes ricinus* that also transmits *Borrelia*, *Anaplasma*, and Kemerovo group viruses has prevented establishment of a clear interpretation.

Pathophysiology

Experimental infection of rhesus monkeys, rodents, and human marrow cells in vitro has shown that CTF virus infects the erythropoietic elements (erythroblasts to reticulocytes) at an early stage of infection.* After the infected cells mature and are released into the peripheral circulation, the virus persists intracellularly, where it has been identified by electron microscopy.[16,39] CTF virus has been cultured from circulating erythrocytes for up to 120 days after the onset of illness.[25] Red cell survival evidently is not shortened, and infected cells circulate in the presence of neutralizing antibody. Prolonged viremia has not been associated with either a protracted or a more severe course of illness. Infected CD34$^+$ progenitor cells and impaired mononuclear cell production of colony-stimulating factors may contribute to the acute aregenerative cytopenia.[40] High levels of serum interferon-γ correlate with fever during the acute phase of illness.[1]

Laboratory Diagnosis

Direct immunofluorescent examination of blood smears for intraerythrocytic viral antigen is an accessible and rapid approach to laboratory diagnosis.[14] However, polymerase chain reaction (PCR) analysis and viral culture of blood or serum samples are more sensitive.[27] Of all cases diagnosed by seroconversion, 96 percent are identified by isolation of virus from acute or convalescent blood samples in baby mice or Vero or BHK cells.[10,14] Freezing and thawing of the clot should be avoided. Virus has been isolated from a blood clot that had been refrigerated for

*References 14, 16, 21, 27, 39, 41.

14 months. Serologic diagnosis by demonstration of fourfold rises in neutralizing, complement-fixation, immunofluorescent, or enzyme-linked immunosorbent assay (ELISA) antibody is confirmatory. ELISAs that distinguish between CTF and Eyach virus antibodies are available. Neutralizing antibody rises slowly; only a third of patients had detectable neutralizing antibody 10 days after onset, but fourfold rises appeared within 30 days of onset in 92 percent.

Treatment

No specific therapy is available. Because thrombocytopenia may occur and hemorrhage is a reported complication in children, antipyretics that interfere with coagulation should not be used.

Prevention

Repellents containing permethrin should be sprayed on clothing, and repellents containing diethyltoluamide (DEET) or picaridin should be applied to exposed skin. Long pants should be tucked into socks and shirts tucked into slacks. Clothing, gear, and skin should be inspected frequently for attached ticks. Light-colored clothing facilitates such inspections. One case of human-to-human transmission by transfusion of infected blood has been reported.[7] Persons with documented CTF should be prohibited from donating blood until the often prolonged viremia has cleared.[41]

BANNA VIRUS

Banna virus, the type species of *Seadornavirus*, has been isolated from patients with febrile illness and encephalitis in China and is believed to be a major contributor to previously unexplained cases of viral encephalitis in that country and, possibly, within a broader region of East and Southeast Asia.[3,29,31,43] Although its transmission cycle has not been defined, a mosquito-borne–vertebrate cycle and possibly tickborne-vertebrate cycle have been surmised by its isolation from various mosquito and tick vectors and from pigs and other vertebrates that could act as intermediate hosts. A common ancestry and taxonomic link to rotaviruses have been indicated by similarities in the viruses' gene organization and certain gene segment sequences (e.g., for VP9), as well as their morphology.[36] Two geno/serotypes (A and B) have been defined with broadly overlapping geographic distributions. The virus has been isolated from far-northern China, Mongolia, southern China, Vietnam, and Indonesia and from *Culex tritaeniorhynchus*, *Culex pipiens pallens*, and other mosquito vectors that also transmit Japanese encephalitis virus, and, in one study, the virus was implicated as the cause of 14 percent of cases in a nominal Japanese encephalitis outbreak.[31,38] Clinical symptoms in confirmed cases have included fever, arthralgia, and encephalitis, but few details have been published. The potential public health impact of Banna virus infection could be substantial, but means to confirm a specific diagnosis are not widely available; broader application of real-time PCR assays that have been developed and field studies are needed to define the burden of disease and need for public health intervention. In addition, several related seadornaviruses that have been described in China (e.g., mosquito-borne Liao Ning virus) are suspected to cause human illness but currently remain orphan agents.

KEMEROVO AND RELATED VIRUSES

The Kemerovo serogroup comprises tick-borne Kemerovo, Tribec, and Lipovnik viruses, which may have descended from ancestral mosquitoborne orbiviruses.[9] Kemerovo virus was isolated from the cerebrospinal fluid of two patients with encephalitis and from ticks in the Kemerovo region of Russia; seroconversion was demonstrated in 10 other patients with meningoencephalitis with tick bites and in whom Russian spring-summer encephalitis was excluded.[8,30] The virus is transmitted in an *Ixodes persulcatus*–rodent cycle. Lipovnik virus has been reported in neurologic infections in the former Czechoslovakia, where flaviviral tick-borne encephalitis is endemic; one study showed seroconversion to Lipovnik virus in half of the patients with encephalitis who were studied, including some with suspected dual infection with tick-borne encephalitis virus.[30] This finding seems plausible because both viruses are transmitted by *I. ricinus* ticks, and multiple exposure to singly infected ticks or infection with a dually infected tick may be possible. In addition, serologic evidence of Lipovnik or Tribec infection was reported in patients with chronic polyradiculoneuritis; however, spirochetal infection was not ruled out in these cases. The increasing geographic range of *I. ricinus* ticks in Europe to more northerly latitudes and higher elevations, attributed to global warming, could lead to increased transmission of these orbiviruses, as has been observed with tick-borne encephalitis virus.

On the basis of serologic rises in patients with acute febrile illness diagnosed clinically as Rocky Mountain spotted fever, a Kemerovo-related virus is suspected to occur in the southwestern region of the United States. Patients with a history of a tick bite or tick exposure, whose sera were negative for *R. rickettsii*, demonstrated fourfold or greater changes in immunofluorescent titers to Lipovnik and Six Gun City viruses (Kemerovo group).[45] Rises in immunofluorescent antibody titers to 128 to 512 suggested recent infection with a Kemerovo-related virus, possibly a novel agent or related to rabbit syncytium virus, which is enzootic in the United States. The patients had acute febrile illnesses with myalgia, vomiting, and severe abdominal pain, along with leukopenia, thrombocytopenia, and anemia, similar to Rocky Mountain spotted fever. No agent has been isolated, but neither was the possibility of *Ehrlichia* infection excluded.

ORUNGO VIRUS

Orungo virus is unrelated antigenically to the other orbiviruses. Infection is prevalent in western, central, and eastern Africa and apparently is transmitted in a sylvatic

monkey, with an *Aedes* mosquito cycle similar to that of yellow fever. Human-to-human transmission by *Anopheles* mosquitoes is speculated to occur. The virus has been isolated from patients with fever and headache, and serologic evidence of infection has been reported in outbreaks of illness characterized by fever, headache, myalgia, nausea, and vomiting. Seroconversion has been observed in patients studied during yellow fever outbreaks, presumably because of concurrent transmission of the viruses. The virus also was isolated from the blood of a child with seizures and flaccid paralysis.[17,37]

LEBOMBO VIRUS

Lebombo virus is not grouped antigenically. The virus was isolated first from *Aedes circumluteolus* mosquitoes in South Africa. Subsequently, the virus was recovered from a Nigerian child with nonspecific febrile illness; the virus also was isolated from rodents and mosquitoes in Nigeria.[37]

CHANGUINOLA VIRUS

Changuinola virus belongs to an antigenic complex of 12 principally phlebotomine-borne orbiviruses. The virus is transmitted in Panama among forest mammals and *Phlebotomus* flies. Only one human case has been reported—a nonspecific febrile illness in a mosquito catcher.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Brackney MM, Marfin AA, Staples JE, et al. Epidemiology of Colorado tick fever in Montana, Utah, and Wyoming, 1995-2003. *Vector Borne Zoonotic Dis* 2010;**10**:381–5.
9. Dilcher M, Hasib L, Lechner M, et al. Genetic characterization of Tribec virus and Kemorovo virus, two tick-transmitted human-pathogenic Orbiviruses. *Virology* 2012;**423**:68–76.
31. Liu H, Li MH, Zhai YG, et al. Banna virus, China, 1987-2007. *Emerg Infect Dis J* 2010;**16**:514–17.
38. Nabeshima T, Nga PT, Guilermo P, et al. Isolation and molecular characterization of Banna virus from mosquitoes, Vietnam. *Emerg Infect Dis J* 2008;**14**:1276–9.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 172

Rotavirus

Mary Allen Staat • Monica Malone McNeal • David I. Bernstein

Before the introduction of rotavirus vaccines, rotaviruses were the single most important cause of severe infantile gastroenteritis worldwide.[349,455] In the United States, rotaviruses were estimated to cause 55,000 to 70,000 hospitalizations, 205,000 to 272,000 emergency department visits, and 20 to 60 deaths in young children annually before rotavirus vaccines were approved and universally recommended for use in infants.[80,196,233,285] The total health care system costs associated with rotavirus disease in the United States were estimated to be $319 million, and the total costs to society were $893 million.[516] On a world scale, rotaviruses were estimated to be responsible each year for 2 million hospitalizations and 352,000 to 592,000 deaths in children younger than 5 years.[349-351,455,460] For these reasons, rotaviruses were a high priority for vaccine development, leading to the introduction of two effective vaccines.[74]

Transmission of rotaviruses occurs by the fecal-oral route, providing a highly efficient mechanism for universal exposure that leads to infection of almost all children around the world by the age of 5 years despite socioeconomic and cultural differences. The symptoms associated with rotavirus disease are typically diarrhea and vomiting accompanied by fever, nausea, anorexia, cramping, and malaise that can be mild and of short duration or produce severe dehydration.[227,405,445] Severe disease occurs primarily in young children, most commonly in children younger than 24 months.[362,363,393,447] Treatment of disease is largely supportive and includes nonspecific methods, such as rehydration therapy for replacement of body fluids and electrolytes.[235]

Approximately 90 percent of children in both developed and developing countries experience a rotavirus infection by the time they reach 3 years of age.[393,481] Natural rotavirus infection provides protection especially against subsequent severe illnesses, but reinfection is common and can occur multiple times in young children in poorer socioeconomic settings.[38,85,123,290,481] Further,

REFERENCES

1. Ater JL, Overall JC, Yeh TJ, et al. Circulating interferon and clinical symptoms in Colorado tick fever. *J Infect Dis* 1985;**151**:966.
2. Attoui H, Mohd Jaafar F, de Micco P, de Lamballerie X. Coltiviruses and seadornaviruses in North America, Europe, and Asia. *Emerg Infect Dis* 2005;**11**:1673–9.
3. Attoui H, Billoir F, Biagini P, et al. Complete sequence determination and genetic analysis of Banna virus and Kadipiro virus: proposal for assignment to a new genus (Seadornavirus) within the family Reoviridae. *J Gen Virol* 2000;**81**:1507–15.
4. Brackney MM, Marfin AA, Staples JE, et al. Epidemiology of Colorado tick fever in Montana, Utah, and Wyoming, 1995-2003. *Vector-borne and Zoonotic Dis* 2010;**10**:381–5.
5. Burgdorfer W. Tick-borne diseases in the United States: Rocky Mountain spotted fever and Colorado tick fever: a review. *Acta Trop* 1977;**34**:103.
6. Carey AB, McLean RB, Maupin GO. The structure of a Colorado tick fever ecosystem. *Ecol Monogr* 1980;**50**:131–51.
7. Centers for Disease Control and Prevention. Transmission of Colorado tick fever virus by blood transfusion: Montana. *MMWR Morb Mortal Wkly Rep* 1975;**24**:422–7.
8. Chumakov MP, Karpovich LG, Sarmanova ES, et al. Report on the isolation from *Ixodes persulcatus* ticks and from patients in western Siberia of a virus differing from the agent of tick-borne encephalitis. *Arch Virol* 1993;**7**:82–3.
9. Dilcher M, Hasib L, Lechner M, et al. Genetic characterization of Tribec virus and Kemorovo virus, two tick-transmitted human-pathogenic Orbiviruses. *Virology* 2012;**423**:68–76.
10. Earnest MP, Breckinridge JC, Barr RJ, et al. Colorado tick fever: clinical and epidemiologic features and evaluation of diagnostic methods. *Rocky Mtn Med J* 1971;**68**:60–2.
11. Eklund CM, Kennedy RC, Casey M. Colorado tick fever. *Rocky M Med J* 1961;**58**:21–5.
12. Eklund CM, Kohls GM, Brennan JM. Distribution of Colorado tick fever and virus-carrying ticks. *JAMA* 1955;**157**:335–7.
13. Emmons RW. Ecology of Colorado tick fever. *Annu Rev Microbiol* 1988;**42**:49.
14. Emmons RW, Lennette EH. Immunofluorescent staining in the laboratory diagnosis of Colorado tick fever. *J Lab Clin Med* 1966;**68**:923–9.
15. Emmons RW, Schade HI. Colorado tick fever simulating acute myocardial infarction. *JAMA* 1972;**222**:87–8.
16. Emmons RW, Oshiro LS, Johnson HN, et al. Intra-erythrocytic location of Colorado tick fever virus. *J Gen Virol* 1972;**17**:185–95.
17. Familusi JB, Moore DL, Fomufod AK, et al. Virus isolates from children with febrile convulsions in Nigeria: a correlation study of clinical and laboratory observations. *Clin Pediatr (Phila)* 1972;**11**: 272–6.
18. Florio L, Mugrage ER, Stewart MO. Colorado tick fever. *Ann Intern Med* 1946;**25**:466–71.
19. Florio L, Stewart MO, Mugrage ER. The etiology of Colorado tick fever. *J Exp Med* 1946;**83**:1–10.
20. Fraser CH, Schiff DW. Colorado tick fever encephalitis. *Pediatrics* 1962;**29**:187–90.
21. Gerloff RK, Larson CL. Experimental infection of rhesus monkeys with Colorado tick fever virus. *Am J Pathol* 1959;**35**:1043–54.
22. Goodpasture HC, Poland JD, Francy DB, et al. Colorado tick fever: clinical, epidemiologic and laboratory aspects of 228 cases in Colorado in 1973-1974. *Ann Intern Med* 1978;**88**:303–10.
23. Harris RE, Morahan P, Coleman P. Teratogenic effects of Colorado tick fever virus in mice. *J Infect Dis* 1975;**131**:397–402.
24. Hierholzer WJ Jr, Barry DW. Colorado tick fever pericarditis. *JAMA* 1971;**217**:825.
25. Hughes LE, Casper EA, Clifford CM. Persistence of Colorado tick fever virus in red blood cells. *Am J Trop Med Hyg* 1974;**23**:530–2.
26. Johnson ES, Napoli VM, White WC. Colorado tick fever as a hematologic problem. *Am J Clin Pathol* 1960;**34**:118–24.
27. Lambert AJ, Kosoy O, Velez JO, et al. Detection of Colorado tick fever viral RNA in acute human serum samples by a quantitative real-time RT-PCR assay. *J Virol Methods* 2007;**140**:43–8.
28. Karabatsos N, Poland JD, Emmons RW, et al. Antigenic variants of Colorado tick fever virus. *J Virol* 1987;**68**:1463.
29. Li QP, Shei ST, Hua C, et al. First isolation of 8 strains of new orbivirus (Banna) from patients with innominate fever in Xinjiang. *Endemic Dis Bull* 1993;**7**:77–82.
30. Libikova H, Heinz F, Ujhazyova D, et al. Orbiviruses of the Kemerovo complex and neurological diseases. *Med Microbiol Immunol* 1978;**116**:255–63.
31. Liu H, Li MH, Zhai YG, et al. Banna virus, China, 1987-2007. *Emerg Infect Dis J* 2010;**16**:514–17.
32. Loge RV. Acute hepatitis associated with Colorado tick fever. *West J Med* 1985;**142**:91.
33. Markovitz A. Thrombocytopenia in Colorado tick fever. *Arch Intern Med* 1963;**111**:307–8.
34. McLean RG, Shriner RB, Pokorny KS, et al. The ecology of Colorado tick fever in Rocky Mountain National Park in 1974. III. Habitats supporting the virus. *Am J Trop Med Hyg* 1989;**40**:86.
35. Midoneck SR, Richard J, Murray HW. Colorado tick fever in a resident of New York City. *Arch Fam Med* 1994;**3**:731–2.
36. Mohd Jaafar F, Attoui H, Mertens PEPC, deMicco P, de Lamballerie X. Structural organization of an encephalitic human isolate of *Banna virus* (genus *Seadornavirus*, family *Reovridae*). *J Gen Virol* 2005;**66**:1147–57.
37. Moore DE, Causey OR, Carey DE. Arthropod-borne viral infections of man in Nigeria, 1964-1970. *Ann Trop Med Parasitol* 1975;**69**:49–64.
38. Nabeshima T, Nga PT, Guilermo P, et al. Isolation and molecular characterization of Banna virus from mosquitoes, Vietnam. *Emerg Infect Dis J* 2008;**14**:1276–9.
39. Oshiro LS, Dondero DV, Emmons RW, et al. The development of Colorado tick fever virus within cells of the haemopoietic system. *J Gen Virol* 1978;**39**:73–9.
40. Philip CS, Callaway C, Chu MC, et al. Replication of Colorado tick fever virus within human hematopoietic progenitor cells. *J Virol* 1993;**67**:2389–95.
41. Philip RN, Casper EA, Cory J, et al. The potential for transmission of arboviruses by blood transfusion with particular references to Colorado tick fever. In: Greenwalt TJ, Jamieson GA, editors. *Transmissible disease and blood transfusion*. New York: Grune & Stratton; 1975. p. 175–95.
42. Silver HK, Meiklejohn G, Kempe CH. Colorado tick fever. *Am J Dis Child* 1961;**101**:30–6.
43. Song LT, Chen BQ, Zhao ZJ. Isolation and identification of new members of Coltivirus from mosquitoes collected in *China J Clin Exp Virol China* 1995;**9**:7–10.
44. Spruance SL, Bailey A. Colorado tick fever. *Arch Intern Med* 1973;**131**:288–93.
45. Tsai TF. Arboviral infections in the United States. *Infect Dis Clin North Am* 1991;**5**:73–102.

infection and illness in adults and the elderly occur commonly and can be acquired within families with an ill infant.*

Because of the frequent occurrence of rotavirus infections and the reduced severity of illness typically associated with sequential infections, rotavirus vaccines were developed to protect against severe disease in early childhood. The first vaccine licensed in 1998[1,329] was effective but was quickly withdrawn because of its association with intussusception.[71,75] Two new vaccines were licensed in the United States for use years later, the first in 2006 and the second in 2008.[2,3,108,348] Since the introduction of these two vaccines, there has been a remarkable decrease in rotavirus disease wherever these vaccines have been used.† However, in resource-poor countries, the decreased efficacy of both vaccines is striking and will need to be further studied.‡

HISTORY

Viruses with morphologic features later associated with rotaviruses were observed first by electron microscopy in 1963 in intestinal tissues and rectal swab specimens from mice and monkeys.[7] These agents, called epizootic diarrhea of infant mice virus and simian agent 11, respectively, were described as 70-nm particles that had a wheel-like appearance. Hence, they were later designated "rota" viruses from the Latin word for wheel. The correlation between these viruses and human diarrheal disease was reported first in 1973 by Bishop and colleagues, who used electron microscopy to examine biopsy specimens of duodenal mucosa from children with acute gastroenteritis.[40] Within a short time, these and other investigators confirmed the association between the presence of rotavirus in feces and acute gastroenteritis.[39,120] In addition to their distinctive morphologic features, these human viruses along with their animal rotavirus counterparts were later shown to share a group antigen[520] and have been classified as members of the *Rotavirus* genus within the Reoviridae family. In 1980, particles that were indistinguishable morphologically from established rotavirus strains but lacked the common group antigen were discovered in pigs.[53,415] This finding subsequently led to the identification of rotaviruses belonging to six additional groups (B to G) on the basis of common group antigens, with the original rotavirus strains classified as group A.[416]

CLASSIFICATION OF ROTAVIRUS

Rotavirus Groups

Rotaviruses have been classified into groups on the basis of antigenic properties of the VP6 protein and more recently the amino acid sequences of this inner capsid protein.[416] There are currently five groups (A to E) and evidence to establish three additional groups (F to H). Only groups A to C have been associated with human diseases, and most known cases of rotavirus gastroenteritis are caused by group A strains. Group A rotaviruses are the strains to which vaccine development has been directed.

Rotavirus Genotypes

The two outer capsid proteins, VP7 and VP4, contain neutralization epitopes.[178,201] Early classifications of rotaviruses were based on cross-neutralization assays with panels of monoclonal antibodies to characterize the VP7 or G type, referring to the glycosylated structure of this protein, or P type, referring to the protease sensitivity of the VP4 protein. Most classification is currently based on sequence analyses or genotypes of the VP4 and VP7 gene,[157,169] and 27 G types and 35 P types have been described to date.[293] The genetic analysis of all 11 gene segments of rotavirus was recently used to classify and to compare rotavirus strains with the scheme detailed in Table 172-1.[293] The nomenclature for rotaviruses includes the G type and P type. The numeric designation for G serotypes and G genotypes is identical. Therefore, only a single number for G types, such as G1, is used. However, P serotypes and P genotypes are not the same. P types are classified into both serotypes (e.g., P1A, P1B, P2A, P3, P4, P5, P8) and their respective genotypes (e.g., P1A and genotype 8, P1B and genotype 4, P2A and genotype 6, P4 and genotype 10, P5 and genotype 3, P8 and genotype 11). Designation of each P genotype is included in square brackets after the term P. The P genotype classification is most commonly used. The G and P combinations may be written as either G1P[8] or P[8]G1.

Rotaviruses, similar to other viruses with segmented genomes, have the ability to form reassortants. When a cell is coinfected with two different rotaviruses, gene segments from each parent virus can be incorporated into new progeny viruses. The gene segments from different viruses can segregate independently, and the new viruses produced can contain genes from both parents. Rotaviruses have an extremely wide host range, but natural cross-species infections, particularly between animals and humans, may be rare. Nevertheless, numerous human isolates appear to be animal strains or animal-human rotavirus reassortants as determined by sequence analyses.[112,118,119,159,294] This property of reassortment has contributed to the diversity of G and P types found throughout the world. As more and more sequences are determined for all 11 genes of rotavirus isolates, more information will be gained on the origin and evolution of human and animal strains.[359]

Most human surveillance studies initially determined the G and P types for rotavirus isolates. In the past, the most common G types were G1 through G4 and G9.[158,161,180,207,420] More recently, other G types have emerged, including G5, G8, G10, and G12 in various regions around the world.* The most common types for

*References 11, 13, 72, 202, 234, 286, 287.
†References 12, 64, 70, 74, 79, 92, 93, 97, 107, 109, 116, 131, 132, 163, 313, 334, 364, 401, 456-459, 522, 525, 527.
‡References 17, 275, 281, 288, 325, 353, 356, 358, 433, 460, 534.

*References 22, 112, 125, 207, 210, 225, 263, 268, 309, 310, 315, 319, 365, 420, 468.

the P protein are P[8] and P[4] in addition to the more recently described P[6]. The global distribution of G and P genotypes can vary greatly by location and time or, as was found in some studies, may vary little over sequential seasons.[382] A review by Santos and Hoshino has summarized rotavirus genotypes from 124 studies performed between 1989 and 2004 and included data on 45,571 strains collected from 52 countries on five continents.[420] The four common G types, G1, G2, G3, and G4, with either P[8] or P[4], represented more than 88 percent of the strains identified worldwide during this period. Genotype G9, containing either P[8] or P[6], was found to be an emerging strain in a number of different locations.[161,420] A study of circulating strains in the United States during 1996-2005 found that G1P[8] was the most prevalent strain during this period.[160] Lower frequencies of the other common strains (G2P[4], G3P[8], and G4P[8]) varied during different years with some unusual combinations of G and P types (e.g., G12P[6] , G6P[9], and G3P[9]). G9 strains, which emerged in 1995, were detected for several seasons but then were not detected for four subsequent seasons. However, in some sites during those same years, a high prevalence of G2 and G9 strains was detected.[158]

The impact of rotavirus vaccines on circulating rotavirus strains is beginning to be studied. International and national surveillance programs around the world isolate and characterize circulating strains. Recent reports have shown that the strains isolated are still the most common (G1-4, G9 with P[8], P[4], and P[6]) but with a growing number of G8 and G12 isolates.* A recent report on strain surveillance in the United States found that G1strains were the dominant G type from 2005 to 2007 but that G3 was the most frequently detected strain in the 2007-2008 season.[207] Unusual combinations or reassortants with animal strains and even vaccine strains have been described.[62,352,432,454] Of interest are the strain surveillance studies in Australia during the period of vaccine introduction. G1P[8] was the most common strain from 2007 to 2010, followed by G2P[4], with G9P[8], G3P[8], and G4P[8] detected at lower frequencies. Overall, there seemed to be an increase in G2P[4] strains in areas using Rotarix vaccine and an increase in G3P[8] in areas using RotaTeq. In the 2011 season, G2P[4] became the dominant strain (51%), with G1P[8] still representing 26 percent of the detected isolates in Australia.[237,238] It is still unclear if vaccines are exerting selection pressure on circulating strains or if the recent findings represent the normal variation of strain diversity that was seen even before vaccine introduction.

Non–Group A Rotavirus Strains

Whereas most rotavirus disease in humans is caused by group A rotaviruses (the focus of this chapter), disease in humans may also occur from infection with group B and C strains of rotavirus. Epidemics of group B rotavirus have been reported in China, particularly in adults.[81,145,208,322,368] Seroprevalence studies have identified group B rotavirus infections in other areas, such as Hong Kong, Myanmar, Thailand, Australia, Bangladesh, Canada, England, Sweden, Africa, and the United States, although the prevalence is lower than in the epidemic regions of China.[416] Group C infections have been reported largely from Japan,[81,256,377,477] but they also have been detected in other parts of Asia, Australia, Europe, Central and South America, and Africa* and in the United States.[216] Serologic evidence has shown group C infection rates of 30 to 43 percent in England[214] and the United States.[403] Unlike group A rotavirus, group B and C rotaviruses appear to cause disease predominantly in children older than 3 years and in adults.[145,256,330] If infection is suspected, the diagnosis is suggested by electron microscopic detection of the typical rotavirus particles with a negative test result by the routine rotavirus assays that detect only group A rotaviruses. The detection of typical RNA migration patterns by polyacrylamide gel electrophoresis of RNA is also suggestive. Polymerase chain reaction (PCR) analysis and enzyme-linked immunosorbent assay (ELISA) have been described.[168,213,330,473] Outbreaks caused by waterborne or food-borne spread of group B rotavirus in China[209] and group C rotavirus in Japan[254,257,377] have been reported, although person-to-person spread also has been implicated.[145] Outbreaks usually involve older children and adults, but infants can be infected as well.[216] Group C rotaviruses have been associated with extrahepatic biliary atresia.[403,428] In one study, group C rotavirus RNA was detected in liver specimens from 10 of 20 patients with biliary atresia; however, there were no controls for comparison.[403] As the prevalence of group A rotavirus infections declines with the implementation of rotavirus vaccine programs, it will be important to monitor the burden of group B and C rotaviruses in the future.

PROPERTIES OF ROTAVIRUSES

Early visualization of the rotavirus particle by cryoelectron microscopy revealed a double-shelled structure with icosahedral symmetry and a T = 13 icosahedral lattice for the two outer layers. The outer shell is composed of two structural proteins, VP4 and VP7, which form capsomers that radiate from the inner capsid composed of the major structural protein, VP6.[386] This inner shell surrounds a core containing the segmented, double-stranded RNA viral genome and three additional structural proteins, VP1, VP2, and VP3 (Fig. 172-1).

Detailed analysis of the outer layer by subnanometer-resolution cryoelectron microscopy suggests that it is composed of 780 molecules or 260 trimers of the VP7 glycoprotein and is ~35 Å thick. Sixty trimers of the VP4 protein, 120 Å in length, are anchored to the VP6 layer. The VP6 layer is also composed of trimers, with one trimer of VP7 sitting on top of a VP6 trimer. The VP4 protein forms spikelike projections as they extend through and beyond the VP7 layer.[136,528] Thus, the full diameter of the mature rotavirus particle, including the VP4 spikes, is approximately 1000 Å. There are 132 aqueous channels in the outer layer that are positioned over 132

*References 68, 210, 237, 410, 468, 498, 513.

*References 5, 69, 255, 330, 368, 416, 449, 473.

channels within the perforated VP6 intermediate shell. These channels are ~140 Å deep and are classified into three types on the basis of their location relative to the icosahedral symmetry axes. Besides interactions between the two layers of VP7 and VP6, both proteins interact with VP4 in these aqueous channels.[265] Further examination of the rotavirus structure by cryoelectron microscopy has indicated that the inner layer composed of 120 subunits of VP2 molecules not only encloses the viral genome but interacts with it as well.[385] This interaction has been found to cause significant conformational changes in the VP2 structure. Within the VP2 layer there are also 11 or 12 copies of VP1, the viral polymerase, and VP3, the viral capping enzyme.[296]

The genome of rotavirus is composed of 11 segments of double-stranded RNA that encode the six structural proteins—VP1 to VP4, VP6, and VP7—and six nonstructural proteins designated NSP1 to NSP6 (Table 172-1). Each segment encodes one known rotavirus protein except segment 11, which encodes both NSP5 and NSP6 using alternative open reading frames. The genome segments range in size from approximately 660 to 3300 base pairs, and their encoded proteins have molecular weights of approximately 12,000 to 125,000. The functions of the structural and nonstructural viral proteins have been at least partially determined and are briefly listed in Table 172-1. The structure and function of the nonstructural proteins are detailed in a review by Hu and colleagues.[205]

The double-stranded RNA genome segments of rotavirus can be extracted from viral particles and separated by polyacrylamide gel electrophoresis into 11 distinct bands visualized by silver staining (Fig. 172-2). Each rotavirus strain has a characteristic RNA profile or electropherotype, a property that has been used extensively in epidemiologic studies of these viruses. The characteristic RNA electrophoretic pattern of group A rotaviruses consists of four size classes containing segments 1 to 4, 5 and 6, 7 to 9, and 10 and 11. RNA segments of strains belonging to less well characterized rotavirus groups (i.e., groups B to G) also can be separated into four size classes, but the distribution of segments within these classes differs from group to group.[415,416]

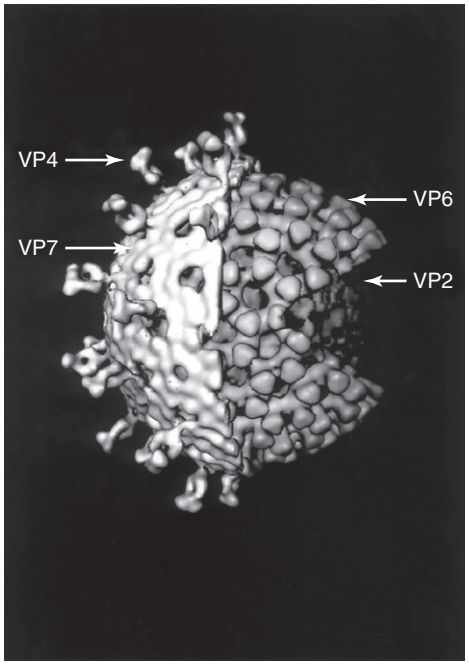

FIGURE 172-1 ■ Computer-generated image of the triple-shelled rotavirus particle obtained by cryoelectron microscopy. The cutaway diagram shows the outer capsid composed of VP4 spikes and VP7 shell, intermediate VP6 shell, and inner VP2 shell surrounding the core containing the 11 double-stranded RNA segments and VP1 and VP3 proteins. (Courtesy Dr. B. V. V. Prasad, Baylor College of Medicine, Houston, Texas.)

Entry and Replication

The major recognition protein for host cell receptors is the outer capsid VP4 protein of rotavirus. Rotaviruses are activated by cleavage of the VP4 protein by trypsin-like

TABLE 172-1 **Rotavirus Gene Segments and Properties of the Encoded Proteins**

RNA Segment	Encoded Protein	Properties of Protein
1	VP1	Structural inner core protein RNA transcriptase and RNA binding
2	VP2	Structural inner core protein RNA binding
3	VP3	Structural inner core protein Complexes with VP1; RNA binding; guanylyltransferase and methyltransferase activity
4	VP4	Outer capsid protein Receptor binding; hemagglutinin and neutralization protein
5	NSP1	Nonstructural protein RNA binding; possible suppression of host innate antiviral response
6	VP6	Intermediate capsid protein Group and subgroup antigen; necessary for transcription
7	NSP3	Nonstructural protein RNA binding and control of viral translation; inhibits host translation
8	NSP2	Nonstructural protein Multifunctional enzyme activity; interacts with NSP5 in formation of viroplasm
9	VP7	Outer capsid protein Neutralization protein
10	NSP4	Nonstructural protein Multifunctional protein; essential for replication, transcription, and morphogenesis; enterotoxin
11	NSP5	Nonstructural protein Binding to NSP2 to form viroplasm
	NSP6	Nonstructural protein Not encoded by all rotavirus strains; RNA binding; role not clearly determined

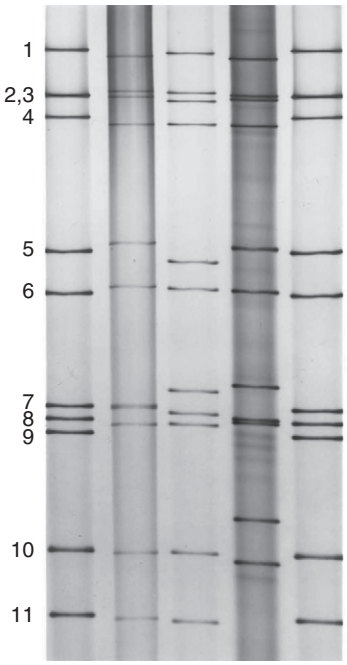

FIGURE 172-2 ■ Polyacrylamide gel electrophoretic patterns of genomic RNAs obtained from group A human rotaviruses and visualized by silver staining. The patterns demonstrate the characteristic four size classes of RNA separated into groups of 4, 2, 3, and 2 segments each. Human rotavirus strains included (from left to right) lane 1, Wa; lane 2, 248 strain; lane 3, 456 strain; lane 4, DS-1; lane 5, Wa.

proteases into VP5* and VP8* proteins, which remain virus associated.[15,24,172,236] Determining cell receptors for rotavirus has been an area of extensive study. Early studies determined that some animal but not human rotavirus strains use sialic acid on the cell surface and that attachment is mediated by VP8*.[305] Additional studies have indicated that multiple molecules are probably involved in virus attachment and entry, including heat shock cognate protein 70 (hsc70), ganglioside compounds, and integrins including $\alpha_2\beta_1$. VP7 has been shown also to interact with cellular integrins.* Another study, using a human rotavirus strain with a specific VP4 type (P[14]), has shown binding of the VP8* protein to a glycan characteristic of A-type histo-blood group antigens (HBGAs).[204] Two additional reports have shown that rotaviruses with different VP4 proteins, including the VP4 type of the study cited, can also bind to different HBGAs including types O, A, and AB. In addition, binding to human saliva, milk, and synthetic HBGA oligosaccharides was demonstrated.[206,272] HBGAs have been shown to be important receptors for noroviruses and are genetically controlled, which may have importance in determining susceptibility to rotavirus in the human population.

Studies to determine how rotavirus enters a cell after attachment have suggested endocytosis or a process similar to enveloped virus fusion as the mechanism for viral internalization, but again the process is not fully elucidated.[273,431,518] From a number of studies, it has been shown that the outer capsid proteins VP5* and VP8* undergo conformational changes that may also involve the VP7 protein during attachment and entry into the cell.[215,431,518,529]

For replication to begin, the outer capsid proteins must be removed to yield a VP2-VP6 double-layered particle. This particle becomes transcriptionally active when the viral RNA-dependent RNA polymerase (VP1) and the RNA capping enzyme (VP3), attached to the VP2 shell, start to synthesize the 11 viral mRNAs.[215,360] The capped non–poly A plus-strand RNAs are extruded from the virus cores through channels in the VP2 and VP6 protein layers at the 12 vertices of the viral particles and subsequently are translated into viral proteins in the cell cytosol. A number of studies using short interfering RNAs (siRNAs) have helped elucidate the various steps of viral replication and how the different viral proteins are involved.[435] Two nonstructural proteins, NSP2 and NSP5, have been found to be involved in the formation of large inclusions or viroplasms.[67] It is in the viroplasms that the assembly of virion precursors is initiated. Particle assembly may be initiated by the formation of complexes within the viroplasm that contain plus-strand RNAs from the 11 genome segments along with VP1 and VP3. Although the mechanism is not fully known, the virus assembles and packages one of each of the plus-strand RNAs within individual precursor viral complexes. On interactions with VP2, a single round of minus-strand RNA is synthesized and the 11 double-stranded RNA genome segments are produced.[297,298] VP6 trimers interact with the VP2 core, and a double-layer particle is completed.[189,297,298,341] The double-layer particles then bud into the rough endoplasmic reticulum after their association with the NSP4 transmembrane glycoprotein.[436] VP4 is added either before entry into the endoplasmic reticulum or sometime thereafter. The other rotavirus glycoprotein VP7, which becomes sequestered within the rough endoplasmic reticulum, is added to complete the formation of a mature viral particle.[469,470] These mature viruses accumulate within the lumen of the rough endoplasmic reticulum until cell lysis occurs.

Immunity

Much has been learned about the immune response to rotavirus, but the key question of what provides protection from infection or disease remains unanswered. Rotavirus infections clearly induce a humoral immune response beginning with production of IgM antibodies and later including IgA and IgG antibody.[121,182,299,402] Infection also induces local, intestinal antibodies that are predominantly IgA but also initially include IgG and IgM.* Cell-mediated immunity, including lymphoproliferative responses, also can be transiently detected after infection in adults.[211,283,339,340,408]

An obvious place to begin to understand rotavirus immunity is to determine the effectiveness of previous rotavirus infections in prevention of subsequent infections and disease. The important questions relate not

*References 21, 148, 187, 188, 273, 536.

*References 29, 111, 121, 182, 211, 283, 299, 339, 340, 402, 408.

only to the degree of protection but also to whether protection is serotype specific. As discussed before, there are multiple serotypes of human rotavirus based on neutralization epitopes on the VP4 and VP7 outer capsid proteins. Thus, protection may be limited to those strains that share neutralization epitopes, or it may be associated with the development of other B- or T-cell immune responses to shared epitopes.

Many investigators have reported that natural rotavirus infections produce incomplete protection, but little doubt exists that previous infections protect against severe disease associated with reinfection. In a large study, protection from both rotavirus reinfection and rotavirus diarrhea increased with each new infection,[481] but sequential infections even with the same serotype have been reported. In the initial study reporting rotavirus disease with reinfection by the same serotype, the investigators noted that protection of young children in a Japanese orphanage lasted 6 months then declined after 1 year.[84] This study noted a close correlation between titers of serotype-specific antibody and protection. Some animal studies also support a role for serotype-specific protection. Thus, in a study with piglets, immunization with reassortant viruses containing either the VP4 or the VP7 protein of the same serotype was protective, whereas immunization with reassortants containing heterotypic genes for these proteins was not protective.[200] In other studies using mice, this association was not as clear,[511] and protection was better correlated to serum and intestinal levels of IgA.[300,303]

Although reinfections with rotavirus appear to be common, some studies have shown protection that lasts at least 1 year.[31,38,502] In one of the first studies of protection, neonates infected within the first 2 weeks of life were protected against severe disease but not against reinfection.[38] In another study, infants who developed a symptomatic or an asymptomatic rotavirus infection during the first year of study were protected against contracting a subsequent rotavirus illness or even an asymptomatic reinfection during the following year.[31] Similarly, when the placebo recipients of a large vaccine trial were observed, a natural rotavirus infection in the first year was found to be 93 percent protective against a symptomatic reinfection in the second year.[502] This protection occurred even though G1 strains that circulated during the first year were responsible for only 66 percent of rotavirus disease in the second year. Other studies conducted in less developed countries and in daycare centers have not shown the same degree of protection.[99,165,481] Differences in these studies may be due to the variation in circulating strains, the magnitude of exposure, the overall health of the infants, or the duration of protection.

Protection has been correlated to both serum and stool antibody titers produced after natural rotavirus infection.[99,110,335,504] In one study, serum antibody levels, especially IgA, were found to be a marker for protection.[482] Reports of a correlation with serotype-specific neutralizing antibody and protection[335] have not been supported in other larger studies.[505] In the largest study, which was conducted in Bangladesh during a 2-year period when four major G genotypes circulated, the titers of both homologous and heterologous neutralizing antibody were significantly lower in patients with acute rotavirus disease than in matched control subjects. However, further analysis could not find a correlation with serotype-specific neutralizing antibody. Thus, protection seemed to correlate better with the magnitude of the response than with specific neutralizing responses.[99,505]

Some results from rotavirus vaccine trials also fail to support a role for serum neutralizing antibody and protection. Thus, immunization with heterologous animal rotavirus vaccines has provided protection in some studies without inducing serum neutralizing antibody to human serotypes.[90,485] In other studies that failed to demonstrate overall efficacy, protection was seen in those who developed the highest antibody titers to the heterologous vaccine,[32,509] again implicating the magnitude of the response rather than specific neutralizing antibody titers. It is possible, however, that serum antibodies are merely markers for the true protective responses in the intestine. Although it is not clear what provides protection, vaccine evaluations of the G1P[8] monovalent vaccine show that heterotypic protection is robust.[128,523]

Animal models also have proved useful for examination of protective immune responses. Initially, large animals, such as piglets and calves, were used, whereas mouse models were limited to the study of passive protection because mice are susceptible to rotavirus diarrhea only for the first 15 days of life. However, the development of an adult infection model in which protection against fecal shedding of rotavirus antigen rather than protection against diarrhea was used as the indicator of active immunity provided much information.[511] Active immunization against both homotypic and heterotypic challenge has been demonstrated in mice, calves, pigs, and rabbits.* Protection has been seen after both oral and parenteral immunization, although what provides protection is not clear. In studies of mice, although no correlation could be found between protection and either serum or intestinal neutralizing antibody, levels of serum or fecal rotavirus IgA did correlate with protection.[300]

The use of these models has been extended to gene knockout mice to distinguish the role of $CD8^+$ cells and antibody in protection. These studies indicated that $CD8^+$ cytolytic cells are important for resolution of an infection, but only antibody could provide protection from a subsequent challenge.[151-153] Other reports have indicated that antibody may be important for complete resolution of a rotavirus infection as well as for protection against subsequent infection.[301,478] A study in mice revealed that oral immunization of mice with a rotavirus that was fully heterotypic to the challenge strain provided nearly complete protection against fecal rotavirus shedding, but this protection was dependent on the ability of IgA antibody to be transported through intestinal epithelial cells.[479] Another report has shown that in IgA-deficient mice, resolution of rotavirus infection is delayed and mice are not protected from subsequent infection with the same virus.[46] These results support the suggestion that protection after live virus immunization may be due to rotavirus-specific IgA, either in the intracellular intestinal cells or in the intestinal lumen, at least in mice.[429,479]

*References 56, 102, 103, 200, 302, 511, 520.

Taken together, these results indicate that the levels, location, and targets of protective antibody as well as the proteins against which it is directed are of immediate importance, provided the results found in mice are relevant to larger animals and humans.

Information on protective immune responses continues to become available and should prove useful in further development of rotavirus vaccines. The absence, to date, of a reliable immunologic marker of protection, however, continues to render efficacy vaccine trials necessary.

Age-Dependent Susceptibility to Rotavirus Disease

In addition to restrictions in interspecies transmission of rotaviruses, age restrictions are associated with rotavirus disease. In animals, rotavirus illness appears to be limited to the first days or weeks of life. Mice are susceptible to rotavirus disease only for their first 15 days of life but can experience a rotavirus infection for their entire lifetime. Similarly, piglets and calves are most susceptible to rotavirus diarrhea during their first days of life.[54,303] In contrast, severe human rotavirus disease occurs most commonly in infants younger than 24 months, but milder rotavirus illnesses can occur throughout our lifetimes.[363,407,447,505,506]

The onset of rotavirus disease in infants has been reported to coincide with the decline of maternal IgG antibody titers.[537] Furthermore, excellent correlations have been observed between responsiveness to the live rotavirus vaccine Rotarix and transplacental neutralizing antibody titers to the vaccine strains (unpublished results). Therefore, early protection from rotavirus disease including asymptomatic neonatal rotavirus infections[38,371] may be due, at least partially, to protection from transplacental antibody that may persist for the first months of life.[32] Mechanisms by which transplacental maternal antibody might protect against intestinal infection are unclear. Passive transfer of neutralizing antibody to the intestine of both humans and animals is associated with protection,* but circulating antirotavirus IgG appears to confer little if any protection in animals.[337,441,442] Possibly, maternal IgG in humans is taken into the intestine, where it neutralizes rotaviruses before infection. Regardless of why rotavirus infection of neonates typically is asymptomatic, these infections have been found to reduce the severity of rotavirus illnesses in older infants.[34,38] For these reasons, two rotavirus strains obtained from neonates have been developed as vaccine candidates,[23,307] and two others obtained more recently from India have been evaluated in a safety and immunogenicity study.[35,118,119]

The reduced severity of rotavirus disease in older children and adults is probably due primarily to immune responses stimulated by previous rotavirus infections. In developed as well as in developing countries, almost all humans experience at least one rotavirus infection by the time they reach 3 years of age, and circulating rotavirus antibody remains detectable indefinitely.[227] Protection against rotavirus infection and disease in children and adults has been correlated with titers of both circulating and intestinal rotavirus antibody.* Although these antibodies have not been established as the effectors of protection, their presence indicates a natural infection that has elicited a protective immune response.

Protection from Breast Milk

Cohort studies have found that the highest incidence of rotavirus-associated diarrhea is in children aged 4 to 6 months, which coincides with the age when weaning from breast-feeding began.[343,393] In addition, several studies have shown that breast-feeding protects against symptomatic rotavirus infections.[98,320,326] In one study, exclusive breast-feeding was found to be protective against severe rotavirus diarrhea, but there was no overall protection during the first 2 years of life, suggesting that breast-feeding simply postpones rotavirus disease to an older age.[98] In another study, breast-feeding was protective against hospitalization for acute gastroenteritis due to rotavirus in infants younger than 6 months.[130] Although breast-feeding alone could be the protective factor, other factors, such as presence of maternal antibody and lack of opportunity for exposure, could also account for this period of protection. Supporting a role of human milk—and factors other than breast milk antibody—in protection against rotavirus is a study demonstrating that infants who received human milk with higher concentrations of the glycoprotein lactadherin were more likely to have asymptomatic rotavirus infections compared with those receiving milk with lower concentrations.[326]

On the other hand, breast-feeding could play a role in limiting the protection induced by rotavirus vaccination.[19,77,314] It is clear that the immune response and protection provided by live oral rotavirus vaccines are less in underdeveloped than in developed countries.[218,219] It has been speculated that this could in part be due to breast milk antibody and other constituents limiting the replication of the vaccine strains as the levels of antibody are higher in women in developing countries.

LABORATORY DIAGNOSIS

Rotavirus infection cannot be reliably diagnosed on the basis of clinical presentation, even in combination with stool examination and nonspecific laboratory tests. Therefore, specific tests have been developed. In the initial epidemiologic studies, electron microscopy was used for identification because of the large number of particles present in stools (>10^{10}) and the characteristic appearance of rotavirus. This technique largely has been replaced by enzyme immunoassay–based and latex agglutination tests, for which kits are available commercially. With the advent of molecular methods, reverse transcription PCR (RT-PCR) to detect conserved genes or real-time RT-PCR has been developed to detect rotavirus and to quantify viral load.[174,226] The use of RT-PCR for the diagnosis of rotavirus disease is discouraged. Because

*References 54, 122, 138, 195, 417, 427, 440, 442.

*References 41, 84, 99, 110, 175, 230, 292, 335, 481, 482, 503, 504.

children may shed rotavirus for prolonged periods and asymptomatic infection commonly occurs, the high sensitivity of RT-PCR will be positive in children without disease in as many as 20 percent of children.[452]

Rotavirus infection, both symptomatic and asymptomatic, also can be identified by changes in rotavirus antibody. ELISAs are used most commonly to measure serum rotavirus-specific IgM, IgA, and IgG levels as well as stool and intestinal antibodies. Specific neutralizing titers also can be measured for each serotype of rotavirus by plaque reduction or focus reduction assays. One ELISA-based antigen reduction neutralization assay has been found to be better suited for the analyses of large numbers of specimens.[240] Serologic detection of infection is more difficult in the first months of life because of the presence of maternal antibodies. Detection of rotavirus IgA, which does not cross the placenta, has been used as a marker for previous infection in the first months of life and also to evaluate immune responses to vaccines.

PATHOLOGY AND PATHOGENESIS

Histologic and Structural Changes in Intestinal Villi

After fecal-oral transmission of rotavirus, infection is initiated in the upper intestine and typically leads to a series of histologic and physiologic changes. These changes have been examined extensively, particularly through experimental infections of animals. Studies in calves revealed that rotavirus infection caused the villus epithelium to change from columnar to cuboidal, which resulted in shortening and stunting of the villi.[304,367] The cells at the villus tips became denuded, whereas in the underlying lamina propria, the numbers of reticulum-like cells increased and mononuclear cell infiltration was observed. The infection started at the proximal end of the small intestine and advanced distally. The most pronounced changes usually but not always[467] were associated with the proximal small intestine. When bovine rotaviruses of different virulence were compared, a low-virulence strain infected the proximal small intestine poorly but infected more villus enterocytes in the mid and distal intestine than did the high-virulence strain.[55,193] Although the low-virulence strain replicated in these cells and caused cytopathic effects, it did not damage intestinal structure or affect function. Similar observations were made after piglets were infected with rotavirus.[100,176,366,462]

The pathologic process of murine rotavirus infection also has been examined in several studies, and the results are similar to those found in larger animals.[6,252,271] Many of these studies have been conducted with heterologous rotavirus strains that require infection with much greater quantities of virus because of the restricted replication of these viruses in mice.[26,50,179,338] The histologic changes induced by these heterologous strains are similar to those found after murine rotavirus infection, even though viral replication is limited after oral inoculation with these viruses. Mice infected during their first 2 weeks of life develop diarrhea.[50] The infection produces time-dependent histologic changes in the intestine, shortening of intestinal villi, induction of apoptosis, and alterations in cell migration kinetics.[50]

A few studies have examined the pathologic changes in the intestines of humans, and the results appeared to be similar to those found in animals.[66,453] Tissue tropism for rotavirus infection in humans appeared to be restricted normally to villi of the small intestine, but it is now clear that infection routinely produces both antigenemia (presence of rotavirus protein in the blood) and viremia (presence of live rotavirus in the blood) in healthy and immunocompromised children[43-45,83,147,164,389] as well as spread to other organs and induction of disease, including meningoencephalitis,[134] elevated liver enzymes,[250] and other systemic diseases as discussed later. It is not clear what virus factors account for increased virulence or induction of extraintestinal disease. Offit and coworkers[336] reported that the virulence of reassortants generated between heterologous rotaviruses tested in a mouse model correlated with the presence of the VP4 protein from the more virulent virus, but a later study with murine-simian rotavirus reassortants revealed no association between the VP4 protein and virulence.[58] In that study, the strongest association between virulence and a gene product was with NSP1, a nonstructural protein. In a study using porcine rotavirus, virulence was associated with VP4, VP7, VP3, and VP5.[199]

Associations between virulence[57] and specific gene segments also were examined in piglets, in which VP4 appeared to correlate best with virulence. In another study with reassortants between a virulent porcine virus and a human strain attenuated for piglets, investigators found that the porcine rotavirus genes encoding VP3, VP4, VP7, and NSP4 were required for virulence in piglets.[199] Whether either of these observations has general applicability or pertains only to a limited combination of rotavirus strains because of specific interactions between their proteins remains to be determined. Until very recently, it was not possible to make specific nucleotide changes within rotavirus genes and to reincorporate the modified gene into new infectious rotavirus particles. However, a system of reverse genetics for rotavirus has now been reported.[242,243,472] Expansion of this system of reverse genetics should permit identification of specific amino acid and protein structural changes that are responsible for rotavirus virulence and attenuation.

Mechanisms of Diarrhea

Although rotaviruses cause severe diarrhea in numerous species, including humans, the mechanisms responsible are not completely understood and include multiple factors. There is evidence that malabsorption due to destruction of enterocytes, villous ischemia, toxin (NSP4), and activation of the enteric nervous system all contribute to diarrhea.[177,390] Infection of mature enterocytes of the small intestines leads to damage and functional abnormalities. However, in animal models, the degree of disease (histologic changes including villous blunting and crypt hyperplasia) is limited and often does not correlate with the severity of diarrhea. Further, diarrhea is often seen before evidence of intestinal disease. Thus, although damage to the mature enterocytes leading to loss of the

enzymatic activities of the brush border decreases absorption of electrolytes, glucose, and amino acids and contributes to rotavirus diarrhea, other mechanisms are also involved.

Secretory mechanisms that contribute to early diarrhea have recently been described. The identification of a viral enterotoxin, NSP4, has increased our understanding of the pathogenesis of diarrhea, as has evidence implicating stimulation of the enteric nervous system.[278,463] NSP4 binding to surface receptors initiates signal transduction pathways, leading to mobilization of intracellular calcium and chloride secretion. NSP4 increases the levels of intracellular calcium[465] by activating a calcium-dependent signal transduction pathway that mobilizes transport of this ion from the endoplasmic reticulum.[135,464] Other reports suggest that NSP4 possesses membrane destabilization activity[59,463] that may result from increased intracellular calcium concentrations, resulting in cytoskeleton disorganization and cell death.[60,61,373,374] Thus, NSP4 binding to intestinal epithelium after its release from infected cells may contribute to altered ion transport and diarrhea. Another possible target for secreted NSP4 is the enteric nervous system, which lies under the villus epithelium. It has been reported that rotavirus infection can activate this system in mice, and drugs that block nerve activity attenuate rotavirus-induced fluid secretion in vitro and attenuate diarrhea in vivo.[278] NSP4 also disrupts tight junctions, allowing paracellular flow of water and electrolytes. Antibody against NSP4 can attenuate rotavirus disease in mice, providing additional evidence for its role in the pathogenesis of disease. More recently, an association between NSP4, secretion of serotonin by enterochromaffin cells, and vomiting was reported.

EPIDEMIOLOGY

Disease Burden

Before the introduction of rotavirus vaccines, rotaviruses were the leading cause of severe acute gastroenteritis in young children worldwide. In children younger than 5 years, an estimated 527,000 children died of rotavirus gastroenteritis each year. The majority of these deaths occurred in children from resource-poor countries in Africa and Asia.[350] Rotaviruses were responsible for approximately 5 percent of all deaths in children younger than 5 years. In addition, in this same age group, there were an estimated 2.4 million hospitalizations, 24 million clinic visits, and 114 million episodes of illnesses attributed to rotavirus each year.[166] The World Health Organization Rotavirus Surveillance Networks estimated that worldwide, 36 percent of diarrhea hospitalizations in children younger than 5 years were due to rotavirus infection.[515]

In the United States, before rotavirus vaccine introduction, rotavirus was estimated to infect nearly every child in the United States by the age of 5 years. Among children younger than 5 years, rotavirus gastroenteritis accounted for 55,000 to 70,000 hospitalizations, 205,000 to 272,000 emergency department visits, and 410,000 physician visits each year.[80,196,233,285] In the pre-vaccine era in the United States, the majority of hospitalizations occurred in young children, with the highest rates in children younger than 3 months (51.8/10,000), slightly lower rates in the 3- to 23-month age group (30.0-34.6/10,000), and the lowest rates in the older age groups of 24 to 35 months (20.5/10,000) and 36 to 59 months (4.1/10,000).[447] Symptomatic disease is less commonly seen in older children and adults because natural infection confers protection against subsequent infection and disease. However, rotavirus still may cause gastroenteritis in these age groups, especially in households with young children or in adults who care for young children with rotavirus.[139,321]

Initial and subsequent rotavirus infections are often asymptomatic. In cohort studies in which infants were observed from birth, as many as 50 percent of initial rotavirus infections were asymptomatic.[190,393,407,481,537] Reports of asymptomatic neonatal infections are common.[34,38,181,192,370] After an initial rotavirus infection, 38 percent of children are protected against a subsequent rotavirus infection, 77 percent are protected against subsequent rotavirus gastroenteritis, and 87 percent are protected against severe gastroenteritis due to rotavirus.[481] Second and third infections due to rotavirus provide greater protection against rotavirus gastroenteritis.

Transmission

Rotaviruses are spread through fecal-oral contamination, generally by close person-to-person contact. Rotaviruses can also be transmitted through contaminated fomites, especially in child care settings and hospitals.[14,65,380,381] Factors facilitating the transmission of rotaviruses include that few infectious virions are needed to cause disease in susceptible hosts (10^3), rotaviruses are shed in high concentrations in the stool (10^{11} particles/gram of human feces),[507] and rotaviruses retain their infectivity for long periods.[143,232,312] Whereas transmission through airborne droplets has been hypothesized, this has not been proved.[387,424] Waterborne transmission has been described,[145,198,209,248] but is unlikely to be a common mode of transmission.

Rotavirus Seasonality and Source of Epidemic Strains

Before the introduction of rotavirus vaccines, in temperate climates there were distinct winter and spring seasonal peaks of rotavirus disease.* In the United States, rotavirus activity typically began in the early winter in the southwest and moved across the country, ending in the northeastern states in the spring.[466] In tropical climates, rotavirus circulates year-round but frequently has peaks during the cool or dry months.† One study, however, suggested that the relative lack of seasonality seen in tropical countries may be due to the high birth rates and transmission rates found in developing countries rather than to environmental conditions.[383] The cause for the

*References 51, 291, 413, 466, 474, 494.
†References 52, 105, 114, 191, 223, 319.

seasonality of rotavirus disease is a topic of considerable interest but remains unknown.

Whereas the transmission of rotavirus infections is thought to be fecal-oral, with little evidence of airborne transmission, a unique pattern of rotavirus infections that follows the general direction of the prevailing winds was observed annually in North America.[260] Rotavirus activity would begin in Mexico and the southwestern United States in mid to late fall and then travel systematically across the continent, ending in the northeastern United States and Maritime Provinces of Canada in the spring. A similar pattern of rotavirus spread was reported for western Europe, where the seasonal peak started in Spain in January and ended in the more northern countries in March.[247] These annual events have no satisfactory explanations. The phenomenon appears to be independent of latitude, which argues against temperature-dependent associations and humidity. Furthermore, the electropherotypes and serotypes of isolates found in different geographic locations can vary, a counterindication for a gradual, physical transmission of rotavirus infections as a wave to the north and east. The introduction of rotavirus vaccines appears to have disrupted this pattern of spread.[343]

Because rotavirus illnesses occurred with seasonal regularity and decreased to almost undetectable levels during the off-season, the virus must be retained in a less active state during most of each year. Retention of human rotavirus in animal reservoirs between seasons is unlikely because of the low interspecies transmissibility associated with this virus, as already discussed. Therefore, the virus may continue to replicate at low levels in humans until conditions are favorable for the annual epidemic. It is also possible that the virus survives in the environment, which provides continuous exposure throughout the year but results in sustained rotavirus illnesses only during seasonal epidemics. Because rotaviruses are shed in extremely high concentrations and can retain their infectivity for long periods, the environment could be a reservoir for human rotavirus and a possible source for the initiation of seasonal epidemics.

To provide clues about the origin of rotavirus strains responsible for epidemics, many extensive studies have been performed to characterize the circulating viruses, primarily by use of electropherotypes and serotypes. From these studies, investigators have determined that rotavirus strains in a specific locale can vary little over sequential seasons or change dramatically, even within a single season.* Furthermore, multiple strains often are present within a region at any period during an epidemic. Because gene reassortment can be extensive after coinfection with rotavirus,† identification of the source of new strains within a defined geographic area is difficult. They could be derived from outside sources, they could be obtained from local reservoirs, or they could arise by gene reassortment of circulating strains.

Since the introduction of rotavirus vaccines in the United States, the traditional pattern of rotavirus seasons in the United States has become more variable, failing to demonstrate the typical southeast to northeast spread of rotavirus activity.[115] In the 2007-2008 and 2008-2009 seasons, the onset of the season was delayed and duration was shortened compared with the 2000-2006 pre-vaccine season.[70] In addition, during the 2009-2010 season, the threshold for the start of the rotavirus season was not observed for the first time in the United States.[457,458]

CLINICAL DISEASE

The incubation period for rotavirus is approximately 1 to 3 days.[230] The onset of rotavirus disease is often abrupt, with fever and vomiting followed by explosive, watery diarrhea.[249,405,475] Vomiting may precede the diarrhea in approximately half the cases. Stools are nonbloody and generally lack fecal leukocytes, but mucus may be found in 20 percent. The disease is usually self-limited, lasting 4 to 8 days, although the duration of symptoms ranged between 2 and 22 days in a Guatemalan study.[521] In a cohort study in children with symptomatic disease, 62 percent had mild disease, 35 percent had moderate disease, and 3 percent had severe disease; 7 percent of children required hospitalization.[393] In another study, 64 percent of children with rotavirus diarrhea had vomiting, 64 percent had fever, 14 percent were dehydrated, and 18 percent were hospitalized.[372] About two thirds of children hospitalized with rotavirus disease will have diarrhea, vomiting, and fever, but children may also have only one or two of these symptoms.[445] When hospitalization is required, the stay usually is brief, with an average of 4 days and a range of 2 to 14 days.[405] Recovery generally is complete, but persistent diarrhea associated with lactose intolerance has been described.[25,267] Complications and death are generally due to dehydration, electrolyte imbalances, and acidosis.

In general, rotavirus infections are more severe than those caused by other viral gastrointestinal agents and therefore are more likely to result in hospitalization.[42,405] In developing countries, 20 to 40 percent of hospitalizations for diarrhea are due to rotavirus infections,[125,222,319,324] whereas in the United States, rotavirus is estimated to account for between one third and two thirds of admissions for diarrhea in children.[406] Vomiting, dehydration, and hospitalization occur significantly more often in patients infected with rotavirus than in those with other causes of diarrhea.[405,475] In one study, vomiting and diarrhea also lasted longer in patients infected with rotavirus (2.6 vs. 0.9 days and 5.0 vs. 2.6 days, respectively).[405] In another study, the severity score for rotavirus diarrhea was almost twice that of disease having other causes.[412] In one study, children with acute gastroenteritis due to rotavirus compared with children who were rotavirus negative were significantly more likely to have a severe clinical score (68% vs. 32%), to be severely dehydrated (63% vs. 37%), and to receive rehydration therapy (56% vs. 33%).[289]

The peak viral shedding in stools occurs on about day 3 of illness.[496] In a study evaluating shedding by PCR, the duration of rotavirus shedding ranged from 4 to 57 days in hospitalized patients.[399] Approximately half of the children shed for fewer than 10 days and 70 percent for fewer

*References 94, 158, 207, 291, 365, 382, 506.
†References 113, 169, 262, 270, 295, 391, 512.

than 20 days. In a study conducted in Guatemala, prolonged viral shedding was reported for as long as 5 weeks.[290] Prolonged shedding has been reported in children with immunocompromised states.[426]

Laboratory findings are related mostly to the extent of dehydration and can include elevated blood urea nitrogen and evidence of mild metabolic acidosis. In children with dehydration caused by rotavirus, dehydration was more likely to be isotonic than was dehydration caused by other agents in one study,[405] whereas in another study, one fourth of the patients infected with rotavirus presented with hypernatremic dehydration.[249] In a study examining the complications of community-acquired rotavirus in hospitalized children, of the 723 children in the study, 1.7 percent had severe dehydration requiring intensive care, 9.1 percent had hypertonic dehydration, 4.0 percent had seizures, and 0.1 percent died.[221]

Symptoms and viral shedding in children with rotavirus disease can be prolonged in immunocompromised patients either as a result of primary immunodeficiencies or from bone marrow or solid-organ transplantation.* More severe infection has not been seen in children with human immunodeficiency virus (HIV) infection.[239,448]

Other clinical manifestations associated either etiologically or incidentally with rotavirus infection have been described. Necrotizing enterocolitis has also been associated with rotavirus,[127] but the possibility of false-positive reactions obtained with some ELISA tests in neonates must be considered. Rotavirus antigen and infectious virus have also been reported in the blood of immunocompetent children with confirmed rotavirus gastroenteritis.† Most children infected with rotavirus have been viremic, independent of the presence of diarrhea. Central nervous system involvement was first associated with rotavirus infections by use of electron microscopy and antigen detection methods in children with aseptic meningitis[476,519] and encephalopathy.[231] However, more recently, cases have been reported by identification of rotavirus RNA in the cerebrospinal fluid with use of RT-PCR analysis. Rotavirus RNA has been found in the cerebrospinal fluid in rotavirus-infected children with seizures,[279,331,344,347] encephalitis,[167,197,279,328,530] encephalopathy,[134,167,284] and flaccid paralysis.[87] Of the 23 cases described, 15 were reported to have full recovery, whereas 6 had significant neurologic complications and 2 children died.

The relationship between intussusception and natural rotavirus infection is of great interest because of the reported link between the tetravalent RRV vaccine and intussusception (discussed in the section on vaccines).[71,75,241] Several studies have investigated the possible infectious etiology of intussusception.‡ Given the rarity of intussusception, these studies have had small sample sizes, they have typically been case series, and a number of different pathogens have been identified. Adenoviruses have been identified most often. Whereas the lack of seasonal variation for intussusception may suggest that wild-type rotavirus does not cause intussusception,[78,398] the possibility remains that many intestinal pathogens, including rotavirus, may cause intussusception, and multiple agents may account for the lack of seasonality. Three case-control studies, however, did not find an association with wild-type rotavirus and intussusception.[20,37,480]

TREATMENT

Treatment of rotavirus gastroenteritis is aimed largely at restoration of the proper fluid balance in dehydrated patients.[235] Continuation or early reintroduction of oral feeding is important. Breast-fed infants should continue to be breast-fed and formula-fed infants should continue with their usual formula after being rehydrated.[235] Emphasis has shifted from intravenous rehydration to oral rehydration with glucose-electrolyte solutions.[323] The glucose in the solution enhances sodium and therefore water absorption in the intestine. Several studies have shown the utility of this approach for treatment of rotavirus gastroenteritis.[414,421,422] The solution accepted by the World Health Organization contains 30 mEq/L of sodium, 30 mEq/L of potassium, and 30 mEq/L of bicarbonate, which is similar to other oral rehydrating solutions available commercially. Should oral rehydration efforts fail or in cases of severe dehydration and shock, fluid should be administered intravenously.

Other experimental approaches to treatment are based on the success of passive oral therapy in animals. Chronic rotavirus shedding has been treated successfully in immunocompromised children with human milk that contained rotavirus antibody.[426] Similarly, immune colostrum, immunoglobulin, or milk from rotavirus-immunized cows and human immunoglobulin have had some success.[122,184,195,311,425] A Cochrane Review, however, did not find sufficient evidence to warrant use of oral immunoglobulin in low-birth-weight infants.[345,346] A hyperimmune immunoglobulin Y (IgY) was evaluated in a randomized placebo-controlled trial in pediatric patients with rotavirus-associated diarrhea.[388,423] Children who received the IgY had significantly lower mean oral rehydration fluid intake, mean duration of intravenous fluid administration, mean duration of diarrhea, and mean duration of rotavirus clearance from the stool compared with placebo.[388]

Trials of bismuth subsalicylate have also been conducted and demonstrated a shortened duration of illness.[146,443,444] Probiotics have been shown to have beneficial albeit modest effects.[183,185] The use of probiotics has been shown to decrease rotavirus shedding in one study[144] and in a meta-analysis was shown to reduce the duration of diarrhea by about one day.[8] The effectiveness of probiotics appears to be strain and dose dependent.[185] In a comparative trial, both a single-species product and one with four strains were found to decrease the duration of diarrhea compared with oral rehydration alone.[173] In another trial, zinc plus a probiotic was found to decrease the length of hospitalization.[117]

Racecadotril (acetorphan), an enkephalinase inhibitor with antisecretory and antidiarrheal properties that

*References 140, 164, 224, 266, 394, 426, 450, 451.
†References 43-45, 83, 147, 354, 389, 390.
‡References 9, 18, 27, 36, 37, 47, 96, 141, 155, 156, 186, 203, 212, 244-246, 258, 316, 327, 332, 342, 384, 430 ,532, 533.

decreases intestinal hypersecretion but not motility by preventing the breakdown of endogenous enkephalinase in the gastrointestinal tract, has been shown to be effective in the treatment of diarrhea in adults and children.[194,261,418] In one study, oral treatment with racecadotril decreased stool output up to 50 percent in children with watery diarrhea.[76] A similar decrease was seen in boys with rotavirus infection.[418] Another drug, nitazoxanide, licensed for treatment of *Giardia intestinalis* and *Cryptosporidium parvum* infections, has shown some effectiveness in treatment of rotavirus infection.[43,461] In one study, 7.5 mg/kg of nitazoxanide administered orally twice a day for 3 days reduced the duration of illness in children with rotavirus gastroenteritis from 75 hours to 31 hours.[43]

VACCINES

The development of rotavirus vaccines has been a major priority because of the high burden of disease, and cost-effectiveness analyses have shown that a rotavirus immunization program would be cost-effective from the perspective of society and the health care system.[514,517] Because natural rotavirus infections induce excellent protection against severe rotavirus disease, vaccine efforts have been largely directed at the development of live attenuated rotavirus vaccines. However, there is still a continued interest in killed vaccines, primarily because of the reduced efficacy seen in resource-poor countries.[217,355]

Whereas the development of rotavirus vaccines has had a relatively short time line, there have been several obstacles to overcome. Just 10 years after the identification of rotavirus as an agent of severe diarrhea, the first vaccine trials were conducted.[485] Efforts initially concentrated on the use of animal rotavirus strains, labeled the Jennerian approach[228] because it relies on the natural attenuation of animal viruses in humans for safety or the attenuation of human strains. The initial efforts with bovine rotavirus vaccines RIT 4237[124,423,483,484] and WC3[32,307] and rhesus monkey vaccines RRV[150,282,397,423,493] and MMU 18006[396] yielded inconsistent results. Therefore, in an attempt to make the vaccines more closely related to human strains, human rotavirus genes coding for the proteins that induce neutralizing antibody VP7 or VP4 were introduced into these animal strains by creating reassortant viruses as described earlier. The segmented genome of rotavirus renders production of these reassortant rotaviruses relatively easy. Reassortant vaccines were developed with RRV[306,308] and WC3[32,91,471] and later UK[229,491] as the animal strain. This approach ultimately produced the first licensed rotavirus vaccine, RotaShield,[1,329] and one of the two currently licensed rotavirus vaccines, RotaTeq.[2,348] The attempt to develop rotavirus vaccines with use of attenuated human strains began with the neonatal human viruses M37 and RV3 that were thought to be naturally attenuated.[23,149,495] The attenuation of a human strain resulted in the licensure of the other rotavirus vaccine now licensed for use, Rotarix.[3,101]

Past and Currently Licensed Rotavirus Vaccines (Fig. 172-3)

RotaShield

History and Development of RotaShield. The development of RotaShield, a reassortant vaccine based on rhesus monkey rotavirus, began with evaluations of RRV. RRV replicates well in humans but produced mild side effects, including low-grade fever and mild diarrhea, especially when it was given to older children who had lost maternal antibodies.[276,371,486] Protection with this vaccine was inconsistent, ranging from greater than 50 percent, even in developing countries,[150,282,396] to moderate (20% to 50%),[397,493] to no protection.[88,423] Therefore, reassortants were developed containing the VP7 genes of G1, G2, or G4 serotype and the 10 other genes from RRV. The G3 serotype representative for the RRV tetravalent vaccine (RRV-TV) was RRV. Thus, four of the common VP7 serotypes were incorporated into the vaccine. Vaccination produced a rotavirus IgG and IgA serum antibody response, but neutralizing antibodies were produced predominantly to RRV rather than to the human serotypes.[28,369,395] This finding appears to be consistent with the experiments showing that VP4 rather than VP7 is more immunogenic after natural infection.[508,510]

Safety and Efficacy of RotaShield. Extensive evaluations of RRV-TV were completed before licensure.[220,372,395] In a large trial conducted at centers across the United States,[395] the vaccine was found to be safe; however, the number of subjects with fever after the first dose was increased compared with children receiving placebo. The vaccine was found to be effective and had the highest

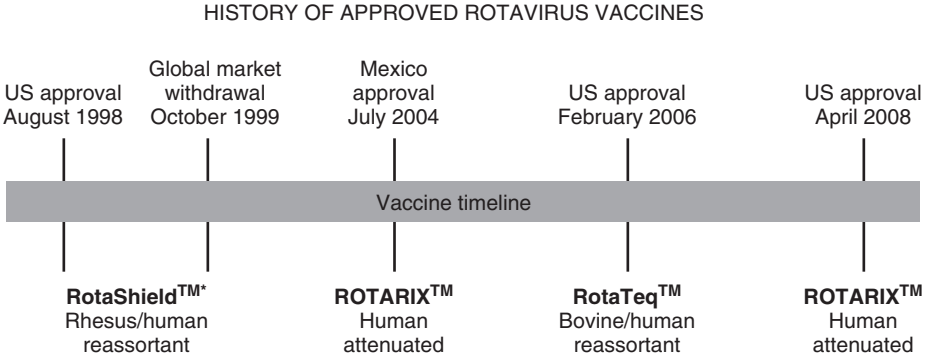

FIGURE 172-3 ■ Time line of first approvals for rotavirus vaccines.

efficacy against more severe disease. Similar results were reported from Finnish studies, except that fever occurred somewhat more commonly and efficacy was somewhat enhanced.[220]

Post-Licensure Effectiveness and Safety. For these reasons, the RRV-TV vaccine, RotaShield, was licensed in the United States in 1998 and recommended for general use in all infants.[1] However, less than 1 year after licensing, 15 cases of intussusception occurring shortly after vaccination were reported to the Vaccine Adverse Events Reporting System.[71] Subsequent analyses led the American Academy of Pediatrics and the Advisory Committee on Immunization Practices to conclude that an association existed between receipt of the vaccine and the development of intussusception and to withdraw the recommendation for use.[4,75] The vaccine was therefore withdrawn by the manufacturer.

The initial publication describing the association reported an increased risk for development of intussusception from 3 to 14 days after receipt of the first dose of vaccine (adjusted odds ratio, 21.7).[318] A smaller risk also existed after receipt of the second dose of vaccine. Researchers estimated that one case of intussusception attributable to vaccination with RRV would occur for every 4670 to 9474 infants vaccinated. Ecologic studies that followed reported a lower vaccine-attributable risk.[317,437] The exact excess burden of intussusception during the life of the child has yet to be defined, but the best estimates are 1 per 10,000 to 12,000.[241,251,376,437] Further analysis revealed that the risk was age related and substantially increased in those older than 90 days.[409,438] The pathogenic mechanism for this association has yet to be defined.[280] The relationship between wild-type rotavirus infection and intussusception is unclear and is discussed in the clinical section.

Despite the withdrawal of RotaShield due to the association with intussusception, the vaccine was found to be effective. In one study, RotaShield was 100 percent effective (95% CI, 75%, 100%) in preventing hospitalization due to rotavirus in children receiving three doses of vaccine. When two doses of vaccine were given, the vaccine was also 100 percent effective (95% CI, 62%, 100%), whereas one dose was 89 percent effective (49%, 97%). A second vaccine effectiveness study in a pediatric practice setting yielded similar results.[375]

RotaTeq (RV5)

History and Development of RotaTeq. The development of RotaTeq began with evaluations of WC3 rotavirus vaccine. WC3 is of bovine origin and appeared to be free of side effects. It replicates poorly in humans, but infants develop a neutralizing antibody response to WC3 and both rotavirus IgA and IgG antibody.[32] The initial studies conducted in Philadelphia appeared promising,[90] but later trials in Cincinnati[52,204] and in less developed countries[162] did not show significant protection. WC3 was therefore used to make reassortants containing rotavirus genes encoding the VP7 and VP4 proteins of human serotypes, which became RotaTeq.

After the WC3 vaccine was shown to be safe but not consistently effective,[32,162] a monovalent vaccine containing the VP7 protein of a human G1 rotavirus was developed. This vaccine was reported to be effective especially against more severe disease during a predominantly serotype G1 outbreak in Rochester.[471] Next, a WC3-based reassortant quadrivalent vaccine including both VP7 and VP4 human rotavirus gene substitutions with G1, G2, G3, or P[8] substitutions and most other segments from WC3 was evaluated. In studies conducted at multiple centers in the United States, this vaccine was shown to be safe and effective against all cases of rotavirus gastroenteritis (75%) and especially against severe cases (100%).[89] In the multiple trials of WC3 and WC3 reassortant vaccines, the viruses replicated less well than RRV and did not induce fever as was noted with RRV and RRV reassortants.

Safety and Efficacy of RotaTeq. The final WC3 reassortant vaccine developed, the pentavalent RotaTeq vaccine, contains five different viruses: one each containing the VP7 of G1, G2, G3, and G4 human rotaviruses and one containing the most common VP4 (P8) gene (Fig. 172-4).[95]

The large pivotal trial of RotaTeq was conducted in more than 70,000 infants in 11 countries. It showed that the vaccine was effective and safe and, unlike RotaShield, did not induce fever.[492] Most important, there was no association with intussusception. Intussusception occurred in six vaccine recipients and five placebo recipients within 42 days after any dose (relative risk, 1.6; 95% CI, 0.4 to 6.4). The vaccine was also highly effective, reducing all G1-G4 rotavirus gastroenteritis by 74.0 percent, severe gastroenteritis by 98.0 percent, and hospitalizations and emergency department visits by 94.5percent. Type-specific efficacy against hospitalization and emergency department visits for rotavirus infections was as follows: G1, 95.1 percent; G2, 87.6 percent; G3, 93.4 percent; G4, 89.1 percent; G9, 100 percent; and G12, 100 percent.

Since the pivotal trial was published, multiple trials of RotaTeq have been conducted around the world.[219] Efficacy has been shown to be sustained for up to 3 years.[487,488] Safety and efficacy were also reported for premature infants.[171] As has been apparent for all rotavirus vaccines, the vaccine appears to be less effective in less developed areas of the world (Fig. 172-5).[17,433,534,535] In the most resource-poor settings, such as Africa and Asia, efficacy has been only 40 to 50 percent in clinical trials.[17,534,535] In general, this correlates with the lower immune response in these settings.[16,535] The reasons for this decrease are not well understood. Possible contributing factors include higher levels of transplacental and breast milk antibody that could inhibit vaccine virus replication, malnutrition, micronutrient deficiencies, interfering gut flora, and differences in the epidemiology.[355] One study evaluated the safety of RotaTeq in HIV-infected and noninfected infants in Kenya and found no significantly increased serious adverse events in HIV-infected children compared with those without infection.[259] However, given the small sample size in the study, there was not sufficient power to fully assess safety in this study.

Post-Licensure Effectiveness and Safety. The effectiveness of RotaTeq vaccine has been substantial wherever

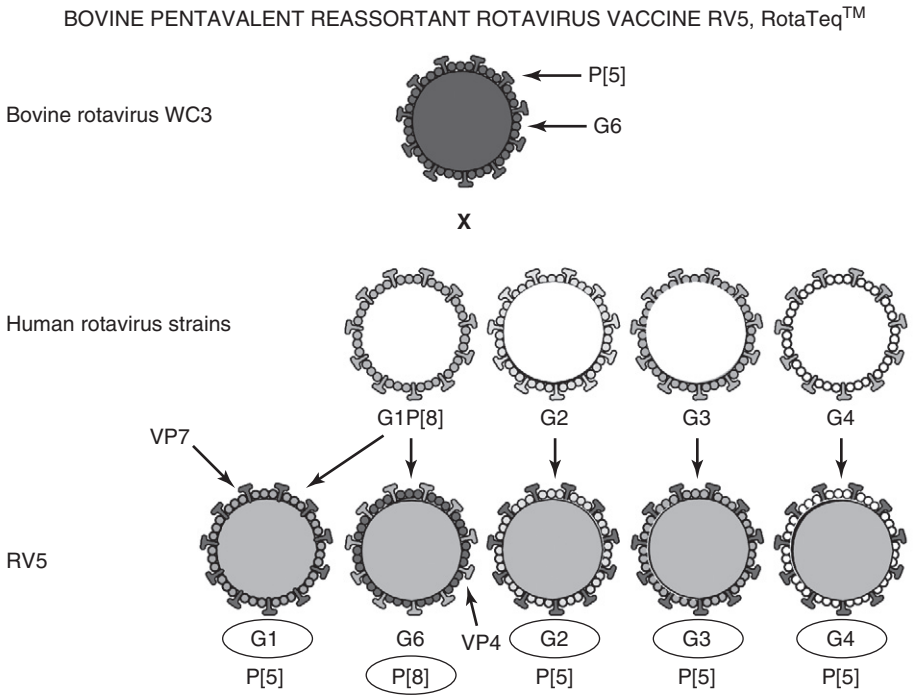

FIGURE 172-4 ■ RotaTeq is composed of five reassortant viruses: one each containing the VP7 of G1, G2, G3, and G4 human rotaviruses and one containing the most common VP4 (P8) gene. The remaining gene segments are derived from the bovine WC3 strain of rotavirus.

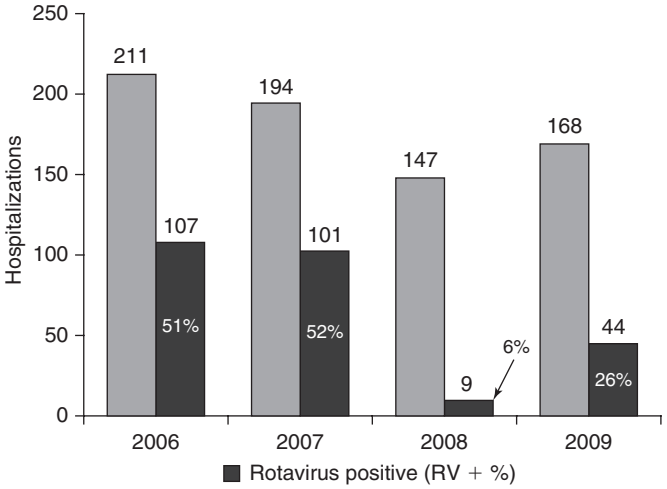

FIGURE 172-5 ■ Impact of rotavirus vaccines in the United States. The number and proportion of hospitalized children younger than 3 years with acute gastroenteritis who tested positive for rotavirus are shown by year from the New Vaccine Surveillance Network.

it has been evaluated.[163,170] Surveillance studies and laboratory monitoring conducted in the United States have shown remarkable reductions in rotavirus disease every year since vaccine introduction (Fig. 172-5).* In fact, in 2009-2010, the threshold for defining a rotavirus season was never achieved.[457] What has been particularly remarkable is the magnitude of the indirect or herd effects observed.[10,274,364] In 2008, shortly after vaccine licensure, reductions of rotavirus disease were seen not only in age-eligible vaccine recipients but in older children as well.[364] Rates of hospitalization for rotavirus gastroenteritis were reduced 87 percent in the 6- to 11-month age group (coverage, 77%), 96 percent in the 12- to 23-month age group (coverage, 46%), and 92 percent in the 24- to 35-month age group (1% coverage). Recent data further suggest that benefits in even older children and adults will be seen.[10,274] Post-licensure vaccine effectiveness studies have shown high levels of protection provided after each dose.[48,49,137,446,499] The large multicenter U.S. study found the effectiveness of RV5 against rotavirus-associated hospitalizations and emergency department visits to be 73 to 74 percent for one dose and similar at 88 percent and 85 to 87 percent for two and three doses, respectively, depending on the comparison group used.[446] In two post-licensure studies conducted in Nicaragua, the three-dose effectiveness of RV5 against severe disease ranged from 58 to 87 percent, depending on the control group chosen for comparison and the definitions used for severity.[288,353]

Monitoring for safety, including intussusception, has been in place since licensure, and the safety of RotaTeq vaccine continues to be monitored in the United States and abroad.* Whereas most studies have found no increased risk of intussusception to date, in one Australian study, there was an excess risk of intussusception in infants 1 month to younger than 3 months of age at 1 to 7 days (relative risk, 5.3) and 1 to 21 days (relative risk, 3.5) after dose one,[63] which is less than was seen in studies of RotaShield. Safety monitoring will continue to

*References 74, 107, 364, 456, 457, 459.

*References 63, 133, 142, 277, 434, 526, 538.

determine if an increased risk of intussusception exists in other countries, including the United States, although, given the large number of doses already used in the United States, any increased risk of intussusception would have to be low.

Rotarix (RV1)

History and Development of Rotarix. Rotarix, the other licensed rotavirus vaccine, is a live oral vaccine based on the attenuated human strain 89-12. Strain 89-12 is a G1P[8] virus, initially obtained from an infant with rotavirus gastroenteritis in Cincinnati, Ohio.[33] The isolate was attenuated by multiple passages in tissue culture; thus all genes are of human origin (Fig. 172-6). Results of the initial multicenter efficacy trial showed that two doses of this vaccine provided 89 percent protection against any rotavirus disease and 100 percent protection from more serious disease.[30]

The 89-12 strain was then purified by limiting dilution and further passaged in tissue culture. The final product, initially called RIX 4414 and later marketed as Rotarix, was evaluated in many studies. Reports from Singapore,[379] Finland,[490] Latin America,[269,411,419] and the United States[129] confirmed the safety and immunogenicity of the vaccine. In the initial efficacy trial of RIX 4414 conducted in Finland over two rotavirus seasons, the vaccine was 72 percent protective against all rotavirus gastroenteritis and 85 percent protective against severe gastroenteritis despite the relatively low dose of vaccine used.[490]

Safety and Efficacy of Rotarix. As with RotaTeq, Rotarix was evaluated in a large safety and efficacy trial. In a study of more than 63,000 infants conducted primarily in several countries in Latin America, the vaccine was found to be effective and safe.[411] The vaccine did not induce fever and, most important, was not associated with intussusception. During the 31-day window after each dose, six vaccine recipients and seven placebo recipients had definite intussusception with no evidence of clustering. Over the duration of the study, there were 16 cases of intussusception in the placebo group compared with 9 in the vaccine group.

In this large study, efficacy was 84.7 percent against severe rotavirus gastroenteritis and 85 percent against rotavirus-related hospitalizations. Efficacy was 91.8 percent against G1[P8] strains and 87.3 percent against the VP4-related G3P[8], G4P[8], and G9P[8] strains. Efficacy against G2P[4] strains in the few subjects infected with these viruses was lower (41.0%).

In a trial of 3994 infants conducted in six European countries, a population more similar to that used for evaluation of RotaTeq, protection was 78.1 percent against rotavirus of any severity, 90.4 percent against severe rotavirus gastroenteritis, and 96 percent against hospitalization due to rotavirus during the combined follow-up period.[489] There was a slightly higher efficacy of 87.1 percent against any severity, 95.8 percent against severe rotavirus, and 100 percent against rotavirus-associated hospitalizations. Efficacies against severe disease due to G3 (93.7%), G4 (95.4%), and G9 (85.0%) rotaviruses were similar to efficacy against G1 (96.4%), and the efficacy against the G2 strain was 85.5 percent.

Because Rotarix is a monovalent G1[P8] vaccine and contains neither G2 nor G9 [P4], there has been interest in examining the efficacy of Rotarix against heterologous strains such as G2[P4] and G9[P4]. In an integrated analysis, efficacy during the combined study period against G2[P4] was 58.3 percent against disease of any severity and 85.5 percent against severe disease.[128,489] More recently, a study in Mexico showed efficacy of 94 percent against rotavirus hospitalizations due to a fully heterotypic G9P[4] rotavirus strain in Mexico.[523] In addition, high rates of efficacy against severe rotavirus disease due to G1 (100%) and non-G1 (94.9%) types were demonstrated in a trial in Singapore, Hong Kong, and Taiwan during a 3-year follow-up period.[378]

When trials of Rotarix have been conducted in resource-poor countries, efficacy has been decreased, similar to what has been reported for RotaTeq (Fig. 172-7). For example, in a study of African infants, overall efficacy against severe disease was 61.2 percent, but it was even lower in infants from Malawi (49.4%) compared with South African infants (76.9%).[281] Despite the lower efficacy seen for both vaccines in these poorer countries, the impact of rotavirus vaccines is still substantial in that more lives can be saved in countries with high rotavirus mortality even with the decreased efficacy of these vaccines. Thus, in the example, more lives would be saved in Malawi compared with South Africa despite the lower efficacy of the rotavirus vaccine in Malawi.

Post-Licensure Effectiveness and Safety of Rotarix. The effectiveness of Rotarix has been verified in several studies.[219,334] In El Salvador, a low- to middle-income country, effectiveness was 76 percent against rotavirus hospitalization.[126] In Brazil, effectiveness was 77 percent against rotavirus hospitalizations or emergency department treatments.[106] Effectiveness was markedly reduced in those older than 12 months. Of interest, in a G9 rotavirus outbreak in Australia, effectiveness was 85 percent.[439] Perhaps most important, vaccination has been associated

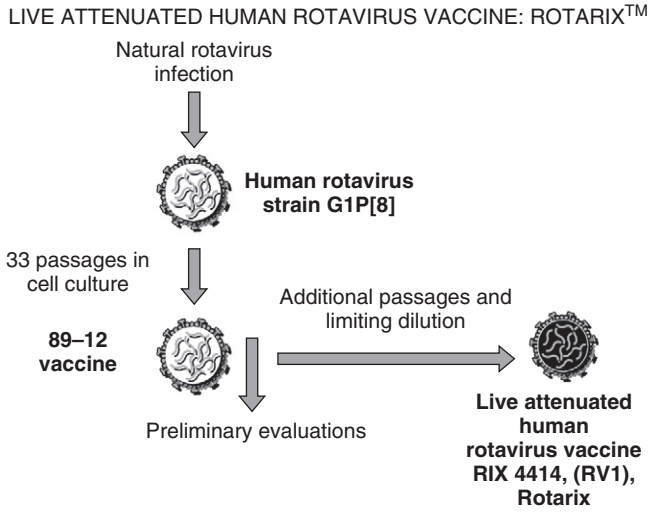

FIGURE 172-6 ■ Rotarix was derived from a human G1P[8] strain obtained from an infant with rotavirus disease. It was passaged 33 times in tissue culture and then evaluated as strain 89-12. After initial studies indicated it was safe and effective, it was purified by limiting dilution and evaluated further as RIX 4414.

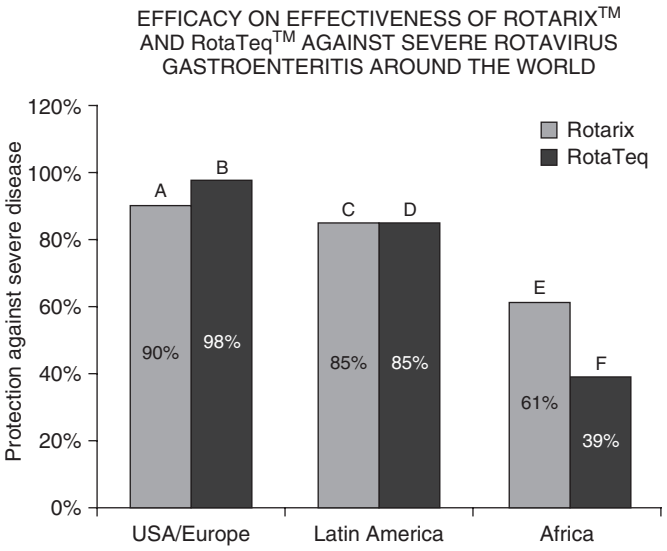

FIGURE 172-7 ■ The efficacy of rotavirus vaccines differs remarkably around the world, decreasing as the socioeconomic conditions decline. Selective studies include Vesikari et al,[489,492] Ruiz-Palacios et al,[411] Mast et al,[288] Madhi et al,[281] and Armah et al.[17]

with reduced mortality from diarrhea-associated disease. In a study in Mexico,[400] diarrhea-related mortality fell from an annual median of 18.1 deaths per 100,000 children younger than 5 years before the introduction of Rotarix vaccination to 11.8 per 100,000 children in 2008 (about 2 years after introduction of the vaccine). Among infants who were 11 months of age or younger, diarrhea-related mortality fell from 61.5 deaths per 100,000 children at baseline to 36.0 per 100,000 children in 2008. These findings were recently extended to show reductions for 3 full years.[401]

Subsequent to licensure and widespread use, several studies evaluating intussusception have been reported. Studies conducted in Mexico and Brazil[357] revealed an increase rate within 7 days after the first dose in Mexico, whereas in Brazil there was no increase after the first dose but a small increase after the second dose. Overall, there was an increased rate of intussusception of about 1 per 51,000 infants in Mexico and 1 per 68,000 infants in Brazil. The authors concluded that "the absolute number of deaths and hospitalizations averted because of vaccination far exceeded the number of intussusception cases that may have been associated with vaccination." In an Australian study that evaluated both RotaTeq and Rotarix, there was an increased relative risk for intussusception of 3.5 and 1.5 for the 1 to 7 days and 1 to 21 days after Rotarix immunization, respectively, in infants 1 month to younger than 3 months of age.[63] At this time, there has not been sufficient time in the United States to assess the risk for intussusception with Rotarix after licensure.

Vaccine Schedule for Both Vaccines

RotaTeq is administered orally in a three-dose series, at ages 2, 4, and 6 months, with the first dose administered by 12 weeks with completion of the series by 32 weeks. Rotarix is administered orally in a two-dose series, at ages 2 and 4 months. Rotarix requires administration of the first dose by 20 weeks and completion of the series by 24 weeks. To simplify and harmonize the schedules, the Advisory Committee on Immunization Practices recommended administration of the first dose of either vaccine by 14 weeks, 6 days and completion of the series by 8 months, with a minimum interval of 4 weeks between doses.[108]

Other Safety Issues for Both Licensed Vaccines

Transmission of Vaccine Virus

Both licensed rotavirus vaccines are live attenuated vaccines and are therefore able to replicate in the intestine of the vaccinee and to be shed in the stool.[379,524] Transmission of both Rotarix in a study of twins[404] and a reassortant virus derived from RotaTeq[361] has been documented.

Porcine Circovirus

In 2010, nucleic acids from an adventitious virus were identified in Rotarix during evaluations of novel viral detection techniques.[497] The detected virus shared 98 percent identity to porcine circovirus (PCV) 1 and included the complete genome. PCV commonly infects pigs; it has been reported to be detected in 5 percent of stool samples from U.S. adults and is thought to be due to dietary consumption of pork products.[497] PCV is not believed to cause illness among humans.[253,264,497] In early 2010, this finding was reported to the U.S. Food and Drug Administration (FDA), which led to the temporary suspension of Rotarix even though there was no evidence of any safety risk.[73] Shortly thereafter, the FDA reported preliminary findings that RotaTeq also contained detectable PCV material from both PCV-1 and PCV-2. Subsequently, on the basis of the available evidence regarding a theoretical risk of PCV infection among humans compared with the observed benefits of rotavirus vaccines in preventing severe acute gastroenteritis among infants, the FDA recommended that clinicians resume use of Rotarix and continue to use RotaTeq.[253] Further investigation suggested that PCV material had been introduced into both rotavirus vaccines through porcine-derived trypsin. This reagent was used in the cell culture growth process of vaccine production, which commenced several years before randomized, controlled, multicenter phase III clinical vaccine trials were conducted for either vaccine.[154,392]

NON-LIVE VACCINES

Because live oral rotavirus vaccines have been much less immunogenic and effective among infants in resource-limited settings, there is continued interest in non-live vaccines. Preclinical studies have included the use of virus-like particles[104,333] that appeared to be protective in mice but not in pigs,[531] DNA vaccines,[82,86] and subunit vaccines, including VP7, VP4, and VP6.[85,500] The

excellent protection in mice, particularly with virus-like particles or VP6 protein, remains to be verified in human studies. The immunogenicity and protection of a candidate inactivated rotavirus vaccine, human strain CDC-9 (G1P[8]) formulated with aluminum phosphate, were also recently evaluated in gnotobiotic piglets. The vaccine was immunogenic and protected animals after oral challenge with a homologous virulent human strain Wa (G1P[8]).[501] It remains to be seen whether these strategies will have a role as alternative approaches.

NEW REFERENCES SINCE THE SIXTH EDITION

3. AAP. Prevention of rotavirus disease: updated guidelines for use of rotavirus vaccine. *Pediatrics* 2009;**123**:1412–20.
8. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. *Cochrane Database Syst Rev* 2010;(11):CD003048.
9. Aminu M, Ameh EA, Geyer A, et al. Role of astrovirus in intussusception in Nigerian infants. *J Trop Pediatr* 2009;**55**:192–4.
10. Anderson EJ, Katz BZ, Polin JA, et al. Rotavirus in adults requiring hospitalization. *J Infect* 2012;**64**:89–95.
11. Anderson EJ, Reddy S, Katz BZ, Noskin GA. Indirect protection and indirect measures of protection from rotavirus in adults. *J Infect Dis* 2012;**205**:1762–4; author reply 1764–5.
12. Anderson EJ, Rupp A, Shulman ST, et al. Impact of rotavirus vaccination on hospital-acquired rotavirus gastroenteritis in children. *Pediatrics* 2011;**127**:e264–70.
16. Armah GE, Breiman RF, Tapia MD, et al. Immunogenicity of the pentavalent rotavirus vaccine in African infants. *Vaccine* 2012; **30**(Suppl. 1):A86–93.
17. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010;**376**:606–14.
20. Bahl R, Saxena M, Bhandari N, et al. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among Indian children. *J Infect Dis* 2009;**200**(Suppl. 1):S277–81.
21. Baker M, Prasad BV. Rotavirus cell entry. *Curr Top Microbiol Immunol* 2010;**343**:121–48.
22. Banyai K, Laszlo B, Duque J, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. *Vaccine* 2012;**30**(Suppl. 1): A122–30.
46. Blutt SE, Miller AD, Salmon SL, et al. IgA is important for clearance and critical for protection from rotavirus infection. *Mucosal Immunol* 2012;**5**:712–19.
48. Boom JA, Tate JE, Sahni LC, et al. Effectiveness of pentavalent rotavirus vaccine in a large urban population in the United States. *Pediatrics* 2010;**125**:e199–207.
49. Boom JA, Tate JE, Sahni LC, et al. Sustained protection from pentavalent rotavirus vaccination during the second year of life at a large, urban United States pediatric hospital. *Pediatr Infect Dis J* 2010;**29**:1133–5.
62. Bucardo F, Rippinger CM, Svensson L, Patton JT. Vaccine-derived NSP2 segment in rotaviruses from vaccinated children with gastroenteritis in Nicaragua. *Infect Genet Evol* 2012;**12**:1282–94.
63. Buttery JP, Danchin MH, Lee KJ, et al. Intussusception following rotavirus vaccine administration: post-marketing surveillance in the National Immunization Program in Australia. *Vaccine* 2011;**29**:3061–6.
64. Buttery JP, Lambert SB, Grimwood K, et al. Reduction in rotavirus-associated acute gastroenteritis following introduction of rotavirus vaccine into Australia's National Childhood vaccine schedule. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S25–9.
67. Carreno-Torres JJ, Gutierrez M, Arias CF, et al. Characterization of viroplasm formation during the early stages of rotavirus infection. *Virology J* 2010;**7**:350.
68. Cashman O, Collins PJ, Lennon G, et al. Molecular characterization of group A rotaviruses detected in children with gastroenteritis in Ireland in 2006-2009. *Epidemiol Infect* 2012;**140**:247–59.
72. CDC. Notes from the field: outbreaks of rotavirus gastroenteritis among elderly adults in two retirement communities—Illinois, 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1456.
73. CDC. Recommendation to temporarily suspend usage of Glaxo-SmithKline Rotarix (Rotavirus Vaccine); March 22, 2010.
74. CDC. Reduction in rotavirus after vaccine introduction—United States, 2000-2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**: 1146–9.
77. Chan J, Nirwati H, Triasih R, et al. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? *Vaccine* 2011;**29**:1242–7.
79. Chang HG, Smith PF, Tserenpuntsag B, et al. Reduction in hospitalizations for diarrhea and rotavirus infections in New York State following introduction of rotavirus vaccine. *Vaccine* 2010; **28**:754–8.
92. Clark HF, Lawley D, Mallette LA, et al. Decline in cases of rotavirus gastroenteritis presenting to The Children's Hospital of Philadelphia after introduction of a pentavalent rotavirus vaccine. *Clin Vaccine Immunol* 2009;**16**:382–6.
93. Clark HF, Lawley D, Matthijnssens J, et al. Sustained decline in cases of rotavirus gastroenteritis presenting to the Children's Hospital of Philadelphia in the new rotavirus vaccine era. *Pediatr Infect Dis J* 2010;**29**:699–702.
97. Clarke MF, Davidson GP, Gold MS, Marshall HS. Direct and indirect impact on rotavirus positive and all-cause gastroenteritis hospitalisations in South Australian children following the introduction of rotavirus vaccination. *Vaccine* 2011;**29**:4663–7.
106. Correia JB, Patel MM, Nakagomi O, et al. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. *J Infect Dis* 2010;**201**:363–9.
107. Cortes JE, Curns AT, Tate JE, et al. Rotavirus vaccine and health care utilization for diarrhea in U.S. children. *N Engl J Med* 2011;**365**:1108–17.
108. Cortese MM, Parashar UD. Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommen Rep* 2009; **58**(RR-2):1–25.
109. Cortese MM, Tate JE, Simonsen L, et al. Reduction in gastroenteritis in United States children and correlation with early rotavirus vaccine uptake from national medical claims databases. *Pediatr Infect Dis J* 2010;**29**:489–94.
115. Curns AT, Panozzo CA, Tate JE, et al. Remarkable postvaccination spatiotemporal changes in United States rotavirus activity. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S54–5.
116. Curns AT, Steiner CA, Barrett M, et al. Reduction in acute gastroenteritis hospitalizations among US children after introduction of rotavirus vaccine: analysis of hospital discharge data from 18 US states. *J Infect Dis* 2010;**201**:1617–24.
117. Dalgic N, Sancar M, Bayraktar B, et al. Probiotic, zinc and lactose-free formula in children with rotavirus diarrhea: are they effective? *Pediatr Int* 2011;**53**:677–82.
125. de Oliveira LH, Danovaro-Holliday MC, Andrus JK, et al. Sentinel hospital surveillance for rotavirus in Latin American and Caribbean countries. *J Infect Dis* 2009;**200**(Suppl. 1):S131–9.
126. de Palma O, Cruz L, Ramos H, et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. *BMJ* 2010;**340**:c2825.
127. de Villiers FP, Driessen M. Clinical neonatal rotavirus infection: association with necrotising enterocolitis. *S Afr Med J* 2012;**102**: 620–4.
128. De Vos B, Han HH, Bouckenooghe A, et al. Live attenuated human rotavirus vaccine, RIX4414, provides clinical protection in infants against rotavirus strains with and without shared G and P genotypes: integrated analysis of randomized controlled trials. *Pediatr Infect Dis J* 2009;**28**:261–6.
131. Desai R, Curns AT, Steiner CA, et al. All-cause gastroenteritis and rotavirus-coded hospitalizations among US children, 2000-2009. *Clin Infect Dis* 2012;**55**:e28–34.
132. Desai R, Haberling D, Holman RC, et al. Impact of rotavirus vaccine on diarrhea-associated disease burden among American Indian and Alaska Native children. *Pediatrics* 2012;**129**:e907–13.
133. Desai R, Parashar UD, Lopman B, et al. Potential intussusception risk versus health benefits from rotavirus vaccination in Latin America. *Clin Infect Dis* 2012;**54**:1397–405.

134. Dickey M, Jamison L, Michaud L, et al. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. *Pediatr Infect Dis J* 2009;**28**:318–21.
137. Eberly MD, Gorman GH, Eide MB, et al. The effect of rotavirus immunization on rotavirus gastroenteritis hospitalization rates in military dependents. *Vaccine* 2011;**29**:650–9.
141. El-Hodhod MA, Nassar MF, Ezz El-Arab S, Ahmed EF. Rotavirus fecal antigen retrieval in infantile intussusception. *Eur J Clin Microbiol Infect Dis* 2008;**27**:879–81.
142. Eng PM, Mast TC, Loughlin J, et al. Incidence of intussusception among infants in a large commercially insured population in the United States. *Pediatr Infect Dis J* 2012;**31**:287–91.
144. Fang SB, Lee HC, Hu JJ, et al. Dose-dependent effect of *Lactobacillus rhamnosus* on quantitative reduction of faecal rotavirus shedding in children. *J Trop Pediatr* 2009;**55**:297–301.
148. Fleming FE, Graham KL, Takada Y, Coulson BS. Determinants of the specificity of rotavirus interactions with the $\alpha_2\beta_1$ integrin. *J Biol Chem* 2011;**286**:6165–74.
158. Gentsch JR, Hull JJ, Teel EN, et al. G and P types of circulating rotavirus strains in the United States during 1996-2005: nine years of prevaccine data. *J Infect Dis* 2009;**200**(Suppl. 1):S99–105.
160. Gentsch JR, Parashar UD, Glass RI. Impact of rotavirus vaccination: the importance of monitoring strains. *Future Microbiol* 2009;**4**:1231–4.
163. Giaquinto C, Dominiak-Felden G, Van Damme P, et al. Summary of effectiveness and impact of rotavirus vaccination with the oral pentavalent rotavirus vaccine: a systematic review of the experience in industrialized countries. *Hum Vaccin* 2011;**7**:734–48.
165. Gladstone BP, Ramani S, Mukhopadhya I, et al. Protective effect of natural rotavirus infection in an Indian birth cohort. *N Engl J Med* 2011;**365**:337–46.
170. Goveia MG, Ciarlet M, Owen KE, Ranucci CS. Development, clinical evaluation, and post-licensure impact of RotaTeq, a pentavalent rotavirus vaccine. *Ann N Y Acad Sci* 2011;**1222**:14–18.
173. Grandy G, Medina M, Soria R, et al. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. *BMC Infect Dis* 2010;**10**:253.
174. Gray J, Iturriza-Gomara M. Rotaviruses. *Methods Mol Biol* 2011;**665**:325–55.
177. Greenberg HB, Estes MK. Rotaviruses: from pathogenesis to vaccination. *Gastroenterology* 2009;**136**:1939–51.
183. Guandalini S. Probiotics for prevention and treatment of diarrhea. *J Clin Gastroenterol* 2011;**45**(Suppl.):S149–53.
185. Guarino A, Lo Vecchio A, Canani RB. Probiotics as prevention and treatment for diarrhea. *Curr Opin Gastroenterol* 2009;**25**:18–23.
189. Guglielmi KM, McDonald SM, Patton JT. Mechanism of intraparticle synthesis of the rotavirus double-stranded RNA genome. *J Biol Chem* 2010;**285**:18123–8.
204. Hu L, Crawford SE, Czako R, et al. Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature* 2012;**485**:256–9.
205. Hu L, Crawford SE, Hyser JM, et al. Rotavirus non-structural proteins: structure and function. *Curr Opin Virol* 2012;**2**:380–8.
206. Huang P, Xia M, Tan M, et al. Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. *J Virol* 2012;**86**:4833–43.
207. Hull JJ, Teel EN, Kerin TK, et al. United States rotavirus strain surveillance from 2005 to 2008: genotype prevalence before and after vaccine introduction. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S42–7.
210. Iturriza-Gomara M, Dallman T, Banyai K, et al. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. *Epidemiol Infect* 2011;**139**:895–909.
218. Jiang B, Patel M, Parashar U. Rotavirus vaccines for global use: what are the remaining issues and challenges? *Hum Vaccin* 2010;**6**:425–7.
219. Jiang V, Jiang B, Tate J, et al. Performance of rotavirus vaccines in developed and developing countries. *Hum Vaccin* 2010;**6**:532–42.
222. Kamiya H, Nakano T, Inoue M, et al. A retrospective evaluation of hospitalizations for acute gastroenteritis at 2 sentinel hospitals in central Japan to estimate the health burden of rotavirus. *J Infect Dis* 2009;**200**(Suppl. 1):S140–6.
225. Kang G, Arora R, Chitambar SD, et al. Multicenter, hospital-based surveillance of rotavirus disease and strains among Indian children aged <5 years. *J Infect Dis* 2009;**200**(Suppl. 1):S147–53.
237. Kirkwood CD, Roczo S, Boniface K, et al. Australian Rotavirus Surveillance Group. Australian Rotavirus Surveillance Program annual report, 2010/11. *Commun Dis Intell Q Rep* 2011;**35**:281–7.
238. Kirkwood CD, Boniface K, Bishop RF, Barnes GL. Australian rotavirus surveillance program: annual report, 2009/2010. *Commun Dis Intell Q Rep* 2010;**34**:427–34.
239. Kiulia NM, Nyaundi JK, Peenze I, et al. Rotavirus infections among HIV-infected children in Nairobi, Kenya. *J Trop Pediatr* 2009;**55**:318–23
248. Koroglu M, Yakupogullari Y, Otlu B, et al. A waterborne outbreak of epidemic diarrhea due to group A rotavirus in Malatya, Turkey. *New Microbiol* 2011;**34**:17–24.
253. Kuehn BM. FDA: Benefits of rotavirus vaccination outweigh potential contamination risk. *JAMA* 2010;**304**:30–1.
258. Lappalainen S, Ylitalo S, Arola A, et al. Simultaneous presence of human herpesvirus 6 and adenovirus infections in intestinal intussusception of young children. *Acta Paediatr* 2012;**101**:663–70.
259. Laserson KF, Nyakundi D, Feikin DR, et al. Safety of the pentavalent rotavirus vaccine (PRV), RotaTeq, in Kenya, including among HIV-infected and HIV-exposed infants. *Vaccine* 2012; **30**(Suppl. 1):A61–70.
261. Lehert P, Cheron G, Calatayud GA, et al. Racecadotril for childhood gastroenteritis: an individual patient data meta-analysis. *Dig Liver Dis* 2011;**43**:707–13.
264. Li L, Kapoor A, Slikas B, et al. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. *J Virol* 2010;**84**:1674–82.
265. Li Z, Baker ML, Jiang W, et al. Rotavirus architecture at subnanometer resolution. *J Virol* 2009;**83**:1754–66.
268. Linhares AC, Stupka JA, Ciapponi A, et al. Burden and typing of rotavirus group A in Latin America and the Caribbean: systematic review and meta-analysis. *Rev Med Virol* 2011; Mar 7.
272. Liu Y, Huang P, Tan M, et al. Rotavirus VP8*: phylogeny, host range, and interaction with histo-blood group antigens. *J Virol* 2012;**86**:9899–910.
274. Lopman BA, Curns AT, Yen C, Parashar UD. Infant rotavirus vaccination may provide indirect protection to older children and adults in the United States. *J Infect Dis* 2011;**204**:980–6.
275. Lopman BA, Pitzer VE, Sarkar R, et al. Understanding reduced rotavirus vaccine efficacy in low socio-economic settings. *PloS One* 2012;**7**:e41720.
277. Loughlin J, Mast TC, Doherty MC, et al. Postmarketing evaluation of the short-term safety of the pentavalent rotavirus vaccine. *Pediatr Infect Dis J* 2012;**31**:292–6.
281. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010;**362**:289–98.
288. Mast TC, Khawaja S, Espinoza F, et al. Case-control study of the effectiveness of vaccination with pentavalent rotavirus vaccine in Nicaragua. *Pediatr Infect Dis J* 2011;**30**:e209–15.
289. Mast TC, Walter EB, Bulotsky M, et al. Burden of childhood rotavirus disease on health systems in the United States. *Pediatr Infect Dis J* 2010;**29**:e19–25.
293. Matthijnssens J, Ciarlet M, Heiman E, et al. Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1–like and bovine rotavirus strains. *J Virol* 2008;**82**:3204–19.
296. McClain B, Settembre E, Temple BR, et al. X-ray crystal structure of the rotavirus inner capsid particle at 3.8 Å resolution. *J Mol Biol* 2010;**397**:587–99.
297. McDonald SM, Patton JT. Assortment and packaging of the segmented rotavirus genome. *Trends Microbiol* 2011;**19**:136–44.
298. McDonald SM, Patton JT. Rotavirus VP2 core shell regions critical for viral polymerase activation. *J Virol* 2011;**85**:3095–105.
309. Miles MG, Lewis KD, Kang G, et al. A systematic review of rotavirus strain diversity in India, Bangladesh, and Pakistan. *Vaccine* 2012;**30**(Suppl. 1):A131–9.
310. Mirzayeva R, Cortese MM, Mosina L, et al. Rotavirus burden among children in the newly independent states of the former Union of Soviet Socialist Republics: literature review and first-year results from the rotavirus surveillance network. *J Infect Dis* 2009;**200**(Suppl. 1):S203–14.
313. Molto Y, Cortes JE, De Oliveira LH, et al. Reduction of diarrhea-associated hospitalizations among children aged <5 years in

Panama following the introduction of rotavirus vaccine. *Pediatr Infect Dis J* 2011;**30**():S16–20.
314. Moon SS, Wang Y, Shane AL, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J* 2010;**29**:919–23.
315. Mukherjee A, Chattopadhyay S, Bagchi P, et al. Surveillance and molecular characterization of rotavirus strains circulating in Manipur, north-eastern India: increasing prevalence of emerging G12 strains. *Infect Genet Evol* 2010;**10**:311–20.
319. Mwenda JM, Ntoto KM, Abebe A, et al. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. *J Infect Dis* 2010;**202**(Suppl.):S5–11.
324. Nelson EA, Widdowson MA, Kilgore PE, et al. A decade of the Asian Rotavirus Surveillance Network: achievements and future directions. *Vaccine* 2009;**27**(Suppl. 5):F1–3.
325. Neuzil KM, Parashar UD, Steele AD. Rotavirus vaccines for children in developing countries: understanding the science, maximizing the impact, and sustaining the effort. *Vaccine* 2012; **30**(Suppl. 1):A1–2.
334. O'Ryan M, Lucero Y, Linhares AC. Rotarix: vaccine performance 6 years postlicensure. *Expert Rev Vaccines* 2011;**10**:1645–59.
341. Ogden KM, Ramanathan HN, Patton JT. Residues of the rotavirus RNA-dependent RNA polymerase template entry tunnel that mediate RNA recognition and genome replication. *J Virol* 2011; **85**:1958–69.
342. Okimoto S, Hyodo S, Yamamoto M, et al. Association of viral isolates from stool samples with intussusception in children. *Int J Infect Dis* 2011;**15**:e641–5.
345. Pammi M, Haque KN. Oral immunoglobulin for the prevention of rotavirus infection in low birth weight infants. *Cochrane Database Syst Rev* 2011;(11):CD003740.
346. Pammi M, Haque KN. Oral immunoglobulin for the treatment of rotavirus diarrhea in low birth weight infants. *Cochrane Database Syst Rev* 2011;(10):CD003742.
349. Parashar UD, Burton A, Lanata C, et al. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis* 2009;**200**(Suppl. 1):S9–15.
352. Park SI, Matthijnssens J, Saif LJ, et al. Reassortment among bovine, porcine and human rotavirus strains results in G8P[7] and G6P[7] strains isolated from cattle in South Korea. *Vet Microbiol* 2011;**152**:55–66.
353. Patel M, Pedreira C, De Oliveira LH, et al. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. *JAMA* 2009;**301**:2243–51.
354. Patel M, Rench MA, Boom JA, et al. Detection of rotavirus antigenemia in routinely obtained serum specimens to augment surveillance and vaccine effectiveness evaluations. *Pediatr Infect Dis J* 2010;**29**:836–9.
355. Patel M, Shane AL, Parashar UD, et al. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis* 2009;**200**(Suppl. 1):S39–48.
356. Patel MM, Clark AD, Glass RI, et al. Broadening the age restriction for initiating rotavirus vaccination in regions with high rotavirus mortality: benefits of mortality reduction versus risk of fatal intussusception. *Vaccine* 2009;**27**:2916–22.
357. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med* 2011;**364**:2283–92.
358. Patel MM, Steele D, Gentsch JR, et al. Real-world impact of rotavirus vaccination. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S1–5.
359. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med* 2012;**13**:85–97.
361. Payne DC, Edwards KM, Bowen MD, et al. Sibling transmission of vaccine-derived rotavirus (RotaTeq) associated with rotavirus gastroenteritis. *Pediatrics* 2010;**125**:e438–41.
364. Payne DC, Staat MA, Edwards KM, et al. Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US counties, 2006-2009. *Clin Infect Dis* 2011;**53**:245–53.
365. Payne DC, Szilagyi PG, Staat MA, et al. Secular variation in United States rotavirus disease rates and serotypes: implications for assessing the rotavirus vaccination program. *Pediatr Infect Dis J* 2009;**28**:948–53.
378. Phua KB, Lim FS, Lau YL, et al. Rotavirus vaccine RIX4414 efficacy sustained during the third year of life: a randomized clinical trial in an Asian population. *Vaccine* 2012;**30**:4552–7.
380. Pickering LK. The day care center diarrhea dilemma. *Am J Public Health* 1986;**76**:623–4.
383. Pitzer VE, Viboud C, Lopman BA, et al. Influence of birth rates and transmission rates on the global seasonality of rotavirus incidence. *J R Soc Interface* 2011;**8**:1584–93.
388. Rahman S, Higo-Moriguchi K, Htun KW, et al. Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients. *Vaccine* 2012;**30**:4661–9.
392. Ranucci C. RotaTeq (Rotavirus Vaccine, Live, Oral, Pentavalent): Update on Porcine Circovirus (PCV). Presented at Advisory Committee on Immunization Practices; Atlanta, Georgia; 2010.
400. Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *N Engl J Med* 2010;**362**:299–305.
401. Richardson V, Parashar U, Patel M. Childhood diarrhea deaths after rotavirus vaccination in Mexico. *N Engl J Med* 2011;**365**: 772–3.
404. Rivera L, Pena LM, Stainier I, et al. Horizontal transmission of a human rotavirus vaccine strain—a randomized, placebo-controlled study in twins. *Vaccine* 2011;**29**:9508–13.
410. Ruggeri FM, Delogu R, Petouchoff T, et al. Molecular characterization of rotavirus strains from children with diarrhea in Italy, 2007-2009. *J Med Virol* 2011;**83**:1657–68.
431. Settembre EC, Chen JZ, Dormitzer PR, et al. Atomic model of an infectious rotavirus particle. *EMBO J* 2011;**30**:408–16.
432. Shim JO, Baek IH, Le VP, et al. Molecular characterization of rotavirus diarrhea among children in South Korea: detection of an unusual G11 strain. *Arch Virol* 2011;**156**:887–92.
433. Shin S, Anh DD, Zaman K, et al. Immunogenicity of the pentavalent rotavirus vaccine among infants in two developing countries in Asia, Bangladesh and Vietnam. *Vaccine* 2012;**30**(Suppl. 1): A106–13.
434. Shui IM, Baggs J, Patel M, et al. Risk of intussusception following administration of a pentavalent rotavirus vaccine in US infants. *JAMA* 2012;**307**:598–604.
439. Snelling TL, Schultz R, Graham J, et al. Rotavirus and the indigenous children of the Australian outback: monovalent vaccine effective in a high-burden setting. *Clin Infect Dis* 2009;**49**:428–31.
446. Staat MA, Payne DC, Donauer S, et al. Effectiveness of pentavalent rotavirus vaccine against severe disease. *Pediatrics* 2011; **128**:e267–75.
447. Staat MA, Rice MA, Donauer S, et al. Estimating the rotavirus hospitalization disease burden and trends, using capture-recapture methods. *Pediatr Infect Dis J* 2010;**29**:1083–6.
448. Steele AD, Cunliffe N, Tumbo J, et al. A review of rotavirus infection in and vaccination of human immunodeficiency virus–infected children. *J Infect Dis* 2009;**200**(Suppl. 1):S57–62.
454. Tapisiz A, Karahan ZC, Ciftci E, et al. Changing patterns of rotavirus genotypes in Turkey. *Curr Microbiol* 2011;**63**:517–22.
455. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:136–41.
456. Tate JE, Cortese MM, Payne DC, et al. Uptake, impact, and effectiveness of rotavirus vaccination in the United States: review of the first 3 years of postlicensure data. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S56–60.
457. Tate JE, Mutuc JD, Panozzo CA, et al. Sustained decline in rotavirus detections in the United States following the introduction of rotavirus vaccine in 2006. *Pediatr Infect Dis J* 2011;**30**(Suppl.): S30–34.
458. Tate JE, Panozzo CA, Payne DC, et al. Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine. *Pediatrics* 2009;**124**:465–71.
459. Tate JE, Parashar UD. Monitoring impact and effectiveness of rotavirus vaccination. *Expert Rev Vaccines* 2011;**10**:1123–5.
460. Tate JE, Patel MM, Cortese MM, et al. Remaining issues and challenges for rotavirus vaccine in preventing global childhood diarrheal morbidity and mortality. *Expert Rev Vaccines* 2012;**11**: 211–20.
461. Teran CG, Teran-Escalera CN, Villarroel P. Nitazoxanide vs. probiotics for the treatment of acute rotavirus diarrhea in children: a randomized, single-blind, controlled trial in Bolivian children. *Int J Infect Dis* 2009;**13**:518–23.

468. Tra My PV, Rabaa MA, Vinh H, et al. The emergence of rotavirus G12 and the prevalence of enteric viruses in hospitalized pediatric diarrheal patients in southern Vietnam. *Am J Trop Med Hyg* 2011;**85**:768–75.
469. Trask SD, McDonald SM, Patton JT. Structural insights into the coupling of virion assembly and rotavirus replication. *Nat Rev Microbiol* 2012;**10**:165–77.
470. Trask SD, Ogden KM, Patton JT. Interactions among capsid proteins orchestrate rotavirus particle functions. *Curr Opin Virol* 2012;**2**:373–9.
472. Troupin C, Dehee A, Schnuriger A, et al. Rearranged genomic RNA segments offer a new approach to the reverse genetics of rotaviruses. *J Virol* 2010;**84**:6711–19.
487. Vesikari T, Karvonen A, Ferrante SA, Ciarlet M. Efficacy of the pentavalent rotavirus vaccine, RotaTeq, in Finnish infants up to 3 years of age: the Finnish Extension Study. *Eur J Pediatr* 2010; **169**:1379–86.
488. Vesikari T, Karvonen A, Ferrante SA, et al. Sustained efficacy of the pentavalent rotavirus vaccine, RV5, up to 3.1 years following the last dose of vaccine. *Pediatr Infect Dis J* 2010;**29**:957–63.
497. Victoria JG, Wang C, Jones MS, et al. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. *J Virol* 2010;**84**:6033–40.
498. Vizzi E, Pineros O, Gonzalez GG, et al. Genotyping of human rotaviruses circulating among children with diarrhea in Valencia, Venezuela. *J Med Virol* 2011;**83**:2225–32.
499. Wang FT, Mast TC, Glass RJ, et al. Effectiveness of the pentavalent rotavirus vaccine in preventing gastroenteritis in the United States. *Pediatrics* 2010;**125**:e208–13.
501. Wang Y, Azevedo M, Saif LJ, et al. Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets. *Vaccine* 2010;**28**:5432–6.
513. Weinberg GA, Payne DC, Teel EN, et al. First reports of human rotavirus G8P[4] gastroenteritis in the United States. *J Clin Microbiol* 2012;**50**:1118–21.
515. WHO. Rotavirus surveillance worldwide—2009. *Wkly Epidemiol Rec* 2011;**86**:174–6.
517. Widdowson MA, Steele D, Vojdani J, et al. Global rotavirus surveillance: determining the need and measuring the impact of rotavirus vaccines. *J Infect Dis* 2009;**200**(Suppl. 1):S1–8.
518. Wolf M, Vo PT, Greenberg HB. Rhesus rotavirus entry into a polarized epithelium is endocytosis dependent and involves sequential VP4 conformational changes. *J Virol* 2011;**85**:2492–503.
522. Yen C, Armero Guardado JA, Alberto P, et al. Decline in rotavirus hospitalizations and health care visits for childhood diarrhea following rotavirus vaccination in El Salvador. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S6–10.
523. Yen C, Figueroa JR, Uribe ES, et al. Monovalent rotavirus vaccine provides protection against an emerging fully heterotypic G9P[4] rotavirus strain in Mexico. *J Infect Dis* 2011;**204**:783–6.
524. Yen C, Jakob K, Esona MD, et al. Detection of fecal shedding of rotavirus vaccine in infants following their first dose of pentavalent rotavirus vaccine. *Vaccine* 2011;**29**:4151–5.
525. Yen C, Tate JE, Patel MM, et al. Rotavirus vaccines: update on global impact and future priorities. *Hum Vaccin* 2011;**7**:1282–90.
526. Yen C, Tate JE, Steiner CA, et al. Trends in intussusception hospitalizations among US infants before and after implementation of the rotavirus vaccination program, 2000-2009. *J Infect Dis* 2012;**206**:41–8.
527. Yen C, Tate JE, Wenk JD, et al. Diarrhea-associated hospitalizations among US children over 2 rotavirus seasons after vaccine introduction. *Pediatrics* 2011;**127**:e9–15.
529. Yoder JD, Trask SD, Vo TP, et al. VP5* rearranges when rotavirus uncoats. *J Virol* 2009;**83**:11372–7.
534. Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010;**376**:615–23.
535. Zaman K, Yunus M, Arifeen SE, et al. Methodology and lessons-learned from the efficacy clinical trial of the pentavalent rotavirus vaccine in Bangladesh. *Vaccine* 2012;**30**(Suppl. 1):A94–100.
538. Zickafoose JS, Benneyworth BD, Riebschleger MP, et al. Hospitalizations for intussusception before and after the reintroduction of rotavirus vaccine in the United States. *Arch Pediatr Adolesc Med* 2012;**166**:350–5.

The full reference list for this chapter is available at expertconsult.com.

RNA VIRUSES

SUBSECTION 4 *TOGAVIRIDAE*

CHAPTER 173

RUBELLA VIRUS

James D. Cherry • Kristina Adachi

Rubella (German measles) is a generally mild, exanthematous, infectious illness in which morbidity and mortality in children usually are minimal. However, infection in pregnancy may result in fetal infection, which usually is associated with considerable adversity for the developing infant. The rubella virus has only one known type.

HISTORY

In ancient history, rubella as a disease is lost among the other prominent exanthematous diseases (e.g., scarlet fever, measles, and smallpox). In an extensive review, Griffith[229] suggested that rubella was known to the early

REFERENCES

1. AAP. Prevention of rotavirus disease: guidelines for use of rotavirus vaccine. *Pediatrics* 1998;**102**:1483–91.
2. AAP. Prevention of rotavirus disease: guidelines for use of rotavirus vaccine. *Pediatrics* 2007;**119**:171–82.
3. AAP. Prevention of rotavirus disease: updated guidelines for use of rotavirus vaccine. *Pediatrics* 2009;**123**:1412–20.
4. Abramson JS, Baker CJ, Fisher MC, et al. Possible association of intussusception with rotavirus vaccination. American Academy of Pediatrics. Committee on Infectious Diseases. *Pediatrics* 1999; **104**(Pt 1):575.
5. Adah MI, Wade A, Oseto M, et al. Detection of human group C rotaviruses in Nigeria and sequence analysis of their genes encoding VP4, VP6, and VP7 proteins. *J Med Virol* 2002;**66**:269–75.
6. Adams WR, Kraft LM. Electron-microscopic study of the intestinal epithelium of mice infected with the agent of epizootic diarrhea of infant mice (EDIM virus). *Am J Pathol* 1967;**51**:39–60.
7. Adams WR, Kraft LM. Epizootic diarrhea of infant mice: identification of the etiologic agent. *Science* 1963;**141**:359–60.
8. Allen SJ, Martinez EG, Gregorio GV, Dans LF. Probiotics for treating acute infectious diarrhoea. *Cochrane Database Syst Rev* 2010;(11):CD003048.
9. Aminu M, Ameh EA, Geyer A, et al. Role of astrovirus in intussusception in Nigerian infants. *J Trop Pediatr* 2009;**55**:192–4.
10. Anderson EJ, Katz BZ, Polin JA, et al. Rotavirus in adults requiring hospitalization. *J Infect* 2012;**64**:89–95.
11. Anderson EJ, Reddy S, Katz BZ, Noskin GA. Indirect protection and indirect measures of protection from rotavirus in adults. *J Infect Dis* 2012;**205**:1762–4; author reply 1764–5.
12. Anderson EJ, Rupp A, Shulman ST, et al. Impact of rotavirus vaccination on hospital-acquired rotavirus gastroenteritis in children. *Pediatrics* 2011;**127**:e264–70.
13. Anderson EJ, Weber SG. Rotavirus infection in adults. *Lancet Infect Dis* 2004;**4**:91–9.
14. Ansari SA, Springthorpe VS, Sattar SA. Survival and vehicular spread of human rotaviruses: possible relation to seasonality of outbreaks. *Rev Infect Dis* 1991;**13**:448–61.
15. Arias CF, Romero P, Alvarez V, Lopez S. Trypsin activation pathway of rotavirus infectivity. *J Virol* 1996;**70**:5832–9.
16. Armah GE, Breiman RF, Tapia MD, et al. Immunogenicity of the pentavalent rotavirus vaccine in African infants. *Vaccine* 2012; **30**(Suppl. 1):A86–93.
17. Armah GE, Sow SO, Breiman RF, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in sub-Saharan Africa: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010;**376**:606–14.
18. Asano Y, Yoshikawa T, Suga S, et al. Simultaneous occurrence of human herpesvirus 6 infection and intussusception in three infants. *Pediatr Infect Dis J* 1991;**10**:335–7.
19. Asensi MT, Martinez-Costa C, Buesa J. Anti-rotavirus antibodies in human milk: quantification and neutralizing activity. *J Pediatr Gastroenterol Nutr* 2006;**42**:560–7.
20. Bahl R, Saxena M, Bhandari N, et al. Population-based incidence of intussusception and a case-control study to examine the association of intussusception with natural rotavirus infection among Indian children. *J Infect Dis* 2009;**200**(Suppl. 1):S277–81.
21. Baker M, Prasad BV. Rotavirus cell entry. *Curr Top Microbiol Immunol* 2010;**343**:121–48.
22. Banyai K, Laszlo B, Duque J, et al. Systematic review of regional and temporal trends in global rotavirus strain diversity in the pre rotavirus vaccine era: insights for understanding the impact of rotavirus vaccination programs. *Vaccine* 2012;**30**(Suppl. 1):A122–30.
23. Barnes GL, Lund JS, Adams L, et al. Phase 1 trial of a candidate rotavirus vaccine (RV3) derived from a human neonate. *J Paediatr Child Health* 1997;**33**:300–4.
24. Bass DM, Mackow ER, Greenberg HB. Identification and partial characterization of a rhesus rotavirus binding glycoprotein on murine enterocytes. *Virology* 1991;**183**:602–10.
25. Beattie RM, Vieira MC, Phillips AD, et al. Carbohydrate intolerance after rotavirus gastroenteritis: a rare problem in the 1990s. *Arch Dis Child* 1995;**72**:466.
26. Bell LM, Clark HF, O'Brien EA, et al. Gastroenteritis caused by human rotaviruses (serotype three) in a suckling mouse model. *Proc Soc Exp Biol Med* 1987;**184**:127–32.
27. Bell TM, Steyn JH. Viruses in lymph nodes of children with mesenteric adenitis and intussusception. *Br Med J* 1962;**2**: 700–2.
28. Bernstein DI, Glass RI, Rodgers G, et al. Evaluation of rhesus rotavirus monovalent and tetravalent reassortant vaccines in US children. US Rotavirus Vaccine Efficacy Group. *JAMA* 1995;**273**: 1191–6.
29. Bernstein DI, McNeal MM, Schiff GM, Ward RL. Induction and persistence of local rotavirus antibodies in relation to serum antibodies. *J Med Virol* 1989;**28**:90–5.
30. Bernstein DI, Sack DA, Rothstein E, et al. Efficacy of live, attenuated, human rotavirus vaccine 89-12 in infants: a randomised placebo-controlled trial. *Lancet* 1999;**354**:287–90.
31. Bernstein DI, Sander DS, Smith VE, et al. Protection from rotavirus reinfection: 2-year prospective study. *J Infect Dis* 1991;**164**: 277–83.
32. Bernstein DI, Smith VE, Sander DS, et al. Evaluation of WC3 rotavirus vaccine and correlates of protection in healthy infants. *J Infect Dis* 1990;**162**:1055–62.
33. Bernstein DI, Smith VE, Sherwood JR, et al. Safety and immunogenicity of live, attenuated human rotavirus vaccine 89-12. *Vaccine* 1998;**16**:381–7.
34. Bhan MK, Lew JF, Sazawal S, et al. Protection conferred by neonatal rotavirus infection against subsequent rotavirus diarrhea. *J Infect Dis* 1993;**168**:282–7.
35. Bhandari N, Sharma P, Glass RI, et al. Safety and immunogenicity of two live attenuated human rotavirus vaccine candidates, 116E and I321, in infants: results of a randomised controlled trial. *Vaccine* 2006;**24**:5817–23.
36. Bhisitkul DM, Todd KM, Listernick R. Adenovirus infection and childhood intussusception. *Am J Dis Child* 1992;**146**: 1331–3.
37. Bines JE, Liem NT, Justice FA, et al. Risk factors for intussusception in infants in Vietnam and Australia: adenovirus implicated, but not rotavirus. *J Pediatr* 2006;**149**:452–60.
38. Bishop RF, Barnes GL, Cipriani E, Lund JS. Clinical immunity after neonatal rotavirus infection. A prospective longitudinal study in young children. *N Engl J Med* 1983;**309**:72–6.
39. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Detection of a new virus by electron microscopy of faecal extracts from children with acute gastroenteritis. *Lancet* 1974;**1**:149–51.
40. Bishop RF, Davidson GP, Holmes IH, Ruck BJ. Virus particles in epithelial cells of duodenal mucosa from children with acute non-bacterial gastroenteritis. *Lancet* 1973;**2**:1281–3.
41. Black RE, Greenberg HB, Kapikian AZ, et al. Acquisition of serum antibody to Norwalk virus and rotavirus and relation to diarrhea in a longitudinal study of young children in rural Bangladesh. *J Infect Dis* 1982;**145**:483–9.
42. Black RE, Merson MH, Huq I, et al. Incidence and severity of rotavirus and *Escherichia coli* diarrhoea in rural Bangladesh. Implications for vaccine development. *Lancet* 1981;**1**:141–3.
43. Blutt SE, Conner ME. Rotavirus: to the gut and beyond! *Curr Opin Gastroenterol* 2007;**23**:39–43.
44. Blutt SE, Kirkwood CD, Parreno V, et al. Rotavirus antigenaemia and viraemia: a common event? *Lancet* 2003;**362**:1445–9.
45. Blutt SE, Matson DO, Crawford SE, et al. Rotavirus antigenemia in children is associated with viremia. *PLoS Med* 2007;**4**:e121.
46. Blutt SE, Miller AD, Salmon SL, et al. IgA is important for clearance and critical for protection from rotavirus infection. *Mucosal Immunol* 2012;**5**:712–19.
47. Bode CO, Omilabu SA. Viral isolates of intussusception in Nigerian infants. *S Afr J Surg* 2002;**40**:57–8.
48. Boom JA, Tate JE, Sahni LC, et al. Effectiveness of pentavalent rotavirus vaccine in a large urban population in the United States. *Pediatrics* 2010;**125**:e199–207.
49. Boom JA, Tate JE, Sahni LC, et al. Sustained protection from pentavalent rotavirus vaccination during the second year of life at a large, urban United States pediatric hospital. *Pediatr Infect Dis J* 2010;**29**:1133–5.
50. Boshuizen JA, Reimerink JH, Korteland-van Male AM, et al. Changes in small intestinal homeostasis, morphology, and gene expression during rotavirus infection of infant mice. *J Virol* 2003;**77**:13005–16.
51. Brandt CD, Kim HW, Rodriguez WJ, et al. Pediatric viral gastroenteritis during eight years of study. *J Clin Microbiol* 1983; **18**:71–8.

52. Bresee J, Fang ZY, Wang B, et al. First report from the Asian Rotavirus Surveillance Network. *Emerg Infect Dis* 2004;**10**:988–95.
53. Bridger JC. Detection by electron microscopy of caliciviruses, astroviruses and rotavirus-like particles in the faeces of piglets with diarrhoea. *Vet Rec* 1980;**107**:532–3.
54. Bridger JC, Brown JF. Development of immunity to porcine rotavirus in piglets protected from disease by bovine colostrum. *Infect Immun* 1981;**31**:906–10.
55. Bridger JC, Hall GA, Parsons KR. A study of the basis of virulence variation of bovine rotaviruses. *Vet Microbiol* 1992;**33**:169–74.
56. Bridger JC, Oldham G, Howard C, et al. In vivo depletion of CD8+ or BOWCI+ lymphocytes increases primary rotavirus excretion in calves. Fourth International Symposium of Double-Stranded RNA Viruses; Scottsdale, Arizona; 1987.
57. Bridger JC, Tauscher GI, Desselberger U. Viral determinants of rotavirus pathogenicity in pigs: evidence that the fourth gene of a porcine rotavirus confers diarrhea in the homologous host. *J Virol* 1998;**72**:6929–31.
58. Broome RL, Vo PT, Ward RL, et al. Murine rotavirus genes encoding outer capsid proteins VP4 and VP7 are not major determinants of host range restriction and virulence. *J Virol* 1993;**67**:2448–55.
59. Browne EP, Bellamy AR, Taylor JA. Membrane-destabilizing activity of rotavirus NSP4 is mediated by a membrane-proximal amphipathic domain. *J Gen Virol* 2000;**81**(Pt 8):1955–9.
60. Brunet JP, Cotte-Laffitte J, Linxe C, et al. Rotavirus infection induces an increase in intracellular calcium concentration in human intestinal epithelial cells: role in microvillar actin alteration. *J Virol* 2000;**74**:2323–32.
61. Brunet JP, Jourdan N, Cotte-Laffitte J, et al. Rotavirus infection induces cytoskeleton disorganization in human intestinal epithelial cells: implication of an increase in intracellular calcium concentration. *J Virol* 2000;**74**:10801–6.
62. Bucardo F, Rippinger CM, Svensson L, Patton JT. Vaccine-derived NSP2 segment in rotaviruses from vaccinated children with gastroenteritis in Nicaragua. *Infect Genet Evol* 2012;**12**:1282–94.
63. Buttery JP, Danchin MH, Lee KJ, et al. Intussusception following rotavirus vaccine administration: post-marketing surveillance in the National Immunization Program in Australia. *Vaccine* 2011;**29**:3061–6.
64. Buttery JP, Lambert SB, Grimwood K, et al. Reduction in rotavirus-associated acute gastroenteritis following introduction of rotavirus vaccine into Australia's National Childhood vaccine schedule. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S25–9.
65. Butz AM, Fosarelli P, Dick J, Cusack T, Yolken R. Prevalence of rotavirus on high-risk fomites in day-care facilities. *Pediatrics* 1993;**92**:202–5.
66. Cameron DJ, Bishop RF, Davidson GP, et al. Rotavirus infections in obstetric hospitals [letter]. *Lancet* 1975;**2**:124–5.
67. Carreno-Torres JJ, Gutierrez M, Arias CF, et al. Characterization of viroplasm formation during the early stages of rotavirus infection. *Virology J* 2010;**7**:350.
68. Cashman O, Collins PJ, Lennon G, et al. Molecular characterization of group A rotaviruses detected in children with gastroenteritis in Ireland in 2006-2009. *Epidemiol Infect* 2012;**140**:247–59.
69. Castello AA, Arguelles MH, Villegas GA, et al. Incidence and prevalence of human group C rotavirus infections in Argentina. *J Med Virol* 2002;**67**:106–12.
70. CDC. Delayed onset and diminished magnitude of rotavirus activity—United States, November 2007–May 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:697–700.
71. CDC. Intussusception among recipients of rotavirus vaccine—United States, 1998-1999. *MMWR Morb Mortal Wkly Rep* 1999;**48**:577–81.
72. CDC. Notes from the field: outbreaks of rotavirus gastroenteritis among elderly adults in two retirement communities—Illinois, 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1456.
73. CDC. Recommendation to temporarily suspend usage of Glaxo-SmithKline Rotarix (Rotavirus Vaccine); March 22, 2010.
74. CDC. Reduction in rotavirus after vaccine introduction—United States, 2000-2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1146–9.
75. CDC. Withdrawal of rotavirus vaccine recommendation. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1007.
76. Cezard JP, Duhamel JF, Meyer M, et al. Efficacy and tolerability of racecadotril in acute diarrhea in children. *Gastroenterology* 2001;**120**:799–805.
77. Chan J, Nirwati H, Triasih R, et al. Maternal antibodies to rotavirus: could they interfere with live rotavirus vaccines in developing countries? *Vaccine* 2011;**29**:1242–7.
78. Chang EJ, Zangwill KM, Lee H, Ward JI. Lack of association between rotavirus infection and intussusception: implications for use of attenuated rotavirus vaccines. *Pediatr Infect Dis J* 2002;**21**:97–102.
79. Chang HG, Smith PF, Tserenpuntsag B, et al. Reduction in hospitalizations for diarrhea and rotavirus infections in New York State following introduction of rotavirus vaccine. *Vaccine* 2010;**28**:754–8.
80. Charles MD, Holman RC, Curns AT, et al. Hospitalizations associated with rotavirus gastroenteritis in the United States, 1993-2002. *Pediatr Infect Dis J* 2006;**25**:489–93.
81. Chen CM, Hung T, Bridger JC, McCrae MA. Chinese adult rotavirus is a group B rotavirus. *Lancet* 1985;**2**:1123–4.
82. Chen SC, Fynan EF, Robinson HL, et al. Protective immunity induced by rotavirus DNA vaccines. *Vaccine* 1997;**15**:899–902.
83. Chiappini E, Azzari C, Moriondo M, et al. Viraemia is a common finding in immunocompetent children with rotavirus infection. *J Med Virol* 2005;**76**:265–7.
84. Chiba S, Yokoyama T, Nakata S, et al. Protective effect of naturally acquired homotypic and heterotypic rotavirus antibodies. *Lancet* 1986;**2**:417–21.
85. Choi AH, Basu M, McNeal MM, et al. Antibody-independent protection against rotavirus infection of mice stimulated by intranasal immunization with chimeric VP4 or VP6 protein. *J Virol* 1999;**73**:7574–81.
86. Choi AH, Basu M, Rae MN, et al. Particle-bombardment-mediated DNA vaccination with rotavirus VP4 or VP7 induces high levels of serum rotavirus IgG but fails to protect mice against challenge. *Virology* 1998;**250**:230–40.
87. Chou IC, Tsai CH, Tsai FJ. Rotavirus associated with poliomyelitis-like syndrome. *Pediatr Infect Dis J* 1998;**17**:930–1.
88. Christy C, Madore HP, Pichichero ME, et al. Field trial of rhesus rotavirus vaccine in infants. *Pediatr Infect Dis J* 1988;**7**:645–50.
89. Clark HF, Bernstein DI, Dennehy PH, et al. Safety, efficacy, and immunogenicity of a live, quadrivalent human-bovine reassortant rotavirus vaccine in healthy infants. *J Pediatr* 2004;**144**:184–90.
90. Clark HF, Borian FE, Bell LM, et al. Protective effect of WC3 vaccine against rotavirus diarrhea in infants during a predominantly serotype 1 rotavirus season. *J Infect Dis* 1988;**158**:570–87.
91. Clark HF, Borian FE, Plotkin SA. Immune protection of infants against rotavirus gastroenteritis by a serotype 1 reassortant of bovine rotavirus WC3. *J Infect Dis* 1990;**161**:1099–104.
92. Clark HF, Lawley D, Mallette LA, et al. Decline in cases of rotavirus gastroenteritis presenting to The Children's Hospital of Philadelphia after introduction of a pentavalent rotavirus vaccine. *Clin Vaccine Immunol* 2009;**16**:382–6.
93. Clark HF, Lawley D, Matthijnssens J, et al. Sustained decline in cases of rotavirus gastroenteritis presenting to the Children's Hospital of Philadelphia in the new rotavirus vaccine era. *Pediatr Infect Dis J* 2010;**29**:699–702.
94. Clark HF, Lawley DA, Schaffer A, et al. Assessment of the epidemic potential of a new strain of rotavirus associated with the novel G9 serotype which caused an outbreak in the United States for the first time in the 1995-1996 season. *J Clin Microbiol* 2004;**42**:1434–8.
95. Clark HF, Offit PA, Plotkin SA, Heaton PM. The new pentavalent rotavirus vaccine composed of bovine (strain WC3)–human rotavirus reassortants. *Pediatr Infect Dis J* 2006;**25**:577–83.
96. Clarke EJ Jr, Phillips IA, Alexander ER. Adenovirus infection in intussusception in children in Taiwan. *JAMA* 1969;**208**:1671–4.
97. Clarke MF, Davidson GP, Gold MS, Marshall HS. Direct and indirect impact on rotavirus positive and all-cause gastroenteritis hospitalisations in South Australian children following the introduction of rotavirus vaccination. *Vaccine* 2011;**29**:4663–7.
98. Clemens J, Rao M, Ahmed F, et al. Breast-feeding and the risk of life-threatening rotavirus diarrhea: prevention or postponement? *Pediatrics* 1993;**92**:680–5.
99. Clemens JD, Ward RL, Rao MR, et al. Seroepidemiologic evaluation of antibodies to rotavirus as correlates of the risk of clinically

significant rotavirus diarrhea in rural Bangladesh. *J Infect Dis* 1992;**165**:161–5.
100. Collins JE, Benfield DA, Duimstra JR. Comparative virulence of two porcine group-A rotavirus isolates in gnotobiotic pigs. *Am J Vet Res* 1989;**50**:827–35.
101. Committee on Infectious Diseases, American Academy of Pediatrics. Prevention of rotavirus disease: updated guidelines for use of rotavirus vaccine. *Pediatrics* 2009;**123**:1412–20.
102. Conner ME, Crawford SE, Barone C, Estes MK. Rotavirus vaccine administered parenterally induces protective immunity. *J Virol* 1993;**67**:6633–41.
103. Conner ME, Gilger MA, Estes MK, Graham DY. Serologic and mucosal immune response to rotavirus infection in the rabbit model. *J Virol* 1991;**65**:2562–71.
104. Conner ME, Zarley CD, Hu B, et al. Virus-like particles as a rotavirus subunit vaccine. *J Infect Dis* 1996;**174**(Suppl. 1): S88–92.
105. Cook SM, Glass RI, LeBaron CW, Ho MS. Global seasonality of rotavirus infections. *Bull World Health Organ* 1990;**68**:171–7.
106. Correia JB, Patel MM, Nakagomi O, et al. Effectiveness of monovalent rotavirus vaccine (Rotarix) against severe diarrhea caused by serotypically unrelated G2P[4] strains in Brazil. *J Infect Dis* 2010;**201**:363–9.
107. Cortes JE, Curns AT, Tate JE, et al. Rotavirus vaccine and health care utilization for diarrhea in U.S. children. *N Engl J Med* 2011;**365**:1108–17.
108. Cortese MM, Parashar UD. Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommen Rep* 2009; **58**(RR-2):1–25.
109. Cortese MM, Tate JE, Simonsen L, et al. Reduction in gastroenteritis in United States children and correlation with early rotavirus vaccine uptake from national medical claims databases. *Pediatr Infect Dis J* 2010;**29**:489–94.
110. Coulson BS, Grimwood K, Hudson IL, et al. Role of coproantibody in clinical protection of children during reinfection with rotavirus. *J Clin Microbiol* 1992;**30**:1678–84.
111. Coulson BS, Grimwood K, Masendycz PJ, et al. Comparison of rotavirus immunoglobulin A coproconversion with other indices of rotavirus infection in a longitudinal study in childhood. *J Clin Microbiol* 1990;**28**:1367–74.
112. Cunliffe NA, Gentsch JR, Kirkwood CD, et al. Molecular and serologic characterization of novel serotype G8 human rotavirus strains detected in Blantyre, Malawi. *Virology* 2000;**274**:309–20.
113. Cunliffe NA, Gondwe JS, Broadhead RL, et al. Rotavirus G and P types in children with acute diarrhea in Blantyre, Malawi, from 1997 to 1998: predominance of novel P[6]G8 strains. *J Med Virol* 1999;**57**:308–12.
114. Cunliffe NA, Kilgore PE, Bresee JS, et al. Epidemiology of rotavirus diarrhoea in Africa: a review to assess the need for rotavirus immunization. *Bull World Health Organ* 1998;**76**:525–37.
115. Curns AT, Panozzo CA, Tate JE, et al. Remarkable postvaccination spatiotemporal changes in United States rotavirus activity. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S54–5.
116. Curns AT, Steiner CA, Barrett M, et al. Reduction in acute gastroenteritis hospitalizations among US children after introduction of rotavirus vaccine: analysis of hospital discharge data from 18 US states. *J Infect Dis* 2010;**201**:1617–24.
117. Dalgic N, Sancar M, Bayraktar B, et al. Probiotic, zinc and lactose-free formula in children with rotavirus diarrhea: are they effective? *Pediatr Int* 2011;**53**:677–82.
118. Das BK, Gentsch JR, Hoshino Y, et al. Characterization of the G serotype and genogroup of New Delhi newborn rotavirus strain 116E. *Virology* 1993;**197**:99–107.
119. Das M, Dunn SJ, Woode GN, et al. Both surface proteins (VP4 and VP7) of an asymptomatic neonatal rotavirus strain (I321) have high levels of sequence identity with the homologous proteins of a serotype 10 bovine rotavirus. *Virology* 1993;**194**:374–9.
120. Davidson GP, Bishop RF, Townley RR, Holmes IH. Importance of a new virus in acute sporadic enteritis in children. *Lancet* 1975;**1**:242–6.
121. Davidson GP, Hogg RJ, Kirubakaran CP. Serum and intestinal immune response to rotavirus enteritis in children. *Infect Immun* 1983;**40**:447–52.
122. Davidson GP, Whyte PB, Daniels E, et al. Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. *Lancet* 1989;**2**:709–12.
123. De Champs C, Laveran H, Peigue-Lafeuille H, et al. Sequential rotavirus infections: characterization of serotypes and electrophoretypes. *Res Virol* 1991;**142**:39–45.
124. De Mol P, Zissis G, Butzler JP, et al. Failure of live, attenuated oral rotavirus vaccine. *Lancet* 1986;**2**:108.
125. de Oliveira LH, Danovaro-Holliday MC, Andrus JK, et al. Sentinel hospital surveillance for rotavirus in Latin American and Caribbean countries. *J Infect Dis* 2009;**200**(Suppl. 1):S131–9.
126. de Palma O, Cruz L, Ramos H, et al. Effectiveness of rotavirus vaccination against childhood diarrhoea in El Salvador: case-control study. *BMJ* 2010;**340**:c2825.
127. de Villiers FP, Driessen M. Clinical neonatal rotavirus infection: association with necrotising enterocolitis. *S Afr Med J* 2012;**102**: 620–4.
128. De Vos B, Han HH, Bouckenooghe A, et al. Live attenuated human rotavirus vaccine, RIX4414, provides clinical protection in infants against rotavirus strains with and without shared G and P genotypes: integrated analysis of randomized controlled trials. *Pediatr Infect Dis J* 2009;**28**:261–6.
129. Dennehy PH, Brady RC, Halperin SA, et al. Comparative evaluation of safety and immunogenicity of two dosages of an oral live attenuated human rotavirus vaccine. *Pediatr Infect Dis J* 2005;**24**:481–8.
130. Dennehy PH, Cortese MM, Begue RE, et al. A case-control study to determine risk factors for hospitalization for rotavirus gastroenteritis in U.S. children. *Pediatr Infect Dis J* 2006;**25**:1123–31.
131. Desai R, Curns AT, Steiner CA, et al. All-cause gastroenteritis and rotavirus-coded hospitalizations among US children, 2000-2009. *Clin Infect Dis* 2012;**55**:e28–34.
132. Desai R, Haberling D, Holman RC, et al. Impact of rotavirus vaccine on diarrhea-associated disease burden among American Indian and Alaska Native children. *Pediatrics* 2012;**129**:e907–13.
133. Desai R, Parashar UD, Lopman B, et al. Potential intussusception risk versus health benefits from rotavirus vaccination in Latin America. *Clin Infect Dis* 2012;**54**:1397–405.
134. Dickey M, Jamison L, Michaud L, et al. Rotavirus meningoencephalitis in a previously healthy child and a review of the literature. *Pediatr Infect Dis J* 2009;**28**:318–21.
135. Dong Y, Zeng CQ, Ball JM, et al. The rotavirus enterotoxin NSP4 mobilizes intracellular calcium in human intestinal cells by stimulating phospholipase C–mediated inositol 1,4,5-trisphosphate production. *Proc Natl Acad Sci U S A* 1997;**94**:3960–5.
136. Dormitzer PR, Nason EB, Prasad BV, Harrison SC. Structural rearrangements in the membrane penetration protein of a non-enveloped virus. *Nature* 2004;**430**:1053–8.
137. Eberly MD, Gorman GH, Eide MB, et al. The effect of rotavirus immunization on rotavirus gastroenteritis hospitalization rates in military dependents. *Vaccine* 2011;**29**:650–9.
138. Ebina T, Ohta M, Kanamaru Y, et al. Passive immunizations of suckling mice and infants with bovine colostrum containing antibodies to human rotavirus. *J Med Virol* 1992;**38**:117–23.
139. Echeverria P, Blacklow NR, Cukor GG, et al. Rotavirus as a cause of severe gastroenteritis in adults. *J Clin Microbiol* 1983;**18**: 663–7.
140. Eiden J, Losonsky GA, Johnson J, Yolken RH. Rotavirus RNA variation during chronic infection of immunocompromised children. *Pediatr Infect Dis* 1985;**4**:632–7.
141. El-Hodhod MA, Nassar MF, Ezz El-Arab S, Ahmed EF. Rotavirus fecal antigen retrieval in infantile intussusception. *Eur J Clin Microbiol Infect Dis* 2008;**27**:879–81.
142. Eng PM, Mast TC, Loughlin J, et al. Incidence of intussusception among infants in a large commercially insured population in the United States. *Pediatr Infect Dis J* 2012;**31**:287–91.
143. Estes MK, Graham DY, Smith EM, Gerba CP. Rotavirus stability and inactivation. *J Gen Virol* 1979;**43**:403–9.
144. Fang SB, Lee HC, Hu JJ, et al. Dose-dependent effect of *Lactobacillus rhamnosus* on quantitative reduction of faecal rotavirus shedding in children. *J Trop Pediatr* 2009;**55**:297–301.
145. Fang ZY, Ye Q, Ho MS, et al. Investigation of an outbreak of adult diarrhea rotavirus in China. *J Infect Dis* 1989;**160**:948–53.
146. Figueroa-Qunitanilla D, Salazar-Lindo E, Sack RB, et al. A controlled trial of bismuth subsalicylate in infants with acute watery diarrheal disease. *N Engl J Med* 1993;**328**:1653–8.

147. Fischer TK, Ashley D, Kerin T, et al. Rotavirus antigenemia in patients with acute gastroenteritis. *J Infect Dis* 2005;**192**:913–19.
148. Fleming FE, Graham KL, Takada Y, Coulson BS. Determinants of the specificity of rotavirus interactions with the $\alpha_2\beta_1$ integrin. *J Biol Chem* 2011;**286**:6165–74.
149. Flores J, Perez-Schael I, Blanco M, et al. Comparison of reactogenicity and antigenicity of M37 rotavirus vaccine and rhesus-rotavirus-based quadrivalent vaccine. *Lancet* 1990;**336**:330–4.
150. Flores J, Perez-Schael I, Gonzalez M, et al. Protection against severe rotavirus diarrhoea by rhesus rotavirus vaccine in Venezuelan infants. *Lancet* 1987;**1**:882–4.
151. Franco MA, Greenberg HB. Immunity to rotavirus infection in mice. *J Infect Dis* 1999;**179**(Suppl. 3):S466–9.
152. Franco MA, Greenberg HB. Role of B cells and cytotoxic T lymphocytes in clearance of and immunity to rotavirus infection in mice. *J Virol* 1995;**69**:7800–6.
153. Franco MA, Tin C, Greenberg HB. $CD8^+$ T cells can mediate almost complete short-term and partial long-term immunity to rotavirus in mice. *J Virol* 1997;**71**:4165–70.
154. Friedland L. Rotarix (Rotavirus Vaccine, Live Oral): GSK's PCV1 Investigation. Presented at Advisory Committee on Immunization Practices; Atlanta, Georgia; 2010.
155. Gardner PS. Adenovirus and intussusception. *Br Med J* 1961;**2**:495–6.
156. Gardner PS, Knox EG, Court SD, Green CA. Virus infection and intussusception in childhood. *Br Med J* 1962;**2**:697–700.
157. Gentsch JR, Glass RI, Woods P, et al. Identification of group A rotavirus gene 4 types by polymerase chain reaction. *J Clin Microbiol* 1992;**30**:1365–73.
158. Gentsch JR, Hull JJ, Teel EN, et al. G and P types of circulating rotavirus strains in the United States during 1996-2005: nine years of prevaccine data. *J Infect Dis* 2009;**200**(Suppl. 1):S99–105.
159. Gentsch JR, Laird AR, Bielfelt B, et al. Serotype diversity and reassortment between human and animal rotavirus strains: implications for rotavirus vaccine programs. *J Infect Dis* 2005; **192**(Suppl. 1):S146–59.
160. Gentsch JR, Parashar UD, Glass RI. Impact of rotavirus vaccination: the importance of monitoring strains. *Future Microbiol* 2009;**4**:1231–4.
161. Gentsch JR, Woods PA, Ramachandran M, et al. Review of G and P typing results from a global collection of rotavirus strains: implications for vaccine development. *J Infect Dis* 1996;**174**(Suppl. 1):S30–6.
162. Georges-Courbot MC, Monges J, Siopathis MR, et al. Evaluation of the efficacy of a low-passage bovine rotavirus (strain WC3) vaccine in children in Central Africa. *Res Virol* 1991;**142**:405–11.
163. Giaquinto C, Dominiak-Felden G, Van Damme P, et al. Summary of effectiveness and impact of rotavirus vaccination with the oral pentavalent rotavirus vaccine: a systematic review of the experience in industrialized countries. *Hum Vaccin* 2011;**7**:734–48.
164. Gilger MA, Matson DO, Conner ME, et al. Extraintestinal rotavirus infections in children with immunodeficiency. *J Pediatr* 1992;**120**:912–17.
165. Gladstone BP, Ramani S, Mukhopadhya I, et al. Protective effect of natural rotavirus infection in an Indian birth cohort. *N Engl J Med* 2011;**365**:337–46.
166. Glass RI, Bresee J, Jiang B, et al. Rotavirus and rotavirus vaccines. *Adv Exp Med Biol* 2006;**582**:45–54.
167. Goldwater PN, Rowland K, Thesinger M, et al. Rotavirus encephalopathy: pathogenesis reviewed. *J Paediatr Child Health* 2001;**37**:206–9.
168. Gouvea V, Allen JR, Glass RI, et al. Detection of group B and C rotaviruses by polymerase chain reaction. *J Clin Microbiol* 1991;**29**:519–23.
169. Gouvea V, de Castro L, Timenetsky MC, et al. Rotavirus serotype G5 associated with diarrhea in Brazilian children. *J Clin Microbiol* 1994;**32**:1408–9.
170. Goveia MG, Ciarlet M, Owen KE, Ranucci CS. Development, clinical evaluation, and post-licensure impact of RotaTeq, a pentavalent rotavirus vaccine. *Ann N Y Acad Sci* 2011;**1222**:14–18.
171. Goveia MG, Rodriguez ZM, Dallas MJ, et al. Safety and efficacy of the pentavalent human-bovine (WC3) reassortant rotavirus vaccine in healthy premature infants. *Pediatr Infect Dis J* 2007; **26**:1099–104.
172. Graham KL, Halasz P, Tan Y, et al. Integrin-using rotaviruses bind $\alpha_2\beta_1$ integrin α_2 I domain via VP4 DGE sequence and recognize $\alpha_X\beta_2$ and $\alpha_V\beta_3$ by using VP7 during cell entry. *J Virol* 2003; **77**:9969–78.
173. Grandy G, Medina M, Soria R, et al. Probiotics in the treatment of acute rotavirus diarrhoea. A randomized, double-blind, controlled trial using two different probiotic preparations in Bolivian children. *BMC Infect Dis* 2010;**10**:253.
174. Gray J, Iturriza-Gomara M. Rotaviruses. *Methods Mol Biol* 2011;**665**:325–55.
175. Green KY, Kapikian AZ. Identification of VP7 epitopes associated with protection against human rotavirus illness or shedding in volunteers. *J Virol* 1992;**66**:548–53.
176. Greenberg HB, Clark HF, Offit PA. Rotavirus pathology and pathophysiology. *Curr Top Microbiol Immunol* 1994;**185**:255–83.
177. Greenberg HB, Estes MK. Rotaviruses: from pathogenesis to vaccination. *Gastroenterology* 2009;**136**:1939–51.
178. Greenberg HB, Valdesuso J, van Wyke K, et al. Production and preliminary characterization of monoclonal antibodies directed at two surface proteins of rhesus rotavirus. *J Virol* 1983;**47**:267–75.
179. Greenberg HB, Vo PT, Jones R. Cultivation and characterization of three strains of murine rotavirus. *J Virol* 1986;**57**:585–90.
180. Griffin DD, Kirkwood CD, Parashar UD, et al. Surveillance of rotavirus strains in the United States: identification of unusual strains. The National Rotavirus Strain Surveillance System collaborating laboratories. *J Clin Microbiol* 2000;**38**:2784–7.
181. Grillner L, Broberger U, Chrystie I, Ransjo U. Rotavirus infections in newborns: an epidemiological and clinical study. *Scand J Infect Dis* 1985;**17**:349–55.
182. Grimwood K, Lund JC, Coulson BS, et al. Comparison of serum and mucosal antibody responses following severe acute rotavirus gastroenteritis in young children. *J Clin Microbiol* 1988.
183. Guandalini S. Probiotics for prevention and treatment of diarrhea. *J Clin Gastroenterol* 2011;**45**(Suppl.):S149–53.
184. Guarino A, Canani RB, Russo S, et al. Oral immunoglobulins for treatment of acute rotaviral gastroenteritis. *Pediatrics* 1994;**93**: 12–16.
185. Guarino A, Lo Vecchio A, Canani RB. Probiotics as prevention and treatment for diarrhea. *Curr Opin Gastroenterol* 2009;**25**: 18–23.
186. Guarner J, de Leon-Bojorge B, Lopez-Corella E, et al. Intestinal intussusception associated with adenovirus infection in Mexican children. *Am J Clin Pathol* 2003;**120**:845–50.
187. Guerrero CA, Bouyssounade D, Zarate S, et al. Heat shock cognate protein 70 is involved in rotavirus cell entry. *J Virol* 2002;**76**:4096–102.
188. Guerrero CA, Mendez E, Zarate S, et al. Integrin $\alpha_v\beta_3$ mediates rotavirus cell entry. *Proc Natl Acad Sci U S A* 2000;**97**:14644–9.
189. Guglielmi KM, McDonald SM, Patton JT. Mechanism of intraparticle synthesis of the rotavirus double-stranded RNA genome. *J Biol Chem* 2010;**285**:18123–8.
190. Gurwith M, Wenman W, Gurwith D, et al. Diarrhea among infants and young children in Canada: a longitudinal study in three northern communities. *J Infect Dis* 1983;**147**:685–92.
191. Haffejee IE. The epidemiology of rotavirus infections: a global perspective. *J Pediatr Gastroenterol Nutr* 1995;**20**:275–86.
192. Haffejee IE. Neonatal rotavirus infections. *Rev Infect Dis* 1991; **13**:957–62.
193. Hall GA, Bridger JC, Parsons KR, Cook R. Variation in rotavirus virulence: a comparison of pathogenesis in calves between two rotaviruses of different virulence. *Vet Pathol* 1993;**30**:223–33.
194. Hamza H, Ben Khalifa H, Baumer P, et al. Racecadotril versus placebo in the treatment of acute diarrhoea in adults. *Aliment Pharmacol Ther* 1999;**13**(Suppl. 6):15–19.
195. Hilpert H, Brussow H, Mietens C, et al. Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. *J Infect Dis* 1987;**156**:158–66.
196. Ho MS, Glass RI, Pinsky PF, Anderson LJ. Rotavirus as a cause of diarrheal morbidity and mortality in the United States. *J Infect Dis* 1988;**158**:1112–16.
197. Hongou K, Konishi T, Yagi S, et al. Rotavirus encephalitis mimicking afebrile benign convulsions in infants. *Pediatr Neurol* 1998;**18**:354–7.
198. Hopkins RS, Gaspard GB, Williams FP Jr, et al. A community waterborne gastroenteritis outbreak: evidence for rotavirus as the agent. *Am J Public Health* 1984;**74**:263–5.
199. Hoshino Y, Saif LJ, Kang SY, et al. Identification of group A rotavirus genes associated with virulence of a porcine rotavirus and

host range restriction of a human rotavirus in the gnotobiotic piglet model. *Virology* 1995;**209**:274–80.
200. Hoshino Y, Saif LJ, Sereno MM, et al. Infection immunity of piglets to either VP3 or VP7 outer capsid protein confers resistance to challenge with a virulent rotavirus bearing the corresponding antigen. *J Virol* 1988;**62**:744–8.
201. Hoshino Y, Wyatt RG, Greenberg HB, et al. Serotypic similarity and diversity of rotaviruses of mammalian and avian origin as studied by plaque-reduction neutralization. *J Infect Dis* 1984;**149**:694–702.
202. Hrdy DB. Epidemiology of rotaviral infection in adults. *Rev Infect Dis* 1987;**9**:461–9.
203. Hsu HY, Kao CL, Huang LM, et al. Viral etiology of intussusception in Taiwanese childhood. *Pediatr Infect Dis J* 1998;**17**:893–8.
204. Hu L, Crawford SE, Czako R, et al. Cell attachment protein VP8* of a human rotavirus specifically interacts with A-type histo-blood group antigen. *Nature* 2012;**485**:256–9.
205. Hu L, Crawford SE, Hyser JM, et al. Rotavirus non-structural proteins: structure and function. *Curr Opin Virol* 2012;**2**:380–8.
206. Huang P, Xia M, Tan M, et al. Spike protein VP8* of human rotavirus recognizes histo-blood group antigens in a type-specific manner. *J Virol* 2012;**86**:4833–43.
207. Hull JJ, Teel EN, Kerin TK, et al. United States rotavirus strain surveillance from 2005 to 2008: genotype prevalence before and after vaccine introduction. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S42–7.
208. Hung T, Chen GM, Wang CG, et al. Seroepidemiology and molecular epidemiology of the Chinese rotavirus. *Ciba Found Symp* 1987;**128**:49–62.
209. Hung T, Chen GM, Wang CG, et al. Waterborne outbreak of rotavirus diarrhoea in adults in China caused by a novel rotavirus. *Lancet* 1984;**1**:1139–42.
210. Iturriza-Gomara M, Dallman T, Banyai K, et al. Rotavirus genotypes co-circulating in Europe between 2006 and 2009 as determined by EuroRotaNet, a pan-European collaborative strain surveillance network. *Epidemiol Infect* 2011;**139**:895–909.
211. Jaimes MC, Rojas OL, Gonzalez AM, et al. Frequencies of virus-specific $CD4^+$ and $CD8^+$ T lymphocytes secreting gamma interferon after acute natural rotavirus infection in children and adults. *J Virol* 2002;**76**:4741–9.
212. Jakab F, Peterfai J, Verebely T, et al. Human astrovirus infection associated with childhood intussusception. *Pediatr Int* 2007;**49**:103–5.
213. James VL, Lambden PR, Caul EO, Clarke IN. Enzyme-linked immunosorbent assay based on recombinant human group C rotavirus inner capsid protein (VP6) to detect human group C rotaviruses in fecal samples. *J Clin Microbiol* 1998;**36**:3178–81.
214. James VL, Lambden PR, Caul EO, et al. Seroepidemiology of human group C rotavirus in the UK. *J Med Virol* 1997;**52**:86–91.
215. Jayaram H, Estes MK, Prasad BV. Emerging themes in rotavirus cell entry, genome organization, transcription and replication. *Virus Res* 2004;**101**:67–81.
216. Jiang B, Dennehy PH, Spangenberger S, et al. First detection of group C rotavirus in fecal specimens of children with diarrhea in the United States. *J Infect Dis* 1995;**172**:45–50.
217. Jiang B, Gentsch JR, Glass RI. Inactivated rotavirus vaccines: a priority for accelerated vaccine development. *Vaccine* 2008;**26**:6754–8.
218. Jiang B, Patel M, Parashar U. Rotavirus vaccines for global use: what are the remaining issues and challenges? *Hum Vaccin* 2010;**6**:425–7.
219. Jiang V, Jiang B, Tate J, et al. Performance of rotavirus vaccines in developed and developing countries. *Hum Vaccin* 2010;**6**:532–42.
220. Joensuu J, Koskenniemi E, Pang XL, Vesikari T. Randomised placebo-controlled trial of rhesus-human reassortant rotavirus vaccine for prevention of severe rotavirus gastroenteritis. *Lancet* 1997;**350**:1205–9.
221. Johansen K, Hedlund KO, Zweygberg-Wirgart B, Bennet R. Complications attributable to rotavirus-induced diarrhoea in a Swedish paediatric population: report from an 11-year surveillance. *Scand J Infect Dis* 2008;**40**:958–64.
222. Kamiya H, Nakano T, Inoue M, et al. A retrospective evaluation of hospitalizations for acute gastroenteritis at 2 sentinel hospitals in central Japan to estimate the health burden of rotavirus. *J Infect Dis* 2009;**200**(Suppl. 1):S140–6.
223. Kane EM, Turcios RM, Arvay ML, et al. The epidemiology of rotavirus diarrhea in Latin America. Anticipating rotavirus vaccines. *Rev Panam Salud Publica* 2004;**16**:371–7.
224. Kanfer EJ, Abrahamson G, Taylor J, et al. Severe rotavirus-associated diarrhoea following bone marrow transplantation: treatment with oral immunoglobulin. *Bone Marrow Transplant* 1994;**14**:651–2.
225. Kang G, Arora R, Chitambar SD, et al. Multicenter, hospital-based surveillance of rotavirus disease and strains among Indian children aged <5 years. *J Infect Dis* 2009;**200**(Suppl. 1):S147–53.
226. Kang G, Iturriza-Gomara M, Wheeler JG, et al. Quantitation of group A rotavirus by real-time reverse-transcription-polymerase chain reaction: correlation with clinical severity in children in South India. *J Med Virol* 2004;**73**:118–22.
227. Kapikian AZ. Viral gastroenteritis. *JAMA* 1993;**269**:627–30.
228. Kapikian AZ, Flores J, Hoshino Y, et al. Rotavirus: the major etiologic agent of severe infantile diarrhea may be controllable by a "Jennerian" approach to vaccination. *J Infect Dis* 1986;**153**:815–22.
229. Kapikian AZ, Simonsen L, Vesikari T, et al. A hexavalent human rotavirus–bovine rotavirus (UK) reassortant vaccine designed for use in developing countries and delivered in a schedule with the potential to eliminate the risk of intussusception. *J Infect Dis* 2005;**192**(Suppl. 1):S22–9.
230. Kapikian AZ, Wyatt RG, Levine MM, et al. Oral administration of human rotavirus to volunteers: induction of illness and correlates of resistance. *J Infect Dis* 1983;**147**:95–106.
231. Keidan I, Shif I, Keren G, Passwell JH. Rotavirus encephalopathy: evidence of central nervous system involvement during rotavirus infection. *Pediatr Infect Dis J* 1992;**11**:773–5.
232. Keswick BH, Pickering LK, DuPont HL, Woodward WE. Survival and detection of rotaviruses on environmental surfaces in day care centers. *Appl Environ Microbiol* 1983;**46**:813–16.
233. Kilgore PE, Holman RC, Clarke MJ, Glass RI. Trends of diarrheal disease–associated mortality in US children, 1968 through 1991. *JAMA* 1995;**274**:1143–8.
234. Kim HW, Brandt CD, Kapikian AZ, et al. Human reovirus-like agent infection. Occurrence in adult contacts of pediatric patients with gastroenteritis. *JAMA* 1977;**238**:404–7.
235. King CK, Glass R, Bresee JS, Duggan C. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recommen Rep* 2003;**52**(RR-16):1–16.
236. Kirkwood CD, Bishop RF, Coulson BS. Attachment and growth of human rotaviruses RV-3 and S12/85 in Caco-2 cells depend on VP4. *J Virol* 1998;**72**:9348–52.
237. Kirkwood CD, Roczo S, Boniface K, et al. Australian Rotavirus Surveillance Group. Australian Rotavirus Surveillance Program annual report, 2010/11. *Commun Dis Intell Q Rep* 2011;**35**:281–7.
238. Kirkwood CD, Boniface K, Bishop RF, Barnes GL. Australian rotavirus surveillance program: annual report, 2009/2010. *Commun Dis Intell Q Rep* 2010;**34**:427–34.
239. Kiulia NM, Nyaundi JK, Peenze I, et al. Rotavirus infections among HIV-infected children in Nairobi, Kenya. *J Trop Pediatr* 2009;**55**:318–23
240. Knowlton DR, Spector DM, Ward RL. Development of an improved method for measuring neutralizing antibody to rotavirus. *J Virol Methods* 1991;**33**:127–34.
241. Kombo LA, Gerber MA, Pickering LK, et al. Intussusception, infection, and immunization: summary of a workshop on rotavirus. *Pediatrics* 2001;**108**:E37.
242. Komoto S, Kugita M, Sasaki J, Taniguchi K. Generation of recombinant rotavirus with an antigenic mosaic of cross-reactive neutralization epitopes on VP4. *J Virol* 2008;**82**:6753–7.
243. Komoto S, Sasaki J, Taniguchi K. Reverse genetics system for introduction of site-specific mutations into the double-stranded RNA genome of infectious rotavirus. *Proc Natl Acad Sci U S A* 2006;**103**:4646–51.
244. Komura E, Hashida T, Otsuka T, et al. Human herpesvirus 6 and intussusception. *Pediatr Infect Dis J* 1993;**12**:788–9.
245. Konno T, Suzuki H, Kutsuzawa T, et al. Human rotavirus and intussusception. *N Engl J Med* 1977;**297**:945.

246. Konno T, Suzuki H, Kutsuzawa T, et al. Human rotavirus infection in infants and young children with intussusception. *J Med Virol* 1978;**2**:265–9.
247. Koopmans M, Van Asperen I. Epidemiology of rotavirus infections in The Netherlands. *Acta Paediatr Suppl* 1999;**88**:31–7.
248. Koroglu M, Yakupogullari Y, Otlu B, et al. A waterborne outbreak of epidemic diarrhea due to group A rotavirus in Malatya, Turkey. *New Microbiol* 2011;**34**:17–24.
249. Kovacs A, Chan L, Hotrakitya C, et al. Rotavirus gastroenteritis. Clinical and laboratory features and use of the Rotazyme test. *Am J Dis Child* 1987;**141**:161–6.
250. Kovacs A, Chan L, Hotrakitya C, et al. Serum transaminase elevations in infants with rotavirus gastroenteritis. *J Pediatr Gastroenterol Nutr* 1986;**5**:873–7.
251. Kramarz P, France EK, Destefano F, et al. Population-based study of rotavirus vaccination and intussusception. *Pediatr Infect Dis J* 2001;**20**:410–16.
252. Kubelka V, Altmann F, Staudacher E, et al. Primary structures of the N-linked carbohydrate chains from honeybee venom phospholipase A_2. *Eur J Biochem* 1993;**213**:1193–204.
253. Kuehn BM. FDA: Benefits of rotavirus vaccination outweigh potential contamination risk. *JAMA* 2010;**304**:30–1.
254. Kuzuya M, Fujii R, Hamano M, Ogura H. Outbreak of acute gastroenteritis caused by human group C rotavirus in a youth educational center in Okayama Prefecture [in Japanese]. *Kansenshogaku Zasshi* 2003;**77**:53–9.
255. Kuzuya M, Fujii R, Hamano M, et al. Seroepidemiology of human group C rotavirus in Japan based on a blocking enzyme-linked immunosorbent assay. *Clin Diagn Lab Immunol* 2001;**8**:161–5.
256. Kuzuya M, Fujii R, Hamano M, et al. Survey of human group C rotaviruses in Japan during the winter of 1992 to 1993. *J Clin Microbiol* 1998;**36**:6–10.
257. Kuzuya M, Hamano M, Nishijima M, et al. An outbreak of acute gastroenteritis caused by human group C rotavirus in a welfare institution in Okayama prefecture. *Jpn J Infect Dis* 2005;**58**: 255–7.
258. Lappalainen S, Ylitalo S, Arola A, et al. Simultaneous presence of human herpesvirus 6 and adenovirus infections in intestinal intussusception of young children. *Acta Paediatr* 2012;**101**:663–70.
259. Laserson KF, Nyakundi D, Feikin DR, et al. Safety of the pentavalent rotavirus vaccine (PRV), RotaTeq, in Kenya, including among HIV-infected and HIV-exposed infants. *Vaccine* 2012; **30**(Suppl. 1):A61–70.
260. LeBaron CW, Lew J, Glass RI, et al. Annual rotavirus epidemic patterns in North America. Results of a 5-year retrospective survey of 88 centers in Canada, Mexico, and the United States. Rotavirus Study Group. *JAMA* 1990;**264**:983–8.
261. Lehert P, Cheron G, Calatayud GA, et al. Racecadotril for childhood gastroenteritis: an individual patient data meta-analysis. *Dig Liver Dis* 2011;**43**:707–13.
262. Leite JP, Alfieri AA, Woods PA, et al. Rotavirus G and P types circulating in Brazil: characterization by RT-PCR, probe hybridization, and sequence analysis. *Arch Virol* 1996;**141**:2365–74.
263. Leite JP, Carvalho-Costa FA, Linhares AC. Group A rotavirus genotypes and the ongoing Brazilian experience: a review. *Mem Inst Oswaldo Cruz* 2008;**103**:745–53.
264. Li L, Kapoor A, Slikas B, et al. Multiple diverse circoviruses infect farm animals and are commonly found in human and chimpanzee feces. *J Virol* 2010;**84**:1674–82.
265. Li Z, Baker ML, Jiang W, et al. Rotavirus architecture at subnanometer resolution. *J Virol* 2009;**83**:1754–66.
266. Liakopoulou E, Mutton K, Carrington D, et al. Rotavirus as a significant cause of prolonged diarrhoeal illness and morbidity following allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2005;**36**:691–4.
267. Lin LH, See M, Wang NK. Breath hydrogen test for assessment of lactose malabsorption following rotavirus gastroenteritis. *J Formos Med Assoc* 1990;**89**:1072–6.
268. Linhares AC, Stupka JA, Ciapponi A, et al. Burden and typing of rotavirus group A in Latin America and the Caribbean: systematic review and meta-analysis. *Rev Med Virol* 2011; Mar 7.
269. Linhares AC, Velazquez FR, Perez-Schael I, et al. Efficacy and safety of an oral live attenuated human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in Latin American infants: a randomised, double-blind, placebo-controlled phase III study. *Lancet* 2008;**371**:1181–9.
270. Linhares AC, Verstraeten T, Wolleswinkel–van den Bosch J, et al. Rotavirus serotype G9 is associated with more-severe disease in Latin America. *Clin Infect Dis* 2006;**43**:312–14.
271. Little LM, Shadduck JA. Pathogenesis of rotavirus infection in mice. *Infect Immun* 1982;**38**:755–63.
272. Liu Y, Huang P, Tan M, et al. Rotavirus VP8*: phylogeny, host range, and interaction with histo-blood group antigens. *J Virol* 2012;**86**:9899–910.
273. Lopez S, Arias CF. Multistep entry of rotavirus into cells: a Versaillesque dance. *Trends Microbiol* 2004;**12**:271–8.
274. Lopman BA, Curns AT, Yen C, Parashar UD. Infant rotavirus vaccination may provide indirect protection to older children and adults in the United States. *J Infect Dis* 2011;**204**:980–6.
275. Lopman BA, Pitzer VE, Sarkar R, et al. Understanding reduced rotavirus vaccine efficacy in low socio-economic settings. *PloS One* 2012;**7**:e41720.
276. Losonsky GA, Rennels MB, Kapikian AZ, et al. Safety, infectivity, transmissibility and immunogenicity of rhesus rotavirus vaccine (MMU 18006) in infants. *Pediatr Infect Dis* 1986;**5**:25–9.
277. Loughlin J, Mast TC, Doherty MC, et al. Postmarketing evaluation of the short-term safety of the pentavalent rotavirus vaccine. *Pediatr Infect Dis J* 2012;**31**:292–6.
278. Lundgren O, Peregrin AT, Persson K, et al. Role of the enteric nervous system in the fluid and electrolyte secretion of rotavirus diarrhea. *Science* 2000;**287**:491–5.
279. Lynch M, Lee B, Azimi P, et al. Rotavirus and central nervous system symptoms: cause or contaminant? Case reports and review. *Clin Infect Dis* 2001;**33**:932–8.
280. Lynch M, Shieh WJ, Bresee JS, et al. Intussusception after administration of the rhesus tetravalent rotavirus vaccine (Rotashield): the search for a pathogenic mechanism. *Pediatrics* 2006;**117**: e827–32.
281. Madhi SA, Cunliffe NA, Steele D, et al. Effect of human rotavirus vaccine on severe diarrhea in African infants. *N Engl J Med* 2010;**362**:289–98.
282. Madore HP, Christy C, Pichichero M, et al. Field trial of rhesus rotavirus or human-rhesus rotavirus reassortant vaccine of VP7 serotype 3 or 1 specificity in infants. The Elmwood, Panorama, and Westfall Pediatric Groups. *J Infect Dis* 1992;**166**:235–43.
283. Makela M, Marttila J, Simell O, Ilonen J. Rotavirus-specific T-cell responses in young prospectively followed-up children. *Clin Exp Immunol* 2004;**137**:173–8.
284. Makino M, Tanabe Y, Shinozaki K, et al. Haemorrhagic shock and encephalopathy associated with rotavirus infection. *Acta Paediatr* 1996;**85**:632–4.
285. Malek MA, Curns AT, Holman RC, et al. Diarrhea- and rotavirus-associated hospitalizations among children less than 5 years of age: United States, 1997 and 2000. *Pediatrics* 2006;**117**:1887–92.
286. Marrie TJ, Lee SH, Faulkner RS, et al. Rotavirus infection in a geriatric population. *Arch Intern Med* 1982;**142**:313–16.
287. Marshall J, Botes J, Gorrie G, et al. Rotavirus detection and characterisation in outbreaks of gastroenteritis in aged-care facilities. *J Clin Virol* 2003;**28**:331–40.
288. Mast TC, Khawaja S, Espinoza F, et al. Case-control study of the effectiveness of vaccination with pentavalent rotavirus vaccine in Nicaragua. *Pediatr Infect Dis J* 2011;**30**:e209–15.
289. Mast TC, Walter EB, Bulotsky M, et al. Burden of childhood rotavirus disease on health systems in the United States. *Pediatr Infect Dis J* 2010;**29**:e19–25.
290. Mata L, Simhon A, Urrutia JJ, et al. Epidemiology of rotavirus in a cohort of 45 Guatemalan Mayan Indian children observed from birth to the age of three years. *J Infect Dis* 1980;**148**:452–61.
291. Matson DO, Estes MK. Impact of rotavirus infection at a large pediatric hospital. *J Infect Dis* 1990;**162**:598–604.
292. Matson DO, O'Ryan ML, Herrera I, et al. Fecal antibody responses to symptomatic and asymptomatic rotavirus infections. *J Infect Dis* 1993;**167**:577–83.
293. Matthijnssens J, Ciarlet M, Heiman E, et al. Full genome-based classification of rotaviruses reveals a common origin between human Wa-like and porcine rotavirus strains and human DS-1–like and bovine rotavirus strains. *J Virol* 2008;**82**:3204–19.
294. Matthijnssens J, Rahman M, Martella V, et al. Full genomic analysis of human rotavirus strain B4106 and lapine rotavirus strain 30/96 provides evidence for interspecies transmission. *J Virol* 2006;**80**:3801–10.

295. Maunula L, Von Bonsdorff CH. Frequent reassortments may explain the genetic heterogeneity of rotaviruses: analysis of Finnish rotavirus strains. *J Virol* 2002;**76**:11793–800.
296. McClain B, Settembre E, Temple BR, et al. X-ray crystal structure of the rotavirus inner capsid particle at 3.8 Å resolution. *J Mol Biol* 2010;**397**:587–99.
297. McDonald SM, Patton JT. Assortment and packaging of the segmented rotavirus genome. *Trends Microbiol* 2011;**19**:136–44.
298. McDonald SM, Patton JT. Rotavirus VP2 core shell regions critical for viral polymerase activation. *J Virol* 2011;**85**:3095–105.
299. McLean B, Sonza S, Holmes IH. Measurement of immunoglobulin A, G, and M class rotavirus antibodies in serum and mucosal secretions. *J Clin Microbiol* 1980;**12**:314–19.
300. McNeal MM, Broome RL, Ward RL. Active immunity against rotavirus infection in mice is correlated with viral replication and titers of serum rotavirus IgA following vaccination. *Virology* 1994;**204**:642–50.
301. McNeal MM, Rae MN, Ward RL. Evidence that resolution of rotavirus infection in mice is due to both CD4 and CD8 cell-dependent activities. *J Virol* 1997;**71**:8735–42.
302. McNeal MM, Sheridan JF, Ward RL. Active protection against rotavirus infection of mice following intraperitoneal immunization. *Virology* 1992;**191**:150–7.
303. McNeal MM, Ward RL. Long-term production of rotavirus antibody and protection against reinfection following a single infection of neonatal mice with murine rotavirus. *Virology* 1995;**211**:474–80.
304. Mebus CA, Wyatt RG, Sharpee RL, et al. Diarrhea in gnotobiotic calves caused by the reovirus-like agent of human infantile gastroenteritis. *Infect Immun* 1976;**14**:471–4.
305. Mendez E, Arias CF, Lopez S. Binding to sialic acids is not an essential step for the entry of animal rotaviruses to epithelial cells in culture. *J Virol* 1993;**67**:5253–9.
306. Midthun K, Greenberg HB, Hoshino Y, et al. Reassortant rotaviruses as potential live rotavirus vaccine candidates. *J Virol* 1985;**53**:949–54.
307. Midthun K, Halsey NA, Jett-Goheen M, et al. Safety and immunogenicity of human rotavirus vaccine strain M37 in adults, children, and infants. *J Infect Dis* 1991;**164**:792–6.
308. Midthun K, Hoshino Y, Kapikian AZ, Chanock RM. Single gene substitution rotavirus reassortants containing the major neutralization protein (VP7) of human rotavirus serotype 4. *J Clin Microbiol* 1986;**24**:822–6.
309. Miles MG, Lewis KD, Kang G, et al. A systematic review of rotavirus strain diversity in India, Bangladesh, and Pakistan. *Vaccine* 2012;**30**(Suppl. 1):A131–9.
310. Mirzayeva R, Cortese MM, Mosina L, et al. Rotavirus burden among children in the newly independent states of the former Union of Soviet Socialist Republics: literature review and first-year results from the rotavirus surveillance network. *J Infect Dis* 2009;**200**(Suppl. 1):S203–14.
311. Mitra AK, Mahalanabis D, Ashraf H, et al. Hyperimmune cow colostrum reduces diarrhoea due to rotavirus: a double-blind, controlled clinical trial. *Acta Paediatr* 1995;**84**:996–1001.
312. Moe K, Shirley JA. The effects of relative humidity and temperature on the survival of human rotavirus in faeces. *Arch Virol* 1982;**72**:179–86.
313. Molto Y, Cortes JE, De Oliveira LH, et al. Reduction of diarrhea-associated hospitalizations among children aged <5 years in Panama following the introduction of rotavirus vaccine. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S16–20.
314. Moon SS, Wang Y, Shane AL, et al. Inhibitory effect of breast milk on infectivity of live oral rotavirus vaccines. *Pediatr Infect Dis J* 2010;**29**:919–23.
315. Mukherjee A, Chattopadhyay S, Bagchi P, et al. Surveillance and molecular characterization of rotavirus strains circulating in Manipur, north-eastern India: increasing prevalence of emerging G12 strains. *Infect Genet Evol* 2010;**10**:311–20.
316. Mulcahy DL, Kamath KR, de Silva LM, et al. A two-part study of the aetiological role of rotavirus in intussusception. *J Med Virol* 1982;**9**:51–5.
317. Murphy BR, Morens DM, Simonsen L, et al. Reappraisal of the association of intussusception with the licensed live rotavirus vaccine challenges initial conclusions. *J Infect Dis* 2003;**187**:1301–8.
318. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. *N Engl J Med* 2001;**344**:564–72.
319. Mwenda JM, Ntoto KM, Abebe A, et al. Burden and epidemiology of rotavirus diarrhea in selected African countries: preliminary results from the African Rotavirus Surveillance Network. *J Infect Dis* 2010;**202**(Suppl.):S5–11.
320. Naficy AB, Abu-Elyazeed R, Holmes JL, et al. Epidemiology of rotavirus diarrhea in Egyptian children and implications for disease control. *Am J Epidemiol* 1999;**150**:770–7.
321. Nakajima H, Nakagomi T, Kamisawa T, et al. Winter seasonality and rotavirus diarrhoea in adults. *Lancet* 2001;**357**:1950.
322. Nakata S, Estes MK, Graham DY, et al. Detection of antibody to group B adult diarrhea rotaviruses in humans. *J Clin Microbiol* 1987;**25**:812–18.
323. Nalin DR, Levine MM, Mata L, et al. Comparison of sucrose with glucose in oral therapy of infant diarrhoea. *Lancet* 1978;**2**:277–9.
324. Nelson EA, Widdowson MA, Kilgore PE, et al. A decade of the Asian Rotavirus Surveillance Network: achievements and future directions. *Vaccine* 2009;**27**(Suppl. 5):F1–3.
325. Neuzil KM, Parashar UD, Steele AD. Rotavirus vaccines for children in developing countries: understanding the science, maximizing the impact, and sustaining the effort. *Vaccine* 2012;**30**(Suppl. 1):A1–2.
326. Newburg DS, Peterson JA, Ruiz-Palacios GM, et al. Role of human-milk lactadherin in protection against symptomatic rotavirus infection. *Lancet* 1998;**351**:1160–4.
327. Nicolas JC, Ingrand D, Fortier B, Bricout F. A one-year virological survey of acute intussusception in childhood. *J Med Virol* 1982;**9**:267–71.
328. Nigrovic LE, Lumeng C, Landrigan C, Chiang VW. Rotavirus cerebellitis? *Clin Infect Dis* 2002;**34**:130.
329. NIH. FDA licenses rotavirus vaccine for infants. *Am J Health Syst Pharm* 1998;**55**:2226, 2228.
330. Nilsson M, Svenungsson B, Hedlund KO, et al. Incidence and genetic diversity of group C rotavirus among adults. *J Infect Dis* 2000;**182**:678–84.
331. Nishimura S, Ushijima H, Nishimura S, et al. Detection of rotavirus in cerebrospinal fluid and blood of patients with convulsions and gastroenteritis by means of the reverse transcription polymerase chain reaction. *Brain Dev* 1993;**15**:457–9.
332. Numazaki Y, Yano N, Ikeda M, et al. Adenovirus infection in intussusception of Japanese infants. *Jpn J Microbiol* 1973;**17**:87–9.
333. O'Neal CM, Crawford SE, Estes MK, Conner ME. Rotavirus virus-like particles administered mucosally induce protective immunity. *J Virol* 1997;**71**:8707–17.
334. O'Ryan M, Lucero Y, Linhares AC. Rotarix: vaccine performance 6 years postlicensure. *Expert Rev Vaccines* 2011;**10**:1645–59.
335. O'Ryan ML, Matson DO, Estes MK, Pickering LK. Anti-rotavirus G type–specific and isotype-specific antibodies in children with natural rotavirus infections. *J Infect Dis* 1994;**169**:504–11.
336. Offit PA, Blavat G, Greenberg HB, Clark HF. Molecular basis of rotavirus virulence: role of gene segment 4. *J Virol* 1986;**57**:46–9.
337. Offit PA, Clark HF. Protection against rotavirus-induced gastroenteritis in a murine model by passively acquired gastrointestinal but not circulating antibodies. *J Virol* 1985;**54**:58–64.
338. Offit PA, Clark HF, Kornstein MJ, Plotkin SA. A murine model for oral infection with a primate rotavirus (simian SA11). *J Virol* 1984;**51**:233–6.
339. Offit PA, Hoffenberg EJ, Pia ES, et al. Rotavirus-specific helper T cell responses in newborns, infants, children, and adults. *J Infect Dis* 1992;**165**:1107–11.
340. Offit PA, Hoffenberg EJ, Santos N, Gouvea V. Rotavirus-specific humoral and cellular immune response after primary, symptomatic infection. *J Infect Dis* 1993;**167**:1436–40.
341. Ogden KM, Ramanathan HN, Patton JT. Residues of the rotavirus RNA-dependent RNA polymerase template entry tunnel that mediate RNA recognition and genome replication. *J Virol* 2011;**85**:1958–69.
342. Okimoto S, Hyodo S, Yamamoto M, et al. Association of viral isolates from stool samples with intussusception in children. *Int J Infect Dis* 2011;**15**:e641–5.
343. Oyejide CO, Fagbami AH. An epidemiological study of rotavirus diarrhoea in a cohort of Nigerian infants: II. Incidence of

diarrhoea in the first two years of life. *Int J Epidemiol* 1988;**17**: 908–12.
344. Pager C, Steele D, Gwamanda P, Driessen M. A neonatal death associated with rotavirus infection—detection of rotavirus dsRNA in the cerebrospinal fluid. *S Afr Med J* 2000;**90**:364–5.
345. Pammi M, Haque KN. Oral immunoglobulin for the prevention of rotavirus infection in low birth weight infants. *Cochrane Database Syst Rev* 2011;(11):CD003740.
346. Pammi M, Haque KN. Oral immunoglobulin for the treatment of rotavirus diarrhea in low birth weight infants. *Cochrane Database Syst Rev* 2011;(10):CD003742.
347. Pang XL, Joensuu J, Vesikari T. Detection of rotavirus RNA in cerebrospinal fluid in a case of rotavirus gastroenteritis with febrile seizures. *Pediatr Infect Dis J* 1996;**15**:543–5.
348. Parashar UD, Alexander JP, Glass RI. Advisory Committee on Immunization Practices, Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommen Rep* 2006; **55**(RR-12):1–13.
349. Parashar UD, Burton A, Lanata C, et al. Global mortality associated with rotavirus disease among children in 2004. *J Infect Dis* 2009;**200**(Suppl. 1):S9–15.
350. Parashar UD, Gibson CJ, Bresee JS, Glass RI. Rotavirus and severe childhood diarrhea. *Emerg Infect Dis* 2006;**12**:304–6.
351. Parashar UD, Hummelman EG, Bresee JS, et al. Global illness and deaths caused by rotavirus disease in children. *Emerg Infect Dis* 2003;**9**:565–72.
352. Park SI, Matthijnssens J, Saif LJ, et al. Reassortment among bovine, porcine and human rotavirus strains results in G8P[7] and G6P[7] strains isolated from cattle in South Korea. *Vet Microbiol* 2011;**152**:55–66.
353. Patel M, Pedreira C, De Oliveira LH, et al. Association between pentavalent rotavirus vaccine and severe rotavirus diarrhea among children in Nicaragua. *JAMA* 2009;**301**:2243–51.
354. Patel M, Rench MA, Boom JA, et al. Detection of rotavirus antigenemia in routinely obtained serum specimens to augment surveillance and vaccine effectiveness evaluations. *Pediatr Infect Dis J* 2010;**29**:836–9.
355. Patel M, Shane AL, Parashar UD, et al. Oral rotavirus vaccines: how well will they work where they are needed most? *J Infect Dis* 2009;**200**(Suppl. 1):S39–48.
356. Patel MM, Clark AD, Glass RI, et al. Broadening the age restriction for initiating rotavirus vaccination in regions with high rotavirus mortality: benefits of mortality reduction versus risk of fatal intussusception. *Vaccine* 2009;**27**:2916–22.
357. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med* 2011;**364**:2283–92.
358. Patel MM, Steele D, Gentsch JR, et al. Real-world impact of rotavirus vaccination. *Pediatr Infect Dis J* 2011;**30**(Suppl.): S1–5.
359. Patton JT. Rotavirus diversity and evolution in the post-vaccine world. *Discov Med* 2012;**13**:85–97.
360. Patton JT, Vasquez–Del Carpio R, Spencer E. Replication and transcription of the rotavirus genome. *Curr Pharm Des* 2004;**10**: 3769–77.
361. Payne DC, Edwards KM, Bowen MD, et al. Sibling transmission of vaccine-derived rotavirus (RotaTeq) associated with rotavirus gastroenteritis. *Pediatrics* 2010;**125**:e438–41.
362. Payne DC, Parashar UD. Epidemiological shifts in severe acute gastroenteritis in US children: will rotavirus vaccination change the picture? *J Pediatr* 2008;**153**:737–8.
363. Payne DC, Staat MA, Edwards KM, et al. Active, population-based surveillance for severe rotavirus gastroenteritis in children in the United States. *Pediatrics* 2008;**122**:1235–43.
364. Payne DC, Staat MA, Edwards KM, et al. Direct and indirect effects of rotavirus vaccination upon childhood hospitalizations in 3 US counties, 2006-2009. *Clin Infect Dis* 2011;**53**: 245–53.
365. Payne DC, Szilagyi PG, Staat MA, et al. Secular variation in United States rotavirus disease rates and serotypes: implications for assessing the rotavirus vaccination program. *Pediatr Infect Dis J* 2009;**28**:948–53.
366. Pearson GR, McNulty MS. Ultrastructural changes in small intestinal epithelium of neonatal pigs infected with pig rotavirus. *Arch Virol* 1979;**59**:127–36.
367. Pearson GR, McNulty MS, Logan EF. Pathological changes in the small intestine of neonatal calves naturally infected with reo-like virus (rotavirus). *Vet Rec* 1978;**102**:454–8.
368. Penaranda ME, Ho MS, Fang ZY, et al. Seroepidemiology of adult diarrhea rotavirus in China, 1977 to 1987. *J Clin Microbiol* 1989;**27**:2180–3.
369. Perez-Schael I, Blanco M, Vilar M, et al. Clinical studies of a quadrivalent rotavirus vaccine in Venezuelan infants. *J Clin Microbiol* 1990;**28**:553–8.
370. Perez-Schael I, Daoud G, White L, et al. Rotavirus shedding by newborn children. *J Med Virol* 1984;**14**:127–36.
371. Perez-Schael I, Gonzalez M, Daoud N, et al. Reactogenicity and antigenicity of the rhesus rotavirus vaccine in Venezuelan children. *J Infect Dis* 1987;**155**:334–8.
372. Perez-Schael I, Guntinas MJ, Perez M, et al. Efficacy of the rhesus rotavirus-based quadrivalent vaccine in infants and young children in Venezuela. *N Engl J Med* 1997;**337**:1181–7.
373. Perez JF, Chemello ME, Liprandi F, et al. Oncosis in MA104 cells is induced by rotavirus infection through an increase in intracellular Ca^{2+} concentration. *Virology* 1998;**252**:17–27.
374. Perez JF, Ruiz MC, Chemello ME, Michelangeli F. Characterization of a membrane calcium pathway induced by rotavirus infection in cultured cells. *J Virol* 1999;**73**:2481–90.
375. Perez Mato S, Perrin K, Scardino D, Begue RE. Evaluation of rotavirus vaccine effectiveness in a pediatric group practice. *Am J Epidemiol* 2002;**156**:1049–55.
376. Peter G, Myers MG. Intussusception, rotavirus, and oral vaccines: summary of a workshop. *Pediatrics* 2002;**110**:e67.
377. Phan TG, Nishimura S, Okame M, et al. Virus diversity and an outbreak of group C rotavirus among infants and children with diarrhea in Maizuru city, Japan during 2002-2003. *J Med Virol* 2004;**74**:173–9.
378. Phua KB, Lim FS, Lau YL, et al. Rotavirus vaccine RIX4414 efficacy sustained during the third year of life: a randomized clinical trial in an Asian population. *Vaccine* 2012;**30**:4552–7.
379. Phua KB, Quak SH, Lee BW, et al. Evaluation of RIX4414, a live, attenuated rotavirus vaccine, in a randomized, double-blind, placebo-controlled phase 2 trial involving 2464 Singaporean infants. *J Infect Dis* 2005;**192**(Suppl. 1):S6–16.
380. Pickering LK. The day care center diarrhea dilemma. *Am J Public Health* 1986;**76**:623–4.
381. Pickering LK, Bartlett AV, Woodward WE. Acute infectious diarrhea among children in day care: epidemiology and control. *Rev Infect Dis* 1986;**8**:539–47.
382. Pipittajan P, Kasempimolporn S, Ikegami N, et al. Molecular epidemiology of rotaviruses associated with pediatric diarrhea in Bangkok, Thailand. *J Clin Microbiol* 1991;**29**:617–24.
383. Pitzer VE, Viboud C, Lopman BA, et al. Influence of birth rates and transmission rates on the global seasonality of rotavirus incidence. *J R Soc Interface* 2011;**8**:1584–93.
384. Porter HJ, Padfield CJ, Peres LC, et al. Adenovirus and intranuclear inclusions in appendices in intussusception. *J Clin Pathol* 1993;**46**:154–8.
385. Prasad BV, Rothnagel R, Zeng CQ, et al. Visualization of ordered genomic RNA and localization of transcriptional complexes in rotavirus. *Nature* 1996;**382**:471–3.
386. Prasad BV, Wang GJ, Clerx JP, Chiu W. Three-dimensional structure of rotavirus. *J Mol Biol* 1988;**199**:269–75.
387. Prince DS, Astry C, Vonderfecht S, et al. Aerosol transmission of experimental rotavirus infection. *Pediatr Infect Dis* 1986;**5**: 218–22.
388. Rahman S, Higo-Moriguchi K, Htun KW, et al. Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients. *Vaccine* 2012;**30**:4661–9.
389. Ramig RF. Pathogenesis of intestinal and systemic rotavirus infection. *J Virol* 2004;**78**:10213–20.
390. Ramig RF. Systemic rotavirus infection. *Expert Rev Anti Infect Ther* 2007;**5**:591–612.
391. Ramig RF, Ward RL. Genomic segment reassortment in rotaviruses and other reoviridae. *Adv Virus Res* 1991;**39**:163–207.
392. Ranucci C. RotaTeq (Rotavirus Vaccine, Live, Oral, Pentavalent): Update on Porcine Circovirus (PCV). Presented at Advisory Committee on Immunization Practices; Atlanta, Georgia; 2010.
393. Raul Velazquez F, Calva JJ, Lourdes Guerrero M, et al. Cohort study of rotavirus serotype patterns in symptomatic and

asymptomatic infections in Mexican children. *Pediatr Infect Dis J* 1993;**12**:54–61.
394. Rayani A, Bode U, Habas E, et al. Rotavirus infections in paediatric oncology patients: a matched-pairs analysis. *Scand J Gastroenterol* 2007;**42**:81–7.
395. Rennels MB, Glass RI, Dennehy PH, et al. Safety and efficacy of high-dose rhesus-human reassortant rotavirus vaccines—report of the National Multicenter Trial. United States Rotavirus Vaccine Efficacy Group. *Pediatrics* 1996;**97**:7–13.
396. Rennels MB, Losonsky GA, Levine MM, Kapikian AZ. Preliminary evaluation of the efficacy of rhesus rotavirus vaccine strain MMU 18006 in young children. *Pediatr Infect Dis* 1986;**5**:587–8.
397. Rennels MB, Losonsky GA, Young AE, et al. An efficacy trial of the rhesus rotavirus vaccine in Maryland. The Clinical Study Group. *Am J Dis Child* 1990;**144**:601–4.
398. Rennels MB, Parashar UD, Holman RC, et al. Lack of an apparent association between intussusception and wild or vaccine rotavirus infection. *Pediatr Infect Dis J* 1998;**17**:924–5.
399. Richardson S, Grimwood K, Gorrell R, et al. Extended excretion of rotavirus after severe diarrhoea in young children. *Lancet* 1998;**351**:1844–8.
400. Richardson V, Hernandez-Pichardo J, Quintanar-Solares M, et al. Effect of rotavirus vaccination on death from childhood diarrhea in Mexico. *N Engl J Med* 2010;**362**:299–305.
401. Richardson V, Parashar U, Patel M. Childhood diarrhea deaths after rotavirus vaccination in Mexico. *N Engl J Med* 2011;**365**: 772–3.
402. Riepenhoff-Talty M, Bogger-Goren S, Li P, et al. Development of serum and intestinal antibody response to rotavirus after naturally acquired rotavirus infection in man. *J Med Virol* 1981;**8**: 215–22.
403. Riepenhoff-Talty M, Gouvea V, Evans MJ, et al. Detection of group C rotavirus in infants with extrahepatic biliary atresia. *J Infect Dis* 1996;**174**:8–15.
404. Rivera L, Pena LM, Stainier I, et al. Horizontal transmission of a human rotavirus vaccine strain—a randomized, placebo-controlled study in twins. *Vaccine* 2011;**29**:9508–13.
405. Rodriguez WJ, Kim HW, Arrobio JO, et al. Clinical features of acute gastroenteritis associated with human reovirus–like agent in infants and young children. *J Pediatr* 1977;**91**:188–93.
406. Rodriguez WJ, Kim HW, Brandt CD, et al. Rotavirus gastroenteritis in the Washington, DC, area: incidence of cases resulting in admission to the hospital. *Am J Dis Child* 1980;**134**:777–9.
407. Rodriguez WJ, Kim HW, Brandt CD, et al. Longitudinal study of rotavirus infection and gastroenteritis in families served by a pediatric medical practice: clinical and epidemiologic observations. *Pediatr Infect Dis J* 1987;**6**:170–6.
408. Rojas OL, Gonzalez AM, Gonzalez R, et al. Human rotavirus specific T cells: quantification by ELISPOT and expression of homing receptors on $CD4^+$ T cells. *Virology* 2003;**314**:671–9.
409. Rothman KJ, Young-Xu Y, Arellano F. Age dependence of the relation between reassortant rotavirus vaccine (RotaShield) and intussusception. *J Infect Dis* 2006;**193**:898; author reply 898–9.
410. Ruggeri FM, Delogu R, Petouchoff T, et al. Molecular characterization of rotavirus strains from children with diarrhea in Italy, 2007-2009. *J Med Virol* 2011;**83**:1657–68.
411. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006;**354**:11–22.
412. Ruuska T, Vesikari T. Rotavirus disease in Finnish children: use of numerical scores for clinical severity of diarrhoeal episodes. *Scand J Infect Dis* 1990;**22**:259–67.
413. Ryan MJ, Ramsay M, Brown D, et al. Hospital admissions attributable to rotavirus infection in England and Wales. *J Infect Dis* 1996;**174**(Suppl. 1):S12–18.
414. Sack DA, Chowdhury AM, Eusof A, et al. Oral hydration rotavirus diarrhoea: a double blind comparison of sucrose with glucose electrolyte solution. *Lancet* 1978;**2**:280–3.
415. Saif LJ. Nongroup A rotaviruses. In: Saif LJ, Theil KW, editors. *Viral diarrheas of man and animals*. Boca Raton, Fla: CRC Press; 1990.
416. Saif LJ, Jiang B. Nongroup A rotaviruses of humans and animals. *Curr Top Microbiol Immunol* 1994;**185**:339–71.
417. Saif LJ, Redman DR, Smith KL, Theil KW. Passive immunity to bovine rotavirus in newborn calves fed colostrum supplements from immunized or nonimmunized cows. *Infect Immun* 1983;**41**: 1118–31.
418. Salazar-Lindo E, Santisteban-Ponce J, Chea-Woo E, Gutierrez M. Racecadotril in the treatment of acute watery diarrhea in children. *N Engl J Med* 2000;**343**:463–7.
419. Salinas B, Perez Schael I, Linhares AC, et al. Evaluation of safety, immunogenicity and efficacy of an attenuated rotavirus vaccine, RIX4414: a randomized, placebo-controlled trial in Latin American infants. *Pediatr Infect Dis J* 2005;**24**:807–16.
420. Santos N, Hoshino Y. Global distribution of rotavirus serotypes/genotypes and its implication for the development and implementation of an effective rotavirus vaccine. *Rev Med Virol* 2005;**15**: 29–56.
421. Santosham M, Burns B, Nadkarni V, et al. Oral rehydration therapy for acute diarrhea in ambulatory children in the United States: a double-blind comparison of four different solutions. *Pediatrics* 1985;**76**:159–66.
422. Santosham M, Daum RS, Dillman L, et al. Oral rehydration therapy of infantile diarrhea: a controlled study of well-nourished children hospitalized in the United States and Panama. *N Engl J Med* 1982;**306**:1070–6.
423. Santosham M, Letson GW, Wolff M, et al. A field study of the safety and efficacy of two candidate rotavirus vaccines in a Native American population. *J Infect Dis* 1991;**163**:483–7.
424. Santosham M, Yolken RH, Wyatt RG, et al. Epidemiology of rotavirus diarrhea in a prospectively monitored American Indian population. *J Infect Dis* 1985;**152**:778–83.
425. Sarker SA, Casswall TH, Mahalanabis D, et al. Successful treatment of rotavirus diarrhea in children with immunoglobulin from immunized bovine colostrum. *Pediatr Infect Dis J* 1998;**17**:1149–54.
426. Saulsbury FT, Winkelstein JA, Yolken RH. Chronic rotavirus infection in immunodeficiency. *J Pediatr* 1980;**97**:61–5.
427. Schaller JP, Saif LJ, Cordle CT, et al. Prevention of human rotavirus–induced diarrhea in gnotobiotic piglets using bovine antibody. *J Infect Dis* 1992;**165**:623–30.
428. Schreiber RA, Kleinman RE. Genetics, immunology, and biliary atresia: an opening or a diversion? *J Pediatr Gastroenterol Nutr* 1993;**16**:111–13.
429. Schwartz-Cornil I, Benureau Y, Greenberg H, et al. Heterologous protection induced by the inner capsid proteins of rotavirus requires transcytosis of mucosal immunoglobulins. *J Virol* 2002; **76**:8110–17.
430. Selvaraj G, Kirkwood C, Bines J, Buttery J. Molecular epidemiology of adenovirus isolates from patients diagnosed with intussusception in Melbourne, Australia. *J Clin Microbiol* 2006;**44**: 3371–3.
431. Settembre EC, Chen JZ, Dormitzer PR, et al. Atomic model of an infectious rotavirus particle. *EMBO J* 2011;**30**:408–16.
432. Shim JO, Baek IH, Le VP, et al. Molecular characterization of rotavirus diarrhea among children in South Korea: detection of an unusual G11 strain. *Arch Virol* 2011;**156**:887–92.
433. Shin S, Anh DD, Zaman K, et al. Immunogenicity of the pentavalent rotavirus vaccine among infants in two developing countries in Asia, Bangladesh and Vietnam. *Vaccine* 2012;**30**(Suppl. 1): A106–13.
434. Shui IM, Baggs J, Patel M, et al. Risk of intussusception following administration of a pentavalent rotavirus vaccine in US infants. *JAMA* 2012;**307**:598–604.
435. Silvestri LS, Taraporewala ZF, Patton JT. Rotavirus replication: plus-sense templates for double-stranded RNA synthesis are made in viroplasms. *J Virol* 2004;**78**:7763–74.
436. Silvestri LS, Tortorici MA, Vasquez–Del Carpio R, Patton JT. Rotavirus glycoprotein NSP4 is a modulator of viral transcription in the infected cell. *J Virol* 2005;**79**:15165–74.
437. Simonsen L, Morens D, Elixhauser A, et al. Effect of rotavirus vaccination programme on trends in admission of infants to hospital for intussusception. *Lancet* 2001;**358**:1224–9.
438. Simonsen L, Viboud C, Elixhauser A, et al. More on RotaShield and intussusception: the role of age at the time of vaccination. *J Infect Dis* 2005;**192**(Suppl. 1):S36–43.
439. Snelling TL, Schultz R, Graham J, et al. Rotavirus and the indigenous children of the Australian outback: monovalent vaccine effective in a high-burden setting. *Clin Infect Dis* 2009;**49**:428–31.
440. Snodgrass DR, Fahey KJ, Wells PW, et al. Passive immunity in calf rotavirus infections: maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. *Infect Immun* 1980;**28**:344–9.

441. Snodgrass DR, Wells PW. The immunoprophylaxis of rotavirus infections in lambs. *Vet Rec* 1978;**102**:146–8.
442. Snodgrass DR, Wells PW. Rotavirus infection in lambs: studies on passive protection. *Arch Virol* 1976;**52**:201–5.
443. Soriano-Brucher H, Avendano P, O'Ryan M, et al. Bismuth subsalicylate in the treatment of acute diarrhea in children: a clinical study. *Pediatrics* 1991;**87**:18–27.
444. Soriano-Brucher HE, Avendano P, O'Ryan M, Soriano HA. Use of bismuth subsalicylate in acute diarrhea in children. *Rev Infect Dis* 1990;**12**(Suppl. 1):S51–6.
445. Staat MA, Azimi PH, Berke T, et al. Clinical presentations of rotavirus infection among hospitalized children. *Pediatr Infect Dis J* 2002;**21**:221–7.
446. Staat MA, Payne DC, Donauer S, et al. Effectiveness of pentavalent rotavirus vaccine against severe disease. *Pediatrics* 2011; **128**:e267–75.
447. Staat MA, Rice MA, Donauer S, et al. Estimating the rotavirus hospitalization disease burden and trends, using capture-recapture methods. *Pediatr Infect Dis J* 2010;**29**:1083–6.
448. Steele AD, Cunliffe N, Tumbo J, et al. A review of rotavirus infection in and vaccination of human immunodeficiency virus–infected children. *J Infect Dis* 2009;**200**(Suppl. 1):S57–S62.
449. Steele AD, James VL. Seroepidemiology of human group C rotavirus in South Africa. *J Clin Microbiol* 1999;**37**:4142–4.
450. Stelzmueller I, Dunst KM, Hengster P, et al. A cluster of rotavirus enteritis in adult transplant recipients. *Transplant Int* 2005;**18**: 470–4.
451. Stelzmueller I, Wiesmayr S, Swenson BR, et al. Rotavirus enteritis in solid organ transplant recipients: an underestimated problem? *Transpl Infect Dis* 2007;**9**:281–5.
452. Stockman LJ, Staat MA, Holloway M, et al. Optimum diagnostic assay and clinical specimen for routine rotavirus surveillance. *J Clin Microbiol* 2008;**46**:1842–3.
453. Suzuki H, Konno T. Reovirus-like particles in jejunal mucosa of a Japanese infant with acute infectious non-bacterial gastroenteritis. *Tohoku J Exp Med* 1975;**115**:199–211.
454. Tapisiz A, Karahan ZC, Ciftci E, et al. Changing patterns of rotavirus genotypes in Turkey. *Curr Microbiol* 2011;**63**:517–22.
455. Tate JE, Burton AH, Boschi-Pinto C, et al. 2008 estimate of worldwide rotavirus-associated mortality in children younger than 5 years before the introduction of universal rotavirus vaccination programmes: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:136–41.
456. Tate JE, Cortese MM, Payne DC, et al. Uptake, impact, and effectiveness of rotavirus vaccination in the United States: review of the first 3 years of postlicensure data. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S56–60.
457. Tate JE, Mutuc JD, Panozzo CA, et al. Sustained decline in rotavirus detections in the United States following the introduction of rotavirus vaccine in 2006. *Pediatr Infect Dis J* 2011;**30**(Suppl.): S30–4.
458. Tate JE, Panozzo CA, Payne DC, et al. Decline and change in seasonality of US rotavirus activity after the introduction of rotavirus vaccine. *Pediatrics* 2009;**124**:465–71.
459. Tate JE, Parashar UD. Monitoring impact and effectiveness of rotavirus vaccination. *Expert Rev Vaccines* 2011;**10**:1123–5.
460. Tate JE, Patel MM, Cortese MM, et al. Remaining issues and challenges for rotavirus vaccine in preventing global childhood diarrheal morbidity and mortality. *Expert Rev Vaccines* 2012;**11**:211–20.
461. Teran CG, Teran-Escalera CN, Villarroel P. Nitazoxanide vs. probiotics for the treatment of acute rotavirus diarrhea in children: a randomized, single-blind, controlled trial in Bolivian children. *Int J Infect Dis* 2009;**13**:518–23.
462. Theil KW, Bohl EH, Cross RF, et al. Pathogenesis of porcine rotaviral infection in experimentally inoculated gnotobiotic pigs. *Am J Vet Res* 1978;**39**:213–20.
463. Tian P, Ball JM, Zeng CQ, Estes MK. The rotavirus nonstructural glycoprotein NSP4 possesses membrane destabilization activity. *J Virol* 1996;**70**:6973–81.
464. Tian P, Estes MK, Hu Y, et al. The rotavirus nonstructural glycoprotein NSP4 mobilizes Ca^{2+} from the endoplasmic reticulum. *J Virol* 1995;**69**:5763–72.
465. Tian P, Hu Y, Schilling WP, et al. The nonstructural glycoprotein of rotavirus affects intracellular calcium levels. *J Virol* 1994;**68**: 251–7.
466. Torok TJ, Kilgore PE, Clarke MJ, et al. Visualizing geographic and temporal trends in rotavirus activity in the United States, 1991 to 1996. National Respiratory and Enteric Virus Surveillance System Collaborating Laboratories. *Pediatr Infect Dis J* 1997;**16**: 941–6.
467. Torres-Medina A. Effect of rotavirus and/or *Escherichia coli* infection on the aggregated lymphoid follicles in the small intestine of neonatal gnotobiotic calves. *Am J Vet Res* 1984;**45**:652–60.
468. Tra My PV, Rabaa MA, Vinh H, et al. The emergence of rotavirus G12 and the prevalence of enteric viruses in hospitalized pediatric diarrheal patients in southern Vietnam. *Am J Trop Med Hyg* 2011;**85**:768–75.
469. Trask SD, McDonald SM, Patton JT. Structural insights into the coupling of virion assembly and rotavirus replication. *Nat Rev Microbiol* 2012;**10**:165–77.
470. Trask SD, Ogden KM, Patton JT. Interactions among capsid proteins orchestrate rotavirus particle functions. *Curr Opin Virol* 2012;**2**:373–9.
471. Treanor JJ, Clark HF, Pichichero M, et al. Evaluation of the protective efficacy of a serotype 1 bovine-human rotavirus reassortant vaccine in infants. *Pediatr Infect Dis J* 1995;**14**:301–7.
472. Troupin C, Dehee A, Schnuriger A, et al. Rearranged genomic RNA segments offer a new approach to the reverse genetics of rotaviruses. *J Virol* 2010;**84**:6711–19.
473. Tsunemitsu H, Jiang B, Saif LJ. Detection of group C rotavirus antigens and antibodies in animals and humans by enzyme-linked immunosorbent assays. *J Clin Microbiol* 1992;**30**:2129–34.
474. Turcios RM, Curns AT, Holman RC, et al. Temporal and geographic trends of rotavirus activity in the United States, 1997-2004. *Pediatr Infect Dis J* 2006;**25**:451–4.
475. Uhnoo I, Olding-Stenkvist E, Kreuger A. Clinical features of acute gastroenteritis associated with rotavirus, enteric adenoviruses, and bacteria. *Arch Dis Child* 1986;**61**:732–8.
476. Ushijima H, Bosu K, Abe T, Shinozaki T. Suspected rotavirus encephalitis. *Arch Dis Child* 1986;**61**:692–4.
477. Ushijima H, Honma H, Mukoyama A, et al. Detection of group C rotaviruses in Tokyo. *J Med Virol* 1989;**27**:299–303.
478. VanCott JL, McNeal MM, Flint J, et al. Role for T cell–independent B cell activity in the resolution of primary rotavirus infection in mice. *Eur J Immunol* 2001;**31**:3380–7.
479. VanCott JL, Prada AE, McNeal MM, et al. Mice develop effective but delayed protective immune responses when immunized as neonates either intranasally with nonliving VP6/LT(R192G) or orally with live rhesus rotavirus vaccine candidates. *J Virol* 2006;**80**:4949–61.
480. Velazquez FR, Luna G, Cedillo R, et al. Natural rotavirus infection is not associated to intussusception in Mexican children. *Pediatr Infect Dis J* 2004;**23**(Suppl.):S173–8.
481. Velazquez FR, Matson DO, Calva JJ, et al. Rotavirus infections in infants as protection against subsequent infections. *N Engl J Med* 1996;**335**:1022–8.
482. Velazquez FR, Matson DO, Guerrero ML, et al. Serum antibody as a marker of protection against natural rotavirus infection and disease. *J Infect Dis* 2000;**182**:1602–9.
483. Vesikari T. Clinical trials of live oral rotavirus vaccines: the Finnish experience. *Vaccine* 1993;**11**:255–61.
484. Vesikari T, Isolauri E, D'Hondt E, et al. Protection of infants against rotavirus diarrhoea by RIT 4237 attenuated bovine rotavirus strain vaccine. *Lancet* 1984;**1**:977–81.
485. Vesikari T, Isolauri E, Delem A, et al. Immunogenicity and safety of live oral attenuated bovine rotavirus vaccine strain RIT 4237 in adults and young children. *Lancet* 1983;**2**:807–11.
486. Vesikari T, Kapikian AZ, Delem A, Zissis G. A comparative trial of rhesus monkey (RRV-1) and bovine (RIT 4237) oral rotavirus vaccines in young children. *J Infect Dis* 1986;**153**:832–9.
487. Vesikari T, Karvonen A, Ferrante SA, Ciarlet M. Efficacy of the pentavalent rotavirus vaccine, RotaTeq, in Finnish infants up to 3 years of age: the Finnish Extension Study. *Eur J Pediatr* 2010; **169**:1379–86.
488. Vesikari T, Karvonen A, Ferrante SA, et al. Sustained efficacy of the pentavalent rotavirus vaccine, RV5, up to 3.1 years following the last dose of vaccine. *Pediatr Infect Dis J* 2010;**29**:957–63.
489. Vesikari T, Karvonen A, Prymula R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet* 2007;**370**:1757–63.

490. Vesikari T, Karvonen A, Puustinen L, et al. Efficacy of RIX4414 live attenuated human rotavirus vaccine in Finnish infants. *Pediatr Infect Dis J* 2004;**23**:937–43.
491. Vesikari T, Karvonen AV, Majuri J, et al. Safety, efficacy, and immunogenicity of 2 doses of bovine-human (UK) and rhesus-rhesus-human rotavirus reassortant tetravalent vaccines in Finnish children. *J Infect Dis* 2006;**194**:370–6.
492. Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006;**354**:23–33.
493. Vesikari T, Rautanen T, Varis T, et al. Rhesus rotavirus candidate vaccine. Clinical trial in children vaccinated between 2 and 5 months of age. *Am J Dis Child* 1990;**144**:285–9.
494. Vesikari T, Rautanen T, Von Bonsdorff CH. Rotavirus gastroenteritis in Finland: burden of disease and epidemiological features. *Acta Paediatr Suppl* 1999;**88**:24–30.
495. Vesikari T, Ruuska T, Koivu HP, et al. Evaluation of the M37 human rotavirus vaccine in 2- to 6-month-old infants. *Pediatr Infect Dis J* 1991;**10**:912–17.
496. Vesikari T, Sarkkinen HK, Maki M. Quantitative aspects of rotavirus excretion in childhood diarrhoea. *Acta Paediatr Scand* 1981; **70**:717–21.
497. Victoria JG, Wang C, Jones MS, et al. Viral nucleic acids in live-attenuated vaccines: detection of minority variants and an adventitious virus. *J Virol* 2010;**84**:6033–40.
498. Vizzi E, Pineros O, Gonzalez GG, et al. Genotyping of human rotaviruses circulating among children with diarrhea in Valencia, Venezuela. *J Med Virol* 2011;**83**:2225–32.
499. Wang FT, Mast TC, Glass RJ, et al. Effectiveness of the pentavalent rotavirus vaccine in preventing gastroenteritis in the United States. *Pediatrics* 2010;**125**:e208–13.
500. Wang L, Huang JA, Nagesha HS, et al. Bacterial expression of the major antigenic regions of porcine rotavirus VP7 induces a neutralizing immune response in mice. *Vaccine* 1999;**17**:2636–45.
501. Wang Y, Azevedo M, Saif LJ, et al. Inactivated rotavirus vaccine induces protective immunity in gnotobiotic piglets. *Vaccine* 2010; **28**:5432–6.
502. Ward RL, Bernstein DI. Protection against rotavirus disease after natural rotavirus infection. US Rotavirus Vaccine Efficacy Group. *J Infect Dis* 1994;**169**:900–4.
503. Ward RL, Bernstein DI, Shukla R, et al. Protection of adults rechallenged with a human rotavirus. *J Infect Dis* 1990;**161**: 440–5.
504. Ward RL, Bernstein DI, Shukla R, et al. Effects of antibody to rotavirus on protection of adults challenged with a human rotavirus. *J Infect Dis* 1989;**159**:79–88.
505. Ward RL, Clemens JD, Knowlton DR, et al. Evidence that protection against rotavirus diarrhea after natural infection is not dependent on serotype-specific neutralizing antibody. *J Infect Dis* 1992;**166**:1251–7.
506. Ward RL, Clemens JD, Sack DA, et al. Culture adaptation and characterization of group A rotaviruses causing diarrheal illnesses in Bangladesh from 1985 to 1986. *J Clin Microbiol* 1991;**29**: 1915–23.
507. Ward RL, Knowlton DR, Pierce MJ. Efficiency of human rotavirus propagation in cell culture. *J Clin Microbiol* 1984;**19**:748–53.
508. Ward RL, Knowlton DR, Schiff GM, et al. Relative concentrations of serum neutralizing antibody to VP3 and VP7 proteins in adults infected with a human rotavirus. *J Virol* 1988;**62**:1543–9.
509. Ward RL, Knowlton DR, Zito ET, et al. Serologic correlates of immunity in a tetravalent reassortant rotavirus vaccine trial. US Rotavirus Vaccine Efficacy Group. *J Infect Dis* 1997;**176**: 570–7.
510. Ward RL, McNeal MM, Sander DS, et al. Immunodominance of the VP4 neutralization protein of rotavirus in protective natural infections of young children. *J Virol* 1993;**67**:464–8.
511. Ward RL, McNeal MM, Sheridan JF. Evidence that active protection following oral immunization of mice with live rotavirus is not dependent on neutralizing antibody. *Virology* 1992;**188**:57–66.
512. Watanabe M, Nakagomi T, Koshimura Y, Nakagomi O. Direct evidence for genome segment reassortment between concurrently-circulating human rotavirus strains. *Arch Virol* 2001;**146**:557–70.
513. Weinberg GA, Payne DC, Teel EN, et al. First reports of human rotavirus G8P[4] gastroenteritis in the United States. *J Clin Microbiol* 2012;**50**:1118–21.
514. WHO. Research priorities for diarrhoeal disease vaccines; memorandum from a WHO meeting. *Bull World Health Organ* 1991;**69**:667–76.
515. WHO. Rotavirus surveillance worldwide—2009. *Wkly Epidemiol Rec* 2011;**86**:174–6.
516. Widdowson MA, Meltzer MI, Zhang X, et al. Cost-effectiveness and potential impact of rotavirus vaccination in the United States. *Pediatrics* 2007;**119**:684–97.
517. Widdowson MA, Steele D, Vojdani J, et al. Global rotavirus surveillance: determining the need and measuring the impact of rotavirus vaccines. *J Infect Dis* 2009;**200**(Suppl. 1):S1–8.
518. Wolf M, Vo PT, Greenberg HB. Rhesus rotavirus entry into a polarized epithelium is endocytosis dependent and involves sequential VP4 conformational changes. *J Virol* 2011;**85**:2492–503.
519. Wong CJ, Price Z, Bruckner DA. Aseptic meningitis in an infant with rotavirus gastroenteritis. *Pediatr Infect Dis* 1984;**3**:244–6.
520. Woode GN, Bridger JC, Jones JM, et al. Morphological and antigenic relationships between viruses (rotaviruses) from acute gastroenteritis of children, calves, piglets, mice and foals. *Infection and Immunity* 1976;**14**:804–10.
521. Wyatt RG, Yolken RH, Urrutia JJ, et al. Diarrhea associated with rotavirus in rural Guatemala: a longitudinal study of 24 infants and young children. *Am J Trop Med Hyg* 1979;**28**:325–8.
522. Yen C, Armero Guardado JA, Alberto P, et al. Decline in rotavirus hospitalizations and health care visits for childhood diarrhea following rotavirus vaccination in El Salvador. *Pediatr Infect Dis J* 2011;**30**(Suppl.):S6–10.
523. Yen C, Figueroa JR, Uribe ES, et al. Monovalent rotavirus vaccine provides protection against an emerging fully heterotypic G9P[4] rotavirus strain in Mexico. *J Infect Dis* 2011;**204**:783–6.
524. Yen C, Jakob K, Esona MD, et al. Detection of fecal shedding of rotavirus vaccine in infants following their first dose of pentavalent rotavirus vaccine. *Vaccine* 2011;**29**:4151–5.
525. Yen C, Tate JE, Patel MM, et al. Rotavirus vaccines: update on global impact and future priorities. *Hum Vaccin* 2011;**7**:1282–90.
526. Yen C, Tate JE, Steiner CA, et al. Trends in intussusception hospitalizations among US infants before and after implementation of the rotavirus vaccination program, 2000-2009. *J Infect Dis* 2012;**206**:41–8.
527. Yen C, Tate JE, Wenk JD, et al. Diarrhea-associated hospitalizations among US children over 2 rotavirus seasons after vaccine introduction. *Pediatrics* 2011;**127**:e9–15.
528. Yoder JD, Dormitzer PR. Alternative intermolecular contacts underlie the rotavirus VP5* two- to three-fold rearrangement. *EMBO J* 2006;**25**:1559–68.
529. Yoder JD, Trask SD, Vo TP, et al. VP5* rearranges when rotavirus uncoats. *J Virol* 2009;**83**:11372–7.
530. Yoshida A, Kawamitu T, Tanaka R, et al. Rotavirus encephalitis: detection of the virus genomic RNA in the cerebrospinal fluid of a child. *Pediatr Infect Dis J* 1995;**14**:914–16.
531. Yuan L, Geyer A, Hodgins DC, et al. Intranasal administration of 2/6-rotavirus-like particles with mutant *Escherichia coli* heat-labile toxin (LT-R192G) induces antibody-secreting cell responses but not protective immunity in gnotobiotic pigs. *J Virol* 2000;**74**: 8843–53.
532. Yunis EJ, Atchison RW, Michaels RH, DeCicco FA. Adenovirus and ileocecal intussusception. *Lab Invest* 1975;**33**:347–51.
533. Yunis EJ, Hashida Y. Electron microscopic demonstration of adenovirus in appendix vermiformis in a case of ileocecal intussusception. *Pediatrics* 1973;**51**:566–70.
534. Zaman K, Dang DA, Victor JC, et al. Efficacy of pentavalent rotavirus vaccine against severe rotavirus gastroenteritis in infants in developing countries in Asia: a randomised, double-blind, placebo-controlled trial. *Lancet* 2010;**376**:615–23.
535. Zaman K, Yunus M, Arifeen SE, et al. Methodology and lessons-learned from the efficacy clinical trial of the pentavalent rotavirus vaccine in Bangladesh. *Vaccine* 2012;**30**(Suppl. 1):A94–100.
536. Zarate S, Espinosa R, Romero P, et al. Integrin $\alpha_2\beta_1$ mediates the cell attachment of the rotavirus neuraminidase-resistant variant nar3. *Virology* 2000;**278**:50–4.
537. Zheng BJ, Lo SK, Tam JS, et al. Prospective study of community-acquired rotavirus infection. *J Clin Microbiol* 1989;**27**:2083–90.
538. Zickafoose JS, Benneyworth BD, Riebschleger MP, et al. Hospitalizations for intussusception before and after the reintroduction of rotavirus vaccine in the United States. *Arch Pediatr Adolesc Med* 2012;**166**:350–5.

Arabian physicians under the name *al-hamikah;* they considered rubella a form of measles, however. Two German physicians, de Bergen in 1752 and Orlow in 1758, usually are credited with providing the first clinical descriptions of rubella as a specific entity.[229,607] In early writings, rubella generally was called *Röteln.*[182,607] However, because of the great interest of German physicians in the disease during the period from the mid-18th to the mid-19th centuries, the name *German measles* frequently was used in other countries.

In 1866, a Scottish physician named Veale[584] described 30 cases of German measles. In his paper, he gave the illness its present name, *rubella*. His opinion was that the German name Röteln was too harsh and foreign and that other possible names—*rubeola notha* and *rosalia idiopathica*—were too long for general use and could be confused with measles.[183] Other historical synonyms of rubella include rubeola, rubeola sine catarrho, rubeola epidemica, rubeola morbillosa, rubeola scarlatinosa, rosania, roseola, roseola epidemica, rosalia, scarlatina morbillosa, scarlatina hybrida, morbilli scarlatinosi, feuer masern, roséole, roséole idiopathique, rubéole, rougéole fausse, French measles, false measles, bastard measles, hybrid measles, and bastard scarlatina.[229]

In 1881, at the International Congress of Medicine in London, a consensus was reached that rubella is a distinct disease. Rubella was thought to be similar in some respects but not identical to measles or scarlatina. By the beginning of the 20th century, the clinical description of rubella was complete, except that joint manifestations had received curiously little notation.*

Rubella gained its present-day importance in 1941 when Gregg,[228] an Australian ophthalmologist, reported congenital defects in babies of mothers who had rubella during early pregnancy. In spite of considerable skepticism, Gregg's observations were confirmed quickly by Swan and colleagues[549,550] in Australia and other investigators in the United States and the United Kingdom.[164,459,466,607] By 1947, 28 communications describing 500 children with severe congenital defects associated with maternal rubella had appeared in the literature.[607]

In 1938, Hiro and Tasaka[269] demonstrated that rubella is a disease of viral etiology by transmission of disease to humans through the subcutaneous injection of filtered nasal washings. In 1942, Habel[237] was able to infect monkeys with nasal washings and blood from human cases. In 1962, rubella virus first was propagated in the laboratory; two investigative teams, Weller and Neva[606] and Parkman and colleagues,[422] using different techniques, reported the growth of rubella virus in tissue culture.

The isolation of rubella virus in 1962 paved the way for definitive study of the 1964 rubella pandemic.[358,422,606] The results of extensive virologic, serologic, and epidemiologic investigation were presented at a Rubella Symposium in May 1965.[319] After the rubella virus was isolated in tissue culture, an intensive worldwide effort to develop vaccines was mounted. The accumulation of these experiences resulted in an extensive body of knowledge related to rubella and rubella immunization that was presented at the International Conference on Rubella Immunization in February 1969.[320] Live attenuated rubella virus vaccines were licensed for use in mid-1969 in the United States.[317,371,603] In the 38-year period since vaccine licensure and its universal use in the United States occurred, the yearly occurrence of rubella fell from 40,000 cases to 11 cases in 2005.*

At present, sustained transmission of rubella no longer occurs in the United States.[86,455]

*References 18, 125, 183, 229, 372, 486, 568.

PROPERTIES

Classification

Rubella virus is placed in the *Rubivirus* genus of the family *Togaviridae*.[174,495,618] At present, it is the only species in this genus. The virus is similar physiochemically to the other member of its family (alphavirus) but is unrelated serologically. Rubella virus has no invertebrate host (a characteristic of all alphaviruses), and humans are the only known vertebrate host.

In 2005, the World Health Organization (WHO) adopted a systematic nomenclature to categorize the distinct genetic variants of rubella virus. Rubella virus genotypes were divided into 2 phylogenetic groups, Clade 1 and Clade 2, differing by only 8 to 10 percent at the nucleotide level. Clade 1 includes 10 genotypes (1a, 1B, 1C, 1D, 1E, 1F, 1G, 1h, 1i, and 1j), and Clade 2 includes 3 genotypes (2A, 2B, and 2C). Recognized genotypes are denoted with uppercase in contrast to provisional genotypes designated with lowercase letters. Of these 13 genotypes, only 3 (1E, 1G, and 1B) are noted to have a large global distribution. This classification has been especially important in the WHO's virologic surveillance and has greatly aided elimination efforts.[2,623]

Physical Properties

The rubella virion is spherical, with a diameter of 60 to 70 nm, and consists of a capsid protein (C) and two glycoproteins (E1 and E2).† E1 (relative molecular weight, 58,000) and E2 (relative molecular weight, 42,000 to 47,000) are glycosylated and are located on the viral surface membrane. E1 is the viral hemagglutinin that is found on 5- to 6-nm surface projections.[272,431] The nucleocapsid has a diameter of 30 to 40 nm and is composed of a polypeptide (C protein) and the genomic RNA. The nucleic acid of rubella virus is single-stranded RNA with a molecular weight of 3.2 to 3.8×10^7 and a genome of 9762 nucleotides.[505] Its genome encodes five protein products, including two nonstructural proteins (P90 and P150) and three virion proteins (the capsid, E1, and E2).[2] The outer coat of the virus (envelope) is lipoprotein with host-cell lipid and virus-specified polypeptides.

*References 77, 79, 81, 83, 85, 87, 437, 445, 456, 613.
†References 94, 335, 354, 431, 495, 618.

Rubella virus is relatively sensitive to heat; it generally has been found to lose infectivity within 30 minutes at 56° C (132.8° F).[170,423,436] However, Kistler and Sapatino[309] observed that some infectivity persists even after heating for 60 minutes at 70° C (158° F). At 37° C (98.6° F) in the presence of 2 percent serum, 90 percent is inactivated in 3 hours.[423] At 4° C (39.2° F), with protein stabilization, viral titers are maintained for 7 or more days. The virus is stable indefinitely at –60° C (–76° F) and below but labile at normal (–10° to –20° C [14° to –4° F]) refrigeration temperatures. When it is stabilized with protein, the virus can survive several rapid freeze–thaw cycles without significant loss of titer.[489]

Rubella virus is sensitive to ultraviolet light. In 1 hour, a high-titer cell-free virus suspension was inactivated by an intensity of 1350 μW/cm^2; on the other hand, a tissue culture suspension of virus was not inactivated completely when it was exposed to a similar intensity of radiation.[309] Rubella virus is sensitive to visible light, and this photosensitivity can be potentiated by the basic dye proflavine.[50]

The virus also is sensitive to pH extremes of less than 6.8 and greater than 8.1.[91] The following chemicals rapidly inactivate rubella virus: ether, acetone, chloroform, deoxycholate, formalin, β-propiolactone, ethylene oxide, free chlorine, and 70 percent alcohol.[436] It is resistant to thimerosal.

Antigenic Composition

Rubella virus infection of tissue culture cells results in the production of infectious virus that can be neutralized by specific antiserum. Specific viral antibodies can be identified by hemagglutination inhibition (HI), complement fixation (CF), precipitation in gel, platelet aggregation, passive hemagglutination, single radial hemolysis, latex agglutination, enzyme-linked immunosorbent assay (ELISA), and immunofluorescence.* Neutralization and HI identify antibodies that inhibit specific biologic functions of the virus, whereas the other assays identify only the formation of antigen-antibody complexes. The E1 glycoprotein is the predominant erythrocyte-binding and neutralization site of the virus.[570,601] Weak neuraminidase activity also has been associated with purified rubella virus.[27]

In 1967, Stewart and associates[540] reported that tissue culture–grown rubella virus produced hemagglutination of erythrocytes from chickens that were younger than 1 day old and from one goose and one lamb, but no hemagglutination was observed with adult chicken, guinea pig, and other red cell preparations commonly used. Subsequently, techniques involving careful control of test system diluents have revealed that red cells from many different animals are agglutinated by rubella virus.[340,500] Viral hemagglutinin is stable at –20° C (–4° F) for months and at 4° C (39.2° F) for several weeks but is destroyed rapidly by heat.[202,241]

Sever and colleagues[515] first demonstrated that supernatant fluid from primary African green monkey kidney (AGMK) and rabbit kidney epithelial cells (RK-13) tissue cultures contained useful complement-fixing antigens. Two distinct rubella complement-fixing antigens exist.[501] One of the antigens is similar in size and weight to both the hemagglutinin and infectious virus; the other "soluble" antigen is smaller, with a buoyant density of 1.08 g/mL. The soluble antigen is noninfectious and does not contain nucleic acid.[436] Rubella complement-fixing antigens retain their antigenicity after either treatment.

Two major small-particle antigens have been identified in the medium of tissue culture–infected cells by immunodiffusion.[330,337,499] These two soluble antigens are structural components of the virion, and natural infection with rubella virus results in the formation of serum precipitating antibodies. These antigens have been designated *theta* and *iota*. Their importance lies in the fact that antibody to the iota antigen rarely is noted in the serum of recipients of some rubella vaccines; therefore, they may be of value in studying vaccine-induced immunity.[69,332]

The E1 glycoprotein is the dominant surface molecule of rubella virus.[95] It is the main target of the humoral antibody response for both detection and elimination of the virus. HI and neutralization sites have been localized to a small segment of the E1 glycoprotein ($E1_{245}$ to $E1_{285}$). A recent study identified the myelin oligodendrocyte glycoprotein (MOG) as the host cell receptor for the rubella virus that interacts with the E1 glycoprotein. Blocking this MOG attachment site of rubella virus may also prove to be a potential way to prevent rubella virus infection of cells.[114]

Seventeen T-cell epitopes have been identified with the lymphoproliferation assay.[94,384] They involve the capsid protein and E1 and E2 glycoproteins.

Molecular analysis of rubella virus epidemiology from 1961 to 1997 in North America, Europe, and Asia found no major antigenic variations.[195] Further phylogenetic analysis of a collection of 103 E1 gene sequences from rubella viruses isolated from 17 countries from 1961 to 2000 found the existence of at least two genotypes.[628] Previous work noted rubella genotype 1 isolates predominantly in Europe, Japan, and the Western Hemisphere, whereas genotype 2 isolates were limited to Asia and Europe. As noted earlier, in 2005, the WHO adopted new nomenclature to categorize all of the new genetic variants of rubella found.[2]

Tissue Culture Growth

Rubella virus grows in many different tissue cultures, including cell strains, cell lines, and primary cells.* Cell sources include mature and embryonic tissue from humans and other primates, rabbits, swine, dogs, birds, hamsters, and cattle. In tissue culture, growth of rubella virus can be identified by either its cytopathic effect or its ability to interfere with the growth of another tissue culture–susceptible virus.

The method used most commonly for primary isolation of rubella virus from clinical material is the

*References 35, 43, 93, 97, 98, 162, 163, 176, 192, 265, 330, 331, 332, 355, 427, 436, 482, 481, 581, 618, 629.

*References 132, 357, 360, 423, 436, 617, 618.

interference technique with primary AGMK cells.[422] In this system, nonadapted rubella virus grows readily but does not produce a cytopathic effect. Infection is demonstrated in AGMK tissue culture by the failure of a typical enterovirus cytopathic effect to occur after the culture is challenged with echovirus 11 or another suitable enterovirus. A common alternative to the AGMK–echovirus 11 interference system for primary isolation of rubella virus is use of the RK-13 cell line, in which infection can be identified by cytopathic effect. For laboratory study and determination of neutralizing antibody, many different cell lines (e.g., RK-13, baby hamster kidney cell [BHK]-21, and rhesus monkey kidney epithelial cells [LLC-MK2]) can be used. The highest titers of rubella virus are produced in the BHK-21 and Vero cell lines.

Kinetic studies in tissue culture indicate that virus adsorption is complete within 90 minutes and that the eclipse period lasts approximately 12 hours. The first new virus noted is cell associated; it is followed in 2 to 4 hours by extracellular virus. In primary cell culture, titers of both cell-associated and free virus reach 10^3 tissue culture infectious dose $(TCID)_{50}$/mL by the fourth day; peak titers are not attained until approximately the 17th day. In all cell systems, chronic infection occurs but is limited in some cultures by the cytopathic effect. Rubella virus induces the formation of plaques in several cell lines.

Animal Susceptibility

Although natural infection is known to occur only in humans, several other primates have been infected experimentally.[357,423,436] In addition to primates, rabbits, hamsters, ferrets, guinea pigs, and suckling mice have been infected with rubella virus.

EPIDEMIOLOGY

In contrast to measles and other diseases having clearly apparent dramatic cycles, knowledge of rubella epidemiology has been acquired primarily during the last 75 years. Major events during this period that stimulated interest in its epidemiology were the observation of teratogenicity in 1941,[228] the isolation of the virus in the early 1960s,[422,606] and the pandemic of 1964. Unfortunately, rubella was not a reportable disease in the United States until 1966, so considerable gaps exist in the available information. At present, we are in a new epidemiologic era because of the widespread use of rubella vaccine. Predicting the incidence of rubella today must take into account the extent and method of vaccine use in the population under surveillance.

Incidence and Prevalence

Epidemic Behavior

The epidemic pattern of rubella in selected areas of the United States in the prevaccine and early vaccine eras is presented in Figure 173-1. The number of reported cases of rubella and congenital rubella syndrome by year in the vaccine era is presented in Figure 173-2. The rubella epidemic cycle usually is described as one of 6- to 9-year intervals, with each cycle consisting of a build-up and fall in incidence during the course of a 3- to 4-year period.[279,280] However, a close look at Figure 173-1 suggests that the basic pattern in the prevaccine era was a 3.6-year cycle. Of the 11 peaks from 1928 to 1968, all but 2 occurred in a 2- to 4-year span, with a median of 3 years. Over and above the 3-year cycle is the better known 6- to 9-year cycle of major disease. Pandemics occurred in the periods 1941 to 1944 and 1963 to 1965.

The U.S. rubella pandemic of 1964 to 1965, which initially began in Europe in 1963, exemplifies the extent of morbidity that rubella has the potential to cause.[436] At that time, there were an estimated 12.5 million cases of rubella, including more than 2000 cases of encephalitis, 11,250 fetal deaths, 20,000 cases of congenital rubella syndrome, 3580 deaf-blind children, and 1800 children with mental retardation.[623]

Since the introduction and widespread use of rubella vaccine in the United States, epidemic rubella has occurred only once on a national scale (see Fig. 173-2).[84,85,454]

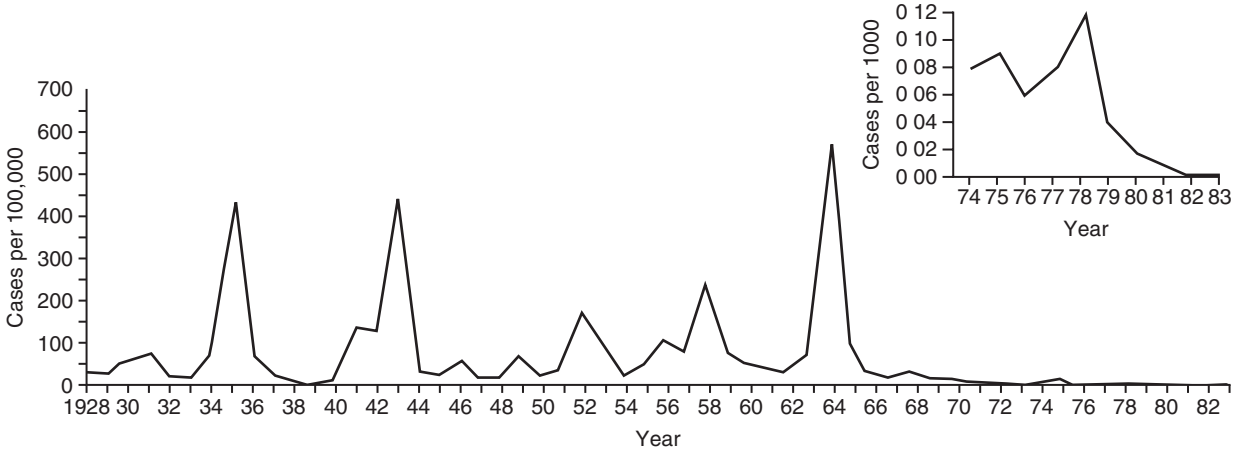

FIGURE 173-1 ■ Rubella incidence in 10 selected areas (Maine, Rhode Island, Connecticut, New York City, Ohio, Illinois, Wisconsin, Maryland, Washington, and Massachusetts) of the United States, 1928 to 1983. (From Williams NM, Preblud SR. Rubella and congenital rubella surveillance, 1983. *MMWR Surveill Summ* 1984;33:1SS-10SS.)

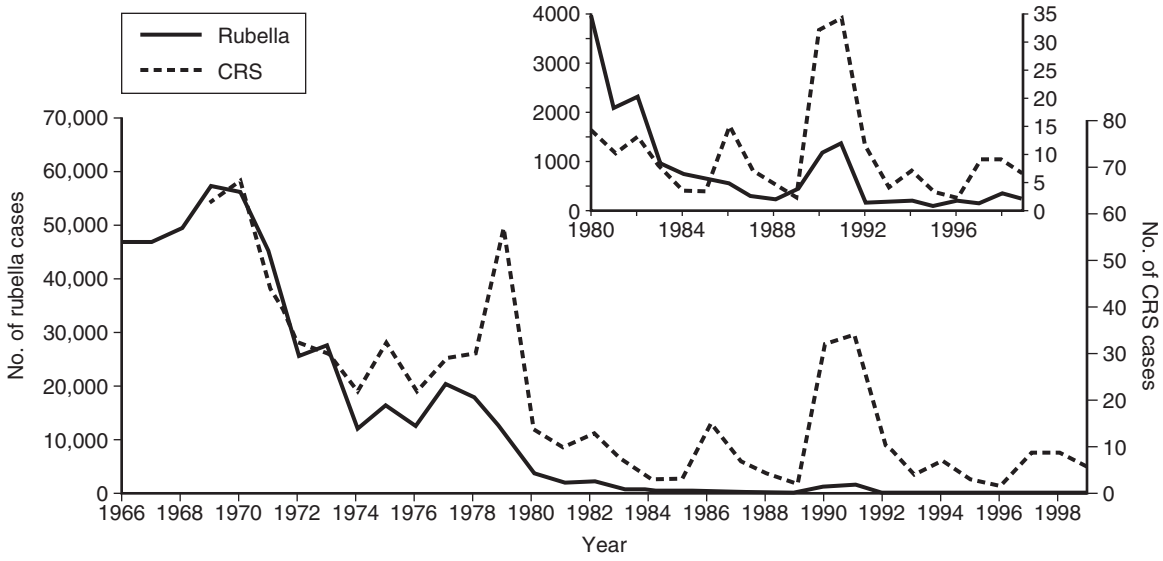

FIGURE 173-2 ■ Number of reported cased of rubella and congenital rubella syndrome *(CRS)* in the United states from 1966 to 1998. (From Reef SE, Frey TK, Theal K, et al: The changing epidemiology of rubella in the 1990s: on the verge of elimination and new challenges for control and prevention. *JAMA* 2002;287:464–72. Copyright 2002, American Medical Association.)

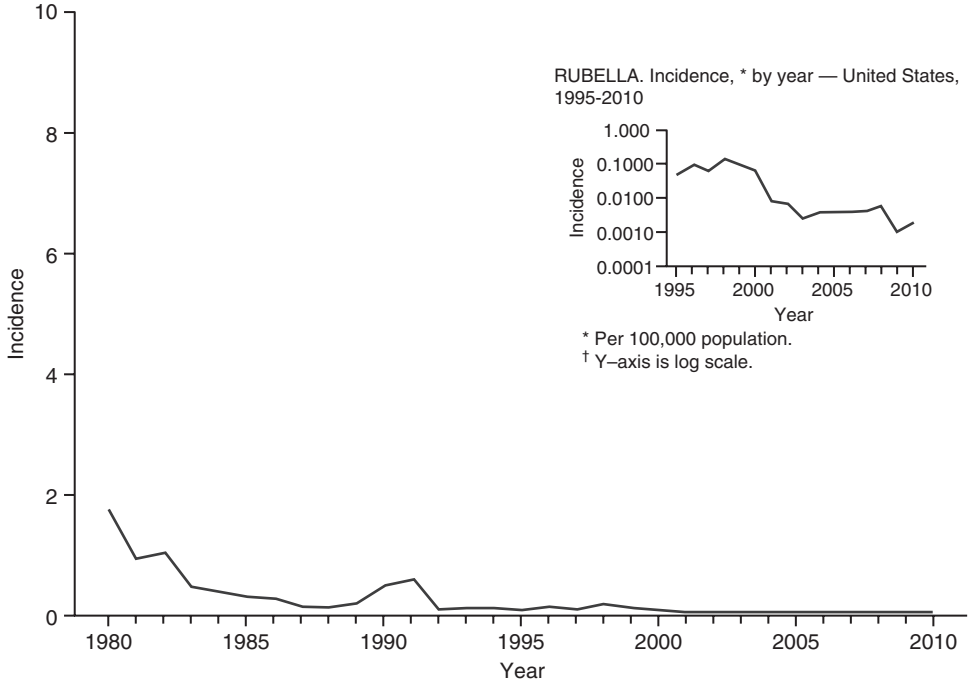

FIGURE 173-3 ■ Rubella. Incidence per 100,000 by year: United States, 1980-2010. (From Centers for Disease Control. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;59:1–111.)

In 1991, 1401 cases of rubella and 47 and congenital rubella syndrome were reported.[87] At present, rubella virus is no longer circulating in the United States, and the cases that occurred in the more recent years were importations.[82,455]

In fact, on October 29, 2004, an expert review panel from the Centers for Disease Control and Prevention (CDC) declared that the rubella virus has not been an endemic threat since 2001 in the United States. Recent data collected from 2005 to 2007 has shown the annual incidence of rubella to be 0.005/100,000 population, with a median number of reported cases as 11, which were primarily import associated.[457] Data from the *Morbidity and Mortality Weekly Report (MMWR)* from 2010 showed an annual incidence rate of 0/100,000 population. Reported rubella cases from 2007 to 2010 were 12, 16, 3, and 5, respectively. During this 4-year period only 2 cases of congenital rubella were reported. The annual incidence of rubella in the United States from 1980 to 2010 is illustrated in Figure 173-3.[90]

Another recent accomplishment has been rubella eradication from the Americas. Elimination of rubella

from the Americas was a remarkable achievement, especially because as many as 135,947 cases were confirmed in the Americas back in 1998.[70,88] In 2003, the Pan American Health Organization strengthened efforts to eliminate rubella and congenital rubella syndrome from the Americas by 2010. They accomplished this goal with high immunization coverage with programs targeting over 450 million people, including mass vaccination of adolescents and adults, and increased surveillance for rubella and congenital rubella syndrome that was integrated into existing measles surveillance programs. As a result of these comprehensive efforts, the last endemic rubella case documented in the Americas was reported in Argentina in February of 2009.[70,88]

In countries in which effective universal childhood immunization had not been achieved, epidemics continue to occur. While the number of rubella cases on a global scale continues to decrease, rubella still remains a problem in many areas of the world. Based on data from 2009 collected by the WHO, 121,344 rubella cases from 167 countries were reported, which is an 82 percent decrease from the 670,894 cases reported in 2000.[545,622] From 2000 to 2009, the percentage of reported rubella cases decreased by nearly 100 percent in the Americas region (39,228 to 18), 98 percent in the European region (621,039 to 11,623), and 35 percent in the Eastern Mediterranean region (3122 to 2030).[458] In contrast, reported rubella cases increased in the Western Pacific, Southeast Asian, and African regions during this period. There was a 12-fold increase (5475 to 73,077 cases) in the Western Pacific region, a 14-fold increase (1165 to 17,208 cases) in the Southeast Asian region, and a 20-fold increase (865 to 17,388 cases) in the African region.[458]

Epidemic rubella was documented in the former Czechoslovakia in 1972; in Australia in 1969 to 1970, 1975 to 1976, 1993 to 1996, and 2003; in Israel in 1972, 1979, and 1983; in Japan in 1975 to 1977 and 1982 to 1998; in Brazil in 1981 and 1997 to 2001; in Argentina in 2008; in the United Kingdom in 1971 to 1973, 1978, and 1983; and recently in some European countries.* A notable outbreak occurred in the Netherlands from 2004 to 2005, which primarily affected individuals unvaccinated secondary to religious beliefs. Three hundred eighty-seven cases of rubella were reported in the Netherlands and then spread to Canada, where an additional 309 cases were noted. An unfortunate consequence of vaccine refusal in these populations included 14 reported infants with congenital rubella infection and 2 fetal deaths.[238] Other recently reported outbreaks were also noted in Georgia in 2004 to 2005; northeastern Italy in 2008; Austria in 2008 to 2009; and Bosnia and Herzegovina in 2009, which were thought to be the result of vaccination failure rates during the war period in the early 1990s.†

The incidence of rubella varies with the epidemic cycle, the number of susceptible people within a population group, and the intrapersonal contact within the group. In closed populations such as military training centers and institutions for the mentally handicapped, the attack rate after the disease is introduced approaches 100 percent in susceptible individuals.[282,283,336] Introduction of disease in the family also affects virtually all susceptible persons.[204,205] In community epidemics, attack rates in susceptible people are estimated to range from 50 to 90 percent.

Age Groups

The age distribution of reported rubella cases and estimated incidence rates in Illinois, Massachusetts, and New York City for 1966 through 1968 and the entire United States for 1985 through 1987[79] are presented in Table 173-1. In the period immediately before the vaccine was introduced (1966 to 1968), the attack rate was highest in the 5- to 9-year-old age group, and the incidence was high in preschool-aged children. The overall reduction in the rate of rubella from the prevaccine era to 1987 was 99.2 percent. However, 50.2 percent of the cases reported in the period 1985 through 1988 were in persons older than 19 years; in the prevaccine period (1966 through 1968), the percentage in this age group was 10.2. In 1999, 75 percent of the reported cases occurred in persons older than 19 years.[456] During the period 1980 to 2002, outbreaks of rubella have occurred in prisons,[78] in colleges and universities,[76] in hospitals,[75,444,542] at work sites,[77,219,456] in communities with high foreign-born populations,[456] and among the Amish in six areas of the United States.[83]

TABLE 173-1 **Age Distribution of Reported Rubella Cases and Estimated Incidence Rates*: Illinois, Massachusetts, and New York City, 1966-1968,† and Total United States, 1985-1987†**

Age Group (yr)	*1966-1968 Average‡*		*1985-1987 Average§*		Rate Change¶ (%) 1966-1987
	%	Rate	%	Rate	
<5	21.6	63.3	24.8	0.6	–99.1
5-9	38.5	101.3	11.8	0.3	–99.7
10-14	17.0	44.0	5.2	0.1	–99.7
15-19	12.7	35.7	8.0	0.2	–99.5
≥20	10.2	3.7	50.2	0.1	–96.5
Total	100.0	24.3	100.0	0.2	–99.2

From Centers for Disease Control and Prevention: Rubella and congenital rubella syndrome: United States 1985-1988. *MMWR Morb Mortal Wkly Rep* 1989;38:173–8.
*Reported cases per 100,000 population. Patients with unknown age are excluded.
†Average annual figures during a 3-year period.
‡Represents prevaccine years. National age data were not available before 1975 and were not reported consistently (i.e., >75% of cases) until 1980.
§Total United States data (1986 population projections) are used for 1985-1987; because the overall number of reported rubella cases currently is small, fluctuations (such as the epidemic in New York City in 1985) in only these three reporting areas skewed the data for this period.
¶Based on actual rates.

*References 171, 182, 187, 207, 218, 239, 249, 278, 301, 325, 326, 366, 461, 487, 528, 551, 552, 582.
†References 133, 154, 286, 300, 406, 497.

TABLE 173-2 **Age-Specific Attack Rates in Two Communities During Epidemic Rubella in 1964**

	Doraville, Georgia			*Kingston, Tennessee*		
Age Group (yr)	**Total Population**	**Cases**	**Attack Rate (%)**	**Total Population**	**Cases**	**Attack Rate (%)**
0-4	87	32	36.8	69	30	43.5
5-9	206	104	50.5	127	90	70.9
10-14	208	59	28.4	127	68	53.5
15-19	78	9	11.5	90	25	27.8
20+	427	11	2.6	487	19	3.9
Unknown	8	—	—			
Total	1014	215	21.2	900	232	25.8

From Communicable Disease Center. Rubella Surveillance-1964. *Morb Mortal Wkly Rep* 1964;13:349–60.

Because rubella was not a reportable disease in the United States until 1966, few age-specific incidence or prevalence data relating to epidemic disease are available. In Table 173-2, age-specific attack rates are presented for two communities during epidemic rubella in 1964.[401] The attack rate curves during epidemic rubella in 1964 are similar to the curve for the prevaccine nonepidemic period from 1966 to 1968 seen in Table 173-1. The overall attack rate during the 1964 epidemic in the two communities was 23 percent. Eighty-six percent of the cases occurred in children younger than 15 years of age. In 1999, 272 cases of rubella were reported in the United States, and of the 269 with known ages, only 14 percent occurred in children younger than 15 years.[456]

Antibody prevalence by age group in the prevaccine era in the St. Louis, Missouri area is presented in Figure 173-4.[101] As can be seen, rubella HI antibody prevalence went from less than 10 percent in children younger than 3 years of age to almost 80 percent in preadolescents. Surveys of adolescents and young women of child-bearing age conducted before 1969 generally have indicated an immunity rate (HI antibody titer ≥1:8) of approximately 75 to 85 percent.[402]

Immunity to rubella as indicated by the curve in Figure 173-4 obviously is affected by epidemic periods. With epidemic disease, the curve itself most probably maintains the same slope, but it moves to the left; prevalence of antibody in each age group of children increases significantly (perhaps 10% to 30%). However, studies in the prevaccine era on sera from young adults indicate that the percentage of susceptible persons in this age group is affected only slightly by epidemic disease.[182,402,510,513,617] In a survey of sera from 600 pregnant women in 1962, Sever and associates[510] noted that 17.5 percent had no detectable antibody. In a similar study in 1966 in which the mean age was slightly less (23.6 vs. 25.6 years), the percentage without detectable antibody was 7.8. Other surveys of rubella antibody in the sera of young adults acquired after the 1964 pandemic indicate a susceptibility rate in the 15- to 20-percent range, similar to pre-1964 data.[402,506,510]

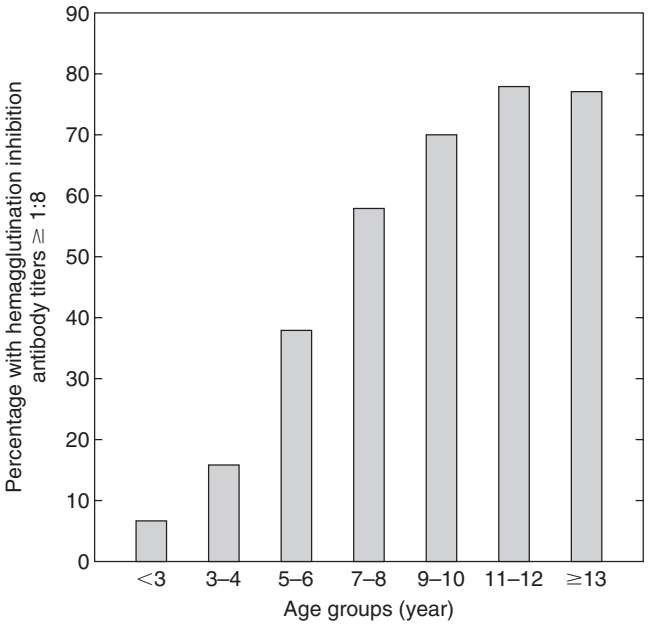

FIGURE 173-4 ■ **Percentage of children with rubella hemagglutination inhibition antibody titers of 1:8 or greater in a St. Louis, Missouri study in 1969.** (From Cherry JD. Rubella: past, present and future. *Volta Rev* 1974;76:461–5. Reprinted with permission from the Volta Review. Copyright 1974 by the Alexander Graham Bell Association for the Deaf, 3417 Volta Place, NW, Washington, DC 20007.)

Effect of Vaccination

Rubella vaccine was licensed for use in the United States and many other countries in 1969, and it has been used extensively for more than 40 years.* However, before the 1990s, rubella-containing vaccines (RCV) were primarily limited to developed countries.[458] Significant progress has been made in increasing the number of countries that vaccinate routinely against rubella since 1996. As of 2009, the WHO estimates that 130/193 Member States use rubella vaccines in their national immunization schedules.[623] As a result of successful rubella vaccination efforts, endemic rubella elimination has been achieved in the United States since 2001 and in many other developed countries. In addition, the success of elimination of rubella from the region of the Americas, with the last reported case in 2009, provides hope that rubella could

*References 84, 115, 446, 457, 508, 516, 601.

be eradicated globally. However at the present, the risk for importation of rubella into the United State remains high because rubella continues to be endemic in many parts of the world.[40] Thus, it has been imperative to maintain rubella vaccination coverage greater than 90 percent in the United States, and recent data have supported achievement of these continued efforts. It has been estimated from the National Immunization Survey (NIS) in the United States that vaccination coverage for children 19 to 35 months of age was greater than 90 percent during 2005 to 2007 and data from school surveys estimate coverage for kindergarten and/or first-grade children to be greater than 95 percent.[457]

It has been estimated by mathematical models that when vaccine coverage is low through suboptimal childhood-only immunization efforts, viral circulation may be decreased such that children normally infected during childhood may remain susceptible until adolescence and adulthood. Thus, the paradoxical effect of sustained low levels of rubella immunization in childhood can actually increase the risk for congenital rubella syndrome more than without vaccine introduction. Thus, the WHO recommends rubella immunization coverage of 80 percent or greater, with at least one dose of rubella vaccine, because the effectiveness of one dose of vaccine is 95 percent or greater.[623]

The immunization effort in the United States initially focused on children.[142,315,317] A secondary goal was to immunize seronegative postpubertal girls and women of child-bearing age, but little effort was extended in this area until 1978. The overall effect of the immunization effort in the United States on the young adult population is difficult to interpret. As noted in Table 173-1 and subsequent data, the reported number of cases and the incidence of rubella decreased significantly from the prevaccine period until the present.[85,87,456,600] A marked reduction in the number of reported cases of congenital rubella also has occurred since 1969 (see Fig. 173-2). However, the actual number of cases and the incidence of rubella in adolescents and young adults did not decrease until 1981, and 58.2 percent of all patients in the period 1985 through 1987 were 15 years of age (see Table 173-1). Also alarming was the marked upswing in the number of congenital rubella cases in 1990 and 1991.[75,456] Antibody survey data reported between 1981 and 1993 indicate that 5 to 25 percent of the adolescent and young adult populations in the United States were susceptible to rubella.*

In four studies in which prevalence of rubella antibody was analyzed by the vaccination status of the participants, differences were significant.[328,377,412,465] Between 87 and 96 percent of vaccinated persons had rubella antibody, whereas only 70 to 80 percent of nonvaccinated persons had antibody.

U.S. serosurvey data for the period 1988 to 1994 found the following seropositive rates by age group: 6 to 11 years, 91.8 percent; 12 to 19 years, 82.6 percent; 20 to 29 years, 84.6 percent; 30 to 39 years, 88.7 percent; 40 to 49 years, 92.5 percent; 50 to 59 years, 93.7 percent; and 60 years and older, 95.7 percent.[160] In this survey, persons born from 1970 to 1974 were found to have the lowest rate of seropositivity (78%). For the period 1999 to 2004, the overall U.S. seropositivity level was 91.3 percent. Selected age-related rates in children aged 6 to 11 years and adolescents aged 12 to 19 years were 96.2 percent and 93.7 percent, respectively.[288] Seropositivity was 91.5 percent among women and 88.0 percent among men.

In contrast to the immunization program in the United States, which focused on children and elimination of epidemic rubella, immunization efforts in the United Kingdom and many other countries initially were aimed at girls aged 11 to 14 years, with selective immunization of women of child-bearing age. This approach would not be expected to disrupt the epidemic pattern of rubella but only to decrease disease in young adult women. Serologic surveys in the United Kingdom indicated a significant reduction in the number of seronegative persons in the target population.[109,242,377-379,450,470,565] In one study in which 10,000 serum samples were analyzed, 93 to 96 percent of females born after 1956 (who would have been offered rubella vaccine in school) were found to have antibody, whereas only 80 to 89 percent of those born before 1954 were found to have antibody.[109] In spite of the high level of antibody prevalence in women of child-bearing age, rubella infection in pregnancy and congenital rubella continued to be a major problem in the United Kingdom.[378,565] From 1971 to 1982, 625 cases of congenital rubella were reported, and from 1974 to 1981, 3273 women had their pregnancies terminated because they were infected with rubella or had been in contact with a person with rubella in England and Wales.[565]

In Finland, where initial immunization in 1975 involved 11- to 13-year-old girls and after 1982 involved a two-dose program for all children, the susceptibility rate in 1992 for 16- to 19-year-old girls was 3 to 5 percent, whereas for boys it was 30 percent.[577]

In Sweden, the initial immunization program, which began in 1973, targeted school girls, susceptible women after pregnancy, and women at special risk; their second program, which began in 1982, was a universal two-dose schedule at 18 months and 12 years.[53] The rate of susceptibility in pregnant women in Sweden decreased from 12 percent in 1975 to below 2 percent in 1994.

Recent serosurveys from around the world have noted varied rates of seropositivity in adults.[112,139,151,294,571] In Jordan, 90.9 percent of women of child-bearing age were immune, but only 83 percent of younger women aged 15 to 19 years were immune.[294] In Catalonia, Spain, 98.1 percent of adults had antibody to rubella[151]; in Israel, approximately 95 percent of women of child-bearing age were immune to rubella, whereas only approximately 85 percent of men of similar age were immune.[112] In this serosurvey, as many as 40 percent of adolescent men were rubella nonimmune, but only approximately 5 percent of similarly aged women were nonimmune. In Taiwan, the prevalence of rubella antibody in women varied markedly by birth cohort.[571] Rubella antibody was undetectable in 19.9 to 29.2 percent, 7.1 to 7.3 percent, and 8.3 to 10.4 percent of cohorts born before 1971, between 1971 and 1976, and after 1976,

*References 135, 153, 192, 304, 328, 412, 413, 465, 503, 538, 543, 592.

respectively.[342] In Argentina, in 2002, 91.2 percent of women of child-bearing age had antibody to rubella.[139] A survey of 17 European countries and Australia from 1996 to 2003 showed seronegativity among women of children-bearing age without protective immunity to rubella ranging from 1.4 to 13.4 percent, lowest among the Czech Republic and highest in Belgium.[399] Data from pregnant women in Canada from 2002 to 2005 found that 8.8 percent of tested women were seronegative for rubella and younger women were more likely to be seronegative.[302] A study from 2000 looking at women of child-bearing age in Morocco found that 16.6 percent were seronegative for rubella immunoglobulin (Ig)G antibodies.[66] Seroprevalence data from Nepal in 2008 demonstrated that 90.8 percent of women of child-bearing age were seropositive for rubella.[579]

Congenital Rubella

Rubella infection during pregnancy, especially during the first 12 weeks of gestation, can lead to miscarriage, intrauterine fetal death, and a variety of birth defects associated with congenital rubella syndrome.[158,517] As a result of rubella vaccine implementation, the number of cases of congenital rubella has decreased dramatically, especially in developed countries. However, in many regions of the world, immunization against rubella is not standard practice, and congenital rubella infection and its devastating consequences remain an ongoing problem. In many developing countries that have still not implemented mass rubella immunization campaigns, congenital rubella syndrome annual rates are actually similar to those over 15 years ago in 1996. Congenital rubella syndrome at that time was estimated at 110,000 cases annually (range, 14,000 to 308,000 cases), with an estimated 22,000 cases in Africa; 46,000 cases in Southeast Asia; and 12,634 cases in the western Pacific regions.[545,623] Unfortunately, accurate data have been severely limited by underreporting. Thus, in 2009, only 165 cases of congenital rubella syndrome were reported and 67 of these were localized to the eastern Mediterranean region.[545] Seroprevalence data and models also attest to the issue of underreporting. These models have predicted approximately 46,621 infants annually with congenital rubella syndrome in the Southeast Asian region alone, although recently only approximately 13 cases were reported annually in this region.[622]

Congenital rubella was not a reportable disease in the United States until 1966, so good data on incidence and prevalence during epidemics of rubella are not available. In the 1964 to 1965 rubella epidemic in the United States, 20,000 cases of congenital rubella occurred, 5000 therapeutic abortions were performed, fetal wastage in excess of 6250 was reported, and more than 2100 neonatal deaths occurred.[402] Estimates of the risk for acquiring congenital rubella after maternal infection vary considerably across studies. In general, studies performed before 1964, which included nonepidemic periods, tended to underestimate the risk, whereas early retrospective studies after epidemics resulted in high incidence values.[183] Clearly, however, the individual risk for acquiring congenital rubella depends on the month of pregnancy in which maternal infection occurs. Sallomi[480] analyzed eight published studies that met his rigid criteria and noted the following rates of anomalies by gestational age when maternal infection occurred: weeks 1 to 4, 61 percent; weeks 5 to 8, 26 percent; and weeks 9 to 12, 8 percent. In pregnancies complicated by rubella in weeks 1 to 8, only 36 percent ended in normal live births, 39 percent ended in abortion or stillbirth, and 25 percent produced gross fetal anomalies. Peckham[426] noted that 85 percent of infants born to mothers infected during the first 8 weeks of pregnancy had detectable defects during the first 4 years of life. Infection at other times during pregnancy revealed the following rates of detectable defects: 9 to 12 weeks, 52 percent; 13 to 20 weeks, 16 percent; and after 20 weeks, no defect. Other studies indicate a risk for malformation of 3 percent and a risk for abortion and stillbirth of 4 percent when intervals from conception to acquisition of rubella are greater than 12 weeks.[111]

Infection with rubella virus confers lifelong immunity against clinical illness, but asymptomatic reinfection does occur. Asymptomatic reinfection is noted frequently in pregnant women, but it generally has not been considered a risk to the fetus. However, in rare instances, reinfection has resulted in severely damaged babies.*

Transmission

Rubella infection generally is thought to be spread by the respiratory route. Although definitive evidence supporting this assumption is not available, data from volunteer projects and studies of natural disease strongly support this view.[227,263,493] Infected persons regularly shed large concentrations of virus in the nose and throat, and droplets of secretions are released into the environment, which allows respiratory-to-respiratory transmission. Also possible is that the initial hosts may contaminate their own hands and then transmit the infectious agent to environmental surfaces or directly to contacts. In this circumstance, new hosts can acquire infection through the fomite-hand-respiratory or hand-hand-respiratory route.

In experimental transmission studies, Green and colleagues[227] noted that efficient transmission of infection to susceptible persons required prolonged, repeated contact; after a brief, single contact, only 1/5 children acquired disease, whereas all but 1/17 patients with prolonged, repeated contact were infected. Although the period of communicability has never been determined accurately, almost 100 years ago the period of infectivity was noted to precede the eruptive phase of illness.[229] Volunteer studies indicate that virus is present in nasopharyngeal secretions from 7 days before to 14 days after the onset of rash.[227,263] Maximal shedding and presumably maximal transmissibility occur for an 11-day period of 5 days before to 6 days after the appearance of rash. Infants with congenital rubella shed virus from the nose and throat for many months and have been responsible for the spread of virus to susceptible contacts.[281,491]

*References 3, 26, 29, 64, 137, 464, 602.

Seasonal Patterns

Rubella is a disease of winter and spring, with the largest number of cases in the United States occurring in March, April, and May.[401,402] This seasonal pattern occurs in years with both a high and a low incidence of rubella. Presumably, some transmission and sporadic illness occur throughout the year in large urban areas.

Geographic Distribution

Rubella has been recognized in many countries throughout the world. In recent years, more information about circulating wild-type rubella viruses has been uncovered largely because of global virologic surveillance efforts for measles, which incorporates surveillance for rubella as well. The WHO Global Measles and Rubella Network (LabNet), which was developed in 2000, serves over 183 countries and includes over 690 laboratories, which helps confirm suspected cases of rubella and monitors rubella transmission patterns.[172,473] Nine of the 13 viral genotypes were identified during surveillance efforts from 2005 to 2010. Viruses 1E, 1G, and 2B have the widest geographic distribution and are the most frequently identified. 1E rubella viruses were found in the Middle East, Europe, Southeast Asia, Africa, and Western Pacific countries. This genotype is also the dominant one found in China. 1G genotype viruses were found in Europe and Africa. The 2B genotype viruses were found in the Middle East, Europe, Southeast Asia, and South and Central America, Africa, and Western Pacific countries.[2] In remote islands, rubella may not be endemic, so large segments of the population may be susceptible.[57,267] In these locales, the introduction of rubella results in epidemic infection that involves approximately 90 percent of the susceptible population. In populated areas of the world in the prevaccine era, rubella was both endemic and epidemic, and between 80 and 90 percent of the adult population had serum rubella antibody.[111] An interesting note is that in the prevaccine era in several well-populated large islands that were not remote (Jamaica, Taiwan, Barbados, Trinidad, Hawaii, and Japan), a smaller percentage of the total population had antibody and rubella was not endemic.[111,169,205,224]

Other Factors

Sex

The incidence of clinical rubella is similar in boys and girls.[401,402] In adults, more cases of rubella are reported in women.[402] This finding possibly is the result of interest and concern related to congenital rubella rather than a true difference on the basis of sex. Of interest is that in rubella vaccine trials, girls have been noted to have geometric mean convalescent-phase antibody titers higher than those of boys.[375,530]

Mitchell and associates[383] studied the IgG, IgM, and IgA antibody responses in men and women to rubella virus structural proteins (E1, E2, and C). IgA E2 antibodies did not develop in men, but they did in women. Men had lower IgG antibody to E2, an earlier onset of E1-specific IgG and IgM antibodies, and a greater proportion of total antibody against E1 than women did. In another study, Mitchell[382] reported that men had a more rapid cell-mediated immune response to whole, inactivated rubella virus and a panel of rubella virus peptides after reimmunization than women did.

Genetics

Hattis and associates[259] showed that individuals differ in their ability to transmit rubella. During a rubella epidemic, they noted a small number of persons who had high potential for transmitting virus to susceptible persons ("spreaders"). Most persons demonstrated only minimal virus transmission ("nonspreaders"). Honeyman and colleagues[273,275] suggest that the ability to spread rubella virus is favored by the cell surface antigen HLA or the combination of HLA and HLA. In a rubella vaccine trial, Spencer and associates[530] noted that 44 percent of persons with high rubella HI antibody titer (≥1:512) responses had HLA. In this study, a high convalescent-phase geometric mean antibody titer also was noted to occur in subjects with the AB blood type.

Ovsyannikova and associates[417] recently studied HLA haplotypes in the genetic control of immune response to measles-mumps-rubella (MMR) vaccine. They found that DRB1*04-DQB1*03-DPB1*03 was associated with high lymphoproliferative responses to rubella vaccine virus. They found that specific class II HLA haplotypes (DRB1*04-DQB1*03-DPB1*03 and DRB1*13/14-DQB1*06-DPB1*03) were associated with higher lymphoproliferative responses to rubella virus vaccine and the haplotype DRB1*07-DQB1*02-DPB1*11 was associated with lower lymphoproliferative responses.[418] Kennedy and associates[305] found that homozygosity within HLA DPB1 locus is associated with increased levels of rubella-specific IgG. Haralambieva and colleagues[250] found associations between polymorphisms within the 2′-5′-oligoadenylate synthetase *(OAS)* gene cluster and rubella virus–specific cytokine secretion (IL-2, IL-10, and IL-6) and antibody levels.

PATHOLOGY AND PATHOGENESIS

Viral Infection

The sequence of events in uncomplicated, postnatally acquired rubella is presented in Table 173-3. Although much is known about rubella infection in humans, considerable gaps regarding specific events exist.[227,261-263,493] Estimates for the timing of events in rubella infection (see Table 173-3) have come from volunteer inoculation studies. In many instances, artificial inoculation has resulted in a reduction in the length of the incubation period of clinical disease. This finding suggests that the size of inoculum is important in the initial generation of human infection. It also helps explain the rather wide boundaries of the incubation period noted in many clinical studies.[229,372,486]

The primary site of infection is the respiratory epithelium of the nasopharynx. Initial infection of the

TABLE 173-3 **Sequence of Rubella Viral Infection in Uncomplicated Primary Disease**

Day	Event
0	Rubella virus from the respiratory secretions of an infected person comes in contact with the epithelial surface of the nasopharynx of a susceptible person. Localized infection in the respiratory epithelium is established, and virus spreads through lymphatics and possibly by transient viremia to regional lymph nodes
1-22	Viral replication in localized areas of the nasopharynx and regional lymph nodes
3-8	First evidence of nasopharyngeal viral shedding
6-20	Viremia (virus free in serum and associated with leukocytes)
8-14	Establishment of infection in the skin and other viremic sites, including generalized nasopharyngeal involvement
10-17	Maximal viremia and viruria
10-24	Maximal nasopharyngeal viral shedding
17-19	Viremia decreases and then ceases. Viral content at viremic sites rapidly diminishes

From references 227, 261-263, 493.

respiratory epithelium apparently is minor; a more important event is early spread of virus to the regional lymphatics. In volunteers given 100 $TCID_{50}$ of rubella virus intranasally, viral multiplication at the respiratory site was noted on the third day.[493] After viremia develops, extensive nasopharyngeal infection occurs. In persons who have received either attenuated or unattenuated virus by the subcutaneous route, nasopharyngeal shedding in varying concentrations always occurs.[102,104,227,493] Concentrations of virus generally are greater in specimens collected from the nose than in those from the throat.

Viremia peaks just before the onset of exanthem and disappears shortly thereafter. In contrast, virus continues to be present consistently in the nasopharynx for a 6-day period after the onset of rash and occasionally for an additional week thereafter.[227] In addition to the blood and nasopharynx, other sites from which rubella virus has been recovered are the lymph nodes,[201] urine,[493] cerebrospinal fluid,[534] conjunctival sac,[262] breast milk,[62] synovial fluid,[266] and lungs.[518] In patients with rubella, the virus was recovered from the skin at sites both with rash and without rash.[261,262]

Immunologic Events

Antibody

After having natural or vaccine rubella viral infection, patients regularly have an antibody response. Serum antibodies to different rubella viral antigens can be measured by HI, CF, neutralization, immunofluorescence, precipitation, radioimmunoassay, ELISA, single radial hemolysis, passive hemagglutination, latex agglutination, and platelet aggregation.*

In natural postnatal rubella infection, HI and neutralizing antibodies appear 14 to 18 days after exposure, at the time of the rash. HI antibody titers usually peak approximately 2 weeks after the onset of clinical illness, stay at a high level for several weeks, decrease about fourfold during the course of a year's time, and then generally persist for life. Immunofluorescence, ELISA, radioimmunoassay, and latex agglutination reveal antibody patterns similar to those determined by HI.[265] Antibody detected by passive hemagglutination does not appear until 3 to 4 weeks after the onset of illness, and that detected by single radial hemolysis is delayed until 1 to 2 weeks after onset. In an Amazon Indian tribe, the geometric mean rubella HI antibody titer 12 years after infection, with no intercurrent rubella exposure, was 1:33.[44] The pattern of the neutralizing antibody response is similar to the HI antibody response, except that the peak is delayed slightly. Brody and colleagues[57] noted that all but 10 percent of an island population had rubella neutralizing antibody 22 years after the time of epidemic illness.

CF antibody first appears approximately a week after HI and neutralizing antibody, peaks approximately 1 month after illness, and in general does not persist as long as does either HI or neutralizing antibody. On occasion, the CF antibody response is delayed and appears 1 month after the exanthem, with peak titers occurring 2 to 5 months later. Sever and colleagues[516] noted CF antibodies in only 44 percent of persons with neutralizing antibody who were studied 10 to 20 years after illness. The use of an antigen prepared by alkaline extraction has increased the sensitivity of CF, but low levels of antibody still are best identified by HI, ELISA, or neutralization.[436,498]

After natural infection occurs, precipitating antibodies develop to both theta and iota antigen.[330] Antibody to theta antigen appears early, parallels HI antibody, and is persistent. In contrast, the response to iota antigen is delayed, with a slow rise in concentration during a 2- to 3-month period. Five years after infection has occurred, anti-iota antibody cannot be detected.

After immunization has been administered, the antibody response pattern varies according to the type of vaccine used.* With RA 27/3 vaccine, the serum antibody response is similar to that after natural disease, except that the peak HI and neutralizing antibody titers attained usually are lower. Serum antibody responses noted after HPV-77 and Cendehill vaccine viral infection are different from those found after natural infection in that CF

*References 17, 43, 124, 148, 175, 212, 222, 243, 265, 271, 332, 338, 355, 369-371, 396, 427, 436, 514, 515, 565, 585, 588, 614, 629.

*References 45, 330, 411, 439, 440, 490, 595.

and anti-iota antibodies are noted only irregularly and then in minimal concentration.

Primary rubella virus infection, either naturally acquired or vaccine induced, is characterized by the initial appearance of antibody in the IgM and IgG serum components.* In general, the IgM-specific response is short-lived and not detectable more than 8 weeks after the onset of infection. On occasion, it has been detected in the serum for extended periods.

IgA nasal HI, ELISA, and neutralizing antibodies also occur regularly after natural viral infection. After immunization, the nasal antibody response varies with the type of vaccine and the route of administration.† After subcutaneous immunization with human papillomavirus (HPV)-77 vaccine is given, rubella-specific nasal IgA antibody is a rare finding; it occurs in most subjects who receive RA 27/3 vaccine administered intranasally and in approximately half of those given this vaccine by the subcutaneous route.

Specific Cell-Mediated Responses

Rubella-specific, cell-mediated lymphocyte responses regularly occur after infection with rubella virus.‡

Steele and associates,[537] using an in vitro lymphocyte-mediated cytotoxicity assay, first noted that lymphocytes from persons who previously had rubella caused cell destruction in a tissue culture chronically infected with rubella virus. Rubella antigen–specific, cell-mediated immunity also has been demonstrated in lymphocyte cultures by blast transformation, production of migration-inhibition factor, and production of interferon (IFN).§ With vaccination, rubella-specific, cell-mediated immunity first was noted to occur 7 days after immunization, with peak responses at 3 weeks.[587,589] Honeyman and associates[276] noted that the rubella antigen–specific, cell-mediated response commenced 1 week before the humoral immune response occurred in both natural and vaccine-induced rubella viral infection. They also noted that the cell-mediated response was of greater magnitude and duration after natural disease than after immunization. Rossier and associates[472] studied cloistered nuns and noted that specific cell-mediated immunity to rubella virus persisted until they reached 79 years of age in the probable absence of reinfection.

Morag and colleagues[392] demonstrated the specific appearance of cell-mediated immunity in tonsillar lymphoid tissue after natural infection or intranasal immunization with rubella vaccine. This responsiveness was conspicuously low after vaccination was administered by the subcutaneous route. In most instances, the presence of cell-mediated responsiveness correlates with the presence of antibody; specific rubella lymphocyte transformation has been noted in the absence of antibody, however.[527] The magnitude of the rubella-specific, cell-mediated response is suppressed during pregnancy.[559]

McCarthy and colleagues[359] identified potential determinants of human cellular immunity to rubella virus by using synthetic peptides representing well-defined sequences of rubella virus structural proteins. They used the following peptide subsequences: two capsid domains (C_1 to C_{29} and C_{64} to C_{97}), a glycoprotein E1 domain ($E1_{202}$ to $E1_{283}$), and a glycoprotein E2 domain ($E2_{31}$ to $E2_{105}$). All but the C_{64} to C_{97} subsequences stimulated specific lymphoproliferative responses in peripheral blood mononuclear cells in 25 to 50 percent of immune subjects. The immunodominant T-proliferative epitope (C_{14} to C_{29}) was recognized by only 50 percent of the peripheral blood mononuclear cells of the study population. Relatively immunodominant T-cell epitopes vary among different persons.

Using a lymphocyte proliferation assay, Mitchell and associates[385] reported positive cell-mediated immune responses to 16 peptides, including 6 that contained antibody neutralization domains after revaccination.

Nonspecific Responses

A large number of nonspecific, immunologically related responses can be demonstrated during rubella virus infections. Niwa and Kanoh[403] performed a comprehensive study of these responses in 85 children and adults during an epidemic of rubella. They noted a decreased number of total leukocytes, neutrophils, and T cells initially, which returned to normal values within 1 week. Some patients had slightly elevated levels of serum IgM, and total hemolytic complement was elevated in 12/30 patients. Marked increases in C4 and C9 also were present. In addition, they noted a marked insensitivity to dinitrochlorobenzene and purified protein derivatives in many patients. Atypical lymphocytes, autoantibodies, and reduced blastogenesis as measured by phytohemagglutinin (PHA) stimulation were noted in some patients. Other studies consistently have demonstrated a reduction in the lymphocyte response to PHA.* In general, this reduction lasts less than 1 month, and infections with attenuated strains of rubella vaccine virus are less immunosuppressive than are infections with unattenuated rubella virus.

Hyypiä and colleagues[290] noted that during rubella virus infection, the proportion of suppressor-cytotoxic T cells was increased and the proportion of helper-inducer T cells was decreased. Polyclonal activation of B cells was associated with these findings.[291]

Zaknun and coworkers[626] noted a marked increase in urine neopterin levels in two children with acute rubella. Their levels increased dramatically 4 days before the onset of exanthem.

Fetal Events

Viral Infection

A considerable amount of information about fetal infection became available from extensive studies during the

*References 32, 42, 128, 147, 236, 424, 425, 535.
†References 7, 8, 126, 130, 411, 436.
‡References 95, 264, 276, 299, 346, 349, 359, 382-385, 392, 416, 417, 471, 527, 537, 587, 589.
§References 61, 276, 392, 527, 559, 587, 589.

*References 60, 68, 206, 290, 323, 349, 361, 403.

1964 epidemic of rubella,* and further information has been obtained more recently from both natural and vaccine viral infections.† In spite of the number and extent of investigations performed to date, we know little about transmission of the virus to the fetus in maternal infections in the latter half of pregnancy.

With maternal infection during the first trimester, placental infection regularly occurs and often persists throughout the remainder of the pregnancy. In the therapeutic abortion studies of Alford and associates,[11] fetal infection occurred in approximately 50 percent of placental infections. However, other studies have revealed almost identical isolation rates from both placental and fetal tissue.[453,558] Persistent infection is the usual outcome of first-trimester fetal infection. This fetal infection usually involves multiple organs, and virus can be isolated at birth regularly from the throat, rectum, and urine.[281,434]

Little is known about events in second- and third-trimester maternal rubella infection. Most probably, placental infection is a regular occurrence, and transmission of virus to the infant in utero also may occur regularly. Because few infants have defects when they are born after maternal rubella infection in the second and third trimesters, a careful search for rubella infection in these infants by virologic or serologic methods rarely has been conducted. Random studies seem to indicate that rubella virus often infects the fetus after the first trimester and occasionally the infection becomes persistent.‡ Other studies have failed to show virologic or serologic evidence of infection in infants in whom maternal rubella occurred in the second and third trimesters.[103,390,541]

With maternal rubella infection, the cervix also is involved, so fetal infection could occur by the ascending route as well as by primary placental infection.[103,390,507,541,580] In addition, fetal infection has resulted from maternal disease that occurred before conception.[183,611,624]

Rubella virus can be recovered regularly after birth from infants with congenital rubella. The percentage of infants with persistent infection decreases during the course of the first year of life; by their first birthdays, between 10 and 20 percent of children still shed virus in nasopharyngeal secretions.[118,453] Rawls and colleagues[453] were unable to isolate virus from the throats of 15 congenitally infected infants after they reached 18 months of age, and Sever and Monif[509] and Cooper and Krugman[118] were unable to demonstrate persistence of nasopharyngeal virus in older children. A 4.5-year-old boy with congenital rubella was found on one occasion to be shedding rubella virus in the throat.[519]

Immunologic Findings

Specific Antibody. Humoral antibody in a congenitally infected fetus is acquired transplacentally from the mother and is produced actively by the fetus. In a normal maternal-fetal relationship, transport of antibody to the fetus is minimal until the midpoint of the second trimester (16 to 20 weeks).[10,11] With first-trimester maternal infection (transplacentally acquired), rubella antibody titers in serum amount to only approximately 5 percent of maternal values. The fetal immune system becomes functional during the second trimester,[329] and small amounts of specific rubella fetal IgM antibody can be detected. From the midpoint of pregnancy, antibody levels in the developing fetus rise so that at birth, the maternal and infant values are similar. Although the values of total antibody are similar, the composition is different. Maternal antibody at the time of delivery usually is composed entirely of IgG. In contrast, the infant titer consists of fetal IgM, presumably fetal IgG, and occasionally fetal IgA and transplacentally derived maternal IgG.

Long-term rubella antibody patterns in congenitally infected infants after birth are different from those of their mothers or from those of a group of children with acquired disease.[119,248,301,571] Cooper and colleagues[116] monitored a group of 223 mothers of children with congenital rubella and noted that at the end of 5 years, all still had detectable HI antibody and the geometric mean titer for the group had undergone a fourfold reduction. In contrast, 5-year follow-up of the congenitally infected infants revealed a 16-fold decline in geometric mean titer; 8/29 infants had serum HI antibody titers less than 1:8 when they were examined at 5 years of age. Other investigators observed similar declines in rubella antibody titers of congenitally infected infants.[251,307,573]

Another unique aspect of rubella antibody in congenitally infected infants is the persistence of specific IgM. Cradock-Watson and colleagues[127] studied 40 infants with congenital rubella and noted that IgM antibody persisted for approximately 6 months in most cases and for up to 2 years in a few children. de Mazancourt and colleagues[140] studied the antibody response to rubella virus structural proteins in infants with congenital rubella syndrome and found that the immunoprecipitation patterns were different from those in sera from postnatally infected adults. The sera from congenitally infected infants had little or no C-specific antibody, occasionally only antibody to E1 was precipitated, E1 protein was precipitated in relative excess of E2 protein, and the relative amount of E2 antibody was greater than antibody to E1.

Specific Cell-Mediated Immunity. Rubella-specific, cell-mediated immune responses have been studied in children with congenital rubella by the following assays: lymphocyte-mediated cytotoxicity, lymphocyte transformation, lymphocyte IFN production, and leukocyte migration-inhibition factor production.[63,200] By all methods of study, infants with congenital rubella have decreased rubella-specific, cell-mediated responses in contrast to persons who previously had acquired rubella postnatally. Buimovici-Klein and associates[63] noted that the degree of suppression correlated with the time of in utero infection: the earlier in pregnancy the maternal infection, the greater is the depression of specific cell-mediated responses. In the study of an infant with late-onset congenital rubella syndrome, Verder and associates[586] noted decreased activity of killer and natural

*References 11, 73, 103, 118, 120, 156, 248, 252-255, 281, 387-390, 434, 453, 491, 494, 541, 611.
†References 47, 149, 161, 327, 433, 507, 582, 624.
‡References 129, 149, 255, 281, 388, 590, 610.

killer cells and alloreactive direct cytotoxic cells. Their data indicated that defective cytotoxic effector cell function was the primary cause for failure to eliminate virus in the illness.

Nonspecific Responses. Desmyter and colleagues[145] noted that infants with congenital rubella produced normal amounts of IFN after receiving measles immunization. They also found that the clinical response and antibody development in these measles-vaccinated children were similar to those in normal children. Lebon and associates[334] noted that sera collected from rubella-infected fetuses and infants with congenital rubella contain an acid-labile IFN. Michaels[373] observed that infants with congenital rubella who still were shedding virus in their throat or urine had depressed antibody responses to diphtheria and tetanus toxoids. White and colleagues[609] reported decreased in vitro lymphocyte blast transformation responses to vaccinia and diphtheria toxoid antigens in children with congenital rubella in contrast to normal children. They also noted depressed skin reactivity to intradermal *Candida* antigen in the congenital rubella group. Buimovici-Klein and associates[63] observed a marked reduction in lymphocyte transformation after PHA stimulation in their congenital rubella group. The most marked defect was seen in children in whom maternal rubella occurred during the first 8 weeks of pregnancy. Pukhalsky and associates[447] noted that rubella immunization resulted in defective lymphocyte response to a mitogen (PHA). After immunization, serum IFN-α was slightly increased at day 7 and then fell significantly so that it was not measurable at day 30 in several subjects. The IFN-α/interleukin-10 ratio significantly decreased after immunization.

Pathology

Postnatally Acquired Disease

Almost no data on the histologic findings in uncomplicated rubella are available, but occasionally, postmortem tissue has been studied from patients with encephalitis. Giuliani and associates[216] studied lymph nodes from patients with rubella and noted edema, reticulum cell hyperplasia, and loss of the usual follicular morphologic features. Sherman and associates[518] reported six cases of rubella encephalitis and noted the autopsy findings in three cases. They specifically searched all organs for inclusion bodies, syncytial giant cells, focal cellular necrosis, and unusual proliferative changes, but none of these was found. Only mild, nonspecific, follicular hyperplasia in the spleen and lymph nodes was seen. Histologic examination of the brain of a 7-year-old girl who died of encephalitis revealed diffuse swelling, nonspecific degeneration, and a sparse, mononuclear perivascular and meningeal exudate.

A synovial biopsy specimen in a woman with rubella arthritis revealed scattered areas of fibrinopurulent exudate and synovial cell hyperplasia; inflammatory cell infiltration composed mainly of lymphocytic cells was present, and vascularity was increased.[625]

Congenital Infection

In contrast to postnatal rubella, the pathologic process of congenital infection has been studied extensively.*

Table 173-4 summarizes the main pathologic findings by anatomic location or system in congenital rubella. As noted, defects in congenital rubella result from both specific cell damage and cellular deficiency. Although specific cellular necrosis is important in certain early lesions such as in the inner ear, of greater overall importance are the secondary effects of generalized vascular damage. Also of presumed major importance is the noncytolytic cellular infection characteristic of rubella virus. It results in mitotic arrest and a reduction in the total number of cells in many organs.

CLINICAL MANIFESTATIONS

Postnatal Illness

Although clinical rubella is a distinctive exanthematous disease, its features are not as clearly discernible as are those of measles or chickenpox.† The exanthematous illnesses caused by enteroviruses, adenoviruses, and other common respiratory viruses often are clinically similar or identical to those of rubella (see Chapter 58). Because of these other viral illnesses that simulate rubella, descriptions of clinical rubella made before modern virologic diagnostic techniques became available are not always accurate, particularly when rubella in infants and young children is described because exanthems caused by other viruses occur most commonly in these age groups. Unfortunately, in spite of the availability of a vast amount of clinical material collected during the rubella epidemic of 1964, most clinical knowledge relating to postnatally acquired rubella was formulated before the present virologic era.

Incubation Period

Although prodromal complaints and lymphadenopathy frequently precede the development of exanthem in rubella, the incubation period in most studies has been calculated as being from the time of exposure to the onset of rash. Almost a century ago, Michael[373] reviewed the incubation periods in 59 different reports and noted a variation of 5 days to 4 weeks. However, in most reports, the minimal incubation time was 14 days or more and the maximum was 17 to 21 days. The mean incubation period from modern reviews is considered to be 18 ± 3 days.

In carefully controlled studies, Green and colleagues[227] noted an incubation time of 13 to 15 days to the onset of rash after the intramuscular inoculation of serum from

*References 6, 9, 33, 58, 72, 108, 120, 155, 165-168, 189, 196, 197, 232, 246-248, 303, 310, 313, 314, 343, 387, 389, 397, 414, 432, 441, 442, 452, 454, 462, 467, 468, 476, 506, 523, 524, 531, 539, 544, 560, 567, 598, 601, 608, 621.

†References 99, 180, 183, 227, 228, 320, 324, 373, 486, 493, 572, 607; Cherry JD, unpublished data, 1963-1973.

TABLE 173-4 **Pathologic Findings in Congenital Rubella**

Anatomic Location or System	Gross and Microscopic Findings	References
Placenta	Perivascular mononuclear cellular infiltration in the deciduas Edema, fibrosis, and necrosis of villi; cytoplasm inclusion bodies noted in swollen Hofbauer cells in villous stroma	414
Generalized growth retardation	Subnormal number of cells in many organs	397
Nervous system	Chronic meningitis with infiltrates of large mononuclear cells, lymphocytes, and plasma cells in the leptomeninges Vascular degeneration, ischemic lesions, and retardation of myelinization throughout brain	467, 468, 523, 560
Eye	Lens: Cataract, cortical liquefaction, and spherophakia Iris and ciliary body: Necrosis of ciliary body, iridocyclitis, iris atrophy, and pigmentation defects Retina: Posterior pigmentary disturbances Cornea: Usually normal; occasional endothelial degeneration Optic nerve: Posterior bowing	49
Ear	Hemorrhage in fetal cochlea resulting in epithelial necrosis Inflammatory cells in stria vascularis Adhesions between Reissner's membrane and tectorial membrane Sacculocochlear degeneration of Scheibe (strial atrophy, collapse of Reissner's membrane, atrophy of organ of Corti, rolled-up tectorial membranes, and collapse and degeneration of sacculus) noted after birth	196, 197, 598
Cardiovascular	Common heart defects in order of frequency: Patent ductus arteriosus, pulmonary artery stenosis, ventricular septal defect, and atrial septal defect (These rubella-induced lesions do not differ from similar non–rubella-induced lesions.) Myocarditis with swelling of muscle fibers and loss of striations; necrosis Intimal proliferation of major arteries	6, 165, 189, 313, 316, 415, 601
Pulmonary	Chronic interstitial pneumonia with large mononuclear cells, lymphocytes, and plasma cells within interstitial spaces and alveoli	313, 432, 523
Liver	Hyalinization and swelling of hepatocytes, hematopoiesis, and multinucleated giant cells	167, 168, 539, 544
Skin	Purpuric lesion: Focal areas of erythropoiesis in dermis and upper subcutaneous adipose tissue Chronic reticulated rash: Acute and chronic inflammatory cells and histiocytes in dermis Edema in dermal papillae	72, 310
Bone	Thinning of metaphyseal trabeculae and decrease in number of osteoblasts and osteoclasts Many plasma cells in metaphyses and cartilaginous epiphyses and around vessels Occasional giant cells with cytoplasmic inclusions Thinning of cartilage	454, 475, 506, 608
Muscle	Focal abnormalities: Very small fibers with darkly staining nuclei and muscle bundles containing empty connective tissue tubes	530
Teeth	Necrosis of enamel-forming epithelial cells	232, 567
Hematologic	Transient thrombocytopenia with decreased megakaryocytes in bone marrow; increased platelet adhesiveness and platelet agglutinins Lymph node consistent with histiocytosis; unorganized cell mass made up of mononuclear cells with dense round nuclei and irregularly shaped cytoplasm	33, 108, 442, 452
Immunologic	Spleen: Fibrosis Loss of normal architecture and absence of germinal centers in spleen and lymph nodes Dysgammaglobulinemia usually with decreased IgG and IgA and elevated IgM	108, 245, 441, 462

rubella-infected patients and a longer incubation time (16 to 21 days) in cases acquired by contact with ill patients. In similar volunteer studies in young adults, Schiff and colleagues[493] noted that the onset of rash occurred 11 to 12 days after the administration of 100 $TCID_{50}$ of tissue culture–grown rubella virus. The investigators attributed this shorter incubation period to a larger inoculum than that occurring in natural transmission.

Prodromal Period

Symptoms reported before the onset of rash in rubella vary with age. In young children, the first evidence of disease usually is the appearance of rash. On occasion, mild coryza and diarrhea precede the development of exanthem in younger patients. In contrast to the lack of prodrome in children, adolescents and adults usually have

symptoms before the onset of rash (Cherry JS, unpublished data, 1963-1973).[180,231] In one study, 94 percent of college students with rubella had prodromal symptoms. In decreasing order of frequency, the reported symptoms were eye pain, sore throat, headache, swollen glands, fever, aches, chills, anorexia, and nausea.[180] Gross and associates[231] reported prodromal upper respiratory tract complaints, including malaise, cough, sore throat, red eyes, and runny nose, in 65 percent of an infected adolescent study group.

Prodromal symptoms usually precede the onset of rash by 1 to 5 days. In the studies of Green and associates[227] involving volunteers, the onset of lymphadenopathy commonly was noted 5 to 7 days before the onset of rash. In contrast, Schiff and colleagues[493] observed the appearance of lymphadenopathy only 1 day before the rash appeared; fever was noted 1 to 4 days before the onset of rash, and most of the volunteers also had malaise and sore throat. Pain on lateral and upward eye movement occurs frequently and occasionally is distressing (Cherry JD, unpublished data, 1963-1976).[180]

Exanthem Period

The rubella exanthem appears first on the face. Spread of the rash is centrifugal from the head toward the hands and feet. The progression, extent, and duration of the exanthem vary considerably. In a typical case, the rash involves the entire body during the first 24 hours, begins to fade on the face during the second day, and has disappeared from the body by the end of the third day. The characteristic rash is erythematous, maculopapular, and discrete (Fig. 173-5). Its appearance on an adolescent's face occasionally is confused initially with an exacerbation of acne. Frequently, the rash is only macular with a scarlatiniform appearance. In some patients, the rash is present for less than a day, although sometimes it persists for 5 days or longer. Particularly in adults, the exanthem frequently is pruritic. This complaint is troublesome because it often leads the patient as well as the physician to attribute the rash to an allergic cause rather than rubella virus infection.

FIGURE 173-5 ■ Rubella exanthem. The rash is erythematous, maculopapular, and discrete. (From Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;16:233–86.)

The exanthem occasionally progresses to confluence with a morbilliform appearance. In these cases, the rash usually is less coppery and pinker than that in measles and heals without desquamation or brownish discoloration. The typical picture of erythema infectiosum (slapped-cheek appearance and reticular rash) has been observed in rubella-infected patients. Balfour and associates[24] described eight children with erythema infectiosum from whom rubella virus was recovered concurrently and two additional children with serologic evidence of rubella infection. The preliminary results of volunteer studies with a virus recovered from one of the patients in this study produced a slapped-cheek appearance but a nonreticulated rash in four of five men. One 3-year-old child from whom the author recovered rubella virus had typical erythema infectiosum (Cherry JD, unpublished data, 1972). A 14-month-old girl had a roseola-like illness and arthritis.[266]

In the volunteer studies of Schiff and associates,[493] the rash was noted to be pink-red and maculopapular. It appeared initially on the face, chest, upper part of the arms, and shoulders and then spread rapidly over the abdomen, back, and thighs. It developed into an erythematous blush on the face and abdomen. The median duration was 3 days, with extremes of 2 and 5 days. No pruritus was noted.

Rubella infection without rash is a rather common occurrence. In some patients, the infection is without symptoms; in other persons, careful questioning reveals prodromal symptoms, and lymphadenopathy is found on examination. Green and associates[227] noted that approximately 25 percent of exposed children who became infected had subclinical infection. In an intensive study of 46 susceptible children and adults, all but one subject had clinical symptoms with infection[512]; 60 percent of the group had both rash and characteristic posterior auricular or suboccipital lymphadenopathy, and 40 percent had lymphadenopathy without rash. In another study of rubella in an institution for intellectually challenged children, Horstmann and associates[284] noted that only approximately half of the children who became infected had a rash. Of nine children without rash, significant posterior auricular lymph node enlargement developed in five. Buescher[59] reported a subclinical-to-clinical infection ratio in a military recruit population of 6.5 : 1.

Lymphadenopathy is a major clinical manifestation of rubella. The most characteristic enlargement occurs in the suboccipital and posterior auricular nodes, but generalized involvement occurs as well. In the volunteer studies of Schiff and colleagues,[493] the lymph node enlargement usually lasted between 5 and 8 days. In two outbreak studies involving adolescents and young adults, posterior auricular and suboccipital lymphadenopathy was noted in all patients with rash.[180,231] In contrast to these findings, Landrigan and associates[324] noted that only 47 percent of children and 58 percent of adolescents had similar lymph node enlargement during epidemic rubella illness. Although a frequent suggestion is that the finding of exanthem and suboccipital lymphadenopathy is pathognomonic for rubella, this suggestion is incorrect. In young children, similar involvement is seen frequently with enteroviral and adenoviral infections. In adolescents

and young adults, the association more strongly indicates rubella, but infectious mononucleosis, *Mycoplasma pneumoniae* infection, acquired toxoplasmosis, and other possibilities also must be considered.

The occurrence of fever in rubella varies; when it does develop, the temperature usually is elevated only minimally. In children with experimentally induced rubella, Krugman and Ward[318] noted that 5/13 had temperatures of 38° C (100.4° F) or higher. Two children had maximal temperatures of 38.5° C (101.6° F). Schiff and colleagues[493] noted that all 9 infected volunteers had fever with a median duration of 5 days. Landrigan and associates[324] found fever in 74 percent of children and only 47 percent of adolescents; Gross and coworkers[231] observed fever in only 6/17 adolescents. On occasion, children with apparent rubella have been noted to have markedly elevated temperatures. Few such cases have undergone virologic study, so some doubt must be raised regarding whether the illnesses were induced by rubella virus or were caused by other viral agents more commonly associated with marked febrile responses, such as enteroviruses and adenoviruses. I have seen an 8-year-old boy with virologically and serologically confirmed rubella with a temperature of 40° C (104° F) on the day before the appearance of his rash. The 14-month-old girl with arthritis described by Hildebrandt and Maassab[266] had a temperature as high as 40.5° C (105° F).

In 1898, Forchheimer[184] described what he thought was the enanthem of German measles. He described pinhead-sized macular lesions with a rose-red color on the soft palate and uvula that appeared at approximately the time of the exanthem and lasted less than 24 hours. This exanthem has not been identified in children I have examined; however, petechial lesions on the soft palate and uvula have been seen occasionally. Mild pharyngitis is not an uncommon occurrence. Other signs and symptoms in rubella include mild conjunctivitis, sore throat, coryza, cough, and headache.

The duration of illness in uncomplicated rubella varies considerably. Most patients would continue normal activity if the rash were not present. In general, full return to normal activity occurs within 3 days. A small number of adults are bothered by persistent headache, eye pain, and pruritus for 7 to 10 days.

The white blood cell count in rubella tends to be low. Schiff and colleagues[493] found leukopenia in all nine volunteers. In these subjects, leukopenia paralleled the pattern of fever, with onset occurring 24 hours before the rash was manifested and persisting for 4 to 5 days. Before rubella could be confirmed by both specific serologic and virologic methods, many experts thought that rubella could be confirmed accurately by characteristics of the white blood cell count.[268,220] Leukopenia was found at the onset of disease; the total count rose to a high-normal value during a 10-day period. Relative neutropenia was noted by Hynes[289] in many patients; one patient had a neutrophil count of 868 cells/mm^3 on the first day of illness. Plasma cells, Türk cells, or both were noted in acute rubella in all cases studied by Hynes[289] and Hillenbrand.[268] A Türk cell is a developing plasma cell that is 25 to 40 µm in diameter and contains a 15- to 30-µm nucleus. The nucleus has two to five prominent nucleoli and a well-defined, light reticulum. The cytoplasm often is vacuolated. Twenty-five percent of the patients studied by Hynes[289] had elevated erythrocyte sedimentation rates during the first week of illness.

Complications

Joint Involvement. The incidences of reported cases of arthritis and arthralgia vary considerably across studies.* In general, both arthralgia and arthritis occur more commonly in adults than in prepubertal children. Women are afflicted more often than are men. In a large outbreak in Bermuda in 1971, 42 percent of 125 patients studied complained of joint pain or discomfort.[298] Three patients had swelling of the joints. The prevalence of joint symptoms increased from 18 percent in the 0- to 9-year-old age group by approximately 20 percent increments per decade; 73 percent of those older than 30 years had symptoms. Joint complaints generally were more common in females than in males older than 10 years of age. This difference was most marked in the 10- to 20-year-old age group. Landrigan and associates[324] studied the location of joint symptoms in adolescents and found that the fingers were involved most often; the knees and wrists also were implicated commonly.

Yanez and associates[625] studied 11 patients with rubella arthritis. In all instances, multiple joints were involved. The onset of arthritis occurred 1 to 6 days after the beginning of the exanthem and lasted 3 to 28 days (mean, 9 days). The erythrocyte sedimentation rate was elevated in three of seven cases, and one patient had markedly positive latex test results. The white blood cell count was below 5000 cells/mm^3 in five of seven patients. One woman had bilateral carpal tunnel syndrome. Four children with transient carpal tunnel syndrome accompanying rubella virus infection have been described.[46] Panush[420] noted serum hypocomplementemia with rubella arthritis in a 25-year-old woman.

The possibility that rubella viral infection is related to rheumatoid arthritis has been studied on several occasions.† Martenis and colleagues[353] described a 21-year-old woman in whom rheumatoid arthritis developed after typical rubella with arthritis. Deinard and associates[142] found that all serum specimens from 80 patients with rheumatoid arthritis contained rubella HI antibody. In contrast, only 86 percent of an equal number of nonarthritic controls and a group of persons with other forms of arthritis had measurable rubella antibody titers. Ogra and associates[410] noted that patients with juvenile rheumatoid arthritis had IgM and IgG serum rubella antibody levels that were four to six times higher than those observed in controls during rubella infection. They also noted specific staining for rubella virus antigen in the synovial fluid of 33 percent of these patients with juvenile rheumatoid arthritis. Grahame and associates[221] repeatedly recovered rubella virus from the synovial fluid of six patients with inflammatory oligoarthritis or polyarthritis during a 2-year period.

*References 38, 92, 208, 231, 298, 324, 522, 576, 607.
†References 14, 22, 52, 353, 410, 417.

Neurologic Manifestations. Encephalitis is a rare complication of rubella.* Sherman and colleagues[518] noted six cases of encephalitis in an epidemic during the spring of 1964 that involved approximately 30,000 children. This rate of encephalitis (1/5000 cases) is similar to the rate of 1/6000 noted in Detroit in 1942.[352] Rubella encephalitis is clinically similar to encephalitis from measles virus infection but is thought to be less severe. Overall, it is estimated that rubella encephalitis mortality is approximately 20 percent.[179] However, mortality and morbidity rates have varied considerably. Although rubella encephalitis is a rare complication of rubella infection, rubella should still be considered in the differential of encephalitis in unvaccinated individuals. Sherman and colleagues[518] noted that three of six children studied in Pittsburgh died of this complication during the spring of 1964, whereas in Atlanta during the same epidemic period, six patients recovered uneventfully. Rubella encephalitis generally is a self-limited illness with a favorable prognosis. Of survivors, 80 percent tend to recover without any permanent sequelae.[233] Guler and colleagues[233] report a case of rubella encephalitis from Turkey in a 9-year-old unvaccinated boy admitted with headache, fever, and loss of consciousness. He had bilateral retroauricular lymphadenopathy but never developed a rash. Serum and cerebrospinal fluid (CSF) rubella IgM antibodies were positive, and serum IgG became positive 3 weeks later.[233] Figueiredo and associates[179] report a case of rubella encephalitis from Brazil in an 18-year-old unvaccinated man who presented with fever, nausea, severe headache, confusion, aggression, and a generalized tonic-clonic seizure. Eight days earlier he had a rash and fever. Serum and CSF tested positive for rubella virus IgM and IgG antibodies. Rubella virus was also isolated and confirmed with polymerase chain reaction (PCR) positivity from CSF and peripheral blood mononuclear cells. Phylogenetic analysis showed that it was rubella virus of the 1a genotype.[179]

The onset of encephalitis usually occurs 2 to 4 days after the rash appears, but occasionally, rash and neurologic symptoms occur at the same time; in other instances, the appearance of encephalitis is delayed as much as 1 week after the onset of illness. Examination of CSF usually reveals mild pleocytosis (20 to 100 cells/mm^3), with most cells being lymphocytes. The protein content is normal or slightly elevated, and the sugar concentration is normal.

Kenny and associates[306] studied seven survivors of rubella encephalitis 1 year after their illnesses and could find no significant loss of intellectual function. Five of the seven had abnormalities on electroencephalography, and two patients had minor neurologic abnormalities. Gibbs and colleagues[211] found abnormal electroencephalographic tracings in 6/45 children with uncomplicated rubella.

Other neurologic complications associated with rubella include progressive panencephalitis, carotid artery thrombosis, myelitis, optic neuritis, Guillain-Barré syndrome, and peripheral neuritis.* Figueiredo and colleagues[178] also report a case of Guillain-Barré syndrome associated with rubella from Brazil. They reported an 18-year-old woman with fever, emesis, headache, neck pain, arthralgias, paresthesias, progressive weakness of her lower limbs, and left-sided facial paralysis. Six days earlier she had rash, fever, cervical lymphadenopathy, and hepatomegaly. Rubella virus, again genotype 1a, was isolated from CSF and peripheral blood mononuclear cells. Serum IgM and IgG and CSF IgG antibodies against rubella were positive.[178]

Of particular interest is the common occurrence of numbness, tingling, and other symptoms consistent with neuritis during rubella infection. Cuetter and John[131] studied 20 patients with complaints of neuritis accompanying rubella and could find no objective sensory deficits or nerve conduction abnormalities.

Wolinsky and colleagues,[619] Lebon and Lyon,[333] and others have described a slowly progressive and fatal nervous system disorder with rubella that was similar to subacute sclerosing panencephalitis. This illness has occurred as a late manifestation of congenital rubella and also has occurred in children who acquired their initial infection in childhood.[56]

Thrombocytopenia. Thrombocytopenic purpura occurs in rubella at an incidence of 1/3000 cases.[33] Children are afflicted more frequently than adults, and girls are affected more often than boys.† The median interval between the onset of exanthem and the occurrence of purpura is approximately 4 days. On occasion, rash and purpura develop simultaneously; often, however, the hemorrhagic manifestations do not become apparent until 2 weeks after the exanthem develops. The illness usually is self-limited, but its duration varies from a few days to several months; although deaths caused by hemorrhagic complications have occurred, recovery is the general rule.

Other Complications. Myocarditis and pericarditis are rare complications of rubella.[201] A 30-year-old woman was noted to have erythema multiforme exudativum and arthritis with apparent clinical rubella.[198] In an outbreak that involved 46 military recruits, testicular pain was a complaint in 25 percent.[496]

During a rubella epidemic in Japan in 1976 in which 79 patients were studied, 71 percent were noted to have mild catarrhal or follicular conjunctivitis.[246] Six patients had epithelial keratitis that persisted for 2 to 7 days. Seventeen patients had preauricular lymph node swelling in association with their eye findings. During the same epidemic, 13 cases of hemolytic anemia (including 2 cases of hemolytic-uremic syndrome) were noted after the patients had infection with rubella virus.[575] During an epidemic of rubella in Japan, Sugaya and colleagues[547] found that 7.5 percent of 241 patients had liver involvement.

*References 5, 31, 54, 107, 115, 138, 159, 353, 368, 392, 469, 518, 534, 594, 614.

*References 5, 22, 67, 92, 115, 199, 256, 260, 270, 479, 566, 616, 619, 620.

†References 24, 33, 235, 295, 345, 393, 419, 536, 599.

Rubella has been noted to cause hepatitis in congenital rubella infections as well as in acute infections in both children and adults. Previously, severe hepatitis secondary to rubella had been noted only in neonatal rubella infections. However, Figueiredo and colleagues[177] report for the first time a case of acute liver failure necessitating liver transplant that was associated with rubella virus infection in a child. Serum IgM and IgG antibodies against rubella were positive, and peripheral blood mononuclear cells showed cytopathic effects characteristic of rubella. The rubella virus genome was also detected in liver fragments, and rubella virus antigen was found in the cytoplasm of hepatic cells, which suggests that the virus was replicating in hepatic tissue. The genotype of rubella virus identified was 1a. Through this case report, it is suggested that rubella can be an unrecognized cause of acute liver failure in children and should be included in the differential even if there is no prior exanthem history.

Rubella virus also has been reported to cause uveitis. De Visser and associates[141] report that rubella virus can cause a clinical spectrum of ocular symptoms that are similar to those of Fuchs heterochromic uveitis (FHU) and may be involved in the pathogenesis of FHU. Ruokonen and colleagues[477] and Suzuki and associates[548] have shown a strong association between FHC and intraocular antibody synthesis against rubella virus.

Congenital Rubella

From Gregg's original observation in 1941 of congenital defects in babies born to mothers who had rubella during early pregnancy until the pandemic of 1964, congenital rubella syndrome was considered to include only some combination of abnormalities involving the eyes, ears, brain, and heart. However, observations in 1964, supported by new virologic and serologic techniques, revealed a far more complex congenital rubella syndrome picture, and rubella syndrome was expanded to include many new anatomic findings and to acknowledge the reality of chronic persistent infection.

Congenital rubella is the result of in utero fetal infection, which usually occurs during the first 12 weeks of pregnancy. The fetal infection generally is subacute or chronic and may result in abortion, stillbirth, congenital malformations, active processes at birth (e.g., thrombocytopenia, encephalitis, or hepatitis), and, rarely, infected infants without defects. Table 173-5 summarizes the clinical findings in congenital rubella, an estimation of their frequency, and their main characteristics.

General: Infant Death and Growth Restriction

The most common manifestation of congenital rubella, readily apparent at birth, is generalized restriction of growth.* Between 50 and 85 percent of all babies weigh less than 2500 g, although gestational age is normal. Virtually all babies with intrauterine growth restriction have one or more other stigmata of congenital rubella. After birth, babies with intrauterine growth restriction often demonstrate continued growth restriction. In some instances, the failure to thrive is severe. Others show a normal growth pattern, but the child is proportionally small. The mortality rate of children with congenital rubella is high during the first year of life, with death specifically related to congenital pneumonia, heart defects and myocarditis, hepatitis, thrombocytopenia, encephalitis, immune deficiency, and failure to thrive.

Eye Findings

Approximately a third of all babies with congenital rubella have cataracts.* Cataracts may be bilateral or unilateral and are either central in location with a surrounding clear zone or diffuse. In most instances, cataracts are present at birth, but occasionally they are not observed until later in infancy. Retinopathy consisting of pigmentary defects occurs commonly in congenital rubella and is useful diagnostically, but it rarely adversely affects visual acuity. Microphthalmos occurs relatively frequently and usually is unilateral. Cataracts frequently are associated with microphthalmos.

Congenital glaucoma occurs in approximately 5 percent of congenitally infected infants. This defect usually is present at birth, but it often is overlooked. Diagnosis must be established early if sight is to be preserved.

Auditory Defects

Sensorineural deafness is the most common manifestation of congenital rubella; almost all patients have some degree of hearing impairment.† The hearing loss usually is bilateral but may be unilateral. Frequently, the only manifestation of congenital infection is deafness. An important note is that deafness is overlooked frequently in infancy, and children incorrectly are considered to be developmentally delayed. All children born to mothers who had rubella during the first half of pregnancy should undergo evaluation of their hearing several times during the first 5 years of life, regardless of whether they have other manifestations of congenital infection.

Neurologic Findings

Between 10 and 20 percent of all infants with congenital rubella have active meningoencephalitis at birth.‡ Manifestations of this infection include one or more of the following: a full anterior fontanelle, irritability, hypotonia, seizures, lethargy, and head retraction and arching of the back. Examination of the CSF reveals elevated protein and mild pleocytosis. Later neurologic disease, such as

*References 20, 121, 191, 225, 251, 281, 314, 337, 347, 364, 374, 424, 435, 476, 478, 480, 494, 514, 524, 550.

*References 20, 36, 49, 113, 116, 118, 166, 191, 210, 217, 225, 251, 254, 255, 281, 313-316, 362, 395, 408, 474-476, 488, 494, 504, 514, 524, 560, 567, 572, 574.

†References 20, 51, 143, 166, 191, 225, 234, 248, 251, 254, 322, 364, 380, 430, 435, 463, 494, 514, 572, 574, 578.

‡References 20, 22, 116, 117, 143, 144, 173, 225, 248, 251, 254, 281, 313, 314, 364, 430, 432, 434, 468, 469, 474, 476, 480, 483, 494, 514, 523, 546, 560, 569, 597, 604, 605, 612, 615.

TABLE 173-5 **Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Virus Infection**

Clinical Findings	Frequency (%)	Main Characteristics	Selected References
General			
In utero death	10-30	Spontaneous abortion; stillbirth	225, 251, 480
Intrauterine growth restriction	50-85	Generalized effect	106, 189, 191, 251, 254, 281, 314, 337, 347, 364, 441, 475, 476, 494, 524, 560
Extrauterine growth restriction	10	Failure to thrive	254, 337, 364, 374, 423, 560
Neonatal and infant deaths	10	Resulting from pneumonia, heart disease, hepatitis, thrombocytopenia, failure to thrive, immune deficiency, encephalitis	121, 225, 251, 314, 476, 494, 560
Eye			
Cataracts	35	Present at birth	20, 36, 49, 121, 191, 210, 225, 251, 254, 281, 313, 314, 364, 395, 476, 488, 494, 523, 560, 572, 574
Retinopathy	35	Present at birth; usually does not cause problems with vision	49, 113, 120, 210, 225, 251, 281, 315, 316, 367, 572, 574
Microphthalmos	5	Usually associated with cataract	49, 166, 210, 281, 314, 476, 504, 560
Glaucoma	5	Usually present at birth	49, 120, 251, 254, 281, 314, 476, 504, 514
Cloudy cornea	Rare	Usually present at birth; resolves spontaneously	49, 254
Severe myopia	Rare	Usually present at birth; defect may progress	116
Hypoplasia of the iris	Rare	Present at birth	49, 474
Strabismus	5	Associated with other eye defects	255, 364, 408
Iridocyclitis	Rare	Transient; associated with other eye defects	523
Auditory			
Nerve deafness	80-90	May be bilateral or unilateral; moderate or severe; often not recognized early	20, 51, 143, 166, 191, 225, 234, 251, 254, 322, 364, 380, 429, 435, 494, 514, 572, 574, 579
Central deafness	5	Often associated with other central nervous system defects	251
Middle ear damage	5	Usually associated with nerve deafness	463
Intraoral, Nasal, and Facial			
Cleft palate or lip	Rare		166, 225, 494
Dental abnormalities	Rare		72, 234, 364
Micrognathia	Rare		279, 494
Chronic rhinitis	Rare	Transitory finding	436
High-arched palate	Rare		251
Neurologic			
Motor defects	10	Associated with mental and other neurologic defects	364, 514, 627
Hyperirritability (tremors)	Rare	Transitory finding	468
Microcephaly	Rare		20, 254, 281, 364, 514, 560, 597, 612
Mental disabilities	10-20	Associated with other stigmata	116, 225, 248, 251, 362, 514, 546
Full anterior fontanelle	10	Transitory finding related to meningoencephalitis	117, 476
Meningoencephalitis	10-20	Transitory finding but may last for 1 year	144, 254, 313, 314, 435, 476, 523, 560
Spastic diplegia and quadriparesis	Rare	Associated with other stigmata	117, 144

TABLE 173-5 **Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Virus Infection** (Continued)

Clinical Findings	Frequency (%)	Main Characteristics	Selected References
Seizures	Rare	Frequently transitory and related to meningoencephalitis	144, 252, 364, 435
Hypotonia	Rare	Transitory defect	143, 144
Brain calcification	Rare		430, 432, 523, 600
Cerebral arterial stenosis	Rare		248
Anencephaly	Rare		494
Encephalocele	Rare		494
Meningomyelocele	Rare		546
Behavior disorders	10-20	Frequently related to deafness	117, 144
Central language disorders	5		173, 225, 605
Autism	5		116, 173, 248
Aqueductal occlusion or hydrocephalus	Rare		225, 483
Poor balance	Rare		143, 627
Progressive panencephalitis	Very rare	Has onset during adolescence	569, 604
Cardiovascular			
Patent ductus arteriosus	30	Frequently associated with other defects	20, 36, 167, 191, 251, 254, 258, 296, 313, 388, 514, 523, 560, 574, 601
Pulmonary arterial hypoplasia, supravalvular stenosis, valvular stenosis, and peripheral branch stenosis	25	Frequently associated with other defects	191, 251, 254, 258, 296, 514, 521, 574, 601
Aortic stenosis	2-5		189, 258, 520, 591, 601
Ventricular and atrial septal defects	2-5		36, 514, 574, 601
Tetralogy of Fallot	2-5		166, 254
Myocarditis and myocardial necrosis	10		6, 166, 314, 388, 560, 601
Intimal fibromuscular proliferation of many arteries	5		165
Ventricular aneurysm	Rare		220
Pulmonary			
Interstitial pneumonitis	5-10	May be acute, subacute, or chronic	48, 166, 254, 313, 314, 388, 430, 432, 435, 494, 523, 560, 612
Tracheoesophageal fistula	Rare		494
Respiratory distress	Rare	Secondary to acute pneumonia	364
Gastrointestinal			
Esophageal atresia	Rare		494
Hepatitis	5-10	Associated with other evidence of disseminated disease	20, 166, 168, 254, 387, 539, 560
Obstructive jaundice	5		313, 314, 388, 468, 523
Chronic diarrhea	Rare	Related to failure to thrive and immune deficiency	435, 560
Pancreatitis	Rare	May lead to diabetes in later life	65, 152
Duodenal stenosis	Rare		146
Jejunal or rectal atresia	Rare		166
Genitourinary			
Undescended testicle	Rare	Cause-and-effect relationship with rubella infection in doubt	364, 523
Polycystic kidney, ectopic kidney, renal agenesis, or bilobed kidney	Rare	Cause-and-effect relationship with rubella infection in doubt	165, 166, 367
Hypospadias	Rare	Cause-and-effect relationship with rubella infection in doubt	49, 191, 523
Duplication of ureter	Rare	Cause-and-effect relationship with rubella infection in doubt	36
Renal artery stenosis	Rare		363

Continued on following page

TABLE 173-5 **Frequency and Main Characteristics of Clinical Findings in Congenital Rubella Virus Infection** (Continued)

Clinical Findings	Frequency (%)	Main Characteristics	Selected References
Hydroureter and hydronephrosis	Rare	Cause-and-effect relationship with rubella in doubt	252, 430, 514, 523
Inguinal hernia	Rare	Cause-and-effect relationship with rubella in doubt	252, 430, 514, 523
Nephritis and nephrocalcinosis	Rare		523, 560
Testicular agenesis	Rare	Cause-and-effect relationship with rubella in doubt	166
Orthopedic			
Bone radiolucencies	10-20	Radiolucencies in metaphyses of long bones	254, 364, 430, 442, 451, 454, 475, 476, 506, 597, 608, 612
Pathologic fractures	Rare		474
Bone deformities	Rare		225, 344*
Clubfoot	Rare		494
Myositis	Rare	Transitory defect	531
Skin			
Dermal erythropoiesis (blueberry muffin syndrome)	5	Transitory defect; usually associated with severe disease	310
Chronic rash	Rare		72, 246
Dermatoglyphic abnormalities	5		4, 12, 448
Dimples	Rare		244
Endocrine			
Diabetes mellitus	Rare		188, 297, 365
Thyroid disorder	Rare		116
Precocious puberty	Rare		116
Growth hormone deficiency	Rare		446
Hematologic			
Thrombocytopenic purpura	5-10	Usually associated with severe disease with high death rate; transitory	20, 33, 36, 39, 120, 191, 254, 281, 314, 321, 364, 430, 435, 442, 451, 452, 475, 494, 514, 593
Hemophagocytic syndrome	Rare		34
Hemolytic anemia	Rare	Transitory	386, 430, 452
Hypoplastic anemia	Rare	Transitory	117, 321
Extramedullary hematopoiesis	5-10	Usually associated with severe disease	548
Immunologic			
Thymic hypoplasia	Rare		248
Dysgammaglobulinemia	Rare		111, 245, 441, 460, 524
Asplenia	Rare		280
Reticuloendothelial			
Generalized lymphadenopathy	10		117, 191, 246, 560
Hepatosplenomegaly	10-20	Usually associated with severe disease; transitory	36, 314, 442, 476, 494, 514
Genetic			
Chromosomal abnormalities	Rare	Cause-and-effect relationship with rubella not established	16, 407

*Cherry JD, unpublished data.

intellectual disabilities and motor retardation, can be related to the severity and persistence of the initial meningoencephalitis. Active central nervous system infection has been demonstrated for a year or more.

Behavior disorders occur commonly in children with deafness and often cannot be associated with apparent meningoencephalitis. Children with congenital rubella who have generalized retardation of growth and a proportionally small head often have normal intelligence. In contrast, the prognosis for mental development in a child with true microcephaly is poor.

Chronic progressive panencephalitis has developed in a small number of adolescents with congenital rubella, similar to measles-related subacute sclerosing panencephalitis.

Cardiovascular Findings

In severe congenital rubella with multisystem involvement, myocarditis occurs and often is a cause of death.* Of the structural defects of the heart, patent ductus arteriosus (PDA) has been previously reported to be the most common. Although it may be the only lesion noted, two thirds of patients have other lesions as well. Historically, pulmonary artery stenosis has been considered the next most common defect and may involve the main pulmonary artery or its branches. Pulmonary valvular stenosis is the third most frequent defect. Pulmonic valvular or arterial stenosis and PDA commonly occur together. However, a recent study by Oster and associates[415] that reviewed studies from 1941 to 2008 that used cardiac catheterization or echocardiography to evaluate cardiovascular malformations in congenital rubella syndrome reported different findings. They noted that branch pulmonary artery stenosis was actually more common (78%) than PDA (62%) in those patients who had been evaluated by catheterization. In addition, the combination of branch pulmonary artery stenosis and PDA (49%) was more frequently found than either branch pulmonary artery stenosis (29%) or PDA (13%) alone.

Other Manifestations

The other manifestations of congenital rubella can be separated into three categories: manifestations related to active persistent infection, structural defects, and delayed manifestations of congenital rubella.†

Manifestations Related to Active Persistent Infection. This category encompasses a broad constellation of clinical events that largely were unknown before the pandemic of 1964. Collectively, they frequently are called the *expanded congenital rubella syndrome* and include interstitial pneumonitis, hepatitis, nephritis, bone radiolucencies, myositis, dermal erythropoiesis, chronic rash, thrombocytopenic purpura, hemolytic and hypoplastic anemia, immunologic deficiency, generalized lymphadenopathy, hepatosplenomegaly, meningoencephalitis, and myocarditis. Most infants with the expanded rubella syndrome clinically have low birth weight, exanthem caused by thrombocytopenia or dermal erythropoiesis or both, hepatosplenomegaly, and jaundice. Radiographs usually reveal long-bone radiolucencies. Respiratory distress caused by both diffuse pulmonary disease and myocarditis occurs commonly, and meningoencephalitis usually is evident. The duration of chronic infection in these babies varies. Approximately 20 percent of survivors still are shedding virus at 1 year of age. Between 10 and 20 percent of babies with hepatosplenomegaly and thrombocytopenia die during the first year of life.

Structural Defects. Other than deafness, eye defects, and cardiac anomalies, which have been discussed previously, the association of other malformations with congenital rubella infection is less well established. Malformations such as tracheoesophageal fistula, jejunal atresia, inguinal hernia, and others recorded in Table 173-5 occur frequently without evidence of in utero infection. Because they are noted only sporadically in babies born after maternal rubella, they possibly are chance associations rather than cause-and-effect relationships.

Delayed Manifestations. Noted delayed manifestations of congenital rubella that were not present in early life are deafness, ocular damage, progressive rubella panencephalitis, and vascular effects such as hypertension.[511] Endocrine delayed manifestations noted in congenital rubella cases include diabetes, thyroid disorders, early menopause, osteoporosis, and even possible growth hormone deficiency.[136] Of particular importance is the association between endocrine abnormalities and autoimmunity.[110] In one study of 201 deaf adolescents with congenital rubella, 23.3 percent had positive thyroid microsomal or thyroglobulin antibodies, and, of these patients, 19.6 percent had thyroid gland dysfunction. Patients with congenital rubella have an increased incidence of insulin-dependent diabetes mellitus.[214] In a 40-year observation study of 280 Japanese patients with congenital rubella syndrome, only 3 of the patients developed insulin-dependent diabetes.[554] HLA associations with diabetes in congenital rubella syndrome have also been reported. In particular, HLA A1-B8, which has been associated with insulin-dependent diabetes, has been found in many patients with congenital rubella who have diabetes (44%).[274] A recent review of literature by Gale,[203] supports prior reports that congenital rubella syndrome predisposes to diabetes. However, Gale's review suggests that only 1% of patients with congenital rubella as opposed to 20 percent will end up developing diabetes.

Psychiatric disorders are also often found in patients with congenital rubella syndrome and may be seen in up to half of patients. Rates of intellectual disability have been reported to be as high as 42 percent, and 4.12 to 7.3 percent of patients with congenital rubella syndrome also may have autism. In addition, higher rates of psychosis have been found, as well as increased rates of behavioral problems, including impulsivity, self-injury, and aggression.[287]

DIAGNOSIS

Differential Diagnosis

Postnatally Acquired Disease

Because no pathognomonic finding in rubella exists, the clinical diagnosis in an individual case often is difficult to establish (see also Chapter 58). However, as with other exanthematous diseases, the key to establishing a

*References 6, 20, 165, 166, 189, 191, 251, 254, 256, 282, 296, 313, 314, 388, 514, 520, 521, 523, 560, 574, 591, 601.

†References 6, 12, 16, 20, 33, 36, 39, 48, 49, 65, 72, 110, 111, 116, 117, 121, 152, 165,166, 168, 188, 191, 214, 225, 244-246, 248, 252, 254, 281, 297, 310, 313, 314, 321, 344, 365, 386-389, 407, 430, 432, 435, 441, 442, 446, 452, 454, 462, 468, 475, 476, 478, 494, 506, 511, 514, 523, 524, 531, 539, 544, 560, 593, 597, 608, 612; Cherry JD, unpublished data.

diagnosis is careful elicitation of historical data. Rubella is an epidemic disease with a high clinical rate of expression of exanthem. Therefore, it is unusual, when proper investigation is performed, not to find the contact case or other cases in the community. Season also is an important consideration. Rubella generally occurs in the winter and spring, whereas enteroviral exanthems, which are the greatest masqueraders in young children, occur mainly in the summer and fall.

The incubation period also is important in separating rubella from exanthems caused by common enteroviruses or respiratory viruses. In rubella, the incubation period is long (18 ± 3 days), whereas the period in the other illnesses usually is much shorter (3 to 7 days). Age also is important. Today, rubella mainly is an illness of adolescents and young adults, and enteroviral exanthems are uncommon findings in patients at these ages.

The nature of fever also is useful in establishing the diagnosis of rubella. Temperature higher than 38.5° C (101.5° F) is an unusual occurrence in rubella but common with enteroviral exanthems, measles, and *M. pneumoniae* infection. In general, a past history of rubella infection is not particularly reliable. However, if a past illness can be documented by year, season, and symptoms, accurate information may be obtained. Useful characteristics of the rubella exanthem are its mild, erythematous, maculopapular, and discrete nature; marked pruritus in adolescents and adults; and an acneiform appearance on the face in adolescents.

Although suboccipital and posterior auricular lymphadenopathy often is thought by some investigators to be pathognomonic, its presence in nonrubella exanthems often leads to undue concern. In general, in a young child, suboccipital and posterior auricular lymphadenopathy occurs as commonly with enteroviral illnesses as with rubella. In young adults, however, this lymphadenopathy is much more useful because the enteroviral differential consideration is less of a problem. Similar lymphadenopathy does occur with acquired toxoplasmosis, infectious mononucleosis, and *M. pneumoniae* infection. A major problem in the differential diagnosis in adults is allergy. However, fever (even low grade), lymphadenopathy, headache, and eye pain, which are common events in rubella, should occur rarely in contact or other simple allergies.

Congenital Rubella

Establishing the diagnosis of congenital rubella in infants with known maternal exposure generally is not difficult. However, examining an apparently normal child at periodic intervals during the first few years of life is important so that deafness and subtle neurologic defects are not missed. The diagnosis of congenital rubella after an uneventful pregnancy is more difficult to make. All babies with evidence of intrauterine growth restriction or stigmata suggestive of congenital infection should undergo virologic and serologic study for rubella as well as for other infectious agents. Determination of the amount of serum IgM also can be useful in the study of babies with intrauterine growth restriction or babies born to mothers in whom rubella or other infection was suspected to have occurred during pregnancy.[541] Values greater than 21 mg/dL during the first week of life strongly indicate congenital infection; normal values do not rule out congenital infection, however.

Specific Diagnosis

Postnatally Acquired Disease

Rubella viral infection can be diagnosed specifically by isolation of virus from nasal or throat specimens in AGMK or other sensitive tissue culture systems or by PCR[120,123]; by the observation of a significant change in value of HI, ELISA, immunofluorescence, CF, or neutralizing antibody in two sequential serum samples or by the demonstration of specific rubella IgM antibody in a single serum sample. Most often today, the diagnosis of rubella is attempted by the use of a single serum test for identifying rubella IgM antibody.* Commercial rubella IgM immunoassays are now available, and in general their sensitivity and specificity ranges vary from 75 to 95 percent and from 85 to 100 percent, respectively.[145,563] Determination of rubella-specific IgM antibody in saliva 7 to 42 days after the onset of illness also has been shown to be both sensitive and specific.[450] Although these tests are practical, one should realize that the specificity and sensitivity of all routinely used tests are not 100 percent accurate. False-positive results occur all too frequently and commonly lead to unnecessary interventions. When the diagnosis is critical, such as with suspected rubella in pregnancy, a wise approach is to study IgG antibody in paired sera collected 1 to 2 weeks apart in addition to determining IgM antibody. One of the problems encountered with diagnosing rubella infection with serum IgM antibodies is that only 50 percent of serum samples from rubella cases collected on the day of rash onset will be positive. Work by Abernathy and associates[1] compared detection of rubella IgM from sera and oral fluids with detection of rubella RNA from oral fluids by RT-PCR taken within 4 days after rash onset from suspected rubella cases during a rubella outbreak in Peru in 2004 to 2005. They found that RT-PCR testing of oral fluid confirmed more rubella cases than IgM testing of either serum or oral fluid during days 1 and 2 after rash onset. However, the methods confirmed approximately the same number of cases on days 3 and 4 after rash onset.

Unfortunately, a rise in IgG antibody titer and an IgM-positive test result can occur in reinfection, as well as in primary infection. Because reinfection in a pregnant woman is very unlikely to lead to damage to the in utero fetus, distinguishing primary infection from reinfection is important and can be done by measuring the avidity of rubella IgG antibody.[243,271,449] Rubella IgG avidity assays focus on the strength of antigen-antibody interaction, which can differ with first exposure to an antigen as with primary infection in contrast to previous infection. In primary infection, this antigen-antibody interaction is low (i.e., low avidity), whereas in reinfection or prior

*References 15, 25, 35, 37, 74, 93, 98, 126, 148, 163, 176, 186, 215, 277, 350, 370, 394, 425, 502, 525, 564, 585.

infections, this antigen-antibody interaction is stronger (i.e., high avidity).[516]

Congenital Rubella

The best method for establishing a definitive diagnosis of congenital rubella is viral isolation or PCR. Specimens for viral culture or PCR should be obtained from the nose, throat, urine, buffy coat of blood, and CSF. Because of transplacental passage of maternal IgG, establishment of the diagnosis of congenital rubella in the neonatal period by serologic methods is fraught with difficulty. Usually, specific rubella IgM antibody can be demonstrated with presently available techniques. In questionable cases, follow-up studies comparing infant and maternal antibody values often establish the diagnosis. If the infant's value is the result solely of transplacentally acquired antibody, it should drop fourfold to eightfold by the time the infant reaches 3 months of age and continue to fall to nondetectable values by 6 to 8 months of age. However, the antibody value in some congenital infections also may fall, so disappearance of antibody in serum does not rule out in utero infection completely. Also, in questionable cases, the study of IgG rubella antibody avidity may be useful.[264,449]

The retrospective diagnosis of congenital rubella in late infancy and the second year of life has been difficult to make. However, researchers have shown that affected children have low avidity of specific IgG antibody, and, therefore, a retrospective diagnosis of congenital rubella can be made by specific avidity assays.[264] Also useful for the serologic diagnosis of congenital rubella during the prenatal and newborn periods are rubella IgG peptide–based enzyme immunoassay and rubella immunoblot assay.[362] Newborns who were infected during the first 12 weeks of gestation have reduced levels of antibodies directed at both the linear E1 epitope (SP15) and the topographic E2 epitope.

Fetal rubella can be diagnosed in amniotic fluid samples by RT-PCR.[460,555]

Qualitative Demonstration of Rubella Antibody

The original screening method for rubella antibody was HI. Today, HI has been replaced by more rapid and easier tests involving enzyme immunoassay, erythrocyte agglutination, and latex agglutination. In general, all are both highly sensitive and specific.[97,175]

TREATMENT

Postnatally Acquired Disease

Uncomplicated Rubella

No specific therapy is necessary or indicated for uncomplicated rubella. Starch baths may be useful in adults with troublesome pruritus. Of importance is that affected patients understand that they are contagious and that transmission of infection to a pregnant woman could have serious consequences.

Complications of Rubella

On occasion, arthritis can be severe in adults. When weight-bearing joints are affected, rest is encouraged. Symptoms readily respond to aspirin therapy; corticosteroids are not indicated. In rubella encephalitis, care is supportive, with adequate maintenance of fluids and electrolytes.

Thrombocytopenia usually is self-limited; however, severe bleeding has occurred on occasion. Splenectomy is not indicated. Corticosteroid therapy often is used, but with little evidence of specific benefit in rubella-infected patients. In patients who do not recover rapidly and in those with severe bleeding, treatment with intravenous immunoglobulin should be considered.

Management of Exposed Pregnant Women

Ideally, all pregnant women should have received rubella vaccine previously or been shown to have rubella antibody by an appropriate serologic test. If a pregnant woman is exposed to a person with rubella and the history of previous immunization or antibody presence is unknown, an immediate blood specimen should be obtained and a rubella antibody test performed. If antibody to rubella is demonstrated, no action is necessary.

If IgG antibody is not detectable in pregnant woman exposed to rubella, she is not rubella immune. Thus, a second blood specimen should be obtained 2 to 3 weeks later and tested for rubella antibody concurrently with the first specimen. If this second test result is negative, a third blood specimen should be obtained 6 weeks after exposure and also tested again for antibody concurrently with the first specimen. A negative result in both second and third specimens indicates that infection has not occurred. However, a positive test result in the second or third specimen but not found in the first indicates seroconversion and provides evidence for recent rubella infection.[14] Susceptible rubella-exposed women should undergo careful clinical observation for fever, lymphadenopathy, or exanthem for a 4-week period. If illness occurs, a nasal specimen should be cultured for rubella virus and serum should be examined for rubella IgM antibody. A second serum specimen should be submitted for rubella antibody examination. If rubella antibody seroconversion is noted or specific IgM antibody is demonstrated, the risk for fetal infection and malformation is considerable. Because false-positive rubella IgM antibody test results are not rare occurrences, all tests that yield positive results should be repeated for confirmation (by another assay and in another laboratory if possible). Because IgM antibody can occur in reinfections and fetal risk is then minimal, determining the avidity of IgG antibody also may be useful.[243,271,449] The patient should be so advised, and therapeutic abortion should be discussed.

In situations of known exposure of a susceptible pregnant woman in which therapeutic abortion is not possible and exposure can be documented to have taken place within 72 hours, our opinion is that 20 mL of immunoglobulin should be administered immediately. The use of

immunoglobulin is controversial, but in certain controlled situations, it has been effective in preventing disease.[57,376,490,580]

Management of Pregnant Women with an Exanthem Thought To Be Rubella

In this circumstance, if previous rubella serologic study results are available, they are extremely useful. If previous serum antibody has been noted, the mother should be reassured that the present illness is not likely to be rubella. However, because false-positive rubella screening results do occur, a wise approach is to carry out rubella serologic study and, when possible, also viral culture. An acute-phase serum sample should be examined for rubella-specific IgM antibody. A second serum specimen should be collected 1 to 2 weeks after the disappearance of the rash. If rubella antibody has risen significantly or IgM antibody is demonstrated, it is highly likely that congenital infection has occurred and that anomalies may result. Again, a wise approach is to confirm IgM positive test results and, if available, do IgG rubella antibody avidity study. In this circumstance, the woman should be counseled about therapeutic abortion.

If a previous serum rubella antibody value is not available, a serum sample should be collected immediately and another collected 2 to 3 weeks later. These sera should be examined as paired specimens for rubella antibody and analyzed for specific rubella IgM antibody. If a rubella antibody rise is demonstrated or the presence of rubella IgM is noted, one must assume that rubella viral infection has occurred, and the patient should be advised about the risk for congenital infection and the possibility of therapeutic abortion.

Management of Children with Congenital Rubella

Isolation Procedures

Most babies with congenital rubella remain actively infected at the time of birth, are contagious, and therefore should be placed in isolation. Room isolation and urine precautions are the major necessities. The isolated baby should be cared for only by persons known to be seropositive for rubella. Because rubella viral shedding has been known to occur for a year or more in some babies, isolation of infants with congenital rubella should be continued for this duration unless repeated viral cultures have proved negative. Current specific recommendations from the 2012 *Red Book* indicate that contact isolation in the hospitalized patient is indicated for children with proved or suspected congenital rubella until they are at least 1 year of age, unless two cultures of clinical specimens obtained 1 month apart after 3 months of age are negative for rubella virus.[14]

After the child is discharged from the hospital, no special precautions are necessary in the household setting. However, the parents should be advised of the potential risk to pregnant visitors.

Neonatal Period

As noted, the clinical manifestations of congenital rubella are varied, and in many infants, no symptoms are manifested during the first few months of life. In these apparently asymptomatic infants, no particular management problems are encountered. In other neonates, symptoms of continued viral infection are readily apparent and frequently are severe. In these infants, the following findings are important: pneumonia, thrombocytopenia, eye findings, heart defects, hyperbilirubinemia, and hepatosplenomegaly.

Although purpura and petechiae secondary to thrombocytopenia may be impressively severe in these infants, true hemorrhagic difficulties have not been a major problem. Corticosteroid therapy does not seem to be indicated, but considering treatment with intravenous immunoglobulin might be worthwhile. Careful evaluation of the eyes is important. Of immediate concern is the search for corneal clouding because its presence probably indicates infantile glaucoma. Cataracts and retinopathy also should be sought carefully. Infants with glaucoma should be referred immediately for ophthalmologic evaluation and therapy. Children with cataracts or retinopathy also should be referred, but therapy for cataracts is best delayed until the child reaches a later age.

Respiratory distress secondary to extensive viral involvement should be managed similar to other neonatal respiratory disease—assisted ventilation and careful attention to arterial blood gas values and pH. Although jaundice secondary to congenital rubella infection rarely is severe, standard criteria for the treatment of hyperbilirubinemia should be followed. Hepatosplenomegaly may be marked in some instances but is of no therapeutic concern.

Cardiac evaluation should be the same as in affected infants without rubella. Specifically, congestive cardiac failure should be treated vigorously; in malignant conditions (patent ductus arteriosus, coarctation of the aorta), lifesaving surgery should be contemplated.

Long-term Problems

Deafness. Hearing disability is the most frequent abnormality after infection with congenital rubella; more than 80 percent of infected infants have some degree of hearing disability. In many instances, deafness is the only clinical manifestation of congenital rubella, and because the establishment of this diagnosis in early infancy is difficult, the diagnosis frequently is delayed. However, early diagnosis of deafness and institution of proper educational programs are the most productive measures in the long-term management of children with congenital rubella. All too frequently, poor medical advice has been responsible for the delay in making an appropriate diagnosis and providing therapy. Any time that a mother suspects that her child is deaf, specific audiometric testing should be performed. Many general practitioners, pediatricians, and even otolaryngologists think that hearing cannot be tested in infants. This concept must be discouraged vigorously; at proper centers, a severely deaf child can be recognized in virtually all instances.

If deafness is diagnosed, the child should be referred immediately to a training program. Information about training programs can be obtained from the Alexander Graham Bell Association for the Deaf* and the John Tracy Clinic.† In virtually all instances, severely deaf children should be enrolled in an education program before or during the second year of life and the child should be fitted with a proper auditory amplification device. Although deafness in congenital rubella is sensorineural, a surprising finding is that conduction defects also are noted in many older children. For these children, other aspects of otolaryngologic care may be indicated.

Eye Problems. All children with eye problems (i.e., cloudy cornea, glaucoma, cataracts, retinitis, or strabismus) should be referred at an early age for ophthalmologic evaluation. Glaucoma requires immediate attention. Decisions about cataract surgery should be left to the discretion of the ophthalmologist but, in general, are well deferred until after the end of the first year. Retinopathy, although frequently impressive on ophthalmoscopic examination, rarely causes much visual defect. Strabismus is managed as it is in children without rubella. Advice on eye problems in congenital rubella can be obtained from the American Foundation for the Blind.‡

Heart Problems. Congenital heart disease secondary to in utero rubella infection should be managed as heart disease is in children without rubella. Of importance is that the children be referred to cardiac centers where sophisticated diagnostic techniques and cardiac surgery facilities are available for correctable lesions.

Musculoskeletal Problems. Isolated musculoskeletal defects are relatively uncommon findings in congenital rubella. However, when the symptoms indicate, referral to a cerebral palsy clinic is useful, both for specific therapeutic modalities and for the camaraderie of group therapy for the children as well as the parents.

Central Nervous System Problems. Careful analysis of the available data suggests that many infants who have been labeled intellectually disabled actually are children with auditory or visual defects who have not had proper diagnosis and training for their handicaps. No child with congenital rubella should be labeled mentally subnormal until extensive audiologic and ophthalmologic investigations and perhaps specific therapy have been performed.

Immunologic Defects. A small number of children with congenital rubella have been noted to have specifically low levels of serum IgG. These infants have systemic continued viral infection and in general do poorly. Although outcome studies are not available, administration of immune serum globulin (intramuscular or intravenous immunoglobulin) to these infants periodically seems prudent.

Multiple Handicaps. All too frequently, children infected in utero with rubella virus suffer from one or more of the handicaps mentioned. Care of these infants and children requires many different resources and modalities of therapy. Frequently, the physician is the one who must coordinate both the diagnostic and the long-term educational efforts that are necessary for optimal progress of an affected child. In addition to the Alexander Graham Bell Association for the Deaf, the John Tracy Clinic, and the American Foundation for the Blind, already mentioned, the following agencies may be helpful to physicians or the parents of congenital rubella children: United Cerebral Palsy Research and Educational Foundation,* Easter Seal Research Foundation,† The Arc of the United States,‡ and Maternal and Child Health Division, Department of Health and Human Services,§

PREVENTION

Active Immunization: Live Attenuated Rubella Virus Vaccine

In 1969 three rubella vaccines were licensed for use in the United States: HPV-77: DE-5 (duck embryo), HPV-77:DK-12 (dog kidney), and GMK-3:RK33 Cendevax (rabbit kidney) strains.[603] The HPV-77:DK-12 vaccine was later removed from usage because a higher rate of joint complaints were noted after vaccination with this strain. In 1979 the RA 27/3 (human diploid fibroblast) strain (Meruvax-II) was licensed for use in the United States. At present, the RA 27/3 rubella virus vaccine is the only vaccine currently licensed for usage in the United States. The RA 27/3 rubella vaccine is a live-attenuated virus vaccine. The virus used was first isolated from a rubella-infected aborted fetus from the Wistar Institute in 1965. Attenuation of the virus was achieved by 25 to 30 passages in tissue culture, using human diploid fibroblasts. This vaccine does not contain any duck, chicken, or egg protein. The rubella vaccine currently is available in combination with measles and mumps vaccines as MMR or combined mumps, measles, and varicella vaccine as MMRV (ProQuad). Currently, the single-antigen rubella vaccine is not available for usage in the United States. Both the MMR and MMRV vaccines are distributed as a lyophilized (freeze-dried) powder and require reconstitution with sterile, preservative-free water. These vaccines both contain

*Alexander Graham Bell Association for the Deaf, 3417 Volta Place, NW, Washington, DC 20007, http://www.agbell.org.
†John Tracy Clinic, 806 West Adams Boulevard, Los Angeles, CA 90007, http://www.johntracyclinic.org.
‡American Foundation for the Blind, 2 Penn Plaza, Suite 1102, New York, NY, 10121, http://www.afb.org.

*United Cerebral Palsy Research and Educational Foundation, 1600 L Street, NW, Suite 700, Washington, DC 20036-5602, http://www.ucp.org or http://affnet.ucp.org.
†Easter Seal Research Foundation, 233 South Wacker Drive, Suite 2400, Chicago, IL 60606, http://easterseals.com.
‡The Arc of the United States, 1825 K Street, NW, Suite 1200, Washington, DC 20006, http://www.thearc.org.
§Maternal and Child Health Division, Department of Health and Human Services, Parklawn Building, 5600 Fishers Lane, Rockville, MD 20857, http://mchb.hrsa.gov.

trace amounts of human albumin, neomycin, sorbitol, and gelatin.[89] Vaccination can be expected to produce antibodies in more than 95 percent of those immunized.* Antibody titers after RA 27/3 vaccination are slightly lower than those after natural infection, but they have been demonstrated to persist for an 11- to 15-year period with a pattern similar to that occurring after natural infection, even in the absence of reexposure to rubella virus.[46,284,438]

Because of universal immunization practices, indigenous rubella has been eliminated in the United States and more recently from the Americas.[70,86,88,455,457] Finland's vigorous two-dose immunization program also resulted in the elimination of indigenous rubella.[428,429]

Recommendations for Use

For complete information about rubella immunization, the reader is referred to the most recent recommendations of the Immunization Practices Advisory Committee of the U.S. Public Health Service,[80,85] the recommendations of the Committee on Infectious Diseases of the American Academy of Pediatrics,[13] and the vaccine manufacturer's product information.

Rubella vaccine is recommended for all children 12 months of age or older, adolescents, and adults, particularly women, unless otherwise contraindicated. Vaccination of children protects them from rubella and thus prevents them from subsequently spreading it. Vaccination of susceptible postpubertal women confers individual protection from rubella-induced fetal injury. Vaccination of adolescents or adults in population groups such as those in colleges, places of employment, or military bases protects them from rubella and reduces the chance of epidemics occurring in partially immune groups.

Rubella vaccine should not be administered to infants younger than 1 year of age because persisting maternal antibodies may interfere with seroconversion. When rubella vaccine is part of a combination vaccine that includes measles antigen, it should be administered to children approximately 12 to 15 months of age. A second dose of rubella-containing vaccine is recommended at school entry, typically at age 4 through 6 years.[89] Children who have not received rubella vaccine at the optimal age should be vaccinated promptly. Because a history of rubella is not a reliable indicator of immunity, all children for whom vaccine is not contraindicated should be vaccinated.

Vaccination of all unimmunized prepubertal children and adolescents as well as of adult women in the childbearing age group must be emphasized. Because of the theoretical risk to the fetus, women of child-bearing age should receive vaccine only if they are not pregnant and understand that they should not become pregnant for 28 days after receiving the vaccination.[14,89]

Special attention also should be placed on targeted immunization of at-risk postpubertal males and females, especially college students, military recruits, recent immigrants, health care professionals, teachers, and child care providers. People born in 1957 or after who have not received at least one dose of vaccine or who have no serologic evidence of immunity to rubella should be immunized against rubella.[14] Educational and training institutions such as colleges, universities, and military bases should seek proof of rubella immunity (a positive serologic test result or documentation of previous rubella vaccination) from all students and employees in the childbearing age. Nonpregnant women who lack proof of immunity should be vaccinated unless contraindications exist.

For the protection of susceptible female patients and female employees, persons working in hospitals and clinics who might contract rubella from infected patients or who, if infected, might transmit rubella to pregnant patients either should have serologically demonstrated immunity to rubella or should receive the vaccine.

Routine premarital serologic testing for rubella immunity would enhance efforts to identify susceptible women before pregnancy. Prenatal or antepartum screening for rubella susceptibility should be undertaken and vaccine administered in the immediate postpartum period—before hospital discharge. Previous administration of anti-Rh_0(D) immunoglobulin (human) or blood products is not a contraindication to vaccination; however, 6- to 8-week postvaccination serologic testing should be performed for confirmation of seroconversion in those few who have received the globulin or blood products. Obtaining laboratory evidence of seroconversion in other vaccines is not necessary. Please refer to the 2012 *Red Book* updated guidelines for the minimum recommended interval for rubella vaccination after receiving immunoglobulin.

No evidence has shown that live rubella virus vaccine given after exposure prevents illness or that vaccination of a person incubating rubella is harmful. Because postexposure vaccination would theoretically prevent illness if administered within 3 days of exposure and protect a person in the event of future exposure, postexposure vaccination is generally recommended unless otherwise contraindicated.[14]

Adverse Reactions

Vaccination in young children rarely is associated with any symptoms. However, on occasion, mild fever, rash, lymphadenopathy, and upper respiratory tract symptoms have been observed. In children receiving the MMR or MMRV vaccines, fever has been noted to develop 6 to 12 days after immunization in approximately 5 to 15 percent of children. Rash may occur in approximately 5 percent of those immunized, and mild lymphadenopathy has been frequently observed. A twofold increase in febrile seizure risk has been noted in children vaccinated with the combined MMRV vaccine 5 to 12 days after immunization. In children 12 through 23 months of age, febrile seizures have been noted more frequently after administration of the MMRV vaccine (7 to 9/10,000) in contrast to the MMR and varicella vaccines (3 to 4/10,000) given separately. One additional febrile seizure

*References 23, 341, 438, 492, 530, 595, 600, 601.

may be expected to occur per approximately 2300 to 2600 of these children immunized with the MMRV vaccine.[14,293,311]

More severe reactions have been noted rarely in children but have been an occasional problem in adults. Most of these complications were reported in association with the previously available vaccines, but complications with the RA 27/3 vaccine also have been noted.[23,157] Particularly troublesome are arthralgia and arthritis.*

These symptoms occur most commonly in adults. Arthralgia and arthritis occur most commonly in adults and have been seen more often in women than men.[89] Joint pain, which is typically localized to smaller, peripheral joints, has been found in approximately 0.5% of young children. Joint involvement typically begins 7 to 21 days after immunization and generally is considered to be a transient reaction.[14,89] Arthralgia develops in approximately 25 percent of susceptible postpubertal females after they have received the RA 27/3 vaccination, and approximately 10 percent have been reported to have arthritis-like signs and symptoms.[41,230,444] Infrequently, chronic or recurrent arthralgia, sometimes with arthritis or neurologic symptoms, including paresthesias, carpal tunnel syndrome, and blurred vision, reportedly have developed in susceptible vaccinees, primarily adult women.[80,285,533] One group of investigators reported the frequency of chronic joint symptoms and signs in adult women to be as high as 5 to 11 percent[561-563]; however, other data from the United States and other countries suggest that such complications caused by RA 27/3 vaccine are rare or perhaps nonexistent.† Patients with juvenile idiopathic arthritis (JIA) have been shown to be safely vaccinated with the rubella vaccine. However, a case report from Korematsu and colleagues[312] describes an 11-year-old girl with systemic JIA, previously in remission for 4 years, who then developed an abrupt and severe relapse with congestive heart failure 5 days after receiving the rubella vaccine. The authors suggest that the exacerbation of the patient's systemic JIA may be secondary to molecular mimicry between the rubella virus and her JIA.

Other complications, mainly with vaccines other than RA 27/3, include polyneuropathies (catcher's crouch, carpal tunnel syndrome, neuritis, and myeloradiculoneuritis),‡ marked lymphadenopathy (Cherry JD, unpublished data, 1967-1976), vasculitis, and myositis.[247]

Geiger and associates[209] noted a persistent rubella virus infection in a 16-year-old boy with acute lymphoblastic leukemia in remission who was vaccinated. The virus was identified in the patient's peripheral blood mononuclear cells 8 months after immunization. In contrast, persistent infection could not be demonstrated in 10 children with symptomatic human immunodeficiency virus type 1 infection.[193]

Cheong and colleagues[96] report a fatal case of disseminated tuberculosis in a 14-year-old girl, which occurred immediately after a measles-rubella vaccination. Previously, the exacerbation of tuberculosis infection related to measles-rubella vaccine has been reported only in severely immunocompromised patients. Although natural measles infection has been known to potentiate other infections such as tuberculosis, no prior evidence has shown that measles-rubella vaccinations may accelerate the progression of tuberculosis.

Other adverse reactions that occur after immunization with MMR or MMRV have been primarily associated with the measles, mumps, and varicella components of the vaccine. In recent years, particular focus has been placed on the risk for immune thrombocytopenia purpura after MMR immunization.[190,351] Please refer to Chapter 244 for further detail of other adverse reactions associated with the MMR and MMRV vaccines.

Contraindications

Administration of live rubella virus vaccine is contraindicated in women who are pregnant; in individuals with altered immune states, such as immunodeficiency, leukemia, lymphoma, and generalized malignant disease; or in patients treated with high doses of corticosteroids (≥2 mg/kg/day or >20 mg/day for 14 days or more), alkylating drugs, antimetabolites, and radiation. Fever is not a contraindication to immunization, and children with mild illnesses such as upper respiratory tract infections may still receive immunizations. However, immunization should be delayed until recovery in cases of moderate to severe acute illnesses.[14,89] A personal or family (i.e., sibling or parent) history of seizures of any etiology is a precaution for MMRV vaccination because these children may have an increased risk for febrile seizures. This caveat does not apply for children vaccinated with MMR and varicella vaccines administered separately.[89] Rubella immunization also, in most instances, should be deferred after the administration of blood products, including immune serum globulin (see Chapters 244 and 245).

Inadvertent Rubella Immunization in Pregnancy

From January 1971 to April 1989, the CDC observed to term 321 known rubella-susceptible pregnant women who had been vaccinated with rubella vaccine within 3 months before or 3 months after conception.[80,181] Ninety-four women received HPV-77 or Cendehill vaccine, 1 received a vaccine of unknown strain, and 226 received RA 27/3 vaccine. None of the 324 infants born to these women had malformations compatible with congenital rubella, but five of the infants (fewer than ~2%) had serologic evidence of subclinical infection. From these data, the CDC estimated a 1.3 percent maximal theoretical risk for congenital rubella among women vaccinated with the RA 27/3 rubella virus vaccine within 1 to 2 weeks before and 4 to 6 weeks after conception.[14] Thus, women who may have been inadvertently immunized with rubella during pregnancy should be counseled extensively regarding these potential risks to the fetus. However, these observations of outcomes do not necessitate pregnancy

*References 19, 30, 105, 122, 223, 339, 391, 409, 443, 532, 553, 556, 557, 561-563, 596.
†References 122, 185, 194, 257, 405, 526, 532.
‡References 100, 105, 213, 240, 308, 404, 485.

termination as a result of rubella vaccine receipt during pregnancy.[14]

Multiple other studies have investigated the risk for congenital rubella among women who have received the rubella vaccine during pregnancy and have confirmed the safety of this vaccine among pregnant women. Castillo-Solorzano and associates[71] collected data from 2001 to 2008 from more than six countries of 30,139 women who were pregnant or became pregnant within 1 month after receiving the rubella vaccine. Seventy infants (3.5%) showed positive rubella serology, but none of the infants had features of congenital rubella syndrome. The authors concluded that the maximum theoretical risk for congenital rubella syndrome after rubella vaccination of susceptible pregnant women was 0.2%. A large study by Soares and colleagues[529] from several states in Brazil investigated 22,708 women vaccinated during pregnancy and found 67 newborns (4.1%) showed evidence of congenital infection but none with congenital rubella syndrome. Da Silva e Sa and associates,[134] Sato and associates,[484] and Minussi and associates[381] reviewed cases from Brazil, finding low rates of congenital rubella infection (2%, 4.7%, and 6.7%), but no cases of vaccine-associated congenital rubella syndrome. Another, smaller study from 2006 in Argentina by Pardon and associates[421] also did not show evidence of congenital rubella syndrome in infants born to women unknowingly pregnant during their mass rubella vaccine campaign. Nasiri and colleagues[400] and Namaei and associates[398] looked at pregnant women inadvertently vaccinated with rubella during pregnancy during Iran's 2003 mass vaccination campaign and found no cases of congenital rubella syndrome.[292]

In conjunction with a nationwide measles-rubella vaccination campaign in 2001, the fetal risk associated with rubella vaccination during pregnancy was studied in Costa Rica.[21] The study involved 1191 mother and child pairs, and no adverse pregnancy outcomes or cases of congenital rubella syndrome occurred.

Passive Immunization

The use of immunoglobulin for the prevention of rubella has been controversial for many years.* However, our opinion is that its use is indicated in certain circumstances. As previously noted, the specific indication for immune serum globulin is for the prevention of rubella in a woman thought to be susceptible to rubella who is in the first 20 weeks of pregnancy. If the exposure can be documented clearly as one to a specific, single person with rubella and immunoglobulin is given within 72 hours of that exposure, both maternal disease and congenital infection likely can be prevented. On the other hand, if the exposure was more general (e.g., a schoolteacher exposed to a child or children in the school setting), the woman probably was exposed for a considerable time before she realized the exposure. Therefore, because immunoglobulin probably will be administered too late (well into the incubation period of her disease and after viremia), congenital infection is not likely to be prevented. The dose of immunoglobulin for the prevention of rubella during pregnancy is 20 mL intramuscularly.

*References 28, 55, 150, 226, 348, 356, 360, 376, 490, 580.

Quarantine and Disease Containment

Patients with rubella should not have contact with susceptible persons until the rash has disappeared. Containment of rubella is a vital part of the prevention policy in the United States today. Rubella is a reportable disease, and compliance is the obligation of all physicians and other health care professionals. Reporting cases of rubella enables public health workers to organize vaccination programs so that small outbreaks of disease can be prevented from developing into major epidemics. During an outbreak, those without evidence of immunity should be immunized or excluded from school or child care attendance.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abernathy E, Cabezas C, Sun H, et al. Confirmation of rubella within 4 days of rash onset: comparison of rubella virus RNA detection in oral fluid with immunoglobulin M detection in serum or oral fluid. *J Clin Microbiol* 2009;**47**:182–8.
2. Abernathy ES, Hubschen JM, Muller CP, et al. Status of global virologic surveillance for rubella viruses. *J Infect Dis* 2011;**204**(Suppl. 1):S524–32.
14. American Academy of Pediatrics. Rubella. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 629–34.
40. Berger BE, Omer SB. Could the United States experience rubella outbreaks as a result of vaccine refusal and disease importation? *Hum Vaccin* 2010;**6**:1016–20.
66. Caidi H, Bloom S, Azilmaat M, et al. Rubella seroprevalence among women aged 15-39 years in Morocco. *East Mediterr Health J* 2009;**15**:526–31.
70. Castillo-Solorzano C, Marsigli C, Bravo-Alcantara P, et al. Elimination of rubella and congenital rubella syndrome in the Americas. *J Infect Dis* 2011;**204**(Suppl. 2):S571–8.
71. Castillo-Solorzano C, Reef SE, Morice A, et al. Rubella vaccination of unknowingly pregnant women during mass campaigns for rubella and congenital rubella syndrome elimination, the Americas 2001-2008. *J Infect Dis* 2011;**204**(Suppl. 2):S713–17.
89. Centers for Disease Control and Prevention. Epidemiology and prevention of vaccine-preventable diseases. Chapter 19 Rubella In: Atkinson W, Wolfe S, Hamborsky J, editors. 12th ed. Washington DC: Public Health Foundation; 2012.
90. Centers for Disease Control. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
96. Cheong HK, Yang BG, Sohn YM, et al. A fatal case of disseminated tuberculosis coincident with measles-rubella vaccination. *Epidemiol Health* 2010;**32**:e2010002.
114. Cong H, Jiang Y, Tien P. Identification of the myelin oligodendrocyte glycoprotein as a cellular receptor for rubella virus. *J Virol* 2011;**85**:11038–47.
133. D'Agaro P, Dal Molin G, Zamparo E, et al. Epidemiological and molecular assessment of a rubella outbreak in North-Eastern Italy. *J Med Virol* 2010;**82**:1976–82.
134. da Silva e Sa GR, Camacho LA, Stavola MS, et al. Pregnancy outcomes following rubella vaccination: a prospective study in the state of Rio de Janeiro, Brazil, 2001-2002. *J Infect Dis* 2011;**204**(Suppl. 2):S722–8.
136. Dammeyer J. Congenital rubella syndrome and delayed manifestations. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1067–70.

154. Doshi S, Khetsuriani N, Zakhashvili K, et al. Ongoing measles and rubella transmission in Georgia, 2004-05: implications for the national and regional elimination efforts. *Int J Epidemiol* 2009;**38**:182–91.
158. Duszak RS. Congenital rubella syndrome: major review. *Optometry* 2009;**80**:36–43.
172. Featherstone DA, Rota PA, Icenogle J, et al. Expansion of the global measles and rubella laboratory network 2005-09. *J Infect Dis* 2011;**204**(Suppl. 1):S491–8.
178. Figueiredo CA, Cordovani NT, Castrignano SB, et al. Acute liver failure associated with rubella virus in a child. *Pediatr Infect Dis J* 2010;**29**:573–4.
179. Figueiredo CA, Oliveira MI, Afonso AM, et al. Rubella encephalitis in a young adult male: isolation and genotype analysis. *Infection* 2011;**39**:73–5.
233. Guler E, Davutoglu M, Guler S, et al. Encephalitis in a child during atypical course of rubella. *Infection* 2009;**37**:65–6.
238. Hahne S, Macey J, van Binnendijk R, et al. Rubella outbreak in the Netherlands, 2004-2005: high burden of congenital infection and spread to Canada. *Pediatr Infect Dis J* 2009;**28**:795–800.
250. Haralambieva IH, Dhiman N, Ovsyannikova IG, et al. 2′-5′-Oligoadenylate synthetase single-nucleotide polymorphisms and haplotypes are associated with variations in immune responses to rubella vaccine. *Hum Immunol* 2010;**71**:383–91.
274. Honeyman MC, Harrison LC. Congenital rubella, diabetes and HLA. *Diabetologia* 2009;**52**:371–2; author reply 373.
286. Hukic M, Hubschen JM, Seremet M, et al. An outbreak of rubella in the Federation of Bosnia and Herzegovina between December 2009 and May 2010 indicates failure to vaccinate during wartime (1992-1995). *Epidemiol Infect* 2012;**140**:447–53.
287. Hwang SJ, Chen YS. Congenital rubella syndrome with autistic disorder. *J Chin Med Assoc* 2010;**73**:104–7.
293. Jacobsen SJ, Ackerson BK, Sy LS, et al. Observational safety study of febrile convulsion following first dose MMRV vaccination in a managed care setting. *Vaccine* 2009;**27**:4656–61.
300. Kasper S, Allerberger F, Aberle S, et al. Rubella in Austria 2008-2009: no longer a typical childhood disease. *Pediatr Infect Dis J* 2010;**29**:448–52.
302. Kearns MJ, Plitt SS, Lee BE, et al. Rubella immunity among pregnant women in a Canadian provincial screening program. *Can J Infect Dis Med Microbiol* 2009;**20**:73–7.
305. Kennedy RB, Ovsyannikova IG, Vierkant RA, et al. Effect of human leukocyte antigen homozygosity on rubella vaccine-induced humoral and cell-mediated immune responses. *Hum Immunol* 2010;**71**:128–35.
311. Klein NP, Fireman B, Yih WK, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;**126**:e1–8.
312. Korematsu S, Miyahara H, Kawano T, et al. A relapse of systemic type juvenile idiopathic arthritis after a rubella vaccination in a patient during a long-term remission period. *Vaccine* 2009;**27**:5041–2.
342. Lin CC, Yang CY, Shih YL, et al. Rubella seroepidemiology and estimations of the catch-up immunisation rate and persistence of antibody titers in pregnant women in Taiwan. *BJOG* 2011;**118**:706–12.
351. Mantadakis E, Farmaki E, Buchanan GR. Thrombocytopenic purpura after measles-mumps-rubella vaccination: a systematic review of the literature and guidance for management. *J Pediatr* 2010;**156**:623–8.
400. Nasiri R, Yoseffi J, Khajedaloe M, et al. Congenital rubella syndrome after rubella vaccination in 1-4 weeks periconceptional period. *Indian J Pediatr* 2009;**76**:279–82.
406. Novo A, Huebschen JM, Muller CP, et al. Ongoing rubella outbreak in Bosnia and Herzegovina, March-July 2009: preliminary report. *Euro Surveill* 2009;**14**:19343.
415. Oster ME, Riehle-Colarusso T, Correa A. An update on cardiovascular malformations in congenital rubella syndrome. *Birth Defects Res A Clin Mol Teratol* 2010;**88**:1–8.
418. Ovsyannikova IG, Vierkant RA, Pankratz VS, et al. HLA haplotype and supertype associations with cellular immune responses and cytokine production in healthy children after rubella vaccine. *Vaccine* 2009;**27**:3349–58.
421. Pardon F, Vilarino M, Barbero P, et al. Rubella vaccination of unknowingly pregnant women during 2006 mass campaign in Argentina. *J Infect Dis* 2011;**204**(Suppl. 2):S745–7.
457. Reef SE, Redd SB, Abernathy E, et al. Evidence used to support the achievement and maintenance of elimination of rubella and congenital rubella syndrome in the United States. *J Infect Dis* 2011;**204**(Suppl. 2):S593–7.
458. Reef SE, Strebel P, Dabbagh A, et al. Progress toward control of rubella and prevention of congenital rubella syndrome: worldwide, 2009. *J Infect Dis* 2011;**204**(Suppl. 1):S24–7.
471. Rota PA, Brown KE, Hubschen JM, et al. Improving global virologic surveillance for measles and rubella. *J Infect Dis* 2011;**204**(Suppl. 1):S506–13.
477. Ruokonen PC, Metzner S, Ucer A, et al. Intraocular antibody synthesis against rubella virus and other microorganisms in Fuchs' heterochromic cyclitis. *Graefes Arch Clin Exp Ophthalmol* 2010;**248**:565–71.
484. Sato HK, Sanajotta AT, Moraes JC, et al. Rubella vaccination of unknowingly pregnant women: the São Paulo experience, 2001. *J Infect Dis* 2011;**204**(Suppl. 2):S737–44.
497. Schmid D, Kasper S, Kuo HW, et al. Ongoing rubella outbreak in Austria, 2008-2009. *Euro Surveill* 2009;**14**:19184.
517. Shah I, Bhatnagar S. Antenatal diagnostic problem of congenital rubella. *Indian J Pediatr* 2010;**77**:450–1.
529. Soares RC, Siqueira MM, Toscano CM, et al. Follow-up study of unknowingly pregnant women vaccinated against rubella in Brazil, 2001-2002. *J Infect Dis* 2011;**204**(Suppl. 2):S729–36.
545. Strebel PM, Gacic-Dobo M, Reef S, et al. Global use of rubella vaccines, 1980-2009. *J Infect Dis* 2011;**204**(Suppl. 2):S579–84.
548. Suzuki J, Goto H, Komase K, et al. Rubella virus as a possible etiological agent of Fuchs heterochromic iridocyclitis. *Graefes Arch Clin Exp Ophthalmol* 2010;**248**:1487–91.
583. Valinotto LE, Viegas M, Acevedo ME, et al. Phylogenetic analysis of rubella viruses isolated in 2008 outbreak in Argentina. *J Clin Virol* 2009;**46**:286–9.
623. World Health Organization. Rubella vaccines: WHO position paper. *Wkly Epidemiol Rec* 2011;**86**:301–16.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abernathy E, Cabezas C, Sun H, et al. Confirmation of rubella within 4 days of rash onset: comparison of rubella virus RNA detection in oral fluid with immunoglobulin M detection in serum or oral fluid. *J Clin Microbiol* 2009;**47**:182–8.
2. Abernathy ES, Hubschen JM, Muller CP, et al. Status of global virologic surveillance for rubella viruses. *J Infect Dis* 2011;**204**(Suppl. 1):S524–32.
3. Aboudy Y, Fogel A, Barnea B, et al. Subclinical rubella reinfection during pregnancy followed by transmission of virus to the fetus. *J Infect* 1997;**34**:273–6.
4. Achs R, Harper RG, Siegel M. Unusual dermatoglyphic findings associated with rubella embryopathy. *N Engl J Med* 1966;**274**: 148–50.
5. Aguado JM, Posada I, Gonzalez M, et al. Meningoencephalitis and polyradiculoneuritis in adults: don't forget rubella. *Clin Infect Dis* 1993;**17**:785–6.
6. Ainger LE, Lawyer NG, Fitch CW. Neonatal rubella myocarditis. *Br Heart J* 1966;**28**:691–7.
7. Akingbade D, Cohen BJ, Brown DW. Detection of low-avidity immunoglobulin G in oral fluid samples: new approach for rubella diagnosis and surveillance. *Clin Diagn Lab Immunol* 2003;**10**: 189–90.
8. Al-Nakib W, Best JM, Banatvala JE. Rubella-specific serum and nasopharyngeal immunoglobulin responses following naturally acquired and vaccine-induced infection: prolonged persistence of virus-specific IgM. *Lancet* 1975;**1**:182–5.
9. Alford BR. Rubella-la bete noire de la medecine. *Laryngoscope* 1968;**78**:1623–59.
10. Alford CA Jr. Studies on antibody in congenital rubella infections. I. Physicochemical and immunologic investigations of rubella neutralizing antibody. *Am J Dis Child (1960)* 1965;**110**:455–63.
11. Alford CA Jr, Neva FA, Weller TH. Virologic and serologic studies on human products of conception after maternal rubella. *N Engl J Med* 1964;**271**:1275–81.
12. Alter M, Schulenberg R. Dermatoglyphics in the rubella syndrome. *JAMA* 1966;**197**:685–8.
13. American Academy of Pediatrics. Rubella. In: Pickering LK, editor. *2006 Red book: Report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 574–9.
14. American Academy of Pediatrics. Rubella. In: Pickering LK, editor. *Red book: 2012 Report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 629–34.
15. Ankerst J, Christensen P, Kjellen L, et al. A routine diagnostic test of IgA and IgM antibodies to rubella virus: absorption of IgG with *Staphylococcus aureus*. *J Infect Dis* 1974;**130**:268–73.
16. Ansari BM, Mason MK. Chromosomal abnormality in congenital rubella. *Pediatrics* 1977;**59**:13–15.
17. Appleton PN, Macrae AD. Comparison of radial haemolysis with haemagglutination inhibition in estimating rubella antibody. *J Clin Pathol* 1978;**31**:479–82.
18. Atkinson IE. Rubella (Rötheln). *Am J Med Sci* 1887;**93**:17–34.
19. Austin SM, Altman R, Barnes EK, et al. Joint reactions in children vaccinated against rubella: Study I. Comparison of two vaccines. *Am J Epidemiol* 1972;**95**:53–8.
20. Avery GB, Monif GG, Sever JL, et al. Rubella syndrome after inapparent maternal illness. *Am J Dis Child* 1965;**110**: 444–6.
21. Badilla X, Morice A, Avila-Aguero ML, et al. Fetal risk associated with rubella vaccination during pregnancy. *Pediatr Infect Dis J* 2007;**26**:830–5.
22. Bailey G. Carpal-tunnel syndrome. *Br Med J* 1962;**1**:1207.
23. Balfour HH Jr, Balfour CL, Edelman CK, et al. Evaluation of Wistar RA27/3 rubella virus vaccine in children. *Am J Dis Child* 1976;**130**:1089–91.
24. Balfour HH Jr, May DB, Rotte TC, et al. A study of erythema infectiosum: recovery of rubella virus and echovirus-12. *Pediatrics* 1972;**50**:285–90.
25. Banatvala JE, Best JM, Bertrand J, et al. Serological assessment of rubella during pregnancy. *Br Med J* 1970;**3**:247–50.
26. Banerji A, Ford-Jones EL, Kelly E, et al. Congenital rubella syndrome despite maternal antibodies. *CMAJ* 2005;**172**:1678–9.
27. Bardeletti G, Kessler N, Aymard-Henry M. Morphology, biochemical analysis and neuraminidase activity of rubella virus. *Arch Virol* 1975;**49**:175–86.
28. Barenberg LH, Levy W, Greenstein NM, et al. Prophylactic use of human serum against contagion in a pediatric ward: further observations, with special reference to measles and rubella. *Arch Pediatr Adolesc Med* 1942;**63**:1101–9.
29. Barfield W, Gardner R, Lett S, et al. Congenital rubella reinfection in a mother with anti-cardiolipin and anti-platelet antibodies. *Pediatr Infect Dis J* 1997;**16**:249–51.
30. Barnes EK, Altman R, Austin SM, et al. Joint reactions in children vaccinated against rubella: Study II. Comparison of two vaccines. *Am J Epidemiol* 1972;**95**:59–66.
31. Barraclough WW. German measles encephalomyelitis. *Can Med Assoc J* 1937;**36**:511–13.
32. Baublis JV, Brown GC. Specific response of the immunoglobulins to rubella infection. *Proc Soc Exp Biol Med* 1968;**128**:206–10.
33. Bayer WL, Sherman FE, Michaels RH, et al. Purpura in congenital and acquired rubella. *N Engl J Med* 1965;**273**:1362–6.
34. Baykan A, Akcakus M, Deniz K. Rubella-associated hemophagocytic syndrome in an infant. *J Pediatr Hematol Oncol* 2005;**27**: 430–1.
35. Bellamy K, Rousseau SA, Gardner PS. The development of an M antibody capture ELISA for rubella IgM. *J Virol Meth* 1986;**14**: 243–51.
36. Bellanti JA, Artenstein MS, Olson LC, et al. Congenital rubella: clinicopathologic, virologic, and immunologic studies. *Am J Dis Child* 1965;**110**:464–72.
37. Bellin E, Safyer S, Braslow C. False positive IgM-rubella enzyme-linked immunoassay in three first trimester pregnant patients. *Pediatr Infect Dis J* 1990;**9**:671–2.
38. Bennett RA, Copeman WS. Notes on rubella. with special reference to certain rheumatic sequelae. *Br Med J* 1940;**1**: 924–6.
39. Berge T, Brunnhage F, Nilsson LR. Congenital hypoplastic thrombocytopenia in rubella embryopathy. *Acta Paediatr* 1963;**52**: 349–52.
40. Berger BE, Omer SB. Could the United States experience rubella outbreaks as a result of vaccine refusal and disease importation? *Hum Vaccin* 2010;**6**:1016–20.
41. Best JM, Banatvala JE, Bowen JM. New Japanese rubella vaccine: comparative trials. *Br Med J* 1974;**3**:221–4.
42. Best JM, Banatvala JE, Watson D. Serum IgM and IgG responses in postnatally acquired rubella. *Lancet* 1969;**2**:65–8.
43. Birch CJ, Glaun BP, Hunt V, et al. Comparison of passive haemagglutination and haemagglutination-inhibition techniques for detection of antibodies to rubella virus. *J Clin Pathol* 1979;**32**: 128–31.
44. Black FL, Lamm SH, Emmons JE, et al. Reactions to rubella vaccine and persistence of antibody in virgin-soil populations after vaccination and wild-virus-induced immunization. *J Infect Dis* 1976;**133**:393–8.
45. Black FL, Lamm SH, Emmons JE, et al. Durability of antibody titers induced by RA27/3 rubella virus vaccine. *J Infect Dis* 1978;**137**:322–3.
46. Blennow G, Bekassy AN, Eriksson M, et al. Transient carpal tunnel syndrome accompanying rubella infection. *Acta Paediatr Scand* 1982;**71**:1025–8.
47. Bolognese RJ, Corson SL, Fuccillo DA, et al. Evaluation of possible transplacental infection with rubella vaccination during pregnancy. *Am J Obstet Gynecol* 1973;**117**:939–41.
48. Boner A, Wilmott RW, Dinwiddie R, et al. Desquamative interstitial pneumonia and antigen-antibody complexes in two infants with congenital rubella. *Pediatrics* 1983;**72**:835–9.
49. Boniuk M, Zimmerman LE. Ocular pathology in the rubella syndrome. *Arch Ophthalmol* 1967;**77**:455–73.
50. Booth JC, Stern H. Photodynamic inactivation of rubella virus. *J Med Microbiol* 1972;**5**:515–28.
51. Borton TE, Stark EW. Audiological findings in hearing loss secondary to maternal rubella. *Pediatrics* 1970;**45**:225–9.
52. Bosma TJ, Etherington J, O'Shea S, et al. Rubella virus and chronic joint disease: is there an association? *J Clin Microbiol* 1998;**36**:3524–6.
53. Bottiger M, Forsgren M. Twenty years' experience of rubella vaccination in Sweden: 10 years of selective vaccination (of

12-year-old girls and of women postpartum) and 13 years of a general two-dose vaccination. *Vaccine* 1997;**15**:1538–44.
54. Bradford RI. Rubella Meningo-encephalitis. *Br Med J* 1943;**1**: 312–13.
55. Britt WJ. Slow viruses. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*, vol. 2. 4th ed. Philadelphia: W. B. Saunders; 1998. p. 1646–65.
56. Brody JA, Sever JL, Schiff GM. Prevention of rubella by gamma globulin during an epidemic in Barrow, Alaska, in 1964. *N Engl J Med* 1965;**272**:127–9.
57. Brody JA, Sever JL, McAlister R, et al. Rubella epidemic on St. Paul Island in the Pribilofs, 1963. I. Epidemiologic, clinical, and serologic findings. *JAMA* 1965;**191**:619–23.
58. Brookhouser PE, Bordley JE. Congenital rubella deafness: pathology and pathogenesis. *Arch Otolaryngol* 1973;**98**:252–7.
59. Buescher EL. Behavior of Rubella Virus in Adult Populations. *Arch Gesamte Virusforsch* 1965;**16**:470–6.
60. Buimovici-Klein E, Cooper LZ. Immunosuppression and isolation of rubella virus from human lymphocytes after vaccination with two rubella vaccines. *Infect Immun* 1979;**25**:352–6.
61. Buimovici-Klein E, Weiss KE, Cooper LZ. Interferon production in lymphocyte cultures after rubella infection in humans. *J Infect Dis* 1977;**135**:380–5.
62. Buimovici-Klein E, Hite RL, Byrne T, et al. Isolation of rubella virus in milk after postpartum immunization. *J Pediatr* 1977; **91**:939–41.
63. Buimovici Klein E, Lang PB, Ziring PR, et al. Impaired cell mediated immune response in patients with congenital rubella: correlation with gestational age at time of infection. *Pediatrics* 1979;**64**: 620–6.
64. Bullens D, Smets K, Vanhaesebrouck P. Congenital rubella syndrome after maternal reinfection. *Clin Pediatr (Phila)* 2000;**39**: 113–16.
65. Bunnell CE, Monif GR. Interstitial pancreatitis in the congenital rubella syndrome. *J Pediatr* 1972;**80**:465–6.
66. Caidi H, Bloom S, Azilmaat M, et al. Rubella seroprevalence among women aged 15-39 years in Morocco. *East Mediterr Health J* 2009;**15**:526–31.
67. Capasso M, Di Muzio A, Caporale CM, et al. Demyelinating motor Guillain-Barré syndrome following rubella. *Neurology* 2005;**64**:390.
68. Cappel R. Cell mediated immunity in experimental rubella infections. *Arch Virol* 1975;**47**:375–9.
69. Cappel R, Schluederberg A, Horstmann DM. Large scale production of rubella precipitinogens and their use in the diagnostic laboratory. *J Clin Microbiol* 1975;**1**:201–5.
70. Castillo-Solorzano C, Marsigli C, Bravo-Alcantara P, et al. Elimination of rubella and congenital rubella syndrome in the Americas. *J Infect Dis* 2011;**204**(Suppl. 2):S571–8.
71. Castillo-Solorzano C, Reef SE, Morice A, et al. Rubella vaccination of unknowingly pregnant women during mass campaigns for rubella and congenital rubella syndrome elimination, the Americas 2001-2008. *J Infect Dis* 2011;**204**(Suppl. 2:)S713–17.
72. Castrow FF, 2nd, De Beukelaer M. Congenital rubella syndrome: unusual cutaneous manifestations. *Arch Dermatol* 1968;**98**:260–2.
73. Catalano LW Jr, Fuccillo DA, Traub RG, et al. Isolation of rubella virus from placentas and throat cultures of infants: a prospective study after the 1964-65 epidemic. *Obstet Gynecol* 1971;**38**:6–14.
74. Caul EO, Smyth GW, Clarke SK. A simplified method for the detection of rubella-specific IgM employing sucrose density fractionation and 2-mercaptoethanol. *J Hyg (Lond)* 1974;**73**: 329–40.
75. Centers for Disease Control and Prevention. Rubella in hospitals: California. *MMWR Morb Mortal Wkly Rep* 1983;**32**:37–9.
76. Centers for Disease Control and Prevention. Rubella in colleges: United States, 1983-1984. *MMWR Morb Mortal Wkly Rep* 1985; **34**:228–31.
77. Centers for Disease Control and Prevention. Rubella outbreak among office workers: New York City. *MMWR Morb Mortal Wkly Rep* 1985;**34**:455–9.
78. Centers for Disease Control and Prevention. Rubella outbreaks in prisons: New York City, West Virginia, California. *M M W R Morb Mortal Wkly Rep* 1985;**34**:615–18.
79. Centers for Disease Control and Prevention. Rubella and congenital rubella syndrome: United States, 1985-1988. *M M W R Morb Mortal Wkly Rep* 1989;**38**:173–8.
80. Centers for Disease Control. Rubella prevention: recommendations of the Immunization Practices Advisory Committee (ACIP). *M M W R Recomm Rep* 1990;**39**:1–18.
81. Centers for Disease Control and Prevention. Rubella in colleges: United States, 1983-1984. *MMWR Morb Mortal Wkly Rep* 1985: 228–31.
82. Centers for Disease Control and Prevention. Increase in rubella and congenital rubella syndrome: United States, 1988-1990. *M M W R Morb Mortal Wkly Rep* 1991;**40**:93–9.
83. Centers for Disease Control. Outbreaks of rubella among the Amish-United States, 1991. *M M W R Morb Mortal Wkly Rep* 1991;**40**:264–5.
84. Centers for Disease Control and Prevention. Rubella and congenital rubella syndrome: United States, 1994-1997. *M M W R Morb Mortal Wkly Rep* 1997;**46**:350–4.
85. Centers for Disease Control and Prevention. Control and prevention of rubella: evaluation and management of suspected outbreaks, rubella in pregnant women, and surveillance for congenital rubella syndrome. *M M W R Recomm Rep* 2001;**50**:1–23.
86. Centers for Disease Control and Prevention. Elimination of rubella and congenital rubella syndrome: United States, 1969-2004. *M M W R Morb Mortal Wkly Rep* 2005;**54**:279–82.
87. Centers for Disease Control and Prevention. Summary of notifiable disease: United States, 2005. *M M W R Morb Mortal Wkly Rep* 2005;**54**:77.
88. Centers for Disease Control and Prevention. Progress toward elimination of rubella and congenital rubella syndrome: the Americas, 2003-2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**: 1176–9.
89. Centers for Disease Control and Prevention. *Epidemiology and prevention of vaccine-preventable diseases*. Chapter 19 Rubella In: Atkinson W, Wolfe S, Hamborsky J, editors. 12th ed. Washington DC: Public Health Foundation; 2012.
90. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
91. Chagnon A, Laflamme P. Effect of acidity on rubella virus. *Can J Microbiol* 1964;**10**:501–2.
92. Chambers RJ, Bywaters EG. Rubella synovitis. *Ann Rheum Dis* 1963;**22**:263–8.
93. Champsaur H, Fattal-German M, Arranhado R. Sensitivity and specificity of viral immunoglobulin M determination by indirect enzyme-linked immunosorbent assay. *J Clin Microbiol* 1988;**26**: 328–32.
94. Chantler J, Wolinsky JS, Tingle A. Rubella virus. In: Knipe DM, Howley PM, editors. *Fields' virology*, Vol. 1. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 963–90.
95. Chaye H, Ou D, Chong P, et al. Human T- and B-cell epitopes of E1 glycoprotein of rubella virus. *J Clin Immunol* 1993;**13**: 93–100.
96. Cheong HK, Yang BG, Sohn YM, et al. A fatal case of disseminated tuberculosis coincident with measles-rubella vaccination. *Epidemiol Health* 2010;**32**:e2010002.
97. Chernesky MA, DeLong DJ, Mahony JB, et al. Differences in antibody responses with rapid agglutination tests for the detection of rubella antibodies. *J Clin Microbiol* 1986;**23**:772–6.
98. Chernesky MA, Wyman L, Mahony JB, et al. Clinical evaluation of the sensitivity and specificity of a commercially available enzyme immunoassay for detection of rubella virus-specific immunoglobulin M. *J Clin Microbiol* 1984;**20**:400–4.
99. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.
100. Cherry JD. Peripheral pain syndromes following rubella immunization. *J Pediatr* 1972;**80**:541–2.
101. Cherry JD. Rubella: past, present, and future. *Volta Rev* 1974;**76**: 461–5.
102. Cherry JD, Bobinski JE, Comerci GD. A clinical trial with live attenuated rubella virus vaccine (Cendehill 51 strain). *J Pediatr* 1969;**75**:79–86.
103. Cherry JD, Soriano F, Jahn CL. Search for perinatal viral infection: a prospective, clinical, virologic, and serologic study. *Am J Dis Child* 1968;**116**:245–50.
104. Cherry JD, Horvath FL, Comerci GD, et al. Clinical trials with Cendehill strain attenuated rubella virus vaccine. *Antimicrob Agents Chemother* 1969;**9**:357–63.
105. Chin J, Werner SB. Neuritis and arthritis following rubella immunization. *JAMA* 1971;**215**:485–6.

106. Chiriboga-Klein S, Oberfield SE, Casullo AM, et al. Growth in congenital rubella syndrome and correlation with clinical manifestations. *J Pediatr* 1989;**115**:251–5.
107. Cifarelli PS, Freireich AW. Rubella encephalitis. *N Y State J Med* 1966;**66**:1117–22.
108. Claman HN, Suvatte V, Githens JH, et al. Histiocytic reaction in dysgammaglobulinemia and congenital rubella. *Pediatrics* 1970; **46**:89–96.
109. Clarke M, Schild GC, Boustred J, et al. Effect of rubella vaccination programme on serological status of young adults in United Kingdom. *Lancet* 1979;**1**:1224–6.
110. Clarke WL, Shaver KA, Bright GM, et al. Autoimmunity in congenital rubella syndrome. *J Pediatr* 1984;**104**:370–3.
111. Cockburn WC. World aspects of the epidemiology of rubella. *Am J Dis Child* 1969;**118**:112–22.
112. Cohen D, Muhsen K, Aboudy Y, et al. Use of rubella seroepidemiological data for assessment of previous vaccination policy and for decision making in response to epidemics in Israel. *Vaccine* 2006;**24**:5604–8.
113. Collis WJ, Cohen DN. Rubella retinopathy: a progressive disorder. *Arch Ophthalmol* 1970;**84**:33–5.
114. Cong H, Jiang Y, Tien P. Identification of the myelin oligodendrocyte glycoprotein as a cellular receptor for rubella virus. *J Virol* 2011;**85**:11038–47.
115. Connolly JH, Hutchinson WM, Allen IV, et al. Carotid artery thrombosis, encephalitis, myelitis and optic neuritis associated with rubella virus infections. *Brain* 1975;**98**:583–94.
116. Cooper LZ. Congenital rubella in the U.S. *Prog Clin Biol Res* 1975;**3**:1–22.
117. Cooper LZ, Krugman S. Diagnosis and management: congenital rubella. *Pediatrics* 1966;**37**:335–8.
118. Cooper LZ, Krugman S. Clinical manifestations of postnatal and congenital rubella. *Arch Ophthalmol* 1967;**77**:434–9.
119. Cooper LZ, Florman AL, Ziring PR, et al. Loss of rubella hemagglutination inhibition antibody in congenital rubella: failure of seronegative children with congenital rubella to respond to HPV-77 rubella vaccine. *Am J Dis Child* 1971;**122**:397–403.
120. Cooper LZ, Preblud SR, Alford CA Jr. Rubella. In: Remington JS, Klein JO editors. *Infectious diseases of the fetus and newborn infants*. Philadelphia: W. B. Saunders; 1995. p. 268–311.
121. Cooper LZ, Ziring PR, Ockerse AB, et al. Rubella: clinical manifestations and management. *Am J Dis Child* 1969;**118**:18–29.
122. Cooper LZ, Ziring PR, Weiss HJ, et al. Transient arthritis after rubella vaccination. *Am J Dis Child* 1969;**118**:218–25.
123. Cooray S, Warrener L, Jin L. Improved RT-PCR for diagnosis and epidemiological surveillance of rubella. *J Clin Virol* 2006; **35**:73–80.
124. Corcoran C, Hardie DR. Serologic diagnosis of congenital rubella: a cautionary tale. *Pediatr Infect Dis J* 2005;**24**:286–7.
125. Corlett WT. *A Treatise on the acute infectious exanthemata, including rubeola, rubella, scarlatina, varicella and vaccinia, with especial reference to diagnosis and treatment*. Philadelphia: F. A. Davis; 1902. p. 348–71.
126. Cradock-Watson JE, Bourne MS, Vandervelde EM. IgG, IgA and IgM responses in acute rubella determined by the immunofluorescent technique. *J Hyg (Lond)* 1972;**70**:473–85.
127. Cradock-Watson JE, Ridehalgh MK. Specific immunoglobulins in infants with the congenital rubella syndrome. *J Hyg (Lond)* 1976;**76**:109–23.
128. Cradock Watson JE, Macdonald H, Ridehalgh MKS. Specific immunoglobulin responses in serum and nasal secretions after the administration of attenuated rubella vaccine. *J Hyg (Lond)* 1974;**73**:127–41.
129. Cradock-Watson JE, Ridehalgh MK, Anderson MJ, et al. Fetal infection resulting from maternal rubella after the first trimester of pregnancy. *J Hyg (Lond)* 1980;**85**:381–91.
130. Cradock-Watson JE, Ridehalgh MK, Bourne MS, et al. Nasal immunoglobulin responses in acute rubella determined by the immunofluorescent technique. *J Hyg (Lond)* 1973;**71**:603–17.
131. Cuetter AC, John JF Jr. Nerve conduction studies in natural rubella. *Ann Neurol* 1977;**1**:199–200.
132. Cunningham AL, Fraser JR. Persistent rubella virus infection of human synovial cells cultured in vitro. *J Infect Dis* 1985;**151**: 638–45.
133. D'Agaro P, Dal Molin G, Zamparo E, et al. Epidemiological and molecular assessment of a rubella outbreak in North-Eastern Italy. *J Med Virol* 2010;**82**:1976–82.
134. da Silva e Sa GR, Camacho LA, Stavola MS, et al. Pregnancy outcomes following rubella vaccination: a prospective study in the state of Rio de Janeiro, Brazil, 2001-2002. *J Infect Dis* 2011; **204**(Suppl. 2):S722–8.
135. Dales LG, Chin J. Public health implications of rubella antibody levels in California. *Am J Public Health* 1982;**72**:167–72.
136. Dammeyer J. Congenital rubella syndrome and delayed manifestations. *Int J Pediatr Otorhinolaryngol* 2010;**74**:1067–70.
137. Das BD, Lakhani P, Kurtz JB, et al. Congenital rubella after previous maternal immunity. *Arch Dis Child* 1990;**65**:545–6.
138. Davison C, Friedfeld L. Acute encephalomyelitis following German measles. *Arch Pediatr Adolesc Med* 1938;**55**:496–510.
139. Dayan GH, Panero MS, Urquiza A, et al. Rubella and measles seroprevalence among women of childbearing age, Argentina, 2002. *Epidemiol Infect* 2005;**133**:861–9.
140. De Mazancourt A, Waxham MN, Nicolas JC, et al. Antibody response to the rubella virus structural proteins in infants with the congenital rubella syndrome. *J Med Virol* 1986;**19**:111–22.
141. de Visser L, Braakenburg A, Rothova A, et al. Rubella virus-associated uveitis: clinical manifestations and visual prognosis. *Am J Ophthalmol* 2008;**146**:292–7.
142. Deinard AS, Venters HD, Bilka PJ, et al. Rubella-antibody titres in rheumatoid arthritis. *Lancet* 1974;**1**:526–8.
143. Desmond MM, Fisher ES, Vorderman AL, et al. The longitudinal course of congenital rubella encephalitis in nonretarded children. *J Pediatr* 1978;**93**:584–91.
144. Desmond MM, Wilson GS, Melnick JL, et al. Congenital rubella encephalitis: course and early sequelae. *J Pediatr* 1967;**71**:311–31.
145. Desmyter J, Rawls WE, Melnick JL, et al. Interferon in congenital rubella: response to live attenuated measles vaccine. *J Immunol* 1967;**99**:771–7.
146. Diamanti A, Pietrobattista A, Bevivino E, et al. Duodenal stenosis, a new finding on congenital rubella syndrome: case description and literature review. *J Infect* 2006;**53**:e207–210.
147. Dibbert HJ. [Diagnosis of rubella by demonstrating rubella-specific 19 S and 7 S antibodies (author's transl)]. *Zentralbl Bakteriol Orig A* 1976;**234**:145–58.
148. Dimech W, Panagiotopoulos L, Marler J, et al. Evaluation of three immunoassays used for detection of anti-rubella virus immunoglobulin M antibodies. *Clin Diagn Lab Immunol* 2005;**12**:1104–8.
149. Division of Maternal and Perinatal Studies, Department of Health, Sydney. Report on pregnancy complicated by wild rubella: spring, 1971. *Med J Aust* 1973;**2**:545–7.
150. Doege TC, Kim KS. Studies of rubella and its prevention with immune globulin. *JAMA* 1967;**200**:584–90.
151. Dominguez A, Plans P, Costa J, et al. Seroprevalence of measles, rubella, and mumps antibodies in Catalonia, Spain: results of a cross-sectional study. *Eur J Clin Microbiol Infect Dis* 2006;**25**: 310–17.
152. Donowitz M, Gryboski JD. Pancreatic insufficiency and congenital rubella syndrome. *J Pediatr* 1975;**87**:241–3.
153. Dorfman SF, Bowers CH Jr. Rubella susceptibility among prenatal and family planning clinic populations. *Mount Sinai Journal of Medicine* 1985;**52**:248–52.
154. Doshi S, Khetsuriani N, Zakhashvili K, et al. Ongoing measles and rubella transmission in Georgia, 2004-05: implications for the national and regional elimination efforts. *Int J Epidemiol* 2009;**38**:182–91.
155. Driscoll SG. Histopathology of gestational rubella. *Am J Dis Child* 1969;**118**:49–53.
156. Dudgeon JA. Congenital rubella. Pathogenesis and immunology. *Am J Dis Child* 1969;**118**:35–44.
157. Dudgeon JA, Marshall WC, Peckham CS. Rubella vaccine trials in adults and children: comparison of three attenuated vaccines. *Am J Dis Child* 1969;**118**:237–43.
158. Duszak RS. Congenital rubella syndrome: major review. *Optometry* 2009;**80**:36–43.
159. Dwyer DE, Hueston L, Field PR, et al. Acute encephalitis complicating rubella virus infection. *Pediatr Infect Dis J* 1992;**11**: 238–40.
160. Dykewicz CA, Kruszon-Moran D, McQuillan GM, et al. Rubella seropositivity in the United States, 1988-1994. *Clin Infect Dis* 2001;**33**:1279–86.

161. Ebbin AJ, Wilson MG, Chandor SB, et al. Inadvertent rubella immunization in pregnancy. *Am J Obstet Gynecol* 1973;**117**: 505–12.
162. Enders G, Knotek F. Detection of IgM antibodies against rubella virus: comparison of two indirect ELISAs and an anti-IgM capture immunoassay. *J Med Virol* 1986;**19**:377–86.
163. Enders G, Knotek F, Pacher U. Comparison of various serological methods and diagnostic kits for the detection of acute, recent, and previous rubella infection, vaccination, and congenital infections. *J Med Virol* 1985;**16**:219–32.
164. Erickson CA. Rubella early in pregnancy causing congenital malformations of eyes and heart. *J Pediatr* 1944;**25**:281–3.
165. Esterly JR, Oppenheimer EH. Vascular lesions in infants with congenital rubella. *Circulation* 1967;**36**:544–54.
166. Esterly JR, Oppenheimer EH. Pathological lesions due to congenital rubella. *Arch Pathol* 1969;**87**:380–8.
167. Esterly JR, Oppenheimer EH. The pathologic manifestations of intrauterine rubella infection. *Arch Otolaryngol* 1973;**98**: 246–8.
168. Esterly JR, Slusser RJ, Ruebner BH. Hepatic lesions in the congenital rubella syndrome. *J Pediatr* 1967;**71**:676–85.
169. Evans A, Cox F, Nankervis G, et al. A health and seroepidemiological survey of a community in Barbados. *Int J Epidemiol* 1974; **3**:167–75.
170. Fabiyi A, Sever JL, Ratner N, et al. Rubella virus: growth characteristics and stability of infectious virus and complement-fixing antigen. *Proc Soc Exp Biol Med* 1966;**122**:392–6.
171. Falkensammer B, Walder G, Busch D, et al. Epidemiology of rubella infections in Austria: Important lessons to be learned. *Eur J Clin Microbiol Infect Dis* 2004;**23**:502–5.
172. Featherstone DA, Rota PA, Icenogle J, et al. Expansion of the global measles and rubella laboratory network 2005-09. *J Infect Dis* 2011;**204**(Suppl. 1):S491–8.
173. Feldman RB, Pinsky L, Mendelson J, et al. Can language disorder not due to peripheral deafness be an isolated expression of prenatal rubella? *Pediatrics* 1973;**52**:296–9.
174. Fenner F. Classification and nomenclature of viruses. Second report of the International Committee on Taxonomy of Viruses. *Intervirology* 1976;**7**:1–115.
175. Ferraro MJ, Kallas WM, Welch KP, et al. Comparison of a new, rapid enzyme immunoassay with a latex agglutination test for qualitative detection of rubella antibodies. *J Clin Microbiol* 1987;**25**:1722–4.
176. Field PR, Gong CM. Diagnosis of postnatally acquired rubella by use of three enzyme-linked immunosorbent assays for specific immunoglobulins G and M and single radial hemolysis for specific immunoglobulin G. *J Clin Microbiol* 1984;**20**:951–8.
177. Figueiredo CA, Cordovani NT, Castrignano SB, et al. Acute liver failure associated with rubella virus in a child. *Pediatr Infect Dis J* 2010;**29**:573–4.
178. Figueiredo CA, Klautau GB, Afonso AM, et al. Isolation and genotype analysis of rubella virus from a case of Guillain-Barré syndrome. *J Clin Virol* 2008;**43**:343–5.
179. Figueiredo CA, Oliveira MI, Afonso AM, et al. Rubella encephalitis in a young adult male: isolation and genotype analysis. *Infection* 2011;**39**:73–5.
180. Finklea JF, Sandifer SH, Moore GT. Epidemic rubella at the Citadel. *Am J Epidemiol* 1968;**87**:367–72.
181. Fleet WF Jr, Benz EW Jr, Karzon DT, et al. Fetal consequences of maternal rubella immunization. *JAMA* 1974;**227**:621–7.
182. Fogel A, Gerichter CB, Rannon L, et al. Serologic studies in 11,460 pregnant women during the 1972 rubella epidemic in Israel. *Am J Epidemiol* 1976;**103**:51–9.
183. Forbes JA. Rubella: historical aspects. *Am J Dis Child* 1969; **118**:5–11.
184. Forchheimer P. The enanthem of German measles. *Am J Med Sci* 1898;**116**.
185. Ford DK, Reid GD, Tingle AJ, et al. Sequential follow up observations of a patient with rubella associated persistent arthritis. *Ann Rheum Dis* 1992;**51**:407–10.
186. Forghani B, Schmidt NJ, Lennette EH. Demonstration of rubella IgM antibody by indirect fluorescent antibody staining, sucrose density gradient centrifugation and mercaptoethanol reduction. *Intervirology* 1973;**1**:48–59.
187. Forrest JM, Burgess M, Donovan T. A resurgence of congenital rubella in Australia? *Commun Dis Intell* 2003;**27**:533–6.
188. Forrest JM, Menser MA, Harley JD. Diabetes mellitus and congenital rubella. *Pediatrics* 1969;**44**:445–7.
189. Fortuin NJ, Morrow AG, Roberts WC. Late vascular manifestations of the rubella syndrome: a roentgenographic-pathologic study. *Am J Med* 1971;**51**:134–40.
190. France EK, Glanz J, Xu S, et al. Risk of immune thrombocytopenic purpura after measles-mumps-rubella immunization in children. *Pediatrics* 2008;**121**:e687–92.
191. Franco SA, Riley HD Jr, Chitwood LA. The congenital rubella syndrome. *South Med J* 1970;**63**:825–30.
192. Fraser V, Spitznagel E, Medoff G, et al. Results of a rubella screening program for hospital employees: a five-year review (1986-1990). *Am J Epidemiol* 1993;**138**:756–64.
193. Frenkel LM, Nielsen K, Garakian A, et al. A search for persistent measles, mumps, and rubella vaccine virus in children with human immunodeficiency virus type 1 infection. *Arch Pediatr Adolesc Med* 1994;**148**:57–60.
194. Frenkel LM, Nielsen K, Garakian A, et al. A search for persistent rubella virus infection in persons with chronic symptoms after rubella and rubella immunization and in patients with juvenile rheumatoid arthritis. *Clin Infect Dis* 1996;**22**:287–94.
195. Frey TK, Abernathy ES, Bosma TJ, et al. Molecular analysis of rubella virus epidemiology across three continents, North America, Europe, and Asia, 1961-1997. *J Infect Dis* 1998;**178**: 642–50.
196. Friedmann I. Cochlear pathology in viral disease. *Adv Otorhinolaryngol* 1973;**20**:155–77.
197. Friedmann I, Wright MI. Histopathological changes in the foetal and infantile inner ear caused by maternal rubella. *Br Med J* 1966;**2**:20–3.
198. Fruehan AE. Erythema multiforme exudativum and arthritis following infection with rubella. *N Y State J Med* 1963;**63**: 859–63.
199. Fry J, Dillane JB, Fry L. Rubella, 1962. *Br Med J* 1962;**2**:833–4.
200. Fuccillo DA, Steele RW, Hensen SA. Impaired cellular immunity to rubella virus in congenital rubella. *Infect Immun* 1974;**9**:81–4.
201. Fujimoto T, Katoh C, Hayakawa H, et al. Two cases of rubella infection with cardiac involvement. *Jpn Heart J* 1979;**20**:227–35.
202. Furukawa T, Plotkin SA, Sedwick WD, et al. Studies on hemagglutination by rubella virus. *Proc Soc Exp Biol Med* 1967; **126**:745–50.
203. Gale EA. Congenital rubella: citation virus or viral cause of type 1 diabetes? *Diabetologia* 2008;**51**:1559–66.
204. Gale JL, Detels R, Kim KSW, et al. The epidemiology of rubella on Taiwan III, family studies in cities of high and low attack rates. *Int J Epidemiol* 1972;**1**:261–5.
205. Gale JL, Grayston JT, Beasley RP, et al. The epidemiology of rubella on Taiwan II. 1968-1969 epidemic. *Int J Epidemiol* 1972;**1**:253–60.
206. Ganguly R, Cusumano CL, Waldman RH. Suppression of cell-mediated immunity after infection with attenuated rubella virus. *Infect Immun* 1976;**13**:464–9.
207. Garcia L. Outbreak of rubella in the Madrid region, Spain, 2005. *Euro Surveill* 2005;**10**:E050707 050702.
208. Geiger JC. Epidemic of German measles in a city adjacent to an army cantonment, and its probable relation thereto. *JAMA* 1918;**70**:1818–20.
209. Geiger R, Fink FM, Solder B, et al. Persistent rubella infection after erroneous vaccination in an immunocompromised patient with acute lymphoblastic leukemia in remission. *J Med Virol* 1995;**47**:442–4.
210. Geltzer AI, Guber D, Sears ML. Ocular manifestations of the 1964-65 rubella epidemic. *Am J Ophthalmol* 1967;**63**:221–9.
211. Gibbs FA, Gibbs EL, Carpenter PR, et al. Electroencephalographic abnormality in "uncomplicated" childhood diseases. *JAMA* 1959;**171**:1050–5.
212. Giessauf A, Letschka T, Walder G, et al. A synthetic peptide ELISA for the screening of rubella virus neutralizing antibodies in order to ascertain immunity. *J Immunol Methods* 2004;**287**: 1–11.
213. Gilmartin RC Jr, Jabbour JT, Duenas DA. Rubella vaccine myeloradiculoneuritis. *J Pediatr* 1972;**80**:406–12.
214. Ginsberg-Fellner F, Witt ME, Fedun B, et al. Diabetes mellitus and autoimmunity in patients with the congenital rubella syndrome. *Rev Infect Dis* 1985;**7**(Suppl. 1):S170–6.

215. Gispen R, Nagel J, Brand-Saathof B, et al. Immunofluorescence test for IgM rubella antibodies in whole serum after absorption with anti-gammaFc. *Clin Exp Immunol* 1975;**22**:431–7.
216. Giuliani G, Angela GC, Baglione L, et al. German measles: acute benign viral lymphoreticulosis: the haemato-humoral and histological aspects of rubella. *Panminerva Med* 1960;**2**:585–602.
217. Givens KT, Lee DA, Jones T, et al. Congenital rubella syndrome: ophthalmic manifestations and associated systemic disorders. *Br J Ophthalmol* 1993;**77**:358–63.
218. Goldwater PN, Quiney JR, Banatvala JE. Maternal rubella at St. Thomas' Hospital: is there a need to change British vaccination policy? *Lancet* 1978;**2**:1298–300.
219. Goodman AK, Friedman SM, Beatrice ST, et al. Rubella in the workplace: the need for employee immunization. *Am J Public Health* 1987;**77**:725–6.
220. Gotsman MS, Van der Horst RL. Left ventricular aneurysm in rubella heart disease. *Am J Dis Child* 1970;**120**:248–51.
221. Grahame R, Armstrong R, Simmons N, et al. Chronic arthritis associated with the presence of intrasynovial rubella virus. *Ann Rheum Dis* 1983;**42**:2–13.
222. Granberg C, Meurman O. Performance of two new enzyme immunoassays for the detection of IgM and IgG antibodies to rubella. *Eur J Clin Microbiol Infect Dis* 1994;**13**:512–15.
223. Grand MG, Wyll SA, Gehlbach SH, et al. Clinical reactions following rubella vaccination: a prospective analysis of joint, muscular, and neuritic symptoms. *JAMA* 1972;**220**:1569–72.
224. Grayston JT, Gale JL, Watten RH. The epidemiology of rubella on Taiwan. I. Introduction and description of the 1957-1958 epidemic. *Int J Epidemiol* 1972;**1**:245–52.
225. Grayston JT, Peng JY, Lee GCY. Congenital abnormalities following gestational rubella in Chinese: report of a prospective study including five-year follow-up examinations after the 1957-1958 rubella epidemic on Taiwan (Formosa). *JAMA* 1967;**202**:1–6.
226. Green RH, Balsamo MR, Giles JP, et al. Experimental studies with rubella: evaluation of gamma globulin for prophylaxis. *Arch Gesamte Virusforsch* 1965;**16**:513–16.
227. Green RH, Balsamo MR, Giles JP, et al. Studies of the natural history and prevention of rubella. *Am J Dis Child* 1965;**110**: 348–65.
228. Gregg NM. Congenital cataract following German measles in the mother. *Trans Ophthalmol Soc Aust* 1941;**3**:35–46.
229. Griffith JPC. Rubella (Rötheln: German measles): with a report of one hundred and fifty cases. *Med Rec* 1887;**32**:11–41.
230. Grillner L, Hedstrom CE, Bergstrom H, et al. Vaccination against rubella of newly delivered women. *Scand J Infect Dis* 1973;**5**: 237–41.
231. Gross PA, Portnoy B, Mathies AW Jr, et al. A rubella outbreak among adolescent boys. *Am J Dis Child* 1970;**119**:326–31.
232. Guggenheimer J, Nowak AJ, Michaels RH. Dental manifestations of the rubella syndrome. *Oral Surg Oral Med Oral Pathol* 1971; **32**:30–7.
233. Guler E, Davutoglu M, Guler S, et al. Encephalitis in a child during atypical course of rubella. *Infection* 2009;**37**:65–6.
234. Gumpel SM, Hayes K, Dudgeon JA. Congenital perceptive deafness: role of intrauterine rubella. *Br Med J* 1971;**2**:300–4.
235. Gunn W. A case of rubella complicated by purpura haemorrhagica. *Br J Child Dis* 1933;**30**:111–17.
236. Gupta JD, Peterson VJ, Murphy AM. Differential immune response to attenuated rubella virus vaccine. *Infect Immun* 1972;**5**:151–4.
237. Habel K. Transmission of rubella to *Macaca mulatta* monkeys. *Public Health Rep* 1942;**57**:1126–39.
238. Hahné S, Macey J, van Binnendijk R, et al. Rubella outbreak in the Netherlands, 2004-2005: high burden of congenital infection and spread to Canada. *Pediatr Infect Dis J* 2009;**28**:795–800.
239. Hahné S, Ward M, Abbink F, et al. Large ongoing rubella outbreak in religious community in the Netherlands since September 2004. *Euro surveillance* 2005;**10**.
240. Hale MS, Ruderman JE. Carpal tunnel syndrome associated with rubella immunization. *Am J Phys Med* 1973;**52**:189–94.
241. Halonen PE, Ryan JM, Stewart JA. Rubella hemagglutinin prepared with alkaline extraction of virus grown in suspension culture of BHK-21 cells. *Proc Soc Exp Biol Med* 1967;**125**:162–7.
242. Hambling MH. Effect of a vaccination programme on the distribution of rubella antibodies in women of childbearing age. *Lancet* 1975;**1**:1130–3.
243. Hamkar R, Jalilvand S, Mokhtari-Azad T, et al. Assessment of IgM enzyme immunoassay and IgG avidity assay for distinguishing between primary and secondary immune response to rubella vaccine. *J Virol Meth* 2005;**130**:59–65.
244. Hammond K. Skin dimples and rubella. *Pediatrics* 1967;**39**: 291–2.
245. Hancock MP, Huntley CC, Sever JL. Congenital rubella syndrome with immunoglobulin disorder. *J Pediatr* 1968;**72**:636–45.
246. Hanissian AS, Hashimoto K. Paramyxovirus-like inclusions in rubella syndrome. *J Pediatr* 1972;**81**:231–7.
247. Hanissian AS, Martinez AJ, Jabbour JT, et al. Vasculitis and myositis secondary to rubella vaccination. *Arch Neurol* 1973;**28**: 202–4.
248. Hanshaw JB, Dudgeon JA. Rubella. In: *Viral diseases of the fetus and newborn*. Philadelphia: W. B. Saunders; 1978. p. 17–96.
249. Hara J, Fujimoto F, Ishibashi T, et al. Ocular manifestations of the 1976 rubella epidemic in Japan. *Am J Ophthalmol* 1979; **87**:642–5.
250. Haralambieva IH, Dhiman N, Ovsyannikova IG, et al. 2′-5′-Oligoadenylate synthetase single-nucleotide polymorphisms and haplotypes are associated with variations in immune responses to rubella vaccine. *Hum Immunol* 2010;**71**:383–91.
251. Hardy JB. Clinical and developmental aspects of congenital rubella. *Arch Otolaryngol* 1973;**98**:230–6.
252. Hardy JB, Sever JL. Indirect inguinal hernia in congenital rubella. *J Pediatr* 1968;**73**:416–18.
253. Hardy JB, Sever JL, Gilkeson MR. Declining antibody titers in children with congenital rubella. *J Pediatr* 1969;**75**:213–20.
254. Hardy JB, Monif GRG, Sever JL. Studies in congenital rubella, Baltimore, 1964-65. *Bull Johns Hopkins Hosp* 1966;**118**:97–108.
255. Hardy JB, McCracken GH Jr, Gilkeson MR, et al. Adverse fetal outcome following maternal rubella after the first trimester of pregnancy. *JAMA* 1969;**207**:2414–20.
256. Harrison BL. Neuritis following rubella. *Br Med J* 1940;**1**:637.
257. Hart H, Marmion BP. Rubella virus and rheumatoid arthritis. *Ann Rheum Dis* 1977;**36**:3–12.
258. Hastreiter AR, Joorabchi B, Pujatti G, et al. Cardiovascular lesions associated with congenital rubella. *J Pediatr* 1967;**71**:59–65.
259. Hattis RP, Halstead SB, Herrmann KL, et al. Rubella in an immunized island population. *JAMA* 1973;**223**:1019–21.
260. Heathfield KWG. Carpal-tunnel syndrome. *Br Med J* 1962;**2**:58.
261. Heggie AD. Pathogenesis of the rubella exanthem: isolation of rubella virus from the skin. *N Engl J Med* 1971;**285**:664–6.
262. Heggie AD. Pathogenesis of the rubella exanthem: distribution of rubella virus in the skin during rubella with and without rash. *J Infect Dis* 1978;**137**:74–7.
263. Heggie AD, Robbins FC. Natural rubella acquired after birth: clinical features and complications. *Am J Dis Child* 1969;**118**: 12–17.
264. Herne V, Hedman K, Reedik P. Immunoglobulin G avidity in the serodiagnosis of congenital rubella syndrome. *Eur J Clin Microbiol Infect Dis* 1997;**16**:763–6.
265. Herrmann KL. Available rubella serologic tests. *Rev Infect Dis* 1985;**7**(Suppl. 1):S108–12.
266. Hildebrandt HM, Maassab HF. Rubella synovitis in a one-year-old patient. *N Engl J Med* 1966;**274**:1428–30.
267. Hillenbrand FK. Rubella in a remote community. *Lancet* 1956;**271**:64–6.
268. Hillenbrand FK. The blood picture in rubella: its place in diagnosis. *Lancet* 1956;**271**:66–8.
269. Hiro VY, Tasaka S. Die Rotheln sind eine Viruskrankheit. *Monatsschr Kinderheilk* 1938;**1**:328–32.
270. Hodges GM. Neuritis following rubella. *Br Med J* 1940;**1**: 830–1.
271. Hofmann J, Liebert UG. Significance of avidity and immunoblot analysis for rubella IgM-positive serum samples in pregnant women. *J Virol Meth* 2005;**130**:66–71.
272. Holmes IH, Wark MC, Warburton MF. Is rubella an arbovirus? II. Ultrastructural morphology and development. *Virology* 1969; **37**:15–25.
273. Honeyman MC, Dorman DC, Menser MA, et al. HL-A antigens in congenital rubella and the role of antigens 1 and 8 in the epidemiology of natural rubella. *Tissue Antigens* 1975;**5**:12–18.
274. Honeyman MC, Harrison LC. Congenital rubella, diabetes and HLA. *Diabetologia* 2009;**52**:371–2; author reply 373.

275. Honeyman MC, Menser MA. Ethnicity is a significant factor in the epidemiology of rubella and Hodgkin's disease. *Nature* 1974; **251**:441–2.
276. Honeyman MC, Forrest JM, Dorman DC. Cell-mediated immune response following natural rubella and rubella vaccination. *Clin Exp Immunol* 1974;**17**:665–71.
277. Hornsleth A, Leerhoy J, Grauballe P, et al. Rubellavirus-specific IgM- and IgA-antibodies: the indirect immunofluorescence (IF)-technique applied to sera with reduced IgG-concentration. *Acta Pathol Microbiol Scand B Microbiol Immunol* 1974;**82**:742–4.
278. Hornstein L, Ben-Porath E. Rubella antibodies in women of childbearing age during an epidemic and the two years thereafter. *Isr J Med Sci* 1976;**12**:1189–93.
279. Horstmann DM. Problems in measles and rubella. *Dis Mon* 1978;**24**:1–52.
280. Horstmann DM. Rubella. In: Evans AS, editor. *Viral infections of humans: epidemiology and control*. 3rd ed. New York: Plenum Medical; 1990. p. 617–31.
281. Horstmann DM, Banatvala JE, Riordan JT, et al. Maternal rubella and the rubella syndrome in infants: epidemiologic, clinical, and virologic observations. *Am J Dis Child* 1965;**110**:408–15.
282. Horstmann DM, Liebhaber H, Le Bouvier GL, et al. Rubella: reinfection of vaccinated and naturally immune persons exposed in an epidemic. *N Engl J Med* 1970;**283**:771–8.
283. Horstmann DM, Riordan JT, Ohtawara M, et al. A natural epidemic of rubella in a closed population: virological and epidemiological observations. *Arch Gesamte Virusforsch* 1965;**16**:483–7.
284. Horstmann DM, Schluederberg A, Emmons JE, et al. Persistence of vaccine-induced immune responses to rubella: comparison with natural infection. *Rev Infect Dis* 1985;**7**(Suppl. 1):S80–5.
285. Howson CP, Katz M, Johnston RB Jr, et al. Chronic arthritis after rubella vaccination. *Clin Infect Dis* 1992;**15**:307–12.
286. Hukic M, Hubschen JM, Seremet M, et al. An outbreak of rubella in the Federation of Bosnia and Herzegovina between December 2009 and May 2010 indicates failure to vaccinate during wartime (1992-1995). *Epidemiol Infect* 2012;**140**:447–53.
287. Hwang SJ, Chen YS. Congenital rubella syndrome with autistic disorder. *J Chin Med Assoc* 2010;**73**:104–7.
288. Hyde TB, Kruszon-Moran D, McQuillan GM, et al. Rubella immunity levels in the United States population: has the threshold of viral elimination been reached? *Clin Infect Dis* 2006;**43**:146–50.
289. Hynes M. Leucocyte count in rubella. *Lancet* 1940;**236**:679–80.
290. Hyypia T, Eskola J, Laine M, et al. B-cell function in vitro during rubella infection. *Infect Immun* 1984;**43**:589–92.
291. Hyypia T, Eskola J, Laine M, et al. Polyclonal activation of B cells during rubella infection. *Scand J Immunol* 1985;**21**:615–17.
292. Jacob John T. The safety of rubella vaccine in pregnancy. *Indian J Med Res* 2008;**127**:519–20.
293. Jacobsen SJ, Ackerson BK, Sy LS, et al. Observational safety study of febrile convulsion following first dose MMRV vaccination in a managed care setting. *Vaccine* 2009;**27**:4656–61.
294. Jarour N, Hayajneh WA, Balbeesi A, et al. Seroprevalence of rubella among Jordanian women of childbearing age. *Vaccine* 2007;**25**:3615–18.
295. Jeffries DJ, Johnson AH, Mowbray JF. Abnormal responses to rubella infection. *J Clin Pathol* 1976;**29**:1003–6.
296. Jeresaty RM, Russell W. Hepatosplenomegaly and heart disease in the congenital rubella syndrome: report of eight cases. *Pediatrics* 1967;**39**:36–42.
297. Johnson GM, Tudor RB. Diabetes mellitus and congenital rubella infection. *Am J Dis Child* 1970;**120**:453–5.
298. Judelsohn RG, Wyll SA. Rubella in Bermuda: termination of an epidemic by mass vaccination. *JAMA* 1973;**223**:401–6.
299. Kanra GY, Vesikari T. Cytotoxic activity against rubella-infected cells in the supernatants of human lymphocyte cultures stimulated by rubella virus. *Clin Exp Immunol* 1975;**19**:17–32.
300. Kasper S, Allerberger F, Aberle S, et al. Rubella in Austria 2008-2009: no longer a typical childhood disease. *Pediatr Infect Dis J* 2010;**29**:448–52.
301. Katow S. Surveillance of congenital rubella syndrome in Japan, 1978-2002: effect of revision of the immunization law. *Vaccine* 2004;**22**:4084–91.
302. Kearns MJ, Plitt SS, Lee BE, et al. Rubella immunity among pregnant women in a Canadian provincial screening program. *Can J Infect Dis Med Microbiol* 2009;**20**:73–7.
303. Kelemen G. Rubella and deafness. *Arch Otolaryngol* 1966;**83**: 520–32.
304. Kelley PW, Petruccelli BP, Stehr-Green P, et al. The susceptibility of young adult Americans to vaccine-preventable infections: a national serosurvey of US Army recruits. *JAMA* 1991;**266**: 2724–9.
305. Kennedy RB, Ovsyannikova IG, Vierkant RA, et al. Effect of human leukocyte antigen homozygosity on rubella vaccine-induced humoral and cell-mediated immune responses. *Hum Immunol* 2010;**71**:128–35.
306. Kenny FM, Michaels RH, Davis KS. Rubella encephalopathy: later psychometric, neurologic, and encephalographic evaluation of seven survivors. *Am J Dis Child* 1965;**110**:374–80.
307. Kenrick KG, Slinn RF, Dorman DC, et al. Immunoglobulins and rubella-virus antibodies in adults with congenital rubella. *Lancet* 1968;**1**:548–51.
308. Kilroy AW, Schaffner W, Fleet WF Jr, et al. Two syndromes following rubella immunization: clinical observations and epidemiological studies. *JAMA* 1970;**214**:2287–92.
309. Kistler GS, Sapatino V. Temperature- and UV-light resistance of rubella virus infectivity. *Arch Gesamte Virusforsch* 1972;**38**: 11–16.
310. Klein HZ, Markarian M. Dermal erythropoiesis in congenital rubella: description of an infected newborn who had purpura associated with marked extramedullary erythropoiesis in the skin and elsewhere. *Clin Pediatr (Phila)* 1969;**8**:604–7.
311. Klein NP, Fireman B, Yih WK, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;**126**:e1–8.
312. Korematsu S, Miyahara H, Kawano T, et al. A relapse of systemic type juvenile idiopathic arthritis after a rubella vaccination in a patient during a long-term remission period. *Vaccine* 2009;**27**: 5041–2.
313. Korones SB, Ainger LE, Monif GRG, et al. Congenital rubella syndrome: new clinical aspects with recovery of virus from affected infants. *J Pediatr* 1965;**67**:166–81.
314. Korones SB, Ainger LE, Monif GR, et al. Congenital rubella syndrome: study of 22 infants: myocardial damage and other new clinical aspects. *Am J Dis Child* 1965;**110**:434–40.
315. Kresky B, Nauheim JS. Rubella retinitis. *Am J Dis Child* 1967;**113**:305–10.
316. Krill AE. The retinal disease of rubella. *Arch Ophthalmol* 1967; **77**:445–9.
317. Krugman S. Present status of measles and rubella immunization in the United States: a medical progress report. *J Pediatr* 1977; **90**:1–12.
318. Krugman S, Ward R. The rubella problem: clinical aspects, risk of fetal abnormality, and methods of prevention. *J Pediatr* 1954;**44**:489–98.
319. Krugman SE. Rubella symposium. *Am J Dis Child* 1965;**110**: 345–476.
320. Krugman SE. International Conference on Rubella Immunization. *Am J Dis Child* 1969;**118**:2–410.
321. Lafer CZ, Morrison AN. Thrombocytopenic purpura progressing to transient hypoplastic anemia in a newborn with rubella syndrome. *Pediatrics* 1966;**38**:499–501.
322. Laguaite JK, Joseph M. A study of children with communication problems associated with maternal rubella. *South Med J* 1965;**58**: 231–5.
323. Lalla M, Vesikari T, Virolainen M. Lymphoblast proliferation and humoral antibody response after rubella vaccination. *Clin Exp Immunol* 1973;**15**:193–202.
324. Landrigan PJ, Stoffels MA, Anderson E, et al. Epidemic rubella in adolescent boys: clinical features and results of vaccination. *JAMA* 1974;**227**:1283–7.
325. Lanzieri TM, Parise MS, Siqueira MM, et al. Incidence, clinical features and estimated costs of congenital rubella syndrome after a large rubella outbreak in Recife, Brazil, 1999-2000. *Pediatr Infect Dis J* 2004;**23**:1116–22.
326. Lanzieri TM, Segatto TC, Siqueira MM, et al. Burden of congenital rubella syndrome after a community-wide rubella outbreak, Rio Branco, Acre, Brazil, 2000 to 2001. *Pediatr Infect Dis J* 2003;**22**:323–9.
327. Larson HE, Parkman PD, Davis WJ, et al. Inadvertent rubella virus vaccination during pregnancy. *N Engl J Med* 1971;**284**: 870–3.

328. Lawless MR, Abramson JS, Harlan JE, et al. Rubella susceptibility in sixth graders: effectiveness of current immunization practice. *Pediatrics* 1980;**65**:1086–9.
329. Lawton AR, Self KS, Royal SA, et al. Ontogeny of B-lymphocytes in the human fetus. *Clin Immunol Immunopathol* 1972;**1**:84–93.
330. Le Bouvier GL. Precipitinogens of rubella virus-infected cells. *Proc Soc Exp Biol Med* 1969;**130**:51–4.
331. LeBouvier GL. Rubella precipitins. In: *International Symposium on Rubella Vaccines*, London, 1968 *Symposium series in immunobiological standardization*, **vol. 11**. New York: Karger; 1969. p. 113–38.
332. Le Bouvier GL, Plotkin SA. Precipitin responses to rubella vaccine RA 27-3. *J Infect Dis* 1971;**123**:220–3.
333. Lebon P, Lyon G. Letter: Non-congenital rubella encephalitis. *Lancet* 1974;**2**:468.
334. Lebon P, Daffos F, Checoury A, et al. Presence of an acid-labile alpha-interferon in sera from fetuses and children with congenital rubella. *J Clin Microbiol* 1985;**21**:775–8.
335. Lee JY, Bowden DS. Rubella virus replication and links to teratogenicity. *Clin Microbiol Rev* 2000;**13**:571–87.
336. Lehane DE, Newberg N, Beam WE Jr. Evaluation of rubella herd immunity during an epidemic. *JAMA* 1970;**213**:2236–9.
337. Lejarraga H, Peckham CS. Birthweight and subsequent growth of children exposed to rubella infection in utero. *Arch Dis Child* 1974;**49**:50–4.
338. Lennette EH, Schmidt NJ, Magoffin RL. The hemagglutination inhibition test for rubella: a comparison of its sensitivity to that of neutralization, complement fixation and fluorescent antibody tests for diagnosis of infection and determination of immunity status. *J Immunol* 1967;**99**:785–93.
339. Lerman SJ, Nankervis GA, Heggie AD, et al. Immunologic response, virus excretion, and joint reactions with rubella vaccine: a study of adolescent girls and young women given live attenuated virus vaccine (HPV-77:DE-5). *Ann Intern Med* 1971;**74**:67–73.
340. Liebhaber H. Measurement of rubella antibody by hemagglutination inhibition. I. Variables affecting rubella hemagglutination. *J Immunol* 1970;**104**:818–25.
341. Liebhaber H, Ingalls TH, Le Bouvier GL, et al. Vaccination with RA 27-3 rubella vaccine: persistence of immunity and resistance to challenge after two years. *Am J Dis Child* 1972;**123**:133–6.
342. Lin CC, Yang CY, Shih YL, et al. Rubella seroepidemiology and estimations of the catch-up immunisation rate and persistence of antibody titers in pregnant women in Taiwan. *BJOG* 2011; **118**:706–12.
343. Lindquist JM, Plotkin SA, Shaw L, et al. Congenital rubella syndrome as a systemic infection: studies of affected infants born in Philadelphia, U.S.A. *Br Med J* 1965;**5475**:1401–6.
344. Lock FR, Gatling HB, Wells HB. Difficulties in the diagnosis of congenital abnormalities: experience in a study of the effect of rubella on pregnancy. *JAMA* 1961;**178**:711–14.
345. Lokietz H, Reynolds FA. Postrubella thrombocytopenic purpura. *J Lancet* 1965;**85**:226–30.
346. Lovett AE, Hahn CS, Rice CM, et al. Rubella virus-specific cytotoxic T-lymphocyte responses: identification of the capsid as a target of major histocompatibility complex class I- restricted lysis and definition of two epitopes. *J Virol* 1993;**67**:5849–58.
347. Macfarlane DW, Boyd RD, Dodrill CB, et al. Intrauterine rubella, head size, and intellect. *Pediatrics* 1975;**55**:797–801.
348. Macrae AD, Mogford H, Reid D, et al. Studies of the effect of immunoglobulin on rubella in pregnancy: report of the Public Health Laboratory Service Working Party on Rubella. *Br Med J* 1970;**2**:497–500.
349. Maller R, Fryden A, Soren L. Mitogen stimulation and distribution of T- and B-lymphocytes during natural rubella infection. *Acta Pathol Microbiol Scand C* 1978;**86**:93–8.
350. Mallinson H, Roberts C, Bruce White GB. Staphylococcal protein A; its preparation and an application to rubella serology. *J Clin Pathol* 1976;**29**:999–1002.
351. Mantadakis E, Farmaki E, Buchanan GR. Thrombocytopenic purpura after measles-mumps-rubella vaccination: a systematic review of the literature and guidance for management. *J Pediatr* 2010;**156**:623–8.
352. Margolis FJ, Wilson JL, Top FH. Postrubella encephalomyelitis: report of cases in Detroit and review of literature. *J Pediatr* 1943;**23**:158–65.
353. Martenis TW, Bland JH, Phillips CA. Rheumatoid arthritis after rubella. *Arthritis Rheum* 1968;**11**:683–7.
354. Matsumoto A, Higashi N. Electron microscopic studies on the morphology and the morphogenesis of togaviruses. *Ann Rep Inst Virus Res Kyoto Univ* 1974;**17**:11–22.
355. Matter L, Gorgievski-Hrisoho M, Germann D. Comparison of four enzyme immunoassays for detection of immunoglobulin M antibodies against rubella virus. *J Clin Microbiol* 1994;**32**:2134–9.
356. McCallin PF, Fuccillo DA, Ley AC, et al. Gammaglobulin as prophylaxis against rubella-induced congenital anomalies. *Obstet Gynecol* 1972;**39**:185–9.
357. McCarthy K, Taylor-Robinson CH. Rubella. *Br Med Bull* 1967;**23**:185–91.
358. McCarthy K, Taylor-Robinson CH, Pillinger SE. Isolation of rubella virus from cases in Britain. *Lancet* 1963;**2**:593–8.
359. McCarthy M, Lovett A, Kerman RH, et al. Immunodominant T-cell epitopes of rubella virus structural proteins defined by synthetic peptides. *J Virol* 1993;**67**:673–81.
360. McDonald JC, Peckham CS. Gammaglobulin in prevention of rubella and congenital defect: a study of 30,000 pregnancies. *Br Med J* 1967;**3**:633–7.
361. McMorrow LE, Vesikari T, Wolman SR, et al. Suppression of the response of lymphocytes to phytohemagglutinin in rubella. *J Infect Dis* 1974;**130**:464–9.
362. Meitsch K, Enders G, Wolinsky JS, et al. The role of rubella-immunoblot and rubella-peptide-EIA for the diagnosis of the congenital rubella syndrome during the prenatal and newborn periods. *J Med Virol* 1997;**51**:280–3.
363. Menser MA, Dorman DC, Reye RD, et al. Renal-artery stenosis in the rubella syndrome. *Lancet* 1966;**1**:790–2.
364. Menser MA, Forrest JM. Rubella: high incidence of defects in children considered normal at birth. *Med J Aust* 1974;**1**:123–6.
365. Menser MA, Forrest JM, Bransby RD. Rubella infection and diabetes mellitus. *Lancet* 1978;**1**:57–60.
366. Menser MA, Hudson JR, Murphy AM, et al. Epidemiology of congenital rubella and results of rubella vaccination in Australia. *Rev Infect Dis* 1985;**7**(Suppl. 1):S37–41.
367. Menser MA, Robertson SE, Dorman DC, et al. Renal lesions in congenital rubella. *Pediatrics* 1967;**40**:901–4.
368. Merritt HH, Koskoff YD. Encephalomyelitis following German measles. *Am J Med Sci* 1936;**191**:690–6.
369. Meurman OH. Antibody responses in patients with rubella infection determined by passive hemagglutination, hemagglutination inhibition, complement fixation, and solid-phase radioimmunoassay tests. *Infect Immun* 1978;**19**:369–72.
370. Meurman OH, Viljanen MK, Granfors K. Solid-phase radioimmunoassay of rubella virus immunoglobulin M antibodies: comparison with sucrose density gradient centrifugation test. *J Clin Microbiol* 1977;**5**:257–62.
371. Meyer HM Jr, Hopps HE, Parkman PD, et al. Control of measles and rubella through use of attenuated vaccines. *Am J Clin Pathol* 1978;**70**:128–35.
372. Michael M. Rubella: a report of an epidemic of eighty cases. *Arch Pediatr* 1908;**25**:598–606.
373. Michaels RH. Suppression of antibody response in congenital rubella. *J Pediatr* 1972;**80**:583–8.
374. Michaels RH, Kenny FM. Postnatal growth retardation in congenital rubella. *Pediatrics* 1969;**43**:251–9.
375. Michaels RM, Rogers KD. A sex difference in immunologic responsiveness. *Pediatrics* 1971;**47**:120–3.
376. Miller CH, Dowd JM, Rytel MW, et al. Prevention of rubella with γ-globulin. *JAMA* 1967;**201**:560–1.
377. Miller CL, Miller E, Sequeira PJ, et al. Effect of selective vaccination on rubella susceptibility and infection in pregnancy. *Br Med J (Clin Res Ed)* 1985;**291**:1398–401.
378. Miller CL, Miller E, Waight PA. Rubella susceptibility and the continuing risk of infection in pregnancy. *Br Med J (Clin Res Ed)* 1987;**294**:1277–8.
379. Miller KA, Zager TD. Rubella susceptibility in an adolescent female population. *Mayo Clin Proc* 1984;**59**:31–4.
380. Miller MH, Rabinowitz M, Cohen M. Pure-tone audiometry in prenatal rubella. *Arch Otolaryngol* 1971;**94**:25–9.
381. Minussi L, Mohrdieck R, Bercini M, et al. Prospective evaluation of pregnant women vaccinated against rubella in southern Brazil. *Reprod Toxicol* 2008;**25**:120–3.
382. Mitchell LA. Sex differences in antibody- and cell-mediated immune response to rubella re-immunisation. *J Med Microbiol* 1999;**48**:1075–80.

383. Mitchell LA, Zhang T, Tingle AJ. Differential antibody responses to rubella virus infection in males and females. *J Infect Dis* 1992;**166**:1258–65.
384. Mitchell LA, Décarie D, Tingle AJ, et al. Use of synthetic peptides to map regions of rubella virus capsid protein recognized by human T lymphocytes. *Vaccine* 1994;**12**:639–45.
385. Mitchell LA, Tingle AJ, Decarie D, et al. Identification of rubella virus T-cell epitopes recognized in anamnestic response to RA27/3 vaccine: associations with boost in neutralizing antibody titer. *Vaccine* 1999;**17**:2356–65.
386. Miyazaki S, Ohtsuka M, Ueda K, et al. Coombs positive hemolytic anemia in congenital rubella. *J Pediatr* 1979;**94**:759–60.
387. Monif GR, Asofsky R, Sever JL. Hepatic dysfunction in the congenital rubella syndrome. *Br Med J* 1966;**1**:1086–8.
388. Monif GR, Avery GB, Korones SB, et al. Postmortem isolation of rubella virus from three children with rubella-syndrome defects. *Lancet* 1965;**1**:723–4.
389. Monif GR, Sever JL, Schiff GM, et al. Isolation of rubella virus from products of conception. *Am J Obstet Gynecol* 1965;**91**: 1143–6.
390. Monif GRG, Hardy JB, Sever JL. Studies in congenital rubella, Baltimore 1964-65. I. Epidemiologic and virologic. *Bull Johns Hopkins Hosp* 1966;**118**:85–96.
391. Monto AS, Cavallaro JJ, Whale EH. Frequency of arthralgia in women receiving one of three rubella vaccines. *Arch Intern Med* 1970;**126**:635–9.
392. Morag A, Morag B, Bernstein JM, et al. In vitro correlates of cell-mediated immunity in human tonsils after natural or induced Rubella virus infection. *J Infect Dis* 1975;**131**:409–16.
393. Morse EE, Zinkham WH, Jackson DP. Thrombocytopenic purpura following rubella infection in children and adults. *Arch Intern Med* 1966;**117**:573–9.
394. Murphy AM, Field PR, Collins E. The value of specific IgM tests in the early diagnosis of congenital rubella. *Med J Aust* 1978; **2**:290–3.
395. Murphy AM, Reid RR, Pollard I, et al. Rubella cataracts: further clinical and virologic observations. *Am J Ophthalmol* 1967;**64**: 1109–19.
396. Myllylä G, Vaheri A, Vesikari T, et al. Interaction between human blood platelets, viruses and antibodies. IV. Post-rubella thrombocytopenic purpura and platelet aggregation by rubella antigen-antibody interaction. *Clin Exp Immunol* 1969;**4**:323–32.
397. Naeye RL, Blanc W. Pathogenesis of congenital rubella. *JAMA* 1965;**194**:1277–83.
398. Namaei MH, Ziaee M, Naseh N. Congenital rubella syndrome in infants of women vaccinated during or just before pregnancy with measles-rubella vaccine. *Indian J Med Res* 2008;**127**:551–4.
399. Nardone A, Tischer A, Andrews N, et al. Comparison of rubella seroepidemiology in 17 countries: progress towards international disease control targets. *Bull World Health Organ* 2008;**86**:118–25.
400. Nasiri R, Yoseffi J, Khajedaloe M, et al. Congenital rubella syndrome after rubella vaccination in 1-4 weeks periconceptional period. *Indian J Pediatr* 2009;**76**:279–82.
401. National Communicable Disease Center. *Rubella surveillance*. Bethesda, Md: US Department of Health, Education, and Welfare, Centers for Disease Control and Prevention; 1969.
402. National Communicable Disease Center. Rubella surveillance summary. *M M W R Morb Mortal Wkly Rep* 1964;**13**:351–4.
403. Niwa Y, Kanoh T. Immunological behaviour following rubella infection. *Clin Exp Immunol* 1979;**37**:470–6.
404. Noble JS, Wand M. Catcher's crouch and rubella immunization. *JAMA* 1971;**217**:212.
405. Norval M, Smith C. Search for viral nucleic sequences in rheumatoid cells. *Ann Rheum Dis* 1979;**38**:456–62.
406. Novo A, Huebschen JM, Muller CP, et al. Ongoing rubella outbreak in Bosnia and Herzegovina, March-July 2009: preliminary report. *Euro Surveill* 2009;**14**.
407. Nusbacher J, Hirschhorn K, Cooper LZ. Chromosomal abnormalities in congenital rubella. *N Engl J Med* 1967;**276**:1409–13.
408. O'Neill JF. Strabismus in congenital rubella: management in the presence of brain damage. *Arch Ophthalmol* 1967;**77**:450–4.
409. Ogra PL, Herd JK. Arthritis associated with induced rubella infection. *J Immunol* 1971;**107**:810–13.
410. Ogra PL, Chiba Y, Ogra SS, et al. Rubella-virus infection in juvenile rheumatoid arthritis. *Lancet* 1975;**1**:1157–61.
411. Ogra PL, Kerr-Grant D, Umana G, et al. Antibody response in serum and nasopharynx after naturally acquired and vaccine-induced infection with rubella virus. *N Engl J Med* 1971;**285**: 1333–9.
412. Orenstein WA, Herrmann KL, Holmgreen P, et al. Prevalence of rubella antibodies in Massachusetts schoolchildren. *Am J Epidemiol* 1986;**124**:290–8.
413. Orenstein WA, Heseltine PN, LeGagnoux SJ, et al. Rubella vaccine and susceptible hospital employees: poor physician participation. *JAMA* 1981;**245**:711–13.
414. Ornoy A, Segal S, Nishmi M, et al. Fetal and placental pathology in gestational rubella. *Am J Obstet Gynecol* 1973;**116**:949–56.
415. Oster ME, Riehle-Colarusso T, Correa A. An update on cardiovascular malformations in congenital rubella syndrome. *Birth Defects Res A Clin Mol Teratol* 2010;**88**:1–8.
416. Ou D, Chong P, Tripet B, et al. Analysis of T- and B-cell epitopes of capsid protein of rubella virus by using synthetic peptides. *J Virol* 1992;**66**:1674–81.
417. Ovsyannikova IG, Pankratz VS, Vierkant RA, et al. Human leukocyte antigen haplotypes in the genetic control of immune response to measles-mumps-rubella vaccine. *J Infect Dis* 2006; **193**:655–63.
418. Ovsyannikova IG, Vierkant RA, Pankratz VS, et al. HLA haplotype and supertype associations with cellular immune responses and cytokine production in healthy children after rubella vaccine. *Vaccine* 2009;**27**:3349–58.
419. Ozsoylu S, Kanra G, Savas G. Thrombocytopenic purpura related to rubella infection. *Pediatrics* 1978;**62**:567–9.
420. Panush RS. Serum hypocomplementemia with rubella arthritis: case report. *Mil Med* 1975;**140**:117–18.
421. Pardon F, Vilarino M, Barbero P, et al. Rubella vaccination of unknowingly pregnant women during 2006 mass campaign in Argentina. *J Infect Dis* 2011;**204**(Suppl. 2):S745–7.
422. Parkman PD, Buescher EL, Artenstein MS. Recovery of rubella virus from army recruits. *Proc Soc Exp Biol Med* 1962;**111**: 225–30.
423. Parkman PD, Buescher EL, Artenstein MS, et al. Studies of rubella. I. Properties of the virus. *J Immunol* 1964;**93**:595–607.
424. Pattison JR. Persistence of specific IgM after natural infection with rubella virus. *Lancet* 1975;**1**:185–7.
425. Pattison JR, Mace JE. The detection of specific IgM antibodies following infection with rubella virus. *J Clin Pathol* 1975;**28**: 377–82.
426. Peckham CS. Clinical and laboratory study of children exposed in utero to maternal rubella. *Arch Dis Child* 1972;**47**:571–7.
427. Pedneault L, Zrein M, Robillard L, et al. Comparison of novel synthetic peptide-based DETECT-RUBELLA enzyme immunoassays with Enzygnost and IMx for detection of rubella-specific immunoglobulin G. *J Clin Microbiol* 1994;**32**:1085–7.
428. Peltola H, Davidkin I, Paunio M, et al. Mumps and rubella eliminated from Finland. *JAMA* 2000;**284**:2643–7.
429. Peltola H, Heinonen OP, Valle M, et al. The elimination of indigenous measles, mumps, and rubella from Finland by a 12-year, two-dose vaccination program. *N Engl J Med* 1994; **331**:1397–402.
430. Peters ER, Davis RL. Congenital rubella syndrome: cerebral mineralizations and subperiosteal new bone formation as expressions of this disorder. *Clin Pediatr (Phila)* 1966;**5**:743–6.
431. Pettersson RF, Oker-Blom C, Kalkkinen N, et al. Molecular and antigenic characteristics and synthesis of rubella virus structural proteins. *Rev Infect Dis* 1985;**7**(Suppl. 1):S140–9.
432. Phelan P, Campbell P. Pulmonary complications of rubella embryopathy. *J Pediatr* 1969;**75**:202–12.
433. Phillips CA, Maeck JV, Rogers WA, et al. Intrauterine rubella infection following immunization with rubella vaccine. *JAMA* 1970;**213**:624–5.
434. Phillips CA, Melnick JL, Yow MD, et al. Persistence of virus in infants with congenital rubella and in normal infants with a history of maternal rubella. *JAMA* 1965;**193**:1027–9.
435. Pineda RG, Desmond MM, Rudolph AJ, et al. Impact of the 1964 rubella epidemic on a clinic population. *Am J Obstet Gynecol* 1968;**100**:1139–46.
436. Plotkin SA. Rubella virus. In: Lennette EH, Schmidt NJ, editors. *Diagnostic procedures for viral and rickettsial infections*. New York: American Public Health Association; 1969. p. 364–413.

437. Plotkin SA. The history of rubella and rubella vaccination leading to elimination. *Clin Infect Dis* 2006;**43**(Suppl. 3):S164–8.
438. Plotkin SA, Buser F. History of RA27/3 rubella vaccine. *Rev Infect Dis* 1985;**7**(Suppl. 1):S77–8.
439. Plotkin SA, Farquhar JD. Immunity to rubella: comparison between naturally and artificially induced resistance. *Postgrad Med J* 1972;**48**(Suppl. 3):47–54.
440. Plotkin SA, Farquhar JD, Ogra PL. Immunologic properties of RA27-3 rubella virus vaccine: a comparison with strains presently licensed in the United States. *JAMA* 1973;**225**:585–90.
441. Plotkin SA, Klaus RM, Whitely JP. Hypogammaglobulinemia in an infant with congenital rubella syndrome; failure of 1-adamantanamine to stop irus excretion. *J Pediatr* 1966;**69**: 1085–91.
442. Plotkin SA, Oski FA, Hartnett EM, et al. Some recently recognized manifestations of the rubella syndrome. *J Pediatr* 1965; **67**:182–91.
443. Polk BF, Modlin JF, White JA, et al. A controlled comparison of joint reactions among women receiving one or two rubella vaccines. *Am J Epidemiol* 1982;**115**:19–25.
444. Polk BF, White JA, DeGirolami PC, et al. An outbreak of rubella among hospital personnel. *N Engl J Med* 1980;**303**:541–5.
445. Preblud SR, Alford CA Jr. Rubella. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 3rd ed. Philadelphia: W. B. Saunders; 1990. p. 196–240.
446. Preece MA, Kearney PJ, Marshall WC. Growth-hormone deficiency in congenital rubella. *Lancet* 1977;**2**:842–4.
447. Pukhalsky AL, Shmarina GV, Bliacher MS, et al. Cytokine profile after rubella vaccine inoculation: evidence of the immunosuppressive effect of vaccination. *Mediators Inflamm* 2003;**12**: 203–7.
448. Purvis-Smith SG, Howard PR, Menser MA. Dermatoglyphic defects and rubella teratogenesis. *JAMA* 1969;**209**:1865–8.
449. Pustowoit B, Liebert UG. Predictive value of serological tests in rubella virus infection during pregnancy. *Intervirology* 1998;**41**: 170–7.
450. Ramsay ME, Brugha R, Brown DW, et al. Salivary diagnosis of rubella: a study of notified cases in the United Kingdom, 1991-4. *Epidemiol Infect* 1998;**120**:315–19.
451. Rausen AR, London RD, Mizrahi A, et al. Generalized bone changes and thrombocytopenic purpura in association with intrauterine rubella. *Pediatrics* 1965;**36**:264–8.
452. Rausen AR, Richter P, Tallal L, et al. Hematologic effects of intrauterine rubella. *JAMA* 1967;**199**:75–8.
453. Rawls WE, Phillips A, Melnick JL, et al. Persistent virus infection in congenital rubella. *Arch Ophthalmol* 1967;**77**:430–3.
454. Reed GB Jr. Rubella bone lesions. *J Pediatr* 1969;**74**:208–13.
455. Reef SE, Cochi SL. The evidence for the elimination of rubella and congenital rubella syndrome in the United States: a public health achievement. *Clin Infect Dis* 2006;**43**(Suppl. 3): S123–5.
456. Reef SE, Frey TK, Theall K, et al. The changing epidemiology of rubella in the 1990s: on the verge of elimination and new challenges for control and prevention. *JAMA* 2002;**287**:464–72.
457. Reef SE, Redd SB, Abernathy E, et al. Evidence used to support the achievement and maintenance of elimination of rubella and congenital rubella syndrome in the United States. *J Infect Dis* 2011;**204**(Suppl. 2):S593–7.
458. Reef SE, Strebel P, Dabbagh A, et al. Progress toward control of rubella and prevention of congenital rubella syndrome: worldwide, 2009. *J Infect Dis* 2011;**204**(Suppl. 1):S24–7.
459. Reese AB. Congenital cataract and other anomalies following German measles in the mother. *Am J Ophthalmol* 1944;**27**: 483–7.
460. Revello MG, Baldanti F, Sarasini A, et al. Prenatal diagnosis of rubella virus infection by direct detection and semiquantitation of viral RNA in clinical samples by reverse transcription-PCR. *J Clin Microbiol* 1997;**35**:708–13.
461. Revello MG, Gorini G, Zavattoni M, et al. Congenital rubella infection following rubella outbreak in northern Italy, 2002: need for an effective vaccination programme. *Eur J Clin Microbiol Infect Dis* 2004;**23**:780–3.
462. Ribon A, Wasserman E. Immunodeficiency with congenital rubella. *Ann Allergy* 1974;**32**:35–40.
463. Richards CS. Middle ear changes in rubella deafness. *Arch Otolaryngol* 1964;**80**:48–59.
464. Robinson J, Lemay M, Vaudry WL. Congenital rubella after anticipated maternal immunity: two cases and a review of the literature. *Pediatr Infect Dis J* 1994;**13**:812–15.
465. Robinson RG, Dudenhoeffer FE, Holroyd HJ. Rubella immunity in older children, teenagers, and young adults: a comparison of immunity in those previously immunized with those unimmunized. *J Pediatr* 1982;**101**:188–91.
466. Rones B. The relationship of German measles during pregnancy to congenital ocular defects. *Med Ann DC* 1944;**13**:285–7.
467. Rorke LB. Nervous system lesions in the congenital rubella syndrome. *Arch Otolaryngol* 1973;**98**:249–51.
468. Rorke LB, Spiro AJ. Cerebral lesions in congenital rubella syndrome. *J Pediatr* 1967;**70**:243–55.
469. Rose HD. Fatal rubella encephalitis. *Am J Med Sci* 1974; **268**:287–90.
470. Ross AC, McCartney A. Progress of rubella immunity in pregnant women. *Br Med J* 1979;**1**:1636.
471. Rossier E, Phipps PH, Polley JR, et al. Absence of cell-mediated immunity to rubella virus 5 years after rubella vaccination. *Can Med Assoc J* 1977;**116**:481–4.
472. Rossier E, Phipps PH, Weber JM, et al. Persistence of humoral and cell-mediated immunity to rubella virus in cloistered nuns and in schoolteachers. *J Infect Dis* 1981;**144**:137–41.
473. Rota PA, Brown KE, Hubschen JM, et al. Improving global virologic surveillance for measles and rubella. *J Infect Dis* 2011; **204**(Suppl. 1):S506–13.
474. Roy FH, Hiatt RL, Korones SB, et al. Ocular manifestations of congenital rubella syndrome: recovery of virus from affected infants. *Arch Ophthalmol* 1966;**75**:601–7.
475. Rudolph AJ, Singleton EB, Rosenberg HS, et al. Osseous manifestations of the congenital rubella syndrome. *Am J Dis Child* 1965;**110**:428–33.
476. Rudolph AJ, Yow MD, Phillips A, et al. Transplacental rubella infection in newly born infants. *JAMA* 1965;**191**:843–5.
477. Ruokonen PC, Metzner S, Ucer A, et al. Intraocular antibody synthesis against rubella virus and other microorganisms in Fuchs' heterochromic cyclitis. *Graefes Arch Clin Exp Ophthalmol* 2010;**248**:565–71.
478. Sacks R, Habermann ET. Pathological fracture in congenital rubella: a case report. *J Bone Joint Surg Am* 1977;**59**:557–9.
479. Saeed AA, Lange LS. Guillain-Barré syndrome after rubella. *Postgrad Med J* 1978;**54**:333–4.
480. Sallomi SJ. Rubella in pregnancy: a review of prospective studies from the literature. *Obstet Gynecol* 1966;**27**:252–6.
481. Salmi AA. Gel precipitation reactions between alkaline extracted rubella antigens and human sera. *Acta Pathol Microbiol Scand* 1969;**76**:271–8.
482. Salmi AA. Characterization of a structural antigen of rubella virus reacting by gel precipitation. *Acta Pathol Microbiol Scand B Microbiol Immunol* 1972;**80**:534–44.
483. Sarwar M, Azar-Kia B, Schechter MM, et al. Aqueductal occlusion in the congenital rubella syndrome. *Neurology* 1974;**24**:198–201.
484. Sato HK, Sanajotta AT, Moraes JC, et al. Rubella vaccination of unknowingly pregnant women: the São Paulo experience, 2001. *J Infect Dis* 2011;**204**(Suppl. 2):S737–44.
485. Schaffner W, Fleet WF, Kilroy AW, et al. Polyneuropathy following rubella immunization: a follow-up study and review of the problem. *Am J Dis Child* 1974;**127**:684–8.
486. Schamberg JF, Kolmer JA. The treatment of measles. In: *Acute infectious diseases*. 2nd ed. Philadelphia: Lea & Febiger; 1928. p. 545–73.
487. Schatzmayr HG. Aspects of rubella infection in Brazil. *Rev Infect Dis* 1985;**7**(Suppl. 1):S53–5.
488. Scheie HG, Schaffer DB, Plotkin SA, et al. Congenital rubella cataracts: surgical results and virus recovery from intraocular tissue. *Arch Ophthalmol* 1967;**77**:440–4.
489. Schell K, Wong KT. Stability and storage of rubella complement fixing antigen. *Nature* 1966;**212**:621–2.
490. Schiff GM. Titered lots of immune globulin (Ig). Efficacy in the prevention of rubella. *Am J Dis Child* 1969;**118**:322–7.
491. Schiff GM, Dine MS. Transmission of rubella from newborns: a controlled study among young adult women and report of an unusual case. *Am J Dis Child (1960)* 1965;**110**:447–51.
492. Schiff GM, Linnemann CC Jr, Shea L. Evaluation of RA 2713 rubella vaccine. *J Pediatr* 1974;**85**:379–81.

493. Schiff GM, Sever JL, Huebner RJ. Experimental rubella: clinical and laboratory findings. *Arch Intern Med* 1965;**116**:537–43.
494. Schiff GM, Sutherland JM, Light IJ, et al. Studies on congenital rubella: preliminary results on the frequency and significance of presence of rubella virus in the newborn and the effect of gamma-globulin in preventing congenital rubella. *Am J Dis Child* 1965;**110**:441–3.
495. Schlesinger S, Schlesinger MJ. Togaviridae: The viruses and their replication. In: Knipe DM, Howley PM, editors: *Fields' virology*, Vol. 1. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 909–11.
496. Schlossberg D, Topolosky MK. Military rubella. *JAMA* 1977; **238**:1273–4.
497. Schmid D, Kasper S, Kuo HW, et al. Ongoing rubella outbreak in Austria, 2008-2009. *Euro Surveill* 2009;**14**.
498. Schmidt NJ, Lennette EH. Complement-fixing and fluorescent antibody responses to an attenuated rubella virus vaccine. *Am J Epidemiol* 1970;**91**:351–4.
499. Schmidt NJ, Styk B. Immunodiffusion reactions with rubella antigens. *J Immunol* 1968;**101**:210–16.
500. Schmidt NJ, Dennis J, Lennette EH. Rubella virus hemagglutination with a wide variety of erythrocyte species. *Appl Microbiol* 1971;**22**:469–70.
501. Schmidt NJ, Lennette EH, Gee PS. Demonstration of rubella complement-fixing antigens of two distinct particle sizes by gel filtration on sephadex G-200. *Proc Soc Exp Biol Med* 1966;**123**: 758–62.
502. Schmitz H, Shimizu H, Kampa D, et al. Rapid method to detect rubella immunoglobulin M and immunoglobulin A antibodies. *J Clin Microbiol* 1975;**1**:132–5.
503. Schum TR, Nelson DB, Duma MA, et al. Increasing rubella seronegativity despite a compulsory school law. *Am J Public Health* 1990;**80**:66–9.
504. Sears ML. Congenital glaucoma in neonatal rubella. *Br J Ophthalmol* 1967;**51**:744–8.
505. Sedwick WD, Sokol F. Nucleic acid of rubella virus and its replication in hamster kidney cells. *J Virol* 1970;**5**:478–89.
506. Sekeles E, Ornoy A. Osseous manifestations of gestational rubella in young human fetuses. *Am J Obstet Gynecol* 1975;**122**:307–12.
507. Seppala M, Vaheri A. Natural rubella infection of the female genital tract. *Lancet* 1974;**1**:46–7.
508. Sever JL. The epidemiology of rubella. *Arch Ophthalmol* 1967; **77**:427–9.
509. Sever JL, Monif G. Limited persistence of virus in congenital rubella. *Am J Dis Child* 1965;**110**:452–4.
510. Sever JL, Schiff GM, Huebner RJ. frequency of rubella antibody among pregnant women and other human and animal populations: a report from the collaborative study of cerebral palsy. *Obstet Gynecol* 1964;**23**:153–9.
511. Sever JL, South MA, Shaver KA. Delayed manifestations of congenital rubella. *Rev Infect Dis* 1985;**7**(Suppl. 1):S164–9.
512. Sever JL, Brody JA, Schiff GM, et al. Rubella epidemic on St. Paul Island in the Pribilofs, 1963. II. Clinical and laboratory findings for the intensive study population. *JAMA* 1965;**191**:624–6.
513. Sever JL, Fuccillo DA, Gilkeson MR, et al. Changing susceptibility to rubella. *Obstet Gynecol* 1968;**32**:365–9.
514. Sever JL, Hardy JB, Nelson KB, et al. Rubella in the collaborative perinatal research study. II. Clinical and laboratory findings in children through 3 years of age. *Am J Dis Child* 1969;**118**:123–32.
515. Sever JL, Huebner RJ, Castellano GA, et al. Rubella complement fixation test. *Science* 1965;**148**:385–7.
516. Sever JL, Huebner RJ, Fabiyi A, et al. Antibody responses in acute and chronic rubella. *Proc Soc Exp Biol Med* 1966;**122**:513–16.
517. Shah I, Bhatnagar S. Antenatal diagnostic problem of congenital rubella. *Indian J Pediatr* 2010;**77**:450–1.
518. Sherman FE, Michaels RH, Kenny FM. Acute encephalopathy (encephalitis) complicating rubella. report of cases with virologic studies, cortisol-production determinations, and observations at autopsy. *JAMA* 1965;**192**:675–81.
519. Shewmon DA, Cherry JD, Kirby SE. Shedding of rubella virus in a 4 1/2-year-old boy with congenital rubella. *Pediatr Infect Dis* 1982;**1**:342–3.
520. Siassi B, Klyman G, Emmanouilides GC. Hypoplasia of the abdominal aorta associated with the rubella syndrome. *Am J Dis Child* 1970;**120**:476–9.
521. Simpson JW, Nora JJ, Singer DB, et al. Multiple valvular sclerosis in Turner phenotypes and rubella syndrome. *Am J Cardiol* 1969;**23**:94–7.
522. Simpson REH. Rubella and polyarthritis. *Br Med J* 1940;**1**: 830–1.
523. Singer DB, Rudolph AJ, Rosenberg HS, et al. Pathology of the congenital rubella syndrome. *J Pediatr* 1967;**71**:665–75.
524. Singer DB, South MA, Montgomery JR, et al. Congenital rubella syndrome: lymphoid tissue and immunologic status. *Am J Dis Child* 1969;**118**:54–61.
525. Skaug K, Gaarder PI. An indirect immunofluorescent antibody test for determination of rubella virus specific IgM antibodies: elimination of secondary IgM rheumatoid factor staining after absorption of serum IgG with staphylococcal protein A. *Acta Pathol Microbiol Scand C* 1978;**86**:33–5.
526. Slater PE, Ben-Zvi T, Fogel A, et al. Absence of an association between rubella vaccination and arthritis in underimmune postpartum women. *Vaccine* 1995;**13**:1529–32.
527. Smith KA, Chess L, Mardiney MR Jr. The relationship between rubella hemagglutination inhibition antibody (HIA) and rubella induced in vitro lymphocyte tritiated thymidine incorporation. *Cell Immunol* 1973;**8**:321–7.
528. Smithells RW, Sheppard S, Holzel H, et al. National Congenital Rubella Surveillance Programme 1 July 1971-30 June 1984. *Br Med J (Clin Res Ed)* 1985;**291**:40–1.
529. Soares RC, Siqueira MM, Toscano CM, et al. Follow-up study of unknowingly pregnant women vaccinated against rubella in Brazil, 2001-2002. *J Infect Dis* 2011;**204**(Suppl. 2):S729–36.
530. Spencer MJ, Cherry JD, Powell KR. Antibody responses following rubella immunization analyzed by HLA and ABO types. *Immunogenetics* 1977;**4**:365–72.
531. Spiro AJ, Rorke LB. Skeletal muscle lesions in congenital rubella syndrome. *Am J Dis Child* 1966;**112**:427–8.
532. Spruance SL, Smith CB. Joint complications associated with derivatives of HPV-77 rubella virus vaccine. *Am J Dis Child* 1971;**122**:105–11.
533. Spruance SL, Metcalf R, Smith CB, et al. Chronic arthropathy associated with rubella vaccination. *Arthritis Rheum* 1977;**20**: 741–7.
534. Squadrini F, Taparelli F, De Rienzo B, et al. Rubella virus isolation from cerebrospinal fluid in postnatal rubella encephalitis. *Br Med J* 1977;**2**:1329–30.
535. Stallman ND, Allan BC, Sutherland CJ. Prolonged rubella IgM antibody response. *Med J Aust* 1974;**2**:629–31.
536. Staub HP. Postrubella thrombocytopenic purpura: a report of eight cases with discussion of hemorrhagic manifestations of rubella. *Clin Pediatr (Phila)* 1968;**7**:350–6.
537. Steele RW, Hensen SA, Vincent MM, et al. A 51 Cr microassay technique for cell-mediated immunity to viruses. *J Immunol* 1973;**110**:1502–10.
538. Stehr-Green PA, Cochi SL, Preblud SR, et al. Evidence against increasing rubella seronegativity among adolescent girls. *Am J Public Health* 1990;**80**:88.
539. Stern H, Williams BM. Isolation of rubella virus in a case of neonatal giant-cell hepatitis. *Lancet* 1966;**1**:293–5.
540. Stewart GL, Parkman PD, Hopps HE, et al. Rubella-virus hemagglutination-inhibition test. *N Engl J Med* 1967;**276**: 554–7.
541. Stiehm ER, Ammann AJ, Cherry JD. Elevated cord macroglobulins in the diagnosis of intrauterine infections. *N Engl J Med* 1966;**275**:971–7.
542. Storch GA, Gruber C, Benz B. A Rubella outbreak among dental students: description of the outbreak and analysis of control measures. *Infection Control* 1985;**6**:150–6.
543. Strassburg MA, Imagawa DT, Fannin SL, et al. Rubella outbreak among hospital employees. *Obstet Gynecol* 1981;**57**:283–8.
544. Strauss L, Bernstein J. Neonatal hepatitis in congenital rubella: a histopathological study. *Arch Pathol* 1968;**86**:317–27.
545. Strebel PM, Gacic-Dobo M, Reef S, et al. Global use of rubella vaccines, 1980-2009. *J Infect Dis* 2011;**204**(Suppl. 2):S579–84.
546. Streissguth AP, Vanderveer BB, Shepard TH. Mental development of children with congenital rubella syndrome: a preliminary report. *Am J Obstet Gynecol* 1970;**108**:391–9.
547. Sugaya N, Nirasawa M, Mitamura K, et al. Hepatitis in acquired rubella infection in children. *Am J Dis Child* 1988;**142**:817–18.

548. Suzuki J, Goto H, Komase K, et al. Rubella virus as a possible etiological agent of Fuchs heterochromic iridocyclitis. *Graefes Arch Clin Exp Ophthalmol* 2010;**248**:1487–91.
549. Swan C, Tostevin AL, Mayo H, et al. Further observations on congenital defects in infants following infectious diseases during pregnancy, with special reference to rubella. *Med J Aust* 1944; **1**:409–13.
550. Swan C, Tostevin AL, Moore B, et al. Congenital defects in infants following infectious diseases during pregnancy: with special reference to the relationship between German measles and cataract, deaf-mutism, heart disease and microcephaly, and to the period of pregnancy in which the occurrence of rubella is followed by congenital abnormalities. *Med J Aust* 1943;**11**:201–10.
551. Swartz TA. An extensive rubella epidemic in Israel, 1972: selected epidemiologic characteristics. *Am J Epidemiol* 1976;**103**:60–6.
552. Swartz TA, Hornstein L, Epstein I. Epidemiology of rubella and congenital rubella infection in Israel, a country with a selective immunization program. *Rev Infect Dis* 1985;**7**(Suppl. 1):S42–6.
553. Swartz TA, Klingberg W, Goldwasser RA, et al. Clinical manifestations, according to age, among females given hpv-77 duck rubella vaccine. *Am J Epidemiol* 1971;**94**:246–51.
554. Takasu N, Ikema T, Komiya I, et al. Forty-year observation of 280 Japanese patients with congenital rubella syndrome. *Diabetes Care* 2005;**28**:2331–2.
555. Tanemura M, Suzumori K, Yagami Y, et al. Diagnosis of fetal rubella infection with reverse transcription and nested polymerase chain reaction: a study of 34 cases diagnosed in fetuses. *Am J Obstet Gynecol* 1996;**174**:578–82.
556. Thompson GR, Ferreyra A, Brackett RG. Acute arthritis complicating rubella vaccination. *Arthritis Rheum* 1971;**14**:19–26.
557. Thompson GR, Weiss JJ, Shillis JL, et al. Intermittent arthritis following rubella vaccination: a three-year follow-up. *Am J Dis Child* 1973;**125**:526–30.
558. Thompson KM, Tobin JO. Isolation of rubella virus from abortion material. *Br Med J* 1970;**2**:264–6.
559. Thong YH, Steele RW, Vincent MM, et al. Impaired in vitro cell-mediated immunity to rubella virus during pregnancy. *N Engl J Med* 1973;**289**:604–6.
560. Thorburn MJ, Miller CG. Pathology of congenital rubella in Jamaica. *Arch Dis Child* 1967;**42**:389–96.
561. Tingle AJ, Allen M, Petty RE. Rubella-associated arthritis. I. Comparative study of joint manifestations associated with natural rubella infection and RA 27/3 rubella immunisation. *Ann Rheum Dis* 1986;**45**:110–14.
562. Tingle AJ, Chantler JK, Pot KH, et al. Postpartum rubella immunization: association with development of prolonged arthritis, neurological sequelae, and chronic rubella viremia. *J Infect Dis* 1985;**152**:606–12.
563. Tingle AJ, Yang T, Allen M, et al. Prospective immunological assessment of arthritis induced by rubella vaccine. *Infect Immun* 1983;**40**:22–8.
564. Tipples GA, Hamkar R, Mohktari-Azad T, et al. Evaluation of rubella IgM enzyme immunoassays. *J Clin Virol* 2004;**30**:233–8.
565. Tobin JO, Sheppard S, Smithells RW, et al. Rubella in the United Kingdom, 1970-1983. *Rev Infect Dis* 1985;**7**(Suppl. 1):S47–52.
566. Tomlinson IW. Rubella polyneuropathy. *Postgrad Med J* 1975;**51**:30–2.
567. Tondury G, Smith DW. Fetal rubella pathology. *J Pediatr* 1966;**68**:867–79.
568. Townsend CW. Concerning German measles. *Boston Med Surg J* 1904;**150**:403–4.
569. Townsend JJ, Baringer JR, Wolinsky JS, et al. Progressive rubella panencephalitis: late onset after congenital rubella. *N Engl J Med* 1975;**292**:990–3.
570. Trudel M, Nadon F, Seguin C. E1 glycoprotein of rubella virus carries an epitope that binds a neutralizing antibody. *J Virol Meth* 1985;**12**:243–50.
571. Tseng HF, Chang CK, Tan HF, et al. Seroepidemiology study of rubella antibodies among pregnant women from seven Asian countries: evaluation of the rubella vaccination program in Taiwan. *Vaccine* 2006;**24**:5772–7.
572. Ueda K, Nishida Y, Kano M, et al. Clinical studies of patients with rubella syndrome occurring in a high incidence in the Ryukyu Islands in 1965: on the diagnostic significance of clinical manifestations. *Pediatr Int* 1972;**14**:9–16.
573. Ueda K, Nishida Y, Oshima K, et al. Seven-year follow-up study of rubella syndrome in Ryukyu with special reference to persistence of rubella hemagglutination inhibition antibodies. *Jpn J Microbiol* 1975;**19**:181–5.
574. Ueda K, Nishida Y, Oshima K, et al. Congenital rubella syndrome: correlation of gestational age at time of maternal rubella with type of defect. *J Pediatr* 1979;**94**:763–5.
575. Ueda K, Shingaki Y, Sato T, et al. Hemolytic anemia following postnatally acquired rubella during the 1975-1977 rubella epidemic in Japan. *Clin Pediatr (Phila)* 1985;**24**:155–7.
576. Ueno Y. Rubella arthritis: an outbreak in Tokyo. *J Rheumatol* 1994;**21**:874–6.
577. Ukkonen P. Rubella immunity and morbidity: impact of different vaccination programs in Finland 1979-1992. *Scand J Infect Dis* 1996;**28**:31–5.
578. Upfold LJ. Deafness following rubella in pregnancy. *Med J Aust* 1970;**1**:420–4.
579. Upreti SR, Thapa K, Pradhan YV, et al. Developing rubella vaccination policy in Nepal: results from rubella surveillance and seroprevalence and congenital rubella syndrome studies. *J Infect Dis* 2011;**204**(Suppl. 1):S433–8.
580. Urquhart GE, Crawford RJ, Wallace J. Trial of high-titre human rubella immunoglobulin. *Br Med J* 1978;**2**:1331–2.
581. Vaheri A, Vesikari T. Small size rubella virus antigens and soluble immune complexes: analysis by the platelet aggregation technique. *Arch Gesamte Virusforsch* 1971;**35**:10–24.
582. Vaheri A, Vesikari T, Oker-Blom N, et al. Isolation of attenuated rubella-vaccine virus from human products of conception and uterine cervix. *N Engl J Med* 1972;**286**:1071–4.
583. Valinotto LE, Viegas M, Acevedo ME, et al. Phylogenetic analysis of rubella viruses isolated in 2008 outbreak in Argentina. *J Clin Virol* 2009;**46**:286–9.
584. Veale H. History of an epidemic of Rötheln: with observations on its pathology. *Edinburgh Med J* 1866;**12**.
585. Vejtorp M, Fanoe E, Leerhoy J. Diagnosis of postnatal rubella by the enzyme-linked immunosorbent assay for rubella IgM and IgG antibodies. *Acta Pathol Microbiol Scand B* 1979;**87**:155–60.
586. Verder H, Dickmeiss E, Haahr S, et al. Late-onset rubella syndrome: coexistence of immune complex disease and defective cytotoxic effector cell function. *Clin Exp Immunol* 1986;**63**:367–75.
587. Vesikari T, Buimovici-Klein E. Lymphocyte responses to rubella antigen and phytohemagglutinin after administration of the RA 27/3 strain of live attenuated rubella vaccine. *Infect Immun* 1975;**11**:748–53.
588. Vesikari T, Vaheri A, Leinikki P. Antibody response to rubella virion (V) and soluble (S) antigens in rubella infection and following vaccination with live attenuated rubella virus. *Arch Gesamte Virusforsch* 1971;**35**:25–37.
589. Vesikari T, Kanra GY, Buimovici-Klein E, et al. Cell-mediated immunity in rubella assayed by cytotoxicity of supernatants from rubella virus-stimulated human lymphocyte cultures. *Clin Exp Immunol* 1975;**19**:33–43.
590. Vesikari T, Vaheri A, Pettay O, et al. Congenital rubella: immune response of the neonate and diagnosis by demonstration of specific IgM antibodies. *J Pediatr* 1969;**75**:658–64.
591. Vince DJ. The role of rubella in the etiology of supravalvular aortic stenosis. *Can Med Assoc J* 1970;**103**:1157–60.
592. Vogt RL, Clark SW. Premarital rubella vaccination program. *Am J Public Health* 1985;**75**:1088–9.
593. Vossaugh P, Leikin S, Avery G, et al. Neonatal thrombocytopenia in association with rubella. *Acta Haematol Acta Haematologica* 1966;**35**:158–62.
594. Walker JM, Nahmias AJ. Neurologic sequelae of rubella infection. *Clin Pediatr (Phila)* 1966;**5**:699–702.
595. Wallace RB, Isacson P. Comparative trial of HPV-77, DE-5 and RA 27-3 live-attenuated rubella vaccines. *Am J Dis Child* 1972;**124**:536–8.
596. Wallace RB, Libert P, Ibrahim M, et al. Joint symptoms following an area-wide rubella immunization campaign: report of a survey. *Am J Public Health* 1972;**62**:658–61.
597. Walls WL, Altman DH, Gair DR, et al. Roentgenological findings in congenital rubella. *Clin Pediatr (Phila)* 1965;**4**:704–8.
598. Ward PH, Honrubia V, Moore BS. Inner ear pathology in deafness due to maternal rubella. *Arch Otolaryngol (Chicago) 1960* 1968;**87**:22–8.

599. Warren HD, Rogliand FT, Potsubay SF. Thrombocytopenic purpura following rubella. *Med Clin North Am* 1946;**30**:401–4.
600. Watson JC, Hadler SC, Dykewicz CA, et al. Measles, mumps, and rubella: vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1998;**47**:1–57.
601. Way RC. Cardiovascular defects and the rubella syndrome. *Can Med Assoc J* 1967;**97**:1329–34.
602. Weber B, Enders G, Schlosser R, et al. Congenital rubella syndrome after maternal reinfection. *Infection* 1993;**21**:118–21.
603. Weibel RE, Stokes J Jr, Buynak EB, et al. Live rubella vaccines in adults and children: HPV-77 and Merck-Benoit strains. *Am J Dis Child* 1969;**118**:226–9.
604. Weil ML, Itabashi H, Cremer NE, et al. Chronic progressive panencephalitis due to rubella virus simulating subacute sclerosing panencephalitis. *N Engl J Med* 1975;**292**:994–8.
605. Weinberger MM, Masland MW, Asbed RA, et al. Congenital rubella presenting as retarded language development. *Am J Dis Child* 1970;**120**:125–8.
606. Weller TH, Neva FA. Propagation in tissue culture of cytopathic agents from patients with rubella-like illness. *Proc Soc Exp Biol Med* 1962;**111**:215–25.
607. Wesselhoeft C. Rubella (German measles). *N Engl J Med* 1947;**236**:978–88.
608. Whalen JP, Winchester P, Krook L, et al. Neonatal transplacental rubella syndrome: its effect on normal maturation of the diaphysis. *Am J Roentgenol Radium Ther Nucl Med* 1974;**121**:166–72.
609. White LR, Leikin S, Villavicencio O, et al. Immune competence in congenital rubella: lymphocyte transformation, delayed hypersensitivity, and response to vaccination. *J Pediatr* 1968;**73**:229–34.
610. White LR, Sever JL, Alepa FP. Maternal and congenital rubella before 1964: frequency, clinical features, and search for isoimmune phenomena. *J Pediatr* 1969;**74**:198–207.
611. Whitehouse WL. Rubella before conception as a cause of foetal abnormality. *Lancet* 1963;**281**:139.
612. Williams HJ, Carey LS. Rubella embryopathy: roentgenologic features. *Am J Roentgenol Radium Ther Nucl Med* 1966;**97**:92–9.
613. Williams NM, Preblud SR. Rubella and congenital rubella surveillance, 1983. *MMWR Surveill Summ* 1984;**33**:1SS–10SS.
614. Wilson KM, Di Camillo C, Doughty L, et al. Humoral immune response to primary rubella virus infection. *Clin Vaccine Immunol* 2006;**13**:380–6.
615. Wingo SM. Encephalomyelitis complicating rubella: report of a case. *US Naval Med Bull* 1945;**45**:546–7.
616. Witney EW. Neuritis following rubella. *Br Med J* 1940;**1**: 830–831.
617. Witte JJ, Karchmer AW, Case G, et al. Epidemiology of rubella. *Am J Dis Child* 1969;**118**:107–11.
618. Wolinsky JS. Rubella. In: Fields BN, Knipe DM, Howley PM, et al, editors. *Fields' virology*. 2nd ed. New York: Raven Press; 1990. p. 815–38.
619. Wolinsky JS, Berg BO, Maitalnd CH. Progressive rubella panencephalitis. *Arch Neurol* 1976;**33**:722–3.
620. Wolinsky JS, Dau PC, Buimovici-Klein E, et al. Progressive rubella panencephalitis: immunovirological studies and results of isoprinosine therapy. *Clin Exp Immunol* 1979;**35**:397–404.
621. Wolman SR, McMorrow LE, Ziring PR, et al. Lack of chromosomal breakage in congenital rubella. *Pediatrics* 1973;**52**:213–20.
622. World Health Organization. Controlling rubella and preventing congenital rubella syndrome: global progress, 2009. *Wkly Epidemiol Rec* 2010;**85**:413–18.
623. World Health Organization. Rubella vaccines: WHO position paper. *Wkly Epidemiol Rec* 2011;**86**:301–16.
624. Wyll SA, Herrmann KL. Inadvertent rubella vaccination of pregnant women: fetal risk in 215 cases. *JAMA* 1973;**225**:1472–6.
625. Yanez JE, Thompson GR, Mikkelsen WM, et al. Rubella arthritis. *Ann Intern Med* 1966;**64**:772–7.
626. Zaknun D, Weiss G, Glatzl J, et al. Neopterin levels during acute rubella in children. *Clin Infect Dis* 1993;**17**:521–2.
627. Zausmer E. Congenital rubella: pathogenesis of motor deficits. *Pediatrics* 1971;**47**:16–26.
628. Zheng DP, Frey TK, Icenogle J, et al. Global distribution of rubella virus genotypes. *Emerg Infect Dis* 2003;**9**:1523–30.
629. Zrein M, Joncas JH, Pedneault L, et al. Comparison of a whole-virus enzyme immunoassay (EIA) with a peptide-based EIA for detecting rubella virus immunoglobulin G antibodies following rubella vaccination. *J Clin Microbiol* 1993;**31**:1521–4.

CHAPTER 174

ALPHAVIRUSES

174A ■ EASTERN EQUINE ENCEPHALITIS

Theodore F. Tsai • Gail J. Harrison

Eastern equine encephalitis (EEE) is an arthropod-borne viral infection of humans, horses, and other vertebrates that occurs in North and South America. In North America, infections recur in highly focal, primarily coastal locations in association with the habitat of *Culiseta melanura*, the enzootic vector. In the United States, Florida continues to have the highest occurrence of human cases.[36] Birds are the amplifying hosts, and humans and horses are infected incidentally. Because it is a highly pathogenic virus in humans that may be disseminated in the population, it is considered a possible biologic agent, category B threat, that could be used against civilian populations.[53,59]

ETIOLOGIC AGENT

EEE virus is an antigenically distinct member of the *Alphavirus* genus in the *Togaviridae* family. On the basis of nucleotide differences, it has been inferred that EEE and Venezuelan equine encephalitis diverged 1000 to 2000 years ago, with a subsequent division of EEE virus into North American and South American varieties.[8,50,52] South American strains can be differentiated by monoclonal antibodies and by short-incubation hemagglutination-inhibition tests.

North American strains collected over a broad geographic and temporal span demonstrate remarkable genetic stability in one or two major lineages, with minor local divergences occurring within isolated geographic loci. South American strains are genetically heterogeneous. They have been isolated in northward-migrating birds captured in the United States, but they have never been shown to become established.

ECOLOGY

Equine cases have been reported as far north as Quebec, Ontario, and Alberta provinces; in South America, EEE viral activity has been reported from the Caribbean, Mexico, Guatemala, Honduras, Panama, Colombia, Venezuela, Peru, Guyana, and Brazil and as far south as Argentina.[5,9,25,58] The viral transmission cycles in the Caribbean and South America are not well characterized but apparently involve small mammals, birds, and *Culex (Melanoconion)* mosquitoes.[1,25]

In North America, the distribution of virus activity closely follows the distribution of freshwater swamps on the Eastern Seaboard, Gulf Coast, and other inland areas. Florida, with abundant fresh water wetlands, is the state in the United States with the most cases in humans.[36] The distribution of virus corresponds to the distribution of the principal enzootic vector, *C. melanura*.[11,21,25] *C. melanura* feeds nearly exclusively on birds, so various epizootic (bridging) vectors are responsible for infecting humans and horses.* Numerous species may be involved, including *Coquillettidia perturbans*, *Aedes sollicitans*, *Aedes vexans*, and *Aedes canadensis*.

Infection and viremia are subclinical in most native birds, whereas whooping cranes and exotic birds such as emus, house sparrows, ring-necked pheasants, Pekin ducks, and chukar partridges may become ill and die of infection.† Outbreaks resulting in thousands of deaths have occurred in commercial pheasant flocks, in some instances perpetuated by cannibalistic pecking or preening of persistently infected quills.[4] Illness and deaths in pigs, goats, calves, rodents, and other mammals also have been reported.[26,45]

C. melanura is found in and near freshwater swamps, where larval stages breed in acidic waters associated with mucky peat soils. These foci (from north to south) are found in upland red maple, coastal white cedar, and southern loblolly bay biotypes.[11,25]

The viral overwintering mechanism has not been elucidated, but the remarkable permanence of endemic foci is a strong argument for overwintering in local reservoirs, such as reptiles and amphibians.[11,34,35,38,45] Serosurveillance in amphibians and reptiles have shown snakes, including garter snakes, to have high seropositive and to support viremia with EEE virus, whereas frogs, turtles, and lizards had little or low rates of infection.[30]

EPIDEMIOLOGY

EEE is a rare sporadic infection; a median of three cases occurs annually in the United States[40] (Fig. 174A-1). The states with the highest rates of infection show an average annual incidence of less than 1 per 10 million

*References 20, 22, 29-31, 36, 38, 41, 42, 56.
†References 4, 13, 14, 42, 57, 62.

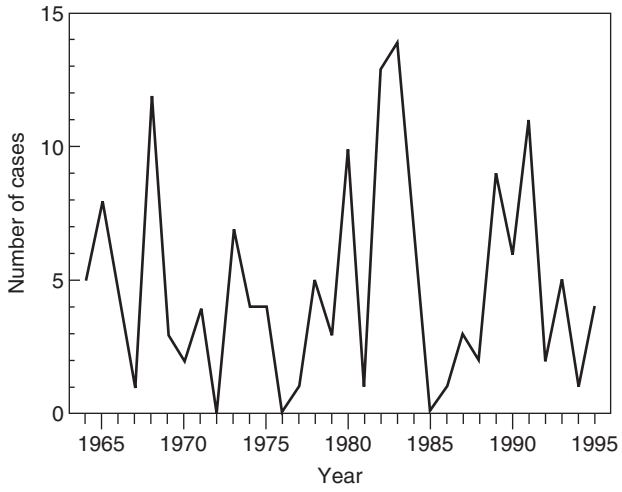

FIGURE 174A-1 ■ Reported cases of eastern equine encephalitis by year, 1964 to 1995.

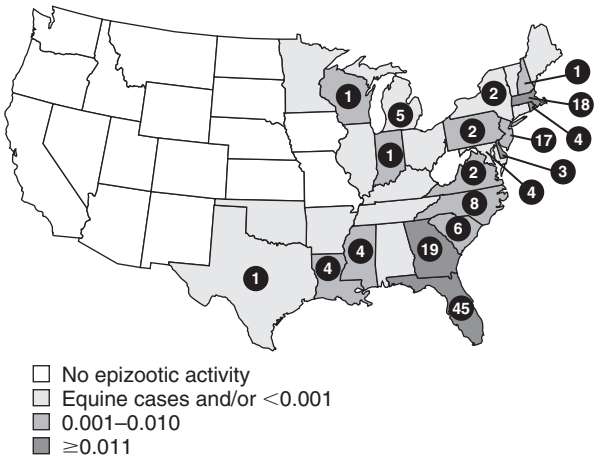

FIGURE 174A-2 ■ Reported cases and average annual incidence (per 100,000) of eastern equine encephalitis by state, 1964 to 1995.

(Fig. 174A-2). However, these estimates obscure the remarkably consistent and focal distribution of cases on the Atlantic and Gulf Coasts, from Ontario to Texas, and in isolated pockets of activity inland.[5,9,20] For example, until 1995, all human cases in Massachusetts had occurred east of Highway 495 in Essex, Norfolk, Plymouth, Bristol, and Middlesex counties; the six southernmost counties account for most of the epizootic activity in New Jersey; and foci of EEE viral activity recur in upstate New York counties near Syracuse, southwestern Michigan, northeastern Indiana, and northeastern Florida.[12,20,29,31,35] Outbreaks of human cases caused by North American viral strains have been reported from Jamaica, Trinidad, and the Dominican Republic.[6,17,18] Isolated epizootics with sporadic human cases have occurred in South America.[58] The unpredictability of risk is underscored by the occurrence of a case in a United Kingdom tourist who had a brief itinerary in Massachusetts.[32] Therefore, history of travel should be obtained in patients with possible EEE who are diagnosed in nonendemic areas.

Viral transmission, reflected in equine cases, occurs all year in Florida, although the peak incidence is from May to September.[7] Human cases have appeared as early as February and as late as December in Florida, with a peak occurring from June to August. In the Northeast, cases usually appear in late summer, from August to September, and as late as the third week in October.[40]

Cases occur chiefly at the extremes of age. However, serologic studies performed during a New Jersey outbreak disclosed that infection occurs with equal frequency in all age groups, thus indicating that biologic responses to infection rather than factors associated with exposure were responsible for the lower attack rates in young and middle-aged adults.[27] The ratio of inapparent infections to cases was highest in the middle years of life (29:1) and lowest in children younger than 4 years (8:1) and adults older than 55 years (16:1).[27]

Family clusters were observed in this epidemic and in the 1947 Louisiana outbreak.[33] In the New Jersey study, family members of cases had twice the rate of inapparent infections as the general population did; and in the southern Louisiana outbreak, two fatal cases and two seropositive members were observed in the same family.[27,33] Clusters of equine cases on a single premise are reported frequently.

Asymptomatic infections are uncommon occurrences, and long-term residence in an enzootic area leads to only a slight increase in population immunity. For example, even in an unusually active focus in southern New Jersey, only 7 percent of persons who had resided there 45 years or more had neutralizing antibody.[27] In serosurveys of endemic foci in Massachusetts, evidence of past infections was found in 0.5 to 0.7 percent of residents[51] and a postepizootic serosurvey of at-risk Connecticut pheasant farmers showed no evidence of infection.[43]

Specific behavioral risk factors have not been described; however, residence, travel, and outdoor activity near swampy habitats have been reported anecdotally as possible contributing factors.

CLINICAL MANIFESTATIONS

EEE is a fulminant encephalitis with rapid progression to coma and death in one third of patients. In infants and children, an abrupt onset of fever, irritability, and headache is followed closely by lethargy, confusion, seizures, and coma. The often desperately ill infants may have a bulging fontanelle, meningismus, high temperature, and generalized flaccid or spastic paralysis.[2,19,24,61,63] Some patients are evaluated initially for status epilepticus. The prodrome in adults and older children usually is brief, with nonspecific symptoms of fever, headache, and dizziness followed by clouding of the sensorium and rapid deterioration to coma. Remarkably, some patients have a prolonged prodrome lasting longer than a week, with a waxing and waning course of nonspecific symptoms, which may be associated with a better prognosis.[49,51,61] Various neurologic deficits have been described; in some cases, unilateral seizures, hemiparesis, hemiplegia, and aphasia have indicated focal areas of involvement.[16,46,49]

The peripheral leukocyte count usually is elevated with a shift to the left. White blood cell counts of 20,000/μL are typical, ranging as high as 60,000 with 55 to 89 percent neutrophils.[51] Cerebrospinal fluid (CSF) usually shows a polymorphonuclear pleocytosis with a total leukocyte count ranging from 500 to 2,000/μL. The initial neutrophilic pleocytosis of 60 to 100 percent may persist into the second week before shifting to a predominance of mononuclear cells.[52] CSF protein level is elevated and glucose concentration is reduced in most cases. Erythrocyte counts higher than 500/μL are found in some cases.

Imaging studies disclose only cerebral edema in three fourths of patients. However, focal rim-enhancing lesions and areas of low alteration with mass effect have been observed on computed tomographic scans in the frontal cortex, thalami, midbrain, and lentiform nuclei.[16,46,49] Isointense diffusion-weighted images and hyperintensity on apparent diffusion coefficient images early in the course of illness in one case suggested vasogenic edema in the T2-weighted hyperintense areas.[28] Magnetic resonance imaging also shows focal lesions in basal ganglia and thalami in patients with EEV.[18] Electroencephalographic tracings with focal or background slowing have been associated with a favorable outcome, whereas disorganized background activity, a burst-suppression pattern, and high-voltage delta slowing have been associated with a poor prognosis.[51]

Mild nonencephalitic illnesses usually are not diagnosed. However, in the 1959 outbreak in New Jersey, fever, headache, nausea, vomiting, and sore throat were common symptoms in 19 patients who had serologic evidence of infection. A third of the patients had illnesses sufficiently severe to motivate them to consult their physicians.[27] Other patients with bladder dysfunction, dysesthesias, weakness, and signs of myelitis have been described.[10,39] One case of infection during pregnancy has been reported. The third-trimester infection was severe, but the woman recovered from encephalitis and coma and was delivered of an apparently normal neonate. Serologic studies of the neonate's blood were not performed. Infection with EEV also was reported to trigger fatal hemophagocytic lymphohistiocytosis in an infant.[43]

PATHOLOGY

Pathologic changes in the brain are characterized by lesions in the cortical and deep gray matter, with varying degrees of neuronal loss from mild to extensive focal necrosis.[15,47] Viral antigen is found predominantly in neurons and only occasionally in astrocytes.[23] Rare viral particles have been identified by electron microscopy in principally extracellular locations.[3,37] Neutrophils predominate in cellular infiltrates in the meninges, vascular cuffs, and foci of tissue damage in the cortex and brain nuclei of patients dying acutely; at later stages, neutrophilic infiltrates are replaced by mononuclear cells.[3,15,23,47] Immunohistochemical examination of one patient disclosed a predominance of helper T cells in perivascular infiltrates, with some B lymphocytes. The most intense inflammatory reaction occurred in areas where antigen-positive neurons were absent, presumably where cell lysis already had occurred. Perivascular macrophages contained cleared viral antigen, but antigen could not be demonstrated in vascular endothelial cells.[23]

PATHOGENESIS

After peripheral inoculation of experimental animals, local viral replication occurs at a low level or may be undetectable. Viremia develops after this eclipse period, with disseminated infection of the spleen, liver, and kidneys noted in monkeys and guinea pigs and infection of the spleen, heart, and lungs observed in mice. Infectivity can be demonstrated in the brain only after viremia and infection in the viscera are established, thus suggesting that invasion of the central nervous system occurs by hematogenous spread.[47] Neurologic injury is associated with vasogenic edema in the earliest stages and subsequently with virus-induced cytolysis and host inflammatory responses.[28]

The immune response in humans presumably is similar to that observed in experimental alphavirus infections in mice, in which host resistance depends on rapid elaboration of a humoral immune response.

PROGNOSIS AND SEQUELAE

The case-fatality ratio is 33 percent among reported cases and is highest in the elderly; outcome is best in young adults aged 20 to 59 years, in whom the case-fatality rate is 24 percent.[35] Patients with a long prodromal illness (>4 days) have a better prognosis, which is consistent with the protective effect of a peripheral antibody response in the pre-neuroinvasive phase.[44]

Residual neurologic damage is observed more often in young children. In a Florida series, serious sequelae were seen in 4 of 7 survivors younger than 5 years and in 1 of 10 survivors in other age groups.[7] Similar findings were reported in a follow-up of epidemic cases in Massachusetts: 7 of 8 survivors younger than 3 years of age had neurologic sequelae, and only 1 of 4 surviving adults had residua.[2,21] Neurologic impairment ranged from mild unilateral spasticity to profound mental retardation, seizure disorders, and quadriplegia.

DIAGNOSIS

A specific laboratory diagnosis usually is made from serologic studies. Isolation of virus from CSF is unusual. The virus should be sought from brain biopsy tissue or autopsy material. EEE virus grows rapidly in a variety of cell lines, including Vero A549 and MRC-5 cells, and causes widespread cytopathic effect in several days.[55] Specific identification of viral isolates can be accomplished rapidly by immunofluorescent or immunoperoxidase techniques. EEE virus has been identified by immunofluorescence and by electron microscopy in brain tissue.[3,23,37] Viral antigen in viremic bird blood, infected mosquito pools,

and infected equine brains can be detected directly by antigen capture enzyme-linked immunosorbent assay. Polymerase chain reaction analysis of CSF has been reported but has not been evaluated extensively for routine clinical diagnosis.[52,54]

Serologic testing is available in many state and reference laboratories and is currently the preferred method for diagnosis in patients. Virus-specific IgM usually can be detected in acute serum and CSF samples.[44] Indirect immunofluorescence, neutralization, and hemagglutination inhibition also are sensitive procedures. Antibodies often are present in the first week of illness. Neutralizing antibody appears 3 to 4 days after the onset of illness, and hemagglutination-inhibition (HI) antibody appears with almost equal rapidity.[27] Both HI and neutralizing antibodies appear to be long lived.[27] Complement-fixing (CF) antibody is slower to rise and can be noted 11 days after onset, with diagnostic fourfold rises often appearing only in the third week after onset.[27] The peaks of both HI and CF titers are observed 3 to 4 weeks after onset.[28] CF antibody declines more rapidly. Measurable CF antibodies were found in approximately 50 percent of persons infected 8 years earlier in one study, although the effects of reexposure could not be ruled out in this endemic area.[27]

The low prevalence of EEE antibody in the general population suggests that detection of EEE viral antibody in the acute serum of a patient with encephalitis indicates a high probability of that diagnosis. Applying the Bayes theorem, if the "rate" of EEE is 1 in every 2000 cases of encephalitis, if HI antibody to EEE is present in 100 percent of cases in the first week of illness, and if the prevalence of HI antibody in the general population is 0.05 percent, the presence of EEE in a patient with encephalitis who has demonstrable EEE antibody in a single serum specimen is a certainty.[59] Thus, the probability of the diagnosis is high when specific antibody is found in any (i.e., acute) serum specimen.

DIFFERENTIAL DIAGNOSIS

The fulminant clinical course of EEE, the laboratory findings of neutrophilic leukocytosis and polymorphonuclear pleocytosis in the CSF, and focal findings on brain imaging may suggest bacterial cerebritis or meningitis. Because no specific therapy is available, EEE should be a diagnosis of exclusion after every feasible effort has been made to diagnose and to treat empirically against bacterial and herpes viral infections, as well as exclusion of other herpesvirus and arbovirus infections, influenza virus, enterovirus, rabies virus, and other causes of encephalitis in humans.

TREATMENT

Specific treatment is unavailable. Therapy aimed at supporting cardiorespiratory function, homeostasis of fluid, electrolyte balance, and control of cerebral edema and convulsions may be lifesaving. Antiviral research and development initiatives have focused on agents with activity against EEV, but no specific antiviral agent is licensed for treatment of EEV in humans.[53] In a single case, intravenous immunoglobulin and glucocorticoids, given for their potential immunomodulatory effects, were associated with survival in an elderly patient.[28]

PREVENTION

An effective killed vaccine is licensed for horses, but no human vaccine is licensed. An investigational killed vaccine, available under investigatory permit, is used to protect laboratory personnel.[52] Other vaccine candidates, such as a Sindbis/EEE virus chimeric vaccine and a recombinant EEE virus attenuated by internal ribosomal entry site control, have been tested in vitro and in animal models.[48,60] Vaccination of the general public is not feasible as a public health measure because of the low incidence of disease.

Climatologic studies have shown a correlation between outbreaks and heavy rainfall in the summer of an epidemic year and in the preceding fall.[34,38,40] Although such predictors would have considerable utility in guiding control measures, outbreaks of EEE have been too few for their validity to be tested. Isolation of Highlands J virus, which shares a common enzootic cycle with EEE virus, often peaks 2 to 3 weeks before the appearance of EEE virus in *C. melanura*.

Surveillance and public health interventions to prevent EEE in endemic areas have been shown to be economic when they are balanced against the direct and indirect costs of even one human case.[60] Infection rates in *C. melanura* of 0.39/1000 have been highly predictive of risk for human cases in areas of Massachusetts surveyed during a 26-year interval.[32] Larviciding of swampland to control *C. melanura* is difficult because of the large areas involved, the potential toxic effects in fish and other wildlife, and the relative inaccessibility of the larvae. Emergency application of adulticides to control epizootic vectors is indicated when viral, mosquito, and animal surveillance suggests a risk for epizootic transmission. Public health advisories to avoid outdoor activity near enzootic foci and closure of campgrounds and parks in these locations may be necessary when viral transmission indices suggest a high level of risk. The use of repellents and avoidance of outdoor activity 1 to 2 hours after sunset, when many mosquitoes are most active, may reduce the risk of exposure; however, some vector *Aedes* spp. are daytime biters.

NEW REFERENCES SINCE THE SIXTH EDITION

18. Ethier M, Rogg J. Eastern equine encephalitis: MRI findings in two patients. *Med Health RI* 2012;**95**:227–9.
30. Graham S, Hassan H, Chapman T, et al. Serosurveillance of eastern equine encephalitis virus in amphibians and reptiles from Alabama, USA. *Am J Trop Med Hyg* 2012;**86**:540–4.
32. Harvala H, Bremner J, Kealey S, et al. Case report: eastern equine encephalitis virus imported to the UK. *J Med Virol* 2009;**81**:305–8.
36. Kelen P, Downs J, Burkett-Cadena N, et al. Habitat associations of eastern equine encephalitis transmission in Walton County Florida. *J Med Entomol* 2012;**49**:746–56.

43. Mancao, M. Imran H, Chandra S, et al. Eastern equine encephalitis virus infection and hemophagocytic lymphohistiocytosis in a 5-month old infant. *Pediatr Infect Dis J* 2009;**28**:543–5.
48. Pandya J, Gorchakov R, Wang E, et al. A vaccine candidate for eastern equine encephalitis virus based on IRES-mediated attenuation. *Vaccine* 2012;**30**:1276–82.
52. Reisler R, Gibbs P, Danner, D, et al. Immune interference in the setting of same day-administration of two similar inactivated alphavirus vaccines: eastern equine and western equine encephalitis. *Vaccine* 2012;**30**:7271–7.
53. Sampath A, Metz M, Stundick M, et al. State-of-the-art therapeutic medical countermeasures for viral threat agents. *Biosecur Bioterror* 2011;**9**:351–60.
60. Wang E, Petrakova O, Adams A, et al. Chimeric Sindbis/eastern equine encephalitis vaccine candidates are highly attenuated and immunogenic in mice. *Vaccine* 2007;**25**:7573–81.

The full reference list for this chapter is available at expertconsult.com.

174B ■ WESTERN EQUINE ENCEPHALITIS

Theodore F. Tsai • Gail J. Harrison

Western equine encephalitis (WEE) is an endemic and enzootic acute central nervous system (CNS) infection of humans and horses in the western part of the United States, Canada, Mexico, and parts of South America. In North America, *Culex tarsalis*, the principal mosquito vector, also maintains the virus in an avian enzootic cycle.[77] WEE virus is currently classified as a Biological Safety Level 3 (BSL-3) agent because it is highly infectious and potentially may be transmitted via aerosol and a Category B bioterrorism agent based on its moderate ability to disseminate in the population and produce serious disease in humans.[64]

ETIOLOGIC AGENT

WEE and other alphaviruses (group A arboviruses) form a genus of principally mosquito-borne viruses in the family *Togaviridae*.[47,68] Three of the eight viruses constituting the WEE antigenic complex are found in North America: Highlands J, Fort Morgan, and WEE viruses; among them, only WEE virus is a human pathogen. Much of what is known about the molecular biology of alphaviruses has been inferred from studies of the Sindbis (the type species and a member of the WEE complex) and Semliki Forest viruses.

Alphaviruses are small, enveloped, positive-stranded RNA viruses. Virions are spherical and 69 nm in diameter (including the length of their glycoprotein spikes), with a lipid bilayer enveloping a nucleocapsid core containing the 11.7-kb RNA viral genome.[64] Glycoprotein spikes embedded in the viral envelope bind to cell membrane receptors and initiate infection by endocytotic fusion. The viral and lysosomal membranes fuse in a pH-dependent step, and the viral nucleocapsid is released into the cell cytoplasm, where RNA and protein synthesis occurs. The 5′ terminal two thirds of the RNA genome encodes four nonstructural proteins, and the 3′ terminal third of the RNA genome encodes the three structural proteins. The 30-kd nucleocapsid and two 50-kd glycoproteins—E1 and E2—are translated as a polyprotein from subgenomic 26S RNA. The envelope proteins—E1 and E2—are assembled in trimers of a single E2 protein and an E1 dimer. Eighty such trimer spikes are arranged in an icosahedral lattice on the virion surface.[10] Capsid proteins assemble with the viral genome in the cytoplasm and then bud through the virally modified cell membrane and acquire an envelope.

The E1 glycoprotein possesses a group-reactive hemagglutinin and group-reactive epitopes linked to cross-protection and cell-mediated cytotoxic effects. E1 also mediates viral cellular membrane fusion and has been explored as a vaccine target.[69] Epitopes on E2 are linked to cell receptor recognition, viral neutralization and clearance, and neurovirulence and cellular apoptosis.[73]

Molecular genetic studies indicate that WEE virus arose more than 1000 years ago as a recombinant of eastern equine virus and an ancestral, now extinct, Sindbis-like virus, with the recombinant having acquired the neurotropic potential of eastern equine encephalitis (EEE) virus while retaining the antigenic and nucleocapsid characteristics of the non-neurotropic Sindbis virus.*

Alphaviruses are thought to have originated in the New World with separate introductions, presumably by birds, to the Old World, resulting in establishment of the present-day Sindbis-like viruses and Semliki Forest–related viruses. A slow rate of evolution, circa 10^{-4} nucleotide changes per year (vs. 10^{-2}/year for other RNA viruses), probably reflects the natural pressure on viruses constrained to replicate in both insect and vertebrate cells.[71]

ECOLOGY

WEE virus is transmitted in an enzootic cycle to mosquitoes, birds, and other vertebrate hosts.[50,51,55] Horses and humans are dead-end hosts, but severe CNS infection may develop. Although WEE has been a public health and veterinary problem primarily in the western United States and Canada, the geographic range of the virus includes Mexico, Guyana, Brazil, Uruguay, and Argentina. Epizootics in horses, accompanied by small numbers of human cases, were reported in 1972 and 1983 in Argentina, where the virus apparently is transmitted by *Aedes albifasciatus* to introduced European hares and

*References 21, 47, 60, 70, 71, 64.

REFERENCES

1. Arrigo N, Adams A, Watts D, et al. Cotton rats and house sparrows as hosts for North and South American strains of eastern equine encephalitis virus. *Emerg Infect Dis* 2010;**16**:1373–80.
2. Ayres JC, Feemster RF. The sequelae of eastern equine encephalomyelitis. *N Engl J Med* 1949;**240**:960–2.
3. Bastian FO, Wende RD, Singer DB, et al. Eastern equine encephalomyelitis: histopathologic and ultrastructural changes with isolation of the virus in a human case. *Am J Clin Pathol* 1975;**64**: 10–13.
4. Beaudette FR, Black JJ, Hudson CB, et al. Equine encephalomyelitis in pheasants from 1947 to 1951. *J Am Vet Med Assoc* 1952;**121**:478–83.
5. Bellavance R, Rossier E, Le Maitre MP. Eastern equine encephalitis in eastern Canada: 1972. *Can J Public Health* 1973;**64**:189–90.
6. Belle EA, Grant LS, Thorburn MJ. An outbreak of eastern equine encephalomyelitis in Jamaica, part II: laboratory diagnosis and pathology of eastern equine encephalomyelitis in Jamaica. *Am J Trop Med Hyg* 1964;**13**:335–41.
7. Bigler WJ, Lassing EB, Buff EE, et al. Endemic eastern equine encephalomyelitis in Florida: a twenty-year analysis, 1955-1974. *Am J Trop Med Hyg* 1976;**25**:884–90.
8. Brault AC, Powers AM, Chavez CL, et al. Genetic and antigenic diversity among eastern equine encephalitis viruses from North, Central, and South America. *Am J Trop Med Hyg* 1999;**6**: 579–86.
9. Carman PS, Artsob H, Emery S, et al. Eastern equine encephalitis in a horse from southwestern Ontario. *Can Vet J* 1995;**36**:170–2.
10. Clarke DH. Two nonfatal human infections with the virus of eastern encephalitis. *Am J Trop Med Hyg* 1961;**10**:67–70.
11. Crans WJ, Caccamise DF, McNelly JR. Eastern equine encephalomyelitis virus in relation to the avian community of a coastal cedar swamp. *J Med Entomol* 1994;**31**:711–28.
12. Davenport DS, Batts DH, Carter JW. Eastern equine encephalitis in Michigan. *Arch Neurol* 1982;**39**:322–3.
13. Day JF, Stark LM. Eastern equine encephalitis transmission to emus *(Dromaius novaehollandiae)* in Volusia County, Florida: 1992 through 1994. *J Am Mosq Control Assoc* 1996;**12**:429–36.
14. Dein FJ, Carpenter JW, Clark GG, et al. Mortality of captive whooping cranes caused by eastern equine encephalitis virus. *J Am Vet Med Assoc* 1986;**9**:1006–10.
15. de la Monte SM. Selective vulnerability of particular central nervous system regions to eastern equine encephalitis virus in humans. *J Neuropathol Exp Neurol* 1985;**44**:358.
16. Deresiewicz RL, Thaler SJ, Hsu L, Zamani AA. Clinical and neuroradiographic manifestations of eastern equine encephalitis. *N Engl J Med* 1997;**336**:1867–74.
17. Eklund CM, Bell JF, Brennan JM. Antibody survey following an outbreak of human and equine disease in the Dominican Republic, caused by the eastern strain of equine encephalomyelitis virus. *Am J Trop Med Hyg* 1951;**31**:312–28.
18. Ethier M, Rogg J. Eastern equine encephalitis: MRI findings in two patients. *Med Health RI* 2012;**95**:227–9.
19. Farber S, Hill A, Connerly ML, et al. Encephalitis in infants and children. *JAMA* 1940;**114**:1725–31.
20. Feemster RF. Equine encephalitis in Massachusetts. *N Engl J Med* 1957;**257**:701–4.
21. Feemster RF, Getting VA. Distribution of the vectors of equine encephalomyelitis in Massachusetts. *Am J Public Health* 1941;**31**: 791–802.
22. Feemster RF, Wheeler RE, Daniels JB, et al. Field and laboratory studies on equine encephalitis. *N Engl J Med* 1958;**259**:107–13.
23. Garen PD, Tsai TF, Powers JM. Human eastern equine encephalitis: immunohistochemistry and ultrastructure. *Mod Pathol* 1999; **12**:646–52.
24. Getting VA. Equine encephalomyelitis in Massachusetts. *N Engl J Med* 1941;**24**:999–1006.
25. Gibbs PJ, Tsai T. Eastern encephalitis. In: Beran G, editor. *Handbook of zoonoses: section B, viral*. Boca Raton, Fla.: CRC Press; 1994.
26. Goldfield M, Sussman O, Black HC, et al. Arbovirus infection of animals in New Jersey. *J Am Vet Med Assoc* 1968;**153**:1780–7.
27. Goldfield M, Taylor BF, Welsh JN. The 1959 outbreak of eastern encephalitis in New Jersey. *Am J Epidemiol* 1968;**87**:18–57.
28. Golomb MR, Durand ML, Schaefer PW, et al. A case of immunotherapy-responsive eastern equine encephalitis with diffusion-weighted imaging. *Neurology* 2001;**56**:420–1.
29. Grady GF, Maxfield HK, Hildreth SW, et al. Eastern equine encephalitis in Massachusetts, 1957-1976: a prospective study centered upon analyses of mosquitoes. *Am J Epidemiol* 1978;**107**: 170–8.
30. Graham S, Hassan H, Chapman T, et al. Serosurveillance of eastern equine encephalitis virus in amphibians and reptiles from Alabama, USA. *Am J Trop Med Hyg* 2012;**86**:540–4.
31. Hachiya M, Osborne M, Stinson C, et al. Human eastern equine encephalitis in Massachusetts: predictive indicators from mosquitoes collected at 10 long-term trap sites, 1979-2004. *Am J Trop Med Hyg* 2007;**76**:285–92.
32. Harvala H, Bremner J, Kealey S, et al. Case report: eastern equine encephalitis virus imported to the UK. *J Med Virol* 2009;**81**: 305–8.
33. Hauser GH. Human equine encephalomyelitis, eastern type, in Louisiana. *New Orleans Med Surg J* 1948;**100**:551–8.
34. Hayes RO, Hess AD. Climatological conditions associated with outbreaks of eastern encephalitis. *Am J Trop Med Hyg* 1964;**13**: 851–8.
35. Howard JJ, Grayson MA, White DJ, Oliver J. Evidence for multiple foci of eastern equine encephalitis virus *(Togaviridae: Alphavirus)* in central New York State. *J Med Entomol* 1996;**33**:421–32.
36. Kelen P, Downs J, Burkett-Cadena N, et al. Habitat associations of eastern equine encephalitis transmission in Walton County Florida. *J Med Entomol* 2012;**49**:746–56.
37. Kim JH, Booss J, Manvelidis EE, et al. Human eastern equine encephalitis: electron microscopic study of a brain biopsy. *Am J Clin Pathol* 1985;**84**:223–7.
38. Komar N, Spielman A. Emergence of eastern encephalitis in Massachusetts. *Ann N Y Acad Sci* 1995;**740**:157–68.
39. Lavoie SR, Markowitz S, Kapadia SJ. Eastern equine encephalomyelitis with hematuria and bladder dysfunction. *South Med J* 1993;**86**:812–14.
40. Letson GW, Bailey RE, Pearson J, et al. Eastern equine encephalitis (EEE): a description of the 1989 outbreak, recent epidemiologic trends, and the association of rainfall with EEE occurrence. *Am J Trop Med Hyg* 1993;**49**:677–85.
41. Levitt LP, Lovejoy FH, Daniels JB. Eastern equine encephalitis in Massachusetts: first human case in 14 years. *N Engl J Med* 1971;**284**:540.
42. Liao SJ. Eastern equine encephalitis in Connecticut: a serological survey of pheasant farmers. *Yale J Biol Med* 1955;**27**:287–96.
43. Mancao M, Imran H, Chandra S, et al. Eastern equine encephalitis virus infection and hemophagocytic lymphohistiocytosis in a 5-month old infant. *Pediatr Infect Dis J* 2009;**28**:543–5.
44. Martin DA, Muth DA, Brown T, et al. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000;**38**: 1823–6.
45. McLean RG, Frier G, Parham GL, et al. Investigations of the vertebrate hosts of eastern equine encephalitis during an epizootic in Michigan. *Am J Trop Med Hyg* 1985;**34**:1190–202.
46. Morse RP, Bennish ML, Darras BT. Eastern equine encephalitis presenting with a focal brain lesion. *Pediatr Neurol* 1992;**8**: 473–5.
47. Nathanson N, Stolley PD, Boolukos PJ. Eastern equine encephalitis: distribution of central nervous system lesions in man and rhesus monkeys. *J Comp Pathol* 1969;**79**:109–15.
48. Pandya J, Gorchakov R, Wang E, et al. A vaccine candidate for eastern equine encephalitis virus based on IRES-mediated attenuation. *Vaccine* 2012;**30**:1276–82.
49. Piliero PJ, Brody J, Zamani A, et al. Eastern equine encephalitis presenting as focal neuroradiographic abnormalities: case report and review. *Clin Infect Dis* 1994;**18**:985–8.
50. Powers AM, Brault AC, Shirako Y, et al. Evolutionary relationships and systematics of the alphaviruses. *J Virol* 2001;**75**:10118–31.
51. Przelomski MM, O'Rourke E, Grady GF, et al. Eastern equine encephalitis in Massachusetts: a report of 16 cases 1970-1984. *Neurology* 1988;**38**:736–9.
52. Reisler R, Gibbs P, Danner D, et al. Immune interference in the setting of same day-administration of two similar inactivated

alphavirus vaccines: eastern equine and western equine encephalitis. *Vaccine* 2012;**30**:7271–7.
53. Sampath A, Metz M, Stundick M, et al. State-of-the-art therapeutic medical countermeasures for viral threat agents. *Biosecur Bioterror* 2011;**9**:351–60.
54. Sanchez-Seco MP, Rosario D, Quiroz E, et al. A generic nested-RT-PCR followed by sequencing for detection and identification of members of the alphavirus genus. *J Virol Methods* 2001;**95**: 153–61.
55. Sotomayor EA, Josephson SL. Isolation of eastern equine encephalitis virus in A549 and MRC-5 cell cultures. *Clin Infect Dis* 1999; **29**:193–5.
56. Sudia WD, Stamm DD, Chamberlain RW, et al. Transmission of eastern equine encephalitis to horses by *Aedes sollicitans* mosquitoes. *Am J Trop Med Hyg* 1956;**5**:802–8.
57. Tengelsen LA, Bowen RA, Royals MA, et al. Response to and efficacy of vaccination against eastern equine encephalomyelitis virus in emus. *J Am Vet Med Assoc* 2001;**218**:1469–73.
58. Tikasingh ES, Ardoin P, Everard COR, et al. Eastern equine encephalitis in Trinidad, epidemiological investigations following two human cases of South American strain in Santa Cruz. *Trop Geogr Med* 1973;**25**:355–61.
59. Villari P, Spielman A, Komar N, et al. The economic burden imposed by a residual case of eastern encephalitis. *Am J Trop Med Hyg* 1995;**52**:8–13.
60. Wang E, Petrakova O, Adams A, et al. Chimeric Sindbis/eastern equine encephalitis vaccine candidates are highly attenuated and immunogenic in mice. *Vaccine* 2007;**25**:7573–81.
61. Wesselhoeff C, Smith EC, Branch CF. Human encephalitis: eight fatal cases with four due to virus of equine encephalomyelitis. *JAMA* 1938;**111**:1735–41.
62. Williams SM, Fulton RM, Patterson JS, et al. Diagnosis of eastern equine encephalitis by immunohistochemistry in two flocks of Michigan ring-neck pheasants. *Avian Dis* 2000;**44**:1012–16.
63. Winter WD Jr. Eastern equine encephalomyelitis in Massachusetts in 1955. *N Engl J Med* 1956;**255**:262–70.

possibly birds. In addition, a distinct sylvatic subtype of WEE virus is transmitted in the subtropical Chaco province.[4,7]

In the United States, the geographic distribution of the virus and *C. tarsalis*, its principal vector, includes the western and central United States, southern Canada, and Mexico. A related virus—Highlands J virus, isolated from *Culiseta melanura* in the eastern United States—overlaps in its range in the east central part of the United States. Highlands J virus causes encephalitis in horses and possibly in humans.[38]

In the western region of the United States, *C. tarsalis* breeds in ground pools found on pasture lands; in irrigation wastewater; and at the margins of lakes, ponds, marshes, and flooded riversides. Some of these aquatic habitats are shared by birds that participate in the amplification of virus in nature.[50,51,55]

The female mosquito becomes infected after feeding on a viremic bird (or mammal or possibly reptile). After an extrinsic incubation period of 7 to 9 days, when the virus propagates in the mosquito and the salivary glands become infected, the mosquito can transmit virus to other birds or mammals or reptiles (amplifying hosts) or to humans and horses (dead-end hosts).

Passerine (perching) birds, especially sparrows and finches, have proved to be particularly important in amplification of the virus. In midsummer, the mosquito shifts its host-seeking activity to mammals. The shift may be influenced by an increase in the defensive behavior of nestling birds, which are its preferred host. The shift corresponds temporally to the appearance of cases in horses and people and appears to be a critical element enabling *C. tarsalis* to function as both an enzootic and an epizootic vector. In California, an auxiliary *Aedes melanimon*–jackrabbit *(Lepus californicus)* cycle has been demonstrated.

The overwintering mechanism for WEE virus has not been elucidated. However, virus has been recovered from adult *Aedes dorsalis* mosquitoes collected as larvae from a coastal area of California, thus indicating a possible role for vertical transmission of the virus in mosquitoes in some locations. Arguments also have been advanced for viral overwintering in adult mosquitoes and in persistently infected mammals, birds, and poikilotherms (snakes, frogs, and turtles). Experimental infection of snakes (garter snakes) and tortoises show them to be susceptible to chronic infection with WEE virus.[3] Seroepidemiology studies in the field show high prevalence of WEE virus antibody in reptile hosts, including snakes, lizards, turtles, and crocodiles. Reptiles, because of their low metabolic rate, may function as a reservoir host over the winter and transmit WEE virus to humans via an arthropod vector.[3] In Canada, *Aedes* spp. and *Culiseta inornata*, which emerge in the spring before *C. tarsalis*, have been proposed as early amplifying vectors.[37]

Numerous climatologic and biologic indices with various degrees of predictive value have been shown to correlate with the occurrence of WEE outbreaks; these indices include vector population size, mosquito infection rates, and virus transmission rates to sentinel chickens or wild-caught sparrows. Such transmission indices are monitored in surveillance activities by public health agencies; however, their sensitivity, specificity, and predictive value for forecasting epidemics have been difficult to evaluate because of the sporadic nature of outbreaks.[72] A retrospective analysis of a 21-year experience in California showed correlation between average daily numbers of *C. tarsalis* females and the occurrence of human encephalitis.[44] In a Texas study, house sparrow infection and antibody rates were the best predictors of human disease, and *C. tarsalis* light trap indices were of borderline significance.[27]

Physical measures associated with the incidence of disease in humans or transmission of virus to sentinel species include the ambient air temperature, the snow pack in mountains providing runoff water, the river flow rate, the soil temperature inversion date (the date that the surface soil temperature exceeds the subsurface soil temperature), and the date when 50 or more days of 21°C (70°F) temperature have accumulated. Snow pack and river flow rate are associated with an abundance of irrigation water and flooding, which in turn are associated with the availability of breeding habitats and mosquito population size.[8,26,50,51,52]

Longitudinal and intraseasonal observations show that high temperatures are associated with a reduced risk for human disease. High ambient air temperature (32°F [>89.6°F]) decreases mosquito survival, adversely affects the competence of *C. tarsalis* to become infected with and to transmit WEE virus, and limits host-seeking activity.[56] In a model of global warming, higher temperatures are predicted to move the range of WEE viral transmission northward.[51,52]

EPIDEMIOLOGY

WEE occurs sporadically and in epidemic form, principally in Canadian provinces and states west of the Mississippi River (Figs. 174B-1 and 174B-2). Infections occur mainly in rural areas, where water impoundments, irrigated farmland, and naturally flooded sites

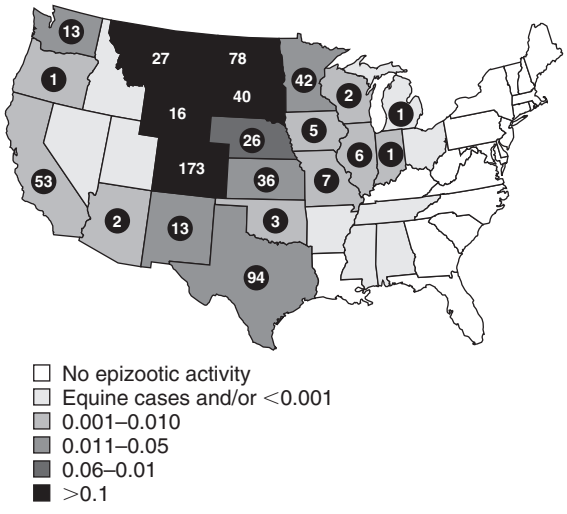

FIGURE 174B-1 ■ Reported cases and average annual incidence (per 100,000) of western equine encephalitis by state, 1964 to 1995.

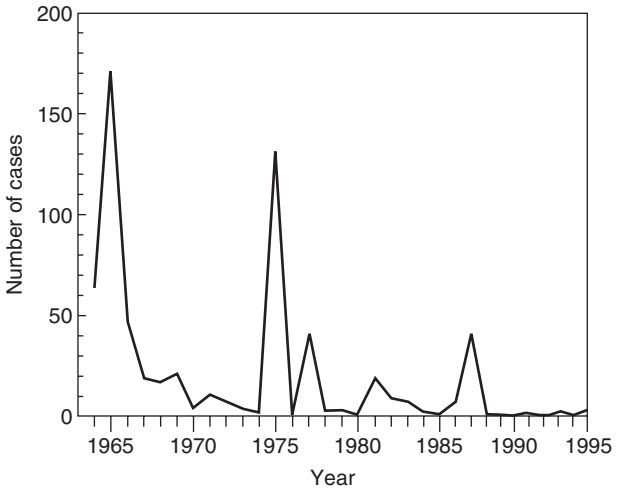

FIGURE 174B-2 ■ Reported cases of western equine encephalitis by year, 1964 to 1995.

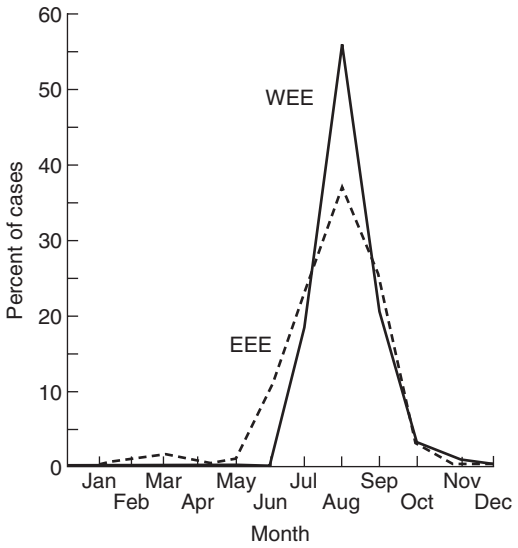

FIGURE 174B-3 ■ Reported cases of western and eastern equine encephalitis by month, 1972 to 1989.

provide breeding habitats for *C. tarsalis;* however, the increasingly rare interface between vector mosquitoes and humans has resulted in a point seroprevalence of 1 to 2 percent even in locations where the virus is transmitted in an enzootic cycle.[56] The annual median number of cases between 1964 and 1995 was only four, and only four cases were reported between 1988 and 1997, a lower incidence than that for EEE. Historically, however, periodic outbreaks have led to scores or hundreds of cases (Fig. 174B-3).

Recurrent endemic and epidemic transmission was recorded in the Yakima Valley, Washington (1939 to 1942); California's Central Valley (1939 to 1952); the north central states and Canadian provinces, including Minnesota, North and South Dakota, Alberta, Manitoba, and Saskatchewan (1941 and 1975); and the high plains panhandle of Texas (1963 to 1966).[15,22,28,49,54] The largest outbreak on record, in 1941, resulted in more than

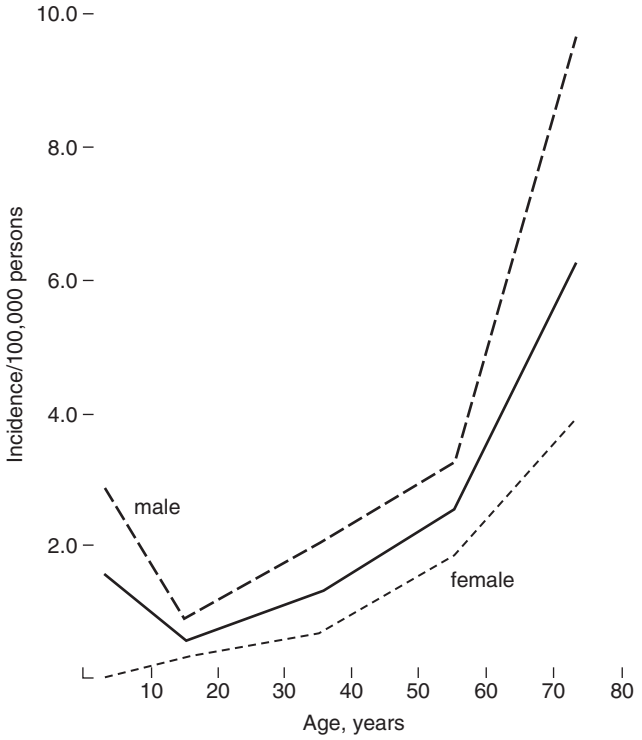

FIGURE 174B-4 ■ Age- and sex-specific incidence of western equine encephalitis, Colorado, 1987. ––, Male; —, total; ---, female.

3400 human cases in Minnesota, North and South Dakota, Nebraska, Montana, Alberta, Manitoba, and Saskatchewan; equine cases were estimated to number in the hundreds of thousands. The outbreak centered in North Dakota, where the attack rate for cases in the state was 167/100,000.[15,32] In 1952, an outbreak in California's Central Valley led to 348 reported cases, an incidence of 36/100,000 residents.[28] More recent outbreaks resulted in 277 cases in the central United States and Manitoba in 1975 and 40 cases in the central and mountain states in 1987.[13,33,37]

Most cases occur between June and September (see Fig. 174B-3), often preceded by cases occurring in horses several weeks earlier. Surveillance of equine cases is a widely used approach to assess the risk for epidemic transmission. However, the low frequency of laboratory-confirmed diagnoses, vaccination, and underreporting limit the precision of equine surveillance as a predictive marker.[46]

Several risk factors for acquiring WEE have been identified:

1. Attack rates usually are highest at the extremes of age[2] (Fig. 174B-4). The experience from the 1952 California outbreak showed that a third of cases occurred in infants younger than 1 year of age.[28,51] Other reports confirm the bimodal pattern of an elevated risk in infants, a declining risk in children and young adults, and a gradual increase in risk in the elderly.
2. Attack rates in males are twofold higher than those in females in every age group[2] (see Fig. 174B-2). Biologic differences in susceptibility to infection

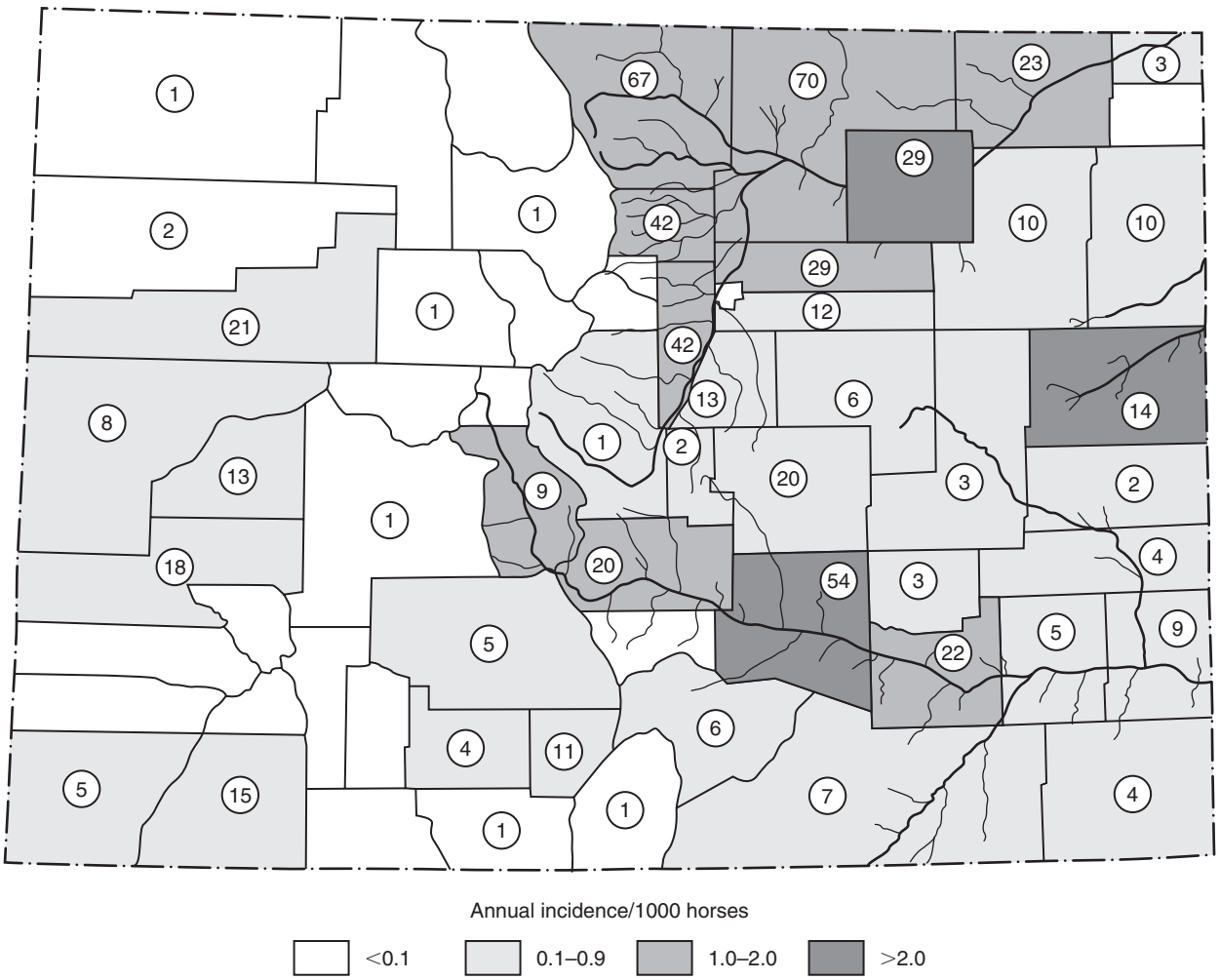

FIGURE 174B-5 ■ Reported equine cases and incidence of western equine encephalitis by county, Colorado, 1975 to 1988.

TABLE 174B-1 **Western Equine Encephalitis Epidemic Attack Rates by Population Density***

Population Area	Minnesota, 1941	Kern County, Calif, 1952	Hale County, Tex, 1963-1966	Manitoba, 1975
Rural	15.8-22.0	149.2	2.62	10.8
Small town	2.3-5.6			2.4
Urban		28.5	1.0	0.9

*Per 100,000.

may account for the observed disparity in infancy, and greater occupational and recreational exposure outdoors might be responsible for the differences in adults. In the 1981 outbreak in Manitoba, 21/25 cases were in males; the four affected women were widows who maintained their premises alone.[13]

3. Rural residence is associated with attack rates 1.5 to 5 times higher than those for urban residence (Table 174B-1). Counties lying in major river drainage areas and with more irrigated acreage have had the highest incidence of equine and human disease[2] (Fig. 174B-5).
4. Agricultural occupation has been suggested as a risk factor in several studies.
5. Length of residence in areas where WEE is endemic is associated inversely with a risk for developing illness. Acquired immunity, through asymptomatic or mild infection, accumulates with length of residence; in endemic areas, the point prevalence of specific antibody previously approached 20 percent by adulthood.[50,51]

CLINICAL MANIFESTATIONS

The clinical illness ranges from a nonspecific syndrome of headache and fever to aseptic meningitis, meningoencephalitis, and encephalitis with fatal outcome. The

estimated case-infection ratio is 1:58 in children 1 to 4 years of age, but it declines to 1:1150 in adults.[12,18,51,55]

The onset of illness typically is abrupt, with sudden fever, headache, malaise, chills, and nausea and vomiting,[5,15,25,49] occasionally preceded by signs of an upper respiratory tract infection. Signs of CNS infection gradually become evident as dizziness, drowsiness, increasing headache, stiff neck, and disorientation develop during the course of hours or days. Infants typically have a sudden cessation in feeding, fussiness, fever, and protracted vomiting. The prodromal interval is abbreviated, and seizures and a lethargic unresponsive state develop rapidly.

On examination, patients appear somnolent and may have signs of meningeal irritation. The sensorium is depressed, and patients may alternate between agitation and somnolence. Generalized muscle weakness is present, and deep tendon reflexes are diminished. Focal neurologic signs suggesting herpes encephalitis have been reported in anecdotal cases[1,6] however, in the 1941 Minnesota outbreak, only 3/226 cases reported had unilateral weakness.[15]

In infants, the fontanelle may be tense or bulging, often accompanied by spastic paresis and generalized seizures. The frequency of seizures is related inversely to age; they occur with greatest frequency in infants younger than 3 months (75% to 80% of cases), whereas in 2- to 4-year-old children, seizures have been reported in 15 percent of cases.[36]

The peripheral leukocyte count is unremarkable. Cerebrospinal fluid (CSF) obtained at an early stage in the illness generally exhibits normal glucose concentration, elevated protein level, and a leukocyte count between 10 and 300/mm^4.[33] Mononuclear cells usually predominate.

Five instances of late third-trimester infection in pregnant women have been reported to result in perinatal illness or encephalitis.[11,12,36] The women's illnesses had an onset 0 to 10 days before delivery, and the infants became ill on the fifth and sixth postpartum days. Teratogenic effects of infection occurring earlier in gestation have not been reported for WEE virus but are suspected for other group A arboviruses.[39]

PATHOLOGY

Specific pathologic changes are confined to the CNS.[5,33,42] On gross examination, the brain appears normal or may be congested and swollen, with minimal changes observed in the meninges. Microscopic lesions affecting gray and white matter appear throughout the brain but predominate in the basal ganglia. Disseminated small focal abscesses infiltrated with neutrophils are a distinctive feature. The vessels appear congested, and small focal hemorrhages or diffuse extravasations of erythrocytes are present along with neutrophilic infiltration of the vascular wall and endarteritis. Vascular lumina may be occluded by endothelial proliferation and swelling.

Extensive patchy areas of demyelination are found throughout the brain. Older lesions appear as sharply circumscribed, punched-out plaques. Secondary microglial reaction in demyelinated areas is minimal.

The spinal cord is affected in the same fashion, with focal, perivascular, and diffuse lesions attracting both polymorphonuclear and mononuclear cells. Lesions predominate in the central gray matter.

PATHOGENESIS

Peripheral inoculation of experimental animals with WEE virus is followed by viral replication in various extraneural sites before invasion of the CNS (including the peripheral site of inoculation, viscera, muscle, and perhaps vascular endothelial cells) occurs.[29,34,43,75]

Host resistance and recovery from infection depend chiefly on an effective antibody response. Antibody contributes to recovery by a variety of means, including viral neutralization and antibody-mediated restriction of viral gene expression.[20,73] Interferon may contribute to containment of local viral spread in the CNS. Antibodies are protective and may be cross-protective to other alphaviruses. Antibodies to the E2 glycoprotein typically are virus specific and associated with neutralization and protection. Antibodies to E1 exhibit greater cross-reactivity with other alphaviruses.[68] Immune serum given to monkeys prophylactically protects them from challenge with WEE virus, and passive protection has been demonstrated in monkeys inoculated with WEE virus and treated with immune serum within 24 hours.[75] However, immunotherapy in monkeys is uniformly unsuccessful in preventing death once signs of CNS infection have appeared. In guinea pigs peripherally inoculated with WEE virus, immunotherapy given within 24 to 48 hours of inoculation leads to survival of some animals and delayed death in others.[43] In one human case, passive immunization led to a delayed antibody response and was followed by the development of parkinsonism.[19]

The age-dependent virulence of specific Sindbis virus strains has been linked to the immaturity of the suckling mouse T- and B-cell repertoire. Fibroblasts from newborn but not weanling mice are susceptible to Sindbis virus infection, and viral infection of immature mouse neurons leads to apoptotic cell death. Mature neurons are protected against apoptosis by induction of the *bcl-2* oncogene, whose products convert the infection to a persistent nonlytic infection. Viral persistence in the brain of recovered mice is modulated by antibody.[20]

PROGNOSIS

Major neurologic sequelae of WEE, including quadriplegia, hemiplegia, spasticity, intracerebral calcifications, developmental delay in children, and epilepsy, have been reported in approximately 13 percent of cases.[14,16,25,78] The risk for development of serious neurologic sequelae in infants is triple that of other age groups, and 30 percent of recovered infants younger than 1 year remain seriously impaired.[16,18,67] In infants younger than 1 year, seizures during the acute phase of illness were associated with a

greater risk for poor long-term outcome, including continued seizures. Multiple intracranial calcifications were observed in one recovered infant with persistent seizures.[66] Minor psychiatric or neurologic deficits remained in approximately one fourth of children and adults monitored for 18 months after infection.[16] Central apnea has been reported as a complication of encephalitis in two adult cases.[74]

Parkinson syndrome has been a residual effect in at least 15 cases of adults surviving WEE.[19,41,62,63]

Its onset may be immediate or delayed for several years after recovery from the illness. Two retrospective investigations of patients with idiopathic Parkinson disease found no difference between cases and controls in seroprevalence to several arboviruses, including WEE virus.

The case-fatality rate in reported cases in the United States from 1955 to 1978 was 3 to 4 percent. In outbreaks in the central portion of the United States and Canada in 1941, 1975, and 1981, approximately 7 to 9 percent of patients died, with higher fatality rates in the elderly.

DIAGNOSIS

Virus rarely can be recovered from blood or CSF, but it can be isolated readily from the brain of fatal patients. In a few attempts, WEE virus has been isolated from brain biopsy material; in principle, rapid diagnosis can be made by immunofluorescent identification of WEE viral antigen in brain biopsy tissue. The virus can be isolated readily, under BSL-3 containment precautions, in Vero cell culture or in suckling mice.[63] Nucleic acid–based sequence amplification and real-time reverse-transcriptase polymerase chain reaction (RT-PCR) assays of CSF appear to be sensitive and specific. Duplex real-time RT PCR assays that not only detect but also discern between alphaviruses have recently been developed.[30]

Immunoglobulin (Ig)M capture enzyme-linked immunosorbent assay (ELISA) is the preferred serologic procedure and is available from public health laboratories and commercial reference laboratories.[35] IgM antibody usually is present in acute serum within a week after the onset of illness, and its presence is presumptive evidence of recent infection. IgM often can be detected in CSF before it can be identified in serum, and its presence, which indicates intrathecal production of antibody, confirms a recent CNS infection.[35] Identification of virus-specific IgM in serum and CSF by immunofluorescence also is possible; however, this approach is somewhat less sensitive than IgM capture ELISA and may be confounded by the effects of rheumatoid factor.

Hemagglutination-inhibition and neutralizing antibodies are elevated in the first week of illness in most cases, and a diagnostic fourfold rise in titer usually is observed in the second week. Complement-fixing antibody is slower to rise, with fourfold changes delayed until the third to fifth week after onset. The complement-fixation response may be blunted in older patients.[67]

Neutralizing and hemagglutination-inhibition antibody titers have considerable longevity, with minimal decay noted 30 months after onset. In contrast, complement-fixing antibody is relatively short-lived, and its presence is a useful indicator of recent infection, particularly in endemic areas where long-term residents may have neutralizing and hemagglutination-inhibition antibody from previous exposure. Only two thirds of patients are estimated to show complement-fixing antibody 2 years after onset, and fewer than 15 percent have residual complement-fixing antibody after 5 years.[67]

DIFFERENTIAL DIAGNOSIS

The clinical findings in patients with encephalitis seldom are sufficiently characteristic that a specific diagnosis can be made on clinical grounds alone. Fever, vomiting, signs of meningeal irritation, and confusion are nonspecific features of encephalitis and cerebral edema. Early symptoms in infants, such as fever, lethargy, and vomiting, may be even less specific.[31]

Furthermore, the peak occurrence of arboviral infection in midsummer to late summer temporally overlaps the seasonal occurrence of other CNS infections by enteroviruses (e.g., echovirus 13, which recently has become epidemic in the United States), leptospires, free-living amebae, and mosquito-borne West Nile and St. Louis encephalitis.[54] CNS signs may be prominent in other summertime diseases, such as Rocky Mountain spotted fever, heat stroke, shigellosis, and lead encephalopathy. High priority should be given to establishing a specific diagnosis of treatable conditions, such as herpes simplex virus and enteroviral infection, while bearing in mind that dual arboviral and enteroviral infection has been reported.[6]

Other causes of CNS infection without an established summertime seasonality that also should be considered in the differential diagnosis include fungal, mycobacterial, and partially treated bacterial meningitis; brain abscess and infected subdural collections; bacterial endocarditis; toxoplasmosis; cat-scratch disease; and infections with rabies virus, mumps virus, herpes simplex virus, Epstein-Barr virus, human herpesvirus 6, adenovirus, lymphocytic choriomeningitis virus, and human immunodeficiency virus. Parainfectious disorders such as postinfectious viral encephalitis, acute cerebellar ataxia of childhood, mycoplasmal infection, neuroblastoma with opsoclonus, and Reye syndrome also may enter into the differential diagnosis. Vascular disorders formed the largest single group of disorders mimicking herpes encephalitis in several studies. Drugs (e.g., trimethoprim-sulfamethoxazole, penicillin, isoniazid, phenazopyridine, ibuprofen, tolmetin, sulindac, and carbamazepine), high doses of bismuth, OKT3 antibody, and vidarabine also may cause aseptic meningitis or encephalopathy.

Because of the potential for their overlapping circumstances of exposure, diethyltoluamide (DEET) encephalopathy should be excluded. DEET-containing insect repellents have been implicated as a cause of seizures and encephalopathy after brief cutaneous exposure (see later discussion).

Valuable clues to the diagnosis of an arbovirus infection are gleaned from a history of travel, residence, or occupational and recreational activities within the appropriate incubation period. Suspected cases should be reported to public health officials.

TREATMENT

Specific U.S. Food and Drug Administration–approved antiviral therapy is not available for WEE. However, novel small molecule inhibitors of neurotropic alphaviruses, including WEE virus, are in preclinical development.[45,53,65] Supportive treatment of acutely ill patients includes monitoring of cardiorespiratory function, fluid and electrolyte balance, and intracranial pressure. A case of laboratory infection treated with immune equine serum resulted in recovery; however, acute Parkinson syndrome developed in this patient and coincided temporally with serum sickness resulting from immunotherapy.[19]

PREVENTION

Killed vaccine prepared in chick embryo fibroblast culture is available in the United States under an investigatory permit for laboratory and field personnel who are at risk for occupational exposure. Effective killed vaccines are available for horses and usually are administered in bivalent or multivalent formulations (i.e., with EEE, Venezuelan equine encephalitis, and influenza viruses). Potential vaccine targets for WEE are under investigation, and include E1 envelope protein targets and adenovirus vectored vaccines.[69,76] Vaccination of the general public, even in endemic areas, is not a practical consideration because of the low risk for disease.

Personal protective measures are encouraged when travel or living in endemic areas, and include avoidance of outdoor activity during the hours of peak mosquito activity at dusk and use of repellents.

The Centers for Disease Control and Prevention (CDC) recommends repellents containing DEET or picaridin (KBR3023) as active ingredients.[8,9,17,23,48] DEET is absorbed readily through the skin, and its absorption and absorption of oxybenzone, a common sunscreen preparation, are increased when they are used in combination. Some authorities recommend that sunscreens be applied first and more liberally, followed by insect repellent applied sparingly because of concerns about the potential neurotoxicity of DEET.[8,58,59] Three cases were fatal. In one recovered child, encephalopathy and seizures developed after only two applications. In an additional child, seizures and encephalopathy occurred after the child had cutaneous and respiratory exposure to DEET in an automobile with closed windows. Additional cases of seizures in patients who used small quantities of DEET have been reported, but whether these events were coincidental in populations in which the prevalence of DEET use is high is unclear.[9] DEET may have an effect on ammonia metabolism; in one case, encephalopathy occurred in a patient with partial ornithine carbamoyltransferase deficiency.[24] DEET is a proved neurotoxin when it is ingested; however, the incidence of neurologic side effects after cutaneous exposure is unknown. Nonetheless, avoiding formulations containing DEET in high concentration is prudent; when it is applied simultaneously with sunscreen, DEET repellency is unhampered but ultraviolet protection may be reduced. Precautions for the prudent use of repellents as recommended by the CDC and U.S. Environmental Protection Agency (EPA) are listed in Table 174B-2.

TABLE 174B-2 Precautions to Reduce Potential Adverse Reactions from Repellents

DEET is approved for use on children older than 2 months. Read and follow label instructions.
Repellents should be applied only to exposed skin and clothing (as directed on the product label). Do not use under clothing.
Never use repellents over cuts, wounds, or irritated skin.
Avoid mucosal contact—do not apply to the eyes and mouth, and apply sparingly around the ears. When using sprays, do not spray directly onto the face; spray on the hands first and then apply to the face.
Do not allow children to handle the product, and do not apply to children's hands. When using on children, apply to your own hands and then put it on the child.
Do not spray in enclosed areas. Avoid breathing a repellent spray.
Avoid ingestion, and do not use the product near food.
Use just enough repellent to cover exposed skin and clothing.
Heavy application and saturation are unnecessary for effectiveness; if biting insects do not respond to a thin film of repellent, apply a bit more.
After returning indoors, wash treated skin with soap and water or bathe. This precaution is particularly important when repellents are used repeatedly in a day or on consecutive days. Also, wash treated clothing before wearing it again.
Pregnant and nursing women should minimize the use of repellents.
If you suspect that you or your child is reacting to an insect repellent, discontinue use, wash treated skin, and then call your local poison control center. If you go to a physician, take the repellent with you.
Specific medical information about the active ingredients in repellents and other pesticides is available at the National Pesticide Information Center at 1-800-858-7378.

Modified from U.S. Environmental Protection Agency recommendations. Available at http://www.epa.gov/pesticides/factsheets/chemicals/deet/htm.

Repellents containing oil of lemon eucalyptus (but not "pure" oil of lemon eucalyptus) and registered with the EPA as repellents also may provide a similar level of protection.

Permethrin, a synthetic pyrethroid available as a 0.5 percent aerosol (Permanone), is both insecticidal and a repellent.[9,17,61] Permethrin is extremely effective in reducing the incidence of mosquito and tick bites when sprayed on clothing and shoes, and it also can be applied to tents, mosquito nets, and other gear.[17,60] Its use on the skin is not approved, except as treatment of scabies and head lice.

Prevention is focused primarily on interruption of the transmission of virus from mosquitoes to humans. In

many localities where WEE is endemic, mosquito abatement districts monitor mosquito populations, virus infection rates in the vector population, or evidence of transmission of virus to wild or sentinel birds and chickens to provide a basis for predicting epidemic transmission and for emergency mosquito control.[40,57]

As a general rule, *C. tarsalis* densities exceeding 10 to 15 females per trap night and mosquito infection rates greater than 5 to 10/1000 signal a risk for epizootic transmission. Evidence of seroconversion in sentinel chickens and observations of equine cases are further indications that human cases may occur.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Ariel E. Viruses in reptiles. *Vet Res* 2011;**43**:100–12.
12. Delfraro A, Burgueno A, Morel N, et al. Fatal human case of western equine encephalitis, Uruguay. *Emerg Infect Dis* 2011;**17**: 952–4.
30. Kang X, Li Y, Liu H, et al. A duplex real-time reverse transcriptase polymerase chain reaction assay for detecting western equine and eastern equine encephalitis viruses. *Virol J* 2010;**7**:284–94.
45. Peng W, Peltier D, Larsen M, et al. Identification of thieno[3,2-b] pyrrole derivatives as novel small molecule inhibitors of neurotropic alphaviruses. *J Infect Dis* 2009;**199**:950–7.
53. Reichert E, Clase A, Bacetty A, Larsen J. Alphavirus antiviral drug development: scientific gap analysis and prospective research areas. *Biosecur Bioterror* 2009;**7**:413–27.
64. Sherman M, Weaver S. Structure of the recombinant alphavirus Western equine encephalitis virus revealed by cryoelectron microscopy. *J Virol* 2010;**84**:9775–82.
65. Sindac J, Yestrepsky B, Barraza S, et al. Novel inhibitors of neurotropic alphavirus replication that improve host survival in a mouse model of acute viral encephalitis. *J Med Chem* 2012;**55**: 3535–45.
69. Swayze R, Bhogal H, Barabe N, et al. Envelope protein E1 as vaccine target for western equine encephalitis virus. *Vaccine* 2011; **29**:813–20.
76. Wu J, Barabe N, Chau D, et al. Complete protection of mice against a lethal dose of challenge of western equine encephalitis virus after immunization with an adenovirus-vectored vaccine. *Vaccine* 2007;**25**:4368–75.
77. Zacks M, Paessler S. Encephalitic alphaviruses. *Vet Microbiol* 2010;**140**:281–6.

The full reference list for this chapter is available at expertconsult.com.

174C ■ VENEZUELAN EQUINE ENCEPHALITIS

Theodore F. Tsai • Gail J. Harrison

Mosquito-borne Venezuelan equine encephalitis (VEE) arguably is the most important viral zoonosis in Latin America. The disease, known locally as *peste loca*, has occurred in combined epizootics and epidemics at regular intervals since the 1920s, sometimes leading to hundreds of thousands of human and equine cases. Between 1935 and 1961, 11 outbreaks were reported, and from 1962 to 1973, epizootics recurred in every year except 1965.[1,4,19,42] The virus was isolated from horse brain in 1938 during an outbreak in Venezuela. Although outbreaks have arisen principally in northern South America, especially from Colombia and Venezuela, in a remarkable period between 1969 and 1971, epizootics and epidemics were reported from Colombia, Venezuela, Ecuador, Peru, all the countries of Central America (except Panama), Mexico, and the state of Texas.[1,4,14,29,42] Subsequently, no major epizootics or epidemics were recognized until a large outbreak emerged in Venezuela and Colombia in 1995.[7,8,34,46] Molecular phylogenetic analyses performed in the wake of that outbreak suggested that epizootic strains arise spontaneously from sylvatic viral strains circulating silently in nature.[3,6,24,46] VEE virus is classified as a Category B bioterrorism agent and a Biosafety Level 3 (BSL-3) agent, based on its ability to cause disease in humans, ease of production and stability, and potential for aerosol dissemination in the population.[49]

ETIOLOGIC AGENT

VEE virus is antigenically distinct from other alphaviruses in the Togaviridae family, but is a complex of antigenically and ecologically distinct viral subtypes (Table 174C-1).[32,38,44,45,48] Epizootic subtypes IAB and IC are so named because they have been associated with major outbreaks in horses. Sylvatic viral subtypes circulate in silent cycles of rodent- or bird-mosquito transmission and in general do not cause encephalitis in equines. Both epizootic and sylvatic viral strains cause human illness. VEE virus is an enveloped virus with a nonsegmented, positive-sense RNA genome of approximately 11 kb in length.

VEE virus is thought to have evolved approximately 1400 years ago from a now extinct ancestral alphavirus in one of two extant lineages of New World alphaviruses represented by eastern equine encephalomyelitis and VEE viruses.[32] Because epizootic VEE viral strains had never been isolated from nature except during epizootics, their reservoirs and the mechanisms by which they emerge to cause outbreaks had remained a puzzle. However, recent molecular phylogenetic analyses have found a close genetic relationship between the epizootic IC and the sylvatic ID strains, thus suggesting that these epizootic strains might arise spontaneously by mutation from naturally circulating ID virus.[1,3,6,45] Other outbreaks have had an iatrogenic source from improperly inactivated equine vaccine.*

Comparisons of the attenuated TC-83 vaccine strain of VEE virus and the epizootic IAB parent virus and mutations of virulent infectious clones have shown that changes associated with attenuation occur principally in

*References 20, 21, 32, 35, 45, 46.

REFERENCES

1. Anderson BA. Focal neurologic signs in western equine encephalitis. *Can Med Assoc J* 1984;**130**:1019–21.
2. Arboviral infections of the central nervous system: United States, 1987. *MMWR Morb Mortal Wkly Rep* 1988;**37**:506–8, 513–15.
3. Ariel E. Viruses in reptiles. *Vet Res* 2011;**43**:100–12.
4. Aviles G, Sabattini MS, Mitchell CJ. Transmission of western equine encephalomyelitis virus by Argentine *Aedes albifasciatus*. *J Med Entomol* 1992;**29**:850–3.
5. Baker AB, Noran HH. Western variety of equine encephalitis in man: a clinicopathologic study. *Arch Neurol Psychiatry* 1942;**47**: 565–87.
6. Bia FJ, Thornton GF, Main AJ, et al. Western equine encephalitis mimicking herpes simplex encephalitis. *J A M A* 1980;**244**: 367–9.
7. Bianchi TI, Aviles G, Monath TP, et al. Western equine encephalomyelitis: virulence markers and their epidemiologic significance. *Am J Trop Med Hyg* 1993;**49**:322–8.
8. Briassoulis G, Narlioglov M, Hatzis T. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. *Hum Exp Toxicol* 2001;**20**:8–14.
9. Brown M, Herbert AM. Insect repellents: an overview. *J Am Acad Dermatol* 1997;**35**:243–9.
10. Cheng RH, Kuhn RJ, Olson NH, et al. Nucleocapsid and glycoprotein organization in an enveloped virus. *Cell* 1995;**80**:621–30.
11. Copps SC, Giddings LE. Transplacental transmission of western equine encephalitis. *Pediatrics* 1959;**24**:31–3.
12. Delfraro A, Burgueno A, Morel N, et al. Fatal human case of western equine encephalitis, Uruguay. *Emerg Infect Dis* 2011;**17**: 952–4.
13. Eadie JA, Friesen B. Epidemiological study of western equine encephalitis. In: Sekla L, editor. *Western Equine Encephalitis in Manitoba*. Winnipeg: Manitoba; 1982. p. 142–55.
14. Earnest MP, Goolishian HA, Calverley JR, et al. Neurologic, intellectual, and psychologic sequelae following western encephalitis: a follow-up study of 35 cases. *Neurology* 1971;**21**:969–74.
15. Eklund CM. Human encephalitis of the western equine type in Minnesota in 1941: clinical and epidemiological study of serologically positive cases. *Am J Hyg* 1946;**43**:171–93.
16. Finley KH, Longshore WA Jr, Palmer RJ, et al. Western equine and St. Louis encephalitis: preliminary report of a clinical follow-up study in California. *Neurology* 1955;**5**:223–35.
17. Fradin MS. Mosquitoes and mosquito repellents: a clinician's guide. *Ann Intern Med* 1998;**128**:931–40.
18. Froeschle JE, Reeves WC. Serologic epidemiology of western equine and St. Louis encephalitis virus infection in California. II. Analysis of inapparent infections in residents of an endemic area. *Am J Epidemiol* 1965;**81**:44–51.
19. Gold H, Hampil B. Equine encephalomyelitis in a laboratory technician with recovery. *Ann Intern Med* 1942;**16**:556–69.
20. Gu X, Wang T, Collins D, et al. In vitro evaluation of concurrent use of commercially available insect repellent and sunscreen preparations. *Br J Dermatol* 2005;**152**:1263–7.
21. Hahn CS, Lustig S, Strauss EG, et al. Western equine encephalitis virus is a recombinant virus. *Proc Natl Acad Sci U S A* 1988; **85**:5997–6001.
22. Hammon WM, Reeves WC, Benner SR, et al. Human encephalitis in the Yakima Valley, Washington, 1942. *JAMA* 1945;**128**: 1133–9.
23. Hampers LC, Oker E, Leikin JB. Topical use of DEET insect repellent as a cause of severe encephalopathy in a healthy adult male. *Acad Emerg Med* 1999;**6**:1295–7.
24. Health Canada Pest Management Regulatory Agency. *Pesticides and pest management*. Available at http://www.pmra-arla.gc.ca/english/consum/insectrepellents-e.html.
25. Herzon H, Shelton JT, Bruyn HB. Sequelae of western equine and other arthropod-borne encephalitides. *Neurology* 1957;**7**:535–48.
26. Hess AD, Cherubin CE, LaMotte LC. Relation of temperature to activity of western and St. Louis encephalitis viruses. *Am J Trop Med Hyg* 1963;**12**:657–67.
27. Holden P, Hayes RO, Mitchell CJ, et al. House sparrows, *Passer domesticus* (L.), as hosts of arboviruses in Hale County, Texas. I. Field studies, 1965-1969. *Am J Trop Med Hyg* 1973;**22**:244–53.
28. Hollister AC, Longshore WA Jr, Dean BH, et al. The 1952 outbreak of encephalitis in California: epidemiologic aspects. *Calif Med* 1953;**79**:84–90.
29. Hurst W. Infection of the Rhesus monkey *(Macaca mulatta)* and the guinea pig with the virus of equine encephalomyelitis. *J Pathol Bacteriol* 1936;**42**:271–302.
30. Kang X, Li Y, Liu H, et al. A duplex real-time reverse transcriptase polymerase chain reaction assay for detecting western equine and eastern equine encephalitis viruses. *Virol J* 2010;**7**:284–94.
31. Kokernot RH, Shinefield HR, Longshore WA. The 1952 outbreak of encephalitis in California: differential diagnosis. *Calif Med* 1953; **79**:73.
32. Leake JP. Epidemic of infectious encephalitis. *Public Health Rep* 1941;**56**:1902–5.
33. Leech RW, Harris JC, Johnson RM. 1975 Encephalitis epidemic in North Dakota and western Minnesota: an epidemiologic, clinical, and neuropathologic study. *Minn Med* 1981;**64**:545–8.
34. Liu C, Voth DW, Rodina P, et al. A comparative study of the pathogenesis of western equine encephalitis and eastern equine encephalomyelitis viral infections in mice by intracerebral and subcutaneous inoculations. *J Infect Dis* 1970;**122**:53–63.
35. Martin DA, Muth DA, Brown T, et al. Standardization of immunoglobulin M capture enzyme-linked immunosorbent assays for routine diagnosis of arboviral infections. *J Clin Microbiol* 2000; **38**:1823–6.
36. Medovy H. Western equine encephalomyelitis in infants. *J Pediatr* 1943;**22**:308–18.
37. Medovy H. The history of western encephalomyelitis in Manitoba. *Can J Public Health* 1976;**67**(Suppl. 1):13–14.
38. Meehan PJ, Wells DL, Paul W, et al. Epidemiological features of and public health response to a St. Louis encephalitis epidemic in Florida, 1990-1991. *Epidemiol Infect* 2000;**125**:181–8.
39. Milner AR, Marshall ID. Pathogenesis of in utero infections with abortigenic and nonabortigenic alphaviruses in mice. *J Virol* 1984;**50**:66–72.
40. Mitchell CJ, Hayes RO, Holden P, et al. Effects of ultra-low volume applications of malathion in Hale County, Texas. I. Western encephalitis virus activity in treated and untreated towns. *J Med Entomol* 1969;**6**:155–62.
41. Mulder DW, Parrott M, Thaler M. Sequelae of western equine encephalitis. *Neurology* 1951;**1**:318–27.
42. Noran HH, Baker AB. Sequels of equine encephalomyelitis. *Arch Neurol Psychiatr* 1943;**49**:398–413.
43. Olitsky PK, Schlesinger RW, Morgan IM. Induced resistance of the central nervous system to experimental infection with equine encephalomyelitis virus. *J Exp Med* 1943;**77**:359–75.
44. Olson JG, Reeves WC, Emmons RW, et al. Correlation of *Culex tarsalis* population indices with the incidence of St. Louis encephalitis and western equine encephalomyelitis in California. *Am J Trop Med Hyg* 1979;**28**:335–43.
45. Peng W, Peltier D, Larsen M, et al. Identification of thieno[3,2-b] pyrrole derivatives as novel small molecule inhibitors of neurotropic alphaviruses. *J Infect Dis* 2009;**199**:950–7.
46. Potter ME, Currier RW II, Pearson JE, et al. Western equine encephalomyelitis in horses in the northern Red River Valley, 1975. *J Am Vet Med Assoc* 1977;**170**:1396–9.
47. Powers AM, Brault AC, Shirako Y, et al. Evolutionary relationships and systematics of the alphaviruses. *J Virol* 2001;**75**:10118–31.
48. Qiu H, Jun HW, McCall JW. Pharmacokinetics, formulations and safety of insect repellent *N,N*-diethyl-3-methylbenzamide (DEET): a review. *J Am Mosq Control Assoc* 1998;**14**:12–27.
49. Ray CG, Sciple GW, Holden P, et al. Acute, febrile CNS illnesses in an endemic area of Texas: epidemiologic and serologic findings, 1965. *Public Health Rep* 1967;**82**:785–93.
50. Reeves WC. *Epidemiology and control of mosquito borne arboviruses in California*, 1943-1987. Sacramento, Calif: Mosquito Vector Control Association; 1990.
51. Reeves WC, Hammon WM. *Epidemiology of the arthropod-borne viral encephalitides in Kern County, California, 1943-1952*. Berkeley: University of California Press; 1962. p. 1–257.
52. Reeves WC, Hardy JL, Reisen WK, et al. Potential effect of global warming on mosquito-borne arboviruses. *J Med Entomol* 1994;**31**:323–32.
53. Reichert E, Clase A, Bacetty A, et al. Alphavirus antiviral drug development: scientific gap analysis and prospective research areas. *Biosecur Bioterror* 2009;**7**:413–27.

54. Reisen WK, Chiles RE. Prevalence of antibodies to western equine encephalomyelitis and St. Louis encephalitis viruses in residents of California exposed to sporadic and consistent enzootic transmission. *Am J Trop Med Hyg* 1997;**57**:526–9.
55. Reisen WK, Monath TP. Western equine encephalitis. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 5. Boca Raton, Fla: CRC Press; 1990. p. 89–137.
56. Reisen WK, Meyer RP, Presser SB, et al. Effect of temperature on the transmission of western equine encephalomyelitis and St. Louis encephalitis viruses by *C. tarsalis*. *J Med Entomol* 1993;**30**:151–60.
57. Reisen WK, Yoshimura G, Reeves WC, et al. The impact of aerial applications of ultra-low volume adulticides on *Culex tarsalis* populations (Diptera: Culicidae) in Kern County, California, USA, 1982. *J Med Entomol* 1984;**21**:573–85.
58. Robbins PJ, Cherniack MG. Review of the biodistribution and toxicity of the insect repellent *N,N*-diethyl-*m*-toluamide (DEET). *J Toxicol Environ Health* 1986;**18**:503–25.
59. Roland EH, Jan JE, Rigg JM. Toxic encephalopathy in a child after brief exposure to insect repellents. *Can Med Assoc J* 1985;**132**:155–6.
60. Rumenapf T, Strauss EG, Strauss JH. Aura virus is a new world representative of Sindbis-like viruses. *Virology* 1995;**208**:621.
61. Schreck CE, Kline DL. Personal protection afforded by controlled-release topical repellents and permethrin-treated clothing against natural populations of *Aedes taeniorhynchus*. *J Am Mosq Control Assoc* 1989;**5**:77–80.
62. Schultz DR, Barthal JS, Garrett C. Western equine encephalitis with rapid onset of parkinsonism. *Neurology* 1977;**27**:1095–6.
63. Sciple GW, Ray G, LaMotte LC, et al. Western encephalitis with recovery of virus from cerebrospinal fluid. *Neurology* 1967;**17**: 169–71.
64. Sherman M, Weaver S. Structure of the recombinant alphavirus Western equine encephalitis virus revealed by cryoelectron microscopy. *J Virol* 2010;**84**:9775–82.
65. Sindac J, Yestrepsky B, Barraza S, et al. Novel inhibitors of neurotropic alphavirus replication that improve host survival in a mouse model of acute viral encephalitis. *J Med Chem* 2012;**55**:3535–45.
66. Somekh E, Glode MP, Reilly TT, et al. Multiple intracranial calcifications after western equine encephalitis. *Pediatr Infect Dis* 1991; **10**:408.
67. Stallones RA, Reeves WC, Lennette EH. Serologic epidemiology of western equine and St. Louis encephalitis virus infection in California. I. Persistence of complement-fixing antibody following clinical illness. *Am J Hyg* 1964;**79**:16–28.
68. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication and evolution. *Microbiol Rev* 1994;**58**:491.
69. Swayze R, Bhogal H, Barabe N, et al. Envelope protein E1 as vaccine target for western equine encephalitis virus. *Vaccine* 2011; **29**(4):831–20.
70. Weaver SC, Hagenbaugh A, Bellow LA, et al. A comparison of the nucleotide sequences of eastern and western equine encephalomyelitis viruses with those of other alphaviruses and related RNA viruses. *Virology* 1993;**197**:375–90.
71. Weaver SC, Kang W, Shirako Y, et al. Recombinational history and molecular evolution of western equine encephalitis complex alphaviruses. *J Virol* 1997;**71**:613–23.
72. Wegbreit J, Reisen WK. Relationships among weather, mosquito abundance, and encephalitis virus activity in California: Kern County 1990-98. *J Am Mosq Control Assoc* 2000;**16**:22–7.
73. Wesseling SL, Griffin DE. Local cytokine responses during acute and chronic viral infections to the central nervous system. *Semin Virol* 1994;**5**:457.
74. White DP, Miller F, Erickson RW. Sleep apnea and nocturnal hypoventilation after western equine encephalitis. *Am Rev Respir Dis* 1983;**127**:132–3.
75. Wyckoff RWG, Tesar WC. Equine encephalomyelitis in monkeys. *J Immunol* 1939;**37**:329–43.
76. Wu J, Barabe N, Chau D, et al. Complete protection of mice against a lethal dose of challenge of western equine encephalitis virus after immunization with an adenovirus-vectored vaccine. *Vaccine* 2007;**25**:4368–75.
77. Zacks M, Paessler S. Encephalitic alphaviruses. *Vet Microbiol* 2010;**140**:281–6.
78. Zeifert M, Pennell WH, Finley KH, et al. The electroencephalogram following western and St. Louis encephalitis. *Neurology* 1962;**12**:311–19.

TABLE 174C-1 **Viruses in the Venezuelan Equine Encephalomyelitis Complex**

Subtype	Variety	Pattern of Transmission	Location	Transmission Cycle
I	AB	Epizootic	South, Central, and North America	Various mosquitoes and biting insects: equines
	C	Epizootic	Northern South America	Various mosquitoes and biting insects: equines
	D	Enzootic	Ecuador, Panama, Colombia, and Venezuela	*Culex (Melanoconion) ocossa* and *panocossa:* rodents, aquatic birds
	E	Enzootic	Central America	*Culex (Melanoconion) taeniopus:* rodents
	F	Enzootic	Brazil	Unknown
II (Everglades)		Enzootic	Southern Florida	*Culex (Melanoconion) cedecei:* rodents
III	A			
	Mucambo	Enzootic	South America	*Culex (Melanoconion) portesi:* rodents
	B			
	Tonate	Enzootic	South America	Unknown
	Bijou Bridge	Unknown	Western North America	*Oeciacus vicarius:* birds
	C	Enzootic	Peru	Unknown
	D	Enzootic	Peru	*Culex (Melanoconion): Proechimys* spp.
IV (Pixuna)		Enzootic	Brazil	Unknown
V (Cabassou)		Enzootic	French Guiana	Unknown
VI		Enzootic	Argentina	Unknown

genes encoding the E2 and E1 glycoproteins but also in the 5′ nontranslated region.[3,6,20,38] Attenuation is associated with reduced neuroinvasiveness (i.e., faster clearance from blood and lower viremia levels) and reduced neurovirulence (i.e., minimal histopathologic changes after intracerebral inoculation of horses).

VEE virus rapidly produces cytopathic effects in a variety of cell cultures, including Vero, LLC-MK2, and BHK-21 and in primary chicken and duck embryo cells. Epizootic strains cause lethal infections in horses, donkeys, mules, rabbits, and dogs. In certain guinea pig strains, pathogenicity is correlated with equine virulence.

EPIDEMIOLOGY AND ECOLOGY

Epizootics and concurrent epidemics of VEE caused by the IAB and IC strains typically have led to thousands and, on at least one occasion, hundreds of thousands of cases in humans and equines. Most such outbreaks have occurred in Venezuela and Colombia and have been caused by IC viruses (see earlier discussion) (Fig. 174C-1).[34,37,45,46] The circumstances leading to the emergence of outbreaks are poorly understood, but outbreaks often have occurred in arid areas during years of heavy rainfall and flooding, especially during the dry season.[42] The importance of a nonimmune horse population to amplify the virus was underscored in the most recent outbreak in 1995, which occurred in areas of Colombia and Venezuela where equine immunizations had lapsed.[7,34,46] Equines are the most important vertebrate amplifying host because high levels of viremia develop and are sustained in equines, and they provide a large surface area

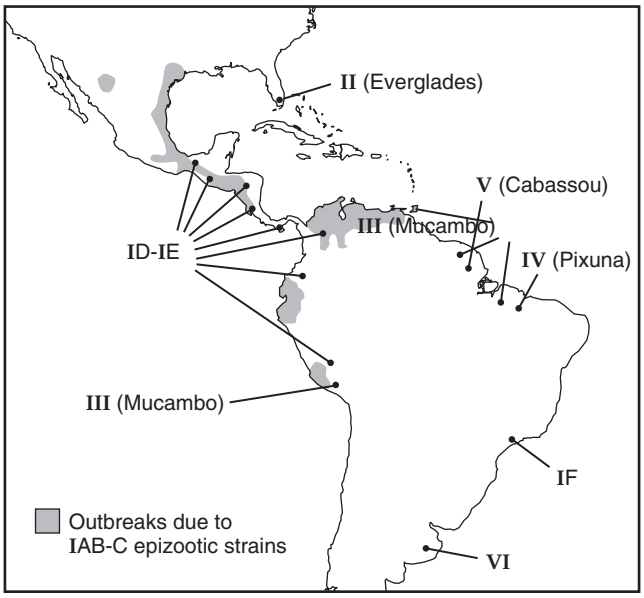

FIGURE 174C-1 ■ Locations of Venezuelan equine encephalitis (VEE) outbreaks caused by type IAB-IC epizootic strains and locations where VEE sylvatic subtypes have been recognized.

for biting mosquitoes. Numerous species of mosquitoes and other blood-feeding insects, among them *Aedes taeniorhynchus*, a salt marsh mosquito, and *Psorophora confinnis*, found in ground pools, can transmit the virus from horse to horse and from horse to human.[40]

Infections are transmitted rapidly among animals and to people such that outbreaks can disseminate at rates of several miles per day. The epidemic curve of human cases usually follows the equine epizootic by several weeks, and

epidemic transmission ceases when the number of susceptible horses has been exhausted by immunization or natural infection.[16,42]

The role of other animals, including humans, in sustaining epidemic viral transmission has been investigated in urban outbreaks, during which household clustering of cases has been observed, and in recent outbreaks, during which few horses were kept in the community. VEE virus levels in human blood are sufficiently high to infect mosquitoes, and virus also has been isolated from the pharynx of ill persons, indicating the possibility of person-to-person transmission by mosquitoes such as *Aedes aegypti* or by direct close contact.[3,39] Although such transmission mechanisms may account for some cases, a household survey found that rates of apparent secondary transmission were no higher than the underlying community attack rate.[7,34] Community attack rates of 20 to 50 percent have been recorded, and the brief course of epidemics in intervals as short as 1 month underscores the considerable force of epidemic transmission.

The series of epizootics beginning in Guatemala in 1969 and reaching Texas in 1971 was caused by an IAB virus that is almost genetically identical to the 1943 Trinidad donkey (epizootic IAB) virus used in inactivated vaccines, thus leading to the conclusion that these outbreaks were iatrogenic and caused by the use of inadequately inactivated equine vaccine.[4,21,45] Another outbreak in Argentina was traced to a similar cause. Serologic serosurveillance in the Gulf Coast region of Mexico conducted between 2003 and 2010 showed the Gulf Coast lineage of VEEV continues to actively circulate and be responsible for infection of animals and humans in this region.[34]

In contrast to the epizootic strains, sylvatic VEE viruses are avirulent in horses and cause a low level of viremia after infection, subclinical or mild illness, and minimal inflammatory change after direct intracerebral inoculation.[43] However, outbreaks of enzootic IE-like viruses rarely have caused outbreaks and deaths in horses (e.g., in Chiapas, Mexico, in 1993). The viruses are maintained in mosquitoes, principally *Culex (Melanoconion) taeniopus* in marshy coastal lowlands, in rodents, and in aquatic birds. In coastal lagoons in Panama, ibises *(Endocinus albus)* and spoon-billed ducks *(Cochlearius cochlearius)* appear to serve as intermediate hosts.[2] VEE subtype II virus, the Everglades virus, is enzootic among cotton rats and other small mammals in the Everglades swamp and probably over a much larger area of Florida but rarely has caused human illness.[9,12] The other VEE virus found in the United States, Bijou Bridge virus, is a nonpathogenic virus thought to have evolved from South American subtype III virus transferred as recently as 40 years ago, probably by a migrating bird.

VEE infections caused by sylvatic strains can be considered "diseases of place" that occur sporadically in persons who enter sylvatic habitats where the viruses are maintained.[2,9] Sporadic cases and rare outbreaks among soldiers in field bivouacs have been reported, but seroprevalence rates in local populations range as high as 75 percent in some locations (e.g., Chiapas, Mexico).[17,36] Many infections are undoubtedly undiagnosed. Bridge vectors, mosquitoes that feed on mammals in the enzootic transmission cycle and subsequently on humans, usually are responsible for these infections.

Numerous cases and outbreaks of VEE have occurred in laboratories when infective aerosols were generated in laboratory procedures.[22] It also has been developed as a biological weapon in the United States and the former Soviet Union.[49] Laboratory manipulations should be undertaken only in BL-3 laboratories and by immunized personnel.

Travelers to Central or South America may return to nonendemic areas with signs and symptoms of VEE. Careful travel history is therefore important in patients who seek medical care with signs and symptoms of encephalitis.[26]

CLINICAL MANIFESTATIONS

The incubation period is brief—2 to 5 days—and in many accounts, the onset of illness was so sudden that it was timed to an exact hour.[22] Fever, chills, headache, myalgia, and malaise are the earliest symptoms, and illness quickly leads to prostration. Photophobia, neck stiffness, backache, conjunctivitis, and sore throat are also common symptoms that occur in approximately one quarter or more of cases. Gastrointestinal complaints, especially nausea and vomiting, and, to a lesser degree, loose stools or diarrhea, are reported frequently.[5]

Physical examination discloses severe prostration and few specific findings. The face may appear hyperemic; inflammation of the pharynx is common; and, occasionally, tonsillitis, palatal ulcers, or petechiae are observed. The cervical lymph nodes may be enlarged and tender. Conjunctivitis and conjunctival suffusion are noted frequently. Nuchal rigidity can be elicited in 10 percent of cases, more often in children. Despite the disease's name, confusion, agitation, and mild disturbances in consciousness suggesting encephalitis are present in only 5 to 10 percent of cases, and patients with significant neurologic findings, such as cranial nerve palsy, motor weakness and paralysis, seizures, and coma, usually account for fewer than 5 percent of all cases.[5,34,35] In epidemics, neurologic findings and encephalitis occurred more commonly in children; however, sporadic cases, including encephalitis caused by VEE subtype II (Everglades) and III (Tonate) viruses, have occurred in middle-aged or elderly adults.[12,17] The fatality rate among patients with encephalitis is 10 to 25 percent, or about 0.5 percent of all cases.[34,35]

In many patients, the illness has an apparently biphasic course; seizures, projectile vomiting, and ataxia occur several days after the onset of fever, followed rapidly by a complete resolution of symptoms. Sequelae such as nervousness, forgetfulness, recurrent headache, and easy fatigability are common occurrences and may persist for months or even up to 1 year. Motor abnormalities usually resolve without residual deficit; however, rarely, sensory and motor abnormalities may persist. Long-term effects on psychometric examination have been reported.[23]

Experimental observations in animals suggest that congenital infection may lead to central nervous system (CNS) anomalies such as porencephaly, micrencephaly,

and hydrocephalus.[24] A similar pattern of structural brain abnormalities has been observed in virus culture–positive fetuses aborted during outbreaks.[46,47] Pancreatic β-cell infection occurs in experimentally infected animals and has led to speculation that VEE may be followed by diabetes, but epidemiologic studies have failed to find an association.[23]

The principal clinical laboratory finding is leukopenia (<4500/mm^3), the nadir of which occurs on the fourth day of illness.[5,11] After the onset of illness, the absolute neutrophil count declines from normal values to 500 to 2000; total lymphocytes are depressed at the onset of illness and gradually recover after 1 week. The platelet count may be diminished below 100,000 mm^3, and lactate dehydrogenase and hepatic transaminases may be elevated moderately. Cerebrospinal fluid (CSF) shows a lymphocytic pleocytosis of up to several hundred cells, moderately elevated protein level, and normal or slightly depressed glucose concentration.

PATHOLOGY AND PATHOGENESIS

VEE virus is both lymphotropic and neurotropic.[10,41] Pathologic changes are observed consistently in the lymph nodes, spleen, lungs, liver, gastrointestinal lymphoid tissue, and brain, with inflammatory infiltrates of mononuclear and polymorphonuclear cells. The lymph nodes and spleen show pronounced lymphoid depletion and necrosis of the germinal centers, along with neutrophilic infiltration and lymphophagocytosis. The selective depletion of lymphoid follicles, with sparing of the paracortical areas and the thymus, suggests that VEE virus principally destroys B cells. These histopathologic changes are reflected in the early lymphopenia seen in the peripheral blood. The liver shows patchy hepatocellular degeneration typical of viral hepatitis. A diffuse interstitial pneumonia with a mixed intraseptal inflammatory cell infiltrate is a consistent finding, and some cases also exhibit intra-alveolar hemorrhage, secondary bronchopneumonia, or both. The brain shows only congestion and edema in most cases. Mild, often focal meningitis and changes associated with encephalitis, perivascular inflammatory infiltrates, and neuronal degeneration are found in a large number of cases.[10] The consistent presence of congestion and edema in various organs and the necrotizing vasculitis seen in some cases suggest that vascular endothelial cells may be a target of infection. This speculation is supported by the observation of VEE viral antigen in the vascular endothelial cells of experimentally infected animals. Secondary immune-mediated destruction of infected vascular endothelia and lymphoid cells may account for delayed clinical manifestations.

LABORATORY DIAGNOSIS

VEE virus can be recovered and grown in cell culture from blood during the first 3 days of illness in at least 75 percent of cases, although isolates have been made after 6 days.[3,4] Virus also has been recovered from throat swabs in approximately 25 percent of cases.[23] Specimens should be inoculated onto Vero or other susceptible cell cultures, but only in a laboratory with BL-3 level containment because of the risk for laboratory aerosol-associated infection. Virus in samples collected and transported in commercial transport media may not support the growth of VEE, but will preserve the nucleic acid and protein integrity of the virus.[18] As an alternative to viral isolation, genomic sequences in acute viremic blood and other samples can be detected rapidly and with high sensitivity by polymerase chain reaction in reference laboratories, and genotyped using reverse-transcription nested PCR assay.[30] Establishing the subtype of VEE viral isolates is vital because subtypes IAB and IC have a potential for epidemic spread. Isolates can be identified rapidly with subtype-specific monoclonal antibodies.

Immunoglobulin (Ig)M capture enzyme-linked immunosorbent assay is the recommended serologic procedure. Both serum and CSF specimens can be tested by this means. Elevated virus-specific IgM in a single serum or CSF specimen is diagnostic and often obviates the need for a second paired serum. An epitope-blocking assay can differentiate antibody responses among serotypes.[44]

DIFFERENTIAL DIAGNOSIS

The self-limited acute febrile illness that characterizes most VEE cases resembles infection caused by dengue, Oropouche, Mayaro, group C bunyaviruses, and various other arboviruses. The epidemic occurrence of cases may suggest dengue or Oropouche fever; however, the absence of rash and hemorrhagic manifestations and an association with equine deaths strongly indicate VEE until it is disproved. Eastern and western equine encephalitis overlap with VEE in some areas of Latin America, but only sporadic human cases have been recognized. Clinical recognition of sporadic VEE caused by sylvatic viruses is difficult; the diagnosis should be entertained in patients with CNS infection and an appropriate exposure history.

TREATMENT

No specific U.S. Food and Drug Administration approved antiviral treatment is available for VEE. Symptomatic treatment with antipyretics and fluids alone is sufficient in most cases. In patients with encephalitis, anticonvulsants may be needed, and intensive supportive care, especially early recognition and treatment of secondary pneumonia, may improve the outcome. Combinations of short interfering RNAs and passive immunization with humanized monoclonal antibodies are under preclinical development and investigation as therapeutic interventions.[15,27,33]

PREVENTION

Immunization with experimental live attenuated TC-83 vaccine is indicated for laboratory and field personnel with a high risk for exposure to VEE virus.[31] However,

the vaccine is immunogenic in only 85 percent of vaccinees, and significant side effects consisting of fever, stiff neck, malaise, and myalgia develop in approximately 20 percent of vaccinees.[13] Previous vaccination with alphaviral vaccines may interfere with response to TC-83, but after immunization with experimental inactivated TC-84 vaccine, antibody will develop in approximately 75 percent of persons who failed to respond to TC-83.[13,25] Safer candidate vaccines that may provide better protection against respiratory infection are being investigated.

Immunization of equines with TC-83 vaccine is the best approach to preventing the emergence of outbreaks. Inadequately inactivated vaccine prepared from epizootic IAB strains poses a danger of producing iatrogenic outbreaks and should be used only if appropriate safety and quality assurance standards of vaccine production have been met. The original TC-83 vaccine virus also may be transmitted by mosquito vectors, efforts to improve its safety by preventing its ability to infect insects were done, through manipulation of structural protein gene expression to produce further attenuation and render it incapable of replicating in insects.[28] Because the live vaccine provides rapid immunity after a single dose, it is the most effective approach in combating outbreaks. Attenuated vaccine is available commercially in certain Latin American countries, but in the United States, only inactivated vaccine formulated with western and eastern equine encephalitis and equine influenza and tetanus antigens is licensed. An improved candidate vaccine, V3526, has been shown to be efficacious in horses.[13] Preclinical development of new, novel VEE vaccines includes Sindbis virus and VEE chimeric viruses, alphavirus replicon particles, DNA vaccines, and adenovirus vector vaccines.[28]

Mosquito control with a combination of larvicides and adulticides may be indicated to mitigate large outbreaks. Taking precautions against mosquito bites, such as using repellents, staying in well-screened or air-conditioned areas when possible, and wearing long-sleeved shirts and long pants, is advised for persons who cannot avoid traveling to areas experiencing epidemics.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Adams AP, Navarro-Lopez R, Ramirez-Aguilar F, et al. Venezuelan equine encephalitis virus activity in the Gulf Coast Region of Mexico, 2003-2010. *PLoS Negl Trop Dis* 2012;**6**:e1675–85.
15. Goodchild S, O'Brien L, Steven J, et al. A humanized murine monoclonal antibody with broad serogroup specificity protects mice from challenge with Venezuelan equine encephalitis virus. *Antiviral Res* 2011;**90**:1–8.
18. Hubbard K, Pellar G, Emanuel P. Suitability of commercial transport media for biological pathogens under nonideal conditions. *Int J Microbiol* 2011;**2011**:463096.
26. Muniz A. Venezuelan equine encephalitis in a teenager visiting Central America. *Pediatr Emerg Care* 2012;**28**:372–5.
27. O'Brien L, Goodchild S, Phillpotts R, et al. A humanized murine monoclonal antibody protects mice from Venezuelan equine encephalitis virus, Everglades virus and Mucambo virus when administered up to 48h after airborne exchange. *Virology* 2012; **426**:100–5.
28. Paessler S, Weaver S. Vaccines for Venezuelan equine encephalitis. *Vaccine* 2009;**27**(S4):D80–5.
33. Reichert E, Clase A, Bacetty A, et al. Alphavirus antiviral drug development: scientific gap analysis and prospective research areas. *Biosecur Bioterror* 2009;**7**:413–27.
49. Zacks M, Paessler S. Encephalitic alphaviruses. *Vet Microbiol* 2010;**140**:281–6.

The full reference list for this chapter is available at expertconsult.com.

174D ■ CHIKUNGUNYA

Scott B. Halstead

Chikungunya is a benign, dengue-like syndrome characterized in adults by the abrupt onset of fever, arthralgia, maculopapular rash, and leukopenia. The term in Makonde means "that which bends up" and refers to the characteristic symptom of arthralgia.[67] In historical times, the terms *knokkel koorts*, *abu rokab*, *mal de genoux*, *dengue*, *dyenga*, and *3-day fever* have been given to epidemics probably caused by chikungunya virus.[12]

The classic account, widely cited as being the initial description of epidemic dengue fever, is that of David Bylon, who was *staads chirurgyn* ("city surgeon") to the city of Batavia (Jakarta) in the year 1779.[9] Dr. Bylon, who himself contracted the illness, wrote the following:

> *It was last May 25, in the afternoon at 5:00 when I noted while talking with two good friends of mine, a growing pain in my right hand, and the joints of the lower arm, which step by step proceeded upward to the shoulder and then continued onto all my limbs; so much so that at 9:00 that same evening I was already in my bed with a high fever. …*
>
> *It's now been three weeks since I … was stricken by the illness, and because of that had to stay home for 5 days; but even until today I have continuously pain and stiffness in the joints of both feet, with swelling of both ankles; so much so, that when I get up in the morning, or have sat up for a while and start to move again, I can not do so very well and going up and down stairs is very painful.*

This account of a febrile illness of acute onset with involvement of the joints clearly suggests that the disease was chikungunya (see discussion of clinical manifestations).

In the same year in Cairo and Alexandria, Egypt, another outbreak of disease occurred that bears a close resemblance to chikungunya.[12,72]

An important pandemic of "dengue" occurred in the years 1870 to 1873; it appeared first on the East African coast and then on the Arabian coast and in Port Said,

REFERENCES

1. Adams AP, Navarro-Lopez R, Ramirez-Aguilar F, et al. Venezuelan equine encephalitis virus activity in the Gulf Coast Region of Mexico, 2003-2010. *PLoS Negl Trop Dis* 2012;**6**:e1675–85.
2. Aguilar PV, Greene IP, Coffey LL, et al. Endemic Venezuelan equine encephalitis in northern Peru. *Emerg Infect Dis* 2004;**10**: 880–8.
3. Anishchenko M, Bowen RA, Paessler S, et al. Venezuelan encephalitis emergence mediated by a phylogenetically predicted viral mutation. *Proc Natl Acad Sci U S A* 2006;**103**:4994–9.
4. Bowen GS, Calisher CH. Virological and serological studies in Venezuelan equine encephalomyelitis in humans. *J Clin Microbiol* 1976;**4**:22–7.
5. Bowen GS, Fashinell TR, Dean PB, et al. Clinical aspects of human Venezuelan equine encephalitis in Texas. *Bull Pan Am Health Org* 1976;**10**:46–57.
6. Brault AC, Powers AM, Ortiz D, et al. Venezuelan equine encephalitis emergence: enhanced vector infection from a single amino acid substitution in the envelope glycoprotein. *Proc Natl Acad Sci U S A* 2004;**101**:11344–9.
7. Centers for Disease Control and Prevention Update. Venezuelan equine encephalitis—Colombia, 1995. *MMWR Morb Mortal Wkly Rep* 1995;**44**:775–7.
8. Centers for Disease Control and Prevention. Venezuelan equine encephalitis: Colombia, 1995. *MMWR Morb Mortal Wkly Rep* 1995;**44**:721–4.
9. Coffey LL, Crawford C, Dee J, et al. Serologic evidence of widespread Everglades virus activity in dogs, Florida. *Emerg Infect Dis J* 2006;**12**:1873–9.
10. de la Monte SM, Castro F, Bonilla NJ, et al. The systemic pathology of Venezuelan equine encephalitis virus infection in humans. *Am J Trop Med Hyg* 1985;**34**:194–202.
11. Dietz WH, Peralta PH, Johnson KM. Ten clinical cases of human infection with Venezuelan equine encephalomyelitis virus, subtype I-D. *Am J Trop Med Hyg* 1979;**28**:329–34.
12. Ehrenkranz NJ, Ventura AK. Venezuelan equine encephalitis virus infection in man. *Annu Rev Med* 1974;**25**:9–14.
13. Fine DL, Roberts BA, Teehee ML, et al. Venezuelan equine encephalitis virus vaccine candidate (V3526) safety, immunogenicity and efficacy in horses. *Vaccine* 2007;**26**:1868–76.
14. Franck PT, Johnson KM. An outbreak of Venezuelan equine encephalomyelitis in Central America. *Am J Epidemiol* 1971;**94**: 487–95.
15. Goodchild S, O'Brien L, Steven J, et al. A humanized murine monoclonal antibody with broad serogroup specificity protects mice from challenge with Venezuelan equine encephalitis virus. *Antiviral Res* 2011;**90**:1–8.
16. Gutierrez VE, Monath TP, Alava AA, et al. Epidemiologic investigations of the 1969 epidemic of Venezuelan encephalitis in Ecuador. *Am J Epidemiol* 1975;**102**:400–13.
17. Hommell D, Heraud JM, Hulin A, et al. Association of Tonate virus (subtype IIIB of the Venezuelan equine encephalitis complex) with encephalitis in a human. *Clin Infect Dis* 2000;**30**: 188–90.
18. Hubbard K, Pellar G, Emanuel P. Suitability of commercial transport media for biological pathogens under nonideal conditions. *Int J Microbiol* 2011;**2011**:463096.
19. Johnson KM, Martin DH. Venezuelan equine encephalitis. *Adv Vet Sci Comp Med* 1974;**18**:79–116.
20. Kinney RM, Chang G-J, Tsuchiya KR, et al. Attenuation of Venezuelan equine encephalitis virus strain TC-83 is encoded by the 5′-noncoding region and the E2 envelope glycoprotein. *J Virol* 1993;**67**:1269–77.
21. Kinney RM, Tsuchiya KR, Sneider JM, et al. Molecular evidence for the origin of the widespread Venezuelan equine encephalitis epizootic of 1969 to 1972. *J Gen Virol* 1992;**73**:3301–5.
22. Koprowski H, Cox HR. Human laboratory infection with Venezuelan equine encephalomyelitis virus. *N Engl J Med* 1947;**236**:647–54.
23. Leon CA, Jaramillo R, Martinez A, et al. Sequelae of Venezuelan equine encephalitis in humans: a four-year follow-up. *Int J Epidemiol* 1975;**4**:131–40.
24. London WT, Levitt NH, Kent SG, et al. Congenital cerebral and ocular malformations induced in Rhesus monkeys by Venezuelan equine encephalitis virus. *Teratology* 1977;**16**:285–96.
25. McClain DJ, Pittman PR, Ramsburg HH, et al. Immunologic interference from sequential administration of live attenuated alphavirus vaccines. *J Infect Dis* 1998;**177**:634–41.
26. Muniz A. Venezuelan equine encephalitis in a teenager visiting Central America. *Pediatr Emerg Care* 2012;**28**:372–5.
27. O'Brien L, Goodchild S, Phillpotts R, Perkins S. A humanized murine monoclonal antibody protects mice from Venezuelan equine encephalitis virus, Everglades virus and Mucambo virus when administered up to 48h after airborne exchange. *Virology* 2012;**426**:100–5.
28. Paessler S, Weaver S. Vaccines for Venezuelan equine encephalitis. *Vaccine* 2009;**27**(S4):D80–5.
29. Pan American Health Organization. Venezuelan encephalitis. Scientific publication no. 243. Proceedings of the Workshop Symposium on Venezuelan Encephalitis Virus, Washington, DC; 1972. p. 1–416.
30. Pisano M, Seco M, Ré V, et al. Specific detection of all members of the Venezuelan equine encephalitis complex: development of RT-nested PCR. *J Virol Methods* 2012;**186**:203–6.
31. Pittman PR, Makuch RS, Mangiafico JA, et al. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine* 1996;**14**:337–43.
32. Powers AM, Brault AC, Shirako Y, et al. Evolutionary relationships and systematics of the alphaviruses. *J Virol* 2001;**75**:10118–31.
33. Reichert E, Clase A, Bacetty A, Larsen J. Alphavirus antiviral drug development: scientific gap analysis and prospective research areas. *Biosecur Bioterror* 2009;**7**:413–27.
34. Rivas F, Diaz LA, Cardenas VM, et al. Epidemic Venezuelan equine encephalitis in La Guajira, Colombia, 1995. *J Infect Dis* 1997;**175**: 828–32.
35. Rossi ALB. Rural epidemic encephalitis in Venezuela caused by a group A arbovirus (VEE). *Prog Med Virol* 1967;**9**:176–203.
36. Sanchez JL, Lednar WM, Macasaet FF, et al. Venezuelan equine encephalomyelitis: report of an outbreak associated with jungle exposure. *Mil Med* 1984;**149**:618–21.
37. Sanmartin-Barberi C, Groot H, Osorno-Mesa E. Human epidemic in Colombia caused by the Venezuelan equine encephalomyelitis virus. *Am J Trop Med Hyg* 1954;**3**:283–93.
38. Strauss JH, Strauss EG. The alphaviruses: gene expression, replication and evolution. *Microbiol Rev* 1994;**58**:491–562.
39. Suarez OM, Bergold GH. Investigations of an outbreak of Venezuelan equine encephalitis in towns of eastern Venezuela. *Am J Trop Med Hyg* 1968;**17**:875–80.
40. Sudia WD, Newhouse VF. Epidemic Venezuelan equine encephalitis in North America: a summary of virus-vector-host relationships. *Am J Epidemiol* 1975;**101**:1–58.
41. Walker DH, Harrison A, Murphy K, et al. Lymphoreticular and myeloid pathogenesis of Venezuelan equine encephalitis in hamsters. *Am J Pathol* 1976;**84**:351–70.
42. Walton TE, Grayson MA. Venezuelan equine encephalitis. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 4. Boca Raton, Fla: CRC Press; 1989. p. 203–31.
43. Walton TE, Alvarez O, Buckwalter RM, et al. Experimental infection of horses with enzootic and epizootic strains of Venezuelan equine encephalomyelitis virus. *J Infect Dis* 1973; **128**:271–82.
44. Wang E, Paessler S, Aguilar PV, et al. A novel, rapid assay for detection and differentiation of serotype-specific antibodies to Venezuelan equine encephalitis complex alphaviruses. *Am J Trop Med Hyg* 2005;**72**:805–10.
45. Weaver SC, Ferro C, Barrera R, et al. Venezuelan equine encephalitis. *Annu Rev Entomol* 2004;**49**:141–74.
46. Weaver SC, Salas R, Rico-Hesse R, et al. Re-emergence of epidemic Venezuelan equine encephalomyelitis in South America. VEE Study Group. *Lancet* 1999;**348**:436–40.
47. Wenger F. Venezuelan equine encephalitis. *Teratology* 1977;**16**: 359–62.
48. Young NA, Johnson KM. Antigenic variants of Venezuelan equine encephalitis virus: their geographic distribution and epidemiologic significance. *Am J Epidemiol* 1969;**89**:286–307.
49. Zacks M, Paessler S. Encephalitic alphaviruses. *Vet Microbiol* 2010;**140**:281–6.

Egypt. From there, it was carried to Bombay and Calcutta, India, and Java. The 1870 outbreak led to the recognition that the Swahili words for this disease were *ki-dinga pepo*.[18] The term *denga* or *dyenga* had been used to designate the disease in Africa in an earlier outbreak in 1823. In this pandemic, dengue had spread with the slave trade to the Caribbean, where in 1827 and 1828 an extensive outbreak occurred in the West Indies. In Cuba, the Spanish homonym "dengue" was used first. Written or serologic evidence exists of chikungunya pandemics in India in 1824 and 1825, 1871 and 1872, 1923, 1964 to 1965, and 2006.[12,40]

In 2005 to 2006 a notable epidemic occurred on Reunion, Mauritius, Madagascar, Mayotte, and Seychelles.[65] This soon spread to India and to Southeast Asia.[40,63] The Reunion outbreak was notable because *Aedes albopictus* was the vector.[5,225] This virus was brought by tourists to Europe, with modest autochthonous outbreaks occurring in southeastern France and northeastern Italy.[26,61]

Chikungunya virus was isolated first by inoculation of suckling mice from an explosive dengue-like epidemic in the Makonde district on the border between Tanganyika and Mozambique in 1952.[43,51,67,68]

ETIOLOGIC AGENT

Classification

According to epidemiologic criteria, chikungunya virus is arthropod-borne because it is transmitted by several species of mosquito. With the use of antigenic relationships demonstrated by hemagglutination inhibition and complement fixation, chikungunya is placed in the *Alphavirus* genus of the family Togaviridae.[38] Antisera prepared to chikungunya virus show strong cross reactions with *o'nyong nyong* by complement fixation and virus-dilution neutralization. Mayaro and Semliki Forest viruses, however, demonstrate only weak cross-reactions by hemagglutination inhibition and no reactions by complement fixation with chikungunya antigen. Cross-comparisons by plaque-reduction neutralization tests have shown little relatedness among alphaviruses.[75] Similar results have been obtained by fluorescent antibody technique.

Phylogenetic analysis of the E1 envelope glycoprotein from 18 strains divides chikungunya virus into two distinct genotypes corresponding to their geographic origin.[62,82] One chikungunya genotype consists of viruses from Senegal and Nigeria and represents the West Africa genotype. These viruses had only 78 to 85 percent nucleotide sequence identity to viruses of the South, Central, and East Africa genotype and the closely related Asian viruses. The phyletic grouping is consistent with the introduction of East African strains into Asia within a period 50 to 310 years ago.[62,80] The African and Asian strains of chikungunya virus are not antigenically separable with the use of mouse immune sera.[56] Although differences in plaque size and heat stability have been described between these strains, these differences might be due to the high number of mouse passages by the prototype African strain. Wild-type strains from all genotypes share the property of autointerference and production of hemorrhagic enteritis (Halstead, S. B., unpublished data, 1965).[28]

Morphology

Chikungunya virions are spherical particles approximately 42 nm in diameter.[34] They possess a lipid-containing envelope with fine projections. The central core, approximately 25 to 30 nm in diameter, is roughly hexagonal in cross section and contains a nucleocapsid of uncertain symmetry. Together with other alphaviruses, the genome is a positive-sense, single-stranded RNA of 12 kb that is messenger active and specifies the viral structural and nonstructural protein, including the polymerase. After an initial round of RNA replication, a subgenomic RNA of approximately 4 kb (26S RNA) is produced. This RNA encodes the viral structural proteins, which include two envelope and capsid proteins.[35] The gene is translated from the 5′ end, where genes for nonstructural proteins are located. Assembly of virus particles at the cell surface occurs by a budding process involving incorporation of the "core" virus precursor into virus particles.[34] Host-cell membranes are modified during infection and contain viral antigen when incorporated into viral envelopes. The protein hemagglutinin spikes are mounted on a phospholipid envelope.

Growth

Chikungunya virus produces death in infant mice, rats, and hamsters after intracerebral inoculation. Serial passage of the virus in mice has resulted in selection of a strain with a short incubation period that is lethal to weanling mice.[68] Virus grows to titers of 10^8 to 10^9 infectious doses per milliliter. Low-passage material is highly infectious for humans during routine laboratory handling, and therefore appropriate precautions should be taken by laboratory workers.

If a mouse brain seed suspension of low-passage virus is prepared and inoculated into other mice at a 1:5 or 1:10 final concentration, death may be delayed significantly, may be sporadic, or may not occur at all. This difference is due to autointerference.[28] Low-passage strains recovered in suckling mice characteristically demonstrate autointerference when inoculated at dilutions below 1:100.

Chikungunya virus produces a cytopathic effect in primary hamster kidney cells and in BHK-21, BSC-1, Vero, FL, HeLa, and rhesus kidney cells.[38] Virus multiplies in *A. albopictus*, *Aedes aegypti*, *Aedes vittatus*, *Anopheles stephensi*, and *Culex fatigans* continuous cell lines and in a cell line derived from *Drosophila*. Plaque assays have been described in LLC-MK2, Vero, and BHK-21 cell and in duck and chick embryos.[38]

TRANSMISSION

Chikungunya virus has been recovered from wild *A. aegypti* in Tanzania, Nigeria, India, and Thailand; from *Aedes africanus* in Uganda and Bangui; from *Aedes*

furcifer-taylori in South Africa and Senegal; and from *Aedes luteocephalus* and *Aedes dalzieli* in Senegal.* Occasional isolates have been recovered from *Mansonia fuscopennata* in Uganda and from *C. fatigans* in Thailand and Tanzania.

Transmission to humans has been demonstrated with the *A. furcifer-taylori* group; transmission to monkeys or mice has been demonstrated with *A. aegypti*, *A. albopictus*, *Aedes calceatus*, *Aedes triseriatus*, *Aedes togoi*, *Aedes pseudoscutellaris*, *Aedes polynesiensis*, *Anopheles albinanus*, *Mansonia africana*, *Erethmapodites chrysogaster*, and *Aedes apicoargenteus*.[38,48,60,71]

Using East African and related Asian chikungunya viruses, Tesh and colleagues[74] examined *A. albopictus* strains from 13 geographic locations from Hawaii to Africa and found considerable variation in susceptibility to infection by oral feeding. The 50 percent oral infective dose (ID_{50}) of a Hawaiian *A. albopictus* strain for a wild-type virus from India was $10^{5.4}$ plaque-forming units per milliliter. The amount of virus replicated by different mosquito strains varied between $10^{4.6}$ and $10^{7.4}$ plaque-forming units per mosquito. These observations, plus a mathematic model of chikungunya virus transmission developed by deMoor and Steffens,[24] suggest that major factors in determining the endemicity of chikungunya may be arthropod related. Tesh and associates[74] suggested that susceptibility to oral infection and the amount of virus replicated may be under genetic control, whereas deMoor and Steffens[24] found mosquito longevity to be the most important determinant in epidemic transmission of chikungunya. The 2005 to 2006 Indian Ocean pandemic evolved from an East African strain of chikungunya in which an A226V mutation enhanced ability of this virus to be biologically transmitted by *A. albopictus*. Subsequently, a further mutation occurred in E2-L210Q that enhanced fitness in *A. albopictus* and resulted in a second epidemic thrust.[79,80]

Serologic studies have demonstrated repeatedly the presence of antibodies in humans throughout the moist forests and semi-arid savannas of East and West Africa.[1,25,53,69] Until recently, most recorded chikungunya infections in humans have occurred in areas infested with *A. aegypti*. The epidemiology in these areas is similar to that of other *A. aegypti*–borne diseases and parallels the distribution of the vector. When *A. aegypti* mosquitoes are abundant in occupied dwellings, infection rates can be expected to be highest in women and children who are at home during daylight hours. When *A. aegypti* is found in public buildings, schools, and hospitals, outbreaks may involve persons in occupational patterns. Characteristically, *A. aegypti* chikungunya pandemics are explosive. Studying *A. aegypti* in laboratory mice, Rao and colleagues[64] documented mechanical transmission. Viremia in humans may be as high as 10^8 infectious doses per milliliter.[64] Because the extrinsic incubation period in *A. aegypti* is relatively long, the explosive nature of chikungunya outbreaks is explained best by mechanical transmission. The 2005 to 2006 outbreak on Reunion Island was unique in that *A. aegypti* was not found. This *A. albopictus*–borne outbreak, with a duration of around 4 months, affected an estimated 35 percent of a population of 800,000. [66]

*References 2, 19, 25, 37, 51, 53.

EPIDEMIOLOGY

Host Range

Chikungunya virus has the ability to replicate in a broad spectrum of vertebrate species. Newborn mice, hamsters, rats, rabbits, guinea pigs, and kittens all can be infected by subcutaneous inoculation of field strains of chikungunya virus,[14] which produces viremia, sickness, and, in most instances, death. Adult rabbits, mice, rats, and chickens inoculated peripherally have an asymptomatic viremia followed by an antibody response. Neutralizing or hemagglutination-inhibition antibodies to chikungunya have been recovered occasionally from sera obtained from ungulates. Attempts to infect cattle, goats, sheep, or horses experimentally failed to produce either viremia or an antibody response.[50] Vervet monkeys and baboons are infected readily. Rhesus monkeys were infected by intravenous and intramuscular inoculation and had viremia titers in excess of 10^7 mouse LD_{50}. *A. aegypti* transmitted virus to rhesus monkeys and could be infected by biting viremic animals.

Chikungunya antibodies have been found in vervet monkeys, baboons, chimpanzees, and red-tailed monkeys in Zimbabwe, South Africa, and Uganda.[47] The zoonotic status of chikungunya virus in Asia has not been studied carefully.

In Africa, zoonotic transmission to subhuman primates is surmised to take place in a wide variety of habitats, with transmission occurring in the forest canopy, at ground level, or both.[49] Chikungunya appears to be enzootic throughout much of eastern, central, southern, and western Africa.[38] Subhuman primate populations are affected in epizootics, which involve critical numbers of the susceptible population, followed by disappearance of the virus. Thus, chikungunya may maintain itself in wildlife populations by constantly moving epizootic activity, in much the same fashion as respiratory tract and enteric virus infections are maintained in humans. To expect that intercurrent and epidemic human infections in Africa are related to epizootic activity seems reasonable. Many putative or identified chikungunya vectors feed on humans as well as subhuman primates. Historically, chikungunya has emerged from its East African zoonotic focus to initiate Indian Ocean epidemics at intervals of approximately 30 to 50 years.[12] Although the 2005 pandemic was associated with genomic changes that increased vectoral capacity for *A. albopictus*, earlier pandemic viruses showed no such mutations.[80] A second series of outbreaks originated from Central-South African lineage strains causing outbreaks in Cameroon and Gabon in 2007.[81]

Geographic Distribution

When susceptible human and *A. aegypti* or *A. albopictus* populations are above the threshold level required for transmission, a person-mosquito-person cycle is established. This cycle probably is responsible for most

of the large urban outbreaks of chikungunya studied during the past 40 years. In Africa, chikungunya outbreaks have been reported from Uganda, Tanzania, Zimbabwe, South Africa, Angola, Zaire, Nigeria, Senegal, Madagascar, and Reunion.[38,64] This distribution best fits the present location of virus research laboratories. A reasonable assumption is that chikungunya is endemic throughout sub-Saharan Africa.

According to historical evidence, chikungunya has spread from the African enzootic focus and caused large pandemics throughout both the Asian and American tropics.[12,18,72] Pandemics swept India in 1824, 1871, 1902, 1923, 1963 to 1964, and 2006, with Sri Lanka (formerly Ceylon) involved in 1965.[12,40] In the 18th and 19th centuries, chikungunya epidemics were reported in the Indonesian archipelago. An extensive serologic survey using the plaque-reduction neutralization test has suggested chikungunya activity possibly during World War II in Kalimantan and Sulawesi, Indonesia. During the late 1950s and early 1960s, chikungunya appears to have established itself endemically in Southeast Asia and was transmitted continuously in urban populations in Thailand, Cambodia, and South Vietnam, possibly into the 1970s.[15,30,83] Involvement of urban populations in Burma appears to have been intermittent, with outbreaks recorded in 1963 and from 1970 to 1973.[52] A large chikungunya epidemic affected much of Indonesia in 1983 and 1984 and Burma in 1984 and 1985.[77] Chikungunya virus was isolated in Australia in 1989.[31] Serologic evidence of chikungunya virus infection has been found throughout the Philippines, possibly during World War II; since then, localized outbreaks have occurred in Manila, Philippines, in 1967 and Negros, Philippines, in 1968[3,11,44] and as recently as 1986.[17] From 1990 to 1995, chikungunya remained endemic at low levels in Thailand, Myanmar (formerly Burma), and Indonesia.[76] Little or no chikungunya virus infection was reported in the 20th century in Papua New Guinea, the Solomon Islands, Vanuatu (formerly New Hebrides), the Caroline Islands, the Pacific Islands, or any of the American tropics.[74]

A pertinent question to ask is why chikungunya and dengue viruses, both transmitted by *A. aegypti*, do not have an identical geographic distribution? The question may be answered partially by differences in the transmission of chikungunya and dengue. The threshold for infection of chikungunya virus in *A. aegypti* is relatively high, with approximately $10^{5.6}$ mouse infectious doses required to infect 50 percent of adult females.[74] The infection threshold of female *A. aegypti* for dengue virus is rather similar. However, *A. aegypti* mosquitoes infected with chikungunya virus transmit virus to vertebrates poorly, whereas dengue-infected mosquitoes transmit with great regularity.[27] Thus, transmission of chikungunya virus would be expected to occur only in areas with high human susceptibility rates and consistently high densities of *A. aegypti* and possibly by mechanical transmission.

CLINICAL MANIFESTATIONS

The incubation period of chikungunya fever is usually 2 to 4 days. In infants, the disease typically begins with the abrupt onset of fever, followed by flushing of the skin. Febrile convulsions may occur in as many as one third of patients. After 3 to 5 days of fever, a generalized maculopapular rash and lymphadenopathy are noted. Conjunctival injection, swelling of the eyelids, pharyngitis, and signs and symptoms of upper respiratory tract disease are common. No enanthem occurs. Febrile convulsions are noted frequently. Some infants have a biphasic fever curve, and arthralgia may be quite severe, although it is not seen frequently.[8,13,29,36,58]

In older children, fever develops acutely and is accompanied by headache, myalgia, and arthralgia involving various joints. Residual arthralgia is uncommon. An early macular blush and a maculopapular rash accompany or immediately precede defervescence. At the same time, marked lymphadenopathy occurs. Febrile convulsions are observed commonly. Hemorrhagic findings, including a positive tourniquet test, are rare.[7,13,29,36,58]

Arthralgia or arthritis is the most conspicuous feature of chikungunya in adults. Usually, patients can identify the precise time and the joint first affected in a chikungunya illness. Swelling and redness of joints and even the pinnae of the ear may occur.[13] The distal lower joints are most frequently involved.[6] During the Reunion Island outbreak excess deaths in the elderly accompanied chikungunya infections.[65]

Although dengue and chikungunya illnesses are similar, important distinguishing features are summarized in Tables 174D-1 through 174D-4 from clinical data obtained from children in Thailand.[29,58]

Table 174D-1 shows the abrupt onset and early severity of chikungunya versus dengue illnesses, many of which came to medical attention only several days after the onset of fever.

Chikungunya virus infections are shorter in duration than dengue virus infections (see Table 174D-2). Almost half of children with chikungunya had a fever that ended within 72 hours after onset, whereas the median duration of dengue fever was 2 days longer.

Many constitutional signs and symptoms occur with similar frequency in chikungunya and dengue viral infections and cannot be used to differentiate the illnesses clinically (see Table 174D-3). However, a terminal maculopapular rash, arthralgia or arthritis, and conjunctival injection were more common symptoms in chikungunya than in dengue (see Table 174D-4). Shock has been reported infrequently in chikungunya.[13,70,78] It was not observed in Thai cases.[58] Changes in taste perception and bradycardia, depression, or asthenia after illness are found rarely in chikungunya; these manifestations are distinctive findings in patients with dengue.

Hemorrhagic phenomena rarely occur with chikungunya virus infection. The frequency of hemorrhagic findings in chikungunya and primary and secondary dengue viral infections in Thai children is compared in Table 174D-5. The frequency of minor hemorrhagic manifestations in outpatient and inpatient dengue did not differ significantly from that in chikungunya cases. However, petechial rash and spontaneous hematemesis or melena developed in only hospitalized dengue cases.

TABLE 174D-1 **Chikungunya and Dengue in Children*: Comparison of Onset of Illness**

	Hospitalized				*Outpatient*			
	Chikungunya (32 cases)		*Dengue (523 cases)†*		*Chikungunya (17 cases)*		*Primary Dengue (29 cases)*	
Day of Illness	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
0	8	25	2	0.4	0	0	0	0
1	15	47	44	8	11	65	8	27
2	5	16	67	13	1	6	5	17
3	2	6	145	28	2	12	3	10
4	1	3	148	28	0	0	7	24
5	1	3	84	16	3	17	4	14
6			20	4			2	
7			11	2			7	
≥8			3	0.6				

Data from Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 1969;**18**:954-71, 1969; and Halstead SB, Nimmannitya S, Margiota MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. *Am J Trop Med Hyg* 1969;**18**:972-83.
*Patients with simultaneous dengue and chikungunya are excluded from analysis.
†Includes primary and secondary infections.

TABLE 174D-2 **Chikungunya and Dengue in Children: Comparison of Duration of Illness**

	Chikungunya (32 cases)		*Dengue (241 cases)**	
Duration of Fever (days)	**No.**	**%**	**No.**	**%**
2	11	34.4	8	3.3
3	4	12.5	16	6.6
4	5	15.6	33	13.7
5	5	15.6	53	21.6
6	2	6.3	53	21.6
7	3	9.4	38	15.8
≥8	2	6.3	42	17.4
	Mean, 4 days		Mean, 5.85 days	

Modified from Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 1969;**18**:954-71, 1969
*Includes primary and secondary dengue infections.

PATHOGENESIS AND PATHOLOGY

The pathologic process of fatal human chikungunya illness has not been studied extensively.[78] Wild-type chikungunya strains produce a hemorrhagic enteritis in mice and hamsters.[28] Recently, a C57BL/6J mouse model involving arthritis, tenosynovitis, and myositis and a cynomolgus monkey model with long-duration chikungunya joint infection have been described.[54,39] In monkeys, chikungunya virus replicated in primary monocyte derived macrophages and joints, muscles, lymphoid organs, and liver.[39] Chikungunya antigens have been detected by immunohistochemistry in muscle satellite cells of muscle biopsies from two patients with a myositic syndrome[59] and in fibroblasts of the joint capsule, skeletal muscle, and dermis from a fatal neonatal case.[20] Chikungunya virus infects and persists in human cornea tissue, and viral infection has been transmitted surgically.[21] In vitro studies have shown that other cell types, such as human epithelial and endothelial cells, primary fibroblasts, and, to a lesser extent, monocyte-derived macrophages, are also able to sustain productive CHIKV infections.[20,39]

DIAGNOSIS

The differential diagnosis includes the viral causes of dengue fever syndrome.[41] In Australia and the western Pacific area, Ross River fever is a frequent cause of epidemic, arthropod-borne, viral arthralgia.

With the use of classic test methods, the diagnosis depends on demonstrating a significant increase in antibody after an illness. Ordinarily, a serum sample collected within 5 days of the onset of fever will not contain antibodies measured by IgM-capture enzyme-linked immunosorbent assay, hemagglutination-inhibition, complement-fixation, or neutralization tests.[4] However, virus or viral RNA usually can be identified in blood taken before 7 days after onset of fever.[4] Neutralizing and hemagglutination-inhibition antibodies generally are present in samples collected 2 weeks or more after the onset of fever. From a single serum sample collected 7 or more days after onset, detection of antibodies of the IgM class is possible with IgM capture enzyme-linked immunosorbent assays.[4,10,77] Neutralizing antibody can be

TABLE 174D-3 Chikungunya and Dengue in Children: Comparison of Frequency of Clinical Findings

	Hospitalized				Outpatient			
	Chikungunya (32 Cases)		Dengue (142 Cases)*		Chikungunya (17 Cases)		Primary Dengue (27 Cases)*	
Day of Illness	No.	%	No.†	%	No.	%	No.	%
Headache	13/19	68	37/83	45	2	12	4	15
Injected pharynx	28/31	90	121/125	97	12	71	27	100
Enanthem	3/27	11	7/84	8	0	24	0	22
Rhinitis	3/31	6	6/47	13	4	6	6	41
Cough	7/30	22	17/79	22	1	35	11	56
Vomiting	19/32	59	73/126	58	6	6	15	15
Constipation	12/30	40	16/30	53	0	18	4	4
Diarrhea	5/32	16	5/78	6	1		1	7
Abdominal pain	6/19	32	38/76	50	3		2	
Lymphadenopathy	8/26	31	32/79	41				
Restlessness	10/30	33	17/79	22				

Data from Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hyg 1969;**18**:954-71, 1969; and Halstead SB, Nimmannitya S, Margiota MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. Am J Trop Med Hyg 1969;**18**:972-83.
*Includes primary and secondary dengue infections.
†Number with finding/number with observations recorded.

TABLE 174D-4 Chikungunya and Dengue in Children: Clinical Findings Occurring with Different Frequency

	Chikungunya (32 Cases)		Dengue* (32 Cases)		
Manifestation	Number†	Percent	Number†	Percent	Significance
Maculopapular rash	19/32	59.4	16/132	12.1	$p < .001$
Conjunctival injection	15/27	55.6	20/61	32.8	$.05 > p < .01$
Myalgias, arthralgias	8/20	40.0	9/75	12.0	$.05 > p < .01$

From Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. Am J Trop Med Hyg 1969;**18**:954-71, 1969
*Includes primary and secondary dengue infections.
†Number positive/number of observations.

TABLE 174D-5 Chikungunya and Dengue in Children: Comparison of Frequency of Clinical Findings

	Chikungunya				Primary Dengue		Secondary Dengue	
	Outpatients (17 Cases)		Inpatients (32 Cases)		Outpatients (27 Cases)		Inpatients (135 Cases)	
Day of Illness	No.*	%	No.	%	No.	%	No.	%
Positive tourniquet test result	3/17	18.0	24/31	77.4	4/27	14.8	94/112	83.9
Petechiae, scattered	0/17	0	10/32	31.2	4/27	7.4	60/129	46.5
Petechial rash	0/17	0	0/32	0	2/27	7.4	13/129	10.1
Maculopapular rash	0/17	0	19/32	59.4	1/27	3.7	16/132	12.1
Epistaxis	0/17	0	4/32	12.5	0/27	0	20/106	18.9
Gum bleeding			0/32	0			2/135	1.5
Melena, hematemesis			0/32	0			14/119	11.8

From Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 1969;**18**:954-71, 1969; and Halstead SB, Nimmannitya S, Margiota MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. *Am J Trop Med Hyg* 1969;**18**:972-83.
*Number positive/number of observations.

measured by the virus-dilution method in suckling mice (or weanling mice with use of the Ross high–mouse passage strain) or by the serum-dilution method in a variety of tissue cultures or plaque assay techniques.

Virus may be isolated by inoculating acute-phase serum or other suspect materials intracerebrally in 1- to 2-day-old mice or in tissue cultures. On initial passage, death may occur within 2 to 5 days after inoculation. An autointerference phenomenon is noted if low-dilution passages of infected mouse brain are performed. Passage at a 10^{-3} dilution or higher avoids this effect.

Vero cells and suckling mice are equally effective for primary isolation.

TREATMENT

Treatment is supportive. Antirheumatoid drugs may be effective for management of chronic arthritis; however, chloroquine phosphate 250 mg/day, once touted for management of acute arthritic pain, had no effect in a double-blinded trial.[23]

Bed rest is advised during the febrile period. Antipyretics or cold sponging should be used to keep the body temperature below 40° C (104° F).

Analgesics or mild sedation may be required to control pain. Arthritis after illness may require continued treatment with anti-inflammatory agents and graduated physiotherapy. Salicylates, because of their hemorrhagic potential, are contraindicated.

Febrile convulsions are treated with phenobarbital given intravenously or orally and continued until the temperature is normal. Severe or intractable convulsions may respond to intravenous diazepam.

Children who have lost excessive fluid because of vomiting, fasting, or thirsting and who cannot take oral fluids may require intravenous rehydration. Individuals with severe hemorrhagic phenomena should be studied for underlying hemostatic disorders.

PROGNOSIS

In a few instances, isolation or serologic evidence of recent infection has been obtained in persons with severe hemorrhagic findings and in individuals dying during an acute febrile illness.* In addition to hemorrhage, neurologic and myocardial involvement have been reported during chikungunya infection in adults.[13,16,20] In adults, arthralgia may persist for weeks, and exercise may prolong this symptom. Typically, pain shifts from joint to joint and is worse in the morning and on first use of the joint. Swelling of ankles, wrists, and fingers occurs frequently. In older patients, the sequelae may resemble rheumatoid arthritis.[6] In the Reunion Island outbreak, 57 percent of adult patients experienced long-duration symptoms, half of which impaired daily activities.[73] Chronic rheumatic manifestations were associated with age, severity of initial acute illness pain, and presence of osteoarthritis.[73] A destructive arthropathy after illness has been reported.[7,46] Chikungunya virus infection might coincide with other pathologic processes and result in death of the individual.[55,65] Carefully studied, virologically documented cases have shown neither thrombocytopenia nor severe neutropenia.[36,46] Until more is known of the pathogenesis of chikungunya virus infection, estimating the frequency with which death can be attributed directly to chikungunya fever will be difficult.

Infants with chikungunya may experience residual neurologic deficits after febrile convulsions.[20]

PREVENTION

Formalin-treated chikungunya virus (Ross strain) grown in African green monkey kidney cells produces a satisfactory immune response and resistance to challenge when administered in three divided doses in monkeys.[32] A vaccine prepared under similar conditions produced hemagglutination-inhibition, complement-fixation, and neutralizing antibody responses in susceptible human volunteers.[33] A comparative study from the same laboratory was made of formalin-inactivated chikungunya vaccines prepared from the virus propagated in African green monkey kidney monolayers and concentrated chick embryo suspension cultures.[84] The latter vaccine was significantly more protective to mice against live homologous virus challenge and stimulated the production of four to five times more circulating antibodies than did the vaccine prepared with virus grown in African green monkey kidney cultures. Nakao and Hotta,[57] studying chikungunya grown in BHK-21 cells, found that ultraviolet-inactivated preparations were significantly more immunogenic than formalin-treated virus. Tween-ether–extracted virus preparations also have been found to be immunogenic. A live attenuated chikungunya vaccine has been developed,[42] as have DNA and chimeric vaccines.[22,45] In view of sporadic outbreaks and low mortality associated with chikungunya virus infection, commercial production of a chikungunya vaccine is likely to have a low priority.

At present, prevention consists of avoiding mosquito bites. For urban outbreaks in most of the Asian and African tropics, the regimen for individual protection and for chronic and emergency control is the same as has been described for dengue. When other vectors are involved, measures designed to combat *A. aegypti* may fail. In such outbreaks, expert entomologic advice will be needed to design appropriate preventive measures.

For discussion of control of mosquitoes, epidemic measures, and health education, see Chapter 175.

*References 13, 36, 55, 57, 65, 70.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Blacksell SD, Tanganuchitcharnchai A, Jarman RG, et al. Poor diagnostic accuracy of commercial antibody-based assays for the diagnosis of acute Chikungunya infection. *Clin Vaccine Immunol* 2011;**18**:1773–5.

5. Borgherini G, Poubeau P, Staikowsky F, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis* 2007;**44**:1401–7.
6. Bouquillard E, Combe B. A report of 21 cases of rheumatoid arthritis following Chikungunya fever: a mean follow-up of two years. *Joint Bone Spine* 2009;**76**:654–7.
17. Chikungunya fever among U.S. Peace Corps volunteers. Republic of the Philippines. *MMWR Morb Mortal Wkly Rep* 1986;**35**: 573–4.
20. Couderc T, Lecuit M. Focus on Chikungunya pathophysiology in human and animal models. *Microbes Infect* 2009;**11**:1197–205.
21. Couderc T, Gangneux N, Chretien F, et al. Chikungunya virus infection of corneal grafts. *J Infect Dis* 2012;**206**:851–9.
22. Darwin JR, Kenney JL, Weaver SC. Transmission potential of two chimeric Chikungunya vaccine candidates in the urban mosquito vectors, *Aedes aegypti* and *Ae. albopictus*. *Am J Trop Med Hyg* 2011;**84**:1012–15.
23. De Lamballerie X, Boisson V, Reynier JC, et al. On chikungunya acute infection and chloroquine treatment. *Vector Borne Zoonotic Dis* 2008;**8**:837–9.
26. Grandadam M, Caro V, Plumet S, et al. Chikungunya virus, southeastern France. *Emerg Infect Dis* 2011;**17**:910–13.
39. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Invest* 2010;**120**(3):894–906.
40. Lahariya C, Pradhan SK. Emergence of chikungunya virus in Indian subcontinent after 32 years: a review. *J Vector Borne Dis* 2006;**43**:151–60.
41. Laoprasopwattana K, Kaewjungwad L, Jarumanokul R, Geater A. Differential diagnosis of chikungunya, dengue viral infection and other acute febrile illnesses in children. *Pediatr Infect Dis J* 2012; **31**:456–63.
45. Mallilankaraman K, Shedlock DJ, Bao H, et al. A DNA vaccine against chikungunya virus is protective in mice and induces neutralizing antibodies in mice and nonhuman primates. *PLoS Negl Trop Dis* 2011;**5**:e928.
46. Manimunda SP, Vijayachari P, Uppoor R, et al. Clinical progression of chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging. *Trans R Soc Trop Med Hyg* 2010;**104**:392–9.
48. McIntosh BM, Jupp PG. Attempts to transmit chikungunya virus with six species of mosquito. *J Med Entomol* 1970;**7**:615–18.
51. McIntosh BM, Paterson HE, McGillivray G, et al. Further studies on the chikungunya outbreak in Southern Rhodesia in 1962. I. Mosquitoes, wild primates and birds in relation to the epidemic. *Ann Trop Med Parasitol* 1964;**58**:45–51.
54. Morrison TE, Oko L, Montgomery SA, et al. A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence. *Am J Pathol* 2011;**178**:32–40.
59. Ozden S, Huerre M, Riviere JP, et al. Human muscle satellite cells as targets of chikungunya virus infection. *PLoS One* 2007;**2**: e527.
61. Poletti P, Messeri G, Ajelli M, et al. Transmission potential of chikungunya virus and control measures: the case of Italy. *PLoS One* 2011;**6**:e18860.
63. Pulmanausahakul R, Roytrakul S, Auewarakul P, Smith DR. Chikungunya in Southeast Asia: understanding the emergence and finding solutions. *Int J Infect Dis* 2011;**15**:e671–6.
65. Renault P, Balleydier E, D'Ortenzio E, Baville M, Filleul L. Epidemiology of chikungunya infection on Reunion Island, Mayotte, and neighboring countries. *Med Mal Infect* 2012;**42**(3):93–101.
66. Renault P, Solet JL, Sissoko D, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. *Am J Trop Med Hyg* 2007;**77**:727–31.
67. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-1953. I. Clinical features. *Trans R Soc Trop Med Hyg* 1955;**49**:28–32.
73. Sissoko D, Malvy D, Ezzedine K, et al. Post-epidemic Chikungunya disease on Reunion Island: course of rheumatic manifestations and associated factors over a 15-month period. *PLoS Negl Trop Dis* 2009;**3**:e389.
79. Tsetsarkin KA, Weaver SC. Sequential adaptive mutations enhance efficient vector switching by Chikungunya virus and its epidemic emergence. *PLoS Pathog* 2011;**7**(12):e1002412.
80. Tsetsarkin KA, Chen R, Sherman MB, Weaver SC. Chikungunya virus: evolution and genetic determinants of emergence. *Curr Opin Virol* 2011;**1**:310–17.
81. Tsetsarkin KA, Chen R, Leal G, et al. Chikungunya virus emergence is constrained in Asia by lineage-specific adaptive landscapes. *Proc Natl Acad Sci U S A* 2011;**108**:7872–7.
82. Volk SM, Chen R, Tsetsarkin KA, et al. Genome-scale phylogenetic analyses of chikungunya virus reveal independent emergences of recent epidemics and various evolutionary rates. *J Virol* 2010;**84**:6497–504.

The full reference list for this chapter is available at expertconsult.com.

174E ■ ROSS RIVER VIRUS ARTHRITIS

John G. Aaskov

"Epidemics" of a benign disease causing polyarthralgia and rash were first described in Australia in 1927.[63] Following the recovery of the causative agent and the advent of serologic tests able to diagnose Ross River virus infection, epidemic polyarthritis has been recognized as endemic in Australia and has occurred as epidemics in numerous Pacific nations. Approximately 4000 cases of epidemic polyarthritis are reported in Australia each year, with a peak of 7800 cases in 1996.

Some confusion has been generated recently by use of the term *Ross River fever* to describe clinical Ross River virus infections because fever does not develop in more than half of those with clinical disease.[59] Additional confusion has been generated by efforts to describe any polyarthritis caused by an Australian arbovirus as epidemic polyarthritis. The term *epidemic polyarthritis* should be used to describe only clinical disease caused by the Ross River virus.

ETIOLOGIC AGENT

Investigations of an epidemic of polyarthritis in southern Australia in 1956[13,74] suggested that an alphavirus was the causative agent. This was confirmed when Ross River virus was isolated from *Aedes vigilax* mosquitoes collected beside Ross River, near Townsville in northern Australia, and shown, on serologic grounds, to be the causative agent of epidemic polyarthritis.[24,27] Although the first isolation of virus from a human was from a febrile child

REFERENCES

1. Adesina OA, Odelola HA. Ecological distribution of chikungunya haemagglutination-inhibition antibodies in human and domestic animals in Nigeria. *Trop Geogr Med* 1991;**43**:271–5.
2. Anderson CR, Singh KRP, Sarkar JK. Isolation of chikungunya virus from *Aedes aegypti* fed on naturally infected humans in Calcutta. *Curr Sci* 1965;**34**:579–80.
3. Basaca-Sevilla V, Halstead SB. Recent virological studies of haemorrhagic fever and other arthropod-borne virus infections in the Philippines. *J Trop Med Hyg* 1966;**69**:203–8.
4. Blacksell SD, Tanganuchitcharnchai A, Jarman RG, et al. Poor diagnostic accuracy of commercial antibody-based assays for the diagnosis of acute Chikungunya infection. *Clin Vaccine Immunol* 2011;**18**(10):1773–5.
5. Borgherini G, Poubeau P, Staikowsky F, et al. Outbreak of chikungunya on Reunion Island: early clinical and laboratory features in 157 adult patients. *Clin Infect Dis* 2007;**44**:1401–7.
6. Bouquillard E, Combe B. A report of 21 cases of rheumatoid arthritis following Chikungunya fever: a mean follow-up of two years. *Joint Bone Spine* 2009;**76**:654–7.
7. Brighton SW, Simson IW. A destructive arthropathy following chikungunya virus arthritis: a possible association. *Clin Rheumatol* 1984;**3**:253–8.
8. Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infection: a retrospective study of 107 cases. *S Afr Med J* 1983; **63**:313–15.
9. Bylon D. Korte aatekening, wegens eene algemeene ziekte, doorgans genaamd de knokkel-koorts. *Verhandelungen van het Bataviaasch Genootschop der Konsten in Wetenschappen* 1780;**2**:17–30.
10. Calisher CH, el-Kafrawi AO, Al-Deen Mahmud MI, et al. Complex-specific immunoglobulin M antibody patterns in humans infected with alphaviruses. *J Clin Microbiol* 1986;**23**:155–9.
11. Campos LE, San Juan A, Cenabre LC, et al. Isolation of chikungunya virus in the Philippines. *Acta Med Philippina* 1969; **5**:152–5.
12. Carey DE. Chikungunya and dengue: a case of mistaken identity? *J Hist Med Allied Sci* 1971;**26**:243–62.
13. Carey DE, Myers RM, Deranitz CM, et al. The 1964 chikungunya epidemic at Vellore, South India, including observations on concurrent dengue. *Trans R Soc Trop Med Hyg* 1969;**63**:434–45.
14. Chakravarty SK, Sarkar JK. Susceptibility of newborn and adult laboratory animals to chikungunya virus. *Indian J Med Res* 1969; **57**:1157–64.
15. Chastel C. Human infections in Cambodia with chikungunya or a closely allied virus. III. *Epidemiology Bull Soc Pathol Exot* 1964; **57**:65–82.
16. Chatterjee SN, Chakravarti SK, Mitra AC, et al. Virological investigation of cases with neurological complications during the outbreak of haemorrhagic fever in Calcutta. *J Indian Med Assoc* 1965; **45**:314–16.
17. Chikungunya fever among U.S. Peace Corps volunteers. Republic of the Philippines. *MMWR Morb Mortal Wkly Rep* 1986;**35**: 573–4.
18. Christie J. Remarks on "kidinga Pepo": A peculiar form of exanthematous disease. *Br Med J* 1872;**1**:577–9.
19. Cornet M, Chateau R. Quelques données biologiques sur *Aedes* (Stegomyia) *luteocephalus* (Newstead) en zone de savane soudanienne dans l'ouest du Senegal. *Cah Off Recherche Sci Tech Outre-Mer Entomol Méd Parasitol* 1974;**12**:97–109.
20. Couderc T, Lecuit M. Focus on Chikungunya pathophysiology in human and animal models. *Microbes Infect* 2009;**11**:1197–205.
21. Couderc T, Gangneux N, Chretien F, et al. Chikungunya virus infection of corneal grafts. *J Infect Dis* 2012;**206**:851–9.
22. Darwin JR, Kenney JL, Weaver SC. Transmission potential of two chimeric Chikungunya vaccine candidates in the urban mosquito vectors, *Aedes aegypti* and *Ae. albopictus*. *Am J Trop Med Hyg* 2011; **84**(6):1012–15.
23. De Lamballerie X, Boisson V, Reynier JC, et al. On chikungunya acute infection and chloroquine treatment. *Vector Borne Zoonotic Dis* 2008;**8**:837–9.
24. deMoor PP, Steffens FE. A computer-simulated model of an arthropod-borne virus transmission cycle, with special reference to chikungunya virus. *Trans R Soc Trop Med Hyg* 1970;**64**:927–34.
25. Diallo M, Thonnon J, Traore-Laminzana M, et al. Vectors of chikungunya virus in Senegal: current data and transmission cycles. *Am J Trop Med Hyg* 1999;**60**:281–6.
26. Grandadam M, Caro V, Plumet S, et al. Chikungunya virus, southeastern France. *Emerg Infect Dis* 2011;**17**:910–13.
27. Gubler DJ, Nalim S, Tan R, et al. Variation in susceptibility to oral infection with dengue viruses among geographic strains of *Aedes aegypti*. *Am J Trop Med Hyg* 1979;**28**:1045–52.
28. Halstead SB, Buescher EL. Hemorrhagic disease in rodents infected with virus associated with Thai hemorrhagic fever. *Science* 1961;**134**:475–6.
29. Halstead SB, Nimmannitya S, Margiotta MR. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. II. Observations on disease in outpatients. *Am J Trop Med Hyg* 1969;**18**: 972–83.
30. Halstead SB, Scanlon JE, Umpaivit P, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. IV. Epidemiologic studies in the Bangkok metropolitan area. *Am J Trop Med Hyg* 1969;**18**:987–1021.
31. Harnett GB, Bucens MR. Isolation of chikungunya virus in Australia. *Med J Aust* 1990;**152**:328–9.
32. Harrison VR, Binn LN, Randall R. Comparative immunogenicities of chikungunya vaccines prepared in avian and mammalian tissues. *Am J Trop Med Hyg* 1967;**16**:786–91.
33. Harrison VR, Eckels KH, Bartelloni PJ, et al. Production and evaluation of a formalin-killed chikungunya vaccine. *J Immunol* 1971;**107**:643–7.
34. Higashi N, Matsumoto A, Tabata K, et al. Electron microscope study of development of chikungunya virus in green monkey kidney stable (VERO) cells. *Virology* 1967;**35**:55–9.
35. Igarashi A, Fukuoka T, Nithiuthai P, et al. Structural components of chikungunya virus. *Biken J* 1970;**13**:93–110.
36. Jadhav M, Namboodripad M, Carman RH, et al. Chikungunya disease in infants and children in Vellore: a report on clinical and haematological features of virologically proved cases. *Indian Med Res* 1965;**53**:764–76.
37. Jupp PG, McIntosh BM. *Aedes furcifer* and other mosquitoes as vectors of chikungunya virus at Mica, northeastern Transvaal, South Africa. *J Am Mosq Control Assoc* 1990;**6**:415–20.
38. Karabatsos N, editor. *International catalog of arbovirus 1985*. Ft. Collins, Colo: American Society of Tropical Medicine and Hygiene; 1985.
39. Labadie K, Larcher T, Joubert C, et al. Chikungunya disease in nonhuman primates involves long-term viral persistence in macrophages. *J Clin Invest* 2010;**120**(3):894–906.
40. Lahariya C, Pradhan SK. Emergence of chikungunya virus in Indian subcontinent after 32 years: a review. *J Vector Borne Dis* 2006;**43**:151–60.
41. Laoprasopwattana K, Kaewjungwad L, Jarumanokul R, Geater A. Differential diagnosis of chikungunya, dengue viral infection and other acute febrile illnesses in children. *Pediatr Infect Dis J* 2012; **31**:456–63.
42. Levitt NH, Ramsburg HH, Hasty SE, et al. Development of an attenuated strain of chikungunya virus for use in vaccine production. *Vaccine* 1986;**4**:157–62.
43. Lumsden WHR. An epidemic of virus disease in Southern Province Tanganyika Territory in 1952-53. II. General description and epidemiology. *Trans R Soc Trop Med Hyg* 1955;**49**:33–53.
44. Macasaet FF, Villamil PT, Wexler S, et al. Epidemiology of arbovirus infections in Negros Oriental. II. Serologic findings of the epidemic in Amlan. *J Philippine Med Assoc* 1969;**45**:311–17.
45. Mallilankaraman K, Shedlock DJ, Bao H, et al. A DNA vaccine against chikungunya virus is protective in mice and induces neutralizing antibodies in mice and nonhuman primates. *PLoS Negl Trop Dis* 2011;**5**:e928.
46. Manimunda SP, Vijayachari P, Uppoor R, et al. Clinical progression of chikungunya fever during acute and chronic arthritic stages and the changes in joint morphology as revealed by imaging. *Trans R Soc Trop Med Hyg* 2010;**104**:392–9.
47. McIntosh BM. Antibody against chikungunya virus in wild primates in southern Africa. *S Afr J Med Sci* 1970;**35**:65–74.
48. McIntosh BM, Jupp PG. Attempts to transmit chikungunya virus with six species of mosquito. *J Med Entomol* 1970;**7**:615–18.
49. McIntosh BM, Jupp PG, DeSouza J. Mosquitoes feeding at two horizontal levels in gallery forest in Natal, South Africa, with

reference to possible vectors of chikungunya virus. *J Entomol Soc S Afr* 1972;**35**:81–90.
50. McIntosh BM, Paterson HE, Donaldson JM, et al. Chikungunya virus: viral susceptibility and transmission studies with some vertebrates and mosquitoes. *S Afr J Med Sci* 1963;**28**:45–52.
51. McIntosh BM, Paterson HE, McGillivray G, et al. Further studies on the chikungunya outbreak in Southern Rhodesia in 1962. I. Mosquitoes, wild primates and birds in relation to the epidemic. *Ann Trop Med Parasitol* 1964;**58**:45–51.
52. Ming CK, Thein S, Thaung UT, et al. Clinical laboratory studies on haemorrhagic fever in Burma, 1970-1972. *Bull World Health Organ* 1974;**51**:227–36.
53. Moore DL, Causey OR, Reddy S, et al. Arthropod-borne viral infections of many in Nigeria, 1964-1970. *Ann Trop Med Parasitol* 1975;**69**:49–64.
54. Morrison TE, Oko L, Montgomery SA, et al. A mouse model of chikungunya virus-induced musculoskeletal inflammatory disease: evidence of arthritis, tenosynovitis, myositis, and persistence. *Am J Pathol* 2011;**178**:32–40.
55. Munasinghe DR, Rajasuriya K. Haemorrhage in Christmas disease following dengue-like fever. *Ceylon Med J* 1966;**11**:39–40.
56. Nakao E. Biological and immunological studies on chikungunya virus: a comparative observation of two strains of African and Asian origins. *Kobe J Med Sci* 1972;**18**:133–41.
57. Nakao E, Hotta S. Immunogenicity of purified, inactivated chikungunya virus in monkeys. *Bull World Health Organ* 1973;**48**: 559–62.
58. Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 1969;**18**:954–71.
59. Ozden S, Huerre M, Riviere JP, et al. Human muscle satellite cells as targets of chikungunya virus infection. *PLoS One* 2007;**2**: e527.
60. Paterson HE, McIntosh BM. Further studies on the chikungunya outbreak in southern Rhodesia in 1962. II. Transmission experiments with the *Aedes furcifertaylori* group of mosquitoes and with a member of the *Anopheles gambiae* complex. *Ann Trop Med Parasitol* 1964;**58**:52–5.
61. Poletti P, Messeri G, Ajelli M, Vallorani R, Rizzo C, Merler S. Transmission potential of chikungunya virus and control measures: the case of Italy. *PLoS One* 2011;**6**:e18860.
62. Powers AM, Brault AC, Tesh RB, et al. Re-emergence of chikungunya and o'nyong-nyong viruses: evidence for distinct geographical lineages and distant evolutionary relationships. *J Gen Virol* 2000;**81**:471–9.
63. Pulmanausahakul R, Roytrakul S, Auewarakul P, Smith DR. Chikungunya in Southeast Asia: understanding the emergence and finding solutions. *Int J Infect Dis* 2011;**15**:e671–6.
64. Rao TR, Devi PS, Singh KRP. Experimental studies on the mechanical transmission of chikungunya virus by *Aedes aegypti*. *Mosq News* 1968;**28**:406–8.
65. Renault P, Balleydier E, D'Ortenzio E, Baville M, Filleul L. Epidemiology of chikungunya infection on Reunion Island, Mayotte, and neighboring countries. *Med Mal Infect* 2012;**42**: 93–101.
66. Renault P, Solet JL, Sissoko D, et al. A major epidemic of chikungunya virus infection on Reunion Island, France, 2005-2006. *Am J Trop Med Hyg* 2007;**77**:727–31.
67. Robinson MC. An epidemic of virus disease in Southern Province, Tanganyika Territory, in 1952-1953. I. Clinical features. *Trans R Soc Trop Med Hyg* 1955;**49**:28–32.
68. Ross RW. The Newala epidemic. III. The virus: Isolation, pathogenic properties and relationship to the epidemic. *J Hyg* 1956; **54**:177–91.
69. Salim AR, Porterfield JS. A serological survey on arbovirus antibodies in the Sudan. *Trans R Soc Trop Med Hyg* 1973;**76**:206–10.
70. Sarkar JK, Chatterjee SN, Chakrevarti SK, et al. Chikungunya virus infection with haemorrhagic manifestations. *Indian J Med Res* 1965;**53**:921–5.
71. Shah KV, Gilotra SK, Gibbs CJ Jr, et al. Laboratory studies of transmission of chikungunya virus by mosquitoes. *Indian J Med Res* 1964;**52**:703–9.
72. Siler JF, Hall MW, Hitchens AP. Dengue: Its history, epidemiology, mechanisms of transmission, etiology, clinical manifestations, immunity and prevention. *Philippine J Sci* 1926;**29**:1–305.
73. Sissoko D, Malvy D, Ezzedine K, et al. Post-epidemic Chikungunya disease on Reunion Island: course of rheumatic manifestations and associated factors over a 15-month period. *PLoS Negl Trop Dis* 2009;**3**:e389.
74. Tesh RB, Gubler DJ, Rosen L. Variation among geographic strains of *Aedes albopictus* in susceptibility to infection with chikungunya virus. *Am J Trop Med Hyg* 1976;**25**:326–35.
75. Tesh RB, Gadjusek DC, Garruto RM, et al. The distribution and prevalence of group A arbovirus neutralizing antibodies among human populations in Southeast Asia and the Pacific Islands. *Am J Trop Med Hyg* 1975;**24**:664–75.
76. Thaikruea L, Charearnsook O, Reanphumkarnkit S, et al. Chikungunya in Thailand: a re-emerging disease? *Southeast Asian J Trop Med Public Health* 1997;**28**:359–64.
77. Thein S, La Linn M, Aaskov J, et al. Development of a simple indirect enzyme-linked immunosorbent assay for the detection of immunoglobulin M antibody in serum from patients following an outbreak of chikungunya virus infection in Yangon, Myanmar. *Trans R Soc Trop Med Hyg* 1992;**86**:438–42.
78. Thiruvengadam KV, Kalyanasundaram V, Rajgopal J. Clinical and pathological studies on chikungunya fever in Madras City. *Indian J Med Res* 1965;**53**:720–8.
79. Tsetsarkin KA, Weaver SC. Sequential adaptive mutations enhance efficient vector switching by Chikungunya virus and its epidemic emergence. *PLoS Pathog* 2011;**7**:e1002412.
80. Tsetsarkin KA, Chen R, Sherman MB, Weaver SC. Chikungunya virus: evolution and genetic determinants of emergence. *Curr Opin Virol* 2011;**1**:310–17.
81. Tsetsarkin KA, Chen R, Leal G, et al. Chikungunya virus emergence is constrained in Asia by lineage-specific adaptive landscapes. *Proc Natl Acad Sci U S A* 2011;**108**:7872–7.
82. Volk SM, Chen R, Tsetsarkin KA, et al. Genome-scale phylogenetic analyses of chikungunya virus reveal independent emergences of recent epidemics and various evolutionary rates. *J Virol* 2010;**84**: 6497–504.
83. Vu-Qui D, Nguyen-Thi KT, Ly QB. Antibodies to chikungunya virus in Vietnamese children in Saigon. *Bull Soc Pathol Exot* 1967;**60**:353–9.
84. White A, Berman S, Lowenthal JP. Comparative immunogenicities of chikungunya vaccines propagated in monkey kidney monolayers and chick embryo suspension cultures. *Appl Microbiol* 1972;**23**: 951–2.

without arthritis,[23] subsequent use of mosquito cell lines enabled Ross River virus to be recovered regularly from epidemic polyarthritis patients.[11,28,68,77]

Ross River virus is related serologically to Getah and Bebaru viruses in the Semliki Forest virus subgroup.[21] Nucleotide sequencing has confirmed this close relationship among Ross River, Getah, and Semliki Forest viruses.[67] Phylogenetic analyses have identified four lineages of Ross River virus, three of which appear to have become extinct.[45] The appearance of the present lineage was associated with major genetic arrangements in the *NSP3* gene of Ross River virus.[7] No association has been observed between any particular virus genotype and disease in humans, but this may be due to most studies employing consensus nucleotide sequences for what genetically are very diverse viral populations.[53]

Cryoelectron microscopic studies[17,66,79] suggest that the nucleocapsid of Ross River virus is approximately 400 Å in diameter and has a T = 4 quaternary structure. It is surrounded by a membrane bilayer that is penetrated by 80 spikes, also arranged in a T = 4 lattice. Each spike is a trimer of heterodimers of the envelope glycoproteins E1 and E2. The E2 protein makes contact with heparin on the surface of host cells, and antibodies that combine with this protein neutralize infection in vitro.[2,75,78]

The virus can be adapted to grow to high titer in the muscle and brain of day-old mice, in which it causes paralysis and death.[56,60] Ross River virus also grows to high titer in vertebrate and mosquito cell lines.[11]

TRANSMISSION AND EPIDEMIOLOGY

Ross River virus is endemic in all mainland states of Australia and in Papua New Guinea. The incidence of epidemic polyarthritis is highest in the Australian states of Queensland, Northern Territory, and Western Australia, with other states experiencing outbreaks of infection against a background of low incidence (http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-surveil-nndss-nndssintro.htm). No transmission of Ross River virus had been reported from the Pacific island states involved in the 1979 to 1980 epidemic (i.e., Fiji, Samoa, Tonga, Cook Islands)[8,28,49,68,77] until 2003, when cases of epidemic polyarthritis began to be detected in travelers who had visited Fiji.[50] Sporadic cases, without local virus transmission, have occurred in Europe and the United States in tourists and military personnel returning from Australia.

Isolation of Ross River virus from more than 20 species of mosquitoes,[69] in particular *A. vigilax*[27,39] and *Culex annulirostris*,[26,39] and the presence of antibody to the virus in almost as many species of animals, particularly mammals,[24,25] suggested a mammal-mosquito cycle with humans as an incidental host. However, improved laboratory diagnostic services have shown clinical infection in humans occurs year-round, although most cases are seen in the late summer and in autumn, and most patients are city dwellers.[10,59] Taken together with the explosive spread of disease during the 1979 to 1980 epidemic of Ross River virus infection in the Pacific,[8,28,68,77] this virus appears to be maintained in either of two cycles, mammal-mosquito-mammal or human-mosquito-human, with movement of virus between the two. Evidence also has been obtained of transovarial transmission of Ross River virus in *Aedes* mosquitoes.[48,52]

Patients may be viremic for up to 7 days after the onset of symptoms.[10,68] The incubation time from infection to the onset of symptoms may vary from 1 to 27 days, 7 to 9 days being the usual interval in endemic areas.[32]

In endemic areas, the ratio of subclinical to clinical infection may be as high as 50:1,[10] but during outbreaks, the ratio can be reduced to 4:1 or less.[8,41] Approximately 1.5 percent of humans living in northern Australia are infected with Ross River virus each year,[10] but the rates are much higher in some areas.[19,20]

Infection rates are the same in both sexes, and early reports that clinical disease was more common in females than in males[22,59] are not supported by Australian national data for the past decade. An association between human leukocyte antigen (HLA)-DR7 and clinical disease also has been observed.[38]

Ross River virus has been shown to cross the placenta in mice[6] and humans.[9] In mice, the result is extensive postpartum mortality,[6] but in humans, no evidence of morbidity or mortality in children infected in utero has been found.

CLINICAL MANIFESTATIONS

Epidemic polyarthritis occurs as a mild to severe illness characterized by joint pain, particularly in the knees and the small joints of the hands and feet.* Frequently, the joint pain is accompanied by a maculopapular or vesicular rash on the trunk and limbs and sometimes by fever, chills, or both. Sore throat, lymphadenopathy, paresthesia, tenderness of the palms and soles, exanthems, and, more rarely, petechiae have been observed. Most patients experience several weeks of painful arthritis followed by a slow decrease in the severity of symptoms during the 30 to 40 weeks required by most for recovery.[5] Infrequently, symptoms may persist for a year or more,[30] and some patients may experience episodes of severe arthritis during convalescence. One study has linked Ross River virus infection to a chronic fatigue syndrome.[44]

In a small proportion of patients (<0.1%), clinical disease may develop without arthritis.[23] Rare cases of glomerulonephritis,[35] hematuria,[14] and central nervous system symptoms[1,54,65,70] accompanying Ross River virus infection in humans also have been reported.

The severity and duration of symptoms are age related. Most clinical disease occurs in adults, who also have the most severe and prolonged symptoms. Teenagers may be incapacitated for only a few days and asymptomatic after 1 to 2 weeks.

The only other species known to develop clinical disease (musculoskeletal symptoms) after a natural infection with Ross River virus is equines.[15]

*References 13, 22, 24, 29, 30, 40, 59, 61.

PATHOLOGY

The pathogenesis of Ross River virus disease in humans is not understood. The literature describing the pathologic process of Ross River virus infection in mice is extensive,* but almost invariably, clinical symptoms are observed only in mice infected when less than 4 weeks of age, which have key immunologic genes silenced, or which are infected by routes that could not occur in nature and with strains of virus that have been passaged extensively in cell culture.

Synovial tissue from patients with epidemic polyarthritis may show a marked mononuclear leukocyte infiltrate with small amounts of fibrin deposition and synovial cell hyperplasia.[42,76] No virus or viral antigen has been detected in synovial tissue from patients with epidemic polyarthritis, but a 250-nucleotide fragment of the viral genome was detected by reverse-transcription polymerase chain reaction (RT-PCR) in synovium from 2/12 patients 5 weeks after the onset of symptoms.[76] Ross River virus has been shown to replicate for a similar period in human synovial cells maintained at 35° C (95° F) in vitro.[46] Whether virus persists in joints as long as symptoms are present remains unclear, and ethical and technical constraints prevent this issue being explored in a systematic manner.

Fluid from affected joints consists almost entirely of mononuclear leukocytes at all stages of disease.[18,31,33] Human synovial fibroblasts infected with Ross River virus in vitro produce elevated levels of messenger RNA (mRNA) coding for chemoattractants and other mediators, such as monocyte chemoattractant protein 1, interleukin-8, granulocyte-macrophage colony-stimulating factor, and tumor necrosis factor–α, but elevated levels of the proteins for which they code have not been demonstrated.[55]

No evidence of immune complexes or complement activation in arthritic joints has been found, and viral antigen can be detected (in macrophages) for only 5 to 7 days after the onset of symptoms, despite the persistence of arthritis for 30 to 40 weeks.[34] No significant levels of anticollagen antibodies could be detected in serum from epidemic polyarthritis patients.[37] Neither anti–Ross River virus antibody levels nor a primary, virus-specific, cell-mediated immune response to Ross River virus infection correlated with the presence or absence of arthritis in human infections.[3] In patients with epidemic polyarthritis, a nonspecific immunologic response (natural killer cells) correlated well with the presence or absence of arthritic symptoms.[3,5] Functional natural killer cells have been recovered from the knee of a patient with epidemic polyarthritis,[42] and natural killer cells have been found to kill autologous synovial tissue in vitro.[5]

A rash develops in approximately 30 percent of patients with epidemic polyarthritis.[59] The dermis underlying these lesions contains a perivascular infiltrate of CD8+ T cells and some monocytes and macrophages.[36] No immunoglobulin or complement deposition has been observed in these lesions, although Ross River virus antigen was detected in basal epidermal and eccrine duct epithelial cells.[36]

*References 43, 51, 56-58, 60, 71.

DIAGNOSIS

The signs and symptoms of a Ross River virus infection (see earlier discussion) are too nonspecific to permit establishment of a diagnosis on clinical grounds alone. Most clinical diagnoses of epidemic polyarthritis are confirmed by use of an indirect enzyme-linked immunosorbent assay (ELISA)[64] to detect Ross River virus–specific IgM antibodies in serum collected 7 to 10 days after the onset of symptoms. Various forms of this assay are available from commercial suppliers. Immunoglobulin (Ig)M can be detected for approximately 3 months after the onset of disease.[10]

In rare cases (perhaps <1/5000), anti–Ross River virus IgM antibody production may persist for years. Assays for serum anti–Ross River virus IgA might be an alternative to IgM assays because the duration of IgA production is shorter than that for IgM.[16] However, such IgA assays have not been adopted by routine diagnostic laboratories. More recently, indirect ELISA assays that measure the avidity of anti–Ross River virus antibody have been shown to be able to discriminate between recent and old infections with this virus.[47]

Although virus sometimes can be isolated from seronegative, acute-phase sera,[8,11,28,68,77] this procedure is not performed in most routine diagnostic laboratories. RT-PCR protocols,[73] mostly unpublished, have been developed for the detection of Ross River virus RNA, but they are less sensitive than is virus isolation for the detection of virus and are not used routinely for the diagnosis of human disease.

Because epidemic polyarthritis is an arthritic disease, care must be taken to avoid false-positive results caused by the presence of rheumatoid factor when indirect ELISA is used to detect anti–Ross River virus IgM antibodies. Such precautions include removal of IgG or rheumatoid factor before testing sera or performing tests for rheumatoid factor in parallel with ELISA.

Another source of false-positive diagnoses is the production of anti–Ross River virus IgM antibody caused by polyclonal B-cell activation after infection of a Ross River virus–immune host with Epstein-Barr virus, cytomegalovirus, *Coxiella burnetii*, or *Plasmodium* spp.

Other viral infections to be considered when a patient is suspected of having epidemic polyarthritis include infections with Barmah Forest, Sindbis, Kunjin, or rubella viruses.

TREATMENT AND PROGNOSIS

No specific antiviral therapy is available, although experimental evidence suggests that interferon-γ may ameliorate acute disease.[72] Nonsteroidal anti-inflammatory medication or steroids have been used to treat patients with severe disease or prolonged symptoms, but the efficacy of such treatment has not been evaluated in any

systematic manner. A small study found that patients taking corticosteroids recovered more quickly than those using nonsteroidal anti-inflammatory medication.[62] Rest, while maintaining mobility and muscle tone, appears to provide significant relief.

Epidemic polyarthritis has not been shown to progress to chronic joint disease.

PREVENTION

A killed virus vaccine[4] was found to be safe and immunogenic in phase I and II clinical trials[12] and completed phase III trials in 2013. In urban areas, public health mosquito control programs reduce mosquito numbers, but prevention of mosquito exposure for those with outdoor occupations and pastimes is impossible. Personal protection (i.e., insect screening of houses, wearing of protective clothing, and application of insect repellents) is the only reliable way to avoid the risk for being infected.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Aaskov J, Fokine A, Liu W. Ross River virus evolution: implications for vaccine development. *Future Virol* 2012;**7**:173–8.
7. Aaskov J, Jones A, Choi W, et al. Lineage replacement accompanying duplication and rapid fixation of an RNA element in the nsP3 gene in a species of alphavirus. *Virology* 2011;**410**:353–9.
12. Aichinger G, Ehrlich HJ, Aaskov JG, et al. Safety and immunogenicity of an inactivated whole virus Vero cell-derived Ross River virus vaccine: a randomized trial. *Vaccine* 2011;**29**:9376–84.
43. Herrero LJ, Nelson M, Srikiathachorn A, et al. Critical role for macrophage migration inhibitory factor (MIF) in Ross River virus-induced arthritis and myositis. *Proc Natl Acad Sci USA* 2011;**108**:12048–53.
45. Jones A, Lowry K, Aaskov J, et al. Molecular evolutionary dynamics of Ross River virus and implications for vaccine efficacy. *J Gen Virol* 2011;**91**:182–8.
53. Liu W, Rourke M, Holmes EC, et al. Persistence of multiple genetic lineages within intrahost populations of Ross River virus. *J Virol* 2011;**85**:5674–8.

The full reference list for this chapter is available at expertconsult.com.

174F ■ OTHER ALPHAVIRAL INFECTIONS

Theodore F. Tsai

O'NYONG-NYONG

The virus name *o'nyong-nyong* is derived from the Acholi term meaning "very painful and weak," which describes the acute constitutional symptoms and polyarthritis associated with the disease of the same name.[14] The virus is related closely to chikungunya virus, but unlike most arboviruses, it is transmitted by anopheline mosquitoes in an interhuman cycle analogous to that of malaria in rural Africa. Apart from the human-to-human epidemic cycle, the natural transmission cycle and involvement of other vertebrate hosts are largely unknown. The disease first came to attention in, arguably, the largest arboviral epidemic ever recorded. After emerging in Uganda in 1959, the epidemic spread to South and West Africa and produced an estimated 2 million cases before the outbreak died out spontaneously 3 years later. The disease apparently had disappeared from East Africa, although the virus was isolated sporadically from mosquitoes until 1996, when only the second ever recognized outbreak appeared, in Uganda.[14] In retrospect, the virus had been circulating at low levels in the area several years before this epidemic occurred. Human and animal infections have been documented serologically in Nigeria, Sierra Leone, Ghana, and Chad. Factors underlying emergence of the virus, its natural reservoir, and the reasons for its disappearance can only be surmised, but exhaustion of susceptible human hosts is a likely explanation for the cessation of epidemic transmission. Clinically, the disease is similar to chikungunya, although lymphadenopathy is more pronounced and mimics, in some cases, the enlarged cervical lymph nodes of sleeping sickness.[2] Virus can be isolated from acute-phase blood samples, or the diagnosis can be confirmed serologically by detection of specific immunoglobulin (Ig)M. Personal protective measures against malaria (e.g., use of bed nets) should be effective against acquiring infection.

IGBO-ORA FEVER

Igbo-Ora virus has been determined genetically to be a subtype of o'nyong-nyong virus and, like it, is transmitted by anopheline mosquitoes and (without adaptation) is nonpathogenic in suckling mice.[21] The viral transmission cycle has not been defined. Sporadic cases and small outbreaks have been reported from Igbo-Ora and Ibadan, Nigeria, the Ivory Coast, and the Central African Republic. Based on a single case description, the illness consists of fever, polyarthritis, and pharyngitis.

BARMAH FOREST FEVER

Barmah Forest fever occurs only in Australia, where it has been a sporadic and occasionally hyperendemic infection leading to a polyarthropathy-rash illness indistinguishable from that of Ross River fever.[6,15,23] The mosquito-borne viral transmission cycle shares some features with that of Ross River virus; however, independent outbreaks have occurred in locations where both viruses are enzootic, thus indicating differences in their

REFERENCES

1. Aaskov JG. Ross River virus disease. In: St. George TD, French EL, editors. *Arbovirus research in Australia*. Paper presented at the Second Symposium of the Commonwealth Scientific and Industrial Organisation and Queensland Institute of Medical Research, July 17–19, 1979. p. 166.
2. Aaskov J, Fokine A, Liu W. Ross River virus evolution: implications for vaccine development. *Future Virol* 2012;**7**:173–8.
3. Aaskov JG, Fraser JRE, Dalglish DA. Specific and nonspecific immunological changes in epidemic polyarthritis in patients. *Aust J Exp Biol Med Sci* 1981;**55**:599–608.
4. Aaskov JG, Williams L, Yu S. A candidate Ross River virus vaccine: pre-clinical evaluation. *Vaccine* 1997;**15**:1396–404.
5. Aaskov JG, Dalglish DA, Harper JJ, et al. Natural killer (NK) cells in viral arthritis. *Clin Exp Immunol* 1987;**68**:23–32.
6. Aaskov JG, Davies CEA, Tucker M, et al. Effect on mice of infection during pregnancy with three Australian arboviruses. *Am J Trop Med Hyg* 1981;**30**:198–203.
7. Aaskov J, Jones A, Choi W, et al. Lineage replacement accompanying duplication and rapid fixation of an RNA element in the nsP3 gene in a species of alphavirus. *Virology* 2011;**410**:353–9.
8. Aaskov JG, Mataika JU, Lawrence GW, et al. An epidemic of Ross River virus infection in Fiji, 1979. *Am J Trop Med Hyg* 1981; **30**:1053–9.
9. Aaskov JG, Nair K, Lawrence GW, et al. Evidence for transplacental transmission of Ross River virus in humans. *Med J Aust* 1981;**2**: 20–1.
10. Aaskov JG, Ross P, Davies CEA, et al. Epidemic polyarthritis in northeastern Australia, 1978-1979. *Med J Aust* 1981;**2**:17–19.
11. Aaskov JG, Ross PV, Harper JJ, et al. Isolation of Ross River virus from epidemic polyarthritis patients in Australia. *Aust J Exp Biol Med Sci* 1985;**63**:587–97.
12. Aichinger G, Ehrlich HJ, Aaskov JG, et al. Safety and immunogenicity of an inactivated whole virus Vero cell-derived Ross River virus vaccine: a randomized trial. *Vaccine* 2011;**29**:9376–84.
13. Anderson SG, French EL. An epidemic exanthem associated with polyarthritis in the Murray Valley, 1956. *Med J Aust* 1957;**2**: 113–17.
14. Anstey N, Currie B, Tai KS. Ross River virus disease presenting with hematuria. *Southeast Asian J Trop Med Public Health* 1991;**22**: 281–3.
15. Azoulas JK, Wishart E, Ainsworth C. Isolation of Ross River virus from horses with signs of musculoskeletal disease. *Aust Vet J* 2003; **81**:344–7.
16. Carter IWJ, Fraser JRE, Cloonan MJ. Specific IgA antibody response in Ross River virus infection. *Immunol Cell Biol* 1987;**65**: 511–13.
17. Cheng RH, Kuhn RJ, Olson NH, et al. Nucleocapsid and glycoprotein organisation in an enveloped virus. *Cell* 1995;**80**:621–30.
18. Clarris BJ, Doherty RL, Fraser JRE, et al. Epidemic polyarthritis: a cytological, virological and immunochemical study. *Aust N Z J Med* 1975;**5**:450–7.
19. Doherty RL. Arboviruses of Australia. *Aust Vet J* 1972;**48**:172–80.
20. Doherty RL. Surveys of haemagglutination-inhibiting antibody to arboviruses in Aborigines and other population groups in northern Australia. *Trans R Soc Trop Med Hyg* 1973;**67**:197–205.
21. Doherty RL. Ross River virus. In: Karabatsos N, editor. *International catalogue of arboviruses including certain other viruses of vertebrates*. 3rd ed. San Antonio, Tex: American Society of Tropical Medicine and Hygiene; 1985.
22. Doherty RL, Barrett EJ, Gorman BM, et al. Epidemic polyarthritis in eastern Australia 1959-1970. *Med J Aust* 1971;**1**:5–8.
23. Doherty RL, Carley JG, Best JC. Isolation of Ross River virus from man. *Med J Aust* 1972;**1**:1083–4.
24. Doherty RL, Gorman BM, Whitehead RH, et al. Studies of epidemic polyarthritis: the significance of three group A arboviruses isolated from mosquitoes in Queensland. *Australas Ann Med* 1964; **13**:322–7.
25. Doherty RL, Gorman BM, Whitehead RH, et al. Studies of arthropod-borne virus infections in Queensland. V. Survey of the antibodies to group A arboviruses in man and animals. *Aust J Exp Biol Med Sci* 1966;**44**:365–77.
26. Doherty RL, Standfast HA, Domrow R, et al. Studies of the epidemiology of arthropod-borne virus infections at Mitchell River Mission, Cape York Peninsula, North Queensland. IV. Arbovirus infections of mosquitoes and mammals, 1967-1969. *Trans R Soc Trop Med Hyg* 1971;**65**:504–13.
27. Doherty RL, Whitehead RH, Gorman BM, et al. The isolation of a third group A arbovirus in Australia, with preliminary observations on its relationship to epidemic polyarthritis. *Aust J Sci* 1963; **26**:183–4.
28. Fauran P, Donaldson M, Harper J, et al. Characterisation of Ross River viruses isolated from patients with polyarthritis in New Caledonia and Wallis and Futuna islands. *Am J Trop Med Hyg* 1984;**33**:1228–31.
29. Feeney KT, Murray KJ, Whittle AJ, et al. Reactive arthritis and vasculitis in a child due to Ross River virus infection. *Med J Aust* 2004;**181**:710.
30. Fraser JRE. Epidemic polyarthritis and Ross River virus disease. *Clin Rheum Dis* 1986;**12**:369–88.
31. Fraser JRE, Becker GJ. Mononuclear cell types in chronic synovial effusions of Ross River virus disease. *Aust N Z J Med* 1984;**14**:505.
32. Fraser JRE, Cunningham AL. Incubation time of epidemic polyarthritis. *Med J Aust* 1980;**1**:550–1.
33. Fraser JRE, Cunningham AL, Clarris BJ, et al. Cytology of synovial effusions in epidemic polyarthritis. *Aust N Z J Med* 1981;**11**: 168–73.
34. Fraser JRE, Cunningham AL, Mathews JD, et al. Immune complexes and Ross River virus disease (epidemic polyarthritis). *Rheumatol Int* 1988;**8**:113–17.
35. Fraser JRE, Cunningham AL, Muller HK, et al. Glomerulonephritis in the acute phase of Ross River virus disease (epidemic polyarthritis). *Clin Nephrol* 1988;**29**:149–52.
36. Fraser JRE, Ratnamohan AM, Dowling JPG, et al. The exanthem of Ross River virus infection: histology, location of virus antigen and the nature of inflammatory infiltrate. *J Clin Pathol* 1983;**36**: 1256–63.
37. Fraser JRE, Rowley MJ, Tait B. Collagen antibodies in Ross River virus disease (epidemic polyarthritis). *Rheumatol Int* 1987;**7**:267–9.
38. Fraser JRE, Tait T, Aaskov JG, et al. Possible genetic determinants in epidemic polyarthritis caused by Ross River virus infection. *Aust N Z J Med* 1980;**10**:597–603.
39. Gard G, Marshall ID, Woodroofe GM. Annually recurrent epidemic polyarthritis and Ross River virus activity in a coastal area of New South Wales. II. Mosquitoes, viruses and wildlife. *Am J Trop Med Hyg* 1973;**22**:551–60.
40. Halliday JH, Horan JP. An epidemic of polyarthritis in the Northern Territory. *Med J Aust* 1943;**2**:293–5.
41. Hawkes RA, Boughton CR, Naim HM, et al. A major outbreak of epidemic polyarthritis in New South Wales during the summer of 1983-1984. *Med J Aust* 1985;**143**:330–3.
42. Hazelton RA, Hughes C, Aaskov JG. The inflammatory response in the synovium of a patient with Ross River arbovirus infection. *Aust N Z J Med* 1985;**15**:336–9.
43. Herrero LJ, Nelson M, Srikiathachorn A, et al. Critical role for macrophage migration inhibitory factor (MIF) in Ross River virus-induced arthritis and myositis. *Proc Natl Acad Sci USA* 2011;**108**: 12048–53.
44. Hickie I, Davenport T, Wakefield D, et al. Post-infective and chronic fatigue syndromes precipitated by viral and non-viral pathogens: prospective cohort study. *B M J* 2006;**333**:575.
45. Jones A, Lowry K, Aaskov J, et al. Molecular evolutionary dynamics of Ross River virus and implications for vaccine efficacy. *J Gen Virol* 2011;**91**:182–8.
46. Journeaux SF, Brown WB, Aaskov JG. Prolonged infection of human synovial cells with Ross River virus. *J Gen Virol* 1987; **68**:3165–9.
47. Kapeleris J, Lowe P, Phillips D, et al. IgG avidity in the diagnosis of acute Ross River virus infection. *Dis Markers* 1996;**12**:279–82.
48. Kay BH. Three modes of transmission of Ross River virus by *Aedes vigilax* (Skuse). *Aust J Exp Biol Med Sci* 1982;**60**:339–44.
49. Kay BH. *Assignment report*. Suva, Fiji: WHO Regional Office for the South Pacific; 1983.
50. Klapsing P, MacLean JD, Glaze S, et al. Ross River virus disease re-emergence, Fiji, 2003-2004. *Emerg Infect Dis* 2005;**11**:613–15.
51. Lidbury BA, Ruli NE, Suhrbier A, et al. Macrophage derived proinflammatory factors contribute to the development of arthritis and myositis after infection with an arthrogenic alphavirus. *J Infect Dis* 2008;**197**:1585–93.
52. Lindsay MDA, Broom AK, Wright AE, et al. Ross River virus isolations from mosquitoes in arid regions of Western Australia:

implication of vertical transmission as a means of persistence of the virus. *Am J Trop Med Hyg* 1993;**49**:686–96.
53. Liu W, Rourke M, Holmes EC, et al. Persistence of multiple genetic lineages within intrahost populations of Ross River virus. *J Virol* 2011;**85**:5674–8.
54. Lucas RE, Qiao M. A case of encephalitis in central Australia due to Ross River virus? *Aust N Z J Med* 1999;**29**:268–70.
55. Mateo L, Linn ML, McColl SR, et al. An arthrogenic alphavirus induces monocyte chemoattractant protein-1 and interleukin-8. *Intervirology* 2000;**43**:55–60.
56. Mims CA, Murphy FA, Taylor WP, et al. Pathogenesis of Ross River virus infection in mice. I. Ependymal infection, cortical thinning, and hydrocephalus. *J Infect Dis* 1973;**127**:121–8.
57. Morrison TE, Simmons JD, Heise MT. Complement receptor 3 promotes severe Ross River virus induced disease. *J Virol* 2008;**82**:11263–72.
58. Morrison TE, Whitmore AC, Shabman RS, et al. Characterisation of Ross River virus trophism and virus-induced inflammation in a mouse model of viral arthritis and myositis. *J Virol* 2006;**80**:737–49.
59. Mudge PR, Aaskov JG. Epidemic polyarthritis in Australia, 1980-1981. *Med J Aust* 1983;**2**:269–73.
60. Murphy FA, Taylor WP, Mims CA, et al. Pathogenesis of Ross River virus infection in mice. II. Muscle, heart and brown fat lesions. *J Infect Dis* 1973;**127**:129–38.
61. Mylonas AD, Brown AM, Carthew TL, et al. Natural history of Ross River virus–induced epidemic polyarthritis. *Med J Aust* 2002;**177**:356–60.
62. Mylonas AD, Harley D, Purdie DM, et al. Corticosteroid therapy in an alphavirus arthritis. *J Clin Rheumatol* 2004;**10**:326–30.
63. Nimmo JR. An unusual epidemic. *Med J Aust* 1928;**1**:549–50.
64. Oseni RA, Donaldson MD, Dalglish DA, et al. Detection by ELISA of IgM antibodies to Ross River virus in serum from patients with suspected epidemic polyarthritis. *Bull World Health Organ* 1983;**61**:703–8.
65. Penna JE, Irving LG. Evidence for meningitis in Ross River virus infection. *Med J Aust* 1993;**159**:492–3.
66. Pletnev SV, Zhang W, Mukhopadhyay S, et al. Locations of carbohydrate sites on alphavirus glycoproteins show that E1 forms an icosahedral scaffold. *Cell* 2001;**105**:127–36.
67. Powers AA, Brault AC, Shirako Y, et al. Evolutionary relationships and systematics of the alphaviruses. *J Virol* 2001;**75**:10118–31.
68. Rosen L, Gubler DJ, Bennett PH. Epidemic polyarthritis (Ross River) virus infection in the Cook Islands. *Am J Trop Med Hyg* 1981;**30**:1294–302.
69. Russell RC. Ross River virus: ecology and distribution. *Annu Rev Entomol* 2002;**47**:1–31.
70. Scrimgeour EM. Suspected Ross River virus encephalitis in Papua New Guinea. *Aust N Z J Med* 1999;**29**:559.
71. Seay AR, Griffin DE, Johnson RT. Experimental viral polymyositis: age dependency and immune response to Ross River virus infection in mice. *Neurology* 1981;**31**:656–60.
72. Seay AR, Kern ER, Murray RS. Interferon treatment of experimental Ross River virus polymyositis. *Neurology* 1987;**37**:1189–93.
73. Sellner LN, Coelen RJ, Mackenzie JS. Detection of Ross River virus in clinical samples using a nested reverse transcriptase polymerase chain reaction. *Clin Diagn Virol* 1995;**49**:257–67.
74. Shope RE, Anderson SG. The virus aetiology of epidemic exanthem and polyarthritis. *Med J Aust* 1960;**1**:156–8.
75. Smith TJ, Cheng RH, Olson NH, et al. Putative receptor binding sites on alphaviruses as visualized by cryo-electron microscopy. *Proc Natl Acad Sci U S A* 1995;**92**:10648–52.
76. Soden M, Vasudevan H, Roberts B, et al. Detection of viral ribonucleic acid and histologic analysis of inflamed synovium in Ross River virus infection. *Arthritis Rheum* 2000;**43**:365–9.
77. Tesh RB, McLean RG, Shrover DA, et al. Ross River virus (Togaviridae: *Alphavirus*) infection (epidemic polyarthritis) in American Samoa. *Trans R Soc Trop Med Hyg* 1981;**75**:426–31.
78. Vrati S, Fernon CA, Dalgarno L, et al. Location of a major antigenic site involved in Ross River virus neutralisation. *Virology* 1988;**162**:346–53.
79. Zhang W, Heil M, Kuhn RJ, et al. Heparin binding sites on Ross River virus revealed by electron cryo-microscopy. *Virology* 2005;**332**:511–18.

transmission cycles or human susceptibility. Seroprevalence to Barmah Forest virus is generally lower. Anecdotal cases complicated by central nervous system signs and glomerulonephritis have been reported.[13,23] Serologic diagnosis is straightforward in most cases, with little cross reaction between the viruses.

SINDBIS FEVER

Sindbis virus was named after a district north of Cairo, Egypt, where it first was isolated. It is the prototype of an antigenic complex that also includes western equine encephalitis virus and several Sindbis viral subtypes, including Ockelbo and related strains isolated from Scandinavia and the Babanki strain from West Africa. Two genotypes consisting of the South African and Scandinavian strains and the Australian and Asian strains have been defined. The close genetic relationship of the South African and Scandinavian strains indicates a recent introduction to Europe, possibly by a migrating bird.

The virus is distributed widely over four continents, although only sporadic cases have been reported from Asia and Australia. Transmission is endemic and occasionally hyperendemic in Africa and Scandinavia. The virus is transmitted to birds by various species of *Culex* mosquitoes, and humans are infected when virus transmission "spills out" of the enzootic cycle by the intervention of bridging vectors that feed on viremic birds and later on humans.

Endemic infections occur with varying intensity in areas of Africa.[11,19] Seroprevalence rates range from a few percent to 20 percent in some areas. Outbreaks producing hundreds to thousands of cases have been described. Concurrent transmission with West Nile virus, transmitted in the same avian cycle, is common. In South Africa, transmission occurs during the austral summer from December to April. Infections rarely may be acquired during travel.[4]

An endemic focus in Scandinavia (between 60 and 63 degrees latitude in Sweden) was recognized after a series of outbreaks in 1981 led to several hundred cases in Sweden, Norway, Finland, and adjacent areas of western Russia called, locally, Ockelbo fever, Pogosta disease, and Karelian fever.[5,16,27] Seroprevalence is low, around 5 to 10 percent. By estimation from seroprevalence rates, 600 to 1200 clinical cases may occur annually in Sweden alone. Serosurveys indicate a wider distribution of unrecognized infection in Northern and Central Europe. Most cases occur from July to September in middle-aged adults exposed to forested areas while picking berries or mushrooms or during other recreational activities. Outbreaks in Finland seem to peak at 7-year intervals, associated with the size of grouse populations.

Acute arthralgia and rash are the principal features of the illness. They may be preceded by mild fever, headache, and myalgia, but patients may have arthralgia alone, and children may exhibit fever and nonspecific symptoms alone. The joints, especially the ankles, wrists, knees, fingers, and toes and less often the hips, shoulders, elbows, neck, and back, are involved symmetrically. The joints appear swollen, and movement is limited. The Achilles and wrist tendons may be inflamed, as well, sometimes causing nerve entrapment and paresthesias. Some patients are confined to bed and unable to walk, but typically, the joint pain and stiffness lead to a lesser degree of discomfort and compromised function. A fine papular and sometimes pruritic rash appears on the trunk and extremities (including the palms and soles) but usually spares the face and head, and after a few days the rash develops a stained appearance and disappears. Lesions on the hands and feet may vesiculate. Joint symptoms resolve within 3 to 4 months in approximately 60 percent of cases; however, in the remainder, symptoms may persist for 3 to 4 years.[12,20,24,25] A higher prevalence of *HLA-DRB1*01* and autoantibodies has been reported in patients with continued joint symptoms 3 years after the initial illness in contrast to a reference population.[25] Muscle biopsy from a patient with prolonged weakness and in vitro experiments suggest that the virus can infect and destroy myotubes and myoblasts.[24] Serologic evidence of infection in patients with central nervous system signs has been reported from China.[8]

The illness is not differentiated easily from West Nile fever, which is transmitted under similar epidemiologic circumstances, and has been mistaken for rubella. The diagnosis is confirmed serologically by detection of specific IgM in acute-phase serum samples. IgM is detectable for months in some patients. The virus can be isolated, but not reliably, from acute-phase capillary blood and skin lesions. Viral genomic products can be detected in skin biopsy samples.[10]

Symptomatic treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) is sufficient in most patients. Anti–tumor necrosis factor antibodies have exacerbated alphaviral infection in experimental animals and should be avoided. Persons who choose to enter sylvatic transmission foci in the summer should apply mosquito repellents and dress appropriately.

MAYARO FEVER

Endemic infections are highly prevalent in the forested areas of South and Central America, where Mayaro virus is transmitted among *Haemagogus* mosquitoes, marsupials, and small mammals, somewhat analogous to the jungle cycle of yellow fever.[7,28] Seroprevalence rates increase with age to higher than 50 percent in native populations in some locations. The virus was isolated first from sporadic fever cases on Trinidad and named after the island's Mayaro district.[1] Outbreaks have occurred in Bolivia, Pará (a state in Brazil), and Surinam, principally in men occupationally exposed to forested sites and in residents of adjoining villages, as well as in travelers returning from Central America.[3,22,26] Cases have been reported in travelers returning to the United States, Netherlands, France, and Germany. Epidemic transmission was reported in the city of Manaus, Brazil and is a risk in other *Aedes aegypti*–infested cities.[17] The virus is placed in the Semliki Forest antigenic complex; its Una subtype causes arthritis in horses.

The illness consists of acute polyarthritis and rash.[22] Joint swelling, pain, and stiffness principally involve the

hands, wrists, ankles, toes, elbows, and knees. The hands may be so swollen that they cannot be closed, and joints of the lower extremities may be so painful that patients limp or are unable to walk. Milder cases may occur, however. The morbilliform rash is difficult to distinguish from rubella, with individual or coalescent papular lesions occurring on the trunk and extremities and usually sparing the face. Rash occurs more often in children than in adults. Mild fever, pharyngitis, conjunctivitis, headache, lymphadenitis, and minor mucosal and petechial bleeding occur in some patients. The constitutional symptoms resolve rapidly, but the joint symptoms may wax and wane for several weeks or months. Pneumonitis and renal dysfunction that have been described in some cases may have been caused by concurrent infections. Transient leukopenia usually develops; some patients have had mild elevations in liver enzyme activities and bilirubin concentration. A fatal case of yellow fever was reported in a patient recovering from Mayaro fever.

The illness is differentiated from other tropical febrile illnesses with rash and musculoskeletal pain by the specific involvement of the joints and the sylvatic setting of exposure. Some patients have features closely resembling those of rubella. Virus can be isolated from acute-phase blood specimens, but laboratory confirmation by IgM serology is sensitive, specific, and more practical. Symptoms are treated with NSAIDs and rest. Repellents should be used when sylvatic-enzootic foci cannot be avoided.

SEMLIKI FOREST VIRAL INFECTION

Febrile illnesses with severe persistent headache have been reported from Central and West Africa, where Semliki Forest virus is transmitted principally by forest *Aedes* mosquitoes.[18] The virus appears to be a common cause of infection in horses, as well. The MeTri subtype has been implicated serologically in encephalitis cases in Vietnam; its transmission cycle has not been described. A fatal case of encephalitis in a laboratory worker has been reported.[9]

NEW REFERENCES SINCE THE SIXTH EDITION

7. Forshey BM, Guevara C, Laguna-Torres VA, et al. Arboviral etiologies of acute febrile illnesses in western South America, 2000-2007. *PLoS* 2010;**4**:e787.
24. Sane J, Kurkela S, Desdouits M, et al. Prolonged myalgia in Sindbis virus infection: case description and in vitro infection of myotubes and myoblasts. *J Infect Dis* 2012.
25. Sane J, Kurkela S, Lokki ML, et al. Clinical Sindbis alphavirus infection is associated with *HLA-DRB1**01allele and production of autoantibodies. *Clin Infect Dis* 2012.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 5 *FLAVIVIRIDAE*

CHAPTER 175

FLAVIVIRUSES

175A ■ ST. LOUIS ENCEPHALITIS

Kimberly C. Martin • José R. Romero • Theodore F. Tsai

St. Louis encephalitis (SLE) virus was first identified in 1933 after an epidemic of encephalitis in St. Louis, Missouri during August 1933.[81] Before the 1999 introduction of West Nile virus, St. Louis encephalitis was the most important arboviral infection in the United States because of its leading role as a cause of widespread epidemics. Encephalitis is the principal clinical manifestation, although milder central nervous system (CNS) syndromes do occur, especially in children. The virus is transmitted between birds and *Culex* mosquitoes with humans serving only as incidental "dead end" hosts.

REFERENCES

1. Anderson CR, Downs WG, Wattley GH, et al. Mayaro virus: a new human disease agent. II. Isolation from blood of patients in Trinidad, B. W. I. *Am J Trop Med Hyg* 1957;**6**:1012.
2. Brighton SW, Prozesky OW, de la Harpe AL. Chikungunya virus infections: a retrospective study of 107 cases. *S Afr Med J* 1983;**63**:313–15.
3. Causey OR, Maroja OM. Mayaro virus: a new human disease agent. III. Investigation of an epidemic of acute febrile illness on the River Guama in Pará, Brazil, and isolation of Mayaro virus as causative agent. *Am J Trop Med Hyg* 1957;**6**:1017.
4. Eisenhut M, Schwarz TF, Hegenscheid B. Seroprevalence of dengue, chikungunya and Sindbis virus in German aid workers. *Infection* 1999;**27**:82–5.
5. Espmark A, Niklasson B. Ockelbo disease in Sweden: epidemiological, clinical and virological data from the 1982 outbreak. *Am J Trop Med Hyg* 1984;**33**:1203.
6. Flexman JP, Smith DW, Mackenzie JS, et al. A comparison of the diseases caused by Ross River virus and Barmah Forest virus. *Med J Aust* 1998;**169**:159–63.
7. Forshey BM, Guevara C, Laguna-Torres VA, et al. Arboviral etiologies of acute febrile illnesses in western South America, 2000-2007. *PLoS* 2010;**4**:e787.
8. Gu HX, Artsob H, Lin YZ, et al. Arboviruses as aetiological agents of encephalitis in the People's Republic of China. *Trans R Soc Trop Med Hyg* 1992;**86**:198.
9. Ha DQ, Calisher CH, Tien PH, et al. Isolation of a newly recognized alphavirus from mosquitoes in Vietnam and evidence for human infection and disease. *Am J Trop Med Hyg* 1995; **53**:100.
10. Horling J, Vene S, Franzen C, et al. Detection of Ockelbo virus RNA in skin biopsies by polymerase chain reaction. *J Clin Microbiol* 1993;**31**:2004.
11. Jupp PG, Blackburn NK, Thompson DL, et al. Sindbis and West Nile virus infection in the Witwatersrand-Pretoria region. *S Afr Med J* 1986;**70**:218.
12. Laine M, Luukkainen R, Jalava J, et al. Prolonged arthritis associated with Sindbis-related (Pogosta) virus infection. *Rheumatology (Oxford)* 2000;**39**:1272–4.
13. Katz IA, Hale GE, Hudson BJ, et al. Glomerulonephritis secondary to Barmah Forest virus infection. *Med J Aust* 1997;**167**: 21–3.
14. Kiwanuka N, Sanders EJ, Rwaguma EB, et al. O'nyong-nyong fever in south-central Uganda, 1996-1997: clinical features and validation of a clinical case definition for surveillance purposes. *Clin Infect Dis* 1999;**29**:1243–50.
15. Lindsay MDA, Johansen CA, Smith DW, et al. An outbreak of Barmah Forest virus disease in the south-west of Western Australia. *Med J Aust* 1995;**162**:291.
16. Lundstrom JO, Vene S, Espmark A, et al. Geographical and temporal distribution of Ockelbo disease in Sweden. *Epidemiol Infect* 1991;**106**:567.
17. Mourao MPG, de Souza Basos M, de Figueiredo RP, et al. Mayaro fever in the city of Manaus, Brazil, 2007-2008. *Vector-borne Zoonotic Dis* 2012;**12**:42.
18. Mathiot CC, Grimaud G, Bouquety GP, et al. An outbreak of human Semliki Forest virus infections in Central African Republic. *Am J Trop Med Hyg* 1990;**42**:386.
19. McIntosh BM, McGuilivray GM, Dickinson DB. Illness caused by Sindbis and West Nile viruses in South Africa. *S Afr Med J* 1964;**38**:219.
20. Niklasson B, Espmark A, Lundstrom J. Occurrence of arthralgia and specific IgM antibodies three to four years after Ockelbo disease. *J Infect Dis* 1988;**157**:832.
21. Olaleye OD, Omilabu SA, Fagbami AH. Igbo-Ora virus (an alphavirus isolated in Nigeria): a serological survey for haemagglutination inhibiting antibody in humans and domestic animals. *Trans R Soc Trop Med Hyg* 1988;**82**:905.
22. Pinheiro FP, Freitas RB, Travassos da Rosa JFS, et al. An outbreak of Mayaro virus disease in Belterra, Brazil. I. Clinical and virological findings. *Am J Trop Med Hyg* 1981;**30**:674.
23. Quinn HE, Gatton ML, Hall G, et al. Analysis of Barmah Forest virus disease activity in Queensland, Australia, 1993-2003: identification of a large, isolated outbreak of disease. *J Med Entomol* 2005;**42**:882–90.
24. Sane J, Kurkela S, Desdouits M, et al. Prolonged myalgia in Sindbis virus infection: case description and in vitro infection of myotubes and myoblasts. *J Infect Dis* 2012.
25. Sane J, Kurkela S, Lokki ML, et al. Clinical Sindbis alphavirus infection is associated with *HLA-DRB1**01allele and production of autoantibodies. *Clin Infect Dis* 2012.
26. Schaeffer M, Gajdusek DC, Lema AB, et al. Epidemic jungle fevers among Okinawan colonists in Bolivian rain forest. I. Epidemiology. *Am J Trop Med Hyg* 1959;**8**:372.
27. Suhrbier A, Jaffar-Bandjee MC, Gasque P. Arthritogenic alphaviruses: an overview. *Nat Rev Rheumatol* 2012;**8**:420.
28. Tesh RB, Watts PM, Russell KL, et al. Mayaro virus disease: an emerging mosquito-borne zoonosis in tropical South America. *Clin Infect Dis* 1999;**28**:67–73.

ETIOLOGIC AGENT

The SLE virus is a member of the genus *Flavivirus* within the Flaviviridae family of viruses. SLE belongs to the Japanese encephalitis virus antigenic complex, which also includes such important human pathogens as Japanese encephalitis virus, West Nile virus (WNV), and Murray Valley encephalitis virus.

Phylogenetic analysis of the E glycoprotein sequences from 62 strains from the United States and Central and South America identified seven genotypes corresponding to origins in the United States and South America.[52] Most recently, analysis of 133 strains, including 30 novel strains from Brazil, has identified an eighth putative genotype.[67] Individual clades can be identified within each genotype.

Genotype II is more prevalent in North America, and genotypes I and III to VIII are found distributed throughout the Americas.[67] The most genetically diverse strains differ by only 10.1 percent nucleotide divergence (2.8% amino acid variation) in the total E gene nucleotide sequence while strains within each lineage show less than 5.5 percent nucleotide divergence, thus indicating a highly constrained viral adaptation to vector and vertebrate hosts.[42] The nucleotide diversity exhibited by SLE is substantially less than that seen by other members of the Japanese encephalitis virus antigenic complex. Strains from the three principal genotypes in the United States (I, II, and V) differ in biologic characteristics; epidemic-associated strains from the Ohio and Mississippi river basins and Florida display greater virulence in mice than do strains from the western United States associated with an endemic pattern of transmission.[15,48] Multiple viral genotypes with distinct biologic characteristics may be transmitted in relatively delimited areas within a region as small as a county in some years and more widely in others.[15,31]

ECOLOGY

SLE virus is transmitted in an enzootic cycle among birds and *Culex* mosquitoes in three distinct cycles associated with its four principal vector species: *Culex tarsalis* in the western and central United States, *Culex pipiens* in the eastern and western United States, *Culex quinquefasciatus* in the east central and Atlantic states, and *Culex nigripalpus* in Florida. *Culex salinarius* and *Culex restuans* may have accessory roles in viral amplification and transmission (Fig. 175A-1).[12,36,39,46,77]

In the western United States, *C. tarsalis* serves as both an enzootic and epizootic vector. This species feeds chiefly on birds early in the summer and switches to mammalian hosts, including humans, in midsummer. The transmission cycles of SLE virus and WNV overlap throughout the United States. Sparrows, finches, and other small birds, and especially their nestlings, are the principal amplifying hosts.[44] In the West, human infections result from encounters with the vector along natural and artificial waterways, irrigated farmland, and other breeding sites found chiefly in rural agricultural areas.

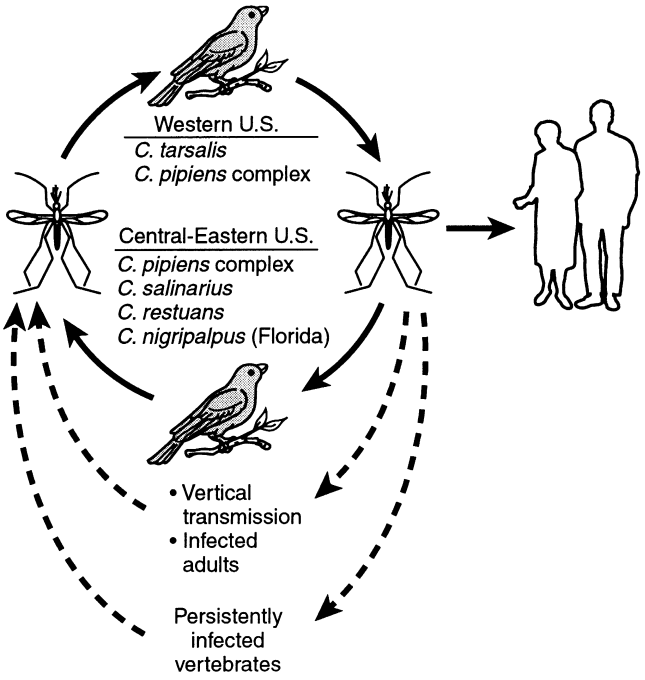

FIGURE 175A-1 ■ Transmission cycles of St. Louis encephalitis virus in North America. *Culex* mosquito vectors differ geographically: *C. tarsalis* in the West, *C. pipiens* and *C. quinquefasciatus* in the Ohio and Mississippi valleys, and *C. nigripalpus* in Florida. Epidemics occur when intense viral transmission in the enzootic cycle "spills over" and results in human infections. The viral overwintering mechanism, possibly in mosquitoes or persistently infected vertebrates, has not been proved *(broken lines).*

The recent introduction of West Nile virus into an overlapping transmission cycle may have an effect of modulating SLE virus transmission because West Nile virus–infected birds are more refractory to development of infection and viremia with SLE virus than the reverse.[24]

In the Ohio and Mississippi valleys and on the Gulf Coast, infections are acquired in the peridomestic environment, where *C. pipiens* and *C. quinquefasciatus* are, respectively, the enzootic vectors in northern and southern states. The mechanism by which the virus overwinters is not known; however, SLE virus has been shown to persist in overwintering *C.* tarsalis and *C. pipiens* for more than a month.[4] Additionally, vertical or transovarial transmission has been detected under laboratory conditions in *Culex* species.[4,26,42]

The intermittent occurrence of urban SLE outbreaks has been associated with climatic factors, such as a severe winter freeze in the 1 to 2 years before an outbreak or an antecedent mild winter, wet spring, and dry hot summer.[19,46] Snowpack and the resultant availability of irrigation water and an area supportive of mosquito breeding appear to be highly predictive of viral transmission in southern California.[82] In a model of global warming, SLE transmission is predicted to move to northern latitudes and, in existing endemic areas, to become seasonal in the spring and fall.[61] Periods of drought followed by a briefer wet interval have been associated with viral transmission in Florida.[18,70]

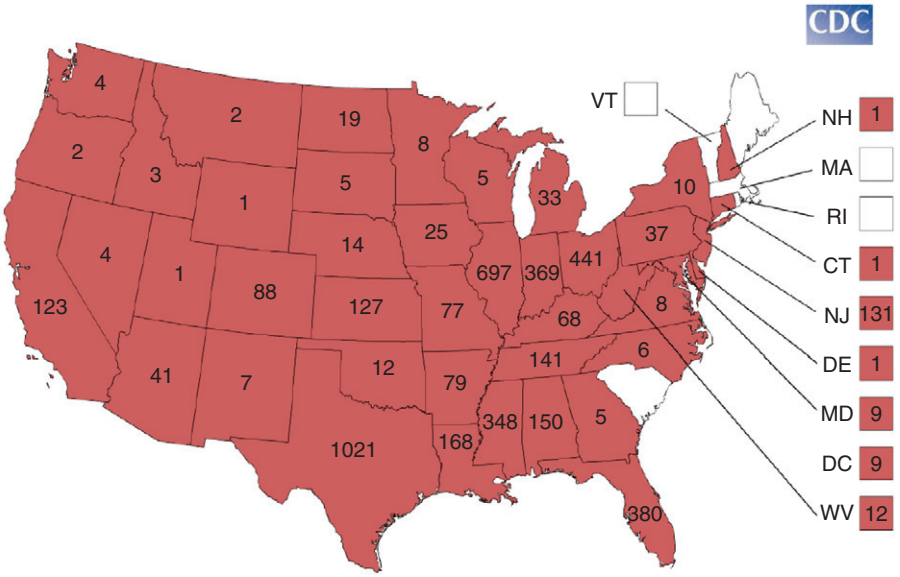

FIGURE 175A-2 ■ Reported case of St. Louis encephalitis virus neuroinvasive disease* by state, 1964 to 2010. Cases reported by state of residence. (See companion Expert Consult website for color version.)
*Neuroinvasive disease includes encephalitis, meningoencephalitis, or meningitis.

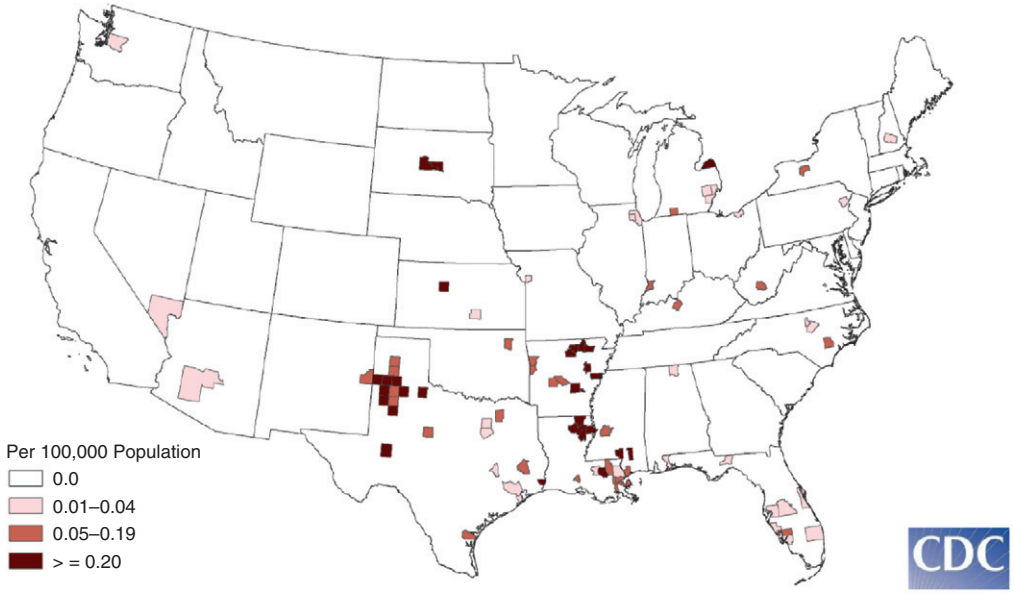

FIGURE 175A-3 ■ Reported case of St. Louis encephalitis virus neuroinvasive disease* by state, 1964 to 2010. Cases reported by county of residence. (See companion Expert Consult website for color version.)
*Neuroinvasive disease includes encephalitis, meningoencephalitis, or meningitis.

EPIDEMIOLOGY

The epidemiologic patterns of SLE virus transmission reflect human interactions with reservoirs of the virus and its principal mosquito vectors.[35,37,46,77] In western states (Fig. 175A-2 and 175A-3), perennial transmission of SLE virus leads to an endemic pattern of transmission, but epidemics are limited by a high level of immunity in the population.[63,79] Infections occur chiefly in rural areas in association with the habitat of the vector. SLE outbreaks have taken place typically in rural agricultural areas and their small towns.[34] Studies in Texas indicated that an overlap of urban and rural cycles contributed to an urban outbreak in Dallas after introduction of the virus to the urban *C. quinquefasciatus* cycle from rural *C. tarsalis*.[28,35,36]

Epidemics frequently have occurred in urban locations or their peripheries. In 1975, a nationwide epidemic led to outbreaks in Houston, Chicago, Memphis, and Detroit, as well as smaller outbreaks in rural towns throughout the South and Midwest (Fig. 175A-4). Outbreaks in large urban centers generally have been associated with attack rates of fewer than 40 cases/100,000 residents and often have clustered in low socioeconomic status areas, the most recent example occurring in Louisiana in 2001.* In recent urban outbreaks, homelessness and infection with

*References 17, 28, 30, 46, 58, 77, 78, 84.

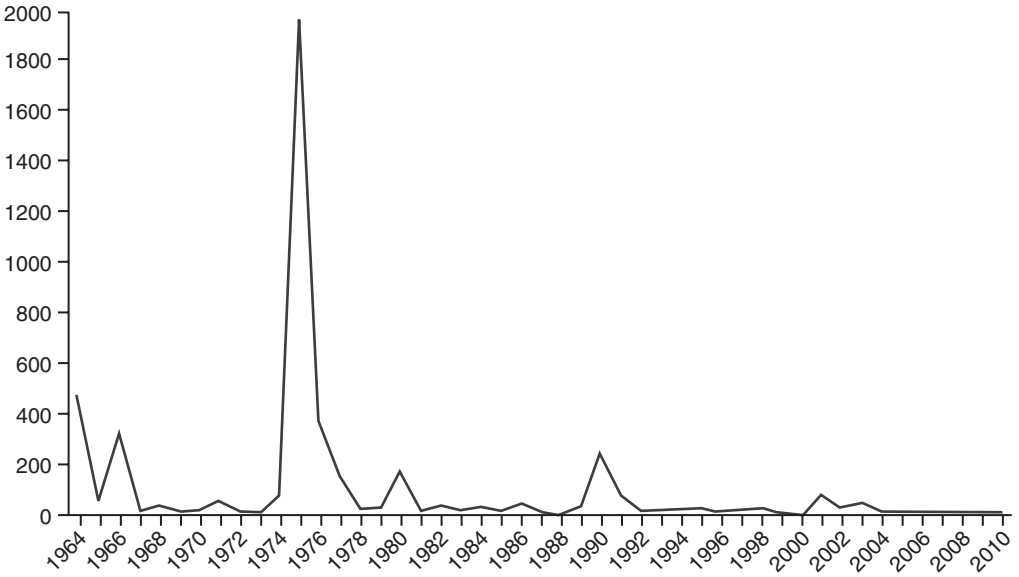

FIGURE 175A-4 ■ Reported St. Louis encephalitis cases by year, United States, 1964 to 2010. A nationwide epidemic in 1975 produced more than 2000 cases.

human immunodeficiency virus (HIV) have been the principal risk factors for acquiring SLE.[55,80] However, in the 1933 St. Louis outbreak, attack rates were highest in wealthy areas where open sewers, streams, ponds, and weeds were prevalent; and in other outbreaks, lush vegetation around houses, which provides shelter for *C. nigripalpus*, and closed sewer systems clogged with grass clippings were factors associated with high attack rates in upper socioeconomic areas.[18,45]

In outbreaks of disease from *C. pipiens* and *C. quinquefasciatus*, epidemic attack rates are 1.2 to 3 times higher in females than in males, possibly reflecting increased exposure of females to these peridomestic vectors,[41,46,78] whereas in western *C. tarsalis*–borne outbreaks, attack rates are usually higher in males.[34,63] A similar observation has been made in Florida, where SLE attack rates are highest in working-age males.[20,45,53] *C. nigripalpus* and *C. tarsalis* are most active in the twilight hours, and outdoor activity during these periods is associated with a greater risk for exposure.

Most cases (>80%) occur during the months of July through September.[62] However, in Florida, epidemics have continued through mid-December.[45,69] In the 1975 nationwide epidemic, outbreaks appeared first in the southeastern states in June, followed by an appearance in northern foci later in the summer and fall.[46]

From the introduction of WNV into the United States in 1999 through 2010, only 215 cases of neuroinvasive SLE virus disease were reported to the CDC.[11,62] Five states accounted for nearly 90 percent of cases during this period—Louisiana, Texas, Arizona, Michigan, and Mississippi (see Fig. 175A-2).[13,62] However, within states reporting SLE infections, cases are geographically restricted (see Fig. 175A-3). A median of eight cases per year were reported nationwide from 2002 to 2011. The annual incidence of neuroinvasive SLE peaked in 2001 (0.27/100,000 population) and fell to an average low of 0.001/100,000 in 2005 to 2007.[62] Reemergence of SLE in areas where it had been absent for decades has been noted. In 2010, the District of Colombia reported two cases of non-neuroinvasive SLE. These were the first case reports from this jurisdiction since 1975.[13] Only Alabama and Arkansas reported cases of neuroinvasive disease (N = 4) in 2011. It is unknown if the recent decrease in the number of reported cases of SLE is due to normal periodicity in SLE virus activity, surveillance artifact, or environmental displacement of the virus by West Nile virus.

Although a case of neuroinvasive SLE in an infant as young as 19 days has been reported, the most important risk factor for acquiring neuroinvasive disease is advanced age.[37,46,83] Age-specific attack rates are lowest in children and rise steadily in adulthood, with attack rates 5 to 40 times higher in persons older than 60 years than in those younger than 10 years. During outbreaks, infections occur uniformly in all age groups, with as many as 300 asymptomatic infections for each clinically apparent case. Therefore, the higher clinical attack rates in the elderly are a function of susceptibility and not exposure.[37,46] The clinical expression of illness is more severe and the case-fatality rate is also highest in the elderly. The biologic basis for this age-related risk probably reflects aging of the immune system, as well as comorbidities compromising cerebrovascular integrity.[10]

In the West, where SLE infections are endemic, an increasing level of immunity associated with length of residence leads to an adult population with lower susceptibility (11% in the most recent serosurvey[63]). Consequently, cases often are seen in children and young adults. In a Florida outbreak, immunity to other flaviviruses (mainly from previous dengue infection) was also shown to protect against the acquisition of SLE.[7]

Cases of SLE have been reported in tropical America (Jamaica, Suriname, French Guiana, northern Argentina, and Brazil).[1,20,72] Increasing seroprevalence rates observed with increasing age indicates a higher frequency of endemic infection than is clinically apparent.[1,73] SLE is an emerging cause of arboviral disease in South America

and has been responsible for both sporadic cases and several outbreaks.[20,50,72] These sporadic SLE outbreaks principally have resulted in mild febrile illnesses that have been clinically confused with dengue. The largest outbreak reported to date occurred in 2005 in Cordoba, Argentina and resulted in 47 laboratory-confirmed or probable cases, 11 of which occurred in children. Nine adults died in this outbreak.[73]

CLINICAL MANIFESTATIONS

Clinical manifestations range from a mild influenza-like illness to fatal encephalitis.[8,60,66,84] A case definition that stratifies cases into clinical syndromes of encephalitis, aseptic meningitis, and febrile headache[8] (Table 175A-1) has proved useful in surveillance (Fig. 175A-5). Although children as a group exhibit milder symptoms, encephalitis develops in more than half of confirmed and presumptive pediatric cases, and 95 percent have objective clinical signs of CNS infection.[5,6,73,84]

SLE cannot be easily differentiated from other viral CNS infections on the basis of clinical features. Photophobia, headache, fever, nausea, vomiting, malaise, and neck stiffness are typical early symptoms. In approximately half of reported cases, an abrupt onset of weakness, incoordination, disturbed sensorium, or other neurologic signs may occur; but equally often, patients have nonspecific symptoms with subtle changes in coordination or mentation during a prodromal phase lasting several days to more than a week. Seizures have been reported in a third of adult cases.[80]

In addition to fever of abrupt onset and signs of meningeal irritation, patients nearly uniformly exhibit alterations in state of consciousness, such as restlessness, confusion, lethargy, delirium, or coma. Neurologic examination usually reveals general weakness, hyperreflexia, and tremulousness, but focal deficits are unusual findings. Cranial nerve palsies, especially those involving nerves VII, IX, and X, occur in 10 to 25 percent of cases.[8] Clinical signs of increased intracranial pressure have been reported in a few patients. Unusual localizing neurologic signs have been described in patients with involvement of the midbrain, thalamus, or brain stem. In a 4-year-old girl, paralysis of upward gaze was associated with a brain stem infection.[31] A 14-year-old boy with acquired parkinsonism attributed to SLE infection has been reported.[59] Ataxia has been observed in a fourth of the cases in children, and opsoclonus has been reported in several cases in young adults.[22,23,80] In a series of 26 children with SLE, seizures were observed in 8, 6 of whom had focal seizures; however, enteroviruses were isolated concurrently from the stool in 5 of the 8 patients.[5,57] It is not known whether they had enteroviral CNS infection. A confirmed case of SLE causing a seizure has been reported in a neonate.[83] In most cases, the fever abates 4 to 7 days after onset, and clinical improvement is evident early in the second week of illness. Polyradiculopathy and transverse myelitis have been reported.[8,68]

The peripheral leukocyte count is elevated modestly, with a shift to the left in most patients. Cerebrospinal fluid (CSF) usually contains fewer than 100 leukocytes/mm^3, with a median value between 100 and 200. However, in some cases, fewer than 10 cells and occasionally no cells are discovered in the CSF. An increasing ratio of mononuclear to polymorphonuclear cells is observed on successive examinations. CSF protein rarely exceeds 200 mg/dL, and hypoglycorrhachia is unusual.[8]

TABLE 175A-1 Definitions of Clinical Syndromes Caused by St. Louis Encephalitis

Encephalitis* (including meningoencephalitis and encephalomyelitis)
Acute febrile illness (oral temperature ≥37.8°C (≥100°F)
One or more signs in either of the following categories:
Altered level of consciousness (i.e., confusion, disorientation, delirium, lethargy, stupor, coma)
Objective signs of neurologic dysfunction (i.e., seizure, cranial nerve palsy, dysarthria, rigidity, paresis, paralysis, abnormal reflexes, tremor)
Aseptic meningitis*
Acute febrile illness
Signs of meningeal irritation (i.e., stiff neck with or without Kernig or Brudzinski sign)
No objective signs of neurologic dysfunction
Febrile headache*
Acute febrile illness
Headache (also may have other systemic symptoms, such as nausea or vomiting)
No signs of meningeal irritation or neurologic dysfunction

Modified from Brinker KR, Monath TP. The acute disease. In: Monath TP, editor. *St. Louis encephalitis.* Washington, DC: American Public Health Association; 1980, p 503-534.
*Cerebrospinal fluid pleocytosis is present in patients with encephalitis and aseptic meningitis; it also may be found in patients with the syndrome of febrile headache.

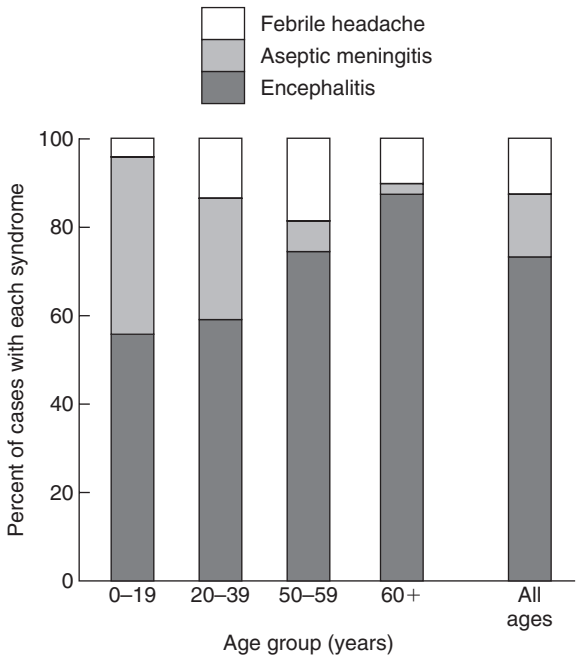

FIGURE 175A-5 ■ Distribution of St. Louis encephalitis cases by clinical syndrome and age. Although mortality rates and disease severity increase with age, most cases in children are clinical encephalitis. (Data from Zweighaft RM, Rasmussen C, Brolnitsky O, et al. St. Louis encephalitis: the Chicago experience. *Am J Trop Med Hyg* 1979;**28**:114-118.)

In one series, elevated serum aldolase levels suggested a myopathic process, and muscle biopsy and electromyography disclosed lower motor neuron dysfunction, possibly from spinal root involvement. Moderate transaminase elevations have been reported in 19 to 48 percent of adult patients. Decreased serum osmolality attributed to inappropriate secretion of antidiuretic hormone was observed in a fourth to a third of cases in two series.[8,9]

Urinary incontinence, frequency, or retention and pyuria or proteinuria have been reported in adult patients, and viral antigen was detected on cells in the urinary sediment by immunofluorescence in one report.[38] Lower motor neuron lesions may have been the mechanism for bladder symptoms in some patients.

Electroencephalographic abnormalities have included focal discharges, including periodic lateralized epileptiform discharges, but more consistently show diffuse generalized slowing.[5,9,80] Computed tomography scans may be normal, but magnetic resonance images have disclosed T2-weighted hyperintense abnormalities in the substantia nigra, consistent with edema.[14]

PATHOLOGY

Pathologic changes are found predominantly in the midbrain, thalamus, and brain stem but also in the cerebral cortex and cerebellum. Lesions are observed in the spinal cord in some cases.[65,71,75] The leptomeninges are affected to a variable degree with edema, small hemorrhages, and round-cell infiltration.

Perivascular and parenchymal inflammation is prevalent in the brain nuclei and scattered in the white matter of the brain stem and spinal cord and subcortical and deep cerebral areas. Nodular collections of inflammatory cells composed of lymphocytes, microglial cells, and monocytes are found in proximity to or surrounding degenerating neurons characterized by eccentric nuclear displacement, nuclear pyknosis, and cellular contraction. Vascular infiltrations and thrombosis have not been described, as they have in western equine encephalitis (WEE) and eastern equine encephalomyelitis (EEE). Focal demyelination, which is observed in WEE, has not been characteristic of SLE. Transmission electron microscopy may provide a pathologic diagnosis on tiny tissue fragments.[16]

PATHOGENESIS

After infection, viral replication occurs locally and in regional lymph nodes. After this eclipse phase, bloodborne (viremic) dissemination ensues, with secondary sites of replication occurring in muscle, endocrine, lymphoreticular, and other tissues and, in less than 1 percent of infections, in the CNS. Vascular endothelial cells may have a role in actively transporting virus from the capillary lumen to the brain and in supporting secondary viral replication.[21] Conditions that compromise cerebrovascular integrity and disrupt the blood-brain barrier may predispose to neuroinvasion; for example, concurrent CNS cysticercosis may be a risk factor for acquiring Japanese encephalitis, a related flaviviral infection. Although some experimental studies suggest that CNS invasion occurs through the olfactory epithelium, pathologic studies of Japanese encephalitis cases indicate widespread involvement of the brain stem, deep nuclei, and cortex, most consistent with hematogenous infection.

Recovery from flaviviral encephalitis depends on early intrathecal synthesis of antibodies and viral clearance by macrophages. The inflammatory response in related CNS infections consists of helper-inducer T cells and, to a lesser degree, B lymphocytes infiltrating the perivascular space and parenchyma from the blood. Macrophages and activated microglial cells in the perivascular space and parenchyma, respectively, are responsible for viral clearance. Outcome is determined by the comparative rates of viral spread and neuronal infection, the migration of inflammatory cells into the CNS, and the rapidity of the antibody response. Production of interferon is elicited in the brain of human SLE patients, but its role in limiting the spread of virus in the CNS is unclear.[39] The clinical course of SLE in HIV-infected persons has not been significantly different from that of other cases; however, few patients have been studied.

Resistance and susceptibility to some flaviviral infections in mice have been linked to genes associated with permissiveness to infection and the immune response. Environmental factors that influence susceptibility to flaviviral infection in experimental models include stress from cold or isolation, reticuloendothelial cell blockade, and heavy metal intoxication.

DIAGNOSIS

A laboratory diagnosis of SLE usually is made serologically, although virus rarely has been recovered from blood in the acute phase of illness.[35] Virus can be sought from brain and other organs obtained by biopsy or at autopsy by inoculation of Vero cell cultures and suckling mice intracerebrally.[33] Viral antigen has been demonstrated in brain sections and in urinary cell sediment.[33,64] It should be borne in mind that the clinical sensitivity of nucleic acid amplification tests (NAAT) is greater than that of antigen detection and viral culture using cell culture or suckling mouse inoculation.[33] The ultimate sensitivity of NAATs depends on the dynamics of viral replication and host tropism.

Serologic studies comparing antibody titers in paired specimens collected during the first week illness and 2 to 3 weeks later are considered the most appropriate for establishing the diagnosis.[33] Hemagglutination-inhibition, complement-fixation, and neutralizing antibody assays are used primarily in reference laboratories. A single immunoglobulin (Ig)M assay may be sufficient to establish the diagnosis in many cases. SLE-specific IgM capture enzyme-linked immunosorbent assay (ELISA) is the preferred serologic screening procedure.[33,49] Both serum and CSF samples should be tested. Intrathecal production of virus-specific IgM reflects recent infection because virus-specific IgM can be detected in serum or CSF in 75 percent or more of specimens obtained within 4 days of onset. In most patients, IgM levels decline in the convalescent sample and disappear approximately 4 months after onset, although IgM persisted at high

titer in a few patients for 6 months.[79] In areas where SLE is endemic, virus-specific IgM carried over from the previous transmission season could potentially lead to an erroneous diagnosis. The indirect immunofluorescent antibody procedure offers a similar capacity for rapid diagnosis; however, it is less sensitive than ELISA and IgM rheumatoid factor can lead to a false-positive result. Differentiation of antibody responses to West Nile virus, particularly during simultaneous outbreaks of the infections, has been aided by development of a duplex immunoassay.[29,41]

The serologic response in a primary flavivirus infection is usually type specific, but repeated infections lead to broad heterologous responses that are often difficult to interpret. Human infections with indigenous flaviviruses (e.g., Rio Bravo and Powassan) other than SLE are rare occurrences; however, in patients with antecedent yellow fever immunization or infection with dengue virus acquired in areas of the United States where dengue was previously endemic and recently reemergent (e.g., Texas and Florida) or acquired during travel or residence abroad, a broadly reactive flavivirus antibody response may obfuscate the diagnosis. Recent immigrants with preexisting dengue antibodies are the most common source of flaviviral antibodies in most areas of the United States.

DIFFERENTIAL DIAGNOSIS

In an endemic setting, clinicians should consider SLE principally in the differential diagnosis of patients with aseptic meningitis or acute encephalitis. However, in the context of an outbreak, the diagnostic threshold should be lowered to include patients with less specific findings, especially acute febrile illnesses with headache, and patients who exhibit confusion or are encephalopathic without fever. Increased suspicion of SLE in mildly ill patients should be directed particularly at children and young adults, in whom the infection often is manifested without meningoencephalitis. As mentioned earlier, in South America, SLE is a sporadic cause of acute febrile illness that, as a syndrome, has many non–vector-borne as well as vector-borne causes, including dengue.[40,76]

The clinical findings of SLE cannot be easily distinguished from those of other CNS infections. However, focal neurologic deficits are less characteristic of SLE and should suggest other diagnoses.[51] The combination of global confusion and tremulousness in SLE may suggest a metabolic encephalopathy, and in elderly patients, the initial manifestations of SLE can overlap the signs and symptoms of a cerebrovascular accident.

West Nile encephalitis and SLE are virtually identical in their clinical findings, their predilection for the elderly, and other epidemiologic features, including late summer seasonality, urban locus of epidemic transmission, and similar *Culex*-bird transmission cycles, such that individual cases cannot be differentiated except by specific laboratory testing.[29,41,43] In Brazil, Rocio virus, an antigenically related mosquito-borne flavivirus that was the cause of a single acute encephalitis outbreak in 1976, must be excluded, particularly because ELISA and other serological assays may cross react (see the discussion on other flaviviruses).

The progression of symptoms and localizing signs associated with herpes simplex viral encephalitis contrasts sharply with the clinical findings in SLE, in which localizing signs are less often demonstrated. Enteroviral infections have an overlapping summer seasonality but often are associated with clustering of illnesses in families and other epidemiologic clues of person-to-person spread. Skin eruptions, respiratory symptoms, pericarditis, myocarditis, and conjunctivitis are helpful distinguishing characteristics. Concurrent enterovirus infection and SLE may be associated with an increased risk for seizures during the acute phase of illness.[5,57] Enteroviral infections can be confirmed by detecting viral genomic sequences in CSF or blood by polymerase chain reaction or by recovery of virus from CSF or blood. Primary HIV, human herpesvirus 6, and mumps virus infections are other common infections that should be entertained in the differential diagnosis. A third of patients with mumps encephalitis do not have associated parotid gland swelling.

Adenoviral encephalitis is a rare infection in children, more severe than SLE, and sometimes associated with hepatic and other extraneural sites of infection. The presence of lymphoreticular involvement and typical hematologic features should suggest the clinical diagnosis of Epstein-Barr virus infection. Rabies virus encephalitis should be considered if the clinical picture is one of a progressive encephalitis.

Partially treated bacterial meningitis and parameningeal pyogenic infections are the principal bacterial causes to exclude. Cat-scratch fever can manifest as fever and encephalopathy mimicking arboviral encephalitis.[54] Seizures associated with *Shigella* enteritis and ataxia associated with typhoid fever potentially could be interpreted as a primary CNS infection. Tuberculous meningitis is typically a subacute illness; evidence of active extraneural sites of infection should be sought. A low CSF glucose level is a clue to the diagnosis.

In urban areas or where parental occupational exposure may occur, lead intoxication remains an important potential cause of encephalopathy in summer. Hyperthermia associated with sustained exposure to elevated environmental temperature likewise may lead to encephalopathy with seizures, signs of raised intracranial pressure, and evidence of hepatic dysfunction. This constellation of symptoms also may suggest Reye syndrome, although this disease typically occurs in the winter and spring after a respiratory viral infection, in particular influenza virus, and is characterized by hypoglycemia and an elevated blood ammonia level. Patients with salicylism exhibit similar clinical findings, but a history of salicylate ingestion, an elevated anion gap early in the course of illness, and an elevated blood salicylate level should suggest that diagnosis.

TREATMENT

Supportive therapy, as outlined in the earlier sections, should be aimed at maintaining cardiorespiratory function and fluid and electrolyte balance, controlling seizures,

and monitoring and maintaining normal intracranial pressure.[43] No specific antiviral therapy is available.

PROGNOSIS

The principal risk factor for a fatal outcome is advanced age (see Fig. 175A-5).* Among all cases reported to the Centers for Disease Control and Prevention from 1955 to 1971, as well as recent data from 2003 to 2007, 8 percent died, but the age-specific mortality rate in persons 60 years of age or older was 19.5 percent. Fatality rates as high as 38 to 80 percent were recorded in the 1933 St. Louis outbreak in patients who were 60 to 89 years of age. Mortality rates in children have ranged from 2 to 5 percent, with the highest risk in children younger than 5 years of age. The overall case-fatality rate in an outbreak in Hermosillo, Mexico (involving principally children), was 20 percent.[17] The high fatality rate in this instance is anomalous because fatality rates are usually lower in *C. tarsalis*–borne SLE outbreaks in the West.

In adults, coma, a low CSF leukocyte count (<100 cells/mm^3), and underlying hypertensive vascular disease have been factors associated with a fatal outcome.[10] Risk factors for mortality in children have not been described.

Recovery from SLE usually is complete or associated with soft sequelae such as emotional disturbances, dizziness, headache, memory impairment, and tremor.[3,25,27,52] In one study of 193 cases, 25 percent of children who were 1 to 4 years of age at the time of infection had serious neurologic sequelae, the highest rate of any age group.[56] The incidence of sequelae was 10 percent in children aged 5 to 9 years. Children who were younger than 1 year of age at the time of infection appeared to be spared any serious sequelae. Deficits in motor and intellectual function were reported in some cases. Seizures were not a significant residual abnormality. The same cohort monitored at intervals of 6 months to 14 years had no perceptible residual differences in intelligence quotient in contrast to the normal population. In a case report, persistent ataxia of the extremities and trunk and dysarthria were reported as residual findings in a 54-year-old patient secondary to postinfectious encephalomyelitis temporally associated with SLE.[29,69]

No deleterious effects of SLE on the outcome of pregnancy have been reported. However, infection with Japanese encephalitis virus in the second trimester resulted in transplacental viral transmission and abortion, whereas infection in the third trimester was not associated with fetal damage. In experimentally infected mice, vertical transmission led to learning deficits in congenitally infected pups.[2]

PREVENTION

Preventive public health efforts have focused on surveillance of viral activity in the enzootic cycle to predict epidemic activity. In many east central states, weekly serologic surveys of captured wild birds and sentinel chickens are conducted to detect rising seroprevalence rates that reflect viral amplification[47] and an increased risk for human infection. The abundance of female vector mosquitoes and increased viral infection rates in vectors also have been correlated with a risk for epidemic transmission.[82]

When viral activity is elevated, ground and aerial application of insecticides is aimed at reducing the infected adult vector population. If insecticides are applied early enough, viral amplification can be potentially attenuated and epidemic transmission aborted.

Avoidance of outdoor activity during the twilight and evening hours, use of repellents appropriately, and repair of screens or installation of air conditioners in residences are simple but effective measures that reduce exposure to adult mosquitoes. Improvement of drainage and removal of containers that could serve as mosquito breeding sites are important steps in preventing the disease.

A vaccine is not available, nor is vaccination of the general public a realistic preventive measure because of low attack rates and the intermittent and focal nature of outbreaks. However, if a vaccine or chemoprophylactic agent were available, selective administration to a targeted population, particularly the elderly, might be appropriate in an outbreak.

*References 8, 35, 37, 46, 62, 78, 79.

NEW REFERENCES SINCE THE SIXTH EDITION

11. Centers for Disease Control. *Saint Louis encephalitis*. n.d. Available from http://www.cdc.gov/sle/technical/epi.html#casesbyyear. n.d. Accessed on June 5, 2012.
12. Centers For Disease Control. *Saint Louis encephalitis*. Available from http://www.cdc.gov/sle/technical/transmission.html. Accessed on June 1, 2012.
26. Flores FS, Diaz LA, Batallán GP, et al. Vertical transmission of St. Louis encephalitis virus in *Culex quinquefasciatus* (Diptera: Culicidae) in Córdoba, Argentina. *Vector Borne Zoonotic Dis* 2010; **10**:999–1002.
32. Kramer LD, Chandler LJ. Phylogenetic analysis of the envelope gene of St. Louis encephalitis virus. *Arch Virol* 2001;**146**:2341–55.
33. Lanciotti RS, Tsai TF. Arboviruses. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2011. p. 1488–503.
40. Manock SR, Jacobsen KH, deBravo NB, et al. Etiology of acute undifferentiated febrile illness in the Amazon basin of Ecuador. *Am J Trop Med Hyg* 2009;**81**:146–51.
42. May FJ, Li L, Zhang S, et al. Genetic variation in St. Louis encephalitis virus. *J Gen Virol* 2008;**89**:1901–10.
50. Mondini A, Cardeal IL, Lázaro E, et al. Saint Louis encephalitis virus, Brazil. *Emerg Infect Dis* 2007;**13**:176–8.
62. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg* 2008;**79**:974–9.
67. Rodrigues SG, Nunes MR, Casseb SM, et al. Molecular epidemiology of Saint Louis encephalitis virus in the Brazilian Amazon: genetic divergence and dispersal. *J Gen Virol* 2010;**91**:2420–7.
81. Webster LT, Fite GL. A virus encountered in the study of material from cases of encephalitis in the St. Louis and Kansas City epidemics of 1933. *Science* 1933;**78**:463–5.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adames AJ, Dutary B, Tejera H, et al. Relocion entre mosquitos vectores y aves acuaticas en la transmission potencial de dos arbovirus. *Rev Med Panama* 1993;**18**:106–19.
2. Andersen AA, Hanson RP. Intrauterine infection of mice with St. Louis encephalitis virus: immunological, physiological, neurological, and behavioral effects on progeny. *Infect Immun* 1975;**12**: 1173–83.
3. Azar GJ, Bond JO, Chappell GL, et al. Follow up studies of St. Louis encephalitis in Florida: sensorimotor findings. *Am J Public Health* 1966;**56**:1074–81.
4. Bailey CL, Eldridge BF, Hayes DE, et al. Isolation of St. Louis encephalitis virus from overwintering *Culex pipiens* mosquitoes. *Science* 1978;**199**:1346–9.
5. Barrett FF, Yow MD, Phillips CA. St. Louis encephalitis in children during the 1964 epidemic. *JAMA* 1965;**193**:381–5.
6. Blattner RJ, Heys FM. St. Louis encephalitis: occurrence in children in the St. Louis area during nonepidemic years 1939-1944. *JAMA* 1945;**129**:854–7.
7. Bond JO, Hammon WM. Epidemiologic studies of possible cross protection between dengue and St. Louis encephalitis arboviruses in Florida. *Am J Epidemiol* 1970;**92**:321–9.
8. Brinker KR, Monath TP. The acute disease. In: Monath TP, editor. *St. Louis encephalitis*. Washington, DC: American Public Health Association; 1980. p. 503–34.
9. Brinker KR, Paulson G, Monath TP, et al. St. Louis encephalitis in Ohio, September 1975: clinical and EEG studies in 16 cases. *Arch Intern Med* 1979;**139**:561–6.
10. Broun GO. Relationship of hypertensive vascular disease to mortality in cases of St. Louis encephalitis. *Med Bull St Louis Univ* 1952;**4**: 32–7.
11. Centers for Disease Control. *Saint Louis Encephalitis*. n.d. Available at http://www.cdc.gov/sle/technical/epi.html#casesbyyear. Accessed on June 5, 2012.
12. Centers For Disease Control. *Saint Louis Encephalitis*. n.d. Available at http://www.cdc.gov/sle/technical/transmission.html. Accessed on June 1, 2012.
13. Centers for Disease Control. Nile virus disease and other arboviral diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011; **60**:1009–13.
14. Cerna F, Mehrad B, Luby JP, et al. St. Louis encephalitis and the substantia nigra: MR imaging evaluation. *AJNR Am J Neuroradiol* 1999;**20**:1281–3.
15. Chandler LJ, Parsons R, Randle Y. Multiple genotypes of St. Louis encephalitis virus (Flaviviridae: Flavivirus) circulate in Harris County, Texas. *Am J Trop Med Hyg* 2001;**64**:12–19.
16. Chu CT, Howell DN, Morgenlander JC, et al. Electron microscopic diagnosis of human flavivirus encephalitis: use of confocal microscopy as an aid. *Am J Surg Pathol* 1999;**23**:1217–26.
17. Cortés AG, Aquino MLZ, Bahena JG, et al. St. Louis encephalomyelitis in Hermosillo, Sonora, Mexico. *Pan Am Health Organ Bull* 1975;**9**:306–16.
18. Day JF. Predicting St. Louis encephalitis: lessons from recent, and not so recent, outbreaks. *Annu Rev Entomol* 2001;**46**:111–38.
19. Day JF, Shaman J. Severe winter freezes enhance St. Louis encephalitis virus amplification and epidemic transmission in peninsular Florida. *J Med Entomol* 2009;**46**:1498–506.
20. Díaz LA, Ré V, Almiron WR, et al. Genotype III Saint Louis encephalitis virus outbreak, Argentina, 2005. *Emerg Infect Dis* 2006; **12**:1752–4.

20a. Division of Notifiable Diseases and Healthcare Information, Office of Surveillance, Epidemiology, and Laboratory Services, CDC. Summary of notifiable diseases—United States, 2011. *MMWR Morb Motal Wkly Rep* 2013;**60**:1–117.

21. Dropulic B, Masters CL. Entry of neurotropic arboviruses into the central nervous system: an in vitro study using mouse brain endothelium. *J Infect Dis* 1990;**161**:685–91.
22. Estrin WJ. The serological diagnosis of St. Louis encephalitis in a patient with the syndrome of opsoclonia, body tremulousness, and benign encephalitis. *Ann Neurol* 1977;**1**:596–8.
23. Evans RW, Welch K. Opsoclonus in a confirmed case of St. Louis encephalitis. *J Neurol Neurosurg Psychiatry* 1982;**45**:660–1.
24. Fang Y, Reisen WK. Previous infection with West Nile or St. Louis encephalitis viruses provides cross protection during reinfection in house finches. *Am J Trop Med Hyg* 2006;**75**:480–5.
25. Finley KH, Riggs N. Convalescence and sequelae. In: Monath TP, editor. *St. Louis encephalitis*. Washington, DC: American Public Health Association; 1980. p. 535–50.
26. Flores FS, Diaz LA, Batallán GP, et al. Vertical transmission of St. Louis encephalitis virus in *Culex quinquefasciatus* (Diptera: Culicidae) in Córdoba, Argentina. *Vector Borne Zoonotic Dis* 2010;**10**: 999–1002.
27. Greve KW, Houston RJ, Adams D. The neurobehavioural consequences of St. Louis encephalitis infection. *Brain Inj* 2002;**16**: 917–27.
28. Hopkins CC, Hollinger FB, Johnson RF, et al. The epidemiology of St. Louis encephalitis in Dallas, Texas, 1966. *Am J Epidemiol* 1975;**102**:1–15.
29. Johnson AJ, Noga AJ, Kosoy O, et al. Duplex microsphere-based immunoassay for detection of anti–West Nile virus and anti–St. Louis encephalitis virus immunoglobulin M antibodies. *Clin Diagn Lab Immunol* 2005;**12**:566–74.
30. Jones SC, Morris J, Hill G, et al. St. Louis encephalitis outbreak in Louisiana in 2001. *La State Med Soc* 2002;**154**:303–6.
31. Kaplan AM, Koveleski JT. St. Louis encephalitis with particular involvement of the brain stem. *Arch Neurol* 1978;**35**:45–6.
32. Kramer LD, Chandler LJ. Phylogenetic analysis of the envelope gene of St. Louis encephalitis virus. *Arch Virol* 2001;**146**:2341–55.
33. Lanciotti RS, Tsai TF. Arboviruses. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, DC: American Society for Microbiology; 2011. p. 1488–503.
34. Longshore WA, Stevens IM, Hollister AC, et al. Epidemiologic observations on acute infectious encephalitis in California, with special reference to the 1952 outbreak. *Am J Hyg* 1956;**63**:69–86.
35. Luby JP. St. Louis encephalitis. *Epidemiol Rev* 1979;**1**:55–73.
36. Luby JP. St. Louis encephalitis. In: Beran GW, editor. *Handbook of zoonoses. Section B: Viral*. 2nd ed. Boca Raton, FL: CRC Press; 1994. p. 47.
37. Luby JP, Miller G, Gardner P, et al. The epidemiology of St. Louis encephalitis in Houston, Texas, 1964. *Am J Epidemiol* 1967;**86**: 584–97.
38. Luby JP, Murphy FK, Gilliam JN, et al. Antigenuria in St. Louis encephalitis. *Am J Trop Med Hyg* 1980;**29**:265–8.
39. Luby JP, Stewart WE, Sulkin SE, et al. Interferon in human infections with St. Louis encephalitis virus. *Ann Intern Med* 1969;**71**:703–9.
40. Manock SR, Jacobsen KH, deBravo NB, et al. Etiology of acute undifferentiated febrile illness in the Amazon basin of Ecuador. *Am J Trop Med Hyg* 2009;**81**:146–51.
41. Martin DA, Noga A, Kosoy O, et al. Evaluation of a diagnostic algorithm using immunoglobulin M enzyme-linked immunosorbent assay to differentiate human West Nile Virus and St. Louis encephalitis virus infections during the 2002 West Nile virus epidemic in the United States. *Clin Diagn Lab Immunol* 2004;**11**: 1130–3.
42. May FJ, Li L, Zhang S, et al. Genetic variation in St. Louis encephalitis virus. *J Gen Virol* 2008;**89**:1901–10.
43. McCarthy M. St. Louis encephalitis and West Nile virus encephalitis. *Curr Treat Options Neurol* 2001;**3**:433–8.
44. McLean RG. Arboviruses of wild birds and mammals. *Bull Soc Vector Ecol* 1991;**16**:3–16.
45. Meehan PJ, Wells DL, Paul W, et al. Epidemiological features of and public health response to a St. Louis encephalitis epidemic in Florida, 1990-1991. *Epidemiol Infect* 2000;**125**:181–8.
46. Monath TP. Epidemiology. In: Monath TP, editor. *St. Louis encephalitis*. Washington, DC: American Public Health Association; 1980. p. 239–312.
47. Monath TP, Tsai TF. St. Louis encephalitis: lessons from the last decade. *Am J Trop Med Hyg* 1987;**37**(Suppl.):40–59.
48. Monath TP, Cropp CB, Bowen GS, et al. Variation in virulence for mice and rhesus monkeys among St. Louis encephalitis virus strains of different origin. *Am J Trop Med Hyg* 1980;**29**:948–62.
49. Monath TP, Nystrom RR, Bailey RE, et al. Immunoglobulin M antibody capture enzyme-linked immunosorbent assay for diagnosis of St. Louis encephalitis. *J Clin Microbiol* 1984;**20**:784–90.
50. Mondini A, Cardeal IL, Lázaro E, et al. Saint Louis encephalitis virus, Brazil. *Emerg Infect Dis* 2007;**13**:176–8.
51. Naidech A, Elliott D. St. Louis encephalitis with focal neurological signs. *Clin Infect Dis* 1999;**29**:1334–5.

52. Neel JL. Neuropsychiatric sequelae in a case of St. Louis encephalitis. *Gen Hosp Psychiatry* 2000;**22**:126–8.
53. Nelson DB, Kappus KD, Janowski JT, et al. St. Louis encephalitis, Florida 1977: patterns of a widespread outbreak. *Am J Trop Med Hyg* 1983;**32**:412–18.
54. Noah DL, Bresee JS, Gorensek MJ, et al. Cluster of five children with acute encephalopathy associated with cat-scratch disease in South Florida. *Pediatr Infect Dis J* 1995;**14**:866–9.
55. Okhuysen PC, Crane JK, Pappas J. St. Louis encephalitis in patients with human immunodeficiency virus infection. *Clin Infect Dis* 1993;**17**:140–1.
56. Palmer RJ, Finley KH. Sequelae of encephalitis: report of a study after the California epidemic. *Calif Med* 1956;**84**:98–100.
57. Phillips CA, Melnick JL, Barrett FF, et al. Dual virus infections: simultaneous enteroviral disease and St. Louis encephalitis. *JAMA* 1966;**197**:169–72.
58. Powell KE, Blakey DL. St. Louis encephalitis: the 1975 epidemic in Mississippi. *JAMA* 1977;**237**:2294–8.
59. Pranzatelli MR, Mott SH, Pavalakis SG, et al. Clinical spectrum of secondary parkinsonism in childhood: a reversible disorder. *Pediatr Neurol* 1994;**10**:131–40.
60. Quick DT, Thompson JM, Bond JO, et al. The 1962 epidemic of St. Louis encephalitis in Florida. II. Clinical features of cases in the Tampa Bay area. *Am J Epidemiol* 1965;**81**:415–24.
61. Reeves WC, Hardy JL, Reisin WK, et al. Potential effect of global warming on mosquito-borne arboviruses. *J Med Entomol* 1994;**31**:323–32.
62. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg* 2008;**79**:974–9.
63. Reisen WK, Chiles RE. Prevalence of antibodies to western equine encephalomyelitis and St. Louis encephalitis viruses in residents of California exposed to sporadic and consistent enzootic transmission. *Am J Trop Med Hyg* 1997;**57**:526–9.
64. Reisen WK, Kramer LD, Chiles RE, et al. Encephalitis virus persistence in California birds: preliminary studies with house finches. *J Med Entomol* 2001;**38**:393–9.
65. Reyes MG, Gardner JJ, Poland JD, et al. St. Louis encephalitis: quantitative histologic and immunofluorescent studies. *Arch Neurol* 1981;**38**:329–34.
66. Riggs S, Smith DL, Phillips CA. St. Louis encephalitis in adults during the 1964 Houston epidemic. *JAMA* 1965;**193**:284.
67. Rodrigues SG, Nunes MR, Casseb SM, et al. Molecular epidemiology of Saint Louis encephalitis virus in the Brazilian Amazon: genetic divergence and dispersal. *J Gen Virol* 2010;**91**:2420–7.
68 Sanders M, Blumberg A, Haymaker W. Polyradiculopathy in man produced by St. Louis encephalitis (SLE). *South Med J* 1976;**69**:1121–5.
69. Sejvar JJ, Bode AV, Curiel M, et al. Post-infectious encephalomyelitis associated with St. Louis encephalitis virus infection. *Neurology* 2004;**63**:1719–21.
70. Shaman J, Day JF, Stieglitz M, et al. Seasonal forecast of St. Louis encephalitis virus transmission, Florida. *Emerg Infect Dis* 2004;**10**:802–9.
71. Shinner JJ. St. Louis virus encephalomyelitis. *Arch Pathol* 1963;**75**:311–22.
72. Spence LP. St. Louis encephalitis in tropical America. In: Monath TP, editor: *St. Louis encephalitis*. Washington, DC: American Public Health Association; 1980. p. 451–71.
73 Spinsanti LI, Díaz LA, Glatstein N, et al. Human outbreak of St. Louis encephalitis detected in Argentina, 2005. *J Clin Virol* 2008;**42**:27–33.
74. Spinsanti LI, Ré VE, Díaz MP, et al. Age-related seroprevalence study for St. Louis encephalitis in a population from Cordoba, Argentina. *Rev Inst Med Trop Sao Paulo* 2002;**44**:59–62.
75. Suzuki M, Phillips CA. St. Louis encephalitis: a histopathologic study of the fatal cases from the Houston epidemic in 1964. *Arch Pathol* 1966;**81**:47–54.
76. Terzian AC, Mondini A, Bronzoni RV, et al. Detection of Saint Louis encephalitis virus in dengue-suspected cases during a dengue 3 outbreak. *Vector Borne Zoonotic Dis* 2011;**11**:291–300.
77. Tsai TF. Arboviral infections in the United States. *Infect Dis Clin North Am* 1991;**5**:73–102.
78. Tsai TF, Canfield MA, Reed CM, et al. Epidemiologic aspects of a St. Louis encephalitis outbreak in Harris County, Texas, 1986. *J Infect Dis* 1988;**157**:351–6.
79. Tsai TF, Cobb WB, Bolin RA, et al. Epidemiologic aspects of a St. Louis encephalitis outbreak in Mesa County. *Am J Epidemiol* 1987;**126**:460–73.
80. Wasay M, Diaz-Arrastia R, Suss RA, et al. St. Louis encephalitis: a review of 11 cases in a 1995 Dallas, Texas, epidemic. *Arch Neurol* 2000;**57**:114–18.
81. Webster LT, Fite GL. A virus encountered in the study of material from cases of encephalitis in the St. Louis and Kansas City epidemics of 1933. *Science* 1933;**78**:463–5.
82. Wegbreit J, Reisen WK. Relationships among weather, mosquito abundance, and encephalitis virus activity in California: Kern County 1990-1998. *J Am Mosq Control Assoc* 2000;**16**:22–7.
83. Wootton SH, Kaplan SL, Perrotta DM, et al. St. Louis encephalitis in early infancy. *Pediatr Infect Dis J* 2004;**23**:951–4.
84. Zweighaft RM, Rasmussen C, Brolnitsky O, et al. St. Louis encephalitis: the Chicago experience. *Am J Trop Med Hyg* 1979;**28**:114–18.

175B ■ WEST NILE VIRUS

José R. Romero

HISTORY

West Nile virus (WNV) was isolated first from the blood of a 37-year-old African woman in December 1937 in Omongo, West Nile district, in the Northern Province of Uganda while researchers were attempting to isolate yellow fever virus.[188] In the same report, these investigators demonstrated that sera from patients recovering from Japanese encephalitis virus could neutralize WNV. Shortly thereafter, this finding was confirmed and expanded to document that WNV was antigenically related not only to Japanese encephalitis virus but also to St. Louis encephalitis virus.[186]

In 1950, Melnick and coworkers[130] isolated WNV from the blood of three healthy children in Egypt, thereby demonstrating that WNV was not confined to a single geographic location. Before 1999, WNV was known to be endemic to Africa, the Middle East, Europe, west and central Asia, and Oceania. With its incursion into the United States in 1999[8,144] and subsequent spread throughout North America, Central America, and the Caribbean,[54,106,163] WNV has established a worldwide distribution.

The first reports of WNV as a cause of outbreaks of febrile illness came from Israel in the early 1950s and ultimately led to its being associated with a clinical entity coined West Nile fever.[18,72] Studies performed by Taylor and colleagues[194] at the U.S. Naval Medical Research Unit in Egypt in the early 1950s provided information about the epidemiology of WNV infection and clinical picture of West Nile fever in children. This information was augmented by the seminal report by Marberg and colleagues[125] describing the clinical characteristics of serologically and virologically confirmed cases of West Nile fever among adolescent and young adult military personnel during a 1953 epidemic in Israel. Sporadic reports of central nervous system (CNS) complications associated with WNV infection followed closely after the early clinical descriptions of West Nile fever and pointed to the neuropathogenic potential of WNV.* Further characterization of West Nile neuroinvasive disease (encephalitis, meningitis, acute flaccid paralysis) followed as additional outbreaks of WNV were reported.

During the past half century, outbreaks of WNV have occurred worldwide. Notable human outbreaks include Israel, 1951 to 1954, 1957, and 2000; France, 1962; South Africa, 1974; Algeria, 1994; Tunisia, 1997; Romania, 1996; and Russia, 1999.† Two of the largest epidemics recorded occurred in South Africa in 1974 and the United States in 2003. During the former, an estimated 18,000 cases of West Nile fever occurred.[54,81]

After the introduction of WNV into the North American continent in 1999, cases of West Nile fever and neuroinvasive disease remained at relatively low levels through 2001 and exploded in 2002 and 2003 when 4156 and 9862 cases, respectively, of WNV infection were reported to the Centers for Disease Control and Prevention (CDC)[32] The 2002 epidemic of WNV in the United States resulted in the largest epidemic of neuroinvasive West Nile disease ever recorded, with nearly 3000 cases reported.[150] Currently, WNV has superseded all autochthonous North American arboviruses as the single most important cause of arboviral disease in the United States.[44] The magnitude of the number of WNV infections is exemplified by an estimate, based on 2003 national blood donor screening data for WNV infection, that 735,000 cases of WNV infection occurred that year.[25]

VIROLOGY

WNV is a member of the Flaviviridae family of RNA viruses.[119] The viral family is composed of three genera: *Flavivirus*, *Pestivirus*, and *Hepacivirus*. WNV is a member of the *Flavivirus* genus and falls within the Japanese encephalitis virus serocomplex, along with Cacipacore, Japanese encephalitis, Kunjin, Murray Valley encephalitis, St. Louis encephalitis, Usutu, and Yaoundé viruses. Kunjin virus, which is endemic to Australia and Asia, is a subtype of WNV. Members within the Japanese encephalitis virus serogroup are antigenically related to each other.

WNV is a spherical, enveloped virus measuring approximately 50 nm in diameter. The viral envelope is derived from the lipid bilayer of the host cell and surrounds the approximately 25-nm nucleocapsid core of the virion. The host cell–derived envelope is modified by the insertion of two viral proteins: E (envelope protein) and M (membrane protein).

The genome of WNV is a single-stranded, positive-sense (messenger sense) RNA molecule measuring 11,029 nucleotides in length (Fig. 175B-1).[112,119] Two noncoding regions (5′ and 3′) flank the 10,302-nucleotide viral single open reading frame and measure 96 and 631 nucleotides, respectively. The open reading frame is organized as 5′-C-prM-E-NS1-NS2A-NS3-NS4A-NS4B-NS5-3′ and is transcribed into a single polyprotein of 3434 amino acids encoding three structural and seven nonstructural proteins.[112,119] The N-terminal portion of the polyprotein consists of viral structural proteins, whereas the remainder of the protein contains the nonstructural (NS) proteins. The viral polyprotein is cotranslationally and posttranslationally processed by host proteases and the viral protease NS2B-NS3 to yield three structural (i.e., E, prM, and C) and seven NS (i.e., 1, 2A, 2B, 3, 4A, 4B, and 5) proteins.

Viral attachment, endocytosis, membrane fusion, and viral assembly are mediated by the E protein. The search

*References 17, 81, 161, 189, 190, 192.
†References 18,46, 54, 58, 62, 81, 153, 158, 196.

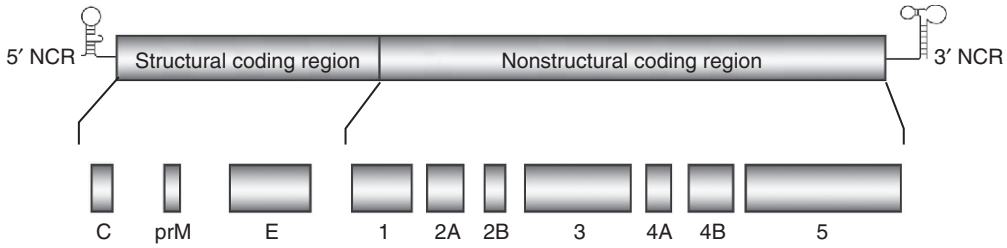

FIGURE 175B-1 ■ Organization and protein products of the West Nile genome.

for the cellular receptors of WNV has identified the integrin $\alpha_v\beta_3$ and the lectins DC-SIGNR and DC-SIGN as capable of binding WNV in vitro.[47,55] However, whether they are physiologically relevant in vivo remains to be documented. Once bound to its receptor, WNV gains entry into the cell through endocytosis; after a pH-dependent change occurs in the conformation of the E protein, the viral and host endosomal membranes fuse. The viral nucleocapsid is then released into the cytoplasm of the cell. Viral replication is thought to occur on the membrane of the rough endoplasmic reticulum. The C or capsid protein binds to the viral RNA genome and forms a ribonucleoprotein complex required for packaging of the genome.[139] The immature virions are assembled, accumulate in vesicles, and bud into the endoplasmic reticulum. The immature virions are processed as they traverse the secretory pathway. Immature viral particles contain a premembrane (prM) protein that blocks premature viral fusion by the E protein. The prM protein is cleaved to the M protein by the trans-Golgi protein furin during the egress of the mature virion from the secretory pathway. Virions are transported to the cell membrane in vesicles and ultimately released through exocytosis or budding.

The NS proteins regulate viral transcription and translation, as well as attenuate host immune responses to WNV.[119,173] The NS1 protein serves as a cofactor for the viral replicase and modulates the host innate immune response by inhibition of TLR3 signal transduction.[210] Host interferon (IFN) responses are inhibited by NS2A through inhibition of IFN-β promoter activity. This protein also may play a role in viral assembly. The NS3 protein is a serine protease and requires NS2B as a cofactor for activity. It also possesses NTPase and helicase activities. NS proteins 4A and B modulate IFN signaling. The NS5 functions as the RNA-dependent RNA polymerase and a methyltransferase. The NS5 protein from the virulent WNV strain NY99 is an antagonist of type 1 IFN-mediated JAK-STAT signal transduction.[113]

Phylogenetic analysis of WNV isolates from around the world has demonstrated the existence of up to seven lineages.[112,157] Lineages 1 and 2 constitute the major lineages. Strains that comprise lineage 2 are found exclusively in sub-Saharan Africa and Madagascar. In contrast, lineage 1 strains are found in geographically dispersed regions around the world and can be subdivided into three clades.[112] WNV strains from Africa, Europe, the United States, the Middle East, and Russia cluster within clade 1a. Kunjin virus and India WNV strains form clades 1b and 1c, respectively. WNV strains isolated from birds and a human during the 1999 outbreak in the United States and a WNV strain isolated in 1998 from a dead goose in Israel have been shown to be closely related.[111] Phylogenetic analysis of the prM protein and E glycoprotein demonstrated that they share more than 99.8 percent nucleotide homology, indicating that the North American strain of WNV almost certainly originated in the Middle East. Analysis of U.S. WNV isolates has demonstrated a slowly evolving nucleotide divergence. The initial WNV strain in the United States, NY99, was displaced in less than 4 years by the current dominant genotype WN02.

Genetic correlations appear to exist between genotype/lineage and virulence/fitness phenotype.[90,112,157] Viruses from lineage 1 have been associated with outbreaks of West Nile neuroinvasive disease, whereas those from lineage 2 appear to cause only asymptomatic infection or West Nile fever. Neurovirulence data by use of a murine model corroborate this finding, indicating that lineage 1 strains of WNV are more neuroinvasive than are those of lineage 2.[14] Nucleotide changes in the 3′ noncoding region and amino acid substitutions in the nonstructural and envelope proteins have been associated with increased morbidity and viral load in American crows, decreased incubation time for infection until transmission of WNV by *Culex pipiens* mosquitos, and modification of the host IFN signaling cascade. These changes have given rise to attenuated genotypes of WNV as it has spread across the United States.

EPIDEMIOLOGY

In temperate climates, WNV infections occur seasonally during the summer months, coinciding with periods of increased activity of its mosquito vectors. The peak period of activity for transmission of WNV to humans in the United States, Europe, and the Mediterranean basin generally is from July through October.[150,212] However, as WNV has spread southward in the United States, the period of reported transmissions has increased, with human cases being reported as early as April and as late as December.[84] Year-round transmission may be possible in tropical climates.

Infections of humans (i.e., epizootic infection) by WNV are incidental to its enzootic life cycle and, with rare exception, result in a "dead-end" infection without subsequent human-to-human transmission. Epizootic transmission of WNV occurs when sufficient numbers of mosquitoes that bite both birds and humans become

infected to allow transmission to humans. Important risk factors for the acquisition of human WNV infection are the amount of time spent outdoors when mosquitoes are biting; the presence of dead birds in the neighborhood; the exposure to mosquitoes; and the land cover, water, and soil suitable for harboring potentially infected mosquitoes.[24,48,80,138] Marked increases in WNV infections have been associated with economic factors, such as the downturn in the housing market and delinquent mortgages, which has led to large numbers of neglected residential swimming pools in some areas.[166]

The enzootic cycle of the WNV requires replicative phases in its mosquito vectors and avian (or other) hosts. After WNV-infected blood meal has been ingested, the virus penetrates the mosquito's gut.[70] Viral replication then takes place in the salivary glands and nervous system of the mosquito. In mosquito tissues, WNV is nonlytic and results in persistent infection for the lifetime of the vector.

Mosquitoes play an important role in transgenerational and transseasonal maintenance of the WNV enzootic cycle. Laboratory and field observations had documented that female *C. pipiens* mosquitoes can transmit WNV vertically to F1 progeny.[60,199] In addition, WNV can survive the winter by overwintering in hibernating female mosquitoes, as documented with *C. pipiens*, which thereby serve as a source of WNV to initiate the enzootic cycle the following spring.[143]

Although numerous mosquito species have been found to be infected with WNV, ornithophilic members in the *Culex* genus are the principal vectors for the transmission of WNV around the world. These include *C. pipens* and *Culex quinquefasciatus* in Europe, *Culex perexiguus* in North Africa and the Middle East, *Culex univittatus* in sub-Saharan Africa, *Culex tritaeniorhynchus* in south Asia, *Culex annulirostris* in Australia, and species of the *Culex vishnu* complex in India.[105]

Although WNV has been found in 59 of the 173 North American species of mosquitoes, only about a half-dozen are considered to be important vectors for transmission to humans.[84] As in other regions of the world, *Culex* spp. of mosquitoes are important vectors[84]; *C. pipens* (the northern house mosquito) and *C. quinquefasciatus* (the southern house mosquito) are considered to be the predominant vectors in the northeast, southern, and southwestern United States, respectively. *Culex tarsalis* is a predominant vector west of the Mississippi River. *Culex nigripalpus* and *Culex salinarius* may be important vectors in regions where they are abundant. Last, *Culex restuans* also has been found to be a significant contributor to WNV-positive mosquito pools. Although arthropods can be infected with WNV, they are not thought to be important in transmission of WNV.

Birds are thought to be the principal amplifying hosts for WNV. Nearly 200 species of North American birds are susceptible to infection by WNV.[105] However, only birds capable of sustaining high-titer viremia for significant periods are capable of infecting the mosquitoes that feed on them. In vivo experiments using *C. tarsalis* have shown that 74 to 100 percent of mosquitoes consuming blood meals containing $10^{7.1}$ plaque-forming units (PFUs) per milliliter become infected. In contrast, 36 percent or fewer mosquitoes become infected after the consumption of blood containing $10^{4.9}$ PFU/mL.[73] As a point of comparison, on the basis of blood tested from viremic blood donors, the maximum viral load in human blood is approximately $10^{3.2}$ PFU/mL.[84] This finding would indicate that humans fail to produce blood viral titers of sufficient magnitude to infect mosquitoes. Most mammals also fail to have sufficient levels of viremia to permit transmission to mosquitoes.

Songbirds (Passeriformes), shorebirds (Charadriiformes), owls (Stringiformes), and hawks (Falconiformes) develop adequate viremia to infect mosquitoes feeding on them (pigeons and ducks do not). House finches, house sparrows, common grackles, and, in particular, corvids such as crows, magpies, and jays have been shown to be highly infectious to mosquitoes.[84,105] In addition, they have a high mortality rate after acquiring infection with WNV. Die-offs of avian species serve as epidemiologic markers for WNV activity in the environment.[75,138]

Unlike mammals, some amphibians appear to be capable of sustaining levels of viremia that potentially could be sufficient to infect mosquitoes and may serve as competent reservoirs for their infection. In the United States, alligators *(Alligator mississippiensis)* infected with WNV produced sufficient viral loads to infect mosquitoes.[104] Detection of WNV RNA in mosquitoes, alligators, and alligator-derived blood meals collected at alligator farms in Louisiana support the role of alligators as competent hosts for WNV amplification and transmission.[198] In Russia, the lake frog *(Rana ridibunda)* may be a host for WNV.[84,107]

In areas where WNV traditionally has been endemic, seroprevalence studies document that most individuals acquire WNV infection during childhood. Such early acquisition of WNV infection results in large portions of the adult population with serologic evidence of immunity to WNV and may attenuate the occurrence of large outbreaks of WNV infection. Studies conducted in the 1950s in Egypt documented that a rapid rise in the presence of antibodies occurred in children 1 to 4 years of age such that by the time children were 4 years of age, 50 to 80 percent had neutralizing or complement-fixing antibodies to WNV.[130,194] Similar patterns of acquisition of WNV-specific antibodies have been documented in the Sudan, Central Africa, and Nigeria.[188,194] Interestingly, more recent studies conducted in Egypt indicate that the seroprevalence rates in children and young adults, although still high, may be decreasing.[50,53] One can speculate that mosquito abatement programs and, possibly, changes in lifestyle have resulted in decreasing numbers of infections in children.

Seroepidemiologic studies conducted in areas where initial WNV has resulted in large outbreaks of West Nile fever and neuroinvasive disease document low seroprevalence rates among the general population.[124,137,140,196] Investigation of the seroprevalence of WNV infection after an outbreak of WNV in Romania after the first major WNV epidemic in Europe found it to be 4.6 percent.[196] After the initial North American outbreak of West Nile encephalitis in New York in 1999, the seroprevalence at the outbreak's epicenter was found to be approximately 2.6 percent.[137] Based on data derived from

the screening of the United States blood supply, the estimated prevalence of past WNV infection among the United States population is approximately 1 percent.[161] In areas where postepidemic seroprevalence has been conducted, it has been found to range from 2.6 to 14 percent; too low to protect against future large-scale outbreaks of WNV disease.[54,140,161]

Multiple novel, non–vector-borne modes of WNV transmission were identified during the North American epidemics. Transfusion-associated transmission of WNV was reported for the first time in 2002, when 23 cases were confirmed.[134,156] WNV virus infection has resulted from the transfusion of red blood cells, platelets, fresh frozen plasma, and granulocytes.[42,43,131,133,156] Of the 32 cases, 5 (16%) occurred in children younger than 18 years[42,133,156] Three of the children had immunocompromising conditions (i.e., acute myeloid leukemia, rhabdomyosarcoma, stem-cell transplant). The three children received platelet infusions and developed West Nile meningoencephalitis; all survived. The sole adolescent was a victim of a motor vehicle accident and received a transfusion of red blood cells. This patient subsequently became an organ donor and was implicated in four cases of transplantation-associated transmission of WNV.[36,94] A sole case of acquisition of WNV via granulocyte transfusion has been reported.[131]

As a result of transfusion-associated WNV transmission, national screening of blood donations for the presence of WNV by nucleic acid amplification–based testing was initiated in June 2003. Although active screening has identified 2557 presumably viremic blood doners, 11 probable or confirmed cases of transfusion-acquired WNV infection occurred through 2010 (none of which occurred in children); they most likely were attributable to donations containing very low levels of virus.[32,40,121,131,133]

In addition to transmission that occurs through transfusion of blood products, transmission through organ transplantation has been documented worldwide.[36,41,43,94,167] However, no cases in pediatric transplant patients have been reported.[121] The risk for acquiring West Nile neuroinvasive disease is approximately 40 times greater in organ transplant recipients than in the general population.[108] Unlike blood donations, organ donations currently are not required to be screened by nucleic acid amplification for WNV infection, nor is it performed routinely.

Confirmed or suspected maternal-fetal and maternal-infant vertical transmission of WNV has been reported.[4,35,34,83,149] In the most rigorously documented case,[4,34,83] confirmed maternal WNV infection occurred during the 27th week of gestation. The infant, delivered at 38 weeks of gestation, was found to have bilateral chorioretinitis, severe bilateral white matter loss in the temporal and occipital lobes, and tissue destruction in a temporal lobe. The presence of WNV-specific immunoglobulin (Ig)M was confirmed in cord blood, infant serum, and cerebrospinal fluid (CSF), although the CSF contained red blood cells. Maternal serum also contained WNV-specific IgM at the time of birth. The WNV genome was detected in the umbilical cord tissue and placenta by reverse-transcription polymerase chain reaction (RT-PCR). No WNV genome was detected in the infant's CSF.

Evaluation of 71 pregnancies reported to the Center for Disease Control and Prevention's (CDC's) WNV pregnancy registry failed to identify any confirmed cases of congenital WNV infection.[149] However, three infants who lacked evidence of anti-WNV IgM in cord blood or infant serum at the time of delivery subsequently were found to have serologic evidence of infection that may have been acquired congenitally. A breast-fed term infant, whose mother had onset of West Nile neuroinvasive disease 6 days before delivery, developed West Nile meningitis at 10 days of age. Another breast-fed infant, born to a woman with acute West Nile fever at the time of delivery, was noted to have a transient rash at birth. No serum or cord blood was available for testing. However, at 1 month of age, the infant was found to have WNV-specific IgM antibodies. In the final case, the infant's mother had a febrile illness 3 weeks before delivery. The infant appeared to be healthy at birth, but at 7 days of age developed seizures. Evaluation of the infant at 17 days of age found lissencephaly and West Nile encephalitis with WNV-specific IgM antibodies detectable in the CSF. Because two of the infants were breast-fed, the possibility exists that WNV was transmitted in that manner. In addition, in none of the cases could the possibility of mosquito-borne transmission of WNV be excluded conclusively. This report also failed to link WNV infection during pregnancy with an increased frequency of spontaneous abortion, premature birth, or low birth weight.

WNV has been detected in the breast milk of infected women.[35,149] At least one case of possible transmission of WNV associated with breast-feeding has been reported.[35,82,149] The mother received packed red blood cell transfusions on the first two postpartum days. Ten days post partum, the mother developed headache followed by fever. On evaluation, she was found to have CSF pleocytosis and the presence of WNV-specific IgM in the CSF. The infant was breast-fed for the first 17 days of life, and although the infant was clinically asymptomatic, a serum sample obtained at 25 days of age was found to have WNV-specific IgM. Because of the paucity of additional case reports, it appears that the risk for WNV transmission from mother to infant through breast-feeding is low. The current state of knowledge about transmission of WNV through breast milk does not warrant a change in breast-feeding recommendations.

Additional nontraditional modes of confirmed or possible transmission of WNV has been reported through laboratory exposure, animal autopsies, percutaneous injury, conjunctival inoculation, aerosol inhalation, and dialysis.[37-39,64,79,200,201]

With the exceptions of Alaska and Hawaii, every state in the United States has reported human, mosquito, avian, or animal WNV. From 1999 to 2011, 31,414 cases of WNV infection were reported to the CDC (Table 175B-1).[32] Neuroinvasive syndromes accounted for 13,241 cases (42.1%), and non-neuroinvasive syndromes were reported in 18,173 cases (57.9%). During the same period, 1282 deaths (4%) were reported.[32]

TABLE 175B-1 **West Nile Virus Infections in the United States: 1999-2011**

1 Year	Total Cases	Deaths	Neuroinvasive Disease	Nonneuroinvasive Disease	Other Specified
1999	62	7	59	3	
2000	21	2	19	2	
2001	66	9	64	2	
2002	4156	284	2,946	1,160	50
2003	9862	264	2,866	6,830	166
2004	2539	100	1,142	1,269	128
2005	3000	119	1,294	1,607	99
2006	4269	177	1,459	2,616	194
2007	3630	124	1,217	2,350	63
2008	1356	44	687	624	45
2009	720	32	373	322	25
2010	1021	57	629	392	
2011	712	42	486	226	
Total	31,414	1,261 (4%)*	13,241 (42.1%)	17,403 (55.4%)	770

*Percent of total cases

TABLE 175B-2 **Epidemiologic Features of West Nile Virus Neuroinvasive Disease Cases Reported in Children and Adults: 1999-2007**

Variable	Children (<18 y), N = 443	Adults (18-49 y), N = 3634	Adults ≥50 y), N = 7004
Median cases/yr, n (range)	49 (1-137)	377 (4-1057)	826 (15-1879)
Median incidence/yr, n (range)*	0.07 (0.00-0.19)	0.28 (<0.01-0.78)	0.92 (0.02-2.32)
Clinical syndrome, n (%)			
Encephalitis or meningoencephalitis	163 (37)	1230 (34)	4160 (59)
Meningitis	208 (47)	1847 (51)	1640 (23)
Acute flaccid paralysis†	5 (1)	23 (<1)	37 (<1)
Unspecified neuroinvasive disease	67 (15)	534 (15)	1167 (17)
Fatality, n (%)	3 (1)	48 (1)	949 (14)
Hospitalized, n/N (%)‡	166/206 (81)	1255/1585 (79)	2941/3344 (88)

From Lindsey NP, Hayes EB, Staples E, Fischer, M. West Nile disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084-1089.
Data include cases reported as encephalitis, meningoencephalitis, meningitis, and/or acute flaccid paralysis with age data.
*Data show the incidence per 100,000 persons.
†Data include cases reported as acute flaccid paralysis only (not those reported as encephalitis or meningitis with acute flaccid paralysis). Data were only available from 2004 to 2007.
‡This includes data from 2004 to 2007 only.

Pediatric-specific epidemiologic and clinical data regarding WNV infections has become available.[82-84,121] However, it should be borne in mind that available data are most likely incomplete because of underreporting of cases. After an Ohio outbreak of WNV, the county-wide seroprevalence rate was found to be 1.9 percent. However, among children younger than 17 years, the seroprevalence rate was nearly 3.5 times higher: 6.4 percent.[124] From 1999 to 2007, 1478 cases of West Nile infection were reported to the CDC. For that period, the median annual incidence of West Nile neuroinvasive disease was 0.07 cases/100,000 children. By way of comparison, for the same period the median annual incidence for adults 18 to 49 years of age and adults older than 50 years was 4 times and 13 times higher, respectively (Table 175B-2). The five states with the highest state-level median annual pediatric incidences were South Dakota (1.02/100,000), Wyoming (0.82 cases/100,000), New Mexico (0.59/100,000), and Nebraska (0.45/100,000) (Fig. 175B-2). The Great Plains and mountain states had the highest cumulative incidences.[121]

The distribution of WNV syndromes among children has been difficult to define because of underreporting of pediatric cases of West Nile infection.[82] A recent report of pediatric cases from 1999 through 2007 provides the clearest picture of the disease in children (see Table 175B-2).[82,121] For that period, a total of 27,605 cases of West Nile infection were reported. During that same period, 1478 (5%) cases of West Nile infection in children younger than 18 years of age were reported. Pediatric cases were reported from 41 states. West Nile fever occurred in 1009 (68%) and neuroinvasive disease in 443 (30%). For 26 cases, disease was not specified. Children accounted for 4 percent of all cases of neuroinvasive disease. For those children with neuroinvasive disease, 208 (47%) had meningitis, 163 (37%) encephalitis or

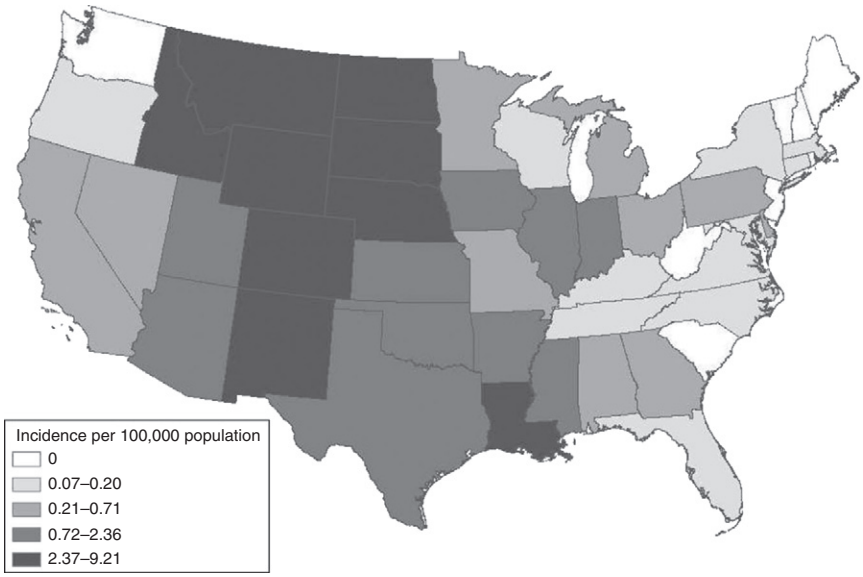

FIGURE 175B-2 ■ The Great Plains and mountain states had the highest cumulative incidences.[121]

TABLE 175B-3 **Epidemiologic Features of West Nile Virus Disease Cases Reported in Children, by Clinical Syndrome**

Variable	Neuroinvasive Diseases (N = 443)*	Fever (N = 1009)	Unspecified (N = 26)
Boys, n (%)	272 (61)	588 (58)	14 (54)
Median age (range)	12 yr (4 days to 17 yr)	14 yr (1 days to 17 yr)	14 yr (1-17 yr)
Age group, n (%)			
<1 yr	17 (4)	11 (1)	1 (4)
1-4 yr	49 (11)	67 (7)	3 (12)
5-9 yr	99 (22)	141 (14)	4 (15)
10-14 yr	137 (31)	327 (32)	7 (27)
15-17 yr	141 (32)	463 (46)	11 (42)
Fatality, n (%)	3 (1)	0 (0)	0 (0)
Hospitalized, n/N (%)†	166/206 (81)	72/450 (16)	1/26 (4)

From Lindsey NP, Hayes EB, Staples E, Fischer, M. West Nile disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084-1089.
*Data include encephalitis, meningoencephalitis, meningitis, and/or acute flaccid paralysis.
†This includes data from 2004 to 2007 only.

meningoencephalitis, and 5 (1%) acute flaccid paralysis alone. It should be noted, however, that 2 patients each reported as having encephalitis and meningitis had associated acute flaccid paralysis. For 15 percent, the specific neuroinvasive syndrome was not specified.

The median age of the reported children with West Nile fever was 14 years (range, 1 day to 17 years). The median age of children with West Nile neuroinvasive disease was slightly younger, at 12 years (Table 175B-3).[121] The 78 percent of cases of West Nile fever and 63 percent of neuroinvasive disease occurred in children 10 years of age or older. Congenital infection appears to have occurred in two children. Only three deaths were reported—1-month-boy, a 14-year-old boy, and a 16-year-old girl. Two of the children who died of neuroinvasive disease had underlying medical conditions; one had an immunodeficiency and the other lissencephaly.[83,121,147]

Comparison of pediatric and adult cases of West Nile neuroinvasive disease from 1999 to 2007 demonstrated that adults 50 years of age and older more frequently had encephalitis or meningoencephalitis than did children or adults younger than 50 years.[121] The case-fatality rate among children was similar to that observed among adults younger than 50 years—0.6 and 1.3 percent, respectively. The fatality rate among adults 50 years of age and older was 14 percent.

Taken together, the available information indicates that WNV infection in children is typically mild, resulting in West Nile fever in more than two thirds of children with symptomatic infection. The distribution of West Nile neuroinvasive disease in children indicates that in individuals younger than 18 years, the predominant West Nile neuroinvasive syndrome is meningitis followed by encephalitis.

PATHOGENESIS

The pathogenesis of West Nile encephalitis has been explored in animal model systems. Subcutaneous inoculation of WNV is thought to result in WNV infection and replication in Langerhans dendritic cells.[26] Domain III of the WNV envelope (E) protein is believed to interact with DC-SIGN or DC-SIGNR to mediate cellular attachment. Under control of interleukin (IL)-1β, activated Langerhans cells migrate to regional lymph nodes and result in a primary viremia that develops after the virus reaches the systemic circulation through the

thoracic duct.[26] In mice, peak viremia occurs by the second day after infection and clears by the sixth day. Virus subsequently can be detected in spleen, brain, and spinal cord. Because of the simultaneous appearance of virus in the brain and spinal cord, a hematogenous route for the CNS infection has been postulated. In humans, WNV can be detected in the neurons of the cerebral cortex, thalamus, brain stem, basal ganglia, cerebellum, and spinal cord, primarily in the anterior horn. Similar locations have been seen in mice and hamsters. Infection of the neurons in these sites results in meningoencephalitis and acute flaccid paralysis. Several mechanisms have been postulated for the neuronal injury seen in WNV infection[183]: viral injury to infected neurons through apoptosis, targeting of infected neurons by cytotoxic T lymphocytes, and neuronal death as a result of bystander injury. Cell death of WNV-infected neurons has been shown to be caspase-3 dependant.

The mechanism by which WNV gains access to the CNS is not fully understood. WNV binding to Toll-like receptor (TLR)3 has been shown to result in TLR3 receptor–dependent inflammatory response with resultant production of tumor necrosis factor (TNF)-α and IL-6.[204] TNF-α may modulate the ability of WNV to invade the CNS, inducing breakdown of the blood-brain barrier. Macrophage migration inhibition factor, induced endothelial cell adhesion molecule (ICAM)-1 and matrix metalloproteinase 9 also have been shown to play roles in increased brain capillary epithelial permeability.[5,52,203] Additional postulated mechanisms through which WNV may breach the blood-brain barrier include infection or passive transport through the choroid plexus epithelial cells or the endothelium, infection of olfactory neurons, transport within infected immune cells that traffic into the CNS (Trojan horse mechanism), and retrograde axonal transport in infected peripheral neurons.[173]

Cessation of the viremia occurs by the sixth day in mice and is coincident with the appearance of type-specific antibody response. Viral clearance from all tissues is complete by 2 to 3 weeks after infection. Humoral immunity is essential to the recovery from WNV infection, as demonstrated by the uniform death of B cell–deficient mice.[59] B cell–deficient mice could be protected from lethal infection by the passive transfer of serum from infected and immune wild-type mice. Mice deficient in $CD4^+$ T cells exhibited prolonged detection of WNV in the CNS, lower WNV-specific levels of IgM and IgG, as well as uniform lethality, indicating that the principal protective role of these cells is to assist in antibody responses. γ/δ T cells that secrete IFN-γ may control viral replication through direct antiviral mechanisms and contribute to adaptive immune responses.[173]

Although clearance of WNV from the CNS and other tissues depends primarily on humoral immunity, cytotoxic T cells also play a role.[118,197] Increased mortality rates in association with higher and sustained WNV loads in the CNS and spleen were observed in mice deficient in $CD8^+$ T cells or class I major histocompatibility complex molecules.[182] In addition, control of WNV infection in the CNS requires IFN-α/β. IFN-α/β also may help increase the survival of neurons. Finally, chemokines such as CXCL10 and CCL5 and their ligands CXCR3 and CCR5 aid in the recruitment of $CD4^+$ and $CD8^+$ T cells and monocytes to the CNS.[173]

CLINICAL MANIFESTATIONS

Most WNV infections in children and adults result in subclinical disease. In general, WNV infection in children more frequently results in asymptomatic infection or milder disease than that in adults.* One report documented that children were 4.5 times more likely to become infected with WNV during an epidemic but 110 times less likely to develop neuroinvasive disease.[124] In another, the estimated incidence of neuroinvasive disease was 1.4/100,000 in children aged 5 to 17 years in contrast to 14.3/100,000 in individuals aged 18 years or older.[109] In adults, multiple reports have identified increasing age as the single most significant risk factor for the development of neuroinvasive disease, as well as adverse outcome.[121,141,158] The incidence of severe neurologic disease is 10 times higher in individuals aged 50 to 59 years and 43 times higher in persons aged 80 years or older in contrast to those 20 years of age or younger. Chronic illnesses, such as hypertension, diabetes mellitus, cardiovascular disease, and alcohol abuse, also have been identified as risk factors for severe disease.[20,141,144,158] These factors have not been linked to an increased risk for the development of severe disease in children.

In the early 1950s, therapeutic inoculation of WNV into patients with malignant neoplasms in advanced stages resulted in a high incidence of development of encephalitis among recipients and pointed to the role of immunosuppression as a risk factor for West Nile neuroinvasive disease.[190] This finding subsequently was substantiated by the recognition that transplant recipients were 40 times more likely to develop West Nile neuroinvasive disease.[108] Reports of West Nile neuroinvasive disease in immunocompromised children are consistent with immunosuppression as a risk factor in this population also.[6,65,88,165,191] Reports also have indicated that the use of immunomodulating agents such as infliximab, rituximab, methotrexate, and fludarabine may predispose to the development of severe WNV infection.[11,45,116,128]

Approximately 20 percent of infected adults develop West Nile fever. Because of the fever's mild nature, only about half of individuals with West Nile fever ever seek medical attention. WNV neuroinvasive syndromes (e.g., encephalitis, meningitis, meningoencephalitis, and acute flaccid paralysis) develop in less than 1 percent (1/140 to 320) of adults infected with WNV.† The occurrence of West Nile neuroinvasive disease in children is significantly less.[109,124] In one study, it was calculated to occur in approximately 1/4200 infected children.[124]

The incubation period of WNV infection ranges from 2 to 14 days but usually is 2 to 6 days.[27,72,105,125] Viremia may be present from approximately 2 days before to approximately 4 days after the onset of the illness.[71] In

*References 46, 72, 121, 144, 175, 192.
†References 137, 144, 158, 196.

immunosuppressed individuals, incubation periods up to 3 weeks have been reported and may exhibit prolonged periods of viremia.[94,156]

In children* and adults, West Nile fever is an influenza-like febrile illness characterized by an abrupt onset of fever that typically ranges from 38° to 40° C (100.4° to 104° F). It is accompanied by fatigue, malaise, anorexia, headache, myalgias, and weakness.[27,72,125,158,206] Ocular pain on eye movement has been reported.[72,125] A diffuse, nonpruritic, macular, papular, maculopapular or morbilliform exanthem and diffuse lymphadenopathy also may be seen. On occasion, it has been described as petechial.[22,49,169] The rash spares the palms and soles and generally involves the chest, back, and arms.[169] The exanthem may be present more frequently in children than in adults, regardless of the WNV syndrome, occurring in 50 percent.[23,49,169] The exanthem generally is present for less than a week. Neck pain and photosensitivity often are reported. Gastrointestinal complaints such as nausea, vomiting, abdominal pain, and diarrhea may occur. Sore throat and cough also have been reported. The illness generally is short-lived, lasting only 3 to 6 days.[72,92,125] Convalescence, however, may take many weeks and is characterized by fatigue.[72,125,206]

Other nonneurologic WNV syndromes that have been reported in adults include cardiac dysrhythmias, myocarditis, pancreatitis, hepatitis, rhabdomyolysis, myositis, and orchitis.† Myocarditis and hepatitis have been reported in children with West Nile infection.

Although cases of West Nile neuroinvasive disease have been reported in children,‡ most of the information about these clinical syndromes has been derived from reports that have involved predominantly adults.§ However, on the basis of a limited number of pediatric cases and series, the clinical presentation in children appears to parallel that of adults.[175] Although discussion of each of the neuroinvasive syndromes individually (i.e., meningitis, encephalitis, and acute flaccid paralysis) is convenient, one should recognize that patients may have components of each in their clinical presentation. Finally, the clinical findings in West Nile neuroinvasive disease in children and adults are not sufficiently distinct to distinguish them from those of other viral meningitides or encephalitides.

In adults, the onset of West Nile meningitis tends to be abrupt, with fever, headache, and neck stiffness and meningeal signs (Kernig and Brudzinski signs).¶ Photophobia and phonophobia may be present. The headache pain may be severe enough to warrant the use of opioid analgesics. Weakness frequently is reported.[49,65] Additional associated symptoms may include those seen with West Nile fever: gastrointestinal and respiratory symptoms (i.e., nausea, vomiting, diarrhea, sore throat, and cough), rash, and lymphadenopathy. Dyskinesias in the form of tremors, myoclonus, or parkinsonism also may occur.

The reported clinical findings of West Nile encephalitis in children* are similar to those described in adults.† The onset of frank encephalitis may be preceded by a brief period, or prodrome, of fever, headache, rash, and malaise consistent with West Nile fever. In some cases, the appearance of fever is coincident with the onset of mental status changes. The mental status changes may range from a mild confusional state to severe encephalopathy and coma. Generalized weakness is a common component of the clinical spectrum of encephalitis. Paresis or palsies may be present. Ataxia, dysmetria, tremors, myoclonus, and parkinsonism may be seen.[49,135,146] Signs of bulbar dysfunction, such as dysarthria, dysphagia, and respiratory failure, have been reported. If a component of meningitis is present (i.e., meningoencephalitis), signs and symptoms described before for meningitis also may be present. Seizures have been reported in children‡ and may be focal with associated neurologic signs similar to those of herpes simplex virus encephalitis.[168,202]

Acute flaccid paralysis in association with WNV infection was reported first in 1979[67] and may occur in the absence of meningitis or encephalitis. Several reports of acute flaccid paralysis, alone or in association with encephalitis, developing after the occurrence of WNV infection in children have been published.[22,65,76,86,171] The onset of the paralysis occurs early in the illness, usually within the first 72 hours.[176] Pain or paresthesia in the affected limb preceding the onset of weakness is reported in most cases.[76,176] Patients exhibit asymmetric weakness, hypotonia, and absent or diminished deep tendon reflexes. Pain or sensory function in the affected limbs remains intact.§ However, at least one report has documented electrophysiologic evidence of sensory fiber loss in association with severe spinal cord involvement.[61] It is postulated that this finding could be explained by damage to neurons in the dorsal root ganglia. Tremor, myoclonus, and parkinsonism are common findings.[177,179] In severe cases, changes in bowel and bladder function or quadriplegia may occur. Involvement of the brain stem motor nuclei of the vagus and glossopharyngeal nerves can result in dysphagia, dysarthria, and acute respiratory failure requiring assisted mechanical ventilation.[22] Patients with dysphagia and dysarthria are at greater risk for the development of respiratory failure.[176]

Ocular manifestations of WNV infection include chorioretinitis, vitritis, and uveitis.[9,175] Chorioretinitis has been described in congenital and postnatal WNV infections[4,9,175] and may be more common than is realized.[85] Postnatal cases of West Nile chorioretinitis have been reported solely in adults. The lesions are reported to cluster in the temporal and nasal regions of the peripheral retina and have been described as multifocal and "target-like." One suggestion is that the distribution and

*References 49, 65, 72, 83, 92, 125, 211.
†References 8, 22, 85, 87, 129, 134, 144, 211.
‡References 6, 22, 29, 49, 57, 58, 63, 65, 69, 82, 86, 88, 89, 109, 135, 146, 147, 155, 162, 165, 168, 191, 202, 208, 211.
§References 8, 20, 27, 30, 45, 46, 96, 103, 117, 144, 158, 171, 176, 177, 179, 209.
¶References 2, 20, 27, 46, 49, 55, 65, 82, 83, 89, 103, 109, 144, 158, 175, 179, 196, 209.

*References 6, 22, 29, 49, 57, 58, 65, 82, 83, 88, 102, 135, 147, 165, 168, 191, 202.
†References 20, 27, 46, 54, 144, 158, 175.
‡References 6, 29, 65, 147, 168, 191.
§References 8, 96, 103, 117, 171, 176.

appearance of the lesions are sufficiently distinct to be considered characteristic for WNV infection.[87] In the sole reported case of congenital WNV infection, the infant had a large chorioretinal scar involving the retina of the right eye. Mild chorioretinal scarring was observed in the far temporal periphery.[4,9] Vitritis also has been reported in children and can lead to blurring and loss of vision.[211]

Other rarely reported neurologic manifestations of West Nile neuroinvasive disease in adults include optic neuritis, myelitis, Guillain-Barré syndrome,[2] and polyradiculitis.[155]

Recently, the possibility of long-term persistence of West Nile virus infection has been raised.[142] WNV genome was detected by RT-PCR in the urine in 20 percent of cohort of patients with neuroinvasive disease 1.6 to 6.7 years after initial infection. All patients reported persistence of neurologic symptoms or signs. This finding is consistent with findings in the hamster model WNV infection and suggests ongoing replication in renal tissue. However, attempts to isolate virus from the urine were unsuccessful. In addition, a higher than expected prevalence of chronic kidney disease associated with proteinuria and hematuria has been documented in a cohort of patients with a history of neuroinvasive WNV.[148]

OUTCOME

Based on cases reported to the CDC from 1999 to 2007, the mortality rate in children is less than 1 percent.[121] Although reports of death in children as a result of WNV infection are rare,[31,69,82,88] WNV outbreaks associated with significant pediatric mortality rates (12.9%) have been documented.[58,114] From 2002 to 2011 the case-fatality rates in adults ranged from 2.6 to 6.8 percent (mean, 4.6%).[32] Hospitalized persons 75 years or older are nine times more likely to die than are younger patients. Other reported independent risk factors for death in adults are change in level of consciousness and anemia at presentation.[46] In addition to early mortality, delayed mortality, occurring years after infection, has been reported.[122]

Although complete recovery from West Nile fever is expected, it may take several weeks before children and adults return to preillness levels of health.[72,155] Watson and colleagues[207] found that the median time to complete recovery in adults was 60 days. Postillness fatigue was reported in 98 percent, and the median duration of fatigue was 36 days. Sixty-one percent of patients reported muscle weakness of a median duration of 28 days. Other symptoms that persisted for longer than 7 days were reported in more than half of the subjects and included headache, muscle pain and aches, difficulty concentrating, joint pain and aches, and sensitivity to light. Of those who worked or attended school, nearly 60 percent reported absenteeism of more than 7 days.

Neurologic outcome of children after West Nile neuroinvasive disease has not been assessed systematically. The published case reports of West Nile neuroinvasive disease in children are most likely biased by the disease severity or outcome and limited by a lack of long-term follow-up of patients. As such, reliable information about the outcome of West Nile encephalitis and acute flaccid paralysis is lacking. However, on the basis of the review of 45 reported cases of West Nile neuroinvasive disease in children and adolescents for whom clinical and outcome information was provided,* recovery from meningitis appears to be complete in children (N = 20). Among cases of encephalitis, meningoencephalitis, and acute flaccid paralysis (N = 25), approximately 50 percent of children were reported to have neurologic sequelae at the time of last medical encounter or at the time the report was submitted. The reported sequelae among children include seizures, weakness, paralysis, gait disturbances, motor difficulties, language and speech deficits, dysphagia, behavior problems, cognitive deficits, mental retardation, difficulty concentrating, difficulties with memory and comprehension, facial palsy, hypertonicity, and persistent vegetative state.†

Between 50 and 65 percent of individuals will have evidence of neuropsychiatric dysfunction at the time of discharge.[209] Longer term outcomes have been reported by Sejvar and colleagues.[178] Comparison of neurocognitive and quality-of-life outcomes of adults returning to independent living after hospitalization for West Nile fever, meningitis, and encephalitis to patients with chronic fatigue syndrome and a normative control group. Fifty percent of patients recovering from West Nile fever reported persistent symptoms, diminished quality of life, and functional impairment. In persons recovering from West Nile meningitis and encephalitis, 75 percent of individuals in each group reported the same. Twenty-two percent of recovering individuals had persistent WNV-associated neurologic dysfunction (i.e., focal weakness or movement disorders). Interestingly, overall, no significant differences in objective neurocognitive performance were observed among the three groups. However, it should be noted that for some neurocognitive subtests sensitive for visual processing, executive function, and maintenance of vigilance the WNV group performed below the 15th percentile in contrast to the normative control. Taken together, these findings indicate that most adults returning to independent living after hospitalization for the three major West Nile syndromes had persistent subjective complaints but had normal cognitive function. The reported sequelae of West Nile neuroinvasive disease in adults include fatigue, fever, weakness, difficulty concentrating, memory loss, depression, paresis, tremor, myoclonus, and parkinsonism.[27,30,209]

Patients recovering from West Nile acute flaccid paralysis exhibited persistent associated neurologic signs, poor physical recovery, and atrophy of affected limbs.[97,177] However, like patients with other WNV neuroinvasive syndromes, mental health outcomes are favorable.[97] Although the degree of initial paralysis was not correlated with long-term outcome in an early report,[28] a more recent publication indicates that the degree of initial paralysis may be an important indicator of long-term

*References 6, 22, 29, 46, 57, 65, 86, 88, 89, 109, 147, 191, 202, 208, 211.

†References 6, 22, 29, 65, 86, 89, 147, 191, 202, 208, 211.

outcome.[177] The greatest recovery in strength occurred during the first 4 months after paralysis.[177]

LABORATORY FINDINGS

As is the case with the clinical findings of WNV infections, routine laboratory studies are nonspecific and do not provide conclusive evidence about the etiologic agent. Similar to those in adults, hematologic findings in children include leukocytosis, leukopenia, normal total white cell count, and lymphopenia.* Pediatric and adult cases of encephalitis hyponatremia have been reported.[22,46,209] Elevations of transaminases and creatine kinase may be seen in children with hepatitis and myositis.[22,211]

Cytochemical evaluation of the CSF generally reveals an abnormal white cell count and protein concentration.† A predominantly lymphocytic pleocytosis with, generally, fewer than 200 cells/mm^3 is a frequent finding. Cell counts of more than 2000 cells/mm^3 have been reported. Early in the infection, the CSF may show a polymorphonuclear cellular predominance that later shifts to a lymphocytic pleocytosis.[51] A mildly increased protein concentration of less than 100 mg/dL generally is seen. However, considerable variation in CSF protein concentration has been reported in patients with West Nile encephalitis. A normal CSF glucose concentration generally is observed.

ELECTRODIAGNOSTIC STUDIES

Electrodiagnostic study findings are abnormal in most patients with West Nile encephalitis.‡ Electroencephalographic abnormalities have included diffuse irregular slow waves, focal sharp waves, and subclinical electrographic seizures. An anterior preponderance of slow waves has been suggested as characteristic of West Nile encephalitis.[68] Reports of focal electroencephalographic findings suggestive of herpes simplex virus encephalitis have been reported in children.[168,202]

In patients with acute flaccid paralysis, electromyography, and nerve conduction studies document normal nerve conduction velocities, normal or reduced compound muscle action potentials, and normal sensory nerve action potentials consistent with anterior horn cell disease.§ Electrophysiologic documentation of severe loss of sensory fibers exists.[61]

NEUROIMAGING

Magnetic resonance imaging (MRI) is more sensitive than computed tomography in identifying CNS abnormalities in patients with West Nile neuroinvasive disease. However, even in cases of severe encephalopathy, MRI may fail to find any abnormalities.[23] Reported MRI findings in cases of West Nile neuroinvasive disease appear as hyperintense signal on T2-weighted and fluid-attenuated inversion recovery images and are nonenhancing and of normal intensity on T1 weighting.[96,160] In children and adults, lesions tend to be seen more frequently in the deep gray matter of the brain and, in particular, the basal ganglia, posterior thalami, and substantia nigra.* Leptomeningeal and paraventricular enhancement may also be seen.[22,165] Focal abnormalities occasionally may be seen, as noted in two cases of children with encephalitis in whom focal enhancement of the right temporal lobe was noted.[57,66,202]

In cases of acute flaccid paralysis, abnormal T2-weighted signals may be seen in the spinal cord gray matter.[3,86,96,117] In a case of a child with acute flaccid paralysis, magnetic resonance imaging of the spine documented edema of the anterior horns of the cervical spinal cord.[86]

VIRAL CULTURE AND NUCLEIC ACID AMPLIFICATION DETECTION OF WEST NILE VIRUS

Multiple reports have documented that WNV can be detected from the blood and brain of infected individuals by use of mouse inoculation or cell culture.† However, this approach to establishing the diagnosis of WNV infections is of limited utility because of lack of sensitivity resulting from the brevity of the viremia, which begins approximately 2 days before and persists until approximately 4 days after the onset of the illness.[72,158,190] In addition, because of the potential risk to laboratory personnel, attempts to isolate WNV from human specimens should be undertaken only in an appropriate biocontainment environment (i.e., biosafety level 3 containment).

Numerous nucleic amplification assays (i.e., RT-PCR, RT loop-mediated isothermal amplification) for the detection of WNV have been developed.[110,154] Diagnosis of acute WNV infections by nucleic acid amplification–based detection of WNV genome from blood and CSF clinical specimens also has limitations because of the short period of viremia.[110,158] Although genome could be detected in all brain tissue specimens tested, only 57 percent of CSF specimens and 14 percent of sera from patients with serologically confirmed infection were positive by a real-time RT-PCR assay.[110] Nucleic acid amplification–based detection of WNV has not been proved to be useful for the clinical diagnosis of WNV infections, but it has been extremely valuable in the screening and detection of asymptomatic viremia in blood donors.[134]

*References 6, 49, 57, 109, 168, 191, 202, 208, 211.
†References 6, 8, 23, 27, 29, 49, 51, 57, 65, 86, 88, 93, 103, 109, 144, 146, 147, 158, 165, 168, 179, 180, 185, 191, 202, 208, 209, 211.
‡References 3, 6, 23, 33, 57, 86, 88, 96, 103, 115, 116, 144, 147, 168, 179, 180, 191, 202.
§References 3, 33, 61, 76, 86, 103, 115, 117, 144, 179, 180.

*References 6, 21, 23, 65, 144, 158, 179, 209.
†References 18, 69, 71, 72, 79, 91, 130, 188, 190.

DETECTION OF WEST NILE VIRUS–SPECIFIC ANTIBODIES

Given the limitations of virologic and nucleic acid amplification–based diagnostic assays, detection of virus-specific antibodies forms the mainstay for establishing the diagnosis of WNV infections. In cases of neuroinvasive disease, the presence of WNV-specific IgM in serum and CSF increases by 10 percent per day the first week of illness such that it is usually detectable in 70 to 80 percent of patients by the eighth day after onset of illness.[85,193] Interestingly, WNV-specific IgM may persist for long periods in serum and CSF of acutely infected individuals.[170] Fifty-eight percent of samples obtained approximately 500 or more days from onset of West Nile encephalitis were in the positive or equivocal range for WNV-specific IgM.[170] Similarly, WNV-specific IgM has been detected in the CSF for up to 199 days after onset of illness.[100]

The interflavivirus cross reactivity of antiflaviviral IgG antibodies is well recognized. Traditionally, confirmation of anti-flaviviral antibody specificity requires the use of a functional antibody assay such as the plaque reduction neutralization test.[120] Although multiple reports have documented greater specificity of antiflaviviral IgM antibodies and, in particular, those of WNV-specific IgM antibodies,[126,127,193] cross reactions may still occur. On the basis of cross reactivity with related flaviviruses (i.e., Japanese encephalitis virus and St. Louis encephalitis virus), an algorithm for the use of CDC-developed IgM capture enzyme-linked immunosorbent assay (MAC-ELISA) has been developed to differentiate human WNV and St. Louis encephalitis virus infections.[127] The algorithm compares the positive-to-negative ratios of WNV-specific and St. Louis encephalitis virus–specific IgM determined in a CDC-standardized MAC-ELISA and has a positive predictive value of 97.8 percent for detection of WNV in the midst of an epidemic. This algorithm has not been validated for commercial MAC-ELISA kits. In the absence of use of the CDC MAC-ELISA, confirmation of WNV infection may require documentation of presence of WNV functional (i.e., neutralizing or hemagglutination inhibition) antibodies.

The diagnosis of WNV infection currently relies on the detection of WNV-specific antibodies in CSF or paired serum samples.[85,175] The MAC-ELISA is the most sensitive and commonly used diagnostic assay. It is capable of detecting IgM antibodies in CSF 3 to 5 days into the clinical illness and 3 or more days earlier than detectable serum antibody.[193] The finding of WNV-specific IgM in the CSF of a child with a clinically compatible CNS syndrome generally is considered diagnostic of acute neuroinvasive infection. However, as discussed previously, persistence of WNV-specific IgM antibodies for as long as 199 days in the CSF has been reported.[100]

Detection of WNV-specific IgM antibodies in serum from a single specimen may not be diagnostic of WNV infection because of its prolonged persistence in serum.[170,175] When only serum is used for establishing a diagnosis, a second serum sample should be obtained at least 2 weeks later to document a fourfold increase in specific antibody titers with use of a functional assay such as neutralization or hemagglutination inhibition.[180] Serologic cross reactions among St. Louis encephalitis virus, WNV, and Powassan virus may occur[195] and can confound the diagnosis in regions where multiple viruses are endemic. Serologic testing ideally should include a battery of region-specific arboviral antigens. Confirmatory assays, such as viral neutralization with the plaque reduction neutralization test, are available from the CDC's Division of Vector-Borne Infectious Diseases, but only 33 percent of commercial diagnostic laboratories and 24 percent of state public health laboratories have the capacity to perform the test.[95] The sensitivities and specificities of commercially available tests for the detection of WNV-specific antibodies may vary. An attenuated immune response may occur in immunocompromised hosts and result in a delayed antibody response.[156] Therefore, serologic assays should be interpreted with caution in this population and adjuncts such as nucleic acid amplification should be used to establish the diagnosis.

NEUROPATHOLOGY

Gross examination of the brain, spinal cord, and meninges generally is unremarkable but may demonstrate evidence of edema. In severe cases, the leptomeninges may show a perivascular monocytic infiltrate on microscopic examination.[196] Although the parenchymal findings of West Nile encephalitis can be seen distributed in nearly all areas of the CNS, the deep nuclei (i.e., thalamus, caudate, and lentiform nuclei), the substantia nigra, and other regions of the brain stem (in particular the pons and medulla), cerebellum, and spinal cord* may be more severely involved.

The CNS pathologic changes are the result of direct viral replication in neuronal and, less so, glial cells as well as the cytotoxic immune response to infected cells.[27,118] WNV demonstrates a penchant for infecting neurons. Viral antigen is detectable in neurons of fatal cases.[8,27,74,191] Neuronal pyknosis or neuronophagia is seen. Features in fatal cases of West Nile encephalitis include perivascular mononuclear inflammatory infiltrates composed predominantly of B lymphocytes, $CD8^+$ T lymphocyte–dominant microglial nodules in the brain parenchyma, and focal mononuclear inflammation along the cranial nerve roots of the medulla.† In the cerebellum, the Purkinje cell layer demonstrates inflammation, neuronal loss, and gliosis.

In cases of acute flaccid paralysis, WNV demonstrates a penchant for infection of the anterior motor neurons. The anterior horns of cervical and lumbar regions are the most severely involved areas.[21,66,74] Evidence of neuronophagia, neuronal loss, and perivascular monocytic infiltrates have been found.[21] Loss of ganglionic neurons, nodules of Nageotte, and perivascular lymphocytic

*References 27, 74, 101, 144, 152, 172, 196.
†References 8, 21, 66, 74, 144, 172.

aggregates have been seen in the dorsal roots and sympathetic ganglia.[21,66]

TREATMENT

Treatment of West Nile fever and neuroinvasive syndromes is primarily supportive. In the case of West Nile encephalitis and meningoencephalitis, close monitoring of respiratory status is essential. Patients with severe muscle weakness, paralysis, dysphagia, or dysarthria may require mechanical ventilation.

No effective antiviral therapy currently is available for the treatment of WNV syndromes. Ribavirin, a broadly active antiviral, has been shown to be active against WNV in vitro and may have as its mechanism of action error-prone replication.[56,98] In the sole pediatric case report, ribavirin was administered orally to a 14-year-old with Hodgkin lymphoma and West Nile meningoencephalitis who recovered completely.[192] Although encouraging, a report of ribavirin in an unblinded, uncontrolled manner for the treatment of WNV infection in 37 patients during an outbreak in Israel in 2000[46] associated its use with a higher mortality rate. Bivariate analysis indicated that its use was associated with a greater likelihood of death (odds ratio, 6.7; 95% confidence interval, 3.0 to 15.2). Because this was an uncontrolled trial, selection bias for its use in more severely ill patients cannot be excluded. However, in a hamster model of WNV infection, ribavirin treatment alone increased the mortality rate.[136] These reports indicate that the use of ribavirin for the treatment of WNV infections appears to be unwarranted and unsupported at this time.

In cell culture, animal models, and a pilot study, IFN-α has been shown to be beneficial against some flaviviruses.[136,164] Although anecdotal reports[99,174] in humans suggest that the use of interferon alfa-2b may improve recovery from West Nile encephalitis, an open label, nonblinded trial did not indicate benefit.[175]

Anecdotal reports of the use of an intravenous immunoglobulin preparation containing a high neutralizing titer of anti-WNV antibodies (Omr-IgG-am) for the treatment of West Nile neuroinvasive disease in adults* and children[22,88] have been published. The limited information suggests a benefit from their use in some but not all cases. A case series suggested that use of hyperimmune gammaglobulin as early as possible in the course of encephalitis may be associated with better outcome.[123]

A humanized monoclonal antibody directed to domain III of the WNV E protein has been developed. It has been demonstrated to be neutralizing and to penetrate the CNS, protecting mice and hamsters against lethal challenge with WNV.[151] In a phase I study it was demonstrated to have mild adverse effects and be well tolerated in healthy adults.[15]

A report of the treatment of macular edema associated with WNV chorioretinitis with bevacizumab has been published.[1] Bevacizumab is a humanized monoclonal antibody that inhibits angiogenesis by inhibition of vascular endothelial growth factor A.

*References 16, 77, 78, 116, 123, 167, 181.

PREVENTION

Prevention of WNV infections requires both community-based public health programs and personal measures. Mosquito abatement through elimination of mosquito breeding sites, use of larvicides to breeding areas, and application of pesticides targeted to adult mosquitoes are approaches used to reduce the abundance of mosquitoes in the environment.

The use and maintenance of door and window screens as physical barriers to prevent mosquitoes gaining access to homes can limit exposure to mosquitoes. Similarly, during engagement in outdoor activities where mosquitoes are prevalent, the use of mosquito netting in sleeping quarters also may decrease exposure to mosquito bites. Individuals should use insect repellent on skin and clothes when exposure to mosquitoes is probable or possible. Repellents such as N,N-diethyl-meta-toluamide (DEET), picaridin, and lemon eucalyptus oil have been shown to be effective and provide long-lasting protection.[10] Soybean oil also has been shown to be effective as a mosquito repellent. Permethrin and DEET have been shown to be effective protection against mosquitoes when applied to clothing.

Finally, avoidance of environments where mosquitoes are found to be in high concentrations and avoidance of outdoor activities during dusk and dawn, when mosquito activity is the highest, should be encouraged. Efforts to prevent transmission of WNV through blood transfusions have led to the use of nucleic acid amplification–based tests for the screening of donated blood. At this time, screening of organ donors for the possible presence of West Nile infection has not been recommended.[102]

The development of WNV vaccines for use in humans has taken various approaches: live attenuated virus, inactivated virus, viral subunits, DNA, vectored virus, pseudoinfectious virions, and chimeric viruses.[13] To date, seven phase I or II vaccine trials have been completed in the United States using a DNA vaccine containing the prM and E coding sequences (NIAID), soluble E protein lacking the C-terminal transmembrane domains, and two chimeric vaccines derived from dengue 4 virus (NIAID) and yellow fever virus (Sanofi-Aventis).[145]

Only the latter has progressed to phase II trials. An infectious clone of yellow fever 17D virus was used as the background for the homologous exchange and insertion of the WNV prM and E genes.[132] The WNV E gene was mutated at three sites to reduce neurovirulence.[7] A phase I chimeric WNV–yellow fever virus vaccine was well tolerated, and the incidences of adverse events were similar in both the vaccine and the placebo groups.[132,184] A transient viremia was observed in 93 percent of recipients of chimeric vaccine. High levels of neutralizing antibodies were detected in 100 percent of the recipients. $CD8^+$ responses were induced in 93 to 100 percent of vaccine recipients. The phase II study[19,12] used a small plaque variant of the vaccine strain, which exhibited reduced viremia in a hamster model. It contained an

additional amino acid mutation in the membrane protein. In part one of the study a short-lived mean viremia was observed but was lower than that seen in the phase I study, regardless of dose of given. Dose ranging studies demonstrated that more than 96 percent of all vaccine recipients developed neutralizing antibodies. The highest dose (3.7×10^5 PFUs) was used for the second part of the study. The mean peak titer of the viremia and its duration was greater in the cohort 65 years of age and older than in the 41- to 64-year-old cohort. Older patients exhibited higher neutralizing titers. Both cohorts had detectable neutralizing antibodies at 12 months and were higher in the 65 years and older cohort.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Afshar AR, Hariprasad SM, et al. Use of intravitreous bevacizumab to treat macular edema in West Nile virus chorioretinitis. *Arch Ophthalmol* 2012;**130**:396–8.
5. Arjona A, Foellmer HG, Town T, et al. Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion. *J Clin Invest* 2007;**117**:3059–66.
12. Beasley DW. Safety and immunogenicity of a chimeric vaccine for West Nile virus in aged subjects. *Expert Rev Vaccines* 2011;**10**:601–4.
13. Beasley DW. Vaccines and immunotherapeutics for the prevention and treatment of infections with West Nile virus. *Immunotherapy* 2011;**3**:269–85.
15. Beigel JH, Nordstrom JL, Pillemer SR, et al. Safety and pharmacokinetics of single intravenous dose of MGAWN1, a novel monoclonal antibody to West Nile virus. *Antimicrob Agents Chemother* 2010;**54**:2431–6.
16. Ben-Nathan D, Gershoni-Yahalom O, Samina I, et al. Using high titer West Nile intravenous immunoglobulin from selected Israeli donors for treatment of West Nile virus infection. *BMC Infect Dis* 2009;**9**:1–18.
19. Biedenbender R, Bevilacqua J, Gregg AM, et al. Phase II, randomized, double-blind, placebo-controlled, multicenter study to investigate the immunogenicity and safety of a West Nile virus vaccine in healthy adults. *J Infect Dis* 2011;**203**:75–84.
32. Centers for Disease Control and Prevention. *West Nile virus*. http://www.cdc.gov/ncidod/dvbid/westnile/surv&control_archive.htm.
43. Centers for Disease Control and Prevention. West Nile virus transmission via organ transplantation and blood transfusion: Louisiana, 2008. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1263–7.
44. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
48. Chuang TW, Hockett CW, Kightlinger L, et al. Landscape-level spatial patterns of West Nile virus risk in the northern Great Plains. *Am J Trop Med Hyg* 2012;**86**:724–31.
52. Dai J, Wang P, Bai F, et al. Icam-1 participates in the entry of west nile virus into the central nervous system. *J Virol* 2008;**82**:4164–8.
64. Fonseca K, Prince GD, Bratvold J, et al. West Nile virus infection and conjunctival exposure. *Emerg Infect Dis* 2005;**11**:1648–9.
76. Hainline ML, Kincaid JC, Carpenter DL, et al. West Nile poliomyelitis in a 7-year-old child. *Pediatr Neurol* 2008;**39**:350–4.
90. Hofmeister EK. West Nile virus: North American experience. *Integrative Zoology* 2011;**6**:279–89.
95. Janusz KB, Lehman JA, Panella AJj, et al. Laboratory testing practices for West Nile virus in the United States. *Vector Borne Zoonotic Dis* 2011;**11**:597–9.
97. Johnstone J, Hanna SE, Nicolle LE, et al. Prognosis of West Nile virus associated acute flaccid paralysis: a case series. *J Med Case Rep* 2011;**5**:395.
113. Laurent-Rolle M, Boer EF, Lubick KJ, et al. The NS5 protein of the virulent West Nile virus NY99 strain is a potent antagonist of type I interferon-mediated JAK-STAT signaling. *J Virol* 2010;**84**:3503–15.
116. Levi ME, Quan D, Ho JT, et al. Impact of rituximab-associated B-cell defects on West Nile virus meningoencephalitis in solid organ transplant recipients. *Clin Transplant* 2010;**24**:223–8.
118. Lim SM, Koraka P, Osterhaus AD, et al. West Nile virus: immunity and pathogenesis. *Viruses* 2011;**3**:811–28.
121. Lindsey NP, Hayes EB, Staples E, Fischer M. West Nile disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084–9.
122. Lindsey NP, Sejvar JJ, Bode AV, et al. Delayed mortality in a cohort of persons hospitalized with West Nile virus disease in Colorado in 2003. *Vector Borne Zoonotic Dis* 2012;**12**:230–5.
123. Makhoul B, Braun E, Herskovitz M, Ramadan R, et al. Hyperimmune gammaglobulin for the treatment of West Nile virus encephalitis. *Isr Med Assoc J* 2009;**11**:151–3.
131. Meny GM, Santos-Zabala L, Szallasi A. West Nile virus infection transmitted by granulocyte transfusion. *Blood* 2011;**117**:5778–9.
142. Murray K, Walker C, Herrington E, et al. Persistent infection with West Nile virus years after initial infection. *J Infect Dis* 2011;**201**:2–4.
145. National Institutes of Health. ClincalTrials.gov. http://clinicaltrials.gov/ct2/results?term=west+nile+virus&pg=1.
148. Nolan MS, Podoll AS, Hause AM, et al. Prevalence of chronic kidney disease and progression of disease over time among patients enrolled in the Houston West Nile virus cohort. *PloS One* 2012;**7**:e40374.
151. Oliphant T, Engle M, Nybakken GE, et al. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. *Nat Med* 2005;**11**:522–30.
157. Pesko KN, Ebel GD. West Nile virus population genetics and evolution. *Infect Genet Evol* 2012;**12**:181–90.
161. Planitzer CB, Modrof J, Yu MY, et al. West Nile virus infection in plasma of blood and plasma donors, United States. *Emerg Infect Dis* 2009;**15**:1668–70.
166. Reisen WK, Takahashi RM, Carroll BD, et al. Delinquent mortgages, neglected swimming pools, and West Nile virus, California. *Emerg Infect Dis* 2008;**14**:1747–9.
167. Rhee C, Eaton EF, Concepcion W, et al. West Nile virus encephalitis acquired via liver transplantation and clinical response to intravenous immunoglobulin: case report and review of the literature. *Transpl Infect Dis* 2011;**13**:312–17.
169. Rizzo C, Esposito S, Azzari C, et al. West Nile Virus infections in children: a disease pediatricians should think about. *Pediatr Infect Dis J* 2011;**30**:65–6.
178. Sejvar JJ, Curns AT, Welburg L, et al. Neurocognitive and functional outcomes in persons recovering from West Nile virus illness. *J Neuropsychol* 2008;**2**:477–99.
184. Smith HL, Monath TP, Pazoles P, et al. Development of antigen-specific memory CD8+ T cells following live-attenuated chimeric West Nile virus vaccination. *J Infect Dis* 2011;**203**:513–22.
197. Ulbert S. West Nile virus: the complex biology of an emerging pathogen. *Intervirology* 2011;**54**:171–84.
198. Unlu I, Kramer WL, Roy AF, Foil LD. Detection of West Nile virus RNA in mosquitoes and identification of mosquito blood meals collected at alligator farms in Louisiana. *J Med Entomol* 2010;**47**:625–33.
199. Unlu I, Mackay AJ, Roy A, et al. Evidence of vertical transmission of West Nile virus in field-collected mosquitoes. *J Vector Ecol* 2010;**35**:95–9.
200. Venter M, Burt FJ, Blumberg L, et al. Cytokine induction after laboratory-acquired West Nile virus infection. *N Engl J Med* 2009;**360**:1260–2.
201. Venter M, Steyl J, Human S, et al. Transmission of West Nile virus during horse autopsy. *Emerg Infect Dis* 2010;**16**:573–5.
203. Wang P, Dai J, Bai F, et al. Matrix metalloproteinase 9 facilitates West Nile virus entry into the brain. *J Virol* 2008;**82**:8978–85.
210. Wilson JR, de Sessions PF, Leon MA, et al. West Nile virus nonstructural protein 1 inhibits TLR3 signal transduction. *J Virol* 2008;**82**:8262–71.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Afshar AR, Hariprasad SM, et al. Use of intravitreous bevacizumab to treat macular edema in West Nile virus chorioretinitis. *Arch Ophthalmol* 2012;**130**:396–8.
2. Ahmed S, Libman R, Wesson K, et al. Guillain-Barré syndrome: an unusual presentation of West Nile virus infection. *Neurology* 2000;**55**:144–6.
3. Al-Shekhlee A, Katirji B. Electrodiagnostic features of acute paralytic poliomyelitis associated with West Nile virus infection. *Muscle Nerve* 2004;**29**:376–80.
4. Alpert SG, Fergerson J, Noel LP. Intrauterine West Nile virus: ocular and systemic findings. *Am J Ophthalmol* 2004;**136**:733–5.
5. Arjona A, Foellmer HG, Town T, et al. Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion. *J Clin Invest* 2007;**117**:3059–66.
6. Arnold JC, Revivo GA, Senac MO, et al. West Nile virus encephalitis with thalamic involvement in an immunocompromised child. *Pediatr Infect Dis J* 2005;**24**:932–4.
7. Arroyo J, Miller C, Catalan J, et al. ChimeriVax–West Nile virus live-attenuated vaccine: preclinical evaluation of safety, immunogenicity, and efficacy. *J Virol* 2004;**78**:12497–507.
8. Asnis DS, Conetta R, Teixeira AA, et al. The West Nile Virus outbreak of 1999 in New York: the Flushing Hospital experience. *Clin Infect Dis* 2000;**30**:413–18.
9. Bakri SJ, Kaiser PK. Ocular manifestations of West Nile virus. *Curr Opin Ophthalmol* 2004;**15**:537–40.
10. Barnard DR, Xue RD. Laboratory evaluation of mosquito repellants against *Aedes albopictus*, *Culex nigripalpus*, and *Ochlerotatus triseriatus* (Diptera: Culicidae). *J Med Entomol* 2004;**41**:726–30.
11. Batsis JA, Phy MP. West Nile virus meningitis in a chronic immunosuppressed patient with rheumatoid arthritis. *Clin Rheumatol* 2005;**24**:548–50.
12. Beasley DW. Safety and immunogenicity of a chimeric vaccine for West Nile virus in aged subjects. *Expert Rev Vaccines* 2011;**10**:601–4.
13. Beasley DW. Vaccines and immunotherapeutics for the prevention and treatment of infections with West Nile virus. *Immunotherapy* 2011;**3**:269–85.
14. Beasley DW, Li L, Suderman MT, et al. Mouse neuroinvasive phenotype of West Nile virus strains varies depending upon virus genotype. *Virology* 2002;**296**:17–23.
15. Beigel JH, Nordstrom JL, Pillemer SR, et al. Safety and pharmacokinetics of single intravenous dose of MGAWN1, a novel monoclonal antibody to West Nile virus. *Antimicrob Agents Chemother* 2010;**54**:2431–6.
16. Ben-Nathan D, Gershoni-Yahalom O, Samina I, et al. Using high titer West Nile intravenous immunoglobulin from selected Israeli donors for treatment of West Nile virus infection. *BMC Infect Dis* 2009;**9**:1–18.
17. Benyesh M, Goldblum N. On the etiology of non-bacterial meningoencephalitis in Israel: preliminary report. *Harefuah* 1955; **48**:58–62.
18. Bernkopf H, Levine S, Nerson R. Isolation of West Nile virus in Israel. *J Infect Dis* 1953;**93**:207–18.
19. Biedenbender R, Bevilacqua J, Gregg AM, et al. Phase II, randomized, double-blind, placebo-controlled, multicenter study to investigate the immunogenicity and safety of a West Nile virus vaccine in healthy adults. *J Infect Dis* 2011;**203**:75–84.
20. Bode V, Sejvar JJ, Pape WJ, et al. West Nile virus disease: a descriptive study of 228 patients hospitalized in a 4-county region of Colorado in 2003. *Clin Infect Dis* 2006;**42**:1234–40.
21. Bosanko CM, Gilroy J, Wang AM, et al. West Nile virus encephalitis involving the substantia nigra: neuroimaging and pathologic findings with literature review. *Arch Neurol* 2003;**60**:1448–52.
22. Braun LE, Tsuchida T, Spiegel H. Meningoencephalitis in a child complicated by myocarditis, quadriparesis and respiratory failure. *Pediatr Infect Dis J* 2006;**25**:853–6.
23. Brilla R, Block M, Geremia G, et al. Clinical and neuroradiologic features of 39 consecutive cases of West Nile Virus meningoencephalitis. *J Neurol Sci* 2004;**220**:37–40.
24. Brownstein JS, Rosen H, Purdy D, et al. Spatial analysis of West Nile virus: rapid risk assessment of an introduced vector-borne zoonosis. *Vector Borne Zoonotic Dis* 2002;**2**:157–64.
25. Busch MP, Wright DJ, Custer B, et al. West Nile virus infections projected from blood donor screening data, United States, 2003. *Emerg Infect Dis* 2006;**12**:395–402.
26. Byrne SN, Halliday GM, Johnston LJ, et al. Interleukin-1 beta but not tumor necrosis factor is involved in West Nile virus–induced Langerhans cell migration from the skin in C57BL/6 mice. *J Investig Dermatol* 2001;**117**:702–9.
27. Campbell GL, Marfin AA, Lanciotti RS, et al. West Nile virus. *Lancet Infect Dis* 2002;**2**:519–29.
28. Cao NJ, Ranganathan C, Kupsky W, et al. Recovery and prognosticators of paralysis in West Nile virus infection. *J Neurol Sci* 2005;**236**:73–80.
29. Carey DE, Rodrigues FM, Myers RM, et al. Arthropod-borne viral infections in children in Vellore, South India, with particular reference to dengue and West Nile viruses. *Indian Pediatr* 1968; **5**:285–96.
30. Carson PJ, Konewko P, Wold KS, et al. Long-term clinical and neuropsychological outcomes of West Nile virus infection. *Clin Infect Dis* 2006;**43**:723–30.
31. Ceianu CS, Unguranu A, Nicolescu G, et al. West Nile virus surveillance in Romania: 1997-2000. *Viral Immunol* 2001;**14**: 251–62.
32. Centers for Disease Control and Prevention. *West Nile virus*. http://www.cdc.gov/ncidod/dvbid/westnile/surv&control_archive.htm.
33. Centers for Disease Control and Prevention. Acute flaccid paralysis syndrome associated with West Nile virus infection: Mississippi and Louisiana, July-August 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:825–8.
34. Centers for Disease Control and Prevention. Intrauterine West Nile virus infection: New York, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:1135–6.
35. Centers for Disease Control and Prevention. Possible West Nile virus transmission to an infant through breast-feeding: Michigan, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:877–8.
36. Centers for Disease Control and Prevention. Public health dispatch: West Nile virus infection in organ donor and transplant recipients—Georgia and Florida, 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:790.
37. Centers for Disease Control and Prevention. Laboratory-acquired West Nile virus infections: United States, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**51**:1133–5.
38. Centers for Disease Control and Prevention. West Nile virus infection among turkey breeder farm workers: Wisconsin, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:1017–19.
39. Centers for Disease Control and Prevention. Possible dialysis-related West Nile virus transmission: Georgia, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:738–9.
40. Centers for Disease Control and Prevention. Transfusion-associated transmission of West Nile virus: Arizona, 2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:842–4.
41. Centers for Disease Control and Prevention. West Nile virus infections in organ transplant recipients: New York and Pennsylvania, August-September, 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1021–3.
42. Centers for Disease Control and Prevention. West Nile virus transmission through blood transfusion: South Dakota, 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:76–9.
43. Centers for Disease Control and Prevention. West Nile virus transmission via organ transplantation and blood transfusion: Louisiana, 2008. *MMWR Morb Mortal Wkly Rep* 2009;**58**: 1263–7.
44. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
45. Chan-Tack KM, Forrest G. West Nile virus meningoencephalitis and acute flaccid paralysis after infliximab treatment. *J Rheumatol* 2006;**33**:191–2.
46. Chowers MY, Lang R, Nassar F, et al. Clinical characteristics of the West Nile fever outbreak, Israel, 2000. *Emerg Infect Dis* 2001;**7**:675–8.
47. Chu JJ, Ng ML. Interaction of West Nile virus with alpha v beta 3 integrin mediates virus entry into cells. *J Biol Chem* 2004;**279**: 54533–41.
48. Chuang TW, Hockett CW, Kightlinger L, et al. Landscape-level spatial patterns of West Nile virus risk in the northern Great Plains. *Am J Trop Med Hyg* 2012;**86**:724–31.
49. Civen R, Villacorte F, Robles DT, et al. West Nile virus infection in the pediatric population. *Pediatr Infect Dis J* 2006;**25**:75–8.

50. Corwin A, Habib M, Olson J, et al. The prevalence of arboviral, rickettsial and Hantaan-like viral antibody among schoolchildren in the Nile river delta of Egypt. *Trans R Soc Trop Med Hyg* 1992;**86**:677–9.
51. Crichlow R, Bailey J, Gardner C. Cerebrospinal fluid pleocytosis in hospitalized West Nile virus patients. *J Am Board Fam Pract* 2004;**17**:470–2.
52. Dai J, Wang P, Bai F, et al. ICAM-1 participates in the entry of west nile virus into the central nervous system. *J Virol* 2008;**82**: 4164–8.
53. Darwish MA, Ibrahim AH. Prevalence of antibodies to arboviruses in Egypt: results of a serologic survey among 1,113 university students. *Am J Trop Med Hyg* 1975;**24**:981–5.
54. Dauphin G, Zientara S, Zeller H, et al. West Nile: Worldwide current situation in animals and humans. *Comp Immunol Microbiol Infect Dis* 2004;**27**:343–55.
55. Davis CW, Nguyen HY, Hanna SL, et al. West Nile virus discriminates between DC-SIGN and DC-SIGNR for cellular attachment and infection. *J Virol* 2006;**80**:1290–301.
56. Day CW, Smee DF, Julander JG, et al. Error-prone replication of West Nile virus caused by ribavirin. *Antiviral Res* 2005;**67**: 38–45.
57. DeBiasi L, Parsons JA, Grabert BE. West Nile virus meningoencephalitis in an immunocompetent adolescent. *Pediatr Neurol* 2005;**33**:217–19.
58. Depoortere E, Kavle J, Keus K, et al. Outbreak of West Nile virus causing severe neurological involvement in children, Nuba Mountains, Sudan, 2002. *Trop Med Int Health* 2004;**9**:730–6.
59. Diamond MS, Shrestha B, Marri A, et al. B cells and antibody play critical roles in the immediate defense of disseminated infection by West Nile encephalitis virus. *J Virol* 2003;**77**:2578–86.
60. Dohm DJ, Sardelis MR, Turell MJ. Experimental vertical transmission of West Nile virus by *Culex pipiens* (Diptera: Culicidae). *J Med Entomol* 2002;**39**:640–4.
61. Doron SI, Dashe JF, Adelman LS, et al. Histopathologically proven poliomyelitis with quadriplegia and loss of brainstem function due to West Nile virus infection. *Clin Infect Dis* 2003;**37**: e74–7.
62. Feki I, Marrakchi C, Hmida MB, et al. Epidemic West Nile virus encephalitis in Tunisia. *Neuroepidemiology* 2005;**24**:1–7.
63. Flatau E, Kohn D. West Nile fever encephalitis. *Isr J Med Sci* 1981;**17**:1057–9.
64. Fonseca K, Prince GD, Bratvold J, et al. West Nile virus infection and conjunctival exposure. *Emerg Infect Dis* 2005;**11**:1648–9.
65. Francisco AM, Glaser C, Frykman E, et al. 2004 California pediatric West Nile virus case series. *Pediatr Infect Dis J* 2006;**25**: 81–4.
66. Fratkin JD, Leis AA, Stokic DS, et al. Spinal cord neuropathology in human West Nile virus infection. *Arch Pathol Lab Med* 2004;**128**: 533–7.
67. Gadoth N, Weitzman S, Lehmann EE. Acute anterior myelitis complicating West Nile fever. *Arch Neurol* 1979;**36**:172–3.
68. Gandelman-Marton R, Kimiagar I, Itzhaki A, et al. Electroencephalography findings in adult patients with West Nile virus–associated meningitis and meningoencephalitis. *Clin Infect Dis* 2003;**37**:1573–8.
69. George S, Gourie-Devi M, Rao JA, et al. Isolation of West Nile virus from the brains of children who had died of encephalitis. *Bull World Health Organ* 1984;**62**:879–82.
70. Girard YA, Popov V, Wen J, et al. Ultrastructural study of West Nile virus pathogenesis in *Culex pipiens quinquefasciatus* (Diptera: Culicidae). *J Med Entomol* 2005;**42**:429–44.
71. Goldblum N, Sterk VV, Jasinskaklingberg W. The natural history of West Nile fever. II. Virological findings and the development of homologous and heterologous antibodies in West Nile infection in man. *Am J Hyg* 1957;**66**:363–80.
72. Goldblum N, Sterk VV, Paderski B. West Nile fever. the clinical features of the disease and the isolation of West Nile virus from the blood of nine human cases. *Am J Hyg* 1954;**59**:89–103.
73. Goddard LB, Roth AE, Reisen WK, et al. Vector competence of California mosquitoes for West Nile virus. *Emerg Infect Dis* 2002;**8**:1385–91.
74. Guarner J, Shieh WJ, Hunter S, et al. Clinicopathologic study and laboratory diagnosis of 23 cases of West Nile virus encephalomyelitis. *Hum Pathol* 2004;**35**:983–90.
75. Guptill SC, Julian KG, Campbell GL. Early-season avian deaths from West Nile virus as warnings of human infection. *Emerg Infect Dis* 2003;**9**:483–4.
76. Hainline ML, Kincaid JC, Carpenter DL, et al. West Nile poliomyelitis in a 7-year-old child. *Pediatr Neurol* 2008;**39**:350–4.
77. Haley M, Retter AS, Fowler D, et al. The role for intravenous immunoglobulin in the treatment of West Nile virus encephalitis. *Clin Infect Dis* 2003;**37**:e88–90.
78. Hamdan A, Green P, Mendelson E, et al. Possible benefit of intravenous immunoglobulin therapy in a lung transplant recipient with West Nile virus encephalitis. *Transpl Infect Dis* 2002;**4**: 160–2.
79. Hamilton PK, Taylor RM. Report of clinical case of West Nile virus infection probably acquired in the laboratory. *Am J Trop Med Hyg* 1954;**3**:51–3.
80. Han LL, Popovici F, Alexander JP Jr. Risk factors for West Nile virus infection and meningoencephalitis, Romania, 1996. *J Infect Dis* 1999;**179**:230–3.
81. Hayes CG. West Nile fever. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 5. Boca Raton, FL: CRC Press; 1989. p. 59.
82. Hayes EB. West Nile virus disease in children. *Pediatr Infect Dis J* 2006;**25**:1065–6.
83. Hayes EB, O'Leary DR. West Nile virus infection: a pediatric perspective. *Pediatrics* 2004;**113**:1375–81.
84. Hayes EB, Komar N, Nasci RS, et al. Epidemiology and transmission dynamics of West Nile virus disease. *Emerg Infect Dis* 2005; **11**:1167–73.
85. Hayes EB, Sejvar JJ, Zaki SR, et al. Virology, pathology, and clinical manifestations of West Nile virus disease. *Emerg Infect Dis* 2005;**11**:1174–9.
86. Heresi GP, Mancias P, Mazur LJ, et al. Poliomyelitis-like syndrome in a child with West Nile virus infection. *Pediatr Infect Dis J* 2004;**23**:788–9.
87. Hershberger VS, Augsburger JJ, Hutchins RK, et al. Chorioretinal lesions in nonfatal cases of West Nile virus infection. *Ophthalmology* 2003;**110**:1732–6.
88. Hindo H, Buescher ES, Frank LM, et al. West Nile virus infection in a teenage boy with acute lymphocytic leukemia in remission. *J Pediatr Hematol Oncol* 2005;**27**:659–62.
89. Horga MA, Fine A. West Nile virus. *Pediatr Infect Dis J* 2001; **20**:801–2.
90. Hofmeister EK. West Nile virus: North American experience. *Integrative Zoology* 2011;**6**:279–89.
91. Huang C, Slater B, Rudd R. First isolation of West Nile virus from a patient with encephalitis in the United States. *Emerg Infect Dis* 2002;**8**:1367–71.
92. Hubalek Z, Halouzka J, Juricova Z. West Nile fever in Czechland. *Emerg Infect Dis* 1999;**5**:594–5.
93. Huhn GD, Austin C, Langkop C, et al. The emergence of West Nile virus during a large outbreak in Illinois in 2002. *Am J Trop Med Hyg* 2005;**72**:768–76.
94. Iwamoto M, Jernigan DB, Guasch A, et al. Transmission of West Nile virus from an organ donor to four transplant recipients. *N Engl J Med* 2003;**348**:2196–203.
95. Janusz KB, Lehman JA, Panella AJj, et al. Laboratory testing practices for West Nile virus in the United States. *Vector Borne Zoonotic Dis* 2011;**11**:597–9.
96. Jeha LE, Sila CA, Lederman RJ. West Nile virus infection: a new acute paralytic illness. *Neurology* 2003;**61**:55–9.
97. Johnstone J, Hanna SE, Nicolle LE, et al. Prognosis of West Nile virus associated acute flaccid paralysis: a case series. *J Med Case Rep* 2011;**5**:395.
98. Jordan I, Briese T, Fischer N, et al. Ribavirin inhibits West Nile virus replication and cytopathic effect in neural cells. *J Infect Dis* 2000;**182**:1214–17.
99. Kalil AC, Devetten MP, Singh S, et al. Use of interferon-alpha in patients with West Nile encephalitis: report of 2 cases. *Clin Infect Dis* 2005;**40**:764–6.
100. Kapoor H, Signs K, Somsel P, et al. Persistence of West Nile virus (WNV) IgM antibodies in cerebrospinal fluid from patients with CNS disease. *J Clin Virol* 2004;**31**:289–91.
101. Kelley TW, Prayson RA, Ruiz AI, et al. The neuropathology of West Nile virus meningoencephalitis: a report of two cases and review of the literature. *Am J Clin Pathol* 2003;**119**:749–53.

102. Kiberd BA, Forward K. Screening for West Nile in organ transplantation: a medical decision and analysis. *Am J Transplant* 2004;**4**:1296–301.
103. Klein C, Kimiagar I, Pollak L, et al. Neurological features of West Nile virus infection during the 2000 outbreak in a regional hospital in Israel. *J Neurol Sci* 2002;**200**:63–6.
104. Klenk K, Snow J, Morgan K. Alligators as West Nile virus amplifiers. *Emerg Infect Dis* 2004;**10**:2150–5.
105. Komar N. West Nile virus: epidemiology and ecology in North America. *Adv Virus Res* 2003;**61**:185–234.
106. Komar N, Clark GG. West Nile virus activity in Latin America and the Caribbean. *Rev Panam Salus Publica* 2006;**19**:112–17.
107. Kostiukov MA, Gordeeva ZE, Bulychev VP, et al. The lake frog *(Rana ridibunda):* one of the food hosts of blood-sucking mosquitoes in Tadzhikistan—a reservoir of the West Nile fever virus. *Med Parazitol (Mosk)* 1985;**3**:49–50.
108. Kumar D, Prasad GV, Zaltzman J, et al. Community-acquired West Nile virus infection in solid-organ transplant recipients. *Transplantation* 2004;**77**:399–402.
109. LaBeaud AD, Lisgaris MV, King CH, et al. Pediatric West Nile virus infection: Neurologic disease presentations during the 2002 epidemic in Cuyahoga County, Ohio. *Pediatr Infect Dis J* 2006;**25**: 751–3.
110. Lanciotti RS, Kerst AJ, Nasci RS, et al. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase–PCR assay. *J Clin Microbiol* 2000;**38**:4066–71.
111. Lanciotti RS, Roehrig JT, Deubel V, et al. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. *Science* 1999;**286**:2333–7.
112. Lanciotti RS, Roehrig JT, Deubel V, et al. Complete genome sequences and phylogenetic analysis of West Nile virus strains isolated from the United States, Europe, and the Middle East. *Virology* 2002;**298**:96–105.
113. Laurent-Rolle M, Boer EF, Lubick KJ, et al. The NS5 protein of the virulent West Nile virus NY99 strain is a potent antagonist of type I interferon-mediated JAK-STAT signaling. *J Virol* 2010; **84**:3503–15.
114. Le Guenno B, Bougermouth A, Azzam T, et al. West Nile: A deadly virus? *Lancet* 1996;**348**:1315.
115. Leis AA, Stokic DS, Polk JL. A poliomyelitis-like syndrome from West Nile virus infection. *N Engl J Med* 2002;**347**:1279–80.
116. Levi ME, Quan D, Ho JT, et al. Impact of rituximab-associated B-cell defects on West Nile virus meningoencephalitis in solid organ transplant recipients. *Clin Transplant* 2010;**24**:223–8.
117. Li J, Loeb JA, Shy ME, et al. Asymmetric flaccid paralysis: a neuromuscular presentation of West Nile virus infection. *Ann Neurol* 2003;**53**:703–10.
118. Lim SM, Koraka P, Osterhaus AD, et al. West Nile virus: immunity and pathogenesis. *Viruses* 2011;**3**:811–28.
119. Lindenbach BD, Rice CM. Flaviviridae: The viruses and their replication. In: Knipe DM, Howley PM, editors. *Fields' virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 991.
120. Lindsey HS, Calisher CH, Matthews JH. Serum dilution neutralization test for California group virus identification and serology. *J Clin Microbiol* 1976;**4**:503–10.
121. Lindsey NP, Hayes EB, Staples E, Fischer M. West Nile disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084–9.
122. Lindsey NP, Sejvar JJ, Bode AV, et al. Delayed mortality in a cohort of persons hospitalized with West Nile virus disease in Colorado in 2003. *Vector Borne Zoonotic Dis* 2012;**12**:230–5.
123. Makhoul B, Braun E, Herskovitz M, Ramadan R, et al. Hyperimmune gammaglobulin for the treatment of West Nile virus encephalitis. *Isr Med Assoc J* 2009;**11**:151–3.
124. Mandalakas AM, Kippes C, Sedransk J, et al. West Nile virus epidemic: Northeast Ohio, 2002. *Emerg Infect Dis* 2005;**11**: 1774–7.
125. Marberg K, Goldblum N, Sterk VV, et al. The natural history of West Nile fever: clinical observations during an epidemic in Israel. *Am J Hyg* 1956;**64**:259–69.
126. Martin DA, Biggerstaff BJ, Allen B, et al. Use of immunoglobulin M cross-reactions in differential diagnosis of human flaviviral encephalitis infections in the United States. *Clin Diagn Lab Immunol* 2002;**9**:544–9.
127. Martin DA, Noga A, Kosoy O, et al. Evaluation of a diagnostic algorithm using immunoglobulin M enzyme-linked immunosorbent assay to differentiae human West Nile virus and St. Louis encephalitis virus infections during the 2002 West Nile virus epidemic in the United States. *Clin Diagn Lab Immunol* 2004;**11**: 1130–3.
128. Mawhorter SD, Sierk A, Staugaitis SM, et al. Fatal West Nile virus infection after rituximab/fludarabine-induced remission for non-Hodgkin's lymphoma. *Clin Lymphoma Myeloma* 2005;**6**:248–50.
129. Medarov BI, Multz AS, Brown W, et al. West Nile meningoencephalitis and rhabdomyolysis. *Lancet Infect Dis* 2005;**5**:2.
130. Melnick JL, Paul JR, Riordan JT, et al. Isolation from human sera in Egypt of a virus apparently identical to West Nile virus. *Proc Soc Exp Biol Med* 1951;**77**:661–5.
131. Meny GM, Santos-Zabala L, Szallasi A. West Nile virus infection transmitted by granulocyte transfusion. *Blood* 2011;**117**:5778–9.
132. Monath TP, Liu J, Kanesa-Thasan N, et al. A live, attenuated recombinant West Nile virus vaccine. *Proc Natl Acad Sci U S A* 2006;**103**:6694–9.
133. Montgomery SP, Brown JA, Kuehnert M, et al. 2003 West Nile Virus Transfusion-Associated Transmission Investigation Team. Transfusion-associated transmission of West Nile virus, United States 2003 through 2005. *Transfusion* 2006;**46**:2038–46.
134. Montgomery SP, Chow CC, Smith SW, et al. Rhabdomyolysis in patients with West Nile encephalitis and meningitis. *Vector Borne Zoonotic Dis* 2005;**5**:252–7.
135. Moon TD, Nadimpalli A, Martin EB. Balance and gait abnormalities of a child with West Nile virus infection. *Pediatr Infect Dis J* 2005;**24**:568–70.
136. Morrey JD, Day CW, Julander JG, et al. Effect of interferon-alpha and interferon-inducers on West Nile virus in mouse and hamster animal models. *Antivir Chem Chemother* 2004;**15**:101–9.
137. Mostashari F, Bunning ML, Kitsutani PT, et al. Epidemic West Nile encephalitis, New York, 1999: results of a household-based seroepidemiological survey. *Lancet* 2001;**358**:261–4.
138. Mostashari F, Kulldorff M, Hartman JJ, et al. Dead bird clusters as an early warning system for West Nile virus activity. *Emerg Infect Dis* 2003;**9**:641–6.
139. Mukhopadhyay S, Kuhn RJ, Rossmann MG. A structural perspective of the flavivirus life cycle. *Nat Rev Microbiol* 2005;**3**:13–22.
140. Murphy TD, Grandpre J, Novick SL, et al. West Nile virus infections among health-fair participants, Wyoming 2003: assessment of symptoms and factors. *Vector Borne Zoonotic Dis* 2005;**5**: 246–51.
141. Murray K, Baraniuk S, Resnick M, et al. Risk factors for encephalitis and death from West Nile virus infection. *Epidemiol Infect* 2006;**134**:1325–32.
142. Murray K, Walker C, Herrington E, et al. Persistent infection with West Nile virus years after initial infection. *J Infect Dis* 2011;**201**:2–4.
143. Nasci RS, Savage HM, White DJ, et al. West Nile virus in overwintering *Culex* mosquitoes, New York City, 2000. *Emerg Infect Dis* 2001;**7**:742–4.
144. Nash D, Mostashari F, Fine A, et al; West Nile Outbreak Response Working Group. The outbreak of West Nile virus infection in the New York City area in 1999. *N Engl J Med* 2001;**344**:1807–14.
145. National Institutes of Health. ClincalTrials.gov. http://clinicaltrials.gov/ct2/results?term=west+nile+virus&pg=1.
146. Nichter CA, Pavlakis SG, Shaikh U, et al. Rhombencephalitis caused by West Nile fever virus. *Neurology* 2000;**55**:153.
147. Nisenbaum C, Wallis K. Meningo-encephalitis due to West Nile fever: reports of 2 cases. *Helv Paediatr Acta* 1965;**20**:392–402.
148. Nolan MS, Podoll AS, Hause AM, et al. Prevalence of chronic kidney disease and progression of disease over time among patients enrolled in the Houston West Nile virus cohort. *PloS One* 2012;**7**: e40374.
149. O'Leary DR, Kuhn S, Kniss KL, et al. Birth outcomes following West Nile virus infection of pregnant women in the United States: 2003-2004. *Pediatrics* 2006;**117**:e537–45.
150. O'Leary DR, Marfin AA, Montgomery SP, et al. The epidemic of West Nile virus in the United States, 2002. *Vector Borne Zoonotic Dis* 2004;**4**:61–70.
151. Oliphant T, Engle M, Nybakken GE, et al. Development of a humanized monoclonal antibody with therapeutic potential against West Nile virus. *Nat Med* 2005;**11**:522–30.

152. Omalu BI, Ghakir AA, Wang G, et al. Fatal fulminant pan-meningopolioencephalitis due to West Nile virus. *Brain Pathol* 2003;**13**:465–72.
153. Reference deleted in proofs.
154. Parida M, Posodas G, Inoue S, et al. Real-time reverse transcription loop-mediated isothermal amplification for rapid detection of West Nile virus. *J Clin Microbiol* 2004;**42**:257–63.
155. Park M, Hui JS, Bartt RE. Acute anterior radiculitis associated with West Nile virus infection. *J Neurol Neurosurg Psychiatry* 2003;**74**:823–5.
156. Pealer LN, Marfin AA, Petersen LR, et al. Transmission of West Nile virus through blood transfusion in the United States in 2002. *N Engl J Med* 2003;**349**:1236–45.
157. Pesko KN, Ebel GD. West Nile virus population genetics and evolution. *Infect Genet Evol* 2012;**12**:181–90.
158. Petersen LR, Marfin AA. West Nile virus: a primer for the clinician. *Ann Intern Med* 2002;**137**:173–9.
159. Reference deleted in proofs.
160. Petropoulou KA, Gordon SM, Prayson RA, et al. West Nile virus meningoencephalitis: MR imaging findings. *Am J Neuroradiol* 2005;**26**:1986–95.
161. Planitzer CB, Modrof J, Yu MY, et al. West Nile virus infection in plasma of blood and plasma donors, United States. *Emerg Infect Dis* 2009;**15**:1668–70.
162. Pruzanski W, Altman R. Encephalitis due to West Nile fever virus. *World Neurol* 1962;**3**:524–8.
163. Pupo M, Guzman MG, Fernandez R, et al. West Nile Virus infection in humans and horses, Cuba. *Emerg Infect Dis* 2006;**12**: 1022–4.
164. Rahal JJ, Anderson J, Rosenberg C, et al. Effect of interferon-α2b therapy on St. Louis viral meningoencephalitis: clinical and laboratory results of a pilot study. *J Infect Dis* 2004;**190**:1084–7.
165. Ravindra KV, Freifeld AG, Kalil AC, et al. West Nile virus–associated encephalitis in recipients of renal and pancreas transplants: case series and literature review. *Clin Infect Dis* 2004;**38**: 1257–60.
166. Reisen WK, Takahashi RM, Carroll BD, et al. Delinquent mortgages, neglected swimming pools, and West Nile virus, California. *Emerg Infect Dis* 2008;**14**:1747–9.
167. Rhee C, Eaton EF, Concepcion W, et al. West Nile virus encephalitis acquired via liver transplantation and clinical response to intravenous immunoglobulin: case report and review of the literature. *Transpl Infect Dis* 2011;**13**:312–17.
168. Rimon A, Straussberg R, Amir J. West Nile encephalitis mimicking herpes encephalitis. *Pediatr Neurol* 2006;**35**:62–4.
169. Rizzo C, Esposito S, Azzari C, et al. West Nile Virus infections in children: a disease pediatricians should think about. *Pediatr Infect Dis J* 2011;**30**:65–6.
170. Roehrig JT, Nash D, Maldin B, et al. Persistence of virus-reactive serum immunoglobulin M antibody in confirmed West Nile virus encephalitis cases. *Emerg Infect Dis* 2003;**9**:376–9.
171. Saad M, Youssef S, Kirschke D, et al. Acute flaccid paralysis: the spectrum of a newly recognized complication of West Nile virus infection. *J Infect Dis* 2005;**51**:120–7.
172. Sampson BA, Ambrosi C, Charlot A, et al. The pathology of human West Nile virus infection. *Hum Pathol* 2000;**31**:527–31.
173. Samuel MA, Diamond MS. Pathogenesis of West Nile Virus infection: a balance between virulence, innate and adaptive immunity, and viral evasion. *J Virol* 2006;**80**:9349–60.
174. Sayao AL, Suchowersky O, Al-Khathaami A, et al. Calgary experience with West Nile virus neurological syndrome during the late summer of 2003. *Can J Neurol Sci* 2004;**31**:194–203.
175. Sejvar JJ, Marfin AA. Manifestations of West Nile neuroinvasive disease. *Rev Med Virol* 2006;**16**:209–24.
176. Sejvar JJ, Bode AV, Marfin AA, et al. West Nile virus–associated flaccid paralysis. *Emerg Infect Dis* 2005;**11**:1021–7.
177. Sejvar JJ, Bode AV, Marfin AA, et al. West Nile virus–associated flaccid paralysis outcome. *Emerg Infect Dis* 2006;**12**:514–16.
178. Sejvar JJ, Curns AT, Welburg L, et al. Neurocognitive and functional outcomes in persons recovering from West Nile virus illness. *J Neuropsychol* 2008;**2**:477–99.
179. Sejvar JJ, Haddad MB, Tierney BC, et al. Neurologic manifestations and outcome of West Nile virus infection. *J A M A* 2003; **290**:511–15.
180. Sejvar JJ, Leis AA, Stokic DS, et al. Acute flaccid paralysis and West Nile virus infection. *Emerg Infect Dis* 2003;**9**:788–93.
181. Shimoni Z, Niven MJ, Pitlick S, et al. Treatment of West Nile virus encephalitis with intravenous immunoglobulin. *Emerg Infect Dis* 2001;**7**:759.
182. Shrestha B, Diamond MS. Role of CD8 T-cells in control of West Nile virus infection. *J Virol* 2004;**78**:8312–21.
183. Shrestha B, Gottlieb D, Diamond MS. Infection and injury of neurons by West Nile encephalitis virus. *J Virol* 2003;**77**: 13203–13.
184. Smith HL, Monath TP, Pazoles P, et al. Development of antigen-specific memory CD8+ T cells following live-attenuated chimeric West Nile virus vaccination. *J Infect Dis* 2011;**203**: 513–22.
185. Smith RD, Konoplev S, DeCourten-Myers G, et al. West Nile virus encephalitis with myositis and orchitis. *Hum Pathol* 2004;**35**: 254–8.
186. Smithburn KC. Differentiation of the West Nile virus from the viruses of St. Louis and Japanese B encephalitis. *J Immunol* 1942;**44**:25–31.
187. Reference deleted in proofs.
188. Smithburn KC, Hughes TP, Burke AW, et al. A neurotropic virus isolated from the blood of a native of Uganda. *Am J Trop Med* 1940;**20**:471–92.
189. Southam CM, Moore AE. West Nile, Ilheus, and Bunyamwera virus infections in man. *Am J Trop Med Hyg* 1951;**31**:724–41.
190. Southam CM, Moore AE. Induced virus infections in man by the Egypt isolates of West Nile virus. *Am J Trop Med Hyg* 1954;**3**:19–50.
191. Spiegel R, Miron D, Gavriel H, et al. West Nile virus meningoencephalitis complicated by motor aphasia in Hodgkin's lymphoma. *Arch Dis Child* 2002;**86**:441–2.
192. Spigland I, Jasinska-Klingberg W, Hofsbi E, et al. Clinical and laboratory observations in an outbreak of West Nile fever in Israel. *Harefuah* 1958;**54**:275–81.
193. Tardei G, Ruta S, Chitu V, et al. Evaluation of immunoglobulin M (IgM) and IgG enzyme immunoassays in serologic diagnosis of West Nile Virus infection. *J Clin Microbiol* 2000;**38**: 2232–9.
194. Taylor RM, Work TH, Hurlbut HS, et al. A study of the ecology of West Nile virus in Egypt Am. *J Trop Med Hyg* 1956;**5**:579–620.
195. Tsai TF, Chandler LJ. Arboviruses. In: Murray PR, Baron EJ, Jorgensen JH, editors. *Manual of clinical microbiology*. 8th ed. Washington, DC: American Society for Microbiology Press; 2003. p. 1553.
196. Tsai TF, Popovici F, Cernescu C, et al. West Nile encephalitis epidemic in southeastern Romania. *Lancet* 1998;**352**:767–71.
197. Ulbert S. West Nile virus: the complex biology of an emerging pathogen. *Intervirology* 2011;**54**:171–84.
198. Unlu I, Kramer WL, Roy AF, Foil LD. Detection of West Nile virus RNA in mosquitoes and identification of mosquito blood meals collected at alligator farms in Louisiana. *J Med Entomol* 2010;**47**:625–33.
199. Unlu I, Mackay AJ, Roy A, et al. Evidence of vertical transmission of West Nile virus in field-collected mosquitoes. *J Vector Ecol* 2010;**35**:95–9.
200. Venter M, Burt FJ, Blumberg L, et al. Cytokine induction after laboratory-acquired West Nile virus infection. *N Engl J Med* 2009;**360**:1260–2.
201. Venter M, Steyl J, Human S, et al. Transmission of West Nile virus during horse autopsy. *Emerg Infect Dis* 2010;**16**: 573–5.
202. Vidwan G, Bryant KK, Puri V, et al. West Nile virus encephalitis in a child with left-side weakness. *Clin Infect Dis* 2003;**37**: 91–4.
203. Wang P, Dai J, Bai F, et al. Matrix metalloproteinase 9 facilitates West Nile virus entry into the brain. *J Virol* 2008;**82**:8978–85.
204. Wang T, Town T, Alexopoulou L, et al. Toll-like receptor 3 mediates West Nile virus entry into the brain causing lethal encephalitis. *Nat Med* 2004;**10**:1366–73.
205. Wang Y, Lobigs M, Lee E, et al. CD8 T cells mediate recovery and immunopathology in West Nile virus encephalitis. *J Virol* 2003;**77**:13323–34.
206. Watson JT, Gerber SI. West Nile virus: a brief review. *Pediatr Infect Dis J* 2004;**23**:357–8.
207. Watson JT, Pertel PE, Jones RC, et al. Clinical characteristics and functional outcomes of West Nile fever. *Ann Intern Med* 2004;**141**:360–5.

208. Weinstein M. Atypical West Nile virus infection in a child. *Pediatr Infect Dis J* 2003;**22**:842–4.
209. Weiss D, Carr D, Kellachan J, et al; West Nile Virus Outbreak Response Working Group. Clinical findings of West Nile virus infection in hospitalized patients, New York and New Jersey, 2000. *Emerg Infect Dis* 2000;**7**:654–8.
210. Wilson JR, de Sessions PF, Leon MA, et al. West Nile virus nonstructural protein 1 inhibits TLR3 signal transduction. *J Virol* 2008;**82**:8262–71.
211. Yim R, Posfay-Barbe KM, Nolt D, et al. Spectrum of clinical manifestations of West Nile virus infection in children. *Pediatrics* 2004;**4**:1673–5.
212. Zeller HG, Schuffenecker I. West Nile virus: An overview of its spread in Europe and the Mediterranean basin in contrast to its spread in the Americas. *Eur J Clin Microbiol Infect Dis* 2004;**23**:147–56.

175C ■ YELLOW FEVER

Duane J. Gubler • Gail J. Harrison

Yellow fever is a mosquito-borne viral disease of humans and lower primates that occurs naturally in tropical Africa and the Americas (Fig. 175C-1). Epidemics, which can occur in both urban and rural areas, often are associated with severe hemorrhagic disease and high fatality rates. It is considered a reemerging disease, with increased incidence documented in the last 25 years.[1] Globally, yellow fever virus infects over 200,000 people every year and causes approximately 30,000 deaths annually.

HISTORY

Yellow fever was described as a disease entity first in 1648 in the Yucatan, Mexico. It apparently was part of a larger regional epidemic that affected the Caribbean islands and Central America from Barbados to Mexico from 1647 to 1649.[14,15] Although it was first described in the Americas, yellow fever virus, along with its principal urban epidemic mosquito vector, *Aedes aegypti*, most likely originated in Africa and was introduced to the New World by the slave trade. During the 17th, 18th, 19th, and early 20th centuries, epidemic yellow fever was a major public health problem in the Western Hemisphere. Large epidemics occurred in tropical America, as well as in the United States (as far north as Boston) and Europe (as far north as England).[24] Epidemics occurred primarily in port cities as a result of spread by sailing vessels and commerce.

After transmission by mosquitoes was documented by Reed and the Yellow Fever Commission in Cuba,[21] major efforts were undertaken to keep the disease in check by mosquito control. The first yellow fever virus was isolated in 1927. By 1938, an effective live attenuated virus vaccine had been developed. Yellow fever was controlled in the Americas by eradication of the mosquito vector of urban disease, *A. aegypti*, from most Central and South American countries. In Francophone countries of West Africa, yellow fever was controlled by mass vaccination programs. The result was the disappearance of major urban epidemics of yellow fever in both Africa and the Americas during the 1950s, 1960s, and 1970s. In the mid-1980s, however, the urban form of disease reemerged in West Africa, with major epidemics in Nigeria and increased transmission in other countries.[14] Kenya experienced its first epidemic in history in 1993. In the Americas, *A. aegypti* has reinfested most Central and South American countries from which it had been eradicated, and urban centers of the American tropics are at their highest risk for epidemic urban yellow fever in more than 60 years.[7] Thus, the disease continues to be an important public health problem in both Africa and the Americas. Yellow fever transmission has never been recognized in Asia or the Pacific.

ETIOLOGIC AGENT

Yellow fever is caused by the yellow fever virus, the prototype of the genus *Flavivirus*, family Flaviviridae. The genus *Flavivirus* contains 53 viruses, which are small (40 to 50 nm in diameter), spherical, and enveloped, with single-stranded RNA approximately 11 kb in length.[5] Many of the flaviviruses are very closely related antigenically, which has resulted in extensive cross reactivity in most serologic tests, so a laboratory diagnosis is difficult

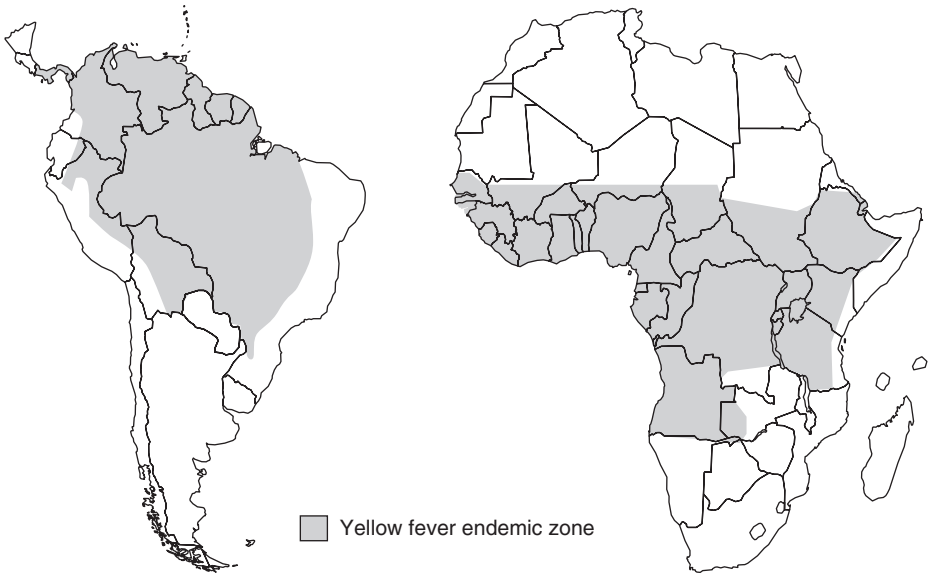

FIGURE 175C-1 ■ Geographic distribution of yellow fever in Africa and the Americas.

to make. Yellow fever virus has seven genotypes that are geographically separated, and outbreaks of disease are more associated with specific genotypes.[1] The virus is part of an antigenic complex that is unique from other members of the family Flaviviridae.[8]

EPIDEMIOLOGY

Yellow fever viruses are maintained in natural zoonotic cycles involving lower primates and canopy-dwelling mosquitoes that breed in tree holes in the rain forests of both Africa and the Americas, called the jungle or sylvatic cycle of yellow fever.[14,16,24] Humans become involved accidentally when they encroach on this forest cycle. Humans thus infected may transport the virus back to a village or city during the incubation period of 2 to 14 days. If infected persons are fed on by urban *A. aegypti*, the virus then can be transmitted to other humans by mosquito bite after an extrinsic incubation of 8 to 10 days. Major urban epidemics are transmitted by this highly domesticated mosquito, which lives in close association with humans in most tropical cities of the world.[1,7]

Yellow fever occurs throughout much of sub-Saharan Africa and tropical America in the sylvatic cycle (see Fig. 175C-1). In Africa, cercopithecoid and celobid monkeys are the main vertebrate hosts; infection rarely causes illness and death in these species. Year-round enzootic transmission by *Aedes africanus* occurs in the rain forests. In wet savanna areas bordering the rain forests of western and central Africa, transmission increases during the rainy season and decreases during the dry season. *Aedes furcifer*, *A. africanus*, and *Aedes leuteocephalus* are the main mosquito vectors in this "zone of emergence," and the virus is transmitted from monkey to monkey, monkey to human, and human to human. In the dry savanna zones, yellow fever activity is intermittent and takes place mainly during the rainy season, but it also occurs in major epidemics in urban areas, where stored water provides the ideal larval habitat for *A. aegypti*. In East Africa, *Aedes simpsoni (Aedes bromeliae)* provides the link between the *A. africanus* sylvatic cycle and humans in areas bordering gallery forests.

In tropical America, howler, spider, squirrel, owl, capuchin, and wooly monkeys all act as vertebrate hosts for yellow fever virus. Mosquitoes of the genus *Haemagogus* are the principal vectors in tropical American rain forests, where they feed in the canopy as well as at ground level. Other mosquito species, such as *Sabethes chloropterus*, *Aedes leucocelaenus*, and *Aedes fulvus*, may play secondary roles. Although most cases of yellow fever have been reported from Bolivia, Brazil, Venezuela, and Peru in recent years, the enzootic zone probably includes the rain forests of at least 10 countries (i.e., Colombia, Ecuador, Peru, Bolivia, Brazil, Venezuela, Guyana, Suriname, French Guiana, and Trinidad[27]) and involves "wandering" enzootic transmission among the monkey populations. The humans involved are mainly adult men who work in the forest.

Vertical transmission of yellow fever virus from an infected female mosquito through the eggs to her offspring plays an important role in survival and maintenance of yellow fever virus in enzootic cycles and has been demonstrated in nature in West Africa by isolation of the virus from male *A. furcifer* mosquitoes. Experimentally, *Haemagogus* and *A. aegypti* mosquitoes have been shown to be capable of vertical transmission. This mechanism is thought to be of major importance in survival of the virus during prolonged dry periods in both enzootic regions.

Urban epidemics of yellow fever have reappeared in West Africa in the past 17 years.[20] Unfortunately, surveillance is very poor, and the actual number of cases reported is thought to be grossly underestimated. For example, in Nigeria in 1986 and 1987, the number of reported cases and deaths during epidemics was 2612 and 973, respectively. Seroepidemiologic studies, however, estimated that the actual number of cases and deaths was 130,000 and 29,000, respectively.[20]

In the Americas, the last urban yellow fever epidemic occurred in 1942. Reinvasion of American tropical urban centers by *A. aegypti*, however, has placed more than 300 million susceptible individuals at risk. As might be expected, urban transmission has been reported recently. In 1998, urban transmission of yellow fever was documented in Santa Cruz, Bolivia.[26] Although not comprising a large number of cases, this outbreak underscores the high risk that many tropical urban centers have at the beginning of the 21st century. In addition, six fatal cases of yellow fever in travelers who visited South America (four cases) or Africa (two cases) in recent years have been confirmed. Three of these cases were U.S. citizens, and three were European. Why contemporary yellow fever epidemics have not occurred in the Americas, despite the high risk, is not known. The dramatic increase in transmission of dengue in urban centers of the American tropics in recent years possibly has resulted in a high prevalence of heterotypic flavivirus antibody that protects against severe yellow fever clinical illness. Another possibility is that the enzootic virus strains are not well adapted to urban transmission by *A. aegypti*.

Epidemic yellow fever has never been reported in Asia or the Pacific. The reason is not known, but both variation in mosquito vector competence and partial protection by heterotypic flavivirus antibody have been suggested as reasons that this virus has never become established in that part of the world. If urban epidemics do occur in the American tropics, however, the virus is expected to move very quickly to this and other permissive areas where urban *A. aegypti* mosquitoes are found; this movement undoubtedly would cause a major international public health emergency.

CLINICAL MANIFESTATIONS

Infection with yellow fever virus causes a spectrum of illness ranging from inapparent infection to severe yellow fever with the classic triad of jaundice, hemorrhage, and albuminuria, which is associated with a high case-fatality rate. Most yellow fever infections are manifested clinically as a mild to severe viral syndrome without

symptoms of intoxication; 10 to 20 percent of infections may result in classic yellow fever.[13,16,17]

The incubation period may be as long as 13 days but generally is 3 to 6 days.[16] The onset of illness is abrupt, with fever, headache, backache, myalgia, nausea, and other nonspecific signs and symptoms. In mild cases, the illness will last for several days, after which recovery is uneventful and complete. In severe cases, prostration occurs commonly, and examination reveals congestion of the skin, conjunctivae, and mucous membranes. The pulse rate usually increases early in the illness, and blood pressure is normal. Leukopenia is a frequent finding, and mild albuminuria may be noted. The temperature generally ranges between 38.5° and 40°C (101.3° and 104°F). Nausea and vomiting are common manifestations. Minor hemorrhagic manifestations such as epistaxis and bleeding gums may be observed. This period of infection may last for approximately 3 days, at which time the congestion declines and a relative bradycardia may occur despite the elevated temperature (Faget sign). The temperature falls to or below normal, and the patient feels better.

In most patients, this period of remission signals the beginning of convalescence; but in severe cases, it may last only a few hours, after which the patient enters a period of intoxication characterized by venous congestion and extreme bradycardia, despite a secondary rise in temperature. Nausea and vomiting are severe and associated with epigastric pain. Prostration, jaundice, marked albuminuria, and anuria are present. Hemorrhagic manifestations include hematemesis and melena.

The jaundice in some patients is not striking; it is difficult to detect in early disease and often is not detected until after death. The severity of hemorrhagic manifestations also varies greatly, but some hemorrhage can be found in most cases. As indicated earlier, minor hemorrhagic manifestations may be observed in the early stage of illness; severe hemorrhage usually develops late in the illness, although it may occur in fulminant cases as early as the second or third day. Hemorrhage may be so severe that it causes shock and death from blood loss. Albuminuria is one of the most common findings in yellow fever, and it occurs in all but very mild cases. It is present early in the illness and may increase rapidly. The albuminuria probably is related to renal involvement during the period of infection. Anuria, on the other hand, appears to be related to hepatic involvement and never is seen in the absence of other signs of liver infection. These terminal events may be related to "cytokine storm."

Death may ensue as early as 2 to 4 days after onset but usually occurs after 7 to 10 days of illness in 20 to 50 percent of severe cases. In patients in whom death occurs later, autopsy generally reveals a cause other than yellow fever. Patients with severe infection often have lowered resistance to secondary infection, which may develop at the time of convalescence. Other complications include kidney abscess, pneumonia, suppurative parotitis, and skin infection. Convalescence usually is rapid and complete except for a general weakness that may last for several weeks. Permanent damage to the liver or kidneys is not apparent.

PATHOLOGY

The gross pathologic findings in fatal cases of yellow fever is not striking.[17] The skin, sclerae, serosa, some internal organs, and subcutaneous fat usually have moderate icterus. Serous effusions, edema, and hemorrhages, including petechiae and purpuric lesions on the skin, conjunctivae, mucous membranes, stomach, duodenum, and bladder, often are present. Gastrointestinal hemorrhage may be prominent.

The most characteristic lesions caused by yellow fever virus are seen in the liver, although liver failure is not generally the cause of death. The liver may have a yellowish color and be enlarged and fatty in consistency. In typical cases, marked necrosis of the midzone of the lobule is present. The necrosis extends both centrally and peripherally and on average involves 80 percent of the lobule in fatal cases.[13,17] The cells bordering the central vein and portal areas usually are spared. Councilman and Torres bodies can be observed in hepatocytes. Little or no inflammatory response occurs, and the reticulin framework is preserved.

The kidneys generally are tense and swollen. Glomerular changes are minor, but acute tubular necrosis and fatty metamorphosis may be significant. Cloudy swelling, degeneration, and fatty infiltration may occur in the myocardial fibers. The spleen and lymph nodes are depleted of lymphocytes, and mononucleocytes or histiocytes accumulate in the follicles of the spleen. Edema and petechiae may be observed in the brain.

LABORATORY FINDINGS

Leukopenia and albuminuria are common findings in early stages of the disease. In severe cases, prolonged prothrombin and partial thromboplastin times, thrombocytopenia, fibrin split products, and elevated liver enzymes are observed. Total and conjugated serum bilirubin levels are elevated. Albumin levels in urine usually are less than 5 g/L, but in rare cases they may reach 40 g/L. The urine contains bile. Cerebrospinal fluid usually is normal but may be under increased pressure.

DIFFERENTIAL DIAGNOSIS

Clinically, yellow fever is difficult to differentiate from many other viral, bacterial, and parasitic infections, including other viral hemorrhagic fevers such as Lassa, Ebola, Marburg, and Rift Valley in Africa; the illnesses caused by arenaviruses in the Americas; and dengue hemorrhagic fever in both continents. Other diseases that cause fever and jaundice, such as viral hepatitis, leptospirosis, falciparum malaria, tick-borne relapsing fever, typhus, typhoid, and Q fever, also should be considered in the differential diagnosis. A definitive diagnosis of yellow fever can be made only by use of the appropriate laboratory test.

LABORATORY DIAGNOSIS

Specific laboratory diagnosis requires isolation of the virus or serologic, nucleic acid amplification, or immunohistochemical tests. Virus can be isolated most easily from acute-phase serum taken during the first 4 days of illness, but it has been isolated as long as 14 days after the onset of illness and from the liver after death.[17] The most sensitive method of virus isolation is inoculation of mosquitoes followed by AP-61 cell culture from *Aedes pseudoscutellaris* mosquitoes. Vero cells and inoculation of suckling mice also can be used but are less sensitive. Polymerase chain (PCR) reaction is highly sensitive, and yellow fever RNA can be amplified from these same tissues with a sensitivity higher than that of virus isolation.[7,11] Viral antigen can be demonstrated in the liver by immunohistochemical methods.[9] Either fresh or formalin-fixed tissue can be used with these techniques, which may be performed to establish a virologic diagnosis after virus has been cleared from the blood.

Serologic diagnosis depends on the collection of properly timed acute- and convalescent-phase serum samples to demonstrate a rise in specific antibody. Serologic tests commonly used to diagnose yellow fever include hemagglutination inhibition, complement fixation, and the plaque reduction neutralization test (PRNT) as well as newer tests, such as enzyme-linked immunosorbent assay (ELISA) for both immunoglobulin (Ig)G and IgM antibodies.[8] The immunofluorescent assay is used in some laboratories.

Antibodies detected by hemagglutination inhibition, PRNT, immunofluorescent assay, and IgM capture ELISA appear within 5 to 7 days after the onset of illness, whereas complement-fixation antibodies appear later, usually after 10 to 14 days. Hemagglutination-inhibition and PRNT antibodies persist at detectable levels for many years (>50) in most patients, whereas the duration of complement-fixation and IgM antibodies is uncertain, but they probably wane to undetectable levels after 12 to 18 months.

PRNT is the most sensitive and specific of the serologic tests.[23] In patients who have had no previous flavivirus infection, this test can be used to make a specific diagnosis of yellow fever, as can IgM capture ELISA. Cross reaction between yellow fever antigen and antibodies to other related flaviviruses complicates the serodiagnosis of this and other flavivirus diseases. Hemagglutination inhibition, immunofluorescent assay, and IgG ELISA are nonspecific tests in which considerable cross reactivity with heterologous flavivirus antibodies occurs.

The use of yellow fever 17D vaccine in disease-endemic areas also may complicate serologic diagnosis. Vaccination induces low-titer (1:10 to 1:40) hemagglutination-inhibition and neutralizing antibodies but no detectable immunofluorescent or complement-fixation antibodies. Vaccination also induces IgM antibody, which may remain at detectable levels for as long as 3 to 4 years.[6,22] Vaccination of individuals who have had a previous flavivirus infection induces an anamnestic response of heterotypic flavivirus antibodies at high titer (≥1:1280).[12]

Yellow fever virus genome also may be detected by molecular methods such as quantitative real-time PCR and isothermal protocols that have been simplified and used in reference laboratories and point-of-care field situations.[4,11]

TREATMENT

Treatment of yellow fever is primarily supportive. No specific antiviral therapy is licensed for treatment of yellow fever.[13,15] However, ribavirin has activity against yellow fever virus and has been used to treat other *Flavivirus* infections.[18] In addition, investigations on the use of compounds such as T-705 and T-1106, as well as derivatives of the antibiotics doxorubicin and teicoplanin, as inhibitors of yellow fever virus replication are reported.[3,10] Patients with severe disease requiring hospitalization should have complete bed rest with good nursing care and close monitoring of vital organ functions. Salicylates should be avoided, but mild sedatives may be helpful. Maintenance of fluid and electrolyte balance is critical. Guidelines for intensive care of severe yellow fever cases have not been established. Secondary bacterial infections may occur and should be treated with appropriate antibiotics.

PROGNOSIS

Mortality in all yellow fever infections is low (<5%), but in severe cases requiring hospitalization, it may be 20 to 50 percent.[13,17] The prognosis is poor for patients who enter a period of intoxication with rapidly increasing albuminuria, jaundice, fever, and severe hemorrhage. Patients in the terminal stage of illness usually are somnolent, have below-normal temperatures, and may have intractable hiccups.

PREVENTION AND CONTROL

The most practical and cost-effective method of preventing yellow fever is vaccination. A single dose of 17D vaccine provides effective, long-term (10 years) protection and should be used in the World Health Organization Expanded Program of Immunization in enzootic countries of Africa and the Americas.

The live attenuated 17D vaccine is prepared from infected chicken embryos and produces effective immunity in more than 95 percent of recipients.[2,17] It is used to provide protection for travelers to and residents of endemic areas of tropical South America, South Africa, and Africa. The decision to administer yellow fever vaccine should include the geographic destination, season of the year, local evidence for yellow fever transmission, likelihood of exposure to vector mosquitoes, and individual risk factors for adverse events.[2,17,19] Adverse reactions rarely occur, but may be categorized as neurotropic or viscerotropic. Adverse events may occur more often in elderly persons, infants, and pregnant women.[2,12,17,19] The elderly appear to have a delayed antibody response and a

prolonged viremia after vaccination, possibly leading to the higher incidence of adverse events in the older age group.[12,22] Infants younger than 4 months of age have a high risk for development of encephalitis and should not be vaccinated with the live attenuated 17D vaccine until they are 9 months of age. Pregnant women also should avoid being vaccinated because vaccine virus may infect the developing fetus, although the risk for adverse events associated with congenital infection is unknown. The live attenuated 17D vaccine should not be given to persons who are allergic to eggs or to those who are immunodeficient or receiving immunosuppressive drugs. Detection of yellow fever IgG antibody persists, and detection of yellow fever IgM antibodies may persist up to 3 to 4 years after vaccination, confounding serologic diagnosis of yellow fever in vaccinated travelers.[6]

The other method of preventing yellow fever is mosquito control, especially during epidemic activity. The principal mosquito vector of urban yellow fever, *A. aegypti*, is a highly domesticated species that lives in and around the houses of humans.[1,7] It breeds primarily in artificial containers that collect rainwater or in domestic water storage containers, and is notoriously difficult to control. The most sustainable and effective prevention, therefore, is to control, discard, or chemically treat larval habitats in the domestic environment.[1,7] This process is labor-intensive but can be done with the help of the citizens in the community.

Some authorities recommend adult mosquito control with insecticide space sprays, primarily ultra-low-volume sprays. Recent field trials, however, have shown this approach to be ineffective unless portable sprayers are used to treat the inside of each dwelling. Because of the excessive cost and lack of efficacy of ultra-low-volume application of insecticides, this method is not recommended.

Recently, the ability of the intracellular bacterium *Wolbachia* to reduce the replication of yellow fever virus in the mosquito vector *Aedes aegypti* shows promise for a novel biocontrol strategy for yellow fever.[25]

Patients suspected of having yellow fever should be protected from mosquitoes. The most effective protection is to use a mosquito net on the bed during the acute febrile period of illness. Alternatively, patients can be kept in screened rooms. Effective repellents are available to treat clothing and exposed skin.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Barrett A, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annu Rev Entomol* 2007;**52**:209–29.
3. De Burghgraeve T, Kaptein S, Ayala-Nunez N, et al. An analogue of the antibiotic teicoplanin prevents flavivirus entry in vitro. *PLoS One* 2012;**7**:e37244.
4. Domingo C, Patel P, Yilah J, et al. Advanced yellow fever virus genome detection in point-of-care facilities and reference laboratories. *J Clin Microbiol* 2012, E pub ahead of print.
6. Gibney K, Edupuganti S, Panella A, et al. Detection of anti-yellow fever virus immunoglobulin M antibodies at 3-4 years following yellow fever vaccination. *Am J Trop Med* 2012;**87**:1112–15.
10. Kaptein S, De Burghgraeve T, Froeyen M, et al. A derivative of the antibiotic doxorubicin is a selective inhibitor of dengue and yellow fever virus replication in vitro. *Antimicrob Agents Chemother* 2010;**54**:5269–80.
18. Monath TP. Treatment of yellow fever. *Antiviral Res* 2008;**78**:116–24.
19. Monath TP. Review of the risks and benefits of yellow fever vaccination including some new analyses. *Expert Rev Vaccines* 2012;**11**:427–48.
22. Roukens A, Soonawala D, Joosten S, et al. Elderly subjects have a delayed antibody response and prolonged viraemia following yellow fever vaccination: a prospective controlled cohort study. *PLoS One* 2011;**6**:e27753.
23. Simoes M, Camacho L, Yamamura A, et al. Evaluation of accuracy and reliability of the plaque reduction neutralization test (micro-PRNT) in detection of yellow fever virus antibodies. *Biologicals* 2012;**40**:399–404.
25. van den Hurk A, Hall-Mendelin S, Pyke A, et al. Impact of *Wolbachia* on infection with Chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. *PLoS Negl Trop Dis* 2012;**6**:e1892.

The full reference list for this chapter is available at expertconsult.com.

175D ■ DENGUE, DENGUE HEMORRHAGIC FEVER, AND SEVERE DENGUE

Scott B. Halstead

Dengue fever is an acute febrile illness syndrome caused by several arthropod-borne viruses and characterized by biphasic fever, myalgia or arthralgia, rash, leukopenia, and lymphadenopathy. Synonyms are dengue and breakbone fever. Dengue hemorrhagic fever (DHF), a febrile disease syndrome caused by dengue viruses, is characterized by abnormalities in hemostasis and leakage of fluid and protein from capillaries, which in severe cases results in shock (dengue shock syndrome [DSS]). It is thought to have an immunopathologic basis. Synonyms are hemorrhagic dengue; acute infectious thrombocytopenic purpura; and Philippine, Thai, Singapore hemorrhagic fever and recently introduced World Health Organization (WHO) case definitions, dengue with warning signs and severe dengue. Useful general reviews are available.[66,150]

The dengue subgroup is composed of four antigenically distinct members.[36,74,88,142] In the second decade of the 21st century, dengue viruses were epidemic and endemic in 110 tropical countries according to reports received by the WHO and other sources, with nearly 3 billion people at risk for infection, an estimated annual 10 million cases of dengue fever, 250,000 children and adults hospitalized, and case fatalities in some countries of 5 percent.[13,58] In tropical Asian countries, dengue is among the 10 leading causes of death in children ranging in age from 1 to 15 years.[64] Small indigenous outbreaks of dengue 1 occurred in Hawaii in 2001 and Key West,

REFERENCES

1. Barrett A, Higgs S. Yellow fever: a disease that has yet to be conquered. *Annu Rev Entomol* 2007;**52**:209–29.
2. Centers for Disease Control and Prevention. Yellow fever vaccine: recommendations of the Advisory Committee on Immigration Practices (ACIP), 2010. *MMWR Recomm Rep* 2010;**59**:1–27.
3. De Burghgraeve T, Kaptein S, Ayala-Nunez N, et al. An analogue of the antibiotic teicoplanin prevents flavivirus entry in vitro. *PLoS One* 2012;**7**:e37244.
4. Domingo C, Patel P, Yilah J, et al. Advanced yellow fever virus genome detection in point-of-care facilities and reference laboratories. *J Clin Microbiol* 2012;**50**:4054–60.
5. Fauquet CM, Mayo MA, Maniloff J, et al, editors: *Virus taxonomy: classification and nomenclature of viruses*. Eighth Report of the International Committee on Taxonomy of Viruses. San Diego: Academic Press; 2005.
6. Gibney K, Edupuganti S, Panella A, et al. Detection of anti-yellow fever virus immunoglobulin M antibodies at 3-4 years following yellow fever vaccination. *Am J Trop Med* 2012;**87**:1112–15.
7. Gubler DJ. *Aedes aegypti* and *Aedes aegypti*–borne disease control in the 1990's: top down or bottom up. *Am J Trop Med Hyg* 1989;**40**: 571–8.
8. Gubler DJ, Kuno G, Markhoff L. Flaviviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007.
9. Hall WC, Crowell TP, Watts DM, et al. Demonstration of yellow fever and dengue antigens in formalin-fixed paraffin-embedded human liver by immunohistochemical analysis. *Am J Trop Med Hyg* 1991;**145**:408–17.
10. Kaptein S, De Burghgraeve T, Froeyen M, et al. A derivative of the antibiotic doxorubicin is a selective inhibitor of dengue and yellow fever virus replication in vitro. *Antimicrob Agents Chemother* 2010;**54**:5269–80.
11. Lanciotti RS. Molecular amplification assays for the detection of flaviviruses. *Adv Virus Res* 2003;**61**:67–99.
12. Martin M, Weld L, Tsai T, et al. Advanced age as a risk factor for adverse events temporally associated with yellow fever vaccination. *Emerg Infect Dis* 2001;**7**:945–51.
13. McKee KT, Monath TP. Arboviruses of Africa. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*, vol. 2. 3rd ed. Philadelphia: W. B. Saunders; 1990. p. 1435–56.
14. Meegan JM. Yellow fever. In: Beran GW, Steele JH, editors. *Handbook of zoonoses*. 2nd ed. Boca Raton, Fla: CRC Press; 1994. p. 111–24.
15. Monath TP. Yellow fever: a medically neglected disease—report on a seminar. *Rev Infect Dis* 1987;**9**:165–75.
16. Monath TP. Yellow fever. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 5. Boca Raton, Fla: CRC Press; 1988. p. 139–231.
17. Monath TP. Yellow fever. In: Strickland GT, editor. *Hunter's tropical medicine*. Philadelphia: W. B. Saunders; 1991. p. 233–8.
18. Monath TP. Treatment of yellow fever. *Antiviral Res* 2008;**78**: 116–24.
19. Monath TP. Review of the risks and benefits of yellow fever vaccination including some new analyses. *Expert Rev Vaccines* 2012;**11**: 427–48.
20. Nasidi A, Monath TP, DeCock K, et al. Urban yellow fever epidemic in western Nigeria, 1987. *Trans R Soc Trop Med Hyg* 1989;**83**:401.
21. Reed W. Recent researches concerning etiology, propagation and prevention of yellow fever, by the United States Army Commission. *J Hyg* 1902;**2**:101–19.
22. Roukens A, Soonawala D, Joosten S, et al. Elderly subjects have a delayed antibody response and prolonged viraemia following yellow fever vaccination: a prospective controlled cohort study. *PLoS One* 2011;**6**:e27753.
23. Simoes M, Camacho L, Yamamura A, et al. Evaluation of accuracy and reliability of the plaque reduction neutralization test (micro-PRNT) in detection of yellow fever virus antibodies. *Biologicals* 2012;**40**:399–404.
24. Strode GK. *Yellow fever*. New York: McGraw-Hill; 1951.
25. Van den Hurk A, Hall-Mendelin S, Pyke A, et al. Impact of *Wolbachia* on infection with Chikungunya and yellow fever viruses in the mosquito vector *Aedes aegypti*. *PLoS Negl Trop Dis* 2012;**6**:e1892.
26. Van der Stuyft P, Gianella A, Pirard M, et al. Urbanization of yellow fever in Santa Cruz, Bolivia. *Lancet* 1999;**353**:1558–62.
27. World Health Organization. Yellow fever situation in Africa and South America, 2005. *Wkly Epidemiol Rec* 2006;**81**:317–24.

Florida in 2009 to 2011.[41,125] Dengue infections are major causes of febrile illnesses in tourists to tropical destinations, some of whom develop life-threatening disease.[175,176]

The term *dengue*, of Spanish origin, came into use in 1828 during a Cuban outbreak that undoubtedly was chikungunya.[22] The Swahili term for chikungunya, *ki denga pepo*, had been used to describe an 1823 outbreak on Zanzibar.[29] The first outbreak that resembled a disease now recognized as dengue fever was described by Benjamin Rush in Philadelphia, Pennsylvania, in 1780.[22,148] Epidemics probably caused by dengue viruses were common from the 18th to the 20th centuries in inhabitants of the Atlantic coastal areas of the United States and South America, the Caribbean islands, and the Mississippi basin.[148] Dengue viruses almost certainly were the cause of the 5- and 7-day fevers that occurred in European colonists in tropical Asia. Similar epidemics occurred commonly in settlers in tropical Australia, where in 1905 *Aedes aegypti* was identified as a dengue vector by Bancroft.[10] Ashburn and Craig[7] found the etiologic agent in human blood and showed that it could pass through a diatomaceous earth filter. An intrinsic incubation period of 3 to 8 days in humans, an extrinsic incubation period of 8 to 11 days in mosquitoes, immunity in humans and monkeys, and the nonsusceptibility of most domestic animals were demonstrated in the classic studies of Siler and associates[148] and Simmons and coworkers[151] between 1924 and 1930. When dengue viruses were isolated in laboratory mice in 1943 and 1944, the modern era of dengue research began.[91,144] Two strains from Hawaii and Papua New Guinea failed to cross protect humans. From this experiment, researchers recognized the existence of at least two different dengue viruses; they were named dengue viruses type 1 and type 2.[143,144]

During most of the previrologic era, dengue viruses were thought to be the cause of a generally benign, self-limited febrile exanthem. However, death, shock, and severe hemorrhagic manifestations accompanied classic dengue fever outbreaks in Australia in 1897 and thereafter for 15 years. Similar phenomena were recorded in Greece in 1928 and in Formosa in 1931.[55] This "new" syndrome was recognized again in Manila in 1954. It was called Philippine hemorrhagic fever because of a resemblance to the epidemic hemorrhagic fever then occurring in United Nations troops in the Korean Peninsula.[74] In 1956, Philippine hemorrhagic fever was associated with dengue when types 3 and 4 were recovered.[74] All four dengue viruses are now endemic in tropical countries throughout the globe.[64,65] Since 1966, the terms *dengue hemorrhagic fever* and *dengue shock syndrome* (DSS) came into general use.[33,55] In 2009, a WHO committee advised that these terms be subsumed under the diagnostic categories, "dengue with warning signs" and "severe dengue."[184]

By epidemiologic criteria, dengue viruses are arthropod-borne (arboviruses) because they are transmitted biologically by various members of the genus *Stegomyia*.[111] Gene structure, replicative strategy, and antigenic relatedness place the dengue viruses in the family Flaviviridae.[88,170] At present, 68 members of the flavivirus family have been identified; 29 of them are established as human pathogens.[88] Cross comparisons by plaque reduction neutralization tests have shown dengue viruses to be an antigenic subgroup.[35] In addition to their antigenic relatedness and their ability to be transmitted by *Stegomyia*, each type of dengue virus produces a closely similar clinical syndrome in susceptible humans.[57,143,148,151]

Dengue virions are spherical particles approximately 50 nm in diameter. The central core, approximately 25 nm in diameter, has icosahedral symmetry and contains a single plus strand of RNA. Dengue RNA consists of approximately 11,000 nucleotides coding from the 5′ end for core, premembrane, envelope, and five nonstructural proteins.[54] Large amounts of nonstructural protein 1 (NS1) are released from infected cells.[104,188] The envelope, which is studded with poorly resolved projections, is composed of many replicates of the envelope protein (54 kDa) embedded in a lipid bilayer. When it is assembled on the virion, the envelope protein bears some epitopes unique to serotypes. Antibodies to these epitopes neutralize by hindering viral attachment or entry into cells.[123] Other epitopes are shared between dengue viruses (dengue subgroup antigens) and other flaviviruses (group antigens).

Four clearly defined types exist, as determined by plaque reduction neutralization tests using antibodies raised by infection of monkeys or fluorescent antibody tests using monoclonal antibodies raised in mice.[34,141] Presumably, virion structures differ for each dengue serotype with the result that infection is blocked by homotypic neutralizing antibody.[35,76] The four types have distinctive genetic structures.[174] Phylogenetic studies suggest that human dengue viruses diverged from four zoonotic dengue types relatively recently, whereas the four zoonotic types evolved from a common ancestor in the more remote past.[78,163,170] Sylvatic dengue viruses retain the capacity to infect and cause disease in humans.[21,164] Different dengue strains cluster in groups that differ genetically (genotypes).[30,31,129,130,160] Genotypes consist of viruses of similar genetic structure that usually circulate within one geographic area.[160] Genotyping can be used to trace the movement of dengue viruses between persons over short and long distances.[84] Of particular interest, a strain of dengue type 2 isolated in Jamaica in 1981 and the dengue 2 viruses recovered from 1981 Cuban DHF patients belong to a Southeast Asian genotype.[129,145] The most sharply divergent genetic differences are among human viruses and strains from Asian and African zoonotic cycles.[129,163,170]

Dengue virus can be grown in 1- to 2-day-old mice or hamsters by intracerebral inoculation or in various mosquitoes by oral or parenteral inoculation. High mouse-passaged virus grows and produces deaths in weanling mice. Various tissue cultures of vertebrate and invertebrate origin support dengue virus growth in vitro, as reviewed in the following section.

TRANSMISSION

A. aegypti, a crepuscular daytime-biting mosquito, is the principal vector. All four virus types have been recovered from naturally infected *A. aegypti*.[55] In most tropical

areas, *A. aegypti* is highly domesticated and breeds in water stored for drinking, washing, or bathing or in any container collecting fresh water. Dengue viruses also have been recovered from naturally infected *Aedes albopictus*, which breeds outdoors in vegetation.[41,55] Outbreaks in the Pacific area have been attributed to *Aedes scutellaris* and *Aedes polynesiensis*.

Transmission of dengue by *A. aegypti* may be explosive and involve as much as 70 to 80 percent of the population.[148] Because *A. aegypti* has a limited flight range, spread of virus is mainly by mobile viremic humans.[105,187] Transmission of dengue viruses by *A. albopictus* results in indolent, long-duration small outbreaks, as exemplified by the introduction of dengue virus type 1 into Hawaii in 2001.[41]

Dengue viruses replicate in the gut, brain, and salivary glands of infected mosquitoes without apparent harm to adult mosquitoes.[135] Mosquitoes are infectious for a lifetime and as long as 70 days in experimental circumstances.[151] Because female mosquitoes take repeated blood meals, long-lived female mosquitoes have great potency as vectors. Several species of *Stegomyia* are infected readily by intrathoracic inoculation, although the threshold for infection by oral feeding is higher.[134,135] *A. aegypti* and *Culex quinquefasciatus* can transmit dengue mechanically by interrupted feeding.[62,151] The contribution of mechanical feeding to the spread of dengue virus during epidemics has never been measured. Because of the "skittishness" of *A. aegypti* and its habit of feeding during the day when its intended victim is awake and often moving, interrupted feeding with simultaneous transmission to multiple hosts within a household must be a common occurrence.

A. aegypti preferentially feeds on people and hence is most abundant in and around human habitations. The mosquito breeds in clean water. Biting activity is reduced at temperatures below a wet bulb temperature of 14° C (57.2° F).[36] Transmission of dengue in temperate countries is interrupted during winter weather, and dengue has not established itself endemically at latitudes above 25 degrees north or south. Breeding sites may be provided by humans through living habits, as in Thailand, where water is stored in and around homes in large earthenware jars.[55,62] In contrast, *A. aegypti* is not abundant in some parts of India because only small amounts of water are brought to homes from village wells for immediate use. Water in flower vases, household offerings, ant traps, coconut husks, tin cans, and rubber tires may supply breeding sites for *A. aegypti*.[47]

In the tropics, outbreaks of dengue generally coincide with the monsoon season. Eggs, which resist desiccation, are deposited inside water containers above the water line.[47] With the beginning of monsoon rains, a large number of eggs laid outdoors are hatched.[68] Indoor populations do not show seasonal change. Temperature is important in controlling viral transmission. Evidence indicates that the extrinsic incubation period shortens with increasing mean temperatures; mosquito biting rates increase with increased temperature and relative humidity.[147]

In sylvan settings, dengue virus has been isolated from three subgenera of *Aedes*, namely, *Stegomyia*, *Diceromyia*, and *Finlaya*, some in circumstances suggesting the occurrence of transovarial transmission. This phenomenon has been demonstrated experimentally, but its contribution to maintenance of virus in a habitat is unknown.[137]

EPIDEMIOLOGY

Host Range

Inoculation of strains of dengue with known human pathogenicity does not produce demonstrable infection in adult chickens, lizards, guinea pigs, rabbits, hamsters, or cotton rats.[146,151] Subhuman primates generally are susceptible to infection by dengue viruses. Numerous species belonging to *Macaca*, *Cercopithecus*, *Cercocebus*, *Papio*, *Hylobates*, and *Pan* can be infected by the bite of virus-infected mosquitoes or by injection of infectious virus preparations.[60,146,151] Infection is essentially asymptomatic. Viremia occurs at levels sufficient to infect mosquitoes. Simmons and colleagues were the first to note that wild-caught *Macaca philippinensis* resisted dengue infection, whereas *Macaca fuscatus* (Japanese macaque) was susceptible.[151] Work by Rudnick[140] in Malaysia has revealed a jungle cycle of dengue transmission involving canopy-feeding monkeys and *Aedes niveus*, a species that feeds on both monkeys and humans. Although the existence of a jungle dengue cycle in the Malaysian rain forest has been documented, the full geographic range of the subhuman primate zoonotic reservoir is not known. In the 1980s and 1990s, extensive epizootics of dengue virus type 2 involved subhuman primates over wide areas of West Africa.[132,166] Genetic and epidemiologic studies have shown that urban human dengue and jungle monkey dengue are only relatively compartmentalized.[129,165,170] Urban dengue is vectored by anthropophilic mosquitoes, and the virus travels along routes of transportation.[105] *A. aegypti* and susceptible humans are so abundant and so widespread that should dengue viruses be exchanged between humans and monkeys, detection would be extremely difficult. If, in the future, urban dengue is eliminated but vector mosquito populations are unabated, dengue viruses may once again be introduced from jungle cycles.

GEOGRAPHIC DISTRIBUTION

Outbreaks of dengue fever have been documented on every continent except Antarctica.[55] Evidence suggests that human dengue may have originated from enzootic foci in tropical Asia.[170,172] The probable spread of *A. aegypti* during historical times from Africa throughout the world provided an ecologic niche quickly occupied by several human viral pathogens: yellow fever, chikungunya, and the dengue viruses. During the 18th and 19th centuries, epidemics occurred in newly settled lands, largely because of the necessity for storage of domestic water in frontier areas. Isolated shipboard or garrison outbreaks often confined to nonindigenous settlers or visitors were reported in Africa, the Indian subcontinent, and Southeast Asia.[24,107,148] During World War II, dengue

virus infections occurred commonly in combatants of the Pacific War and spread to staging areas not normally infected—Japan, Hawaii, and Polynesia.[64,67,143] DHF-like disease was described clinically in Thailand beginning in 1950 and in the Philippines from 1953; cases were confirmed etiologically as dengue in 1958 and 1956, respectively.[74] DHF first was described in Singapore and Malaysia in 1962, Vietnam in 1963, India in 1963, Ceylon (Sri Lanka) in 1965, Indonesia in 1969, Burma (Myanmar) in 1970, China in 1985, and Kampuchea and Laos from about 1985; major outbreaks have occurred in Sri Lanka and India since 1988, in French Polynesia since 1990, in Pakistan since 1998, and in Bangladesh since 1999.[59,64,67] DHF has occurred at consistently high endemicity in Thailand, Burma, Vietnam, Cambodia, Laos, Philippines, India, and Indonesia.[67] Since 2008, repeated outbreaks have occurred in Pakistan.[81] In Thailand, DHF is the third ranking cause of hospitalization and death in children. Intermittent epidemics have involved the Maldives, Singapore, and Bangladesh.

During the past 20 years, major epidemics of all four dengue serotypes have occurred on several Pacific islands, with repeated severe epidemics on Tahiti.[31,48,64,79]

American genotype dengue 2 virus circulated in the American hemisphere for hundreds of years and was the only virus to survive an intensive period of control of *A. aegypti* designed to eradicate urban yellow fever.[5] In 1963, an Asian strain dengue 3 was introduced into the Caribbean and died out in the 1980s. Dengue viruses became fully established in the hemisphere after the introduction of a Southeast Asian dengue 1 in 1977.[65,121] Dengue 1 spread rapidly and now is endemic on the larger Caribbean islands and in Mexico, coastal Central America, and the tropical areas of Guyana, Venezuela, Colombia, Ecuador, Peru, Bolivia, and Brazil.[65] A sharp dengue virus type 2 epidemic in Cuba in 1981 involving 116,000 hospitalizations in a 3-month period led to apparent eradication of *A. aegypti*, but reintroduction of dengue type 2 into Santiago de Cuba in 1997 and type 3 in Havana in 2001 resulted in particularly severe outbreaks of DHF.[2,53,95] In 1981, an Asian dengue 4 was introduced into the Caribbean, spread widely, and now is endemic throughout the Caribbean basin. In 1986 and 1987, dengue virus type 1 spread through most of coastal Brazil and from there to Paraguay, Peru, and Ecuador.[126] In 1990, more than 9000 dengue cases were reported from Venezuela; 2600 of them were classified as DHF, and 74 deaths were associated with the epidemic.[126] Dengue virus types 1, 2, and 4 were isolated.[126] Shortly thereafter, DHF/DSS caused by dengue type 2 was reported from Brazil and French Guiana.[118,127] In 1994, dengue virus 3 was introduced into the region.[65] Dengue virus types 1 to 3 are now endemic in most of Brazil. Initially, in the Americas most dengue infections produced disease in adults, occasionally severe but of low mortality. As two or more dengue viruses became endemically established, classic DHF was recognized throughout most of South and Central America with severe disease increasingly involving children.[65,133]

In the 1960s, dengue viruses 1 and 2 were recovered from humans with mild clinical illnesses in Nigeria in the absence of epidemic disease.[23] In 1983, dengue virus type 3 was isolated in Mozambique.[64] Since then, all four dengue virus types have been recovered from individuals with febrile diseases in much of Africa or travelers returning from Africa.[3] Dengue virus type 3 infections were notable in the past decade with a sharp outbreak of classic dengue fever observed in adults on the Cape Verde islands, and cases and deaths resulting from classic DHF/DSS in Kassala, Sudan, and Port Sudan on the Gulf of Aden.[1,6,108] In Sub-Saharan Africa, DHF/DSS have not been reported and even dengue fever outbreaks are rare. In this respect, Sub-Saharan Africa resembles the situation in Haiti, where multiple dengue serotypes are transmitted at high rates among a predominantly black population but severe disease is not recognized.[73,131]

CLINICAL MANIFESTATIONS

Dengue Fever

In classic dengue fever (seen most frequently in adults), after an incubation period of 2 to 7 days, patients experience a sudden onset of fever, which rapidly rises to 39.5° to 41.4°C (103°F to 106°F) and usually is accompanied by frontal or retro-orbital headache. On occasion, back pain precedes the fever. A transient, macular, generalized rash that blanches under pressure may be seen during the first 24 to 48 hours of fever. The pulse rate may be slow in proportion to the degree of fever. Myalgia or bone pain occurs soon after onset and increases in severity. During the second to sixth day of fever, nausea and vomiting are likely to occur, and generalized lymphadenopathy, cutaneous hyperesthesia or hyperalgesia, aberrations in taste, and pronounced anorexia may develop.

One or 2 days after defervescence, a generalized morbilliform, maculopapular rash appears, with sparing of the palms and soles. It disappears in 1 to 5 days. In some cases, edema of the palms and soles may be noted, and desquamation may occur. About the time of appearance of this second rash, the body temperature, which has fallen to normal, may become elevated slightly and establish the biphasic temperature curve.

Epistaxis, petechiae, and purpuric lesions are uncommon manifestations but may occur at any stage of the disease. Swallowed blood from epistaxis may be passed per rectum or be vomited and could be interpreted as bleeding of gastrointestinal origin. In adults, gastrointestinal bleeding, menorrhagia, and bleeding from other organs have been observed in some dengue fever outbreaks.[128,161,173] Very clear evidence argues that dengue infections in individuals with peptic ulcer predisposes to gastrointestinal hemorrhage; in some cases, patients may exsanguinate during an otherwise normal dengue fever.[161] This syndrome may be confused with DSS (see later discussion) and contribute to a misunderstanding of its pathogenesis. The mechanism of the hemorrhagic diathesis that commonly occurs with dengue virus infection is not known, but speculation centers on platelet abnormalities. The DHF syndrome is differentiated from dengue fever by its association with hemostatic abnormalities and capillary leakage.[155] After the febrile stage,

prolonged asthenia, mental depression, bradycardia, and ventricular extrasystoles are noted commonly in adults.[107]

Primary infections with dengue virus types 2 and 4 are thought to be largely inapparent, particularly in children.[53,87,167,168] Primary infections with dengue virus types 1 and 3 in children often result in mild to moderate febrile disease, sometimes accompanied by low-grade vascular permeability. Infections with these two viruses in adults produce biphasic fever and rash as the most characteristic features of the dengue fever syndrome.[42,151] Manifestations vary with age and among patients. In infants and young children, the disease may be undifferentiated or characterized by a 1- to 5-day fever, pharyngeal inflammation, rhinitis, and mild cough.[71] A distinctive mean incubation period, duration of illness, or spectrum of clinical findings could characterize disease with different dengue types, although these factors have not been studied carefully. As dengue infections spread around the world, persons of all ages are experiencing their first or successive dengue infection as adults. In some cases, dengue infections, whether primary or secondary, in adults or the elderly, particularly those with diabetes, asthma, or hypertension, are serious and pose a risk for death.[98-100,103,122]

Differences in mild dengue syndromes, hospitalized dengue cases (predominantly during secondary dengue virus infections), and chikungunya illnesses are illustrated in the section on chikungunya. Chikungunya virus infections produce a dengue fever syndrome. Because chikungunya and the dengue viruses are transmitted by the same vector mosquitoes, the diseases frequently are observed in the same patient populations. Chikungunya illnesses begin more abruptly than dengue illnesses and are of shorter duration. Maculopapular rash, conjunctival injection, and myalgia or arthralgia occur more frequently in chikungunya than in dengue illnesses, but other features associated with both viruses are remarkably similar.[22,116]

Dengue Hemorrhagic Fever/Dengue Shock Syndrome

DHF/DSS is an acute vascular permeability syndrome accompanied by abnormal hemostasis. The incubation period of DHF/DSS is unknown but is presumed to be the same as that of dengue fever. In children, progression of the illness is characteristic.[33,115,116,177] A relatively mild first phase with an abrupt onset of fever, malaise, vomiting, headache, anorexia, and cough may be followed after 2 to 5 days by rapid deterioration and physical collapse. In Thailand, the median day of admission to the hospital after the onset of fever is day 4. In this second phase, the patient usually has cold and clammy extremities, a warm trunk, a flushed face, and diaphoresis. Patients are restless and irritable and complain of midepigastric pain. Frequently, scattered petechiae appear on the forehead and extremities, spontaneous ecchymoses may develop, and easy bruisability and bleeding at sites of venipuncture are common findings. Circumoral and peripheral cyanosis may occur. Respirations are rapid and often labored. The pulse is weak, rapid, and thready, and the heart sounds are faint. Systolic pressure may remain normal or even elevated, and the patient appears deceptively well, retaining full consciousness. Once hypotension ensues, the pulse pressure becomes narrow (<20 mm Hg) and systolic and diastolic pressure fall rapidly and may become unobtainable. The liver may become palpable two or three fingerbreadths below the costal margin and usually is firm and nontender. Chest radiographs show unilateral (right) or bilateral pleural effusions. Ultrasonographic evidence of plasma leakage may be detected in some cases within 3 days after fever onset. Pleural effusion is the most common ultrasonographic sign of plasma leakage (62% of DHF cases 1 day after defervescence). Thickening of the gallbladder wall, postvesicular edema, and ascites are detected less frequently and resolve more rapidly than pleural effusions.[153] Approximately 10 percent of patients have gross ecchymosis or gastrointestinal bleeding. During the transition from the febrile to the critical phase, it is crucial for the clinician to be aware of warning signs of impending vascular permeability: persistent vomiting, increasingly severe abdominal pain, rising hematocrit, and falling platelet count. After a 24- or 36-hour period of crisis, convalescence is fairly rapid in children who recover. The temperature may return to normal before or during the stage of shock.

PATHOLOGY AND PATHOGENESIS

In rhesus monkeys experimentally infected with dengue virus by subcutaneous inoculation, virus replicates initially in the skin. Virus disseminates rapidly to regional lymph nodes and then to lymphatic tissue throughout the body.[109] Early in the viremic period, virus can be recovered only from the skin inoculation site and lymph nodes, whereas 2 to 3 days later, evidence of general dissemination to skin and other tissues is found. Virus is recovered from the skin, lymph nodes, and several leukocyte-rich tissues for up to 3 days after termination of viremia. Virus can be recovered from circulating leukocytes and from the skin only at the end of the viremic period. The number of sites from which virus can be recovered increase as the infection progresses. Intracellular infection is terminated abruptly 2 to 3 days after viremia ceases.

Monkeys infected initially with dengue viruses 1, 3, or 4 and then with dengue virus 2 had higher viremia than when the same strain was inoculated into susceptible animals.[60,63] A similar phenomenon was observed in monkeys given diluted dengue antibodies and then challenged with dengue virus 2.[58] This phenomenon, in vivo antibody-dependent enhancement of dengue infection, provides an explanatory hypothesis of the immunopathogenesis of dengue in humans.

Models have been developed in mice that lack interferon (INF) receptors in which early infection events result in severe vascular permeability.[186,190] Of importance, dengue antibodies modulate infection in Fc-receptor–bearing cells all the way from protection to enhancement.[8,9,189] To date, mutant dengue virus 2 is required to produce DHF-like disease in immunocompromised mice

after peripheral or intravenous infection. These mutations affect replication or infection efficiency. A single amino acid change in NS4B modulates replicative efficiency, and two amino acid changes in the envelope protein affect interactions with heparan receptors for dengue infection.[45,124] The operative correlate of vascular permeability is the inoculum dose. Enhancing antibodies increase viremia and disease severity when infection is established using a low dose.

If tissue cultures or suckling mice are used for recovery of virus, dengue usually is absent in tissues at the time of death.[117] If patients experienced a second dengue infection, their tissue suspensions contain large quantities of dengue-neutralizing antibodies. The use of mosquito inoculation techniques improves viral isolation rates.[134,136,157] Genetic probes increase viral detection sensitivity still further.[80] In humans, dengue viruses infect and replicate efficiently in intracutaneous Langerhans cells in vitro and in tissue explants.[185] Virus ultimately targets liver parenchymal cells, where infection produces apoptosis, but hepatocytes may not serve as replicative hosts.[80,110] Late in infection, virus is found associated in circulating monocytes.[40] Fluorescent antibody, virus isolation, electron-microscopic, and in situ hybridization studies suggest tissue mononuclear phagocytes as major infection hosts.[8,15,60,70,85]

On pathologic examination, usually no gross or microscopic lesions are found that might account for death.[15] In rare instances, death may be caused by gastrointestinal or intracranial hemorrhage. Minimal to moderate hemorrhage is seen in the upper gastrointestinal tract, and petechial hemorrhage occurs frequently in the intraventricular septum of the heart, on the pericardium, and on the subserosal surfaces of major viscera. Focal hemorrhaging is seen occasionally in the lungs, liver, adrenals, and subarachnoid space. The liver usually is enlarged, often with fatty changes. Yellow, watery, at times blood-tinged effusions are present in serous cavities in approximately three fourths of patients. Retroperitoneal tissues are markedly edematous.

On microscopic examination, perivascular edema in soft tissues and widespread diapedesis of red blood cells can be seen. Maturational arrest of megakaryocytes may be noted in the bone marrow,[96] and increased numbers of such megakaryocytes are seen in the capillaries of the lungs, in the renal glomeruli, and in the sinusoids of the liver and spleen. Proliferation of lymphocytoid and plasmacytoid cells, lymphocytolysis, and lymphophagocytosis occur in the spleen and lymph nodes.[14] In the spleen, evidence shows infected tissue macrophages and malpighian corpuscle germinal centers are necrotic.[7,112] Depletion of lymphocytes occurs in the thymus. In the liver, widespread evidence of infection with varying degrees of fatty metamorphosis, focal midzonal necrosis, and hyperplasia of Kupffer cells present.[7,14,84] Dengue infection of hepatocytes may result in apoptosis, with evidence of assembled virions lacking.[84,110] Non-nucleated cells with vacuolated acidophilic cytoplasm resembling Councilman bodies (apoptotic hepatocytes[80] are seen in the sinusoids. A mild, proliferative glomerulonephritis is present. Biopsy specimens of the rash reveal swelling and minimal necrosis of endothelial cells, subcutaneous deposits of fibrinogen, and, in a few cases, dengue antigen in extravascular mononuclear cells and on blood vessel walls.[20]

Epidemiologic, clinical, and virologic studies of DHF/DSS in humans have shown a significant association between severe vascular permeability and dengue virus infection in the presence of circulating dengue antibody, whether it is passively acquired from the mother or actively acquired from previous infection. Risk for developing vascular permeability syndrome is inversely correlated with age; the youngest children are at highest intrinsic risk.[4,52] In humans, circulating antibody appears to have two biologic activities: neutralization of virus and enhancement of infection.[61,63] In Thailand and Vietnam, DHF/DSS developed in infants during dengue virus type 2 infection only when maternal neutralizing antibody had catabolized to low titer[27] and infection-enhancing antibodies were left in circulation.[72,92,114,149] Similarly, in a prospective study of dengue virus infection in Thai children, DHF/DSS occurred in children who had circulating enhancing antibodies from a previous single dengue virus infection, but it did not occur in children whose first infection left them with low levels of cross-reactive dengue virus type 2 neutralizing antibody at the time of the second dengue virus infection.[93] A similar mechanism explains the failure of secondary infections to produce DHF/DSS with the American genotype dengue type 2.[171] American genotype dengue 2 viruses are significantly neutralized by human anti–dengue 1, whereas Southeast Asian dengue 2 viruses are not.[94] The full-length sequences of the American and Southeast Asian dengue 2 genomes reveal few amino acid differences.[102]

In vitro studies of dengue virus type 2 demonstrated enhanced growth in cultures of human mononuclear phagocytes that were supplemented with very small quantities of dengue antibodies.[69] Dengue infection of primary human monocytes or macrophages by enhancing immune complexes increased infection productivity by 100-fold.[19] Infection of human myeloid cells by dengue virus–immunoglobulin (Ig)G antibody complexes appears to suppress innate immunity via type I INF or an alternative mechanism involving interleukin (IL)-10.[26,162] Investigators have proposed that the infected mass of mononuclear phagocytes in dengue-infected humans circulating enhancing antibodies, whether passively or actively acquired, is greater than in nonimmune individuals.[56,61,63,66] In serial blood samples taken early in the illness in children experiencing secondary dengue infections, enhanced viremia levels or enhanced levels of dengue NS1 predicted severe disease.[104,168] Because of its late appearance, vascular permeability is thought to result when infected cells are attacked by activated T lymphocytes with the release of vasoactive cytokines.[61,138] As yet, no cytokine or group of cytokines is the proved cause of vascular permeability, although blockade of tumor necrosis factor (TNF-α) prevents vascular permeability in a mouse model.[9] The reduced risk for DHF/DSS in protein-calorie malnourished children[158] and the increased risk for DHF/DSS in girls versus boys are consistent with the hypothesis that a competent immune elimination

system must be available to generate the mediators of DHF/DSS.[5,116]

Evidence indicates the existence of a human dengue resistance gene. Epidemiologic studies of the 1981 Cuban outbreak demonstrated a higher risk for DHF/DSS in white than in black individuals.[51,95] A search for DHF/DSS in black children in Haiti revealed no cases, despite the presence of high dengue type 1, 2, and 4 infection rates and circulation of the Southeast Asian genotype dengue 2 viruses.[73] Human leukocyte antigens and other genetic factors have shown differing frequencies in DHF/DSS cases and controls.[28,150] Early in the acute stage of secondary dengue virus infection, rapid activation of the complement system occurs.[18,182] During shock, blood levels of C1q, C3, C4, C5, C6, C7, C8, and C3 proactivators are depressed and C3 catabolic rates are elevated. The blood clotting and fibrinolytic systems are activated.[177,180] As yet, neither the mediator of vascular permeability nor the complete mechanism of bleeding has been identified unequivocally. The kinin system apparently is not involved. Recent studies suggest a role for TNF, IL-2, and INF-γ.[96,139] Capillary damage allows fluid, electrolytes, protein, and, in some instances, red blood cells to leak into intravascular spaces.[179] This internal redistribution of fluid, together with deficits caused by fasting, thirsting, and vomiting, results in hemoconcentration, hypovolemia, increased cardiac work, tissue hypoxia, metabolic acidosis, and hyponatremia. A mild degree of disseminated intravascular coagulation, plus liver damage and thrombocytopenia, could contribute additively to produce hemorrhage.[150]

TABLE 175D-1 WHO 1997 Case Definition for Dengue Hemorrhagic Fever and Dengue Shock Syndrome[183]

Dengue hemorrhagic fever (DHF):
- Fever, or history of acute fever, lasting 2 to 7 days, occasionally biphasic
- Hemorrhagic tendencies, evidenced by at least one of the following:
  - A positive tourniquet test
  - Petechiae, ecchymosis, or purpura
  - Bleeding from the mucosa, gastrointestinal tract, injection sites, or other locations
  - Hematemesis or melena
- Thrombocytopenia (100,000 cells per mm^3 or less)
- Evidence of plasma leakage due to increased vascular permeability, manifested by at least one of the following:
  - A rise in the hematocrit equal to or greater than 20% above average for age, sex, and population
  - A drop in the hematocrit following volume replacement treatment equal to or greater than 20% of baseline
  - Signs of plasma leakage such as pleural effusion, ascites, and hypoproteinemia

Dengue shock syndrome (DSS):

All of the above four criteria for DHF must be present and evidence of circulatory failure manifested by the following:
- Rapid and weak pulse, and
- Narrow pulse pressure (<20 mm Hg) or manifested by:
- Hypotension for age, and
- Cold, clammy skin and restlessness

WHO, World Health Organization.

DIAGNOSIS

Dengue Fever

A clinical diagnosis can be made by having a high index of suspicion and knowledge of the geographic distribution and ecology of dengue viruses. Activities of the patient during the period preceding the onset of illness may give important clues to the possibility of infection.

The differential diagnosis includes many viral, respiratory, and influenza-like diseases and the early stages of malaria, typhoid fever, scrub typhus, hepatitis, and leptospirosis. Abortive forms of these diseases may never evolve beyond a dengue-like stage. Four arbovirus diseases are similar to dengue: chikungunya and o'nyong-nyong fever (togaviruses), West Nile fever (flavivirus), and Oropouche (bunyavirus). Four other diseases are dengue-like but without rash: Colorado tick fever, sandfly fever, Ross River fever, and the mild form of Rift Valley fever. Because of the variation in clinical findings and the multiplicity of possible causative agents, the descriptive term *dengue-like disease* should be used until a specific etiologic diagnosis is provided by the laboratory.

Dengue Hemorrhagic Fever/Dengue Shock Syndrome/Severe Dengue

DHF is a dengue illness syndrome in which an acute fever is accompanied by thrombocytopenia (<100,000/mm^3), prolonged bleeding time, elevated activated partial thromboplastin time, and hemoconcentration (hematocrit >20% of the recovery value).[33,154,155] For case diagnostic criteria, see Table 175D-1. Early detection of vascular permeability remains a diagnostic problem; however, the use of strain-gauge plethysmography documents up to 50 percent higher microvascular permeability in DHF/DSS patients than in controls.[14] Pleural or peritoneal effusions observed by ultrasonography or radiography are virtually pathognomonic.[153] DSS is diagnosed when these manifestations are accompanied by hypotension or narrow pulse pressure (<20 mm Hg). In areas endemic for dengue, hemorrhagic fever should be suspected in children with a febrile illness who exhibit shock and hemoconcentration with thrombocytopenia. Hypoproteinemia, hemorrhagic manifestations, and hepatic enlargement are frequent accompanying findings. Because many rickettsial diseases, meningococcemia, and other severe illnesses caused by a variety of agents may produce a similar clinical picture, the diagnosis should be made only when epidemiologic or serologic evidence suggests the possibility of dengue. Hemorrhagic manifestations have been described in other diseases of viral origin, including the arenavirus hemorrhagic fevers of Argentina, Bolivia, and West Africa (Lassa fever); the tick-borne hemorrhagic fevers of India and the former Soviet Union; hemorrhagic fever with renal syndrome, which occurs across northern Eurasia, specifically from Scandinavia to Korea; and Marburg and Ebola virus infections in central Africa.[86]

TABLE 175D-2 WHO 2009 Dengue Case Definitions

Probable dengue:

Live in or travel to dengue-endemic area, fever, and two of the following:

- Nausea, vomiting
- Rash
- Aches and pains
- Positive tourniquet test
- Leucopenia
- Any "warning sign"

Dengue with warning signs:

- Abdominal pain or tenderness
- Persistent vomiting
- Clinical fluid accumulation
- Mucosal bleed
- Lethargy, restlessness
- Liver enlargement >2 cm
- Laboratory increase in HCT concurrent with rapid decrease in platelet count

Severe dengue (short form):

- Severe plasma leakage
  - Shock (DSS)
  - Fluid accumulation with respiratory distress
- Severe bleeding (as evaluated by clinician)
- Severe organ involvement
  - Liver AST or ALT ≥1000
  - CNS-impaired consciousness
  - Heart and other organs

Severe dengue (long form):

- There is evidence of plasma leakage, such as the following
  - High or progressively rising hematocrit
  - Pleural effusions or ascites
  - Circulatory compromise or shock (tachycardia, cold and clammy extremities, capillary refill time greater than 3 seconds, weak or undetectable pulse, narrow pulse pressure, or, in late shock, unrecordable blood pressure)
- There is significant bleeding
- There is an altered level of consciousness (lethargy or restlessness, coma, convulsions)
- There is severe gastrointestinal involvement (persistent vomiting, increasing or intense abdominal pain, jaundice)
- There is severe organ impairment (acute liver failure, acute renal failure, encephalopathy or encephalitis, or other unusual manifestations, cardiomyopathy) or other unusual manifestations.

ALT, alanine aminotransferase; *AST,* aspartate aminotransferase; *CNS,* central nervous system; *DSS,* dengue shock syndrome; *HCT,* hematocrit;

Severe Dengue

In 2009, the WHO released *Dengue: Guidelines for Diagnosis, Treatment, Prevention and Control.*[184] New case definitions were promulgated for use by clinicians and public health workers. These were intended to reduce the diagnostic requirement on laboratory studies.[183] The earlier and new WHO case definitions are listed in Tables 175D-1 and 175D-2, respectively. A useful focus of the new case definitions is the effort to alert clinicians to recognize the clinical signs of dengue vascular permeability (Dengue with Warning Signs). However, the clinical category Severe Dengue includes patients with altered levels of consciousness, severe liver disease, acute renal failure, cardiomyopathy, respiratory distress, and severe bleeding. Many of these may accompany prolonged shock or are due to comorbidity. Severe bleeding, however, does accompany dengue infections in 10 to 15 percent of cases. Particularly severe is gastrointestinal bleeding in adults with peptic ulcer disease. Treatment may require surgical intervention and aggressive replacement of blood losses. But, these patients may not have increased vascular permeability. Labeling patients who are experiencing fluid loss and blood loss shock using the same diagnostic term could lead to errors in diagnosis and management. More serious is using Severe Dengue to identify patients who are accumulating fluid and are in respiratory distress. This may confuse a common iatrogenic complication caused by excess intravenous fluid treatment with the vascular permeability shock syndrome. The new case definitions were designed to facilitate triage and better management of dengue patients, an assertion that has resulted in vigorous debate.[11,12,113,155]

LABORATORY STUDIES

An etiologic diagnosis can be made by serologic study of properly collected serum samples, by isolation of the virus, and by identification of viral RNA or the nonstructural protein NS1 in acute-phase sera.[52,121,122] The acute-phase serum or plasma collected for isolation of virus should be stored optimally at less than 65° C (149° F). Serologic diagnosis depends on a fourfold or greater increase in antibody titer by hemagglutination inhibition, complement fixation, radioimmunoassay, enzyme-linked immunosorbent assay (ELISA), or neutralization. IgM capture ELISA has revolutionized dengue serology, and commercial kits are available.[17,82,83] Primary and sequential (secondary) dengue virus infections result in the production of dengue-reactive IgM antibodies, which appear during the acute phase and disappear within 60 days of infection.[83] Secondary or primary dengue virus infections can be confirmed in a single serum specimen by quantitating IgM/IgG antibody ratios. IgG antibody concentrations are abundant in secondary but minimal in primary dengue virus infection. Dengue NS1 proteins can be detected in blood during the acute illness phase by dengue group–specific antibodies, and these have been formatted into commercial ELISA or rapid immunochromatographic tests.[16,43] By combining IgM-capture ELISA and NS1 detection, point-of-care tests are available to diagnose acute dengue infections. Accurate use of diagnostic tests requires an understanding of the kinetics of primary and secondary dengue virus infections. Dengue IgM antibodies are seldom detected until 5 or more days after onset of fever. By contrast, dengue NS1 protein circulates in blood during and shortly after febrile period, with higher detection in primary and secondary dengue infections.[16,17,81]

Numerous techniques are available for the recovery and identification of dengue viruses.[184] Recommendations for general use have been made by a WHO expert committee.[184] Diagnostic testing for dengue virus infection is available through commercial reference laboratories, selected public health laboratories, and the Dengue Branch of the Centers for Disease Control and

Prevention (http://www.cdc.gov/dengue). Acute-phase serum, mosquito suspensions, or other materials thought to contain dengue virus may be inoculated into suckling mice, which may be examined for sickness or subtle neurologic signs or challenged at 14 days with a neurovirulent dengue virus. Repeated subpassage markedly increases the neurovirulence of dengue virus. Alternatively, materials may be inoculated into any of several tissue cultures of mammalian or mosquito origin and examined for plaques under agar or methylcellulose overlay, for cytopathic effect, resistance to a challenge cytopathic virus by use of a fluid overlay, or fluorescence or other markers with the use of an appropriate detection system. Intrathoracic inoculation of *A. albopictus*, *A. aegypti*, or *Toxorhynchites* spp. is a highly sensitive dengue virus recovery system.[134,135]

TREATMENT

Dengue Fever

Treatment is supportive. Bed rest is advised during the febrile period. Antipyretics or cold sponging should be used to keep the body temperature below 40° C (104° F). Paracetamol (10 to 15 mg/kg every 4 to 6 hours) is the preferred antipyretic agent. Analgesics or mild sedation may be required to control pain. Fluid and electrolyte replacement therapy is required when deficits caused by sweating, fasting, thirsting, vomiting, or diarrhea are present. Because of the risk for Reye syndrome and the dengue hemorrhagic diathesis, aspirin should not be given to reduce fever or to control pain. Adult patients may develop severe menorrhagia or gastrointestinal bleeding during acute dengue fever. Treatment may require blood transfusions and, in the case of peptic ulcer disease, surgical intervention.

Dengue Hemorrhagic Fever (Compensated Shock)/Dengue Shock Syndrome

Explicit recommendations for management of DHF (compensated shock) and DSS have been made by a WHO expert committee.[184] These and research observations are the basis of this section.[33,38,178]

Outpatients

No specific antiviral treatment exists, but in DHF/DSS, symptomatic and supportive measures are effective.

The major pathophysiologic abnormality seen in DHF/DSS is an acute increase in vascular permeability that leads to leakage of fluid and albumin. Plasma volume studies revealed a reduction of more than 20 percent in severe cases. Supporting evidence of plasma leakage (and consequent hypovolemia) includes a rapid, weak pulse; diaphoresis; cool, pale skin of the extremities; decreased urine output; and direct measurement by strain-gauge plethysmography,[14] pleural effusion on chest radiography or ultrasonography, hemoconcentration, and hypoproteinemia. Pleural effusion may not be evident until after fluid resuscitation is started. In the absence of increased vascular permeability, clinically significant hemoconcentration may result from thirst, dehydration, fever, anorexia, and vomiting. Fluid intake by mouth should be as ample as tolerated. Electrolyte and dextrose solution (as used in diarrheal disease), fruit juice, or both are preferable to plain water. With high fever, a risk for seizures exists, so antipyretic drugs may be indicated. Salicylates should be avoided because they are known to cause bleeding and acidosis. Acetaminophen is preferable at the following doses: younger than 1 year, 60 mg per dose; 1 to 3 years of age, 60 to 120 mg per dose; 3 to 6 years of age, 120 mg per dose; and 6 to 12 years of age, 240 mg per dose.

Children should be observed closely for early signs of shock. The critical period is the transition from the febrile to the afebrile phase. Frequent hematocrit determinations are essential because they reflect the degree of plasma leakage and the need for administration of intravenous fluid. Hemoconcentration usually precedes changes in blood pressure and pulse. The hematocrit should be determined daily from the third day until the temperature becomes normal for 1 or 2 days. Patients who tolerate oral fluids and pass urine at least once in 6 hours should be sent home, but instructed to return with onset of any warning sign. Patients should be clinically monitored until after defervescence. Patients with social situations not permitting observation and those living alone or far from clinical facilities may be admitted for observation.

- Oral or parenteral fluid therapy can be administered in an outpatient rehydration unit for correction of dehydration or acidosis or when signs of hemoconcentration are present. The volume of fluid and its composition are similar to the fluids used for the treatment of diarrhea with moderate dehydration. The fluids should consist of the following:
- One third to half of the total fluid as physiologic saline solution.
- Half to two thirds of the remainder as 5 percent glucose in water.
- For acidosis: One fourth of the total fluids should be one-sixth molar sodium bicarbonate.
- Solution for fluid therapy in DHF: Lactated Ringer solution, 5 percent glucose in half normal physiologic saline solution, 5 percent glucose in one half lactated Ringer solution, 5 percent glucose in one-third physiologic saline solution.
- Fluids as listed are calculated to be given during a 24-hour period. If the child seems severely dehydrated, half the calculated fluid is given in the first 8 hours and the second half in the next 16 hours. During rapid administration of fluids, watching for signs of cardiac failure is especially important.

Written orders should be explicit about the type of solution and the rate of administration.

Inpatients

Patients should be hospitalized and immediately treated when they have any of the following signs and symptoms

of shock: restlessness or lethargy, cold extremities and circumoral cyanosis, rapid and feeble pulses, narrowing of pulse pressure (<20 mm Hg) or hypotension, and sudden rise in hematocrit or continuously elevated hematocrit despite the administration of intravenous fluid.

Shock is a medical emergency and requires access to intensive care facilities if available. A baseline hematocrit should be obtained. Immediate administration of intravenous fluid to expand plasma volume is essential. In children, shock may develop or subside during the course of a 48-hour period, so close observation 24 hours per day is imperative. Patients with similar degrees of severity should be grouped together. Those with shock require intensive 24-hour care by nurses and physicians. Paramedical workers or parents can assist in provision of oral fluid therapy or in surveillance of the rate of intravenous fluid administration and general status of the patient. Critical care management of dengue vascular permeability syndrome is a major challenge to pediatricians who care for more than half the world's children; therefore, detailed guidelines, including recognition and management of hemorrhage and of fluid overload (an all too frequent complication and cause of death), are given in the following section.

Treatment of Patients with Nonshock Dengue Hemorrhagic Fever (Compensated Shock). Treatment for patients with nonshock dengue hemorrhagic fever is as follows:

- Start intravenous fluid resuscitation with isotonic crystalloid solution at 5 to 10 mL/kg/hr over 1 hour. Then reassess the patient's condition (i.e., vital signs, capillary refill time, hematocrit, urine output).
- If the patients' condition improves, intravenous fluids should be gradually reduced to 5 to 7 mL/kg/hr for 1 to 2 hours, then to 3 to 5 mL/kg/hr for 2 to 4 hours, and then to 2 to 3 mL/kg/hr and then further depending on hemodynamic status.
- If vital signs are still unstable, check hematocrit after first bolus. If the hematocrit increases or is still high (>50%), administer a second bolus of crystalloid solution at 10 to 20 ml/kg/hr for 1 hour. After the second bolus, if there is improvement, reduce the rate to 7 to 10 mL/kg/hr for 1 to 2 hours. If the hematocrit decreases from the initial reference hematocrit, this indicates bleeding. Immediately obtain cross match and transfuse blood.
- Further boluses of crystalloid solutions may be necessary for the next 1 to 2 days.

Treatment of Patients with Shock. Treatment for patients with shock is as follows:

- For patients with narrow pulse pressure or systolic hypotension, initiate intravenous fluid resuscitation with crystalloid or colloid (gelatin has the least effect on blood coagulation, dextran 40 and 70 are in wide use) solution at 20 mL/kg as a bolus over 15 minutes to bring the patient out of shock as quickly as possible.
- If the patient's condition improves, give a crystalloid/colloid infusion of 10 mL/kg/hr for 1 hour. Then continue with crystalloid infusion and gradually reduce to 5 to 7 mL/kg/hr for 1 to 2 hours, then to 3 to 5 mL/kg/hr for 2 to 4 hours, and then to 2 to 3 mL/kg/hr or less, which can be maintained for up to 24 to 48 hours.
- If vital signs are still unstable, review the hematocrit obtained before the first bolus. If the hematocrit is low, this indicates bleeding and the need for blood transfusion.
- If the hematocrit was high in contrast to the baseline value, change intravenous fluids to colloid solutions at 10 to 20 mL/kg/hr as a second bolus given over 30 minutes to 1 hour. After the second bolus, reassess the patient. If the condition improves, reduce the rate to 7 to 10 mL/kg/hr for 1 to 2 hours, then change back to crystalloid solution and reduce the rate of infusion as mentioned earlier. If the condition is still unstable, repeat the hematocrit after the second bolus.
- If hematocrit decreases from the previous value, this indicates bleeding and the need for blood transfusion. If the hematocrit increases from the previous value or remains very high, continue colloid solutions at 10 to 20 mL/kg/hr as a third bolus over 1 hour. After this dose, reduce the rate to 7 to 10 mL/kg/hr for 1 to 2 hours, then change back to crystalloid solution and reduce the rate of infusion as mentioned earlier.
- Further boluses may need to be given during the next 24 hours. The rate and volume of each bolus infusion should be titrated to the clinical response.

General Management. If resources permit, patients with severe shock syndrome should have an arterial catheter placed. The reason for this is that in shock states, estimation of blood pressure using a cuff is commonly inaccurate. An indwelling arterial catheter allows for continuous and reproducible blood pressure measurements and frequent blood sampling on which decisions regarding therapy can be made. Monitoring of electrocardiogram and pulse oximetry should be available. Urine output should be checked regularly (i.e., hourly until patient is out of shock, then every 1 to 2 hours. Hematocrit should be monitored before and after fluid boluses until stable and then every 4 to 6 hours. In addition, there should be monitoring of arterial or venous blood gases, lactate, total carbon dioxide/bicarbonate every 30 minutes to 1 hour until stable, and blood glucose before fluid resuscitation and afterward as indicated.

Treatment of Hemorrhagic Complications

- Patients in danger of bleeding are those with prolonged or refractory shock, hypotensive shock and renal or liver failure or persistent metabolic acidosis; those who were given nonsteroidal anti-inflammatory agents; those with preexisting peptic ulcer disease; those who are on anticoagulant therapy; and those who have any form of trauma, including intramuscular injections. Patients with hemolytic conditions are at risk for acute hemolysis with hemoglobinuria and will require blood transfusion.
- Severe bleeding can be recognized by refractory shock that fails to respond to consecutive fluid

resuscitation of 40 to 60 mL/kg, hypotensive shock with low or normal hematocrit before fluid resuscitation, or persistent or worsening metabolic acidosis despite well-maintained systolic blood pressure, especially if accompanied by severe abdominal tenderness and distention.
- Give 5 to 10 mL/kg of fresh-packed red cells or 10 to 20 mL/kg of fresh whole blood at an appropriate rate and observe the clinical response.
- Consider repeating the blood transfusion if there is further blood loss or no appropriate rise in hematocrit after blood transfusion.
- There are no evidence-based data supporting the use of platelet concentrates or fresh-frozen plasma as a treatment for profound thrombocytopenia or severe bleeding. This treatment option is widely practiced in dengue-endemic countries and may contribute to fluid overload.

Recognizing Fluid Overload Begins with Knowing When to Decrease or Stop Intravenous Fluids. Intravenous fluids should be discontinued or reduced when there are signs of cessation of plasma leakage; stable blood pressure, pulse, and peripheral perfusion; hematocrit decreases in the presence of good pulse volume; no fever for more than 24 to 48 hours; resolving bowel and abdominal symptoms, and improving urine output.

Causes of fluid overload are excessive or too rapid intravenous fluids; incorrect use of hypotonic rather than isotonic crystalloid solutions; inappropriate use of large volumes of intravenous fluids in patients with unrecognized severe bleeding; inappropriate transfusion of fresh-frozen plasma, platelet concentrates or cryoprecipitates; continuation of intravenous fluids after plasma leakage has stopped; and comorbid conditions, such as congenital or ischemic heart disease and chronic lung or renal disease.

Early clinical features of fluid overload are respiratory distress, difficulty in breathing, rapid breathing, chest wall in-drawing, wheezing (rather than crepitations), large pleural effusions, tense ascites, and increased jugular venous pressure.

Late clinical features are pulmonary edema (cough with pink or frothy sputum with or without crepitations; irreversible shock (i.e., heart failure with ongoing hypovolemia); chest radiograph shows cardiomegaly, pleural effusion, upward displacement of the diaphragm by the ascites, and varying degrees of "bat wings" appearance with or without Kerley B lines suggestive of fluid overload and pulmonary edema.

Action Plan for Treatment of Fluid Overload. Treatment of fluid overload is as follows:
- Oxygen therapy should be given immediately.
- Stop intravenous fluids.
- Check physiologic status: ECG to exclude ischemic changes and arrhythmia; arterial blood gases; echocardiogram for assessment of left ventricular function and regional wall dyskinesia that may suggest underlying ischemic heart disease; and cardiac enzymes.
- If necessary, give oral or intravenous furosemide 0.1 to 0.5 mg/kg/dose once or twice daily, or a continuous infusion of furosemide 0.1 mg/kg/hr. Monitor serum potassium and correct the ensuing hypokalemia.
- Patients in shock with low or normal hematocrit levels but who show signs of fluid overload may have occult hemorrhage. Further infusion of large volumes of intravenous fluids will lead only to a poor outcome. Careful transfusion of fresh whole blood should be initiated as soon as possible. Repeated small boluses of colloid solution may be helpful.

REGULATORY MEASURES

Dengue diseases are not subject to international quarantine or surveillance regulations. Dengue is a nationally notifiable disease in the United States and suspected cases should be reported to the local health department. An intensive and effective voluntary reporting system has been devised by the regional offices of the WHO. People traveling to areas where dengue is endemic should assess the risk (http://www.healthmap.org/dengue/) and take necessary precautions to protect themselves against mosquito exposure.

PROGNOSIS

Not all patients suspected of having DHF need to be hospitalized because circulatory failure and shock may develop in only approximately a third of patients. Mild and moderate cases may be treated on an outpatient basis. For the purpose of early recognition of shock, parents should be advised to bring the patient back if evidence of clinical deterioration is noted or such warning signs as restlessness with or without lethargy, severe abdominal pain, cold extremities, and skin congestion occur on or after the third day after the onset of fever.

In most cases, early and effective replacement of lost plasma with plasma, plasma expanders, or fluid and electrolyte solutions (or any combination of these products) results in a favorable outcome. The acute onset of shock and the rapid, often dramatic clinical recovery, together with the fact that no destructive or inflammatory vascular lesions are observed, suggest that the disease is produced by transient functional vascular changes caused by short-acting pharmacologic mediators.

Sequelae in dengue and DHF have not been studied systematically. Common sequelae of mild and uncomplicated dengue virus infection include bradycardia and ventricular extrasystoles during the convalescent stage, often persisting for several weeks. Profound asthenia with or without mental depression has been described. In patients with DHF/DSS, great care must be taken to reduce use of invasive procedures for managing shock. Nosocomial infections such as gram-negative sepsis can masquerade as DHF/DSS. Overhydration during the shock resuscitation phase may lead to heart failure and a complicated,

stormy postshock stage. Infrequently, residual brain damage occurs, apparently as a result of either prolonged shock or, occasionally, intracranial hemorrhage. Children in whom profound shock develops rapidly with no detectable diastolic pressure or with unobtainable blood pressure, children in shock with delayed admission to the hospital, or children in shock with gastrointestinal hemorrhage have a poor prognosis. Mortality rates may exceed 50 percent in these groups.

PREVENTION

Three safe and immunogenic tissue culture–based vaccines for dengue virus types 1, 2, 3, and 4 are in phase II or III human clinical trials, one of which is nearing licensure.[39,50,120,159,169] The lead candidate, manufactured by Sanofi Pasteur, is a chimera of the nonstructural genes of yellow fever 17D vaccine spliced with structural genes of the four dengue viruses.[49] It is designed as a three-dose vaccine to be given at months 0, 6, and 12. At present, prophylaxis depends on killing vector mosquitoes or avoiding mosquito bites. This involves the use of insecticides, repellents, protective body clothing, and screens on houses to avoid mosquito bites. Destruction of *A. aegypti* breeding sites also is effective.[184] If water storage is mandatory, a tight-fitting lid or a thin layer of oil may prevent eggs from being deposited or hatching. A larvicide such as temefos (Abate), which is available as a 1 percent sand granule formulation and effective at a concentration of 1 ppm, may be added safely to drinking water.

Epidemic Measures

The WHO recommends integrated vector management, which requires training, preparation, and application of appropriate technologies.[184] Control of *A. aegypti* is mainly achieved by eliminating container habitats that are favorable oviposition sites permitting development of aquatic stages. In emergencies, space sprays can be applied either as thermal fogs at 10 to 50 L/ha or as ultra-low-volume applications of undiluted or slightly diluted technical-grade insecticide in the form of a cold aerosol or droplets of controlled size (15 to 25 μm) at a rate of 0.5 to 2.0 L/ha. Portable or vehicle-mounted thermal or cold-fog generators can be used for ground application. If the target area exceeds 1000 ha or cannot be covered for ground equipment within 10 days, aerial cold fog application is sometimes used. Applications vary with the susceptibility of the target species and environmental conditions, such as wind speed. Cold-fog applications from large fixed-wing aircraft are made at approximately 240 km/h and 60 m above the ground.

Eradication and Control

A. aegypti was eradicated successfully from countries and whole continents with use of the techniques pioneered by the Rockefeller Foundation in its worldwide program to control urban yellow fever.[156] With time, the species successfully reestablished itself in much of its former range. An eradication campaign in the United States was abandoned and replaced by a program of disease surveillance and containment of introduced virus.

Mosquito control or eradication programs require the simultaneous use of two approaches: a reduction in breeding sites and the application of larvicides. Alternatively, a significant reduction in population may be effected by closely spaced applications of adulticide.[46]

Source reduction requires public support by either legal sanctions or voluntary actions (see the following section). Source reduction campaigns should be well organized, supervised, and evaluated. Proper disposal of discarded cans, bottles, tires, and other potential breeding sites not used for storage of drinking or bathing water should be performed. Sides of water storage containers should be scrubbed to remove eggs when the water level is low. Water storage containers for drinking and bathing and flower vases should be emptied completely once weekly. Water containers that cannot be emptied should be treated with Abate 1 percent sand granules at a dosage of 1 ppm (e.g., 10 g of sand to 100 L of water). Treatments should be repeated at intervals of 2 to 3 months.

Vehicle-mounted or portable ultra-low-volume aerosol generators or mist blowers can be used to apply technical-grade malathion or fenitrothion at 438 mL/hectare. Three applications made at 1-week intervals can suppress *A. aegypti* populations for approximately 2 months.

In dengue-endemic countries, little effort has been made to adopt building codes or waste collection methods to reduce the number of mosquito breeding sites.[101] Furthermore, almost no way has been found to use the private sector to implement vector control despite ample evidence of long-term successful programs in the United States.[25,32]

A new generation of vector control strategies is under active development and testing. *Mesocyclops*, an antilarval insect predator, has been introduced into water storage containers in rural Vietnam with near eradication of *A. aegypti* larvae.[89,90,152,169] Infection of *A. aegypti* by *Wolbachia*, a species of rickettsial-like organism, blocks replication of dengue virus and vectoral capacity of the mosquito.[112] *Wolbachia* has been successfully introduced into wild populations of *A. aegypti* with sufficient spread to prevent dengue transmission.[77] Various methods of achieving and spreading sterile *A. aegypti* males or flightless females are being tested in the field.[44,75,181]

Health Education

Control of *A. aegypti* has been maintained effectively in some tropical areas through the simple expedient of emptying water containers once per week. During the yellow fever campaigns, strong sanitary laws made the breeding of mosquitoes on premises a crime punishable by fine or jail.[156] In the modern era, Singapore and Cuba have adopted these measures with moderate degrees of success.[119] Health education through mass media and schools has been attempted in Myanmar, Thailand, Malaysia, and Indonesia, but without spectacular success.[37] The goals of health education and community

participation approaches are to make the population aware of the identity of the vector of DHF, to describe its biting habits (daytime feeding) and its breeding habits (containers holding clean water), and to motivate people to reduce breeding sources by emptying water from containers on a regular basis.[62] The use of piped water rather than water storage should be encouraged. Studies in Malaysia after the 1973 epidemic of DHF indicated a very low level of functional knowledge among the inhabitants of Kuala Lumpur, Malaysia, about the vector of DHF.[37] Discouragingly, persons who were informed correctly, in most instances, took no action to protect themselves against mosquito breeding in their homes. Extensive effort is being made to apply social science methods to gain the voluntary participation of the population in sustained mosquito control programs.[106]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdallah TM, Ali AA, Karsany MS, et al. Epidemiology of dengue infections in Kassala, Eastern Sudan. *J Med Virol* 2012; **84**:500–3.
3. Amarasinghe A, Kuritsk JN, Letson GW, et al. Dengue virus infection in Africa. *Emerg Infect Dis* 2011;**17**:1349–54.
4. Anders KL, Nguyet NM, Chau NV, et al. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg* 2011;**84**:127–34.
6. Anonymous. Dengue fever, Cape Verde. *Wkly Epidemiol Rec* 2009;**84**:469.
8. Balsitis SJ, Coloma J, Castro G, et al. Tropism of dengue virus in mice and humans defined by viral nonstructural protein 3-specific immunostaining. *Am J Trop Med Hyg* 2009;**80**:416–24.
9. Balsitis SJ, Williams KL, Lachica R, et al. Lethal antibody enhancement of dengue disease in mice is prevented by Fc modification. *PLoS Pathog* 2010;**6**:e1000790.
11. Barniol J, Gaczkowski R, Barbato EV, et al. Usefulness and applicability of the revised dengue case classification by disease: multicentre study in 18 countries. *BMC Infect Dis* 2011;**11**:106.
12. Basuki P, Budiyanto S, Puspitasari D, et al. Application of revised dengue classification criteria as a severity marker of dengue viral infection in Indonesia. *Southeast Asian J Trop Med Public Health* 2010;**41**:1088–94.
13. Beatty ME, Stone A, Fitzsimons DW, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. *PLoS Negl Trop Dis* 2010;**4**:e890.
16. Blacksell SD, Jarman RG, Bailey MS, et al. Evaluation of six commercial point-of-care tests for the diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. *Clin Vaccine Immunol* 2011;**18**:2095–101.
17. Blacksell SD, Jarman RG, Gibbons RV, et al. Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. *Clin Vaccine Immunol* 2012;**19**:804–10.
19. Boonnak K, Dambach KM, Donofrio GC, et al. Cell type specificity and host genetic polymorphisms influence antibody dependent enhancement of dengue virus infection. *J Virol* 2011;**85**:1671–83.
21. Cardosa J, Ooi MH, Tio PH, et al. Dengue virus serotype 2 from a sylvatic lineage isolated from a patient with dengue hemorrhagic fever. *PLoS Negl Trop Dis* 2009;**3**:e423.
34. de Alwis R, Smith SA, Olivarez NP, et al. Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proc Natl Acad Sci U S A* 2012;**109**:7439–44.
39. Durbin AP, Kirkpatrick BD, Pierce KK, et al. Development and clinical evaluation of multiple investigational monovalent DENV vaccines to identify components for inclusion in a live attenuated tetravalent DENV vaccine. *Vaccine* 2011;**29**:7242–50.
42. Fox A, Hoa le NM, Simmons CP, et al. Immunological and viral determinants of dengue severity in hospitalized adults in Hanoi, Vietnam. *PLoS Negl Trop Dis* 2011;**5**:e967.
43. Fry SR, Meyer M, Semple MG, et al. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis* 2011;**5**:e1199.
44. Fu G, Lees RS, Nimmo D, et al. Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci U S A* 2010; **107**:4550–4.
45. Grant D, Tan GK, Qing M, et al. A single amino acid in nonstructural protein NS4B confers virulence to dengue virus in AG129 mice through enhancement of viral RNA synthesis. *J Virol* 2011;**85**:7775–87.
50. Guy B, Barrere B, Malinowski C, et al. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* 2011;**29**:7229–41.
75. Harris AF, Nimmo D, McKemey AR, et al. Field performance of engineered male mosquitoes. *Nat Biotechnol* 2011;**29**:1034–7.
77. Hoffmann AA, Montgomery BL, Popovici J, et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 2011;**476**:454–7.
79. Hubert B, Halstead SB. Dengue 1 virus and dengue hemorrhagic fever, French Polynesia, 2001. *Emerg Infect Dis* 2009;**15**:1265–70.
81. Humayoun MA, Waseem T, Jawa AA, et al. Multiple dengue serotypes and high frequency of dengue hemorrhagic fever at two tertiary care hospitals in Lahore during the 2008 dengue virus outbreak in Punjab, Pakistan. *Int J Infect Dis* 2010;**14**(Suppl. 3): e54–9.
82. Hunsperger EA, Yoksan S, Buchy P, et al. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. *Emerg Infect Dis* 2009;**15**:436–40.
90. Kay BH, Tuyet Hanh TT, Le NH, et al. Sustainability and cost of a community-based strategy against *Aedes aegypti* in northern and central Vietnam. *Am J Trop Med Hyg* 2010;**82**:822–30.
98. Lee IK, Liu JW, Yang KD. Clinical characteristics, risk factors, and outcomes in adults experiencing dengue hemorrhagic fever complicated with acute renal failure. *Am J Trop Med Hyg* 2009;**80**:651–5.
100. Lee VJ, Lye DC, Sun Y, et al. Decision tree algorithm in deciding hospitalization for adult patients with dengue haemorrhagic fever in Singapore. *Trop Med Int Health* 2009;**14**:1154–9.
103. Leo YS, Thein TL, Fisher DA, et al. Confirmed adult dengue deaths in Singapore: 5-year multi-center retrospective study. *BMC Infect Dis* 2011;**11**:123.
105. Liebman KA, Stoddard ST, Morrison AC, et al. Spatial dimensions of dengue virus transmission across interepidemic and epidemic periods in Iquitos, Peru (1999-2003). *PLoS Negl Trop Dis* 2012;**6**:e1472.
108. Malik A, Earhart K, Mohareb E, et al. Dengue hemorrhagic fever outbreak in children in Port Sudan. *J Infect Public Health* 2011;**4**:1–6.
109. Marchette NJ, Halstead SB, Falkler WA Jr, et al. Studies on the pathogenesis of dengue infection in monkeys. III. Sequential distribution of virus in primary and heterologous infections. *J Infect Dis* 1973;**128**:23–30.
112. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* 2009;**139**:1268–78.
113. Narvaez F, Gutierrez G, Perez MA, et al. Evaluation of the traditional and revised WHO classifications of dengue disease severity. *PLoS Negl Trop Dis* 2012;**5**:e1397.
120. Osorio JE, Huang CY, Kinney RM, et al. Development of DENVax: a chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever. *Vaccine* 2011;**29**:7251–60.
122. Pang J, Salim A, Lee VJ, et al. Diabetes with hypertension as risk factors for adult dengue hemorrhagic fever in a predominantly dengue serotype 2 epidemic: a case control study. *PLoS Negl Trop Dis* 2012;**6**:e1641.
125. Radke EG, Gregory CJ, Kintziger KW, et al. Dengue outbreak in Key West, Florida, USA, 2009. *Emerg Infect Dis* 2012;**18**:135–7.
131. Rioth M, Beauharnais CA, Noel F, et al. Serologic imprint of dengue virus in urban Haiti: characterization of humoral immunity to dengue in infants and young children. *Am J Trop Med Hyg* 2011;**84**:630–6.
133. Rodriguez-Barraquer I, Cordeiro MT, Braga C, et al. From re-emergence to hyperendemicity: the natural history of the dengue epidemic in Brazil. *PLoS Negl Trop Dis* 2011;**5**:e935.

138. Rothman AL. Cellular immunology of sequential dengue virus infection and its role in disease pathogenesis. *Curr Top Microbiol Immunol* 2010;**338**:83–98.
150. Simmons CP, Farrar JJ, Nguyen V, et al. Dengue. *N Engl J Med* 2012;**366**:1423–32.
152. Sinh Nam V, Thi Yen N, Minh Duc H, et al. Community-based control of *Aedes aegypti* by using *Mesocyclops* in southern Vietnam. *Am J Trop Med Hyg* 2012;**86**:850–9.
154. Srikiatkhachorn A, Gibbons RV, Green S, et al. Dengue hemorrhagic fever: the sensitivity and specificity of the world health organization definition for identification of severe cases of dengue in Thailand, 1994-2005. *Clin Infect Dis* 2010;**50**:1135–43.
155. Srikiatkhachorn A, Rothman AL, Gibbons RV, et al. Dengue: how best to classify it. *Clin Infect Dis* 2011;**53**:563–7.
159. Thomas SJ, Endy TP. Vaccines for the prevention of dengue: development update. *Hum Vaccin* 2011;**7**:674–84.
162. Ubol S, Halstead SB. How innate immune mechanisms contribute to antibody-enhanced viral infections. *Clin Vaccine Immunol* 2010; **17**:1829–35.
164. Vasilakis N, Cardosa J, Diallo M, et al. Sylvatic dengue viruses share the pathogenic potential of urban/endemic dengue viruses. *J Virol* 2010;**84**:3726–7; author reply 3727–8.
165. Vasilakis N, Cardosa J, Hanley KA, et al. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat Rev Microbiol* 2011;**9**: 532–41.
177. Wills B, Tran VN, Nguyen TH, et al. Hemostatic changes in Vietnamese children with mild dengue correlate with the severity of vascular leakage rather than bleeding. *Am J Trop Med Hyg* 2009;**81**:638–44.
181. Wise de Valdez MR, Nimmo D, Betz J, et al. Genetic elimination of dengue vector mosquitoes. *Proc Natl Acad Sci U S A* 2011; **108**:4772–5.
184. World Health Organization. *Dengue: guidelines for diagnosis, treatment, prevention and control*. Geneva, Switzerland: WHO; 2009.
187. Yoon IK, Rothman AL, Tannitisupawong D, et al. underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *J Infect Dis* 2012;**206**:389–98.
189. Zellweger RM, Prestwood TR, Shresta S. Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. *Cell Host Microbe* 2010;**7**:128–39.
190. Zompi S, Harris E. Animal models of dengue virus infection. *Viruses* 2012;**4**:62–82.

The full reference list for this chapter is available at expertconsult.com.

175E ■ JAPANESE ENCEPHALITIS

Theodore F. Tsai

Japanese encephalitis (JE), a mosquito-borne flaviviral infection, is the leading vaccine-preventable viral encephalitis in Asia.

HISTORY

JE first was recognized after an outbreak in Japan in 1924 led to an alarming 6125 cases of encephalitis. In retrospect, similar summer and autumn outbreaks were recognized as early as 1871 to 1873.[37] In 1933, Hayashi recovered the virus from monkeys and the brain of a patient, and in 1938, Mitamura confirmed its mosquito-borne mode of transmission by isolating the virus from *Culex tritaeniorhynchus*. Inactivated vaccine prepared from infected mouse brain was licensed in Japan in 1956, and its use successfully controlled the disease in Asian countries where it was deployed. New inactivated cell culture–derived and live attenuated vaccines are now displacing the efficacious but reactogenic 50-year-old mouse brain–derived product.[29]

ETIOLOGIC AGENT

JE virus is the prototypic member of the flaviviral antigenic complex that includes St. Louis encephalitis, Murray Valley encephalitis, and West Nile viruses. Five viral genotypes have been described. Within the last 3 decades, viruses in genotype I have displaced previously predominant genotype III strains and genotype V viruses also may be emerging.[14,15,49,68,95] Because humans are incidental to the viral transmission cycle, the changing distribution of viral genotypes indicates evolving viral adaptation to vertebrate hosts and mosquito vectors responsible for viral maintenance. However, the virus exists as a single serotype and all vaccines are made from genotype III strains, with no evidence that they fail to prevent disease caused by viruses in other genotypes.

Strains isolated from nature exhibit a range of virulence in mice. With repeated cell culture passage, biologically derived neuroattenuated strains have been produced for use as vaccines.[94] As with other flaviviruses, important biologic functions, including attenuation and viral neutralization, are associated with the E glycoprotein. Combinations of mutations in genes of the E protein, nonstructural proteins, and noncoding regions have been associated with attenuation of the live vaccine strain SA 14-14-2 and so have been selectively incorporated with others into a chimeric JE vaccine using nonstructural proteins from the itself attenuated yellow fever 17D vaccine strain.[29]

JE virus is transmitted between birds, especially egrets and herons, and *Culex* mosquitoes; however, pigs, when present, are the most important source of viral amplification (Fig. 175E-1).[3,5,79,80] Pigs maintain high sustained viremias and may be hosts to thousands of mosquitoes in a single night, thereby serving as prolific sources of infected vectors capable of transmitting the virus onward. In rural Asia, the onset of human cases each summer occurs shortly after pigs become infected, following which the epidemic curves of human and pig infections evolve in parallel.[70] However, outbreaks of JE have occurred in areas devoid of pigs, where birds, including

REFERENCES

1. Abdallah TM, Ali AA, Karsany MS, et al. Epidemiology of dengue infections in Kassala, Eastern Sudan. *J Med Virol* 2012;**84**:500–3.
2. Alvarez M, Rodriguez R, Bernardo L, et al. Dengue hemorrhagic fever caused by sequential dengue 1-3 infections at a long interval: Havana epidemic, 2001-2002. *Am J Trop Med Hyg* 2006;**75**: 1113–17.
3. Amarasinghe A, Kuritsk JN, Letson GW, et al. Dengue virus infection in Africa. *Emerg Infect Dis* 2011;**17**:1349–54.
4. Anders KL, Nguyet NM, Chau NV, et al. Epidemiological factors associated with dengue shock syndrome and mortality in hospitalized dengue patients in Ho Chi Minh City, Vietnam. *Am J Trop Med Hyg* 2011;**84**:127–34.
5. Anderson CR, Downs WG. Isolation of dengue virus from a human being in Trinidad. *Science* 1956;**124**:224–5.
6. Anonymous. Dengue fever, Cape Verde. *Wkly Epidemiol Rec* 2009; **84**:469.
7. Ashburn PM, Craig CF. Experimental investigations regarding the etiology of dengue fever. *J Infect Dis* 1907;**4**:440–75.
8. Balsitis SJ, Coloma J, Castro G, et al. Tropism of dengue virus in mice and humans defined by viral nonstructural protein 3-specific immunostaining. *Am J Trop Med Hyg* 2009;**80**:416–24.
9. Balsitis SJ, Williams KL, Lachica R, et al. Lethal antibody enhancement of dengue disease in mice is prevented by Fc modification. *PLoS Pathog* 2010;**6**:e1000790.
10. Bancroft TL. On the etiology of dengue fever. *Australasian Med Gazette* 1906;**25**:17–18.
11. Barniol J, Gaczkowski R, Barbato EV, et al. Usefulness and applicability of the revised dengue case classification by disease: multicentre study in 18 countries. *BMC Infect Dis* 2011;**11**:106.
12. Basuki P, Budiyanto S, Puspitasari D, et al. Application of revised dengue classification criteria as a severity marker of dengue viral infection in Indonesia. *Southeast Asian J Trop Med Public Health* 2010;**41**:1088–94.
13. Beatty ME, Stone A, Fitzsimons DW, et al. Best practices in dengue surveillance: a report from the Asia-Pacific and Americas Dengue Prevention Boards. *PLoS Negl Trop Dis* 2010;**4**:e890.
14. Bethell DB, Gamble J, Pham PL, et al. Noninvasive measurement of microvascular leakage in patients with dengue hemorrhagic fever. *Clin Infect Dis* 2001;**32**:243–53.
15. Bhamarapravati N, Tuchinda P, Boonyapaknavik V. Pathology of Thailand haemorrhagic fever: a study of 100 autopsy cases. *Ann Trop Med Parasitol* 1967;**61**:500–10.
16. Blacksell SD, Jarman RG, Bailey MS, et al. Evaluation of six commercial point-of-care tests for the diagnosis of acute dengue infections: the need for combining NS1 antigen and IgM/IgG antibody detection to achieve acceptable levels of accuracy. *Clin Vaccine Immunol* 2011;**18**:2095–101.
17. Blacksell SD, Jarman RG, Gibbons RV, et al. Comparison of seven commercial antigen and antibody enzyme-linked immunosorbent assays for detection of acute dengue infection. *Clin Vaccine Immunol* 2012;**19**:804–10.
18. Bokisch VA, Top FH Jr, Russell PK, et al. The potential pathogenic role of complement in dengue hemorrhagic shock syndrome. *N Engl J Med* 1973;**289**:996–1000.
19. Boonnak K, Dambach KM, Donofrio GC, et al. cell type specificity and host genetic polymorphisms influence antibody dependent enhancement of dengue virus infection. *J Virol* 2011;**85**: 1671–83.
20. Boonpucknavig S, Boonpucknavig V, Bhamarapravati N, et al. Immunofluorescence study of skin rash in patients with dengue hemorrhagic fever. *Arch Pathol Lab Med* 1979;**103**:463–6.
21. Cardosa J, Ooi MH, Tio PH, et al. Dengue virus serotype 2 from a sylvatic lineage isolated from a patient with dengue hemorrhagic fever. *PLoS Negl Trop Dis* 2009;**3**:e423.
22. Carey DE. Chikungunya and dengue: a case of mistaken identity? *J Hist Med Allied Sci* 1971;**26**:243–62.
23. Carey DE, Causey OR, Reddy S, et al. Dengue viruses from febrile patients in Nigeria, 1964-68. *Lancet* 1971;**1**:105–6.
24. Carey DE, Myers RM, Reuben R, et al. Studies on dengue in Vellore, South India. *Am J Trop Med Hyg* 1966;**15**:580–7.
25. Challet GL. Mosquito abatement district programs in the United States. *Kaohsiung J Med Sci* 1994;**10**:S67–73.
26. Chareonsirisuthigul T, Kalayanarooj S, Ubol S. Dengue virus (DENV) antibody-dependent enhancement of infection upregulates the production of anti-inflammatory cytokines, but suppresses anti-DENV free radical and pro-inflammatory cytokine production, in THP-1 cells. *J Gen Virol* 2007;**88**:365–75.
27. Chau TN, Quyen NT, Thuy TT, et al. Dengue in Vietnamese infants: results of infection-enhancement assays correlate with age-related disease epidemiology, and cellular immune responses correlate with disease severity. *J Infect Dis* 2008;**198**:516–24.
28. Chiewsilp P, Scott RM, Bhamarapravati N. Histocompatibility antigens and dengue hemorrhagic fever. *Am J Trop Med Hyg* 1981;**30**:1100–5.
29. Christie J. Remarks on "kidinga Pepo" a peculiar form of exanthematous disease. *Br Med J* 1872;**1**:577–79.
30. Chungue E, Cassar O, Drouet MT, et al. Molecular epidemiology of dengue-1 and dengue-4 viruses. *J Gen Virol* 1995;**76**:1877–84.
31. Chungue E, Deubel V, Cassar O, et al. Molecular epidemiology of dengue 3 viruses and genetic relatedness among dengue 3 strains isolated from patients with mild or severe form of dengue fever in French Polynesia. *J Gen Virol* 1993;**74**(Pt 12):2765–70.
32. Clarke JL. Privatization of mosquito control services in urban areas. *Kaohsiung J Med Sci* 1994;**10**:S74–7.
33. Cohen SN, Halstead SB. Shock associated with dengue infection. I. Clinical and physiologic manifestations of dengue hemorrhagic fever in Thailand, 1964. *J Pediatrics* 1966;**68**:448–56.
34. de Alwis R, Smith SA, Olivarez NP, et al. Identification of human neutralizing antibodies that bind to complex epitopes on dengue virions. *Proc Natl Acad Sci U S A* 2012;**109**:7439–44.
35. DeMadrid AT, Porterfield JS. The flaviviruses (group B arboviruses): a cross-neutralization study. *J Gen Virol* 1974;**23**:91–6.
36. Derrick EH, Bicks VA. The limiting temperature for the transmission of dengue. Presented at the Proceedings of the Ninth Pacific Science Congress of the Pacific Science Association; 1962. p. 40–1.
37. Dobbins JG, Else JG. Knowledge, attitudes and practices related to control of dengue hemorrhagic fever in an urban Malay kampung. *South East Asian J Trop Med Pub Health* 1975;**6**: 120–6.
38. Dung N, Day M, Tam NPJ, et al. Fluid replacement in dengue shock syndrome: a randomized, double-blind comparison of four intravenous-fluid regimens. *Clin Infect Dis* 1999;**29**:787–94.
39. Durbin AP, Kirkpatrick BD, Pierce KK, et al. Development and clinical evaluation of multiple investigational monovalent DENV vaccines to identify components for inclusion in a live attenuated tetravalent DENV vaccine. *Vaccine* 2011;**29**:7242–50.
40. Durbin AP, Vargas MJ, Wanionek K, et al. Phenotyping of peripheral blood mononuclear cells during acute dengue illness demonstrates infection and increased activation of monocytes in severe cases compared to classic dengue fever. *Virology* 2008;**376**: 429–35.
41. Effler PV, Pang L, Kitsutani P, et al. Dengue fever, Hawaii, 2001-2002. *Emerg Infect Dis* 2005;**11**:742–9.
42. Fox A, Hoa le NM, Simmons CP, et al. Immunological and viral determinants of dengue severity in hospitalized adults in Hanoi, Vietnam. *PLoS Negl Trop Dis* 2011;**5**:e967.
43. Fry SR, Meyer M, Semple MG, et al. The diagnostic sensitivity of dengue rapid test assays is significantly enhanced by using a combined antigen and antibody testing approach. *PLoS Negl Trop Dis* 2011;**5**:e1199.
44. Fu G, Lees RS, Nimmo D, et al. Female-specific flightless phenotype for mosquito control. *Proc Natl Acad Sci U S A* 2010;**107**: 4550–4.
45. Grant D, Tan GK, Qing M, et al. A single amino acid in nonstructural protein NS4B confers virulence to dengue virus in AG129 mice through enhancement of viral RNA synthesis. *J Virol* 2011;**85**:7775–87.
46. Gratz NG. Emergency control of *Aedes aegypti* as a disease vector in urban areas. *J Am Mosq Control Assoc* 1991;**7**:353–65.
47. Gubler DJ. *Aedes aegypti* and *Aedes aegypti*-borne disease control in the 1990s: top down or bottom up. Charles Franklin Craig Lecture. *Am J Trop Med Hyg* 1989;**40**:571–8.
48. Gubler DJ, Reed D, Rosen L, et al. Epidemiological, clinical, and virologic observations on dengue in The Kingdom of Tonga. *Am J Trop Med Hyg* 1978;**27**:581–9.
49. Guirakhoo F, Weltzin R, Chambers TJ, et al. Recombinant chimeric yellow fever-dengue type 2 virus is immunogenic and protective in nonhuman primates. *J Virol* 2000;**74**:5477–85.

50. Guy B, Barrere B, Malinowski C, et al. From research to phase III: preclinical, industrial and clinical development of the Sanofi Pasteur tetravalent dengue vaccine. *Vaccine* 2011;**29**:7229–41.
51. Guzman MG, Kouri GP, Bravo J, et al. Dengue hemorrhagic fever in Cuba, 1981: a retrospective seroepidemiologic study. *Am J Trop Med Hyg* 1990;**42**:179–84.
52. Guzman MG, Kouri G, Bravo J, et al. Effect of age on outcome of secondary dengue 2 infections. *Int J Infect Dis* 2002;**6**:118–24.
53. Guzman MG, Kouri G, Valdes L, et al. Epidemiologic studies on dengue in Santiago de Cuba, 1997. *Am J Epidemiol* 2000;**152**: 793–9.
54. Hahn CS, Dalrymple JM, Strauss JH, et al. Comparison of the virulent Asibi strain of yellow fever virus with the 17D vaccine strain derived from it. *Proc Natl Acad Sci USA* 1987;**84**:2019–23.
55. Halstead SB. Mosquito-borne haemorrhagic fevers of South and South-East Asia. *Bull World Health Organ* 1966;**35**:3–15.
56. Halstead SB. Observations related to pathogenesis of dengue hemorrhagic fever. VI. Hypotheses and discussion. *Yale J Biol Med* 1970;**42**:350–62.
57. Halstead SB. Etiologies of the experimental dengues of Siler and Simmons. *Am J Trop Med Hyg* 1974;**23**:974–82.
58. Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *J Infect Dis* 1979;**140**:527–33.
59. Halstead SB. Dengue haemorrhagic fever: a public health problem and a field for research. *Bull World Health Organ* 1980;**58**:1–21.
60. Halstead SB. Immunological parameters of Togavirus disease syndromes. In: Schlesinger RW, editor. *The togaviruses: biology, structure, replication*. New York: Academic Press; 1980. p. 107–73.
61. Halstead SB. The Alexander D. Langmuir Lecture. The pathogenesis of dengue: molecular epidemiology in infectious disease. *Am J Epidemiol* 1981;**114**:632–48.
62. Halstead SB. Selective primary health care: strategies for control of disease in the developing world. XI. *Dengue Rev Infect Dis* 1984;**6**:251–64.
63. Halstead SB. Pathogenesis of dengue: challenges to molecular biology. *Science* 1988;**239**:476–81.
64. Halstead SB. Epidemiology of dengue and dengue hemorrhagic fever. In: Gubler DJ, Kuno G, editors. *Dengue and dengue hemorrhagic fever*. Wallingford, UK: CAB; 1997. p. 23–44.
65. Halstead SB. Dengue in the Americas and Southeast Asia: do they differ? *Rev Panam Salud Publica* 2006;**20**:407–15.
66. Halstead SB. Dengue. *Lancet* 2007;**370**:1644–52.
67. Halstead SB. Dengue: overview and history. In: Halstead SB, editor. *Dengue*, vol. 5. London: Imperial College Press; 2008. p. 1–28.
68. Halstead SB. Dengue virus-mosquito interactions. *Annu Rev Entomol* 2008;**53**:273–91.
69. Halstead SB, O'Rourke EJ. Dengue viruses and mononuclear phagocytes. I. Infection enhancement by non-neutralizing antibody. *J Exp Med* 1977;**146**:201–17.
70. Halstead SB, O'Rourke EJ, Allison AC. Dengue viruses and mononuclear phagocytes. II. Identity of blood and tissue leukocytes supporting in vitro infection. *J Exp Med* 1977;**146**:218–28.
71. Halstead SB, Lan NT, Myint TT, et al. Infant dengue hemorrhagic fever: research opportunities ignored. *Emerg Infect Dis* 2002;**12**:1474–9.
72. Halstead SB, Nimmannitya S, Yamarat C, et al. Hemorrhagic fever in Thailand; recent knowledge regarding etiology. *Jpn J Med Sci Biol* 1967;**20s**:96–103.
73. Halstead SB, Streit TG, Lafontant JG, et al. Haiti: Absence of dengue hemorrhagic fever despite hyperendemic dengue virus transmission. *Am J Trop Med Hyg* 2001;**65**:180–3.
74. Hammon WM, Rudnick A, Sather GE. Viruses associated with epidemic hemorrhagic fevers of the Philippines and Thailand. *Science* 1960;**131**:1102–3.
75. Harris AF, Nimmo D, McKemey AR, et al. Field performance of engineered male mosquitoes. *Nat Biotechnol* 2011;**29**:1034–7.
76. He RT, Innis BL, Nisalak A, et al. Antibodies that block virus attachment to Vero cells are a major component of the human neutralizing antibody response against dengue virus type 2. *J Med Virol* 1995;**45**:451–61.
77. Hoffmann AA, Montgomery BL, Popovici J, et al. Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. *Nature* 2011;**476**:454–7.
78. Holmes EC, Twiddy SS. The origin, emergence and evolutionary genetics of dengue virus. *Infect Genet Evol* 2003;**3**:19–28.
79. Hubert B, Halstead SB. Dengue 1 virus and dengue hemorrhagic fever, French Polynesia, 2001. *Emerg Infect Dis* 2009;**15**:1265–70.
80. Huerre MR, Lan NT, Marianneau P, et al. Liver histopathology and biological correlates in five cases of fatal dengue fever in Vietnamese children. *Virchows Arch* 2001;**438**:107–15.
81. Humayoun MA, Waseem T, Jawa AA, et al. Multiple dengue serotypes and high frequency of dengue hemorrhagic fever at two tertiary care hospitals in Lahore during the 2008 dengue virus outbreak in Punjab, Pakistan. *Int J Infect Dis* 2010;**14**(Suppl. 3): e54–9.
82. Hunsperger EA, Yoksan S, Buchy P, et al. Evaluation of commercially available anti-dengue virus immunoglobulin M tests. *Emerg Infect Dis* 2009;**15**:436–40.
83. Innis BL, Nisalak A, Nimmannitya S, et al. An enzyme-linked immunosorbent assay to characterize dengue infections where dengue and Japanese encephalitis co-circulate. *Am J Trop Med Hyg* 1989;**40**:418–27.
84. Jarman RG, Holmes EC, Rodpradit P, et al. Microevolution of Dengue viruses circulating among primary school children in Kamphaeng Phet, Thailand. *J Virol* 2008;**82**:5494–500.
85. Jessie K, Fong MY, Devi S, et al. Localization of dengue virus in naturally infected human tissues, by immunohistochemistry and in site hybridization. *J Infect Dis* 2004;**189**:1411–18.
86. Johnson KM, Halstead SB, Cohen SN. Hemorrhagic fevers of Southeast Asia and South America: a comparative appraisal. *Prog Med Virol* 1967;**9**:105–58.
87. Kalayanarooj S, Vaughn DW, Nimmannitya S, et al. Early clinical and laboratory indicators of acute dengue illness. *J Infect Dis* 1997;**176**:313–21.
88. Karabatsos NE. *International catalog of arboviruses*. Ft. Collins, Colo: American Society of Tropical Medicine and Hygiene; 1985.
89. Kay BH, Nam VS, Tien TV, et al. Control of *Aedes* vectors of dengue in three provinces of Vietnam by use of *Mesocyclops* (Copepoda) and community-based methods validated by entomologic, clinical and serological surveillance. *Amer J Trop Med Hyg* 2002;**66**:40–8.
90. Kay BH, Tuyet Hanh TT, Le NH, et al. Sustainability and cost of a community-based strategy against *Aedes aegypti* in northern and central Vietnam. *Am J Trop Med Hyg* 2010;**82**:822–30.
91. Kimura R, Hotta S. On the inoculation of dengue virus into mice. *Nippon Igaku* 1944;**3379**:629–33.
92. Kliks SC, Nimmannitya S, Nisalak A, et al. Evidence that maternal dengue antibodies are important in the development of dengue hemorrhagic fever in infants. *Am J Trop Med Hyg* 1988;**38**: 411–19.
93. Kliks SC, Nisalak A, Brandt WE, et al. Antibody-dependent enhancement of dengue virus growth in human monocytes as a risk factor for dengue hemorrhagic fever. *Am J Trop Med Hyg* 1989;**40**:444–51.
94. Kochel TJ, Watts DM, Halstead SB, et al. Effect of dengue-1 antibodies on American dengue-2 viral infection and dengue haemorrhagic fever. *Lancet* 2002;**360**:310–12.
95. Kouri GP, Guzman MG, Bravo JR, et al. Dengue haemorrhagic fever/dengue shock syndrome: lessons from the Cuban epidemic, 1981. *Bull World Health Organ* 1989;**67**:375–80.
96. Kurane I, Ennis FA. Cytokines in dengue virus infections: role of cytokines in the pathogenesis of dengue hemorrhagic fever. *Semin Virol* 1994;**5**:443–8.
97. La Russa VF, Innis BL. Mechanisms of dengue virus-induced bone marrow suppression. *Baillieres Clin Haematol* 1995;**8**:249–70.
98. Lee IK, Liu JW, Yang KD. Clinical characteristics, risk factors, and outcomes in adults experiencing dengue hemorrhagic fever complicated with acute renal failure. *Am J Trop Med Hyg* 2009; **80**:651–5.
99. Lee VJ, Lye DC, Sun Y, et al. Predictive value of simple clinical and laboratory variables for dengue hemorrhagic fever in adults. *J Clin Virol* 2008;**42**:34–9.
100. Lee VJ, Lye DC, Sun Y, et al. Decision tree algorithm in deciding hospitalization for adult patients with dengue haemorrhagic fever in Singapore. *Trop Med Int Health* 2009;**14**:1154–9.
101. Lee YF. Urban planning and vector control in Southeast Asian cities. *Kaohsiung J Med Sci* 1994;**10**:S39–51.

102. Leitmeyer KC, Vaughn DW, Watts DM, et al. Dengue virus structural differences that correlate with pathogenesis. *J Virol* 1999;**73**:4738–47.
103. Leo YS, Thein TL, Fisher DA, et al. Confirmed adult dengue deaths in Singapore: 5-year multi-center retrospective study. *BMC Infect Dis* 2011;**11**:123.
104. Libraty DH, Young PR, Pickering D, et al. High circulating levels of the dengue virus nonstructural protein NS1 early in dengue illness correlate with the development of dengue hemorrhagic fever. *J Infect Dis* 2002;**186**:1165–8.
105. Liebman KA, Stoddard ST, Morrison AC, et al. Spatial dimensions of dengue virus transmission across interepidemic and epidemic periods in Iquitos, Peru (1999-2003). *PLoS Negl Trop Dis* 2012;**6**:e1472.
106. Lloyd LS, Winch P, Ortega-Canto J, et al. The design of a community-based health education intervention for the control of *Aedes aegypti*. *Am J Trop Med Hyg* 1994;**50**:401–11.
107. Lumley GF, Taylor FH. *Dengue: service publications*, vol. 3. Glebe N.S.W., Australia: University of Sydney; 1943.
108. Malik A, Earhart K, Mohareb E, et al. Dengue hemorrhagic fever outbreak in children in Port Sudan. *J Infect Public Health* 2011; **4**:1–6.
109. Marchette NJ, Halstead SB, Falkler WA Jr, et al. Studies on the pathogenesis of dengue infection in monkeys. III. Sequential distribution of virus in primary and heterologous infections. *J Infect Dis* 1973;**128**:23–30.
110. Marianneau P, Cardona A, Edelman L, et al. Dengue virus replication in human hepatoma cells activates NF-kappaB which in turn induces apoptotic cell death. *J Virol* 1997;**71**:3244–9.
111. Mattingly PF. Symposium on the evolution of arbovirus diseases. II. Ecological aspects of the evolution of mosquito-borne virus diseases. *Trans Roy Soc Trop Med Hyg* 1960;**54**:97–112.
112. Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, et al. A *Wolbachia* symbiont in *Aedes aegypti* limits infection with dengue, chikungunya, and *Plasmodium*. *Cell* 2009;**139**:1268–78.
113. Narvaez F, Gutierrez G, Perez MA, et al. Evaluation of the traditional and revised WHO classifications of Dengue disease severity. *PLoS Negl Trop Dis* 2012;**5**:e1397.
114. Nelson ER. Hemorrhagic fever in children in Thailand: report of 69 cases. *J Pediatr* 1960;**56**:101–8.
115. Nguyen TH, Nguyen TL, Lei HY, et al. Association between sex, nutritional status, severity of dengue hemorrhagic fever, and immune status in infants with dengue hemorrhagic fever. *Am J Trop Med Hyg* 2005;**72**:370–4.
116. Nimmannitya S, Halstead SB, Cohen SN, et al. Dengue and chikungunya virus infection in man in Thailand, 1962-1964. I. Observations on hospitalized patients with hemorrhagic fever. *Am J Trop Med Hyg* 1969;**18**:954–71.
117. Nisalak A, Halstead SB, Singharaj P, et al. Observations related to pathogenesis of dengue hemorrhagic fever. III. Virologic studies of fatal disease. *Yale J Biol Med* 1970;**42**:293–310.
118. Nogueira RM, Zagner SM, Martins IS, et al. Dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) caused by serotype 2 in Brazil. *Mem Inst Oswaldo Cruz* 1991;**86**:269.
119. Ooi EE, Goh KT, Gubler DJ. Dengue prevention and 35 years of vector control in Singapore. *Emerg Infect Dis* 2006;**12**:887–93.
120. Osorio JE, Huang CY, Kinney RM, et al. Development of DENVax: a chimeric dengue-2 PDK-53-based tetravalent vaccine for protection against dengue fever. *Vaccine* 2011;**29**:7251–60.
121. Pan American Health Organization. *Dengue in the Caribbean, 1977*. No. 375. Washington, D.C.: PAHO Scientific Publication; 1979.
122. Pang J, Salim A, Lee VJ, et al. Diabetes with hypertension as risk factors for adult dengue hemorrhagic fever in a predominantly dengue serotype 2 epidemic: a case control study. *PLoS Negl Trop Dis* 2012;**6**:e1641.
123. Pierson TC, Fremont DH, Kuhn RJ, Diamond MS. Structural insights into the mechanisms of antibody-mediated neutralization of flavivirus infection: implications for vaccine development. *Cell Host Microbe* 2008;**4**:229–38.
124. Prestwood TR, Prigozhin DM, Sharar KL, et al. A mouse-passaged dengue virus strain with reduced affinity for heparan sulfate causes severe disease in mice by establishing increased systemic viral loads. *J Virol* 2008;**82**:8411–21.
125. Radke EG, Gregory CJ, Kintziger KW, et al. Dengue outbreak in Key West, Florida, USA, 2009. *Emerg Infect Dis* 2012;**18**:135–7.
126. Ramirez-Ronda CH, Garcia CD. Dengue in the Western hemisphere. *Infect Dis Clin North Am* 1994;**8**:107–28.
127. Reynes JM, Laurent A, Deubel V, et al. The first epidemic of dengue hemorrhagic fever in French Guiana. *Am J Trop Med Hyg* 1994;**51**:545–53.
128. Rice L. A clinical report of the Galveston epidemic of 1922. *Am J Trop Med* 1923;**3**:73–90.
129. Rico-Hesse R. Molecular evolution and distribution of dengue viruses type 1 and 2 in nature. *Virology* 1990;**174**:479–93.
130. Rico-Hesse R, Harrison LM, Nisalak A, et al. Molecular evolution of dengue type 2 virus in Thailand. *Am J Trop Med Hyg* 1998; **58**:96–101.
131. Rioth M, Beauharnais CA, Noel F, et al. Serologic imprint of dengue virus in urban Haiti: characterization of humoral immunity to dengue in infants and young children. *Am J Trop Med Hyg* 2011;**84**:630–6.
132. Roche JC, Cordellier R, Hervy JP, et al. Isolement de 96 souches de virus dengue 2 a partir de moustiques captures en Cote d'Ivoire et Haute Volta. *Ann Virol* 1983;**143**:233–44.
133. Rodriguez-Barraquer I, Cordeiro MT, Braga C, et al. From re-emergence to hyperendemicity: the natural history of the dengue epidemic in Brazil. *PLoS Negl Trop Dis* 2011;**5**:e935.
134. Rosen L. The use of toxorhynchites mosquitoes to detect and propagate dengue and other arboviruses. *Am J Trop Med Hyg* 1981;**30**:177–83.
135. Rosen L. Use of mosquitoes to detect and propagate viruses. *Methods Virol* 1984;**8**:281–92.
136. Rosen L, Gubler DJ. The use of mosquitoes to detect and propagate dengue viruses. *Am J Trop Med Hyg* 1974;**23**:1153–60.
137. Rosen L, Shroyer DA, Tesh RB, et al. Transovarial transmission of dengue viruses by mosquitoes: *Aedes albopictus* and *Aedes aegypti*. *Am J Trop Med Hyg* 1983;**32**:1108–19.
138. Rothman AL. Cellular immunology of sequential dengue virus infection and its role in disease pathogenesis. *Curr Top Microbiol Immunol* 2010;**338**:83–98.
139. Rothman AL, Ennis FA. Immunopathogenesis of dengue hemorrhagic fever. *Virology* 1999;**257**:1–6.
140. Rudnick A. Ecology of dengue virus. *Asian J Infect Dis* 1978;**2**: 156–60.
141. Russell PK, Nisalak A. Dengue virus identification by the plaque reduction neutralization test. *J Immunol* 1967;**99**:291–6.
142. Sabin AB. The dengue group of viruses and its family relationships. *Bact Rev* 1950;**14**:225–32.
143. Sabin AB. Research on dengue during World War II. *Am J Trop Med Hyg* 1952;**1**:30–50.
144. Sabin AB, Schlesinger RW. Production of immunity to dengue with virus modified by propagation in mice. *Science* 1945;**101**: 640–2.
145. Sariol CA, Pelegrino JL, Martinez A, et al. Detection and genetic relationship of dengue virus sequences in seventeen-year-old paraffin-embedded samples from Cuba. *Am J Trop Med Hyg* 1999;**61**:994–1000.
146. Schlesinger RW, editor. *The togaviruses: biology, structure, replication*. New York: Academic Press; 1980.
147. Sheppard PM, MacDonald WW, Tonn RJ, et al. The dynamics of an adult population of *Aedes aegypti* in relation to dengue hemorrhagic fever in Bangkok. *J Anim Ecol* 1969;**38**:661–702.
148. Siler JF, Hall MW, Hitchens AP. Dengue: its history, epidemiology, mechanism of transmission, etiology, clinical manifestations, immunity, and prevention. *Philippine J Sci* 1926;**29**:1–304.
149. Simmons CP, Chau TN, Thuy TT, et al. Maternal antibody and viral factors in the pathogenesis of dengue virus in infants. *J Infect Dis* 2007;**196**:416–24.
150. Simmons CP, Farrar JJ, Nguyen V, et al. Dengue. *N Engl J Med* 2012;**366**:1423–32.
151. Simmons JS, St John JH, Reynolds FHK. Experimental studies of dengue. *Philippine J Sci* 1931;**44**:1–252.
152. Sinh Nam V, Thi Yen N, Minh Duc H, et al. Community-based control of *Aedes aegypti* by using *Mesocyclops* in southern Vietnam. *Am J Trop Med Hyg* 2012;**86**:850–9.
153. Srikiatchachorn A, Krautrachue A, Ratanaprakarn W, et al. Natural history of plasma leakage in dengue hemorrhagic fever: a serial ultrasonographic study. *Ped Infect Dis J* 2007;**26**:283–90.
154. Srikiatkhachorn A, Gibbons RV, Green S, et al. Dengue hemorrhagic fever: the sensitivity and specificity of the World Health

Organization definition for identification of severe cases of dengue in Thailand, 1994-2005. *Clin Infect Dis* 2010;**50**:1135–43.
155. Srikiatkhachorn A, Rothman AL, Gibbons RV, et al. Dengue: how best to classify it. *Clin Infect Dis* 2011;**53**:563–7.
156. Strode GK, editor. *Yellow fever*. New York: McGraw-Hill Book Company; 1951.
157. Sumarmo H, Wulur E, Jahja DJ, et al. Clinical observations on virologically confirmed fatal dengue infections in Jakarta, Indonesia. *Bull World Health Organ* 1983;**61**:693–701.
158. Thisyakorn U, Nimmannitya S. Nutritional status of children with dengue hemorrhagic fever. *Clin Infect Dis* 1993;**16**:295–7.
159. Thomas SJ, Endy TP. Vaccines for the prevention of dengue: development update. *Hum Vaccin* 2011;**7**:674–84.
160. Trent DW, Grant JA, Monath TP, et al. Genetic variation and microevolution of dengue 2 virus in Southeast Asia. *Virology* 1989;**172**:523–35.
161. Tsai CJ, Kuo CH, Chen PC, et al. Upper gastrointestinal bleeding in dengue fever. *Am J Gastroenterol* 1991;**86**:33–5.
162. Ubol S, Halstead SB. How innate immune mechanisms contribute to antibody-enhanced viral infections. *Clin Vaccine Immunol* 2010; **17**:1829–35.
163. Vasilakis N, Weaver SC. The history and evolution of human dengue emergence. *Adv Virus Res* 2008;**72**:1–76.
164. Vasilakis N, Cardosa J, Diallo M, et al. Sylvatic dengue viruses share the pathogenic potential of urban/endemic dengue viruses. *J Virol* 2010;**84**:3726–7; author reply 3727–8.
165. Vasilakis N, Cardosa J, Hanley KA, et al. Fever from the forest: prospects for the continued emergence of sylvatic dengue virus and its impact on public health. *Nat Rev Microbiol* 2011;**9**: 532–41.
166. Vasilakis N, Fokam EB, Hanson CT, et al. Genetic and phenotypic characterization of sylvatic dengue virus type 2 strains. *Virology* 2008;**377**:296–307.
167. Vaughn DW. Invited commentary: dengue lessons from Cuba. *Am J Epidemiol* 2000;**152**:800–3.
168. Vaughn DW, Green S, Kalayanarooj S, et al. Dengue viremia titer, antibody response pattern, and virus serotype correlate with disease severity. *J Infect Dis* 2000;**181**:2–9.
169. Vu SN, Nuguyen TY, Tran TP, et al. Elimination of dengue by community programs using *Mesocyclops (Copepoda)* against *Aedes aegypti* in central Vietnam. *Amer J Trop Med Hyg* 2005;**72**: 67–73.
170. Wang E, Ni H, Xu R, et al. Evolutionary relationships of endemic/epidemic and sylvatic dengue viruses. *J Virol* 2000;**74**: 3227–34.
171. Watts DM, Porter KR, Putvatana P, et al. Failure of secondary infection with American genotype dengue 2 to cause dengue haemorrhagic fever [see comments]. *Lancet* 1999;**354**:1431–4.
172. Weaver SC, Vasilakis N. Molecular evolution of dengue viruses: contributions of phylogenetics to understanding the history and epidemiology of the preeminent arboviral disease. *Infect Genet Evol* 2009;**9**:523–40.
173. Wen KH, Sheu MM, Chung CB, et al. [The ocular fundus findings in dengue fever]. *Kao-Hsiung I Hsueh Ko Hsueh Tsa Chih (Kaohsiung J Med Sci)* 1989;**5**:24–30.
174. Westaway EG, Brinton MA, Gaidamovich SY, et al. Flaviviridae. *Intervirology* 1985;**24**:183–92.
175. Wichmann O, Jelinek T. Dengue in travelers: a review. *J Travel Med* 2004;**11**.
176. Wichmann O, Gascon J, Schunk M, et al. Severe dengue virus infection in travelers: risk factors and laboratory indicators. *J Infect Dis* 2007;**195**:1089–96.
177. Wills B, Tran VN, Nguyen TH, et al. Hemostatic changes in Vietnamese children with mild dengue correlate with the severity of vascular leakage rather than bleeding. *Am J Trop Med Hyg* 2009;**81**:638–44.
178. Wills BA, Dung NM, Loan HT, et al. Comparison of three fluid solutions for resuscitation in dengue shock syndrome. *N Engl J Med* 2005;**353**:877–89.
179. Wills BA, Oragui EE, Dung NM, et al. Size and charge characteristics of the protein leak in dengue shock syndrome. *J Infect Dis* 2004;**190**:810–18.
180. Wills BA, Oragui EE, Stephens AC, et al. Coagulation abnormalities in dengue hemorrhagic fever: serial investigations in 167 Vietnamese children with dengue shock syndrome. *Clin Infect Dis* 2002;**35**:277–85.
181. Wise de Valdez MR, Nimmo D, Betz J, et al. Genetic elimination of dengue vector mosquitoes. *Proc Natl Acad Sci U S A* 2011; **108**:4772–5.
182. World Health Organization. Pathogenic mechanisms in dengue hemorrhagic fever: report of an international collaborative study. *Bull World Health Organ* 1973;**48**:117–32.
183. World Health Organization. *Dengue haemorrhagic fever: diagnosis, treatment, prevention and control*. 2nd ed. Geneva: World Health Organization; 1997.
184. World Health Organization. *Dengue: guidelines for diagnosis, treatment, prevention and control*. Geneva: World Health Organization; 2009.
185. Wu SJ, Grouard-Vogel G, Sun W, et al. Human skin Langerhans cells are targets of dengue virus infection. *Nat Med* 2000;**6**: 816–20.
186. Yauch LE, Shresta S. Mouse models of dengue virus infection and disease. *Antiviral Res* 2008;**80**:87–93.
187. Yoon IK, Rothman AL, Tannitisupawong D, et al. Underrecognized mildly symptomatic viremic dengue virus infections in rural Thai schools and villages. *J Infect Dis* 2012;
188. Young PR, Hilditch PA, Bletchly C, et al. An antigen capture enzyme-linked immunosorbent assay reveals high levels of the dengue virus protein NS1 in the sera of infected patients. *J Clin Microbiol* 2000;**38**:1053–7.
189. Zellweger RM, Prestwood TR, Shresta S. Enhanced infection of liver sinusoidal endothelial cells in a mouse model of antibody-induced severe dengue disease. *Cell Host Microbe* 2010;**7**:128–39.
190. Zompi S, Harris E. Animal models of dengue virus infection. *Viruses* 2012;**4**:62–82.

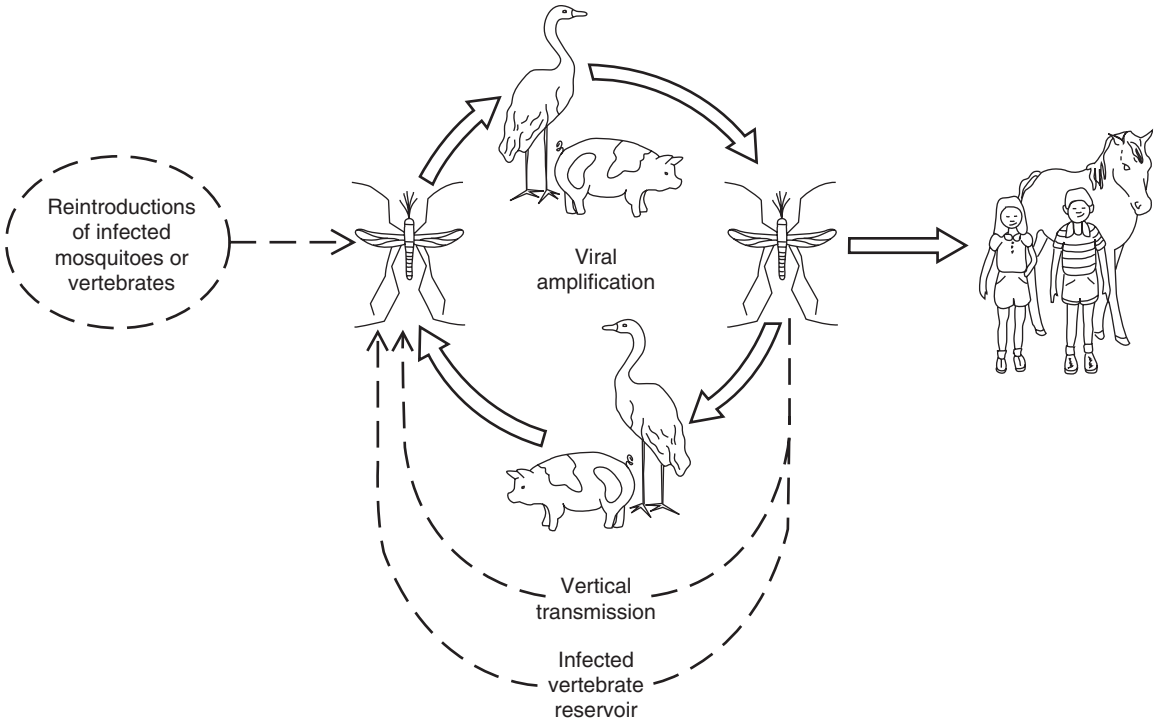

FIGURE 175E-1 ■ Transmission cycle of Japanese encephalitis virus. Speculative portions of the cycle are shown in *broken lines.*

ducklings, pigeons, sparrows, and possibly other small birds found near human residences, have served as amplifying hosts; furthermore, transmission in other sylvatic cycles is possible.[3,5,25,79]

C. tritaeniorhynchus is the major mosquito vector in most areas of Asia, and ecologic modeling of its predicted distribution in niches below 1000 m elevation (valleys in Tibet and Kathmandu are conspicuous exceptions), along with other environmental and climatic features, corresponds well to the distribution of human cases.[60,80] That distribution also overlaps the land cover used for rice paddies. *C. tritaeniorhynchus* exploit ground pools and especially rice paddies in their preadult stages, emerging in immense numbers from the flooded rice paddies responsible for 80 percent of the land cover in some areas and that frequently surround individual residences and villages. The vector is zoophilic and prefers to feed on large animals such as pigs and less so on humans, but with the custom of keeping pigs near or inside houses in rural Asia, residents live in the midst of the viral transmission cycle and are at high risk to be exposed to infected mosquitoes, starting at an early age. Living in proximity to a rice field and pig ownership understandably are risk factors for acquiring the illness.[55] Although *C. tritaeniorhychnus* is most active in the evening and night, because adults feed outdoors, bed nets are not entirely protective.

In various regions, other species *(Culex pseudovishnui*, *Culex vishnui*, *Culex gelidus*, *Culex fuscocephalus*, and *Culex bitaeniorhynchus)* with different habitats also may serve as vectors.[3,80] *Culex annulirostris* is the principal vector in northern Australia and the Pacific, and anopheline mosquitoes may contribute to transmission in northeastern India. The viral overwintering mechanism for JE virus has been a matter of speculation; however, vertical infection of mosquito eggs has been demonstrated as a means by which transmission is reestablished.[23,80] Viral persistence in local mammalian reservoirs such as bats, and reintroduction from external sources by migrating birds or windblown mosquitoes also may contribute to annual outbreaks and, the latter, to geographical spread of the virus.[5]

Domestic animals such as dogs and cattle can be infected, but their viremias are insufficient to support further transmission. Because they are attractive hosts to JE vectors, they may divert mosquitoes from humans, thereby protecting them from infection (zooprophylaxis). Horses can develop encephalitis, and outbreaks and economically significant loss of prize horses have led to routine use of equine vaccines in China, Mongolia, and Japan. Sows also are vaccinated, not to prevent illness in the animals, which remain asymptomatic after infection, but to prevent congenital infection of piglets, which may die in utero or may be born with lethal neurologic malformations.

Ecologic factors have been modeled to describe the seasonality of infection and to predict the occurrence and severity of outbreaks; surveillance tools include monitoring infections in pigs, birds, dogs, and horses, as well as precipitation and temperature. The potential impact of global climate on JE seasonality has been speculated, because warmer environmental temperature shortens the extrinsic incubation period of related flaviviruses, thereby speeding the transmission cycle. In support of this hypothesis, a secular trend toward an earlier onset of JE transmission in Taiwan has been noted, from June to

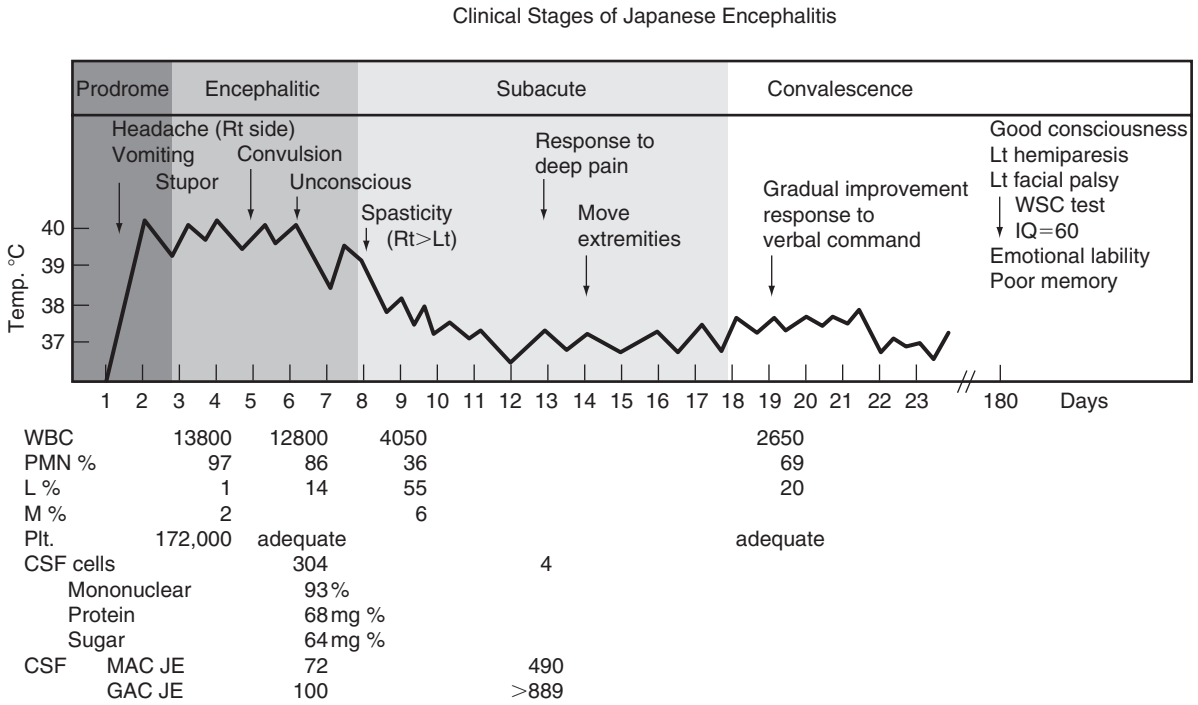

FIGURE 175E-2 ■ Clinical stages of a typical Japanese encephalitis case.

May, while high summertime temperatures have been associated with the occurrence and severity of West Nile and St. Louis encephalitis outbreaks.

EPIDEMIOLOGY

The disease occurs mainly in rural locations, where high levels of virus transmission lead to infection at an early age. In areas where children are not routinely vaccinated, cases occur mainly in children after the first year of life, with a slight preponderance in boys. More than 99 percent of infections are subclinical, and cumulative exposure with age leads to seroprevalence rates of 80 percent or more by adulthood.[27] In Japan and other developed Asian countries, where children are protected by routine vaccination, adult cases now predominate.[2,43,48] A majority of cases occur in adults over 45 years of age, because of immunosenescence and age-related chronic diseases that may be risk factors.

Transmission is seasonal, during late summer and early fall in temperate regions (July to October) and over a longer interval in southern China and Southeast Asia (April to November).[95] Transmission patterns are more complex in tropical areas, where mosquitoes proliferate after monsoon rains, and multiple epidemic seasons or year-round transmission is possible in some tropical locations. Although mosquito abundance usually corresponds to the rainy season, in many locations, vector populations now follow irrigation-controlled schedules of rice field flooding and other patterns dictated by mechanized rice production.[67]

JE transmission is endemic in nearly every country in Asia (Fig. 175E-2), with an estimated 60,000 cases reported annually from the region.[10] Annual incidence rates range widely from less than 0.01 to 5/100,000, depending on vaccine coverage and environmental conditions, and epidemic attack rates as high as 100/100,000 have been described. Incidence is underestimated because the unavailability of laboratory confirmation limits specific diagnosis and surveillance. Rare outbreaks have occurred in Oceania, on Guam and Saipan in 1949 and 1989, respectively, and in far northern Australia in 1995 and 1998.[31,69] These self-limited outbreaks occurred after the virus was introduced by migrating birds or other mechanisms, coupled with unusual conditions of human activity or weather patterns, but transmission was not sustained after the exhaustion of susceptible hosts. Establishment of JE virus on the Australian mainland appears to have been limited by the zooprophylaxis provided by dead-end hosts, as well as an immune barrier resulting from infections with other flaviviruses.[90] Few cases are reported from countries where vaccination rates are high (e.g., Japan, Korea, and Taiwan) and where development through urbanization, decreased land under cultivation, and improved standards of living have reduced human exposure. The use of agricultural pesticides and centralized pig rearing also may have contributed to lowering risk. However, the small number of human cases belies the persistence of enzootic viral transmission; for example, in Japan, more than 80 percent of pigs and 2.6 percent of the human population still are infected each year.[2,44]

Although economic development has paralleled a decline in JE, in some areas, development in the form of deforestation, construction of dams, and irrigation schemes has led to increases in transmission of JE virus or its emergence in areas where the disease had not occurred previously. Examples include development

projects in the Terai in southern Nepal and the Mahaweli Valley in Sri Lanka, where JE and malaria have become hyperendemic after large-scale programs of deforestation and agricultural development.

Travelers to areas where JE is endemic may be at risk for acquiring the illness.[33] Updated information may be obtained from the Centers for Disease Control and Prevention (CDC) at the website http://www.cdc.gov/travel/. Approximately 80 cases have been reported in travelers and expatriates (including military personnel and their family members) from North America, Europe, Russia, and Australia in the past 20 years, but the number undoubtedly is larger because of underreporting. The risk for acquiring the disease can best be described as serendipitous—cases have been reported in travelers who were infected within days of arriving in Asia, who never visited a rural area, and who had none of the risk issues cited in recommendations. The generally low but difficult-to-predict risk can be understood by factoring the probability of development of an illness after a single mosquito bite—only certain vector species transmit the virus, typically less than 3 percent of vector mosquitoes are infected, and only one in several hundred infections leads to clinical illness. Other factors entering into the risk for acquiring infection and illness are described in Table 175E-1)

CLINICAL MANIFESTATIONS

Because the overwhelming majority of infections are inapparent or manifested as mild self-limited illnesses, occasional cases of JE viremia are identified in serologic studies of children with undifferentiated fever, which might otherwise have been diagnosed clinically as dengue or another viral syndrome. However, patients who develop neurologic infection usually are profoundly ill and 5 to 25 percent of cases are fatal. After an incubation period of 4 to 14 days, the illness begins with lethargy, nausea or abdominal pain and vomiting, headache, and feverishness (Fig. 175E-3).[4,45,46,52,78] The prodrome is followed over several days by increasing lethargy or uncharacteristic patterns of behavior and motor disturbances, leading eventually to deep depression of consciousness and coma. The initial presentation varies, however; in some cases, it may be a sudden seizure, whereas in others, there may be a long prodrome of a week or more, with periods of confusion or agitation and unsteadiness of gait and other motor abnormalities. Unusual manifestations, such as acute psychosis and Guillain-Barré syndrome, also have been reported; the latter enter into the diagnosis of acute flaccid paralysis in areas of Asia where acute flaccid paralysis surveillance for polio still is maintained.[77]

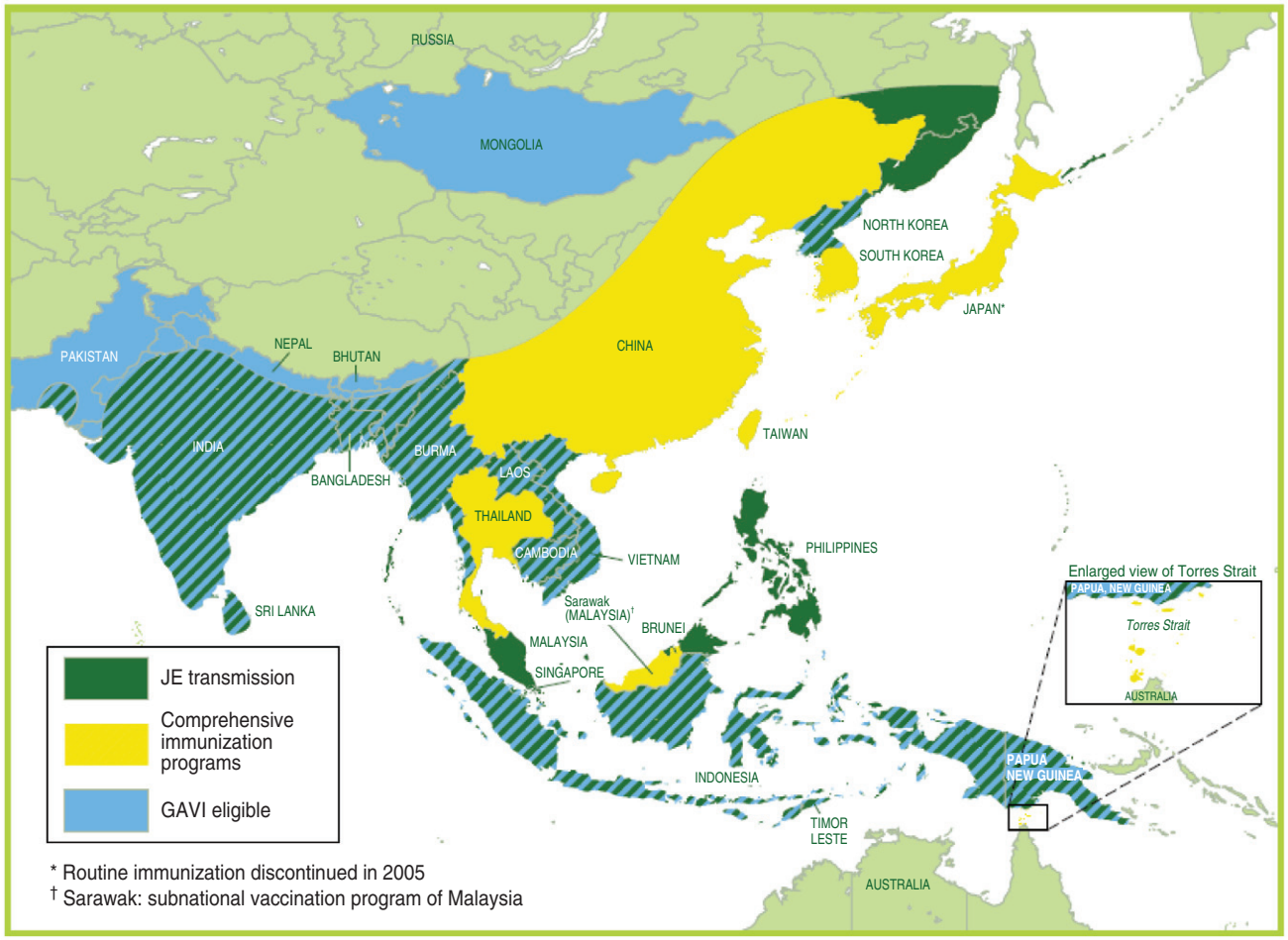

FIGURE 175E-3 ■ Locations where Japanese encephalitis is prevalent. (See companion Expert Consult website for color version.)

TABLE 175E-1 **Factors to Consider in Recommending Japanese Encephalitis Vaccine for Travelers to Asia**

Factor	Comment
Expatriates	During extended assignments, expatriates likely will make unanticipated trips within the region, including to areas where the risk for acquiring Japanese encephalitis (JE) is high or unknown. In many countries, local companions will have been vaccinated and/or will have naturally acquired immunity; an expatriate child could be alone in being unprotected among the local population if unvaccinated.
Visiting friends or relatives	Returning families may be more likely to visit ancestral villages or other locations that pose a greater risk for viral transmission. Parents may have been vaccinated or have acquired natural infections before they emigrated and may not realize that their own children are unprotected.
Travelers with open itineraries	Wanderjahr and other travelers who have open itineraries may journey to locations where risk for disease is higher, including rural areas.
Frequent travelers	Travelers who have second homes in the region or who travel frequently to Asia, even if individual trips are brief, have a cumulative risk for exposure that may justify vaccination, particularly because seroprotective antibodies may persist for years after one booster immunization.
Season	Only generalizations can be made about the seasonality of transmission within the region. In East Asia, which has a temperate or subtropical climate, transmission usually is described as extending from April to October, but within individual countries even the size of Thailand, transmission periods may differ by several months from north to south, or, as in China, from West to East. In the tropical zone, the virus may be transmitted year-round with superimposed patterns of higher risk. In many locations, seasonal monsoons dictate the periods when mosquito vectors are prevalent and those patterns can differ within surprisingly delimited geographic bounds, such as for Southeastern versus Southwestern India. Historical seasonal patterns may not be predictive in any given year. As rice cultivation has become more industrialized, the timing of rice field flooding and resulting blooms of vector mosquitoes may not correspond only to natural patterns of rainfall.
Activity out of doors	Persons with occupational exposure outdoors or whose activities during travel take them outdoors, especially at twilight and evening, are more likely to be exposed to the principal JE mosquito vectors, which are mainly exophilic (prefer to bite outdoors) and crepuscular (most active at twilight and evening). In contrast, the principal dengue vector, *Aedes aegypti*, is frequently encountered indoors and is active during the day. Even brief episodes outdoors (e.g., an elephant ride, have been reported in case histories of travel-associated JE).
Exposure to vector mosquito habitat	Most recommendations cite "rural exposure" as a risk factor for acquiring JE. However, "rural" is used in this context as a proxy for exposure to rice paddies that are highly productive habitats for JE vectors. Rice paddies, however, are found at the edges or even within major Asian cities (e.g., in Denpasar, Indonesia, the city greenbelts are rice fields). Moreover, other pools of water (e.g., flooded ditches) can support species capable of transmitting JE virus; thus infected mosquitoes and seroconverting sentinel animals have been detected in urban or suburban surveillance sites. Sporadic cases in local residents and cases in travelers have occurred in persons who stayed exclusively in cities. A 2.6% incidence of natural infection is estimated in the population of Japan, an industrialized country with shrinking agricultural lands.
Exposure to pigs	Because pigs are highly important in amplifying JE virus in the usual transmission cycle, locations with few or no pigs may pose a lower risk for encountering viral-infected mosquitoes. However, windblown mosquitoes can travel miles in a single evening. In addition, birds, ducks, and potentially other vertebrates, also can amplify the virus and have been implicated in outbreaks.
Age	Infants and older adults are at an increased risk for developing neurologic disease after infection in contrast to young adults. This epidemiologic pattern of age-specific risk is seen in nearly every other flaviviral infection that causes encephalitis, including St. Louis encephalitis, West Nile encephalitis, tick-borne encephalitis, and Rocio encephalitis.
Chronic disease	Immunocompromising conditions, including HIV infection, increase risk for illness. Thymus disorders and thymectomy have been associated with increased risk for vaccine-associated disease following yellow fever (another flavivirus) vaccination. Other chronic diseases, including cardiovascular disease, hypertension, and metabolic conditions have been associated with risk for illness or increased severity of illness for other flaviviruses. As observational and experimental data indicate that certain underlying neurologic diseases (e.g., neurocysticercosis and other concurrent neurologic infections) can facilitate neuroinvasion, it seems possible that CSF shunts, cochlear implants, baclofen pumps, and other implanted devices as well as other neurologic disorders could pose a similar risk.
Pregnancy	JE acquired in pregnancy has been associated with miscarriage and fetal death. Data are insufficient to assess risk by stage of pregnancy. No safety or immunogenicity data are available for use of the only available JE vaccine in the United States, an inactivated cell culture–derived vaccine, in pregnant women (Pregnancy category C).

Continued on following page

TABLE 175-E1 **Factors to Consider in Recommending Japanese Encephalitis Vaccine for Travelers to Asia** (Continued)

Factor	Comment
Genetic factors	Several studies suggest that persons homozygous for *CCR5*Δ32 are at increased risk for clinical illness from the flaviviruses, West Nile virus and tick-borne encephalitis virus. This deletion is so uncommon in Asia that it is unlikely to be unimportant as a risk factor among the indigenous population; however, it is a consideration for persons with other ancestries.
Concurrent medication	Because maraviroc is an inhibitor for CCR5, treated patients hypothetically may be at increased risk for developing JE or for more severe illness.
Previous flaviviral infection or vaccination	No protection has been confirmed from either yellow fever or tick-borne encephalitis vaccination. The contribution of previous dengue infection in modifying risk for JE is unclear, despite the presence of cross-reactive antibodies that can be demonstrated in dengue patients.
Vaccine safety	The only available JE vaccine in the United States, an inactivated Vero cell–derived vaccine, is well-tolerated and has not been associated with any safety issues since its licensure in the United States, Europe, and Australia in 2009. Studies of the vaccine that included a hepatitis A vaccine comparator indicate a similar level of acute safety.
Vaccine cost	As a rule, travel vaccines are not reimbursed by health insurance schemes, so the decision to take the JE vaccine is a decision for an out-of-pocket discretionary "purchase." Therefore, highly personal factors, which should be explored and respected, may enter into that decision. Individual judgments on events with great impact or that are potentially catastrophic, such as JE, may be assessed by certain individuals as more important to prevent than more mundane but more likely risks.

The principal physical findings are high fever and alterations in consciousness, ranging from mild mental clouding, frank disorientation and delirium, to coma. Some children exhibit bizarre behavior, including mutism, shouting, spitting, and personality changes. Signs of meningeal irritation are present. Cranial nerve palsies, mainly disconjugate gaze and central facial paralysis, are observed in a third of cases, and generalized or focal motor weakness can usually be demonstrated, presenting with either flaccid or spastic paralysis. Some patients exhibit erratic flailing movements, tremor, rigidity, expressionless facies, or thick slurred speech; frequently, choreoathetosis and other extrapyramidal signs become evident only in the second week of illness. Focal or generalized seizures develop in 50 to 75 percent of patients. Papilledema is seen in 10 percent of cases, along with hypertension, indicating increased intracranial pressure. In more than a third of patients, coma and respiratory failure necessitate ventilatory support. Patients with fulminant infections may die within the first 5 days of illness.

Recovery is slow, with defervescence of fever followed by gradual improvement of neurologic function over several weeks. Convalescence frequently is complicated by stasis ulcers, urinary tract infections, pneumonia, and bacteremia that may be secondary causes of death. In anecdotal cases, a biphasic pattern of illness has been described, with a recurrence of motor and behavioral abnormalities 2 to 5 weeks after initial improvement, accompanied by larger and more numerous associated lesions on magnetic resonance imaging (MRI). Recovery of motor function and adaptation to sequelae may progress over a period of months to years, although in some cases, residual neurologic abnormalities may not declare themselves until after hospital discharge.

Routine laboratory studies initially show a peripheral leukocytosis, often with a left shift and a total leukocyte count as high as 30,000/mm^3. Opening pressures on lumbar puncture usually are normal or slightly elevated. Cerebrospinal fluid (CSF) pleocytosis ranges from fewer than 10 cells to several thousand, with a median of several hundred per cubic millimeter, potentially leading to a misdiagnosis of bacterial meningitis. Lymphocytic pleocytosis follows, and CSF glucose and protein levels frequently are normal (80% of cases); when elevated, protein levels rarely exceed 100 mg/dL.

Electroencephalograms typically show diffuse theta to delta wave slowing, whereas spike and seizure discharges are relatively uncommon. MRI is more sensitive using T2-weighted and fluid attenuation inversion recovery (FLAIR) sequences in early stages of illness, with a relatively lower sensitivity from diffusion-weighted signals.[30,59] A distinct pattern of hyperintensity in the thalamus, basal ganglia, and brain stem has been noted, although cortical, cerebellar, and spinal cord involvement also are seen. JE-associated abnormalities have been reported to appear more frequently in the ipsilateral hemisphere affected by neurocysticercosis-related changes. Electromyograms show a neurogenic pattern consistent with anterior horn cell involvement.[86] Central motor conduction times are prolonged, indicative of diffuse subcortical damage.

PATHOLOGY

Although JE presents clinically as a central nervous system (CNS) infection, often overlooked is that the infection is pantropic, with pathologic changes found in the lungs and viscera, in addition to the brain.* On gross examination, the brain appears swollen and the meninges

*References 20, 35, 38, 50, 84, 96.

may be congested with macroscopically visible punctate hemorrhages. Microscopic examination discloses a moderate inflammatory response in the meninges. The parenchyma shows foci of neuronal degeneration and parenchymal and perivascular inflammatory responses principally in the thalamus and brain stem, but also more widely, in the hippocampus, temporal cortex, cerebellum, and spinal cord. Areas of neuronophagia may be surrounded by microglial nodules, but sharply defined round areas of softening or necrolysis without an inflammatory response also are seen. Purkinje cells and cerebellar glial shrubs also may be lost.[96]

PATHOPHYSIOLOGY

After virus is introduced by a mosquito bite, replication locally and within regional lymphatic tissue leads to a secondary amplified viremia and disseminated infection that includes the CNS. Neuroinvasion is thought to occur through cerebral capillaries as virions cross from the vascular side of the endothelial cell to the perivascular space, with subsequent neuronal infection, or by infected inflammatory cells crossing the brain barrier. Neurons show evidence of viral antigen in the cell body, axons, and dendrites, indicating cell-to-cell viral spread within the brain.[19,40,49] CSF levels of catecholamines and their metabolites are acutely lowered.[41] Infiltrating T cells elicit a broad inflammatory response, with B cells, T cells, and macrophages found in perivascular cuffs and macrophages and T cells in the parenchyma.[40,92] Neuronophagia proceeds with the formation of microglial nodules and the eventual disappearance of neurons, leaving ghostlike remnants with antigen accumulated within macrophages.

Relatively low levels of circulating neutralizing antibodies, approximately 1:10, correlate with protection and the rapidity of the neutralizing antibody response in naïve individuals is a principal determinant of outcome.[8,9,40,64] Most fatal cases occurring within approximately 5 days after the onset of illness have no detectable CSF antibody response while virus is recoverable from the CSF, indicating unimpeded viral replication.[8,9] In experimentally infected animals, passive immunization reduces mortality, even when it is given 4 to 5 days after inoculation.[64] However, the persistence of viral antigen in neurons for extended periods in the presence of intrathecal antibody and immune complexes in some cases, and development of antineurofilament antibodies, indicate the importance of viral clearance and potential for immunopathology in delayed or incomplete resolution of illness.[19,20] The role of innate immune responses is not well characterized, but they may contribute toward both control of infection and immunopathology.[89,92] The contribution of interferon and CCR5 ligands in susceptibility may be inferred from studies of other flaviviral infections that associated risk with oligoadenylate synthetase polymorphisms and *CCR5*Δ32 gene deletions.[8,51] Elevated CXCL8 levels in serum and CSF have been associated with increased disease severity and duration.

Why most JE virus infections are subclinical or lead to no signs of infection in the CNS is unclear. Epidemiologic observations indicate an elevated risk for JE in the elderly and increased severity in young children, but the biologic basis for age-related susceptibility remains poorly understood. Tumor necrosis factor–α promoter polymorphism also has been suggested as a specific risk factor.[74] Pathologic observations have shown a higher prevalence of neurocysticercosis in fatal JE cases than in deaths from other causes, which suggests that physical or physiologic disruption of the brain architecture by infection or other mechanisms could facilitate neuroinvasion (see earlier discussion).[18,54,86] Experimental dual infection of animals with JE virus and other agents supports this hypothesis. In experimental animals, other host factors associated with a risk for acquiring illness and poor outcome include flavivirus-specific susceptibility genes, age, levels of sex hormones, and cold and stress responses. Because dengue infections are prevalent across much of the JE-endemic region, the role of cross-reactive flaviviral immunity in the clinical outcome of JE is of considerable interest; unfortunately, no clear pattern of protection or increased risk has been confirmed.[1,24]

COMPLICATIONS

The principal risk is secondary bacterial infection occurring in the acute and subacute phases of illness. In addition, concurrent malaria and other parasitic or bacterial infections that are endemic in Asia may complicate management. Although JE occurs in areas of Asia where human immunodeficiency virus (HIV) infection is prevalent, data are insufficient to conclude differences in outcome in coinfected patients. Stress-induced gastrointestinal hemorrhage and hyponatremia caused by inappropriate antidiuretic hormone secretion also can complicate treatment.

Interestingly, cases of clinical relapse with seizures, coma, and weakness, several occurring 6 to 9 months after recovery from the acute illness, have been reported.[73,76] These and other patients experiencing relapse had evidence of persistent JE virus infection in peripheral blood mononuclear cells.[85] A study of 253 patients found laboratory evidence of subacute CNS infection in 5 percent of cases, with persistent intrathecal production of JE virus–specific immunoglobulin (Ig)M beyond 50 to 180 days or CSF containing JE virus antigen or virus more than 3 weeks after recovery.[76] These observations suggest that some infections may be followed by viral persistence but additional confirmatory studies are needed.

JE acquired during the first two trimesters of pregnancy may lead to fetal infection and miscarriage, which has been confirmed by viral isolation from products of conception in a few cases.[13,57] Infections acquired during the third trimester, however, have not been associated with adverse outcomes to the pregnancy. It is still unknown whether sublethal malformations can result from congenital infection and whether subclinical infections acquired during pregnancy pose a risk for fetal infection. Because JE virus is a proved teratogen in pigs, producing fetal death and CNS malformations, there is a need for closer studies in humans.

LABORATORY DIAGNOSIS

A specific diagnosis can be made serologically by identifying JE virus–specific IgM antibody in serum or CSF by enzyme-linked immunosorbent assay (ELISA); often, however, results need to be confirmed by demonstrating fourfold titer changes in neutralizing antibodies between acute-phase and convalescent-phase serum samples to resolve cross reactions to dengue and other flaviviruses. Several IgM detection kits exhibiting good sensitivity and specificity are commercially available.[61,75] IgM can be detected in serum or CSF (or in both) in nearly all cases by 1 week after the onset of illness. Detection of NS1 in CSF also has been reported to augment the sensitivity of serologic diagnosis. Although real-time polymerase chain reaction (PCR) and reverse transcription–loop-mediated isothermal amplification (RT-LAMP) assays have been described, their limited availability in the endemic situation has limited opportunities for evaluation. Available reports indicate that as a result of the sensitivity of these methods, both acute serum samples and CSF should be tested, because viremia may be detected early in infection in the preneuroinvasive phase of illness, although usually no later than 6 to 7 days after onset. Viral infection frequently is cleared by the time lumbar punctures are performed; therefore CSF should be tested both for IgM and by PCR to maximize the chances of making a specific diagnosis.

Virus can be recovered from brain biopsy and autopsy material by intracerebral inoculation of baby mice and various cell cultures, such as primary chick or duck embryo cells, and in Vero, LLCMK-2, C6-36, and AP-61 cell lines.

DIFFERENTIAL DIAGNOSIS

Other mosquito-borne arboviruses that also cause encephalitis—West Nile, Banna, and Chandipura viruses—and that, in some circumstances, are transmitted by the vectors that also spread JE virus, are frequently mixed among JE cases in seasonal endemic and even epidemic settings.[28] As specific diagnostic assays are even less accessible for these infections than for JE virus itself, they may be responsible for some of the overall burden of seasonal encephalitis syndrome cases currently attributed to JE. Infections caused by West Nile, and also, dengue virus, which frequently produces encephalopathy, are particularly difficult to differentiate by routine serologic methods because of the antigenic relatedness of the viruses. JE has been misdiagnosed as tubercular or pyogenic meningitis, but more often cannot be differentiated clinically from other causes of viral encephalitis.[47,66] Enterovirus 71, Nipah virus, and hepatitis E virus—the latter two associated with pig contact—are transmitted in Asia in epidemiologic contexts similar to those of JE and can present with CNS signs and symptoms. Other infections that may enter the differential diagnosis include typhoid fever, which can present with tremor and lethargy similar to the prodrome of JE, and scrub typhus, which rarely can present with meningitis, although rash, adenopathy, and an eschar, if present, are helpful diagnostic signs. Acute encephalitis with seizures is encountered in two thirds of patients with neurocysticercosis, a prevalent chronic condition in some areas of Asia that may increase the risk for acquiring JE (see the discussion on pathophysiology). Where poliomyelitis still has not been confirmed to be eradicated, JE should be included among the possible causes of acute flaccid paralysis.

Noninfectious causes of acute encephalopathy to be considered include heat stroke, autoimmune encephalitis, vascular occlusion and intracranial hemorrhage, acute electrolyte disturbances, Reye syndrome, lead encephalopathy and other poisonings (including insect repellent toxicity), and inherited metabolic disorders.

TREATMENT

No specific antiviral therapy is available. A few patients have been treated with interferon-α, but its efficacy has not been evaluated in wider trials.[32] Supportive care and control of increased intracranial pressure are critical for a good outcome. Mannitol or hypertonic saline is used routinely to control raised intracranial pressure, and early high-dose dexamethasone therapy was shown to have no clinical efficacy in a prospective controlled clinical trial.[36] Corticosteroids also have been given, without apparent benefit, in the late stages of illness as empirical therapy for late neurologic changes that were presumed to have an immunopathologic basis. Other supportive measures, including control of fever and seizures, attention to fluid balance, respiratory support, and prevention and treatment of secondary infections, have contributed to increased survival and improved outcomes. Small interfering and hairpin RNA molecules, and minocycline, administered as an immunomodulator, are being investigated as therapeutic agents.

PROGNOSIS

The case-fatality ratio varies from 5 to 35 percent, depending on the accessibility and quality of supportive care. Younger children (<10 years of age) are more likely to die of the infection and to have more serious neurologic complications acutely and as sequelae. Gross neurologic impairment, such as paralysis, weakness, abnormal muscle tone, seizures, ataxia, and extrapyramidal movement disorders, are found in approximately a third to half of recovered patients several months to a year after onset.[34,56,62,82] Electroencephalographic abnormalities have been detected in more than 50 percent of surviving children 1 year after recovery. The graver clinical consequences of JE in contrast to other forms of encephalitis necessitating hospitalization were clearly shown in a follow-up study 6 to 27 years after hospitalization that disclosed neurologic deficits on examination in 22 versus 2 percent of the subjects and subnormal intelligent quotients in 28 versus 2 percent, respectively.[23] Thus, in areas where the disease is prevalent, JE may account for

substantial disability in the resident population. Optic atrophy and chronic seizures have been reported to have a delayed onset of 3 to 17 years after hospital discharge, indicating the need for long-term follow-up.[23] On the other hand, another study suggested that compensatory improvements of acute deficits continue even after a decade.[81]

PREVENTION

JE vaccine is administered routinely under the World Health Organization's Expanded Programme on Immunization and national schedules in most Asian countries (Fig. 175E-3). Several JE vaccines are used, of which the most widely distributed is a live attenuated vaccine made from the SA14-14-2 strain, produced in China.[88,94] Efficacious inactivated mouse brain–derived vaccines largely have been discarded because of associated allergic and neurologic adverse events and are being displaced by Vero cell–derived inactivated vaccines, including in the United States and Europe.[71,72] Another live vaccine, derived as a chimera of the yellow fever 17D vaccine strain and the envelope protein of the SA14-14-2 virus, has been licensed in several countries but has not yet been distributed widely.[29] An inactivated vaccine grown in primary hamster kidney cells is used to a limited degree and only in China. Currently no JE vaccine is licensed for use in children in the United States. Pediatric clinical trials are ongoing, and information on these clinical trials may be obtained at the website http://www.clinicaltrials.gov. In addition, a list of clinics for international travelers that may be administering the JE vaccine to children is available at the CDC website http://www.cdc.gov/ncidod/dvbid/jencephalitis/children.htm.

An inactivated Vero cell–derived JE vaccine, made from the SA14-14-2 strain, is licensed in the United States for adults and internationally for use in travelers; others produced with different viral seeds are licensed in Asia for local use.[42] Two intramuscular doses of 6 μg separated by 28 days produced seroconversion to protective (>10) neutralizing antibody titers in 96 percent of adult subjects with few local and systemic reactions and was noninferior to three doses of the mouse brain–derived vaccine. Protective antibody titers were maintained in 48 to 82 percent of subjects at 24 months with geometric mean titer (GMT) of 16 to 44. When subjects were vaccinated 15 months after primary immunization, all responded to a booster dose with a GMT of 900 that remained elevated at a GMT of 361 at 1 year later.[12] A single booster dose in subjects who previously had been vaccinated with the inactivated mouse brain vaccine produced a response in more than 95 percent, indicating interchangeability of the two inactivated vaccines. Limited data in children indicate 100 percent seroconversion to a protective antibody titer after two 3 μg (children <3 years of age) or 6 μg doses (children 3 to 18 years of age), with GMTs of 216 to 332 and that persisted at titers of 48 to 84 at 6 months later. Reactogenicity was similar to that of the PCV7 control vaccine, and no safety signals have emerged in postmarketing surveillance.[83] Compatible coadministration of the vaccine with hepatitis A vaccine has been demonstrated in adult subjects.

More than 100 JE cases have been reported in travelers from Europe, including Russia; North America; Australia; and Asia; therefore, vaccination of certain travelers is recommended.[6] Current U.S. recommendations specify vaccination for expatriates, for travelers spending 30 days or more in an endemic area during the transmission season, and for persons with briefer itineraries if they have a high risk for exposure (see Table 175E-1).[11,33] The course of immunization should be completed 7 to 10 days before the onset of travel. These recommendations are highly conservative, however, because cases in travelers have been reported in individuals with briefer itineraries and without the cited risk factors, and who later died or survived with serious neurologic damage. The narrow criteria defined in the recommendation are a legacy of committee efforts to limit use of the highly reactogenic mouse brain–derived vaccine, which was the only licensed vaccine when recommendations initially were formulated.[11] With the availability of a safer inactivated vaccine, the changed risk-benefit of vaccination has prompted reconsideration of these recommendations.[6,7,58,91] Because the illness has such a high clinical impact and the risk for acquiring the infection is serendipitous—although low—thoughtful counseling is needed while discussing the risks for the disease, vaccine cost, and other factors, so that travelers can make an informed decision (see Table 175E-1).

The live attenuated SA 14-14-2 vaccine is used in areas of China, India, Nepal, Sri Lanka, North Korea, Thailand, and Cambodia. Although the recommended schedule of immunization is three doses given at 1, 2, and 6 years of age, case-control effectiveness studies suggest that two doses could be 98 percent effective. Some case-control studies suggest that one dose could be effective; however, primary vaccine failure was shown in 5 percent of 9- to 15-month-old children who failed to respond serologically to the first dose of vaccine.[17,65] Although the strain is highly attenuated, with low reactogenicity, safety studies are limited. A report of four JE cases occurring in temporal relationship to vaccination could not distinguish vaccine failure from vaccine-associated encephalitis, indicating a need for additional safety surveillance.[39] No data are available on its use in HIV-infected or other immunocompromised patients. Coadministration with measles vaccine showed measles antibody titers were lowered, but not significantly so.

The live chimeric vaccine led to seroconversion to a GMT of 230 in 97 percent of adults 6 months after a single dose, with 93 percent retaining protective antibody levels at a GMT of 70 at 5 years.[87] Antibody persistence was modeled to show that 90 percent of subjects would remain seroprotected at 8 years.[21] Well-tolerated, compatible or sequential administration with yellow fever 17D vaccine was shown.[63] In 12- to 19-month-old toddlers, 92 to 95 percent seroconverted, with persistence of antibodies to a GMT of approximately 80 in 84 to 90 percent after 2 years.[16,26] A low level of viremia was detected in 5 percent of vaccinated children on the fourth day after vaccination; the vaccine was as well tolerated as

the comparator hepatitis A vaccine. Cautions associated with the use of the live vaccine in immunocompromised persons, at the extremes of age, and in other populations have not been specifically defined.

Although vaccination is recommended to prevent infection and illness, avoidance of outdoor activities during the evening hours, staying in screened or air-conditioned quarters, and sleeping under a bed net also will lower the risk for acquiring the illness by reducing exposures to vector mosquitoes. Wearing long-sleeved shirts and long pants and using mosquito repellents on clothing and exposed skin are recommended for outdoor activities.

From a public health perspective, routine childhood vaccination with the live SA14-14-2 vaccine has been shown to be highly cost-effective.[53,93] Pig vaccination is ineffective and impractical, although moving pigs sties and pig production facilities at a distance from the human population can reduce disease risk. Mosquito control is impractical, but agricultural practices, such as schedules of rice production and changes in water levels, and non-targeted effects of agricultural pesticides have had secondary effects in reducing the vector population and cannot be excluded to have reduced human infections.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Anderson KB, Gibbons RV, Thomas SJ, et al. Preexisting Japanese encephalitis virus neutralizing antibodies and increased symptomatic dengue illness in a school-based cohort in Thailand. *PLoS Negl Trop Dis* 2011;**5**:e1311.
4. Borah J, Dutta Pl, Khan SA, et al. A comparison of clinical features of Japanese encephalitis virus infection in the adult and pediatric age group with acute encephalitis syndrome. *J Clin Virol* 2011; **52**:45–9.
6. Buhl MR, Lindquist L. Japanese encephalitis in travelers: review of cases and seasonal risk. *J Travel Med* 2009;**16**:217–19.
7. Burchard GD, Caumes E, Connor BA, et al. Expert opinion on vaccination of travelers against Japanese encephalitis. *J Travel Med* 2009;**16**:204–16.
10. Campbell GL, Hils SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* 2011;**89**:766–74.
11. Centers for Disease Control and Prevention. Japanese encephalitis vaccines: recommendations of the ACIP. *MMWR Morb Mortal Wkly Rep Recomm Rep* 2010;**59**(RR-1):1–27.
12. Centers for Disease Control and Prevention. Recommendations for use of a booster dose of inactivated Vero cell culture-derived Japanese encephalitis vaccine: advisory committee on immunization practices, 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 661–3.
14. Chen SP. Molecular phylogenetic and evolutionary analysis of Japanese encephalitis virus in China. *Epidemiol Infect* 2011.
15. Chen YY, Fan YC, Tu WC, et al. Japanese encephalitis virus genotype replacement, Taiwan, 2009-10. *Emerg Infect Dis* 2011;**17**: 2354–6.
16. Chokephaibulkit K, Sirivichayakul C, Thisyakorn U, et al. safety and immunogenicity of a single administration of live-attenuated Japanese encephalitis vaccine in previously primed 2-5 year–olds and naïve 12-24 month-olds: multicenter randomized controlled trial. *Pediatr Infect Dis J* 2010;**29**:1111–17.
17. Chokephaibulkit K, Sohn YM, Yoksan S, et al. Immunizing children aged 9-15 months with live attenuated SA14-14-2 Japanese encephalitis vaccine in Thailand. *J Med Assoc Thai* 2011;**94S**: S195–203.
21. Desai K, Coudeville L, Bailleux F. Modelling the long-term persistence of neutralizing antibody in adults after one dose of live attenuated Japanese encephalitis chimeric vaccine. *Vaccine* 2012;**20**: 2510–15.
26. Feroldi E, Pancharoen C, Kosalarsaksa P, et al. Single-dose, live-attenuated Japanese encephalitis vaccine in children aged 12-18 months: randomized, controlled phase 3 immunogenicity and safety trial. *Hum Vaccin Immunother* 2012;**8**:929–37.
28. Guarav YK, Tandale BV, Jadi RS. Chandipura virus encephalitis outbreak among children in Nagpur division, Maharashtra, 2007. *Indian J Med Res* 2010;**132**:395–9.
29. Halstead SB, Thomas SJ. New Japanese encephalitis vaccines: alternatives to production in mouse brain. *Expert Rev Vaccines* 2011;**10**:355–64.
33. Hills SL, Griggs AC, Fischer M. Japanese encephalitis in travelers from non-endemic countries, 1973-2008. *Am J Trop Med Hyg* 2010;**82**:930–6.
34. Hills SL, Van Cuong N, Touch S. Disability from Japanese encephalitis in Cambodia and Viet Nam. *J Trop Pediatr* 2011;**57**: 241–4.
39. Jia N, Zhao QM, Guo XF, et al. Encephalitis temporally associated with live attenuated Japanese encephalitis vaccine: four case reports. *BMC Infect Dis* 2011;**11**:344.
42. Kikukawa A, Gomi Y, Akechi M, et al. Superior immunogenicity of a freeze-dried, cell culture-derived Japanese encephalitis vaccine (inactivated). *Vaccine* 2012;**16**:2329–35.
44. Konishi E, Kitai Y, Tabei Y, et al. Natural Japanese encephalitis virus infection among humans in west and east Japan shows the need to continue a vaccination program. *Vaccine* 2010;**28**:2664–70.
47. Le VT, Phan TQ, Do QH, et al. Viral etiology of encephalitis in children in southern Vietnam: results of a one-year prospective descriptive study. *PLoS Negl Trop Dis* 2011;**4**:e854.
49. Li MH, Fu SH, Chen WX, et al. Genotype V Japanese encephalitis virus is emerging. *PLoS Negl Trop Dis* 2011;**5**:e1231.
51. Lim JK, McDermott DH, Lisco A. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. *J Infect Dis* 2010;**201**:178–85.
55. Lu W, Gibbons RV, Kari K, et al. Risk factors for Japanese encephalitis: a case-control study. *Epidemiol Infect* 2010;**138**:1292–7.
56. Maha SM, Moniaga VA, Hills SL, et al. Outcome and extent of disability following Japanese encephalitis in Indonesian children. *Int J Infect Dis* 2009;**13**:e389–93.
58. Mayer CA, Nielson AA. Japanese encephalitis: prevention in travellers. *Aust Fam Physician* 2010;**39**:389–94.
59. Misra UK, Kalita J, Phadke RV, et al. Usefulness of various MRI sequences in the diagnosis of viral encephalitis. *Acta Trop* 2010; **116**:206–11.
60. Miller RH, Masuoka P, Klein TA, et al. Ecological niche modeling to estimate the distribution of Japanese encephalitis virus in Asia. *PLoS Negl Trop Dis* 2012;**6**:e1678.
61. Moore CE, Blacksell SD, Taojaikong T, et al. A prospective assessment of the accuracy of commercial IgM ELISAs in diagnosis of Japanese encephalitis virus infections in patients with suspected central nervous system infection in Laos. *Am J Trop Med* 2012; **87**:171–8.
63. Nasveld PE, Marajson J, Bennett S, et al. Concomitant or sequential administration of live attenuated Japanese encephalitis chimeric virus vaccine and yellow fever 17D vaccine: randomized double-blind phase II evaluation of safety and immunogenicity. *Hum Vaccin* 2011;**6**:906–14.
66. Olsen SJ, Supawat K, Campbell AP, et al. Japanese encephalitis virus remains an important cause of encephalitis in Thailand. *Int J Infect Dis* 2010;**14**:e888–92.
68. Pan XL, Liu H, Wang HY, et al. Emergence of genotype I of Japanese encephalitis virus as the dominant genotype in Asia. *J Viol* 2011;**85**:9847–53.
74. Pujhari SK, Ratho RK, Prabhakar S, et al. TNF-α promoter polymorphism: a factor contributing to the different immunological and clinical phenotypes in Japanese encephalitis. *BMC Infect Dis* 2012.
81. Sarkari NB, Thacker AK, Barthwal SP, et al. Japanese encephalitis (JE). II. 14 years' follow-up of survivors. *J Neurol* 2012;**259**: 58–69.
83. Schuller E, Klingler A, Dubischar-Kastner K, et al. Safety profile of the Vero cell-derived Japanese encephalitis (JEV) vaccine IXIARO®. *Vaccine* 2011;**29**:8669–76.
87. Torresi J, McCarthy K, Feroldi E, et al. Immunogenicity, safety and tolerability in adults of a new single-dose, live-attenuated vaccine against Japanese encephalitis: randomised controlled phase 3 trials. *Vaccine* 2010;**28**:7993–8000.

89. Turtle L, Griffiths JJ, Solomon T. Encephalitis caused by flaviviruses. *Q J Med* 2012;**105**:219–23.
91. Wilder-Smith A, Halstead SB. Japanese encephalitis: update on vaccines and vaccine recommendations. *Curr Opin Infect Dis* 2011;**23**:426–31.
93. Yin Z, Beeler Asay GR, Zhang L, et al. An economic evaluation of the use of Japanese encephalitis vaccine in the expanded program of immunization in Guizhou province, China. *Vaccine* 2012;**30**: 5569–77.
94. Yu YX. Phenotypic and genotypic characteristics of Japanese encephalitis attenuated live vaccine virus SA14-14-2 and their stabilities. *Vaccine* 2010;**28**:3635–41.
95. Zheng YL, Li M, Wang H, et al. Japanese encephalitis and Japanese encephalitis virus in mainland China. *Rev Med Virol* 2012.

The full reference list for this chapter is available at expertconsult.com.

175F ■ MURRAY VALLEY ENCEPHALITIS

John G. Aaskov

Outbreaks of an acute, severe, encephalitic illness, clinically similar to Japanese and St. Louis encephalitis, occurred in rural areas of southeastern Australia in 1917, 1918, 1922, 1925, 1951, and 1974[1,9,14-16] and in north and northwestern Australia in 1981, 1993, and 2000.[8,12,41] Approximately 420 cases were reported in these nine outbreaks.[41] They are thought to represent a single entity for which various names (Australian X disease, Murray Valley encephalitis, Australian encephalitis) have been used. Twenty-two cases were diagnosed in the 5 years between 2007 and 2011; three were fatal, and one of the fatalities occurred in a Canadian tourist on return from a holiday in northern Australia.

Case-fatality rates, as high as 70 percent in the early years,[9,11] declined to 20 percent in the 1974 outbreak and have remained at about this level since then.[5,10,12] However, significant residual neurologic disability occurs in as many as 50 percent of survivors.[10,12]

The presence of this disease in Papua New Guinea was confirmed in 1956.[20] The causative virus was transmitted to experimental animals as early as 1918,[6,11] although those strains could not be maintained. The definitive isolation and characterization of Murray Valley encephalitis virus in 1951[19] led to epidemiologic studies that suggested its survival in bird-mosquito cycles in northern Australia but not in the area of epidemic occurrence in southern Australia.[1]

Murray Valley encephalitis is caused by Murray Valley encephalitis virus. In an effort to dissociate a disease from a specific locality, the term *Australian encephalitis* was proposed by residents of Murray Valley for the disease caused by Murray Valley encephalitis virus. Some researchers subsequently have attempted to expand the term *Australian encephalitis* to include encephalitis caused by any Australian arbovirus. Because the term *Australian encephalitis* has no scientific validity and is ambiguous, it should not be used.

ETIOLOGIC AGENT

Murray Valley encephalitis virus was isolated post mortem from the brains of patients in 1951 and 1974 and shown to be related antigenically to Japanese encephalitis virus.[19,28]

A partial nucleotide sequence of Murray Valley encephalitis virus[13] confirmed the previous classification of this virus, on serologic grounds, as being a member of the Japanese encephalitis, St. Louis encephalitis, West Nile fever subgroup of flaviviruses.[37] Four genotypes of Murray Valley encephalitis have been identified, two exclusively from Papua New Guinea.[24,29] However, the most recent isolates from Papua New Guinea are indistinguishable from Australian strains in genotype 1.[23]

TRANSMISSION AND EPIDEMIOLOGY

The mosquito *Culex annulirostris* is thought to be the major vector of Murray Valley encephalitis virus,[16,17,31] although other mosquitoes, such as *Aedes normanensis*, *Aedes tremulus*, and *Culex quinquefasciatus*, also may play a significant role in transmission of the virus.[7]

Murray Valley encephalitis virus is thought to survive in cycles of infection between birds and mosquitoes in northern Australia and Papua New Guinea,[1] where regular infection of humans and other animals is indicated by seroconversion in the summer-autumn "wet" season[16] as well as by clinical disease.[7,41] Studies also suggest that the virus might survive, after vertical transmission, in desiccation-resistant eggs of *A. tremulus* mosquitoes.[7] Epidemics that occurred in more populous areas of southeastern Australia are thought to have followed the introduction of virus when abnormal spring rainfall allowed chains of bird-mosquito transmission to occur through northern Australia.[2] However, several studies also have suggested that interepidemic survival of virus occurs in southern Australia.[21]

Although disease occurs most commonly in children, clinical infection occurs in individuals of all ages.* Data are insufficient to determine whether the lower incidence of disease in adults is due to immunity acquired from previous subclinical infections.

*References 1, 4, 8, 10, 12, 16, 30.

REFERENCES

1. Anderson KB, Gibbons RV, Thomas SJ, et al. Preexisting Japanese encephalitis virus neutralizing antibodies and increased symptomatic dengue illness in a school-based cohort in Thailand. *PLoS Negl Trop Dis* 2011;**5**:e1311.
2. Aria S, Matsunga Y, Takasaki T, et al. Japanese encephalitis: surveillance and elimination effort in Japan from 1982 to 2004. *Jpn J Infect Dis* 2008;**61**:333–8.
3. Bhattacharya S, Chakraborty SK, Chakraborty S, et al. Density of *Culex vishnui* and appearance of JE antibody in sentinel chicks and wild birds in relation to Japanese encephalitis cases. *Trop Geogr Med* 1986;**38**:46–50.
4. Borah J, Dutta PL, Khan SA, et al. A comparison of clinical features of Japanese encephalitis virus infection in the adult and pediatric age group with acute encephalitis syndrome. *J Clin Virol* 2011;**52**: 45–9.
5. Buescher EL, Scherer WF. Ecologic studies of Japanese encephalitis virus in Japan. *Am J Trop Med Hyg* 1959;**8**:719–22.
6. Buhl MR, Lindquist L. Japanese encephalitis in travelers: review of cases and seasonal risk. *J Travel Med* 2009;**16**:217–19.
7. Burchard GD, Caumes E, Connor BA, et al. Expert opinion on vaccination of travelers against Japanese encephalitis. *J Travel Med* 2009;**16**:204–16.
8. Burke DS, Morrill JC. Levels of interferon in the plasma and cerebrospinal fluid of patients with acute Japanese encephalitis. *J Infect Dis* 1987;**155**:797–9.
9. Burke DS, Lorsomrudee W, Leake CJ, et al. Fatal outcome in Japanese encephalitis. *Am J Trop Med Hyg* 1985;**34**:1203–10.
10. Campbell GL, Hils SL, Fischer M, et al. Estimated global incidence of Japanese encephalitis: a systematic review. *Bull World Health Organ* 2011;**89**:766–74.
11. Centers for Disease Control and Prevention. Japanese encephalitis vaccines: recommendations of the ACIP. *MMWR Morb Mortal Wkly Rep Recomm Rep* 2010;**59**(RR-1):1–27.
12. Centers for Disease Control and Prevention. Recommendations for use of a booster dose of inactivated Vero cell culture-derived Japanese encephalitis vaccine: advisory committee on immunization practices, 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 661–3.
13. Chaturvedi UC, Mathur A, Chandra A, et al. Transplacental infection with Japanese encephalitis virus. *J Infect Dis* 1980;**141**: 712–15.
14. Chen SP. Molecular phylogenetic and evolutionary analysis of Japanese encephalitis virus in China. *Epidemiol Infect* 2011.
15. Chen YY, Fan YC, Tu WC, et al. Japanese encephalitis virus genotype replacement, Taiwan, 2009-10. *Emerg Infect Dis* 2011;**17**: 2354–6.
16. Chokephaibulkit K, Sirivichayakul C, Thisyakorn U, et al. safety and immunogenicity of a single administration of live-attenuated Japanese encephalitis vaccine in previously primed 2-5 year–olds and naïve 12-24 month-olds: multicenter randomized controlled trial. *Pediatr Infect Dis J* 2010;**29**:1111–17.
17. Chokephaibulkit K, Sohn YM, Yoksan S, et al. Immunizing children aged 9-15 months with live attenuated SA14-14-2 Japanese encephalitis vaccine in Thailand. *J Med Assoc Thai* 2011;**94S**: S195–203.
18. Das SK, Nityanand S, Sood K, et al. Japanese B encephalitis with neurocysticercosis. *J Assoc Physicians India* 1991;**39**:643–4.
19. Desai A, Ravi V, Guru SC, et al. Detection of autoantibodies to neural antigens in the CSF of Japanese encephalitis patients and correlation of findings with the outcome. *J Neurol Sci* 1994;**122**: 109–16.
20. Desai A, Shankar SK, Ravi V, et al. Japanese encephalitis virus antigen in the human brain and its topographic distribution. *Acta Neuropathol* 1995;**89**:368–73.
21. Desai K, Coudeville L, Bailleux F. Modelling the long-term persistence of neutralizing antibody in adults after one dose of live attenuated Japanese encephalitis chimeric vaccine. *Vaccine* 2012;**20**: 2510–15.
22. Dhanda V, Mourya DT, Mishra AC, et al. Japanese encephalitis virus infection in mosquito reared from field-collected immatures and in wild-caught males. *Am J Trop Med Hyg* 1989;**41**:732–6.
23. Ding D, Zhen H, Zhao SJ, et al. Long-term disability from acute childhood Japanese encephalitis in Shanghai, China. *Am J Trop Med Hyg* 2007;**77**:528–33.
24. Edelman R, Schneider RJ, Chieowanich P, et al. The effect of dengue virus infection on the clinical sequelae of Japanese encephalitis: a one-year follow-up study in Thailand. *Southeast Asian J Trop Med Public Health* 1975;**6**:308–15.
25. Fang R, Hus DR, Lim TW. Investigation of a suspected outbreak of Japanese encephalitis in Pulau Langkawi. *Malaysia J Pathol* 1980;**3**:23–30.
26. Feroldi E, Pancharoen C, Kosalarsaksa P, et al. Single-dose, live-attenuated Japanese encephalitis vaccine in children aged 12-18 months: randomized, controlled phase 3 immunogenicity and safety trial. *Hum Vaccin Immunother* 2012;**8**:929–37.
27. Gajanana A, Thenmozhi V, Samuel PP, et al. A community-based study of subclinical flavivirus infections in children in an area of Tamil Nadu, India, where Japanese encephalitis is endemic. *Bull World Health Organ* 1995;**73**:237–44.
28. Guarav YK, Tandale BV, Jadi RS. Chandipura virus encephalitis outbreak among children in Nagpur division, Maharashtra, 2007. *Indian J Med Res* 2010;**132**:395–9.
29. Halstead SB, Thomas SJ. New Japanese encephalitis vaccines: alternatives to production in mouse brain. *Expert Rev Vaccines* 2011;**10**:355–64.
30. Handique SK, Das RR, Barman K, et al. Temporal involvement in Japanese encephalitis: problems in differential diagnosis. *Am J Neuroradiol* 2006;**27**:1027–31.
31. Hanna JN, Ritchie SA, Phillips DA, et al. Japanese encephalitis in north Queensland, Australia, 1998. *Med J Aust* 1999;**170**:533–6.
32. Harinasuta C, Nimmanitya S, Titsyakorn U. The effect of interferon-alpha A on two cases of Japanese encephalitis in Thailand. *Southeast Asian J Trop Med Public Health* 1985;**16**: 332–6.
33. Hills SL, Griggs AC, Fischer M. Japanese encephalitis in travelers from non-endemic countries, 1973-2008. *Am J Trop Med Hyg* 2010;**82**:930–6.
34. Hills SL, Van Cuong N, Touch S. Disability from Japanese encephalitis in Cambodia and Viet Nam. *J Trop Pediatr* 2011;**57**:241–4.
35. Hiyake M. The pathology of Japanese encephalitis. *Bull World Health Organ* 1964;**30**:153–60.
36. Hoke CH, Vaughn DW, Nisalak A, et al. Effect of high-dose dexamethasone on the outcome of acute encephalitis due to Japanese encephalitis virus. *J Infect Dis* 1992;**165**:631–7.
37. Inada R. Compte rendu des recherches sur l'encephalite epidemique au Japon. *Offic Int D'Hyg Pub Bull Mens* 1937;**29**:1389–401.
38. Ishii T, Matsushita M, Hamada S. Characteristic residual neuropathological features of Japanese B encephalitis. *Acta Neuropathol* 1977;**38**:181–6.
39. Jia N, Zhao QM, Guo XF, et al. Encephalitis temporally associated with live attenuated Japanese encephalitis vaccine: four case reports. *BMC Infect Dis* 2011;**11**:344.
40. Johnson RT, Burke DS, Elwell M, et al. Japanese encephalitis: Immunocytochemical studies of viral antigen and inflammatory cells in fatal cases. *Ann Neurol* 1985;**18**:567–73.
41. Kalita J, Kumar S, Viriaykuma K, et al. A study of CSF catecholamine and its metabolites in acute and convalescent period of encephalitis. *J Neurol Sci* 2007;**252**:62–6.
42. Kikukawa A, Gomi Y, Akechi M, et al. Superior immunogenicity of a freeze-dried, cell culture-derived Japanese encephalitis vaccine (inactivated). *Vaccine* 2012;**16**:2329–35.
43. Kitaoka M. Shift of age distribution of cases of Japanese encephalitis in Japan during the period 1950 to 1967. In: Hammon MW, Kitaoka M, Downs WG, editors. *Immunization for Japanese encephalitis*. Amsterdam: Excerpta Medica; 1972. p. 285–91.
44. Konishi E, Kitai Y, Tabei Y, et al. Natural Japanese encephalitis virus infection among humans in west and east Japan shows the need to continue a vaccination program. *Vaccine* 2010;**28**:2664–70.
45. Kumar R, Selvan AS, Sharma S, et al. Clinical predictors of Japanese encephalitis. *Neuroepidemiology* 1994;**13**:97–102.
46. Kumar R, Tripathi P, Singh S, et al. Clinical features in children hospitalized during the 2005 epidemic of Japanese encephalitis in Uttar Pradesh, India. *Clin Infect Dis* 2006;**42**:123–31.
47. Le VT, Phan TQ, Do QH, et al. Viral etiology of encephalitis in children in southern Vietnam: results of a one-year prospective descriptive study. *PLoS Negl Trop Dis* 2011;**4**:e854.
48. Lee DW, Choe YJ, Kim JH, et al. Epidemiology of Japanese encephalitis in South Korea, 2007-2010. *Int J Infect Dis* 2012; **16**:e448–52.

49. Li MH, Fu SH, Chen WX, et al. Genotype V Japanese encephalitis virus is emerging. *PLoS Negl Trop Dis* 2011;**5**:e1231.
50. Li ZS, Hong SF, Gong NL. Immunohistochemical study of Japanese B encephalitis. *Chin Med J (Engl)* 1988;**101**:768–71.
51. Lim JK, McDermott DH, Lisco A. CCR5 deficiency is a risk factor for early clinical manifestations of West Nile virus infection but not for viral transmission. *J Infect Dis* 2010;**201**:178–85.
52. Lincoln AF, Sivertson SE. Acute phase of Japanese B encephalitis: two hundred and one cases in American soldiers, Korea, 1960. *J Med Assoc* 1952;**150**:268–73.
53. Liu W, Clemens JD, Kari K, et al. Cost-effectiveness of Japanese encephalitis (JE) immunization in Bali, Indonesia. *Vaccine* 2008;**26**: 4456–60.
54. Liu YF, Teng CL, Liu K. Cerebral cysticercosis as a factor aggravating Japanese B encephalitis. *Chin Med J (Engl)* 1957;**75**:1010.
55. Lu W, Gibbons RV, Kari K, et al. Risk factors for Japanese encephalitis: a case-control study. *Epidemiol Infect* 2010;**138**:1292–7.
56. Maha SM, Moniaga VA, Hills SL, et al. Outcome and extent of disability following Japanese encephalitis in Indonesian children. *Int J Infect Dis* 2009;**13**:e389–93.
57. Mathur A, Tandon HO, Mathur KR, et al. Japanese encephalitis infection during pregnancy. *Indian J Med Res* 1985;**81**:9–12.
58. Mayer CA, Nielson AA. Japanese encephalitis: prevention in travellers. *Aust Fam Physician* 2010;**39**:389–94.
59. Misra UK, Kalita J, Phadke RV, et al. Usefulness of various MRI sequences in the diagnosis of viral encephalitis. *Acta Trop* 2010; **116**:206–11.
60. Miller RH, Masuoka P, Klein TA, et al. Ecological niche modeling to estimate the distribution of Japanese encephalitis virus in Asia. *PLoS Negl Trop Dis* 2012;**6**:e1678.
61. Moore CE, Blacksell SD, Taojaikong T, et al. A prospective assessment of the accuracy of commercial IgM ELISAs in diagnosis of Japanese encephalitis virus infections in patients with suspected central nervous system infection in Laos. *Am J Trop Med* 2012; **87**:171–8.
62. Murgod UA, Muthane UB, Ravi V, et al. Persistent movement disorders following Japanese encephalitis. *Neurology* 2001;**57**: 2313–15.
63. Nasveld PE, Marajson J, Bennett S. At al: Concomitant or sequential administration of live attenuated Japanese encephalitis chimeric virus vaccine and yellow fever 17D vaccine: randomized double-blind phase II evaluation of safety and immunogenicity. *Hum Vaccin* 2011;**6**:906–14.
64. Nathanson N, Cole GA. Fatal Japanese encephalitis virus infection in immunosuppressed spider monkeys. *Clin Exp Immunol* 1970;**6**: 161–6.
65. Ohrr H, Tandan JB, Sohn YM, et al. Effect of single dose of SA 14-14-2 vaccine 1 year after immunisation in Nepalese children with Japanese encephalitis: a case-control study. *Lancet* 2005;**366**: 1375–8.
66. Olsen SJ, Supawat K, Campbell AP, et al. Japanese encephalitis virus remains an important cause of encephalitis in Thailand. *Int J Infect Dis* 2010;**14**:e888–92.
67. Olson JG, Ksiazek TG, Tan R, et al. Correlation of population indices of female *Culex tritaeniorhynchus* with Japanese encephalitis viral activity in Kapuk, Indonesia. *Southeast Asian J Trop Med Public Health* 1985;**16**:337–40.
68. Pan XL, Liu H, Wang HY, et al. Emergence of genotype I of Japanese encephalitis virus as the dominant genotype in Asia. *J Viol* 2011;**85**:9847–53.
69. Paul WS, Moore PS, Karabatsos N, et al. Outbreak of Japanese encephalitis on the island of Saipan, 1990. *J Infect Dis* 1993;**167**: 1053–8.
70. Peiris JSM, Amerasinghe FP, Amerasinghe PH, et al. Japanese encephalitis in Sri Lanka. I. The study of an epidemic-vector incrimination, porcine infection and human disease. *Trans R Soc Trop Med Hyg* 1992;**86**:307–23.
71. Plesner AM. Allergic reactions to Japanese encephalitis vaccine. *Immunol Allergy Clin North Am* 2003;**23**:665–97.
72. Plesner AM, Arlien-Soborg P, Herning M. Neurological complications to vaccination against Japanese encephalitis. *Eur J Neurol* 1998;**5**:479–85.
73. Pradhan S, Gupta RK, Singh MB, et al. Biphasic illness pattern due to early relapse in Japanese-B virus encephalitis. *J Neurol Sci* 2001;**183**:13–18.
74. Pujhari SK, Ratho RK, Prabhakar S, et al. TNF-α promoter polymorphism: a factor contributing to the different immunological and clinical phenotypes in Japanese encephalitis. *BMC Infect Dis* 2012.
75. Ravi V, Desai A, Balaji J, et al. Development and evaluation of a rapid IgM capture ELISA (JE-CHEX) for the diagnosis of Japanese encephalitis. *J Clin Virol* 2006;**35**:429–34.
76. Ravi V, Desai AS, Shenoy PK, et al. Persistence of Japanese encephalitis virus in the human nervous system. *J Med Virol* 1993;**40**: 326–9.
77. Ravi V, Taly AB, Shankar SK, et al. Association of Japanese encephalitis virus infection with Guillain-Barré syndrome in endemic areas of South India. *Acta Neurol Scand* 1994;**90**:67–72.
78. Rayamajhi A, Singh R, Prasad R, et al. Clinico-laboratory profile and outcome of Japanese encephalitis in Nepali children. *Ann Trop Paediatr* 2006;**26**:293–301.
79. Rodrigues FM, Guttikar SN, Pinto BD. Prevalence of antibodies to Japanese encephalitis and West Nile viruses among wild birds in the Krishna-Godavari Delta, Andhra Pradesh, India. *Trans R Soc Trop Med Hyg* 1981;**75**:258–62.
80. Rosen L. The natural history of Japanese encephalitis virus. *Annu Rev Microbiol* 1986;**40**:395–414.
81. Sarkari NB, Thacker AK, Barthwal SP, et al. Japanese encephalitis (JE). II. 14 years' follow-up of survivors. *J Neurol* 2012;**259**: 58–69.
82. Schneider RJ, Fireston MH, Edelman R, et al. Japanese encephalitis: a one-year follow-up study in Thailand. *Southeast Asian J Trop Med Public Health* 1974;**5**:560–8.
83. Schuller E, Klingler A, Dubischar-Kastner K, et al. Safety profile of the Vero cell-derived Japanese encephalitis (JEV) vaccine IXIARO®. *Vaccine* 2011;**29**:8669–76.
84. Shankar SK, Rao TV, Mruthyunjayanna BP, et al. Autopsy study of brains during an epidemic of Japanese encephalitis in Karnataka. *Indian J Med Res* 1983;**78**:431–40.
85. Sharma S, Mathur A, Prakash R, et al. Japanese encephalitis virus latency in peripheral blood lymphocytes and recurrence of infection in children. *Clin Exp Immunol* 1991;**85**:85–9.
86. Singh P, Kalra N, Ratho RK, et al. Coexistent neurocysticercosis and Japanese B encephalitis: MR imaging correlation. *AJNR Am J Neuroradiol* 2001;**22**:1131–6.
87. Torresi J, McCarthy K, Feroldi E, et al. Immunogenicity, safety and tolerability in adults of a new single-dose, live-attenuated vaccine against Japanese encephalitis: randomised controlled phase 3 trials. *Vaccine* 2010;**28**:7993–8000.
88. Tsai TF. New initiatives for the control of Japanese encephalitis by vaccination: minutes of a WHO/CVI meeting, Bangkok, Thailand, 13-15 October, 1998. *Vaccine* 2000;**18**(Suppl. 2):1–25.
89. Turtle L, Griffiths JJ, Solomon T. Encephalitis caused by flaviviruses. *Q J Med* 2012;**105**:219–23.
90. Van Den Hurk AF, Johansen CA, Zborowski P, et al. Mosquito host-feeding patterns and implications for Japanese encephalitis virus transmission in northern Australia and Papua New Guinea. *Med Vet Entomol* 2003;**17**:403–11.
91. Wilder-Smith A, Halstead SB. Japanese encephalitis: update on vaccines and vaccine recommendations. *Curr Opin Infect Dis* 2011;**23**:426–31.
92. Winter PM, Dung NM, Loan HT, et al. Proinflammatory cytokines and chemokines in humans with Japanese encephalitis. *J Infect Dis* 2004;**190**:1618–26.
93. Yin Z, Beeler Asay GR, Zhang L, et al. An economic evaluation of the use of Japanese encephalitis vaccine in the expanded program of immunization in Guizhou province, China. *Vaccine* 2012;**30**: 5569–77.
94. Yu YX. Phenotypic and genotypic characteristics of Japanese encephalitis attenuated live vaccine virus SA14-14-2 and their stabilities. *Vaccine* 2010;**28**:3635–41.
95. Zheng YL, Li M, Wang H, Liang G. Japanese encephalitis and Japanese encephalitis virus in mainland China. *Rev Med Virol* 2012.
96. Zimmerman HM. The pathology of Japanese B encephalitis. *Am J Pathol* 1946;**22**:965–91.

The recent report of a clinical Murray Valley encephalitis virus infection in a horse, with pathologic changes in the brain and spinal cord similar to those seen in humans,[23] confirms a previous, unconfirmed report of possible clinical Murray Valley encephalitis infections in horses[22] and raises the possibility that both wild and domestic horses could play a role in the transmission and dissemination of this virus.

CLINICAL MANIFESTATIONS*

An initial period of nonspecific prodromal symptoms and signs, such as fever, headache, nausea, vomiting, muscle pain, and photophobia, is followed within 2 to 5 days by drowsiness, mental obtundation, confusion, disorientation, incongruous behavior, ataxia, speech disturbance, or convulsions, sometimes with a grand mal character. Neurologic signs were present in most patients on admission to the hospital, and additional signs appeared as the disease progressed. In some cases, the signs fluctuated from hour to hour. Bennett[5] recognized three groups of patients according to eventual clinical outcome:

1. Patients with mild disease commonly had disturbed mentation short of coma, incoherent or slurred speech, aphasia, speech perseveration, incontinence, neck stiffness, intention tremor, and limb hypertonicity but rarely required assistance for respiration. The neurologic changes stabilized in 5 to 10 days, and the patients' clinical conditions improved.
2. Patients with severe disease showed more profound central nervous system (CNS) involvement consisting of impairment of consciousness to coma, more marked signs of upper motor neuron involvement, and pharyngeal or respiratory paralysis requiring artificial respiration.
3. In fatal cases, patients had either spastic quadriplegia progressing to almost complete loss of nervous function or severe disease with superimposed infection.

There also may be a fourth group with even milder signs and symptoms than Bennett's group 1. These are patients with mild or no encephalitis whose principal features are fever and headache.[40]

PATHOLOGY

A period of viremia probably precedes infection of the CNS, during the 1- to 3-week incubation period,[1] but it has not been demonstrated. Pathologic changes in fatal cases were restricted to the CNS and included extensive perivascular cuffing (especially in the cortex), lymphocytic infiltration of the meninges, neuron degeneration and neuronophagia in the cerebellum and spinal cord, and thalamic necrosis. Evidence of repair, including calcification, was described in patients who died late in the course of the disease.[6,10,11,36,39] These pathologic changes do not distinguish Murray Valley encephalitis from other arthropod-borne encephalitides.

In rodents infected peripherally with Murray Valley encephalitis virus, the virus enters the CNS through the olfactory pathway after replicating in regional lymph nodes. From the olfactory lobe, it spreads through interconnected neural circuits to the cortex, hippocampus, thalamus, cerebellum, medulla oblongata, and spinal cord. Extensive neuronal necrosis was observed in the olfactory bulb and hippocampus. The severity of the subsequent encephalitis correlated with the magnitude of mononuclear and polymorphonuclear cell infiltrates. Infiltration of neutrophils was preceded by increased expression of tumor necrosis factor-α and the chemokine N51/KC in the CNS. Previous depletion of neutrophils or inhibition of inducible nitric oxide synthetase resulted in prolonged survival and decreased incidence of mortality in mice infected with Murray Valley encephalitis virus.[3,32] However, the pronounced mononuclear cell content of cerebrospinal fluid in some patients with Murray Valley encephalitis[10,19] contrasts with the picture in rodents.[32]

DIAGNOSIS

Clinical and epidemiologic features may suggest the diagnosis of Murray Valley encephalitis, especially during recognized epidemics, but individual cases may be difficult to distinguish from cases of encephalitis or encephalopathy of other cause (e.g., herpesvirus or Japanese encephalitis or Kunjin viruses).[5,34,42] A differential diagnosis of Murray Valley encephalitis may be assisted by observing signs attributable to spinal cord involvement and cranial nerve palsies, tremor, and frequency of seizures (seizures rarely develop in adults with Murray Valley encephalitis, whereas they may occur in up to 90% of children). Although computed tomographic scans in these patients may be normal and electroencephalograms may not show focal features,[10] magnetic resonance imaging reveals changes similar to those reported in the brains of patients with Japanese encephalitis[25]—thalamic edema, hypointensity on T1-weighted images, and hyperintensity on T2-weighted images.[18,26,27] Changes also were noted in the reticular formation, the substantia nigra, and the cervical spinal cord.[18] In the absence of serology, the magnetic resonance images might be regarded as diagnostic of an infection with herpes simplex virus rather than with Murray Valley encephalitis virus.[42] Magnetic resonance imaging may provide a presumptive diagnosis within a week of onset of symptoms.

Establishment of a specific diagnosis depends on serologic evidence of infection concurrent with disease. Detection of IgM antibody that reacts with Murray Valley encephalitis virus in enzyme-linked immunosorbent assays is the most useful indication of recent infection. Other flaviviruses, especially Kunjin virus, may cause encephalitis, subclinical infection, or minor illness during epidemics of Murray Valley encephalitis,[41] and

*References 5, 9, 10, 12, 27, 40.

interpretation of serologic cross-reactions between Kunjin and Murray Valley encephalitis viruses may require specific tests (e.g., neutralization) with several viruses.[16] Serologic tests for the laboratory diagnosis of Murray Valley encephalitis are not available commercially and so are performed only at larger regional laboratories. Isolation of virus has not been successful for antemortem diagnosis. However, some reports[18,33] document the detection of Murray Valley encephalitis viral RNA in serum or cerebrospinal fluid from patients by use of the reverse transcription–polymerase chain reaction (RT-PCR). Subsequent serology confirmed these RT-PCR data. In response to the need for rapid clinical intervention in this disease, a number of laboratories now employ "in-house" RT-PCR assays as part of routine diagnosis.[33,38]

TREATMENT AND PROGNOSIS

No specific antiviral therapy is available. Administration of corticosteroids has been recommended during the acute phase of the illness to reduce brain edema. Artificial respiration has been lifesaving, and all patients should be transported to base hospitals with facilities for the management of patients with respiratory paralysis.[5] Most patients also require parenteral nutrition or nasogastric feeding.[10]

The early epidemics left some patients with neurologic and psychiatric sequelae, but the lower case-fatality rate in recent outbreaks, presumably because of use of modern intensive care techniques, has been associated with a high rate of residual disability.[10,12] Bennett[5] observed 18 patients up to 16 months after they experienced infection. Four of 11 patients with mild disease had emotional problems and mild degrees of impaired motor coordination and mental acuity. All 7 patients with severe disease had serious defects, including paraplegia or quadriplegia and mental disturbance.

This pattern of high residual disability has continued to the present.[10,12]

PREVENTION

Because of the irregularity of outbreaks and the large areas over which they occur, implementation of measures to prevent Murray Valley encephalitis is difficult. Several Australian states have established sentinel flocks of chickens to provide warning of transmission of Murray Valley encephalitis virus, but there is no evidence that this information serves other than to alert residents to the need to enhance their mosquito avoidance measures.

There is no vaccine against infection with Murray Valley encephalitis virus, but there may be value in assessing the capacity of the live attenuated Japanese encephalitis virus vaccine that was recently licensed in Australia[35] to provide cross-protection against infection with Murray Valley encephalitis virus.[29]

NEW REFERENCES SINCE THE SIXTH EDITION

27. Knox J, Cowan RU, Doyle JS, et al. Murray Valley encephalitis: a review of clinical features, diagnosis and treatment. *Med J Aust* 2012;**196**:322–6.
35. Nasveld PE, Ebringer A, Elmes N, et al. Long term immunity to live attenuated Japanese encephalitis chimeric vaccine: randomized, double blind, 5 year phase II study in healthy adults. *Hum Vaccin* 2010;**6**:1038–46.

The full reference list for this chapter is available at expertconsult.com.

175G ■ TICK-BORNE ENCEPHALITIS

Andrea Duppenthaler • Christoph Aebi • Theodore F. Tsai

Tick-borne encephalitis (TBE) refers here to the neurotropic tick-transmitted flaviviral infection that occurs across the Eurasian land mass from the Far East to western Europe. The Far Eastern form of the disease frequently is called Russian spring-summer encephalitis (RSSE); in Europe, where the disease is distinctly milder and often biphasic, it is called tick-borne encephalitis, spring-summer meningoencephalitis, central European encephalitis, or, because sometimes it is transmitted by raw infected milk, biphasic milk fever.[26,35,70,107]

HISTORY

After an outbreak of encephalitis in the Far Eastern region of Russia in 1932, Zilber isolated the virus from viremic humans and from *Ixodes persulcatus* ticks. A milder form of the disease with a similar seasonality was described previously in Sweden and Austria, but its etiology was not defined until 1948, when the virus was isolated in the Czech Republic and Slovakia. Milk-borne transmission of TBE virus (TBEV) from infected livestock animals first was recognized in an outbreak in 1951 and 1952.

ETIOLOGIC AGENT

TBEV and the closely related viruses of RSSE and Powassan encephalitis are flaviviruses placed antigenically within a complex of tick-borne flaviviruses that also includes the agents of Kyasanur Forest disease, Omsk hemorrhagic fever, and an encephalomyelitis syndrome

REFERENCES

1. Anderson SG. Murray Valley encephalitis and Australian X disease. *J Hyg (Camb)* 1954;**52**:447–68.
2. Anderson SG, Eagle M. Murray Valley encephalitis: the contrasting epidemiological picture in 1951 and 1952. *Med J Aust* 1953;**1**: 478–81.
3. Andrews DM, Matthews VB, Sammels LM, et al. The severity of Murray Valley encephalitis in mice is linked to neutrophil infiltration and inducible nitric oxide synthetase activity in the central nervous system. *J Virol* 1999;**73**:8781–90.
4. Annual report of the National Notifiable Diseases Surveillance System, 1993. *Commun Dis Intell Bull* 1994;**18**:518–47.
5. Bennett NM. Murray Valley encephalitis, 1974: clinical features. *Med J Aust* 1976;**2**:446–50.
6. Breinl A. Clinical, pathological, and experimental observations on the "mysterious disease," a clinically aberrant form of acute poliomyelitis. *Med J Aust* 1918;**1**:209–13.
7. Broom AK, Lindsay MDA, Johansen CA, et al. Two possible mechanisms for survival and initiation of Murray Valley encephalitis virus activity in the Kimberley region of Western Australia. *Am J Trop Med Hyg* 1995;**53**:95–9.
8. Bucens M. Arbovirus infections diagnosed in Perth, 1981. In: St. George TD, Kay BH, editors. *Arbovirus research in Australia. Proceedings, 3rd Symposium, February 15-17, 1982*. Brisbane, Queensland: Commonwealth Scientific and Industrial Research Organisation and Queensland Institute of Medical Research; 1982. p. 171.
9. Burnell GH. The Broken Hill epidemic. *Med J Aust* 1917;**2**: 157–61.
10. Burrow JNC, Whelan PI, Kilburn CJ, et al. Australian encephalitis in the Northern Territory: clinical and epidemiological features, 1987-1996. *Aust N Z J Med* 1998;**28**:590–6.
11. Cleland JB, Campbell AW, Bradley B. *The Australian epidemics of an acute polioencephalomyelitis (X disease)*. Report of the Director-General of Public Health NSW; 1918. p. 150–280.
12. Cordova SP, Smith DW, Broom AK, et al. Murray Valley encephalitis in Western Australia in 2000, with evidence of southerly spread. *Commun Dis Intell* 2000;**24**:368–72.
13. Dalgarno L, Trent DW, Strauss JH, et al. Partial nucleotide sequence of the Murray Valley encephalitis virus genome: comparison of the encoded polypeptides with yellow fever virus structural and non-structural proteins. *J Mol Biol* 1986;**187**:309–29.
14. Doherty RL. Arthropod-borne viruses in Australia and their relation to infection and disease. *Prog Med Virol* 1974;**17**:136–92.
15. Doherty RL, Carley JG, Cremer MR, et al. Murray Valley encephalitis in eastern Australia, 1971. *Med J Aust* 1972;**2**:1170–3.
16. Doherty RL, Carley JG, Filippich C, et al. Murray Valley encephalitis in Australia, 1974: antibody response in cases and community. *Aust N Z J Med* 1976;**6**:446–53.
17. Doherty RL, Carley JG, Kay BH, et al. Murray Valley encephalitis virus infection in mosquitoes and domestic fowls in Queensland, 1974. *Aust J Exp Biol Med Sci* 1976;**54**:237–43.
18. Einsiedel L, Kat E, Ravindran J, et al. MR findings in Murray Valley encephalitis. *Am J Neuroradiol* 2003;**24**:1379–82.
19. French EL. Murray Valley encephalitis: isolation and characterisation of aetiological agent. *Med J Aust* 1952;**1**:100–3.
20. French EL, Anderson SG, Price AVG, et al. Murray Valley encephalitis in New Guinea. 1. Isolation of Murray Valley encephalitis virus from the brain of a fatal case of encephalitis occurring in a Papuan native. *Am J Trop Med Hyg* 1957;**6**:827–34.
21. Gard GP, Giles JR, Dwyer-Gray RJ, et al. Serological evidence of interepidemic infection of feral pigs in New South Wales with Murray Valley encephalitis virus. *Aust J Exp Biol Med Sci* 1976;**54**: 297–302.
22. Gard GP, Marshall ID, Walker KH, et al. Association of Australian arboviruses with nervous disease in horses. *Aust Vet J* 1977;**53**: 61–6.
23. Gordon AN, Marbach CR, Oakey J et al. Confirmed case of encephalitis caused by Murray Valley encephalitis infection in a horse. *J Vet Diagn Invest* 2012;**24**:431–6.
24. Johansen CA, Susai V, Hall RA, et al. Genetic and phenotypic differences between isolates of Murray valley encephalitis virus in Western Australia, 1972-2003. *Virus Genes* 2007;**35**:147–54.
25. Kalita J, Misra UK. Comparison of CT scan and MRI findings in the diagnosis of Japanese encephalitis. *J Neurol Sci* 2000;**174**: 3–8.
26. Kienzle N, Boyes L . Murray Valley encephalitis: case report and review of neuroradiological features. *Australas Radiol* 2003;**47**: 61–3.
27. Knox J, Cowan RU, Doyle JS, et al. Murray Valley encephalitis: a review of clinical features, diagnosis and treatment. *Med J Aust* 2012;**196**:322–6.
28. Lehmann NI, Gust ID, Doherty R. Isolation of Murray Valley encephalitis virus from the brains of three patients with encephalitis. *Med J Aust* 1976;**2**:450–4.
29. Lobigs M, Marshall ID, Weir RC, et al. Murray Valley encephalitis virus field strains from Australia and Papua New Guinea: studies on the sequence of the major envelope protein gene and virulence for mice. *Virology* 1988;**165**:245–55.
30. Mackenzie JS, Smith DW, Broom AK, et al. Australian encephalitis in Western Australia, 1979-1991. *Med J Aust* 1993;**158**:591–5.
31. Marshall ID, Woodroofe GM, Hirsch S. Viruses recovered from mosquitoes and wildlife serum collected in the Murray Valley of southeastern Australia, February, 1974, during an epidemic of encephalitis. *Aust J Exp Biol Med Sci* 1982;**60**:457–70.
32. Matthews V, Robertson T, Kendrick T, et al. Morphological features of Murray Valley encephalitis virus infection in the central nervous system of Swiss mice. *Int J Exp Pathol* 2000;**81**:31–40.
33. McMinn PC, Carman PG, Smith DW. Early diagnosis of Murray Valley encephalitis by reverse transcriptase–polymerase chain reaction. *Pathology* 2000;**32**:49–51.
34. Merritt A, Phillips DA, Carney I, et al. A presumptive case of fatal Murray Valley encephalitis acquired in Alice Springs. *Commun Dis Intell* 1998;**22**:103–4.
35. Nasveld PE, Ebringer A, Elmes N, et al. Long term immunity to live attenuated Japanese encephalitis chimeric vaccine: randomized, double blind, 5 year phase II study in healthy adults. *Hum Vaccin* 2010;**6**:1038–46.
36. Pedrau JR. The Australian epidemics of encephalomyelitis (X-disease). *J Pathol Bacteriol* 1936;**42**:59–65.
37. Pond WL, Russ SB, Rogers NG, et al. Murray Valley encephalitis virus: its serological relationship to the Japanese–West Nile–St. Louis encephalitis group of viruses. *J Immunol* 1955;**75**:78–84.
38. Pyke AT, Smith IL, van den Hurk AF, et al. Detection of Australasian flavivirus encephalitic viruses using rapid fluorogenic TaqMan assays. J. Virol. *Methods* 2004;**117**:161–7.
39. Robertson EG. Murray Valley encephalitis: pathological aspects. *Med J Aust* 1952;**1**:107–10.
40. Robertson EG, McLorinan H. Murray Valley encephalitis: clinical aspects. *Med J Aust* 1952;**1**:103–7.
41. Russell RC, Dwyer DE. Arboviruses associated with human disease in Australia. *Microbes Infect* 2000;**2**:1693–704.
42. Wong SH, Smith DW, Fallon MJ, et al. Murray Valley encephalitis mimicking herpes simplex encephalitis. *J Clin Neurosci* 2005;**12**: 822–4.

in sheep variously called louping ill in the British Isles and Spanish, Greek, or Turkish sheep encephalomyelitis in their respective countries.[43] Molecular taxonomic studies based on nucleotide sequence differences in the E glycoprotein gene of the virus show the early divergence of a mammal-associated clade from seabird-associated agents. Viruses in the mammal-associated clade exhibit a continuous east-to-west cline consistent with an evolutionary origin of TBEV in the Far East and dispersion westward to Europe and the British Isles (Fig. 175G-1).[31,88]

ECOLOGY

The viruses of RSSE and TBE are transmitted principally by hard ticks in the *Ixodes ricinus* complex—*I. ricinus* in Europe and *I. persulcatus* in the Far East.[35,70,107] Other tick vectors include *Ixodes arboricola*, *Ixodes hexagonus*, *Haemaphysalis punctata*, *Haemaphysalis concinna*, *Dermacentor marginatus*, and *Dermacentor reticulatus*. Viral circulation is maintained by continuous horizontal infection between ticks and animals and through the winter by vertical transmission in vector ticks and by latent infection in hibernating animals. The viruses are transmitted transstadially from larval to nymphal to adult tick stages and also transovarially. All stages of the tick and both male and female ticks transmit infection to animals and humans. Ixodid ticks feed on three hosts, one for each of the stages, during the typical 3-year life cycle. Larval and nymphal ticks feed preferentially on birds and small mammals, such as wild mice, voles, and dormice; adult ticks feed on larger mammals, such as roe deer, hedgehogs, foxes, hares, badgers, deer, domestic livestock (i.e., pigs, goats, sheep, and cows), dogs, cats, and humans. RNA sequence heterogeneity of the 5′ noncoding region in different TBE virus isolates recovered from the same biotope suggest that virus populations consist of subtypes with specificities for individual vertebrate hosts.[13] Infections in animals, except occasionally in dogs, are asymptomatic, and the viremia is of brief duration. Therefore, a large population of susceptible vertebrate hosts is needed to maintain viral transmission. Human infections are incidental to the natural cycle of transmission. Birds and large mammals contribute to the spread of vector ticks and viral foci.[80]

Ticks in the *I. ricinus* complex require high soil and ambient humidity (>80% to 90% relative humidity) and moderate temperature, within the 8°C (46.4°F) isotherm. They typically are found in the transitional vegetation zone from the forest edge to fields or meadows or in areas where dense brush or ground vegetation provides a sheltered microenvironment. Vector ticks are absent from mountainous areas with an elevation above 1200 to 1500 m. Foci of TBE transmission are restricted geographically and ecologically to these biotopes and have tended to be highly stable over years. However, global warming has been speculated to underlie the changes, both expansion and contraction of transmission foci, in northern and central-southern Europe,

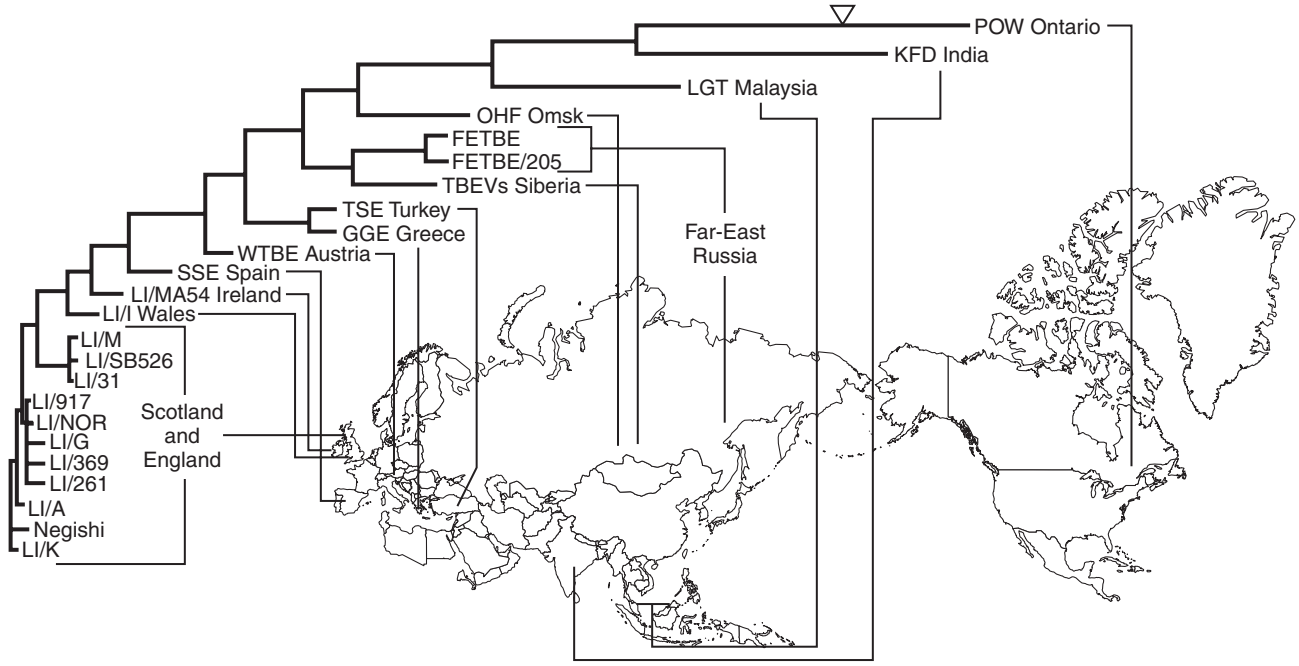

FIGURE 175G-1 ■ Cladogram of tick-borne encephalitis complex viruses based on E gene sequences presented from west to east with respect to louping ill *(LI)* virus. *FETBE,* Far Eastern tick-borne encephalitis; *GGE,* Greek goat encephalitis; *KFD,* Kyasanur Forest disease; *LGT,* Langat; *OHF,* Omsk hemorrhagic fever; *POW,* Powassan encephalitis; *SSE,* Spanish sheep encephalitis; *TSE,* Turkish sheep encephalitis; *WTBE,* Western tick-borne encephalitis. The phylogenetics of these and other mosquito-borne flaviviruses are rooted in the Powassan branch indicated by the triangle. (From Zanotto PM, Gao GF, Gritsun T, et al. An arbovirus cline across the Northern Hemisphere. Virology 1995;**210**:152-159.)

respectively.[79,107] New data also suggest a shift of tick activity to higher altitudes in recent years, potentially allowing vertical expansions of TBE foci.[21]

Tick activity varies with seasonal temperature and humidity. In central Europe, activity begins in March and April, reaches a peak in May, and declines during the summer in July and August. With the return of cooler temperatures, a second peak of activity occurs in September. In temperate regions, tick activity begins later and is greatest in the summer months. In Mediterranean climates, ticks are most active from November to January.

In foci with hyperendemic transmission, tick density may exceed 1/mm^2. Viral infection rates in ticks generally are in the range of 0.1 to 5 percent.[14] These rates typically are 10-fold lower than *Borrelia burgdorferi* (sensu lato) infection rates in *I. ricinus* in the same areas, although *I. persulcatus* infection rates greater than 10 percent have been reported.[63] The mechanisms underlying this difference are unclear but may include the following[100]:

- The variable infectiousness of the agents and their interactions with modulatory factors in tick saliva
- The relatively brief duration of TBE viremia in animals, which lasts only a few days and thus results in a reduced chance of transmission of virus to feeding ticks, as opposed to the persistent *B. burgdorferi* infections in rodents, with tick feeding more likely to result in infection
- The potential differences in the principal reservoir hosts for the respective infectious agents, with a more limited and focal distribution of important hosts for TBEV, such as goats.

The two agents evidently do not interfere with each other in their infection of *I. persulcatus* ticks; dually infected ticks and singly TBE-infected and *Borrelia*-infected ticks stand in a ratio of approximately 1 : 4 : 14.[63] *I. ricinus* also can be infected with and transmit *Francisella tularensis* and *Anaplasma phagocytophilum*, the agent of human granulocytic ehrlichiosis.[138]

The geographic distributions of RSSE and European TBE correspond to the ranges of their principal tick vectors; however, transmission is highly focal within this range because of the locations of biotopes that support viral circulation (Fig. 175G-2). New foci are reported periodically as a result of better recognition of the disease, its natural spread, human modifications of the landscape, and environmental and climate changes (e.g., global warming).[29,54,79,107]

EPIDEMIOLOGY

TBE has been recognized in all countries of Europe except Portugal and the Benelux countries, but endemic transmission is most intense in central Europe.[70,107] Human TBE has increased over the last 20 to 30 years in all endemic European countries except Austria, where a well-organized vaccination campaign was initiated.[6,52,79] The incidence of TBE previously ranged as high as 50/100,000 population in Austria, Poland, Hungary, Russia, the Czech Republic, Slovakia, and former Yugoslavia; in certain areas, similar levels of transmission still may prevail. Vaccination has reduced the incidence of disease regionally, especially in Austria, where a national program of immunization since 1981 has reduced the incidence to less than 1/100,000 population.[142] Currently, isolated cases are reported from Norway, Denmark,[128] France,[39] Greece, and Liechtenstein, and fewer than 200 cases are reported annually in Sweden, Germany, and Italy. The Swedish Institute for Communicable Disease Control recently reported the highest annual incidence of human TBE in 2011 and 2012.[52] Switzerland reported a threefold increase in human TBE cases between 2004 and 2006,[143] which resulted both from more infections in endemic areas and from infections in newly recognized foci. Since then, the number of newly diagnosed cases in Switzerland has lowered again, but remained higher than before 2004. In the Far East, cases of RSSE occur principally in forest workers. The disease is recognized in Russia and China, and the first cases acquired in Japan (transmitted by *Ixodes ovatus*) were reported recently.

Within each country, the distribution of cases is highly focal in certain areas and may vary with each year. Local seroprevalence may exceed 20 percent, but the general seroprevalence usually is less than 1/100,000 population. Frequent exposure during long-term residence leads to a general trend of increasing seroprevalence with age. Seroprevalence rates as high as 50 percent have been observed in groups at high risk for exposure, such as farmers and forestry workers. Although, in general, rates are lower (1% to 5%) and in certain groups are similar to the seroprevalence of hantaviruses, lymphocytic choriomeningitis virus, and *Anaplasma* spp., *B. burgdorferi* seroprevalence rates are 10-fold higher or more.[92,98] The frequency of clinical cases of TBE, borreliosis, and dual infection closely approximates the relative frequency of tick infection, singly and dually. Adults 20 to 50 years of age characteristically accounted for most of the cases. In some studies, cases in males (adults and children) predominate by a ratio of 2 : 1.[27,40,78]

Cases have occurred in children as young as 3 weeks,[50,55] but generally, risk in children increases with age as a result of their increased mobility and activity in the sylvatic environment.[78] These epidemiologic patterns are changing in areas with high immunization coverage.[142] Vaccination effort has focused principally on hyperendemic areas and on high-risk occupational groups such as forestry workers. The low number of cases currently reported from areas where vaccination coverage is high belies the continued transmission of virus in these locations.

Cases may be acquired during outdoor activities, such as berry picking and mushroom gathering, and infection occasionally has been acquired from ticks brought from endemic areas on Christmas trees and other objects. One study found that the seroprevalence in Swedish orienteers (1%) was not substantially different from the general seroprevalence in residents of Stockholm County (5%), an area where TBE is endemic. The absence of a higher risk for acquiring TBE in persons with occasional sylvatic exposure reflects the low infection rate of ticks and the generally low risk to persons with sporadic or short-term exposure. Neither of two studies of American

FIGURE 175G-2 ■ The geographic distributions of Russian spring-summer encephalitis and European tick-borne encephalitis correspond to the ranges of their principal tick vectors; however, transmission is highly focal within this range because of the locations of biotopes that support viral circulation.

soldiers stationed in central Europe found a clinical case, although one seroconversion in 3297 person-months of exposure, an infection rate of 0.9/1000 person-months, and four seroconversions in 959 persons (0.4%) were detected.[90,117] With the dissolution of the former Soviet Union and increasing commerce with eastern Europe, interest in the risk for TBE being acquired by travelers to Europe and Russia has increased. The available data suggest that the risk is low for most travelers and that vaccination is not indicated except for unusual circumstances of prolonged stay in an endemic area.

The seasonal distribution of cases lags roughly 1 month behind that of tick activity and extends from April until November. The peak incidence in Sweden is in August; in Austria, the peak occurs in June and July, with a secondary rise in October.

Milk-borne TBE previously accounted for 10 to 20 percent of all cases in central Europe. Infections

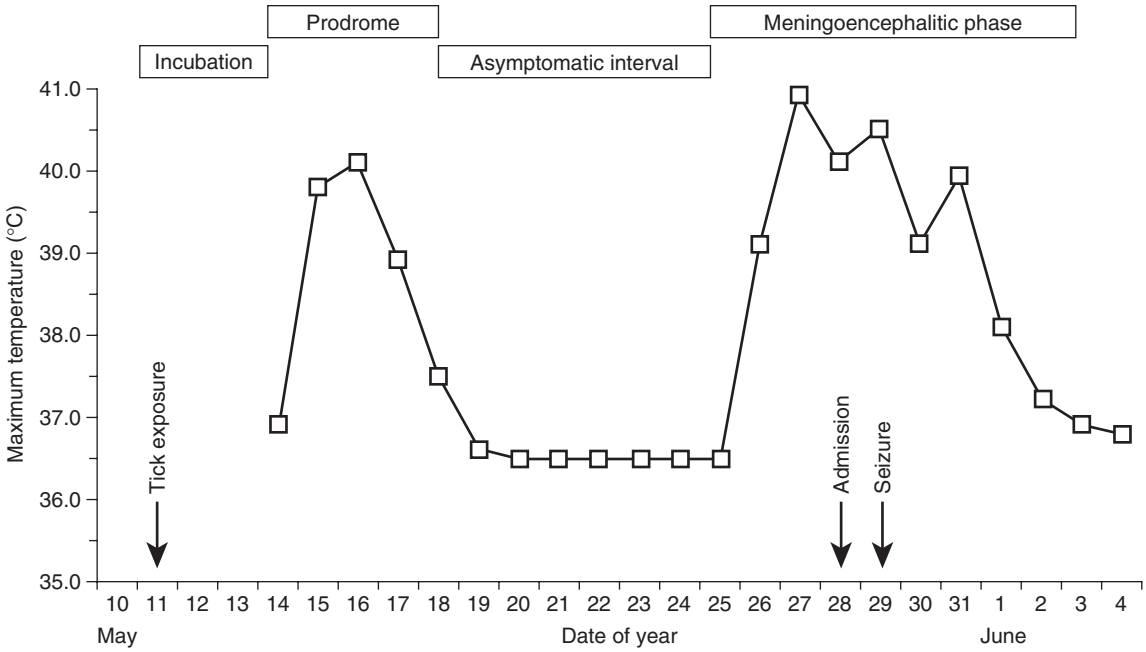

FIGURE 175G-3 ■ Clinical course of tick-borne encephalitis (TBE) in a 5-year-old boy exposed to a tick bite in a known endemic area for TBE in the pre-alpine region of central Switzerland. Lumbar puncture on admission revealed mild cerebrospinal fluid (CSF) pleocytosis (14×10^6/L, 50% polymorphonuclear cells). Serum anti-TBE virus IgM and IgG were present; serum and CSF antibodies against *Borrelia burgdorferi* were absent. CSF enteroviral polymerase chain reaction was negative. Recovery was uneventful.

frequently were acquired from consuming unpasteurized milk or cheese from infected goats, sheep, and cows, and outbreaks resulting in thousands of cases have been reported. Transmission from infected milk now is a rare occurrence, but as recently as 1994, an outbreak in Slovakia led to 7 cases in a group that regularly drank raw milk from a family goat.[63] Also, an outbreak of 21 human cases caused by contaminated cheese produced from unpasteurized sheep milk has been reported.[139] Contact infection, acquired during slaughter of an infected goat, also has been observed.

CLINICAL MANIFESTATIONS

Seroepidemiologic studies indicate that as many as 90 percent of human infections with TBEV remain asymptomatic or result in a nonspecific illness.[2,7,38,59] The classic manifestation of the European form of TBE is an acute febrile illness characterized by a biphasic course consisting of a nonspecific prodromal syndrome followed by central nervous system (CNS) disease (Fig. 175G-3).[34,136] Infection with TBEV may occur solely as the primary, nonspecific phase without the secondary CNS phase.[26,84] Infection with Far Eastern strains of TBEV results in a more severe, monophasic illness that progresses directly to neurologic involvement with a poorer prognosis for survival and full recovery. TBE has been observed in all age groups. The median age in pediatric case series is 8 to 10 years (range, 0 to 17 years).[17,40,78,81,134] The youngest patient with serologically and virologically documented TBE described in the literature was a 17-day-old infant.[55] Congenital infection has not been reported.

The clinical features of TBE in children are summarized in Table 175G-1. The diagnosis of TBE should be considered in all acutely ill patients with fever, CNS abnormalities, and a history of potential tick exposure or ingestion of raw milk in an area endemic for TBE. A high degree of diagnostic awareness is required because the clinical presentation of TBE is nonspecific. After an incubation period of 2 to 28 days, in most cases 7 to 14 days,[27,40,78,81,136] the patient may have a prodromal illness consisting of fever, malaise, nausea and vomiting, headache, myalgia, and, occasionally, upper respiratory tract symptoms.[27,40,65,136] Defervescence occurs after 2 to 7 days, and the patient subsequently remains asymptomatic for 1 to 20 days, usually 2 to 10 days.[27,40,65,78,134] This prodromal illness may be absent. In various case series, a biphasic course was reported in 30 to 90 percent of children with TBE.[40,57,78,81,134] An abrupt onset of fever, headache, emesis, and symptoms of meningeal irritation heralds the beginning of the second phase of disease.[81] In adults, no association exists between the length of the incubation period and the severity of clinical illness.[57] The typical evolution of fever in a child with TBE is shown in Figure 175G-4.

In this second phase of illness, most affected children (50% to 90%) have meningitis without clinical evidence of parenchymal CNS involvement.* In adult patients, by

*References 40, 64, 68, 81, 95, 112, 134.

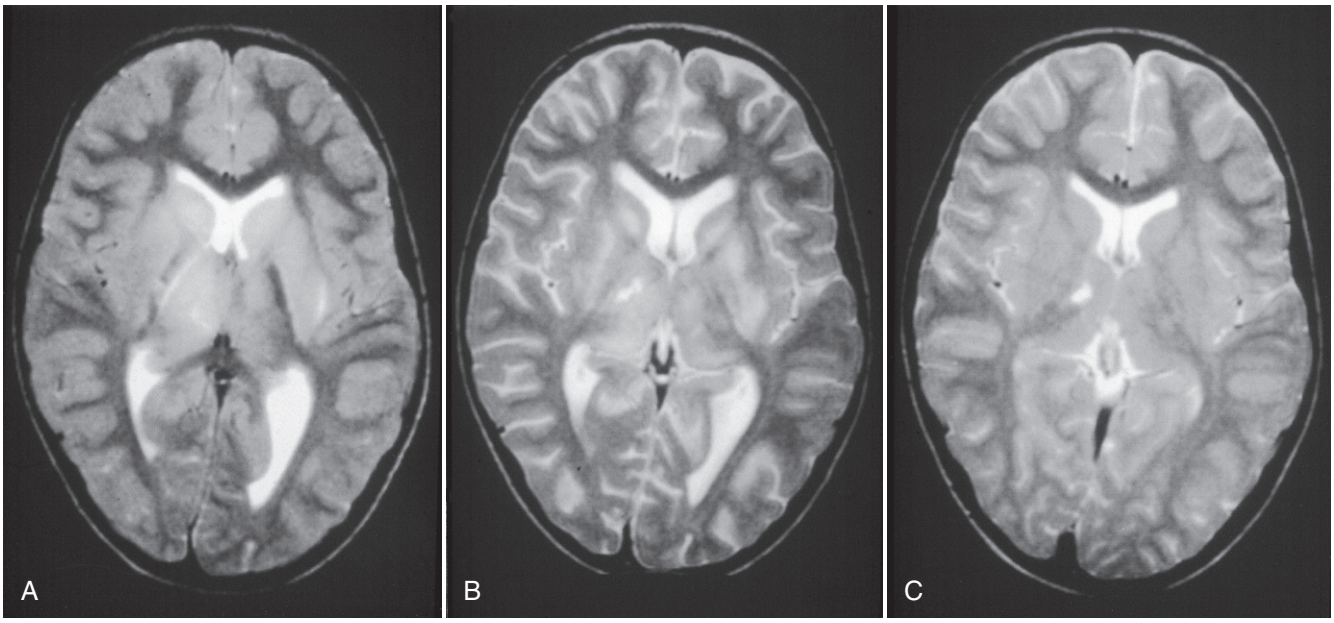

FIGURE 175G-4 ■ T2-Weighted magnetic resonance imaging series of a 5-year-old girl with a severe course of tick-borne encephalitis. **A,** Acute phase. Note the T2 hyperintensity in the right side of the thalamus, basal ganglia, and diencephalon. **B,** One month later, partial recovery of T2 hyperintensity and enlargement of cerebrospinal fluid spaces can be seen. **C,** Three months later, normal T2 intensity and a cerebrospinal fluid–filled cavity in the right side of the thalamus are apparent. (Courtesy David Nadal, MD, University Children's Hospital, Zurich, Switzerland.)

TABLE 175G-1 **Clinical Features of Serologically Documented Tick-Borne Encephalitis in Five Pediatric Case Series (333 Children)**

Clinical Features	Rate (%)
History	
Tick exposure	47-78
Biphasic illness	77-90
Major Symptoms at Presentation	
Fever >38.0°C (100.4°F)	100
Headache	90-100
Vomiting	60-90
Central Nervous System Signs	
Nuchal rigidity	74-90
Photophobia	10-25
Impaired consciousness	9-11
Ataxia or tremor	5-26
Seizures	0-5
Paresis	0-5
Extent of Central Nervous System Involvement	
Meningitis alone	49-89
Meningoencephalitis	9-48
Meningoencephalomyelitis	0-5
Fatal outcome	0
Cerebrospinal Fluid Parameters	
Pleocytosis >15 × 10^6/L	98-100
Glucose normal	100
Protein elevated	9-55
Abnormal electroencephalogram	80-87

Data from references 16, 36, 52, 74, 76, 127.

contrast, meningitis without encephalitis or myelitis occurs only in 20 to 60 percent of cases.[2,57,65,108,127] On physical examination, fever, signs of meningeal irritation, and photophobia are the most common features.[81,134] In uncomplicated cases, patients defervesce within 5 to 10 days.[40,78,81,134] Meningoencephalitis, meningoencephalomyelitis, or both manifested by impaired consciousness, seizures, and focal neurologic signs, including limb paresis, occurs in 0 to 34 percent of children.* In a large series of 139 pediatric patients with TBE meningoencephalitis, Lesnicar and coworkers[78] observed tremor in all patients, impaired consciousness in 27 (7%), ataxia in 18 (5%), behavioral changes in 16 (4%), cranial nerve paresis in 14 (4%), and limb paresis in 11 (3%). In a series of 13 children, Harasek[40] observed ataxia in 10 patients, somnolence in 4, paresthesias and seizures in 2 each, and central facial palsy and nystagmus in 1 each. In 2 of these patients, transient unilateral shoulder girdle weakness that occurred during the second week of CNS disease suggested involvement of the cervical anterior horn or radiculitis. Overall, limb or cranial nerve paresis occur in 0 to 5 percent of children with TBE (see Table 175-1). In a series of 133 children with TBE, Cizman and colleagues[17] reported transient paresis in 5 patients and irreversible hemiparesis in 1.

In large case series of predominantly adult patients, a paralytic course secondary to bulbar, spinal, or radicular injury was observed in 5 to 25 percent of cases.† Unilateral, flaccid paresis of an upper extremity is the most common manifestation of lower motor neuron disease

*References 17, 40, 57, 78, 81, 134.
†References 57, 61, 90, 127, 131, 136.

complicating adult TBE.[90,131,136] Involvement of the cranial nerves occurs somewhat less frequently and is revealed most commonly by external ocular muscle paralysis (usually cranial nerve VI), peripheral facial palsy (VII), otovestibular manifestations (VIII), or involvement of the pharyngeal muscles (IX, X, and XI).[27,57,90,129] Lower extremity weakness and, occasionally, autonomic nervous system affliction manifested as bladder dysfunction also may occur.[27,58,129] Whereas most manifestations of parenchymal CNS involvement evolve during the acute stage of TBE, paralysis resulting from radiculitis may develop up to 14 days after the onset of CNS disease.[40,70]

As many as 5 percent of children with TBE experience seizures during the acute stage of TBE.[17,40,134] Because most patients are older than 6 years and thus are unlikely to suffer from febrile seizures, these episodes probably reflect encephalitis. In adults, seizures have been observed in less than 2 percent of patients.[27,57]

Extracerebral manifestations of TBE seldom are reported and are of minor clinical relevance. Mild hepatitis[45,57] and electrocardiographic abnormalities have been described in adults.[132] A single case of myopericarditis in a child with TBE has been reported.[28]

The peripheral white blood cell (WBC) count is not altered in a characteristic way. Although it is usually in the normal range, both leukopenia and moderate leukocytosis may be found.[40,81,129] In adult patients, several investigators observed leukopenia during the viremic prodrome and normal or moderately elevated WBC counts during the second phase of illness.[57,65,81,85] In a report describing hematologic values during the prodromal phase of TBE, 23/28 (82%) patients had mild to moderate thrombocytopenia (60 to 130 $\times$ 10^9/L), with values returning to normal during the second phase of illness.[85] Cerebrospinal fluid (CSF) analysis in children with serologically proven TBE reveals predominantly mononuclear pleocytosis in all patients. However, as with enteroviral meningitis, neutrophils may predominate during the first 1 to 3 days of illness.[53] Typically, the CSF WBC count is 100 to 1000 $\times$ 10^6/L.* On occasion, lower values occur, as reported by Krausler and associates,[64] who found less than 100 $\times$ 10^6/L in 24/75 (32%) pediatric cases. Wahlberg and colleagues[136] reported an absence of pleocytosis in 18 percent of 94 adult patients examined. Harasek[40] could not find any correlation between the CSF leukocyte count and the severity of clinical disease. In contrast, some investigators[57,58,61] reported that adult patients with a high CSF white blood cell count (>300 $\times$ 10^6/L) were more likely to experience a severe course of TBE and persistent neurologic sequelae. CSF pleocytosis disappears within 4 to 5 weeks of the onset of acute CNS disease. The CSF glucose concentration is normal,[81] and the protein concentration is normal or moderately elevated (<1000 mg/L),[40,65,81,129] with evidence of dysfunction of the blood-brain barrier in most patients.[58] Electroencephalographic examination findings during acute TBE usually are abnormal and characterized by a nonspecific reduction in rhythmic background activity, bilateral periodic slowing, and, rarely, focal abnormalities.[40,56,57] Attenuation of background activity with periodic delta groups has been shown to correlate with parenchymal CNS involvement in adult TBE patients.[56] Reorganization of electroencephalographic activity commonly lags behind clinical improvement, and abnormalities may persist for months to years.[40,56,134]

The limited information available on magnetic resonance imaging in children with severe TBE indicates that parenchymal lesions characteristically are located in the thalamus (see Fig. 175G-4).[17,57,60,137] The diagnostic and prognostic value of neuroimaging studies in pediatric TBE has not been established. In adults, magnetic resonance imaging studies may detect abnormality in approximately 20 percent of patients.[57] Focal lesions are more likely to be found in those with severe neurologic abnormalities[57] and are confined mainly to the thalamus.* Less frequently, lesions are located in the basal ganglia,[5,57,82,105] cerebellum,[10,57] brain stem,[11,17,57] and anterior portions of the cervical spine.[10,17]

PATHOLOGY

CNS findings are mainly those of acute meningeal inflammation and focal gray matter encephalomyelitis. The white matter rarely is involved. Macroscopic findings include congestion of the leptomeninges and swelling and hyperemia of the cerebral parenchyma, particularly in the brain stem and cervical region of the spinal cord.[35,97] Petechial hemorrhages are seen in the brain stem, the anterior horns of the spinal cord, and, less consistently, the cerebellum and the anterior central region of the cortex. Histologic changes are dominated by infiltration and ganglion cell damage in the gray matter of these same areas. Changes are particularly pronounced in the anterior horns of the cervical spine (consistent with the poliomyelitis-like manifestations of paralytic courses of TBE),[121] the medulla oblongata, the cranial nerve nuclei of the pons, and the Purkinje cell layer of the cerebellum.[35] Inflammatory foci are characterized by lymphocytic perivascular infiltration and various stages of degenerative changes in neuronal cells. Areas of neuronal necrosis are characterized by perifocal edema, lymphocytic and neutrophilic infiltration, and, at a later stage, nodular microglial proliferation at sites of complete neuronophagia. The presence of granzyme, B-expressing, cytotoxic T cells in close contact with TBE-expressing neurons indicates that immune mechanisms contribute to tissue injury in these areas.[32] Rarely, the spinal nerve roots, spinal ganglia, and peripheral nerves are involved. Spongiform changes, particularly after protracted illness, appear as sharply defined areas of softening with minimal inflammatory reaction. In the Far Eastern form of the disease, extensive poliomyelitis of the spinal cord with destruction of anterior horn cells, particularly in the upper cervical and lower lumbar areas, and poliomyelitis of the brain stem are noted.

*References 40, 67, 78, 81, 129, 134.

*References 5, 10, 57, 82, 105, 135, 137.

PATHOGENESIS

Tick-mediated inoculation of TBEV and viral replication within local dermal cells are followed by lymphatic spread to regional lymph nodes, where further replication occurs.[58] Subsequently, viremia leads to generalized infection, especially of reticuloendothelial cells, followed by secondary rounds of viremia that result in neuroinvasion. Viral penetration of the CNS occurs through capillary endothelia and by TBEV-infected, infiltrating mononuclear cells. Envelope glycoprotein E, the immunodominant TBEV-encoded surface protein, mediates attachment to and fusion with the host cellular membrane.[87] The molecular mechanisms of neural invasion by TBEV have not been elucidated in detail. Current knowledge of the molecular pathogenesis of TBEV has been reviewed elsewhere.[41-43]

LABORATORY DIAGNOSIS

Because neither clinical nor CSF findings differentiate TBE from other causes of meningitis or meningoencephalitis, the diagnosis of TBE rests on demonstration of specific antibody by serologic testing. The presence of specific serum immunoglobulin (Ig)M or a significant rise in titer of specific IgG antibody in paired sera (or both) is diagnostic of TBE[48,49,58] unless the patient previously received TBE vaccine doses. Enzyme-linked immunosorbent assay (ELISA) has replaced complement-fixation, hemagglutination-inhibition, and neutralization assays in the routine laboratory diagnosis of TBE.[47,49] ELISA technology offers increased sensitivity, reliably differentiates between IgM and IgG antibodies, and, in contrast to neutralization, uses nonviable viral antigens.[44,112] For the detection of specific anti-TBEV IgM, an IgM-capture ELISA system has proved to be more sensitive and specific than the conventional three-layer ELISA system because high titers of specific IgG and rheumatoid factor do not interfere with IgM binding.[44,112] With this method, specific IgM virtually always can be demonstrated during the acute illness and thereafter may persist for as long as 9 months.[48,58,112] The highly sensitive capture ELISA format allows a determination of anti-TBEV IgM, even in serum samples obtained late during the acute illness, when high titers of specific IgG already are present. A potential problem of TBEV IgG ELISA systems is cross reactivity with other flaviviruses, notably yellow fever virus, dengue virus, and Japanese encephalitis virus.[24,25,99] This limitation should be considered in the interpretation of seroprevalence studies and in patients with a relevant travel or immunization history.

Specific IgM and IgG also can be measured in CSF.[44,112] Detection of TBEV-specific intrathecal antibody production by ELISA is highly specific for the diagnosis of TBE but somewhat less sensitive than is determination of serum IgM during the first several days of CNS disease.[49,58] Intrathecal TBEV antibody assays are less widely used in clinical practice, but they may be useful if serum antibody titers fail to differentiate between infection- and vaccine-induced immunity.

Detection of virus by culture is technically feasible, but it is not used routinely in clinical practice. TBEV can be recovered from blood by viral culture, but because detectable viremia occurs during the prodromal stage (when the diagnosis of TBE seldom is considered), culture is not useful in clinical practice. TBEV may be recovered from the brain tissue of patients who died at an early stage of disease.[18] Amplification of TBEV-specific RNA by reverse transcription–polymerase chain reaction (RT-PCR) has been established for detection of TBEV in ticks, in tick surveys as part of national surveillance systems,[30,106] and, in humans, for individual patient diagnosis[126] Primers that amplify the highly conserved 5′ noncoding region of the TBEV genome have been shown to be sensitive and specific.[125] Successful amplification of TBEV-specific RNA from blood, CSF, or brain tissue has been reported.[19,126,133,137] However, like culture, RT-PCR usually is positive only during the prodromal stage and thus generally is not useful in clinical practice.[55]

DIFFERENTIAL DIAGNOSIS

The clinical course of TBE is nonspecific in most cases, and a history of exposure to ticks in an endemic area can be elicited in 40 to 75 percent of pediatric patients.* Because serologic confirmation of the diagnosis is not available immediately in most cases, the differential diagnosis includes a wide spectrum of diseases causing fever and CNS manifestations.

Enteroviruses are the most common cause of symptomatic CNS infection in children during the warm seasons in regions where TBE is prevalent. Fever in enteroviral infection may occasionally be biphasic,[93] although the duration of both the first phase and the asymptomatic interval usually is shorter than that noted in TBE.[15] Routine tests of CSF do not differentiate between the two entities. Skin and mucosal manifestations are indicative of enteroviral infection rather than TBE, whereas encephalitis and myelitis can occur in both. Enteroviral meningitis is diagnosed most readily by PCR of CSF[118] or by culture of virus from CSF and mucosal surfaces. Mumps meningitis is to be considered in the differential diagnosis in cases without parotid enlargement, particularly in areas with low rates of mumps immunization.[130] The diagnosis of mumps meningitis is made by viral culture of CSF or detection of specific serum IgM antibody. Although it is a rare occurrence in childhood, herpes simplex virus (HSV) encephalitis always should be considered in the differential diagnosis. Because the case-fatality rate of HSV encephalitis is greater than 90 percent if it is left untreated, therapy with intravenous acyclovir should be initiated without delay, if that diagnosis is thought possible. The combination of fever, which may be biphasic,[113] and localizing signs observed by neurologic examination, electroencephalographic studies, or magnetic resonance imaging[51] suggests HSV encephalitis and rarely occurs in pediatric TBE, particularly if the temporal lobe or the orbital portion of the frontal lobe is affected. Meningeal

*References 40, 57, 78, 81, 95, 124, 134.

irritation usually is absent in HSV encephalitis. The diagnosis of HSV encephalitis is confirmed by PCR of CSF.[9,76] Epstein-Barr virus encephalitis can occur in immunocompetent children, and Epstein-Barr virus shares with TBEV a preference for causing thalamic and basal ganglia lesions.[57,102] Other viral causes include common respiratory tract viruses such as influenza, parainfluenza, and adenovirus.

Among bacterial infections, partially treated pyogenic meningitis, encephalitis in association with *Mycoplasma pneumoniae* infection,[115] and encephalopathy caused by *Bartonella henselae* may resemble TBE. Tuberculous meningitis runs a subacute course, and although near-normal CSF chemistry may be recorded very early in the disease, hypoglycorrhachia invariably develops and CSF protein rises to high levels. CNS infection by *B. burgdorferi* is particularly important in the differential diagnosis because in Europe this pathogen and TBEV are transmitted by the same tick vectors—*I. ricinus* and *I. persulcatus*—and because one of the most prevalent European genospecies, *Borrelia garinii*, is more neurotropic than *B. burgdorferi* sensu stricto.[114] In contrast to TBE, the incubation period for early neuroborreliosis in children generally is 4 to 10 weeks, high-grade fever is unusual, and most patients have cranial nerve paresis. However, several cases of concomitant infection with TBEV and *B. burgdorferi* have been reported in the literature,[1,16,66,101] and coexistence of TBEV and *B. burgdorferi* in ticks has been documented.[63] In some cases of TBE, persistent or late-appearing limb paralysis has been suggested to be attributed to concomitant, but undiagnosed, borrelial radiculoneuritis rather than TBE-related myelitis with anterior horn involvement.[66] In this situation, diagnostic evidence of Lyme borreliosis should be sought because this condition requires antimicrobial therapy. Seroepidemiologic and clinical evidence indicates that concomitant infection with TBEV and *Anaplasma phagocytophilum*, the agent of human granulocytic ehrlichiosis, also may occur.[63,83]

TREATMENT

No specific antiviral therapy is available to treat TBE. However, recent studies suggest tick-borne encephalitis virus is inhibited by LCTA-949, an analog of the antibiotic teicoplanin, and the anthelminthic compound ivermectin.[22,89] In severe cases, supportive therapy is aimed at preventing the development of sequelae related to increased intracranial pressure, seizures, and bulbar dysfunction. After the acute stage, neurorehabilitation may be necessary for patients with motor, cognitive, or emotional disturbances. Because TBE rarely causes a chronic seizure disorder, prolonged anticonvulsive therapy seldom is indicated in patients who had convulsions during acute disease.

PROGNOSIS

The main risk factor for neurologic residua and a fatal outcome of TBE is advanced age.[2,57,127] Substantial sequelae appear to be infrequent in children,* although no prospective long-term follow-up studies of pediatric TBE patients have been performed to date. Individual cases of children, presenting more likely with meningoencephalitis in the acute phase, show persistent neurologic damage, including hemiparesis,[17,55,60,137] unilateral arm paresis,[136] epilepsy,[55,60,111] and extrapyramidal movement disorder.[60] Recent evidence, however, indicates that subtle abnormalities in attention and psychomotor skills may persist in many children for prolonged periods.[122] In adult patients, long-term neurologic defects are observed in 2 to 10 percent of patients with clinical evidence of parenchymal CNS involvement during the acute illness.[2,34,57,61,136] Risk factors for persistent sequelae have been assessed by Kaiser[57] and, by univariate analysis, include impaired consciousness (i.e., Glasgow Coma Scale score <7), ataxia, paresis, abnormal findings on magnetic resonance imaging, CSF pleocytosis greater than 300×10^6/L, and CSF protein concentration of more than 600 mg/L. Gunther and colleagues[37] reported that encephalitic symptoms were associated with low levels of intrathecal IgM in early stages of TBE. Paresis of the extremities and ataxia are the most common persistent findings on neurologic examination.† Much more common, however, are ill-defined manifestations such as chronic fatigue, headache, sleep disorders, memory dysfunction, and emotional disturbances, which are reported by most adults recovering from TBE and persist for months to years.[27,77,127] In some patients, electroencephalographic findings may remain abnormal for prolonged periods, with seizure activity occasionally demonstrated.[40,122] Most of these patients are asymptomatic, although epilepsy secondary to TBE has been described infrequently.[40,96] The reported case-fatality rate in adult TBE patients is approximately 1 percent in large series,‡ with most deaths being attributed to severe bulbar encephalitis or related to underlying cardiovascular disease in elderly patients.[27] Fatal outcome of TBE in children is exceedingly rare.[91]

PREVENTION

General preventive principles include avoidance of known endemic areas of TBE, use of protective clothing, and rapid removal of ticks attached to the skin. The topical repellent *N,N*-diethyl-*m*-toluamide (DEET) has a definite albeit moderate effect against ticks.[116] Because of its potential neurotoxicity, the content of DEET in products used for children should not exceed 15 percent.[13,103] These measures are effective in reducing tick exposure and transmission of TBEV to some degree. Pasteurization of raw milk prevents enteric transmission of TBEV.[4,62]

Reliable protection, however, requires active immunization against TBE. Inactivated whole-virus vaccines are produced in Austria (Baxter), Germany (Chiron), and Russia. The vaccines are made from different central

*References 40, 57, 68, 81, 122, 134.
†References 2, 27, 57, 61, 86, 127.
‡References 2, 27, 34, 57, 61, 86.

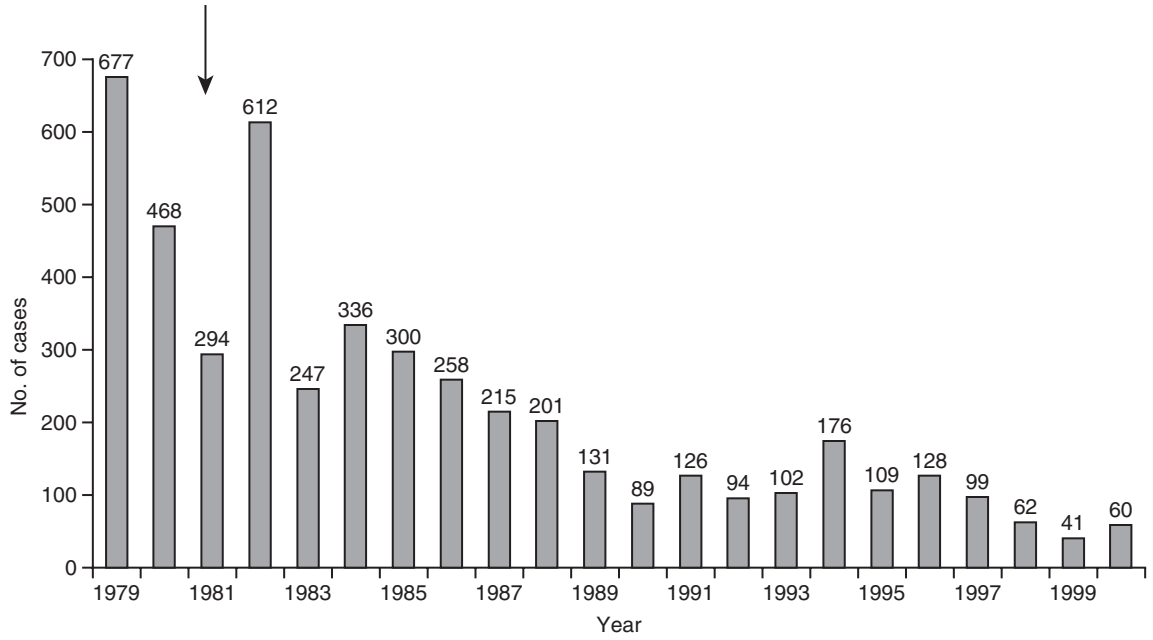

FIGURE 175G-5 ■ Annual number of cases of tick-borne encephalitis in Austria before and after the introduction of mass immunization in 1981 *(arrow).* (Data obtained from the International Scientific Working Group on Tick-Borne-Encephalitis, http://www.tbe-info.com/report.)

European TBEV strains and are produced by concentrating and purifying cell culture fluid from infected chick embryo cells. Adjuvants (aluminum hydroxide) are added to the formalin-inactivated cell culture fluid. The Russian vaccine is an inactivated, unconcentrated cell culture fluid from infected African green monkey kidney cells. TBE vaccine is available in the United States under investigational drug exemption to military personnel deployed in endemic areas.[12,20]

Currently approved TBE vaccines are approved for children 12 months of age and older. Basic immunization consists of three doses given at 0, 1 to 3, and 5 to 12 months. Rapid immunization schedules also are available. The vaccines are immunogenic and safe.* Seroconversion rates for neutralizing antibodies after the first, second, and third dose are 70, 95, and 99 percent, respectively.[69,73] Reliable protection is achieved 2 weeks after administration of the second dose.[141] Data on the kinetics of vaccine-induced serum antibodies led to the first recommendation for booster doses every 3 years.[69,71] Recent data on the decay of neutralizing antibodies, however, indicate that protection may last considerably longer[109,110]; therefore, booster doses are recommended only every 5 to 10 years in some European countries. Minor adverse events (i.e., fever and local reactions) are reported by 4 to 15 percent of vaccinees after receiving the first dose and less frequently after receiving subsequent doses.[71,104,140] Mostly transient neurologic adverse events temporally associated with the administration of TBE vaccine have been reported rarely.[33,34,119,120,123] Their true incidence has not been established. Based on passive notification of adverse events, an incidence of one neurologic illness in 1 million doses of TBE vaccine has been calculated.[46] Placebo-controlled trials of vaccine efficacy have not been conducted. Observational studies in Austria using historical controls indicate that the effectiveness of the vaccine is greater than 90 percent.[72,75]

In Austria, the TBE vaccine is recommended for mass immunization of all potentially exposed individuals. Since the beginning of the vaccination campaign in 1981, a dramatic reduction in nationally notified cases has been observed (Fig. 175G-5).[75,142] Mass immunization is the probable cause of this decrease because neighboring countries (e.g., Switzerland, southern Germany, and Slovenia) reported stable or increasing numbers of cases of TBE during the same time. In these and other endemic countries of central and eastern Europe, authorities recommend immunization of school-aged children and adults who live in endemic areas and have a risk for exposure to ticks.[17,98] In younger children, TBE vaccination is not recommended routinely because neurologic complications are exceedingly rare.[17,78]

Specific IgG has been used exclusively for passive immunization within 96 hours of exposure to ticks in endemic regions. Because of reports from some patients[3,74] who developed a severe form of TBE disease after immunoglobulin administration, these products have been withdrawn in many countries. These severe cases raised concern about a causative role of TBE immunoglobulin in enhancement of disease.* Antibody-dependent enhancement, a mechanism of pathogenicity proposed in dengue hemorrhagic fever,[94] was discussed as a possible explanation,[60] but experimental data in support of this hypothesis in TBE are lacking.[60]

*References 23, 33, 34, 36, 104, 123.

*References 8, 57, 60, 82, 135, 137.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Arnez M, Avsic-Zupanc T. Tick-borne encephalitis in children: an update on epidemiology and diagnosis. *Expert Rev Anti Infect Ther* 2009;**7**:1251–60.
22. De Burghgraeve T, Kaptein S, Ayala-Nunez N, et al. An analogue of the antibiotic teicoplanin prevents flavivirus entry in vitro. *PLos One* 2012;**7**:e37244.
23. Demicheli V, Debalini MG, Rivetti A. Vaccines for preventing tick-borne encephalitis. *Cochrane Databse Syst Rev* 2009;**21**(1):CD000977.
30. Gaumann R, Muhlemann K, Strasser M, et al. High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. *Appl Environ Microbiol* 2010;**76**:4241–9.
52. Jaenson T, Hjiertqvist M, Bergstrom T, et al. Why is tick-borne encephalitis increasing? A review of the key factors causing the increasing incidence of human TBE in Sweden. *Parasit Vectors* 2012;**5**:184–95.
80. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet* 2008;**371**:1861–71.
88. Mansfield KL, Johnson N, Phipps L, et al. Tick-borne encephalitis virus-a review of an emerging zoonosis. *J Gen Virol* 2009;**90**: 1781–94.
89. Mastrangelo E, Pezzullo M, De Burghgraeve T, et al. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J Antimicrob Chemother* 2012;**67**:1884–94.

The full reference list for this chapter is available at expertconsult.com.

175H ■ OTHER FLAVIVIRAL INFECTIONS

Theodore F. Tsai

POWASSAN ENCEPHALITIS

Powassan virus was isolated from a fatally infected patient and named after the patient's Ontario town of residence.[7] The virus is classified within the antigenic complex of tick-borne flaviviruses, with North American viruses circulating in two genetic lineages in distinct enzootic cycles.[9] More than half of surviving cases suffer from residual neurologic deficits, frequently associated with upper cervical damage leading to upper extremity muscle weakness and wasting.

Epidemiology and Ecology

Fewer than 50 sporadic cases of naturally acquired Powassan encephalitis have been reported from North America and others from Russia, as well as two laboratory-acquired cases. Approximately one fourth of the cases from North America have occurred in children younger than 15 years, and the preponderance of cases have been in males. Most cases have occurred in the summer or early fall, and one patient had an onset in December.[14,30]

Reported cases have been acquired chiefly in eastern and upper midwestern U.S. states and Canadian provinces; however, the geographic distribution of the virus is far wider, with strains recorded from West Virginia, Colorado, South Dakota, and California, and serologic evidence of infection in humans or animals reported from Wyoming, North Dakota, British Columbia, Alberta, and Sonora, Mexico.[9,20] Viral isolates also have been recovered from ticks, mosquitoes, and birds from southeastern Russia, and evidence of viral transmission in China and Southeast Asia has been reported.[26]

In North America, viruses in lineage 1 are transmitted to small mammals by *Ixodes cookei* ticks, chiefly to groundhogs, skunks, and weasels; by *Ixodes marxi* ticks mainly to red squirrels; and by *Dermacentor andersoni* ticks to chipmunks.[9] The virus is transmitted trans-stadially in *D. andersoni* and *Ixodes pacificus*, and transovarial transmission has been shown in other species. Lineage 2 viruses (deer tick virus) are transmitted by *Ioxdes scapularis;* however, although infections have been demonstrated in *Peromyscus* mice, details of the viral transmission cycle are poorly defined. Because *I. scapularis* also is the vector of *Borrelia burgdorferi*, *Babesia microti*, and *Anaplasma phagocytophilum*, it is of interest that in a collection of adults ticks from Long Island and Westchester County, New York, 2 percent were infected with Powassan virus, whereas infection rates for the other pathogens were 64, 20, and 20 percent, respectively.[38] The absence of a Powassan epidemic paralleling that of Lyme disease suggests differences in the agents' transmission cycles. Because the deer tick virus has been implicated in two fatal Powassan encephalitis cases, it seems unlikely that the strain is less virulent to humans than lineage 1 strains.[14,37] Russian strains of the virus are identical to lineage 1 viruses, suggesting an introduction from North America within the last century, possibly with the importation of farmed minks.[23] *Ixodes persulcatus* and various *Haemaphysalis* ticks are the principal vectors and *Apodemus* mice and *Microtus* voles are the main vertebrate hosts.

Seroprevalence in humans is low, from less than 1 percent to 3 percent. Human infections probably are rare because *I. cookei* ticks usually confine themselves to animal host burrows, limiting opportunities for human exposure. Patients usually give a history of outdoor exposure but, in one case involving a 13-month-old infant, an infected tick was brought into the home by a domestic cat.[41] Animal serosurveys indicate that infections occur in dogs, and exposure to ticks on domestic animals may be an alternative source of infection. In a survey of New York goats, 2 percent had serologic evidence of past Powassan virus infection, which indicates the possibility of raw milk-borne Powassan virus infection in the United States, similar to the alimentary transmission of tick-borne encephalitis from infected raw milk products in Europe.[43] Although no such cases of Powassan cases infection have been reported, experimental studies have shown that domestic goats can be infected with Powassan virus and can shed the virus into milk.

REFERENCES

1. Abshagen R, Bahr J. Simultaneous infection with *Borrelia burgdorferi*–FSME virus [in German]. *Kinderarztl Prax* 1992;**60**: 103–4.
2. Ackermann R, Kruger K, Roggendorf M, et al. Spread of early-summer meningoencephalitis in the Federal Republic of Germany [in German]. *Dtsch Med Wochenschr* 1986;**111**:927–33.
3. Aebi C, Schaad UB. TBE-immunoglobulins: a critical assessment of efficacy [in German]. *Schweiz Med Wochenschr* 1994;**124**:1837–40.
4. Aendekerk RP, Schrivers AN, Koehler PJ. Tick-borne encephalitis complicated by a polio-like syndrome following a holiday in central Europe. *Clin Neurol Neurosurg* 1996;**98**:262–4.
5. Alkadhi H, Kollias SS. MRI in tick-borne encephalitis. *Neuroradiology* 2000;**42**:753–5.
6. Arnez M, Avsic-Zupanc T. Tick-borne encephalitis in children: an update on epidemiology and diagnosis. *Expert Rev Anti Infect Ther* 2009;**7**:1251–60.
7. Arnez M, Luznik-Bufon T, Avsic-Zupanc T, et al. Causes of febrile illnesses after a tick bite in Slovenian children. *Pediatr Infect Dis J* 2003;**22**:1078–83.
8. Arras C, Fescharek R, Gregersen JP. Do specific hyperimmunoglobulins aggravate clinical course of tick-borne encephalitis? *Lancet* 1996;**347**:1331.
9. Atkins JT. HSV PCR for CNS infections: pearls and pitfalls. *Pediatr Infect Dis J* 1999;**18**:823–4.
10. Beer S, Brune N, Kesselring J. Detection of anterior horn lesions by MRI in central European tick-borne encephalomyelitis. *J Neurol* 1999;**246**:1169–71.
11. Bender A, Schulte-Altedorneburg G, Walther EU, et al. Severe tick borne encephalitis with simultaneous brain stem, bithalamic, and spinal cord involvement documented by MRI. *J Neurol Neurosurg Psychiatry* 2005;**76**:135–7.
12. Birmingham K. Pentagon breaks FDA rules. *Nat Med* 1998;**4**:255.
13. Briassoulis G, Narlioglou M, Hatzis T. Toxic encephalopathy associated with use of DEET insect repellents: a case analysis of its toxicity in children. *Hum Exp Toxicol* 2001;**20**:8–14.
14. Casati S, Gern L, Piffaretti JC. Diversity of the population of tick-borne encephalitis virus infecting *Ixodes ricinus* ticks in an endemic area of central Switzerland (Canton Bern). *J Gen Virol* 2006;**87**: 2235–41.
15. Cherry JD. Enteroviruses: Coxsackieviruses, echoviruses, and polioviruses. In: Feigin RD, Cherry JD, editors. *Textbook of Pediatric Infectious Diseases*. 7th ed. Philadelphia: W. B. Saunders; 2013. p. 1787–839.
16. Cimperman J, Maraspin V, Lotric-Furlan S, et al. Concomitant infection with tick-borne encephalitis virus and *Borrelia burgdorferi* sensu lato in patients with acute meningitis or meningoencephalitis. *Infection* 1998;**26**:160–4.
17. Cizman M, Rakar R, Zakotnik B, et al. Severe forms of tick-borne encephalitis in children. *Wien Klin Wochenschr* 1999;**111**:484–7.
18. Clarke DH, Casals J. Arboviruses: Group B. In: Horsfall FL, Tamm I, editors. *Viral and Rickettsial Infections of Man*. Philadelphia: Lippincott; 1965. p. 606–58.
19. Clement J, Heyman P. PCR for diagnosis of viral infections of the central nervous system. *Lancet* 1997;**349**:1256.
20. Craig SC, Pittman PR, Lewis TE, et al. An accelerated schedule for tick-borne encephalitis vaccine: the American Military experience in Bosnia. *Am J Trop Med Hyg* 1999;**61**:874–8.
21. Daniel M, Danielova V, Kriz B, et al. Shift of the tick *Ixodes ricinus* and tick-borne encephalitis to higher altitudes in central Europe. *Eur J Clin Microbiol Infect Dis* 2003;**22**:327–8.
22. De Burghgraeve T, Kaptein S, Ayala-Nunez N, et al. An analogue of the antibiotic teicoplanin prevents flavivirus entry in vitro. *PLos One* 2012;**7**:e37244.
23. Demicheli V, Debalini MG, Rivetti A. Vaccines for preventing tick-borne encephalitis. *Cochrane Database Syst Rev* 2009;**21**(1): CD000977.
24. Dobler G, Jelinek T, Frosner G, et al. Cross reactions of patients with acute dengue fever to tick-borne encephalitis [in German]. *Wien Med Wochenschr* 1997;**147**:463–4.
25. Dobler G, Treib J, Kiessig ST, et al. Diagnosis of tick-borne encephalitis: evaluation of sera with borderline titers with the TBE-ELISA. *Infection* 1996;**24**:405–6.
26. Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. *Clin Infect Dis* 1999;**28**:882–90.
27. Duniewicz M. Clinical picture of Central European tick-borne encephalitis (author's transl) [in German]. *M M W Munch Med Wochenschr* 1976;**118**:1609–12.
28. Duppenthaler A, Pfammatter JP, Aebi C. Myopericarditis associated with central European tick-borne encephalitis. *Eur J Pediatr* 2000;**159**:854–6.
29. Floris R, Altobelli A, Boemo B, et al. First detection of TBE virus sequences in *Ixodes ricinus* from Friuli Venezia Giulia (Italy). *New Microbiol* 2006;**29**:147–50.
30. Gaumann R, Muhlemann K, Strasser M, et al. High-throughput procedure for tick surveys of tick-borne encephalitis virus and its application in a national surveillance study in Switzerland. *Appl Environ Microbiol* 2010;**76**:4241–9.
31. Gaunt MW, Sall AA, de Lamballerie X, et al. Phylogenetic relationships of flaviviruses correlate with their epidemiology, disease association and biogeography. *J Gen Virol* 2001;**82**:1867–76.
32. Gelpi E, Preusser M, Laggner U, et al. Inflammatory response in human tick-borne encephalitis: analysis of postmortem brain tissue. *J Neurovirol* 2006;**12**:322–7.
33. Goerre S, Kesselring J, Hartmann K, et al. Neurological side effects following vaccination of early-summer meningoencephalitis: case report and experiences of the Swiss Center for Adverse Drug Effects [in German]. *Schweiz Med Wochenschr* 1993;**123**: 654–7.
34. Gold R, Wietholter H, Rihs I, et al. Early summer meningoencephalitis vaccination: the indications and a critical assessment of the neurological vaccination complications [in German]. *Dtsch Med Wochenschr* 1992;**117**:112–16.
35. Grinschgl G. Virus meningo-encephalitis in Austria. II. Clinical features, pathology, and diagnosis. *Bull World Health Organ* 1955;**12**: 535–64.
36. Grzeszczuk A, Sokolewicz-Bobrowska E, Prokopowicz D. Adverse reactions to tick-borne encephalitis vaccine: FSME-Immun. *Infection* 1998;**26**:385–8.
37. Gunther G, Haglund M, Lindquist L, et al. Intrathecal IgM, IgA and IgG antibody response in tick-borne encephalitis: long-term follow-up related to clinical course and outcome. *Clin Diagn Virol* 1997;**8**:17–29.
38. Gustafson R, Forsgren M, Gardulf A, et al. Clinical manifestations and antibody prevalence of Lyme borreliosis and tick-borne encephalitis in Sweden: a study in five endemic areas close to Stockholm. *Scand J Infect Dis* 1993;**25**:595–603.
39. Hansmann Y, Pierre GJ, Remy V, et al. Tick-borne encephalitis in eastern France. *Scand J Infect Dis* 2006;**38**:520–6.
40. Harasek G. Tick-borne encephalitis in children (author's transl) [in German]. *Dtsch Med Wochenschr* 1974;**99**:1965–70.
41. Heinz FX. Tick-borne encephalitis virus: advances in molecular biology and vaccination strategy in the next century. *Zentralbl Bakteriol* 1999;**289**:506–10.
42. Heinz FX. Molecular aspects of TBE virus research. *Vaccine* 2003;**21**(Suppl. 1):S3–10.
43. Heinz FX, Mandl CW. The molecular biology of tick-borne encephalitis virus. Review article. *APMIS* 1993;**101**:735–45.
44. Heinz FX, Roggendorf M, Hofmann H, et al. Comparison of two different enzyme immunoassays for detection of immunoglobulin M antibodies against tick-borne encephalitis virus in serum and cerebrospinal fluid. *J Clin Microbiol* 1981;**14**:141–6.
45. Hofbauer K. On a case of spring-summer meningoencephalitis with participation of the liver [in German]. *Wien Klin Wochenschr* 1966;**78**:514–17.
46. Hofmann H. After vaccination for tick-borne encephalitis must onset of neurologic disorders be expected? [in German]. *Wien Klin Wochenschr* 1995;**107**:509–15.
47. Hofmann H, Frisch-Niggemeyer W, Heinz F. Rapid diagnosis of tick-borne encephalitis by means of enzyme linked immunosorbent assay. *J Gen Virol* 1979;**42**:505–11.
48. Hofmann H, Kunz C, Heinz FX, et al. Detectability of IgM antibodies against TBE virus after natural infection and after vaccination. *Infection* 1983;**11**:164–6.
49. Holzmann H. Diagnosis of tick-borne encephalitis. *Vaccine* 2003; **21**(Suppl. 1):S36–40.
50. Iff T, Meier R, Olah E, et al. Tick-borne meningo-encephalitis in a 6-week-old infant. *Eur J Pediatr* 2005;**164**:787–8.

51. Ito Y, Ando Y, Kimura H, et al. Polymerase chain reaction–proved herpes simplex encephalitis in children. *Pediatr Infect Dis J* 1998; **17**:29–32.
52. Jaenson T, Hjiertqvist M, Bergstrom T, et al. Why is tick-borne encephalitis increasing? A review of the key factors causing the increasing incidence of human TBE in Sweden. *Parasit Vectors* 2012; **5**:184–95.
53. Jeren T, Vince A. Cytologic and immunoenzymatic findings in CSF from patients with tick-borne encephalitis. *Acta Cytol* 1998;**42**: 330–4.
54. Johan F, Asa L, Rolf A, et al. Tick-borne encephalitis (TBE) in Skane, southern Sweden: a new TBE endemic region? *Scand J Infect Dis* 2006;**38**:800–4.
55. Jones N, Sperl W, Koch J, et al. Tick-borne encephalitis in a 17-day-old newborn resulting in severe neurologic impairment. *Pediatr Infect Dis J* 2007;**26**:185–6.
56. Juhasz C, Szirmai I. Spectral EEG parameters in patients with tick-borne encephalitis: a follow-up study. *Clin Electroencephalogr* 1993; **24**:53–8.
57. Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994-98: a prospective study of 656 patients. *Brain* 1999;**122**(pt. 11):2067–78.
58. Kaiser R, Holzmann H. Laboratory findings in tick-borne encephalitis: correlation with clinical outcome. *Infection* 2000;**28**:78–84.
59. Kaiser R, Kern A, Kampa D, et al. Prevalence of antibodies to *Borrelia burgdorferi* and tick-borne encephalitis virus in an endemic region in southern Germany. *Zentralbl Bakteriol* 1997;**286**:534–41.
60. Kluger G, Schottler A, Waldvogel K, et al. Tickborne encephalitis despite specific immunoglobulin prophylaxis. *Lancet* 1995;**346**: 1502.
61. Kock T, Stunzner D, Freidl W, et al. Clinical aspects of early summer meningoencephalitis in Styria [in German]. *Nervenarzt* 1992;**63**:205–8.
62. Kohl I, Kozuch O, Eleckova E, et al. Family outbreak of alimentary tick-borne encephalitis in Slovakia associated with a natural focus of infection. *Eur J Epidemiol* 1996;**12**:373–5.
63. Korenberg EI, Gorban LY, Kovalevskii YV, et al. Risk for human tick-borne encephalitis, borrelioses, and double infection in the pre-Ural region of Russia. *Emerg Infect Dis* 2001;**7**:459–62.
64. Krausler J, Kraus P, Moritsch H. Clinical and virological-serological study results of early summer meningoencephalitis and other virus infections of the central nervous system in the Neunkirchen area [in German]. *Wien Klin Wochenschr* 1958;**70**:634–7.
65. Krech U, Jung F, Jung M. Central European tick-borne encephalitis in Switzerland [in German]. *Schweiz Med Wochenschr* 1969; **99**:282–5.
66. Kristoferitsch W, Stanek G, Kunz C. Double infection with early summer meningoencephalitis virus and *Borrelia burgdorferi* [in German]. *Dtsch Med Wochenschr* 1986;**111**:861–4.
67. Kunz C. Arbovirus B-Infektionen. In: Grumbach A, Bonin O, editors. *Die Infektionskrankheiten des Menschen und ihre Erreger*. Stuttgart: Thieme Verlag; 1969.
68. Kunz C. Die Fruehsommer-Meningoenzephalitis. *Paediatr Prax* 1974;**14**:189–92.
69. Kunz C. Preventive vaccination against early summer meningoencephalitis [in German]. *Ther Umsch* 1983;**40**:236–8.
70. Kunz C. Tick-borne encephalitis in Europe. *Acta Leiden* 1992; **60**:1–14.
71. Kunz C, Heinz FX, Hofmann H. Immunogenicity and reactogenicity of a highly purified vaccine against tick-borne encephalitis. *J Med Virol* 1980;**6**:103–9.
72. Kunz C, Hofmann H, Dippe H. Early summer meningoencephalitis vaccination, a preventive medicine measure with high acceptance in Austria [in German]. *Wien Med Wochenschr* 1991;**141**: 273–6.
73. Kunz C, Hofmann H, Heinz FX, et al. Efficacy of vaccination against tick-borne encephalitis [in German]. *Wien Klin Wochenschr* 1980;**92**:809–13.
74. Kunz C, Hofmann H, Kundi M, et al. Efficacy of specific immunoglobulin against TBE (author's transl) [in German]. *Wien Klin Wochenschr* 1981;**93**:665–7.
75. Kunze U, Bernhard G, Bohm G, et al. Early-summer meningoencephalitis (ESME) and ESME-vaccination: status 2000 [in German]. *Wien Med Wochenschr* 2000;**150**:103–8.
76. Lakeman FD, Whitley RJ. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. National Institute of Allergy and Infectious Diseases Collaborative Antiviral Study Group. *J Infect Dis* 1995;**171**:857–63.
77. Lammli B, Muller A, Ballmer PE. Late sequelae of early summer meningoencephalitis [in German]. *Schweiz Med Wochenschr* 2000; **130**:909–15.
78. Lesnicar G, Poljak M, Seme K, et al. Pediatric tick-borne encephalitis in 371 cases from an endemic region in Slovenia, 1959 to 2000. *Pediatr Infect Dis J* 2003;**22**:612–17.
79. Lindgren E, Gustafson R. Tick-borne encephalitis in Sweden and climate change. *Lancet* 2001;**358**:16–18.
80. Lindquist L, Vapalahti O. Tick-borne encephalitis. *Lancet* 2008; **371**:1861–71.
81. Logar M, Arnez M, Kolbl J, et al. Comparison of the epidemiological and clinical features of tick-borne encephalitis in children and adults. *Infection* 2000;**28**:74–7.
82. Lorenzl S, Pfister HW, Padovan C, et al. MRI abnormalities in tick-borne encephalitis. *Lancet* 1996;**347**:698–9.
83. Lotric-Furlan S, Strle F. Thrombocytopenia: a common finding in the initial phase of tick-borne encephalitis. *Infection* 1995;**23**: 203–6.
84. Lotric-Furlan S, Avsic-Zupanc T, Strle F. Is an isolated initial phase of a tick-borne encephalitis a common event? *Clin Infect Dis* 2000; **30**:987–8.
85. Lotric-Furlan S, Petrovec M, Avsic-Zupanc T, et al. Concomitant tickborne encephalitis and human granulocytic ehrlichiosis. *Emerg Infect Dis* 2005;**11**:485–8.
86. Mamoli B, Pelzl G. Residualschaden ach FSME. *Oesterr Aerzteztg* 1990;**45**:51.
87. Mandl CW, Kroschewski H, Allison SL, et al. Adaptation of tick-borne encephalitis virus to BHK-21 cells results in the formation of multiple heparan sulfate binding sites in the envelope protein and attenuation in vivo. *J Virol* 2001;**75**:5627–37.
88. Mansfield KL, Johnson N, Phipps L, et al. Tick-borne encephalitis virus-a review of an emerging zoonosis. *J Gen Virol* 2009;**90**: 1781–94.
89. Mastrangelo E, Pezzullo M, De Burghgraeve T, et al. Ivermectin is a potent inhibitor of flavivirus replication specifically targeting NS3 helicase activity: new prospects for an old drug. *J Antimicrob Chemother* 2012;**67**:1884–94.
90. McNair AN, Brown JL. Tick-borne encephalitis complicated by monoplegia and sensorineural deafness. *J Infect* 1991;**22**:81–6.
91. Messner H. Pediatric problems of TBE. In: Kunz C, editor. *Tick-borne Encephalitis*. Vienna: Facultas Verlag; 1981. p. 25–7.
92. Moll van Charante AW, Groen J, Mulder PG, et al. Occupational risks of zoonotic infections in Dutch forestry workers and muskrat catchers. *Eur J Epidemiol* 1998;**14**:109–16.
93. Moore M, Kaplan MH, McPhee J, et al. Epidemiologic, clinical, and laboratory features of Coxsackie B1-B5 infections in the United States, 1970-79. *Public Health Rep* 1984;**99**:515–22.
94. Morens DM. Antibody-dependent enhancement of infection and the pathogenesis of viral disease. *Clin Infect Dis* 1994;**19**:500–12.
95. Moritsch H. Arthropod-transmitted virus infections of the central nervous system in Europe [in German]. *Ergeb Inn Med Kinderheilkd* 1962;**17**:1–57.
96. Moritsch H. Die Arboviren. In: Haas R, Vivell O, editors. *Die Virus- und Rickettsieninfektionen des Menschen*. Munich: J. F. Lehmanns Verlag; 1965.
97. Moritsch H, Krausler J. Endemic spring-summer meningoencephalomyelitis in the Viennese area (Schneider's disease) [in German]. *Wien Klin Wochenschr* 1957;**69**:965–70.
98. Muller A. Active vaccination against early summer meningoencephalitis [in German]. *Schweiz Med Wochenschr* 1998;**128**:1110–16.
99. Niedrig M, Vaisviliene D, Teichmann A, et al. Comparison of six different commercial IgG-ELISA kits for the detection of TBEV-antibodies. *J Clin Virol* 2001;**20**:179–82.
100. Nuttall PA. Pathogen-tick-host interactions: *Borrelia burgdorferi* and TBE virus. *Zentralbl Bakteriol* 1999;**289**:492–505.
101. Oksi J, Viljanen MK, Kalimo H, et al. Fatal encephalitis caused by concomitant infection with tick-borne encephalitis virus and *Borrelia burgdorferi*. *Clin Infect Dis* 1993;**16**:392–6.
102. Ono J, Shimizu K, Harada K, et al. Characteristic MR features of encephalitis caused by Epstein-Barr virus: a case report. *Pediatr Radiol* 1998;**28**:569–70.

103. Osimitz TG, Murphy JV. Neurological effects associated with use of the insect repellent *N,N*-diethyl-*m*-toluamide (DEET). *J Toxicol Clin Toxicol* 1997;**35**:435–41.
104. Pavlova BG, Loew-Baselli A, Fritsch S, et al. Tolerability of modified tick-borne encephalitis vaccine FSME-IMMUN "NEW" in children: results of post-marketing surveillance. *Vaccine* 2003;**21**: 742–5.
105. Pfister HW, Lorenzl S, Yousry T. Neuroradiographic manifestations of encephalitis. *N Engl J Med* 1997;**337**:1393–4.
106. Ramelow C, Suss J, Berndt D, et al. Detection of tick-borne encephalitis virus RNA in ticks *(Ixodes ricinus)* by the polymerase chain reaction. *J Virol Methods* 1993;**45**:115–19.
107. Randolph SE. The shifting landscape of tick-borne zoonoses: Tick-borne encephalitis and *Lyme borreliosis* in Europe. *Philos Trans R Soc Lond B Biol Sci* 2001;**356**:1045–56.
108. Rehse-Kupper B, Danielova V, Klenk W, et al. Epidemiology of Central European encephalitis in the Federal Republic of Germany [in German]. *M M W Munch Med Wochenschr* 1976; **118**:1615–16.
109. Rendi-Wagner P, Kundi M, Zent O, et al. Immunogenicity and safety of a booster vaccination against tick-borne encephalitis more than 3 years following the last immunisation. *Vaccine* 2004; **23**:427–34.
110. Rendi-Wagner P, Kundi M, Zent O, et al. Persistence of protective immunity following vaccination against tick-borne encephalitis: longer than expected? *Vaccine* 2004;**22**:2743–9.
111. Roggendorf M. Epidemiology of tick-borne encephalitis virus in Germany. *Infection* 1996;**24**:465–6.
112. Roggendorf M, Heinz F, Deinhardt F, Kunz C. Serological diagnosis of acute tick-borne encephalitis by demonstration of antibodies of the IgM class. *J Med Virol* 1981;**7**:41–50.
113. Rosenfeld EA, Radkowski MA, Rowley AH. Biphasic course of illness with disparate outcomes in herpes simplex encephalitis in children. *Abstract Pediatr Res* 1994;**35**:193.
114. Ryffel K, Peter O, Rutti B, et al. Scored antibody reactivity determined by immunoblotting shows an association between clinical manifestations and presence of *Borrelia burgdorferi* sensu stricto, *B. garinii*, *B. afzelii*, and *B. valaisiana* in humans. *J Clin Microbiol* 1999;**37**:4086–92.
115. Sakoulas G. Brainstem and striatal encephalitis complicating *Mycoplasma pneumoniae* pneumonia: Possible benefit of intravenous immunoglobulin. *Pediatr Infect Dis J* 2001;**20**:543–5.
116. Salafsky B, He YX, Li J, et al. Study on the efficacy of a new long-acting formulation of *N,N*-diethyl-*m*-toluamide (DEET) for the prevention of tick attachment. *Am J Trop Med Hyg* 2000;**62**: 169–72.
117. Sanchez JL Jr, Craig SC, Kohlhase K, et al. Health assessment of U.S. military personnel deployed to Bosnia-Herzegovina for operation joint endeavor. *Mil Med* 2001;**166**:470–4.
118. Sawyer MH. Enterovirus infections: diagnosis and treatment. *Curr Opin Pediatr* 2001;**13**:65–9.
119. Schabet M, Wiethoelter H, Grodd W, et al. Neurological complications after simultaneous immunisation against tick-borne encephalitis and tetanus. *Lancet* 1989;**1**:959–60.
120. Schattenfroh C. Acute disseminated encephalomyelitis after active immunization against early summer encephalitis [in German]. *Nervenarzt* 2004;**75**:776–9.
121. Schellinger PD, Schmutzhard E, Fiebach JB, et al. Poliomyelitic-like illness in central European encephalitis. *Neurology* 2000;**55**: 299–302.
122. Schmolck H, Maritz E, Kletzin I, et al. Neurologic, neuropsychologic, and electroencephalographic findings after European tick-borne encephalitis in children. *J Child Neurol* 2005;**20**:500–8.
123. Scholz E, Wietholter H. Postvaccinal neuritis following prophylactic vaccination against early-summer meningo-encephalitis [in German]. *Dtsch Med Wochenschr* 1987;**112**:544–6.
124. Scholz H, Summer K. *Tick-borne encephalitis 1970 in Styria. XIII Symposium de l'Association Européenne contre la polio-myélite, Helsinki, 1971*. Brussels: Imprimerie des Sciences; 1972.
125. Schrader C, Suss J. A nested RT-PCR for the detection of tick-borne encephalitis virus (TBEV) in ticks in natural foci. *Zentralbl Bakteriol* 1999;**289**:319–28.
126. Schultze D, Dollenmaier G, Rohner A, et al. Benefit of detecting tick-borne encephalitis viremia in the first phase of illness. *J Clin Virol* 2007;**38**:172–5.
127. Schwanda M, Oertli S, Frauchiger B, et al. Tick-borne meningoencephalitis in Thurgau Canton: a clinical and epidemiological analysis [in German]. *Schweiz Med Wochenschr* 2000;**130**:1447–55.
128. Skarpaas T, Golovljova I, Vene S, et al. Tickborne encephalitis virus, Norway and Denmark. *Emerg Infect Dis* 2006;**12**:1136–8.
129. Spiess H, Mumenthaler M, Burkhardt S, et al. Central European encephalitis ("tick-borne encephalitis") in Switzerland [in German]. *Schweiz Med Wochenschr* 1969;**99**:277–82.
130. Stohrer-Draxl P, Amstad H, Grize L, et al. Measles, mumps and rubella: vaccination rate and seroprevalence in 8th grade students of 8 different sites in Switzerland 1995/96 [in German]. *Schweiz Rundsch Med Prax* 1999;**88**:1069–77.
131. Svedmyr A, von Zeipel G, Holmgren B, et al. Tick-borne meningoencephalomyelitis in Sweden. *Arch Gesamte Virusforsch* 1959;**8**: 565–76.
132. Tesarova-Magrova J, Kroo AH. Cardiac disease in patients with tick-borne encephalitis. *G Mal Infett Parasit* 1966;**18**:803–6.
133. Tomazic J, Poljak M, Popovic P, et al. Tick-borne encephalitis: possibly a fatal disease in its acute stage—PCR amplification of TBE RNA from postmortem brain tissue. *Infection* 1997;**25**: 41–3.
134. Torm S, Zilmer K, Jagoo K, et al. *Clinical features of tick-borne encephalitis in children in Estonia [Abstract]*. European Society for Paediatric Infectious Diseases; 1996.
135. Valdueza JM, Weber JR, Harms L, et al. Severe tick borne encephalomyelitis after tick bite and passive immunisation. *J Neurol Neurosurg Psychiatry* 1996;**60**:593–4.
136. Wahlberg P, Saikku P, Brummer-Korvenkontio M. Tick-borne viral encephalitis in Finland: the clinical features of Kumlinge disease during 1959-1987. *J Intern Med* 1989;**225**:173–7.
137. Waldvogel K, Bossart W, Huisman T, et al. Severe tick-borne encephalitis following passive immunization. *Eur J Pediatr* 1996; **155**:775–9.
138. Wicki R, Sauter P, Mettler C, et al. Swiss Army Survey in Switzerland to determine the prevalence of *Francisella tularensis*, members of the *Ehrlichia phagocytophila* genogroup, *Borrelia burgdorferi* sensu lato, and tick-borne encephalitis virus in ticks. *Eur J Clin Microbiol Infect Dis* 2000;**19**:427–32.
139. Zeman P, Januska J, Orolinova M, et al. High seroprevalence of granulocytic ehrlichiosis distinguishes sheep that were the source of an alimentary epidemic of tick-borne encephalitis. *Wien Klin Wochenschr* 2004;**116**:614–16.
140. Zent O, Banzhoff A, Hilbert AK, et al. Safety, immunogenicity and tolerability of a new pediatric tick-borne encephalitis (TBE) vaccine, free of protein-derived stabilizer. *Vaccine* 2003;**21**:3584–92.
141. Zent O, Jilg W, Plentz A, et al. Kinetics of the immune response after primary and booster immunization against tick-borne encephalitis (TBE) in adults using the rapid immunization schedule. *Vaccine* 2003;**21**:4655–60.
142. Zenz W, Pansi H, Zoehrer B, et al. Tick-borne encephalitis in children in Styria and Slovenia between 1980 and 2003. *Pediatr Infect Dis J* 2005;**24**:892–6.
143. Zimmermann H. Tickborne encephalitis in Switzerland: significant increase in notified cases, 2005. *Euro Surveill* 2005;**10**: E051006.

Clinical and pathologic signs of encephalitis have been produced in experimentally infected horses. Unlike Lyme disease and some rickettsial infections, Powassan virus infection can be transmitted after a brief period of tick attachment.[10]

Clinical Manifestations

The incubation period may be several weeks after known exposure to a tick. Early symptoms of fever, headache, lethargy, vomiting, retro-orbital pain, and photophobia are followed by changes in sensorium, confusion, generalized or focal seizures, paresis, and paralysis, that may lead to coma and require ventilator support and intensive care.* Magnetic resonance imaging has shown restricted diffusion and abnormal enhancement in parietal and temporal lobes and thalamic hemorrhage, which is often seen in other forms of flaviviral encephalitis. Approximately 10 percent of cases have been fatal; however, significant neurologic sequelae follow in the majority of survivors, including hemiplegia, quadriplegia, aphasia, and cognitive impairment. Residual shoulder girdle atrophy and weakness analogous to sequelae that occur after European and Far Eastern tick-borne encephalitis, are common.[5] In another patient, wasting and weakness of a leg consistent with lumbosacral poliomyelitis were reported.[7,21] On the other hand, a laboratory confirmed case was reported in a febrile patient without neurologic signs.[20]

Clinical and experimental observations of tick-borne encephalitis suggest that chronic central nervous system infection characterized by a seizure disorder (i.e., epilepsia partialis continua), weakness, and dementia may occur after recovery from the acute phase of illness. A retrospective study of 22 Canadian patients with a similar clinical syndrome and in whom histologic changes in the brain resembled those of chronic tick-borne encephalitis showed no evidence of Powassan virus infection. However, none of these patients had a history of acute encephalitis.

Laboratory Diagnosis

Virus has been recovered from the brains of patients with fatal cases of the disease. A serologic diagnosis can be achieved more rapidly by detecting specific immunoglobulin (Ig)M antibody in acute-phase serum or spinal fluid. Heterologous antibodies from other flavivirus infections (e.g., dengue and West Nile and St. Louis encephalitis) or vaccinations (e.g., tick-borne encephalitis and yellow fever) may interfere with interpretation.

Differential Diagnosis

The clinical findings of encephalitis in a patient with a history of a tick bite acquired in an endemic area should suggest the possibility of Powassan encephalitis. However, because ixodid tick bites may be inconspicuous, a negative history does not exclude the diagnosis. Other viral agents of encephalitis, especially eastern equine encephalitis virus and California group viruses, which are prevalent in New York State and eastern Canada, should be considered in the differential diagnosis. Lyme disease, because of its known geographic distribution and association with a tick vector, also may be associated with neurologic complications; however, a history of having a typical rash and arthritis should differentiate the conditions. In a series of 145 clinical encephalitis cases at a Canadian tertiary pediatric hospital, one was attributed to Powassan virus.[22] Imported travel-associated cases of tick-borne encephalitis acquired in Europe have been reported, underscoring the value of obtaining a travel history.

Treatment and Prevention

No specific therapy for Powassan encephalitis is available. Personal protective measures to avoid tick bites are advised. Consumption of unpasteurized goat milk should be avoided because of the theoretical risk for contracting Powassan virus infection and the well-documented risk for acquiring other infections associated with raw milk. Inactivated tick-borne encephalitis vaccines licensed in Europe do not cross protect against Powassan virus infection.

ROCIO ENCEPHALITIS

Rocio encephalitis virus was isolated from the brain of a fatal case of encephalitis in a patient during an outbreak in 1975.[12,24] The virus is antigenically to viruses in the Japanese encephalitis virus complex. The disease has been reported exclusively in São Paulo State and adjacent Paraná State, Brazil, principally in the Ribiera Valley and Santista lowlands. More than 1000 cases were documented in outbreaks between 1975 and 1977; only one symptomatic case (in an infant) has been recognized subsequently, although recent infections (IgM in asymptomatic individuals) were documented in later serosurveys in northeastern and southeastern Brazil. Thus, the virus may be transmitted undetected in an area beyond the Ribiera Valley. The viral transmission cycle has not been elucidated; however, field and laboratory observations suggest transmission between birds and *Psorophora* or *Aedes* mosquitoes. Humans are dead-end hosts. Human cases have occurred principally in men with outdoor occupations, especially fishermen.

The incubation period is estimated to be 7 to 14 days. Prodromal symptoms of fever, headache, malaise, vomiting, and conjunctivitis are followed by mental status changes, meningismus, and motor impairment. Cerebellar signs occur commonly. Signs of bulbar involvement also are seen. Coma and a fatal outcome occur most commonly in children (30%) and in seniors; overall, 10 percent of cases are fatal and 20 percent of patients have had neuropsychiatric sequelae. Pathologic findings of encephalitis principally involve the thalamus, cerebellar dentate nucleus, brain nuclei, brain stem, and spinal cord.

The diagnosis should be suspected in patients with acute encephalitis acquired in South America. The

*References 4, 7, 11, 14, 16, 19-21, 30, 34, 36, 37, 41.

presence of virus-specific IgM in cerebrospinal fluid or a fourfold change in serum antibody titer confirms a case, but cross reactions with St. Louis encephalitis, dengue, and other flaviviruses should be ruled out. Treatment is supportive. Emergency application of adulticides and larvicides has been used to control epidemics.

LOUPING ILL VIRUS

Louping ill virus derives its name from an old Scottish term describing the leaping motions of encephalitic sheep. Historical accounts of the disease in sheep date from 1795, and the virus was isolated in 1931. The virus is a member of the antigenic complex of tick-borne flaviviruses and is transmitted by *Ixodes ricinus* ticks.[6,15,28] Sheep, deer, mountain hares, and grouse are the principal amplifying hosts. Grouse may become infected by ingesting infected ticks. The disease is enzootic in pasturelands of Scotland, England, Wales, and Ireland, where human infections have occurred mainly in sheep farmers, veterinarians, and abattoir workers or butchers who had direct contact with animals or their meat. Antibody prevalence is 10 percent in abattoir workers in enzootic areas. Laboratory-acquired infections are common and account for half of all reported human cases. These observations suggest that infections are transmitted easily by direct mucous membrane or respiratory infection. Hospital surveillance in an enzootic area found louping ill virus to be a rare occurrence that was responsible for fewer than 0.5 percent of encephalitis cases. Related tick-borne viruses in Spain, Greece, Norway, and Turkey have not been associated with human disease. The virus is shed in sheep and goat milk, but unlike tick-borne encephalitis, milk-transmitted cases have not been reported. Sheep in the United Kingdom are routinely vaccinated with a crude inactivated vaccine.

The incubation period can be as short as 3 days. Three clinical syndromes have been described.[6] Approximately a third of patients have a self-limited influenza-like illness with fever, headache, dizziness, and myalgias. The febrile illness is followed by clinical improvement and a second encephalitic phase in more than half of the cases. Neurologic symptoms include meningismus, severe headache, vomiting, drowsiness, and tremor; one fatal case was reported. A poliomyelitis syndrome with muscle weakness or paralysis has been described in a few cases; the final presentation is hemorrhagic fever, which was reported in one atypical laboratory-acquired case.

The diagnosis should be suspected in febrile patients with occupational or other exposure, especially if central nervous system symptoms are present. The diagnosis can be confirmed by polymerase chain reaction or, serologically, by demonstrating virus-specific IgM in cerebrospinal fluid or serum or by a fourfold rise in antibody titer. Treatment is symptomatic. Consumption of unpasteurized milk products should be avoided. Persons with outdoor exposure in enzootic areas are advised to use repellents and other protective measures against tick bites. Licensed human vaccines against tick-borne encephalitis virus are likely to be protective.

KYASANUR FOREST DISEASE

Kyasanur Forest virus was isolated in 1957 after an outbreak of hemorrhagic fever, initially suspected to be the first outbreak of yellow fever in Asia, appeared in India in the Kyasanur Forest of Mysore (now Karnataka).[2,31-33] The virus subsequently was identified as a member of the tick-borne flaviviral antigenic complex and shown to be transmitted by *Haemaphysalis spinigera* (among numerous other ixodid ticks). Forest rodents, insectivores, and possibly bats, participate in the transmission cycle, and Langur monkeys sicken in epizootics and die of the infection. Human cases occur in dry-season epidemics, chiefly in persons who have contact with tick-infested forests. The disease has spread geographically as villagers clear forests for pastureland. Between 1982 and 1988, 1847 cases were reported, 254 of which were fatal.[31] Hyperendemic transmission in Karnataka State continues despite the introduction of mass vaccination with a locally produced, inactivated chick embryo fibroblast–derived vaccine.[31] The geographic distribution of the virus is now known to be much broader, with virologic or serologic evidence of infection throughout China and on the Andaman and Nicobar Islands.[40]

Additionally, a viral variant of the virus that has caused outbreaks of hemorrhagic fever in Saudia Arabia, Alkhumra virus, was transmitted to Italian tourists visiting the Egypt–Sudan border, indicating a distribution possibly encompassing a broad swath of Northern Africa, the Middle East, and into Central, South, and East Asia.[4,27] Molecular epidemiologic studies support the ancestral eastward spread of tick-borne flaviviruses, possibly along camel trade routes or by migrating birds carrying infected ticks.[18] Alkhumra virus has been acquired principally by persons who had close contact with livestock animals or their meat during or after slaughter, although mosquito-borne transmission from infected animals also has been suspected.[27] Tick-borne transmission was strongly suspected in the case of the Italian tourists.[4]

After an incubation period of 3 to 8 days, illness begins abruptly with fever, headache, myalgias, chills, and gastrointestinal symptoms.[2,32,33] Patients are seriously ill with facial hyperemia, conjunctival suffusion, lymphadenopathy, and hepatomegaly, and they may be bradycardic and hypotensive. A papulovesicular enanthem and petechiae are seen, and mucosal bleeding from the gums, epistaxis, hemoptysis, and gastrointestinal and vaginal bleeding may be prominent. Bronchopneumonia and hemorrhagic pulmonary edema complicate the illness in 40 percent of cases. Renal failure may develop. After the resolution of symptoms and an afebrile interval of 1 to 3 weeks, fever, recurrence of symptoms, and meningoencephalitis develop in 15 to 50 percent of cases, as occur in tick-borne encephalitis. Although biphasic febrile illness also occurs in patients infected with Alkhumra virus, encephalitis, which complicates one fourth of cases, usually is a component of the primary illness.[27] Leukopenia with a left shift, thrombocytopenia, and an elevated hematocrit reflecting hemoconcentration are seen, and, in some cases, evidence of disseminated intravascular coagulation. Elevations in liver enzymes are present in the majority of

cases. The case-fatality rate is 3 to 15 percent. Keratitis and iritis have been reported to occur as sequelae. The virus frequently can be isolated from acute-phase blood specimens (<12 days after onset), or the diagnosis can be confirmed serologically.

OMSK HEMORRHAGIC FEVER

Omsk hemorrhagic fever virus was isolated from a viremic human during a series of outbreaks in the Omsk region of western Siberia from 1945 to 1949.[25,35] Between 1945 and 1958, approximately 1500 cases were reported in the forest-steppe zones within the Omsk, Novosibirsk, Kurgan, and Tjumen regions; however, the distribution of enzootic and human infections has shrunk considerably and human cases now are reported sporadically only from Novosibirsk. The virus is related antigenically to other tick-borne flaviviruses and is spread by *Dermacentor marginatus*, *Dermacentor reticulatus*, and *I. persultcatus* ticks that may be dually infected with tick-borne encephalitis virus. Numerous small mammals, especially *Microtus* voles, are infected in the transmission cycle, but it was the introduction of muskrats *(Ondatra zibethicus)* into the region from Canada in the early 20th century that resulted in an expansion of viral transmission and large outbreaks in trappers, despite high mortality in the infected muskrats. Seasonal peaks of infection are in May to June and August to September, but recent cases, occurring principally among hunters and trappers, occur through the end of the year. Infections are transmitted by tick bites, directly from infected muskrats during skinning, and potentially from streams where the virus survives for months after being shed by infected animals. Alimentary infection from consumption of infected goat milk is possible but has not been reported. Laboratory-associated cases frequently have occurred. The incubation period may be as brief as 2 to 4 days. The illness is similar to Kyasanur Forest disease, but with an earlier onset of hemorrhagic phenomena (e.g., epistaxis). Hemorrhages tend to be less severe, and the overall mortality rate is lower (<3%). A second phase of illness develops after an asymptomatic period of 1 to 2 weeks in 30 to 50 percent of cases and frequently is dominated by neurologic symptoms. Cases coming to attention at that point can be diagnosed only serologically. Vaccine produced against tick-borne encephalitis virus is reported to provide some degree of cross protection.

ZIKA FEVER

Although Zika virus was described to cause sporadic acute febrile infections in Uganda in 1947, only sporadic cases of the self-limited fever, usually accompanied by a maculopapular rash and arthralgias were reported from Africa and Asia until an explosive outbreak occurred on the Micronesian island of Yap in 2007.[8,17] More than 70 percent of the island's population was infected, and, in addition to the previously described symptoms, conjunctivitis featured prominently in the cases. The virus is transmitted by many of the same forest and urban vectors as yellow fever virus, and in this outbreak *Aedes hensilli* was strongly suspected to be the vector. The means by

TABLE 175H-1 **Less Commonly Recognized Flaviviral Infections**

Virus	Clinical Syndrome	Geographic Distribution	Transmission Cycle	Mode of Transmission
Alfuy	Fever	Australia	Unknown	V
Apoi	Encephalitis	Japan	Rodent: ?	L
Banzi	Nonspecific febrile illness	South, East Africa	*Culex rubinotus:* Rodent	V
Bussuquara	Fever, arthralgias	Brazil, Colombia, Panama	*Culex melaconion:* Rodent	V
Edge Hill	Fever, polyarthritis	Australia	*Aedes vigilax:* Marsupial	V
Ilheus	Fever, myalgia, encephalitis	Argentina, Brazil, Colombia, Guatemala, Panama, Trinidad	*Psorophora ferox:* bird	V, E
Karshi	Nonspecific febrile illness	Uzbekistan	Various ticks: Rodent	V
Kokobera	Fever, polyarthralgia	Australia, Papua New Guinea	*Culex annulirostris:* ? Marsupial	V
Koutango	Fever, rash, arthralgia	Western and central Africa	Tick: Rodent	L
Langat	Fever, encephalitis	Malaysia, Thailand, Russia	*Ixodes* tick: Rodent	L
Modoc	Aseptic meningitis	Western United States, Canada	Rodent: Rodent	Z
Negishi	Encephalitis	Japan, China, Russia	Tick: Unknown	L, V
Rio Bravo	Nonspecific febrile illness, meningitis	Western United States, Canada	Bat: Bat	Z, L
Sepik	Nonspecific febrile illness	Papua New Guinea	*Mansonia* spp.: ?	V
Spondweni	Fever, arthralgia, rash	Southern and western Africa	*Aedes* spp.: ?	L, V
Usutu	Fever, rash, encephalitis	Southern and central Africa, Europe	*Culex* spp.: Bird	V
Wesselsbron	Fever, arthralgia, rash, encephalitis	Sub-Saharan Africa, Thailand	*Aedes* spp.: ?	V, L, DC?

DC, Direct contact with infected sheep; *E,* experimental infection; *L,* laboratory-acquired infection; *S,* sexual tranmission; *V,* vector-borne infection; *Z,* zoonotic infection.

which the virus was introduced to the island are unclear, although introduction by a viremic traveler is possible—a mechanism that was underscored when two entomologists returned to the United States from Senegal while incubating the illness and fell ill after arriving home.[13] One of the index cases then transmitted the infection directly to his partner, possibly venereally. The nonspecific febrile illness should be considered in the differential diagnosis of dengue and other tropical fevers.

OTHER FLAVIVIRAL INFECTIONS

Flaviviral infections of lesser public health importance or that are recognized less frequently are listed in Table 175H-1.[1,3,26,29,42] Several of the viruses are known to cause human illness only after laboratory exposure, and others, like Usutu virus, have been reported to cause illness only in highly immunocompromised patients.[39] The clinical manifestations of these infections may differ from naturally acquired infection because of their mode of transmission by respiratory, mucosal, or other routes of infection. Other viruses that cause nonspecific syndromes of febrile illness were discovered through fever surveys; although few cases may have been reported, their prevalence may be underestimated because few cases are recognized.[1,3,26,29,42] Because immune responses to repeated flaviviral infections lead to broadly cross-reactive responses, serologic diagnosis may be misleading unless extensive cross-neutralization assays are undertaken. Of the zoonotic flaviviruses (transmitted from animal to animal without an arthropod vector), only Rio Bravo and Modoc viruses are known to cause human illness.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Carletti Fl, Castilletti D, DiCaro A, et al. Alkhurma hemorrhagic fever in travelers returning from Egypt, 2010. *Emerg Infect Dis J* 2010;**16**:1979–82.
8. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap island, Federated States of Micronesia. *N Engl J Med* 2009;**360**:2536–43.
9. Ebel GD. Update on Powassan virus: emergence of a North American tick-borne flavivirus. *Ann Rev Entomol* 2010;**55**:95–110.
13. Foy BD, Kobylinski KC, Foy JL, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis J* 2011;**5**:880–2.
17. Hayes EB. Zika virus outside Africa. *Emerg Infect Dis J* 2009;**15**:1347–50.
18. Heinze DM, Gould EA, Forrester NL. Revisiting the clinical concept of evolution and dispersal for the tick-borne flaviviruses using phylogenetic and biogeographic analyses. *J Virol* 2012.
19. Hicar MD, Edwards K, Bloch K. Powassan virus infection presenting as acute disseminated encephalomyelitis in Tennessee. *Pediatr Infect Dis J* 2010;**30**:86–8.
26. Mackenzie JS, Williams DT. The zoonotic flaviviruses of southern, southeastern and eastern Asia, and Australasia: the potential for emergent viruses. *Zoonoses Public Health* 2009;**56**:338–56.
35. Ruzek D, Yakimenko VV, Karan LS, Tkachev SE. Omsk hemorrhagic fever. *Lancet* 2010;**376**:2104–13.
37. Tavakoli NP, Wang H, Dupuis M, et al. Fatal case of deer tick virus encephalitis. *N Engl J Med* 2009;**360**:2099–107.
38. Tokarz R, Jain K, Bennett A, et al. Assessment of polymicrobial infections in ticks in New York State. *Vector-borne Zoonotic Dis* 2010;**10**:217–21.
39. Vazquez A, Jimenez-Clavero M, Franco L, et al. Usutu virus: potential risk of human disease in Europe. *Euro Surveill* 2011;**16**: pii: 19935.
40. Wang JL, Zhang HL, Fu SH, et al. Isolation of Kyasanur Forest disease virus from febrile patient, Yunnan, China. *Emerg Infect Dis J* 2009;**15**:326–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aaskov JG, Phillips DA, Wiemers MA. Possible clinical infection with Edge Hill virus. *Trans R Soc Trop Med Hyg* 1993;**87**:452–3.
2. Adhikari Prabha MR, Prabhu MG, Raghuveer CV, et al. Clinical study of 100 cases of Kyasanur Forest disease with clinicopathological correlation. *Indian J Med Sci* 1993;**47**:124–30.
3. Boughton CR, Hawkes RA, Naim HM. Illness caused by a Kokobera-like virus in southeastern Australia. *Med J Aust* 1986; **145**:90–7.
4. Carletti Fl, Castilletti D, DiCaro A, et al. Alkhurma hemorrhagic fever in travelers returning from Egypt, 2010. *Emerg Infect Dis J* 2010;**16**:1979–82.
5. Conway D, Rossier E, Spence L, et al. A case report: Powassan virus encephalitis with shoulder girdle involvement. *Can Dis Wkly Rep* 1976;**2**:85–7.
6. Davidson MM, Williams H, MacLeod AJ. Louping ill in man: a forgotten disease. *J Infect* 1991;**23**:241–9.
7. Deibel R, Srihongse S, Woodall JP. Arboviruses in New York State. *Am J Trop Med Hyg* 1979;**28**:577–82.
8. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap island, Federated States of Micronesia. *N Engl J Med* 2009; **360**:2536–43.
9. Ebel GD. Update on Powassan virus: emergence of a North American tick-borne flavivirus. *Ann Rev Entomol* 2010;**55**:95–110.
10. Ebel GD, Kramer LD. Short report: duration of tick attachment required for transmission of Powassan virus by deer ticks. *Am J Trop Med Hyg* 2004;**71**:268–71.
11. Embil J, Camfield P, Artsob H, et al. Powassan virus encephalitis resembling herpes simplex encephalitis. *Arch Intern Med* 1983; **143**:341–3.
12. Figueiredo LTM. The Brazilian flaviviruses. *Microbes Infect* 2000;**2**:1643–9.
13. Foy BD, Kobylinski KC, Foy JL, et al. Probable non-vector-borne transmission of Zika virus, Colorado, USA. *Emerg Infect Dis J* 2011;**5**:880–2.
14. Gholam BI, Puksa S, Provias JP. Powassan encephalitis: a case report with neuropathology and literature review. *CMAJ* 1999; **161**:1419–22.
15. Gilbert L, Jones LD, Laurenson MD, et al. Ticks need not bite their red grouse hosts to infect them with louping ill virus. *Proc Biol Sci* 2004;**271**(Suppl. 4):S202–5.
16. Goldfield M, Austin SM, Black HC, et al. A nonfatal human case of Powassan virus encephalitis. *Am J Trop Med Hyg* 1973;**22**: 78–81.
17. Hayes EB. Zika virus outside Africa. *Emerg Infect Dis J* 2009; **15**:1347–50.
18. Heinze DM, Gould EA, Forrester NL. Revisiting the clinical concept of evolution and dispersal for the tick-borne flaviviruses using phylogenetic and biogeographic analyses. *J Virol* 2012.
19. Hicar MD, Edwards K, Bloch K. Powassan virus infection presenting as acute disseminated encephalomyelitis in Tennessee. *Pediatr Infect Dis J* 2010;**30**:86–8.
20. Hinten SR, Beckett GA, Gensheimer KF, et al. Increased recognition of Powassan encephalitis in the United States, 1999-2005. *Vector Borne Zoonotic Dis* 2008;**8**:733–40.
21. Jackson AC. Leg weakness associated with Powassan virus infection: Ontario [in English, French]. *Can Dis Wkly Rep* 1989;**15**:123.
22. Kolski H, Ford-Jones EL, Richardson S, et al. Etiology of acute childhood encephalitis at The Hospital for Sick Children, Toronto, 1994-1995. *Clin Infect Dis* 1998;**26**:398–409.
23. Leonova GN, Kondratov IG, Ternovoi VA, et al. Characterization of Powassan viruses from Far Eastern Russia. *Arch Virol* 2009; **154**:811–20.
24. Lopes O, de Abreu Sacchetta L, Coimbra TLM, et al. Emergence of a new arbovirus disease in Brazil. II. Epidemiologic studies on 1975 epidemic. *Am J Epidemiol* 1978;**108**:394–401.
25. Lvov DK. Arboviral zoonoses of northern Eurasia (Eastern Europe and The Commonwealth of Independent States). In: Beran GW, editor. *Handbook of zoonoses*. 2nd ed. Boca Raton, Fla: CRC Press; 1994. p. 237–60.
26. Mackenzie JS, Williams DT. The zoonotic flaviviruses of southern, southeastern and eastern Asia, and Australasia: the potential for emergent viruses. *Zoonoses Public Health* 2009;**56**:338–56.
27. Madani TA. Alkhumra virus infection, a new viral hemorrhagic fever in Saudi Arabia. *J Infect* 2005;**51**:91–7.
28. Marriott L, Willoughby K, Chianini F, et al. Detection of louping ill virus in clinical specimens from mammals and birds using TaqMan RT-PCR. *J Virol Methods* 2006;**137**:21–8.
29. Nassar ES, Coimbra TL, Rocco IM, et al. Human disease caused by an arbovirus closely related to Ilheus virus: report of 5 cases. *Intervirology* 1997;**40**:249–52.
30. Partington MW, Thomson V, O'Shaughnessy MV. Powassan virus encephalitis in southeastern Ontario. *Can Med Assoc J* 1980; **123**:603–4.
31. Pattnaik P. Kyasanur forest disease: an epidemiological view in India. *Rev Med Virol* 2006;**16**:151–65.
32. Pavri K. Clinical, clinicopathologic, and hematologic features of Kyasanur Forest disease. *Rev Infect Dis* 1989;**4**(Suppl. 4):854–9.
33. Prabha A, Prabhu MG, Raghuvcer CV, et al. Clinical study of 100 cases of Kyasanur Forest disease with clinicopathological correlation. *Indian J Med Sci* 1993;**47**:124–30.
34. Rossier E, Harrison RJ, Lemieux B. A case of Powassan virus encephalitis. *Can Med Assoc J* 1974;**110**:1173–80.
35. Ruzek D, Yakimenko VV, Karan LS, et al. Omsk hemorrhagic fever. *Lancet* 2010;**376**:2104–13.
36. Smith R, Woodall JP, Whitney E, et al. Powassan virus infection: a report of three human cases of encephalitis. *Am J Dis Child* 1974;**127**:691–3.
37. Tavakoli NP, Wang H, Dupuis M, et al. Fatal case of deer tick virus encephalitis. *N Engl J Med* 2009;**360**:2099–107.
38. Tokarz Rl, Jain K, Bennett A, et al. Assessment of polymicrobial infections in ticks in New York State. *Vector Borne Zoonotic Dis* 2010;**10**:217–21.
39. Vazquez A, Jimenez-Clavero M, Franco L, et al. Usutu virus: potential risk of human disease in Europe. *Euro Surveill* 2011;**16**: pii: 19935.
40. Wang JL, Zhang HL, Fu SH, et al. Isolation of Kyasanur Forest disease virus from febrile patient, Yunnan, China. *Emerg Infect Dis J* 2009;**15**:326–8.
41. Wilson MS, Wherrett BA, Mahdy MS. Powassan virus meningoencephalitis: a case report. *Can Med Assoc J* 1979;**121**:320–3.
42. Wolff MS, Calishan CH, McGuire K. Spondweni virus infection in a foreign resident of Upper Volta. *Lancet* 1982;**2**:1306–7.
43. Woodall JP, Roz A. Experimental milk-borne transmission of Powassan virus in the goat. *Am J Trop Med Hyg* 1977;**26**:190–2.

HISTORY

After reliable testing for both hepatitis A and B was developed, it became clear that a significant number of bloodborne, transfusion-related cases of hepatitis existed. These patients appeared to be infected with an agent that tested negative for both hepatitis A and B and thus was deemed "non-A, non-B hepatitis." The agent was suspected to be viral because transfer of blood from affected patients into naïve chimpanzees resulted in hepatitis. After a great deal of effort, the virus was finally identified in 1989.[25] A group at Chiron used molecular methods to identify RNA sequences from infected chimpanzees that were distinct from any known virus. HCV was the first pathogen to be described using only molecular methods, a practice that has now become the standard form of pathogen discovery. After a great deal of validation work, they named the virus hepatitis C virus.[63]

VIROLOGY

HCV is classified in its own *Hepacivirus* genus within the Flaviviridae family.[68,102] It is an enveloped single-stranded, plus-sense RNA virus organized into one long open reading frame of approximately 9500 nucleotides.[69] It has the structural protein genes at its 5′ end and its nonstructural proteins toward the 3′ end.[35,95] Figure 176-1 illustrates how the HCV genome is organized and lists the names of individual genes and their main role during infection. The details of the molecular and structural biology of HCV are reviewed elsewhere,[35,69,95] but several unique features of the HCV genome are worth noting here. The HCV RNA 5′ untranslated region (5′UTR) contains an internal ribosomal entry site (IRES), a feature that no other flavivirus contains.[28] The IRES allows for direct binding of host ribosomes to the viral RNA using a set of proteins different from those required for conventional capP-dependent translation. The 3′UTR does not feature a poly-A tail as many flaviviruses do.[35] The HCV E2 protein contains a hypervariable region that responds to immune-mediated pressure with hypermutation.[35] The resulting "quasispecies" of HCV allow for rapid escape of antibody neutralization in a fashion very similar to that observed with human immunodeficiency virus (HIV), with the similar effect of making vaccine development a difficult proposition.[45] The HCV NS3 is a multifunctional protein, with both helicase and protease domains.[68,69] The HCV NS5A protein is an important cofactor for many viral processes, including RNA replication and viral assembly.[68,69] The HCV NS5B is the RNA-dependent RNA polymerase.[68,69]

In vitro systems used to study HCV include subgenomic replicons, which are RNA constructs containing only the nonstructural region of the virus that can replicate themselves but produce no functional virus.[13,70] Pseudoviruses using a retrovirus or Semliki Forest virus as a backbone have been used to study virus-receptor interaction.[12,46] In 2005, a one-of-a-kind isolate of HCV was discovered in Japan, deemed "JFH1," which was found to replicate in vitro and produce infectious virus

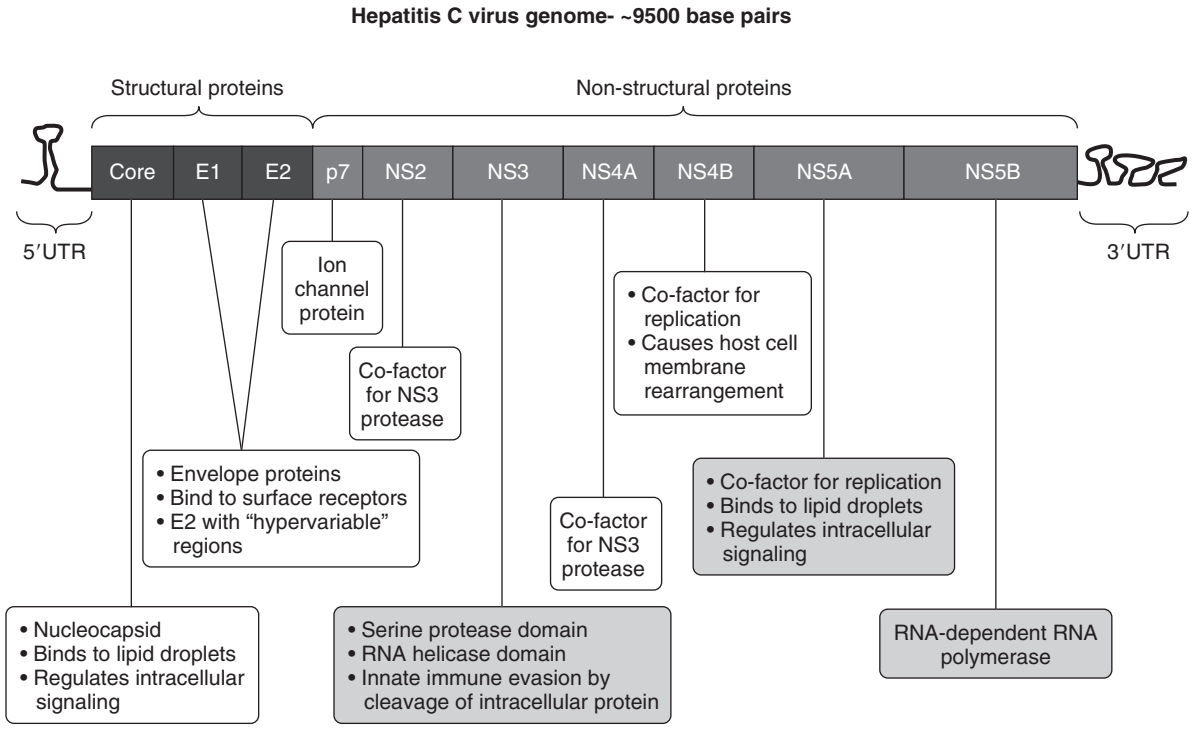

FIGURE 176-1 ■ Genomic organization of hepatitis C virus (HCV). HCV is organized as a single open reading frame coding for one polyprotein that is subsequently cleaved into multiple individual proteins. Structural proteins are shaded in *dark gray,* nonstructural proteins in *light gray.* The name and most important functions of each protein are provided. Proteins that serve a current or near future direct antiviral targets are indicated by a *shaded* function box.

in several liver cell lines.[126] This discovery has since allowed for incredibly rapid progress in understanding the HCV viral life cycle.

HCV exists in the bloodstream in several different forms: "naked" virus, which would be the enveloped virus alone; HCV bound to antibody in higher density fractions; HCV bound to low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL).[3,6,7] These last two fractions are deemed "lipo-viro-particles" (LVPs) and have been shown to be the most infectious form of HCV.[3,7,11,39]

PATHOGENESIS

After gaining access to the bloodstream, the virus targets the liver by poorly understood mechanisms. Receptor density as a potential contributing factor was discussed earlier.[68,100] The virus replicates within the infected cell but does not cause cytolysis.[68,69] HCV replicates to very high levels within liver cells, and a significant proportion of the virus is released into the bloodstream. The exact levels of virus in the liver are unknown.[65] At some point that differs from patient to patient based on age, genetics, and other host factors, the interaction among virus, host liver cells, and host immune cells results in either resolution of infection or progression to chronic infection.[24] Resolved acute HCV has no long-term consequences. During chronic infection, inflammatory signals are released from the infected cells that, over time, contribute to the fibrosis and necrosis that is observed with long-standing infection.[111]

Viral Life Cycle

HCV has been shown to interact with several proteins that serve as primary receptors or coreceptors depending on whether it is the initial infection or whether cell to cell spread has occurred within an already infected liver. The first interaction appears to be when LVPs interact with the scavenger-receptor B1 (SR-B1) protein.[18] This interaction is not required, and other lipid-related proteins such as the low-density lipoprotein receptor (LDLR) may substitute for SR-B1.[84] Given the enrichment of these proteins on liver cells, this step likely provides much of the tissue specificity for the virus. After this initial binding step, a more specific interaction takes place between the HCV E2 protein and the CD81 molecule.[59,133] This interaction is thought to be a critical one for initial infections. This E2-CD81 complex then traffics to the tight junction of the cell, where interactions with both tight junction proteins claudin-1 and occludin-1 mediate the final steps in viral entry.[33,43,97] It has been shown that for direct cell to cell spread, the tight junction proteins can mediate infection alone, bypassing the need for CD81 or SR-B1.[121]

After cell entry, HCV relies on a specific interaction with a liver-specific microRNA, miR-122, as a necessary cofactor.[56] The HCV RNA has two sites in the 5′UTR that are complementary to miR-122.[55] This is the first instance of a microRNA enhancing the function of an RNA, because in almost every other circumstance, microRNAs serve to silence gene expression by causing degradation of mRNA.

Another key step in the HCV life cycle is the association of the core protein with lipid droplets as part of viral assembly and release.[15,82] When first discovered, this was unique among flaviviruses, although it is now understood that dengue virus also associates with lipid droplets.[107] When bound to lipid droplets, the core protein recruits other viral proteins and newly synthesized RNA to form new virions.[82] Disruption of this association prevents the release of infectious virus.[15,112] HCV has evolved to co-opt many host lipid synthesis and trafficking pathways—binding to lipid droplets, requiring several apolipoproteins for assembly, and using VLDL export pathways for viral release.*

EPIDEMIOLOGY

The epidemiology of HCV varies significantly based on age and geography. In the general U.S. population, approximately 4 million have HCV antibody–positive status and approximately two-thirds of those have chronic infection.[5,8] In children, between 0.1 and 0.4 percent have evidence of HCV antibody.[5,31] These studies were performed before major demographic shifts in the U.S. population over the last decade, so these rates may have increased.

Certain countries are known to have exceedingly high rates of HCV seroprevalence, largely as a result of unsafe injection practices and a contaminated blood supply. Pakistan is known to have HCV seroprevalence rates of 4 to 10 percent.[61,124] In Egypt, 10 to 15 percent of the general population, including children, have HCV antibody–positive status.[1,113] This the highest rate in the world and is largely due to widespread inoculation of many individuals during a public health intervention to control schistosomiasis.[1] After screening for the parasite in the stool and urine, patients were treated with an intravenous tartar preparation.[116] Needles used during these treatments were not sterile during the approximate 20-year period when these practices were in place. Once oral praziquantel replaced the intravenous treatment, the rates of HCV infection from this intervention were reduced significantly. However, the seroprevalence in the population was already high and other modes of transmission serve to perpetuate the epidemic.[83,96]

VIRAL GENETICS

HCV has six major genotypes of HCV that very in their geographic distribution.[35,80,115] Genotypes 1a and 1b are the most prevalent genotypes in the United States and Western Europe. Genotypes 2 and 3 are minority genotypes in this region, but are more frequently observed in Asia and Australia. Genotype 4 is frequently observed in Africa, particularly in Egypt. Genotypes 5 and 6 are minority genotypes, mostly in Africa.

*References 7, 11, 49, 58, 82, 106, 112, 114.

The different genotypes are associated with important clinical phenotypes. Response to interferon (IFN)-based therapy varies widely based on genotype.[36] Genotypes 2 and 3 are exceedingly responsive to IFN, with sustained response rates between 80 to 90 percent. Genotypes 1a, 1b, and 4 all respond to IFN at much lower rates, closer to 40 to 50 percent. Genotype 3 is associated with more frequent and more severe steatosis (fatty liver) in contrast to other genotypes.[48,92]

TRANSMISSION

HCV is transmitted efficiently by exposure for blood and blood-containing solutions, tissue, and equipment.[68,130] Before effective tests for screening blood and blood products in the early 1990s, virtually all patients dependent on regular transfusions, such as hemophiliacs and those with hemoglobinopathies, became chronically infected with HCV.[4,128] In the current era, in which blood supplies are rigorously screened, the primary route of bloodborne transmission is among injection drug users. Nosocomial transmission from medical and dental equipment is a constant issue that manifests when intentional and unintentional breach of sterile techniques occur.[19,21,22,103,118]

For children, the major route of transmission is mother to infant (vertical transmission).[9] It is clear that only mothers with active viremia will transmit HCV, but beyond that, no clear risk factors for transmission have been defined.[20,74,101,113] Although higher viral loads increase risk, no threshold level has been defined. Caesarean section has not been demonstrated to be protective of transmission.[19,32,74,79] Studies from countries with different prevailing genotypes demonstrate comparable rates of transmission.[20,74,113] Overall transmission rates vary between 5 and 10 percent depending on the study. In the era before highly active antiretroviral therapy (HAART), HIV coinfection raised the vertical transmission rate to 20 to 25 percent.[89,101,120] However, in the current HAART era, if a woman's HIV disease is suppressed or well controlled, the risk for HCV vertical transmission is comparable to that with HCV monoinfection.[22a]

For reasons that are not entirely clear, a significant rate of spontaneous resolution of HCV infection occurs among young children such that up to 50 percent of those with an initial transmission resolve their HCV viremia, leaving a rate of chronic infection after vertical transmission of 3 to 5 percent.[19,113]

Other, less frequent modes of transmission deserve some discussion. Sexual transmission is an inefficient and uncommon route of transmission.[73,86] This has been demonstrated largely in long-term studies of serodiscordant couples, in whom the infection rate was only 1 to 2 percent and many of those who seroconverted had independent risk factors for HCV infection. The exception to this is for men who have sex with men, which is a high-risk exposure.[64] Tattooing is not considered a high-risk exposure, particularly in the current era when sterile techniques are commonplace.[122] This may not be true for a war veteran or prisoner where sterile techniques were not used. Breast-feeding has never been documented as a source of infection and is not contraindicated in new mothers who have HCV infection.[32,62,67] Other microscopic blood exposures from razors and toothbrushes are possible and are targets for prevention measures.[20] In a fraction of patients, no obvious risk factor for HCV exists. It is presumed that some of these microscopic exposures may have been the cause in these cases or that patients may either not recall their high-risk exposure or may not be entirely forthcoming.

CLINICAL MANIFESTATIONS

HCV in most cases is an asymptomatic infection.[4,54] Patients are often diagnosed when seeking medical attention for other reasons and are found to have elevated liver enzymes. In the minority of cases, patients will have acute signs of hepatitis, including malaise, jaundice, abdominal pain, and discomfort.[127] This is often the sign of a robust immune response that may portend clearance of the acute infection. In most patients, there are no acute symptoms and the infection quietly progresses from acute to chronic over the course of several months. The specific determinants of this progression are still largely unknown.

For children infected with HCV, these same observations largely hold true.[54] Infants infected at birth manifest no symptoms for many years.[123] They are often diagnosed only because the mother is known to be infected or has several high-risk factors that warrant screening. Some infants may have elevated transaminases early in life, but most do not. HCV does not alter physical growth or cognitive development. This is an important point to emphasize with parents in counseling during early visits.

Children are also frequently diagnosed during their teenage years during screening for other reasons. Commonly encountered scenarios include testing of those who were preterm infants born in the late 1980s or early 1990s or teenagers who were screened as part of a school blood donation drive and tested positive for HCV antibody.

The major risk for chronic HCV infection is the progression to chronic liver disease, which includes both cirrhosis and subsequent hepatocellular carcinoma (HCC).[111] The rate of these complications in children is low, approximately 5 percent of the time, but it increases with duration of infection. A European study demonstrated that cirrhosis was observed only in children infected for more than 10 years.[53] A large U.S. study demonstrated that many infected children who underwent biopsy had either mild or no fibrosis.[42] Teenagers can have HCV-associated cirrhosis or HCC requiring transplant.[105]

HCV has a variety of extrahepatic manifestations that are primarily observed in adults, but may be possible in children.[17,50] These are skin and joint manifestations such as porphyria cutanea tarda, mixed cryoglobulinemia, and vasculitis that are due to immune complex deposition in small vessels. HCV has also been associated with insulin-resistance and resulting diabetes.[88] Although the specific mechanism of this is unknown, it is likely due to effects of the HCV core protein on regulation of glucose metabolism at multiple levels within liver cells.[87,93] HCV also

has been associated with extreme levels of fatigue and altered mood and concentration.[108] It has recently been demonstrated that HCV can infect brain endothelial cells in vitro, and immunohistochemistry has shown HCV to be present in the brain tissue of chronically infected patients.[34] This raises the possibility that HCV may have some direct effect on the brain.

IMMUNITY

HCV infection is associated with both a cellular and humoral immune response.[16] Responses can be detected after approximately 6 to 8 weeks of infection. Patients with acute infection have evidence of a robust cell-mediated response, with readily detected HCV specific $CD8^+$ T cells.[127] This response mediates clearance of acute infection in these patients. Most patients, however, have specific T cells detected, but the response is weak and ineffective. These patients progress to chronic infection, and over time their T cells display an exhausted phenotype that has little impact on the course of infection.

HCV antibodies are detectable throughout the course of infection, but their role in preventing infection is unclear.[16] As mentioned earlier, neutralizing antibodies to HCV are not observed because of the hypermutation observed in the HCV E2 protein generating many "quasispecies" of HCV that escape neutralization. However, the presence of antibody may alter progression of infection over time in ways not yet understood.

HCV also interacts with the innate immune system to subvert host response to viral infection.[44] Multiple proteins have been demonstrated to directly cleave IFN-induced mediators of host protection.[117] A more specific discussion of these effects can be found in several in depth reviews.[37,44]

DIAGNOSIS

HCV infection can be diagnosed by demonstration of HCV-specific antibody, the presence of HCV RNA from serum or blood samples, or direct demonstration of HCV proteins in serum or blood samples.[57]

First-pass screening of at-risk children is usually done with enzyme-linked immunosorbent assay (ELISA)-based testing. If positive, patients can be tested using a highly specific recombinant immunoblot assay, which detects the presence of antibodies to several HCV proteins. However, this is very rarely necessary. Most practitioners would move immediately to testing for HCV RNA, because the presence of RNA demonstrates chronic infection, which is the most important biomarker of infection. HCV RNA is now done using a real-time polymerase chain reaction (PCR)-based assay that offers the advantages of both reliability for presence of viral RNA and a broad dynamic range for quantitation.[23] If HCV RNA is detected, a frequent next step is determination of HCV genotype, which is done by characterizing the sequence of the HCV polymerase gene.[115] As has been discussed previously, knowledge of genotype can have a major impact on the choice of how and when to treat a patient. An algorithm for the evaluation of a child with HCV-positive antibody testing is outlined in Figure 176-2.

The testing of those infants who are vertically exposed is an altogether different matter. Because of the persistence of maternal antibody and the significant potential for spontaneous resolution of infection in young children, simple ELISA or single-point HCV RNA can be misleading.[20,113] There is no consensus on the best time to test these infants. The approach of this author is outlined in Figure 176-3.

Because no treatment is initiated during infancy, testing is not recommended in the first year. The possible exception to this is if a parent has considerable anxiety over the possibility of infection, performing an HCV RNA test in the first 6 months of life can be considered. If the test is negative, it is very reassuring although not absolute that the child is not infected. A positive result indicates infection but still leaves open the possibility that the infant may spontaneously resolve the infection over the next 1 to 2 years. Preferred timing for testing would be after the first birthday and should include both an HCV antibody and HCV RNA PCR. A repeat result several months later confirming a negative test should be performed before declaring a child uninfected. If the initial test result is positive, a repeat in 6 to 12 months should be performed, and, if the result is still positive, an annual monitoring plan that includes a careful history and physical, liver function testing, and HCV RNA testing should be initiated.

Assessment for Severity of Disease

In addition to a careful physical examination to determine if signs of chronic liver disease are present, assessment of laboratory, radiologic, and pathologic specimens should be performed. The severity of transaminase elevation, if any, should be determined. These tests are notoriously insensitive for disease of mild and moderate severity but are helpful when normal or significantly elevated.[99] Other laboratory tests should be performed to assess the synthetic function of the liver (albumin, prothrombin time, partial thromboplastin time, etc.).[72]

Routine radiologic studies are not necessary for all HCV-infected patients. Ultrasound of the liver can be used at an initial visit for an assessment of any anatomic factors that may complicate future assessments.[72] Unlike hepatitis B virus, with which the risk for HCC is independent of liver damage, with rare exception, HCC with HCV occurs only after cirrhosis is present.[47] If a patient is known to have HCV-induced cirrhosis, annual ultrasounds are justified. If not, periodic testing every 5 years or so may be more appropriate.

Definitive assessment of HCV-induced liver damage is done using a liver biopsy.[72] Certainly all teenagers and adults should have a liver biopsy performed as part of their initial assessment, with the exception of those known to be infected with genotypes that are exceptionally responsive to current standard of care (genotypes 2 or 3). In that case, many providers would proceed to therapy to achieve a definitive cure. In the case of the

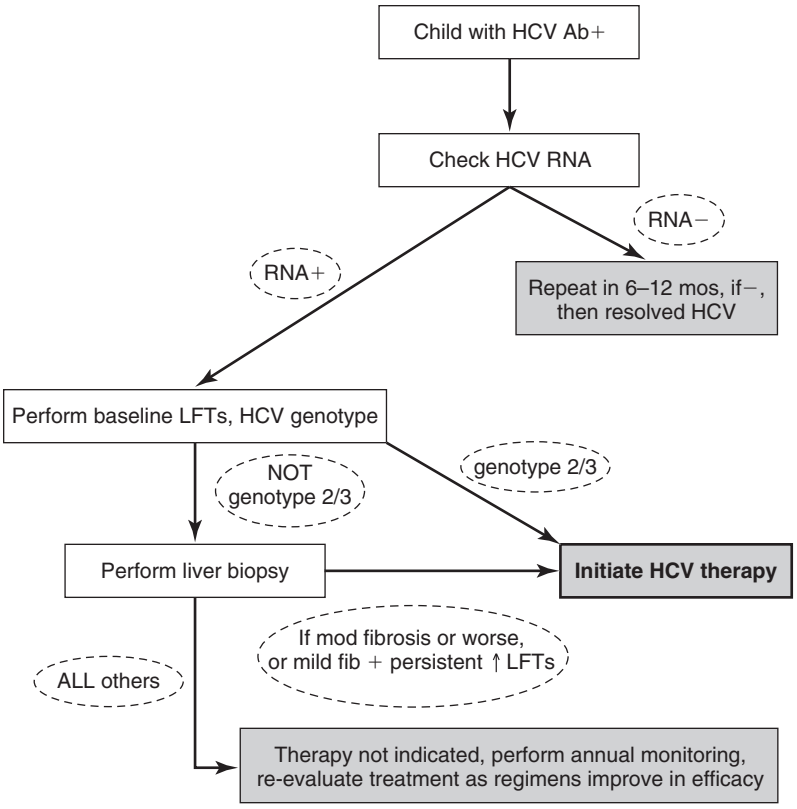

FIGURE 176-2 ■ Suggested algorithm for evaluation of a child with evidence of HCV antibody. End points are highlighted by a *shaded box,* while key result branch points in the decision tree are highlighted in the *ovals* with *dashed borders.*

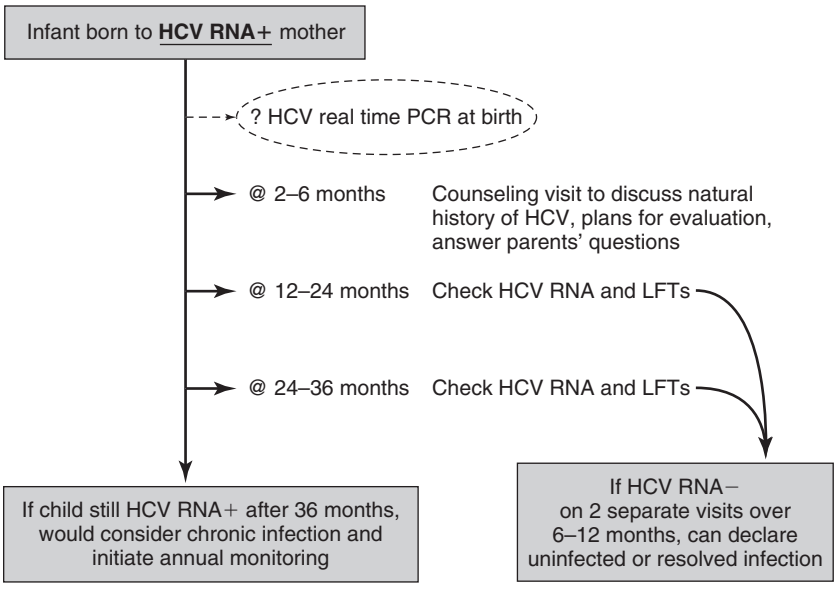

FIGURE 176-3 ■ Suggested algorithm for evaluation of the infant vertically exposed to HCV. End points are highlighted by a *shaded box,* while testing that has not yet been studied are highlighted with *dashed borders.*

child in the first decade of life where rates of significant fibrosis and cirrhosis are exceedingly low, liver biopsy is not necessary, particularly because the timing of potential therapy in these patients has not been determined. It is my opinion that in most cases liver biopsy not be performed until a child is 8 to 10 years of age, unless evidence demonstrates ongoing liver injury (i.e., persistent liver enzyme elevation, complicating risk factors such as obesity, or history of liver disease). However, no consensus exists on this issue.

Many studies have been performed to determine if combinations of laboratory or radiologic tests can be used to assess for liver fibrosis in the absence of a liver biopsy. These so-called noninvasive markers of fibrosis consist of a variety of blood tests that are used to create a score or index that allows for subsequent selection of those more likely to have severe liver damage. Most of these tests perform reasonably well at the ends of the disease severity spectrum, but very poorly in the middle.[90] This is true in children as well.[75] More sensitive ultrasound-based

methods evaluate liver stiffness, which more closely examines for liver fibrosis.[27] These methods are becoming more prevalent in adults and have been piloted in children.[30,91] However, they have not yet supplanted liver biopsy as the primary mode of assessment.

Host Genetics

Studies performed in the last several years have now demonstrated that host genetics play a significant role in the course of HCV disease. As part of a genome-wide associated study, polymorphisms within the *IL28b* gene were initially shown to have an exceedingly high correlation to response to treatment with IFN-based therapy.[40] In examination of the rates of the favorable polymorphisms in different ethnic groups, these polymorphisms potentially accounted for most of the racial and ethnic differences seen using IFN-based therapy. Further studies demonstrated that these polymorphisms also correlated very highly with patients resolving acute HCV in contrast to those whose disease progressed to chronic infection.[119] The mechanism underlying these significant differences has yet to be determined, but because *IL28b* encodes for a lambda-IFN that then regulates subsequent host antiviral responses, studies are being conducted to closely examine the initial lambda-IFN dynamics. No studies have thus far been performed in children, but because these are genetic differences, they likely will have the same impact in children. One study searched for a potential role of this polymorphism in predicting and determining vertical transmission, but no correlation was found.[104] It is important to emphasize that although this polymorphism on a population level is an exceedingly powerful predictor of HCV clearance and response to therapy, it is still unclear how to use this information for the individual patient. The polymorphism is not absolutely predictive of response in either direction; therefore, most practitioners evaluate patients for therapy based on disease status, not factoring in the patient's IL28b status.[26]

TREATMENT

The North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition recently issued practice guidelines for the monitoring and treatment of HCV-infected children.[72] The reader is referred there for a detailed discussion of drug doses, adjustments, and side effects.

HCV therapy is undergoing a significant transition as more agents against specific viral proteins emerge from preclinical studies. This section will attempt to cover currently approved information for HCV, as well as what is anticipated in the next several years. Specific attention will be focused on the existing data for treating children. The goal of HCV treatment is the eradication from the bloodstream of viral RNA that persists after the discontinuation of therapy. This is in stark contrast to HIV therapy, which is suppressive only while the medications are continued. It is thought that treatment for HCV reduces the burden of virus in the liver such that either HCV is completely eradicated from the system or the immune system is able to control the virus to such low levels as to be undetectable in the bloodstream. Rare cases have been reported of viral reemergence long after treatment response when immunosuppression is initiated for an unrelated reason, supporting the latter as a likely scenario.[66] For clinical studies, the end point of HCV therapy is the sustained viral response (SVR), which is defined as the absence of detectable HCV RNA at 6 months after the discontinuation of therapy.[36,77] If patients do not achieve SVR, they are categorized as nonresponders (i.e., patients who never suppressed virus during treatment), partial responders (i.e., patients who partially suppressed virus early but then had recrudescence) or relapsers (i.e., patients who completely suppressed virus for some amount of time but then had recrudescence at some point later). Knowledge of this terminology is helpful in interpreting the HCV treatment literature and important because each group has a different prognosis if new treatment regimens are attempted later.

IFN-α and its chemically modified derivatives have long been the backbone for HCV therapy. Old preparations given subcutaneously three times per week offered SVR rates of only 15 to 20 percent.[29] In an attempt to improve the poor pharmacokinetics of traditional IFN, a polyethylene glycol (PEG-IFN) moiety was added to allow for much higher serum concentrations combined with less frequent weekly subcutaneous dosing. The use of PEG-IFN improved SVR rates by almost 20 percent.[36] The mechanism of IFN against HCV is still poorly understood. It most likely acts by an enhancement of host antiviral responses that were otherwise at levels too low to be effective. It is interesting to note that studies examining patients with the favorable and unfavorable IL28b polymorphisms have shown that HCV patients who achieve SVR are those with low levels of IFN-induced gene expression that are readily enhanced with IFN administration, whereas patients who do not respond to treatment have high IFN-induced gene expression that is ineffective in counteracting the virus and incapable of being augmented with exogenous IFN.[2,125] It has long been known that significant racial and ethnic differences exist in rates of response to IFN therapy. African-American and Hispanic/Latino patients achieve SVR at rates significantly lower than those of Caucasian or Asian patients.[78,85] Much of this may be due to differences in IL28b status, but not entirely.

Ribavirin is a current mainstay of HCV therapy. When traditional IFN was still being used with very low SVR rates, other agents were sought to boost its effects. With the addition of orally administered ribavirin, SVR rates increased to almost 40 percent for patients infected with genotypes 1a or 1b.[76] When PEG-IFN was developed, the combination of PEG-IFN and ribavirin resulted in SVR rates of 40 to 50 percent for genotype 1a and 1b patients and 80 to 90 for genotype 2 and 3 patients.[36] The mechanism of action of ribavirin is still unknown but is thought to inhibit viral replication efficiency enough to make the virus more susceptible to other agents. Ribavirin monotherapy has no effect on HCV RNA levels and does not lead to SVR.[14]

PEG-IFN and ribavirin have been studied and approved for use in children at reduced doses.[52,109,110,129] Small pharmacokinetic studies determined that either dosing of PEG-IFN based on weight or body surface area led to serum drug levels comparable to those in adult administration.[110] Ribavirin is given on a weight-based dose for both children and adults.[41] The largest single study of HCV treatment in children was the Pediatric Study of Hepatitis C (PEDS-C) trial, which compared PEG-IFN alone to PEG-IFN plus ribavirin.[109] The rationale for this design was that many thought that PEG-IFN alone may be sufficient in younger children with the absence of many other adverse risk factors. The PEDS-C trial demonstrated that children had overall SVR rates comparable to their adult counterparts (53%) when treated with both drugs, significantly better in contrast to when PEG-IFN was used alone (37%).

Several factors must be considered when treating children with these agents.[72,109] Side effects of PEG-IFN include flulike symptoms and malaise. For children attending school who require maximal function during the school week, PEG-IFN administration on Friday with coadministration of a nonsteroidal anti-inflammatory agent has been effective in alleviating these effects. IFN can also cause neutropenia and hemolytic anemia, both of which can be managed with dose reduction. PEG-IFN has been shown to cause depression and suicidal ideation, dangerous at any age but particularly worrisome during the usually emotionally turbulent adolescent years. A side effect unique to children is the associated arrest of linear growth during PEG-IFN treatment. Although children do experience catch-up growth once the drug is discontinued, long-term follow up is still being conducted to determine whether children actually attain their predicted midparental height.[60]

The issue of when to initiate therapy for a child infected with HCV is still under contention.[72] There is no controversy in the case of a child who has clear evidence of liver compromise from long-standing HCV infection, but these cases are the minority. Because natural history studies of children with HCV do indicate that many of them will do well with only minimal progression of liver fibrosis, treatment initiation remains a controversial issue. Proponents of therapy argue the many potential benefits of treating children: the cost advantage of using less of an expensive drug when treating a smaller person; the number of years of "virus-free" life still ahead for a child; the absence of comorbidities in a child that may complicate treatment; eradicating virus before entering adolescence, when high-risk behavior that can potentially transmit the virus traditionally begins; and for girls, eliminating the potential for vertical transmission. At this point, the decision about when to treat is one that should be individualized on a case-by-case basis.

In May 2011, the first of the HCV-specific antiviral agents were approved. Boceprevir and telaprevir both target the NS3 protease domain of HCV genotype 1a and 1b isolates. When used in combination with PEG-IFN and ribavirin, they are able to boost SVR rates to almost 70 percent in adults.[51,98] These agents also offer an SVR rate of about 70 percent when combined with PEG-IFN and ribavirin for those patients who were relapsers or partial responders to prior IFN-based combination therapy.[10,131] Side effects include a rash that spontaneously resolves in most cases but may require discontinuation in severe cases. They can also cause anemia that can be handled with dose reduction. These agents require a lead in dosing period before initiating PEG-IFN and ribavirin, so their use should be limited to experienced providers. Studies examining the dose and efficacy of these agents in children are currently in the planning and organizational phase.

Several other classes of agents in advanced preclinical development deserve mention. More potent and multigenotype NS3 inhibitors are in phase II and III development.[81] Agents that target the NS5A protein are extremely potent antiviral agents that achieve 3 to 4 log reductions in HCV RNA with single doses.[38,94] HCV NS5B polymerase inhibitors are also in phase II and III development.[132] Studies looking at combinations of multiple agents offer hope of highly potent regimens that offer SVR to the majority of patients. One such pilot study examined adults who were previous nonresponders to PEG-IFN and ribavirin and separated them into two study groups.[71] In one group, 10 patients were retreated with PEG-IFN and ribavirin, along with two orally administered investigational agents, daclatasvir, an anti-NS5A drug, and asunaprevir, a potent NS3 protease inhibitor. In the other group, 11 patients just received the two investigational agents. The results were striking in that 100 percent of patients (10/10) who previously had no response to PEG-IFN achieved SVR when treated with all four drugs. What is also striking is that almost 40 percent of the patients (4/11) given a regimen of just the two investigational agents also achieved SVR. These results affirm the hope of many that HCV therapy will eventually become free of subcutaneous IFN injections and the associated side effects and involve only orally administered, highly potent agents.

What also becomes possible in the future is interruption of vertical transmission using these specific antiviral agents in and around the time of delivery in a manner similar to treatment of HIV.[9] Given the much lower risk for transmission and lower morbidity and mortality in contrast to HIV, further studies into understanding better which mothers transmit HCV and when they transmit the virus would allow more selective drug use.

PREVENTION AND COUNSELING

No specific prevention measures are currently available against HCV infection. Attempts to create a vaccine have been frustrated by the heterogeneity of the virus and an incomplete understanding of how potent cellular responses are generated. Prevention largely rests on avoiding high-risk behaviors, rigorous sterile technique to avoid nosocomial transmission, and screening to identify HCV-infected adults and children so patients can be evaluated and treated. Counsel for the child who is already infected with HCV should include prompt care of bloody injuries, vaccination against other forms of viral hepatitis to avoid potentiated injury to the liver, being

aware of prescription and nonprescription drugs that cause liver toxicity, being aware of the risks of alcohol use when chronically infected with HCV and that no known safe level of alcohol consumption exists for HCV-infected patients.[72] It is also important to reiterate to parents that HCV-infected children grow and develop normally and do not present a risk to classmates. It is also important to advocate for HCV-infected children because some schools and other agencies needlessly continue to try to restrict the attendance and activities of HCV-infected children.

SUMMARY

Children with HCV infection often do very well with few or no long-term effects, but a few children do develop significant liver disease. A working knowledge of how to monitor and manage these patients can assist in mitigating the severity of disease. The treatment of HCV is in the midst of major changes that will revolutionize the field. Although the new anti-HCV agents are still far from being ready to be used for children, familiarity with what will ultimately become available will assist providers in discussions with their patients.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abe H, Hayes CN, Ochi H, et al. IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. *J Hepatol* 2011;**54**:1094–101.
9. Arshad M, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period: are they opportunities for treatment? *J Viral Hepat* 2011;**18**:229–36.
10. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**:1207–17.
11. Bartenschlager R, Penin F, Lohmann V, et al. Assembly of infectious hepatitis C virus particles. *Trends Microbiol* 2011;**19**:95–103.
21. Centers for Disease Control and Prevention. Acute hepatitis C virus infections attributed to unsafe injection practices at an endoscopy clinic: Nevada, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:513–17.
22. Centers for Disease Control and Prevention. Hepatitis C virus transmission at an outpatient hemodialysis unit: New York, 2001-2008. *MMWR Morb Mortal Wkly Rep* 2009;**58**:189–94.
23. Chevaliez S. Virological tools to diagnose and monitor hepatitis C virus infection. *Clin Microbiol Infect* 2011;**17**:116–21.
26. Clark PJ, Muir AJ. Lost in translation? IL28B's discovery and the journey back to the patient. *Hepatology* 2012;**56**:5–8.
27. Colletta C, Smirne C, Fabris C, et al. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. *Hepatology* 2005;**42**:838–45.
34. Fletcher NF, Wilson GK, Murray J, et al. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. *Gastroenterology* 2012;**142**:634–43 e6.
38. Gao M, Nettles RE, Belema M, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010;**465**:96–100.
39. Gastaminza P, Cheng G, Wieland S, et al. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. *J Virol* 2008;**82**:2120–9.
40. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;**461**:399–401.
42. Goodman ZD, Makhlouf HR, Liu L, et al. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. *Hepatology* 2008;**47**:836–43.
43. Harris HJ, Farquhar MJ, Mee CJ, et al. CD81 and claudin 1 coreceptor association: role in hepatitis C virus entry. *J Virol* 2008;**82**:5007–20.
44. Horner SM, Gale M Jr. Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. *J Interferon Cytokine Res* 2009;**29**:489–98.
47. Huang YT, Jen CL, Yang HI, et al. Lifetime risk and sex difference of hepatocellular carcinoma among patients with chronic hepatitis B and C. *J Clin Oncol* 2011;**29**:3643–50.
49. Icard V, Diaz O, Scholtes C, et al. Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins. *PLoS One* 2009;**4**:e4233.
50. Indolfi G, Bartolini E, Olivito B, et al. Autoimmunity and extrahepatic manifestations in treatment-naive children with chronic hepatitis C virus infection. *Clin Dev Immunol* 2012; **2012**:785627.
51. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011;**364**:2405–16.
52. Jara P, Hierro L, de la Vega A, et al. Efficacy and safety of peginterferon-alpha2b and ribavirin combination therapy in children with chronic hepatitis C infection. *Pediatr Infect Dis J* 2008; **27**:142–8.
55. Jopling CL, Schutz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 2008;**4**:77–85.
57. Kamili S, Drobeniuc J, Araujo AC, et al. Laboratory diagnostics for hepatitis C virus infection. *Clin Infect Dis* 2012;**55**(suppl 1): S43–8.
60. Kelly DA, Haber B, Gonzalez-Peralta RP, et al. Durability of sustained response shown in paediatric patients with chronic hepatitis C who were treated with interferon alfa-2b plus ribavirin. *J Viral Hepat* 2012;**19**:263–70.
61. Khattak MN, Akhtar S, Mahmud S, et al. Factors influencing hepatitis C virus sero-prevalence among blood donors in north west Pakistan. *J Public Health Policy* 2008;**29**:207–25.
64. Larsen C, Chaix ML, Le Strat Y, et al. Gaining greater insight into HCV emergence in HIV-infected men who have sex with men: the HEPAIG Study. *PLoS One* 2011;**6**:e29322.
65. Liang Y, Shilagard T, Xiao SY, et al. Visualizing hepatitis C virus infections in human liver by two-photon microscopy. *Gastroenterology* 2009;**137**:1448–58.
66. Lin A, Thadareddy A, Goldstein MJ, et al. Immune suppression leading to hepatitis C virus re-emergence after sustained virological response. *J Med Virol* 2008;**80**:1720–2.
71. Lok AS, Gardiner DF, Lawitz E, et al. Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012;**366**:216–24.
72. Mack CL, Gonzalez-Peralta RP, Gupta N, et al. NASPGHAN practice guidelines: diagnosis and management of hepatitis C infection in infants, children, and adolescents. *J Pediatr Gastroenterol Nutr* 2012;**54**:838–55.
75. McGoogan KE, Smith PB, Choi SS, et al. Performance of the AST-to-platelet ratio index as a noninvasive marker of fibrosis in pediatric patients with chronic viral hepatitis. *J Pediatr Gastroenterol Nutr* 2010;**50**:344–6.
78. McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009;**361**:580–93.
79. McMenamin MB, Jackson AD, Lambert J, et al. Obstetric management of hepatitis C-positive mothers: analysis of vertical transmission in 559 mother-infant pairs. *Am J Obstet Gynecol* 2008;**199**: 315 e1–5.
81. McPhee F, Sheaffer AK, Friborg J, et al. Preclinical profile and characterization of the hepatitis C virus NS3 protease inhibitor asunaprevir (BMS-650032). *Antimicrob Agents Chemother* 2012;**56**: 5387–96.
90. Patel K, Benhamou Y, Yoshida EM, et al. An independent and prospective comparison of two commercial fibrosis marker panels (HCV FibroSURE and FIBROSpect II) during albinterferon alfa-2b combination therapy for chronic hepatitis C. *J Viral Hepat* 2009;**16**:178–86.
91. Patel K, Friedrich-Rust M, Lurie Y, et al. FibroSURE and FibroScan in relation to treatment response in chronic hepatitis C virus. *World J Gastroenterol* 2011;**17**:4581–9.

94. Pelosi LA, Voss S, Liu M, et al. Effect on HCV replication by combinations of direct acting antivirals including NS5A inhibitor daclatasvir. *Antimicrob Agents Chemother* 2012;**56**:5230–9.
96. Plancoulaine S, Mohamed MK, Arafa N, et al. Dissection of familial correlations in hepatitis C virus (HCV) seroprevalence suggests intrafamilial viral transmission and genetic predisposition to infection. *Gut* 2008;**57**:1268–74.
97. Ploss A, Evans MJ, Gaysinskaya VA, et al. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009;**457**:882–6.
98. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;**364**:1195–206.
103. Ross RS, Viazov S, Khudyakov YE, et al. Transmission of hepatitis C virus in an orthopedic hospital ward. *J Med Virol* 2009;**81**:249–57.
104. Ruiz-Extremera A, Munoz-Gamez JA, Salmeron-Ruiz MA, et al. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011;**53**:1830–8.
107. Samsa MM, Mondotte JA, Iglesias NG, et al. Dengue virus capsid protein usurps lipid droplets for viral particle formation. *PLoS Pathog* 2009;**5**:e1000632.
108. Sarkar S, Jiang Z, Evon DM, et al. Fatigue before, during and after antiviral therapy of chronic hepatitis C: results from the Virahep-C study. *J Hepatol* 2012.
109. Schwarz KB, Gonzalez-Peralta RP, Murray KF, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. *Gastroenterology* 2011;**140**:450–8 e1.
113. Shebl FM, El-Kamary SS, Saleh DA, et al. Prospective cohort study of mother-to-infant infection and clearance of hepatitis C in rural Egyptian villages. *J Med Virol* 2009;**81**:1024–31.
119. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009;**461**:798–801.
121. Timpe JM, Stamataki Z, Jennings A, et al. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 2008;**47**:17–24.
122. Tohme RA, Holmberg SD. Transmission of hepatitis C virus infection through tattooing and piercing: a critical review. *Clin Infect Dis* 2012;**54**:1167–78.
124. Umar M, Khaar HT, Khurram M, et al. Anti-HCV antibody positivity of various sections of Pakistani patients. *J Coll Physicians Surg Pak* 2009;**19**:737–41.
125. Urban TJ, Thompson AJ, Bradrick SS, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. *Hepatology* 2010;**52**:1888–96.
129. Wirth S, Ribes-Koninckx C, Calzado MA, et al. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. *J Hepatol* 2010;**52**:501–7.
131. Zeuzem S, Andreone P, Pol S, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011;**364**:2417–28.
132. Zeuzem S, Asselah T, Angus P, et al. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011;**141**:2047–55; quiz e14.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdel-Wahab MF, Zakaria S, Kamel M, et al. High seroprevalence of hepatitis C infection among risk groups in Egypt. *Am J Trop Med Hyg* 1994;**51**:563–7.
2. Abe H, Hayes CN, Ochi H, et al. IL28 variation affects expression of interferon stimulated genes and peg-interferon and ribavirin therapy. *J Hepatol* 2011;**54**:1094–101.
3. Agnello V, Abel G, Elfahal M, et al. Hepatitis C virus and other flaviviridae viruses enter cells via low density lipoprotein receptor. *Proc Natl Acad Sci U S A* 1999;**96**:12766–71.
4. Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol* 2007;**13**:2436–41.
5. Alter MJ, Kruszon-Moran D, Nainan OV, et al. The prevalence of hepatitis C virus infection in the United States, 1988 through 1994. *N Engl J Med* 1999;**341**:556–62.
6. Andre P, Komurian-Pradel F, Deforges S, et al. Characterization of low- and very-low-density hepatitis C virus RNA-containing particles. *J Virol* 2002;**76**:6919–28.
7. Andre P, Perlemuter G, Budkowska A, et al. Hepatitis C virus particles and lipoprotein metabolism. *Semin Liver Dis* 2005;**25**: 93–104.
8. Armstrong GL, Wasley A, Simard EP, et al. The prevalence of hepatitis C virus infection in the United States, 1999 through 2002. *Ann Intern Med* 2006;**144**:705–14.
9. Arshad M, El-Kamary SS, Jhaveri R. Hepatitis C virus infection during pregnancy and the newborn period: are they opportunities for treatment? *J Viral Hepat* 2011;**18**:229–36.
10. Bacon BR, Gordon SC, Lawitz E, et al. Boceprevir for previously treated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**:1207–17.
11. Bartenschlager R, Penin F, Lohmann V, et al. Assembly of infectious hepatitis C virus particles. *Trends Microbiol* 2011;**19**: 95–103.
12. Blanchard E, Brand D, Trassard S, et al. Hepatitis C virus-like particle morphogenesis. *J Virol* 2002;**76**:4073–9.
13. Blight KJ, Kolykhalov AA, Rice CM. Efficient initiation of HCV RNA replication in cell culture. *Science* 2000;**290**:1972–4.
14. Bodenheimer HC Jr, Lindsay KL, Davis GL, et al. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. *Hepatology* 1997;**26**:473–7.
15. Boulant S, Targett-Adams P, McLauchlan J. Disrupting the association of hepatitis C virus core protein with lipid droplets correlates with a loss in production of infectious virus. *J Gen Virol* 2007;**88**:2204–13.
16. Bowen DG, Walker CM. Adaptive immune responses in acute and chronic hepatitis C virus infection. *Nature* 2005;**436**: 946–52.
17. Cacoub P, Poynard T, Ghillani P, et al. Extrahepatic manifestations of chronic hepatitis C. MULTIVIRC Group. Multidepartment Virus C. *Arthritis Rheum* 1999;**42**:2204–12.
18. Catanese MT, Graziani R, von Hahn T, et al. High-avidity monoclonal antibodies against the human scavenger class B type I receptor efficiently block hepatitis C virus infection in the presence of high-density lipoprotein. *J Virol* 2007;**81**:8063–71.
19. Ceci O, Margiotta M, Marello F, et al. Vertical transmission of hepatitis C virus in a cohort of 2,447 HIV-seronegative pregnant women: a 24-month prospective study. *J Pediatr Gastroenterol Nutr* 2001;**33**:570–5.
20. Centers for Disease Control and Prevention. *STD & TB prevention: viral hepatitis C fact sheet* 2007. Atlanta, Ga, 2007.
21. Centers for Disease Control and Prevention. Acute hepatitis C virus infections attributed to unsafe injection practices at an endoscopy clinic: Nevada, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:513–17.
22. Centers for Disease Control and Prevention. Hepatitis C virus transmission at an outpatient hemodialysis unit: New York, 2001-2008. *MMWR Morb Mortal Wkly Rep* 2009;**58**:189–94.

22a. Checa Cabo CA, Stoszek SK, Quarleri J, et al. Mother -to-child transmission of hepatitis C virus (HCV) among HIV/HCV coinfected women. *J of Pediatr Infect Dis Soc* 2013;**2**:126–35.

23. Chevaliez S. Virological tools to diagnose and monitor hepatitis C virus infection. *Clin Microbiol Infect* 2011;**17**:116–21.
24. Chisari FV. Unscrambling hepatitis C virus-host interactions. *Nature* 2005;**436**:930–2.
25. Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;**244**:359–62.
26. Clark PJ, Muir AJ. Lost in translation? IL28B's discovery and the journey back to the patient. *Hepatology* 2012;**56**:5–8.
27. Colletta C, Smirne C, Fabris C, et al. Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferases. *Hepatology* 2005;**42**:838–45.
28. Das S, Ott M, Yamane A, et al. Inhibition of internal entry site (IRES)-mediated translation by a small yeast RNA: a novel strategy to block hepatitis C virus protein synthesis. *Front Biosci* 1998; **3**:D1241–52.
29. Davis GL, Balart LA, Schiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alfa: a multicenter randomized, controlled trial. Hepatitis Interventional Therapy Group. *N Engl J Med* 1989;**321**:1501–6.
30. de Ledinghen V, Le Bail B, Rebouissoux L, et al. Liver stiffness measurement in children using FibroScan: feasibility study and comparison with Fibrotest, aspartate transaminase to platelets ratio index, and liver biopsy. *J Pediatr Gastroenterol Nutr* 2007;**45**: 443–50.
31. El-Kamary SS, Serwint JR, Joffe A, et al. Prevalence of hepatitis C virus infection in urban children. *J Pediatr* 2003;**143**:54–9.
32. European Paediatric Hepatitis C Virus Network. Effects of mode of delivery and infant feeding on the risk of mother-to-child transmission of hepatitis C virus. *BJOG* 2001;**108**:371–7.
33. Evans MJ, von Hahn T, Tscherne DM, et al. Claudin-1 is a hepatitis C virus co-receptor required for a late step in entry. *Nature* 2007;**446**:801–5.
34. Fletcher NF, Wilson GK, Murray J, et al. Hepatitis C virus infects the endothelial cells of the blood-brain barrier. *Gastroenterology* 2012;**142**:634–43 e6.
35. Forns X, Bukh J. The molecular biology of hepatitis C virus: genotypes and quasispecies. *Clin Liver Dis* 1999;**3**:693–716, vii.
36. Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;**347**:975–82.
37. Gale M Jr, Foy EM. Evasion of intracellular host defence by hepatitis C virus. *Nature* 2005;**436**:939–45.
38. Gao M, Nettles RE, Belema M, et al. Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. *Nature* 2010;**465**:96–100.
39. Gastaminza P, Cheng G, Wieland S, et al. Cellular determinants of hepatitis C virus assembly, maturation, degradation, and secretion. *J Virol* 2008;**82**:2120–9.
40. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009;**461**:399–401.
41. Gonzalez-Peralta RP, Kelly DA, Haber B, et al. Interferon alfa-2b in combination with ribavirin for the treatment of chronic hepatitis C in children: efficacy, safety, and pharmacokinetics. *Hepatology* 2005;**42**:1010–18.
42. Goodman ZD, Makhlouf HR, Liu L, et al. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. *Hepatology* 2008;**47**:836–43.
43. Harris HJ, Farquhar MJ, Mee CJ, et al. CD81 and claudin 1 coreceptor association: role in hepatitis C virus entry. *J Virol* 2008;**82**:5007–20.
44. Horner SM, Gale M Jr. Intracellular innate immune cascades and interferon defenses that control hepatitis C virus. *J Interferon Cytokine Res* 2009;**29**:489–98.
45. Houghton M, Abrignani S. Prospects for a vaccine against the hepatitis C virus. *Nature* 2005;**436**:961–6.
46. Hsu M, Zhang J, Flint M, et al. Hepatitis C virus glycoproteins mediate pH-dependent cell entry of pseudotyped retroviral particles. *Proc Natl Acad Sci U S A* 2003;**100**:7271–6.
47. Huang YT, Jen CL, Yang HI, et al. Lifetime risk and sex difference of hepatocellular carcinoma among patients with chronic hepatitis B and C. *J Clin Oncol* 2011;**29**:3643–50.
48. Hui JM, Kench J, Farrell GC, et al. Genotype-specific mechanisms for hepatic steatosis in chronic hepatitis C infection. *J Gastroenterol Hepatol* 2002;**17**:873–81.
49. Icard V, Diaz O, Scholtes C, et al. Secretion of hepatitis C virus envelope glycoproteins depends on assembly of apolipoprotein B positive lipoproteins. *PLoS One* 2009;**4**:e4233.

50. Indolfi G, Bartolini E, Olivito B, et al. Autoimmunity and extrahepatic manifestations in treatment-naive children with chronic hepatitis C virus infection. *Clin Dev Immunol* 2012;**2012**:785627.
51. Jacobson IM, McHutchison JG, Dusheiko G, et al. Telaprevir for previously untreated chronic hepatitis C virus infection. *N Engl J Med* 2011;**364**:2405–16.
52. Jara P, Hierro L, de la Vega A, et al. Efficacy and safety of peginterferon-alpha2b and ribavirin combination therapy in children with chronic hepatitis C infection. *Pediatr Infect Dis J* 2008;**27**:142–8.
53. Jara P, Resti M, Hierro L, et al. Chronic hepatitis C virus infection in childhood: clinical patterns and evolution in 224 white children. *Clin Infect Dis* 2003;**36**:275–80.
54. Jonas MM. Children with hepatitis C. *Hepatology* 2002;**36**: S173–8.
55. Jopling CL, Schutz S, Sarnow P. Position-dependent function for a tandem microRNA miR-122-binding site located in the hepatitis C virus RNA genome. *Cell Host Microbe* 2008;**4**:77–85.
56. Jopling CL, Yi M, Lancaster AM, et al. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. *Science* 2005;**309**:1577–81.
57. Kamili S, Drobeniuc J, Araujo AC, et al. Laboratory diagnostics for hepatitis C virus infection. *Clin Infect Dis* 2012;**55**(suppl 1): S43–8.
58. Kapadia SB, Chisari FV. Hepatitis C virus RNA replication is regulated by host geranylgeranylation and fatty acids. *Proc Natl Acad Sci U S A* 2005;**102**:2561–6.
59. Kapadia SB, Barth H, Baumert T, et al. Initiation of hepatitis C virus infection is dependent on cholesterol and cooperativity between CD81 and scavenger receptor B type I. *J Virol* 2007;**81**: 374–83.
60. Kelly DA, Haber B, Gonzalez-Peralta RP, et al. Durability of sustained response shown in paediatric patients with chronic hepatitis C who were treated with interferon alfa-2b plus ribavirin. *J Viral Hepat* 2012;**19**:263–70.
61. Khattak MN, Akhtar S, Mahmud S, et al. Factors influencing hepatitis C virus sero-prevalence among blood donors in north west Pakistan. *J Public Health Policy* 2008;**29**:207–25.
62. Kumar RM, Shahul S. Role of breast-feeding in transmission of hepatitis C virus to infants of HCV-infected mothers. *J Hepatol* 1998;**29**:191–7.
63. Kuo G, Choo QL, Alter HJ, et al. An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989;**244**:362–4.
64. Larsen C, Chaix ML, Le Strat Y, et al. Gaining greater insight into HCV emergence in HIV-infected men who have sex with men: the HEPAIG Study. *PLoS One* 2011;**6**:e29322.
65. Liang Y, Shilagard T, Xiao SY, et al. Visualizing hepatitis C virus infections in human liver by two-photon microscopy. *Gastroenterology* 2009;**137**:1448–58.
66. Lin A, Thadareddy A, Goldstein MJ, et al. Immune suppression leading to hepatitis C virus re-emergence after sustained virological response. *J Med Virol* 2008;**80**:1720–2.
67. Lin HH, Kao JH, Hsu HY, et al. Absence of infection in breast-fed infants born to hepatitis C virus-infected mothers. *J Pediatr* 1995; **126**:589–91.
68. Lindenbach BD, Rice CM. Flaviviruses. In: Knipe DM, Howley PM, editors. *Fields virology*. 4th ed. Philadelphia, Pa: Lippincott Williams & Wilkins; 2001. p. 991–1042.
69. Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. *Nature* 2005;**436**:933–8.
70. Lohmann V, Korner F, Koch J, et al. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. *Science* 1999; **285**:110–13.
71. Lok AS, Gardiner DF, Lawitz E, et al. Preliminary study of two antiviral agents for hepatitis C genotype 1. *N Engl J Med* 2012;**366**:216–24.
72. Mack CL, Gonzalez-Peralta RP, Gupta N, et al. NASPGHAN practice guidelines: diagnosis and management of hepatitis C infection in infants, children, and adolescents. *J Pediatr Gastroenterol Nutr* 2012;**54**:838–55.
73. Marincovich B, Castilla J, del Romero J, et al. Absence of hepatitis C virus transmission in a prospective cohort of heterosexual serodiscordant couples. *Sex Transm Infect* 2003;**79**:160–2.
74. Mast EE, Hwang LY, Seto DS, et al. Risk factors for perinatal transmission of hepatitis C virus (HCV) and the natural history of HCV infection acquired in infancy. *J Infect Dis* 2005;**192**: 1880–9.
75. McGoogan KE, Smith PB, Choi SS, et al. Performance of the AST-to-platelet ratio index as a noninvasive marker of fibrosis in pediatric patients with chronic viral hepatitis. *J Pediatr Gastroenterol Nutr* 2010;**50**:344–6.
76. McHutchison JG, Fried MW. Current therapy for hepatitis C: pegylated interferon and ribavirin. *Clin Liver Dis* 2003;**7**:149–61.
77. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;**339**:1485–92.
78. McHutchison JG, Lawitz EJ, Shiffman ML, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009;**361**:580–93.
79. McMenamin MB, Jackson AD, Lambert J, et al. Obstetric management of hepatitis C-positive mothers: analysis of vertical transmission in 559 mother-infant pairs. *Am J Obstet Gynecol* 2008;**199**: 315 e1–5.
80. McOmish F, Yap PL, Dow BC, et al. Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. *J Clin Microbiol* 1994;**32**:884–92.
81. McPhee F, Sheaffer AK, Friborg J, et al. Preclinical profile and characterization of the hepatitis C virus NS3 protease inhibitor asunaprevir (BMS-650032). *Antimicrob Agents Chemother* 2012;**56**: 5387–9.
82. Miyanari Y, Atsuzawa K, Usuda N, et al. The lipid droplet is an important organelle for hepatitis C virus production. *Nat Cell Biol* 2007;**9**:1089–97.
83. Mohamed MK, Magder LS, Abdel-Hamid M, et al. Transmission of hepatitis C virus between parents and children. *Am J Trop Med Hyg* 2006;**75**:16–20.
84. Molina S, Castet V, Fournier-Wirth C, et al. The low-density lipoprotein receptor plays a role in the infection of primary human hepatocytes by hepatitis C virus. *J Hepatol* 2007;**46**:411–19.
85. Muir AJ, Bornstein JD, Killenberg PG. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N Engl J Med* 2004;**350**:2265–71.
86. Neumayr G, Propst A, Schwaighofer H, et al. Lack of evidence for the heterosexual transmission of hepatitis C. *QJM* 1999;**92**: 505–8.
87. Okuda M, Li K, Beard MR, et al. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002;**122**:366–75.
88. Ozyilkan E, Arslan M. Increased prevalence of diabetes mellitus in patients with chronic hepatitis C virus infection. *Am J Gastroenterol* 1996;**91**:1480–1.
89. Paccagnini S, Principi N, Massironi E, et al. Perinatal transmission and manifestation of hepatitis C virus infection in a high risk population. *Pediatr Infect Dis J* 1995;**14**:195–9.
90. Patel K, Benhamou Y, Yoshida EM, et al. An independent and prospective comparison of two commercial fibrosis marker panels (HCV FibroSURE and FIBROSpect II) during albinterferon alfa-2b combination therapy for chronic hepatitis C. *J Viral Hepat* 2009;**16**:178–86.
91. Patel K, Friedrich-Rust M, Lurie Y, et al. FibroSURE and FibroScan in relation to treatment response in chronic hepatitis C virus. *World J Gastroenterol* 2011;**17**:4581–9.
92. Patton HM, Patel K, Behling C, et al. The impact of steatosis on disease progression and early and sustained treatment response in chronic hepatitis C patients. *J Hepatol* 2004;**40**:484–90.
93. Pazienza V, Clement S, Pugnale P, et al. The hepatitis C virus core protein of genotypes 3a and 1b downregulates insulin receptor substrate 1 through genotype-specific mechanisms. *Hepatology* 2007;**45**:1164–71.
94. Pelosi LA, Voss S, Liu M, et al. Effect on HCV replication by combinations of direct acting antivirals including NS5A inhibitor daclatasvir. *Antimicrob Agents Chemother* 2012;**56**:5230–9.
95. Penin F, Dubuisson J, Rey FA, et al. Structural biology of hepatitis C virus. *Hepatology* 2004;**39**:5–19.
96. Plancoulaine S, Mohamed MK, Arafa N, et al. Dissection of familial correlations in hepatitis C virus (HCV) seroprevalence suggests

intrafamilial viral transmission and genetic predisposition to infection. *Gut* 2008;**57**:1268–74.
97. Ploss A, Evans MJ, Gaysinskaya VA, et al. Human occludin is a hepatitis C virus entry factor required for infection of mouse cells. *Nature* 2009;**457**:882–6.
98. Poordad F, McCone J Jr, Bacon BR, et al. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011;**364**: 1195–206.
99. Puoti C, Castellacci R, Montagnese F, et al. Histological and virological features and follow-up of hepatitis C virus carriers with normal aminotransferase levels: the Italian prospective study of the asymptomatic C carriers (ISACC). *J Hepatol* 2002;**37**: 117–23.
100. Randall G, Panis M, Cooper JD, et al. Cellular cofactors affecting hepatitis C virus infection and replication. *Proc Natl Acad Sci U S A* 2007;**104**:12884–9.
101. Resti M, Azzari C, Mannelli F, et al. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. Tuscany Study Group on Hepatitis C Virus Infection. *BMJ* 1998;**317**:437–41.
102. Robertson B, Myers G, Howard C, et al. Classification, nomenclature, and database development for hepatitis C virus (HCV) and related viruses: proposals for standardization. International Committee on Virus Taxonomy. *Arch Virol* 1998;**143**:2493–503.
103. Ross RS, Viazov S, Khudyakov YE, et al. Transmission of hepatitis C virus in an orthopedic hospital ward. *J Med Virol* 2009;**81**: 249–57.
104. Ruiz-Extremera A, Munoz-Gamez JA, Salmeron-Ruiz MA, et al. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. *Hepatology* 2011;**53**:1830–8.
105. Rumbo C, Fawaz RL, Emre SH, et al. Hepatitis C in children: a quaternary referral center perspective. *J Pediatr Gastroenterol Nutr* 2006;**43**:209–16.
106. Sagan SM, Rouleau Y, Leggiadro C, et al. The influence of cholesterol and lipid metabolism on host cell structure and hepatitis C virus replication. *Biochem Cell Biol* 2006;**84**:67–79.
107. Samsa MM, Mondotte JA, Iglesias NG, et al. Dengue virus capsid protein usurps lipid droplets for viral particle formation. *PLoS Pathog* 2009;**5**:e1000632.
108. Sarkar S, Jiang Z, Evon DM, et al. Fatigue before, during and after antiviral therapy of chronic hepatitis C: results from the Virahep-C Study. *J Hepatol* 2012;**57**:946–52.
109. Schwarz KB, Gonzalez-Peralta RP, Murray KF, et al. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. *Gastroenterology* 2011;**140**:450–8 e1.
110. Schwarz KB, Mohan P, Narkewicz MR, et al. Safety, efficacy and pharmacokinetics of peginterferon alpha2a (40 kd) in children with chronic hepatitis C. *J Pediatr Gastroenterol Nutr* 2006;**43**: 499–505.
111. Seeff LB, Hollinger FB, Alter HJ, et al. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: a National Heart, Lung, and Blood Institute collaborative study. *Hepatology* 2001;**33**:455–63.
112. Shavinskaya A, Boulant S, Penin F, et al. The lipid droplet binding domain of hepatitis C virus core protein is a major determinant for efficient virus assembly. *J Biol Chem* 2007;**282**:37158–69.
113. Shebl FM, El-Kamary SS, Saleh DA, et al. Prospective cohort study of mother-to-infant infection and clearance of hepatitis C in rural Egyptian villages. *J Med Virol* 2009;**81**:1024–31.
114. Shi ST, Polyak SJ, Tu H, et al. Hepatitis C virus NS5A colocalizes with the core protein on lipid droplets and interacts with apolipoproteins. *Virology* 2002;**292**:198–210.
115. Simmonds P, Holmes EC, Cha TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; **74**(Pt 11):2391–9.
116. Strickland GT. Liver disease in Egypt: hepatitis C superseded schistosomiasis as a result of iatrogenic and biological factors. *Hepatology* 2006;**43**:915–22.
117. Sumpter R Jr, Loo YM, Foy E, et al. Regulating intracellular antiviral defense and permissiveness to hepatitis C virus RNA replication through a cellular RNA helicase, RIG-I. *J Virol* 2005; **79**:2689–99.
118. Tallis GF, Ryan GM, Lambert SB, et al. Evidence of patient-to-patient transmission of hepatitis C virus through contaminated intravenous anaesthetic ampoules. *J Viral Hepat* 2003;**10**: 234–9.
119. Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009;**461**:798–801.
120. Thomas DL, Villano SA, Riester KA, et al. Perinatal transmission of hepatitis C virus from human immunodeficiency virus type 1-infected mothers. Women and Infants Transmission Study. *J Infect Dis* 1998;**177**:1480–8.
121. Timpe JM, Stamataki Z, Jennings A, et al. Hepatitis C virus cell-cell transmission in hepatoma cells in the presence of neutralizing antibodies. *Hepatology* 2008;**47**:17–24.
122. Tohme RA, Holmberg SD. Transmission of hepatitis C virus infection through tattooing and piercing: a critical review. *Clin Infect Dis* 2012;**54**:1167–78.
123. Tovo PA, Pembrey LJ, Newell ML. Persistence rate and progression of vertically acquired hepatitis C infection. European Paediatric Hepatitis C Virus Infection. *J Infect Dis* 2000;**181**: 419–24.
124. Umar M, Khaar HT, Khurram M, et al. Anti-HCV antibody positivity of various sections of Pakistani patients. *J Coll Physicians Surg Pak* 2009;**19**:737–41.
125. Urban TJ, Thompson AJ, Bradrick SS, et al. IL28B genotype is associated with differential expression of intrahepatic interferon-stimulated genes in patients with chronic hepatitis C. *Hepatology* 2010;**52**:1888–96.
126. Wakita T, Pietschmann T, Kato T, et al. Production of infectious hepatitis C virus in tissue culture from a cloned viral genome. *Nat Med* 2005;**11**:791–6.
127. Wang CC, Krantz E, Klarquist J, et al. Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. *J Infect Dis* 2007;**196**:1474–82.
128. Williams I. Epidemiology of hepatitis C in the United States. *Am J Med* 1999;**107**:2S–9S.
129. Wirth S, Ribes-Koninckx C, Calzado MA, et al. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. *J Hepatol* 2010;**52**: 501–7.
130. Yap PL, McOmish F, Webster AD, et al. Hepatitis C virus transmission by intravenous immunoglobulin. *J Hepatol* 1994;**21**: 455–60.
131. Zeuzem S, Andreone P, Pol S, et al. Telaprevir for retreatment of HCV infection. *N Engl J Med* 2011;**364**:2417–28.
132. Zeuzem S, Asselah T, Angus P, et al. Efficacy of the protease inhibitor BI 201335, polymerase inhibitor BI 207127, and ribavirin in patients with chronic HCV infection. *Gastroenterology* 2011;**141**:2047–55; quiz e14.
133. Zhang J, Randall G, Higginbottom A, et al. CD81 is required for hepatitis C virus glycoprotein-mediated viral infection. *J Virol* 2004;**78**:1448–55.

indicate that illness attack rates and hospitalization rates associated with influenza are high in young children. Sporadic infections with novel influenza A viruses of nonhuman origin, usually by avian or swine influenza A viruses, can occur, with highest frequency in children. Uncommonly, the emergence of an influenza pandemic can have profound impact on morbidity and mortality among a wide range of age groups, especially children, throughout the world. Because influenza viruses are dynamic and continually evolving, epidemics are of variable and unpredictable severity, and influenza research is also rapidly evolving, clinicians are referred to sources such as the Centers for Disease Control and Prevention (CDC)* and the World Health Organization (WHO)† for the latest information on influenza surveillance, novel influenza A viruses, and guidance on treatment, prevention, and control of influenza.

HISTORY

Although the authenticity of influenza in medical antiquity is difficult to establish, the disease has existed for more than 2000 years. The epidemic in 412 BCE described by Hippocrates and Livy probably was influenza.[677] Epidemic influenza-like disease occurred in Europe in the 6th and 10th centuries, but the first generally accepted influenza epidemic occurred in December 1173.[292] Hirsch and Creighton[292] noted 299 epidemics of influenza between 1173 and 1875. The first pandemic involving Europe, Asia, and North Africa occurred in 1580, and the first epidemic recorded in the Western Hemisphere occurred in 1647. From 1580 until 1918, at least eight pandemics of influenza occurred.

In more recent times, pandemics of influenza caused by different influenza A subtypes occurred in 1874, 1889, 1900, 1918, 1957, 1968, and 1977. The most noteworthy of all pandemics of influenza occurred in 1918. This event has the dubious distinction of producing the greatest morbidity and mortality rates of all time—more than 40 million deaths occurred in the world.[34,376]

The term *influenza* may have come from the Latin word *influo*, "to flow in," perhaps indicating its airborne transmission. It may be Italian, relating to an "influence," such as the weather or mystical astrologic causes.[376]

VIRUS CHARACTERISTICS

Isolation of influenza viruses from human respiratory tract specimens was first described in 1933.[633] Influenza viruses are negative-sense, single-stranded, segmented RNA viruses encased in a host-derived lipid membrane envelope, belonging to the Orthomyxoviridae family, and are classified antigenically into types, A, B, and C.[537] Morphologically, influenza viruses can have spherical or filamentous shapes (Fig. 177-1). The genome of

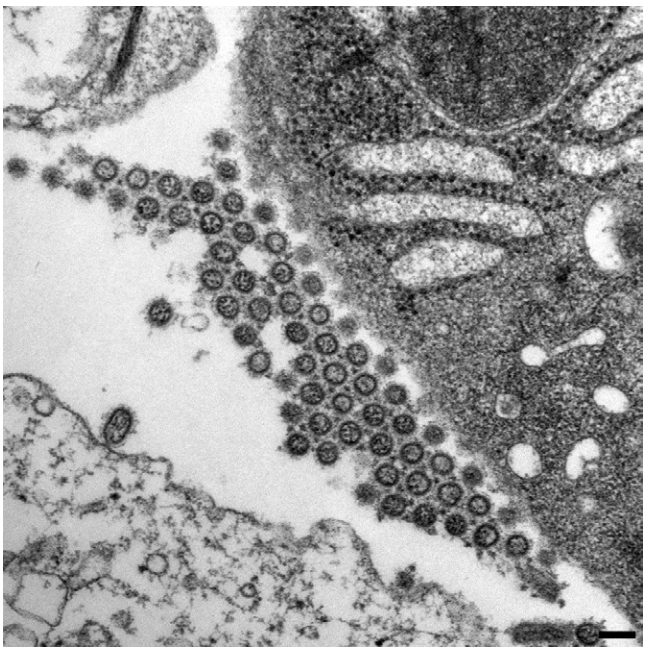

FIGURE 177-1 ■ Influenza A(H1N1)pdm09 viruses budding from infected cells in tissue cell culture. (Electron micrograph provided courtesy Cynthia Goldsmith, CDC. Micron bar indicates 100 nm.)

influenza A and B viruses comprises eight RNA segments, versus seven segments for influenza C virus. The eight gene segments of influenza A viruses range from approximately 850 to 2350 nucleotides, with a total length of approximately 13,500 nucleotides.[297] Influenza A and B viral RNA segments code for up to 12 proteins identified to date, including PB1 (basic polymerase 1; comprises RNA polymerase and associated with endonuclease activity, elongation), PB1-F2 (associated with virulence and proapoptotic activity), PB2 (basic polymerase 2; comprises RNA polymerase, cap recognition), PA (acidic polymerase; comprises RNA polymerase, protease), HA (hemagglutinin), NA (neuraminidase), NP (nucleoprotein; RNA binding, synthesis, nuclear import), M1 (matrix; viral particle assembly), M2 (matrix 2; only found in influenza A viruses; uncoating during virus entry, ion channel function), NS1 (nonstructural; innate immunity, antagonist), and NEP (nuclear export protein, only found in influenza B viruses).[536] A recently discovered influenza A virus protein, PA-X, has multiple functions, including repressing cellular gene expression and modulating the host response (innate immunity) to virus infection.[327]

Influenza viruses have four notable antigens, most importantly the two major surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), which are spike-like projections from the surface of influenza A and B viruses (Fig. 177-2), whereas influenza C viruses lack neuraminidase.[220] HA is the most important protein because it is the site of attachment to the host cell membrane and therefore is essential in receptor binding affinity, is involved in cell fusion, and is the major influenza virus antigen.[436] Virus strain–specific HA antibodies are induced by infection or vaccination. The NA has an enzymatic catalytic function that results in cleavage of sialic

*Centers for Disease and Control and Prevention: http://www.cdc.gov/flu/professionals/index.htm.

†World Health Organization: http://www.who.int/influenza/en/index.html.

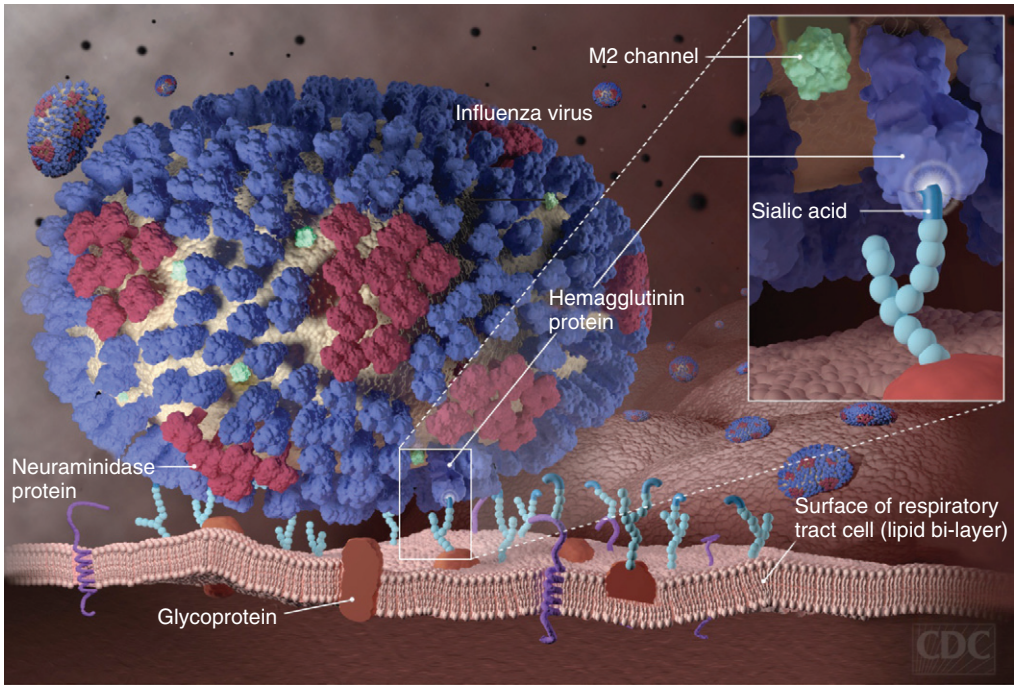

FIGURE 177-2 ■ The two major influenza virus surface proteins, hemagglutinin (HA) and neuraminidase (NA), are shown projecting from the virus surface membrane. HA and NA are also the two main viral antigens. The site of viral HA binding to sialic acid receptors on the surface of the host's epithelial cells is also shown. (See companion Expert Consult website for color version.)

acid receptors from the infected cell, allowing release of infected viral particles (virions). Antibodies to the NA are thought to reduce disease severity, and NA inhibition is the goal of one class of antiviral drugs. Induction of antibodies directed against the matrix proteins is the target of one approach to universal influenza A vaccine development.[200,344] Antibodies targeted at the NP are used in some influenza virus detection assays such as rapid influenza diagnostic tests.

Influenza A and B viruses are most important for public health because of their ability to cause seasonal epidemics. Influenza C virus infections have been described sporadically, usually in children, and because most surveillance systems and clinical laboratories do not conduct influenza C testing, the impact of type C viruses may be underestimated.[84,242,460,461] Influenza A viruses are further classified into subtypes on the basis of antigenic differences in the HA and NA proteins. Designation of influenza A virus subtypes is performed using "H" to denote the HA subtype followed by a specific number for the order first described. Similarly, "N" is used to denote NA subtypes. Sixteen influenza A virus hemagglutinin subtypes have been identified in birds. Based on phylogenetic analyses, the HA subtypes are divided into group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16) and group 2 (H3, H4, H7, H10, H14, H15), which has implications for development of "universal" influenza vaccines that aim to induce broad heterosubtypic cross immune protection against a range of influenza A viruses subtypes.[120,135,176,177,589] Recently a 17th HA subtype was identified in bats.[682] Nine influenza A virus neuraminidase subtypes have been identified in birds.[736] Phylogenetic analyses divide NA subtypes into group 1 (N1, N4, N5, N8) and group 2 (N2, N3, N6, N7, N9), which has implications for antiviral drug resistance.[391] Group 2 NA subtypes are less likely to develop NAI resistance in contrast to group 1 NA subtypes.[391]

Influenza A viruses are commonly referred to by HA and NA subtype. For example, during 2012 and 2013, two subtypes of influenza A viruses circulated among humans worldwide, A (H3N2), and A (H1N1)pdm09 viruses. The most detailed level of influenza virus classification is by strain based on phylogenetic characteristics, such as the HA nucleotide sequences. Strains are grouped by phylogenetic sequence analysis into clades and genotypes. Nomenclature for human influenza viruses is ordered by virus type, geographic location where the virus was identified, strain number, and year of isolation, with subtype designation for influenza A viruses (e.g., B/Wisconsin/1/2010 and A/Victoria/361/2011 [H3N2]).

Both influenza A and B viruses can undergo random point mutations to the hemagglutinin and neuraminidase genes, with resultant changes to the HA and NA proteins, respectively. These changes are generally gradual, but unpredictable, and may result in minor antigenic changes. Antigenic classification is by relationship to vaccine strains or other reference strains on the basis of ability to cause hemagglutination of red blood cells in a hemagglutination inhibition (HAI) assay using postinfection antisera from infected animals, usually ferrets. For example, a virus strain might be characterized antigenically as similar to a reference virus or vaccine virus strain (e.g., A/Victoria/361/2011 [H3N2]-like) if the cross-reactive HAI titer was within a twofold range. A virus strain with a fourfold or greater difference in cross-reactive HAI

titer using ferret antisera to a reference virus strain is significant and considered antigenically distinct. "Antigenic drift" refers to significant antigenic changes in the HA protein, and to a lesser extent in the NA protein, which are thought to be a major factor in driving seasonal influenza epidemics. Influenza A viruses appear to evolve more rapidly than influenza B viruses, and influenza A (H3N2) viruses have been observed to evolve more rapid antigenic drift than A (H1N1) viruses.[274,588] Reinfection in young children has been observed more frequently with influenza B viruses, even without antigenic drift, than for influenza A viruses.[259] Infection with influenza A and B viruses can occur in the same season, and infection with different influenza A virus subtypes also can occur in the same season.[215] Multiple groups or clades have been identified in the two circulating influenza A virus subtypes (H3N2 and H1N1), whereas only two major antigenically distinct groups or lineages of influenza B viruses have co-circulated (i.e., B/Victoria/2/87-like and B/Yamagata/16/88-like) since 1983.[584]

Influenza A viruses have the propensity to undergo genetic reassortment that can generate new viruses.[274,537] This is believed to occur when an infected animal host (e.g., pig) is coinfected with different influenza A subtype viruses and exchange of RNA virus segments occurs.[74,438] *Antigenic shift* refers to human infection with an influenza A virus of nonhuman origin, which is also referred to as infection with a novel influenza A virus. By definition, this refers to either human infection with a different virus subtype containing a new HA (e.g., H5N1, H7N7, H7N9, H9N2) or infection with a virus with significantly different genetic and antigenic properties (e.g., H3N2 variant virus of swine origin) than viruses of the same HA subtype (e.g., seasonal H3N2 virus) circulating among humans.[631,632] Antigenic shift can result in "dead-end" human infection with no further spread or limited nonsustained human-to-human transmission, or an influenza pandemic can ensue if the virus causes disease, acquires the ability to transmit in a sustained manner, and a high proportion of the global population lacks immunity.[252]

Seasonality

In temperate climate countries, influenza epidemics are associated with relatively cooler temperature periods, such as during fall, winter, and spring. Low influenza activity in temperate countries is generally observed during the summer months, although outbreaks can occur. Various studies have suggested that low relative or absolute humidity and low temperatures are associated with enhanced transmission of influenza viruses, including in animal models.[431,604-606,666] However, exact determinants of influenza seasonality remain unknown. Surveillance data indicate that in subtropical and tropical climates, influenza viruses may be detected year-round, with increases during cooler or rainy periods.[27,380,505,619,719] However, in some areas, increases in influenza activity can occur after the rainy season or in the dry season. Bimodal peaks in influenza activity and associated morbidity and mortality can occur in subtropical countries such as Hong Kong and Singapore, as well as regions of large geographic countries such as southern China.[197,404,749,779] In countries with large areas or with diverse climates, the epidemiology and seasonality of influenza activity may vary widely. One study reported that seasonality of influenza in Brazil was linked to a southbound "wave" of activity from equatorial low-population areas to temperate, more densely populated regions, and suggested that temperature and humidity may be important factors.[10]

Ecology, Hosts, and Infected Animals

The natural reservoir for 16 HA and 9 NA subtypes of influenza A viruses is wild aquatic birds such as geese, ducks, and shorebirds.[736] Genetic reassortment among avian influenza A viruses from different bird species occurs frequently.[251,400,765,772,773] Wild waterfowl can be infected asymptomatically with avian influenza A viruses. Avian influenza A viruses generally infect receptors bearing sialic acids with ∝2,3 galactose linkages ("avian-like receptors"), which are found in the respiratory and gastrointestinal tracts of birds. Avian influenza A viruses are classified as *highly pathogenic* or *low pathogenic* on the basis of molecular and pathogenicity criteria—possession or absence of a highly basic amino acid sequence at the hemagglutinin cleavage site, and mortality in 4-week-old infected chickens.[765] Most avian influenza A viruses are classified as low pathogenic viruses, and only H5 and H7 subtypes have been identified as highly pathogenic avian influenza A viruses; however, most H5 and H7 viruses are low pathogenic. Low-pathogenic avian influenza (LPAI) A viruses generally cause asymptomatic infection in most wild birds, and can cause either asymptomatic infection or mild illness in infected domestic poultry.[206,519,576] Highly pathogenic avian influenza (HPAI) A viruses can cause a spectrum of illness in infected wild birds (asymptomatic infection to rapid death) and domestic poultry (ducks may experience asymptomatic infection more commonly than chickens; rapid spread with high mortality can occur in chickens). Influenza A viruses have been documented to infect a wide range of mammals, including horses, camels, dogs, cats, leopards, cheetahs, tigers, civet cats, and guinea pigs.* Avian influenza A viruses can be transmitted from birds to other animals such as pigs, humans, and marine mammals (e.g., seals and whales). Avian influenza A viruses continue to evolve among birds worldwide.

Swine influenza A virus infections are endemic among pigs worldwide.[74,386,600,721,799] Swine influenza A viruses can bind to receptors bearing sialic acids with ∝2,3 galactose linkages ("avian-like receptors") and ∝2,6 galactose linkages ("human-like receptors"), which are found in the respiratory (primarily ∝2,6 galactose) and gastrointestinal (∝2,3 galactose) tracts of pigs. Because pigs can also be infected with avian and human influenza A viruses, including coinfection with swine influenza A viruses, there is potential for genetic reassortment. Reassortment with other influenza A viruses can occur, including with avian or human viruses, to create new viruses (e.g., triple-reassortant swine influenza A viruses with genes from human, avian, and swine influenza A viruses).[520] These

*References 141, 225, 409, 437, 478, 577, 579, 671, 735, 777.

new viruses can continue to circulate among pigs and may represent a public health threat of zoonotic infection and pose pandemic potential. Thus, pigs have been viewed as "mixing vessels" in which new influenza A viruses can arise, including viruses of pandemic potential.[438,738] A recent example was the emergence of a novel influenza A (H1N1) virus from a swine reservoir to cause the 2009 H1N1 pandemic.[222] Another example is the emergence of a swine influenza A (H3N2) virus with avian, human, and swine influenza A virus gene segments and containing the matrix gene from the 2009 H1N1 virus, in the United States during 2011.[493] This quadruple reassortant virus, referred to as H3N2 variant (H3N2v) virus, acquired the matrix gene through human-to-pig transmission of the 2009 H1N1 virus. Evidence of human-to-swine transmission of the 2009 H1N1 virus to create different reassortant swine influenza A viruses has been documented in pigs in other regions of the world.[191,685,720] Outbreaks of swine influenza A viruses occur seasonally in some countries, but because of poor surveillance, limited data exist on the prevalence and evolution of swine influenza A viruses in pigs worldwide.[632]

Canine influenza A (H3N8) viruses are circulating among dogs and are related to equine influenza A (H3N8) viruses.[18,140] Although equine influenza A (H7N7) viruses have circulated among horses in the past, currently, equine influenza A (H3N8) viruses are circulating among horses.[145,408] Outbreaks of highly pathogenic influenza A (H3N8) viruses among seals have been reported; these viruses are closely related to avian influenza A (H3N8) viruses.[20] Other marine mammals such as whales have been infected with influenza A viruses, but it is unclear whether such viruses continue to circulate among these animals.[291,437,508]

Transmission of human influenza A viruses to other animals without establishing an animal reservoir for these viruses has been documented. Transmission of seasonal or pandemic influenza A viruses to household pets has been reported (e.g., dogs, cats, and cheetah) and to other animals that are raised for consumption (e.g., guinea pigs).[141,146,409,422,465] The host for influenza B viruses is humans, but other animals also can be infected with influenza B viruses (e.g., seals).[527]

Surveillance Among Animals

Surveillance of influenza viruses among animals remains a major gap worldwide. Since 2004, stimulated by concerns of a pandemic arising from an avian reservoir, increased surveillance has yielded data on highly pathogenic avian influenza A (H5N1) viruses and low-pathogenic avian influenza A viruses circulating among domestic poultry and wild birds. A major gap is the lack of monitoring and data for swine influenza A viruses worldwide. The World Organization for Animal Health (OIE) receives reports of outbreaks of animal diseases by member states, including highly pathogenic avian influenza A (H5) and (H7) viruses. Outbreaks of swine influenza A viruses among pigs are not a reportable disease. OIE and the United Nations Food and Agriculture Organization (FAO) global network of expertise on animal influenza (OFFLU) is focused on coordinating, exchanging scientific data and biologic materials (including virus strains), assisting in prevention and control of animal influenza, and collaborating with the WHO Global Influenza Surveillance and Response System on issues at the animal–human interface (http://www.offlu.net/index.php?id=1). OFFLU-participating laboratories include reference laboratories for diagnosis of avian, equine, and swine influenza. Influenza A viruses circulating among animals is the focus of one WHO Collaborating Center for Studies on the Ecology of Influenza in Animals.[754]

Pandemic Influenza Viruses

Influenza pandemics have probably been occurring for millennia.[415] Only influenza A viruses have the ability to cause rare human influenza pandemics. Three conditions are required for an influenza pandemic to occur: the novel influenza A virus must cause disease in humans, have the ability to transmit from person to person in a sustained manner, and a large proportion of the world's population must lack sufficient protective immunity. Pandemic influenza A viruses are thought to emerge through two primary mechanisms: adaptation of an animal influenza A virus that has acquired the ability to infect and transmit among humans (e.g., 1918 H1N1 virus and 2009 H1N1 virus) or through genetic reassortment between a human influenza A virus and an animal influenza A virus (e.g., 1957 H2N2 virus and 1968 H3N2 virus).[495] A theory of recycling of pandemic viruses has been proposed to include H1, H2, and H3 viruses,[801] but the determinants for the emergence of a pandemic virus are unknown.

During the 20th century, three pandemic influenza viruses emerged. The origin of the 1918 H1N1 virus has been debated. Some have argued that the 1918 H1N1 virus was of avian origin, but other researchers believe that a virus of swine origin adapted or acquired the ability for sustained human-to-human transmission.[19,252,566,631,710] The 1957 H2N2 virus was a reassortant virus possessing three RNA segments (HA, NA, PB1) from a low-pathogenic avian influenza A virus and five from H1N1 virus circulating among humans.[252,301,302] Similarly, the 1968 H3N2 virus was a reassortant event between two genes (HA, PB1) from a low-pathogenic avian influenza A virus and six RNA segments from the circulating H2N2 virus.[252,301,302] The 2009 H1N1 ([H1N1]pdm09) virus can be considered a quadruple reassortant virus based on origin from a triple reassortant swine influenza A (H1N1) virus with genes of avian, swine, and human origin (six genes) and a Eurasian swine influenza A (H1N1) virus (NA, M).[222] Before 2009, it was believed that a requirement for a pandemic virus was that it had to possess a different HA subtype than circulating human influenza A viruses. However, the 2009 H1N1 pandemic virus emerged during global circulation of seasonal influenza A (H1N1) virus, but was sufficiently antigenically distinct so that preexisting cross-protective immunity was observed only in elderly persons exposed to the 1918 H1N1 virus or thereafter until significant antigenic drift occurred (e.g., glycosylation of the HA "head" domain) in approximately the late 1920s or early 1930s or in

persons who had received the 1976 swine influenza A (H1N1) vaccine.[265,739,774]

Once a pandemic virus emerges, distinct waves of disease are often observed. The 1918 H1N1 pandemic virus was associated with three "waves" of disease during 1918 through 1920.[477] At some point, a pandemic virus will acquire antigenic changes through antigenic drift and circulate as a seasonal influenza virus. Replacement of a circulating influenza A virus by a pandemic virus has been observed. For example, the 1957 H2N2 virus replaced the H1N1 virus, and the 1968 H3N2 virus replaced the H2N2 virus. An H1N1 virus identical to pre-1957 virus reappeared in circulation in 1977, and is suspected to have had a laboratory source.[363] Some researchers have referred to the reemergence of H1N1 virus in 1977 as the fourth pandemic of the 20th century, whereas others have viewed it as a "pseudo-pandemic." With the emergence of the 2009 H1N1 virus, seasonal influenza A (H1N1) virus has not been detected since 2010. During the first wave of 2009 H1N1 virus circulation, seasonal influenza A and B viruses were rarely detected in many temperate climate countries, although this varied worldwide. Beginning in 2010, the 2009 H1N1 virus co-circulated with seasonal influenza viruses, and in the United States the 2010 to 2011 influenza season had similar proportions of 2009 H1N1, influenza A (H3N2), and influenza B viruses detected.

Virologic Surveillance

In the United States, influenza surveillance comprises multiple components, including virologic and disease-based monitoring.[92] Virologic surveillance is conducted year-round, with respiratory specimens collected from persons with influenza-like illness (temperature ≥38.0° C [≥100.4° F] with either cough or sore throat) tested at public health and private laboratories. Approximately 80 WHO collaborating laboratories, including all state and some local public health and other government laboratories and 60 laboratories participating in the National Respiratory and Enteric Surveillance System (primarily hospital clinical laboratories), submit weekly data on respiratory specimens tested and positive results of influenza testing to the CDC. State public health and other laboratories perform influenza testing using viral culture and molecular assays. All state public health laboratories use the CDC real-time reverse transcription polymerase chain reaction assay (CDC Flu rRT-PCR Dx Panel) that uses primers and probes to detect influenza A, B, H1, H3, H5, influenza A pdm09, and influenza A(H1)pdm09 viral RNA. A subset of influenza viruses that are isolated at WHO collaborating laboratories are sent to the CDC for more detailed characterization, including antigenic characterization, genetic sequencing, phylogenetic analyses, and antiviral susceptibility testing. As part of pandemic preparedness efforts, state public health laboratories submit clinical specimens and extracted RNA from suspected cases of novel influenza A virus infection (human infection with a virus of nonhuman origin, e.g., of avian or swine origin) to CDC for confirmatory testing. This includes specimens testing positive for influenza A viruses that cannot be subtyped by available primers and probes, produce indeterminate results, or are presumptive positive for variant (swine origin) influenza A viruses using the CDC FLU rRT-PCR Dx Panel assay. The CDC issues a weekly influenza surveillance report that is accessible at http://www.cdc.gov/flu/weekly/.

Globally, influenza virologic surveillance is coordinated year-round through the WHO Global Influenza Surveillance and Response System (GISRS). The WHO's influenza surveillance network was established in 1952, and comprises approximately 138 national influenza centers in at least 108 member states, and 6 WHO Collaborating Centers (located in Melbourne, Tokyo, Beijing, London, Atlanta, and Memphis) and 4 WHO Essential Regulatory Laboratories.[752,754] Within a country, influenza surveillance is conducted by one or more national influenza centers, which perform influenza testing, virus typing, and subtyping of influenza A viruses by molecular assays and viral culture.[753] Specimens collected from patients with influenza-like illness at health care facilities participating in sentinel surveillance are submitted to national influenza centers. These national influenza centers send influenza viruses to five WHO Collaborating Center Laboratories for more detailed analyses (i.e., antigenic characterization, genetic sequencing, phylogenetic analyses, and antiviral susceptibility testing). The Influenza Division of the CDC serves as the National Influenza Center for the United States and is a WHO Collaborating Center for the Surveillance, Epidemiology and Control of Influenza. In addition, a network of WHO H5 Reference Laboratories, including WHO Collaborating Centers, was established for laboratory confirmation and detailed analyses of highly pathogenic avian influenza A (H5N1) viruses isolated from human cases.[802] The WHO Collaborating Centers share antigenic and genetic data on influenza viruses identified through surveillance for vaccine strain selection, including seasonal influenza vaccines and prepandemic vaccine candidate development.[17,274]

Currently, influenza A (H3N2), A(H1N1)pdm09 (2009 H1N1 virus), and B virus strains are co-circulating worldwide among humans. Two antigenically distinct lineages of influenza B viruses comprising B/Yamagata and B/Victoria strains continue to co-circulate. Historically, a major gap in influenza surveillance has existed in tropical and subtropical low-resource countries.[226,622] Recently, influenza surveillance was established or expanded using RT-PCR in Asian and African countries, which has contributed to better understanding of the epidemiology of influenza viruses in developing countries[380,505,793] and allowed for detection and monitoring of the spread of 2009 H1N1 virus.[340,360]

Virus Evolution and Spread

Genetic and antigenic evolution of influenza viruses are monitored by various analytical methods. Phylogenetic analyses assess the molecular relationship of strains and classify strain groups into lineages, clades, and subclades. Antigenic analyses assess the antigenic similarity of viruses to reference strains and vaccine strains using antisera, generally from inoculated ferrets, and HAI antibody titers. Antigenic differences resulting in a fourfold or

greater HAI titer from a reference strain are considered significantly different. Recent use of modeling techniques and antigenic cartography techniques to analyze antigenic differences in circulating influenza viruses based on influenza virus surveillance data has provided insight into the evolution and spread of influenza viruses worldwide.[630] One theory, based on data for H3N2 virus strains, suggests that greater evolution occurs in subtropical regions of Southeast Asia ("source"), which then "seed" other regions of the world where less evolution is occurring ("sink"), such as in South America.[116,569,587] Another study suggested that evolution of influenza A (H3N2) viruses during 2003 to 2006 was more complex and that tropical regions of Southeast Asia showed limited genetic diversity and did not maintain a source for seeding seasonal epidemics in temperate climates.[29] This theory proposes that many different localities can be the source of seasonal epidemics in temperate climate countries, with high rates of viral migration occurring between urban centers, based on global travel patterns.[29]

Disease Surveillance

Influenza disease surveillance in the United States comprises multiple components that report data regularly to the CDC.[68,92] Weekly monitoring of the percentage of nonspecific influenza-like illness visits to sentinel primary care providers allows establishment of a seasonal baseline and determination of peaks in influenza-like illness activity, which usually is correlated with peak influenza virus activity. This allows determination of regional and national peaks in influenza-like illness activity. Weekly monitoring of reported deaths from pneumonia and influenza in 122 jurisdictions, comprising approximately one third of the U.S. population, allows comparison to a projected "epidemic threshold" using modeling techniques and to provide a relative assessment of epidemic severity from year to year. During October through May, state and territorial epidemiologists report a weekly qualitative assessment of influenza activity. Hospitalizations for laboratory-confirmed influenza among all ages are monitored through population-based surveillance in the Emerging Infections Program; this allows for determination of rates of laboratory-confirmed influenza hospitalizations by age group.[147,149] Since 2004, pediatric deaths with laboratory testing evidence of influenza virus infection are nationally notifiable to CDC. All of the disease surveillance components are analyzed together with influenza virologic surveillance data to inform an assessment of the impact of influenza in the United States.

Recently, global efforts have been directed to assess the burden of severe influenza disease by conducting syndromic surveillance for severe acute respiratory infection (SARI) requiring hospitalization; when combined with testing of respiratory specimens for influenza viruses, the proportion of SARI cases with influenza can be determined. Use of standard case definitions for SARI allows relative comparisons across countries and regions by season, climate, region, country, age group, and influenza virus.[538,759] When SARI surveillance or influenza virus surveillance is conducted on a population basis (with a defined population denominator), calculation of incidence rates can be determined in outpatients and hospitalized patients to facilitate assessment of the relative severity of influenza seasons.[149,323,558] Health utilization survey data can augment influenza-like illness and SARI surveillance to yield influenza disease burden estimates.[26]

Epidemiology: Seasonal Influenza

In most of the United States, seasonal influenza epidemics occur typically during October to May and peak during January to March, but regional differences may exist in activity, including differences in the predominant circulating influenza viruses.[91] Community influenza activity generally lasts 6 to 8 weeks, but activity may persist longer, especially if antigenically drifted strains emerge. Influenza activity may start earlier and last longer in Alaska and may be detected year-round in Hawaii's tropical climate.[5,273] During the summer months, sporadic influenza outbreaks may occur—for example, in institutions such as long-term care facilities, in summer camps, or among organized tour groups and cruise ship passengers.[94,377,705]

Most people with influenza may not seek medical care, and very few persons with influenza virus infection are tested; thus, the number of symptomatic cases and incidence of influenza virus infection is unknown. It is estimated that an average of 5 to 20 percent of the U.S. population develops influenza illness each year. Community studies of seasonal influenza epidemics have established that the highest infection rates and illness attack rates for laboratory-confirmed influenza or influenza-like illness are typically in young children. Many of these studies were conducted over multiple seasons in the 1970s and 1980s and used different laboratory methods, including serology, to assess influenza virus infection. These community studies have documented high infection and illness attack rates during seasonal influenza epidemics among preschool and school-age children.* Outbreaks of influenza among schoolchildren usually are noted in the early weeks of community influenza activity.[233] Preschool and young school-aged children may introduce influenza to households and are therefore an important group to target for preventing and controlling community influenza activity during seasonal epidemics and pandemics.[28,233,258] During early periods of community influenza activity, absenteeism in schoolchildren peaks, whereas during school holidays, reductions in influenza activity in the community are observed, further suggesting the role of schoolchildren in driving influenza epidemics.[208,235,236,659]

To estimate the national disease burden of influenza, indirect methods such as modeling studies are conducted that use influenza surveillance data and hospitalization discharge or mortality data during periods when influenza virus activity is high (influenza period) in contrast to low (peri-influenza or baseline) periods.† These modeling studies generate excess influenza-attributable hospitalizations or deaths. Some models, including the

*References 28, 208, 210, 233, 423, 473, 659.
†References 97, 324, 672, 674-676, 796.

CDC's projections, incorporate surveillance data for influenza viruses and respiratory syncytial virus. It is estimated that an average of more than 200,000 excess influenza-attributable hospitalizations occur each year in the United States, with the highest rates in persons 65 years of age and older and in young children.[675] Because of the variability of the severity of influenza epidemics, a wide range of mortality estimates has been generated.[97] Estimated hospitalizations and mortality attributable to influenza are highest during seasons predominated by influenza A (H3N2) viruses,[512,674] and seasons predominated by influenza B viruses are generally less severe, although illness attack rates are high among children.[474,512] Approximately 3400 to 49,000 excess influenza-attributable deaths are estimated to occur annually in the United States, with nearly 90 percent of deaths occurring in persons 65 years of age and older.[97] During severe seasons, excess influenza-associated mortality is estimated to exceed the numbers of estimated deaths during the 1968 H3N2 pandemic, likely because of the continued aging of the U.S. population. Although mortality during influenza pandemics attracts heightened attention, some seasonal influenza epidemics result in higher numbers of deaths, and because pandemic influenza A viruses undergo antigenic drift to circulate as seasonal influenza A viruses for many years, the cumulative mortality of seasonal influenza epidemics can exceed all but the most severe influenza pandemics.

High-Risk Pediatric Groups

Population-based studies indicate high rates of outpatient and emergency department visits for influenza in children in the United States.[67,498,500,516,558] Retrospective studies using seasonal influenza surveillance data estimate that the highest hospitalization rates among children in the United States are in those younger than 5 years of age; rates are higher in infants younger than 2 years in contrast to those 24 to 59 months and highest in those younger than 12 months, with high hospitalization rates in those with chronic medical conditions.* Direct methods using laboratory-confirmed influenza data to determine seasonal influenza incidence and population-based studies of laboratory-confirmed influenza hospitalizations in the United States further indicate that the highest hospitalization rates among children are in those younger than 2 years of age (highest rates in infants younger than 6 months), followed by those 24 to 59 months of age,† and that a high proportion of hospitalized influenza patients have chronic medical conditions. Using laboratory-confirmed influenza hospitalizations may underestimate the burden of influenza because of testing biases and limitations of rapid influenza diagnostic tests.[247]

Overall, these disease burden studies and surveillance data indicate that children at highest risk for influenza complications are those younger than 5 years of age, particularly those younger than 2 years, with the risk increasing by decreasing age.[567] Additionally, children with certain underlying medical conditions are considered at highest risk for influenza complications because of exacerbation of chronic disease or severe respiratory illness, including those with chronic pulmonary disease (e.g., asthma or cystic fibrosis); cardiac disease (e.g., congenital cardiac disease, congestive heart failure, or coronary artery disease); metabolic disorders (e.g., diabetes mellitus, inherited metabolic disorders, or mitochondrial disorders); hematologic disease (e.g., sickle cell anemia); renal disease; hepatic disease; immunosuppression; neurologic and neurodevelopmental conditions (including disorders of the brain, spinal cord, peripheral nerve, and muscle such as cerebral palsy, epilepsy [seizure disorders], stroke, intellectual disability (mental retardation), moderate to severe developmental delay, muscular dystrophy, or spinal cord injury); children receiving chronic aspirin therapy; morbid obesity; and persons who are American Indian or Alaska Natives.[111,499]

Economic Impact

The economic impact of influenza in children includes the direct medical care costs of illness (for medications, tests, a clinic or emergency department visit, health care provider consultation fees, or hospitalization costs), out-of-pocket costs (for over the counter medications or travel costs to medical care), as well as possible associated indirect costs, such as work time loss for a parent or guardian to care for a sick child.* Measuring the economic impact depends on whether the societal or individual perspective is assessed and may vary by whether assessments are conducted in developed countries versus middle-income or low-resource settings, different climates, health care–seeking behavior, and the structure of the health care system (e.g., national health insurance vs. private insurance). Overall, the direct medical costs, out-of-pocket costs, and indirect costs of seasonal influenza in young children are substantial, even for outpatient and emergency department care.[524] One study estimated that the total burden of seasonal influenza epidemics in the United States was $87 billion.[472]

Global Disease Burden

Outside of developed countries, the burden of influenza is less well understood, but recent studies indicate that influenza is a major public health problem in temperate, subtropical, and tropical regions.[226,622] Influenza virus infections occur worldwide in children, resulting in medically attended outpatient visits, although the burden may be underestimated by surveillance data because many ill persons may not seek medical care.[226,416,622] Therefore, household population–based surveillance may be more informative about influenza disease burden, especially in developing countries.[77,572] Use of serosurveys in community-based studies can further enhance determination of the burden of influenza virus infection in developing countries.[299] Such studies consistently show a high burden of influenza in school-aged children. Data from

*References 324, 485, 498, 516, 674, 796.
†References 15, 149, 150, 427, 485, 500, 558, 599.

*References 124, 184, 189, 357, 472, 524.

other countries consistently indicate that young children are hospitalized with complications of influenza more frequently than older children.[167,350,441,619,787] Influenza viruses can be detected in respiratory specimens from young children with acute lower respiratory tract disease worldwide.* Syndromic surveillance for severe acute respiratory infection (SARI) indicate that influenza virus infection causes SARI in young children in many diverse settings.[26,33,195,196] One study reported a high incidence of influenza virus infection in infants younger than 6 months of age.[285] A review of studies from sub-Saharan Africa reported that among children hospitalized for acute respiratory tract infections, a median of 6.3 person were associated with influenza.[226] One study estimated that during 2008, 90 million new cases of influenza occurred among children younger than 5 years of age, resulting in 20 million cases of acute lower respiratory tract infection (ALRI) and 1 million cases of severe ALRI.[492]

Influenza-Associated Mortality

Death is an uncommon outcome of influenza virus infection, but data on pediatric mortality associated with seasonal influenza are limited. One modeling study estimated that 38 to 92 pneumonia or respiratory and circulatory deaths attributable to influenza in children younger than 5 years of age occurred each year in the United States.[674] During 2003 and 2004, a severe influenza A (H3N2) virus season, 153 deaths in children with laboratory evidence of influenza virus infection were reported to CDC through enhanced passive surveillance.[56] Pediatric influenza-associated deaths were also noted during the 2003 and 2004 season in the United Kingdom.[336] Pediatric influenza-associated deaths became a nationally notifiable condition in the United States during 2004, and from 2004 to 2008, a range of 46 to 88 deaths were reported to CDC.[201,546] These data indicate that influenza virus infection causes the highest number of pediatric deaths of any vaccine-preventable disease in the United States. A high proportion of deaths occurred in children with underlying chronic comorbidities, most deaths occurred in children who were not vaccinated, and a substantial proportion died at home or in an emergency department with a short median time from illness onset to death. This suggests that some deaths have occurred unexpectedly, after a fulminant progression that followed an initially mild illness, as a result of influenza virus infection alone or with secondary invasive bacterial coinfection.[56,201] Although the median age has been increasing in recent years, one study reported that the highest mortality rate was in children younger than 6 months of age.[56] Since the 2009 H1N1 pandemic, the number of pediatric seasonal influenza-associated deaths has ranged from 34 to 122 per year.[93] Deaths have been associated with complications of influenza A or influenza B virus infections and are reflective of the circulating influenza virus strains. It is likely that reported deaths are an underestimate of all pediatric influenza-associated deaths in the United States, because some deaths related to influenza virus infection are not suspected or tested for influenza or may not yield evidence of influenza virus infection when tested. One study estimated that during 2008, a range of 28,000 to 111,500 influenza-attributable ALRI deaths occurred worldwide among children younger than 5 years of age.[492]

*References 33, 55, 77, 123, 129, 195, 196, 227.

Zoonotic Influenza

Sporadic human infections with influenza A viruses of nonhuman origin have been identified and usually are associated with recent direct or close exposure to animals such as poultry or pigs that are infected with influenza A viruses. A human case of conjunctivitis with H7N7 virus from a seal was reported in a marine mammal worker.[737] Human infections with influenza A viruses from animals are referred to as cases of novel influenza A virus infection. Novel influenza A virus infection represents antigenic shift and is an event of public health importance because of the pandemic potential, but does not necessarily result in an influenza pandemic unless sustained human-to-human transmission occurs. The frequency and incidence of novel influenza A virus infections is unknown in the United States and worldwide because of surveillance and testing limitations. Although novel influenza A virus infections in the United States have been a nationally notifiable condition since 2007, the reported number of cases is likely a large underestimate of all cases that have occurred, because most such novel influenza A virus infections do not result in severe disease, few cases may be suspected or tested, and testing may not detect or specifically identify a suspect novel influenza A virus infection unless detailed molecular testing using specific primers and probes, viral culture, or genetic sequencing is performed. For example, antigen detection tests (e.g., rapid influenza diagnostic tests and immunofluorescence) do not distinguish between human infection with seasonal influenza A viruses and animal influenza A viruses.

Avian Influenza A Virus Infections

Human infections with different avian influenza A virus subtypes resulting in respiratory illness have been identified to date, including low-pathogenic avian influenza (LPAI)A (H7N2, H7N3, H7N7, H7N9, H9N2, and H10N7)[23,387,411,529,548,628] and highly pathogenic avian influenza (HPAI) A (H5N1, H7N3, and H7N7) viruses.[1,109,378,694] Whereas many avian influenza A virus infections identified to date have been in adults, most of the LPAI H9N2 virus infections identified to date have been in children.[122,548] LPAI virus infections generally have resulted in uncomplicated influenza illness with fever, myalgia, and upper respiratory tract symptoms (i.e., nonproductive cough, sore throat, rhinorrhea, nasal and congestion). Some children with LPAI H9N2 virus infections have been hospitalized with uncomplicated influenza, with full recovery.[702] Both LPAI and HPAI H7 virus infections can cause conjunctivitis with or without upper respiratory tract symptoms, and H7 viruses have been shown to cause ocular tropism.[42] Sporadic cases of lower respiratory tract disease with LPAI H7N2, H7N3, or H9N2 virus infection have been reported in adults,

including in immunosuppressed persons.[122,628] Although most cases of LPAI virus infection are associated with recent direct or close exposure to poultry, in some cases the exposure was not determined. However, LPAI viruses circulate widely among wild birds and domestic poultry and are likely the source of transmission. No human-to-human transmission of LPAI viruses has been reported, but limited human-to-human transmission should be considered in cases without identified direct or indirect poultry exposure. In the United States only a few cases of LPAI H7N2 virus infection have been reported.[529]

A wide spectrum of illness has been reported with HPAI virus infections, including conjunctivitis alone with HPAI H7N3 or H7N7 virus infection and relatively mild uncomplicated febrile upper respiratory tract symptoms with or without conjunctivitis (HPAI H7N7 virus).[109,378,694] During the spring of 2003, a large outbreak of HPAI H7N7 virus among domestic poultry occurred at farms in the Netherlands.[378] This outbreak was associated with one human fatal adult case with acute respiratory distress syndrome (ARDS) in a veterinarian and other cases of conjunctivitis with or without uncomplicated influenza illness.[207,378] Limited human-to-human transmission of HPAI H7N7 virus was documented in some households.[378] Limited, nonsustained HPAI H5N1 virus infections have caused both clinically mild and critical to fatal illness.[1]

The first human case of poultry-to-human transmission of HPAI H5N1 virus was identified in a child in Hong Kong during 1997.[650] Eighteen human cases of HPAI H5N1 virus infection were identified during May to December 1997, with 6 deaths.[117] The spectrum of disease in 11 pediatric patients included uncomplicated influenza illness, ARDS, and multiorgan failure. Additional H5N1 cases were identified in early 2003.[547] Since November 2003, more than 600 human cases of HPAI H5N1 virus infection with approximately 60 percent case-fatality proportion have been reported from 15 countries to the WHO.[751] These cases are associated with the ongoing epizootic of HPAI H5N1 viruses among poultry. Currently, the United Nations Food and Agricultural Organization (FAO) considers six countries endemic for HPAI H5N1 virus among poultry, and outbreaks have occurred among nearby nonendemic countries.[697] HPAI H5N1 viruses continue to evolve among poultry and some wild birds and have been diversifying into distinct clades (groups), subclades, and genotypes worldwide.[757,758] To date, human infections have been documented with clade 0, 1, 2, and 7 viruses, including subclades 2.1, 2.2, and 2.3 viruses.[1] In 2012, HPAI H5N1 viruses transmitting to humans included clade 1.1, 2.2.1, 2.3.2.1, and 2.3.4.1 viruses. HPAI H5N1 viruses generally bind to receptors bearing sialic acids with ∝2,3 galactose linkages, usually found in the lower respiratory tract (type II pneumocytes).[457,458,613] However, HPAI H5N1 viruses have been shown to bind to tissues (in ex vivo culture) found in the upper respiratory tract, including tissues without known receptors.[507] HPAI H5N1 viruses have also been found to infect the gastrointestinal tract, brain, placental tissue, and fetal lung cells.[1] Both upper and lower respiratory tract infections with HPAI H5N1 viruses have been identified in pediatric patients.

Risk factors for HPAI H5N1 virus infection include recent direct or close contact with sick or dead poultry or visiting a live poultry market, although in some cases, the source of infection was not determined.[1,601,798] Sporadic clusters of human HPAI H5N1 cases have been identified in some countries. Nearly all clusters have occurred among blood-related family members.[300,342] In most clusters, a common poultry exposure was the likely source of infection, but some cases of limited, nonsustained human-to-human transmission following close, prolonged unprotected exposure to a symptomatic HPAI H5N1 patient, including nosocomial transmission, have been reported.[342,696,726] For HPAI H5N1 virus and other avian influenza A viruses to transmit efficiently among humans, it is believed that adaption in receptor-binding affinity to human influenza A virus receptors is needed.[314] Two recent studies suggest that only a small number of viral mutations are needed for HPAI H5N1 viruses currently circulating among poultry to transmit well in a mammalian (ferret) model.[286,315,586]

Although most adult HPAI H5N1 patients have experienced severe or fatal disease, clinically mild illnesses have been identified in young children, including some patients identified through routine surveillance for influenza-like illness.[76,316,522] In addition to febrile upper respiratory tract illness and viral pneumonia, some HPAI H5N1 patients have had vomiting and diarrhea. Early clinical presentation includes febrile respiratory or gastrointestinal illness, and such patients have been diagnosed with more common diseases (e.g., dengue or typhoid), even in endemic countries.[1] Atypical cases in children have presented with encephalitis or gastroenteritis preceding viral pneumonia.[21,154] Eliciting a history of recent exposure to sick or dead poultry and clinical suspicion may facilitate diagnosis in endemic countries. Findings in some HPAI H5N1 patients at hospital admission include leukopenia, lymphopenia, moderate thrombocytopenia, bilateral pneumonia, dyspnea, tachypnea, and hypoxia.[701] Diagnosis is by testing respiratory specimens with RT-PCR using H5-specific primers and probes. Early clinical presentation and early initiation of antiviral treatment with the neuraminidase inhibitor oseltamivir is associated with survival in pediatric HPAI H5N1 virus infection in retrospective cohort studies.[3,522,701] Clinical management includes early NA inhibitor treatment, supportive management of complications (i.e., shock, viral sepsis, respiratory failure, ARDS, and renal failure), and adherence to recommended infection control measures (i.e., standard, droplet, and contact precautions; eye protection; and respiratory protection with N95 or equivalent respirators if available or surgical masks).[3,701,741] Few cases have been identified with secondary invasive bacterial coinfection, although nosocomial infection has been reported.[36,696,726]

Swine Influenza A Virus Infections

Human infection with swine influenza A viruses is referred to as variant virus infection and designated with a "v" following the subtype identification (e.g., H3N2v virus infection). Swine influenza A viruses are endemic among pigs in the United States and worldwide, and

sporadic transmission to humans has been identified, especially in the United States since novel influenza A virus infections were made a nationally notifiable condition in 2007.[148,490,504,611,670] However, because surveillance for swine influenza A viruses among pigs is poor and limited, understanding of the evolution of swine influenza A viruses and the public health risk is unknown. Undoubtedly, variant virus infections are underestimated, because most human infections do not result in severe illness and influenza testing is not performed. Most cases of variant virus infection have occurred after recent direct or close exposure to pigs. Examples of variant virus infections with different influenza A virus subtypes have been identified, including with classic H1N1, triple-reassortant H1N2, triple-reassortant H3N2, and triple-reassortant H3N2 virus with the M gene from influenza A(H1N1) pdm09 virus.[110] The majority of variant virus infections have occurred among children. The clinical spectrum of variant virus infection ranges from uncomplicated influenza (e.g., febrile upper respiratory tract illness) to exacerbation of underlying chronic disease (e.g., asthma), to severe and fatal illness. Most hospitalized patients have been at increased risk for influenza complications because of age or underlying comorbidities.[104] Most variant virus patients have experienced self-limited influenza illness with full recovery. Some patients have experienced vomiting and diarrhea. In 1976, human infections with a novel swine influenza A (H1N1) virus (A/New Jersey/76 [Hsw1N1]) caused severe respiratory illness in 13 military trainees, with 1 death at Fort Dix, New Jersey.[224] Because A/New Jersey/76 virus was similar to the 1918 H1N1 pandemic virus, and human-to-human transmission had been observed at Ft. Dix, a large vaccination program was implemented.[602] However, no influenza pandemic occurred and an increase in cases of Guillain-Barré syndrome (GBS) was found in vaccinees.[454,602]

Before 2011, variant virus infections were sporadically identified in the United States, with 12 cases identified in 2011. However, beginning in July 2012, cases of human infection with an H3N2v virus containing the M gene from influenza A(H1N1)pdm09 virus were increasingly identified in the United States.[102,110] As of September 2012, more than 300 confirmed H3N2v patients had been identified, with 16 hospitalizations and 1 death.[104] Of 11 hospitalized H3N2v patients, 10 were in children, with a median age of 6 years, most hospitalizations were brief, and only 1 case was severe. Of the 11 hospitalized H3N2v patients, 6 were persons at high risk for complications because of age younger than 5 years or underlying chronic comorbidities.[105] It is possible that acquisition of the M gene from the influenza A(H1N1) pdm09 virus may facilitate increased transmission among pigs, from pigs to humans, and among humans. Most H3N2v cases were identified in children who raised and exhibited pigs at agricultural fairs, especially in 2012, because H3N2 viruses circulated more widely among pigs in the United States[102,110] Some cases were identified among visitors who entered a swine barn at an agricultural fair. Limited, nonsustained human-to-human transmission of H3N2v viruses has been reported in persons without swine exposure, including young children at a child care center. Limited serologic data suggest that children lack immunity to H3N2v virus infections and may be at greater risk for infection after exposure to infected pigs than older adults.[101,627,722]

No commercially available tests can specifically identify variant virus infection. Clinical suspicion depends on eliciting a history of recent direct or indirect contact with pigs or with an ill person who had recent swine exposure. In suspected cases, respiratory tract specimens should be collected for testing at a state health department laboratory using the CDC FLU rRT-PCR Dx Panel assay, which can identify presumptive probable H3N2v virus infection, which is classified as a confirmed H3N2v case.[102] Further testing, sequencing, and viral characterization are performed at the CDC. Empirical NA inhibitor antiviral treatment is recommended to be started as soon as possible for any hospitalized patients, those with severe or progressive disease, or outpatients at increased risk for influenza complications if H3N2v virus infection is suspected.[104] Early antiviral treatment (within 48 hours of illness onset) of non–high-risk patients is encouraged.[104] For hospitalized patients, infection control precautions such as for seasonal influenza should be followed. In addition to H3N2v cases, other human infections with variant influenza A viruses containing the M gene from the 2009 H1N1 virus were identified in the United States during 2012, including H1N1v and H1N2v viruses.[113]

Pandemic Influenza

The epidemiology of pandemic influenza is different from that for seasonal influenza because many people, especially children and young adults, lack immunity to a pandemic influenza A virus, whereas older adults may have some cross-reactive antibodies from prior infection with antigenically similar influenza A viruses. Although the severity of influenza pandemics is variable, illness attack rates are highest among children and among household members, although hospitalization rates and mortality rates may be higher in adults.[35] Substantial outbreaks of pandemic influenza typically occur among schoolchildren.[406] Pregnant women are also disproportionately affected during influenza pandemics in terms of severe disease and mortality in contrast to nonpregnant women.[169,623] In contrast to seasonal influenza epidemics, in which mortality is highest among elderly persons, during influenza pandemics, an age shift toward deaths occurring in persons younger than 65 years of age is often observed.

The 1918 H1N1 pandemic was the most severe pandemic of the 20th and 21st centuries to date, with estimates of a case-fatality proportion of 2 percent, 600,000 deaths in the United States, and 50 to 100 million deaths worldwide.[363,477,668,733] This pandemic has been called "Spanish influenza," but did not begin in Spain. Although some think this pandemic started in China, some epidemiologic evidence suggests that it may have had an impact in the United States first in the spring of 1918. Severity was higher in the second wave of the 1918 H1N1 pandemic than in the first wave. Mortality rates were high in young children, the elderly, and persons between 15 and 35 years of age, resulting in the "W"-shaped mortality curve.[217,363,477,668,733] The 1918 H1N1 virus caused rapid

progression to pneumonia, and it has been proposed that secondary invasive bacterial coinfection in the preantibiotic era contributed to the high mortality, but this is based on only very limited autopsy tissue data.[479,480,607] Studies of the 1918 H1N1 virus in experimentally infected animals and molecular studies indicate that this virus possessed molecular markers associated with severe virulence and caused severe lung pathologic conditions with viral-induced and inflammatory damage.[347,541,692] The 1957 H2N2 virus caused a relatively moderately severe pandemic, with an estimated 70,000 excess deaths in the United States.[512] Viral pneumonia also was observed in children and nonelderly adults during the 1957 H2N2 pandemic.[430] The 1957 H2N2 pandemic is referred to as *Asian influenza* and may have originated in China.[363] The 1968 H3N2 virus caused a relatively mild pandemic that is referred to as *Hong Kong influenza*, with an estimated 34,000 excess U.S. deaths.[363,512] The 1968 H3N2 pandemic was the first pandemic with some availability of antiviral agents for treatment (amantadine). Regional differences in impact of the 1968 H3N2 pandemic have been reported, with Asia and the Americas being affected before sub-Saharan Africa.[803]

In 2009, a novel influenza A (H1N1) virus of swine origin emerged and caused the first influenza pandemic of the 21st century.[152] Epidemiologic evidence suggests that southeastern Mexico may have been affected first, with wider spread in Mexico and to the United States and Canada before spreading among humans worldwide.[35] The epidemiology of 2009 H1N1 indicated a younger age shift in contrast to seasonal influenza epidemics with higher hospitalization rates in school-aged children and young adults.[707] Hospitalization rates were highest in infants and young children, whereas mortality rates were highest in persons 50 years of age and older and the elderly. Hospitalization rates were substantially higher for 2009 H1N1 among children 5 to 17 years of age and adults 18 to 49 years in contrast to seasonal influenza.[139] Among hospitalized patients, a higher proportion of adults had underlying medical conditions than children. In some patients, more commonly in adults, a rapidly progressive viral pneumonia led to respiratory failure, ARDS, and refractory hypoxemia and was sometimes accompanied by vasopressor-dependent shock and multiorgan failure.[35] Autopsy tissue studies indicated that 2009 H1N1 virus can bind to receptors such as type II pneumocytes deep in the lower respiratory tract, and that up to 30 percent of fatal cases had evidence of bacterial pneumonia *(Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes)*.[610]

2009 H1N1 was the first pandemic in which antiviral drugs, primarily oseltamivir, were widely available in many countries, although not in low-resource settings. One study estimated that antivirals may have prevented 8400 to 12,600 hospitalizations, mainly in adults, in the United States.[25] Mortality was low in Japan, where early antiviral treatment was implemented widely.[656] In the United States, more than 300 children (median age, 9.4 years, many with underlying medical conditions) died of complications related to 2009 H1N1 virus infection.[138] Children in the United Kingdom who died of 2009 H1N1-related complications before hospital admission were more likely to have been previously healthy or have mild preexisting disorders in contrast to children who died after hospital admission.[590] Risk factors for severe disease included the same high-risk groups for seasonal influenza complications. As in previous pandemics, indigenous persons, such as aboriginal people, First Nations Canadians, American Indians, and Native Alaskans experienced high attack rates. Notably, obesity, particularly morbid obesity, was associated with critical illness and death worldwide.[707] One study estimated that approximately 200,000 respiratory deaths and an additional 83,000 cardiovascular deaths occurred worldwide, with 80 percent occurring in persons younger than 65 years, during the 2009 H1N1 pandemic.[151] In the United States, monovalent 2009 H1N1 vaccine did not become available until after the fall second wave had peaked but was still cost-effective in children.[563]

Transmission and Viral Shedding

Influenza viruses (seasonal and pandemic influenza viruses) are believed to be transmitted among humans primarily through large droplets and small particle droplet nuclei (aerosols) that are expelled by a symptomatic person with influenza virus infection to susceptible persons in close proximity (~1 to 2 meters).[69,70,669] Generally, large and small droplets are not observed to spread for longer distances because of desiccation and drop off. Although influenza viral RNA has been detected in exhaled breath, it is uncertain whether this can lead to transmission at very close distances.[188] Some studies have reported transmission of influenza viruses aboard planes.[30,523] The influence of air currents (i.e., ventilation, fans, and ventilators) in closed spaces is unknown. One study of nosocomial influenza virus transmission on two adjacent open general medical wards found an association with noninvasive ventilation in the index patient and an air jet from an air purifier, suggesting the role of aerosols.[748] Contact transmission is believed to be possible, but the relative contribution to influenza virus transmission is unknown. Experimental human challenge studies may provide insight on the relative contribution of influenza virus transmission modalities.[364,365] Influenza viruses can persist on nonporous surfaces, and influenza viral RNA was detected on the fingertips of children in one household study.[70,620] A randomized controlled trial (RCT) of hand washing with soap and water in Egyptian schoolchildren found significant reduction in rapid influenza test–confirmed influenza cases.[661] One U.S. RCT of hand sanitizer use and cough hygiene in schoolchildren reported a reduction of influenza A but not influenza B illness.[641] Other studies in schoolchildren or households have suggested a reduction in acute respiratory tract infection, influenza-like illness, and laboratory-confirmed influenza by hand washing during the influenza season.[394,432]

Data from animal models suggests the role of viral factors in influencing influenza virus transmission.[41] Single or two mutations in the HA gene can change or enhance pandemic influenza A virus receptor binding affinity and transmissibility in the ferret model.[330,693] One study suggested that the NA influenced increased

transmissibility of the 2009 H1N1 virus in the ferret model in contrast to closely related swine influenza A viruses with a different NA.[784] Switching the receptor binding affinity from avian-like to human-like in ferrets experimentally infected with mutated HPAI H5N1 virus did not enhance transmissibility, suggesting that other viral factors play a role in transmissibility.[443]

In household studies, the serial interval (time from illness onset of an index case to onset of a secondary case) is approximately 3 days.[395] This correlates with influenza viral load in the upper respiratory tract, which peaks during the first 2 days after illness onset and decreases substantially by about 4 to 5 days after illness onset.[410] The incubation period for influenza virus infection ranges from 1 to 4 days and is typically 1.5 days.[405] Influenza viruses can be detected the day before illness onset. Rare cases of a longer incubation period in which influenza virus was isolated up to 6 days before illness onset in young children have been reported.[215] It is unlikely that asymptomatically infected persons transmit influenza viruses to close contacts, and asymptomatic infection is not believed to contribute to community transmission, although transmission during the incubation period before illness onset has been reported.[250] Asymptomatic infection is important for ascertaining the denominator of all infected persons among those exposed in household and community studies.[211,326,545] Older studies that used serology suggest seasonal influenza virus infection attack rates of 17 to 46 percent in familes.[209,334,659]

In infants and young children, influenza viral replication may be detected for 7 to 10 days and up to 21 days.[250,256] School-aged children may have viable influenza virus detected for approximately 5 days after illness onset. Molecular assays can detect influenza viral RNA for longer periods, but this may not necessarily represent viable virus. Higher influenza viral load is correlated with longer viral shedding.[410] Children who are immunocompromised or immunosuppressed (especially severely immunosuppressed) may have detectable influenza viral replication for weeks to months.[223,375,581] Although it is generally believed that young children can have high levels of influenza viruses in the upper respiratory tract and shed influenza viruses for longer periods than adults, some studies have found no differences in viral load levels in children versus adults.[266,421] Hospitalized patients with pneumonia and respiratory failure can experience prolonged viral replication in the lower respiratory tract, although most data are from critically ill adults.[35,63,410,574]

PATHOGENESIS AND PATHOLOGY

A combination of many viral and host immune and host genetic factors affects the pathogenesis of influenza virus infection. The application of reverse genetics methodology to influenza virus research has facilitated understanding of viral molecular factors associated with pathogenesis.[205,494,496] The receptor binding affinity of influenza viruses is an important determinant of the host species range and may also influence disease severity. Human seasonal A and B and pandemic influenza A viruses bind preferentially to receptors on upper respiratory tract epithelial cells bearing a terminal sialic acid linked to galactose by an ∝2,6 linkage ("human-like" receptor binding affinity).[775] In contrast, influenza viruses that bind primarily to receptors on lower respiratory tract epithelial cells bearing a terminal sialic acid linked to galactose by an ∝2,3 linkage ("avian-like" receptor binding affinity) rarely infect humans and transmit inefficiently from human to human but can cause severe disease.[457,458,613,708] However, both seasonal and pandemic influenza viruses can bind to lower respiratory tract tissues in some individuals and cause severe lower respiratory tract disease, suggesting a role of other viral and host factors. Single mutations in the *HA* gene can change or enhance influenza virus receptor binding affinity and transmissibility in the ferret model.[330] Although the 2009 H1N1 virus did not exhibit predominant receptor binding affinity in the lower respiratory tract, this virus caused increased morbidity and replicated to higher titers in lung tissues in contrast to seasonal influenza A (H1N1) virus in experimentally infected ferrets, mice, and nonhuman primates.[321,444,487] In lower respiratory tract tissues of fatal human cases, 2009 H1N1 virus was found to infect alveolar lining cells, including type I and II pneumocytes.[610] Although seasonal influenza A and B viruses have tropism for the upper respiratory tract, influenza viral pneumonia can occur more commonly in elderly, immunosuppressed individuals and young children, especially with underlying chronic comorbidities, although middle-aged adults can be affected.[150,293,318,489]

Examples of virulence factors include mutations in *HA* (e.g., D222G and D225G),[40,127,419] which also can enhance viral tropism for lower respiratory tract tissues, and presence of the PB1-F2 protein, which can inhibit the induction of type I interferon (IFN) and cause a proinflammatory effect[712]; some PB1-F2 inflammatory phenotypes were associated with pulmonary infection with *S. pneumoniae* or *S. aureus* in the murine model.[11,322] Expression of the 1918 H1N1 virus PB1-F2 protein enhanced pathogenesis and secondary bacterial pneumonia in the murine model.[462] The HA protein of influenza viruses has to be cleaved by host proteases for the virus to be infectious.[373] Presence of a highly basic amino acid sequence in the HA cleavage site of highly pathogenic avian influenza A (H7N7) and (H5N1) viruses is associated with pathogenicity in avian and mammalian species, including humans. Mutations in PA and PB2 can result in enhanced replication of avian influenza A viruses in mammalian cells.[158,488] The NS1 protein of avian influenza A (H1N1) and (H5N1) viruses can induce suppression of innate immune responses and promote apoptosis in human kidney cells in vitro.[484]

Influenza virus infection of respiratory cells triggers activation of innate and adaptive immune responses. Toll-like receptors (TLRs), retinoic acid inducible gene-I *(RIG-I)*, and NOD- nucleotide-binding oligomerization domain (NOD)-like receptor family pyrin domain containing 3 protein (NLRP3) recognize influenza viral RNA.[381] TLR7, TLR3, and RIG-I bind influenza viral RNA and induce production of proinflammatory cytokines (tumor necrosis factor–α, interleukin [IL]-1, IL1β,

and IL-6,) and chemokines (monocyte chemoattractant protein [MCP-1], regulated upon activation normal T cell expressed and presumably secreted [RANTES], and macrophage inflammatory protein [MIP]-1αβ, IP-10, IL-8).[219] Type 1 IFNs (IFN-β, IFN-α) are stimulated, resulting in an antiviral effect on viral replication and by inhibiting protein synthesis in host cells and also stimulate dendritic cells to increase antigen presentation to $CD4^+$ and $CD8^+$ T cells.[219] $CD4^+$, $CD8^+$, and regulatory T cells (Tregs) are induced by influenza virus infection. $CD4^+$ T helper (Th) cells (Th1, Th2) are important components of the adaptive immune response. Th1 cells produce IFN-γ and IL-2 and are involved in cellular immune responses.[381] Th2 cells produce IL-4 and IL-13 and promote B cell responses.[381] Tregs and Th17 cells regulate T helper cell and $CD8^+$ response, and, when induced, Th17 cells produce IL-6 to inhibit Treg function.[381] $CD8^+$ cells are activated in lymphoid tissues and recruited to the site of infection, where they function as cytotoxic lymphocytes and recognize and lyse cells infected by influenza viruses. Natural killer cells can recognize and lyse infected cells bound to antibody. Memory $CD4^+$ and $CD8^+$ T cells contribute to promote the immune response to subsequent influenza virus infection.[168,381] Memory $CD8^+$ T cells can be distributed in the lung, liver, spleen, and bone marrow.[168]

Influenza viral factors allow escape from host innate immunity, and other factors can accelerate tissue damage from influenza virus infection. Binding of viral RNA by NS1 protein helps avoid recognition by TLRs and RIG-I, inhibiting type 1 IFN production.[381] NS1 also can inhibit RIG-I, and NS1, NP, and M2 contribute toward blocking host inhibition of influenza virus protein synthesis and replication.[381] PA-X, a newly described influenza A virus protein, modulates the host innate immune response.[327] Mutations can arise in viral epitopes that are targeted by cytotoxic T lymphocytes, resulting in escape from cellular immunity.[381] Release of chemokines induces neutrophils and T cells to migrate to infected respiratory tissues, which can further contribute to lung injury. MCP-1 mediates migration of alveolar macrophages and monocytes to infected lung tissues, and influenza virus infection of alveolar macrophages leads to apoptosis, whereas infection of epithelial cells causes necrosis.[390] Infected macrophages also can contribute to lung injury by releasing excessive amounts of reactive nitrogen intermediates and reactive oxygen species.[390] In the ferret model, local innate immune responses were temporally correlated with transmission, peak viral shedding, and virulence for influenza viruses that cause a range of disease and transmissability.[442] Cytokine dysregulation ("cytokine storm") has been demonstrated in vitro, and high serum and plasma cytokine levels are often correlated with high viral levels (delayed viral clearance) in the lower respiratory tract, suppression of adaptive and cellular immunity, critical illness (which can include multiorgan failure and hemophagocytosis), and fatal pulmonary disease (e.g., ARDS, pulmonary edema) in patients with seasonal influenza, 2009 H1N1, or HPAI H5N1 virus infections.*

*References 4, 52, 53, 155, 402, 403, 678.

Recently, differences in cytokine production in human macrophages and virulence in mice from highly pathogenic avian influenza A (H5N1) virus infection were attributed to the acidic polymerase (PA) protein, suggesting a viral molecular basis for induction of the host response and complementing the findings of another study that identified a new protein (PA-X) that modulates the host response to influenza A virus infection.[327,593]

Antibodies to the influenza virus HA protein are produced by plasma cells after influenza virus infection and are also induced by influenza vaccination. Memory B cells are virus-specific and are of long duration. The HA inhibition antibodies produced are virus strain-specific and of variable duration, depending on host age, and underlying immune function. Other aspects of the humoral immune response to influenza virus infection are the production of local immunoglobulin (Ig)A and systemic IgG neutralizing antibodies.[168] The concept of "original antigenic sin" was first proposed by Thomas Francis, Jr., to refer to the humoral immune response to the initial influenza virus infection of early childhood that is virus-specific and influences the subsequent humoral immune response to subsequent influenza virus infections.[213] Infection with an antigenically drifted virus stimulates memory B cells to produce antibodies to the initial virus, which may not clear the new virus infection as effectively. However, it has been observed that heterosubtypic immunity can be produced through production of cross-reactive influenza A virus antibodies and T cells. Antibodies to influenza A viruses have been identified that bind broadly to the conserved stem region epitopes of group 1 HA or group 2 HA proteins. This is an area of active research for development of immunotherapy and broadly protective "universal" vaccines.[381]

Host genetic factors may contribute to susceptibility to severe disease outcomes, although most studies have been underpowered. A study using genealogic records linked to death certificate records for a 100-year period in the state of Utah reported that first-, second, and third-degree relatives of a person who died of an influenza-attributable condition had a significantly increased risk for death attributable to influenza than expected.[6] One study of fatal seasonal influenza cases reported that those with low-producing *MBL2* genotypes had a sevenfold risk for invasive methicillin-resistant *S. aureus* (MRSA) than fatal cases with higher producing *MBL2* genotypes.[198] Other family clustering of influenza deaths has been observed with MRSA coinfection[804] and with HPAI H5N1 virus infection.[300,342,601,798] Case reports have identified thermolabile carnitine palmitoyltransferase II (CPT-II) variants as a possible risk factor in influenza-associated encephalopathy in Japanese and Chinese children.[384,445,782] One study reported that *CD55* gene polymorphisms were associated with severe illness from 2009 H1N1 virus infection in Chinese patients.[797] A case-control study reported four single-nucleotide polymorphisms that were associated with severe pneumonia from 2009 H1N1 virus infection located on chromosomes 1 and 17.[800] Evidence of a host genetic susceptibility factor was reported through in vitro, in vivo, and genetic studies of hospitalized influenza patients in the United Kingdom.

A significant number of hospitalized influenza patients had a single-nucleotide polymorphism identified in the IFN-inducible transmembrane *(IFITM3)* gene in contrast to matched European controls.[187] The topic of host genetic factors associated with severe and fatal influenza outcomes is under active investigation.

Histopathologic findings on evaluation of tissues from fatal cases with laboratory-confirmed seasonal or pandemic influenza suggest primary pulmonary and extrapulmonary causes of death for seasonal influenza virus infection in children (Fig. 177-3A to E). One study of tissues from cases of pediatric influenza-associated death identified lung damage (diffuse alveolar damage [26%], extensive secondary bacteria pneumonia [24%], extensive intra-alveolar hemorrhage [22%], viral pneumonitis [22%]), and myocarditis (30%) as causes of death of tested cases.[253] In trachea and bronchi, additional findings included submucosal congestion (90%), submucosal mononuclear inflammation (73%), bronchial epithelium necrosis (50%), submucosal hemorrhage (50%), and eosinophils (40%).[253] In lung tissues, mononuclear interstitial inflammation (67%), hyaline membrane formation (67%), intra-alveolar hemorrhage (54%), and neutrophilic bronchopneumonia were identified.[253] Hemophagocytosis was noted in 53 percent of lymph nodes examined.[253] In a study of fatal influenza B cases, myocardial injury was noted in 69 percent of tested patients, mostly in children; of these, 50 percent had myocarditis and 50 percent had evidence of ischemic myocardial necrosis.[534] In a study of autopsy tissues from 100 fatal cases of 2009 H1N1 virus infection, 80 percent of whom were 20 years of age or older, lung findings included edema (63%), hyaline membranes (59%), fibrin (58%), hemorrhage (58%), inflammation (48%), and neutrophilic bronchopneumonia (29%).[610] Seasonal or pandemic influenza viruses have rarely been isolated or detected outside of the respiratory tract. Very rare isolation of influenza virus has been reported from cerebrospinal fluid (CSF), stool, and myocardium.[166,178,453,557] Infrequent detection of influenza viral RNA from blood, urine, and stool has been reported, but may not necessarily represent viable virus. Detection of viral RNA in stool, including in patients without gastrointestinal symptoms, may represent swallowed virus.[662,679,786]

Clinical Issues

Clinical Characteristics of Uncomplicated Influenza

A wide spectrum of illness in children is associated with seasonal influenza virus infection, ranging from asymptomatic infection to critical illness and death. Asymptomatic infection may vary by age and prior immunity to antigenically similar influenza viruses. Household and seroepidemiologic studies have identified asymptomatic seasonal influenza virus infections in approximately 10 to 30 percent of persons studied,[208,545] and in one study approximately 60 percent of infections were estimated to be asymptomatic.[474] Asymptomatic infection is likely to be less common with pandemic influenza virus infection because of the lower prevalence of prior immunity in contrast to seasonal influenza virus infection.[85,128,209,334,413] Clinical manifestations are influenced by age, underlying immune status, and presence of chronic comorbidities.

Influenza in neonates and young infants may not always be accompanied by temperature elevation, but young infants can have high fever and a sepsis-like syndrome.[49,143,238,425,618] Neonates may manifest apnea and experience symptoms ranging from mild illness to respiratory failure.[455,706,790] In one study over three influenza seasons, one third of hospitalized children with laboratory-confirmed influenza were younger than 6 months of age.[567] Infants younger than 1 year of age can have fever and pneumonia, bronchiolitis, or croup.[346] Premature infants and those with chronic medical conditions may be at increased risk for severe illness.[783]

Common clinical features of influenza in young children, older children, adolescents, and adults are presented in Tables 177-1, 177-2, and 177-3. Diarrhea and vomiting are more common in young children than in older children.[358,417,617] Older children may have sudden onset of fever, nonproductive cough, rhinorrhea or nasal congestion, myalgia, headache, and pharyngitis. Sneezing is uncommon, but can occur. Conjunctivitis can occur infrequently and rashes are rare.[144] Influenza A and B virus infections cannot be distinguished on the basis of clinical signs and symptoms, illness severity, or other respiratory virus infections.[144] High fever is the only consistent sign or symptom that may be suggestive of influenza.[283,517] Vomiting and diarrhea are observed more frequently with influenza B than influenza A.[358,561,766] Atypical presentations in school-age children may include abdominal pain with or without fever.[358]

Influenza Complications

Influenza virus infection of the respiratory tract can result in a wide range of clinical complications. Otitis media is a frequent complication of influenza in young children.* Otitis media, bronchitis, and sinusitis can be managed on an outpatient basis. Mild to moderate exacerbation of underlying medical conditions may be managed on an outpatient basis, but worsening of chronic disease may require hospitalization, including intensive care (e.g., asthma, acute chest syndrome or other sickle cell crises, epilepsy, or cystic fibrosis).† Children with certain comorbidities such as chronic cardiac, pulmonary, neurologic, and neuromuscular diseases are at higher risk for severe pulmonary complications and prolonged hospitalization.‡ However, previously healthy children also can experience fulminant progression of severe influenza complications, including from influenza virus infection alone or from secondary invasive bacterial coinfection. Severe complications require hospitalization and can progress rapidly to death.[56] Epidemiologic studies, hospital cohort studies, and fatal case series have consistently identified children younger than 2 years of age to be at highest risk for hospitalization and death among children.

*References 60, 126, 144, 282-284, 346, 358, 417, 500, 517, 550, 561, 617, 690, 746, 766, 781, 783.
†References 82, 132, 153, 199, 337, 469, 525, 618, 649, 732.
‡Reference 58, 131, 356, 426, 570, 582.

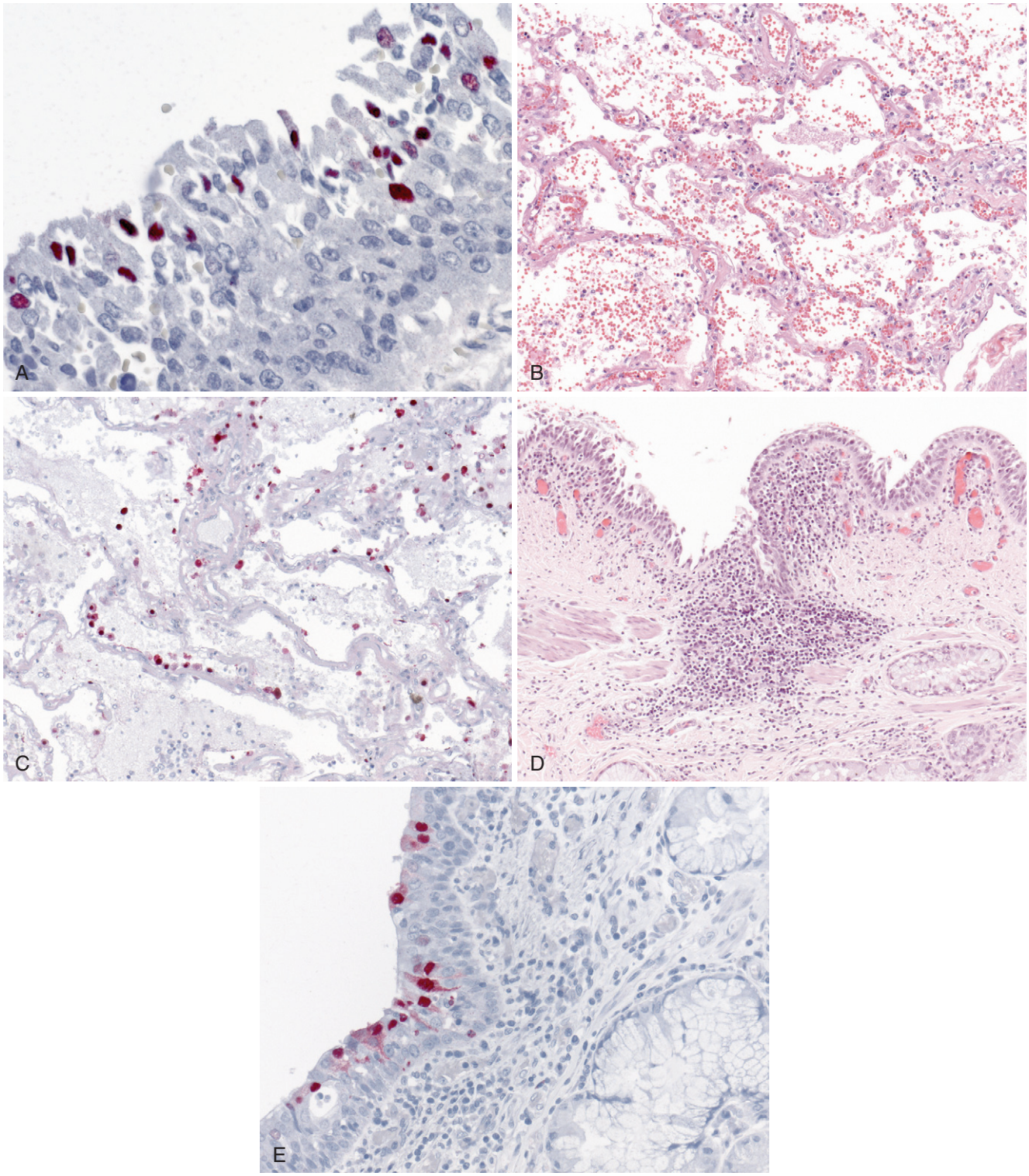

FIGURE 177-3 ■ **A,** Influenza B virus antigens (*dark stain*) in respiratory epithelial cells lining a bronchus from a fatal pediatric case (original magnification × 100, immunoalkaline phosphatase with naphthol-fast red and hematoxylin counterstain). **B,** Histologic section of lung tissue obtained from fatal case of influenza A(H1N1)pdm09 virus infection showing early diffuse alveolar damage (original magnification × 25, hematoxylin and eosin stain). **C,** Immunohistochemical staining of influenza A(H1N1)pdm09 virus antigens (*dark stain*) in pneumocytes in the lung of the same patient as in B (original magnification × 40, immunoalkaline phosphatase with naphthol-fast red and hematoxylin counterstain). **D,** Histologic appearance of bronchial mucosa from a fatal case of seasonal influenza A virus infection showing partially denuded epithelium and lymphoplasmacytic inflammatory cell infiltrates in the lamina propria and submucosa (original magnification × 40, hematoxylin and eosin stain). **E,** Influenza A virus antigens (*dark stain*) in respiratory epithelial cells lining a bronchus of a fatal pediatric case of seasonal influenza A virus infection (original magnification × 50, immunoalkaline phosphatase with naphthol-fast red and hematoxylin counterstain). (See companion Expert Consult website for color version.) (All images courtesy Dr. Christopher D. Paddock, CDC.)

TABLE 177-1 Frequency of Symptoms and Signs of Proven Influenza A (H2N2) in Children (0-14 Years of Age) and Adults*

Symptoms and Signs	Percentage with Symptoms or Signs	
	Children (N = 95)	Adults (N = 30)
Symptoms		
Sudden onset	66*	46
Systemic symptoms		
Feverishness	93	71
Headache	81	72
Anorexia	69*	37
Malaise	68	67
Chilliness	37	64†
Myalgia	33	62†
Respiratory symptoms		
Cough	86	90
Nasal discharge	67	82
Sore throat	62	62
Nasal obstruction	54	52
Sneezing	38	67†
Hoarseness	22	37
Sputum production	19	41†
Other symptoms		
Abdominal pain	31*	0
Vomiting	26*	7
Nausea	23*	4
Diarrhea	2	0
Signs		
Maximum temperature		
≤37.7°C (≤88.86°F)	11	13
37.8°-38.8°C (100.04°-101.84°F)	29	58†
≤38.9°C (≤102.02°F)	60*	29
Conjunctival abnormalities	61	56
Pharyngeal injection	60	68
Nasal injection/edema	50	64
Nasal discharge	38	20
Cervical adenopathy	38*	8
Rhonchi or rales	2	0
Pharyngeal exudates	1	0

Data from Jordan WS, Denny FW, Badger GF, et al. A study of illness in a group of Cleveland families. XVII. The occurrence of Asian influenza. *Am J Hyg* 1958;**68**:190-212.
*Significantly more frequent in children ($p < .05$, Fisher exact test).
†Significantly more frequent in adults ($p < .05$, Fisher exact test).

TABLE 177-2 Relative Frequency of Symptoms and Signs During Classic Influenza in Older Children and Adolescents

Symptoms and Signs	Occurrence*
Symptoms	
Chilly sensation	++++
Cough	+++
Headache	+++
Sore throat	+++
Prostration	++
Nasal stuffiness	++
Dizziness	+
Eye irritation or pain	+
Vomiting	+
Myalgia	+
Signs	
Fever	++++
Pharyngitis	+++
Conjunctivitis (mild)	++
Rhinitis	++
Cervical adenitis	+
Pulmonary rales, wheezes, or rhonchi	+

From Cherry JD. Influenza viral infections. In: Vaughan V, McKay R, Behrman R, editors. *Nelson textbook of pediatrics.* 14th ed. Philadelphia W.B. Saunders, 1987, p. 675-678.
*++++, 76%-100%; +++, 51%-75%; ++, 26%-50%; and +, 1%-25%.

Respiratory

In young children, influenza virus infection of the respiratory tract can result in bronchiolitis and croup,[73,346,367,535,582,637] although school-age children also can experience croup with influenza.[160] Tracheitis may occur uncommonly, usually with bacterial coinfection.[54,298] Viral pneumonitis and viral pneumonia can occur in all ages, may occur more frequently in immunosuppressed patients, and may vary by virus characteristics. For example, influenza A(H1N1)pdm09 virus is more often associated with viral pneumonitis and pneumonia than other seasonal influenza A or B viruses.[481,683] Radiographic findings of influenza viral pneumonia include bilateral interstitial or patchy infiltrates, diffuse infiltrates, multifocal opacities, and segmental or lobar consolidation (Fig. 177-4A to E])[329,776] Secondary bacterial pneumonia can be associated with fulminant progression to respiratory failure within 2 to 4 days of influenza onset.[201] The most common bacterial pathogens associated with influenza and secondary bacterial pneumonia in the United States are *S. pneumoniae*, *S. aureus* (methicillin-sensitive MSSA, or MRSA), and *S. pyogenes* (group A streptococcus).[201,253,331,515] In other countries, *Haemophilus influenzae* (type B or nontypable) also may be identified. The most fulminant clinical course is usually observed with *S. aureus* coinfection, either *MSSA or MRSA*, and can result in rapid progression to respiratory failure. MRSA was a mortality risk factor in previously healthy children who were critically ill with 2009 H1N1 virus infection.[570] In animal models, a synergistic effect of influenza virus with pneumococcal infections and *S. aureus* infections have been reported.[322,463,464] A report of chest pain in a child with influenza is concerning for secondary bacterial pneumonia. Parapneumonic empyema has been reported with *S. pneumoniae*, *S. aureus*, and 2009 H1N1 virus infection.[16] Status asthmaticus can occur in patients without a prior history of asthma, but is more common in asthmatic children. Spontaneous

TABLE 177-3 **Relative Frequency of Clinical Manifestations of Influenza Viral Infections in Children Younger Than 5 Years**

Clinical Manifestation	Occurrence*
Major Clinical Category	
Upper respiratory tract illness	++++
Laryngotracheitis	+
Bronchitis	+
Bronchiolitis	+
Pneumonia	+
Symptoms	
Cough	++++
Anorexia	++
Coryza	++
Vomiting	++
Diarrhea	+
Sore throat	+
Signs	
Fever	++++
Pharyngitis	+++
Cervical adenitis	++
Otitis media	++
Seizures	+
Exanthem	+
Generalized adenitis	+

From Cherry JD. Influenza viral infections. In: Vaughan V, McKay R, Behrman R, editors. *Nelson textbook of pediatrics.* 14th ed. Philadelphia W.B. Saunders, 1987, p. 675-678.
*++++, 76%-100%; +++, 51%-75%; ++, 26%-50%; and +, 1%-25%.

pneumomediastinum with pneumonia has been reported.[270,776] Children with second-hand tobacco smoke exposure may be at risk of more severe influenza illness.[745]

Cardiac

Rare complications such as myocarditis and pericarditis are associated with influenza more commonly in young children, but cases can occur in children of all ages with either influenza A or B virus infection of the respiratory tract.* Myocarditis can cause critical and fatal illness, including sudden death, especially in young children. Influenza virus is almost never isolated or detected in the myocardium and is most likely due to an immune-mediated inflammatory process.[253,534]

Neurologic

A wide range of neurologic complications associated with seasonal and pandemic influenza has been reported, ranging from moderate to fatal illness.† Simple febrile seizures may occur in young children, as well as complex partial seizures and status epilepticus.* Transient encephalopathy may be manifested by confusion, incoherence, mutism, and lethargy, with or without fever.[453,503] Influenza-associated encephalopathy (IAE), including with neurologic sequelae or death, has been reported in some countries, most frequently through national surveillance in Japan.[14,482,680] One study reported that treatment with diclofenac (nonsteroidal anti-inflammatory drug), elevated aspartate aminotransferase, blood glucose level of 150 mg/dL or greater, and hematuria or proteinuria, were associated with mortality.[491] A reversible splenial lesion has been reported in some encephalopathy patients.[660] Delirium and visual hallucinations have been described in IAE patients.[351] IAE is thought to be a result of cytokine dysregulation and inflammation and not viral invasion of central nervous system tissue. Encephalopathy and encephalitis may undergo fulminant clinical progression, with hyperpyrexia, elevated intracranial pressure, and uncal herniation observed in some fatal cases. Neuroimaging findings can be normal or show diffuse cerebral edema on computed tomography or magnetic resonance imaging (MRI). A mild pleocytosis may be observed, but the CSF is often normal. Cerebellitis has been described. A subset of encephalopathy, acute necrotizing encephalitis is often manifested by high fever, seizures, altered mental status to loss of consciousness and fulminant progression, with poor clinical outcomes, including neurologic sequelae or death.[470,482,652] Classic findings in acute necrotizing encephalitis are bilateral thalamic lesions on MRI, and brain stem also may be involved. Electroencephalogram may be abnormal.

Influenza-associated encephalopathy is distinguished from Reye syndrome by the absence of hypoglycemia and usually mild to moderate elevated hepatic transaminases.[482] Cases of encephalitis were noted during the 1957 H2N2 pandemic,[755] and encephalopathy with 1957 H2N2 virus infection with fatal outcomes was described in children.[526] Encephalopathy and encephalitis associated with the 2009 H1N1 pandemic were reported worldwide among children of all ages.[218,351,361] Postinfectious influenza encephalopathy with meningismus also has been reported.[502,805] Acute disseminated encephalomyelitis with influenza is uncommon, but has been reported.[532,575,729,785] Guillain-Barre syndrome (GBS) has been reported rarely in children with influenza virus infection and is associated with influenza in adults.[713] Transverse myelitis with influenza also is rare.[393] Rare cases of cerebrovascular accident, including secondary to hypotension, also have been reported.[38,296,502] Isolation of influenza virus from CSF is extremely rare, but has been reported.[453] Similarly, detection of influenza viral RNA in CSF has been reported infrequently, but may not represent viable virus. Aseptic meningitis with influenza has been described.[531] Influenza virus infection of the upper respiratory tract may precipitate secondary bacterial infection, bacteremia, and meningitis, with or without pneumonia. Influenza has been observed to precede outbreaks of meningitis with *Neisseria meningitidis*.[79,87,269]

*References 253, 379, 514, 534, 565, 568, 570, 695.
†References 32, 216, 232, 354, 361, 453.

*References 125, 267, 389, 510, 533, 560, 561, 618.

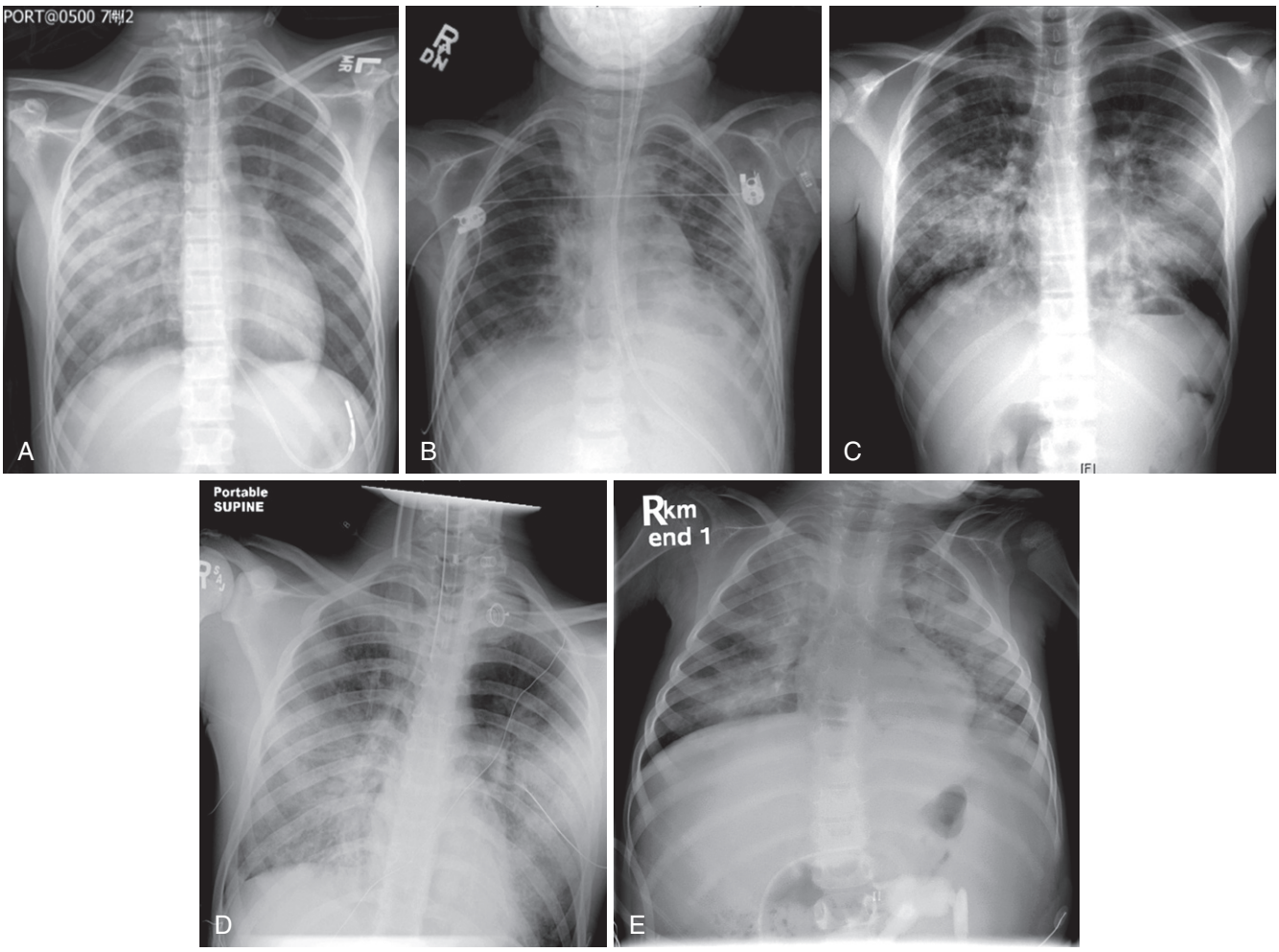

FIGURE 177-4 ■ **A,** Chest x-ray (CXR) demonstrating right lower lobe (RLL) and right middle lobe (RML) pneumonia in previously healthy adolescent presenting with fever, diarrhea, and altered mental status and diagnosed with acute necrotizing encephalitis associated with influenza A(H1N1)pdm09 (2009 H1N1) virus infection. Lung tissue was positive for 2009 H1N1 viral RNA by rRT-PCR; blood, bacterial cultures, and special lung stains were all negative for bacterial infection. Brain tissue was negative for 2009 H1N1 viral RNA. **B,** CXR showing bilateral infiltrates and opacities in previously healthy child presenting with fever and hypoxia, diffuse expiratory wheezing, and rales associated with 2009 H1N1 virus infection. Upper respiratory tract specimens were positive by rRT-PCR for 2009 H1N1 viral RNA; blood and lower respiratory tract specimens were negative for bacterial infection. **C** and **D,** Progression of bilateral infiltrates and opacities in a previously healthy adolescent presenting with fever, cough, dyspnea, tachypnea, chest pain, and pneumonia associated with 2009 H1N1 virus infection and secondary invasive MRSA infection. Respiratory specimens were positive by rRT-PCR for 2009 H1N1 viral RNA and blood cultures were positive for MRSA. **E,** Diffuse bilateral infiltrates and opacities in child with underlying comorbidities who had respiratory failure due to influenza A (H3N2) virus infection. (**A,** Courtesy Dr. Jaime Fergie, MD; **B,** courtesy Dr. Emily Dobyns, MD; **C** and **D,** courtesy Dr. K. J .S. Anand, MD; **E,** courtesy Dr. Andy Pavia, MD, and Dr. Krow Ampofo, MB, ChB.)

Musculoskeletal

Myositis with mild to moderate elevations in creatine phosphokinase is common in hospitalized influenza patients. Schoolchildren typically 6 to 9 years of age, and boys more frequently than girls, may experience acute onset of bilateral gastrocnemius and soleus myositis.* Rhabdomyolysis and myoglobinuria can occur.[767] A common complaint is severe bilateral calf muscle pain and inability to walk. Myositis appears to be more commonly associated with influenza B than influenza A.[194,358] Severe myositis can precipitate rhabdomyolysis, acute renal failure, sepsis, disseminated intravascular coagulation (DIC), and compartment syndrome, requiring emergency fasciotomy to prevent muscle necrosis.[625,657]

*References 83, 134, 194, 306, 358, 447.

Hepatic

Mild to moderate elevation of hepatic transaminases can be observed with influenza, and rare cases of acute hepatitis and liver failure have been reported, including in previously healthy children.[743] Elevated transaminases and steatosis are observed with influenza and Reye syndrome, and hepatic failure and death can occur.[37,294,509,640] Reye syndrome with influenza is associated with salicylate exposure and occurs more commonly with influenza B than influenza A virus infection.[37,261,294,509,640] Chronic hepatic disease has been associated with pneumonia from 2009 H1N1 virus infection.[771] One study conducted primarily in adults suggested that hepatic involvement was greater with 2009 H1N1 in contrast to seasonal influenza and was likely the result of hypoxia and hypoxemia.[540]

Renal

Influenza can precipitate renal abnormalities through different mechanisms.[608] Cytokine dysregulation, hypotension, and rhabdomyolysis with myoglobinuria may contribute to acute renal dysfunction and failure.[159,239,435,608] Patients who are critically ill with influenza viral pneumonia, respiratory failure, and refractory hypoxemia may experience multiorgan dysfunction, including renal failure.[570,734]

Bacterial Coinfection

Most influenza virus infections do not result in secondary invasive bacterial infection. Uncommonly, young infants may experience urinary tract bacterial infection,[383,425] and serious bacterial infections are less frequent in nonhospitalized febrile young children with influenza in contrast to those without influenza.[634] However, secondary invasive bacterial infections associated with seasonal or pandemic influenza may result in bacteremia and sepsis and cause critical illness and fatal outcomes.[667,791] Secondary bacterial pneumonia is the most common serious bacterial complication associated with influenza. In the United States, the most common bacterial pathogens causing pneumonia, bacteremia, and sepsis associated with influenza virus infection reflect nasopharyngeal carriage and subsequent invasive infection: *S. pneumoniae*, *S. aureus* (MSSA or MRSA), and *S. pyogenes* (group A streptococcus).* In other countries, *H. influenzae* (type B or nontypable) also may be identified. The most fulminant clinical course is usually observed with invasive *S. aureus* infection, either MSSA or MRSA.[201] *N. meningitidis* infection resulting in meningococcemia or meningitis can follow influenza virus infection.[79,87,194,201,269]

Sepsis and Disseminated Intravascular Coagulation

Sepsis and DIC can be precipitated by influenza virus infection of the respiratory tract, with or without bacterial infection, leading to critical and often fatal outcomes.[435,689] Toxic shock syndrome with influenza and toxin-producing *S. aureus*, with tracheitis or pneumonia, can lead to critical illness and death.[440]

Viremia

Viremia with influenza virus infection has been poorly studied. However, studies of blood donated to blood banks during pandemics and other limited studies suggest that seasonal or pandemic influenza viremia is extremely rare in uncomplicated disease and critical illness. Isolation of influenza A (H3N2) virus was reported from blood and throat specimens of an individual collected 12 hours before illness onset during the 1968 H3N2 pandemic.[359] Isolation of 1957 H2N2 virus from extrapulmonary tissues (i.e., spleen, liver, kidney, and heart) in two fatal cases suggested viremia.[341] The 1957 H2N2 virus was isolated from myocardial suspension without histologic changes except in the arterioles.[526] Viremia with seasonal influenza virus infection has been reported rarely.[341,359,440] Viremia was identified during the incubation period in experimentally infected volunteers.[691] Detection of 2009 H1N1 viral RNA in blood specimens of hospitalized patients during the 2009 H1N1 pandemic was reported.[530,688] Studies that tested banked blood for evidence of 2009 H1N1 viral RNA during the pandemic did not find evidence of viremia.[459,635,646] The largest autopsy case series of fatal 2009 H1N1 patients did not find evidence of extrapulmonary dissemination.[610] Viremia has been identified in fatal human infection with highly pathogenic avian influenza A (H5N1) virus.[81,154,155,806]

Vertical Transmission

Seasonal influenza virus infection of pregnant women does not appear to result in vertical transmission.[317] Rare case reports suggesting vertical transmission have not conclusively proved that seasonal or pandemic influenza viremia has occurred, with subsequent transmission to the fetus.[115,172,345] Vertical transmission has been demonstrated in human infection with highly pathogenic avian influenza A (H5N1) virus.[806]

Sudden Death

Sudden death can occur after a brief mild influenza illness.[56] For some children without underlying comorbidities, autopsy findings may include evidence of diffuse alveolar damage without bacterial infection or myocarditis.[253] In some cases, encephalitis or cerebral edema with concomitant pneumonia are identified. Autopsy findings may not identify a clear etiology for death, suggesting the possibility of an arrhythmia or other cause of cardiac failure. Fulminant cases of bacterial sepsis with influenza also have been reported.

CLINICAL MANAGEMENT

Clinical Diagnosis

Influenza may not always be accurately diagnosed based on clinical presentation from other etiologies of acute febrile respiratory illness with or without fever, if other pathogens, especially noninfluenza respiratory viruses, are co-circulating in the community.[549] Signs and symptoms associated with higher odds ratios or positive predictive values for influenza in children include high fever (temperature >39.0° C [>102.0° F]) and cough.[283,517,617] Many other co-circulating respiratory viruses can cause influenza-like illness, including rhinoviruses, coronaviruses, adenoviruses, parainfluenza viruses, bocaviruses, human metapneumoviruses, respiratory syncytial viruses, and others. Although testing is not necessary to diagnose influenza in every patient, especially during high influenza activity in a community, some testing can be beneficial to establish that influenza virus infections are present in the patient population. Influenza A and B virus

*References 8, 56, 201, 331, 610, 667.

infections cannot be distinguished by clinical signs and symptoms.[144]

Diagnostic Testing

Several diagnostic tests for the qualitative detection of influenza viruses are commercially available,[385] although the sensitivities to detect influenza viruses can vary widely and clinicians should be aware of many factors influencing testing results and the limitations of available diagnostic tests.

Specimens for Influenza Testing

For symptomatic outpatients with suspected influenza, upper respiratory tract specimens should be collected within 3 to 4 days of illness onset for influenza testing. Collection of respiratory specimens later in the clinical course still may yield detection of influenza viruses from young infants, immunosuppressed patients, and those with severe illness resulting from prolonged influenza viral replication, by use of molecular assays. For detection of seasonal or pandemic influenza A and B viruses, the optimal upper respiratory tract specimens are nasopharyngeal aspirates from infants and young children and nasopharyngeal swabs from school-age children.[281] Nasal washes or combined nasal and throat swabs also may have high yield for influenza viruses.[156,280,366] The lowest yield for influenza viruses for viral isolation is from throat swabs alone. However, molecular assays may detect influenza viral RNA for longer periods than other assays, including in throat swabs. For hospitalized patients with respiratory failure, lower respiratory tract specimens (e.g., endotracheal aspirates) should be collected for influenza testing, especially if testing of upper respiratory tract specimens is negative, because influenza viral replication is longer in the lower respiratory tract than in the upper respiratory tract in patients with lower respiratory tract disease.[35,699] Bronchioalveolar lavage fluid, if obtained for other diagnostic purposes, also can be tested. Clinicians should consult laboratory colleagues or the test manufacturer's package inserts for the acceptable swabs and approved clinical specimens. Swabs used for specimen collection should have a Dacron tip and an aluminum or plastic shaft. In general, swabs with calcium alginate or cotton tips and wooden shafts are not recommended for any influenza testing because specimens collected with these swabs may produce inaccurate results.

Available Assays

For outpatients and emergency department patients, the most common commercially available assays are rapid influenza diagnostic tests (RIDTs) that detect influenza virus antigens in respiratory specimens. The majority of RIDTs are enzyme immunoassays that use monoclonal antibodies against influenza viral nucleoprotein (a conserved protein) to produce a result in approximately 10 to 15 minutes. The advantages of RIDTs are that they are simple to perform and produce a quick result. Some tests may not distinguish between influenza A and B viruses, although most tests can differentiate between influenza A and B using separate type-specific monoclonal antibodies. None of the RIDTs can differentiate between influenza A virus subtypes, whether of human (i.e., seasonal influenza viruses) or animal origin (i.e., swine or avian influenza A viruses). Most RIDTs are available in kits that contain antigen extraction reagents, swabs, and indicator devices that generally fit in the palm of a hand. The disadvantages are that RIDTs have generally poor to moderate sensitivities to detect seasonal, pandemic, and novel influenza A viruses in contrast to molecular assays or viral culture and produce limited information (influenza virus type).* In the United States, most RIDTs are approved for point-of-care use, but some must be performed in a moderately complex clinical laboratory. Recently, RIDTs have become available that use a reader device to improve test sensitivity. One fluorescent lateral flow immunoassay employs a cartridge and analyzer device to improve test sensitivity. Use of influenza RITDs in outpatients yielding positive results have reduced patient time spent in the emergency department, ordering of other diagnostic tests and antibiotics, and cost.[65,511,556] Other studies have reported that empirical antiviral treatment is more cost-effective than using RIDTs for antiviral treatment decisions.[396,585]

Other antigen detection tests include immunofluorescent assays (direct florescent antibody staining [DFA], and indirect florescent antibody staining [IFA]) that use influenza virus type-specific florescent-tagged monoclonal antibodies visualized through a florescent microscope. Turnaround time to results is approximately 2 to 3 hours and is more commonly available in hospital clinical laboratories. Some respiratory virus immunofluorescence panels are available that detect influenza A and B virus antigens and other respiratory viruses. Collection of a good-quality respiratory specimen containing epithelial tissue is important to improve test performance. Sensitivity to detect influenza A viral antigen is generally slightly higher than for detecting influenza B viral antigen, and most studies of immunofluorescence assays have reported a range of sensitivities of 70 to 90 percent versus viral culture.[700] Neither DFA nor IFA can produce results other than identifying influenza virus type.

Molecular assays that can detect influenza virus nucleic acid using different platforms are becoming more commonly available in hospital clinical laboratories.[714] Some molecular assays can differentiate between influenza A and B viral RNA, and some may use different primers and probes to distinguish between some influenza A virus subtypes. The commonly available assays use RT-PCR, including some assays that detect only influenza viral RNA, and other multipathogen assays that may detect a variety of respiratory viruses; some assays may detect many viruses and bacteria associated with respiratory illness. State public health laboratories in the United States use the CDC FLU rRT-PCR FLU Dx Panel, which can detect influenza A, B, H1, H3, H5, influenza A(H1N1)pdm09, and A/pdm09.[615] Deployment of this assay worldwide allowed detection of influenza A(H1N1) pdm09 virus infections throughout the 2009 H1N1

*References 31, 102, 119, 190, 699, 700, 704.

pandemic.[335] In general, molecular assays such as rRT-PCR have the highest sensitivities to detect influenza viruses in respiratory specimens in contrast to other influenza tests.

Viral culture of respiratory specimens is the most specific influenza test, but the sensitivity of viral culture to isolate influenza viruses, although high, is lower than the sensitivity of molecular assays to detect influenza viral RNA. This is because viral culture can isolate only viable influenza viruses, whereas molecular assays can detect the presence of virus, but not necessarily replicating virus. Viral culture may be done at some hospital clinical laboratories, but is generally performed at public health laboratories. Some clinical laboratories may perform shell vial tissue cell culture to produce results in 24 to 48 hours. Routine tissue cell viral culture generally requires 3 to 7 days. Most viral culture for influenza viruses is performed by inoculation of Madin-Darby canine kidney (MDCK) tissue cells, but other tissue cell lines are used for culture of a wider range of respiratory viruses.

Public health laboratories such as national influenza centers and WHO Collaborating Center laboratories also isolate influenza viruses using embryonated eggs for vaccine candidates because most influenza vaccine strains are selected and prepared from egg-grown viruses. Isolation of viruses allows for detailed virologic characterization, including antigenic properties, genetic analyses, antiviral biologic assays, structural analyses (e.g., receptor binding analyses). Pyrosequencing assays can be used for determination of the presence of known virulence markers for research purposes.[407] Full genomic sequencing is essential to assessing genetic reassortment and presence of known and unknown mutations associated with virulence, antiviral resistance, transmissibility, receptor binding affinity, and other viral characteristics.

Antiviral resistance testing is important for public health and for clinical management of influenza patients who are at increased risk for emergence of antiviral resistance. Routine testing of viruses for antiviral susceptibility is an important component of influenza surveillance worldwide. During 2003 to 2006, a substantial global increase in the prevalence of influenza A viruses resistant to the adamantane antivirals (i.e., amantadine and rimantadine) was identified through pyrosequencing, leading to the recommendation not to use these antiviral drugs for influenza A.[71,72,163] During 2007 and 2008, influenza A (H1N1) viruses with an H275Y mutation in viral neuraminidase associated with oseltamivir resistance became widely prevalent; these viruses are no longer circulating and were replaced by the emergence of influenza A(H1N1)pdm09 virus.[272,391,468] During the 2009 H1N1 pandemic, oseltamivir resistance was identified sporadically, most often during or after oseltamivir treatment in immunosuppressed patients or during oseltamivir chemoprophylaxis.[310] Some human-to-human spread and community transmission of influenza A(H1N1)pdm09 viruses with the H275Y mutation in neuraminidase have been identified since 2009.[121,310,311,397,476]

Current public health laboratory monitoring for antiviral susceptibility in circulating influenza viruses includes pyrosequencing for known markers associated with antiviral resistance and functional phenotypic assays such as the neuraminidase inhibition assay using live viruses combined with full genetic sequencing to identify known and new markers of antiviral resistance.* Such assays can be performed only at specialized laboratories, national influenza centers, and WHO Collaborating Center laboratories. The WHO has published criteria for classifying neuraminidase inhibitor resistance on the basis of relative differences in inhibitory concentration $(IC)_{50}$ determined in neuraminidase inhibition assays.[764] However, for clinical management purposes, especially for severely immunosuppressed patients at higher risk for prolonged influenza, viral replication and emergence of antiviral resistance during or after treatment, antiviral resistance testing is not widely available. A limited number of laboratories can perform pyrosequencing testing for known markers of antiviral resistance such as H275Y in viral neuraminidase.

Influenza virus serologic testing currently has no role in the clinical management of influenza patients and should not be ordered for many reasons. No validated commercially available IgG or IgM serologic assays, including enzyme-linked immunosorbent assay, exist for detection of influenza virus antibodies. Furthermore, serologic results of a single acute serum specimen are uninterpretable for clinical use. Standard serologic testing for seasonal influenza viruses requires appropriately timed, paired acute and convalescent serum specimens to determine virus strain–specific HAI antibodies and must be performed at special influenza research or public health laboratories. Research laboratories and some public health laboratories can perform microneutralization assays to determine neutralizing antibodies to specific influenza viruses. Seropositive results for either HAI or neutralizing antibodies are considered when a fourfold rise in antibodies is observed in paired serum specimens. Serologic testing can establish a retrospective diagnosis of influenza virus infection, but must be performed by a specialized laboratory. Applications of influenza serologic testing include seroepidemiologic investigations, as well as vaccine immunogenicity and vaccine effectiveness studies. Seroepidemiologic public health investigations can yield data on seroprevalence and seroincidence of strain specific and cross-protective antibodies for infections with seasonal, animal-origin, and pandemic influenza viruses.[101,265,768]

Interpretation of Testing Results

Clinicians should consider many factors that can influence testing results and understand the inherent limitations of influenza tests to properly interpret results, especially from rapid influenza diagnostic tests or other antigen detection tests. These factors include the time from illness onset to specimen collection and the likelihood that influenza viral replication is occurring and viruses can be detected, the kind of specimen that is collected (i.e., swab, aspirate, or wash), the kind of swab used (e.g., Dacron, cotton, or flocked or nonflocked), the specimen source (i.e., nasopharynx, nasal, throat, or

*References 161, 162, 254, 309, 312, 518, 609.

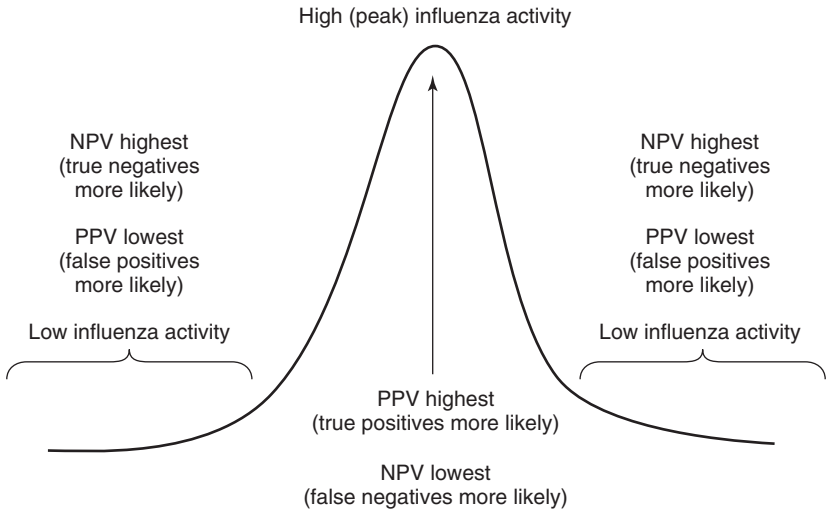

FIGURE 177-5 ■ Prevalence of influenza viruses (influenza activity in the community) and relationship to positive predictive value (*PPV*) and negative predictive value (*NPV*) of influenza diagnostic testing results.

lower respiratory tract), the quality of the specimen collected, and the time from specimen collection to testing, including how specimens are stored and processed (e.g., freeze-thawing) before testing.[700] Inherent fixed properties of tests are the sensitivity and specificity compared with a gold standard assay such as RT-PCR. Furthermore, antigen detection tests (e.g., RIDTs and immunofluorescence) cannot differentiate among influenza A virus subtypes or among seasonal, pandemic, or novel influenza A virus infections. Although specificity may be high, sensitivity of rapid influenza diagnostic tests to detect seasonal, pandemic, and novel influenza A viruses may be poor to moderate, resulting in many false negative results.* One meta-analysis of RIDTs reported that although overall sensitivity to detect influenza viruses was moderate (62.3%) with high specificity (98.2%) in contrast RT-PCR or viral culture, sensitivity was higher for detection of influenza A virus (64.6%) than for influenza B virus (52.2%) and sensitivity was higher in specimens collected from young children (66.6%) than in adults (53.9%).[119]

The prevalence of seasonal influenza viruses circulating in the population being tested is variable.[248,700] The predictive values of tests are calculated based on sensitivity, specificity, and prevalence. Because predictive values vary by influenza virus prevalence, the positive predictive value (PPV) of an influenza test is highest (likely to be "true positives") during peak influenza activity and the PPV is lowest (likely to be "false positives") during periods when influenza viruses are not circulating or activity is very low (e.g., summer in temperate climate countries)[248,700] (Fig. 177-5). Conversely, the negative predictive value (NPV) of an influenza test is highest (likely to be "true negatives") during periods when influenza viruses are not circulating or activity is very low, and the NPV is lowest (likely to be "false negatives") during peak influenza activity. Testing results can vary by influenza virus type, influenza A virus subtype, virus strains, and novel influenza A viruses of animal origin that sporadically infect humans.* Clinicians also should realize that the respiratory tract in a patient with influenza virus infection comprises a population of replicating viruses (quasispecies), and that sampling, amplification, and genetic sequencing may identify a mixed population or a dominant population that is amplified or isolated from the respiratory sample collected.

Antiviral Treatment

In the United States, four prescription influenza antiviral drugs are approved, with different dosing and age-based recommendations.[202] The adamantane antivirals, amantadine and rimantadine, are chemically similar drugs that appear to block the ion channels of the M2 protein of influenza A viruses, but they do not have any activity against influenza B viruses. Both amantadine and rimantadine are approved for chemoprophylaxis of influenza A in children 1 year of age or older. Amantadine is approved for treatment of influenza A in children 1 year of age and older, and rimantadine is approved for treatment of influenza in adults. Gastrointestinal and neuropsychiatric adverse effects have been reported with both drugs, more frequently with amantadine.[202] When used for treatment of susceptible influenza A virus infection, a reduction of viral shedding is observed with early treatment (within 48 hours after illness onset) versus placebo, but rapid emergence of antiviral resistance to amantadine and rimantadine can develop in treated patients.† Since 2006, the CDC has recommended that amantadine and rimantadine not be used for influenza A because of the high prevalence of circulating influenza A viruses that are resistant to these drugs. Similarly, the WHO does not recommend use of amantadine or rimantadine for treatment of influenza A virus infections. One exception is treatment of severe illness resulting from novel influenza A virus infection with documented susceptibility to

*References 31, 102, 119, 190, 700, 704.

*References 31, 102, 119, 190, 700, 704.
†References 48, 257, 276, 277, 303, 592.

TABLE 177-4 **Recommended Dosage and Schedule of Influenza Antiviral Medications* for Treatment† and Chemoprophylaxis‡**

Antiviral Agent		Age Group (yr) 0-6	7-9	10-12	13-64	≥65
Zanamivir	Treatment, influenza A and B	NA	10 mg (2 inhalations) twice daily	10 mg (2 inhalations) twice daily	10 mg (2 inhalations) twice daily	10 mg (2 inhalations) twice daily
	Chemoprophylaxis, influenza A and B	NA for ages 1-4	Ages 5-9 10 mg (2 inhalations) once daily	10 mg (2 inhalations) once daily	10 mg (2 inhalations) once daily	10 mg (2 inhalations) once daily
Oseltamivir§	Treatment,‖ influenza A and B	Dose varies by child's weight‖	Dose varies by child's weight‖	Dose varies by child's weight‖ >40 kg = adult dose	75 mg twice daily	75 mg twice daily
	Chemoprophylaxis, influenza A and B	Dose varies by child's weight¶	Dose varies by child's weight¶	Dose varies by child's weight¶ >40 kg = adult dose	75 mg once daily	75 mg once daily

Modified from Fiore AE, Fry A, Shay D, et al; Centers for Disease Control and Prevention (CDC). Antiviral agents for the treatment and chemoprophylaxis of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**:1-24.

*Zanamivir is manufactured by GlaxoSmithKline (Relenza: inhaled powder). Zanamivir is approved for treatment of persons aged ≥7 years and approved for chemoprophylaxis of persons aged ≥5 years. Zanamivir is administered through oral inhalation by using a plastic device included in the medication package. Patients will benefit from instruction and demonstration of the correct use of the device. Zanamivir is not recommended for those persons with underlying airway disease. Oseltamivir is manufactured by Roche Pharmaceuticals (Tamiflu: tablet). Oseltamivir is approved for treatment of persons 14 days of age and older and for chemoprophylaxis of persons aged ≥1 year. Oseltamivir is available for oral administration in 30-, 45-, and 75-mg capsules and liquid suspension. This information is based on data published by the Food and Drug Administration, available at http://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm100228.htm.

†Recommended duration for antiviral treatment is 5 days. Longer treatment courses can be considered for patients who remain severely ill after 5 days of treatment.

‡For control of outbreaks in long-term care facilities and hospitals, the CDC recommends antiviral chemoprophylaxis for a minimum of 2 weeks and up to 1 week after the most recent known case was identified.

§The CDC and ACIP recommend oseltamivir treatment for children of all ages, including from birth. The recommended oseltamivir treatment dosing for infants <1 year is 3 mg/kg/dose twice daily. A reduction in the dose of oseltamivir is recommended for persons with creatinine clearance <30 mL/min.

‖The treatment dosing recommendation for oseltamivir for children aged ≥1 year who weigh ≤15 kg is 30 mg twice per day. For children who weigh >15 kg and up to 23 kg, the dose is 45 mg twice per day. For children who weigh >23 kg and up to 40 kg, the dose is 60 mg twice per day. For children who weigh >40 kg, the dose is 75 mg twice per day.

¶For children ages 3 months to <1 year, the recommended chemoprophylaxis dosing for oseltamivir is 3 mg/kg/dose once daily. The chemoprophylaxis dosing recommendation for oseltamivir for children aged ≥1 year who weigh ≤15 kg is 30 mg once per day. For children who weigh >15 kg and up to 23 kg, the dose is 45 mg once per day. For children who weigh >23 kg and up to 40 kg, the dose is 60 mg once per day. For children who weigh >40 kg, the dose is 75 mg once per day.

NA, Not approved.

amantadine and rimantadine in which one of these drugs may be used as combination antiviral treatment with an NA inhibitor drug.[760]

Two NA inhibitor drugs, oseltamivir and zanamivir, are approved for use in children (Table 177-4). These drugs bind to the NA protein of influenza A and B viruses, inhibiting the release of influenza virions from the infected host cell. Oseltamivir is administered orally and is available in a suspension for young children and in capsules for older children. Oseltamivir is approved for treatment (twice daily) of influenza in children 14 days of age and older and chemoprophylaxis (once daily) of influenza in those 1 year of age and older in the United States. The CDC and WHO recommend oseltamivir for treatment of influenza in children of all ages. Small case series have reported use of oseltamivir treatment or chemoprophylaxis in premature infants and neonates with few serious adverse events.* After oral absorption, the metabolite oseltamivir carboxylate is metabolized by renal clearance. The most common adverse events in children are nausea and vomiting.[742] Rare episodes of neuropsychiatric events have been reported in Japanese adolescents, although it is unclear whether such events are associated with influenza virus infection or oseltamivir.[62,89,664,698] Resistance to oseltamivir can emerge frequently in seasonal influenza A (H1N1) viruses (no longer circulating) and infrequently in influenza A (H3N2) viruses during or after treatment in children.[370,645,663] Resistance is more likely to emerge in severely immunosuppressed patients with prolonged viral replication.[223,309] The most common mutation associated with oseltamivir resistance is the H275Y mutation in viral neuraminidase.[310] Reduced oseltamivir susceptibility and lower clinical effectiveness has been noted in some influenza B virus infections.[271,654]

RCTs have reported efficacy of oral oseltamivir versus placebo in treatment of uncomplicated influenza in pediatric outpatients when treatment is started within 48

*References 2, 450, 467, 539, 639, 686.

hours after illness onset. Systematic reviews and meta-analyses of RCTs have reported approximately a 21-hour to 1.5-day reduction in the duration of influenza illness of early oseltamivir treatment.[133,332,727] Early oral oseltamivir treatment of influenza in children can reduce the risk for otitis media.[284,742] A meta-analysis of 11 RCTs of early oseltamivir treatment in adolescents and adults with uncomplicated influenza found a 37 percent reduced risk for lower respiratory tract complications requiring antibiotic treatment in patients with confirmed influenza versus placebo.[287] As of 2012, no data from placebo-controlled RCTs were available for oseltamivir treatment of hospitalized pediatric influenza patients. Observational studies of oseltamivir treatment of hospitalized children with influenza have reported a reduction in duration of hospitalization and lower risk for severe complications requiring mechanical ventilation.[130,182] Critically ill patients with respiratory failure can be administered oseltamivir enterically via a nasogastric or orogastric tube. Very limited data suggest that oseltamivir may be absorbed in such patients.[230,744] Observational studies of oseltamivir effectiveness in influenza patients of all ages and in adults, including pregnant women, have consistently reported a significant reduction in the risk for critical illness and death when oseltamivir treatment was started up to 5 days after seasonal or pandemic influenza illness onset, with the greatest clinical benefit observed when oseltamivir treatment was started within 48 hours after illness onset.[305,428,623,780,788] This suggests that although early treatment is most effective, later initiation of oseltamivir treatment, including for hospitalized influenza patients, can still have some clinical benefit.

The CDC recommends empirical oseltamivir treatment of hospitalized patients of all ages with suspected influenza as soon as possible without waiting for influenza testing results.[202] Empirical NA inhibitor treatment (with oseltamivir) is also recommended as soon as possible for pediatric outpatients of all ages with suspected influenza who are considered at high risk for influenza complications (i.e., children younger than 5 years; those of any age with chronic pulmonary, cardiac, metabolic, hematologic, renal, neurologic, neuromuscular, or neurodevelopmental conditions; and those receiving chronic aspirin therapy). Although children younger than 5 years of age are considered at high risk for influenza complications, the highest risk is for children younger than 2 years, and clinical judgment is advised when treating children 2 through 4 years of age with suspected influenza who are otherwise healthy.[202] Children 5 years of age and older with suspected influenza who are not considered to be in a high-risk group can be treated based on clinical judgment if oseltamivir treatment can be started within 48 hours after illness onset.[202] Pediatric patients with highly suspected or documented oseltamivir-resistant influenza virus infection should receive inhaled zanamivir treatment, if treatment is indicated and not contraindicated by age, underlying chronic pulmonary disease, or intubation. Limited data suggest that severe illness with oseltamivir-resistant influenza virus infection (influenza A[H1N1]pdm09 virus) may respond to treatment with the investigational drug intravenous zanamivir. For hospitalized children in whom inhaled zanamivir is contraindicated, the investigational drug intravenous zanamivir should be considered.

One RCT of oseltamivir chemoprophylaxis for 12 weeks in liver, kidney, or liver and kidney transplant or allogeneic hematopoietic stem cell transplant recipients 1 year of age or older, primarily adults, reported a reduction of 80 to 90 percent versus placebo in laboratory-confirmed influenza virus infection.[320] Oseltamivir was well-tolerated, and one oseltamivir-resistant 2009 H1N1 virus infection was identified in each of the treatment and placebo groups. Although routine antiviral chemoprophylaxis is not recommended, oseltamivir chemoprophylaxis of some schoolchildren during the 2009 H1N1 pandemic had variable compliance because of adverse events.[371,648,709,723]

Zanamivir is a powdered drug and is administered using an inhaler device. Zanamivir is approved for treatment (two inhalations twice daily) of influenza in children 7 years of age and older and for chemoprophylaxis (two inhalations once daily) of influenza in those 5 years of age and older in the United States. In some countries, inhaled zanamivir is approved for treatment of influenza in children 5 years of age and older. In younger children, inhaled zanamivir cannot be effectively delivered using the proprietary disk inhaler device. RCTs of early inhaled zanamivir treatment in children and adolescents with uncomplicated influenza have shown a reduction of 1.25 to 2.5 days in illness duration in contrast to placebo.[278,446,727] Although nausea and vomiting can occur with inhaled zanamivir, no significant differences in adverse events versus placebo were reported in RCTs.[278,446] Postmarketing studies have reported bronchospasm in some inhaled zanamivir recipients, and zanamivir is contraindicated in patients with underlying chronic pulmonary disease.[362] The inhaled zanamivir formulation should not be administered to intubated patients because it may precipitate and clog the ventilator tubing, resulting in death.[362,642] As of 2012, no data from placebo-controlled RCTs were available for inhaled zanamivir treatment of hospitalized pediatric influenza patients. Emergence of influenza viruses resistant to zanamivir during or after treatment is rare and much less frequent than the frequency of oseltamivir resistance during and after oseltamivir treatment of influenza.[663] Laninamivir is a long-acting inhaled NA inhibitor that is chemically similar to zanamivir and approved for treatment of influenza in Japan. Treatment with inhaled laninamivir consists of a single dose, which is equivalent to a 5-day twice daily treatment course of oral oseltamivir or inhaled zanamivir.[349,655]

No intravenous NA inhibitors are approved in the United States, but some have been approved in a few other countries. Investigational intravenous NA inhibitors in development include intravenous zanamivir and intravenous peramivir. Intravenous zanamivir is available for compassionate use through an emergency investigational new drug approval application to U.S. Food and Drug Administration (FDA) and the manufacturer. In the United States, intravenous zanamivir and intravenous peramivir may be available through clinical trials through an investigational new drug approval application. Intravenous NA inhibitors have been administered to hospitalized seasonal and pandemic influenza patients,

especially those who are critically ill, although data for pediatric patients are limited.* The WHO suggests that intravenous zanamivir be considered where available for treatment of highly suspected or demonstrated oseltamivir-resistant influenza virus infection.[171,223,760] Dosing with intravenous NA inhibitors may need adjustment for weight, reduced creatinine clearance, hemodialysis, hemofiltration, or extracorporeal membrane oxygenation (ECMO).

Corticosteroids

Corticosteroids are not indicated for the routine clinical management of uncomplicated or complicated influenza, although most data are derived from studies in adults. Observational studies have reported no clinical benefit of corticosteroids in treatment of influenza. Early administration of glucocorticoids was associated with increased risk for severe illness and mortality with influenza A(H1N1)pdm09 virus infection in China.[264] In critically ill influenza patients with respiratory failure or ARDS, administration of corticosteroids did not improve survival, increased the risk for hospital-acquired pneumonia, and increased the risk for death in some studies.† Corticosteroid therapy is associated with prolonged influenza viral shedding.[66,228,401] Although administered for a potential immunomodulatory effect, high-dose corticosteroids can result in immunosuppression with increased risk for severe complications, including nosocomial bacterial or fungal infections. However, low-dose corticosteroids may be indicted for septic shock or refractory, vasopressor-dependent shock with suspected adrenal insufficiency. Corticosteroids have been associated with either no benefit in reducing disease progression or increased mortality in patients with highly pathogenic avian influenza A (H5N1) virus infection.[290,412] Pulsed high-dose corticosteroids have been administered to patients with influenza-associated encephalitis, although clinical trial data are unavailable.[352] Corticosteroids should not be withheld from influenza patients who are receiving chronic corticosteroid therapy, such as for chronic pulmonary, rheumatologic, or other disorders.

Salicylates

Salicylates (aspirin) and salicylate-containing medications should not be administered to children with suspected or confirmed influenza because of the risk for Reye syndrome.[509,640] The incidence of Reye syndrome has declined markedly in the United States since the 1980s, when the association between aspirin ingestion and influenza resulting in Reye syndrome was recognized.[37,261] Children receiving short-term or long-term aspirin therapy for underlying disease (e.g., Kawasaki disease, cerebrovascular ischemia, or congenital cardiac disease) should receive annual influenza vaccination; if influenza is suspected, early antiviral treatment should be administered, with close monitoring for the potential development of Reye syndrome.

*References 118, 288, 429, 636, 653, 789.
†References 78, 164, 368, 369, 456, 770.

Investigational Therapies

Investigational agents in development include monotherapy with an oral polymerase inhibitor (favipiravir T-705), inhaled sialidase (Fludase DAS101), long-acting inhaled NA inhibitor (laninamivir), intravenous NA inhibitors (peramivir or zanamivir), and triple combination antivirals (amantadine, oseltamivir, or ribavirin).[64,275,304] Optimal dosing and duration of antiviral treatment for hospitalized and critically ill children of all ages with influenza has not been determined, and more data are especially needed for treatment of neonates, premature infants, and children younger than 1 year of age. The most appropriate clinical and virologic outcomes in trials of antiviral treatment of hospitalized influenza patients are unclear.[319] Observational studies of immunomodulator therapy of influenza have shown mixed results, and no clinical trial data are available.[325,388,711] Immunotherapy with convalescent plasma, hyperimmune plasma, monoclonal or polyclonal antibodies with broad-spectrum neutralizing activity are in development.*

Supportive Care

Although data from randomized clinical trials are generally lacking, early administration of supplemental oxygen is recommended for hypoxia in influenza patients with acute respiratory distress. Close monitoring of respiratory status is critical because influenza patients with pneumonia or pneumonitis can progress very rapidly to respiratory failure. Pulse oximetry monitoring should be done in the emergency department or clinic at presentation, and continued on inpatient wards. Fluid management is important because some influenza patients may be severely dehydrated, leading to hypovolemic shock. Vasopressors may be required for septic shock and DIC, with or without secondary invasive bacterial infection. Low-dose glucocorticoids may be indicated in refractory septic shock with suspected adrenal insufficiency. Hypovolemic shock may result from vascular permeability (i.e., capillary leakage) caused by cytokine dysregulation (cytokine storm) triggered by influenza virus infection of the respiratory tract. Cardiogenic shock may occur from rare cases of myocarditis or exacerbation of underlying congenital cardiac disease, requiring management by pediatric cardiologists and intensivists.

Management of Complications: Special Issues

Suspected Invasive Bacterial Infection

If secondary invasive bacterial infection is suspected (e.g., bacteremia, sepsis, or pneumonia), empirical intravenous antibiotic treatment should be administered immediately to cover the most likely bacterial pathogens based on underlying host conditions (e.g., cystic fibrosis, asplenia, or immunosuppression), local microbiologic data, carriage data, and antibiotic susceptibilities. In the United

*References 135, 176, 308, 382, 434, 741, 750, 795.

States, the most frequently identified invasive bacterial infections associated with influenza in children without underlying chronic conditions are *S. aureus*, *S. pneumoniae*, and *S. pyogenes*. Initial empirical antibiotic coverage should treat MRSA.[418] In other countries, initial treatment for *H. influenzae* also should be considered in patients with secondary bacterial pneumonia. If meningitis is suspected, empirical antibiotics should also cover *N. meningitidis*.

Renal Insufficiency or Failure

In critically ill children with influenza, renal insufficiency or renal failure may develop, requiring close monitoring of fluid and electrolyte status. Patients requiring renal replacement therapy with hemofiltration or dialysis may need adjustments to antiviral and antibiotic dosing.

Respiratory Failure and Acute Respiratory Distress Syndrome

Although noninvasive ventilation may be attempted initially, influenza viral pneumonia, especially resulting from influenza A(H1N1)pdm09 virus infection, is likely to progress to require invasive mechanical ventilation.[35,193] Progression to ARDS with refractory hypoxemia should be managed according to evidence-based guidelines. During the 2009 H1N1 pandemic, many ARDS cases were treated with a range of rescue strategies (e.g., prone positioning, high-frequency oscillatory ventilation, inhaled nitric oxide, and ECMO).[35,193,570] Without controlled trials, the relative benefits of such rescue strategies for influenza-associated ARDS are unknown. Corticosteroids and other immunomodulators have not been shown to be of clinical benefit in most observational studies of ARDS with influenza. Plasma exchange was used for the purpose of cytokine reduction in a small number of critically ill pediatric patients with 2009 H1N1 virus infection and ARDS.[544] However, the effectiveness of this investigational therapy is unknown.

Encephalopathy and Encephalitis

Currently, no data from RCTs are available for treatment of IAE or encephalitis. Progression from mild, uncomplicated influenza illness to altered mental status with or without seizures may occur rapidly or within 1 to 4 days after illness onset.[352,482] In fulminant cases, progression to increased intracranial pressure (ICP), uncal herniation, and brain death can occur within 1 to 3 days after initial onset of fever.[353] Clinical management is largely supportive, including anticonvulsant therapy for status epilepticus, and intensive care unit management of possible increased ICP. A range of uncontrolled therapies have been administered to children with influenza-associated encephalopathy or encephalitis in Japan, including antivirals, intravenous pulse methylprednisolone, hypothermia induction intravenous immunoglobulin, plasmaphoresis, edaravone, cyclosporine, antithrombin III, and other treatments, but the relative effectiveness of these interventions is unknown.[352,353,482,680] Most Japanese pediatric patients with influenza-associated encephalitis, including acute necrotizing encephalitis, are administered pulse methylprednisolone as soon as possible.[352] Early initiation of antiviral treatment with NA inhibitor drugs is recommended, although the effectiveness of antiviral treatment in patients with neurologic complications is unknown. IAE may be accompanied by viral pneumonia (including occult pneumonia).

Infection Control

Infection control for prevention of nosocomial influenza is a fundamental part of clinical management in all health care settings, including chronic care facilities.* All health care personnel should receive annual influenza vaccination. For the 2011 to 2012 season, one U.S. survey reported that influenza vaccination coverage of health care personnel was 66.9 percent.[105] Patients with suspected or confirmed influenza should be isolated in single-patient rooms or cohorted with other influenza patients if single-room isolation is not possible. Standard, contact, and droplet precautions (i.e., gown, gloves, and surgical mask) are recommended for health care personnel providing care for patients with seasonal influenza and should be changed between patients. For aerosol-generating procedures, use of a fit-tested N95 respirator or equivalent is recommended.[114,756] Antiviral treatment of ill patients and postexposure antiviral chemoprophylaxis of exposed patients and health care personnel may be implemented, along with other prevention and control measures during nosocomial influenza outbreaks.[268,612] For patients with suspected or confirmed novel influenza A virus infection (i.e., zoonotic influenza resulting from infection with avian or swine influenza A viruses), a higher level of respiratory protection and use of eye protection may be indicated for routine clinical care if available, especially for patients with highly pathogenic avian influenza A (H7) or (H5) virus infections.[1] Avian influenza A (H7) viruses appear to have ocular tropism, and eye protection with goggles is recommended when providing care for patients with low-pathogenic or highly pathogenic avian influenza A (H7) viruses (e.g., H7N2, H7N3, and H7N7).[42] For patients with suspected or confirmed highly pathogenic avian influenza A (H7) or (H5) virus infections, especially severely ill patients, health care personnel should wear gowns, gloves, goggles, N95 respirator, or equivalent protection.[759] Close follow-up of contacts exposed within 2 m of a case of highly pathogenic avian influenza A (H7) or (H5) virus infection, including health care personnel, is recommended for at least 7 days after the last known exposure.[107] Severely ill patients with highly pathogenic avian influenza A (H5N1) virus infection can be isolated in a negative-pressure room, if available.[108,756] For all influenza patients, family members and visitors should be restricted, and caregivers should be equipped with the same level of personal protective equipment as indicated for health care personnel.

Outbreaks

In addition to nosocomial outbreaks, seasonal and pandemic influenza outbreaks affecting children can occur in

*References 80, 112, 192, 256, 268, 398, 449, 486, 591, 594, 612.

almost any setting. Outbreaks of influenza occur frequently among schoolchildren, resulting in high absenteeism.* Influenza outbreaks can occur in childcare centers.[372] Outbreaks in pediatric long-term care facilities can result in severe complications and deaths in children with disabilities.[112] During the summer months, influenza outbreaks among children attending camps can occur.[170,580] Cruise ship outbreaks of influenza can occur any time of the year and affect a wide age range.[705,730] Prevention and control of influenza outbreaks require a high index of suspicion for influenza and active surveillance for influenza-like illness and acute respiratory illness among children, adult caregivers, and visitors; influenza testing to confirm the outbreak; prompt implementation of infection control measures (i.e., isolation of ill persons and quarantine and surveillance of exposed); pharmaceutical (i.e., antiviral treatment of ill persons, antiviral chemoprophylaxis of exposed persons, and influenza vaccination of unvaccinated persons) and nonpharmaceutical measures (e.g., use of appropriate personal protective equipment); and close collaboration with public health personnel.

Influenza Vaccines

Vaccine Strain Selection

The WHO conducts biannual meetings to select the antigenic composition of the seasonal influenza vaccine virus strains for the Northern (February) and Southern (September) Hemispheres.[17,587] These decisions are based on antigenic characterization data on recently circulating influenza A and B virus strains worldwide produced by WHO Collaborating Centers, the availability of egg-grown virus isolates to serve as vaccine seed strains, growth properties, and other factors.[17,374] Vaccine manufacturers may substitute antigenically equivalent strains for those recommended by the WHO because of growth properties. Individual countries make the final vaccine strain decisions for licensed influenza vaccines. In the United States, influenza vaccine strain selection recommendations are made by the FDA's Vaccines and Related Biological Products Advisory Committee each February for the coming fall and winter influenza season. Vaccine strain selection is done in advance of the typical influenza season in temperate climate countries because of the long lead time needed for vaccine production using traditional methods of virus growth in embryonated hen's eggs. The process from influenza vaccine production to vaccine availability takes approximately 5 to 8 months for egg-grown vaccine viruses, and less time for tissue cell culture–derived vaccine viruses.[475,551]

Pediatric Influenza Vaccines

Most seasonal influenza vaccines produced worldwide to date are split virion or subunit, unadjuvanted, trivalent inactivated influenza vaccine (ITIV3). They contain two subtypes of type A viruses (H1N1 and H3N2) and at least one type B virus antigen. These are produced by growing a virus derived from an egg-adapted influenza virus (e.g., PR8 for type A and B/Lee for type B) that is genetically reassorted with two genes from the seasonal vaccine virus (HA and NA). Each of the three or more reassortant vaccine seed viruses are individually inoculated and grown in the allantoic sac of embryonated eggs, through essentially the same process that has been used for many decades. The viruses harvested from the allantoic fluid are inactivated, purified, disrupted into split virions or subunits, and then combined to produce vaccine containing two influenza A virus antigens (an H1N1 and an H3N2) and one influenza B virus antigen (or two influenza B virus antigens for quadrivalent vaccines). Each dose of vaccine contains a minimum of 15 μg of hemagglutinin for each of the three or four components and is given by intramuscular injection. In the United States, quadrivalent inactivated and quadrivalent live attenuated influenza vaccines are approved for use in children.

Inactivated influenza vaccine (IIV) is available as "preservative-free," containing zero or very low thimerosal levels, in prefilled syringes or small-volume vials, and in thimerosal-containing multidose vials. Whole virus vaccines are associated with greater reactogenicity versus split virion vaccines and are no longer available in the United States. It is impossible to become infected with influenza viruses from receipt of inactivated influenza vaccine. As of July 2013, quadrivalent inactivated influenza vaccine (IIV4) containing two influenza A and two influenza B virus antigens from two manufacturers was approved for use in children. An inactivated vaccine from tissue cell culture–derived viruses was approved in Europe in 2007 and for adults in the United States in 2012. Vaccines produced using tissue cell culture–derived viruses can be manufactured more rapidly than vaccine produced using egg-grown viruses. Advantages include higher purity yield of virus antigens, more reliable production, and avoidance of ovalbumin—eliminating the potential for severe egg-related allergic reactions in persons with egg allergies. However, use of tissue culture raises the issue of potential contamination with adventitious agents, including known and novel viruses that may not have a cytopathic effect, and the need to conduct a range of testing strategies to assess the presence of such adventitious agents.

Use of adjuvants enhances immunogenicity and antibody response, especially in young infants and for influenza B antigens, allows for use of lower vaccine antigen concentration (dose-sparing), and promotes a broader cross-protective immune response.[183,718] Induction of broader cross-immunity may provide an advantage during seasonal influenza epidemics in which significant antigenic drift occurs (i.e., mismatch between vaccine strains and circulating strains). As of 2013, no adjuvanted influenza vaccines were approved in the United States, but an adjuvanted inactivated trivalent influenza vaccine has been available in some European, Latin American, and Asian countries. Vaccines containing oil and water adjuvants, MF59 or ASO3, are available from different manufacturers.

Live attenuated influenza vaccine (LAIV) is produced from genetic reassortment using separate influenza type A and B "master" strains that were serially passaged in eggs and attenuated at lower temperatures

*References 86, 88, 233, 235, 236, 406, 471, 778, 794.

("cold-adapted") and the HA and NA of seasonal vaccine viruses and grown in the allantoic sac of embryonated eggs.[439] The three or four vaccine strain components are then combined into trivalent (LAIV) or quadrivalent (LAIV4) vaccines. LAIV viruses replicate only at temperatures typical of the nasal passages, which are lower than the internal body temperature in the lower respiratory tract. Each dose of LAIV contains a minimum of 15 μg of HA of each of the strain components and is administered as an intranasal spray. Replication of the live attenuated viruses in the nasopharynx induces local mucosal IgA immunity and a systemic immune response (i.e., subtype-specific neutralizing antibodies). LAIV viruses can replicate and be detected in nasal specimens for several days to longer periods (i.e., 7 to 21 days),[61,448] but secondary transmission is rare. Therefore, testing of nasal specimens collected within a few days or longer after LAIV administration may be positive for influenza viruses of LAIV origin.[7] Transmission of LAIV viruses is rare, but theoretically possible. A quadrivalent LAIV (LAIV4) was approved in 2012 for healthy children 2 years of age and older to be available during the 2013-2014 influenza season in the United States and will replace trivalent LAIV. This quadrivalent vaccine contains two influenza A (an H1N1 and an H3N2) and two influenza B (a B/Victoria lineage, and a B/Yamagata lineage) viruses. An advantage of the quadrivalent vaccine is that virus antigens from both currently circulating lineages of influenza B viruses (i.e., B/Victoria-like and B/Yamagata-like) are included versus only one B virus lineage antigen for trivalent vaccines.[573]

Monovalent pandemic influenza vaccine was used in many countries during the 2009 H1N1 pandemic, including unadjuvanted and adjuvanted inactivated vaccines (both tissue cell culture-derived and egg-grown) and LAIV. In the United States, inactivated unadjuvanted egg-derived monovalent 2009 H1N1 split virion vaccine became available after the second pandemic wave had peaked in the fall of 2009. The same 2009 H1N1 virus antigen used in the monovalent pandemic vaccine was included in the trivalent seasonal influenza vaccines for the Southern and Northern Hemispheres beginning in 2010.

Vaccine Immunogenicity

Correlates of humoral immune protection for inactivated influenza vaccination include measurement of serum HAI antibodies or, less commonly, neutralizing antibodies. In general, an HAI titer of 40 or greater is considered to correlate with immunity. However, in one study, much higher HAI antibody titers were found to correlate with immune protection after TIV (IIV3).[57] Inactivated influenza vaccines are less immunogenic in very young infants in contrast to those 6 months of age and older, especially when maternally derived antibody is present, and are more immunogenic for influenza A versus B viruses in young children.[179,255,724] Immunogenicity in inactivated influenza vaccine studies can be assessed by the postvaccination geometric mean antibody titer rise relative to the prevaccination geometric mean antibody titer, which can provide a meaningful estimation of the robustness of response to vaccination. In persons with prevaccination antibodies, immunogenicity is generally assessed by the mean fold increase in HAI antibody titer (i.e., postvaccination divided by prevaccination titer), or seroresponse rate (i.e., proportion of vaccinated individuals with a fourfold or greater rise in antibody titer from prevaccination to postvaccination sampling), where seronegative individuals achieve an antibody titer of 40 or greater. The proportion of vaccinated individuals that achieve an HAI antibody titer of 40 or greater is often used to evaluate vaccine immunogenicity for regulatory purposes.

Two doses of inactivated influenza vaccine administered the same season 1 month or longer apart are needed for priming in previously unvaccinated children 6 months through 8 years of age. Two doses given 1 month apart are immunogenic in infants 6 to 23 months of age. When vaccine strains are changed, children 6 months to 9 years of age who received only one dose in their first year of vaccination and one dose the following year are less likely to achieve protective antibody titers in contrast to children who received two doses their first vaccination year.[181,497,725] When vaccine strains are unchanged in consecutive seasons, priming children 6 to 23 months of age with one dose in the spring and one dose in the fall has similar immunogenicity to two doses given in the fall,[180] but estimated vaccine effectiveness in one study was higher for the fall two-dose regimen.[9] Immunogenicity, safety, and reactogenicity of a mammalian cell culture–derived IIV in children 9 to 17 years of age were comparable to those of egg-grown TIV (IIV3).[715]

Vaccination with LAIV may produce a minimal HAI antibody response and the correlates of cellular immunity are not well-established. LAIV induces $CD4^+$, $CD8^+$, and T helper cell responses.[295] Data for LAIV also suggest that two doses 1 month apart are also needed for priming in young children who have not received any influenza vaccination. Immunogenicity and safety of quadrivalent LAIV was shown to be noninferior to trivalent LAIV.[59]

Vaccine Safety

Influenza vaccination is generally safe in children, including in infants 6 months of age and older, with few serious adverse events in randomized controlled trials or postmarketing surveillance studies.* The most common adverse events reported are self-limited local injection site pain and induration. In a comprehensive review of the evidence on influenza vaccine–associated adverse effects, a U.S. Institute of Medicine committee concluded that the evidence was insufficient to assess the risk for most serious influenza vaccine–associated adverse events, but that anaphylaxis may occur, TIV (IIV3) in children and adults is not associated with exacerbation of asthma or reactive airway disease and is not associated with Bell palsy.[647] Potential serious vaccine-associated adverse events are reported to the Vaccine Adverse Event Reporting System.[483] No increases were noted in neuropsychological outcomes among children at 7 to 10 years who had been exposed to thimerosal-containing vaccines,

*References 212, 231, 241, 262, 466, 483, 583, 629.

including some influenza vaccines.[673] The risk for Guillain-Barre syndrome (GBS) after influenza vaccination was very low, but a slight increase was noted in recipients of monovalent 2009 H1N1 vaccine,[157,244,747] but not in 2009 H1N1 antigen-containing seasonal influenza vaccine.[681] No increased risk for GBS from adujvanted 2009 H1N1 vaccine was found in a European case-control study.[165] Although GBS was observed after the 1976 and 1977 swine influenza vaccination in the United Sates at a case frequency of 1/100,000 vaccinated persons, GBS has rarely been observed in children associated with influenza vaccination.[597,598] Influenza vaccination has been shown to have a protective association for GBS.[807] Notably, influenza virus infection has been associated with GBS in adults.[624]

Egg-derived influenza vaccines may contain a very small amount of ovalbumin and are thought to be safe to administer to children who have experienced only hives after egg exposure, with monitoring for at least 30 minutes after vaccination by a health care provider who is familiar with and can treat manifestations of egg allergy.[111] Children with more serious allergic reactions to egg should be referred to a physician with expertise in management of allergic conditions for risk assessment before vaccine administration.[111]

In 2010, an increase in febrile reactions and febrile seizures in children younger than 5 years of age was first noted in Western Australia after receipt of an Australian TIV (IIV3).[240] Studies indicated that this was associated with two IIV3s, Fluvax and Fluvax junior, and febrile reactions in young children were also noted in New Zealand with these vaccines.[22,355,552] In the United States, use of IIV3 produced from the same manufacturer was not recommended since 2010 for use in children younger than 9 years of age.[98] No evidence of any increase in febrile seizure events related to seasonal influenza vaccination of young children before 2010 was identified in the United States.[75] A slight increase in febrile seizures was noted with first-dose IIV3 in children 6 to 59 months of age in the United States, but the risk for febrile seizures was substantially higher when TIV was coadministered with PCV13.[687] Given the benefit of these vaccines to prevent morbidity resulting from influenza and pneumococcal disease, no changes in recommendations were made for IIV3 or PCV13. A previous study had reported an increase in local injection site and systemic reactions with IIV3 and pneumococcal conjugate vaccines versus either vaccine plus placebo.[328]

During the 2000 and 2001 influenza season in Canada, cases of self-limited bilateral red eyes, facial edema, or respiratory symptoms (i.e., oculorespiratory syndrome) were observed, beginning within 24 hours of receipt of one manufacturer's IIV3.[626] This syndrome has not been observed in the United States.

Vaccination with one manufacturer's adjuvanted (ASO3) monovalent inactivated 2009 H1N1 vaccine was associated with narcolepsy in children and adolescents in Finland and Sweden, but not with adjuvanted or unadjuvanted 2009 H1N1 antigen-containing vaccines in other countries.[185,186,513,542] Narcolepsy seasonality has been observed with an increase in cases during the 2009 H1N1 pandemic.[263]

LAIV is well tolerated, with few serious adverse events in young and older children and slight increases in nasal discharge or congestion, vomiting, abdominal pain, fever, and muscle aches generally associated with the first dose in young children.[47,555] A community-based nonrandomized study in healthy children 18 months to 18 years of age found no increased risk for medically attended post-vaccination events in 42 days after vaccination or for asthma in 14 days after vaccination.[554] One RCT reported an increase in reactive airway disease in children younger than 36 months of age.[51] One study of LAIV versus TIV (IIV3) in children 2 to 5 years of age with mild to moderate asthma or prior wheezing from two multinational RCTs did not find any increases in wheezing, lower respiratory tract illness, or hospitalization in LAIV recipients, but increases were noted in upper respiratory tract symptoms and irritability.[12]

Influenza Vaccine Efficacy

Results of influenza vaccine efficacy studies can vary depending on the defined outcomes (e.g., virus culture–confirmed or RT-PCR confirmed influenza, otitis media, or clinically diagnosed influenza illness). An updated systematic review concluded that influenza vaccines are efficacious in prevention of influenza in children 2 years of age and older but that limited evidence was available for those younger than 2 years.[333] Most RCTs have shown lower efficacy of TIV in children younger than 2 years of age and higher efficacy of IIV3 in children 2 years or older. Another review reported that the efficacy of influenza vaccination against laboratory-confirmed influenza was 67 percent and against otitis media 51 percent.[451] Both LAIV and IIV3 have efficacy versus placebo, respectively, and have similar efficacy to each other against influenza-associated otitis media.[60]

A systematic review and meta-analysis of LAIV reported pooled efficacy of 83 percent (95% confidence interval, 69%-91%) against laboratory-confirmed influenza in children 6 months to 7 years of age.[528] In the pivotal RCT, LAIV was 93 percent efficacious versus placebo against culture-confirmed influenza in children 15 to 71 months of age.[46] LAIV was more efficacious than TIV (IIV3) in RCTs among children 6 to 59 months of age[44] and those 6 to 71 months against culture-confirmed influenza.[13,24] One RCT in children 6 to less than 36 months found very high efficacy of LAIV against the influenza viruses studied, with higher efficacy against culture-confirmed influenza A versus influenza B virus infections.[716] One RCT in children 6 to less than 36 months of age attending daycare found an efficacy of 85 to 89 percent versus placebo.[716] In one open-label RCT of LAIV versus IIV3 among asthmatic children 6 to 17 years of age, LAIV showed greater efficacy than IIV3 against virus culture–confirmed influenza and was well tolerated, with no evidence of increases in adverse pulmonary outcomes.[204] LAIV was substantially more efficacious versus placebo in children 15 to 71 months of age against culture-confirmed influenza.[43] In one study, LAIV provided protection against an antigenically drifted H3N2 virus strain not included in the vaccine.[45]

Influenza Vaccine Effectiveness

The effectiveness of seasonal influenza vaccines varies from year to year and depends especially on the antigenic match between vaccine strains and the predominant circulating influenza A and B virus strains.[39,279] Ideally, multiyear assessments of influenza vaccine effectiveness are more informative than studies based on a single season and should use an outcome of laboratory-confirmed influenza virus infection. The current gold standard outcome for such studies is RT-PCR–confirmed influenza versus a RT-PCR influenza "test negative" comparison group with influenza-like illness. A case-control study design has been used to assess influenza vaccine effectiveness for outpatients and hospitalized children. Young children who received only one dose of TIV (IIV3) in the first year of vaccination did not have reductions in outpatient visits for influenza-like illness or laboratory-confirmed influenza.[9,578,616] IIV3 had effectiveness of 56 percent in fully vaccinated children 6 to 59 months of age who were not previously vaccinated, but was not effective in children who had received only one dose.[638] One study reported vaccine effectiveness of 42 to 69 percent in Japanese children 6 months to 6 years of age over a 6-year period for positive rapid influenza test outcomes.[348] One case-control study reported a substantially lower risk for medically attended laboratory-confirmed influenza in vaccinated versus unvaccinated U.S. children 6 to 59 months of age during 1999 to 2007.[338] During seasons with a suboptimal antigenic match between vaccine strains and circulating influenza virus strains, IIV3 effectiveness was poor in children 6 to 59 months of age.[658] In seasons in which influenza vaccines were well matched to all three circulating influenza virus strains, vaccine effectiveness of 69 percent (95% CI, 56% to 77%) was found in children 6 months to 8 years of age using a case-control test-negative study design with RT-PCR–confirmed influenza as the outcome.[684] Unadjuvanted, inactivated monovalent 2009 H1N1 effectiveness in the United States was 62 percent (95% CI, 25%-81%) in all ages and 32 percent in children 6 months to 9 years of age.[246]

Economic Studies of Influenza Vaccination

Multiple factors influence the economic impact (e.g., cost-effectiveness, cost-benefit, and cost-utility analyses) of pediatric influenza vaccination, including the cost of vaccine (e.g., inactivated versus LAIV); vaccine administration costs; the costs associated with vaccine adverse events; the clinical illness attack rate, which is highest in young children (variable from year-to-year); whether a child is at higher risk for severe illness complications (i.e., has underlying comorbidities or is very young); vaccine effectiveness (varies from year-to-year depending on the match between circulating strains and vaccine strains); and the direct medical care costs associated with treatments.[433,501,506,564,629] Although death is a rare outcome of influenza among children, the value placed on a death or a severe complication of influenza in a child can be an important factor in overall economic costs of influenza, and high costs are associated with hospitalization, although the greatest direct cost is from outpatient clinic and emergency medical care for young children.[189,260,357,562] Most economic studies have found that influenza vaccination of healthy children is cost-saving or cost-effective, with higher benefit in children who are at increased risk for influenza complications.* Few economics studies have considered the indirect benefits of pediatric influenza vaccination to prevent influenza in secondary household members, school and social contacts, and the community, suggesting that economic benefits may be underestimated.[234,237,313,420,553]

Maternal Vaccination and Benefits

One RCT and multiple observational studies have reported the clinical benefit of maternal influenza vaccination on birth outcomes and prevention of influenza in infants up to 6 months of age who were born to women vaccinated during pregnancy.[50,175,644,792] Infants born to women who did not receive influenza vaccination during pregnancy had lower birth weight and higher likelihood of prematurity than babies born to mothers who had received influenza vaccination during pregnancy.[521,644] The clinical benefit observed in infants born to vaccinated mothers was correlated with higher levels of maternally derived HAI antibodies in cord blood and with longer duration of detection of HAI antibodies in infant sera.[175,643] Although the kinetics of passively transferred antibodies is not well understood, existing data suggest a drop off of HAI antibodies from 2 to 3 months to 6 months of life. The role of breast-feeding and contribution of IgA antibodies is unclear. In the United States, influenza vaccination coverage of pregnant women during the 2011 and 2012 season was 47 percent in one survey, with 36.5 percent vaccinated during pregnancy.[106] Overall, available data suggest that in addition to prevention of influenza in pregnant women, maternal influenza vaccination also results in improved birth outcomes and reduction of influenza in infants to 6 months of life. This suggests that one important strategy to prevent influenza in infants younger than 6 months of age who are contraindicated for influenza vaccination is to administer influenza vaccine to pregnant women during influenza season.

Prevention of Influenza in the Intrapartum Setting and Breast-Feeding

A pregnant woman symptomatic with influenza in the intrapartum period poses a risk for influenza virus transmission to the newborn infant through direct and close exposure. Although data are lacking on the risk and best prevention measures, guidance has been developed to reduce transmission while balancing the importance of preserving the maternal–infant bond and breast-feeding.[571] These emphasize adhering to recommended infection control precautions (i.e., isolation and droplet precautions) for influenza prevention in the labor, delivery, recovery, and postpartum settings and consideration of temporary separation of the newborn infant on

*References 229, 289, 433, 452, 595, 596.

a case-by-case basis until at least 24 hours after fever resolution. A healthy caregiver can feed the infant the mother's expressed breast milk or formula. If co-location is unavoidable, the infant should be kept at least 2 m away from the mother. Limited data suggest that breast milk from a woman treated with oseltamivir will contain low levels of oseltamivir and oseltamivir carboxylate (metabolite) which do not pose a risk for the infant.[245,665,740] The infant should be monitored closely for signs and symptoms of influenza. At discharge, the family should be educated about prevention of influenza virus transmission to the infant at home. Influenza vaccination of all household members 6 months of age and older and caregivers of an infant younger than 6 months ("cocooning strategy") is recommended.[571]

Vaccine Production and Availability

Once the WHO issues vaccine strain selection recommendations for the upcoming hemisphere's influenza season, manufacturers commence with production of influenza vaccines. For egg-grown vaccine viruses, the manufacturing process takes approximately 5 to 8 months until vaccine is available, depending on growth properties of vaccine virus strains, quantity produced, vaccine lot approvals, and other factors. During the 2004 and 2005 season in the United States, one of the two manufacturers experienced problems with bacterial contamination and did not provide vaccine; therefore 50 percent of the projected vaccine supply for that season was lost and high-risk groups were prioritized to receive available vaccine.[95,96] In the United States, influenza vaccine generally becomes available in the late summer and early fall, with production and distribution continuing into the late fall and winter. In the United States, influenza vaccine is given an expiration date of June 30 each year. In Northern Hemisphere temperate climate countries, the optimal period for influenza vaccination is during the fall, starting in September to December. In Southern Hemisphere temperate climate countries, the vaccination period is typically March to June. Influenza vaccination can continue in a community for longer periods if local influenza activity has not peaked. In tropical and subtropical regions with different influenza virus seasonality, including countries with bimodal peaks in influenza activity or with year-round activity, decisions about when to start influenza vaccination and which vaccine formulation to use should be based on analyses of multiple years of local surveillance data. A wide range of influenza vaccines are available from various manufacturers, with different age approvals.

Vaccine Recommendations

Annual influenza vaccine recommendations in the United States are issued by the Advisory Committee on Immunization Practices (ACIP).[100] The United States is the only country that has recommended annual influenza vaccination of all persons 6 months of age and older since 2010.[99,111,203] Before 2010, influenza vaccination of children was recommended for children 6 to 59 months of age and for children 6 months of age and older with certain chronic medical conditions. In other countries, priority is placed on vaccination of high-risk groups such as elderly persons, and children and adults with certain chronic underlying conditions. In 2012, the WHO Strategic Advisory Group of Experts recommended that all member states should prioritize annual influenza vaccination of pregnant women and children 6 to 59 months of age and especially those 6 to 23 months.[763] This recommendation was based on available global influenza burden data.

Influenza vaccine coverage among children in the United States has increased in recent years as recommendation for pediatric age groups expanded, with higher coverage in younger children, but remains suboptimal.[603] For the 2011 and 2012 season, estimated overall influenza vaccination coverage in children 6 months to 17 years of age was 51.5 percent, including 74.6 percent for those 6 to 23 months of age, 63.3 percent for those 2 to 4 years, 54.2 percent for those 5 to 12 years, and 33.7 percent for those 13 to 17 years.[103] School-based vaccination is a strategy to increase influenza vaccine coverage among schoolchildren,[142,174,307] which may have direct and indirect benefits.[237,249,559]

Influenza vaccine is not approved for infants younger than 6 months of age because of poor immunogenicity. Standard-dose preparations of inactivated vaccine contain 7.5 μg HA antigen per vaccine strain (for <36 months) or 15 μg of HA antigen (for ≥36 months) per vaccine strain. For young children 6 months through 8 years of age who have not previously received influenza vaccine, two doses of inactivated seasonal influenza vaccine administered 1 month apart are recommended. For otherwise vaccine-naïve healthy children 2 years through 8 years of age, two doses of seasonal LAIV 1 month apart are recommended. For children younger than 9 years of age, if they have previously received two doses in 1 year, a single dose of seasonal influenza vaccine is recommended each year. Any child 9 years of age or older, even if not previously vaccinated, requires a single dose. Children aged 6 months to less than 3 years require 0.25 mL per dose (7.5 μg of H antigen content per vaccine strain), and those 3 years of age and older require 0.5 mL per dose (15 μg of HA antigen content per vaccine strain). Children with a history of severe egg allergies (anaphylaxis) should be evaluated by an allergist before egg-derived influenza vaccine is administered. All influenza vaccines approved for persons 6 months of age and older in the United States are unadjuvanted vaccines.[99,111]

Vaccines in Development

Influenza vaccines approved for adults in the United States include intradermal inactivated vaccine for persons 18 to 64 years of age (approved in 2011), tissue cell culture–derived trivalent inactivated vaccine (ccIIV3) for persons 18 years of age and older (approved in 2012), a recombinant DNA hemagglutinin vaccine (RIV) using an insect cell line and a baculovirus vector for persons 18 to 49 years of age (approved in 2013), a live attenuated vaccine for healthy nonpregnant persons up to 49 years of age (approved in 2003), and a high-dose antigen vaccine for persons 65 years of age and older (approved

in 2009). Adjuvanted inactivated influenza vaccines are approved for persons 65 years of age and older in some countries. In addition to adjuvanted vaccine and tissue cell culture–produced vaccines,[551,717] novel vaccines in development include virosomal vaccines, virus-like particles, insect cell culture–derived vaccines, recombinant plant cell–derived vaccines, adenovirus-vectored vaccines, DNA vaccines, and universal vaccines that target conserved epitopes such as the M2 extra domain or HA stalk domain.*

Nonpharmaceutical Interventions

Nonpharmaceutical interventions can be an adjunct to pharmaceutical interventions in the prevention of influenza or can be the only preventive measures if influenza vaccine and antiviral medications are not available, for both seasonal influenza epidemics and pandemic influenza.[136,137,621,651] Use of facemasks to prevent secondary household influenza virus transmission is of variable efficacy in a small number of studies, with benefit when mask-wearing is started by exposed family members within 36 hours after an index case's illness onset.[136,137,621,651] Soap and water and alcohol-based hand rub preparations are effective at reducing influenza A viruses on human hands.[243] Overall, the effectiveness of hand hygiene to reduce influenza virus transmission likely varies by the setting, context, and compliance.[731] One RCT in Egyptian schoolchildren found that a twice-daily hand washing intervention resulted in a reduction of rapid influenza diagnostic test confirmed influenza by 50 percent in contrast to controls.[661] A cluster-randomized hand washing study in U.S. elementary schoolchildren did not find a reduction in overall RT-PCR–confirmed influenza, but reported a reduction of total absences by 26 percent and in laboratory-confirmed influenza A by 52 percent versus the nonintervention group.[641] Hand washing has been demonstrated to have efficacy in reducing acute lower respiratory tract infections in young Pakistani children.[432] Other nonpharmaceutical community interventions include school closures, but must be implemented early for greatest effectiveness.[90,173,769]

Pandemic Preparedness Countermeasures

Pharmaceutical (medical) countermeasures for pandemic influenza preparedness include antiviral drugs and vaccines (and adjuvants) against novel influenza A viruses. Many countries have stockpiled oral formulations of the NA inhibitor antiviral drug oseltamivir, and some have also stockpiled inhaled zanamivir. Such antiviral drug stockpiles need to be maintained and replenished, depending on shelf-life.[543] Some countries have stockpiled vaccines against different H5N1 viruses. Because of the dynamic nature of H5N1 viruses circulating among poultry and other birds worldwide, such H5N1 vaccine candidates need updating.[762] Similarly, candidate vaccines against different avian (H7, H9) and variant (H3N2v) viruses are under development.[761,762] Technologies to rapidly expand vaccine production capacity (e.g., tissue cell culture) in the event of an influenza pandemic are also in development.

*References 183, 339, 343, 392, 398, 414, 424, 614, 728.

Update on H7N9 at Time of Printing

On March 31, 2013, the first human infections with a novel avian-origin influenza A (H7N9) virus were reported in China to the WHO, raising global public health concerns.[221,703] These infections were identified in critically ill adults with severe pneumonia in eastern China. As of July 2013, 132 confirmed cases of H7N9 virus infection with 43 deaths had been identified in 10 different areas of China, and one additional case was identified in Taiwan. Most cases experienced febrile respiratory illness that evolved into a rapidly progressive pneumonia and respiratory failure, and some patients experienced multiorgan dysfunction. Most human infections were linked to recent poultry exposures, including at live animal markets. Although some clusters of illness were identified in family contacts of confirmed cases in which limited, nonsustained human-to-human transmission of H7N9 virus likely occurred, no evidence was found of sustained human-to-human transmission.[411] The latest information on human infections with H7N9 virus from the WHO is available at http://www.who.int/influenza/human_animal_interface/influenza_h7n9/en/index.html.

Latest Information on Influenza

For updated information on influenza surveillance and circulating influenza viruses, novel influenza A viruses, and recommendations for prevention and control of influenza, including guidance on the use of influenza vaccines and approved antivirals, clinicians are referred to sources such as the CDC (http://www.cdc.gov/flu/professionals/index.htm) and WHO (http://www.who.int/influenza/en/index.html).

NEW REFERENCES SINCE THE SIXTH EDITION

2. Acosta EP, Jester P, Gal P, et al. Oseltamivir dosing for influenza infection in premature neonates. *J Infect Dis* 2010;**202**:563–6.
3. Adisasmito W, Chan PK, Lee N, et al. Effectiveness of antiviral treatment in human influenza A(H5N1) infections: analysis of a global patient registry. *J Infect Dis* 2010;**202**:1154–60.
4. Agrati C, Gioia C, Lalle E, et al. Association of profoundly impaired immune competence in H1N1v-infected patients with a severe or fatal clinical course. *J Infect Dis* 2010;**202**:681–9.
8. Allard R, Couillard M, Pilon P, et al. Invasive bacterial infections following influenza: a time-series analysis in Montreal, Canada, 1996-2008. *Influenza Other Respi Viruses* 2012;**6**:268–75.
11. Alymova IV, Green AM, van de Velde N, et al. Immunopathogenic and antibacterial effects of H3N2 influenza A virus PB1-F2 map to amino acid residues 62, 75, 79, and 82. *J Virol* 2011;**85**:12324–33.
12. Ambrose CS, Dubovsky F, Yi T, et al. The safety and efficacy of live attenuated influenza vaccine in young children with asthma or prior wheezing. *Eur J Clin Microbiol Infect Dis* 2012;**31**:2549–57.
13. Ambrose CS, Levin MJ, Belshe RB. The relative efficacy of trivalent live attenuated and inactivated influenza vaccines in children and adults. *Influenza Other Respi Viruses* 2011;**5**:67–75.
16. Ampofo K, Herbener A, Blaschke AJ, et al. Association of 2009 pandemic influenza A (H1N1) infection and increased

hospitalization with parapneumonic empyema in children in Utah. *Pediatr Infect Dis J* 2010;**29**:905–9.
17. Ampofo WK, Baylor N, Cobey S, et al. Improving influenza vaccine virus selection: report of a WHO informal consultation held at WHO headquarters, Geneva, Switzerland, June 14-16, 2010. *Influenza Other Respi Viruses* 2012;**6**:142–52, e141–5.
18. Anderson TC, Bromfield CR, Crawford PC, et al. Serological evidence of H3N8 canine influenza-like virus circulation in USA dogs prior to 2004. *Vet J* 2012;**191**:312–16.
19. Anhlan D, Grundmann N, Makalowski W, et al. Origin of the 1918 pandemic H1N1 influenza A virus as studied by codon usage patterns and phylogenetic analysis. *RNA* 2011;**17**:64–73.
20. Anthony SJ, St Leger JA, Pugliares K, et al. Emergence of fatal avian influenza in New England harbor seals. *MBio* 2012;**3**: e00166–00112.
22. Armstrong PK, Dowse GK, Effler PV, et al. Epidemiological study of severe febrile reactions in young children in Western Australia caused by a 2010 trivalent inactivated influenza vaccine. *BMJ Open* 2011;**1**:e000016.
23. Arzey GG, Kirkland PD, Arzey KE, et al. Influenza virus A (H10N7) in chickens and poultry abattoir workers, Australia. *Emerg Infect Dis* 2012;**18**:814–16.
25. Atkins CY, Patel A, Taylor TH Jr, et al. Estimating effect of antiviral drug use during pandemic (H1N1) 2009 outbreak, United States. *Emerg Infect Dis* 2011;**17**:1591–8.
26. Azziz-Baumgartner E, Alamgir AS, Rahman M, et al. Incidence of influenza-like illness and severe acute respiratory infection during three influenza seasons in Bangladesh, 2008-2010. *Bull World Health Organ* 2012;**90**:12–19.
27. Azziz Baumgartner E, Dao CN, Nasreen S, et al. Seasonality, timing, and climate drivers of influenza activity worldwide. *J Infect Dis* 2012;**206**:838–46.
35. Bautista E, Chotpitayasunondh T, Gao Z, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med* 2010;**362**:1708–19.
29. Bahl J, Nelson MI, Chan KH, et al. Temporally structured metapopulation dynamics and persistence of influenza A H3N2 virus in humans. *Proc Natl Acad Sci U S A* 2011;**108**:19359–64.
30. Baker MG, Thornley CN, Mills C, et al. Transmission of pandemic A/H1N1 2009 influenza on passenger aircraft: retrospective cohort study. *BMJ* 2010;**340**:c2424.
31. Balish A, Garten R, Klimov A, et al. Analytical detection of influenza A(H3N2)v and other A variant viruses from the USA by rapid influenza diagnostic tests. *Influenza Other Respi Viruses* 2012, doi: 10.1111/irv.12017 (Epub ahead of print).
32. Baltagi SA, Shoykhet M, Felmet K, et al. Neurological sequelae of 2009 influenza A (H1N1) in children: a case series observed during a pandemic. *Pediatr Crit Care Med* 2010;**11**:179–84.
33. Barakat A, Ihazmad H, Benkaroum S, et al. Influenza surveillance among outpatients and inpatients in Morocco, 1996-2009. *PLoS One* 2011;**6**:e24579.
40. Belser JA, Jayaraman A, Raman R, et al. Effect of D222G mutation in the hemagglutinin protein on receptor binding, pathogenesis and transmissibility of the 2009 pandemic H1N1 influenza virus. *PLoS One* 2011;**6**:e25091.
41. Belser JA, Maines TR, Tumpey TM, et al. Influenza A virus transmission: contributing factors and clinical implications. *Expert Rev Mol Med* 2010;**12**:e39.
42. Belser JA, Zeng H, Katz JM, et al. Ocular tropism of influenza A viruses: identification of H7 subtype-specific host responses in human respiratory and ocular cells. *J Virol* 2011;**85**:10117–25.
49. Bender JM, Ampofo K, Gesteland P, et al. Influenza virus infection in infants less than three months of age. *Pediatr Infect Dis J* 2010;**29**:6–9.
50. Benowitz I, Esposito DB, Gracey KD, et al. Influenza vaccine given to pregnant women reduces hospitalization due to influenza in their infants. *Clin Infect Dis* 2010;**51**:1355–61.
52. Bermejo-Martin JF, Martin-Loeches I, Rello J, et al. Host adaptive immunity deficiency in severe pandemic influenza. *Crit Care* 2010;**14**:R167.
53. Bermejo-Martin JF, Ortiz de Lejarazu R, Pumarola T, et al. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. *Crit Care* 2009;**13**:R201.
57. Black S, Nicolay U, Vesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. *Pediatr Infect Dis J* 2011;**30**:1081–5.
58. Blanton L, Peacock G, Cox C, et al. Neurologic disorders among pediatric deaths associated with the 2009 pandemic influenza. *Pediatrics* 2012;**130**:390–6.
59. Block SL, Falloon J, Hirschfield JA, et al. Immunogenicity and safety of a quadrivalent live attenuated influenza vaccine in children. *Pediatr Infect Dis J* 2012;**31**:745–51.
60. Block SL, Heikkinen T, Toback SL, et al. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. *Pediatr Infect Dis J* 2011;**30**: 203–7.
68. Brammer L, Blanton L, Epperson S, et al. Surveillance for influenza during the 2009 influenza A (H1N1) pandemic-United States, April 2009-March 2010. *Clin Infect Dis* 2011;**52**(Suppl. 1): S27–35.
75. Broder KR, Martin DB, Vellozzi C. In the heat of a signal: responding to a vaccine safety signal for febrile seizures after 2010-11 influenza vaccine in young children, United States. *Vaccine* 2012;**30**:2032–4.
76. Brooks WA, Alamgir AS, Sultana R, et al. Avian influenza virus A (H5N1), detected through routine surveillance, in child, Bangladesh. *Emerg Infect Dis* 2009;**15**:1311–13.
77. Brooks WA, Goswami D, Rahman M, et al. Influenza is a major contributor to childhood pneumonia in a tropical developing country. *Pediatr Infect Dis J* 2010;**29**:216–21.
78. Brun-Buisson C, Richard JC, Mercat A, et al. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2011;**183**:1200–6.
80. Buchbinder N, Dumesnil C, Pinquier D, et al. Pandemic A/H1N1/2009 influenza in a paediatric haematology and oncology unit: successful management of a sudden outbreak. *J Hosp Infect* 2011;**79**:155–60.
82. Bundy DG, Strouse JJ, Casella JF, et al. Burden of influenza-related hospitalizations among children with sickle cell disease. *Pediatrics* 2010;**125**:234–43.
83. Buss BF, Shinde VM, Safranek TJ, et al. Pediatric influenza-associated myositis: Nebraska, 2001-2007. *Influenza Other Respi Viruses* 2009;**3**:277–85.
86. Carrillo-Santisteve P, Renard-Dubois S, Cheron G, et al. 2009 pandemic influenza A(H1N1) outbreak in a complex of schools in Paris, France, June 2009. *Euro Surveill* 2010;**15**.
89. Casscells SW, Granger E, Kress AM, et al. The association between oseltamivir use and adverse neuropsychiatric outcomes among TRICARE beneficiaries, ages 1 through 21 years diagnosed with influenza. *Int J Adolesc Med Health* 2009;**21**:79–89.
90. Cauchemez S, Ferguson NM, Wachtel C, et al. Closure of schools during an influenza pandemic. *Lancet Infect Dis* 2009;**9**: 473–81.
97. Centers for Disease Control and Prevention. Estimates of deaths associated with seasonal influenza: United States, 1976-2007. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1057–62.
98. Centers for Disease Control and Prevention. Update: recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL seasonal influenza vaccine (Afluria) in the United States during 2010-11. *MMWR Morb Mortal Wkly Rep* 2010;**59**:989–92.
99. Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1128–32.
100. Centers for Disease Control and Prevention. *Advisory Committee on Immunization Practices*. 2012. Available at: http://www.cdc.gov/vaccines/acip/index.html.
101. Centers for Disease Control and Prevention. Antibodies cross-reactive to influenza A (H3N2) variant virus and impact of 2010-11 seasonal influenza vaccine on cross-reactive antibodies: United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**:237–41.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdel-Ghafar AN, Chotpitayasunondh T, Gao Z, et al. Update on avian influenza A (H5N1) virus infection in humans. *N Engl J Med* 2008;**358**:261–73.
2. Acosta EP, Jester P, Gal P, et al. Oseltamivir dosing for influenza infection in premature neonates. *J Infect Dis* 2010;**202**:563–6.
3. Adisasmito W, Chan PK, Lee N, et al. Effectiveness of antiviral treatment in human influenza A(H5N1) infections: analysis of a Global Patient Registry. *J Infect Dis* 2010;**202**:1154–60.
4. Agrati C, Gioia C, Lalle E, et al. Association of profoundly impaired immune competence in H1N1v-infected patients with a severe or fatal clinical course. *J Infect Dis* 2010;**202**:681–9.
5. Alaska Department of Health and Social Services. *Alaska influenza surveillance report*. Juneau, n.d. Available at: http://www.epi.alaska.gov/id/influenza/influenza.jsp.
6. Albright FS, Orlando P, Pavia AT, et al. Evidence for a heritable predisposition to death due to influenza. *J Infect Dis* 2008;**197**:18–24.
7. Ali T, Scott N, Kallas W, et al. Detection of influenza antigen with rapid antibody-based tests after intranasal influenza vaccination (FluMist). *Clin Infect Dis* 2004;**38**:760–2.
8. Allard R, Couillard M, Pilon P, et al. Invasive bacterial infections following influenza: a time-series analysis in Montreal, Canada, 1996-2008. *Influenza Other Respi Viruses* 2012;**6**:268–75.
9. Allison MA, Daley MF, Crane LA, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. *J Pediatr* 2006;**149**:755–62.
10. Alonso WJ, Viboud C, Simonsen L, et al. Seasonality of influenza in Brazil: a traveling wave from the Amazon to the subtropics. *Am J Epidemiol* 2007;**165**:1434–42.
11. Alymova IV, Green AM, van de Velde N, et al. Immunopathogenic and antibacterial effects of H3N2 influenza A virus PB1-F2 map to amino acid residues 62, 75, 79, and 82. *J Virol* 2011;**85**:12324–33.
12. Ambrose CS, Dubovsky F, Yi T, et al. The safety and efficacy of live attenuated influenza vaccine in young children with asthma or prior wheezing. *Eur J Clin Microbiol Infect Dis* 2012;**31**:2549–57.
13. Ambrose CS, Levin MJ, Belshe RB. The relative efficacy of trivalent live attenuated and inactivated influenza vaccines in children and adults. *Influenza Other Respi Viruses* 2011;**5**:67–75.
14. Amin R, Ford-Jones E, Richardson SE, et al. Acute childhood encephalitis and encephalopathy associated with influenza: a prospective 11-year review. *Pediatr Infect Dis J* 2008;**27**:390–5.
15. Ampofo K, Gesteland PH, Bender J, et al. Epidemiology, complications, and cost of hospitalization in children with laboratory-confirmed influenza infection. *Pediatrics* 2006;**118**:2409–17.
16. Ampofo K, Herbener A, Blaschke AJ, et al. Association of 2009 pandemic influenza A (H1N1) infection and increased hospitalization with parapneumonic empyema in children in Utah. *Pediatr Infect Dis J* 2010;**29**:905–9.
17. Ampofo WK, Baylor N, Cobey S, et al. Improving influenza vaccine virus selection: report of a WHO informal consultation held at WHO headquarters, Geneva, Switzerland, 14-16 June 2010. *Influenza Other Respi Viruses* 2012;**6**:142–52, e141–5.
18. Anderson TC, Bromfield CR, Crawford PC, et al. Serological evidence of H3N8 canine influenza-like virus circulation in USA dogs prior to 2004. *Vet J* 2012;**191**:312–16.
19. Anhlan D, Grundmann N, Makalowski W, et al. Origin of the 1918 pandemic H1N1 influenza A virus as studied by codon usage patterns and phylogenetic analysis. *RNA* 2011;**17**:64–73.
20. Anthony SJ, St Leger JA, Pugliares K, et al. Emergence of fatal avian influenza in New England harbor seals. *MBio* 2012;**3**:e00166–00112.
21. Apisarnthanarak A, Kitphati R, Thongphubeth K, et al. Atypical avian influenza (H5N1). *Emerg Infect Dis* 2004;**10**:1321–4.
22. Armstrong PK, Dowse GK, Effler PV, et al. Epidemiological study of severe febrile reactions in young children in Western Australia caused by a 2010 trivalent inactivated influenza vaccine. *BMJ Open* 2011;**1**:e000016.
23. Arzey GG, Kirkland PD, Arzey KE, et al. Influenza virus A (H10N7) in chickens and poultry abattoir workers, Australia. *Emerg Infect Dis* 2012;**18**:814–16.
24. Ashkenazi S, Vertruyen A, Aristegui J, et al. Superior relative efficacy of live attenuated influenza vaccine compared with inactivated influenza vaccine in young children with recurrent respiratory tract infections. *Pediatr Infect Dis J* 2006;**25**:870–9.
25. Atkins CY, Patel A, Taylor TH Jr, et al. Estimating effect of antiviral drug use during pandemic (H1N1) 2009 outbreak, United States. *Emerg Infect Dis* 2011;**17**:1591–8.
26. Azziz-Baumgartner E, Alamgir AS, Rahman M, et al. Incidence of influenza-like illness and severe acute respiratory infection during three influenza seasons in Bangladesh, 2008-2010. *Bull World Health Organ* 2012;**90**:12–19.
27. Azziz Baumgartner E, Dao CN, Nasreen S, et al. Seasonality, timing, and climate drivers of influenza activity worldwide. *J Infect Dis* 2012;**206**:838–46.
28. Badger GF, Dingle JH, Feller AE, et al. A study of illness in a group of Cleveland families. II. Incidence of the common respiratory diseases. *Am J Hyg* 1953;**58**:31–40.
29. Bahl J, Nelson MI, Chan KH, et al. Temporally structured metapopulation dynamics and persistence of influenza A H3N2 virus in humans. *Proc Natl Acad Sci U S A* 2011;**108**:19359–64.
30. Baker MG, Thornley CN, Mills C, et al. Transmission of pandemic A/H1N1 2009 influenza on passenger aircraft: retrospective cohort study. *BMJ* 2010;**340**:c2424.
31. Balish A, Garten R, Klimov A, et al. Analytical detection of influenza A(H3N2)v and other A variant viruses from the USA by rapid influenza diagnostic tests. *Influenza Other Respi Viruses* 2012, doi: 10.1111/irv.12017 (Epub ahead of print).
32. Baltagi SA, Shoykhet M, Felmet K, et al. Neurological sequelae of 2009 influenza A (H1N1) in children: a case series observed during a pandemic. *Pediatr Crit Care Med* 2010;**11**:179–84.
33. Barakat A, Ihazmad H, Benkaroum S, et al. Influenza surveillance among outpatients and inpatients in Morocco, 1996-2009. *PLoS One* 2011;**6**:e24579.
34. Barry JM. *The great influenza*. New York: Viking Penguin Group; 2004.
35. Bautista E, Chotpitayasunondh T, Gao Z, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. *N Engl J Med* 2010;**362**:1708–19.
36. Beigel JH, Farrar J, Han AM, et al. Avian influenza A (H5N1) infection in humans. *N Engl J Med* 2005;**353**:1374–85.
37. Belay ED, Bresee JS, Holman RC, et al. Reye's syndrome in the United States from 1981 through 1997. *N Engl J Med* 1999;**340**:1377–82.
38. Bell ML, Buchhalter JR. Influenza A-associated stroke in a 4-year-old male. *Pediatr Neurol* 2004;**31**:56–8.
39. Belongia EA, Kieke BA, Donahue JG, et al. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004-2005 season to the 2006-2007 season. *J Infect Dis* 2009;**199**:159–67.
40. Belser JA, Jayaraman A, Raman R, et al. Effect of D222G mutation in the hemagglutinin protein on receptor binding, pathogenesis and transmissibility of the 2009 pandemic H1N1 influenza virus. *PLoS One* 2011;**6**:e25091.
41. Belser JA, Maines TR, Tumpey TM, et al. Influenza A virus transmission: contributing factors and clinical implications. *Expert Rev Mol Med* 2010;**12**:e39.
42. Belser JA, Zeng H, Katz JM, et al. Ocular tropism of influenza A viruses: identification of H7 subtype-specific host responses in human respiratory and ocular cells. *J Virol* 2011;**85**:10117–25.
43. Belshe RB, Gruber WC. Safety, efficacy and effectiveness of cold-adapted, live, attenuated, trivalent, intranasal influenza vaccine in adults and children. *Philos Trans R Soc Lond B Biol Sci* 2001;**356**:1947–51.
44. Belshe RB, Edwards KM, Vesikari T, et al. Live attenuated versus inactivated influenza vaccine in infants and young children. *N Engl J Med* 2007;**356**:685–96.
45. Belshe RB, Gruber WC, Mendelman PM, et al. Efficacy of vaccination with live attenuated, cold-adapted, trivalent, intranasal influenza virus vaccine against a variant (A/Sydney) not contained in the vaccine. *J Pediatr* 2000;**136**:168–75.
46. Belshe RB, Mendelman PM, Treanor J, et al. The efficacy of live attenuated, cold-adapted, trivalent, intranasal influenzavirus vaccine in children. *N Engl J Med* 1998;**338**:1405–12.
47. Belshe RB, Nichol KL, Black SB, et al. Safety, efficacy, and effectiveness of live, attenuated, cold-adapted influenza vaccine in an indicated population aged 5-49 years. *Clin Infect Dis* 2004;**39**:920–7.

48. Belshe RB, Smith MH, Hall CB, et al. Genetic basis of resistance to rimantadine emerging during treatment of influenza virus infection. *J Virol* 1988;**62**:1508–12.
49. Bender JM, Ampofo K, Gesteland P, et al. Influenza virus infection in infants less than three months of age. *Pediatr Infect Dis J* 2010; **29**:6–9.
50. Benowitz I, Esposito DB, Gracey KD, et al. Influenza vaccine given to pregnant women reduces hospitalization due to influenza in their infants. *Clin Infect Dis* 2010;**51**:1355–61.
51. Bergen R, Black S, Shinefield H, et al. Safety of cold-adapted live attenuated influenza vaccine in a large cohort of children and adolescents. *Pediatr Infect Dis J* 2004;**23**:138–44.
52. Bermejo-Martin JF, Martin-Loeches I, Rello J, et al. Host adaptive immunity deficiency in severe pandemic influenza. *Crit Care* 2010;**14**:R167.
53. Bermejo-Martin JF, Ortiz de Lejarazu R, Pumarola T, et al. Th1 and Th17 hypercytokinemia as early host response signature in severe pandemic influenza. *Crit Care* 2009;**13**:R201.
54. Bernstein T, Brilli R, Jacobs B. Is bacterial tracheitis changing? A 14-month experience in a pediatric intensive care unit. *Clin Infect Dis* 1998;**27**:458–62.
55. Bharaj P, Sullender WM, Kabra SK, et al. Respiratory viral infections detected by multiplex PCR among pediatric patients with lower respiratory tract infections seen at an urban hospital in Delhi from 2005 to 2007. *Virol J* 2009;**6**:89.
56. Bhat N, Wright JG, Broder KR, et al. Influenza-associated deaths among children in the United States, 2003-2004. *N Engl J Med* 2005;**353**:2559–67.
57. Black S, Nicolay U, Vesikari T, et al. Hemagglutination inhibition antibody titers as a correlate of protection for inactivated influenza vaccines in children. *Pediatr Infect Dis J* 2011;**30**:1081–5.
58. Blanton L, Peacock G, Cox C, et al. Neurologic disorders among pediatric deaths associated with the 2009 pandemic influenza. *Pediatrics* 2012;**130**:390–6.
59. Block SL, Falloon J, Hirschfield JA, et al. Immunogenicity and safety of a quadrivalent live attenuated influenza vaccine in children. *Pediatr Infect Dis J* 2012;**31**:745–51.
60. Block SL, Heikkinen T, Toback SL, et al. The efficacy of live attenuated influenza vaccine against influenza-associated acute otitis media in children. *Pediatr Infect Dis J* 2011;**30**:203–7.
61. Block SL, Yogev R, Hayden FG, et al. Shedding and immunogenicity of live attenuated influenza vaccine virus in subjects 5-49 years of age. *Vaccine* 2008;**26**:4940–6.
62. Blumentals WA, Song X. The safety of oseltamivir in patients with influenza: analysis of healthcare claims data from six influenza seasons. *MedGenMed* 2007;**9**:23.
63. Blyth CC, Iredell JR, Dwyer DE. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;**361**:2493.
64. Boltz DA, Aldridge JR Jr, Webster RG, et al. Drugs in development for influenza. *Drugs* 2010;**70**:1349–62.
65. Bonner AB, Monroe KW, Talley LI, et al. Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial. *Pediatrics* 2003;**112**: 363–7.
66. Boudreault AA, Xie H, Leisenring W, et al. Impact of corticosteroid treatment and antiviral therapy on clinical outcomes in hematopoietic cell transplant patients infected with influenza virus. *Biol Blood Marrow Transplant* 2011;**17**:979–86.
67. Bourgeois FT, Valim C, Wei JC, et al. Influenza and other respiratory virus-related emergency department visits among young children. *Pediatrics* 2006;**118**:e1–8.
68. Brammer L, Blanton L, Epperson S, et al. Surveillance for influenza during the 2009 influenza A (H1N1) pandemic: United States, April 2009-March 2010. *Clin Infect Dis* 2011;**52**(Suppl. 1): S27–35.
69. Brankston G, Gitterman L, Hirji Z, et al. Transmission of influenza A in human beings. *Lancet Infect Dis* 2007;**7**:257–65.
70. Bridges CB, Kuehnert MJ, Hall CB. Transmission of influenza: implications for control in health care settings. *Clin Infect Dis* 2003;**37**:1094–101.
71. Bright RA, Medina MJ, Xu X, et al. Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. *Lancet* 2005;**366**: 1175–81.
72. Bright RA, Shay DK, Shu B, et al. Adamantane resistance among influenza A viruses isolated early during the 2005-2006 influenza season in the United States. *JAMA* 2006;**295**:891–4.
73. Brocklebank JT, Court SD, McQuillin J, et al. Influenza-A infection in children. *Lancet* 1972;**2**:497–500.
74. Brockwell-Staats C, Webster RG, Webby RJ. Diversity of influenza viruses in swine and the emergence of a novel human pandemic influenza A (H1N1). *Influenza Other Respi Viruses* 2009;**3**: 207–13.
75. Broder KR, Martin DB, Vellozzi C. In the heat of a signal: responding to a vaccine safety signal for febrile seizures after 2010-11 influenza vaccine in young children, United States. *Vaccine* 2012;**30**:2032–4.
76. Brooks WA, Alamgir AS, Sultana R, et al. Avian influenza virus A (H5N1), detected through routine surveillance, in child, Bangladesh. *Emerg Infect Dis* 2009;**15**:1311–13.
77. Brooks WA, Goswami D, Rahman M, et al. Influenza is a major contributor to childhood pneumonia in a tropical developing country. *Pediatr Infect Dis J* 2010;**29**:216–21.
78. Brun-Buisson C, Richard JC, Mercat A, et al. Early corticosteroids in severe influenza A/H1N1 pneumonia and acute respiratory distress syndrome. *Am J Respir Crit Care Med* 2011;**183**:1200–6.
79. Brundage JF. Interactions between influenza and bacterial respiratory pathogens: implications for pandemic preparedness. *Lancet Infect Dis* 2006;**6**:303–12.
80. Buchbinder N, Dumesnil C, Pinquier D, et al. Pandemic A/H1N1/2009 influenza in a paediatric haematology and oncology unit: successful management of a sudden outbreak. *J Hosp Infect* 2011;**79**:155–60.
81. Buchy P, Mardy S, Vong S, et al. Influenza A/H5N1 virus infection in humans in Cambodia. *J Clin Virol* 2007;**39**:164–8.
82. Bundy DG, Strouse JJ, Casella JF, et al. Burden of influenza-related hospitalizations among children with sickle cell disease. *Pediatrics* 2010;**125**:234–43.
83. Buss BF, Shinde VM, Safranek TJ, et al. Pediatric influenza-associated myositis: Nebraska, 2001-2007. *Influenza Other Respi Viruses* 2009;**3**:277–85.
84. Calvo C, Garcia-Garcia ML, Centeno M, et al. Influenza C virus infection in children, Spain. *Emerg Infect Dis* 2006;**12**: 1621–2.
85. Carey DE, Dunn FL, Robinson RQ, et al. Community-wide epidemic of Asian strain influenza; clinical and subclinical illnesses among school children. *J Am Med Assoc* 1958;**167**:1459–63.
86. Carrillo-Santisteve P, Renard-Dubois S, Cheron G, et al. 2009 pandemic influenza A(H1N1) outbreak in a complex of schools in Paris, France, June 2009. *Euro Surveill* 2010;**15**(25):19599.
87. Cartwright KA, Jones DM, Smith AJ, et al. Influenza A and meningococcal disease. *Lancet* 1991;**338**:554–7.
88. Cashman P, Massey P, Durrheim D, et al. Pneumonia cluster in a boarding school: implications for influenza control. *Commun Dis Intell* 2007;**31**:296–8.
89. Casscells SW, Granger E, Kress AM, et al. The association between oseltamivir use and adverse neuropsychiatric outcomes among TRICARE beneficiaries, ages 1 through 21 years diagnosed with influenza. *Int J Adolesc Med Health* 2009;**21**:79–89.
90. Cauchemez S, Ferguson NM, Wachtel C, et al. Closure of schools during an influenza pandemic. *Lancet Infect Dis* 2009;**9**: 473–81.
91. Centers for Disease Control and Prevention. *Flu activity and surveillance*. n.d. Available at: http://www.cdc.gov/flu/weekly/fluactivitysurv.htm.
92. Centers for Disease Control and Prevention. *Overview of influenza surveillance in the United States*. n.d. Available at: http://www.cdc.gov/flu/weekly/overview.htm.
93. Centers for Disease Control and Prevention. *Past weekly surveillance reports*. n.d. Available at: http://www.cdc.gov/flu/weekly/pastreports.htm.
94. Centers for Disease Control and Prevention. Outbreak of influenza-like illness in a tour group: Alaska. *MMWR Morb Mortal Wkly Rep* 1987;**36**:697–8, 704.
95. Centers for Disease Control and Prevention. Updated interim influenza vaccination recommendations: 2004-05 influenza season. *MMWR Morb Mortal Wkly Rep* 2004;**53**:1183–4.
96. Centers for Disease Control and Prevention. Tiered use of inactivated influenza vaccine in the event of a vaccine shortage. *MMWR Morb Mortal Wkly Rep* 2005;**54**:749–50.

97. Centers for Disease Control and Prevention. Estimates of deaths associated with seasonal influenza: United States, 1976-2007. *MMWR Morb Mortal Wkly Rep* 2010;**59**:1057–62.
98. Centers for Disease Control and Prevention. Update: recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL seasonal influenza vaccine (Afluria) in the United States during 2010-11. *MMWR Morb Mortal Wkly Rep* 2010;**59**:989–92.
99. Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1128–32.
100. Centers for Disease Control and Prevention. *Advisory Committee on Immunization Practices*. 2012. Available at: http://www.cdc.gov/vaccines/acip/index.html.
101. Centers for Disease Control and Prevention. Antibodies cross-reactive to influenza A (H3N2) variant virus and impact of 2010-11 seasonal influenza vaccine on cross-reactive antibodies: United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**:237–41.
102. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for influenza A (H3N2)v virus and updated case count: United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:619–21.
103. Centers for Diseases Control and Prevention. *Flu vaccination coverage, United States, 2011-12 Influenza Season*. 2012. Available at: http://www.cdc.gov/flu/professionals/vaccination/coverage_1112estimates.htm.
104. Centers for Disease Control and Prevention. Influenza A (H3N2) variant virus-related hospitalizations: Ohio, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:764–7.
105. Centers for Disease Control and Prevention. Influenza vaccination coverage among health-care personnel: 2011-12 influenza season, United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**:753–7.
106. Centers for Disease Control and Prevention. Influenza vaccination coverage among pregnant women: 2011-12 influenza season, United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**:758–63.
107. Centers for Disease Control and Prevention. *Interim guidance for follow-up of contacts of persons with suspected infection with highly pathogenic avian influenza A (H5N1) virus*. 2012. Available at: http://www.cdc.gov/flu/avianflu/guidance-followup.htm.
108. Centers for Disease Control and Prevention. *Interim guidance for laboratory testing of persons with suspected infection with highly pathogenic avian influenza A (H5N1) virus in the United States*. 2012. Available at: http://www.cdc.gov/flu/avianflu/guidance-labtesting.htm.
109. Centers for Disease Control and Prevention. Notes from the field: Highly pathogenic avian influenza A (H7N3) virus infection in two poultry workers: Jalisco, Mexico, July 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:726–7.
110. Centers for Disease Control and Prevention. Notes from the field: outbreak of influenza A (H3N2) virus among persons and swine at a county fair—Indiana, July 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:561.
111. Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2012-13 influenza season. *MMWR Morb Mortal Wkly Rep* 2012;**61**:613–18.
112. Centers for Disease Control and Prevention. Severe influenza among children and young adults with neurologic and neurodevelopmental conditions: Ohio, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**60**:1729–33.
113. Centers for Disease Control and Prevention. Update: influenza activity—United States and Worldwide, May 20–September 22, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:785–9.
114. Centers for Disease Control and Prevention. *Prevention strategies for seasonal influenza in healthcare settings*. 2013. Available at: http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm.
115. Cetinkaya M, Ozkan H, Celebi S, et al. Human 2009 influenza A (H1N1) virus infection in a premature infant born to an H1N1-infected mother: placental transmission? *Turk J Pediatr* 2011;**53**:441–4.
116. Chan J, Holmes A, Rabadan R. Network analysis of global influenza spread. *PLoS Comput Biol* 2010;**6**:e1001005.
117. Chan PK. Outbreak of avian influenza A(H5N1) virus infection in Hong Kong in 1997. *Clin Infect Dis* 2002;**34**(Suppl. 2):S58–64.
118. Chan-Tack KM, Gao A, Himaya AC, et al. Clinical Experience with intravenous zanamivir under an emergency investigational new drug program in the United States. *J Infect Dis* 2012;**207**:196–8.
119. Chartrand C, Leeflang MM, Minion J, et al. Accuracy of rapid influenza diagnostic tests: a meta-analysis. *Ann Intern Med* 2012;**156**:500–11.
120. Chen GL, Subbarao K. Attacking the flu: neutralizing antibodies may lead to 'universal' vaccine. *Nat Med* 2009;**15**:1251–2.
121. Chen LF, Dailey NJ, Rao AK, et al. Cluster of oseltamivir-resistant 2009 pandemic influenza A (H1N1) virus infections on a hospital ward among immunocompromised patients: North Carolina, 2009. *J Infect Dis* 2011;**203**:838–46.
122. Cheng VC, Chan JF, Wen X, et al. Infection of immunocompromised patients by avian H9N2 influenza A virus. *J Infect* 2011;**62**:394–9.
123. Chidlow GR, Laing IA, Harnett GB, et al. Respiratory viral pathogens associated with lower respiratory tract disease among young children in the highlands of Papua New Guinea. *J Clin Virol* 2012;**54**:235–9.
124. Chiu SS, Chan KH, So LY, et al. The population based socioeconomic burden of pediatric influenza-associated hospitalization in Hong Kong. *Vaccine* 2012;**30**:1895–900.
125. Chiu SS, Tse CY, Lau YL, et al. Influenza A infection is an important cause of febrile seizures. *Pediatrics* 2001;**108**:E63.
126. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis* 2008;**46**:815–23.
127. Chutinimitkul S, Herfst S, Steel J, et al. Virulence-associated substitution D222G in the hemagglutinin of 2009 pandemic influenza A(H1N1) virus affects receptor binding. *J Virol* 2010;**84**:11802–13.
128. Clarke SK, Heath RB, Sutton RN, et al. Serological studies with Asian strain of influenza A. *Lancet* 1958;**1**:814–18.
129. Coelho MC, Tsuchiya LR, Nogueira MB, et al. Impact of respiratory infections by influenza viruses A and B in pediatrics patients from Federal University of Parana, Brazil. *Braz J Infect Dis* 2007;**11**:220–3.
130. Coffin SE, Leckerman K, Keren R, et al. Oseltamivir shortens hospital stays of critically ill children hospitalized with seasonal influenza: a retrospective cohort study. *Pediatr Infect Dis J* 2011;**30**:962–6.
131. Coffin SE, Zaoutis TE, Rosenquist AB, et al. Incidence, complications, and risk factors for prolonged stay in children hospitalized with community-acquired influenza. *Pediatrics* 2007;**119**:740–8.
132. Colombatti R, Perrotta S, Masera N, et al. Lessons learned from the H1N1 pandemic: the need to improve systematic vaccination in sickle cell disease children: a multi center survey in Italy. *Vaccine* 2011;**29**:1126–8.
133. Cooper NJ, Sutton AJ, Abrams KR, et al. Effectiveness of neuraminidase inhibitors in treatment and prevention of influenza A and B: systematic review and meta-analyses of randomised controlled trials. *BMJ* 2003;**326**:1235.
134. Corey L, Rubin RJ, Thompson TR, et al. Influenza B-associated Reye's syndrome: incidence in Michigan and potential for prevention. *J Infect Dis* 1977;**135**:398–407.
135. Corti D, Voss J, Gamblin SJ, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science* 2011;**333**:850–6.
136. Cowling BJ, Chan KH, Fang VJ, et al. Facemasks and hand hygiene to prevent influenza transmission in households: a cluster randomized trial. *Ann Intern Med* 2009;**151**:437–46.
137. Cowling BJ, Zhou Y, Ip DK, et al. Face masks to prevent transmission of influenza virus: a systematic review. *Epidemiol Infect* 2010;**138**:449–56.
138. Cox CM, Blanton L, Dhara R, et al. 2009 Pandemic influenza A (H1N1) deaths among children: United States, 2009-2010. *Clin Infect Dis* 2011;**52**(Suppl. 1):S69–74.
139. Cox CM, D'Mello T, Perez A, et al. Increase in rates of hospitalization due to laboratory-confirmed influenza among children and adults during the 2009-10 influenza pandemic. *J Infect Dis* 2012;**206**:1350–8.

140. Crawford PC, Dubovi EJ, Castleman WL, et al. Transmission of equine influenza virus to dogs. *Science* 2005;**310**:482–5.
141. Crossley B, Hietala S, Hunt T, et al. Pandemic (H1N1) 2009 in captive cheetah. *Emerg Infect Dis* 2012;**18**:315–17.
142. Cummings GE, Ruff E, Guthrie SH, et al. Successful use of volunteers to conduct school-located mass influenza vaccination clinics. *Pediatrics* 2012;**129**(Suppl. 2):S88–95.
143. Dagan R, Hall CB. Influenza A virus infection imitating bacterial sepsis in early infancy. *Pediatr Infect Dis* 1984;**3**:218–21.
144. Daley AJ, Nallusamy R, Isaacs D. Comparison of influenza A and influenza B virus infection in hospitalized children. *J Paediatr Child Health* 2000;**36**:332–5.
145. Daly JM, MacRae S, Newton JR, et al. Equine influenza: a review of an unpredictable virus. *Vet J* 2011;**189**:7–14.
146. Damiani AM, Kalthoff D, Beer M, et al. Serological survey in dogs and cats for influenza A(H1N1)pdm09 in Germany. *Zoonoses Public Health* 2012;**59**:549–52.
147. Dao CN, Kamimoto L, Nowell M, et al. Adult hospitalizations for laboratory-positive influenza during the 2005-2006 through 2007-2008 seasons in the United States. *J Infect Dis* 2010;**202**: 881–8.
148. Dawood FS, Dong L, Liu F, et al. A pre-pandemic outbreak of triple-reassortant swine influenza virus infection among university students, South Dakota, 2008. *J Infect Dis* 2011;**204**:1165–71.
149. Dawood FS, Fiore A, Kamimoto L, et al. Burden of seasonal influenza hospitalization in children, United States, 2003 to 2008. *J Pediatr* 2010;**157**:808–14.
150. Dawood FS, Fiore A, Kamimoto L, et al. Influenza-associated pneumonia in children hospitalized with laboratory-confirmed influenza, 2003-2008. *Pediatr Infect Dis J* 2010;**29**:585–90.
151. Dawood FS, Iuliano AD, Reed C, et al. Estimated global mortality associated with the first 12 months of 2009 pandemic influenza A H1N1 virus circulation: a modelling study. *Lancet Infect Dis* 2012;**12**:687–95.
152. Dawood FS, Jain S, Finelli L, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;**360**:2605–15.
153. Dawood FS, Kamimoto L, D'Mello TA, et al. Children with asthma hospitalized with seasonal or pandemic influenza, 2003-2009. *Pediatrics* 2011;**128**:e27–32.
154. de Jong MD, Bach VC, Phan TQ, et al. Fatal avian influenza A (H5N1) in a child presenting with diarrhea followed by coma. *N Engl J Med* 2005;**352**:686–91.
155. de Jong MD, Simmons CP, Thanh TT, et al. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006;**12**:1203–7.
156. de la Tabla VO, Masia M, Antequera P, et al. Comparison of combined nose-throat swabs with nasopharyngeal aspirates for detection of pandemic influenza A/H1N1 2009 virus by real-time reverse transcriptase PCR. *J Clin Microbiol* 2010;**48**:3492–5.
157. De Wals P, Deceuninck G, Toth E, et al. Risk of Guillain-Barré syndrome following H1N1 influenza vaccination in Quebec. *JAMA* 2012;**308**:175–81.
158. de Wit E, Munster VJ, van Riel D, et al. Molecular determinants of adaptation of highly pathogenic avian influenza H7N7 viruses to efficient replication in the human host. *J Virol* 2010;**84**: 1597–606.
159. Dell KM, Schulman SL. Rhabdomyolysis and acute renal failure in a child with influenza A infection. *Pediatr Nephrol* 1997;**11**: 363–5.
160. Denny FW, Murphy TF, Clyde WA Jr, et al. Croup: an 11-year study in a pediatric practice. *Pediatrics* 1983;**71**:871–6.
161. Deyde VM, Okomo-Adhiambo M, Sheu TG, et al. Pyrosequencing as a tool to detect molecular markers of resistance to neuraminidase inhibitors in seasonal influenza A viruses. *Antiviral Res* 2009;**81**:16–24.
162. Deyde VM, Sheu TG, Trujillo AA, et al. Detection of molecular markers of drug resistance in 2009 pandemic influenza A (H1N1) viruses by pyrosequencing. *Antimicrob Agents Chemother* 2010;**54**: 1102–10.
163. Deyde VM, Xu X, Bright RA, et al. Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. *J Infect Dis* 2007;**196**:249–57.
164. Diaz E, Martin-Loeches I, Canadell L, et al. Corticosteroid therapy in patients with primary viral pneumonia due to pandemic (H1N1) 2009 influenza. *J Infect* 2012;**64**:311–18.
165. Dieleman J, Romio S, Johansen K, et al. Guillain-Barré syndrome and adjuvanted pandemic influenza A (H1N1) 2009 vaccine: multinational case-control study in Europe. *BMJ* 2011;**343**:d3908.
166. Dilantika C, Sedyaningsih ER, Kasper MR, et al. Influenza virus infection among pediatric patients reporting diarrhea and influenza-like illness. *BMC Infect Dis* 2010;**10**:3.
167. Do AH, van Doorn HR, Nghiem MN, et al. Viral etiologies of acute respiratory infections among hospitalized Vietnamese children in Ho Chi Minh City, 2004-2008. *PLoS One* 2011;**6**: e18176.
168. Doherty PC, Turner SJ, Webby RG, et al. Influenza and the challenge for immunology. *Nat Immunol* 2006;**7**:449–55.
169. Dolan GP, Myles PR, Brett SJ, et al. The comparative clinical course of pregnant and non-pregnant women hospitalised with influenza A(H1N1)pdm09 infection. *PLoS One* 2012;**7**:e41638.
170. Doyle TJ, Hopkins RS. Low secondary transmission of 2009 pandemic influenza A (H1N1) in households following an outbreak at a summer camp: relationship to timing of exposure. *Epidemiol Infect* 2011;**139**:45–51.
171. Dulek DE, Williams JV, Creech CB, et al. Use of intravenous zanamivir after development of oseltamivir resistance in a critically Ill immunosuppressed child infected with 2009 pandemic influenza A (H1N1) virus. *Clin Infect Dis* 2010;**50**:1493–6.
172. Dulyachai W, Makkoch J, Rianthavorn P, et al. Perinatal pandemic (H1N1) 2009 infection, Thailand. *Emerg Infect Dis* 2010;**16**: 343–4.
173. Earn DJ, He D, Loeb MB, et al. Effects of school closure on incidence of pandemic influenza in Alberta, Canada. *Ann Intern Med* 2012;**156**:173–81.
174. Effler PV, Chu C, He H, et al. Statewide school-located influenza vaccination program for children 5-13 years of age, Hawaii, USA. *Emerg Infect Dis* 2010;**16**:244–50.
175. Eick AA, Uyeki TM, Klimov A, et al. Maternal influenza vaccination and effect on influenza virus infection in young infants. *Arch Pediatr Adolesc Med* 2011;**165**:104–11.
176. Ekiert DC, Friesen RH, Bhabha G, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science* 2011; **333**:843–50.
177. Ekiert DC, Kashyap AK, Steel J, et al. Cross-neutralization of influenza A viruses mediated by a single antibody loop. *Nature* 2012;**489**:526–32.
178. Engblom E, Ekfors TO, Meurman OH, et al. Fatal influenza A myocarditis with isolation of virus from the myocardium. *Acta Med Scand* 1983;**213**:75–8.
179. Englund JA, Walter E, Black S, et al. Safety and immunogenicity of trivalent inactivated influenza vaccine in infants: a randomized double-blind placebo-controlled study. *Pediatr Infect Dis J* 2010; **29**:105–10.
180. Englund JA, Walter EB, Fairchok MP, et al. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. *Pediatrics* 2005;**115**:1039–47.
181. Englund JA, Walter EB, Gbadebo A, et al. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. *Pediatrics* 2006;**118**:e579–85.
182. Eriksson CO, Graham DA, Uyeki TM, et al. Risk factors for mechanical ventilation in U.S. children hospitalized with seasonal influenza and 2009 pandemic influenza A*. *Pediatr Crit Care Med* 2012;**13**:625–31.
183. Esposito S, Principi N. Different influenza vaccine formulations and adjuvants for childhood influenza vaccination. *Vaccine* 2011; **29**:7535–41.
184. Esposito S, Cantarutti L, Molteni CG, et al. Clinical manifestations and socio-economic impact of influenza among healthy children in the community. *J Infect* 2011;**62**:379–87.
185. European Centre for Disease Prevention and Control. *Narcolepsy in association with pandemic influenza vaccination (a multi-country European epidemiological investigation)*. 2012. Available at: http://www.ecdc.europa.eu/en/publications/Publications/Vaesco%20report%20FINAL%20with%20cover.pdf.
186. Eurosurveillance editorial team. Swedish Medical Products Agency publishes report from a case inventory study on Pandemrix vaccination and development of narcolepsy with cataplexy. *Euro Surveill* 2011;**16**(26):19904.
187. Everitt AR, Clare S, Pertel T, et al. IFITM3 restricts the morbidity and mortality associated with influenza. *Nature* 2012;**484**: 519–23.

188. Fabian P, McDevitt JJ, DeHaan WH, et al. Influenza virus in human exhaled breath: an observational study. *PLoS One* 2008; **3**:e2691.
189. Fairbrother G, Cassedy A, Ortega-Sanchez IR, et al. High costs of influenza: direct medical costs of influenza disease in young children. *Vaccine* 2010;**28**:4913–19.
190. Faix DJ, Sherman SS, Waterman SH. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;**361**:728–9.
191. Fan X, Zhu H, Zhou B, et al. Emergence and dissemination of a swine H3N2 reassortant influenza virus with 2009 pandemic H1N1 genes in pigs in China. *J Virol* 2012;**86**:2375–8.
192. Fanella ST, Pinto MA, Bridger NA, et al. Pandemic (H1N1) 2009 influenza in hospitalized children in Manitoba: nosocomial transmission and lessons learned from the first wave. *Infect Control Hosp Epidemiol* 2011;**32**:435–43.
193. Farias JA, Fernandez A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. *Intensive Care Med* 2010;**36**:1015–22.
194. Farrell MK, Partin JC, Bove KE. Epidemic influenza myopathy in Cincinnati in 1977. *J Pediatr* 1980;**96**:545–51.
195. Feikin DR, Njenga MK, Bigogo G, et al. Viral and bacterial causes of severe acute respiratory illness among children less than 5 years old in a high malaria prevalence area of Western Kenya, 2007-2010. *Pediatr Infect Dis J* 2012.
196. Feikin DR, Ope MO, Aura B, et al. The population-based burden of influenza-associated hospitalization in rural western Kenya, 2007-2009. *Bull World Health Organ* 2012;**90**:256–63A.
197. Feng L, Shay DK, Jiang Y, et al. Influenza-associated mortality in temperate and subtropical Chinese cities, 2003-2008. *Bull World Health Organ* 2012;**90**:279–88B.
198. Ferdinands JM, Denison AM, Dowling NF, et al. A pilot study of host genetic variants associated with influenza-associated deaths among children and young adults. *Emerg Infect Dis* 2011;**17**: 2294–302.
199. Ferson MJ, Morton JR, Robertson PW. Impact of influenza on morbidity in children with cystic fibrosis. *J Paediatr Child Health* 1991;**27**:308–11.
200. Fiers W, De Filette M, El Bakkouri K, et al. M2e-based universal influenza A vaccine. *Vaccine* 2009;**27**:6280–3.
201. Finelli L, Fiore A, Dhara R, et al. Influenza-associated pediatric mortality in the United States: increase of *Staphylococcus aureus* coinfection. *Pediatrics* 2008;**122**:805–11.
202. Fiore AE, Fry A, Shay D, et al. Antiviral agents for the treatment and chemoprophylaxis of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**:1–24.
203. Fiore AE, Uyeki TM, Broder K, et al. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Recomm Rep* 2010;**59**:1–62.
204. Fleming DM, Crovari P, Wahn U, et al. Comparison of the efficacy and safety of live attenuated cold-adapted influenza vaccine, trivalent, with trivalent inactivated influenza virus vaccine in children and adolescents with asthma. *Pediatr Infect Dis J* 2006; **25**:860–9.
205. Fodor E, Devenish L, Engelhardt OG, et al. Rescue of influenza A virus from recombinant DNA. *J Virol* 1999;**73**:9679–82.
206. Fouchier RA, Munster VJ. Epidemiology of low pathogenic avian influenza viruses in wild birds. *Rev Sci Tech* 2009;**28**:49–58.
207. Fouchier RA, Schneeberger PM, Rozendaal FW, et al. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. *Proc Natl Acad Sci U S A* 2004;**101**:1356–61.
208. Fox JP, Cooney MK, Hall CE, et al. Influenza virus infections in Seattle families, 1975-1979. II. Pattern of infection in invaded households and relation of age and prior antibody to occurrence of infection and related illness. *Am J Epidemiol* 1982;**116**: 228–42.
209. Fox JP, Hall CE, Cooney MK, et al. Influenza virus infections in Seattle families, 1975-1979. I. Study design, methods and the occurrence of infections by time and age. *Am J Epidemiol* 1982; **116**:212–27.
210. Fox JP, Hall CE, Cooney MK, et al. The Seattle virus watch. II. Objectives, study population and its observation, data processing and summary of illnesses. *Am J Epidemiol* 1972;**96**:270–85.
211. Foy HM, Cooney MK, Allan ID, et al. Influenza B in households: virus shedding without symptoms or antibody response. *Am J Epidemiol* 1987;**126**:506–15.
212. France EK, Glanz JM, Xu S, et al. Safety of the trivalent inactivated influenza vaccine among children: a population-based study. *Arch Pediatr Adolesc Med* 2004;**158**:1031–6.
213. Francis T. On the doctrine of original antigenic sin. *Proc Am Philos Soc* 1960;**104**:572–8.
214. Frank AL, Taber LH, Wells JM. Individuals infected with two subtypes of influenza A virus in the same season. *J Infect Dis* 1983;**147**:120–4.
215. Frank AL, Taber LH, Wells CR, et al. Patterns of shedding of myxoviruses and paramyxoviruses in children. *J Infect Dis* 1981; **144**:433–41.
216. Frobert E, Sarret C, Billaud G, et al. Pediatric neurological complications associated with the A(H1N1)pdm09 influenza infection. *J Clin Virol* 2011;**52**:307–13.
217. Frost WH. The epidemiology of influenza. 1919. *Public Health Rep* 2006;**121**(Suppl. 1):149–59; discussion 148.
218. Fuchigami T, Imai Y, Hasegawa M, et al. Acute encephalopathy with pandemic (H1N1) 2009 virus infection. *Pediatr Emerg Care* 2012;**28**:998–1002.
219. Fukuyama, bS., Kawaoka Y. The pathogenesis of influenza virus infections: the contributions of virus and host factors. *Curr Opin Immunol* 2011;**23**:481–6.
220. Gamblin SJ, Skehel JJ. Influenza hemagglutinin and neuraminidase membrane glycoproteins. *J Biol Chem* 2010;**285**: 28403–9.
221. Gao HN, Lu HZ, Cao B, et al. Clinical findings in 111 cases of influenza A (H7N9) virus infection. *N Engl J Med* 2013;**368**: 2277–85.
222. Garten RJ, Davis CT, Russell CA, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. *Science* 2009;**325**:197–201.
223. Gaur AH, Bagga B, Barman S, et al. Intravenous zanamivir for oseltamivir-resistant 2009 H1N1 influenza. *N Engl J Med* 2010; **362**:88–9.
224. Gaydos JC, Top FH Jr, Hodder RA, et al. Swine influenza a outbreak, Fort Dix, New Jersey, 1976. *Emerg Infect Dis* 2006;**12**: 23–8.
225. Geraci JR, St Aubin DJ, Barker IK, et al. Mass mortality of harbor seals: pneumonia associated with influenza A virus. *Science* 1982; **215**:1129–31.
226. Gessner BD, Shindo N, Briand S. Seasonal influenza epidemiology in sub-Saharan Africa: a systematic review. *Lancet Infect Dis* 2011;**11**:223–35.
227. Ghani AS, Morrow BM, Hardie DR, et al. An investigation into the prevalence and outcome of patients admitted to a pediatric intensive care unit with viral respiratory tract infections in Cape Town, South Africa. *Pediatr Crit Care Med* 2012;**13**: e275–81.
228. Giannella M, Alonso M, Garcia de Viedma D, et al. Prolonged viral shedding in pandemic influenza A(H1N1): clinical significance and viral load analysis in hospitalized patients. *Clin Microbiol Infect* 2011;**17**:1160–5.
229. Giglio N, Gentile A, Lees L, et al. Public health and economic benefits of new pediatric influenza vaccination programs in Argentina. *Hum Vaccin Immunother* 2012;**8**:312–22.
230. Giraud C, Manceau S, Oualha M, et al. High levels and safety of oseltamivir carboxylate plasma concentrations after nasogastric administration in critically ill children in a pediatric intensive care unit. *Antimicrob Agents Chemother* 2011;**55**:433–5.
231. Glanz JM, Newcomer SR, Hambidge SJ, et al. Safety of trivalent inactivated influenza vaccine in children aged 24 to 59 months in the vaccine safety datalink. *Arch Pediatr Adolesc Med* 2011;**165**: 749–55.
232. Glaser CA, Winter K, DuBray K, et al. A population-based study of neurologic manifestations of severe influenza A(H1N1)pdm09 in California. *Clin Infect Dis* 2012;**55**:514–20.
233. Glezen WP. Serious morbidity and mortality associated with influenza epidemics. *Epidemiol Rev* 1982;**4**:25–44.
234. Glezen WP. Universal influenza vaccination and live attenuated influenza vaccination of children. *Pediatr Infect Dis J* 2008;**27**: S104–9.
235. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-76. *N Engl J Med* 1978;**298**:587–92.

236. Glezen WP, Couch RB, Taber LH, et al. Epidemiologic observations of influenza B virus infections in Houston, Texas, 1976-1977. *Am J Epidemiol* 1980;**111**:13–22.
237. Glezen WP, Gaglani MJ, Kozinetz CA, et al. Direct and indirect effectiveness of influenza vaccination delivered to children at school preceding an epidemic caused by 3 new influenza virus variants. *J Infect Dis* 2010;**202**:1626–33.
238. Glezen WP, Taber LH, Frank AL, et al. Influenza virus infections in infants. *Pediatr Infect Dis J* 1997;**16**:1065–8.
239. Goebel J, Harter HR, Boineau FG, et al. Acute renal failure from rhabdomyolysis following influenza A in a child. *Clin Pediatr (Phila)* 1997;**36**:479–81.
240. Gold MS, Effler P, Kelly H, et al. Febrile convulsions after 2010 seasonal trivalent influenza vaccine: implications for vaccine safety surveillance in Australia. *Med J Aust* 2010;**193**:492–3.
241. Goodman MJ, Nordin JD, Harper P, et al. The safety of trivalent influenza vaccine among healthy children 6 to 24 months of age. *Pediatrics* 2006;**117**:e821–6.
242. Gouarin S, Vabret A, Dina J, et al. Study of influenza C virus infection in France. *J Med Virol* 2008;**80**:1441–6.
243. Grayson ML, Melvani S, Druce J, et al. Efficacy of soap and water and alcohol-based hand-rub preparations against live H1N1 influenza virus on the hands of human volunteers. *Clin Infect Dis* 2009;**48**:285–91.
244. Greene SK, Rett M, Weintraub ES, et al. Risk of confirmed Guillain-Barré syndrome following receipt of monovalent inactivated influenza A (H1N1) and seasonal influenza vaccines in the Vaccine Safety Datalink Project, 2009-2010. *Am J Epidemiol* 2012;**175**:1100–9.
245. Greer LG, Leff RD, Rogers VL, et al. Pharmacokinetics of oseltamivir in breast milk and maternal plasma. *Am J Obstet Gynecol* 2011;**204**:524.e521–4.
246. Griffin MR, Monto AS, Belongia EA, et al. Effectiveness of non-adjuvanted pandemic influenza A vaccines for preventing pandemic influenza acute respiratory illness visits in 4 U.S. communities. *PLoS One* 2011;**6**:e23085.
247. Grijalva CG, Craig AS, Dupont WD, et al. Estimating influenza hospitalizations among children. *Emerg Infect Dis* 2006;**12**:103–9.
248. Grijalva CG, Poehling KA, Edwards KM, et al. Accuracy and interpretation of rapid influenza tests in children. *Pediatrics* 2007;**119**:e6–11.
249. Grijalva CG, Zhu Y, Simonsen L, et al. The population impact of a large school-based influenza vaccination campaign. *PLoS One* 2010;**5**:e15097.
250. Gu Y, Komiya N, Kamiya H, et al. Pandemic (H1N1) 2009 transmission during presymptomatic phase, Japan. *Emerg Infect Dis* 2011;**17**:1737–9.
251. Guan Y, Shortridge KF, Krauss S, et al. Molecular characterization of H9N2 influenza viruses: were they the donors of the "internal" genes of H5N1 viruses in Hong Kong? *Proc Natl Acad Sci U S A* 1999;**96**:9363–7.
252. Guan Y, Vijaykrishna D, Bahl J, et al. The emergence of pandemic influenza viruses. *Protein Cell* 2010;**1**:9–13.
253. Guarner J, Paddock CD, Shieh WJ, et al. Histopathologic and immunohistochemical features of fatal influenza virus infection in children during the 2003-2004 season. *Clin Infect Dis* 2006;**43**:132–40.
254. Gubareva LV, Trujillo AA, Okomo-Adhiambo M, et al. Comprehensive assessment of 2009 pandemic influenza A (H1N1) virus drug susceptibility in vitro. *Antivir Ther* 2010;**15**:1151–9.
255. Halasa NB, Gerber MA, Chen Q, et al. Safety and immunogenicity of trivalent inactivated influenza vaccine in infants. *J Infect Dis* 2008;**197**:1448–54.
256. Hall CB, Douglas RG Jr. Nosocomial influenza infection as a cause of intercurrent fevers in infants. *Pediatrics* 1975;**55**:673–7.
257. Hall CB, Dolin R, Gala CL, et al. Children with influenza A infection: treatment with rimantadine. *Pediatrics* 1987;**80**:275–82.
258. Hall CE, Cooney MK, Fox JP. The Seattle virus watch. II. Objectives, study population and its observation, data processing and summary of illnesses. *Am J Epidemiol* 1973;**98**:365–80.
259. Hall CE, Cooney MK, Fox JP. The Seattle virus watch. IV. Comparative epidemiologic observations of infections with influenza A and B viruses, 1965-1969, in families with young children. *Am J Epidemiol* 1973;**98**:365–80.
260. Hall JL, Katz BZ. Cost of influenza hospitalization at a tertiary care children's hospital and its impact on the cost-benefit analysis of the recommendation for universal influenza immunization in children age 6 to 23 months. *J Pediatr* 2005;**147**:807–11.
261. Halpin TJ, Holtzhauer FJ, Campbell RJ, et al. Reye's syndrome and medication use. *JAMA* 1982;**248**:687–91.
262. Hambidge SJ, Glanz JM, France EK, et al. Safety of trivalent inactivated influenza vaccine in children 6 to 23 months old. *JAMA* 2006;**296**:1990–7.
263. Han F, Lin L, Warby SC, et al. Narcolepsy onset is seasonal and increased following the 2009 H1N1 pandemic in China. *Ann Neurol* 2011;**70**:410–17.
264. Han K, Ma H, An X, et al. Early use of glucocorticoids was a risk factor for critical disease and death from pH1N1 infection. *Clin Infect Dis* 2011;**53**:326–33.
265. Hancock K, Veguilla V, Lu X, et al. Cross-reactive antibody responses to the 2009 pandemic H1N1 influenza virus. *N Engl J Med* 2009;**361**:1945–52.
266. Hara M, Morihara M, Takao S, et al. Influenza viral load and rapid influenza diagnostic tests in children and adults. *Diagn Microbiol Infect Dis* 2012;**73**:99–100.
267. Harder KM, Molbak K, Glismann S, et al. Influenza-associated illness is an important contributor to febrile convulsions in Danish children. *J Infect* 2012;**64**:520–4.
268. Harper SA, Bradley JS, Englund JA, et al. Seasonal influenza in adults and children: diagnosis, treatment, chemoprophylaxis, and institutional outbreak management— clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:1003–32.
269. Harrison LH, Armstrong CW, Jenkins SR, et al. A cluster of meningococcal disease on a school bus following epidemic influenza. *Arch Intern Med* 1991;**151**:1005–9.
270. Hasegawa M, Hashimoto K, Morozumi M, et al. Spontaneous pneumomediastinum complicating pneumonia in children infected with the 2009 pandemic influenza A (H1N1) virus. *Clin Microbiol Infect* 2010;**16**:195–9.
271. Hatakeyama S, Sugaya N, Ito M, et al. Emergence of influenza B viruses with reduced sensitivity to neuraminidase inhibitors. *JAMA* 2007;**297**:1435–42.
272. Hauge SH, Dudman S, Borgen K, et al. Oseltamivir-resistant influenza viruses A (H1N1), Norway, 2007-08. *Emerg Infect Dis* 2009;**15**:155–62.
273. Hawaii Department of Health. *Influenza in Hawaii at a glance*. 2012. Available at: http://healthadmin.hawaii.gov/STAGING/health/family-child-health/contagious-disease/influenza/Flu_Glance.htm.
274. Hay AJ, Gregory V, Douglas AR, et al. The evolution of human influenza viruses. *Philos Trans R Soc Lond B Biol Sci* 2001;**356**:1861–70.
275. Hayden F. Developing new antiviral agents for influenza treatment: what does the future hold? *Clin Infect Dis* 2009;**48**(Suppl. 1):S3–13.
276. Hayden FG, Hay AJ. Emergence and transmission of influenza A viruses resistant to amantadine and rimantadine. *Curr Top Microbiol Immunol* 1992;**176**:119–30.
277. Hayden FG, Sperber SJ, Belshe RB, et al. Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. *Antimicrob Agents Chemother* 1991;**35**:1741–7.
278. Hedrick JA, Barzilai A, Behre U, et al. Zanamivir for treatment of symptomatic influenza A and B infection in children five to twelve years of age: a randomized controlled trial. *Pediatr Infect Dis J* 2000;**19**:410–17.
279. Heikkinen T, Heinonen S. Effectiveness and safety of influenza vaccination in children: European perspective. *Vaccine* 2011;**29**:7529–34.
280. Heikkinen T, Salmi AA, Ruuskanen O. Comparative study of nasopharyngeal aspirate and nasal swab specimens for detection of influenza. *BMJ* 2001;**322**:138.
281. Heikkinen T, Marttila J, Salmi AA, et al. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol* 2002;**40**:4337–9.
282. Heikkinen T, Silvennoinen H, Peltola V, et al. Burden of influenza in children in the community. *J Infect Dis* 2004;**190**:1369–73.
283. Heinonen S, Peltola V, Silvennoinen H, et al. Signs and symptoms predicting influenza in children: a matched case-control analysis of prospectively collected clinical data. *Eur J Clin Microbiol Infect Dis* 2012;**31**:1569–74.

284. Heinonen S, Silvennoinen H, Lehtinen P, et al. Early oseltamivir treatment of influenza in children 1-3 years of age: a randomized controlled trial. *Clin Infect Dis* 2010;**51**:887–94.
285. Henkle E, Steinhoff MC, Omer SB, et al. Incidence of influenza virus infection in early infancy: a prospective study in South Asia. *Pediatr Infect Dis J* 2011;**30**:170–3.
286. Herfst S, Schrauwen EJ, Linster M, et al. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* 2012;**336**: 1534–41.
287. Hernan MA, Lipsitch M. Oseltamivir and risk of lower respiratory tract complications in patients with flu symptoms: a meta-analysis of eleven randomized clinical trials. *Clin Infect Dis* 2011;**53**: 277–9.
288. Hernandez JE, Adiga R, Armstrong R, et al. Clinical experience in adults and children treated with intravenous peramivir for 2009 influenza A (H1N1) under an Emergency IND program in the United States. *Clin Infect Dis* 2011;**52**:695–706.
289. Hibbert CL, Piedra PA, McLaurin KK, et al. Cost-effectiveness of live-attenuated influenza vaccine, trivalent in preventing influenza in young children attending day-care centres. *Vaccine* 2007; **25**:8010–20.
290. Hien ND, Ha NH, Van NT, et al. Human infection with highly pathogenic avian influenza virus (H5N1) in northern Vietnam, 2004-2005. *Emerg Infect Dis* 2009;**15**:19–23.
291. Hinshaw VS, Bean WJ, Geraci J, et al. Characterization of two influenza A viruses from a pilot whale. *J Virol* 1986;**58**:655–6.
292. Hirsch A, Creighton CT. *Handbook of geographical and historical pathology*. Vol. 1. London: New Sydenham Society; 1883.
293. Ho YC, Wang JL, Wang JT, et al. Prognostic factors for fatal adult influenza pneumonia. *J Infect* 2009;**58**:439–45.
294. Hochberg FH, Nelson K, Janzen W. Influenza type B-related encephalopathy: the 1971 outbreak of Reye syndrome in Chicago. *JAMA* 1975;**231**:817–21.
295. Hoft DF, Babusis E, Worku S, et al. Live and inactivated influenza vaccines induce similar humoral responses, but only live vaccines induce diverse T-cell responses in young children. *J Infect Dis* 2011;**204**:845–53.
296. Honorat R, Tison C, Sevely A, et al. Influenza A(H1N1)-associated ischemic stroke in a 9-month-old child. *Pediatr Emerg Care* 2012;**28**:368–9.
297. Hoper D, Hoffmann B, Beer M. A comprehensive deep sequencing strategy for full-length genomes of influenza A. *PLoS One* 2011;**6**:e19075.
298. Hopkins BS, Johnson KE, Ksiazek JM, et al. H1N1 influenza A presenting as bacterial tracheitis. *Otolaryngol Head Neck Surg* 2010;**142**:612–14.
299. Horby P, Mai le Q, Fox A, et al. The epidemiology of interpandemic and pandemic influenza in Vietnam, 2007-2010: the Ha Nam household cohort study I. *Am J Epidemiol* 2012;**175**: 1062–74.
300. Horby P, Sudoyo H, Viprakasit V, et al. What is the evidence of a role for host genetics in susceptibility to influenza A/H5N1? *Epidemiol Infect* 2010;**138**:1550–8.
301. Horimoto T, Kawaoka Y. Pandemic threat posed by avian influenza A viruses. *Clin Microbiol Rev* 2001;**14**:129–49.
302. Horimoto T, Kawaoka Y. Influenza: lessons from past pandemics, warnings from current incidents. *Nat Rev Microbiol* 2005;**3**: 591–600.
303. Houck P, Hemphill M, LaCroix S, et al. Amantadine-resistant influenza A in nursing homes. Identification of a resistant virus prior to drug use. *Arch Intern Med* 1995;**155**:533–7.
304. Howard WA, Peiris M, Hayden FG. Report of the 'mechanisms of lung injury and immunomodulator interventions in influenza.' Workshop, 21 March 2010, Ventura, California, USA. *Influenza Other Respi Viruses* 2011;**5**:453–4, e458–75.
305. Hsu J, Santesso N, Mustafa R, et al. Antivirals for treatment of influenza: a systematic review and meta-analysis of observational studies. *Ann Intern Med* 2012;**156**:512–24.
306. Hu JJ, Kao CL, Lee PI, et al. Clinical features of influenza A and B in children and association with myositis. *J Microbiol Immunol Infect* 2004;**37**:95–8.
307. Hull HF, Frauendienst RS, Gundersen ML, et al. School-based influenza immunization. *Vaccine* 2008;**26**:4312–13.
308. Hung IF, To KK, Lee CK, et al. Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection. *Clin Infect Dis* 2011;**52**:447–56.
309. Hurt AC, Okomo-Adhiambo M, Gubareva LV. The fluorescence neuraminidase inhibition assay: a functional method for detection of influenza virus resistance to the neuraminidase inhibitors. *Methods Mol Biol* 2012;**865**:115–25.
310. Hurt AC, Chotpitayasunondh T, Cox NJ, et al. Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. *Lancet Infect Dis* 2012;**12**:240–8.
311. Hurt AC, Hardie K, Wilson NJ, et al. Community transmission of oseltamivir-resistant A(H1N1)pdm09 influenza. *N Engl J Med* 2011;**365**:2541–2.
312. Hurt AC, Hardie K, Wilson NJ, et al. Characteristics of a widespread community cluster of H275Y oseltamivir-resistant A(H1N1)pdm09 influenza in Australia. *J Infect Dis* 2012;**206**: 148–57.
313. Hurwitz ES, Haber M, Chang A, et al. Effectiveness of influenza vaccination of day care children in reducing influenza-related morbidity among household contacts. *JAMA* 2000;**284**:1677–82.
314. Imai M, Kawaoka Y. The role of receptor binding specificity in interspecies transmission of influenza viruses. *Curr Opin Virol* 2012;**2**:160–7.
315. Imai M, Watanabe T, Hatta M, et al. Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 2012;**486**: 420–8.
316. Institute of Epidemiology Disease Control and Research. Ministry of Health and Family Welfare, Government of The People's Republic of Bangladesh (GoB), The Programme on Infectious Diseases and Vaccine Sciences. Outbreak of mild respiratory disease caused by H5N1 and H9N2 infections among young children in Dhaka, Bangladesh. *Health and Science Bulletin* 2011;**9**: 1–11.
317. Irving WL, James DK, Stephenson T, et al. Influenza virus infection in the second and third trimesters of pregnancy: a clinical and seroepidemiological study. *BJOG* 2000;**107**:1282–9.
318. Ison MG. Epidemiology, prevention, and management of influenza in patients with hematologic malignancy. *Infect Disord Drug Targets* 2011;**11**:34–9.
319. Ison MG, de Jong MD, Gilligan KJ, et al. End points for testing influenza antiviral treatments for patients at high risk of severe and life-threatening disease. *J Infect Dis* 2010;**201**:1654–62.
320. Ison MG, Szakaly P, Shapira MY, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**:955–64.
321. Itoh Y, Shinya K, Kiso M, et al. In vitro and in vivo characterization of new swine-origin H1N1 influenza viruses. *Nature* 2009; **460**:1021–5.
322. Iverson AR, Boyd KL, McAuley JL, et al. Influenza virus primes mice for pneumonia from *Staphylococcus aureus*. *J Infect Dis* 2011; **203**:880–8.
323. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. *Pediatrics* 2004;**113**:1758–64.
324. Izurieta HS, Thompson WW, Kramarz P, et al. Influenza and the rates of hospitalization for respiratory disease among infants and young children. *N Engl J Med* 2000;**342**:232–9.
325. Jackson LA, Nelson JC. Association between statins and mortality. *J Infect Dis* 2012;**206**:303–4; author reply 304–5.
326. Jackson ML, France AM, Hancock K, et al. Serologically confirmed household transmission of 2009 pandemic influenza A (H1N1) virus during the first pandemic wave–New York City, April-May 2009. *Clin Infect Dis* 2011;**53**:455–62.
327. Jagger BW, Wise HM, Kash JC, et al. An overlapping protein-coding region in influenza A virus segment 3 modulates the host response. *Science* 2012;**337**:199–204.
328. Jansen AG, Sanders EA, Smulders S, et al. Adverse reactions to simultaneous influenza and pneumococcal conjugate vaccinations in children: randomized double-blind controlled trial. *Pediatr Allergy Immunol* 2008;**19**:552–8.
329. Jartti A, Rauvala E, Kauma H, et al. Chest imaging findings in hospitalized patients with H1N1 influenza. *Acta Radiol* 2011;**52**: 297–304.
330. Jayaraman A, Pappas C, Raman R, et al. A single base-pair change in 2009 H1N1 hemagglutinin increases human receptor affinity and leads to efficient airborne viral transmission in ferrets. *PLoS One* 2011;**6**:e17616.

331. Jean C, Louie JK, Glaser CA, et al. Invasive group A streptococcal infection concurrent with 2009 H1N1 influenza. *Clin Infect Dis* 2010;**50**:e59–62.
332. Jefferson T, Jones MA, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. *Cochrane Database Syst Rev* 2012;(1):CD008965.
333. Jefferson T, Rivetti A, Di Pietrantonj C, et al. Vaccines for preventing influenza in heathy children. *Cochrane Database Syst Rev* 2012;(8):CD004879.
334. Jennings LC, Miles JA. A study of acute respiratory disease in the community of Port Chalmers. II. Influenza A/Port Chalmers/1/73: intrafamilial spread and the effect of antibodies to the surface antigens. *J Hyg (Lond)* 1978;**81**:67–75.
335. Jernigan DB, Lindstrom SL, Johnson JR, et al. Detecting 2009 pandemic influenza A (H1N1) virus infection: availability of diagnostic testing led to rapid pandemic response. *Clin Infect Dis* 2011;**52**(Suppl. 1):S36–43.
336. Johnson BF, Wilson LE, Ellis J, et al. Fatal cases of influenza a in childhood. *PLoS One* 2009;**4**:e7671.
337. Johnston SL, Ferrero F, Garcia ML, et al. Oral oseltamivir improves pulmonary function and reduces exacerbation frequency for influenza-infected children with asthma. *Pediatr Infect Dis J* 2005;**24**:225–32.
338. Joshi AY, Iyer VN, St Sauver JL, et al. Effectiveness of inactivated influenza vaccine in children less than 5 years of age over multiple influenza seasons: a case-control study. *Vaccine* 2009;**27**:4457–61.
339. Jul-Larsen A, Madhun AS, Brokstad KA, et al. The human potential of a recombinant pandemic influenza vaccine produced in tobacco plants. *Hum Vaccin Immunother* 2012;**8**:653–61.
340. Kadjo HA, Ekaza E, Coulibaly D, et al. Sentinel surveillance for influenza and other respiratory viruses in Cote d'Ivoire, 2003-2010. *Influenza Other Respi Viruses* 2013;**7**:296–303.
341. Kaji M, Oseasohn R, Jordan WS Jr, et al. Isolation of Asian virus from extrapulmonary tissues in fatal human influenza. *Proc Soc Exp Biol Med* 1959;**100**:272–5.
342. Kandun IN, Wibisono H, Sedyaningsih ER, et al. Three Indonesian clusters of H5N1 virus infection in 2005. *N Engl J Med* 2006;**355**:2186–94.
343. Kang SM, Kim MC, Compans RW. Virus-like particles as universal influenza vaccines. *Expert Rev Vaccines* 2012;**11**:995–1007.
344. Kang SM, Song JM, Compans RW. Novel vaccines against influenza viruses. *Virus Res* 2011;**162**:31–8.
345. Kanmaz HG, Erdeve O, Ogz SS, et al. Placental transmission of novel pandemic influenza a virus. *Fetal Pediatr Pathol* 2011;**30**:280–5.
346. Kao HT, Huang YC, Lin TY. Influenza A virus infection in infants. *J Microbiol Immunol Infect* 2000;**33**:105–8.
347. Kash JC, Tumpey TM, Proll SC, et al. Genomic analysis of increased host immune and cell death responses induced by 1918 influenza virus. *Nature* 2006;**443**:578–81.
348. Katayose M, Hosoya M, Haneda T, et al. The effectiveness of trivalent inactivated influenza vaccine in children over six consecutive influenza seasons. *Vaccine* 2011;**29**:1844–9.
349. Katsumi Y, Otabe O, Matsui F, et al. Effect of a single inhalation of laninamivir octanoate in children with influenza. *Pediatrics* 2012;**129**:e1431–6.
350. Katz MA, Tharmaphornpilas P, Chantra S, et al. Who gets hospitalized for influenza pneumonia in Thailand? Implications for vaccine policy. *Vaccine* 2007;**25**:3827–33.
351. Kawashima H, Morichi S, Okumara A, et al. National survey of pandemic influenza A (H1N1) 2009-associated encephalopathy in Japanese children. *J Med Virol* 2012;**84**:1151–6.
352. Kawashima H, Morichi S, Okumara A, et al. Treatment of pandemic influenza A (H1N1) 2009-associated encephalopathy in children. *Scand J Infect Dis* 2012;**44**:941–7.
353. Kawashima H, Togashi T, Yamanaka G, et al. Efficacy of plasma exchange and methylprednisolone pulse therapy on influenza-associated encephalopathy. *J Infect* 2005;**51**:E53–6.
354. Kedia S, Stroud B, Parsons J, et al. Pediatric neurological complications of 2009 pandemic influenza A (H1N1). *Arch Neurol* 2011;**68**:455–62.
355. Kelly HA, Skowronski DM, De Serres G, et al. Adverse events associated with 2010 CSL and other inactivated influenza vaccines. *Med J Aust* 2011;**195**:318–20.
356. Keren R, Zaoutis TE, Bridges CB, et al. Neurological and neuromuscular disease as a risk factor for respiratory failure in children hospitalized with influenza infection. *JAMA* 2005;**294**:2188–94.
357. Keren R, Zaoutis TE, Saddlemire S, et al. Direct medical cost of influenza-related hospitalizations in children. *Pediatrics* 2006;**118**:e1321–7.
358. Kerr AA, McQuillin J, Downham MA, et al. Gastric 'flu influenza B causing abdominal symptons in children. *Lancet* 1975;**1**:291–5.
359. Khakpour M, Saidi A, Naficy K. Proved viraemia in Asian influenza (Hong Kong variant) during incubation period. *Br Med J* 1969;**4**:208–9.
360. Khamphaphongphane B, Ketmayoon P, Lewis HC, et al. Epidemiological and virological characteristics of seasonal and pandemic influenza in Lao PDR, 2008-2010. *Influenza Other Respi Viruses* 2013;**7**:304–11.
361. Khandaker G, Zurynski Y, Buttery J, et al. Neurologic complications of influenza A(H1N1)pdm09: surveillance in 6 pediatric hospitals. *Neurology* 2012;**79**:1474–81.
362. Kiatboonsri S, Kiatboonsri C, Theerawit P. Fatal respiratory events caused by zanamivir nebulization. *Clin Infect Dis* 2010;**50**:620.
363. Kilbourne ED. Influenza pandemics of the 20th century. *Emerg Infect Dis* 2006;**12**:9–14.
364. Killingley B, Enstone J, Booy R, et al. Potential role of human challenge studies for investigation of influenza transmission. *Lancet Infect Dis* 2011;**11**:879–86.
365. Killingley B, Enstone JE, Greatorex J, et al. Use of a human influenza challenge model to assess person-to-person transmission: proof-of-concept study. *J Infect Dis* 2012;**205**:35–43.
366. Kim C, Ahmed JA, Eidex RB, et al. Comparison of nasopharyngeal and oropharyngeal swabs for the diagnosis of eight respiratory viruses by real-time reverse transcription-PCR assays. *PLoS One* 2011;**6**:e21610.
367. Kim HW, Brandt CD, Arrobio JO, et al. Influenza A and B virus infection in infants and young children during the years 1957-1976. *Am J Epidemiol* 1979;**109**:464–79.
368. Kim SH, Hong SB, Yun SC, et al. Corticosteroid treatment in critically ill patients with pandemic influenza A/H1N1 2009 infection: analytic strategy using propensity scores. *Am J Respir Crit Care Med* 2011;**183**:1207–14.
369. Kinikar AA, Kulkarni RK, Valvi CT, et al. Predictors of mortality in hospitalized children with pandemic H1N1 influenza 2009 in Pune, India. *Indian J Pediatr* 2012;**79**:459–66.
370. Kiso M, Mitamura K, Sakai-Tagawa Y, et al. Resistant influenza A viruses in children treated with oseltamivir: descriptive study. *Lancet* 2004;**364**:759–65.
371. Kitching A, Roche A, Balasegaram S, et al. Oseltamivir adherence and side effects among children in three London schools affected by influenza A(H1N1)v, May 2009: an internet-based cross-sectional survey. *Euro Surveill* 2009;**14**:19287.
372. Klein JD, Collier AM, Glezen WP. An influenza B epidemic among children in day-care. *Pediatrics* 1976;**58**:340–5.
373. Klenk HD, Garten W. Host cell proteases controlling virus pathogenicity. *Trends Microbiol* 1994;**2**:39–43.
374. Klimov AI, Garten R, Russell C, et al. WHO recommendations for the viruses to be used in the 2012 Southern Hemisphere Influenza Vaccine: epidemiology, antigenic and genetic characteristics of influenza A(H1N1)pdm09, A(H3N2) and B influenza viruses collected from February to September 2011. *Vaccine* 2012;**30**:6461–71.
375. Klimov AI, Rocha E, Hayden FG, et al. Prolonged shedding of amantadine-resistant influenzae A viruses by immunodeficient patients: detection by polymerase chain reaction-restriction analysis. *J Infect Dis* 1995;**172**:1352–5.
376. Knight V, Kasel JA. Influenza viruses. In: Knight V, editor. *Viral and mycoplasmal infections of the respiratory tract*. Philadelphia: Lea & Febiger; 1973. p. 87–123.
377. Kohn MA, Farley TA, Sundin D, et al. Three summertime outbreaks of influenza type A. *J Infect Dis* 1995;**172**:246–9.
378. Koopmans M, Wilbrink B, Conyn M, et al. Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands. *Lancet* 2004;**363**:587–93.
379. Koranyi K, Yontz D, Rohrer Z, et al. Pericardial effusion complicating novel influenza A (H1N1) infection in an infant. *Pediatr Infect Dis J* 2010;**29**:782–3.

380. Kosasih H, Roselinda, Nurhayati, et al. Surveillance of influenza in Indonesia, 2003-2007. *Influenza Other Respi Viruses* 2012. doi: 10.1111/j.1750-2659.2012.00403.x (Epub ahead of print).
381. Kreijtz JH, Fouchier RA, Rimmelzwaan GF. Immune responses to influenza virus infection. *Virus Res* 2011;**162**:19–30.
382. Kreil TR, Mc Vey JK, Lei LS, et al. Preparation of commercial quantities of a hyperimmune human intravenous immunoglobulin preparation against an emerging infectious disease: the example of pandemic H1N1 influenza. *Transfusion* 2012;**52**:803–9.
383. Krief WI, Levine DA, Platt SL, et al. Influenza virus infection and the risk of serious bacterial infections in young febrile infants. *Pediatrics* 2009;**124**:30–9.
384. Kubota M, Chida J, Hoshino H, et al. Thermolabile CPT II variants and low blood ATP levels are closely related to severity of acute encephalopathy in Japanese children. *Brain Dev* 2012;**34**:20–7.
385. Kumar S, Henrickson KJ. Update on influenza diagnostics: lessons from the novel H1N1 influenza A pandemic. *Clin Microbiol Rev* 2012;**25**:344–61.
386. Kuntz-Simon G, Madec F. Genetic and antigenic evolution of swine influenza viruses in Europe and evaluation of their zoonotic potential. *Zoonoses Public Health* 2009;**56**:310–25.
387. Kurtz J, Manvell RJ, Banks J. Avian influenza virus isolated from a woman with conjunctivitis. *Lancet* 1996;**348**:901–2.
388. Kwong JC, Li P, Redelmeier DA. Influenza morbidity and mortality in elderly patients receiving statins: a cohort study. *PLoS One* 2009;**4**:e8087.
389. Kwong KL, Lam SY, Que TL, et al. Influenza A and febrile seizures in childhood. *Pediatr Neurol* 2006;**35**:395–9.
390. La Gruta NL, Kedzierska K, Stambas J, et al. A question of self-preservation: immunopathology in influenza virus infection. *Immunol Cell Biol* 2007;**85**:85–92.
391. Lackenby A, Thompson CI, Democratis J. The potential impact of neuraminidase inhibitor resistant influenza. *Curr Opin Infect Dis* 2008;**21**:626–38.
392. Lambert LC, Fauci AS. Influenza vaccines for the future. *N Engl J Med* 2010;**363**:2036–44.
393. Landau YE, Grisaru-Soen G, Reif S, et al. Pediatric neurologic complications associated with influenza A H1N1. *Pediatr Neurol* 2011;**44**:47–51.
394. Lau CH, Springston EE, Sohn MW, et al. Hand hygiene instruction decreases illness-related absenteeism in elementary schools: a prospective cohort study. *BMC Pediatr* 2012;**12**:52.
395. Lau LL, Nishiura H, Kelly H, et al. Household transmission of 2009 pandemic influenza A (H1N1): a systematic review and meta-analysis. *Epidemiology* 2012;**23**:531–42.
396. Lavelle TA, Uyeki TM, Prosser LA. Cost-effectiveness of oseltamivir treatment for children with uncomplicated seasonal influenza. *J Pediatr* 2012;**160**:67–73 e66.
397. Le QM, Wertheim HF, Tran ND, et al. A community cluster of oseltamivir-resistant cases of 2009 H1N1 influenza. *N Engl J Med* 2010;**362**:86–7.
398. Leckerman KH, Sherman E, Knorr J, et al. Risk factors for healthcare-associated, laboratory-confirmed influenza in hospitalized pediatric patients: a case-control study. *Infect Control Hosp Epidemiol* 2010;**31**:421–4.
399. Ledgerwood JE, Hu Z, Gordon IJ, et al. Influenza virus h5 DNA vaccination is immunogenic by intramuscular and intradermal routes in humans. *Clin Vaccine Immunol* 2012;**19**:1792–7.
400. Lee HJ, Lee DH, Lee YN, et al. Generation of reassortant influenza viruses within the non-industrial poultry system. *Infect Genet Evol* 2012;**12**:933–46.
401. Lee N, Chan PK, Hui DS, et al. Viral loads and duration of viral shedding in adult patients hospitalized with influenza. *J Infect Dis* 2009;**200**:492–500.
402. Lee N, Wong CK, Chan PK, et al. Hypercytokinemia and hyperactivation of phospho-p38 mitogen-activated protein kinase in severe human influenza A virus infection. *Clin Infect Dis* 2007;**45**:723–31.
403. Lee N, Wong CK, Chan PK, et al. Cytokine response patterns in severe pandemic 2009 H1N1 and seasonal influenza among hospitalized adults. *PLoS One* 2011;**6**:e26050.
404. Lee VJ, Yap J, Ong JB, et al. Influenza excess mortality from 1950-2000 in tropical Singapore. *PLoS One* 2009;**4**:e8096.
405. Lessler J, Reich NG, Brookmeyer R, et al. Incubation periods of acute respiratory viral infections: a systematic review. *Lancet Infect Dis* 2009;**9**:291–300.
406. Lessler J, Reich NG, Cummings DA, et al. Outbreak of 2009 pandemic influenza A (H1N1) at a New York City school. *N Engl J Med* 2009;**361**:2628–36.
407. Levine M, Sheu TG, Gubareva LV, et al. Detection of hemagglutinin variants of the pandemic influenza A (H1N1) 2009 virus by pyrosequencing. *J Clin Microbiol* 2011;**49**:1307–12.
408. Lewis NS, Daly JM, Russell CA, et al. Antigenic and genetic evolution of equine influenza A (H3N8) virus from 1968 to 2007. *J Virol* 2011;**85**:12742–9.
409. Leyva-Grado VH, Mubareka S, Krammer F, et al. Influenza virus infection in guinea pigs raised as livestock, Ecuador. *Emerg Infect Dis* 2012;**18**:1135–8.
410. Li CC, Wang L, Eng HL, et al. Correlation of pandemic (H1N1) 2009 viral load with disease severity and prolonged viral shedding in children. *Emerg Infect Dis* 2010;**16**:1265–72.
411. Li Q, Zhou L, Zhou M, et al. Preliminary Report: Epidemiology of the Avian Influenza A (H7N9) Outbreak in China. *N Engl J Med* 2013. [Epub ahead of print]
412. Liem NT, Tung CV, Hien ND, et al. Clinical features of human influenza A (H5N1) infection in Vietnam: 2004-2006. *Clin Infect Dis* 2009;**48**:1639–46.
413. Lim KA, Smith A, Hale JH, et al. Influenza outbreak in Singapore. *Lancet* 1957;**273**:791–6.
414. Lin SC, Lin YF, Chong P, et al. Broader neutralizing antibodies against H5N1 viruses using prime-boost immunization of hyperglycosylated hemagglutinin DNA and virus-like particles. *PLoS One* 2012;**7**:e39075.
415. Lina B. History of influenza pandemics. In: Raoult D, Drancourt M, editors. *Paleomicrobiology: past human infections*. Berlin: Springer; 2008. p. 199–211.
416. Lindblade KA, Johnson AJ, Arvelo W, et al. Low usage of government healthcare facilities for acute respiratory infections in guatemala: implications for influenza surveillance. *BMC Public Health* 2011;**11**:885.
417. Liou YS, Barbour SD, Bell LM, et al. Children hospitalized with influenza B infection. *Pediatr Infect Dis J* 1987;**6**:541–3.
418. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the infectious diseases society of america for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011;**52**:285–92.
419. Liu Y, Childs RA, Matrosovich T, et al. Altered receptor specificity and cell tropism of D222G hemagglutinin mutants isolated from fatal cases of pandemic A(H1N1) 2009 influenza virus. *J Virol* 2010;**84**:12069–74.
420. Loeb M, Russell ML, Moss L, et al. Effect of influenza vaccination of children on infection rates in Hutterite communities: a randomized trial. *JAMA* 2010;**303**:943–50.
421. Loeb M, Singh PK, Fox J, et al. Longitudinal study of influenza molecular viral shedding in Hutterite communities. *J Infect Dis* 2012;**206**:1078–84.
422. Lohr CV, DeBess EE, Baker RJ, et al. Pathology and viral antigen distribution of lethal pneumonia in domestic cats due to pandemic (H1N1) 2009 influenza A virus. *Vet Pathol* 2010;**47**:378–86.
423. Longini IM Jr, Koopman JS, Monto AS, et al. Estimating household and community transmission parameters for influenza. *Am J Epidemiol* 1982;**115**:736–51.
424. Lopez-Macias C. Virus-like particle (VLP)-based vaccines for pandemic influenza: performance of a VLP vaccine during the 2009 influenza pandemic. *Hum Vaccin Immunother* 2012;**8**:411–14.
425. Lopez-Medina E, Ardura MI, Siegel JD, et al. 2009 influenza A in infants hospitalized at younger than 6 months. *J Pediatr* 2012;**160**:626–31 e621.
426. Louie JK, Gavali S, Acosta M, et al. Children hospitalized with 2009 novel influenza A(H1N1) in California. *Arch Pediatr Adolesc Med* 2010;**164**:1023–31.
427. Louie JK, Schechter R, Honarmand S, et al. Severe pediatric influenza in California, 2003-2005: implications for immunization recommendations. *Pediatrics* 2006;**117**:e610–18.
428. Louie JK, Yang S, Acosta M, et al. Treatment with neuraminidase inhibitors for critically ill patients with influenza A (H1N1) pdm09. *Clin Infect Dis* 2012;**55**:1198–204.
429. Louie JK, Yang S, Yen C, et al. Use of intravenous peramivir for treatment of severe influenza A(H1N1)pdm09. *PLoS One* 2012;**7**:e40261.

430. Louria DB, Blumenfeld HL, Ellis JT, et al. Studies on influenza in the pandemic of 1957-1958. II. Pulmonary complications of influenza. *J Clin Invest* 1959;**38**:213–65.
431. Lowen AC, Mubareka S, Steel J, et al. Influenza virus transmission is dependent on relative humidity and temperature. *PLoS Pathog* 2007;**3**:1470–6.
432. Luby SP, Agboatwalla M, Feikin DR, et al. Effect of handwashing on child health: a randomised controlled trial. *Lancet* 2005;**366**: 225–33.
433. Luce BR, Nichol KL, Belshe RB, et al. Cost-effectiveness of live attenuated influenza vaccine versus inactivated influenza vaccine among children aged 24-59 months in the United States. *Vaccine* 2008;**26**:2841–8.
434. Luke TC, Kilbane EM, Jackson JL, et al. Meta-analysis: convalescent blood products for Spanish influenza pneumonia: a future H5N1 treatment? *Ann Intern Med* 2006;**145**:599–609.
435. Luksza AR, Jones DK. Influenza B virus infection complicated by pneumonia, acute renal failure and disseminated intravascular coagulation. *J Infect* 1984;**9**:174–6.
436. Luo M. Influenza virus entry. *Adv Exp Med Biol* 2012;**726**: 201–21.
437. Lvov DK, Zdanov VM, Sazonov AA, et al. Comparison of influenza viruses isolated from man and from whales. *Bull World Health Organ* 1978;**56**:923–30.
438. Ma W, Lager KM, Vincent AL, et al. The role of swine in the generation of novel influenza viruses. *Zoonoses Public Health* 2009;**56**:326–37.
439. Maassab HF, Bryant ML. The development of live attenuated cold-adapted influenza virus vaccine for humans. *Rev Med Virol* 1999;**9**:237–44.
440. MacDonald KL, Osterholm MT, Hedberg CW, et al. Toxic shock syndrome: a newly recognized complication of influenza and influenzalike illness. *JAMA* 1987;**257**:1053–8.
441. Madhi SA, Ramasamy N, Bessellar TG, et al. Lower respiratory tract infections associated with influenza A and B viruses in an area with a high prevalence of pediatric human immunodeficiency type 1 infection. *Pediatr Infect Dis J* 2002;**21**:291–7.
442. Maines TR, Belser JA, Gustin KM, et al. Local innate immune responses and influenza virus transmission and virulence in ferrets. *J Infect Dis* 2012;**205**:474–85.
443. Maines TR, Chen LM, Van Hoeven N, et al. Effect of receptor binding domain mutations on receptor binding and transmissibility of avian influenza H5N1 viruses. *Virology* 2011;**413**:139–47.
444. Maines TR, Jayaraman A, Belser JA, et al. Transmission and pathogenesis of swine-origin 2009 A(H1N1) influenza viruses in ferrets and mice. *Science* 2009;**325**:484–7.
445. Mak CM, Lam CW, Fong NC, et al. Fatal viral infection-associated encephalopathy in two Chinese boys: a genetically determined risk factor of thermolabile carnitine palmitoyltransferase II variants. *J Hum Genet* 2011;**56**:617–21.
446. Makela MJ, Pauksens K, Rostila T, et al. Clinical efficacy and safety of the orally inhaled neuraminidase inhibitor zanamivir in the treatment of influenza: a randomized, double-blind, placebo-controlled European study. *J Infect* 2000;**40**:42–8.
447. Mall S, Buchholz U, Tibussek D, et al. A large outbreak of influenza B-associated benign acute childhood myositis in Germany, 2007/2008. *Pediatr Infect Dis J* 2011;**30**:e142–6.
448. Mallory RM, Yi T, Ambrose CS. Shedding of Ann Arbor strain live attenuated influenza vaccine virus in children 6-59 months of age. *Vaccine* 2011;**29**:4322–7.
449. Maltezou HC, Drancourt M. Nosocomial influenza in children. *J Hosp Infect* 2003;**55**:83–91.
450. Maltezou HC, Drakoulis N, Siahanidou T, et al. Safety and pharmacokinetics of oseltamivir for prophylaxis of neonates exposed to influenza H1N1. *Pediatr Infect Dis J* 2012;**31**:527–9.
451. Manzoli L, Schioppa F, Boccia A, et al. The efficacy of influenza vaccine for healthy children: a meta-analysis evaluating potential sources of variation in efficacy estimates including study quality. *Pediatr Infect Dis J* 2007;**26**:97–106.
452. Marchetti M, Kuhnel UM, Colombo GL, et al. Cost-effectiveness of adjuvanted influenza vaccination of healthy children 6 to 60 months of age. *Hum Vaccin* 2007;**3**:14–22.
453. Maricich SM, Neul JL, Lotze TE, et al. Neurologic complications associated with influenza A in children during the 2003-4 influenza season in Houston, Texas. *Pediatrics* 2004;**114**:e626–33.
454. Marks JS, Halpin TJ. Guillain-Barré syndrome in recipients of A/New Jersey influenza vaccine. *JAMA* 1980;**243**:2490–4.
455. Martic J, Savic N, Minic P, et al. Novel H1N1 influenza in neonates: from mild to fatal disease. *J Perinatol* 2011;**31**:446–8.
456. Martin-Loeches I, Lisboa T, Rhodes A, et al. Use of early corticosteroid therapy on ICU admission in patients affected by severe pandemic (H1N1)v influenza A infection. *Intensive Care Med* 2011;**37**:272–83.
457. Matrosovich MN, Gambaryan AS, Teneberg S, et al. Avian influenza A viruses differ from human viruses by recognition of sialyloligosaccharides and gangliosides and by a higher conservation of the HA receptor-binding site. *Virology* 1997;**233**:224–34.
458. Matrosovich MN, Matrosovich TY, Gray T, et al. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. *Proc Natl Acad Sci U S A* 2004;**101**: 4620–4.
459. Matsumoto C, Sobata R, Uchida S, et al. Risk for transmission of pandemic (H1N1) 2009 virus by blood transfusion. *Emerg Infect Dis* 2010;**16**:722–3.
460. Matsuzaki Y, Abiko C, Mizuta K, et al. A nationwide epidemic of influenza C virus infection in Japan in 2004. *J Clin Microbiol* 2007;**45**:783–8.
461. Matsuzaki Y, Katsushima N, Nagai Y, et al. Clinical features of influenza C virus infection in children. *J Infect Dis* 2006;**193**: 1229–35.
462. McAuley JL, Hornung F, Boyd KL, et al. Expression of the 1918 influenza A virus PB1-F2 enhances the pathogenesis of viral and secondary bacterial pneumonia. *Cell Host Microbe* 2007;**2**:240–9.
463. McCullers JA, Bartmess KC. Role of neuraminidase in lethal synergism between influenza virus and Streptococcus pneumoniae. *J Infect Dis* 2003;**187**:1000–9.
464. McCullers JA, McAuley JL, Browall S, et al. Influenza enhances susceptibility to natural acquisition of and disease due to *Streptococcus pneumoniae* in ferrets. *J Infect Dis* 2010;**202**:1287–95.
465. McCullers JA, Van De Velde LA, Schultz RD, et al. Seroprevalence of seasonal and pandemic influenza A viruses in domestic cats. *Arch Virol* 2011;**156**:117–20.
466. McMahon AW, Iskander J, Haber P, et al. Adverse events after inactivated influenza vaccination among children less than 2 years of age: analysis of reports from the vaccine adverse event reporting system, 1990-2003. *Pediatrics* 2005;**115**:453–60.
467. McPherson C, Warner B, Hunstad DA, et al. Oseltamivir dosing in premature infants. *J Infect Dis* 2012;**206**:847–50.
468. Meijer A, Lackenby A, Hungnes O, et al. Oseltamivir-resistant influenza virus A (H1N1), Europe, 2007-08 season. *Emerg Infect Dis* 2009;**15**:552–60.
469. Miller EK, Griffin MR, Edwards KM, et al. Influenza burden for children with asthma. *Pediatrics* 2008;**121**:1–8.
470. Mizuguchi M, Yamanouchi H, Ichiyama T, et al. Acute encephalopathy associated with influenza and other viral infections. *Acta Neurol Scand Suppl* 2007;**186**:45–56.
471. Mizuta K, Oshitani H, Mpabalwani EM, et al. An outbreak of influenza A/H3N2 in a Zambian school dormitory. *East Afr Med J* 1995;**72**:189–90.
472. Molinari NA, Ortega-Sanchez IR, Messonnier ML, et al. The annual impact of seasonal influenza in the US: measuring disease burden and costs. *Vaccine* 2007;**25**:5086–96.
473. Monto AS, Kioumehr F. The Tecumseh Study of Respiratory Illness. IX. Occurence of influenza in the community, 1966-1971. *Am J Epidemiol* 1975;**102**:553–63.
474. Monto AS, Koopman JS, Longini IM Jr. Tecumseh study of illness. XIII. Influenza infection and disease, 1976-1981. *Am J Epidemiol* 1985;**121**:811–22.
475. Montomoli E, Khadang B, Piccirella S, et al. Cell culture-derived influenza vaccines from Vero cells: a new horizon for vaccine production. *Expert Rev Vaccines* 2012;**11**:587–94.
476. Moore C, Galiano M, Lackenby A, et al. Evidence of person-to-person transmission of oseltamivir-resistant pandemic influenza A(H1N1) 2009 virus in a hematology unit. *J Infect Dis* 2011; **203**:18–24.
477. Morens DM, Fauci AS. The 1918 influenza pandemic: insights for the 21st century. *J Infect Dis* 2007;**195**:1018–28.
478. Morens DM, Taubenberger JK. Historical thoughts on influenza viral ecosystems, or behold a pale horse, dead dogs, failing fowl, and sick swine. *Influenza Other Respi Viruses* 2010;**4**:27–337.

479. Morens DM, Taubenberger JK. 1918 influenza, a puzzle with missing pieces. *Emerg Infect Dis* 2012;**18**:332–5.
480. Morens DM, Taubenberger JK, Fauci AS. Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. *J Infect Dis* 2008;**198**:962–70.
481. Mori T, Morii M, Terada K, et al. Clinical characteristics and computed tomography findings in children with 2009 pandemic influenza A (H1N1) viral pneumonia. *Scand J Infect Dis* 2011;**43**: 47–54.
482. Morishima T, Togashi T, Yokota S, et al. Encephalitis and encephalopathy associated with an influenza epidemic in Japan. *Clin Infect Dis* 2002;**35**:512–17.
483. Muhammad RD, Haber P, Broder KR, et al. Adverse events following trivalent inactivated influenza vaccination in children: analysis of the Vaccine Adverse Event Reporting System. *Pediatr Infect Dis J* 2010.
484. Mukherjee S, Majumdar S, Vipat VC, et al. Non structural protein of avian influenza A (H11N1) virus is a weaker suppressor of immune responses but capable of inducing apoptosis in host cells. *Virol J* 2012;**9**:149.
485. Mullooly JP, Barker WH. Impact of type A influenza on children: a retrospective study. *Am J Public Health* 1982;**72**:1008–16.
486. Munoz FM, Campbell JR, Atmar RL, et al. Influenza A virus outbreak in a neonatal intensive care unit. *Pediatr Infect Dis J* 1999;**18**:811–15.
487. Munster VJ, de Wit E, van den Brand JM, et al. Pathogenesis and transmission of swine-origin 2009 A(H1N1) influenza virus in ferrets. *Science* 2009;**325**:481–3.
488. Munster VJ, de Wit E, van Riel D, et al. The molecular basis of the pathogenicity of the Dutch highly pathogenic human influenza A H7N7 viruses. *J Infect Dis* 2007;**196**:258–65.
489. Murata Y, Walsh EE, Falsey AR. Pulmonary complications of interpandemic influenza A in hospitalized adults. *J Infect Dis* 2007;**195**:1029–37.
490. Myers KP, Olsen CW, Gray GC. Cases of swine influenza in humans: a review of the literature. *Clin Infect Dis* 2007;**44**: 1084–8.
491. Nagao T, Morishima T, Kimura H, et al. Prognostic factors in influenza-associated encephalopathy. *Pediatr Infect Dis J* 2008;**27**: 384–9.
492. Nair H, Brooks WA, Katz M, et al. Global burden of respiratory infections due to seasonal influenza in young children: a systematic review and meta-analysis. *Lancet* 2011;**378**:1917–30.
493. Nelson MI, Vincent AL, Kitikoon P, et al. Evolution of novel reassortant A/H3N2 influenza viruses in North American swine and humans, 2009-2011. *J Virol* 2012;**86**:8872–8.
494. Neumann G, Kawaoka Y. Reverse genetics of influenza virus. *Virology* 2001;**287**:243–50.
495. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. *Nature* 2009; **459**:931–9.
496. Neumann G, Watanabe T, Ito H, et al. Generation of influenza A viruses entirely from cloned cDNAs. *Proc Natl Acad Sci U S A* 1999;**96**:9345–50.
497. Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. *J Infect Dis* 2006; **194**:1032–9.
498. Neuzil KM, Mellen BG, Wright PF, et al. The effect of influenza on hospitalizations, outpatient visits, and courses of antibiotics in children. *N Engl J Med* 2000;**342**:225–31.
499. Neuzil KM, Wright PF, Mitchel EF Jr, et al. The burden of influenza illness in children with asthma and other chronic medical conditions. *J Pediatr* 2000;**137**:856–64.
500. Neuzil KM, Zhu Y, Griffin MR, et al. Burden of interpandemic influenza in children younger than 5 years: a 25-year prospective study. *J Infect Dis* 2002;**185**:147–52.
501. Newall AT, Jit M, Beutels P. Economic evaluations of childhood influenza vaccination: a critical review. *Pharmacoeconomics* 2012; **30**:647–60.
502. Newland JG, Laurich VM, Rosenquist AW, et al. Neurologic complications in children hospitalized with influenza: characteristics, incidence, and risk factors. *J Pediatr* 2007;**150**: 306–10.
503. Newland JG, Romero JR, Varman M, et al. Encephalitis associated with influenza B virus infection in 2 children and a review of the literature. *Clin Infect Dis* 2003;**36**:e87–95.
504. Newman AP, Reisdorf E, Beinemann J, et al. Human case of swine influenza A (H1N1) triple reassortant virus infection, Wisconsin. *Emerg Infect Dis* 2008;**14**:1470–2.
505. Nguyen HT, Dharan NJ, Le MT, et al. National influenza surveillance in Vietnam, 2006-2007. *Vaccine* 2009;**28**:398–402.
506. Nichol KL. Cost-effectiveness and socio-economic aspects of childhood influenza vaccination. *Vaccine* 2011;**29**:7554–8.
507. Nicholls JM, Chan MC, Chan WY, et al. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nat Med* 2007;**13**:147–9.
508. Nielsen O, Clavijo A, Boughen JA. Serologic evidence of influenza A infection in marine mammals of arctic Canada. *J Wildl Dis* 2001;**37**:820–5.
509. Ninove L, Daniel L, Gallou J, et al. Fatal case of Reye's syndrome associated with H3N2 influenza virus infection and salicylate intake in a 12-year-old patient. *Clin Microbiol Infect* 2011;**17**: 95–7.
510. Nishiyama I, Ohtsuka Y, Tsuda T, et al. An epidemiological study of children with status epilepticus in Okayama, Japan: incidence, etiologies, and outcomes. *Epilepsy Res* 2011;**96**:89–95.
511. Nitsch-Osuch A, Stefanska I, Kuchar E, et al. Influence of rapid influenza test on clinical management of children younger than five with febrile respiratory tract infections. *Adv Exp Med Biol* 2013;**755**:237–41.
512. Noble GR. In: Beare AS, editor. *Basic and applied influenza research: epidemiological and clinical aspects of influenza*. Boca Raton, Flda: CRC Press; 1982. p. 11–50.
513. Nohynek H, Jokinen J, Partinen M, et al. AS03 adjuvanted AH1N1 vaccine associated with an abrupt increase in the incidence of childhood narcolepsy in Finland. *PLoS One* 2012;**7**:e33536.
514. Nolte KB, Alakija P, Oty G, et al. Influenza A virus infection complicated by fatal myocarditis. *Am J Forensic Med Pathol* 2000;**21**:375–9.
515. O'Brien KL, Walters MI, Sellman J, et al. Severe pneumococcal pneumonia in previously healthy children: the role of preceding influenza infection. *Clin Infect Dis* 2000;**30**:784–9.
516. O'Brien MA, Uyeki TM, Shay DK, et al. Incidence of outpatient visits and hospitalizations related to influenza in infants and young children. *Pediatrics* 2004;**113**:585–93.
517. Ohmit SE, Monto AS. Symptomatic predictors of influenza virus positivity in children during the influenza season. *Clin Infect Dis* 2006;**43**:564–8.
518. Okomo-Adhiambo M, Hurt AC, Gubareva LV. The chemiluminescent neuraminidase inhibition assay: a functional method for detection of influenza virus resistance to the neuraminidase inhibitors. *Methods Mol Biol* 2012;**865**:95–113.
519. Olsen B, Munster VJ, Wallensten A, et al. Global patterns of influenza a virus in wild birds. *Science* 2006;**312**:384–8.
520. Olsen CW. The emergence of novel swine influenza viruses in North America. *Virus Res* 2002;**85**:199–210.
521. Omer SB, Goodman D, Steinhoff MC, et al. Maternal influenza immunization and reduced likelihood of prematurity and small for gestational age births: a retrospective cohort study. *PLoS Med* 2011;**8**:e1000441.
522. Oner AF, Dogan N, Gasimov V, et al. H5N1 avian influenza in children. *Clin Infect Dis* 2012;**55**:26–32.
523. Ooi PL, Lai FY, Low CL, et al. Clinical and molecular evidence for transmission of novel influenza A(H1N1/2009) on a commercial airplane. *Arch Intern Med* 2010;**170**:913–15.
524. Ortega-Sanchez IR, Molinari NA, Fairbrother G, et al. Indirect, out-of-pocket and medical costs from influenza-related illness in young children. *Vaccine* 2012;**30**:4175–81.
525. Ortiz JR, Neuzil KM, Victor JC, et al. Influenza-associated cystic fibrosis pulmonary exacerbations. *Chest* 2010;**137**:852–60.
526. Oseasohn R, Adelson L, Kaji M. Clinicopathologic study of thirty-three fatal cases of Asian influenza. *N Engl J Med* 1959; **260**:509–18.
527. Osterhaus AD, Rimmelzwaan GF, Martina BE, et al. Influenza B virus in seals. *Science* 2000;**288**:1051–3.
528. Osterholm MT, Kelley NS, Sommer A, et al. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:36–44.

529. Ostrowsky B, Huang A, Terry W, et al. Low pathogenic avian influenza A (H7N2) virus infection in immunocompromised adult, New York, USA, 2003. *Emerg Infect Dis* 2012;**18**:1128–31.
530. Oughton M, Dascal A, Laporta D, et al. Evidence of viremia in 2 cases of severe pandemic influenza A H1N1/09. *Diagn Microbiol Infect Dis* 2011;**70**:213–17.
531. Ozdemir H, Karbuz A, Ciftci E, et al. Aseptic meningitis in a child due to 2009 pandemic influenza A (H1N1) infection. *Turk J Pediatr* 2011;**53**:91–3.
532. Ozkale Y, Erol I, Ozkale M, et al. Acute disseminated encephalomyelitis associated with influenza A H1N1 infection. *Pediatr Neurol* 2012;**47**:62–4.
533. Ozkan M, Tuygun N, Erkek N, et al. Neurologic manifestations of novel influenza A (H1N1) virus infection in childhood. *Pediatr Neurol* 2011;**45**:72–6.
534. Paddock CD, Liu L, Denison AM, et al. Myocardial injury and bacterial pneumonia contribute to the pathogenesis of fatal influenza B virus infection. *J Infect Dis* 2012;**205**:895–905.
535. Paisley JW, Bruhn FW, Lauer BA, et al. Type A2 influenza viral infections in children. *Am J Dis Child* 1978;**132**:34–6.
536. Palese P, Shaw M. Orthomyxoviridae: the viruses and their replication. In: Knipe DM, Shaw NL, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1647–89.
537. Palese P, Young JF. Variation of influenza A, B, and C viruses. *Science* 1982;**215**:1468–74.
538. Pan American Health Organization Health Surveillance and Disease Management Area Communicable Disease Unit. *PAHO-CDC Generic Protocol for Influenza Surveillance*. 15 December 2006. Available at: http://new.paho.org/hq/images/stories/AD/HSD/CD/INFLUENZA/flu-snl-gpis.pdf.
539. Pannaraj PS, Tam B, Akan D. Oseltamivir treatment and prophylaxis in a neonatal intensive care unit during a 2009 H1N1 influenza outbreak. *J Perinatol* 2011;**31**:487–93.
540. Papic N, Pangercic A, Vargovic M, et al. Liver involvement during influenza infection: perspective on the 2009 influenza pandemic. *Influenza Other Respi Viruses* 2012;**6**:e2–5.
541. Pappas C, Aguilar PV, Basler CF, et al. Single gene reassortants identify a critical role for PB1, HA, and NA in the high virulence of the 1918 pandemic influenza virus. *Proc Natl Acad Sci U S A* 2008;**105**:3064–9.
542. Partinen M, Saarenpaa-Heikkila O, Ilveskoski I, et al. Increased incidence and clinical picture of childhood narcolepsy following the 2009 H1N1 pandemic vaccination campaign in Finland. *PLoS One* 2012;**7**:e33723.
543. Patel A, Gorman SE. Stockpiling antiviral drugs for the next influenza pandemic. *Clin Pharmacol Ther* 2009;**86**:241–3.
544. Patel P, Nandwani V, Vanchiere J, et al. Use of therapeutic plasma exchange as a rescue therapy in 2009 pH1N1 influenza A: an associated respiratory failure and hemodynamic shock. *Pediatr Crit Care Med* 2011;**12**:e87–9.
545. Patrozou E, Mermel LA. Does influenza transmission occur from asymptomatic infection or prior to symptom onset? *Public Health Rep* 2009;**124**:193–6.
546. Peebles PJ, Dhara R, Brammer L, et al. Influenza-associated mortality among children: United States, 2007-2008. *Influenza Other Respi Viruses* 2011;**5**:25–31.
547. Peiris JS, Yu WC, Leung CW, et al. Re-emergence of fatal human influenza A subtype H5N1 disease. *Lancet* 2004;**363**:617–19.
548. Peiris M, Yuen KY, Leung CW, et al. Human infection with influenza H9N2. *Lancet* 1999;**354**:916–17.
549. Peltola V, Reunanen T, Ziegler T, et al. Accuracy of clinical diagnosis of influenza in outpatient children. *Clin Infect Dis* 2005;**41**:1198–200.
550. Peltola V, Ziegler T, Ruuskanen O. Influenza A and B virus infections in children. *Clin Infect Dis* 2003;**36**:299–305.
551. Perdue ML, Arnold F, Li S, et al. The future of cell culture-based influenza vaccine production. *Expert Rev Vaccines* 2011;**10**:1183–94.
552. Petousis-Harris H, Poole T, Booy R, et al. Fever following administration of two inactivated influenza vaccines: a survey of parents of New Zealand infants and children 5 years of age and under. *Vaccine* 2011;**29**:2933–7.
553. Piedra PA, Gaglani MJ, Kozinetz CA, et al. Herd immunity in adults against influenza-related illnesses with use of the trivalent-live attenuated influenza vaccine (CAIV-T) in children. *Vaccine* 2005;**23**:1540–8.
554. Piedra PA, Gaglani MJ, Riggs M, et al. Live attenuated influenza vaccine, trivalent, is safe in healthy children 18 months to 4 years, 5 to 9 years, and 10 to 18 years of age in a community-based, nonrandomized, open-label trial. *Pediatrics* 2005;**116**:e397–407.
555. Piedra PA, Yan L, Kotloff K, et al. Safety of the trivalent, cold-adapted influenza vaccine in preschool-aged children. *Pediatrics* 2002;**110**:662–72.
556. Pierron S, Haas H, Berlioz M, et al. [Impact of rapid influenza test during influenza epidemic in all febrile children less than 6 years old in a pediatric emergency department]. *Arch Pediatr* 2008;**15**:1283–8.
557. Pinsky BA, Mix S, Rowe J, et al. Long-term shedding of influenza A virus in stool of immunocompromised child. *Emerg Infect Dis* 2010;**16**:1165–7.
558. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. *N Engl J Med* 2006;**355**:31–40.
559. Poehling KA, Talbot HK, Williams JV, et al. Impact of a school-based influenza immunization program on disease burden: comparison of two Tennessee counties. *Vaccine* 2009;**27**:2695–700.
560. Polkinghorne BG, Muscatello DJ, Macintyre CR, et al. Relationship between the population incidence of febrile convulsions in young children in Sydney, Australia and seasonal epidemics of influenza and respiratory syncytial virus, 2003-2010: a time series analysis. *BMC Infect Dis* 2011;**11**:291.
561. Price DA, Postlethwaite RJ, Longson M. Influenzavirus A2 infections presenting with febrile convulsions and gastrointestinal symptoms in young children. *Clin Pediatr (Phila)* 1976;**15**:361–7.
562. Prosser LA, Bridges CB, Uyeki TM, et al. Values for preventing influenza-related morbidity and vaccine adverse events in children. *Health Qual Life Outcomes* 2005;**3**:18.
563. Prosser LA, Lavelle TA, Fiore AE, et al. Cost-effectiveness of 2009 pandemic influenza A(H1N1) vaccination in the United States. *PLoS One* 2011;**6**:e22308.
564. Prosser LA, Meltzer MI, Fiore A, et al. Effects of adverse events on the projected population benefits and cost-effectiveness of using live attenuated influenza vaccine in children aged 6 months to 4 years. *Arch Pediatr Adolesc Med* 2011;**165**:112–18.
565. Puzelli S, Buonaguro FM, Facchini M, et al. Cardiac tamponade and heart failure due to myopericarditis as a presentation of infection with the pandemic H1N1 2009 influenza A virus. *J Clin Microbiol* 2010;**48**:2298–300.
566. Qi L, Davis AS, Jagger BW, et al. Analysis by single-gene reassortment demonstrates that the 1918 influenza virus is functionally compatible with a low-pathogenicity avian influenza virus in mice. *J Virol* 2012;**86**:9211–20.
567. Quach C, Piche-Walker L, Platt R, et al. Risk factors associated with severe influenza infections in childhood: implication for vaccine strategy. *Pediatrics* 2003;**112**:e197–201.
568. Quandt D, Valsangiacomo Buechel ER, Knirsch W. Pericarditis constrictiva in a 10-year-old boy after influenza A virus infection. *Pediatr Cardiol* 2011;**32**:525–6.
569. Rambaut A, Pybus OG, Nelson MI, et al. The genomic and epidemiological dynamics of human influenza A virus. *Nature* 2008;**453**:615–19.
570. Randolph AG, Vaughn F, Sullivan R, et al. Critically ill children during the 2009-10 influenza pandemic in the United States. *Pediatrics* 2011;**128**:e1450–8.
571. Rasmussen SA, Kissin DM, Yeung LF, et al. Preparing for influenza after 2009 H1N1: special considerations for pregnant women and newborns. *Am J Obstet Gynecol* 2011;**204**:S13–20.
572. Razuri H, Romero C, Tinoco Y, et al. Population-based active surveillance cohort studies for influenza: lessons from Peru. *Bull World Health Organ* 2012;**90**:318–20.
573. Reed C, Meltzer MI, Finelli L, et al. Public health impact of including two lineages of influenza B in a quadrivalent seasonal influenza vaccine. *Vaccine* 2012;**30**:1993–8.
574. Rello J, Rodriguez A, Ibanez P, et al. Intensive care adult patients with severe respiratory failure caused by Influenza A (H1N1)v in Spain. *Crit Care* 2009;**13**:R148.
575. Rellosa N, Bloch KC, Shane AL, et al. Neurologic manifestations of pediatric novel h1n1 influenza infection. *Pediatr Infect Dis J* 2011;**30**:165–7.
576. Reperant LA, Kuiken T, Osterhaus AD. Influenza viruses: from birds to humans. *Hum Vaccin Immunother* 2012;**8**:7–16.

577. Reperant LA, Rimmelzwaan GF, Kuiken T. Avian influenza viruses in mammals. *Rev Sci Tech* 2009;**28**:137–59.
578. Ritzwoller DP, Bridges CB, Shetterly S, et al. Effectiveness of the 2003-2004 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. *Pediatrics* 2005;**116**:153–9.
579. Roberton SI, Bell DJ, Smith GJ, et al. Avian influenza H5N1 in viverrids: implications for wildlife health and conservation. *Proc Biol Sci* 2006;**273**:1729–32.
580. Robinson S, Averhoff F, Kiel J, et al. Pandemic influenza a in residential summer camps: Maine, 2009. *Pediatr Infect Dis J* 2012;**31**: 547–50.
581. Rocha E, Cox NJ, Black RA, et al. Antigenic and genetic variation in influenza A (H1N1) virus isolates recovered from a persistently infected immunodeficient child. *J Virol* 1991;**65**:2340–50.
582. Rojo JC, Ruiz-Contreras J, Fernandez MB, et al. Influenza-related hospitalizations in children younger than three years of age. *Pediatr Infect Dis J* 2006;**25**:596–601.
583. Rosenberg M, Sparks R, McMahon A, et al. Serious adverse events rarely reported after trivalent inactivated influenza vaccine (TIV) in children 6-23 months of age. *Vaccine* 2009;**27**:4278–83.
584. Rota PA, Wallis TR, Harmon MW, et al. Cocirculation of two distinct evolutionary lineages of influenza type B virus since 1983. *Virology* 1990;**175**:59–68.
585. Rothberg MB, Fisher D, Kelly B, et al. Management of influenza symptoms in healthy children: cost-effectiveness of rapid testing and antiviral therapy. *Arch Pediatr Adolesc Med* 2005;**159**: 1055–62.
586. Russell CA, Fonville JM, Brown AE, et al. The potential for respiratory droplet-transmissible A/H5N1 influenza virus to evolve in a mammalian host. *Science* 2012;**336**:1541–7.
587. Russell CA, Jones TC, Barr IG, et al. Influenza vaccine strain selection and recent studies on the global migration of seasonal influenza viruses. *Vaccine* 2008;**26**(Suppl. 4):D31–4.
588. Russell CA, Jones TC, Barr IG, et al. The global circulation of seasonal influenza A (H3N2) viruses. *Science* 2008;**320**: 340–6.
589. Russell RJ, Kerry PS, Stevens DJ, et al. Structure of influenza hemagglutinin in complex with an inhibitor of membrane fusion. *Proc Natl Acad Sci U S A* 2008;**105**:17736–41.
590. Sachedina N, Donaldson LJ. Paediatric mortality related to pandemic influenza A H1N1 infection in England: an observational population-based study. *Lancet* 2010;**376**:1846–52.
591. Sagrera X, Ginovart G, Raspall F, et al. Outbreaks of influenza A virus infection in neonatal intensive care units. *Pediatr Infect Dis J* 2002;**21**:196–200.
592. Saito R, Oshitani H, Masuda H, et al. Detection of amantadine-resistant influenza A virus strains in nursing homes by PCR-restriction fragment length polymorphism analysis with nasopharyngeal swabs. *J Clin Microbiol* 2002;**40**:84–8.
593. Sakabe S, Takano R, Nagamura-Inoue T, et al. Differences in cytokine production in human macrophages and in virulence in mice are attributable to the acidic polymerase protein of highly pathogenic influenza a virus subtype H5N1. *J Infect Dis* 2012, doi: 10.1093/infdis/jis523. (Epub ahead of print).
594. Salgado CD, Farr BM, Hall KK, et al. Influenza in the acute hospital setting. *Lancet Infect Dis* 2002;**2**:145–55.
595. Salo H, Kilpi T, Sintonen H, et al. Cost-effectiveness of influenza vaccination of healthy children. *Vaccine* 2006;**24**:4934–41.
596. Savidan E, Chevat C, Marsh G. Economic evidence of influenza vaccination in children. *Health Policy* 2008;**86**:142–52.
597. Schonberger LB, Bregman DJ, Sullivan-Bolyai JZ, et al. Guillain-Barré syndrome following vaccination in the National Influenza Immunization Program, United States, 1976–1977. *Am J Epidemiol* 1979;**110**:105–23.
598. Schonberger LB, Hurwitz ES, Katona P, et al. Guillain-Barré syndrome: its epidemiology and associations with influenza vaccination. *Ann Neurol* 1981;**9**(Suppl):31–8.
599. Schrag SJ, Shay DK, Gershman K, et al. Multistate surveillance for laboratory-confirmed, influenza-associated hospitalizations in children: 2003-2004. *Pediatr Infect Dis J* 2006;**25**:395–400.
600. Schultz-Cherry S, Olsen CW, Easterday BC. History of swine influenza. *Curr Top Microbiol Immunol* 2012, doi: 10.1093/infdis/jis523 (Epub ahead of print).
601. Sedyaningsih ER, Isfandari S, Setiawaty V, et al. Epidemiology of cases of H5N1 virus infection in Indonesia, July 2005-June 2006. *J Infect Dis* 2007;**196**:522–7.
602. Sencer DJ, Millar JD. Reflections on the 1976 swine flu vaccination program. *Emerg Infect Dis* 2006;**12**:29–33.
603. Setse RW, Euler GL, Gonzalez-Feliciano AG, et al. Influenza vaccination coverage: United States, 2000-2010. *MMWR Surveill Summ* 2011;**60**(Suppl):38–41.
604. Shaman J, Goldstein E, Lipsitch M. Absolute humidity and pandemic versus epidemic influenza. *Am J Epidemiol* 2011;**173**: 127–35.
605. Shaman J, Kohn M. Absolute humidity modulates influenza survival, transmission, and seasonality. *Proc Natl Acad Sci U S A* 2009;**106**:3243–8.
606. Shaman J, Pitzer VE, Viboud C, et al. Absolute humidity and the seasonal onset of influenza in the continental United States. *PLoS Biol* 2010;**8**:e1000316.
607. Sheng ZM, Chertow DS, Ambroggio X, et al. Autopsy series of 68 cases dying before and during the 1918 influenza pandemic peak. *Proc Natl Acad Sci U S A* 2011;**108**:16416–21.
608. Shenouda A, Hatch FE. Influenza A viral infection associated with acute renal failure. *Am J Med* 1976;**61**:697–702.
609. Sheu TG, Deyde VM, Garten RJ, et al. Detection of antiviral resistance and genetic lineage markers in influenza B virus neuraminidase using pyrosequencing. *Antiviral Res* 2010;**85**:354–60.
610. Shieh WJ, Blau DM, Denison AM, et al. 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States. *Am J Pathol* 2010;**177**:166–75.
611. Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. *N Engl J Med* 2009;**360**:2616–25.
612. Shinjoh M, Takano Y, Takahashi T, et al. Postexposure prophylaxis for influenza in pediatric wards oseltamivir or zanamivir after rapid antigen detection. *Pediatr Infect Dis J* 2012;**31**:1119–23.
613. Shinya K, Ebina M, Yamada S, et al. Avian flu: influenza virus receptors in the human airway. *Nature* 2006;**440**:435–6.
614. Shoji Y, Farrance CE, Bautista J, et al. A plant-based system for rapid production of influenza vaccine antigens. *Influenza Other Respi Viruses* 2012;**6**:204–10.
615. Shu B, Wu KH, Emery S, et al. Design and performance of the CDC real-time reverse transcriptase PCR swine flu panel for detection of 2009 A (H1N1) pandemic influenza virus. *J Clin Microbiol* 2011;**49**:2614–19.
616. Shuler CM, Iwamoto M, Bridges CB, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. *Pediatrics* 2007;**119**: e587–95.
617. Silvennoinen H, Peltola V, Lehtinen P, et al. Clinical presentation of influenza in unselected children treated as outpatients. *Pediatr Infect Dis J* 2009;**28**:372–5.
618. Silvennoinen H, Peltola V, Vainionpaa R, et al. Admission diagnoses of children 0-16 years of age hospitalized with influenza. *Eur J Clin Microbiol Infect Dis* 2012;**31**:225–31.
619. Simmerman JM, Chittaganpitch M, Levy J, et al. Incidence, seasonality and mortality associated with influenza pneumonia in Thailand: 2005-8. *PLoS One* 2009;**4**:e7776.
620. Simmerman JM, Suntarattiwong P, Levy J, et al. Influenza virus contamination of common household surfaces during the 2009 influenza A (H1N1) pandemic in Bangkok, Thailand: implications for contact transmission. *Clin Infect Dis* 2010;**51**:1053–61.
621. Simmerman JM, Suntarattiwong P, Levy J, et al. Findings from a household randomized controlled trial of hand washing and face masks to reduce influenza transmission in Bangkok, Thailand. *Influenza Other Respi Viruses* 2011;**5**:256–67.
622. Simmerman JM, Uyeki TM. The burden of influenza in East and South-East Asia: a review of the English language literature. *Influenza Other Respi Viruses* 2008;**2**:81–92.
623. Siston AM, Rasmussen SA, Honein MA, et al. Pandemic 2009 influenza A(H1N1) virus illness among pregnant women in the United States. *JAMA* 2010;**303**:1517–25.
624. Sivadon-Tardy V, Orlikowski D, Porcher R, et al. Guillain-Barre syndrome and influenza virus infection. *Clin Infect Dis* 2009;**48**: 48–56.
625. Skellett SC, Dhesi R. Myositis, rhabdomyolysis and compartment syndrome complicating influenza A in a child. *BMJ Case Rep* 2009:**2009**.
626. Skowronski DM, Bjornson G, Husain E, et al. Oculorespiratory syndrome after influenza immunization in children. *Pediatr Infect Dis J* 2005;**24**:63–9.

627. Skowronski DM, Janjua NZ, De Serres G, et al. Cross-reactive and vaccine-induced antibody to an emerging swine-origin variant of influenza A virus subtype H3N2 (H3N2v). *J Infect Dis* 2012; **206**:1852–61.
628. Skowronski DM, Tweed SA, Petric M, et al. Human illness and isolation of low-pathogenicity avian influenza virus of the H7N3 subtype in British Columbia, Canada. *J Infect Dis* 2006;**193**:899–900; author reply 900–891.
629. Skowronski DM, Woolcott JC, Tweed SA, et al. Potential cost-effectiveness of annual influenza immunization for infants and toddlers: experience from Canada. *Vaccine* 2006;**24**:4222–32.
630. Smith DJ, Lapedes AS, de Jong JC, et al. Mapping the antigenic and genetic evolution of influenza virus. *Science* 2004;**305**: 371–6.
631. Smith GJ, Bahl J, Vijaykrishna D, et al. Dating the emergence of pandemic influenza viruses. *Proc Natl Acad Sci U S A* 2009;**106**: 11709–12.
632. Smith GJ, Vijaykrishna D, Bahl J, et al. Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* 2009;**459**:1122–5.
633. Smith W, Andrewes CH, Laidlaw PP. A virus obtained from influenza patients. *Lancet* 1933;**2**:66–8.
634. Smitherman HF, Caviness AC, Macias CG. Retrospective review of serious bacterial infections in infants who are 0 to 36 months of age and have influenza A infection. *Pediatrics* 2005;**115**: 710–18.
635. Sobata R, Matsumoto C, Igarashi M, et al. No viremia of pandemic (H1N1) 2009 was demonstrated in blood donors who had donated blood during the probable incubation period. *Transfusion* 2011;**51**:1949–56.
636. Sorbello A, Jones SC, Carter W, et al. Emergency use authorization for intravenous peramivir: evaluation of safety in the treatment of hospitalized patients infected with 2009 H1N1 influenza A virus. *Clin Infect Dis* 2012;**55**:1–7.
637. Spelman DW, McHardy CJ. Concurrent outbreaks of influenza A and influenza B. *J Hyg (Lond)* 1985;**94**:331–9.
638. Staat MA, Griffin MR, Donauer S, et al. Vaccine effectiveness for laboratory-confirmed influenza in children 6-59 months of age, 2005-2007. *Vaccine* 2011;**29**:9005–11.
639. Standing JF, Nika A, Tsagris V, et al. Oseltamivir pharmacokinetics and clinical experience in neonates and infants during an outbreak of H1N1 influenza A virus infection in a neonatal intensive care unit. *Antimicrob Agents Chemother* 2012;**56**:3833–40.
640. Starko KM, Ray CG, Dominguez LB, et al. Reye's syndrome and salicylate use. *Pediatrics* 1980;**66**:859–64.
641. Stebbins S, Cummings DA, Stark JH, et al. Reduction in the incidence of influenza A but not influenza B associated with use of hand sanitizer and cough hygiene in schools: a randomized controlled trial. *Pediatr Infect Dis J* 2011;**30**:921–6.
642. Steel HM, Peppercorn AF. Fatal respiratory events caused by zanamivir nebulization. *Clin Infect Dis* 2010;**51**:121.
643. Steinhoff MC, Omer SB, Roy E, et al. Influenza immunization in pregnancy: antibody responses in mothers and infants. *N Engl J Med* 2010;**362**:1644–6.
644. Steinhoff MC, Omer SB, Roy E, et al. Neonatal outcomes after influenza immunization during pregnancy: a randomized controlled trial. *CMAJ* 2012;**184**:645–53.
645. Stephenson I, Democratis J, Lackenby A, et al. Neuraminidase inhibitor resistance after oseltamivir treatment of acute influenza A and B in children. *Clin Infect Dis* 2009;**48**:389–96.
646. Stramer SL, Collins C, Nugent T, et al. Sensitive detection assays for influenza RNA do not reveal viremia in US blood donors. *J Infect Dis* 2012;**205**:886–94.
647. Stratton K, Ford A, Rusch E, et al. *Adverse effects of vaccines: evidence and causality*. Washington, DC: Institute of Medicine, National Academies Press; 2012. p. 293–420.
648. Strong M, Burrows J, Stedman E, et al. Adverse drug effects following oseltamivir mass treatment and prophylaxis in a school outbreak of 2009 pandemic influenza A(H1N1) in June 2009, Sheffield, United Kingdom. *Euro Surveill* 2010;**15**(pii):19565.
649. Strouse JJ, Reller ME, Bundy DG, et al. Severe pandemic H1N1 and seasonal influenza in children and young adults with sickle cell disease. *Blood* 2010;**116**:3431–4.
650. Subbarao K, Klimov A, Katz J, et al. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. *Science* 1998;**279**:393–6.
651. Suess T, Remschmidt C, Schink SB, et al. The role of facemasks and hand hygiene in the prevention of influenza transmission in households: results from a cluster randomised trial; Berlin, Germany, 2009-2011. *BMC Infect Dis* 2012;**12**:26.
652. Sugaya N. Influenza-associated encephalopathy in Japan: pathogenesis and treatment. *Pediatr Int* 2000;**42**:215–18.
653. Sugaya N, Kohno S, Ishibashi T, et al. Efficacy, safety, and pharmacokinetics of intravenous peramivir in children with 2009 pandemic H1N1 influenza A virus infection. *Antimicrob Agents Chemother* 2012;**56**:369–77.
654. Sugaya N, Mitamura K, Yamazaki M, et al. Lower clinical effectiveness of oseltamivir against influenza B contrasted with influenza A infection in children. *Clin Infect Dis* 2007;**44**: 197–202.
655. Sugaya N, Ohashi Y. Long-acting neuraminidase inhibitor laninamivir octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus infection. *Antimicrob Agents Chemother* 2010;**54**:2575–82.
656. Sugaya N, Shinjoh M, Mitamura K, et al. Very low pandemic influenza A (H1N1) 2009 mortality associated with early neuraminidase inhibitor treatment in Japan: analysis of 1000 hospitalized children. *J Infect* 2011;**63**:288–94.
657. Swaringen JC, Seiler JG 3rd, Bruce RW Jr. Influenza A induced rhabdomyolysis resulting in extensive compartment syndrome. *Clin Orthop Relat Res* 2000:243–9.
658. Szilagyi PG, Fairbrother G, Griffin MR, et al. Influenza vaccine effectiveness among children 6 to 59 months of age during 2 influenza seasons: a case-cohort study. *Arch Pediatr Adolesc Med* 2008;**162**:943–51.
659. Taber LH, Paredes A, Glezen WP, et al. Infection with influenza A/Victoria virus in Houston families, 1976. *J Hyg (Lond)* 1981;**86**: 303–13.
660. Takanashi J, Tada H, Kuroki H, et al. Delirious behavior in influenza is associated with a reversible splenial lesion. *Brain Dev* 2009;**31**:423–6.
661. Talaat M, Afifi S, Dueger E, et al. Effects of hand hygiene campaigns on incidence of laboratory-confirmed influenza and absenteeism in schoolchildren, Cairo, Egypt. *Emerg Infect Dis* 2011;**17**:619–25.
662. Tamura D, Fujino M, Ozawa M, et al. Significance of seasonal influenza viruses in the stool of pediatric patients. *Pediatr Infect Dis J* 2010;**29**:578–9.
663. Tamura D, Sugaya N, Ozawa M, et al. Frequency of drug-resistant viruses and virus shedding in pediatric influenza patients treated with neuraminidase inhibitors. *Clin Infect Dis* 2011;**52**:432–7.
664. Tanabe T, Hara K, Nakajima M, et al. Oseltamivir treatment for children showing abnormal behavior during influenza virus infection. *Brain Dev* 2010;**32**:440–4.
665. Tanaka T, Nakajima K, Murashima A, et al. Safety of neuraminidase inhibitors against novel influenza A (H1N1) in pregnant and breastfeeding women. *CMAJ* 2009;**181**:55–8.
666. Tang JW, Lai FY, Nymadawa P, et al. Comparison of the incidence of influenza in relation to climate factors during 2000-2007 in five countries. *J Med Virol* 2010;**82**:1958–65.
667. Tasher D, Stein M, Simoes EA, et al. Invasive bacterial infections in relation to influenza outbreaks, 2006-2010. *Clin Infect Dis* 2011; **53**:1199–207.
668. Taubenberger JK, Morens DM. 1918 Influenza: the mother of all pandemics. *Emerg Infect Dis* 2006;**12**:15–22.
669. Tellier R. Aerosol transmission of influenza A virus: a review of new studies. *J R Soc Interface* 2009;**6**(Suppl. 6):S783–90.
670. Terebuh P, Olsen CW, Wright J, et al. Transmission of influenza A viruses between pigs and people, Iowa, 2002-2004. *Influenza Other Respi Viruses* 2010;**4**:387–96.
671. Thiry E, Zicola A, Addie D, et al. Highly pathogenic avian influenza H5N1 virus in cats and other carnivores. *Vet Microbiol* 2007;**122**:25–31.
672. Thompson WW, Moore MR, Weintraub E, et al. Estimating influenza-associated deaths in the United States. *Am J Public Health* 2009;**99**(Suppl. 2):S225–30.
673. Thompson WW, Price C, Goodson B, et al. Early thimerosal exposure and neuropsychological outcomes at 7 to 10 years. *N Engl J Med* 2007;**357**:1281–92.
674. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;**289**:179–86.

675. Thompson WW, Shay DK, Weintraub E, et al. Influenza-associated hospitalizations in the United States. *JAMA* 2004; **292**:1333–40.
676. Thompson WW, Weintraub E, Dhankhar P, et al. Estimates of US influenza-associated deaths made using four different methods. *Influenza Other Respi Viruses* 2009;**3**:37–49.
677. Thomson D. Influenza. Part I. With special reference to the part played by Pfeiffer's bacillus, streptococci, pneumococci, etc. and the virus theory. *Ann Picket-Thomson Res Lab* 1933;**9**:142.
678. Tisoncik JR, Korth MJ, Simmons CP, et al. Into the eye of the cytokine storm. *Microbiol Mol Biol Rev* 2012;**76**:16–32.
679. To KK, Chan KH, Li IW, et al. Viral load in patients infected with pandemic H1N1 2009 influenza A virus. *J Med Virol* 2010;**82**: 1–7.
680. Togashi T, Matsuzono Y, Narita M, et al. Influenza-associated acute encephalopathy in Japanese children in 1994-2002. *Virus Res* 2004;**103**:75–8.
681. Tokars JI, Lewis P, DeStefano F, et al. The risk of Guillain-Barré syndrome associated with influenza A (H1N1) 2009 monovalent vaccine and 2009-2010 seasonal influenza vaccines: results from self-controlled analyses. *Pharmacoepidemiol Drug Saf* 2012;**21**: 546–52.
682. Tong S, Li Y, Rivailler P, et al. A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci U S A* 2012;**109**:4269–74.
683. Tran D, Vaudry W, Moore DL, et al. Comparison of children hospitalized with seasonal versus pandemic influenza A, 2004-2009. *Pediatrics* 2012;**130**:397–406.
684. Treanor JJ, Talbot HK, Ohmit SE, et al. Effectiveness of seasonal influenza vaccines in the United States during a season with circulation of all three vaccine strains. *Clin Infect Dis* 2012;**55**: 951–9.
685. Trevennec K, Leger L, Lyazrhi F, et al. Transmission of pandemic influenza H1N1 (2009) in Vietnamese swine in 2009-2010. *Influenza Other Respi Viruses* 2012;**6**:348–57.
686. Tsagris V, Nika A, Kyriakou D, et al. Influenza A/H1N1/2009 outbreak in a neonatal intensive care unit. *J Hosp Infect* 2012; **81**:36–40.
687. Tse A, Tseng HF, Greene SK, et al. Signal identification and evaluation for risk of febrile seizures in children following trivalent inactivated influenza vaccine in the Vaccine Safety Datalink Project, 2010-2011. *Vaccine* 2012;**30**:2024–31.
688. Tse H, To KK, Wen X, et al. Clinical and virological factors associated with viremia in pandemic influenza A/H1N1/2009 virus infection. *PLoS One* 2011;**6**:e22534.
689. Tselios K, Tsioka R, Sarantopoulos A, et al. Influenza A/H1N1 septic shock in a patient with systemic lupus erythematosus: a case report. *BMC Infect Dis* 2011;**11**:358.
690. Tsolia MN, Logotheti I, Papadopoulos NG, et al. Impact of influenza infection in healthy children examined as outpatients and their families. *Vaccine* 2006;**24**:5970–6.
691. Tsuruoka H, Xu H, Kuroda K, et al. [Viremia in influenza: detection by polymerase chain reaction]. *Nihon Rinsho* 1997;**55**: 2714–18.
692. Tumpey TM, Garcia-Sastre A, Taubenberger JK, et al. Pathogenicity of influenza viruses with genes from the 1918 pandemic virus: functional roles of alveolar macrophages and neutrophils in limiting virus replication and mortality in mice. *J Virol* 2005;**79**:14933–44.
693. Tumpey TM, Maines TR, Van Hoeven N, et al. A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission. *Science* 2007;**315**:655–9.
694. Tweed SA, Skowronski DM, David ST, et al. Human illness from avian influenza H7N3, British Columbia. *Emerg Infect Dis* 2004; **10**:2196–9.
695. Ukimura A, Izumi T, Matsumori A. A national survey on myocarditis associated with the 2009 influenza A (H1N1) pandemic in Japan. *Circ J* 2010;**74**:2193–9.
696. Ungchusak K, Auewarakul P, Dowell SF, et al. Probable person-to-person transmission of avian influenza A (H5N1). *N Engl J Med* 2005;**352**:333–40.
697. United Nations Food and Agriculture Organization. Approaches to controlling, preventing, and eliminating H5N1 highly pathogenic avian influenza in endemic countries. Animal Production and Health Paper. No. 171. Rome. 2011.
698. Urushihara H, Doi Y, Arai M, et al. Oseltamivir prescription and regulatory actions vis-a-vis abnormal behavior risk in Japan: drug utilization study using a nationwide pharmacy database. *PLoS One* 2011;**6**:e28483.
699. Uyeki T. Diagnostic testing for 2009 pandemic influenza A (H1N1) virus infection in hospitalized patients. *N Engl J Med* 2009;**361**:e114.
700. Uyeki TM. Influenza diagnosis and treatment in children: a review of studies on clinically useful tests and antiviral treatment for influenza. *Pediatr Infect Dis J* 2003;**22**:164–77.
701. Uyeki TM. Human infection with highly pathogenic avian influenza A (H5N1) virus: review of clinical issues. *Clin Infect Dis* 2009;**49**:279–90.
702. Uyeki TM, Chong YH, Katz JM, et al. Lack of evidence for human-to-human transmission of avian influenza A (H9N2) viruses in Hong Kong, China 1999. *Emerg Infect Dis* 2002;**8**: 154–9.
703. Uyeki TM, Cox NJ. Global concerns regarding novel influenza A (H7N9) virus infections. *N Engl J Med* 2013;**368**:1862–4.
704. Uyeki TM, Prasad R, Vukotich C, et al. Low sensitivity of rapid diagnostic test for influenza. *Clin Infect Dis* 2009;**48**:e89–92.
705. Uyeki TM, Zane SB, Bodnar UR, et al. Large summertime influenza A outbreak among tourists in Alaska and the Yukon Territory. *Clin Infect Dis* 2003;**36**:1095–102.
706. van den Dungen FA, van Furth AM, Fetter WP, et al. Fatal case of influenza B virus pneumonia in a preterm neonate. *Pediatr Infect Dis J* 2001;**20**:82–4.
707. Van Kerkhove MD, Vandemaele KA, Shinde V, et al. Risk factors for severe outcomes following 2009 influenza A (H1N1) infection: a global pooled analysis. *PLoS Med* 2011;**8**:e1001053.
708. van Riel D, den Bakker MA, Leijten LM, et al. Seasonal and pandemic human influenza viruses attach better to human upper respiratory tract epithelium than avian influenza viruses. *Am J Pathol* 2010;**176**:1614–18.
709. van Velzen E, Hutchinson S, Penrice G, et al. Compliance to oseltamivir and subsequent occurrence of self-reported adverse drug reactions among nursery and primary school children following exposure to Influenza A(H1N1)v. *Scott Med J* 2011;**56**:120.
710. Vana G, Westover KM. Origin of the 1918 Spanish influenza virus: a comparative genomic analysis. *Mol Phylogenet Evol* 2008; **47**:1100–10.
711. Vandermeer ML, Thomas AR, Kamimoto L, et al. Association between use of statins and mortality among patients hospitalized with laboratory-confirmed influenza virus infections: a multistate study. *J Infect Dis* 2012;**205**:13–19.
712. Varga ZT, Ramos I, Hai R, et al. The influenza virus protein PB1-F2 inhibits the induction of type I interferon at the level of the MAVS adaptor protein. *PLoS Pathog* 2011;**7**:e1002067.
713. Vasconcelos A, Abecasis F, Monteiro R, et al. A 3-month-old baby with H1N1 and Guillain-Barré syndrome. *BMJ Case Rep* 2012;**2012**, doi: 10.1136/bcr.12.2011.5462.
714. Vernet G. Use of molecular assays for the diagnosis of influenza. *Expert Rev Anti Infect Ther* 2007;**5**:89–104.
715. Vesikari T, Block SL, Guerra F, et al. Immunogenicity, safety and reactogenicity of a mammalian cell-culture-derived influenza vaccine in healthy children and adolescents three to seventeen years of age. *Pediatr Infect Dis J* 2012;**31**:494–500.
716. Vesikari T, Fleming DM, Aristegui JF, et al. Safety, efficacy, and effectiveness of cold-adapted influenza vaccine-trivalent against community-acquired, culture-confirmed influenza in young children attending day care. *Pediatrics* 2006;**118**:2298–312.
717. Vesikari T, Knuf M, Wutzler P, et al. Oil-in-water emulsion adjuvant with influenza vaccine in young children. *N Engl J Med* 2011;**365**:1406–16.
718. Vesikari T, Pellegrini M, Karvonen A, et al. Enhanced immunogenicity of seasonal influenza vaccines in young children using MF59 adjuvant. *Pediatr Infect Dis J* 2009;**28**:563–71.
719. Viboud C, Alonso WJ, Simonsen L. Influenza in tropical regions. *PLoS Med* 2006;**3**:e89.
720. Vijaykrishna D, Poon LL, Zhu HC, et al. Reassortment of pandemic H1N1/2009 influenza A virus in swine. *Science* 2010;**328**: 1529.
721. Vincent AL, Ma W, Lager KM, et al. Swine influenza viruses a North American perspective. *Adv Virus Res* 2008;**72**:127–54.
722. Waalen K, Kilander A, Dudman SG, et al. Age-dependent prevalence of antibodies cross-reactive to the influenza A(H3N2) variant virus in sera collected in Norway in 2011. *Euro Surveill* 2012;**17**.

723. Wallensten A, Oliver I, Lewis D, et al. Compliance and side effects of prophylactic oseltamivir treatment in a school in South West England. *Euro Surveill* 2009;**14**(30):19285.
724. Walter EB, Englund JA, Blatter M, et al. Trivalent inactivated influenza virus vaccine given to two-month-old children: an off-season pilot study. *Pediatr Infect Dis J* 2009;**28**:1099–104.
725. Walter EB, Neuzil KM, Zhu Y, et al. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming? *Pediatrics* 2006;**118**:e570–8.
726. Wang H, Feng Z, Shu Y, et al. Probable limited person-to-person transmission of highly pathogenic avian influenza A (H5N1) virus in China. *Lancet* 2008;**371**:1427–34.
727. Wang K, Shun-Shin M, Gill P, et al. Neuraminidase inhibitors for preventing and treating influenza in children. *Cochrane Database Syst Rev* 2012;(1):CD002744.
728. Wang S, Hackett A, Jia N, et al. Polyvalent DNA vaccines expressing HA antigens of H5N1 influenza viruses with an optimized leader sequence elicit cross-protective antibody responses. *PLoS One* 2011;**6**:e28757.
729. Wang YH, Huang YC, Chang LY, et al. Clinical characteristics of children with influenza A virus infection requiring hospitalization. *J Microbiol Immunol Infect* 2003;**36**:111–16.
730. Ward KA, Armstrong P, McAnulty JM, et al. Outbreaks of pandemic (H1N1) 2009 and seasonal influenza A (H3N2) on cruise ship. *Emerg Infect Dis* 2010;**16**:1731–7.
731. Warren-Gash C, Fragaszy E, Hayward AC. Hand hygiene to reduce community transmission of influenza and acute respiratory tract infection: a systematic review. *Influenza Other Respi Viruses* 2012, doi: 10.1111/irv.12015 (Epub ahead of print).
732. Wat D, Gelder C, Hibbitts S, et al. The role of respiratory viruses in cystic fibrosis. *J Cyst Fibros* 2008;**7**:320–8.
733. Watanabe T, Kawaoka Y. Pathogenesis of the 1918 pandemic influenza virus. *PLoS Pathog* 2011;**7**:e1001218.
734. Watanabe T, Yoshikawa H, Abe Y, et al. Renal involvement in children with influenza A virus infection. *Pediatr Nephrol* 2003;**18**:541–4.
735. Webster RG. Influenza virus: transmission between species and relevance to emergence of the next human pandemic. *Arch Virol Suppl* 1997;**13**:105–13.
736. Webster RG, Bean WJ, Gorman OT, et al. Evolution and ecology of influenza A viruses. *Microbiol Rev* 1992;**56**:152–79.
737. Webster RG, Geraci J, Petursson G, et al. Conjunctivitis in human beings caused by influenza A virus of seals. *N Engl J Med* 1981;**304**:911.
738. Webster RG, Wright SM, Castrucci MR, et al. Influenza: a model of an emerging virus disease. *Intervirology* 1993;**35**:16–25.
739. Wei CJ, Boyington JC, Dai K, et al. Cross-neutralization of 1918 and 2009 influenza viruses: role of glycans in viral evolution and vaccine design. *Sci Transl Med* 2010;**2**:24ra21.
740. Wentges-van Holthe N, van Eijkeren M, van der Laan JW. Oseltamivir and breastfeeding. *Int J Infect Dis* 2008;**12**:451.
741. White NJ, Webster RG, Govorkova EA, et al. What is the optimal therapy for patients with H5N1 influenza? *PLoS Med* 2009;**6**:e1000091.
742. Whitley RJ, Hayden FG, Reisinger KS, et al. Oral oseltamivir treatment of influenza in children. *Pediatr Infect Dis J* 2001;**20**:127–33.
743. Whitworth JR, Mack CL, O'Connor JA, et al. Acute hepatitis and liver failure associated with influenza A infection in children. *J Pediatr Gastroenterol Nutr* 2006;**43**:536–8.
744. Wildschut ED, de Hoog M, Ahsman MJ, et al. Plasma concentrations of oseltamivir and oseltamivir carboxylate in critically ill children on extracorporeal membrane oxygenation support. *PLoS One* 2010;**5**:e10938.
745. Wilson KM, Pier JC, Wesgate SC, et al. Second-hand tobacco smoke exposure and severity of influenza in hospitalized children. *J Pediatr* 2013;**162**:16–21.
746. Winther B, Block SL, Reisinger K, et al. Impact of oseltamivir treatment on the incidence and course of acute otitis media in children with influenza. *Int J Pediatr Otorhinolaryngol* 2010;**74**:684–8.
747. Wise ME, Viray M, Sejvar JJ, et al. Guillain-Barré syndrome during the 2009-2010 H1N1 influenza vaccination campaign: population-based surveillance among 45 million Americans. *Am J Epidemiol* 2012;**175**:1110–19.
748. Wong BC, Lee N, Li Y, et al. Possible role of aerosol transmission in a hospital outbreak of influenza. *Clin Infect Dis* 2010;**51**:1176–83.
749. Wong CM, Yang L, Chan KP, et al. Influenza-associated hospitalization in a subtropical city. *PLoS Med* 2006;**3**:e121.
750. Wong HK, Lee CK, Hung IF, et al. Practical limitations of convalescent plasma collection: a case scenario in pandemic preparation for influenza A (H1N1) infection. *Transfusion* 2010;**50**:1967–71.
751. World Health Organization. *Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO*. n.d. Available at: http://www.who.int/influenza/human_animal_interface/H5N1_cumulative_table_archives/en/index.html.
752. World Health Organization. *Global Influenza Surveillance and Response System (GISRS)*. n.d. Available at: http://www.who.int/influenza/gisrs_laboratory/en/.
753. World Health Organization. *Manual for the laboratory diagnosis and virological surveillance of influenza*. n.d. Available at: http://whqlibdoc.who.int/publications/2011/9789241548090_eng.pdf.
754. World Health Organization. *WHO Collaborating centres for influenza and essential regulatory laboratories*. n.d. Available at: http://www.who.int/influenza/gisrs_laboratory/collaborating_centres/list/en/index.html.
755. World Health Organization. *Expert Committee on Respiratory Virus Diseases*. First Report No. 170. Geneva: WHO; 1959.
756. World Health Organization. *Clinical management of human infection with avian influenza A (H5N1) virus: updated advice 15 August 2007*. 2007. Available at: http://www.who.int/influenza/resources/documents/ClinicalManagement07.pdf.
757. World Health Organization/OIE/FAO H5N1 Evolution Working Group. Toward a unified nomenclature system for highly pathogenic avian influenza virus (H5N1). *Emerg Infect Dis* 2008;**14**:e1.
758. World Health Organization/OIE/FAO H5N1 Evolution Working Group. Continuing progress towards a unified nomenclature for the highly pathogenic H5N1 avian influenza viruses: divergence of clade 2.2 viruses. *Influenza Other Respi Viruses* 2009;**3**:59–62.
759. World Health Organization. *WHO Interim global epidemiological surveillance standards for influenza*. 2012. Available at: http://www.who.int/influenza/resources/documents/INFSURVMANUAL.pdf.
760. World Health Organization. *WHO guidelines for pharmacological management of pandemic (H1N1) 2009 influenza and other influenza viruses*. 2010. Available at: http://www.who.int/csr/resources/publications/swineflu/h1n1_guidelines_pharmaceutical_mngt.pdf.
761. World Health Organization. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness. *Wkly Epidemiol Rec* 2011;**86**:469–80.
762. World Health Organization. Antigenic and genetic characteristics of zoonotic influenza viruses and development of candidate vaccine viruses for pandemic preparedness. *Wkly Epidemiol Rec* 2012;**87**:401–12.
763. World Health Organization. Meeting of the Strategic Advisory Group of Experts on immunization, April 2012: conclusions and recommendations. *Wkly Epidemiol Rec* 2012;**21**:201–16.
764. World Health Organization. Meetings of the WHO working group on surveillance of influenza antiviral susceptibility: Geneva, November 2011 and June 2012. *Wkly Epidemiol Rec* 2012;**87**:369–74.
765. World Organization for Animal Health (OIE). *Avian influenza*. 2008. Available at: http://web.oie.int/eng/normes/mmanual/2008/pdf/2.03.04_AI.pdf.
766. Wright PF, Bryant JD, Karzon DT. Comparison of influenza B/Hong Kong virus infections among infants, children, and young adults. *J Infect Dis* 1980;**141**:430–5.
767. Wu CT, Hsia SH, Huang JL. Influenza B-associated rhabdomyolysis in Taiwanese children. *Acta Paediatr* 2010;**99**:1701–4.
768. Wu JT, Ho A, Ma ES, et al. Estimating infection attack rates and severity in real time during an influenza pandemic: analysis of serial cross-sectional serologic surveillance data. *PLoS Med* 2011;**8**:e1001103.
769. Wu P, Cowling BJ, Wu JT, et al. The epidemiological and public health research response to 2009 pandemic influenza A(H1N1):

experiences from Hong Kong. *Influenza Other Respi Viruses* 2013;**7**:367–82.
770. Xi X, Xu Y, Jiang L, et al. Hospitalized adult patients with 2009 influenza A(H1N1) in Beijing, China: risk factors for hospital mortality. *BMC Infect Dis* 2010;**10**:256.
771. Xu CL, Yuan F, Chen M, et al. [Risk factors for influenza A (H1N1)-associated pneumonia on hospitalized people less than 18 years old in China, 2009-2010]. *Zhonghua Liu Xing Bing Xue Za Zhi* 2012;**33**:404–8.
772. Xu KM, Li KS, Smith GJ, et al. Evolution and molecular epidemiology of H9N2 influenza A viruses from quail in southern China, 2000 to 2005. *J Virol* 2007;**81**:2635–45.
773. Xu KM, Smith GJ, Bahl J, et al. The genesis and evolution of H9N2 influenza viruses in poultry from southern China, 2000 to 2005. *J Virol* 2007;**81**:10389–401.
774. Xu R, Ekiert DC, Krause JC, et al. Structural basis of preexisting immunity to the 2009 H1N1 pandemic influenza virus. *Science* 2010;**328**:357–60.
775. Xu R, McBride R, Nycholat CM, et al. Structural characterization of the hemagglutinin receptor specificity from the 2009 H1N1 influenza pandemic. *J Virol* 2012;**86**:982–90.
776. Yamada K, Shinmoto H, Hamamoto M, et al. Pneumonia induced by swine-origin influenza A (H1N1) infection: chest computed tomography findings in children. *Jpn J Radiol* 2011;**29**:712–17.
777. Yamnikova SS, Mandler J, Bekh-Ochir ZH, et al. A reassortant H1N1 influenza A virus caused fatal epizootics among camels in Mongolia. *Virology* 1993;**197**:558–63.
778. Yan L, Gao Y, Zhang Y, et al. Epidemiological and virological characteristics of pandemic influenza A (H1N1) school outbreaks in China in 2009. *PLoS One* 2012;**7**:e45898.
779. Yang L, Ma S, Chen PY, et al. Influenza associated mortality in the subtropics and tropics: results from three Asian cities. *Vaccine* 2011;**29**:8909–14.
780. Yang SG, Cao B, Liang LR, et al. Antiviral therapy and outcomes of patients with pneumonia caused by influenza A pandemic (H1N1) virus. *PLoS One* 2012;**7**:e29652.
781. Yano H, Okitsu N, Hori T, et al. Detection of respiratory viruses in nasopharyngeal secretions and middle ear fluid from children with acute otitis media. *Acta Otolaryngol* 2009;**129**:19–24.
782. Yao D, Mizuguchi H, Yamaguchi M, et al. Thermal instability of compound variants of carnitine palmitoyltransferase II and impaired mitochondrial fuel utilization in influenza-associated encephalopathy. *Hum Mutat* 2008;**29**:718–27.
783. Yen CJ, Louie JK, Schechter R. Infants hospitalized in intensive care units with 2009 H1N1 influenza infection, California, 2009-2010. *Pediatr Infect Dis J* 2012;**31**:e52–5.
784. Yen HL, Liang CH, Wu CY, et al. Hemagglutinin-neuraminidase balance confers respiratory-droplet transmissibility of the pandemic H1N1 influenza virus in ferrets. *Proc Natl Acad Sci U S A* 2011;**108**:14264–9.
785. Yildizdas D, Kendirli T, Arslankoylu AE, et al. Neurological complications of pandemic influenza (H1N1) in children. *Eur J Pediatr* 2011;**170**:779–88.
786. Yoo SJ, Moon SJ, Kuak EY, et al. Frequent detection of pandemic (H1N1) 2009 virus in stools of hospitalized patients. *J Clin Microbiol* 2010;**48**:2314–15.
787. Yoshida LM, Suzuki M, Yamamoto T, et al. Viral pathogens associated with acute respiratory infections in central vietnamese children. *Pediatr Infect Dis J* 2010;**29**:75–7.
788. Yu H, Feng Z, Uyeki TM, et al. Risk factors for severe illness with 2009 pandemic influenza A (H1N1) virus infection in China. *Clin Infect Dis* 2011;**52**:457–65.
789. Yu Y, Garg S, Yu PA, et al. Peramivir use for treatment of hospitalized patients with influenza A(H1N1)pdm09 under emergency use authorization, October 2009-June 2010. *Clin Infect Dis* 2012;**55**:8–15.
790. Yusuf K, Soraisham AS, Fonseca K. Fatal influenza B virus pneumonia in a preterm neonate: case report and review of the literature. *J Perinatol* 2007;**27**:623–5.
791. Zakikhany K, Degail MA, Lamagni T, et al. Increase in invasive *Streptococcus pyogenes* and *Streptococcus pneumoniae* infections in England, December 2010 to January 2011. *Euro Surveill* 2011;**16**(5).
792. Zaman K, Roy E, Arifeen SE, et al. Effectiveness of maternal influenza immunization in mothers and infants. *N Engl J Med* 2008;**359**:1555–64.
793. Zaman RU, Alamgir AS, Rahman M, et al. Influenza in outpatient ILI case-patients in national hospital-based surveillance, Bangladesh, 2007-2008. *PLoS One* 2009;**4**:e8452.
794. Zhao H, Joseph C, Phin N. Outbreaks of influenza and influenza-like illness in schools in England and Wales, 2005/06. *Euro Surveill* 2007;**12**:E3–4.
795. Zhou B, Zhong N, Guan Y. Treatment with convalescent plasma for influenza A (H5N1) infection. *N Engl J Med* 2007;**357**:1450–1.
796. Zhou H, Thompson WW, Viboud CG, et al. Hospitalizations associated with influenza and respiratory syncytial virus in the United States, 1993-2008. *Clin Infect Dis* 2012;**54**:1427–36.
797. Zhou J, To KK, Dong H, et al. A functional variation in CD55 increases the severity of 2009 pandemic H1N1 influenza A virus infection. *J Infect Dis* 2012;**206**:495–503.
798. Zhou L, Liao Q, Dong L, et al. Risk factors for human illness with avian influenza A (H5N1) virus infection in China. *J Infect Dis* 2009;**199**:1726–34.
799. Zhu H, Webby R, Lam TT, et al. History of swine influenza viruses in Asia. *Curr Top Microbiol Immunol* 2011.
800. Zuniga J, Buendia-Roldan I, Zhao Y, et al. Genetic variants associated with severe pneumonia in A/H1N1 influenza infection. *Eur Respir J* 2012;**39**:604–10.
801. Dowdle WR. Influenza pandemic periodicity, virus recycling, and the art of risk assessment. *Emerg Infect Dis* 2006;(**1**):34–9.
802. World Health Organization. *WHO reference laboratories for diagnosis of influenza A/H5 infection 25 September* 2012. Available at: http://www.who.int/influenza/gisrs_laboratory/h5_reflabs/list/en/index.html.
803. Ortiz JR, Lafond KE, Wong TA, et al. Pandemic influenza in Africa, lessons learned from 1968: a systematic review of the literature. *Influenza Other Respi Viruses* 2012;**6**:11–24.
804. Centers for Disease Control and Prevention (CDC). Severe coinfection with seasonal influenza A (H3N2) virus and Staphylococcus aureus—Maryland, February-March 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:289–91.
805. Steininger C, Popow-Kraupp T, Laferl H, et al. Acute encephalopathy associated with influenza A virus infection. *Clin Infect Dis* 2003;**36**:567–74.
806. Gu J, Xie Z, Gao Z, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study. *Lancet* 2007;**370**:1137–45.
807. Tam CC, O'Brien SJ, Petersen I, et al. Guillain-Barré syndrome and preceding infection with campylobacter, influenza and Epstein-Barr virus in the general practice research database. *PLoS One* 2007;**2**:e344.

SUBSECTION 7
PARAMYXOVIRIDAE

CHAPTER 178

Parainfluenza Viruses

Ram Yogev • Caroline Breese Hall

Farewell to a Giant!

Only rarely one has the fortune to have a mentor who is a larger-than-life figure. Caroline Hall was that figure for many of us. As a member of the Red Book Committee under her leadership, I quickly appreciated her vast knowledge of infectious diseases and her creativity and critical thinking. Caren was always gracious and generous yet challenged us to achieve the highest standards during our deliberations. While she championed the art of consensus-reaching, she encouraged independent thinking and was excited to hear and support new ideas. Caren's unique characteristics both as a scientist and a human being made her a towering figure in my career and in those of many others. There are no words to express my appreciation for the enormous influence she had on it. Although my generation benefitted from the short time Caren shared her talents with us, future generations will never know what they missed.

As to the unfortunate word croup, it would be well to expunge it from British scientific medicine, or be careful to employ it unambiguously which can always be done by the use of a descriptive prefix such as diphtheritic, herpetic, spasmodic, etc.

SIR JOHN ROSE CORMACK, MD, FRSE, *THE BRITISH MEDICAL JOURNAL,* APRIL 24, 1875

The human parainfluenza viruses (hPIVs) are ubiquitous agents with well-earned recognition as being among the most important viral respiratory pathogens of humans. The three major hPIVs (1 to 3) have been estimated as being among the three most frequent causes of hospitalizations for young children with respiratory tract illnesses.[88,89,96] The impact of these three hPIVs on our health care resources is well illustrated in the estimates of their causing among children under 5 years of age approximately 16,000 to 100,000 hospitalizations each year.[45] Hospitalizations from hPIV-3 alone are estimated to range from about 9,000 to 52,000 each year. The rates of outpatient visits for hPIV infections among young children are even higher, estimated to be 10-fold the hospitalization rates.[59] One study estimated that the cost of emergency visits and hospitalization from croup resulting from parainfluenza alone was $20 to $56 million.[89] The taxes imposed by the burden of all the hPIVs, types 1 to 4, and at all ages are unestimated.

Respiratory tract illnesses from the hPIVs may occur and recur throughout life, and they are subordinate only to respiratory syncytial virus (RSV) in the morbidity caused by acute lower respiratory tract disease in infants and young children.[206] Among the agents of acute laryngotracheitis or "croup," the hPIVs are second to none.[167] The frequently epidemic nature of hPIV types 1 and 2, the prolonged seasonal occurrence of type 3, and the ability of all types to cause repeated infections indicate why they continue to be important despite the technological advances available toward control of viral diseases.

HISTORY

The historical delineation of the parainfluenza viral family is intertwined with the discovery of the related animal viruses and marked by the colorful names descriptive of their origin (Table 178-1). The first strain of PIV was discovered in Japan and named *Sendai*, or the hemagglutinating virus of Japan (HVJ).[115] This agent was recovered from mice that had been inoculated with postmortem lung tissue of infants with pneumonia. The first PIV from human sources was recovered several years later by Chanock[33] from infants with croup, and thus it became known as the croup-associated (CA) virus. Subsequently, three additional hPIVs that were distinct antigenically were isolated from children with acute respiratory illness.[35,100] In contrast to the CA virus, which was recognized by its ability to produce syncytia in tissue culture, the strains of the next two types of PIVs produced few or no cytopathic effects but were recognized by their ability to cause hemadsorption of guinea pig erythrocytes to infected tissue culture. These viruses thus were called hemadsorption type 1 (HA-1) and hemadsorption type 2 (HA-2) viruses.

Once the similarity of the familial characteristics of the CA virus with those of the other three viruses was recognized, the viruses were renamed the hPIVs, type 1 (HA-2), type 2 (CA), and type 3 (HA-1).[4] The fourth

TABLE 178-1 **Parainfluenza Viruses**

Parainfluenza Virus Type/Related Animal Virus	Natural Host	Experimental Infection	Preferred Tissue Culture for Initial Isolation	Cytopathic Effect	
				Initial Isolation	Passage
Parainfluenza type 1, hemadsorption type 2 (HA-2)	Human, guinea pig, rabbit, monkey, marmoset	Hamster, ferret	Monkey kidney, human diploid, human embryonic kidney	±	Rounding, cell destruction
Sendai, hemagglutinating virus of Japan (HVJ)	Mouse, pig				
Parainfluenza type 2	Human, monkey, rabbit, guinea pig	Hamster, dog	Monkey kidney, human embryonic kidney	+ (syncytial on monkey and human cells)	Syncytial, "Swiss cheese"
Simian virus 5 (SV 5)	Monkey				
Simian virus 41 (SV 41)	Monkey				
Parainfluenza type 3, hemadsorption type 1 (HA-1)	Human, guinea pig, monkey	Hamster, ferret, cotton rat, mouse, lamb	Monkey kidney, human embryonic kidney	±	In monkey cells: elongated, detachment of cell sheet In human cells: syncytial
Shipping fever (SF-4) Parainfluenza type 4	Cow Human	Hamster, guinea pig	Monkey kidney	±	Rounding granular, vacuolated

hPIV, discovered subsequent to this reclassification, is without a sobriquet and simply is termed hPIV-4, with two subtypes, A and B. The first type 4 strain was recovered in monkey kidney cell culture from a college student afflicted with a common cold.[100]

Several animal species appear to be natural hosts to the PIVs (see Table 178-1), with the exception of hPIV-4, which has been found only in humans. In animals, PIVs may be important pathogens. Bovine PIV (bPIV), similar to hPIV-3, often in combination with infection by *Pasteurella* organisms, causes an illness of considerable morbidity and cost in cattle termed *shipping fever*. Naturally acquired antibodies to the PIVs are found not only in cows but also in rodents, monkeys, rabbits, and other mammals. Various rodents, along with ferrets, dogs, and lambs, have been used for experimental infection. The virus previously named *equine Morbillivirus* has been demonstrated to be in the Paramyxoviridae family and named *Hendra virus*. This virus, which causes lethal illness in horses, was recognized to cause illness in humans also. Individuals in close contact with horses in Australia contracted the virus and two died.[155] Subsequently, in 1998, an outbreak of severe, often fatal encephalitis occurred in Malaysia and Singapore in individuals who had close contact with pigs.[40,69] The agent of this outbreak, Nipah virus, is phenotypically and antigenically similar to Hendra virus (80% genomic homology).

CHARACTERIZATION OF PARAINFLUENZA VIRUSES

Classification and Structure

The five hPIVs, hPIV serotypes 1, 2, 3, 4A, and 4B, are members of the Paramyxoviridae family, belonging to the *Respirovirus* and *Rubulavirus* genera (Fig. 178-1). The paramyxoviride family also includes other human viral pathogens transmitted via the respiratory route, such as RSV, measles, mumps, and metapneumovirus. The PIVs are distinguishable from the orthomyxoviruses (i.e., influenza viruses) in their size, nucleocapsid structure, antigenic composition, and laboratory growth characteristics.

hPIVs are nonsegmented, single-stranded, negative-sense, enveloped RNA viruses appearing as pleomorphic particles of medium size, with an average diameter of 150 to 200 nm.[103,187] The genomes of all the hPIVs, consisting of an average of approximately 15,000 nucleotides, code for at least six structural proteins (3′-N-P-M-F-HN-L-5′) and one or more nonstructural proteins (C, V, D) (Fig. 178-2). The viral ribonucleoprotein (RNP) is the viral RNA, tightly encapsulated by the nucleoprotein (NP). It is surrounded by the envelope, derived from the host cell, which is studded with spikes of surface glycoproteins, the hemagglutinin-neuraminidase (HN) and fusion (F) proteins, which are the major protective antigens. The large polymerase (L) protein and the phosphorylated nucleocapsid-associated (P) protein also form clusters within the nucleocapsid structure. The sixth structural protein is the nonglycosylated matrix (M) protein, located between the nucleocapsid and the envelope.

Replication and assembly of the virus occur solely in the cytoplasm of the host cell.[103,188] The NP, along with the P and L proteins, is responsible for the primary transcription that occurs sequentially, beginning from the 3′ end of the genome, producing predominately nonoverlapping subgenomic messenger RNAs from which the viral proteins are translated. The genome also is replicated from the formation of a full-length antigenome with positive-sense RNA. The NP and the genomic RNA

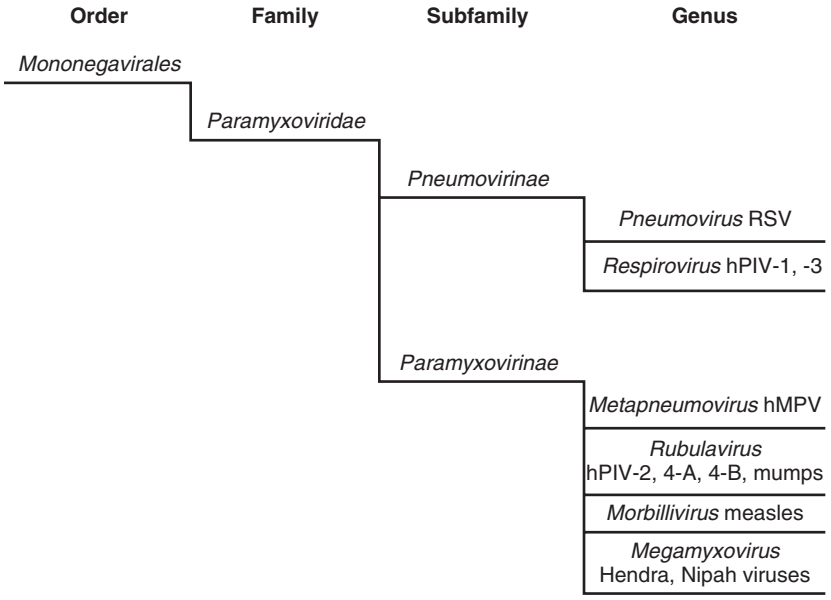

FIGURE 178-1 ■ Classification of parainfluenza viruses that cause human infection.

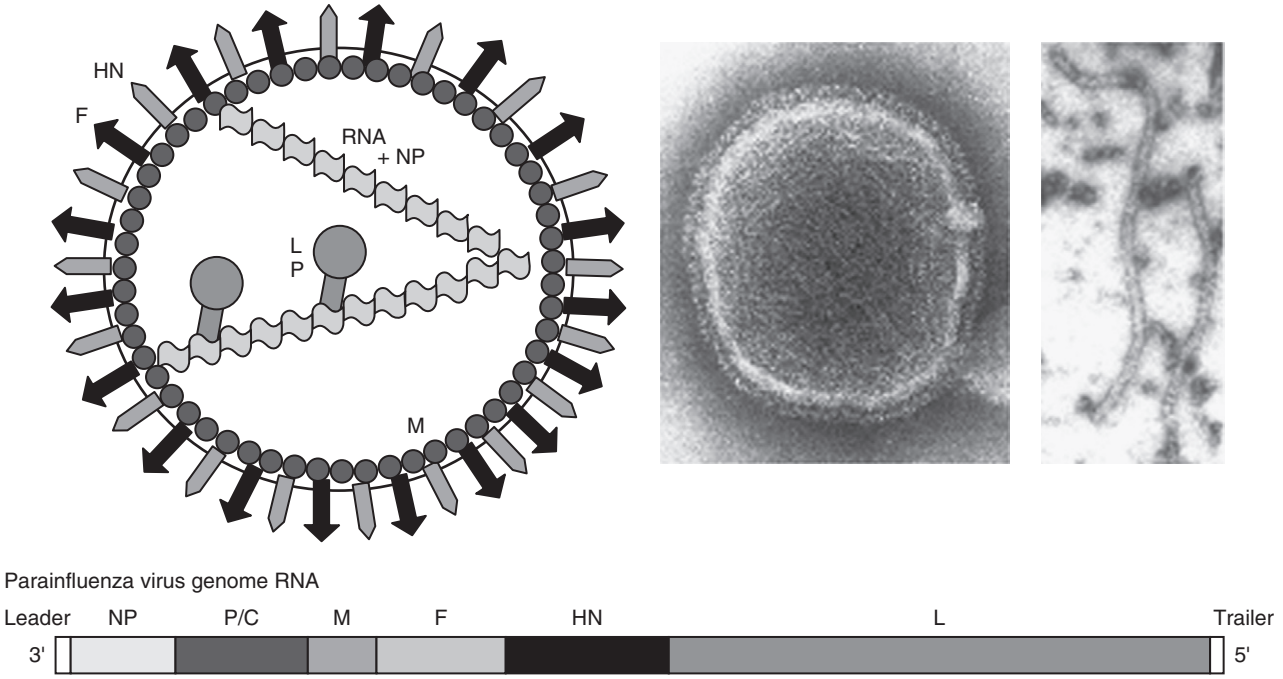

FIGURE 178-2 ■ Structure of parainfluenza virus (Sendai virus, *top left*) and its genomic RNA *(bottom).* Electron micrograph of Sendai virus *(top middle)* and its helical nucleocapsid *(top right).* (From Takimoto T. Parainfluenza viruses. In: Harper D, editor. *Encyclopedia of life sciences.* Chichester, UK: John Wiley & Sons; 2006, p. 678-84.)

comprise the helical structure, and with the subsequent addition of the P and L protein complexes, the nucleocapsid is formed. The envelope proteins are assembled at the cell surface. The M protein is the smallest of the major structural proteins and is the most abundant of the virion's proteins. The M protein facilitates the interaction between the nucleocapsid and the surface glycoprotein by the attachment and the insertion of the surface glycoproteins to the host cell and recruitment of the completed NP to the budding site of new virions, which are released from the cell surface by evagination of the plasma membrane.

The HN protein is a dimer of two polypeptide portions held together by a disulfide link in the hydrophilic region and at the bases by hydrophobic bonds. The HN is the major immunogenic and type-specific determinant. It is important in multiple viral functions, including mediating the attachment of the virus to the host cell by binding to sialic acid (N-acetylneuraminic acid), a terminal saccharide receptor present on the cell glycans. The

hemagglutination component of the HN protein mediates the hemagglutination of mammalian erythrocytes. The neuraminidase component of the HN protein functions during late infection to release progeny virions by dislodging the cellular bond by cleaving the sialic acid residues and prevents reattachment of the viral particles to the cell surface.

The F protein effects entry of virus by fusing the host and viral cellular membranes. Once adsorption of the virus occurs, the F protein precursor, F0, is cleaved by host proteases into the active F1 and F2 protein fragments, which mediate penetration of the virus into the cell, with subsequent fusion of the viral and cell membranes and hemolysis. The N-terminal residues of the F1 protein (the fusion peptide) promote the penetration and fusion of the virus with the cellular membrane. In contrast to the foregoing proteins, the nonstructural C, V, and D proteins are not encoded by all the hPIVs but are produced variably by the different hPIV types and appear to have roles in transcription, replication, production, and pathogenicity of the virions. At least with hPIV-3, the C protein has been shown to inhibit viral transcription.[47,86,103,128] Recent structural studies led to a better understanding of the various mechanisms by which hPIVs and other Paramyxovirinae attachment glycoproteins use cell-surface receptors to facilitate viral entry[23] and form particles that assemble and bud from the cellular membranes.[83]

hPIVs share common antigens but also may be differentiated into two antigenic groups. hPIV-1 and hPIV-3 are within the Paramyxovirinae genus *Respirovirus*, and hPIV-2 and hPIV-4A and hPIV-4B are within the Paramyxovirinae genus *Rubulavirus*[103] (see Fig. 178-1). Their common antigenicity is illustrated by serologic heterotypic antibody responses to infection with hPIV-1, hPIV-2, and hPIV-3 and mumps viruses. Although the hPIVs do not undergo major antigenic alterations in their surface antigens similar to influenza virus, genetic and antigenic variations within all four types have been detected, and their evolution can be determined by analyses of strains obtained over time.[86,103,210]

Isolation and Identification

The hPIVs are inactivated rapidly by acid at a pH of 3.0 and by exposure to lipid solvents, such as ether and chloroform, which destroy the envelope of the virus. They also are relatively labile to temperatures higher than 37°C (98.6°F).[49,50] Media containing protein, however, tend to protect against loss of infectivity when the virus is exposed to heat and when it is frozen at −70°C (−94°F).[49,94]

Isolation of the hPIVs occurs in permissive cell cultures after an incubation period of 3 to 7 days at 35° to 37°C (95° to 98.6°F). The preferred cell cultures for the isolation of the hPIVs are primary or continuous monkey kidney cells, such as LLC-MK and human embryonic kidney cells, along with a continuous line of mucoepidermoid human lung carcinoma cells, NCI-H292.[60,86,103] The production and characteristic type of cytopathic effect in tissue culture vary according to the hPIV strain (see Table 178-1).[159] hPIV-1 and hPIV-3 on initial isolation are unlikely to demonstrate distinctive cytopathic effects, but, depending on the cell line, they may on passage. hPIV-4 is the most difficult hPIV to grow and identify in tissue culture because the cytopathic effect is not evident, and 2 to 4 weeks may be required before identification by hemadsorption is possible. Identification of hPIVs in tissue culture traditionally has relied on the ability of the hPIVs to hemadsorb erythrocytes, most commonly guinea pig, human group O, or chicken erythrocytes, at 4° or 25°C (39.2° or 77°F). However, the sensitivity of this method is variable, especially for the rubulaviruses hPIV2 and hPIV4.[86]

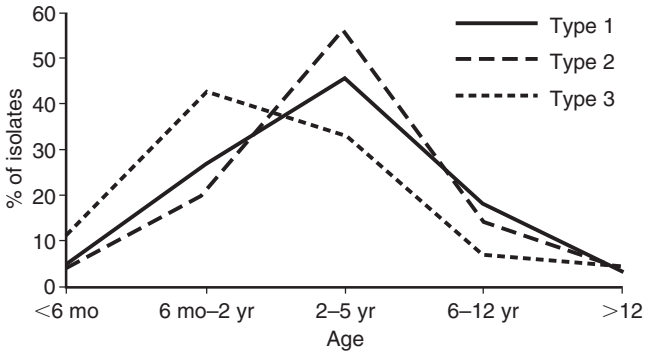

FIGURE 178-3 ■ Age distribution of infections with human parainfluenza virus types 1, 2, and 3 in outpatient children in Rochester, New York, from 1976 to 1992. (From Knott A, Long CE, Hall CB. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. *Pediatr Infect Dis J* 1994;13:269-73.)

EPIDEMIOLOGY

Geographic Distribution

The ubiquitous nature of the hPIVs has been illustrated among diverse populations, in many parts of the world, and in differing climates.* Despite these wide variations, experience with the hPIVs is similar in most countries.

Prevalence and Age at Infection

The frequency and impact of hPIV infections are greatest among preschool-aged children and are estimated to account for approximately one third of the lower respiratory tract infections in this age group.[65,86,89,114,165] Among the hPIVs, the type most commonly isolated is hPIV3, which usually is acquired first, followed by hPIV-1 and hPIV-2 (Fig. 178-3).

Like RSV infection, hPIV-3 infection is a common occurrence during the first few months of life. Half to two thirds of infants have acquired infection by the time they reach 12 months of age.[65,138,142] Experience with hPIV-1 and hPIV-2 usually occurs later, most commonly during the preschool years.[65,66,138,165] In a study in

*References 65, 133, 138, 150, 185, 201.

Tecumseh, Michigan, the annual isolation rate of hPIV per 1000 person-years was 53.8 for children birth to 4 years of age, 14.3 for those 5 to 19 years of age, 3.9 for adults 20 to 39 years of age, and 2.3 for those 40 years of age or older.[137]

The frequency of hPIV infection among young children was well illustrated by the Houston Family Studies, in which infections were detected by both viral isolation and serology among children observed from birth.[65,66] By the time they reached 2 years of age, 92 percent of the children had experienced at least one infection with hPIV-3 and 32 percent had been infected more than once. hPIV-3 is the most frequent cause among hospitalizations associated with hPIV infections. hPIV-1 and hPIV-2 are associated primarily with hospitalizations of children 2 to 6 years old. The age distribution of children with hPIV-1 and hPIV-3 viral infections cared for in private pediatric practices, however, may overlap substantially (see Fig. 178-3). Among outpatients in Rochester, New York, approximately half of the hPIV-3 viral infections occurred among children younger than 24 months, in contrast to approximately one third of the hPIV-1 infections.[114] Among children 2 to 5 years of age, the reverse was true, with half of the hPIV-1 infections and approximately one third of the hPIV-3 infections occurring in this older age group.

Until recently, the epidemiology of hPIV-4 was less well described, in part because the clinical disease is mild and its recovery from cell cultures is inherently low, and thus its prevalence was underestimated.[117,118,170] More recent studies using sensitive reverse-transcriptase polymerase chain reaction (RT-PCR) methods show that hPIV-4 infection is more frequent and sometimes can cause more serious illnesses than previously recognized.[28,57,200] Serologic studies have suggested that infection with hPIV-4 is particularly frequent during the preschool years, with between 50 and 90 percent of 5-year-old children possessing antibodies.[112,118,119]

Seasonal Occurrence

The seasonal patterns of the hPIVs vary according to location and year. In temperate regions, the incidence of parainfluenza viral infections appears to correlate with seasonal weather conditions, but in tropical regions it is less well-defined. Different hPIV strains have different seasonal activities.[61] hPIV-1 tends to occur in the fall and commonly causes outbreaks every other year.[65,114,165] The behavior of hPIV-2 has been more erratic.[65,86,114,165] During 20 years of surveillance in Rochester, New York, hPIV-2 appeared sporadically in low numbers, usually at the end of the fall outbreaks of hPIV-1 in the odd-numbered years.[77,114] hPIV-3 may be present in a community in low numbers for many months, but swells of more epidemic activity occur in the spring to fall.[65,77,114] During long-term surveillance, approximately 75 percent of hPIV-3 isolates were recovered in the spring and summer and most of the remainder were obtained in the autumns of the even-numbered years, when hPIV-1 was absent.[114] hPIV-4 usually occurs during late fall and winter in temperate countries,[118,119] but in China it is more prevalent during the spring and summer.[166] Studies from a more diverse geographic range are needed to better understand the global seasonal patterns of hPIV-4.

PATHOGENESIS

Transmission

Experimental studies using Sendai viruses have shown that even a low inoculum (70 plaque-forming units) to the upper respiratory tract resulted in robust growth and efficient contact transmission.[26] Of note, the efficiency and timing of transmission were independent of clinical symptoms and severity, which is consistent with data from animal models and with the epidemiology of hPIV outbreaks.[77,86] Furthermore, more severe infection involving the lower respiratory tract was a poor predictor of transmission.[26] Several clinical and experimental observations also have indicated that the hPIVs are able to spread readily and effectively.[5,44,81,104,142] Close contact with infected individuals or their secretions appears necessary, which suggests infection is spread through aerosols of large particles (>10 μm mass median diameter) that travel short distances (<0.9 m) or by touching infectious secretions on fomites followed by self-inoculation.[78] Contagiousness, therefore, is related to how effectively the infectious secretions are propelled into the environment, such as through sneezing and coughing, and how well the infectious virus can survive in the environment.[24]

Children with primary infection caused by hPIV-1 have considerable quantities of infectious virus in their nasal secretions, an average of approximately 1000 tissue culture infective doses ($TCID_{50}$) per milliliter.[81] Because hPIVs cause illnesses associated with frequent sneezing, coughing, and profuse nasal discharge, transmission and environmental contamination of infectious secretions is likely to occur readily, especially among young children. Although the routes of inoculation that occur naturally have not been studied adequately, adults have been infected experimentally by intranasal and oropharyngeal inoculation. The eye as a site of inoculation has not been examined similarly, but it may be an important portal of infection from self-inoculation with contaminated hands.

Experimental data suggest that hPIV secretions survive well on hard, nonporous surfaces, such as those found in hospitals, workplaces, and homes. The hPIVs remain infectious on nonporous surfaces for as long as 10 hours and on porous surfaces for as long as 4 hours, but survival may be diminished by drying.[24] hPIVs may remain infectious after being transferred to hands from environmental surfaces, a finding further suggesting that fomites are an important mode of dissemination. A curious conundrum is the survival and persistence of hPIV-1 and hPIV-3 infections among a group of individuals isolated in the frigid environs of the South Pole for 8.5 months.[141,154] Despite no outside contacts, infection with hPIV-1 and hPIV-3 occurred repetitively in the quarantined individuals. This phenomenon raised the possibility that persistent infection may be engendered not only in tissue culture but also in humans under such circumstances as extreme environmental conditions.

Pathology

Inoculation of the hPIVs into the upper respiratory tract results in infection of the nasal epithelium and nasopharynx and the appearance of clinical signs after an incubation period of 2 to 4 days. The hPIVs appear to replicate preferentially in the superficial ciliated epithelial cells lining the airways of the upper and lower respiratory tract. The factors determining the tropism and the severity of the viral infection are incompletely understood. Such factors may include host genetics, age, and immunity; the protease activity of various tissues; and virus-specific determinants, such as the cleavage phenotype of the F_0 protein.[103,139]

The magnitude of viral replication appears important in the pathogenesis, as evidenced by the correlation of viral loads with severity of disease.[22,75,81] This has been confirmed by the development of live attenuated PIV vaccines using reverse genetics, which demonstrated precise viral mutations correlated with clinical attenuation.[15,52,105,149] Viral shedding is most abundant and prolonged in young children with primary and severe disease and may begin several days to a week before the onset of symptoms.[81] Children with hPIV-1 infections shed the virus for an average of 4 to 7 days, but may shed for as long as 12 days. With hPIV-3 infections, shedding tends to be longer, occasionally for 2 to 3 weeks, and in adults with chronic lung disease, shedding may be both prolonged and intermittent.[74,84]

The pathophysiology of hPIVs has been explored in animal models.[103,153,163] Inoculation of rodents with hPIV-3 produced histologically evident, but usually asymptomatic, interstitial pneumonitis or bronchiolitis. Ferrets also can be silently infected, but in newborn ferrets, PIV infection may be progressive and fatal.[136] Chimpanzees and several species of monkeys may be infected, but clinical manifestations generally are lacking, except with hPIV-3 infection of chimpanzees and African green monkeys. Inoculation of hPIV-2 into the respiratory tract of a canine model produced clinical signs of cough and rhinitis accompanied by histologic changes in the airways.[198]

Pathologic studies of children with hPIV infections are limited. The few cases of confirmed hPIV infection examined have shown that in the young child with primary infection, inflammation, marked primarily by necrosis of the epithelium, is evident throughout the respiratory tract. The subglottic tissues in croup appear particularly involved, but with primary infection, the conducting airways at all levels and the alveoli may be affected.[34,213] Lung tissue samples from 200 young children who died from severe respiratory disease, which were examined for parainfluenza viruses types 1, 2, and 3, respiratory syncytial virus, influenza, and adenoviruses, showed no distinctive histologic changes that would allow identification of a specific virus.[51] hPIV was identified as the sole pathogen in 4 percent of the 200 samples tested and was the sole pathogen in 10 percent of virus-positive samples. Of the hPIVs identified, hPIV-3 was the most common serotype. hPIV infections caused bronchopneumonia and interstitial pneumonitis, which had infiltrates consisting mainly of mononuclear inflammatory cells in the bronchial lumen. Most of the children with bronchopneumonia had cellular necrosis and destruction of the bronchial pseudostratified columnar epithelium and diffuse alveolar damage. Immunohistochemical assessment on tissue microarray slides showed the virus in bronchial and bronchiole epithelial cells and in alveolar cells.[51]

Immune Response: Role in Pathogenesis and Protection

Innate immunity's role in controlling PIV infection and how it is elicited are of increasing interest but currently of limited clarity. Better understanding of the pathogenesis of the innate response and its effect on the transition to adaptive immunity may lead to novel and better means of controlling the ubiquitous hPIV infections that occur during infancy and thereafter.

The innate antiviral responses to infection of viruses of the Paramyxoviridae family have been studied in experimental models. The response is elicited within the respiratory tract once the viral genome enters and replicates in the cytoplasm of the host cell and is characterized by the production of a cascade of immunomodulatory mediators, chemokines, and proinflammatory cytokines.[46,64,103,151,152] Toll-like receptors (TLRs), a class of pattern-recognition receptors, appear to be important early in the innate antiviral defense.[46,134] The products of viral replication trigger and activate specific TLRs that allow the binding of the virus to the host cell and the expression of the genes associated with the host immune response. TLRs may help mediate the immune response, both by their interaction with infected respiratory epithelial cells and by the induction of dendritic cell maturation that is integral in the subsequent development of adaptive immunity with the production of effector and memory T cell and B cells.[123,172] Although TLRs appear to have a pivotal place in innate antiviral defense, experimental evidence suggests that replicating infection of paramyxoviruses and other respiratory viruses may induce and enhance the innate immune response by other pathways that are not dependent on TLR-mediated signaling.[121,123,125]

The major components of the adaptive immune response to hPIV infection that play important and independent roles in primary infection and reinfection are both humoral and cellular. Rodent models using Sendai virus have shown that $CD8^+$ cytotoxic T lymphocytes are necessary for clearing the virus from the lung.[93,110] The viral antigen is carried by dendritic cells and macrophages to the draining lymph nodes, and activated cytotoxic T lymphocytes subsequently migrate to the site of pulmonary inflammation, resulting in viral clearance. The cellular immune response of memory T cells, which was established during the primary infection, persists in high titers in the spleen and rapidly responds to a repeated infection. In addition, virus-specific interferon (IFN)-γ–producing $CD4^+$ T cells have been shown to increase in the airways of infected mice during viral clearance, and IFN-α and IFN-β may be produced by experimental PIV infection.[71,103] IFN production also has been demonstrated during natural parainfluenza infection in young

children.[80,209] However, some hPIVs may be able to evade this protective response by inhibiting IFN production through accessory proteins (C and V) expressed from the P gene.[71,103,111]

In contrast to infection with hPIV-1 and hPIV-2, the most serious illness caused by hPIV-3 infection usually occurs during the first year of life, when infants possess maternally derived specific antibody. This finding has raised the question whether interactions of maternal antibody and virus may be detrimental.[133] In animal models, however, passively administered PIV antibody and monoclonal antibodies against the F and HN proteins appeared protective. Furthermore, the trials with an inactivated trivalent hPIV vaccine in the 1960s did not suggest a pathogenic role for antibody.[63] Although not protective, the vaccine was immunogenic and produced serum antibody in the vaccinees. Subsequently, the vaccinees did not experience augmented disease, as observed with the simultaneously tested formalin-inactivated RSV vaccine.

In animal models the specific antibody response is generated only during the later part of the primary infection and does not play an important role in clearing the infection.[212] However, specific antibodies are protective against secondary challenge with the same parainfluenza virus. Clinical observations also show that the more severe infections involving the lower respiratory tract occur during initial infection in young, seronegative children, but recurrent infections usually are mild. Naturally acquired immunity, therefore, although not complete and of variable durability, does provide protection against more severe disease.[66,103] Antigenic variation in the hPIVs is not sufficient to explain the lack of complete or durable immunity. The cause is more likely the waning of both humoral and cellular immune responses that occurs during the intervals between repeated exposure to one of the hPIVs.

Serum hemagglutination-inhibition antibody and neutralizing antibody are detectable within 1 to 2 weeks.[109] Neutralizing antibody is directed primarily to epitopes on the HN and F proteins. The importance of neutralizing antibody in providing protection against infection of the lower respiratory tract, and to some extent of the upper respiratory tract, has been well established in animal models. Infants with primary hPIV infection usually produce a greater antibody response toward HN than F, and the response is relatively specific to the PIV serotype. With repeated infections, an anamnestic and broader humoral antibody response occurs, producing antibodies with cross reactivity against the different hPIV serotypes. In young children with primary hPIV-3 infection, the antibody response to the HN protein has been shown to be directed at multiple neutralizing antigenic sites.[192] The response to the F protein, whether with primary or recurrent infection, appears to be more variable in magnitude and restricted in terms of antigenic sites. The presence of maternal antibody, however, inhibits the infant's ability to produce specific humoral antibody.

Primary and secondary infections result in secretory immunoglobulin (Ig)G, IgM, and IgA antibodies that peak at 2 weeks after onset of symptoms.[46,209] Infection induces relatively incomplete protection in infants because of the lower titer amount of antibodies and the different IgG subclasses produced.[46] IgG_1 and IgG_3 antibodies are the predominant subclasses, contrary to the IgG_2 antibodies generated in older children and adults. Protection of the upper respiratory tract is mediated by secretory IgA and that of the lower tract by local IgG antibodies.[46] The transient nature of the induced secretory IgA antibodies may partially explain the common occurrence of reinfections throughout life.

Understanding the cell-mediated immune response to hPIV infection is important for vaccine development, and observations about the functions of $CD4^+$ and $CD8^+$ T cells in the recall response to PIV infection suggest that this system plays a major role in protection from primary and recurrent infections.[181,207] The increased severity and prolonged period of viral shedding in immunocompromised hosts, such as those receiving organ transplants, indicate the importance of intact cellular immunity in the control of disease and recovery from hPIV infection.[36,55,193]

CLINICAL MANIFESTATIONS

Primary Infection

The types of illnesses caused by hPIV infections have characteristic associations with the age of the child, the season, and the hPIV serotype (see Figs. 178-3 and 178-4).* Most primary infections are symptomatic, and many affect the tracheobronchial and lower respiratory tract. Of the primary infections that involve only the upper respiratory tract, fever and laryngeal or tracheal signs are common occurrences. Even an influenza-like illness with sudden onset of high fever, chills, cough, headache, myalgia, or fatigue has been reported to occur with hPIV infection.[161,176] Reinfections occur frequently at all ages and usually are mild and limited to the upper respiratory tract, or sometimes they are asymptomatic.[66]

The hPIVs cause a greater proportion of acute respiratory tract illnesses among outpatients than among hospitalized children.[114,119] Of all viral respiratory illnesses examined in a pediatric practice, the hPIVs caused approximately two thirds of the croup cases, one fourth of the tracheobronchitis cases, and approximately half of the upper respiratory tract illnesses, including colds, laryngitis, pharyngitis, and otitis media.

hPIV infections account for almost 7 percent of all hospitalizations for respiratory illnesses with or without fever among children younger than 5 years of age.[201] The proportion of children with hPIV infection who require hospitalization, mainly those with croup, has markedly diminished in recent years, primarily because of better outpatient management and therapy of croup (see discussion of management and therapy).[177,208] Currently, most children hospitalized with hPIV infections are infants with pneumonia and bronchiolitis from hPIV-3, who are most likely to be admitted during the spring.

*References 66, 68, 96, 114, 119, 165, 201, 208.

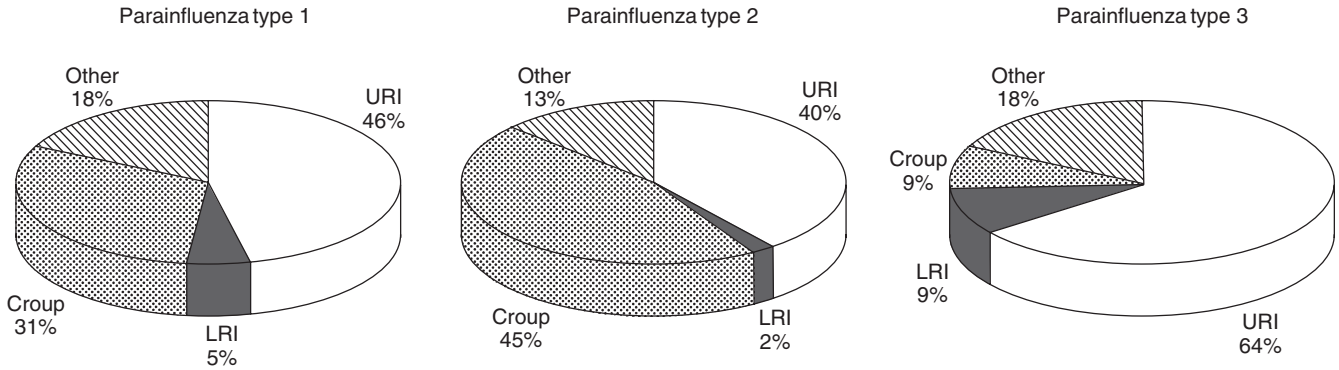

FIGURE 178-4 ■ Clinical manifestations of parainfluenza virus infections according to serotype in pediatric outpatients in Rochester, New York (1976 to 1992). *LRI,* Lower respiratory tract infection other than croup; *URI,* upper respiratory tract infection (including otitis, colds, and pharyngitis). (From Knott A, Long CE, Hall CB. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. *Pediatr Infect Dis J* 1994;13:269-73.)

The onset of the typical primary hPIV infection generally is acute and associated with mild fever and upper respiratory tract signs of the common cold, such as rhinitis, sore throat, and cough. Laryngeal involvement manifesting as hoarseness is apt to be more prominent than with other viruses that commonly produce colds in young children. After 3 to 4 days, these upper respiratory tract symptoms may progress, primarily in infants with hPIV-3 infection, to involve the lower respiratory tract. The characteristic manifestations of pneumonia or bronchiolitis, including dyspnea, crackles, wheezing, and hyperaeration, follow. Children with hPIV-1 or hPIV-2 infection, who usually are 2 years of age or older, also have acute onset of fever and upper respiratory tract signs, but if the infection progresses in this age group, it is more likely to evolve into croup. Children with hPIV-1 infections who were seen in a private pediatric practice during a community outbreak of hPIV-1 predominantly had fever, upper respiratory tract signs, and tracheobronchial or tracheolaryngeal signs of croup.[81]

In children with croup, upper respiratory tract signs, such as coryza, rhinorrhea, and sore throat, which may be accompanied by low-grade fever, are followed by a harsh, barking cough (the "seal's bark"), which heralds the classic manifestations of stridor, nasal flaring, retractions of the chest wall, and dyspnea.[184] The course of croup is unpredictable, with waxing and waning in the intensity of the stridor and dyspnea. The acute symptoms usually last for 3 to 4 days, although cough may continue for longer.[20] Fewer than 10 percent of children with croup have hypoxemia from viral involvement of the lung parenchyma, which, although important in management, may not be recognized because of the focus on the apparent major site of inflammation, the subglottic area. The most reliable findings to assess illness severity are the presence of stridor and the degree of retractions.[32,37] Very few patients need intubation, and the mortality is low.[169] Recurrent (spasmodic) croup usually occurs without fever or other respiratory tract symptoms. It has a viral etiology similar to that of acute laryngotracheobronchitis,[199] but allergic reactions or gastroesophageal reflux may cause a similar syndrome.[37]

Otitis media may complicate hPIV upper respiratory tract infection among young infants and older children.* hPIVs are commonly isolated from children with and without a history of otitis media, and thus their role in acute and recurrent otitis media is unclear. However, of the otitis media cases that have been associated with hPIV, the serotypes most frequently identified were hPIV-1 and hPIV-3. hPIVs have also been detected in middle ear aspirates as the sole agent or with a bacterial agent, suggesting that hPIVs may play both primary and secondary roles in predisposing children to middle ear infection.

The association of acute hPIV infection with a nonrespiratory illness or the isolation of hPIV from sites other than the respiratory tract occurs rarely. Case reports have associated hPIV infections with a variety of diseases, including adult respiratory distress syndrome, pertussis-like disease, parotitis, myopericarditis, and diseases of the central nervous system.† Anecdotal reports also have associated hPIV infection with collagen vascular diseases and sporadic severe hepatitis, characterized histologically by syncytial giant hepatocytes.[157,158]

The clinical manifestations associated with hPIV-4 are less well described, which may be explained partially by the technical difficulty of isolating hPIV-4.‡ Most have been associated with a mild, usually afebrile, upper respiratory tract infection, but more severe disease and lower respiratory tract illness also have been reported.[122]

Reinfection

Immunity to PIV infection is transient and incomplete; therefore, reinfections commonly develop among healthy older children and adults. hPIV reinfections usually are mild, with symptoms of the common cold. In adults, hPIV-1, hPIV-2, and hPIV-3 cause approximately 2 to 5 percent of the upper respiratory tract infections, but one fifth or more of these infections may be asymptomatic.[21,90]

*References 39, 85, 114, 140, 164, 203.
†References 7, 47, 92, 97, 116, 196, 205, 214.
‡References 28, 57, 90, 118, 122, 200.

Of those with clinical illness, approximately 30 to 92 percent develop fever, which is often associated with malaise, sore throat, and cough. Rhinorrhea occurs in fewer than 25 percent of infections among adults and usually less among children.

hPIV infection may cause more severe disease in elderly persons and also may exacerbate symptoms in both children and adults with chronic lung disease, including chronic bronchitis, asthma, and cystic fibrosis.* On occasion, pneumonia has been described with hPIV infections in previously healthy adults, such as among military recruits, but is a rare development in normal, healthy populations of adults and school-aged children.[56,202]

INFECTION IN IMMUNOCOMPROMISED PATIENTS

As demonstrated by multiple reports of outbreaks of hPIV infections occurring on transplant units, children and adults with compromised immunity, especially those with deficient cellular immunity, are at high risk for development of severe, and sometimes fatal, hPIV disease.† Some of these hPIV outbreaks are not associated with a concurrent increase in hPIV infections in the community, which suggests that many hPIV infections are nosocomial.[120,146] Most of the hPIV infections occur more than 1 year after transplantation. Close to half of the hPIV infections involve the lower respiratory tract, and approximately 15 to 40 percent of these infections are fatal.[12,194] In some studies, hPIV infection is as common as RSV infection in causing pneumonia in these patients.[29] Corticosteroid administration for graft-versus-host disease (GVHD) increases the risk for developing lower respiratory tract infection.[145] Recipients of nonmyeloablative conditioning regimens appear to have a lower risk for developing lower respiratory tract disease.[174]

Very high viral loads have been found in bone marrow transplant patients with pneumonia,[29] and the duration of shedding of hPIV is prolonged. These two features significantly augment the risk for an outbreak of infection among patients and personnel in transplant units. Furthermore, hPIV infection often is not considered among the opportunistic infections that plague these patients, and the diagnosis is confounded by the observation that hPIV infection may lead to bacterial and fungal pneumonia.[53,145] In addition, the sensitivity of detecting the virus in specimens from the upper respiratory tract is poor.

The incidence of hPIV infections among lung transplant recipients has been estimated to be 1.6 to 11.9 percent and is most often associated with hPIV-3.[193,195] Among these patients the initial manifestations of hPIV infections are usually nondistinctive upper respiratory tract symptoms, such as cough and sore throat, and, less commonly, rhinorrhea and fever.[193] Progression to involvement of the lower respiratory tract or rejection of the transplant follows in approximately 10 to 70 percent of cases, depending on multiple host factors, including being a child, presence of GVHD, degree of immunosuppression, and corticosteroid or antilymphocyte therapy.[95] The risk for developing lower airway disease in transplant recipients is further enhanced because of mechanical factors present in transplanted lungs and because of altered or absent mucosal immunity.[193] The bronchus-associated lymphoid tissue in the lung, which is integral to the protective immune response against pulmonary infections, is injured during transplantation, thus resulting in diminished control and clearance of the viral infection.

Therefore, the high rate of symptomatic infection (95%) with hPIV infections among lung transplant recipients is not surprising.[193] Cough has been reported as present in 70 percent, dyspnea in 66 percent, fever in 16 percent, and lower respiratory tract disease, which is diagnosed as viral pneumonia, in 16 percent. hPIV and other viral infections may lead not only to graft rejection but also to severe long-term complications. Thirty-two percent of lung transplant recipients have developed acute bronchiolitis obliterans, which has occurred an average of 6 months (range, 1 to 14 months) after their hPIV infection.[55,193,194] hPIV infection occasionally has been reported also to be complicated by pancytopenia, Guillain-Barré syndrome, parotitis, and acute disseminated encephalitis.[8,116,168]

Among children with immunodeficiency syndromes who have diminished cellular immunity, especially children with severe combined immunodeficiency syndrome, hPIV has been notably severe and frequently fatal.[98] Complicated disease and lower respiratory tract involvement are observed less frequently with hPIV infection among children with less severely compromised cellular immunity, such as patients with human immunodeficiency virus (HIV) infection.[102,126,135] The clinical severity of hPIV infection among patients with HIV infection is variable, but even among those with mild disease, the shedding of hPIV usually is prolonged and may persist beyond the period of clinical symptoms.

DIAGNOSIS

The diagnosis of hPIV infections may be surmised on clinical and epidemiologic grounds, such as the patient's age, the type of illness, and the seasonal patterns of hPIV in the community. However, the nonspecific clinical presentation of many other viral respiratory infections makes the specific laboratory diagnosis of hPIV necessary. During the acute phase of the illness, hPIV may be diagnosed in specimens of nasal secretions by the following methods:

1. Detection of viral RNA using RT-PCR in single or multiplex assays.[1,13,204] RT-PCR has markedly increased the detection and specific identification of the hPIVs in patient specimens.[25,86,88,201] RT-PCR assays can detect a single respiratory virus or multiple viruses simultaneously and provide sensitive, specific, and rapidly available identification of hPIVs from clinical specimens.[27,204]
2. Isolation of the virus by culture or enhanced by shell viral culture techniques.[159]

*References 43, 67, 101, 147, 189, 190.
†References 19, 36, 55, 129-131, 160, 193, 195.

3. Serologic assays during the patient's convalescence (see discussion of isolation and identification).

Numerous serologic assays exist, including complement fixation, hemagglutination-inhibition, hemadsorption inhibition, neutralizing, enzyme immunoassay, and immunofluorescence.[58,87] However, serologic diagnosis is confounded by heterotypic antibody responses (see discussion of immune response). During reinfection, both homotypic and heterotypic antibody rises may occur, or sometimes little antibody response is detected, despite shedding of the virus from the nasopharynx.[103] Less is known about the antibody response to hPIV-4 infection, but during primary infection, a homotypic response usually occurs.[112] Detection of specific IgM antibodies, which usually persist for 2 to 11 weeks after infection, has been of limited success as a diagnostic technique.[197]

PCR-based methods should be the preferred diagnostic tool for hPIV infection over culture or serologic assays because they have a sensitivity of 95 to 100 percent and a specificity of 97 to 100 percent in contrast to viral isolation.[6,86] Although most hPIV infections do not require specific diagnosis, among more severely ill and hospitalized patients the diagnosis using these newer molecular techniques, if rapidly available, offers several advantages in addition to their high sensitivity and specificity. Among these are providing the health care provider a known specific viral diagnosis, a diminished use of unnecessary antibiotics, and the implementation of effective infection control procedures, potentially reducing the risk for nosocomial infection with a different virus from patients with the same clinical presentation.

DIFFERENTIAL DIAGNOSIS

Most of the other major respiratory viral pathogens can mimic the illnesses produced by hPIV infections. Clinical differentiation rarely is possible. More helpful are such epidemiologic clues as the differing seasonal patterns and age-related attack rates of the common viral agents of respiratory illnesses in children.[79]

Croup from hPIVs or any other virus must be differentiated from bacterial tracheitis, retropharyngeal abscess, and epiglottitis, which have rapidly progressive courses and a potentially fatal outcome. With the widespread use of the *Haemophilus influenzae* type b vaccine, epiglottitis now rarely occurs. Retropharyngeal abscess is also less common because of the widespread use of antibiotics for upper respiratory tract infections. However, bacterial tracheitis has been increasingly observed.[91] Croup associated with hPIV usually has a more gradual onset, with a preceding upper respiratory tract infection. Its fluctuating course and prominent "seal's bark" cough without drooling help differentiate it from epiglottitis and bacterial tracheitis. Children with croup associated with hPIV infection usually have peripheral white blood cell counts that are normal or initially slightly elevated, with a mild left shift. In contrast, among children with stridor from epiglottitis, retropharyngeal abscess, or bacterial tracheitis, the left shift usually is marked.

In countries that continue to have diphtheria, laryngeal diphtheria may be a consideration in an unimmunized child. However, laryngeal diphtheria usually is gradual in onset and manifests with characteristic membranous pharyngitis. Stridor and other signs of croup also may be present in children with congenital or acquired conditions that obstruct the laryngeal airflow, such as aspiration of a foreign body, tracheomalacia, stenosis, and acute angioneurotic edema.

MANAGEMENT AND THERAPY

Most hPIV infections are self-limited and require no treatment. Specific therapy currently is not available for hPIV infections. In vitro studies have shown that ribavirin inhibits hPIV, but clinical studies produced mixed results. A few small studies showed some benefit in controlling hPIV infections among a limited number of immunocompromised patients,[31,42,54,132,186] but larger studies have not shown such an effect and the duration of viral shedding was not shortened.[53,145] Controlled trials, however, are lacking. Thus, until more data are available, aerosolized ribavirin currently should be considered only for high-risk patients with severe lower respiratory tract disease. Among potential therapies being explored are sialic acid inhibitors, such as those used for treating influenza, which inhibit hPIV F and HN activities.[1,73,144,162] Other therapeutic approaches include interference with viral attachment and replication with the use of inhibitors of protein and nucleic acid synthesis, synthetic peptides, and antivirals inhibiting *S*-adenosyl-L-homocysteine hydrolase[16,86,103,188] or protein kinase B (AKT), which is critical for viral replication.[62]

Management of croup in the home is primarily supportive.[208] The armamentarium used during the past century has been varied and usually anecdotal, ranging from cold night air to humidification or putting the child in a comfortable position. No such home therapies have proved beneficial. Most children with croup improve spontaneously within a few days.

Children who are more severely affected require outpatient evaluation, usually in an emergency department. With the current management offered in emergency departments, few children's symptoms progress such that hospitalization is required.[45,177,208] Intravenous immunoglobulin (IVIG) is not recommended; a retrospective analysis of its administration did not find any benefit.[145] In addition, the specific antibody content in pooled IVIG preparations is highly variable, confounding the evaluation of its efficacy. The current consensus and recommendations for the management of acute viral laryngotracheitis have afforded a more consistent beneficial outcome.[2,37,208] Administration of 0.05 mL/kg (maximum 5 mL) nebulized 2.25% racemic epinephrine (a 1:1 mixture of D-and L-isomers of epinephrine) has been demonstrated to provide rapid relief for the airway obstruction. The relief from nebulized epinephrine is transient, usually lasting no longer than 2 to 3 hours. Because of concern for a rebound phenomenon, such as bronchospasm, worsening of respiratory distress, or persistent tachycardia, patients should be observed for at least 3 hours after the last racemic epinephrine dose. Furthermore, hypoxemia, if present, and the

outcome are not affected by treatment with nebulized epinephrine.[180,208]

Administration of corticosteroids has become the cornerstone of management for the more severely affected child who fails to improve with the usual supportive care. Corticosteroids administered intramuscularly, intravenously or, orally have been shown to provide significant and lasting benefit on the clinical course and outcome, in contrast to placebo or other therapies, and also decreased the need for nebulized epinephrine.[2,3,171] Although studies have shown that any dexamethasone dose between 0.15 mg/kg and 0.6 mg/kg (with a maximum of 10 mg/day) will be effective in relieving the symptoms and diminishing the severity of disease and need for hospitalization,[41,99] the recommended dose most commonly used is a single dose of dexamethasone 0.6 mg/kg given intramuscularly or intravenously.[171,208] Prednisolone 1 mg/kg is less effective than dexamethasone 0.15 mg/kg, probably because of its shorter half-life (36 hours vs. 54 hours, respectively).[187] Although nebulized steroids (e.g., budesonide) have been shown to be effective, they are expensive and less effective than oral dexamethasone.[30] The treatment of croup is discussed more thoroughly in Chapter 18. Antibiotic therapy should be reserved for documented episodes of secondary bacterial infection, which infrequently are associated with hPIV infections.

PROGNOSIS

Most previously healthy children recover from hPIV infections completely and without complication. Follow-up studies of normal children with hPIV infections support the generally good prognosis. Some children who have had croup have pulmonary function abnormalities detected subsequently, but proof that these abnormalities were caused by their hPIV infection is lacking.[76,124]

The potential for development of acute complications, morbidity, and mortality is related to the presence of a comorbid condition such as immunodeficiency and diseases that affect cardiopulmonary function, including congenital malformations and acquired conditions obstructing the airway.[208] Of children hospitalized with hPIV infections who were studied during a 4-year period, 35 percent had preexisting pulmonary or cardiac abnormalities, prematurity, or asthma.[84] In contrast to the children who previously were normal, the children with underlying conditions had significantly more severe disease and more complicated courses requiring longer hospitalization. Immunodeficient patients, as described previously, have particular difficulty in controlling and clearing hPIV infections, and, thus, these children have complicated, prolonged, and recurrent respiratory tract infections. Prolonged or persistent infection with hPIVs, especially with hPIV-3, has been noted to occur in some patients with chronic bronchitis, possibly related to the lack of a sufficient specific antibody response in the sputum.[74] The possibility that hPIV may cause central nervous system involvement has been suggested by the occasional detection of such viruses in the central nervous system in patients with previous transplantation.[8,168]

PREVENTION

Infection control measures to prevent the spread of hPIV through direct contact with fomites or other infected objects are integral in preventing infection. Hand washing and droplet precautions should be implemented as soon as hPIV infection is suspected and continued throughout the period of shedding, which in immunocompromised patients may be for extended periods (>100 days).[53] Nonetheless, infection control measures may fail because many hPIV infections are mild and prolonged asymptomatic shedding may occur among immunocompromised patients[156] and adults with chronic bronchitis.

For decades, attempts to prevent hPIV infections have focused on the development of effective vaccines. The current lack of a licensed vaccine for hPIV infections attests to the problematic nature of infection with hPIVs and of the response of the human host at different ages. Many of the hurdles encountered in the development of an effective vaccine for hPIV are similar to those for RSV. The vaccine, at least for hPIV-3, would have to be administered during the first few months of life when the infant's immunologic system is not fully developed[48,211] and when the potential inhibitory effect of passive maternal antibody exists. Furthermore, to elicit durable protection, the vaccine would have to produce an immunologic response superior to that derived from natural infection, because recurrent infections occur throughout life.

Hence, the goals of immunization for hPIVs, like those for RSV, currently may need to be modified toward prevention of more serious disease and attenuating disease associated with primary infection and reinfections by restricting viral replication. Because the greatest clinical and economic burdens have been estimated to occur with hPIV-3 infection among infants, compromised patients, and elderly individuals, many of the candidate vaccines have been aimed at providing protection against hPIV-3 infection.[3,52,143,188]

Initially formalin-inactivated, parenteral hPIV vaccines, along with formalin-inactivated vaccines for RSV, were developed and evaluated in clinical trials.[38,63,113,183] In general, these vaccines, whether monovalent, trivalent, or combined with other respiratory vaccines, were immunogenic, inducing a variable humoral and mucosal antibody response, but they were not protective.[38,63] In many cases the serum antibody titers were insufficient, and nasal neutralizing antibodies were not induced.[183]

Development of candidate vaccines currently focuses primarily on live-attenuated and subunit vaccines.[52,72,182] Live-attenuated vaccines, in contrast to inactivated or subunit vaccines, have the potential advantages of inducing immunity more closely mimicking natural infection by eliciting both humoral and mucosal immunity and a longer duration of protection. Furthermore, because significant yearly antigenic change, as observed with the influenza viruses, does not occur with hPIVs, a single monovalent or polyvalent vaccine may suffice, although later booster doses may be required. The potential disadvantages of a live vaccine are more adverse or symptomatic reactions, the possible transmission of the vaccine virus, and insufficient genetic stability to prevent

reversion to the more virulent virus from which it was derived.

Cold-passage hPIV-3 mutant strains derived from a wild-type hPIV-3 strain produced in cell culture (by 45 passages at low temperature; cold passages *[cp]*) were demonstrated to be stable and to grow well at the lower temperatures of the nasal passages, but not at the higher temperatures of the lower respiratory tract, and they were attenuated clinically.[52] The *cp*45 mutant strain was developed into a candidate hPIV-3 vaccine (hPIV-3 *cp*45) containing 20-point mutation with the desired characteristics of cold adaptation *(ca)*, temperature sensitivity *(ts)*, attenuation *(att)*, and genetic stability.[14,15,178,179] A second candidate hPIV-3 vaccine was developed from a closely related bovine hPIV-3 (bPIV-3). The protective surface glycoproteins, HN and F, of bPIV-3 and hPIV-3 share amino acid identities of 75 and 78 percent, respectively.[192] In contrast to hPIV, the bPIV-3 has restricted replication in humans and thus is attenuated. The hPIV-3 *cp*45 was evaluated in phase I and II clinical trials in adults, seropositive children, and seronegative infants as young as 1 month of age. In seropositive children and adults, the vaccines were not sufficiently immunogenic or protective.[105] In some trials of seronegative infants, the hPIV3 *cp*45 vaccine was too reactogenic and was associated with otitis media.[52,105] Nonetheless, the vaccine readily produced infection and was immunogenic. Most toddlers and infants developed both humoral IgG and IgA anti-HN antibody responses, and the presence of maternal antibodies did not affect vaccine virus replication in the upper airway. Low titers of the attenuated virus were shed for 2 to 3 weeks after vaccination. The observation that a higher percentage of infants shed the virus after receiving the second dose 3 months after the first dose (62%), in contrast to those receiving the second dose 1 month after the first dose (24%), suggests a rapid waning of protective immunity.[105] In a subsequent trial of hPIV3 *cp*45 vaccine involving seronegative children 6 to 18 months old, the vaccine was found to be well-tolerated, safe, and immunogenic.[15] No significant difference in frequency of side-effects (including otitis media) between the vaccine and placebo was observed. An attempt to combine the hPIV3 *cp*45 with live-attenuated RSV (*cpts* 248/404) found that replication of the attenuated RSV interfered with that of the attenuated hPIV-3.[14]

When a bovine PIV-3 (bPIV-3) vaccine was evaluated as a live-attenuated vaccine against hPIV-3, it was demonstrated to be safe and immunogenic against bPIV-3, but because of its antigenic differences from hPIV-3, it induced only a modest immune response against hPIV-3 in seronegative infants and children.[72,107]

The use of reverse genetics is particularly applicable and beneficial in advancing the development of hPIV vaccines because the molecular virology of the PIVs has been relatively well deciphered.[143] This technology has allowed the development of such PIV candidate vaccines as those produced by recovering infectious hPIV and bPIV from cDNA. The systematic introduction of desired mutations into the viral genome with a well-defined passage history and with minimal risk for biologic contamination resulted in recombinant and chimeric vaccines with more precise and promising profiles. In a phase I trial with the recombinant virus (9r) hPIV-3 *cp*45 in seronegative infants 6 to less than 12 months of age, the vaccine appeared safe, well tolerated, and immunogenic.[18] After three vaccine doses administered at 2-month intervals, 76.9 percent of the infants had over a fourfold increase in hemagglutination inhibition antibody. Shedding of the vaccine virus was restricted in titer and in duration after the second and third doses, suggesting an immune response was elicited. The cDNA-derived virus was also shown to be comparable to previously observed results with the biologically derived hPIV-3 *cp*45 vaccine.[106] In an attempt to improve the immunogenicity, cDNA-derived chimeric human-bovine vaccines for PIV-3 were constructed by replacing the HN and F glycoproteins of bPIV-3 with those of hPIV-3, and substituting the N of hPIV-3 with that of bPIV-3.[9,82,103] The chimeric vaccine was evaluated in adults and in hPIV-3 seropositive and seronegative children. Although the vaccine's viral replication was highly restricted in both adults and seropositive children, it remained infectious in seronegative children and antibody was produced after a single dose.[108] However, the titer was lower than that observed among children with natural hPIV-3 infection, suggesting that a series of vaccinations, such as three injections 2 months apart, may improve the antibody response.

The need to protect against both hPIV and RSV early in life led to further modifications of the chimeric rB/hPIV3 that also allowed the expression of the G and/or F proteins of RSV.[175] The vaccine, when administered to seropositive children 1 to 9 years of age, appeared to be safe, but with restricted replication, and was minimally immunogenic.[70] Further investigation among seronegative infants (6 to 24 months of age) showed seroconversion occurred for RSV in 67 percent and for hPIV-3 in 100 percent of the infants.[17] Subunit vaccines containing HN and F proteins from hPIV-1 in hPIV-3 have not been encouraging, but the introduction of amino acid substitutions in various hPIV-1 genes was successful in creating an attenuated virus. A vaccine containing three attenuating elements that protected against hPIV-1 infection of both the upper and lower respiratory tract of African green monkeys[11] is now being tested in seronegative children. Although the need for a vaccine against hPIV-2 is less because of its lower prevalence and clinical significance in the first 6 months of life, several candidate hPIV-2 live-attenuated vaccines have been developed. Mutations in the L protein produced strains that were both temperature sensitive and attenuated. One of these mutant strains was found to be attenuated in an in vitro model of human ciliated airway epithelium and to provide significant protection against wild-type hPIV-2 challenge in African green monkeys.[148,173]

Multiple other approaches to develop candidate hPIV vaccines through recombinant DNA technology are being explored and include chimeric PIV vaccines with heterologous paramyxoviruses and with viruses for which concurrent immunization would be desirable, such as RSV and influenza.[10,127] Approaches combined with these creative techniques, such as novel adjuvants, immunomodulators, and vehicles for incorporation and administration of the vaccine, are evolving and may enhance

further the protective immune response from PIV immunization.

NEW REFERENCES SINCE THE SIXTH EDITION

2. American Academy of Pediatrics. Parainfluenza. In: Pickering L, Baker C, Kimberlin DW, Long S, editors. *Red book 2009: Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 485–7.
6. Arens MQ, Buller RS, Rankin A, et al. Comparison of the Eragen multi-code respiratory virus panel with conventional viral testing and real-time multiplex PCR assays for detection of respiratory viruses. *J Clin Microbiol* 2010;**48**:2387–95.
10. Bartlett EJ, Boonyaratanakornkit J, Collins PL, et al. Progress in development of human parainfluenza virus vaccines. *Exp Rev Respiratory Med* 2011;**5**:515–31.
13. Beck ET, Henrickson KJ. Molecular diagnosis of respiratory viruses. *Future Microbiol* 2010;**5**:901–16.
17. Bernstein DI, Malkin E, Charenkavanich S, Dubovsky F. *Safety profile, immunogenicity and viral shedding of MEDI-534, a live, attenuated respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) vaccine in RSV/PIV3 seronegative children*. Rotterdam, Netherlands: International RSV Symposium; 2010.
18. Bernstein DI, Falloon J, Yi T. A randomized, double-blind, placebo-controlled, phase 1/2a study of the safety and immunogenicity of a live attenuated human parainfluenza virus type 3 vaccine in healthy infants. *Vaccine* 2011;**29**:7042–8.
20. Bjornson CL, Johnson DW. Croup. *Lancet* 2008;**371**:329–39.
26. Burke CW, Mason JN, Shurman SL, et al. Illumination of parainfluenza virus infection and transmission in living animals reveals a tissue-specific dichotomy. *PLoS* 2011;**7**:e1002134.
27. Caliendo AM. Multiplex PCR and emerging technologies for the detection of respiratory pathogens. *Clin Infect Dis* 2011;**52**(suppl 4):S326–30.
28. Calvo C, Garcia-Garcia ML, Ambrona P, et al. The burden of infections by parainfluenza virus in hospitalized children in Spain. *Ped Infect Dis J* 2011;**30**:792–4.
29. Campbell AP, Chien JW, Kuypers J, et al. Respiratory virus pneumonia after hematopoietic cell transplantation (HCT): associations between viral load in bronchoalveolar lavage samples, viral RNA detection in serum samples and clinical outcomes of HCT. *J Infect Dis* 2010;**201**:1404–13.
36. Chemaly RF, Hanmod SS, Rathod DB, et al. The characteristics and outcomes of parainfluenza virus infections in 200 patients with leukemia or recipients of hematopoietic stem cell transplantation. *Blood* 2012;**119**:2738–45.
37. Cherry JD. Clinical practice. *Croup N Engl J Med* 2008;**358**:384–91.
47. Cunha BA, Mickall N, Schoch P. Human parainfluenza virus type 3 (HPIV 3) community-acquired pneumonia (CAP) mimicking pertussis in an adult: the diagnostic importance of hoarseness and monocytosis. *Heart Lung* 2011;**40**:569–73.
48. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009;**15**:34–41.
51. doCarmo Debur M, Raboni SM, Flizikowski FBZ. Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal and non-pandemic seasonal respiratory infections. *J Clin Pathol* 2010;**63**:930–4.
57. Fairchok MP, Martin ET, Kuypers J, Englund JA. A prospective study of parainfluenza virus type 4 infections in children attending daycare. *Ped Infect Dis J* 2011;**30**:714–16.
62. Fuentes SM, He B, Sun D, Schmitt AP. Phosphorylation of paramyxovirus phosphoprotein and its role in viral gene expression. *Future Microbiol* 2010;**5**:9–13.
64. Gitlin L, Benoit L, Song C, et al. Melanoma differentiation-associated gene 5 (MDA5) is involved in the innate immune response to Paramyxoviridae infection in vivo. *PLoS Pathog* 2010;**6**:e1000734.
70. Gomez M, Mufson MA, Dubovsky F, et al. Phase-I study MEDI-534, of a live, attenuated intranasal vaccine against respiratory syncytial virus and parainfluenza-3 virus in seropositive children. *Pediatr Infect Dis J* 2009;**28**:655–8.
83. Harrison MS, Sakaguchi T, Schmitt AP. Paramyxovirus assembly and budding: building particles that transmit infections. *Intl J Biochem Cell Biol* 2010;**42**:1416–29.
117. Lau SK, Li KS, Chau KY, et al. Clinical and molecular epidemiology of human parainfluenza virus 4 infections in Hong Kong; subtype 4B as common as subtype 4A. *J Clin Microbiol* 2009;**47**:1549–52.
131. Maziarz RT, Sridharan P, Slater S, et al. Control of an outbreak of human parainfluenza virus 3 in hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transpl* 2010;**16**:192–8.
160. Piralla A, Percivalle E, Di Cesare-Merlone A, et al. Multicluster nosocomial outbreak of parainfluenza virus type 3 infection in a pediatric oncohematology unit: a phylogenetic study. *Haematologica* 2009;**94**:833–9.
161. Pogka V, Kossivakis A, Kalliaropoulos A, et al. Respiratory viruses involved in influenza-like illness in a Greek pediatric population during the winter period of the years 2005-2008. *J Med Virol* 2011;**83**:1841–8.
166. Ren L, Gonzalez R, Xie Z, et al. Human parainfluenza virus type 4 infection in Chinese children with lower respiratory tract infections: a comparison study. *J Clin Virol* 2011;**51**:209–12.
167. Rihkanen H, Ronkko E, Nieminen T, et al. Respiratory viruses in laryngeal croup of young children. *J Pediatr* 2008;**152**:661–5.
169. Rosychuk JJ, Klassen TP, Metes D, et al. Croup presentations to emergency departments in Alberta, Canada. *Pediatr Pulmonol* 2010;**45**:83–91.
173. Schaap-Nutt A, Scull MA, Schmidt AC, et al. Growth restriction of an experimental live attenuated human parainfluenza virus type 2 vaccine in human ciliated airway epithelium *in vitro* parallels attenuation in African green monkeys. *Vaccine* 2010;**28**:2788–98.
176. Schnepf N, Resche-Rigon M, Chaillon A, et al. High burden of non-influenza viruses in influenza-like illness in the early weeks of H1N1v epidemic in France. *PLoS ONE* 2011;**6**:e23514.
184. Sobol SE, Zapata S. Epiglottitis and croup. *Otolaryngol Clin North Am* 2008;**41**:551–66.
190. van Asten L, van den Wijngaard C, van Pelt W, et al. Mortality attributable to 9 common infections: significant effect of influenza A, RSV, influenza B, norovirus and parainfluenza in the elderly. *J Infect Dis* 2012;**206**:628–39.
199. Wall SR, Wat D, Spiller OB, et al. The viral aetiology of croup and recurrent croup. *Arch Dis Child* 2009;**94**:359–60.
200. Wang CYT, Arden KE, Greer R, et al. A novel duplex real-time PCR for HPIV-4 detects co-circulation of both viral subtypes among ill children during 2008. *J Clin Virol* 2012;**54**:83–5.
204. Wiertsema SP, Chidlow GR, Kirkham LS, et al. High detection rates of nucleic acids of a wide range of respiratory viruses in the nasopharynx and the middle ear of children with a history of recurrent acute otitis media. *J Med Virol* 2011;**83**:2008–17.
206. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. *J Infect Dis* 2010;**201**:1890–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alymova IV, Taylor G, Takimoto T, et al. Efficacy of novel hemagglutinin-neuraminidase inhibitors BCX 2798 and BCX 2855 against human parainfluenza viruses in vitro and in vivo. *Antimicrob Agents Chemother* 2004;**48**:1495–502.
2. American Academy of Pediatrics. Parainfluenza. In: Pickering L, Baker C, Kimberlin DW, Long S, editors. *Red book 2009: Report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 485–7.
3. Amir L, Hbermann H, Halevi A, et al. Oral dexamethasone versus intramuscular dexamethasone for the treatment of mild to moderate viral croup: a prospective, randomized trial. *Pediatr Emerg Care* 2006;**22**:541–4.
4. Andrewes CH, Bang FB, Chanock RM, Zhdanov VM. Parainfluenza viruses 1, 2, and 3: suggested names for recently described myxoviruses. *Virology* 1959;**8**:129–30.
5. Ansari SA, Springthorpe VS, Sattar SA, et al. Potential role of hands in the spread of respiratory viral infections: studies with human parainfluenza virus 3 and rhinovirus 14. *J Clin Microbiol* 1991;**29**:2115–19.
6. Arens MQ, Buller RS, Rankin A, et al. Comparison of the Eragen multi-code respiratory virus panel with conventional viral testing and real-time multiplex PCR assays for detection of respiratory viruses. *J Clin Microbiol* 2010;**48**:2387–95.
7. Arisoy ES, Demmler GJ, Thakar S, Doerr C. Meningitis due to parainfluenza virus type 3: report of two cases and review. *Clin Infect Dis* 1993;**17**:995–7.
8. Au WY, Lie AKW, Cheung RTF, et al. Acute disseminated encephalomyelitis after para-influenza infection post bone marrow transplantation. *Leuk Lymphoma* 2002;**43**:455.
9. Bailly JE, McAuliffe JM, Durbin AP, et al. Recombinant human parainfluenza virus type 3 (PIV3) in which the nucleocapsid N protein has been replaced by that of bovine PIV3 is attenuated in primates. *J Virol* 2000;**74**:3188–95.
10. Bartlett EJ, Boonyaratanakornkit J, Collins PL, et al. Progress in development of human parainfluenza virus vaccines. *Exp Rev Respir Med* 2011;**5**:515–31.
11. Bartlett EJ, Castano A, Surman SR, et al. Attenuation and efficacy of human parainfluenza virus type 1 (HPIV1) vaccine candidates containing stabilized mutations in the P/C and L genes. *Virol J* 2007;**4**:67.
12. Barton TD, Blumberg EA. Viral pneumonias other than cytomegalovirus in transplant recipients. *Clin Chest Med* 2005;**26**: 707–20.
13. Beck ET, Henrickson KJ. Molecular diagnosis of respiratory viruses. *Future Microbiol* 2010;**5**:901–16.
14. Belshe RB, Newman FK, Anderson EL, et al. Evaluation of combined live, attenuated respiratory syncytial virus and parainfluenza 3 virus vaccines in infants and young children. *J Infect Dis* 2004;**190**:2096–103.
15. Belshe RB, Newman FK, Tsai TF, et al. Phase 2 evaluation of parainfluenza type 3 cold passage mutant 45 live attenuated vaccine in healthy children 6-18 months old. *J Infect Dis* 2004;**189**: 462–70.
16. Beppu Y, Imamura Y, Tashiro M, et al. Human mucus protease inhibitor in airway fluids is a potential defensive compound against infection with influenza A and Sendai viruses. *J Biochem* 1997; **121**:309–16.
17. Bernstein DI, Malkin E, Charenkavanich S, Dubovsky F. *Safety profile, immunogenicity and viral shedding of MEDI-534, a live, attenuated respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) vaccine in RSV/PIV3 seronegative children*. Rotterdam, Netherlands: International RSV Symposium; 2010.
18. .Bernstein DI, Falloon J, Yi T. A randomized, double-blind, placebo-controlled, phase 1/2a study of the safety and immunogenicity of a live attenuated human parainfluenza virus type 3 vaccine in healthy infants. *Vaccine* 2011;**29**:7042–8.
19. Billings JL, Hertz MI, Savik K, Wendt CH. Respiratory viruses and chronic rejection in lung transplant recipients. *J Heart Lung Transplant* 2002;**21**:559–66.
20. Bjornson CL, Johnson DW. Croup. *Lancet* 2008;**371**:329–39.
21. Bloom HH, Johnson KM, Jacobsen R. Recovery of parainfluenza viruses from adults with upper respiratory illness. *Am J Hyg* 1961; **74**:50–9.
22. Bousse T, Chambers RL, Scroggs RA, et al. Human parainfluenza virus type 1 but not Sendai virus replicates in human respiratory cells despite IFN treatment. *Virus Res* 2006;**121**:23–32.
23. Bowden TA, Crispin M, Jones Y, Stuart DI. Shared paramyxoviral glycoprotein architecture is adapted for diverse attachment strategies. *Biochem Soc Trans* 2010;**38**:1349–55.
24. Brady MT, Evans J, Cuartas J. Survival and disinfection of parainfluenza viruses on environmental surfaces. *Am J Infect Control* 1990;**18**:18–23.
25. Briese T, Palacios G, Kokoris M, et al. Diagnostic system for rapid and sensitive differential detection of pathogens. *Emerg Infect Dis* 2005;**11**:310–13.
26. Burke CW, Mason JN, Shurman SL, et al. Illumination of parainfluenza virus infection and transmission in living animals reveals a tissue-specific dichotomy. *PLoS* 2011;**7**:e1002134.
27. Caliendo AM. Multiplex PCR and emerging technologies for the detection of respiratory pathogens. *Clin Infect Dis* 2011; **52**(suppl 4):S326–30.
28. Calvo C, Garcia-Garcia ML, Ambrona P, et al. The burden of infections by parainfluenza virus in hospitalized children in Spain. *Ped Infect Dis J* 2011;**30**:792–4.
29. Campbell AP, Chien JW, Kuypers J, et al. Respiratory virus pneumonia after hematopoietic cell transplantation (HCT): associations between viral load in bronchoalveolar lavage samples, viral RNA detection in serum samples and clinical outcomes of HCT. *J Infect Dis* 2010;**201**:1404–13.
30. Cetinkaya F, Tufekci BS, Kutluck GA. A comparison of nebulized budesonide and intramuscular, and oral dexamethasone for treatment of croup. *Int J Pediatr Otolaryngol* 2004;**68**:453–6.
31. Chakrabarti S, Collingham KE, Holder K, et al. Parainfluenza virus 3 infection after stem cell transplant relevance to outcome of rapid diagnosis and ribavirin treatment. *Clin Infect Dis* 2001; **32**:413–18.
32. Chan A, Langley J, Leblanc J. Interobserver variability of croup scoring in clinical practice. *Paediatr Child Health* 2001;**6**: 347–51.
33. Chanock RM. Association of a new type of cytopathogenic myxovirus with infantile croup. *J Exp Med* 1965;**104**:555–76.
34. Chanock RM, Bell JA, Parrott RH. Natural history of parainfluenza infection. *Perspect Virol* 1961;**2**:126–39.
35. Chanock RM, Parrott RH, Cook K, et al. Newly recognized myxoviruses from children with respiratory disease. *N Engl J Med* 1958;**5**:207–13.
36. Chemaly RF, Hanmod SS, Rathod DB, et al. The characteristics and outcomes of parainfluenza virus infections in 200 patients with leukemia or recipients of hematopoietic stem cell transplantation. *Blood* 2012;**119**:2738–45.
37. Cherry JD. Clinical practice: croup. *N Engl J Med* 2008;**358**: 384–91.
38. Chin J, Magoffin RL, Shearer LA. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. *Am J Epidemiol* 1969;**89**:449–63.
39. Chonmaitree T, Henrickson KJ. Detection of respiratory viruses in the middle ear fluids of children with acute otitis media by multiplex reverse transcription polymerase chain reaction assay. *Pediatr Infect Dis J* 2000;**19**:258–60.
40. Chua KB, Bellini WJ, Rota PA, et al. Nipah virus: a recently emergent deadly paramyxovirus. *Science* 2000;**288**:1432–5.
41. Chub-Uppakarn S, Sangsupawanich P. A randomized comparison of dexamethasone 0.15 mg/kg versus 0.6 mg/kg for the treatment of moderate to severe croup. *Int J Pediatr Otolaryngol* 2007;**71**: 473–7.
42. Cobian L, Houston S, Greene J, Sinnott JT. Parainfluenza virus respiratory infection after heart transplantation: successful treatment with ribavirin. *Clin Infect Dis* 1995;**21**:1040–1.
43. Collinson J, Nicholson KG, Cancio E, et al. Effects of upper respiratory tract infections in patients with cystic fibrosis. *Thorax* 1996;**51**:1115–22.
44. Cortez KJ, Erdman DD, Peret TCT, et al. Outbreak of human parainfluenza virus 3 infections in a hematopoietic stem cell transplant population. *J Infect Dis* 2001;**184**:1093–7.
45. Counihan ME, Shay DK, Holman RC, et al. Human parainfluenza virus–associated hospitalizations among children less than five years of age in the United States. *Pediatr Infect Dis J* 2001; **20**:646–53.

46. Crowe JE, Williams JV. Immunology of viral respiratory tract infection in infancy. *Paediatr Respir Rev* 2003;**4**:112–19.
47. Cunha BA, Mickall N, Schoch P. Human parainfluenza virus type 3 (HPIV 3) community-acquired pneumonia (CAP) mimicking pertussis in an adult: the diagnostic importance of hoarseness and monocytosis. *Heart Lung* 2011;**40**:569–73.
48. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009;**15**:34–41.
49. Denny FW. Certain biologic characteristics of myxovirus parainfluenza 3. *Fed Proc* 1960;**19**:409.
50. Dick EC, Mogabgab WJ. Characteristics of parainfluenza 1 (HA-2) virus. III. Antigenic relationships, growth, interaction with erythrocytes, and physical properties. *J Bacteriol* 1962;**83**:561–71.
51. doCarmo Debur M, Raboni SM, Flizikowski FBZ. Immunohistochemical assessment of respiratory viruses in necropsy samples from lethal and non-pandemic seasonal respiratory infections. *J Clin Pathol* 2010;**63**:930–4.
52. Durbin AP, Karron RA. Progress in the development of respiratory syncytial virus and parainfluenza virus vaccines. *Clin Infect Dis* 2003;**37**:1668–77.
53. Elizaga J, Olavarria E, Apperley J, et al. Parainfluenza virus 3 infection after stem cell transplant: relevance to outcome of rapid diagnosis and ribavirin treatment. *Clin Infect Dis* 2001;**32**:413–18.
54. Englund JA, Piedra PA, Whimbey E. Prevention and treatment of respiratory syncytial virus and parainfluenza viruses in immunocompromised patient. *Am J Med* 1997;**102**:61–70.
55. Erard V, Chien JW, Kim HW, et al. Airflow decline after myeloablative allogeneic hematopoietic cell transplantation: the role of community respiratory viruses. *J Infect Dis* 2006;**193**:1619–25.
56. Evans AS, Brobst M. Bronchitis, pneumonitis and pneumonia in University of Wisconsin students. *N Engl J Med* 1961;**265**:401–9.
57. Fairchok MP, Martin ET, Kuypers J, Englund JA. A prospective study of parainfluenza virus type 4 infections in children attending daycare. *Ped Infect Dis J* 2011;**30**:714–16.
58. Fedova D, Novotny J, Kubinova I. Serological diagnosis of parainfluenza virus infections: verification of the sensitivity and specificity of the haemagglutination-inhibition (HI), complement-fixation (CF), immunofluorescence (IFA) tests and enzyme immunoassay (ELISA). *Acta Virol* 1992;**36**:304–12.
59. Forster J, Ihorst G, Rieger C, et al. Prospective population-based study of viral lower respiratory tract infections in children under 3 years of age (the PRI.DE study). *Eur J Pediatr* 2004;**163**:709–16.
60. Fraser KB, Anderson J. Persistent non-cytocidal infection of BHK 21 cells by human parainfluenza type 2 virus. *J Gen Microbiol* 1966;**44**:47–58.
61. Fry AM, Curns AT, Harbour K, et al. Seasonal trends of human para-influenza viral infections: United States. *Clin Infect Dis* 2006;**43**:1016.
62. Fuentes SM, He B, Sun D, Schmitt AP. Phosphorylation of paramyxovirus phosphoprotein and its role in viral gene expression. *Future Microbiol* 2010;**5**:9–13.
63. Fulginiti VA, Eller JJ, Sieber OF, et al. Respiratory virus immunization. I. A field trial of two inactivated respiratory virus vaccines: an aqueous trivalent parainfluenza virus vaccine, and an alum-precipitated respiratory syncytial virus vaccine. *Am J Epidemiol* 1969;**89**:435–48.
64. Gitlin L, Benoit L, Song C, et al. Melanoma differentiation-associated gene 5 (MDA5) is involved in the innate immune response to Paramyxoviridae infection in vivo. *PLoS Pathog* 2010;**6**:e1000734.
65. Glezen WP. Morbidity associated with the major respiratory viruses. *Pediatr Ann* 1990;**19**:535–42.
66. Glezen WP, Frank AL, Taber LH, Kasel JA. Parainfluenza virus type 3: seasonality and risk of infection and reinfection in young children. *J Infect Dis* 1984;**150**:851–7.
67. Glezen WP, Greenberg SB, Atmar RL, et al. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000;**283**:499–505.
68. Glezen WP, Loda FA, Clyde WA Jr, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
69. Goh KJ, Tan CT, Chew NK, et al. Clinical features of Nipah virus encephalitis among pig farmers in Malaysia. *N Engl J Med* 2000;**342**:1229–35.
70. Gomez M, Mufson MA, Dubovsky F, et al. Phase-I study MEDI-534, of a live, attenuated intranasal vaccine against respiratory syncytial virus and parainfluenza-3 virus in seropositive children. *Pediatr Infect Dis J* 2009;**28**:655–8.
71. Gotoh B, Komatsu T, Takeuchi K, Yokoo J. Paramyxovirus strategies for evading the interferon response. *Rev Med Virol* 2000;**12**:337–57.
72. Greenberg DP, Walker RE, Lee MS, et al. A bovine parainfluenza virus type 3 vaccine is safe and immunogenic in early infancy. *J infect Dis* 2005;**191**:1116–22.
73. Greengard O, Poltoratskaia N, Leikina E, et al. The anti-influenza virus agent 4-GU-DANA (zanamivir) inhibits cell fusion mediated by human parainfluenza virus and influenza virus HA. *J Virol* 2000;**74**:11108–14.
74. Gross PA, Green RH, Curren MGM. Persistent infection with parainfluenza type 3 virus in man. *Am Rev Respir Dis* 1973;**108**:894–8.
75. Grubor B, Gallup JM, Meyerholz DK, et al. Enhanced surfactant protein and defensin mRNA levels and reduced viral replication during parainfluenza virus type 3 pneumonia in neonatal lambs. *Clin Diagn Lab Immunol* 2004;**11**:599–607.
76. Gurwitz D, Corey M, Levison H. Pulmonary function and bronchial reactivity in children after croup. *Am Rev Respir Dis* 1980;**122**:95–9.
77. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001;**344**:1917–28.
78. Hall CB. Green schools: attributes for health and learning. In: N.R.C.O.T.N. Academies, editor. *Building Characteristics and the Spread of Infectious Diseases*, *in Green Schools: Attributes for Health and Learning*. Washington, DC: National Academies Press; 2007. p. 105–19.
79. Hall CB, Douglas RG. Respiratory syncytial virus and influenza: practical community surveillance. *Am J Dis Child* 1976;**130**:615–20.
80. Hall CB, Douglas RG Jr, Simons RL, Geiman JM. Interferon production in children with respiratory syncytial, influenza, and parainfluenza virus infections. *J Pediatr* 1978;**93**:28–32.
81. Hall CB, Geiman JM, Breese BB, Douglas RG Jr. Parainfluenza viral infections in children: correlation of shedding with clinical manifestations. *J Pediatr* 1977;**91**:194–8.
82. Haller AA, Miller T, Mitiku M, Coelingh K. Expression of the surface glycoproteins of human parainfluenza virus type 3 by bovine parainfluenza virus type 3, a novel attenuated virus vaccine vector. *J Virol* 2000;**74**:11626–35.
83. Harrison MS, Sakaguchi T, Schmitt AP. Paramyxovirus assembly and budding: Building particles that transmit infections. *Intl J Biochem Cell Biol* 2010;**42**:1416–29.
84. Heidemann SM. Clinical characteristics of parainfluenza virus infection in hospitalized children. *Pediatr Pulmonol* 1992;**13**:86–9.
85. Henderson FW, Collier AM, Sanyal MA, et al. A longitudinal study of respiratory viruses and bacteria in the etiology of acute otitis media with effusion. *N Engl J Med* 1982;**306**:1377–83.
86. Henrickson KJ. Parainfluenza viruses. *Clin Microbiol Rev* 2003;**16**:242–64.
87. Henrickson KJ. Advances in the laboratory diagnosis of viral respiratory disease. *Pediatr Infect Dis J* 2004;**23**(suppl.):S6–10.
88. Henrickson KJ, Hoover S, Kehl KS, Hua W. National disease burden of respiratory viruses detected in children by polymerase chain reaction. *Pediatr Infect Dis J* 2004;**23**(suppl.):S11–18.
89. Henrickson KJ, Kuhn SM, Savatski LL. Epidemiology and cost of infection with human parainfluenza virus types 1 and 2 in young children. *Clin Infect Dis* 1994;**18**:770–9.
90. Herrmann EC, Hable KA. Experiences in laboratory diagnosis of parainfluenza viruses in routine medical practice. *Mayo Clin Proc* 1970;**45**:177–88.
91. Hopkins A, Lahiri T, Salerno R, Heath B. Changing epidemiology of life-threatening upper airway infections: the reemergence of bacterial tracheitis. *Pediatrics* 2006;**118**:1418–21.
92. Hotez PJ, Goldstein B, Ziegler J, et al. Adult respiratory distress syndrome associated with parainfluenza virus type 1 in children. *Pediatr Infect Dis J* 1990;**9**:750–2.
93. Hou S, Doherty PC, Zijlstra M, et al. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8+ T cells. *J Immunol* 1992;**149**:1319–25.

94. Hurrell JM. Methods of storing viruses at low temperatures with particular reference to the Myxovirus group. *J Med Lab Technol* 1967;**24**:30–41.
95. Ison MG. Respiratory viral infections in transplant recipients. *Antiviral Ther* 2007;**12**:627–38.
96. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. *Pediatrics* 2004;**113**:1758–64.
97. Jackson MA, Olson LC, Burry VF. Parainfluenza virus and neurologic signs. *Pediatr Infect Dis J* 1994;**13**:759–80.
98. Jarvis WR, Middleton PJ, Gelfand EW. Parainfluenza pneumonia in severe combined immunodeficiency disease. *J Pediatr* 1979;**94**: 423–5.
99. Johnson D. Croup. *Clin Evid* 2005;**14**:310–27.
100. Johnson KM, Chanock RM, Cook MK, Huebner RJ. Studies of a new human hemadsorption virus. I. Isolation, properties and characterization. *Am J Hyg* 1960;**71**:81–92.
101. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;**310**:1225–9.
102. Josephs S, Kim HW, Brandt CD, Parrott RH. Parainfluenza 3 virus and other common respiratory pathogens in children with human immunodeficiency virus infection. *Pediatr Infect Dis J* 1988;**7**:207–9.
103. Karron RA, Collins PL. Parainfluenza viruses. In: Rields BN, Knipe DM, Howley PM, editors. *Field's virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 1497–526.
104. Karron RA, O'Brien KL, Froehlich JL, Brown VA. Molecular epidemiology of a parainfluenza type 3 virus outbreak on a pediatric ward. *J Infect Dis* 1993;**167**:1441–5.
105. Karron RA, Belshe RB, Wright PF, et al. A live human parainfluenza type 3 virus vaccine is attenuated and immunogenic in young infants. *Pediatr Infect Dis J* 2003;**22**:394–405.
106. Karron RA, Casey R, Thumar B, et al. The cDNA-derived investigational human parainfluenza virus type 3 vaccine rcp45 is well-tolerated, infectious, and immunogenic in infants and young children. *Pediatr Infect Dis J* 2011;**30**:e186–9.
107. Karron RA, Makhene M, Gay K, et al. Evaluation of a live attenuated bovine parainfluenza type 3 vaccine in two- to six- month old infants. *Pediatr Infect Dis J* 1996;**15**:650–4.
108. Karron RA, Thumor B, Schappell E, et al. Evaluation of two chimeric bovine-human parainfluenza virus type 3 vaccines in infants and young children. *Vaccine* 2012;**30**:3975–81.
109. Kasel JA, Frank AL, Keitel WA, et al. Acquisition of serum antibodies to specific viral glycoproteins of parainfluenza virus 3 in children. *J Virol* 1984;**52**:828–32.
110. Kast WM, Roux L, Curren J, et al. Protection against lethal Sendai virus infection by in vivo priming of virus-specific cytotoxic T lymphocytes with a free synthetic peptide. *Proc Natl Acad Sci U S A* 1991;**88**:2283–7.
111. Kato A. Sendai virus proteins counteracting the host innate immunity. *Uirusu* 2004;**54**:179–88.
112. Killgore GE, Dowdle WR. Antigenic characterization of parainfluenza 4A and 4B by the hemagglutination-inhibition test and distribution of HI antibody in human sera. *Am J Epidemiol* 1970; **91**:308–16.
113. Kim HW, Canchola JG, Vargosko AJ, et al. Immunogenicity of inactivated parainfluenza type 1, type 2, and type 3 vaccines in infants. *JAMA* 1966;**196**:819–24.
114. Knott AM, Long CE, Hall CB. Parainfluenza viral infections in pediatric outpatients: seasonal patterns and clinical characteristics. *Pediatr Infect Dis J* 1994;**13**:269–73.
115. Kuroya M, Ishida N. New born virus pneumonitis (type Sendai). II. The isolation of a new virus possessing hemagglutination activity. *Yokohama Med Bull* 1953;**4**:217–33.
116. Lange T, Franke G, Niederwieser D. Parotitis associated with a para-influenza virus type 3 infection during aplasia after unrelated allogeneic stem cell transplantation. *Leuk Lymphoma* 2006;**47**:1714.
117. Lau SK, Li KS, Chau KY, et al. Clinical and molecular epidemiology of human parainfluenza virus 4 infections in Hong Kong; subtype 4B as common as subtype 4A. *J Clin Microbiol* 2009;**47**: 1549–52.
118. Lau SK, To WK, Tse PW, et al. Human parainfluenza virus 4 outbreak and the role of diagnostic tests. *J Clin Microbiol* 2005;**43**:4515–21.
119. Laurichesse H, Dedman D, Watson JM, Zambon MC. Epidemiological features of parainfluenza virus infections: laboratory surveillance in England and Wales, 1975-1997. *Eur J Epidemiol* 1999; **15**:475–84.
120. Lee I, Barton TD. Viral respiratory tract infections in transplant patients. *Drugs* 2007;**67**:1411–27.
121. Levroney EL, Aguilar HC, Fulcher JA, et al. Novel innate immune functions for galectin-1: galectin-1 inhibits cell fusion by Nipah virus envelope glycoproteins and augments dendritic cell secretion of proinflammatory cytokines. *J Immunol* 2005;**175**:413–20.
122. Lindquist SW, Darnule A, Istas A, Demmler GJ. Parainfluenza virus type 4 infections in pediatric patients. *Pediatr Infect Dis J* 1997;**16**:34–8.
123. Lopez CB, Moltedo B, Alexopoulou L, et al. TLR-independent induction of dendritic cell maturation and adaptive immunity by negative-strand RNA viruses. *J Immunol* 2004;**173**:6882–9.
124. Loughlin G, Taussig LM. Pulmonary function in children with a history of laryngotracheobronchitis. *J Pediatr* 1979;**94**:365–9.
125. Lu G, Reinert JT, Pitha-Rowe I, et al. ISG15 enhances the innate antiviral response by inhibition of IRF-3 degradation. *Cell Mol Biol (Noisy-le-grand)* 2006;**52**:29–41.
126. Madhi SA, Schoub B, Simmank K, et al. Increased burden of respiratory viral associated severe lower respiratory tract infections in children infected with human immunodeficiency virus type-1. *J Pediatr* 2000;**137**:78–84.
127. Maeda Y, Hatta M, Takada A, et al. Live bivalent vaccine for parainfluenza and influenza virus infections. *J Virol* 2005;**79**: 6674–9.
128. Malur AG, Hoffman MA, Banerjee AK. The human parainfluenza virus type 3 (HPIV) C protein inhibits viral transcription. *Virus Res* 2004;**99**:199–204.
129. Martino R, Rámila E, Rabella N, et al. Respiratory virus infections in adults with hematologic malignancies: a prospective study. *Clin Infect Dis* 2003;**36**:1–8.
130. Matar LD, McAdams HP, Palmer SM, et al. Respiratory viral infections in lung transplant recipients: radiologic findings with clinical correlation. *Radiology* 1999;**213**:735–42.
131. Maziarz RT, Sridharan P, Slater S, et al. Control of an outbreak of human parainfluenza virus 3 in hematopoietic stem cell transplant recipients. *Biol Blood Marrow Transpl* 2010;**16**:192–8.
132. McCurdy LH, Milstone A, Dummer S. Clinical features and outcomes of paramyxoviral infection in lung transplant recipients treated with ribavirin. *J Heart Lung Transplant* 2003;**22**:745–53.
133. McIntosh K. Pathogenesis of severe acute respiratory infections in the developing world: respiratory syncytial virus and parainfluenza virus. *Rev Infect Dis* 1991;**13**(suppl.):S492–500.
134. Melchjorsen J, Jensen SB, Malmgaard L, et al. Activation of innate defense against a paramyxovirus is mediated by RIG-I and TLR7 and TLR8 in a cell-type-specific manner. *J Virol* 2005;**79**: 12944–51.
135. Mendoza Sanchez MC, Ruiz-Contreras J, Vivanco JL, et al. Respiratory virus infections in children with cancer or HIV infection. *J Pediatr Hematol Oncol* 2006;**28**:154–9.
136. Metzgar DP, Gower TA, Larson EF, et al. The effect of parainfluenza virus type 3 on newborn ferrets. *J Biol Stand* 1974;**2**: 273–82.
137. Monto AS. Epidemiology of viral respiratory infections. *Am J Med* 2002;**112**:4S–12S.
138. Monto AS. Occurrence of respiratory virus: time, place and person. *Pediatr Infect Dis J* 2004;**23**(suppl.):S58–64.
139. Moscona A. Interaction of human parainfluenza virus type 3 with the host cell surface. *Pediatr Infect Dis J* 1997;**16**:917–24.
140. Moyse E, Lyon M, Cordier G, et al. Viral RNA in middle ear mucosa and exudates in patients with chronic otitis media with effusion. *Arch Otolaryngol Head Neck Surg* 2000;**126**:1105–10.
141. Muchmore HG, Parkinson AJ, Humphries JE, et al. Persistent parainfluenza virus shedding during isolation at the South Pole. *Nature* 1981;**289**:187–9.
142. Mufson MA, Mocega HE, Krause HE. Acquisition of parainfluenza 3 virus infection by hospitalized children. I. Frequencies, rates, and temporal data. *J Infect Dis* 1973;**128**:141–7.
143. Murphy BR, Collins PL. Live-attenuated virus vaccines for respiratory syncytial and parainfluenza viruses: applications of reverse genetics. *J Clin Invest* 2002;**110**:21–7.
144. Murrell M, Porotto M, Weber T, et al. Mutations in human parainfluenza virus type 3 hemagglutinin-neuraminidase causing

increased receptor binding activity and resistance to the transition state sialic acid analog 4-GU-DANA (Zanamivir). *J Virol* 2003;**77**: 309–17.
145. Nichols WG, Corey L, Gooley T, et al. Parainfluenza virus infections after hematopoietic stem cell transplantation: risk factors, response to antiviral therapy, and effect on transplant outcome. *Blood* 2001;**98**:573–8.
146. Nichols WG, Erdman DD, Han A, et al. Prolonged outbreak of human parainfluenza virus 3 infection in a stem cell transplant outpatient department: insights from molecular epidemiologic analysis. *Biol Blood Marrow Transplant* 2004;**10**:58–64.
147. Nicholson KG, Kent J, Ireland DC. Respiratory viruses and exacerbations of asthma in adults. *BMJ* 1993;**307**:982–6.
148. Nolan SM, Skiadopoulous MH, Bradley K, et al. Recombinant human parainfluenza virus type 2 vaccine candidates containing a 3' genomic promoter mutation and L polymerase mutations are attenuated and protective in non-human primates. *Vaccine* 2007;**25**:6409–22.
149. Nolan SM, Surman SR, Amaro-Carambot E, et al. Live-attenuated intranasal parainfluenza virus type 2 vaccine candidates developed by reverse genetics containing L polymerase protein mutations imported from heterologous paramyxoviruses. *Vaccine* 2005;**23**: 4765–74.
150. Noyola DE, Rodriguez-Moreno G, Sanchez-Alvarado J, et al. Viral etiology of lower respiratory tract infections in hospitalized children in Mexico. *Pediatr Infect Dis J* 2004;**23**:118–23.
151. Openshaw PJ. Potential therapeutic implications of new insights into respiratory syncytial virus disease. *Respir Res* 2002;**3**(suppl.): S15–20.
152. Openshaw PJ. Antiviral immune responses and lung inflammation after respiratory syncytial virus infection. *Proc Am Thorac Soc* 2005;**2**:121–5.
153. Ottolini MG, Porter DD, Hemming VG, et al. Semi-permissive replication and functional aspects of the immune response in a cotton rat model of human parainfluenza virus type 3 infection. *J Gen Virol* 1996;**77**:1739–43.
154. Parkinson AJ, Muchmore HG, Scott EN, Scott LV. Survival of human parainfluenza viruses in the South Polar environment. *Appl Environ Microbiol* 1983;**46**:901–5.
155. Patterson DL, Murray PK, McCormack JG. Zoonotic disease in Australia caused by a novel member of the Paramyxoviridae. *Clin Infect Dis* 1998;**27**:112–18.
156. Peck AJ, Englund JA, Kuypers J, et al. Respiratory virus infection among hematopoietic cell transplant recipients: evidence for asymptomatic parainfluenza virus infection. *Blood* 2007;**110**: 1681–8.
157. Phillips MJ, Blendis LM, Poucell S, et al. Sporadic hepatitis with distinctive pathological features, a severe clinical course, and paramyxoviral features. *N Engl J Med* 1991;**324**:455–60.
158. Phillips PE, Christian CL. Myxovirus antibody increases in human connective tissue disease. *Science* 1970;**168**:982–4.
159. Piedra PA, Englund JA, Glezen WP. Respiratory syncytial virus and parainfluenza viruses. In: Specter S, Hodinka R, Young S, editors. *Clinical virology manual*. 3rd ed. Washington, D.C.: ASM Press; 2000.
160. Piralla A, Percivalle E, Di Cesare-Merlone A, et al. Muticluster nosocomial outbreak of parainfluenza virus type 3 infection in a pediatric oncohematology unit: a phylogenetic study. *Haematologica* 2009;**94**:833–9.
161. Pogka V, Kossivakis A, Kalliaropoulos A, et al. Respiratory viruses involved in influenza-like illness in a Greek pediatric population during the winter period of the years 2005-2008. *J Med Virol* 2011;**83**:1841–8.
162. Porotto M, Greengard O, Poltoratskaia N, et al. Human parainfluenza virus type 3 HN-receptor interaction: effect of 4-guanidino-Neu5Ac2en on a neuraminidase-deficient variant. *J Virol* 2001;**75**:7481–8.
163. Porter DD, Prince GA, Hemming VG, Porter HG. Pathogenesis of human parainfluenza virus 3 infection in two species of cotton rats: *Sigmodon hispidus* develops bronchiolitis, while *Sigmodon fulviventer* develops interstitial pneumonia. *J Virol* 1991;**65**:103–11.
164. Raty R, Ziegler T, Kleemola M. The value of virus serology in epidemiological studies of acute otitis media in children. *J Clin Virol* 2004;**29**:315–19.
165. Reed G, Jewett PH, Thompson J, et al. Epidemiology and clinical impact of parainfluenza virus infections in otherwise healthy infants and young children <5 years old. *J Infect Dis* 1997;**175**: 807–13.
166. Ren L, Gonzalez R, Xie Z, et al. Human parainfluenza virus type 4 infection in Chinese children with lower respiratory tract infections: a comparison study. *J Clin Virol* 2011;**51**:209–12.
167. Rihkanen H, Ronkko E, Nieminen T, et al. Respiratory viruses in laryngeal croup of young children. *J Pediatr* 2008;**152**: 661–5.
168. Rodriguez V, Kuehnle I, Heslop HE, et al. Guillain-Barré syndrome after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant* 2002;**29**:515.
169. Rosychuk JJ, Klassen TP, Metes D, et al. Croup presentations to emergency departments in Alberta, Canada. *Pediatr Pulmonol* 2010;**45**:83–91.
170. Rubin EE, Quennec P, McDonald JC. Infection due to parainfluenza virus type 4 in children. *Clin Infect Dis* 1993;**17**: 998–1002.
171. Russell K, Wiebe N, Saenz A, et al. Glucocorticoids for croup. *Cochrane Database Syst Rev* 2004;**1**:1–40.
172. Sato A, Iwasaki A. Induction of antiviral immunity requires Toll-like receptor signaling in both stromal and dendritic cell compartments. *Proc Natl Acad Sci U S A* 2004;**46**:16274–9.
173. Schaap-Nutt A, Scull MA, Schmidt AC, et al. Growth restriction of an experimental live attenuated human parainfluenza virus type 2 vaccine in human ciliated airway epithelium *in vitro* parallels attenuation in African green monkeys. *Vaccine* 2010;**28**: 2788–98.
174. Schiffer J, Kirby K, Sandmaier B, et al. *Community acquired respiratory virus (CRV) infection after myeloablative versus non-myeloablative (NM) hematopoietic cell transplantation (HCT)*. Toronto, Ontario, Canada: Forty-Fourth Annual meeting of the Infectious Disease Society of America; 2006.
175. Schmidt AC, Wenzke DR, McAuliffe JM, et al. Mucosal immunization of rhesus monkeys against respiratory syncytial virus subgroups A and B and human parainfluenza type 3 by using a live cDNA-derived vaccine based on a host range-attenuated bovine parainfluenza virus type 3 vector backbone. *J Virol* 2002;**76**: 1089–99.
176. Schnepf N, Resche-Rigon M, Chaillon A, et al. High burden of non-influenza viruses in influenza-like illness in the early weeks of H1N1v epidemic in France. *PLoS ONE* 2011;**6**:e23514.
177. Segal AO, Crighton EJ, Moineddin R, et al. Croup hospitalizations in Ontario: a 14-year time-series analysis. *Pediatrics* 2005; **116**:51–5.
178. Skiadopoulos MH, Surman S, Tatem JM, et al. Identification of mutations contributing to the temperature-sensitive, cold-adapted and attenuation phenotypes of the live-attenuated cold-passage 45 (cp45) human parainfluenza virus 3 candidate vaccine. *J Virol* 1999;**73**:1374–81.
179. Skiadopoulos MH, Tao T, Surman SR, et al. Generation of a parainfluenza virus type 1 vaccine candidate by replacing the HN and F glycoproteins of the live-attenuated PIV3 cp45 vaccine virus with their PIV1 counterparts. *Vaccine* 1999;**18**:503–10.
180. Skolnik NS. Treatment of croup. *Am J Dis Child* 1989;**143**: 1045–9.
181. Slobod KS, Allan JE. Parainfluenza type 1 virus–infected cells are killed by both $CD8^+$ and $CD4^+$ cytotoxic T cell precursors. *Clin Exp Immunol* 1993;**93**:363–9.
182. Slobod KS, Shenep JL, Lujan-Zilbermann J, et al. Safety and immunogenicity of intranasal murine parainfluenza virus type 1 (Sendai virus) in healthy human adults. *Vaccine* 2004;**22**:3182–6.
183. Smith CB, Bellanti JA, Chanock RM. Immunoglobulins in serum and nasal secretions following infection with type 1 parainfluenza virus and injection of inactivated vaccines. *J Immunol* 1967;**99**: 133–41.
184. Sobol SE, Zapata S. Epiglottitis and croup. *Otolaryngol Clin North Am* 2008;**41**:551–66.
185. Sonoda S, Gotoh Y, Bann F, Nakayama T. Acute lower respiratory infections in hospitalized children over a 6 year period in Tokyo. *Pediatr Int* 1999;**41**:519–24.
186. Sparrelid E, Ljungman P, Ekelof-Andstrom E, et al. Ribavirin therapy in bone marrow transplant recipients with viral respiratory tract infections. *Bone Marrow Transplant* 1997;**19**:905–8.
187. Sparrow A, Geelhoed G. Prednisolone versus dexamethasone in coup: a randomized equivalence trial. *Arch Dis Child* 2006;**91**: 580–3.

188. Takimoto T. Parainfluenza viruses. In: Harper D, editor. *Encyclopedia of life sciences*. Chichester, U. K.: John Wiley & Sons; 2006. p. 678–84.
189. Treanor J, Falsey A. Respiratory viral infections in the elderly. *Antiviral Res* 1999;**44**:79–102.
190. van Asten L, van den Wijngaard C, van Pelt W, et al. Mortality attributable to 9 common infections: significant effect of influenza A, RSV, influenza B, norovirus and parainfluenza in the elderly. *J Infect Dis* first published on line, June 21 2012.
191. van Wyke Coelingh KL, Winter CC, Tierney EL, et al. Attenuation of bovine parainfluenza virus type 3 in nonhuman primates and its ability to confer immunity to human parainfluenza virus type 3 challenge. *J Infect Dis* 1988;**157**:655–62.
192. van Wyke Coelingh KL, Winter CC, Tierney EL, et al. Antibody responses of humans and nonhuman primates to individual antigenic sites of the hemagglutinin-neuraminidase and fusion glycoproteins after primary infection or reinfection with parainfluenza type 3 virus. *J Virol* 1990;**64**:3833–43.
193. Vilchez RA, Dauber J, McCurry K, et al. Parainfluenza virus infection in adult lung transplant recipients: An emergent clinical syndrome with implications on allograft function. *Am J Transplant* 2003;**3**:116–20.
194. Vilchez RA, McCurry K, Dauber J, et al. The epidemiology of parainfluenza virus infection in lung transplant recipients. *Clin Infect Dis* 2001;**33**:2004.
195. Vilchez RA, McCurry K, Dauber J, et al. Influenza and parainfluenza respiratory viral infection requiring admission in adult lung transplant recipients. *Transplantation* 2002;**73**:1075–8.
196. Vreede RW, Schellekens H, Zuijderwijk M. Isolation of parainfluenza virus type 3 from cerebrospinal fluid. *J Infect Dis* 1992;**165**: 1166.
197. Vuorinen T, Meurman O. Enzyme immunoassays for detection of IgG and IgM antibodies to parainfluenza types 1, 2 and 3. *J Virol Methods* 1989;**23**:63–70.
198. Wagener JS, Minnich L, Sobonya R, et al. Parainfluenza type II infection in dogs: a model for viral lower respiratory tract infection in humans. *Am Rev Respir Dis* 1987;**127**:771–5.
199. Wall SR, Wat D, Spiller OB, et al. The viral aetiology of croup and recurrent croup. *Arch Dis Child* 2009;**94**:359–60.
200. Wang CYT, Arden KE, Greer R, et al. A novel duplex real-time PCR for HPIV-4 detects co-circulation of both viral subtypes among ill children during 2008. *J Clin Virol* 2012;**54**:83–5.
201. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. *J Pediatr* 2009;**154**:694–9.
202. Welliver RC, Wong DT, Choi TS, Ogra PL. Natural history of parainfluenza virus infection in childhood. *J Pediatr* 1982;**101**: 180–7.
203. Wenzel RP, McCormick DP, Beam WE Jr. Parainfluenza pneumonia in adults. *JAMA* 1972;**221**:294–5.
204. Wiertsema SP, Chidlow GR, Kirkham LS, et al. High detection rates of nucleic acids of a wide range of respiratory viruses in the nasopharynx and the middle ear of children with a history of recurrent acute otitis media. *J Med Virol* 2011;**83**:2008–17.
205. Wilks D, Burns SM. Myopericarditis associated with parainfluenza virus type 3 infection. *Eur J Clin Microbiol* 1998;**17**: 363–5.
206. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. *J Infect Dis* 2010;**201**:1890–8.
207. Woodland DL, Hogan RJ, Zhong W. Cellular immunity and memory to respiratory virus infections. *Immunol Res* 2001;**24**: 53–67.
208. Woods CR. *Management of croup*. UptoDate 2006. Available at http://www.uptodate.com.
209. Yanagihara R, McIntosh K. Secretory immunological response in infants and children to parainfluenza virus types 1 and 2. *Infect Immun* 1980;**30**:23–8.
210. Yurlova TI, Sverkunova MV, Furaeva VA, et al. Studies of natural population variability of parainfluenza viruses during their epidemic circulation. *Acta Virol* 1991;**35**:64–70.
211. Zemlin M, Hoersch G, Zemlin C, et al. The postnatal maturation of the immunoglobulin heavy chain IgG repertoire in human preterm neonates is slower than in terms neonates. *J Immunol* 2007;**178**:1180–8.
212. Zhong W, Marshall D, Coleclough C, Woodland DL. CD4+ T cell priming accelerates the clearance of Sendai virus in mice, but has a negative effect on CD8+ T cell memory. *J Immunol* 2000; **164**:3274–82.
213. Zinserling A. Peculiarities of lesions in viral and mycoplasma infections of the respiratory tract. *Virchows Arch A Pathol Anat* 1972;**356**:259–73.
214. Zollar LM, Mufson MA. Acute parotitis associated with parainfluenza 3 virus infection. *Am J Dis Child* 1970;**119**:147–8.

CHAPTER 179

Measles Virus

James D. Cherry

Measles virus is a singular agent that causes a relatively distinct, exanthematous disease characterized by fever, cough, coryza, conjunctivitis, an erythematous maculopapular confluent rash, and a pathognomonic enanthem. Clinical measles is an epidemic disease, the incidence of which in the United States was reduced by the use of attenuated vaccines from 315 reported cases per 100,000 persons in the pre-vaccine era to fewer than 1 per 100,000 persons in 1992. At present, measles no longer is an endemic disease in the United States. However, measles is still a major problem because of its worldwide prevalence, associated morbidity and mortality, and changing epidemiologic patterns in countries where vaccine use has been less than optimal.

HISTORY

In antiquity, measles and smallpox frequently were confused with each other, as well as with other exanthematous diseases.[26,340] Major epidemics of both measles and smallpox occurred 1800 years ago in the Roman Empire and China.[131,294] The first written record of measles generally is credited to Rhazes, a 10th century Persian physician.[26,131,230,459] However, Rhazes quoted writers, including El Yahudi, a famous Hebrew physician, who lived 300 years earlier.[131] Rhazes identified measles as an entity distinct from smallpox.

During the Middle Ages in Europe, smallpox and measles continued to be confused. By the beginning of the 17th century, however, differentiation between the two diseases was relatively clear; death reports by London parish clerks in 1629 listed measles and smallpox separately.[459] Repeated epidemics of measles were described in the English medical literature during the 17th and 18th centuries.[230]

The first account of measles in America was given by John Hall, who described epidemic disease in Boston in the fall of 1657.[65] The next epidemic in colonial America was reported in 1687. During the next 150 years, the interval between epidemics in Boston gradually decreased from 30 years to approximately 3 years. Epidemics during the 17th and 18th centuries involved persons of all ages, including neonates; coincident with a reduction in the interval between epidemics was a reduction in the age-specific incidence of measles. The reduction in time between epidemics can be attributed to the increased incidence of importation of measles that occurred as more and faster ships crossed the Atlantic and the population density in North America gradually increased. By the turn of the 19th century, both Boston and Philadelphia had sufficient populations for measles to propagate itself.

The first recognition of the contagiousness of measles is unclear. Shakespeare in *Coriolanus* was aware of its human-to-human transmission.[459] Home,[203] in 1758, attempted to immunize against measles by applying a technique similar to the variolation used in smallpox.[366] In 1911, Goldberger and Anderson[166] produced clinical measles in monkeys by injecting filtered material from acute cases of human disease.

The classic epidemiologic study of measles is the account described by Panum[354] of the 1846 epidemic in the Faroe Islands. In this study, Panum confirmed that spread occurred solely through human-to-human contagion by the respiratory route, the incubation period was 14 days, and infection conveyed lifelong immunity.

The enanthem of measles, which is pathognomonic, was described carefully and presented by Koplik[246-248] in 1896, 1898, and 1899.[51] However, Koplik spots clearly were recognized specifically about a century earlier by John Quier, a physician in Jamaica,[51,164] as well as by Richard Hazeltine, a general practitioner in rural Maine.[65]

Although Plotz[367] reported cultivation of measles virus in 1938 and Rake and Shaffer[372] noted similar findings in 1940, reliable tissue culture methods were not available until approximately 10 years later. In 1954, Enders and Peebles[129] isolated eight agents (from cases of measles) in human or simian renal cell cultures. These investigators also demonstrated the ability of convalescent-phase serum from a patient with measles to neutralize the viral cytopathogenic effect. The stage for vaccine development was set by recovery in tissue culture of the virus,[129] adaptation of viral growth in chicken embryos,[300] and, finally, cultivation of the virus in chicken embryo tissue culture cells.[232]

After extensive trials were conducted from 1958 through 1962, tissue culture–grown, inactivated ("killed"), and attenuated ("live") measles viral vaccines became available for general use in 1963.[91,229,252] In the United States, a nationwide immunization effort instituted in 1965 and 1966 led to a dramatic reduction in the incidence of epidemic measles for several years. Epidemic measles recurred in 1971, 1977, and 1989, but at overall levels less than those in the pre-vaccine era.[22,66,67,74,91,253] After initiation of the Childhood Immunization Initiative in 1977 and the Measles Elimination Program in 1978, the incidence of measles in the United States fell in 1981 to fewer than 1.5 cases per 100,000 population and remained at this low level until 1986.[22,72,73] In 1990, the

rate was 11.2 cases per 100,000 population, which was the highest it had been since 1977. After 1990, a substantial decline in the incidence of measles occurred in the United States, to a low of 86 confirmed cases in 2000.[75] Currently, endemic transmission of measles no longer is occurring in the United States.[349] However, in spite of increased vaccination efforts, measles morbidity and mortality is still a worldwide problem.[82,160,348]

PROPERTIES

Classification

Measles virus is a relatively large virus with helical capsid symmetry and an RNA genome.[36,133,260] It is a singular virus, but antigenically it is related closely to canine distemper. These agents, as well as peste des petits ruminants virus, dolphin morbillivirus, phocine distemper virus, and rinderpest virus, currently are included in the genus *Morbillivirus;* they are members of the *Paramyxoviridae*. Measles virus differs from the other paramyxoviruses in that it does not possess specific neuraminidase activity and does not adsorb to neuraminic acid–containing cellular receptors.[311,331,363,448] Measles virus hemagglutinates, whereas the other members of its genus do not.

Physical Properties

Measles virus is a roughly spherical but pleomorphic virus that ranges from 100 to 300 nm in diameter.* The virion is composed of an outer lipoprotein envelope and an internal helical nucleocapsid. The virion contains eight proteins. The outer viral envelope is 10 to 22 nm thick, has short surface projections (peplomers), and contains three virus-coded proteins (F, H, and M).[94,311,340] F protein is a dumbbell-shaped peplomer that causes membrane fusion of the virus and host cell and enables penetration of the virus into the host cell. H protein is the hemagglutinin and is a conical peplomer. In infection, H protein reacts with a host cellular receptor. CD46 appears to be the cellular receptor for attenuated measles vaccine strains but not wild-type viral strains.† More recent data suggest that the signaling lymphocyte activation molecule (SLAM) is the cellular receptor for wild-type virus.[118,346,467] M (matrix) protein is nonglycosylated and associated with the inner lipid bilayer of the envelope. It plays an important role in the maturation of the virus. The nucleocapsid (N) protein (Fig. 179-1) is a coiled rod with a diameter of 18 nm and a length of 1 μm that contains the viral genomic RNA.[335,339,340,446,448] N protein has a molecular weight of approximately 60 kDa. Approximately 5 percent of the nucleocapsid is RNA.[189,436,447] The other internal proteins of the virus are the L (large), P (phospho), C, and V proteins.

The virus genome has a molecular weight of 4.5×10^6 daltons. It is a linear, single strand of RNA that contains approximately 15,900 nucleotides.

*References 36, 133, 178, 323, 340, 448, 449.
†References 54, 120, 277, 278, 325, 346.

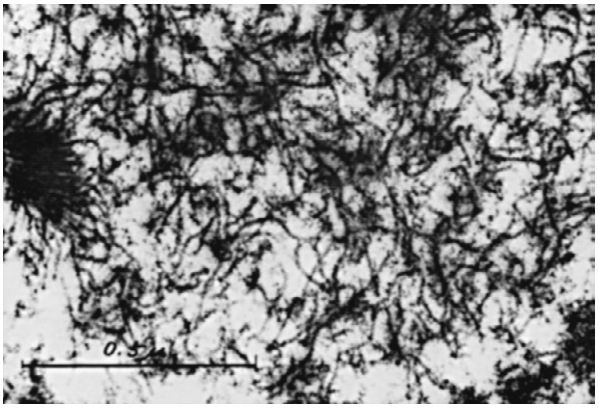

FIGURE 179-1 ■ Nucleocapsid fragments. Electron micrograph. (Courtesy Dr. John M. Adams.)

Measles virus is labile.[230,231,318] It is inactivated rapidly by heat, ultraviolet light, lipid solvents such as ether and chloroform, and extreme degrees of acidity and alkalinity (i.e., pH <5 and >10). Longevity is prolonged when protein is present in the viral suspending medium and when the virus is lyophilized with a protein stabilizer. Protein specifically protects against the adverse effects of heat and light. Protein-stabilized measles virus can be stored at −70° C for 5 or more years without significant loss of infectivity. At room temperature, a 60 percent loss in titer occurs in 3 to 5 days; at 56° C, the virus is inactivated within 30 minutes.[41,240,318]

Antigenic Composition

Clinical and epidemiologic data and early laboratory study suggested antigenic homogeneity of all measles strains.[231] More recent nucleotide sequence analysis identified distinct lineages among wild-type measles virus isolates.[36,317,318,387,428] There are eight clades (A to H) that contain 23 genotypes.

The following properties have been associated with measles virus: a hemagglutinin (for simian cells), complement-fixing antigens, hemolytic activity, and a giant-cell–inducing factor.* Human measles virus infection results in serum antibodies that are capable of neutralizing viral infectivity, fixing complement with viral antigen, and inhibiting viral hemagglutination and hemolysis.

Cross-seroreactivity occurs among members of the genus *Morbillivirus* but not with other members of the *Paramyxoviridae*.[44,60,212] Measles virus serum antibody in humans reacts with distemper virus, but canine serum after a case of distemper does not react with measles virus. A two-way cross between measles and rinderpest viruses has been demonstrated.

Tissue Culture Growth

Measles virus can be propagated in many different primary and cell line tissue cultures.[44,231,288] However, for

*References 56, 190, 230, 231, 333, 334, 338, 363, 397.

isolation of virus from patient specimens, primary human and monkey kidney cultures have been most successful over the years. In one study, an Epstein-Barr virus–transformed marmoset lymphocytic line (B95-8) was found to be superior to primary monkey kidney cell culture for isolation of virus from nasopharyngeal specimens.[242] In tissue culture, measles virus has two distinct cytopathogenic effects. With initial isolation, syncytial formation occurs as a result of cell fusion and the resulting giant cells may contain 10 to 50 or more nuclei (Fig. 179-2). On stained preparations, both the nuclei and cytoplasm contain eosinophilic inclusions. The second type of cytopathogenic effect is characterized by the alteration of single cells into spindle shapes or stellate forms. In general, tissue culture–adapted measles viral strains are more likely to cause this cytopathogenic effect than is giant-cell formation. In most cultures, both forms of cytopathogenic effect are evident and changes in medium composition render one or the other type predominant.

Infection in tissue culture is associated with an attachment-adsorption phase lasting approximately 1 hour and an eclipse period of 6 to 12 hours.[288,311] Antigen first is noted in the perinuclear cytoplasm by 12 hours; by 24 hours, it is distributed throughout the cytoplasm. By 30 hours, most antigen is detected at the cell surface. In mature cultures, more cell-associated virus is present than is found free within the medium.

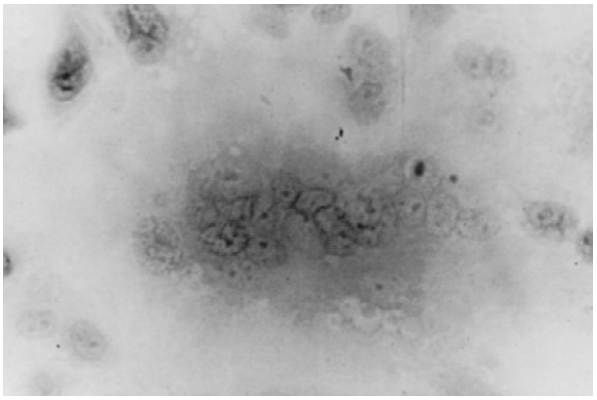

FIGURE 179-2 ■ Measles virus cytopathogenic effect in monkey kidney tissue culture. Giant cell with approximately 20 nuclei.

Animal Susceptibility

Humans are the natural hosts of measles virus, but, with human contact, monkeys also are infected easily.[297] Laboratory strains of measles virus have been adapted to suckling mice and hamsters.[55,59,213,443]

EPIDEMIOLOGY

Prevalence

The prevalence of measles throughout history has been affected markedly by population density and, since the 1960s, by the use of measles vaccine. Reported cases of measles in the United States from 1963 to 1998, analyzed by year, are presented in Figure 179-3. After the universal use of measles vaccine began in 1965, the number of cases in the United States decreased to 22,231 in 1968.[74] In 1971 to 1972 and 1976 to 1977, modest epidemics occurred; then, after a national commitment to measles immunization, the number of cases of measles fell to an all-time low of 1497 cases reported in 1983. Beginning in 1986, the number of measles cases again increased, with 27,786 cases reported in 1990. After 1990, measles in the United States declined, with only 100 cases reported in 1998.[75] Since 1998, the endemic transmission of measles in the United States has been eliminated.[349] In 2005, 66 cases were reported in the United States, and 95 percent of these were linked to importations.[76] Half of all the cases in 2005 were traced to one unvaccinated traveler who was infected in Europe. All but two of the contact cases in this outbreak were unvaccinated because their parents had declined immunization. In 2011, there were 222 measles cases in the United States with 17 different outbreaks.[81] Two hundred (90%) of these cases were associated with importations from other countries;

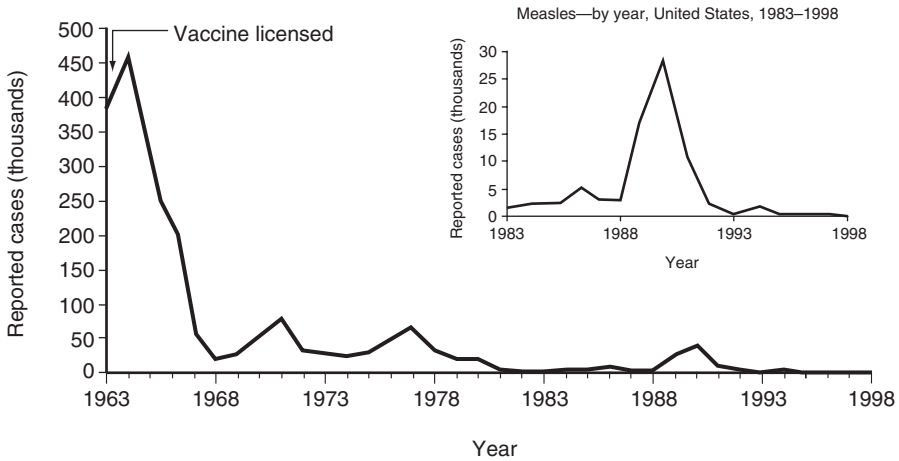

FIGURE 179-3 ■ **Reported measles cases by year in the United States, 1963 to 1998.** (From Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 1998. *MMWR Morb Mortal Wkly Rep* 1999;**47**:48.)

TABLE 179-1 **Incidence and Percent Distribution of Reported Measles Cases by Age Group in Selected Years, United States**

	1960-1964*			1974			1979			1991		
Age Group	CASES	%	INCIDENCE†	CASES	%	INCIDENCE†	CASES	%	INCIDENCE†	CASES	%	INCIDENCE†
<1-4	93,653	37.2	766	5,899	26.7	36	2,331	20.7	18.0	4,756	49.3	24.7
5-9	132,956	52.8	1,237	5,391	24.4	30	2,473	21.9	18.1	991	10.2	5.5
10-14	16,403	6.5	169	7,799	35.3	38	3,054	27.1	20.4	905	9.4	5.3
15-19	8,635	3.4	10	2,475	11.2	12	2,633	23.3	15.2	1,102	11.4	6.2
20+				552	2.5	>1	786	7.0	0.6	1,890	19.6	1.8
Totals	251,647			22,094			11,277			9,643		

*Data from four reporting areas: Washington, D.C., New York City, Illinois (including Chicago), and Massachusetts.[22,66,69,192]
†Incidence, cases per 100,000 population, extrapolated from the age distribution of known cases.

this included United States residents returning from abroad and foreign visitors. Most patients (86%) were unvaccinated or had unknown vaccination status.

In the 20th century, before the widespread use of measles vaccine, between 200,000 and 600,000 cases of measles were reported annually in the United States.[101,262] Careful survey suggested that in the past, reported cases accounted for only approximately 15 percent of the actual number of cases of measles.[91]

In the pre-vaccine era in the United States and other concentrated populations throughout the world, measles epidemics occurred regularly. In the United States, urban-centered measles epidemics took place every 2 to 5 years, with each epidemic lasting 3 to 4 months.* In general, the larger the community, the shorter the interval between epidemics. In the vaccine era, the epidemic pattern has been changed. As noted in Figure 179-3, the total number of cases has been reduced and the cycle between peaks has lengthened.

Age Incidence and Prevalence

In modern times in populous areas, measles has been a disease of children. The age-specific incidence in the United States for selected years is presented in Table 179-1. In the pre-vaccine era during the 20th century, the highest measles attack rate occurred in children aged 5 to 9 years.[26,131,197,458] In the period 1960 to 1964, data from five reporting areas showed that more than half of all measles cases occurred in this age group.[192] Before the present vaccine era, infections and epidemic loci centered in elementary schools. Younger children acquired measles as secondary cases from their older siblings who were attending school. In rural areas, the interval between measles epidemics tended to be greater; therefore, a broader spread in ages occurred in the percentage of measles cases. In a nationwide serum survey of U.S. military recruits in 1962, Black[43] found that 99 percent had measles antibody.

As noted in Table 179-1, the age-related incidence and percentage of measles cases by age group have changed markedly since 1964. In 1991, more than 40 percent of measles cases occurred in persons older than 10 years; in the period from 1960 to 1964, fewer than 10 percent of the patients were older than 10 years. Approximately a third of the cases in 1991 were in adolescents and young adults. Evidence also indicates high primary measles attack rates in neonates to 4-year-old children in areas of suboptimal vaccine utilization.[22,75,91,146,195] In 2005, 66 measles cases were reported.[76] Seven cases (10.6%) were in infants, 4 (6.1%) were in children aged 1 to 4 years, 33 (50%) were in persons aged 5 to 19 years, 7 (10.6%) were in persons aged 20 to 34 years, and 15 (22.7%) were in adults aged 35 years or older. In 2011, the median age of the patients was 14 years; 14 percent were aged younger than 12 months, 26 percent were aged 1 to 4 years, 21 percent were aged 5 to 19 years, and 39 percent were 20 years or older.[81] Most measles cases that have occurred in the United States since 1994 have been imported or were importation associated.[75,386,388] Measles in heavily populated but developing countries has its greatest incidence in children younger than 2 years.[98,139,196,313,341]

Geographic Distribution

In the 20th century, measles occurred regularly throughout the world in all but the most remote areas.[26,131] In island and other isolated populations, intervals between epidemics can be 10 years or more, and the occurrence of disease depends on its introduction from outside the area.

Seasonal Patterns

Epidemic measles is a winter-spring disease in temperate climates, with peak activity in the Northern Hemisphere occurring in March or April. In equatorial regions, epidemics of measles are less marked but tend to occur in the hot, dry seasons.[26,312,381,425]

Host and Social Factors

No difference is found in the incidence of measles in the male and female population. One suggestion, however, is that complication rates are higher in boys or men than in girls or women. Tidstrom[430] found that acute laryngitis occurred more than twice as frequently in male patients

*References 26, 131, 187, 197, 270, 454, 469.

as compared with female patients. In other studies, otitis media, pneumonia, and death occurred slightly more often in male patients.[26,342] Miller,[299] in a large survey in England in 1963, found no difference in the incidence of complications in male and female patients. Christensen and associates,[95] in studies of an epidemic in Greenland, noted a greater number of complications in female patients older than 15 years, attributable primarily to an increased incidence of pneumonia. In a review of 375 confirmed cases of subacute sclerosing panencephalitis (SSPE), the male-to-female ratio was 2.4:1.[308] In one reported study,[131] antibody titers are slightly higher in women than in men. My studies involving young adults in 1976 failed to reveal a similar sex-associated difference in antibody levels, however.[410] Green and associates[170] noted that a group of women had a higher postimmunization geometric mean antibody titer than did vaccinated men.

Although the severity of disease in certain populations appears to suggest differences based on race, this difference may be the result of nutritional and other environmental factors. Deseda-Tous and associates[115] were unable to demonstrate any differences in measles antibody by human leukocyte antigen (HLA) or by ABO blood types. In a more recent study, the findings of Ovsyannikova and coworkers[351] suggested that HLA alleles relate to antibody responses after immunization. The following alleles were positively associated with hyperseropositivity: HLA-B*7, HLA-DQA1*0104, and HLA-DPA1*0202. In contrast, HLA-B*44, HLA-DRB1*01, HLA-DRB1*08, HLA-DQB1*0301, and HLA-DPD1*0401 alleles were negatively associated with hyperseropositivity. In another study, the same group looked for associations between HLA homozygosity and measles antibody levels.[412] They found a negative correlation between homozygosity (= 1 loci) and antibody value. More recently, the same group of investigators found significant associations with class I HLA-B, as well as class II HLA-DPBI and HLA-DPA1 genes for measles vaccine–induced antibody values after the second vaccine dose.[216] This group also noted associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity after measles immunization.[194]

In the pre-vaccine era, the age of patients at the time of acquiring measles infection was related inversely to the number of siblings in the family.[26,131,464] In general, measles occurred at an earlier age in city dwellers and those of lower socioeconomic classes than in rural families and well-educated, upper-income groups.

In the present era of antibiotics and other medical modalities, the greatest factors in measles-related morbidity and mortality are age and nutritional status. The mortality rates for measles are highest in children younger than 2 years and in adults.[26,131] The severity of measles and rates of mortality correlate in general with the severity of malnutrition.[44,337] However, extensive investigations by Aaby and others[3,4,159] since the 1980s suggest that overcrowding and intensive exposure may be more important determinants of measles-related mortality than is nutritional status alone.

Studies of measles conducted early in the 20th century and in developing countries indicated that secondary cases in households were likely to be more severe than were primary cases.[2-4,160] However, more recent studies in the United States found no difference in severity between primary and secondary cases in families.[422]

Spread of Infection

Measles is a highly contagious disease in nonimmune persons. It is spread from an infected ill person to a new host by the respiratory route.[26,131,378,379,382] Although monkeys can acquire measles from humans, practically speaking, no animal reservoir exists.[297] Available evidence suggests that infection is spread by persons ill with measles. Asymptomatic contagious carriers are unknown, and persons with acute asymptomatic infection probably are not contagious or only minimally so. The period of greatest contagion occurs during the prodromal period.[166]

Transmission of measles is thought to occur mainly by aerosolized droplets of respiratory secretions. Acquisition of infection by a new host is by the nose and possibly the conjunctivae.[355] Infection can be initiated by small-droplet nuclei, which stay suspended in air for considerable periods of time, or by direct hits of large droplets at close range.[47,376] Also possible is that spread involves close person-to-person contact in young children, with large virus-containing droplets of nasal secretions being picked up on the hands of the future host and then applied to the nose.

PATHOGENESIS AND PATHOLOGY

Viral Infection

The sequence of viral events in uncomplicated measles is presented in Table 179-2. Although much is known about measles virus infection in humans, considerable gaps exist regarding specific events. Experimental studies in other primates have been performed in an attempt to fill in the gaps and thereby provide a more complete picture.* The primary site of infection appears to be the respiratory epithelium of the nasopharynx. Measles vaccine virus instilled into the nose or by aerosol results in infection.[45,250] Papp[355] reported studies suggesting that infection resulted from conjunctival contact and proposed that this means was the primary portal of entry. However, experiments with vaccine virus generally have been unsuccessful when conjunctival inoculation has been performed.[45] Initial infection of the respiratory epithelium appears to be minimal; a more important event is the early spread of virus to regional lymphatics. A presumption based on data derived from Fenner's ectromelia-mouse experimental model[132] is that after such spread, primary viremia occurs, followed by extensive multiplication of virus in the reticuloendothelial system at both regional and distant sites. Multiplication of virus also continues at the site of initial infection.

During the fifth to seventh days of infection, extensive secondary viremia takes place and results in the establishment of generalized measles viral infection. The skin,

*References 227, 289, 330, 345, 400, 466.

TABLE 179-2 **Sequence of Measles Virus Infection in Uncomplicated Primary Disease**

Day	Event
0	Measles virus in droplet nuclei or large droplet comes in contact with the epithelial surface of the nasopharynx or possibly the conjunctiva. Infection of epithelial cells and virus multiplication
1-2	Extension of infection to regional lymphoid tissue
2-3	Primary viremia
3-5	Multiplication of measles virus in respiratory epithelium at the site of initial infection and in the reticuloendothelial system regionally and at distant sites
5-7	Secondary viremia
7-11	Establishment of infection in the skin and other viremic sites, including the respiratory tract
11-14	Virus in blood, respiratory tract, skin, and other organs
15-17	Viremia decreases and then ceases. Viral content in organs rapidly diminishes.

Data from references 176-178, 181, 185, 227, 233, 238, 266, 330, 345, 362, 382, 390, 400, and 466.

conjunctivae, and respiratory tract are obvious sites of infection, but other organs may be involved as well. From the 11th to the 14th days, the viral content of the blood, respiratory tract, and other organs peaks and then rapidly diminishes over the ensuing 2- to 3-day period.

In immunologically compromised patients with defects in cell-mediated factors, measles virus is not cleared from the secondary infection sites, and progressive, frequently fatal illnesses occur.* During infection, measles virus replicates in endothelial cells, epithelial cells, and monocytes and macrophages.[181,309]

Pathology

The characteristic pathologic feature of measles is the widespread distribution of multinucleated giant cells, which are the result of cell fusion.† Two main types of giant cells occur in measles: (1) Warthin-Finkeldey cells, which are found in the reticuloendothelium; and (2) epithelial giant cells (Fig. 179-4), which occur principally in the respiratory epithelium but also on other epithelial surfaces.[266]

Warthin-Finkeldey giant cells are found throughout the reticuloendothelial system in the adenoids, tonsils, Peyer patches, appendix, lymph nodes, spleen, and thymus. They vary in size and contain as many as 100 or more nuclei. The cells contain both cytoplasmic and intranuclear eosinophilic inclusions, with cytoplasmic inclusions more common than intranuclear lesions. During the prodromal stage of measles, epithelial giant cells regularly are present on respiratory surfaces and frequently are sloughed free (see Fig. 179-4).

*References 8, 128, 302, 304, 315.
†References 44, 227, 230, 266, 288, 330, 345, 383, 400, 466.

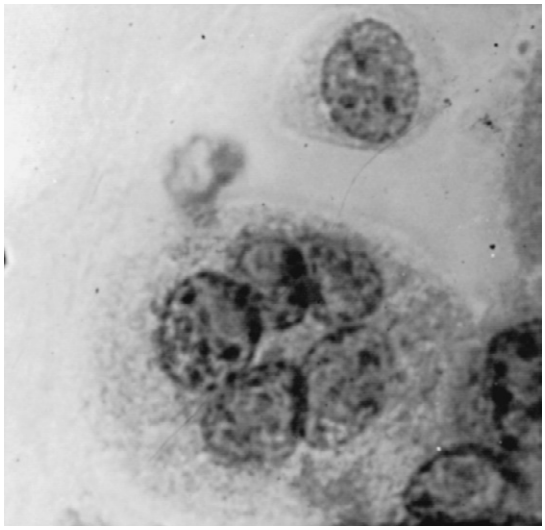

FIGURE 179-4 ■ Epithelial giant cell with five nuclei. Pharyngeal smear. (Courtesy Dr. John M. Adams.)

Measles Exanthem

Hematoxylin and eosin–stained sections of skin biopsy specimens have revealed typical epithelial syncytial giant cells with nuclear and cytoplasmic inclusions.[238,421] The giant cells contain 3 to 26 nuclei. Other findings include the following: focal parakeratosis, dyskeratosis, spongiosis, and intracellular edema. The superficial blood vessels are dilated, with a sparse, surrounding lymphohistiocytic infiltrate.

Koplik Spots

Suringa and colleagues[421] observed that the histopathologic features of Koplik spots were similar to those of the rash. These investigators noted more giant cells with more nuclei, a greater degree of edema, and a lessened inflammatory response in the enanthem biopsy sample, however.

Respiratory Tract

The extent of respiratory involvement in uncomplicated measles is not known. However, clinical symptoms and the extensive radiographic studies of Kohn and Koiransky[244] suggested that pharyngitis, tracheitis, bronchitis, and pulmonary infiltration are the rule rather than the exception. Unfortunately, the human pathologic data available have been obtained mainly from complicated cases, so the findings cannot be considered representative.[113,383] In a study in monkeys, Nii and colleagues[330] noted giant cells in the mucosal epithelium of the trachea, bronchi, and bronchioli. The lumina of these airways contained sloughed syncytial cells. Warthin-Finkeldey cells were found in the adjacent lymphatics.

In the lungs of experimentally infected monkeys, interstitial pneumonitis was observed with giant-cell formation.[330] Infiltration of neutrophils, eosinophils, and mononuclear cells also was noted.

Immunologic Events

After natural or attenuated measles virus infection develops, large numbers of specific and nonspecific immunologic responses occur. Many questions relating to immunity in measles remain unanswered, although a considerable body of knowledge has been gathered since the 1950s.

Antibody

After measles viral infection develops, an antibody response regularly occurs. Serum antibodies to the N, F, H, and M proteins of measles virus can be demonstrated by hemagglutination-inhibition (HI), complement-fixation (CF), neutralization, immune precipitation, hemolysin inhibition, enzyme-linked immunosorbent assay (ELISA), and fluorescent antibody (FA) methods.* Antibody-dependent cellular cytotoxicity and antibody-dependent complement-mediated lysis also have been demonstrated.[144,145,236] In natural infection, HI and neutralizing antibodies appear at approximately the 14th day, peak at around 4 to 6 weeks, and decrease approximately fourfold from the peak over the course of a year. Most naturally infected persons have demonstrable HI and neutralizing antibodies for life. Krugman[253] noted an average 16-fold reduction in HI antibody titer 15 years after natural infection in a group of children who had no measles exposure during the observation period.

After immunization has been administered, both HI and neutralizing antibodies are present by the 14th day.[157,254] CF antibody appears slightly later than does HI antibody and in general does not persist as long as does either HI or neutralizing antibody. Primary infection is characterized by the initial appearance of antibody in the immunoglobulin (Ig) M and IgG serum components.[142,198,398,399,433] The IgM-specific response is short-lived, and rarely can measles antibody in this fraction be demonstrated more than 9 weeks after infection. IgA secretory antibody also occurs regularly after vaccine viral infection and after natural infection.[34,151]

IgG antibody appearing after infection is primarily subclasses IgG1 and IgG4.[286] Antibody detected by neutralization and by HI is mainly to H protein and correlates with clinical protection against illness.[111,181,333] Antibody to N protein is the main antibody detected by CF.[333] Antibody to F protein is demonstrated by inhibition of hemolysis of monkey erythrocytes by measles virus or by immunoprecipitation.[332,334] Antibody to F protein may contribute to neutralization by disrupting the fusion of virus membrane with host cell membranes. Only small amounts of antibody to M protein are elicited after infection with wild virus or vaccine virus.[172]

Specific Cell-Mediated Responses

Investigators recognized in the 1960s that patients with defective cellular resistance factors frequently died of progressive measles virus infections.[322] In the 1970s, techniques became available that demonstrated measles-specific lymphocyte sensitization.* Graziano and colleagues[173] found that lymphocytes from persons who previously had measles had a blastogenic response in vitro when these cells were incubated with measles antigen. Labowskie and associates,[259] using an in vitro lymphocyte-mediated cytotoxicity assay, noted that lymphocytes from persons who previously had measles caused cell destruction in a tissue culture chronically infected with measles virus. Ruckdeschel and colleagues[389] observed that two physicians without detectable measles antibody who had experienced repeated recent exposure to measles without developing illness had strong in vitro cellular responsiveness to measles virus. Krause and associates[249] demonstrated excessive measles-specific lymphocyte blastogenic responses in some persons previously immunized with killed measles vaccine.

Today, T cells are appreciated to be important both in B-cell antiviral antibody responses and as effector cells for the clearance of virus-infected cells from tissues.[181] Both helper ($CD4^+$) and suppressor ($CD8^+$) cells participate in the cellular response.[445] During infection, $CD8^+$ T cells eliminate virus-infected cells by major histocompatibility complex (MHC) class I–restricted cytotoxic mechanisms.[181] $CD4^+$ T cells respond to measles virus infection by the secretion of cytokines. After initial exposure to virus occurs, $CD4^+$ T cells mount a T-helper cell type 1 (Th1) response. Before the rash develops, levels of plasma interferon-γ (IFN-γ) are increased, and, with the rash, interleukin (IL)-2 (IL-2) appears. With clinical recovery, plasma levels of IL-4 increase (a T-helper cell type 2 [Th2] response) and stay elevated for several weeks, whereas the initial Th1 response subsides.

On reexposure, Th1 and Th2 responses occur as indicated by the production of INF-γ, IL-2, tumor necrosis factor-β (TNF-β), IL-4, IL-5, and IL-10. The Th1 cytokine response is important for macrophage activation (through the action of INF-γ), lymphocyte proliferation (through IL-2), and MHC class II–restricted cytotoxicity (through TNF-β). The Th2 cytokine response is important for macrophage deactivation (through the action of IL-4 and IL-10) and B-cell help (through IL-4, IL-5, and IL-10). In studies in rhesus monkeys, researchers found that humoral immunity had only a limited role in the control of measles virus replication in primary infection.[364]

Ovsyannikova and colleagues[352] found a predominant Th1 cytokine pattern after measles vaccine viral infection. This pattern occurred after both primary immunization and revaccination. In this study, no relationship was found between any specific cytokine level and serum measles antibody values. In another study, the same research group found a subset of vaccinees in whom the cytokine responses were skewed toward the Th2 type.[119] These investigators suggested that this response in this subset may not be sufficient for long-term immunity.

*References 107, 157, 172, 239, 254, 326, 332, 333, 438.

*References 108, 158, 173, 174, 249, 259, 389.

Other Responses

Listed in Table 179-3 are nonspecific, immunologically related responses that can be demonstrated during natural or vaccine measles virus infection. Anderson and colleagues[16] demonstrated a temporary defect in neutrophil motility during acute measles that resolved by the 11th day after the onset of rash. Leukopenia has been observed in natural measles[37] and after immunization.[46] With immunization, the numbers of both neutrophils and lymphocytes are reduced; this reduction lasts approximately 1 week, with an onset occurring approximately 7 days after vaccination. Coovadia and colleagues[103,104] showed that the numbers of T, B, and null cells in the lymphocyte population are reduced. With a reduction in the number of T lymphocytes, no change occurs in the ratio of helper and suppressor-cytotoxic cell phenotypes.[10,19,221]

Thrombocytopenia occasionally has been associated with natural measles[209] and vaccination.[11,456] Oski and Naiman[350] noted a mild reduction in the peripheral platelet count during routine measles immunization. Transitory complement defects vary in measles; Charlesworth and associates[84] found evidence of pathologic complement activation in 20 of 50 patents studied. Coovadia and colleagues[104] noted slight but significant reductions in serum IgA levels and elevated IgM values in acute measles virus infections. These investigators found the IgG concentrations to be normal. Increased levels of serum or plasma IgE were noted in two studies.[178,401] Delayed hypersensitivity responses in skin are suppressed in both natural and vaccine measles virus infection.[136,413] Similarly, in vitro lymphocyte blastogenic responses to common antigens are suppressed.[455]

Mechanisms in Recovery from Measles Viral Infection

Acute clinical measles is characterized by viral multiplication in many organs of the body and then rapid subsidence of the infection by the 17th day. As noted earlier, measles virus infection is associated with both serum and secretory antibody responses and specific cell-mediated responses that coincide with clinical recovery. All three factors would seem to be important in recovery from acute measles virus infection, but confusing information clouds the issue. For example, researchers have noted that children with simple agammaglobulinemia in whom measurable measles antibodies do not develop after measles virus infection recover from the disease normally.[168] This finding suggests that development of measles antibody is not important for recovery from acute infection. However, by using a sensitive plaque-reduction measles neutralizing antibody technique, Black[42] found small amounts of antibody in the sera of three agammaglobulinemic patients.

In contrast to antibody data, data on patients with defects in the cell-mediated immune system show that they clearly do poorly with measles virus infection and frequently succumb to progressive infection despite the administration of large doses of measles antibody-containing immunoglobulin. T cells (both $CD4^+$ and $CD8^+$) clear virus-infected cells from tissues by cytotoxicity. INF-α, which is released by a variety of cells, including T cells, inhibits the spread of virus infection.

In studies in a transgenic mouse model system, Tishon and associates[432] showed that CD4, CD8, or B cells alone controlled measles virus infection. However, combinations

TABLE 179-3 **Nonspecific, Immunologically Related Responses During Measles Virus Infection**

Category	Findings	Reference
Leukocytes	Defective neutrophil motility	16
	Leukopenia (both lymphocytes and neutrophils)	37, 131
	Decreased T, B, and null lymphocytes	10, 19, 103, 104, 221
	Decreased natural killer cell activity	184
	Decreased helper T cells	108, 237
	Prolonged suppression of interleukin-12 production	21
	Prolonged depression of virus-specific interferon-α production	324
Interferon-α	Elevated plasma levels	183
Neopterin	Elevated plasma levels	183
Interleukin-2 receptor (soluble)	Elevated plasma levels	182
Platelets	Reduction in peripheral count	350
Complement	Frequent pathologic activation of the complement system; reduction of C1q, C4, C3, and C5	84
Serum immunoglobulins	IgA reduced and IgE and IgM elevated	104, 179, 401
In vitro lymphocyte response to phytohemagglutinin	Suppressed in presence of autologous serum; normal in presence of calf serum	455
In vitro lymphocyte response to *Candida*	Suppressed	455
Cutaneous delayed hypersensitivity	Depressed	136
C-reactive protein	Elevated at onset of rash	180
Circulating immune complexes	Noted in 25% of patients 7 to 13 days after rash onset	473

Ig, immunoglobulin.

of CD4 T cells and B cells or CD4 and CD8 T cells were essential in clearing infection. In contrast, the combination of CD5 T cells and B cells was ineffective.

Mechanisms in Prevention of Repeat Illness in Persons Previously Infected with Measles Virus

In contrast to mechanisms of recovery in acute measles, the role of serum antibody in protection against recurrent disease is solid. Before the present vaccine era, researchers repeatedly demonstrated that the administration of measles antibody-containing immunoglobulin could prevent clinical measles.[217,418] Similarly, infants with transplacentally acquired measles antibody are immune.[254] Whether other factors also are important in protection against reinfection is not clear. Often cited is that patients with agammaglobulinemia do not have repeated measles virus infections, so other factors must be involved.[168]

Of interest is that patients have been described in whom antibody did not prevent acquisition of atypical measles after immunization with killed measles vaccine.[268] The data of Krause and associates[249] suggested that persons in whom the capacity for exaggerated measles-specific lymphocyte activity persists (some previous killed vaccine recipients) may be subject to illness in spite of the presence of antibody. However, the antibody produced after receipt of killed measles vaccine was incomplete; it lacked specific antibody to F protein.[94] Black[42] noted one child with a low measles neutralizing antibody titer, presumably from natural measles virus infection, in whom measles later developed. Chen and coworkers[86] found that some students with measurable but low neutralizing antibody titers were not protected from clinical measles on exposure.

CLINICAL MANIFESTATIONS

Before the present vaccine era, measles was an inevitable disease of childhood that was recognized readily by parents and other laypersons, as well as by physicians. Despite the occasional confusion with other exanthematous diseases,[88] the epidemic character of measles usually resulted in an accurate diagnosis. Currently in North America, few of the new generation of parents, physicians, and other medical personnel have seen measles; therefore, the illness can be misdiagnosed.

Typical Illness

Incubation Period

The incubation period of measles is approximately 10 days (range, 8 to 12 days).* Although extensive virologic and immunologic events occur during this period, the individual has virtually no outward sign of illness. Goodall[169] suggested (and Partington and Quinton[357] presented supporting data) that some patients have mild transient respiratory symptoms and fever shortly after initial acquisition of the virus.

*References 26, 89, 91, 230, 256, 382, 417.

Prodromal Period

The prodrome of measles lasts approximately 3 days (range, 2 to 4 days). Initial symptoms are respiratory and suggest the possibility of a cold, except fever is an early sign. In fact, in situations of close observation, slight temperature elevation has occurred and then subsided for a day or so before the appearance of typical respiratory symptoms.[417]

The onset of clinical measles is characterized by general malaise, fever, coryza, conjunctivitis, and cough. These symptoms worsen during a 2- to 4-day period. Early in the prodromal phase, a transitory rash occasionally has been observed. It has been urticarial or macular, has occurred with the initial onset of fever, and has disappeared before onset of the typical exanthem.

During the prodromal period, the temperature increases gradually to a value of 39.5° ± 1.1° C (103° ± 2° F) over the course of a 4-day period. The nasal symptoms resemble those of other viral respiratory tract infections and are similar to those of the common cold or acute nasopharyngitis. Sneezing, rhinitis, and congestion are common symptoms. The degree of prodromal conjunctivitis varies considerably. Initially, the conjunctival infection is divided by a transverse marginal line across the lower lids.[416] The conjunctivitis is associated with considerable lacrimation, and older patients in particular are bothered by photophobia, which frequently is severe. Slit-lamp examination reveals both corneal and conjunctival lesions.[25,138]

The cough in prodromal measles frequently is troublesome. It worsens throughout the period and often has a brassy quality suggesting laryngeal and tracheal involvement. On day 10 ± 1, Koplik spots, the pathognomonic enanthem of measles, first appear (Fig. 179-5). Koplik[246-248] originally described the lesions as bluish white specks on a bright red mucosal surface. In my experience, the lesions always have appeared white and a blue component has not been observed. Koplik spots first arise on the buccal mucosa opposite the lower molars but usually

FIGURE 179-5 ■ Koplik spots with involvement of the buccal and lower labial mucosa. (See companion Expert Consult website for color version.)

spread quickly to involve most of the buccal and lower labial mucosa. The lesions are approximately 1 mm at first but occasionally seem to coalesce into larger lesions. Initially, only a few lesions appear, but within 12 hours the number usually is uncountable. Of equal importance in establishing the diagnosis is the appearance of the background mucosal surface, which is always bright red and granular. Frequently, 1-mm lesions (Fordyce aphthae), which commonly occur normally in adolescents and adults, are confused with Koplik spots.[87] These lesions, however, can be differentiated easily because they appear on a normal pale mucosal surface rather than the bright red background of measles.

During the prodromal period, erythematous maculopapular lesions also are observed occasionally on the palate. At the end of the prodromal period, the posterior pharyngeal wall usually is erythematous and infected and the patient may complain of a sore throat.

Exanthem Period

In typical measles, the exanthem appears on approximately the 14th day after exposure. The exanthem occurs at approximately the peak of the respiratory symptoms and when the temperature usually is approximately 39.5° C (103° F). At this time, the manifestation of Koplik spots has peaked, and during the next 3 days they disappear. However, after the specific white spots have disappeared, the red, sandpapery mucosal background remains present for a day or so.

The measles exanthem first appears behind the ears and on the forehead at the hair line. Spread of the rash is centrifugal from the head to the feet. By the third day, the rash has involved the face, neck, trunk, upper extremities, buttocks, and lower extremities sequentially. The rash initially is erythematous and maculopapular but progresses to confluence in the same centrifugal manner as it is spread. Confluence always is more prominent on the face; frequently, the lesions on the lower extremities remain discrete. At the height of the rash, the appearance suggests microvesicles on top of a generalized erythematous confluent base (Fig. 179-6).

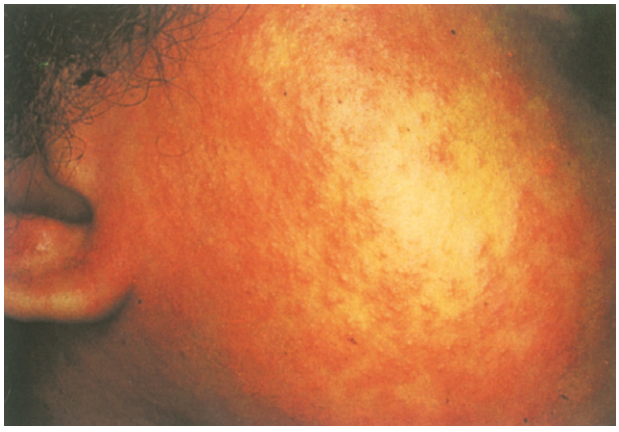

FIGURE 179-6 ■ Measles exanthem. Note the generalized erythematous confluent base supporting small papular and microvesicular lesions. (See companion Expert Consult website for color version.)

The exanthem begins to clear on the third to fourth day, again following the centrifugal course of progression. During the initial stages of the rash, its color is red and it readily blanches on pressure. As the rash fades, it takes on a coppery appearance, after which a brownish discoloration is seen that does not clear with pressure. With healing, a fine desquamation frequently occurs in confluent areas with brownish discoloration. The duration of the exanthem usually is 6 to 7 days.

During the exanthem period, the fever generally peaks on approximately the second or third day of the rash and then falls by lysis during a 24-hour period. Fever that persists after the third or fourth day of exanthem usually is an indication of a complication. Conjunctivitis and nasal symptoms generally subside at about the time of defervescence. Continued nasal discharge, whether purulent or not, suggests bacterial secondary infection. With the appearance of the rash, the cough loosens up, and in older persons it frequently becomes productive. The cough may persist for 10 days or more.

Pharyngitis is a common development during the exanthem period, as is enlargement of cervical lymph nodes. Generalized lymphadenopathy with suboccipital and postauricular involvement is not an uncommon finding, nor is splenomegaly. Young children occasionally have diarrhea, vomiting, laryngitis, and croup. Abdominal pain also can be troublesome.

Laboratory Findings

Laboratory studies rarely are indicated for acute uncomplicated typical measles because the diagnosis can be established on a clinical and epidemiologic basis, and the results of studies rarely affect management of the patient. During the periods of prodrome and rash, the total leukocyte count is low. Numbers of neutrophils and lymphocytes are reduced, but the most marked reduction, when absolute counts are considered, is in the number of lymphocytes.[37] In difficult cases, such as the first apparent case in a particular locality, a specific diagnosis can be made by serologic study, most easily by measles antibody studies performed by ELISA. If an acute serum sample is obtained during the prodrome and a second serum sample is obtained 7 to 10 days later, a significant rise in IgG antibody titer usually is demonstrated. At the time that the rash is manifest, the most useful test for identification of measles antibody is the IgM fraction.

Modified Illness

Modified measles is an infection that occurs in a partially immune person.* It is characterized by a generally mild illness that usually follows the regular sequence of events in measles. The prodromal period is shorter; cough, coryza, and fever are minimal. Koplik spots are few and transient, and they frequently do not occur. The exanthem follows the progression pattern of regular measles, but confluence of the lesions does not occur. Because serologic studies reveal some children with measles antibody who have never had clinical disease suggestive

*References 26, 30, 86, 91, 93, 126, 156, 200, 217, 256, 359, 377, 395.

of measles, some modified infections probably occur without exanthem and perhaps without overt symptoms at all.[42,255]

Modified measles develops under a variety of circumstances, the most important of which historically was the result of intentional alteration of disease by the administration of immune serum globulin to an exposed susceptible child. Naturally occurring modified measles is seen occasionally in infants younger than 9 months old because of the presence of transplacentally acquired maternal measles antibody.

Although the magnitude of the problem is unknown, modified measles also occurs as an occasional manifestation of failure of live vaccine. In these instances, patients have had modified illness but demonstrated a secondary measles serum antibody response (only IgG antibody).[91,93,408] With increasing time from immunization, this response possibly will occur more frequently.[114] However, few data currently support this possibility.

Recurrent measles also rarely results in modified illness. The frequency of recurrent measles is unknown. In general, most authorities have discounted the existence of recurrent measles and suggested that the recorded experiences were the result of confusion with infection by other exanthem-producing agents.[88,252] However, Cherry and colleagues,[91] Schaffner and associates,[395] and Schluederberg[399] have described children with modified illnesses, secondary immunologic responses, and well-documented instances of previous cases of measles.

Atypical Measles

Atypical measles is a clearly defined clinical syndrome that occurs in some previously immunized persons after they have been exposed to natural measles. Most cases have occurred in persons who initially received inactivated (killed) measles viral vaccines, but some cases also have been noted in children who received only live measles vaccines.[92,268,327,411]

Historical Aspects

The initial studies with inactivated measles vaccines in the early 1960s demonstrated that multiple doses were necessary to stimulate an antibody response and that measurable serum antibody levels were short-lived.[156,186,228,254,460] In the initial trials, it soon became apparent that some study participants who had received killed vaccine were still susceptible to measles. On being exposed to natural measles, some children developed typical measles and others a mild, modified illness.[156] As a result, regimens were developed in which children were immunized with two or three doses of killed vaccine at monthly intervals, followed in a month or more by a single dose of Edmonston B live measles vaccine (KKL or KKKL). Studies at the time demonstrated that good antibody levels were achieved after administration of both regimens. After measles vaccine was licensed in 1963, killed-live measles vaccine regimens enjoyed modest popularity in the United States (as well as in other countries) because live Edmonston B strain vaccine frequently was associated with alarming febrile responses and occasional febrile convulsions.

In 1965, Rauh and Schmidt[374] reported an unusual illness after exposure to natural measles in some children who had received killed vaccine 2 years previously. The significance of their findings became more apparent during the following 2 years when Fulginiti and colleagues[154] and Nader and coworkers[320] noted many instances of "atypical measles" in previous recipients of KKK and KKL vaccination regimens. In these two instances, original immunization had taken place 4 to 6 years before the occurrence of atypical illness.

In 1968, killed measles vaccine was taken off the market in the United States after the distribution of approximately 1.8 million doses in the period from 1963 to 1968.[347] During the 12-year period from 1968 to 1980, atypical measles was reported frequently.* I am unaware of reports since 1980 of further cases of atypical measles. However, I observed one 26-year-old physician and a 28-year-old nurse with the syndrome in the 1980s. Sporadic cases in adults may still be occurring but are misdiagnosed because physicians caring for adults are unaware of the syndrome; therefore, the history of killed measles vaccine is not uncovered and specific antibody studies are not performed.

In 1971, during study of an extensive measles epidemic, my colleagues and I[92] observed six children who had relatively mild, atypical measles-like illnesses but had received only live measles vaccine. Linnemann and colleagues[268] similarly noted two children with atypical measles-like illnesses who had received only live measles vaccine. Nichols[327] and St. Geme and associates[411] also have reported bizarre measles illnesses in patients who formerly received live vaccine.

Clinical Characteristics

Atypical measles was a common illness from 1967 to 1978. Because killed measles vaccines have not been available in the United States since 1968, two obvious facts need to be mentioned: with each passing year, potential patients and actual patients with atypical measles will be 1 year older and also 1 year farther from the time of receiving the primary killed measles vaccine immunization series. Both these aspects raise concern about whether the clinical manifestations of the syndrome will remain the same today as when the illness first was described.† It was my opinion in 1981 that the syndrome had changed slightly but still was recognizable from original descriptions of the illness.[89]

The incubation period of atypical measles is similar to that of typical measles—between 7 and 14 days in duration. The prodromal period is characterized by the sudden onset of high fever (39.5° to 40.6° C [103° F to 105° F]) and usually headache. Abdominal pain and myalgia also are common complaints. Dry, nonproductive cough occurs in most patients, and vomiting is noted

*References 18, 85, 92, 155, 165, 188, 201, 207, 263, 292, 301, 327, 329, 337, 452, 453, 470, 471.

†References 53, 85, 89, 92, 154, 155, 165, 188, 201, 207, 263, 292, 301, 320, 327, 329, 336, 337, 374, 452, 453, 470, 471.

in approximately one third of those afflicted. Pleuritic chest pain and weakness also are common complaints. Although few reports to the contrary exist, Koplik spots appear to be rare in atypical measles.

Two to 3 days after onset of the illness, the rash appears. It is unique in that it first develops on the distal ends of the extremities and progresses in a cephalad direction. Usually, the rash initially is erythematous and maculopapular. I have been impressed by a slight yellowish hue of the exanthem when compared with that of typical measles. The rash is particularly prominent on the wrists and ankles; it involves the palms and soles. Spread of the rash varies considerably. In some patients, only the wrists and ankles are involved, whereas in others, the entire extremities as well as the lower part of the trunk are affected. In a peculiar fashion, the rash frequently seems to end its cephalad progression in a line at the level of the nipples. Occasionally, a few erythematous, maculopapular but discrete lesions are found on the face. In some cases, the rash becomes vesicular, with the lesions 2 to 3 mm in diameter; they do not proceed to scab formation as in varicella, but occasionally pruritus is a problem, and excoriation occurs from scratching. The exanthem often has a petechial or purpuric component, and urticaria also occurs frequently. Edema of the extremities has been a common finding.

Although coryza has been noted in a few reports, it is not a prominent feature, nor is conjunctivitis. Respiratory distress with dyspnea and rales occurs commonly, and radiographic examination reveals pulmonary involvement in virtually all cases. Most patients have hilar adenopathy and pneumonia. Pleural effusion also occurs frequently. The pneumonia in atypical measles usually is lobular or segmental, and the lesions often appear nodular (Fig. 179-7). Although initial descriptions of the syndrome suggested an illness of approximately 1 week's duration, later observations indicated illnesses of 2 weeks or more. In one case, fatigue and other symptoms persisted for more than 1.5 years.

FIGURE 179-7 ■ Nodular pulmonary infiltrates in a child with atypical measles.

In the original description by Rauh and Schmidt,[374] one patient had an exanthem that was biphasic; initially, a transitory rash suggested modified measles. Two weeks later, the more characteristic atypical exanthem developed. The same observers also noted a second case with a biphasic exanthem in which the first lesions were vesicular and occurred when the child had little fever. The second exanthem was maculopapular and associated with a febrile response. Zahradnik and colleagues[471] also noted a similar sequence in two young adults. Two patients who had received killed measles vaccine in the past had radiographic evidence of characteristic pulmonary findings, but the typical exanthem did not develop.[337,470]

Other findings in atypical measles include marked hepatosplenomegaly,[374] marked hyperesthesia,[320] weakness,[263] and numbness and paresthesia.[292] Personal observations of cases in adolescents and young adults suggest that the exanthem is less prominent than in past cases, and the fever and overall morbidity are of greater duration.[89] Follow-up radiographic studies demonstrated the persistence of nodular pulmonary lesions for longer than 1 year in several patients and up to 6 years in one patient.[263,301,470]

Measles antibody studies in atypical measles are remarkably diagnostic. If an initial serum sample is obtained before or at onset of the exanthem, CF and HI titers usually are less than 1:5. By the 10th day of illness, both titers are elevated markedly, and most are 1:1280 or greater. In contrast, in typical natural measles at the 10th day of illness, the titer rarely is greater than 1:160.

Measles virus was not recovered from a patient with atypical measles, but only a few adequately performed studies were performed. The epidemiologic data currently available suggest that patients with atypical measles are not contagious. Other laboratory studies are not particularly useful in atypical measles. The erythrocyte sedimentation rate is elevated. When serial blood cell counts have been performed, slight early leukopenia and late eosinophilia have been noted.[154]

The pathogenesis of atypical measles was studied by several investigators, and several possible mechanisms were suggested,[368] including a generalized Arthus reaction, induction of abnormal measles virus–specific, delayed-type hypersensitivity, and an imbalance in antibody responses to H and F proteins caused by denaturation of F protein during formalin inactivation of the vaccine virus. In a study in monkeys, Polack and associates[368] found that atypical measles resulted from previous priming for a nonproductive type 2 $CD4^+$ T-cell response and not from the lack of functional antibody against F protein. In revaccination studies performed by my colleagues and I in the 1970s, we found that vaccinees with severe local reactions had marked lymphocyte reactivity to inactivated measles virus and absent or minimal HI antibody.[249]

Unusual Manifestations and Complications of Measles

In addition to typical measles, modified measles, and atypical measles, many other clinical manifestations and complications occur at a broad range of frequency. By

definition, unusual manifestations are a direct result of the primary viral infection, whereas complications are a result of damage by a secondary infection with another microorganism. In many instances, determining whether a particular manifestation is just viral or involves a second agent is difficult. Combinations of infections are common occurrences.

In general, complications resulting from secondary infection are not as common today as they were before the antibiotic era; however, no evidence suggests that unusual manifestations of illness occur less frequently today than formerly. To demonstrate the magnitude of the problem today, the findings in a 1970 to 1971 hospital survey in St. Louis are revealing.[90,91] In this period, an extensive epidemic occurred, with 10,000 cases of measles. In eight area hospitals, 130 children (1.3%) were admitted; 66 cases of pneumonia and six fatalities occurred, and 6 children had encephalitis.

The records of measles patients in three hospitals were reviewed carefully. Of this group of 71 patients, 53 had pneumonia; 37 of the patients with pneumonia had either previous cardiorespiratory or other chronic systemic disorders. Two children had mediastinal and subcutaneous emphysema. All six deaths were caused by fulminant pneumonia. Of the 6 children with encephalitis, severe residual neurologic damage developed in 3. One child had acute measles appendicitis with perforation and peritonitis, and another patient had mesenteric lymphadenitis.

Pneumonia

Pulmonary involvement in measles, as a result of the viral infection, is probably the rule rather than the exception. Kohn and Koiransky[244] performed careful radiographic studies in 130 children with measles and noted that 55 percent had pneumonic infiltration and 74 percent had hilar adenopathy. In most instances, the pneumonia was observed early in the course of the illness, a finding that suggested primary viral involvement rather than secondary bacterial infection. In the 1970 to 1971 St. Louis measles epidemic, approximately 1 in every 150 patients with measles was hospitalized because of pneumonia.

Pneumonia in measles has varied radiographic manifestations.* Clearly, viral pneumonia is characterized by bilateral hyperinflation with diffuse fluffy infiltrates that are more confluent at the hilum. Unilateral, segmental, and lobar pneumonias also are observed. Gremillion and Crawford[175] reviewed 106 cases of pneumonia that occurred in 3220 Air Force recruits with measles between 1976 and 1979. Illnesses were severe, but no deaths occurred. Bacterial superinfection was documented in 30.3 percent of the cases; bronchospasm occurred in 17 percent of the recruits with pneumonia. In one study, seven children with massive and bilateral lung consolidation had clinical findings consistent with adult respiratory distress syndrome.[6]

Clinically, young infants have a picture of bronchiolitis with expiratory distress. In severe cases at all ages, a marked ventilation-perfusion deficit is noted.[269,358] Patients with defects in the cell-mediated immune system are particularly prone to progressive fatal bilateral infection.*

Secondary bacterial pneumonia is the result of common respiratory pathogens, particularly *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Coinfection with other viruses also was noted in an extensive study of measles-associated pneumonia in the Philippines.[371] In this study, parainfluenza virus and adenoviruses were isolated most frequently.

Other Respiratory Manifestations

Otitis media is the most common complication and is age related. In the immediate pre-vaccine era in the United States, otitis media developed in 5 to 15 percent of patients with measles. It now is less a problem because of the change in the age-related incidence of measles. The bacterial pathogens in otitis media associated with measles are similar to those in otitis media in children without measles. Mastoiditis was a common complication of measles in the era before antibiotics, but fortunately today it is rare.

Laryngitis and mild laryngotracheitis occur commonly. Occasionally, frank, severe laryngotracheobronchitis occurs and may require tracheotomy.[384] Measles-associated bacterial tracheitis is not an uncommon finding.[99] Secondary bacterial infection of the cervical lymph nodes and secondary bacterial pharyngitis also are rather frequent complications of measles. Field[134] attributed 3.2 percent of cases of childhood bronchiectasis to former infection with measles virus.[134] Measles also has a deleterious effect on the course of tuberculosis.[414]

Cardiac Manifestations

Myocarditis and pericarditis occasionally occur in cases of measles.[113,135] Nonspecific, transient electrocardiographic abnormalities were noted in more than half of 71 children with measles in one study.[385] In another study, 19 percent of patients had transient but clear-cut abnormalities, including T-wave changes, atrioventricular conduction defects, and premature auricular contractions.[167] Although cardiac involvement appears to occur frequently in measles, clinical consequences from such involvement are rare developments.

Neurologic Manifestations

Neurologic involvement is not an uncommon finding in patients with measles.† Gibbs and associates[163] noted that 51 percent of 680 patients with measles who had no clinical evidence of encephalitis had abnormal electroencephalographic results during acute or immediate postacute illness. Although the incidence varies, clinically evident encephalitis occurs in 0.5 to 1 of every 1000 measles cases.[25,68,91,127,229] From 1962 to 1979, the average measles

*References 6, 90, 175, 244, 269, 276, 285, 342, 423.

*References 128, 243, 302, 304, 406, 409.

†References 5, 26, 38, 127, 130, 163, 171, 258, 272, 291, 353, 369, 435, 472.

encephalitis-to-case ratio was 0.73 : 1000.[68] Both mortality and the incidence of sequelae have varied in the reports available. LaBoccetta and Tornay[258] noted a mortality rate of 32 percent in 50 patients in a group seen before 1947 and a rate of 11.5 percent in a group seen between 1947 and 1957. Ziegra[472] reported only two deaths in a group of 38 cases. Long-term morbidity data also vary considerably. In general, between 20 and 40 percent of patients who recover from measles encephalitis have manifestations of brain damage. Douglas[121] could find no evidence of later subnormal school performance in a group of children who had uncomplicated measles.

Symptoms of encephalitis usually develop during the period of measles exanthem and within 8 days of the onset of illness.[5,258,383] Occasionally, the onset of central nervous system (CNS) signs and symptoms occurs during the prodromal period. LaBoccetta and Tornay[258] noted the following frequencies of signs and symptoms at the onset of measles encephalitis: convulsions, 56 percent; lethargy, 46 percent; coma, 28 percent; and irritability, 26 percent. Patients with encephalitis frequently have multiple findings: headache, abnormalities in respiratory rate and rhythm, twitching and other involuntary movements, and disorientation. Cerebellar ataxia, myelitis, retrobulbar neuritis, transient mental disorders, and hemiplegia are findings noted during the subacute stages of illness. Long-term sequelae include various degrees of retardation and selective brain damage, recurrent seizures, deafness, and hemiplegia and paraplegia. An 8-year-old girl developed pseudotumor cerebri 3 weeks after having a case of measles.[426]

Examination of cerebrospinal fluid (CSF) in measles encephalitis usually reveals mild pleocytosis with a predominance of mononuclear cells, mildly elevated protein values, and a normal glucose level.[258,291,343,435] In one study, in 15 percent of the cases CSF pleocytosis was not found.[258]

Considerable controversy relates to the mechanisms in measles encephalitis.* Some investigators failed to isolate measles virus or to demonstrate measles virus RNA or other viral antigens in the brains of affected patients.[161,225,234,309] These findings led to the widespread consensus that the illness is autoimmune (acute disseminated encephalomyelitis) and that viral invasion of the CSF is unnecessary. However, other investigators have recovered measles virus from the CSF and brain of affected patients, a finding indicating that the virus is involved directly in the process.[130,141,291,429] In the hamster model of measles virus encephalitis, virus can be cultured directly from the brain.[356]

Other Manifestations

Measles has been associated with many other manifestations and complications. Of historical interest was the occurrence of a severe, often fatal, form of measles called *black measles* that was characterized by a confluent hemorrhagic skin eruption.[256] Patients with this illness had signs of both encephalitis or encephalopathy and pneumonia. Extensive bleeding from the mouth, nose, and bowel frequently occurred. Severe hemorrhagic measles rarely is seen today, and little is known about its pathogenesis. Disseminated intravascular coagulation would appear to play a role.

Another complication of measles involving bleeding is thrombocytopenic purpura.[6,209] It is a postinfectious illness and different from hemorrhagic measles. Although bleeding is extensive on occasion, the ultimate prognosis usually is good. Stevens-Johnson syndrome has been noted occasionally in measles.[279] Other manifestations include pneumomediastinum, subcutaneous emphysema, hepatitis, appendicitis, ileocolitis, mesenteric lymphadenitis, cervicitis, acute glomerulonephritis, corneal ulceration, and gangrene of the extremities.* Measles in pregnancy results in significant maternal and fetal morbidity and mortality.[24,125,220,315,415] Jespersen and associates[220] retrospectively reviewed 10 epidemics of measles in Greenland; these investigators obtained adequate data on 327 women infected during pregnancy, and they also were able to examine 252 of the offspring. Thirty-two percent of women infected during the first trimester had spontaneous abortions, and 9 percent of these pregnancies that continued to term resulted in stillbirths. Congenital malformations occurred in 8 of 300 live-born infants.[220] Pneumonia is a frequent maternal complication of measles during pregnancy.[24,125]

Clinical measles shortly before birth in the mother-to-be has resulted in congenital infection and illness in the neonate.[162] Congenital measles is often severe, with death occurring in approximately 32 percent.

Subacute Sclerosing Panencephalitis

SSPE is an uncommon, slowly progressive disease of the CNS, with an almost invariably fatal outcome. The disease was recognized by a number of investigators early in the 20th century.[49,109,391] Several early accounts, especially those published by Dawson in 1933,[111] suggested a viral origin of this disease; however, not until the mid-1960s was measles virus clearly demonstrated to be the causative agent.†

Epidemiology

SSPE is a rare disease that in the pre-vaccine era had an annual incidence of approximately 1 case per 1 million population in most of the developed world, including the United States. The estimated incidence after wild-type measles virus infection in the pre-vaccine era was approximately 1 case per 100,000 population. The incidence of SSPE in the pre-vaccine era in some developing nations greatly exceeded that of developed nations. In southern India the incidence of SSPE was as high as 21 per 100,000 population,[392] and in Pakistan the rate was estimated to be 100 per 100,000.[245] A report from Papua New Guinea suggested a similarly high incidence of SSPE.[271] Several characteristics of natural measles virus infection in developing countries in the pre-vaccine era were associated

*References 7, 137, 141, 161, 181-183, 223-225, 234, 291, 309, 321, 360, 365, 429.

*References 147, 199, 229, 235, 256, 267, 293, 310, 328, 405, 431, 465.
†References 41, 84, 100, 205, 360, 428.

with the development of SSPE and included the high incidence of measles and the frequent occurrence of measles in children younger than 2 years of age. Both these risk factors for SSPE are modifiable, if not preventable, by vaccination.

The disease has been reported in all countries of the world, and, although no outbreaks have been reported, clustering of cases prompted suggestions that environmental factors or unique strains of viruses may contribute to the development of SSPE after measles. The epidemiologic study of SSPE in the Netherlands described 4 cases of SSPE that occurred in 1 year in one city of 120,000 people, a number that calculated to 4 cases of SSPE per 6,000 cases of measles, a rate 10-fold greater than that for the rest of the country.[33]

The incidence of SSPE in the pre-vaccine era was approximately five times higher in the southeastern United States than that in other regions of the country,[191,215,307] also suggesting the possibility of environmental cofactors. In addition, in the pre-vaccine era, a preponderance of cases of SSPE occurred in the United States, as well as in other developed and developing countries, in children from rural areas, independent of the incidence of measles in these areas.[58,59,191,273,306] SSPE appears to have no racial predilection, although the largest number of cases occur in whites.[214,307] The incidence of SSPE consistently is two to three times more common in male than in female patients.[33,214] No relationship between any human leukocyte antigen genotype and the development of SSPE has been demonstrated, and cases of SSPE in only one member of identical twins have been reported.[96,117,208] The usual age at presentation in the pre-vaccine era was between 6 and 10 years, with ranges of 2 to 35 years reported.[33,152,153,191] The youngest case reported was in a 10-month-old child, and the oldest was in a 52-year-old man with depressed cellular immunity.[39,424] In most cases, symptoms attributable to SSPE begin 4 to 8 years after measles virus infection; however, in some series, older patients (>10 years of age) appeared to have experienced a prolonged interval between measles virus infection and the development of SSPE.[33,214,307]

Although several risk factors for the development of SSPE have been demonstrated repeatedly, the most consistent is the acquisition of measles before reaching the second birthday.[33,116,191,307,392] An estimated minimum of 50 percent of those with SSPE have had early measles.[191] In India, an estimated 60 percent of measles infections occurred before the patients reached 2 years of age, a factor possibly contributing to the high incidence of SSPE in this country.[392] Other risk factors have included exposure to animals.[191]

The decreased incidence of SSPE after institution of an effective measles vaccine program was demonstrated in several countries.* The incidence of SSPE in the Netherlands decreased 10-fold after universal vaccination was initiated.[33] The incidence in Japan decreased at least 10-fold and possibly more.[344] Development of SSPE after receipt of measles vaccination has been noted to occur. In a case-control study of patients with SSPE in the United States, 17 of 52 (32.7%) patients with SSPE had received measles vaccine before they developed disease.[191] Similarly, SSPE occurring after measles immunization was noted in the Netherlands, but the incidence of vaccine-related SSPE was less than 1 case per 2.5 million immunizations.[33] In Japan, the estimated incidence of measles vaccine-related SSPE was 0.9 cases per million doses of vaccine.[344] An inherent problem common to all of these studies was the inability to identify patients with subclinical, wild-type measles virus infection before they received vaccination. Subclinical measles virus infections are thought to be common in developing countries, as illustrated by studies from India that suggested that as many as 20 to 40 percent of patients with measles may have had a subclinical infection.[392]

In the United States, the success of measles immunization clearly resulted in a profound reduction in the incidence of SSPE.[48,71,450] In the earlier years of the immunization program in the United States, rare cases of SSPE developed in vaccinees. Some of these children and perhaps all of them had unrecognized measles infection before they were vaccinated, and the SSPE was caused by the natural measles virus infection and not the vaccine virus.[450] I am unaware of any cases of SSPE in the United States since the year 2000 that can be attributed to measles vaccine virus.

Bellini and associates[35] studied brain samples from 11 patients with SSPE during the period 1992 to 2003. Nine of the patients in this group had a history of measles immunization. Nonetheless, in all 11 cases, the measles virus genotype identified was that of wild measles and was not consistent with vaccine virus. These researchers attributed the measles infections to the epidemic that occurred in the United States from 1989 through 1991.

In the period 1989 through 1997, 55,622 measles cases were reported. From these data, a rate of 22 cases of SSPE per 100,000 reported cases of measles was calculated. This rate is approximately 10 times higher than previous estimates in the United States and similar to those noted in developing countries in the pre-vaccine era.[245,271,392]

Pathogenesis and Pathology

Autopsy specimens from patients who died of SSPE reveal mild to striking changes in the gross appearance of the cerebral cortex, with ventriculomegaly in some cases. Histopathologic changes include minimal meningeal cellular infiltrate, with a perivascular accumulation of lymphocytes and plasma cells. Within involved areas of the brain, dense infiltrates of T lymphocytes and marked cellular expression of MHC class II antigens have been described.[52] A prominent microglial and astrocytic hyperplasia often is present. One of the characteristics of SSPE is the presence of intranuclear and cytoplasmic inclusion bodies.

The study of these structures by electron microscopy in 1965 led to the finding of paramyxovirus-like particles in autopsy material.[50,428] Subsequently, Connolly and colleagues[100] described the presence of measles virus antibody in the CSF of patients with SSPE, as well as the presence of measles virus antigens in brain tissue from

*References 33, 48, 71, 298, 344, 450.

autopsy specimens of patients who had SSPE. Shortly thereafter, several laboratories reported the recovery of defective measles virus in specimens of brain from patients with SSPE.[52,204,205] Virus was isolated only by cocultivation and not as cell-free material. Together, these findings suggested that SSPE was caused by a persistent infection of the brain by measles virus.

Several mechanisms, such as an abnormal host immune response to a common infection or a mutant virus, have elicited the most interest. Available data suggest that it may be a combination of these two possibilities. Several observations of the natural history of SSPE are consistent with an abnormal host immune response to a primary measles infection. They include the following: (1) the chronicity of measles virus infection in children with SSPE indicated a failure to eliminate the agent; (2) the importance of early acquisition of measles virus in the development of SSPE suggested that the immature immune system may predispose the host to a persistent infection; and (3) the onset of clinical disease long after a primary infection also suggested a failure of a previously protective immune response. Early on, the consensus was that patients with SSPE had subtle defects in cellular immune responses as measured by decreased cutaneous reactivity to common skin test antigens, decreased lymphocyte proliferative responses to mitogens and measles virus antigens, and reduced production of cytokines. Subsequent studies failed to confirm these conclusions, and most investigators now suggest that patients with SSPE can generate vigorous cellular immune responses after exposure to mitogen and measles-specific antigens.[52] Studies demonstrated the presence of several cytokines, including IL-1b, IL-2, IL-6, TNF, heat-labile toxin, and IFN-γ, as well as other markers of immune activation, in brain lesions from patients with SSPE.[52] Still, some debate continues about the immunocompetence of patients with SSPE. Finally, measles virus is associated with chronic progressive encephalitis in immunocompromised patients (measles inclusion body encephalitis); however, the clinical course and histologic findings of this disease clearly are different from those of SSPE.

In contrast to the questions that surround the cellular response to measles virus in patients with SSPE, antibody responses to measles virus–encoded proteins are well preserved. Antibodies of all isotypes are produced peripherally and within the CNS. Antibodies against all the proteins encoded by measles, including the M (matrix) protein, have been detected in the sera from patients with SSPE.[52] The paradox of elevated levels of circulating anti-measles antibody as well as CSF anti-measles antibody in the presence of persistent infection suggested a possible immune-mediated origin of this disease. Evidence was presented that anti-measles antibody could interfere with spread of the virus and syncytial formation. Fujinami and Oldstone[152] demonstrated that anti-measles antibody reversibly could modulate the intracellular expression of measles virus–encoded proteins, thereby providing a mechanism by which antiviral antibody could convert an acute productive infection into a chronic persistent infection. This decrease in expression of measles antigen also could shelter virus-infected cells from immunologic recognition. This hypothesis also is supported by observations suggesting that early acquisition of measles predisposes the individual to the development of SSPE because the limited quantity of passively acquired maternal anti-measles antibody may be insufficient to prevent development of infection and dissemination of measles virus but adequate to modulate expression of measles virus. This series of events then would predispose the patient to the development of a chronic persistent infection. Animal models consistent with this disease mechanism have been described.[52]

The second general mechanism for the development of SSPE is the generation of viral mutants during acute infection that then can establish a persistent infection. Persistence may result from antigenic variability or a viral mutation leading to decreased production of viral proteins below a level recognizable by the immune system or an extension of cell tropism. That measles viruses from the CNS of patients with SSPE are defective in replication is well documented. Analysis of these isolates does not define a virulence feature of SSPE strains but does suggest several mechanisms that could account for the generation of viral mutants. Much interest has been focused on the decreased expression of the M protein in explants of brain tissue from patients with SSPE.[52] This viral phenotype also was consistent with the decreased production of anti-M antibody in patients with SSPE, a finding suggesting the possibility of subthreshold production of antigen in these patients. Although the decreased expression of M protein was proposed originally as the mechanism for the persistence of mutant measles virus in patients with SSPE, subsequent studies showed that this is only one of many mutant phenotypes in strains of measles virus associated with SSPE.

Further studies of the replication of measles virus revealed several mechanisms that favor the production of mutant viruses. The RNA polymerase of measles viruses, like that of other RNA viruses, does not have proofreading functions and therefore frequently misincorporates nucleotides.[62] Thus, mutant progeny virus arises regularly and can be selected either by host immune responses or because of its extended host-cell tropism. In addition to this general mechanism of genetic diversity in RNA viruses, measles virus also exhibits what has been described originally as biased hypermutation and more recently as A/I hypermutation.[63,64] This mutational event results in clusters of U (uridine) to C (cytidine) or A (adenosine) to G (guanosine) transitions, possibly as the result of novel host-derived enzymatic activity referred to as *double-stranded RNA unwindase*.[52] This activity results in the replacement of A residues with inosines in duplex RNA molecules formed between genomic RNA and mRNA. Replication of the inosine-modified genomic strand then results in the replacement of U by C. Evidence of this proposed mutational event has been found in the genomes of measles viruses from the brains of patients with SSPE, in which up to 132 of a possible 266 U residues in the M coding sequence were converted to C.[62]

Studies clearly demonstrated that the M gene is the most heavily mutated of measles virus genes, often with early stop codons. Researchers stressed that this mechanism of genetic change is operative during lytic as well as persistent infection, and mutants that persist must do

so as the result of growth advantage, such as evasion of the host immune response. The frequent mutations found in the M gene suggest that the measles virus can tolerate extensive genetic change in the M protein yet still replicate within cells of the CNS. The finding of clonal spread of a hypermutated measles virus genome within the brain of a patient with SSPE is consistent with this hypothesis.[28] Other genes of the measles virus also exhibit mutations that alter function, including the gene encoding the hemagglutinin (H) protein. Mutations of this protein were shown to limit cell surface expression but not the function of hemagglutinin in the replicative cycle of measles virus.[40] Cells infected with this phenotype likely are poorly recognized by the immune system because of the limited cell-surface expression of the H protein, yet enough functional activity remains to allow spread of the mutant virus within the CNS by a mechanism of cell-to-cell fusion. Thus, the generation of measles viruses that can induce disease and establish persistent infections results from viral strategies of genetic diversity coupled with selective pressure of the host immune response.

Clinical Manifestations

The initial stage of the disease can be described best as a period of progressive psychointellectual disturbances.[380] These disturbances can include lability of mood, deterioration of school performance, hyperactivity or lethargy, depression, and occasionally altered states of consciousness. Although retrospective analysis often allows precise definition of the onset of SSPE to be established, many of these disturbances are so subtle that they escape parental or physician detection. Physical findings vary and often are nonspecific. A peculiar pigmented retinopathy has been observed in a small number of patients.[60] The duration in stage I varies, depending on the clinical staging system, but in larger series, this stage is relatively short, usually lasting fewer than 6 months.[380] The duration in stage I appeared to be prolonged in older patients, compared with patients younger than 10 years of age, in the series from the Netherlands.[33] Accelerated progression to stage II has been reported.[380]

Stage II is characterized best by a variety of convulsive and motor disorders. The motor disorders are striking, ranging from akinetic drop attacks to violent myoclonic jerks. The motor disturbances have been described consistently as stereotypic and rhythmic. Rigidity or spasticity may be present in the later part of this stage.[52] Extrapyramidal findings, such as choreoathetotic and ballistic movements as well as parkinsonism with abnormalities in the basal ganglia, have been reported. Intellectual functions continue to deteriorate, but patients may retain receptive function during this stage. In one series of patients, as many as 50 percent with stage II disease had abnormal retinal findings, including optic atrophy. The duration of this stage is quite protracted; as many as 50 percent of patients remain in this stage for longer than 6 months and as many as 20 percent remain so for more than 1 year.

Progression to stage III is suggested by increased frequency of myoclonic jerks, development of spasticity or rigidity, and decerebrate and decorticate posturing. Hypothalamic dysfunction becomes prominent, with hyperpyrexia, diaphoresis, and periods of pallor and flushing. Cortical activity rapidly decreases, and patients usually become comatose in this preterminal stage of disease. Duration of this stage is relatively short, lasting fewer than 6 months in most cases. Death usually is associated with complications that accompany the vegetative state, although destruction of essential structures within the brain stem or hypothalamus may result in death.

The previous descriptions account for most patients with SSPE; however, a significant number may exhibit a less predictable course. Some 5 to 10 percent of patients can be expected to have a prolonged survival measured in years. These patients may progress to stage II or III and then remain static without relapse, or they can experience a periodic and sometimes fatal relapse of disease. Conversely, some 10 percent of patients may have a fulminant, rapidly progressive course lasting less than 3 months.[170,274,383]

Laboratory Findings

The definitive diagnosis of SSPE relies on a combination of laboratory findings and a compatible clinical course. Before the association between measles and SSPE was made, the electroencephalogram (EEG) was extremely helpful in establishing the diagnosis.[149,281,380] The classic pattern of the EEG in SSPE is described as periodic, synchronous, bilateral discharges with a frequency of 3 to 20 seconds.[97] The discharge contains high-amplitude polyphasic slow-wave complexes, often consisting of two or more delta waves.[281] Frequently, the background of the EEG is suppressed, thus creating the familiar burst suppression pattern. The EEG provides little help in predicting progression of SSPE. Also of note is that the absence of the classic findings as well as other abnormalities on the EEG has been reported in patients with SSPE.[149,211,222] Alternatively, the finding of periodic generalized bursts of fast waves should prompt consideration of SSPE.[284]

More recent advances in imaging technology have aided greatly in the diagnosis of SSPE. Computed tomographic (CT) scans are abnormal in more than 50 percent of patients with SSPE, but the findings often are nonspecific.[52,103,112,122,316] In the later stages of the disease, cortical atrophy often is a prominent finding on CT scan. Magnetic resonance imaging (MRI) of patients with SSPE has revealed the presence of focal lesions consistent with inflammation, often in the white matter, in multiple areas of the brain.[112] In some cases the location of abnormalities detected by MRI closely reflects the clinical symptoms of the patient, whereas in others the MRI findings do not correlate with clinical findings.[427] Finally, brain biopsy has been used as an important means of establishing the diagnosis in the past and still is of considerable value in atypical cases.

Perhaps the most useful laboratory examination of the patient with suspected SSPE is the examination of the CSF. Normal to slightly increased levels of protein with an absolute increase in the gamma-globulin fraction are a consistent finding in patients with SSPE.[222]

Furthermore, the increase in the gamma-globulin fraction is caused almost exclusively by elevation of immunoglobulin.[407,444] Additional studies showed that 20 to 40 percent of the CSF IgG was oligoclonal and that the oligoclonal fraction contained anti-measles antibodies.[439,440] This finding almost is pathognomonic for SSPE in patients with compatible clinical presentations. All isotypes of antibodies have been found in the CSF, although the finding of IgM anti-measles antibody remains controversial.[52,128,206,446] Because the levels of anti-measles antibodies within the CSF are elevated as well as oligoclonal, most investigators contend that these antibodies are produced locally within the CNS, apparently in response to ongoing measles virus replication. Other CSF findings include normal levels of glucose and slight pleocytosis consisting primarily of lymphocytes. Patients with SSPE have normal to elevated levels of circulating anti-measles antibody; in some cases, this antibody response also appears clonally restricted.[52]

Treatment

In the past, 5-bromo-2-deoxyuridine, transfer factor, ribavirin, and amantadine proved to be of little value.[52] In the 1970s, the antiviral agent inosiplex was introduced and was thought to have antiviral activity in vivo and in vitro, although this claim remains controversial. Some studies suggested that treatment with inosiplex may result in stabilization, prolongation of survival, or actual clinical improvement in as many as 50 to 60 percent of patients. In a large study, 98 patients with SSPE were treated continuously, and their actuarial survival was compared with that of 500 historical controls.[226] The results of this study showed that median survival for the treated patients was 3.2 years, compared with 1.2 years for the historical controls. The use of historical controls, however, raised a number of questions about the validity of the results. The results of this trial also suggested a greater benefit of treatment with inosiplex in patients with more slowly progressive SSPE. Other studies did not demonstrate any efficacy of inosiplex.[52] Inosiplex and intrathecal INF-α seem to elicit transient improvement in some patients.[123,124,257,275,305] INF-β combined with inosiplex also may offer benefit in patients with SSPE.[17] Other treatment approaches that may have shown some benefit in children include levetiracetam, ribavirin, amantadine, cimetidine, corticosteroids, plasmapheresis, and intravenous immunoglobulin.[31]

Measles in Developing Countries

Measles has been and continues to be a staggering problem in developing countries. The mortality rate in much of Africa is approximately 10 percent,[341] and a reasonable assumption is that the rates are similar in some regions of Central and South America and Asia.[110,381,425] In developing countries, measles is a disease of young children. For example, in Kenya in the pre-vaccine era, 25 to 30 percent of children contracted measles before reaching their first birthday and 55 to 60 percent did so before they were 2 years of age; virtually all children had measles by the time they were 4 years old.[196] In Kenyan children, mortality peaked in the 17- to 20-month-old age group and the median age for hospital admission was 14 months.

Although many factors, such as the age at infection, suboptimal medical facilities, and failure to seek medical care, contribute to the excessive morbidity and mortality in children of developing countries, the single overriding factor generally has been thought to be the nutritional status of the infected children.[312,314,341] The data of O'Donovan[341] clearly indicate a direct relationship between malnutrition and hospital admissions for measles and deaths. Studies by Coovadia and colleagues[102] and by Carney and associates[61] indicated both humoral and cell-mediated defects in protein-calorie malnutrition. The clinical picture of measles in children with malnutrition is frequently similar to that in patients with known defects in cell-mediated functions.

Low serum retinol concentrations nearly always are present in children with measles in developing countries.* Low retinol levels correlate directly with measles mortality, and treatment with vitamin A reduces this mortality rate.[29,210,282] Studies performed since the 1980s indicate that the intensive exposure that occurs in children in developing countries is a major factor in measles mortality.[1,3,159]

Clinical Manifestations

Measles in children in developing countries is characterized by two different types of severe disease. One type of illness is a fulminant, toxic illness without apparent localizing complications. The other is a more prolonged illness with obvious complications; the complications may be caused by infection with secondary bacterial or other infectious agents, persistent measles virus infection, or a combination of both.

In a group of 507 hospitalized children, O'Donovan[341] noted that 301 had pneumonia, 96 had gastroenteritis, 36 had croup, 11 had convulsions, 67 had two or more complications, and 140 had nonlocalized systemic toxic effects. The measles rash in malnourished children tends to result in greater confluence and progresses to dark red and then violet.[312] Desquamation is marked and occurs in large scales.[396] After desquamation, patchy depigmentation lasts for some weeks. Other common problems are stomatitis and the resultant sore mouth, which leads to further loss of nutritional intake. Acute corneal ulceration, which occurs after measles in malnourished children, is a common cause of blindness.[393] Multiple skin abscesses and noma (cancrum oris) are rare secondary infectious problems.[229]

Measles in Immunocompromised Hosts

Today, because of the extensive use of immunosuppressive therapeutic modalities and greater duration of survival in certain rapidly fatal diseases, a sizable population of children and adults is immunologically compromised. Measles virus infection in a patient with disease-induced

*References 29, 105, 210, 214, 282, 375, 441.

or iatrogenically caused immune deficiency usually is severe and protracted and frequently fatal.

The most common severe measles virus infection in an immunocompromised host is giant-cell pneumonia.* The mode of manifestation of this illness varies. Some patients initially have severe but otherwise typical measles after a normal incubation period. Clinical findings at the time of the exanthem indicate pulmonary involvement and respiratory distress, and radiographic findings become rapidly worse over a period of approximately a week or less. Other patients initially have rather vague illness, frequently without rash. In these cases, the pulmonary process may progress over the course of a month or longer. Siegel and associates[406] reported a child with leukemia who recovered from typical measles and then died the following year of diffuse interstitial pneumonia in which characteristic measles giant cells were seen.

A unique form of measles encephalitis also is manifested in immunosuppressed patients.† Although the symptoms in different described cases varied, the illness appears to be intermediate between the acute encephalitis occurring in patients without known immune defects and the chronic picture of SSPE. The incubation period has varied between 5 weeks and 6 months.[8] Convulsions frequently are the initial symptom, and they are a prominent aspect of the illness. The seizures have been focal, unilateral, or permanent localized twitching. Other findings include hemiplegia, stupor, coma, hypertonia, and slurred speech. Most cases have been fatal, and the duration of illness has been from 1 week to 2 months.

DIFFERENTIAL DIAGNOSIS

The differential diagnosis of typical measles must include all illnesses in which an erythematous maculopapular rash occurs (see also Chapter 58). The following are most important in establishing the diagnosis of measles: a consideration of possible exposure; the duration of the incubation period; the presence of Koplik spots; the presence of the typical febrile prodrome with cough, coryza, and conjunctivitis; and progression of the rash in a caudal direction. The brown discoloration and the intensity of the measles rash are such that the illness usually should not be confused with rubella, erythema infectiosum, roseola infantum, or enteroviral infection. Of greatest differential difficulty are the exanthems of infectious mononucleosis, *Mycoplasma pneumoniae* infection, and drug eruptions.

In the past, atypical measles was extremely difficult to diagnose. Today, this disease, if it occurs at all, would occur only in adults aged 45 years or older. The key to diagnosing this illness is careful elicitation of an accurate vaccination history. Even if it is not known whether the vaccination that the patient received as a child was live or killed vaccine, it usually can be determined by the number of doses given; if a child received more than one dose of vaccine in a short interval, killed vaccine almost certainly was administered. Differential considerations in atypical measles include Rocky Mountain spotted fever, anaphylactoid purpura, *M. pneumoniae* infection, and drug eruptions.

DIAGNOSIS

Measles virus infection can be diagnosed specifically by the following methods: isolation of virus in an appropriate tissue culture system; demonstration of measles antigen in exfoliated cells and tissues by FA techniques or polymerase chain reaction (PCR)[287]; or the demonstration of a rise in HI, CF, ELISA, FA, or neutralizing antibody titer in two sequential serum samples or specific measles IgM antibody in a single serum sample (see Chapter 252). For practical purposes, most measles cases can be diagnosed by the demonstration of specific IgM antibody in an acute-phase serum specimen. False-positive IgM ELISA results may occur.[79,219,437] Acute measles also can be diagnosed by PCR using throat-swab specimens.[437]

TREATMENT

Uncomplicated Measles

No specific therapy for uncomplicated measles exists. During the febrile period of illness, activity should be discouraged and fluid status should be maintained by the liberal provision of soft drinks and ice. Fever may be controlled with acetaminophen. Cough frequently is distressing and can be managed by the judicious use of common antitussive agents. Room humidification also is useful in controlling the cough and generally can be expected to make the patient more comfortable. As the fever disappears, a gradual return to normal activity is indicated. However, measles virus infection is associated with considerable damage to the ciliated epithelium of the respiratory tract; therefore, resumption of normal activities too soon and exposure to other children and their bacterial pathogens can be associated with severe secondary infection.

Children in developing countries frequently have vitamin A deficiency. Measles morbidity correlates with this deficiency, and treatment with vitamin A is beneficial.* Studies in the United States indicate that vitamin A levels are low in a substantial number of measles cases and that morbidity is increased in these deficient children.[13,20,57,150] Vitamin A supplementation also has been shown to enhance IgG antibody levels and total lymphocyte numbers.[106] In 1993, the Committee on Infectious Diseases of the American Academy of Pediatrics recommended providing vitamin A supplementation for children with measles in selected circumstances.[13] Vitamin A was recommended for children aged 6 months to 2 years who require hospitalization and all patients 6 months or older with immune deficiencies or possible vitamin A deficiency. The dose of vitamin A is 100,000 IU for

*References 128, 243, 295, 302, 303, 373, 406.
†References 8, 148, 193, 296, 316, 319, 370, 434, 461.

*References 29, 105, 210, 214, 282, 375, 441.

children aged 6 months to 1 year and 200,000 IU for children aged 1 year or older. The dose should be repeated 24 hours and 4 weeks after the first dose in children with ophthalmologic evidence of vitamin A deficiency.

Atypical Measles

The most important aspect of therapy for atypical measles is proper diagnosis. In patients with atypical measles, Rocky Mountain spotted fever, other septic conditions, lymphoma, or collagen vascular disease frequently is diagnosed erroneously, and their workup is associated with extensive blood cultures, other diagnostic procedures, and vigorous antibiotic therapy. Careful attention given to a history of previous administration of killed measles vaccine should clarify the diagnosis and preclude the unnecessary trauma associated with extensive diagnostic and therapeutic procedures.

In atypical measles, chest radiographs always should be obtained because the pneumonia that usually develops in these patients frequently is much more extensive than the clinical findings would indicate. Activity should be discouraged in acutely ill patients, and follow-up chest radiographs should be used as a guide to resumption of normal activity. In some patients, pulmonary abnormalities have persisted for a considerable period.

Complications of Measles

Otitis Media

Otitis media is the most frequent complication of measles. The infectious agent of otitis media in measles is no different from that in other children without measles of comparable age, so conventional antibiotic therapy is all that is necessary (see Chapter 16).

Laryngotracheitis

Management of the laryngotracheitis caused by measles virus infection is similar to that in other patients with croup caused by other viral etiologic agents. Administration of corticosteroids is contraindicated in measles, and antibiotics are indicated only in patients with laboratory or clinical evidence of secondary bacterial infection (see Chapter 18).

Pneumonia

Pneumonia is a common complication of measles, and it is the leading cause of death. Pneumonia may be a manifestation of primary viral infection, or it may result from a superimposed bacterial infection. The differential diagnosis between primary viral and superimposed bacterial disease cannot be made with certainty. Because the diagnosis of viral pneumonia often is uncertain, most patients should be treated with antibiotics (see Chapters 22 and 24). In primary viral pneumonia, treatment with aerosolized ribavirin should be considered.

In one uncontrolled study in adult patients, intravenous ribavirin was found to be well tolerated, and its use was associated with clinical improvement.[143] In a study in pregnant women, Atmar and associates[24] were unable to demonstrate clear clinical benefit with the administration of aerosolized ribavirin.

Encephalitis

The course of measles encephalitis is unpredictable, and treatment is symptomatic and supportive. Trained nursing care is essential. Careful attention given to fluid and electrolyte balance is necessary. In prolonged states of coma, parenteral hyperalimentation is indicated. Status epilepticus should be treated vigorously with the use of a structured protocol for ensuring optimal control (see Chapter 36).

Numerous review articles indicate that measles encephalitis is a postinfectious encephalitis and is predominantly a disease of white matter (acute disseminated encephalomyelitis [ADEM]).[178,223,224] This concept resulted from a study of 19 patients with measles encephalitis that was published in 1984 by Johnson and associates.[225] I am aware of only two patients subsequent to this article who had ADEM associated with measles virus infection. Clinical evidence supports the finding that ADEM not related to measles virus infection is responsive to corticosteroid therapy.[140,290]

Considerable evidence indicates that measles encephalitis usually is not ADEM and that active direct CNS infection is involved in the process.[130,141,291,361,429] A controlled trial of 32 children with measles encephalitis failed to find benefit in the corticosteroid-treated group.[472] These data suggest to me that corticosteroids should not be used to treat measles encephalitis unless evidence of ADEM is clear and active measles virus infection has subsided. In patients with severe intractable seizures or other evidence of cerebral edema, the use of intravenous mannitol therapy (0.25 to 1 g/kg of a 20% solution administered over the course of a 30- to 60-minute period) is indicated. In occasional circumstances, respiratory arrest is a problem, and artificial ventilators should be used to tide patients over until respiration becomes normal. Mustafa and coworkers[319] noted improvement in an immunocompromised child with subacute measles encephalitis who was treated with intravenously administered ribavirin.

Appendicitis

Acute abdominal pain occurs occasionally in primary measles, and it can be caused by generalized mesenteric adenitis secondary to measles virus appendicitis. In appendicitis, evidence of measles virus involvement of the appendix is present. However, therapy should be similar to that in other cases of appendicitis; removal of the appendix is indicated because measles appendicitis perforates with a frequency equal to that in non–measles virus infection.

Prophylactic Antibiotics

In the developing world, secondary bacterial infections in measles are a major cause of mortality. Accordingly, interest has arisen in the use of antibiotics

prophylactically.[83,402] A meta-analysis in 1997 of studies involving the use of prophylactic antibiotics noted that the available data were poor and provided only weak evidence for giving antibiotics to all children with measles.[402] The recommendation from this study was that antibiotics should be given only if a child has clinical signs of pneumonia or other evidence of sepsis. Chalmers[83] pointed out the necessity and urgency for performing controlled prophylactic trials.

PREVENTION

Active Immunization: Live Attenuated Measles Virus Vaccine

The original attenuated measles vaccine was developed from the Edmonston strain of measles virus by passage in human kidney and human amnion tissue cultures, then in chicken embryos, and finally in chicken embryo fibroblast tissue culture.[420] Today in the United States, measles vaccines are prepared in chicken embryo fibroblast tissue culture. Vaccines available in other countries are prepared in chicken embryo fibroblast tissue culture, Japanese quail tissue culture, chicken embryo, and WI-38 tissue culture. Vaccination produces a mild or inapparent noncommunicable infection that induces active immunity in more than 95 percent of recipients. Vaccine-induced antibodies persist for many years, and although reinfection with illness has been noted on occasion in apparently successfully immunized children, waning immunity does not appear to be of significant epidemiologic importance.[15,91,93,114,253] Symptoms associated with measles immunization are minimal and are limited to fever, mild malaise, and occasionally a faint rash occurring approximately 1 week after immunization. For complete information and recommendations related to measles immunization, the reader is referred to Chapter 244 and the most recent recommendations of the Advisory Committee on Immunization Practices,[23] the CDC "Pink Book,"[80] the Committee on Infectious Diseases of the American Academy of Pediatrics,[12] and the manufacturer's package insert. The vaccines in routine use in the United States today are measles-mumps-rubella (MMR) or measles-mumps-rubella-varicella (MMRV). Only a summary of recommendations is presented here.

Recommendations for Use

The widespread epidemics of measles in the United States in 1989 to 1991 were the result of a failure to immunize children at the appropriate age and the increased number of susceptible older children and adults because of vaccine failure.[22,72,73,264,283] Therefore, continued eradication of measles in the United States depends on ongoing programs that (1) enroll all children of initial vaccination age and (2) allow revaccination of all persons whose primary vaccine failed. Because routine immunity testing is not a viable public health option, revaccination of primary vaccine failures can be accomplished only by universal reimmunization.

Currently, live measles vaccine is recommended in a two-dose schedule.[12,72,450] In general, live measles vaccine should be administered at 12 to 15 months of age and the second dose at entry into school.* However, the second dose can be given after any interval longer than 1 month after administration of the first dose.[23] Children who have not received vaccine during infancy may be immunized at any age, and adults who have not had natural measles also should be immunized. When measles is endemic or epidemic in a community, all children aged 6 months or older should be immunized. In children who initially were vaccinated before they were 12 months of age, a second vaccination should be administered at 12 to 15 months of age, and a third dose at entry into school is necessary to complete the schedule.

Precautions

Measles vaccination should be deferred at times of febrile illness or when interference from another viral infection could cause failure of measles vaccine. Measles immunization also should be postponed for 3 to 11 months in persons who have received whole blood, blood plasma, or immunoglobulin because these products may contain sufficient measles antibody to neutralize the vaccine virus. The duration of postponement depends on the product administered and the dose.[12,23] Children treated with intravenous immunoglobulin for Kawasaki disease should not receive measles vaccine until 11 months after receiving the immunoglobulin, whereas 3 months' time is adequate for children given immunoglobulin for hepatitis A prophylaxis.

Contraindications

Live measles vaccine should not be administered to pregnant women or to some persons with diseases or therapeutic programs associated with impaired cell-mediated immunity. These conditions in general include the following: leukemia, lymphoma, or other generalized malignant diseases; primary and secondary immunologic disorders; and therapy with corticosteroids, radiation, antimetabolites, or alkylating agents. Measles immunization is recommended for children and adults with asymptomatic human immunodeficiency infection and for symptomatic patients who are not severely immunocompromised.[12,14]

Complications

Serious complications associated with administration of measles vaccine are exceedingly rare. Serious neurologic disease (encephalitis, Reye syndrome, cranial nerve palsy, cerebellar ataxia, and Guillain-Barré syndrome) within 30 days of immunization occurs at a rate of approximately one case per million doses of vaccine administered.[66,261] This rate is lower than the rate of occurrence of encephalitis of unknown cause in children for any 30-day period. However, the clustering of cases on days 8 and 9 after immunization noted from claims submitted to the

*References 9, 12, 23, 70, 251, 403, 450, 457, 468.

National Vaccine Injury Compensation Program suggests that a causal relationship between measles vaccine and encephalopathy may be a rare complication of immunization.[451] In addition, recovery of measles virus from the CSF of a vaccinated child with encephalitis also suggests that rare vaccine-induced neurologic disease may occur.[141] In a large population-based study in Denmark between 1991 and 1998 it was found that MMR immunization was associated with a transient increased rate of febrile seizures.[442] However, the long-term rate of epilepsy was not increased in children who had febrile seizures after receiving vaccination compared with children who had febrile seizures related to other causative events. Studies in the United States also noted an increased risk of febrile seizures during the first 2 weeks after the first dose of MMR.[280] Further study noted that the risk of febrile seizures was significantly greater after MMRV than after MMR. In a recent study, Klein and associates[240] found no increased risk of febrile seizures after the second dose of measles-containing vaccines in children 4 to 6 years of age. Thrombocytopenic purpura, anaphylaxis, hearing loss, and toxic epidermal necrolysis also have been associated with measles immunization.*

In developing countries, high-titer measles vaccines were used to induce seroconversion at a young age.[462] However, follow-up studies in some countries noted that the mortality rate was increased in female recipients of these high-titer vaccines over the course of a 3-year period when compared with children who received conventional doses of vaccine.[202,241] Nonetheless, follow-up studies in other countries showed no increased mortality rates in recipients of high-titer measles vaccine.[3,265]

Global Progress toward Measles Eradication

Measles has been a major cause of death in the developing world, with 750,000 deaths in 2000.[77] A World Health Organization (WHO) global immunization vision meeting in 2005 established a commitment to achieve a 90 percent reduction in measles mortality by 2010 compared with 2000.[463] This commitment focused on 47 priority countries in the developing world. The components of the strategy included achieving and maintaining high coverage (>90%) with routinely scheduled first dose of measles-containing vaccine among children aged 1 year and ensuring that all children receive a second opportunity for measles immunization. In 2007, 82 percent of the target population was vaccinated but the coverage varied substantially by geographic region. During the period 2000 through 2007, the global measles mortality was reduced by 74 percent (from 750,000 deaths in 2000 to 197,000 deaths in 2007).

Endemic measles has been eliminated in North America, Sweden, Finland, and selected other countries in the world. In the WHO European Region (EUR), substantial progress was made between 2003 and 2009 in eliminating measles.[78] However, since late 2009, measles outbreaks have become widespread. In 2010, 30,639 measles cases were reported in the EUR and up to October 26, 2011, an additional 26,074 cases were reported. The majority of the cases occurred in the western European subregion.

In 2011 in the EUR, 49.4 percent of the cases occurred in persons 15 years of age or older, 25 percent occurred in children younger than age 5 years, and 24.7 percent occurred in children 5 to 14 years of age.[78] Of the reported cases in 2011, 54 percent occurred in France and 11 percent in Spain. Nine deaths occurred (six in France), and seven of these occurred in persons older than 10 years. In 2011, 90 percent of the cases occurred in unvaccinated persons or in persons with an unknown vaccination status.

In the EUR, the overall coverage with at least one dose of a measles-containing vaccine is below the target of 95 percent.[78] This is a particular problem in the western European subregion.

There are multiple reasons for the underuse of vaccines in the EUR.[78,317] In contrast to North America, compulsory school immunization laws do not exist in Western Europe. Some degree of herd immunity has allowed unvaccinated children to become susceptible adolescents and adults. Important and serious barriers to increasing population immunity in the EUR and, in particular, the western European subregion, are religious or philosophical objections to vaccination.[317]

Quarantine and Disease Containment

Before the use of measles vaccines became widespread, quarantine measures were practiced widely but were largely ineffective in preventing the spread of measles. However, containment of disease is practical today because the widespread use of measles vaccine has reduced the general number of susceptible young children and thus has decreased the rapidity of epidemic development. Epidemic measles now generally involves a greater age range of the population (cases in adolescents and young adults occur frequently), and progression of disease from one age group to another is slower than in epidemics that involve one uniform, largely susceptible population.

Containment is a vital part of the measles prevention policy in the United States. Measles is a reportable disease throughout the United States, and compliance is the obligation of all physicians. After receiving early reports of sporadic measles, health department workers can organize local immunization clinics so that the disease often can be contained in a small geographic area rather than developing into a widespread epidemic.

Passive Immunization: Immunoglobulin

In the present vaccine era, little need for passive immunization exists. However, when a known susceptible child has had definite exposure to measles, immunoglobulin should be administered in a dose of 0.25 mg/kg (maximal dose, 15 mL). If this treatment is administered within 5 days of exposure, prevention of infection and disease can be expected. Administration of immunoglobulin later in the incubation period may modify the illness but does not prevent it. The use of immunoglobulin is particularly important in children who have not been immunized

*References 11, 27, 32, 218, 394, 404, 419, 456.

because of the contraindications to vaccination mentioned previously. In these children, immunoglobulin (0.5 mL/kg; maximal dose, 15 mL) should be administered when measles is epidemic in the community in which they reside. The dosage should be repeated every 4 weeks until the epidemic subsides. Intravenous immunoglobulin in a dose of 400 mg/kg may be used (also see Chapter 245).

NEW REFERENCES SINCE THE SIXTH EDITION

31. Becker D, Patel A, Abou-Khalil BW, et al. Successful treatment of encephalopathy and myoclonus with levetiracetam in a case of subacute sclerosing panencephalitis. *J Child Neurol* 2009;**24**:763–7.
77. Centers for Disease Control and Prevention. Progress in global measles control and mortality reduction, 2000-2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:1303–6.
78. Centers for Disease Control and Prevention. Increased transmission and outbreaks of measles—European region, 2011. *M M W R Morb Mortal Wkly Rep* 2011;**60**:1605–10.
79. Centers for Disease Control and Prevention. False-positive measles test—Maine, February 2012. *M M W R Morb Mortal Wkly Rep* 2012;**61**:396.
80. Centers for Disease Control and Prevention. Measles. In: Atkinson W, Wolfe S, Hamborsky J, editors. *Epidemiology and prevention of vaccine-preventable diseases*. 12th ed. Washington, D.C.: Public Health Foundation; 2012.
81. Centers for Disease Control and Prevention. Measles—United States, 2012. *M M W R Morb Mortal Wkly Rep* 2012;**61**:253–8.
82. Centers for Disease Control and Prevention. Progress in global measles control, 2009-2010. *M M W R Morb Mortal Wkly Rep* 2012;**61**:73–8.
162. Gershon AA. Chickenpox, measles, and mumps. In: Remington JS, Klein JO, Wilson CB, et al, editors. *Infectious diseases of the fetus and newborn infant*. 7th ed. Philadelphia: Saunders; 2011. p. 685–95.
170. Goraya J, Marks H, Khurana D, et al. Subacute sclerosing panencephalitis (SSPE) presenting as acute disseminated encephalomyelitis in a child. *J Child Neurol* 2009;**24**:899–903.
194. Haralambieva IH, Ovsyannikova IG, Kennedy RB, et al. Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. *Vaccine* 2011;**29**:7883–95.
216. Jacobson RM, Ovsyannikova IG, Vierkant RA, et al. Human leukocyte antigen associations with humoral and cellular immunity following a second dose of measles-containing vaccine: persistence, dampening, and extinction of associations found after a first dose. *Vaccine* 2011;**29**:7982–91.
240. Klein NP, Lewis E, Baxter R, et al. Measles-containing vaccines and febrile seizures in children age 4 to 6 years. *Pediatrics* 2012;**129**:809–14.
274. Mahadevan A, Vaidya SR, Wairagkar NS, et al. Case of fulminant-SSPE associated with measles genotype D7 from India: an autopsy study. *Neuropathology* 2008;**28**:621–6.
280. Marin M, Broder KR, Temte JL, et al; Centers for Disease Control and Prevention (CDC). Use of combination measles, mumps, rubella, and varicella vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-3):1–12.
317. Muscat M. Who gets measles in Europe? *J Infect Dis* 2011;**204**(Suppl 1):S353–65.
420. Strebel PM, Papania MJ, Dayan GH, et al. Measles vaccine. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders; 2008.
467. Yanagi Y, Takeda M, Ohno S, et al. Measles virus receptors. *Curr Top Microbiol Immunol* 2009;**329**:13–30.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 180

MUMPS VIRUS

James D. Cherry • Kevin K. Quinn

Mumps (epidemic parotitis) is an acute communicable disease caused by the mumps virus, a member of the genus *Rubulavirus*. As a result of universal immunization, mumps was an uncommon disease in children in the United States at the end of the 20th century; however, a 2006 epidemic and subsequent outbreaks indicate the need for renewed study of mumps. In addition, mumps continues to be an epidemic problem worldwide.

HISTORY

In the fifth century BCE, Hippocrates described an outbreak on the island of Thasos.[99,188] He noted that most patients had bilateral swelling near the ears and that the others had unilateral swelling. He also noted that some patients had bilateral or unilateral pain and swelling of the testes.

The origin of the name mumps is not known. It may be from the English noun *mump*, which means "a lump," or the English verb *mump*, one of whose definitions is "mumble." This latter possible origin is based on the mumbling speech that patients with significant parotitis may have.

In 1790, Robert Hamilton[74,75] presented an extensive study of mumps in which he noted orchitis, associated the illness with neurologic involvement, and described the neuropathology of a fatal case. In 1886, Hirsch[85]

REFERENCES

1. Aaby P. Malnutrition and overcrowding/intensive exposure in severe measles infection: review of community studies. *Rev Infect Dis* 1988;**10**:478–91.
2. Aaby P. Patterns of exposure and severity of measles infection: Copenhagen 1915-1925. *Ann Epidemiol* 1992;**2**:257–62.
3. Aaby P, Bukh J, Lisse IM, et al. Overcrowding and intensive exposure as determinants of measles mortality. *Am J Epidemiol* 1984;**120**:49–63.
4. Aaby P, Samb B, Simondon F, et al. A comparison of vaccine efficacy and mortality during routine use of high-titre Edmonston-Zagreb and Schwarz standard measles vaccines in rural Senegal. *Trans R Soc Trop Med Hyg* 1996;**90**:326–30.
5. Aarli JA. Nervous complications of measles: clinical manifestations and prognosis. *Eur Neurol* 1974;**12**:79–93.
6. Abramson O, Dagan R, Tal A, et al. Severe complications of measles requiring intensive care in infants and young children. *Arch Pediatr Adolesc Med* 1995;**149**:1237–40.
7. Adams JM, Baird C, Filloy L. Inclusion bodies in measles encephalitis. *JAMA* 1966;**195**:290–8.
8. Aicardi J, Goutieres F, Arsenio-Nunes ML, et al. Acute measles encephalitis in children with immunosuppression. *Pediatrics* 1977;**59**:232–9.
9. Albrecht P, Ennis FA, Saltzman EJ, et al. Persistence of maternal antibody in infants beyond 12 months: mechanism of measles vaccine failure. *J Pediatr* 1977;**91**:715–18.
10. Alpert G, Leibovitz L, Danon YL. Analysis of T-lymphocyte subsets in measles. *J Infect Dis* 1984;**149**:1018.
11. Alter HJ, Scanlon RT, Schechter GP. Thrombocytopenic purpura following vaccination with attenuated measles virus. *Am J Dis Child* 1968;**115**:111–13.
12. American Academy of Pediatrics. Measles. In: Pickering LK, editor. *2006 Red book: report of the Committee on Infectious Diseases*. 25th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2000. p. 385–96.
13. American Academy of Pediatrics, Committee on Infectious Diseases. Vitamin A treatment of measles. *Pediatrics* 1993;**91**:1014–15.
14. American Academy of Pediatrics, Committee on Infectious Diseases and Committee on Pediatric AIDS. Measles immunization in HIV-infected children. *Pediatrics* 1999;**103**:1057–60.
15. Anders JF, Jacobson RM, Poland GA, et al. Secondary failure rates of measles vaccines: a metaanalysis of published studies. *Pediatr Infect Dis J* 1996;**15**:62–6.
16. Anderson R, Rabson AR, Sher R, et al. Defective neutrophil motility in children with measles. *J Pediatr* 1976;**89**:27–32.
17. Anlar B, Aydin OF, Guven A, et al. Retrospective evaluation of interferon-beta treatment in subacute sclerosing panencephalitis. *Clin Ther* 2004;**26**:1890–4.
18. Annunziato D, Kaplan MH, Hall WW, et al. Atypical measles syndrome: pathologic and serologic findings. *Pediatrics* 1982;**70**:203–9.
19. Arneborn P, Biberfeld G. T-lymphocyte subpopulations in relation to immunosuppression in measles and varicella. *Infect Immun* 1983;**39**:29–37.
20. Arrieta AC, Zaleska M, Stutman HR, et al. Vitamin A levels in children with measles in Long Beach, California. *J Pediatr* 1992;**121**:75–8.
21. Atabani SF, Byrnes AA, Jaye A, et al. Natural measles causes prolonged suppression of interleukin-12 production. *J Infect Dis* 2001;**184**:1–9.
22. Atkinson WL, Hadler SC, Redd SB, et al. Measles surveillance—United States, 1991. *MMWR CDC Surveill Summ* 1992;**41**:1–12.
23. Atkinson WL, Pickering LK, Schwartz B, et al. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP). *MMWR Recomm Rep* 2002;**51**(RR-2):1–35.
24. Atmar RL, Englund JA, Hammill H. Complications of measles during pregnancy. *Clin Infect Dis* 1992;**14**:217–26.
25. Azizi A, Krakovsky D. Keratoconjunctivitis as a constant sign of measles. *Ann Paediatr* 1965;**204**:397–405.
26. Babbott FL Jr, Gordon JE. Modern measles. *Am J Med Sci* 1954;**228**:334–61.
27. Bachand AJ, Rubenstein J, Morrison AN. Thrombocytopenic purpura following live measles vaccine. *Am J Dis Child* 1967;**113**:283–5.
28. Baczko K, Pardowitz I, Rima BK, et al. Constant and variable regions of measles virus proteins encoded by the nucleocapsid and phosphoprotein genes derived from lytic and persistent viruses. *Virology* 1992;**190**:469–74.
29. Barclay AJG, Foster A, Sommer A. Vitamin A supplements and mortality related to measles: a randomised clinical trial. *BMJ* 1987;**294**:294–6.
30. Barsegar B, Hofmann H, Zweymuller E. The diagnosis of measles in the newborn and infant age groups. *Z Kinderheilkd* 1972;**113**:175–84.
31. Becker D, Patel A, Abou-Khalil BW, et al. Successful treatment of encephalopathy and myoclonus with levetiracetam in a case of subacute sclerosing panencephalitis. *J Child Neurol* 2009;**24**:763–7.
32. Beeler J, Varricchio F, Wise R. Thrombocytopenia after immunization with measles vaccines: review of the vaccine adverse events reporting system (1990 to 1994). *Pediatr Infect Dis J* 1996;**15**:88–90.
33. Beersma MF, Galama JM, Van Druten HA, et al. Subacute sclerosing panencephalitis in The Netherlands—1976-1990. *Int J Epidemiol* 1992;**21**:583–8.
34. Bellanti JA, Sanga RL, Klutinis B, et al. Antibody responses in serum and nasal secretions of children immunized with inactivated and attenuated measles-virus vaccines. *N Engl J Med* 1969;**280**:628–33.
35. Bellini WJ, Rota JS, Lowe LE, et al. Subacute sclerosing panencephalitis: more cases of this fatal disease are prevented by measles immunization than was previously recognized. *J Infect Dis* 2005;**192**:1686–93.
36. Bellini WJ, Rota JS, Rota PA. Virology of measles virus. *J Infect Dis* 1994;**170**(Suppl 1):S15–23.
37. Benjamin B, Ward SM. Leukocytic response to measles. *Arch Pediatr Adolesc Med* 1932;**44**:921–63.
38. Berkovich S, Schneck L. Ascending paralysis associated with measles. *J Pediatr* 1964;**64**:88–93.
39. Bhettay E, Kipps A, McDonald R. Early onset of subacute sclerosing panencephalitis. *J Pediatr* 1976;**89**:271–2.
40. Billeter MA, Cattaneo R, Spielhofer P, et al. Generation and properties of measles virus mutations typically associated with subacute sclerosing panencephalitis. *Ann N Y Acad Sci* 1994;**724**:367–77.
41. Black FL. Growth and stability of measles virus. *Virology* 1959;**7**:184–92.
42. Black FL. Discussion of paper by Karelitz, S.: Measles vaccine and immunity. *N Y J Med* 1963;**63**:519–28.
43. Black FL. A nationwide serum survey of U.S. military recruits, 1962, part III: measles and mumps antibodies. *Am J Hyg* 1964;**80**:304–7.
44. Black FL, Reissig M, Melnick JL. Measles virus. *Adv Virus Res* 1959;**6**:205–27.
45. Black FL, Sheridan SR. Studies on an attenuated measles-virus vaccine, part IV: administration of vaccine by several routes. *N Engl J Med* 1960;**263**:165–9.
46. Black FL, Sheridan SR. Blood leukocyte response to live measles vaccine. *Am J Dis Child* 1967;**113**:301–4.
47. Bloch AB, Orenstein WA, Ewing WM, et al. Measles outbreak in a pediatric practice: airborne transmission in an office setting. *Pediatrics* 1985;**75**:676–83.
48. Bloch AB, Orenstein WA, Stetler HC, et al. Health impact of measles vaccination in the United States. *Pediatrics* 1985;**76**:524–32.
49. Bodechtel G, Guttmann E. Diffuse Encephalitis mit sklerosierender Entzurdurg des Hemispharemarkes. *Z Gesamte Neurol Psychiatr* 1931;**133**:601–10.
50. Bouteille M, Fontaine C, Vedrenne C, et al. Sur un cas d'encephalite subaigue à l'inclusions étude anatomoclinique et ultrastructurale. *Rev Neurol (Paris)* 1965;**113**:454–74.
51. Brem J. Koplik spots for the record: an illustrated historical note. *Clin Pediatr* 1972;**11**:161–3.
52. Britt WJ. Slow viruses. In: Feigin RD, Cherry JD, editors. *Textbook of pediatric infectious diseases*. 4th ed. Philadelphia: W. B. Saunders; 1998. p. 1646–65.

53. Brodsky AL. Atypical measles. Severe illness in recipients of killed measles virus vaccine upon exposure to natural infection. *JAMA* 1972;**222**:1415–16.
54. Buckland R, Wild TF. Is CD46 the cellular receptor for measles virus? *Virus Res* 1997;**48**:1–9.
55. Burnstein T, Frankel JW, Jensen JH. Adaptation of measles virus to suckling hamsters. *Fed Proc* 1958;**17**:507.
56. Bussell RH, Karzon DT. Measles–canine distemper–rinderpest group. In: Prier JE, editor. *Basic medical virology*. Baltimore: Williams & Wilkins; 1966. p. 313–36.
57. Butler JC, Havens PL, Sowell AL, et al. Measles severity and serum retinol (vitamin A) concentration among children in the U.S. *Pediatrics* 1993;**91**:1176–81.
58. Canal N, Torck P. An epidemiological study of sub-acute sclerosing leucoencephalitis in Belgium. *J Neurol Sci* 1964;**64**:380–9.
59. Canelas HM, Juliano OF, Lefevre AB, et al. Subacute sclerosing leucoencephalitis: an epidemiological, clinical, and biochemical study of 31 cases. *Arq Neuropsiquiatr* 1967;**25**:255–68.
60. Carlstrom G. Relation of measles to other viruses. *Am J Dis Child* 1962;**103**:287–91.
61. Carney J, Stiehm ER, Cherry JD, et al. Unpublished data. 1978.
62. Cattaneo R, Billeter MA. Mutations and A/I hypermutations in measles virus persistent infections. *Curr Top Microbiol Immunol* 1992;**176**:63–74.
63. Cattaneo R, Schmid A, Billeter MA, et al. Multiple viral mutations rather than host factors cause defective measles virus gene expression in a subacute sclerosing panencephalitis cell line. *J Virol* 1988;**62**:1388–97.
64. Cattaneo R, Schmid A, Eschle D, et al. Biased hypermutation and other genetic changes in defective measles viruses in human brain infections. *Cell* 1988;**55**:255–65.
65. Caulfield E. Early measles epidemics in America. *Yale J Biol Med* 1943;**15**:531–56.
66. Centers for Disease Control and Prevention. *Measles surveillance report No. 10, 1973-1976*. Atlanta: Centers for Disease Control; 1977.
67. Centers for Disease Control and Prevention. Measles prevention. *M M W R Morb Mortal Wkly Rep* 1978;**27**:427–37.
68. Centers for Disease Control and Prevention. Measles encephalitis: United States, 1962-1979. *M M W R Morb Mortal Wkly Rep* 1981;**30**:362–4.
69. Centers for Disease Control and Prevention. Recommendations of the Immunization Practices Advisory Committee (ACIP): Measles prevention. *M M W R Morb Mortal Wkly Rep* 1982;**31**:217–24, 229–31.
70. Centers for Disease Control and Prevention. *Measles surveillance, 1977-1981*. Atlanta: Centers for Disease Control; 1982.
71. Centers for Disease Control and Prevention. Subacute sclerosing panencephalitis surveillance: United States. *M M W R Morb Mortal Wkly Rep* 1982;**31**:585–588.
72. Centers for Disease Control and Prevention. Measles prevention. *M M W R Morb Mortal Wkly Rep* 1989;**38**:1–18.
73. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 1994. *M M W R Morb Mortal Wkly Rep* 1995;**43**:1–80.
74. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 1998. *M M W R Morb Mortal Wkly Rep* 1999;**47**:ii–92.
75. Centers for Disease Control and Prevention. Measles: United States, 2000. *M M W R Morb Mortal Wkly Rep* 2002;**51**:120–3.
76. Centers for Disease Control and Prevention. Measles: United States, 2005. *M M W R Morb Mortal Wkly Rep* 2006;**55**: 1348–51.
77. Centers for Disease Control and Prevention. Progress in global measles control and mortality reduction, 2000-2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:1303–6.
78. Centers for Disease Control and Prevention. Increased transmission and outbreaks of measles—European Region, 2011. *M M W R Morb Mortal Wkly Rep* 2011;**60**:1605–10.
79. Centers for Disease Control and Prevention. False-positive measles test—Maine, February 2012. *M M W R Morb Mortal Wkly Rep* 2012;**61**:396.
80. Centers for Disease Control and Prevention. Measles. In: Atkinson W, Wolfe S, Hamborsky J, editors. *Epidemiology and prevention of vaccine-preventable diseases*. 12th ed. Washington, D.C.: Public Health Foundation; 2012.
81. Centers for Disease Control and Prevention. Measles—United States, 2012. *M M W R Morb Mortal Wkly Rep* 2012;**61**:253–8.
82. Centers for Disease Control and Prevention. Progress in global measles control, 2009-2010. *M M W R Morb Mortal Wkly Rep* 2012;**61**:73–8.
83. Chalmers I. Why we need to know whether prophylactic antibiotics can reduce measles-related morbidity. *Pediatrics* 2002;**109**:312–15.
84. Charlesworth JA, Pussell BA, Roy LP, et al. Measles infection: involvement of the complement system. *Clin Exp Immunol* 1976; **24**:401–6.
85. Chatterji M, Mankad V. Failure of attenuated viral vaccine in prevention of atypical measles. *JAMA* 1977;**238**:2635.
86. Chen RT, Markowitz LE, Albrecht P, et al. Measles antibody: reevaluation of protective titers. *J Infect Dis* 1990;**162**:1036–42.
87. Cherry JD. Lesions of the lips and mouth. In: Gellis SS, Kagan BM, editors. *Current pediatric therapy*, vol. 3. Philadelphia: W. B. Saunders; 1968. p. 257–60.
88. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**:233–86.
89. Cherry JD. Personal observations. 1978.
90. Cherry JD, Feigin RD, Lobes LA Jr, et al. Unpublished data. 1971.
91. Cherry JD, Feigin RD, Lobes LA Jr, et al. Urban measles in the vaccine era: a clinical, epidemiologic, and serologic study. *J Pediatr* 1972;**81**:217–30.
92. Cherry JD, Feigin RD, Lobes LA Jr, et al. Atypical measles in children previously immunized with attenuated measles virus vaccines. *Pediatrics* 1972;**50**:712–17.
93. Cherry JD, Feigin RD, Shackelford PG, et al. A clinical and serologic study of 103 children with measles vaccine failure. *J Pediatr* 1973;**82**:802–8.
94. Choppin PW, Richardson CD, Merz DC, et al. The functions and inhibition of the membrane glycoproteins of paramyxoviruses and myxoviruses and the role of the measles virus M protein in subacute sclerosing panencephalitis. *J Infect Dis* 1981;**143**:352–63.
95. Christensen PE, Schmidt H, Bang HO, et al. An epidemic of measles in southern Greenland, 1951; measles in virgin soil, part II: the epidemic proper. *Acta Med Scand* 1953;**144**:430–49.
96. Cianchetti C, Marrosu MG, Manconi PE, et al. Subacute sclerosing panencephalitis in only one of identical twins: case report with study of cell-mediated immunity. *Eur Neurol* 1983;**22**:428–32.
97. Cobb W, Hill D. EEG in subacute progressive encephalitis. *Brain* 1950;**73**:392–404.
98. Cobban K. Measles in Nigerian children. *West Afr Med J* 1963; **12**:18–23.
99. Conley SF, Beste DJ, Hoffmann RG. Measles-associated bacterial tracheitis. *Pediatr Infect Dis J* 1993;**12**:414–15.
100. Connolly JH, Allen IV, Hurwitz LJ, et al. Measles-virus antibody and antigen in subacute sclerosing panencephalitis. *Lancet* 1967;**1**: 542–4.
101. Conrad JL, Wallace R, Witte JJ. The epidemiologic rationale for the failure to eradicate measles in the United States. *Am J Public Health* 1971;**61**:2304–10.
102. Coovadia HM, Parent MA, Loening WE, et al. An evaluation of factors associated with the depression of immunity in malnutrition and in measles. *Am J Clin Nutr* 1974;**27**:665–9.
103. Coovadia HM, Wesley A, Brain P, et al. Immunoparesis and outcome in measles. *Lancet* 1977;**1**:619–21.
104. Coovadia HM, Wesley A, Henderson LG, et al. Alterations in immune responsiveness in acute measles and chronic post-measles chest disease. *Int Arch Allergy Appl Immunol* 1978;**56**:14–23.
105. Coutsoudis A, Broughton M, Coovadia HM. Vitamin A supplementation reduces measles morbidity in young African children: a randomized, placebo-controlled, double-blind trial. *Am J Clin Nutr* 1991;**54**:890–5.
106. Coutsoudis A, Kiepiela P, Coovadia HM, et al. Vitamin A supplementation enhances specific IgG antibody levels and total lymphocyte numbers while improving morbidity in measles. *Pediatr Infect Dis J* 1992;**11**:203–9.
107. Cremer NE, Cossen CK, Shell G, et al. Enzyme immunoassay versus plaque neutralization and other methods for determination of immune status to measles and varicella-zoster viruses and versus complement fixation for serodiagnosis of infections with those viruses. *J Clin Microbiol* 1985;**21**:869–74.
108. Dagan R, Phillip M, Sarov I, et al. Cellular immunity and T-lymphocyte subsets in young children with acute measles. *J Med Virol* 1987;**22**:175–82.

109. Dawson JR. Cellular inclusions in cerebral lesions of lethargic encephalitis. *Am J Pathol* 1933;**9**:7–16, 13.
110. de Moraes N. Medical importance of measles in Brazil. *Am J Dis Child* 1962;**103**:233–6.
111. De Swart RL, Yüksel S, Osterhaus ADME. Relative contributions of measles virus hemagglutinin- and fusion protein–specific serum antibodies to virus neutralization. *J Virol* 2005;**79**:11547–51.
112. Defanti CA, Franza A, D'Angelo, et al. SSPE: Clinical, EEG and neuroradiological findings in a series of 69 cases. In: Bergamini F, Defanti CA, Ferrante P, editors. *Subacute sclerosing panencephalitis: a reappraisal*. Amsterdam: Elsevier; 1986. p. 121–31.
113. Degen JA Jr. Visceral pathology in measles: a clinicopathologic study of 100 fatal cases. *Am J Med Sci* 1937;**194**:104–11.
114. Deseda-Tous J, Cherry JD, Spencer MJ, et al. Measles revaccination: persistence and degree of antibody titer by type of immune response. *Am J Dis Child* 1978;**132**:287–90.
115. Deseda-Tous JE, Spencer MJ, Cherry JD, et al. *Measles antibody in healthy adults analyzed by HLA and ABO blood types*. Abstract. Paper presented at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, 1976.
116. Detels R, Brody JA, McNew J, et al. Further epidemiological studies of subacute sclerosing panencephalitis. *Lancet* 1973;**2**: 11–14.
117. Dhib-Jalbut S, Haddad FS. Subacute sclerosing panencephalitis in one member of identical twins. *Neuropediatrics* 1984;**15**:49–51.
118. Dhiman N, Jacobson RM, Poland GA. Measles virus receptors: SLAM and CD46. *Rev Med Virol* 2004;**14**:217–29.
119. Dhiman N, Ovsyannikova IG, Ryan JE, et al. Correlations among measles virus-specific antibody, lymphoproliferation and Th1/ Th2 cytokine responses following measles-mumps-rubella-II (MMR-II) vaccination. *Clin Exp Immunol* 2005;**142**:498–504.
120. Dorig RE, Marcil A, Chopra A, et al. The human CD46 molecule is a receptor for measles virus (Edmonston strain). *Cell* 1993;**75**: 295–305.
121. Douglas JW. Ability and adjustment of children who have had measles. *BMJ* 1964;**2**:1301–3.
122. Duda EE, Huttenlocher PR, Patronas NJ. CT of subacute sclerosing panencephalitis. *AJNR Am J Neuroradiol* 1980;**1**:35–8.
123. Dunn RA. Subacute sclerosing panencephalitis. *Pediatr Infect Dis J* 1991;**10**:68–72.
124. DuRant RH, Dyken PR, Swift AV. The influence of inosiplex treatment on the neurological disability of patients with subacute sclerosing panencephalitis. *J Pediatr* 1982;**101**:288–93.
125. Eberhart-Phillips JE, Frederick PD, Baron RC, et al. Measles in pregnancy: a descriptive study of 58 cases. *Obstet Gynecol* 1993;**82**: 797–801.
126. Edmonson MB, Addiss DG, McPherson JT, et al. Mild measles and secondary vaccine failure during a sustained outbreak in a highly vaccinated population. *JAMA* 1990;**263**:2467–71.
127. Ehrengut W. Measles encephalitis: age disposition and vaccination. *Arch Gesamte Virusforsch* 1965;**16**:311–14.
128. Enders JF, McCarthy K, Mitus A, et al. Isolation of measles virus at autopsy in cases of giant-cell pneumonia without rash. *N Engl J Med* 1959;**261**:875–81.
129. Enders JF, Peebles TC. Propagation in tissue cultures of cytopathogenic agents from patients with measles. *Proc Soc Exp Biol Med* 1954;**86**:277–86.
130. Esolen LM, Takahashi K, Johnson RT, et al. Brain endothelial cell infection in children with acute fatal measles. *J Clin Invest* 1995;**96**:2478–81.
131. Evans AS, editor. *Viral infections of humans: epidemiology and control*. 3rd ed. New York: Plenum Medical; 1989. p. 451–69.
132. Fenner F. The pathogenesis of the acute exanthems; an interpretation based on experimental investigations with mousepox; infectious ectromelia of mice. *Lancet* 1948;**2**:915–20.
133. Fenner F. Classification and nomenclature of viruses: second report of the International Committee on Taxonomy of Viruses. *Intervirology* 1976;**7**:1–115.
134. Field CE. Bronchiectasis in childhood; clinical survey of 160 cases. *Pediatrics* 1949;**4**:21–46.
135. Finkel HE. Measles myocarditis. *Am Heart J* 1964;**67**:679–83.
136. Fireman P, Friday G, Kumate J. Effect of measles vaccine on immunologic responsiveness. *Pediatrics* 1969;**43**:264–72.
137. Fleischer B, Kreth HW. Clonal expansion and functional analysis of virus-specific T lymphocytes from cerebrospinal fluid in measles encephalitis. *Hum Immunol* 1983;**7**:239–48.
138. Florman AL, Agatston HJ. Keratoconjunctivitis as a diagnostic aid in measles. *JAMA* 1962;**179**:568–70.
139. Foege WH. *Measles control in West and Central Africa*. Paper presented at the Eighth Annual Immunology Conference 1971, Kansas City, Missouri.
140. Ford-Jones EL, Macgregor D, Richardson S, et al. Acute childhood encephalitis and meningoencephalitis: diagnosis and management. *Paediatr Child Health* 1998;**3**:33–40.
141. Foreman ML, Cherry JD. *Isolation of measles virus from the cerebrospinal fluid of a child with encephalitis following measles vaccination*. Abstract. Paper presented at the Program for the American Pediatric Society, 1967.
142. Forghani B, Myoraku CK, Schmidt NJ. Use of monoclonal antibodies to human immunoglobulin M in 'capture' assays for measles and rubella immunoglobulin M. *J Clin Microbiol* 1983;**18**: 652–7.
143. Forni AL, Schluger NW, Roberts RB. Severe measles pneumonitis in adults: evaluation of clinical characteristics and therapy with intravenous ribavirin. *Clin Infect Dis* 1994;**19**:454–62.
144. Forthal DN, Landucci G, Habis A, et al. Measles virus-specific functional antibody responses and viremia during acute measles. *J Infect Dis* 1994;**169**:1377–80.
145. Forthal DN, Landucci G, Katz J, et al. Comparison of measles virus-specific antibodies with antibody-dependent cellular cytotoxicity and neutralizing functions. *J Infect Dis* 1993;**168**: 1020–3.
146. Frank JA Jr, Orenstein WA, Bart KJ, et al. Major impediments to measles elimination: the modern epidemiology of an ancient disease. *Am J Dis Child* 1985;**139**:881–8.
147. Frederique G, Howard RO, Boniuk V. Corneal ulcers in rubeola. *Am J Ophthalmol* 1969;**68**:996–1003.
148. Freeman AF, Jacobsohn DA, Shulman ST, et al. A new complication of stem cell transplantation: measles inclusion body encephalitis. *Pediatrics* 2004;**114**:e657–660.
149. Freeman JM. The clinical spectrum and early diagnosis of Dawson's encephalitis, with preliminary notes on treatment. *J Pediatr* 1969;**75**:590–603.
150. Frieden TR, Sowell AL, Henning KJ, et al. Vitamin A levels and severity of measles. New York City. *Am J Dis Child* 1992;**146**: 182–6.
151. Friedman MG, Phillip M, Dagan R. Virus-specific IgA in serum, saliva, and tears of children with measles. *Clin Exp Immunol* 1989;**75**:58–63.
152. Fujinami RS, Oldstone MB. Alterations in expression of measles virus polypeptides by antibody: molecular events in antibody-induced antigenic modulation. *J Immunol* 1980;**125**:78–85.
153. Fukuyama Y, Nihei K, Matsumoto S, et al. Clinical effects of MND-19 (Inosiplex) on subacute sclerosing panencephalitis—a multi-institutional collaborative study. The Inosiplex-SSPE Research Committee. *Brain Dev* 1987;**9**:270–82.
154. Fulginiti VA, Eller JJ, Downie AW, et al. Altered reactivity to measles virus: atypical measles in children previously immunized with inactivated measles virus vaccines. *JAMA* 1967;**202**:1075–80.
155. Fulginiti VA, Helfer RE. Atypical measles in adolescent siblings 16 years after killed measles virus vaccine. *JAMA* 1980;**244**: 804–6.
156. Fulginiti VA, Kempe CH. Measles exposure among vaccine recipients: response to measles exposure and antibody persistence among recipients of measles vaccines. *Am J Dis Child* 1963;**106**: 450–61.
157. Fulginiti VA, Kempe CH. A comparison of measles neutralizing and hemagglutination-inhibition antibody titers in individual sera. *Am J Epidemiol* 1965;**82**:135–42.
158. Gallagher MR, Welliver R, Yamanaka T, et al. Cell-mediated immune responsiveness to measles: its occurrence as a result of naturally acquired or vaccine-induced infection and in infants of immune mothers. *Am J Dis Child* 1981;**135**:48–51.
159. Garenne M, Aaby P. Pattern of exposure and measles mortality in Senegal. *J Infect Dis* 1990;**161**:1088–94.
160. Gellin BG, Katz SL. Putting a stop to a serial killer: measles. *J Infect Dis* 1994;**170**(Suppl 1):S1–2.
161. Gendelman HE, Wolinsky JS, Johnson RT, et al. Measles encephalomyelitis: lack of evidence of viral invasion of the central nervous system and quantitative study of the nature of demyelination. *Ann Neurol* 1984;**15**:353–60.

162. Gershon AA. Chickenpox, measles, and mumps. In: Remington JS, Klein JO, Wilson CB, et al, editors. *Infectious diseases of the fetus and newborn infant*. 7th ed. Philadelphia: Saunders; 2011. p. 685–95.
163. Gibbs FA, Gibbs EL, Carpenter PR, et al. Electroencephalographic abnormality in "uncomplicated" childhood diseases. *JAMA* 1959;**171**:1050–5.
164. Goerka H. The life and scientific works of Mr. John Quier. *West Indian Med J* 1956;**5**:23.
165. Gokiert JG, Beamish WE. Altered reactivity to measles virus in previously vaccinated children. *Can Med Assoc J* 1970;**103**: 724–7.
166. Goldberger J, Anderson JF. An experimental demonstration of the presence of the virus of measles in the mixed buccal and nasal secretions. *JAMA* 1911;**57**:476–8.
167. Goldfield M, Boyer NH, Weinstein L. Electrocardiographic changes during the course of measles. *J Pediatr* 1955;**46**:30–5.
168. Good RA, Zak SJ. Disturbances in gamma globulin synthesis as experiments of nature. *Pediatrics* 1956;**18**:109–49.
169. Goodall EW. Measles with an "illness of infection. *Clin J* 1925; **54**:69.
170. Goraya J, Marks H, Khurana D, et al. Subacute sclerosing panencephalitis (SSPE) presenting as acute disseminated encephalomyelitis in a child. *J Child Neurol* 2009;**24**:899–903.
171. Grattan-Smith PJ, Procopis PG, Wise GA, et al. Serious neurological complications of measles—a continuing preventable problem. *Med J Aust* 1985;**143**:385–7.
172. Graves M, Griffin DE, Johnson RT, et al. Development of antibody to measles virus polypeptides during complicated and uncomplicated measles virus infections. *J Virol* 1984;**49**:409–12.
173. Graziano KD, Ruckdeschel JC, Mardiney MR Jr. Cell-associated immunity to measles (rubeola): the demonstration of in vitro lymphocyte tritiated thymidine incorporation in response to measles complement fixation antigen. *Cell Immunol* 1975;**15**:347–59.
174. Greenstein JI, McFarland HF. Response of human lymphocytes to measles virus after natural infection. *Infect Immun* 1983;**40**: 198–204.
175. Gremillion DH, Crawford GE. Measles pneumonia in young adults: an analysis of 106 cases. *Am J Med* 1981;**71**:539–42.
176. Gresser I, Chany C. Isolation of measles virus from the washed leucocytic fraction of blood. *Proc Soc Exp Biol Med* 1963;**113**: 695–8.
177. Gresser I, Katz SL. Isolation of measles virus from urine. *N Engl J Med* 1960;**263**:452–4.
178. Griffin DE. Measles virus. In: Knipe DM, Howley PM, editors: *Fields virology*, vol. 1. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1401–41.
179. Griffin DE, Cooper SJ, Hirsch RL, et al. Changes in plasma IgE levels during complicated and uncomplicated measles virus infections. *J Allergy Clin Immunol* 1985;**76**:206–13.
180. Griffin DE, Hirsch RL, Johnson RT, et al. Changes in serum C-reactive protein during complicated and uncomplicated measles virus infections. *Infect Immun* 1983;**41**:861–4.
181. Griffin DE, Ward BJ, Esolen LM. Pathogenesis of measles virus infection: an hypothesis for altered immune responses. *J Infect Dis* 1994;**170**(Suppl 1):S24–31.
182. Griffin DE, Ward BJ, Jauregui E, et al. Immune activation in measles. *N Engl J Med* 1989;**320**:1667–72.
183. Griffin DE, Ward BJ, Jauregui E, et al. Immune activation during measles: interferon-gamma and neopterin in plasma and cerebrospinal fluid in complicated and uncomplicated disease. *J Infect Dis* 1990;**161**:449–53.
184. Griffin DE, Ward BJ, Jauregui E, et al. Natural killer cell activity during measles. *Clin Exp Immunol* 1990;**81**:218–24.
185. Grist NR. The pathogenesis of measles: review of the literature and discussion of the problem. *Glasgow Med J* 1950;**31**:431–41.
186. Guinee VF, Henderson DA, Casey HL, et al. Cooperative measles vaccine field trial, part I: clinical efficacy. *Pediatrics* 1966;**37**: 649–65.
187. Gunn W. Control of common fevers: measles. *Lancet* 1938;**1**: 795–9.
188. Haas EJ, Wendt VE. Atypical measles 14 years after immunization. *JAMA* 1976;**236**:1050.
189. Hall WW, Martin SJ. Purification and characterization of measles virus. *J Gen Virol* 1973;**19**:175–88.
190. Hall WW, Martin SJ. Structure and function relationships of the envelope of measles virus. *Med Microbiol Immunol* 1974;**160**: 143–54.
191. Halsey NA, Modlin JF, Jabbour JT, et al. Risk factors in subacute sclerosing panencephalitis: a case-control study. *Am J Epidemiol* 1980;**111**:415–24.
192. Halsey NA, Nieburg PI, Preblud SR, et al. *Recent trends in reported measles cases and deaths: effectiveness of measles vaccine administered at 12 months of age: simultaneous administration of measles vaccine and DPT antigens*. Prepared for the American Academy of Pediatrics Committee on Infectious Diseases, Red Book Committee and Advisory Committee on Immunization Practices. Atlanta: Centers for Disease Control; 1978.
193. Haltia M, Tarkkanen A, Vaheri A, et al. Measles retinopathy during immunosuppression. *Br J Ophthalmol* 1978;**62**:356–60.
194. Haralambieva IH, Ovsyannikova IG, Kennedy RB, et al. Associations between single nucleotide polymorphisms and haplotypes in cytokine and cytokine receptor genes and immunity to measles vaccination. *Vaccine* 2011;**29**:7883–95.
195. Hardy GE Jr, Kassanoff I, Orbach HG, et al. The failure of a school immunization campaign to terminate an urban epidemic of measles. *Am J Epidemiol* 1970;**91**:286–93.
196. Hayden RJ. The epidemiology and nature of measles in Nairobi before the impact of measles immunization. *East Afr Med J* 1974; **51**:199–205.
197. Hedrich AW. The corrected average attack rate from measles among city children. *Am J Epidemiol* 1930;**11**:576–600.
198. Heffner RR Jr, Schluederberg A. Specificity of the primary and secondary antibody responses to myxoviruses. *J Immunol* 1967;**98**: 668–72.
199. Heimann A, Scanlon R, Gentile J, et al. Measles cervicitis: report of a case with cytologic and molecular biologic analysis. *Acta Cytol* 1992;**36**:727–30.
200. Helfand RF, Kim DK, Gary HE Jr, et al. Nonclassic measles infections in an immune population exposed to measles during a college bus trip. *J Med Virol* 1998;**56**:337–41.
201. Henderson JA, Hammond DI. Delayed diagnosis in atypical measles syndrome. *Can Med Assoc J* 1985;**133**:211–13.
202. Holt EA, Moulton LH, Siberry GK, et al. Differential mortality by measles vaccine titer and sex. *J Infect Dis* 1993;**168**:1087–96.
203. Home F. *Medical facts and experiments*. London: Printed for Millar A, Kincaid A, Bell J, at Edinburgh; 1759.
204. Horta-Barbosa L, Fuccillo DA, London WT, et al. Isolation of measles virus from brain cell cultures of two patients with subacute sclerosing panencephalitis. *Proc Soc Exp Biol Med* 1969;**132**: 272–7.
205. Horta-Barbosa L, Fuccillo DA, Sever JL, et al. Subacute sclerosing panencephalitis: isolation of measles virus from a brain biopsy. *Nature* 1969;**221**:974.
206. Horta-Barbosa L, Krebs H, Ley A, et al. Progressive increase in cerebrospinal fluid measles antibody levels in subacute sclerosing panencephalitis. *Pediatrics* 1971;**47**:782–3.
207. Horwitz MS, Grose C, Fisher M. Letter: Atypical measles rash mimicking Rocky Mountain spotted fever. *N Engl J Med* 1973; **289**:1203–4.
208. Houff SA, Madden DL, Sever JL. Subacute sclerosing panencephalitis in only one of identical twins: a seven-year follow-up. *Arch Neurol* 1979;**36**:854–6.
209. Hudson JB, Weinstein L, Chang TW. Thrombocytopenic purpura in measles. *J Pediatr* 1956;**48**:48–56.
210. Hussey GD, Klein M. A randomized, controlled trial of vitamin A in children with severe measles. *N Engl J Med* 1990;**323**: 160–4.
211. Ibrahim MM, Jeavons PM. The value of electroencephalography in the diagnosis of subacute sclerosing panencephalitis. *Dev Med Child Neurol* 1974;**16**:295–307.
212. Imagawa DT. Relationships among measles, canine distemper and rinderpest viruses. *Prog Med Virol* 1968;**10**:160–93.
213. Imagawa DT, Adams JM. Propagation of measles virus in suckling mice. *Proc Soc Exp Biol Med* 1958;**98**:567–9.
214. Inua M, Duggan MB, West CE, et al. Post-measles corneal ulceration in children in northern Nigeria: the role of vitamin A, malnutrition and measles. *Ann Trop Paediatr* 1983;**3**:181–91.
215. Jabbour JT, Duenas DA, Sever JL, et al. Epidemiology of subacute sclerosing panencephalitis (SSPE). A report of the SSPE registry. *JAMA* 1972;**220**:959–62.

216. Jacobson RM, Ovsyannikova IG, Vierkant RA, et al. Human leukocyte antigen associations with humoral and cellular immunity following a second dose of measles-containing vaccine: persistence, dampening, and extinction of associations found after a first dose. *Vaccine* 2011;**29**:7982–91.
217. Janeway CA. Use of concentrated human serum gamma-globulin in the prevention and attenuation of measles. *Bull N Y Acad Med* 1945;**21**:202–22.
218. Jayarajan V, Sedler PA. Hearing loss following measles vaccination. *J Infect* 1995;**30**:184–5.
219. Jenkerson SA, Beller M, Middaugh JP, et al. False positive rubeola IgM tests. *N Engl J Med* 1995;**332**:1103–4.
220. Jespersen CS, Littauer J, Sagild U. Measles as a cause of fetal defects: a retrospective study of ten measles epidemics in Greenland. *Acta Paediatr Scand* 1977;**66**:367–72.
221. Joffe MI, Sukha NR, Rabson AR. Lymphocyte subsets in measles: depressed helper/inducer subpopulation reversed by in vitro treatment with levamisole and ascorbic acid. *J Clin Invest* 1983;**72**:971–80.
222. Johannes RS, Sever JL. Subacute sclerosing panencephalitis. *Annu Rev Med* 1975;**26**:589–601.
223. Johnson RT. The pathogenesis of acute viral encephalitis and postinfectious encephalomyelitis. *J Infect Dis* 1987;**155**:359–64.
224. Johnson RT. Acute encephalitis. *Clin Infect Dis* 1996;**23**:219–24; quiz 225–16.
225. Johnson RT, Griffin DE, Hirsch RL, et al. Measles encephalomyelitis—clinical and immunologic studies. *N Engl J Med* 1984;**310**:137–41.
226. Jones CE, Dyken PR, Huttenlocher PR, et al. Inosiplex therapy in subacute sclerosing panencephalitis: a multicentre, non-randomised study in 98 patients. *Lancet* 1982;**1**:1034–7.
227. Kamahora J, Nii S. Pathological and immunological studies of monkeys infected with measles virus. *Arch Gesamte Virusforsch* 1965;**16**:161–7.
228. Karzon DT, Rush D, Winkelstein W Jr. Immunization with inactivated measles virus vaccine: effect of booster dose and response to natural challenge. *Pediatrics* 1965;**36**:40–50.
229. Katz SL. Measles—its complications, treatment and prophylaxis. *Med Clin North Am* 1962;**46**:1163–75.
230. Katz SL, Enders JF. Measles virus. In: Horsfall FL Jr, Tamm T, editors. *Viral and rickettsial infections of man*. Philadelphia: J. B. Lippincott; 1965. p. 784–801.
231. Katz SL, Enders JF. Measles virus. In: Lennette EH, Schmidt NJ, editors. *Diagnostic procedures for viral and rickettsial infections*. Washington, D.C.: American Public Health Association; 1969. p. 504–28.
232. Katz SL, Milovanovic MV, Enders JF. Propagation of measles virus in cultures of chick embryo cells. *Proc Soc Exp Biol Med* 1958;**97**:23–9.
233. Kempe CH, Fulginiti VA. The pathogenesis of measles virus infection. *Arch Gesamte Virusforsch* 1965;**16**:103–28.
234. Kennedy CR, Webster AD. Measles encephalitis. *N Engl J Med* 1984;**311**:330–1.
235. Khatib R, Siddique M, Abbass M. Measles associated hepatobiliary disease: an overview. *Infection* 1993;**21**:112–14.
236. Kibler R, Meulen VT. Antibody-mediated cytotoxicity after measles virus infection. *J Immunol* 1975;**114**:93–8.
237. Kiepiela P, Coovadia HM, Coward P. T helper cell defect related to severity in measles. *Scand J Infect Dis* 1987;**19**:185–92.
238. Kimura A, Tosaka K, Nakao T. Measles rash. I. Light and electron microscopic study of skin eruptions. *Arch Virol* 1975;**47**:295–307.
239. Kleiman MB, Blackburn CK, Zimmerman SE, et al. Comparison of enzyme-linked immunosorbent assay for acute measles with hemagglutination inhibition complement fixation, and fluorescent-antibody methods. *J Clin Microbiol* 1981;**14**:147–52.
240. Klein NP, Lewis E, Baxter R, et al. Measles-containing vaccines and febrile seizures in children age 4 to 6 years. *Pediatrics* 2012;**129**:809–14.
241. Knudsen KM, Aaby P, Whittle H, et al. Child mortality following standard, medium or high titre measles immunization in West Africa. *Int J Epidemiol* 1996;**25**:665–73.
242. Kobune F, Sakata H, Sugiura A. Marmoset lymphoblastoid cells as a sensitive host for isolation of measles virus. *J Virol* 1990;**64**:700–5.
243. Koffler D. Giant cell pneumonia; fluorescent antibody and histochemical studies on alveolar giant cells. *Arch Pathol* 1964;**78**:267–73.
244. Kohn JL, Koiransky H. Successive roentgenograms of the chest of children during measles. *Arch Pediatr Adolesc Med* 1929;**38**:258–70.
245. Kondo K, Takasu T, Ahmed A. Neurological diseases in Karachi, Pakistan—elevated occurrence of subacute sclerosing panencephalitis. *Neuroepidemiology* 1988;**7**:66–80.
246. Koplik H. The diagnosis of the invasion of measles from a study of the exanthema as it appears on the buccal mucous membrane. *Arch Pediatr* 1896;**13**:918.
247. Koplik H. A new diagnostic sign of measles. *Med Rec* 1898;**53**:505.
248. Koplik H. The new diagnostic spots of measles on the buccal and labial mucous membrane. *Med News* 1899;**74**:673.
249. Krause PJ, Cherry JD, Naiditch MJ, et al. Revaccination of previous recipients of killed measles vaccine: clinical and immunologic studies. *J Pediatr* 1978;**93**:565–71.
250. Kress S, Schluederberg AE, Hornick RB, et al. Studies with live attenuated measles-virus vaccine, part II: clinical and immunologic response of children in an open community. *Am J Dis Child* 1961;**101**:701–7.
251. Krugman RD, Rosenberg R, McIntosh K. Further attenuated live measles vaccines: the need for revised recommendations. *J Pediatr* 1977;**91**:766–7.
252. Krugman S. Present status of measles and rubella immunization in the United States: a medical progress report. *J Pediatr* 1971;**78**:1–16.
253. Krugman S. Present status of measles and rubella immunization in the United States: a medical progress report. *J Pediatr* 1977;**90**:1–12.
254. Krugman S, Giles JP, Friedman H, et al. Studies on immunity to measles. *J Pediatr* 1965;**66**:471–88.
255. Krugman S, Giles JP, Jacobs AM, et al. Studies with live attenuated measles-virus vaccine: comparative clinical, antigenic, and prophylactic effects after inoculation with and without gamma-globulin. *Am J Dis Child* 1962;**103**:353–63.
256. Krugman S, Ward R, Katz SL. Measles (rubeola). In: Krugman S, Ward R, Katz SL, editors. *Infectious diseases of children*. 6th ed. St. Louis: C. V. Mosby; 1977. p. 132–48.
257. Kurata T, Matsubara E, Yokoyama M, et al. Improvement of SSPE by intrathecal infusion of alpha-IFN. *Neurology* 2004;**63**:398–9.
258. Laboccetta AC, Tornay AS. Measles encephalitis: report of 61 cases. *Am J Dis Child* 1964;**107**:247–55.
259. Labowskie R, Edelman R, Rustigian R, et al. Studies of cell-mediated immunity to measles virus by in vitro lymphocyte-mediated cytotoxicity. *J Infect Dis* 1974;**129**:233–9.
260. Lamb RA, Kolakofsky D. Paramyxoviridae: the viruses and their replication. In: Knipe DM, Howley PM, editors. *Fields virology*, vol. 1. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1305–39.
261. Landrigan PJ, Witte JJ. Neurologic disorders following live measles-virus vaccination. *JAMA* 1973;**223**:1459–62.
262. Langmuir AD. Medical importance of measles. *Am J Dis Child* 1962;**103**:224–6.
263. Laptook A, Wind E, Nussbaum M, et al. Pulmonary lesions in atypical measles. *Pediatrics* 1978;**62**:42–6.
264. Levy DL. The future of measles in highly immunized populations: a modeling approach. *Am J Epidemiol* 1984;**120**:39–48.
265. Libman MD, Ibrahim SA, Omer MIA, et al. No evidence for short or long term morbidity after increased titer measles vaccination in Sudan. *Pediatr Infect Dis J* 2002;**21**:112–19.
266. Lightwood R, Nolan R, Franco M, et al. Epithelial giant cells in measles as an aid in diagnosis. *J Pediatr* 1970;**77**:59–64.
267. Lin CY, Hsu HC. Measles and acute glomerulonephritis. *Pediatrics* 1983;**71**:398–401.
268. Linnemann CC Jr, Rotte TC, Schiff GM, et al. A seroepidemiologic study of a measles epidemic in a highly immunized population. *Am J Epidemiol* 1972;**95**:238–46.
269. Lobes LA Jr, Cherry JD. Fatal measles pneumonia in a child with chickenpox pneumonia. *JAMA* 1973;**223**:1143–4.
270. London WP, Yorke JA. Recurrent outbreaks of measles, chickenpox and mumps, part I: seasonal variation in contact rates. *Am J Epidemiol* 1973;**98**:453–68.

271. Lucas KM, Sanders RC, Rongap A, et al. Subacute sclerosing panencephalitis (SSPE) in Papua New Guinea: a high incidence in young children. *Epidemiol Infect* 1992;**108**:547–53.
272. Lyon G, Ponsot G, Lebon P. Acute measles encephalitis of the delayed type. *Ann Neurol* 1977;**2**:322–7.
273. Mackenzie DJM, Kipps A, McDonald R. Subacute sclerosing panencephalitis in Southern Africa. *S Afr Med J* 1975;**49**:2083–6.
274. Mahadevan A, Vaidya SR, Wairagkar NS, et al. Case of fulminant-SSPE associated with measles genotype D7 from India: an autopsy study. *Neuropathology* 2008;**28**:621–6.
275. Maimone D, Grimaldi LM, Incorpora G, et al. Intrathecal interferon in subacute sclerosing panencephalitis. *Acta Neurol Scand* 1988;**78**:161–6.
276. Makhene MK, Diaz PS. Clinical presentations and complications of suspected measles in hospitalized children. *Pediatr Infect Dis J* 1993;**12**:836–40.
277. Manchester M, Liszewski MK, Atkinson JP, et al. Multiple isoforms of CD46 (membrane cofactor protein) serve as receptors for measles virus. *Proc Natl Acad Sci U S A* 1994;**91**:2161.
278. Manchester M, Valsamakis A, Kaufman R, et al. Measles virus and C3 binding sites are distinct on membrane cofactor protein (CD46). *Proc Natl Acad Sci U S A* 1995;**92**:2303–7.
279. Maretic Z, Stihovic L, Ogrizek M, et al. Stevens-Johnson syndrome in the course of measles. *J Trop Med Hyg* 1965;**68**:50–2.
280. Marin M, Broder KR, Temte JL, et al; Centers for Disease Control and Prevention (CDC). Use of combination measles, mumps, rubella, and varicella vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-3):1–12.
281. Markand ON, Panszi JG. The electroencephalogram in subacute sclerosing panencephalitis. *Arch Neurol* 1975;**32**:719–26.
282. Markowitz LE, Nzilambi N, Driskell WH, et al. Retinol concentration and mortality among hospitalized measles patients, Kinshasa, Zaire. *J Trop Pediatr* 1989;**35**:109–12.
283. Markowitz LE, Preblud SR, Orenstein WA, et al. Patterns of transmission in measles outbreaks in the United States, 1985-1986. *N Engl J Med* 1989;**320**:75–81.
284. Martinovic Z. Periodic generalized bursts of fast waves in subacute sclerosing panencephalitis. *Electroencephalogr Clin Neurophysiol* 1986;**63**:236–8.
285. Mason WH, Ross LA, Lanson J, et al. Epidemic measles in the postvaccine era: evaluation of epidemiology, clinical presentation and complications during an urban outbreak. *Pediatr Infect Dis J* 1993;**12**:42–8.
286. Mathiesen T, Hammarstrom L, Fridell E, et al. Aberrant IgG subclass distribution to measles in healthy seropositive individuals, in patients with SSPE and in immunoglobulin-deficient patients. *Clin Exp Immunol* 1990;**80**:202–5.
287. Matsuzono Y, Narita M, Ishiguro N, et al. Detection of measles virus from clinical samples using the polymerase chain reaction. *Arch Pediatr Adolesc Med* 1994;**148**:289–93.
288. Matumoto M. Multiplication of measles virus in cell cultures. *Bacteriol Rev* 1966;**30**:152–76.
289. McChesney MB, Miller CJ, Rota PA, et al. Experimental measles, part I: pathogenesis in the normal and the immunized host. *Virology* 1997;**233**:74–84.
290. McHugh K, McMenamin JB. Acute disseminated encephalomyelitis in childhood. *Ir Med J* 1987;**80**:412–14.
291. McLean DM, Best JM, Smith PA, et al. Viral infections of Toronto children during 1965, part II: measles encephalitis and other complications. *Can Med Assoc J* 1966;**94**:905–10.
292. McLean DM, Kettyls GD, Hingston J, et al. Atypical measles following immunization with killed measles vaccine. *Can Med Assoc J* 1970;**103**:743–4.
293. McLellan RK, Gleiner JA. Acute hepatitis in an adult with rubeola. *JAMA* 1982;**247**:2000–1.
294. McNeill WH. *Plagues and peoples*. Garden City, NY: Anchor Press-Doubleday; 1976. p. 105, 119.
295. Meadow SR, Weller RO, Archibald RW. Fatal systemic measles in a child receiving cyclophosphamide for nephrotic syndrome. *Lancet* 1969;**2**:876–8.
296. Mellor DH, Purcell M. Unusual encephalitic illnesses in a child with acute leukaemia in remission: possible role of measles virus and *Toxoplasma gondii*. *Neuropadiatrie* 1976;**7**:423–30.
297. Meyer HM Jr, Brooks BE, Douglas RD, et al. Ecology of measles in monkeys. *Am J Dis Child* 1962;**103**:307–13.
298. Miller C, Andrews N, Rush M, et al. The epidemiology of subacute sclerosing panencephalitis in England and Wales 1990-2002. *Arch Dis Child* 2004;**89**:1145–8.
299. Miller DL. Frequency of complications of measles, 1963. Report on a National Inquiry by the Public Health Laboratory Service in Collaboration with the Society of Medical Officers of Health. *BMJ* 1964;**2**:75–8.
300. Milovanovic MV, Enders JF, Mitus A. Cultivation of measles virus in human amnion cells and in developing chick embryo. *Proc Soc Exp Biol Med* 1957;**95**:120–7.
301. Mitnick J, Becker MH, Rothberg M, et al. Nodular residua of atypical measles pneumonia. *AJR Am J Roentgenol* 1980;**134**:257–60.
302. Mitus A, Enders JF, Craig JM, et al. Persistence of measles virus and depression of antibody formation in patients with giant-cell pneumonia after measles. *N Engl J Med* 1959;**261**:882–9.
303. Mitus A, Enders JF, Edsall G, et al. Measles in children with malignancy problems and prevention. *Arch Gesamte Virusforsch* 1965;**16**:331–7.
304. Mitus A, Holloway A, Evans AE, et al. Attenuated measles vaccine in children with acute leukemia. *Am J Dis Child* 1962;**103**:413–18.
305. Miyazaki M, Nishimura M, Toda Y, et al. Long-term follow-up of a patient with subacute sclerosing panencephalitis successfully treated with intrathecal interferon alpha. *Brain Dev* 2005;**27**:301–3.
306. Modlin JF, Halsey NA, Conrad JI, et al. Subacute sclerosing panencephalitis: a report of the National Registry. *J Pediatr* 1979;**94**:231–6.
307. Modlin JF, Halsey NA, Eddins DL, et al. Epidemiology of subacute sclerosing panencephalitis. *J Pediatr* 1979;**94**:231–6.
308. Modlin JF, Jabbour JT, Witte JJ, et al. Epidemiologic studies of measles, measles vaccine, and subacute sclerosing panencephalitis. *Pediatrics* 1977;**59**:505–12.
309. Moench TR, Griffin DE, Obriecht CR, et al. Acute measles in patients with and without neurological involvement: distribution of measles virus antigen and RNA. *J Infect Dis* 1988;**158**:433–42.
310. Monif GR, Hood CI. Ileocolitis associated with measles (rubeola). *Am J Dis Child* 1970;**120**:245–7.
311. Morgan EM, Rapp F. Measles virus and its associated diseases. *Bacteriol Rev* 1977;**41**:636–66.
312. Morley D, Woodland M, Martin WJ. Measles in Nigerian children: a study of the disease in West Africa, and its manifestations in England and other countries during different epochs. *J Hyg (Lond)* 1963;**61**:115–34, 111.
313. Morley DC. Measles in Nigeria. *Am J Dis Child* 1962;**103**:230–3.
314. Morley DC, Martin WJ, Allen I. Measles in East and Central Africa. *East Afr Med J* 1967;**44**:497–508.
315. Moroi K, Saito S, Kurata T, et al. Fetal death associated with measles virus infection of the placenta. *Am J Obstet Gynecol* 1991;**164**:1107–8.
316. Murphy JV, Yunis EJ. Encephalopathy following measles infection in children with chronic illness. *J Pediatr* 1976;**88**:937–42.
317. Muscat M. Who gets measles in Europe? *J Infect Dis* 2011;**204**(Suppl 1):S353–65.
318. Musser SJ, Underwood GE. Studies on measles virus, part II: physical properties and inactivation studies of measles virus. *J Immunol* 1960;**85**:292–7.
319. Mustafa MM, Weitman SD, Winick NJ, et al. Subacute measles encephalitis in the young immunocompromised host: report of two cases diagnosed by polymerase chain reaction and treated with ribavirin and review of the literature. *Clin Infect Dis* 1993;**16**:654–60.
320. Nader PR, Horwitz MS, Rousseau J. Atypical exanthem following exposure to natural measles: eleven cases in children previously inoculated with killed vaccine. *J Pediatr* 1968;**72**:22–8.
321. Nagai K, Mori T. Acute disseminated encephalomyelitis with probable measles vaccine failure. *Pediatr Neurol* 1999;**20**:399–402.
322. Nahmias AJ, Griffith D, Salsbury C, et al. Thymic aplasia with lymphopenia, plasma cells, and normal immunoglobulins: relation to measles virus infection. *JAMA* 1967;**201**:729–34.
323. Nakai M, Imagawa DT. Electron microscopy of measles virus replication. *J Virol* 1969;**3**:187–97.

324. Nakayama T, Urano T, Osano M, et al. Long-term regulation of interferon production by lymphocytes from children inoculated with live measles virus vaccine. *J Infect Dis* 1988;**158**:1386–90.
325. Naniche D, Varior-Krishnan G, Cervoni F, et al. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J Virol* 1993;**67**:6025–32.
326. Neumann PW, Weber JM, Jessamine AG, et al. Comparison of measles antihemolysin test, enzyme-linked immunosorbent assay, and hemagglutination inhibition test with neutralization test for determination of immune status. *J Clin Microbiol* 1985;**22**: 296–8.
327. Nichols EM. Atypical measles syndrome: a continuing problem. *Am J Public Health* 1979;**69**:160–2.
328. Nickell MD, Cannady PB Jr, Schwitzer GA. Subclinical hepatitis in rubeola infections in young adults. *Ann Intern Med* 1979;**90**: 354–5.
329. Nieburg PI, D'Angelo LJ, Hermann KL. Measles in patients suspected of having Rocky Mountain spotted fever. *JAMA* 1980; **244**:808–9.
330. Nii S, Kamahora J, Mori Y, et al. Experimental pathology of measles in monkeys. *Biken J* 1964;**6**:271–97.
331. Norrby E. Hemagglutination by measles virus, part II: properties of the hemagglutinin and of the receptors on the erythrocytes. *Arch Gesamte Virusforsch* 1962;**12**:164–72.
332. Norrby E, Enders Ruckle G, Ter Meulen V. The significance of hemolysing inhibiting antibodies in protection against measles. *Med Microbiol Immunol* 1974;**160**:232.
333. Norrby E, Gollmar Y. Appearance and persistence of antibodies against different virus components after regular measles infections. *Infect Immun* 1972;**6**:240–7.
334. Norrby E, Gollmar Y. Identification of measles virus specific hemolysis inhibiting antibodies separate from hemagglutination inhibiting antibodies. *Infect Immun* 1975;**11**:231–9.
335. Norrby E, Hammarskjold B. Structural components of measles virus. *Microbios* 1972;**5**:17–29.
336. Norrby E, Lagercrantz R, Gard S. Measles vaccination, part IV: responses to two different types of preparations given as a fourth dose of vaccine. *BMJ* 1965;**1**:813–17.
337. Norrby E, Lagercantz R, Gard S. Measles vaccination, part VII: follow-up studies in children immunized with four doses of inactivated vaccine. *Acta Paediatr Scand* 1969;**58**:261–7.
338. Norrby EC, Magnusson P. Some morphological characteristics of the internal component of measles virus. *Arch Gesamte Virusforsch* 1965;**17**:443–7.
339. Norrby EC, Magnusson P, Falksveden LG, et al. Separation of measles virus components by equilibrium centrifugation in Cscl gradients, part II: studies on the large and the small hemagglutinin. *Arch Gesamte Virusforsch* 1964;**14**:462–73.
340. Norrby L, Oxman MN. Measles virus. In: Fields BN, Knipe DM, Chanock RM, et al, editors. *Virology*. 2nd ed. New York: Raven Press; 1990. p. 1013–44.
341. O'Donovan C. Measles in Kenyan children. *East Afr Med J* 1971;**48**:526–32.
342. O'Donovan C, Barua KN. Measles pneumonia. *Am J Trop Med Hyg* 1973;**22**:73–7.
343. Ojala A. On changes in the cerebrospinal fluid during measles. *Ann Med Intern Fenn* 1947;**36**:321–31.
344. Okuno Y, Nakao T, Ishida N, et al. Incidence of subacute sclerosing panencephalitis following measles and measles vaccination in Japan. *Int J Epidemiol* 1989;**18**:684–9.
345. Ono K, Iwa N, Kato S, et al. Demonstration of viral antigen in giant cells formed in monkeys experimentally infected with measles virus. *Biken J* 1970;**13**:329–37.
346. Ono N, Tatsuo H, Hidaka Y, et al. Measles viruses on throat swabs from measles patients use signaling lymphocytic activation molecule (CDw150) but not CD46 as a cellular receptor. *J Virol* 2001;**75**:4399–401.
347. Orenstein WA, Halsey NA, Hayden GF, et al. From the Center for Disease Control: current status of measles in the United States, 1973-1977. *J Infect Dis* 1978;**137**:847–53.
348. Orenstein WA, Markowitz LE, Atkinson WL, et al. Worldwide measles prevention. *Isr J Med Sci* 1994;**30**:469–81.
349. Orenstein WA, Papania MJ, Wharton ME. Measles elimination in the United States. *J Infect Dis* 2004;**189**(Suppl 1):S1–3.
350. Oski FA, Naiman JL. Effect of live measles vaccine on the platelet count. *N Engl J Med* 1966;**275**:352–6.
351. Ovsyannikova IG, Jacobson RM, Vierkant RA, et al. Associations between human leukocyte antigen (HLA) alleles and very high levels of measles antibody following vaccination. *Vaccine* 2004;**22**: 15–16.
352. Ovsyannikova IG, Reid KC, Jacobson RM, et al. Cytokine production patterns and antibody response to measles vaccine. *Vaccine* 2003;**21**:3946–53.
353. Pampiglione G. Prodromal phase of measles: some neurophysiological studies. *BMJ* 1964;**2**:1296–300.
354. Panum PL. Observations made during the epidemic of measles on the Faroe Islands in the year 1846. *Med Classics* 1939;**3**:829–86.
355. Papp K. Expériences prouvant que la voie d'infection de la rougeole est la contamination de la muqueuse conjonctivale. *Rev Immunol Ther Antimicrob* 1956;**20**(1-2):27–36.
356. Parhad IM, Johnson KP, Wolinsky JS, et al. Encephalitis after inhalation of measles virus: a pathogenetic study in hamsters. *Ann Neurol* 1981;**9**:21–7.
357. Partington MW, Quinton JF. The pre-eruptive illness of measles. *Arch Dis Child* 1959;**34**:149–53.
358. Pather M, Wesley AG, Schonland M, et al. Severe measles-associated pneumonia treated with assisted ventilation. *S Afr Med J* 1976;**50**:1600–3.
359. Paunio M, Hedman K, Davidkin I, et al. Secondary measles vaccine failures identified by measurement of IgG avidity: high occurrence among teenagers vaccinated at a young age. *Epidemiol Infect* 2000;**124**:263–71.
360. Payne FE, Baublis JV, Itabashi HH. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. *N Engl J Med* 1969;**281**:585–9.
361. Pearl PL, Abu-Farsakh H, Starke JR, et al. Neuropathology of two fatal cases of measles in the 1988-1989 Houston epidemic. *Pediatr Neurol* 1990;**6**:126–30.
362. Peebles TC. Distribution of virus in blood components during the viremia of measles. *Arch Gesamte Virusforsch* 1967;**22**:43–7.
363. Peries JR, Chany C. Studies on measles viral hemagglutination. *Proc Soc Exp Biol Med* 1962;**110**:477–82.
364. Permar SR, Klumpp SA, Mansfield KG, et al. Limited contribution of humoral immunity to the clearance of measles viremia in rhesus monkeys. *J Infect Dis* 2004;**190**:998–1005.
365. Phillips CA. Inclusion bodies in measles encephalitis. *JAMA* 1966;**195**:307–8.
366. Plotkin SA. Vaccination against measles in the 18th century. *Clin Pediatr (Phila)* 1967;**6**:312–15.
367. Plotz H. Culture "in vitro" du virus de la rougeole. *Bull Acad Med Paris* 1938;**119**:598.
368. Polack FP, Auwaerter PG, Lee SH, et al. Production of atypical measles in rhesus macaques: evidence for disease mediated by immune complex formation and eosinophils in the presence of fusion-inhibiting antibody. *Nat Med* 1999;**5**:629–34.
369. Pollack MA, Grose C, Friend H. Letter: Measles associated with Bell palsy. *Am J Dis Child* 1975;**129**:747.
370. Pullan CR, Noble TC, Scott DJ, et al. Atypical measles infections in leukaemic children on immunosuppressive treatment. *BMJ* 1976;**1**:1562–5.
371. Quiambao BP, Gatchalian SR, Halonen P, et al. Coinfection is common in measles-associated pneumonia. *Pediatr Infect Dis J* 1998;**17**:89–93.
372. Rake G, Shaffer MF. Studies on measles, part I: the use of the chorio-allantois of the developing chicken embryo. *J Immunol* 1940;**38**:177–200.
373. Rand KH, Emmons RW, Merigan TC. Measles in adults: an unforeseen consequence of immunization? *JAMA* 1976;**236**: 1028–31.
374. Rauh LW, Schmidt R. Measles immunization with killed virus vaccine: serum antibody titers and experience with exposure to measles epidemic. *Am J Dis Child* 1965;**109**:232–7.
375. Reddy V, Bhaskaram P, Raghuramulu N. Relationship between measles, malnutrition, and blindness a prospective study in Indian children. *Am J Clin Nutr* 1986;**44**:924–30.
376. Remington PL, Hall WN, Davis IH, et al. Airborne transmission of measles in a physician's office. *JAMA* 1985;**253**:1574–7.
377. Reyes MA, de Borrero MF, Roa J, et al. Measles vaccine failure after documented seroconversion. *Pediatr Infect Dis J* 1987;**6**: 848–51.
378. Riley EC, Murphy G, Riley RL. Airborne spread of measles in a suburban elementary school. *Am J Epidemiol* 1978;**107**:421–32.

379. Riley RL. Airborne infection. *Am J Med* 1974;**57**:466–75.
380. Risk WS, Haddad FS. The variable natural history of subacute sclerosing panencephalitis: a study of 118 cases from the Middle East. *Arch Neurol* 1979;**36**:610–14.
381. Ristori C, Boccardo H, Borgono JM, et al. Medical importance of measles in Chile. *Am J Dis Child* 1962;**103**:236–41.
382. Robbins FC. Measles: clinical features, pathogenesis, pathology and complications. *Am J Dis Child* 1962;**103**:266–73.
383. Roberts GB, Bain AD. The pathology of measles. *J Pathol Bacteriol* 1958;**76**:111–18.
384. Ross LA, Mason WH, Lanson J, et al. Laryngotracheobronchitis as a complication of measles during an urban epidemic. *J Pediatr* 1992;**121**:511–15.
385. Ross LJ. Electrocardiographic findings in measles. *AMA Am J Dis Child* 1952;**83**:282–91.
386. Rota JS, Rota PA, Redd SB, et al. Genetic analysis of measles viruses isolated in the United States, 1995-1996. *J Infect Dis* 1998;**177**:204–8.
387. Rota PA, Bellini WJ. Update on the global distribution of genotypes of wild type measles viruses. *J Infect Dis* 2003;**187**(Suppl 1): S270–276.
388. Rota PA, Rota JS, Bellini WJ. Molecular epidemiology of measles virus. *Virology* 1995;**6**:379–86.
389. Ruckdeschel JC, Graziano KD, Mardiney MR Jr. Additional evidence that the cell-associated immune system is the primary host defense against measles (rubeola). *Cell Immunol* 1975;**17**:11–18.
390. Ruckle G, Rogers KD. Studies with measles virus, part II: isolation of virus and immunologic studies in persons who have had the natural disease. *J Immunol (Baltimore, Md: 1950)* 1957;**78**: 341–55.
391. Rustigian R. Persistent infection of cells in culture by measles virus, part I: development and characteristics of HeLa sublines persistently infected with complete virus. *J Bacteriol* 1966;**92**: 1792–804.
392. Saha V, John TJ, Mukundan P, et al. High incidence of subacute sclerosing panencephalitis in south India. *Epidemiol Infect* 1990; **104**:151–6.
393. Sandford-Smith JH, Whittle HC. Corneal ulceration following measles in Nigerian children. *Br J Ophthalmol* 1979;**63**:720–4.
394. Saxton NL. Thrombocytopenic purpura following the administration of attenuated live measles vaccine. *J Iowa Med Soc* 1967; **57**:1017–18.
395. Schaffner W, Schluederberg AE, Byrne EB. Clinical epidemiology of sporadic measles in a highly immunized population. *N Engl J Med* 1968;**279**:783–9.
396. Scheifele DW, Forbes CE. Prolonged giant cell excretion in severe African measles. *Pediatrics* 1972;**50**:867–73.
397. Schluederberg AE. Separation of measles virus particles in density gradients. *Am J Dis Child* 1962;**103**:291–6.
398. Schluederberg A. Immune globulins in human viral infections. *Nature* 1965;**205**:1232.
399. Schluederberg A. Modification of immune response by previous experience with measles. *Arch Gesamte Virusforsch* 1965;**16**: 347–50.
400. Sergiev PG, Ryazantseva NE, Shroit IG. The dynamics of pathological processes in experimental measles in monkeys. *Acta Virol* 1960;**4**:265–73.
401. Shalit M, Ackerman Z, Wollner S, et al. Immunoglobulin E response during measles. *Int Arch Allergy Appl Immunol* 1984;**75**: 84–6.
402. Shann F. Meta-analysis of trials of prophylactic antibiotics for children with measles: inadequate evidence. *BMJ* 1997;**314**: 334–6.
403. Shasby DM, Shope TC, Downs H, et al. Epidemic measles in a highly vaccinated population. *N Engl J Med* 1977;**296**:585–9.
404. Shoss RG, Rayhanzadeh S. Toxic epidermal necrolysis following measles vaccination. *Arch Dermatol* 1974;**110**:766–70.
405. Siegel D, Hirschman SZ. Hepatic dysfunction in acute measles infection of adults. *Arch Intern Med* 1977;**137**:1178–9.
406. Siegel MM, Walter TK, Ablin AR. Measles pneumonia in childhood leukemia. *Pediatrics* 1977;**60**:38–40.
407. Siemes H, Siegert M, Hanefeld F, et al. Oligoclonal gammaglobulin banding of cerebrospinal fluid in patients with subacute sclerosing panencephalitis: comparison of the electrophoretic pattern with that in multiple sclerosis and congenital infections. *J Neurol Sci* 1977;**32**:395–409.
408. Smith FR, Curran AS, Raciti KA, et al. Reported measles in persons immunologically primed by prior vaccination. *J Pediatr* 1982;**101**:391–3.
409. Sobonya RE, Hiller FC, Pingleton W, et al. Fatal measles (rubeola) pneumonia in adults. *Arch Pathol Lab Med* 1978;**102**:366–71.
410. Spencer MJ, Cherry JD. Unpublished data. 1976.
411. St. Geme JW Jr, George BL, Bush BM. Exaggerated natural measles following attenuated virus immunization. *Pediatrics* 1976; **57**:148–50.
412. St. Sauver JL, Ovsyannikova IG, Jacobson RM, et al. Associations between human leukocyte antigen homozygosity and antibody levels to measles vaccine. *J Infect Dis* 2002;**185**:1545–9.
413. Starr S, Berkovich S. Effects of measles, gamma-globulin-modified measles and vaccine measles on the tuberculin test. *N Engl J Med* 1964;**270**:386–91.
414. Starr S, Berkovich S. The effect of measles, gamma globulin modified measles, and attenuated measles vaccine on the course of treated tuberculosis in children. *Pediatrics* 1965;**35**:97–102.
415. Stein SJ, Greenspoon JS. Rubeola during pregnancy. *Obstet Gynecol* 1991;**78**:925–9.
416. Stimson PM. The earlier diagnosis of measles. *JAMA* 1928;**90**: 660–3.
417. Stokes J Jr. Viral infections, including those presumed to be caused by viruses: measles (rubeola). In: Nelson WE, editor. *Textbook of pediatrics*. 6th ed. Philadelphia: W. B. Saunders; 1954. p. 466–71.
418. Stokes J, Maris EP, Gellis SS. Chemical, clinical, and immunological studies on the products of human plasma fractionation, part XI: the use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prophylaxis and treatment of measles. *J Clin Invest* 1944;**23**:531–40.
419. Stratton KR, Howe CJ, Johnston RB Jr, et al. Adverse events associated with childhood vaccines other than pertussis and rubella: summary of a report from the Institute of Medicine. *JAMA* 1994;**271**:1602–5.
420. Strebel PM, Papania MJ, Dayan GH, et al. Measles vaccine. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders; 2008.
421. Suringa DW, Bank LJ, Ackerman AB. Role of measles virus in skin lesions and Koplik's spots. *N Engl J Med* 1970;**283**:1139–42.
422. Sutter RW, Markowitz LE, Bennetch JM, et al. Measles among the Amish: a comparative study of measles severity in primary and secondary cases in households. *J Infect Dis* 1991;**163**:12–16.
423. Swift JD, Barruga MC, Perkin RM, et al. Respiratory failure complicating rubeola. *Chest* 1993;**104**:1786–7.
424. Tanaka J, Nakamura H, Fukada T. Adult-onset subacute sclerosing panencephalitis: immunocytochemical and electron microscopic demonstration of the viral antigen. *Clin Neuropathol* 1987; **6**:30–7.
425. Taneja PN, Ghai OP, Bhakoo ON. Importance of measles to India. *Am J Dis Child* 1962;**103**:226–9.
426. Tasdemir HA, Dilber C, Totan M, et al. Pseudotumor cerebri complicating measles: a case report and literature review. *Brain Dev* 2006;**28**:395–7.
427. Taylor MJ, Godfrey E, Baczko K, et al. Identification of several different lineages of measles virus. *J Gen Virol* 1991;**72**:83–8.
428. Tellez-Nagel I, Harter DH. Subacute sclerosing leukoencephalitis, part I: clinicopathological, electron microscopic and virological observations. *J Neuropathol Exp Neurol* 1966;**25**:560–81.
429. ter Meulen V, Muller D, Kackell Y, et al. Isolation of infectious measles virus in measles encephalitis. *Lancet* 1972;**2**:1172–5.
430. Tidstrom B. Complications in measles with special reference to encephalitis. *Acta Med Scand* 1968;**184**:411–15.
431. Tishler M, Abramov AL. Liver involvement in measles infection of young adults. *Isr J Med Sci* 1983;**19**:791–3.
432. Tishon A, Lewicki H, Andaya A, et al. CD4 T cell control primary measles virus infection of the CNS: regulation is dependent on combined activity with either CD8 T cells or with B cells: CD4, CD8 or B cells alone are ineffective. *Virology* 2006;**347**:234–45.
433. Tuokko H, Salmi A. Detection of IgM antibodies to measles virus by enzyme-immunoassay. *Med Microbiol Immunol* 1983;**171**: 187–98.
434. Turner A, Jeyaratnam D, Haworth F, et al. Measles-associated encephalopathy in children with renal transplants. *Am J Transplant* 2006;**6**:1459–65.

435. Tyler HR. Neurological complications of rubeola (measles). *Medicine (Baltimore)* 1957;**36**:147–67.
436. Udem SA, Cook KA. Isolation and characterization of measles virus intracellular nucleocapsid RNA. *J Virol* 1984;**49**:57–65.
437. Van Binnendijk RS, Van Den Hof S, Van Den Kerkhof H, et al. Evaluation of serological and virological tests in the diagnosis of clinical and subclinical measles virus infections during an outbreak of measles in The Netherlands. *J Infect Dis* 2003;**188**:898–903.
438. Van den Hof S, Van Gageldonk-Lafeber AB, Van Binnendijk RS, et al. Comparison of measles virus-specific antibody titres as measured by enzyme-linked immunosorbent assay and virus neutralisation assay. *Vaccine* 2003;**21**:4210–14.
439. Vandvik B, Norrby E. Oligoclonal IgG antibody response in the central nervous system to different measles virus antigens in subacute sclerosing panencephalitis. *Proc Natl Acad Sci U S A* 1973;**70**:1060–3.
440. Vandvik B, Norrby E, Nordal HJ, et al. Oligoclonal measles virus-specific IgG antibodies isolated from cerebrospinal fluids, brain extracts, and sera from patients with subacute sclerosing panencephalitis and multiple sclerosis. *Scand J Immunol* 1976;**5**: 979–92.
441. Varavithya W, Stoecker B, Chaiyaratana W, et al. Vitamin A status of Thai children with measles. *Trop Geogr Med* 1986;**38**:359–61.
442. Vestergaard M, Hviid A, Madsen KM, et al. MMR vaccination and febrile seizures: evaluation of susceptible subgroups and long-term prognosis. *JAMA* 2004;**292**:351–7.
443. Waksman BH, Burnstein T, Adams RD. Histologic study of the encephalomyelitis produced in hamsters by a neurotropic strain of measles. *J Neuropathol Exp Neurol* 1962;**21**:25–49.
444. Walker RW, Thompson EJ. The cerebrospinal fluid in subacute sclerosing panencephalitis and multiple sclerosis. *Prog Brain Res* 1983;**59**:375–90.
445. Ward BJ, Johnson RT, Vaisberg A, et al. Spontaneous proliferation of peripheral mononuclear cells in natural measles virus infection: identification of dividing cells and correlation with mitogen responsiveness. *Clin Immunol Immunopathol* 1990;**55**:315–26.
446. Waters DJ, Bussell RH. Isolation and comparative study of the nucleocapsids of measles and canine distemper viruses from infected cells. *Virology* 1974;**61**:64–79.
447. Waters DJ, Hersh RT, Bussell RH. Isolation and characterization of measles nucleocapsid from infected cells. *Virology* 1972;**48**: 278–81.
448. Waterson AP. Measles virus. *Arch Gesamte Virusforsch* 1965;**16**: 57–80.
449. Waterson AP, Cruickshank JG, Laurence GD, et al. The nature of measles virus. *Virology* 1961;**15**:379–82.
450. Watson JC, Hadler SC, Dykewicz CA, et al. Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recommend Rep* 1998;**47**(RR-8): 1–57.
451. Weibel RE, Caserta V, Benor DE, et al. Acute encephalopathy followed by permanent brain injury or death associated with further attenuated measles vaccines: a review of claims submitted to the National Vaccine Injury Compensation Program. *Pediatrics* 1998;**101**:383–7.
452. Weiner LB, Corwin RM, Nieburg PI, et al. A measles outbreak among adolescents. *J Pediatr* 1977;**90**:17–20.
453. Welliver RC, Cherry JD, Holtzman AE. Typical, modified, and atypical measles. *Arch Intern Med* 1977;**137**:39–41.
454. Wells MW. The seasonal patterns of measles and chicken pox. *Am J Epidemiol* 1944;**40**:279–317.
455. Whittle HC, Dossetor J, Oduloju A, et al. Cell-mediated immunity during natural measles infection. *J Clin Invest* 1978;**62**: 678–84.
456. Wilhelm DJ, Paegle RD. Thrombocytopenic purpura and pneumonia following measles vaccination. *Am J Dis Child* 1967;**113**: 534–7.
457. Wilkins J, Wehrle PF. Evidence for reinstatement of infants 12 to 14 months of age into routine measles immunization programs. *Am J Dis Child* 1978;**132**:164–6.
458. Wilson EB, Worcester J. Contact and measles. *Proc Natl Acad Sci U S A* 1941;**27**:7–13.
459. Wilson GS. Measles as a universal disease. *Am J Dis Child* 1962;**103**:219–23.
460. Winkelstein W Jr, Karzon DT, Rush D, et al. A field trial of inactivated measles virus vaccine in young school children: protection during 27 months of follow-up. *JAMA* 1965;**194**:494–8.
461. Wolinsky JS, Swoveland P, Johnson KP, et al. Subacute measles encephalitis complicating Hodgkin's disease in an adult. *Ann Neurol* 1977;**1**:452–7.
462. World Health Organization. Expanded programme on immunization: Global Advisory Group. Part I. *Wkly Epidemiol Rec* 1990;**65**: 5–12.
463. World Health Organization. *Global immunization vision and strategy 2006-15*. Available at http://www.who.int/vaccines-documents/docspdf05/givs_final_en.pdf; 2005.
464. Wright GP, Wright HP. The influence of social conditions upon diphtheria, measles, tuberculosis and whooping cough in early childhood in London. *J Hyg (Lond)* 1942;**42**:451–73.
465. Wynne JM, Williams GL, Ellman BA. Gangrene of the extremities in measles. *S Afr Med J* 1977;**52**:117–21.
466. Yamanouchi K, Egashira Y, Uchida N, et al. Giant cell formation in lymphoid tissues of monkeys inoculated with various strains of measles virus. *Jpn J Med Sci Biol* 1970;**23**:131–45.
467. Yanagi Y, Takeda M, Ohno S, et al. Measles virus receptors. *Curr Top Microbiol Immunol* 2009;**329**:13–30.
468. Yeager AS, Davis JH, Ross LA, et al. Measles immunization: successes and failures. *JAMA* 1977;**237**:347–51.
469. Yorke JA, London WP. Recurrent outbreaks of measles, chickenpox and mumps, part II: Systematic differences in contact rates and stochastic effects. *Am J Epidemiol* 1973;**98**:469–82.
470. Young LW, Smith DI, Glasgow LA. Pneumonia of atypical measles: residual nodular lesions. *Am J Roentgenol Radium Ther Nucl Med* 1970;**110**:439–48.
471. Zahradnik JM, Cherry JD, Rachelefsky G. Atypical measles acquired abroad: foreign travel and pseudoexotic disease. *JAMA* 1979;**241**:1711–12.
472. Ziegra SR. Corticosteroid treatment for measles encephalitis. *J Pediatr* 1961;**59**:322–3.
473. Ziola B, Lund G, Meurman O, et al. Circulating immune complexes in patients with acute measles and rubella virus infections. *Infect Immun* 1983;**41**:578–83.

noted that mumps occurred throughout the world and that it was a major cause of morbidity in Confederate troops during the American Civil War. In the first half of the 20th century, investigators recognized that mumps virus infection involved multiple organs, and the causative agent was shown by Johnson and Goodpasture in 1934[52,93] to be a filterable virus.

The growth of mumps virus in embryonated eggs was reported in 1945, and 10 years later its propagation in tissue culture was noted.[72,78] This latter development led to the development and licensure of a live attenuated mumps vaccine in 1967.

CLASSIFICATION

Mumps virus is a member of the genus *Rubulavirus*, subfamily *Paramyxovirinae*, and family *Paramyxoviridae*.[115] It contains a single-stranded, nonsegmented, negative-sense RNA genome that is surrounded by a helical nucleocapsid and a surface envelope.

PROPERTIES

Physical Properties[19,101,115]

The virus generally is spherical, but marked pleomorphism occurs. Its size varies from 100 to 600 nm. The mumps viral genome is 15,384 nucleotides in length and encodes for nine known viral proteins: a nucleocapsid-associated protein (NP), a phosphoprotein (P), a membrane or matrix protein (M), a fusion protein (F), a hydrophobic membrane-associated protein (SH), a hemagglutinin-neuraminidase (HN), a polymerase protein (L), and nonstructural proteins within the P protein (I and V, which counteracts the interferon [IFN]-induced host antiviral response).[189] The genes for these proteins have been sequenced, and the gene order is 3-NP-P-M-F-SH-HN-L-5. The viral envelope is studded with 12- to 15-nm projections that contain either of the two structural glycoproteins (HN or F).

The P structural protein is associated with the nucleocapsid, and an RNA-dependent RNA polymerase is located within the nucleocapsid structure. The envelope has a high lipid content, and it contains the M protein.

The SH protein gene is the most variable region of the genome and, therefore, can be used to differentiate viral strains.[2,19,107,135,165] Analysis of the variations in SH gene nucleotide sequences has been used to study outbreaks, identify vaccine viruses, study vaccine adverse events, and identify new viral strains. Antigenic differences between vaccine strains result in variable cross-neutralization of unknown significance.[127]

Mumps virus infectivity is destroyed by heat (56° C for 20 minutes), and its infectivity is reduced by ultraviolet light, Tween 80, ether, and formalin. The virus is stable at 4° C for several days; and when placed in a buffered salt solution (such as Hanks) with 1 to 2 percent inactivated fetal calf serum, it can be stored indefinitely at –70° C.

Antigenic Composition

The three major antigenic components of mumps virus are the two glycoproteins (HN or V antigen and F protein) and the nucleocapsid protein (NP or S antigen).[101] The glycoproteins that project from the viral surface are the antigenic target for specific antibodies. Host antibodies to HN and F proteins confer protective immunity against the virus. Mumps viral particles agglutinate erythrocytes of several mammalian and avian species (human, avian, rodent, and simian); at 37° C, the virus causes partial hemolysis of susceptible erythrocytes when it is attached to cellular surface receptors. Specific antibody blocks hemagglutination, hemadsorption, and hemolysis.

Mumps virus is considered to have a single immunotype. However, polyclonal antibodies to parainfluenza and Newcastle disease viral antigens cross react with antibodies to mumps virus in complement-fixation and hemagglutination-inhibition assays. With monoclonal antibodies, an antigenic relationship between the NH and NP proteins of Sendai virus (a murine parainfluenza type 1 virus) and mumps virus has been demonstrated.[136]

Although mumps is a monotypic virus, genetic variation between strains exists. A standardized nomenclature and an analysis protocol have been proposed for the genetic characterization of mumps strains to facilitate the expansion of molecular epidemiologic studies.[92] This nomenclature includes 13 genotypes (A to M) based on the nucleotide sequence of the hydrophobic (SH) gene. The 2004-2005 UK outbreak and the 2006 USA outbreak were of serotype G,[29,148] whereas the F serotype is most prevalent in recent Chinese outbreaks.[118] Historically, C, D, E, G, and H predominate in the Western hemisphere and B, F, and I occur in Asia.[88] The Western hemisphere tends to use vaccines to the A serotype.[189]

Tissue Culture Growth and Animal Susceptibility

Mumps virus can be propagated in many different primary and cell-line tissue cultures.[101] To isolate the virus, primary monkey kidney cells generally are used. Its cytopathic effect is similar to that of other paramyxoviruses. When stained, multinucleated giant cells and cytoplasmic eosinophilic inclusions may be observed. In culture, the addition of erythrocytes results in hemadsorption to surface virus.

Mumps virus infects monkeys, rabbits, dogs, cats, and rodents. The virus is isolated readily after inoculation of the amniotic sac of 7- to 8-day-old chicken embryos.

EPIDEMIOLOGY

Incidence

In the United States, mumps was a reportable disease from 1922 to 1950 and has been again since 1967. Between 1950 and 1967, incidence data were gathered from voluntary reporting by cooperating states. Incidence

data from 1922 to 1982 are presented in Figure 180-1, and vaccine-era data are presented in Figure 180-2.[22,35] In the pre-vaccine era, mumps was a yearly disease, with epidemic peaks occurring approximately every 4 years. The peak epidemic year was 1944, when the rate was 250 per 100,000 population.

In the pre-vaccine era, mumps was a disease predominantly of young children.[22] However, outbreaks of mumps were a significant problem in young adults in the military.[67,123] The age distribution of mumps in the United States during selected years is presented in Table 180-1. After the mumps vaccine was licensed in 1967, mumps remained a disease of predominantly young children from 1967 to 1971. However, by 1981, most reported patients were 10 years or older. In 1987, 76 percent of the cases occurred in persons 10 years or older (see Table 180-1). In 1994, 1537 cases were reported, and of those with age noted, 21.8 percent were in patients 20 years or older. The increase in reported cases that occurred in 1987 (see Fig. 180-2 and Table 180-1) was the result of a marked increase in cases in nonimmunized persons aged 10 to 19 years. This group had been protected for a time by herd immunity because of the rapidly declining incidence of mumps as a result of routine vaccine use beginning in 1977.[41] In 1998, only 666 cases of mumps were reported in the United States.[26] Nineteen percent of the patients were younger than 5 years old, and 36 percent were 15 years or older. In 2010, 54.6 percent of

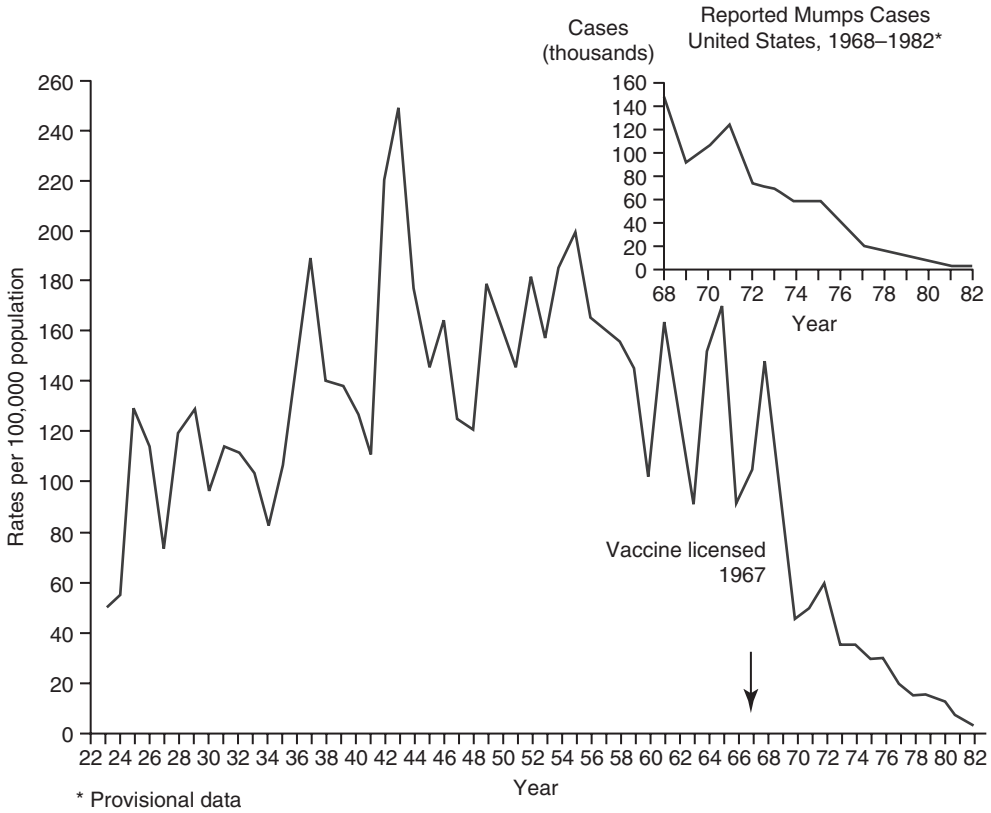

FIGURE 180-1 ■ Incidence of reported mumps in the United States from 1922 to 1982 and the number of reported cases from 1968 to 1982. (From Centers for Disease Control and Prevention: Mumps—United States, 1983-1984. *MMWR Morb Mortal Wkly Rep* 1984;**33**:533-5.)

TABLE 180-1 **Age Distribution of Mumps Cases in the United States During Selected Years**

	*1967-1971**		*1987*		*1994*			*2010*	
Age Group (yr)	CASES	%	CASES	%	CASES	%	**Age Group (yr)**	CASES	%
<5	2,932	17.1	804	6.5	250	17.4	<5	297	11.4
5-9	10,413	60.8	2,196	17.9	473	33.0	5-14	884	33.8
10-14	2,372	13.8	4,567	37.3	271	18.9	15-24	767	29.4
15-19	1,418†	8.3	3,455	28.2	128	8.9	≥25	657	25.2
≥20			1,235	10.1	312	20.8			

*Average annual reported cases for California, Massachusetts, and New York City.
†Includes all reported cases in patients 15 years or older.
Data from references 22, 35, and 174.

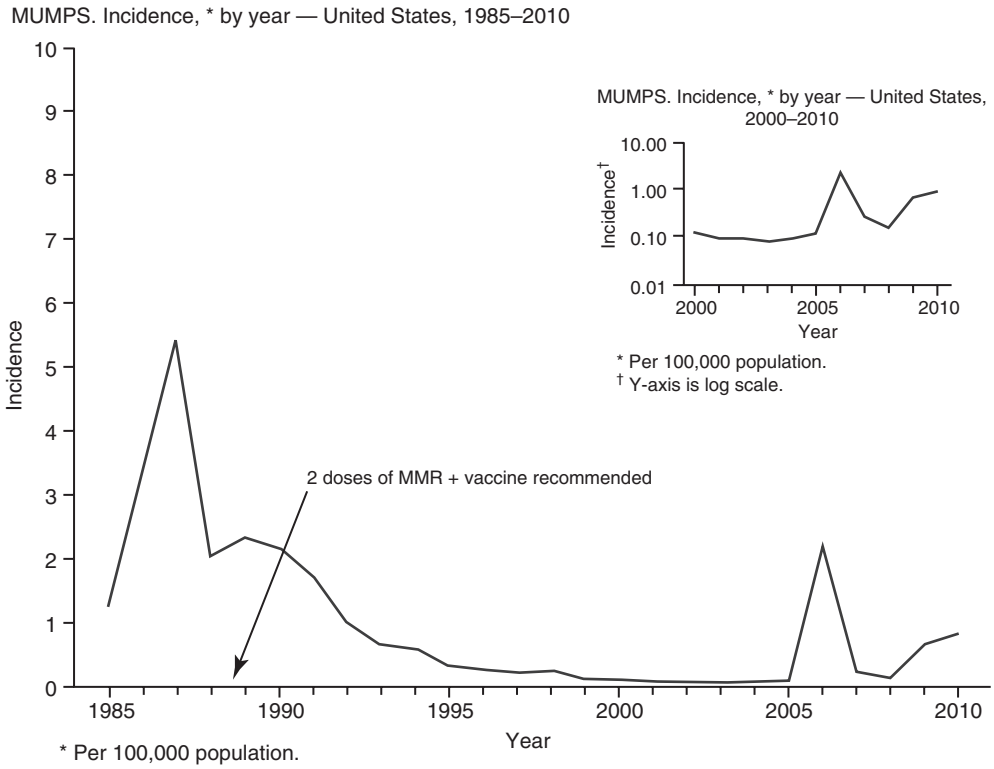

FIGURE 180-2 ■ **Incidence of reported mumps in the United States from 1985 to 2010.** (Modified from Centers for Disease Control and Prevention: Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:74.)

the reported cases were in persons 15 years of age or older (see Table 180-1).

In the pre-vaccine era, the peak incidence of mumps occurred in the winter and spring months, and this peak continued well into the vaccine era.[21,67,105] After licensure of the mumps vaccine, reported cases in the United States dropped from 152,209 cases (87.9 cases per 100,000 persons) in 1968 to less than 300 cases (<0.1 case per 100,000 persons) from 2001 through 2005.[20,28] However, a 2006 outbreak in primarily Midwestern college student populations revealed a dramatic resurgence of disease.[28,30,31] By March 28, 2006, a total of 219 mumps cases were reported in Iowa alone; this was a 44-fold increase in cases from the preceding decade. Of the 219 cases, the median patient age was 21 years (range, 3 to 85 years), with 48 percent of the patients aged 17 to 25 years old; 30 percent of the patients were known to be college students. This outbreak in Iowa in December 2005 was found to involve 10 additional states by May 2, 2006.[31] A total of 6584 cases were reported nationwide.[27,49] There were 85 hospitalizations, 85 percent of patients lived in eight contiguous Midwestern states, and 83 percent were college students. Sixty-three percent of the case patients with known vaccination status had received two doses of either mumps or mumps-measles-rubella (MMR) vaccine, and affected colleges reported 84 percent two-dose MMR vaccine coverage. The year 2006 represented a historical national two-dose coverage peak of 87 percent among adolescents.[10,44,49,120]

In 2004 to 2005, a mumps epidemic occurred in the United Kingdom.[29] There were 56,390 reported cases. In 2004, 79.1 percent of the confirmed cases were in persons aged 15 to 24 years, and two thirds of all cases occurred in persons who had not been vaccinated. In many respects, this epidemic is similar to the one in the United States in 1987; herd immunity had protected this adolescent and young adult cohort for a number of years.

A 2009-2010 outbreak in the New York/New Jersey area revealed 1,494 cases linked to a boy who returned from the United Kingdom in the midst of an epidemic (Fig. 180-3). Among those whose vaccinations were reported, 88 percent had received at least one dose of mumps-containing vaccine and 75 percent had received two doses of mumps vaccine.[34]

Worldwide, recent outbreaks have occurred in the Netherlands (2007-2009[187] and 2009-2010[56,186]), Canada (2007-2009[187] and 2009-2010[179]), Spain (2007-2008[66]), Poland (2009[138]), Bosnia/Herzegovina (2010-2011[87]), Israel (2009-2010),[128,163] Scotland (2010-2011[178]), Moldova (2007-2008[153]), Macedonia (2008-2009[113]), Australia (2007-2008[9]), the United Kingdom (2003-2006[91]), Luxembourg (2008[126]), and Palestinian refugee camps of the West Bank (2003-2005[84]).

Morbidity and Mortality

The most common clinical manifestations of mumps virus infection are fever and parotitis. However, approximately 30 percent of infected persons do not have parotitis and therefore are not recognized.[104] Epididymo-orchitis occurs in 20 to 30 percent of clinical cases in postpubertal male patients. Approximately 60 percent of

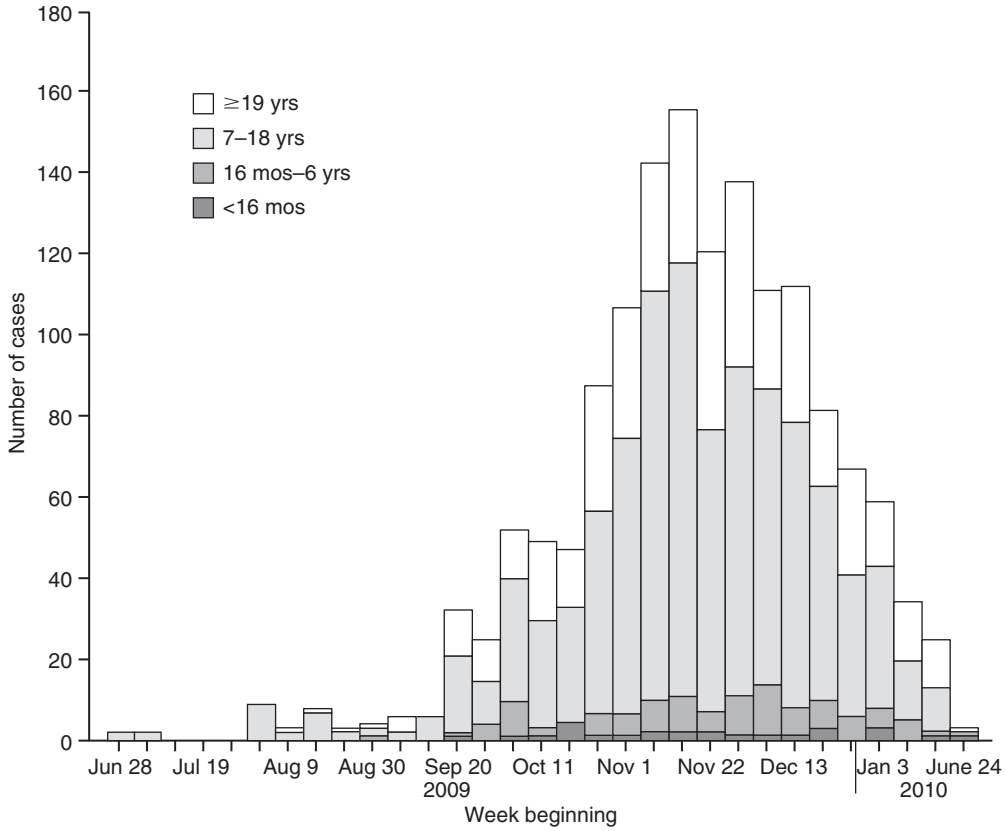

FIGURE 180-3 ■ The number (n = 1494) of reported confirmed or probable mumps cases by week of illness onset and age group in New York and New Jersey during an outbreak that occurred from June 2009 to January 2010. The outbreak-related mumps cases were reported from several counties in New York and New Jersey. (From Centers for Disease Control and Prevention: Update: mumps outbreak—New York and New Jersey, June 2009–January 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:125-9.)

clinical mumps cases involve cerebrospinal fluid (CSF) pleocytosis, but only one sixth of these patients have meningeal symptoms.[8] In 1966, 628 cases of encephalitis (0.5%) were reported, and of these, 10 (1.6%) had a fatal outcome.[23] Encephalitis occurs more frequently in male patients (61%), and the rate of occurrence is greatest in adults. Deafness after a case of mumps has been estimated to occur in 0.5 to 5.0 per 100,000 cases.[55,177]

From 1966 to 1975, 200 mumps-associated deaths were reported in the United States.[23] Of these, 44 (22%) were in patients with encephalitis; in the others, the causes of death were not identified. Forty-one percent of the deaths occurred in adults 40 years or older. During the 10-year period from 1988 through 1997, only 8 deaths caused by mumps were reported.[26]

In the 2006 mumps outbreak in the United States there were 85 hospitalizations (2%) but no deaths were reported. Parotitis or other salivary gland inflammation was reported in 99 percent, nonspecific symptoms (headache, myalgia, or fatigue) in 57 percent, and fever in 29 percent. Overall complications were 5 percent, with orchitis most prevalent. Encephalitis and meningitis occurred in less than 1 percent.[49]

Spread of Infection

Mumps virus is contagious in nonimmune persons. It is spread from an infected person to a new host by the respiratory route. The virus can be isolated from the saliva of infected patients from 7 days before the onset of parotitis to 9 days after onset.[101] Transmission is greatest during a 7-day period beginning 2 days before the onset of parotitis. Asymptomatically infected persons also can transmit the virus. The finding that outbreaks of mumps occurred in young adult populations in the pre-vaccine era suggests that mumps virus is less contagious than is measles; a significant number of persons passed through childhood without being infected with mumps virus. A serologic study reported by Black[13] in 1964 noted that 24 percent of army recruits lacked hemagglutination-inhibition antibody to mumps whereas only 1 percent lacked measles antibody. The incubation period usually is 16 to 18 days, although it can vary from 12 to 25 days.[101]

PATHOGENESIS

After respiratory or perhaps oral acquisition of the virus occurs, primary viral replication occurs in the upper respiratory mucosal epithelium.[57,101,188] Virus multiplies and is spread by drainage to local lymph nodes.[57] Subsequently, viremia develops.[96,137,173] As a result of viremia, infection occurs in multiple secondary infection sites. Most prominent is infection of the salivary glands, which results in inflammation and swelling. This infection

causes virus shedding for 1 to 2 weeks. Other secondary sites of infection include the inner ear (cochlea), pancreas, heart, nervous system (meninges and brain), joints, kidneys, liver, gonads, and thyroid.

PATHOLOGY

In the salivary glands, virus infects the ductal epithelium and causes periductal interstitial edema and a local inflammatory reaction involving lymphocytes and macrophages.[185,188] Tissue damage ensues, and the involved cells desquamate. Virus enters the central nervous system (CNS) by the choroid plexus through infected mononuclear cells. Virus multiplies in choroid and ependymal cells on the ventricular surfaces, and these cells desquamate into the CSF and result in meningitis. In encephalitis, perivascular infiltration with mononuclear cells, scattered foci of neuronophagia, and microglial rod-cell proliferation occur.[16] Periventricular demyelination also takes place. In the male gonad, the primary site of viral replication is the seminiferous tubules, where infection results in lymphocytic infiltration and edema of interstitial tissue.

Immunologic Events

Infection gives rise to serum antibodies to the HN glycoprotein (V antigen), the F antigen, and the NP protein (S antigen).[101] Antibody to NP protein develops first, 3 to 7 days after the onset of symptoms. Antibodies to NP protein are short lived and usually are absent after 6 months; they cross react with parainfluenza viruses. Antibodies to HN glycoprotein develop 2 to 4 weeks after the onset of illness and persist for long periods after infection.

Immunoglobulin (Ig) G and IgM antibody responses (determined by enzyme-linked immunosorbent assay [ELISA]) regularly occur after infection.[172] IgG antibody levels measured by ELISA correlate best with those derived by complement-fixation and hemolysis-in-gel assays. ELISA results are less specific than are those achieved with the plaque-reduction neutralization assay because of the high rate of cross-reacting antibodies against parainfluenza viruses detected by ELISA.[19,121,143] IgM antibodies develop early in the course of infection (second day of illness), peak within the first week of illness, and usually are undetectable 3 months after the onset of illness; occasionally, mumps-specific IgM antibody persists for 5 to 6 months.[101,172] Mumps-specific IgG antibody appears at the end of the first week of illness, peaks 3 weeks later, and persists throughout life. The IgG response is mainly the IgG1 subclass; a minor IgG3 subclass response also occurs.[19,152] Salivary IgA antibodies to mumps virus regularly appear after infection.[61]

During infection with vaccine virus, the cell-mediated response to tuberculin is diminished for up to 4 weeks.[108] During the same period, a mumps-specific, cell-mediated immune response develops.[101,188] This response has been demonstrated by skin test hypersensitivity, in vitro lymphocyte proliferative responses, and cytotoxic T-lymphocyte studies.[15,17,103]Asano and colleagues demonstrated a significant increase in interleukin (IL)-8, IL-10, IL-12, IL-13, and IFN-γ levels isolated from Japanese patients with mumps meningitis when compared with other cases of viral meningitis, suggesting a distinct immunologic response.[5]

In general, immunity against recurrent disease has been thought to be lifelong. However, Gut and associates[71] noted 26 patients with mumps who had a previous history of mumps and antibody response patterns suggestive of reinfection and not primary infection. Specifically, these patients had a rise in IgG but not IgM titers.

CLINICAL MANIFESTATIONS

In epidemics of mumps, cases can be separated into five groups of patients: (1) those with a short course whose signs and symptoms are nonspecific, (2) those in whom the disease is full blown with salivary swelling but no complications, (3) those with severe mumps and complications (epididymo-orchitis or meningoencephalitis, or both, or other complications), (4) those with no apparent symptoms but with typical antibody responses, and (5) those with meningoencephalitis or orchitis but without involvement of the salivary glands.[164] Approximately 75 percent of all cases of apparent mumps in children are of the full-blown type without complications. Involvement of the gonads rarely occurs before puberty.

Typical Mumps Without Complications

The average case in children has a prodromal period of 1 to 2 days that consists of fever, anorexia, headache, vomiting, and generalized aches and pains. The headache often is particularly marked and probably is caused by mild meningoencephalitis.[59] The temperature usually rises slowly to 38.9°C or 39.4°C (102°F or 103°F) as the disease becomes full blown, but at times fever is only slight or absent.[164]

After the prodromal period, one or both parotid glands begin to enlarge (Fig. 180-4). Mumps is bilateral in 70 to 80 percent of cases. A few days to a week or more may intervene between the swelling of the two sides. A distinctive "puckering" sensation is experienced at the angle of the jaw in the early stage, and it may be increased by the application of sour liquids, such as lemon juice or vinegar, to the tongue. This sign, when present, may be useful for early establishment of the diagnosis. The swelling of the gland also is distinctive in that a brawny type of edema occurs about the parotid gland, the borders of which are not discrete, in contrast to the discrete swelling typical of lymphadenitis, in which the node generally is outlined easily. The lobe of the ear is in the center of the swelling, which usually cannot be separated by palpation from the angle of the mandible. Pressure is painful, and opening the jaw often is difficult.

The swelling of an individual gland reaches its maximum in approximately 3 days, remains at its peak for approximately 2 days, and then slowly recedes. The extent of the swelling varies considerably but at times is sufficient to distort the outline of the face and head completely. The submaxillary and sublingual glands may

FIGURE 180-4 ■ Note the swelling on the left side of the face related to parotitis secondary to mumps virus infection. The left ear protrudes from the side of the head.

be involved separately or with the parotids in any combination.

During the prodromal phase, slight redness of the orifices of the Stensen or Wharton ducts, when present, has diagnostic significance. The amount of saliva usually is unchanged, although the mouth may be dry or salivation may be extreme. Gellis and Peters[65] described a few cases with edema over the upper part of the sternum, apparently caused by pressure on the lymphatics in the neck.

In uncomplicated mumps, the white blood cell count usually is low, with a slight relative lymphocytosis. The serum amylase value typically is elevated.

Meningitis, Meningoencephalitis, and Encephalitis

Meningitis and mild meningoencephalitis are the most frequent complications of mumps in children. Azimi and Cramblett[6] reviewed 51 children with mumps meningoencephalitis who were admitted to Columbus Children's Hospital between July 1964 and December 1967. Of this group of patients, fever occurred in 94 percent, vomiting in 84 percent, nuchal rigidity in 71 percent, lethargy in 69 percent, parotid swelling in 47 percent, headache in 47 percent, convulsions in 18 percent, abdominal pain in 14 percent, sore throat in 8 percent, diarrhea in 8 percent, and delirium in 6 percent.

Clinical findings in neurologic illness caused by mumps virus infection differ according to the age of the patient. Meningeal signs are recognized more readily in older children, adolescents, and adults, whereas nonspecific findings such as drowsiness and lethargy occur more frequently in young children.[188] Although seizures develop in 20 to 30 percent of hospitalized patients, electroencephalographic results usually are normal. Even in patients with severe obtundation, the electroencephalogram reveals only diffuse slowing with increased voltage. Focal findings are rare. The outlook in mumps meningoencephalitis generally is good and usually is better than in encephalitis of other viral causes (see Chapter 36). Even patients with profound obtundation generally recover without having residual damage. Rarely, deaths do occur, however.[26]

In the typical case, CSF has normal glucose and elevated protein levels and pleocytosis. Glucose levels are slightly low in approximately 20 percent of cases. At the onset of symptoms, modest mononuclear pleocytosis is present. The CSF cell count peaks on the third day of illness; counts average 250/mm^3, but counts greater than 1000/mm^3 are not uncommon. Mumps meningitis usually develops in patients with parotitis approximately 5 days after the onset of illness, but CNS findings can precede the parotid findings and can develop without any salivary gland involvement.

Herndon and colleagues[80] noted that ependymitis regularly occurs in cases of mumps meningitis. A rare complication of mumps appears to be acquired aqueductal stenosis, and researchers have suggested that it may be caused by the preceding ependymitis.[80,132,162,170] In one reported fatal case, hydrocephalus developed within 5 days of onset of disease.[134] More recently, acute hydrocephalus associated with mumps meningoencephalitis was noted in an 8½-year-old boy.[3] This child received a ventriculoperitoneal shunt and at follow-up had no neurologic deficit.

A 3-year-old boy with mumps cerebellitis, nystagmus, and focal localization of brain lesions noted by electroencephalography was described.[119] Also reported were facial palsy in a 3-year-old Japanese boy and transverse myelitis in an adult.[53,176] A case in a girl aged 4 years and 3 months who had brain stem encephalitis and acute disseminated encephalomyelitis after mumps has been reported.[160] This child responded rather dramatically to treatment with glucocorticoids and intravenous immunoglobulin. Haginoya and associates[73] reported the case of a 14-year-old Japanese girl who had chronic progressive mumps virus encephalitis. This child had an illness similar to subacute sclerosing panencephalitis, and her antibody titers suggested that the infecting virus may have had a defect in the HN protein.

Gonadal Infection (Epididymo-Orchitis and Oophoritis)

Epididymo-orchitis and oophoritis almost never occur before puberty.[164] However, in adolescent boys and adult men, epididymo-orchitis is second only to parotitis as a manifestation of mumps virus infection.[104] Cases of orchitis have been reported in children as young as 3 years old.[147] In postpubertal male patients, orchitis develops in 30 to 38 percent with mumps.[11,142] The rate of orchitis is highest in those 15 to 29 years of age. The greatest number of cases occur during the second, third, and fourth decades of life. Approximately 80 percent of cases of epididymo-orchitis appear during the first 8 days of

involvement of the salivary gland, but a few cases occur a considerable time after the parotitis has subsided.[164]

The onset of testicular involvement usually is manifested as chills, recurrence of fever, and swelling of the testes. Pain over the renal area or in the lower part of the abdomen, bilateral or unilateral, may precede or accompany the orchitis. Occasionally, this pain, if on the right side, may suggest appendicitis.

The orchitis usually is unilateral, but bilateral involvement has been reported in 17 to 38 percent of cases.[11,142] Although atrophy may develop after orchitis, in those with unilateral involvement, sterility is not a concern. Sterility has resulted after some cases of bilateral orchitis, however. A 16-year-old adolescent with mumps epididymo-orchitis was found to have persistent mumps virus RNA in his semen for 40 days.[90] In follow-up studies, he was found to have antisperm antibodies. The development of malignant disease in affected testes also has been reported.[11,95]

Oophoritis occurs in approximately 7 percent of postpubertal female patients. Pelvic pain and tenderness are noted.[154]

Pancreatitis

In a retrospective survey of 2482 hospitalized patients with mumps, pancreatitis was noted in 75 (3%).[1] Cases occurred in children and adults, and pancreatitis was 1.6 times more common in male than in female patients. Severe involvement of the pancreas is a rare occurrence, but mild or subclinical infection may occur more frequently than recognized.[154] It may be unassociated with salivary gland manifestations and be misdiagnosed as gastroenteritis. Epigastric pain and tenderness are suggestive; they may be accompanied by fever, chills, vomiting, and prostration. A child with acute hemorrhagic pancreatitis and a pseudocyst caused by mumps virus infection has been reported.[58]

Diabetes Mellitus

Diabetes mellitus long has been suspected to be associated with antecedent mumps.[23,76] In experimental animals, mumps virus infection has been linked to hyperglycemia and histologic lesions of the pancreatic islets. Mumps virus can invade the human pancreas and can infect and destroy human and rhesus beta cells in vitro,[146] but pancreatic damage has not been documented in reported cases of diabetes that developed after mumps or mumps vaccination.[159]

In humans, many cases of temporal association have been described both in individuals and in siblings,[45,46,100,125,131] and outbreaks of diabetes mellitus a few months or years after outbreaks of mumps have been reported.[70,124,168] Although evidence has not established a causal association in these cases, a study in Surrey, England, suggested that a small proportion, if any, of diabetes cases that start in childhood (only 15 of the 1663 patients in the study [<1%]) may have resulted from a recent mumps virus infection.[63] Antibody studies have shown fewer positive titers for mumps in diabetic patients than in normal subjects, even in children.[151] Infection may contribute to the development of diabetes either by specifically damaging islet cells or by precipitating diabetes in patients whose disease is latent. Teng and colleagues[169] reported the case of an 11-year-old girl with mumps infection complicated by transient hyperinsulinemic hypoglycemia.

Nephritis

Viruria is a common occurrence in uncomplicated mumps, and mild abnormalities in renal function occur.[173] Severe and fatal nephritis was reported as a rare complication of mumps occurring 10 to 14 days after parotitis.[154] Fujieda and associates[62] reported the demonstration of mumps virus genomic RNA by polymerase chain reaction (PCR) in a renal biopsy specimen from a 5-year-old girl with IgA nephropathy. Baas and colleagues reported a case of a 56-year old renal transplant patient who developed acute irreversible transplant failure due to interstitial nephritis with mumps RNA detected in urine and renal biopsy.[7]

Deafness

Deafness is an important but rare complication of mumps virus infection.[21,67,105] Its incidence has been estimated at 0.5 to 5.0 per 100,000 cases of mumps.[41,152] However, the incidence rate of minor degrees of hearing impairment, such as high-tone hearing loss, is probably much higher.[38]

Mumps-associated deafness occurs with or without meningoencephalitis and may develop after asymptomatic infection.[28,55,130] The deafness usually is unilateral and often permanent. Twenty-two of 103 cases (21%) reviewed by Everberg[55] were bilateral. Mumps virus has been isolated from perilymph fluid in a case of sudden-onset, unilateral, complete deafness that began 2 days after the onset of mumps.[14,144] Vertigo also is noted occasionally in patients with mumps; it occurs most commonly in those in whom deafness develops.[89]

Mumps and Pregnancy

The incidence of mumps during pregnancy was estimated at 0.8 to 10 cases per 10,000 pregnancies[155] before vaccine was licensed in the United States. No vaccine-era data are available for comparison. Maternal complications such as mastitis,[142] aseptic meningitis,[14] and fatal glomerulonephritis[51] have been reported. Mumps virus has been isolated from human breast milk.[97]

Increased rates of fetal mortality were reported in women who contracted mumps during the first trimester. In a large prospective case-control study, a 27.3 percent rate of fetal wastage was noted in women with mumps during the first trimester versus a 13.0 percent rate in matched, healthy controls during the first trimester.[158] No significant differences in birth weight were noted among the live births.[157] Because fetal loss usually occurs in such cases within 2 weeks of development of maternal infection, investigators postulated that factors related to maternal gonadal infection with resulting hormonal changes may be responsible. A histopathologic study of

the products of conception in mothers with gestational mumps revealed severe proliferative necrotic villitis and vasculitis in the placenta and viral inclusions, as seen in mumps infection, in fetal tissues.[64] Mumps virus was isolated from a spontaneously aborted 10-week-old human fetus.[110]

No evidence has shown that gestational mumps in humans increases the risk of development of fetal malformations,[156] although a few cases of various congenital malformations with no consistent pattern have been reported.[86]

Other Manifestations

Other rare clinical manifestations include exanthem and enanthem,[37] arthritis,[68] myocarditis,[12] thrombocytopenia,[109,114,144] keratouveitus,[133] lower respiratory tract infection,[60] and other glandular involvement (thyroiditis, mastitis, dacryoadenitis, and bartholinitis).[104,140]

DIAGNOSIS

Differential Diagnosis

Not all patients with mumps have parotid swelling, and mumps virus is not the only cause of parotitis. Mumps virus infection must be considered in all children with aseptic meningitis, meningoencephalitis, and encephalitis (see Chapters 35 and 36). In addition to mumps virus infection, many other infectious agents and noninfectious conditions are associated with parotitis or parotid swelling (see Chapter 13). Other viruses that can cause parotitis include Epstein-Barr virus, coxsackieviruses and echoviruses, influenza A virus, parainfluenza viruses, cytomegalovirus, human herpesvirus–6, and lymphocytic choriomeningitis virus.[18,48] Purulent parotitis can be differentiated from mumps by the exquisite tenderness of the region, an elevated white blood cell count, and the observation of pus coming from the Stensen duct. Other viral causes of parotitis can be differentiated by the respective epidemiologic and clinical characteristics of specific agents and appropriate culture, serologic study, or both.

Enlargement of lymph nodes in proximity to the parotid gland must be differentiated from parotid enlargement (see Chapter 12). The cervical lymph nodes are below the ramus of the mandible. The preparotid nodes generally are anterior to the parotid, and their enlargement usually is associated with conjunctivitis. Occasionally, an enlarged lymph node within the parotid gland may cause some confusion. Lesions of the ramus of the mandible, such as osteomyelitis, occasionally have been mistaken for parotid enlargement. In this case, the enlargement usually is persistent.

DIAGNOSIS

In an epidemic situation, the diagnosis of mumps is clinically straightforward, and performing laboratory tests is unnecessary. The critical points are a history of exposure, an incubation period of 2 to 3 weeks, and a typical clinical picture consisting of fever and parotitis. In a sporadic case or in a previously vaccinated child, confirming the cause by laboratory study is important. Mumps virus, as well as most other viruses that cause parotitis, can be demonstrated readily by culture or nucleic acid detection from saliva, throat swabs, or mouth washings during acute illness. Oral fluid specimens can also be assayed for virus-specific IgM. In patients with meningoencephalitis, virus also can be recovered from CSF. Virus is isolated in primary monkey kidney tissue culture. In unusual cases in which the source of facial swelling is obscure, determination of a serum amylase level may be helpful; a high value indicates parotid involvement.

Mumps virus infection also can be confirmed by demonstrating a significant rise in antibody titer in paired serum specimens by complement fixation, hemagglutination inhibition, or ELISA. However, because mumps cross reacts with parainfluenza viruses, these methods are not ideal.[143] Mumps-specific IgM antibody determined by ELISA is the usual test performed; the presence of this antibody indicates a recent infection. Mumps-specific IgM antibodies determined by enzyme immunoassay in oral fluid also are useful for establishing the diagnosis.[180] Mumps virus RNA also can be detected directly from clinical samples by real-time PCR.[171] In outbreaks today, many cases occur in previously vaccinated individuals. Many of these vaccine failures are secondary and, therefore, there is less likely to be a mumps-specific serum IgM antibody response.[116,149] Therefore, greater diagnostic sensitivity will occur using culture or PCR assay.

TREATMENT

Conservative therapy is indicated in the treatment of mumps. Adequate attention given to hydration and alimentation of patients is important. Patients may have difficulty with acidic foods such as orange juice. In addition, orange juice may cause vomiting in an already nauseated patient. The diet should be light, with a generous offering of fluids.

Occasionally, analgesics are necessary to treat severe headache or discomfort caused by parotitis. Stronger analgesics such as codeine or meperidine (Demerol) rarely are required for headache but may be useful for orchitis. Vomiting seldom is sufficiently severe to require intravenous fluids. In these instances, however, electrolytes lost by vomiting should be repleted.

Although lumbar puncture seldom is necessary for establishing a diagnosis in patients with meningoencephalitis accompanying mumps, patients often indicate that they have experienced relief of headache after undergoing this procedure. No antiviral agent is appropriate or indicated for the treatment of mumps, which is a self-limited illness.

A potential new treatment for mumps orchitis specifically is IFN-α2b. In a small study of 12 patients, both symptom resolution and normospermia were improved versus results in untreated control subjects.[54,106]

PROGNOSIS

The overall prognosis in uncomplicated mumps is excellent. The outlook in meningoencephalitis also generally is favorable, but death and neurologic damage can occur. Deafness and sterility are rare complications.

PREVENTION

Immunization

A summary of the recommendations of the Advisory Committee on Immunization Practices (ACIP) and the Committee on Infectious Diseases of the American Academy of Pediatrics for the use of mumps vaccine follows.[4,32] For more complete information, the reader should consult the most recent ACIP statement or the Report of the Committee on Infectious Diseases of the American Academy of Pediatrics.

The mumps virus vaccine available in the United States (official name: mumps virus vaccine, live) is the Jeryl Lynn strain and is prepared in chick embryo cell culture. The vaccine produces a subclinical, noncommunicable infection with few side effects. Mumps vaccine is available in both a monovalent (mumps only) form and combinations: mumps-rubella, MMR, and measles-mumps-rubella-varicella (MMRV) vaccines.

In initial trials, mumps vaccine was approximately 95 percent efficacious in preventing mumps disease[83,98,166]; and after vaccination, measurable antibody developed in more than 97 percent of persons known to be susceptible to mumps.[184] Vaccine-induced antibody was protective and long lasting,[182,183] although it was of considerably lower titer than antibody resulting from natural infection.[184] The duration of vaccine-induced immunity is unknown, but serologic and epidemiologic data collected during 35 years of use of live vaccine suggested both persistence of antibody and continuing protection against infection. The epidemic occurrence of mumps in 2006 in the United States as well as in other countries resulted in further evaluations of vaccine efficacy and waning immunity.* In a study in England, the effectiveness of vaccine after one dose declined from 96 percent in 2-year-old children to 66 percent in children aged 11 to 12 years old.[42] In those children who had received two doses, the effectiveness declined from 99 percent in children aged 5 to 6 years old to 86 percent in children aged 11 to 12 years old. These data suggest that waning immunity contributes to outbreaks of mumps in older, previously vaccinated populations. Studies in Belgium and Korea also demonstrated waning immunity as contributing to outbreaks of mumps.[36] However, a small study in Wisconsin compared the mumps antibody titers of children who received their second MMR shot at age 4 to 6 (as is typical) with delaying until age 9 to 11. At age 17, both groups had similar titers.[117]

In Finland, a two-dose MMR vaccination program with the Jeryl Lynn mumps vaccine strain was launched in 1982.[141] This program was highly successful, and Finland was the first country documented to be free of indigenous mumps, as well as rubella and measles. In a study in Finland that was published in 2007, researchers found that previous vaccinees who were found to be seronegative nonetheless had mumps antigen-specific lymphoproliferative responses.[94]

In a review of 47 publications documenting the vaccination status of patients during 50 outbreaks from 1977 through 2008, Dayan and Rubin[50] calculated that the effectiveness of prior vaccination with one dose of vaccine ranged from 72.8 to 91 percent for the Jeryl Lynn strain, from 54.4 to 93 percent for the Urabe strain, and from 0 to 33 percent for the Rubini strain. Vaccine effectiveness after two doses of mumps vaccine was reported in three outbreaks and ranged from 91 to 94.6 percent.[150]

Clinical isolates sequenced from recent outbreaks have revealed genotypes distinct from those of vaccine viruses, raising concern that certain mumps virus strains may escape vaccine-induced immunity. Rubin and colleagues performed a study in which sera obtained from children 6 weeks after MMR vaccination were tested for the ability to neutralize genetically diverse mumps virus strains. Although antibody titers varied, all viruses were readily neutralized, arguing against immune escape.

The vaccination rates in the United States in 1999 through 2004 were 90 percent, which is at the low end of the estimated population immunity (90% to 92%) needed for herd immunity.[111] It appears that outbreaks have occurred when vaccination rates drop below that required for herd immunity, and both vaccinated and unvaccinated individuals are at risk of infection. At present, the contribution of waning immunity to outbreaks is not well understood and additional booster vaccinations are not routinely recommended in non-outbreak settings. Notably, the 2006 Midwestern U.S. outbreak occurred during a time of very high two-dose vaccine coverage, leading to the suggestion that a more effective vaccine or an updated vaccination policy was needed.[49]

General Recommendations

Susceptible children, adolescents, and adults should be vaccinated against mumps unless vaccination is contraindicated. Mumps vaccine is of particular value for children approaching puberty and for adolescents and adults who have not had mumps. MMR is the vaccine of choice for routine administration and should be used in all situations in which recipients also are likely to be susceptible to measles, rubella, or both. The favorable benefit-to-cost ratio for routine mumps immunization is more marked when vaccine is administered as MMR.[102,181] Persons should be considered susceptible to mumps unless they have documentation of (1) physician-diagnosed mumps, (2) adequate immunization with live mumps virus vaccine on or after their first birthday, or (3) laboratory evidence of immunity.

Persons who are unsure of their history of mumps disease or mumps vaccination should be vaccinated. No evidence has shown that persons who previously either received mumps vaccine or had mumps are at any

*References 15, 35, 42, 77, 94, 139, 175.

increased risk for developing local or systemic reactions from receiving live mumps vaccine. Testing for susceptibility before administering vaccination, especially in adolescents and young adults, is not necessary. In addition to the expense, some tests (e.g., mumps skin test, complement-fixation antibody test) may be unreliable and tests with established reliability (e.g., neutralization, enzyme immunoassay, radial hemolysis antibody test) are not readily available.

Dosage

Two doses of MMR vaccine separated by at least 1 month in the volume specified by the manufacturer should be administered subcutaneously.

Age

Live mumps virus vaccine is recommended at any age on or after the first birthday for all susceptible persons, unless a contraindication exists. In routine circumstances, mumps vaccine should be given in combination with measles and rubella vaccines as MMR, and the currently recommended schedule for administration of measles vaccine should be followed. It should not be administered to infants younger than 12 months old because persisting maternal antibody may interfere with seroconversion. To ensure that the patient has developed immunity, all persons vaccinated before their first birthday should be revaccinated on or after their first birthday.

Persons Exposed to Mumps

Use of Vaccine

When given after exposure to mumps, live mumps virus vaccine may not provide protection. However, if the exposure did not result in infection, vaccine should induce protection against infection from subsequent exposure. No evidence has indicated that the risk of vaccine-associated adverse events increases if vaccine is administered to persons incubating disease.

Use of Immunoglobulin

Immunoglobulin has not been demonstrated to be of established value in postexposure prophylaxis and is not recommended.

Adverse Effects of Vaccine Use

In field trials in the United States before vaccine was licensed, illnesses did not occur more often in vaccinees than in unvaccinated controls.[82] Reports of illnesses occurring after receipt of mumps vaccination have been episodes mainly of parotitis and low-grade fever. Allergic reactions, including rash, pruritus, and purpura, have been associated temporally with mumps vaccination but are uncommon and usually mild and of brief duration. The reported development of encephalitis within 30 days of receiving a mumps-containing vaccine in the United States (0.4 per million doses) is not greater than the observed background incidence rate of CNS dysfunction in the normal population. Other manifestations of CNS involvement in the United States, such as febrile seizures and deafness, also have been reported infrequently. Complete recovery usually occurs. Reports of nervous system illness occurring after receipt of mumps vaccination do not necessarily denote an etiologic relationship between the illness and the vaccine. In parts of Europe, Canada, and Japan, where different mumps vaccines (Leningrad 3 strain and Urabe Am 9 strain) have been used, rates of vaccine-induced aseptic meningitis have been high.[39,43,122,129,167] Orchitis is a very rare complication of mumps vaccination.[40] Most cases have occurred in adolescents and adults, and five different vaccine strains have been associated with orchitis.

Contraindications to Vaccine Use

Pregnancy

Although mumps vaccine virus has been shown to infect the placenta and fetus,[190] no evidence has indicated that it causes congenital malformations in humans. However, because of the theoretic risk of fetal damage, a prudent approach is to avoid giving live virus vaccine to pregnant women. Women should avoid becoming pregnant for 3 months after receiving vaccination. Routine precautions for vaccinating postpubertal women include asking whether they are or may be pregnant, excluding those who say that they are, and explaining the theoretic risk to those who plan to receive the vaccine. Being vaccinated during pregnancy should not be considered an indication for termination of pregnancy. However, the final decision about interruption of pregnancy must rest with the individual patient and her physician.

Severe Febrile Illness

Administration of vaccine should not be postponed because of minor or intercurrent febrile illnesses such as mild upper respiratory tract infections. However, vaccination of persons with severe febrile illnesses generally should be deferred until they have recovered.

Allergies

Because live mumps vaccine is produced in chick embryo cell culture, persons with a history of anaphylactic reactions (e.g., hives, swelling of the mouth and throat, difficulty breathing, hypotension, shock) after ingesting eggs should be vaccinated only with caution and according to published protocols.[69,79] Children known to be allergic should not leave the vaccination site for 20 minutes after being vaccinated. Evidence indicates that persons are not at increased risk if they have egg allergies that are not anaphylactic. Such persons may be vaccinated in the usual manner. No evidence has demonstrated that persons with allergies to chickens or feathers are at increased risk of having a reaction to the vaccine.

Because mumps vaccine contains trace amounts of neomycin (25 μg), persons who have experienced anaphylactic reactions to topically or systemically

administered neomycin should not receive mumps vaccine. Most often, allergy to neomycin is manifested as contact dermatitis, which is a delayed-type (cell-mediated) immune response rather than anaphylaxis. In such persons, the adverse reaction, if any, to 25 μg of neomycin in the vaccine would be an erythematous, pruritic nodule or papule at 48 to 96 hours. A history of contact dermatitis to neomycin is not a contraindication to receiving mumps vaccine. Live mumps virus vaccine does not contain penicillin.

Recent Immunoglobulin Injection

Passively acquired antibody can interfere with the response to live attenuated virus vaccines. Therefore, mumps vaccine should be given at least 2 weeks before the administration of immunoglobulin or be deferred for 3 to 11 months after the administration of immunoglobulin. The duration of deferral depends on the dose of immunoglobulin administered.[181]

Altered Immunity

In theory, replication of the mumps vaccine virus may be potentiated in patients with immune deficiency disease and by the suppressed immune responses that occur with leukemia, lymphoma, or generalized malignancy or with therapy with corticosteroids, alkylating drugs, antimetabolites, or radiation. In general, patients with such conditions should not be given live mumps virus vaccine. Because vaccinated persons do not transmit mumps vaccine virus, the risk of exposure to mumps in these patients may be reduced by vaccinating their close susceptible contacts.

An exception to these general recommendations is in children infected with human immunodeficiency virus (HIV): all asymptomatic HIV-infected children should receive MMR vaccine at 12 months of age.[24,181] If measles vaccine is administered to symptomatic HIV-infected children, the combination MMR vaccine generally is preferred.[25]

Patients with leukemia in remission whose chemotherapy has been terminated for at least 3 months also may receive live mumps virus vaccine. Short-term (<2 weeks' duration) corticosteroid therapy, topical corticosteroid therapy (e.g., nasal, skin), and intra-articular, bursal, or tendon injection with corticosteroids are not contraindications to the administration of mumps vaccine. However, mumps vaccine should be avoided if systemic immunosuppressive levels are reached by prolonged, extensive, topical application.

Live vaccines have historically been avoided in patients who have received bone marrow, stem cell, and solid-organ transplantation. An international consensus conference recommends two doses of the MMR vaccine for allogeneic hematopoietic stem cell transplant recipients starting 24 months after transplant and once no longer on immunosuppression.[81] A review by Danerseau and colleagues in 2008 concluded that there were insufficient published data to determine the safety and/or efficacy of live viral vaccines in transplant recipients still on immunosuppression.[47]

Containment of Disease

Containment is important in prevention of mumps in the United States. Mumps is a reportable disease, and compliance is the obligation of all physicians. After early reports of sporadic mumps cases, health department workers can organize local immunization clinics and exclude susceptible students from school so that disease can be contained in a small geographic area. Kutty and colleagues have published a discussion of containment strategies in wake of the 2006 U.S. outbreak.[112] Isolation of case patients was the primary outbreak control measure.

A 2008 CDC update has recommended isolation of infected persons for 5 days after onset of parotitis and for contact and droplet precautions during this time.[33] These recommendations were based on data from the 2006 Iowa outbreaks, which yielded information on the duration of virus shedding after symptom onset[145] as well as poor compliance with 9-day as compared with 5-day isolation regimens.[161]

NEW REFERENCES SINCE THE SIXTH EDITION

4. American Academy of Pediatrics. Mumps. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 464–8.
5. Asano T, Ichiki K, Koizumi S, et al. Enhanced expression of cytokines/chemokines in cerebrospinal fluids in mumps meningitis in children. *Pediatr Int* 2011;**53**:143–6.
7. Baas MC, van Donselaar KA, Florquin S, et al. Mumps: not an innocent bystander in solid organ transplantation. *Am J Transplant* 2009;**9**:2186–9.
9. Bangor-Jones RD, Dowse GK, Giele CM, et al. A prolonged mumps outbreak among highly vaccinated Aboriginal people in the Kimberley region of Western Australia. *Med J Aust* 2009; **191**:398–401.
10. Barskey AE, Glasser JW, LeBaron CW. Mumps resurgences in the United States: a historical perspective on unexpected elements. *Vaccine* 2009;**27**:6186–95.
34. Centers for Disease Control and Prevention. Update: mumps outbreak—New York and New Jersey, June 2009–January 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:125–9.
35. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
40. Clifford V, Wadsley J, Jenner B, et al. Mumps vaccine associated orchitis: evidence supporting a potential immune-mediated mechanism. *Vaccine* 2010;**28**:2671–3.
44. Cortese MM, Jordan HT, Curns AT, et al. Mumps vaccine performance among university students during a mumps outbreak. *Clin Infect Dis* 2008;**46**:1172–80.
47. Danerseau AM, Robinson JL. Efficacy and safety of measles, mumps, rubella and varicella live viral vaccines in transplant recipients receiving immunosuppressive drugs. *World J Pediatr* 2008;**4**: 254–8.
49. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. *N Engl J Med* 2008;**358**:1580–9.
50. Dayan GH, Rubin S. Mumps outbreaks in vaccinated populations: are available mumps vaccines effective enough to prevent outbreaks? *Clin Infect Dis* 2008;**47**:1458–67.
56. Fanoy EB, Cremer J, Ferreira JA, et al. Transmission of mumps virus from mumps-vaccinated individuals to close contacts. *Vaccine* 2011;**29**:9551–6.
66. Gonzalez PP, Barrios JA, Morales Serna JC. [Study of a population-wide epidemic outbreak of mumps virus G1 in Jerez de la Frontera (Spain)]. *Aten Primaria* 2012;**44**:320–7. In Spanish.
81. Hilgendorf I, Freund M, Jilg W, et al. Vaccination of allogeneic haematopoietic stem cell transplant recipients: report from the international consensus conference on clinical practice in chronic GVHD. *Vaccine* 2011;**29**:2825–33.

84. Hindiyeh MY, Aboudy Y, Wohoush M, et al. Characterization of large mumps outbreak among vaccinated Palestinian refugees. *J Clin Microbiol* 2009;**47**:560–5.
87. Hukic M, Ravlija J, Dedeic Ljubovic A, et al. Ongoing large mumps outbreak in the Federation of Bosnia and Herzegovina, Bosnia and Herzegovina, December 2010 to July 2011. *Euro Surveill* 2011;**16**(35).
88. Hviid A, Rubin S, Muhlemann K. Mumps. *Lancet* 2008;**371**: 932–44.
91. Jick H, Chamberlin DP, Hagberg KW. The origin and spread of a mumps epidemic: United Kingdom, 2003-2006. *Epidemiology* 2009;**20**:656–61.
111. Kutty PK, Kruszon-Moran DM, Dayan GH, et al. Seroprevalence of antibody to mumps virus in the US population, 1999-2004. *J Infect Dis* 2010;**202**:667–74.
112. Kutty PK, Kyaw MH, Dayan GH, et al. Guidance for isolation precautions for mumps in the United States: a review of the scientific basis for policy change. *Clin Infect Dis* 2010;**50**:1619–28.
113. Kuzmanovska G, Polozhani A, Mikik V, et al. Mumps outbreak in the former Yugoslav Republic of Macedonia, January 2008–June 2009: epidemiology and control measures. *Euro Surveill* 2010; **15**(23).
116. Latner DR, McGrew M, Williams N, et al. Enzyme-linked immunospot assay detection of mumps-specific antibody-secreting B cells as an alternative method of laboratory diagnosis. *Clin Vaccine Immunol* 2011;**18**:35–42.
117. LeBaron CW, Forghani B, Beck C, et al. Persistence of mumps antibodies after 2 doses of measles-mumps-rubella vaccine. *J Infect Dis* 2009;**199**:552–60.
118. Liang Y, Ma S, Liu L, et al. Identification and development of a promising novel mumps vaccine candidate strain. *Microbes Infect* 2010;**12**:1178–87.
120. Marin M, Quinlisk P, Shimabukuro T, et al. Mumps vaccination coverage and vaccine effectiveness in a large outbreak among college students—Iowa, 2006. *Vaccine* 2008;**26**:3601–7.
126. Mossong J, Bonert C, Weicherding P, et al. Mumps outbreak among the military in Luxembourg in 2008: epidemiology and evaluation of control measures. *Euro Surveill* 2009;**14**(7).
128. Muhsen K, Shohat T, Aboudy Y, et al. Sero-prevalence of mumps antibodies in subpopulations subsequently affected by a large scale mumps epidemic in Israel. *Vaccine* 2011;**29**:3878–82.
138. Paradowska-Stankiewicz I, Orlikova H. [Mumps in Poland in 2009]. *Przegl Epidemiol* 2011;**65**:185–7. In Polish.
145. Polgreen PM, Bohnett LC, Cavanaugh JE, et al. The duration of mumps virus shedding after the onset of symptoms. *Clin Infect Dis* 2008;**46**:1447–9.
148. Rota JS, Turner JC, Yost-Daljev MK, et al. Investigation of a mumps outbreak among university students with two measles-mumps-rubella (MMR) vaccinations, Virginia, September-December 2006. *J Med Virol* 2009;**81**:1819–25.
149. Royuela E, Castellanos A, Sanchez-Herrero C, et al. Mumps virus diagnosis and genotyping using a novel single RT-PCR. *J Clin Virol* 2011;**52**:359–62.
150. Rubin SA, Link MA, Sauder CJ, et al. Recent mumps outbreaks in vaccinated populations: no evidence of immune escape. *J Virol* 2012;**86**:615–20.
153. Schwarz NG, Bernard H, Melnic A, et al. Mumps outbreak in the Republic of Moldova, 2007-2008. *Pediatr Infect Dis J* 2010;**29**: 703–6.
161. Soud FA, Cortese MM, Curns AT, et al. Isolation compliance among university students during a mumps outbreak, Kansas 2006. *Epidemiol Infect* 2009;**137**:30–7.
163. Stein-Zamir C, Shoob H, Abramson N, et al. Mumps outbreak in Jerusalem affecting mainly male adolescents. *Euro Surveill* 2009; **14**(50).
178. Walker J, Huc S, Sinka K, et al. Ongoing outbreak of mumps infection in Oban, Scotland, November 2010 to January 2011. *Euro Surveill* 2011;**16**(8).
179. Walkty A, Van Caeseele P, Hilderman T, et al. Mumps in prison: description of an outbreak in Manitoba, Canada. *Can J Public Health* 2011;**102**:341–4.
186. Whelan J, van Binnendijk R, Greenland K, et al. Ongoing mumps outbreak in a student population with high vaccination coverage, Netherlands, 2010. *Euro Surveill* 2010;**15**(17).
187. Wielders CC, van Binnendijk RS, Snijders BE, et al. Mumps epidemic in orthodox religious low-vaccination communities in the Netherlands and Canada, 2007 to 2009. *Euro Surveill* 2011;**16**(41).
189. Xu P, Luthra P, Li Z, et al. The V protein of mumps virus plays a critical role in pathogenesis. *J Virol* 2012;**86**:1768–76.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. A retrospective survey of the complications of mumps. *J R Coll Gen Pract* 1974;**24**:552–6.
2. Afzal MA, Buchanan J, Heath AB, et al. Clustering of mumps virus isolates by SH gene sequence only partially reflects geographical origin. *Arch Virol* 1997;**142**:227–38.
3. Alp H, Tan H, Orbak Z, et al. Acute hydrocephalus caused by mumps meningoencephalitis. *Pediatr Infect Dis J* 2005;**24**:657–8.
4. American Academy of Pediatrics. Mumps. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 464–8.
5. Asano T, Ichiki K, Koizumi S, et al. Enhanced expression of cytokines/chemokines in cerebrospinal fluids in mumps meningitis in children. *Pediatr Int* 2011;**53**:143–6.
6. Azimi PH, Cramblett HG, Haynes RE. Mumps meningoencephalitis in children. *JAMA* 1969;**207**:509–12.
7. Baas MC, van Donselaar KA, Florquin S, et al. Mumps: not an innocent bystander in solid organ transplantation. *Am J Transplant* 2009;**9**:2186–9.
8. Bang HO, Bang J. Involvement of the central nervous system in mumps. *Bull Hyg* 1944;**19**:503–4.
9. Bangor-Jones RD, Dowse GK, Giele CM, et al. A prolonged mumps outbreak among highly vaccinated Aboriginal people in the Kimberley region of Western Australia. *Med J Aust* 2009; **191**:398–401.
10. Barskey AE, Glasser JW, LeBaron CW. Mumps resurgences in the United States: a historical perspective on unexpected elements. *Vaccine* 2009;**27**:6186–95.
11. Beard CM, Benson RC, Jr, Kelalis PP, et al. The incidence and outcome of mumps orchitis in Rochester, Minnesota, 1935 to 1974. *Mayo Clin Proc* 1977;**52**:3–7.
12. Bengtsson E, Orndahl G. Complications of mumps with special reference to the incidence of myocarditis. *Acta Med Scand* 1954; **149**:381–8.
13. Black FL. A nationwide serum survey of United States military recruits, 1962, part 3: measles and mumps antibodies. *Am J Hyg* 1964;**80**:304–7.
14. Bowers D. Mumps during pregnancy. *West J Surg Obstet Gynecol* 1953;**61**:72–3.
15. Bruserud O, Thorsby E. HLA control of the proliferative T lymphocyte response to antigenic determinants on mumps virus: studies of healthy individuals and patients with type 1 diabetes. *Scand J Immunol* 1985;**22**:509–18.
16. Bruyn HB, Sexton HM, Brainerd HD. Mumps meningoencephalitis; a clinical review of 119 cases with one death. *Calif Med* 1957;**86**:153–60.
17. Callaghan JT, Petersen BH, Smith WC, et al. Delayed hypersensitivity to mumps antigen in humans. *Clin Immunol Immunopathol* 1983;**26**:102–10.
18. Caplan CE. Mumps in the era of vaccines. *CMAJ* 1999;**160**: 865–6.
19. Carbone KM, Wolinsky JS. Mumps virus. In: Knipe DM, Howley PM, editors. Fields virology, **vol. 1**. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1381–400.
20. Centers for Disease Control and Prevention. Mumps vaccine: recommendation of Public Health Service Advisory Committee on Immunization Practices. *MMWR Morb Mortal Wkly Rep* 1977; **26**:393–4.
21. Centers for Disease Control and Prevention. Annual summary 1980. Reported morbidity & mortality in the United States. *MMWR Morb Mortal Wkly Rep* 1981;**29**:1–128.
22. Centers for Disease Control and Prevention. Mumps—United States, 1983-1984. *MMWR Morb Mortal Wkly Rep* 1984;**33**: 533–5.
23. Centers for Disease Control and Prevention. Immunization of children infected with human T-lymphotropic virus type III/ lymphadenopathy-associated virus. *MMWR Morb Mortal Wkly Rep* 1986;**35**:595–8,603–6.
24. Centers for Disease Control and Prevention. Immunization of children infected with human immunodeficiency virus: supplementary ACIP statement. Immunization Practices Advisory Committee. *MMWR Morb Mortal Wkly Rep* 1988;**37**:181–3.
25. Centers for Disease Control and Prevention. Mumps prevention. *MMWR Morb Mortal Wkly Rep* 1989;**38**:388–92, 397–400.
26. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States 1998. *MMWR Morb Mortal Wkly Rep* 1999;**47**:ii-92.
27. Centers for Disease Control and Prevention. Brief report: update: mumps activity—United States, January 1–October 7, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1152–3.
28. Centers for Disease Control and Prevention. Mumps epidemic: Iowa, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:366–8.
29. Centers for Disease Control and Prevention. Mumps epidemic: United Kingdom, 2004-2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:173–5.
30. Centers for Disease Control and Prevention. Update: mumps activity: United States, January. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1152–3.
31. Centers for Disease Control and Prevention. Update multistate outbreak of mumps: United States, January 1–May 2 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:559–63.
32. Centers for Disease Control and Prevention. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP) for the control and elimination of mumps. *MMWR Morb Mortal Wkly Rep* 2006;**55**:629–30.
33. Centers for Disease Control and Prevention. Updated recommendations for isolation of persons with mumps. *MMWR Morb Mortal Wkly Rep* 2008;**57**:1103–5.
34. Centers for Disease Control and Prevention. Update: mumps outbreak—New York and New Jersey, June 2009–January 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:125–9.
35. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
36. Chalken BP, Williams NM, Preblud SR, et al. The effect of a school entry law on mumps activity in a school district. *JAMA* 1987;**257**:2455–6.
37. Cherry JD, Jahn CL. Exanthem and enanthem associated with mumps virus infection. *Arch Environ Health* 1966;**12**:518–21.
38. Chuden HG, Michtl W, Stehr K. Hearing loss due to mumps. *Laryngol Rhinol Otol* 1978;**57**:745–50.
39. Cizman M, Mozetic M, Radescek-Rakar R, et al. Aseptic meningitis after vaccination against measles and mumps. *Pediatr Infect Dis J* 1989;**8**:302–8.
40. Clifford V, Wadsley J, Jenner B, et al. Mumps vaccine associated orchitis: evidence supporting a potential immune-mediated mechanism. *Vaccine* 2010;**28**:2671–3.
41. Cochi SL, Preblud SR, Orenstein WA. Perspectives on the relative resurgence of mumps in the United States. *Am J Dis Child* 1988;**142**:499–507.
42. Cohen C, White JM, Savage EJ, et al. Vaccine effectiveness estimates, 2004-2005 mumps outbreak, England. *Emerg Infect Dis* 2007;**13**:12–17.
43. Colville A, Pugh S. Mumps meningitis and measles, mumps, and rubella vaccine. *Lancet* 1992;**340**:786.
44. Cortese MM, Jordan HT, Curns AT, et al. Mumps vaccine performance among university students during a mumps outbreak. *Clin Infect Dis* 2008;**46**:1172–80.
45. Craighead JE. The role of viruses in the pathogenesis of pancreatic disease and diabetes mellitus. *Prog Med Virol* 1975;**19**: 161–214.
46. Dacau-Voutetakis C, Constantinidis M, Moschos A, et al. Diabetes mellitus following mumps. *Am J Dis Child* 1974;**127**:890–1.
47. Danerseau AM, Robinson JL. Efficacy and safety of measles, mumps, rubella and varicella live viral vaccines in transplant recipients receiving immunosuppressive drugs. *World J Pediatr* 2008;**4**: 254–8.
48. Davidkin I, Jokinen S, Paananen A, et al. Etiology of mumps-like illnesses in children and adolescents vaccinated for measles, mumps, and rubella. *J Infect Dis* 2005;**191**:719–23.
49. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. *N Engl J Med* 2008;**358**:1580–9.
50. Dayan GH, Rubin S. Mumps outbreaks in vaccinated populations: are available mumps vaccines effective enough to prevent outbreaks? *Clin Infect Dis* 2008;**47**:1458–67.
51. Dutta PC. A fatal case of pregnancy complicated with mumps. *BJOG* 1935;**42**:869–70.
52. Enders JF. Mumps. In: Rivers TM, Horsfall FL Jr, editors. *Viral and rickettsial infections of man*. 3rd ed. Philadelphia: J. B. Lippincott; 1959. p. 780–9.

53. Endo A, Izumi H, Miyashita M, et al. Facial palsy associated with mumps parotitis. *Pediatr Infect Dis J* 2001;**20**:815–16.
54. Erpenbach KH. Systemic treatment with interferon-alpha 2B: an effective method to prevent sterility after bilateral mumps orchitis. *J Urol* 1991;**146**:54–6.
55. Everberg G. Deafness following mumps. *Acta Otolaryngol* 1957; **48**:397–403.
56. Fanoy EB, Cremer J, Ferreira JA, et al. Transmission of mumps virus from mumps-vaccinated individuals to close contacts. *Vaccine* 2011;**29**:9551–6.
57. Feldman HA. Mumps. In: Evans AS, editor. *Viral infections of humans: epidemiology and control*. 3rd ed. New York: Plenum Press; 1989. p. 471–91.
58. Feldstein JD, Johnson FR, Kallick CA, et al. Acute hemorrhagic pancreatitis and pseudocyst due to mumps. *Ann Surg* 1974;**180**: 85–8.
59. Finkelstein H. Meningo-encephalitis in mumps. *JAMA* 1938;**3**: 17–19.
60. Foy HM, Cooney MK, Hall CE, et al. Isolation of mumps virus from children with acute lower respiratory tract disease. *Am J Epidemiol* 1971;**94**:467–72.
61. Friedman MG. Salivary IgA antibodies to mumps virus during and after mumps. *J Infect Dis* 1981;**143**:617.
62. Fujieda M, Kinoshita A, Naruse K, et al. Mumps associated with immunoglobulin A nephropathy. *Pediatr Infect Dis J* 2000;**19**: 669–71.
63. Gamble DR. Relation of antecedent illness to development of diabetes in children. *BMJ* 1980;**281**:99–101.
64. Garcia AG, Pereira JM, Vidigal N, et al. Intrauterine infection with mumps virus. *Obstet Gynecol* 1980;**56**:756–9.
65. Gellis SS, Peters M. Mumps with presternal edema. *Bull Johns Hopkins Hosp* 1944;**75**:241.
66. Gonzalez PP, Barrios JA, Morales Serna JC. [Study of a population-wide epidemic outbreak of mumps virus G1 in Jerez de la Frontera (Spain)]. *Aten Primaria* 2012;**44**:320–7. In Spanish.
67. Gordon JE. The epidemiology of mumps. *Am J Med Sci* 1940; **200**:412–28.
68. Gordon SC, Lauter CB. Mumps arthritis: a review of the literature. *Rev Infect Dis* 1984;**6**:338–44.
69. Greenberg MA, Birx DL. Safe administration of mumps-measles-rubella vaccine in egg-allergic children. *J Pediatr* 1988;**113**: 504–6.
70. Gundersen E. Is diabetes of infectious origin? *J Infect Dis* 1927; **41**:197–202.
71. Gut JP, Lablache C, Behr S, et al. Symptomatic mumps virus reinfections. *J Med Virol* 1995;**45**:17–23.
72. Habel K. Cultivation of mumps virus in the developing chick embryo and its application to studies of immunity to mumps in man. *Public Health Rep* 1945;**60**:201–12.
73. Haginoya K, Ike K, Iinuma K, et al. Chronic progressive mumps virus encephalitis in a child. *Lancet* 1995;**346**:50.
74. Hamilton R. An account of a distemper, by the common people in England vulgarly called the mumps. *R Soc Edinb Trans* 1790;**2**.
75. Hamilton R. An account of distemper by the common people of England vulgarly called the mumps. *Lond Med J* 1790;**11**: 190–211.
76. Hams HF. A case of diabetes quickly following mumps. *Boston Med Surg J* 1899;**140**:465–9.
77. Harling R, White JM, Ramsay ME, et al. The effectiveness of the mumps component of the MMR vaccine: a case control study. *Vaccine* 2005;**23**:4070–4.
78. Henle G, Deinhardt F. Propagation and primary isolation of mumps virus in tissue culture. *Proc Soc Exp Biol Med* 1955;**89**: 556–60.
79. Herman JJ, Radin R, Schneiderman R. Allergic reactions to measles (rubeola) vaccine in patients hypersensitive to egg protein. *J Pediatr* 1983;**102**:196–9.
80. Herndon RM, Johnson RT, Davis LE, et al. Ependymitis in mumps virus meningitis: electron microscopical studies of cerebrospinal fluid. *Arch Neurol* 1974;**30**:475–9.
81. Hilgendorf I, Freund M, Jilg W, et al. Vaccination of allogeneic haematopoietic stem cell transplant recipients: report from the international consensus conference on clinical practice in chronic GVHD. *Vaccine* 2011;**29**:2825–33.
82. Hilleman MR, Buynak EB, Weibel RE, et al. Live, attenuated mumps-virus vaccine. *N Engl J Med* 1968;**278**:227–32.
83. Hilleman MR, Weibel RE, Buynak EB, et al. Live attenuated mumps-virus vaccine, part IV: Protective efficacy as measured in a field evaluation. *N Engl J Med* 1967;**276**:252–8.
84. Hindiyeh MY, Aboudy Y, Wohoush M, et al. Characterization of large mumps outbreak among vaccinated Palestinian refugees. *J Clin Microbiol* 2009;**47**:560–5.
85. Hirsch A. *Handbook of geographical and historical pathology, 3. Diseases of organ and parts*. London: Sydenham Society; 1886.
86. Holowach J, Thurston DL, Becker B. Congenital defects in infants following mumps during pregnancy: a review of the literature and a report of chorioretinitis due to fetal infection. *J Pediatr* 1957;**50**:689–94.
87. Hukic M, Ravlija J, Dedeic Ljubovic A, et al. Ongoing large mumps outbreak in the Federation of Bosnia and Herzegovina, Bosnia and Herzegovina, December 2010 to July 2011. *Euro Surveill* 2011;**16**(35).
88. Hviid A, Rubin S, Muhlemann K. Mumps. *Lancet* 2008;**371**: 932–44.
89. Hyden D, Odkvist LM, Kylen P. Vestibular symptoms in mumps deafness. *Acta Otolaryngol Suppl* 1979;**360**:182–3.
90. Jalal H, Bahadur G, Knowles W, et al. Mumps epididymo-orchitis with prolonged detection of virus in semen and the development of anti-sperm antibodies. *J Med Virol* 2004;**73**:147–50.
91. Jick H, Chamberlin DP, Hagberg KW. The origin and spread of a mumps epidemic: United Kingdom, 2003-2006. *Epidemiology* 2009;**20**:656–61.
92. Jin L, Rima B, Brown D, et al. Proposal for genetic characterization of wild-type mumps strains: preliminary standardization of the nomenclature. *Arch Virol* 2005;**150**:1903–9.
93. Johnson CD, Goodpasture EW. The etiology of mumps. *Am J Hyg* 1935;**21**:46–57.
94. Jokinen S, Osterlund P, Julkunen I, et al. Cellular immunity to mumps virus in young adults 21 years after measles-mumps-rubella vaccination. *J Infect Dis* 2007;**196**:861–7.
95. Kaufman JJ, Bruce PT. Testicular atrophy following mumps: a cause of testis tumour? *Br J Urol* 1963;**35**:67–9.
96. Kilham L. Isolation of mumps virus from the blood of a patient. *Proc Soc Exp Biol Med* 1948;**69**:99.
97. Kilham L. Mumps virus in human milk and in milk of infected monkey. *JAMA* 1951;**146**:1231–2.
98. Kim-Farley R, Bart S, Stetler H, et al. Clinical mumps vaccine efficacy. *Am J Epidemiol* 1985;**121**:593–7.
99. Kim-Farley RJ. Mumps. In: Kiple KF, editor. *The Cambridge world history of human disease*. Cambridge: Cambridge University Press; 1993. p. 887–9.
100. King RC. Mumps followed by diabetes. *Lancet* 1962;**280**:1055.
101. Kleiman MB, Leland DS. Mumps virus and Newcastle disease virus. In: Lennette EH, Lennette DA, Lennette ET, editors. *Diagnostic procedures for viral, rickettsial, and chlamydial infections*. 7th ed. Washington, D.C.: American Public Health Association; 1995. p. 455–63.
102. Koplan JP, Preblud SR. A benefit-cost analysis of mumps vaccine. *Am J Dis Child* 1982;**136**:362–4.
103. Kress HG, Kreth HW. HLA restriction of secondary mumps-specific cytotoxic T lymphocytes. *J Immunol* 1982;**129**:844–9.
104. Krugman S. Mumps (epidemic parotitis). In: Krugman S, editor. *Infectious diseases of children*. 7th ed. St. Louis: C. V. Mosby; 1981. p. 195–207.
105. Krugman S, Ward R. Mumps (epidemic parotitis). In: Krugman S, editor. *Infectious diseases of children*. 4th ed. St. Louis: CV Mosby; 1968. p. 184–98.
106. Ku JH, Kim YH, Jeon YS, et al. The preventive effect of systemic treatment with interferon-alpha2B for infertility from mumps orchitis. *BJU Int* 1999;**84**:839–42.
107. Kunkel U, Driesel G, Henning U, et al. Differentiation of vaccine and wild mumps viruses by polymerase chain reaction and nucleotide sequencing of the SH gene: brief report. *J Med Virol* 1995; **45**:121–6.
108. Kupers TA, Petrich JM, Holloway AW, et al. Depression of tuberculin delayed hypersensitivity by live attenuated mumps virus. *J Pediatr* 1970;**76**:716–21.
109. Kurekci AE, Atay AA, Demirkaya E, et al. Immune thrombocytopenic purpura in a child with acute lymphoblastic leukemia and mumps. *J Pediatr Hematol Oncol* 2006;**28**:170–2.
110. Kurtz JB, Tomlinson AH, Pearson J. Mumps virus isolated from a fetus. *BMJ (Clin Res Ed)* 1982;**284**:471.

111. Kutty PK, Kruszon-Moran DM, Dayan GH, et al. Seroprevalence of antibody to mumps virus in the US population, 1999-2004. *J Infect Dis* 2010;**202**:667–74.
112. Kutty PK, Kyaw MH, Dayan GH, et al. Guidance for isolation precautions for mumps in the United States: a review of the scientific basis for policy change. *Clin Infect Dis* 2010;**50**:1619–28.
113. Kuzmanovska G, Polozhani A, Mikik V, et al. Mumps outbreak in the former Yugoslav Republic of Macedonia, January 2008–June 2009: epidemiology and control measures. *Euro Surveill* 2010; **15**(23).
114. Lacour M, Maherzi M, Vienny H, et al. Thrombocytopenia in a case of neonatal mumps infection: evidence for further clinical presentations. *Eur J Pediatr* 1993;**152**:739–41.
115. Lamb RA, Kolakofsky D. Paramyxoviridae: the viruses and their replication. In: Knipe DM, Howley PM, editors. Fields virology, **vol. 1**. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1305–40.
116. Latner DR, McGrew M, Williams N, et al. Enzyme-linked immunospot assay detection of mumps-specific antibody-secreting B cells as an alternative method of laboratory diagnosis. *Clin Vaccine Immunol* 2011;**18**:35–42.
117. LeBaron CW, Forghani B, Beck C, et al. Persistence of mumps antibodies after 2 doses of measles-mumps-rubella vaccine. *J Infect Dis* 2009;**199**:552–60.
118. Liang Y, Ma S, Liu L, et al. Identification and development of a promising novel mumps vaccine candidate strain. *Microbes Infect* 2010;**12**:1178–87.
119. Majda-Stanislawska E. Mumps cerebellitis. *Eur Neurol* 2000; **43**:117.
120. Marin M, Quinlisk P, Shimabukuro T, et al. Mumps vaccination coverage and vaccine effectiveness in a large outbreak among college students—Iowa, 2006. *Vaccine* 2008;**26**:3601–7.
121. Mauldin J, Carbone K, Hsu H, et al. Mumps virus-specific antibody titers from pre-vaccine era sera: comparison of the plaque reduction neutralization assay and enzyme immunoassays. *J Clin Microbiol* 2005;**43**:4847–51.
122. McDonald JC, Moore DL, Quennec P. Clinical and epidemiologic features of mumps meningoencephalitis and possible vaccine-related disease. *Pediatr Infect Dis J* 1989;**8**:751–5.
123. McGuinness AC, Gall EA. Mumps at army camps in 1943. *War Med* 1944;**5**:95–104.
124. Melin K, Ursung B. [Diabetes mellitus as a complication of epidemic parotitis.] *Nord Med* 1958;**60**:1715–17. In Swedish.
125. Messaritikas J, Karabula C, Kattamis C, et al. Diabetes following mumps in sibs. *Arch Dis Child* 1971;**46**:561–2.
126. Mossong J, Bonert C, Weicherding P, et al. Mumps outbreak among the military in Luxembourg in 2008: epidemiology and evaluation of control measures. *Euro Surveill* 2009;**14**(7).
127. Muhlemann K. The molecular epidemiology of mumps virus. *Infect Genet Evol* 2004;**4**:215–19.
128. Muhsen K, Shohat T, Aboudy Y, et al. Sero-prevalence of mumps antibodies in subpopulations subsequently affected by a large scale mumps epidemic in Israel. *Vaccine* 2011;**29**:3878–82.
129. Nagai T, Okafuji T, Miyazaki C, et al. A comparative study of the incidence of aseptic meningitis in symptomatic natural mumps patients and monovalent mumps vaccine recipients in Japan. *Vaccine* 2007;**25**:2742–7.
130. Nomura Y, Harada T, Sakata H, et al. Sudden deafness and asymptomatic mumps. *Acta Otolaryngol Suppl* 1988;**456**:9–11.
131. Notkins AL. Virus-induced diabetes mellitus. *Arch Virol* 1977; **54**:1–17.
132. Ogata H, Oka K, Mitsudome A. Hydrocephalus due to acute aqueductal stenosis following mumps infection: report of a case and review of the literature. *Brain Dev* 1992;**14**:417–19.
133. Onal S, Toker E. A rare ocular complication of mumps: keratouveitis. *Ocul Immunol Inflamm* 2005;**13**:395–7.
134. Oran B, Ceri A, Yilmaz H, et al. Hydrocephalus in mumps meningoencephalitis: case report. *Pediatr Infect Dis J* 1995;**14**:724–5.
135. Orvell C, Kalantari M, Johansson B. Characterization of five conserved genotypes of the mumps virus small hydrophobic (SH) protein gene. *J Gen Virol* 1997;**78**(Pt 1):91–5.
136. Orvell C, Rydbeck R, Löve A. Immunological relationships between mumps virus and parainfluenza viruses studied with monoclonal antibodies. *J Gen Virol* 1986;**67**:1929–39.
137. Overman JR. Viremia in human mumps virus infections. *AMA Arch Intern Med* 1958;**102**:354–6.
138. Paradowska-Stankiewicz I, Orlikova H. [Mumps in Poland in 2009]. *Przegl Epidemiol* 2011;**65**:185–7. In Polish.
139. Park DW, Nam MH, Kim JY, et al. Mumps outbreak in a highly vaccinated school population: assessment of secondary vaccine failure using IgG avidity measurements. *Vaccine* 2007;**25**:4665–70.
140. Parmar RC, Bavdekar SB, Sahu DR, et al. Thyroiditis as a presenting feature of mumps. *Pediatr Infect Dis J* 2001;**20**:637–8.
141. Peltola H, Davidkin I, Paunio M, et al. Mumps and rubella eliminated from Finland. *JAMA* 2000;**284**:2643–7.
142. Philip RN, Reinhard KR, Lackman DB. Observations on a mumps epidemic in a virgin population. *Am J Hyg* 1959;**69**:91–111.
143. Pipkin PA, Afzal MA, Heath AB, et al. Assay of humoral immunity to mumps virus. *J Virol Methods* 1999;**79**:219–25.
144. Polat A, Inan M, Cakaloz I, et al. A case of symptomatic idiopathic thrombocytopenic purpura during mumps. *Pediatr Hematol Oncol* 2005;**22**:215–18.
145. Polgreen PM, Bohnett LC, Cavanaugh JE, et al. The duration of mumps virus shedding after the onset of symptoms. *Clin Infect Dis* 2008;**46**:1447–9.
146. Prince GA, Jenson AB, Billups LC, et al. Infection of human pancreatic beta cell cultures with mumps virus. *Nature* 1978;**271**: 158–61.
147. Reed D, Brown G, Merrick R, et al. A mumps epidemic on St. George Island, Alaska. *JAMA* 1967;**199**:113–17.
148. Rota JS, Turner JC, Yost-Daljev MK, et al. Investigation of a mumps outbreak among university students with two measles-mumps-rubella (MMR) vaccinations, Virginia, September-December 2006. *J Med Virol* 2009;**81**:1819–25.
149. Royuela E, Castellanos A, Sanchez-Herrero C, et al. Mumps virus diagnosis and genotyping using a novel single RT-PCR. *J Clin Virol* 2011;**52**:359–62.
150. Rubin SA, Link MA, Sauder CJ, et al. Recent mumps outbreaks in vaccinated populations: no evidence of immune escape. *J Virol* 2012;**86**:615–20.
151. Samantray SK, Christopher S, Mukundan P, et al. Lack of relationship between viruses and human diabetes mellitus. *Aust NZ J Med* 1977;**7**:139–42.
152. Sarnesto A, Julkunen I, Mäkelä O. Proportions of Ig classes and subclasses in mumps antibodies. *Scand J Immunol* 1985;**22**: 345–50.
153. Schwarz NG, Bernard H, Melnic A, et al. Mumps outbreak in the Republic of Moldova, 2007-2008. *Pediatr Infect Dis J* 2010;**29**: 703–6.
154. Scott TFM. Mumps (epidemic parotitis). In: Nelson WE, Vaughan VC, McKay RJ, editors. *Textbook of pediatrics*. 9th ed. Philadelphia: W. B. Saunders; 1969. p. 647–51.
155. Sever J, White LR. Intrauterine viral infections. *Annu Rev Med* 1968;**19**:471–86.
156. Siegel M. Congenital malformations following chickenpox, measles, mumps, and hepatitis: results of a cohort study. *JAMA* 1973;**226**:1521–4.
157. Siegel M, Fuerst HT. Low birth weight and maternal virus diseases: a prospective study of rubella, measles, mumps, chickenpox, and hepatitis. *JAMA* 1966;**197**:680–4.
158. Siegel M, Fuerst HT, Peress NS. Comparative fetal mortality in maternal virus diseases: a prospective study on rubella, measles, mumps, chicken pox and hepatitis. *N Engl J Med* 1966;**274**: 768–71.
159. Sinaniotos CA, Daskalopoulou E, Lapatsanis P, et al. Diabetes mellitus after mumps vaccination. *Arch Dis Child* 1975;**50**:749.
160. Sonmez FM, Odemis E, Ahmetoglu A, et al. Brainstem encephalitis and acute disseminated encephalomyelitis following mumps. *Pediatr Neurol* 2004;**30**:132–4.
161. Soud FA, Cortese MM, Curns AT, et al. Isolation compliance among university students during a mumps outbreak, Kansas 2006. *Epidemiol Infect* 2009;**137**:30–7.
162. Spartaro RF, Lin SR, Horner FA, et al. Aqueductal stenosis and hydrocephalus: rare sequelae of mumps virus infection. *Neuroradiology* 1976;**12**:11–13.
163. Stein-Zamir C, Shoob H, Abramson N, et al. Mumps outbreak in Jerusalem affecting mainly male adolescents. *Euro Surveill* 2009;**14**(50).
164. Stokes J Jr. Mumps (epidemic parotitis). In: Nelson WE, editor. *Textbook of pediatrics*. 7th ed. Philadelphia: W. B. Saunders; 1959. p. 505–8.

165. Ströhle A, Bernasconi C, Germann D. A new mumps virus lineage found in the 1995 mumps outbreak in western Switzerland identified by nucleotide sequence analysis of the SH gene: brief report. *Arch Virol* 1996;**141**:733–41.
166. Sugg WC, Finger JA, Levine RH, et al. Field evaluation of live virus mumps vaccine. *J Pediatr* 1968;**72**:461–6.
167. Sugiura A, Yamada A. Aseptic meningitis as a complication of mumps vaccination. *Pediatr Infect Dis J* 1991;**10**:209–13.
168. Sultz HA, Hart BA, Zielezny M, et al. Is mumps virus an etiological factor in juvenile diabetes mellitus? Preliminary report. *J Pediatr* 1975;**86**:654–6.
169. Teng RJ, Wu TJ, Ho MM. Mumps infection complicated by transient hyperinsulinemic hypoglycemia. *Pediatr Infect Dis J* 1997;**16**:416–17.
170. Thompson JA. Mumps: a cause of acquired aqueductal stenosis. *J Pediatr* 1979;**94**:923–4.
171. Uchida K, Shinohara M, Shimada S, et al. Rapid and sensitive detection of mumps virus RNA directly from clinical samples by real-time PCR. *J Med Virol* 2005;**75**:470–4.
172. Ukkonen P, Granstrom ML, Penttinen K. Mumps-specific immunoglobulin M and G antibodies in natural mumps infection as measured by enzyme-linked immunosorbent assay. *J Med Virol* 1981;**8**:131–42.
173. Utz JP, Houk VN, Alling DW. Clinical and laboratory studies of mumps. *N Engl J Med* 1964;**270**:1283–6.
174. van Loon FP, Holmes SJ, Sirotkin BI, et al. Mumps surveillance, United States, 1988-1993. *MMWR CDC Surveill Summ* 1995;**44**: 1–14.
175. Vandermeulen C, Roelants M, Vermoere M, et al. Outbreak of mumps in a vaccinated child population: a question of vaccine failure? *Vaccine* 2004;**22**:2713–16.
176. Venketasubramanian N. Transverse myelitis following mumps in an adult—a case report with MRI correlation. *Acta Neurol Scand* 1997;**96**:328–31.
177. Vuori M, Lahikainen EA, Peltonen T. Perceptive deafness in connection with mumps: a study of 298 servicemen suffering from mumps. *Acta Otolaryngol* 1962;**55**:231–6.
178. Walker J, Huc S, Sinka K, et al. Ongoing outbreak of mumps infection in Oban, Scotland, November 2010 to January 2011. *Euro Surveill* 2011;**16**(8).
179. Walkty A, Van Caeseele P, Hilderman T, et al. Mumps in prison: description of an outbreak in Manitoba, Canada. *Can J Public Health* 2011;**102**:341–4.
180. Warrener L, Samuel D. Evaluation of a commercial assay for the detection of mumps specific IgM antibodies in oral fluid and serum specimens. *J Clin Virol* 2006;**35**:130–4.
181. Watson JC, Hadler SC, Dykewicz CA, et al. Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1998;**47**(RR-8): 1–57.
182. Weibel RE, Buynak EB, McLean AA, et al. Follow-up surveillance for antibody in human subjects following live attenuated measles, mumps, and rubella virus vaccines. *Proc Soc Exp Biol Med* 1979; **162**:328–32.
183. Weibel RE, Buynak EB, McLean AA, et al. Persistence of antibody in human subjects for 7 to 10 years following administration of combined live attenuated measles, mumps, and rubella virus vaccines. *Proc Soc Exp Biol Med* 1980;**165**:260–3.
184. Weibel RE, Stokes J, Jr, Buynak EB, et al. Live attenuated mumps-virus vaccine, part 3: Clinical and serologic aspects in a field evaluation. *N Engl J Med* 1967;**276**:245–51.
185. Weller TH, Craig JR. Isolation of mumps virus at autopsy. *Am J Pathol* 1949;**25**:1105–25.
186. Whelan J, van Binnendijk R, Greenland K, et al. Ongoing mumps outbreak in a student population with high vaccination coverage, Netherlands, 2010. *Euro Surveill* 2010;**15**(17).
187. Wielders CC, van Binnendijk RS, Snijders BE, et al. Mumps epidemic in orthodox religious low-vaccination communities in the Netherlands and Canada, 2007 to 2009. *Euro Surveill* 2011; **16**(41).
188. Wolinsky JS. Mumps virus. In: Fields BN, Howley PM, editors. *Virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996. p. 1243–65.
189. Xu P, Luthra P, Li Z, et al. The V protein of mumps virus plays a critical role in pathogenesis. *J Virol* 2012;**86**:1768–76.
190. Yamauchi T, Wilson C, Geme JW, Jr. Transmission of live, attenuated mumps virus to the human placenta. *N Engl J Med* 1974; **290**:710–12.

diminutive in stature, Caren was a giant among physicians in setting an example for integrity, selflessness, and commitment to the highest ideals of medicine.

HCM

Respiratory syncytial virus (RSV) is the most important respiratory pathogen of infancy and early childhood and the major cause of hospitalization for bronchiolitis and pneumonia in infants globally.* In the United States, RSV has been estimated to be the leading cause of all hospitalizations for infants, resulting in an estimated 74,000 to 126,000 hospitalizations each year during 1994 to 1996.[349] More recent studies suggest that these figures are lower than the current number of infant hospitalizations associated with RSV infections and that the associated annual costs are approximately $2.6 billion.[174,235,307] Furthermore, the considerable burden of RSV infections among older children, adults, and outpatients has not been recognized.[116,174,401]

RSV is marked by its contagiousness and by its propensity to cause the most severe disease during the first few months of life, despite the uniform presence of specific maternal antibody. Sizable outbreaks occur each year, with the result that most children become infected at least once in their first 2 years of life.[133,184] Nearly all first infections are symptomatic, and many involve the lower respiratory tract. Reinfections occur throughout life and commonly are sufficiently symptomatic to cause absence from work or school.[148,151]

HISTORY

In 1956, Morris and associates[281] noted an outbreak of colds with coryza in a colony of chimpanzees that had been under observation for the previous 3 to 24 weeks. A new virus was recovered from 1 of the 14 afflicted chimpanzees, and it was appropriately named *chimpanzee coryza agent* (CCA). Specific antibody to the CCA agent developed in the remaining 13 animals during convalescence; thus, the attack rate was 100 percent. An upper respiratory tract infection and a serologic antibody response to CCA also developed in a person working with these chimpanzees, but the virus was not isolated from his secretions. However, further evidence of the pathogenicity of CCA was obtained subsequently when the CCA virus isolated from one chimpanzee was inoculated into susceptible chimpanzees and a coryzal illness developed after 3 days.

The suspected human origin of the chimpanzee coryza agent was confirmed a year later when Chanock and Finberg[65] recovered two agents indistinguishable from the CCA virus from the throat swabs of an infant with bronchopneumonia (Long strain) and from a child with laryngotracheobronchitis (Snyder strain). These investigators also observed an antibody response to these viruses in patients with respiratory disease and noted that by the time they reached 4 years of age, 80 percent of children had neutralizing antibody against the Long strain. Nonetheless, these investigators could not determine a definite etiologic association between the virus and lower respiratory tract disease in their young patients. They proposed naming this group of viruses (Long, Snyder, and CCA) *respiratory syncytial virus* because of their similar clinical manifestations and striking viral cytopathic effect of syncytial formation and multinucleated giant cells when the virus was grown in vitro. Confirmation of RSV as a major agent of respiratory disease soon accumulated from studies throughout the United States, and investigators from many countries subsequently documented and further delineated the importance of RSV.[28,44,65,116]

PROPERTIES

A tiny thistle—
of coiled spine
and outer quill

CBH

Classification

The original classification of RSV with the Newcastle disease and parainfluenza group of viruses was based on their similar internal particle structure, eosinophilic inclusions, and syncytial appearance in tissue culture. However, RSV is antigenically distinct and does not hemagglutinate erythrocytes. Subsequently, the diameter of the nucleocapsid of RSV was determined to be between those of the larger paramyxoviruses and those of the smaller influenza viruses. Further study of the structure of RSV resulted in its current classification in the order Mononegavirales, family *Paramyxoviridae*, subfamily *Pneumovirinae* and genus *Pneumovirus*.[79] RSV belongs, along with pneumonia virus of mice, bovine RSV, ovine RSV, caprine RSV, and turkey rhinotracheitis virus, to the subfamily *Pneumovirinae*, which consists of two genera, *Pneumovirus* and *Metapneumovirus* (MPV). The second genus, *Metapneumovirus*, contains human metapneumovirus (hMPV), first described in 2001 by van den Hoogan and colleagues[378] in the Netherlands.[210]

Structural and Antigenic Properties

The virion of RSV consists of a nucleocapsid enclosed within an envelope consisting of a bilipid layer derived from the plasma membrane of host cells. Transmembrane glycoprotein spikes on its surface give the virus the appearance of a thistle on electron microscopy. The genome of RSV (strain A2) has been completely sequenced and is composed of 15,222 nucleotides. It is a nonsegmented, single-stranded, negative-sense RNA genome that encodes 11 proteins. Each of the major messenger RNAs (mRNA) (Fig. 181-1) encodes for one of the major proteins, except for M2 mRNA, which possesses two overlapping open-reading frames that encode for two separate proteins (M2-1 and M2-2).[79,399] Eight of these, including the seven largest (L, G, F, N, P, M, and SH), are structural proteins; two (NS1 and NS2) are nonstructural proteins that interfere with the interferon type I and type III response; M2-1 and M2-2 proteins are involved in RNA synthesis.[80]

*References 44, 129-131, 200, 309, 313.

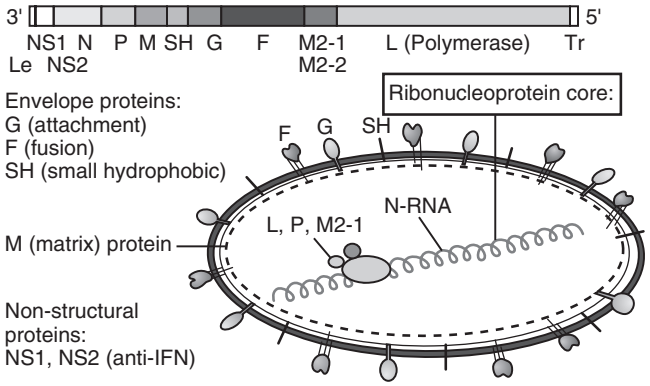

FIGURE 181-1 ■ The genome of respiratory syncytial virus (RSV) and a schematic diagram of an RSV virus particle. Le, leader; Tr, trailer.

Of the structural proteins, three form the viral capsid: N (nucleoprotein), P (phosphoprotein), and L (polymerase). Two are matrix membrane–associated proteins: the nonglycosylated M and M2 (the transcriptional antiterminator protein or transcriptase processivity factor, M2-1). The other three structural proteins, the glycosylated F (fusion) and G (attachment) proteins and the small, nonglycosylated hydrophobic SH protein, are transmembrane surface proteins. The F and G proteins are integral to RSV's infectivity and pathogenicity. The primary function of G, the largest glycoprotein, appears to be in the initial attachment of the virus to the host cell and is unusual compared with the attachment proteins of other paramyxoviruses because it has no hemagglutinin or neuraminidase. Initial penetration of the virus then is mediated by the F protein by fusing the viral and host cellular membranes, a process that allows cell-to-cell spread of the virus and results in the syncytia characteristic of RSV. However, all three of the surface glycoproteins, F, G, and SH, appear to act in concert to enhance the fusion process. Binding to a cellular receptor produces conformational changes in the F protein resulting in fusion of the virion envelope with the membrane of the cell.[264] Previously, G protein was identified as the major attachment protein, but more recently the F protein also has been recognized as having an important role in attachment of the virus.[367]

Significant strain variations among RSV isolates cause strains to be divided into two major groups.[16,399] These two major groups, A and B, have intergroup and intragroup variations in several proteins, including F, G, P, and N. The primary differences reside in the largest surface glycoprotein, the G protein. The amino acid sequence of the G protein varies by approximately 50 percent between the RSV A and RSV B strain groups. Intragroup strain variation also occurs mainly within the G protein. In contrast, within F protein, the amino acid sequence is conserved relatively well, and thus neutralizing antibody to F protein is cross reactive between the two strain groups.[79] The F proteins of prototype strains from groups A and B have greater than 90 percent amino acid homology and a high degree of antigenic relatedness.

Laboratory Growth

RSV is relatively labile and is destroyed rapidly at 55° C. At 37° C, infectivity does not diminish for 1 hour, but by 24 hours, only 10 percent remains. RSV can grow in a variety of human and animal cell cultures. Human heteroploid cells such as HEp-2, HeLa, and A549 cells are used most frequently for primary isolation. Additional cell cultures that may be used, but generally are less sensitive, include diploid fibroblastic, monkey kidney, human kidney, and amnion cells. However, the sensitivity of these cell lines is variable, especially with passage, and must be monitored constantly. The characteristic cytopathic effect of RSV in continuous cell lines is syncytial formation with eosinophilic cytoplasmic inclusions. The syncytia usually are evident 2 to 7 days after inoculation and progress to complete degeneration within about 4 days.[149] The cytopathic effect, however, depends on the strain of virus, the medium, the sensitivity and thickness of the cell cultures, and the number of passages. Syncytia tend to be less evident in fibroblast cell lines; and in some primary cell cultures, RSV may produce rounded, refractile cells.

The growth cycle of RSV has been shown to consist of a period of adsorption, in which 50 percent of the inoculum is adsorbed in 2 hours, followed by an eclipse period of 12 hours. New virus appears shortly thereafter and enters a log phase of replication lasting for approximately 10 hours. Viral antigen can be documented by fluorescent antibody staining 7 to 10 hours after inoculation. Shortly thereafter, cell-free virus may be demonstrated in the culture medium, but 50 to 90 percent of the virus remains associated with the cell surface at the time maximal titers are reached. Most of the cell-associated virus consists of incomplete noninfectious virions that may be released by agitation and sonication. With the laboratory Long strain (group A), peak titers usually are reached in 48 hours. For each infected cell, approximately 10 plaque-forming units generally result. Titers of virus are enhanced by inoculation of cell monolayers that are not yet confluent.

Animal Susceptibility

An animal model of human RSV disease is a critical step in the search for effective antiviral therapy as well as for development of a vaccine. Susceptible animals that could serve as a model for the lower respiratory tract disease of infants have been sought for some time. Each has advantages and disadvantages in replicating primary RSV disease in infants, but none is ideal.[25,82,96] Although RSV grows in the lung of a variety of animals, direct inoculation into their respiratory tract generally produces infection that is clinically and pathologically silent.

The natural hosts for symptomatic RSV infection are primarily humans and chimpanzees and are cows for bovine RSV.[51,96] RSV also has been recovered from asymptomatic goats and sheep. Other domestic animals, such as dogs and cats, have been found to possess antibody to RSV, the significance of which is unclear.

Chimpanzees most closely resemble humans in their clinical response to RSV. However, lung disorders and

some degree of clinical disease have been induced in many other primates.[51,96] Infection in ferrets is age dependent, with limited histopathologic features of the nasal turbinates and trachea developing in adults. In infant ferrets, however, the virus replicates in the lung. The use of the cotton rat model resulted in major progress in understanding the pathogenesis of RSV infection, as demonstrated by the seminal studies of Prince and colleagues.[320] With intranasal inoculation, viral titers peak in the lung and nasal turbinates after 4 to 5 days. Histologic changes in the lung are present but may be inconsistent, and immunologic reagents are limited in comparison with those for mice. Active replication of RSV in other rodents generally is limited. BALB/c mice are among the most susceptible, and marked pulmonary disease may occur with high-titer, large-volume inocula, which in older mice is accompanied by evidence of clinical illness.[96]

A human model of RSV infection has been reported in which healthy adult volunteers are infected intranasally with an attenuated strain of RSV and observed for viral load, immune markers, and clinical symptoms.[94] It is hoped that the availability of a safe and reproducible human model will permit more rapid testing of novel drugs and immunotherapies.

EPIDEMIOLOGY

What occult power pries loose the lid
to give you winter flight,
But with the lengthening light of spring
gives cloak and leaden wing?

—CBH

Geographic Distribution

Experience with RSV infection is worldwide, as is its predominant pathogenicity for the very young and for those with underlying high-risk conditions. The timing, length, and intensity of RSV outbreaks, however, vary geographically. Outbreaks in warm and tropical climates tend to be more prolonged, with less distinctive peaks of activity.

In industrialized countries, RSV lower respiratory tract disease is a leading cause of hospitalization of children in the first year of life. The global burden of RSV disease is incompletely defined, but the morbidity and mortality from RSV in developing countries appears to be high.[29] One report estimated that among children younger than 5 years of age, 3.4 million RSV hospitalizations due to severe lower respiratory tract infection resulted in between 66,000 and 199,000 deaths in 2005. Ninety-nine percent of the deaths were estimated to occur in developing countries.[288] Although these figures may not be precise, little doubt exists that the worldwide burden of lower respiratory tract disease attributable to RSV exceeds that of *Haemophilus influenzae* type b and *Streptococcus pneumoniae*.[295]

Seasonal Patterns

Within the United States, the inevitability of the RSV season is predictable but the severity of the season, the time of onset, the peak of activity, and the end of the season cannot be predicted with precision (Table 181-1). The timing of RSV outbreaks often shows variation from year to year within the same community. This variation, however, occurs within the overall pattern of RSV seasonality, beginning in November or December, peaking in January or February, and ending in March or April. Trends in RSV seasonality are monitored by the National Respiratory and Enteric Virus Surveillance System maintained by the Centers for Disease Control and Prevention.[57,234,304,387] Communities in the southern states tend to experience the earliest onset of RSV activity, and those in the Midwest tend to experience the latest onset. The duration of the season for the western and northeast regions typically occurs between that observed in the south and the Midwest. During the four RSV seasons of 2007 through 2011, the seasonal onset occurred from mid November to early January and the season's end occurred from mid March to late April across all 10 Department of Health and Human Services regions. The

TABLE 181-1 **Summary of Median Values of Respiratory Syncytial Virus Season Characteristics for Entire Nation and U.S. Census Regions from National Respiratory and Enteric Virus Surveillance System Data, 1990 to 2000**

	Median Weeks of Year for RSVs			
Location	ONSET	PEAK	OFFSET	DURATION
Nation	51 (late December)*	5 (early February)	13 (end of March)	15
West	52 (late December)	6 (mid February)	13 (end of March)	14
Midwest	1† (early January)	7 (late February)	13 (end of March)	13†
South	47‡ (late November)	1 (early January)	10 (mid March)	16†
Northeast	49 (early December)	4 (end of January)	12 (late March)	15

*Numbers in parentheses, time of year.
†Statistically different from the rest of the nation, $p < .05$.
‡Statistically different from the rest of the nation, $p < .01$.
From Mullins JA, LaMonte AC, Bresee JS, Anderson LJ: Substantial variability in community respiratory syncytial virus season timing. *Pediatr Infect Dis J* 2003;**22**:857-62.

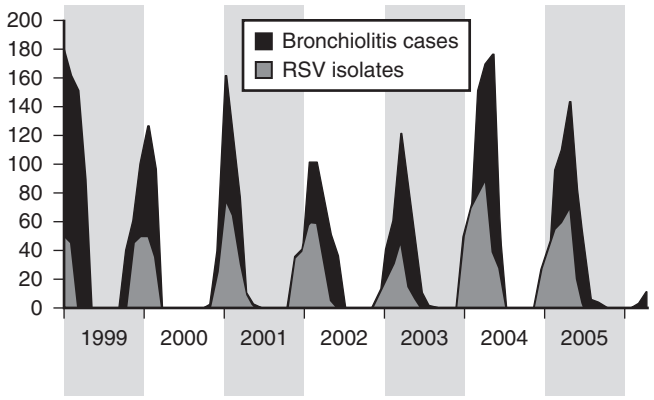

FIGURE 181-2 ■ The number of cases of bronchiolitis and the number of isolates of respiratory syncytial virus from children younger than 5 years of age who were evaluated in private offices and outpatient facilities from 2000 to 2005 identified by a community surveillance program in Monroe County, New York.

median duration ranged from 13 to 23 weeks, and the peak of RSV activity occurred from mid December to early February. Florida is reported separately because it has an earlier onset and longer duration of RSV activity than the rest of the United States. Possible explanations for differences in regional activity include population density, the mean age of residents, and weather patterns. Data on hospitalizations likely associated with RSV suggest that RSV disease correlates with RSV activity in the community and occurs from November through April (Fig. 181-2).[57,244,349]

Specific groups of American Indian/Alaska Native children in certain geographic areas (particularly young children in southwestern Alaska) may experience more severe RSV disease and a longer RSV season than other parts of the United States.[355] Navajo and White Mountain Apache infants and young children may experience RSV hospitalization rates that are two to three times those of similar age children in other parts of the country.[50]

In geographic regions with temperate climates, including both the Northern and Southern hemispheres, periods of RSV activity correlate with cold weather. In equatorial countries, RSV activity continues for much of the year. These patterns suggest the possibility that meterologic conditions influence the dissemination of virus. Efforts have been made to correlate RSV activity in different geographic locations with mean daily temperature, dew point, absolute humidity, ultraviolet B radiance, relative humidity, and precipitation. Results show a complex interaction between RSV circulation and environmental conditions that cannot be correlated easily.[115,394,411] In addition, weather conditions are likely to alter behavior, such that colder temperatures and rainy weather result in more time spent indoors, resulting in closer contact with other individuals and facilitating the spread of virus.

Results from several epidemiologic studies suggest that vitamin D may be protective against RSV lower respiratory tract infection. Vitamin D serum concentrations are lowest during winter months. Plasma concentrations of 25-hydroxyvitamin D concentrations are lower in infants hospitalized with RSV infections compared with healthy controls[267,335] and a low 25-hydroxyvitamin D plasma concentration at birth correlates with a subsequent risk for RSV.[24] Whether the benefits of vitamin D supplementation will outweigh the potential detrimental effects has not been determined.

Strain Variation

Variation in circulating strains of RSV has been suggested as partly accounting for the fluctuating clinical impact of RSV epidemics.[16,305,341] The two major strain groups, A and B, usually circulate simultaneously during an outbreak, but the proportion of strains from each group may vary by season and geography, as does predominance of the subgroups of A and B strains.[16,261,310,341] In most areas, the annual dominant strains appear to be group A. In Rochester, New York, during a 20-year period, group A strains predominated in 11 of the years, group A and B strains were relatively equal in another 5 years, and in four seasons group B strains accounted for more than 75 percent of the year's isolates.[173] A study of strains from 14 cities across the United States demonstrated that the variability of strains occurs both seasonally and geographically.[16] Several distinct but varying genotypes within the A and B strain groups predominate each year in a community, a finding suggesting that local preexisting immunity to previously circulating RSV strains may engender a selective advantage for strains with genotypes most diverse from those recently present in a community.[261,310,399] The relationship between the circulating strain group or subgroup and the size or clinical severity of an RSV outbreak, thus far, has not appeared to be strong or consistent.[278,305] Further understanding of these relationships and the molecular mechanisms, pathogenesis, and clinical importance of differing genotypes may be integral to the development of effective vaccines and the control of RSV.[399]

Acquisition and Ramifications of Infection

The major burden that RSV inflicts on the health care of children is illustrated by its ubiquity and the proportion of lower respiratory tract disease it causes in early childhood, especially among young infants. RSV accounts for 30 to 90 percent of bronchiolitis cases, up to 50 percent of pneumonia admissions during infancy, and 10 to 30 percent of cases of pediatric bronchitis.[44,174,200,220] In contrast, only relatively small proportions, less than 10 percent, of croup cases have been associated with RSV infection. RSV is isolated rarely from patients (<1%) without respiratory disease.[66,220]

During the first year of life, 50 to 70 percent or more of infants acquire RSV infection, despite the presence of maternal specific neutralizing antibody in the sera of all newborns.[132,309] The level of antibody in a term newborn is similar to the maternal level, which gradually declines during the first 6 months of life. In patients older than 7 months of age, detectable specific serum antibody usually is the result of natural infection. Essentially all children by 3 years of age possess specific serum antibody.

The impact of RSV infections on communities around the world has been examined in numerous studies and varies depending on the geographic location, the population, and the methods of assessment. Nonetheless, several generalizations are evident: (1) RSV is highly contagious, resulting in ubiquitous infection by 2 to 3 years of age; (2) in this age group, RSV is the major cause of lower respiratory disease, predominantly bronchiolitis and pneumonia; (3) RSV infection of the lower respiratory tract in normal children almost always results from primary infection; (4) reinfections occur throughout life and tend to be milder and usually are upper respiratory tract infections; (5) the severity of illness from reinfections remains appreciable at any age in persons with comorbid conditions; and (6) medical visits by outpatients with RSV infection comprise an appreciable portion of the burden to health care.

Infants with underlying chronic conditions are at high risk for requiring hospitalization for RSV illness, but numerous environmental and other host factors have been reported to augment the risk for a previously healthy infant's developing more severe RSV infection.[132,234,328,340,357] These factors include becoming infected within the first few months of life, living in crowded and lower socioeconomic circumstances, geographic location, and gender. Although the rate of infection among girls and boys is equal, boys are hospitalized more frequently than are girls, suggesting their propensity when infants to develop more severe RSV infection.

In the United States, an estimated 55,000 to 125,000 RSV associated hospitalizations and 200 to 500 associated deaths occur each year.[174,239,349,350] The annual rate of hospitalizations caused by RSV in comparison with that from influenza or the parainfluenza viruses has been approximately three times greater for children aged 5 years and younger and six to eight times greater for children younger than 1 year of age.[174,200,293] Adding to this burden of disease are emergency department and pediatric office visits, which occur at 5 to 10 times the rates for hospitalization.[174]

Fatal RSV cases are uncommon in industrialized countries.[239,350,370] In the United States, the number of deaths associated with RSV infection each year among children younger than 5 years of age was estimated in 1985 to be 4500.[197] During 1979 to 1997, this number was reported to have declined to an estimated 510 or less each year in children younger than 5 years of age.[174,239,349,350]

In the United Kingdom for more than 11 winter respiratory seasons, RSV was associated with more deaths than was influenza in young children, especially in the first year of life.[114] The rates of hospitalization associated with RSV, however, have not declined but appear to be increasing from the estimate of 110,000 in 1997 in children younger than 5 years of age.[349] The rate of admissions attributable to RSV is markedly higher among infants within the first year of life than among children aged 1 to 4 years. In infants, the estimated rates have varied from 5 to 41 per 1000 each year, depending on the area and the methods used.[42,238,254]

Population-based studies have reported annual hospitalization rates attributable to RSV in the United States of 3.0 to 3.5 per 1000 children younger than 5 years of age.[174,200] These rates were approximately six times greater than those associated with the influenza viruses and three times greater than those associated with the parainfluenza viruses. The rates of hospitalization for RSV among infants were even greater, 12.9 per 1000 children younger than 1 year of age, compared with 1.7 for influenza and 3.2 for the parainfluenza viruses. The youngest infants, those younger than 6 months old, had the greatest risk for requiring admission for their RSV infection, with a yearly rate of 17 per 1000 children.[174]

In other developed countries, population-based studies demonstrated similarly high rates of hospitalizations related to RSV among infants and young children.[106,116,254,293] Annual population-based hospitalization rates attributable to RSV from European countries were reported per 1000 children to be 2.5 for those younger than 4 years of age, 11 for children younger than 3 years of age, and 4 to 7 for those younger than 2 years of age. Among children within the first 12 months of life, 19 to 22 per 1000 children were admitted each year with RSV infection.

The magnitude of ambulatory visits among children resulting from RSV infection has not been well quantified and generally has received less attention. Surveillance conducted in Nashville, Tennessee, and Rochester, New York, for acute respiratory illness occurring among children aged up to 59 months showed that RSV was the agent most frequently identified among ambulatory children evaluated for respiratory illness.[174] RSV accounted for 18 percent of emergency department visits and 15 percent of pediatric practice visits from November to May. The estimated average rate of RSV-associated outpatient visits was 80 per 1000 children younger than 5 years of age and was estimated to be three times the rate of emergency department visits and 27 times the hospitalization rate for RSV. This appreciable impact of RSV outpatient visits on health care resources is further supported by a German study in which the annual incidence rate for RSV illness among outpatients was notably high at 77 per 1000 children younger than 3 years of age.[116]

The economic and clinical costs related to RSV infections among young outpatient children have been poorly characterized. In the United States, the costs for emergency department visits were estimated in 1997 to 2000 as more than $200 million per year for infants and for all children younger than 5 years of age the costs for hospitalizations and other medical visits were $652 million in 2000. The contributions to this economic burden from office practices have not been estimated but are likely significant.[239,307]

Spread of Infection

Each breath's toll is virus spread and shed.[146]

—CBH

RSV spreads effectively through exposed families, and introduction of the virus into the family appears to occur most commonly through a school-aged child.[165] Serious disease in infancy is likely to follow a mild "cold" in an older sibling.[165] In a prospective study of families with an

TABLE 181-2 **Attack Rate of Respiratory Syncytial Virus in Families According to Age**

Age (yr)	Crude Rate*		Rate in RSV-Positive Families		Secondary Rate	
	NO.†	%	NO.†	%	NO.†	%
<1	10/34	29.4	10/16	62.5	5/11	45.4
1-<2	2/7	28.6	2/5	40.0	0/3	0.0
2-<5	9/34	26.4	9/19	47.0	2/12	16.6
5-<17	9/48	18.7	9/24	38.0	4/19	21.0
17-45	9/55	16.8	9/21	43.0	6/18	33.3
Total	39/178	21.9	39/85	45.9	17/63	27.0

*The crude attack rate according to age is shown for all family members studied and for members of RSV-positive families. The secondary attack rate is also shown for members of RSV-positive families, excluding all primary and co-primary cases.

†Number of persons infected with RSV per total number of persons exposed.

Reprinted, by permission from Hall CB, Geiman JM, Biggar R, et al. Respiratory syncytial virus infection within families. *N Engl J Med* 1976;**294**:414-9.

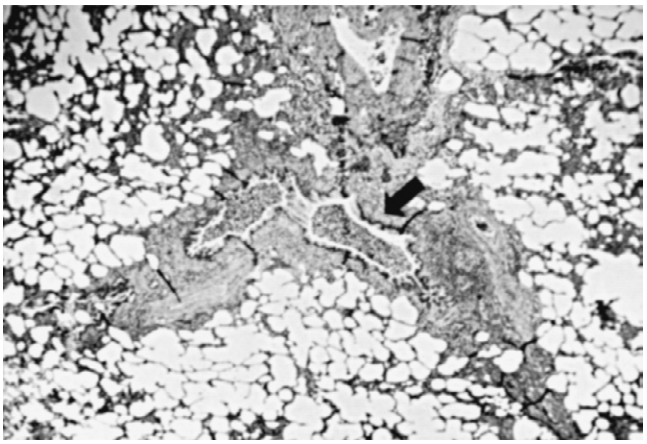

FIGURE 181-3 ■ Histologic examination of an infant dying of respiratory syncytial virus bronchiolitis. The arrow denotes a bronchiole filled with inflammatory exudate and comparatively normal alveoli.

infant and one or more older siblings, 44 percent of the families became infected with RSV during a 3-month epidemic. In almost all these families, older siblings (2 to 16 years of age) introduced the virus into the family, and the infants became secondarily infected (Table 181-2).

These clinical observations and volunteer studies indicate that RSV is predominantly spread by two mode: (1) large particle droplet aerosols (>10 to 100 μm mass median diameter), which generally travel short distances (≤0.9 m) through sneezing, coughing, and even in some individuals by normal quiet breathing, and (2) self-inoculation of infectious secretions contaminating environmental surfaces.[152,153,157] Small particle aerosols (≤10 μm mass median diameter), which can produce distant spread, may also occur with RSV, but their clinical importance is controversial.

These modes of spread have been supported by volunteer and experimental studies. One study demonstrated that infection occurs in volunteers who touch surfaces contaminated by secretions and then their eyes or nasal mucosa.[157] In contrast, no infections developed in volunteers exposed to infected infants at a distance of greater than 6 feet, thus suggesting that small-particle aerosol spread of RSV was not a major mode of transmission. RSV infection, therefore, appears in most instances to require close contact with infected individuals or their secretions.

RSV in the nasal secretions of infants with acute infection remains infectious on countertops for longer than 6 hours and on cloth and tissue paper for approximately 30 minutes.[166] These nasal secretions can remain infectious after transfer from objects or hands to the hands of another person, thus indicating that contact with clothing, furniture, or tissues contaminated by the secretions of infected children can result in spread.[166] Furthermore, the potential for RSV's transmission is augmented by the long duration of shedding and high viral loads commonly observed among young infants with RSV infection.[91,160,161,316,406]

PATHOLOGY AND PATHOGENESIS

A spray that turned to dew
upon the fecund field
of young, unblemished lung;
its innate shield concealed.

—CBH

The incubation period of illness from RSV has been reported variably as being between 2 and 8 days, most frequently as 4 to 6 days. Experimental infection in adult volunteers produced an incubation period of 3 to 6 days, with an average of 5 days.[162,204] With primary infection, the incubation period may be shorter.[162,172] Inoculation is through the upper respiratory tract, followed by infection of the respiratory epithelium. Both the eye and the nose appear to be equally sensitive routes of inoculation.[162] In contrast, inoculation by mouth results in infection much less frequently. Spread along the respiratory tract occurs mainly by cell-to-cell transfer of the virus along intracytoplasmic bridges and may involve the conducting airways at all levels.

The major pathologic findings shown in the lungs of infants dying of RSV bronchiolitis are (1) peribronchiolar mononuclear infiltration, (2) necrosis of the epithelium of the small airways, (3) plugging of the lumina, and (4) hyperinflation and atelectasis (Fig. 181-3).[5,149,381,382] The initial lesions in bronchiolitis occur in the small airways (75 to 300 μm).[382] Lymphocytic peribronchiolar infiltration develops along with edema of the walls, the submucosa, and adventitial tissue. Subsequently, the epithelium of the bronchioles undergoes striking necrosis, sometimes with proliferation of the epithelium into the lumen. Sloughing of necrotic material into the lumina of the small airways impedes the flow of air, which is aggravated by increased mucus secretion.

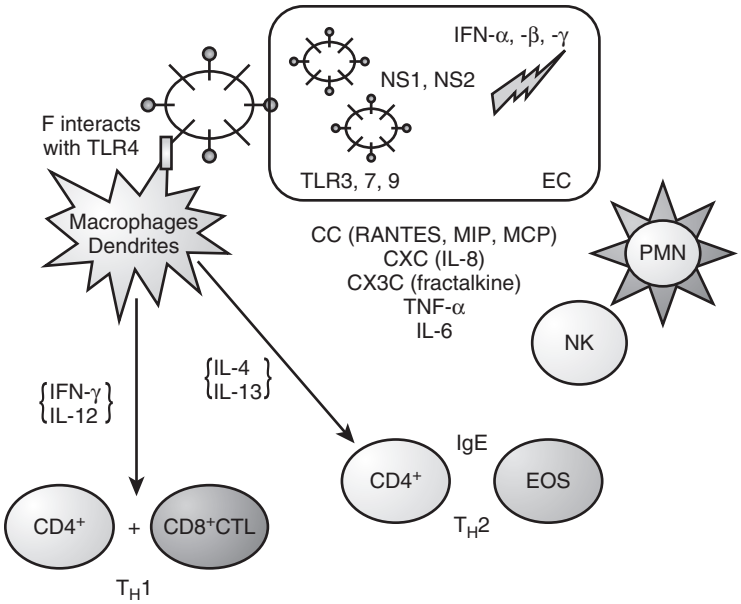

FIGURE 181-4 ■ Schematic of the early innate and adaptive immune responses to respiratory syncytial virus (RSV). RSV's envelope glycoprotein G attaches to the respiratory tract epithelial cells (ECs) via glucosamine glycans expressed on the cell surface, and F interacts with antigen-presenting cells (macrophages, dendritic cells) via Toll-like receptor 4 (TLR4) protein. This triggers the production and release of antiviral interferons (IFN-α, IFN-β, IFN-γ) and cascade of proinflammatory cytokines and chemokines. Two early nonstructural RSV gene products (NS1, NS2) antagonize IFN production. The chemokines recruit polymorphonuclear neutrophils (PMN), natural killer (NK) cells, and $CD4^+$ and $CD8^+$ T cells. A Th1-type cellular response becomes dominant under the influence of IFN-γ and interleukin (IL)-12, whereas under the influence of IL-4 and IL-13 the cellular response is skewed toward Th2 with the production of IgE and eosinophils (EOS). (From Walsh EE. Respiratory syncytial virus. In: Feigin RD, Cherry JD, Demmler-Harrison GJ, Kaplan SL, editors. *Feigin and Cherry's textbook of pediatric infectious diseases.* 6th ed. Philadelphia: Saunders Elsevier; 2009.)

Peripheral to the sites of partial occlusion, air trapping occurs, similar to a ball-and-valve mechanism. During the negative intrapleural pressure of inspiration, air can flow past the site of partial obstruction, but with the positive pressure of expiration the lumen narrows, resulting in more complete obstruction and the hyperinflation characteristic of bronchiolitis. The trapped air may be absorbed and result in multiple areas of focal atelectasis. Functionally, these pathologic changes translate into an increased lung volume and higher expiratory resistance in the infant. Pneumonia may be present concurrently and is marked by interstitial infiltration of mononuclear cells. Lymphocytic infiltration of the bronchiolar walls also may be observed. The lung parenchyma appears edematous, with areas of necrosis leading to alveolar filling, consolidation, and collapse.

Recovery from acute bronchiolitis may be evident in 3 to 4 days, with regeneration of the bronchiolar epithelium, but ciliated cells rarely are present before 2 weeks.[382] Complete histologic and functional recovery may require another 4 to 8 weeks.[175]

IMMUNE RESPONSE: IMMUNITY AND DISEASE PATHOGENESIS

Despite the recent rapid rise in our knowledge of the immune response to RSV infection, unclear is whether disease results primarily from the direct cytotoxic effect of viral replication or from the evoked immune response. Evidence for the direct viral replication being the prime cause has been long suspected by the observation of a correlation between increased RSV concentration in nasal secretions and disease severity.[161] Experimental studies in humans suggest that symptoms begin near the onset of viral shedding in nasal secretions, peak at the time of peak viral shedding, and begin to subside as viral load declines.[94] More recent evidence showed that cytokine concentrations in respiratory secretions of infants with RSV infection were minimal and were lower than in children with influenza infection.[393] In addition, lung tissue from infants with fatal RSV infection demonstrated abundant RSV antigen, but few $CD4^+$ and $CD8^+$ lymphocytes and few natural killer (NK) cells, leading to the conclusion that cytotoxic lymphocyte responses do not have an important role in the pathogenesis of RSV lower respiratory tract disease but rather that severe RSV lower tract disease is characterized by abundant viral replication and an inadequate adaptive immune response.[397] These findings suggest that during acute illness the pathogenesis of RSV disease is related more to the infant's inability to develop an adaptive cytotoxic T-cell response to curb viral replication than to an aberrant immune response that enhances the disease process.

Evidence supporting an immunopathic contribution comes from observations of enhanced disease among recipients of a formalin-inactivated RSV vaccine in the 1960s.[80,300,374] Current research also suggests that the innate and adaptive immune responses are instrumental in producing primary disease and protection against subsequent infection and possibly in whether pulmonary sequelae, such as asthma, will occur. Taken together, this research indicates that it is the balance between T-helper 1 (Th1) and T-helper 2 (Th2) T-cell responses and the patterns of the evoked chemokines and cytokines that determine the clinical outcome (Fig. 181-4). For some infants, disease may result from an exaggerated Th2 cellular response and activated cytotoxic lymphocytes. Most likely, however, is that immune factors of the host and the direct effect of the virus are involved in disease from RSV.[380]

Innate Immunity

RSV targets type I alveolar cells, airway epithelial cells, and alveolar macrophages, resulting in impairment of

ciliary action and sloughing of infected epithelial cells.[203] Dendritic cells scavenge the lung for foreign antigen and are important in generating a viral-specific immune response against RSV. After ingesting viral antigen, dendritic cells migrate to lymph nodes where antigen is presented to naïve T cells.[265] The infant's early innate immune response to the infection of these cells is characterized by the synthesis of chemokines and proinflammatory cytokines and the recruitment of inflammatory cells to the infected pulmonary tissues.[206,343,372] Among these are neutrophils, eosinophils, and interferon-γ (IFN-γ)–secreting NK cells. Toll-like receptors (TLRs) have an important role in mediating and modulating the inflammatory cytokine and chemokine production during RSV infection. This may result from the TLRs' polarization of the cytokine responses toward a Th1 or Th2 profile, which potentially could lead to sensitization of the airway epithelium and subsequent pulmonary hyperreactivity.[134]

RSV's interaction with specific TLRs (TLR3, TLR4, TLR7, TLR8, TLR9) on mononuclear cells and alveolar macrophages results in production of antiviral and immunomodulatory mediators, including type I and III interferons, IFN-α, IFN-β, and IFN-γ, chemokines CXCL8 (interleukin [IL]-8) and CCL5 (RANTES), and cytokines IL-6 and tumor necrosis factor-α (TNF-α) (see Fig. 181-4).* Because experimental evidence indicates that the TLR-mediated immune responses are TLR specific, the function and effectiveness of the components of the elicited immune response will vary according to the interaction of RSV with individual TLRs. This is illustrated in rodent models by TLR4-deficient mice having impaired viral clearance, whereas TLR3-deficient mice develop Th2-polarized responses but continue to eliminate virus with normal kinetics.[179,337]

Passively Acquired Antibody

During the neonatal period, initial protection against RSV infection is from transplacentally acquired IgG, as demonstrated by Glezen and coworkers, who showed that the age at the time of hospitalization for infants with primary RSV infection was directly correlated with the level of cord blood–neutralizing antibody titer.[132] However, with the antibody's half-life of approximately 21 days, the protective effect rapidly wanes. These observations, coupled with studies in experimental animals, provided impetus for the development and use of high-titered RSV intravenous immunoglobulin (RSV-IGIV) and, subsequently, of the monoclonal antibody palivizumab for RSV prophylaxis in high-risk infants.[140,198]

B-Cell Responses

After acquiring RSV infection, infants develop antibodies to many of the 11 RSV-specified proteins, the most important of which are antibodies to F and G proteins because they have viral-neutralizing activity.[408] Serum-neutralizing and F and G protein–specific antibodies are observed in the majority of infected infants, regardless of the severity of their illness.[395] However, neutralizing and F antibody responses are blunted in those younger than 6 months of age and diminished responses to the G protein are associated with high levels of preexisting maternal antibody. No evidence exists that preexisting antibody suppresses RSV-specific, cell-mediated responses. The antibody response to the F protein is broadly cross reactive with both A and B strains because neutralizing epitopes are conserved between the two strain groups. This is in marked contrast to the group- and genotype-specific response to the highly variable G protein. Overall, the serum IgG response to RSV is relatively weak. Titers may become undetectable within a year after infection, thus requiring several additional infections before levels approximate those found in adults.[382] Nonetheless, levels of serum antibody are directly correlated to the degree of protection against natural reinfection in infants and adults, a finding confirmed in experimental challenge studies in adults.[172,240,384] However, solid protection from reinfection is of brief duration, because a substantial proportion of adults can be reinfected within several months of a naturally occurring infection.[172]

During primary RSV infection, RSV-specific IgA antibody is detectable in nasal secretions and coincides with the cessation of viral shedding.[172,356] Using an adult challenge model, high levels of RSV-specific IgA antibody have been correlated with protection.[172] In animal models, the durability of mucosal IgA antibody protection was relatively brief when compared with the lower tract protection afforded by serum IgG antibody.[398] In addition to IgA antibody, RSV-specific IgE antibody has also been detected in nasal secretions during RSV infection, which correlated with an increased risk for the development of wheezing both during acute disease and later in childhood.[396] Elevated levels of cysteinyl leukotrienes in nasal secretions also have been associated with wheezing. Mucosal administration of nonreplicating RSV antigen does not induce a protective mucosal immune response, but if inactivated RSV is supplemented with innate receptor ligands (TLR9/NOD2) before application, an antibody response is stimulated.[348]

Efforts to develop a monoclonal antibody to be used for prophylaxis initially focused on the F protein because of its highly conserved amino acid sequence. By contrast, most portions of the RSV G protein are heavily glycosylated and highly variable except for a short central motif that appears to be invariant. The G protein appears to induce neutralizing antibodies and protective immunity, but the G protein also has been implicated in disease pathogenesis, particularly modulation of the host immune response.[205,301,374] Antibodies to the highly conserved motif on the G protein have shown efficacy in animal models both in prophylaxis and in disease attenuation in postinfection treatment settings. The conserved region of the G protein demonstrates molecular mimicry with fractalkine, the chemokine CX3C. Fractalkine is a low-molecular-weight protein that coordinates leukocyte travel to the site of inflammation. Both membrane-bound and secreted forms of G protein can bind to fractalkine receptor CX3CR1, which interferes in the recruitment of immune cells (NK cells, $CD4^+$ and $CD8^+$ T cells) to

*References 27, 33, 141, 145, 179, 207, 230, 266, 336, 339, 346.

the site of inflammation.[49] Interaction of G protein with the fractalkine receptor may contribute to immune evasion and enhance pathogenesis by reducing antiviral cellular responses, which may augment RSV's replication and persistence. Antibodies that block this motif of the G protein have been shown to reduce both viral replication and disease severity in animals.[178] Whether antibody to G protein (from passively administered monoclonal antibodies or from vaccine stimulation) will have a role in prophylaxis and disease modification in humans remains to be determined.

T-Cell Responses

Our understanding of cell-mediated immunity to RSV has been derived principally from studies in rodent models and from limited information in humans. The major hypothesis that has emerged from these studies is that the severity of disease is controlled by the generation of Th1-dominant responses characterized by the induction of IFN-γ–secreting $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes, neutralizing serum antibody, and mucosal IgA antibody.* Conversely, a Th2-polarized response, involving the productions of IL-4–secreting $CD4^+$ cells, eosinophils, and IgE antibody, is associated with airway hyperreactivity and more severe disease.

RSV infection has been shown to have a wide spectrum of effects on T-cell immune responses, but many have yet to be delineated.[283] Long recognized is RSV's inhibitory effects on lymphocyte proliferative responses in vitro. In addition, RSV-infected dendritic cells have diminished capacity to activate $CD4^+$ T cells.† Nonetheless, RSV infection can induce a full range of T-cell responses, including Th1 $CD4^+$ and $CD8^+$ cytotoxic T lymphocytes as well as Th2 $CD4^+$ cells.[263] Th1 $CD4^+$ cells are primarily characterized by a profile of secretion of IL-2, IFN-γ, and TNF-α, whereas the pattern associated with Th2 $CD4^+$ cells consists primarily of IL-4, IL-5, and IL-13.[300] Human and murine $CD8^+$ cytotoxic T lymphocytes recognize F, M2, M, and NS2 epitopes in the context of class I antigens.‡ Each cell type and its signature pattern of secreted cytokines have been correlated with the severity of illness, the presence of wheezing, and the nature of the inflammatory response.[53,251] Both $CD4^+$ and $CD8^+$ T cells can clear virus from the lungs of infected animals. $CD8^+$ CTLs are the most efficient, but excessive numbers of cytotoxic T lymphocytes can also be detrimental.[53] IFN-γ-secreting $CD4^+$ cells (Th1 pattern) eradicate the virus while inducing minimal disease, whereas IL-4–secreting $CD4^+$ cells (Th2 pattern) are associated with pulmonary eosinophilic infiltration and airway hyperreactivity.

The balance between Th1 and Th2 induction depends on multiple factors, including host genetics, the local cytokine environment, and the antigen.§ Replicating antigens, such as live RSV, generally induce a Th1 response with cytotoxic T lymphocytes, whereas inactivated virus, as was used in the formalin-inactivated vaccine trials in the 1960s, or subunit vaccines induce Th2-dominant responses.[208] The F antigen is associated with a Th1 response, whereas the G protein tends to evoke a Th2-type response. The mechanism by which the RSV G protein induces a Th2 polarized T-cell response is not clear, but a highly conserved central cysteine-rich region with structural analogy to the CX3C chemokine fractalkine may be involved. The CX3C motif on the G protein competes with fractalkine binding on T cells and appears to inhibit pulmonary trafficking by CX3CR-positive cytotoxic T lymphocytes.[177] Additional evidence from studies in both mice and humans suggests that RSV is a poor inducer of central memory T-cell responses that are protective, a finding that may account in part for the failure of long-term immunity.[63,332]

During primary RSV infection, peripheral blood mononuclear cells secrete both IFN-γ (Th1) and IL-4 (Th2).[17,68,375] The presence of a Th2-dominant cytokine pattern in respiratory secretions during RSV infection, or a Th2 response induced by in vitro stimulation of lymphocytes, has been correlated with disease that is more severe and associated with wheezing.[3,37,202,344] Not all studies, however, measured RSV-specific responses, and not all studies found a correlation between Th2 polarization and more severe illness.[17] The patient's age at the time of infection also may influence the T-cell response. Infants younger than 3 months of age have been found to have higher levels of Th2 cytokines in nasal secretions compared with older children, who were more likely to have a Th1 cytokine pattern.[229] Little information exists regarding the role of cellular immune responses in protection from subsequent infection. In one report, RSV-specific lymphoproliferative responses were relatively short lived after primary infection and were not boosted by reinfection.[38]

In summary, the cornucopia of recent information on the immune response to RSV has indicated its singular complexity and conundrums. Nonetheless, some consistent principles have evolved. Humoral and mucosal antibodies provide some protection from infection, although it is variable and often transient. The innate and adaptive T-cell responses elicited by RSV infection are pivotal in determining the disease pathogenesis and in viral clearance. Host and environmental factors favoring the induction of Th1 responses, either during primary infection or with repeated infection or with subsequent immunization, may minimize pathologic changes, disease, and airway hyperreactivity.

CLINICAL MANIFESTATIONS

We view their chests ballooning, with the fears
that they're "pink puffers" of more tender years.[146]

Primary Infection

An infant's first encounter with RSV almost always is apparent, but the symptoms may range from those of a mild cold to severe bronchiolitis or pneumonia. Rarely is

*References 98, 163, 164, 190, 262, 274, 300, 351, 358, 374.
†References 71, 80, 88, 143, 248, 265.
‡References 43, 64, 70, 123, 180, 209, 338.
§References 10, 48, 73, 86, 126, 195, 268, 277, 365, 376, 405.

TABLE 181-3 **Frequency of Respiratory Syncytial Virus Infection Among Children Studied from Birth***

Age (mo)	No. of Child-Years	No. with Respiratory Syncytial Virus				
		PRIMARY	REINFECTION	TOTAL (RATE/100 CHILD-YEARS)	LRD (RATE/100 CHILD-YEARS)	LRD (RATE/100 INFECTIONS)
0-12	125	85	1	86 (68.8)	28 (22.4)	32.6
13-24	92	33	43	76 (82.6)	12 (13.0)	15.8
25-36	65	1	29	30 (46.2)	7 (10.8)	23.3
37-48	39	0	13	13 (33.3)	3 (7.7)	23.1
49-60	24	0	12	12 (50.0)	0 (0)	—
Total	345	119	98	217 (62.9)	50 (14.5)	23.0

**Houston Family Study, 1975-1980.*
LRD, lower respiratory tract disease.
Reprinted with permission from Glezen WP, Taber LH, Frank AL, et al. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986;**140**:543-6. Copyright 1986, American Medical Association.

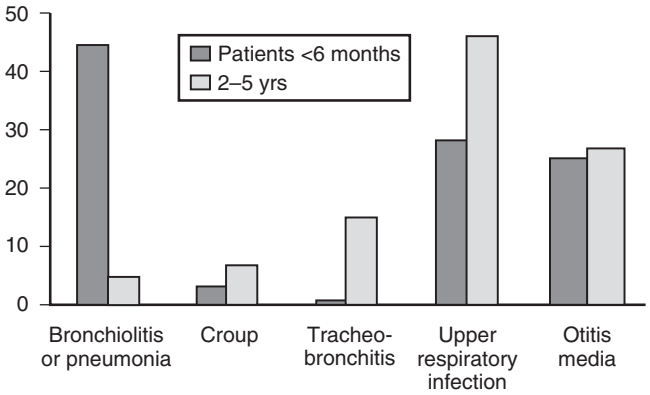

FIGURE 181-5 ■ Clinical diagnoses of children with respiratory syncytial virus (RSV) infection according to age. The proportion of children presenting with each respiratory syndrome who were younger than 6 months of age and had primary RSV infection is shown in comparison with those children aged 2 to 5 years who had a repeated RSV infection. Data were obtained from 415 outpatient infants and children evaluated in Rochester, New York. (From Hall CB, unpublished data.)

FIGURE 181-6 ■ Bronchiolitis from respiratory syncytial virus (RSV) infection. This chest radiograph of a 3-month-old infant with bronchiolitis from RSV infection shows hyperaeration of the lung and area of atelectasis in the right middle lobe.

a child's first encounter with RSV entirely asymptomatic. Among infants examined over the course of a 10-year period with known primary RSV infection, who initially were younger than 6 months of age, 45 percent had the clinical manifestations of bronchiolitis or pneumonia, compared with 5 percent of children 2 to 5 years of age who had experienced two or more outbreaks of RSV infection (Fig. 181-5). Higher rates of lower respiratory tract disease have been documented to occur in children with underlying cardiopulmonary disease, those in closed populations such as daycare centers, and those from certain geographic areas or with particular ethnic backgrounds.[184,250,269,328]

In the longitudinal Houston family studies,[133] 69 percent of children in their first year of life acquired RSV infection and one third of these infections were lower respiratory tract illnesses (Table 181-3). By the time they were 24 months of age, essentially all children had been infected at least once and about half had experienced two infections, such that the rate of infections during the second year of life was 83 percent. The proportion of RSV infections involving the lower respiratory tract remained appreciable during the second, third, and fourth years of life, but the severity decreased. In general, lower respiratory tract illnesses associated with RSV after the second year of life most frequently are tracheobronchitis and reactive airway disease.[133]

Pneumonia and bronchiolitis, the most common forms of RSV lower respiratory tract disease in infants, frequently coexist and may be difficult to differentiate clinically or radiographically. Children with hyperinflation and wheezing, the hallmarks of bronchiolitis, may have densities on a chest film that may appear similar to infiltrates from pneumonia (Fig. 181-6). However, in bronchiolitis, these shadows most likely result from atelectasis rather than from the interstitial inflammation and alveolar filling of pneumonia.

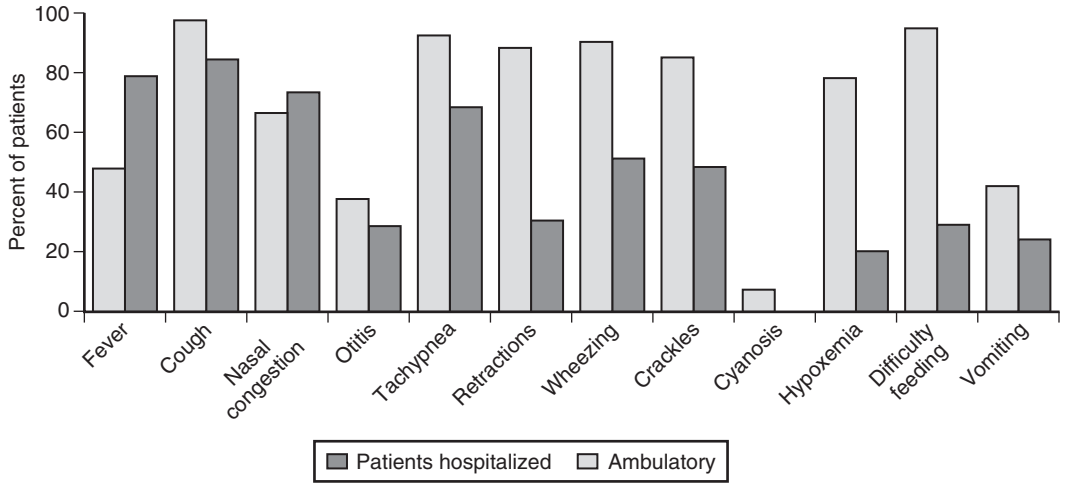

FIGURE 181-7 ■ Clinical manifestations in children with RSV infection who were evaluated in Rochester, New York. Shown are the proportions of 1132 hospitalized children in comparison with the proportions of 489 outpatient children with each clinical sign.

Upper respiratory tract symptoms usually precede lower respiratory tract involvement by several days. Low-grade fever is a common manifestation during this initial phase of the illness, but it may have disappeared by the time of hospitalization (Fig. 181-7). Among 565 children hospitalized with RSV infection, fewer than half had a temperature higher than 38° C (100.4° F) at the time of admission.[171] Although fever occurs more commonly in primary infections, 20 to 40 percent of children with their second, third, or fourth infections are febrile.[184]

Increased coughing commonly heralds involvement of the lower respiratory tract, which subsequently may be indicated by an increased respiratory rate, dyspnea, and retractions of the chest wall (see Fig. 181-7). Variability of the child's clinical findings within minutes to hours is characteristic of RSV lower respiratory tract disease. Auscultatory findings of crackles and wheezing may be present on initial examination but absent on reexamination shortly thereafter. Ill children who elicit concern because they are lethargic and short of breath may have minimal auscultatory signs. The correlation between the findings on auscultation, or on the chest radiograph, with severity of disease or outcome is not consistent.

Radiographic Findings

The radiographic findings in infants hospitalized with RSV lower respiratory tract disease may be variable, but with bronchiolitis, hyperinflation with hyperlucency of the lung, flattened diaphragms, and peribronchiolar thickening usually are evident. Patchy areas of atelectasis also may be present, most often in the right middle and upper lobes. A little less frequent are interstitial infiltrates of pneumonitis, which tend to be present in more than one lobe (see Fig. 181-6). The radiographic findings usually persist beyond the clinical illness.

With tracheobronchitis and upper respiratory tract infections, the accompanying signs and symptoms tend to be more severe in RSV infection than in infections with other common respiratory agents. Cough often is predominant and prolonged, and fever may be present. Otitis media frequently is associated with the initial RSV infection in young children, but it also occurs in older children with recurrent infection.[181,315,318] Among children with RSV infection, the virus has been detected in 30 to 74 percent of middle ear aspirates. Among children presenting with otitis media, RSV is one of the most frequently identified viral pathogens and has been associated with prolonged symptoms and apparent failure of therapy.[23] RSV may be detected as the sole pathogen or simultaneously with a bacterial agent, thus suggesting that RSV may play both a primary and a secondary role in the pathogenesis of otitis media.[315,402]

RSV infection has been associated occasionally with a variety of neurologic, cardiac, and other conditions.[23,216,280,290,364] However, in most of these reports, the causal role of RSV is tenuous.

Infection In Neonates

Symptoms of RSV infection are largely dependent on the age of the host at the time of infection. Infection with RSV in the first 2 to 4 weeks of life has been recognized relatively infrequently. In Washington, D.C., the incidence of RSV infection during the first month of life was noted to be only one third of that during the second month of life.[309] The protected environment of newborns with diminished exposure to others, as well as the high levels of maternal antibody or other early immune factors, may explain in part the generally lower incidence of illness in the neonatal period. RSV infection in neonates may be variable and atypical; nonspecific findings and minimal respiratory signs may result in an incorrect or delayed diagnosis.[168]

In full-term infants, RSV infection in the neonatal period may be manifest primarily as an upper respiratory tract illness, which is usually mild,[150] and often associated with nonspecific findings, such as feeding difficulties, failure to thrive, and generally in the absence of fever, but may also result in periodic breathing and apnea. However,

among premature infants, the morbidity may be more pronounced.[168] During a community outbreak of RSV infection in Rochester, New York, 25 percent of infants kept in a neonatal intensive care unit (ICU) for more than 6 days acquired RSV infection.[168] Infants older than 3 weeks of age tended to have lower respiratory tract disease characteristic of RSV infection and apnea, whereas infants in the first 3 weeks of life were more likely to have nonspecific findings, without clinical evidence of lower respiratory tract disease. Fewer than half had upper respiratory tract signs; nonetheless, four (17%) of the infants died.

Acute Complications

Apnea, a major complication of acute RSV infection in young infants, occurs in 1 to 20 percent of infants hospitalized with RSV infection.[15,167,324] Apnea is most likely to occur among infants born prematurely, in those with a young postnatal age, especially infants who have not yet reached a postconceptional age of 44 weeks, and in infants with a history of apnea of prematurity. The apnea usually develops at the beginning of the RSV infection, often as the first manifestation of illness, and is short lived and nonobstructive.[15] Most infants do not have apnea subsequently, even with later respiratory tract infections.

Simultaneous or secondary bacterial infection appears to be a relatively frequent complication of RSV infection in some countries in the developing world.[60,288,321,373] In the United States and other developed countries, it is an unusual complication, and most clinical and pathologic studies indicate that RSV infection rarely predisposes one to acquisition of a bacterial infection.

In a 9-year prospective study of 565 children hospitalized with RSV lower respiratory tract disease, the rate of subsequent bacterial infection was 1.2 percent in the total group of children infected with RSV and 0.6 percent in the 352 children who received no antibiotics.[171] If a bacterial infection is present simultaneously, it most frequently involves the urinary tract, which is detected by a positive urine culture in about 3 percent of infants.[243,275,326] Concurrent urinary tract infections may be coincidental because they are frequently first detected in this age group. Serious concurrent bacterial infections are unusual enough that evaluations for sepsis and meningitis are not recommended routinely.[13] Controlled studies with randomized antibiotic treatment of lower respiratory tract disease caused mostly by RSV have shown no difference in the severity or duration of illness or in the child's outcome.[13]

More frequently, coinfection with other respiratory viruses occurs.* Studies examining whether coexistent viral infections in normal children augment the severity of infection show mixed results.[19,89,194,256,257] Among the commonly found agents that are simultaneously detected in young children with RSV infection are adenoviruses, rhinoviruses, human metapneumovirus, bocavirus, enteroviruses, influenza viruses, and parainfluenza viruses.[257] Using real-time polymerase chain reaction (PCR) assays, viral coinfection has been reported to occur in more than 15 percent of hospitalized children with acute respiratory tract infection.[39,194] Respiratory disease from bacterial coinfection is uncommon, but bacterial pathogens have been detected simultaneously in the respiratory secretions of children with RSV infection, including *S. aureus*, *Bordetella pertussis*, *B. parapertusis*, *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae*.[294,362,368,386] These bacterial isolates likely represent colonization among infants with RSV infection.

Infants hospitalized with RSV infection also have been noted to be at increased risk for aspiration and subsequently for development of hyperreactive airways.[116,219] One study reported that during a follow-up period of 1 year, 83 percent of the infants hospitalized with RSV bronchiolitis who received no therapy for aspiration or ribavirin developed reactive airway disease, compared with 45 percent of those patients given thickened feedings and early ribavirin therapy.[100] Furthermore, the reduction in the episodes of reactive airway disease was greater with the combined therapy than with either ribavirin or thickened feedings alone.

PROGNOSIS

Thus, if infected at this tender stage
will they become "blue bloaters" as they age?[146]

Prediction at Time of Initial Evaluation

Determining whether an infant with RSV infection who initially presents as an outpatient is likely to require subsequent hospitalization has been problematic. Numerous clinical findings have been evaluated, but most have relatively poor predictive value for individual patients.[13] Among the more consistent and helpful findings at the time of evaluation are oxygen saturation and global assessment of the degree of illness. Fever does not correlate with the severity of disease.[46] Some studies have associated an increased respiratory rate with the risk for developing more severe illness, whereas others have not.[13] However, the lack of tachypnea (≥70 breaths/min) has been correlated with less chance of development of lower respiratory tract infection in infants.[13] Repetitive assessments are helpful, especially considering the rapid fluctuations in clinical manifestations characteristic of RSV lower respiratory tract disease.

Clinical scoring systems commonly are used, but their use is limited by variable design, rendering comparison and standardization impossible.[13] The Respiratory Distress Assessment Instrument has been standardized, but it is not validated in predicting the severity or outcome of children with bronchiolitis. Another report described a scoring system to predict which healthy, term newborns in the Netherlands are at increased risk for RSV lower respiratory tract infection during the first year of life.[191] Independent predictors for RSV lower respiratory tract disease were daycare attendance and/or having siblings, birth weight more than 4000 g, birth in April to September, and a high level of parental education.

*References 120, 194, 217, 227, 242, 256, 257, 272, 329.

Infants hospitalized with RSV bronchiolitis or pneumonia usually show clinical improvement after 3 to 4 days, and most previously normal infants are discharged within 3 to 7 days, with an average of 3 to 4 days.[313,322] However, children with underlying conditions that increase their risk for developing more complicated illness tend to require 2 to 7 days more of hospitalization.[269] Among infants in the first year of life who present to the emergency department with bronchiolitis, the median duration of symptoms was 15 days, and this did not vary in regard to viral etiology.[314]

Patients at Risk for Complicated RSV Infection

Infants most likely to develop severe or fatal RSV infections are those who were premature or have an underlying condition that compromises pulmonary function.* The risk for requiring rehospitalization for infants correlates inversely with birth weight and gestational age. The risk for needing hospitalization with RSV has been reported as three or more times greater for infants of less than 36 weeks' gestation than for those of 36 weeks' gestation or longer.[12,357,361] In an Australian study, the highest risk for developing an RSV infection severe enough to require hospitalization occurred among infants born weighing less than 2500 g and who had older siblings.[328] Other factors reported to be associated with an increased risk for severe disease include impaired neuromuscular function or certain congenital malformations, low socioeconomic status, crowded living conditions, birth during the first half of the RSV season, multiple births, exposure to indoor air pollution including second hand smoke, malnutrition, living at an altitude greater than 2500 meters, Down syndrome, and low antibody concentration in cord blood.† Other chronic diseases, chromosomal abnormalities, and congenital malformations have been associated with an increased risk for RSV hospitalization.[228] However, most of these risk factors either have been inconsistent findings in various reports or they have been associated with only a slightly increased risk for hospitalization.[270]

The highest risk for development of severe and fatal RSV infection occurs among premature infants of very low birth weight (≤1500 g) and of gestational ages of 28 weeks or less.[189] Their high rates of severe RSV infection related to their compromised cardiopulmonary status is augmented further by the lack of maternal neutralizing antibody to RSV in infants of very low birth weight.[90]

Infants with hemodynamically compromising congenital heart disease, such as conditions associated with pulmonary hypertension, have been shown to have a markedly elevated risk for dying.[252] The mortality rate of children with certain functionally important cardiac conditions has declined from greater than 30 to approximately 3 percent.[9,12] The improved outcome of infants with cardiac disease likely is attributable to advances in techniques for surgical correction and medical management, as well as recognition of the risk associated with RSV infection among these patients.

Innate host factors that predispose a child to development of more severe RSV infection have long been assumed but remain mostly undefined. More recent studies, however, show a correlation of genetic polymorphisms with primary RSV disease and severity. Variations within a number of genetic loci potentially associated with controlling immune function, such as the expression of IL-4 and other T-lymphocyte cytokines and chemokines associated with the production of IgE antibody and hyperactive airways, have been shown to be overrepresented in children with RSV lower respiratory tract disease and with disease that is more severe* (see Immune Response: Immunity and Disease Pathogenesis).

Immunocompromised Patients

Recognition of the risk for complicated RSV infection among immunocompromised patients has been heightened with the concurrently increasing numbers of highly immunosuppressed patients, such as recipients of bone marrow and solid organ transplants.[292] A close correlation generally exists between the degree of immunosuppression and the clinical severity of the RSV infection.[78,102,170,247,259] This correlation is illustrated well by the severe and potentially fatal outcome of RSV infection among children with congenital immunodeficiency syndromes who have both defective B-cell and T-cell immunity. Limited information exists about the severity of disease in children with isolated humoral or partial humoral and cellular deficiencies.

RSV infection has been reported as occurring in approximately 50 percent of bone marrow transplant recipients and often is acquired nosocomially.[1,276,323] The morbidity and clinical manifestations associated with RSV in these patients are highly variable.[1,35,36,59,323] Risk factors for development of more severe disease in transplant recipients include not only the degree of immunosuppression but also allogenic transplantation, acquisition of the RSV infection within 2 months of undergoing transplantation but before engraftment, and the presence of graft versus host disease. The associated mortality rate among transplant recipients with lower respiratory tract disease has been reported as 70 to 100 percent, but mortality has been reported more recently as ranging from 0 to 50 percent depending on the patient population.[21,214,218,345] Upper respiratory tract symptoms, sometimes with fever, may be the sole signs of the infection, or they may herald the progression to lower respiratory tract involvement.[36] Findings on the chest radiograph include focal interstitial infiltrates, sometimes associated with lobar consolidation or with hyperinflation, or more severe involvement of generalized alveolar and interstitial infiltration, and marked hypoxemia. The major findings with high-resolution computed tomography (CT) are small centrilobular nodules, airspace consolidation, ground-glass opacities, and bronchial wall thickening. These abnormalities frequently are present in both the

*References 42, 54, 106, 170, 174, 297, 328, 389.
†References 34, 75, 76, 113, 132, 170, 228, 237, 330, 331, 357, 360, 412, 413.

*References 74, 107, 188, 195, 233, 296, 300, 365.

periphery and central areas of the lung, are bilateral, and are asymmetric in distribution.

In this setting, RSV infection often is not recognized or considered because it may occur at times when RSV is not circulating in the community, and the clinical picture may mimic that of other opportunistic agents that commonly infect immunocompromised patients. Furthermore, establishing the diagnosis is problematic because the most frequently available assays—rapid antigen assays applied to swabs of upper respiratory tract secretions—often are inadequate and are relatively insensitive in these patients. A higher yield is obtained with bronchopulmonary lavage specimens. Quantitative PCR assays have detected RSV in bronchoalveolar lavage specimens, as well as in serum specimens, from hematopoietic cell transplant patients with RSV lower respiratory tract disease.[52]

Preemptive therapy with aerosolized ribavirin and anti-RSV antibodies appears to offer minimal benefit to RSV-infected immunocompromised patients.[35,199,218] Because many RSV infections on a hematology-oncology ward are nosocomial, strict enforcement of infection control policies is critical, particularly in the absence of effective antiviral therapy. Strict adherence to an infection control program, particularly during the respiratory virus season, is likely to markedly reduce RSV transmission among patients with hematologic malignancies and stem cell and solid organ transplants.[236]

Patients with Human Immunodeficiency Virus Infection

The effects of coinfection of human immunodeficiency virus (HIV) infection with RSV infection are unclear. Some, but not all, studies suggest that increased morbidity occurs with RSV infection among HIV-infected patients. In a cohort of South African children observed for 5 years, the incidence of hospitalization from RSV lower respiratory tract disease in children with HIV infection was more than two times greater than that in those not infected with HIV (45 vs. 19.4 per 1000 children).

A retrospective Spanish study examined viral respiratory tract infections in children with HIV infection compared with children with cancer and found that RSV was the most common (43%) of the viral agents identified and that 38 percent of the viral infections were acquired nosocomially.[276] The RSV infection involved the lower respiratory tract disease in half of the children with cancer and also in half of those with HIV infection, and hospitalization was required for 12 and 20 percent, respectively. However, the RSV infection was fatal for 21 percent of the hospitalized children with cancer whereas all the HIV-infected patients survived.

Other studies suggested that adverse outcomes in HIV-infected patients are unlikely to occur or are limited to greater or more prolonged viral shedding.[61,81,224] The reported variability in the clinical course in HIV-infected patients who also are infected with RSV likely results from the different ages, degree of immune compromise, location, and therapy for the disease in the HIV-infected populations studied. Nonetheless, the risk for developing severe disease from RSV for most HIV-infected patients appears to be less than that observed among immunosuppressed transplant recipients.

Pulmonary Sequelae

The association between viral lower respiratory tract infection early in life and episodes of recurrent wheezing, reactive airway disease, and pulmonary function abnormalities later in life is well accepted.* A number of respiratory viruses have been implicated in this association, although RSV appears to be the virus most strongly linked with recurrent wheezing. Variations on three basic theories have been proposed to explain the link between early RSV infection and subsequent asthma. One possibility, a causal relationship, is that changes induced by viral replication alter the pattern of normal lung development in such a way that the infant is predisposed to subsequent episodes of wheezing. A second possibility, a triggering relationship, is that certain infants have a preexisting aberration of either airway function or the immune response and early viral infection serves as a triggering event to episodes of acute airway obstruction. For example, several polymorphisms in cytokine genes have been associated with RSV severity and asthma suggesting a shared predisposition.[183,196,296,359,371] A third hypothesis proposes the response to a viral infection is a function of several factors: the genetic makeup of the infant, concomitant exposure to other environmental antigens, and the degree of maturation of the infant's immune system and airway at the time of the infection. This theory proposes no single response to a viral infection; rather, the outcome is a function of several intrinsic and extrinsic factors. If viral lower respiratory tract infections are a major cause of asthma, prevention of infection should reduce the incidence of asthma. Alternatively, if RSV is one of several viral lower respiratory tract infections that can initiate the events leading to subsequent episodes of airway obstruction in a predisposed child, prevention of infection can be expected to reduce the wheezing associated with the acute RSV illness but will have only a modest or no impact on the incidence of asthma. Only intervention studies with a vaccine or other preventive and therapeutic measures are likely to determine whether RSV has a direct, indirect, or no link to the development of recurrent wheezing and asthma among these young wheezers. At the present time, insufficient data are available to answer fully this persistent, but important, question.

Repeated Infection

The most striking proof of RSV's incomplete, inconsistent, and impermanent immune response is the burden of illness levied by the repeated infections it causes. Recurrent infections occur across the age span from toddlers to elderly persons, whether healthy or infirm. Reinfection occurs most frequently among those in close contact with young or school-aged children, as in daycare

*References 8, 101, 108, 127, 135, 185, 187, 197, 258, 299, 353, 354, 366, 409.

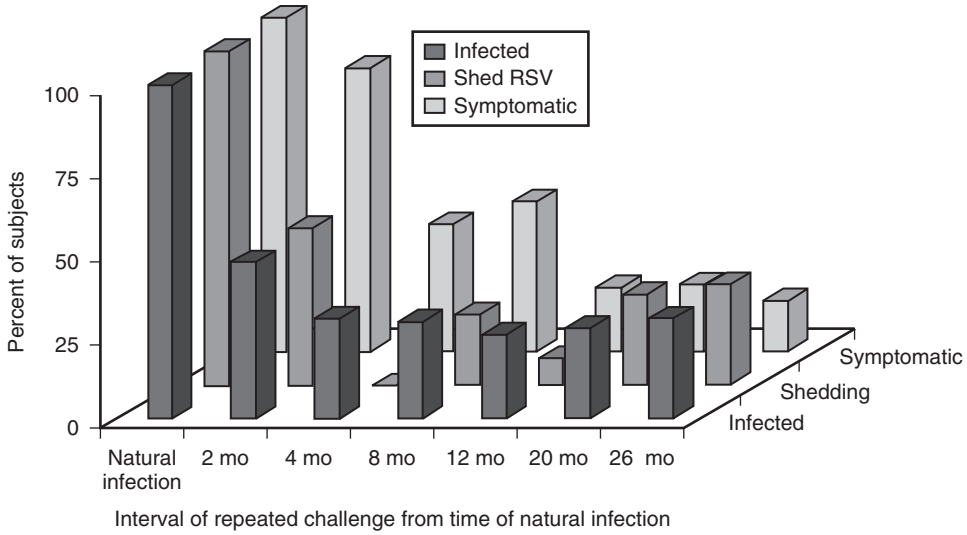

FIGURE 181-8 ■ Reinfection with RSV. Proportion of young adults with natural RSV A infection who became reinfected, shed virus, and were symptomatic when they were subsequently rechallenged intranasally with RSV A after intervals of 2, 4, 8, 12, 20, and 26 months from time of their natural infection. (From Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. *J Infect Dis* 1991;**163**:693-8.)

facilities and primary schools, or among those in confined settings, including military recruits.* Most of these infections are upper respiratory tract illnesses, which often are more severe than is the average cold and often are complicated by sinusitis and otitis media. Recurrent infections involving the lower respiratory tract usually are manifest as tracheobronchitis or as exacerbations of wheezing. Even among young, healthy adults without asthma or other chronic pulmonary conditions, acute RSV infection may produce hyperreactivity of the airways that does not resolve for as long as 8 weeks.[175]

Recurrent Infections in Children

Among longitudinally observed children, up to three fourths have been shown to be reinfected each year. The intervals between infections often are no longer than the period between successive outbreaks of RSV infection or even less, especially among children in daycare, in whom the attack rates have been reported for the first, second, and third infections to be as high as 98, 75, and 65 percent, respectively.[184] Of note is the finding that immunity resulting from a single infection appeared to have no ameliorating effect on illness associated with reinfection 1 year later. Not until the third infection was severity reduced appreciably. Among preschool-aged children, 20 to 50 percent of the repeated RSV infections involved the lower respiratory tract, but the illness tended to be mild.[133] Most children had lower respiratory tract illness only once, and those with repetitive lower respiratory tract infections tended to have reactive airway disease.

RSV Infections in Adults

Healthy adults frequently are exposed to RSV in their workplace and as parents or members of families with preschool-aged and elementary school-aged children. Despite these repetitive exposures, most of them remain susceptible to RSV infection during the subsequent outbreaks and, if infected, are symptomatic.* Recent infection, however, may result in diminished clinical findings and virus shedding, as demonstrated in a study of 15 young healthy adults who became infected with an RSV A strain during a community outbreak and developed upper respiratory tract illnesses (Fig. 181-8).[172] These individuals subsequently were inoculated intranasally with RSV A strain at repetitive intervals over the course of 2 to 26 months. The number reinfected and the proportion manifesting any symptoms continually declined during the 26 months. Concurrently, the quantity and duration of virus shedding also diminished.

Among 211 working adults observed over the course of multiple seasons, RSV reinfection was symptomatic in 84 percent and manifested as an upper respiratory tract illness in 74 percent, two thirds of whom had accompanying fever.[151] The other fourth of the reinfections involved the lower respiratory tract, with the diagnosis of tracheobronchitis or wheezing. Some individuals became infected with RSV and, at a separate time, with both RSV and influenza virus. Comparison of the two illnesses occurring in the same individual revealed that influenza and RSV were clinically similar (Fig. 181-9). Influenza was accompanied more frequently by fever and absence from work, whereas RSV infection was associated significantly more often with sinusitis, otitis, and a longer duration of illness.

The clinical similarity of illness from RSV and from influenza among young adults and the difficulty of differentiating the two also were shown in university students and in military recruits.[249,302] In the close military environment of basic training, new military recruits frequently acquired acute respiratory tract infections,

*References 128, 133, 148, 150, 151, 165, 184, 302.

*References 97, 109, 112, 151, 302, 385.

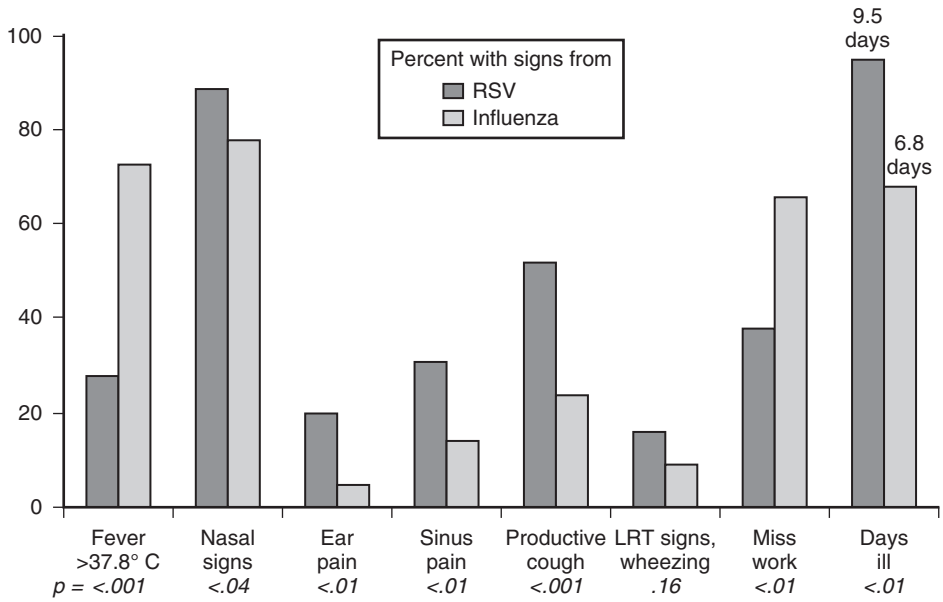

FIGURE 181-9 ■ RSV infection and influenza. Comparison of clinical findings observed during RSV infection compared with influenza infection among 211 prospectively followed previously healthy adults who acquired both infections, RSV infection at one time and influenza infection at a separate time. (From Hall CB. Nosocomial respiratory syncytial virus infections: the "cold war" has not ended. *Clin Infect Dis* 2000;**31**:590-6.)

especially from RSV.[302] RSV infections in these recruits were very similar to those from influenza, except wheezing was associated more frequently with RSV infection. The proportion of patients requiring confinement to a ward was the same for those with RSV infection as for those with influenza.

The importance of RSV infection as a cause of morbidity and mortality in older adults, both those previously healthy and those with underlying conditions, has been recognized increasingly.[97,111,384,403] Between 2 and 9 percent of all lower respiratory tract hospitalizations in elderly persons have been estimated to be caused by RSV, with a cost in 1995 of $150 million to $680 million annually. More recent estimates suggest these costs are over $1 billion.[112] Additional hospitalizations and costs may be incurred from the exacerbations of underlying chronic conditions associated with unrecognized RSV infections.

The impact of RSV infection in elderly individuals was well illustrated in a prospective study of 608 healthy community-dwelling elderly patients and 504 adults with high-risk conditions. An additional 1388 individuals who were hospitalized with acute cardiopulmonary conditions also were studied.[112] The mean age of the individuals studied ranged from 70 to 75 years. Using viral isolation, reverse transcriptase–polymerase chain reaction (RT-PCR), and serology, RSV infection was identified annually in 3 to 7 percent of the healthy cohort, in 4 to 10 percent of the high-risk group, and in 8 to 13 percent of the hospitalized patients. In comparison, influenza A infection was detected annually in 2 to 4 percent of the healthy elderly cohort, in less than 1 to 5 percent of the high-risk group, and in 1 to 20 percent of hospitalized patients. The illnesses from RSV and influenza A could not be distinguished clinically. Among the hospitalized patients, intensive care was required by 15 percent of those with RSV and by 12 percent of those with influenza A infection; 8 percent of the RSV and 7 percent of the influenza A infections were fatal. Although RSV infection generated fewer office visits than did influenza among the healthy, older age group, those with high-risk conditions used health care services similarly if they were infected with RSV or influenza. The impact on health care services and costs by RSV is underscored by the proportion of hospitalizations caused by RSV for pneumonia (10.6%), chronic obstructive pulmonary disease (11.4%), congestive heart failure (5.4%), and asthma (7.2%). The role of RSV in elderly patients with these diagnoses frequently is unrecognized and not diagnosed.

Further evidence of the burden of RSV disease is provided by a prospective, three-season study evaluating patients 50 years of age or older admitted to the hospital with respiratory symptoms caused by infection attributable to RSV, human metapneumovirus, or influenza viruses.[401] The length of hospitalization, admission to the ICU, days in the ICU, the need for mechanical ventilation, and death were no different among patients infected with any of the three viruses. However, the rates of hospitalization during the 3-year period were 15.0, 9.8, and 11.8 per 10,000 residents for infection caused by RSV, human metapneumovirus, and influenza, respectively. For adults 65 years of age or older, rates of hospitalization were statistically higher for those with RSV and human metapneumovirus infections than for those with influenza. RSV accounted for 6.1 percent of hospitalizations for acute respiratory illness in these elderly individuals over the 3 years. In a separate study among high-risk adults 65 years of age or older, hospitalization rates were estimated at 23.4 per 10,000 for infections with both RSV and influenza.[282]

FIGURE 181-10 ■ Simple method for obtaining nasal wash specimens from young children. A 1-oz tapered rubber bulb is used to inject and collect 5 to 10 mL of saline with one squeeze. (From Hall CB, Douglas RG Jr: Clinically useful method for the isolation of respiratory syncytial virus. *J Infect Dis* 1975;**131**:1-5.)

DIAGNOSIS

Too minuscule
for human eyes,
It leaves its scent
and prints precise,
A hidden hare
for hunt and snare …

—CBH

A presumptive diagnosis of RSV lower respiratory tract disease often is made on the basis of the age of the child, the clinical syndrome, especially bronchiolitis, and the usual period of peak RSV seasonal activity, which, if not monitored locally, is available nationally through the Centers for Disease Control and Prevention.[58] An increase in the number of outpatient visits and admissions for bronchiolitis and respiratory illness in children younger than 2 years of age usually is a local indication that RSV is active within the community (see Fig. 181-2).[156]

However, new molecular techniques have shown an increasing number of other viruses may produce in young children illnesses clinically similar to those from RSV, although less frequently among young infants.[255,392,404] Thus, clinical characteristics are seldom sufficiently distinctive to differentiate RSV from non-RSV respiratory tract infections. Routine viral testing of outpatient or hospitalized young children with bronchiolitis is not recommended in the Clinical Practice Guidelines from the American Academy of Pediatrics.[13] However, for epidemiologic monitoring, for evaluating children with an atypical presentation, or for decisions regarding cohorting of patients, particularly high-risk patients, viral testing may be useful.[169]

A specific diagnosis of RSV infection may be made by viral isolation using standard cell culture or shell vials, direct and indirect fluorescent assays, enzyme immunoassays, and methods using nucleic acid amplification PCR.[284] The specimens employed usually are upper respiratory tract secretions from a nasopharyngeal swab or, less frequently, nasal aspirate. A nasal wash or tracheal aspirate is more sensitive than is a nasopharyngeal swab specimen for the recovery of RSV.[155,182] Nasal wash specimens may be obtained with a suction apparatus or, more simply in young children, by the use of a tapered rubber bulb (Fig. 181-10).

In the past, tissue culture isolation of the virus was the standard technique used for establishing the diagnosis of RSV. However, culture techniques now are used less frequently. The disadvantages of viral isolation include the expense, the number of days required until a syncytial cytopathic effect appears (an average of 3 to 5 days), the need for considerable technical expertise and time, and the need for reliably sensitive tissue culture.[186] Viral isolation techniques, however, do have the advantage of being able to grow some coinfecting viruses. Shell viral centrifugation cultures followed by direct fluorescent antibody testing or immunoperoxidase staining with monoclonal antibodies increases the sensitivity of tissue culture techniques and shortens the time until a result is available.[312]

Indirect and direct fluorescent assays applied to nasopharyngeal secretions from children, including neonates, have an average sensitivity and specificity of approximately 95 percent when these tests are performed by experienced personnel.[154,186] However, the ranges of sensitivity and specificity are wide and depend on the laboratory's expertise and on their suitability for certain populations, such as elderly persons, in whom these tests are less sensitive.

Rapid antigen-based assays include direct fluorescent antibody tests, enzyme immunosorbent assays, and chromatographic and optical immunoassays and currently are the most frequently used methods to diagnose RSV infection. Their major advantages are their rapidity, low cost, and simplicity. Nonetheless, they are screening assays and their sensitivity and specificity vary highly, depending on the prevalence of activity of RSV in the community. During times of low prevalence (i.e., non-RSV seasons or at either end of the RSV season when RSV activity is low), their sensitivity, specificity, and positive predictive

value plummet. In addition, for certain patients, such as those who are immunocompromised or elderly, these tests tend to be less sensitive.[186]

PCR techniques are the most sensitive and specific of currently available methods. Although they generally have been used primarily in research laboratories, these assays are becoming more widely available. Nested PCR assays add sensitivity, allow multiple viruses to be identified simultaneously, and help to differentiate RSV group A versus B strains. RT-PCR assays have the advantages of rapidity, from a diminished number of procedural steps, and of a decreased chance of contamination, but most are limited in the number of viruses that may be detected concurrently. Quantitative RT-PCR has the further advantage of determining the viral load, which has been well correlated with viral quantitation by other methods. In addition, higher viral loads appear to correlate with a more severe clinical course.[91,117,192,342] PCR techniques consistently have been shown to be the optimal means for detecting viral infections among populations of children and adults, and they detect 30 percent or more infections than do viral isolation techniques.[45,109,174,249,391] However, the timing and duration of viral isolation have been shown to correlate more closely than PCR results with the acute clinical illness. A considerable proportion of asymptomatic children have specimens positive by PCR to various respiratory viruses, including RSV.[369,379] This may be related to the sensitivity of PCR techniques to detect low levels of shedding during the convalescent phase of a previously symptomatic infection, or indicate a concurrent asymptomatic viral infection, or a persistent infection of the lower airways. It is hoped that future studies will generate a better understanding of the clinical significance of such findings.

Serologic diagnosis usually is reserved for research purposes because it generally is not helpful in the acute diagnosis and management of patients. A detectable serologic response is inconsistent among infants, even among those with severe disease.[172] Furthermore, serologic assays may be falsely negative in 10 to 30 percent of patients.[110,118,186] Serologic assays available include complement-fixation assays, neutralization assays, and enzyme immunoassays, using purified F and G proteins of RSV for IgM and IgG antibodies. The complement-fixation assay tends to be the least sensitive. Serologic diagnosis appears to be more sensitive among elderly than younger subjects.[383] Faster diagnosis by the use of a single high titer has been tried, but it has had variable results and is not well standardized.

TREATMENT

For most children with RSV lower respiratory tract disease, supportive care is sufficient.[420] Ensuring that the child has adequate fluid intake and, when necessary, clearing the nasal passages by gentle bulb suctioning are important in making the infant comfortable.

Supportive care also is integral to the management of the more severely ill infant.[225] Increased production of mucus and secretions in the nasal and lower airways and respiratory rates of more than 60 to 70 breaths per minute are likely to compromise the infant's ability to take fluids and sleep, thus resulting in an increased risk for requiring intravenous hydration and assisted ventilation.[13,77] A Cochrane report assessed the results of nine controlled trials comparing chest physiotherapy (vibration and percussion techniques) to no intervention. No differences were detected between groups in their respiratory parameters, oxygen requirement, length of stay, or severe side effects among hospitalized, nonintubated patients with acute bronchiolitis. In addition, deep pharyngeal or tracheal suctioning has not been shown to be beneficial.[13,291]

The criteria used for determining the need for administration of supplemental oxygen to previously healthy infants are controversial, in part because the usual fluctuating respiratory findings characteristic of RSV lower respiratory tract disease confound the evaluation of the oxyhemoglobin saturation (Spo_2) level at which supplemental oxygen administration provides measurable clinical improvement. Spo_2 levels on room air of less than 95 to 90 percent have been used, but current recommendations suggest that persistent measurements of Spo_2 of less than 90 percent indicate the need for initiating supplemental oxygen.[13]

Conflicting results have come from trials evaluating the role of hypertonic saline inhalation for acute bronchiolitis. Results from some studies suggest a modest reduction in the length of hospitalization among patients treated with nebulized 3 percent saline versus those treated with 0.9 percent saline.[18,142,325,415] Theories suggesting a mechanism of action for hypertonic saline include increased hydration of secretions, reduction of airway edema, stimulation of airway cilia, and enhanced clearance of debris from the airways.

Several randomized trials have evaluated the usefulness of inhaled helium-oxygen therapy among children with bronchiolitis. This approach is based on the theory that helium-oxygen mixtures may improve gas flow through the high-resistance airways that characterize bronchiolitis.[222] However, trial results have been inconsistent and unequivocal benefit has not been demonstrated, perhaps owing to small sample size, inconsistent endpoints, or variation in study design.[144,223,260] A Cochrane review concluded that helium-oxygen therapy may have an early, transient benefit but does not result in a reduced rate of intubation, less mechanical ventilation, or shortened length of stay in a pediatric ICU.[245]

The use of bronchodilating agents, corticosteroids, and antibiotics for the management of infants with RSV lower respiratory tract illness has been highly variable among centers in the United States and other countries.[13,388] Bronchodilators have been administered to an average 75 to 80 percent of infants hospitalized with RSV infection. Corticosteroid therapy has been used in 10 to 40 percent and intravenous antibiotics in 15 to 40 percent of infants admitted with documented RSV infection.[13,420]

Trials evaluating α-adrenergic or β-adrenergic bronchodilating agents have given inconsistent results.[13,77,215] These trials have differed in the agents and outcomes measured. The patients studied have varied in age,

presence of underlying conditions, and history of previous episodes of wheezing, rendering comparisons difficult to make.

A Cochrane review of bronchodilator therapy concluded that a limited transient improvement in assessment scores may be observed in one of every seven children treated, but the clinical benefit was questionable.[215] A second Cochrane review of bronchodilator therapy for hospitalized infants and young children with bronchiolitis found no effect on oxygen saturation or length of stay.[122] Outpatient bronchodilator treatment did not reduce the rate of hospitalization or reduce time to resolution of illness at home. After a comprehensive review of the clinical trials, the American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis[13] recommended that for management of infants with a first episode of wheezing, bronchodilators should not be used routinely. The guidelines note that a carefully monitored trial of inhaled α-adrenergic and β-adrenergic agents was a possible option in some cases. However, bronchodilator administration should be continued only if benefit was documented by objective means of evaluation. Some evidence suggests for trials conducted in emergency departments and hospitals that epinephrine may be the preferred nebulized agent. In the home and outpatient settings, however, racemic epinephrine would not be advisable because of its potential adverse effects and its short duration of action. Anticholinergic medications alone or in addition to bronchodilatory agents have not been shown to improve the course of viral bronchiolitis.[13]

Several reviews analyzing the randomized and controlled trials of administering systemic glucocorticoids in the management of acute viral bronchiolitis concluded that the evidence was not sufficient to support or recommend the use of systemic glucocorticoids on a routine basis.[13,85,124,311] The studies that were included varied in methods and in the type of patients included. Nonetheless, most of them showed no benefit in the clinical scores, the rate of hospitalization after glucocorticoid use during the initial medical visit, the length of stay in those hospitalized, or the rates of readmission or return outpatient visits. One meta-analysis showed a decrease of 0.43 day in hospitalization, but when the population was limited to only first-time wheezers no benefit existed.[124]

Placebo-controlled studies that examined the use of oral glucocorticoid therapy administered in the emergency department to preclude the need for hospitalization showed some benefit of glucocorticoid therapy over placebo.[347] More recently, however, a large study involving 20 emergency departments during the course of three RSV seasons examined the outcomes of 608 children aged 2 to 12 months who presented with a first episode of bronchiolitis in a double-blind, placebo-controlled trial that administered one dose of oral dexamethasone of 1 mg/kg.[83] The rate of hospitalization and the Respiratory Assessment Change Score (RACS) of these groups did not differ significantly. Furthermore, no benefit was observed among the subgroup of children with asthma or with a family history of asthma. Thus, the 2006 American Academy of Pediatrics guidelines[13] concluded that corticosteroid medication should not be used routinely in the management of bronchiolitis and that current data do not support a recommendation for using corticosteroid therapy to prevent hospitalization. However, some evidence exists that certain subsets of children, including older children and those with prior episodes of wheezing and with asthma, may potentially benefit from the administration of systemic glucocorticoid therapy.[7,13,311]

Montelukast is a selective leukotriene-receptor antagonist that inhibits the cysteinyl leukotriene 1 receptor and is an effective treatment for asthma and allergic rhinitis.[32,69] Because increased leukotriene levels are present in nasal secretions during many viral infections, including RSV,[87] montelukast was evaluated for its effectiveness in treating RSV lower respiratory tract disease. Although an initial controlled trial suggested a beneficial effect in infants with RSV bronchiolitis,[30] a large follow-up study randomizing 979 subjects failed to show a difference between the groups in the primary outcome (days with no cough, wheeze, or shortness of breath).[31]

Antibiotics should not be administered prophylactically to children with RSV infection or as therapy unless specific indications of the coexistence of a bacterial infection are present.[13] As noted previously, concurrent or secondary respiratory bacterial infection is an unusual occurrence, except for otitis media, among children with RSV lower respiratory tract disease, and prolonged administration of intravenous broad-spectrum antibiotics may augment the risk for development of secondary bacterial infection.[171] Furthermore, the administration of antibiotics in placebo-controlled trials has not been associated with greater beneficial outcomes.[226,317]

The only specific treatment currently approved for children hospitalized with RSV infection is ribavirin (1-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide). This synthetic nucleoside has been administered as a small-particle aerosol for 12 to 22 hours per day until clinical improvement is evident, usually about 3 days. Much shorter, intermittent courses of higher doses also appear to be an acceptable mode of administration.[104] An analysis of the use of ribavirin for RSV lower respiratory tract disease included 11 randomized trials.[13] Seven of these studies showed benefit in the clinical outcome, and 4 did not. Long-term studies examining subsequent development of pulmonary function abnormalities or recurrent wheezing have had mixed results. However, the total number of patients in these controlled studies is small, the design of the trials is heterogeneous, and the degree of benefit in clinical outcome is unclear. For these reasons, plus its high cost, the American Academy of Pediatrics recommends that ribavirin should not be used routinely in the management of RSV lower respiratory tract disease.[13] Consideration of ribavirin's use should be reserved primarily for infants with or at high risk for developing severe disease, on an individual basis.[13,377] Currently, aerosolized ribavirin is used mainly for treatment of RSV infection in immunocompromised patients, although it is not licensed for this indication.[36]

Investigations continue to find therapeutic compounds that may offer benefit for infants with RSV bronchiolitis. Palivizumab has been evaluated in small, controlled trials of hospitalized infants, but, although antiviral activity has been shown, therapeutic benefit has not been

demonstrated.[67,253,333,334] A small randomized controlled trial of motavizumab also failed to demonstrate therapeutic benefit.[232] Efforts to develop a complementary, antisense sequence of RNA (ALN-RSV01) that suppresses expression of a specific viral gene (nucleocapsid gene) are underway. This product has been evaluated in adult volunteers who received the drug intranasally prior to RSV inoculation, but the differences in the symptom scores between treatment and placebo groups were not significant.[93] ALN-RSV01 is being investigated among lung transplant patients for whom RSV lower respiratory disease may be fatal.[414] Another promising area of investigation is inhibitors of RSV fusion. Inhibition of enzymatic activity associated with several epitopes within the fusion (F) RSV glycoprotein prevents viral entry into the host cell as well as inhibition of cell-to-cell spread.[417] Other small molecule compounds that bind specific epitopes on viral-encoded proteins, including G, L, N and P proteins, interfere with the viral replication cycle.[67] Clinical efficacy of these compounds has not yet been demonstrated.

PREVENTION

With perseverance and
the dreamer's scanning scope,
reality is made of distant hope…

—CBH

Breast-Feeding

The number of infants who have been breast-fed has increased in recent years because of the growing recognition of the correlation of breast-feeding with both general and specific beneficial effects on the health of the infant.[56,60,125] Most studies evaluating the protective effect of breast-feeding against RSV infection show a diminished likelihood of acquiring more severe infection.[13,22,125,308] Although colostrum and breast milk contain IgG and IgA antibodies and neutralizing activity against RSV, the mechanism of the protective effect is complex and may relate more to many other multifunctional components in human milk, which during digestion inhibit infection by viral, bacterial, and other infectious agents.[289] Longer-term protection from breast milk against bronchiolitis may relate to human milk factors that modulate the infant's developing immune system, such as maternally derived human breast milk cells.[47] The degree and duration of breast-feeding necessary to provide protection against RSV infection, however, remain undefined.[231]

Numerous studies performed over the course of several decades have indicated that breast-fed infants are less likely to be hospitalized with RSV and other respiratory tract infections than are those children who are not being breast-fed.[13,22,308] Breast-feeding and the risk for hospitalization for lower respiratory tract disease in healthy, full-term infants were examined in a meta-analysis of 33 studies,[22] all of which indicated a protective association. However, in this meta-analysis, only 9 studies met all the inclusion criteria. Infants who were not breast-fed were almost three times (risk ratio, 0.28) more likely to be hospitalized for lower respiratory tract disease in comparison with those exclusively breast-fed for 4 months. One study estimated that 30 percent of hospitalizations of infants for infection would have been avoided for each additional month of full breast-feeding beyond 3 months.[308] The authors thus estimated that if all 4-month-old infants had been exclusively breast-fed, 56 percent of hospital admissions for infants in the first year of life would have been prevented.

Infection Control and Nosocomial Infection

Preventing RSV infection from being acquired by normal infants and young children, as well as avoiding the RSV infections that frequently occur subsequently, is difficult, if not impossible, considering the prevalence and often covert presence of RSV during the annual outbreaks. Infection control procedures in the home should focus on interrupting the spread of RSV by using effective hand hygiene practices, including frequent hand washing and the use of hand sanitizers.[41,241] If hands are not visibly soiled, alcohol-based sanitizers are preferentially recommended.[41] Their use generally has been more effective, has been better accepted by both children and adults, and has shown significant decreases in the occurrence of respiratory and diarrhea illnesses in daycare facilities and schools.[41,147,400] Objects contaminated with respiratory secretions, which act as fomites, should be cleaned and used tissues carefully disposed.

Within medical settings, infection control procedures are the mainstay of controlling nosocomial infections from RSV.[41] RSV infection acquired nosocomially is recognized increasingly as a problem of magnitude, cost, and concern, considering the current number of patients with high-risk conditions.[147]

More than 70 years ago, in 1941, Adams[4] described an epidemic of pneumonitis that occurred in January through February in the nurseries of two Minneapolis hospitals. Thirty-two infants, mostly in the second and third months of life, were affected, and 29 percent died. Cytoplasmic inclusions in the bronchial epithelium were observed in all fatal cases. The distinctive epidemiology, clinical syndrome, and pathology of these cases led Adams to propose a viral origin. Twenty years later, he and his colleagues described a markedly similar epidemic of respiratory illness in infants in whom they identified RSV as the cause.[5] RSV was thus indicted as the agent of the earlier, first-described nosocomial outbreak.

During community outbreaks of RSV, the risk for the nosocomial spread of RSV infection is high and, if not recognized, results in increased morbidity and prolonged hospitalization. On infant wards and neonatal units, nosocomial RSV infection has been especially hazardous and sometimes fatal for the more compromised infants.[147,159] The risk for acquiring nosocomial infection is related to the child's age, underlying disease, length of hospitalization, and, clearly, adherence to proper infection control procedures.

Among immunocompromised children and adults, nosocomial RSV infections often are serious and difficult to control.[40,105,147,269,378] Outbreaks in transplant units in particular have been associated with a high mortality rate and prolonged and intermittent transmission (see Immunocompromised Patients).

RSV infection frequently is introduced to the ward by hospital staff, families, and visitors who may have only a mild cold. Genetic analysis has demonstrated that nosocomial outbreaks may be caused by one or multiple strains of RSV during a single outbreak or season.[20,341,363] Infection rates may be high among staff members, who often require absence from work.[147] However, even uninfected staff may spread RSV infection by fomites, through contact with infectious secretions contaminating environmental surfaces.

Various infection control procedures have been employed to prevent the nosocomial spread of RSV (Table 181-4). Integral to any effective infection control program are assiduous hand hygiene and education of health care personnel, as well as families and visitors, including frequent reminders to ensure compliance throughout the RSV season.[41,147] Careful and continued education regarding the importance of infection control practices has reduced the incidence of nosocomial RSV infection in many pediatric facilities. These practices generally include (1) early identification of RSV infected patients through laboratory screening of symptomatic patients, (2) cohorting of infected patients and staff, (3) excluding visitors with current or recent respiratory tract infections, (4) excluding staff with respiratory tract illness from caring for susceptible infants, (5) emphasizing hand hygiene before and after direct patient contact or contact with objects in the vicinity of patients, and (6) limiting visitation by young siblings and other young children during the RSV season.[11] The efficacy of additional barrier precautions such as gowns, gloves, and masks has varied in different settings. In two controlled studies, the routine use of gowns and masks did not add further benefit to conscientious hand washing and other infection control procedures.[158,287] Masks that cover the nose and mouth are of limited benefit because touching or rubbing the eyes or nose are the major modes of self-inoculation.[121,157,162] Thus, eye-nose goggles or goggles in addition to a mask appear to be more effective in diminishing nosocomial infection in both staff and infants.[121] With widespread application of infection control practices, the occurrence of nosocomial RSV infection in most neonatal intensive care units has become an uncommon event.[28]

As many as 70 percent of RSV infections on hematology-oncology wards are nosocomial.[1,68] More severe RSV disease is recognized to occur early after transplantation and among patients with persistent lymphopenia, chronic graft versus host disease, and allogeneic stem cell transplantation.[14,68,99,199] Application of the infection control measures just described in conjunction with a private room, central HEPA filtered air supply, and positive-pressure ventilation to a hematology-oncology ward has been shown to significantly reduce RSV transmission.[236]

TABLE 181-4 **Infection Control Procedures, Both Standard Precautions and Contact Precautions for Prevention of Respiratory Syncytial Virus Infection, Recommended by Centers for Disease Control and Prevention**

Category I-B Recommendations*	Comment(s)
Hand washing	Water with soap or antibacterial agent or waterless antiseptic hand rub
Wearing gloves	Combined with hand washing before and after each glove change; may diminish self-inoculation
Wearing gowns	When direct contact with patient or patient secretions is likely
Wearing masks and eye protection	Eyes and nose are major sites for inoculation
Housing patient in private room or in a cohort isolated from other patients	Patients with documented infection can be grouped and isolated from other patients; beds should be separated by >0.9 m
Use of dedicated patient-care equipment sometimes recommended with less or no supporting evidence	Equipment, including toys, assigned to specific patients
Staff assigned according to patient's RSV status	Specific staff care only for patients with RSV infection
Visitor restrictions during RSV season	Some qualify by restricting young children only
Screening visitors for illness during RSV season	Visitor assessed by trained personnel or advised by use of an educational patient information list

**Centers for Disease Control and Prevention I-B recommendations based on "strong rationale and suggestive evidence" and strongly recommended for all hospitals and "reviewed as effective by experts in the field."*

RSV, respiratory syncytial virus.

Modified from Hall CB. Nosocomial respiratory syncytial virus infections: the "cold war" has not ended. *Clin Infect Dis* 2000;**31**:590-6.

Prophylaxis

Interest in immunoprophylaxis against RSV infection for high-risk infants came from the observation that although maternally derived RSV antibodies may not completely protect against RSV disease in young infants, the severity of RSV lower respiratory tract disease was inversely correlated with neutralizing antibody titers. Longitudinal studies indicated that repeated RSV infections are less severe, suggesting attenuation of disease by the immune response. These observations led to immunoprophylaxis trials with infusion of standard intravenous immunoglobulin in high-risk infants. Efficacy was not demonstrated, most likely because the peak titer of RSV neutralizing antibody did not reach the levels necessary to confer

protection. Subsequent trials with an enriched or hyperimmune RSV globulin (RSV-IGIV) demonstrated statistically significant differences in hospitalization rates between RSV-IGIV recipients and control groups. In January 1996, the U.S. Food and Drug Administration (FDA) licensed RSV-IGIV (Respigam) for "the prevention of serious lower respiratory tract infection caused by RSV in children younger than 24 months of age with bronchopulmonary dysplasia or a history of premature birth defined as less than 35 weeks of gestation."[273,319] Disadvantages associated with RSV-IGIV included significant adverse events in children with congenital heart disease, the need for intravenous access, the risk for infectious disease transmission associated with plasma-derived products, supply issues, and interference with live attenuated vaccines. These issues led to the development of monoclonal antibodies. RSV-IGIV is no longer available.

In 1998, the FDA licensed a monoclonal anti-RSV antibody preparation, palivizumab, which targets a highly conserved epitope on the RSV F glycoprotein. Palivizumab was 50 to 100 times more effective than was the polyclonal immunoglobulin and circumvented many of the difficulties and adverse effects of administering a large intravenous dose of gamma globulin to small infants.[303] Palivizumab was developed from a mouse monoclonal antibody that recognizes a protective epitope of the surface glycoprotein, the F protein. Only the antigen recognition site from the mouse monoclonal antibody was inserted into the spine of a human antibody, thus diminishing the chance of having allergic reactions to the mouse-raised antibody.[352] A randomized, multicenter, double-blind, placebo-controlled trial involving 1502 premature infants with or without chronic lung disease demonstrated that monthly intramuscular injections of palivizumab reduce the overall incidence of RSV hospitalization by 55 percent compared with placebo recipients.[198] A second randomized, placebo-controlled trial involving 1287 infants with hemodynamically significant congenital heart disease published in 2003 confirmed the safety and efficacy of immunoprophylaxis in high-risk infants and young children.[11] The rate of hospitalization for RSV illness was reduced by approximately 50 percent among the infants who received prophylaxis in comparison with the placebo group. In different subgroups, the reduction of RSV hospitalization rates ranged from 39 to 78 percent, with the lower rates of protection observed among infants with chronic lung disease and congenital heart disease.[13] Post-licensure studies suggested that reduction rates in the necessity for hospitalization with RSV infection may be even greater.[12] This humanized monoclonal IgG subclass 1 antibody became the first monoclonal antibody introduced into clinical practice for the prevention of an infectious disease.

A second-generation humanized IgG1 monoclonal antibody (motavizumab) derived by in vitro affinity maturation of the complementarity-determining regions of the heavy and light chains of palivizumab demonstrated approximately 18-fold greater neutralizing capacity than palivizumab.[2] Motavizumab was evaluated in a randomized, double-blind, noninferiority trial to palivizumab in 6635 infants with prematurity and/or chronic lung disease of prematurity. Motavizumab proved to be noninferior to palivizumab in terms of reduction in RSV hospitalization rates.[55] However, because of several concerns, including an increased incidence of hypersensitivity reactions among motavizumab recipients, motavizumab was not licensed by the FDA.

The mechanism by which RSV-neutralizing monoclonal antibodies interrupt the viral replicative cycle appears to be through inhibition of viral cellular fusion. Pretreatment of virus with either palivizumab or motavizumab does not inhibit the ability of the F protein to interact with the target cell receptor, but viral transcription does not occur. This suggests these antibodies block virus-to-cell fusion, perhaps by preventing conformational changes in the F protein after attachment.[193]

RSV isolates resistant to palivizumab due to mutations in the palivizumab-binding site on the F protein initially were described in vitro during long-term incubation of infected cells in the presence of antibody.[92,416,419] Subsequent reports have isolated palivizumab-resistant RSV isolates from RSV infected children who were receiving palivizumab prophylaxis.[6] Palivizumab-resistant RSV isolates appear to occur in a subset of RSV breakthrough cases, although these escape mutants appear to have impaired growth characteristics relative to wild-type RSV. The significance of these escape mutants as a cause of breakthrough disease is poorly understood.[418]

Current Guidelines for Immunoprophylaxis

Palivizumab is the only licensed product available to reduce the risk for RSV lower respiratory tract disease in infants and children with chronic lung disease of prematurity, with a history of preterm birth (<35 weeks' gestation), or with hemodynamically significant congenital heart disease. Palivizumab is administered intramuscularly at a dose of 15 mg/kg once every 30 days. For most areas of the United States, monthly prophylaxis should begin in November.[11] The American Academy of Pediatrics recommends prophylaxis for subgroups of high-risk children for different periods of time depending on their gestational and postnatal ages, the severity of their underlying conditions, their need for recent medical therapy, and the presence of other risk factors. Prophylaxis with palivizumab should be considered for children with chronic lung disease who have required therapy within 6 months of the start of the RSV season, for infants born at 28 weeks' or less gestation during their first RSV season, and for infants born at 29 to 32 weeks' gestation if younger than 6 months of age at the start of the RSV season. For infants born at 32 to less than 35 weeks' gestation, prophylaxis currently is recommended to be limited to infants at greatest risk for hospitalization due to RSV. This includes infants younger than 90 days of age at the start of the RSV season or infants who are born during the RSV season and are at increased risk for exposure to RSV. RSV is more likely to lead to hospitalization of infants in this group if the infant attends child care or if the infant lives with a sibling younger than 5 years of age.[11,271] Prophylaxis also is recommended for

children with congenital heart disease if it is hemodynamically significant. Once a child has qualified at the beginning of the season for the administration of prophylaxis, it should be continued throughout the season, but with no more than the recommended five monthly doses.[12] Palivizumab has not been shown to be effective in the treatment of RSV disease.

The use of palivizumab, nonetheless, remains controversial. Economic analyses, in general, have not demonstrated an overall savings in health care costs if all high-risk children receive prophylaxis.[103,327] Cost-to-benefit analyses have been difficult to determine because of considerable geographic variation in the population of infants and hospitalization costs for such infants. Most published cost-effectiveness analyses without pharmaceutical sponsorship have found that the cost of immunoprophylaxis far exceeds the economic benefit of avoiding an RSV hospitalization.[176,201,390,410] Palivizumab immunoprophylaxis has not been correlated with a significantly diminished rate of mortality for infants with RSV infection who receive the prophylaxis, but the mortality rate from RSV currently is very low. The effect of palivizumab prophylaxis on subsequent sequelae, such as recurrent wheezing and pulmonary function abnormalities, is unknown.

Vaccines

Control of RSV has long lured but eluded investigators. Although the outcome of infants with RSV infection has improved greatly in recent years, the burden on health care that RSV imposes yearly remains notable among both children and adults.[110,168] Immunoprophylaxis with palivizumab administration will lower the RSV hospitalization rate among high-risk infants and young children, but the vast majority of hospitalized young children do not have risk factors that justify the use of palivizumab. Thus, immunoprophylaxis will have only a modest impact on the overall burden of RSV disease in the United States. Only effective immunization is likely to alleviate this burden. Certain characteristics of RSV infection, however, continue to pose barriers to the successful development of a vaccine.

The shroud of past experience with the formalin-inactivated vaccines conferred caution and concern regarding the development of candidate vaccines. The initial RSV vaccine trials, with the alum-precipitated, formalin-inactivated vaccine (FI-RSV), were conducted in 1966 to 1967 among seronegative children 2 months of age or older with the administration of an inactivated and trivalent parainfluenza vaccine. When subsequently naturally infected during the subsequent RSV outbreak, the FI-RSV vaccinees experienced an unanticipated increased severity of disease. Almost 80 percent required hospitalization, and two died.* The unfortunate outcomes of these trials stimulated research that has been integral to the evolution of future vaccines. Subsequent examination of the vaccinees' immune response and experiments in rodent models with exaggerated disease indicated that the formalin-inactivated vaccine produced a poor and defective neutralizing antibody response and an excessive Th2 type CD4 T-cell response.[62,80,95] These studies suggested that a successful vaccine should evoke a balanced immune response with both $CD4^+$ and cytotoxic lymphocytes (see Immune Response section).[136,298]

Multiple barriers exist to the development of an effective and safe vaccine. The lack of a model to test candidate vaccines that is reliable, adequate, and predictive of the effect in humans remains a significant hurdle.

An RSV vaccine also should reflect the immune response of natural infection, yet improve its inconsistent and nondurable protection. In addition, an RSV vaccine would need to be administered in the first few weeks of life, when the most severe disease usually occurs. Little experience exists regarding immunization with live viruses during the neonatal period when the immune system is variably developed and maternal antibody is present, both of which may exert unpredictable effects.[298] However, pregnant women respond well to vaccines with resulting placental transfer of maternal antibodies during the third trimester, which may be a potential approach to offer protection to infants early in life. Furthermore, the effect of the multiple antigens from other vaccines administered in the first few months of life must be considered.

Vaccine development subsequently has cautiously taken several approaches with production of live attenuated, protein subunit, and vectored candidate vaccines.[135] Live attenuated vaccines have the advantage over subunit vaccines of producing an immune response that more closely mimics the natural route of inoculation into the upper respiratory tract, thus eliciting more balanced local and systemic humoral and cellular responses and offering potentially broader and more durable protection.

The initial live attenuated candidate vaccines were derived from cold-passaged *mutants* propagated from wild-type RSV (wt RSV) strains grown at progressively lower temperatures, and strains were selected by growth at low temperatures, similar to that of the upper respiratory tract. Subsequent passage in the presence of chemical mutagenic agents produced candidate strains that were temperature sensitive *(ts)* and unable to replicate at the higher body temperatures of the lower respiratory tract.[79] These candidate (*cp* and *ts*) vaccines generally produced promising results in adult volunteers and seropositive children but proved to be unsuitable for infants and young, seronegative children. These vaccines were associated with unacceptable degrees of illness, were overattenuated and not protective, or were genetically unstable; some were accompanied by prolonged shedding of wild-type virus, which most frequently was observed in infants and resulted in transmission of the shed virus to 20 to 25 percent of the unvaccinated contacts.[212]

More recent vaccine candidates have been derived from previously combined *cp* and *ts (cpts)* mutants, which contain mutations generated by reverse genetics that allow the precise desired enhancements of stability, attenuation, and immunogenicity. For example, one candidate, *cpts 248* strain, was further mutated and attenuated into *cpts 248/404*. Each of its mutations was introduced alone or in combination into wt RSV for evaluation in

*References 72, 119, 211, 221, 285, 286.

experimental animals of the mutations' immunogenic and attenuating effects. This candidate *cpts 248/404* vaccine was immunogenic in seropositive and seronegative children older than 6 months, but upper respiratory tract illness with appreciable nasal congestion developed in younger infants 1 and 2 months of age.[407]

Reverse genetics has produced a lineage of further "designer gene" candidate vaccines with one or combined deletions of *NS1*, *NS2*, *M2-2*, and *SH* genes. Two recombinant candidate vaccines, *cpts 248/404/ΔSH* and *cpts 248/404/1030ΔSH*, illustrate this technique's ability to correlate a vaccine's attenuating and protective effect to specific mutations but not to predict the host's mitigating factors such as age. Both appeared highly attenuated, immunogenic, and stable in initial clinical evaluations of adults and children 6 months of age and older.[213] *Cpts 248/404/1030ΔSH* produced more restricted replication in seronegative children, but only 44 percent of infants aged 1 to 2 months developed IgA antibody responses. However, despite the lack of a measurable antibody response, the infants demonstrated some protection against a second dose of the RSV vaccine, a finding suggesting that immunity not reflected in the measured humoral response was evoked. The two vaccines, *cpts 248/404/ΔSH* and *cpts 248/404/1030ΔSH*, differed only in a missense mutation (1030) in the polymerase gene, and this indicated that the 1030 mutation conferred attenuation. Clinical trials with live attenuated intranasal vaccines have demonstrated that achieving an appropriate balance between overattenuation (failure to induce a protective immune response) and underattenuation (production of symptoms in the vaccinee) is difficult.

Many other mutations have been evaluated in experimental animals, not only for their immunogenic, attenuating, and stability characteristics but also for properties required for augmenting the safety, efficacy, and acceptance of potential vaccines, such as increasing the degree and breadth of antigenic expression and the compatibility with different viral antigens that would allow combination vaccines of RSV with parainfluenza and other respiratory viruses.[79,279]

Protein subunit vaccines have raised concern for disease enhancement, particularly in the pediatric population, and thus trials have been largely restricted to older children and adults. Candidate subunit vaccines have used primarily the two major surface glycoproteins, F and G, which are the major targets for the production of neutralizing antibodies.[84,352] Clinical investigation has focused on vaccines of the F protein, which has greater cross reactivity between RSV A and RSV B strains. Three generations of purified F protein vaccines, PFP-1, PFP-2, and PFP-3, that consist of immunoaffinity-purified, alum-absorbed F glycoprotein, have been evaluated in clinical trials. The initial PFP-1 vaccine contained small amounts of the G protein, which did induce an antibody response. PFP-1 and PFP-2 vaccines have been administered to normal adults, institutionalized adults, normal children, and those with chronic underlying conditions including chronic lung disease, cystic fibrosis, and asthma.[84,111,137,139,306] The PFP vaccines generally have been safe and immunogenic, producing good levels of neutralizing antibody, especially in seropositive individuals and in those receiving vaccine that also contained small amounts of the G protein.

The PFP-2 vaccine, administered during the third trimester in a study of maternal immunization, showed no adverse effects on the infants, but it was poorly immunogenic in the mothers. At delivery, only low levels of maternal neutralizing antibody were present. However, increased levels of IgG, but not IgA, antibody to the F protein did occur in breast milk. The protective effect of this approach for breast-fed infants requires further study. A meta-analysis of the PFP vaccine trials concluded that a significant relative risk of 0.55 (95% confidence interval, 0.35 to 0.88) in the overall number of RSV infections existed, but the heterogeneity of the studies cast doubt on the validity of this conclusion. Vaccination with PFP did not show any effect on reducing the incidence of RSV lower respiratory tract disease.

Additional candidate vaccines that have been evaluated include a peptide vaccine, BBG2Na, containing a conserved region of the G protein fused to the albumin-binding domain of the streptococcal G protein, and chimeric vaccines of the F and G proteins.

Novel adjuvants also are being developed, some of which can shift the immune response evoked by subunit vaccines from a Th2- to a Th1-type response in experimental animals. Enhanced immunogenicity appears possible with enterically active adjuvants and carriers, such as liposomes and biodegradable microspheres, and with adjuvants in vesicles of immunostimulating complexes.[84]

A third approach to vaccine development involves engineered live viral vectors, including vaccinia virus, adenovirus, Sendai virus, and parainfluenza virus.[62] Results from animal models indicate these vaccines are immunogenic, suggesting a potential role in the prevention of human disease. DNA plasmid vaccines expressing F or G glycoproteins have shown limited immunogenicity.[26,246]

The novel development of individual vaccines and approaches is paving the road toward successful control of RSV infection. In view of the number of different populations from infants to elders who would benefit from protection against RSV infections, multiple approaches and vaccines may be required to achieve effective control of RSV infection and diminish its associated human and financial cost.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abarca K, Jung E, Fernández P, et al. Safety, tolerability, pharmacokinetics and immunogenicity of motavizumab, a humanized enhanced potency monoclonal antibody for the prevention of respiratory syncytial virus infection in at risk children. *Pediatr Infect Dis J* 2009;**28**:267–72.
6. Adams O, Bonzel L, Kovacevic A, et al. Palivizumab resistant human respiratory syncytial virus infection in infancy. *Clin Infect Dis* 2010;**51**:185–8.
12. American Academy of Pediatrics, Committee on Infectious Diseases. Modified recommendations for use of palivizumab for prevention or respiratory syncytial virus infections. *Pediatrics* 2009;**124**:1694–701.
18. Anil AB, Anil M, Saglam AB, et al. High volume normal saline alone is as effective as nebulized salbutamol-normal saline, epinephrine-normal saline and 3% saline in mild bronchiolitis. *Pediatr Pulmonol* 2010;**45**:41–7.
19. Aramburo A, van Schaik S, Louie J, et al. Role of real-time reverse transcription polymerase chain reaction for detection of

respiratory viruses in critically ill children with respiratory disease: is it time for a change in algorithm? *Pediatr Crit Care Med* 2011; **12**:e160–5.
21. Avetiayan G, Mattsson J, Sparrelid E, Ljungman P. Respiratory syncytial virus infection in recipients of allogeneic stem cell transplantation: a retrospective study of the incidence, clinical features and outcome. *Transplantation* 2009;**27**:122–6.
24. Belderbos ME, Houben ML, Wilbrink B, et al. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics* 2011;**217**:e1513–20.
25. Bem RA, Domachowski JB, Rosenberg HF. Animal models of human respiratory syncytial virus disease. *Am J Physiol Lung Cell Mol Physiol* 2011;**301**:148–56.
26. Bembridge GP, Rodriguez N, Carcia-Beato R, et al. Respiratory syncytial virus infection of gene gun vaccinated mice induces TH2 driven pulmonary eosinophilia even in the absence of sensitization to the fusion or attachment protein. *Vaccine* 2000;**19**:1038–46.
27. Bendelja K, Wojovoda V, Aberle N, et al. Decreased Toll-like receptor 8 expression and lower TNF alpha synthesis in infants with acute RSV infection. *Respir Res* 2010;**11**:143–53.
28. Berger A, Obwegeser E, Aberle S, et al. Nosocomial transmission of respiratory syncytial virus in neonatal intensive care and intermediate care units: a prospective epidemiologic study. *Pediatr Infect Dis J* 2010;**29**:669–70.
31. Bisgaard H, Flores-Nunez A, Goh A, et al. Study of montelukast for the treatment of respiratory symptoms of post respiratory syncytial virus bronchiolitis in children. *Am J Respir Crit Care Med* 2008;**178**:854–60.
34. Bloemers BL, van Furth AM, Weijerman ME, et al. High incidence of recurrent wheeze in children with Down syndrome with and without previous respiratory syncytial virus lower respiratory tract infection. *Pediatr Infect Dis J* 2010;**29**:39–42.
35. Boeckh M. The challenge of respiratory virus infections in hematopoietic cell transplant recipients. *Br J Haematol* 2008;**143**: 455–67.
39. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
45. Britton-Long R, Andersson LM, Olofsson S, et al. Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reaction assay. *Scand J Infect Dis* 2012;**44**:9–17.
49. Bukreyev A, Yang L, Fricke J, et al. The secreted form of respiratory syncytial virus G glycoprotein helps the virus evade antibody mediated restriction of replication by acting as an antigen decoy and through effects on Fc receptor bearing leukocytes. *J Virol* 2008;**82**:12181–204.
52. Campbell AP, Chien JW, Kuypers J, et al. Respiratory virus pneumonia after hematopoietic cell transplantation: associations between viral load in bronchioalveolar lavage samples, viral RNA detection in serum samples and clinical outcomes of HCT. *J Infect Dis* 2010;**201**:1404–13.
57. Centers for Disease Control and Prevention. Respiratory syncytial virus—United States July 2007–June 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1203–6.
58. Centers for Disease Control and Prevention. *National Respiratory and Enteric Virus Surveillance System (NREVSS)*, 2012. Available at http://www.cdc.gov/surveillance/nrevss/rsv/natl-trend.htm.
62. Chang J. Current progress on development of respiratory syncytial virus vaccine. *BMB Rep* 2011;**44**:232–7.
80. Collins PL, Melero JA. Progress in understanding and controlling RSV: still crazy after all these years. *Virus Res* 2011;**162**:80–9.
82. Cormier SA, You D, Honnegowda S. The use of a neonatal mouse model to study respiratory syncytial virus infections. *Expert Rev Anti Infect Ther* 2010;**8**:1371–80.
89. De Paulis M, Gilio AE, Ferraro AA, et al. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011;**87**:307–13.
93. DeVincenzo J, Lambkin-Williams R, Wilkinson T, et al. A randomized, double blind, placebo controlled study of an RNAi based therapy directed against respiratory syncytial virus. *Proc Natl Acad Sci U S A* 2010;**107**:8800–5.
94. DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010;**182**:1305–14.
95. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009;**15**:34–41.
102. El Saleeby CM, Somes GW, DeVincenzo JP, Gaur AH. Risk factors for severe respiratory syncytial virus disease in children with cancer: the importance of lymphopenia and young age. *Pediatrics* 2008;**121**:235–43.
107. Emers MJJ, Jassen R, Onland-Moret C, et al. IL10 family member genes IL19 and IL20 are associated with recurrent wheeze after respiratory syncytial virus bronchiolitis. *Pediatr Res* 2011;**70**: 518–23.
113. Figueras-Aloy J, Carbonell-Estrany X, Quero-Jimenez J, et al. FLIP-2 Study: risk factors linked to respiratory syncytial virus infection requiring hospitalization in premature infants born in Spain at a gestational age of 32 to 35 weeks. *Pediatr infect Dis J* 2008;**27**:788–93.
117. Franz A, Adams O, Willems R, et al. Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. *J Clin Virol* 2010;**48**:239–45.
118. Freitas GR, Silva DA, Yokosawa J, et al. Antibody response and avidity of respiratory syncytial virus specific total IgG, IgG1 and IgG3 in young children. *J Med Virol* 2011;**83**:1826–33.
122. Gadomski AM, Brower M. Bronchodilators for bronchiolitis (review). *Cochrane Database Syst Review* 2010;(12):CD001266.
134. Gonzalez PA, Bueno SM, Carreno LJ, et al. Respiratory syncytial virus infection and immunity. *Rev Med Virol* 2012;**22**:230–44.
135. Graham BS. Biological challenges and technological opportunities for respiratory syncytial virus vaccine development. *Immunol Rev* 2011;**239**:149–66.
142. Grewal S, Ali S, McConnell DW, et al. A randomized trial of nebulized 3% hypertonic saline with epinephrine in the treatment of acute bronchiolitis in the emergency department. *Arch Pediatr Adolesc Med* 2009;**163**:1007–12.
154. Hall CB. Rapid antigen testing for respiratory syncytial virus can be used in neonates with provisos. *J Pediatr* 2011;**159**:516–17.
174. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection among healthy children. *N Engl J Med* 2009;**360**:588–98.
176. Hampp C, Kauf T, Saidi AS, Winterstein AG. Cost effectiveness of respiratory syncytial virus prophylaxis in various indications. *Arch Pediatr Adolesc Med* 2011;**165**:498–505.
178. Haynes LM, Caidi H, Radu GU, et al. Therapeutic monoclonal antibody treatment targeting respiratory syncytial virus G protein mediates viral clearance and reduces the pathogenesis of respiratory syncytial virus infection in BALB/c mice. *J Infect Dis* 2009;**200**:439–47.
191. Houben ML, Bont L, Wilbrink B, et al. Clinical prediction rule for respiratory syncytial virus bronchiolitis in healthy newborns: prognostic birth cohort study. *Pediatrics* 2011;**127**:35–41.
192. Houben ML, Coenjaerts FE, Rossen JW, et al. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. *J Med Virol* 2010;**82**: 1266–71.
193. Huang K, Incognito L, Cheng X, et al. Respiratory syncytial virus neutralizing monoclonal antibodies motavizumab and palivizumab inhibit fusion. *J Virol* 2010;**84**:8132–40.
194. Huguenin A, Moutte L, Renois F, et al. Broad respiratory virus detection in infants hospitalized for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. *J Med Virol* 2012;**84**: 979–85.
199. Ison MG. Respiratory syncytial virus and other respiratory viruses in the setting of bone marrow transplantation. *Curr Opin Oncol* 2009;**21**:171–6.
214. Kassis C, Champlin RE, Hachem RY, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. *Biol Blood Marrow Transplant* 2010;**16**:1265–71.
218. Khanna N, Widmer AF, Decker M, et al. Respiratory syncytial virus infection in patients with hematological diseases: single center study and review of the literature. *Clin Infect Dis* 2008;**46**: 402–12.
222. Kim IK, Corcoran T. Recent developments in heliox therapy for asthma and bronchiolitis. *Clin Pediatr Emerg Med* 2009;**10**: 68–74.

226. Kneyber MC, van Woensel J, Uijtendaal E, et al. Azithromycin does not improve disease course in hospitalized infants with respiratory syncytial virus (RSV) lower respiratory tract disease: a randomized equivalence trial. *Pediatr Pulmonol* 2008;**43**: 142–9.
228. Kristensen K, Hjuler T, Ravn H, et al. Chronic diseases, chromosomal abnormalities and congenital malformations as risk factors for respiratory syncytial virus hospitalization: a population based cohort study. *Clin Infect Dis* 2012;**54**:810–17.
232. Lagos R, DeVincenzo JP, Munoz A, et al. Safety and antiviral activity of motavizumab, a respiratory syncytial virus–specific humanized monoclonal antibody when administered to respiratory syncytial virus–infected children. *Pediatr Infect Dis J* 2009;**28**: 835–7.
234. Langley GF, Anderson LJ. Epidemiology and prevention of respiratory syncytial virus infections among infants and young children. *Pediatr Infect Dis J* 2011;**30**:510–17.
236. Lavergne V, Ghannoum M, Wesiss K, et al. Successful prevention of respiratory syncytial virus nosocomial transmission following an enhanced seasonal infection control program. *Bone Marrow Transplant* 2011;**46**:137–42.
244. Light M, Bauman J, Mavunda K, et al. Correlation between respiratory syncytial virus test data and hospitalization of children for RSV lower respiratory tract illness in Florida. *Pediatr Infect Dis J* 2008;**27**:512–18.
245. Liet JM, Ducruet T, Gupta V, Cambonie G. Heliox inhalation therapy for bronchiolitis in infants. *Cochrane Database Syst Rev* 2010;(4):CD006915.
257. Martin ET, Kuypers J, Wald A, Englund JA. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respi Viruses* 2012;**6**: 71–7.
264. McClellan JS, Yongpink Y, Graham BS, Kwong PS. Structure of RSV fusion glycoprotein in the post fusion conformation reveals preservation of neutralizing epitopes. *J Virol* 2011;**85**: 7788–96.
265. McDermott DS, Weiss KA, Knudson CJ, Varga SM. Central role of dendritic cells in shaping the adaptive immune response during RSV infection. *Future Virol* 2011;**6**:963–73.
267. McNally JD, Leis K, Matheson LA, et al. Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediar Pulmonol* 2009;**44**:981–8.
278. Mlinaric-Galinovic G, Vojnovic G, Cepin-Bogovic J, et al. Does the viral subtype influence the biennial cycle of respiratory syncytial virus? *Virol J* 2009;**6**:133.
283. Munir S, Hillyer P, LeNouen C, et al. Respiratory syncytial virus interferon antagonist NS1 protein suppresses and skews the human T lymphocyte response. *PLoS Pathog* 2011;**7**(4):e1001366.
284. Munywoki PK, Hamid F, Mutunga M, et al. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real time PCR using nasopharyngeal flocked swabs. *J Clin Microbiol* 2011;**49**:3365–7.
288. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010;**378**:1917.
294. Nuolivirta K, Koponen P, He Q, et al. *Bordetella pertussis* infection is common in nonvaccinated infants admitted for bronchiolitis. *Pediatr Infect Dis J* 2010;**29**:1013–15.
295. O'Brien KL, Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* 2009;**374**:893–902.
301. Oshaansky CM, Zhang W, Moore E, et al. The host response and molecular pathogenesis associated with respiratory syncytial virus infection. *Future Microbiol* 2009;**4**:279–97.
304. Panozzo CA, Stockman LJ, Curns AT, Anderson LJ. Use of respiratory syncytial virus surveillance data to optimize the timing of immunoprophylaxis. *Pediatrics* 2010;**126**:e116–23.
314. Petruzela FD, Gorelick MH. Duration of illness in infants with bronchiolitis evaluated in the emergency department. *Pediatrics* 2010;**126**:285–90.
315. Pettigrew MM, Gent JF, Pyles RB, et al. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011;**49**:3750–5.
317. Pinto LA, Pitrez PM, Luisi F, et al. Azithromycin therapy in hospitalized infants with acute bronchiolitis is not associated with better clinical outcomes: a randomized, double-blinded, and placebo-controlled clinical trial. *J Pediatr* 2012;**161**:1104–8.
324. Ralston S, Hill V. Incidence of apnea in infants hospitalized with respiratory syncytial virus bronchiolitis: a systematic review. *J Pediatr* 2009;**155**:728–33.
325. Ralsoton S, Hill V, Martinez M. Nebulized hypertonic saline without adjunctive bronchodilators for children with bronchiolitis. *Pediatrics* 2010;**126**:e520–5.
326. Ralston S, Hill V, Waters A. Occult serious bacterial infection in infants younger than 60 to 90 days with bronchiolitis: a systematic review. *Arch Pediatr Adolesc Med* 2011;**165**:951–6.
329. Resch B, Eibisberger M, Morris N, Muller W. Respiratory syncytial virus and influenza virus associated hospitalization in infants less than 12 months of age. *Pediatr Infect Dis J* 2011;**30**:797–9.
330. Resch B, Manzoni P, Lanari M. Severe respiratory syncytial virus infection in infants with neuromuscular diseases and immune deficiency syndromes. *Paediatr Respir Rev* 2009;**10**:148–53.
335. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. *Acta Paediatr* 2010;**99**:389–93.
342. Scagnolari C, Midulla F, Selvaggi C, et al. Evaluation of viral load in infants hospitalized with bronchiolitis caused by respiratory syncytial virus. *Med Microbiol Immunol* 2012;**201**:311–17.
345. Schiffer JT, Kirby K, Sandmaier B, et al. Timing and severity of community acquired respiratory syncytial virus infections after myeloablative versus non-myeloablative hematopoietic stem cell transplantation. *Haematologica* 2009;**94**:1101–8.
348. Shafique M, Wilschut J, de Haan A. Induction of mucosal and systemic immunity against respiratory syncytial virus by inactivated virus supplemented with TLR9 and NOD2 ligands. *Vaccine* 2012;**30**:597–606.
357. Sommer C, Resch B, Simoes EA. Risk factors for severe respiratory syncytial virus lower respiratory tract infection. *Open Microbiol J* 2011;**5**:144–54.
359. Spycher BD, Silverman M, Brooke AM, et al. Distinguishing phenotypes of childhood wheeze and cough using latent class analysis. *Eur Respir J* 2008;**31**:974–81.
360. Stensballe LG, Ravn H, Kristensen K, et al. Seasonal variation of maternally derived respiratory syncytial virus antibodies and association with infant hospitalizations for respiratory syncytial virus. *J Pediatr* 2009;**154**:296–8.
362. Stockman LJ, Reed C, Kallen AJ, et al. Respiratory syncytial virus and *Staphylococcus aureus* coinfection in children hospitalized with pneumonia. *Pediatr Infect Dis J* 2010;**29**:1048–50.
367. Tayyari F, Marchant D, Moraes TJ, et al. Identification of nucleolin as a cellular receptor for human RSV. *Nat Med* 2011;**17**: 1132–5.
368. Techasaensiri B, Techasaensire C, Mejias A, et al. Viral coinfections in children with invasive pneumococcal disease. *Pediatr Infect Dis J* 2010;**29**:519–23.
369. Thavagnanam S, Christie SN, Doherty GM, et al. Respiratory viral infection in lower airways of asymptomatic children. *Acta Paediatr* 2010;**99**:394–8.
371. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med* 2009;**179**:1091–7.
377. Uckay I, Gasche-Soccal PM, Kaiser L, et al. Low incidence of severe respiratory syncytial virus infections in lung transplant recipients despite the absence of specific therapy. *J Heart Lung Transplant* 2010;**29**:299–305.
379. van der Zalm MM, van Ewijk BE, Wilbrink B, et al. Respiratory pathogens in children with and without respiratory symptoms. *J Pediatr* 2009;**154**:396–400.
380. van Drunen L, Watkiss ER. Pathogenesis of respiratory syncytial virus. *Curr Opin Biol* 2012;**2**:300–5.
386. Walsh PF, Kimmel L, Feola M, et al. Prevalence of *Bordetella pertussis* and *Bordetella parapertussis* in infants presenting to the emergency department with bronchiolitis. *J Emerg Med* 2011;**40**: 256–61.
387. Walton NA, Poynton MR, Gesteland PH, et al. Predicting the start week of respiratory syncytial virus outbreaks using real time weather variables. *BMC Med Inform Decis Mak* 2010;**10**:68.
393. Welliver RC. The immune response to respiratory syncytial virus infection: friend or foe? *Clin Rev Allergy Immunol* 2008;**34**: 163–73.

394. Welliver R. The relationship of meterological conditions to the epidemiologic activity of respiratory syncytial virus. *Paediatr Respir Rev* 2009;**10**(Suppl. 1):6–8.
401. Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for RSV, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012;**206**:56–62.
402. Wiertsema SP, Chidlow GR, Kirkham LA, et al. High detection rates of nucleic acids of a wide range of respiratory viruses in the nasopharynx and the middle ear of children with a history of recurrent acute otitis media. *J Med Virol* 2011;**83**:2008–17.
409. Wu P, Dupont WD, Griffin MR, et al. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. *Am J Respir Crit Care Med* 2008;**178**:1123–9.
412. Zachariah P, Tutterber M, Simões EA. Down syndrome and hospitalizations due to respiratory syncytial virus: a population-based study. *J Pediatr* 2011;**160**:827–31.e1.
413. Zachariah P, Tuttenber M, Simões EA. Hospitalizations due to respiratory syncytial virus in children with congenital malformations. *Pediatr Infect Dis J* 2012;**30**:442–5.
414. Zamora MR, Budev M, Rolfe M, et al. RNA interference therapy in lung transplant patients infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010;**183**:531–38.
415. Zhang L, Mendoza-Sassi RA, Wainwright C, Klassen TP. Nebulized hypertonic saline solution for acute bronchiolitis in infants. *Cochrane Database Syst Rev* 2008;(4):CD006458.
417. Zhao X, Singh M, Malashkevich VN, Kim PS. Structural characterization of the human respiratory syncytial virus fusion protein core. *Proc Natl Acad Sci U S A* 2000;**97**:14172–77.
418. Zhu Q, McAuliffe JM, Patel NK, et al. Analysis of respiratory syncytial virus preclinical and clinical variants resistant to neutralization by monoclonal antibodies palivizumab and or motavizumab. *J Infect Dis* 2011;**203**:674–82.
419. Zhu Q, Patel NK, McAuliffe JM, et al. Natural polymorphisms and resistance associated mutations in the fusion protein of respiratory syncytial virus: effects of respiratory syncytial virus susceptibility to palivizumab. *J Infect Dis* 2012;**205**:635–8.
420. Zorc JJ, Hall CB. Bronchiolitis: recent evidence on diagnosis and management. *Pediatrics* 2010;**125**:342–9.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 182

Human Metapneumovirus

Jennifer E. Schuster • John V. Williams

Human metapneumovirus (MPV, HMPV) is a paramyxovirus that is a ubiquitous respiratory tract pathogen with seropositivity demonstrated in virtually all adults around the world.* The spectrum of disease varies from minor upper respiratory tract illness to significant morbidity and mortality from lower respiratory tract disease.[47,149,160,169] The clinical manifestations of MPV include acute otitis media, asthma exacerbations, bronchiolitis, croup, and pneumonia, similar to other common respiratory viruses. MPV can cause severe disease in children and adults with underlying medical conditions as well as elderly individuals.† Multiple epidemiologic studies have demonstrated that MPV is widely prevalent and is usually second to respiratory syncytial virus (RSV) in incidence.[46,47,112,114] Research groups have begun to elucidate the pathogenesis of and immunity for this virus, facilitating the development of preventive strategies and vaccines.

*References 38, 47, 63, 90-93, 100, 102, 108, 149.
†References 17, 42, 47, 89, 96, 104, 115, 134, 136, 154, 160, 166.

HISTORY

In 2001, van den Hoogen and colleagues[149] identified a novel virus in respiratory specimens collected from 28 Dutch children with acute respiratory tract illness over a 20-year period. Clinical symptoms were similar to those of RSV, and disease severity ranged from mild respiratory tract infection to respiratory failure requiring mechanical ventilation. The unknown virus replicated and produced cytopathic effects, including syncytia, in tertiary monkey kidney cells. Virus particles exhibited pleomorphic, enveloped morphology by electron microscopy, consistent with a paramyxovirus. However, polymerase chain reaction (PCR) assay for known respiratory viruses was negative and the virus did not adsorb red blood cells (hemagglutination). Elegant random reverse-transcriptase (RT)-PCR experiments provided sufficient genomic sequence data to place the virus in the *Pneumovirinae* subfamily of the *Paramyxoviridae*. MPV, like avian metapneumovirus (AMPV), is a member of the *Metapneumovirus* genus, but it is the first described human pathogen in the genus. The most closely related virus genetically

REFERENCES

1. Abdallah A, Rowland KE, Schepetiuk SK, et al. An outbreak of respiratory syncytial virus infection in a bone marrow transplant unit: effect on engraftment and outcome of pneumonia without specific antiviral treatment. *Bone Marrow Transplant* 2003;**32**: 195–203.
2. Abarca K, Jung E, Fernandez P, et al. Safety, tolerability, pharmacokinetics and immunogenicity of motavizumab, a humanized enhanced potency monoclonal antibody for the prevention of respiratory syncytial virus infection in at risk children. *Pediatr Infect Dis J* 2009;**28**:267–72.
3. Aberle JH, Aberle SW, Rebhandl W, et al. Decreased interferon-gamma response in respiratory syncytial virus compared to other respiratory viral infections in infants. *Clin Exp Immunol* 2004;**137**: 146–50.
4. Adams JM. Primary virus pneumonitis with cytoplasmic inclusion bodies: a study of an epidemic involving thirty-two infants with nine deaths. *JAMA* 1941;**116**:925–33.
5. Adams JM, Imagawa DT, Zike K. Epidemic bronchiolitis and pneumonia related to respiratory syncytial virus. *JAMA* 1961; **176**:1037–9.
6. Adams O, Bonzel L, Kovacevic A, et al. Palivizumab resistant human respiratory syncytial virus infection in infancy. *Clin Infect Dis* 2010;**51**:185–8.
7. Agency for Healthcare Research and Quality. Management of bronchiolitis in infants and children. *Evid Rep Technol Assess* 2003;**03-E009**:1–5.
8. Aguinaga OI, Arnedo PA, Bellido J, et al. The prevalence of asthma-related symptoms in 13-14-year-old children from 9 Spanish populations: The Spanish Group of the ISAAC Study (International Study of Asthma and Allergies in Childhood). *Med Clin (Barc)* 1999;**112**:171–5.
9. Altman CA, Englund JA, Demmler G, et al. Respiratory syncytial virus in patients with congenital heart disease: a contemporary look at epidemiology and success of preoperative screening. *Pediatr Cardiol* 2000;**21**:433–8.
10. Amanatidou V, Sourvinos G, Apostolakis S, et al. T280M variation of the CX3C receptor gene is associated with increased risk for severe respiratory syncytial virus bronchiolitis. *Pediatr Infect Dis J* 2006;**25**:410–14.
11. American Academy of Pediatrics. Respiratory syncytial virus. In: Pickering L, Baker C, Kimberlin DW, Long SS, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 609–18.
12. American Academy of Pediatrics, Committee on Infectious Diseases. Modified recommendations for use of palivizumab for prevention or respiratory syncytial virus infections. *Pediatrics* 2009;**124**:1694–701.
13. American Academy of Pediatrics, Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. *Pediatrics* 2006;**118**:1774–93.
14. Anaissie EJ, Mahfouz TH, Aslan T, et al. The natural history of respiratory syncytial virus infection in cancer and transplant patients: implications for management. *Blood* 2004;**103**:1611–17.
15. Anas NG, Boettrich C, Hall CB, Brooks JG. The association of apnea and respiratory syncytial virus infection in infants. *J Pediatr* 1982;**101**:65–8.
16. Anderson LJ, Hendry RM, Pierik LT, et al. Multicenter study of strains of respiratory syncytial virus. *J Infect Dis* 1991;**163**: 687–92.
17. Anderson LJ, Tsou C, Potter C, et al. Cytokine response to respiratory syncytial virus stimulation of human peripheral blood mononuclear cells. *J Infect Dis* 1994;**170**:1201–8.
18. Anil AB, Anil M, Saglam AB, et al. High volume normal saline alone is as effective as nebulized salbutamol-normal saline, epinephrine-normal saline and 3% saline in mild bronchiolitis. *Pediatr Pulmonol* 2010;**45**:41–7.
19. Aramburo A, van Schaik S, Louie J, et al. Role of real-time reverse transcription polymerase chain reaction for detection of respiratory viruses in critically ill children with respiratory disease: is it time for a change in algorithm? *Pediatr Crit Care Med* 2011; **12**:e160–5.
20. Arbiza J, Berois M, Delfraro A, et al. Genetic characterization of respiratory syncytial viruses isolated from consecutive acute respiratory infections in a HIV infected child. *J Clin Virol* 2006; **35**:41–5.
21. Avetiayan G, Mattsson J, Sparrelid E, Ljungman P. Respiratory syncytial virus infection in recipients of allogeneic stem cell transplantation: a retrospective study of the incidence, clinical features and outcome. *Transplantation* 2009;**27**:122–6.
22. Bachrach VR, Schwarz E, Bachrach LR. Breastfeeding and the risk of hospitalization for respiratory disease in infancy: a meta-analysis. *Arch Pediatr Adolesc Med* 2003;**157**:237–43.
23. Bairan AC, Cherry JD, Fagan LF, Codd JR. Complete heart block and respiratory syncytial virus. *Am J Dis Child* 1974;**127**:264–5.
24. Belderbos ME, Houben ML, Wilbrink B, et al. Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics* 2011;**217**:e1513–20.
25. Bem RA, Domachowski JB, Rosenberg HF. Animal models of human respiratory syncytial virus disease. *Am J Physiol Lung Cell Mol Physiol* 2011;**301**:148–56.
26. Bembridge GP, Rodriguez N, Carcia-Beato R, et al. Respiratory syncytial virus infection of gene gun vaccinated mice induces TH2 driven pulmonary eosinophilia even in the absence of sensitization to the fusion or attachment protein. *Vaccine* 2000; **19**:1038–46.
27. Bendelja K, Wojovoda V, Aberle N, et al. Decreased Toll-like receptor 8 expression and lower TNF alpha synthesis in infants with acute RSV infection. *Respir Res* 2010;**11**:143–53.
28. Berger A, Obwegeser E, Aberle S, et al. Nosocomial transmission of respiratory syncytial virus in neonatal internsive care and intermediate care units: a prospective epidemiologic study. *Pediatr Infect Dis J* 2010;**29**:669–70.
29. Berman S. Epidemiology of acute respiratory infections in children of developing countries. *Rev Infect Dis* 1991;**13**(Suppl.): S454–62.
30. Bisgaard H. A randomized trial of montelukast in respiratory syncytial virus postbronchiolitis. *Am J Respir Crit Care Med* 2003;**167**:379–83.
31. Bisgaard H, Flores-Nunez A, Goh A, et al. Study of montelukast for the treatment of respiratory symptoms of post respiratory syncytial virus bronchiolitis in children. *Am J Respir Crit Care Med* 2008;**178**:854–60.
32. Bisgaard H, Zielen S, Garcia-Garcia ML, et al. Montelukast reduces asthma exacerbations in 2 to 5 year old children with intermittent asthma. *Am J Respir Crit Care Med* 2005;**171**: 315–22.
33. Bitko V, Shulyayeva O, Mazumder B, et al. Nonstructural proteins of respiratory syncytial virus suppress premature apoptosis by an NF-kappaB dependent interferon independent mechanism and facilitate virus growth. *J Virol* 2007;**81**:1786–95.
34. Bloemers BL, van Furth AM, Weijerman ME, et al. High incidence of recurrent wheeze in children with Down syndrome with and without previous respiratory syncytial virus lower respiratory tract infection. *Pediatr Infect Dis J* 2010;**29**:39–42.
35. Boeckh M. The challenge of respiratory virus infections in hematopoietic cell transplant recipients. *Br J Haematol* 2008;**143**: 455–67.
36. Boeckh M, Englund J, Li Y, et al. Randomized controlled multicenter trial of aerosolized ribavirin for respiratory syncytial virus upper respiratory tract infection in hematopoietic cell transplant recipients. *Clin Infect Dis* 2007;**44**:245–9.
37. Bont L, Heijnen CJ, Kavelaara A, et al. Monocyte IL-10 production during respiratory syncytial virus bronchiolitis is associated with recurrent wheezing in a one-year follow-up study. *Am J Respir Crit Care Med* 2000;**161**:1518–23.
38. Bont L, Versteegh J, Swelsen WT, et al. Natural reinfection with respiratory syncytial virus does not boost virus-specific T-cell immunity. *Pediatr Res* 2002;**52**:363–7.
39. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
40. Bowden RA. Respiratory virus infection after marrow transplant: The Fred Hutchingson Cancer Research Center experience. *Am J Med* 1997;**102**:27–30.
41. Boyce JM, Pittet D, Healthcare Infection Control Practices Advisory Committee, et al. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/

IDSA Hand Hygiene Task Force. *Infect Control Hosp Epidemiol* 2002;**23**(Suppl.):S3–40.
42. Boyce TG, Mellen BG, Mitchel EF Jr, et al. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. *J Pediatr* 2000;**137**:865–70.
43. Brandenburg AH, de Waal L, Timmerman HH, et al. HLA class I–restricted cytotoxic T-cell epitopes of the respiratory syncytial virus fusion protein. *J Virol* 2000;**74**:10240–4.
44. Brandt CD, Kim HW, Arrobio JO, et al. Epidemiology of respiratory syncytial virus infection in Washington, DC, part III: composite analysis of eleven consecutive yearly epidemics. *Am J Epidemiol* 1973;**98**:355–64.
45. Britton-Long R, Andersson LM, Olofsson S, et al. Seasonal variations of 15 respiratory agents illustrated by the application of a multiplex polymerase chain reation assay. *Scand J Infect Dis* 2012; **44**:9–17.
46. Brooks AM, McBride JT, McConnochie KM, et al. Predicting deterioration in previously healthy infants hospitalized with respiratory syncytial virus infection. *Pediatrics* 1999;**104**:463–7.
47. Bryan DL, Hart PH, Forsyth KD, Gibson RA. Immunomodulatory consitituents of human milk change in response to infant bronchiolitis. *Pediatr Allergy Immunol* 2007;**18**:495–502.
48. Bukreyev A, Belyakov IM, Prince GA, et al. Expression of interleukin-4 by recombinant respiratory syncytial virus is associated with accelerated inflammation and a nonfunctional cytotoxic T-lymphocyte response following primary infection but not following challenge with wild-type virus. *J Virol* 2005;**79**:9515–26.
49. Bukreyev A, Yang L, Fricke J, et al. The secreted form of respiratory syncytial virus G glycoprotein helps the virus evade antibody-mediated restriction of replication by acting as an antigen decoy and through effects on Fc receptor–bearing leukocytes. *J Virol* 2008;**82**:12191–204.
50. Bulkow LR, Singleton RJ, Karron RA, et al. Risk factors for severe respiratory syncytial virus infection among Alaska native children. *Pediatrics* 2002;**109**:210–16.
51. Byrd LG, Prince GA. Animal models of respiratory syncytial virus infection. *Clin Infect Dis* 1997;**25**:1363–8.
52. Campbell AP, Chien JW, Kuypers J, et al. Respiratory virus pneumonia after hematopoietic cell transplantation: associations between viral load in bronchioalveolar lavage samples, viral RNA detection in serum samples and clinical outcomes of HCT. *J Infect Dis* 2010;**201**:1404–13.
53. Cannon MJ, Openshaw PJM, Askonas BA. Cytotoxic T cells clear virus but augment lung pathology in mice infected with respiratory syncytial virus. *J Exp Med* 1988;**163**:1163–8.
54. Carbonell-Estrany X, Figueras-Aloy J, Law BJ. Identifying risk factors for severe respiratory syncytial virus among infants born after 33 through 35 completed weeks of gestation: different methodologies yield consistent findings. *Pediatr Infect Dis J* 2004; **23**(Suppl.):S193–201.
55. Carbonell-Estrany X, Simões EA, Dagan R, et al. Motavizumab for prophylaxis of respiratory syncytial virus in high risk children: a noninferiority trial. *Pediatrics* 2001;**125**:e35–51.
56. Centers for Disease Control and Prevention. Quick stats: percentage of infants born during 1990-1993 and 1997-2000 who were ever breastfed, by race/ethnicity of mother—United States. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1155.
57. Centers for Disease Control and Prevention. Respiratory syncytial virus—United States July 2007–June 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1203–6.
58. Centers for Disease Control and Prevention. *National Respiratory and Enteric Virus Surveillance System (NREVSS)*, 2012. Available at http://www.cdc.gov/surveillance/nrevss/rsv/natl-trend.htm.
59. Champlin RE, Whimbey E. Community respiratory virus infections in bone marrow transplant recipients: the M.D. Anderson Cancer Center experience. *Biol Blood Marrow Transplant* 2001; **7**(Suppl.):8S–10S.
60. Chandra A, Martinez GM, Mosher WD, et al. Fertility, family planning, and reproductive health of U.S. women: data from the 2002 National Survey of Family Growth. *Vital Health Stat* 2005;**23**: 1–160.
61. Chandwani S, Borkowsky W, Krasinski K, et al. Respiratory syncytial virus infection in human immunodeficiency virus–infected children. *J Pediatr* 1990;**117**:251–4.
62. Chang J. Current progress on development of respiratory syncytial virus vaccine. *BMB Rep* 2011;**44**:232–7.
63. Chang J, Choi SY, Jin HT, et al. Improved effector activity and memory CD8 T cell development by IL-2 expression during experimental respiratory syncytial virus infection. *J Immunol* 2004;**172**:503–8.
64. Chang J, Srikiatkhachorn A, Braciale TJ. Visualization and characterization of respiratory syncytial virus F-specific CD8(+) T cells during experimental virus infection. *J Immunol* 2001;**167**: 4254–60.
65. Chanock RM, Finberg L. Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA), part II: epidemiologic aspects of infection in infants and young children. *Am J Hyg* 1957;**66**:291–300.
66. Chanock RM, Kim HW, Vargosko AJ, et al. Respiratory syncytial virus, part I: virus recovery and other observations during 1960 outbreak of bronchiolitis pneumonia, and minor respiratory diseases in children. *JAMA* 1961;**176**:647–53.
67. Chapman J, Abbott E, Alber D, et al. RSV604 a novel inhibitor of respiratory syncytial virus replication. *Antimicrob Agents Chemo* 2007;**51**:3346–53.
68. Chemaly R, Ghosh S, Bodey G, et al. Respiratory viral infections in adults with hematologic malignancies and human stem cell transplantation recipients: a retrospective study at a major cancer center. *Medicine* 2006;**85**:278–87.
69. Chen St, Lu KH, Sun HL, et al. Randomized placebo controlled trial comparing montelukast and cetirizine for treating perennial allergic rhinitis in children aged 2-6 years. *Pediatr Allergy Immunol* 2006;**17**:49–54.
70. Cherrie AH, Anderson K, Wertz GW, Openshaw PJM. Human cytotoxic T cells stimulated by antigen on dendritic cells recognize the N, SH, F, M, 22K, and 1b proteins of respiratory syncytial virus. *J Virol* 1992;**66**:2102–10.
71. Chi B, Dickensheets HL, Spann KM, et al. Alpha and lambda interferon together mediate suppression of CD4 T cells induced by respiratory syncytial virus. *J Virol* 2006;**80**:5032–40.
72. Chin J, Magoffin RL, Shearer LA. Field evaluation of a respiratory syncytial virus vaccine and a trivalent parainfluenza virus vaccine in a pediatric population. *Am J Epidemiol* 1969;**89**:449–63.
73. Choi EH, Lee HJ, Yoo T, Chanock SJ. A common haplotype of interleukin-4 gene IL4 is associated with severe respiratory syncytial virus disease in Korean children. *J Infect Dis* 2002;**186**: 1207–11.
74. Choi KS, Scorpio DG, Dumler JS. *Anaplasma phagocytophilum* ligation to Toll-like receptor (TLR) 2, but not to TLR4, activates macrophages for nuclear factor-kappa B nuclear translocation. *J Infect Dis* 2004;**189**:1921–5.
75. Choudhuri JA, Ogden LG, Tuttenbrt AJ, et al. Effect of altitude on hospitalizations for respiratory syncytial virus infection. *Pediatrics* 2006;**117**:349–56.
76. Cilla G, Sarasua A, Montes M, et al. Risk factors for hospitalization due to respiratory syncytial virus infection among infants in the Basque Country, Spain. *Epidemiol Infect* 2006;**134**: 506–13.
77. Cincinnati Children's Hospital Medical Center. *Evidence-based clinical practice guideline for management of first time bronchiolitis in infants less than 1 year of age*, 2010. Available at http://www.cincinnatichildrens.org/assets/0/78/1067/2709/2777/2793/9199/edf8f194-1a56-48f7-8419-7c5e0a168b5d.pdf.
78. Cole PD, Suh JS, Onel K, et al. Benign outcome of respiratory syncytial virus infection in children with cancer. *Med Pediatr Oncol* 2001;**37**:24–9.
79. Collins PL, Chanock RM, Murphy BR. Respiratory syncytial virus and metapneumoviruses. In: Knipe D, Howley P, Griffin D, et al, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 1601–46.
80. Collins PL, Melero JA. Progress in understanding and controlling RSV: still crazy after all these years. *Virus Res* 2011;**162**:80–9.
81. Cooper S, Lyall H, Walters S, et al. Children with human immunodeficiency virus admitted to a paediatric intensive care unit in the United Kingdom over a 10-year period. *Intensive Care Med* 2004;**30**:113–18.
82. Cormier SA, You D, Honnegowda S. The use of a neonatal mouse model to study respiratory syncytial virus infections. *Expert Rev Anti Infect Ther* 2010;**8**:1371–80.
83. Corneli HM, Zorc JJ, Mahajan P, et al. A multicenter, randomized, controlled trial of dexamethasone for bronchiolitis. *N Engl J Med* 2007;**357**:331–9.

84. Crowe JE. Respiratory syncytial virus vaccine development. *Vaccine* 2002;**20**(Suppl.):S32–7.
85. Csonka P, Kaila M, Laippala P, et al. Oral prednisolone in the acute management of children age 6 to 35 months with viral respiratory infection–induced lower airway disease: a randomized, placebo-controlled trial. *J Pediatr* 2003;**143**:725–30.
86. Culley FJ, Pennycook AM, Tregoning JS, et al. Differential chemokine expression following respiratory virus infection reflects Th1- or Th2-based immunopathology. *J Virol* 2006;**80**:4521–7.
87. Da Dalt L, Callegaro S, Carraro S, et al. Nasal lavage leukotrienes in infants with respiratory syncytial virus bronchiolitis. *Pediatr Allergy Immunol* 2007;**18**:100–4.
88. de Graaff PM, de Jong EC, van Capel TM, et al. Respiratory syncytial virus infection of monocyte-derived dendritic cells decreases their capacity to activate CD4 T cells. *J Immunol* 2005;**175**:5904–11.
89. De Paulis M, Gilio AE, Ferraro AA, et al. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011;**87**:307–13.
90. DeVincenzo JP. Factors predicting childhood respiratory syncytial virus severity: what they indicate about pathogenesis. *Pediatr Infect Dis J* 2005;**24**(Suppl.):S177–83.
91. DeVincenzo JP, El Saleeby CM, Bush AJ. Respiratory syncytial virus load predicts disease severity in previously healthy infants. *J Infect Dis* 2005;**191**:1861–8.
92. DeVincenzo JP, Hall CB, Kimberlin DW, et al. Surveillance of clinical isolates of respiratory syncytial virus for palivizumab (Synagis)–resistant mutants. *J Infect Dis* 2004;**190**:975–8.
93. DeVincenzo J, Lambkin-Williams R, Wilkinson T, et al. A randomized, double blind, placebo controlled study of an RNAi-based therapy directed against respiratory syncytial virus. *Proc Natl Acad Sci U S A* 2010;**107**:8800–5.
94. DeVincenzo JP, Wilkinson T, Vaishnaw A, et al. Viral load drives disease in humans experimentally infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010;**182**:1305–14.
95. Delgado MF, Coviello S, Monsalvo AC, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. *Nat Med* 2009;**15**:34–41.
96. Domachowske JB, Bonville CA, Rosenberg HF. Animal models for studying respiratory syncytial virus infection and its long term effects on lung function. *Pediatr Infect Dis J* 2004;**23**(Suppl.):S228–34.
97. Dowell SF, Anderson LJ, Gary HE, et al. Respiratory syncytial virus is an important cause of community-acquired lower respiratory infection among hospitalized adults. *J Infect Dis* 1996;**174**:456–62.
98. Durbin JE, Durbin RK. Respiratory syncytial virus–induced immunoprotection and immunopathology. *Viral Immunol* 2004;**17**:370–80.
99. Ebbert JO, Limper AH. Respiratory syncytial virus pneumonitis in immunocompromised adults: clinical features and outcome. *Respiration* 2005;**72**:263–9.
100. Edell D, Khoshoo V, Ross G, Salter K. Early ribavirin treatment of bronchiolitis: effect on long-term respiratory morbidity. *Chest* 2002;**122**:935–9.
101. Eder W, Ege MJ, von Mutius E. The asthma epidemic. *N Engl J Med* 2006;**355**:2226–35.
102. El Saleeby CM, Somes GW, DeVincenzo JP, Gaur AH. Risk factors for severe respiratory syncytial virus disease in children with cancer: the importance of lymphopenia and young age. *Pediatrics* 2008;**121**:235–43.
103. Elhassan NO, Sorbero ME, Hall CB, et al. Cost-effectiveness analysis of palivizumab in premature infants without chronic lung disease. *Arch Pediatr Adolesc Med* 2006;**160**:1070–6.
104. Englund JA, Piedra PA, Jefferson LS, et al. High-dose, short-duration ribavirin aerosol therapy in children with suspected respiratory syncytial virus infection. *J Pediatr* 1990;**117**:313–20.
105. Erard V, Chien JW, Kim HW, et al. Airflow decline after myeloablative allogeneic hematopoietic cell transplantation: the role of community respiratory viruses. *J Infect Dis* 2006;**193**:1619–25.
106. Eriksson M, Bennet R, Rotzen-Ostlund M, et al. Population-based rates of severe respiratory syncytial virus infection in children with and without risk factors, and outcome in a tertiary care setting. *Acta Paediatr* 2002;**91**:593–8.
107. Emers MJJ, Jassen R, Onland-Moret C, et al. IL10 family member genes IL19 and IL20 are associated with recurrent wheeze after respiratory syncytial virus bronchiolitis. *Pediatr Res* 2011;**70**:518–23.
108. Everard ML. The relationship between respiratory syncytial virus infections and the development of wheezing and asthma in children. *Curr Opin Allergy Clin Immunol* 2006;**6**:56–61.
109. Falsey AR, Formica MA, Treanor JJ, Walsh EE. Comparison of quantitative reverse transcription-PCR to viral culture for assessment of respiratory syncytial virus shedding. *J Clin Microbiol* 2003;**41**:4160–5.
110. Falsey AR, Hennessey PA, Formica MA, et al. Respiratory syncytial virus infection in elderly and high-risk adults. *N Engl J Med* 2005;**352**:1749–59.
111. Falsey AR, Walsh EE. Safety and immunogenicity of a respiratory syncytial virus subunit vaccine (PFP-2) in the institutionalized elderly. *Vaccine* 1997;**15**:1130–2.
112. Falsey AR, Walsh EE. Respiratory syncytial virus infections in adults. *Clin Microbiol Rev* 2000;**13**:371–84.
113. Figueras-Aloy J, Carbonell-Estrany X, Quero-Jimenez J, et al. FLIP-2 Study: risk factors linked to respiratory syncytial virus infection requiring hospitalization in premature infants born in Spain at a gestational age of 32 to 35 weeks. *Pediatr Infect Dis J* 2008;**27**:788–93.
114. Fleming DM, Pannell RS, Cross KW. Mortality in children from influenza and respiratory syncytial virus. *J Epidemiol Community Health* 2005;**59**:586–90.
115. Florman AL, McLaren LC. The effect of altitude and weather on the occurrence of outbreaks of respiratory syncytial virus infections. *J Infect Dis* 1988;**158**:1401–2.
116. Forster J, Ihorst G, Rieger CH, et al. Prospective population-based study of viral lower respiratory tract infections in children under 3 years of age (the PRIDE study). *Eur J Pediatr* 2004;**163**:709–16.
117. Franz A, Adams O, Willems R, et al. Correlation of viral load of respiratory pathogens and co-infections with disease severity in children hospitalized for lower respiratory tract infection. *J Clin Virol* 2010;**48**:239–45.
118. Freitas GR, Silva DA, Yokosawa J, et al. Antibody response and avidity of respiratory syncytial virus specific total IgG, IgG1 and IgG3 in young children. *J Med Virol* 2011;**83**:1826–33.
119. Fulginiti VA, Eller JJ, Sieber OF, et al. Respiratory virus immunization, part I: a field trial of two inactivated respiratory virus vaccines, an aqueous trivalent parainfluenza virus vaccine, and an alum-precipitated respiratory syncytial virus vaccine. *Am J Epidemiol* 1969;**89**:435–48.
120. Fuller DG, Davie G, Lamb D, et al. Analysis of respiratory viral coinfection and cytomegalovirus coisolation in pediatric inpatients. *Pediatr Infect Dis J* 2005;**24**:195–200.
121. Gala CL, Hall CB, Schnabel KC, et al. The use of eye-nose goggles to control nosocomial respiratory syncytial virus infection. *JAMA* 1986;**256**:2706–8.
122. Gadomski AM, Brower M. Bronchodilators for bronchiolitis (review). *Cochrane Database Syst Rev* 2010;(12):CD001266.
123. Garofalo RP, Patti J, Hintz KA, et al. Macrophage inflammatory protein-1alpha (not T helper type 2 cytokines) is associated with severe forms of respiratory syncytial virus bronchiolitis. *J Infect Dis* 2001;**184**:393–9.
124. Garrison M, Christakis DA, Harvey E, et al. Systemic corticosteroids in infant bronchiolitis: a meta-analysis. *Pediatrics* 2000;**105**:e44.
125. Gartner LM, Morton J, Lawrence RA, et al. Breastfeeding and the use of human milk. *Pediatrics* 2005;**115**:496–506.
126. Gentile DA, Doyle WJ, Zeevi A, et al. Cytokine gene polymorphisms moderate illness severity in infants with respiratory syncytial virus infection. *Hum Immunol* 2003;**64**:338–44.
127. Gern JE, Rosenthal LA, Sorkness RL, Lemanske RF Jr. Effects of viral respiratory infections on lung development and childhood asthma. *J Allergy Clin Immunol* 2005;**115**:668–74.
128. Glezen WP. Viral pneumonia as a cause and result of hospitalization. *J Infect Dis* 1983;**4**:765–70.
129. Glezen WP. The changing epidemiology of respiratory syncytial virus and influenza: impetus for new control measures. *Pediatr Infect Dis J* 2004;**23**(Suppl.):S202–6.
130. Glezen WP, Denny FW. Epidemiology of acute lower respiratory disease in children. *N Engl J Med* 1973;**288**:498–505.

131. Glezen WP, Greenberg SB, Atmar RL, et al. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000;**283**:499–505.
132. Glezen WP, Paredes A, Allison JE, et al. Risk of respiratory syncytial virus infection for infants from low-income families in relationship to age, sex, ethnic group, and maternal antibody level. *J Pediatr* 1981;**98**:708–15.
133. Glezen WP, Taber LH, Frank AL, Kasel JA. Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986;**140**:543–6.
134. Gonzalez PA, Bueno SM, Carreno LJ, et al. Respiratory syncytial virus infection and immunity. *Rev Med Virol* 2012;**22**:230–44.
135. Graham BS. Biological challenges and technological opportunities for respiratory syncytial virus vaccine development. *Immunol Rev* 2011;**239**:149–66.
136. Graham BS, Johnson TR, Peebles RS. Immune-mediated disease pathogenesis in respiratory syncytial virus infection. *Immunopharmacology* 2000;**48**:237–47.
137. Greenberg HB, Piedra PA. Immunization against viral respiratory disease: a review. *Pediatr Infect Dis J* 2004;**23**(Suppl.):S254–61.
138. Greenes DS, Harper MB. Low risk of bacteremia in febrile children with recognizable viral syndromes. *Pediatr Infect Dis J* 1999;**18**:258–61.
139. Groothuis JR, King SJ, Hogerman DA, et al. Safety and immunogenicity of a purified F protein respiratory syncytial virus (PFP-2) vaccine in seropositive children with bronchopulmonary dysplasia. *J Infect Dis* 1998;**177**:467–9.
140. Groothuis JR, Simões EAF, Levin MJ, et al. Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children. *N Engl J Med* 1993;**329**:1524–30.
141. Groskreutz DJ, Monick MM, Powers LS, et al. Respiratory syncytial virus induces TLR3 protein and protein kinase R, leading to increased double-stranded RNA responsiveness in airway epithelial cells. *J Immunol* 2006;**176**:1733–40.
142. Grewal S, Ali S, McConnell DW, et al. A randomized trial of nebulized 3% hypertonic saline with epinephrine in the treatment of acute bronchiolitis in the emergency department. *Arch Pediatr Adolesc Med* 2009;**163**:1007–12.
143. Guerrero-Plata A, Casola A, Suarez G, et al. Differential response of dendritic cells to human metapneumovirus and respiratory syncytial virus. *Am J Respir Cell Mol Biol* 2006;**34**:320–9.
144. Gupta VK, Cheifetz IM. Heliox administration in the pediatric intensive care unit: an evidence based review. *Pediatr Crit Care Med* 2005;**6**:204–11.
145. Haeberle HA, Takizawa R, Casola A, et al. Respiratory syncytial virus–induced activation of nuclear factor-κB in the lung involves alveolar macrophages and Toll-like receptor 4–dependent pathways. *J Infect Dis* 2002;**186**:1199–206.
146. Hall CB. The shedding and spreading of respiratory syncytial virus. *Pediatr Res* 1977;**11**:236–9.
147. Hall CB. Nosocomial respiratory syncytial virus infections: the "cold war" has not ended. *Clin Infect Dis* 2000;**31**:590–6.
148. Hall CB. Respiratory syncytial virus: microbe for all ages, mimic of maladies many. In: Scheld W, Craig W, Hughes J, editors. *Emerging infections 4*. Washington, D.C.: ASM Press; 2000. p. 1–15.
149. Hall CB. Respiratory syncytial viral infections: pathology and pathogenicity. In: Weisman L, Groothuis J, editors. *Contemporary diagnosis and management of respiratory syncytial virus infection*. 5th ed. Newtown, PA: Handbooks in Health Care; 2000. p. 72–93.
150. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001;**344**:1917–28.
151. Hall CB. Respiratory syncytial virus infections in previously healthy working adults. *Clin Infect Dis* 2001;**33**:792–6.
152. Hall CB. The spread of influenza and other respiratory viruses: complexities and conjectures. *Clin Infect Dis* 2007;**45**:353–9.
153. Hall CB. Building characteristics and the spread of infectious diseases. In: *Green schools: attributes for health and learning*. Washington, D.C.: National Academies Press; 2007. p. 105–19.
154. Hall CB. Rapid antigen testing for respiratory syncytial virus can be used in neonates with provisos. *J Pediatr* 2011;**159**:516–17.
155. Hall CB, Douglas RG Jr. Clinically useful method for the isolation of respiratory syncytial virus. *J Infect Dis* 1975;**131**:1–5.
156. Hall CB, Douglas RG Jr. Respiratory syncytial virus and influenza: practical community surveillance. *Am J Dis Child* 1976;**130**:615–20.
157. Hall CB, Douglas RG Jr. Modes of transmission of respiratory syncytial virus. *J Pediatr* 1981;**99**:100–3.
158. Hall CB, Douglas RG Jr. Nosocomial respiratory syncytial virus infections: the role of gowns and masks on prevention. *Am J Dis Child* 1981;**135**:512–16.
159. Hall CB, Douglas RG Jr, Geiman JM, Messner MK. Nosocomial respiratory syncytial virus infections. *N Engl J Med* 1975;**293**:1343–6.
160. Hall CB, Douglas RG Jr, Geiman JM. Quantitative shedding patterns of respiratory syncytial virus in infants. *J Infect Dis* 1975;**132**:151–6.
161. Hall CB, Douglas RG Jr, Geiman JM. Respiratory syncytial virus infections in infants: quantitation and duration of shedding. *J Pediatr* 1976;**89**:11–15.
162. Hall CB, Douglas RG Jr, Schnabel KC, Geiman JM. Infectivity of respiratory syncytial virus by various routes of inoculation. *Infect Immun* 1981;**33**:779–83.
163. Hall CB, Douglas RG Jr, Simons RL, Geiman JM. Interferon production in children with respiratory syncytial, influenza, and parainfluenza virus infections. *J Pediatr* 1978;**93**:28–32.
164. Hall CB, Douglas RG Jr, Simons RL. Interferon production in adults with respiratory syncytial viral infection. *Ann Intern Med* 1981;**94**:53–5.
165. Hall CB, Geiman JM, Biggar R, et al. Respiratory syncytial virus infections within families. *N Engl J Med* 1976;**294**:414–19.
166. Hall CB, Geiman JM, Douglas RG Jr. Possible transmission by fomites of respiratory syncytial virus. *J Infect Dis* 1980;**141**:98–102.
167. Hall CB, Hall WJ, Speers DM. Clinical and physiological manifestations of bronchiolitis and pneumonia: outcome of respiratory syncytial virus. *Am J Dis Child* 1979;**133**:798–802.
168. Hall CB, Kopelman A, Douglas RG. Neonatal respiratory syncytial viral infections. *N Engl J Med* 1979;**300**:393–6.
169. Hall CB, Lieberthal AS. Viral testing and isolation of patients with bronchiolitis. *Pediatrics* 2007;**120**:893–4.
170. Hall CB, Powell KR, MacDonald NE, et al. Respiratory syncytial virus infection in children with compromised immune function. *N Engl J Med* 1986;**315**:77–81.
171. Hall CB, Powell KR, Schnabel KC, et al. Risk of secondary bacterial infection in infants hospitalized with respiratory syncytial viral infection. *J Pediatr* 1988;**113**:266–71.
172. Hall CB, Walsh EE, Long CE, Schnabel KC. Immunity to and frequency of reinfection with respiratory syncytial virus. *J Infect Dis* 1991;**163**:693–8.
173. Hall CB, Walsh EE, Schnabel KC, et al. Occurrence of groups A and B of respiratory syncytial virus over 15 years: associated epidemiologic and clinical characteristics in hospitalized and ambulatory children. *J Infect Dis* 1990;**162**:1283–90.
174. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection among healthy children. *N Engl J Med* 2009;**360**:588–98.
175. Hall WJ, Hall CB, Speers DM. Respiratory syncytial virus infections in adults: clinical, virologic, and serial pulmonary function studies. *Ann Intern Med* 1978;**88**:203–5.
176. Hampp C, Kauf T, Saidi AS, Winterstein AG. Cost effectiveness of respiratory syncytial virus prophylaxis in various indications. *Arch Pediatr Adolesc Med* 2011;**165**:498–505.
177. Harcourt J, Alvarez R, Jones LP, et al. Respiratory syncytial virus G protein and G protein CX3C motif adversely affect $CX3CR1^{+}$ T cell responses. *J Immunol* 2006;**176**:1600–8.
178. Haynes LM, Caidi H, Radu GU, et al. Therapeutic monoclonal antibody treatment targeting respiratory syncytial virus G protein mediates viral clearance and reduces the pathogenesis of respiratory syncytial virus infection in BALB/c mice. *J Infect Dis* 2009;**200**:439–47.
179. Haynes LM, Moore DD, Kurt-Jones E, et al. Involvement of Toll-like receptor 4 in innate immunity to respiratory syncytial virus. *J Virol* 2001;**75**:10730–7.
180. Heidema J, de Bree GJ, De Graaff PM, et al. Human $CD8(^{+})$ T cell responses against five newly identified respiratory syncytial virus–derived epitopes. *J Gen Virol* 2004;**85**:2365–74.
181. Heikkinen T, Chonmaitree T. Importance of respiratory viruses in acute otitis media. *Clin Microbiol Rev* 2003;**16**:230–41.

182. Heikkinen T, Marttila J, Salmi AA, Ruuskanen O. Nasal swab versus nasopharyngeal aspirate for isolation of respiratory viruses. *J Clin Microbiol* 2002;**40**:4337–9.
183. Heinzmann A, Ahlert I, Kurz T, et al. Association study suggests opposite effects of polymorphisms within IL8 on bronchial asthma and respiratory syncytial virus bronchiolitis. *J Allergy Clin Immunol* 2004;**114**:671–6.
184. Henderson FW, Collier AM, Clyde WA, Denny FW. Respiratory syncytial virus infections, reinfections and immunity: a prospective, longitudinal study in young children. *N Engl J Med* 1979; **300**:530–4.
185. Henderson J, Hilliard TN, Sherriff A, et al. Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol* 2005;**16**:386–92.
186. Henrickson KJ. Advances in the laboratory diagnosis of viral respiratory disease. *Pediatr Infect Dis J* 2004;**23**(Suppl.):S6–S10.
187. Heymann PW, Platts-Mills TA, Johnston SL. Role of viral infections, atopy and antiviral immunity in the etiology of wheezing exacerbations among children and young adults. *Pediatr Infect Dis J* 2005;**24**(Suppl.):S217–22.
188. Hoebee B, Bont L, Rietveld E, et al. Influence of promoter variants of interleukin-10, interleukin-9, and tumor necrosis factor-alpha genes on respiratory syncytial virus bronchiolitis. *J Infect Dis* 2004;**189**:239–47.
189. Holman RC, Shay DK, Curns AT, et al. Risk factors for bronchiolitis-associated deaths among infants in the United States. *Pediatr Infect Dis J* 2003;**22**:483–90.
190. Hornsleth A, Loland L, Larsen LB. Cytokines and chemokines in respiratory secretion and severity of disease in infants with respiratory syncytial virus (RSV) infection. *J Clin Virol* 2001;**21**: 163–70.
191. Houben ML, Bont L, Wilbrink B, et al. Clinical prediction rule for respiratory syncytial virus bronchiolitis in healthy newborns: prognostic birth cohort study. *Pediatrics* 2011;**127**:35–41.
192. Houben ML, Coenjaerts FE, Rossen JW, et al. Disease severity and viral load are correlated in infants with primary respiratory syncytial virus infection in the community. *J Med Virol* 2010;**82**: 1266–71.
193. Huang K, Incognito L, Cheng X, et al. Respiratory syncytial virus neutralizing monoclonal antibodies motavizumab and palivizumab inhibit fusion. *J Virol* 2010;**84**:8132–40.
194. Huguenin A, Moutte L, Renois F, et al. Broad respiratory virus detection in infants hospitalized for bronchiolitis by use of a multiplex RT-PCR DNA microarray system. *J Med Virol* 2012;**84**:979–85.
195. Hull J, Rowlands K, Lockhart E, et al. Variants of the chemokine receptor CCR5 are associated with severe bronchiolitis caused by respiratory syncytial virus. *J Infect Dis* 2003;**188**:904–7.
196. Hull J, Rowlands K, Lockhart E, et al. Haplotype mapping of the bronchiolitis susceptibility locus near IL8. *Hum Genet* 2004; **114**:272–9.
197. Hyvarinen M, Piippo-Savolainen E, Korhonen K, Korppi M. Teenage asthma after severe infantile bronchiolitis or pneumonia. *Acta Paediatr* 2005;**94**:1378–83.
198. Impact RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 1998;**102**:531–7.
199. Ison MG. Respiratory syncytial virus and other respiratory viruses in the setting of bone marrow transplantation. *Curr Opin Oncol* 2009;**21**:171–6.
200. Iwane MK, Edwards KM, Szilagyi PG, et al. Population-based surveillance for hospitalizations associated with respiratory syncytial virus, influenza virus, and parainfluenza viruses among young children. *Pediatrics* 2004;**113**:1758–64.
201. Joffe S, Ray GT, Escobar GJ, et al. Cost effectiveness of respiratory syncytial virus prophylaxis among preterm infants. *Pediatrics* 1999;**104**:419–27.
202. John AE, Gerard CJ, Schaller M, et al. Respiratory syncytial virus–induced exaggeration of allergic airway disease is dependent upon CCR1-associated immune responses. *Eur J Immunol* 2005; **35**:108–16.
203. Johnson JE, Gonzales RA, Olson SJ, et al. The histopathology of fatal untreated human respiratory syncytial virus infection. *Mod Pathol* 2007;**20**:108–19.
204. Johnson KM, Chanock RM, Rifkind D, et al. Respiratory syncytial virus, part IV: correlation of virus shedding, serologic response, and illness in adult volunteers. *JAMA* 1961;**176**:663–7.
205. Johnson TR, Johnson JE, Roberts SR, et al. Priming with secreted glycoprotein G of respiratory syncytial virus augments interleukin 5 production and tissue eosinophilia after respiratory syncytial virus challenge. *J Virol* 1998;**72**:2871–80.
206. Johnson TR, Hong S, Van Kaer L, et al. NK T cells contribute to expansion of $CD8^{(+)}$ T cells and amplification of antiviral immune responses to respiratory syncytial virus. *J Virol* 2002;**76**: 4294–303.
207. Johnson TR, Mertz SE, Gitiban N, et al. Role for innate IFNs in determining respiratory syncytial virus immunopathology. *J Immunol* 2005;**174**:7234–41.
208. Johnson TR, Varga SM, Braciale TJ, Graham BS. $Vbeta14^{(+)}$ T cells mediate the vaccine-enhanced disease induced by immunization with respiratory syncytial virus (RSV) G glycoprotein but not with formalin-inactivated RSV. *J Virol* 2004;**78**:8753–60.
209. Johnstone C, de Leon P, Medina F, et al. Shifting immunodominance pattern of two cytotoxic T-lymphocyte epitopes in the F glycoprotein of the Long strain of respiratory syncytial virus. *J Gen Virol* 2004;**85**:3229–38.
210. Kahn JS. Human metapneumovirus: a newly emerging respiratory pathogen. *Curr Opin Infect Dis* 2003;**16**:255–8.
211. Kapikian AZ, Mitchell RH, Chanock RM, et al. An epidemiologic study of altered clinical reactivity to respiratory syncytial (RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. *Am J Epidemiol* 1969;**89**:405–21.
212. Karron RA, Buonagurio DA, Georgiu AF, et al. Respiratory syncytial virus (RSV) SH and G proteins are not essential for viral replication in vitro: clinical evaluation and molecular characterization of a cold-passaged, attenuated RSV subgroup B mutant. *Proc Natl Acad Sci U S A* 1997;**94**:13961–6.
213. Karron RA, Wright PF, Belshe RB, et al. Identification of a recombinant live attenuated respiratory syncytial virus vaccine candidate that is highly attenuated in infants. *J Infect Dis* 2005; **191**:1093–104.
214. Kassis C, Champlin RE, Hachem RY, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. *Biol Blood Marrow Transplant* 2010;**16**:1265–71.
215. Kellner JD, Ohlsson A, Gadomski AM, Wang EE. Bronchodilators for bronchiolitis. *Cochrane Database Syst Rev* 2000;(3): CD001266.
216. Kennedy CR, Chrzanowska K, Robinson RO, et al. A major role for viruses in acute childhood encephalopathy. *Lancet* 1986;**1**: 989–91.
217. Kesebir D, Vazquez M, Weibel C, et al. Human bocavirus infection in young children in the United States: molecular epidemiological profile and clinical characteristics of a newly emerging respiratory virus. *J Infect Dis* 2006;**194**:1276–82.
218. Khanna N, Widmer AF, Decker M, et al. Respiratory syncytial virus infection in patients with hematological diseases: single center study and review of the literature. *Clin Infect Dis* 2008;**46**: 402–12.
219. Khoshoo V, Ross G, Kelly B, et al. Benefits of thickened feeds in previously healthy infants with respiratory syncytial virus bronchiolitis. *Pediatr Pulmonol* 2001;**31**:301–2.
220. Kim HW, Arrobio JO, Brandt CD, et al. Epidemiology of respiratory syncytial virus infection in Washington, D.C., part I: importance of the virus in different respiratory tract disease syndromes and temporal distribution of infection. *Am J Epidemiol* 1973;**98**: 216–25.
221. Kim HW, Canchola JG, Brandt CD, et al. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969;**89**:422–34.
222. Kim IK, Corcoran T. Recent developments in heliox therapy for asthma and bronchiolitis. *Clin Pediatr Emerg Med* 2009;**10**: 68–74.
223. Kim IK, Phrampus E, Sikes K, et al. Helium-oxygen therapy for infants with bronchiolitis: a randomized controlled trial. *Arch Pediatr Adolesc Med* 2011;**165**:1115–22.
224. King JC. Community respiratory viruses in individuals with human immunodeficiency virus infection. *Am J Med* 1997;**102**: 19–26.

225. King VJ, Viswanathan M, Bordley WC, et al. Pharmacologic treatment of bronchiolitis in infants and children: a systematic review. *Arch Pediatr Adolesc Med* 2004;**158**:127–37.
226. Kneyber MC, van Woensel J, Uijtendaal E, et al. Azithromycin does not improve disease course in hospitalized infants with respiratory syncytial virus (RSV) lower respiratory tract disease: a randomized equivalence trial. *Pediatr Pulmonol* 2008;**43**: 142–9.
227. Konig B, Konig W, Arnold R, et al. Prospective study of human metapneumovirus infection in children less than 3 years of age. *J Clin Microbiol* 2004;**42**:4632–5.
228. Kristensen K, Hjuler T, Ravn H, et al. Chronic diseases, chromosomal abnormalities and congenital malformations as risk factors for respiratory syncytial virus hospitalization: a population-based cohort study. *Clin Infect Dis* 2012;**54**:810–17.
229. Kristjansson S, Bjarnarson SP, Wennergren G, et al. Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local TH2-like response. *J Allergy Clin Immunol* 2005;**116**:805–11.
230. Kurt-Jones EA, Popova L, Kwinn L, et al. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat Immunol* 2000;**1**:398–401.
231. Laegreid A, Kolsto Otnaess AB, Orstavik I, Carlsen KH. Neutralizing activity in human milk fractions against respiratory syncytial virus. *Acta Paediatr Scand* 1986;**75**:696–701.
232. Lagos R, DeVincenzo JP, Munoz A, et al. Safety and antiviral activity of motavizumab, a respiratory syncytial virus specific humanized monoclonal antibody when administered to respiratory syncytial virus infected children. *Pediatr Infect Dis J* 2009; **28**:835–7.
233. Lahti M, Lofgren J, Marttila R, et al. Surfactant protein D gene polymorphism associated with severe respiratory syncytial virus infection. *Pediatr Res* 2002;**51**:696–9.
234. Langley GF, Anderson LJ. Epidemiology and prevention of respiratory syncytial virus infections among infants and young children. *Pediatr Infect Dis J* 2011;**30**:510–17.
235. Langley JM, LeBlanc JC, Smith B, Wang EEL. Increasing incidence of hospitalization for bronchiolitis among Canadian children, 1980-2000. *J Infect Dis* 2003;**188**:1764–7.
236. Lavergne V, Ghannoum M, Wesiss K, et al. Successful prevention of respiratory syncytial virus nosocomial transmission following an enhanced seasonal infection control program. *Bone Marrow Transplant* 2011;**46**:137–42.
237. Law BJ, Langley JM, Allen U, et al. The Pediatric Investigator Collaborative Network on Infections in Canada study of predictors of hospitalization for respiratory syncytial virus infection for infants born at 33 through 35 completed weeks of gestation. *Pediatr Infect Dis J* 2004;**23**:806–14.
238. Leader S, Kohlhase K. Respiratory syncytial virus–coded pediatric hospitalizations, 1997-1999. *Pediatr Infect Dis J* 2002;**21**:629–32.
239. Leader S, Kohlhase K. Recent trends in severe respiratory syncytial virus (RSV) among US infants, 1997 to 2000. *J Pediatr* 2003; **143**(Suppl.):S127–32.
240. Lee FE, Walsh EE, Falsey AR, et al. Experimental infection of humans with A2 respiratory syncytial virus. *Antiviral Res* 2004;**63**: 191–6.
241. Lee GM, Salomon JA, Friedman JF, et al. Illness transmission in the home: a possible role for alcohol-based hand gels. *Pediatrics* 2005;**115**:852–60.
242. Legg JP, Warner JA, Johnston SL, Warner JO. Frequency of detection of picornaviruses and seven other respiratory pathogens in infants. *Pediatr Infect Dis J* 2005;**24**:611–16.
243. Levine DA, Platt SL, Dayan PS, et al. Risk of serious bacterial infection in young febrile infants with respiratory syncytial virus infections. *Pediatrics* 2004;**113**:1728–34.
244. Light M, Bauman J, Mavunda K, et al. Correlation between respiratory syncytial virus test data and hospitalization of children for RSV lower respiratory tract illness in Florida. *Pediatr Infect Dis J* 2008;**27**: 512–18.
245. Liet JM, Ducruet T, Gupta V, Cambonie G. Heliox inhalation therapy for bronchiolitis in infants. *Cochrane Database Syst Rev* 2010;(4):CD006915.
246. Li X, Sambhara S, Li CX, et al. Protection against respiratory syncytial virus infection by DNA immunization. *J Exp Med* 1998; **188**:681–8.
247. Ljungman P. Respiratory virus infections in stem cell transplant patients: the European experience. *Biol Blood Marrow Transplant* 2001;**7**(Suppl.):5S–7S.
248. Lo MS, Brazas RM, Holtzman MJ. Respiratory syncytial virus nonstructural proteins NS1 and NS2 mediate inhibition of Stat2 expression and alpha/beta interferon responsiveness. *J Virol* 2005;**79**:9315–19.
249. Louie JK, Hacker JK, Gonzales R, et al. Characterization of viral agents causing acute respiratory infection in a San Francisco University Medical Center Clinic during the influenza season. *Clin Infect Dis* 2005;**41**:822–8.
250. Lowther SA, Shay DK, Holman RC, et al. Bronchiolitis-associated hospitalizations among American Indian and Alaska Native children. *Pediatr Infect Dis J* 2000;**19**:11–17.
251. Lukens MV, Claassen EA, de Graaff PM, et al. Characterization of the $CD8^+$ T cell responses directed against respiratory syncytial virus during primary and secondary infection in C57BL/6 mice. *Virology* 2006;**352**:157–68.
252. MacDonald NE, Hall CB, Suffin SC, et al. Respiratory syncytial viral infection in infants with congenital heart disease. *N Engl J Med* 1982;**307**:397–400.
253. Malley R, DeVinchenzo J, Ramilo O, et al. Reduction of respiratory syncytial virus in tracheal aspirates in intubated infants by use of humanized monoclonal antibody to respiratory syncytial virus F protein. *J Infect Dis* 1998;**178**:1555–61.
254. Mangtani P, Hajat S, Kovats S, et al. The association of respiratory syncytial virus infection and influenza with emergency admissions for respiratory disease in London: an analysis of routine surveillance data. *Clin Infect Dis* 2006;**42**:640–6.
255. Manning A, Russell V, Eastick K, et al. Epidemiological profile and clinical associations of human bocavirus and other human parvoviruses. *J Infect Dis* 2006;**194**:1283–90.
256. Mansbach JM, Piedra PA, Teach SJ, et al. Prospective multicenter study of viral etiology and hospital length of stay in children with severe bronchiolitis. *Arch Pediatr Adolesc Med* 2012;**166**:700–6.
257. Martin ET, Kuypers J, Wald A, Englund JA. Multiple versus single virus respiratory infections: viral load and clinical disease severity in hospitalized children. *Influenza Other Respi Viruses* 2012;**6**: 71–7.
258. Martinez FD. Heterogeneity of the association between lower respiratory illness in infancy and subsequent asthma. *Proc Am Thorac Soc* 2005;**2**:157–61.
259. Martino R, Rámila E, Rabella N, et al. Respiratory virus infections in adults with hematologic malignancies: a prospective study. *Clin Infect Dis* 2003;**36**:1–8.
260. Martinon-Torres F, Rodriguez-Nunez A, Marinon-Sanchez JM. Heliox therapy in infants with acute bronchiolitis. *Pediatrcs* 2002;**109**:68–73.
261. Matheson JW, Rich FJ, Cohet C, et al. Distinct patterns of evolution between respiratory syncytial virus subgroups A and B from New Zealand isolates collected over thirty-seven years. *J Med Virol* 2006;**78**:1354–64.
262. Matthews SP, Tregoning JS, Coyle AJ, et al. Role of CCL11 in eosinophilic lung disease during respiratory syncytial virus infection. *J Virol* 2005;**79**:2050–7.
263. Mbawuike IN, Wells JM, Byrd RP, et al. HLA-restricted $CD8^+$ cytotoxic T lymphocyte, interferon-γ, and interleukin-4 responses to respiratory syncytial virus infection in infants and children. *J Infect Dis* 2001;**183**:687–96.
264. McClellan JS, Yongpink Y, Graham BS, Kwong PS. Structure of RSV fusion glycoprotein in the post fusion conformation reveals preservation of neutralizing epitopes. *J Virol* 2011;**85**:7788–96.
265. McDermott DS, Weiss KA, Knudson CJ, Varga SM. Central role of dendritic cells in shaping the adaptive immune response during RSV infection. *Future Virol* 2011;**6**:963–73.
266. McIntosh K. Interferon in nasal secretions from infants with viral respiratory tract infections. *J Pediatr* 1978;**93**:33–6.
267. McNally JD, Leis K, Matheson LA, et al. Vitamin D deficiency in young children with severe acute lower respiratory infection. *Pediatr Pulmonol* 2009;**44**:981–8.
268. McNamara PS, Flanagan BF, Hart CA, Smyth RL. Production of chemokines in the lungs of infants with severe respiratory syncytial virus bronchiolitis. *J Infect Dis* 2005;**191**:1225–32.
269. Meissner HC. Selected populations at increased risk from respiratory syncytial virus infection. *Pediatr Infect Dis J* 2003;**22**(Suppl.): S40–5.

270 Meissner HC. The unresolved issue of risk factors for hospitalization of infants with respiratory syncytial virus infection born after 33-35 weeks gestation. *Pediatr Infect Dis J* 2004;23821–3.
271. Meissner HC, Anderson LJ, Pickering LK. Annual variation in respiratory syncytial virus season and decisions regarding immunoprophylaxis with palivizumab. *Pediatrics* 2004;**114**:1082–4.
272. Meissner HC, Murray SA, Kiernan MA, et al. A simultaneous outbreak of respiratory syncytial virus and parainfluenza virus type 3 in a newborn nursery. *J Pediatr* 1984;**104**:680–4.
273. Meissner HC, Welliver RC, Chartrand SA, et al. Prevention of respiratory syncytial virus infection in high risk infants: consensus opinion on the role of immunoprophylaxis with hyperimmune globulin. *Pediatr Infect Dis J* 1996;**15**:1059–68.
274. Mejias A, Chavez-Bueno S, Ramilo O. Respiratory syncytial virus pneumonia: mechanisms of inflammation and prolonged airway hyperresponsiveness. *Curr Opin Infect Dis* 2005;**18**:199–204.
275. Melendez E, Harper MB. Utility of sepsis evaluation in infants 90 days of age or younger with fever and clinical bronchiolitis. *Pediatr Infect Dis J* 2003;**22**:1053–6.
276. Mendoza Sanchez MC, Ruiz-Contreras J, Vivanco JL, et al. Respiratory virus infections in children with cancer or HIV infection. *J Pediatr Hematol Oncol* 2006;**28**:154–9.
277. Miller AL, Bowlin TL, Lukacs NW. Respiratory syncytial virus–induced chemokine production: linking viral replication to chemokine production in vitro and in vivo. *J Infect Dis* 2004;**189**: 1419–30.
278. Mlinaric-Galinovic G, Vojnovic G, Cepin-Bogovic J, et al. Does the viral subtype influence the biennial cycle of respiratory syncytial virus? *Virol J* 2009;**6**:133.
279. Moore ML, Peebles RS Jr. Respiratory syncytial virus disease mechanisms implicated by human, animal model, and in vitro data facilitate vaccine strategies and new therapeutics. *Pharmacol Ther* 2006;**112**:405–24.
280. Morichi S, Kawashima H, Ioi H, et al. Classification of acute encephalopathy in respiratory syncytial virus infection. *J Infect Chemother* 2011;**17**:776–81.
281. Morris JA, Blount RE, Savage RE. Recovery of cytopathogenic agent from chimpanzees with coryza. *Proc Soc Exp Biol Med* 1956;**92**:544–9.
282. Mullooly JP, Bridges CB, Thompson WW, et al. Influenza and respiratory syncytial virus associated hospitalizations among adults. *Vaccine* 2007;**25**:846–55.
283. Munir S, Hillyer P, LeNouen C, et al. Respiratory syncytial virus interferon antagonist NS1 protein suppresses and skews the human T lymphocyte response. *PLoS Pathog* 2011;**7**(4):e1001366.
284. Munywoki PK, Hamid F, Mutunga M, et al. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real time PCR using nasopharyngeal flocked swabs. *J Clin Microbiol* 2011;**49**:3365–7.
285. Murphy BR, Prince GA, Walsh EE, et al. Dissociation between serum neutralizing antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. *J Clin Microbiol* 1986;**24**:197–202.
286. Murphy BR, Walsh EE. Formalin-inactivated respiratory syncytial virus vaccine induces antibodies to the fusion glycoprotein that are deficient in fusion-inhibiting activity. *J Clin Microbiol* 1988; **26**:1595–7.
287. Murphy D, Todd JK, Chao RK, et al. The use of gowns and masks to control respiratory illness in pediatric personnel. *J Pediatr* 1981;**99**:746.
288. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet* 2010;**378**:1917.
289. Newburg DS, Walker WA. Protection of the neonate by the innate immune system of developing gut and of human milk. *Pediatr Res* 2007;**61**:2–8.
290. Ng Y, Cox C, Atkins J, Butler IJ. Encephalopathy associated with respiratory syncytial virus bronchiolitis. *J Child Neurol* 2001;**16**: 105–8.
291. Nicholas KJ, Dhouieb MO, Marshal TG, et al. An evaluation of chest physiotherapy in the management of acute bronchiolitis: changing clinical practice. *Physiotherapy* 1999;**85**:669–74.
292. Nichols WG, Gooley T, Boeckh M. Community-acquired respiratory syncytial virus and parainfluenza virus infections after hematopoietic stem cell transplantation: The Fred Hutchinson Cancer Research Center experience. *Biol Blood Marrow Transplant* 2001;**7**(Suppl.):11S–15S.
293. Nicholson KG, McNally T, Silverman M, et al. Rates of hospitalisation for influenza, respiratory syncytial virus and human metapneumovirus among infants and young children. *Vaccine* 2006;**24**:102–8.
294. Nuolivirta K, Koponen P, He Q, et al. *Bordetella pertussis* infection is common in nonvaccinated infants admitted for bronchiolitis. *Pediatr Infect Dis J* 2010;**29**:1013–15.
295. O'Brien KL,Wolfson LJ, Watt JP, et al. Burden of disease caused by *Streptococcus pneumoniae* in children younger than 5 years: global estimates. *Lancet* 2009;**374**:893–902.
296. Ober C. Perspectives on the past decade of asthma genetics. *J Allergy Clin Immunol* 2005;**116**:274–8.
297. Ogra PL. Respiratory syncytial virus: the virus, the disease and the immune response. *Paediatr Respir Rev* 2004;**5**(Suppl. A):S119–28.
298. Openshaw PJ. Antiviral immune responses and lung inflammation after respiratory syncytial virus infection. *Proc Am Thorac Soc* 2005;**2**:121–5.
299. Openshaw PJ, Dean GS, Culley FJ. Links between respiratory syncytial virus bronchiolitis and childhood asthma: clinical and research approaches. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S58–65.
300. Openshaw PJ, Tregoning JS. Immune responses and disease enhancement during respiratory syncytial virus infection. *Clin Microbiol Rev* 2005;**18**:541–55.
301. Oshaansky CM, Zhang W, Moore E, et al. The host response and molecular pathogenesis associated with respiratory syncytial virus infection. *Future Microbiol* 2009;**4**:279–97.
302. O'Shea MK, Ryan MA, Hawksworth AW, et al. Symptomatic respiratory syncytial virus infection among previously healthy young adults living in a crowded military environment. *Clin Infect Dis* 2005;**41**:311–17.
303. Palivizumab Outcomes Registry Study Group. Palivizumab prophylaxis of respiratory syncytial virus disease in 2000-2001: results from the palivizumab outcomes registry. *Pediatr Pulmonol* 2003;**35**:484–9.
304. Panozzo CA, Stockman LJ, Curns AT, Anderson LJ. Use of respiratory syncytial virus surveillance data to optimize the timing of immunoprophylaxis. *Pediatrics* 2010;**126**:e116–23.
305. Papadopoulos NG, Gourgiotis D, Javadyan A, et al. Does respiratory syncytial virus subtype influence the severity of acute bronchiolitis in hospitalized infants? *Respir Med* 2004;**98**:879–82.
306. Paradiso PR, Hildreth SW, Hogerman DA, et al. Safety and immunogenicity of a subunit respiratory syncytial virus vaccine in children 24 to 48 months old. *Pediatr Infect Dis J* 1994;**13**: 792–8.
307. Paramore L, Ciuryla V, Ciesla G, Liu L. Economic impact of RSV related illness in the U.S: an analysis of national databases. *Pharmacoeconomics* 2004;**22**:275–84.
308. Paricio Talayero JM, Lizan-Garcia M, Otero Puime A, et al. Full breastfeeding and hospitalization as a result of infections in the first year of life. *Pediatrics* 2006;**118**:e92–9.
309. Parrott RH, Kim HW, Arrobio JO, et al. Epidemiology of respiratory syncytial virus infection in Washington, DC, part II: infection and disease with respect to age, immunologic status, race, and sex. *Am J Epidemiol* 1973;**98**:289–300.
310. Parveen S, Sullender WM, Fowler K, et al. Genetic variability in the G protein gene of group A and B respiratory syncytial viruses from India. *J Clin Microbiol* 2006;**44**:3055–64.
311. Patel H, Platt R, Lozano JM, Wang EEL. Glucocorticoids for acute viral bronchiolitis in infants and young children. *Cochrane Database Syst Rev* 2004;**4**:1–63.
312. Pedneault L, Robillard L, Turgeon JP. Validation of respiratory syncytial virus enzyme immunoassay and shell vial assay results. *J Clin Microbiol* 1994;**32**:2861–4.
313. Pelletier AJ, Mansbach JM, Camargo CA Jr. Direct medical costs of bronchiolitis hospitalizations in the United States. *Pediatrics* 2006;**118**:2418–23.
314. Petruzela FD, Gorelick MH. Duration of illness in infants with bronchiolitis evaluated in the emergency department. *Pediatrics* 2010;**126**:285–90.
315. Pettigrew MM, Gent JF, Pyles RB, et al. Viral-bacterial interactions and risk of acute otitis media complicating upper respiratory tract infection. *J Clin Microbiol* 2011;**49**:3750–5.
316. Perkins SM, Webb DL, Torrance SA, et al. Comparison of a real-time reverse transcriptase PCR assay and a culture technique for

quantitative assessment of viral load in children naturally infected with respiratory syncytial virus. *J Clin Microbiol* 2005;**43**: 2356–62.
317. Pinto LA, Pitrez PM, Luisi F, et al. Azithromycin therapy in hospitalized infants with acute bronchiolitis is not associated with better clinical outcomes: a randomized, double-blinded, and placebo-controlled clinical trial. *J Pediatr* 2012;**161**:1104–8.
318. Pitkaranta A, Virolainen A, Jero J, et al. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. *Pediatrics* 1998;**102**:291–5.
319. PREVENT Study Group. Reduction of respiratory syncytial virus hospitalization among premature infants and infants with bronchopulmonary dysplasia using respiratory syncytial virus immune globulin prophylaxis. *Pediatrics* 1997;**99**:93–9.
320. Prince GA, Prieels JP, Slaoui M, Porter DD. Pulmonary lesions in primary respiratory syncytial virus infection, reinfection, and vaccine-enhanced disease in the cotton rat *(Sigmodon hispidus)*. *Lab Invest* 1999;**79**:1385–92.
321. Purcell K, Fergie J. Concurrent serious bacterial infections in 912 infants and children hospitalized for treatment of respiratory syncytial virus lower respiratory tract infection. *Pediatr Infect Dis J* 2004;**3**:267–9.
322. Purcell K, Fergie J. Driscoll Children's Hospital respiratory syncytial virus database: risk factors, treatment and hospital course in 3308 infants and young children, 1991 to 2002. *Pediatr Infect Dis J* 2004;**23**:418–23.
323. Raboni SM, Nogueira MB, Tsuchiya LR, et al. Respiratory tract viral infections in bone marrow transplant patients. *Transplantation* 2003;**76**:142–6.
324. Ralston S, Hill V. Incidence of apnea in infants hospitalized with respiratory syncytial virus bronchiolitis: a systemiatic review. *J Pediatr* 2009;**155**:728–33.
325. Ralston S, Hill V, Martinez M. Nebulized hypertonic saline without adjunctive bronchodilators for children with bronchiolitis. *Pediatrics* 2010;**126**:e520–5.
326. Ralston S, Hill V, Waters A. Occult serious bacterial infection in infants younger than 60 to 90 days with bronchiolitis: a systematic review. *Arch Pediatr Adolesc Med* 2011;**165**:951–6.
327. Reeve CA, Whitehall JS, Buettner PG, et al. Cost-effectiveness of respiratory syncytial virus prophylaxis with palivizumab. *J Paediatr Child Health* 2006;**42**:248–52.
328. Reeve CA, Whitehall JS, Buettner PG, et al. Predicting respiratory syncytial virus hospitalisation in Australian children. *J Paediatr Child Health* 2006;**42**:248–52.
329. Resch B, Eibisberger M, Morris N, Muller W. Respiratory syncytial virus and influenza virus associated hospitalization in infants less than 12 months of age. *Pediatr Infect Dis J* 2011;**30**:797–9.
330. Resch B, Manzoni P, Lanari M. Severe respiratory syncytial virus infection in infants with neuromuscular diseases and immune deficiency syndromes. *Paediatr Respir Rev* 2009;**10**:148–53.
331. Resch B, Pasnocht A, Gusenleitner W, Muller W. Rehospitalisations for respiratory disease and respiratory syncytial virus infection in preterm infants of 29-36 weeks gestational age. *J Infect* 2005;**50**:397–403.
332. Richter BW, Onuska JM, Niewiesk S, et al. Antigen-dependent proliferation and cytokine induction in respiratory syncytial virus–infected cotton rats reflect the presence of effector-memory T cells. *Virology* 2005;**337**:102–10.
333. Rodrigiez WJ, Gruber WC, Groothuis JR, et al. Respiratory syncytial virus immune globulin treatment of respiratory syncytial virus lower respiratory tract infection in previously healthy children. *Pediatrics* 1997;**100**:937–42.
334. Rodriguez WJ, Gruber WC, Welliver RC, et al. Respiratory syncytial virus immune globulin intravenous therapy for respiratory syncytial virus lower respiratory tract infection in infants and young children at high risk for severe respiratory syncytial virus infections. *Pediatrics* 1997;**99**:454–61.
335. Roth DE, Shah R, Black RE, Baqui AH. Vitamin D status and acute lower respiratory infection in early childhood in Sylhet, Bangladesh. *Acta Paediatr* 2010;**99**:389–93.
336. Rudd BD, Burstein E, Duckett CS, et al. Differential role for TLR3 in respiratory syncytial virus–induced chemokine expression. *J Virol* 2005;**79**:3350–7.
337. Rudd BD, Smit JJ, Flavell RA, et al. Deletion of TLR3 alters the pulmonary immune environment and mucus production during respiratory syncytial virus infection. *J Immunol* 2006;**176**:1937–42.
338. Rutigliano JA, Rock MT, Johnson AK, et al. Identification of an H-2D(b)-restricted $CD8^+$ cytotoxic T lymphocyte epitope in the matrix protein of respiratory syncytial virus. *Virology* 2005;**337**: 335–43.
339. Saito T, Deskin RW, Casola A, et al. Respiratory syncytial virus induces selective production of the chemokine RANTES by upper airway epithelial cells. *J Infect Dis* 1997;**175**:497–504.
340. Sangare L, Curtis MP, Ahmad S. Hospitalization for respiratory syncytial virus among California infants: disparities related to race, insurance, and geography. *J Pediatr* 2006;**149**:373–7.
341. Sato M, Saito R, Sakai T, et al. Molecular epidemiology of respiratory syncytial virus infections among children with acute respiratory symptoms in a community over three seasons. *J Clin Microbiol* 2005;**43**:36–40.
342. Scagnolari C, Midulla F, Selvaggi C, et al. Evaluation of viral load in infants hospitalized with bronchiolitis caused by respiratory syncytial virus. *Med Microbiol Immunol* 2012;**201**:311–17.
343. Schaller M, Hogaboam CM, Lukacs N, Kunkel SL. Respiratory viral infections drive chemokine expression and exacerbate the asthmatic response. *J Allergy Clin Immunol* 2006;**118**:295–302.
344. Schauer U, Hoffjan S, Rothoeft T, et al. Severe respiratory syncytial virus infections and reduced interferon-gamma generation in vitro. *Clin Exp Immunol* 2004;**138**:102–9.
345. Schiffer JT, Kirby K, Sandmaier B, et al. Timing and severity of community acquired respiratory syncytial virus infections after myeloablative versus non-myeloablative hematopoietic stem cell transplantation. *Haematologica* 2009;**94**:1101–8.
346. Schlender J, Hornung V, Finke S, et al. Inhibition of Toll-like receptor 7- and 9-mediated alpha/beta interferon production in human plasmacytoid dendritic cells by respiratory syncytial virus and measles virus. *J Virol* 2005;**79**:5507–15.
347. Schuh S, Coates AL, Binnie R, et al. Efficacy of oral dexamethasone in outpatients with acute bronchiolitis. *J Pediatr* 2002; **140**:27–32.
348. Shafique M, Wilschut J, de Haan A. Induction of mucosal and systemic immunity against respiratory syncytial virus by inactivated virus supplemented with TLR9 and NOD2 ligands. *Vaccine* 2012;**30**:597–606.
349. Shay DK, Holman RC, Newman RD, et al. Bronchiolitis-associated hospitalizations among US children, 1980-1996. *JAMA* 1999;**282**:1440–6.
350. Shay DK, Holman RC, Roosevelt GE, et al. Bronchiolitis-associated mortality and estimates of respiratory syncytial virus–associated deaths among US children, 1979-1997. *J Infect Dis* 2001;**183**:16–22.
351. Sheeran P, Jafri H, Carubelli C, et al. Elevated cytokine concentrations in the nasopharyngeal and tracheal secretions of children with respiratory syncytial virus disease. *Pediatr Infect Dis J* 1999; **18**:115–22.
352. Sidwell RW, Barnard DL. Respiratory syncytial virus infections: recent prospects for control. *Antiviral Res* 2006;**71**:379–90.
353. Sigurs N, Gustafsson PM, Bjarnason R, et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005;**171**:137–41.
354. Singh AM, Moore PE, Gern JE, et al. Bronchiolitis to asthma: a review and call for studies of gene-virus interactions in asthma causation. *Am J Respir Crit Care Med* 2007;**175**:108–19.
355. Singleton RJ, Bruden D, Bulkow LR. Respiratory syncytial virus season and hospitalizations in the Alaskan Yukon-Kuskokwim Delta. *Pediatr Infect Dis J* 2007;**11**(Suppl):S46–50.
356. Singleton R, Etchart N, Hou S, Hyland L. Inability to evoke a long-lasting protective immune response to respiratory syncytial virus infection in mice correlates with ineffective nasal antibody responses. *J Virol* 2003;**77**:11303–11.
357. Sommer C, Resch B, Simoes EA. Risk factors for severe respiratory syncytial virus lower respiratory tract infection. *Open Microbiol J* 2011;**5**:144–54.
358. Spann KM, Tran KC, Collins PL. Effects of nonstructural proteins NS1 and NS2 of human respiratory syncytial virus on interferon regulatory factor 3, NF-kappaB, and proinflammatory cytokines. *J Virol* 2005;**79**:5353–62.
359. Spycher BD, Silverman M, Brooke AM, et al. Distinguishing phenotypes of childhood wheeze and cough using latent class analysis. *Eur Respir J* 2008;**31**:974–81.

360. Stensballe LG, Ravn H, Kristensen K, et al. Seasonal variation of maternally derived respiratory syncytial virus antibodies and association with infant hospitalizations for respiratory syncytial virus. *J Pediatr* 2009;**154**:296–8.
361. Stevens TP, Sinkin RA, Hall CB, et al. Respiratory syncytial virus and premature infants born at 32 weeks' gestation or earlier: hospitalization and economic implications of prophylaxis. *Arch Pediatr Adolesc Med* 2000;**54**:55–61.
362. Stockman LJ, Reed C, Kallen AJ, et al. Respiratory syncytial virus and *Staphylococcus aureus* coinfection in chidren hospitalized with pneumonia. *Pediatr Infect Dis J* 2010;**29**:1048–50.
363. Storch GA, Hall CB, Anderson LJ, et al. Antigenic and nucleic acid analysis of nosocomial isolates of respiratory syncytial virus. *J Infect Dis* 1993;**167**:562–6.
364. Sweetman LL, Ng YT, Butler IJ, Bodensteiner JB. Neurologic complications associated with respiratory syncytial virus. *Pediatr Neurol* 2005;**32**:307–10.
365. Tal G, Mandelberg A, Dalal I, et al. Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease. *J Infect Dis* 2004;**189**:2057–63.
366. Taussig LM, Wright AL, Holberg CJ, et al. Tucson Children's Respiratory Study: 1980 to present. *J Allergy Clin Immunol* 2003; **111**:661–75.
367. Tayyari F, Marchant D, Moraes TJ, et al. Identification of nucleolin as a cellular receptor for human RSV. *Nat Med* 2011;**17**:1132–5.
368. Techasaensiri B, Techasaensiri C, Mejias A, et al. Viral coinfections in children with invasive pneumococcal disease. *Pediatr Infect Dis J* 2010;**29**:519–23.
369. Thavagnanam S, Christie SN, Doherty GM, et al. Respiratory viral infection in lower airways of asymptomatic children. *Acta Paediatr* 2010;**99**:394–8.
370. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;**289**:179–86.
371. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med* 2009;**179**:1091–7.
372. Tian B, Zhang Y, Luxon BA, et al. Identification of NF-κB–dependent gene networks in respiratory syncytial virus–infected cells. *J Virol* 2002;**76**:6800–14.
373. Titus MO, Wright SW. Prevalence of serious bacterial infections in febrile infants with respiratory syncytial virus infection. *Pediatrics* 2003;**112**:282–4.
374. Tripp RA. Pathogenesis of respiratory syncytial virus infection. *Viral Immunol* 2004;**17**:165–81.
375. Tripp RA, Moore D, Barskey A, et al. Peripheral blood mononuclear cells from infants hospitalized because of respiratory syncytial virus infection express T helper-1 and T helper-2 cytokines and CC chemokine messenger RNA. *J Infect Dis* 2002;**185**: 1388–94.
376. Tripp RA, Oshansky C, Alvarez R. Cytokines and respiratory syncytial virus infection. *Proc Am Thorac Soc* 2005;**2**: 147–9.
377. Uckay I, Gasche-Soccal PM, Kaiser L, et al. Low incidence of severe respiratory syncytial virus infections in lung transplant recipients despite the absence of specific therapy. *J Heart Lung Transplant* 2010;**29**:299–305.
378. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;**7**:719–24.
379. van der Zalm MM, van Ewijk BE, Wilbrink B, et al. Respiratory pathogens in children with and without respiratory symptoms. *J Pediatr* 2009;**154**:396–400.
380. van Drunen, L Watkiss ER. Pathogenesis of respiratory syncytial virus. *Curr Opin Virol* 2012;**2**:300–5.
381. Visscher DW, Myers JL. Bronchiolitis: the pathologist's perspective. *Proc Am Thorac Soc* 2006;**3**:41–7.
382. Wagner DK, Muelenaer P, Henderson FW, et al. Serum immunoglobulin G antibody subclass response to respiratory syncytial virus F and G glycoproteins after first, second, and third infections. *J Clin Microbiol* 1989;**27**:589–92.
383. Walsh EE, Falsey AR. Age-related differences in humoral immune response to respiratory syncytial virus infection in adults. *J Med Virol* 2004;**73**:295–9.
384. Walsh EE, Falsey AR. Humoral and mucosal immunity in protection from natural respiratory syncytial virus infection in adults. *J Infect Dis* 2004;**190**:373–8.
385. Walsh EE, Peterson DR, Falsey AR. Risk factors for severe respiratory syncytial virus infection in elderly persons. *J Infect Dis* 2004;**189**:233–8.
386. Walsh PF, Kimmel L, Feola M, et al. Prevalence of *Bordetella pertussis* and *Bordetella parapertussis* in infants presenting to the emergency department with bronchiolitis. *J Emerg Med* 2011; **40**:256–61.
387. Walton NA, Poynton MR, Gesteland PH, et al. Predicting the start week of respiratory syncytial virus outbreaks using real time weather variables. *BMC Med Inform Decis Mak* 2010;**10**:68.
388. Wang EE, Law BJ, Boucher FD, et al. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study of admission and management variation in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. *J Pediatr* 1996;**129**:390–5.
389. Wang EE, Law BJ, Stephens D. Pediatric Investigators Collaborative Network on Infections in Canada (PICNIC) prospective study of risk factors and outcomes in patients hospitalized with respiratory syncytial viral lower respiratory tract infection. *J Pediatr* 1995;**126**:212–19.
390. Wegner S, Vann JJ, Liu G, et al. Direct cost analyses of palivizumab treatment in a cohort of at risk children: evidence from the North Carolina Medicaid program. *Pediatrics* 2004;**114**:1612–19.
391. Weinberg GA, Erdman DD, Edwards KM, et al. Superiority of reverse-transcription polymerase chain reaction to conventional viral culture in the diagnosis of acute respiratory tract infections in children. *J Infect Dis* 2004;**189**:706–10.
392. Welliver RC. Respiratory syncytial virus and other respiratory viruses. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S6–S12.
393. Welliver RC. The immune response to respiratory syncytial virus infection: friend or foe? *Clin Rev Allergy Immunol* 2008;**34**: 163–73.
394. Welliver R. The relationship of meterological conditions to the epidemiologic activity of respiratory syncytial virus. *Paediatr Respir Rev* 2009;**10**(Suppl 1):6–8.
395. Welliver RC, Kaul TN, Putnam TI, et al. The antibody response to primary and secondary infection with respiratory syncytial virus: kinetics of class-specific responses. *J Pediatr* 1980;**96**: 808–13.
396. Welliver RC, Wong DT, Sun M, et al. The development of respiratory syncytial virus–specific IgE and the release of histamine in nasopharyngeal secretions after infection. *N Engl J Med* 1981; **305**:841–6.
397. Welliver TP, Garofalo RP, Hosakote Y, et al. Severe human lower respiratory tract illness caused by respiratory syncytial virus and influenza virus is characterized by the absence of pulmonary cytotoxic lymphocyte responses. *J Infect Dis* 2007;**195**:1126–36.
398. Weltzin R, Monath TP. Intranasal antibody prophylaxis for protection against viral disease. *Clin Microbiol Rev* 1999;**12**: 383–93.
399. Wertz GW, Moudy RM. Antigenic and genetic variation in human respiratory syncytial virus. *Pediatr Infect Dis J* 2004; **23**(Suppl.):S19–24.
400. White C, Kolble R, Carlson R, et al. The effect of hand hygiene on illness rate among students in university residence halls. *Am J Infect Control* 2003;**31**:364–70.
401. Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for RSV, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012;**206**:56–62.
402. Wiertsema SP, Chidlow GR, Kirkham LA, et al. High detection rates of nucleic acids of a wide range of respiratory viruses in the nasopharynx and the middle ear of children with a history of recurrent acute otitis media. *J Med Virol* 2011;**83**:2008–17.
403. Wilkinson TM, Donaldson GC, Johnston SL, et al. Respiratory syncytial virus, airway inflammation, and FEV_1 decline in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2006;**173**:871–6.
404. Williams JV, Wang CK, Yang CF, et al. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. *J Infect Dis* 2006;**193**:387–95.
405. Wilson J, Rowlands K, Rockett K, et al. Genetic variation at the IL10 gene locus is associated with severity of respiratory syncytial virus bronchiolitis. *J Infect Dis* 2005;**191**:1705–9.

406. Wright PF, Gruber WC, Peters M, et al. Illness severity, viral shedding, and antibody responses in infants hospitalized with bronchiolitis caused by respiratory syncytial virus. *J Infect Dis* 2002;**185**:1011–18.
407. Wright PF, Karron RA, Belshe RB, et al. Evaluation of a live, cold-passaged, temperature sensitive, respiratory syncytial virus vaccine candidate in infancy. *J Infect Dis* 2000;**182**:1331–42.
408. Wu H, Pfarr DS, Tang Y, et al. Ultra-potent antibodies against respiratory syncytial virus: effects of binding kinetics and binding valence on viral neutralization. *J Mol Biol* 2005;**350**:126–44.
409. Wu P, Dupont WD, Griffin MR, et al. Evidence of a causal role of winter virus infection during infancy in early childhood asthma. *Am J Respir Crit Care Med* 2008;**178**:1123–9.
410. Yount LE, Mahle WT. Economic analysis of palivizumab in infants with congenital heart disease. *Pediatrics* 2004;**114**:1606–11.
411. Yusuf S, Peidimonte G, Auais A, et al. The relationship of meteorological conditions to the epidemic activity of respiratory syncytial virus. *Epidemiol Infect* 2007;**135**:1077–90.
412. Zachariah P, Tutterber M, Simões EA. Down syndrome and hospitalizations due to respiratory syncytial virus: a population-based study. *J Pediatr* 2011;**160**:827–31.e1.
413. Zachariah P, Tuttenber M, Simões EA. Hospitalizations due to respiratory syncytial virus in children with congenital malformations. *Pediatr Infect Dis J* 2012;**30**:442–5.
414. Zamora MR, Budev M, Rolfe M, et al. RNA interference therapy in lung transplant patients infected with respiratory syncytial virus. *Am J Respir Crit Care Med* 2010;**183**:531–8.
415. Zhang L, Mendoza-Sassi RA, Wainwright C, Klassen TP. Nebulized hypertonic saline solution for acute bronchiolitis in infants. *Cochrane Database Syst Rev* 2008;(4):CD006458.
416. Zhao X, Chen FP, Megaw AG, Sullender WM. Variable resistance to palivizumab in cotton rats by respiratory syncytial virus mutants. *J Infect Dis* 2004;**190**:1941–6.
417. Zhao X, Singh M, Malashkevich VN, Kim PS. Structural characterization of the human respiratory syncytial virus fusion protein core. *Proc Natl Acad Sci U S A* 2000;**97**:14172–7.
418. Zhu Q, McAuliffe JM, Patel NK, et al. Analysis of respiratory syncytial virus preclinical and clinical variants resistant to neutralization by monoclonal antibodies palivizumab and or motavizumab. *J Infect Dis* 2011;**203**:674–82.
419. Zhu Q, Patel NK, McAuliffe JM, et al. Natural polymorphisms and resistance associated mutations in the fusion protein of respiratory syncytial virus: effects of respiratory syncytial virus susceptibility to palivizumab. *J Infect Dis* 2012;**205**:635–8.
420. Zorc JJ, Hall CB. Bronchiolitis: recent evidence on diagnosis and management. *Pediatrics* 2010;**125**:342–9.

FIGURE 182-1 ■ Genomic map of human metapneumovirus (MPV) compared with human respiratory syncytial virus (RSV). The putative open-reading frames and the approximate nucleotide positions within the genome are indicated.

to MPV is AMPV type C (AMPV-C); evolutionary analyses of viral genes estimate that MPV diverged from AMPV-C 200 to 300 years ago.[31,176] MPV-specific antibodies were present in human sera from 1958,[149] confirming that the virus circulated prior to recent identification.

PROPERTIES

Structural and Antigenic Properties

Similar to other paramyxoviruses, MPV contains a single-stranded, negative-sense RNA genome with a lipid envelope.[149] MPV, like AMPV, lacks the two nonstructural proteins, NS1 and NS2, encoded by RSV (Fig. 182-1). The eight genes are nucleoprotein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), second matrix protein (M2), small hydrophobic protein (SH), and polymerase protein (L). The M2 gene has two open reading frames, M2-1 and M2-2, for a total of nine viral proteins. The gene order in MPV (3′N-P-M-F-M2-SH-G-L-5′) demonstrates a different placement of the F and M2 genes compared with RSV (3′NS1-NS2-N-P-M-SH-G-F-M2-L-5′).The absent nonstructural proteins and the different gene order characterize MPV as a member of the *Metapneumovirus* genus.[148,149] Like all paramyxoviruses, the fusion protein is synthesized as an active precursor cleaved by host proteases.[148] The fusion protein binds to integrins, which serve as entry receptors, as well as heparan sulfate.[24,28]

Electron microscopy demonstrates paramyxovirus-like pleomorphic spherical and filamentous particles of 150 to 600 nm, with short envelope projections 13 to 17 nm representing glycoprotein spikes.[117,149] Nucleocapsid diameter averages 17 nm.[117] The genome is about 13.3 kb.[12,119,148] Sequence analysis demonstrates that MPV is most similar to AMPV-C, with six of the nine proteins having a 70 to 80 percent amino acid identity with AMPV-C.[119,148] The SH and G proteins are the most divergent between the two viruses as well as among MPV genotypes.[12,148] The F protein is highly conserved with 93 to 97 percent amino acid identity among all strains of MPV.[12,18,151,176] Phylogenetic analysis of MPV genes identifies four distinct genetic lineages.[10,18,31,151,176] These lineages are classified into two groups (A and B), each with 2 subgroups (A1, A2, B1, and B2). All four strains have been identified in different global regions over multiple years, demonstrating that they are not exclusive to countries or seasons.

Most evidence suggests that the different genetic lineages of MPV do not represent distinct serotypes. Animal studies in rodents and nonhuman primates show a high degree of cross neutralization and cross protection between subgroups,[92,137,143] although one study in ferrets identified a 16-fold difference in serum neutralizing antibody titer.[151]

Laboratory Growth

MPV requires trypsin for optimal replication in most cell types and is difficult to culture.[149] LLC-MK2 and Vero cells, two monkey kidney epithelial cell lines, are the most permissive, with viral replication enhanced by supplemental trypsin.[144] A low level of replication occurs in LLC-MK2 and MA-104 cells without trypsin, and some strains exhibit a reduced dependence on trypsin.[128] No growth is observed on MDCK or CEF cells even with exogenous trypsin.[117,149] In LLC-MK2 cells, plaques tend to be small and round with occasional syncytia.[15,117] Cytopathic effects are noted between 3 and 23 (average, 17) days.[15] The subtle, late cytopathic effects in primary isolates and trypsin requirement likely account for the failure to detect MPV previously.

Animal Susceptibility

Van den Hoogen and colleagues[80,149] infected turkeys, chickens, and cynomolgus macaques with MPV. No overt clinical symptoms were observed in the birds, but half of the monkeys exhibited upper respiratory tract disease. Viral replication was not detected in poultry but was present in all macaques.[149] African green monkeys appear to be the most permissive nonhuman primate species.[137] Fatal MPV infections have occurred in wild chimpanzees and gorillas, likely transmitted from human sources based on sequencing of the isolates.[71,79,111] Hamsters, ferrets, cotton rats, and mice display viral replication in the upper and lower respiratory tracts, allowing for small animal models.[94,167,174] Cotton rats displayed the highest lung viral titers, whereas Syrian golden hamsters had the highest nasal titers.[167] Ferrets and guinea pigs have been infected for generation of antisera but do not manifest clinical symptoms.[149]

EPIDEMIOLOGY

Geographic Distribution

Epidemiologic studies have verified the presence of MPV worldwide. The incidence of the virus varies between countries and years, but MPV circulates in every year.*

*References 4, 26, 39, 49, 66, 75, 84, 114, 122, 139, 149, 165.

Seasonal Patterns

In temperate regions, MPV is present during all months, although it is predominant in late winter/early spring, typically following the peak RSV incidence.* In subtropical climates such as Hong Kong, a spring/summer season similar to that of RSV occurs.[114] Biannual peaks of seasonality have been described in some European studies.[1,58] In general, rates of MPV acute respiratory tract infection are lower than those of RSV infection and comparable to those of parainfluenza (PIV) and influenza,† although infection with MPV does on occasion surpass that of RSV in incidence.[75]

Strain Variation

The predominant subtype changes between years and locations.[51,73,109,139,169] In Italy, the incidence of MPV varied from 8 to 20 percent over a 3-year period. All four subtypes were identified each season; however, the predominant subtype changed. In 2001-2002, A1 accounted for 59 percent of all strains; the following year B1 and B2 were present equally; and in 2003-2004, 72 percent of strains were A2.[51] Similar variation was observed in a 20-year prospective U.S. study, with multiple genotypes present in most years.[169] Whether groups and subgroups differ in virulence remains unclear. Vicente and colleagues[159] reviewed MPV strains isolated in Spain and noted that children with group A infection had significantly more frequent pneumonia and higher disease severity, whereas in Canada genotype B was associated with more severe disease in hospitalized patients.[112] In France, patients with group B strains were more likely to have abnormal chest radiographs, but they did not have significant differences in oxygen saturation, hospitalizations, or clinical severity scores.[118] Other studies have found no major distinctions in disease severity,[2,39,139] laboratory abnormalities,[100] or symptoms[73] between genotypes. Group A viruses replicate more efficiently in animal models, suggesting some meaningful biologic differences between groups.[137,150]

Incidence and Prevalence

The majority of MPV epidemiologic studies include convenience samples collected in single-center clinical settings from patients with acute respiratory tract infection and report incidence rates of MPV between 5 and 25 percent.‡ Some prospective and multicenter studies exist.

A prospective study of all hospitalized children younger than 18 years old over one winter in Hong Kong detected MPV in 5.5 percent (32/587), slightly less than the rate of 8 percent for both RSV infection and influenza. The estimated incidence of MPV-associated lower respiratory tract illness requiring hospitalization was 4/1000.[114] A systematic study of all lower respiratory tract illnesses in Queensland, Australia, over 4 years detected MPV in 707/10,025 (7%), with seasonal peaks of 15 percent; the rates of RSV infection and influenza in this cohort were 9 percent and 4 percent, respectively.[139] A 25-year prospective study of children with acute lower respiratory tract illness in Tennessee identified MPV in 12 percent of all specimens.[165] A large, population-based, prospective surveillance study in two United States cities found that the incidence of hospitalization for MPV in children younger than 5 years old was 1.2 per 1000, lower than the rate of RSV-associated hospitalization in the same cohort (3/1000) but similar to the rates for influenza (0.9/1000) and PIV type 3 (PIV-3) (0.5/1000).[164] A prospective 1-year study of outpatient children with acute respiratory tract illness in Melbourne, Australia, tested 543 specimens by RT-PCR and detected MPV in 6 percent, RSV in 7 percent, and influenza in 4 percent.[84] A prospective study that enrolled 878 hospitalized children during one winter in China detected MPV in 26 percent and RSV in 36 percent.[25]

A South African group tested specimens from children hospitalized with acute respiratory tract infection who were subjects in a prospective pneumococcal vaccine trial; MPV was present in 126/1409 (9%) and was the most common virus after RSV. The incidence of MPV-associated hospitalization in human immunodeficiency virus (HIV)-negative children was 29/1000.[96] A Japanese 2-year, multicenter study of inpatient and outpatient children with acute respiratory tract infection identified MPV in 57/637 (9%); many of these were proven by serology to be primary infections.[39] A 5-year observational study of Korean children younger than 5 years old found MPV in 24/515 (4.7%), similar to the rates for influenza and PIV-3.[26] In Argentina, MPV was isolated from 11 percent of previously negative specimens in children with respiratory tract illnesses.[49] In Jordanian[4] and Israeli[122] children, MPV without coinfection caused 6 percent and 10 percent of acute respiratory tract infections, respectively.

Children hospitalized with MPV infections tend to be older than those admitted with RSV.* In a prospective analysis of Chinese children with acute respiratory tract infection, children with MPV had an average age of 22 months compared with 10.5 months in children infected with RSV ($p < .001$).[66] Ebihara and colleagues[39] did not identify any patients younger than 6 months or older than 10 years who had acute respiratory tract infection with MPV, suggesting that maternal antibodies may confer protection in young infants and the adaptive immune system may provide protection in older children. A male predominance has been observed in some studies, although this is not always significant.[26,45,66,114,164]

There are fewer studies of adults with MPV infection, but the rate of MPV in studies of adults with acute respiratory tract infection is 2 to 14 percent.† A 4-year prospective study detected MPV in 8.5 percent (range, 4.4% to 13.2%) of adults hospitalized with acute respiratory tract infection.[160] A population-based study found that the incidence of hospitalization with MPV in adults 50 years or older was 1 per 1000, which is similar to

*References 39, 45-47, 66, 84, 109, 139, 149, 165, 169.
†References 26, 66, 84, 106, 114, 139.
‡References 4, 26, 39, 49, 66, 75, 84, 109, 114, 122, 139, 149, 165.

*References 16, 26, 66, 104, 109, 114, 149.
†References 41, 47, 54, 67, 160, 161.

the rates for influenza (1.2/1000) and RSV infection (1.5/1000).[41]

MPV has been implicated in hospital and institutional outbreaks leading to mortality.[17,145] Kim and colleagues[74] report the transmission of MPV to pediatric hematology-oncology patients during a nosocomial outbreak. Two children shared a room with an infected child. Standard, but not droplet, precautions, were used. The incubation period was between 7 and 9 days. In laboratory studies, virus has been isolated from metal and nonporous surfaces for up to 8 hours,[144] although another study suggests that drying of MPV resulted in loss of infective particles while viral RNA could still be detected at day 7.[105] Owing to the significant morbidity and mortality of MPV in high-risk children, isolation precautions are important.

Exposure to MPV is ubiquitous by adulthood. In one Dutch study, 100 percent of patients older than the age of 5 demonstrated a positive antibody response to MPV.[149] Serosurveys testing large sample collections in Canada, China, Croatia, Germany, Israel, Japan, Taiwan, Thailand, the United States, and Uruguay show that 95 to 100 percent of children have antibodies against MPV by the age of 5 years.* In many of these studies, 50 to 75 percent of children are seropositive by age 2 years, suggesting that most acquire primary MPV infection early.

PATHOGENESIS AND PATHOLOGY

The pathogenesis of MPV has been described in animals and humans, although human data are limited. In macaques, the incubation period was short at about 2 days. Necropsy revealed mild erosive and inflammatory changes in the mucosa and submucosa of the airways. Alveolar macrophages were increased, and viral replication was observed in ciliated epithelial cells in the respiratory tract.[80] In a mouse model, lung changes were identified starting 1 day after infection. Inflammatory infiltrates with a lymphocytic and monocytic predominance, with perivascular edema, were seen in the peribronchial areas. Pathology was the most significant between days 5 and 7, with evidence of abnormalities remaining on day 14.[78] Similar patterns of bronchiolitis were observed in the cotton rat model.[167] Sumino and colleagues[141] reviewed the lung pathology of five adults with MPV infection. Histopathology in three patients demonstrated acute, organizing lung injury with diffuse alveolar membrane formation and the presence of smudge cells. The fourth patient had no evidence of lower respiratory tract infection, and the fifth patient had nonspecific acute and chronic inflammation. A similar study in children revealed chronic inflammatory changes of the airways with intra-alveolar macrophages.[155] A major limitation of both these reports of human pathology is that the patients had been mechanically ventilated for a prolonged period before death, and thus, it is impossible to distinguish virus-induced pathology from barotrauma and nonspecific inflammation.

The interaction between MPV and the immune system has been studied in humans and animal models, but the relationship is poorly understood. Laham and colleagues[83] evaluated cytokines from nasal wash specimens of children with acute viral lower respiratory tract infections. After comparing MPV-infected to RSV-infected children, it was found that interleukin (IL)-12, tumor necrosis factor-α (TNF-α), IL-1β, IL-6, and IL-8 values were lower but that of IL-10 was not different. Cytokine profiles of hospitalized versus outpatient children with MPV were not significantly different. Another study evaluated cytokine production induced by MPV in the lungs of mice. IFN-α and IFN-γ were present at higher levels than RSV as well as granulocyte-macrophage colony-stimulating factor.[53] In peripheral blood mononuclear cells, MPV produced more IL-6 compared with RSV but less IFN-γ.[37] MPV lacks the genes that mediate innate immune inhibition in other paramyxoviruses. Nonetheless, MPV is capable of blocking type I interferon responses by an unknown mechanism.[9,34] MPV and other viruses induce $CD8^+$ T-cells in the lungs that fail to secrete IFN-γ or exhibit cytotoxic degranulation in response to viral peptides; these impaired CD8 T cells resemble exhausted CD8 T cells induced by chronic infections such as HIV and hepatitis C virus.[43]

Humans mount robust antibody responses to MPV, and antibodies alone can be protective in animal models. However, immunity likely wanes over time and provides limited cross protection between genotypes because recurrent infections in children and adults are well described[47,161] with genetically different strains[39,73,115,117,165] as well as strains from the same lineage.[169] In vitro, antisera show evidence of cross protection between A and B strains, with neutralization titers generally fourfold higher against homologous versus heterologous strains.[151] Using a mouse model, Kolli and colleagues[78] demonstrated that initial infection with MPV was protective against reinfection 6 weeks later with a homologous strain. Early protection was confirmed in macaques; however, when challenged 12 weeks after initial infection, virus replication was detectable despite the presence of serum antibodies. At 11 months, lower levels of antibody were observed. At that time, all macaques infected with heterologous virus and two of three macaques reinfected with homologous virus had no evidence of protection.[150] These data suggest that in primates, and likely humans, antibody levels wane over time, facilitating reinfection. A prospective study in humans noted that baseline MPV antibodies were lower in patients who subsequently became infected versus those who did not become infected.[48] Thus, cross-protection and duration of antibody responses are important issues for vaccine development.

CLINICAL MANIFESTATIONS

MPV causes upper and lower respiratory tract disease as well as systemic symptoms, although signs and symptoms vary in incidence between studies. Clinically, MPV causes symptoms similar to those of RSV and other respiratory viruses.[16,39,106,165,177] Duration of hospitalization does not differ between children infected with RSV and MPV.[16,114] White blood cell count and C-reactive protein level were

*References 63, 88, 90-93, 100, 102, 113, 172.

not significantly different between patients with RSV and MPV infections.[66,164] Fever is present in the majority of patients* and in some is more prevalent than in RSV disease.[26,66] Transient maculopapular rash has been described in 8 to 12 percent of patients.[75,114,139] Gastrointestinal symptoms are described with low frequency,[39,114,165] although Kim and colleagues[75] noted vomiting and/or diarrhea present in 54 percent of cases. Similarly, abnormalities in aminotransferases are uncommon, although the previous study identified 27 percent of patients with alanine aminotransferase and aspartate aminotransferase values more than twice normal.[75]

MPV is rarely detected in asymptomatic persons,[8,46,110,152,165] although in otherwise healthy young adults MPV infection can be subclinical.[47] The duration of shedding in healthy individuals is 7 to 14 days.[39,82]

Upper Respiratory Tract Manifestations

Rates of upper respiratory tract disease are less than those of lower respiratory tract disease.[44,66,165,169] Hoarseness, laryngitis, sore throat, and croup are less common than pulmonary symptoms, although rhinorrhea is a frequent finding.† In one large study in Nashville, Tennessee, that evaluated children with upper respiratory tract disease, MPV was detected in 5 percent of patients. The incidence was similar to that for RSV, influenza, and PIV but lower than for adenovirus and rhinovirus. In this cohort, fever was present in 54 percent of patients, with coryza in 82 percent, cough in 66 percent, pharyngitis in 44 percent, hoarseness in 8 percent, and conjunctivitis in 3 percent.[169]

MPV is associated with acute otitis media and has been isolated by nasal wash and tympanocentesis.‡ Schildgen and colleagues[129] isolated MPV in nasopharyngeal specimens from 13 percent of children with acute otitis media. Nokso and colleagues[107] noted that 24 percent of children with MPV and upper respiratory tract infection had acute otitis media; the incidence of acute otitis media was less in children with MPV than in those with adenovirus, coronavirus, RSV, influenza, parainfluenza, and rhinovirus. In another study of 118 children with MPV and upper respiratory tract infection, 63 percent had an abnormal tympanic membrane on examination, although this was not significantly different from children infected with RSV, PIV, or influenza virus.[169] Bacterial coinfection is common; however, MPV has been isolated as the sole pathogen in middle ear fluid.[142] In another study, children with MPV had a more common history of acute otitis media necessitating tympanocentesis compared with those infected with RSV.[171]

Lower Respiratory Tract Manifestations

Lower respiratory tract signs and symptoms include cough, wheeze, and rhonchi. In one large study of Chinese children with acute respiratory tract infections, wheezing was notably more common in children with RSV disease.[66]

*References 26, 39, 45, 75, 114, 139, 169.
†References 26, 39, 66, 114, 139, 164.
‡References 107, 129, 142, 165, 168, 169.

TABLE 182-1 **Signs and Symptoms in Young Children with Human Metapneumovirus Acute Respiratory Tract Infections**

Clinical Feature	Frequency Ranges (%)
Acute otitis media	11-26
Asthma exacerbation	9-47
Conjunctivitis	3-9
Coryza	82-88
Cough	67-100
Crackles, rales	4-89
Cyanosis	2-85
Dyspnea	5-91
Febrile seizures	4-16
Fever	36-97
Pharyngitis/sore throat	10-46
Rash	4-13
Rhinorrhea	18-91
Tachypnea	59-81
Vomiting and/or diarrhea	0-54
Wheezing	15-56

Data from references 3, 7, 20, 25, 32, 35, 39, 66, 73, 75, 98, 114, 152, 165, 169, 170, and 177.

Children with MPV were diagnosed with pneumonia significantly more than children with RSV—47 percent versus 31 percent ($p = .002$). Conversely, a larger percentage of children with RSV were diagnosed with bronchiolitis compared with MPV—62 percent versus 42 percent, respectively ($p < .001$). Other studies also note the trend toward a higher percentage of children with MPV and pneumonia compared with RSV,[164,165] although this is not always statistically significant.[114] Frequency ranges of signs and symptoms of MPV infections in young children are presented in Table 182-1. As can be seen, the ranges vary considerably by feature in the studies reviewed. Presented in Figure 182-2 is the chest radiograph of a child with MPV pneumonia.

In one study, 75 percent of children with MPV had no underlying medical conditions.[16] Although mortality is most commonly described in immunocompromised patients and patients with underlying conditions, healthy children do have fatal sequelae from MPV. Donoso and colleagues[36] reported a case of a previously healthy 2-year old with fatal pneumonia, hypotension, and acute respiratory distress syndrome (ARDS) due to MPV. A similar case was reported in a 20-month-old boy with ARDS who developed multiorgan system failure with hepatitis, cytopenia, coagulopathy, and renal failure requiring extracorporeal membrane oxygenation.[131] Severe symptoms have also been reported in healthy adults.[23]

Viral coinfection has been described with MPV. Dual infection with RSV and MPV increased the risk for admission to a pediatric intensive care unit compared with RSV alone in one small study,[132] but this finding was not confirmed by subsequent larger reports.[86,135,153] Other studies demonstrate no significant difference in children with coinfections, including adenovirus,

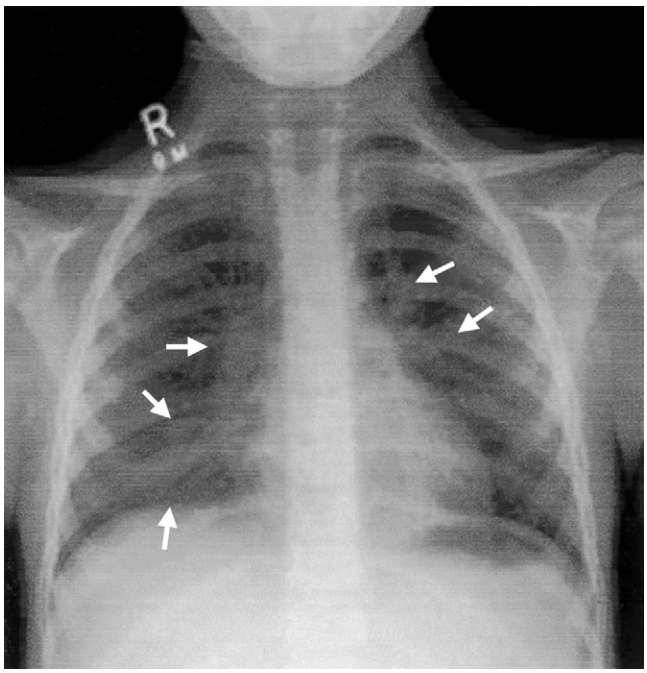

FIGURE 182-2 ■ Chest radiograph of a child infected with human metapneumovirus demonstrating bilateral pneumonic infiltrates *(arrows)* and hyperinflated lung fields.

bocavirus, coronavirus, influenza virus, parainfluenza viruses, or RSV.*

Streptococcus pneumoniae acute otitis media is associated with MPV infection[142,168] and may be a cause of pneumonia. Verkaik and colleagues[157] noted that frequent carriage of *S. pneumoniae* in children increased seroconversion rates of MPV. Pneumococcal vaccination has been shown to decrease the number of children with MPV who have severe pneumonia in both HIV- and non–HIV-infected children.[95] Secondary bacterial pneumonia after MPV infection has been reported,[5] although the frequency of this complication is unknown. Animal models show that MPV infection facilitates bacterial pneumonia with *S. pneumoniae*. Mice infected with MPV or influenza virus and subsequently challenged with sublethal doses of *S. pneumoniae* exhibited more severe lung disease than mice infected with influenza virus, MPV, or pneumococcus alone.[81]

Asthma

MPV has been linked to asthma exacerbations, although the incidence is less frequent than that for RSV or human rhinovirus.[64,72,121] MPV was detected in 10 of 132 hospitalized Finnish children with acute wheezing.[65] Similarly, MPV was isolated from 7 percent of adults hospitalized for an acute asthma exacerbation.[163] Like RSV, infection with MPV within the first 2 years of life is a risk factor for later development of asthma.[50] Broughton and colleagues[21] prospectively followed premature infants who developed MPV bronchiolitis within the first year of life and noted that they had decreased lung function.

*References 26, 66, 69, 73, 75, 87, 139.

Infections in Immunocompromised Persons

Severe and fatal MPV disease has been described in immunocompromised individuals, including solid-organ and stem cell transplant recipients, HIV-infected individuals, chemotherapy patients, and persons with chronic cardiopulmonary disease. In a study of hospitalized South African children, children with underlying medical problems accounted for 88 percent of positive MPV specimens. This was not significantly different than for infection with RSV.[104] A 7-month old with acute lymphocytic leukemia developed shock and respiratory failure ultimately leading to death, and only MPV was detected by RT-PCR.[115] MPV is a cause of mortality in adults with hematologic malignancies[166] and in recipients of stem cell transplants[42]; MPV was detected in bronchoalveolar lavage specimens from 5/163 (3%) patients with acute respiratory tract infection who were stem cell transplant recipients, and 4 of the 5 died.[42] In a South African study, HIV-positive children with MPV were significantly more likely to receive a diagnosis of pneumonia and experience longer hospitalization and have lower mean oxygen saturation and bacteremia. Children with HIV infection were fivefold more likely to be infected with MPV than HIV-negative children.[96]

MPV causes severe disease in children with underlying cardiac and respiratory pathologic processes or prematurity,[76,104,112,156] with some evidence suggesting it is more common than RSV in these children, possibly related to the use of palivizumab.[177] Esper and colleagues[45] noted that 34 percent of patients with MPV had a history of prematurity, chronic lung disease, complex congenital heart disease, or immunodeficiency, emphasizing that these children are at risk for MPV disease. Ulloa-Gutierrez and colleagues[147] described a 3-month-old 27-week premature infant who required 10 days of extracorporeal membrane oxygenation. MPV has been detected in 3 to 12 percent of adults hospitalized for exacerbations of chronic obstructive pulmonary disease.[99,124,158]

Encephalitis and Encephalopathy

MPV has been associated with neurologic complications, including febrile seizures and altered mental status, but there is no conclusive evidence for direct central nervous system infection. Schildgen and colleagues[130] report a case of one patient who died and had MPV isolated by RT-PCR from brain and lung tissue. Glaser and colleagues[52] reviewed cases of encephalitis of unknown etiology. They identified MPV by RT-PCR in nasal specimens from 4 of 1570 cases. Several other reports describe the detection of MPV in a respiratory specimen from patients with encephalitis.[6,57,68,130] MPV also has been reported to have been detected in cerebrospinal fluid.[126]

DIFFERENTIAL DIAGNOSIS

Clinical illness caused by MPV cannot be reliably distinguished from disease caused by other common respiratory viruses, including RSV, parainfluenza viruses, and

influenza. Complications such as acute otitis media and supervening bacterial pneumonia may occur.

DIAGNOSIS

Because detection in tissue culture can require prolonged incubation for viral replication, other diagnostic tests have been employed. Shell viral culture offers increased sensitivity over traditional culture.[85,123] Immunofluorescence assay has demonstrated a sensitivity of 73 percent and specificity of 97 percent with RT-PCR as the gold standard.[40] Direct fluorescent antibody testing has shown similar results.[116] Commercial antibody reagents for immunofluorescent detection of MPV are available. RT-PCR is widely used for detection of the virus in epidemiologic studies; primers have been directed at the F, M, L, P, and N proteins.[15,27,117,149] Real-time RT-PCR directed toward the N gene has a high sensitivity for virus detection and can detect the four subgroups.[77,97] Several commercial molecular assays for MPV are available.

TREATMENT

The mainstay of therapy for MPV infections is supportive care, including oral or intravenous hydration for infants unable to feed, monitoring of respiratory status and oxygen saturation, supplemental oxygen as needed, and mechanical ventilation for respiratory failure. Reports of pharmacologic treatment of MPV are limited to its use in immunocompromised and severely ill patients. Ribavirin, an antiviral agent used in severe RSV infection, has been used in animal models and humans. Hamelin and colleagues[56] demonstrated that ribavirin reduced inflammation and viral replication in mice with MPV infection. Commercial intravenous immunoglobulin (IVIG), ribavirin, and NMSO3 effectively inhibited MPV in vitro.[173,175] Spetch and colleagues[140] noted in a mouse model that NMSO3 decreased viral replication, weight loss associated with infection, and pulmonary inflammation. Ribavirin, with and without IVIG, has been used successfully in immunocompromised adults.[70] Bonney and colleagues[19] reported successful treatment of an immunocompromised child with MPV using intravenous ribavirin and IVIG. Subsequently, oral ribavirin and inhaled ribavirin with IVIG have been successful.[133,134] Alternate therapies currently being explored include the use of plant-derived compounds and short interfering RNA.[30,33,101,120] Human and murine monoclonal antibodies exhibit therapeutic efficacy in animal models.[55,146,162]

PROGNOSIS

Most patients with MPV infection experience a full recovery. Fatalities are uncommon and occur primarily in severely immunocompromised persons (e.g., hematopoietic stem cell or solid-organ transplant recipients, those who have had intensive chemotherapy, or those with HIV infection), persons with significant underlying conditions (e.g., severe COPD), and adults older than age 65 in poor health (the "frail" elderly). MPV may be associated with the subsequent development of asthma.

PREVENTION

Development of candidate MPV vaccines began shortly after identification of the virus. The F protein is highly immunogenic and the only target of neutralizing antibodies; in contrast to RSV, the MPV G protein does not induce neutralizing antibodies and is not a protective antigen.[103,125,138] A recombinant PIV encoding the MPV F protein demonstrated protection against wild-type virus,[143] and soluble F protein vaccines have shown a reduction in viral titers in cotton rats and hamsters.[29,60] Monoclonal antibodies directed at the fusion protein are effective at decreasing viral replication in mouse and cotton rat models.[55,120,146] Reverse genetics has been developed for MPV and has been used to develop recombinant strains for vaccine development.[13,59] Viruses lacking the G, M2-1, M2-2, or SH proteins or with point mutations are attenuated and immunogenic in rodent and primate models.*

NEW REFERENCES SINCE THE SIXTH EDITION

1. Aberle SW, Aberle JH, Sandhofer MJ, et al. Biennial spring activity of human metapneumovirus in Austria. *Pediatr Infect Dis J* 2008;**27**:1065–8.
2. Agapov E, Sumino KC, Gaudreault-Keener M, et al. Genetic variability of human metapneumovirus infection: evidence of a shift in viral genotype without a change in illness. *J Infect Dis* 2006;**193**:396–403.
3. Al-Sonboli N, Hart CA, Al-Aeryani A, et al. Respiratory syncytial virus and human metapneumovirus in children with acute respiratory infections in Yemen. *Pediatr Infect Dis J* 2005;**24**: 734–6.
4. Ali SA, Williams JV, Chen Q, et al. Human metapneumovirus in hospitalized children in Amman, Jordan. *J Med Virol* 2010;**82**: 1012–16.
5. Ampofo K, Bender J, Sheng X, et al. Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics* 2008;**122**:229–37.
6. Arnold JC, Singh KK, Milder E, et al. Human metapneumovirus associated with central nervous system infection in children. *Pediatr Infect Dis J* 2009;**28**:1057–60.
8. Banerjee S, Sullender WM, Choudekar A, et al. Detection and genetic diversity of human metapneumovirus in hospitalized children with acute respiratory infections in India. *J Med Virol* 2011;**83**:1799–810.
9. Bao X, Liu T, Shan Y, et al. Human metapneumovirus glycoprotein G inhibits innate immune responses. *PLoS Pathog* 2008;**4**: e1000077.
10. Bastien N, Normand S, Taylor T, et al. Sequence analysis of the N, P, M and F genes of Canadian human metapneumovirus strains. *Virus Res* 2003;**93**:51–62.
11. Biacchesi S, Pham QN, Skiadopoulos MH, et al. Infection of nonhuman primates with recombinant human metapneumovirus lacking the SH, G, or M2-2 protein categorizes each as a nonessential accessory protein and identifies vaccine candidates. *J Virol* 2005;**79**:12608–13.
12. Biacchesi S, Skiadopoulos MH, Boivin G, et al. Genetic diversity between human metapneumovirus subgroups. *Virology* 2003;**315**: 1–9.
13. Biacchesi S, Skiadopoulos MH, Tran KC, et al. Recovery of human metapneumovirus from cDNA: optimization of growth in vitro and expression of additional genes. *Virology* 2004;**321**:247–59.

*References 11, 14, 22, 61, 62, 127.

14. Biacchesi S, Skiadopoulos MH, Yang L, et al. Recombinant human metapneumovirus lacking the small hydrophobic SH and/or attachment G glycoprotein: deletion of G yields a promising vaccine candidate. *J Virol* 2004;**78**:12877–87.
16. Boivin G, De Serres G, Cote S, et al. Human metapneumovirus infections in hospitalized children. *Emerg Infect Dis* 2003;**9**: 634–40.
18. Boivin G, Mackay I, Sloots TP, et al. Global genetic diversity of human metapneumovirus fusion gene. *Emerg Infect Dis* 2004;**10**: 1154–7.
19. Bonney D, Razali H, Turner A, et al. Successful treatment of human metapneumovirus pneumonia using combination therapy with intravenous ribavirin and immune globulin. *Br J Haematol* 2009;**145**:667–9.
21. Broughton S, Sylvester KP, Fox G, et al. Lung function in prematurely born infants after viral lower respiratory tract infections. *Pediatr Infect Dis J* 2007;**26**:1019–24.
22. Buchholz UJ, Biacchesi S, Pham QN, et al. Deletion of M2 gene open reading frames 1 and 2 of human metapneumovirus: effects on RNA synthesis, attenuation, and immunogenicity. *J Virol* 2005;**79**:6588–97.
23. Chan PK, Tam JS, Lam CW, et al. Human metapneumovirus detection in patients with severe acute respiratory syndrome. *Emerg Infect Dis* 2003;**9**:1058–63.
24. Chang A, Masante C, Buchholz UJ, et al. Human metapneumovirus (HMPV) binding and infection are mediated by interactions between the HMPV fusion protein and heparan sulfate. *J Virol* 2012;**86**:3230–43.
25. Chen X, Zhang ZY, Zhao Y, et al. Acute lower respiratory tract infections by human metapneumovirus in children in Southwest China: a 2-year study. *Pediatr Pulmonol* 2010;**45**:824–31.
27. Cote S, Abed Y, Boivin G. Comparative evaluation of real-time PCR assays for detection of the human metapneumovirus. *J Clin Microbiol* 2003;**41**:3631–5.
28. Cseke G, Maginnis MS, Cox RG, et al. Integrin alphavbeta1 promotes infection by human metapneumovirus. *Proc Natl Acad Sci U S A* 2009;**106**:1566–71.
30. Darniot M, Schildgen V, Schildgen O, et al. RNA interference in vitro and in vivo using DsiRNA targeting the nucleocapsid N mRNA of human metapneumovirus. *Antiviral Res* 2012;**93**: 364–73.
31. de Graaf M, Osterhaus AD, Fouchier RA, et al. Evolutionary dynamics of human and avian metapneumoviruses. *J Gen Virol* 2008;**89**:2933–42.
32. Debur MC, Vidal LR, Stroparo E, et al. Impact of human metapneumovirus infection on in and outpatients for the years 2006-2008 in Southern Brazil. *Mem Inst Oswaldo Cruz* 2010; **105**:1010–18.
33. Deffrasnes C, Cavanagh MH, Goyette N, et al. Inhibition of human metapneumovirus replication by small interfering RNA. *Antiviral Ther* 2008;**13**:821–32.
34. Dinwiddie DL, Harrod KS. Human metapneumovirus inhibits IFN-alpha signaling through inhibition of STAT1 phosphorylation. *Am J Respir Cell Mol Biol* 2008;**38**:661–70.
36. Donoso AF, Leon JA, Camacho JF, et al. Fatal hemorrhagic pneumonia caused by human metapneumovirus in an immunocompetent child. *Pediatr Int* 2008;**50**:589–91.
38. Ebihara T, Endo R, Kikuta H, et al. Seroprevalence of human metapneumovirus in Japan. *J Med Virol* 2003;**70**:281–3.
40. Ebihara T, Endo R, Ma X, et al. Detection of human metapneumovirus antigens in nasopharyngeal secretions by an immunofluorescent-antibody test. *J Clin Microbiol* 2005;**43**: 1138–41.
41. El Sayed Zaki M, Raafat D, El-Metaal AA, et al. Study of human metapneumovirus-associated lower respiratory tract infections in Egyptian adults. *Microbiol Immunol* 2009;**53**:603–8.
43. Erickson JJ, Gilchuk P, Hastings AK, et al. Viral acute lower respiratory infections impair $CD8^+$ T cells through PD-1. *J Clin Invest* 2012;**122**:2967–82.
44. Esper F, Boucher D, Weibel C, et al. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. *Pediatrics* 2003;**111**:1407–10.
46. Falsey AR, Criddle MC, Walsh EE. Detection of respiratory syncytial virus and human metapneumovirus by reverse transcription polymerase chain reaction in adults with and without respiratory illness. *J Clin Virol* 2006;**35**:46–50.
47. Falsey AR, Erdman D, Anderson LJ, et al. Human metapneumovirus infections in young and elderly adults. *J Infect Dis* 2003; **187**:785–90.
48. Falsey AR, Hennessey PA, Formica MA, et al. Humoral immunity to human metapneumovirus infection in adults. *Vaccine* 2010;**28**: 1477–80.
49. Galiano M, Videla C, Puch SS, et al. Evidence of human metapneumovirus in children in Argentina. *J Med Virol* 2004;**72**: 299–303.
51. Gerna G, Campanini G, Rovida F, et al. Changing circulation rate of human metapneumovirus strains and types among hospitalized pediatric patients during three consecutive winter-spring seasons: brief report. *Arch Virol* 2005;**150**:2365–75.
53. Guerrero-Plata A, Casola A, Garofalo RP. Human metapneumovirus induces a profile of lung cytokines distinct from that of respiratory syncytial virus. *J Virol* 2005;**79**:14992–7.
54. Hamelin ME, Cote S, Laforge J, et al. Human metapneumovirus infection in adults with community-acquired pneumonia and exacerbation of chronic obstructive pulmonary disease. *Clin Infect Dis* 2005;**41**:498–502.
55. Hamelin ME, Gagnon C, Prince GA, et al. Prophylactic and therapeutic benefits of a monoclonal antibody against the fusion protein of human metapneumovirus in a mouse model. *Antiviral Res* 2010;**88**:31–7.
58. Heininger U, Kruker AT, Bonhoeffer J, et al. Human metapneumovirus infections—biannual epidemics and clinical findings in children in the region of Basel, Switzerland. *Eur J Pediatr* 2009; **168**:1455–60.
59. Herfst S, de Graaf M, Schickli JH, et al. Recovery of human metapneumovirus genetic lineages a and B from cloned cDNA. *J Virol* 2004;**78**:8264–70.
60. Herfst S, de Graaf M, Schrauwen EJ, et al. Immunization of Syrian golden hamsters with F subunit vaccine of human metapneumovirus induces protection against challenge with homologous or heterologous strains. *J Gen Virol* 2007;**88**: 2702–9.
61. Herfst S, de Graaf M, Schrauwen EJ, et al. Generation of temperature-sensitive human metapneumovirus strains that provide protective immunity in hamsters. *J Gen Virol* 2008;**89**: 1553–62.
62. Herfst S, Schrauwen EJ, de Graaf M, et al. Immunogenicity and efficacy of two candidate human metapneumovirus vaccines in cynomolgus macaques. *Vaccine* 2008;**26**:4224–30.
63. Huang CG, Tsao KC, Lin TY, et al. Estimates of individuals exposed to human metapneumovirus in a community-based Taiwanese population in 1999. *Arch Virol* 2010;**155**:343–50.
64. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004;**10**:1095–101.
66. Ji W, Wang Y, Chen Z, et al. Human metapneumovirus in children with acute respiratory tract infections in Suzhou, China 2005-2006. *Scand J Infect Dis* 2009;**41**:735–44.
67. Johnstone J, Majumdar SR, Fox JD, et al. Human metapneumovirus pneumonia in adults: results of a prospective study. *Clin Infect Dis* 2008;**46**:571–4.
68. Kaida A, Iritani N, Kubo H, et al. Seasonal distribution and phylogenetic analysis of human metapneumovirus among children in Osaka City, Japan. *J Clin Virol* 2006;**35**:394–9.
69. Kaida A, Kubo H, Goto K, et al. Co-infection of human metapneumovirus with adenovirus or respiratory syncytial virus among children in Japan. *Microbiol Immunol* 2007;**51**:679–83.
70. Kamble RT, Bollard C, Demmler G, et al. Human metapneumovirus infection in a hematopoietic transplant recipient. *Bone Marrow Transplant* 2007;**40**:699–700.
71. Kaur T, Singh J, Tong S, et al. Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees *(Pan troglodytes)* at Mahale Mountains National Park, Western Tanzania. *Am J Primatol* 2008; **70**:755–65.
72. Khetsuriani N, Kazerouni NN, Erdman DD, et al. Prevalence of viral respiratory tract infections in children with asthma. *J Allergy Clin Immunol* 2007;**119**:314–21.

73. Kim HR, Cho AR, Lee MK, et al. Genotype variability and clinical features of human metapneumovirus isolated from Korean children, 2007 to 2010. *J Mol Diagn* 2012;**14**:61–4.
74. Kim S, Sung H, Im HJ, et al. Molecular epidemiological investigation of a nosocomial outbreak of human metapneumovirus infection in a pediatric hemato-oncology patient population. *J Clin Microbiol* 2009;**47**:1221–4.
75. Kim YK, Lee HJ. Human metapneumovirus–associated lower respiratory tract infections in Korean infants and young children. *Pediatr Infect Dis J* 2005;**24**:1111–12.
77. Klemenc J, Asad Ali S, Johnson M, et al. Real-time reverse transcriptase PCR assay for improved detection of human metapneumovirus. *J Clin Virol* 2012;**54**:371–5.
78. Kolli D, Bataki EL, Spetch L, et al. T lymphocytes contribute to antiviral immunity and pathogenesis in experimental human metapneumovirus infection. *J Virol* 2008;**82**:8560–9.
79. Kondgen S, Kuhl H, N'Goran PK, et al. Pandemic human viruses cause decline of endangered great apes. *Curr Biol* 2008;**18**: 260–4.
80. Kuiken T, van den Hoogen BG, van Riel DA, et al. Experimental human metapneumovirus infection of cynomolgus macaques *(Macaca fascicularis)* results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. *Am J Pathol* 2004;**164**:1893–900.
81. Kukavica-Ibrulj I, Hamelin ME, Prince GA, et al. Infection with human metapneumovirus predisposes mice to severe pneumococcal pneumonia. *J Virol* 2009;**83**:1341–9.
82. Kunz AN, Englund JA, Kuypers J, et al. Detection of multiple respiratory viruses by real-time polymerase chain reaction in infants attending an outpatient clinic. *Eur J Clin Microbiol Infect Dis* 2008;**27**:1245–8.
85. Landry ML, Ferguson D, Cohen S, et al. Detection of human metapneumovirus in clinical samples by immunofluorescence staining of shell vial centrifugation cultures prepared from three different cell lines. *J Clin Microbiol* 2005;**43**:1950–2.
86. Lazar I, Weibel C, Dziura J, et al. Human metapneumovirus and severity of respiratory syncytial virus disease. *Emerg Infect Dis* 2004;**10**:1318–20.
88. Leung J, Esper F, Weibel C, et al. Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. *J Clin Microbiol* 2005;**43**: 1213–19.
89. Liao RS, Appelgate DM, Pelz RK. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility for the elderly in Oregon. *J Clin Virol* 2012;**53**: 171–3.
90. Liu L, Bastien N, Sidaway F, et al. Seroprevalence of human metapneumovirus (hMPV) in the Canadian province of Saskatchewan analyzed by a recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay. *J Med Virol* 2007;**79**: 308–13.
91. Ljubin Sternak S, Vilibic Cavlek T, Falsey AR, et al. Serosurvey of human metapneumovirus infection in Croatia. *Croat Med J* 2006;**47**:878–81.
92. Lu G, Gonzalez R, Guo L, et al. Large-scale seroprevalence analysis of human metapneumovirus and human respiratory syncytial virus infections in Beijing, China. *Virol J* 2011;**8**:62.
93. Lusebrink J, Wiese C, Thiel A, et al. High seroprevalence of neutralizing capacity against human metapneumovirus in all age groups studied in Bonn, Germany. *Clin Vaccine Immunol* 2010; **17**:481–4.
94. MacPhail M, Schickli JH, Tang RS, et al. Identification of small-animal and primate models for evaluation of vaccine candidates for human metapneumovirus (hMPV) and implications for hMPV vaccine design. *J Gen Virol* 2004;**85**:1655–63.
97. Maertzdorf J, Wang CK, Brown JB, et al. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *J Clin Microbiol* 2004;**42**:981–6.
98. Manoha C, Espinosa S, Aho SL, et al. Epidemiological and clinical features of hMPV, RSV and RVs infections in young children. *J Clin Virol* 2007;**38**:221–6.
99. Martinello RA, Esper F, Weibel C, et al. Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease. *J Infect* 2006;**53**:248–54.
100. Matsuzaki Y, Itagaki T, Abiko C, et al. Clinical impact of human metapneumovirus genotypes and genotype-specific seroprevalence in Yamagata, Japan. *J Med Virol* 2008;**80**:1084–9.
101. Mendes G, Soares AR, Sigiliano L, et al. In vitro anti-HMPV activity of meroditerpenoids from marine alga *Stypopodium zonale (Dictyotales)*. *Molecules* 2011;**16**:8437–50.
102. Mirazo S, Ruchansky D, Blanc A, et al. Serologic evidence of human metapneumovirus circulation in Uruguay. *Mem Inst Oswaldo Cruz* 2005;**100**:715–18.
103. Mok H, Tollefson SJ, Podsiad AB, et al. An alphavirus replicon-based human metapneumovirus vaccine is immunogenic and protective in mice and cotton rats. *J Virol* 2008;**82**:11410–18.
105. Muller A, Tillmann RL, Simon A, et al. Stability of human metapneumovirus and human coronavirus NL63 on medical instruments and in the patient environment. *J Hosp Infect* 2008;**69**: 406–8.
107. Nokso-Koivisto J, Pyles RB, Miller AL, et al. Viral load and acute otitis media development after human metapneumovirus upper respiratory tract infection. *Pediatr Infect Dis J* 2012;**31**:763–6.
108. Okamoto M, Sugawara K, Takashita E, et al. Longitudinal course of human metapneumovirus antibody titers and reinfection in healthy adults. *J Med Virol* 2010;**82**:2092–6.
109. Oliveira DB, Durigon EL, Carvalho AC, et al. Epidemiology and genetic variability of human metapneumovirus during a 4-year-long study in Southeastern Brazil. *J Med Virol* 2009;**81**: 915–21.
111. Palacios G, Lowenstine LJ, Cranfield MR, et al. Human metapneumovirus infection in wild mountain gorillas, Rwanda. *Emerg Infect Dis* 2011;**17**:711–13.
112. Papenburg J, Hamelin ME, Ouhoummane N, et al. Comparison of risk factors for human metapneumovirus and respiratory syncytial virus disease severity in young children. *J Infect Dis* 2012;**206**: 178–89.
113. Pavlin JA, Hickey AC, Ulbrandt N, et al. Human metapneumovirus reinfection among children in Thailand determined by ELISA using purified soluble fusion protein. *J Infect Dis* 2008;**198**: 836–42.
114. Peiris JS, Tang WH, Chan KH, et al. Children with respiratory disease associated with metapneumovirus in Hong Kong. *Emerg Infect Dis* 2003;**9**:628–33.
116. Percivalle E, Sarasini A, Visai L, et al. Rapid detection of human metapneumovirus strains in nasopharyngeal aspirates and shell vial cultures by monoclonal antibodies. *J Clin Microbiol* 2005;**43**:3443–6.
118. Pitoiset C, Darniot M, Huet F, et al. Human metapneumovirus genotypes and severity of disease in young children (n = 100) during a 7-year study in Dijon hospital, France. *J Med Virol* 2010;**82**:1782–9.
119. Piyaratna R, Tollefson SJ, Williams JV. Genomic analysis of four human metapneumovirus prototypes. *Virus Res* 2011;**160**: 200–5.
120. Preston FM, Straub CP, Ramirez R, et al. siRNA against the G gene of human metapneumovirus. *Virol J* 2012;**9**:105.
122. Regev L, Hindiyeh M, Shulman LM, et al. Characterization of human metapneumovirus infections in Israel. *J Clin Microbiol* 2006;**44**:1484–9.
124. Rohde G, Borg I, Arinir U, et al. Relevance of human metapneumovirus in exacerbations of COPD. *Respir Res* 2005;**6**:150.
125. Ryder AB, Tollefson SJ, Podsiad AB, et al. Soluble recombinant human metapneumovirus G protein is immunogenic but not protective. *Vaccine* 2010;**28**:4145–52.
126. Sanchez Fernandez I, Rebollo Polo M, Munoz-Almagro C, et al. Human metapneumovirus in the cerebrospinal fluid of a patient with acute encephalitis. *Arch Neurol* 2012;**69**:649–52.
127. Schickli JH, Kaur J, Macphail M, et al. Deletion of human metapneumovirus M2-2 increases mutation frequency and attenuates growth in hamsters. *Virol J* 2008;**5**:69.
128. Schickli JH, Kaur J, Ulbrandt N, et al. An S101P substitution in the putative cleavage motif of the human metapneumovirus fusion protein is a major determinant for trypsin-independent growth in vero cells and does not alter tissue tropism in hamsters. *J Virol* 2005;**79**:10678–89.
130. Schildgen O, Glatzel T, Geikowski T, et al. Human metapneumovirus RNA in encephalitis patient. *Emerg Infect Dis* 2005;**11**: 467–70.

131. Schlapbach LJ, Agyeman P, Hutter D, et al. Human metapneumovirus infection as an emerging pathogen causing acute respiratory distress syndrome. *J Infect Dis* 2011;**203**:294–5; author reply 296.
133. Shachor-Meyouhas Y, Ben-Barak A, Kassis I. Treatment with oral ribavirin and IVIG of severe human metapneumovirus pneumonia (HMPV) in immune compromised child. *Pediatr Blood Cancer* 2011;**57**:350–1.
134. Shahda S, Carlos WG, Kiel PJ, et al. The human metapneumovirus: a case series and review of the literature. *Transpl Infect Dis* 2011;**13**:324–8.
135. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol* 2010;**82**:1282–90.
136. Sivaprakasam V, Collins TC, Aitken C, et al. Life-threatening human metapneumovirus infections in West of Scotland. *J Clin Virol* 2007;**39**:234–7.
137. Skiadopoulos MH, Biacchesi S, Buchholz UJ, et al. The two major human metapneumovirus genetic lineages are highly related antigenically, and the fusion (F) protein is a major contributor to this antigenic relatedness. *J Virol* 2004;**78**:6927–37.
138. Skiadopoulos MH, Biacchesi S, Buchholz UJ, et al. Individual contributions of the human metapneumovirus F, G, and SH surface glycoproteins to the induction of neutralizing antibodies and protective immunity. *Virology* 2006;**345**:492–501.
140. Spetch L, Bowlin TL, Casola A. Effect of NMSO3 treatment in a murine model of human metapneumovirus infection. *J Gen Virol* 2008;**89**:2709–12.
141. Sumino KC, Agapov E, Pierce RA, et al. Detection of severe human metapneumovirus infection by real-time polymerase chain reaction and histopathological assessment. *J Infect Dis* 2005;**192**:1052–60.
143. Tang RS, Mahmood K, Macphail M, et al. A host-range restricted parainfluenza virus type 3 (PIV3) expressing the human metapneumovirus (hMPV) fusion protein elicits protective immunity in African green monkeys. *Vaccine* 2005;**23**:1657–67.
144. Tollefson SJ, Cox RG, Williams JV. Studies of culture conditions and environmental stability of human metapneumovirus. *Virus Res* 2010;**151**:54–9.
145. Tu CC, Chen LK, Lee YS, et al. An outbreak of human metapneumovirus infection in hospitalized psychiatric adult patients in Taiwan. *Scand J Infect Dis* 2009;**41**:363–7.
152. van den Hoogen BG, van Doornum GJ, Fockens JC, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. *J Infect Dis* 2003;**188**:1571–7.
153. van Woensel JB, Bos AP, Lutter R, et al. Absence of human metapneumovirus co-infection in cases of severe respiratory syncytial virus infection. *Pediatr Pulmonol* 2006;**41**:872–4.
154. Vanspauwen MJ, van Mook WN, Bruggeman CA, et al. Human metapneumovirus in bronchoalveolar lavage fluid of critically ill patients with suspected pneumonia. *Intensive Care Med* 2012;**38**:728–9.
156. Varman M, Truemper EJ, Chatterjee A. Severe human metapneumovirus pneumonia in a child with chronic illness. *Clin Pediatr (Phila)* 2010;**49**:1075–7.
157. Verkaik NJ, Nguyen DT, de Vogel CP, et al. *Streptococcus pneumoniae* exposure is associated with human metapneumovirus seroconversion and increased susceptibility to in vitro HMPV infection. *Clin Microbiol Infect* 2011;**17**:1840–4.
158. Vicente D, Montes M, Cilla G, et al. Human metapneumovirus and chronic obstructive pulmonary disease. *Emerg Infect Dis* 2004;**10**:1338–9.
160. Walsh EE, Peterson DR, Falsey AR. Human metapneumovirus infections in adults: another piece of the puzzle. *Arch Intern Med* 2008;**168**:2489–96.
161. Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012;**206**:56–62.
162. Williams JV, Chen Z, Cseke G, et al. A recombinant human monoclonal antibody to human metapneumovirus fusion protein that neutralizes virus in vitro and is effective therapeutically in vivo. *J Virol* 2007;**81**:8315–24.
163. Williams JV, Crowe JE Jr, Enriquez R, et al. Human metapneumovirus infection plays an etiologic role in acute asthma exacerbations requiring hospitalization in adults. *J Infect Dis* 2005;**192**:1149–53.
164. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. *J Infect Dis* 2010;**201**:1890–8.
166. Williams JV, Martino R, Rabella N, et al. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. *J Infect Dis* 2005;**192**:1061–5.
167. Williams JV, Tollefson SJ, Johnson JE, et al. The cotton rat *(Sigmodon hispidus)* is a permissive small animal model of human metapneumovirus infection, pathogenesis, and protective immunity. *J Virol* 2005;**79**:10944–51.
171. Wolf DG, Greenberg D, Shemer-Avni Y, et al. Association of human metapneumovirus with radiologically diagnosed community-acquired alveolar pneumonia in young children. *J Pediatr* 2010;**156**:115–20.
172. Wolf DG, Zakay-Rones Z, Fadeela A, et al. High seroprevalence of human metapneumovirus among young children in Israel. *J Infect Dis* 2003;**188**:1865–7.
174. Wyde PR, Chetty SN, Jewell AM, et al. Development of a cotton rat-human metapneumovirus (hMPV) model for identifying and evaluating potential hMPV antivirals and vaccines. *Antiviral Res* 2005;**66**:57–66.
175. Wyde PR, Moylett EH, Chetty SN, et al. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by NMSO3 in tissue culture assays. *Antiviral Res* 2004;**63**:51–9.
176. Yang CF, Wang CK, Tollefson SJ, et al. Genetic diversity and evolution of human metapneumovirus fusion protein over twenty years. *Virol J* 2009;**6**:138.
177. Zhang SX, Tellier R, Zafar R, et al. Comparison of human metapneumovirus infection with respiratory syncytial virus infection in children. *Pediatr Infect Dis J* 2009;**28**:1022–4.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aberle SW, Aberle JH, Sandhofer MJ, et al. Biennial spring activity of human metapneumovirus in Austria. *Pediatr Infect Dis J* 2008;**27**:1065–8.
2. Agapov E, Sumino KC, Gaudreault-Keener M, et al. Genetic variability of human metapneumovirus infection: evidence of a shift in viral genotype without a change in illness. *J Infect Dis* 2006;**193**:396–403.
3. Al-Sonboli N, Hart CA, Al-Aeryani A, et al. Respiratory syncytial virus and human metapneumovirus in children with acute respiratory infections in Yemen. *Pediatr Infect Dis J* 2005;**24**:734–6.
4. Ali SA, Williams JV, Chen Q, et al. Human metapneumovirus in hospitalized children in Amman, Jordan. *J Med Virol* 2010;**82**: 1012–16.
5. Ampofo K, Bender J, Sheng X, et al. Seasonal invasive pneumococcal disease in children: role of preceding respiratory viral infection. *Pediatrics* 2008;**122**:229–37.
6. Arnold JC, Singh KK, Milder E, et al. Human metapneumovirus associated with central nervous system infection in children. *Pediatr Infect Dis J* 2009;**28**:1057–60.
7. Baer G, Schaad UB, Heininger U. Clinical findings and unusual epidemiologic characteristics of human metapneumovirus infections in children in the region of Basel, Switzerland. *Eur J Pediatr* 2008;**167**:63–9.
8. Banerjee S, Sullender WM, Choudekar A, et al. Detection and genetic diversity of human metapneumovirus in hospitalized children with acute respiratory infections in India. *J Med Virol* 2011;**83**:1799–810.
9. Bao X, Liu T, Shan Y, et al. Human metapneumovirus glycoprotein G inhibits innate immune responses. *PLoS Pathog* 2008;**4**: e1000077.
10. Bastien N, Normand S, Taylor T, et al. Sequence analysis of the N, P, M and F genes of Canadian human metapneumovirus strains. *Virus Res* 2003;**93**:51–62.
11. Biacchesi S, Pham QN, Skiadopoulos MH, et al. Infection of nonhuman primates with recombinant human metapneumovirus lacking the SH, G, or M2-2 protein categorizes each as a nonessential accessory protein and identifies vaccine candidates. *J Virol* 2005;**79**:12608–13.
12. Biacchesi S, Skiadopoulos MH, Boivin G, et al. Genetic diversity between human metapneumovirus subgroups. *Virology* 2003;**315**: 1–9.
13. Biacchesi S, Skiadopoulos MH, Tran KC, et al. Recovery of human metapneumovirus from cDNA: optimization of growth in vitro and expression of additional genes. *Virology* 2004;**321**: 247–59.
14. Biacchesi S, Skiadopoulos MH, Yang L, et al. Recombinant human metapneumovirus lacking the small hydrophobic SH and/or attachment G glycoprotein: deletion of G yields a promising vaccine candidate. *J Virol* 2004;**78**:12877–87.
15. Boivin G, Abed Y, Pelletier G, et al. Virological features and clinical manifestations associated with human metapneumovirus: a new paramyxovirus responsible for acute respiratory-tract infections in all age groups. *J Infect Dis* 2002;**186**:1330–4.
16. Boivin G, De Serres G, Cote S, et al. Human metapneumovirus infections in hospitalized children. *Emerg Infect Dis* 2003;**9**: 634–40.
17. Boivin G, De Serres G, Hamelin ME, et al. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility. *Clin Infect Dis* 2007;**44**:1152–8.
18. Boivin G, Mackay I, Sloots TP, et al. Global genetic diversity of human metapneumovirus fusion gene. *Emerg Infect Dis* 2004;**10**: 1154–7.
19. Bonney D, Razali H, Turner A, et al. Successful treatment of human metapneumovirus pneumonia using combination therapy with intravenous ribavirin and immune globulin. *Br J Haematol* 2009;**145**:667–9.
20. Bosis S, Esposito S, Niesters HG, et al. Impact of human metapneumovirus in childhood: comparison with respiratory syncytial virus and influenza viruses. *J Med Virol* 2005;**75**:101–4.
21. Broughton S, Sylvester KP, Fox G, et al. Lung function in prematurely born infants after viral lower respiratory tract infections. *Pediatr Infect Dis J* 2007;**26**:1019–24.
22. Buchholz UJ, Biacchesi S, Pham QN, et al. Deletion of M2 gene open reading frames 1 and 2 of human metapneumovirus: effects on RNA synthesis, attenuation, and immunogenicity. *J Virol* 2005;**79**:6588–97.
23. Chan PK, Tam JS, Lam CW, et al. Human metapneumovirus detection in patients with severe acute respiratory syndrome. *Emerg Infect Dis* 2003;**9**:1058–63.
24. Chang A, Masante C, Buchholz UJ, et al. Human metapneumovirus (HMPV) binding and infection are mediated by interactions between the HMPV fusion protein and heparan sulfate. *J Virol* 2012;**86**:3230–43.
25. Chen X, Zhang ZY, Zhao Y, et al. Acute lower respiratory tract infections by human metapneumovirus in children in Southwest China: a 2-year study. *Pediatr Pulmonol* 2010;**45**:824–31.
26. Choi EH, Lee HJ, Kim SJ, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. *Clin Infect Dis* 2006;**43**:585–92.
27. Cote S, Abed Y, Boivin G. Comparative evaluation of real-time PCR assays for detection of the human metapneumovirus. *J Clin Microbiol* 2003;**41**:3631–5.
28. Cseke G, Maginnis MS, Cox RG, et al. Integrin alphavbeta1 promotes infection by human metapneumovirus. *Proc Natl Acad Sci U S A* 2009;**106**:1566–71.
29. Cseke G, Wright DW, Tollefson SJ, et al. Human metapneumovirus fusion protein vaccines that are immunogenic and protective in cotton rats. *J Virol* 2007;**81**:698–707.
30. Darniot M, Schildgen V, Schildgen O, et al. RNA interference in vitro and in vivo using DsiRNA targeting the nucleocapsid N mRNA of human metapneumovirus. *Antiviral Res* 2012;**93**: 364–73.
31. de Graaf M, Osterhaus AD, Fouchier RA, et al. Evolutionary dynamics of human and avian metapneumoviruses. *J Gen Virol* 2008;**89**:2933–42.
32. Debur MC, Vidal LR, Stroparo E, et al. Impact of human metapneumovirus infection on in and outpatients for the years 2006-2008 in Southern Brazil. *Mem Inst Oswaldo Cruz* 2010;**105**: 1010–18.
33. Deffrasnes C, Cavanagh MH, Goyette N, et al. Inhibition of human metapneumovirus replication by small interfering RNA. *Antiviral Ther* 2008;**13**:821–32.
34. Dinwiddie DL, Harrod KS. Human metapneumovirus inhibits IFN-alpha signaling through inhibition of STAT1 phosphorylation. *Am J Respir Cell Mol Biol* 2008;**38**:661–70.
35. Dollner H, Risnes K, Radtke A, et al. Outbreak of human metapneumovirus infection in norwegian children. *Pediatr Infect Dis J* 2004;**23**:436–40.
36. Donoso AF, Leon JA, Camacho JF, et al. Fatal hemorrhagic pneumonia caused by human metapneumovirus in an immunocompetent child. *Pediatr Int* 2008;**50**:589–91.
37. Douville RN, Bastien N, Li Y, et al. Human metapneumovirus elicits weak IFN-gamma memory responses compared with respiratory syncytial virus. *J Immunol* 2006;**176**:5848–55.
38. Ebihara T, Endo R, Kikuta H, et al. Seroprevalence of human metapneumovirus in Japan. *J Med Virol* 2003;**70**:281–3.
39. Ebihara T, Endo R, Kikuta H, et al. Human metapneumovirus infection in Japanese children. *J Clin Microbiol* 2004;**42**:126–32.
40. Ebihara T, Endo R, Ma X, et al. Detection of human metapneumovirus antigens in nasopharyngeal secretions by an immunofluorescent-antibody test. *J Clin Microbiol* 2005;**43**: 1138–41.
41. El Sayed Zaki M, Raafat D, El-Metaal AA, et al. Study of human metapneumovirus-associated lower respiratory tract infections in Egyptian adults. *Microbiol Immunol* 2009;**53**:603–8.
42. Englund JA, Boeckh M, Kuypers J, et al. Brief communication: fatal human metapneumovirus infection in stem-cell transplant recipients. *Ann Intern Med* 2006;**144**:344–9.
43. Erickson JJ, Gilchuk P, Hastings AK, et al. Viral acute lower respiratory infections impair CD8+ T cells through PD-1. *J Clin Invest* 2012;**122**:2967–82.
44. Esper F, Boucher D, Weibel C, et al. Human metapneumovirus infection in the United States: clinical manifestations associated with a newly emerging respiratory infection in children. *Pediatrics* 2003;**111**:1407–10.
45. Esper F, Martinello RA, Boucher D, et al. A 1-year experience with human metapneumovirus in children aged <5 years. *J Infect Dis* 2004;**189**:1388–96.
46. Falsey AR, Criddle MC, Walsh EE. Detection of respiratory syncytial virus and human metapneumovirus by reverse transcription

polymerase chain reaction in adults with and without respiratory illness. *J Clin Virol* 2006;**35**:46–50.
47. Falsey AR, Erdman D, Anderson LJ, et al. Human metapneumovirus infections in young and elderly adults. *J Infect Dis* 2003;**187**: 785–90.
48. Falsey AR, Hennessey PA, Formica MA, et al. Humoral immunity to human metapneumovirus infection in adults. *Vaccine* 2010;**28**: 1477–80.
49. Galiano M, Videla C, Puch SS, et al. Evidence of human metapneumovirus in children in Argentina. *J Med Virol* 2004;**72**: 299–303.
50. Garcia-Garcia ML, Calvo C, Casas I, et al. Human metapneumovirus bronchiolitis in infancy is an important risk factor for asthma at age 5. *Pediatr Pulmonol* 2007;**42**:458–64.
51. Gerna G, Campanini G, Rovida F, et al. Changing circulation rate of human metapneumovirus strains and types among hospitalized pediatric patients during three consecutive winter-spring seasons: brief report. *Arch Virol* 2005;**150**:2365–75.
52. Glaser CA, Honarmand S, Anderson LJ, et al. Beyond viruses: clinical profiles and etiologies associated with encephalitis. *Clin Infect Dis* 2006;**43**:1565–77.
53. Guerrero-Plata A, Casola A, Garofalo RP. Human metapneumovirus induces a profile of lung cytokines distinct from that of respiratory syncytial virus. *J Virol* 2005;**79**:14992–7.
54. Hamelin ME, Cote S, Laforge J, et al. Human metapneumovirus infection in adults with community-acquired pneumonia and exacerbation of chronic obstructive pulmonary disease. *Clin Infect Dis* 2005;**41**:498–502.
55. Hamelin ME, Gagnon C, Prince GA, et al. Prophylactic and therapeutic benefits of a monoclonal antibody against the fusion protein of human metapneumovirus in a mouse model. *Antiviral Res* 2010;**88**:31–7.
56. Hamelin ME, Prince GA, Boivin G. Effect of ribavirin and glucocorticoid treatment in a mouse model of human metapneumovirus infection. *Antimicrob Agents Chemother* 2006;**50**:774–7.
57. Hata M, Ito M, Kiyosawa S, et al. A fatal case of encephalopathy possibly associated with human metapneumovirus infection. *Jpn J Infect Dis* 2007;**60**:328–9.
58. Heininger U, Kruker AT, Bonhoeffer J, et al. Human metapneumovirus infections—biannual epidemics and clinical findings in children in the region of Basel, Switzerland. *Eur J Pediatr* 2009; **168**:1455–60.
59. Herfst S, de Graaf M, Schickli JH, et al. Recovery of human metapneumovirus genetic lineages a and B from cloned cDNA. *J Virol* 2004;**78**:8264–70.
60. Herfst S, de Graaf M, Schrauwen EJ, et al. Immunization of Syrian golden hamsters with F subunit vaccine of human metapneumovirus induces protection against challenge with homologous or heterologous strains. *J Gen Virol* 2007;**88**:2702–9.
61. Herfst S, de Graaf M, Schrauwen EJ, et al. Generation of temperature-sensitive human metapneumovirus strains that provide protective immunity in hamsters. *J Gen Virol* 2008;**89**: 1553–62.
62. Herfst S, Schrauwen EJ, de Graaf M, et al. Immunogenicity and efficacy of two candidate human metapneumovirus vaccines in cynomolgus macaques. *Vaccine* 2008;**26**:4224–30.
63. Huang CG, Tsao KC, Lin TY, et al. Estimates of individuals exposed to human metapneumovirus in a community-based Taiwanese population in 1999. *Arch Virol* 2010;**155**:343–50.
64. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004;**10**:1095–101.
65. Jartti T, van den Hoogen B, Garofalo RP, et al. Metapneumovirus and acute wheezing in children. *Lancet* 2002;**360**:1393–4.
66. Ji W, Wang Y, Chen Z, et al. Human metapneumovirus in children with acute respiratory tract infections in Suzhou, China 2005-2006. *Scand J Infect Dis* 2009;**41**:735–44.
67. Johnstone J, Majumdar SR, Fox JD, et al. Human metapneumovirus pneumonia in adults: results of a prospective study. *Clin Infect Dis* 2008;**46**:571–4.
68. Kaida A, Iritani N, Kubo H, et al. Seasonal distribution and phylogenetic analysis of human metapneumovirus among children in Osaka City, Japan. *J Clin Virol* 2006;**35**:394–9.
69. Kaida A, Kubo H, Goto K, et al. Co-infection of human metapneumovirus with adenovirus or respiratory syncytial virus among children in Japan. *Microbiol Immunol* 2007;**51**:679–83.
70. Kamble RT, Bollard C, Demmler G, et al. Human metapneumovirus infection in a hematopoietic transplant recipient. *Bone Marrow Transplant* 2007;**40**:699–700.
71. Kaur T, Singh J, Tong S, et al. Descriptive epidemiology of fatal respiratory outbreaks and detection of a human-related metapneumovirus in wild chimpanzees *(Pan troglodytes)* at Mahale Mountains National Park, Western Tanzania. *Am J Primatol* 2008;**70**: 755–65.
72. Khetsuriani N, Kazerouni NN, Erdman DD, et al. Prevalence of viral respiratory tract infections in children with asthma. *J Allergy Clin Immunol* 2007;**119**:314–21.
73. Kim HR, Cho AR, Lee MK, et al. Genotype variability and clinical features of human metapneumovirus isolated from Korean children, 2007 to 2010. *J Mol Diagn* 2012;**14**:61–4.
74. Kim S, Sung H, Im HJ, et al. Molecular epidemiological investigation of a nosocomial outbreak of human metapneumovirus infection in a pediatric hemato-oncology patient population. *J Clin Microbiol* 2009;**47**:1221–4.
75. Kim YK, Lee HJ. Human metapneumovirus–associated lower respiratory tract infections in korean infants and young children. *Pediatr Infect Dis J* 2005;**24**:1111–12.
76. Klein MI, Coviello S, Bauer G, et al. The impact of infection with human metapneumovirus and other respiratory viruses in young infants and children at high risk for severe pulmonary disease. *J Infect Dis* 2006;**193**:1544–51.
77. Klemenc J, Asad Ali S, Johnson M, et al. Real-time reverse transcriptase PCR assay for improved detection of human metapneumovirus. *J Clin Virol* 2012;**54**:371–5.
78. Kolli D, Bataki EL, Spetch L, et al. T lymphocytes contribute to antiviral immunity and pathogenesis in experimental human metapneumovirus infection. *J Virol* 2008;**82**:8560–9.
79. Kondgen S, Kuhl H, N'Goran PK, et al. Pandemic human viruses cause decline of endangered great apes. *Curr Biol* 2008;**18**: 260–4.
80. Kuiken T, van den Hoogen BG, van Riel DA, et al. Experimental human metapneumovirus infection of cynomolgus macaques *(Macaca fascicularis)* results in virus replication in ciliated epithelial cells and pneumocytes with associated lesions throughout the respiratory tract. *Am J Pathol* 2004;**164**:1893–900.
81. Kukavica-Ibrulj I, Hamelin ME, Prince GA, et al. Infection with human metapneumovirus predisposes mice to severe pneumococcal pneumonia. *J Virol* 2009;**83**:1341–9.
82. Kunz AN, Englund JA, Kuypers J, et al. Detection of multiple respiratory viruses by real-time polymerase chain reaction in infants attending an outpatient clinic. *Eur J Clin Microbiol Infect Dis* 2008;**27**:1245–8.
83. Laham FR, Israele V, Casellas JM, et al. Differential production of inflammatory cytokines in primary infection with human metapneumovirus and with other common respiratory viruses of infancy. *J Infect Dis* 2004;**189**:2047–56.
84. Lambert SB, Allen KM, Druce JD, et al. Community epidemiology of human metapneumovirus, human coronavirus NL63, and other respiratory viruses in healthy preschool-aged children using parent-collected specimens. *Pediatrics* 2007;**120**:e929–37.
85. Landry ML, Ferguson D, Cohen S, et al. Detection of human metapneumovirus in clinical samples by immunofluorescence staining of shell vial centrifugation cultures prepared from three different cell lines. *J Clin Microbiol* 2005;**43**:1950–2.
86. Lazar I, Weibel C, Dziura J, et al. Human metapneumovirus and severity of respiratory syncytial virus disease. *Emerg Infect Dis* 2004;**10**:1318–20.
87. Lee N, Chan PK, Yu IT, et al. Co-circulation of human metapneumovirus and SARS-associated coronavirus during a major nosocomial SARS outbreak in Hong Kong. *J Clin Virol* 2007;**40**: 333–7.
88. Leung J, Esper F, Weibel C, et al. Seroepidemiology of human metapneumovirus (hMPV) on the basis of a novel enzyme-linked immunosorbent assay utilizing hMPV fusion protein expressed in recombinant vesicular stomatitis virus. *J Clin Microbiol* 2005;**43**: 1213–19.
89. Liao RS, Appelgate DM, Pelz RK. An outbreak of severe respiratory tract infection due to human metapneumovirus in a long-term care facility for the elderly in Oregon. *J Clin Virol* 2012;**53**: 171–3.
90. Liu L, Bastien N, Sidaway F, et al. Seroprevalence of human metapneumovirus (hMPV) in the Canadian province of

Saskatchewan analyzed by a recombinant nucleocapsid protein-based enzyme-linked immunosorbent assay. *J Med Virol* 2007;**79**:308–13.
91. Ljubin Sternak S, Vilibic Cavlek T, Falsey AR, et al. Serosurvey of human metapneumovirus infection in Croatia. *Croat Med J* 2006;**47**:878–81.
92. Lu G, Gonzalez R, Guo L, et al. Large-scale seroprevalence analysis of human metapneumovirus and human respiratory syncytial virus infections in Beijing, China. *Virology journal* 2011;**8**:62.
93. Lusebrink J, Wiese C, Thiel A, et al. High seroprevalence of neutralizing capacity against human metapneumovirus in all age groups studied in Bonn, Germany. *Clin Vaccine Immunol* 2010;**17**:481–4.
94. MacPhail M, Schickli JH, Tang RS, et al. Identification of small-animal and primate models for evaluation of vaccine candidates for human metapneumovirus (hMPV) and implications for hMPV vaccine design. *J Gen Virol* 2004;**85**:1655–63.
95. Madhi SA, Ludewick H, Kuwanda L, et al. Pneumococcal coinfection with human metapneumovirus. *J Infect Dis* 2006;**193**:1236–43.
96. Madhi SA, Ludewick H, Kuwanda L, et al. Seasonality, incidence, and repeat human metapneumovirus lower respiratory tract infections in an area with a high prevalence of human immunodeficiency virus type-1 infection. *Pediatr Infect Dis J* 2007;**26**:693–9.
97. Maertzdorf J, Wang CK, Brown JB, et al. Real-time reverse transcriptase PCR assay for detection of human metapneumoviruses from all known genetic lineages. *J Clin Microbiol* 2004;**42**:981–6.
98. Manoha C, Espinosa S, Aho SL, et al. Epidemiological and clinical features of hMPV, RSV and RVs infections in young children. *J Clin Virol* 2007;**38**:221–6.
99. Martinello RA, Esper F, Weibel C, et al. Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease. *J Infect* 2006;**53**:248–54.
100. Matsuzaki Y, Itagaki T, Abiko C, et al. Clinical impact of human metapneumovirus genotypes and genotype-specific seroprevalence in Yamagata, Japan. *J Med Virol* 2008;**80**:1084–9.
101. Mendes G, Soares AR, Sigiliano L, et al. In vitro anti-HMPV activity of meroditerpenoids from marine alga *Stypopodium zonale (Dictyotales)*. *Molecules* 2011;**16**:8437–50.
102. Mirazo S, Ruchansky D, Blanc A, et al. Serologic evidence of human metapneumovirus circulation in Uruguay. *Mem Inst Oswaldo Cruz* 2005;**100**:715–18.
103. Mok H, Tollefson SJ, Podsiad AB, et al. An alphavirus replicon-based human metapneumovirus vaccine is immunogenic and protective in mice and cotton rats. *J Virol* 2008;**82**:11410–18.
104. Morrow BM, Hatherill M, Smuts HE, et al. Clinical course of hospitalised children infected with human metapneumovirus and respiratory syncytial virus. *J Paediatr Child Health* 2006;**42**:174–8.
105. Muller A, Tillmann RL, Simon A, et al. Stability of human metapneumovirus and human coronavirus NL63 on medical instruments and in the patient environment. *J Hosp Infect* 2008;**69**:406–8.
106. Mullins JA, Erdman DD, Weinberg GA, et al. Human metapneumovirus infection among children hospitalized with acute respiratory illness. *Emerg Infect Dis* 2004;**10**:700–5.
107. Nokso-Koivisto J, Pyles RB, Miller AL, et al. Viral load and acute otitis media development after human metapneumovirus upper respiratory tract infection. *Pediatr Infect Dis J* 2012;**31**:763–6.
108. Okamoto M, Sugawara K, Takashita E, et al. Longitudinal course of human metapneumovirus antibody titers and reinfection in healthy adults. *J Med Virol* 2010;**82**:2092–6.
109. Oliveira DB, Durigon EL, Carvalho AC, et al. Epidemiology and genetic variability of human metapneumovirus during a 4-year-long study in Southeastern Brazil. *J Med Virol* 2009;**81**:915–21.
110. Osterhaus A, Fouchier R. Human metapneumovirus in the community. *Lancet* 2003;**361**:890–1.
111. Palacios G, Lowenstine LJ, Cranfield MR, et al. Human metapneumovirus infection in wild mountain gorillas, Rwanda. *Emerg Infect Dis* 2011;**17**:711–13.
112. Papenburg J, Hamelin ME, Ouhoummane N, et al. Comparison of risk factors for human metapneumovirus and respiratory syncytial virus disease severity in young children. *J Infect Dis* 2012;**206**:178–89.
113. Pavlin JA, Hickey AC, Ulbrandt N, et al. Human metapneumovirus reinfection among children in Thailand determined by ELISA using purified soluble fusion protein. *J Infect Dis* 2008;**198**:836–42.
114. Peiris JS, Tang WH, Chan KH, et al. Children with respiratory disease associated with metapneumovirus in Hong Kong. *Emerg Infect Dis* 2003;**9**:628–33.
115. Pelletier G, Dery P, Abed Y, et al. Respiratory tract reinfections by the new human metapneumovirus in an immunocompromised child. *Emerg Infect Dis* 2002;**8**:976–8.
116. Percivalle E, Sarasini A, Visai L, et al. Rapid detection of human metapneumovirus strains in nasopharyngeal aspirates and shell vial cultures by monoclonal antibodies. *J Clin Microbiol* 2005;**43**:3443–6.
117. Peret TC, Boivin G, Li Y, et al. Characterization of human metapneumoviruses isolated from patients in North America. *J Infect Dis* 2002;**185**:1660–3.
118. Pitoiset C, Darniot M, Huet F, et al. Human metapneumovirus genotypes and severity of disease in young children (n = 100) during a 7-year study in Dijon hospital, France. *J Med Virol* 2010;**82**:1782–9.
119. Piyaratna R, Tollefson SJ, Williams JV. Genomic analysis of four human metapneumovirus prototypes. *Virus Res* 2011;**160**:200–5.
120. Preston FM, Straub CP, Ramirez R, et al. siRNA against the G gene of human metapneumovirus. *Virol J* 2012;**9**:105.
121. Rawlinson WD, Waliuzzaman Z, Carter IW, et al. Asthma exacerbations in children associated with rhinovirus but not human metapneumovirus infection. *J Infect Dis* 2003;**187**:1314–18.
122. Regev L, Hindiyeh M, Shulman LM, et al. Characterization of human metapneumovirus infections in Israel. *J Clin Microbiol* 2006;**44**:1484–9.
123. Reina J, Ferres F, Alcoceba E, et al. Comparison of different cell lines and incubation times in the isolation by the shell vial culture of human metapneumovirus from pediatric respiratory samples. *J Clin Virol* 2007;**40**:46–9.
124. Rohde G, Borg I, Arinir U, et al. Relevance of human metapneumovirus in exacerbations of COPD. *Respir Res* 2005;**6**:150.
125. Ryder AB, Tollefson SJ, Podsiad AB, et al. Soluble recombinant human metapneumovirus G protein is immunogenic but not protective. *Vaccine* 2010;**28**:4145–52.
126. Sanchez Fernandez I, Rebollo Polo M, Munoz-Almagro C, et al. Human metapneumovirus in the cerebrospinal fluid of a patient with acute encephalitis. *Arch Neurol* 2012;**69**:649–52.
127. Schickli JH, Kaur J, Macphail M, et al. Deletion of human metapneumovirus M2-2 increases mutation frequency and attenuates growth in hamsters. *Virol J* 2008;**5**:69.
128. Schickli JH, Kaur J, Ulbrandt N, et al. An S101P substitution in the putative cleavage motif of the human metapneumovirus fusion protein is a major determinant for trypsin-independent growth in vero cells and does not alter tissue tropism in hamsters. *J Virol* 2005;**79**:10678–89.
129. Schildgen O, Geikowski T, Glatzel T, et al. Frequency of human metapneumovirus in the upper respiratory tract of children with symptoms of an acute otitis media. *Eur J Pediatr* 2005;**164**:400–1.
130. Schildgen O, Glatzel T, Geikowski T, et al. Human metapneumovirus RNA in encephalitis patient. *Emerg Infect Dis* 2005;**11**:467–70.
131. Schlapbach LJ, Agyeman P, Hutter D, et al. Human metapneumovirus infection as an emerging pathogen causing acute respiratory distress syndrome. *J Infect Dis* 2011;**203**:294–5; author reply 296.
132. Semple MG, Cowell A, Dove W, et al. Dual infection of infants by human metapneumovirus and human respiratory syncytial virus is strongly associated with severe bronchiolitis. *J Infect Dis* 2005;**191**:382–6.
133. Shachor-Meyouhas Y, Ben-Barak A, Kassis I. Treatment with oral ribavirin and IVIG of severe human metapneumovirus pneumonia (HMPV) in immune compromised child. *Pediatr Blood Cancer* 2011;**57**:350–1.
134. Shahda S, Carlos WG, Kiel PJ, et al. The human metapneumovirus: a case series and review of the literature. *Transpl Infect Dis* 2011;**13**:324–8.
135. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol* 2010;**82**:1282–90.

136. Sivaprakasam V, Collins TC, Aitken C, et al. Life-threatening human metapneumovirus infections in West of Scotland. *J Clin Virol* 2007;**39**:234–7.
137. Skiadopoulos MH, Biacchesi S, Buchholz UJ, et al. The two major human metapneumovirus genetic lineages are highly related antigenically, and the fusion (F) protein is a major contributor to this antigenic relatedness. *J Virol* 2004;**78**:6927–37.
138. Skiadopoulos MH, Biacchesi S, Buchholz UJ, et al. Individual contributions of the human metapneumovirus F, G, and SH surface glycoproteins to the induction of neutralizing antibodies and protective immunity. *Virology* 2006;**345**:492–501.
139. Sloots TP, Mackay IM, Bialasiewicz S, et al. Human metapneumovirus, Australia, 2001-2004. *Emerg Infect Dis* 2006;**12**:1263–6.
140. Spetch L, Bowlin TL, Casola A. Effect of NMSO3 treatment in a murine model of human metapneumovirus infection. *J Gen Virol* 2008;**89**:2709–12.
141. Sumino KC, Agapov E, Pierce RA, et al. Detection of severe human metapneumovirus infection by real-time polymerase chain reaction and histopathological assessment. *J Infect Dis* 2005;**192**:1052–60.
142. Suzuki A, Watanabe O, Okamoto M, et al. Detection of human metapneumovirus from children with acute otitis media. *Pediatr Infect Dis J* 2005;**24**:655–7.
143. Tang RS, Mahmood K, Macphail M, et al. A host-range restricted parainfluenza virus type 3 (PIV3) expressing the human metapneumovirus (hMPV) fusion protein elicits protective immunity in African green monkeys. *Vaccine* 2005;**23**:1657–67.
144. Tollefson SJ, Cox RG, Williams JV. Studies of culture conditions and environmental stability of human metapneumovirus. *Virus Res* 2010;**151**:54–9.
145. Tu CC, Chen LK, Lee YS, et al. An outbreak of human metapneumovirus infection in hospitalized psychiatric adult patients in Taiwan. *Scand J Infect Dis* 2009;**41**:363–7.
146. Ulbrandt ND, Ji H, Patel NK, et al. Isolation and characterization of monoclonal antibodies which neutralize human metapneumovirus in vitro and in vivo. *J Virol* 2006;**80**:7799–806.
147. Ulloa-Gutierrez R, Skippen P, Synnes A, et al. Life-threatening human metapneumovirus pneumonia requiring extracorporeal membrane oxygenation in a preterm infant. *Pediatrics* 2004;**114**: e517–19.
148. van den Hoogen BG, Bestebroer TM, Osterhaus AD, et al. Analysis of the genomic sequence of a human metapneumovirus. *Virology* 2002;**295**:119–32.
149. van den Hoogen BG, de Jong JC, Groen J, et al. A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat Med* 2001;**7**:719–24.
150. van den Hoogen BG, Herfst S, de Graaf M, et al. Experimental infection of macaques with human metapneumovirus induces transient protective immunity. *J Gen Virol* 2007;**88**:1251–9.
151. van den Hoogen BG, Herfst S, Sprong L, et al. Antigenic and genetic variability of human metapneumoviruses. *Emerg Infect Dis* 2004;**10**:658–66.
152. van den Hoogen BG, van Doornum GJ, Fockens JC, et al. Prevalence and clinical symptoms of human metapneumovirus infection in hospitalized patients. *J Infect Dis* 2003;**188**:1571–7.
153. van Woensel JB, Bos AP, Lutter R, et al. Absence of human metapneumovirus co-infection in cases of severe respiratory syncytial virus infection. *Pediatr Pulmonol* 2006;**41**:872–4.
154. Vanspauwen MJ, van Mook WN, Bruggeman CA, et al. Human metapneumovirus in bronchoalveolar lavage fluid of critically ill patients with suspected pneumonia. *Intensive Care Med* 2012;**38**: 728–9.
155. Vargas SO, Kozakewich HP, Perez-Atayde AR, et al. Pathology of human metapneumovirus infection: insights into the pathogenesis of a newly identified respiratory virus. *Pediatr Dev Pathol* 2004;**7**:478–86; discussion 421.
156. Varman M, Truemper EJ, Chatterjee A. Severe human metapneumovirus pneumonia in a child with chronic illness. *Clin Pediatr (Phila)* 2010;**49**:1075–7.
157. Verkaik NJ, Nguyen DT, de Vogel CP, et al. *Streptococcus pneumoniae* exposure is associated with human metapneumovirus seroconversion and increased susceptibility to in vitro HMPV infection. *Clin Microbiol Infect* 2011;**17**:1840–4.
158. Vicente D, Montes M, Cilla G, et al. Human metapneumovirus and chronic obstructive pulmonary disease. *Emerg Infect Dis* 2004; **10**:1338–9.
159. Vicente D, Montes M, Cilla G, et al. Differences in clinical severity between genotype A and genotype B human metapneumovirus infection in children. *Clin Infect Dis* 2006;**42**:e111–13.
160. Walsh EE, Peterson DR, Falsey AR. Human metapneumovirus infections in adults: another piece of the puzzle. *Arch Intern Med* 2008;**168**:2489–96.
161. Widmer K, Zhu Y, Williams JV, et al. Rates of hospitalizations for respiratory syncytial virus, human metapneumovirus, and influenza virus in older adults. *J Infect Dis* 2012;**206**:56–62.
162. Williams JV, Chen Z, Cseke G, et al. A recombinant human monoclonal antibody to human metapneumovirus fusion protein that neutralizes virus in vitro and is effective therapeutically in vivo. *J Virol* 2007;**81**:8315–24.
163. Williams JV, Crowe JE Jr, Enriquez R, et al. Human metapneumovirus infection plays an etiologic role in acute asthma exacerbations requiring hospitalization in adults. *J Infect Dis* 2005;**192**: 1149–53.
164. Williams JV, Edwards KM, Weinberg GA, et al. Population-based incidence of human metapneumovirus infection among hospitalized children. *J Infect Dis* 2010;**201**:1890–8.
165. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004;**350**:443–50.
166. Williams JV, Martino R, Rabella N, et al. A prospective study comparing human metapneumovirus with other respiratory viruses in adults with hematologic malignancies and respiratory tract infections. *J Infect Dis* 2005;**192**:1061–5.
167. Williams JV, Tollefson SJ, Johnson JE, et al. The cotton rat *(Sigmodon hispidus)* is a permissive small animal model of human metapneumovirus infection, pathogenesis, and protective immunity. *J Virol* 2005;**79**:10944–51.
168. Williams JV, Tollefson SJ, Nair S, et al. Association of human metapneumovirus with acute otitis media. *Int J Pediatr Otorhinolaryngol* 2006;**70**:1189–93.
169. Williams JV, Wang CK, Yang CF, et al. The role of human metapneumovirus in upper respiratory tract infections in children: a 20-year experience. *J Infect Dis* 2006;**193**:387–95.
170. Wolf DG, Greenberg D, Kalkstein D, et al. Comparison of human metapneumovirus, respiratory syncytial virus and influenza A virus lower respiratory tract infections in hospitalized young children. *Pediatr Infect Dis J* 2006;**25**:320–4.
171. Wolf DG, Greenberg D, Shemer-Avni Y, et al. Association of human metapneumovirus with radiologically diagnosed community-acquired alveolar pneumonia in young children. *J Pediatr* 2010;**156**:115–20.
172. Wolf DG, Zakay-Rones Z, Fadeela A, et al. High seroprevalence of human metapneumovirus among young children in Israel. *J Infect Dis* 2003;**188**:1865–7.
173. Wyde PR, Chetty SN, Jewell AM, et al. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by ribavirin and immune serum globulin in vitro. *Antiviral Res* 2003;**60**:51–9.
174. Wyde PR, Chetty SN, Jewell AM, et al. Development of a cotton rat–human metapneumovirus (hMPV) model for identifying and evaluating potential hMPV antivirals and vaccines. *Antiviral Res* 2005;**66**:57–66.
175. Wyde PR, Moylett EH, Chetty SN, et al. Comparison of the inhibition of human metapneumovirus and respiratory syncytial virus by NMSO3 in tissue culture assays. *Antiviral Res* 2004;**63**: 51–9.
176. Yang CF, Wang CK, Tollefson SJ, et al. Genetic diversity and evolution of human metapneumovirus fusion protein over twenty years. *Virol J* 2009;**6**:138.
177. Zhang SX, Tellier R, Zafar R, et al. Comparison of human metapneumovirus infection with respiratory syncytial virus infection in children. *Pediatr Infect Dis J* 2009;**28**:1022–4.

SUBSECTION 8 *RHABDOVIRIDAE*

CHAPTER 183

Rabies Virus

Sergio E. Recuenco • Stanley A. Plotkin • Charles E. Rupprecht

Rabies is an acute, progressive viral infection of the central nervous system (CNS) transmitted from mammals to humans. Introduction of the agent by bite, scratch, or aerosol enables the virions to attach to and migrate via the peripheral nerves to the spinal cord and brain. The clinical presentation is characterized by typical nonspecific viral encephalitic signs, classic hydrophobia, and rapid progression to paralysis and coma. Rabies encephalitis is almost always fatal. To understand and implement the ultimate prevention and control of this zoonosis, a basic appreciation of the human–animal interface is needed within a One Health context.

RECENT HISTORY

Rabies is a disease with an ancient record throughout history, often mixed with horrific fact and frightful myth, and associated with all major civilizations. For a detailed historical account of rabies throughout the ages, we refer to a previous version of this chapter, and other relevant publications.[207,247,287] More recently, at the beginning of the 21st century, rabies remains a major public health problem throughout most of the world. Current estimations of the global burden of human rabies suggest that 50,000 to 100,000 victims may succumb each year, with most deaths in Asia and Africa.[146] Canine rabies is the source for the majority of fatalities in the developing world, but has been eliminated in all developed countries. For example, during 2007, the United States was declared free of canine rabies. By 2008, most western European countries were free of classic rabies (excluding the disease in bats). However, even with the control of canine rabies in some regions, importation of infected dogs from rabies-endemic countries was responsible for sporadic reintroduction, such as in France and Italy in recent years.[23,78] Whereas the Old World still has canine rabies as an important public health problem, within Latin America, significant progress was achieved, with a 95 percent decline in human rabies cases since 1983, when a large regional effort for animal rabies elimination was launched.[234]

Important wildlife rabies reservoirs, such as raccoons, skunks, foxes, and bats, remained as the main source of rabies in the United States at the end of the 20th century. Human and animal rabies prevention recommendations in the United States were adjusted to this reality, and a national oral rabies vaccination program targeted to wild carnivores was launched to contain the spread of rabies. Oral rabies vaccination programs for wildlife have increased in the last decade, using baits containing a vaccinia-rabies recombinant vaccine and more recently an adenovirus-rabies recombinant vaccine.[81] Although progress in the development of tools to control terrestrial rabies was notable in the beginning of the 21st century, little progress has occurred regarding rabies transmitted by bats. Bats are the next major challenge for rabies management, because of the extensive global biodiversity of species that are able to come in contact with humans and transmit rabies.[17,202]

Besides basic approaches focused on animals, development of human rabies biologics was also notable in the late 20th century. Both human rabies immunoglobulin and rabies cell culture vaccines, to replace older nervous tissue vaccines (NTVs), were made available.[28,304] Presently, the access to modern rabies biologics is limited for large human populations in many developing countries.[30] Therefore, NTVs are still in use in some countries in Latin America, Asia, and Africa. Social determinants underlying basic health disparities, such as poverty, limited education, and geographic isolation, became relevant factors for focus for more innovative strategies targeted to control and prevent rabies at the local community level.[233] Clearly, globalization in a modern world with intense movement of disenfranchised populations, illegal hunting and trade of animals, and overnight travel threatens the historical progress in rabies prevention, with a need for a global strategy as a response. In this regard, new stakeholders and partners, such as the Global Alliance for Rabies Control and the Partners for Rabies Prevention, were created in the 21st century. These global nongovernmental organizations coordinate efforts for advocacy, education, awareness, and joint human and animal campaigns for improved disease prevention and control in the world, teaming with leading rabies research groups, international philanthropic institutions, and international agencies, such as the United Nations Food and Agriculture Organization, World Organization for Animal Health, Pan American Health Organization, World Health Organization (WHO), and many others.[163]

To this effect, the first global rabies campaign was held in 2007, recognizing World Rabies Day, and September 28 was established officially as a date for focused rabies outreach. Currently, more than 150 countries participate annually in World Rabies Day activities. Another important underlying element for global rabies mitigation in the 21st century was the adoption of a One Health approach, fostering the collaborative work between animal health and human health sectors to accomplish control of zoonotic diseases. Such a basic strategy is a current mainframe for integrated public health actions, especially concentrated on diseases of nature.[137]

ETIOLOGY

All mammals are believed susceptible to infection with the viruses that cause rabies. Animal rabies reports include laboratory confirmation in most of the mammal species in contact with humans, with an obvious surveillance bias.[224] Laboratory propagation of virus may be accomplished readily in mice or other standard laboratory animals; in vitro in neuroblastoma or certain hamster cell cultures; or, after adaptation, in certain cell lines of human or other mammalian origin.[199]

Electron-microscopic studies reveal that the *Lyssavirus* virion is rod- or bullet-shaped and typically matures at cytoplasmic plasma membranes and intracellular membranes of the endoplasmic reticulum in infected cultured cells (Fig. 183-1).[122] Standard virions are approximately 75 nm in diameter and 160 to 180 nm in length. Regular arrays of standard-size virions maturing from plasma membranes are observed in infected salivary glands. Budding of virus from plasma membranes is much less pronounced in neurons of the CNS than in cell culture or salivary gland cells. However, meticulous electron-microscopic examination of experimentally and naturally infected tissues has revealed that budding from membranes of perikarya and dendrites, as well as the presence of virus in intracellular spaces of brain (especially at synaptic junctions), occurs regularly.[124] In addition, CNS neurons often exhibit both typical and bizarre morphologic forms of virus maturing in the cytoplasm. Cytoplasmic forms often develop in proximity to nucleocapsid matrix inclusions (Negri bodies).[176]

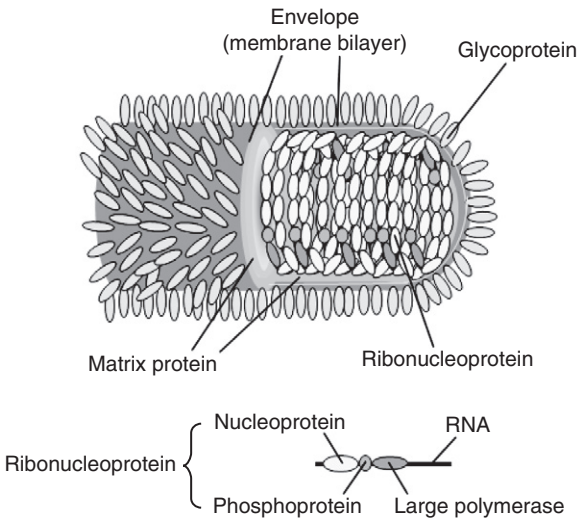

FIGURE 183-1 ■ Structural diagram of a *Lyssavirus*. (Courtesy Lilian Orciari, Centers for Disease Control and Prevention. From Orciari, LA, Rupprecht, CE. 2011. Rabies. In: Versalovic, J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press: 2011; p. 1470-1478.)

The gross morphologic characteristics and biochemical composition of the etiologic agents of rabies place them within the family Rhabdoviridae, genus *Lyssavirus*. Lyssaviruses share antigenic and genetic relationships and are not closely related to other rhabdoviruses on the basis of similarities of the internal proteins detected by fluorescent antibody and complement-fixation tests, reactions with monoclonal antibodies, and later sequencing studies.

The *Lyssavirus* genus contains at least 12 viruses recognized by the International Committee for the Taxonomy of Viruses (ICTV) and all-cause rabies. The 12 species include rabies virus as the type species, Aravan virus, Australian bat *Lyssavirus*, Duvenhage virus, European bat *Lyssavirus* (types 1 and 2), Irkut virus, Khujand virus, Lagos bat virus, Mokola virus, Shimoni virus, and West Caucasian bat virus.[133,152,153,172,224] Figure 183-2 shows the known *Lyssavirus* and the three phylogenetic groups in which they are classified.[17] The lyssaviruses are antigenically variable, with Lagos bat virus and Mokola being distant from rabies virus.[121] Additionally, recently discovered species, named Bokeloh bat virus and Ikoma virus, are pending ICTV recognition, and many more may be discovered in the near future.[38,173,174] Cases of human disease have been recorded at least from Duvenhage, European bat Lyssavirus, and Australian bat *Lyssavirus*.

All lyssaviruses have been associated or suspected with bat reservoirs, except Mokola virus.[229] The Duvenhage virus, Mokola virus, and Lagos bat virus were identified only in Africa; the West Caucasian bat virus was isolated in Eurasia only.[76,279] Many variants of the rabies virus, also known as *Lyssavirus* type 1, exist, and such variants are adaptations to diverse mammal reservoirs and may be specific to certain geographic regions. For example, rabies virus in bats is widely distributed in the New World, and viral variants specific to different bat species exist in North America, as well as to vampire bats in Latin America, and to insectivorous and frugivorous bats recognized in South America, such as in Chile and Brazil.[25,89,205,224,235]

The *Lyssavirus* virion is recognized to consist of a molecular composition somewhat similar to that of the other rhabdoviruses, such as vesicular stomatitis virus. The virion comprises a helical nucleocapsid core, which contains the single-stranded RNA genome coupled to a nucleoprotein, a phosphoprotein, and a large protein representing the RNA-dependent polymerase.[56] This core is surrounded by a lipoprotein membrane, which contains a matrix protein, an external glycoprotein embedded in the envelope, and several lipids (primarily phospholipids and cholesterol).[26,244] The genome is a single-stranded RNA molecule containing approximately 11,932 nucleotides with a molecular mass of approximately 4.0×10^6 Da.[22] The genome is negative-stranded; that is, it

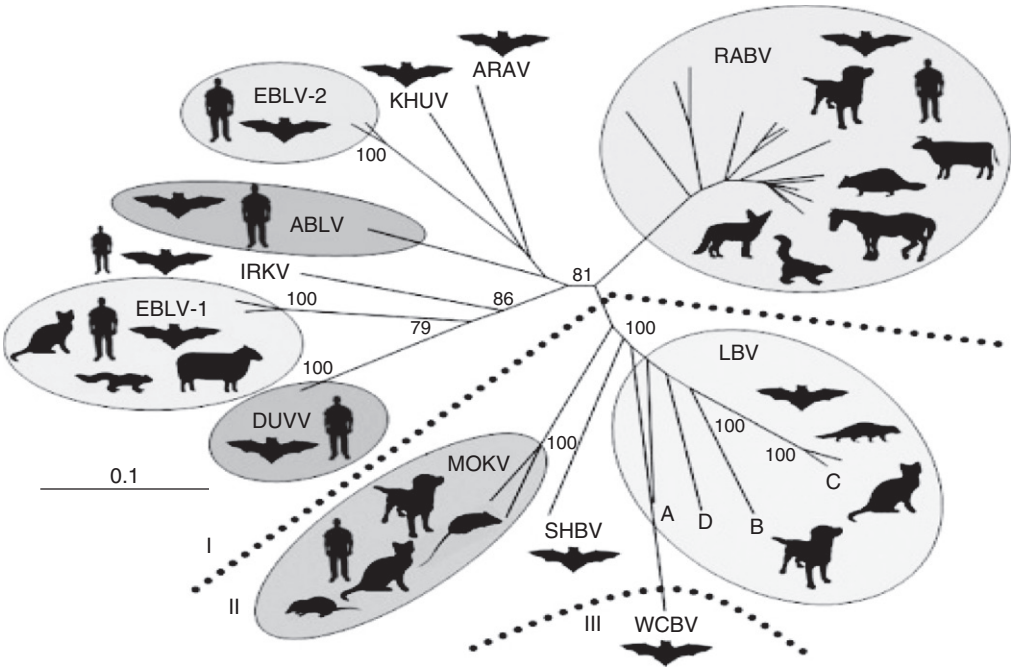

FIGURE 183-2 ■ Phylogenetic analysis of characterized *Lyssavirus* isolates based on 405 nucleotides of the nucleoprotein, grouped by phylogenetic groups I, II, and III and indicating main hosts and reservoirs identified. (From Banyard AC, Hayman D, Johnson N, et al. Bats and lyssaviruses. Adv Virus Res 2011;**79**:239-89. Available at: http://dx.doi.org/10.1016/B978-0-12-387040-7.00012-3.)

must be transcribed to produce messenger RNA necessary for replication. Defective virions containing characteristic (for a given strain) sizes of incomplete RNA molecules have been demonstrated in cell culture–propagated virus populations, but their occurrence or role in natural infections has not been determined.

Important surface antigenic properties are associated primarily with the glycoprotein,[244] a polypeptide containing three major oligosaccharide side chains.[65] The glycoprotein is the sole antigen responsible for induction of and reaction with virus-neutralizing antibodies. Antibodies to the nucleoprotein administered passively have no virus-neutralizing or protective capacity but are an essential antibody for the diagnostic detection of intracellular viral antigens in the CNS by immunofluorescent staining.[294] Active immunization with rabies nucleocapsid may contribute to immune protection, especially to heterotropic lyssaviruses, apparently by induction of a *Lyssavirus*-specific T-cell immune response, but further work is needed to appreciate the role of other viral antigens for prophylaxis.[46,71]

Historically, viral specificity can be recognized on the basis of genomic sequence analysis[263] and differentiation by monoclonal antibodies. By these means, animal-specific variants and geographic distribution of viruses can be discerned and isolates from human cases can be traced to the animal and place of origin.[242] Researchers have suggested that lyssaviruses, like several other RNA virus populations, may exist as a quasispecies. The presence of such viral subpopulations may permit rapid adaptation to new hosts.[144,188]

Antigenic differences between both strains of classic laboratory-fixed virus strains and different field isolates of diverse origin have been detected with the use of monoclonal antibodies[290] directed against viral antigens, including the glycoprotein and nucleoprotein.[70] The use of monoclonal antibodies allowed rapid distinction of rabies virus from the other rabies-related lyssaviruses.[222] Studies with monoclonal antibodies have revealed that, worldwide, clustering of certain rabies virus antigenic subtypes is geographic and may be species-specific.[210] Many genetic groups of rabies viruses with relatively specific geographic localizations exist.[31,242] Within those areas, the viruses may be transmitted by different species, such as dogs or foxes. In the United States, readily distinguishable subtypes of rabies virus have been determined to circulate in each of the major wild mammal reservoir species (primarily skunks and raccoons) and in bats.[25] In all well-studied rabies enzootic areas, researchers have determined that rabies circulates predominantly in a limited number of mammalian species characteristic of that area and a major antigenic phenotype is characteristic of that transmission cycle.[70] However, distinct bat-adapted rabies viruses may circulate independently in these areas or in areas where other animals (such as dogs) are free of rabies. Different geographic subtypes of rabies virus share varying proportions of the immunologically important glycoprotein epitopes with the vaccine strains of virus. Rabies viruses isolated from human cases of rabies in the United States have been shown to differ from vaccine strain virus in their antigenic composition.[291] Despite intense investigation, however, no evidence supports the contention that cross protection between the rabies virus vaccine and antigenically distinguishable wild-type viruses is reduced to a degree justifying inclusion of new virus strains in human vaccines. Physicians should have every reason to believe that present vaccines protect against all classic rabies virus and

other members of the phylogenetic group I, provided the vaccine is sufficiently potent. The same degree of cross reactivity does not exist for the lyssaviruses in the phylogenetic groups II and III. Recent studies using virtual tridimensional models demonstrated that antigenic distances among the known lyssaviruses are the base for antigenic variation and unequal response to rabies biologics.[121]

TRANSMISSION AND EPIDEMIOLOGY

The main mode of rabies transmission is the inoculation of the virus through an animal bite that opens skin, subcutaneous tissue, and muscle and exposes the agent to nervous system tissues. Mucosae from the eyes, nose, and mouth are also potential entry points for the virus, especially given the ease of abrasions and exposure of neural innervations in those areas. After replication occurs in the CNS, many other areas become infected, including the salivary glands. The saliva of a rabid animal contains virus capable of initiating productive infections. Other secretions from rabid animals, such as urine, feces, and milk, are considered low or no risk for viral transmission[41]; however, exposure to those secretions can potentially allow transmission of other diseases.[171] Consuming the meat of rabid animals is not recommended; however, in the developing world it is not a rare event that rabid animals, especially livestock, are killed and eaten, even with the onset neurologic signs. While eating cooked meat of a rabid animal is not considered a risk for viral transmission, the people handling the carcasses and in contact with the head, saliva, brains, nervous tissues, and other organs may be exposed.[273] Besides bite transmission, infection may occur rarely by the nonbite route, such as by scratches or inhalation of virus. Thus, aerosol transmission is controversial except under selected circumstances. Experimental studies with animals exposed to certain caves heavily infested with bats indicate that this form of transmission is possible, although such scenarios are unlikely unless humans may be exposed to such a condition without protective equipment. Concentrated virus aerosols produced in the laboratory setting have caused human rabies cases.[132] Optimum biosafety practices and equipment will prevent this kind of exposure. As a result of the rarity of human rabies in the United States, many cases have not been suspected until diagnosed at autopsy. Transmission by corneal transplant from fatal cases of undiagnosed encephalitis has been reported.[122,246] Several other human rabies cases caused by organs transplanted form unrecognized rabies encephalitis donors have been reported in the United States and Europe, raising concerns about proper diagnostics available for viral encephalitis in the modern health care setting.[170,246]

Domestic animals constitute the largest source of human exposure in most parts of the world. Dogs and cats are the principal transmitters to people in Asian countries, such as India, Indonesia, China, and the Philippines. The magnitude of the problem is illustrated by the observation that approximately 12,000 to 20,000 deaths are attributed to rabies annually in India alone,[303] and as many as 50,000 to 100,000 may occur throughout the entire world.[146] The incidence of rabies deaths in India of 2 to 4/100,000 population may be exceeded only by that of Ethiopia, where the rate may approximate 18/100,000.[106] The total number of people given postexposure prophylaxis is estimated to exceed 10 million or more annually. On the other hand, many areas, including Japan, Taiwan, Singapore, many parts of the Caribbean, New Zealand, Pacific Oceania, and others, are considered to be "rabies-free."[16,29] China has averaged about 1500 cases per year, but in at least one year there were more than 3000 cases. Dogs were by far the most important source of human rabies.[245]

The burden to people is directly related to the perpetuation of disease in local animal populations. For example, in a single year of an urban epizootic of dog rabies in one city in Mexico, an estimated 2.5 percent of the human population received dog bites and 2.7 percent were given postexposure prophylaxis for rabies.[77] In Africa, the dog still is the most important reservoir and vector, although in the southern portion of the continent, jackals and mongooses also are common sources of viral exposures.[21,58,139,310,311] In much of Africa, the incidence of rabies in humans and dogs has increased in recent years because of social and political disruptions.[303,304]

Rabies in Central and South America is related predominantly to dogs, but vampire bats also have considerable importance. Although humans sometimes are bitten, often they also are exposed to cattle infected by bat bites. However, despite common exposure, viral transmission from livestock to humans is an extremely rare event.[63] Most of the human rabies outbreaks in South America were responded to with the application of NTV, which requires painful subcutaneous injections in the abdomen or in the back daily, during 1 week, plus additional doses at 30 and 60 days, in isolated areas of the Andes and Amazon, with a significant proportion of noncompliance.

In the United States and Canada, foxes and skunks once were the primary rabid wild animal that most commonly attacked humans. In more recent years, the raccoon has replaced these other carnivores as the most important potential source of infection among local mammals because of a rapid extension of raccoon rabies northward and southward in North America, in a range now continuous from Canada to Florida and west to Ohio.[42,72] This outbreak stems from introduction of raccoons into the mid-Atlantic states from a long-standing nidus of raccoon infection previously restricted to Florida and southeastern Georgia. Despite massive spread of raccoon rabies engendering great costs in terms of human postexposure prophylaxis and efforts at wildlife control, actual human cases of rabies attributable to raccoons have been rare.[34] The significance of the bat to rabies epidemiology varies globally. In the United States, bats serve as a reservoir in every state except Hawaii. Bats are responsible for a high proportion of all U.S. cases, including those with no antecedent history of animal bite.[24] Between 2002 and 2012, an average of three human rabies cases occurred per year, in contrast to eight imported cases resulting from dog bites and two from suspected bat bites.[24] However, between 1990 and 2005, 42/45

domestic cases (excluding four transplant-transmitted infections)[246] were caused by bat viruses, predominantly from the silver-haired and eastern tricolored bats.[24,80,171]

Viruses isolated from silver-haired bats may be better able to grow in non-neuronal cells, and although prophylactic vaccination is effective when exposure is recognized, the infectiousness of these viruses apparently appears high.[188] In Colorado, 15 percent of laboratory-tested bats were found rabid.[199] As with other animals, clinical signs may not be apparent immediately, even though the animal is infected.[216]

Bats may bite sleeping humans but leave little trace behind.[149,280] Therefore, vaccination may be indicated if a confirmed rabid bat is found in the chamber of a sleeping person and the ability to sense or detect a bite could be limited, and it may be recommended if a bat entered a room but escaped, if there is a reasonable probability of bite exposure. In Europe, bats have been responsible for transmission of other rabies-related lyssaviruses. Despite the rarity of this event, bats and vampirism are linked inextricably in legend with Dracula, the 15th-century despot who lived in current-day Romania.[108] In contrast, true vampire bats in Central and South America are responsible for considerable losses of domestic animals and human rabies cases.[162]

On average, only two to about six cases of human rabies are reported in the United States each year, but thousands of animals are diagnosed rabid by laboratory. All reservoirs are within wildlife hosts, with spillover infection to other animals. The distribution of infection among animals is shown in Table 183-1.[24] The cat now is a more important host of domestic rabies than the dog in the United States. In Puerto Rico, the mongoose is the main reservoir, and almost all of the mongooses that bite people may be expected to be rabid.[194]

TABLE 183-1 **Cases of Animal Rabies in the United States, 2011**

Animal	Number	Percent
Wild		
Raccoons	1981	32.85
Skunks	1627	26.98
Bats	1380	22.88
Foxes	427	7.08
Rodents/lagomorphs	48	0.8
Other	72	1.19
Total	5535	91.78
Domestic		
Cats	303	5.02
Dogs	70	1.16
Cattle	65	1.08
Horses/mules	44	0.73
Sheep/goats	12	0.2
Swine	0	0
Other	2	0.03
Total	**496**	**8.22**

From Rabies Program, Centers for Disease Control and Prevention, Atlanta.

True canine rabies virus transmission has been eliminated in the United States. Moreover, vaccination has curtailed the prevalence of rabies in cats in the United States so well that some cities and other areas may be considered free of rabies in common animals, although the spread of raccoon rabies has reversed this trend in the eastern states. The dominance of wildlife rabies in the United States can be appreciated in the prevalence for 2011 presented in Table 183-1.

Rabies host shifts were recognized during the last decade for multiple species. Although spillover of virus from a primary reservoir to other animals may be common, the transmission within the same species allows for adaptation and differentiation of the virus in a new reservoir, and a host shift could occur, such as from bats to foxes or skunks in Arizona.[154] This is an important observation that warns about the potential for emerging reservoirs that could change epidemiologic dynamics, affecting the local public health system. The importance of these facts is obvious when considering risk assessments and the need for postexposure prophylaxis.

PATHOGENESIS

The initial stages of the *Lyssavirus* infectious process are perhaps the least understood. Disease certainly depends on the entry of the virus into peripheral nerves, after which centripetal spread occurs rapidly toward the CNS. Replication of virus in muscle cells at a peripheral inoculation site has been demonstrated experimentally, and the virus has been suggested to persist in myocytes for many days after inoculation[49] but has not been proved to occur in nonexperimental situations.[50,192] Nonetheless, replication at a low level in myocytes, followed by subsequent infection of nerve cells, would help to explain the occasionally extended incubation periods of disease. Entry of virus into the peripheral nerve endings is mediated by attachment to various receptors, such as the nicotinic acetylcholine receptor and other ganglioside receptors, such as neural cell adhesion molecule.[155,236] Once the virus is in the nervous tissue, spread can occur by cell-to-cell contact.[48,264] In any case, virus is thought to enter peripheral nerves at an extremely variable time after exposure, presumably at the site of neuromuscular or neurotendinal spindles, at motor end plates, or (in the case of aerosol exposure) at olfactory end organs.[193] Viremia is of no known importance in the dissemination of infection.

The fact that virus transits along nervous pathways has been demonstrated by repeated experimental observations, such that the progress of infection may be interrupted by surgical excision or chemical destruction of nerves at sites distal to the inoculation site.[12] Central progression of virus along neuronal pathways has been demonstrated by electron-microscopic, immunofluorescent, and serial infectivity studies. Virus appears to be sequestered within neuronal cells during transmission (by axoplasmic flow) through peripheral nerves, and the virus can, therefore, be used experimentally to trace synaptic circuits.[140] Intra-axonal retrograde transport appears to be aided by interaction of the viral phosphoprotein (P

protein) with the cytoplasmic protein dynein light chains.[128,215] Minimal numbers of virions are observed within neurons, and connective tissue elements of nerve sheaths are not involved. Because little maturation and release of virus from nerve plasma membranes occur during transit, little or no virus is presented to the immune system, which explains the absence of detectable humoral antibody until late in the disease that is characteristic of even prolonged incubation periods with rabies. Nonetheless, neutralizing antibody may enter the neuronal cytosol by endocytosis and block viral transcription.[69] A recent study described an inmmunoevasive strategy triggered by rabies virus to infect the CNS. Moreover, LGP2, a protein that regulates retinoic acid–inducible gene I (RIG-I), mediates immune responses and reacts with negative immune signals in the presence of negative-strand RNA viruses, preventing the elimination of T cells infiltrated by virus.[52]

Rabies virus enters the CNS at the spinal cord and thereafter travels rapidly to the brain. Early selective infection of the limbic system in the brain may cause disease characterized by extreme excitability and agitation (furious rabies), whereas the encephalitic depressive symptoms are associated with early, widespread infection in the brain.[184] In either case, infection rapidly spreads to affect nearly all brain neurons within a few days of the onset of brain entry and CNS symptoms. Brain infection leads rapidly to death by respiratory or cardiac failure, but via mechanisms that are poorly understood. Fatal cases of encephalitis reveal little damage of neuronal cells, despite the ubiquitous neuronal infection.[91] On the other hand, persons kept alive by vigorous supportive therapy for lengthy periods after onset of disease may develop severe histopathologic encephalitic lesions. Studies in mice have shown that apoptotic mechanisms may be induced by laboratory rabies virus[126,267] but that more virulent rabies virus variants induce less if any apoptosis by expressing less G protein in neurons[189] Alternatively, the viral G protein expression may contribute to pathogenesis by downregulating apoptosis.[189] Although ascending infection is specific to neurons, once the CNS is infected, virus spreads centrifugally to peripheral nerve plexuses, salivary glands, muscle fibers, hair follicles, and other innervated sites.[127]

The entire manner of progression of the infecting virus, particularly those points in its transit vulnerable to immune intervention, remains to be determined fully. Clearly, preexisting humoral antibody protects, apparently by inactivating virus before it gains entry into the nervous system. After a naïve person is exposed to virus, disease can be delayed or prevented by passive antibody, interferon treatment, or both,[131] but protection becomes efficient only when active immunization with potent vaccine accompanies such prophylaxis.

That neutralizing antibodies are key to protection against rabies has been obvious for many years. However, studies have demonstrated the complexity of the immune response after infection.[90,195] Although some experimental evidence argues for the importance of cytotoxic T lymphocytes in protection against rabies, Hooper and colleagues[119] infected mice that were unable to mount a cellular response and found no difference in disease with respect to normal mice. Nonetheless, early inflammatory responses, as well as rapid production of antibody, correlated with recovery from rabies. Thus, interferon (IFN) and other mediators may supplement antibody in the prevention of rabies. Interestingly, virus-specific cytotoxic T lymphocytes do not develop during the disease, and street rabies virus may act as an immunosuppressor. Moreover, chemical immunosuppression enhances the development of experimental rabies. IFN[13] and interleukin-2,[197] both important immunomodulators produced by cells, enhance protection against rabies. More sophisticated understanding of rabies pathogenesis is required before ideal combinations of immunotherapeutic procedures, possibly including IFN, can be applied.

The reverse side of the infection-immunity coin is reflected by the "early death" phenomenon, in which exposed animals or humans develop earlier onset of rabies if previously they received incomplete immunization. Sugamata and associates[250] demonstrated that early death depends on the presence of T cells, indicating that it is mediated by an immunopathologic cellular response. In addition, some authors have argued that cytotoxic T lymphocytes actually may contribute to rabies neuritic paralysis.[250,288]

CLINICAL MANIFESTATIONS

In Animals

Rabies encephalitis in animals is expressed in either a paralytic (dumb) or encephalitic (furious) syndrome. Typical infections are characterized by behavioral changes and a rapid clinical course leading to paralysis, coma, and death, but rarely encephalitis can be nonfatal.[10] Because literally millions of animal bites of humans occur annually in the United States, the identification of aberrant behavior and whether animal attacks can be considered provoked or unprovoked are critical factors in many decisions to administer prophylaxis against rabies.[98]

The prodromal stage of disease is marked by nonspecific signs, such as restlessness and malaise. Subsequently, placid dogs, cats, cattle, or horses may become vicious. Fear of humans and of areas frequented by them may be lost by wild animals. Thus, rabid foxes, normally nocturnal, may be seen wandering abroad in daylight, even in populous areas. Similarly, rabid bats often have been encountered flying oddly in daytime hours. Early behavioral changes are not accompanied by paralysis.

The clinical course progresses rapidly to either dumb or furious disease. Dumb rabies typically is a depressed ascending manifestation. In addition to lethargy, selectively severe paralysis of throat muscles may be observed, which causes drooling of saliva because of difficulty in swallowing. Hydrophobia is not noted in animals. Furious rabies is characterized by an unusual state of alertness in which any visual or sound stimulation may incite an attack. Animals may roam indiscriminately, frequently feeding on stones, twigs, and other inanimate objects. Pets alternatively may exhibit unusually affectionate and playful behavior and then viciously bite those playing

with them. Biting behavior may be noted by herbivores such as horses, mules, and cattle, as well as by normal pets and wild animals.

Both dumb and furious rabies have a rapid clinical course in domestic and wild animals. The period between onset of prodromal signs and death caused by respiratory paralysis rarely exceeds 7 to 10 days.[257] Although 10 days usually is given as the limit of virus excretion in the saliva of dogs before death supervenes, rare exceptions involving longer excretion have been reported experimentally and in animals observed in both Africa and Asia.[82,269]

The diagnosis of rabies in an animal depends on the demonstration of postmortem viral antigens in the brain by fluorescent antibody tests or of viral RNA by reverse-transcriptase polymerase chain reaction (RT-PCR) followed by sequencing.

Management of Animal Rabies

Aside from the historical ineffective large-scale removal of potentially rabid animal species, such as community dogs, most modern control methods focus on the induction of herd immunity by vaccination. In domestic animals, immunization is accomplished readily by a variety of veterinary vaccines, although boosters must be given annually or after other stated intervals, depending on the product.[34]

In wild animals, primarily raccoons in the United States and foxes in Europe, oral vaccination with use of baits containing vaccinia-vectored rabies glycoprotein or attenuated live virus has been successful,[72,201] with the more recent addition a rabies-adenovirus recombinant vaccine.[117,240,262]

In Humans

The incubation period of rabies in some cases may be so long as to qualify it as a slow virus disease. Well-documented cases have occurred as long as 6 and 8 years after the bite.[25,276] However, most cases occur within 20 to 90 days after exposure. The shortest incubation period appears to be 7 to 10 days.

That incubation periods are longer after bites on the legs than after bites on the face is well known. The reason for this duration appears to be related not to the length of the nerve that the virus must traverse, because it travels rapidly even from the farthest site, but to the extent of innervation of different parts of the body. Bites on the tips of the fingers or on the genitalia have relatively short incubation periods for this reason. Children in general tend to have shorter incubation periods. Curiously, cases of vaccination failure tend to have shorter incubation periods, a fact that also is observed in experimental trials in animals. This early death effect is discussed in the section on pathogenesis.

Hemachudha[110] published a comprehensive review of human rabies. The first symptoms of rabies usually are vague and insidious. The patient simply may feel unwell or have anxiety or depression. Some fever or nausea may be present. The most striking prodromal symptom is itching, pain, or tingling at the site of the bite. This paresthesia is not present always and may take various forms, but its localization is a definite harbinger of rabies. The prodrome lasts 2 to 10 days, when the acute neurologic phase begins. The symptoms of this second phase are divided into furious versus paralytic rabies, with most cases being in the furious category.

In furious rabies, the emphasis is on agitation, hyperactivity, fluctuating consciousness, bizarre behavior, and perhaps nuchal rigidity. Sore throat and hypersalivation are prominent complaints, and laryngospasm may cause hoarseness.

The signs and symptoms reported from human rabies cases in the United States are listed in Table 183-2. The legendary but all too real pathognomonic sign of furious rabies is hydrophobia. Although pathognomonic, hydrophobia has been reported in only a third of the human cases in the United States[204] and with even less frequency among children affected with a vampire bat rabies variant (Table 183-3). Initial attempts to swallow liquid result in painful spasms of the pharyngeal and laryngeal muscles, with aspiration of the liquid into the trachea. A conditioned response appears to be created, in which fear exacerbates the actual spasms. Warrell and associates[278] hypothesized that brain stem encephalitis leads to destruction of inhibitors of inspiratory motor neurons. Respiratory tract instant reflexes are exaggerated, leading to inspiratory spasms. The hydrophobia has an important psychological element. In an extreme case, spasms occur if the patient merely is approached with water. Also frequently present is aerophobia, in which spasms occur when a current of air is fanned across the face. Priapism and increased libido also have been reported.

The neurologic examination findings in rabies are not uniform. Meningismus is a commonly occurring abnormality. Even more common occurrences are cranial nerve signs, particularly paralysis of the palate and vocal cords. The voice may develop a hoarse, barking quality. The reflexes may vary from hyperactive to absent, and involuntary movements are prominent. Magnetic resonance imaging (MRI) shows involvement of gray matter in the hippocampus, hypothalamus, and brain stem.[112,175] Advanced MRI techniques such as diffusion weighted imaging and diffusion tensor imaging can provide finer imaging about microstructural and macrostructural damage from rabies encephalitis.[160]

A distinct pattern of neurologic involvement is shown by approximately 20 to 30 percent of patients with rabies, particularly those bitten by vampire bats. Flaccid paralysis may start in the limb that was bitten originally and spread to other limbs. The cranial nerves become involved, and the face, rather than showing agitation, becomes expressionless. Hydrophobia is not a feature. Paralytic rabies is confused easily with Guillain-Barré syndrome. Hemachudha and colleagues[115,116] emphasized the importance of fever, intact sensation, urinary incontinence, and percussion myoedema in paralytic rabies.

The cerebrospinal fluid (CSF) is abnormal in a minority of patients, particularly those in whom meningitis is present clinically. When abnormal, the CSF shows a mild pleocytosis, mainly mononuclear. The peripheral white blood cell count shows increased polymorphonuclear cells.

TABLE 183-2 **Comparison of Human Rabies Cases and Nonrabies Cases Sorted by Frequency of Signs and Symptoms in Confirmed Cases: United States, 1960-2010**

Variable	Positive, N = 108	Negative, N = 144	Odds Ratio	P value
	n (%)	n (%)	(95% CI)	
Mean age (range)	34.35 (2-82)	30.72 (<1-78)		0.173
Male gender	83 (76.9)	87 (62.6)	1.98 (1.13-3.49)	0.019*
Fever	90 (83.3)	113 (78.5)	1.37 (0.72-2.61)	0.422
Confusion or delirium	67 (62.0)	123 (85.4)	0.28 (0.15-0.51)	<0.001*
Agitation or combativeness	55 (50.9)	86 (59.7)	0.70 (0.42-1.16)	0.2
Paresthesia or localized pain	54 (50.0)	21 (14.6)	5.86 (3.22-10.64)	<0.001*
Dysphagia	53 (49.1)	43 (29.9)	2.26 (1.35-3.80)	0.003*
Muscle spasm	45 (41.7)	59 (41.0)	1.03 (0.62-1.71)	1
Localized weakness	44 (40.7)	34 (23.6)	2.22 (1.29-3.83)	0.004*
Malaise or fatigue	39 (36.1)	101 (70.1)	0.24 (0.14-0.41)	<0.001*
Nausea or vomiting	38 (35.2)	66 (45.8)	0.64 (0.38-1.07)	0.09
Hydrophobia	36 (33.3)	9 (6.3)	7.50 (3.42-16.43)	<0.001*
Anxiety	33 (30.6)	51 (35.4)	0.80 (0.47-1.37)	0.5
Headache	29 (26.9)	90 (62.5)	0.22 (0.13-0.38)	<0.001*
Autonomic instability	29 (26.9)	47 (32.6)	0.76 (0.44-1.31)	0.34
Hypersalivation	28 (25.9)	38 (26.4)	0.98 (0.55-1.72)	1
Seizures	27 (25.0)	79 (54.9)	0.27 (0.16-0.47)	<0.001*
Hallucinations	26 (24.1)	42 (19.2)	0.77 (0.44-1.36)	0.39
Ataxia	20 (18.5)	38 (26.4)	0.63 (0.34-1.17)	0.17
Anorexia	19 (17.6)	37 (25.7)	0.62 (0.33-1.15)	0.17
Insomnia	11 (10.2)	27 (18.8)	0.49 (0.23-1.04)	0.17
Aerophobia	10 (9.3)	1 (0.7)	14.59 (1.84-115.83)	0.001*
Priapism or spontaneous ejaculation	4 (3.7)	2 (1.4)	2.73 (0.50-15.19)	0.41

Modified from Petersen B, Rupprecht C. Human rabies epidemiology and diagnosis. In: Tkachev S, editor. *Non-flavivirus encephalitis.* New York: InTech, 2011.
*Statistically significant.

The acute neurologic phase lasts 2 to 10 days, with eventual deterioration of the patient's mental status into coma. The patient may survive in this state for 2 weeks, particularly in dumb rabies. Before the final deterioration, the patient may have alternating periods of wild agitation and alert cooperation. During the alert state, patients may be able to discuss their illnesses and express fear of impending death. However, most often, death rapidly follows onset of coma unless intubation and ventilatory assistance are offered, in which case survival may be prolonged for months. During the comatose state, a variety of problems may be present, including cerebral edema, inappropriate antidiuretic hormone secretion, diabetes insipidus, and other manifestations of hypothalamic dysfunction; hypotension or arrhythmia; and pneumonia. Death caused by rabies in the acute stage occurs because of cardiac or respiratory problems. Cardiac arrhythmias with circulatory collapse commonly occur, and virus may be recovered at autopsy from cardiac tissue that shows pathologic evidence of myocarditis. Respiration becomes increasingly labored, and death may occur during a laryngeal spasm or aspiration.

However, cases of spontaneous recovery and survival recently has been documented, challenging current knowledge of rabies fatality.[44,45] Additionally, recent published evidence of survival after bites from rabid vampire bats without clinical rabies among indigenous Amazon communities challenge the paradigm of fatality from viral exposures.[95] Survival also has been documented in a small number of individuals who had been vaccinated[1,39,105,212] and in a 15-year-old girl who was treated with coma induction, midazolam, ribavirin, ketamine, and amantadine.[302] The last two drugs may have rabies receptor–blocking ability. This experimental rabies treatment has been termed the Milwaukee Protocol, and its original version was modified, dropping ribavirin because of its effect delaying immune response, adding tetrahydrobiopterin to cover the deficiency observed in CSF, and including calcium channel blockers to prevent vasospasm.[300] With the Milwaukee Protocol a 15-year-old boy survived rabies in Recife, Brazil, but this patient received rabies vaccine and developed the disease anyway.[198] Additional attempts for the use of the Milwaukee Protocol were not successful for survival but resulted

TABLE 183-3 **Symptoms and Signs Reported in 17 Children from a Vampire Bat Rabies Outbreak: Peru, 2011**

Signs/Symptoms/Factor	Frequency	Percentage
Mean age (range)	5.7(1-14)	
Female gender	14	82.3
Fever	17	100
Somnolence	12	70.6
Dysphagia with solids	12	70.6
Dyspnea	11	64.7
Dysphagia with liquids	10	58.8
Headache	10	58.8
Paralysis	9	52.9
Fatigue	9	52.9
Hypersalivation	9	47.1
Sore throat	8	41.2
Abdominal pain	7	41.2
Vomiting	7	41.2
Spasm, chorea, seizures	6	35.3
Anorexia	5	29.4
Cough	5	29.4
Stridors	5	29.4
Hallucinations	5	29.4
Weakness	4	23.5
Muscle pain	4	23.5
Anxiety	3	17.6
Otalgia	3	17.6
Hydrophobia	3	17.6
Gaze palsy	2	11.8
Diarrhea	2	11.8
Photophobia	2	11.8
Back pain	2	11.8
Paresthesis	2	11.8
Arthralgia	2	11.8
Neck pain	2	5.9
Chest pain	1	5.9
Tachycardia	1	5.9
Tachypnea	1	5.9
Aerophobyia	1	5.9
Restlessness	1	5.9
Polydipsia	1	5.9
Delirium	1	5.9
Altered sensorium	1	5.9
Priapism	1	5.9

From Dirección General de Epidemiología, Ministerio de Salud del **Perú** and Centers for Disease Control and Prevention. Unpublished data, 2011.

in extended survival period, allowing the increase of clinical knowledge of rabies complications and viral clearance.[8,123,219,301] Many failures of the Milwaukee Protocol have been recorded, and the requirement for a high level of intensive care and laboratory handling of cases might not be available in small hospitals or health care systems with limited budgets, such as in the developing world, where more impact can be achieved with rabies prevention activities.[125]

DIAGNOSIS

The diagnosis of rabies in humans begins with its diagnosis in the animal. The technique now most commonly used is to stain the brain of the previously apprehended animal with a fluorescein-labeled antibody to viral antigens. More recently, enzyme-coupled antibodies have been introduced, allowing the colored product to be seen under the light microscope. Rabies RNA also can be detected by dot hybridization or by RT-PCR amplification.[136] Virus isolation is used for confirmation, either by the classic procedure of intracerebral inoculation in mice or by inoculation of neuroblastoma cells followed by fluorescent antibody staining for viral antigens.

Although the clinical expression of the disease in humans often is characteristic and establishment of the diagnosis is simplified by having a history of known exposure to an animal bite, encephalitic illness may occur, in which viral involvement cannot be diagnosed with certainty without laboratory assistance.

When a patient has a history of being bitten by an animal, paresthesia at the wound site, and hydrophobia, determination of a clinical diagnosis of rabies is not difficult. Other diseases in which encephalitis occurs, such as those caused by arboviruses, enteroviruses, and herpes simplex virus, cerebral malaria, and limbic encephalitis, occasionally may cause confusion with diagnosis. However, if one finds signs of brain stem involvement in a patient whose sensorium basically is clear and who has no signs of a space-occupying lesion, the other diagnoses usually can be set aside.

Paralytic rabies may be misdiagnosed as Guillain-Barré syndrome, poliomyelitis, or post–rabies vaccine encephalomyelitis. Careful neurologic examination and analysis of the CSF often help rule out these diagnoses.

The spasms of tetanus can be a distractor for the diagnosis, but trismus is not part of rabies, and hydrophobia is not part of tetanus. Botulism (wound or ingestion) will cause paralysis, but the absence of sensory changes should exclude rabies.

Postvaccination encephalitis after NTV poses a diagnostic problem in countries in which it still is used. It may require the performance of laboratory tests.

Perhaps the most confusing differential problem is hysteria in a person who thinks he or she has rabies. Normal blood gas analyses and the absence of variation in bizarre behavior suggest psychic rabies. Psychiatric and drug-induced reactions also may cause transient diagnostic problems.

Laboratory diagnosis now is possible before death through the denominated rabies antemortem rule out testing. Antemortem rabies testing has increased relevance with the improvement of intensive care and potential new treatment for rabid patients. The rabies antemortem testing requires a set of four samples, saliva, skin biopsy from the back of the neck, CSF, and serum. The virus also may be demonstrated by fluorescent antibody staining of smears of corneal epithelial cells[147,232] or sections of skin from the neck at the hairline.[35,243] Corneal impressions are not routinely used in presence of modern diagnostic techniques. These test results become positive

TABLE 183-4 **Antemortem Diagnostic Test Results for 62 Human Patients with Rabies: United States, 1960-2010**

Test	No. of Patients Positive for Rabies Virus/Total No. Tested (%)	Earliest Positive (Day of Test Illness)
RT-PCR of saliva for rabies virus RNA	27/32 (84)	2
Brain biopsy for rabies virus antigen	4/6 (67)	7
Nuchal skin biopsy for rabies virus antigen	24/41 (59)	2
Virus isolation from saliva	11/23 (48)	3
Antibody to rabies virus in serum	38/53 (72)	4
Rabies virus antigen in touch impression from cornea	9/19 (47)	2
Antibody to rabies virus in cerebrospinal fluid	17/41 (5)	5

Modified from Petersen B, Rupprecht C. Human rabies epidemiology and diagnosis. In: Tkachev S, editor. *Non-flavivirus encephalitis*. New York: InTech, 2011.

once virus migrates down the nerves from the brain, and both the cornea and hair follicles are richly innervated.

Serologic diagnosis also can be obtained if the patient survives beyond the acute period. In persons not given postexposure prophylaxis, only low levels of antibodies appear.[111,115] In contrast, patients who have received vaccine show a rapid rise in titer of virus-neutralizing antibodies between 6 and 10 days after the onset of symptoms.[103] Such antibodies are detected most rapidly by an in vitro rapid fluorescent focus inhibition test or historically by mouse neutralization tests. Rabies may be diagnosed in immunized persons by a rise in titer after the onset of clinical symptoms and is suggested by any antibody titer 1:5000 or greater, a level not usually achieved by vaccines. High antibody levels in CSF are characteristic late in the course of rabies encephalitis; CSF antibody is not induced efficiently by vaccination.[103,277]

Virus has been isolated from human saliva between days 2 and 24 after onset of disease.[103,204] Virus also may be isolated in some rare cases from CSF or from concentrated urine sediment during the first 2 weeks of illness. In persons surviving longer than 2 weeks, isolation of virus from body tissues or fluids (or from postmortem brain) may be difficult, presumably because of virus neutralization by humoral antibody.

Postmortem diagnosis can be confirmed by the presence of pathognomonic cytoplasmic inclusions (Negri bodies) in brain tissue, but they are present in fewer than 80 percent of cases. Rabies virus antigens may be detected by fluorescent antibody examination, with higher frequency in brain tissues of persons dying after a brief, acute course of disease. In postmortem tissues, histochemical staining with monoclonal antibody to viral internal antigens especially may be useful because ribonucleoprotein possesses epitopes resistant to the formalin fixation and the paraffin-embedding process.[180] In studies of paraffin-embedded brain tissues (samples up to 40 years old), digestion of sections with proteinase K followed by immunofluorescence or RT-PCR testing gave 100 percent (300/300) positive results.[275] TaqMan real-time RT PCR is particularly useful.[272]

However, as in the case of virus isolation attempts, identification of viral antigens in brains of persons kept alive for prolonged periods after onset of disease may be extremely difficult.

Petersen and colleagues[204] summarized the results of attempts to diagnose rabies in humans antemortem. Their data are summarized in Table 183-4,[204] which shows that RT-PCR analysis of saliva and brain biopsy is the most accurate method of diagnosis, which can be found positive as early as the second day after onset. Because of the different times in which antemortem tests can be negative in any of the four required samples (i.e., skin, saliva, serum, and CSF), ruling out of rabies can occur definitively only when all samples taken at the same time are negative. Serial sampling may be required if other etiologic explanations fail.

Rabies now has been recognized postmortem in patients whose history included transplantation of corneas and other organs from donors dying of encephalitis that may also be unrecognized. All fatalities of neurologic disease that may be an undiagnosed encephalitis, therefore, should be considered for rabies, and tissue from such patients should not be used for transplantation.[43,211]

POSTEXPOSURE PROPHYLAXIS

Local Treatment

Removal of material, such as saliva containing virus, is a crucial part of care after an animal bite and should be undertaken urgently. The wound should be flushed copiously with soap and water, rubbing vigorously for no less than 10 to 15 minutes. Immediate washing of the wound is crucial to reduce viral load, leading to increased survival after exposures in 50 percent of patients, and this inexpensive but effective promotion is especially useful in economically disadvantaged health care access settings.[138,281] Some authors would suggest following flushing with a local application of povidone-iodine. Others suggest administration of alcohols, in which the concentration of ethanol is important: 43 percent (86 proof) or higher gave the best results.[289]

Experimental data suggest that regardless of the solutions used, thorough irrigation and flushing is important, particularly in deep puncture wounds. Catheters should be inserted into puncture wounds and fluid instilled by means of an attached syringe.[62,239] If this procedure proves

to be too painful, the area may be anesthetized safely with local procaine-type anesthetics.[138,289] Suturing should be done only in line with good surgical practice and avoided when unnecessary so as to minimize the opportunity for driving virus deeper into surrounding tissues.

Passive Immunity

Administration of rabies immunoglobulin (RIG) is an essential component of postexposure prophylaxis. The influx of antibodies enables immediate virus neutralization while the immune system response after rabies vaccination is still insufficient. The production of endogenous antibodies induced by rabies vaccination may take a minimum of 7 days to reach adequate levels. Hence, use of RIG is not indicated after 1 week from initiation of the vaccination and never in individuals previously vaccinated against rabies.[28,99] The role of passive immunity in saving lives after rabies exposures had been demonstrated under field conditions, and it is more relevant when short rabies incubation period is presumed, such as in head and neck exposures.[99,100] Despite the WHO recommendation of RIG use as part of routine postexposure prophylaxis, it is still rarely used in most of the developing world. Delayed initiation of postexposure prophylaxis because of access limitations, as is observed in many developing countries, implies advanced stages of rabies incubation. In those cases, the use of RIG should not be ignored because of increased risk for prophylaxis failure.[281]

As an alternative for RIG, virus-specific monoclonal antibodies have been developed and clinical studies are ongoing in animals and humans. Monoclonal antibody cocktails can be produced in larger specific definable quantities than RIG and would likely improve the access to passive immunity.[15,28,61,190]

Equine Rabies Immunoglobulin

An experimental basis for the desirability of combining antiserum with vaccine was established in 1954.[148] A field test of combined vaccine-serum protection was made possible by a natural outbreak, the attack of a rabid wolf on 29 Iranian villagers, 18 of whom were bitten on the head and neck.[23] Rabies developed in 3/5 persons who were treated with NTV only but in only 1/13 persons who were given antiserum with NTV.

Animal rabies serum is no longer available in the United States and most developed countries. However, an immunoglobulin from the serum of rabies-immunized horses (equine RIG) is used extensively outside of the United States because of its lower price and greater availability (in contrast to human RIG).[298] To reduce allergic reactions, pepsin digestion is used to convert the intact equine globulin to an $F(ab')_2$ preparation. The product is dispensed typically at a concentration of 200 IU/mL, and the dose that should be administered is 40 IU/kg, with as much as possible infiltrated into the wound. Administration of higher doses may dampen the active immune response. Historically, the manufacturers recommend an intradermal skin test with equine products, but the sensitivity and specificity of the test for prediction of subsequent allergic reactions are poor and abandonment of the skin test has been proposed.[248,] At least one preparation of equine RIG was purified sufficiently so that the allergic reaction rate was less than 1 percent,[296,297] whereas another yielded a 6 percent reaction rate.[295] Anaphylaxis with high-quality equine RIG rarely occurs.[230] Products that are heat-reacted to reduce the risk for viral contamination are available.[157] Because of shorter half-lives of fractional $F(ab')_2$ preparations, the WHO recommends human RIG for severe bite exposure.

Human Rabies Immunoglobulin

To avoid possible reactions to animal proteins, gamma globulin was prepared from the plasma of volunteers hyperimmunized with rabies vaccine. Although expensive, human RIG is preferable to equine products for use in postexposure prophylaxis in the United States.[171] Because the gamma globulin is homologous in humans, human RIG persists longer in the circulation of inoculated persons but, for this reason, may have an even greater dampening effect on active immunization. Thus, Hattwick and associates[104] found that 23 doses of a duck embryo vaccine were needed to overcome the suppressive effect on antibody production of 15 to 40 U of human RIG per kilogram. Such indications are not observed so dramatically with modern vaccines of high quality and potency.

Pharmacokinetic measurements[109,169,179,220] resulted in a recommendation to give 20 IU of human RIG per kilogram immediately, with as much as possible being injected locally. Local injection is important because serum levels of antibody after intramuscular injection are not high.[51] Although intravenous administration of human RIG produces higher serum titers,[5] local injection still is preferred. No further dose is necessary or desirable because excessive antibody diminishes the active response to vaccine. Two equivalent preparations are available in the United States, one produced by Sanofi Pasteur and the other by Talecris (Grifols).

If neither equine nor human RIG is available, vaccination should be started immediately, followed by administration of RIG if it arrives within a week.[142] Because of the expense and occasional difficulty in obtaining adequate supplies of human RIG, as well as substantial variability of immunoglobulin products tested in an animal model,[102] the WHO is exploring the possibility of using instead "cocktail" mixtures of neutralizing monoclonal antibodies to rabies virus prepared in either murine[237] or human[68] hybridoma cell cultures. Such cocktails have shown effectiveness in animal studies.[15,28,96,213]

Nerve Tissue Vaccine

Although used for 100 years after the introduction of the original Pasteur vaccine, NTVs are being replaced increasingly by cell culture vaccines in developing countries. Vaccines made in sheep or goat brain were widely used throughout Asia and Africa but were associated with a high incidence of postvaccinal encephalitis.[7] The susceptibility to rabies vaccine–induced encephalomyelitis has been suggested to vary according to the genetic

pattern of major histocompatibility class II alleles in the vaccinated host.[206] Suckling mouse brain vaccines (SMBVs) are still used to some degree in Latin America to decrease sensitization to myelinated nerve tissue, which is thought to be the cause of autoimmune allergic encephalitis.[27,92] Because SMBVs are locally manufactured in each country, no supervisory entity certifies the vaccine quality at the regional level. Differences in purity and potency are also possible, resulting in different rates of adverse reactions. A study in Brazil found no evidence of neuroparalytic effects in more than 1000 patients vaccinated with SMBV,[27] and in the Dominican Republic a cluster of neuroparalytic cases was reported in three members of a family receiving SMBV locally produced after exposure to a rabid cat.

Although mild, mainly local adverse reactions are frequent (125/1000) because of the administration of the 2 mL of SMBV in the subcutaneous tissue[27] either in the abdomen or in the back, serious neurologic reactions are expected in a frequency estimated at 1/16,000 vaccinations.

However, a study from Tunisia found no evidence for humoral response to myelin in patients with encephalitis after receiving NTV. Instead, antibodies to G_{M1} and G_{D1a} gangliosides present on human cells were demonstrated.[161] Thus, the mechanism of encephalitis remains unclear, beyond the conclusion that it results from nonviral antigens in the nervous systems of animals, including sheep, lambs, and mice.

The efficacy of NTV in humans has not been evaluated by controlled studies. The Pasteur Institute of Southern India analyzed the incidence of disease in vaccinated and unvaccinated people bitten by animals that were proved rabid by the transmission of virus to other animals or humans. Fifty-six percent of untreated persons developed rabies, in contrast to only 7 percent of vaccinated persons, for an effectiveness of approximately 88 percent.[268]

On the other hand, vaccination with NTV of persons bitten on the head or neck by rabid wolves in Iran still was followed by a 40 percent overall mortality rate. In comparison, 15/32 villagers (47%) who failed to seek prophylaxis after being attacked by a rabid wolf developed rabies.[118]

Duck Embryo Vaccine

Duck embryo vaccine was developed to overcome the problem of encephalitic reactions to NTV. In this respect, it was successful in that the incidence of neuroparalytic reactions fell from 1/1000 to 1/25,000 with duck embryo vaccine.[208,221,231] Unfortunately, a high price was paid in immunogenicity. In a study of postexposure Americans, Corey and associates[59] found that 8 percent of those given courses of duck embryo vaccine failed to respond with the production of measurable antibody. Even more disturbing was the fact that 23 percent of recipients of duck embryo vaccine plus antiserum did not develop antibody. Duck embryo vaccine contained enough duck protein to produce a significant number of allergic reactions.[221] Several human rabies cases were reported despite the use of duck embryo vaccine combined with serum. Currently, an improved purified duck embryo vaccine is produced in India.

Cell Culture Vaccines

The ideal solution to the problems of immunogenicity and the safety of rabies vaccine lies in the development of vaccines prepared from rabies virus grown in cell culture free of neural tissue. To avoid inclusion of foreign host proteins in the vaccine, the optimal cell cultures needed to be derived from humans.

The basic ingredients for the production of human diploid cell vaccine (HDCV), the first cell culture vaccine widely used, were the development of the WI-38 normal human fibroblast cell line by Hayflick and Moorhead[107] and the adaptation of the Pitman-Moore strain of rabies virus to growth in WI-38 by Wiktor and associates.[292] Virus grown in human fibroblasts was concentrated by ultrafiltration to increase antigen content and inactivated by β-propiolactone.

After various schedules of HDCV were compared, researchers found that three properly spaced intramuscular doses consistently produced an immune response. A similar schedule of duck embryo vaccine resulted in antibody titers 10 to 20 times lower. The excellent immunogenicity of HDCV was confirmed,* and it became a gold standard against which other vaccines were measured.

A crucial test of any rabies vaccine is protection of those actually exposed to virus. In Europe, the HDCV was used to vaccinate thousands of people exposed to rabies. However, those situations in which rabies was confirmed in the biting animal were particularly important to consider. Kuwert and associates,[150,151] in Essen, vaccinated 68 persons after exposure to dogs, cats, cows, or wild animals with laboratory-confirmed rabies virus infection or exposed as a result of a laboratory accident. The schedule used was 1 mL intramuscularly on days 0, 3, 7, 14, 30, and 90. They had no failures of protection, no significant reactions, and excellent neutralizing and complement-fixing antibody responses.

Bahmanyar and associates[14] in Iran conducted another test of HDCV. Forty-five persons who were bitten by rabid wolves or dogs were given rabies antiserum, followed by the same schedule of vaccine used in Germany. Once again, no rabies was seen in vaccinees, despite a 40 percent risk (estimated from previous experience) if they had remained unvaccinated. Antibody measurements showed mean titers as follows: 7 days, 1.1 IU; 14 days, 10.7 IU; 30 days, 49 IU; and 100 days, 312 IU.

Later, the Centers for Disease Control and Prevention (CDC) distributed HDCV of American manufacture for persons whose exposure to rabies was established.[2] No vaccine failures occurred, and all who received the full schedule of five 1-mL doses intramuscularly responded with antibodies, to date.

Although a small number of vaccine failures have been reported in other countries after administration of HDCV, ancillary prophylaxis recommendations were not followed scrupulously in each case.[4] The failure rate is estimated to be at most 1/12,000 courses of vaccination

*References 6, 36, 60, 94, 171, 209, 266, 293.

given in countries with high risk for rabies.[196] General anesthetics given locally while the wound is repaired may increase the risk for vaccine failure.[84] The mechanism of protection afforded by postexposure prophylaxis is uncertain. It may be blockage by antibodies of virus passage from subcutaneous tissue to the CNS, or it may actually be through the induction of innate and adaptive immune responses in the CNS.[120,164]

An effort has been made to produce vaccines with the desirable properties of HDCV at a lower cost that would make them broadly available in the developing nations, where the risk for human rabies is most severe. For example, purified Vero cell rabies vaccine (PVRV) is the Pasteur strain of rabies virus (as used in HDCV) grown in a Vero cell line in industrial fermenters. The virus is concentrated, inactivated, and purified. The PVRV has an immunogenic potency similar to that of HDCV. Successful postexposure experience has been reported in multiple countries, including Tunisia,[47] China,[274] and Thailand, even after severe exposure.[129,252,277] The PVRV has been purified further by chromatography.[158]

Purified chick embryo cell (PCEC) rabies vaccine is produced in primary chick embryo cell culture with use of the Flury low egg passage strain of rabies virus. It is inactivated, concentrated, and purified, yielding a vaccine preparation of antigenic potency similar to that of PVRV and HDCV.[18] The PCEC rabies vaccine was uniformly successful in preventing rabies in postexposure trials in the former Yugoslavia, including subjects who were exposed by wolf bite.[57,270] The PCEC rabies vaccine also was shown to be equivalent to HDCV for postexposure immunization,[214] for boosting previously immunized subjects,[33] and in animal models for protection against bat rabies virus variants.[67]

Today, purified duck embryo cell rabies vaccine is produced in embryonated duck eggs. The Pitman-Moore virus harvested from the eggs is purified, concentrated, and inactivated.[141]

Primary hamster cell kidney vaccine has been produced in China and Russia, and was demonstrated to be immunogenic and provide postexposure protection.[165] It completely replaced Semple vaccine in China.[165]

A theoretical argument has been made that a potent rabies virus might be made at low cost in continuous BHK-21 cells, and if it were inactivated with β-propiolactone, the β-propiolactone would so damage the cell DNA that the theoretical danger posed by cell DNA in vaccine would be eliminated totally. This concept was never broadly used in human vaccination against rabies.[187]

Two types of rabies vaccine are licensed and available in the United States: HDCV and PCEC. A tissue culture vaccine made in fetal rhesus diploid cells previously by the Michigan State Department of Health (and later Bioport), termed *rabies vaccine adsorbed* (RVA), is no longer distributed.[19] Either HDCV or PCEC rabies vaccine is recommended for postexposure prophylaxis when given by the intramuscular route according to the regimens listed in Table 183-5. In 2011, the postexposure regimen in the United States was reduced from five to four intramuscular doses in those with a normal immune system, in recognition of the high immunogenicity of the vaccines. Immunosuppressed patients should continue to receive five doses.[225] The dose should not be reduced for children.[5] Decreased immune responses have been noted in vaccinees given injections in the gluteal area,[87] and therefore all intramuscular injections of rabies vaccine should be given in the deltoids. In the event of reexposure of persons with a prior history of vaccination, two intramuscular booster doses should be given (see Table 183-5). Virtually 100 percent of these patients will have an anamnestic response.[254]

TABLE 183-5 **Regimens for Preexposure and Postexposure Vaccination with Rabies Vaccines**

Vaccination	Route	Days on Which Doses Are Given	Remarks
Preexposure	IM*	0, 7, 21, or 28	Standard U.S. and WHO three-dose regimen
	ID†	0, 7, 21, or 28	Economical, but not to be used in those taking antimalarial medications; unlicensed route in U.S. WHO recommended.
Postexposure‡	IM*	0, 3, 7, 14	Standard U.S. four-dose recommendation
	IM*	0 (two doses), 7, 21	Used in some countries when RIG is not indicated; so-called 2-1-1 schedule
	ID†	0, 3, 7 (two doses each), 28	Used in Thailand with PVRV, PCECV; so-called 2-2-2-0-1 schedule
	ID†,§	0 (8 doses), 7 (4 doses), 28, 90	Used in developing countries with HDCV, PCECV, or PVRV cell culture vaccines; so-called 8-0-4-0-1-1
Booster (for reexposure)	IM*	0, 3	Only after documented vaccination with cell culture vaccine¶
	ID†	0, 3	Only after documented vaccination with cell culture vaccine¶

*Together with rabies immunoglobulin.
†Give 0.1 mL, over the deltoids.
‡Give 0.5 mL (PVRV) or 1 mL, depending on the vaccine, into the deltoid.
§Give 0.1 mL at multiple sites (see text).
¶Or demonstrated presence of virus-neutralizing antibodies after other vaccines.
HDCV, Human diploid cell vaccine; *ID,* intradermal; *IM,* intramuscular; *PCECV,* purified chick embryo cell vaccine; *PVRV,* purified Vero cell rabies vaccine; *RIG,* rabies immune globulin.

Numerous regimens have been developed for rapid immunization of exposed patients in developing countries who may seek medical help late or who may not return for subsequent doses.[253,271,277] To reduce costs, advantage is taken of the smaller volume needed for intradermal administration. Moreover, because the return of patients for subsequent doses is more pragmatic in developing countries, the intradermal schedules are relatively short.[101,238,282,283] Some workers propose a 1-week schedule.[182,251] However, no intradermal vaccine is licensed currently in the United States. These regimens are listed in Table 183-5 and have the advantage of being less expensive. Note that if the 2-1-1 regimen is chosen, RIG may interfere with the response to the vaccine.[305]

The effectiveness of current rabies vaccines to protect against most rabies-related lyssaviruses appears to be adequate, but not for those viruses in phylogroups II or III.[97,191]

Decisions to Vaccinate

A physician must take into account numerous human and biologic factors in deciding when to vaccinate (Table 183-6), although reluctance to use vaccine now is based more on cost than on the pain of injections. A study performed in the United States[186] revealed that administration of rabies prophylaxis was inappropriate in 40 percent of cases in which it was given. However, the study also showed that 6 percent of those not receiving prophylaxis should have received it. Thus, the education of physicians needs improvement, not only for medical reasons but also because some incidents of exposure to rabies result in needless vaccination of many persons, with high cost.[218] The factors discussed in the following section should be considered.

Pregnancy

Pregnant women tolerate rabies vaccination without problem.[54,249]

Geography

Many animal bites in most large urban areas in the developed world are unlikely to be from rabid animals, although Philadelphia, Baltimore, New York, and Washington, DC, were part of the raccoon epizootic, with not infrequent transfer of infection to urban cats and other species.

Type of Animal

In the United States, reservoirs consist of wildlife. Rabies cases in cats now outnumber those in dogs. Cats always are suspect if they go out of their way to bite. Dogs on the U.S.–Mexican border are more suspect than those farther north. Skunks, foxes, and raccoons involved in biting incidents must be considered rabid until proved otherwise. With the exception of woodchucks, rodents such as squirrels are unlikely to be rabid.

Bat Exposures

As discussed, viral transmission from bats has become a threat to humans in the United States because of an apparent increase in exposures to silver-haired and eastern tricolored bats. The in vitro characteristics of these bat rabies viruses, which may allow them to replicate better in non-neural tissues, plus the small and sometimes imperceptible bites these species inflict, led to recommendation that vaccination may be appropriate when bats are handled without gloves and when sleeping

TABLE 183-6 **Rabies Postexposure Prophylaxis Guide: United States**

Animal Type	Evaluation and Disposition of Animal	Postexposure Prophylaxis Recommendations
Dogs, cats, and ferrets	Healthy and available for 10 days of observation	Persons should not begin prophylaxis unless animal develops clinical signs of rabies*
	Rabid or suspected rabid	Immediately vaccinate
	Unknown (e.g., escaped)	Consult public health officials
Skunks, raccoons, foxes, and most other carnivores; bats†	Regarded as rabid unless animal proven negative by laboratory tests‡	Consider immediate vaccination
Livestock, small rodents, lagomorphs (rabbits and hares), large rodents (woodchucks and beavers), and other mammals	Consider individually	Consult public health officials. Bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other small rodents, rabbits, and hares almost never require antirabies postexposure prophylaxis.

From Manning SE, Rupprecht CE, Fishbein D, et al; Advisory Committee on Immunization Practices Centers for Disease Control and Prevention (CDC). Human rabies prevention—United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**:1–28.

*During the 10-day observation period, begin postexposure prophylaxis at the first sign of rabies in a dog, cat, or ferret that has bitten someone. If the animal exhibits clinical signs of rabies, it should be euthanized immediately and tested.

†Postexposure prophylaxis should be initiated as soon as possible after exposure to such wildlife unless the animal is available for testing and public health authorities are facilitating expeditious laboratory testing or it is already known that brain material from the animal has tested negative. Other factors that might influence the urgency of decision-making regarding initiation of postexposure prophylaxis before diagnostic results are known include the species of the animal, the general appearance and behavior of the animal, whether the encounter was provoked by the presence of a human, and the severity and location of bites. Discontinue vaccine if appropriate laboratory diagnostic test (i.e., the direct fluorescent antibody test) is negative.

‡The animal should be euthanized and tested as soon as possible. Holding for observation is not recommended.

individuals awake to find a bat in the room and are uncertain if a bite was sensed or detected.[171]

Circumstances of Bite

The attempt to feed an undomesticated animal should be considered provocative behavior. Invasion of an animal's space or territory may result in an attack, which is less suggestive of rabies than is an attack by an animal that invades human environments. However, judgment as to whether a bite was provoked is poorly predictive of rabies in enzootic areas.[148] If an animal appears clinically rabid, it should be euthanized immediately for confirmation of rabies by examination of the brain. If a dog, cat, or ferret appears normal, it may be kept for 10 days to see if it develops rabies.[183] Meanwhile, exposed humans should or should not be started on prophylaxis, depending on the known prevalence of rabies in the particular region, the species of animal, and the circumstances of the incident.

Animal Vaccination

Rabies is an extremely rare occurrence in properly vaccinated animals. Nevertheless, prophylaxis may be administered if clinical or epidemiologic data suggest rabies, even in a vaccinated animal.

Table 183-6 summarizes recommendations by the Advisory Committee on Immunization Practices (ACIP) on vaccination against rabies.[171] Advice can be sought from state and local health departments (particularly with regard to the occurrence of rabies in animals) and from the CDC directly.

Failure of Rabies Prophylaxis

The most common causes of human rabies are that no prophylaxis was provided or RIG was not used simultaneously with vaccine.[64,93,299] Active immunization will not regularly produce antibodies until 7 to 14 days after the first dose is given. Lack of local infiltration of RIG and the immunosuppressive effect of chloroquine with intradermal immunization may account for other failures. Similarly, delays in prophylaxis provide an advantage to these highly neurotropic agents toward development of productive infection. The presence of B-cell immunodeficiency in a patient requires measurement of antibodies after vaccination. Patients infected with human immunodeficiency virus (HIV) are likely to respond if their $CD4^+$ lymphocytes are higher than 300/μL. If not, their postvaccination antibodies also should be measured.[129] In HIV-infected children, a standard postexposure course of HDCV had no effect on the level of $CD4^+$ lymphocytes or the HIV viral load.[258] However, even with correct prophylaxis, failures may occur, perhaps because in severe exposures, virus is deposited directly onto nerve endings[114] or because of inadequate monitoring of vaccine and RIG potency in developing countries.[11]

PREEXPOSURE IMMUNIZATION

The development of modern vaccines has made effective immunization of persons at risk for coming into contact with virus possible before the actual exposure occurs. Veterinarians, animal handlers, laboratory workers, and spelunkers should receive preexposure immunization (Table 183-7). Large numbers of veterinary students have been vaccinated under a three-dose schedule at 0, 7, and 21 or 28 days. Rabies virus antibody titers were determined on the serum of each veterinary student. Nearly 100 percent developed antibodies, with geometric mean titers of 10 IU or greater, which is equivalent to a neutralizing antibody titer of at least 1:200.[217] The three-dose regimen listed in Table 183-5 induces antibodies in 100 percent of recipients. Preexposure immunization may be given with use of HDCV and PCEC rabies vaccines by intramuscular administration. Outside the United States, any of the alternative cell culture vaccines can be used on the same schedule.[228] Alternatively, and more economically, immunization may be performed intradermally according to the same schedule.

Because rabies in developing countries is primarily a disease of children, exploratory studies have been performed to determine if preexposure vaccination could be incorporated into routine pediatric schedules. Preliminary results from Vietnam show that routine rabies vaccination in infancy is feasible[159] and could even be performed by low-dose intradermal administration.[75] In areas enzootic for canine rabies, universal vaccination of children could be cost-effective if the incidence of dog bite is high.[53] Children at routine risk for vampire bat exposures throughout Amazonia should be considered, as well, for preexposure vaccination. Children infected with HIV can be immunized successfully if their $CD4^+$ cells exceed 15 percent of lymphocytes. Those with fewer $CD4^+$ cells may need at least double doses of rabies vaccine.[258]

Missionaries and Peace Corps personnel operating in rabies-endemic countries should receive preexposure vaccination.[9]

Intradermal Vaccination

For preexposure use, the intradermal route is considered an acceptable alternative to intramuscular injection, with the important proviso that persons receiving antimalarial or other immunosuppressive agents and perhaps older persons should have their titers checked after vaccination or receive the injections by the intramuscular route.

The success of intradermal vaccination depends on a technique that ensures intradermal rather than subcutaneous injection, but a margin of error exists.[20,86]

Today, essentially all intradermal vaccinations are done with PVRV and PCEC rabies vaccine. To reduce the costs of vaccination, intradermal vaccination for postexposure use has become popular in developing countries, but it no longer is approved in the United States. Although single-dose preparations are not available for the intradermal administration of the 0.1-mL dose, extensive experience in Thailand and elsewhere has validated the successful prevention of rabies with vaccine extracted from vials intended for intramuscular use.[75,265,285,284] However, attention must be paid to the correct administration of the dose into the skin, the sterility of unused portions of the vial for only a few hours while maintaining the cold chain, the volume

TABLE 183-7 **Rabies Preexposure Prophylaxis Guide: United States**

Risk Category	Nature of Risk	Typical Populations	Pre-exposure Recommendations
Continuous	Virus present continuously, often in high concentrations	Rabies research laboratory workers*	Primary course
	Specific exposure likely to go unrecognized Bite, nonbite, or aerosol exposure	Rabies biologics production workers	Serologic testing every 6 mo; booster vaccination if antibody titer is below acceptable level†
Frequent	Exposure usually episodic with source recognized, but exposure also might be unrecognized. Bite, nonbite, or aerosol exposure	Rabies diagnostic laboratory workers,* cavers, veterinarians and staff, and animal-control and wildlife workers in areas where rabies is enzootic All persons who frequently handle bats	Primary course Serologic testing every 2 years; booster vaccination if antibody titer is below acceptable level†
Infrequent (greater than in population at large)	Exposure nearly always episodic with source recognized	Veterinarians and animal-control and wildlife workers in areas with low rabies rates	Primary course
	Bite or nonbite exposure	Veterinary students	No serologic testing or booster vaccination
		Travelers visiting areas where rabies is enzootic and immediate access to appropriate medical care, including biologics, is limited	
Rare (population at large)	Exposure always episodic with source recognized	U.S. population at large, including persons in rabies-epizootic areas	No vaccination necessary
	Bite or nonbite exposure		

From Manning SE, Rupprecht CE, Fishbein D, et al; Advisory Committee on Immunization Practices Centers for Disease Control and Prevention (CDC). Human rabies prevention—United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**:1–28.

*Judgment of relative risk and extra monitoring of vaccination status of laboratory workers are responsibilities of the laboratory supervisor.

†Minimum acceptable antibody level is complete virus neutralization at a 1:5 serum dilution by the rapid fluorescent focus inhibition test. A booster dose should be administered if the titer falls below this level.

of vaccine in the ampule, and the antigenic content of the vaccine used. The antigenic content should be at least 0.25 IU/0.1 mL. A popular intradermal schedule is one developed by the Thai Red Cross, consisting of inoculations on days 0, 3, 7, and 28 days, with double doses given in the first three administrations. A former recommendation for a 90-day dose has been abandoned.[143] Poor responses to intradermal vaccine have been noted in those concurrently receiving chloroquine or immunosuppressives, such as corticosteroids.[171,200] Therefore, persons who must be vaccinated while they are taking chloroquine or related antimalarials should be given injections into the deltoid muscle, and postvaccination rabies serologic studies should be obtained on those patients and others who are immunosuppressed.

Alternative Schedules

Although the WHO schedules are firmly established for the induction of optimal immune responses, other schedules have been tested extensively to reduce the number of vaccination visits, particularly in the developing world. One of the most popular of these is the 2-1-1 schedule, in which a double dose is given intramuscularly at day 0, followed by single doses on days 7 and 21[55,181] and the regimen developed by Warrell and associates,[284] consisting of eight intradermal doses on day 0, four intradermal doses on day 7, and single doses on days 28 and 91.

The 2-1-1 schedule may not be reliable if immunoglobulins also are administered.[156,159]

Booster Doses

Even with administration of cell culture vaccine, antibodies fall off rapidly after initial immunization, although most vaccine recipients have some detectable antibody decades after initial vaccination.[79,135] Nonetheless, once an immune response to rabies vaccine has developed, revaccination is almost certain to evoke a rapid response.[254] One booster of cell culture vaccine given to previously vaccinated persons results in a dramatic anamnestic response, with titers in one study rising from 2.8 IU to 94 IU at 14 days and more than 100 IU in 35 days.[210] As mentioned, with exposure to rabies in a previously vaccinated person, two intramuscular booster doses are recommended to provide a margin of safety. Single intramuscular or intradermal boosters are given to maintain immunity in individuals chronically exposed to rabies, according to the recommendations made by the ACIP and WHO in Table 183-7 if serology suggests a waning titer.

In persons who have received preexposure immunization to rabies, the necessity of boosters is an important issue. Persons who definitely were exposed to a rabid animal should receive two booster doses of vaccine by the intramuscular route. Individuals who are likely to be exposed, such as rabies laboratory workers, should receive booster injections to maintain their antibody titers above 0.5 IU (or complete neutralization at a dilution of 1/5). A follow-up study by Briggs and Schwenke[32] was interesting. They found maintenance of an adequate titer (>0.5 IU) at 1.5 to 2 years after vaccination in 99 percent of subjects who received vaccine by the intramuscular route and in 93 percent who received vaccine by the intradermal route. In Thailand, researchers demonstrated that persons who received preexposure rabies vaccination by the intradermal route mounted a slow response to boosters, and the authors suggested that in severe exposures, rabies immunoglobulin should be given despite the prior immunization.[129] However, the intradermal route can be used successfully to boost prior immunity.[256] Thraenhart and associates[260] observed 100 percent positive antibodies in 18 subjects studied between 2 and 14 years after they received vaccination. Thus, if a person is properly vaccinated with modern cell culture vaccines, subsequent boosting with rabies immunoglobulin is not indicated in postexposure prophylaxis.

Adverse Reactions

The available tissue culture vaccines are well tolerated. In more than 1770 human volunteers receiving preexposure immunization with HDCV administered intramuscularly, a sore arm was noted in approximately 20 percent, headache in about 8 percent, malaise in 5 percent, and allergic edema in 0.1 percent.[210] During incidents involving mass postexposure prophylaxis, pain, swelling, and other local symptoms occurred in 30 to 74 percent of individuals.[171] Pregnancy is not a contraindication to receiving modern rabies vaccines.[55] Guillain-Barré syndrome and other neurologic problems have been rare occurrences, and their relationship to HDCV is uncertain.[145] Guillain-Barré syndrome that occurs after administration of NTV is associated with antibodies to myelin basic protein.[113]

In contrast, booster vaccinations with HDCV have been associated with allergic reactions in approximately 6 percent of subjects.[40] These reactions are caused by the presence in the vaccine of human albumin that has been altered by the β-propiolactone used to inactivate the virus.[3,255,286] The reactions are of the immune complex type (type III), with urticaria, edema, joint manifestations, fever, and malaise. CDC data suggest that when primary vaccination is given intramuscularly and booster intradermally, or vice versa, reactions are more common than if all vaccination is by the same route.[88] Because the reaction is associated with the particular formulation of HDCV rather than with the virus antigen itself, additional boosters may be given if necessary with PCEC rabies vaccine, PVRV, or HDCV manufactured in Canada (Sanofi Pasteur).[85] Reactions to PVRV and PCEC cell culture vaccines generally have been mild.[73] A comparative study of PVRV with HDCV showed lower or equivalent rates of local and systemic reactions, with no serious adverse events.[134] Because PCEC rabies vaccine is manufactured in chicken cells, egg allergy is a possible problem, and anaphylactic events as well as some rare and perhaps unrelated neurologic complications have been reported.

FUTURE DEVELOPMENTS

The world of rabies is far from static. Table 183-8 shows the progress of rabies vaccine development for humans. Perhaps the most dramatic future prospect is the development of vaccines in which the gene for rabies glycoprotein has been inserted into a viral vector, such as a poxvirus or adenovirus, or the use of rabies virus as a cloning and expression vector system by reverse genetics.[37,227] These vaccines already have been used extensively in a large variety of animal species, in which they have been found to be safe and highly immunogenic.[226] In field studies, raccoons and other wild animals have been immunized successfully by the oral route with baits containing rabies recombinant vaccines. Such vaccination has eliminated fox rabies from large areas of western Europe[29] and reduced raccoon rabies in the eastern United States.[223,240,241] Other avenues being pursued include the use of potent adjuvants to enhance the immunogenicity of subunit vaccine,[83] the addition of the internal ribonucleoprotein to enhance protection through induction of cellular immune responses and higher

TABLE 183-8 **Some Rabies Vaccines Developed for Humans**

Vaccine Types	Remarks
Pasteur: Dried rabbit spinal cord	Residual live virus
Fermi: Phenolized sheep or goat brain	Residual live virus
Semple: Phenol-inactivated sheep or goat brain	Contains nerve tissue
Fuenzalida: Phenol-inactivated suckling mouse brain	Contains less myelin
Duck embryo: BPL inactivated	Allergy to duck proteins
Human diploid cell (HDCV): BPL-inactivated fetal human cell culture vaccine	Current standard; booster allergic reactions
Rhesus diploid cell (RVA): Fetal rhesus cell culture, BPL inactivated	Fewer allergic reactions
Vero cell (PVRV): BPL-inactivated virus	Purified by density gradient centrifugation; grown in Vero monkey kidney cell line
Chick embryo cell (PCEC rabies vaccine): Inactivated virus grown in chick embryo cells	Purified like PVRV
Vaccine recombinant glycoprotein (VRG): Genetic construct expressing rabies	Probably will be used only in animals (see text)

BPL, β-Propiolactone.

antibody responses,[261] synthetic peptides constructed from epitopes of the single glycoprotein and nucleoprotein,[66] and plasmid vectors containing cDNA of the glycoprotein (G protein) gene.[308]

Particular effort has been directed toward the development of DNA vaccines. They are alleged to be potentially inexpensive and particularly thermostable, but to date most have required an unsatisfactory interval to produce virus-neutralizing antibodies or to have induced virus-neutralizing antibodies of inadequate titer.[166-168] Dogs given two doses of Pasteur strain G protein DNA, administered intramuscularly, were shown to develop virus-neutralizing antibodies to rabies and to European bat lyssaviruses 1 and 2 and to have protection against virus challenge.[203] Jallet and colleagues[130] produced chimeric G DNAs of rabies and EBL1, or rabies and Mokola virus, to generate vaccines designed to induce virus-neutralizing antibodies in dogs to either all European lyssaviruses or all African lyssaviruses, respectively. In primates, rabies G DNA induced virus-neutralizing antibodies only if it was administered by gene gun.[168]

Other approaches have included efforts to express rabies G protein in yeast cells, which failed,[259] and in baculovirus in insect cells, which was successful. Although it is less glycosylated than the virion G protein, the baculovirus-expressed G protein induced virus-neutralizing antibodies efficiently in mice; its immunogenicity was not improved by adding baculovirus-expressed rabies nucleoprotein (N protein).[74] In approaches using the rabies G protein cloned into adenovirus, researchers have shown that a replication-defective recombinant elicited enzyme-linked immunosorbent assay antibodies in mice inoculated onto mucosal surfaces[307] and that an adenovirus incorporating a special promoted-intron expression cassette efficiently induced rabies G protein in cell culture that induced virus-neutralizing antibodies in intraperitoneally inoculated mice.[177] The rabies G protein gene was cloned into canine herpesvirus; this recombinant induced virus-neutralizing antibodies efficiently in dogs inoculated by the intranasal route, suggesting that it might be useful as an oral product to control canine rabies.[309] Similar hopes are offered by a new recombinant canine adenovirus vaccine, but widespread infection by canine adenoviruses may preclude such a possibility.

Molecular biologic approaches have not yet produced an ideal vaccine, especially for human use. In the near future, the most cost-effective and protective vaccines may be virion products propagated to high titer in an easily managed cell culture system, for example, a suitable BHK-21 continuous cell line or transgenic plants.[185] Also, monoclonal antibodies may offer an effective alternative to human rabies immunoglobulin. Regardless of developments in human medicine, the key to future progress is a focus on elimination of canine rabies, with greater global partnerships from a One Health perspective.[178,306]

NEW REFERENCES SINCE THE SIXTH EDITION

8. Aramburo A, Willoughby RE, Bollen AW, et al. Failure of the Milwaukee protocol in a child with rabies. *Clin Infect Dis* 2011; **53**:572–4.
16. Banyard AC, Hartley M, Fooks AR. Reassessing the risk from rabies: a continuing threat to the UK? *Virus Res* 2010;**152**: 79–84.
17. Banyard AC, Hayman D, Johnson N, et al. Bats and lysaviruses. *Adv Virus Res* 2011;**79**:239–89.
24. Blanton JD, Dyer J, McBrayer J, et al. Rabies surveillance in the United States during 2011. *J Am Vet Med Assoc* 2012;**241**: 712–22.
25. Blanton JD, Palmer D, Dyer J, et al. Rabies surveillance in the United States during 2010. *J Am Vet Med Assoc* 2011;**239**: 773–83.
28. Both L, Banyard AC, van Dolleweerd C, et al. Passive immunity in the prevention of rabies. *Lancet Infect Dis* 2012;**12**:397–407.
30. Bourhy H, Dautry-Varsat A, Hotez PJ, Salomon J. Rabies, still neglected after 125 years of vaccination. *PLoS Negl Trop Dis* 2010; **4**:e839.
34. Brown CM, Conti L, Ettestad P, et al. Compendium of animal rabies prevention and control, 2011. *J Am Vet Med Ass* 2011; **239**:609–17.
38. Calisher CH, Ellison JA. The other rabies viruses: the emergence and importance of lyssaviruses from bats and other vertebrates. *Travel Med Infect Dis* 2012;**10**:69–79.
44. Centers for Disease Control and Prevention. Presumptive abortive human rabies: Texas, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:185–90.
45. Centers for Disease Control and Prevention. Recovery of a patient from clinical rabies: California, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:61–5.
52. Chopy D, Pothlichet J, Lafage M, et al. Ambivalent role of the innate immune response in rabies virus pathogenesis. *J Virol* 2011;**85**:6657–68.
76. Dzikwi AA, Kuzmin II, Umoh JU, et al. Evidence of Lagos bat virus circulation among Nigerian fruit bats. *J Wildl Dis* 2010; **46**:267–71.
79. Fayaz A, Simani S, Janani A, et al. Antibody persistence, 32 years after post-exposure prophylaxis with human diploid cell rabies vaccine (HDCV). *Vaccine* 2011;**29**:3742–5.
80. Feder HM Jr, Petersen BW, Robertson KL, Rupprecht CE. Rabies: still a uniformly fatal disease? Historical occurrence, epidemiological trends, and paradigm shifts. *Curr Infect Dis Rep* 2012;**14**:408–22.
81. Fehlner-Gardiner C, Rudd R, Donovan D, et al. Comparing ONRAB(R) AND RABORAL V-RG(R) oral rabies vaccine field performance in raccoons and striped skunks, New Brunswick, Canada, and Maine, USA. *J Wildl Dis* 2012;**48**:157–67.
90. Franka R, Wu X, Jackson FR, et al. Rabies virus pathogenesis in relationship to intervention with inactivated and attenuated rabies vaccines. *Vaccine* 2009;**27**:7149–55.
95. Gilbert AT, Petersen BW, Recuenco S, et al. Evidence of rabies virus exposure among humans in the Peruvian Amazon. *Am J Trop Med Hyg* 2012;**87**:206–15.
98. Green AL, Carpenter LR, Dunn JR. Rabies epidemiology, risk assessment, and pre- and post exposure vaccination. *Vet Clin North Am Exot Anim Pract* 2011;**14**:507–18, vii.
101. Hampson K, Cleaveland S, Briggs D. Evaluation of cost-effective strategies for rabies post-exposure vaccination in low-income countries. *PLoS Negl Trop Dis* 2011;**5**:e982.
117. Henderson H, Jackson F, Bean K, et al. Oral immunization of raccoons and skunks with a canine adenovirus recombinant rabies vaccine. *Vaccine* 2009;**27**:7194–7.
120. Hooper DC, Roy A, Kean RB, et al. Therapeutic immune clearance of rabies virus from the CNS. *Future Virol* 2011;**6**: 387–97.
121. Horton DL, McElhinney LM, Marston DA, et al. Quantifying antigenic relationships among the lyssaviruses. *J Virol* 2010;**84**: 11841–8.
123. Hunter M, Johnson N, Hedderwick S, et al. Immunovirological correlates in human rabies treated with therapeutic coma. *J Med Virol* 2010;**82**:1255–65.
125. Jackson AC. Therapy of human rabies. *Adv Virus Res* 2011;**79**: 365–75.
135. Kamoltham T, Thinyounyong W, Khawplod P, et al. Immunogenicity of simulated PCECV postexposure booster doses 1, 3, and 5 years after 2-dose and 3-dose primary rabies vaccination in schoolchildren. *Adv Prev Med* 2011;**2011**:403201.

137. Kaplan B, Echols M. 'One Health': the Rosetta stone for 21st century health and health providers. *Vet Ital* 2009;**45**:377–82.
153. Kuzmin IV, Mayer AE, Niezgoda M, et al. Shimoni bat virus, a new representative of the *Lyssavirus* genus. *Virus Res* 2010;**149**:197–210.
154. Kuzmin IV, Shi M, Orciari LA, et al. Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001-2009. *PLoS Pathog* 2012;**8**.
160. Laothamatas J, Sungkarat W, Hemachudha T. Neuroimaging in rabies. *Adv Virus Res* 2011;**79**:309–27.
162. Lee DN, Papes M, Van Den Bussche RA. Present and potential future distribution of common vampire bats in the Americas and the associated risk to cattle. *PLoS One* 2012;**7**.
163. Lembo T, Attlan M, Bourhy H, et al. Renewed global partnerships and redesigned roadmaps for rabies prevention and control. *Vet Med Int* 2011;**2011**:923149.
164. Li J, Ertel A, Portocarrero C, et al. Postexposure treatment with the live-attenuated rabies virus (RV) vaccine TriGAS triggers the clearance of wild-type RV from the central nervous system (CNS) through the rapid induction of genes relevant to adaptive immunity in CNS tissues. *J Virol* 2012;**86**:3200–10.
170. Maier T, Schwarting A, Mauer D, et al. Management and outcomes after multiple corneal and solid organ transplantations from a donor infected with rabies virus. *Clin Infect Dis* 2010;**50**:1112–19.
171. Manning SE, Rupprecht CE, Fishbein D, et al. Advisory Committee on Immunization Practices Centers for Disease Control and Prevention. Human rabies prevention: United States, 2008—recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**:1–28.
172. Markotter W, Kuzmin I, Rupprecht CE, Nel LH. Phylogeny of Lagos bat virus: challenges for lyssavirus taxonomy. *Virus Res* 2008;**135**:10–21.
173. Marston DA, Ellis RJ, Horton DL, Kuzmin IV, et al. Complete genome sequence of ikoma lyssavirus. *J Virol* 2012;**86**:10242–3.
174. Marston DA, Horton DL, Ngeleja C, et al. Ikoma lyssavirus, highly divergent novel lyssavirus in an African civet. *Emerg Infect Dis* 2012;**18**:664–7.
182. Mills DJ, Lau CL, Fearnley EJ, Weinstein P. The immunogenicity of a modified intradermal pre-exposure rabies vaccination schedule: a case series of 420 travelers. *J Travel Med* 2011;**18**:327–32.
190. Muller T, Dietzschold B, Ertl H, et al. Development of a mouse monoclonal antibody cocktail for post-exposure rabies prophylaxis in humans. *PLoS Negl Trop Dis* 2009;**3**:e542.
198. Pacheco-Caldas E, Wada MY. Protocolo para tratamento de raiva humana no Brasil. *Epidemiol Serv Saude, Brasilia* 2009;**18**:385–94.
202. Patyk K, Turmelle A, Blanton JD, Rupprecht CE. Trends in national surveillance data for bat rabies in the United States: 2001-2009. *Vector Borne Zoonotic Dis* 2012;**12**:666–73.
204. Petersen B, Rupprecht A. Human rabies epidemiology and diagnosis. In: Tkachev S, editor. *Non-flavivirus encephalitis*. New York, NY: InTech; 2011. p. 247–78.
205. Pinero C, Gury Dohmen F, Beltran F, et al. High diversity of rabies viruses associated with insectivorous bats in Argentina: presence of several independent enzootics. *PLoS Negl Trop Dis* 2012;**6**:e1635.
207. Plotkin S, Clark HF, Rupprecht C. Rabies virus. In: Feigin RD, Cherry JD, Kemmler-Harrion, et al, editors. *Feigin & Cherry's texbook of pediatric infectious diseases*. Philadelphia: Saunders; 2009. p. 2494–510.
219. Rubin J, David D, Willoughby RE, et al. Applying the Milwaukee protocol to treat canine rabies in Equatorial Guinea. *Scand J Infect Dis* 2009;**41**:372–5.
224. Rupprecht CE, Turmelle A, Kuzmin IV. A perspective on lyssavirus emergence and perpetuation. *Curr Opin Virol* 2011;**1**:662–70.
225. Rupprecht CE, Briggs D, Brown CM, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2010;**59**:1–9.
233. Schneider MC, Aguilera XP, Barbosa da Silva Junior J, et al. Elimination of neglected diseases in latin america and the Caribbean: a mapping of selected diseases. *PLoS Negl Trop Dis* 2011;**5**:e964.
235. Schneider MC, Romijn PC, Uieda W, et al. Rabies transmitted by vampire bats to humans: an emerging zoonotic disease in Latin America? *Rev Panam Salud Publica* 2009;**25**:260–9.
236. Schnell MJ, McGettigan JP, Wirblich C, Papaneri A. The cell biology of rabies virus: using stealth to reach the brain. *Nat Rev Microbiol* 2010;**8**:51–61.
238. Shantavasinkul P, Wilde H. Postexposure prophylaxis for rabies in resource-limited/poor countries. *Adv Virus Res* 2011;**79**:291–307.
240. Slate D, Algeo TP, Nelson KM, et al. Oral rabies vaccination in North America: opportunities, complexities, and challenges. *PLoS Negl Trop Dis* 2009;**3**:e549.
245. Song M, Tang Q, Wang DM, et al. Epidemiological investigations of human rabies in China. *BMC Infect Dis* 2009;**9**:210.
248. Sudarshan MK, Ashwath Narayana DH, Ravish HS. Is the skin sensitivity test required for administering equine rabies immunoglobulin? *Natl Med J India* 2011;**24**:80–2.
251. Sudarshan MK, Narayana DH, Madhusudana, et al. Evaluation of a one week intradermal regimen for rabies post-exposure prophylaxis: Results of a randomized, open label, active-controlled trial in healthy adult volunteers in India. *Hum Vaccin Immunother* 2012;**8**:1077–81.
262. Tordo N, Foumier A, Jallet C, et al. Canine adenovirus based rabies vaccines. *Dev Biol (Basel)* 2008;**131**:467–76.
272. Wacharapluesadee S, Phumesin P, Supavonwong P, et al. Comparative detection of rabies RNA by NASBA, real-time PCR and conventional PCR. *J Virol Methods* 2011;**175**:278–82.
279. Warrell M. Rabies and African bat lyssavirus encephalitis and its prevention. *Int J Antimicrob Agents* 2010;**36**(Suppl. 1):S47–52.
282. Warrell MJ. Current rabies vaccines and prophylaxis schedules: preventing rabies before and after exposure. *Travel Med Infect Dis* 2012;**10**:1–15.
283. Warrell MJ. Intradermal rabies vaccination: the evolution and future of pre- and post-exposure prophylaxis. *Curr Top Microbiol Immunol* 2012;**351**:139–57.
287. Wasik B, Murphy M. *Rabid: a cultural history of the world's most diabolical virus*. Viking Penguin; 2012.
289. Willoughby RE, Roy-Burman A, Martin KW, et al. Generalised cranial artery spasm in human rabies. *Dev Biol (Basel)* 2008;**131**:367–75.
290. Willoughby RE, Opladen T, Maier T, et al. Tetrahydrobiopterin deficiency in human rabies. *J Inherit Metab Dis* 2009;**32**:65–72.
306. Wu X, Smith TG, Rupprecht CE. From brain passage to cell adaptation: the road of human rabies vaccine development. *Expert Rev Vaccines* 2011;**10**:1597–608.
310. Zinsstag J, Durr S, Penny MA, et al. Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proc Natl Acad Sci U S A* 2009;**106**:14996–5001.
311. Zinsstag J, Durr S, Penny MA, et al. [Transmission dynamics and cost-effectiveness of rabies control in dogs and humans in an African city]. *Med Trop (Mars)* 2011;**71**:596–604.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alvarez L, Fajardo R, Lopez E, et al. Partial recovery from rabies in a nine-year-old boy. *Pediatr Infect Dis J* 1994;**13**:1154–5.
2. Anderson LJ, Sikes RK, Langkop CW, et al. Postexposure trial of a human diploid cell strain rabies vaccine. *J Infect Dis* 1980; **142**:133–8.
3. Anderson MC, Baer H, Frazier DJ, et al. The role of specific IgE and beta-propiolactone in reactions resulting from booster doses of human diploid cell rabies vaccine. *J Allergy Clin Immunol* 1987;**80**:861–8.
4. Anonymous. Rabies vaccine failures. *Lancet* 1988;**1**:917–18.
5. Aoki FY, Rubin ME, Fast MV. Rabies neutralizing antibody in serum of children compared to adults following post-exposure prophylaxis. *Biologicals* 1992;**20**:283–7.
6. Aoki FY, Tyrrell DA, Hill LE. Immunogenicity and acceptability of a human diploid-cell culture rabies vaccine in volunteers. *Lancet* 1975;**1**:660–2.
7. Appelbaum E, Nelson J. Neurological complications following antirabies vaccination. *J Am Med Assoc* 1953;**151**:188–91.
8. Aramburo A, Willoughby RE, Bollen AW, et al. Failure of the Milwaukee protocol in a child with rabies. *Clin Infect Dis* 2011; **53**:572–4.
9. Arguin PM, Krebs JW, Mandel E, et al. Survey of rabies preexposure and postexposure prophylaxis among missionary personnel stationed outside the United States. *J Travel Med* 2000;**7**:10–14.
10. Arko RJ, Schneider LG, Baer GM. Nonfatal canine rabies. *Am J Vet Res* 1973;**34**:937–8.
11. Arya SC. Therapeutic failures with rabies vaccine and rabies immunoglobulin. *Clin Infect Dis* 1999;**29**:1605.
12. Baer GM, Cleary WF. A model in mice for the pathogenesis and treatment of rabies. *J Infect Dis* 1972;**125**:520–7.
13. Baer GM, Moore SA, Shaddock JH, et al. An effective rabies treatment in exposed monkeys: a single dose of interferon inducer and vaccine. *Bull World Health Organ* 1979;**57**:807–13.
14. Bahmanyar M, Fayaz A, Nour-Salehi S, et al. Successful protection of humans exposed to rabies infection: postexposure treatment with the new human diploid cell rabies vaccine and antirabies serum. 1976. *Wilderness Environ Med* 2000;**11**:42–6.
15. Bakker AB, Marissen WE, Kramer, et al. Novel human monoclonal antibody combination effectively neutralizing natural rabies virus variants and individual in vitro escape mutants. *J Virol* 2005;**79**:9062–8.
16. Banyard AC, Hartley M, Fooks AR. Reassessing the risk from rabies: a continuing threat to the UK? *Virus Res* 2010;**152**: 79–84.
17. Banyard AC, Hayman D, Johnson N, et al. Bats and lysaviruses. *Adv Virus Res* 2011;**79**:239–89.
18. Barth R, Gruschkau H, Bijok U, et al. A new inactivated tissue culture rabies vaccine for use in man: evaluation of PCEC-vaccine by laboratory tests. *J Biol Stand* 1984;**12**:29–46.
19. Berlin BS, Mitchell JR, Burgoyne GH, et al. Rhesus diploid rabies vaccine (adsorbed), a new rabies vaccine. II. Results of clinical studies simulating prophylactic therapy for rabies exposure. *JAMA* 1983;**249**:2663–5.
20. Bernard KW, Mallonee J, Wright JC, et al. Preexposure immunization with intradermal human diploid cell rabies vaccine: risks and benefits of primary and booster vaccination. *JAMA* 1987;**257**: 1059–63.
21. Bingham J, Foggin CM, Wandeler AI, Hill FW. The epidemiology of rabies in Zimbabwe.II. Rabies in jackals (*Canis adustus* and *Canis mesomelas*). *Onderstepoort J Vet Res* 1999;**66**:11–23.
22. Bishop DH, Aaslestad HG, Clark HF. Evidence for the sequence homology and genome size of rhabdovirus RNA's. In: Mahy BWJ, Barry RD, editors. *Negative strand viruses*. New York: Academic Press; 1975. p. 259–92.
23. Blanton JD, Hanlon CA, Rupprecht CE. Rabies surveillance in the United States during 2006. *J Am Vet Med Assoc* 2007;**231**: 540–56.
24. Blanton JD, Dyer J, McBrayer J, et al. Rabies surveillance in the United States during 2011. *J Am Vet Med Assoc* 2012;**241**: 712–22.
25. Blanton JD, Palmer D, Dyer J, et al. Rabies surveillance in the United States during 2010. *J Am Vet Med Assoc* 2011;**239**:773–83.
26. Blough HA, Tiffany JM, Aaslestad HG. Lipids of rabies virus and BHK-21 cell membranes. *J Virol* 1977;**21**:950–5.
27. Bonito RF, de Oliveira NM, Nishioka Sde A. Adverse reactions associated with a Fuenzalida-Palacios rabies vaccine: a quasi-experimental study. *Rev Soc Bras Med Trop* 2004;**37**:7–9.
28. Both L, Banyard AC, van Dolleweerd C, et al. Passive immunity in the prevention of rabies. *Lancet Infect Dis* 2012;**12**:397–407.
29. Bourhy H, Dacheux L, Strady C, et al. Rabies in Europe in 2005. *Euro Surveill* 2005;**10**:213–16.
30. Bourhy H, Dautry-Varsat A, Hotez PJ, et al. Rabies, still neglected after 125 years of vaccination. *PLoS Negl Trop Dis* 2010;**4**:e839.
31. Bourhy H, Reynes JM, Dunham EJ, et al. The origin and phylogeography of dog rabies virus. *J Gen Virol* 2008;**89**:2673–81.
32. Briggs DJ, Schwenke JR. Longevity of rabies antibody titre in recipients of human diploid cell rabies vaccine. *Vaccine* 1992;**10**: 125–9.
33. Briggs DJ, Dreesen DW, Nicolay U, et al. Purified chick embryo cell culture rabies vaccine: interchangeability with human diploid cell culture rabies vaccine and comparison of one versus two-dose post-exposure booster regimen for previously immunized persons. *Vaccine* 2000;**19**:1055–60.
34. Brown CM, Conti L, Ettestad P, et al. Compendium of animal rabies prevention and control, 2011. *J Am Vet Med Ass* 2011;**239**: 609–17.
35. Bryceson AD, Greenwood BM, Warrell DA, et al. Demonstration during life of rabies antigen in humans. *J Infect Dis* 1975;**131**: 71–4.
36. Cabasso VJ, Dobkin MB, Roby RE, et al. Antibody response to a human diploid cell rabies vaccine. *Appl Microbiol* 1974;**27**:553–61.
37. Cadoz M, Strady A, Meignier B, et al. Immunisation with canarypox virus expressing rabies glycoprotein. *Lancet* 1992;**339**: 1429–32.
38. Calisher CH, Ellison JA. The other rabies viruses: the emergence and importance of lyssaviruses from bats and other vertebrates. *Travel Med Infect Dis* 2012;**10**:69–79.
39. Centers for Disease Control and Prevention. Rabies in a laboratory worker: New York. *MMWR Morbid Mortal Wkly Rep* 1977;**26**:183–4.
40. Centers for Disease Control and Prevention. Systemic allergic reactions following immunization with human diploid cell rabies vaccine. *MMWR Morb Mortal Wkly Rep* 1984;**33**:185–7.
41. Centers for Disease Control and Prevention. Mass treatment of humans who drank unpasteurized milk from rabid cows: Massachusetts, 1996-1998. *MMWR Morb Mortal Wkly Rep* 1999; **48**:228–9.
42. Centers for Disease Control and Prevention. Update: raccoon rabies epizootic—United States and Canada, 1999. *MMWR Morb Mortal Wkly Rep* 2000;**49**:31–5.
43. Centers for Disease Control and Prevention. Investigation of rabies infections in organ donor and transplant recipients: Alabama, Arkansas, Oklahoma, and Texas, 2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:586–9.
44. Centers for Disease Control and Prevention. Presumptive abortive human rabies: Texas, 2009. *MMWR Morb Mortal Wkly Rep* 2010;**59**:185–90.
45. Centers for Disease Control and Prevention. Recovery of a patient from clinical rabies: California, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:61–5.
46. Celis E, Ou D, Dietzschold B, et al. Recognition of rabies and rabies-related viruses by T cells derived from human vaccine recipients. *J Virol* 1988;**62**:3128–34.
47. Chadli A, Merieux C, Arrouji A, et al. Study on the efficacy of a vaccine produced from rabies virus cultivated on Vero cells. In: Vodopija I, Nicholson KG, Smerdel S, et al, editors. *Improvements in rabies post-exposure treatment*. Zagreb: Zagreb Institute of Public Health; 1985. p. 120–36.
48. Charlton KM. The pathogenesis of rabies and other lyssaviral infections: recent studies. *Curr Top Microbiol Immunol* 1994;**187**: 95–119.
49. Charlton KM, Casey GA. Experimental rabies in skunks: immunofluorescence light and electron microscopic studies. *Lab Invest* 1979;**41**:36–44.
50. Charlton KM, Nadin-Davis S, Casey GA, et al. The long incubation period in rabies: delayed progression of infection in muscle at the site of exposure. *Acta Neuropathol* 1997;**94**:73–7.
51. Chomchay P, Khawplod P, Wilde H. Neutralizing antibodies to rabies following injection of rabies immune globulin into gluteal fat or deltoid muscle. *J Travel Med* 2000;**7**:187–8.

52. Chopy D, Pothlichet J, Lafage M, et al. Ambivalent role of the innate immune response in rabies virus pathogenesis. *J Virol* 2011;**85**:6657–68.
53. Chulasugandha P, Khawplod P, Havanond P, et al. Cost comparison of rabies pre-exposure vaccination with post-exposure treatment in Thai children. *Vaccine* 2006;**24**:1478–82.
54. Chutivongse S, Wilde H, Benjavongkulchai M, et al. Postexposure rabies vaccination during pregnancy: effect on 202 women and their infants. *Clin Infect Dis* 1995;**20**:818–20.
55. Chutivongse S, Wilde H, Fishbein DB, et al. One-year study of the 2-1-1 intramuscular postexposure rabies vaccine regimen in 100 severely exposed Thai patients using rabies immune globulin and Vero cell rabies vaccine. *Vaccine* 1991;**9**:573–6.
56. Clark HF, Prabhakar BS. Rabies. In: Olsen RG, Krakowka GS, Blakesle JR, editors. *Comparative pathobiology of viral disease*. Boca Raton, Fla: CRC Press; 1985. p. 165–214.
57. Clark HF, Vodopija I. Human vaccination against rabies. In: Baer GM, editor. *The natural history of rabies*. Boca Raton, Fla: CRC Press; 1991. p. 571–95.
58. Cleaveland S. Epidemiology and control of rabies: the growing problem of rabies in Africa. Royal Society of Tropical Medicine and Hygiene meeting at Manson House, London, 20 March 1997. *Trans R Soc Trop Med Hyg* 1998;**92**:131–4.
59. Corey L, Hattwick MA, Baer GM, et al. Serum neutralizing antibody after rabies postexposure prophylaxis. *Ann Intern Med* 1976;**85**:170–6.
60. Cox JH, Schneider LG. Prophylactic immunization of humans against rabies by intradermal inoculation of human diploid cell culture vaccine. *J Clin Microbiol* 1976;**3**:96–101.
61. de Kruif J, Bakker AB, Marissen, et al. A human monoclonal antibody cocktail as a novel component of rabies postexposure prophylaxis. *Annu Rev Med* 2007;**58**:359–68.
62. Dean DJ, Baer GM, Thompson WR. Studies on the local treatment of rabies-infected wounds. *Bull World Health Organ* 1963; **28**:477–86.
63. Delpietro HA, Larghi OP, Russo RG. Virus isolation from saliva and salivary glands of cattle naturally infected with paralytic rabies. *Prev Vet Med* 2001;**48**:223–8.
64. Deshmukh RA, Yemul VL. Fatal rabies encephalitis despite post-exposure vaccination in a diabetic patient: a need for use of rabies immune globulin in all post-exposure cases. *J Assoc Physicians India* 1999;**47**:546–7.
65. Dietzschold B. Oligosaccharides of the glycoprotein of rabies virus. *J Virol* 1977;**23**:286–93.
66. Dietzschold B, Ertl HC. New developments in the pre- and post-exposure treatment of rabies. *Crit Rev Immunol* 1991;**10**: 427–39.
67. Dietzschold B, Hooper DC. Human diploid cell culture rabies vaccine (HDCV) and purified chick embryo cell culture rabies vaccine (PCECV) both confer protective immunity against infection with the silver-haired bat rabies virus strain (SHBRV). *Vaccine* 1998;**16**:1656–9.
68. Dietzschold B, Gore M, Casali P, et al. Biological characterization of human monoclonal antibodies to rabies virus. *J Virol* 1990;**64**: 3087–90.
69. Dietzschold B, Kao M, Zheng YM, et al. Delineation of putative mechanisms involved in antibody-mediated clearance of rabies virus from the central nervous system. *Proc Natl Acad Sci U S A* 1992;**89**:7252–6.
70. Dietzschold B, Rupprecht CE, Tollis M, et al. Antigenic diversity of the glycoprotein and nucleocapsid proteins of rabies and rabies-related viruses: implications for epidemiology and control of rabies. *Rev Infect Dis* 1988;**10**(Suppl. 4):S785–98.
71. Dietzschold B, Wang HH, Rupprecht CE, et al. Induction of protective immunity against rabies by immunization with rabies virus ribonucleoprotein. *Proc Natl Acad Sci U S A* 1987;**84**: 9165–9.
72. Dobson A. Raccoon rabies in space and time. *Proc Natl Acad Sci U S A* 2000;**97**:14041–3.
73. Dreesen DW, Fishbein DB, Kemp DT, Brown J. Two-year comparative trial on the immunogenicity and adverse effects of purified chick embryo cell rabies vaccine for pre-exposure immunization. *Vaccine* 1989;**7**:397–400.
74. Drings A, Jallet C, Chambert B, et al. Is there an advantage to including the nucleoprotein in a rabies glycoprotein subunit vaccine? *Vaccine* 1999;**17**:1549–57.
75. Dutta JK, Warrell MJ, Dutta TK. Intradermal rabies immunization for pre- and post-exposure prophylaxis. *Natl Med J India* 1994;**7**:119–22.
76. Dzikwi AA, Kuzmin II, Umoh JU, et al. Evidence of Lagos bat virus circulation among Nigerian fruit bats. *J Wildl Dis* 2010; **46**:267–71.
77. Eng TR, Fishbein DB, Talamante HE, et al. Urban epizootic of rabies in Mexico: epidemiology and impact of animal bite injuries. *Bull World Health Organ* 1993;**71**:615–24.
78. European Centre for Disease Prevention and Control. *Expert consultation on rabies post-exposure prophylaxis*. Stockholm: ECDC; 2009.
79. Fayaz A, Simani S, Janani A, et al. Antibody persistence, 32 years after post-exposure prophylaxis with human diploid cell rabies vaccine (HDCV). *Vaccine* 2011;**29**:3742–5.
80. Feder HM Jr, Petersen BW, Robertson KL, Rupprecht CE. Rabies: still a uniformly fatal disease? Historical occurrence, epidemiological trends, and paradigm shifts. *Curr Infect Dis Rep* 2012;**14**:408–22.
81. Fehlner-Gardiner C, Rudd R, Donovan D, et al. Comparing ONRAB(R) AND RABORAL V-RG(R) oral rabies vaccine field performance in raccoons and striped skunks, New Brunswick, Canada, and Maine, USA. *J Wildl Dis* 2012;**48**:157–67.
82. Fekadu M. Pathogenesis of rabies virus infection in dogs. *Rev Infect Dis* 1988;**10**(Suppl. 4):S678–83.
83. Fekadu M, Shaddock JH, Ekstrom J, et al. An immune stimulating complex (ISCOM) subunit rabies vaccine protects dogs and mice against street rabies challenge. *Vaccine* 1992;**10**:192–7.
84. Fescharek R, Franke V, Samuel MR. Do anaesthetics and surgical stress increase the risk of post-exposure rabies treatment failure? *Vaccine* 1994;**12**:12–13.
85. Fishbein DB, Dreesen DW, Holmes DF, et al. Human diploid cell rabies vaccine purified by zonal centrifugation: a controlled study of antibody response and side effects following primary and booster pre-exposure immunizations. *Vaccine* 1989;**7**:437–42.
86. Fishbein DB, Pacer RE, Holmes DF, et al. Rabies preexposure prophylaxis with human diploid cell rabies vaccine: a dose-response study. *J Infect Dis* 1987;**156**:50–5.
87. Fishbein DB, Sawyer LA, Reid-Sanden FL, et al. Administration of human diploid-cell rabies vaccine in the gluteal area. *N Engl J Med* 1988;**318**:124–5.
88. Fishbein DB, Yenne KM, Dreesen DW, et al. Risk factors for systemic hypersensitivity reactions after booster vaccinations with human diploid cell rabies vaccine: a nationwide prospective study. *Vaccine* 1993;**11**:1390–4.
89. Franka R, Constantine DG, Kuzmin I, et al. A new phylogenetic lineage of rabies virus associated with western pipistrelle bats *(Pipistrellus hesperus)*. *J Gen Virol* 2006;**87**:2309–21.
90. Franka R, Wu X, Jackson FR, et al. Rabies virus pathogenesis in relationship to intervention with inactivated and attenuated rabies vaccines. *Vaccine* 2009;**27**:7149–55.
91. Fu ZF, Jackson AC. Neuronal dysfunction and death in rabies virus infection. *J Neurovirol* 2005;**11**:101–6.
92. Fuenzalida E, Palacios R, Borgono JM. Antirabies antibody response in man to vaccine made from infected suckling-mouse brains. *Bull World Health Organ* 1964;**30**:431–6.
93. Gacouin A, Bourhy H, Renaud JC, et al. Human rabies despite postexposure vaccination. *Eur J Clin Microbiol Infect Dis* 1999; **18**:233–5.
94. Garner WR, Jones DO, Pratt E. Problems associated with rabies preexposure prophylaxis. *JAMA* 1976;**235**:1131–2.
95. Gilbert AT, Petersen BW, Recuenco S, et al. Evidence of rabies virus exposure among humans in the Peruvian Amazon. *Am J Trop Med Hyg* 2012;**87**:206–15.
96. Goudsmit J, Marissen WE, Weldon WC, et al. Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin. *J Infect Dis* 2006; **193**:796–801.
97. Gould AR, Hyatt AD, Lunt R, et al. Characterisation of a novel lyssavirus isolated from pteropid bats in Australia. *Virus Res* 1998; **54**:165–87.
98. Green AL, Carpenter LR, Dunn JR. Rabies epidemiology, risk assessment, and pre- and post exposure vaccination. *Vet Clin North Am Exot Anim Pract* 2011;**14**:507–18, vii.
99. Habel K. Rabies prophylaxis in man. *Public Health Rep* 1957;**72**: 667–73.

100. Habel K, Koprowski H. Laboratory data supporting the clinical trial of anti-rabies serum in persons bitten by a rabid wolf. *Bull World Health Organ* 1955;**13**:773–9.
101. Hampson K, Cleaveland S, Briggs D. Evaluation of cost-effective strategies for rabies post-exposure vaccination in low-income countries. *PLoS Negl Trop Dis* 2011;**5**:e982.
102. Hanlon CA, Niezgoda M, Morrill PA, et al. The incurable wound revisited: progress in human rabies prevention? *Vaccine* 2001;**19**:2273–9.
103. Hattwick MA, Gregg MB. The disease in man. In: Baer GM, editor. *The natural history of rabies*. New York: Academic Press; 1975. p. 281–304.
104. Hattwick MA, Rubin RH, Music S, et al. Postexposure rabies prophylaxis with human rabies immune globulin. *JAMA* 1974; **227**:407–10.
105. Hattwick MA, Weis TT, Stechschulte CJ, et al. Recovery from rabies: a case report. *Ann Intern Med* 1972;**76**:931–42.
106. Haupt W. Rabies: risk of exposure and current trends in prevention of human cases. *Vaccine* 1999;**17**:1742-9.
107. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res* 1961;**25**:585–621.
108. Heick A. Prince Dracula, rabies, and the vampire legend. *Ann Intern Med* 1992;**117**:172–3.
109. Helmick CG, Johnstone C, Sumner J, et al. A clinical study of Merieux human rabies immune globulin. *J Biol Stand* 1982;**10**: 357–67.
110. Hemachudha T. Human rabies: clinical aspects, pathogenesis, and potential therapy. *Curr Top Microbiol Immunol* 1994;**187**:121–43.
111. Hemachudha T, Wacharapluesadee S. Antemortem diagnosis of human rabies. *Clin Infect Dis* 2004;**39**:1085–6.
112. Hemachudha T, Laothamatas J, Rupprecht CE. Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. *Lancet Neurol* 2002;**1**:101–9.
113. Hemachudha T, Griffin DE, Chen WW, et al. Immunologic studies of rabies vaccination-induced Guillain-Barré syndrome. *Neurology* 1988;**38**:375–8.
114. Hemachudha T, Mitrabhakdi E, Wilde H, et al. Additional reports of failure to respond to treatment after rabies exposure in Thailand. *Clin Infect Dis* 1999;**28**:143–4.
115. Hemachudha T, Phanuphak P, Sriwanthana B, et al. Immunologic study of human encephalitic and paralytic rabies: preliminary report of 16 patients. *Am J Med* 1988;**84**:673–7.
116. Hemachudha T, Wacharapluesadee S, Mitrabhakdi E, et al. Pathophysiology of human paralytic rabies. *J Neurovirol* 2005;**11**: 93–100.
117. Henderson H, Jackson F, Bean K, et al. Oral immunization of raccoons and skunks with a canine adenovirus recombinant rabies vaccine. *Vaccine* 2009;**27**:7194–7.
118. Hildreth EA. Prevention of rabies, or the decline of Sirius. *Ann Intern Med* 1963;**58**:883–96.
119. Hooper DC, Morimoto K, Bette M, et al. Collaboration of antibody and inflammation in clearance of rabies virus from the central nervous system. *J Virol* 1998;**72**:3711–19.
120. Hooper DC, Roy A, Kean RB, et al. Therapeutic immune clearance of rabies virus from the CNS. *Future Virol* 2011;**6**:387–97.
121. Horton DL, McElhinney LM, Marston DA, et al. Quantifying antigenic relationships among the lyssaviruses. *J Virol* 2010; **84**:11841–8.
122. Houff SA, Burton RC, Wilson RW, et al. Human-to-human transmission of rabies virus by corneal transplant. *N Engl J Med* 1979;**300**:603–4.
123. Hunter M, Johnson N, Hedderwick S, et al. Immunovirological correlates in human rabies treated with therapeutic coma. *J Med Virol* 2010;**82**:1255–65.
124. Iwasaki Y, Liu DS, Yamamoto T, et al. On the replication and spread of rabies virus in the human central nervous system. *J Neuropathol Exp Neurol* 1985;**44**:185–95.
125. Jackson AC. Therapy of human rabies. *Adv Virus Res* 2011;**79**: 365–75.
126. Jackson AC, Park H. Apoptotic cell death in experimental rabies in suckling mice. *Acta Neuropathol* 1998;**95**:159–64.
127. Jackson AC, Ye H, Phelan CC, et al. Extraneural organ involvement in human rabies. *Lab Invest* 1999;**79**:945–51.
128. Jacob Y, Badrane H, Ceccaldi PE, Tordo N. Cytoplasmic dynein LC8 interacts with lyssavirus phosphoprotein. *J Virol* 2000;**74**: 10217–22.
129. Jaijaroensup W, Limusanno S, Khawplod P, et al. Immunogenicity of rabies postexposure booster injections in subjects who had previously received intradermal preexposure vaccination. *J Travel Med* 1999;**6**:234–7.
130. Jallet C, Jacob Y, Bahloul C, et al. Chimeric lyssavirus glycoproteins with increased immunological potential. *J Virol* 1999;**73**: 225–33.
131. Janis B, Habel K. Rabies in rabbits and mice: protective effect of polyriboinosinic-polyribocytidylic acid. *J Infect Dis* 1972;**125**: 345–52.
132. Johnson N, Phillpotts R, Fooks AR. Airborne transmission of lyssaviruses. *J Med Microbiol* 2006;**55**:785–90.
133. Johnson N, Freuling C, Marston DA, et al. Identification of European bat lyssavirus isolates with short genomic insertions. *Virus Res* 2007;**128**:140–3.
134. Jones RL, Froeschle JE, Atmar RL, et al. Immunogenicity, safety and lot consistency in adults of a chromatographically purified Vero-cell rabies vaccine: a randomized, double-blind trial with human diploid cell rabies vaccine. *Vaccine* 2001;**19**:4635–43.
135. Kamoltham T, Thinyounyong W, Khawplod P, et al. Immunogenicity of simulated PCECV postexposure booster doses 1, 3, and 5 years after 2-dose and 3-dose primary rabies vaccination in schoolchildren. *Adv Prev Med* 2011;**2011**:403201.
136. Kamolvarin N, Tirawatnpong T, Rattanasiwamoke R, et al. Diagnosis of rabies by polymerase chain reaction with nested primers. *J Infect Dis* 1993;**167**:207–10.
137. Kaplan B, Echols M. "One Health": the Rosetta stone for 21st century health and health providers. *Vet Ital* 2009;**45**:377–82.
138. Kaplan MM, Cohen D, Koprowski H, et al. Studies on the local treatment of wounds for the prevention of rabies. *Bull World Health Organ* 1962;**26**:765–75.
139. Kayali U, Mindekem R, Yemadji N, et al. Incidence of canine rabies in N'Djamena, Chad. *Prev Vet Med* 2003;**61**:227–33.
140. Kelly RM, Strick PL. Rabies as a transneuronal tracer of circuits in the central nervous system. *J Neurosci Methods* 2000;**103**: 63–71.
141. Khawplod P, Glueck R, Wilde H, et al. Immunogenicity of purified duck embryo rabies vaccine (Lyssavac-N) with use of the WHO-approved intradermal postexposure regimen. *Clin Infect Dis* 1995;**20**:646–51.
142. Khawplod P, Wilde H, Chomchey P, et al. What is an acceptable delay in rabies immune globulin administration when vaccine alone had been given previously? *Vaccine* 1996;**14**:389–91.
143. Khawplod P, Wilde H, Sirikwin S, et al. Revision of the Thai Red Cross intradermal rabies post-exposure regimen by eliminating the 90-day booster injection. *Vaccine* 2006;**24**:3084–6.
144. Kissi B, Badrane H, Audry L, et al. Dynamics of rabies virus quasispecies during serial passages in heterologous hosts. *J Gen Virol* 1999;**80**(Pt 8):2041–50.
145. Knittel T, Ramadori G, Mayet WJ, et al. Guillain-Barré syndrome and human diploid cell rabies vaccine. *Lancet* 1989;**1**:1334–5.
146. Knobel DL, Cleaveland S, Coleman PG, et al. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 2005;**83**:360–8.
147. Koch FJ, Sagartz JW, Davidson DE, et al. Diagnosis of human rabies by the cornea test. *Am J Clin Pathol* 1975;**63**:509–15.
148. Koprowski H, Black J. Studies on chick-embryo-adapted rabies virus. V. Protection of animals with antiserum and living attenuated virus after exposure to street strain of rabies virus. *J Immunol* 1954;**72**:85–93.
149. Krebs JW, Smith JS, Rupprecht CE, et al. Mammalian reservoirs and epidemiology of rabies diagnosed in human beings in the United States, 1981-1998. *Ann N Y Acad Sci* 2000;**916**:345–53.
150. Kuwert EK, Marcus I, Hoher PG. Neutralizing and complement-fixing antibody responses in pre- and post-exposure vaccinees to a rabies vaccine produced in human diploid cells. *J Biol Stand* 1976;**4**:249–62.
151. Kuwert EK, Werner J, Marcus I, et al. Immunization against rabies with rabies immune globulin, human (RIGH) and a human diploid cell strain (HDCS) rabies vaccine. *J Biol Stand* 1978;**6**: 211–19.
152. Kuzmin IV, Hughes GJ, Botvinkin AD, et al. Phylogenetic relationships of Irkut and West Caucasian bat viruses within the Lyssavirus genus and suggested quantitative criteria based on the N gene sequence for lyssavirus genotype definition. *Virus Res* 2005;**111**:28–43.

153. Kuzmin IV, Mayer AE, Niezgoda M, et al. Shimoni bat virus, a new representative of the *Lyssavirus* genus. *Virus Res* 2010;**149**: 197–210.
154. Kuzmin IV, Shi M, Orciari LA, et al. Molecular inferences suggest multiple host shifts of rabies viruses from bats to mesocarnivores in Arizona during 2001-2009. *PLoS Pathog* 2012;**8**.
155. Lafon M. Rabies virus receptors. *J Neurovirol* 2005;**11**:82–7.
156. Landry P, Lazzaro M, Darioli R. [Comparative immunogenicity of 2 antirabies vaccines in a 2-1-1 post-exposure vaccination schedule]. *Praxis (Bern 1994)* 1998;**87**:1177–9.
157. Lang J, Attanath P, Quiambao B, et al. Evaluation of the safety, immunogenicity, and pharmacokinetic profile of a new, highly purified, heat-treated equine rabies immunoglobulin, administered either alone or in association with a purified, Vero-cell rabies vaccine. *Acta Trop* 1998;**70**:317–33.
158. Lang J, Cetre JC, Picot N, et al. Immunogenicity and safety in adults of a new chromatographically purified Vero-cell rabies vaccine (CPRV): a randomized, double-blind trial with purified Vero-cell rabies vaccine (PVRV). *Biologicals* 1998;**26**:299–308.
159. Lang J, Duong GH, Nguyen VG, et al. Randomised feasibility trial of pre-exposure rabies vaccination with DTP-IPV in infants. *Lancet* 1997;**349**:1663–5.
160. Laothamatas J, Sungkarat W, Hemachudha T. Neuroimaging in rabies. *Adv Virus Res* 2011;**79**:309–27.
161. Laouini D, Kennou MF, Khoufi S, et al. Antibodies to human myelin proteins and gangliosides in patients with acute neuroparalytic accidents induced by brain-derived rabies vaccine. *J Neuroimmunol* 1998;**91**:63–72.
162. Lee DN, Papes M, Van Den Bussche RA. Present and potential future distribution of common vampire bats in the Americas and the associated risk to cattle. *PLoS One* 2012;**7**.
163. Lembo T, Attlan M, Bourhy H, et al. Renewed global partnerships and redesigned roadmaps for rabies prevention and control. *Vet Med Int* 2011;**2011**:923149.
164. Li J, Ertel A, Portocarrero C, et al. Postexposure treatment with the live-attenuated rabies virus (RV) vaccine TriGAS triggers the clearance of wild-type RV from the central nervous system (CNS) through the rapid induction of genes relevant to adaptive immunity in CNS tissues. *J Virol* 2012;**86**:3200–10.
165. Lin FT, Lina N. Developments in the production and application of rabies vaccine for human use in China. *Trop Doct* 2000;**30**: 14–16.
166. Lodmell DL, Ewalt LC. Rabies vaccination: comparison of neutralizing antibody responses after priming and boosting with different combinations of DNA, inactivated virus, or recombinant vaccinia virus vaccines. *Vaccine* 2000;**18**:2394–8.
167. Lodmell DL, Ewalt LC. Post-exposure DNA vaccination protects mice against rabies virus. *Vaccine* 2001;**19**:2468–73.
168. Lodmell DL, Ray NB, Ulrich JT, et al. DNA vaccination of mice against rabies virus: effects of the route of vaccination and the adjuvant monophosphoryl lipid A (MPL). *Vaccine* 2000;**18**: 1059–66.
169. Loofbourow JC, Cabasso VJ, Roby RE, Anuskiewicz W. Rabies immune globulin (human): clinical trials and dose determination. *JAMA* 1971;**217**:1825–31.
170. Maier T, Schwarting A, Mauer D, et al. Management and outcomes after multiple corneal and solid organ transplantations from a donor infected with rabies virus. *Clin Infect Dis* 2010;**50**: 1112–19.
171. Manning SE, Rupprecht CE, Fishbein D, et al. Advisory Committee on Immunization Practices Centers for Disease Control and Prevention. Human rabies prevention: United States, 2008—recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**:1–28.
172. Markotter W, Kuzmin I, Rupprecht CE, et al. Phylogeny of Lagos bat virus: challenges for lyssavirus taxonomy. *Virus Res* 2008;**135**:10–21.
173. Marston DA, Ellis RJ, Horton DL, et al. Complete genome sequence of ikoma lyssavirus. *J Virol* 2012;**86**:10242–3.
174. Marston DA, Horton DL, Ngeleja C, et al. Ikoma lyssavirus, highly divergent novel lyssavirus in an African civet. *Emerg Infect Dis* 2012;**18**:664–7.
175. Maschke M, Kastrup O, Forsting M, et al. Update on neuroimaging in infectious central nervous system disease. *Curr Opin Neurol* 2004;**17**:475–80.
176. Matsumoto S. Electron microscopy of central nervous system infection. In: Baer GM, editor. *The natural history of rabies*. New York: Academic Press; 1975. p. 33–61.
177. Matthews DA, Cummings D, Evelegh C, et al. Development and use of a 293 cell line expressing lac repressor for the rescue of recombinant adenoviruses expressing high levels of rabies virus glycoprotein. *J Gen Virol* 1999;**80**(Pt 2):345–53.
178. McGettigan JP. Experimental rabies vaccines for humans. *Expert Rev Vaccines* 2010;**9**:1177–86.
179. Mertz GJ, Nelson KE, Vithayasai, et al. Antibody responses to human diploid cell vaccine for rabies with and without human rabies immune globulin. *J Infect Dis* 1982;**145**:720–7.
180. Metze K, Feiden W. Demonstration of rabies virus antigen at autopsy. *Hum Pathol* 1993;**24**:930.
181. Meyer JP, Tissot J, Estavoyer JM, et al. [Efficacy and immunogeneity of reduced rabies vaccination schedules in subjects in contact with rabies-infected animals]. *Presse Med* 1992;**21**:319.
182. Mills DJ, Lau CL, Fearnley EJ, et al. The immunogenicity of a modified intradermal pre-exposure rabies vaccination schedule: a case series of 420 travelers. *J Travel Med* 2011;**18**:327–32.
183. Mitmoonpitak C, Tepsumethanon V, Wilde H. Rabies in Thailand. *Epidemiol Infect* 1998;**120**:165–9.
184. Mitrabhakdi E, Shuangshoti S, Wannakrairot P, et al. Difference in neuropathogenetic mechanisms in human furious and paralytic rabies. *J Neurol Sci* 2005;**238**:3–10.
185. Mlot C. Public health: bat researchers dispute rabies policy. *Science* 2000;**287**:2391–2.
186. Moran GJ, Talan DA, Mower W, et al. Appropriateness of rabies postexposure prophylaxis treatment for animal exposures. Emergency ID Net Study Group. *JAMA* 2000;**284**:1001–7.
187. Morgeaux S, Tordo N, Gontier C, Perrin P. Beta-propiolactone treatment impairs the biological activity of residual DNA from BHK-21 cells infected with rabies virus. *Vaccine* 1993;**11**:82–90.
188. Morimoto K, Hooper DC, Carbaugh H, et al. Rabies virus quasispecies: implications for pathogenesis. *Proc Natl Acad Sci U S A* 1998;**95**:3152–6.
189. Morimoto K, Hooper DC, Spitsin S, et al. Pathogenicity of different rabies virus variants inversely correlates with apoptosis and rabies virus glycoprotein expression in infected primary neuron cultures. *J Virol* 1999;**73**:510–18.
190. Muller T, Dietzschold B, Ertl H, et al. Development of a mouse monoclonal antibody cocktail for post-exposure rabies prophylaxis in humans. *PLoS Negl Trop Dis* 2009;**3**:e542.
191. Muller WW. Review of rabies in Europe. *Med Pregl* 1998; **51**(Suppl. 1):9–15.
192. Murphy FA. Rabies pathogenesis. *Arch Virol* 1977;**54**:279–97.
193. Murphy FA, Bauer SP. Early street rabies virus infection in striated muscle and later progression to the central nervous system. *Intervirology* 1974;**3**:256–68.
194. Nadin-Davis SA, Velez J, Malaga C, et al. A molecular epidemiological study of rabies in Puerto Rico. *Virus Res* 2008;**131**:8–15.
195. Nathanson N, Gonzalez SF. Immune response to rabies virus. In: Baer GM, editor. *The natural history of rabies*. Boca Raton, Fla: CRC Press; 1991. p. 145–61.
196. Nicholson KG. Modern vaccines: rabies. *Lancet* 1990;**335**: 1201–5.
197. Nunberg JH, Doyle MV, York SM, et al. Interleukin 2 acts as an adjuvant to increase the potency of inactivated rabies virus vaccine. *Proc Natl Acad Sci U S A* 1989;**86**:4240–3.
198. Pacheco-Caldas E, Wada MY. Protocolo para tratamento de raiva humana no Brasil. *Epidemiol Serv Saude, Brasilia* 2009;**18**:385–94.
199. Pape WJ, Fitzsimmons TD, Hoffman RE. Risk for rabies transmission from encounters with bats, Colorado, 1977-1996. *Emerg Infect Dis* 1999;**5**:433–7.
200. Pappaioanou M, Fishbein DB, Dreesen DW, et al. Antibody response to preexposure human diploid-cell rabies vaccine given concurrently with chloroquine. *N Engl J Med* 1986;**314**:280–4.
201. Pastoret PP, Brochier B. Epidemiology and elimination of rabies in western Europe. *Vet J* 1998;**156**:83–90.
202. Patyk K, Turmelle A, Blanton JD, et al. Trends in national surveillance data for bat rabies in the United States: 2001-2009. *Vector Borne Zoonotic Dis* 2012;**12**:666–73.
203. Perrin P, Jacob Y, Aguilar-Setien A, et al. Immunization of dogs with a DNA vaccine induces protection against rabies virus. *Vaccine* 1999;**18**:479–86.

204. Petersen B, Rupprecht A. Human rabies epidemiology and diagnosis. In: Tkachev S, editor. *Non-flavivirus encephalitis*. New York, NY: InTech; 2011. p. 247–78.
205. Pinero C, Gury Dohmen F, Beltran F, et al. High diversity of rabies viruses associated with insectivorous bats in Argentina: presence of several independent enzootics. *PLoS Negl Trop Dis* 2012;**6**:e1635.
206. Piyasirisilp S, Schmeckpeper BJ, Chandanayingyong D, et al. Association of HLA and T-cell receptor gene polymorphisms with Semple rabies vaccine-induced autoimmune encephalomyelitis. *Ann Neurol* 1999;**45**:595–600.
207. Plotkin S, Clark HF, Rupprecht C. Rabies virus. In: Feigin RD, Cherry, JD, Kemmler-Harrion, et al, editors. *Feigin & Cherry's texbook of pediatric infectious diseases*. Philadelphia: Saunders; 2009. p. 2494–510.
208. Plotkin SA, Clark HF. Prevention of rabies in man. *J Infect Dis* 1971;**123**:227–40.
209. Plotkin SA, Wiktor TJ, Koprowski H, et al. Immunization schedules for the new human diploid cell vaccine against rabies. *Am J Epidemiol* 1976;**103**:75–80.
210. Plotkin SA, Wiktor T. Rabies vaccination. *Annu Rev Med* 1978;**29**:583–91.
211. Plotkin SA, Koprowski H. Phobia of hydrophobia justified. *N Engl J Med* 1979;**300**:620–2.
212. Porras C, Barboza JJ, Fuenzalida, et al. Recovery from rabies in man. *Ann Intern Med* 1976;**85**:44–8.
213. Prosniak M, Faber M, Hanlon CA, et al. Development of a cocktail of recombinant-expressed human rabies virus-neutralizing monoclonal antibodies for postexposure prophylaxis of rabies. *J Infect Dis* 2003;**188**:53–6.
214. Quiambao BP, Dimaano EM, Ambas C, et al. Reducing the cost of post-exposure rabies prophylaxis: efficacy of 0.1 ml PCEC rabies vaccine administered intradermally using the Thai Red Cross post-exposure regimen in patients severely exposed to laboratory-confirmed rabid animals. *Vaccine* 2005;**23**:1709–14.
215. Raux H, Flamand A, Blondel D. Interaction of the rabies virus P protein with the LC8 dynein light chain. *J Virol* 2000;**74**: 10212–16.
216. Ronsholt L, Sorensen KJ, Bruschke CJ, et al. Clinically silent rabies infection in (zoo) bats. *Vet Rec* 1998;**142**:519–20.
217. Rosanoff E, Tint H. Responses to human diploid cell rabies vaccine: neutralizing antibody responses of vaccinees receiving booster doses of human diploid cell rabies vaccine. *Am J Epidemiol* 1979;**110**:322–7.
218. Rotz LD, Hensley JA, Rupprecht CE, et al. Large-scale human exposures to rabid or presumed rabid animals in the United States: 22 cases (1990-1996). *J Am Vet Med Assoc* 1998;**212**:1198–200.
219. Rubin J, David D, Willoughby RE, et al. Applying the Milwaukee protocol to treat canine rabies in Equatorial Guinea. *Scand J Infect Dis* 2009;**41**:372–5.
220. Rubin RH, Sikes RK, Gregg MB. Human rabies immune globulin: clinical trials and effects on serum anti-globulins. *JAMA* 1973;**224**:871–4.
221. Rubin RH, Hattwick MA, Jones S, et al. Adverse reactions to duck embryo rabies vaccine: range and incidence. *Ann Intern Med* 1973;**78**:643–9.
222. Rupprecht C, Dietzschold B, Wunner WH, et al. Antigenic relationship of lyssaviruses. In: Baer GM, editor. *The natural history of rabies*. Boca Raton, Fla: CRC Press; 1991.
223. Rupprecht CE, Hanlon CA, Slate D. Oral vaccination of wildlife against rabies: opportunities and challenges in prevention and control. *Dev Biol (Basel)* 2004;**119**:173–84.
224. Rupprecht CE, Turmelle A, Kuzmin IV. A perspective on lyssavirus emergence and perpetuation. *Curr Opin Virol* 2011;**1**: 662–70.
225. Rupprecht CE, Briggs D, Brown CM, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2010;**59**:1–9.
226. Rupprecht CE, Hamir AN, Johnston DH, et al. Efficacy of a vaccinia-rabies glycoprotein recombinant virus vaccine in raccoons *(Procyon lotor)*. *Rev Infect Dis* 1988;**10**(Suppl. 4):S803–9.
227. Rupprecht CE, Wiktor TJ, Johnston DH, et al. Oral immunization and protection of raccoons (Procyon lotor) with a vaccinia-rabies glycoprotein recombinant virus vaccine. *Proc Natl Acad Sci U S A* 1986;**83**:7947–50.
228. Sabchareon A, Lang J, Attanath P, et al. A new Vero cell rabies vaccine: results of a comparative trial with human diploid cell rabies vaccine in children. *Clin Infect Dis* 1999;**29**:141–9.
229. Sabeta CT, Markotter W, Mohale DK, et al. Mokola virus in domestic mammals, South Africa. *Emerg Infect Dis* 2007;**13**: 1371–3.
230. Satpathy DM, Sahu T, Behera TR. Equine rabies immunoglobulin: a study on its clinical safety. *J Indian Med Assoc* 2005;**103**:238, 241–2.
231. Schlenska GK. Neurological complications following rabies duck embryo vaccination. *J Neurol* 1976;**214**:71–4.
232. Schneider LG. Spread of virus from the central nervous system. In: Baer GM, editor. *The natural history of rabies*. New York: Academic Press; 1975. p. 273–301.
233. Schneider MC, Aguilera XP, Barbosa da Silva J Jr, et al. Elimination of neglected diseases in latin america and the Caribbean: a mapping of selected diseases. *PLoS Negl Trop Dis* 2011;**5**:e964.
234. Schneider MC, Belotto A, Ade MP, et al. Current status of human rabies transmitted by dogs in Latin America. *Cad Saude Publica* 2007;**23**:2049–63.
235. Schneider MC, Romijn PC, Uieda W, et al. Rabies transmitted by vampire bats to humans: an emerging zoonotic disease in Latin America? *Rev Panam Salud Publica* 2009;**25**:260–9.
236. Schnell MJ, McGettigan JP, Wirblich C, et al. The cell biology of rabies virus: using stealth to reach the brain. *Nat Rev Microbiol* 2010;**8**:51–61.
237. Schumacher CL, Dietzschold B, Ertl HC, et al. Use of mouse anti-rabies monoclonal antibodies in postexposure treatment of rabies. *J Clin Invest* 1989;**84**:971–5.
238. Shantavasinkul P, Wilde H. Postexposure prophylaxis for rabies in resource-limited/poor countries. *Adv Virus Res* 2011;**79**: 291–307.
239. Shaughnessy HJ, Zichis J. Treatment of wounds inflicted by rabid animals. *Bull World Health Organ* 1954;**10**:805–13.
240. Slate D, Algeo TP, Nelson KM, et al. Oral rabies vaccination in north america: opportunities, complexities, and challenges. *PLoS Negl Trop Dis* 2009;**3**:e549.
241. Slate D, Rupprecht CE, Rooney JA, et al. Status of oral rabies vaccination in wild carnivores in the United States. *Virus Res* 2005;**111**:68–76.
242. Smith JS, Orciari LA, Yager PA, et al. Epidemiologic and historical relationships among 87 rabies virus isolates as determined by limited sequence analysis. *J Infect Dis* 1992;**166**:296–307.
243. Smith WB, Blenden DC, Fuh TH, et al. Diagnosis of rabies by immunofluorescent staining of frozen sections of skin. *J Am Vet Med Assoc* 1972;**161**:1495–501.
244. Sokol F. Recent advances in microbiology. In: Perez-Miravete A, Pelez D, editors. *Xth International Congress of Microbiology*. Mexico City: Association Mexicana de Microbiologia; 1971. p. 551–62.
245. Song M, Tang Q, Wang DM, et al. Epidemiological investigations of human rabies in China. *BMC Infect Dis* 2009;**9**:210.
246. Srinivasan A, Burton EC, Kuehnert MJ, et al. Rabies in Transplant Recipients Investigation. Transmission of rabies virus from an organ donor to four transplant recipients. *N Engl J Med* 2005; **352**:1103–11.
247. Steele JH. History of rabies. In: Baer GM, editor. *The natural history of rabies*. New York: Academic Press; 1975. p. 1–29.
248. Sudarshan MK, Ashwath Narayana DH, Ravish HS. Is the skin sensitivity test required for administering equine rabies immunoglobulin? *Natl Med J India* 2011;**24**:80–2.
249. Sudarshan MK, Madhusudana SN, Mahendra BJ, et al. Post exposure rabies prophylaxis with purified Vero cell rabies vaccine: a study of immunoresponse in pregnant women and their matched controls. *Indian J Public Health* 1999;**43**:76–8.
250. Sugamata M, Miyazawa M, Mori S, et al. Paralysis of street rabies virus-infected mice is dependent on T lymphocytes. *J Virol* 1992; **66**:1252–60.
251. Sudarshan MK, Narayana DH, Madhusudana, et al. Evaluation of a one week intradermal regimen for rabies post-exposure prophylaxis: results of a randomized, open label, active-controlled trial in healthy adult volunteers in India. *Hum Vaccin Immunother* 2012;**8**.
252. Suntharasamai P, Chanthavanich P, Warrell MJ, et al. Purified Vero cell rabies vaccine and human diploid cell strain vaccine: comparison of neutralizing antibody responses to post-exposure regimens. *J Hyg (Lond)* 1986;**96**:483–9.

253. Suntharasamai P, Warrell MJ, Warrell DA, et al. Early antibody responses to rabies post-exposure vaccine regimens. *Am J Trop Med Hyg* 1987;**36**:160–5.
254. Suwansrinon K, Wilde H, Benjavongkulchai M, et al. Survival of neutralizing antibody in previously rabies vaccinated subjects: a prospective study showing long lasting immunity. *Vaccine* 2006;**24**: 3878–80.
255. Swanson MC, Rosanoff E, Gurwith M, et al. IgE and IgG antibodies to beta-propiolactone and human serum albumin associated with urticarial reactions to rabies vaccine. *J Infect Dis* 1987;**155**:909–13.
256. Tantawichien T, Benjavongkulchai M, Limsuwan K, et al. Antibody response after a four-site intradermal booster vaccination with cell-culture rabies vaccine. *Clin Infect Dis* 1999;**28**:1100–3.
257. Tepsumethanon V, Lumlertdacha B, Mitmoonpitak C, et al. Survival of naturally infected rabid dogs and cats. *Clin Infect Dis* 2004;**39**:278–80.
258. Thisyakorn U, Pancharoen C, Ruxrungtham K, et al. Safety and immunogenicity of preexposure rabies vaccination in children infected with human immunodeficiency virus type 1. *Clin Infect Dis* 2000;**30**:218.
259. Thisyakorn U, Pancharoen C, Wilde H. Immunologic and virologic evaluation of HIV-1-infected children after rabies vaccination. *Vaccine* 2001;**19**:1534–7.
260. Thraenhart O, Kreuzfelder E, Hillebrandt M, et al. Long-term humoral and cellular immunity after vaccination with cell culture rabies vaccines in man. *Clin Immunol Immunopathol* 1994;**71**: 287–92.
261. Tollis M, Dietzschold B, Volia CB, et al. Immunization of monkeys with rabies ribonucleoprotein (RNP) confers protective immunity against rabies. *Vaccine* 1991;**9**:134–6.
262. Tordo N, Foumier A, Jallet C, et al. Canine adenovirus based rabies vaccines. *Dev Biol (Basel)* 2008;**131**:467–76.
263. Tordo N, Poch O, Ermine A, et al. Completion of the rabies virus genome sequence determination: highly conserved domains among the L (polymerase) proteins of unsegmented negative-strand RNA viruses. *Virology* 1988;**165**:565–76.
264. Tsiang H. Pathophysiology of rabies virus infection of the nervous system. *Adv Virus Res* 1993;**42**:375–412.
265. Turner GS. A review of the world epidemiology of rabies. *Trans R Soc Trop Med Hyg* 1976;**70**:175–8.
266. Turner GS. Brief review of diagnosis, management and immunotherapy of rabies. *Trop Doct* 1982;**12**:204–7.
267. Ubol S, Sukwattanapan C, Utaisincharoen P. Rabies virus replication induces Bax-related, caspase dependent apoptosis in mouse neuroblastoma cells. *Virus Res* 1998;**56**:207–15.
268. Veeraghaven N. *Scientific report for 1968 of the Pasteur Institute of Southern India*. India: Madras; 1969.
269. Veeraraghavan N, Gajanana A, Rangasami R, et al. *Studies on the salivary excretion of rabies virus by the dog from Suandai*. Coonoor: Pasteur Institute; 1970. p. 68–70.
270. Vodopija I, Baklaic Z, Vodopija R. Rabipur: a reliable vaccine for rabies protection. *Vaccine* 1999;**17**:1739-41.
271. Vodopija I, Sureau P, Lafon M, et al. An evaluation of second generation tissue culture rabies vaccines for use in man: a four-vaccine comparative immunogenicity study using a pre-exposure vaccination schedule and an abbreviated 2-1-1 postexposure schedule. *Vaccine* 1986;**4**:245–8.
272. Wacharapluesadee S, Phumesin P, Supavonwong P, et al. Comparative detection of rabies RNA by NASBA, real-time PCR and conventional PCR. *J Virol Methods* 2011;**175**:278–82.
273. Wallerstein C. Rabies cases increase in the Philippines. *BMJ* 1999;**318**:1306.
274. Wang XJ, Lang J, Tao XR, et al. Immunogenicity and safety of purified Vero-cell rabies vaccine in severely rabies-exposed patients in China. *Southeast Asian J Trop Med Public Health* 2000;**31**:287–94.
275. Warner CK, Whitfield SG, Fekadu M, Ho H. Procedures for reproducible detection of rabies virus antigen mRNA and genome in situ in formalin-fixed tissues. *J Virol Methods* 1997;**67**:5–12.
276. Warrell DA. The clinical picture of rabies in man. *Trans R Soc Trop Med Hyg* 1976;**70**:188–95.
277. Warrell DA, Warrell MJ. Human rabies and its prevention: an overview. *Rev Infect Dis* 1988;**10**(Suppl. 4):S726–31.
278. Warrell DA, Davidson NM, Pope HM, et al. Pathophysiologic studies in human rabies. *Am J Med* 1976;**60**:180–90.
279. Warrell M. Rabies and African bat lyssavirus encephalitis and its prevention. *Int J Antimicrob Agents* 2010;**36**(Suppl. 1):S47–52.
280. Warrell MJ. Human deaths from cryptic bat rabies in the USA. *Lancet* 1995;**346**:65–6.
281. Warrell MJ. The challenge to provide affordable rabies post-exposure treatment. *Vaccine* 2003;**21**:706–9.
282. Warrell MJ. Current rabies vaccines and prophylaxis schedules: preventing rabies before and after exposure. *Travel Med Infect Dis* 2012;**10**:1–15.
283. Warrell MJ. Intradermal rabies vaccination: the evolution and future of pre- and post-exposure prophylaxis. *Curr Top Microbiol Immunol* 2012;**351**:139–57.
284. Warrell MJ, Nicholson KG, Warrell DA, et al. Economical multiple-site intradermal immunisation with human diploid-cell-strain vaccine is effective for post-exposure rabies prophylaxis. *Lancet* 1985;**1**:1059–62.
285. Warrell MJ, Warrell DA, Suntharasamai P, et al. An economical regimen of human diploid cell strain anti-rabies vaccine for post-exposure prophylaxis. *Lancet* 1983;**2**:301–4.
286. Warrington RJ, Martens CJ, Rubin M, et al. Immunologic studies in subjects with a serum sickness-like illness after immunization with human diploid cell rabies vaccine. *J Allergy Clin Immunol* 1987;**79**:605–10.
287. Wasik B, Murphy M. *Rabid: a cultural history of the world's most diabolical virus*. Viking Penguin; 2012.
288. Weiland F, Cox JH, Meyer S, et al. Rabies virus neuritic paralysis: immunopathogenesis of nonfatal paralytic rabies. *J Virol* 1992;**66**: 5096–9.
289. Wiktor TJ, Koprowski H. [Local action of various drugs on rabies infection in mice]. *Bull World Health Organ* 1963;**28**:487–94.
290. Wiktor TJ, Koprowski H. Monoclonal antibodies against rabies virus produced by somatic cell hybridization: detection of antigenic variants. *Proc Natl Acad Sci U S A* 1978;**75**:3938–42.
291. Wiktor TJ, Koprowski H. Antigenic variants of rabies virus. *J Exp Med* 1980;**152**:99–112.
292. Wiktor TJ, Fernandes MV, Koprowski H. Cultivation of rabies virus in human diploid cell strain Wi-38. *J Immunol* 1964;**93**: 353–66.
293. Wiktor TJ, Plotkin SA, Grella DW. Human cell culture rabies vaccine: antibody response in man. *JAMA* 1973;**224**:1170–1.
294. Wiktor TJ, Gyorgy E, Schlumberger D, et al. Antigenic properties of rabies virus components. *J Immunol* 1973;**110**:269–76.
295. Wilde H, Chutivongse S. Equine rabies immune globulin: a product with an undeserved poor reputation. *Am J Trop Med Hyg* 1990;**42**:175–8.
296. Wilde H, Chomchey P, Prakongsri S, et al. Safety of equine rabies immune globulin. *Lancet* 1987;**2**:1275.
297. Wilde H, Chomchey P, Prakongsri S, et al. Adverse effects of equine rabies immune gobulin. *Vaccine* 1989;**7**:10–11.
298. Wilde H, Chomchey P, Punyaratabandhu P, et al. Purified equine rabies immune globulin: a safe and affordable alternative to human rabies immune globulin. *Bull World Health Organ* 1989;**67**: 731–6.
299. Wilde H, Sirikawin S, Sabcharoen A, et al. Failure of postexposure treatment of rabies in children. *Clin Infect Dis* 1996;**22**:228–32.
300. Willoughby RE, Opladen T, Maier T, et al. Tetrahydrobiopterin deficiency in human rabies. *J Inherit Metab Dis* 2009;**32**:65–72.
301. Willoughby RE, Roy-Burman A, Martin KW, et al. Generalised cranial artery spasm in human rabies. *Dev Biol (Basel)* 2008;**131**: 367–75.
302. Willoughby RE Jr, Tieves KS, Hoffman GM, et al. Survival after treatment of rabies with induction of coma. *N Engl J Med* 2005;**352**:2508–14.
303. WHO. *Guidelines for dog rabies control*. Geneva World Health Organization; 1984.
304. WHO. *WHO expert consultation on rabies*. First report. WHO tech rep series no 931. Geneva: WHO; 2004. p. 121.
305. WHO. WHO Expert consultation on rabies. *World Health Organ Tech Rep Ser* 2005;**931**:1–88, back cover.
306. Wu X, Smith TG, Rupprecht CE. From brain passage to cell adaptation: the road of human rabies vaccine development. *Expert Rev Vaccines* 2011;**10**:1597–608.
307. Xiang Z, Ertl HC. Induction of mucosal immunity with a replication-defective adenoviral recombinant. *Vaccine* 1999;**17**: 2003–8.

308. Xiang ZQ, Spitalnik S, Tran M, et al. Vaccination with a plasmid vector carrying the rabies virus glycoprotein gene induces protective immunity against rabies virus. *Virology* 1994;**199**:132–40.
309. Xuan X, Tuchiya K, Sato I, et al. Biological and immunogenic properties of rabies virus glycoprotein expressed by canine herpesvirus vector. *Vaccine* 1998;**16**:969–76.
310. Zinsstag J, Durr S, Penny MA, et al. Transmission dynamics and economics of rabies control in dogs and humans in an African city. *Proc Natl Acad Sci U S A* 2009;**106**:14996–5001.
311. Zinsstag J, Durr S, Penny MA, et al. [Transmission dynamics and cost-effectiveness of rabies control in dogs and humans in an African city]. *Med Trop (Mars)* 2011;**71**:596–604.

SUBSECTION 9 *ARENAVIRIDAE AND FILOVIRIDAE*

CHAPTER 184

Lymphocytic Choriomeningitis Virus

Rémi N. Charrel • Xavier de Lamballerie

Lymphocytic choriomeningitis virus (LCMV) is maintained in nature through persistent infection of its reservoir host, the common house mouse (*Mus musculus*). Human infection occurs through direct contact with infected rodents or by inhaling infectious rodent excreta or secreta. Although infection with LCMV usually is asymptomatic or mild and self-limiting, it can be severe and manifest as meningitis and encephalitis. Infection during pregnancy may cause abortion or congenital malformations.

HISTORY

In the 1930s, LCMV was discovered, at about the same time and independently, in three different localities in the United States. Armstrong and Lillie encountered the agent in a monkey when they passaged a recent isolate of St. Louis encephalitis virus.[6,7] In 1935, Rivers and Scott[52] described the first two cases of human infection with LCMV. Both these patients developed febrile illnesses characterized by headache, vomiting, and stiff neck; lumbar puncture revealed 720 and 1700 cells/mm^3, nearly all of which were lymphocytes. These investigators subsequently isolated five strains from patients with meningitis.[52] Traub[57] revealed the virus in a colony of albino mice. Mice were incriminated in these first isolations, and later observations proved beyond a doubt that *M. musculus* is the principal reservoir of LCMV in nature. However, not until 2007 was definitive evidence presented that LCMV-infected rodents caused disease in humans, as established by genetic comparative analysis.[28] For some time after its discovery, LCMV was regarded as the sole etiologic agent of Wallgren acute aseptic meningitis. However, it became clear that Wallgren syndrome had a multitude of viral causes, among which LCMV was of little relevance. In the years after other etiologic agents of acute aseptic meningitis, such as enteroviruses, mumps, or herpesviruses were identified, LCMV progressively disappeared from the sight of virologists and general practitioners. This situation is exemplified by the low numbers of human cases or outbreaks of LCMV infection reported in the medical literature (<60 articles). Meanwhile, LCMV was used as an excellent model for the study of a variety of phenomena of biologic and medical relevance, such as immunologic tolerance, viral immunopathology, slow viral diseases, and latent viral infections, the last demonstrated by the large number of scientific articles on that topic (>3200 references in the PubMed bibliographic database found using "lymphocytic choriomeningitis" as the search criterion).

EPIDEMIOLOGY

LCMV belongs to the genus *Arenavirus*, family *Arenaviridae*. *M. musculus* (the common house mouse) constitutes the reservoir of LCMV in nature, but hamsters also can carry the virus. Humans usually become infected through direct contact with infected rodents or by inhaling infectious rodent excreta or secreta during occupational exposure (laboratory workers, rodent sellers) or household exposure (pet owners). The epidemiologic features vary when animals other than *M. musculus* are involved. Thus, LCMV transmitted from Syrian hamsters has occurred in areas where infected mice are not found. Altogether, 47 human cases have been traced to these pets and may have caused family outbreaks but no epidemics.

Human infection with LCMV is a rare occurrence. Specific tests conducted with diagnostic materials from several large hospitals revealed an incidence seldom exceeding one case per year.[53] Because *M. musculus* is the reservoir from which most human infections can be traced, efforts were made to clarify the geographic distribution of LCMV-carrying mice. Of 1795 house mice trapped in Germany, 65 carried the virus.[1] In 44 of 376

trapping areas, infected mice were found; these 44 positive sites were unevenly distributed, with a majority located in northern and northwestern Germany. In a later serologic survey, the proportion of people with LCMV-specific antibodies was significantly higher in rural areas where infected mice were known to live as compared with zoonosis-free districts. In areas where LCMV-carrier mice are frequently found, 9.1 percent of the rural population had neutralizing antibodies. In contrast, only 1.2 percent of the people residing in southern Germany, which is essentially free of LCMV-carrying mice, had neutralizing antibodies. Extrapolating from these statistics, approximately 1,000 persons are newly infected each year in Germany. Thus, researchers estimate that in western Germany, at least 72,000 persons must have had an infection, based on an average life expectancy of 70 years and an approximate rate of new cases of 1,000 per year. If this estimation is correct, most infections are not diagnosed and many remain unapparent.

The occurrence of LCMV infection in persons from homes where mice have been proved to be carriers of the virus is well known.[7] Researchers have suggested that small, persistent zoonotic foci of infection exist, on the basis of the known natural history of the disease in mice and the circumscribed range of activity of mice. The percentage of infected house mice apparently varies in different communities, and it has been recorded at 21.5 percent in Washington, D.C.[4] and at 4 percent in Boston and New York.[37,61] However, because infected mouse colonies are dispersed unevenly, the proportion of infected animals in a given infected colony can be high, up to 95 percent.[28,31]

Many human cases have been reported in the context of occupational exposure to rodent colonies.* Outbreaks and isolated cases of LCMV infections were reported in laboratory personnel having contact with hamsters, not mice.[12,18,41,42]

The study by real-time polymerase chain reaction (RT-PCR) assay of 130 samples of cerebrospinal fluid (CSF) from patients with acute aseptic meningitis in Switzerland showed no cases of acute infection with LCMV.[22] This suggests that LCMV infections are extremely rare in hospitalized patients. Until 2005, no case of person-to-person transmission had been documented and the general consensus was that LCMV could infect humans only after direct or indirect contact with an infected rodent. The more recent evidence that LCMV can be transmitted through organ donation should lead physicians to evoke LCMV as a possible cause of infection in the absence of established close contact with mice or hamsters.[16,30] In a report of LCMV infection developing after organ transplantation, the fatality rate was much higher (seven deaths in eight cases) than previously noted, perhaps a reflection of the immunosuppressive regimens required by these transplant recipients.[30] Another, similar episode occurred in Australia, where three patients died after receiving an organ transplant from the same donor. Next-generation sequencing provided clues about possible involvement of an arenavirus related to LCMV.[39,50]

*References 5, 12, 14, 15, 25, 27, 33-35, 40, 44, 58.

On April 15, 2008, an organ procurement organization notified the Centers for Disease Control and Prevention (CDC) of severe illness in two kidney transplant recipients from a common donor; at the time of notification, one of the recipients had died. Samples from the donor and both recipients were tested at the CDC; on April 22, test results revealed evidence of acute LCMV infection in the donor and both recipients. This report summarizes the results of the subsequent public health investigation.[17]

Serologic studies conducted in healthy populations of large cities either by immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) found immunoglobulin G (IgG) antibody prevalence varying between 0.3 and 4.7 percent: 0.3 percent in Marseille, France[23]; 1.7 percent in Spain by IFA[43]; 3.3 percent in Argentina[51]; and 4.7 percent in Baltimore.[21] A study conducted in a region where murine typhus is endemic in Croatia reported an unexpectedly high prevalence, 36 percent, through IFA.[26]

Serologic evidence of four acute LCMV infections was retrospectively reported in Spain in serum and CSF samples from 341 patients.[24]

CLINICAL MANIFESTATIONS

In most cases, LCMV infection is minimally symptomatic or asymptomatic and consists of an influenza-like illness with fever, headache, myalgia, fatigue, and general malaise. These symptoms are self-limited and resolve after a few days. These mild illnesses, which usually do not motivate patients to see a physician, most likely represent most LCMV infections, but the exact proportion is unknown. Historical articles reported that respiratory symptoms occur in LCMV infections. In a certain (but unknown) proportion of cases, a second phase is observed during which neurologic manifestations, such as meningitis, encephalitis, or even acquired hydrocephalus, can be recorded.

In 1942, Farmer and Janeway[29] proposed the following classification of LCM into four clinical categories:

1. *Inapparent and subclinical infections:* Immunologic studies suggested that these forms are quite common: 11 percent of 2000 sera tested contained neutralizing antibodies against LCMV.[3]
2. *Nonmeningeal form, non–central nervous system (CNS) infection, systemic infection, influenza-like infection:* This form consists of two stages: systemic illness, then convalescence. The incidence probably is underestimated because of the lack of laboratory documentation and the absence of specific or suggestive signs. Only vague assumptions can be made concerning the number of underdiagnosed cases. The diagnosis of LCMV usually is not suspected unless attention is directed to signs and symptoms suggestive of meningoencephalitis, although researchers have shown that generalized systemic infection may occur without any evidence of involvement of the CNS.[55] How often the disease manifests in this form or how often apparently asymptomatic infection occurs is not known. The

demonstration of neutralizing antibodies in the blood of as many as 10 percent of the adult population suggests that infection with LCMV may occur more frequently than is suspected.[59]

3. *Lymphocytic choriomeningitis, meningeal form:* This form usually consists of three stages: systemic illness, meningitis (possibly delayed up to 2 to 3 weeks), and convalescence.[8]
4. *Lymphocytic choriomeningitis, encephalomyelitic form:* This form has three stages: systemic illness, encephalitis (possibly delayed), and convalescence.

Other CNS manifestations such as transverse myelitis, Guillain-Barré–type syndrome, and transient or permanent acquired hydrocephalus can be associated with LCMV infection.[10] LCMV-associated acquired hydrocephalus has been described only five times since the virus was discovered.[9,19,38,56] In all cases, the clinical picture was severe and patients required ventriculostomy.

A fifth category (congenital LCM) should be added to the previous classification, corresponding to fetal consequences of LCMV infection during pregnancy. LCMV is a fetal teratogen. It can cause hydrocephalus, microcephaly or macrocephaly, intracranial calcifications, chorioretinitis, and nonimmune hydrops. More than 50 cases of congenital LCMV infection have been documented since 1955, with more than two thirds of these cases diagnosed since 1993. Chorioretinitis and hydrocephalus are the predominant characteristics among children diagnosed with congenital LCMV infection.[36] LCMV was isolated for the first time in the CSF of a congenitally infected infant, who had congenital hydrocephalus caused by LCMV infection with severe neurologic sequelae, including, in addition to hydrocephalus, chorioretinitis, blindness, and developmental delay.[54] The differential diagnosis of congenital LCMV infection includes toxoplasmosis, rubella, cytomegalovirus infection, herpes simplex, enteroviral infection, human parvovirus B19 infection, and syphilis.[11,48,60]

Last, immunosuppressed patients, such as organ transplant recipients, can develop fatal hemorrhagic fever–like disease. Transmission of LCMV via organ transplantation has been documented recently in several occurrences.[16,17,30] Of 11 recipients described in those clusters, 10 died of multisystem organ failure, with LCMV-associated hepatitis as a prominent feature. The surviving patient was treated with ribavirin (an antiviral agent with in vitro activity against LCMV) and reduction of immunosuppressive therapy.[17]

DIAGNOSIS

As in other viral diseases, direct diagnosis can be established by virus isolation using cell culture; Vero and L929 cells have proved effective for LCMV isolation, but other mammalian cells also can be used. Usually, either no effect or a mild cytopathic effect is noted. Several molecular techniques using PCR technology have been described.[13,20,28,47,49] RT-PCR assay has recently been described.[22,45] The main problem with all these techniques is that the genetic diversity of LCMV is poorly understood. Sequence data acquired from newer LCMV strains indicate that the genetic heterogeneity could be greater than 20 percent between two strains.[28] Therefore, one must keep in mind that a diagnostic assay able to amplify recognized LCMV strains may provide false-negative results with a new, uncharacterized strain. Further genetic analysis of newly discovered strains therefore will be key to developing better diagnostic tools in the future.

Several techniques have been described for establishing the diagnosis of LCMV infection in patients. Among these techniques, the one that was used commonly in the past, complement fixation assay, has been demonstrated to be poorly sensitive.[41] IFA and ELISA techniques are equivalent in terms of specificity and sensitivity. However, because of broad cross reactivity among members of the genus *Arenavirus*, confirmation should rely on neutralization assays to discriminate between LCMV and other arenaviruses.

Serologic study can be performed with serum or CSF samples. As in other viral diseases, a fourfold difference in titers indicates a recent infection. In a single sample, detection of specific IgM suggests a recent infection. Molecular detection of LCMV genomic RNA, as well as virus isolation, can be performed from CSF during CNS manifestations and possibly from serum or plasma.

TREATMENT

No treatment exists for LCMV infection. Ribavirin has been shown to be efficient for Lassa fever,[46] a viral hemorrhagic fever caused by another arenavirus related to LCMV. Ribavirin was used once in a kidney transplant recipient whose immunosuppressive medications were discontinued and who received intravenous immunoglobulin when the diagnosis was established; the issue was favorable with complete recovery.[17]

CONCLUSION

Since 2000, several reports have shown that LCMV has not disappeared and that it still can cause severe or fatal cases. Renewed attention was drawn to LCMV after seven recipients of solid-organ transplants died of LCMV infection.[30] Historically, this virus was a major cause of aseptic meningitis in the United States; LCMV ranked first during the World War II era and second during the 1947 to 1952 period.[2] At that time, other major causes were mumps, herpes simplex virus, and leptospirosis. The focus on LCMV declined over the intervening decades, even though the rate of unrecognized causes of CNS infections still remains high, at more than 50 percent.[32] The reason for the diminishing interest in LCMV is not clear, but it may be related to the historical prevalence of the disease in rural rather than urban populations of developed countries. However, the growing proportion of urban people living below the poverty level may lead to conditions compatible with contact with mice and, therefore, may boost the incidence of rodent-associated diseases.

In addition, the capacity to diagnose LCMV has declined dramatically since the 1980s. Hence, the decreasing number of reported cases may reflect the decrease in clinical interest and the lack of diagnostic tests, rather than a genuine modification of the epidemiology of the disease. Standard techniques, such as IFA or virus isolation, have been virtually abandoned in clinical microbiology laboratories. In Europe, the situation has been critical since 2000; barely a handful of laboratories are capable of performing recognized serologic or molecular diagnostic tests. LCMV infection still exists, and cases are reported when physicians include LCMV in the differential diagnosis and when appropriate diagnostic tests are conducted. Efforts are essential to determine the epidemiologic landscape of the disease and to implement etiologic investigations of neonates with congenital malformations who have CNS infections. The combination of poor diagnostic testing capacity and a lack of awareness of the virus among physicians may contribute directly to an underestimation of the current role of LCMV in medicine.

NEW REFERENCES SINCE THE SIXTH EDITION

17. Centers for Disease Control and Prevention (CDC). Brief report: lymphocytic choriomeningitis virus transmitted through solid organ transplantation—Massachusetts, 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:799–801.
18. Centers for Disease Control and Prevention (CDC). Notes from the field: lymphocytic choriomeningitis virus infections in employees of a rodent breeding facility—Indiana, May-June 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:622–3.
45. McCausland MM, Crotty S. Quantitative PCR technique for detecting lymphocytic choriomeningitis virus in vivo. *J Virol Methods* 2008;**147**:167–76.
24. De Ory F, Gegúndez MI, Fedele CG, et al. Toscana virus, West Nile virus and lymphochoriomeningitis virus as causing agents of aseptic meningitis in Spain. *Med Clin (Barc)* 2009;**132**:587–90.
22. Cordey S, Sahli R, Moraz ML, et al. Analytical validation of a lymphocytic choriomeningitis virus real-time RT-PCR assay. *J Virol Methods* 2011;**177**:118–22.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 185

Arenaviral Hemorrhagic Fevers

Rémi N. Charrel • Xavier de Lamballerie

HISTORY

In the 1950s, a new disease emerged in the Buenos Aires Province of Argentina, a rich farming region. The causative agent of the Argentine hemorrhagic fever (AHF), named Junín virus, was isolated in 1958.[122] Junín virus is hosted by rodents widely distributed in the region, and human infection occurs through contact with infected excreta, usually by a respiratory pathway. AHF is a severe disease that is fatal in approximately 20 percent of cases in the absence of specific treatment. Intensive deforestation and extensive agricultural practices in this region have increased considerably the number of contacts between humans and rodents and thereby fueled the emergence of severe annual outbreaks. The cases were expanding progressively into north-central Argentina until they finally were controlled by the availability of a live attenuated vaccine developed in the early 1980s. Junín virus is estimated to have caused approximately 30,000 cases of symptomatic disease since its discovery.

Bolivian hemorrhagic fever (BHF) was described first in 1959 in the El Beni Department of eastern Bolivia. The causative agent, named Machupo virus, was isolated in 1963 and was found to be antigenically and genetically distinct from but related to Junín virus.[78] Machupo virus was responsible for large outbreaks with high incidence (up to 21% of the population) and a case-fatality rate of approximately 20 percent during the 1960s. Effective rodent-control efforts interrupted these epidemics, and after the mid-1970s, no cases of BHF were reported. After 20 years of silence, however, a total of 19 additional cases were reported in the same region from 1993 to 1999.[5]

An outbreak of severe hemorrhagic fever began in the state of Portuguesa, located in the central plains of Venezuela, in 1989. Initially thought to be dengue hemorrhagic fever, the causative agent was isolated in 1990, identified as a new arenavirus species, and named Guanarito after the municipality where the first epidemic occurred.[133] Venezuelan hemorrhagic fever (VHF)

REFERENCES

1. Ackermann R, Bloedhorn H, Küpper B, et al. Über die Verbeitung des Virus der Lymphocytären Choriomeningitis unter den Mäusen in West Deutschland. I. Untersuchungen überwiegend an Hausmäusen *(Mus musculus)*. *Zentralbl Bakteriol [Orig]* 1964;**194**:407–30.
2. Adair CV, Gauld RL, Smadel JE. Aseptic meningitis, a disease of diverse etiology: clinical and etiologic studies on 854 cases. *Ann Intern Med* 1953;**39**:675–704.
3. Armstrong C. Studies on choriomeningitis and poliomyelitis. *Harvey Lect* 1940-1941;**36**:39–56.
4. Armstrong C. Some recent research in the field of neurotropic viruses with especial reference to lymphocytic choriomeningitis and herpes simplex. *Mil Surg* 1942;**91**:129–46.
5. Armstrong C, Fortner JG, Rowe WP, et al. Meningitis due to lymphocytic choriomeningitis virus endemic in a hamster colony. *JAMA* 1969;**209**:265–7.
6. Armstrong C, Lillie RD. Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St. Louis encephalitis epidemic. *Public Health Rep* 1934;**49**:1619–27.
7. Armstrong C, Sweet LK. Lymphocytic choriomeningitis: report of two cases, with recovery of the virus from gray mice *(Mus musculus)* trapped in the two infected households. *Public Health Rep* 1939;**54**: 673–83.
8. Baird RD, Rivers TM. Relation of lymphocytic choriomeningitis to acute aseptic meningitis (Wallgren). *Am J Public Health* 1938;**28**:47.
9. Barker LF, Ford FR. Chronic arachnoiditis obliterating the spinal subarachnoid space. *JAMA* 1937;**109**:785–6.
10. Barton LL, Hyndman NJ. Lymphocytic choriomeningitis virus: reemerging central nervous system pathogen. *Pediatrics* 2000;**105**: e35.
11. Barton LL, Mets MB. Congenital lymphocytic choriomeningitis virus infection: decade of rediscovery. *Clin Infect Dis* 2001;**33**: 370–4.
12. Baum SG, Lewis AM Jr, Rowe WP, et al. Epidemic nonmeningitic lymphocytic-choriomeningitis-virus infection: an outbreak in a population of laboratory personnel. *N Engl J Med* 1966;**274**: 934–6.
13. Besselsen DG, Wagner AM, Loganbill JK. Detection of lymphocytic choriomeningitis virus by use of fluorogenic nuclease reverse transcriptase-polymerase chain reaction analysis. *Comp Med* 2003; **53**:65–9.
14. Biggar RJ, Schmidt TJ, Woodall JP. Lymphocytic choriomeningitis in laboratory personnel exposed to hamsters inadvertently infected with LCM virus. *J Am Vet Med Assoc* 1977;**171**:829–32.
15. Bowen GS, Calisher CH, Winkler WG, et al. Laboratory studies of a lymphocytic choriomeningitis virus outbreak in man and laboratory animals. *Am J Epidemiol* 1975;**102**:233–40.
16. Centers for Disease Control and Prevention. Lymphocytic choriomeningitis virus infection in organ transplant recipients—Massachusetts, Rhode Island, 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**:537–9.
17. Centers for Disease Control and Prevention. Brief report: lymphocytic choriomeningitis virus transmitted through solid organ transplantation—Massachusetts, 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:799–801.
18. Centers for Disease Control and Prevention. Notes from the field: lymphocytic choriomeningitis virus infections in employees of a rodent breeding facility—Indiana, May-June 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:622–3.
19. Charrel RN, Retornaz K, Emonet S, et al. Acquired hydrocephalus caused by a variant lymphocytic choriomeningitis virus. *Arch Intern Med* 2006;**166**:2044–6.
20. Chesky M, Scalco R, Failace L, et al. Polymerase chain reaction for the laboratory diagnosis of aseptic meningitis and encephalitis. *Arq Neuropsiquiatr* 2000;**58**:836–42.
21. Childs JE, Glass GE, Ksiazek TG, et al. Human-rodent contact and infection with lymphocytic choriomeningitis and Seoul viruses in an inner-city population. *Am J Trop Med Hyg* 1991;**44**:117–21.
22. Cordey S, Sahli R, Moraz ML, et al. Analytical validation of a lymphocytic choriomeningitis virus real-time RT-PCR assay. *J Virol Methods* 2011;**177**:118–22.
23. de Lamballerie X, Fulhorst CF, Charrel RN. Prevalence of antibodies to lymphocytic choriomeningitis virus in blood donors in southeastern France. *Transfusion* 2007;**47**:172–3.
24. De Ory F, Gegúndez MI, Fedele CG, et al. Toscana virus, West Nile virus and lymphochoriomeningitis virus as causing agents of aseptic meningitis in Spain. *Med Clin (Barc)* 2009;**132**:587–90.
25. Deibel R, Woodall JP, Decher WJ, et al. Lymphocytic choriomeningitis virus in man: serologic evidence of association with pet hamsters. *JAMA* 1975;**232**:501–4.
26. Dobec M, Dzelalija B, Punda-Polic V, et al. High prevalence of antibodies to lymphocytic choriomeningitis virus in a murine typhus endemic region in Croatia. *J Med Virol* 2006;**78**:1643–7.
27. Dykewicz CA, Dato VM, Fisher-Hoch SP, et al. Lymphocytic choriomeningitis outbreak associated with nude mice in a research institute. *JAMA* 1992;**267**:1349–53.
28. Emonet S, Retornaz K, Gonzalez JP, et al. Mouse-to-human transmission of variant lymphocytic choriomeningitis virus. *Emerg Infect Dis* 2007;**13**:472–5.
29. Farmer TW, Janeway CA. Infections with the virus of lymphocytic choriomeningitis. *Medicine (Baltimore)* 1942;**21**:1–63.
30. Fischer SA, Graham MB, Kuehnert MJ, et al. Transmission of lymphocytic choriomeningitis virus by organ transplantation: LCMV in Transplant Recipients Investigation Team. *N Engl J Med* 2006;**354**:2235–49.
31. Foster ES, Signs KA, Marks DR, et al. Lymphocytic choriomeningitis in Michigan. *Emerg Infect Dis* 2006;**12**:851–3.
32. Glaser CA, Gilliam S, Schnurr D, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998-2000. *Clin Infect Dis* 2003;**36**:731–42.
33. Hinman AR, Fraser DW, Douglas RG, et al. Outbreak of lymphocytic choriomeningitis virus infections in medical center personnel. *Am J Epidemiol* 1975;**101**:103–10.
34. Hirsch MS, Moellering RC Jr, Pope HG, et al. Lymphocytic-choriomeningitis-virus infection traced to a pet hamster. *N Engl J Med* 1974;**291**:610–12.
35. Hotchin J, Sikora E, Kinch W, et al. Lymphocytic choriomeningitis in a hamster colony causes infection of hospital personnel. *Science* 1974;**185**:1173–4.
36. Jamieson DJ, Kourtis AP, Bell M, et al. Lymphocytic choriomeningitis virus: an emerging obstetric pathogen? *Am J Obstet Gynecol* 2006;**194**:1532–6.
37. Jungeblut CW, Dalldorf G. Epidemiological and experimental observations of poliomyelitis in New York City (1943-1944). *Am J Hyg* 1946;**43**:49–64.
38. Larsen PD, Chartrand SA, Tomashek KM, et al. Hydrocephalus complicating lymphocytic choriomeningitis virus infection. *Pediatr Infect Dis J* 1993;**12**:528–31.
39. Ledford H. Rapid sequencer puts virus in the frame for deaths. *Nature* 2007;**447**:12–13.
40. Lehmann-Grube F, Ibscher B, Bugislaus E, et al. A serological study concerning the role of the golden hamster *(Mesocricetus auratus)* in transmitting lymphocytic choriomeningitis virus to humans. *Med Microbiol Immunol* 1979;**167**:205–10.
41. Lehmann-Grube F, Kallay M, Ibscher B, et al. Serologic diagnosis of human infection with lymphocytic choriomeningitis virus: a comparative evaluation of seven methods. *J Med Virol* 1979;**4**: 125–36.
42. Lewis AM Jr, Rowe WP, Turner HC, et al. Lymphocytic choriomeningitis virus in hamster tumor: spread to hamsters and humans. *Science* 1965;**150**:363–4.
43. Lledo L, Gegundez MI, Saz JV, et al. Lymphocytic choriomeningitis virus infection in a province of Spain: analysis of sera from the general population and wild rodents. *J Med Virol* 2003;**70**: 273–5.
44. Maetz HM, Sellers CA, Bailey WC, et al. Lymphocytic choriomeningitis from pet hamster exposure: a local public health experience. *Am J Public Health* 1976;**66**:1082–5.
45. McCausland MM, Crotty S. Quantitative PCR technique for detecting lymphocytic choriomeningitis virus in vivo. *J Virol Methods* 2008;**147**:167–76.
46. McCormick JB, King IJ, Webb PA, et al. Lassa fever: effective therapy with ribavirin. *N Engl J Med* 1986;**314**:20–6.
47. McIver CJ, Jacques CF, Chow SS, et al. Development of multiplex PCRs for detection of common viral pathogens and agents of congenital infections. *J Clin Microbiol* 2005;**43**:5102–10.

48. Mets MB, Barton LL, Khan AS, et al. Lymphocytic choriomeningitis virus: an underdiagnosed cause of congenital chorioretinitis. *Am J Ophthalmol* 2000;**130**:209–15.
49. Park JY, Peters CJ, Rollin PE, et al. Development of a reverse transcription polymerase chain reaction assay for diagnosis of lymphocytic choriomeningitis virus infection and its use in a prospective surveillance study. *J Med Virol* 1997;**51**:107–14.
50. ProMED-mail (Program for Monitoring Emerging Diseases): Arenavirus, organ transplants: Australia (Victoria). ProMED-mail 20070423.1325, 2007.
51. Riera L, Castillo E, Del Carmen Saavedra M, et al. Serological study of the lymphochoriomeningitis virus (LCMV) in an inner city of Argentina. *J Med Virol* 2005;**76**:285–9.
52. Rivers TM, Scott TF. Meningitis in man caused by a filterable virus. *Science* 1935;**89**:439–40.
53. Scheid WR, Ackermann R, Bloedhorn H, et al. Über die Verbreitung des Virus der Lymphozytären Choriomeningitis in West-Deutschland. *Dtsch Med Wochenschr* 1964;**89**:325–8.
54. Schulte DJ, Comer JA, Erickson BR, et al. Congenital lymphocytic choriomeningitis virus: an underdiagnosed cause of neonatal hydrocephalus. *Pediatr Infect Dis J* 2006;**25**:560–2.
55. Smadel JE, Wall MJ. Lymphocytic choriomeningitis in the Syrian hamster. *J Exp Med* 1942;**75**:581–91.
56. Tindall GT, Gladstone LA. Hydrocephalus as a sequel to lymphocytic choriomeningitis. *Neurology* 1957;**7**:516–18.
57. Traub E. Persistence of lymphocytic choriomeningitis virus in immune animals and its relation to immunity. *J Exp Med* 1936; **63**:847–61.
58. Vanzee BE, Douglas RG, Betts RF, et al. Lymphocytic choriomeningitis in university hospital personnel: clinical features. *Am J Med* 1975;**58**:803–9.
59. Wooley JG, Stimpert FD, Kessel JF, et al. A study of human sera antibodies capable of neutralizing the virus of lymphocytic choriomeningitis. *Public Health Rep* 1939;**54**:938–44.
60. Wright R, Johnson D, Neumann M, et al. Congenital lymphocytic choriomeningitis virus syndrome: a disease that mimics congenital toxoplasmosis or cytomegalovirus infection. *Pediatrics* 1997; **100**:E9.
61. Yamarat C. *A study of lymphocytic choriomeningitis*. Cambridge, MA: Thesis, Harvard School of Public Health; 1940.

presents a seasonal occurrence clearly related to intense agricultural activity and to human contact with the soil and the rodents hosting Guanarito virus.

Little is known about Sabiá virus, which was responsible for one fatal case of hemorrhagic fever near São Paulo (Brazil) in 1994.[32] Subsequently, two other cases were reported in laboratory workers. The epidemiology and the natural reservoir of Sabiá virus remain unknown.

In 1999 and 2000, three fatal cases of illness were reported in female patients aged 14, 30, and 52 years; two resided in northern California and one in southern California.[26] These cases were associated with Whitewater Arroyo virus infections, a recently described arenavirus indigenous to the southwestern United States and hosted by *Neotoma* rodents.[52]

In the Old World, Lassa fever was described initially in Nigeria in 1969, and the causative virus was identified during that same year.[14] The disease occurs also in Sierra Leone, Guinea, and Liberia and more sparsely in other countries of West Africa.

ETIOLOGIC AGENTS

Arenaviruses are enveloped single-stranded RNA viruses with a genome consisting of two RNA segments, designated large (L) and small (S). The L genomic segment (~7.2 kb) encodes the viral RNA-dependent RNA polymerase and a zinc-binding protein. The S genomic segment (~3.5 kb) encodes the nucleocapsid protein (N) and glycoprotein precursor (GPC; secondarily cleaved into the G1 and G2 envelope glycoproteins) in nonoverlapping open reading frames of opposite polarities.[138] Nucleocapsid antigens are shared by most arenaviruses, and quantitative relationships show a basic split between viruses of Africa and viruses of the Western Hemisphere. Individual viruses immunologically are distinct by neutralization test, which depends on the specificity of epitopes contained in the envelope glycoproteins.[127]

At the time this chapter was written, 38 arenaviruses were recognized (Table 185-1). They have been classified according to their immunologic characteristics. Two groups are recognized (Fig. 185-1): the Tacaribe serocomplex, including the New World viruses; and the Lassa–lymphocytic choriomeningitis (LCM) serocomplex, including the ubiquitous LCM virus and all recognized Old World viruses. Genetic studies are congruent with serologic analyses.[43] They also indicate that the 38 arenaviruses represent four phylogenetic lineages. The Old World (Lassa-LCM serocomplex) lineage comprises 13 viruses (LCM, Lassa, Mopeia, Mobala, Ippy, Lujo, Gbagroube, Menekre, Luna, Morogoro, Merino Walk, Lunk, and Kodoko) and is deeply rooted in the three New World (Tacaribe serocomplex) lineages, designated A, B, and C (Fig. 185-1). The lineage A includes eight North American viruses (Whitewater Arroyo, Tamiami, Bear Canyon, Real de Catorce, Tonto Creek, Skinner Tank, Big Brushy Tank, and Catarina) and five South American viruses (Pirital, Pichindé, Flexal, Paraná, and Allpahuayo). The lineage B includes nine South American viruses (Sabiá, Junín, Machupo, Guanarito, Amapari, Tacaribe, Cupixi, Ocozocoautla de Espinoza, and Chapare). The lineage C comprises three South American viruses (Oliveros, Latino, and Pampa). Phylogenies reconstructed from each of the four genes independently demonstrated that the eight North American arenaviruses possess a chimeric genome, with the glycoprotein gene inherited from a clade B ancestor and the nucleoprotein gene inherited from a clade A ancestor; this mechanism could not be identified within the large genomic segment, with both polymerase and matrix genes more closely related to those of clade A viruses.[27-29]

Lassa and Lujo viruses are the only Old World arenaviruses that cause human hemorrhagic fever, whereas five New World members of the lineage B (Junín, Machupo, Guanarito, Sabiá, and Chapare) have been associated with hemorrhagic fever in South America. Whitewater Arroyo virus has been associated with fatal human cases in North America.

Very recently, novel arenaviruses were discovered in snakes (in the families *Boidae* and *Pythonidae*, i.e., boas and pythons), in which they cause a disease called inclusion body disease that is characterized by behavioral abnormalities, wasting, and secondary infections. The three new viruses were provisionally named California Academy of Sciences virus (CASV), Golden Gate virus (GGV), and Collierville virus (CVV). These viruses have a typical arenavirus genome organization but are highly divergent, belonging to a lineage separate from that of the Old and New World arenaviruses. Interestingly, these viruses encode envelope glycoproteins that are more similar to those of filoviruses than to those of other arenaviruses.[140]

EPIDEMIOLOGY

Specific rodents (usually one or two closely related species) are the principal hosts of the arenaviruses for which natural host relationships have been characterized (Table 185-2); the only exception is Tacaribe virus, associated with bats. The consensus is that the diversity of arenaviruses is the result of a long-term, shared evolutionary relationship (termed co-evolution or co-speciation) between the virus members of the family Arenaviridae and the rodents of the family Muridae.[9,10] Chronic infections of the host appear to be crucial to the long-term persistence of arenaviruses in nature. The infection in rodents is accompanied by a chronic viremia or viruria. Because of this specific association, the geographic area where each arenaviral disease is diagnosed is limited by the geographic distribution of the corresponding rodent host.

The rodent host of Junín virus is *Calomys musculinus*, a small field rodent. Since it was first isolated in 1959, the endemic area has expanded from a region of 16,000 km^2 in the north of Buenos Aires Province to a region that is now 150,000 km^2 (reaching north of Buenos Aires, south of Santa Fé, southeast of Córdoba, and northeast of La Pampa provinces). The human population at risk now is estimated to be almost 5 million.[47] AHF typically is a seasonal disease, with a peak frequency in the corn harvesting season from March to June; during this period, 75 percent of the infected people are male

TABLE 185-1 **List of Arenaviruses**

Virus	Acronym	Lineage	Country	(Possible) Host	Reference
Allpahuayo	ALLV	NW-A	Peru	*Oecomys bicolor, Oecomys paricola*	114
Amapari	AMAV	NW-B	Brazil	*Oryzomys goeldi, Neacomys guianae*	28
Bear Canyon	BCNV	NW-Rec	USA	*Peromyscus californicus, Neotoma macrotis*	56
Big Brushy Tank	BBTV	NW-Rec	Arizona, USA	*Neotoma albigula*	110
Catarina	CTNV	NW-Rec	USA	*Neotoma micropus*	16
Chapare	CHPV	NW-B	Bolivia	Unknown	38
Cupixi	CPXV	NW-B	Brazil	*Oryzomys capito*	28
Flexal	FLEV	NW-A	Brazil	*Oryzomys* spp.	28
Gbagroube		OW	Ivory Coast	*Mus (Nannomys) setulosus*	33
Guanarito	GTOV	NW-B	Venezuela	*Zygodontomys brevicauda, Sigmodon alstoni*	145
Ippy	IPPYV	OW	Central African Republic	*Arvicanthus* spp.	141
Junín	JUNV	NW-B	Argentina	*Callomys musculinus*	123
Kodoko		OW	Guinea	*Mus (Nannomys) minutoides*	
Lassa	LASV	OW	West Africa	*Mastomys natalensis*	14
Latino	LATV	NW-C	Bolivia	*Callomys callosus*	158
Lymphocytic choriomeningitis	LCMV	OW	Ubiquitous	*Mus musculus, Mus domesticus*	119, 131
Lujo	LUJV	OW	South Africa	Unknown	124
Luna	LUNV	OW	Zambia	*Mastomys natalensis*	75, 76
Lunk	LNKV	OW	Zambia	*Mus minutoides*	75, 76
Machupo	MACV	NW-B	Bolivia	*Callomys callosus*	159
Menekre		OW	Ivory Coast	*Hylomyscus* spp.	33
Merino Walk	MWV	OW	South Africa	*Myotomys unisulcatus*	120
Mobala	MOBV	OW	Central African Republic	*Praomys* spp.	108
Mopeia	MOPV	OW	Mozambique, Zimbabwe	*Mastomys natalensis*	
Morogoro	MORV	OW	Tanzania	*Mastomys* spp.	67
Ocozocoautla de Espinoza	OCEV	NW-B	Southern Mexico	*Peromyscus mexicanus*	17
Oliveros	OLVV	NW-C	Argentina	*Bolomys* spp.	8
Pampa		NW-C	Argentina	*Bolomys* spp.	
Paraná	PARV	NW-A	Paraguay	*Oryzomys buccinatus*	157
Pichindé	PICV	NW-A	Colombia	*Oryzomys albigularis*	109,147
Pirital	PIRV	NW-A	Venezuela	*Sigmodon alstoni*	53
Real de Catorce	RCTV	NW-Rec	Mexico	*Neotoma leucodon*	74
Sabiá	SABV	NW-B	Brazil	Unknown	87
Skinner Tank	SKTV	NW-Rec	USA	*Neotoma mexicana*	15
Tonto Creek	TTCV	NW-Rec	USA	*Neotoma* spp.	110
Tacaribe	TCRV	NW-B	Trinidad	*Artibeus* bat	41
Tamiami	TAMV	NW-Rec	USA	*Sigmodon hispidus*	19
Whitewater Arroyo	WWAV	NW-Rec	New Mexico, USA	*Neotoma* spp.	52

agricultural workers who are contaminated by inhalation of infected aerosols produced from rodent excreta or from rodents caught in mechanical harvesters.[95] Since 1958, cases have been recorded annually, with a variation ranging from several hundreds to 3500. The mortality rate for patients with confirmed AHF was 14 to 17 percent before immune plasma was used routinely.[96] Evidence indicates that the number of human cases reflects the proportion of infected *C. musculinus* in a particular area.[112]

Machupo virus, the causative agent of BHF, is hosted by *Calomys laucha* rodents. As for Junín virus, the dynamics of the rodent population determine the epidemiologic features in humans.[106] By contrast to *Calomys callosus*, *C. laucha* invades houses during inundation at the rainy season; this invasion results in human cases occurring with an attack rate identical in men, women, and children. The disease has been recorded only from a sparsely populated region in northeast Bolivia, the El Beni Province. From 1962 to 1964, a series of outbreaks involved more than 1000 patients, 180 of whom died. Nosocomial transmission of Machupo virus was clearly demonstrated,[126] although most of the recorded infections were acquired by direct contact with *C. laucha* or by aerosol contact through infected excreta.

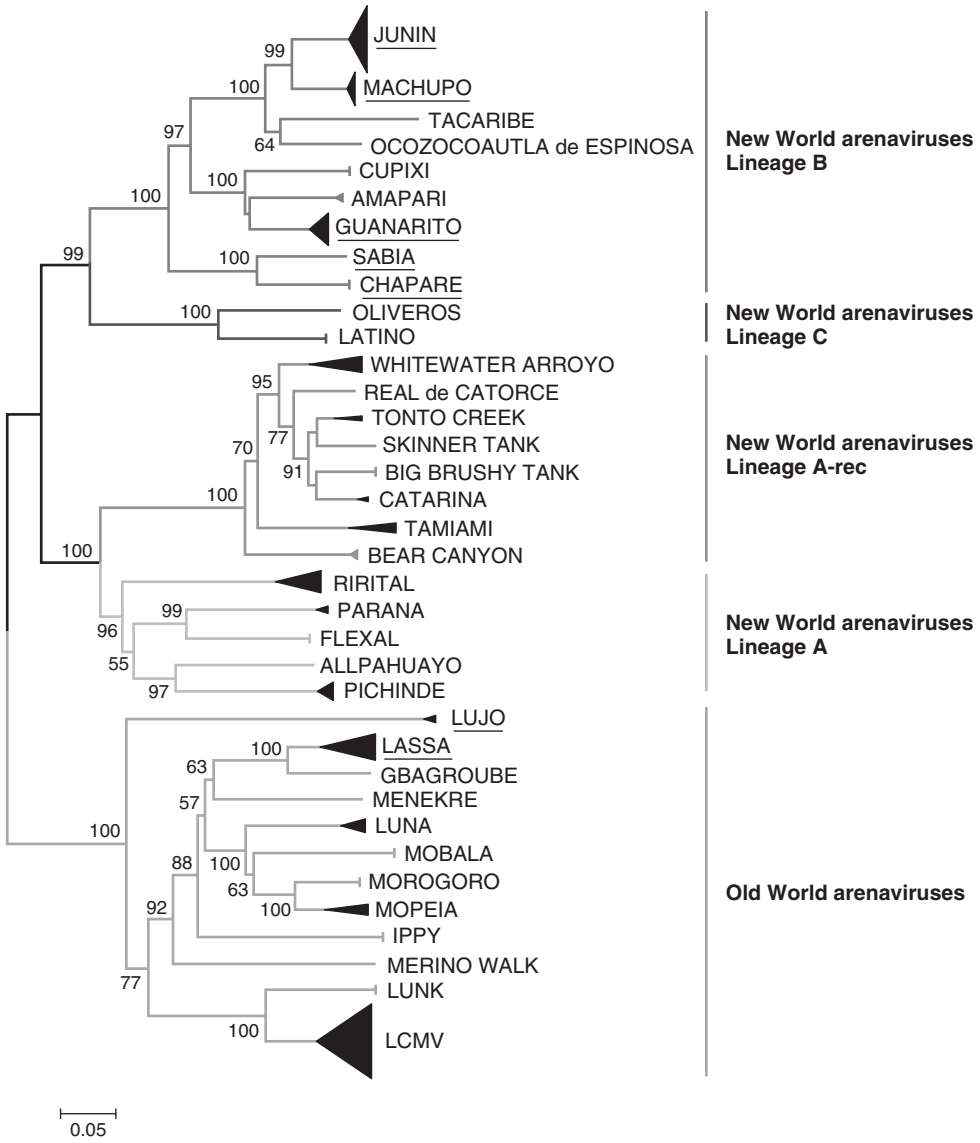

FIGURE 185-1 ■ Phylogeny of arenaviruses based on the analysis of complete sequences of the NP gene. NW, New World arenaviruses; OW, Old World arenaviruses; A, B, and C show the three evolutionary lineages of New World arenaviruses. A/Rec denotes the recombinant lineage including the North American viruses. Bootstrap values above 75 percent are indicated and correspond to 500 replications.

In 1990, new cases of hemorrhagic fever were investigated in Venezuela; the culprit was found to be a new arenavirus, designated Guanarito virus after the region where the outbreak occurred.[133] Natural and experimental data suggest that two different rodent species are involved in the transmission cycle of Guanarito virus in nature: the cane rat, *Zygodontomys brevicauda*; and the cotton rat, *Sigmodon alstoni*.[53,54,144] Since its discovery, Guanarito virus has been responsible for at least 200 cases of VHF. For unknown reasons, the number of reported human cases has dropped spontaneously since 1992, although rodent infection can still be demonstrated easily inside and even outside the original endemic zone.[155]

In December 2003 and January 2004, a small number of hemorrhagic fever cases were reported in rural Bolivia, outside of the known Machupo hemorrhagic fever endemic zone. The man had symptoms similar to those seen with other arenaviral hemorrhagic fever cases—acute febrile illness beginning with headache, joint and muscle pain, and vomiting—and rapidly progressed to shock, bleeding, and death at 14 days after onset of illness. In the serum from one fatal case, a novel arenavirus was isolated and sequenced.[38]

In California, three fatal cases recently were associated with Whitewater Arroyo virus, initially isolated from a wood rat (*Neotoma* spp.).[26,52] The present-day geographic range of rodents of the genus *Neotoma* extends from western Canada southward to Guatemala, Honduras, and Nicaragua. Of the 20 recognized *Neotoma* spp., nine occur in the United States, and evidence of arenavirus infection has been reported for five of them. Field studies have provided strong evidence that Whitewater Arroyo virus circulates in the states of New Mexico, Utah, Texas, Oklahoma, Arizona, and Colorado.[18,55] The abundance

TABLE 185-2 **Some Epidemiologic Features of Arenaviral Hemorrhagic Fevers**

Fever	Virus	Case, Season, Place, Pattern	Annual Incidence	Geographic Distribution	Ecology	Rodent Reservoir	Comment
Argentine hemorrhagic fever	Junín	Males; corn harvest; March-June	20-200	North-central Argentina	Temperate pampa	*Calomys musculinus*	3- to 4-year rodent-disease cycle
Bolivian hemorrhagic fever	Machupo	All ages, both sexes; villages; February-July	<10	Northeast Bolivia	Tropical savanna	*Calomys callosus*	Rodent control successful
Venezuelan hemorrhagic fever	Guanarito	All ages, both sexes equally; house, gardens; no seasonality	0-100	Central Venezuela	Tropical mixed savanna	*Zygodontomys brevicauda* *Sigmodon alstoni*	Recently described
Brazilian hemorrhagic fever	Sabiá			Brazil		Unknown	
	Chapare			Bolivia		Unknown	Recently described
Lassa fever	Lassa	All ages, both sexes; villages; no seasonality	10,000	West Africa	Tropical forest savanna	*Mastomys natalensis*	No long-term cycle; nosocomial infections
	Lujo			South Africa		Unknown	Recently described

and habits of wood rats suggest that potential contact between *Neotoma* rodents and humans is limited. Recent data support the fact that Whitewater Arroyo virus can infect humans and may cause disease.[111]

Lassa virus is associated with rodents belonging to the *Mastomys* genus, widely distributed in sub-Saharan Africa. Documented cases of Lassa fever have been reported in different countries of West Africa, such as Nigeria, Liberia, Sierra Leone, Burkina Faso, Guinea, Ivory Coast, Ghana, Senegal, Gambia, and Mali.* Between 1985 and 1987, 3 of 5000 (0.06%) randomly selected persons living in six central African countries (Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, and Gabon) tested for serologic evidence of Lassa virus infection were found to have antibody.[63] In contrast, a second study conducted with individuals living in different parts of Nigeria found that 21.3 percent of the 1677 tested antibody positive.[146] Lassa fever is a disease of major public health importance in West Africa because it causes an estimated 5000 deaths and as many as 300,000 infections annually. The first epidemic was described in 1969 in Nigeria[14] and soon was followed by several other epidemics in nearby countries. Among hospitalized patients, the mortality rate is estimated at 15 to 20 percent.[156] In hospitals located within the endemic area, 10 to 17 percent of the admissions are caused by Lassa fever. However, serologic surveys suggest that subclinical cases occur and result in a lower overall mortality (<1%).[102] The overall incidence of Lassa fever in West Africa may be tens of thousands to hundreds of thousands annually. The main characteristic of Lassa fever is that it is highly transmissible from human to human through direct contact with blood or secretions from infected patients and by aerosol exposure.[82] Many nosocomial outbreaks have been described. Another characteristic of Lassa fever is that a number of cases are acquired by local residents who seek out rodents for consumption.[142] Because of the prevalence of virus transmission, Lassa fever is a prominent threat of imported hemorrhagic fever cases; such cases have been reported in England,[34] Germany,[66] Japan,[72] the Netherlands,[152] Israel,[136] and the United States.[73] A comprehensive report of imported Lassa fever recorded 24 cases occurring from 1969 to 2004, with a fatality rate of 29 percent.[93]

In Fall 2008, five cases of hemorrhagic fever were reported in South Africa after air transfer of a critically ill index case from Zambia. Four of them were fatal; the fifth case received ribavirin and recovered. Next-generation sequencing from serum and tissue samples detected a novel arenavirus that was subsequently completely sequenced. Lujo virus (LUJV) was named after its geographic origin (Lusaka, Zambia, and Johannesburg, South Africa).[11]

PERSON-TO-PERSON TRANSMISSION

Although human-to-human transmission of BHF has been seen only rarely and then only after intimate contact, Machupo virus clearly was responsible for severe nosocomial outbreaks in which all cases were associated with a single index case who had returned from the endemic region. The only recognized hospital-based outbreak resulted in four secondary cases followed by a tertiary case acquired from a necropsy incident; all but one died. An epidemic was reported in which seven members of the same family were infected, with a fatal outcome for six.[24]

Nosocomial transmission is a common feature of Lassa fever, and many nosocomial outbreaks have been described.[49,82] However, it is apparent that this aspect of Lassa fever has been overestimated in reports based on

*References 20, 58, 59, 71, 102, 134, 146.

infections in hospitals. The additional risk to hospital workers within the endemic zone is not great as judged by serosurveys, provided basic hygiene measures are respected in hospitals in dealing with suspected cases.[70] Good barrier nursing and protection of personnel by use of gloves, gowns, and eye shields have reduced the incidence of such infection in recent years. Broader surveys of community infections have revealed Lassa fever to be an important community-acquired disease, with a wide spectrum of clinical manifestations. Because Lassa virus has been isolated from milk, clearly a risk to nursing children exists.

Concerning LUJV infection, the index patient was airlifted from Zambia to a clinic in South Africa, after infection from an unidentified source. Secondary infections were recognized in a paramedic (case 2) who attended the index case during air transfer from Zambia, in a nurse (case 3) who attended the index case in the intensive care unit in South Africa, and in a member of the hospital staff (case 4) who cleaned the room after the index case died. One case of tertiary infection was recorded in a nurse (case 5) who attended case 2 after his transfer from Zambia.[124]

In conclusion, the data demonstrating nosocomial and human-to-human transmission of several arenaviral diseases highlight the necessity for implementing biocontainment precautions in dealing with arenavirus-infected patients and materials.

CLINICAL MANIFESTATIONS

The clinical picture of the South American arenaviral hemorrhagic fevers is almost identical, regardless of the virus responsible for the disease. The incubation period usually is 7 to 14 days, with an extreme extending from 5 to 21 days. Infection secondary to high-load inoculum may result in the reduction of the incubation period to 2 days. The onset is gradual with fever and malaise, secondarily joined by myalgia, back pain, headache, and dizziness. Hyperesthesia of the skin is a common manifestation. Petechiae of the skin and hemorrhages from the gums, vagina, and gastrointestinal tract beginning about the fourth day of illness herald the advent of hypovolemic clinical shock. Blood loss usually is minor, so the hematocrit generally increases as the capillary leak syndrome, the hallmark of the disease, becomes more severe (Table 185-3). Bleeding and prothrombin time may be prolonged, and reductions of factors II and VII of the coagulation cascade have been noted. Renal function generally is preserved until shock occurs, but urinary protein concentration may be high. Neurologic manifestations are prominent; intention tremor of the hands and inability to swallow or to speak clearly may develop, and these symptoms can progress to grand mal convulsions, coma, and death in the absence of significant capillary leak or hemorrhagic signs. Death usually occurs 7 to 12 days after onset of disease. Those who survive generally recover completely without permanent sequelae, although transient loss of scalp hair and Beau lines in digital nails are a common consequence of the high and sustained fever.

Symptoms that appear to be more specifically associated with one or another of the viruses have been reported.[151] Although the frequency of clinical and laboratory findings is identical for Junín and Machupo virus infections, clear differences exist with Guanarito virus infections. Pharyngitis, vomiting, and diarrhea are observed more frequently with Guanarito virus; in contrast, petechiae, erythema, facial edema, hyperesthesia of the skin, and shock are observed more frequently in the case of Junín or Machupo infection. There seems to be little difference, if any, between clinical manifestations caused by these three viruses and the recently isolated Chapare virus; the patient, a 22-year-old man, had lived for the last 4 years in the same region. He was a tailor and also a farmer. He had no history of travel and no contact with any case with compatible illness for at least 4 weeks before his disease onset. In addition, no members of case household or other close contacts were affected. His clinical course included fever, headache, arthralgia, myalgia, and vomiting with subsequent deterioration and multiple hemorrhagic signs and death 14 days after onset.[38]

Fatal outcome of Junín virus infection is observed more frequently in pregnant women in the last trimester, and a high fetal mortality is associated with Junín and Machupo virus infections.[12,40,79]

The patients infected with Whitewater Arroyo virus were healthy before acquiring the virus infection. In one case, the patient probably acquired the virus by aerosol pathway while cleaning rodent droppings at home. In all cases, no history of travel outside California was found during the 4 weeks preceding the illness. The onset was characterized by nonspecific febrile symptoms including fever, headache, and myalgia. All patients presented with acute respiratory distress syndrome, and two developed liver failure and hemorrhagic manifestations. Death occurred within 8 weeks after the onset.[26]

Concerning Lassa fever, the clinical description is based on signs observed in hospitalized patients.[57,81,127] After having an incubation period that ranges from 7 to

TABLE 185-3 **Some Laboratory Findings in Arenaviral Hemorrhagic Fevers**

Disease	Viremia	RBC Increase	WBC	Urine Protein	AST/ALT
Argentine hemorrhagic fever	+	++	↓↓	+	N-200
Bolivian hemorrhagic fever	+/–	++	↓↓	+	N-200
Venezuelan hemorrhagic fever	++	++	↓↓	+	??
Lassa fever	++++	++	N-↑	++	100-1500

ALT, alanine aminotransferase; AST, aspartate aminotransferase; RBC, red blood cell; WBC, white blood cell count.

14 days, patients suffer an insidious onset characterized by progressive fever, malaise, and generalized myalgia. Retrosternal pain frequently is associated with this phase. These symptoms increase in severity during the following week and are accompanied by nausea, vomiting, diarrhea, chest pain, abdominal pain, headache, sore throat, cough, and dizziness. Patients complain of sore throat, pharyngeal inflammation, and chest pain. As the disease progresses, vomiting, diarrhea, hemorrhagic manifestations, encephalopathy, and evidence of vascular permeability may be noted. During the second week, recovery may begin or the disease progressively worsens until harbingers of a fatal outcome are evident: severe edema, mucosal hemorrhages, pulmonary involvement, encephalopathy (with seizures and coma), or shock; at this stage, pleural and pericardial effusions and ascites are not rare findings. There frequently is liver involvement, but icterus is not usual. Death occurs in 15 to 20 percent of the hospitalized patients, usually in the second or third week of the course, and is associated with sudden cardiovascular collapse caused by hepatic, pulmonary, and myocardial damage. Few patients develop severe central nervous system (CNS) signs, and disturbances in consciousness and convulsions are markers of a poor prognosis. Deafness is an important sequela of Lassa fever; it may be unilateral or bilateral and is a consequence of the eighth cranial nerve dysfunction; it can be observed in as many as 30 percent of the patients.

Clinical disease among children generally is similar to that in adults. Systematic data are available for only Lassa fever patients.[115,156] Fever, vomiting, diarrhea, and cough are common presenting symptoms. Pulmonary signs, including rales and pleural effusion, are seen more commonly in children than in adults. Among very young children, especially those infected ante partum, an unusual condition called swollen baby syndrome has been described. It is marked by abdominal distention, widespread edema, and spontaneous bleeding.

Lassa virus replicates at a very high level in the placenta of pregnant women, which might account for the high mortality rate observed in these patients. The risk for death in pregnant women is estimated to vary from 7 percent in the first two trimesters to 30 percent in the third trimester, compared with the 13 percent mortality in nonpregnant women. A high fetal mortality rate varying from 92 percent in early pregnancy to 75 percent in the last trimester has been observed.[107,113,128]

Although few cases of LUJV infection were reported, it appears that the clinical manifestations are not drastically different from those observed with other arenaviruses. The putative incubation period ranged from 7 to 13 days, and the period of illness varied from 10 to 13 days. All patients presented with nonspecific febrile illness with headache and myalgia. The severity increased for 7 days with diarrhea and pharyngitis. A morbilliform rash was observed on the face and trunk of white patients but was lacking in African patients. Transient subjective improvement after approximately 1 week of illness was reported; afterward, the patients endured a rapid deterioration with respiratory distress, neurologic signs, and circulatory collapse, the terminal features in patients who died. Bleeding was not a prominent feature. However, petechial rash, gingival bleeding, and oozing of blood from venipuncture sites were observed. Chest pain was a prominent symptom for two patients. All patients had thrombocytopenia on admission to the hospital (platelet count range, 20 to 104×10^9 cells/L). Three patients had leukocyte counts in the normal range, and two had leukopenia on admission, whereas leukocytosis developed in four patients during the illness. The highest aspartate aminotransferase values recorded in the patients who died ranged from 549 to 2486 U/L, compared with 240 U/L in the survivor who was treated with ribavirin.[124]

Very little is known about the health consequences of infection with the other arenaviruses. Aerosol infections are common in workers of laboratories where arenaviruses are manipulated. Pichindé virus has resulted in several seroconversions without any notable clinical significance.[13] Flexal virus has resulted in two severe laboratory infections and should be regarded as potentially dangerous (F. Pinheiro, personal data). Tacaribe virus has resulted in a single case of febrile disease with mild CNS symptoms (J. Casals, personal data).

PATHOGENESIS AND PATHOLOGY

Human and nonhuman primate infection initially involves virus replication at the site of infection, usually in the lung after infected aerosol deposition. Replication occurs in the hilar lymph nodes, and despite interstitial infiltration, pneumonic foci usually are not observed. Regardless of the route of infection, the macrophage is a site of important viral replication. With the exception of the hepatitis peculiar to Lassa fever, the pathologic process of arenaviral hemorrhagic fever is notable for the general lack of parenchymal histologic damage. Edema of tissues and focal hemorrhages in mucosal surfaces and fascia of many organs are common events. Rarely are blood clots found that are large enough to be of clinical significance. Thus, the crucial and life-threatening lesion in these diseases appears to be the capillary leak. How this lesion, in which plasma protein and fluid escape the circulation at a much higher rate than erythrocytes do, is produced still is not clear.[127]

Hemorrhages are common findings with New World arenaviruses causing hemorrhagic fever. The prominent thrombocytopenia has a central origin possibly linked to high levels of interferon observed in these patients.[86] Minor foci of necrosis with acidophilic inclusions (Councilman bodies) have been reported in patients with BHF,[31] and erythroid hypoplasia of bone marrow as well as lymphoid depletion of nodes and spleen may be present. Histologic evidence of disseminated intravascular coagulation generally is absent. Inflammatory lesions of the CNS are lacking. Mild inflammation of the myocardium has been noted in patients with BHF. The Argentine and Bolivian fevers may be marked by encephalopathy. Virus is not recovered from cerebrospinal fluid of patients presenting such CNS symptoms with the South American agents. Macrophages and lymphocytes or their precursors are targets for infection, and an activation of the complex cytokine cascade occurs, which can

cause endothelial cells of small capillaries to lose the tight continuity that keeps protein and red cells inside the circulation. As part of this cascade, levels of interferon are elevated. Analysis of the serum levels of hematopoietic growth factors suggested a link between granulocyte colony-stimulating factor serum levels and the severity of the illness in humans.[99] Similarly, interleukin-8 is thought to play an essential role in neutrophil activation.[98] Specific antibodies may not appear until 3 to 4 weeks after the onset of the disease. However, New World arenaviruses causing hemorrhagic fever are neutralized more readily, and immune serum–based therapy has been successful for humans. Preliminary studies of VHF suggest that this disease resembles that caused by other South American relatives.[37,133] Genetic analysis of Junín and Guanarito virus sequences amplified from human cases in Argentina and Venezuela, respectively, failed to identify any genetic marker correlating with the human pathogenicity or with the severity of clinical forms.[60,155]

Although only three cases have been reported to date, Whitewater Arroyo virus infection seems to be associated with profound lymphopenia and thrombocytopenia.[26] To date, no studies have been performed to investigate the pathogenesis of Whitewater Arroyo virus infection. An interesting note is that Whitewater Arroyo virus belongs to the lineage A (based on genetic analysis performed in the nucleocapsid gene), in which no other members have been recognized so far as human pathogens. However, the small genomic segment of Whitewater Arroyo virus has been shown to have a dual origin: the gene encoding the nucleocapsid is inherited from an ancestor belonging to the lineage A, whereas the glycoprotein precursor gene is inherited from an ancestor belonging to the lineage B.[27] Further investigations are needed to clarify whether the lineage B–inherited GPC gene is responsible for the putative human pathogenicity of Whitewater Arroyo virus.

The hepatitis of Lassa fever is represented by different stages successively characterized by hepatocellular injury, necrosis, and regeneration, any of which may be present at death. In no instance is the degree of hepatic damage sufficient to be responsible for hepatic failure; consequently, the hepatitis of Lassa fever is not the primary cause of death.[80,101] For the cases characterized by encephalopathy, correlation could not be established with the presence of Lassa virus in the cerebrospinal fluid or with virus antibodies in cerebrospinal fluid or serum; no evidence shows that the encephalopathy observed in Lassa fever is the consequence of either direct cytopathic or immune-mediated mechanisms.[35] Direct damage to circulating leukocytes is not demonstrated, and profound thrombocytopenia does not occur.

In human cases of Lassa fever, no correlation exists between antibody levels and outcome of the disease.[50] More than one third of patients have antibodies to the nucleocapsid of the virus on admission to the hospital. These antibodies do not neutralize the virus, which is found in blood in concentrations much higher in severe infection than in any of the other arenaviral diseases. During convalescence, viral neutralizing antibodies evolve for months and rarely reach levels even one tenth of those that develop after infection with the other viruses in this group. Recovery from Lassa virus infection usually precedes the appearance of neutralizing antibodies, indicating that cellular immunity plays a primary role in viral clearance. Thus, it seems reasonable to speculate that compromise of cell-mediated immunity is responsible for uncontrolled virus replication and functional capillary collapse. Primates challenged with Lassa virus or naturally infected patients who recovered from acute Lassa fever usually do not exhibit a measurable neutralizing antibody response. Individuals who are seropositive exhibit a strong memory $CD4^+$ T-cell response against the nucleocapsid protein of Lassa virus.[143] Experiments based on inactivated vaccines have shown that in spite of the resulting synthesis of high titers of antibodies, vaccination did not prevent virus replication and death in nonhuman primates.[50] It therefore seems obvious that control of human Lassa fever involves mostly T cells through a Th1-type immune response. In addition, immune response requires epitopes located on both GP1 and GP2 glycoproteins to protect efficiently primates challenged with Lassa virus.[50]

Several animals have provided reasonable models for the clinical disease seen in humans. Junín virus causes fatal hemorrhagic disease in guinea pigs; rhesus monkeys are good models for Lassa[77] and Machupo virus infections; and marmosets (*Callithrix jacchus*) reproduce the pathogenesis of Junín virus. Indeed, rhesus monkeys were used to predict the effectiveness of the antiviral drug ribavirin to treat Lassa fever, and guinea pigs were instrumental in the development of an attenuated vaccine for AHF. The paired Syrian golden hamster and Pirital virus (a nonpathogenic clade A arenavirus) has been shown to be a model to mimic clinical virologic and pathologic changes observed with Lassa fever in humans.[135]

α-Dystroglycan has been identified as a major receptor for LCM and Lassa viruses. In New World arenaviruses, only clade C viruses (Oliveros and Latino) use α-dystroglycan as a major receptor, whereas clade A and clade B arenaviruses use distinct and widely expressed receptors.[130,139] Functional receptors for South American hemorrhagic fever arenaviruses (Guanarito, Junín, and Machupo) were found on most human cell types and cells derived from nonhuman primates and rodents.[132]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

Direct Diagnosis

Serum or heparinized plasma should be collected during the acute febrile stages of the disease; the samples need to be frozen on dry ice or in liquid nitrogen. Storage at temperatures higher than –40°C will result in the progressive loss of infectivity. Classically achieved by virus isolation, direct diagnosis now may be performed by reverse transcription polymerase chain reaction (RT-PCR) assay. Isolation of the virus is achieved readily in cell culture, and Vero cells are a sensitive substrate for such direct diagnosis. Because cytopathic effect is not

always observed, arenavirus-infected cells are detected by immunofluorescence test.

For South American arenaviruses causing hemorrhagic fevers, the delay for isolation on Vero cells is 1 to 5 days, much faster than animal inoculation that requires 7 to 20 days before illness develops. Serum and throat washings collected 3 to 10 days after onset usually yield virus. Virus is isolated less frequently from urine. Specifically, Machupo virus is recovered from only 20 percent of acute-phase sera and even less frequently from throat washings or urine. When serum or throat samples are collected within 2 weeks of clinical onset, the success of isolating Lassa virus is very high. Virus is isolated less frequently from urine.

Viral RNA can be purified from serum, plasma, urine, throat washings, and tissues and can be used as a target for cDNA synthesis, with use of a reverse transcriptase. This cDNA can be amplified by a PCR assay with consensus primers or specific primers. The amplified region finally is sequenced for diagnostic confirmation. RT-PCR–based diagnosis offers the advantages of reducing the delay of response and possessing a greater sensitivity compared with cell culture. This technique has been applied successfully to Lassa fever diagnosis. Specific primers designed to amplify certain arenaviruses have been proposed but still are not used for diagnostic purposes in epidemic conditions. Molecular tests based on the RT-PCR methodology are being developed increasingly for the diagnosis of arenaviral infections.[92] For Junín and Machupo virus infection specifically, this technique is the only one to be early and sensitive enough to detect low viremia encountered during the period in which immune plasma therapy can be used effectively.[89] At the time of admission to the hospital, RT-PCR detected Lassa virus RNA in 79 percent of the patients; for comparison, in these patients, immunofluorescence assay detected antibodies in only 21 percent. By the third day of admission, 100 percent of the patients tested positive by RT-PCR analysis, whereas only 52 percent tested positive by the immunofluorescence assay.[39] The drawbacks of molecular methods consist mostly of the need for sophisticated equipment to be fully applicable in the field conditions when an outbreak occurs.

Additional techniques for direct detection of virus in tissues are being actively developed; they include in situ nucleic acid hybridization techniques. Antigen-detection enzyme-linked immunosorbent assay (ELISA) has been a sensitive tool in a number of patients.

More recently, molecular assays taking advantage of the real-time PCR technology have been reported for the detection of the four South American arenaviruses causing VHF. Apart from assays specific for one virus, a universal clade B PCR assay proved able to detect 5 to 500 $TCID_{50}$ per reaction for Junín, Machupo, Guanarito, and Sabiá viruses and also for Tacaribe, Cupixi, and Amapari viruses.[153] These tests have application to laboratory diagnosis on human specimens but also can be used to detect arenaviruses in rodent tissues. Real-time RT-PCR assays were recently developed and published.[118,121,148,154]

The first quality assurance study on the rapid detection of Lassa virus RNA included 24 participant laboratories from 17 countries; 10-fold dilutions of Lassa virus–inactivated genetic material were detected with success varying from 21 to 85 percent, depending on the concentration in the sample.[117] Of interest is that specimens containing high concentration of viral RNA may lead to false-negative results by the inhibition of enzymatic reaction, suggesting that clinical specimens should be either spiked with internal control to monitor enzymatic reaction efficacy or tested after dilution.[42,117]

Indirect Diagnosis

Serologic diagnosis is made by demonstration of a fourfold rise in the titer of specific antibody. A high IgG antibody titer or the presence of specific IgM is indicative of a probable case. Nucleocapsid antigens are shared by most of these viruses, and quantitative relationships show the basic split between the viruses of Africa and the Western Hemisphere. Individual viruses immunologically are distinct by neutralization test, which depends on the specificity of epitopes contained in the envelope glycoproteins G1 and G2. Samples collected in view of serologic diagnosis can be kept at –20° C. Blood obtained in the early convalescence may be infectious despite the presence of antibodies and therefore should be handled accordingly. Antibodies often are detected by indirect immunofluorescence because it is an inexpensive, rapid, and sensitive test. An alternative is the ELISA, the specificity of which is greater than and the sensitivity of which is on the level of the neutralization test. ELISA is therefore the test of choice for differentiation of viral strains of arenaviruses, for confirmation of unexpected results, and for detection of infection from the distant past. All these tests have been tested in the conditions of an outbreak and revealed effective.

Antibodies specific to South American arenaviruses appear 12 to 30 days after the onset, which often correlates with clinical improvement.[125]

In the case of Lassa fever, antibodies detectable by immunofluorescence appear early and often are present during the acute illness with no apparent relation to viremia or clinical status; 50 percent of patients with Lassa fever develop IgM or IgG antibody during the first week, and more than 66 percent do so during the next week. Antigen-detection ELISA in conjunction with IgM-capture ELISA has shown promise for rapid and sensitive diagnosis of Lassa fever. Neutralizing antibodies usually develop only after 4 to 6 months of convalescence.

Predictive Value

In West Africa, the association of fever, pharyngitis, retrosternal pain, and proteinuria presented a predictive value of 81 percent. In the endemic area of Argentina, the constellation of asthenia and dizziness accompanied by petechiae and conjunctival congestion has been shown to be indicative for the diagnosis of AHF; the presence of leukopenia, thrombocytopenia, and proteinuria further reinforces the diagnostic suspicion based on clinical findings. The association of a platelet count of less than 100,000/mm^3 and a white blood cell count of

less than 2500/mm^3 or less than 4000/mm^3 was reported to have a sensitivity of 87 percent and 100 percent, respectively, and a specificity of 88 percent and 71 percent, respectively.[68]

Differential Diagnosis

Differential diagnosis of Lassa fever should include malaria, typhoid, shigellosis, dengue, and yellow fever. For South American viruses causing hemorrhagic fever, the differential diagnosis includes yellow fever, dengue hemorrhagic fever, hepatitis, leptospirosis, hemorrhagic fever with renal syndrome caused by hantaviruses, rickettsial diseases, typhoid, sepsis with disseminated intravascular coagulation, and, in the case of CNS involvement, viral encephalitis.

Because of biosafety reasons, suspected cases must be reported to the national laboratories having the expertise and capacity for molecular diagnostics of viral hemorrhagic fevers together with a procedure adapted to handling and transportation of specimens. Isolation of virus must be attempted in biosafety level 4 laboratory facilities.

PROGNOSIS AND TREATMENT

Prognosis

The case-fatality ratio of AHF, which is greater than 15 percent without specific treatment, drops spectacularly to less than 1 percent when specific treatment is available.[68] Granulocyte colony-stimulating factor serum level seems to be a marker of severity of the illness.[99]

For Lassa fever, no correlation exists between antibody levels and outcome. Viremia levels in Lassa fever are important in terms of prognostic value; basically, the higher the viremia, the higher the mortality rate. The serum aspartate aminotransferase level also is an accurate prognostic marker; values above 150 U/mL are associated with a 55 percent mortality rate, whereas the overall mortality rate is approximately 15 percent.[80]

Treatment

Arenaviral hemorrhagic fever should be managed by monitoring and correction of fluid, electrolyte, and osmotic imbalances and of metabolic acidosis. Hydration should be done cautiously because of the generalized capillary leak and the possibility of precipitating pulmonary edema. If possible, bleeding should be treated with clotting factor and platelet replacement as guided by laboratory test results.

For Junín virus infection, immune serum therapy is effective in reducing the incidence of mortality when it is given within the first 8 days of illness.[45,96] Experimental evidence suggests that the plasma may work through attacking infected cells as well as viral neutralization. An important note is that 10 percent of the patients who received this treatment secondarily developed cerebellar signs, such as headaches and tremors. These clinical signs are transient; they are thought to be related to immunopathologic mechanisms induced by the treatment with convalescent plasma.

Despite the recognized efficacy of convalescent plasma for treatment of Machupo virus infection, the paucity of survivors and the lack of programs for collection and storage of BHF immune plasma anticipate the problem of shortage in case of a new outbreak. In recent cases, ribavirin was offered to two patients presenting with life-threatening infection; both recovered without sequelae. These promising results suggest the need for performing more extensive clinical studies to assess the usefulness of ribavirin for treatment of BHF.[83]

Junín and Guanarito viruses showed very high in vitro sensitivity to ribavirin, which also showed antiviral effect in patients.[46] One of the laboratory-acquired Sabiá virus infections was treated successfully with ribavirin; the treatment was started early after the onset, and there was no production of neutralizing antibodies.

The late evolution and low titers of Lassa virus neutralizing antibodies render immune serum–based therapy less efficient than in treatment of South American arenaviral fevers.[100] In contrast, clinical studies have shown ribavirin to be of benefit in severe cases and that it therefore should be used as early as possible in the course of the disease. The intravenous regimen that is recommended is as follows:

- a loading dose of 30 mg/kg, then
- 15 mg/kg every 6 hours for 4 days, then
- 7.5 mg/kg every 8 hours for 6 days.

A recognized side effect of ribavirin is hemolytic anemia, reversible when drug administration is interrupted.

The Future of Antivirals in the Domain of Arenaviruses

In the past 5 years, basic research has used reverse genetics to develop replicons and infectious clones that are used to investigate virus replication mechanisms, to test antivirals in vitro, and to better understand arenavirus cycles. The development of reverse genetic systems allows conduction of a detailed molecular characterization of the viral *cis*-acting signals and *trans*-acting factors that control each of the steps of the virus cycle, including RNA synthesis, packaging, and budding. Likewise, the ability to generate predetermined specific mutations in the genome and to analyze their phenotypic expression will contribute to elucidation of the arenavirus-host interactions as well as the mechanisms leading to severe hemorrhagic disease in humans. The development of a mini-replicon system for Lassa virus will help the investigation of replication and transcription and may facilitate the testing of antivirals outside biosafety level 4 laboratories.[69]

Infectious LCM virus has been recovered entirely from cDNA. Intracellular transcription from polymerase I–driven vectors and coexpression of the minimal viral *trans*-acting factors NP and L from polymerase II–driven plasmids resulted in the efficient formation of infectious virus with genetic tags in both genome segments; this cDNA-derived virus behaves identically to wild-type virus in both cell culture and infected mice.[51]

High throughput screening of molecules for their antiviral effects is being performed increasingly by both public laboratories and private companies. From 2003 to 2006, seven articles reporting testing of molecules with antiviral efficacy on arenaviruses were published.* The last 5 years have witnessed an acceleration in the research activity in antivirals against these viruses.†

PREVENTION AND CONTROL

Prevention of arenavirus disease consists of preventing transmission from rodents to humans, from humans to humans, and from infected specimens to laboratory personnel. Strategies for avoiding contact between rodents and humans have been effective in BHF.[94] Simple trapping of *C. callosus* in towns was successful in reducing human contact and thus reducing the disease to essentially zero. This is more difficult in AHF because conditions under which human exposure occurs are different from those in BHF. *C. musculinus* (reservoir of Junín virus) distribution is much wider than that of *C. callosus* (reservoir of Machupo virus), and Argentine agricultural practices continue to place workers at risk for exposure to reservoir hosts.

A collaborative effort conducted by the United States and Argentine governments led to the production of a live attenuated Junín virus vaccine named Candid 1, which has passed safety and immunogenicity tests in U.S. volunteers. Its efficacy was established in a double-blind trial in 15,000 agricultural workers at risk for natural infection in Argentina. Subsequently, more than 100,000 persons were immunized with Junín virus vaccine in Argentina. Animal protection studies suggest that the Junín vaccine could be protective against Machupo virus infections as well. A prospective study conducted during two epidemic seasons among 6500 male agricultural workers in Argentina showed that Candid 1 vaccine efficacy was 84 percent or higher and that no serious adverse effects could be expected.[97]

Neutralizing antibody titers to Candid 1 vaccine against AHF were studied for 2 years after vaccination in 330 volunteers. Of a total of 160 volunteers who received Candid 1, 54 had no detectable preinfection with arenaviruses, 55 had preexisting antibodies to Junín virus, and 51 had preexisting antibodies to LCM virus; the remaining 170 individuals received placebo. Levels of anti–Junín virus antibodies displayed a trend in which titers increased with the virulence of the infecting strain, from Candid 1 through subclinical Junín virus infection, vaccination after subclinical infection, and Junín virus symptomatic infection. The study also demonstrated that the mean titer of neutralizing antibodies to Candid 1 did not vary significantly during the 2 years, was significantly lower than that elicited by wild strains of Junín virus, significantly increased the titers of preexisting anti-Junín antibodies, and was not modified by preexisting anti-LCM virus antibodies.[2]

Comparative sequence analysis of the L RNA segment of Candid 1 strain and the more virulent ancestors (XJ 44 and XJ 13) revealed 12 point mutations in the L polypeptide that are unique to the vaccine strain.[62] Whether these changes are associated with the attenuated phenotype remains to be investigated by the use of the reverse genetic systems.

For VHF, no preventive measures have been developed. Attenuated Junín virus strains do not protect experimental animals against challenge with Guanarito virus. In VHF, the evidence suggests that transmission occurs around houses and in fields as in BHF, so measures to avoid contact between rodents and humans should be effective, as shown for BHF.

For Lassa fever, reducing the contact with *Mastomys natalensis* is a formidable task in West Africa, and this option does not seem to have a promising future. Because person-to-person transmission has been reported in Lassa fever, precautions should be taken to place patients in single rooms. The health care team should be small and adequately trained; they should wear gloves, gowns, and filter masks.[23,25] All secretions should be decontaminated.[88] Laboratory procedures should be done with care by use of inactivating methods of heat, chemicals, or irradiation.

In rhesus monkeys (*Cercopithecus aethiops*), the humoral antibody response measured after they were challenged with purified inactivated Lassa virus, although as high as in humans who recovered from Lassa fever, was insufficient to protect the animals from a fatal outcome.[103] A naturally attenuated strain (Mopeia virus, from Mozambique) protects rhesus monkeys against challenge with Lassa virus, but field studies are required to establish the extent and nature of natural human infection with this virus before it can be considered seriously as a candidate for human vaccine development. Alternative approaches, including the use of vaccinia virus vectors bearing the Lassa virus GPC or N gene, are being investigated actively and show promising preliminary results.[48,50] Two peptides encoded by the glycoprotein precursor were shown to display high-affinity binding to human leukocyte antigen (HLA)–A*0201 transgenic mice; mice immunized with these peptides were protected against challenge with a recombinant vaccinia virus that expressed Lassa virus glycoproteins. These two epitopes represent candidates for inclusion in epitope-based vaccine constructs.[6]

The Future of Vaccine Research

Tremendous advances have been made during the last years for vaccine development.* Many different approaches were used. Reverse genetic exchange of the viral glycoprotein for foreign glycoproteins created attenuated vaccine strains that remained viable although unable to cause disease in infected mice; this phenotype remained stable even after extensive propagation in immunodeficient hosts. The engineered viruses induced T cell–mediated immunity, protecting against systemic infection and severe liver disease on wild-type virus

*References 1, 3, 5, 20, 22, 65, 149.
†References 21, 30, 85, 104, 105, 116, 129, 137, 150.

*References 7, 36, 44, 61, 64, 84.

challenge.[4] The yellow fever vaccine 17D has been used as a vector for the Lassa virus glycoprotein precursor, resulting in construction of recombinant virus, which was replication competent and processed Lassa virus glycoprotein in cell culture. The recombinant virus replicated poorly in guinea pigs but still elicited specific antibodies against Lassa and yellow fever antigens, and single subcutaneous injection protected guinea pigs against fatal Lassa fever.[91] Clone ML29 is a reassortant virus that encodes the nucleocapsid protein and glycoproteins from Lassa virus and the RNA polymerase and matrix protein of Mopeia virus; replication of ML29 was attenuated in guinea pigs and nonhuman primates. Guinea pigs vaccinated with ML29 survived after challenge with Lassa virus without developing either signs of disease or histologic lesions. Rhesus macaques inoculated with clone ML29 developed primary virus-specific T cells capable of secreting interferon in response to homologous and heterologous challenge (the latter with Mopeia virus and LCM virus). Vaccinated monkeys did not present any histologic lesions or signs of disease.[90]

NEW REFERENCES SINCE THE SIXTH EDITION

7. Botten J, Whitton JL, Barrowman P, et al. A multivalent vaccination strategy for the prevention of Old World arenavirus infection in humans. *J Virol* 2010;**84**:9947–56.
11. Briese T, Paweska JT, McMullan LK, et al. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever–associated arenavirus from southern Africa. *PLoS Pathog* 2009;**5**:e1000455.
15. Cajimat MN, Milazzo ML, Borchert JN, et al. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the Mexican woodrat (*Neotoma mexicana*). *Virus Res* 2008;**133**:211–17.
17. Cajimat MN, Milazzo ML, Bradley RD, Fulhorst CF. Ocozocoautla de espinosa virus and hemorrhagic fever, Mexico. *Emerg Infect Dis* 2012;**18**:401–5.
21. Cashman KA, Smith MA, Twenhafel NA, et al. Evaluation of Lassa antiviral compound ST-193 in a guinea pig model. *Antiviral Res* 2011**90**:70–9.
30. Charrel RN, Coutard B, Baronti C, et al. Arenaviruses and hantaviruses: from epidemiology and genomics to antivirals. *Antiviral Res* 2011;**90**:102–14.
33. Coulibaly-N'Golo D, Allali B, Kouassi SK, et al. Novel arenavirus sequences in *Hylomyscus* sp. and *Mus (Nannomys) setulosus* from Côte d'Ivoire: implications for evolution of arenaviruses in Africa. *PLoS One* 2011;**6**:e20893.
36. de la Torre JC. Reverse genetics approaches to combat pathogenic arenaviruses. *Antiviral Res* 2008;**80**:239–50.
38. Delgado S, Erickson BR, Agudo R, et al. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog* 2008;**4**:e1000047.
44. Emonet SF, Seregin AV, Yun NE, et al. Rescue from cloned cDNAs and in vivo characterization of recombinant pathogenic Romero and live-attenuated Candid #1 strains of Junin virus, the causative agent of Argentine hemorrhagic fever disease. *J Virol* 2011;**85**:1473–83.
61. Goicochea MA, Zapata JC, Bryant J, et al. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. *Vaccine* 2012;**30**:1445–52.
64. Grant-Klein RJ, Altamura LA, Schmaljohn CS. Progress in recombinant DNA-derived vaccines for Lassa virus and filoviruses. *Virus Res* 2011;**162**:148–61.
67. Günther S, Hoofd G, Charrel R, et al. Mopeia virus–related arenavirus in natal multimammate mice, Morogoro, Tanzania. *Emerg Infect Dis* 2009;**15**:2008–12.
74. Inizan CC, Cajimat MN, Milazzo ML, et al. Genetic evidence for a Tacaribe serocomplex virus, Mexico. *Emerg Infect Dis* 2010;**16**:1007–10.
75. Ishii A, Thomas Y, Moonga L, et al. Novel arenavirus, Zambia. *Emerg Infect Dis* 2011;**17**:1921–4.
76. Ishii A, Thomas Y, Moonga L, et al. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. *J Gen Virol* 2012;**93**:2247–51.
84. Kotturi MF, Botten J, Sidney J, et al. A multivalent and cross-protective vaccine strategy against arenaviruses associated with human disease. *PLoS Pathog* 2009;**5**:e1000695.
85. Lee AM, Pasquato A, Kunz S. Novel approaches in anti-arenaviral drug development. *Virology* 2011;**411**:163–9.
104. Mendenhall M, Russell A, Juelich T, et al. T-705 (favipiravir) inhibition of arenavirus replication in cell culture. *Antimicrob Agents Chemother* 2011;**55**:782–7.
105. Mendenhall M, Russell A, Smee DF, et al. Effective oral favipiravir (T-705) therapy initiated after the onset of clinical disease in a model of arenavirus hemorrhagic fever. *PLoS Negl Trop Dis* 2011;**5**:e1342.
110. Milazzo ML, Cajimat MN, Haynie ML, et al. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the white-throated woodrat (*Neotoma albigula*) in the southwestern United States. *Vector Borne Zoonotic Dis* 2008;**8**:523–40.
111. Milazzo ML, Campbell GL, Fulhorst CF. Novel arenavirus infection in humans, United States. *Emerg Infect Dis* 2011;**17**:1417–20.
116. Neuman BW, Bederka LH, Stein DA, et al. Development of peptide-conjugated morpholino oligomers as pan-arenavirus inhibitors. *Antimicrob Agents Chemother* 2011;**55**:4631–8.
118. Olschläger S, Günther S. Rapid and specific detection of Lassa virus by reverse transcription–PCR coupled with oligonucleotide array hybridization. *J Clin Microbiol* 2012;**50**:2496–9.
119. Palacios G, Druce J, Du L, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. *N Engl J Med* 2008;**358**:991–8.
120. Palacios G, Savji N, Hui J, et al. Genomic and phylogenetic characterization of Merino Walk virus, a novel arenavirus isolated in South Africa. *J Gen Virol* 2010;**91**:1315–24.
121. Panning M, Emmerich P, Olschläger S, et al. Laboratory diagnosis of Lassa fever, Liberia. *Emerg Infect Dis* 2010;**16**:1041–3.
124. Paweska JT, Sewlall NH, Ksiazek TG, et al. Nosocomial outbreak of novel arenavirus infection, southern Africa. *Emerg Infect Dis* 2009;**15**:1598–602.
129. Radoshitzky SR, Kuhn JH, de Kok–Mercado F, et al. Drug discovery technologies and strategies for Machupo virus and other New World arenaviruses. *Expert Opin Drug Discov* 2012;**7**:613–32.
137. Sepúlveda CS, García CC, Fascio ML, et al. Inhibition of Junin virus RNA synthesis by an antiviral acridone derivative. *Antiviral Res* 2012;**93**:16–22.
140. Stenglein MD, Sanders C, Kistler AL, et al. Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. *MBio* 2012;**3**:e00180–12.
148. Trombley AR, Wachter L, Garrison J, et al. Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and New World hantaviruses. *Am J Trop Med Hyg* 2010;**82**:954–60.
150. Urata S, Yun N, Pasquato A, et al. Antiviral activity of a small-molecule inhibitor of arenavirus glycoprotein processing by the cellular site 1 protease. *J Virol* 2011;**85**:795–803.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Albiol Matanic VC, Castilla V. Antiviral activity of antimicrobial cationic peptides against Junin virus and herpes simplex virus. *Int J Antimicrob Agents* 2004;**23**:382–9.
2. Ambrosio AM, Riera LM, Saavedra Mdel C, Sabattini MS. Immune response to vaccination against Argentine hemorrhagic fever in an area where different arenaviruses coexist. *Viral Immunol* 2006;**19**:196–201.
3. Asper M, Sternsdorf T, Hass M, et al. Inhibition of different Lassa virus strains by alpha and gamma interferons and comparison with a less pathogenic arenavirus. *J Virol* 2004;**78**:3162–9.
4. Bergthaler A, Gerber NU, Merkler D, et al. Envelope exchange for the generation of live-attenuated arenavirus vaccines. *PLoS Pathog* 2006;**2**:e51.
5. Bolken TC, Laquerre S, Zhang Y, et al. Identification and characterization of potent small molecule inhibitor of hemorrhagic fever New World arenaviruses. *Antiviral Res* 2006;**69**:86–97.
6. Botten J, Alexander J, Pasquetto V, et al. Identification of protective Lassa virus epitopes that are restricted by HLA-A2. *J Virol* 2006;**80**:8351–61.
7. Botten J, Whitton JL, Barrowman P, et al. A multivalent vaccination strategy for the prevention of Old World arenavirus infection in humans. *J Virol* 2010;**84**:9947–56.
8. Bowen MD, Peters CJ, Mills JN, Nichol ST. Oliveros virus: a novel arenavirus from Argentina. *Virology* 1996;**217**:362–6.
9. Bowen MD, Peters CJ, Nichol ST. The phylogeny of New World (Tacaribe complex) arenaviruses. *Virology* 1996;**219**:285–90, 1996.
10. Bowen MD, Peters CJ, Nichol ST. Phylogenetic analysis of the Arenaviridae: patterns of virus evolution and evidence for cospeciation between arenaviruses and their rodent hosts. *Mol Phylogenet Evol* 1997;**8**:301–16.
11. Briese T, Paweska JT, McMullan LK, et al. Genetic detection and characterization of Lujo virus, a new hemorrhagic fever–associated arenavirus from southern Africa. *PLoS Pathog* 2009;**5**:e1000455.
12. Briggiler AM, Levis S, Enria D, et al. Argentine hemorrhagic fever in pregnant women. *Medicina (Buenos Aires)* 1990;**50**:443.
13. Buchmeier M, Adam E, Rawls WE. Serologic evidence of infection by Pichinde virus among laboratory workers. *Infect Immun* 1974;**9**:821–3.
14. Buckley SM, Casals J. Lassa fever, a new virus of man from West Africa. III. Isolation and characterization of the virus. *Am J Trop Med Hyg* 1970;**19**:680–91.
15. Cajimat MN, Milazzo ML, Borchert JN, et al. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the Mexican woodrat (*Neotoma mexicana*). *Virus Res* 2008;**133**:211–17.
16. Cajimat MN, Milazzo ML, Bradley RD, Fulhorst CF. Catarina virus, an arenaviral species principally associated with *Neotoma micropus* (southern plains woodrat) in Texas. *Am J Trop Med Hyg* 2007;**77**:732–6.
17. Cajimat MN, Milazzo ML, Bradley RD, Fulhorst CF. Ocozocoautla de espinosa virus and hemorrhagic fever, *Mexico Emerg Infect Dis* 2012;**18**:401–5.
18. Calisher CH, Nabity S, Root JJ, et al. Transmission of an arenavirus in white-throated woodrats *(Neotoma albigula)*, southeastern Colorado, 1995-1999. *Emerg Infect Dis* 2001;**7**:397–402.
19. Calisher CH, Tzianabos T, Lord RD, Coleman PH. Tamiami virus, a new member of the Tacaribe group. *Am J Trop Med Hyg* 1970;**19**:520–6.
20. Carey DC, Kemp GE, White HA, et al. Lassa fever: epidemiological aspects of the 1970 epidemic, Jos, Nigeria. *Trans R Soc Trop Med Hyg* 1972;**66**:402–8.
21. Cashman KA, Smith MA, Twenhafel NA, et al. Evaluation of Lassa antiviral compound ST-193 in a guinea pig model. *Antiviral Res* 2011;**90**:70–9.
22. Castilla V, Larzabal M, Sgalippa NA, et al. Antiviral mode of action of a synthetic brassinosteroid against Junin virus replication. *Antiviral Res* 2005;**68**:88–95.
23. Centers for Disease Control. Management of patients with suspected viral hemorrhagic fever. *MMWR Morb Mortal Wkly Rep* 1988;**37**:1–16.
24. Centers for Disease Control and Prevention. Bolivian hemorrhagic fever—El Beni Department, Bolivia, 1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**:943–6.
25. Centers for Disease Control and Prevention. Update: management of patients with suspected viral hemorrhagic fever. *MMWR Morb Mortal Wkly Rep* 1995;**44**:475–9.
26. Centers for Disease Control and Prevention. Fatal illnesses associated with a New World arenavirus—California, 1999-2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:709–11.
27. Charrel RN, de Lamballerie X, Fulhorst CF. The Whitewater Arroyo virus: natural evidence for genetic recombination among Tacaribe serocomplex viruses (family Arenaviridae). *Virology* 2001;**283**:161–6.
28. Charrel RN, Feldmann H, Fulhorst CF, et al. Phylogeny of New World arenaviruses based on the complete coding sequences of the small genomic segment identified an evolutionary lineage produced by intrasegmental recombination. *Biochem Biophys Res Commun* 2002;**296**:1118–24.
29. Charrel RN, Lemasson JJ, Garbutt M, et al. New insights into the evolutionary relationships between arenaviruses provided by comparative analysis of small and large segment sequences. *Virology* 2003;**317**:191–6.
30. Charrel RN, Coutard B, Baronti C, et al. Arenaviruses and hantaviruses: from epidemiology and genomics to antivirals. *Antiviral Res* 2011;**90**:102–14.
31. Child PL, Mackenzie RB, Valverde LR, et al. Bolivian haemorrhagic fever: a pathologic description. *Arch Pathol* 1967;**83**:434–45.
32. Coimbra TLM, Nassar ES, Burattini MN, et al. New arenavirus isolated in Brazil. *Lancet* 1994;**343**:391–2.
33. Coulibaly-N'Golo D, Allali B, Kouassi SK, et al. Novel arenavirus sequences in *Hylomyscus* sp. and *Mus (Nannomys) setulosus* from Côte d'Ivoire: implications for evolution of arenaviruses in Africa. *PLoS One* 2011;**6**:e20893.
34. Cummins D. Lassa fever. *Br J Hosp Med* 1990;**43**:186–92.
35. Cummins D, Bennett D, Fisher-Hoch SP, et al. Lassa fever encephalopathy: clinical and laboratory findings. *J Trop Med Hyg* 1992;**95**:197–201.
36. de la Torre JC. Reverse genetics approaches to combat pathogenic arenaviruses. *Antiviral Res* 2008;**80**:239–50.
37. de Manzione N, Salas RA, Paredes H, et al. Venezuelan hemorrhagic fever: clinical and epidemiological studies of 165 cases. *Clin Infect Dis* 1998;**26**:308–13.
38. Delgado S, Erickson BR, Agudo R, et al. Chapare virus, a newly discovered arenavirus isolated from a fatal hemorrhagic fever case in Bolivia. *PLoS Pathog* 2008;**4**:e1000047.
39. Demby AH, Chamberlain J, Brown DW, et al. Early diagnosis of Lassa fever by reverse transcription–PCR. *J Clin Microbiol* 1994;**32**:2898–903.
40. Douglas RG, Wiebenga NH, Couch RB, et al. Bolivian hemorrhagic fever probably transmitted by personal contact. *Am J Epidemiol* 1965;**82**:85–91.
41. Downs WG, Anderson CR, Spence L, et al. Tacaribe virus, a new agent isolated from *Artibeus* bats and mosquitoes in Trinidad, West Indies. *Am J Trop Med Hyg* 1963;**12**:640–6.
42. Drosten C, Panning M, Guenther S, Schmitz H. False-negative results of PCR assay with plasma of patients with severe viral hemorrhagic fever. *J Clin Microbiol* 2002;**40**:4394–5.
43. Emonet S, Lemasson JJ, Gonzalez JP, et al. Phylogeny and evolution of old world arenaviruses. *Virology* 2006;**350**:251–7.
44. Emonet SF, Seregin AV, Yun NE, et al. Rescue from cloned cDNAs and in vivo characterization of recombinant pathogenic Romero and live-attenuated Candid #1 strains of Junin virus, the causative agent of Argentine hemorrhagic fever disease. *J Virol* 2011;**85**:1473–83.
45. Enria DA, Briggiler AM, Fernandez NJ, et al. Dose of neutralising antibodies for Argentine hemorrhagic fever. *Lancet* 1984;**2**:255–6.
46. Enria DA, Briggiler AM, Levis S, et al. Tolerance and antiviral effects of ribavirin in patients with Argentine hemorrhagic fever. *Antiviral Res* 1987;**7**:353–9.
47. Enria D, Feuillade MR. Argentine haemorrhagic fever (Junin virus—Arenaviridae): a review on clinical, epidemiological, ecological, treatment and preventive aspects of the disease. In: Travassos da Rosa APA, Vasconcelos PFC, Travassos da Rosa JFS, editors. *An overview of arbovirology in Brazil and neighboring countries*. Belem, Brazil: Instituto Evandro Chaggas; 1998. p. 219–32.
48. Fisher-Hoch SP, McCormick JB, Auperin D, et al. Protection of rhesus monkeys from fatal Lassa fever by vaccination with a

recombinant vaccinia virus containing the Lassa virus glycoprotein gene. *Proc Natl Acad Sci U S A* 1988;**85**:1–6.
49. Fisher-Hoch SP, Tomori O, Nasidi A, et al. Review of cases of nosocomial Lassa fever in Nigeria: the high price of poor medical practice. *BMJ* 1995;**311**:857–9.
50. Fisher-Hoch SP, Hutwagner L, Brown B, et al. Effective vaccine for Lassa fever. *J Virol* 2000;**74**:6777–83.
51. Flatz L, Bergthaler A, de la Torre JC, Pinschewer DD. Recovery of an arenavirus entirely from RNA polymerase I/II–driven cDNA. *Proc Natl Acad Sci U S A* 2006;**103**:4663–8.
52. Fulhorst CF, Bowen MD, Ksiazek TG, et al. Isolation and characterization of Whitewater Arroyo virus, a novel North American arenavirus. *Virology* 1996;**224**:114–20.
53. Fulhorst CF, Bowen MD, Salas RA, et al. Natural rodent host associations of Guanarito and Pirital viruses (family Arenaviridae) in Central Venezuela. *Am J Trop Med Hyg* 1999;**61**:325–30.
54. Fulhorst CF, Ksiazek TG, Peters CJ, et al. Experimental infection of the cane mouse *Zygodontomys brevicauda* (family Muridae) with Guanarito virus (Arenaviridae), the etiologic agent of Venezuelan hemorrhagic fever. *J Infect Dis* 1999;**180**:966–9.
55. Fulhorst CF, Charrel RN, Weaver SC, et al. Geographic distribution and genetic diversity of Whitewater Arroyo virus in the southwestern Unites States. *Emerg Infect Dis* 2001;**7**:403–7.
56. Fulhorst CF, Bennett SG, Milazzo ML, et al. Bear Canyon virus: an arenavirus naturally associated with the California mouse (*Peromyscus californicus*). *Emerg Infect Dis* 2002;**8**:717–21.
57. Frame JD, Baldwin JM Jr, Gocke DJ, et al. Lassa fever, a new virus disease of man from West Africa: clinical description and pathological findings. *Am J Trop Med Hyg* 1970;**19**:670–6.
58. Frame JD. Surveillance of Lassa fever in missionaries stationed in West Africa. *Bull World Health Organ* 1975;**52**:593–8.
59. Frame JD, Jahrling PB, Yalley-Ogunro JE, et al. Endemic Lassa fever in Liberia. II. Serological and virological findings in hospital patients. *Trans R Soc Trop Med Hyg* 1984;**78**:656–60.
60. Garcia JB, Morzunov SP, Levis S, et al. Genetic diversity of the Junin virus in Argentina: geographic and temporal patterns. *Virology* 2000;**272**:127–36.
61. Goicochea MA, Zapata JC, Bryant J, et al. Evaluation of Lassa virus vaccine immunogenicity in a CBA/J-ML29 mouse model. *Vaccine* 2012;**30**:1445–52.
62. Goni SE, Iserte JA, Ambrosio AM, et al. Genomic features of attenuated Junin virus vaccine strain candidate. *Virus Genes* 2006; **32**:37–41.
63. Gonzalez JP, Josse R, Johnson ED, et al. Antibody prevalence against haemorrhagic fever viruses in randomized representative Central African populations. *Res Virol* 1989;**140**:319–31.
64. Grant-Klein RJ, Altamura LA, Schmaljohn CS. Progress in recombinant DNA-derived vaccines for Lassa virus and filoviruses. *Virus Res* 2011;**162**:148–61.
65. Günther S, Asper M, Roser C, et al. Application of real-time PCR for testing antiviral compounds against Lassa virus, SARS coronavirus and Ebola virus in vitro. *Antiviral Res* 2004;**63**:209–15.
66. Günther S, Emmerich P, Laue T, et al. Imported Lassa fever in Germany: molecular characterization of a new Lassa virus strain. *Emerg Infect Dis* 2000;**6**:466–76.
67. Günther S, Hoofd G, Charrel R, et al. Mopeia virus–related arenavirus in natal multimammate mice, Morogoro, Tanzania. *Emerg Infect Dis* 2009;**15**:2008–12.
68. Harrison LH, Halsey NA, McKee KT Jr, et al. Clinical case definitions for Argentine hemorrhagic fever. *Clin Infect Dis* 1999;**28**:1091–4.
69. Hass M, Golnitz U, Muller S, et al. Replicon system for Lassa virus. *J Virol* 2004;**78**:13793–803.
70. Helmick CG, Webb PA, Scribner CL, et al. No evidence for increased risk of Lassa fever infection in hospital staff. *Lancet* 1986;**2**:1202–5.
71. Henderson BE, Gary GW, Kissling RE, et al. Lassa fever. Virological and serological studies. *Trans R Soc Trop Med Hyg* 1972;**66**:409–16.
72. Hirabayashi Y, Oka S, Goto H, et al. An imported case of Lassa fever with late appearance of polyserositis. *J Infect Dis* 1988;**158**: 872–5.
73. Holmes GP, McCormick JB, Trock SC, et al. Lassa fever in the United States: investigation of a case and new guidelines for management. *N Engl J Med* 1990;**323**:1120–3.
74. Inizan CC, Cajimat MN, Milazzo ML, et al. Genetic evidence for a Tacaribe serocomplex virus, *Mexico Emerg Infect Dis* 2010; **16**:1007–10.
75. Ishii A, Thomas Y, Moonga L, et al. Novel arenavirus, Zambia. *Emerg Infect Dis* 2011;**17**:1921–4.
76. Ishii A, Thomas Y, Moonga L, et al. Molecular surveillance and phylogenetic analysis of Old World arenaviruses in Zambia. *J Gen Virol* 2012;**93**:2247–51.
77. Jahrling PB, Peters CJ. Passive antibody therapy of Lassa fever in cynomolgus monkeys: importance of neutralizing antibody and Lassa virus strain. *Infect Immun* 1984;**44**:528–33.
78. Johnson KM, Wiebenga NH, Mackenzie RB, et al. Virus isolations from human cases of hemorrhagic fever in Bolivia. *Proc Soc Exp Biol Med* 1965;**118**:113–18.
79. Johnson KM, Halstead SB, Cohen SN. Hemorrhagic fevers of Southeast Asia and South America: a comparative appraisal. *Prog Med Virol* 1967;**9**:105–58.
80. Johnson KM, McCormick JB, Webb PA, et al. Clinical virology of Lassa fever in hospitalized patients. *J Infect Dis* 1987;**155**: 456–64.
81. Keane E, Gilles HM. Lassa fever in Panguma Hospital, Sierra Leone, 1973-1976. *BMJ* 1977;**1**:1399–402.
82. Keenlyside RA, McCormick JB, Webb PA, et al. Case-control study of *Mastomys natalensis* and humans in Lassa virus–infected households in Sierra Leone. *Am J Trop Med Hyg* 1983;**32**: 829–37.
83. Kilgore PE, Ksiazek TG, Rollin PE, et al. Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin Infect Dis* 1997; **24**:718–22.
84. Kotturi MF, Botten J, Sidney J, et al. A multivalent and cross-protective vaccine strategy against arenaviruses associated with human disease. *PLoS Pathog* 2009;**5**:e1000695.
85. Lee AM, Pasquato A, Kunz S. Novel approaches in anti-arenaviral drug development. *Virology* 2011;**411**:163–9.
86. Levis SC, Saavedra MC, Ceccoli C, et al. Correlation between endogenous interferon and the clinical evolution of patients with Argentine hemorrhagic fever. *J Interferon Res* 1985;**5**:383–9.
87. Lisieux T, Coimbra M, Nassar ES, et al. New arenavirus isolated in Brazil. *Lancet* 1994;**343**:391–2.
88. Lloyd G, Bowen ETW, Slade JHR. Physical and chemical methods of inactivating Lassa virus. *Lancet* 1982;**1**:1046–8.
89. Lozano ME, Enria D, Maiztegui JI, et al. Rapid diagnosis of Argentine hemorrhagic fever by reverse transcriptase PCR–based assay. *J Clin Microbiol* 1995;**33**:1327–32.
90. Lukashevich IS, Patterson J, Carrion R, et al. A live attenuated vaccine for Lassa fever made by reassortment of Lassa and Mopeia viruses. *J Virol* 2005;**79**:13934–42.
91. Lukashevich IS. A recombinant yellow fever 17D vaccine expressing Lassa virus glycoproteins. *Virology* 2006;**345**:299–304.
92. Lunkenheimer K, Hufert FT, Schmitz H. Detection of Lassa virus RNA in specimens from patients with Lassa fever by using the polymerase chain reaction. *J Clin Microbiol* 1990;**28**: 2689–92.
93. Macher AM, Wolfe MS. Historical Lassa fever reports and 30-year clinical update. *Emerg Infect Dis* 2006;**12**:835–7.
94. Mackenzie RB. Epidemiology of Machupo virus infection. I. Pattern of human infection, San Joaquin, Bolivia, 1962-1964. *Am J Trop Med Hyg* 1965;**14**:808–13.
95. Maiztegui JI. Clinical and epidemiological patterns of Argentine haemorrhagic fever. *Bull World Health Organ* 1975;**52**:567–76.
96. Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine hemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet* 1979;**2**:1216–17.
97. Maiztegui JI, McKee KT Jr, Barrera Oro JG, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis* 1998;**177**:277–83.
98. Marta RF, Montero VS, Hack CE, et al. Proinflammatory cytokines and elastase-alpha-1-antitrypsin in Argentine hemorrhagic fever. *Am J Trop Med Hyg* 1999;**60**:85–9.
99. Marta RF, Enria D, Molinas FC. Relationship between hematopoietic growth factors levels and hematological parameters in Argentine hemorrhagic fever. *Am J Hematol* 2000;**64**:1–6.
100. McCormick JB, King IJ, Webb PA, et al. Lassa fever: effective therapy with ribavirin. *N Engl J Med* 1986;**314**:20–6.

101. McCormick JB, Walker DH, King IJ, et al. Lassa virus hepatitis: a study of fatal Lassa fever in humans. *Am J Trop Med Hyg* 1986; **35**:401–7.
102. McCormick JB, Webb PA, Krebs JW, et al. A prospective study of the epidemiology and ecology of Lassa fever. *J Infect Dis* 1987;**155**:437–44.
103. McCormick JB, Mitchell SW, Kiley MP, et al. Inactivated Lassa virus elicits a nonprotective immune response in rhesus monkeys. *J Med Virol* 1992;**37**:1–7.
104. Mendenhall M, Russell A, Juelich T, et al. T-705 (favipiravir) inhibition of arenavirus replication in cell culture. *Antimicrob Agents Chemother* 2011;**55**:782–7.
105. Mendenhall M, Russell A, Smee DF, et al. Effective oral favipiravir (T-705) therapy initiated after the onset of clinical disease in a model of arenavirus hemorrhagic fever. *PLoS Negl Trop Dis* 2011; **5**:e1342.
106. Mercado R. Rodent control programmes in areas affected by Bolivian haemorrhagic fever. *Bull World Health Organ* 1975;**52**: 691–5.
107. Mertens PE, Patton R, Baum JJ, et al. Clinical presentation of Lassa fever during the hospital epidemic of Zorzor, Liberia, March-April 1972. *Am J Trop Med Hyg* 1973;**22**:780–4.
108. Meunier DY, McCormick JB, Georges AJ, et al. Comparison of Lassa, Mobala, and Ippy virus reactions by immunofluorescence test. *Lancet* 1985;**1**:873–4.
109. Mifune K, Carter M, Rawls W. Characterization studies of the Pichinde virus—a member of the arenavirus group. *Proc Soc Exp Biol Med* 1971;**136**:637–44.
110. Milazzo ML, Cajimat MN, Haynie ML, et al. Diversity among Tacaribe serocomplex viruses (family Arenaviridae) naturally associated with the white-throated woodrat (*Neotoma albigula*) in the southwestern United States. *Vector Borne Zoonotic Dis* 2008;**8**: 523–40.
111. Milazzo ML, Campbell GL, Fulhorst CF. Novel arenavirus infection in humans, United States. *Emerg Infect Dis* 2011;**17**: 1417–20.
112. Mills JN, Ellis BA, McKee KT, et al. Junin virus activity in rodents from endemic and nonendemic loci in centrol Argentina. *Am J Trop Med Hyg* 1991;**44**:589–97.
113. Monath TP, Maher M, Casals J, et al. Lassa fever in the Eastern Province of Sierra Leone, 1970-1972. II. Clinical observations and virological studies on selected hospital cases. *Am J Trop Med Hyg* 1974;**23**:1140–9.
114. Moncayo AC, Hice CL, Watts DM, et al. Allpahuayo virus: a newly recognized arenavirus (Arenaviridae) from arboreal rice rats (*Oecomys bicolor* and *Oecomys paricola*) in northeastern Peru. *Virology* 2001;**284**:277–86.
115. Monson MH, Cole AK, Frame JD, et al. Pediatric Lassa fever: a review of 33 Liberian cases. *Am J Trop Med Hyg* 1987;**36**:408–15.
116. Neuman BW, Bederka LH, Stein DA, et al. Development of peptide-conjugated morpholino oligomers as pan-arenavirus inhibitors. *Antimicrob Agents Chemother* 2011;**55**:4631–8.
117. Niedrig M, Schmitz H, Becker S, et al. First international quality assurance study on the rapid detection of viral agents of bioterrorism. *J Clin Microbiol* 2004;**42**:1753–5.
118. Olschläger S, Günther S. Rapid and specific detection of Lassa virus by reverse transcription–PCR coupled with oligonucleotide array hybridization. *J Clin Microbiol* 2012;**50**:2496–9.
119. Palacios G, Druce J, Du L, et al. A new arenavirus in a cluster of fatal transplant-associated diseases. *N Engl J Med* 2008;**358**: 991–8.
120. Palacios G, Savji N, Hui J, et al. Genomic and phylogenetic characterization of Merino Walk virus, a novel arenavirus isolated in South Africa. *J Gen Virol* 2010;**91**:1315–24.
121. Panning M, Emmerich P, Olschläger S, et al. Laboratory diagnosis of Lassa fever, *Liberia Emerg Infect Dis* 2010;**16**:1041–3.
122. Parodi AS, Greenway DJ, Rugiero HR, et al. Sobre la étiologia del brote epidemico de Junin. *Diagn Med* 1958;**30**:2300–1.
123. Parodi AS, Rugiero HR, Greenway DJ, et al. Isolation of the Junin virus (epidemic hemorrhagic fever) from the mites of the epidemic area (*Echinolaelaps echidninus*, Barlese). *Prensa Med Argent* 1959;**46**:2242–4.
124. Paweska JT, Sewlall NH, Ksiazek TG, et al. Nosocomial outbreak of novel arenavirus infection, southern Africa. *Emerg Infect Dis* 2009;**15**:1598–602.
125. Peters CJ, Webb PA. Measurement of antibodies to Machupo virus by the indirect fluorescent technique. *Proc Soc Exp Biol Med* 1973;**142**:526–31.
126. Peters CJ, Kuehne RW, Mercado RR, et al. Hemorrhagic fever in Cochabamba, Bolivia, 1971. *Am J Epidemiol* 1974;**99**:425–33.
127. Peters CJ, Buchmeier M, Rollin PE, Ksiazek TG. Arenaviruses. In: Fields BN, Knipe DM, Howley PM, et al, editors. *Fields' virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996. p. 1521–51.
128. Price ME, Fisher-Hoch SP, Craven RB, et al. A prospective study of maternal and fetal outcome in acute Lassa fever infection during pregnancy. *BMJ* 1988;**297**:584–7.
129. Radoshitzky SR, Kuhn JH, de Kok–Mercado F, et al. Drug discovery technologies and strategies for Machupo virus and other New World arenaviruses. *Expert Opin Drug Discov* 2012;**7**: 613–32.
130. Reignier T, Oldenburg J, Noble B, et al. Receptor use by pathogenic arenaviruses. *Virology* 2006;**353**:111–20.
131. Rivers TM, Scott TF. Meningitis in man caused by a filterable virus: II. Identification of the etiological agent. *J Exp Med* 1936; **63**:415–32.
132. Rojek JM, Spiropoulou CF, Kunz S. Characterization of the cellular receptors for the South American hemorrhagic fever viruses Junin, Guanarito, and Machupo. *Virology* 2006;**349**:476–91.
133. Salas R, de Manzione N, Tesh RB, et al. Venezuelan haemorrhagic fever. *Lancet* 1991;**338**:1033–6.
134. Saluzzo JF, Adam F, McCormick JB, Digoutte JP. Lassa fever in Senegal. *J Infect Dis* 1988;**157**:605.
135. Sbrana E, Mateo RI, Xiao SY, et al. Clinical laboratory, virologic, and pathologic changes in hamsters experimentally infected with Pirital virus (Arenaviridae): a rodent model of Lassa fever. *Am J Trop Med Hyg* 2006;**74**:1096–102.
136. Schlaeffer F, Bar-Lavie Y, Sikuler E, et al. Evidence against the high contagiousness of Lassa fever. *Trans R Soc Trop Med Hyg* 1988;**82**:31.
137. Sepúlveda CS, García CC, Fascio ML, et al. Inhibition of Junin virus RNA synthesis by an antiviral acridone derivative. *Antiviral Res* 2012;**93**:16–22.
138. Southern PJ. Arenaviridae: the viruses and their replication. In: Fields BN, Knipe DM, Howley PM, et al, editors. *Fields' virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996. p. 1505–19.
139. Spiropoulou CF, Kunz S, Rollin PE, et al. New World arenavirus clade C, but not clade A and B viruses, utilizes alpha-dystroglycan as its major receptor. *J Virol* 2002;**76**:5140–6.
140. Stenglein MD, Sanders C, Kistler AL, et al. Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease. *MBio* 2012;**3**: e00180–12.
141. Swanepoel R, Leman PA, Shepherd AJ, et al. Identification of Ippy as a Lassa-fever-related virus. *Lancet* 1985;**1**:639.
142. Ter Meulen J, Lukashevitch I, Sidibe K, et al. Hunting of peridomestic rodents and consumption of their meat as possible risk factors for rodent-to-human transmission of Lassa virus in the Republic of Guinea. *Am J Trop Med Hyg* 1996;**55**:661–6.
143. Ter Meulen J, Badusche M, Kuhnt K, et al. Characterization of human $Cd4^+$ T-cell clones recognizing conserved and variable epitopes of the Lassa virus nucleoprotein. *J Virol* 2000;**74**: 2186–92.
144. Tesh RB, Wilson ML, Salas R, et al. Field studies on the epidemiology of Venezuelan hemorrhagic fever: implication of the cotton rat *Sigmodon alstoni* as the probable rodent reservoir. *Am J Trop Med Hyg* 1993;**49**:227–35.
145. Tesh RB, Jahrling PB, Salas R, Shope RE. Description of Guanarito virus (Arenaviridae: Arenavirus), the etiologic agent of Venezuelan hemorrhagic fever. *Am J Trop Med Hyg* 1994;**50**: 452–9.
146. Tomori O, Fabiyi A, Sorungbe A, et al. Viral hemorrhagic fever antibodies in Nigerian populations. *Am J Trop Med Hyg* 1988;**38**: 407–10.
147. Trapido H, Sanmartín C. Pichindé virus, a new virus of the Tacaribe group from Colombia. *Am J Trop Med Hyg* 1971;**20**: 631–41.
148. Trombley AR, Wachter L, Garrison J, et al. Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses,

and New World hantaviruses. *Am J Trop Med Hyg* 2010;**82**: 954–60.
149. Uckun FM, Venkatachalam TK, Erbeck D, et al. Zidampidine, an aryl phosphate derivative of AZT: in vivo pharmacokinetics, metabolism, toxicity, and anti-viral efficacy against hemorrhagic fever caused by Lassa virus. *Bioorg Med Chem* 2005;**13**: 3279–88.
150. Urata S, Yun N, Pasquato A, et al. Antiviral activity of a small-molecule inhibitor of arenavirus glycoprotein processing by the cellular site 1 protease. *J Virol* 2011;**85**:795–803.
151. Vainrub B, Salas RA. Latin American hemorrhagic fever. *Infect Dis Clin North Am* 1994;**8**:47–59.
152. Van der Heide RM. A patient with Lassa fever from Burkina Faso (formerly Upper Volta), diagnosed in the Netherlands. *Ned Tijdschr Geneeskd* 1982;**126**:1–7.
153. Vieth S, Drosten C, Charrel R, et al. Establishment of conventional and fluorescence resonance energy transfer–based real-time PCR assays for detection of pathogenic New World arenaviruses. *J Clin Virol* 2005;**32**:229–35.
154. Vieth S, Drosten C, Lenz O, et al. RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene. *Trans R Soc Trop Med Hyg* 2007;**101**:1253–64.
155. Weaver SC, Salas RA, de Manzione N, et al. Guanarito virus (Arenaviridae) isolates from endemic and outlying localities in Venezuela: sequence comparisons among and within strains isolated from Venezuelan hemorrhagic fever patients and rodents. *Virology* 2000;**266**:189–95.
156. Webb PA, McCormick JB, King IJ, et al. Lassa fever in children in Sierra Leone, West Africa. *Trans R Soc Trop Med Hyg* 1986;**80**: 577–82.
157. Webb PA, Johnson KM, Hibbs JB, Kuns ML. Parana, a new Tacaribe complex virus from Paraguay. *Arch Gesamte Virusforsch* 1970;**32**:379–88.
158. Webb PA, Justines G, Johnson KM. Infection of wild and laboratory animals with Machupo and Latino viruses. *Bull World Health Organ* 1975;**52**:493–9.
159. Webb PA. Properties of Machupo virus. *Am J Trop Med Hyg* 1965;**14**:799–802.

CHAPTER 186

Filoviral Hemorrhagic Fever: Marburg and Ebola Virus Fevers

Eric Leroy • Gail J. Harrison

Viral hemorrhagic fever, associated with vascular instability, cytokine storm, hemorrhage, and disseminated intravascular coagulation, is caused by viruses from four diverse viral families: Arenaviridae, Bunyaviridae, Flaviviridae, and Filoviridae. *Ebolavirus* and *Marburgvirus* are the only genera of the family Filoviridae, which belongs to the order Mononegavirales.[19] Ebola and Marburg viruses cause the most severe form of viral hemorrhagic fever and are among the most virulent pathogens for humans and great apes. Human outbreaks of acute hemorrhagic fever caused by these viruses are associated with case-fatality rates of up to 88 percent, and Ebola virus also causes concomitant devastating outbreaks in Central African great apes. Based on their high potential for large-scale dissemination, high rates of mortality with potential for major public health effects, public panic, social disruption, and requirement for special preparedness, these two viruses are classified as Biohazard Level 4 and Biothreat Category A agents. Thus, they obviously must be manipulated in maximum security facilities.

ETIOLOGIC AGENTS

Members of the Filoviridae all have similar morphologic features. The viral particles are filamentary and often form bizarre configurations, such as "6"-shaped forms and hairpins (Fig. 186-1), and may be up to 14,000 nm in length.[19,78] In contrast, the filaments are always 80 nm wide. The capsid contains a single negative-stranded RNA genome that encodes seven structural proteins and one nonstructural protein, the soluble glycoprotein.[79,95,96] Filoviruses replicate readily in Vero, MA-104, and SW-13 cells, although specific species seem to prefer particular primate cell lines. These viruses also are highly pathogenic for macaques, producing a hemorrhagic disease and killing more than 75 percent of infected animals.[22,24] The Filoviridae comprise two genera. The only member species of the Marburg-like viruses genus is Marburg virus itself. The Ebola-like viruses genus comprises four antigenically and genetically related species, named *Zaire ebolavirus*, *Sudan ebolavirus*, *Ivory Coast ebolavirus*, and *Reston ebolavirus*. With the possible exception of *Reston ebolavirus*, filoviruses are highly pathogenic organisms that must be handled in special laboratories (Biosafety Level 4) with maximal biologic containment. Guidelines on the management of patients and specimens suspected of harboring these viruses are available from the Centers for Disease Control and Prevention (CDC) in Atlanta (http://www.cdc.gov/ncidod/dvrd/spb). There are virtually no antigenic relationships between Marburg virus and Ebola virus. The four Ebola virus species exhibit extensive cross reactivity in immunofluorescence methods and enzyme-linked immunosorbent assays (ELISAs). Virus neutralization, in contrast, has been virtually impossible to measure for any of the filoviruses, which has hindered epidemiologic and ecologic studies of these agents. Unlike the other three Ebola species, which are confined to Africa, *Reston ebolavirus* is found only in the Philippines among nonhuman primates.[41] Only the three African Ebola virus species appear to be pathogenic for humans.

HISTORY AND EPIDEMIOLOGY

Marburg Virus

In 1967, simultaneous outbreaks of a previously unknown hemorrhagic fever occurred in Marburg and Frankfurt (Germany) and in Belgrade (Serbia, former Yugoslavia) (Table 186-1).[62,64,83] The source was traced to African green monkeys *(Cercopithecus aethiops)* from a primate export facility in Entebbe, Uganda. Thirty-one cases occurred, including six by secondary transmission, and seven deaths occurred among primary cases. An unknown infectious agent was recovered from the blood and organs of these persons and was named Marburg virus, after the German city where the first cases occurred. Sporadic cases since then were diagnosed in the south part of Africa in 1975[28] and in Kenya in 1982.[85] A lengthy outbreak occurred between 1998 and 2000 in Durba (eastern Democratic Republic of the Congo), affecting 154 people and killing 83 percent of victims (Fig. 186-2).[9] Most patients (94%) worked in underground gold mines. Because of the civil war raging in 1997, the cause of the outbreak was not identified until 1999, a year after the first cases occurred. The Durba outbreak was characterized by the circulation of multiple viral strains, pointing to multiple independent introductions from the unknown natural reservoir.[8,9] The largest recorded Marburg outbreak occurred recently, in Uige Province of northern Angola. It was the first recorded outbreak in the west side of Africa and lasted from October 2004 to July 2005, causing 252 cases and 227 deaths (fatality rate, 90%).[16] Complete genomic characterization showed that the

culprit strain, named *Angola marburgvirus*, was closely related to previous East African isolates.[92-101] In 2007, a small outbreak of Marburg hemorrhagic fever among mine workers in Kamwenge District of Uganda was reported. In 2008, a nonfatal case of an American tourist who contracted Marburg hemorrhagic fever after a visit to the Python Cave, a bat cave in Queen Elizabeth National Park in western Uganda, was reported. In 2008, a rapidly fatal, imported case of Marburg hemorrhagic fever was reported from the Netherlands in a 40-year-old woman who had traveled to Uganda, and visited the same bat cave. In November 2012, an outbreak of Marburg Hemorrhagic Fever has been reported, with at least 17 cases and 9 deaths, in the Kabale District of southwest Uganda. The Special Pathogens Branch of the CDC hosts a webpage with archived and current outbreak postings, case counts, and location lists of viral hemorrhagic fevers (http://www.cdc.gov/ncidod/dvrd/spb/outbreaks/index.htm) reported from around the world.

Confirmed cases of Marburg hemorrhagic fever have been reported in Uganda, Zimbabwe, the Democratic Republic of the Congo, Kenya, and Angola. It characteristically occurs in outbreaks. Cases of Marburg hemorrhagic fever outside of Africa have been reported infrequently, in laboratory workers, and in travelers.

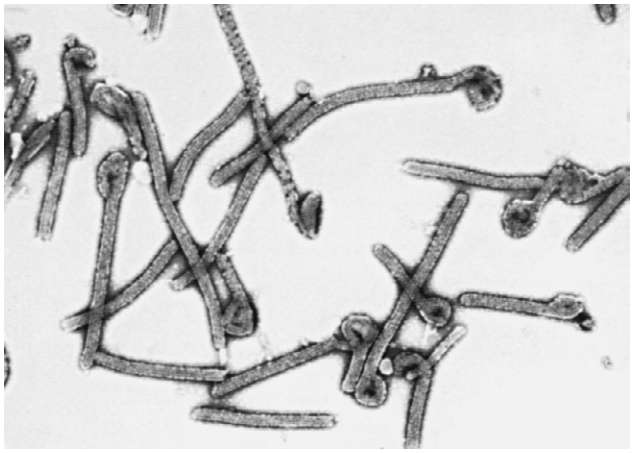

FIGURE 186-1 ■ Electron micrograph of Ebola virus (magnification ×38,750). (Courtesy T. W. Geisbert.)

Ebola Virus

1976 to 1979: The First Recorded Ebola Outbreaks

Ebola first emerged in the form of two nearly simultaneous outbreaks in 1976, one caused by *Sudan ebolavirus* and the other by *Zaire ebolavirus*.

The first outbreak was due to *Sudan ebolavirus* and occurred in Sudan, near the border with the Democratic Republic of the Congo, mainly affecting the towns of Nzara and Maridi.[68,86] The outbreak had 284 cases and the mortality rate was 53 percent, a proportion characteristic of *Sudan ebolavirus* infection. The outbreak due to *Zaire ebolavirus* occurred in the Democratic Republic of the Congo, near the border with Sudan, 2 months later.[47] The epicenter was Yambuku, about 800 km from Nzara. This previously unknown disease was named for the river Ebola, which flows past Yambuku. There were 318 cases and the mortality rate was 89 percent, characteristic of *Zaire ebolavirus* infection. Later, an unconfirmed lethal case involving a 9-year-old girl living in

FIGURE 186-2 ■ Geographic distribution of human outbreaks of Marburg virus fever in Africa.

TABLE 186-1 **Year and Location of Filoviral Hemorrhagic Fever Cases and Outbreaks**

Year	Virus Species	Country	No. of Human Cases	Fatality Rate (%)
1967	*Marburg*	Germany and Serbia	37	19
1975	*Marburg*	South Africa	3	33
1976	*Zaire Ebola*	Zaire	318	88
1976	*Sudan Ebola*	Sudan	284	53
1976	*Sudan Ebola*	England	1	0
1979	*Sudan Ebola*	Sudan	34	65
1980	*Marburg*	Kenya	2	50
1987	*Marburg*	Kenya	1	100
1989	*Reston Ebola**	United States	0	0
1990	*Reston Ebola**	United States	0	0
1992	*Reston Ebola**	Italy	0	0
1994	*Marburg*	Zaire	11	Unknown
1994	*Zaire Ebola*	Gabon	44	63
1994	*Côte d'Ivoire Ebola*	Ivory Coast	1	0
1995	*Zaire Ebola*	Zaire	315	81
1996	*Zaire Ebola*	Gabon	37	57
1996	*Zaire Ebola*	Gabon	60	75
1996	*Zaire Ebola*	South Africa	2	50
1996	*Reston Ebola**	United States	0	0
1996	*Reston Ebola**	Philippines	0	0
1998-2000	*Marburg*	DRC†	130	71
2000	*Sudan Ebola*	Uganda	425	53
2004-2005	*Marburg*	Angola	252	227
2007	*Marburg*	Uganda	2	2
2008	*Marburg*	Netherlands ex Uganda	1	1
2008	*Marburg*	United States ex Uganda	1	0
2008	*Ebola-Reston*†	Philippines	6	0
2011	*Ebola*	Uganda	1	1
2012	*Ebola*	Uganda	24	17
2012	*Marburg*	Uganda	17	9
2012	*Ebola*	DRC	35	12

*Symptomatic infections and fatal cases were observed in nonhuman primates only.
†New strain detected in swine with asymptomatic transmission to humans.
DRC, Democratic Republic of Congo, formerly was known as Zaire.

Tandala, Democratic Republic of Congo, was reported,[34,42] followed by another *Sudan ebolavirus* outbreak in 1979, again in Nzara, with 34 cases and 22 deaths.[6]

1994 to 1997: Ebola Resurgence

After a 15-year period in which no further cases were recorded, Ebola reemerged in 1994 for a 3-year period. This new phase was marked by the identification of a new species, *Ivory Coast*, and by four *Zaire ebolavirus* outbreaks. The only case caused by *Ivory Coast ebolavirus* occurred in 1994, when an ethnologist sickened a few days after performing an autopsy on a chimpanzee found dead in Tai National Park in Ivory Coast.[25,54,55] A large outbreak then occurred in 1995, in and around the town of Kikwit in the southern Democratic Republic of Congo,[71] with 315 cases and a mortality rate of 81 percent. Despite the deployment of more sophisticated scientific and medical resources than were available in 1976, this outbreak was as large as the 1976 one, probably because it affected a town of several hundred thousand inhabitants and person-to-person transmission occurred mainly in two hospitals. Three other outbreaks, all from *Zaire ebolavirus*, struck northeast Gabon between 1996 and 1997.[39] The first occurred in northeastern Gabon, close to the border with Cameroon,[3] in three gold-digger camps located in the heart of the forest. Some of the victims left the camps for the nearest hospital, located in Makokou, where they infected other patients and caused a second wave of virus dissemination. In total, 49 clinical cases and 29 deaths were recorded. The second outbreak hit the village of Mayibout, located south of Mekouka.[39] The first victims were 18 children who had helped carry and butcher a chimpanzee carcass found in the forest. These 18 children infected their families and friends, who in turn transmitted the disease to neighboring villages. In total, this outbreak involved 31 people and caused 21 deaths. The third outbreak in this region occurred between October 1996 and March 1997.[39] It started among hunters, who infected a traditional healer, his assistant, and some of his patients, who in turn transmitted the disease to several towns and villages in Gabon. Fifteen cases and 11 deaths were recorded in Libreville, and a South African nurse was infected by a

Gabonese physician who had traveled to Johannesburg. This outbreak, with 60 cases and 45 deaths occurring in a 6-month period, was noteworthy for its wide geographic range.

2000 to 2004: A Geographic Pattern of *Zaire Ebolavirus* and *Sudan Ebolavirus* Resurgence

Zaire Ebolavirus *Outbreaks*

The first outbreak during this period occurred in northeast Gabon and northwest Democratic Republic of Congo between October 2001 and May 2002.[59] It spread along the main road between Mekambo (Gabon) and Mbomo (Democratic Republic of Congo) and, in fact, involved several independent epidemic chains of human-human transmission with different source animals.[58] These epidemic chains started when local hunters found and manipulated the carcasses of an antelope, chimpanzees, and gorillas. Simultaneously, a second outbreak occurred in the Democratic Republic of Congo, in villages close to the Gabonese border, 200 km south of Mbomo. It then spread to the town of Kelle, about 65 km away. The origin of this outbreak is unknown. The third outbreak also affected the region of Mbomo, between 2002 and 2003.[27] It had two independent sources resulting in two independent chains, one in Mbomo and the other in Kelle. This outbreak involved 143 cases and caused 128 deaths. The fourth outbreak again affected the region of Mbomo, in late 2003; the first cases occurred in Mbanza, a village located about 30 km north of Mbomo. In this outbreak, 35 cases and 29 deaths were recorded.[99] The last outbreak occurred in 2005 in Etoumbi, 60 km south of Mbomo, where 12 cases and 9 deaths were reported. Simultaneously with the human outbreaks, *Zaire ebolavirus* also infected animals belonging to several species, including gorillas, chimpanzees, and duiker antelopes, probably accounting for the sharp declines in animal populations observed in these regions between 2001 and 2005.[43,58,98]

Sudan Ebolavirus *Outbreaks*

Two outbreaks of *Sudan ebolavirus* also occurred during this period (Fig. 186-3). One hit Uganda in 2000, causing 173 deaths among its 425 victims (mortality rate, 40.7%).[99] It was the largest of all recorded outbreaks and comprised three foci, one in the immediate area of Gulu, one in the town of Masindi, and one in Mbarara; the last outbreak was small, and the index patient was a soldier. A second *Sudan ebolavirus* outbreak occurred in Sudan in 2004 in the town of Yambio, located only a few dozen kilometers from Nzara and Maridi, which had been struck in 1976 and 1979. In this outbreak, 17 cases and 7 deaths occurred.[101]

The Particular Case of Reston ebolavirus

In 1989, it became apparent that Ebola virus is not restricted to Africa. A group of cynomolgus monkeys *(Macaca fascicularis)* from the Philippines, quarantined in a laboratory in Reston, Virginia, developed a lethal hemorrhagic disease.[45] Virions antigenically similar to Ebola virions were detected in tissues, and Ebola virus infection was confirmed by culture in Vero cells. In the following weeks, the introduction of new monkeys and the spread

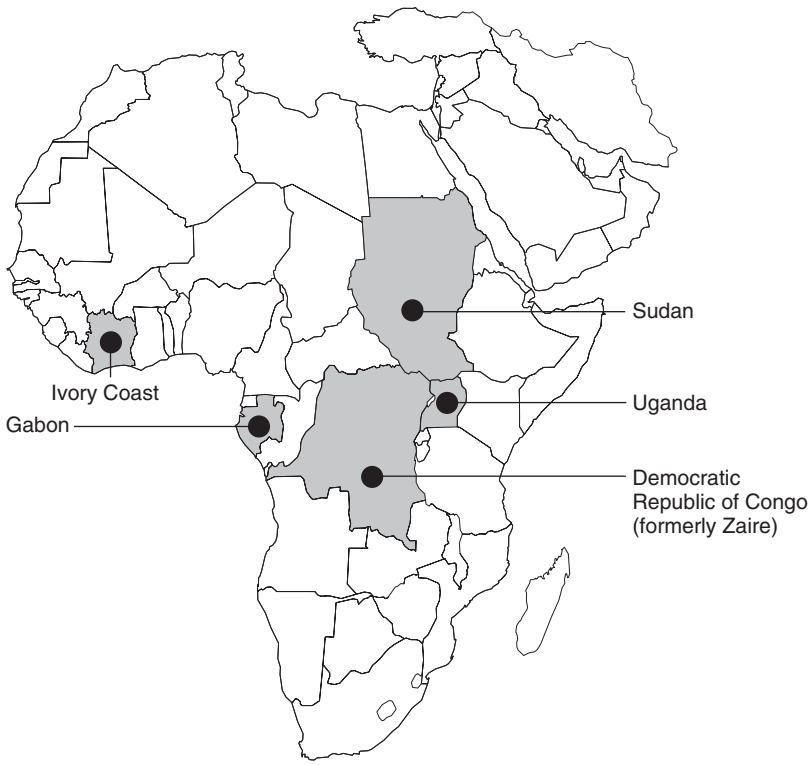

FIGURE 186-3 ■ Geographic distribution of human outbreaks of Ebola virus fever in Africa.

of the infection to several animal rooms meant that the entire cohort of more than 400 animals had to be destroyed. The building was fumigated and abandoned. The virus was recovered from another macaque in Philadelphia and later in a laboratory in Italy.[72] Although no humans fell ill during these episodes, several monkey handlers in the United States were shown to have seroconverted. In 1996, a cynomolgus monkey imported from the Philippines died in an animal facility in Texas, where two additional cases subsequently occurred, all caused by *Reston ebolavirus*.[77]

2007 to 2012: Ebola Outbreaks Continue to Emerge

In 2007, an outbreak of 264 cases of Ebola hemorrhagic fever caused by Ebola-Zaire species was reported in the Democratic Republic of Congo. In this outbreak, 187 deaths occurred and the mortality rate was 71 percent. In Uganda in late 2007 and early 2008, 149 cases of Ebola hemorrhagic fever, caused by a new strain, *Ebola Bundibugyo*, was reported from the Bundibugyo District. In this outbreak, 37 deaths occurred, with a mortality rate of 25 percent. In November 2008, the first known occurrence of Ebola-Reston strain in pigs was reported from the Philippines, with asymptomatic transmission to six human workers (no fatalities) in contact with the pigs.[49,61] In December 2008 through February 2009, 32 human cases of Ebola hemorrhagic fever caused by the Ebola-Zaire strain, resulting in 15 deaths (47% mortality rate), was reported from the Democratic Republic of Congo. In 2011, one case of Ebola hemorrhagic fever was reported from Uganda and quickly contained, with no other known cases from that incident reported. In 2012, Uganda reported an outbreak of Ebola hemorrhagic fever in the Kibaale District with 24 human cases and 17 fatalities, and Democratic Republic of Congo reported 35 confirmed cases of Ebola hemorrhagic fever, 12 of them fatal. The Special Pathogens Branch of the Centers for Disease Control and Prevention (CDC) hosts a webpage with archived and current outbreak postings, case counts, and location lists of viral hemorrhagic fevers (http://www.cdc.gov/ncidod/dvrd/spb/outbreaks/index.htm) reported from around the world.

EBOLA AND MARBURG VIRUS ECOLOGY AND BREAKTHROUGH TO HUMANS

The sources of most human outbreaks of viral hemorrhagic fever, including most Marburg outbreaks, the *Zaire ebolavirus* outbreaks that occurred between 1976 and 1997, and all outbreaks of *Sudan ebolavirus*, have not been identified. A review of the epidemiologic surveillance during Ebola and Marburg hemorrhagic fever epidemics in Africa over a 15-year period delineated several factors that affected transmission. During rural epidemics, multiple introductions of the virus into the human population through wildlife were observed. In contrast, during epidemics in more urban settings, the introduction of a single case in the community was responsible for epidemics. In addition, approximately 9 cases were reported in health care workers.[1]

Several outbreaks have been firmly linked to infected animal carcasses. As mentioned before, an ethnologist became infected by *Ivory Coast ebolavirus* in 1994 while performing an autopsy on a chimpanzee.[55] Similarly, the 1996 Mayibout outbreak in Gabon started among children who found and butchered a chimpanzee carcass in the forest.[39] The 1967 outbreak of Marburg virus infection in Marburg and Belgrade was linked to the handling of organs and tissues from vervet monkeys imported from Uganda.[62,83] Likewise, the sources of the *Zaire ebolavirus* outbreaks that occurred in the border region of Gabon and Democratic Republic of Congo between 2001 and 2003 are well documented; they all occurred after people handled animal carcasses they had found in the forest (mainly gorillas, chimpanzees, and duikers).[58]

Since 1976, 13 outbreaks and two isolated cases of Ebola, totaling about 1850 human cases and 1300 deaths, have struck countries spanning the equatorial forest regions of Africa.[100]

Ebola hemorrhagic fever is thus a zoonotic disease that often is transmitted to humans by direct contact with infected animal carcasses, which are themselves probably infected from the reservoir animal species that harbor the virus, symptomatically or asymptomatically. Since the first recorded human outbreak occurred, many field and laboratory studies have been conducted in attempts to identify Ebola virus reservoir species. Wild animals (vertebrates and invertebrates) were captured in the field, and blood and tissues were used to inoculate Ebola virus–permissive Vero cells. Six field studies, conducted from 1976 to 1995, examined a total of 7000 vertebrates and 30,000 invertebrates, but none was found to harbor Ebola virus.[47,67] Although small Ebola virus nucleotide sequences and Ebola virus–like nucleocapsids were detected in organs of six mice (*Mus musculus* and *Praomys* sp.) and a shrew *(Sylvisorex ollula)* caught in Central African Republic in 1999,[67] no conclusions could be drawn as to their reservoir status.

All attempts at experimental inoculation of rodents, bats, birds, reptiles, mollusks, arthropods, and plants have failed, although some bat species belonging to the genera *Epomophorus* and *Tadarida* developed transient viremia lasting nearly 4 weeks.[90,94] These results did not provide conclusive evidence that bats serve as an Ebola virus reservoir.

Other field collections were conducted during 2002 and 2003 in Gabon and the Republic of Congo, in areas hit by the different outbreaks. A total of 1030 animals were captured, autopsied, and analyzed. The captures and laboratory analyses spanned a period of 4 years. The results showed that three species of fruit bat (*Hypsignathus monstrosus*, *Epomops franqueti*, and *Myonycteris torquata*) were asymptomatically infected by Ebola virus.[57] Indeed, anti–Ebola virus immunoglobulin (Ig)G was detected in the serum of 16 bats (4 *Hypsignathus*, 8 *Epomops*, and 4 *Myonycteris*), but no other animal species, including other bat species, were positive. Likewise, Ebola virus nucleotide sequences were detected in the organs of 13 bats (3 *Hypsignathus*, 5 *Epomops*, and 5 *Myonycteris*). Sequencing

of the amplified fragments confirmed they were specific to Ebola virus. Phylogenetic analysis of the sequences by the Bayesian and maximum parsimony methods showed they belonged to the species *Zaire*. Although the virus itself was not isolated, this work provided the first biologic evidence that some fruit bat species harbor Ebola virus. In addition, these findings were in keeping with certain epidemiologic clues from previous outbreaks and with the transient viremia observed in some bat species after experimental challenge. The distribution of the bat species also matched that of the outbreaks. Recent studies implicate the Egyptian fruit bat *(Rousettus aegyptiacus)* as the reservoir host for Marburg virus. This Egyptian fruit bat is a cave-dwelling species widely distributed across Africa, and carries the virus asymptomatically.

In 2009, domestic pigs in the Philippines were discovered to host *Reston ebolavirus*, and may pose a transmission risk to farm, veterinary, and abattoir workers exposed to infected pigs.[7,61]

CLINICAL MANIFESTATIONS

Marburg virus and the four Ebola virus species all cause severe, usually fatal, hemorrhagic fever, but they differ in terms of the incubation period, clinical severity, and case-fatality rate.[63] After an incubation period ranging from 2 to 21 days (mostly 4 to 9 days), symptoms of Ebola virus fever occur abruptly. Most patients develop high fever, headache, muscle pain, stomach pain, fatigue, and diarrhea; some patients also have sore throat, hiccup, rash, and red and itchy eyes.[14,63] Bloody diarrhea and hematemesis also can occur. After about a week, probable fatal outcome is signaled by the onset of chest pain and shock, together with blindness and bleeding in some cases. The incubation period of Marburg virus fever is almost identical to that of Ebola virus fever; clinical onset also is abrupt, starting with fever, chills, headache, and muscle pain. A week later, some patients develop a maculopapular rash, nausea, vomiting, chest pain, abdominal pain, diarrhea, and sore throat. Clinical status deteriorates gradually, with jaundice, delirium, shock, liver failure, massive hemorrhaging, and multiorgan dysfunction.[14,44,73,84] Although the virus disappears quickly from the bloodstream of survivors, it can persist in the eyes and testicles.[17,64] Marburg virus was recovered from the semen of one patient some weeks after clinical recovery.

Studies of nonhuman primates show that filovirus infection triggers clotting disorders, but the clinical repercussions are variable. In survivors, they are limited to conjunctival hemorrhage, easy bruising, failure of venipuncture sites to clot, and bloody vomiting and diarrhea. Most fatalities are associated with disseminated intravascular coagulation and systemic bleeding.[21,23,33,36,37]

Mild leukopenia is a common development early in filovirus diseases, but leukocytosis may appear later in response to secondary bacterial infection (usually of the lungs). Thrombocytopenia (<100,000 platelets/mm^3) always is present, and coagulation studies are abnormal at the time of onset of bleeding. Fibrin split products indicative of disseminated intravascular coagulation have been detected in humans and experimentally infected primates. Serum transaminase activity is markedly elevated, whereas the bilirubin level is normal and clinical jaundice is a rare development. Serum protein levels are depressed, the hematocrit is increased, and proteinuria is a common finding.

PATHOLOGY

Limited information is available on human filovirus infections, whereas a large body of data has been obtained with experimentally infected rodents and nonhuman primates. Why some people recover from filovirus fevers and others die remains unclear.

No major difference exists in the pathogenesis of the different filovirus diseases. Studies of *Zaire ebolavirus* show that dendritic cells and macrophages are the initial host targets at the entry site.[20,33,37] These infected cells then disseminate through the bloodstream and lymphatics to all organs, where they release virions that infect monocytic cells in lymph nodes, spleen, liver, and other organs and tissues.[2] In the later stages of the disease, the virus also infects other cell types, such as hepatocytes, adrenal cortical cells, and endothelial cells, causing lysis and necrosis.[36] Lymphocytes do not seem to be infected, but they nonetheless undergo bystander apoptotic lysis.[34]

The pathogenetic mechanisms of filovirus infection involve both direct damage to infected cells[69,88,97] and indirect insults through interactions between the virus and the adaptive immune system.[13,66] By contrast with other viruses, filoviruses can infect and kill a wide variety of cells throughout the body. This apparent lack of target cell specificity may be due to the binding of the viral surface glycoprotein to widely distributed cell surface lectins.[82] Infection of macrophages and dendritic cells can suppress various cellular functions, including the production of proinflammatory mediators such as interferon (IFN)-α, interleukin-1β, and tumor necrosis factor-α (TNF-α),[5,11,40] contributing to the suppression of innate antiviral responses and weakening adaptive immune responses. Studies of serial samples from infected nonhuman primates have shown that lymphocytes undergo apoptosis in vivo, probably through the TNF-related apoptosis-inducing ligand (TRAIL) and FAS pathways.[33,34] These findings are compatible with the massive intravascular apoptosis of peripheral monocytic cells observed in patients who died (but not in those who survived) during the 1996 outbreaks in Gabon.[4] This bystander lymphocyte destruction has been suggested to play a major role both in immunosuppression and in the onset of septic shock observed in nonsurvivors. Various studies have shown that filovirus infection of endothelial cells causes their lysis and a loss of vascular integrity, leading to disseminated intravascular coagulation, vascular dysfunction, and finally shock.

DIFFERENTIAL DIAGNOSIS

In the clinical setting, Marburg and Ebola virus fevers must be distinguished from other viral hemorrhagic diseases occurring in Africa, as well as from the many

bacterial, rickettsial, and protozoal diseases that can cause similar initial signs and symptoms. The absence of jaundice helps eliminate yellow fever and Rift Valley fever. For patients seen outside Africa, the travel history is the most important diagnostic sign available to physicians. Imported cases in travelers have been documented, and the increase in global air travel, the rise of tourism in Africa, and the greater dispersion of military troops increases the possibility for importation of filoviruses to nonendemic areas.[80]

DIAGNOSIS

Early laboratory diagnoses of Ebola virus and Marburg virus infection are based on antigen-capture enzyme-linked immunosorbent assay (ELISA), reverse-transcription polymerase chain reaction (RT-PCR), real-time PCR, and virus isolation.[50-52,56,93] These tests are the most accurate tools for diagnosis of acute Ebola virus fever. Later in the course of the disease, or after recovery, diagnosis relies on IgM-capture ELISA or IgG antibody detection. Interestingly, however, specific antibodies do not develop in a significant proportion of patients in whom the disease is fatal. These diagnostic tools also may be used for oral fluid specimens obtained from clinical patients.[26] Because large quantities of Ebola virus are present in dermal tissue, skin necropsy with immunohistochemical analysis has been proposed for postmortem diagnostic confirmation.[105] These specimens are easy to collect, and formalin fixation renders them safe for transport. Skin biopsy testing is not sufficiently sensitive for early diagnosis, however, and thus should be reserved for dead or dying patients.

Cases have been classified into three categories, depending on the patient's clinical manifestations and epidemiologic data:

- *Suspect:* A person with fever and a history of contact with a potential case (i.e., a person with unexplained bleeding, or with fever plus three or more of the following symptoms: headache, vomiting, loss of appetite, diarrhea, weakness or severe fatigue, abdominal pain, body aches or joint pains, difficulty swallowing, difficulty breathing, and hiccups; and any person having died of unknown causes).
- *Probable:* A case that meets the preceding criteria and is assessed and reported by a physician.
- *Confirmed (laboratory-confirmed case):* A case that meets the surveillance case definitions, and the patient has positive test results for Ebola virus antigens, Ebola virus IgG antibodies, or Ebola virus genomic sequences (RT-PCR).

TREATMENT

No specific antiviral treatment for filoviral hemorrhagic fevers is licensed. Ribavirin and IFN have no effect on filoviruses, and no other antiviral compounds have been found to be clinically effective in humans.[46]

Standard medical treatment relies on symptomatic measures aimed at maintaining hydration, reversing cytokine storm and disseminated intravascular coagulation, and treating hemorrhage with transfusions. In addition, maintaining nutritional status and preventing or curing bacterial and parasitic superinfections are important for management. Appropriate control measures for health care workers and family members to prevent secondary transmission are important to follow. Barrier techniques to prevent direct physical contact with the patient and the patient's secretions and blood, including wearing of protective gowns, gloves, and masks; placing the infected patient in strict isolation; and sterilization or incineration of needles, equipment, and patient excretions are recommended. A manual prepared collaboratively by the CDC and World Health Organization is available and addresses early provisional diagnosis, medical response, and measures to prevent spread of filovirus disease and other viral hemorrhagic fevers within a limited infrastructure that is likely in the African health care setting (http://cdc.gov/ncidod/dvrd/spb/mnpages/vhfmanual.htm).

Convalescent plasma has been used for treatment of filovirus infection, but its efficacy has not been proved in randomized clinical trials. In a study conducted during the 1995 Kikwit Ebola epidemic, seven of eight patients who received blood collected from convalescent donors recovered, warranting a thorough evaluation of passive immune therapy.[68] One person infected by *Sudan ebolavirus* after a laboratory accident survived after receiving plasma from *Zaire ebolavirus* survivors. Likewise, several patients who received early treatment with Marburg virus immune plasma appeared to have milder disease. Hyperimmunoglobulin from horses protected baboons in recent experimental studies.[65] Goat immunoglobulin has been tested in laboratory animals and also was given to laboratory researchers suspected of being infected by Ebola virus[53]; one researcher with highly probable infection made a full recovery. More recent studies have investigated the potential therapeutic utility of a variety of monoclonal antibody preparations for treatment and postexposure prophylaxis of Ebola virus infection.[15,91] Mouse and human chimeric monocloncal antibodies active against Ebola virus and produced in Chinese hamster ovary and in whole plant cells have been shown to successfully protect rhesus monkeys against lethal viral challenge. Fucose-free monoclonal antibody against Ebola virus has also been found effective in preclinical trials.[106]

In addition to antibody-based strategies, preclinical development of a variety of antiviral drug strategies for treatment of filovirus infection is in progress. Compound library screening in vitro, small molecules, and in silicon structure–based drug design are being explored to treat filovirus infection.[38,70,81] Administration of recombinant nematode anticoagulant protein c2 (rNAPc2), a potent inhibitor of tissue factor–initiated blood coagulation, attenuated proinflammatory and coagulopathic responses and prolonged the survival of macaques that had received a lethal inoculum of Ebola virus.[32] Administration of positively charged phosphorodiamidate morpholino oligomers (PMOplus) delivered at various dosing strategies, protected more than 60 percent of rhesus monkeys

against lethal Zaire Ebola virus challenge and 100 percent of cynomolgus monkeys against Lake Victoria Marburg virus infection, suggesting PMOplus may be useful for treatment.[38] Even though laboratory-based research has produced antiviral treatments and vaccines that appear effective in laboratory animals and ready for clinical evaluation in humans, significant challenges exist in clinical testing that will need to be considered. For example, most cases of filovirus infection in the wild are outbreaks in remote or resource-poor areas of sub-Saharan Africa, where little infrastructure exists and few personnel experienced in clinical research reside. In addition, significant political, legal, and sociocultural barriers may exist and will need to be addressed before clinical trials or other forms of clinical research for treatment and vaccination against Ebola and Marburg virus infection can be conducted.[10]

PREVENTION

A variety of vaccine platforms, including DNA vaccines, recombinant replication–deficient adenovirus serotype 5 vector vaccines, recombinant human parainfluenza virus 3, vesicular stomatitis virus–based vaccines, and virus-like particle vaccines, have been investigated and shown to be effective against filovirus infection in animal models.[13,18,30,74,87] Successful preclinical developments suggest studies on the safety and efficacy of a filovirus vaccine for human use may be possible.

Several candidate vaccines against Ebola virus are effective in guinea pigs but less so in macaques.[35,87] Experimental vaccines have so far been based on viral particles inactivated by heat, formalin, or γ-irradiation[60]; plasmids coding for the Ebola virus glycoprotein (GP) or nucleoprotein (NP),[103] recombinant GP-expressing Venezuelan equine encephalitis virus,[75] or variola virus; and Ebola virus particles encapsulated in liposomes.[76] In contrast, a vaccine candidate based on three injections of GP-encoding DNA given 4 weeks apart, followed by a booster with recombinant inactivated GP expressing adenovirus, protected macaques from infectious challenge 1 week later.[89] However, the small inoculum used to challenge the macaques (six plaque-forming units [PFU]), together with the lengthy immunization protocol, renders this candidate vaccine impractical.

A new advance was made recently with the development of two candidate vaccines that appear to be effective in both guinea pigs and macaques. The first consists of a single injection of 2×10^{12} particles of recombinant inactivated adenovirus 5 expressing both the GP and the NP (ADV-GP/NP). A variant consisting of two injections 9 weeks apart also has been tested. The two variants gave total protection and induced IFN-γ production by $CD8^+$ T cells, as well as a humoral response, after both low-dose challenge (13 PFU) and high-dose challenge (1500 PFU) 1 or 4 weeks later. No change in $CD4^+$ T lymphocyte status was observed. The single injection offers faster but shorter protection than does the dual injection.[69] The second candidate vaccine consists of a single dose of 10^7 PFU of live attenuated, recombinant, vesicular stomatitis virus expressing the Ebola GP (VSVΔG/*Zaire ebolavirus*). This immunization fully protected macaques from a high-dose challenge (1000 PFU) 28 days later. It induced a moderate increase in anti-GP IgG (mainly neutralizing antibodies) and a strong increase in IFN-γ^+ and TNF-α^+ CD8 T cells and IFN-γ^+ and TNF-α^+ CD4 T cells. Nearly 30 macaques so far have received this immunization, and none of them has developed any clinical signs after infectious challenge.[48]

Virus-like particles have shown promise and protect small laboratory animals and nonhuman primates against lethal challenge by Ebola or Marburg viruses.[104] Also, a vaccine system based on recombinant vesicular stomatitis virus that expresses filovirus glycoprotein in place of the vesicular stomatitis virus glycoprotein has shown protection against infection with Marburg and Ebola virus.[29-31,87] Protection of nonhuman primates against Ebola virus infection by both parenteral and aerosol route challenges also has been documented by vaccination with a single complex adenovirus vector vaccine.[12,74]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Allaranga Y, Kone M, Formenty P, et al. Lessons learned during active epidemiological surveillance of Ebola and Marburg viral hemorrhagic fever epidemics in Africa. *East Afr J Public Health* 2010;**7**:30–6.
7. Barrette R, Metwally S, Rowland J, et al. Discovery of swine as a host for the Reston ebolavirus. *Science* 2009;**325**:204–6.
10. Bausch D, Sprecher A, Jeffs B, et al. Treatment of Marburg and Ebola hemorrhagic fevers: a strategy for testing new drugs and vaccines under outbreak conditions. *Antiviral Res* 2008;**78**: 150–61.
12. Bradfute A, Bavari S. Correlates of immunity to filovirus infection. *Viruses* 2011;**3**:982–1000.
13. Bradfute S, Dye J, Bavaria S. Filovirus vaccines. *Hum Vaccin* 2011;**7**:701–11.
15. Castilho A, Bohorova N, Grass J, et al. Rapid high yield production of different glycoforms of Ebola virus monoclonal antibody. *PLoS One* 2011;**6**:e26040.
18. Falzarano D, Geisbert T, Feldmann H. Progress in filovirus vaccine development: evaluating the potential for clinical use. *Expert Rev Vaccines* 2011;**10**:63–77.
29. Geisbert T, Feldmann H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. *J Infect Dis* 2011;**204**(Suppl. 3):S1075–81.
30. Geisbert T, Bausch D, Feldmann H. Prospects for immunization against Marburg and Ebola viruses. *Rev Med Virol* 2010;**20**: 344–57.
31. Gesibert T, Geisbert J, Leug A, et al. Single-infection vaccine protects nonhuman primates against infection with Marburg virus and three species of Ebola virus. *J Virol* 2009;**83**:7296–304.
38. Gene O, Julia B, Vanessa M, et al. Drug targets in infections with Ebola and Marburg viruses. *Infect Discord Drug Targets* 2009;**9**:191–200.
49. Kobinger G, Leung A, Neufeld J, et al. Replication, pathogenicity, shedding and transmission of Zaire ebolavirus in pigs. *J Infect Dis* 2011;**204**:200–8.
61. Marsh G, Haining J, Robinson R, et al. Ebola Reston virus infection of pigs: clinical significance and transmission potential. *J Infect Dis* 2011;**204**(Suppl. 3):S804–9.
69. Paessler S, Walker D. Pathogenesis of the viral hemorrhagic fevers. *Annu Rev Pathol* 2012 (Epub ahead of print).
70. Panchal R, Reid S, Trans J, et al. Identification of an antioxidant small-molecule with broad-spectrum antiviral activity. *Antiviral Res* 2012;**93**:23–9.
74. Pratt W, Wang D, Nichols D.,et al. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vaccine Immunol* 2010;**1**: 572–81.

80. Sarwar U, Sitar S, Ledgerwood J. Filovirus emergence and vaccine development: a perspective for health care practitioners in travel medicine. *Travel Med Infect Dis* 2011;**9**:126–34.
81. Shurtleff A, Nguyen T, Kingery D, et al. Therapeutics for filovirus infection: traditional approaches and progress towards in silico drug design. *Expert Opin Drug Discov* 2012;**7**:935–54.
91. Takada A, Ebihara H, Jones S, et al. Protective efficacy of neutralizing antibodies against Ebola virus infection. *Vaccine* 2007;**25**: 993–6.
104. Yang C, Ye L, Compans R. Protection against filovirus infection: virus-like particle vaccines. *Expert Rev Vaccines* 2008;**7**:333–44.
106. Zeitlin L, Petitt J, Scully C, et al. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc Natl Acad Sci USA* 2011;**108**:20690–4.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 10 *CORONAVIRIDAE* AND *TOROVIRIDAE*

CHAPTER 187

Coronaviruses and Toroviruses, Including Severe Acute Respiratory Syndrome

Kenneth McIntosh • Janet A. Englund

The family Coronaviridae contains two subfamilies, Coronavirinae and Torovirinae. The subfamily Coronavirinae is currently divided into three genera, the alphacoronaviruses, betacoronaviruses, and gammacoronaviruses; the human coronaviruses (HCoVs) belong in the first two of these. Until recently, HCoVs were considered to cause upper respiratory tract illness and probably also some undetermined fraction of viral diarrhea. In the final months of 2002 and extending into 2003, a new coronavirus, the SARS-related coronavirus (SARSr-CoV), was found to be the cause of an acute, severe, frequently fatal respiratory disease with prominent systemic symptoms (severe acute respiratory syndrome, or SARS). The outbreak spread worldwide from its origin in southern China and probably represented the transmission from animal (possibly via the palm civet but originating in bats) to human.[69,85] More recently, in 2012 a highly pathogenic coronavirus, the Middle East Respiratory Syndrome coronavirus (MERS-CoV) appeared in Saudi Arabia and neighboring countries.[35a,188a] This virus, with a case-fatality rate of about 50% and an ability to spread at least to a limited extent in families and among patients and caretakers in hospitals, may also have originated in bats.[2a] In retrospect, the emergence of SARS and the MERS-CoV is consistent with what was already known about coronaviruses: they are widespread pathogens in the animal kingdom, they evolve rapidly through mutation and recombination, and they jump species barriers.[84] Coronaviruses cause a wide variety of important diseases in animals, including infectious bronchitis and nephrosis in chickens; gastroenteritis and encephalitis in young piglets; enteritis in turkeys, dogs, and calves; hepatitis and encephalitis in mice; pneumonitis and sialodacryoadenitis in rats; infectious peritonitis in cats; and pneumonia and hepatitis in whales. Multiple strains of unknown pathogenicity have been recovered from numerous species of bats.[180]

Since the first report of HCoV isolation in 1965,[161] the HCoV group has been repeatedly confirmed as an important and frequent cause of the common cold and a likely cause of more serious respiratory tract infections in the elderly.[49,60,174] They also have been implicated as contributors to lower respiratory tract infections in children and adults. The role of coronaviruses

REFERENCES

1. Allaranga Y, Kone M, Formenty P, et al. Lessons learned during active epidemiological surveillance of Ebola and Marburg viral hemorrhagic fever epidemics in Africa. *East Afr J Public Health* 2010;**7**:30–6.
2. Aman MJ, Bosio CM, Panchal RG, et al. Molecular mechanisms of filovirus cellular trafficking. *Microbes Infect* 2003;**5**:639–49.
3. Amblard J, Obiang P, Edzang S, et al. Identification of the Ebola virus in Gabon in 1994. *Lancet* 1997;**349**:181–2.
4. Baize S, Leroy EM, Georges-Courbot M-C, et al. Defective humoral responses and extensive intravascular apoptosis are associated with fatal outcome in Ebola virus–infected patients. *Nat Med* 1999;**5**:1–5.
5. Baize S, Leroy EM, Georges AJ, et al. Inflammatory responses in Ebola virus–infected patients. *Clin Exp Immunol* 2002;**128**:163–8.
6. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 1983;**61**:997–1003.
7. Barrette R, Metwally S, Rowland J, et al. Discovery of swine as a host for the Reston ebolavirus. *Science* 2009;**325**:204–6.
8. Bausch DG, Borchert M, Grein T, et al. Risk factors for Marburg hemorrhagic fever, Democratic Republic of the Congo. *Emerg Infect Dis* 2003;**9**:1531–7.
9. Bausch DG, Nichol ST, Muyembe-Tamfum JJ, et al. Marburg hemorrhagic fever associated with multiple genetic lineages of virus. *N Engl J Med* 2006;**355**:909–19.
10. Bausch D, Sprecher A, Jeffs B, Boumandouki P. Treatment of Marburg and Ebola hemorrhagic fevers: a strategy for testing new drugs and vaccines under outbreak conditions. *Antiviral Res* 2008;**78**:150–61.
11. Bosio CM, Aman MJ, Grogan C, et al. Ebola and Marburg viruses replicate in monocyte-derived dendritic cells without inducing the production of cytokines and full maturation. *J Infect Dis* 2003;**188**:1630–8.
12. Bradfute A, Bavari S. Correlates of immunity to filovirus infection. *Viruses* 2011;**3**:982–1000.
13. Bradfute S, Dye J, Bavaria S. Filovirus vaccines. *Hum Vaccin* 2011;**7**:701–11.
14. Bwaka MA, Bonnet M-J, Calain P, et al. Ebola hemorrhagic fever in Kikwit, Democratic Republic of the Congo: clinical observations in 103 patients. *J Infect Dis* 1999;**179**:S1–7.
15. Castilho A, Bohorova N, Grass J, et al. Rapid high yield production of different glycoforms of Ebola virus monoclonal antibody. *PLoS One* 2011;**6**:e26040.
16. Centers for Disease Control and Prevention. Outbreak of Marburg virus hemorrhagic fever: Angola, October 1, 2004–March 9, 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**:308–9.
17. Emond RT, Evans B, Bowen ET, et al. A case of Ebola virus infection. *Br Med J* 1977;**2**:541–4.
18. Falzarano D, Geisbert T, Feldmann H. Progress in filovirus vaccine development: evaluating the potential for clinical use. *Expert Rev Vaccines* 2011;**10**:63–77.
19. Feldmann H, Kiley MP. Classification, structure, and replication of filoviruses. *Curr Top Microbiol Immunol* 1999;**235**:1–21.
20. Feldmann H, Bugany H, Mahner F, et al. Filovirus-induced endothelial leakage triggered by infected monocytes/macrophages. *J Clin Invest* 1996;**70**:2208–14.
21. Fisher-Hoch SP, Lloyd G, Platt GS, et al. Hematological and biochemical monitoring of Ebola infection in rhesus monkeys: implications for patient management. *Lancet* 1983;**2**:1055–8.
22. Fisher-Hoch SP, Perez-Oronoz GI, Jackson EL, et al. Filovirus clearance in non-human primates. *Lancet* 1992;**340**:451–3.
23. Fisher-Hoch SP, Platt GS, Neild GH, et al. Pathophysiology of shock and hemorrhage in a fulminating viral infection (Ebola). *J Infect Dis* 1985;**152**:887–94.
24. Fonseca AM, Porto G, Uchida K, et al. Red blood cells inhibit activation-induced cell death and oxidative stress in human peripheral blood T lymphocytes. *Blood* 2001;**97**:3152–60.
25. Formenty P, Hatz C, Le Guenno B, et al. Human infection due to Ebola virus, subtype Côte d'Ivoire: clinical and biologic presentation. *J Infect Dis* 1999;**179**:S48–53.
26. Formenty P, Leroy EM, Epelboin A, et al. Detection of Ebola virus in oral fluid specimens during outbreaks of Ebola virus hemorrhagic fever in the Republic of Congo. *Clin Infect Dis* 2006;**42**:1521–6.
27. Formenty P, Libama F, Epelboin A, et al. L'épidémie de fièvre hémorragique à virus ebola en République du Congo, 2003: une nouvelle stratégie? *Med Trop* 2003;**63**:291–5.
28. Gear JS, Cassel GA, Gear AJ, et al. Outbreak of Marburg virus disease in Johannesburg. *Br Med J* 1975;**4**:489–93.
29. Geisbert T, Feldmann H. Recombinant vesicular stomatitis virus-based vaccines against Ebola and Marburg virus infections. *J Infect Dis* 2011;**204**(Suppl. 3):S1075–81.
30. Geisbert T, Bausch D, Feldmann H. Prospects for immunization against Marburg and Ebola viruses. *Rev Med Virol* 2010;**20**: 344–57.
31. Gesibert T, Geisbert J, Leug A, et al. Single-infection vaccine protects nonhuman primates against infection with Marburg virus and three species of Ebola virus. *J Virol* 2009;**83**:7296–304.
32. Geisbert TW, Hensley L, Jahrling PB, et al. Treatment of Ebola virus infection with a recombinant inhibitor of factor VII/tissue factor: a study in rhesus monkeys. *Lancet* 2003;**362**:1953–8.
33. Geisbert TW, Hensley L, Larsen T, et al. Pathogenesis of Ebola hemorrhagic fever in cynomolgus macaques. *Am J Pathol* 2003; **163**:2347–70.
34. Geisbert TW, Hensley LE, Gibb TR, et al. Apoptosis induced in vitro and in vivo during infection by Ebola and Marburg viruses. *Lab Invest* 2000;**80**:171–86.
35. Geisbert TW, Pushko P, Anderson K, et al. Evaluation in nonhuman primates of vaccines against Ebola virus. *Emerg Infect Dis* 2002;**8**:503–7.
36. Geisbert TW, Young HA, Jahrling PB, et al. Mechanisms underlying coagulation abnormalities in Ebola hemorrhagic fever: overexpression of tissue factor in primate monocytes/macrophages is a key event. *J Infect Dis* 2003;**188**:1618–29.
37. Geisbert TW, Young HA, Jahrling PB, et al. Pathogenesis of Ebola hemorrhagic fever in primate models: evidence that hemorrhage is not a direct effect of virus-induced cytolysis of endothelial cells. *Am J Pathol* 2003;**163**:2371–82.
38. Gene O, Julia B, Vanessa M, et al. Drug targets in infections with Ebola and Marburg viruses. *Infect Discord Drug Targets* 2009;**9**(2):191–200.
39. Georges AJ, Leroy EM, Renaut AA, et al. Ebola hemorrhagic fever outbreaks in Gabon, 1994-1997: epidemiologic and health control issues. *J Infect Dis* 1999;**179**:S65–75.
40. Gupta M, Mahanty S, Ahmed R, et al. Monocyte-derived human macrophage and peripheral blood mononuclear cells infected with Ebola virus secrete MIP-1α and TNF-α and inhibit poly-IC–induced IFN-α in vitro. *Virology* 2001;**284**:20–5.
41. Hayes CG, Burans JP, Ksiazek TG, et al. Outbreak of fatal illness among captive macaques in the Philippines caused by an Ebola-related filovirus. *Am J Trop Med Hyg* 1992;**46**:664–71.
42. Heymann DL, Weisfeld JS, Webb PA, et al. Ebola hemorrhagic fever: Tandala, 1977-1978. *J Infect Dis* 1980;**142**:372–6.
43. Huijbregts B, De Wachter P, Ndong Obiang S, et al. Ebola and the decline of gorilla *Gorilla gorilla* and chimpanzee *Pan troglodytes* populations in Minkebe forest, north-eastern Gabon. *Oryx* 2003;**37**:437–43.
44. Isaacson M, Sureau P, Courteille G, et al. Clinical aspects of Ebola virus disease as the Ngaliema hospital, Kinshasa, Zaire, 1976. In: Pattyn SR, editor. *Ebola virus haemorrhagic fever*. New York: Elsevier/North-Holland Biomedical Press; 1978. p. 15–20.
45. Jahrling PB, Geisbert TW, Dalgard DW, et al. Preliminary report: isolation of Ebola virus from monkeys imported to USA. *Lancet* 1990;**335**:502–5.
46. Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferon-α2b for treatment of experimental Ebola virus infections. *J Infect Dis* 1999;**179**: S224–34.
47. Johnson KM. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health Organ* 1978;**56**:271–93.
48. Jones SM, Feldmann H, Stroher U, et al. Live attenuated recombinant vaccine protects nonhuman primates against Ebola and Marburg viruses. *Nat Med* 2005;**11**:786–90.
49. Kobinger G, Leung A, Neufeld J, et al. Replication, pathogenicity, shedding and transmission of Zaire ebolavirus in pigs. *J Infect Dis* 2011;**204**:200–8.
50. Ksiazek TG, Rollin, PE, Jahrling PB, et al. Enzyme immunosorbent assay for Ebola virus antigens in tissues of infected primates. *J Clin Microbiol* 1992;**30**:947–50.

51. Ksiazek TG, Rollin PE, Williams AJ, et al. Clinical virology of Ebola hemorrhagic fever (EHF): virus, virus antigen, and IgG and IgM antibody findings among EHF patients in Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999;**179**: S177–87.
52. Ksiazek TG, West CP, Rollin PE, et al. ELISA for the detection of antibodies to Ebola viruses. *J Infect Dis* 1999;**179**:S192–8.
53. Kudoyarova-Zubavichene NM, Sergeyev NN, Chepurnov AA, et al. Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. *J Infect Dis* 1999; **179**(suppl. 1):S218–23.
54. Le Guenno B, Formenty P, Boesch C. Ebola virus outbreaks in Ivory Coast and Liberia, 1994-1995. *Curr Top Microbiol Immunol* 1999;**235**:77–84.
55. Le Guenno B, Formenty P, Wyers M, et al. Isolation and partial characterisation of a new strain of Ebola. *Lancet* 1995;**345**: 1271–4.
56. Leroy EM, Baize S, Lu C-Y, et al. Diagnosis of Ebola haemorrhagic fever by RT-PCR in an epidemic setting. *J Med Virol* 2000;**60**:463–7.
57. Leroy EM, Kumulungui B, Pourrut X, et al. Fruit bats as reservoirs of Ebola virus. *Nature* 2005;**438**:575–6.
58. Leroy EM, Rouquet P, Formenty P, et al. Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 2004;**303**:387–90.
59. Leroy EM, Souquière S, Rouquet P, et al. Re-emergence of Ebola haemorrhagic fever in Gabon. *Lancet* 2002;**359**:712.
60. Lupton HW, Lambert RD, Bumgardner DL, et al. Inactivated vaccine for Ebola virus efficacious in guinea pig model. *Lancet* 1980;**2**:1294–5.
61. Marsh G, Haining J, Robinson R, et al. Ebola Reston virus infection of pigs: clinical significance and transmission potential. *J Infect Dis* 2011;**204**(Suppl. 3):S804–9.
62. Martini GA. Marburg agent disease in man. *Trans R Soc Trop Med Hyg* 1969;**63**:295–302.
63. Martini GA. Clinical syndrome. In: Martini GA, Siegert R, editors. *Marburg virus disease*. New York: Springer-Verlag; 1971. p. 1–9.
64. Martini GA, Siegert R. *Marburg virus disease*. New York: Springer-Verlag; 1971.
65. Mikhailov VV, Borisevich IV, Chernikova NK, et al. Evaluation of possibility of Ebola fever specific prophylaxis in baboons *(Papio hamadryas)*. *Vopr Virusol* 1994;**39**:82–4.
66. Mohamadzadeh M, Chen L, Olinger GG, et al. Filoviruses and the balance of innate, adaptive, and inflammatory responses. *Viral Immunol* 2006;**19**:602–12.
67. Morvan JM, Deubel V, Gounon P, et al. Identification of Ebola virus sequences present as RNA or DNA in organs of terrestrial small mammals of the Central African Republic. *Microbes Infect* 1999;**1**:1193–201.
68. Mupapa K, Massamba M, Kibadi K, et al. Treatment of Ebola hemorrhagic fever with blood transfusions from convalescent patients. *J Infect Dis* 1999;**179**:S18–23.
69. Paessler S, Walker D. Pathogenesis of the viral hemorrhagic fevers. *Annu Rev Pathol* 2012 (Epub ahead of print).
70. Panchal R, Reid S, Trans J, et al. Identification of an antioxidant small-molecule with broad-spectrum antiviral activity. *Antiviral Res* 2012;**93**:23–9.
71. Pearlman E, Heinzel FP, Hazlett FE, et al. IL-12 modulation of T helper responses to the filarial helminth, *Brugia malayi*. *J Immunol* 1995;**154**:4658–64.
72. Peters CJ, Johnson ED, Jahrling PB. Filoviruses. In: Morse SS, editor. *Emerging viruses*. New York: Oxford University Press; 1992. p. 159–75.
73. Piot P, Sureau P, Breman G. Clinical aspects of Ebola virus infection in Yambuku area, Zaire, 1976. In: Pattyn SR, editor. *Ebola virus haemorrhagic fever*. New York: Elsevier/North-Holland Biomedical Press; 1978. p. 7–14.
74. Pratt W, Wang D, Nichols D, et al. Protection of nonhuman primates against two species of Ebola virus infection with a single complex adenovirus vector. *Clin Vaccine Immunol* 2010;**17**: 572–81.
75. Pushko P, Bray M, Ludwig GV, et al. Recombinant RNA replicons derived from attenuated Venezuelan equine encephalitis virus protect guinea pigs and mice from Ebola hemorrhagic fever virus. *Vaccine* 2000;**19**:142–53.
76. Rao M, Bray M, Alving CR, et al. Induction of immune responses in mice and monkeys to Ebola virus after immunization with liposome-encapsulated irradiated Ebola virus: protection in mice requires $CD4^+$ T cells. *J Virol* 2002;**76**:9176–85.
77. Rollin PE, Williams RJ, Bressler DS, et al. Ebola (subtype Reston) virus among quarantined nonhuman primates recently imported from the Philippines to the United States. *J Infect Dis* 1999;**179**: S108–14.
78. Sanchez A, Khan A, Zaki SR, et al. Filoviridae: Marburg and Ebola viruses. In: Knipe DM, Howley PM, editors. *Fields virology*. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1279–304.
79. Sanchez A, Trappier SG, Mahy BWJ, et al. The virion glycoproteins of Ebola viruses are encoded in two reading frames and are expressed through transcriptional editing. *Proc Natl Acad Sci U S A* 1996;**93**:3602–7.
80. Sarwar U, Sitar S, Ledgerwood J. Filovirus emergence and vaccine development: a perspective for health care practitioners in travel medicine. *Travel Med Infect Dis* 2011;**9**:126–34.
81. Shurtleff A, Nguyen T, Kingery D, et al. Therapeutics for filovirus infection: traditional approaches and progress towards in silico drug design. *Expert Opin Drug Discov* 2012;**7**:935–54.
82. Simmons G, Reeves JD, Grogan CC, et al. DC-SIGN and DC-SIGNR bind Ebola glycoproteins and enhance infection of macrophages and endothelial cells. *Virology* 2003;**305**:115–23.
83. Smith CEG, Simpson DIH, Bowen ETW. Fatal human disease from vervet monkeys. *Lancet* 1967;**2**:1119–21.
84. Smith DH, Francis F, Simpson DIH. African haemorrhagic fever in Southern Sudan, 1976: the clinical manifestations. In: Pattyn SR, editor. *Ebola virus haemorrhagic fever*. New York: Elsevier/ North-Holland Biomedical Press; 1978. p. 21–6.
85. Smith DH, Isaacson M, Johnson KM, et al. Marburg-virus disease in Kenya. *Lancet* 1982;**1**:816–20.
86. Smith DIH. Ebola haemorrhagic fever in Sudan, 1976. *Bull World Health Organ* 1978;**56**:247–70.
87. Sullivan NJ, Geisbert TW, Geisbert JB, et al. Accelerated vaccination for Ebola virus haemorrhagic fever in non-human primates. *Nature* 2003;**424**:681–4.
88. Sullivan NJ, Peterson M, Yang ZY, et al. Ebola virus glycoprotein toxicity is mediated by a dynamin-dependent protein-trafficking pathway. *J Virol* 2005;**79**:547–53.
89. Sullivan NJ, Sanchez A, Rollin PE, et al. Development of a preventive vaccine for Ebola virus infection in primates. *Nature* 2000;**408**:605–9.
90. Swanepoel R, Leman PA, Burt FJ. Experimental inoculation of plants and animals with Ebola virus. *Emerg Infect Dis* 1996;**2**: 321–5.
91. Takada A, Ebihara H, Jones S, et al. Protective efficacy of neutralizing antibodies against Ebola virus infection. *Vaccine* 2007;**25**: 993–6.
92. Towner JS, Khristova ML, Sealy TK, et al. Marburg virus genomics and association with a large hemorrhagic fever outbreak in Angola. *J Virol* 2006;**80**:6497–516.
93. Towner JS, Rollin PE, Bausch DG, et al. Rapid diagnosis of Ebola hemorrhagic fever by reverse transcription–PCR in an outbreak setting and assessment of patient viral load as a predictor of outcome. *J Virol* 2004;**78**:4330–41.
94. Turell MJ, Bressler DS, Rossi CA. Lack of virus replication in arthropods after intrathoracic inoculation of Ebola Reston virus. *Am J Trop Med Hyg* 1996;**55**:89–90.
95. Volchkov VE. Processing of the Ebola virus glycoprotein. *Curr Top Microbiol Immunol* 1999;**235**:35–47.
96. Volchkov VE, Feldmann H, Volchkova VA, et al. Processing of the Ebola virus glycoprotein by the proprotein convertase furin. *Proc Natl Acad Sci U S A* 1998;**95**:5762–7.
97. Volchkov VE, Volchkova VA, Mühlberger E, et al. Recovery of infectious Ebola virus from complementary DNA: RNA editing of the GP gene and viral cytotoxicity. *Science* 2001;**291**: 1965–9.
98. Walsh PD, Abernethy KA, Bermejo M, et al. Catastrophic ape decline in western equatorial Africa. *Nature* 2003;**422**:611–14.
99. World Health Organization. Outbreak of Ebola haemorrhagic fever, Uganda, August 2000–January 2001. *Wkly Epidemiol Rec* 2001;**76**:41–8.
100. World Health Organization. Ebola haemorrhagic fever: fact sheet revised in May 2004. *Wkly Epidemiol Rec* 2004;**79**:435–9.

101. World Health Organization. Ebola haemorrhagic fever in south Sudan: update. *Wkly Epidemiol Rec* 2004;**79**:253.
102. World Health Organization. Marburg virus disease. *Wkly Epidemiol Rec* 2005;**80**:115–17.
103. Xu L, Sanchez A, Yang Z-Y, et al. Immunization for Ebola virus infection. *Nat Med* 1998;**4**:37–42.
104. Yang C, Ye L, Compans R. Protection against filovirus infection: virus-like particle vaccines. *Expert Rev Vaccines* 2008;**7**:333–44.
105. Zaki SR, Shieh W-J, Greer PW, et al. A novel immunohistochemical assay for the detection of Ebola virus in skin: implications for diagnosis, spread, and surveillance of Ebola hemorrhagic fever. *J Infect Dis* 1999;**179**:S36–47.
106. Zeitlin L, Petitt J, Scully C, et al. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc Natl Acad Sci USA* 2011;**108**:20690–4.

in acute or chronic gastroenteritis in humans is less clear.

The first HCoV was cultivated by Tyrrell and Bynoe at the Common Cold Unit in Salisbury, England, with the use of human embryonic tracheal and nasal epithelial mucosal organ cultures.[161] The authors were able to produce colds regularly in volunteers inoculated with organ culture fluid from the first and later passages of an agent, B814, obtained from a boy with a cold.[12]

Working independently, Hamre and Procknow in 1966 described the isolation of five viruses, including the prototype strain HCoV-229E, in primary human embryonic kidney cell cultures.[63] Four of these agents were obtained from medical students with upper respiratory tract illnesses and one from a healthy student. The growth of six additional HCoVs, including the second human prototype HCoV-OC43, was reported in 1967 by McIntosh and associates, who used human embryonic tracheal organ cultures inoculated with secretions from adults with upper respiratory tract infections.[103,105]

The relationship of HCoVs to similar agents known to infect animals soon became evident. By electron microscopy, 229E and B814 were demonstrated to be morphologically identical to each other as well as to avian infectious bronchitis virus.[2] Subsequently, mouse hepatitis virus was demonstrated to be very similar morphologically and to be antigenically related to HCoV-OC43.[103,107] Shortly thereafter, these and similar agents were given the name "coronaviruses" owing to the crown-like appearance of their surface projections on electron microscopy.[160]

Poor growth and a lack of cytopathic effect in cell culture were, until recently, major deterrents to research on HCoV. With the development of new molecular diagnostic techniques including the polymerase chain reaction (PCR), and after the dramatic emergence of SARS in 2002 and 2003[22,88,132,159,191] and the discovery of the SARSr-CoV,[39,81,126] the field of coronavirology has developed widely and rapidly. Besides the SARS-related coronavirus, three more HCoVs have been described, NL63 in 2004,[47,52,166] HKU1 in 2005,[86,179] and MERS-CoV in 2013. NL63 and HKU1 are related to and clinically resemble traditional respiratory coronaviruses 229E and OC43 more than the SARSr-CoV. It seems likely that the full spectrum of viruses in the human host is still to be delineated because several HCoVs, including the very first isolate, HCoV-B814, remain antigenically and genetically uncharacterized.[11,13,107,161]

Toroviruses were, like coronaviruses, first described in animals. They were detected in the 1970s and 1980s in the feces of cattle (Breda virus) and horses (Berne virus).[175,182] Shortly thereafter, Beards and colleagues reported finding particles with a similar appearance in feces of adults and children with gastroenteritis. These particles aggregated in the presence of antiserum to the bovine and equine viruses.[8]

ETIOLOGIC AGENTS

Coronaviruses

Coronaviruses are medium to large (80 to 220 nm), pleomorphic, spherical or elliptical enveloped RNA viruses

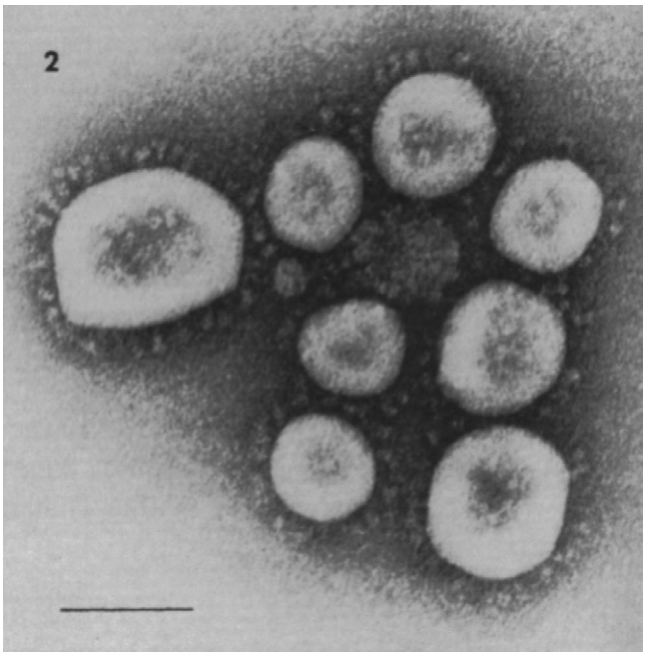

FIGURE 187-1 ■ A human coronavirus. Electron micrograph of one of the earliest coronaviruses found from an adult with a cold (OC16), negatively stained with phosphotungstic acid. The bar is 100 nm.[105]

with widely spaced, petal-shaped, 20-nm-long surface projections giving the virus the appearance of a solar corona[101] (Fig. 187-1). They are labile to heat, lipid solvents, and acid pH. The genomes of multiple coronaviruses have been sequenced completely, including those of HCoVs 229E, OC43, NL63, HKU1, and MERS-CoV and multiple strains of the SARSr-CoV.* The genome and its surrounding capsid are arranged in helical symmetry and enclosed within a lipoprotein envelope.

Within the coronavirus particle, a nucleoprotein (N) surrounds the RNA genome, and together they appear as a coiled tubular helix within the bilayer lipid-containing envelope. The envelope contains two or three glycoproteins: (1) a matrix protein M, which is embedded in the envelope; (2) a surface component S, which is the structural protein of the petal-shaped spikes; and (3) a hemagglutinin esterase HE, which is found in several of the betacoronaviruses, including OC43 and HKU1, and contains sequences closely related to influenza C hemagglutinin. The four respiratory HCoV species plus the SARSr-CoV and MERS-CoV, each along with several other mammalian coronaviruses, have been placed in the alphacoronavirus genus (229E and NL63) or the betacoronavirus genus (OC43, HKU1, SARSr-CoV, and MERS-CoV); avian infectious bronchitis virus and several other coronaviruses have been placed in the gammacoronavirus genus. Recent discovery of several new avian coronaviruses has opened the possibility of a fourth group, the deltacoronaviruses.[178]

Coronaviruses contain a positive-sense, single-stranded RNA genome of molecular weight 26 to 31 kilobases, the largest of any RNA virus group.[84] In coronavirus replication, all processes take place in the

*References 33a, 52, 61, 67, 135, 166, 172, 173, 179.

cytoplasm. In the first step, the virus attaches to the cell membrane by using its HE or S protein in the petal-shaped spike. HCoV-229E uses aminopeptidase N (CD-13) as a cellular receptor,[186] and both the SARSr-CoV and NL63 use angiotensin-converting enzyme 2,[68,93] whereas the receptor for HCoV-OC43 still has not been identified definitively. Penetration occurs as a result of S protein–mediated fusion of the viral envelope with the plasma membrane. The large viral replicase gene, which occupies two thirds of the genome, then codes for a large polyprotein. This polyprotein is cleaved into 15 or 16 nonstructural proteins by two or three virally encoded proteases, and a replication complex is formed. Within this complex, an RNA-dependent RNA polymerase initiates the transcription of subgenomic mRNA through a negative-stranded intermediary. The mRNA molecules, as in other members of the virus order Nidovirales, form a nested set, with the sequences of the first open reading frame at the 5′ end (after a leader sequence) containing the coding region and subsequent sequences through to the polyadenylated 3′ end being untranslated.[123] Virions then are assembled by budding into cytoplasmic vesicles in the rough endoplasmic reticulum and Golgi region. Virus particles are released by cell lysis or fusion of post-Golgi, virion-containing vesicles with the plasma membrane.

Toroviruses

Almost all the published information on human toroviruses (HToVs) is based on their detection by electron microscopy, by which their morphology has been difficult to distinguish from either coronaviruses or artifacts. Unlike for bovine, equine, and porcine toroviruses,[80,153,182] there are no reports of their detection in human feces by PCR or their growth in any in vitro system. In addition, one of the two reports of partial nucleic acid sequences for HToVs has been retracted because of contamination with a bovine torovirus,[41] and concerns have been raised about the possibility of equine torovirus contamination in the other report.[40] Moreover, since the last edition of this textbook, no further publications or reports on HToVs have appeared. For this reason, there is some concern about their role in human gastrointestinal diseases. The reader is referred to the sixth edition for further information on HToVs.

EPIDEMIOLOGY

Early epidemiologic surveys of HCoV infections were carried out by serologic methods of complement fixation and hemagglutination inhibition assays with 229E or OC43 viruses as antigens.[17,79,108] Enzyme immunoassays have also been useful in serosurveys.[37,97] More recently, epidemiologic surveys of 229E, OC43, NL63, and HKU1 infection have been based on PCR of respiratory tract samples. Many of these studies have been carried out retrospectively from specimens archived in freezer collections or hospital laboratories. Such surveys reflect the limitations or biases of their sampling strategies as o btaining clinical specimens longitudinally or from asymptomatic patients is not generally performed. In addition, because the four known HCoV species show considerable geographic and year-to-year variability, because molecular diagnostic techniques often pick up signals in asymptomatic individuals, and because duration of shedding of coronaviruses as detected by PCR is not well characterized, inferences about incidence and disease causation must be made with care.

SARSr-CoV epidemiology has been described both by PCR testing of respiratory tract and stool samples and by serology. The subject is reviewed in the fifth and sixth editions of this textbook and in the later section devoted to the outbreak.

Because enteric HCoVs rarely have been propagated in culture, epidemiologic study of these agents has been hindered by lack of antigens for serology. Moreover, studies that look for HCoVs by PCR in stool have been limited in number. The epidemiology of enteric HCoVs is outlined in the later section on clinical manifestations.

Geographic Prevalence

Respiratory coronavirus infections occur worldwide. Seroprevalence studies using 229E and OC43 antigens have been conducted in the United States, Europe, Brazil, and Iraq.* With enzyme-linked immunosorbent assay (ELISA), antibody prevalence in adults from all areas where they have been examined approaches 90 to 100 percent. Coronavirus NL63 has been found by PCR analysis in Europe, the United States, Canada, Australia, Japan, Korea, Taiwan, and Hong Kong.† Coronavirus HKU1 has been found in Hong Kong, the United States, Europe, and Australia.[46,83,152,165,181]

Seasonal Incidence and Annual Recycling Pattern

Although HCoV infection may occur at any time of the year (Fig. 187-2), most are seen in midwinter to early spring. The irregular year-to-year pattern that has been described for OC43 and 229E, namely, that individual species predominate for one or more years, followed by one or more years of lower activity,[76,77,112,113] appears also to hold for NL63 and HKU1.[35,133,157,165] Thus, because one of the four known HCoV species may be the only or at least the predominant HCoV found in a particular area during a particular season, and all four types have been demonstrated to circulate simultaneously, epidemiologically adequate coronavirus screening must include tests that are sensitive for all four species.[83]

During outbreaks, individual coronavirus types may be responsible for a large fraction of respiratory tract infections. At the University of Chicago in 1966 and 1967, 229E was associated with a significant outbreak in medical students; 66 (35%) of 191 sampled students were infected.[62] In Tecumseh, Michigan, a large 229E outbreak that occurred between January and April 1967 affected 68 percent of 38 families and 34 percent of 159

*References 13, 21, 65, 66, 76, 77, 108.
†References 4, 7, 10, 24, 30, 31, 42, 47, 52, 138, 166.

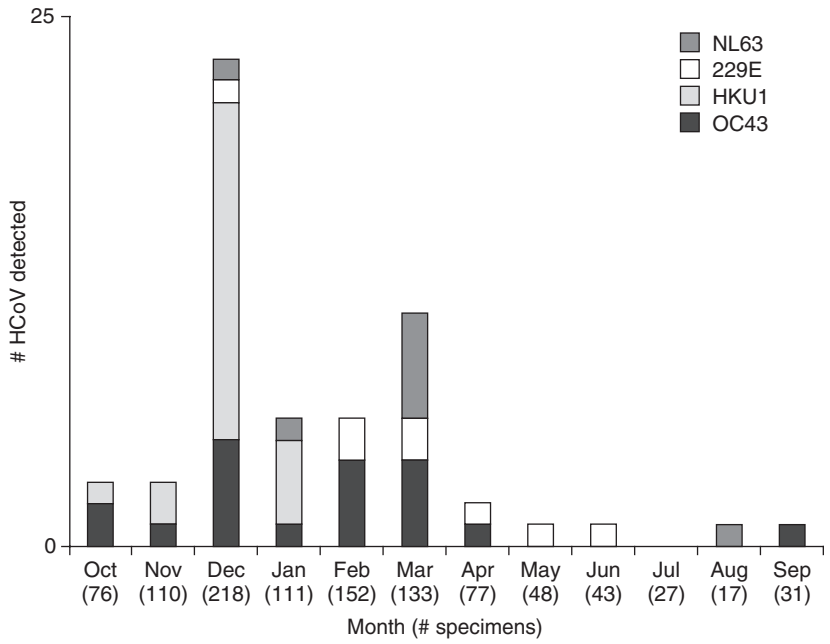

FIGURE 187-2 ■ Distribution of four types of human coronavirus in respiratory specimens from symptomatic hospitalized pediatric patients during 1 year. (Modified from Kuypers J, Martin ET, Heugel J, et al. Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics* 2007;**119**:e70-6.)

individuals tested. Sharp outbreaks also can occur in hospitalized infants; at National Jewish Hospital in Denver, 16 of 20 hospitalized asthmatic infants were infected with HCoV-OC43 in December 1968,[106] and smaller outbreaks have been seen in neonatal intensive care units with OC43,[151] 229E,[55] and NL63.[47]

Ratio of Clinical to Subclinical Illness

Although HCoVs clearly caused symptomatic upper respiratory tract disease in adult volunteers, asymptomatic infections were also seen in these volunteer studies. Early surveys of 229E and OC43 that identified infection in infants and children by a rise in antibody indicated that at least 50 percent of infections were subclinical.[76,77,108] In contrast, in the Denver children hospitalized with atopic asthma, 19 were infected with either 229E or OC43 and 17 were symptomatic.[106] A 2-year prospective PCR-based study of respiratory illness in more than 600 rural Alaskan children demonstrated similar rates of coronavirus infection in hospitalized children and community controls.[147] Other surveys of HCoVs in both symptomatic and asymptomatic children using detection by PCR have shown almost identical rates of positivity, indicating that in otherwise normal children, these viruses may be of low pathogenicity or are shed during long intervals.[10,35,82,133]

The importance of coronavirus in severely immunocompromised patients, such as hematopoietic stem cell transplant (HSCT) patients, has been investigated. In a 2-year prospective study conducted in HSCT recipients of all ages who were sampled weekly before and for 100 days after transplantation, the rates of detection of any of the four types of HCoV in symptomatic versus asymptomatic patients were similar.[111] Of note, the duration of shedding of coronaviruses in immunocompromised patients varied from less than 2 weeks (35%) to more than 10 weeks (17%).

Age Specificity of Infection

Antibody to the HCoVs appears in early childhood, with a tendency for antibody to the two betacoronaviruses (OC43 and NL63) to appear earlier and more frequently.[37] Prevalence increases rapidly with age. Asymptomatic and symptomatic infection occurs at all ages, including in the elderly. In the Tecumseh families studied during a community-wide 229E epidemic in 1967, only 3 of 54 infections occurred in children younger than 4 years. The attack rate then rose to a peak of 14 percent in individuals 15 to 29 years of age and subsequently fell with increasing age.[21] The results were different in an outbreak of HCoV-OC43 infection. With this virus, the highest rate, 29 percent, occurred in children 4 years of age or younger, and rates decreased very little even into adult years, when the incidence was 22 percent.[113]

Prospective studies during the first year of life of viruses found by PCR in nasopharyngeal samples obtained during acute respiratory symptoms have found coronaviruses, including OC43, 229E, NL63, and HKU1, in 6 to 16 percent of episodes.[82,91,138,168] In a birth cohort study that included samples obtained from asymptomatic as well as symptomatic infants, the proportions in the two groups were not significantly different (4.4% in asymptomatics vs. 5.5% in symptomatics).[82] In a prospective study of symptomatic children younger than 2 years attending daycare, coronavirus was detected as a sole pathogen in only 4 percent of upper respiratory tract illnesses but in an additional 11 percent of illnesses when more than one virus was present.[48] In the same study, the incidence of coronavirus detection by age cohort decreased from a rate of 0.6 infection per child-year for children observed beginning at 0 to 6 months of age to 0.5 infection per child-year for those observed from 7 to 12 months of age and further to 0.2 for those observed from 1 to 2 years of age.

Several studies have emphasized the importance of coronavirus infection in the elderly and the burden of infection, particularly in individuals in chronic care

facilities and those with underlying cardiopulmonary disease.[121]

Transmission

Human volunteers can be infected readily by nose drops, and a typical cold develops 2 to 3 days later; therefore, natural infections are assumed to occur through the respiratory route. Nosocomial transmission of coronavirus respiratory tract infection has been reported. One study took place in a neonatal intensive care unit, where prospective surveillance by immunofluorescence testing of nasal aspirates performed for 15 months detected 10 infections with coronavirus OC43, all of them nosocomially acquired.[151] All infants had apnea, bradycardia, or abdominal distention, or a combination of these pathologic effects, at the time of infection. Another, conducted similarly but using PCR analysis for HCoV-229E, found that during 1 year, three outbreaks occurred involving 28 percent of neonates (43 of 152, most of whom were symptomatic) and 52 percent of staff members (most of whom were asymptomatic).[56] The mode of transmission was not demonstrated in either study.

INFECTION AND IMMUNITY

Pathogenesis, Incubation Period, and Serologic Response

In healthy adults, HCoVs seem to replicate only in the upper respiratory tract and to produce little direct cytopathologic effect. In human embryonic tracheal organ culture, a decline in ciliary activity after serial passage was the only cytopathic effect observed.[105,161] Very similar events appear to take place in vivo. Electron microscopy of nasal epithelial biopsy specimens from a young girl with chronic rhinitis and bronchitis showed preservation of cellular structures and cilia despite replication of coronavirus particles.[1]

OC43 and 229E have been detected in nasopharyngeal cells,[109] and 229E virus titers from 10 to more than 1000 $TCID_{50}$ (median tissue culture infective dose) were found for a week or more in nasopharyngeal washings.[117] Bende and associates studied the course of 229E colds in 24 volunteers and delineated the typical signs, symptoms, and virus shedding patterns; 8 volunteers were asymptomatic.[9] The incubation period of HCoV colds is, on average, 2 days, and they usually last approximately 1 week.[12] In small infants, virus may be detectable in respiratory secretions for 3 weeks and longer.[138] Recovery is likely to be dependent on cell-mediated immunity. Infections in immunocompromised subjects may be associated with severe and prolonged disease.[4,50,127]

Little is known about the pathogenesis or immunity of HCoV infection of the gastrointestinal tract. There have, in contrast, been numerous studies devoted to the pathogenesis of SARS,[128] and this subject is briefly discussed later.

Reinfection

Evidence from human volunteer experiments demonstrates that strain-specific antibody can be protective. Reed infected volunteers with one of several 229E-like viruses. She found that immunity to homotypic challenge endured for at least 1 year but that immunity to heterotypic HCoV-229E strains in these same volunteers was much lower.[137] IgA antibody may play an important protective role.[16]

CLINICAL MANIFESTATIONS OF RESPIRATORY TRACT INFECTIONS

The Common Cold and Other Upper Respiratory Tract Illnesses

A significant association of HCoVs 229E and OC43 with respiratory tract illnesses—most of them cold-like—has been demonstrated in prospective serologic studies of adults and families with children. In the Chicago medical students described earlier, HCoV-229E attack rates were 31 percent during "illness periods" and only 9 percent in "wellness periods" ($p < .001$); the illnesses did not differ significantly from undifferentiated acute respiratory tract infections caused by respiratory syncytial virus, parainfluenza viruses, or rhinoviruses.[62] These findings have been confirmed in several other seroepidemiologic studies.[21,76,77]

Symptoms in the adults in the two investigations cited earlier were much like those in human volunteers inoculated with HCoV.[9,12,16,17,137] Infected volunteers contracted typical common colds, with perhaps more rhinorrhea than occurs in rhinovirus colds; sore throat, cough, malaise, and headache were noted in approximately 50 percent of volunteers. Twenty percent had fever.

The proportion of total respiratory tract disease or colds attributable to HCoV varies by season, from year to year, and also by the methodology used. Most serologic surveys have concluded that approximately 8 to 10 percent of all colds are associated with coronavirus infection.[98] A well-controlled study in the Netherlands used PCR for 229E and OC43 to estimate the contribution of coronaviruses to illness at all ages leading to a visit to general practitioners. Coronaviruses were found in 8.0 percent of subjects with acute respiratory tract infection, in comparison to 5.5 percent of nonrespiratory controls. Rhinoviruses were found most commonly in this group (24.7% vs. 16.6% of controls).[169] One survey of first respiratory tract illnesses in the first year of life that used PCR with primers for 229E, OC43, and NL63 found coronaviruses in 13 of 82 (16%) infants.[138] As more studies are performed that span longer periods, sample properly matched asymptomatic controls, and include all four known respiratory HCoV strains, the true incidence is likely to become clearer.

The possible role of coronaviruses in the etiology of otitis media with effusion has been the subject of several studies that used PCR for 229E and OC43 in both nasal secretions and middle ear fluid. In one study, 92 children with acute otitis media were investigated. Coronavirus sequences were found in 16 children (17%), in the nasopharynx in 14 and in middle ear fluid in 7. This prevalence was less than that of both respiratory syncytial virus (26%) and rhinoviruses (32%).[129] Another 3½-year survey of viral upper respiratory tract infections with or without

otitis media used PCR for OC43, NL63, and 229E as well as for other respiratory viruses. Otitis complicated approximately 50 percent of upper respiratory tract infections in which respiratory syncytial virus, HCoVs, and adenoviruses were found and a smaller proportion in which other viruses (influenza, rhinovirus, metapneumovirus, and parainfluenza viruses) were detected.[32]

Lower Respiratory Tract Disease

Pneumonia and Other Severe Lower Respiratory Tract Infections

The first evidence of a possible causative role of coronaviruses in lower respiratory tract disease was found in a serologic study of hospitalized infants during 1967 to 1970.[104] Infections with both HCoV-229E and HCoV-OC43 were noted, and HCoV was the third most frequently occurring virus (behind respiratory syncytial virus and parainfluenza virus type 3), both in incidence and in specific association with pneumonia and bronchiolitis. Many of these infants required oxygen. HCoV-229E was grown in tissue culture from oropharyngeal swabs obtained from two of the infants with pneumonia and rises in antibody.

With the advent of PCR for detection of coronaviruses and the addition of two new HCoV strains, NL63 and HKU1, that can be detected by PCR, the information about presence of these viruses in children with serious respiratory tract disease has expanded greatly. A large number of surveys have indicated that HCoVs can be found in the respiratory tract of 4 to 8 percent of children hospitalized with community-acquired pneumonia, often accompanied by coinfection with other respiratory tract viruses.* A much smaller number of surveys have included age- and season-matched control children without respiratory symptoms. When these controls are properly chosen and studied, however, the HCoV detection rates in well and ill children have been found to be similar.[35,133,147] It appears likely that coronaviruses are pathogenic in some instances whereas in others they are not, or persistent shedding of these viruses may occur, and it is difficult to assign an etiologic role in individual cases.[102]

In newborns, apnea, bradycardia, and increased demand for oxygen have been described during coronavirus infection.[35,56,150,151] One prospective nursery study, with sampling at regular intervals regardless of symptoms, associated HCoV-229E infection with symptomatic disease in high-risk neonates.[54] Another outbreak study in a neonatal intensive care setting implicated NL63 in acute symptomatic disease.[47]

There is a suggestion that NL63 is seen particularly in croup.[31,156,167] Several children with either NL63 or HKU1 have had prominent gastrointestinal symptoms.[163,164]

Infections in Children with Underlying Respiratory Disease or Immunodeficiency

Substantial evidence indicates that HCoVs can precipitate asthma attacks,[70,73,106,110,124] although their role in this regard is clearly secondary to that of rhinoviruses and respiratory syncytial virus.[71] In a 2-year surveillance (1967 to 1969) of 32 mostly atopic children 1 to 5 years of age hospitalized in Denver for severe, recurrent bouts of wheezing, 19 HCoV infections were diagnosed.[106] Six children were infected simultaneously with either parainfluenza virus or respiratory syncytial virus. Of the remaining 13 patients, all were symptomatic: 3 had mild wheezing, and 7 had acute asthma attacks, 2 of whom required intravenous therapy.

A study of HCoVs in children with cystic fibrosis sought all four species by PCR during 1 year.[34] HCoVs were found in 19 of 408 samples, and although there was some correlation of NL63 infection with exacerbations of respiratory symptoms, this did not hold up in a logistic regression model for independent association.

Coronavirus has been detected as the sole respiratory pathogen in upper and lower respiratory tract samples in immunocompromised children with pneumonia receiving chemotherapy or having received a solid-organ transplant as well as in adults undergoing HSCT.[83,162] Respiratory disease in six immunocompromised children with coronavirus and no other viral, bacterial, or fungal pathogen detected by sensitive testing began as fever and rhinorrhea; only two children had cough at the time of first medical encounter.[83] Only one of three children had evidence of abnormalities on chest radiographs, although all six children required hospitalization for coronavirus-linked illness for 2 to 12 days. A prospective study with weekly sampling in 215 asymptomatic and symptomatic HSCT recipients of all ages during the first 100 days after transplantation did not find coronavirus as a sole pathogen associated with pneumonia, demonstrating that coronavirus-associated pneumonia is relatively infrequent in immunocompromised patients and that the detection of coronavirus is probably insufficient evidence to assess causality.[111]

Carefully conducted epidemiologic studies do not link coronavirus infection or disease with Kawasaki syndrome.[6]

Severe Acute Respiratory Syndrome (SARS)

Severe acute respiratory syndrome (SARS) in children was covered in detail in the sixth edition of this textbook, and readers are referred to that chapter for greater coverage. SARS was a new infectious disease that had its origin in Foshan in Guangdong Province, China, in November 2002. After a few sporadic cases, the virus spread at first within China and then, through Hong Kong, to Vietnam, Singapore, Europe, Canada, and the United States. By the time the epidemic ended in the summer of 2003, there were 8096 probable cases in 29 countries and areas with 774 deaths (9.6% mortality).[185] The total number of health care workers affected was 1706 (21.1% of all probable cases). Globally, it is estimated that children younger than 18 years accounted for only about 5% of cases. Interestingly, the severity of the syndrome appears to have been greater in adults and adolescents than in young children, and there was no reported mortality in children.[184]

Etiology

It is now accepted that the cause of SARS is a novel coronavirus, the SARSr-CoV, identified independently

*References 30, 31, 57, 83, 136, 157, 171.

and collaboratively by multiple investigators.[39,81,126,132] The virus almost undoubtedly originated in animals, probably bats, and then spread to exotic animals kept for human consumption in cages in parts of southeastern China, and then to humans.[28,61,74,85,94] The genome of the SARSr-CoV is about 29,700 nucleotides in length and has a genetic organization similar to that of other coronaviruses. The virus is classified as a betacoronavirus.

The SARSr-CoV uses angiotensin-converting enzyme 2 as a key functional cellular receptor. The virus also binds to the C-type lectin CD209L (also known as L-SIGN) and DC-SIGN.[68,87,93]

Epidemiology

Through case identification and rigorous implementation of barrier methods, the SARS epidemic was quenched after only about 8 months of activity. This is in spite of the fact that the virus could, under the right circumstances, spread widely through aerosols, with one well-described airplane cluster and several explosive community epidemics.[122,188]

Children with SARS are apparently less infectious than their adult counterparts are.

Clinical Presentation

There are two large series of serologically proven pediatric cases of SARS.[29,92] In Leung's series, the median age was 12 years, with more than 90 percent being older than 5 years. The disease had many features similar to that in adults: onset with fever and, particularly in adolescents, systemic, influenza-like symptoms. Coryza was significantly more common in children younger than 13 years (61%) and much more common than in adults. The prodromal period was followed with cough in 64 percent and diarrhea in 20 percent, but the signs and symptoms of progressive pneumonia, common in adults, appeared in a small minority of children and mostly in adolescents. Cough was not invariably present during the illness, even in severely affected children.[92]

Chest radiography and high-resolution computed tomography findings were similar to those found in adults, showing peripheral consolidations and ground-glass opacities, with pleural effusions being rare.[5] The most prominent laboratory findings were leukopenia and, particularly, lymphopenia, with reduction in all lymphocyte types.

Mortality in children has not been reported worldwide, although the disease can be severe in young infants or even life-threatening in adolescents.[27,51,148]

In spite of the apparent universality of viremia, there was no evidence of perinatal transmission to infants born to infected pregnant women.* However, SARSr-CoV infection has been associated with both maternal and fetal morbidity and mortality.[119,120,146,176,177]

Specific Diagnosis

For surveillance purposes, both the World Health Organization and the Centers for Disease Control and Prevention developed and revised case definitions as the SARS epidemic evolved.[23,183]

In the absence of known cases since 2004 anywhere in the world, the laboratory takes a particularly important place in specific diagnosis. The "gold standard" for laboratory diagnosis is a rise in antibody titer during illness. Rapid diagnosis can be accomplished by reverse transcription PCR of either plasma or respiratory tract secretions (e.g., nasopharyngeal aspirate) obtained during the first 2 weeks of illness.[118,131]

Treatment

No specific treatment is now recommended other than careful supportive care.

Prevention

The mainstay of prevention of SARS is effective hospital infection control practices and quarantine procedures outside hospitals. Patients with respiratory illnesses consistent with SARS should be isolated at triage and placed in a negative air pressure room. Health care workers should wear appropriate masks and goggles or face shields while tending patients, especially when performing procedures in which splashing and aerosolization are likely. Adequate hand washing before and after contact with patients is critical.[33]

Current vaccine research is concentrated on the S protein, which contains important epitopes that stimulate production of neutralizing antibody.[44]

Middle East Respiratory Syndrome (MERS)

In September 2012, a man hospitalized with severe pneumonia in Jeddah, Saudi Arabia, was found to be excreting a new coronavirus, subsequently termed the Middle East Respiratory Syndrome coronavirus (MERS-CoV).[188a] In retrospect, cases were identified in a hospital outbreak that occurred in Jordan in June 2012, and subsequent cases have been recorded, primarily in Saudi Arabia with all but a few presenting with severe pneumonia. As of August 2013, over 90 proven infections have been reported with a mortality of about 50%. Most cases have been in adults with underlying chronic disease or immunosuppression.[4a]

Etiology

The virus is a beta-coronavirus, like the SARSr-CoV.[35a] Although person-to-person spread has been clearly proven, the origin of the MERS-CoV, which is presumably an animal source, has not been identified. Very similar viruses have been found by sequencing RNA in the feces of bats of the Vespertilionidae family in Europe and Africa, so bats appear to be a likely original source, but other unidentified animal intermediaries may also be involved.[2a] Like several other CoVs, including SARSr-CoV, the MERS-CoV uses a protease (dipeptidyl peptidase 4) as its cellular receptor.[135a] The virus grows and produces cytopathic effect in many tissue culture types.

*References 119, 120, 143, 146, 177, 189.

Epidemiology

The epidemiology of MERS-CoV infection has not been well described. Evidence points to its origin in bats because closely related CoV RNA sequences have been found in bat feces in eastern Bulgaria, Ukraine, and South Africa.[2a] No intermediate animal species has been identified. Limited person-to-person spread in families and health care settings has been demonstrated. Calculations from available data suggest a reproductive coefficient less than 1, suggesting that, in its present form, the virus does not have pandemic potential.[13a] All known infections can be traced to countries in the Middle East, primarily Saudi Arabia.

Clinical Presentation

Most but not all identified infections have presented with fever, cough (often with hemoptysis), shortness of breath, and rapidly developing pneumonia. Some of those infected, including children and adolescents, have been asymptomatic or only mildly affected. Other common symptoms have been diarrhea, sore throat, myalgias, and headache. The mortality is around 50%.[4a]

Diagnosis

The World Health Organization and the United States Centers for Disease Control have published case definitions and urge the early submission of respiratory samples, preferably from the lower respiratory tract, for PCR diagnosis using laboratories with approved protocols and reagents. Serologic diagnosis has not been standardized.[21a,184a]

Prevention

In the absence of specific information about the mode of spreading MERS-CoV and because of the high lethality of infection, full barrier precautions are recommended for all contacts, with details very similar to the precautions that were used in the SARS epidemic.

Enteric Human Coronavirus

From the earliest descriptions of HCoVs, because of the prominence of coronaviruses as a cause of diarrhea in young calves and pigs, attempts have been made to find coronaviruses in the stool and to associate them with enteric disease. The particles that have been seen, often called coronavirus-like particles (CVLPs), have appeared as frequently in stools from well subjects as in those with diarrhea in many studies, and at times they have been difficult to distinguish from cellular membrane fragments. Indeed, their authenticity as viruses has been called into doubt on occasion.[38,96,114,125]

The first reports of HCoV as a possible cause of human gastroenteritis appeared in 1975; CVLPs were found by electron microscopy in the stools of English adults in three sharp outbreaks of nonbacterial gastroenteritis.[18,20] In the same year, CVLPs were reported in the stools of healthy adults in India.[100] These rather contrasting publications from England and India heralded the beginning of a continuing controversy on the etiologic importance of these agents as enteric pathogens.[96,114]

The firmest link of CVLPs to human enteric disease is with gastroenteritis in the very young, especially neonatal necrotizing enterocolitis (NEC). A controlled epidemiologic investigation of NEC was conducted in two hospitals in France, one with and one without an NEC outbreak.[25] Within each hospital, newborns with "no pathologic occurrence" were used as controls. In the NEC-free hospital, no CVLPs were found in the stools of 21 controls, but 2 patients with mild diarrhea had CVLPs. In the NEC hospital, 23 of the 32 (72%) NEC patients had fecal CVLPs, whereas only 3 of 26 (11.5%) controls were positive ($p < .02$). Similarly, CVLPs were observed in infants who were part of an NEC outbreak in a special care nursery in Texas.[139] This outbreak yielded a virus that subsequently has been adapted to growth in tissue culture and to some extent characterized.[95]

In September 1979, an episode of acute, severe (bloody stools, bilious gastric aspirates, abdominal distention) gastroenteritis occurred in a neonatal intensive care unit in Arizona, and several clinical signs were associated significantly with CVLPs in patients' stools.[170] Two children died in this outbreak, and another infant death, with careful electron microscopic description of coronavirus infection in the distal end of the small bowel, occurred in Oklahoma.[140] In Italy, a case-control study of infants and young children with enteritis found a significant difference in fecal CVLP presence between the ill and control groups: 16.3 percent in ill children and 1.6 percent in controls ($p < .01$).[58]

With the development of molecular methods for detection of HCoVs and the discovery of two new HCoV species, it is surprising that PCR has not been more widely used in study of enteric coronaviruses. Two surveys have been published, one of which included asymptomatic control samples. In the latter, 878 samples from children with gastrointestinal complaints were tested during 2 years, and all four HCoV species were found (OC43-10, HKU1-6, NL63-4, 229E-2).[142] In all but 4 of 22 cases, either rotavirus or norovirus was also found. About half of the children had respiratory as well as gastrointestinal symptoms. In the same study, 112 asymptomatic controls were sampled, and 2 samples were positive. The other study sampled 151 children with diarrhea, abdominal pain, or vomiting, and 2 were found to have HKU1 RNA.[45]

Thus, despite some evidence for a causal relationship between CVLPs and gastrointestinal disease, reservation about the role of HCoVs in children's enteric disease remains. CVLPs are found often in the feces of healthy children,* and PCR data indicate that most symptomatic children with HCoV RNA in the stool also have evidence of coinfection by viruses with known gastrointestinal pathogenicity.[142]

Attempts have been made to cultivate CVLPs in cell and organ culture and to compare the resultant virus suspensions antigenically with other HCoVs or animal

*References 78, 99, 100, 134, 145, 149.

coronaviruses. As mentioned earlier, a possible enteric HCoV was cultured from a child with NEC, and this virus has been adapted further to growth in a mouse macrophage cell line and mosquito cells.[95] This virus is antigenically unrelated to HCoV-229E and HCoV-OC43. A hemagglutinating coronavirus antigenically and genetically related to a bovine coronavirus (and therefore, presumably, OC43), BCV-LY138, has been isolated from a 6-year-old child with severe diarrhea.[190]

Neurologic Diseases

As described earlier, coronaviruses are the cause of some animal neurologic disorders, including a murine demyelinating disease with some features resembling multiple sclerosis. In humans, a serosurvey of HCoV infection in southern Finland discovered evidence of HCoV-OC43 infection in six patients with acute neurologic episodes, including one with polyradiculitis.[141] A case of acute demyelinating encephalitis accompanying OC43 respiratory tract infection was described in which OC43 RNA was detected by PCR in the spinal fluid.[187] Several observations have suggested a possible role of coronavirus infection in multiple sclerosis: (1) isolation of coronaviruses (SK + SD) from the central nervous system tissue of two patients with multiple sclerosis,[15] (2) demonstration that coronavirus SD can cause demyelination in a primate model,[116] (3) direct visualization of CVLPs in the brain of a patient with multiple sclerosis,[158] and (4) identification by in situ hybridization or PCR of coronavirus RNA in the brains of patients with multiple sclerosis.[3,115,155] However, coronaviruses SK and SD are antigenically and genetically very similar to mouse hepatitis virus and were isolated with the use of mouse tissues; the possibility that these isolates were of mouse origin has not been excluded. In addition, other studies have failed to demonstrate coronavirus RNA in the central nervous system tissue of patients with multiple sclerosis[154] or have found such RNA in the same proportion of patients with demyelinating diseases as in controls.[36] Further investigation will be needed to establish whether HCoVs are related causally to any neurologic disease in humans.

LABORATORY DIAGNOSIS

Virus Isolation

Respiratory Coronaviruses

Human embryo tracheal or nasal mucosal organ cultures were first used for primary isolation of multiple HCoV strains, including B814, OC43, and several less well characterized strains.[105,161] OC43 was subsequently adapted to growth in monkey kidney, BSC-1, and rhabdomyoma cells.[14,144] The 229E-type strains were shown to replicate and produce a cytopathic effect in secondary human embryo kidney[64] or certain diploid cell lines: WI-38, MRC-5, and MA-177.[62,75] There has been a report of the growth and cytopathic effect of OC43-like strains in the HuH7 hepatocarcinoma cell line.[53] This line has also been used for primary isolation of HKU1 strains.[163] NL63 strains grow directly from clinical samples in tertiary monkey kidney cells, with subsequent growth in LLC or Vero cells.[52,166] The SARSr-CoV was isolated in and grows well in Vero-E6 cells.[39,81]

Enteric Coronavirus-like Particles

Growth of these agents in cell or organ culture has been reported,[19,139,190] and one isolate has been adapted to growth in tissue culture.[95]

Virus Detection Techniques

Respiratory Coronaviruses

Respiratory coronaviruses can be found in clinical samples by direct antigen detection techniques or by nucleic acid detection. Both immunofluorescence[72,109,151] and antigen-detecting ELISA have been used successfully for the diagnosis of HCoV infection.[70,97,110]

During the past decade, PCR has opened up the field of coronavirus diagnosis. Not only is the method the most sensitive for all the known strains of HCoV, but it can also be designed to detect new strains.[47] Attempts to design a set of "pan-coronavirus" primers have, however, always led to suboptimal sensitivity.[47,57] The exact design of the tests also influences greatly their sensitivity and specificity.

Enteric Coronaviruses

Enteric coronaviruses have been detected primarily by electron microscopy of negatively stained preparations from clarified stool specimens.

An antigen detection ELISA for enteric coronavirus has been described and applied to fecal specimens from healthy and diarrheal subjects in Thailand.[89,90] PCR has not been widely used to detect coronaviruses in diarrhea, although HKU1 was found in both the respiratory tract and the stool in two patients as part of a larger study of respiratory disease,[163] and all four HCoV respiratory virus types have been found in the stool of children with diarrhea, usually accompanied by other known gastrointestinal viruses.[142]

Serodiagnosis

A rise in antibody titer after day 28 of onset of symptoms is the gold standard for diagnosis of SARSr-CoV infection, most commonly by ELISA or immunofluorescence assay in Vero-E6 cells. The finding of antibody in convalescent serum is highly specific for SARSr-CoV infection, although there are one-way cross-reactivities with both 229E and, especially, OC43.[26] For respiratory coronaviruses, complement fixation,[21,104,108,113,141] hemagglutination inhibition (for HCoV-OC43 only),[77] neutralization,[43] ELISA,[17,37,59,79,97] indirect hemagglutination,[76] and Western blot[130] have been used.

Serodiagnostic techniques specific for enteric coronaviruses have not been described.

PREVENTION AND TREATMENT

Barrier methods were extraordinarily successful in the limitation and final elimination of the SARS epidemic,

and it seems likely that contact precautions would serve to reduce the spread of respiratory and gastrointestinal coronaviruses as well.

The high reinfection rate with the respiratory HCoVs as well as the number of related strains suggests that a vaccine may be ineffective in preventing HCoV-caused respiratory illness.

There are no known effective antivirals for the respiratory coronaviruses.

Acknowledgments

Dr. McIntosh gratefully acknowledges the support of The Bruce R. and Jolene M. McCaw Fund.

NEW REFERENCES SINCE THE SIXTH EDITION

2a. Annan A, Baldwin HJ, Corman VM, et al. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. *Emerg Infect Dis* 2013;**19**:456–9.

4a. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis* 2013. Available at http://dx.doi.org/10.1016/S1473-3099(13)70204-4.

6. Baker SC, Shimizu C, Shike H, et al. Human coronavirus–NL63 infection is not associated with acute Kawasaki disease. *Adv Exp Med Biol* 2006;**581**:523–6.

13a. Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 2013. Available at http://dx.doi.org/10.1016/S0140-6736(13)61492–0.

21a. Centers for Disease Control and Prevention. *Coronavirus. Update, Case Definitions, and Guidance - MERS update*. http://www.cdc.gov/coronavirus/mers/case-def.html. Accessed August 12, 2013.

32. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis* 2008;**46**:815–23.

33a. Cotten M, Lam TT, Watson SJ, et al. Full-genome deep sequencing and phylogenetic analysis of novel human betacoronavirus. *Emerg Infect Dis* 2013;**19**:736–42B.

34. da Silva Filho LV, Zerbinati RM, Tateno AF, et al. The differential clinical impact of human coronavirus species in children with cystic fibrosis. *J Infect Dis* 2012;**206**:384–8.

35. Dare RK, Fry AM, Chittaganpitch M, et al. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis* 2007;**196**:1321–8.

35a. de Groot RJ, Baker SC, Baric RS, et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. *J Virol* 2013;**87**:7790–2.

37. Dijkman R, Jebbink MF, Gaunt E, et al. The dominance of human coronavirus OC43 and NL63 infections in infants. *J Clin Virol* 2012;**53**:135–9.

41. Duckmanton L, Tellier R, Richardson C, Petric M. Notice of retraction to "The novel hemagglutinin-esterase genes of human torovirus and Breda virus" [*Virus Res* 1999;64:137–49]. *Virus Res* 2001;**81**:167.

44. Enjuanes L, Dediego ML, Alvarez E, et al. Vaccines to prevent severe acute respiratory syndrome coronavirus-induced disease. *Virus Res* 2008;**133**:45–62.

45. Esper F, Ou Z, Huang YT. Human coronaviruses are uncommon in patients with gastrointestinal illness. *J Clin Virol* 2010;**48**:131–3.

47. Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. *J Infect Dis* 2005;**191**:492–8.

54. Gagneur A, Dirson E, Audebert S, et al. Materno-fetal transmission of human coronaviruses: a prospective pilot study. *Eur J Clin Microbiol Infect Dis* 2008;**27**:863–6.

56. Gagneur A, Vallet S, Talbot PJ, et al. Outbreaks of human coronavirus in a pediatric and neonatal intensive care unit. *Eur J Pediatr* 2008;**167**:1427–34.

60. Graat JM, Schouten EG, Heijnen ML, et al. A prospective, community-based study on virologic assessment among elderly people with and without symptoms of acute respiratory infection. *J Clin Epidemiol* 2003;**56**:1218–23.

69. Hon CC, Lam TY, Shi ZL, et al. Evidence of the recombinant origin of a bat severe acute respiratory syndrome (SARS)–like coronavirus and its implications on the direct ancestor of SARS coronavirus. *J Virol* 2008;**82**:1819–26.

83. Kuypers J, Martin ET, Heugel J, et al. Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics* 2007;**119**:e70–6.

84. Lai MM, Holmes KV. Coronaviridae: the viruses and their replication. In: Knipe D, Howley PM, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott-Raven; 2007.

102. McIntosh K. Proving etiologic relationships to disease: the particular problem of human coronaviruses. *Pediatr Infect Dis J* 2012;**31**:241–2.

111. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;**115**:2088–94.

133. Prill MM, Iwane MK, Edwards KM, et al. Human coronavirus in young children hospitalized for acute respiratory illness and asymptomatic controls. *Pediatr Infect Dis J* 2011;**31**:235–40.

135. Pyrc K, Berkhout B, van der Hoek L. The novel human coronaviruses NL63 and HKU1. *J Virol* 2007;**81**:3051–7.

135a. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 2013;**495**:251–4.

136. Raymond F, Carbonneau J, Boucher N, et al. Comparison of automated microarray detection with real-time PCR assays for detection of respiratory viruses in specimens obtained from children. *J Clin Microbiol* 2009;**47**:743–50.

138. Regamey N, Kaiser L, Roiha HL, et al. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J* 2008;**27**:100–5.

142. Risku M, Lappalainen S, Rasanen S, Vesikari T. Detection of human coronaviruses in children with acute gastroenteritis. *J Clin Virol* 2010;**48**:27–30.

147. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol* 2010;**82**:1282–90.

156. Sung JY, Lee HJ, Eun BW, et al. Role of human coronavirus NL63 in hospitalized children with croup. *Pediatr Infect Dis J* 2010;**29**:822–6.

157. Talbot HK, Shepherd BE, Crowe JE Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J* 2009;**28**:682–7.

162. Uhlenhaut C, Cohen JI, Pavletic S, et al. Use of a novel virus detection assay to identify coronavirus HKU1 in the lungs of a hematopoietic stem cell transplant recipient with fatal pneumonia. *Transpl Infect Dis* 2012;**14**:79–85.

165. van der Hoek L, Ihorst G, Sure K, et al. Burden of disease due to human coronavirus NL63 infections and periodicity of infection. *J Clin Virol* 2010;**48**:104–8.

168. van der Zalm MM, Uiterwaal CS, Wilbrink B, et al. Respiratory pathogens in respiratory tract illnesses during the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2009;**28**:472–6.

171. Venter M, Lassauniere R, Kresfelder TL, et al. Contribution of common and recently described respiratory viruses to annual hospitalizations in children in South Africa. *J Med Virol* 2011;**83**:1458–68.

178. Woo PC, Huang Y, Lau SK, Yuen KY. Coronavirus genomics and bioinformatics analysis. *Viruses* 2010;**2**:1804–20.

184a. World Health Organization. *Interim surveillance recommendations for human infection with Middle East respiratory syndrome coronavirus* http://www.who.int/csr/disease/coronavirus_infections/Interim-RevisedSurveillanceRecommendations_nCoVinfection_27Jun13.pdf. Accessed August 12, 2013.

188a. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012;**367**:1814–20.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Afzelius BA. Ultrastructure of human nasal epithelium during an episode of coronavirus infection. *Virchows Arch* 1994;**424**: 295–300.
2. Almeida JD, Tyrrell DAJ. The morphology of three previously uncharacterized human respiratory viruses that grow in organ culture. *J Gen Virol* 1967;**1**:175–8.

2a. Annan A, Baldwin HJ, Corman VM, et al. Human betacoronavirus 2c EMC/2012-related viruses in bats, Ghana and Europe. *Emerg Infect Dis* 2013;**19**:456–9.

3. Arbour N, Day R, Newcombe J, Talbot PJ. Neuroinvasion by human respiratory coronaviruses. *J Virol* 2000;**74**:8913–21.
4. Arden KE, Nissen MD, Sloots TP, Mackay IM. New human coronavirus, HCoV-NL63, associated with severe lower respiratory tract disease in Australia. *J Med Virol* 2005;**75**: 455–62.

4a. Assiri A, Al-Tawfiq JA, Al-Rabeeah AA, et al. Epidemiological, demographic, and clinical characteristics of 47 cases of Middle East respiratory syndrome coronavirus disease from Saudi Arabia: a descriptive study. *Lancet Infect Dis* 2013. Available at http://dx.doi.org/10.1016/S1473-3099(13)70204–4.

5. Babyn PS, Chu WC, Tsou IY, et al. Severe acute respiratory syndrome (SARS): chest radiographic features in children. *Pediatr Radiol* 2004;**34**:47–58.
6. Baker SC, Shimizu C, Shike H, et al. Human coronavirus–NL63 infection is not associated with acute Kawasaki disease. *Adv Exp Med Biol* 2006;**581**:523–6.
7. Bastien N, Anderson K, Hart L, et al. Human coronavirus NL63 infection in Canada. *J Infect Dis* 2005;**191**:503–6.
8. Beards GM, Hall C, Green J, et al. An enveloped virus in stools of children and adults with gastroenteritis that resembles the Breda virus of calves. *Lancet* 1984;**1**:1050–2.
9. Bende M, Barrow I, Heptonstall J, et al. Changes in human nasal mucosa during experimental coronavirus common colds. *Acta Otolaryngol* 1989;**107**:262–9.
10. Boivin G, Baz M, Cote S, et al. Infections by human coronavirus–NL in hospitalized children. *Pediatr Infect Dis J* 2005;**24**: 1045–8.
11. Bradburne AF. Antigenic relationships amongst coronaviruses. *Arch Gesamte Virusforsch* 1970;**31**:352–64.
12. Bradburne AF, Bynoe ML, Tyrrell DA. Effects of a "new" human respiratory virus in volunteers. *Br Med J* 1967;**3**:767–9.
13. Bradburne AF, Somerset BA. Coronavirus antibody titres in sera of healthy adults and experimentally infected volunteers. *J Hyg (Lond)* 1972;**70**:235–44.

13a. Breban R, Riou J, Fontanet A. Interhuman transmissibility of Middle East respiratory syndrome coronavirus: estimation of pandemic risk. *Lancet* 2013. Available at http://dx.doi.org/10.1016/S0140-6736(13)61492-0.

14. Bruckova M, McIntosh K, Kapikian AZ, Chanock RM. The adaptation of two human coronavirus strains (OC38 and OC43) to growth in cell monolayers. *Proc Soc Exp Biol Med* 1970;**135**: 431–5.
15. Burks JS, DeVald BL, Jankovsky LD, Gerdes JC. Two coronaviruses isolated from central nervous system tissue of two multiple sclerosis patients. *Science* 1980;**209**:933–4.
16. Callow KA. Effect of specific humoral immunity and some non-specific factors on resistance of volunteers to respiratory coronavirus infection. *J Hyg (Lond)* 1985;**95**:173–89.
17. Callow KA, Parry HF, Sergeant M, Tyrrell DA. The time course of the immune response to experimental coronavirus infection of man. *Epidemiol Infect* 1990;**105**:435–46.
18. Caul EO, Clarke SK. Coronavirus propagated from patient with non-bacterial gastroenteritis. *Lancet* 1975;**2**:953–4.
19. Caul EO, Egglestone SI. Further studies on human enteric coronaviruses. *Arch Virol* 1977;**54**:107–17.
20. Caul EO, Paver WK, Clarke SK. Coronavirus particles in faeces from patients with gastroenteritis [letter]. *Lancet* 1975;**1**: 1192.
21. Cavallaro JJ, Monto AS. Community-wide outbreak of infection with a 229E-like coronavirus in Tecumseh, Michigan. *J Infect Dis* 1970;**122**:272–9.

21a. Centers for Disease Control and Prevention. *Coronavirus. Update, Case Definitions, and Guidance—MERS update*. http://www.cdc.gov/coronavirus/mers/case-def.html. Accessed August 12, 2013.

22. Centers for Disease Control and Prevention. Outbreak of severe acute respiratory syndrome—worldwide. *MMWR Morb Mortal Wkly Rep* 2003;**52**:226–8.
23. Centers for Disease Control and Prevention. *Severe acute respiratory syndrome: revised CSTE SARS surveillance case definition*. 2004. Available at http://www.cdc.gov/ncidod/SARS/guidance/b/app1.htm.
24. Chang LY, Chiang BL, Kao CL, et al. Lack of association between infection with a novel human coronavirus (HCoV), HCoV-NH, and Kawasaki disease in Taiwan. *J Infect Dis* 2006;**193**:283–6.
25. Chany C, Moscovici O, Lebon P, Rousset S. Association of coronavirus infection with neonatal necrotizing enterocolitis. *Pediatrics* 1982;**69**:209–14.
26. Che XY, Qiu LW, Liao ZY, et al. Antigenic cross-reactivity between severe acute respiratory syndrome–associated coronavirus and human coronaviruses 229E and OC43. *J Infect Dis* 2005; **191**:2033–7.
27. Cheng FW, Ng EK, Li AM, et al. Clinical, virologic and immunologic profiles of a young infant with severe acute respiratory syndrome. *Pediatr Infect Dis J* 2005;**24**:567–8.
28. Chinese SARS Molecular Epidemiology Consortium. Molecular evolution of the SARS coronavirus during the course of the SARS epidemic in China. *Science* 2004;**303**:1666–9.
29. Chiu WK, Cheung PC, Ng KL, et al. Severe acute respiratory syndrome in children: experience in a regional hospital in Hong Kong. *Pediatr Crit Care Med* 2003;**4**:279–83.
30. Chiu SS, Chan KH, Chu KW, et al. Human coronavirus NL63 infection and other coronavirus infections in children hospitalized with acute respiratory disease in Hong Kong, China. *Clin Infect Dis* 2005;**40**:1721–9.
31. Choi EH, Lee HJ, Kim SJ, et al. The association of newly identified respiratory viruses with lower respiratory tract infections in Korean children, 2000-2005. *Clin Infect Dis* 2006;**43**:585–92.
32. Chonmaitree T, Revai K, Grady JJ, et al. Viral upper respiratory tract infection and otitis media complication in young children. *Clin Infect Dis* 2008;**46**:815–23.
33. Chow CB. Post-SARS infection control in the hospital and clinic. *Paediatr Respir Rev* 2004;**5**:289–95.

33a. Cotten M, Lam TT, Watson SJ, et al. Full-genome deep sequencing and phylogenetic analysis of novel human betacoronavirus. *Emerg Infect Dis* 2013;**19**:736–42B.

34. da Silva Filho LV, Zerbinati RM, Tateno AF, et al. The differential clinical impact of human coronavirus species in children with cystic fibrosis. *J Infect Dis* 2012;**206**:384–8.
35. Dare RK, Fry AM, Chittaganpitch M, et al. Human coronavirus infections in rural Thailand: a comprehensive study using real-time reverse-transcription polymerase chain reaction assays. *J Infect Dis* 2007;**196**:1321–8.

35a. de Groot RJ, Baker SC, Baric RS, et al. Middle East Respiratory Syndrome Coronavirus (MERS-CoV): Announcement of the Coronavirus Study Group. *J Virol* 2013;**87**:7790–2.

36. Dessau RB, Lisby G, Frederiksen JL. Coronaviruses in spinal fluid of patients with acute monosymptomatic optic neuritis. *Acta Neurol Scand* 1999;**100**:88–91.
37. Dijkman R, Jebbink MF, Gaunt E, et al. The dominance of human coronavirus OC43 and NL63 infections in infants. *J Clin Virol* 2012;**53**:135–9.
38. Dourmashkin RR, Davies HA, Smith H, Bird RG. Are coronavirus-like particles seen in diarrhoea stools really viruses? *Lancet* 1980;**2**: 971–2.
39. Drosten C, Gunther S, Preiser W, et al. Identification of a novel coronavirus in patients with severe acute respiratory syndrome. *N Engl J Med* 2003;**348**:1967–76.
40. Duckmanton L, Luan B, Devenish J, et al. Characterization of torovirus from human fecal specimens. *Virology* 1997;**239**: 158–68.
41. Duckmanton L, Tellier R, Richardson C, Petric M. Notice of retraction to "The novel hemagglutinin-esterase genes of human torovirus and Breda virus" [*Virus Res* 1999;64:137-49]. *Virus Res* 2001;**81**:167.
42. Ebihara T, Endo R, Ma X, et al. Detection of human coronavirus NL63 in young children with bronchiolitis. *J Med Virol* 2005; **75**:463–5.
43. El-Sahly HM, Atmar RL, Glezen WP, Greenberg SB. Spectrum of clinical illness in hospitalized patients with "common cold" virus infections. *Clin Infect Dis* 2000;**31**:96–100.

44. Enjuanes L, Dediego ML, Alvarez E, et al. Vaccines to prevent severe acute respiratory syndrome coronavirus–induced disease. *Virus Res* 2008;**133**:45–62.
45. Esper F, Ou Z, Huang YT. Human coronaviruses are uncommon in patients with gastrointestinal illness. *J Clin Virol* 2010;**48**: 131–3.
46. Esper F, Weibel C, Ferguson D, et al. Coronavirus HKU1 infection in the United States. *Emerg Infect Dis* 2006;**12**:775–9.
47. Esper F, Weibel C, Ferguson D, et al. Evidence of a novel human coronavirus that is associated with respiratory tract disease in infants and young children. *J Infect Dis* 2005;**191**:492–8.
48. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
49. Falsey AR, McCann RM, Hall WJ, et al. The "common cold" in frail older persons: impact of rhinovirus and coronavirus in a senior daycare center. *J Am Geriatr Soc* 1997;**45**:706–11.
50. Folz RJ, Elkordy MA. Coronavirus pneumonia following autologous bone marrow transplantation for breast cancer. *Chest* 1999; **115**:901–5.
51. Fong NC, Kwan YW, Hui YW, et al. Adolescent twin sisters with severe acute respiratory syndrome (SARS). *Pediatrics* 2004;**113**: e146–9.
52. Fouchier RA, Hartwig NG, Bestebroer TM, et al. A previously undescribed coronavirus associated with respiratory disease in humans. *Proc Natl Acad Sci U S A* 2004;**101**:6212–16.
53. Freymuth F, Vabret A, Rozenberg F, et al. Replication of respiratory viruses, particularly influenza virus, rhinovirus, and coronavirus in HuH7 hepatocarcinoma cell line. *J Med Virol* 2005;**77**: 295–301.
54. Gagneur A, Dirson E, Audebert S, et al. Materno-fetal transmission of human coronaviruses: a prospective pilot study. *Eur J Clin Microbiol Infect Dis* 2008;**27**:863–6.
55. Gagneur A, Sizun J, Vallet S, et al. Coronavirus-related nosocomial viral respiratory infections in a neonatal and paediatric intensive care unit: a prospective study. *J Hosp Infect* 2002;**51**:59–64.
56. Gagneur A, Vallet S, Talbot PJ, et al. Outbreaks of human coronavirus in a pediatric and neonatal intensive care unit. *Eur J Pediatr* 2008;**167**:1427–34.
57. Gerna G, Campanini G, Rovida F, et al. Genetic variability of human coronavirus OC43-, 229E-, and NL63-like strains and their association with lower respiratory tract infections of hospitalized infants and immunocompromised patients. *J Med Virol* 2006;**78**:938–49.
58. Gerna G, Passarani N, Battaglia M, Rondanelli EG. Human enteric coronaviruses: antigenic relatedness to human coronavirus OC43 and possible etiologic role in viral gastroenteritis. *J Infect Dis* 1985;**151**:796–803.
59. Gill EP, Dominguez EA, Greenberg SB, et al. Development and application of an enzyme immunoassay for coronavirus OC43 antibody in acute respiratory illness. *J Clin Microbiol* 1994;**32**: 2372–6.
60. Graat JM, Schouten EG, Heijnen ML, et al. A prospective, community-based study on virologic assessment among elderly people with and without symptoms of acute respiratory infection. *J Clin Epidemiol* 2003;**56**:1218–23.
61. Guan Y, Zheng BJ, He YQ, et al. Isolation and characterization of viruses related to the SARS coronavirus from animals in southern China. *Science* 2003;**302**:276–8.
62. Hamre D, Beem M. Virologic studies of acute respiratory disease in young adults. V. Coronavirus 229E infections during six years of surveillance. *Am J Epidemiol* 1972;**96**:94–106.
63. Hamre D, Kindig DA, Mann J. Growth and intracellular development of a new respiratory virus. *J Virol* 1967;**1**:810–16.
64. Hamre D, Procknow JJ. A new virus isolated from the human respiratory tract. *Proc Soc Exp Biol Med* 1966;**121**:190–3.
65. Hasony HJ, Macnaughton MR. Prevalence of human coronavirus antibody in the population of southern Iraq. *J Med Virol* 1982;**9**: 209–16.
66. Hendley JO, Fishburne HB, Gwaltney JM. Coronavirus infections in working adults. Eight-year study with 229 E and OC 43. *Am Rev Respir Dis* 1972;**105**:805–11.
67. Herold J, Raabe T, Siddell S. Molecular analysis of the human coronavirus (strain 229E) genome. *Arch Virol Suppl* 1993;**7**: 63–74.
68. Hofmann H, Pyrc K, van der Hoek L, et al. Human coronavirus NL63 employs the severe acute respiratory syndrome coronavirus receptor for cellular entry. *Proc Natl Acad Sci U S A* 2005;**102**: 7988–93.
69. Hon CC, Lam TY, Shi ZL, et al. Evidence of the recombinant origin of a bat severe acute respiratory syndrome (SARS)–like coronavirus and its implications on the direct ancestor of SARS coronavirus. *J Virol* 2008;**82**:1819–26.
70. Isaacs D, Flowers D, Clarke JR, et al. Epidemiology of coronavirus respiratory infections. *Arch Dis Child* 1983;**58**:500–3.
71. Jartti T, Lehtinen P, Vuorinen T, et al. Respiratory picornaviruses and respiratory syncytial virus as causative agents of acute expiratory wheezing in children. *Emerg Infect Dis* 2004;**10**:1095–101.
72. Jennings LC, Anderson TP, Werno AM, et al. Viral etiology of acute respiratory tract infections in children presenting to hospital: role of polymerase chain reaction and demonstration of multiple infections. *Pediatr Infect Dis J* 2004;**23**:1003–7.
73. Johnston SL, Pattemore PK, Sanderson G, et al. Community study of role of viral infections in exacerbations of asthma in 9-11 year old children. *BMJ* 1995;**310**:1225–9.
74. Kan B, Wang M, Jing H, et al. Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus–like virus in palm civets at an animal market and on farms. *J Virol* 2005;**79**:11892–900.
75. Kapikian AZ, James HD, Kelly SJ, et al. Isolation from man of "avian infectious bronchitis virus–like" viruses (coronaviruses) similar to 229E virus, with some epidemiological observations. *J Infect Dis* 1969;**119**:282–90.
76. Kaye HS, Dowdle WR. Seroepidemiologic survey of coronavirus (strain 229E) infections in a population of children. *Am J Epidemiol* 1975;**101**:238–44.
77. Kaye HS, Marsh HB, Dowdle WR. Seroepidemiologic survey of coronavirus (strain OC 43) related infections in a children's population. *Am J Epidemiol* 1971;**94**:43–9.
78. Kidd AH, Esrey SA, Ujfalusi MJ. Shedding of coronavirus-like particles by children in Lesotho. *J Med Virol* 1989;**27**:164–9.
79. Kraaijeveld CA, Reed SE, Macnaughton MR. Enzyme-linked immunosorbent assay for detection of antibody in volunteers experimentally infected with human coronavirus strain 229 E. *J Clin Microbiol* 1980;**12**:493–7.
80. Kroneman A, Cornelissen LA, Horzinek MC, et al. Identification and characterization of a porcine torovirus. *J Virol* 1998;**72**: 3507–11.
81. Ksiazek TG, Erdman D, Goldsmith CS, et al. A novel coronavirus associated with severe acute respiratory syndrome. *N Engl J Med* 2003;**348**:1953–66.
82. Kusel MM, de Klerk NH, Holt PG, et al. Role of respiratory viruses in acute upper and lower respiratory tract illness in the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2006;**25**: 680–6.
83. Kuypers J, Martin ET, Heugel J, et al. Clinical disease in children associated with newly described coronavirus subtypes. *Pediatrics* 2007;**119**:e70–6.
84. Lai MM, Holmes KV. Coronaviridae: the viruses and their replication. In: Knipe D, Howley PM, editors. *Fields virology*. 5th ed. Philadelphia: Lippincott-Raven; 2007.
85. Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus–like virus in Chinese horseshoe bats. *Proc Natl Acad Sci U S A* 2005;**102**:14040–5.
86. Lau SK, Woo PC, Yip CC, et al. Coronavirus HKU1 and other coronavirus infections in Hong Kong. *J Clin Microbiol* 2006;**44**: 2063–71.
87. Lau YL, Peiris JS. Pathogenesis of severe acute respiratory syndrome. *Curr Opin Immunol* 2005;**17**:404–10.
88. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2003;**348**: 1986–94.
89. Leechanachai P, Yoosook C, Matangkasombut P. Epidemiological study of enteric coronavirus excretion by an enzyme-linked immunosorbent assay. *J Med Assoc Thai* 1989;**72**:452–7.
90. Leechanachai P, Yoosook C, Saguanwongse S, Matangkasombut P. Comparison of a modified enzyme-linked immunosorbent assay with immunosorbent electron microscopy to detect coronavirus in human faecal specimens. *J Diarrhoeal Dis Res* 1987;**5**: 24–9.

91. Legg JP, Warner JA, Johnston SL, Warner JO. Frequency of detection of picornaviruses and seven other respiratory pathogens in infants. *Pediatr Infect Dis J* 2005;**24**:611–16.
92. Leung CW, Kwan YW, Ko PW, et al. Severe acute respiratory syndrome among children. *Pediatrics* 2004;**113**:e535–43.
93. Li W, Moore MJ, Vasilieva N, et al. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003;**426**:450–4.
94. Li W, Shi Z, Yu M, et al. Bats are natural reservoirs of SARS-like coronaviruses. *Science* 2005;**310**:676–9.
95. Luby JP, Clinton R, Kurtz S. Adaptation of human enteric coronavirus to growth in cell lines. *J Clin Virol* 1999;**12**:43–51.
96. Macnaughton MR, Davies HA. Human enteric coronaviruses. Brief review. *Arch Virol* 1981;**70**:301–13.
97. Macnaughton MR, Flowers D, Isaacs D. Diagnosis of human coronavirus infections in children using enzyme-linked immunosorbent assay. *J Med Virol* 1983;**11**:319–25.
98. Makela MJ, Puhakka T, Ruuskanen O, et al. Viruses and bacteria in the etiology of the common cold. *J Clin Microbiol* 1998;**36**: 539–42.
99. Marshall JA, Birch CJ, Williamson HG, et al. Coronavirus-like particles and other agents in the faeces of children in Efate, Vanuatu. *J Trop Med Hyg* 1982;**85**:213–15.
100. Mathan M, Mathan VI, Swaminathan SP, Yesudoss S. Pleomorphic virus-like particles in human faeces. *Lancet* 1975;**1**:1068–9.
101. McIntosh K. Coronaviruses: a comparative review. *Curr Top Microbiol Immunol* 1974;**63**:83–129.
102. McIntosh K. Proving etiologic relationships to disease: the particular problem of human coronaviruses. *Pediatr Infect Dis J* 2012; **31**:241–2.
103. McIntosh K, Becker WB, Chanock RM. Growth in suckling-mouse brain of "IBV-like" viruses from patients with upper respiratory tract disease. *Proc Natl Acad Sci U S A* 1967;**58**:2268–73.
104. McIntosh K, Chao RK, Krause HE, et al. Coronavirus infection in acute lower respiratory tract disease of infants. *J Infect Dis* 1974;**130**:502–7.
105. McIntosh K, Dees JH, Becker WB, et al. Recovery in tracheal organ cultures of novel viruses from patients with respiratory disease. *Proc Natl Acad Sci U S A* 1967;**57**:933–40.
106. McIntosh K, Ellis EF, Hoffman LS, et al. The association of viral and bacterial respiratory infections with exacerbations of wheezing in young asthmatic children. *J Pediatr* 1973;**82**:578–90.
107. McIntosh K, Kapikian AZ, Hardison KA, et al. Antigenic relationships among the coronaviruses of man and between human and animal coronaviruses. *J Immunol* 1969;**102**:1109–18.
108. McIntosh K, Kapikian AZ, Turner HC, et al. Seroepidemiologic studies of coronavirus infection in adults and children. *Am J Epidemiol* 1970;**91**:585–92.
109. McIntosh K, McQuillin J, Reed SE, Gardner PS. Diagnosis of human coronavirus infection by immunofluorescence: method and application to respiratory disease in hospitalized children. *J Med Virol* 1978;**2**:341–6.
110. Mertsola J, Ziegler T, Ruuskanen O, et al. Recurrent wheezy bronchitis and viral respiratory infections. *Arch Dis Child* 1991;**66**: 124–9.
111. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;**115**:2088–94.
112. Monto AS. Medical reviews. Coronaviruses. *Yale J Biol Med* 1974;**47**:234–51.
113. Monto AS, Lim SK. The Tecumseh study of respiratory illness. VI. Frequency of and relationship between outbreaks of coronavirus infection. *J Infect Dis* 1974;**129**:271–6.
114. Mortensen ML, Ray CG, Payne CM, et al. Coronaviruslike particles in human gastrointestinal disease. Epidemiologic, clinical, and laboratory observations. *Am J Dis Child* 1985;**139**:928–34.
115. Murray RS, Brown B, Brian D, Cabirac GF. Detection of coronavirus RNA and antigen in multiple sclerosis brain. *Ann Neurol* 1992;**31**:525–33.
116. Murray RS, Cai GY, Hoel K, et al. Coronavirus infects and causes demyelination in primate central nervous system. *Virology* 1992; **188**:274–84.
117. Myint S, Siddell S, Tyrrell D. Detection of human coronavirus 229E in nasal washings using RNA:RNA hybridisation. *J Med Virol* 1989;**29**:70–3.
118. Ng EK, Ng PC, Hon KL, et al. Serial analysis of the plasma concentration of SARS coronavirus RNA in pediatric patients with severe acute respiratory syndrome. *Clin Chem* 2003;**49**:2085–8.
119. Ng PC, Lam CW, Li AM, et al. Chemokine response in children with SARS. *Arch Dis Child* 2005;**90**:422–3.
120. Ng PC, Leung CW, Chiu WK, et al. SARS in newborns and children. *Biol Neonate* 2004;**85**:293–8.
121. Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. *BMJ* 1997;**315**:1060–4.
122. Olsen SJ, Chang HL, Cheung TY, et al. Transmission of the severe acute respiratory syndrome on aircraft. *N Engl J Med* 2003;**349**:2416–22.
123. Pasternak AO, Spaan WJ, Snijder EJ. Nidovirus transcription: how to make sense …? *J Gen Virol* 2006;**87**:1403–21.
124. Pattemore PK, Johnston SL, Bardin PG. Viruses as precipitants of asthma symptoms. I. Epidemiology. *Clin Exp Allergy* 1992;**22**: 325–36.
125. Payne CM, Ray CG, Borduin V, et al. An eight-year study of the viral agents of acute gastroenteritis in humans: ultrastructural observations and seasonal distribution with a major emphasis on coronavirus-like particles. *Diagn Microbiol Infect Dis* 1986;**5**: 39–54.
126. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. *Lancet* 2003;**361**:1319–25.
127. Pene F, Merlat A, Vabret A, et al. Coronavirus 229E–related pneumonia in immunocompromised patients. *Clin Infect Dis* 2003; **37**:929–32.
128. Perlman S, Dandekar AA. Immunopathogenesis of coronavirus infections: implications for SARS. *Nat Rev Immunol* 2005;**5**: 917–27.
129. Pitkaranta A, Virolainen A, Jero J, et al. Detection of rhinovirus, respiratory syncytial virus, and coronavirus infections in acute otitis media by reverse transcriptase polymerase chain reaction. *Pediatrics* 1998;**102**:291–5.
130. Pohl-Koppe A, Raabe T, Siddell SG, ter Meulen V. Detection of human coronavirus 229E-specific antibodies using recombinant fusion proteins. *J Virol Methods* 1995;**55**:175–83.
131. Poon LL, Chan KH, Wong OK, et al. Detection of SARS coronavirus in patients with severe acute respiratory syndrome by conventional and real-time quantitative reverse transcription–PCR assays. *Clin Chem* 2004;**50**:67–72.
132. Poutanen SM, Low DE, Henry B, et al. Identification of severe acute respiratory syndrome in Canada. *N Engl J Med* 2003;**348**: 1995–2005.
133. Prill MM, Iwane MK, Edwards KM, et al. Human coronavirus in young children hospitalized for acute respiratory illness and asymptomatic controls. *Pediatr Infect Dis J* 2011;**31**:235–40.
134. Puel JM, Orillac MS, Bauriaud RM, et al. Occurrence of viruses in human stools in the Ahaggar (Alberia). *J Hyg (Lond)* 1982;**89**: 171–4.
135. Pyrc K, Berkhout B, van der Hoek L. The novel human coronaviruses NL63 and HKU1. *J Virol* 2007;**81**:3051–7.

135a. Raj VS, Mou H, Smits SL, et al. Dipeptidyl peptidase 4 is a functional receptor for the emerging human coronavirus-EMC. *Nature* 2013;**495**:251–4.

136. Raymond F, Carbonneau J, Boucher N, et al. Comparison of automated microarray detection with real-time PCR assays for detection of respiratory viruses in specimens obtained from children. *J Clin Microbiol* 2009;**47**:743–50.
137. Reed SE. The behaviour of recent isolates of human respiratory coronavirus in vitro and in volunteers: evidence of heterogeneity among 229E-related strains. *J Med Virol* 1984;**13**:179–92.
138. Regamey N, Kaiser L, Roiha HL, et al. Viral etiology of acute respiratory infections with cough in infancy: a community-based birth cohort study. *Pediatr Infect Dis J* 2008;**27**:100–5.
139. Resta S, Luby JP, Rosenfeld CR, Siegel JD. Isolation and propagation of a human enteric coronavirus. *Science* 1985;**229**: 978–81.
140. Rettig PJ, Altshuler GP. Fatal gastroenteritis associated with coronaviruslike particles. *Am J Dis Child* 1985;**139**:245–8.
141. Riski H, Hovi T. Coronavirus infections of man associated with diseases other than the common cold. *J Med Virol* 1980;**6**: 259–65.

142. Risku M, Lappalainen S, Rasanen S, Vesikari T. Detection of human coronaviruses in children with acute gastroenteritis. *J Clin Virol* 2010;**48**:27–30.
143. Robertson CA, Lowther SA, Birch T, et al. SARS and pregnancy: a case report. *Emerg Infect Dis* 2004;**10**:345–8.
144. Schmidt OW, Cooney MK, Kenny GE. Plaque assay and improved yield of human coronaviruses in a human rhabdomyosarcoma cell line. *J Clin Microbiol* 1979;**9**:722–8.
145. Schnagl RD, Holmes IH, Mackay-Scollay EM. Coronavirus-like particles in Aboriginals and non-Aboriginals in Western Australia. *Med J Aust* 1978;**1**:307–9.
146. Shek CC, Ng PC, Fung GP, et al. Infants born to mothers with severe acute respiratory syndrome. *Pediatrics* 2003;**112**:e254.
147. Singleton RJ, Bulkow LR, Miernyk K, et al. Viral respiratory infections in hospitalized and community control children in Alaska. *J Med Virol* 2010;**82**:1282–90.
148. Sit SC, Yau EK, Lam YY, et al. A young infant with severe acute respiratory syndrome. *Pediatrics* 2003;**112**:e257.
149. Sitbon M. Human-enteric-coronaviruslike particles (CVLP) with different epidemiological characteristics. *J Med Virol* 1985;**16**: 67–76.
150. Sizun J, Soupre D, Giroux JD, et al. Nasal colonization with coronavirus and apnea of the premature newborn. *Acta Paediatr* 1993;**82**:238.
151. Sizun J, Soupre D, Legrand MC, et al. Neonatal nosocomial respiratory infection with coronavirus: a prospective study in a neonatal intensive care unit. *Acta Paediatr* 1995;**84**:617–20.
152. Sloots TP, McErlean P, Speicher DJ, et al. Evidence of human coronavirus HKU1 and human bocavirus in Australian children. *J Clin Virol* 2006;**35**:99–102.
153. Smits SL, Lavazza A, Matiz K, et al. Phylogenetic and evolutionary relationships among torovirus field variants: evidence for multiple intertypic recombination events. *J Virol* 2003;**77**:9567–77.
154. Sorensen O, Collins A, Flintoff W, et al. Probing for the human coronavirus OC43 in multiple sclerosis. *Neurology* 1986;**36**: 1604–6.
155. Stewart JN, Mounir S, Talbot PJ. Human coronavirus gene expression in the brains of multiple sclerosis patients. *Virology* 1992;**191**:502–5.
156. Sung JY, Lee HJ, Eun BW, et al. Role of human coronavirus NL63 in hospitalized children with croup. *Pediatr Infect Dis J* 2010;**29**:822–6.
157. Talbot HK, Shepherd BE, Crowe JE Jr, et al. The pediatric burden of human coronaviruses evaluated for twenty years. *Pediatr Infect Dis J* 2009;**28**:682–7.
158. Tanaka R, Iwasaki Y, Koprowski H. Intracisternal virus-like particles in brain of a multiple sclerosis patient. *J Neurol Sci* 1976;**28**:121–6.
159. Tsang KW, Ho PL, Ooi GC, et al. A cluster of cases of severe acute respiratory syndrome in Hong Kong. *N Engl J Med* 2003; **348**:1977–85.
160. Tyrrell DA, Almeida JD, Cunningham CH, et al. Coronaviridae. *Intervirology* 1975;**5**:76–82.
161. Tyrrell DAJ, Bynoe ML. Cultivation of a novel type of common-cold virus in organ cultures. *Br Med J* 1965;**1**:1467–70.
162. Uhlenhaut C, Cohen JI, Pavletic S, et al. Use of a novel virus detection assay to identify coronavirus HKU1 in the lungs of a hematopoietic stem cell transplant recipient with fatal pneumonia. *Transpl Infect Dis* 2012;**14**:79–85.
163. Vabret A, Dina J, Gouarin S, et al. Detection of the new human coronavirus HKU1: a report of 6 cases. *Clin Infect Dis* 2006;**42**: 634–9.
164. Vabret A, Mourez T, Dina J, et al. Human coronavirus NL63, France. *Emerg Infect Dis* 2005;**11**:1225–9.
165. van der Hoek L, Ihorst G, Sure K, et al. Burden of disease due to human coronavirus NL63 infections and periodicity of infection. *J Clin Virol* 2010;**48**:104–8.
166. van der Hoek L, Pyrc K, Jebbink MF, et al. Identification of a new human coronavirus. *Nat Med* 2004;**10**:368–73.
167. van der Hoek L, Sure K, Ihorst G, et al. Croup is associated with the novel coronavirus NL63. *PLoS Med* 2005;**2**:e240.
168. van der Zalm MM, Uiterwaal CS, Wilbrink B, et al. Respiratory pathogens in respiratory tract illnesses during the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2009;**28**:472–6.
169. van Gageldonk–Lafeber AB, Heijnen ML, Bartelds AI, et al. A case-control study of acute respiratory tract infection in general practice patients in The Netherlands. *Clin Infect Dis* 2005;**41**: 490–7.
170. Vaucher YE, Ray CG, Minnich LL, et al. Pleomorphic, enveloped, virus-like particles associated with gastrointestinal illness in neonates. *J Infect Dis* 1982;**145**:27–36.
171. Venter M, Lassauniere R, Kresfelder TL, et al. Contribution of common and recently described respiratory viruses to annual hospitalizations in children in South Africa. *J Med Virol* 2011; **83**:1458–68.
172. Vijgen L, Keyaerts E, Lemey P, et al. Circulation of genetically distinct contemporary human coronavirus OC43 strains. *Virology* 2005;**337**:85–92.
173. Vijgen L, Keyaerts E, Moes E, et al. Complete genomic sequence of human coronavirus OC43: molecular clock analysis suggests a relatively recent zoonotic coronavirus transmission event. *J Virol* 2005;**79**:1595–604.
174. Walsh EE, Falsey AR, Hennessey PA. Respiratory syncytial and other virus infections in persons with chronic cardiopulmonary disease. *Am J Respir Crit Care Med* 1999;**160**:791–5.
175. Weiss M, Steck F, Horzinek MC. Purification and partial characterization of a new enveloped RNA virus (Berne virus). *J Gen Virol* 1983;**64**:1849–58.
176. Wong SF, Chow KM, de Swiet M. Severe acute respiratory syndrome and pregnancy. *BJOG* 2003;**110**:641–2.
177. Wong SF, Chow KM, Leung TN, et al. Pregnancy and perinatal outcomes of women with severe acute respiratory syndrome. *Am J Obstet Gynecol* 2004;**191**:292–7.
178. Woo PC, Huang Y, Lau SK, Yuen KY. Coronavirus genomics and bioinformatics analysis. *Viruses* 2010;**2**:1804–20.
179. Woo PC, Lau SK, Chu CM, et al. Characterization and complete genome sequence of a novel coronavirus, coronavirus HKU1, from patients with pneumonia. *J Virol* 2005;**79**:884–95.
180. Woo PC, Lau SK, Li KS, et al. Molecular diversity of coronaviruses in bats. *Virology* 2006;**351**:180–7.
181. Woo PC, Lau SK, Tsoi HW, et al. Clinical and molecular epidemiological features of coronavirus HKU1-associated community-acquired pneumonia. *J Infect Dis* 2005;**192**:1898–907.
182. Woode GN, Reed DE, Runnels PL, et al. Studies with an unclassified virus isolated from diarrheic calves. *Vet Microbiol* 1982; **7**:221–40.
183. World Health Organization. *Case definitions for surveillance of severe acute respiratory syndrome (SARS)*. 2003. Available at http://www.who.int/csr/sars/casedefinition/en/.
184. World Health Organization. *Consensus document on the epidemiology of severe acute respiratory syndrome (SARS)*. 2003. Available at http://www.who.int/csr/sars/en/WHOconsensus.pdf. Accessed September 14, 2006.
184a. World Health Organization. *Interim surveillance recommendations for human infection with Middle East respiratory syndrome coronavirus* http://www.who.int/csr/disease/coronavirus_infections/InterimRevisedSurveillanceRecommendations_nCoVinfection_27Jun13.pdf. Accessed August 12, 2013.
185. World Health Organization. *Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003*. 2004. Available at http://www.who.int/csr/sars/country/table2004_04_21/en/index.html.
186. Yeager CL, Ashmun RA, Williams RK, et al. Human aminopeptidase N is a receptor for human coronavirus 229E. *Nature* 1992; **357**:420–2.
187. Yeh EA, Collins A, Cohen ME, et al. Detection of coronavirus in the central nervous system of a child with acute disseminated encephalomyelitis. *Pediatrics* 2004;**113**:e73–6.
188. Yu IT, Li Y, Wong TW, et al. Evidence of airborne transmission of the severe acute respiratory syndrome virus. *N Engl J Med* 2004;**350**:1731–9.
188a. Zaki AM, van Boheemen S, Bestebroer TM, et al. Isolation of a novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012;**367**:1814–20.
189. Zhang JP, Wang YH, Chen LN, et al. Clinical analysis of pregnancy in second and third trimesters complicated severe acute respiratory syndrome [in Chinese]. *Zhonghua Fu Chan Ke Za Zhi* 2003;**38**:516–20.
190. Zhang XM, Herbst W, Kousoulas KG, Storz J. Biological and genetic characterization of a hemagglutinating coronavirus isolated from a diarrhoeic child. *J Med Virol* 1994;**44**:152–61.
191. Zhao Z, Zhang F, Xu M, et al. Description and clinical treatment of an early outbreak of severe acute respiratory syndrome (SARS) in Guangzhou, PR China. *J Med Microbiol* 2003;**52**:715–20.

SUBSECTION 11
BUNYAVIRIDAE

CHAPTER 188

Hantaviruses

Louisa E. Chapman • C. J. Peters • James N. Mills • Kelly T. McKee, Jr.

HISTORICAL PERSPECTIVE

Hantaviruses are the etiologic agents of a diverse group of rodent-borne hemorrhagic fevers that are responsible for considerable morbidity and mortality worldwide. Although recognized by so-called modern medicine only relatively recently, clinical syndromes now known to be associated with these viruses have been described by traditional practitioners across the globe from antiquity.[31,125,150] Soviet scientists documented sporadic outbreaks of febrile renal failure with hemorrhage in the eastern Soviet Union between 1913 and 1930,[22] and Japanese and Soviet scientists recognized annual outbreaks of a similar syndrome in Manchuria and Siberia between 1932 and 1935.[48,149,203,220]

In 1934, Swedish scientists described a novel disorder, characterized by fever, abdominal and back pain, and renal abnormalities.[158,247] Epidemic disease with these features occurred among German and Finnish troops stationed in Lapland during World War II.[92,211] Physicians called this syndrome *nephropathia epidemica*.

North American medical practitioners initially became acquainted with a similar syndrome in the early 1950s when a previously unrecognized febrile illness characterized by shock, hemorrhage, and renal failure developed in thousands of soldiers serving with United Nation forces during the Korean War. Physicians called this syndrome, which had a case-fatality rate of 5 to 15 percent, *epidemic hemorrhagic fever*.[50,151]

In 1953, Gajdusek[60] noted similarities among epidemic hemorrhagic fever, the severe and frequently fatal Far Eastern diseases described by Soviet and Japanese scientists, and the Scandinavian disorder and proposed a common origin. Subsequent validation of this hypothesis (see The Organism) ultimately prompted adoption of the collective term *hemorrhagic fever with renal syndrome* (HFRS) to describe this clinical entity characterized by varying degrees of renal dysfunction and hemorrhage with fever.

In June 1993, investigation of a cluster of unexplained respiratory deaths in previously healthy young adult residents of rural areas in the southwestern United States led to recognition of a "new" febrile disorder in North America. Application of sophisticated serologic and virologic tools to diagnostic specimens from patients with this disease quickly pointed to a hantavirus related to, but distinct from, those causing HFRS.[117,160] This disorder, now called *hantavirus pulmonary syndrome* (HPS) or *hantavirus cardiopulmonary syndrome* (HCPS), proved to be different clinically from classic HFRS; however, as virologic and epidemiologic studies of the etiologic agent and its reservoirs were completed, similarities to other hantaviruses became apparent. Subsequent studies searching for hantaviruses associated with febrile pulmonary rather than renal disease established HPS as an endemic panhemispheric disease and identified a large number of unique hantaviruses associated with rodents indigenous to the Americas, not all of which have been associated with human disease.[143] Andes virus, discovered in association with an outbreak in southwestern Argentina in 1995, is the only hantavirus to have been associated with human-to-human transmission (Fig. 188-1).[91,146,236]

THE ORGANISM

Classification and Antigenic Composition

The genus *Hantavirus* of the family *Bunyaviridae* was defined in 1985.[191-194] Members of the genus *Hantavirus* are classified among the bunyaviruses because of their shared morphologic, physicochemical, and molecular properties. Like other members of the *Bunyaviridae*, hantaviruses are negative-stranded, lipid-enveloped RNA viruses with tripartite genomes; genomic segments are designated as large (L, approximately 6500 nucleotides, encodes the virus L protein, an RNA-dependent RNA polymerase), medium (M, approximately 3600 nucleotides long, encodes the envelope glycoprotein precursor [GPC] which is processed into two transmembrane glycoproteins mature Gn and Gc glycoproteins), and small

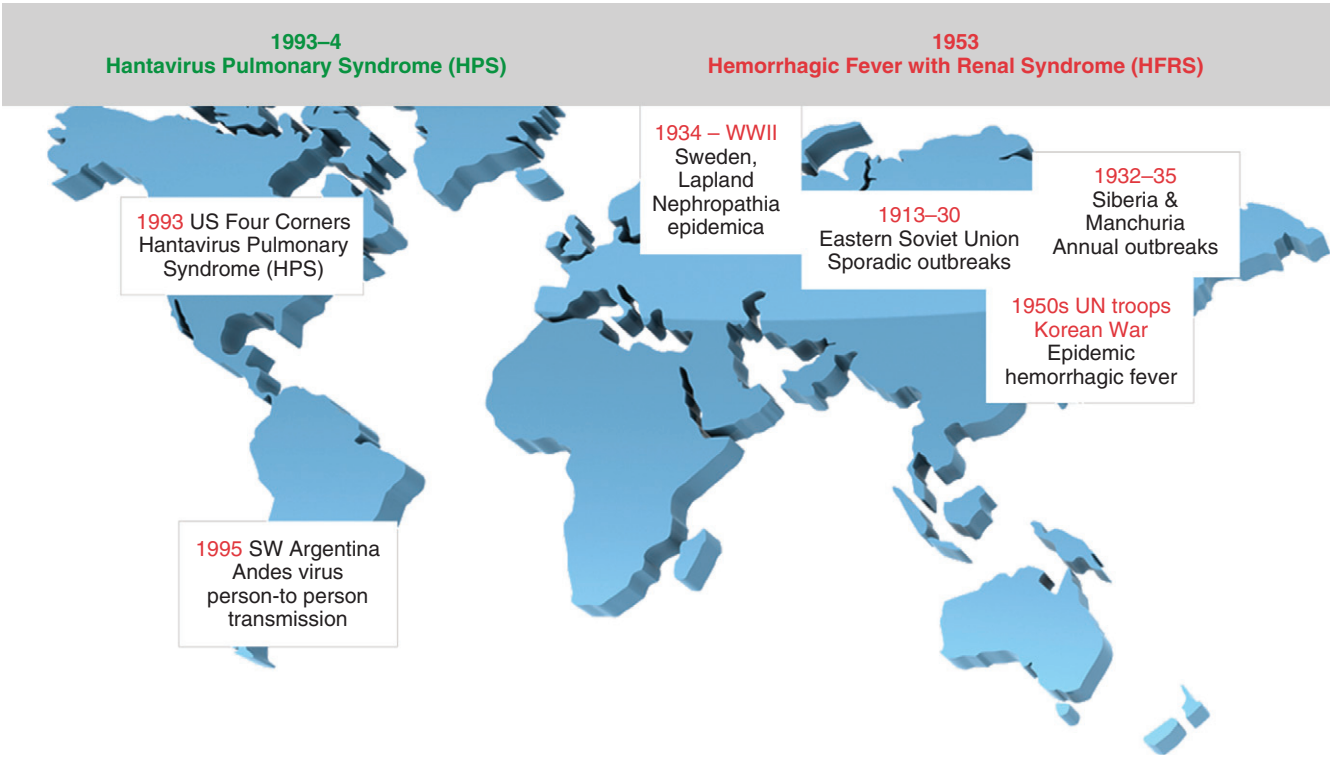

FIGURE 188-1 ■ Map demonstrating the temporal and geographic recognition of human diseases associated with hantavirus infections. (See companion Expert Consult website for color version.)

(S, 1600 to 2004 nucleotides long, encodes an RNA-binding nucleocapsid protein, the N protein).[89,143,195]

Also like other bunyaviruses, hantaviruses display Golgi-associated morphogenesis and usually acquire their envelopes by budding into intracytoplasmic vacuoles.[194] However, they are serologically distinct from other family members and possess unique terminal genomic sequences.[193,195] The genus *Hantavirus* contains the only members of *Bunyaviridae* that lack arthropod vectors. As a general rule, each hantavirus is highly adapted to a specific rodent species and depends on persistent inapparent infection in wild rodents for maintenance in nature.

Physical Properties

Morphologically, hantaviruses are spherical, 70 to 210 nm in diameter, and have a surface glycoprotein complex consisting of Gn tetramers interconnected with GC dimers embedded in a lipid bilayer envelope.[82,143] Elongated particles (110 to 120 nm long) often are observed, and they display a characteristic gridlike pattern on their surfaces.[72,191,194]

Integrins have been shown to serve as host cell surface receptors for hantaviruses, with β3 integrin implicated as a receptor of pathogenic hantaviruses and β1 integrin as a receptor for nonpathogenic hantaviruses.[143] The viral surface glycoproteins (Gn and Gc) mediate virus attachment to cells. After virion uptake, the fusion of the virus and endosomal membranes resulting in release of the virus ribonucleocapsid (RNP) cores into the cell cytoplasm is dependent on low pH and mediated by Gc glycoproteins. The virus RNP core particles contain S, M, and L RNA segments.[143]

The virion associated L polymerase transcribes negative-sense RNA segments to produce functional virus mRNAs (N protein, L polymerase protein, and the glycoprotein precursor or GPC) and plays roles in viral replication, translation, and viral assembly.[89] L functions as an RNA transcriptase, replicase, and endonuclease, which are functions fundamental for virus mRNA transcription, viral replication, and acquisition of 5′ end capped primers from host mRNAs.[143] The GPC mRNA is translated by membrane-bound ribosomes. The N and L mRNAs are translated by free ribosomes.[143] The N protein, a structural component of the viral nucleocapsid plus genomic viral RNA segments, is an RNA-binding protein that engages the hairpin termini of the genomic segments with high affinity, helping to keep viral replication a closed process within the cytoplasm.[89] The multifunctional N protein protects the virus genomic RNA from nuclease degradation by limiting access of RNA to host nucleases and is involved in encapsidation of virus genomic (negative sense) and antigenomic (positive sense) RNA, but not viral mRNA. The N protein plays an active role in virus RNA transcription, interacts with ribosomal protein RPS19 to facilitate the loading of the 40S ribosomal subunit onto the virus mRNA, and plays an active role in virus assembly.[143] N protein activities are linked to fundamental requirements of viral replication but also to interference with intracellular processes of the host cell, including inflammatory processes.[89,111]

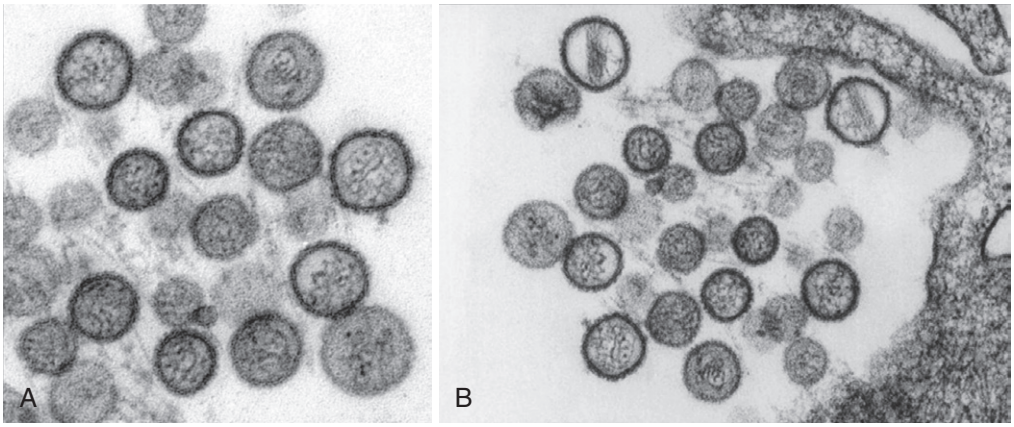

FIGURE 188-2 ■ Transmission electron micrographs reveal the ultrastructural appearance of a number of virus particles, or virions, of a hantavirus known as the Sin Nombre virus (SNV). SNV is the hantavirus most commonly responsible for HPS in the United States. In November 1993, the specific hantavirus that caused the Four Corners outbreak was isolated. Using tissue from a deer mouse that had been trapped near the New Mexico home of a person who had gotten the disease, the Special Pathogens Branch at CDC grew the virus in the laboratory. Shortly afterward, and independently, the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID) also grew the virus from a person in New Mexico who had gotten the disease as well as from a mouse trapped in California. (Courtesy Centers for Disease Control [CDC]/Cynthia Goldsmith, Luanne H. Elliott, Brian W.J. Mahy and the CDC Viral Special Pathogens Branch.)

Hantavirus assembly involves the RNP core (containing the vRNA and N and L proteins) and the acquired host cell membrane containing the embedded virus Gn and Gc glycoproteins. Direct interaction of the virus Gn or its cytoplasmic tail with N protein and RNA has been reported for the Old World hantaviruses. The cytoplasmic tail of the virus Gn is thought to replace the function of a matrix protein encoded by other viruses in facilitating virus assembly and budding.[143] Virus glycoproteins are believed to determine the site of virus assembly and budding. The Gn and Gc proteins of New World hantaviruses accumulate in the Golgi but eventually can also be detected at the cell surface, identifying two sites of New World hantaviral maturation.[143] During infection in polarized epithelial cells Black Creek Canal virus, a New World pathogenic hantavirus, has been shown to be released from the apical membrane.[181] In contrast, Andes virus infection of primary hamster epithelium resulted in both apical and basolateral release of virus (Fig. 188-2).[185]

Susceptibility

That hantavirus transmission is possible through indirect contact with contaminated environmental surfaces was demonstrated through a study in which Puumala virus excreted by experimentally infected bank voles into bedding maintained infectivity to recipient voles at room temperature for 12 to 15 days.[100] Knowledge about the survival time in the environment in the absence of disinfection is somewhat limited, but survival appears to be temperature dependent. Studies have shown persistent infectivity in dried cell culture medium for as long as 2 days and in neutral solutions for several hours at 37° C or several days at lower temperatures.[24] Infectious Hantaan virus was detected after 96 days under "wet" conditions at 4° C, and it remained viable for 2 minutes in 30 percent ethanol.[78] Cell culture supernatants of Puumala and Tula hantaviruses remained infectious for 5 to 11 days at room temperature and for as long as 18 days at 4° C but were inactivated after 24 hours at 37° C.[191]

However, hantaviruses are inactivated readily by lipid solvents and most disinfectants, including dilute hypochlorite solutions, detergents, ethyl alcohol (70%), most general-purpose household disinfectants, and β-propiolactone (0.1% at 4° C for 3 days).[73,113,180] Limited studies with Hantaan virus have shown sensitivity to a pH of 5 or less and to temperatures of 56° C or higher.[73]

Laboratory Propagation and Tissue Culture Growth

Hantaviruses are fastidious but can be grown in culture through serial blind passage. They routinely establish persistent, noncytolytic infections and generally do not replicate to high titer.[73,194] The prototype member of the group, Hantaan virus, was isolated in 1978[130] and propagated successfully in cell culture in the A-549 cell line in 1981.[58] However, Vero E-6 cells subsequently were found to be a better cell culture system for this and other hantaviruses.[73] Hantaviruses have been isolated and propagated successfully through direct inoculation of homogenates of infected tissue from wild-caught rodent hosts onto cell lines or after amplification through serially infected, colonized rodent hosts.* Recovery of hantaviruses from human specimens has been difficult and infrequent. More than 25 hantaviruses currently are recognized as causing human disease, although many of them have not been fully characterized, and the reservoirs of a few have not been clearly determined. Some hantaviruses have been isolated in cell culture, but many are known only from genetic material amplified from human or

*References 51, 58, 70, 73, 130, 162, 189, 241.

rodent tissue. The major named genotypes for known pathogenic hantaviruses and many of those that are not known to cause disease are listed in Table 188-1. This list will continue to be expanded and modified as our knowledge of classification and new viruses increases. Laboratory infection of persons working with cell culture–adapted Hantaan virus has occurred occasionally, but laboratory transmission of hantaviruses from infected rodents to humans is a particularly common occurrence.* Consequently, attempts to propagate the virus should be performed only when using biosafety level 3 or 4 facilities and practices.[24,28]

*References 42, 47, 102, 108, 126, 127, 138, 222.

TABLE 188-1 **Named Hantavirus Genotypes**

Virus Strain	Principal Reservoir	Known Distribution of Virus	Disease Association*
Old World Rodent-Borne			
Hantaan	*Apodemus agrarius manchuricus*	Far East Northern Asia	HFRS (severe)
Seoul	*Rattus norvegicus*	Worldwide	HFRS (mild/moderate)
Dobrava	*Apodemus flavicollis*	Balkans	HFRS (severe)
Puumala	*Myodes (Clethrionomys) glareolus*	Scandinavia, Northern Europe, Balkans	HFRS (mild; nepropathia epidemica)
Tula	*Microtus arvalis/Microtus levis*	Western Russia, eastern Europe	HFRS
Saaremaa	*Apodemus agrarius agrarius*	Europe	HFRS (mild)
Amur	*Apodemus peninsulae*	Far Eastern Russia, Korea, China	HFRS
Muju	*Myodes regulus*	South Korea	HFRS
Thailand	*Bandicota indica*	Thailand	HFRS
Sangassou	*Hylomyscus alleni*	West Africa	None recognized
Hokkaido	*Myodes rufocanus*	Japan	None recognized
Khabarovsk	*Microtus* spp.	Far eastern Russia, China	None recognized
Vladivostok	*Microtus fortis*	Far eastern Russia, China	None recognized
Topografov	*Lemmus sibiricus*	Siberia	None recognized
New World Rodent-Borne			
Sin Nombre	*Peromyscus maniculatus*	North America	HPS
New York	*Peromyscus leucopus*	Eastern USA	HPS
Monongahela	*Peromyscus maniculatus nubiterrae*	Eastern USA	HPS
Black Creek Canal	*Sigmodon hispidus spadicipygus*	Florida	HPS
Bayou	*Oryzomys palustris*	Southeastern USA	HPS
Andes	*Oligoryzomys longicaudatus*	Southern Chile, Argentina	HPS
Oran	*Oligoryzomys* chacoensis	Northern Argentina	HPS
Laguna Negra	*Calomys laucha*	Central South America	HPS
Choclo	*Oligoryzomys fulvescens costaricensis*	Panama	HPS
Juquitiba	*Oligoryzomys nigripes*	Southeastern Brazil	HPS
Araraquara	*Necromys lasiurus*	Southeastern Brazil	HPS
Castelo dos Sonhos	Unknown	Northern Brazil	HPS
Lechiguanas	*Oligoryzomys flavescens*†	Central Argentina, Southwest Uruguay	HPS
Central Plata	*Oligoryzomys flavescens*†	Southern Uruguay	HPS
Hu39694	*Oligoryzomys flavescens*†	Central Argentina	
Bermejo	*Oligoryzomys chacoensis*	Northwestern Argentina, Central Brazil	HPS
Anajatuba	*Oligoryzomys fornesi*	Northeastern Brazil	HPS
Alto Paraguay	*Holochilus chacarius*	Northwestern Paraguay	None recognized
Calabazo	*Zygodontomys brevicauda*	Southwestern Panama	None recognized
Muleshoe	*Sigmodon hispidus texianus*	Texas, Louisiana, USA	None recognized
Caño Delgadito	*Sigmodon alstoni*	Venezuela	None recognized
Prospect Hill	*Microtus pennsylvanicus*	USA	None recognized
Bloodland Lake	*Microtus ochrogaster*	Central USA and Canada	None recognized
Isla Vista	*Microtus californicus*	Western North America	None recognized
Limestone Canyon	*Peromyscus boylii*	Southwestern USA	None recognized
El Moro Canyon	*Reithrodontomys megalotis*	Western USA, Mexico	None recognized
Río Segundo	*Reithrodontomys mexicanus*	Costa Rica, Panama	None recognized

Continued on following page

TABLE 188-1 **Named Hantavirus Genotypes** (Continued)

Virus Strain	Principal Reservoir	Known Distribution of Virus	Disease Association*
Pergamino	*Akodon azarae*	Central Argentina	None recognized
Ape Aime	*Akodon montensis*	Eastern Paraguay	None recognized
Jaborá	*Akodon montensis*	Southern Brazil, Paraguay	None recognized
Maciel	*Necromys benefactus*	Central Argentina	None recognized
Alto Paraguay	*Holochilus chacarius*	Northwestern Paraguay	None recognized
Río Mearim	*Holochilus sciureus*	Northeastern Brazil	None recognized
Catacamas	*Oryzomys couesi couesi*	Eastern Honduras	None recognized
Playa de Oro	*Oryzomys couesi mexicanus*	Colima, Mexico	None recognized
Río Mamoré	*Oligoryzomys microtis*	Bolivia, Peru	None recognized
Maporal	*Oligoryzomys fulvescens*	Western Venezuela	None recognized
Itapua	*Oligoryzomys nigripes*	Eastern Paraguay	None recognized
Insectivore-Borne			
Thottapalayam	*Suncus murinus* (insectivore)	India	None recognized
Tanganya	*Crocidura theresae*	Guinea	None recognized
Camp Ripley	*Blarina brevicauda*	Minnesota, USA	None recognized
Cao Bang	*Anourosorex squamipes*	Vietnam	None recognized
Ash River	*Sorex cinereus*	Minnesota, USA	None recognized
Jemez Springs	*Sorex monticolus*	New Mexico, USA	None recognized
Seewis	*Sorex araneus*	Switzerland	None recognized
Asama	*Urotrichus talpoides*	Japan	None recognized

*HFRS, hemorrhagic fever with renal syndrome; HPS, hantavirus pulmonary syndrome.
†The taxonomy of the genus *Oligoryzomys* in general and *O. flavescens* in particular is still unresolved. The uniqueness of the viruses these rodents carry is also debated.
Data from references 154A, 164, and 168.

Transmission

Hantaviruses are zoonotic; with few exceptions, each hantavirus is naturally associated with a distinct rodent species or subspecies in which it establishes a chronic, inapparent infection (see Table 188-1). Hantavirus infection of rodents produces brief viremia that results in dissemination of virus to the lungs, salivary glands, and kidneys. The infected rodent sheds virus in urine, saliva, and (to a lesser extent) feces throughout its life, despite the development of neutralizing antibodies.[128] In contrast to early laboratory data from several hantaviruses that infection does not adversely affect host longevity or reproduction, field evidence is beginning to accumulate that deer mice infected with Sin Nombre virus have reduced survivorship in nature.[2,45,46,140] Vertical transmission has not been demonstrated, and pups born to infected dams appear to be protected by maternal antibody.[44,101,156] Enzootic infection appears to be maintained by exposure to nesting materials contaminated with infectious secretions, by grooming, and by intraspecies biting.[46,127,128]

Humans typically become infected with hantaviruses after contact with saliva or excreta from infected rodents (or material contaminated by these) by inhalation of small-particle aerosols, by direct contact of infectious materials with broken skin or mucous membranes, or by percutaneous inoculation of infectious materials (e.g., rodent bite). The primacy of respiratory droplets or airborne particles as the mode of transmission to humans is supported by evidence from outbreaks of disease among laboratory workers and sporadic cases resulting from brief exposure to rodent-infested habitats.

The risk of human hantavirus infection is a function of the density of the local rodent reservoir population, the prevalence of infection among rodents, and the frequency of activities that result in contact between humans and rodents or rodent excreta. Individual hantavirus infections usually occur when humans disturb rodent habitats or rodents enter human housing.[246] The risk for contracting disease after rodent exposure remains unquantified; however, one serosurvey suggested that persons in North America whose occupation entails close physical contact with rodents have a low risk for acquiring hantavirus infection.[59] Epidemics generally are associated with changes in the behavior of human populations that result in large-scale exposure of persons to rodent-infested areas (e.g., military maneuvers, agricultural or forestry activities) or environmental changes that result in rapid increases in the density of rodent populations (e.g., increased abundance of food items).[155,242]

Person-to-person transmission of hantaviruses associated with HFRS has not been demonstrated. The experience with HPS has been similar in North and Central America.[234] However, interpersonal spread clearly occurred during a 1996 outbreak in southern Argentina.[54,236] Subsequent independent investigations support this observation by having identified clusters in Argentina and Chile that suggest person-to-person transmission associated with Andes virus.[121,147,232] Interpersonal transmission thus appears to be an infrequent but clear feature peculiar to Andes virus.[29,54,147]

EPIDEMIOLOGY

Geographic Distribution

Hantaviruses have been found on every continent except Antarctica. All rodent-borne hantaviruses are associated with three subfamilies of rodents in the large superfamily Muroidea. These are the Old World rats and mice (subfamily Murinae), the New World rats and mice (subfamilies Sigmodontinae and Neotominae), and the voles and lemmings (subfamily Arvicolinae). With the exception of HFRS caused by Seoul virus, hantavirus diseases occur almost exclusively in rural areas. Hantaan virus, the cause of classic HFRS, is carried by the striped field mouse *Apodemus agrarius manchuricus*. This rodent is distributed across eastern Russia, China, and the Korean peninsula.[239] The principal reservoir for Puumala virus, the etiologic agent of a milder HFRS variant found in Scandinavia, northern Europe, and Russia west of the Ural Mountains, is the bank vole, *Myodes glareolus*.[130,197]

In Europe, at least four viruses have been linked etiologically with HFRS: Puumala, Tula, Saaremaa, and Dobrava; Tula virus is associated with voles (*Microtus* spp.); Saaremaa virus is hosted by a subspecies of the striped field mouse *(Apodemus agrarius agrarius);* Dobrava virus is associated with *Apodemus flavicollis*, the yellow-necked field mouse.* *Rattus* spp. (primarily *Rattus norvegicus*) serve as reservoirs for the Seoul-like viruses identified worldwide; human infections have been seen most frequently in eastern Asia. Outbreaks of severe and occasionally fatal HFRS among animal handlers and laboratory scientists have been caused by inapparent infection of laboratory rats with Seoul-like viruses.[126]

Genome sequences of a novel hantavirus species were detected in an African wood mouse *(Hylomyscus alleni)* in Guinea.[110] This agent, provisionally named "Sangassou" virus, was more similar to Eurasian hantaviruses than to those causing HPS in the Western Hemisphere. It was sufficiently distinct to represent a separate clade, but has not been linked to clinical illness.

Numerous hantaviruses associated with native muroid rodents are known in the Americas (see Table 188-1). Several, all associated with New World rats and mice, are of medical importance in the United States.[174] The deer mouse, *Peromyscus maniculatus*, is the reservoir for Sin Nombre virus, the agent associated most frequently with human disease in the United States and Canada.[34,51,142] This rodent is distributed widely over the United States, Canada, and parts of Mexico. In addition, HPS cases in the eastern United States have been associated with New York and Monongahela viruses, which are closely related to Sin Nombre virus[86,206] Rare cases of HPS also have been associated with Black Creek Canal virus and Bayou virus.† Several hantaviruses associated with voles (Arvicolinae) in North America are not associated with human disease: Prospect Hill virus has been recovered from meadow voles *(Microtus pennsylvanicus)* in Maryland,[239,240] and related but distinct hantaviruses (Bloodland Lake and Isla Vista) are present in other voles by detection of genetic sequences by reverse transcriptase-polymerase chain reaction (RT-PCR).[85,209] The Old World, murine-borne Seoul virus, introduced by the Norway rat *Rattus norvegicus*, has been associated with human infections in several large cities throughout the Americas, but acute (HFRS-like) disease has been recognized and confirmed only in Recife, Brazil (Fig. 188-3).[37,68,69,122]

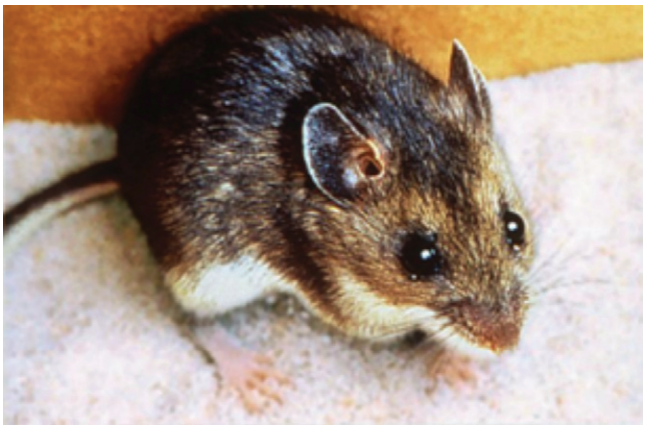

FIGURE 188-3 ■ The deer mouse, *Peromyscus maniculatus,* the carrier of Sin Nombre virus, the most common agent of HPS in North America. (See companion Expert Consult website for color version.) (Courtesy Centers for Disease Control and Prevention Special Pathogens Branch.)

South America clearly has surpassed North America in terms of numbers of human HPS cases and numbers of hantaviruses and host species identified.[14,88,233,235] All the HPS-causing hantaviruses in South America are associated with sigmodontine rodents. Some of the most important viruses include Andes virus in southern Argentina and Chile; Lechiguanas virus in central Argentina; Laguna Negra virus in northern Argentina, Paraguay, and Bolivia; Juquitiba virus in Brazil and eastern Paraguay; and Choclo virus in Panama. Numerous other hantaviruses, both pathogenic and not known to be pathogenic, occur throughout South America (see Table 188-1). Although HPS is clearly a pan-hemispheric disease, all HPS agents identified or suspected to date belong to a single genetic group of hantaviruses and are associated with two subfamilies of rodents, the Neotominae (North America) and the Sigmodontinae (primarily Central and South America). These rodent species are restricted to the Americas, in accordance with the findings that HPS has been an exclusively Western Hemisphere disease.[157]

A new finding has been the apparently widespread occurrence of a divergent clade of hantaviruses associated with a group of small mammals unrelated to rodents, the insectivores (shrews and moles, order Soricomorpha).[7-9,111,207,208] Although Thottapalayam virus and its association with the common house shrew *(Suncus marinus)* has been recognized for more than 40 years,[21] it was thought to be a unique exception. At least seven new insectivore-borne hantaviruses have been recently described from North America, Asia, Europe, and Africa. Clearly, the association of hantaviruses with insectivores

*References 4, 11, 12, 70, 177, 178.
†References 84, 104, 107, 159, 184, 218.

is ancient, and understanding their distribution and evolution will be a challenge. None of these viruses has so far been associated with human disease.

Seasonal Patterns

The natural population cycles of rodents and the seasonal nature of certain human behavior result in a pattern of human hantavirus disease that varies both seasonally and annually.* Although the incidence of disease varies by season, cases of hantavirus-associated human disease are recognized year round in all disease-endemic areas.[25,105,124,197]

In eastern Asia and Russia east of the Ural Mountains, HFRS occurs primarily during the late fall and early winter, with smaller peaks during the spring and summer. Most Scandinavian HFRS occurs between the late summer and early spring, whereas European HFRS in warmer regions (e.g., France, Belgium) tends to peak in the spring.[231] In the Balkans, the presence of multiple viral strains results in a more diffuse seasonal distribution of human disease. Persons whose occupations or avocations bring them to rural settings, such as agricultural workers, foresters, biologists, hunters, campers, and soldiers stationed in the field, are at greatest risk for contracting HFRS.

HFRS caused by Seoul virus tends to occur throughout the year, but there are conflicting descriptions of a seasonal occurrence of Seoul virus infection as well.[33,131] Cases of Seoul virus disease also have a more even age and sex distribution than do rural hantavirus infections, presumably because of the peri-domestic nature of the reservoir.

The temporal distribution of HPS cases in the United States displays strong seasonality. Maximum numbers of cases occur in May, June, and July whereas minimum case numbers are seen during December, January, and February.[142] However, environmental and geographic factors influence this pattern[154]; HPS cases have been identified throughout the winter and early spring.[25,26,52,67,242] Seasonal patterns vary by region in the United States; in the midwestern and northwestern regions the highest proportion of cases occur in May, whereas cases peak in July in the southwestern region.[142]

Prevalence

Worldwide, human hantavirus infections may number in the hundreds of thousands of cases annually.[239] Because of the predominantly rural nature of the disease and its prevalence in developing regions of the Eurasian land mass (e.g., rural China), accurate case reporting (and statistical data) for HFRS is challenging. Researchers have estimated that 100,000 or more cases of HFRS may occur each year in China.[204,239] One report suggested an incidence of 1.6 to 29.6 per 100,000 population during 1980.[98] In the former Soviet Union, more than 4000 cases per year were recorded between 1978 and 1989, 96 percent of which occurred in "European" republics. Rates in western regions near the Ural Mountains were higher (20 to 40 per 100,000 population) than were those in eastern districts (2 to 5 per 100,000 population).[217] In Korea, approximately 500 persons with HFRS, approximately half of whom are soldiers, are hospitalized annually.[217] In central and northern Sweden, a mean annual incidence of 4.3 per 100,000 population has been reported, although northern locales have rates of more than 20 per 100,000 population.[197] In Finland between 1995 and 2008, the average annual incidence of Puumala virus infection was 31/100,000 population.[144]

As of September 2011, 581 cases of HPS had been laboratory confirmed in the United States. Although 30 cases were identified retrospectively, most recognized have occurred since 1993.[143] HPS is rare in the United States; the annual incidence is estimated to range from 0.04 to 0.19 cases/million persons.[142] Case counts were relatively stable across a 17-year period in the northwestern and midwestern regions of the United States, but greater variability in the southwestern part of the country drove fluctuations in the national HPS case counts.[142] Updated U.S. surveillance data can be found on the Centers for Disease Control and Prevention website.* HPS is less common in Canada, between 2 and 8 reported cases per year, but more common in South America. More than 3500 cases were documented in South America between 1993 and 2011, including more than 1400 cases identified in Brazil alone.

Demographic Features

Hantavirus infections are recognized relatively infrequently in the pediatric age group.† The disease occurs principally in adults, with a significant minority (≤15%) of cases diagnosed in children. Reports from Argentina, China, Sweden, and the United States confirm a male predominance in reported incidence of disease,[90,109,142,143,146] despite serologic evidence that similar proportions of men and women are infected with Puumala virus in Sweden.[3] In a recent survey from Finland, 85 percent of patients were aged 20 to 64 years; 62 percent were male; 52 percent were hospitalized; 3 percent were registered as having occupational diseases; and the case-fatality proportion was 0.08 percent.[144] Case-fatality rates appear to be higher among females with HRFS in China (Hantaan and Seoul viruses),[109] women infected with Andes-associated HPS in Argentina,[146] and women with Puumala virus–associated nephropathia epidemica in Sweden.[90] In all studies, these sex-based differences were most marked among populations at peak reproductive ages (20s and 30s) and older than 50 years.[90,109,142,143,146] Analysis of U.S. data did not support a similar sex-based difference in case-fatality rates.[143] Interpretations conflict regarding the likelihood that these differences are biologically based. The peak incidence of HFRS in Europe, the Balkans, and the Far East and of HPS in the United States occurs in individuals who are 20 to 50 years of

*References 22, 28, 31, 61, 125, 150, 220.

*Centers for Disease Control and Prevention: http://www.cdc.gov/hantavirus/surveillance/.

†References 1, 4, 10, 62, 105, 106, 197, 217, 243.

age.[118,124,142,171,220] Few cases have been reported in children younger than 10 years. Both HFRS and HPS cases in young children often are recognized in association with cases in other family members.[10,62,118,158,169] A male predominance has been observed for both the severe (Korean) and milder (European) forms of HFRS in children.[1,4,112,158,243] Although this pattern, also seen in adults with HFRS and with HPS in the United States, suggests differential susceptibility or risk for exposure by gender, the number of recorded cases, particularly in the youngest age groups, is too small to be able to be used to draw firm conclusions.

In the United States, fewer than 7 percent of reported HPS cases occur in children younger than 17 years of age[27]; in South America, the proportion of cases in the pediatric age group may be somewhat higher.[57] The underrepresentation of children recognized to have HFRS and HPS may not be explained by age-related avoidance of activities resulting in exposure. The limited data available (see Clinical Manifestations) suggest that hantavirus infections in children may induce milder HFRS and NE syndromes than those seen in adults. In contrast, typical HPS has been repeatedly described in children, particularly teenagers. Comprehensive population-based serosurveys are sparse and have been inadequate to define age-associated infection rates. The apparently immune-mediated pathologic process of this disease (see Pathogenesis and Pathology) is possibly more evident in persons experiencing infection in adulthood.

CLINICAL MANIFESTATIONS

Hemorrhagic Fever with Renal Syndrome

Two major clinical variants of HFRS have been recognized traditionally. Severe disease associated with high morbidity and mortality rates occurs primarily in areas of the world where Hantaan and Dobrava viruses are endemic: across the northern half of Asia, China, the Korean Peninsula, and the Balkan nations. Milder illness with little mortality occurs in areas where Puumala and related viruses have been recovered; this latter disease, known also as nephropathia epidemica, is found throughout northern Europe, Scandinavia, and western areas of the former Soviet Union.[119] In the Balkans, the coexistence of Puumala virus–like strains with viruses causing more serious disease has resulted in a mixture of clinical findings in the former Yugoslavia and neighboring countries. Benign manifestations of HFRS are well recognized in many regions where Hantaan virus is found, however, and clinically severe disease occasionally results from Puumala virus infection.[129,175] Hence, it is important to appreciate the protean nature of this disorder and to recognize the potential for disease of any severity whenever and wherever human infection with hantavirus occurs.

Severe HFRS, such as that associated with Hantaan or Dobrava virus, is a complex, multiphasic disorder that presents a substantial challenge in patient management. The clinical course of HFRS caused by Hantaan virus in adults spans a wide spectrum from mildly symptomatic disease to severe hemorrhagic fever and death. Subclinical infections probably occur infrequently.[179,243] In most cases, the clinical course is relatively benign, with severe disease developing in 20 to 30 percent of patients. Modern-day case-fatality rates range from 2 to 7 percent. In Korea, approximately a third of recognized Hantaan virus infections follow a clinical course consisting of progression through five clinically and pathophysiologically defined stages: febrile, hypotensive, oliguric, diuretic, and convalescent.[131,198] Phases often blur, however; and in milder cases, one or more phases may not be discernible.

After an incubation period of 2 to 3 weeks (range, 4 to 42 days), most patients report the abrupt onset of high fever, headache, chills, dizziness, myalgia, anorexia, and backache. Approximately one third of patients experience prodromal mild respiratory or gastrointestinal symptoms. Nausea, vomiting, abdominal pain, and intense thirst may be evident at initial evaluation but increase in severity in succeeding days. Photophobia, blurred vision, and eyeball pain are reported frequently. Physical examination reveals a restless, acutely ill patient with flushing of the face, neck, and upper thoracic region. Relative bradycardia is present. Conjunctival and pharyngeal injection, together with facial puffiness, is characteristic. In more than 90 percent of patients, petechiae develop on the soft palate, axillae, lateral aspect of the thorax, conjunctivae, or face, generally between the third and sixth days of illness. Tenderness occurs commonly over the costovertebral angles and diffusely throughout the abdomen.

Hematologic studies in the first 3 to 4 days of illness reveal leukocytosis with a left shift in more than 90 percent of patients, as well as almost universal thrombocytopenia; the hematocrit at this stage generally is normal or slightly increased. Proteinuria develops by the third to fourth day, and microscopic hematuria, hyposthenuria, and mild pyuria are reported in most patients; fibrin clots in urine are a characteristic finding.

This febrile phase generally lasts approximately 1 week, followed by abrupt defervescence. Approximately 40 percent of patients then become hypotensive; in most cases, the drop in blood pressure is mild and brief, but in severely ill persons (30% to 50% of hypotensive patients), clinical shock develops. Tachycardia replaces bradycardia, the pulse pressure narrows, and cyanosis and mental confusion may be seen. The hypotensive phase may last from a few hours to 3 days, and 30 to 40 percent of deaths occur during this period.

As patients recover from hypotension, they enter a period of oliguria. This phase occurs in approximately 60 percent of patients and generally lasts for several days. Anuria develops in approximately 10 percent of patients. Blood pressure normalizes, and hypertension often develops. Clinical manifestations of uremia, including protracted vomiting and hiccups, may be seen. More extensive hemorrhagic manifestations such as ecchymoses, hemoptysis, hematemesis, melena, gross hematuria, and, rarely, bleeding in the central nervous system (CNS) become evident during the oliguric phase. Striking elevations in blood urea nitrogen and creatinine levels are common

findings. Biochemical disturbances (electrolyte derangements, metabolic acidosis, uremia) may be severe, and, in such cases, dialysis may be lifesaving. Approximately half of fatalities occur during the oliguric phase.

Between 10 and 14 days into the illness, renal function is restored spontaneously in most patients and a period of diuresis follows. Polyuria may be substantial, and urine output frequently exceeds 3 to 6 L/day. With the onset of diuresis, clinical recovery is initiated; however, the rapid change in fluid status may precipitate further electrolyte disturbances, so close monitoring remains necessary.

Convalescence typically lasts from 3 to 6 weeks, but in many cases a longer period passes before health is restored completely. Weight gain and strength are recovered slowly. Proteinuria resolves, but hyposthenuria persists for months. Most patients recover completely, although permanent sequelae may result from such complications as anterior pituitary or other CNS hemorrhage.

The disease has been reported infrequently in children.[62,112,243] However, the data available indicate that clinical manifestations are similar to and perhaps somewhat less severe than those observed in adults. In a series of 63 children identified retrospectively over a 15-year period in Korea, fever was universal, whereas abdominal pain, headache, and vomiting were present in 73 percent or more of patients (Table 188-2).[243] Proteinuria (100%), leukocytosis (71%), thrombocytopenia (80%), hypocholesterolemia (87%), and elevations in creatinine (94%), blood urea nitrogen (94%), and alanine aminotransferase (80%) were the laboratory abnormalities found most commonly. Petechiae and hypotension occurred infrequently (38% and 11%, respectively), and frank hemorrhage did so rarely. Eleven patients (18%) required dialysis. The mortality rate was 5 percent, and the remaining patients recovered without sequelae.

HFRS occurring in adults after they are infected with Seoul virus resembles that described for Hantaan virus infection, but the clinical manifestations generally are milder.[131] Fever and constitutional symptoms are similar in the two types of infection, but hypotension occurs infrequently in persons infected with Seoul virus (10% of cases), and clinical shock is a rare event. The frequency and severity of thrombocytopenia are less with Seoul virus infection, whereas elevations of aminotransferases occur commonly (>60% of cases). The mortality rate for HFRS caused by Seoul virus is 1 percent or less.

Nephropathia Epidemica

The Scandinavian or European form of HFRS (nephropathia epidemica) generally is a more benign disease than that attributed to Hantaan virus; fatal outcomes are observed, but the case-fatality rate is less than 1 percent.[96]

TABLE 188-2 **Prominent Clinical and Laboratory Features of Hemorrhagic Fever with Renal Syndrome in Children**

Features	Korea[172] (%)	Sweden[1] (%)	Finland[109] (%)	Austria[1] (%)
Fever	100	100	100/NR	89
Headache	76	100	59/62	58
Anorexia	33	100	NR/NR	NR
Nausea	62	86	81/NR	89
Vomiting	73	91	72/NR	84
Abdominal pain	91	93	59/100	100
Back/costovertebral angle pain	35	76	63/92	NR
Dizziness	21	73	9/NR	NR
Thirst	NR	75	NR/NR	NR
Polyuria	NR	57	NR/100	NR
Diarrhea	NR	57	9/NR	NR
Petechiae	38	NR	NR/0	NR
Conjunctival hemorrhage	35	NR	3/0	NR
Proteinuria	100	100	97/100	100
Hematuria	67	80	73/NR	100
Pyuria	8	43	44/NR	NR
Glucosuria	NR	26	12/NR	NR
Casts	NR	33	NR/NR	NR
Leukocytosis	71	22	41/NR	NR
Thrombocytopenia	80	68	87/100	84
Elevated hemoglobin	39	NR	28/NR	NR
Elevated C-reactive protein	NR	28	89/100	100
Elevated erythrocyte sedimentation rate	NR	58	74/NR	NR
Elevated alanine aminotransferase	80	NR	53/83	63
Elevated creatinine	94	76	84/92	95

NR, not recorded.

In contrast to the findings with Hantaan virus, subclinical infection apparently occurs commonly with Puumala virus; one report suggested a case-to-infection ratio of 1:10.[163]

In adults, nephropathia epidemica typically is a biphasic disease of 1 to 3 weeks' duration.[38,171,229] The onset usually is abrupt, with no apparent prodrome. High fever is the initial symptom in 95 percent of patients. On examination, a facial flush is a usual finding. This febrile phase lasts from 3 to 6 days. The development of nausea, vomiting, abdominal pain, back pain, somnolence, and, occasionally, joint pain heralds the onset of the second, or renal, phase. The abdominal pain may be of such severity and character that an acute abdomen is suspected, and many patients have undergone surgery for suspected appendicitis before nephropathia epidemica was diagnosed. Visual disturbances are common manifestations (about 43%).[79,96] In one prospective study, 70 percent of patients hospitalized in Finland with acute Puumala infection reported ocular symptoms. Examinations identified decreased intraocular pressure (88%), reduced visual acuity and conjunctival chemosis (87%), thickening of the lens (82%), myopic shift (78%), and shallowing of the anterior chamber (64%) and of the vitreous length (52%) during the acute phase compared with measurements taken after clinical recovery. That myopic shift was less common than diminished visual acuity suggested that multiple mechanisms, possibly including extraocular CNS involvement, contribute to visual disturbances during acute nephropathia epidemica.[80] Hypotension, if it develops, usually is mild. Petechiae are seen relatively infrequently (11%).[96] Severe internal bleeding is rare, seen almost exclusively in adults, and frequently associated with fatal outcome.[96] The renal stage generally lasts 1 to 2 weeks and is characterized by the development of oliguria, proteinuria, hematuria, and hyposthenuria. Modest elevations in blood urea nitrogen and creatinine accompany the oliguria, which typically lasts for no more than a few days before diuresis begins. Mild leukocytosis and thrombocytopenia occur during the renal phase, whereas electrolyte disturbances sufficient to require dialysis are infrequent events. As with other forms of HFRS, convalescence may be prolonged and hyposthenuria may persist for many months. Recovery typically is complete, although minor abnormalities in renal function and blood pressure have been described.[118,161]

As with Hantaan virus–associated HFRS, nephropathia epidemica in children is recognized relatively infrequently. Clinically, the disease also appears to be similar to, though milder, than that seen in adults. Abdominal pain and vomiting are more frequently observed among children than adults, and children are more often hypertensive, thrombocytopenic, and oliguric.[96] Arthralgias, myalgias, and visual disturbances are more commonly reported in adults, as are epistaxis, leukocytosis, polyuria, and proteinuria.[96] Chest radiographs are more frequently abnormal in pediatric patients.[96] Among 32 Swedish cases reported (18 identified retrospectively and 14 prospectively), fever (100%), headache (100%), anorexia (100%), abdominal pain (93%), vomiting (91%), nausea (86%), and back pain (76%) were the most prevalent symptoms (see Table 188-2).[1] Proteinuria (100%), microscopic hematuria (80%), elevated serum creatinine (76%), and thrombocytopenia (68%) were the laboratory abnormalities most frequently found. Leukocytosis was a relatively uncommon finding (22%) in this series. Six children (19%) had hemorrhagic manifestations, and 1 complained of blurred vision. In a 32-patient series from Finland, fever (100%), nausea (81%), vomiting (72%), and back pain (63%) again were prevalent, but headache and abdominal pain (59%) were reported less frequently.[158] Proteinuria (97%), hematuria (73%), and elevated serum creatinine level (84%) were common findings. In a separate 13-patient retrospective series from the same country, abdominal pain was universal as a presenting symptom, with temperature elevation, oliguria, polyuria, proteinuria, thrombocytopenia, and elevated serum creatinine level ultimately reported in all (see Table 188-2).[120] Among 19 Austrian children 6 to 18 years old who were identified retrospectively, abdominal pain (100%), fever (89%), nausea (89%), vomiting (84%), and headache (58%) were the most frequent signs and symptoms reported.[1,4] Ninety-five percent of these children developed acute renal failure, but all apparently recovered fully. Thrombocytopenia was reported more prominently among Austrian and Finnish than Swedish children. Up to one fourth of Austrian and Finnish children displayed hemorrhagic manifestations. The proportion of transient visual blurring was identical to that seen in adults (25%). Some disease manifestations (e.g., thrombocytopenia, renal function abnormalities) may be absent, however, and the disease should be considered in the differential diagnosis of "fever of unknown origin" in endemic areas.[226] Three children with Puumala virus nephropathy in the Czech Republic were admitted with interstitial nephritis after a flulike prodrome. All were mildly febrile, with anemia and elevated C-reactive protein value, erythrocyte sedimentation rate, and serum creatinine level and with proteinuria. Biopsy-confirmed renal abnormalities returned to normal within 4 weeks after corticosteroid treatment.[49] No children in any of these series required dialysis.

Hantavirus Pulmonary Syndrome

Classic HPS in adults is a biphasic illness that challenges the diagnostic acumen and clinical management skills of physicians.[23,166,168,174] The incubation period was 7 to 39 days (median, 18 days) for 20 people with defined exposure to Andes virus in a high-risk area,[232] an observation consistent with the incubation period of 9 to 33 days (median, 14 to 17 days) for 11 cases of patients with HPS and well-defined exposure in the United States[244] and the 3-week incubation period observed in two boys with HPS bitten by the same mouse.[202]

The clinical features of the prodrome phase are not pathognomonic, and the diagnosis rarely is suspected before the abrupt clinical deterioration occurs that heralds onset of the cardiopulmonary phase. HPS characteristically begins with a prodrome that lasts on average 3 to 4 days but may extend as long as a week or more.[30,48,134,174,231] This phase is typified by fever and myalgia, particularly of the back or lower extremities. Although a cough may develop as the prodrome

progresses, illnesses initially characterized predominantly by upper respiratory tract symptoms, such as cough and coryza, are unlikely to be HPS.[30,134,147,157] More than half of patients with HPS also have gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea) that usually are mild.[30,48] Occasionally, HPS-associated gastrointestinal symptoms have been mistaken for an acute surgical abdomen or another intra-abdominal process. The presence of thrombocytopenia in the context of a compatible prodrome is highly suggestive of HPS.[30,48,114,157,200]

The onset of the cardiopulmonary phase is abrupt and often life threatening. Patients usually are hospitalized within 12 to 24 hours of initial medical evaluation, and most deaths occur during the first 24 to 48 hours in the hospital.[30,32,48,134] Among fatal HPS cases identified in the United States between 1993 and 2009, the mean time from onset of symptoms to death was 6.4 days (median, 5 days) and the 35 percent case-fatality rate was similar across demographic characteristics and geographic regions.[142] Clear lungs shown by radiography have progressed to diffuse bilateral pulmonary involvement during the course of several hours. Interstitial edema, present in only 5 percent of patients with adult respiratory distress syndrome, is present in most patients with HPS on initial radiography, and alveolar flooding that is usually indistinguishable from the peripheral pattern seen in the acute phase of adult respiratory distress syndrome develops in most patients (Fig. 188-4).[103] This noncardiogenic pulmonary edema, resulting from a diffuse pulmonary capillary leak, can be differentiated from cardiac (hydrostatic) pulmonary edema by the presence of low pulmonary artery occlusion pressure and an increased protein content in edema fluid.[77] Secretions recovered when patients with HPS are intubated generally are acellular, resemble plasma or pulmonary edema fluid, and have been observed to clot in severe cases.[48,77] The presence of significant numbers of polymorphonuclear leukocytes in pulmonary secretions suggests an alternative cause.

The pulmonary decline usually is accompanied by the onset of shock caused by myocardial dysfunction and relative hypovolemia.[30,77,134] In severe cases, hemodynamic measurements show high systemic vascular resistance combined with low cardiac and stroke volume indices. Progression to death is associated with worsening cardiac dysfunction unresponsive to treatment, despite provision of adequate oxygenation.[77] Increased hematocrit, creatinine levels, and leukocyte counts; requirement for supplemental oxygen; and necessity for intubation are all associated with fatal outcome, and thrombocytopenia, although common, is more pronounced among patients who die compared with survivors.[142] At autopsy, the myocardium of patients who died of Araraquara virus–associated HPS contained endothelial cells and interstitial macrophage-type cells harboring hantavirus antigen and viral particles accompanied by a mononuclear cell inflammatory interstitial infiltrate that met the international consensus criteria for infectious inflammatory myocarditis and was absent from the autopsy myocardium of persons who died after having either necrotizing pancreatitis associated with acute lung injury or nonthoracic trauma.[182,187] This infectious inflammatory cardiomyopathy may be responsible for HPS-associated myocardial depression and shock. In one South American study, the absence of clinical and laboratory signs of circulatory shock on admission was associated with a favorable outcome.[188]

Thrombocytopenia may be present in the late prodrome and almost always is found during the cardiopulmonary phase. Additional laboratory abnormalities in hospitalized patients include hemoconcentration, prolonged prothrombin and partial thromboplastin times, elevated serum lactate and serum lactate dehydrogenase concentration, decreased serum protein concentration, mild leukocytosis with a marked left shift, and the frequent presence of myeloid precursors on the peripheral smear (Fig. 188-5).* Proteinuria is a common occurrence.

*References 30, 48, 114, 134, 174.

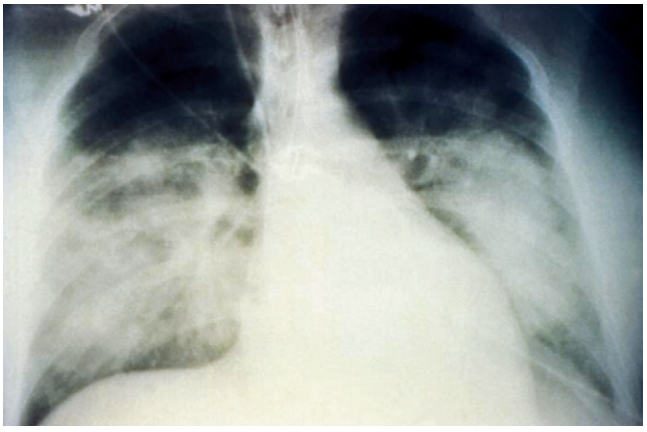

FIGURE 188-4 ■ Anteroposterior chest radiograph reveals the mid-staged bilateral pulmonary effusion due to HPS. The radiologic evolution begins with minimal changes of interstitial pulmonary edema, progressing to alveolar edema with severe bilateral involvement. Pleural effusions are common and are often large enough to be evident radiographically. (See companion Expert Consult website for color version.) (Courtesy Centers for Disease Control and Prevention and D. Loren Ketai, M.D.)

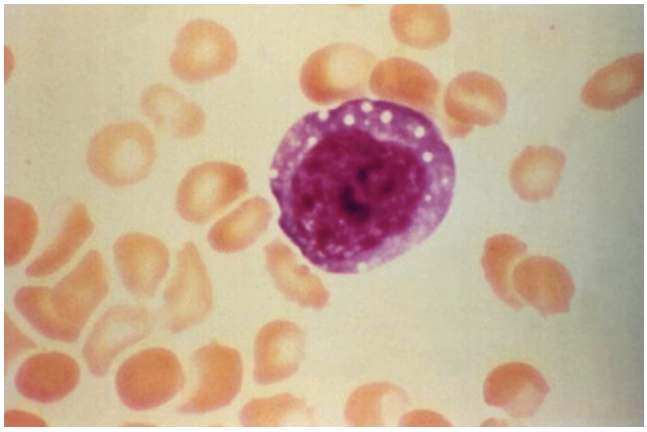

FIGURE 188-5 ■ Micrograph shows an atypical enlarged lymphocyte found in the blood smear from a patient with HPS. The large atypical lymphocyte shown here is an example of one of the laboratory findings, which when combined with a bandemia and dropping platelet count, is characteristic of HPS. (See companion Expert Consult website for color version.) (Courtesy Centers for Disease Control and Prevention.)

The serum creatinine value often is elevated modestly in severe cases, although renal failure is not characteristic of infection with Sin Nombre virus, despite episodes of hypotension and other predisposing conditions that are present in many patients. Renal failure was more prominent in two reported North American cases of HPS caused by infection with Black Creek Canal virus and Bayou virus.[46,107]

Patients who survive enter a diuretic phase after recovery from cardiovascular collapse and improve rapidly thereafter. Surviving patients may be extubated within 3 to 7 days after admission to the intensive care unit and may be discharged from the hospital within 2 weeks.[22,174,187]

Subclinical or mild disease is rarely recognized after Sin Nombre virus infection.[199] However, although initial case-fatality rates of 70 to 80 percent were reported in 1993, a wider clinical spectrum became recognized once clinicians developed heightened suspicion and diagnostic assays became more readily available. As of 2011, the overall case-fatality rate for HPS cases in the United States was 35 percent[142,143]; case-fatality rates are similar throughout the Americas.[143] This apparent decline in the incidence of mortality compared with initial reports in the early 1990s may reflect improvements in survival attributed to improved clinical management. However, it undoubtedly also reflects a decrease in the tendency to suspect this diagnosis only with the fatal and near-fatal disease that existed early in the clinical understanding of this syndrome.

The hantavirus disease pulmonary manifestations associated with Andes virus[139] in Argentina and Chile and that from other related virus infections in Brazil[159] and Paraguay[237] closely resemble Sin Nombre virus disease. However, hemorrhagic and renal symptoms are more often associated with HPS in South America.* Renal failure accompanied some cases in a focus in northern Argentina,[169] and both viral antigen and infectious virus have been isolated from urine of Andes virus–infected HPS patients.[71] The unique association of person-to-person transmission with Andes virus infections influences infection control requirements.

HPS is relatively uncommon (7% to 15% of reported cases) in children younger than age 17 years. Several case reports and reviews of North American pediatric patients with HPS indicated that the geographic distribution, clinical course, and mortality rates in children and adolescents are similar to those described for adults.† In South America (predominantly Chile and Argentina), clinical manifestations of HPS in children appear to be similar to those described in North America.[57,121] Fever, headache, dyspnea or cough, gastrointestinal disturbances, and myalgia are common manifestations in the prodromal phase. Tachypnea and fever are frequent on hospital admission; in one case series, however, hypotension at presentation was an uncommon finding (33%).[181] In one patient, dizziness with an apparent vestibular component was described.[106] Thrombocytopenia, a left shift in the leukocyte differential count, elevated levels of hepatic aminotransferases and lactate dehydrogenase, and hypoalbuminemia typically are noted on admission. Although leukocytosis and hemoconcentration are not found early reliably, they may appear later in the course of illness. Investigators suggested that the mortality rate among prepubertal children may be somewhat lower than that seen in older individuals,[20] but as patient numbers have accumulated over time, overall case-fatality rates in pediatric and adolescent patients seem to be similar to those seen in adults (30% to 40%). In one series, hypotension and absence of fever at admission were found to be predictive of respiratory failure, whereas elevated prothrombin time (≥14 seconds) at admission was predictive of mortality.[181] Overall mortality among 82 children with HPS contracted in Chile was 37 percent[27]; in one small case series from Argentina, however, the case-fatality rate for HPS in 5- to 11-year-old children was higher (60%).[176] In the same report, the authors noted evidence of passive hantavirus antibody transfer from a pregnant mother to her fetus, without subsequent illness in the infant. Additionally, a woman who nursed a 7-month-old infant contracted HPS and subsequently died. Hantavirus antibodies were detected to high titer (>1:6,400) 8 and 15 months later in the infant (who remained healthy), a finding suggesting possible asymptomatic mother-to-child transmission of the infection through breast-feeding.[176]

COMPLICATIONS

In general, human infection with HFRS-associated hantaviruses results in an acute illness with prolonged incapacitation followed by complete recovery. Unless complicated by organ hemorrhage (e.g., CNS bleeding), residua generally have not been observed.

In one Finnish study of 58 hospitalized Puumala virus–infected patients, 87 percent suffered symptoms suggestive of CNS involvement (headache, light sensitivity, nausea/vomiting, or dizziness); 2 experienced symptoms indicative of encephalitis (vomiting, headache, somnolence, confusion). Two patients experienced an abrupt complete loss of vision with spontaneous recovery, associated with severe headache, vomiting, dizziness, and pituitary hemorrhage demonstrated by magnetic resonance imaging.[81] One of the 2 patients with demonstrated pituitary hemorrhage recovered spontaneously within 3 months.[81] Young male patients may be at increased risk of developing serious CNS complications during acute Puumala virus infections.[79] Epidemiologic associations between previous infections with Seoul virus in the United States and hypertensive renal disease have been reported.[68,69] In one study, 5 years after recovery, 50 percent of patients who had HFRS were hypertensive, with a higher glomerular filtration rate and urinary protein excretion compared with 21 percent of controls. These differences resolved by 10 years.[153] These data require further study to determine their significance.

Among HPS patients hospitalized in North America, although prolonged prothrombin and partial thromboplastin times occur commonly, overt disseminated intravascular coagulation and overt hemorrhage occur

*References 23, 167, 183, 219, 237.
†References 10, 20, 27, 106, 123, 165, 181.

infrequently among Sin Nombre–infected patients.[30,245] Patients in whom disseminated intravascular coagulation is established rarely survive longer than 48 hours. One patient who did survive a cardiopulmonary phase accompanied by overt disseminated intravascular coagulation died 3 weeks later of gangrenous complications.

One 5-year follow-up study of 30 North American HPS survivors (29 infected with Sin Nombre and one with Bayou virus) identified proteinuria of more than 150 mg by 24-hour urine collection in more than half the cohort; 17 percent also had decreased creatinine clearance. Proteinuria prevalence appeared to increase over time and to be associated with increased severity of HPS disease, although treatment by extracorporeal membrane oxygenation (ECMO) may have lowered risk.[172]

The South American HPS-associated viruses have been associated with more extrapulmonary manifestations than have the Sin Nombre infections in the United States. Evidence of disseminated intravascular coagulation, bleeding, and renal manifestations are reported with greater frequency among Andes virus–infected patients.[23]

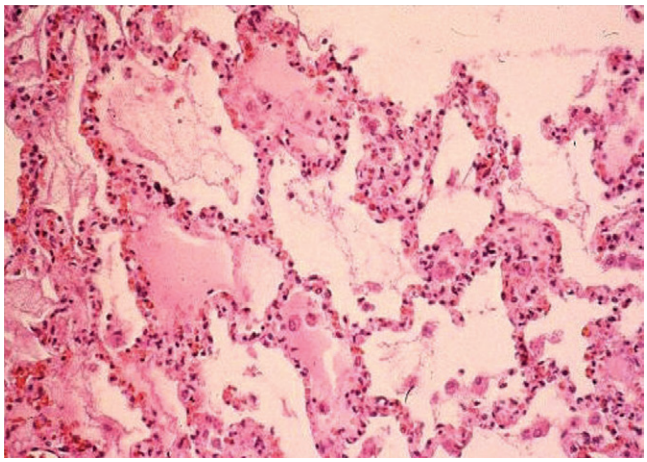

FIGURE 188-6 ■ First micrograph depicts the histopathology of the lung in HPS, with interstitial pneumonitis and intraalveolar edema. Second micrograph depicts a histopathologic comparison between HPS *(right)* and acute respiratory distress syndrome *(left)*. HPS reveals interstitial lymphocytic infiltrates and alveolar edema, while acute respiratory distress syndrome reveals neutrophilic presence, and damage to pneumocytes. (See companion Expert Consult website for color version.) (Courtesy Centers for Disease Control and Prevention and Dr. Sherif R. Zaki.)

PATHOGENESIS AND PATHOLOGY

Disease development after hantavirus infection is likely due to immune-mediated factors as well as to direct effects of the virus. Early clinical events are presumed and with Andes virus demonstrated to be accompanied by viremia[56]; however, significant signs and symptoms develop in temporal association with the onset of a measurable antibody response.

In HPS, Andes viral RNA (detected by RT-PCR) has been observed up to 2 weeks before symptom onset.[56] A statistically significant association has been demonstrated between plasma viral RNA levels at hospital admission and the severity of HPS disease.[238] Survivors rapidly clear virus.[215]

Hantaviruses infect alveolar macrophages and follicular dendritic cells, but primarily target and replicate in human endothelial cells, without apparent cytopathic effect.[245] However, virus replication has been shown to result in increased permeability and loss of integrity of the endothelial cell barrier in association with early increase in secreted vascular endothelial growth factor (VEGF) and concomitant decreased vascular endothelial (VE)-cadherin during HPS-associated hantavirus infection of human primary lung endothelial cells.[65,199] VEGF is a potent vascular permeability agent and when present, it binds to vascular endothelial growth factor receptor-2 (VEGFR2), initiating the internalization and degradation of VE-cadherin and the disruption of the adherens junctions of the endothelial cell barrier.[199] This cascade of VEGF/VEGFR2/VE-cadherin has been shown to be involved in the increase of vascular permeability in hantavirus-infected endothelial cells.[74,75,199]

Pathologic studies of fatal HFRS cases indicated that multiple organ systems are involved, but a triad of lesions consisting of hemorrhagic necrosis of the renal medulla, anterior pituitary, and cardiac right atrium is described as characteristic.[95] In HPS, multiple organ involvement with variable degrees of vascular congestion is noted in all fatal cases. However, the predominant findings at autopsy have involved the lungs, with pulmonary edema and serous effusions seen grossly and interstitial pneumonitis with a mononuclear cell infiltrate, intra-alveolar edema, and focal hyaline membrane formation described microscopically (Fig. 188-6). Immunohistochemical studies demonstrated the widespread presence of hantavirus antigens in endothelial cells of the microvasculature, particularly in the lungs but also in the kidneys, heart, spleen, pancreas, lymph nodes, skeletal muscle, intestine, adrenal gland, adipose tissue, urinary bladder, and brain. However, few histologic changes have been noted in the kidney, brain, and heart of autopsied patients who died with HPS.[245]

Endothelial cell mRNA responses to infection by pathogenic and nonpathogenic hantaviruses, observed by DNA array analyses, showed differential patterns of gene activation.[66] Pathogenic Hantaan and New York-1 viruses appeared to suppress early interferon (IFN) responses that were activated by nonpathogenic Prospect Hill virus. Hantaan virus uniquely induced multiple chemokines (interleukin-8 [IL-8], IL-6, growth-regulated oncogene-beta [GRO-β]), cell adhesion molecules (intercellular cell adhesion molecule [ICAM]), and complement cascade–associated factors that could play a role in HFRS immunopathogenesis. Although New York-1 virus failed to induce most of the cellular chemokines activated by Hantaan virus, it uniquely induced β_3 integrin–linked potassium channels, an action that could be important to HPS-induced vascular permeability. The differential use of β_3 integrins as receptors by pathogenic hantaviruses may be relevant to pathogenesis, because β_3 integrins are present on platelets and endothelial cells and interaction with these proteins can regulate vascular permeability, platelet activation, and adhesion.[143]

Evidence exists that hantaviruses work to subvert host innate immune responses.[64,212] Hantaviruses use a transcription mechanism that avoids activation of the IFN pathway.[63,76] Hantavirus Gn and Gc glycoproteins can downregulate the host IFN response and contribute, along with the nucleocapsid protein, to inhibition of the IFN signaling pathway.[5,133,210] Pathogenic and nonpathogenic hantaviruses both downregulate the IFN induction, but pathogenic hantaviruses do not induce the early IFN responses activated by nonpathogenic hantaviruses described earlier.[66,143] This difference in innate immune activation has been speculated to contribute to differences in pathogenicity.

Whereas the mechanisms underlying the observed pathologic lesions are incompletely understood, both cellular and humoral immune-mediated mechanisms have been implicated.[15,39,50,95,221] Serum antibodies develop within 3 to 7 days of the onset of illness in patients infected with hantaviruses. In HPS patients, N protein–reactive antibodies are present in serum soon after onset of disease; neutralizing antibodies directed against Gn and Gc glycoproteins follow.[97,225] High-titer neutralizing antibodies in serum correlate with milder disease and higher hantavirus-specific IgG levels early in disease are associated with survival.[13,141]

The abrupt onset of noncardiogenic pulmonary edema in HPS occurs after the development of an immune response directed against viral antigen present throughout the endothelial cells lining the pulmonary capillaries. This temporal association suggests an immune-mediated capillary leak syndrome in addition to direct effects of virus replication and indirect effects of increased VEGF and decreased VE-cadherin on the permeability and integrity of the endothelial cell barrier.[65,74,75,173,199]

Virus-specific cytotoxic T lymphocytes have been observed during acute phases of HFRS,[93] and elevated levels of inflammatory cytokines (IFN-γ, tumor necrosis factor [TNF]-α, TNF-β, interleukin [IL]-6, and IL-10) have been found in sera and renal biopsy specimens of patients.[93,115,136,213] Animal studies suggested a role for T-helper cells and antibodies as well.[41] $CD4^+$ and $CD8^+$ T-cell clones that recognize Hantaan virus N, Gn, and Gc proteins are present in acute and convalescent patient blood samples.* Several studies suggest that cytotoxic T lymphocytes play a major role in induction of HPS. Patients with more severe HPS had higher frequencies of Sin Nombre virus–specific $CD8^+$ T cells compared with those with milder disease, and the presence of Sin Nombre virus–specific $CD8^+$ T cells has been shown to increase permeability of infected HLA-matched human endothelial cells in vitro.[82,214]

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

A high index of suspicion is essential for recognition of hantavirus infection in persons living or traveling in disease-endemic areas and participating in activities that might provide appropriate exposures. The protean nature of the disease in children is such that almost any ill-defined febrile disease (or fever of unknown origin) associated with abdominal or back pain in an appropriate geographic setting should stimulate consideration of the diagnosis, particularly if the clinical syndrome is accompanied by myalgia, if thrombocytopenia or proteinuria is present, or if a history of exposure to rodents or rural environments in disease-endemic areas is elicited.[200]

The differential diagnosis of HFRS includes rickettsial disease, leptospirosis, meningococcemia, other viral diseases, post-streptococcal syndromes, pyelonephritis, leukemia, and hemolytic-uremic syndrome. Differentiating from an acute intra-abdominal or pelvic process, such as appendicitis, may be extremely difficult on clinical grounds.

The differential diagnosis of HPS includes rickettsial diseases, leptospirosis, influenza, streptococcal pneumonia, legionellosis, *Yersinia pestis* infection (plague), meningococcemia, brucellosis, mycoplasmal and fungal pneumonias (including *Coccidioides immitis* and *Histoplasma* pneumonias), tularemia, psittacosis, pancreatitis accompanied by adult respiratory distress syndrome, and autoimmune disorders (including thrombotic thrombocytopenia purpura). A prominent cough or sore throat or a localized infiltrate on a chest radiograph that does not generalize within hours argues for a cause other than hantaviral.[30,83,158] A constellation of the absence of a cough in the presence of dizziness, nausea or vomiting, a low platelet count, a low serum bicarbonate level, and an elevated hematocrit discriminated HPS from similar patients with unexplained adult respiratory distress syndrome in two studies.[30,157] Thrombocytopenia or a falling platelet count provides a valuable clue late in the prodrome. After the onset of pulmonary edema, the presence of four of five findings (thrombocytopenia, myelocytosis, hemoconcentration, lack of significant toxic granulations in neutrophils, and more than 10% of lymphocytes with immunoblastic morphologic findings) has sensitivity for HPS of 96 percent and specificity of 99 percent.[114] Frequent use of pulse oximetry also is of assistance.

Laboratory Studies

Serologic testing remains the mainstay of clinical diagnosis and is widely available through commercial laboratories or, in the United States, state public health laboratories and the Centers for Disease Control and Prevention (CDC).[27,195,201] IgM antibodies to nucleocapsid antigen are universally present in symptomatic HPS patients and almost universally in HFRS patients; seroconversion takes place up to 5 days after onset of disease in 2 to 5 percent of Puumala virus–infected patients with nephropathia epidemica.[89,99,201,224] The extent of viremia, and therefore the utility of testing for viral RNA, varies depending on the infecting hantavirus. Plasma viral RNA correlates with disease severity for both HFRS and HPS, and a high viral load is generally identifiable in the blood circulation of severe HFRS and HPS disease (i.e., infections with Hantaan, Dobrava, Sin Nombre or Andes viruses).[89,224] Prospective surveillance of household contact of Andes virus–infected HPS patients detected hantaviral

*References 53, 108, 216, 227, 228, 237.

RNA 5 to 15 days before symptom onset or detection of IgM antibodies.[224] Although the viremia level is lower in Puumala virus–associated nephropathica epidemica cases, viral RNA detection may be successful on blood samples collected within the first 7 days after symptom onset.[224] Detection of viral RNA in urine or cerebrospinal fluid of hantavirus-infected patients helps describe pathophysiology but has little diagnostic utility, although detection of hantavirus RNA in saliva may prove useful for diagnostic applications.[224] Kidney biopsies were used for diagnosis of HFRS in earlier years, but findings were generally nonspecific; interstitial hemorrhage was found in only 25 percent of HFRS cases, and identification of viral markers was rare.[224] Routine immunohistologic methods can detect hantavirus antigens in autopsy samples.[224] Laboratory diagnosis of HFRS or HPS is made by demonstrating specific anti-hantavirus immunoglobulin M (IgM) antibodies in acute-phase serum by enzyme immunoassay in the IgM capture format. A fourfold or greater rise in specific IgG antibodies by enzyme immunoassay or immunofluorescence[117,132] in sequential sera (ideally obtained ≥2 weeks apart) also is useful. The availability of purified recombinant hantavirus antigens has enhanced diagnostic specificity in both the enzyme immunoassay and Western blot assay systems.[55,97,99,224,249] Enzyme-linked immunosorbent assay and indirect fluorescent antibody tests are broadly cross reactive, particularly among viruses from rodents of the same subfamily.[35,36] For example, a recombinant Sin Nombre virus antigen detects antibodies against Bayou, Black Creek Canal, Andes, and several other viruses from sigmodontine rodents and it maintains reactivity with antibodies directed against arvicolid rodent–associated viruses (e.g., Puumala and Prospect Hill viruses). Neutralizing antibody assays are more specific, but technical requirements preclude their routine use.[99,224] Immunohistochemical techniques have been applied to tissue samples from patients infected with hantaviruses.[224,245] Nucleic acid primers from several hantavirus strains have been generated, and they have enabled nucleotide sequences from fresh or frozen tissues to be amplified by RT-PCR. RT-PCR usually is successful on whole blood or blood clots obtained within the first 7 to 10 days of illness. However, the expense and effort of performing the method are not justified unless the resulting genetic sequence information is needed for definition of the viral strain or for epidemiologic studies.[6,160] Attempts to isolate hantaviruses from human specimens are generally unrewarding.

TREATMENT

Cautious fluid management and hemodynamic and intensive care unit support are the most important aspects of the clinical management of any hantavirus disease.[77,134] Early hospitalization and avoidance of even minor trauma are essential to maintain the integrity of damaged vascular beds in these patients. Transport of patients should be minimized; the barotrauma associated with transport in underpressurized aircraft may be particularly hazardous.[19] Attention to fluid management and metabolic status is especially critical.

In patients with HFRS, restriction of fluids may be necessary early in the course of disease as renal function diminishes, but large input may be required later during diuresis to correct massive losses. Electrolyte abnormalities and metabolic acidosis occur commonly. Peritoneal dialysis or hemodialysis may be lifesaving in severe cases.[19]

In patients with HPS, the nature of the pulmonary disease predisposes to iatrogenic pulmonary edema; careful attention must be paid to maintaining appropriate central venous and pulmonary arterial pressure to avoid such complications. Tissue perfusion and adequate oxygenation are the goals of supportive therapy with this syndrome. Oxygen supplementation and mechanical ventilation are required nearly always. Inotropic agents may be necessary to maintain tissue perfusion.[77,134] ECMO has been used in severe HPS cases; some studies suggest this procedure may improve survival.[40,43] One study suggested ECMO may also be associated with a lower risk of proteinuria after acute disease.[71]

Studies have suggested that cytotoxic T-lymphocyte activity and other aspects of inflammatory response contribute to the capillary leakage observed in patients with HFRS or HPS, and case reports have attributed clinical improvement to treatment with corticosteroids and venovenous hemodiafiltration.[82,196] The safety and efficacy of corticosteroid treatment have not been confirmed through controlled clinical trials. Immunotherapeutic approaches that may inhibit the vascular permeability induced by hantavirus infection are of current clinical investigational interest.[143]

Hantaan, Sin Nombre, and a number of both Old and New World hantaviruses exhibit similar in vitro sensitivity to ribavirin. One prospective, placebo-controlled trial suggested that intravenous ribavirin was effective in reducing the mortality and morbidity rates associated with HFRS in China.[94] In contrast, although 30 patients with HPS who received investigational, open-label intravenous ribavirin generally tolerated it well, treatment was accompanied by a low frequency of early drug-associated adverse events, most significantly anemia and resulting transfusion, and no clear evidence of benefit was obtained.[30,32] A subsequent randomized placebo-controlled trial was stopped short of a definitive end point because of small enrollment without trends supporting either efficacy or adverse events.[152] However, the study sample size was insufficient to exclude adverse events that occurred at a rate of less than 27 percent.[145] Similar doses of intravenous ribavirin resulted in hemolysis in 76 percent, a 2 g/dL hemoglobin level decrease in 49 percent, and discontinuation because of toxicity in 18 percent of 126 patients treated for severe acute respiratory syndrome, findings consistent with observations during the open-label trial.[16,30,32,145] This contrast in clinical experience is not explained by differences in dosing schedules; patients in all three protocols received identical doses.[16,32,94] Because all hantaviruses have been sensitive to the antiviral effects of ribavirin in vitro, the lack of any dramatic effect by intravenous ribavirin in HPS is probably the result of the rapid progression of the disease; HPS-associated deaths usually occur within the first 48 hours after admission and therapeutic

intervention.* Although ribavirin administered at 3 days after infection provided complete protection from disease in a hamster animal model,[186] the 2- to 3-week incubation period associated with Andes and Sin Nombre–virus infections leading to HPS precludes administration of ribavirin comparably early in human infection.

Administration of 5,000 or more neutralizing antibody units (NAU) of human polyclonal antibody obtained from fresh frozen plasma of an HPS survivor per kilogram or of 12,000 NAU/kg of a de-speciated (Fc removed) polyclonal immunoglobulin-based product purified from the eggs of ducks vaccinated with a DNA vaccine protected against lethal disease when given up to 8 days after intranasal Andes virus challenge in the same hamster model.[17] Therapeutic approaches attempting to combine ribavirin with other therapeutic agents, including immune modulators, may be more efficacious. Ribavirin is not licensed for intravenous use in the United States. Teratogenic concerns mandate careful informed consent if the use of this drug is considered in children, pregnant women, or nursing mothers.

PREVENTION

Primary Prevention

The most effective preventive measure available for hantavirus infection is the avoidance of rodents and their habitats. However, eradication of rodent reservoir hosts in disease-endemic areas is not feasible. Prevention efforts are directed more appropriately toward reducing the frequency of rodent-human interactions through environmental hygiene practices that minimize rodent abundance in home and work environments and avoidance of known rodent-infested areas and activities that increase the risk of human exposure to aerosolized infectious rodent excreta. Such avoidance may be particularly important during times of high rodent populations in specific localities. In fact, in the southwestern United States, remote sensing patterns from the year before have predicted local areas of increased risk for HPS, allowing precisely targeted public health messages and interventions.[67]

Detailed guidelines on measures appropriate to eliminate rodents from homes in disease-endemic areas, to clean rodent-contaminated areas safely, and to minimize the risk for workers occupationally exposed to rodents and participants in outdoor recreational activities have been published.[155] Although rodent ectoparasites do not transmit hantaviruses, in the southwestern United States, several rodent species are also hosts to fleas that transmit *Yersinia pestis*. In such areas, insecticides should be used in conjunction with rodent extermination measures because eradication of rodents without concurrent control of the associated fleas may increase the risk of human plague.[155]

One study[230] reported a strong association between smoking and infection with Puumala virus, but more study is needed to demonstrate cause and effect. As the authors hypothesize, one potential mechanism would be that smoking impairs respiratory ciliary action, thereby increasing the likelihood that Puumala virus in an aerosol exposure would reach the alveoli and remain long enough to cause an infection.

Vaccine Prospects

Both rodent brain–derived and cell culture–derived inactivated vaccines to Hantaan and Seoul viruses have been developed in South Korea and China. Some of these vaccines induce neutralizing antibody responses in humans and are being tested in field studies, but none has been approved in the United States.[170,190,204,205] A commercial suckling mouse brain–derived vaccine marketed in Korea for more than a decade, Hantavax, has no reported safety issues but placebo-controlled trials have not been conducted and a case-control study did not have sufficient statistical power to demonstrate efficacy.[188] Monovalent and bivalent cell culture–based Hantaan virus– and Seoul virus–inactivated vaccines produced in China elicited neutralizing antibodies in more than 90 percent of persons tested who received three doses.[190]

A recombinant vaccinia strain expressing Hantaan virus antigens has been tested in human volunteers in the United States and has been shown to induce a serum neutralizing antibody response.[192] Because only 26 percent of vaccinia virus–immune volunteers developed neutralizing antibodies, this vaccine has not been pursued.[190] A plasmid DNA platform vaccine delivered by gene gun was developed because the platform allows ready construction of combination vaccines and because the absence of a protein component means that preexisting vector immunity is not a problem. DNA vaccines for Hantaan and Puumala viruses have been developed, tested in animals, and moved into phase I clinical trials.[190] Various other approaches to vaccine development against hantaviruses across the globe are underway,* but none has yet progressed to clinical trials. An immediate need exists for a vaccine to Hantaan virus and perhaps other hantaviruses, but lack of a realistic animal model of human disease has hampered efforts to perform full preclinical evaluation of candidates. This is particularly important because the diseases are immunopathologic and some monoclonal antibodies can enhance macrophage infection in vitro. The discovery that hamsters infected with Andes virus provide a reproducible model for HPS that mimics the human disease very closely[91] provides an avenue to explore mechanisms of disease and also vastly improves preclinical evaluation of hantavirus vaccines. Nonetheless, even more than with other viral vaccines, double-blind, placebo-controlled trials are needed urgently to provide definitive evidence of protection and to exclude adverse effects.

Hospital Infection Control

The use of universal precautions in handling the blood and body fluids of all patients is prudent practice. In addition, a certified biologic safety cabinet should be used for

*References 31, 32, 48, 77, 94, 134.

*References 18, 116, 135, 137, 148, 248.

all handling of human body fluids in situations in which splatter or aerosolization is possible.[24] Viral antigens can be detected in necropsy specimens, and RT-PCR readily detects viral genetic material in necropsy tissue and in blood and plasma obtained from hantavirus-infected persons early in the course of the disease.[87,245] However, secondary transmission of hantaviruses associated with HFRS has not been documented after contact with acutely ill persons or exposure to their clinical laboratory specimens.[223] The experience with North American hantaviruses linked to HPS has been similar.[24,234]

However, a well-documented episode of person-to-person spread during an Andes virus outbreak in South America and subsequent molecular and epidemiologic studies have demonstrated numerous occurrences of person-to-person transmission of Andes virus. Person-to-person spread has been reported to result in secondary cases among up to 3.4 percent of household contacts of primary HPS cases, with sexual partners at greatest risk.* With the possible exception of Andes virus infections, the many years of experience with HFRS and HPS indicate that once the diagnosis has been confirmed, isolation of hospitalized patients to prevent nosocomial transmission generally is not required. In plague-endemic areas, respiratory isolation pending confirmation of hantavirus infection may be a wise precaution. Sera and other specimens from hantavirus-infected persons can be handled safely by using biosafety level 2 facilities and practices. Higher biosafety levels are recommended for attempts to propagate hantaviruses.[24,31]

The full reference list for this chapter is available at expertconsult.com.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Acham-Roschitz B, Aberle SW, Pivker N, et al. Nephropathia epidemica (Puumala virus infection) in Austrian Children. *Pediatr Infect Dis J* 2010;**29**:874–6.
2. Adler FR, Pearce-Duvet JM, Dearing MD. How host population dynamics translate into time-lagged prevalence: an investigation of Sin Nombre virus in deer mice. *Bul Math Biol* 2008;**60**: 236–52.
7. Arai S, Bennett SN, Sumibcay L, et al. Phylogenetically distinct hantaviruses in the masked shrew *(Sorex cinereus)* and dusky shrew *(Sorex monticolus)* in the United States. *Am J Trop Med Hyg* 2008;**78**:348–51.
8. Arai S, Ohdachi SD, Asakawa M, et al. Molecular phylogeny of a newfound hantavirus in the Japanese shrew mole (*Urotrichus talpoides*). *Proc Natl Acad Sci USA* 2008;**21**:16296–301.
17. Brocato R, Joslyn M, Ballantyne J, et al. DNA vaccine-generated duck polyclonal antibodies as a postexposure prophylactic to prevent hantavirus pulmonary syndrome (HPS). *PLoS One* 2012;**7**:e35996.
18. Brown KS, Safronetz D, Marzi A, et al. Vesicular stomatitis virus–based vaccine protects hamsters against lethal challenge with Andes virus. *J Virol* 2011;**85**:12781–91.
27. Centers for Disease Control and Prevention. Hantavirus pulmonary syndrome in five pediatric patients—four states. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1409–12.
28. Centers for Disease Control and Prevention/National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. HHS publication No. (CDC) 21-1112. Washington, D.C.: U.S. Department of Health and Human Services, Public Health Service; 2009.
57. Ferris M, Sandoval C, Delgado I, et al. Hantavirosis: caracterización clinica-epidemiológica de pacientes pediátricas en Chile. *Rev Chil Infect* 2010;**27**:52–9.
65. Gavrilovskaya IN, Gorbunova EE, Mackow NA, et al. Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine-1-phosphate inhibit hantavirus-directed permeability. *J Virol* 2008;**82**:5797–806.
71. Godoy P, Marsac D, Stefas E, et al. Andes virus antigens are shed in urine of patients with acute hantavirus cardiopulmonary syndrome. *J Virol* 2009;**83**:5046–55.
74. Gorbunova EE, Gavrilovskaya IN, Pepini T, et al. VEGFR2 and Src kinase inhibitors suppress Andes virus–induced endothelial cell permeability. *J Virol* 2011;**85**:2296–303.
75. Gorbunova E, Gavrilovskaya IN, Mackow ER. Pathogenic hantaviruses Andes virus and Hantaan virus induce adherens junction disassembly by directing vascular endothelial cadherin internalization in human endothelial cells. *J Virol* 2010;**84**:7405–11.
76. Habjan M, Andersson I, Klingstrom J, et al. Processing of genome 5′ termini as a strategy of negative-strand RNA viruses to avoid RIGI-dependent interferon induction. *PLoS One* 2008;**3**:e2032.
79. Hautala T, Hautala N, Mähönen S-M, et al. Young male patients are at elevated risk of developing serious central nervous system complications during acute Puumala hantavirus infection. *BMC Infect Dis* 2011;**11**:217.
80. Hautala N, Kauma H, Vapalahti O, et al. Prospective study on ocular findings in acute Puumala hantavirus infection in hospitalized patients. *Br J Ophthalmol* 2011;**95**:559–62.
81. Hautala T, Mähönen S-M, Sironen T, et al. Central nervous system–related symptoms and findings are common in acute Puumala hantavirus infection. *Ann Med* 2010;**42**:344–51.
89. Hjelle B, Torres-Pérez F. Hantaviruses in the Americas and their role as emerging pathogens. *Viruses* 2010;**2**:2559–86.
90. Hjertqvist M, Klein SL, Ahlm C, et al. Mortality rate patterns for hemorrhagic fever with renal syndrome caused by Puumula virus. *Emerg Infect Dis* 2010;**16**:1584–6.
96. Huttunen N-P, Makela S, Pokka T, et al. Systematic literature review of symptoms, signs, and severity of serologically confirmed nephropathia epidemica in pediatric and adult patients. *Scand J Infect Dis* 2011;**43**:405–10.
99. Jonsson CB, Figueiredo LTM, Vapalahti O. A global perspective on hantavirus ecology, epidemiology, and disease. *Clin Microbiol Rev* 2010;**23**:412–41.
109. Klein SL, Marks MA, Li W, et al. Sex differences in the incidence and case fatality rates from hemorrhagic fever with renal syndrome in China, 2004-2008. *Clin Infect Dis* 2011;**52**:1414–21.
116. Kruger DH, Schonrich G, Klempa B. Human pathogenic hantaviruses and prevention of infection. *Hum Vaccine* 2011;**7**:685–93.
133. Levine JR, Prescott J, Brown KS, et al. Antagonism of type I interferon responses by new world hantaviruses. *J Virol* 2010;**84**:11790–801.
135. Li PY, Yu L, Wu XA, et al. Modification of the adenoviral transfer vector enhances expression of the hantavirus fusion protein GNS0.7 and induces a strong immune response in C57BL/6 mice. *J Virol Methods* 2012;**179**:90–6.
137. Liu F, Liang M, Cao S, et al. Fusion with extracellular domain of cytotoxic T-lymphocyte–associated antigen 4 leads to enhancement of immunogenicity of Hantaan virus DNA vaccines in C57Bl/6 mice. *Virol J* 2011;**8**:448.
140. Luis AD, Douglass RJ, Hudson PJ. Sin Nombre hantavirus decreases survival of male deer mice. *Oecologia* 2012;**169**:431–9.
141. MacNeil A, Comer JA, Ksiazek TG, et al. Sin Nombre virus–specific immunoglobulin M and G kinetics in hantavirus pulmonary syndrome and the role played by serologic responses in predicting disease outcome. *J Infect Dis* 2010;**202**:242–6.
142. MacNeil A, Ksiazek TG, Rollin PE. Hantavirus pulmonary syndrome United States, 1993-2009. *Emerg Infect Dis* 2011;**17**: 1195–201.
143. MacNeil A, Nichol ST, Spiropoulou CF. Hantavirus pulmonary syndrome. *Virus Res* 2011;**162**:138–47.
144. Makary P, Kanerva M, Ollgren J, et al. Diseases burden of Puumala virus infections, 1995-2008. *Epidemiol Infect* 2010;**138**:1484–92.
146. Martinez VP, Bellomo CM, Cacace ML, et al. Hantavirus pulmonary syndrome in Argentina, 1995-2008. *Emerg Infect Dis* 2010;**16**:1853–60.

*References 54, 56, 121, 147, 167, 219, 236.

148. Martinez VP, Padula PJ. Induction of protective immunity in a Syrian hamster model against a cytopathogenic strain of Andes virus. *J Med Virol* 2012;87–95.
154. Mills JN, Amman BR, Glass GE. Ecology of hantaviruses and their hosts in North America. *Vector Borne Zoonotic Dis* 2010;**10**:563–74.
164. Olsson GE, Leirs H, Henttonen H. Hantaviruses and their hosts in Europe: reservoirs here and there, but not everywhere? *Vector Borne Zoonotic Dis* 2010;**10**:549–61.
168. Palma RE, Polop JJ, Owen RN, et al. Ecology of rodent-associated hantaviruses in the Southern Cone of South America: Argentina, Chile, Paraguay, and Uruguay. *J Wildl Dis* 2012;**48**:267–81.
172. Pergam SA, Schmidt DQ, Nofchissey RA, et al. Potential renal sequelae in survivors of hantavirus cardiopulmonary syndrome. *Am J Trop Med Hyg* 2009;**80**:279–85.
186. Safronetz D, Haddock E, Feldmann F, et al. In vitro and in vivo activity of ribavirin against Andes virus infection. *PLoS One* 2011;**6**:e23560.
190. Schmaljohn C. Vaccines for hantaviruses. *Vaccine* 2009;**27**:D61–4.
199. Shrivastava-Ranjan P, Rollin PE, Spiropoulou CF. Andes virus disrupts the endothelial cell barrier by induction of vascular endothelial growth factor and down regulation of VE-cadherin. *J Virol* 2010;**84**:11227–34.
201. Simpson SQ, Spikes L, Patel S, et al. Hantavirus pulmonary syndrome. *Infect Dis Clin North Am* 2010;**24**:159–73.
212. Takeuchi O, Akira S. Innate immunity to virus infection. *Immunol Rev* 2009;**227**:75–86.
224. Vaheri A, Vapalahti O, Plyusnin A. How to diagnose hantavirus infections and detect them in rodents and insectivores. *Rev Med Virol* 2008;**18**:277–88.
230. Vapalahti K, Virtala AM, Vaheri A, et al. Case-control study on Puumala virus infection: smoking is a risk factor. *Epidemiol Infect* 2010;**138**:576–84.
248. Zhao C, Sun Y, Wang S, et al. Immunogenicity of a multi-epitope DNA vaccine against hantavirus. *Hum Vaccin Immunother* 2012;**8**:208–15.

CHAPTER 189

La Crosse Virus and Other California Serogroup Viruses

W. Garrett Hunt • James E. McJunkin

La Crosse virus (LACV) is a member of the California serogroup viruses within the family Bunyaviridae and the genus *Orthobunyavirus*. The term *California serogroup* does not describe the widespread geographic distribution of viruses in the serogroup but simply reflects the name of the prototype virus, California encephalitis virus (CEV), initially discovered in California in 1943.[67] LACV, the most prevalent and pathogenic member of the serogroup, causes disease in the upper midwestern, mid-Atlantic, and southeastern United States, particularly in Appalachia in more recent reports, but not in the West Coast (Fig. 189-1).* LACV is estimated to cause 8 to 30 percent of all cases of encephalitis in the United States annually, and in 2011, the most recent year for which data were available from the Centers for Disease Control and Prevention (CDC) at the time of the writing of this chapter, LACV was the most common arboviral infection of children in North America, a finding consistent with previous reports.[26,27,123] From 2003 to 2007, the incidence risk for all counties reporting probable and confirmed cases of LACV infection in children 15 years and younger to the CDC was 7.2 per 100,000 persons and 1.6 per 100,000 persons for the total population, including a group of states that reported no cases.[64] In a separate analysis, the average annual reported incidence of neuroinvasive disease (meningitis, encephalitis, and meningoencephalitis) caused by the California serogroup viruses as a whole from 1999 to 2007 was 0.035 case per 100,000 population per year (all of those patients for whom the etiology was tested had confirmed or probable California serogroup–associated disease).[121]

The medical, psychosocial, and economic costs to affected individuals in endemic areas are significant. Acute morbidity includes seizures in roughly half of hospitalized cases, mental status changes in one third, cerebral edema in 16 percent, and cerebral herniation in 2 to 3 percent. One fourth of all hospitalized patients require mechanical ventilation.[99] Long-term morbidity after

*References 4, 21, 54, 59, 64, 92, 97, 121, 138, 139.

REFERENCES

1. Acham-Roschitz B, Aberle SW, Pivker N, et al. Nephropathia epidemica (Puumala virus infection) in Austrian Children. *Pediatr Infect Dis J* 2010;**29**:874–6.
2. Adler FR, Pearce-Duvet JM, Dearing MD. How host population dynamics translate into time-lagged prevalence: an investigation of Sin Nombre virus in deer mice. *Bul Math Biol* 2008;**60**: 236–52.
3. Ahlm C, Linderholm M, Juto P, et al. Prevalence of serum IgG antibodies to Puumula virus (haemorrhagic fever with renal syndrome) in northern Sweden. *Epidemiol Infect* 1994;**113**:129–36.
4. Ahlm C, Settergren B, Gothefors L, et al. Nephropathia epidemica (hemorrhagic fever with renal syndrome) in children: clinical characteristics. *Pediatr Infect Dis J* 1994;**13**:45.
5. Alff PJ, Gavrilovskaya IN, Gorbunova E, et al. The pathogenic NY-1 hantavirus G1 cytoplasmic tail inhibits RIG-1– and TBK-1–directed interferon response. *J Virol* 2006;**80**:9676–86.
6. Antoniadis A, Stylianakis A, Papa A, et al. Direct genetic detection of Dobrava virus in Greek and Albanian haemorrhagic fever with renal syndrome (HFRS) patients. *J Infect Dis* 1996;**174**:407.
7. Arai S, Bennett SN, Sumibcay L, et al. Phylogenetically distinct hantaviruses in the masked shrew *(Sorex cinereus)* and dusky shrew *(Sorex monticolus)* in the United States. *Am J Trop Med Hyg* 2008;**78**:348–51.
8. Arai S, Ohdachi SD, Asakawa M, et al. Molecular phylogeny of a newfound hantavirus in the Japanese shrew mole *(Urotrichus talpoides)*. *Proc Natl Acad Sci USA* 2008;**21**:16296–301.
9. Arai S, Song JW, Sumibcay L, et al. Hantavirus in northern short-tailed shrew, United States. *Emerg Infect Dis* 2007;**13**:1420–2.
10. Armstrong LR, Bryan RT, Sarisky J, et al. Mild hantaviral disease caused by Sin Nombre virus in a four-year-old child. *Pediatr Infect Dis J* 1995;**14**:1108.
11. Avsic-Zupnac T, Likar M, Novakovic S, et al. Evidence of the presence of two hantaviruses in Slovenia. *Arch Virol* 1990;**115**(Suppl. 1):87.
12. Avsic-Zupanc T, Xiao SY, Stojanovic R, et al. Characterization of Dobrava virus: a hantavirus from Slovenia. *J Med Virol* 1992;**38**:132.
13. Bharadqaj M, Nofchissey R, Goade D, et al. Humoral immune responses in the hantavirus cardiopulmonary syndrome. *J Infect Dis* 2000;**182**:43–8.
14. Bohlman MC, Morzumor SP, Meissner J, et al. Analysis of hantavirus genetic diversity in Argentina: S segment–derived phylogeny. *J Virol* 2002;**76**:3765.
15. Borges AA, Campos GM, Moreli ML, et al. Hantavirus cardiopulmonary syndrome: immune response and pathogenesis. *Microbes Infect* 2006;**8**:2324.
16. Booth CC, Matukas LM, Tomlinson GA, et al. Clinical features and short-term outcomes of 144 patients with SARS in the greater Toronto area. *JAMA* 2003;**289**:2801.
17. Brocato R, Joslyn M, Ballantyne J, et al. DNA vaccine-generated duck polyclonal antibodies as a postexposure prophylactic to prevent hantavirus pulmonary syndrome (HPS). *PLoS One* 2012;**7**:e35996.
18. Brown KS, Safronetz D, Marzi A, et al. Vesicular stomatitis virus–based vaccine protects hamsters against lethal challenge with Andes virus. *J Virol* 2011;**85**:12781–91.
19. Bruno P, Hassell LH, Brown J, et al. The protean manifestations of hemorrhagic fever with renal syndrome: a retrospective review of 26 cases from Korea. *Ann Intern Med* 1990;**113**:385.
20. Bryan RT, Doyle TJ, Moolenaar RL, et al. Hantavirus pulmonary syndrome in children. *Semin Pediatr Infect Dis* 1997;**8**:1–7.
21. Carey DE, Reuben R, Panicker KN, et al. Thottapalayam virus: a presumptive arbovirus isolated from a shrew in India. *Indian J Med Res* 1971;**59**:1758–60.
22. Casals J, Henderson BE, Hoogstraal H, et al. A review of Soviet viral hemorrhagic fevers, 1969. *J Infect Dis* 1970;**122**:437–53.
23. Castillo C, Naranjo J, Sepulveda A, et al. Hantavirus pulmonary syndrome due to Andes virus in Temuco Chile: clinical experience with 16 adults. *Chest* 2001;**120**:548–54.
24. Centers for Disease Control and Prevention. Laboratory management of agents associated with hantavirus pulmonary syndrome: interim biosafety guidelines. *MMWR Recomm Rep* 1994;**43**:1–7.
25. Centers for Disease Control and Prevention. Hantavirus pulmonary syndrome: United States, 1995 and 1996. *MMWR Morb Mortal Wkly Rep* 1996;**45**:291–5.
26. Centers for Disease Control and Prevention. Hantavirus pulmonary syndrome: five states, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:627.
27. Centers for Disease Control and Prevention. Hantavirus pulmonary syndrome in five pediatric patients—four states. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1409–12.
28. Centers for Disease Control and Prevention/National Institutes of Health. *Biosafety in Microbiological and Biomedical Laboratories*. 5th ed. HHS publication No. (CDC) 21-1112. Washington, D.C.: U.S. Department of Health and Human Services, Public Health Service; 2009.
29. Chaparro J, Vega J, Terry W, et al. Assessment of person-to-person transmission of hantavirus pulmonary syndrome in a Chilean hospital setting. *J Hosp Infect* 1998;**40**:281–5.
30. Chapman LE, Ellis BA, Koster FT, et al. Discriminators between hantavirus-infected and uninfected persons enrolled in a trial of intravenous ribavirin for presumptive hantavirus pulmonary syndrome. *Clin Infect Dis* 2002;**34**:293–304.
31. Chapman LE, Khabbaz RF. Review: etiology and epidemiology of the Four Corners hantavirus outbreak. *Infect Agents Dis* 1994;**3**:234.
32. Chapman LE, Mertz GJ, Peters CJ, et al. Safety and tolerance during one year of open label experience. *Antiviral Ther* 1999;**4**:211–19.
33. Chen HX, Qiu FX, Dong BJ, et al. Epidemiological studies on hemorrhagic fever with renal syndrome in China. *J Infect Dis* 1986;**154**:394.
34. Childs JE, Ksiazek TG, Spiropoulou CF, et al. Serologic and genetic identification of *Peromyscus maniculatus* as the primary rodent reservoir for a new hantavirus in the southwestern United States. *J Infect Dis* 1994;**169**:1271.
35. Chu YK, Jennings G, Schmaljohn A, et al. Cross-neutralization of hantaviruses with immune sera from experimentally infected animals and from hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome patients. *J Infect Dis* 1995;**172**:1581.
36. Chu YK, Rossi C, LeDuc JW, et al. Serological relationships among viruses in the *Hantavirus* genus, family *Bunyaviridae*. *Virology* 1994;**198**:196.
37. Clement J, Neild GH, Maes P, et al. Symptomatic human hantavirus in the Americas [letter]. *Emerg Infect Dis* 2007;**13**:345.
38. Collan Y, Mihatsch MJ, Lahdevirta J, et al. Nephropathia epidemica: Mild variant of hemorrhagic fever with renal syndrome. *Kidney Int* 1991;**40**(Suppl. 35):62.
39. Cosgriff TM. Mechanisms of disease in hantavirus infection: pathophysiology of hemorrhagic fever with renal syndrome. *Rev Infect Dis* 1991;**13**:97.
40. Crowley MR, Katz RW, Kessler R, et al. Successful treatment of adults with severe hantavirus pulmonary syndrome with extracorporeal membrane oxygenation. *Crit Care Med* 1998;**26**:409–14.
41. de Carvalho Nicacio C, Sallberg M, Hultgren C, et al. T-helper and humoral responses to Puumala hantavirus nucleocapsid protein: identification of T-helper epitopes in a mouse model. *J Gen Virol* 2001;**82**:129–38.
42. Desmyter J, LeDuc JW, Johnson KM, et al. Laboratory rat associated outbreak of haemorrhagic fever with renal syndrome due to Hantaan-like virus in Belgium. *Lancet* 1983;**2**:445.
43. Dietl CA, Wernly JA, Pett SB, et al. Extracorporeal membrane oxygenation support improves survival of patients with severe hantavirus cardiopulmonary syndrome. *J Thorac Cardiovasc Surg* 2008;**135**:579–84.
44. Dohmae K, Koshimizu U, Nishimune Y. In utero and mammary transfer of hantavirus antibody from dams to infant rats. *Lab Anim Sci* 1993;**43**:557–61.
45. Douglass RJ, Calisher CH, Wagoner KD. Sin Nombre virus infection of deer mice in Montana: characteristics of newly infected mice, incidence, and temporal pattern of infection. *J Wildl Dis* 2007;**43**:12–22.
46. Douglass RJ, Wilson T, Semmens WJ, et al. Longitudinal studies of Sin Nombre virus in deer mouse–dominated ecosystems of Montana. *Am J Trop Med Hyg* 2001;**65**:33–41.
47. Dournon E, Moriniere B, Matheron S, et al. HFRS after a wild rodent bite in the Haute-Savoie and risk of exposure to Hantaan-like virus in a Paris laboratory. *Lancet* 1984;**1**:676.
48. Duchin JS, Koster FT, Peters CJ, et al. Hantavirus pulmonary syndrome: a clinical description of 17 patients with a newly recognized disease. *N Engl J Med* 1994;**330**:949.

49. Dusek J, Pejcoch M, Kolsky A, et al. Mild course of Puumula nephropathy in children in an area with sporadic occurrence hantavirus infection. *Pediatr Nephrol* 2006;**21**:1889.
50. Earle DP. Symposium on epidemic hemorrhagic fever. *Am J Med* 1954;**16**:619.
51. Elliott L, Ksiazek TG, Rollin PE, et al. Isolation of the causative agent of hantavirus pulmonary syndrome. *Am J Trop Med Hyg* 1994;**51**:102.
52. Engelthaler DM, Mosley DG, Cheek JE, et al. Climatic and environmental patterns associated with hantavirus pulmonary syndrome, Four Corners region, United States. *Emerg Infect Dis* 1999;**5**:87.
53. Ennis FA, Cruz J, Spiropoulou CF, et al. Hantavirus pulmonary syndrome: $CD8^+$ and $CD4^+$ cytotoxic T lymphocytes to epitopes on Sin Nombre virus nucleocapsid protein isolated during acute illness. *Virology* 1997;**238**:380–90.
54. Enria D, Padula P, Segura EL, et al. Hantavirus pulmonary syndrome in Argentina: possibility of person-to-person transmission. *Medicina (Bueno Aires)* 1996;**56**:709–11.
55. Feldmann H, Sanchez A, Morzunov S, et al. Utilization of autopsy RNA for the synthesis of the nucleocapsid antigen of a newly recognized virus associated with hantavirus pulmonary syndrome. *Virus Res* 1993;**30**:351.
56. Ferres M, Vial P, Marco C, et al. Prospective evaluation of household contacts of persons with hantavirus cardiopulmonary syndrome in Chile. *J Infect Dis* 2007;**195**:1563–71.
57. Ferris M, Sandoval C, Delgado I, et al. Hantavirosis: caracterización clinica-epidemiológica de pacientes pediátricas en Chile. *Rev Chil Infect* 2010;**27**:52–9.
58. French GR, Foulke RS, Brand OA, et al. Korean hemorrhagic fever: propagation of the etiologic agent in a cell line of human origin. *Science* 1981;**211**:1046.
59. Fulhorst CF, Milazzo ML, Armstrong LR, et al. Hantavirus and arenavirus antibodies in persons with occupational rodent exposure, North America. *Emerg Infect Dis* 2007;**13**:532.
60. Gajdusek DC. *Acute infectious hemorrhagic fevers and mycotoxicoses in the Union of Soviet Socialist Republics. Medical science publication No. 2*. Washington, D.C.: Army Medical Service Graduate School, Walter Reed Army Medical Center; 1953.
61. Gajdusek DC. Hemorrhagic fevers in Asia: a problem in medical ecology. *Geogr Rev* 1956;**41**:20.
62. Gajdusek DC. Virus hemorrhagic fevers: Special reference to hemorrhagic fever with renal syndrome (epidemic hemorrhagic fever). *J Pediatr* 1962;**60**:841.
63. Garcin D, Lezzi M, Dobbs M, et al. The 5′ ends of Hantaan virus *(Bunyaviridae)* RNAs suggest a prime-and-realign mechanism for the initiation of RNA synthesis. *J Virol* 1995;**69**: 5754–62.
64. Garcia-Sastre A, Biron CA. Type I interferons and the virus-host relationship: a lesson in détente. *Science* 2006;**312**:879–82.
65. Gavrilovskaya IN, Gorbunova EE, Mackow NA, et al. Hantaviruses direct endothelial cell permeability by sensitizing cells to the vascular permeability factor VEGF, while angiopoietin 1 and sphingosine-1-phosphate inhibit hantavirus-directed permeability. *J Virol* 2008;**82**: 5797–806.
66. Geimonen E, Neff S, Raymond T, et al. Pathogenic and nonpathogenic hantaviruses differentially regulate endothelial cell responses. *Proc Natl Acad Sci U S A* 2002;**99**:13,837–42.
67. Glass GE, Cheek JE, Patz JA, et al. Using remotely sensed data to identify areas at risk for hantavirus pulmonary syndrome. *Emerg Infect Dis* 2000;**6**:238–47.
68. Glass GE, Watson AJ, LeDuc JW, et al. Infection with rat borne hantavirus in U.S. residents is consistently associated with hypertensive renal disease. *J Infect Dis* 1993;**167**:614.
69. Glass GE, Watson AJ, LeDuc JW, et al. Domestic cases of hemorrhagic fever with renal syndrome in the United States. *Nephron* 1994;**68**:48.
70. Gligic A, Dimkovic N, Xiao SY, et al. Belgrade virus: a new hantavirus causing severe hemorrhagic fever with renal syndrome in Yugoslavia. *J Infect Dis* 1992;**166**:113.
71. Godoy P, Marsac D, Stefas E, et al. Andes virus antigens are shed in urine of patients with acute hantavirus cardiopulmonary syndrome. *J Virol* 2009;**83**:5046–55.
72. Goldsmith CS, Elliot LH, Peters CJ, et al. Ultrastructural characteristics of Sin Nombre virus, causative agent of hantavirus pulmonary syndrome. *Arch Virol* 1995;**140**:2107.
73. Gonzalez-Scarano F, Nathanson N. *Bunyaviridae*. In: Fields BN, Knipe DM, Howley PM, et al, editors. *Fields virology*. 3rd ed. Philadelphia: Lippincott-Raven; 1996. p. 1473–504.
74. Gorbunova E, Gavrilovskaya IN, Pepini T, et al. VEGFR2 and Src kinase inhibitors suppress Andes virus–induced endothelial cell permeability. *J Virol* 2011;**85**:2296–303.
75. Gorbunova E, Gavrilovskaya IN, Mackow ER. Pathogenic hantaviruses Andes virus and Hantaan virus induce adherens junction disassembly by directing vascular endothelial cadherin internalization in human endothelial cells. *J Virol* 2010;**84**:7405–11.
76. Habjan M, Andersson I, Klingstrom J, et al. Processing of genome 5′ termini as a strategy of negative-strand RNA viruses to avoid RIGI-dependent interferon induction. *PLoS One* 2008;**3**: e2032.
77. Hallin GW, Simpson SQ, Crowell RE, et al. Cardiopulmonary manifestations of hantavirus pulmonary syndrome. *Crit Care Med* 1996;**24**:252.
78. Hardestam J, Simon M, Hedlund KO, et al. Ex-vivo stability of the rodent-borne Hantaan virus in comparison to arthropod-borne members of the *Bunyaviridae* family. *Appl Environ Microbiol* 2007;**73**:2547–55.
79. Hautala T, Hautala N, Mähönen S-M, et al. Young male patients are at elevated risk of developing serious central nervous system complications during acute Puumala hantavirus infection. *BMC Infect Dis* 2011;**11**:217.
80. Hautala N, Kauma H, Vapalahti O, et al. Prospective study on ocular findings in acute Puumala hantavirus infection in hospitalized patients. *Br J Ophthalmol* 2011;**95**:559–62.
81. Hautala T, Mähönen S-M, Sironen T, et al. Central nervous system-related symptoms and findings are common in acute Puumala hantavirus infection. *Ann Med* 2010;**42**:344–51.
82. Hayasaka D, Maeda K, Ennis FA, et al. Increased permeability of human endothelial cell line EA.hy926 induced by hantavirus-specific cytotoxic T lymphocytes. *Virus Res* 2007;**123**:120.
83. Hinojosa M, Villagra E, Mora J, et al. Identification of infectious agents in cases suspicious of hantavirus infection but with negative serology. *Rev Med Chil* 2006;**134**:332.
84. Hjelle B, Goade D, Torrez-Martinez N, et al. Hantavirus pulmonary syndrome, renal insufficiency and myositis associated with infection by Bayou hantavirus. *Clin Infect Dis* 1996;**23**:495–500.
85. Hjelle B, Jenison SA, Goade DE, et al. Hantaviruses: clinical microbiologic, and epidemiologic aspects. *Crit Rev Clin Lab Sci* 1995;**32**:469–508.
86. Hjelle B, Krolikowski J, Torrez-Martinez N, et al. Phylogenetically distinct hantavirus implicated in a case of hantavirus pulmonary syndrome in the northeastern United States. *J Med Virol* 1995;**46**:21.
87. Hjelle B, Spiropoulou CF, Torrez-Martinez N, et al. Detection of Muerto Canyon virus RNA in peripheral blood mononuclear cells from patients with hantavirus pulmonary syndrome. *J Infect Dis* 1994;**170**:1013.
88. Hjelle B, Torrez-Martinez N, Koster FT. Hantavirus pulmonary syndrome–related virus from Bolivia. *Lancet* 1996;**347**:57.
89. Hjelle B, Torres-Pérez F. Hantaviruses in the Americas and their role as emerging pathogens. *Viruses* 2010;**2**:2559–86.
90. Hjertqvist M, Klein SL, Ahlm C, et al. Mortality rate patterns for hemorrhagic fever with renal syndrome caused by Puumula virus. *Emerg Infect Dis* 2010;**16**:1584–6.
91. Hooper JW, Larsen T, Duster DM, Schmaljohn CS. A lethal disease model for hantavirus pulmonary syndrome. *Virology* 2001;**289**:6–14.
92. Hortling H. En epidemi av falteben in finska Lappland. *Nord Med* 1946;**30**:1001.
93. Huang C, Jim B, Wang M, et al. Hemorrhagic fever with renal syndrome: relationship between pathogenesis and cellular immunity. *J Infect Dis* 1994;**169**:868–70.
94. Huggins JW, Hsiang CM, Cosgriff TM, et al. Prospective, double-blind, concurrent, placebo-controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis* 1991;**164**:1119.
95. Hullinghorst RL, Steer A. Pathology of epidemic hemorrhagic fever. *Ann Intern Med* 1953;**38**:77.
96. Huttunen N-P, Makela S, Pokka T, et al. Systematic literature review of symptoms, signs, and severity of serologically confirmed nephropathia epidemica in pediatric and adult patients. *Scand J Infect Dis* 2011;**43**:405–10.

97. Jenison S, Yamada T, Morris C, et al. Characterization of human antibody responses to Four Corners hantavirus infections among patients with hantavirus pulmonary syndrome. *J Virol* 1994;**68**:3000.
98. Jiang YT. A preliminary report on hemorrhagic fever with renal syndrome in China. *Chin Med J* 1983;**96**:265.
99. Jonsson CB, Figueiredo LTM, Vapalahti O. A global perspective on hantavirus ecology, epidemiology, and disease. *Clin Microbiol Rev* 2010;**23**:412–41.
100. Kallio ER, Klingstrom J, Gustafsson E, et al. Prolonged survival of Puumala hantavirus outside the host: evidence for indirect transmission via the environment. *J Gen Virol* 2006;**87**:2127.
101. Kallio ER, Poikonen A, Vaheri A, et al. Maternal antibodies postpone hantavirus infection and enhance individual breeding success. *Proc Biol Sci* 2006;**273**:2771–6.
102. Kawamata J, Yamanouchi T, Dohmae K, et al. Control of laboratory acquired hemorrhagic fever with renal syndrome (HFRS) in Japan. *Lab Anim Sci* 1987;**37**:431.
103. Ketai LH, Williamson MR, Telepak RJ, et al. Hantavirus pulmonary syndrome: radiographic findings in 16 patients. *Radiology* 1994;**191**:665.
104. Khan AS, Gaviria M, Rollin PE, et al. Hantavirus pulmonary syndrome in Florida: association with the newly identified Black Creek Canal virus. *Am J Med* 1996;**100**:46.
105. Khan AS, Khabbaz RF, Armstrong LR, et al. Hantavirus pulmonary syndrome: the first 100 U.S. cases. *J Infect Dis* 1996;**173**:1297.
106. Khan AS, Ksiazek TG, Zaki SR, et al. Fatal hantavirus pulmonary syndrome in an adolescent. *Pediatrics* 1995;**95**:276.
107. Khan AS, Spiropoulou CF, Morzunov S, et al. Fatal illness associated with a new hantavirus in Louisiana. *J Med Virol* 1995;**46**:281.
108. Kilpatrick ED, Terajima M, Koster FT, et al. Role of specific $CD8^+$ T cells in the severity of a fulminant zoonotic viral hemorrhagic fever, hantavirus pulmonary syndrome. *J Immunol* 2004;**172**: 3297–304.
109. Klein SL, Marks MA, Li W, et al. Sex differences in the incidence and case fatality rates from hemorrhagic fever with renal syndrome in China, 2004-2008. *Clin Infect Dis* 2011;**52**:1414–21.
110. Klempa B, Fichet-Clavet E, Lecompte E, et al. Hantavirus in African wood mouse, Guinea. *Emerg Infect Dis* 2006;**12**:838–40.
111. Klempa B, Fichet-Clavet E, Lecompte E, et al. Novel hantavirus sequences in shrew, Guinea. *Emerg Infect Dis* 2007;**13**:520–2.
112. Ko KW. Korean hemorrhagic fever in Korean children. In: Murakami K, Sakai Y, editors. *Recent advances in pediatric nephrology*. Amsterdam: Elsevier; 1987. p. 643–6.
113. Kolman J. Some physical and chemical properties of Uukuniemi virus, strain Potepli-63. *Acta Virol* 1970;**14**:159.
114. Koster F, Foucar K, Hjelle B, et al. Rapid presumptive diagnosis of hantavirus cardiopulmonary syndrome by peripheral blood smear review. *Am J Clin Pathol* 2001;**116**:665.
115. Krakauer T, Leduc JW, Morrill JC, et al. Serum levels of alpha and gamma interferons in hemorrhagic fever with renal syndrome. *Viral Immunol* 1994;**7**:97–101.
116. Kruger DH, Schonrich G, Klempa B. Human pathogenic hantaviruses and prevention of infection. *Hum Vaccine* 2011;**7**: 685–93.
117. Ksiazek TG, Peters CJ, Rollin PE, et al. Identification of a new North American hantavirus that causes acute pulmonary insufficiency. *Am J Trop Med Hyg* 1995;**52**:117.
118. Lahdevirta J. Nephropathia epidemica in Finland: a clinical, histological and epidemiological study. *Ann Clin Res* 1971;**3**(Suppl. 8):1.
119. Lahdevirta J, Collan YK, Jokinen EJ, et al. Renal sequelae to nephropathia epidemica. *Acta Pathol Microbiol Scand* 1978;**86**:265.
120. Lautala P, Uhari M. Epidemic nephropathy in children. *Am J Dis Child* 1991;**145**:1181–3.
121. Lazaro ME, Cantoni GE, Calanni LM, et al. Clusters of hantavirus infection, southern Argentina. *Emerg Infect Dis* 2007;**13**:104.
122. LeDuc JW, Childs JE, Glass GE. The hantaviruses, etiologic agents of hemorrhagic fever with renal syndrome: a possible cause of hypertension and chronic renal disease in the United States. *Annu Rev Public Health* 1992;**13**:79.
123. Lee BE, Joffe AR, Vaudry W. Hantavirus pulmonary syndrome: report of the first Canadian pediatric case. *Can J Infect Dis* 1998;**9**:319.
124. Lee HW. Epidemiology. In: Lee HW, Dalrymple JM, editors. *Manual of hemorrhagic fever with renal syndrome*. Seoul: World Health Organization Collaborating Center for Virus Reference and Research Institute for Viral Diseases; 1989. p. 39–48.
125. Lee HW. *Epidemiologic features of Korean hemorrhagic fever and research activities on this disease in the Republic of Korea*. Paper presented by the World Health Organization Working Group on Hemorrhagic Fever with Renal Syndrome, Tokyo, February 1982.
126. Lee HW, Johnson KM. Laboratory-acquired infections with Hantaan virus, the etiologic agent of Korean hemorrhagic fever. *J Infect Dis* 1982;**146**:645.
127. Lee HW, French GR, Lee PW, et al. Observations on natural and laboratory infection of rodents with the etiologic agent of Korean hemorrhagic fever. *Am J Trop Med Hyg* 1981;**30**:477.
128. Lee HW, Lee PW, Baek LJ, et al. Intraspecific transmission of Hantaan virus, etiologic agent of Korean hemorrhagic fever, in the rodent *Apodemus agrarius*. *Am J Trop Med Hyg* 1981;**30**:1106.
129. Lee HW, Lee PW, Baek LJ, et al. Geographical distribution of hemorrhagic fever with renal syndrome and hantaviruses. *Arch Virol* 1990;**115**(Suppl. 1):5.
130. Lee HW, Lee PW, Johnson KM. Isolation of the etiologic agent of Korean hemorrhagic fever. *J Infect Dis* 1978;**137**:298.
131. Lee JS. Clinical features of hemorrhagic fever with renal syndrome in Korea. *Kidney Int* 1991;**40**(Suppl. 35):88.
132. Lee PW, Meegan JM, LeDuc JW, et al. Serologic techniques for detection of Hantaan virus infection, related antigens and antibodies. In: Lee HW, Dalrymple JM, editors. *Manual of hemorrhagic fever with renal syndrome*. Seoul: World Health Organization Collaborating Center for Virus Reference and Research Institute for Viral Diseases; 1989. p. 36–8.
133. Levine JR, Prescott J, Brown KS, et al. Antagonism of type I interferon responses by new world hantaviruses. *J Virol* 2010;**84**:11790–801.
134. Levy H, Simpson SQ. Hantavirus pulmonary syndrome. *Am J Respir Crit Care Med* 1994;**149**:1710.
135. Li PY, Yu L, Wu XA, et al. Modification of the adenoviral transfer vector enhances expression of the Hantavirus fusion protein GNS0.7 and induces a strong immune response in C57BL/6 mice. *J Virol Methods* 2012;**179**:90–6.
136. Linderholm M, Ahlm C, Settergren B, et al. Elevated plasma levels of tumor necrosis factor (TNF)-alpha, soluble TNF receptors, interleukin (IL)-6, and IL-10 in patients with hemorrhagic fever with renal syndrome. *J Infect Dis* 1996;**173**:298–308.
137. Liu F, Liang M, Cao S, et al. Fusion with extracellular domain of cytotoxic T-lymphocyte-associated–antigen 4 leads to enhancement of immunogenicity of Hantaan virus DNA vaccines in C57Bl/6 mice. *Virol J* 2011;**8**:448.
138. Lloyd G, Bowen ETW, Jones N, et al. HFRS outbreak associated with laboratory rats in UK. *Lancet* 1984;**1**:175.
139. Lopez N, Padula P, Rossi C, et al. Genetic identification of a new hantavirus causing severe pulmonary syndrome in Argentina. *Virology* 1996;**220**:223.
140. Luis AD, Douglass RJ, Hudson PJ. Sin Nombre hantavirus decreases survival of male deer mice. *Oecologia* 2012;**169**:431–9.
141. MacNeil A, Comer JA, Ksiazek TG, et al. Sin Nombre virus-specific immunoglobulin M and G kinetics in hantavirus pulmonary syndrome and the role played by serologic responses in predicting disease outcome. *J Infect Dis* 2010;**202**:242–6.
142. MacNeil A, Ksiazek TG, Rollin PE. Hantavirus pulmonary syndrome United States, 1993-2009. *Emerg Infect Dis* 2011;**17**: 1195–201.
143. MacNeil A, Nichol ST, Spiropoulou CF. Hantavirus pulmonary syndrome. *Virus Res* 2011;**162**:138–47.
144. Makary P, Kanerva M, Ollgren J, et al. Diseases burden of Puumala virus infections, 1995-2008. *Epidemiol Infect* 2010;**138**:1484–92.
145. Marcus KA, Johann-Liang R, Powers JH. Ribavirin trials and hantavirus: what we should not conclude. *Clin Infect Dis* 2005;**40**:1550.
146. Martinez VP, Bellomo CM, Cacace ML, et al. Hantavirus pulmonary syndrome in Argentina, 1995-2008. *Emerg Infect Dis* 2010;**16**:1853–60.
147. Martinez VP, Bellomo C, San Juan J, et al. Person-to-person transmission of Andes virus. *Emerg Infect Dis* 2005;**11**:1848.
148. Martinez VP, Padula PJ. Induction of protective immunity in a Syrian hamster model against a cytopathogenic strain of Andes virus. *J Med Virol* 2012;87–95.
149. Mayer CF. Epidemic hemorrhagic fever of the Far East, or endemic hemorrhagic nephrosonephritis, a short outline of the

disease, with supplemental data on the results of experimental inoculation of human volunteers. *Mil Surg* 1952;**110**:276.
150. McKee KT Jr, LeDuc JW, Peters CJ. Hantaviruses. In: Belshe RB, editor. *Textbook of human virology*. 2nd ed. Littleton, MA: PSG Publishing; 1991. p. 615–32.
151. McNinch JH. Far East command conference on epidemic hemorrhagic fever. *Ann Intern Med* 1953;**38**:53.
152. Mertz GJ, Miedzinski L, Goade D, et al. Placebo-controlled, double-blind trial of intravenous ribavirin for the treatment of hantavirus cardiopulmonary syndrome in North America. *Clin Infect Dis* 2004;**39**:1307.
153. Miettinen MH, Makela SM, Ala-Houhala IO, et al. Ten-year prognosis of Puumala hantavirus–induced acute interstitial nephritis. *Kidney Int* 2006;**69**:2043.
154. Mills JN, Amman BR, Glass GE. Ecology of hantaviruses and their hosts in North America. *Vector Borne Zoonotic Dis* 2010;**10**:563–74.
155. Mills JN, Corneli A, Young JC, et al. Hantavirus pulmonary syndrome—United States: updated recommendations for risk reduction. *MMWR Morb Mortal Wkly Rep* 2002;**51**:1–12.
156. Mills JN, Ksiazek TG, Ellis BA, et al. Patterns of association with host and habitat: antibody reactive with Sin Nombre virus in small mammals in the major biotic communities of the southwestern United States. *Am J Trop Med Hyg* 1997;**56**:273–81.
157. Moolenaar RL, Dalton C, Lipman HB, et al. Clinical features that differentiate hantavirus pulmonary syndrome from three other acute respiratory illnesses. *Clin Infect Dis* 1995;**21**:643.
158. Mustonen J, Huttunen NP, Brummer-Korvenkontio M, et al. Clinical picture of nephropathia epidemica in children. *Acta Pediatr* 1994;**83**:526.
159. Nichol ST, Rollin PE, Ksiazek TG, et al. Hantavirus pulmonary syndrome and newly described hantaviruses in the United States. In: Elliott RH, editor. *Bunyaviridae*. New York: Plenum Press; 1996. p. 269–80.
160. Nichol ST, Spiropoulou CF, Morzunov S, et al. Genetic identification of a hantavirus associated with an outbreak of acute respiratory illness. *Science* 1993;**262**:914.
161. Niklasson B, Hellsten G, LeDuc J. Hemorrhagic fever with renal syndrome: a study of sequelae following nephropathia epidemica. *Arch Virol* 1994;**137**:241.
162. Niklasson B, LeDuc JW. Isolation of the nephropathia epidemica agent in Sweden. *Lancet* 1984;**1**:1012.
163. Niklasson B, LeDuc J, Nystrom K, et al. Nephropathia epidemica: incidence of clinical cases and antibody prevalence in an endemic area of Sweden. *Epidemiol Infect* 1987;**99**:559.
164. Olsson GE, Leirs H, Henttonen H. Hantaviruses and their hosts in Europe: reservoirs here and there, but not everywhere? *Vector Borne Zoonotic Dis* 2010;**10**:549–61.
165. Overturf GD. Clinical Sin Nombre hantaviral infections in children. *Pediatr Infect Dis J* 2005;**24**:373.
166. Padula P, Edelstein A, Miguel SD, et al. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* 1998;**241**:323–30.
167. Padula P, Martinez VP, Bellomo C, et al. Pathogenic hantaviruses, northeastern Argentina and eastern Paraguay. *Emerg Infect Dis* 2007;**13**:1211–14.
168. Palma RE, Polop JJ, Owen RN, et al. Ecology of rodent-associated hantaviruses in the Southern Cone of South America: Argentina, Chile, Paraguay, and Uruguay. *J Wildl Dis* 2012;**48**:267–81.
169. Parisi MDN, Enria DA, Piri NC, et al. Retrospective detection of clinical infection caused by hantavirus in Argentina. *Medicina (Bueno Aires)* 1996;**56**:1.
170. Park K, Kim CS, Moon KT. Protective effectiveness of hantavirus vaccine. *Emerg Infect Dis* 2004;**10**:2218–20.
171. Peco-Antic A, Popovic-Rolovic M, Gligic A, et al. Clinical characteristics of haemorrhagic fever with renal syndrome in children. *Pediatr Nephrol* 1992;**6**:335.
172. Pergam SA, Schmidt DQ, Nofchissey RA, et al. Potential renal sequelae in survivors of hantavirus cardiopulmonary syndrome. *Am J Trop Med Hyg* 2009;**80**:279–85.
173. Peters CJ. Pathogenesis of viral hemorrhagic fevers. In: Nathanson N, Ahmed R, Gonzalez-Scarano F, et al, editors. *Viral pathogenesis*. Philadelphia: Lippincott-Raven; 1996. p. 779–800.
174. Peters CJ, Khan AS. Hantavirus pulmonary syndrome: the new American hemorrhagic fever. *Clin Infect Dis* 2002;**34**:1224–31.
175. Pilaski J, Feldman H, Morzunov S, et al. Genetic identification of a new Puumala virus strain causing severe hemorrhagic fever with renal syndrome in Germany. *J Infect Dis* 1994;**170**:1456.
176. Pini NC, Resa A, del Jesus Laime G, et al. Hantavirus infection in children in Argentina. *Emerg Infect Dis* 1998;**4**:85–7.
177. Plyusnin A. Genetics of hantaviruses: implications to taxonomy. *Arch Virol* 2002;**147**:665–82.
178. Plyusnin A, Vapalahti O, Lankinen H, et al. Tula virus: a newly detected hantavirus carried by European common voles. *J Virol* 1994;**68**:7833–9.
179. Pon E, McKee KT Jr, Diniega BM, et al. Outbreak of hemorrhagic fever with renal syndrome among U.S. Marines in Korea. *Am J Trop Med Hyg* 1990;**42**:612.
180. Prince HN, Prince DL, Prince RN. Principles of viral control and transmission. In: Block SS, editor. *Disinfection, sterilization, and preservation*. 4th ed. Philadelphia: Lea & Febiger; 1991. p. 411–44.
181. Ramos MM, Overturf GD, Crowley MR, et al. Infection with Sin Nombre hantavirus: clinical presentation and outcome in children and adolescents. *Pediatrics* 2001;**108**:e27.
182. Richardson P, McKenna W, Bristow M, et al. Report of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force on the Definition and Classification of Cardiomyopathies. *Circulation* 1996;**93**:841–2.
183. Riquelme R, Riquelme M, Torres A, et al. Hantavirus pulmonary syndrome, southern Chile. *Emerg Infect Dis* 2003;**9**:1438–43.
184. Rollin PE, Ksiazek TG, Elliot LH, et al. Isolation of Black Creek Canal virus, a new hantavirus from *Sigmodon hispidus* in Florida. *J Med Virol* 1995;**46**:35.
185. Rowe RK, Pekosz A. Bidirectional virus secretion and non-ciliated cell tropism following Andes virus infection of primary airway epithelial cell culture. *J Virol* 2006; **80**:1087–97.
186. Safronetz D, Haddock E, Feldmann F, et al. In vitro and in vivo activity of ribavirin against Andes virus infection. *PLoS One* 2011;**6**:e23560.
187. Saggioro FP, Rossi MA, Duarte MIS, et al. Hantavirus infection induces a typical myocarditis that may be responsible for myocardial depression and shock in hantavirus pulmonary syndrome. *J Infect Dis* 2007;**195**:1541–9.
188. Santana RD, Campos GM, Figueiredo LTM, et al. Clinical and laboratory findings related to a favorable evolution of hantavirus pulmonary syndrome. *Rev Soc Bras Med Trop* 2006;**39**:237.
189. Schmaljohn A, Li D, Negley DL, et al. Isolation and initial characterization of a new found hantavirus from California. *Virology* 1995;**206**:963.
190. Schmaljohn C. Vaccines for hantaviruses. *Vaccine* 2009;**27**: D61–4.
191. Schmaljohn CS, Dalrymple JM. Analysis of Hantaan virus RNA: evidence for a new genus of *Bunyaviridae*. *Virology* 1983;**131**:482.
192. Schmaljohn CS, Hasty SE, Dalrymple JM. Preparation of candidate vaccinia-vectored vaccines for haemorrhagic fever with renal syndrome. *Vaccine* 1992;**10**:10.
193. Schmaljohn CS, Hasty SE, Dalrymple JM, et al. Antigenic and genetic properties of viruses linked to hemorrhagic fever with renal syndrome. *Science* 1985;**227**:1041.
194. Schmaljohn CS, Hooper JW. *Bunyaviridae:* the viruses and their replication. In: Knipe DM, Howley PM, Griffin DE, editors. *Fields virology*. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1635–68.
195. Schmaljohn CS, Huggins J, Calisher CH. Laboratory and field safety. In: Lee HW, Calisher CH, Schmaljohn CS, editors. *Manual of hemorrhagic fever with renal syndrome and hantavirus pulmonary syndrome*. Seoul: World Health Organization Collaborating Center for Virus Reference and Research (Hantaviruses), Asian Institute for Life Sciences; 1999. p. 192–8.
196. Seitsonen E, Hynninen M, Kolho E, et al. Corticosteroids combined with continuous veno-venous hemodiafiltration for treatment of hantavirus pulmonary syndrome caused by Puumala virus infection. *Eur J Clin Microbiol Infect Dis* 2006;**25**:261.
197. Settergren B. Nephropathia epidemica (hemorrhagic fever with renal syndrome) in Scandinavia. *Rev Infect Dis* 1991;**13**:736.
198. Sheedy JA, Froed BF, Batson HA, et al. The clinical course of epidemic hemorrhagic fever. *Am J Med* 1954;**16**:619.
199. Shrivastava-Ranjan P, Rollin PE, Spiropoulou CF. Andes virus disrupts the endothelial cell barrier by induction of vascular

endothelial growth factor and down regulation of VE-cadherin. *J Virol* 2010;**84**:11227–34.
200. Simonsen L, Dalton MJ, Breiman RF, et al. Evaluation of the magnitude of the 1993 hantavirus outbreak in the southwestern United States. *J Infect Dis* 1995;**172**:729.
201. Simpson SQ, Spikes L, Patel S, et al. Hantavirus pulmonary syndrome. *Infect Dis Clin North Am* 2010;**24**:159–73.
202. Smith JJ. Three-week incubation period for hantavirus infection. *Pediatr Infect Dis J* 2004;**23**:974.
203. Smorodintsev AA, Kazbintsev LI, Chudakov VG. *Viral hemorrhagic fevers*. Washington, D.C.: Office of Technical Services, U.S. Department of Commerce; 1964. p. 1.
204. Song G. Epidemiological progresses of hemorrhagic fever with renal syndrome in China. *Chin Med J* 1999;**112**:472–7.
205. Song G, Huang YC, Hang CS, et al. Preliminary human trial of inactivated golden hamster kidney cell (GHKC) vaccine against haemorrhagic fever with renal syndrome. *Vaccine* 1992;**10**: 214–16.
206. Song JW, Back LJ, Gajdusek DC, et al. Isolation of pathogenic hantavirus from white-footed mouse *(Peromyscus leucopus)*. *Lancet* 1994;**344**:1637.
207. Song JW, Gu SH, Bennett SN, et al. Seewis virus, a genetically distinct hantavirus in the Eurasian common shrew *(Sorex aranus)*. *Virol J* 2007;**4**:114.
208. Song JW, Kang HJ, Song KJ, et al. Newfound hantavirus in Chinese mole shrew, Vietnam. *Emerg Infect Dis* 2007;**13**: 1784–7.
209. Song WM, Torrez-Martinez N, Irwin W, et al. Isla Vista virus: a genetically novel hantavirus of the California vole *Microtus californicus*. *J Gen Virol* 1995;**76**:3195–9.
210. Spiropoulou CF, Albarino CG, Ksiazek TG, et al. Andes and Prospect Hill hantaviruses differ in early induction of interferon although both can down regulate interferon signaling. *J Virol* 2007;**81**:2769–76.
211. Stuhlfauth K. Bericht über ein neues schlammtieberahnliches Krankheitsbild bei deutschen Truppen in Lappland. *Dtsch Med Wochenschr* 1973;**69**:439.
212. Takeuchi O, Akira S. Innate immunity to virus infection. *Immunol Rev* 2009;**227**:75–86.
213. Temonen M, Mustonen J, Helin H, et al. Cytokines, adhesion molecules, and cellular infiltration in nephropathia epidemica kidneys: an immunochemical study. *Clin Immunol Immunopathol* 1996;**78**:47–55.
214. Terajima M, Hayasaka D, Maeda K, et al. Immunopathologenesis of hantavirus pulmonary syndrome and hemorrhagic fever with renal syndrome: do $CD8^+$ T cells trigger capillary leakage in viral hemorrhagic fevers? *Immunol Lett* 2007;**113**:117–20.
215. Terajima M, Hendershot JD 3rd, Kariwa H, et al. High levels of viremia in patients with the Hantavirus pulmonary syndrome. *J Infect Dis* 1999;**180**:2030–4.
216. Terajima M, Van Epps HL, Li D, et al. Generation of recombinant vaccinia viruses expressing Puumala virus proteins and use in isolating cytolyic T cells specific for Puumala virus. *Virus Res* 2002;**84**: 67–77.
217. Tkachenko EA, Lee HW. Etiology and epidemiology of hemorrhagic fever with renal syndrome. *Kidney Int* 1991;**40**(Suppl. 35):54.
218. Torrez-Martinez N, Hjelle B. Enzootic of Bayou hantavirus in rice rats *(Oryzomys palustris)* in 1983. *Lancet* 1995;**346**:780.
219. Torro J, Vega JD, Khan AS, et al. An outbreak of hantavirus pulmonary syndrome, Chile, 1997. *Emerg Infect Dis* 1998;**4**:687–94.
220. Trencseni T, Keleti B. *Clinical aspects and epidemiology of haemorrhagic fever with renal syndrome*. Budapest: Akademiai Kiado; 1971.
221. Tsai TF. Hemorrhagic fever with renal syndrome: clinical aspects. *Lab Anim Sci* 1987;**37**:419.
222. Tsai TF. Hemorrhagic fever with renal syndrome: mode of transmission to humans. *Lab Anim Sci* 1987;**37**:428.
223. Umenai T, Lee HW, Lee PW, et al. Korean haemorrhagic fever in staff in an animal laboratory. *Lancet* 1979;**1**:314.
224. Vaheri A, Vapalahti O, Plyusnin A. How to diagnose hantavirus infections and detect them in rodents and insectivores. *Rev Med Virol* 2008;**18**:277–88.
225. Valdivieso F, Vial P, Ferres M, et al. Neutralizing antibodies in survivors of Sin Nombre and Andes hantavirus infection. *Emerg Infect Dis* 2006;**12**:166–8.
226. van der Werff ten Bosch J, Heyman P, Potters D, et al. Hantavirus Puumala infection as a cause of fever of unknown origin in a child. *Acta Paediatr* 2004;**93**:1120–2.
227. Van Epps HL, Schmaljohn CS, Ennis FA. Human memory cytotoxic T-lymphocyte (CTL) responses to Hantaan virus infection: identification of virus-specific and cross-reactive CD8(+) CTL epitopes on nucleocapsid protein. *J Virol* 1999;**73**:5301–8.
228. Van Epps HL, Terajima M, Mustonen J, et al. Long-lived memory T lymphocyte response after hantavirus infection. *J Exp Med* 2002;**196**:579–88.
229. van Ypersele de Strihou C. Clinical features of hemorrhagic fever with renal syndrome in Europe. *Kidney Int* 1991;**40**(Suppl. 35):80.
230. Vapalahti K, Virtala AM, Vaheri A, et al. Case-control study on Puumala virus infection: smoking is a risk factor. *Epidemiol Infect* 2010;**138**:576–84.
231. Verity R, Prasad E, Grimsrud K, et al. Hantavirus pulmonary syndrome in northern Alberta, Canada: clinical and laboratory findings for 19 cases. *Clin Infect Dis* 2000;**31**:942.
232. Vial PA, Valdivieso F, Mertz G, et al. Incubation period of hantavirus cardiopulmonary syndrome. *Emerg Infect Dis* 2006;**12**:1271.
233. Vincent MJ, Quiroz E, Gracia F, et al. Hantavirus pulmonary syndrome in Panama: identification of novel hantaviruses and their likely reservoirs. *Virology* 2000;**277**:14.
234. Vitek CR, Breiman RF, Ksiazek TG, et al. Evidence against person-to-person transmission of hantavirus to health-care workers. *Clin Infect Dis* 1996;**22**:824.
235. Weissenbacher MC, Cura E, Segura EL, et al. Serological evidence of human hantavirus infection in Argentina, Bolivia, and Uruguay. *Medicina (Bueno Aires)* 1996;**56**:17.
236. Wells RM, Sosa Estani S, Yadon ZE, et al. An unusual hantavirus outbreak in southern Argentina: person-to-person transmission? *Emerg Infect Dis* 1997;**3**:171–4.
237. Williams RJ, Bryan RT, Mills JN, et al. An outbreak of hantavirus pulmonary syndrome among Mennonite colonies in western Paraguay. *Am J Trop Med Hyg* 1997;**57**:274–82.
238. Xiao RB, Yang S, Koster F, et al. Sin Nombre viral RNA load in patients with hantavirus cardiopulmonary syndrome. *J Infect Dis* 2006;**194**:1403.
239. Yanagihara R. Hantavirus infection in the United States: epizootiology and epidemiology. *Rev Infect Dis* 1990;**12**:449.
240. Yanagihara R, Gajdusek DC, Gibbs CJ Jr, et al. Prospect Hill virus: serological evidence for infection in mammologists. *N Engl J Med* 1984;**310**:1325.
241. Yanagihara R, Goldgaber D, Lee PW, et al. Propagation of nephropathia epidemica in cell culture. *Lancet* 1984;**1**:1013.
242. Yates TL, Mills JN, Parmenter C, et al. The ecology and evolutionary history of an emergent disease: hantavirus pulmonary syndrome. *Bioscience* 2002;**52**:989–98.
243. Yoo KH, Choi Y. Haemorrhagic fever with renal syndrome in Korean children: Korean Society of Pediatric Nephrology. *Pediatr Nephrol* 1994;**8**:540–4.
244. Young JC, Hansen GR, Graves TK, et al. The incubation period of hantavirus pulmonary syndrome. *Am J Trop Med Hyg* 2000;**62**:714–17.
245. Zaki SR, Greer PW, Coffield LM, et al. Hantavirus pulmonary syndrome: pathogenesis of an emerging infectious disease. *Am J Pathol* 1995;**146**:552.
246. Zeitz PS, Butler JC, Cheek JE, et al. A case-control study of hantavirus pulmonary syndrome during an outbreak in the southwestern United States. *J Infect Dis* 1995;**171**:864.
247. Zetterholm SG. Akuta mefriter simulerande akuta bukfall. *Svenska Lakartidningen* 1934;**31**:425.
248. Zhao C, Sun Y, Wang S, et al. Immunogenicity of a multi-epitope DNA vaccine against hantavirus. *Hum Vaccin Immunother* 2012;**8**:208–15.
249. Zoller L, Yang S, Gott P, et al. Use of recombinant nucleocapsid proteins of the Hantaan and nephropathia epidemica serotypes of hantaviruses as immunodiagnostic antigens. *J Med Virol* 1993;**39**:200.

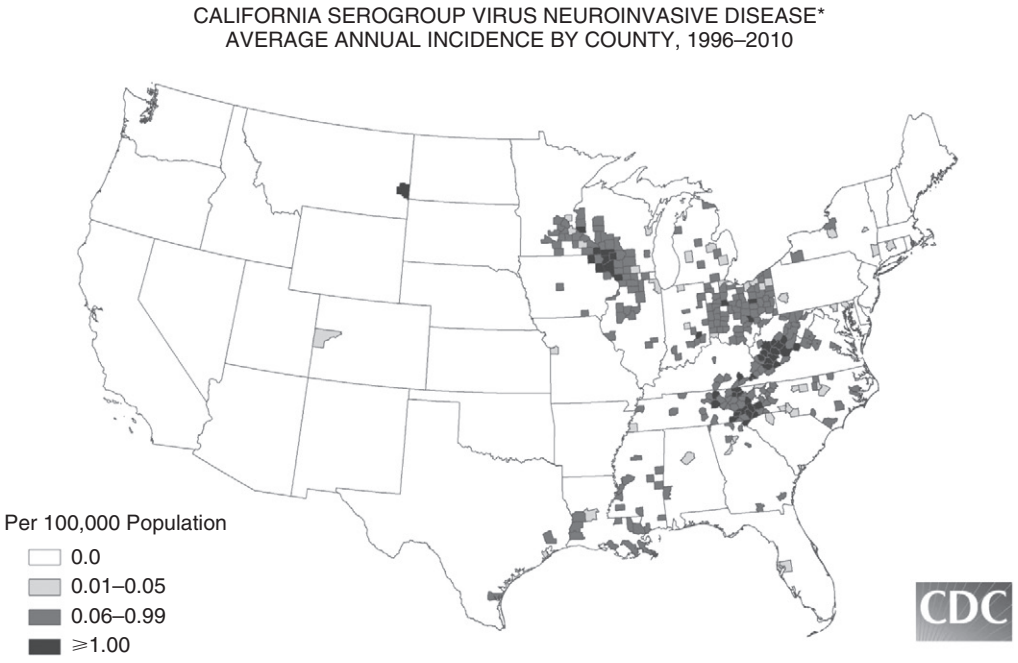

* Neuroinvasive disease includes cases reported as encephalitis, meningoencephalitis, or meningitis. Most reported cases of California serogroup virus neuroinvasive disease are due to La Crosse encephalitis virus. Cases are reported by county of residence.

Data table: This map shows the distribution of California serogroup virus neuroinvasive disease (encephalitis and/or meningitis) average annual incidence from 1996 through 2010. Counties are shaded according to incidences ranging from less than 0.06, 0.06 to 0.99, and greater than 1.00 per 100,000 population. Shaded counties are primarily distributed in the eastern half of the country. Most of the highest incidence counties are along the Appalachian range and the upper Mississippi river basin (in Minnesota, Michigan, Iowa, and Indiana)

FIGURE 189-1 ■ California serogroup virus neuroinvasive disease, average annual incidence by county, 1996-2010.

severe disease may include cognitive delays in up to one third and attention-deficit/hyperactivity disorder in up to two thirds of all patients.[99] Although the mortality from La Crosse virus encephalitis (LACVE) is relatively low compared with certain other arboviral illnesses, such as West Nile virus or eastern equine encephalitis, the first risk assessment mapping of LACVE cases, reported to the CDC from 2003 to 2007, found a mortality rate of 1.9 percent among confirmed cases, higher than previously reported (0.3%).[64,121,123] In a study of the economic and social impact of LACVE in an endemic region of western North Carolina, the average direct and indirect medical costs per patient (from a group of 24 patients) were roughly $33,000 (≤$35,000); the projected cost for five patients with neurologic sequelae ranged from approximately $50,000 to $3 million per patient.[141] Although the number of confirmed cases of LACV-associated neuroinvasive disease in the United States is usually 80 to 100 per year, with an approximate range of 30 to 170 per year from 1964 to 2010, underrecognition and underreporting within a voluntary national reporting system are common problems (Fig. 189-2).[92] Because LACV is the most clinically significant member of the California serogroup viruses, it is the focus of this chapter, but other members of the serogroup are addressed briefly.

ETIOLOGIC AGENT

In 1960, a 4-year-old girl died of "rural encephalitis" in a hospital in La Crosse County, Wisconsin. In 1965, Thompson and colleagues reported the isolation of a novel virus from frozen homogenates of the child's brain by intracerebral inoculation of suckling mice.[135,137] Soon after its discovery, LACV was identified as a member of the California serogroup viruses on the basis of complement fixation and virus neutralization tests; it is one of five such viruses causing human disease in North America. The prototype, CEV, is one of 16 viruses in the *Orthobunyavirus* genus. CEV was discovered in mosquitoes from Kern County in the San Joaquin Valley of California in 1943 and linked to human cases of encephalitis in the same region in 1945.[66,67] The other four viruses of this serogroup in North America include LACV, snowshoe hare virus (SSHV), Jamestown Canyon virus (JCV), and trivittatus virus (TV). Inkoo virus (INKV) and Tahyna virus (TAHV) are members of the California serogroup viruses distributed in Europe.

The LACV virion is pleomorphic, roughly 90 to 100 nm in diameter, and has a host-derived viral envelope with virus-encoded glycoproteins, Gc (formerly G1) and

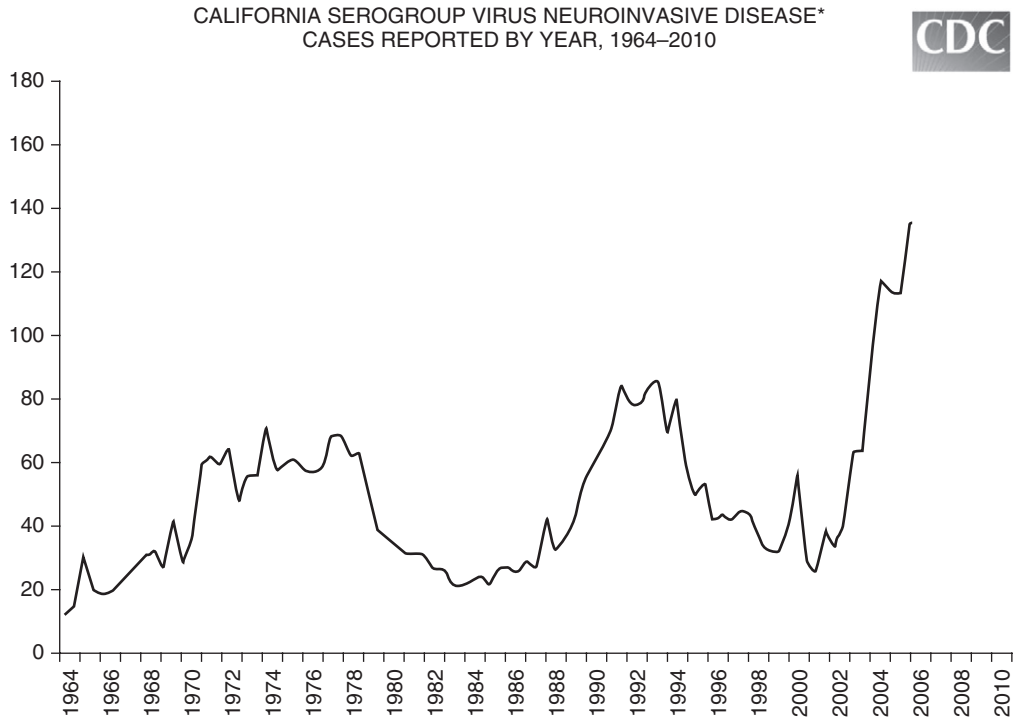

* Neuroinvasive disease includes cases reported as encephalitis, meningoencephalitis, or meningitis. Most reported cases of California serogroup virus neuroinvasive disease are due to La Crosse encephalitis virus.

Data table: In the United States, approximately 80–100 La Crosse encephalitis virus (LACV) neuroinvasive disease cases are reported annually. This graph demonstrates how the number of cases can vary markedly from year to year.

FIGURE 189-2 ■ California serogroup virus neuroinvasive disease, cases reported by year, 1964-2010.

Gn (formerly G2), located on spikes 5 to 10 nm long. The virus replicates in the cytoplasm and buds from the Golgi apparatus. Of the three segments of the RNA genome, the large (L) RNA segment encodes the polymerase, the medium (M) RNA segment encodes the Gc and Gn glycoproteins and a nonstructural protein (NSm), and the small (S) RNA segment encodes the nucleocapsid (N) protein and nonstructural protein NSs.[16,118]

The M segment that encodes the Gc and Gn glycoproteins is a major determinant of viral neuroinvasiveness in mice and susceptibility of *Ochlerotatus triseriatus* mosquitoes to oral and intrathoracic infection.[53,54,125] Neuroinvasiveness may be linked to the cell receptor and fusion functions associated with glycoproteins.[53,55] The Gc protein of LACV is involved in viral attachment to mammalian cell receptors, functioning as a type II fusion protein,[117,118] and is involved in hemagglutination and viral neutralization.[53,54,85,86,125] Group- and LACV-specific epitopes are present on Gc. Gc is also required for infection of mosquito cells in vitro and in vivo.[63] The Gn glycoprotein mediates viral attachment to insect cells.[94] Whereas the M segment genome has been defined in great detail as far as its neuroinvasiveness, that is, the ability to invade the central nervous system (CNS) from an extraneural site through its glycoprotein products Gc and Gn, viral reassortants have implicated the L segment as a major factor in determining neurovirulence, which is the ability to cause CNS disease after direct cerebral injection.[42,129] Furthermore, studies of Gc fusion peptide mutants showed significantly reduced replication but persisting ability to cause neuronal death in culture, implicating the native Gc fusion protein as important in neuroinvasion but not necessarily in neurovirulence.[129] Finally, a viral component encoded by the S segment also seems to serve a role in pathogenesis. NSs was shown in insect and mammalian cells to inhibit the RNA interference pathway, thus inhibiting a mechanism of innate immune response to viral infection. NSs also is involved in apoptosis, the main mechanism by which LACV causes cell death.[16,128] Investigators have shown that a truncated Gc protein preparation induces a protective immune response in suckling mice through neutralizing antibody (see Prevention).[113] Apparently, neutralizing antibody can prevent neuroinvasion by interruption of transient viremia, which occurs just after virus inoculation.

Oligonucleotide maps of LACV isolates from various areas of the United States have grouped the viruses into three types. Upper Midwest viral strains are of two types,

A and B, and type C varieties are found in scattered areas of the eastern part of the United States.[45,88] Variations in strains indicate that changes in the viral genome occur by genetic drift. Genetic reassortment through exchange of RNA segments also has been demonstrated in nature; one proposed mechanism involves dually infected mosquitoes, particularly those with initial transovarial infection preceding superinfection with a second closely related virus.[17]

ECOLOGY

La Crosse Virus

Borucki and coworkers have provided an in-depth review of LACV replication in vertebrate and invertebrate hosts.[18] *Ochlerotatus triseriatus* (formerly known as *Aedes triseriatus*), the eastern tree hole mosquito, is the reservoir of LACV in nature and the vector for transmission of infection to humans (Fig. 189-3).[59,99,143,144] Humans do not maintain prolonged viremia and therefore are dead-end hosts. The mosquito is distributed principally in eastern hardwood deciduous forests, where it breeds in tree holes; however, it also is adapted to breeding in small artificial containers that hold rainwater, such as discarded cans, bottles, and tires. The mosquito is diurnal (i.e., it feeds actively during the day). Although these insects disperse over a wide area, fairly permanent foci of infected mosquitoes are observed in sharply delimited areas, and both isolation of virus and detection of human cases recur in established foci each summer.[10,59,87,133]

The most important mechanism for maintenance of LACV in nature is vertical (transovarial) transmission in *O. triseriatus*. The virus overwinters in infected eggs, which give rise to infected mosquito progeny the following year, thereby providing the mechanism of recurrent disease each summer in endemic areas.[104,132,144] Transovarial transmission of LACV in *O. triseriatus* is controlled by a single gene locus.[57] LACV also is maintained in nature through horizontal transmission by venereal propagation in *O. triseriatus* and through amplification of the virus in the vertebrate hosts on which vector mosquitoes feed.[104,143,144] Vector competence, related in part to the ability of the female mosquito to become infected in the first place (only female mosquitoes take blood meals), may be associated with malnutrition of the mosquito, which compromises its mesenteric barrier and allows infection to occur after the mosquito takes an LACV-infected blood meal.[57,111] The principal amplifying hosts involved in horizontal transmission are chipmunks and squirrels, specifically the eastern chipmunk, *Tamias striatus*, the gray squirrel, *Sciurus carolinensis*, and the fox squirrel, *Sciurus niger*.[90,107,149] Other wild vertebrates, such as foxes and woodchucks, may contribute to amplification. Although domestic livestock and pets are thought not to contribute to horizontal amplification of the virus,

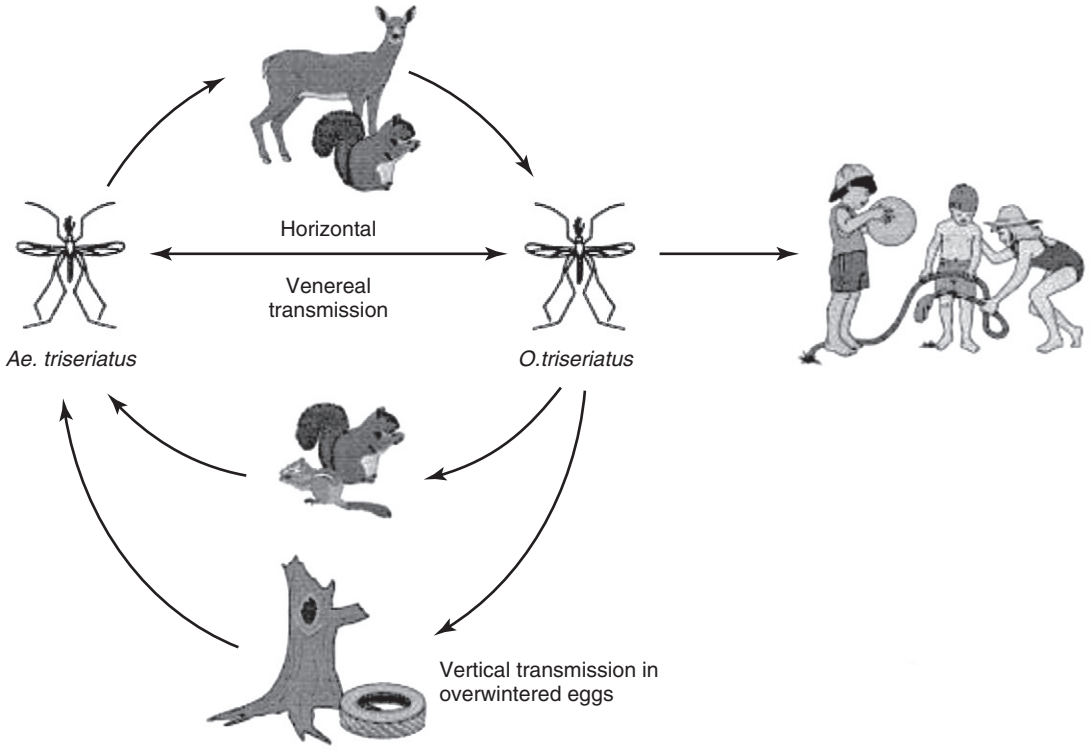

FIGURE 189-3 ■ La Crosse virus transmission cycle. The virus is maintained by vertical transmission in *Ochlerotatus triseriatus* mosquitoes; the virus overwinters in infected eggs that usually are deposited in tree holes or in artificial containers holding rainwater. Horizontal transmission by viral amplification in small vertebrates, such as squirrels and chipmunks, or by the venereal route, among adult mosquitoes, is required to supplement vertical transmission. The role of deer in viral amplification is uncertain. Human infections are incidental to the transmission cycle.[101]

certain species do show seroconversion to California serogroup viruses, and these species may prove to be useful markers of viral presence in a given area.[52] Concern that *Aedes albopictus*, the Asian tiger mosquito, has entered the transmission cycle for LACV was heightened in 2001 when LACV-infected *A. albopictus* mosquitoes were isolated in nature in areas proximate to human cases.* In addition, an elevated burden of *A. albopictus* near the residence of patients with LACVE was associated significantly with LACV infection in cases versus controls.[48] This mosquito species, which was first discovered in the United States in 1985 in Houston, Texas, has since dispersed widely throughout the southeastern United States. The presence of LACV in *A. albopictus* mosquitoes in Dallas County, Texas, in the late summer of 2009 signified the possible expansion of the geographic range of LACV within this invasive mosquito species in Texas.[92] *A. albopictus* has significant further potential to expand the geographic range and frequency of transmission of LACV and possibly other California serogroup viruses.

Other California Serogroup Viruses

A brief discussion of the ecology of other California serogroup viruses follows. Although JCV was isolated first in Colorado and is distributed widely in the West and Midwest, most cases of human illness have been reported in New York, New England, Ontario, and the upper Midwest. Seroconversion to JCV also has been found frequently (18%) in native Alaskans.[142] Various *Aedes* mosquitoes, including *Aedes communis* in the West, *Aedes stimulans* in the upper Midwest, and *Aedes abserratus* in Connecticut, function as vectors.[40,59-61] As opposed to LACV, for which large mammals do not play a role in viral propagation in nature, JCV's horizontal amplification includes deer as the primary amplifying host, with up to 80 percent seroconversion found in adult deer populations in endemic areas.[109,110,150] The overwintering mechanism of JCV has not been elucidated, although transovarial transmission in *Aedes provocans* has been demonstrated.[15]

SSHV is distributed throughout Canada, including the Yukon and Northwest Territories, and in Russia and China. *Culiseta inornata* and various *Aedes* spp. transmit the virus to snowshoe hares (*Lepus americanus*), ground squirrels (*Citellus undulatus*), and other mammals. Transovarial transmission in vector mosquitoes has been demonstrated.[69]

TV is transmitted and maintained through vertical transmission by *Aedes trivittatus* in the Midwest and by *Aedes infirmatus* in the South. CEV is distributed in the western part of the United States, where *Aedes melanimon* and *Aedes dorsalis* are the principal vectors.[57,66,119,136]

INKV and TAHV cause a febrile illness typified by CNS or respiratory tract symptoms, respectively, within the same regions: Scandinavia, central Europe, and western Russia. INKV also has been found in native Alaskans,[142] whereas TAHV has additional endemicity in Asia and Africa.[58,65] Viral recombinants of these viruses have been demonstrated.[42]

*References 7, 8, 35, 36, 51, 60, 74.

EPIDEMIOLOGY

LACVE principally is a disease of children. From 1999 to 2007, 895 neuroinvasive California serogroup disease cases were reported from 25 states within the United States, and 88% of all cases occurred in persons younger than 20 years.[121] Incidence also was 1.5 times higher among males than among females and 4.2 times higher among whites than among blacks. Although the specific viral etiology of California serogroup disease cases from 1999 to 2002 was not reported, 407 (99%) of 412 cases reported between 2003 and 2007 were caused by LACV; the remaining 5 cases were not differentiated further.[64,121] From 2003 to 2007, the incidence risk for all counties reporting probable and confirmed cases in children 15 years and younger to the CDC was 7.2 per 100,000 persons (county range, 0.2 to 228.7) and 1.6 per 100,000 persons for the total population, including a group of states that reported no cases.[64] LACV infections are endemic in the United States. Infections typically occur from July through October, chiefly in rural areas of the east-central states.[83,138] The medical and economic burden to affected individuals and society in endemic areas is significant.[141] Table 189-1 summarizes demographic data and clinical characteristics for a large series of hospitalized children.[99]

The geographic distribution of LACV infection corresponds to natural divisions in which beech, oak, and maple woodlots are prevalent.[59] Although LACVE has been considered a disease of the midwestern and mid-Atlantic states (with highly endemic zones along the upper Mississippi River Valley, specifically in Minnesota, Wisconsin, Illinois, and Iowa, and in Appalachia, particularly in Ohio and West Virginia), cases have been found in 30 states, mostly east of the Mississippi River (Fig. 189-1). One highly endemic focus has been recognized in western North Carolina for many years,[132] and sporadic cases have been found as far south as Louisiana and as far east as Connecticut (Fig. 189-1). Although certain foci of infection appear to remain well localized,[105,132] investigations in eastern Tennessee showed a low seroprevalence in the general population (only 0.5%) despite a recent marked increase in the number of pediatric cases in that region, suggesting that true increases in the incidence of LACV infection (indicating a newly established endemic focus) do occur in some areas.[80,81] For true increases in case incidence to be detected, it may be necessary to track cases at a county level as opposed to a state level. The variability of case incidence within a state is apparent in the more contemporaneous CDC county incidence data cited previously, for which the county incidence varied from 0.2 to 228.7 cases per 100,000 persons, with both confirmed and probable cases combined. Therefore, there is a wide variation that occurs because of the focal nature of the virus between counties reporting LACV infections, and this phenomenon highlights the need to report the range of incidence risk in relatively smaller zones (e.g., county) rather than only the mean incidence risk (e.g., state), which may not provide an accurate assessment of risk within focal areas.[64]

TABLE 189-1 **Epidemiologic and Clinical Data of 127 Children with La Crosse Encephalitis***

Variable	Value
Sex: No. (%)	
Male	90 (71)
Female	37 (29)
Age in Years: No. (%)	
0.5-2	9 (7)
3-5	30 (24)
6-8	42 (33)
9-11	26 (20)
12-14	19 (15)
15	1 (1)
Month of Presentation: No. (%)	
June	3 (2)
July	31 (24)
August	38 (30)
September	44 (35)
October	11 (9)
Symptoms on Presentation: No. with Findings/Total No. (%)†	
Headache	105/126 (83)
Fever	107/125 (86)
Vomiting	89/127 (70)
Disorientation	50/119 (42)
Seizures‡	58/127 (46)
Signs on Admission: No. with Finding/ Total No. (%)	
Nuchal rigidity	31/120 (26)
Glasgow Coma Scale score ≤12	42/127 (33)
Focal neurologic signs	23/126 (18)

*For symptoms on presentation and signs on admission, data were missing for some patients. Because of rounding, not all percentages total 100.
†Typically, the patients with headache or fever were admitted 3 or 4 days after presentation; those with vomiting were admitted 1 or 2 days after presentation; and those with disorientation or seizures were admitted on the day of presentation.
‡Seizures were generalized in 22 patients, partial in 24, and partial with secondary generalization in 22.
Data from McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med* 2001;**344**:801-7.

Most LACV infections are asymptomatic. Clinical disease ranges from mild febrile illness to severe encephalitis. For the purposes of surveillance and reporting, cases of LACV-associated clinical disease, like all cases of arboviral disease, are often categorized into either neuroinvasive (meningitis, encephalitis, or meningoencephalitis) or non-neuroinvasive disease. Confirmed cases of LACV infections are required to meet both the clinical and laboratory requirements set by the CDC's case definition for arboviral disease.[22] Because LACV in the cerebrospinal fluid (CSF) is difficult to isolate by culture and to identify by detection of antigen or nucleic acid, the most typically employed "gold standard" for diagnosis is serology. In the absence of a more likely clinical explanation, confirmed neuroinvasive cases must meet all of the following criteria:

1. fever (≥38° C or 100.4° F);
2. meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician; and
3. isolation of virus from or demonstration of specific viral antigen or nucleic acid in tissue, blood, CSF, or other body fluid; or fourfold or greater change in virus-specific quantitative antibody titers in paired sera; or virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen; or virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.[22]

A probable case is one that meets the clinical definition for neuroinvasive disease and has LACV-specific IgM antibodies in the CSF or serum but lacks convalescent titers, neutralization titers, or testing to help exclude other endemic arboviruses. The clinical case definition for non-neuroinvasive disease requires fever (≥38° C or 100.4° F) as well as the absence of neuroinvasive disease and any more likely clinical explanation; the laboratory criteria are the same as for neuroinvasive disease. Although the clinical case definition for arboviral disease, whether neuroinvasive or non-neuroinvasive, requires the presence of fever, in practical terms the absence of fever does not exclude LACV-associated clinical disease. For example, most clinical case series of LACVE include patients for whom fever did not occur (Table 189-1).[99]

Active surveillance efforts can increase case findings markedly, as seen in West Virginia,[84] where after the death of a child from LACVE in 1987, more than 150 cases were diagnosed during the next 8 years (versus only 15 cases ever previously reported in West Virginia).[101] CDC records from 1987 to 1997 reveal that West Virginia became the most highly endemic state in the United States.[28] This trend has continued, with West Virginia highest among the states with 235 (26%) of the 895 suspected or confirmed cases of neuroinvasive California serogroup–associated neuroinvasive disease from 1999 to 2007 (see Fig. 189-1 for relative case rates by county).[121] A complete knowledge of the public health importance of LACVE is not available because the virus is distributed discontinuously in the eastern part of the United States, where the disease could be endemic but underrecognized. Nevertheless, more recent use of spatial mapping techniques has increased the understanding of regional transmission patterns.[13,64,75,121,146] Also, the high ratio of inapparent to apparent infections (see Clinical Manifestations) implies that the majority of persons who are infected will not come to medical attention and thus will not be reported.[81] Even the presentation of symptomatic patients occurs along a continuum from fever and headache to alteration of consciousness and seizures. To improve case diagnosis and reporting to state health departments, clinicians should cultivate an appreciation for the epidemiology of LACV, the range of clinical presentations of LACVE, and the appropriate diagnostic tests. The virus typically is not recoverable on culture of

CSF and therefore requires specific serologic testing (see Laboratory and Radiologic Diagnosis).

Seroprevalence rises with age in endemic areas.[132,133] Serosurveys have shown that point prevalence rates vary in endemic locations from 30 percent in rural areas to 15 percent in urban locations.[106] Although risk appears to be highest in rural areas, many cases occur in suburban residential locations. Some patients with clinical disease have reported travel to forested recreational areas in endemic regions.[41,75,136] A case-control study performed in a highly endemic county found several characteristics of the peridomestic environment to be associated with a risk of acquiring LACVE: tree holes on the residential premise, proximity of the house to the forest edge, and presence of artificial containers and large numbers of discarded tires.[146] A case-control study in eastern Tennessee also showed an association of LACVE with proximity (within 100 m) of tree holes to the residence, increased time of patients spent outdoors, and increased burden of *A. albopictus* in the peridomestic environment.[48] A more recent noncontrolled study in West Virginia that investigated the residences of LACVE cases found the presence of a wooded area in 92.0 percent and outside containers in 70.5 percent.[65] The presence of standing water was observed at almost half of the case residences (49.4%), and the majority of case residences (76.7%) were located within 45.6 m (149.0 feet) of a wooded area. In general, these epidemiologic data support use of public health and spatial mapping techniques to identify hyperendemic areas where preventive efforts should be focused.

With regard to California serogroup viruses in the United States other than LACV, JCV seroprevalence rates of 5 to 40 percent have been found in the upper Midwest. Most reported cases (21 of 29 in one series) have occurred in male patients.[130] In contrast to LACV, JCV infections occur earlier, in May or June, and continue through the end of the summer. In addition, JCV is more likely to cause illness in adults than in children.[42,65] As for SSHV, human infections have been documented by serosurveys in Alaska and throughout Canada.[59] In these serosurveys, antibody prevalence rates were more than 30 percent in some areas; seroprevalence in the male population was twice the rate in the female population. Sporadic infection with California serogroup viruses other than LACV is likely to occur frequently in the western part of the United States.[119] However, symptomatic cases of infection due to CEV occur rarely.[46]

PATHOGENESIS

LACV, which infects the salivary glands of the mosquito vector, is introduced into the host's skin and subcutaneous tissue during feeding. Probing alone is sufficient for infection. Completion of a blood meal is not necessary for transmission to occur. Local viral replication in adjacent muscle tissue leads to systemic viremia that seeds the reticuloendothelial system, muscle, and chondrocytes. This seeding results in further amplification of viremia and allows infection of vascular endothelial cells abutting the CNS.[54,55,76,78] Entrance into the CNS probably is initiated by infection of these vascular endothelial cells, which provide a route for infection of neurons and glial cells.[76,78] In a murine model, LACV probably gained direct access to the CNS through the bloodstream or olfactory neurons, or both.[14] The ability to efficiently infect mice intranasally raises the possibility that LACV might use this route to infect its natural hosts. Case reports suggest that vascular involvement in human LACVE may be important in the pathogenesis of focal deficits (from stroke) and more generalized cerebral edema (possibly from vasogenic edema).[93,102] Polymerase chain reaction (PCR) studies of the location of LACV within the CNS found evidence of viral RNA within the cortex but not in other CNS tissues.[30] This is consistent with the results of magnetic resonance imaging (MRI) in one study that showed findings consistent with lesions predominantly in cortical areas.[99]

Most studies indicate that the extraneural phase of viral replication and neuroinvasiveness are mediated largely by the M segment of the genome, primarily through Gc glycoprotein, which appears to promote binding to and entry into cells.[53-55,117,125] A study in adult mice indicated that once the virus has gained access to the CNS, replication in CNS tissue (i.e., neurovirulence) is mediated largely by the L RNA segment, probably through the viral polymerase.[47,54] Previous work implicated the M segment in neurovirulence, but in that study neurovirulence was defined as time to death after intracutaneous inoculation.[125] The host immune response involves neutralizing antibody, which mediates viral aggregation, inhibition of viral attachment to cells, and inhibition of viral penetration and uncoating.[86] Other aspects of the immune response shown to play a role in protection against LACV infection of the CNS in animal models include $CD4^+$ T cells and MxA protein, which is induced by the interferon-α/β system.[69,89,120,124] Susceptibility to and complications of LACVE may have an immunogenic component, as indicated by an association of illness and seizures with certain human leukocyte antigens.[23] Neuronal cell death may be mediated largely by apoptosis (programmed cell death), as determined by studies in mice,[114] and the nonstructural protein NSs has been found to promote apoptosis as well as to interrupt the RNAi pathway.[16,128] It is interesting that bc1-2 expression can inhibit apoptosis in certain cell lines.[114]

CLINICAL MANIFESTATIONS

The clinical spectrum of illness in LACV infections ranges from inapparent infection or mild febrile illness to aseptic meningitis and fatal encephalitis.* The ratio of inapparent to clinically apparent infection ranges from 26:1 to 322:1.[83] Most clinically apparent infections are associated with signs and symptoms of meningoencephalitis and occur within an average incubation period of 5 to 15 days after inoculation. Severe cases tend to mimic herpes simplex virus encephalitis (HSVE) at initial evaluation, whereas mild cases may manifest similarly to enterovirus-associated aseptic meningitis. For 282 probable and confirmed cases of LACV infections

*References 5, 6, 31, 32, 34, 62, 70, 99, 134, 148.

reported to the CDC from 2003 to 2007, the clinical classifications were as follows: meningoencephalitis (56.3%), encephalitis (20.7%), meningitis (17.2%), uncomplicated fever (5%), and undefined (other or not reported, 1.8%).[64] The case-fatality rate in this study was 1.9 percent of all confirmed cases. In the most recent and largest series of hospitalized children published in 2001, three children had the near-lethal complication of cerebral herniation.[112] In addition, nearly half of all patients required admission to intensive care, and one fourth required mechanical ventilation.[64,99,121,134]

Children typically have a 3- to 4-day prodrome of fever, headache, and vomiting; diarrhea is usually absent. The illness often progresses such that disorientation (42%) or seizures (46%), or both, may be seen on the day of admission. Most seizures are partial or partial with secondary generalization (58%), and the remainder of seizures are generalized. Status epilepticus develops in approximately one fourth of children with seizures. Rarely, patients may have sudden onset of seizures with little or no preceding fever or headache.[99]

On examination, signs of meningeal irritation and changes in mental status are found in approximately one quarter and one third of patients, respectively.[99] Focal neurologic signs, principally focal seizures, paresis, aphasia, and abnormal reflexes, are seen in 16 to 25 percent of patients with LACVE. Focal and generalized seizures occur in 42 to 62 percent of cases, and status epilepticus develops in 10 to 15 percent.[31,32,98,99] Focal neurologic signs, whether they are found on physical examination or on electroencephalographic (EEG) or imaging studies, should be taken as evidence of presumptive HSVE, thus indicating the need to start empirical acyclovir until diagnostic studies have been completed.[147] Table 189-1 summarizes the clinical characteristics and demographic data of a large series of hospitalized children with LACVE.[99]

From the same study, the hospital course was complicated by hyponatremia (21%), generally consistent with the syndrome of inappropriate antidiuretic hormone secretion; recurrent or de novo seizures (13%); signs of increased intracranial pressure (ICP; 13%); and, rarely, cerebral herniation (2.3%).[112] Approximately 1 in 10 patients (including the 3 with herniation and 10 others with recurrent seizures, coma, or both) experienced neurologic deterioration after hospital admission, usually between 36 and 72 hours after admission. Risk factors associated with deterioration were the presence of disorientation or vomiting at admission and a trend toward seizures at admission. Neurologic deterioration appeared to be related temporally to a decrease in serum sodium concentration or an increase in body temperature, suggesting that these conditions may be risk factors for in-hospital deterioration as well. The tendency for 1 in 10 hospitalized children with LACVE to deteriorate neurologically after admission represents a significant challenge for clinicians (see Treatment).

Clinical findings in patients infected with other members of the California serogroup viruses deserve brief mention. JCV infections often are associated with prodromal respiratory symptoms in conjunction with aseptic meningitis or clinical encephalitis. Among 39 patients with proven or suspected CNS infection with JCV, 10 had encephalitis, and 7 reported upper respiratory symptoms or pneumonia.[59,130]

The few patients described with SSHV infection have had manifestations ranging from an influenza-like illness to aseptic meningitis or fatal encephalitis. One fatality was reported in a 14-year-old girl who had symptoms and signs that suggested Reye syndrome.[43] In a 59-year-old man, radiologic and EEG findings showed a temporal lobe focus, which led to performance of a brain biopsy and a clinical and pathologic diagnosis of herpes encephalitis, even though herpes virus was not isolated. Serologic examination later confirmed the diagnosis of SSHV infection. Mild febrile illness attributed to infection with TV has been described in some patients, and CNS infections due to TV have been identified in others.[106] Three cases of symptomatic infection caused by CEV have been reported. All three patients had signs and symptoms of encephalitis.[66]

LABORATORY AND RADIOLOGIC DIAGNOSIS

Despite the lack of an effective antiviral agent for the treatment of LACV, early diagnosis of LACVE is important in guiding triage and management decisions. Initially, such a diagnosis is often probable or presumptive rather than definitive because at best a single elevated IgM or IgG antibody titer is available in the acute phase of illness, meaning that clinicians should continue to be vigilant for alternative diagnoses. In addition, when choosing diagnostic tests for encephalitis, clinicians should communicate with virology laboratory colleagues so that the limitations of these tests are understood. For example, viral culture of CSF is usually negative. There have been only two reports of successful growth of LACV from CSF.[9,95] Isolation from brain tissue has occurred on four occasions.[9,82,100] Improvements in the detection of LACV RNA,[29,71,91] including nucleic acid–based amplification and reverse transcription PCR, have been tested in mosquitoes and human brain tissue,[92] but clinical diagnostic capabilities with blood or CSF samples remain experimental. Therefore, at this time, serologic methods to detect LACV antibody continue to be the best method for diagnosis, with fourfold rise between acute and convalescent titers remaining the gold standard. IgM-specific antibody to LACV can be detected reliably in serum and CSF by two methods: (1) indirect immunofluorescent antibody (IFA), which is a Food and Drug Administration (FDA)–approved test with rapid turnaround available for use by clinical laboratories; and (2) enzyme immunoassay (EIA), which is available only through the CDC and some state laboratories. The IFA assay is a two-stage "sandwich" procedure. In the first stage, the patient's serum is diluted, added to appropriate slide wells bearing the antigen, and incubated. After incubation, unbound antibodies are washed away, and then each antigen well is overlaid with fluorescein-labeled antihuman goat antibody to IgG or IgM. The slide is incubated a second time, allowing complexes of antigen and either LACV IgM or LACV IgG antibody to react with the

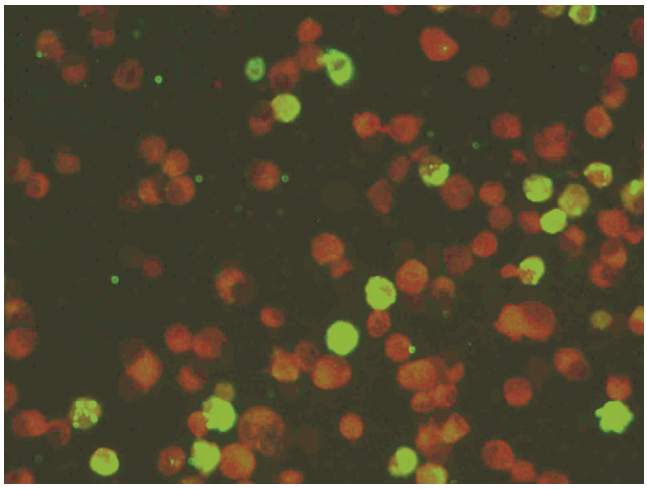

FIGURE 189-4 ■ California serogroup viruses immunofluorescent antibody. The indirect immunofluorescent antibody (IFA) assay is a two-stage "sandwich" procedure. In the first stage, the patient's serum is diluted, added to appropriate slide wells bearing the LACV antigen, and incubated. After incubation, unbound antibodies are washed away, and then each antigen well is overlaid with fluorescein-labeled antibody to IgG. The slide is incubated a second time, allowing complexes of antigen and antibody to react with the fluorescein-labeled anti-IgG. After the slide is washed, dried, and mounted, it is examined by fluorescence microscopy. Positive reactions appear as cells exhibiting bright apple-green cytoplasmic fluorescence against a background of red negative control cells. The so-called starry night pattern of stippling along the cell membrane and cytoplasm is a finding unique to the California serogroup viruses. Semiquantitative end-point titers are obtained by testing serial dilutions of positive specimens.[3,19] (See companion Expert Consult website for color version.)

fluorescein-labeled anti-IgG. After the slide is washed, dried, and mounted, it is examined by fluorescence microscopy. Positive reactions appear as cells exhibiting bright apple-green cytoplasmic fluorescence against a background of red negative control cells (Fig. 189-4). The cutoff for a positive IgM or IgG IFA is a titer of ≥1:16. Recent work, employing the standard fourfold rise in antibody titer as the gold standard for comparison to diagnosis by single acute-phase LACV titers, reported that a single elevated acute LACV IgM or LACV IgG titer by IFA has a sensitivity and specificity of 75 percent and 98 percent, respectively.[108] The available IFA kit also provides tests for St. Louis encephalitis virus (SLEV), eastern equine encephalitis virus (EEEV), and western equine encephalitis virus (WEEV).[3] The IFA test for LACV IgM has the advantage of providing a rapid diagnosis within 24 to 48 hours, but a single titer defines only a probable case under the CDC case definition. For a probable case to become a confirmed case, it is necessary to obtain positive serum convalescent titers by either IFA or EIA (i.e., a fourfold rise) or confirmatory neutralization titers performed at the CDC, done either on a serum sample (acute or convalescent) or CSF (see Epidemiology).

IgM capture EIA is employed by the CDC as a sensitive serologic test that detects virus-specific IgM in 83 to 100 percent of cases (Fig. 189-4).* An IgG capture EIA method has been developed as well, but preliminary work suggests that it lacks reliability at this time.[77,96] The main advantage of IgM capture is that it is less operator dependent than the IFA method, which requires careful observance of visual reading criteria by experienced personnel. For this reason, IgM capture EIA is the method used by the CDC for detection of LACV IgM. In the near future, the CDC plans to replace the EIA capture method with a microsphere immunofluorescence assay (MIA) for the concurrent detection of either IgM or IgG antibody to LACV, WNV, SLEV, WEEV, EEEV, and Powassan virus (a tick-transmitted flavivirus).[9] An MIA is similar to an EIA, except that viral antigens are either directly attached to beads instead of a plate or attached indirectly by an intermediate antibody. Results are read by use of a modified flow cytometer. Relative advantages of the MIA in comparison to EIA include decreased time to test completion, improved technical ease, and decreased sample volume. Confirmatory testing of patients with a positive IgM by IFA or EIA involves the detection of LACV-specific neutralizing antibodies by plaque reduction assay, performed by the CDC. Despite the current CDC recommendations that a probable case should be confirmed with additional testing, recent work suggests that a single acute serologic sample tested by IFA might eventually be considered sufficient for clinical purposes if not also epidemiologic ones.[108] For more on surveillance and reporting of cases, see also the CDC case definition of arboviral disease in the Epidemiology section. Instructions for sending diagnostic specimens to the CDC Division of Vector-Borne Diseases (DVBD) Arbovirus Diagnostic Laboratory can be found at the following site: http://www.cdc.gov/ncezid/dvbd/specimensub/index.html. In fatal cases, nucleic acid amplification, histopathologic examination with immunohistochemistry, and virus culture of autopsy tissues can also be useful. Only a few state laboratories or other specialized laboratories, including those at CDC, are capable of doing this specialized testing.

In all diagnostic tests for LACVE, even a minimal decrease in specificity (a false-positive result) can present a key clinical dilemma, with the potential to distract clinicians from diagnosis of treatable conditions. Unfortunately, neither currently available serologic method for LACV IgM detection can guarantee 100 percent specificity, especially because in rare instances LACV IgM reportedly has remained elevated in serum for more than 1 year. Therefore, even in the context of positive acute titers for LACV, it may be prudent to consider continuation of empirical therapy for HSVE with intravenous acyclovir until a negative CSF PCR assay for herpes simplex virus is obtained. Although the occurrence of false-positive test results for LACV is the major concern clinically, false-negative results may occur as well, particularly within the context of a rapidly progressive acute infection. IgM antibodies are generally first detectable at 3 to 8 days after onset of illness and usually persist for 30 to 90 days. Serum collected within 8 days of illness onset may not have detectable IgM.[22] In patients with a negative result for IgM and a clinical syndrome compatible with LACVE, it may be helpful to obtain convalescent-phase IgM and IgG titers in 1 to 2 weeks and in some cases in as little as 3 or 4 days from initial testing at clinical presentation.

*References 3, 11, 12, 19, 20, 42, 44.

TABLE 189-2 **Laboratory Values at Admission in Patients with La Crosse Encephalitis**

Variable	Mean Value ± SD	Range	Percentile					Remarks
			10th	25th	50th	75th	90th	
Cerebrospinal Fluid								
White blood cell count (per mm^3)	130 ± 151	2-867	10	26	75	184	316	<200/mm^3 in most cases
Differential count (% lymphocytes)	—	2-100	10	27	62	77	90	Predominance of lymphocytes
Red blood cell count (per mm^3)	71 ± 213	0-1,500	0	1	5	20	177	Elevated (≥20/mm^3) in 25%
Glucose (mg/dL)*	75 ± 20	37-149	56	62	71	83	105	Normal
Protein (mg/dL)	37 ± 15	10-85	20	27	34	45	56	Rarely elevated
Peripheral Blood								
White blood cell count (per mm^3)	15,700 ± 5900	6,800-49,700	8,900	11,500	14,800	19,000	22,600	Usually elevated (>15,000/mm^3)
Differential count (% polymorphonuclear leukocytes)	—	17-94	58	66	76	82	86	Predominance of polymorphonuclear leukocytes

*To convert values for glucose to millimoles per liter, multiply by 0.05551.
Data from McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med* 2001;**344**:801-7.

Ancillary laboratory examinations for LACV infection have been well outlined in one series (Table 189-2).[99] The total CSF leukocyte count was elevated only moderately (the median white blood cell [WBC] count in the CSF was 75 cells/mm^3), and 25 percent of patients had sparsely elevated red blood cells in the CSF.[99] The 75th percentile for the CSF protein level was 45 mg/dL (normal range, 5 to 40 mg/dL), consistent with numerous reports in which elevated protein levels were observed in less than one third of cases.[5,31,34,70,99] Approximately 10 percent of children had negative or equivocal findings on initial CSF analysis, only to have numerous WBCs in the CSF within 24 to 48 hours on repeated lumbar puncture. The peripheral WBC count was elevated (≥15,000 WBCs/mm^3) in half of the cases at admission and was frequently associated with neutrophilia, which in general may raise suspicion for bacterial meningitis or partially treated bacterial meningitis. Serum electrolyte values should be monitored for hyponatremia, which occurs frequently and has been associated with worsening clinical status in LACVE.[99]

Even though the Infectious Diseases Society of America recommends that all children and adolescents with encephalitis should have brain MRI with and without contrast enhancement, optimally with diffusion-weighted imaging, at this time most available data in children with LACVE are from computed tomography (CT) scans (see Treatment for basic recommendations on obtaining imaging in this clinical setting).[140] In 127 hospitalized children with LACVE, 92 had non–contrast-enhanced CT scans. Only 11 of these scans had abnormal findings; 8 showed generalized edema and 3 showed focal findings, all in supratentorial locations.[112] In 3 children whose courses were complicated by cerebral herniation, CT scans detected an abnormality only after the herniation event (i.e., CT scans on admission were normal). From this same series, each of 10 children had an MRI of the brain; 4 of these children had abnormal MRI findings that showed focal areas of gadolinium enhancement, predominantly in cortical areas (Fig. 189-5).[99] Case reports of LACVE suggest that MRI may reveal lesions not detected by CT. In a 20-month-old infant with right hemiparesis, CT findings on admission were normal, but MRI on day 1 showed evidence of acute infarction of the left basal ganglia.[93] In a 12-month-old child with clinical evidence of worsening neurologic status, MRI showed areas of increased signal intensity in the periventricular white matter, even though the initial CT scan had been normal.[50]

EEG abnormalities have been found in 71 to 90 percent of patients tested. Focal EEG findings, usually slowing, may appear in as many as 44 percent of abnormal EEG tracings.[31,32,98,99] Periodic lateralizing epileptiform discharges (PLEDs), previously thought to be virtually pathognomonic for HSVE, also occur in LACVE. In one case series of LACVE, 8 of 90 EEG tracings were positive for PLEDs[37,99]; in 4 of these 8 cases, patients required continuous EEG surveillance to monitor treatment of nonconvulsive status epilepticus.[99] Patients with PLEDs represent a worrisome subgroup of all patients with LACVE because they have longer, more complicated hospitalizations and are likely to have worse long-term outcomes.[38]

DIFFERENTIAL DIAGNOSIS

The principal consideration in the differential diagnosis of LACVE is HSVE.[99,127] Clinical descriptions of LACVE consistently disclose a high rate of focal neurologic findings or focal seizures, or both (~30%). The frontal and temporal lobe locations of abnormalities detected by

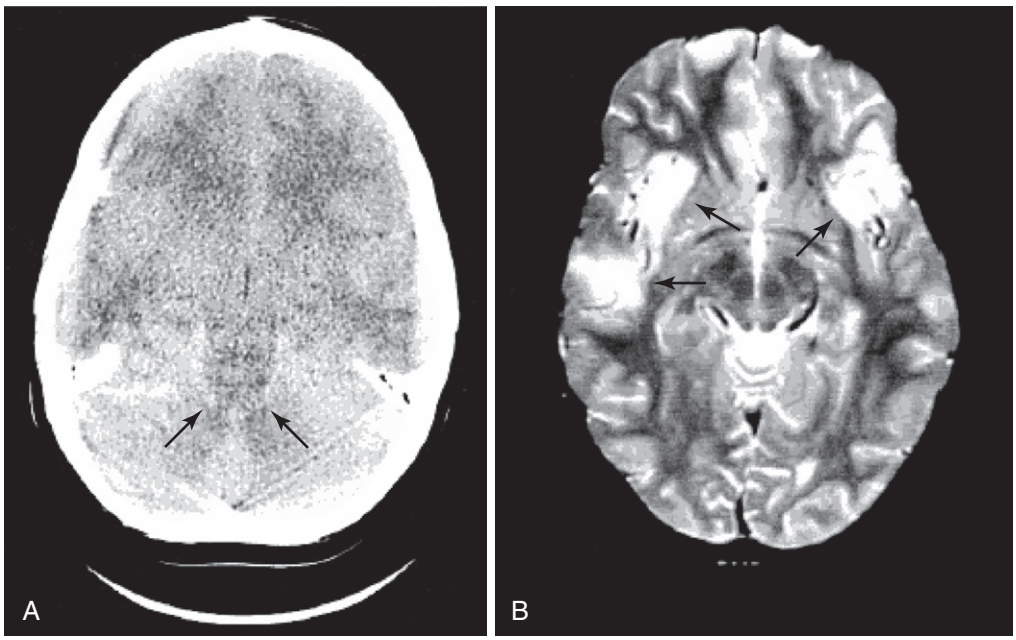

FIGURE 189-5 ■ Radiographic studies in patients with severe La Crosse encephalitis. In **A**, a CT scan (obtained on the second hospital day) of an 8-year-old boy with severe La Crosse encephalitis complicated by uncal herniation reveals brain edema with associated obliteration of perimesencephalic cisterns (*arrows*). In **B**, a T2-weighted magnetic resonance image obtained in a 7-year-old boy with severe La Crosse encephalitis shows focal areas of increased signal intensity in the right temporoparietal and left frontotemporal regions (*arrows*). A CT scan obtained at the time of uncal herniation 7 days earlier showed hypodensity in the same areas.[99]

electroencephalography (including PLEDs) and by brain imaging studies in some cases of LACVE initially may suggest a presumptive diagnosis of HSVE.[5,99] Hemorrhagic pleocytosis, which can occur in both HSVE and LACVE, also may favor presumptive diagnosis of and therapy for HSVE. Because delay of therapy for HSVE is associated with a poor outcome, a reasonable approach in cases of encephalitis with focal findings or in severe encephalitis is to treat empirically for HSVE with intravenous acyclovir pending definitive diagnosis.[140,145]

In patients with less fulminant signs of CNS infection, enteroviral aseptic meningitis is a common consideration, especially because it often occurs with similar seasonality, in summer and early fall. The presence of rash, pharyngitis, conjunctivitis, or myocarditis is a clue to enterovirus and is not characteristic of LACV infection. In a study comparing enteroviral CNS infection with LACVE, patients with LACVE were significantly more likely to have aphasia, loss of consciousness, seizure, and admission to the pediatric intensive care unit than were patients with enteroviral CNS infection.[68] For undervaccinated persons and communities, poliomyelitis remains an important diagnostic consideration. In September 2005, a 7-month-old Amish child from Minnesota with failure to thrive, diarrhea, and recurrent infections was diagnosed with type 1 vaccine-derived poliovirus (VDPV) in the context of newly diagnosed severe combined immunodeficiency.[25] Although 8 of 23 children (including the infant) in the index community tested positive for VDPV, none had paralytic disease.[1] Mumps encephalitis is another consideration, even in the absence of parotid swelling, and low or low-normal CSF glucose levels may be a clue to this diagnosis.[92]

Patients with Rocky Mountain spotted fever may exhibit signs of CNS disturbance, and one should remember that rash may be absent in 20 percent of patients. *Bartonella henselae* infection (cat-scratch disease) or *Mycoplasma pneumoniae*–associated encephalopathy may be valid considerations in the differential diagnosis. Certainly, other Arboviridae should be considered, and many laboratories now test simultaneously for Arboviridae known to occur in the United States (WNV, SLEV, EEEV, and WEEV) in patients with severe seasonal (summer or fall) encephalitis. A rare and tragic consideration in a child presenting with encephalitis is rabies, which may occur in the absence of a known animal bite. Certainly, a wide array of viruses, bacteria, and even amebae and parasites can cause CNS infection. These etiologic agents are reviewed in other chapters of this text as well as in the Infectious Diseases Society of America practice guidelines for the management of encephalitis.[140]

TREATMENT

Because no specific FDA-approved antiviral treatment of LACVE is currently available, careful supportive care is the mainstay of therapy. A reasonable general supportive strategy is to assess these patients as one would assess children with closed head injury. In both instances, (1) changes in the level of consciousness cannot be assumed

to be caused by a normal need to sleep and therefore require serial monitoring, and (2) changes in the level of consciousness generally are the best and earliest indicator of evolving intracranial disease, as opposed to later brain stem signs such as pupillary abnormalities or bradycardia with hypertension. Therefore, serial monitoring of the level of consciousness is the most important aspect of neurologic monitoring, that is, use of the Glasgow Coma Scale (GCS), even if the child has minimal changes in the level of consciousness at the time of admission.

Three particularly important decision points in management depend on the presence or absence of (1) disorientation, (2) further deterioration in GCS scores, and (3) focal findings. Once the child becomes disoriented (GCS score typically < 13), as shown by changes in mental status in an older child or perhaps by lack of recognition of parents in an infant, the child is monitored best in an intensive care setting. Children presenting with seizures on admission or those documented to have elevated opening pressure on lumbar puncture also deserve consideration for admission to intensive care, as do those with unremitting vomiting or headache or both despite appropriate medical care on the general pediatric ward.[99] Further deterioration in the GCS score to 8 or lower generally indicates that the patient no longer is able to protect the airway adequately, and appropriate intervention is needed. Cases suggesting HSVE because of focal findings or coma or both warrant presumptive treatment with intravenous acyclovir pending definitive diagnosis (see Diagnosis).

Many of the same strategies that apply in treating children at risk for increased ICP are used in treating those with LACVE. A reasonable approach is to perform fundoscopy and to consider a non–contrast-enhanced CT scan of the brain before lumbar puncture and, when possible, measurement of the opening pressure. In patients with encephalitis, brain MRI with and without gadolinium contrast and with diffusion-weighted imaging is the recommended imaging modality, and this should be performed as soon as it is feasible, but practical considerations (such as the need to perform lumbar puncture with minimal delay and the lack of the patient's cooperation due to young age or disorientation) still often favor CT over MRI in the early phase of illness. General treatment strategies in patients at risk for increased ICP include the following: airway and (if necessary) ventilatory management designed to avoid hypercapnia; hemodynamic management to optimize mean arterial pressure (and presumably cerebral perfusion pressure); neurologic strategies to optimize control of seizure; and strategies designed to avoid hypo-osmolality, hyperthermia, and other factors that may exacerbate intracranial hypertension or avoidable increases in the cerebral metabolic rate. Intubated patients should be sedated adequately and may need additional analgesia or sedation before noxious procedures such as endotracheal suctioning are performed.

In a large series of hospitalized patients with LACVE, 1 in 10 patients with LACVE deteriorated neurologically after admission. Risk factors identified to occur more often before in-hospital deterioration were the occurrence of disorientation (consistent with a GCS score ≤13), vomiting, and seizures.[99] In addition, hyponatremia and hyperthermia may be related temporally to clinical deterioration in patients with LACVE.[99] Regarding therapeutic implications of the risk for development of hyponatremia, isotonic fluids (normal saline or 5% dextrose in normal saline) should be used at maintenance rates, in addition to deficit replacement, to minimize the tendency for the development of hyponatremia while maintaining intravascular volume.[99] Serum sodium concentration should be monitored approximately every 8 hours during the acute phase of the illness. If evidence of the syndrome of inappropriate antidiuretic hormone secretion is documented and cerebral salt wasting is ruled out by careful monitoring of fluid status as well as sodium level and osmolality of the urine and serum, careful restriction of fluids may be considered. Because hyperthermia may be associated with neurologic deterioration in some patients, having a low threshold for prevention or treatment of fever with antipyretics is recommended. In critically ill patients with evidence of increased ICP, methods other than antipyretics (e.g., cooling blankets) may be considered to address persistent hyperthermia, provided shivering and discomfort are controlled.

Patients suffering neurologic deterioration may need to have repeated EEG or brain imaging studies or both because nonconvulsive status epilepticus can occur and because the initial brain imaging results may be normal, but later imaging may show evidence of cerebral edema. Some experience has been gained in the monitoring of ICP in LACVE.[82] In one particular study, high ICP was documented in three of six patients in whom monitors were placed, and such monitoring was considered by clinicians to be a useful adjunct in these cases.[99] Establishment of clinical and imaging criteria for which patients might benefit from insertion of an ICP monitoring device remains a dilemma, particularly because ictal and postictal events in LACVE can confound the neurologic examination regarding possible signs of increased ICP.

No specific antiviral therapy is approved by the FDA for the treatment of LACV infection or LACVE. Although previous laboratory studies suggested the potential of ribavirin (RBV) as a therapeutic agent,* a report summarizing the largest study of antiviral treatment of LACVE documented overall poor clinical efficacy and a narrow therapeutic index.[103] This report summarized three studies of children treated with intravenous RBV during a 10-year period from 1994 to 2003, each one exploring a different phase (I, IIA, and IIB) of clinical trial development. In a group of 15 children treated in phase I and phase IIA, RBV appeared safe at moderate dosing, but on the basis of steady-state RBV levels of 9.3 μM, estimated CSF levels were less than 20 percent of the EC_{50} of RBV for LACV. No trends to suggest efficacy of intravenous RBV could be detected in the statistically underpowered phase IIA randomized controlled trial. At the escalated dose used in phase IIB, each of the first two enrollees experienced adverse effects (elevated serum ammonia and elevated serum pancreatic enzymes, respectively) considered likely to be related to RBV, and therefore the trial was discontinued. In view of the limitations of RBV for LACVE, it is encouraging to note a group of novel broad-spectrum

*References 24, 33, 72, 73, 102, 116, 126, 131.

RNA virus inhibitors known as the pyrazinecarboxamide derivatives: T-705 (favipiravir), T-1105, and T-1106.[49] These compounds have demonstrated good activity in treating laboratory animals with viral infections caused by various RNA viruses, including influenza virus, arenaviruses, bunyaviruses, and others. Whether these agents will prove capable of treating viral infection in the CNS, however, remains yet to be investigated. Most recently, research on inhibition of certain cellular enzymes called deubiquitinases, important for cellular processes such as the response to unfolded proteins in the endoplasmic reticulum, implicates this pathway as a possible target for the inhibition of replication of certain RNA viruses, including LACV.[115] Even though this finding is certainly interesting, the mechanism of inhibition of viral replication has not been elucidated. In summary, the search for antiviral treatment of LACVE remains important because of the high morbidity caused by this virus as well as its potential to increase in prevalence and severity.[51,99]

OUTCOME

LACV infections are associated with a case-fatality rate of up to 1.9%.[64] A series of hospitalized patients ($n = 127$) showed near-lethal disease from cerebral herniation in three cases. Furthermore, in this study a sizable proportion of patients required mechanical ventilation (25%) or intensive care (50%).[99]

In the 1960s and 1970s, psychometric evaluation of patients who had recovered from their acute LACV–associated encephalitis failed to show significant differences in standard tests of cognitive ability compared with controls or normative data in the general population.[98,122] In 2001, however, a study of 28 recovered patients, most of whom had severe LACVE (e.g., associated with seizures or coma, including three patients with cerebral herniation), showed a mean full-scale intelligence quotient (IQ) of 87.8 (95% CI, 82.2 to 93.2). Thirty-five percent of these patients had an IQ below 80, and 46 percent demonstrated significant disparities between verbal and performance IQ scores. In addition, 60 percent of this group had test results indicative of attention-deficit/hyperactivity disorder.[99] This study lacked a contemporaneous, matched control group, but certainly compared with normative data, these findings strongly suggest that neurocognitive and behavioral deficits do occur after LACVE, particularly with severe disease. In addition, previous data indicating changes in performance on tests administered before and after illness suggested effects in children who were more seriously ill.[98] In another small series, visual-motor function abnormalities and intellectual impairment were observed more often in patients who had focal abnormalities during the acute illness.[98]

Six to 15 percent of patients who recover from LACVE have recurrent seizures.[31,39] The risk of having a recurrent convulsive disorder is approximately 25 percent in patients who experienced a seizure during the acute phase of illness.[39,56] The interval between the onset of recurrent seizures and recovery from acute infection ranges from a few days to years (mean, 4 years).[39] Persistent hemiparesis was a residual abnormality in 2 of 151 patients monitored for up to 6 years after recovery from LACVE, but one patient had a brain biopsy performed, which may have accounted for some of his neurologic deficit.[31] Unilateral infarction of the basal ganglia and hemiparesis have been described in the case of an infant.[93] Another infant with a presumed LACV infection initially had an apparent neurodegenerative disease secondary to acute disseminated encephalomyelitis.[50] See the description at the beginning of this chapter regarding the economic costs of LACVE.

PATHOLOGY

The original model of LACV disease in LACVE proposed that the virus first replicates in muscle cells, leading to viremia with subsequent hematogenous spread to the vascular endothelium and ultimately to access in the CNS.[14,79] In a more recent study, weanling mice inoculated intraperitoneally with 100 LD_{50} of LACV/human/1960 had histopathologic lesions on day 6 after inoculation in the (1) brain, including olfactory bulb, cerebral cortex, thalamus, hippocampus, and medulla oblongata, and (2) spinal cord, including perivascular cuffing, neuronal degeneration, necrosis (either single cell or small foci), and apoptosis.[14] Histopathologic changes were minimal outside the CNS. Viral antigens were not observed in the nasal turbinates, muscle, spleen, or pancreas. However, viral antigen was detected in the olfactory bulb of the brain, thalamus, cerebrum, medulla oblongata, and spinal cord. Therefore, this more recent study suggests that LACV can enter the CNS through the olfactory neurons from nasal olfactory epithelium, although the virus may alternatively or additionally enter through the bloodstream and vascular endothelium.

Pathologic descriptions of two fatal cases have been reported.[82] In gross appearance, the brain was swollen, and the meninges were congested. The principal brain lesions were neuronal degeneration, patchy inflammatory lesions, and vasculitis. The cerebrum and basal ganglia were the principal sites of involvement, but petechial hemorrhages and edema were noted in the spinal cord of one patient. In both cases, lesions in the cerebrum were confined to the frontal, parietal, and temporal lobes, and the cerebellum was not involved. Focal inflammatory lesions and perivascular reactions were composed primarily of mononuclear cells. Neuronolysis and neuronophagia were observed in foci of inflammation and necrosis, along with reactive polymorphonuclear, mononuclear, and microglial responses. Small extravasations of erythrocytes may appear as well. Lymphocytic perivascular cuffs were seen, but inclusion bodies were not. In one case, a focal area of necrosis, hemorrhage, and formation of a hematoma was present in the temporal lobe, a finding corresponding to a mass lesion seen on CT. From a patient in a separate case report, brain biopsy material examined by indirect immunofluorescence demonstrated LACV antigen in neurons and perhaps in endothelial cells. The same biopsy material from this patient showed minimal necrosis and perivascular cuffing on light microscopy.[100] The finding of minimal necrosis despite abnormal CT findings and deep coma is interesting in view of the

relatively benign outcome of LACVE in most patients, even in those who experience deep coma or exhibit CT abnormalities. This finding is in marked contradistinction to HSVE, in which the presence of either coma or CT lesions is a poor prognostic factor, consistent with the extensive necrosis seen on pathologic examination.[145]

PREVENTION

Reduction of exposure to mosquito bites is the best method for prevention of infection by LACV or other mosquito-borne viruses. To accomplish this goal, community campaigns have emphasized using repellent, using barriers (mosquito nets, screens, or protective clothing), avoiding peak biting hours (dawn until dusk), and eliminating mosquito breeding sites. Large-scale spraying of insecticides is not an effective intervention for elimination of *O. triseriatus* because this tree-dwelling mosquito tends to be protected by the leaf canopy of its habitat. Public health prevention has focused on commonsense methods of elimination of breeding sites of *O. triseriatus*. Such efforts in endemic areas have included removal of used tires and other containers that hold small pools of water and (less commonly) sealing of tree holes with cement or gypsum wool insulation.[136] Other methods of minimizing standing water include drilling holes in tire swings so that water drains out; emptying standing water from flower pots, buckets, and barrels; sealing nearby tree holes; replacing water in bird baths weekly; and emptying children's wading pools, storing them on their side after use.[92] Home public health or mosquito control program visits to patients with new cases of LACVE may identify risk factors around the home or in neighboring areas, with implications for uninfected siblings of the index case and possibly for other children in the community. Counties identified with cases may be targeted during the subsequent spring for educational town meetings or television coverage or interventions such as tire removal campaigns. Such targeted interventions have been tracked in highly endemic areas of West Virginia in recent years. The proper use of insect repellents and avoidance of tree-shaded areas during the vector's active feeding period (from dawn until dusk) may confer a reduction in risk by minimizing exposure to the vector.

Repellants are synthetic compounds or derivatives of plant oils that can be used during outdoor activities when mosquitoes are present to discourage mosquitoes from biting. The most active insect repellants for use on skin are products that contain either *N,N*-diethyl-*m*-toluamide or *N,N*-diethyl-3-methyl-benzamide (DEET), picaridin (KBR 3023), IR3535 (3-[*N*-butyl-*N*-acetyl]-aminopropionic acid, ethyl ester), or the plant-based oil of lemon eucalyptus (OLE) and its synthetic equivalent, *p*-menthane-3,8-diol.[2] Insect repellents acceptable for children may contain up to 30 percent DEET per recommendations of the American Academy of Pediatrics (and up to 50% DEET per recommendations of the CDC), but DEET-containing products should not be used on infants younger than 2 months. As of 2005, the American Academy of Pediatrics and the CDC revised recommendations such that repellants containing picaridin are considered an acceptable alternative to DEET.[2] OLE should not be used in children younger than 3 years according to specifications of the Environmental Protection Agency. A possible advantage of DEET and permethrin is that each has been proven to repel ticks (and to kill ticks in the case of permethrin). DEET is an effective solvent and should be used with caution next to clothing because it may dissolve some plastics, rayon, spandex, or other synthetic fibers. In contrast, permethrin is a repellant and insecticide registered by the Environmental Protection Agency for use on clothing and will provide excellent protection through multiple washes. It should not be applied to skin. All products should be used as directed by the manufacturer.

The potential for development of a vaccine comes from work with a truncated Gc protein preparation that induces a protective immune response in suckling mice through generation of neutralizing antibodies.[113] Protection against neuroinvasion was achieved by interruption of transient viremia, which occurred just after inoculation of the virus. A DNA-based vaccine that encodes viral glycoproteins (Gc and Gn) also induced a protective neutralizing antibody response in mice.[112,124] Should ecologic preventive measures fail to lower disease rates in endemic areas, development of a vaccine for use in persons living in these areas may prove worthwhile.

Acknowledgments

We extend many thanks to Lisa Breedlove Chaffee, Assistant in the Section of Infectious Diseases, Nationwide Children's Hospital (NCH), Columbus, Ohio, for her efforts in obtaining relevant literature and in creating the reference list for this chapter revision. In addition, regarding the interpretation of diagnostic studies for La Crosse virus, we would like to recognize the valuable input of Kathy Mack, Supervisor of the Virology and Immunoserology Laboratories at NCH; Linda Minnich, Virology Supervisor at CAMC Memorial–Charleston; and Robert Lanciotti, PhD, Section Chief at the CDC Arbovirus Diseases Branch, DVBD. Last, we would like to thank Marc Fischer, MD, MPH, medical officer at the CDC DVBD and liaison to the American Academy of Pediatrics Committee on Infectious Diseases from 2010 to 2012, for his insight into the epidemiologic investigation of arboviruses.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Alexander JP, Ehresmann K, Seward J, et al. Transmission of imported vaccine-derived poliovirus in an undervaccinated community in Minnesota. *J Infect Dis* 2009;**199**:391–7.
2. American Academy of Pediatrics. Prevention of mosquitoborne infections. In: Pickering LK, editor. *Red Book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 209–11.
3. *Arbovirus IFA IgG [package insert]*. Cypress, Calif: Focus Diagnostics; 2011.
9. Basile AJ, microbiologist, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention. Personal communication.
14. Bennett R, Cress C, Ward J, et al. La Crosse virus infectivity, pathogenesis, and immunogenicity in mice and monkeys. *Virology* 2008;**5**:5–25.

19. *California serogroup viruses immunofluorescent antibody [package insert]*. Cypress, Calif: Focus Diagnostics; 2011.
22. Case Definitions. *Nationally notifiable conditions infectious and non-infectious case*. Atlanta, Ga: Centers for Disease Control and Prevention; 2012.
25. Centers for Disease Control and Prevention. Poliovirus infections in four unvaccinated children—Minnesota, August-October. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1053–5.
26. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1009–13.
27. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:510–14.
40. Deibel R, Srihongse S, Grayson MA. Jamestown Canyon virus: the etiologic agent of an emerging human disease? In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 313–25.
42. Demikhov VG, Chaitsev VG, Butenko AM, et al. California serogroup virus infections in the Ryazan region of the USSR. *Am J Trop Med Hyg* 1991;**45**:371–6.
49. Furuta Y, Takahashi K, Shiraki K, et al. T-705 (favipiravir) and related compounds: novel broad-spectrum inhibitors of RNA viral infections. *Antiviral Res* 2009;**82**:95–102.
58. Grayson MA, Srihongse S, Deibel R, Calister CH. California serogroup viruses in New York State: a retrospective analysis of subtype distribution patterns and their epidemiologic significance, 1965-1981. *Prog Clin Biol Res* 1983;**123**:257–67.
64. Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. *PLoS One* 2009;**4**:e6145.
65. Haddow AD, Schuh AJ. The demographic and socioeconomic factors predictive for populations at high-risk for La Crosse virus infection in West Virginia. *PLoS One* 2011;**6**:e6145.
79. Johnson RT. Pathogenesis of La Crosse virus in mice. *Prog Clin Biol Res* 1983;**123**:139–44.
90. Ksiazek TG, Yuill TM. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciuris carolinensis*) and chipmunks (*Tamias striatus*). *Am J Trop Med Hyg* 1977;**26**:815–21.
92. Lambert A, Blair CD, D'Anton M, et al. La Crosse virus in *Aedes albopictus* mosquitoes, Texas, USA, 2009. *Emerg Infect Dis* 2010;**16**:856–8.
95. Mack K, Salamon D, Dible M, Marcon M. *First reported recovery of La Crosse virus from CSF: a case study*. Clearwater Beach, Fla: Clinical Virology Symposium; 1997.
96. Mack KE, Bowersock C, Zamastil C, et al. *Clinical utility of detection of IgG by IFA to La Crosse virus in a pediatric hospital*. Clearwater Beach, Fla: Clinical Virology Symposium; 2012.
103. McJunkin JE, Nahata MC, de los Reyes EC, et al. Safety and pharmacokinetics of ribavirin for the treatment of La Crosse encephalitis. *Pediatr Infect Dis J* 2011;**30**:860–5.
106. Monath TP, Nuckolls JG, Berall J, et al. Studies on California encephalitis in Minnesota. *Am J Epidemiol* 1970;**92**:40–50.
107. Moulton DW, Thompson WH. California group virus infections in small, forest-dwelling mammals of Wisconsin. Some ecological considerations. *Am J TropMed Hyg* 1971;**20**:474–82.
108. Murphree R, Dunn JR, Schaffner W, Jones TF. La Crosse encephalitis surveillance using single versus paired serologic testing. *Zoonoses Public Health* 2012;**59**:181–3.
115. Perry JW, Ahmed M, Chang KO, et al. Antiviral activity of a small molecule deubiquitinase inhibitor occurs via induction of the unfolded protein response. *PLoS Pathog* 2012;**8**:e1002783.
117. Plassmeyer ML, Soldan SS, Stachelek KM, et al. California serogroup Gc (G1) glycoprotein is the principal determinant of pH-dependent cell fusion and entry. *Virology* 2005;**338**:121–32.
118. Plassmeyer ML, Soldan SS, Stachelek KM, et al. Mutagenesis of the La Crosse Virus glycoprotein supports a role for Gc (1066-1087) as the fusion peptide. *Virology* 2007;**358**:273–82.
120. Reichelt M, Stertz S, Krijnse-Locker J, et al. Missorting of LaCrosse virus nucleocapsid protein by the interferon-induced MxA GTPase involves smooth ER membranes. *Traffic* 2004;**5**:772–84.
121. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg* 2008;**79**:974–9.
127. Sokol DK, Kleiman MB, Garg BP. LaCrosse viral encephalitis mimics herpes simplex viral encephalitis. *Pediatr Neurol* 2001;**25**:413–15.
129. Soldan SS, Wagner V, Weber F, et al. La Crosse virus (LACV) Gc fusion peptide mutants have impaired growth and fusion phenotypes, but remain neurotoxic. *Virology* 2010;**404**:139–47.
139. Tsai TF. Arboviruses. In: Murray PR, editor. *Manual of clinical microbiology*. Washington, D.C.: ASM Press; 1995.
140. Tunkel I, Glaser C, Bloch K, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**47**:303–27.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alexander JP, Ehresmann K, Seward J, et al. Transmission of imported vaccine-derived poliovirus in an undervaccinated community in Minnesota. *J Infect Dis* 2009;**199**:391–7.
2. American Academy of Pediatrics. Prevention of mosquitoborne infections. In: Pickering LK, editor. *Red Book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 209–11.
3. *Arbovirus IFA IgG [package insert]*. Cypress, Calif: Focus Diagnostics; 2011.
4. Artsob H, Spence L. California encephalitis: *Quebec Can Dis Wkly Rep* 1981;**7**:194–5.
5. Balfour HH Jr, Siem RA, Bauer H, Quie PG. California arbovirus (La Crosse) infections. I. Clinical and laboratory findings in 66 children with meningoencephalitis. *Pediatrics* 1973;**52**:680–91.
6. Balkhy HH Jr. Schreiber: Severe La Crosse encephalitis with significant neurologic sequelae. *Pediatr Infect Dis J* 2000;**19**: 77–80.
7. Barker CM, Brewster CC, Paulson SL. Spatiotemporal oviposition and habitat preferences of *Ochlerotatus triseriatus* and *Aedes albopictus* in an emerging focus of La Crosse virus. *J Am Mosq Control Assoc* 2003;**19**:382–91.
8. Barker CM, Paulson SL, Cantrell S, Davis BS. Habitat preferences and phenology of *Ochlerotatus triseriatus* and *Aedes albopictus* (Diptera: Culicidae) in southwestern Virginia. *J Med Entomol* 2003;**40**:403–10.
9. Basile AJ, microbiologist, Division of Vector-Borne Diseases, National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention. Personal communication.
10. Beaty BJ, Bishop DH. Bunyavirus-vector interactions. *Virus Res* 1988;**10**:289–301.
11. Beaty BJ, Casals J, Brown KL, et al. Indirect fluorescent-antibody technique for serological diagnosis of La Crosse (California) virus infections. *J Clin Microbiol* 1982;**15**:429–34.
12. Beaty BJ, Jamnback TL, Hildreth SW, et al. Rapid diagnosis of La Crosse virus infections: evaluation of serologic and antigen detection techniques for the clinically relevant diagnosis of La Crosse encephalitis. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 293–302.
13. Beghi E, Nicolosi A, Kurland LT, et al. Encephalitis and aseptic meningitis, Olmsted County, Minnesota, 1950-1981: I. Epidemiology. *Ann Neurol* 1984;**16**:283–94.
14. Bennett R, Cress C, Ward J, et al. La Crosse virus infectivity, pathogenesis, and immunogenicity in mice and monkeys. *Virology* 2008;**5**:5–25.
15. Berry RL, Weigert JL, Calisher CH, et al. Evidence for transovarial transmission of Jamestown Canyon virus in Ohio. *Mosq News* 1997;**37**:494–6.
16. Blakqori G, Weber F. Efficient cDNA-based rescue of La Crosse bunyaviruses expressing or lacking the nonstructural protein NSs. *J Virol* 2005;**79**:10420–8.
17. Borucki MK, Chandler LJ, Parker BM, et al. Bunyavirus superinfection and segment reassortment in transovarially infected mosquitoes. *J Gen Virol* 1999;**80**(Pt 12):3173–9.
18. Borucki MK, Kempf BJ, Blitvich BJ, et al. La Crosse virus: replication in vertebrate and invertebrate hosts. *Microbes Infect* 2002;**4**:341–50.
19. *California serogroup viruses immunofluorescent antibody [package insert]*. Cypress, Calif: Focus Diagnostics; 2011.
20. Calisher CH, Pretzman CI, Muth DJ, et al. Serodiagnosis of La Crosse virus infections in humans by detection of immunoglobulin M class antibodies. *J Clin Microbiol* 1972;**23**:667–71.
21. Campbell GL, Reeves WD, Hardy JL, et al. Seroepidemiology of California and Bunyamwera serogroup bunyavirus infections in humans in California. *Am J Epidemiol* 1992;**136**:308–19.
22. Case Definitions. *Nationally notifiable conditions infectious and noninfectious case*. Atlanta, Ga: Centers for Disease Control and Prevention; 2012.
23. Case KL, West RM, Smith MJ. Histocompatibility antigens and La Crosse encephalitis. *J Infect Dis* 1993;**168**:358–60.
24. Cassidy LF, Patterson JL. Mechanism of La Crosse virus inhibition by ribavirin. *Antimicrob Agents Chemother* 1989;**33**:2009–11.
25. Centers for Disease Control and Prevention. Poliovirus infections in four unvaccinated children—Minnesota, August-October. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1053–5.
26. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1009–13.
27. Centers for Disease Control and Prevention. West Nile virus disease and other arboviral diseases—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:510–14.
28. Centers for Disease Control and Prevention. *California serogroup viral (mainly La Crosse) encephalitis cases in humans, 1964-2005*. Atlanta, Ga: Centers for Disease Control and Prevention; 2006.
29. Chandler LJ, Beaty BJ, Bishop DHL, et al. Detection of La Crosse and snowshoe hare viral nucleic acids by in situ hybridization. *Am J Trop Med Hyg* 1989;**40**:561–8.
30. Chandler LJ, Borucki MK, Dobie DK, et al. Characterization of La Crosse virus RNA in autopsied central nervous system tissues. *J Clin Microbiol* 1998;**36**:3332–6.
31. Chun RW, Thompson WH, Grabow JD, et al. California arbovirus encephalitis in children. *Neurology* 1968;**18**:369–75.
32. Chun RWM. Clinical aspects of La Crosse encephalitis: neurological and psychological sequelae. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 193–201.
33. Connor E, Morrison S, Lane J, et al. Safety, tolerance, and pharmacokinetics of systemic ribavirin in children with human immunodeficiency virus infection. *Antimicrob Agents Chemother* 1993;**37**:532–9.
34. Cramblett HG, Stegmiller H, Spencer C. California encephalitis virus infections in children: clinical and laboratory studies. *JAMA* 1966;**198**:128–32.
35. Craven RB, Eliason DA, Francy DB, et al. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. *J Am Mosq Control Assoc* 1988;**4**:138–42.
36. Cully JF Jr, Streit TG, Heard PB. Transmission of La Crosse virus by four strains of *Aedes albopictus* to and from the eastern chipmunk (*Tamias striatus*). *J Am Mosq Control Assoc* 1992;**8**:237–40.
37. de los Reyes E, Glauser TA, McJunkin JE, et al. Periodic lateralizing epileptiform discharges and La Crosse encephalitis: diagnostic and public health implications. *Neurology* 1996;**46**:150–1.
38. de los Reyes EC, McJunkin JE, Glauser TA, et al. Periodic lateralized epileptiform discharges in La Crosse encephalitis, a worrisome subgroup: clinical presentation, electroencephalogram (EEG) patterns, and long-term neurologic outcome. *J Child Neurol* 2008;**23**:167–72.
39. Deering WM. Neurologic aspects and treatment of La Crosse encephalitis. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 187–91.
40. Deibel R, Srihongse S, Grayson MA. Jamestown Canyon virus: the etiologic agent of an emerging human disease? In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 313–25.
41. Deibel R, Srihongse S, Woodall JP. Arboviruses in New York State: an attempt to determine the role of arboviruses in patients with viral encephalitis and meningitis. *Am J Trop Med Hyg* 1979;**28**:577–82.
42. Demikhov VG, Chaitsev VG, Butenko AM, et al. California serogroup virus infections in the Ryazan region of the USSR. *Am J Trop Med Hyg* 1991;**45**:371–6.
43. Disease Control and Epidemiology Service, Ontario Ministry of Health. Surveillance of arboviruses in Ontario in 1983: the increased detection of seropositive cases to the California group viruses (CGV). *Ont Dis Surveill Rep* 1984;**5**:394–400.
44. Dykers TI, Brown KL, Gundersen CB, et al. Rapid diagnosis of LaCrosse encephalitis: detection of specific immunoglobulin M in cerebrospinal fluid. *J Clin Microbiol* 1985;**22**:740–4.
45. El Said LH, Vorndam V, Gentsch JR, et al. A comparison of La Crosse virus isolates obtained from different ecological niches and an analysis of the structural components of California encephalitis serogroup viruses and other bunyaviruses. *Am J Trop Med Hyg* 1979;**28**:364–86.
46. Eldridge BF, Glaser C, Pedrin RE, Chiles RE. The first reported case of California encephalitis in more than 50 years. *Emerg Infect Dis* 2001;**7**:451–2.

47. Endres MJ, Griot C, Gonzalez-Scarano F, et al. Neuroattenuation of an avirulent bunyavirus variant maps to the L RNA segment. *J Virol* 1991;**65**:5465–70.
48. Erwin PC, Jones TF, Gerhardt RR, et al. La Crosse encephalitis in Eastern Tennessee: clinical, environmental, and entomological characteristics from a blinded cohort study. *Am J Epidemiol* 2002;**155**:1060–5.
49. Furuta Y, Takahashi K, Shiraki K, et al. T-705 (favipiravir) and related compounds: novel broad-spectrum inhibitors of RNA viral infections. *Antiviral Res* 2009;**82**:95–102.
50. Garg BP, Kleiman MB. Acute disseminated encephalomyelitis presenting as a neurodegenerative disease in infancy. *Pediatr Neurol* 1994;**11**:57–8.
51. Gerhardt RR, Gottfried KL, Apperson CS, et al. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. *Emerg Infect Dis* 2001;**7**:807–11.
52. Godsey MS Jr, Amoo F, Yuill TM, et al. California serogroup virus infections in Wisconsin domestic animals. *Am J Trop Med Hyg* 1988;**39**:409–16.
53. Gonzalez-Scarano F, Beaty BJ, Sudin D, et al. Genetic determinants of the virulence and infectivity of La Crosse virus. *Microb Pathog* 1988;**4**:1–7.
54. Gonzalez-Scarano F, Pobjecky N, Nathanson N. Bunyaviruses. In: Fields BN, Knipe DM, editors. *Virology*. New York: Raven Press; 1990. p. 1195–228.
55. Gonzalez-Scarano F, Pobjecky N, Nathanson N. La Crosse bunyavirus can mediate pH-dependent fusion from without. *Virology* 1984;**132**:222–5.
56. Grabow JD, Matthews CG, Chun RWM, et al. The electroencephalogram and clinical sequelae of California arbovirus encephalitis. *Neurology* 1969;**19**:394–404.
57. Graham DH, Holmes JL, Higgs S, et al. Selection of refractory and permissive strains of *Aedes triseriatus* (Diptera: Culicidae) for transovarial transmission of La Crosse virus. *J Med Entomol* 1999;**36**:671–8.
58. Grayson MA, Srihongse S, Deibel R, Calister CH. California serogroup viruses in New York State: a retrospective analysis of subtype distribution patterns and their epidemiologic significance, 1965-1981. *Prog Clin Biol Res* 1983;**123**:257–67.
59. Grimstad PR. California group virus disease. In: Monath TP, editor. *The arbovirus: epidemiology and ecology*, **vol. 2**. Boca Raton, Fla: CRC Press; 1988. p. 99–136.
60. Grimstad PR, Kobayashi JF, Zhand M, et al. Recently introduced *Aedes albopictus* in the United States: potential vector of La Crosse virus (Bunyaviridae: California serogroup). *J Am Mosq Control Assoc* 1989;**5**:422–7.
61. Grimstad PR, Shabino CL, Calisher CH, et al. A case of encephalitis in a human associated with a serologic rise to Jamestown Canyon virus. *Am J Trop Med Hyg* 1982;**31**:1238–44.
62. Gundersen CB, Brow KL. Clinical aspects of La Crosse encephalitis: preliminary report. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 169–77.
63. Hacker JK, Volkman LE, Hardy JL. Requirement for the G1 protein of California encephalitis virus in infection in vitro and in vivo. *Virology* 1995;**206**:945–53.
64. Haddow AD, Odoi A. The incidence risk, clustering, and clinical presentation of La Crosse virus infections in the eastern United States, 2003-2007. *PLoS One* 2009;**4**:e6145.
65. Haddow AD, Schuh AJ. The demographic and socioeconomic factors predictive for populations at high-risk for La Crosse virus infection in West Virginia. *PLoS One* 2011;**6**:e6145.
66. Hammon WM, Reeves WC. California encephalitis virus, a newly described agent. *Calif Med* 1952;**77**:303–9.
67. Hammon WM, Reeves WC, Sather G. California encephalitis virus, a newly described agent. II. Isolations and attempts to identify and characterize the agent. *J Immunol* 1952;**69**:493–510.
68. Hardin SG, Erwin PC, Patterson L, et al. Clinical comparisons of La Crosse encephalitis and enteroviral central nervous system infections in a pediatric population: 2001 surveillance in East Tennessee. *Am J Infect Control* 2003;**31**:508–10.
69. Hefti HP, Frese M, Landis H, et al. Human MxA protein protects mice lacking a functional alpha/beta interferon system against La Crosse virus and other lethal viral infections. *J Virol* 1999;**73**:6984–91.
70. Hilty MD, Haynes RE, Azimi PH, et al. California encephalitis in children. *Am J Dis Child* 1972;**124**:530–3.
71. Huang C, Thompson WH, Campbell WP. Comparison of the M RNA genome segments of two human isolates of La Crosse virus. *Virus Res* 1995;**36**:177–85.
72. Huggins JW, Jahrling P, Kende M, et al. Efficacy of ribavirin against virulent RNA virus infections. In: Smith RA, Knight V, Smith JAD, editors. *Clinical applications of ribavirin*. Orlando, Fla: Academic Press; 1984. p. 49–63.
73. Huggins JW, Kim GR, Brand OM, et al. Ribavirin therapy for Hantaan virus infection in suckling mice. *J Infect Dis* 1986;**153**:489–97.
74. Hughes MT, Gonzalez JA, Reagan KL, et al. Comparative potential of *Aedes triseriatus*, *Aedes albopictus*, and *Aedes aegypti* (Diptera: Culicidae) to transovarially transmit La Crosse virus. *J Med Entomol* 2006;**43**:757–61.
75. Hurwitz ES, Schell W, Nelson D, et al. Surveillance for California encephalitis group virus illness in Wisconsin and Minnesota, 1978. *Am J Trop Med Hyg* 1983;**32**:595–601.
76. Janssen R, Gonzalez-Scarano F, Nathanson N. Mechanisms of bunyavirus virulence. Comparative pathogenesis of a virulent strain of La Crosse and an avirulent strain of Tahyna virus. *Lab Invest* 1984;**50**:447–55.
77. Johnson AJ, Martin DA, Karabatsos N, et al. Detection of anti-arboviral immunoglobulin G by using a monoclonal antibody–based capture enzyme-linked immunosorbent assay. *J Clin Microbiol* 2000;**38**:1827–31.
78. Johnson KP, Johnson RT. California encephalitis. II. Studies of experimental infection in the mouse. *J Neuropathol Exp Neurol* 1968;**27**:390–400.
79. Johnson RT. Pathogenesis of La Crosse virus in mice. *Prog Clin Biol Res* 1983;**123**:139–44.
80. Jones TF, Craig AS, Nasci RS, et al. Newly recognized focus of La Crosse encephalitis in Tennessee. *Clin Infect Dis* 1999;**28**:93–7.
81. Jones TF, Erwin PC, Craig AS, et al. Serological survey and active surveillance for La Crosse virus infections among children in Tennessee. *Clin Infect Dis* 2000;**31**:1284–7.
82. Kalfayan B. Pathology of La Crosse virus infection in humans. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 179–86.
83. Kappus KD, Monath TP, Kaminski RM, et al. Reported encephalitis associated with California serogroup virus infections in the United States, 1963-1981. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 31–41.
84. Kindle AA, McJunkin JE, Meek JR, et al. La Crosse encephalitis in West Virginia. *MMWR Morb Mortal Wkly Rep* 1988;**37**: 1449–53.
85. Kingsford L, Boucquey KH. Monoclonal antibodies specific for the G1 glycoprotein of La Crosse virus that react with other California serogroup viruses. *J Gen Virol* 1990;**71**(Pt 3):523–30.
86. Kingsford L, Boucquey KH, Cardoso TP. Effects of specific monoclonal antibodies on La Crosse virus neutralization: aggregation, inactivation by Fab fragments, and inhibition of attachment to baby hamster kidney cells. *Virology* 1991;**180**:591–601.
87. Kitron U, Michael J, Swanson J, et al. Spatial analysis of the distribution of LaCrosse encephalitis in Illinois, using a geographic information system and local and global spatial statistics. *Am J Trop Med Hyg* 1997;**57**:469–75.
88. Klimas RA, Thompson WH, Calisher CH, et al. Genotypic varieties of La Crosse virus isolated from different geographic regions of the continental United States and evidence for a naturally occurring intertypic recombinant La Crosse virus. *Am J Epidemiol* 1981;**114**:112–31.
89. Kochs G, Janzen C, Hohenberg H, Haller O. Antivirally active MxA protein sequesters La Crosse virus nucleocapsid protein into perinuclear complexes. *Proc Natl Acad Sci U S A* 2002;**99**:3153–8.
90. Ksiazek TG, Yuill TM. Viremia and antibody response to La Crosse virus in sentinel gray squirrels (*Sciuris carolinensis*) and chipmunks (*Tamias striatus*). *Am J Trop Med Hyg* 1977;**26**: 815–21.
91. Kuno G, Mitchell CJ, Chang GJ, et al. Detecting bunyaviruses of the Bunyamwera and California serogroups by a PCR technique. *J Clin Microbiol* 1996;**34**:1184–8.

92. Lambert A, Blair CD, D'Anton M, et al. La Crosse virus in *Aedes albopictus* mosquitoes, Texas, USA, 2009. *Emerg Infect Dis* 2010;**16**:856–8.
93. Leber SM, Brunberg JA, Pavkovic IM. Infarction of basal ganglia associated with California encephalitis virus. *Pediatr Neurol* 1995;**12**:346–9.
94. Ludwig GV, Israel BA, Christensen BM, et al. Role of La Crosse virus glycoproteins in attachment of virus to host cells. *Virology* 1991;**181**:564–71.
95. Mack K, Salamon D, Dible M, Marcon M. *First reported recovery of La Crosse virus from CSF: a case study*. Clearwater Beach, Fla: Clinical Virology Symposium; 1997.
96. Mack KE, Bowersock C, Zamastil C, et al. *Clinical utility of detection of IgG by IFA to La Crosse virus in a pediatric hospital*. Clearwater Beach, Fla: Clinical Virology Symposium; 2012.
97. Mancao MY, Law IM, Roberson-Trammell K. California encephalitis in Alabama. *South Med J* 1996;**89**:992–3.
98. Matthews CG, Chun RWM, Grabow JD, et al. Psychological sequelae in children following California arbovirus encephalitis. *Neurology* 1968;**18**:1023–30.
99. McJunkin JE, de los Reyes EC, Irazuzta JE, et al. La Crosse encephalitis in children. *N Engl J Med* 2001;**344**:801–7.
100. McJunkin JE, Khan R, de los Reyes EC, et al. Treatment of severe La Crosse encephalitis with intravenous ribavirin following diagnosis by brain biopsy. *Pediatrics* 1997;**99**:261–7.
101. McJunkin JE, Khan RR, Tsai TF. California–La Crosse encephalitis. *Infect Dis Clin North Am* 1998;**12**: 83–93.
102. McJunkin JE, Minnich LL, Huang E. *Evaluation of the efficacy of ribavirin in treatment of experimentally induced La Crosse encephalitis in mice*. Baltimore, Md: Pediatric Academic Societies Annual Meeting; 2002.
103. McJunkin JE, Nahata MC, de los Reyes EC, et al. Safety and pharmacokinetics of ribavirin for the treatment of La Crosse encephalitis. *Pediatr Infect Dis J* 2011;**30**:860–5.
104. Miller BR, DeFoliart GR, Yuill TM. Vertical transmission of La Crosse virus (California encephalitis group): transovarial and filial infection rates in *Aedes triseriatus* (Diptera: Culicidae). *J Med Entomol* 1977;**14**:437–40.
105. Mitchell CJ, Hramis LD, Karabatsos N, et al. Isolation of La Crosse, Cache Valley, and Potosi viruses from *Aedes* mosquitoes (Diptera: Culicidae) collected at used-tire sites in Illinois during 1994-1995. *J Med Entomol* 1998;**35**:573–7.
106. Monath TP, Nuckolls JG, Berall J, et al. Studies on California encephalitis in Minnesota. *Am J Epidemiol* 1970;**92**:40–50.
107. Moulton DW, Thompson WH. California group virus infections in small, forest-dwelling mammals of Wisconsin. Some ecological considerations. *Am J TropMed Hyg* 1971;**20**:474–82.
108. Murphree R, Dunn JR, Schaffner W, Jones TF. La Crosse encephalitis surveillance using single versus paired serologic testing. *Zoonoses Public Health* 2012;**59**:181–3.
109. Neitzel DF, Grimstad PR. Serological evidence of California group and Cache Valley virus infection in Minnesota white-tailed deer. *J Wildl Dis* 1991;**27**:230–7.
110. Osorio JE, Godsey MS, Defoliart GR, et al. La Crosse viremias in white-tailed deer and chipmunks exposed by injection or mosquito bite. *Am J Trop Med Hyg* 1996;**54**:338–42.
111. Paulson SL, Hawley WA. Effect of body size on the vector competence of field and laboratory populations of *Aedes triseriatus* for La Crosse virus. *J Am Mosq Control Assoc* 1991;**7**:170–5.
112. Pavlovic J, Schultz J, Hefti HP, et al. DNA vaccination against La Crosse virus. *Intervirology* 2000;**43**:312–21.
113. Pekosz A, Griot C, Stillmock K, et al. Protection from La Crosse virus encephalitis with recombinant glycoproteins: role of neutralizing anti-G1 antibodies. *J Virol* 1995;**69**:3475–81.
114. Pekosz A, Phillips J, Pleasure D, et al. Induction of apoptosis by La Crosse virus infection and role of neuronal differentiation and human bcl-2 expression in its prevention. *J Virol* 1996;**70**: 5329–35.
115. Perry JW, Ahmed M, Chang KO, et al. Antiviral activity of a small molecule deubiquitinase inhibitor occurs via induction of the unfolded protein response. *PLoS Pathog* 2012;**8**:e1002783.
116. Peters CJ, Reynolds JA, Slone TW, et al. Prophylaxis of Rift Valley fever with antiviral drugs, immune serum, an interferon inducer, and a macrophage activator. *Antiviral Res* 1986;**6**:285–97.
117. Plassmeyer ML, Soldan SS, Stachelek KM, et al. California serogroup Gc (G1) glycoprotein is the principal determinant of pH-dependent cell fusion and entry. *Virology* 2005;**338**:121–32.
118. Plassmeyer ML, Soldan SS, Stachelek KM, et al. Mutagenesis of the La Crosse virus glycoprotein supports a role for Gc (1066-1087) as the fusion peptide. *Virology* 2007;**358**:273–82.
119. Reeves WC, Emmons RW, Hardy JL. Historical perspectives on California encephalitis virus in California. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 19–29.
120. Reichelt M, Stertz S, Krijnse-Locker J, et al. Missorting of LaCrosse virus nucleocapsid protein by the interferon-induced MxA GTPase involves smooth ER membranes. *Traffic* 2004;**5**: 772–84.
121. Reimann CA, Hayes EB, DiGuiseppi C, et al. Epidemiology of neuroinvasive arboviral disease in the United States, 1999-2007. *Am J Trop Med Hyg* 2008;**79**:974–9.
122. Rie HE, Hilty MD, Cramblett HG. Intelligence and coordination following California encephalitis. *Am J Dis Child* 1973;**125**:824–7.
123. Rust RS, Thompson WH, Matthews CG, et al. La Crosse and other forms of California encephalitis. *J Child Neurol* 1999;**14**:1–14.
124. Schuh T, Schultz J, Moelling K, et al. DNA-based vaccine against La Crosse virus: protective immune response mediated by neutralizing antibodies and $CD4^+$ T cells. *Hum Gene Ther* 1999;**10**: 1649–58.
125. Shope RE, Rozhon EJ, Bishop DH. Role of the middle-sized bunyavirus RNA segment in mouse virulence. *Virology* 1981;**114**: 273–6.
126. Sidwell RW, Huffman JH, Barnett BB, et al. In vitro and in vivo Phlebovirus inhibition by ribavirin. *Antimicrob Agents Chemother* 1988;**32**:331–6.
127. Sokol DK, Kleiman MB, Garg BP. LaCrosse viral encephalitis mimics herpes simplex viral encephalitis. *Pediatr Neurol* 2001;**25**: 413–15.
128. Soldan SS, Plassmeyer ML, Matukonis MK, Gonzalez-Scarano F. La Crosse virus nonstructural protein NSs counteracts the effects of short interfering RNA. *J Virol* 2005;**79**:234–44.
129. Soldan SS, Wagner V, Weber F, et al. La Crosse virus (LACV) Gc fusion peptide mutants have impaired growth and fusion phenotypes, but remain neurotoxic. *Virology* 2010;**404**:139–47.
130. Srihongse S, Grayson MA, Deibel R. California serogroup viruses in New York State: the role of subtypes in human infections. *Am J Trop Med Hyg* 1984;**33**:1218–27.
131. Stephen EL, Jones DE, Peters CJ, et al. Ribavirin treatment of toga-, arena-, and bunyavirus infection in subhuman primates and other laboratory animal species. In: Smith RA, Kirkpatrick W, editors. *Ribavirin: a broad-spectrum antiviral agent*. New York: Academic Press; 1980, p. 169–83.
132. Szumlas DE, Apperson CS, Hartig PC, et al. Seroepidemiology of La Crosse virus infection in humans in western North Carolina. *Am J Trop Med Hyg* 1996;**54**:332–7.
133. Szumlas DE, Apperson CS, Powell EF. Seasonal occurrence and abundance of *Aedes triseriatus* and other mosquitoes in a La Crosse virus–endemic area in western North Carolina. *J Am Mosq Control Assoc* 1996;**12**(Pt 1):184–93.
134. Taylor MR, Carpenter DE, Currier RD, et al. California encephalitis virus causes subacute encephalomyelitis in an adult. *Arch Neurol* 1985;**42**:88–9.
135. Thompson WH, Evans AS. California encephalitis virus studies in Wisconsin. *Am J Epidemiol* 1965;**81**:230–44.
136. Thompson WH, Gundersen CB. La Crosse encephalitis: occurrence of disease and control in a suburban area. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 225–36.
137. Thompson WH, Kalfayan B, Anslow RO. Isolation of California encephalitis group virus from a fatal human illness. *Am J Epidemiol* 1965;**81**:245–53.
138. Tsai TF. Arboviral infections in the United States. *Infect Dis Clin North Am* 1991;**5**:73–102.
139. Tsai TF. Arboviruses. In: Murray PR, editor. *Manual of clinical microbiology*. Washington, DC: ASM Press; 1995.
140. Tunkel I, Glaser C, Bloch K, et al. The management of encephalitis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**47**:303–27.

141. Utz JT, Apperson CS, MacCormack JN, et al. Economic and social impacts of La Crosse encephalitis in western North Carolina. *Am J Trop Med Hyg* 2003;**69**:509–18.
142. Walters LL, Tirrell SJ, Shope RE. Seroepidemiology of California and Bunyamwera serogroup (Bunyaviridae) virus infections in native populations of Alaska. *Am J Trop Med Hyg* 1999;**60**: 806–21.
143. Watts DM, Pantuwatana S, Yuill TM, et al. Transovarial transmission of LaCrosse virus in *Aedes triseriatus*. *Ann N Y Acad Sci* 1975;**266**:135–43.
144. Watts DM, Thompson WH, Yuill TM, et al. Overwintering of La Crosse virus in *Aedes triseriatus*. *Am J Trop Med Hyg* 1974;**23**: 694–700.
145. Whitley RJ. Viral encephalitis. *N Engl J Med* 1990;**323**:242–50.
146. Woodruff BA, Baron RC, Tsai TF. Symptomatic La Crosse virus infections of the central nervous system: a study of risk factors in an endemic area. *Am J Epidemiol* 1992;**136**:320–7.
147. Wurtz R, Paleologos N. La Crosse encephalitis presenting like herpes simplex encephalitis in an immunocompromised adult. *Clin Infect Dis* 2000;**31**:1113–14.
148. Young DJ. California encephalitis virus. Report of three cases and review of the literature. *Ann Intern Med* 1966;**65**:419–28.
149. Yuill TM. The role of mammals in the maintenance and dissemination of La Crosse virus. In: Thompson WH, Calisher CH, editors. *California serogroup viruses*. New York: Alan R. Liss; 1983. p. 77–87.
150. Zamparo JM, Andreadis TG, Shope RE. Serologic evidence of Jamestown Canyon virus infection in white-tailed deer populations from Connecticut. *J Wildl Dis* 1997;**33**:623–7.

CHAPTER 190

Other Bunyaviridae

190A ■ RIFT VALLEY FEVER

C. J. Peters • Gail J. Harrison

Rift Valley fever (RVF) is primarily a disease of sheep and cattle. It is transmitted by mosquitoes, and outbreaks usually occur during years of heavy rainfall. It is caused by a virus with selective affinity for the parenchymal cells of the liver, which undergo characteristic eosinophilic degeneration. Infection with RVF virus causes a short but severe disease in sheep and cattle. Most pregnant ewes and cows abort, and more than 90 percent of newborn lambs die. Case-fatality rates in older sheep and cattle are lower but nonetheless significant. People usually acquire the infection from direct contact or aerosols generated from the body fluids and tissues of animals dying of the disease and, less commonly, from bites of infected mosquitoes, especially during epidemics.[3,9,16] The public health importance of this disease in humans is emerging because recent outbreaks in Africa, sub-Saharan Africa, Madagascar, Saudi Arabia, Yemen, and Egypt have resulted in an increase in human fatalities (Fig. 190a-1). Recently, RVF virus has been classified as a potential bioterrorist agent.[3,9]

HISTORY

RVF probably has occurred for many years in Africa and was recognized first at the beginning of the 20th century with the introduction of intensive livestock husbandry. In 1912, large numbers of newborn lambs died of an unknown disease in the Rift Valley of Kenya, and in the following year the clinical features were described. Daubney and associates[5] proved that the causal agent was a filterable virus, which they suspected was transmitted by mosquitoes because animals protected by screens did not contract the disease. In 1944, the virus was isolated from mosquitoes caught in the Semliki Forest in western Uganda, and it was proved later that *Erethmapodites chrysogaster* was able to transmit the infection under experimental conditions. Between 1950 and 1974, at least 15 major epizootics of RVF occurred in livestock in various areas of sub-Saharan Africa. During 1975, an extensive epizootic of RVF took place in South Africa, with many human cases and several deaths documented.[7]

An extensive epizootic of RVF also occurred in lower Egypt in 1977. Hundreds of thousands of domestic animals, including cattle, buffaloes, goats, and sheep, were lost. Associated with this epizootic was the largest human epidemic of the disease ever known, in which 200,000 humans were infected and 600 died.[11] This outbreak emphasized the increasing threat of RVF to humans and domestic animals and showed that RVF virus was an important cause of hemorrhagic fever in Africa. In 1987, an epidemic of RVF occurred in Mauritania after flooding of the Senegal River basin following completion of the Diama Dam; at least 1200 human cases and 200 deaths occurred in one affected area alone. Epidemics and epizootics were reported in Madagascar in 1990,[13] in Egypt in 1993,[2] in eastern Africa in 1997 to 1998,[1] and in Yemen and Saudi Arabia in 2000. In November 2006 through January 2007, Kenya experienced an outbreak of RVF, with a total of 404 cases, 118 deaths, and a case-fatality rate of 29 percent. Similar to previous outbreaks, this most recent outbreak was associated with a heavy rainfall, and most cases occurred in young men who herded and slaughtered livestock and in young women, who likely handled uncooked animal products as they prepared meals for the family.[4] The cases in the Arabian peninsula are the first outside Africa and demonstrate the potential of RVF to spread. Based on isolation of the virus, RVF probably occurs sporadically throughout most of sub-Saharan Africa. Zinga virus, previously described as a cause of sporadic human disease in central Africa, has been shown to be a strain of RVF virus. With increase in international trade and tourist travel, as well as climate changes affecting northern Africa and southern Europe, it is likely that future outbreaks of VF may soon affect agricultural industry and humans in Europe and Asia.[3]

ETIOLOGIC AGENT

RVF virus is a member of the genus *Phlebovirus*, belonging to the *Bunyaviridae* family of viruses. It is a negative-stranded RNA virus, with tripartite S, M, and L segments of its RNA. It is a Biosafety Level-4 pathogen that is easily cultivated in Vero cells or mice from blood and tissue specimens of infected animals and human patients. It also is classified as a Category A overlap select agent by the Centers for Disease Control and Prevention.[3] The virus is destroyed by solvents such as ether and is inactivated readily by formalin; it is destroyed by heating at 56° C for 40 minutes. When stored at 4° C or –10° C, the virus loses its infectivity in about 3 months. The virus can

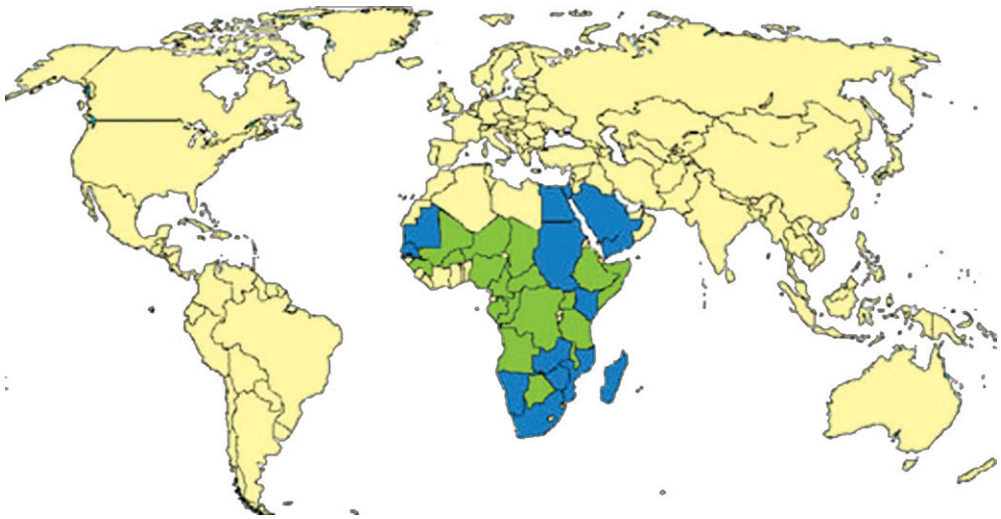

FIGURE 190A-1 ■ Rift Valley fever world distribution map. (See companion Expert Consult website for color version.) (From Centers for Disease Control and Prevention Special Pathogens Branch: http://www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/rvf/rvfmap.htm.)

be preserved indefinitely on dry ice at –70° C or in lyophilized form.

Fully formed virions are spherical and approximately 94 nm in diameter. They mature in the cytoplasm, although intranuclear inclusions occur in vivo and in cell culture. The virus multiplies readily in a variety of cell lines of animal and human origin. The virus is highly pathogenic for mice, young rats, and hamsters, and death occurs in 95 to 100 percent of these animals 36 to 96 hours after inoculation.

VECTORS AND EPIDEMIOLOGY

In studies in South Africa, the virus has been transmitted experimentally by a variety of mosquitoes, including *Culex theileri*, *Culex zombaensis*, *Culex neavei*, *Aedes juppi*, *Anopheles*, and *Erethmapodites quinquevittatus*. Epizootics of RVF have occurred in years of unusually heavy rains that filled natural depressions in the land (pans or dambos), thereby favoring the proliferation of floodwater mosquitoes. Studies in Kenya have shown that transovarial transmission of the virus in pan-breeding *Aedes* of the subgenus *Neomelanoconion* is the probable mechanism of virus maintenance and periodic recrudescence. *Culex pipiens* was implicated as a vector in Egypt during the 1977 to 1978 epidemic. During epizootics, domestic livestock serve as viremic, amplifying hosts in the transmission cycle. Although currently the virus is limited to Africa and the Middle East, regions in Europe and North America have mosquito genera capable of transmitting RVF virus. Prolonged periods of rainfall, which allow mosquito eggs to hatch, may increase chances for RVF outbreaks, and "early warning systems" using satellite measurements of sea surface temperature, rainfall, and vegetation have been used to predict human and livestock outbreaks of RVF in Africa.[3,9] In addition to arthropod-borne transmission from domestic livestock to humans by mosquitoes, RVF virus also is transmitted by aerosol and by direct contact with infected tissues from aborted livestock or carcasses of freshly slaughtered animals infected with RVF virus. Therefore, individuals who herd, care for, or slaughter livestock as well as individuals who prepare raw meats for cooking and consumption are at greatest risk for RVF. Laboratory worker–acquired cases also occur. The person-to-person transmission of RVF has not been documented, but infected travelers may spread the virus to nonendemic countries.[3,9]

CLINICAL MANIFESTATIONS

Humans are very susceptible to RVF virus. During the epizootics in South Africa, most veterinarians and many farmers engaged in work with sick sheep and cattle became infected. In most cases, infection was linked to direct contact with the carcasses, tissues, and organs of animals that died of RVF. Transmission probably occurred by the aerosolized body fluids of the animals. Some patients gave no history of such contact; in these cases, the infection is presumed to have been transmitted by mosquitoes or possibly acquired by drinking infected milk. The virus can be transmitted readily to laboratory personnel by direct contact with infected animals or by the respiratory route from aerosol droplet infection. Travelers from endemic areas may also acquire RVF, so a careful travel history should be ascertained.[3,9]

The incubation period of RVF is 3 to 7 days. Infection may be asymptomatic. Illness with RVF onset is sudden, with chills, myalgia, joint pain, dizziness, headache, and an influenza-like illness. A biphasic fever that lasts approximately 1 week may occur. Patients often feel nauseated and may vomit or complain of abdominal fullness and pain. The face is flushed, the conjunctivae are injected, and the tongue is furred. Icterus may develop. Bradycardia is present, and slight tenderness over the liver, which may be enlarged, can be present. Many patients become delirious, and some have hallucinations. In a small proportion (1% to 10%) of patients, the

infection is complicated by retinitis. Late in the course of the illness or early in convalescence, unclear vision may be noted, and the patient may have a central blind spot. This visual defect is associated with a cotton-wool exudate on the macula, with permanent vision loss. Both eyes are involved occasionally, and the loss of vision is a severe handicap. These lesions gradually resolve, and the patient's vision returns to normal in most cases. Meningoencephalitis manifested as intense headache, confusion, and stupor may occur as a complication in fewer than 1 percent of patients during or after the second wave of fever. Lumbar puncture relieves the headache. Cerebrospinal fluid shows slight pleocytosis, mostly of lymphocytes, a normal glucose level, and a slightly increased protein content. Antibody to RVF virus also may be demonstrated in the cerebrospinal fluid. Few patients with encephalitis die. Recovery usually is complete but convalescence may be prolonged. Occasionally, the patient is left with permanent neurologic sequelae or vision loss. Hemorrhagic fever, a complication with a case-fatality rate of 15 percent, develops in approximately 1 percent of patients with RVF. The mortality rate was disproportionately lower in children than in adults during the 1977 Egyptian epidemic. In cases of severe illness, a hemorrhagic diathesis may develop that includes epistaxis, hematemesis, melena, and, sometimes, cerebral hemorrhage. Profuse gastrointestinal hemorrhage may be fatal. Jaundice may be evident.

LABORATORY FINDINGS

The patient has initial leukocytosis that is followed by leukopenia. Profound thrombocytopenia and other defects in coagulation may be observed. Disturbance in liver and kidney function also may be documented. The diagnosis of RVF is clinically suggested when human beings suffer from an acute, severe, but short febrile illness at the same time that an epizootic with high mortality is occurring in sheep.

The laboratory diagnosis usually can be confirmed by isolation of the virus in mice or cell culture from blood during the early days of illness and, in fatal cases, from the liver. Immunohistochemistry of tissues from necropsy or biopsy also may provide the diagnosis. Since the virus is a Biosafety Level-4 pathogen, virus cultivation should only be attempted in restricted laboratories. Detection using polymerase chain reaction (PCR) assays also may be performed, under appropriate safety precautions in specialized laboratories. Quantitative real-time PCR detection and quantification of RVF virus in serum appears sensitive and useful as a routine diagnostic test for identifying the virus in the early stage of the disease in individual humans, for identification of cases during outbreaks, and for follow-up of antiviral therapy.[6,15] The development of serum antibodies can be demonstrated by immunofluorescence, IgM enzyme-linked immunosorbent assay, hemagglutination inhibition, complement fixation, and neutralization. In patients with encephalitis, RVF virus–specific IgM and IgG antibodies are detectable in CSF.

TREATMENT

Treatment is symptomatic and supportive, with appropriate isolation precautions. When a hemorrhagic diathesis develops, treatment should be directed toward controlling bleeding. Transfusions of fresh-frozen plasma and platelets may be beneficial for bleeding. Convalescent-phase plasma from survivors of RVF may also provide benefit and improve survival. There is no licensed specific antiviral therapy for RVF. Ribavirin has activity against *Bunyaviridae* and is used to treat disease caused by hemorrhagic fever viruses.[3,9] In addition, treatment with amantadine, rifampicin, and dexamethasone in one patient with encephalitis is reported.[3,9]

PREVENTION

RVF is mainly a disease of adults and usually is acquired occupationally. It is a serious hazard faced by veterinarians, ranchers, and laboratory personnel in the course of their work. Because RVF usually is acquired by direct contact with the tissues of infected sheep and cattle, the risk for acquiring infection can be reduced by wearing gloves, protective masks, and goggles when postmortem examinations are carried out on animals that have died of unknown causes. Because of the value of domestic animals in many economically depressed areas of Africa, sheep and cattle often are housed within family compounds. Sick or dying animals usually are killed to salvage their meat. In this peri-domestic environment, children also can be exposed to virus aerosols and readily become infected. Infection from a mosquito bite is possible as well; and for this reason, the use of repellents and bed nets is recommended.

The primary strategy for preventing RVF in both humans and animals relies on vaccination of sheep and cattle, which are the amplifying hosts, and vaccination of humans in high-risk situations. Attenuated strains of the virus have been developed and used successfully on a mass scale to immunize livestock. These attenuated vaccines are associated with some abortions in pregnant ewes and cows. An inactivated vaccine for livestock is safe and is in widespread use in Africa.[14]

A live attenuated vaccine designated MP-12 was developed by passage of RVF virus in the presence of the mutagen 5-fluorouracil.[12] The vaccine has proved safe for use in domestic livestock, does not produce abortions, and offers considerable promise as a veterinary and human vaccine. Recently an attenuated strain, designated clone 13, has been investigated and shown effective in protecting sheep without any noticeable adverse effects. Another strain, designated R566, derived from both clone 13 and MP-12 through reassortment, is also under investigation.[3,8,10] Formalin-inactivated vaccine RVFV TSI-GSD-200 is being used to vaccinate military and laboratory personnel who are in contact with RVF virus.[3,4,8,10] Virus-like particles generated by reverse genetics or expression vectors, as well as DNA vaccines, are in development.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Boshra H, Lorenz G, Busquets N, et al. Rift Valley fever: recent insights into pathogenesis and prevention. *J Virol* 2011;**85**: 6098–105.
6. Garcia S, Crance JM, Billecocq A, et al. Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. *J Clin Microbiol* 2001;**39**:4456–61.
8. Ikegami T, Makino S. Rift Valley fever vaccines. *Vaccine* 2009;**27**(Suppl. 4):D69–72.
9. Ikegami T, Makino S. The pathogenesis of Rift Valley fever. *Viruses* 2011;**3**:493–519.
10. Kortekaas J, Antonis A, Kant J, et al. Efficacy of three candidate Rift Valley fever vaccines in sheep. *Vaccine* 2012;**30**:3423–9.
15. Sall A, Thonnon J, Sene O, et al. Single-tube and nested reverse transcriptase-polymerase chain reaction for detection of Rift Valley fever virus in human and animal sera. *J Virol Methods* 2001;**91**: 85–92.

The full reference list for this chapter is available at expertconsult.com.

190B ■ CRIMEAN-CONGO HEMORRHAGIC FEVER

Robert B. Tesh • Gail J. Harrison

Crimean-Congo hemorrhagic fever (CCHF) is a serious and potentially fatal viral illness that often has hemorrhagic manifestations. The clinical entity as a tick-borne hemorrhagic fever, and its viral origin were described first in Russia in the Crimean Peninsula by Chumakov in 1945. The agent was not propagated or available for study until 1967 and was further studied in 1969 in the Congo.[21,23,24] Since then, CCHF virus has been reported from many countries in eastern Europe, central Asia, and Africa.[6,21,23] The virus currently can be found in Africa, Asia, the Middle East, and Eastern Europe. During 2001, CCHF has been diagnosed in patients in Kosovo, Albania, Iran, Pakistan, and South Africa. In the 21st century, outbreaks have become more frequent in the region of the former Yugoslavia, Turkey, and Iran. In 2010, an outbreak in India was reported.[1] Exposure to ticks and contact with the blood of infected livestock or people are the usual sources of human infection.

ETIOLOGIC AGENT

CCHF virus, the etiologic agent, is a member of the genus *Nairovirus*, in the family *Bunyaviridae*. It is lethal to newborn mice after intracerebral inoculation. CCHF virus replicates in some vertebrate cell lines, but isolation in mice is more sensitive because the virus usually does not cause any discernible cytopathic effect under fluid overlay. Thus, indirect methods such as immunofluorescence or reverse-transcriptase polymerase chain reaction (RT-PCR) must be used to detect CCHF viral antigen or nucleic acid in the cells. The complete genome of CCHF fever viruses isolated from geographically and climate diverse areas have indicated that distinct geographic lineages exist but also that within lineages these viruses are highly variable.[4,9,18,20,24]

A relatively high level of viremia develops in persons with CCHF and persists for 6 to 8 days after onset of the illness.[21] Blood from acutely ill CCHF patients is quite infectious, and numerous nosocomial infections have occurred in hospital personnel caring for patients with this disease.[3,6,14] CCHF virus is relatively stable in blood and serum and has been recovered from specimens stored as long as 2 or 3 weeks at 4° C.

EPIDEMIOLOGY

CCHF virus is known to occur over a wide geographic area, including eastern and southern Europe, central Asia, western China, the Middle East, India, and Africa (Fig. 190b-1).* The global distribution of CHFF virus appears to be migrating and expanding. Because it is expected future outbreaks may extend to new areas, increased surveillance and geographic mapping of this potentially lethal viral infection is needed. Most cases of the disease probably are unrecognized because of its sporadic occurrence and largely rural distribution or its occurrence in developing countries. It is often considered an occupational disease of health care workers and individuals who work with or around animals. It also is considered a possible bioterrorism agent.[24]

The epidemiology of CCHF is complex and not fully understood because of the variety of tick and mammalian species that have been found to be naturally infected with the virus.[23] The *Hyalomma* spp. ticks have been incriminated in transmission of CCHF virus in the field, but the role of other ticks in transmission is also possible.[4] The basic transmission cycle of CCHF virus varies from region to region, depending on the developmental stage and species of ticks involved and their preferred mammalian hosts.[23] In addition, transovarial and transstadial transmissions of CCHF virus also have been demonstrated in some tick vectors. In general, human cases of CCHF usually are associated with periods of tick abundance and feeding activity.[6] For example, in eastern Europe, cases of CCHF typically occur between May and August, when the tick population is active. Models of global warming climate scenarios project the northern limit of the tick range may increase and change the distribution of the disease.[4,9] Humans usually acquire CCHF from a tick bite or from contact with blood or other tissues of infected livestock.[6,21,23] Most patients have a history of contact with large domestic animals (farmers, abattoir workers, and veterinarians). Health care workers are another high-risk group.

Numerous CCHF health care associated outbreaks have occurred in hospital workers and laboratory

*References 1, 4, 6, 9, 18, 24.

REFERENCES

1. An outbreak of Rift Valley fever, eastern Africa, 1997-1998. *Wkly Epidemiol Rep* 1998;**73**:105–9.
2. Arthur RR, el-Sharkawy MS, Cope SE, et al. Recurrence of Rift Valley fever in Egypt. *Lancet* 1993;**342**:1149–50.
3. Boshra H, Lorenz G, Busquets N, et al. Rift Valley fever: recent insights into pathogenesis and prevention. *J Virol* 2011;**85**: 6098–105.
4. Centers for Disease Control and Prevention. Rift Valley fever outbreak—Kenya, November 2006—January 2007. *MMWR Morb Mortal Wkly Rep* 2007;**56**:73–6.
5. Daubney R, Hudson JR, Garnham PC. Enzootic hepatitis or Rift Valley fever: an undescribed virus disease of sheep, cattle and man from East Africa. *J Pathol Bacteriol* 1931;**34**:545–79.
6. Garcia S, Crance JM, Billecocq A, et al. Quantitative real-time PCR detection of Rift Valley fever virus and its application to evaluation of antiviral compounds. *J Clin Microbiol* 2001;**39**:4456–61.
7. Gear J. Hemorrhagic fevers in South Africa: an account of two recent outbreaks. *J S Afr Vet Assoc* 1977;**48**:5–8.
8. Ikegami T, Makino S. Rift Valley fever vaccines. *Vaccine* 2009;**27** (Suppl. 4):D69–72.
9. Ikegami T, Makino S. The pathogenesis of Rift Valley fever. *Viruses* 2011;**3**:493–519.
10. Kortekaas J, Antonis A, Kant J, et al. Efficacy of three candidate Rift Valley fever vaccines in sheep. *Vaccine* 2012;**30**:3423–9.
11. Meegan JM. Rift Valley fever in Egypt: an overview of the epizootics in 1977 and 1978. *Contrib Epidemiol Biostat* 1981;**3**:100–13.
12. Morrill JC, Jennings GB, Caplen H, et al. Pathogenicity and immunogenicity of a mutagen-attenuated Rift Valley fever virus immunogen in pregnant ewes. *Am J Vet Res* 1987;**48**:1042–7.
13. Morvan J, Rollin PE, Laventure S, et al. Rift Valley fever epizootic in the central highlands of Madagascar. *Res Virol* 1992;**143**: 407–15.
14. Randall R, Gibbs CJ, Anlisio CG, et al. The development of a formalin-killed Rift Valley fever vaccine for use in man. *J Immunol* 1962;**89**:660–71.
15. Sall A, Thonnon J, Sene O, et al. Single-tube and nested reverse transcriptase-polymerase chain reaction for detection of Rift Valley fever virus in human and animal sera. *J Virol Methods* 2001;**91**: 85–92.
16. Swanepoel R, Coetzer JAW. Rift Valley fever. In: Coetzer JAW, Thomson GR, Tustin RC, editors. *Infectious diseases of livestock*. Cape Town, South Africa: Oxford University Press; 1994. p. 688–717.

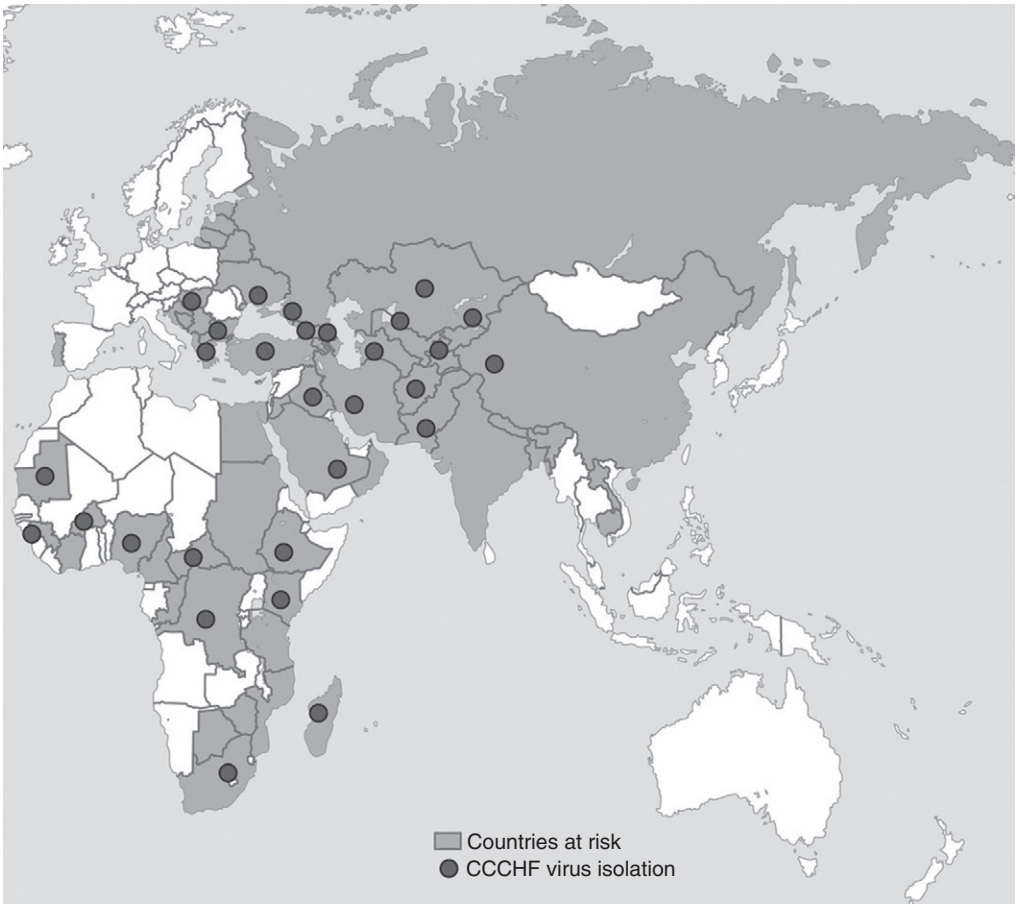

FIGURE 190B-1 ■ Known distribution of Crimean-Congo hemorrhagic fever (CCHF) virus in the world. (From Ergonul O. Crimean-Congo hemorrhagic fever. *Lancet Infect Dis* 2006;**6**:203-14.)

technicians.[3,6,14] These persons usually have had direct contact with the blood of patients with CCHF. The mortality rate reported in secondary cases has been high, particularly in persons with needle-stick injury or those involved in management of patients with gastrointestinal bleeding.[6]

CLINICAL MANIFESTATIONS

The course of CCHF infection typically has four distinct phases: incubation, prehemorrhagic, hemorrhagic, and convalescent (Fig. 190b-2).[6,7,22] The incubation period generally is 3 to 7 days. The prehemorrhagic phase begins suddenly with fever (39° C to 41° C [102.2° F to 105.8° F]), nausea, diarrhea, vomiting, myalgia, and general toxemia. Patients usually are flushed, with conjunctival and scleral injection. Hepatosplenomegaly occurs in as many as 30 percent of cases. This second phase lasts approximately 3 days (range, 1 to 7 days). The hemorrhagic phase usually begins between the third and fifth days of the disease and lasts only 2 to 3 days. The appearance is sudden and can vary from petechiae to large hematomas on the skin and mucous membranes. Profuse bleeding from the nose, gums, gastrointestinal and genitourinary tracts, and lungs can occur at this stage, resulting in tachycardia, shock, and, sometimes, death. Not infrequently, intra-abdominal bleeding occurs during this third phase and causes acute pain; some of these patients are subjected to surgery because of suspected appendicitis or intestinal perforation. Fatality is higher in patients with diarrhea, melena, hematemesis, hematuria, and confusion at presentation.[12] If the patient survives the hemorrhagic phase, convalescence begins about the 10th day, with a slow convalescent period lasting 2 to 6 weeks.[6] Viremia is intense and prolonged in CCHF, especially in fatal cases, so blood from these patients should be treated with extreme care.

Leukopenia and thrombocytopenia are consistent features of CCHF and occur early in the disease.[6,7,12] Other abnormal laboratory findings usually include elevated levels of serum aspartate and alanine aminotransferase, lactate dehydrogenase, bilirubin, creatinine, and urea. Fatality is associated with higher levels of aspartate aminotransferase, alanine aminotransferase, and C-reactive protein levels and with prolonged coagulation tests such as prothrombin time and activated prothrombin time.[12]

Fibrinogen may be decreased, and fibrin degradation products may be increased. These laboratory tests generally return to normal levels with the onset of convalescence.

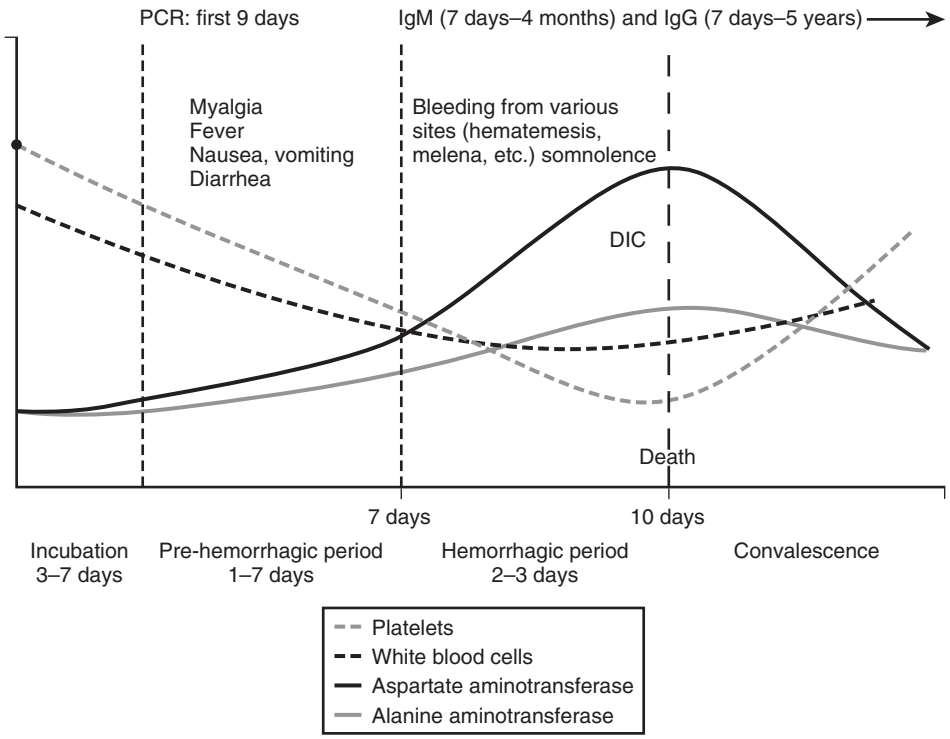

FIGURE 190B-2 ■ Clinical and laboratory course of Crimean-Congo hemorrhagic fever. DIC, disseminated intravascular coagulation; IgM, immunoglobulin M; PCR, polymerase chain reaction. (From Ergonul O. Crimean-Congo hemorrhagic fever. *Lancet Infect Dis* 2006;**6**:203-14.)

PATHOGENESIS

The pathogenesis of CCHF is not well understood, but the available evidence suggests that endothelial damage and dysfunction play an important role in the hemostatic failure.[6,14,22] Excessive proinflammatory cytokine production, cytokine storm, and dysfunction of the innate immune response also appear to contribute to the disease process. Lymphocyte depletion also appears to occur.[2] CCHF sometimes leads to reactive hemophagocytic histiocytosis in children.[5,17] Fluorescent antibody studies on organs in fatal cases of CCHF show a concentration of viral antigen in the reticuloendothelial cells of the liver and spleen, a finding suggesting that these cells are major sites of virus replication.[22] Other pathologic changes observed at autopsy include generalized vascular lesions with endothelial damage that give rise to scattered focal hemorrhage and edema in most organs.[6] In severe cases, dysfunction of the coagulation system and disseminated intravascular coagulation appear to be prominent features of the disease.

DIAGNOSIS

A clinical diagnosis of CCHF is difficult to establish before the onset of hemorrhagic manifestations because the initial symptoms are nonspecific, and cases usually occur sporadically. As a result of the frequency and severity of the abdominal pain associated with the prehemorrhagic and hemorrhagic phases of CCHF, the disease sometimes is misdiagnosed as appendicitis or gastric ulcer. Such patients may be subjected to unnecessary surgery, which can be fatal to both the patient and the attending medical personnel.[3,6,21] Once hemorrhagic manifestations appear, the differential diagnosis should include erythema multiforme (Stevens-Johnson syndrome), leptospirosis, hemorrhagic fever with renal syndrome, Ebola virus infection, Lassa fever, Rift Valley fever, Q fever, and yellow fever, depending on the region of the world where the patient was exposed.

A definitive diagnosis of CCHF can be made by isolating the virus from the patient's serum or detecting viral nucleic acids by PCR during the first week of illness. Molecular methods for diagnosis are being applied by many laboratories in endemic areas for accurate and timely diagnosis. However, performance of these molecular methods may vary, depending on method used, type of CCHF virus strain tested, and the concentration of the virus in the sample.[8] Immunoglobulin (Ig) G and IgM antibodies do not appear until 7 to 10 days after the onset of symptoms in nonfatal cases.[21,22] Establishing the diagnosis early is critical for proper management of the patient and prevention of potential transmission to health care workers caring for the patient.

TREATMENT

Treatment of CCHF is largely supportive and should include immediate hospitalization and strict bed rest.[6,21] High mortality rates (average, 30 percent) have been reported. Because death usually results from acute blood loss and shock, patients may require transfusions of fresh

blood, platelets, or plasma (or all three). Administration of immune plasma or CCHF-negative hyperimmune globulin obtained from convalescent donors can be used early in the course of the illness or for persons, such as hospital personnel, who are exposed to the virus inadvertently, especially if they reside in endemic areas.[5,17,24] Therapeutic plasma exchange has also been successfully performed in selected patients. Ribavirin may be beneficial for treatment of CCHF, especially if treatment is begun within the first 4 days of illness.[6,7,10] The mechanism of action is unknown. Ribavirin also has been shown to protect mice from lethal CCHF virus challenge.[13,16,17] A World Health Organization report recommended intravenous ribavirin treatment for 10 days, with 30 mg/kg as an initial loading dose, then 15 mg/kg every 6 hours for 4 days, and then 7.5 mg/kg every 8 hours for 6 days.[6,10,13,16] Oral ribavirin also has been reported to have potential benefit.[10,13,16] In patients with CCHF associated with reactive hemophagocytic histiocytosis, administration of high-dose methylprednisolone 5 to 30 mg/kg/day, fresh frozen plasma, and intravenous immunoglobulin has been shown possibly to be effective.[5]

PROGNOSIS

The mortality rates reported for CCHF have estimated a 30 percent mortality rate, and estimates have varied from 15 to 70 percent.[6,21,23] These figures may be high, because milder cases of the disease may not be recognized or reported. Factors predictive of fatality include clinical signs of melena, hemorrhage, and confusion, and laboratory findings of elevated transferase levels, elevated C-reactive protein value, and prolonged coagulation factors.[12] These factors may help clinicians in countries with limited resources decide which patients may benefit from care in a tertiary care center. If the patient survives the hemorrhagic phase of the disease, recovery is complete and permanent immunity results. Survivors may experience posttraumatic stress disorder, especially if they were critically ill and required care in an intensive care unit.[11]

PREVENTION

No vaccine of proven safety or efficacy for prevention of CCHF is licensed. However, an inactivated, suckling mouse brain–derived Bulgarian CCHF vaccine has been shown to produce anti–CCHF-specific T-cell activity and high levels of CCHF-specific antibodies.[15,19] Personal protection measures, including insect repellents containing DEET, are recommended for people living or working in heavily tick-infested areas. People exposed occupationally to potentially infected animal blood should wear protective clothing and gloves to prevent skin contact with infected tissue or blood. Hospital personnel caring for patients with CCHF should follow barrier nursing and isolation protocols, which include use of gloves, gowns, face shields, and goggles.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Baipai S, Nadkar MY. Crimean Congo hemorrhagic fever: requires vigilance and not panic. *J Assoc Physicians India* 2011;**59**:164–7.
2. Bente D, Alimonti J, Shieh W, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol* 2010;**84**(21):11089–100.
5. Erduran E, Bahadir A, Palanci N, Gedik Y. The treatment of Crimean-Congo hemorrhagic fever with high-dose methylprednisolone, intravenous immunoglobulin, and fresh frozen plasma. *J Pediatr Hematol Oncol* 2013;**35**(1):e19–24.
8. Escadafal C, Olschlager S, Avsic-Zuanc T, et al. First international external quality assessment of molecular detection of Crimean-Congo hemorrhagic fever virus. *PLoS Negl Trop Dis* 2012;**6**(6):e1706.
9. Estrada-Pena A, Jameson L, Medlock J, et al. Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the western Palearctic. *Vector Borne Zoonotic Dis* 2012;**12**(9):743–52.
11. Gul S, Gul E, Yesilyurt M, et al. Health-related quality of life and the prevalence of post-traumatic stress disorder among Crimean-Congo hemorrhagic fever survivors. *Jpn J Infect Dis* 2012;**64**(5):392–5.
12. Hatipoglu C, Bulut C, Yetkin M, et al. Evaluation of clinical and laboratory predictors of fatality in patients with Crimean-Congo hemorrhagic fever in a tertiary care hospital in Turkey. *Scand J Infect Dis* 2010;**42**(6):516–21.
13. Izadi S, Salehi M. Evaluation of the efficacy of ribavirin therapy on survival of Crimean-Congo hemorrhagic fever patients: a case-control study. *Jpn J Infect Dis* 2009;**64**(1):11–15.
15. Keshtkar-Jahromi M, Kun J, Christova I, et al. Crimean-Congo hemorrhagic fever: current and future prospective of vaccines and therapies. *Antiviral Res* 2011;**90**(2):85–92.
17. Mardani M, Rahnavardi M, Sharifi-Mood B. Current treatment of Crimean-Congo hemorrhagic fever in children. *Expert Rev Anti Infect Ther* 2010;**8**(8):911–18.
18. Mild M, Simon M, Albert J, Mirazimi A. Towards an understanding of the migration of Crimean-Congo hemorrhagic fever virus. *J Gen Virol* 2010;**91**:199–207.
19. Mousavi-Jazi M, Karlberg H, Pap A, et al. Healthy individuals; immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. *Vaccine* 2012;**30**(44):6225–9.
20. Ozkaya A, Dincer E, Carhan A, et al. Molecular epidemiology of Crimean-Congo hemorrhagic fever virus in Turkey: occurrence of local topotype. *Virus Res* 2010;**149**(1):64–70.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Baipai S, Nadkar MY. Crimean Congo hemorrhagic fever: requires vigilance and not panic. *J Assoc Physicians India* 2011;**59**:164–7.
2. Bente D, Alimonti J, Shieh W, et al. Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *J Virol* 2010;**84**(21):11089–100.
3. Burney MI, Ghafoor A, Saleen M, et al. Nosocomial outbreak of viral hemorrhagic fever caused by Crimean hemorrhagic fever–Congo virus in Pakistan, January 1976. *Am J Trop Med Hyg* 1980;**29**: 941–7.
4. Deyde V, Khristova M, Rollin P, et al. Crimean-Congo hemorrhagic fever virus genomics and global diversity. *J Virol* 2006;**80** (17):8834–42.
5. Erduran E, Bahadir A, Palanci N, Gedik Y. The treatment of Crimean-Congo hemorrhagic fever with high-dose methylprednisolone, intravenous immunoglobulin, and fresh frozen plasma. *J Pediatr Hematol Oncol* 2013;**35**(1):e19–24.
6. Ergonul O, Whitehead CA, editors. *Crimean-Congo hemorrhagic fever. A global perspective*. Amsterdam: Springer; 2007.
7. Ergonul O, Celikbas A, Dokuzoguz B, et al. Characteristics of patients with Crimean-Congo hemorrhagic fever in a recent outbreak in Turkey and impact of ribavirin therapy. *Clin Infect Dis* 2004;**39**:284–7.
8. Escadafal C, Olschlager S, Avsic-Zuanc T, et al. First international external quality assessment of molecular detection of Crimean-Congo hemorrhagic fever virus. *PLoS Negl Trop Dis* 2012;**6**(6):e1706.
9. Estrada-Pena A, Jameson L, Medlock J, et al. Unraveling the ecological complexities of tick-associated Crimean-Congo hemorrhagic fever virus transmission: a gap analysis for the western Palearctic. *Vector Borne Zoonotic Dis* 2012;**12**(9):743–52.
10. Fisher-Hoch SP, Khan JA, Rehman S, et al. Crimean-Congo haemorrhagic fever treated with oral ribavirin. *Lancet* 1995;**346**:472–5.
11. Gul S, Gul E, Yesilyurt M, et al. Health-related quality of life and the prevalence of post-traumatic stress disorder among Crimean-Congo hemorrhagic fever survivors. *Jpn J Infect Dis* 2012;**64**(5): 392–5.
12. Hatipoglu C, Bulut C, Yetkin M, et al. Evaluation of clinical and laboratory predictors of fatality in patients with Crimean-Congo hemorrhagic fever in a tertiary care hospital in Turkey. *Scand J Infect Dis* 2010;**42**(6):516–21.
13. Izadi S, Salehi M. Evaluation of the efficacy of ribavirin therapy on survival of Crimean-Congo hemorrhagic fever patients: a case-control study. *Jpn J Infect Dis* 2009;**64**(1):11–15.
14. Joubert JR, King JB, Rossouw DJ, et al. A nosocomial out-break of Crimean-Congo hemorrhagic fever at Tygerberg Hospital, part III: clinical pathology and pathogenesis. *S Afr Med J* 1985;**68**:772–8.
15. Keshtkar-Jahromi M, Kun J, Christova I, et al. Crimean-Congo hemorrhagic fever: current and future prospective of vaccines and therapies. *Antiviral Res* 2011;**90**(2):85–92.
16. Mardani M, Jahromi M, Naieni K, Zeinali M. The efficacy of oral ribavirin in the treatment of Crimean-Congo hemorrhagic fever in Iran. *Clin Infect Dis* 2003;**36**(12):1613–18.
17. Mardani M, Rahnavardi M, Sharifi-Mood B. Current treatment of Crimean-Congo hemorrhagic fever in children. *Expert Rev Anti Infect Ther* 2010;**8**(8):911–18.
18. Mild M, Simon M, Albert J, Mirazimi A. Towards an understanding of the migration of Crimean-Congo hemorrhagic fever virus. *J Gen Virol* 2010;**91**:199–207.
19. Mousavi-Jazi M, Karlberg H, Pap A, et al. Healthy individuals; immune response to the Bulgarian Crimean-Congo hemorrhagic fever virus vaccine. *Vaccine* 2012;**30**(44):6225–9.
20. Ozkaya A, Dincer E, Carhan A, et al. Molecular epidemiology of Crimean-Congo hemorrhagic fever virus in Turkey: occurrence of local topotype. *Virus Res* 2010;**149**(1):64–70.
21. Swanepoel R. Nairovirus infections. In: Porterfield JS, editor. *Exotic viral infections*. London: Chapman & Hall; 1995. p. 285–93.
22. Swanepoel R, Gill DE, Shepherd AJ, et al. The clinical pathology of Crimean-Congo hemorrhagic fever. *Rev Infect Dis* 1989;**11**(Suppl. 4):794–800.
23. Watts DM, Ksiazek TG, Linthicum KJ, et al. Crimean-Congo hemorrhagic fever. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 2. Boca Raton, Fla.: CRC Press; 1989. p. 177–222.
24. Whitehouse CA. Crimean-Congo hemorrhagic fever. *Antiviral Res* 2004;**64**(3):145–60.

190C ■ PHLEBOTOMUS FEVER (SANDFLY FEVER)

Robert B. Tesh • Gail J. Harrison

Phlebotomus fever is an acute, self-limited febrile illness of 2 to 4 days' duration that usually is acquired from the bite of infected phlebotomine sandflies. Phlebotomus fever also is known as sandfly fever, pappataci fever, and 3-day fever. Sandflies have sporadically been in Central Europe since the Holocene climate optima (ca. 4500 BC). The disease is currently endemic in many areas of central Asia, northern Africa, and southern Europe. Global warming is predicted to extend the distribution of sandfly fever into Central Europe.[1,10] Both historically and currently, phlebotomus fever has been largely of military interest because the introduction of large numbers of susceptible troops into endemic areas often has resulted in epidemics of the disease.[4,8] More recently, the disease has been reported with increasing frequency in civilian populations and in tourists visiting endemic areas. An outbreak of sandfly fever virus has been reported in Turkey.[9] Travelers with sandfly fever virus infection imported from Malta into Switzerland also recently have been reported.[14] In 1909, Doerr and associates first demonstrated that the causative agent of the illness was a virus transmitted by *Phlebotomus papatasi*. In 1954, Sabin successfully adapted the agent to mice and described two distinct serologic types, designated the Naples and Sicilian strains.[16,17] Subsequently, 44 additional serotypes were isolated from various regions of the world; all these agents are included in the genus *Phlebovirus*, family *Bunyaviridae*. Although most of the phleboviruses probably are capable of producing human illness, the discussion here is limited to the three (Naples, Sicilian, and Toscana) that are associated most commonly with human illness. Rift Valley fever virus, a severe disease acquired from bite of infected mosquitoes or aerosol, and Toscana virus, a phlebovirus that causes meningoencephalitis, also are members of this genus and frequently cause a phlebotomus fever–like illness in infected persons. These diseases are covered in more detail in other chapters.[3,5,13,15]

ETIOLOGIC AGENT

The phleboviruses are RNA-containing viruses, spherical, and 90 to 100 nm in diameter.[16] The Naples, Sicilian, and Toscana viruses produce a cytopathic effect, as well as plaques in Vero cells. A novel sandfly fever virus variant of the Sicilian virus, termed *sandfly fever turkey virus* has been identified via consensus polymerase chain reaction (PCR) and sequencing methodology.[9] Most laboratory animals are not susceptible to infection with these viruses, although the viruses usually can be adapted to newborn mice by serial passage intracerebrally. For this reason, tissue culture is recommended for primary isolation of these agents and molecular detection using real-time reverse-transcriptase PCR (RT-PCR) is useful for rapid detection and characterization.[9,12]

EPIDEMIOLOGY

The geographic distribution of the Naples and Sicilian virus types in the Old World closely parallels that of their presumed vector, *P. papatasi* (Fig. 190c-1).[16] Toscana virus has been isolated in Portugal, Spain, France, Italy, Turkey, and Cyprus and has been associated with two other peridomestic sandfly species, *Phlebotomus perniciosus* and *Phlebotomus perfiliewi*.[5,16]

These phlebotomine species are tiny, sand-colored, biting flies that are 2 to 3 mm long. Because of their small size, they have little difficulty squeezing through ordinary screens and mosquito netting. They usually are nocturnal, and only the female bites. Sandflies move in short hops and usually do not travel more than a few hundred meters from their resting and breeding sites. During the day, peridomestic species such as *P. papatasi* and *P. perniciosus* rest in dark corners and crevices, often within houses. The larvae develop in loose soil and organic debris in stone walls, animal sheds, privies, open wells, and gardens. Because of their indoor habits, these peridomestic species are quite vulnerable to residual insecticides.

In central Asia and the Mediterranean region, sandflies are active during the late spring and summer. The incidence of phlebotomus fever follows the same seasonal pattern. Global warming patterns are predicted to alter the epidemiology of sandfly fever in the future.[1,10]

Many phlebotomus fever viruses appear to be maintained in the sandfly population by transovarial (vertical) transmission.[16] Thus, sandflies appear to serve as both vectors and reservoirs of these viruses. Unlike most other arboviruses, whose activity depends on the presence of susceptible and viremic vertebrate hosts, phlebotomus fever viruses are probably active continuously during each sandfly season, regardless of the immune status of the local human population. Serologic studies of persons living in endemic areas of the disease indicate that most of the residents are infected early in life.[16,17] In these communities, sporadic cases of phlebotomus fever occur in children because most of the adult population is already immune. However, because of the benign nature of the disease, its sporadic occurrence, and its similarity to many other viral diseases of childhood, most cases are unrecognized. When large numbers of susceptible persons (e.g., soldiers, tourists, refugees) enter an endemic area of phlebotomus fever, however, bite transmission occurs and an epidemic of the disease quickly ensues.[7,16]

CLINICAL MANIFESTATIONS

The incubation period for phlebotomus fever averages 3 to 5 days.[2,16] The illness begins suddenly with fever, severe frontal headache, retro-orbital pain, conjunctival

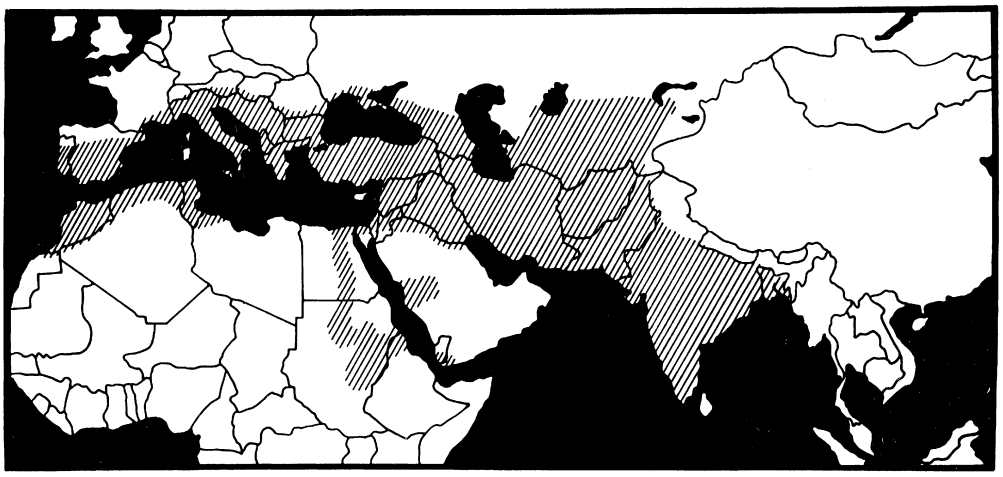

FIGURE 190C-1 ■ The known geographic distribution of *Phlebotomus papatasi*, the presumed vector of sandfly fever (Naples and Sicilian types), in central Asia, the Mediterranean region, and adjacent areas of Africa. (Modified from Tesh RB, Saidi S, Gajdamovic SJ, et al. Serological studies on the epidemiology of sandfly fever in the Old World. *Bull World Health Organ* 1976;**54**:663-74.)

injection, photophobia, malaise, anorexia, nausea, vomiting, myalgia, and lower back pain. The face may be flushed, but a true rash is absent. The disease is self-limited, and symptoms usually disappear within 1 to 3 days; however, a general feeling of weakness and depression often occurs for a week or more after the illness.

Meningitis and meningoencephalitis with Toscana virus infection have been described with increasing frequency in southern Europe.[2,3,5] In addition to the classic symptoms of phlebotomus fever, these patients exhibit nuchal rigidity, a positive Kernig sign, a clouded sensorium, and, occasionally, nystagmus and tremor. To date, no deaths have been recorded. Childhood cases of the meningoencephalitic form of Toscana virus infection have been reported,[2] but this form of the infection occurs more commonly in adults.

One attack of phlebotomus fever usually confers lifelong immunity against the infecting virus type but not against heterologous serotypes.[8] For this reason, second cases of the disease have been reported in the same individual in areas where two or more virus serotypes are active.

Marked leukopenia usually is observed in patients with phlebotomus fever.[1] It is characterized by initial lymphopenia, followed by protracted neutropenia (Fig. 190c-2). In patients with central nervous system involvement, pleocytosis and an elevated protein content are observed in the cerebrospinal fluid.

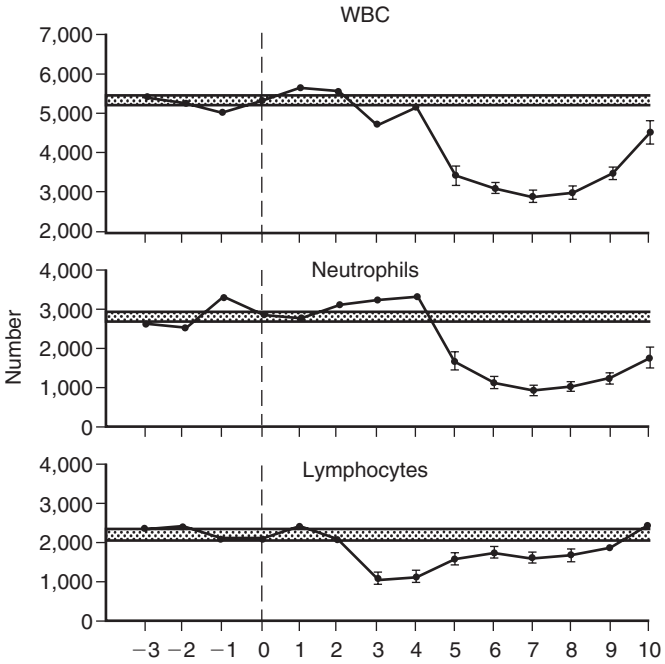

FIGURE 190C-2 ■ Mean total white blood cell (WBC) count as well as absolute neutrophil and lymphocyte counts in 11 adult volunteers inoculated (day 0) with the Sicilian strain of sandfly fever virus. The average incubation period in the subjects was approximately 70 hours. (Modified from Bartelloni PJ, Tesh RB. Clinical and serologic responses of volunteers infected with phlebotomus fever virus [Sicilian type]. *Am J Trop Med Hyg* 1976;**25**:456-62.)

PATHOLOGY

Fatalities caused by phlebotomus fever have not been reported, and little is known of the pathologic changes produced by these viruses.

DIAGNOSIS

The diagnosis of phlebotomus fever often can be made on the basis of clinical and epidemiologic evidence. A sudden outbreak during the summer months of a short febrile illness with severe headache and marked leukopenia in visitors or other newcomers to an endemic area where sandflies are abundant should suggest the disease. Depending on the region, the differential diagnosis may include dengue, West Nile fever, malaria, influenza, and numerous other respiratory tract and enteroviral infections.

Transient (24 to 36 hours), low-level viremia occurs during this illness; therefore, isolation of the virus from

blood is unusual. In a few instances, Toscana virus has been isolated directly from the cerebrospinal fluid of persons with central nervous system symptoms.[3] Molecular techniques (RT-PCR) have been described for direct detection of Toscana and Sicilian virus and a novel Turkey variant virus in clinical samples.[5,9,12] However, one of the problems with using molecular methods to detect phleboviruses directly in clinical material is the wide genetic diversity among members of each serotype. In addition, limited sequence data are available for many of the phleboviruses. At present, serologic tests offer the simplest method for establishing a specific diagnosis of phlebotomus fever. Antibodies are present in serum 7 to 14 days after infection and can be demonstrated by immunofluorescence,[2] enzyme immunoassay,[8] or neutralization testing.[2] A fourfold rise in antibody titer from acute to convalescent sera or the presence of specific immunoglobulin M antibodies provides presumptive evidence of a recent phlebotomus fever virus infection. At present, these serologic tests are not generally available and usually are performed in only a few arbovirus laboratories.

TREATMENT

There is no licensed specific antiviral treatment available for sandfly fever. However, ribavirin inhibits a late step of the virus replicative cycle and, when combined with interferon-α, has shown a synergistic antiviral effect.[6] In addition, more recent studies have evaluated the pyrazine carboxamide derivatives T-705 (favipiravir), T-1105, and T-1106, which are compounds with broad activity against RNA viruses, for treatment of phlebovirus infections.[11] Treatment is symptomatic, and hospitalization usually is not necessary, except in more severe cases of Toscana virus infection. Occasionally, pain relievers and narcotics are required to relieve the severe headache associated with the disease.

PROGNOSIS

Phlebotomus fever is a self-limited, nonfatal illness. Recovery is complete.

PREVENTION

Control measures are directed primarily against the vector. Household spraying with residual insecticides is quite effective in reducing peridomestic sandfly vectors and controlling the disease. The use of personal insect repellents (e.g., diethyltoluamide) and fine-mesh bed nets is also effective in avoiding sandfly bites.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Aspock H, Gerersdorfer T, Formayer H, Walochnik J. Sandflies and sandfly-borne infections of humans in Central Europe in the light of climate change. *Wien Klin Wochenschr* 2008;**120**(Suppl. 19-20):24–9.
4. Brett-Major DM, Claborn DM. Sandfly fever: what have we learned in one hundred years? *Mil Med* 2009;**174**(4):426–31.
9. Ergunay K, Ismayilova V, Colpak IA, et al. A case of central nervous system infection due to a novel Sandfly Fever Virus (SFV) variant: Sandfly Fever Turkey Virus (SFTV). *J Clin Virol* 2012;**54**(1): 79–82.
10. Fischer D, Moeller P, Thomas SM, et al. Combining climatic projections and dispersal ability: a method for estimating the responses of sandfly vector species to climate change. *PloS Negl Trop Dis* 2011;**5**(11):e1407.
11. Gowen B, Wong M, Jung K, et al. Efficacy of favipiravir (T-705) and T-1106 pyrazine derivatives in phlebovirus disease models. *Antiviral Res* 2010;**86**(2):121–7.
12. Ibrahim SM, Aitichou M, Hardick J, et al. Detection of Crimean-Congo hemorrhagic fever, Hanta, and sandfly fever viruses by real-time RT-PCR. *Methods Mol Biol* 2011;**665**:357–68.
14. Schultze D, Korte W, Rafeiner P, Niedrig M. First report of sandfly fever virus infection imported from Malta into Switzerland, October 2011. *Euro Surveill* 2012;**17**(27):20209.

The full reference list for this chapter is available at expertconsult.com.

190D ■ OROPOUCHE FEVER

Francisco P. Pinheiro • Amelia P. A. Travassos da Rosa • Pedro Fernando da C. Vasconcelos

Oropouche (ORO) fever is an arbovirus infection that causes an acute febrile episode accompanied by headache, myalgia, arthralgia, and other systemic symptoms. The symptoms usually recur a few days after the end of the first febrile episode, at which time they generally are less severe. Aseptic meningitis may develop in some patients. Patients make a full recovery, without any apparent aftereffects, even in the most serious cases. No fatalities have been confirmed as being attributable to ORO fever. One of the most striking characteristics of ORO virus (OROV) is its ability to produce epidemics in urban population centers, most of which reportedly have occurred in the Brazilian Amazon region, although some have occurred in other countries in northern South America, Central America (Panama), and the Caribbean region (Trinidad and Tobago). Many of these outbreaks have had a major impact on the stricken cities.

The first case of the disease was described in 1955 in a resident of Vega de Oropouche, Trinidad, from whose blood the agent was isolated.[2] The disease was detected again in 1961, this time in the city of Belém, in northern Brazil, where it caused an epidemic that affected at least 11,000 people.[21] This epidemic was followed by many other epidemics, several of an explosive nature, in urban population centers throughout the Brazilian states of Pará, Amapá, Amazonas, Tocantins, Maranhão,

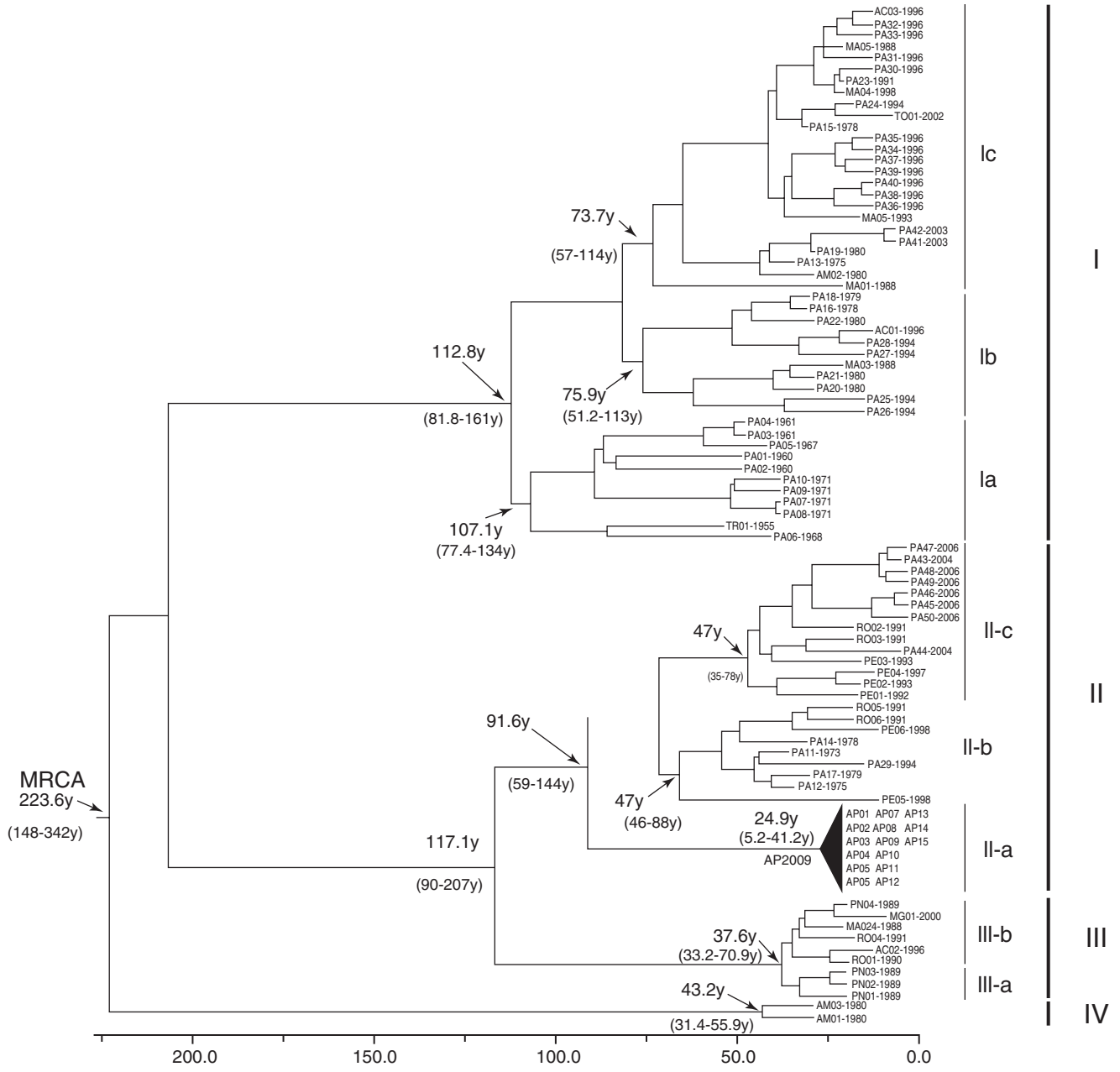

FIGURE 190D-1 ■ Phylogenetic tree based on the complete nucleotide sequence of the N gene (693 nt) of 96 OROV strains isolated from different hosts, locations, and periods. The main phylogenetic groups are represented by the genotypes (I, II, III, and IV). The values above the main nodes represent the dates of emergence of common ancestors expressed in years from the current date (year 2009). The arrows indicate the probable date of emergence of genotypes I, II, III, and IV. MRCA, most recent common ancestor. The numbers inside the parentheses are its value for high population density (HPD) 95 percent. The numerical data in the bar represent the time scale of molecular dating. (From Vasconcelos HB, Nunes MRT, Casseb LMN, et al. Oropouche fever virus: molecular epidemiology and evolution. *Emerg Infect Dis* 2011;**17**:800-6.)

Rondônia, and Acre.* Outside Brazil, epidemics of ORO fever were reported in Panama in 1989† and in the Amazon region of Peru in 1992[5] and in 1994‡ (Fig. 190d-1). On June 22, 2010, the Pan American Health Organization reported an outbreak of OROV in the northern Amazonian region of Peru. As of mid June 2010 the Peruvian Ministry of Health reported a total of 282 cases, of which 41 cases have been confirmed and 241 were probable. The outbreak has centered on Bagazán, in the district of Pachiza in the department of San Martín, and is the first outbreak to be reported in the region.[18]

ETIOLOGIC AGENT

ORO fever is caused by OROV, which belongs to the genus *Orthobunyavirus* of the family *Bunyaviridae*.[27] The

*References 9, 14, 21, 25, 26, 28, 31, 34.
†Quiroz E and associates, Panama, unpublished data, 1989.
‡Ministry of Health, Peru, and U.S. Naval Medical Research Institute Detachment [NAMRID], Lima, unpublished data, 1994.

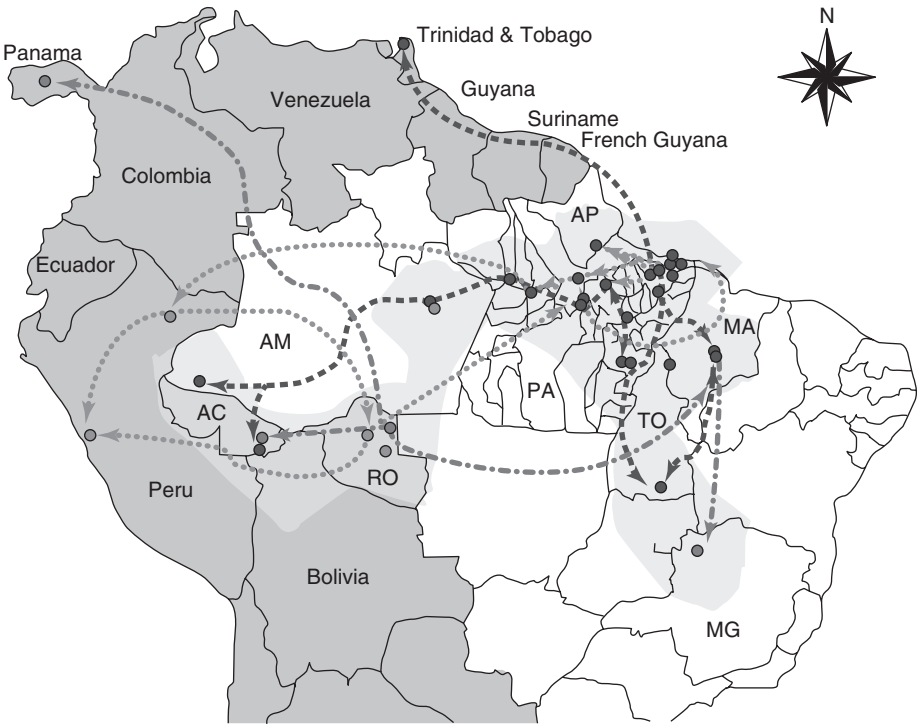

Source: Vasconcelos et al., 2011. *Emerg Infect Dis* 17(5): 800–806.

FIGURE 190D-2 ■ Geographic dispersion of OROV genotypes in the Americas between 1955 and 2009 based on the N gene genetic data. Dispersion route for genotype I (*dashed gray line*); dispersion route for genotype II (*dotted gray line*); dispersion route for genotype III (*dashed and dotted gray line*); genotype IV (*gray dot*). Coverage area of the OROV in Brazil: *AC,* Acre; *AP,* Amapá; *AM,* Amazonas; *MA,* Maranhão; *MG,* Minas Gerais; *PA,* Pará; *RO,* Rondônia; *TO,* Tocantins. (See companion Expert Consult website for color version.) (From Vasconcelos HB, Nunes MRT, Casseb LMN, et al. Oropouche fever virus: molecular epidemiology and evolution. *Emerg Infect Dis* 2011;**17**:800-6.)

virus has enveloped spherical particles 90 to 100 nm in diameter; the capsid has helical symmetry; and the single-stranded, negative-sense RNA contains three segments, designated large (L), medium (M), and small (S) RNA.[13,27] Complete nucleotide sequences have been determined for all three RNA segments.[3,30,35] Phylogenetic analysis based on S RNA (N gene) has revealed that all OROV strains form a monophylogenetic group consisting of four distinct lineages (see Fig. 190d-2).[33] Lineage I contains the prototype strain from Trinidad and most of the Brazilian strains; lineage II contains the Peruvian strains isolated between 1992 and 1998 and strains from western Brazil isolated in 1991, as well as isolates obtained more recently from Amapá and Pará states in the eastern Amazon; lineage III comprises strains isolated from Panama during 1989[30] and strains isolated in the Brazilian states of Acre, Pará, and Rondônia (Amazon region) and Minas Gerais (Southeast region); and, finally, lineage IV was only detected in a strain isolated during an outbreak that occurred in Manaus, Brazil, in 1981.[33] Antigenically, OROV belongs to the Simbu group, which, in turn, is part of the Bunyamwera supergroup of arboviruses. The virus has a hemagglutinin that is active against geese erythrocytes, and it can be recovered from infected hamster serum treated with acetone.* Intracerebral and intraperitoneal inoculation of OROV into baby mice and intracerebral, intraperitoneal, and subcutaneous inoculation of the virus into adult hamsters produce lethal infections. The virus replicates in numerous cell cultures, including Vero, BHK-21, and primary chicken embryo fibroblast, and it causes a cytopathic effect.[27] The agent is sensitive to the action of sodium deoxycholate.[12]

*Travassos da Rosa APA, Belém, unpublished data, 1969.

EPIDEMIOLOGY

Geographic Distribution

Thus far, the only reported cases of ORO fever have occurred in Argentina, Brazil, Panama, Peru, and Trinidad (see Fig. 190d-2). However, most cases have been limited to the Brazilian Amazon region, with none reported in other areas of Brazil.

With a few exceptions, all episodes of ORO fever have been in the form of urban epidemics, including those in Belém and Manaus, the largest cities in the Brazilian Amazon region. The city of Belém, capital of Pará, was struck by three major epidemics during a 20-year period. The city of Santarém and surrounding villages also were affected by a major epidemic in 1974 and 1975.[25] The first epidemics that occurred outside Pará, those striking the cities of Manaus and Barcelos in Amazonas[6] and the city of Mazagão in what was then the Amapá Territory, were reported early in the 1980s.[19] After a period of

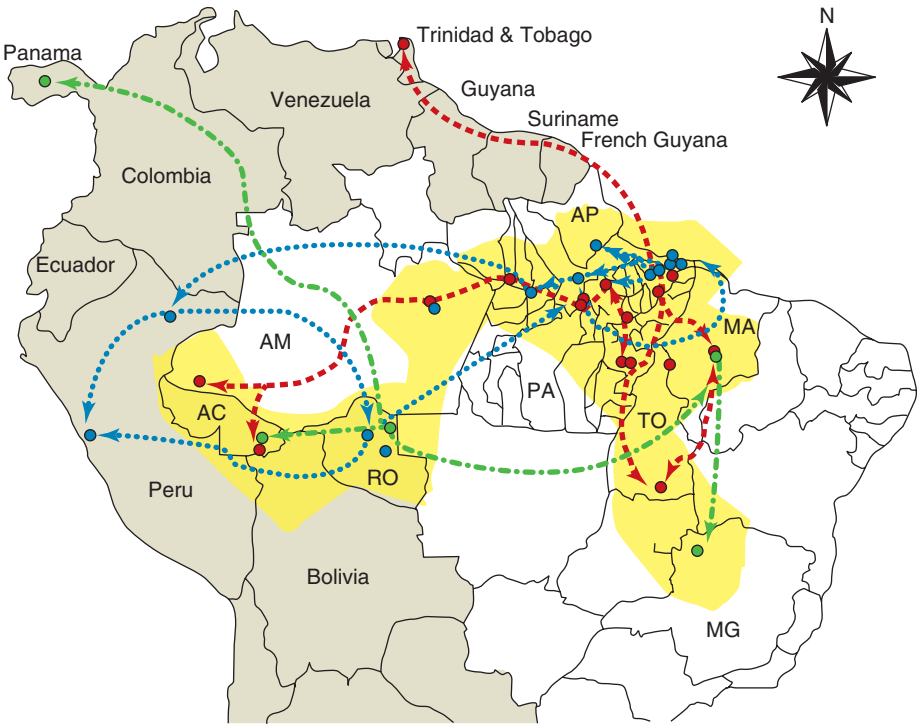

Source: Vasconcelos et al., 2011. *Emerg Infect Dis* 17(5): 800–806.

FIGURE 190D-2 ■ Geographic dispersion of OROV genotypes in the Americas between 1955 and 2009 based on the N gene genetic data. *Red line:* dispersion route for genotype I; *blue line:* dispersion route for genotype II; *green line:* dispersion route for genotype III; *light blue dot:* genotype IV. Coverage area of the OROV in Brazil: *AC,* Acre; *AP,* Amapá; *AM,* Amazonas; *MA,* Maranhão; *MG,* Minas Gerais; *PA,* Pará; *RO,* Rondônia; *TO,* Tocantins. (See companion Expert Consult website for color version.) (From Vasconcelos HB, Nunes MRT, Casseb LMN, et al. Oropouche fever virus: molecular epidemiology and evolution. *Emerg Infect Dis* 2011;**17**:800-6.)

quiescence lasting until 1988, new outbreaks of the disease struck the cities of Porto Franco and Tocantinópolis in the states of Maranhão and Tocantins, respectively.[34] The next reported epidemics occurred in 1991, this time in more distant locations, namely, in the cities of Ariquemes and Ouro Preto D'Oeste in Rondônia; the epidemic's impact on these cities was so great that it was reported in the national press. In 1994, another outbreak involving at least 6000 people was recorded in Serra Pelada in Pará.[31] Other outbreaks were recorded in 1996 and affected at least five urban centers in the states of Pará, Amazonas, and Acre.[26] Thus, during 1961 to 1996, more than 30 epidemics of ORO fever were recorded in Brazil. Several small outbreaks also were recognized in Canãa dos Carajás in 2003 and in Tapará Village at Porto de Moz in 2004, both municipalities in Pará.[4,17] In April of 2006, a large epidemic of ORO fever occurred in northeastern Pará that affected several municipalities. Autochthonous cases of ORO fever also were diagnosed in the municipalities of Magalhães Barata, Maracanã, Marapanim, Igarapé-Açu, São Francisco do Pará, and Viseu. A retrospective serologic study conducted among inhabitants of urban and rural communities of the municipality of Magalhães Barata showed that 320 of 631 persons had immunoglobulin (Ig) M OROV antibodies detected by enzyme-linked immunosorbent assay (ELISA), thus suggesting that approximately half of the 8000 inhabitants had been infected. In this episode, several isolates were obtained and sequenced and the OROV genotype II was recognized.[32] The last recorded epidemic in those municipalities occurred in 1980.[9]

Serologic surveys[9,19,23,26] estimated that more than 360,000 people were infected during this period.* However, this estimate actually is quite conservative because the incidence of this viral disease was not computed in many major outbreaks (Belém, 1968; Porto Franco and Tocantinópolis, 1988). In 2009 a large epidemic of Oropouche fever was reported in Mazagão, Amapá, and was caused by genotype III†; the last report of Oropouche fever in the municipality occurred in 1980. Possibly more than a half million people in the Brazilian Amazon region may have been infected with OROV since the beginning of the 1960s.

In addition to the aforementioned epidemic areas, countless small villages scattered throughout virtually the entire Amazon region have residents who show hemagglutination-inhibition antibodies against OROV. In general, the prevalence of these antibodies is less than 3 percent, with the exception of Ilha de Gurupá, where it is 10.7 percent.[26] An OROV strain was isolated from a novel primate host (*Callithrix* spp.) in Arinos in Minas Gerais in southeastern Brazil,[16] thus extending the enzootic area farther to southern Brazil.

Outside Brazil, outbreaks were reported in Panama and Peru. The outbreak in Panama occurred in 1989 in the village of Bejuco, which is located approximately 50 km west of the capital.‡ The first epidemic in Peru was reported in 1992 in the city of Iquitos in the Peruvian Amazon region.[7] Subsequently, an outbreak occurred in Puerto Maldonado, Madre de Dios, also in the Peruvian Amazon region.* Studies performed in Peru suggested that transmission of OROV occurs continuously in the population of the city of Iquitos and surrounding villages.[36] Evidence of immunity to OROV was detected in nonhuman primates in Colombia, a finding thus suggesting the presence of OROV in that country as well.[12] OROV has been detected by reverse-transcriptase polymerase chain reaction assay (RT-PCR) from febrile persons in Jujuy, northern Argentina.† The nucleotide sequencing of S RNA showed that the Argentinean strains belonged to the genotype IV.

Incidence

A significant characteristic of ORO fever is the exceptionally high attack rates seen during several outbreaks. Although incidence rates varied in different outbreaks, a rate of 30 percent was quite common. The proportion of those infected who suffered overt disease is not known with certainty, but in one epidemic clinical disease developed in 63 percent of those infected.[9]

Gender-specific attack rates vary. Rates in female patients were slightly higher than those in male patients in villages in the Bragantina area, in eastern Pará, which was struck by the virus in 1979,[9] and the opposite was true in the outbreak in Belém that same year. However, in the reported epidemics in Santarém, the infection struck female patients twice as often as male patients.[8] ORO fever affects all age groups, although in certain outbreaks the incidence was higher in children and young adults.

Diffusion of Epidemics

As indicated earlier, ORO fever epidemics have struck different locations at varying intervals. However, many outbreaks have been marked by bona fide epidemic sweeps, with countless numbers of villages within a particular geographic area affected by the virus. This diffusion phenomenon was observed in Bragança in 1967, in Santarém in 1974 and 1975, and even more dramatically in Belém and in the Bragantina area from 1978 to 1980 (where at least 10 towns were stricken), as well as in Rondônia in 1991. Spread of the virus most likely is attributable to the movement of viremic individuals throughout areas in which the virus vector is present.

Seasonal Fluctuation

Most epidemics of ORO fever typically occur during the rainy season, which, in the case of Pará in Brazil, corresponds to the period between January and June. However, many epidemics have extended into the dry season, although with less intensity. The seasonal nature of ORO

*Vasconcelos PFC, Nunes MRT, unpublished data, 2006.
†Vasconcelos PFC, unpublished data, 2009.
‡Quiroz E and associates, Panama, unpublished data, 1989.

*Ministry of Health, Peru, and NAMRID, Lima, unpublished data, 1994.
†Fabbri C, Nunes MRT, Vasconcelos PFC, Enria D, unpublished data, 2006.

fever most likely is linked to the higher density of populations of *Culicoides paraensis*, commonly known as the biting midge, the urban virus vector, in months with higher levels of rainfall, combined with a higher concentration of exposed individuals. Downward trends in epidemics of ORO fever generally are associated with the arrival of the dry season and the resulting lower density of biting midge populations and smaller numbers of exposed individuals.[13,28]

Endemic Transmission

During interepidemic periods, isolated cases of ORO fever undoubtedly occur in Brazil but remain undiagnosed. Although no systematic studies have been conducted to investigate endemic transmission of OROV in Brazil, seroepidemiologic investigations have indicated that the prevalence of anti-OROV antibodies in humans is 0 to 2 percent in areas where endemic transmission has not been reported,[26] but it increases to 17 to 44 percent after outbreaks.[8,13,27] These data suggest that endemic transmission of OROV is quite low in Brazil.

Transmission Mechanism

Laboratory studies and broad-based surveys conducted by the Evandro Chagas Institute of Belém during the course of epidemics point to the importance of the insect *C. paraensis* in the Ceratopogonidae as the urban vector for OROV.[20,24] These tiny insects, commonly known as maruins (biting midges) in the Amazon region, are active during the day, particularly in the late afternoon hours. They crave human blood and bite people inside as well as outside their homes.[11,25,29] The disease is transmitted by inoculation of the virus into exposed individuals by bites of infected midges.

Transmission Cycles

Studies conducted by the Evandro Chagas Institute[26] suggest that OROV is perpetuated in nature through two different cycles, namely, an urban cycle and a wild cycle. In the urban or epidemic cycle, the virus is transmitted from person to person by the bite of *C. paraensis*. One of the most conclusive pieces of evidence attesting to this assertion lies in demonstration of the ability of *C. paraensis*, after feeding on the blood of viremic patients, to transmit the virus to hamsters bitten by the midges 5 or more days later.[24] Moreover, these midges typically are found in high densities during periods of epidemics. They breed mostly in the decomposing trunks of felled banana trees, in rotting husks of cocoa beans,[10] and in piles of detritus formed in tree hollows.[15] They are scattered throughout tropical and subtropical areas of the Americas.[15]

Attempts to transmit the virus from one hamster to another through the bite of the *Culex quinquefasciatus* mosquito (a species commonly found in urban areas throughout the Amazon region) demonstrated that the virus was transmitted only in the presence of extremely high levels of viremia, which rarely occurs in infected humans.[20] Thus, this finding virtually rules out all likelihood that the epidemic vector is *C. quinquefasciatus*. Curiously, the virus isolation rate from *C. paraensis* during periods of epidemics is only 1:12,500,[13] a finding suggesting that we are dealing with a low-efficiency vector. Apparently, humans are the only vertebrates involved in the urban cycle of OROV because studies of domestic animals conducted during the course of numerous outbreaks ruled out the possibility that these animals play an amplifying role.

As far as the wild, silent cycle of the virus is concerned, evidence suggests that among vertebrates, the *Edentata* (sloth), nonhuman primates, and possibly certain species of wild birds serve as hosts. Although to date OROV has been isolated from mosquitoes (from a single pool of *Aedes serratus* in Brazil and once from *Coquillettidia venezuelensis* in Trinidad[2,22]), the possible involvement of biting midges in the wild cycle of the virus nonetheless should be investigated.

The link between the two cycles most likely is humans themselves, who, after contracting the infection in enzootic forested areas and then returning to an urban setting during the viremic phase, become a source of infection for biting midges. The virus replicates in the tissues of biting midges, which, after the extrinsic incubation period, bite and infect exposed individuals. These individuals, in turn, serve as a source of infection for other midges, thereby forming a chain of transmission resulting in the unleashing of an epidemic.

Incubation Period

Observations conducted during numerous epidemics suggest that the incubation period ranges from 4 to 8 days. A laboratory worker who accidentally was infected orally exhibited symptoms of the viral disease 3 days later, and another technician fell ill 4 days after being infected, probably through the respiratory route.[26]

Transmissibility Period

The blood of infected patients is infectious to *C. paraensis* for the first 3 or 4 days after the onset of symptoms, when the level of viremia is high enough to infect the midges. Experimental studies have shown the length of the extrinsic incubation period to be 5 days or more.[24] The virus is not transmitted directly from one person to another.

Ratio of Symptomatic Cases

A prospective study conducted in the city of Santa Izabel in Pará in Brazil during the course of the epidemic of 1979 showed the ratio of symptomatic to asymptomatic cases to be roughly 2:1.[9] The study was performed during the period from March through June of that year. It involved 274 individuals exposed to the virus who were monitored by clinical examination and laboratory testing on a weekly basis throughout the study. By the end of the study period, 78 (28.5%) of these individuals had serologic evidence of OROV infection, with clinical manifestations of the disease developing in 49 (63%) of the 78.

CLINICAL MANIFESTATIONS

In most cases of ORO fever, the infection takes the form of an acute febrile episode, which runs its course. However, certain patients may show typical signs and symptoms of aseptic meningitis, which also runs its course without complications.

Classic Febrile Form

This form of the disease is characterized by the sudden onset of symptoms after an incubation period ranging from 4 to 8 days. The first symptoms to appear are fever, headache, chills, dizziness, muscular pain, arthralgia, and photophobia. Retro-ocular pain and conjunctival congestion also may be present. In addition, some patients suffer from nausea, which may be accompanied by episodes of vomiting. Not uncommonly, patients have severe anorexia and insomnia. At times, cough and coryza are present as well, although these manifestations may be attributable to intercurrent infections. Certain patients complain of fleeting burning or stinging sensations in different parts of their body. The presence of an exanthem is a rare finding. Two laboratory workers who were infected accidentally reported a longer and heavier menstrual flow than usual.[25,26] The fever can be quite high, 39°C or 40°C (102.2°F or 104°F), and in some cases it may be higher than 40°C. The headache usually is localized in the front or back part of the head, although it also may be diffuse. It generally is severe and, in some cases, may not respond readily to common analgesics. Generalized myalgia is present and sharpest in the neck, along the vertebral column, and in the area of the sacrum. The pain may be extremely severe. Patients generally describe feeling as though their body had been crushed or they had been beaten. Usually, generalized arthralgia also is present. Certain patients have dizzy spells so severe that in some cases they collapse. Any epigastric pain generally is mild. Patients have no sign of jaundice, hepatomegaly, or splenomegaly. Occasionally, swollen lymph nodes are detected in the submaxillary and occipital regions, and these findings could be totally unrelated to the viral infection.

The intensity of the clinical symptoms varies. In some cases, the symptoms are quite severe and even may cause prostration, whereas in others, they can be rather mild. Many patients are bedridden and, during epidemics, flood area hospitals, thus causing serious overcrowding.

The acute phase of the disease generally lasts 2 to 5 days but can be as long as a week. The myalgia, conversely, may persist for 3 to 5 days after the fever has disappeared. Some patients report having prolonged asthenia for as long as a month. Certain patients complain of a persistent headache lasting up to several weeks. No human deaths have been attributed to OROV infection.[13]

Nearly 60 percent of all patients have one or more recurrences in the first or second week after disappearance of the manifestations of the acute phase of the disease.[9,19,26] Relapses may take the form of reappearance of all the acute-phase symptoms of the disease, or they may be limited strictly to fever, asthenia, and dizziness. In some reported patients, relapses were accompanied by a urinary tract infection of bacterial origin. An abscess, most likely bacterial, developed in the oropharynx of one particular patient approximately 10 days after recovery from the original febrile condition. In some cases, patients may suffer a series of relapses during a period of 2 to 3 weeks.[26] All attempts to isolate OROV during relapses have failed.

Observations made during the course of the 1980 outbreak in Belém revealed that an exanthem developed in approximately 5 percent of all laboratory-confirmed cases.[19] The exanthem appeared between the third and sixth day after onset of the fever, disappeared 2 or 3 days later, and mainly involved the thorax, back, arms, and legs.[19,23] During the outbreak in Manaus, many patients exhibited a maculopapular exanthem beginning on the torso and spreading to the upper and lower extremities.[5] In another rare case, a 4-year-old child whose infection was confirmed by serodiagnosis experienced nystagmus, generalized tremors, and somnolence.[9] These symptoms lasted approximately 8 days, and the child apparently made a full recovery.

The effects of ORO fever on pregnancy are unknown. The only available data in this regard come from studies conducted in Manaus of nine pregnant patients, two of whom, both in the second month of pregnancy at the time, suffered miscarriages.[5]

Aseptic Meningitis

At first, patients exhibit manifestations typical of the initial acute phase of the infection. As the illness progresses, a few days later the headache and dizziness become increasingly severe, and some patients begin to experience other neurologic symptoms, generally during the second week of the illness, that lead them to seek medical care. The main complaints cited by patients are fever, extremely severe headache in the back of the head, and dizziness. Approximately one third of all patients complain of nausea and vomiting. Some patients suffer from moderate lethargy. They also may have trouble holding themselves upright. Some patients complain of double vision or diplopia. They generally try to keep from moving their heads to avoid aggravating their pain. In most cases, physical examination of these patients reveals varying degrees of stiffness of the neck but no signs of paresis or paralysis. Some patients experience nystagmus. Despite the seriousness of these neurologic symptoms, patients make a full recovery. Electroencephalograms taken of four patients showed no abnormalities. The incidence of meningitis in patients who seek medical care is less than 5 percent.[22]

PATHOGENESIS

Little is known about the pathogenesis of ORO fever. Apparently, the agent produces a systemic infection in humans that induces a viremic phase, but the organs in which the virus replicates have not been identified. Virtually all infected patients exhibit viremia during the first 2 days of their illness. By the third day, the rate of viremia

drops to 72 percent, and it falls to 44 and 23 percent by the fourth and fifth days, respectively. Viremia titers generally are higher than 3.0 $\log_{10}$ median lethal dose per 0.02 mL in mice, with approximately 10 percent of patients exhibiting virus titers as high as 5.0 to 5.3 during the course of the first 2 days of their illness. By the third day, virus titers are 1 log lower than in the first 2 days, and titers plummet by the fourth day.[24]

Similarly, little is known about the pathogenesis of relapses, which occur commonly. That no sign of viremia could be detected in any of the countless patients examined while they are suffering relapses is noteworthy.

The finding that OROV is capable of causing aseptic meningitis, combined with isolation of the virus from the cerebrospinal fluid (CSF) in one patient with meningitis,[22] suggests that the virus has the ability to penetrate the blood-brain barrier. With no known confirmed fatalities attributable to OROV, no data are available on the possible organic lesions caused by this agent in humans.

Laboratory tests on young hamsters inoculated with OROV showed that the virus has essentially hepatoviscerotropic properties, with isolated necrosis of hepatocytes or focal necrosis and the involvement of Kupffer cells exhibiting reactive hyperplasia; animals invariably succumb to the infection. In newborn mice the virus exhibits marked neurotropism, and animals show signs of focal encephalitis within 24 to 48 hours after being inoculated.[4]

LABORATORY FINDINGS

Leukopenia associated with neutropenia is found commonly, although in certain cases moderate leukocytosis may be present. The leukopenia can be severe, with reports of leukocyte counts as low as 2000/mm^3. No signs of cell abnormalities are present. Aspartate and alanine aminotransferase levels are normal or may show a moderate increase, but in no case do they exceed 135 units/mL of serum. Platelet counts usually are normal but occasionally may be slightly low. The erythrocyte sedimentation rate and levels of urea, creatinine, and glucose in blood are normal, as are urine tests.[24]

The CSF of patients with aseptic meningitis shows pleocytosis and an increased concentration of protein.[22] The cell count varies from 7 to 310 cells/mm^3 of CSF; both segmented and mononuclear cells are present, with a predominance of segmented cells. In one case the cell count in CSF fell from 130 to 30 in a 1-week period, and another patient's cell count fell from 70 to 10 cells over a 3-week interval. In general, a moderate increase in protein levels occurs in the CSF, although one patient's protein level was more than 100 mg/mL. Glucose levels remain normal.

DIAGNOSIS

Specific confirmation of the infection is made by isolating the virus from a patient's blood or by performing OROV-specific serologic assays.[27] To isolate the virus, blood samples need to be taken during the first 5 days of the illness, preferably in the first 2 days, when viremia is present in virtually all cases. The virus can be isolated by intracerebral or intraperitoneal inoculation of serum from infected patients into baby mice or young hamsters (in this case, subcutaneous inoculation can be used as well). Viral isolates also can be recovered in different cell cultures, such as Vero and BHK-21. The virus is identified by complement fixation or neutralization using OROV-specific ascitic fluid or antisera. Serodiagnosis is accomplished by the demonstration of an increase in antibody in paired serum samples taken during the acute and convalescent phases of the disease by hemagglutination inhibition, complement fixation, or neutralization. A positive IgM antibody capture ELISA (MAC-ELISA) on a single serum sample provides a presumptive diagnosis of recent infection, particularly in the presence of a clinical picture consistent with the disease; the test usually is positive after the fifth day of illness. A rapid and highly sensitive one-step TaqMan RT-PCR assay has been described for detection of OROV and certain other orthobunyaviruses; it was found to be more sensitive than the established nested PCR system.[37]

Recently, a comprehensive and large molecular study was performed with OROV isolates focusing on phylogenetic analysis, as well as on dispersal and evolutionary aspects of it (see Figs. 190d-1 and 190d-2), and the conclusions of the study were that OROV emerged in the Brazilian Amazon approximately 223 years ago and that based on N gene (S RNA) data, the genotype I was the first genetic lineage to emerge and it was responsible for the emergence of the other genetic lineages, as well as for virus dispersal in northern South America.[34]

DIFFERENTIAL DIAGNOSIS

Because of the nonspecific nature of the symptoms, a clinical diagnosis of ORO fever is difficult to make, and often the disease is mistaken for other febrile illnesses such as dengue fever and malaria. In fact, malaria and dengue fever initially were suspected as the cause of numerous epidemics of ORO fever. Detailed clinical records combined with epidemiologic data can help establish a differential diagnosis, the certainty of which hinges on the absence of plasmodia in blood samples and the lack of laboratory evidence of dengue infection. Other viral and bacterial febrile diseases must be considered in the differential diagnosis. Accordingly, clinical and epidemiologic data and nonspecific tests will need to be taken into account, although establishing an accurate diagnosis requires specific tests.

Febrile forms of the disease accompanied by an exanthem need to be distinguished from other exanthematous febrile symptoms caused by dengue, measles, parvovirus B19 infection, infection with human herpesvirus type 6 or 5, enteroviral infections, and allergies to medication. A potential problem to differentiate ORO fever was observed in Peru after the emergence of a new orthobunyavirus named Iquitos virus (IQTV), which is a reassortant virus containing the S and L segments of OROV and the M segment of an unknown Simbu serogroup virus, but clinically the febrile disease caused by IQTV is

identical to ORO fever.[1] Finally, differentiating cases of aseptic meningitis associated with OROV infection from cases of aseptic meningitis associated with other causative agents requires a specific etiologic diagnosis.

TREATMENT

Because ORO fever has no specific treatment, management is symptomatic. Rest is important and should be continued for several days after disappearance of the initial acute manifestations because relapses are thought to occur more often in patients who prematurely resume regular activities, particularly strenuous ones. Aspirin or another antipyretic should be taken to lower the fever, and the use of ordinary analgesics is recommended for headache, myalgia, and arthralgia. However, certain reported patients whose headaches failed to respond to this treatment were treated with morphine derivatives. Also recommended are fruit juices or glucose solutions. Severely dehydrated patients may be treated with intravenous fluids.

PREVENTION AND CONTROL

The most effective way to prevent, avert, or curb the impact of epidemics of ORO fever is to combat its vector, *C. paraensis*. To be effective, vector control effort needs to focus on the midge's adult and larval forms. Given that *C. paraensis* is habitually active during the day, application of insecticides to its habitats by thermonebulization or ultra-low-volume aerosolization may help to reduce populations of adult biting midges. Because this *Culicoides* species is most active during the late afternoon hours,[29] ultra-low-volume spraying may be more effective during this period. However, carefully planned studies are needed to assess how to maximize the effectiveness of spraying by determining the type and concentration of insecticide to be used, the necessary volume of insecticide per treatment area, the size of the droplets, the frequency and timing of applications, and other factors. At the same time, making an effort to control the larvae by applying larvicides to corresponding habitats or, better yet, by conducting drives to eliminate or burn breeding sites such as rotting cocoa bean husks and the decomposing trunks of felled banana trees is essential.[10] Obviously, the success of these measures will depend largely on community involvement. Providing proper community education is important. Individuals can protect themselves by applying insect repellent directly to the skin. However, these types of products provide only temporary action, and they may be unaffordable to the poor.

No vaccine against ORO fever exists at this time. In light of the relatively benign nature of this viral disease, developing a general-purpose vaccine for at-risk populations living in areas where they are exposed to the disease is difficult to justify.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Aguilar PV, Barrett AD, Saeed MF, et al. Iquitos virus: a new reassortant Orthobunyavirus associated with human illness in Peru. *PLoS Negl Trop Dis* 2011;**5**(9):e1315.
18. Pan American Health Organization (PAHO). *Epidemiological alert: outbreak of Oropouche fever: 22 June 2010*. Available at http://new.paho.org/hq/index.php?option=com_content&task=view&id=3084&Itemid=2206. Accessed July 9, 2010.
33. Vasconcelos HB, Nunes MRT, Casseb LMN, et al. Oropouche fever virus: molecular epidemiology and evolution. *Emerg Infect Dis* 2011;**17**:800–6.

The full reference list for this chapter is available at expertconsult.com.

190E ■ TOSCANA VIRUS

Rémi N. Charrel • Xavier de Lamballerie • Laurence Bichaud

HISTORY

Toscana virus (TOSV) was isolated first in 1971 from the sandfly *Phlebotomus perniciosus* collected in Monte Argentario (Grosseto Province, central Italy).[71-73] Three strains were isolated from female sandflies. So far, most clinical and epidemiologic studies have been conducted in Italy, although studies from other Mediterranean countries have been published more recently. Results of these studies indicate that TOSV is a major viral pathogen involved in aseptic meningitis during summer months in these countries. A bibliographic search using "Toscana virus" as keywords in the PubMed database retrieved 218 research and review articles. Fewer than 50 percent of them reported imported or autochthonous human cases acquired in Italy, Spain, Portugal, France, Croatia, Cyprus, and Turkey.[2f,33,55] Recently, TOSV was isolated in Northern Africa, demonstrating its presence on the southern shore of the Mediterranean.[1a,7a] Despite increasing evidence of its major role in human disease, TOSV remains poorly studied, and most physicians have little awareness concerning its potential to cause central nervous system (CNS) infections. Moreover, TOSV cases were reported from few countries where sandflies are prevalent; whether this factor is an accurate reflection of the reality or whether it relates to a lack of investigation is unknown. However, recent evidence for the presence of TOSV in a growing number of countries favors the second hypothesis.

REFERENCES

1. Aguilar PV, Barrett AD, Saeed MF, et al. Iquitos virus: a new reassortant Orthobunyavirus associated with human illness in Peru. *PLoS Negl Trop Dis* 2011;**5**(9):e1315.
2. Anderson CR, Spence L, Downs WG, et al. Oropouche virus: a new human disease agent from Trinidad, West Indies. *Am J Trop Med Hyg* 1961;**10**:574–8.
3. Aquino VH, Moreli ML, Moraes Figueiredo LT. Analysis of Oropouche virus L protein amino acid sequence showed the presence of an additional conserved region that could harbor an important role for the polymerase activity. *Arch Virol* 2003;**148**:19–28.
4. Araújo R, Pinheiro FP, Araújo MT, et al. Patogenia das lesões hepáticas na infecção experimental com o vírus Oropouche (BeAn 19991): análise comparativa das curvas virémica e de infectividade com as alterações ultra-estruturais. *Hil Med Belem* 1979;**1**:7–12.
5. Azevedo RSS, Nunes MRT, Chiang JO, et al. Reemergence of Oropouche fever, Northern Brazil. *Emerg Infect Dis* 2007;**13**(6): 912–15.
6. Borborema CAT, Pinheiro FP, Albuquerque BC, et al. Primeiro registro de epidemias causadas pelo vírus Oropouche no Estado do Amazonas. *Rev Inst Med Trop São Paulo* 1982;**24**:132–9.
7. Chavez R, Colan E, Phillips I. Fiebre de Oropouche en Iquitos: reporte preliminar de 5 casos. *Rev Farmacol Ter* 1992;**2**:12–14.
8. Dixon KE, Travassos da Rosa APA, Travassos da Rosa JFS, et al. Oropouche virus, part II: epidemiological observations during an epidemic in Santarém, Pará, Brazil, in 1975. *Am J Trop Med Hyg* 1981;**30**:161–4.
9. Freitas RB, Pinheiro FP, Santos MAV, et al. Epidemia de virus Oropouche no leste do Estado do Pará, 1979. In: Pinheiro FP, editor. *International symposium on tropical arboviruses and haemorrhagic fevers*. Rio de Janeiro: Academia Brasileira de Ciências; 1982. p. 419–39.
10. Hoch AL, Roberts DR, Pinheiro FP. Breeding sites of *Culicoides paraensis* and other options for control by environmental management. *Bull Pan Am Health Org* 1986;**20**:284–93.
11. Hoch AL, Roberts DR, Pinheiro FP. Host-seeking behavior and seasonal abundance of *Culicoides paraensis* (Diptera: Ceratopogonidae) in Brazil. *J Am Mosq Control Assoc* 1990;**6**:110–14.
12. Karabatsos N, editor. *International catalogue of arboviruses including certain other viruses of vertebrates*. San Antonio: American Society of Tropical Medicine and Hygiene; 1985.
13. LeDuc JW, Pinheiro FP. Oropouche fever. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 4. Boca Raton, Fla.: CRC Press; 1986. p. 1–14.
14. LeDuc JW, Hoch AL, Pinheiro FP, et al. Epidemic Oropouche virus disease in northern Brazil. *Bull Pan Am Health Org* 1981;**15**: 97–103.
15. Linley JR, Hoch AL, Pinheiro FP. Biting midges (Diptera: Ceratopogonidae) and human health. *J Med Entomol* 1983;**20**:347–64.
16. Nunes MRT, Martins LC, Rodrigues SG, et al. Oropouche virus isolation, southeast Brazil. *Emerg Infect Dis* 2005;**11**:1610–13.
17. Nunes MRT, Vasconcelos HB, Medeiros DBA, et al. A febre do Oropouche: uma revisão dos aspectos epidemiológicos e moleculares na Amazônia Brasileira. *Cad Saúde Colet* 2007;**15**:303–18.
18. Pan American Health Organization (PAHO). *Epidemiological alert: outbreak of Oropouche fever: 22 June 2010*. Available at http://new.paho.org/hq/index.php?option=com_content&task=view&id=3084&Itemid=2206. Accessed July 9, 2010.
19. Pinheiro FP. Febre do Oropouche. *J Bras Med* 1983;**44**:46–62.
20. Pinheiro FP, Hoch AL, Gomes MLC, et al. Oropouche virus, part IV: laboratory transmission by *Culicoides paraensis*. *Am J Trop Med Hyg* 1981;**30**:172–6.
21. Pinheiro FP, Pinheiro M, Bensabath G, et al. Epidemia de vírus Oropouche em Belém. *Rev Serv Esp Saude Publ* 1962;**12**:15–23.
22. Pinheiro FP, Rocha AG, Freitas RB, et al. Meningite associada às infecções por vírus Oropouche. *Rev Inst Med Trop São Paulo* 1982;**24**:246–51.
23. Pinheiro FP, Travassos da Rosa APA, Freitas RB, et al. Arbovírus: Aspectos clínico-epidemiológicos. In: *Instituto Evandro Chagas: 50 anos de contribuição às ciências biológicas e à medicina tropical*. Belém: Fundação Serviços de Saúde Pública; 1986. p. 349–57.
24. Pinheiro FP, Travassos da Rosa APA, Gomes MLC, et al. Transmission of Oropouche virus from man to hamster by the midge *Culicoides paraensis*. *Science* 1982;**215**:1251–3.
25. Pinheiro FP, Travassos da Rosa APA, Travassos da Rosa JF, et al. An outbreak of Oropouche virus disease in the vicinity of Santarém, Pará, Brazil. *Tropenmed Parasitol* 1976;**27**:213–23.
26. Pinheiro FP, Travassos da Rosa APA, Travassos da Rosa JF, et al. Oropouche virus, part I: a review of clinical, epidemiological and ecological findings. *Am J Trop Med Hyg* 1981;**30**:149–60.
27. Pinheiro FP, Travassos da Rosa APA, Vasconcelos PFC. Arboviral zoonoses of Central and South America, part G: Oropouche fever. In: Beran GW, editor. *Handbook of zoonoses*. 2nd ed. Boca Raton, Fla.: CRC Press; 1994. p. 214–17.
28. Pinheiro FP, Travassos da Rosa APA, Vasconcelos PFC. An overview of Oropouche fever epidemics in Brazil and neighbour countries. In: Travassos da Rosa APA, Vasconcelos PFC, Travassos da Rosa JFS, editors. *An overview of arbovirology in Brazil and neighbouring countries*. Belém: Evandro Chagas Institute; 1998. p. 186–92.
29. Roberts DR, Hoch AL, Dixon KE, et al. Oropouche virus, part III: entomological observations from three epidemics in Pará, Brazil, 1975. *Am J Trop Med Hyg* 1981;**30**:165–71.
30. Saeed MF, Wang H, Nunes MR, et al. Nucleotide sequences and phylogeny of the nucleocapsid gene of Oropouche virus. *J Gen Virol* 2000;**81**:743–8.
31. Travassos da Rosa APA, Rodrigues SG, Nunes MR, et al. Epidemia de febre do Oropouche em Serra Pelada, Município de Curionópolis, Pará, 1994. *Rev Soc Bras Med Trop* 1996;**29**:537–41.
32. Vasconcelos HB, Azevedo RSS, Nunes MRT, et al. Oropouche fever epidemics in Northern Brazil: epidemiology and molecular characterization of isolates. *J Clin Virol* 2007;**44**:129–33.
33. Vasconcelos HB, Nunes MRT, Casseb LMN, et al. Oropouche fever virus: molecular epidemiology and evolution. *Emerg Infect Dis* 2011;**17**:800–6.
34. Vasconcelos PFC, Travassos da Rosa JFS, Guerreiro SC, et al. Primeiro registro de epidemias causadas pelo vírus Oropouche nos estados do Maranhão e Goiás, Brasil. *Rev Inst Med Trop São Paulo* 1989;**31**:271–8.
35. Wang H, Beasley DW, Holbrook MR, Barrett AD. Nucleotide sequence and deduced amino acid sequence of the medium RNA segment of Oropouche, a Simbu serogroup virus: comparison with the middle RNA of Bunyamwera and California serogroup viruses. *Virus Res* 2001;**73**:153–62.
36. Watts DM, Phillips I, Callahan JD, et al. Oropouche virus transmission in the Amazon River basin of Peru. *Am J Trop Med Hyg* 1997;**56**:148–52.
37. Weidmann M, Rudaz V, Nunes MRT, et al. Rapid detection of human pathogenic orthobunyaviruses. *J Clin Microbiol* 2003;**41**: 3299–305.

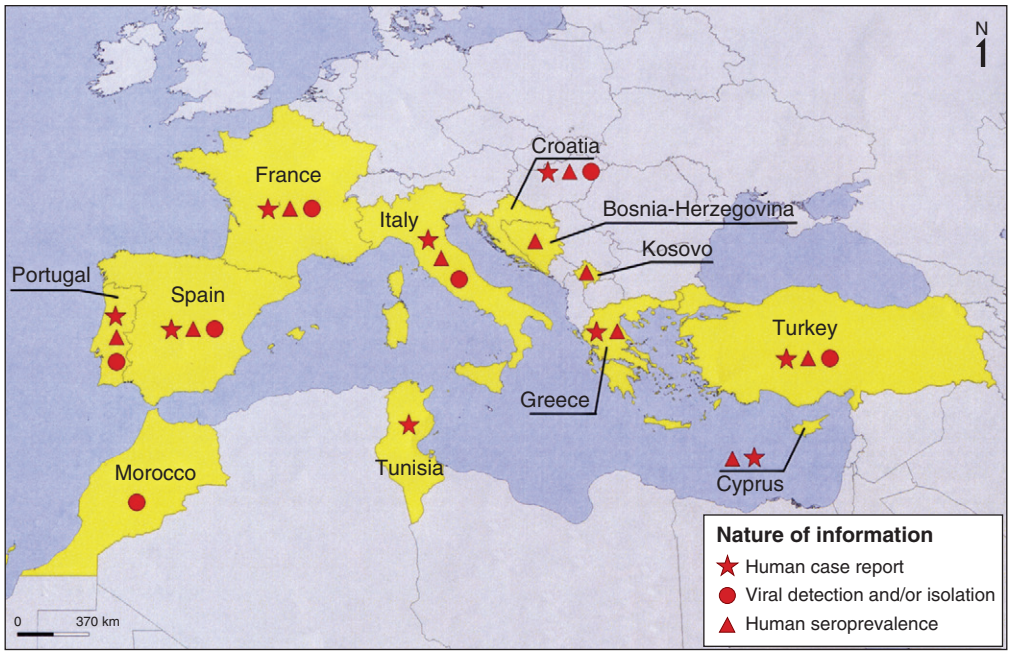

FIGURE 190E-1 ■ Geographic distribution of Toscana virus in Europe. (See companion Expert Consult website for color version.) Updated version submitted by Dr. Charrel in Toscana Virus chapter.

ETIOLOGIC AGENT

Phleboviruses are arthropod-borne viruses that contain a negative-sense, single-stranded RNA genome that consists of three segments, designated large, medium, and small, that encode the RNA-dependent RNA polymerase,[32] the envelope glycoproteins, and the nucleoprotein, respectively. Phylogenetic analyses performed using the medium segment identified three major lineages separate from Rift Valley fever virus and Uukuniemi virus. Lineage 1 includes sandfly fever Sicilian virus and Sicilian-like viruses isolated from Italy, Cyprus, India, Iran, and Greece. Lineage 2 includes sandfly fever Naples, Naples-like viruses, and genotypes (Tehran and TOSV) isolated from Italy, Portugal, Iran, the former Yugoslavia, Egypt, Cyprus, and India. Lineage 3 includes phleboviruses from the New World, such as Punta Toro, Buenaventura, and related viruses.[42] However, the recent availability of a large number of complete genome sequences will allow genetic classification of phleboviruses. According to the 9th report of the International Committee on Taxonomy of Viruses (ICTV), TOSV is a serotype of the species *Sandfly fever Naples virus* within the genus *Phlebovirus* in the Bunyaviridae family.[53] TOSV is an arthropod-borne virus. By using other serologic tests (indirect immunofluorescence assay [IFA], hemagglutination inhibition, complement fixation, enzyme-linked immunosorbent assay [ELISA]), serologic cross-reactions are observed between the viruses included in the Sandfly fever Naples virus antigenic group, such as TOSV, Naples, Tehran, and the recently discovered viruses (i.e., Massilia, Granada, and Punique).[16,18,76] Therefore, studies based on ELISA or IFA techniques must be interpreted carefully because of possible misinterpretations as a result of antigenic cross reactivity between distinct viruses. Confirmation of the results obtained by these broadly reactive tests is necessary using neutralization assays.

EPIDEMIOLOGY

Phlebotomus fever viruses have been isolated from sandflies in southern Europe, Africa, central Asia, and the Americas, and evidence exists for the presence of different viruses in the same sandfly population. The simultaneous occurrence of Sandfly fever Sicilian virus, Karimabad virus, and Isfahan virus (a *Vesiculovirus*) was documented in Iran,[66] and Verani and colleagues[72] isolated TOSV (associated with human disease) and Arbia (not yet shown to cause infection in humans) viruses in Italy in the same sandfly population. Sandfly fever Naples and Sicilian viruses have the widest geographic distribution, parallel to their vector's *(Phlebotomus papatasi)* distribution. Recent data indicate that the same may apply to TOSV and *P. perniciosus* (or *Phlebotomus perfiliewi*). Until recent years, the known distribution of TOSV was limited to Italy and Portugal.[28,48] In Italy, the virus was isolated both from the vectors (*P. perniciosus* and *P. perfiliewi*) and from humans, whereas the presence of the virus in Portugal was suspected on the basis of a strain isolated from the cerebrospinal fluid (CSF) of a Swedish patient returning to his home country from Portugal. More recently, the geographic distribution of the virus has been extended to Portugal, France, Spain, Croatia, Greece, Cyprus, Turkey, and Northern Africa, according to results from viral isolation and serologic surveys.[27,31,37,43,52] The currently recognized geographic distribution of TOSV virus is shown in Figure 190E-1.

Italy

Preliminary clues pointing to the role of TOSV in CNS infections in Italy were provided by case reports of imported cases diagnosed in the United States[12] and Germany.[63] Italy was the first country where TOSV activity was demonstrated, and subsequently most studies concerning the epidemiology of TOSV were conducted there. A large study carried out between 1977 and 1988 showed that the virus was the cause of meningitis in two different regions of Italy, Tuscany and Marche, with a seasonal peak occurring in August, corresponding to the peak of sandfly activity.[48] Since then, the virus has been isolated in many other regions of southern and northern Italy. Striking evidence that TOSV was the most prominent viral cause of summertime meningitis was reported in the late 1990s.[67,69] A study of children living in rural or suburban areas of Siena (central Italy) showed that 40 percent of meningitis or encephalitis cases could be linked to infection with TOSV.[10] A 7-year study (1990 to 1996) performed in Siena showed that 52 percent of aseptic meningitis cases in adults were associated with TOSV.[8] All studies are in agreement regarding the monthly distribution of human cases of TOSV infections—the highest risk for acquiring TOSV is in August (++++), then July and September (++), and finally June and October (+). Many reports describe TOSV cases in other regions of Italy, such as northern Italy, Emilia Romagna, Naples, Sicily, and Sardinia.* Seroprevalence studies report rates that vary between 9 and 25 percent, indicating different levels of exposure according to regional and local environmental conditions.

Spain

The first case of TOSV infection reported from Spain occurred in the late 1980s in a Swedish tourist after visiting Catalonia and was documented by plaque reduction neutralization test (PRNT).[29] Subsequently, Spanish researchers and physicians reported many cases of TOSV infection and conducted several comprehensive epidemiologic studies demonstrating that TOSV was a major cause of meningitis in Spain.[45] A total of 15 strains of TOSV were isolated between 1988 and 1996 from the CSF of patients with acute aseptic meningitis in Granada, Spain.[43] A total of 724 CSF samples from patients with suspected aseptic meningitis were tested through viral isolation in cell culture. TOSV was isolated from the CSF of 17 patients (7% of all viral isolates), 15 of whom were previously reported in the foregoing reference. TOSV was ranked as the third most common cause of aseptic meningitis after enteroviruses and mumps virus, a finding suggesting that its etiologic role in CNS infections in Spain is not as high as observed in central Italy. These data suggest that the situation in Spain is similar to that observed in France, with lower prevalence of CNS infections in contrast to those observed in central Italy.

Sequence data obtained from Spanish isolates of TOSV and other phleboviruses indicate that natural genome variability may hamper establishing the diagnosis of these agents by molecular methods. Generic nested RT-PCR, followed by sequencing of the amplified products, was developed for the detection and specific identification of every member of the *Phlebovirus* genus, including TOSV. The testing of clinical samples with these assays confirmed the role of TOSV as a causative agent of acute aseptic meningitis in the central region of Spain.[57] Of a collection of 88 serum and 53 CSF samples (taken from 81 patients with acute aseptic meningitis of unknown origin), living in Madrid or on the southern Mediterranean coast of Spain, 7 were found to have acute TOSV infection (three residents from the Mediterranean region and four from the Madrid region).[27] A large study conducted in different regions of Spain showed the presence of IgG antibodies to TOSV (26.2%), Sandfly fever Naples virus (2.2%), and Sandfly fever Sicilian virus (11.9%) in 1181 adults and 87 children.[43] A total of 457 serum samples taken from healthy individuals, 2 to 60 years of age, from the south of the Madrid region was investigated for anti-TOSV immunoglobulin (Ig)G. The overall prevalence of anti-TOSV was 5 percent.[27]

In the Granada region, the overall seroprevalence rate was 24.9 percent. A statistically significant increase in the seroprevalence rate was observed with age (9.4% in individuals younger than 15 years of age in contrast to 60.4% in individuals older than 65 years). In addition, several cases of TOSV were documented in numerous geographic areas of Spain (i.e., Badajoz, Málaga, Granada, Almería, Murcia, Alicante, Valencia, Madrid, and Barcelona), mostly in the southern, central, and Mediterranean areas of the country (EVITAR network, Tenorio, A., personal communication, 2003).

France

The first reported case of TOSV infection acquired in France was in a German traveler returning from southern France.[26] Five of 205 CSF samples retrospectively tested for TOSV RNA were positive and corresponded to patients living in Marseille city in southeastern France with no history of travel abroad. Sequence analysis indicated that these strains were most closely related to TOSV circulating in Italy.[15] A total of 13/72 serum samples collected from April to September of 2003 in patients admitted for meningitis contained specific IgM using an ELISA test.[22] Twelve percent (n = 11) of 92 healthy blood donors from Marseille were positive for specific IgG.[22] Similar prevalence rates were reported later.[11]

During the national surveillance for West Nile virus in southern France, many cases of acute TOSV infections were diagnosed by the National Reference center for Arboviruses and the virology laboratory of the Public Hospitals of Marseille. Together these findings indicate that strains of TOSV belonging to both the Italian and Spanish genotypes are co-circulating in the region, and both cause human infections.[15,37,52] Together, these data confirm that TOSV circulates in southeastern France and ranks third as etiologic agent of aseptic meningitis.

*References 19, 24, 35, 41, 54, 70, 74.

Cyprus

Several studies were conducted in Swedish United Nations soldiers based in Cyprus in 1985. Blood samples were obtained from the 362-soldier battalion just before and immediately after their 6-month tour of duty. Of 298 serum pairs available, seroconversion for TOSV was observed in a single case without any clinical manifestations.[31] Seroprevalence studies showed that 20 percent (96/479) of the healthy population of the island had TOSV IgG.[30]

Greece

Phleboviruses are present in Greece.[64] Results of a study showed that the prevalence of neutralizing antibodies for Sandfly fever Naples virus and Sandfly fever Sicilian virus among Athens residents older than 30 years of age was 36 percent and 13 percent, respectively, whereas 4 percent of younger persons had Sandfly fever Naples virus antibodies and none had antibodies to Sandfly fever Sicilian virus.[66] These differences were the result of the insecticide-spraying malaria eradication campaigns.[64] A phlebovirus, strain Corfu (Pa Ar 814), was isolated in 1981, on Corfu island from *Phlebotomus major*.[56] Using spot slides with Vero E-6 cells infected with this strain, researchers found a seroprevalence of 4 percent among healthy farmers, woodcutters, and shepherds.[4] Studies of populations living on two Ionian Islands and in northern Greece showed a seroprevalence of 11 and 48 percent, respectively.[3,49]

Portugal

One imported case of TOSV was reported in Portugal, in a man who presented with severe headache and fever without neck stiffness. Viral isolation was successful, and identification was performed by plaque neutralization.[28] In addition, one German patient returning from vacation in Portugal developed meningitis; the diagnosis was established by ELISA and confirmed by immunoblot assay.[62]

A recent seroepidemiologic study, performed with IFA and ELISA, showed that TOSV IgG was found in 2 percent of blood donors (n = 150), in 1.3 percent of sera from symptomatic individuals with laboratory diagnostic request for vector-borne viruses without neurologic signs, and in 4.2 percent of serum samples from symptomatic individuals with laboratory diagnostic request for vector-borne viruses with neurologic signs. Interestingly, only 5/29 serum samples positive through IFA or ELISA were confirmed by plaque reduction neutralization assay with the Italian strain of TOSV. This suggests that phleboviruses other than TOSV may circulate in Portugal and infect human populations.[2]

In the north of Portugal, of 106 CSF samples collected between May and September from 2002 to 2005 in patients younger than 30 years of age who were tested for TOSV RNA; six were positive, and all had a benign course and self-limited disease.[58]

Germany

In a seroepidemiologic survey of 859 health care workers and medical students, anti-TOSV IgG was detected in 1.0 percent of samples by IFA and in 0.7 percent by ELISA. In 2034 German patients hospitalized for various diseases, 1.6 percent tested positive for anti-TOS IgG by IFA and 0.8 percent by ELISA. Although the seroprevalence of antibodies to TOSV is low in Germany, TOSV infection should be considered in patients returning from endemic areas who complain of fever and headaches and who have symptoms of meningitis.[61]

North Africa (Tunisia, Algeria, and Morocco)

Several studies suggest that TOSV circulates in the countries located on the southern shore of the Mediterranean Sea. Present data consist of serologic studies,[5] which are prone to cross reaction that may confuse between TOSV and genetically related phleboviruses that have been recently discovered.[44,76] TOSV has been recently detected or isolated from sandflies collected in Morocco[34a] and in Tunisia.[7a]

Turkey

TOSV is present in Turkey, where it causes human infections.[33,34] Neutralizing antibodies were found in sera of blood donors from the Ankara, Konya, Eskişehir, and Zonguldak provinces. In addition, 16/102 patients with CNS infections of unknown etiology were positive by real-time reverse-transcription polymerase chain reaction (RT-PCR) for TOSV, which was subsequently isolated and demonstrated to belong to the Italian genotype.

CYCLE IN NATURE

The Vectors of Toscana Virus

TOSV was isolated first from *P. perniciosus*. Other strains have been isolated from *P. perfiliewi* but never from *P. papatasi*. TOSV also has been isolated from the brains of the bat *Pipistrellus kublii* trapped in areas where *P. perniciosus* and *P. perfiliewi* were present.[71-73] Isolation from bats has been reported for a large number of mosquito-borne arboviruses, including flaviviruses such as yellow fever virus, dengue virus, West Nile virus, and St. Louis encephalitis virus, with no clear information regarding implications for the ecologic cycle of these viruses. Transovarial transmission of TOSV in sandflies has been demonstrated in the laboratory and by viral isolation from male *Phlebotomus* species. Venereal transmission from infected males to uninfected females also has been demonstrated. *P. perniciosus* is distributed throughout the Mediterranean region as two races. The typical *P. perniciosus* race occurs in Italy, as well as in Malta, Tunisia, and Morocco. It is replaced in southern Spain by the Iberian race (with the pni mtDNA sublineage).[51] TOSV was

detected in both male and female pools of phlebotomine sandflies captured during the summer seasons of 2003 and 2004 in Granada. The most probable transmission vector for TOSV in Spain is *P. perniciosus*, because approximately 70 percent of captured individuals corresponded to this species. Detection in pools of males suggests vertical or sexual transmission of TOSV among sandflies. TOSV RNA was detected in *Sergentomyia minuta*, a sandfly known to feed on reptiles and assumed not to feed on humans; this property may prevent *S. minuta* from being a vector of human infection.[14] However, *Sergentomyia* flies have been reported to be infected with a variety of arboviruses.[14]

The Reservoir of Toscana Virus

The reservoir of TOSV is most likely the vector. Neither mammals nor birds have been recognized as a potential reservoir, although few studies have been carried out on mammals and almost none on birds. Whether humans can play a role in the virus cycle by infecting naïve sandflies is not known.

Although numerous phleboviruses have been isolated from the blood of sick persons and from wild animals, the importance of vertebrates in the maintenance of the transmission cycle of these viruses remains unclear. Transient and low-level viremia is present after phlebovirus infection in humans and in susceptible laboratory animals.[7,20,65] Moreover, a large quantity of virus must be ingested to infect sandflies.[17] It seems unlikely that a biting sandfly would often come into contact with an animal with viremia high enough to infect the insect orally. Verani and colleagues[72] examined different species of wild vertebrates (i.e., wild mouse, bank vole, stone marten, coypus, porcupine, bat, fox, and hedgehog) by serology and viral isolation; one virus strain was isolated from the brain of a bat *P. khulii*, whereas no hemagglutination-inhibition antibodies were found in sera. Recently, a study investigated the potential of domestic animals (i.e., horses, goats, pigs, cats, dogs, sheep, and cows) to act as reservoir of TOSV during the cold months. High seroprevalence rates were found in cats (60%) and dogs (48%). However, virus isolation was negative in all animals and RT-PCR detection was obtained in one goat only, suggesting that these animals do not play a significant role as reservoir of TOSV.[46]

CLINICAL MANIFESTATIONS

Asymptomatic or Minimally Symptomatic Infection

Seroprevalence studies suggest that some infections caused by TOSV are asymptomatic or minimally symptomatic. Additional studies will be necessary to evaluate the ratio of symptomatic to asymptomatic or minimally symptomatic infections.

Febrile Illness

In some cases, TOSV infection causes a self-limiting febrile illness without CNS manifestations. These patients usually are neither hospitalized nor investigated further and may account for the probable underestimation of TOSV infection rates. The proportion of this clinical form is unknown because of the lack of systematic documentation of fever of unknown origin in regions where TOSV is present.

Meningitis

After an incubation period ranging from a few days to a maximum of 2 weeks, the onset of disease is brutal (70%), with headache (100%, 1 to 5 days), fever (76% to 97%), nausea and vomiting (67% to 88%), photophobia (86%), malaise (62%), and myalgias (18%). On physical examination, stiff neck (53% to 95%), Kernig signs (87%), encephalitis (44%), focal neurologic defect (21%), seizures (12%), poor levels of consciousness (12%), paresis (1.7%), and nystagmus (5.2%) are apparent.[39,48] In most cases reported thus far, CSF samples contained more than 5 to 10 cells, with normal levels of glucose and protein. Leukocytosis (29%) or leukopenia (6%) may be observed in the blood. The mean duration of the disease is 7 days, and the outcome usually is favorable.

Other Central Nervous System Manifestations

Although TOSV infection in most cases is a mild disease with a favorable outcome, a few severe cases have been reported in the literature. Two young brothers and a sister living in Umbria experienced TOSV infection in the form of severe meningoencephalitis with stiff neck, deep coma, maculopapular rash, diffuse lymphadenopathy, hepatosplenomegaly, renal involvement, skin rash with lamellar desquamation, tendency for bleeding, and diffuse intravascular coagulopathy. CNS manifestations occurred after 3 weeks of fever. Convalescence was marked by hydrocephalus that required a ventriculoatrial shunt. The diagnosis was established by serologic means and PCR sequencing. The occurrence of such severe manifestations in relatives suggests the existence of an immune defect, although this possibility was not tested.[6] Two cases of encephalitis without meningitis were diagnosed by serology and the detection of TOSV sequences in the CSF; for both patients, the outcome was favorable, without late manifestations.[25] One case of meningitis complicated by abducens nerve palsy was reported in a German tourist returning from Italy.[59]

Other Disease Manifestations Not Involving the Central Nervous System

To date, no published data exist to suggest that TOSV could cause manifestations other than those previously mentioned. However, to our knowledge no study has investigated the potential of TOSV to cause other manifestations in humans.

DIAGNOSIS AND DIFFERENTIAL DIAGNOSIS

For diagnosis of suspected cases of TOSV, it is important to follow the clinical criteria and laboratory tests as recommended for the detection of arboviral infections.[13] A clinically compatible case of TOSV infection is defined as follows:

- Fever (≥38° C [≥100.4° F]) as reported by the patient or a health care provider, *and* absence of neuroinvasive disease, *or*
- Fever (≥38° C [≥100.4° F]) as reported by the patient or a health care provider, *and*
- Meningitis, encephalitis, acute flaccid paralysis, or other acute signs of central or peripheral neurologic dysfunction, as documented by a physician, *and*
- Absence of a more likely clinical explanation.

The laboratory tests necessary for virological documentation are as follows:

For confirmed cases:

A case that meets the above clinical criteria and one or more of the following laboratory criteria for a confirmed case:

- Isolation of virus from, or demonstration of specific viral antigen or nucleic acid in, tissue, blood, CSF, or other body fluid, *or*
- Fourfold or greater change in virus-specific quantitative antibody titers in paired sera, *or*
- Virus-specific IgM antibodies in serum with confirmatory virus-specific neutralizing antibodies in the same or a later specimen, *or*
- Virus-specific IgM antibodies in CSF and a negative result for other IgM antibodies in CSF for arboviruses endemic to the region where exposure occurred.

For probable cases:

A case that meets the above clinical criteria for neuroinvasive disease and the following laboratory criteria:

- Virus-specific IgM antibodies in CSF or serum but with no other testing.

Serology

Immunofluorescence Assay

Seroconversion and the detection of IgG or IgM can be achieved in cells infected with TOSV. However, cross reactivity exists among members of the genus *Phlebovirus* and specifically between TOSV and other viruses within the Sandfly fever Naples virus group (i.e., Naples, Tehran, Massilia, Granada, and Punique).

Enzyme-Linked Immunosorbent Assay

ELISA tests have been developed with either crude antigens or purified virus obtained from infected cells. The advantage of ELISA resides in its capacity to test a large number of specimens rapidly; however, cross reactions most likely also are observed. An ELISA test based on a recombinant nucleoprotein gene has been developed and is now available commercially from an Italian company. More recent seroprevalence studies were based on this test.[27,43] They must therefore be interpreted with caution because other antigenically related viruses (within the sandfly fever Naples virus group) may have elicited cross-reactive antibodies. The same applies to IFA. Thus, confirmation using a more specific test should be performed.

Rapid Immunochromatographic test

Recently, an immunochromatographic assay was developed for human anti-TOSV IgG or IgM detection with performances that are similar to those observed with the commercial ELISA test.[38]

Plaque Reduction Neutralization Test

PRNT is the test of choice when confirmation of the causing virus is necessary. Therefore, careful interpretation of seroprevalence data is necessary because, in most cases, these data were obtained with ELISA or IFA tests that cannot discriminate below the species (or antigenic complex) level.

Although seroconversion remains the serology gold standard for establishing the diagnosis of viral diseases, the presence of specific IgM in a single serum sample is indicative of a recent infection. This finding must be interpreted very carefully because, as demonstrated with West Nile virus and certain alphaviruses, TOSV infection may elicit specific IgM lasting for a very long time; Calisher and colleagues[12] reported 418-day persistence in a 66-year-old immunocompetent patient. TOSV serologic and molecular markers could be investigated concurrently with the screening of blood donors in the regions where the vector circulates and where human cases are reported. Long-term surveillance of TOSV-infected patients could indicate whether persistence of IgM is a common feature of TOSV infection or is rare. With this in mind, the presence of IgM in asymptomatic patients could result from an old infection instead of indicating an asymptomatic recent infection.[9] The major immune response elicited by TOSV infection is against the nucleoprotein. However, all serum samples that tested positive for IgG against TOSV also exhibited neutralizing properties.

Viral Isolation

Isolation from clinical samples can be achieved by using CSF. The clinical specimens from which TOSV was isolated were collected in the first 2 to 4 days of the disease. To date, there is no report of TOSV isolation from serum sample. TOSV replicates in a variety of animals; intracerebral, intraperitoneal, and subcutaneous routes lead to death in the newborn mouse; intracerebral and intraperitoneal routes lead to death in the weanling mouse; in the guinea pig and rabbit, intracerebral inoculation results in paralysis and irregular death, whereas intraperitoneal inoculation is not fatal and results in antibody synthesis. *Macaca fascicularis* succumbs after

intracerebral inoculation, but recovers with antibody production after intraperitoneal inoculation.[40] TOSV replicates in Vero, BHK-21, CV-1, and SW13 cells with cytopathic effect but not in C6/36 cells.[40,71] However, cell culture appears to have a low sensitivity for detecting TOSV because only 14 percent of PCR-positive CSF specimens inoculated onto Vero cells led to viral isolation.

Genome Amplification by Reverse-Transcription Polymerase Chain Reaction Assays

Three different classical PCR protocols for the diagnosis of TOSV have been developed. Studies in the molecular detection of TOSV RNA in CSF of patients with meningitis or other CNS manifestations have used classic PCR detection through single-round or nested protocols. Single-round RT-PCR protocols are not sensitive enough to detect the low number of copies present in the CSF or serum of infected patients.[68] Two of the three molecular detection systems[60,68] use specific primers for amplification of regions of the S segment, and one is based on degenerate oligonucleotides designed in the L segment.[57] The latter is a nested RT-PCR for detecting all phleboviruses. The method is a modification of the generic method used for detecting phleboviruses and is able to detect all the members of the genus, including TOSV. The replacement of one of the generic primers used in the nested reaction by one able to detect only TOSV strains renders the diagnosis of this virus more specific. This method has been used in establishing the diagnosis of Spanish cases of TOSV infection. However, these techniques are time-consuming and require sequence determination to confirm the diagnosis. One of the major drawbacks of molecular techniques, principally those requiring the manipulation of PCR tubes for gel loading and nested PCR reactions, is the risk for contamination. Real-time PCR systems, including a fluorescent dye–labeled probe, have reduced dramatically the risk for contamination because these systems rely on one-round RT-PCR assays without the need for gel loading or sequencing. The sensitivity of clinical tests for human viruses using real-time RT-PCR is close to that obtained by nested PCR protocols, and the results are obtained within 3 hours.[50,75] Therefore, such technology must now be applied to TOSV diagnosis.

GENETIC DIVERSITY OF TOSCANA VIRUS

TOSV has been isolated from sandflies of two different species, *P. perniciosus* and *P. perfiliewi;* from humans; and once from a bat *(P. khulii)*. The prototype TOSV strain, ISS Phl.3, was isolated from sandflies *P. perniciosus* in 1971. This strain has been sequenced completely and is used as reference for further genetic studies.[1,23,36] A total of 84 virus strains was obtained from 16,374 male and female sandflies (*P. perniciosus* and *P. perfiliewi*) collected in two localities of the Tuscany region of Italy between 1980 and 1985. Thirty-seven (44%) strains were identified as TOSV, and 47 (56%) were identified as a new member of the *Phlebotomus* fever serogroup, Arbia virus. The overall virus isolation rate from sandflies was 0.5 percent. Viral isolation rates for both viruses were similar in different years and in the two localities, a finding suggesting that the two virus types were active in the sandfly population simultaneously (maximum activity in July).[71] Seventeen strains of TOSV have been isolated in Spain from clinical cases.[45] Several strains have been isolated in southeastern France from clinical cases.

Many strains from Italy have been partially sequenced, and only minor differences in nucleoprotein sequences were found among strains isolated in the early 1980s from both species of sandflies, from the bat, and from humans, with no more than one amino acid substitution.[47] Similar results were described in a study on some variants in the nucleoprotein gene of strains isolated from humans in the years 1995 to 1998.[68] Studies conducted with partial sequences in the polymerase gene indicate that a distinct genotype of TOSV is present in Spain.[57] This finding suggests the presence of at least two topotypes of TOSV. Both genetic populations of TOSV circulate in France, geographically located between Italy and Spain.[15] To date, sequence data are too scarce to perform significant phylogenetic analyses. Very recently, a strain of TOSV detected in Croatia (but not isolated) appeared to belong to a third lineage.[55] However, this result needs to be confirmed by complete genome sequence analysis after virus isolation. The recent easy access to next-generation sequencing technologies will provide the opportunity to substitute studies based on short partial sequences for studies using complete genome sequences. Next-generation sequencing programs must be fueled with new TOSV strains isolated from clinical grounds and by field virus discovery programs in countries surrounding the Mediterranean, where sandfly vectors are circulating, to understand more clearly the genetic diversity, phylogenetic relationships, and mechanisms driving the evolution of TOSV.

Acknowledgments

We wish to thank Pierre Gallian, Jose-Maria Navarro-Mari, Loredana Nicoletti, Anna Papa, Maria Paz Sanchez-Seco, and Antonio Tenorio. We also thank Laurence Bichaud for the map.

NEW REFERENCES SINCE THE SIXTH EDITION

1a. Alkan C, Bichaud L, de Lamballerie X, et al. Sandfly-borne phleboviruses of Eurasia and Africa: Epidemiology, genetic diversity, geographic range, control measures. *Antiviral Res* 2013; doi:pii: S0166-3542(13)00192-7. [Epub ahead of print]

2. Amaro F, Luz T, Parreira P, Marchi A, et al. Serological evidence of Toscana virus infection in Portuguese patients. *Epidemiol Infect* 2012;**140**:1147–50.

3. Anagnostou V, Papa A. Prevalence of antibodies to phleboviruses within the sand fly fever Naples virus species in humans, northern Greece. *Clin Microbiol Infect* 2013;**19**:566–70.

5. Bahri O, Fazaa O, Ben Alaya-Bouafif N, et al. Role of Toscana virus in meningo-encephalitis in Tunisia. *Pathol Biol (Paris)* 2011;**59**: e125–7.

7a. Bichaud L, Dachraoui K, Piorkowski G, et al. Toscana virus isolated from sandflies, Tunisia. *Emerg Infect Dis* 2013;**19**:322–4.

11. Brisbarre N, Attoui H, Gallian P, et al. Seroprevalence of Toscana virus in blood donors, France, 2007. *Emerg Infect Dis* 2011;**17**: 941–3.
13. Centers for Disease Control and Prevention. *Nationally notifiable conditions: infectious and non-infectious case, 2012: case definitions*. Atlanta, Ga: 2012. (http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/arboviral_current.htm).
16. Charrel RN, Moureau G, Temmam S, et al. Massilia virus, a novel phlebovirus (Bunyaviridae) isolated from sandflies in the Mediterranean. *Vector Borne Zoonotic Dis* 2009;**9**:519–30.
18. Collao X, Palacios G, de Ory F, et al. Granada virus: a natural phlebovirus reassortant of the sandfly fever Naples serocomplex with low seroprevalence in humans. *Am J Trop Med Hyg* 2010; **83**:760–5.
19. Colomba C, Saporito L, Ciufolini MG, et al. Prevalence of Toscana sandfly fever virus antibodies in neurological patients and control subjects in Sicily. *New Microbiol* 2012;**35**:161–5.
21. Cusi MG, Savellini GG, Zanelli G. Toscana virus epidemiology: from Italy to beyond. *Open Virol J* 2010;**4**:22–8.
22. De Lamballerie X, Tolou H, Durand JP, et al. Prevalence of Toscana virus antibodies in volunteer blood donors and patients with central nervous system infections in southeastern France. *Vector Borne Zoonotic Dis* 2007;**7**:275–7.
33. Ergünay K, Aydoğan S, Ilhami Ozcebe O, et al. Toscana Virus (TOSV) exposure is confirmed in blood donors from Central, North and South/Southeast Anatolia, Turkey. *Zoonoses Public Health* 2012;**59**:148–54.
34. Ergünay K, Saygan MB, Aydoğan S, et al. Sandfly fever virus activity in central/northern Anatolia, Turkey: first report of Toscana virus infections. *Clin Microbiol Infect* 2011;**17**:575–81.
38. Houghton R, Savellini GG, Chen H, et al. Comparison of a new prototype immunochromatographic assay and a commercial enzyme-linked immunosorbent assay for the detection of serum antibodies against Toscana virus. *J Virol Methods* 2013;**187**:182–4.
39. Jaijakul S, Arias CA, Hossain M, et al. Toscana meningoencephalitis: a comparison to other viral central nervous system infections. *J Clin Virol* 2012;**55**:204–8.
41. Kay MK, Gibney KB, Riedo FX, et al. Toscana virus infection in American traveler returning from Sicily, 2009. *Emerg Infect Dis* 2010;**16**:1498–500.
44. Moureau G, Bichaud L, Salez N, et al. Molecular and serological evidence for the presence of novel phleboviruses in sandflies from northern Algeria. *Open Virol J* 2010;**4**:15–21.
46. Navarro-Marí JM, Palop-Borrás B, Pérez-Ruiz M, et al. Serosurvey study of Toscana virus in domestic animals, Granada, Spain. *Vector Borne Zoonotic Dis* 2011;**11**:583–7.
49. Papa A, Andriotis V, Tzilianos M. Prevalence of Toscana virus antibodies in residents of two Ionian islands, Greece. *Travel Med Infect Dis* 2010;**8**:302–4.
50. Pérez-Ruiz M, Collao X, Navarro-Marí JM, et al. Reverse transcription, real-time PCR assay for detection of Toscana virus. *J Clin Virol* 2007;**39**:276–81.
53. Plyusnin A, Beaty BJ, Elliott RM, et al. Genus phlebovirus. In: King AMQ, Adams MJ, Carstens EB, et al, editors. *Virus taxonomy*. Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Academic Press; 2005. p. 735–7.
55. Punda-Polić V, Mohar B, Duh D, et al. Evidence of an autochthonous Toscana virus strain in Croatia. *J Clin Virol* 2012;**55**: 4–7.
58. Santos L, Simões J, Costa R, et al. Toscana virus meningitis in Portugal, 2002-2005. *Euro Surveill* 2007;**12**:E3–4.
70. Venturi G, Madeddu G, Rezza G, et al. Detection of Toscana virus central nervous system infections in Sardinia Island, Italy. *J Clin Virol* 2007;**40**:90–1.
74. Vocale C, Bartoletti M, Rossini G, et al. Toscana virus infections in northern Italy: laboratory and clinical evaluation. *Vector Borne Zoonotic Dis* 2012;**12**:526–9.
75. Weidmann M, Sanchez-Seco MP, Sall AA, et al. Rapid detection of important human pathogenic Phleboviruses. *J Clin Virol* 2008;**41**:138–42.
76. Zhioua E, Moureau G, Chelbi I, et al. Punique virus, a novel phlebovirus, related to sandfly fever Naples virus, isolated from sandflies collected in Tunisia. *J Gen Virol* 2010;**91**:1275–83.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Accardi L, Gro MC, Di Bonito P, et al. Toscana virus genomic L segment: molecular cloning, coding strategy and aminoacid sequence in comparison with other negative strand RNA viruses. *Virus Res* 1993;**27**:119–31.

1a. Alkan C, Bichaud L, de Lamballerie X, et al. Sandfly-borne phleboviruses of Eurasia and Africa: Epidemiology, genetic diversity, geographic range, control measures. *Antiviral Res* 2013;doi: pii:S0166-3542(13)00192–7. [Epub ahead of print]

2. Amaro F, Luz T, Parreira P, et al. Serological evidence of Toscana virus infection in Portuguese patients. *Epidemiol Infect* 2012;**140**: 1147–50.

3. Anagnostou V, Papa A. Prevalence of antibodies to phleboviruses within the sand fly fever Naples virus species in humans, northern Greece. *Clin Microbiol Infect* 2013;**19**:566–70.

4. Antoniadis A, Alexiou-Daniel S, Malissiovas N, et al. Seroepidemiological survey for antibodies to arboviruses in Greece. *Arch Virol Suppl* 1990;**1**:277–85.

5. Bahri O, Fazaa O, Ben Alaya-Bouafif N, et al. Role of Toscana virus in meningo-encephalitis in Tunisia. *Pathol Biol (Paris)* 2011;**59**: e125–7.

6. Baldelli F, Ciufolini MG, Francisci D, et al. Unusual presentation of life-threatening TOSV meningoencephalitis. *Clin Infect Dis* 2004;**38**:515–20.

7. Bartelloni PJ, Tesh RB. Clinical and serologic responses of volunteers infected with phlebotomus fever virus (Sicilian type). *Am J Trop Med Hyg* 1976;**25**:456–62.

7a. Bichaud L, Dachraoui K, Piorkowski G, et al. Toscana virus isolated from sandflies, Tunisia. *Emerg Infect Dis* 2013;**19**:322–4.

8. Braito A, Ciufolini MG, Pippi L, et al. Phlebotomus-transmitted Toscana virus infections of the central nervous system: a seven-year experience in Tuscany. *Scand J Infect Dis* 1998;**30**:505–8.

9. Braito A, Corbisiero R, Corradini S, et al. Evidence of Toscana virus infections without central nervous system involvement: a serological study. *Eur J Epidemiol* 1997;**13**:761–4.

10. Braito A, Corbisiero R, Corradini S, et al. Toscana virus infections of the central nervous system in children: a report of 14 cases. *J Pediatr* 1998;**132**:144–8.

11. Brisbarre N, Attoui H, Gallian P, et al. Seroprevalence of Toscana virus in blood donors, France, 2007. *Emerg Infect Dis* 2011;**17**: 941–3.

12. Calisher CH, Weinberg A, Muth DJ, et al. Toscana virus infection in United States citizen returning from Italy. *Lancet* 1987;**17**: 165–6.

13. Centers for Disease Control and Prevention. *Nationally notifiable conditions infectious and non-infectious case, 2012: case definitions.)*. Atlanta, 2012. (http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/arboviral_current.htm).

14. Charrel RN, Izri A, Temmam S, et al. Toscana virus RNA in *Sergentomyia minuta* flies. *Emerg Infect Dis* 2005;**12**:1299–300.

15. Charrel RN, Izri A, Temmam S, et al. Co-circulation of two genotypes of Toscana virus in southeastern France. *Emerg Infect Dis* 2007;**13**:465–8.

16. Charrel RN, Moureau G, Temmam S, et al. Massilia virus, a novel phlebovirus (Bunyaviridae) isolated from sandflies in the Mediterranean. *Vector Borne Zoonotic Dis* 2009;**9**:519–30.

17. Ciufolini MG, Maroli M, Verani P. Growth of two phleboviruses after experimental infection of their suspected sand fly vector, *Phlebotomus perniciosus* (Diptera: Psychodidae). *Am J Trop Med Hyg* 1985;**34**:174–9.

18. Collao X, Palacios G, de Ory F, et al. Granada virus: a natural phlebovirus reassortant of the sandfly fever Naples serocomplex with low seroprevalence in humans. *Am J Trop Med Hyg* 2010;**83**: 760–5.

19. Colomba C, Saporito L, Ciufolini MG, et al. Prevalence of Toscana sandfly fever virus antibodies in neurological patients and control subjects in Sicily. *New Microbiol* 2012;**35**:161–5.

20. Cusi MG, Gori Savellini G, Terrosi C, et al. Development of a mouse model for the study of Toscana virus pathogenesis. *Virology* 2005;**333**:66–73.

21. Cusi MG, Savellini GG, Zanelli G. Toscana virus epidemiology: from Italy to beyond. *Open Virol J* 2010;**4**:22–8.

22. De Lamballerie X, Tolou H, Durand JP, et al. Prevalence of Toscana virus antibodies in volunteer blood donors and patients with central nervous system infections in southeastern France. *Vector Borne Zoonotic Dis* 2007;**7**:275–7.

23. Di Bonito P, Mochi S, Gro MC, et al. Organization of the M genomic segment of Toscana phlebovirus. *J Gen Virol* 1997;**78**: 77–81.

24. Di Nicuolo G, Pagliano P, Battisti S, et al. Toscana virus central nervous system infections in southern Italy. *J Clin Microbiol* 2005;**43**:6186–8.

25. Dionisio D, Valassina M, Ciufolini MG, et al. Encephalitis without meningitis due to sandfly fever virus serotype Toscana. *Clin Infect Dis* 2001;**32**:1241–3.

26. Dobler G, Treibl J, Haass A, et al. Toscana virus infection in German travellers returning from the Mediterranean. *Infection* 1997;**25**:325–8.

27. Echevarria JM, de Ory F, Guisasola ME, et al. Acute meningitis due to Toscana virus infection among patients from both the Spanish Mediterranean region and the region of Madrid. *J Clin Virol* 2003;**26**:79–84.

28. Ehrnst A, Peters CJ, Niklasson B, et al. Neurovirulent Toscana virus (a sandfly fever virus) in Swedish man after visit to Portugal. *Lancet* 1985;**25**:1212–13.

29. Eitrem R, Niklasson B, Weiland O. Sandfly fever among Swedish tourists. *Scand J Infect Dis* 1991;**23**:451–7.

30. Eitrem R, Stylianou M, Niklasson B. High prevalence rates of antibody to three sandfly fever viruses (Sicilian, Naples, and Toscana) among Cypriots. *Epidemiol Infect* 1991;**107**:685–91.

31. Eitrem R, Vene S, Niklasson B. Incidence of sand fly fever among Swedish United Nations soldiers on Cyprus during 1985. *Am J Trop Med Hyg* 1990;**43**:207–11.

32. Elliott RM, Dunn E, Simons JF, et al. Nucleotide sequence and coding strategy of the Uukuniemi virus L RNA segment. *J Gen Virol* 1992;**73**:1745–52.

33. Ergünay K, Aydoğan S, Ilhami Ozcebe O, et al. Toscana Virus (TOSV) exposure is confirmed in blood donors from Central, North and South/Southeast Anatolia, Turkey. *Zoonoses Public Health* 2012;**59**:148–54.

34. Ergünay K, Saygan MB, Aydoğan S, et al. Sandfly fever virus activity in central/northern Anatolia, Turkey: first report of Toscana virus infections. *Clin Microbiol Infect* 2011;**17**:575–81.

34a. Es-Sette N, Nourlil J, Hamdi S, et al. First detection of Toscana virus RNA from sand flies in the genus Phlebotomus (Diptera: Phlebotomidae) naturally infected in Morocco. *J Med Entomol* 2012;**49**:1507–9.

35. Francisci D, Papili R, Camanni G, et al. Evidence of Toscana virus circulation in Umbria: first report. *Eur J Epidemiol* 2003;**18**: 457–9.

36. Giorgi C, Accardi L, Nicoletti L, et al. Sequences and coding strategies of the S RNAs of Toscana and Rift Valley fever viruses compared to those of Punta Toro, Sicilian sandfly fever, and Uukuniemi viruses. *Virology* 1991;**180**:738–53.

37. Hemmersbach-Miller M, Parola P, Charrel RN, et al. Sandfly fever due to Toscana virus: an emerging infection in southern France. *Eur J Intern Med* 2004;**15**:316–17.

38. Houghton R, Savellini GG, Chen H, et al. Comparison of a new prototype immunochromatographic assay and a commercial enzyme-linked immunosorbent assay for the detection of serum antibodies against Toscana virus. *J Virol Methods* 2013;**187**: 182–4.

39. Jaijakul S, Arias CA, Hossain M, et al. Toscana meningoencephalitis: a comparison to other viral central nervous system infections. *J Clin Virol* 2012;**55**:204–8.

40. Karabatsos N. *International catalogue of arboviruses including certain other viruses of vertebrates*. 3rd ed. San Antonio: American Society of Tropical Medicine and Hygiene; 1985.

41. Kay MK, Gibney KB, Riedo FX, et al. Toscana virus infection in American traveler returning from Sicily, 2009. *Emerg Infect Dis* 2010;**16**:1498–500.

42. Liu DY, Tesh RB, Travassos Da Rosa AP, et al. Phylogenetic relationships among members of the genus *Phlebovirus (Bunyaviridae)* based on partial M segment sequence analyses. *J Gen Virol* 1993;**84**: 465–73.

43. Mendoza-Montero J, Gámez-Rueda MI, Navarro-Marí JM, et al. Infections due to sandfly fever virus serotype Toscana in Spain. *Clin Infect Dis* 1998;**27**:434–6.

44. Moureau G, Bichaud L, Salez N, et al. Molecular and serological evidence for the presence of novel phleboviruses in sandflies from northern Algeria. *Open Virol J* 2010;**4**:15–21.
45. Navarro JM, Fernández-Roldán C, Pérez-Ruiz M, et al. Meningitis by Toscana virus in Spain: description of 17 cases. *Med Clin (Barc)* 2004;**122**:420–2.
46. Navarro-Marí JM, Palop-Borrás B, Pérez-Ruiz M, et al. Serosurvey study of Toscana virus in domestic animals, Granada, Spain. *Vector Borne Zoonotic Dis* 2011;**11**:583–7.
47. Nicoletti L, Ciufolini MG, Giorni C, et al. Studies on variabilità of Toscana virus N protein. *ARBO Information Exchange* 1993.
48. Nicoletti L, Verani P, Caciolli S, et al. Central nervous system involvement during infection by Phlebovirus Toscana of residents in natural foci in central Italy (1977-1988). *Am J Trop Med Hyg* 1991;**45**:429–34.
49. Papa A, Andriotis V, Tzilianos M. Prevalence of Toscana virus antibodies in residents of two Ionian islands, Greece. *Travel Med Infect Dis* 2010;**8**:302–4.
50. Pérez-Ruiz M, Collao X, Navarro-Marí JM, et al. Reverse transcription, real-time PCR assay for detection of Toscana virus. *J Clin Virol* 2007;**39**:276–81.
51. Pesson B, Ready JS, Benabdennbi I, et al. Sandflies of the *Phlebotomus perniciosus* complex: mitochondrial introgression and a new sibling species of *P. longicuspis* in the Moroccan Rif. *Med Vet Entomol* 2004;**18**:25–37.
52. Peyrefitte CN, Devetakov I, Pastorino B, et al. Toscana virus and acute meningitis, France. *Emerg Infect Dis* 2005;**11**:778–80.
53. Plyusnin A, Beaty BJ, Elliott RM, et al. Genus phlebovirus. In: King AMQ, Adams MJ, Carstens EB, et al, editors. *Virus taxonomy.* Ninth Report of the International Committee on Taxonomy of Viruses. San Diego: Academic Press; 2005. p. 735–7.
54. Portolani M, Sabbatini AM, Beretti F, et al. Symptomatic infections by Toscana virus in the Modena province, 1999-2001. *Microbiologica* 2002;**25**:485–8.
55. Punda-Polić V, Mohar B, Duh D, et al. Evidence of an autochthonous Toscana virus strain in Croatia. *J Clin Virol* 2012;**55**:4–7.
56. Rodhain F, Madulo-Leblond G, Hannoun C, et al. Le virus Corfou: un nouveau phlebovirus isolé de phlébotomes en Grèce. *Ann Inst Pasteur Virol* 1985;**136E**:161–6.
57. Sánchez Seco MP, Echevarría JM, Hernández L, et al. Detection and identification of Toscana and other phleboviruses by RT-PCR assays with degenerated primers. *J Med Virol* 2003;**71**:140–9.
58. Santos L, Simões J, Costa R, et al. Toscana virus meningitis in Portugal, 2002-2005. *Euro Surveill* 2007;**12**:E3–4.
59. Schwarz TF, Gilch S, Jager G. Aseptic meningitis caused by sandfly fever virus, serotype Toscana. *Clin Infect Dis* 1995;**21**:669–71.
60. Schwarz TF, Jager G, Gilch S, et al. Nested RT-PCR for detection of sandfly fever virus, serotype Toscana, in clinical specimens, with confirmation by nucleotide sequence analysis. *Res Virol* 1995;**146**: 355–62.
61. Schwarz TF, Jäger S, Gilch S, et al. Serosurvey and laboratory diagnosis of imported sandfly fever virus, serotype Toscana, infection in Germany. *Epidemiol Infect* 1995;**114**:501–10.
62. Schwarz TF, Jager G, Gilch S, et al. Travel-related vector-borne virus infections in Germany. *Arch Virol Suppl* 1996;**11**:57–65.
63. Schwarz TF, Sabine G, Jäger G. Travel-related Toscana virus infection. *Lancet* 1993;**342**:803–4.
64. Tesh R, Saidi S, Javadian E, et al. Studies on the epidemiology of sandfly fever in Iran. I. Virus isolates obtained from Phlebotomus. *Am J Trop Med Hyg* 1977;**26**:282–7.
65. Tesh RB, Duboise SM. Viremia and immune response with sequential phlebovirus infections. *Am J Trop Med Hyg* 1987;**36**:662–8.
66. Tesh RB, Papaevangelou G. Effect of insecticide spraying for malaria control on the incidence of sandfly fever in Athens, Greece. *Am J Trop Med Hyg* 1977;**26**:163–6.
67. Valassina M, Cusi MG, Valensin PE. Rapid identification of Toscana virus by nested PCR during an outbreak in the Siena area of Italy. *J Clin Microbiol* 1996;**34**:2500–2.
68. Valassina M, Cuppone AM, Bianchi S, et al. Evidence of Toscana virus variants circulating in Tuscany, Italy, during the summers of 1995 to 1997. *J Clin Microbiol* 1998;**36**:2103–4.
69. Valassina M, Meacci F, Valensin PE, et al. Detection of neurotropic viruses circulating in Tuscany: the incisive role of Toscana virus. *J Med Virol* 2000;**60**:86–90.
70. Venturi G, Madeddu G, Rezza G, et al. Detection of Toscana virus central nervous system infections in Sardinia Island, Italy. *J Clin Virol* 2007;**40**:90–1.
71. Verani P, Nicoletti L, Ciufolini MG. Antigenic and biological characterization of Toscana virus, a new phlebotomus fever group virus isolated in Italy. *Acta Virol* 1984;**28**:39–47.
72. Verani P, Ciufolini MG, Caciolli S, et al. Ecology of viruses isolated from sand flies in Italy and characterized of a new Phlebovirus (Arbia virus). *Am J Trop Med Hyg* 1988;**38**:433–9.
73. Verani P, Ciufolini MG, Nicoletti L, et al. [Ecological and epidemiological studies of Toscana virus, an arbovirus isolated from Phlebotomus.] *Ann Ist Super Sanita* 1982;**18**:397–9.
74. Vocale C, Bartoletti M, Rossini G, et al. Toscana virus infections in northern Italy: laboratory and clinical evaluation. *Vector Borne Zoonotic Dis* 2012;**12**:526–9.
75. Weidmann M, Sanchez-Seco MP, Sall AA, et al. Rapid detection of important human pathogenic Phleboviruses. *J Clin Virol* 2008;**41**: 138–42.
76. Zhioua E, Moureau G, Chelbi I, et al. Punique virus, a novel phlebovirus, related to sandfly fever Naples virus, isolated from sandflies collected in Tunisia. *J Gen Virol* 2010;**91**:1275–83.

REFERENCES

1. Aspock H, Gerersdorfer T, Formayer H, Walochnik J. Sandflies and sandfly-borne infections of humans in Central Europe in the light of climate change. *Wien Klin Wochenschr* 2008;**120**(Suppl. 19-20):24–9.
2. Bartelloni PJ, Tesh RB. Clinical and serologic responses of volunteers infected with phlebotomus fever virus (Sicilian type). *Am J Trop Med Hyg* 1976;**25**:456–62.
3. Braito A, Grazia Ciufolini M, Pippi L. Phlebotomus-transmitted Toscana virus infections of the central nervous system: a seven-year experience in Tuscany. *Scand J Infect Dis* 1998;**30**:505–8.
4. Brett-Major DM, Claborn DM. Sandfly fever: what have we learned in one hundred years? *Mil Med* 2009;**174**(4):426–31.
5. Charrel RN, Gallian P, Navarro-Mari JM, et al. Emergence of Toscana virus in Europe. *Emerg Infect Dis* 2005;**11**:1657–63.
6. Crance JM, Gratier D, Guimet J, Jouan A. Inhibition of sandfly fever Sicilian virus (Phlebovirus) replication in vitro by antiviral compounds. *Res Virol* 1997;**148**(5):353–65.
7. Eitrem R, Niklasson B, Weiland O. Sandfly fever among Swedish tourists. *Scand J Infect Dis* 1991;**23**:451–7.
8. Eitrem R, Vene S, Niklasson B. ELISA for detection of IgM and IgG antibodies to sandfly fever Sicilian virus. *Res Virol* 1991;**142**: 387–94.
9. Ergunay K, Ismayilova V, Colpak IA, et al. A case of central nervous system infection due to a novel Sandfly Fever Virus (SFV) variant: Sandfly Fever Turkey Virus (SFTV). *J Clin Virol* 2012;**54**(1): 79–82.
10. Fischer D, Moeller P, Thomas SM, et al. Combining climatic projections and dispersal ability: a method for estimating the responses of sandfly vector species to climate change. *PloS Negl Trop Dis* 2011;**5**(11):e1407.
11. Gowen B, Wong M, Jung K, et al. Efficacy of favipiravir (T-705) and T-1106 pyrazine derivatives in phlebovirus disease models. *Antiviral Res* 2010;**86**(2):121–7.
12. Ibrahim SM, Aitichou M, Hardick J, et al. Detection of Crimean-Congo hemorrhagic fever, Hanta, and sandfly fever viruses by real-time RT-PCR. *Methods Mol Biol* 2011;**665**:357–68.
13. Meagan JM, Bailey CL. Rift Valley fever. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 4. Boca Raton, Fla.: CRC Press; 1989. p. 51–76.
14. Schultze D, Korte W, Rafeiner P, Niedrig M. First report of sandfly fever virus infection imported from Malta into Switzerland, October 2011. *Euro Surveill* 2012;**17**(27):20209.
15. Schwarz TF, Gilch S, Jager G. Aseptic meningitis caused by sandfly fever virus, serotype Toscana. *Clin Infect Dis* 1995;**21**:669.
16. Tesh RB. Phlebotomus fever. In: Monath TP, editor. *The arboviruses: epidemiology and ecology*, vol. 4. Boca Raton, Fla.: CRC Press; 1989. p. 15–27.
17. Tesh RB, Saidi S, Gajdamovic SJ, et al. Serological studies on the epidemiology of sandfly fever in the Old World. *Bull World Health Organ* 1976;**54**:663–74.

SUBSECTION 12
RETROVIRIDAE

CHAPTER 191

Human Retroviruses

191A ■ ONCOVIRUSES (HUMAN T-CELL LYMPHOTROPIC VIRUSES) AND LENTIVIRUSES (HUMAN IMMUNODEFICIENCY VIRUS TYPE 2)

Michael A. Tolle • Susan L. Gillespie

The discovery of human T-cell lymphotropic virus type 1 (HTLV-1) in 1980 demonstrated for the first time that humans could be infected by retroviruses, which previously were known as animal pathogens that could cause malignant disease.[414] HTLV-1 subsequently was found to cause adult T-cell leukemia/lymphoma (ATLL) and now is recognized to be associated with the following spectrum of disease manifestations: a chronic degenerative neurologic disease (HTLV-1–associated myelopathy [HAM]/tropical spastic paraparesis [TSP]); relapsing, severe generalized dermatitis in children (infective dermatitis); and numerous inflammatory or autoimmune conditions, or both, such as uveitis, arthropathy, polymyositis, bronchoalveolitis, and Sjögren syndrome.[428] In 1982, a second antigenically related retrovirus of the oncovirus subfamily, HTLV-2, was identified from a patient with a T-cell variant of hairy-cell leukemia.[246] The pathogenicity of this virus remains unclear, although the virus has been found in some individuals with $CD8^+$ lymphoproliferative diseases and neuromyopathies.[49,81,476] The more recent discovery of HTLV-3 and HTLV-4 demonstrated that diversity among these viruses is greater than previously understood.

CLASSIFICATION

Retroviruses form a large and diverse family of single-stranded RNA viruses that are unusual because they contain a diploid RNA genome that replicates by flow of genetic information through a DNA intermediate, a process known as reverse transcription. The name *retrovirus* is derived from this characteristic. This unique capability results from the presence of a virally encoded RNA-dependent DNA polymerase, reverse transcriptase, which catalyzes transcription of viral RNA into a double-stranded DNA copy. This viral DNA intermediary becomes integrated into host-cell DNA, where it then resides as a provirus; this process occurs by a specialized recombination mechanism requiring another viral protein, integrase. This capacity for genomic integration correlates with the capability of retroviruses to cause lifelong infection, evade the usual mechanisms of immune clearance, and produce chronic diseases in the host that manifest only after a long asymptomatic period that may last years to decades. Retroviruses infect both animals and humans.

Retroviruses can be categorized as being either exogenous or endogenous. Exogenous retroviruses such as HIV and HTLV are transmitted between individuals and across species, whereas endogenous retroviruses have become part of the genetic material of their host species through the chromosomal integration of viral DNA in the host germ cells, which then allows for vertical transmission of viral genetic material and fixation in the host population. Through whole-genome sequencing, it is estimated that 1% of the human genome consists of human endogenous retrovirus sequences resulting from infections millions of years ago.

The classification of retroviruses has been complicated by the ongoing discovery of new viruses and elucidation of their genetic organization and replication. At one time, retroviruses were divided historically into three subfamilies on the basis of nucleotide sequences and genetic organization. The subfamilies were the Oncovirinae (oncogenic or transforming viruses, which include HTLV), the Lentivirinae (slow viruses with cytopathic effects, which include human immunodeficiency virus types 1 [HIV-1] and 2 [HIV-2]), and the Spumavirinae (foamy viruses). Retroviruses have been also classified according to morphologic and biological criteria based on the observance of retroviral particles in infected

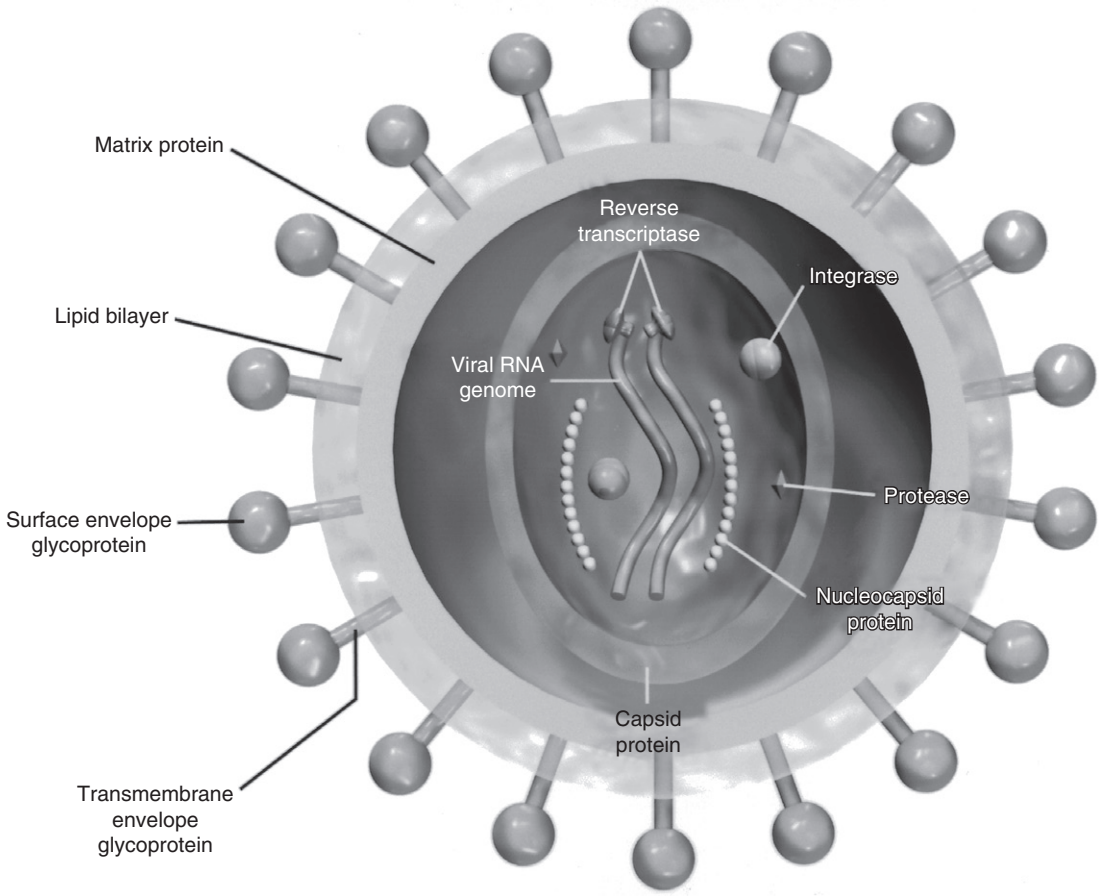

FIGURE 191A-1 ■ Retroviral structure. The mature retroviral virion is spherical; the central viral core is spherical in human T-cell lymphotropic virus oncoviruses and cylindric in human immunodeficiency virus lentiviruses. The core is surrounded by a lipid bilayer envelope derived from the host-cell membrane during bussing, the surface projections consisting of the viral surface and transmembrane envelope proteins. The viral matrix protein surrounds the virion core and is associated with the viral transmembrane envelope glycoproteins. The virion core is a structural shell composed of the viral capsid proteins. Within the shell are two copies of single-stranded viral RNA and multiple copies of the virally encoded reverse transcriptase, protease, and integrase enzymes. The viral nucleocapsid protein is bound to the RNA copies and may serve to condense the viral RNA into the capsid shell during virion assembly.

cells. Currently, human retroviruses have been broadly classified in to five groups, three of which include only endogenous viruses (betaretroviruses, spumaviruses, and gammaretroviruses) and two groups for exogenous viruses (lentiviruses [e.g., HIV]and deltaretroviruses [e.g., HTLV]).

MORPHOLOGY AND GENOMIC STRUCTURE

Retroviruses have a distinct morphology. They are enveloped RNA viruses that have diameters of 80 to 120 nm, a thin, electron-dense outer envelope, and an electron-dense core that is either spherical (HTLV) or cylindrical (HIV). The envelope of all retroviruses comprises a lipid bilayer derived from the host-cell plasma membrane during budding of the virus from the cell surface, with surface projections consisting of the viral envelope proteins (Fig. 191A-1). The retroviral core protein encloses a ribonucleoprotein of genomic RNA complexed with viral reverse transcriptase and integrase.[95] The genome is a messenger-sense, linear, single-stranded RNA comprising two identical subunits held together by hydrogen bonds at their 5′ ends. The 5′ and 3′ ends of the RNA contain repeated sequences that give rise to elements in viral DNA called long terminal repeats (LTRs).

The LTRs are composed of nucleic acid sequences that provide signals critical for structural transformation of the viral genome, including initiation and progression of reverse transcription of viral RNA into DNA, integration of viral DNA into the host genome by viral integrase, and initiation of viral mRNA transcription from the integrated provirus and binding sites for viral and cellular proteins and that positively and negatively influence mRNA transcription.[432,503,528,569] Between the LTRs are the genes that encode the major structural proteins of the virus, the enzymes found in the viral particles, and additional proteins with specialized intracellular functions.

As noted earlier, all retroviruses contain a minimum of three genes: *gag*, *pol*, and *env*. They are arranged in a 5′ to 3′ order, with LTRs at each end (Fig. 191A-2). The *gag* gene encodes the structural protein products that form the core particle of the virus, including

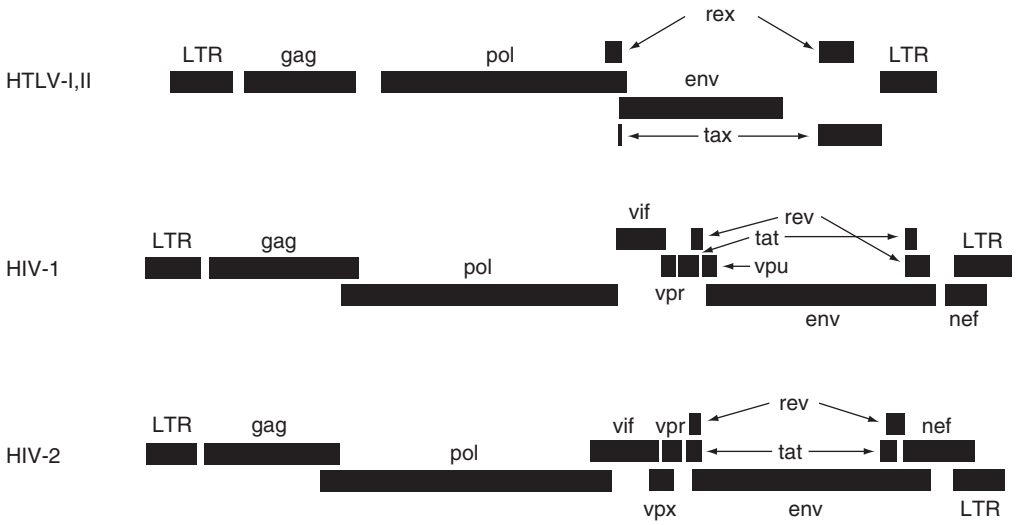

FIGURE 191A-2 ■ Retroviral genome. This schematic depiction of the human T-cell lymphotropic virus (HTLV) and human immunodeficiency virus (HIV) genomes shows that HTLV has a more complex genome than most animal retroviruses do and that HIV, in turn, has additional regulatory genes. Details of the functions of these genes are summarized in the text. *LTR,* Long terminal repeat. (Reprinted by permission from Gallo RC, Wong-Staal F, Montagnier L, et al. HIV/HTLV gene nomenclature. *Nature* 1988;333:504. Copyright Macmillan Magazines, Ltd.)

nucleocapsid, capsid, and matrix proteins. The *pol* gene products include the enzymes required for genome replication (viral RNA-dependent DNA polymerase and ribonuclease H), proviral integration (integrase), and polyprotein processing (protease). The *env* gene encodes the major components of the viral coat, the surface and transmembrane glycoproteins. Retroviral genes generally are expressed first as large overlapping polyproteins that undergo processing into functional peptide products by viral or cellular proteases.

Oncoviral Regulatory and Accessory Genes

In addition to the standard retroviral genes, the oncoviruses HTLV-1 and HTLV-2 contain several regulatory and accessory genes important for viral replication and activation of host genes that are encoded in open reading frames (ORFs) in a unique region at the 3′ end of the genome called the pX region.[5,228] The HTLV-1 pX region contains four ORFs, whereas HTLV-2 contains five ORFs. Two of these ORFs common to both viruses (ORF III and ORF IV) encode the regulatory proteins Rex and Tax.

The regulator of expression of virion proteins gene *(rex)* encodes a nucleolus-localizing phosphoprotein that affects mRNA splicing and export from the nucleus to the cytoplasm. For cellular genes, mRNA splicing and export are tightly coupled. After cellular genes are transcribed into mRNA, splicing occurs in the cell nucleus before mRNA can be exported into the cytoplasm for translation into proteins. Incompletely spliced mRNA molecules are retained in the nucleus.[336,503] However, for retroviral reproduction, the export of full-length, unspliced viral RNA into the cytoplasm is required to serve as genomic RNA for integration into progeny virions and mRNA for the production of viral structural proteins. Thus, retroviruses had to develop a mechanism to bypass the cellular regulatory process; for HTLV, the Rex protein serves this function. This protein is expressed predominantly at an early stage of viral gene expression and enhances the transport of single-spliced and unspliced viral mRNA coding for structural proteins, as well as transport of the viral genome from the nucleus to the cytoplasm. The Rex protein also exerts a negative effect on the transport of multiply spliced viral mRNA to the cytoplasm. These multiply spliced mRNA molecules code for regulatory proteins, including Rex and Tax.[528]

The transactivator gene *(tax)* encodes a nuclear protein that plays a critical role in the regulation of viral replication and also stimulates a large number of cellular genes involved in activation and proliferation of T cells, including lymphokines, lymphokine receptors, and nuclear proto-oncogenes.[582,583] The Tax protein does not bind directly to DNA but rather interacts with host-cell transcriptional factors to produce a multifaceted array of molecular effects.[42] The Tax protein activates mRNA transcription by binding to several different host-cell transcription-enhancing factors, including those of the nuclear factor (NF)-κB family. In addition to activating transcription of the HTLV genome, the Tax transcription factor complex stimulates transcription of many cellular genes, including those encoding interleukin (IL)-2, IL-2 receptor-α, tumor necrosis factor (TNF), cyclooxygenase 2 (a prostaglandin synthetase), and some nuclear oncogenes.[350,582,583] Tax also represses transcription of tumor suppression genes and the expression of cellular DNA polymerase-β, a key enzyme for repair of damaged DNA. Additionally, Tax can bind directly to and inhibit some cell cycle regulation and tumor suppressor proteins, thereby interfering with cell cycle regulation and resulting in abnormal promotion of the cell cycle and enhancement of cellular proliferation.[583] Finally, Tax increases the genetic instability of the cell by impairing cellular DNA repair mechanisms, a process that leads to an accumulation of mutations and an increase in chromosomal

anomalies. In vitro, Tax protein can immortalize human T cells and induce tumors in transgenic mice. However, in vivo, malignant disease develops in fewer than 5 percent of HTLV-1–infected individuals. Thus, although the *tax* gene probably is involved in malignant transformation of infected T cells, it is not sufficient by itself to explain the final development of malignancy.[8,533,582,583]

Less is known about the function of the HTLV accessory proteins that are encoded by the remaining ORFs in the pX region (e.g., p12, p13, and p30 in HTLV-1; and p10, p11, p22/20, and p28 in HTLV-2). The best characterized accessory protein is HTLV-1 p12 from ORF I, which is a conserved, hydrophobic protein localized to the internal cell plasma membrane and perinuclear regions of the cell.[88] The p12 protein contains amino acid motifs commonly found in proteins involved in intracellular signaling pathways and probably interacts with cellular proteins, perhaps cellular kinases, to modulate intracellular signaling in infected cells. The protein also interacts with the β and γ chains of the IL-2 receptor. This protein may be required for activation of host cells during the early stages of infection, for cellular transformation, and for efficient viral infectivity, similar to the function of the *nef* gene of the lentivirus HIV-1.[3] The corresponding ORF I protein in HTLV-2, p10, does not bind the IL-2 receptor and is not associated with the constitutive activation of the IL-2 signaling pathway that is seen with HTLV-1–transformed cells.[88] These differences may be associated with differences in pathogenicity between the two oncoviruses.

Lentiviral Regulatory and Accessory Genes

More detailed data are available about the function of lentiviral genes.[141] The lentiviruses HIV-1 and HIV-2 have more complex genomes containing at least six regulatory genes in addition to *env*, *gag*, and *pol*. The lentiviral genes corresponding to the oncoviral *tax* and *rex* are called *tat* and *rev*; these genes are found in all known human and animal lentiviruses and appear to be essential for replication.[336]

Like the oncoviral *rex* gene, *rev* is expressed early in the viral replication cycle and undergoes splicing in the nucleus; multiply spliced *rev* mRNA transcripts then are exported to produce Rev protein in the cytoplasm. This protein contains a nuclear localization signal that facilitates entry of the protein back into the nucleus. Rev then binds to unspliced viral mRNA at a *cis*-acting Rev response element (RRE) located on the mRNA. A nuclear export signal sequence on the carboxy-terminal domain of Rev next targets the unspliced viral mRNA for export to the cytoplasm by binding to the cellular protein CRM1 (exportin 1) and exiting the nucleus through the host-cell export pathway.[106] The full-length unspliced viral mRNA then serves as a translational template for expression of the Gag and Pol proteins and also serves as genomic RNA for incorporation into new virions.

In contrast to the oncoviral Tax protein, which binds to host-cell proteins and has multiple effects in addition to promoting initiation of HTLV gene transcription, the lentiviral Tat protein binds to an RNA target, the Tat activation region (TAR), located immediately 3′ to the LTR transcription start site, and plays a primary role in the expression of HIV viral transcripts by enhancing mRNA elongation. At least two host-cell cofactors are involved in this process.[586] Cellular cyclin T binds to the activation domain of Tat, which increases both the affinity and specificity of the resulting complex for TAR. A host-cell encoded kinase then is recruited to the Tat-cyclin T-TAR complex and phosphorylates the carboxyl-terminal domain of the host-cell RNA polymerase II, which in turn enhances mRNA transcript elongation.

The remaining lentiviral accessory regulatory genes *nef*, *vpr*, and *vif* provide fine-tuning by enhancing or, less commonly, by diminishing viral replication. Another accessory gene, *vpu*, is found only in HIV-1, whereas HIV-2 and simian immunodeficiency virus lack the *vpu* gene but contain a gene called *vpx*. The *nef* gene, like *tat* and *rev*, is expressed early in the replication cycle. Similar to the HTLV-1 p12 protein, *nef* encodes a hydrophobic, membrane-associated protein that probably interacts with and modulates cellular signaling pathways and is required for optimal infectivity in vivo and for activation of infected host cells. Nef protein significantly enhances the cytoplasmic delivery of viral particles entering the cell through fusion at the plasma membrane, possibly by enhancing phosphorylation of the viral matrix protein and thereby allowing dissociation of the matrix and associated preintegration nucleoprotein complex from the virion capsid proteins.[459,503] Nef also downregulates cell surface expression of $CD4^+$ by accelerating $CD4^+$ receptor endocytosis through interaction with the cytoplasmic tail of $CD4^+$ and a protein complex (i.e., AP-2 adaptor complex) responsible for recruiting membrane-associated proteins to clathrin-coated pits for endocytosis.[141] This process reduces the potential interference of membrane-associated $CD4^+$ with the release of budding virions. Major histocompatibility complex (MHC) class I receptor expression also is downregulated by Nef.

The Vpr protein is present in the virion itself, may act to regulate cellular events after penetration and uncoating of the virus to facilitate viral replication, and is involved in efficient localization of the nucleoprotein preintegration complex into the nucleus.[95,208] Vpr appears to interact with a specific site on nucleoporins in the nuclear pore complex to facilitate nuclear entry.[503] Similar to the Vpr protein, the HIV-2 Vpx protein is found in the virion and appears to affect the efficiency of early replication events, but the precise mechanism is unknown.[95,288]

The virion infectivity factor *(vif)* accessory gene is expressed late, along with structural and enzymatic proteins. The Vif protein is predominantly cytoplasmic but also exists in a membrane-associated form.[336] Vif protein is phosphorylated by a host-cell protein kinase, which seems to be important for viral growth in primary cells.[196] Vif appears to stabilize the preintegration provirus; viral particles that are produced in the absence of Vif are incapable of incorporating the provirus into host-cell chromosomes.[336] Vif also may function to prevent premature processing of Gag precursor proteins by viral protease in the cytoplasm, thereby ensuring that the Gag-derived peptides that form the viral nucleoprotein core

are available at the plasma membrane for assembly with other viral components.

The HIV-1 *vpu* gene also is expressed late. The Vpu protein is associated with the endoplasmic reticulum in infected cells, where it binds the $CD4^+$ molecule and prevents it from translocating to the plasma membrane; instead, the $CD4^+$ molecule is targeted for proteolysis through the cytoplasmic ubiquitin-proteasome pathway.[141,503] This process prevents trapping and subsequent degradation of HIV envelope proteins by $CD4^+$ in the cytoplasm, thus increasing the ability of the viral envelope proteins to reach the cell membrane. Vpu, like Nef, also downregulates MHC class I receptor expression. In addition, Vpu may be important in virion assembly and release of particles.[90,174,552]

VIRAL REPLICATION

The lentiviruses can infect and replicate in nondividing, terminally differentiated cells, and transmission can occur by cell-free or cell-associated virus. HIV-1 has high replication rates in vivo, with an average of 10^9 to 10^{10} viral particles produced each day. Free viral particles are estimated to have a half-life of less than 6 hours, and productively infected cells have a half-life of approximately 1 day.[225,380,557] The relentless rounds of reverse transcription rapidly generate extensive viral genetic variation within a single individual that results in a genetically related "swarm," or quasi-species, of viruses and provides the ability to escape the host immune response rapidly.

In contrast, the oncoviruses require that host cells undergo division for productive infection to be established, and they replicate in vivo largely by mitosis rather than by reverse transcription.[315] In addition, transmission is predominantly, if not solely, accomplished by means of cell-cell contact.[123] The events that occur during primary HTLV infection are reverse transcription of the viral genome, integration of the provirus into DNA, transcription of mRNA, synthesis of viral proteins, and a burst of viral expression. However, after a period of active replication, viral persistence is facilitated by a prolonged phase of clonal proviral expansion produced by transactivation of host-cell genes by Tax, which results in cellular DNA replication and the production of new cells containing the HTLV proviral genome.[353] Unlike viral nucleic acid replication by the error-prone viral reverse transcriptase, cellular mitosis is much less prone to mutation and generates little viral genetic variation. Moreover, the provirus remains hidden from immune surveillance. Thus, oncoviruses have extraordinary genetic stability in contrast to lentiviruses. The genetic variability of the HIV-1 envelope protein that exists within the viral quasi-species in a single individual 5 years after infection is greater than the genetic variability found in all HTLV-1 proteins identified worldwide to date.[556]

These differences are related to divergence among the retroviral subfamilies in genetic organization, transcription, and function of the major regulatory genes (*tax* and *rex* for the HTLVs and *tat* and *rev* for HIVs). The Tax and Tat proteins both are transactivating enhancer proteins. However, the HTLV Tax protein interacts with cellular proteins and activates cellular genes, thereby resulting in disruption of the cell cycle and induction of cell proliferation. In contrast, the HIV Tat protein has more focal effects; it recognizes a small RNA domain in the HIV LTR and serves primarily to transactivate HIV replication. The Rex and Rev proteins have similar functions; both interact with genomic mRNA structures (the Rex responsive element and Rev responsive element) to enhance transport of the unspliced and partly spliced viral mRNA molecules from the nucleus to the cytoplasm, and both also downregulate the transport of multiply spliced mRNA transcripts that encode for early regulatory proteins, including the mRNA molecules coding for themselves. In the oncoviruses, Tax and Rex are derived from the same mRNA, and, therefore, downregulation of Rex mRNA transport results in decreased Tax mRNA transport and protein production. This process leads to diminished transactivation of HTLV genome transcription and prevents massive viral production. In contrast, the HIV Tat protein comes in two functionally equivalent forms, a 72- and an 86-residue protein. The 72-residue Tat protein is derived from a joint Rev/Tat mRNA, but the 86-residue Tat protein comes from a small Rev-independent mRNA. Although Rev, like Rex, has a negative effect on its own expression, as well as that of Tat, this effect is mitigated by the expression of a Tat mRNA that is not dependent on expression of Rev mRNA, thereby resulting in continued production of the Tat transactivating protein and constant and massive transcription of the HIV genome.

Retroviral Life Cycle

All retroviruses have two phases in their life cycle: an infection phase (including viral attachment, entry, reverse transcription, and proviral integration) and an expression phase (including transcription, translation, assembly, and budding of the virion) (Fig. 191A-3).[95] In general, more detail is known about replication in lentiviruses than in oncoviruses.

Infection Phase of Retroviral Replication

Retroviruses attach to cells through recognition and binding of viral outer surface envelope proteins to specific proteins on the surface of the host cell; such cell surface receptor specificity probably accounts for the species and cellular tropism of retroviruses.[243,376] The surface glycoprotein of the retroviral envelope has three structural and functional domains separated by conserved hinges and is noncovalently linked to the transmembrane protein by disulfide bonds. The envelope transmembrane protein anchors the entire envelope glycoprotein complex on the virion surface and is responsible for the fusogenic capacity of the viral envelope. The transmembrane protein of all retroviruses has several conserved motifs, including a "leucine zipper" coiled motif followed by the N-terminal hydrophobic "fusion peptide" responsible for fusion with the host-cell membrane. After binding of the viral surface protein to the host-cell receptor occurs, conformational changes permit correct exposure of the transmembrane fusion peptide to the host-cell

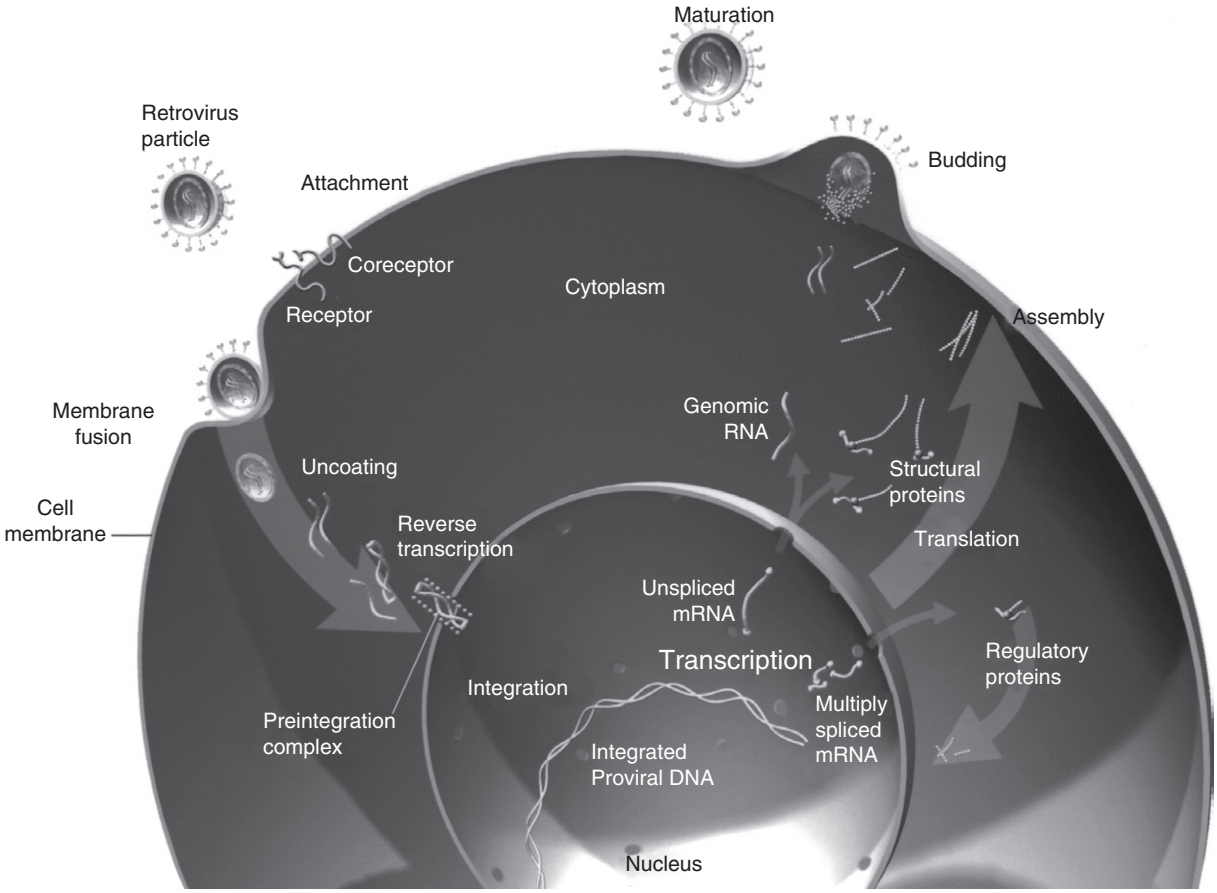

FIGURE 191A-3 ■ **Life cycle of retroviruses. Details of viral replication are discussed in the text.** (Modified from Furtado MR, Callaway DS, Phair JP, et al. Persistence of HIV-1 transcription in peripheral blood mononuclear cells in patients receiving potent antiretroviral therapy. *N Engl J Med* 1999;340:1620.)

membrane. The interaction of several fusion peptides may serve to destabilize the lipid bilayer of the target-cell membrane by forming a "fusion pore" between the two bilayers[77] to facilitate fusion of the viral and cellular membranes and release of the viral RNA–protein complex into the cytoplasm.[193,488,533]

The cell surface receptor for HTLV-1 and HTLV-2 has not been characterized completely. Oncoviruses transmit infection primarily by fusion of infected cells with uninfected cells and the subsequent formation of syncytia. In HTLV-1, fusion is thought to be mediated by the HTLV envelope proteins expressed on the surface of infected cells and by cell adhesion molecules, such as vascular cell adhesion molecule type 1 (VCAM-1), intracellular adhesion molecule type 1 (ICAM-1), ICAM-3, and the membrane permease CD98, on target-cell surfaces.[42] Although HTLV-1 envelope–expressing infected cells can form syncytia with cells from most cell lines, in vivo, HTLV-1 is found only in $CD4^+$ lymphocytes.[123] In contrast, HTLV-2 displays a preferential tropism for cells of the $CD8^+$ T-lymphocyte phenotype.[237,279]

The lentiviruses HIV-1 and HIV-2 infect cells that express $CD4^+$, the human leukocyte antigen (HLA) class II receptor, on their cell surface.[376] These cells include $CD4^+$ T lymphocytes, cells of the monocyte and macrophage lineage, and microglia in the brain. A high-affinity interaction between the surface glycoprotein and the $CD4^+$ receptor produces a conformational change that results in exposure of another site with high affinity for a secondary coreceptor required for the entry of HIV-1 and some HIV-2 isolates into the cell. The chemokine receptor CCR5 is the coreceptor used by monocyte-tropic and macrophage-tropic HIV-1, whereas the CXCR4 chemokine receptor is used by T-lymphocyte–tropic HIV-1; although most HIV-1 strains use only one receptor, some strains of HIV-1 are dually tropic and can use both receptors.[123] Additional coreceptors may support the entry of more restricted types of HIV isolates. For example, the chemokine receptor CCR3 is used by some macrophage-tropic HIV-1 strains and mediates entry of the virus into microglia; CCR2b, CR8, STLR/STRL-22, GPR15, GPR1, and the cytomegalovirus protein US28 also support entry by various HIV-1 and HIV-2 strains.[201,503] Whereas nearly all strains of HIV-2 can use $CD4^+$ together with the chemokine receptor CCR5 or CXCR4 (or both), most HIV-2 isolates also can interact with multiple other coreceptors.[201,503] Additionally, some HIV-2 isolates can use CXCR4 or other receptors, such as CCR3, as a primary receptor and infect cells lacking the $CD4^+$ molecule.[142]

Early postentry events include uncoating of the virus, which appears to require interaction of viral structural proteins with host-cell components that may have been incorporated into the virion during viral assembly. In

HIV-1, interaction of the viral capsid protein with the host-cell cyclophilin A that is associated with the virion core during viral assembly may destabilize the multimeric capsid complex.[295,503] Additionally, a virion-associated host-cell protein kinase phosphorylates the viral matrix protein and thereby allows dissociation of the capsid and nucleocapsid proteins from the rest of the virus at the cell membrane. The HIV-1 Vif protein, which colocalizes with the Gag-derived proteins, is thought to confer enhanced stability to the remaining cytoplasmic viral nucleoprotein complex.

Retroviral RNA-to-DNA transcriptional events are initiated in the cytoplasm through the action of the viral enzymes reverse transcriptase (RNA-directed DNA polymerase) and ribonuclease. Viral single-stranded RNA is transcribed into a viral RNA/DNA hybrid complex by the DNA polymerase; the ribonuclease then destroys the original RNA and permits the DNA polymerase to complete transcription of a second DNA strand to form a linear double-stranded DNA copy of the original viral RNA.[193] Retroviral integration is mediated by a large nucleoprotein complex that forms in the cytoplasm and is called the *preintegration complex*. This complex includes the viral nucleic acids and a subset of viral proteins, including the matrix protein, reverse transcriptase, integrase, and, in the lentiviruses, Vpr.[503] The viral integrase enzyme component of this complex recognizes specific sequences within the viral LTR and cleaves two nucleotides from the 3′ ends as an initial processing step for integration; this process occurs while the preintegration complex is still in the cytoplasm.[18] Integrase appears to be the principal viral determinant of integration specificity.[290] In the lentiviruses, which can infect nondividing cells, the preintegration complex is imported actively into the nucleus during interphase. Nuclear transport is mediated by nuclear localization sequences on the matrix protein and on integrase and by interactions of Vpr with nucleoporins in the nuclear pore complex.

Once nuclear entry has been gained, the integrase joins the recessed 3′ ends to phosphates in the host DNA for insertion of the DNA duplex into the host genome.[18,98] A final step in integration is thought to involve cellular factors that participate in DNA repair, in particular, a DNA-dependent protein kinase. This kinase is hypothesized to repair gaps left by the integrase around the sequence inserted into the host DNA; the result is a fully integrated provirus.[111]

Expression Phase of Retroviral Replication

Once viral DNA is integrated into the host cell, the proviral genome resembles other cellular genes and becomes highly dependent on the host cell for further replication, with the host-cell machinery used for replication, expression, and production of protein. Regulation of transcription is a complex process requiring interaction among the integrated proviral LTRs, host-cell DNA transcription factors, and the lentiviral Tat protein or oncoviral Tax protein.

The early phase of viral gene expression is characterized by the presence of spliced and unspliced viral mRNA in the nucleus, but only multiply spliced mRNA in the cytoplasm. In HTLVs, these mRNA molecules serve to direct production of the Tax and Rex regulatory proteins; in HIVs, they direct production of the Tat, Rev, and Nef proteins. The transition from early regulatory to late structural gene expression is characterized by the selective transport of unspliced and partially spliced mRNA into the cytoplasm. As discussed previously, in lentiviruses, this process depends on sufficient amounts of the Rev protein and, in oncoviruses, the Rex protein.[95,533] The unspliced or partially spiced mRNA transcripts that enter the cytoplasm encode for structural proteins and are first expressed as large overlapping polyproteins. These polyproteins undergo processing into functional peptide viral structural components by the action of virally encoded and host-cell protease enzymes.

The retroviral Env protein is synthesized as a large precursor protein; it undergoes initial glycosylation in the endoplasmic reticulum, with subsequent disulfide bonding, folding, and oligomerization, and then is transported to the Golgi complex, where it is cleaved by a host-cell protease into surface and transmembrane proteins, which undergo further glycosylation.[123] The two mature glycoproteins are noncovalently associated through disulfide bonds. After cleavage occurs, the complexed proteins are transported and inserted into the plasma cell membrane by the cellular secretory pathway.[155]

The retroviral *gag* and *pol* genes are transcribed into a single mRNA transcript, which subsequently is translated into two separate polyprotein precursors, the Gag and Gag-Pol precursor proteins; the Gag-Pol polyprotein is translated by a ribosomal frame shift between the Gag and Pol mRNA reading frames. The Pol component of the Gag-Pol precursor protein is cleaved to form the viral enzymes reverse transcriptase, ribonuclease, and protease. The Gag precursor encodes the major structural proteins of the virion core, which do not undergo proteolytic processing into mature proteins until after assembly of the immature virion at the plasma membrane occurs.

Virion assembly and budding occur through targeting, accumulation, and association of different domains of the Gag and Gag-Pol precursor proteins at the inner face of the plasma membrane where the envelope proteins are being expressed.[155,156,550] The M domain, an amino acid sequence contained in the N-terminal area of the matrix portion of the Gag protein, has a membrane-targeting sequence that directs the Gag and Gag-Pol polyproteins to the cytoplasmic face of the plasma membrane. Myristylation of the N-terminal portion of some (lentiviruses) or all (oncoviruses) of the Gag precursor protein matrix domain is required for membrane targeting to occur and to allow Gag protein assembly at the membrane. In lentiviruses, the matrix domain interacts with the long cytoplasmic tail of the viral envelope transmembrane protein inserted into the membrane of the cell.[156] The I domain, an amino acid sequence contained in the nucleocapsid portion of the Gag polyprotein, participates in the Gag–Gag interactions to promote protein polymerization and also appears to be required for incorporation of the Gag-Pol precursor into virions.[155] The nucleocapsid domain of the Gag polyprotein interacts with viral

genomic RNA through specific (sequence-specific nucleic acid binding) and nonspecific (interaction of basic residues with RNA) mechanisms and plays an important role in RNA binding, dimerization, and encapsidation and in facilitating Gag protein interactions and packaging into tight complexes.[93,155]

The interactions of these three domains in the precursor protein thus lead to assembly and emergence (but not release) of the viral "bud" from the cell surface.[402] On electron microscopy, large, electron-dense patches of Gag multimers, visualized under the plasma membrane, deform the membrane outward as they grow. More advanced intermediates appear as spheres connected to the cell by a thin stalk. During the process of budding, substantial amounts of cellular surface antigens, such as β_2-microglobulin and human leukocyte antigen (HLA)-DR, are incorporated into the viral envelope. Additionally, during the process of virion assembly, host-cell proteins may be incorporated into the virion. For example, in HIV-1, a host-cell protein, cyclophilin A, interacts with the Gag polyprotein to form a complex in the virion core.[154,510] This host factor appears to be required for the formation of infectious virions, and it may play a role in early events after viral entry, as discussed earlier.[503]

In addition to the capsid, nucleocapsid, and matrix protein domains, other polypeptide segments that are required during the late stages of viral assembly are contained in the Gag polyprotein. They are referred to collectively as "late assembly" or "L" domains, and they are found in both lentiviruses and oncoviruses. In lentiviruses, the C-terminal portion of Gag (p6) includes a highly conserved amino acid motif that is needed to recruit the cellular machinery required for efficient release of budding virus from the cell membrane.[402,464,484,550] The Gag protein of oncoviruses also contains a late domain, although it is located in the N-terminal region of the protein.[484] Some investigators have suggested that these domains engage in interactions with host proteins that are located at the plasma membrane and that facilitate final release of the budding virus from the cell.

In HIV-1, the late domain is thought to interact with the cellular protein ubiquitin. Ubiquitin is a small protein present in cells that, together with cellular proteasomes, is involved in collecting or destroying cellular proteins that are damaged or no longer needed; it exists in cells as a free molecule or is covalently attached to lysines in a variety of proteins. When a protein is linked to multiple ubiquitin molecules (polyubiquitination), the protein becomes targeted for degradation by cellular proteasomes. However, when a protein is linked to a single ubiquitin molecule (mono-ubiquitination), instead of degradation, protein function is modulated; for example, mono-ubiquitination of plasma membrane receptor proteins promotes internalization and downregulation of the receptor through endocytosis in a nonproteolytic process that has some similarities to virus budding. After protein degradation occurs, the ubiquitin molecule is recycled as a free molecule. HIV-1 particles have been shown to contain ubiquitin, predominantly as a free molecule, but 2 to 5 percent of the Gag-derived, L domain protein p6 is mono-ubiquitinated.[484] One hypothesis is that the late domain of Gag contains an amino acid sequence that binds to residues on the ubiquitin ligase enzyme and thereby results in mono-ubiquitination of the Gag protein. The Gag-ubiquitin conjugate then may attract undefined cellular factors, perhaps those normally involved in endocytosis, that trigger release of the retrovirus from the cell membrane.[402] In the absence of the late domain, an infected cell becomes covered with virus that remains tethered to the plasma membrane by narrow stalks.

Maturation of the virion requires cleavage of the Gag precursor by the virally encoded protease and triggers structural changes that produce mature virions with the characteristic spherical oncoviral or cylindric lentiviral capsid. The Gag polyprotein gives rise to the following: the matrix protein, which lines the inner face of the viral membrane; the capsid protein, which forms a core shell surrounding the viral RNA genome and its associated proteins; and the nucleocapsid protein, which coats and condenses the RNA genome. This step occurs concomitantly with or immediately after the external budding process.

DIAGNOSIS

Detection of retroviral antibody has been the test most widely used to diagnose infection in older children and adults. However, because of transplacental passage of maternal antibody, antibody testing during infancy (children younger than 18 months of age) is not diagnostic of infection, and direct viral detection methods are necessary for establishing the diagnosis. Additionally, during primary infection, a window period exists during which ongoing viral replication is detectable by virologic tests, but antibody tests will be negative or indeterminant because a detectable antibody response has not yet developed.

Antibody testing for retroviruses most frequently involves the use of an initial screening test followed by performance of a more specific confirmatory test. Initial screening generally involves the detection of viral antigens from disrupted whole virus or synthetic or recombinant viral antigens in an enzyme-linked immunosorbent assay (ELISA). A positive ELISA is confirmed by the more specific immunoblot (or western blot), which measures the presence of antibodies to various virus-associated proteins, both structural and nonstructural. Viral antigens (from disrupted whole virus or synthetic or recombinant antigens) undergo electrophoresis through polyacrylamide gel to separate the antigens by size. The separated antigens then are transferred to nitrocellulose paper, incubated with the patient's serum, and subsequently incubated with enzyme-linked antihuman antibody and chromogenic substrate, which results in visible bands where patient antibodies are bound by antigens. Analysis of band patterns permits more specific identification of the virus. Other techniques used to measure antibodies to retroviruses include indirect immunofluorescence assay (IFA), radioimmunoprecipitation assay (RIPA), and biologic assays for antibody to envelope

glycoproteins (neutralization or syncytium inhibition assays).

Because antigenic differences exist between HIV-1 and HIV-2, HIV-1 screening tests are not reliable for the detection of HIV-2. Commercial HIV-1 and HIV-2 combination assays are available that have high sensitivity for detection of both viruses; a supplemental immunoblot assay is used to confirm the viral subtype.[86,319] Serologic diagnosis of HTLV infection is similar to that of HIV and involves the use of a screening enzyme immunoassay (EIA) and confirmatory immunoblot or RIPA. However, these tests do not distinguish between antibodies directed at HTLV-1 or HTLV-2.[85] In patients with positive serology, virologic detection tests, such as proviral amplification by polymerase chain reaction (PCR) or viral isolation, have been used.[113] Assays containing several synthetic peptides and recombinant proteins have been developed and appear to be capable of differentiating between HTLV-1 and HTLV-2.[62,442]

Direct detection of virus by culture is intensive, expensive, and time-consuming; several weeks often are required for results. The ability to isolate HTLV and HIV depends on the patient's disease state, immune status, and viral load. The ability to culture retroviruses has been improved by co-cultivation of patient cells with human peripheral blood mononuclear cells that have been stimulated in vitro with mitogens (e.g., phytohemagglutinin) and growth factors (e.g., IL-2), as well as by removal of patient $CD8^+$ (suppressor) cells from the co-culture. In infants and children, the small volume of blood available for culture and the low virus load in some cases may render virus isolation challenging.

Other methods used for detecting virus include (1) antigen capture assays to detect free circulating viral antigens (e.g., p24 HIV-1 core antigen), (2) IFA and immunohistochemistry to detect viral antigens in tissue, (3) detection of viral nucleic acids by Southern blot analysis, (4) in situ hybridization or dot blots, (5) PCR to detect proviral DNA in leukocytes or tissue, and (6) assays to detect viral RNA in plasma or other fluids. DNA PCR can be used to quantitate virus and is highly sensitive because it is capable of amplifying tiny quantities of viral nucleic acids enzymatically to detectable levels with a system of specific nucleotide primers and probes. This technique can be modified to detect viral RNA by using reverse transcription to convert viral RNA to DNA and by performing PCR on the DNA product or by using other techniques such as branched-chain amplification or nucleic acid sequence-based amplification.[71,397,477]

ONCOVIRUSES: HUMAN T-CELL LYMPHOTROPIC VIRUS TYPES

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE I

In 1980, human T-cell lymphotropic virus type 1 (HTLV-1) was reported as having been isolated from lymphocytes from a patient with cutaneous T-cell lymphoma, becoming the first oncoretrovirus to be described as infecting and causing disease in humans.[414] HTLV-1 is now recognized as the causative agent of not only ATLL but also HTLV-associated HAM/TSP and uveitis and is strongly associated with a variety of inflammatory and infectious disorders[428] (Table 191A-1), including autoimmune disorders, pneumonitis, tuberculoisis, bronchiectasis, and, particularly in children, a severe infectious dermatitis. Unlike HTLV-2, in which coinfection with HIV-1 is associated with a survival benefit, HIV-1 and HTLV-1 coinfections are associated with reduced survival and possible increased risk for malignancies, HAM/TSP, and other neurologic disease.[35]

EPIDEMIOLOGY

Although its disease implications are clear, the vast majority of HTLV-1 infections are asymptomatic. It is estimated that 10 to 20 million persons are infected globally.[547] HTLV-1 is found in human populations around the world; however, its distribution is highly clustered, with highest prevalence in Japan, the Caribbean, and parts of tropical Africa and South America.[428] Southwestern Japan, in particular, has the world's highest HTLV-1 prevalence, with rates as high as 37 percent in some communities,[355,572] whereas in much of Europe and North America HTLV-1 rates are very low, 0.0039 percent in one group of French blood donors.[103]

Seven subtypes of HTLV-1 have been identified[541]: HTLV-1a is termed Cosmopolitan, and although its origins are in Africa, it is most widely distributed globally. Other subtypes include Australo-Melanesian subtype HTLV-1c and Central African subtypes HTLV-1b, HTLV-1d, HTLV-1e, HTLV-1f, and HTLV-1g.[541,547] The Cosmopolitan group has been divided further into five subtypes based on LTR sequencing: Transcontinental (A), Japanese (B), West African (C), North African (D), and Black Peruvian (E).[73] Transcontinental, subtype A, the most widespread, has been isolated from Japan, the Caribbean basin, South America, India, Iran, far-eastern Russia, and South Africa. Subtypes B and C are more restricted geographically, with subtype B found primarily in Japan and rarely in India and subtype C found in West Africa and the Caribbean basin. Subtype D has been identified in Morocco, Algeria, and elsewhere in North Africa. Subtype E, the most rare of the Cosmopolitan subtypes, was characterized from two Peruvians of African heritage.[73]

TABLE 191A-1 **Basis for and Lifetime Risk for Diseases Associated with Human T-Cell Lymphotropic Virus–1**

	Epidemiologic Evidence			*Biologic Evidence*		
	Case Reports or Case Series	**Case Control Studies**	**Cohort Studies**	**HTLV-1 Present in Lesions**	**Animal model**	**Lifetime Risk for Developing (for HTLV-1 Carriers) (%)**
Malignant Diseases						
ATLL	+	+	+	+	+	1-5*
Cutaneous T-cell lymphoma	+			+		
Inflammatory Syndromes						
HAM/TSP	+	+	+	+	+	0.3-4*
Uveitis	+	+		+	+	
Pulmonary disease	+					
Arthritis/arthropathy	+	+		+	+	
Polymyositis	+			+	+	
Sjögren syndrome	+			+	+	
Infectious Complications						
Infective dermatitis	+					
Tuberculosis	+	+				
Strongyloides stercoralis	+	+	+			
Crusted scabies	+					

Modified from Verdonck K, Gonzalez E, Van Dooren S, et al. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* 2007;7:266-81.

*For HTLV-1–associated diseases in general, including ATLL, HAM/TSP, uveitis, arthritis/arthropathy, and polymyositis, lifetime risk approaches 10%.

Phylogenetic data indicate substantial similarity between all HTLV-1 subtypes, with the exception of the Australo-Melanesian subtype HTLV-1c, which appears to have undergone a lengthy independent evolution separate from the other strains.[541] Molecular clock studies suggest that HTLV-1a, HTLV-1b, HTLV-1d, and HTLV-1e appear to have diverged in Africa from precursor Primate T-cell lymphotropic virus type 1 (PTLV-1) between approximately 21,100 and 5300 years ago, with HTLV-1f a more recent emergent approximately 3000 years ago.[541] Phylogenetic studies,[539,545] along with the fact that Africa is the only continent where all PTLVs have been found (HTLV types 1 to 4 and their simian counterparts simian T-lymphotropic viruses [STLV] types 1 to 3),[547] suggest PTLV originated in central Africa.[539,545,547]

Although information on HTLV-1 prevalence in most African countries is scarce, West African data from Benin and Guinea-Bissau indicate relatively high rates, up to 5 percent.[7,134,175,456] Data from equatorial Africa indicate a north–south gradient exists for HTLV-1 prevalence, with rates of 0 to 2 percent in Chad, the northern Central African Republic, and northern Cameroon to 5 to 10 percent in forested areas of Gabon, Equatorial Guinea, southern Cameroon, and southern Central African Republic.[298] Indeed, a recent cross-sectional survey in southern Central African Republic yielded a 7.4 percent prevalence of HTLV-1,[408] as well as suggestions that HTLV-1 was likely transmitted in Central Africa during the 1940s and 1950s when prophylactic injections of pentamidine were used as chemoprophylaxis for African trypanosomiasis.[407,408]

The broad dissemination of HTLV-1 beyond Africa was likely driven by the slave trade, along with increased mobility amongst populations, with HTLV-1a being introduced to Japan, the Middle East, North Africa, and the Americas.[167,176,480,540,547] Japan's southern islands of Kyushu and Okinawa have the world's highest HTLV-1 prevalence, more than 10 percent in the general population,[264,328,352,494] although transmission rates to infants have fallen greatly with the institution of exclusive bottle-feeding by HTLV-1 carrier mothers.[494] Recent data from blood donors in Japan showed 2.0 and 1.8 percent HTLV-1 prevalence in females and males, respectively, with significantly higher rates in the 40- to 64-year-old age group in contrast to younger individuals.[145] Information on HTLV-1 rates in other parts of Asia is scarce, with available data showing rates of 0.1 to 1.0 percent in Iran, Taiwan, and parts of China.[1,300,555] In Oceania, HTLV-1 is endemic to Irian Jaya, Papua New Guinea, Vanuatu, and the Solomon Islands, with between 1 and 2 percent of the indigenous population carrying HTLV-1c.[77,162,497] In Australia, HTLV-1 is relatively common in Aboriginal populations, particularly in central Australia.[137]

The origins of HTLV-1 in the Americas have been controversial, with some data, including HTLV-1 isolated from an Andean mummy, suggesting introduction of HTLV-1 in ancient times through migration of persons across the land bridge once connecting Asia with Alaska.[73,541] Molecular clock estimates and phylogenetic studies, however, suggest HTLV-1 arrived in the Americas with the slave trade.[73] Indeed, where HTLV-1 is prevalent in the region, including Jamaica and Trinidad

and Tobago (up to 6% in both),[73,357] Martinique, Guyana, French Guiana, Suriname, Colombia, and northern Brazil, HTLV-1 is especially frequent among descendants of African slaves.[55,423,516] Studies of blood donors in Argentina and Peru demonstrate HTLV-1 rates up to 2 percent,[171,258,284] with higher prevalence in indigenous peoples.[186,455,588] HTLV-1 is generally rare in Central and North America.[186,547]

In western Europe, HTLV-1 infection is generally rare,[547] with infections clustered in specific risk groups. In a British sexual health clinic, 0.7 percent of an HIV-infected population was coinfected with HTLV-1, with 80 percent of HIV-1 or HITLV-1 coinfected persons being females born in either sub-Saharan Africa or the Caribbean.[101] In a comprehensive survey of individuals infected with HTLV-1 in England and Wales between 2002 and 2004, more than 50 percent were of Afro-Caribbean origin.[133] In some parts of western Europe, HTLV-1 is nearly nonexistent; in a recent survey of young heroin users in Spain, no HTLV-1 infections were recorded.[117] Eastern European rates may be higher, between 0.5 and 1.0 percent,[403] with Romania considered an endemic area for HTLV-1 and its associated pathologies, particularly ATLL.[306]

Factors that influence HTLV-1 seropositivity are age, race, sex, and geography. Seroprevalence rates in blood donors in the United States in the year after initiation of blood screening were 0.016 to 0.021 percent. Female donors and blacks, Hispanics, and Asians were more likely to be seropositive than were male donors and whites.[84] Similar to the demographics observed for seropositive blood donors, the prevalence in black applicants was more than 30 times that in white applicants, and a disproportionate rate of seropositivity in female applicants was observed. Clusters of HTLV-1 infection also have been reported in blacks in the southern and southeastern United States and in immigrants from HTLV-1–endemic areas.[127,130,226,558]

With the development of advanced immunoassays, differentiating the epidemiology of HTLV-1 and HTLV-2 infection has been possible. In a study of 1.7 million blood donors in the United States, the prevalence of HTLV-1 and HTLV-2 infection was 9.1 and 22.3/100,000 donors, respectively. HTLV-1 seroprevalence was associated with older age; female sex; hepatitis C seropositivity; black, Hispanic, or Asian race/ethnicity; and birth outside the United States.[361] In the United States, HTLV-1 infection is concentrated primarily in persons born in or having sexual contact with persons from HTLV-1–endemic areas of Africa, the Caribbean, and Japan. In contrast, HTLV-2 has been found primarily in injection drug users and their sexual contacts, and this phenomenon has been seen globally.[252]

Modes of Transmission

Unlike HIV, HTLV-1 is not produced by infected T cells in appreciable quantities. Plasma HTLV-1 viral loads are undetectable,[547] and its efficiency of transmission is accordingly much lower than that of HIV. HTLV-1 is transmitted in a cell-associated provirus.[21] Hence, its modes of transmission are those in which T cells themselves are transmitted—via sexual intercourse; from mother-to-child, chiefly through breast-feeding; and parenterally through transfusion of infected cellular blood products or reuse of needles and syringes. In areas endemic for HTLV-1, infection generally clusters in communities. This, along with the observation that seroprevalence declines in subsequent generations of people migrating from endemic to nonendemic areas,[335] suggest that biologic or social cofactors exist that affect HTLV-1 transmission.[312]

Sexual Transmission

Sexual transmission is a significant source for acquisition of HTLV-1 in adults. In serodiscordant couples, incident infection is low, with one cohort of 30 couples followed for 10 years yielding an HTLV-1 infection incidence estimate of 0.9/100 person-years.[448] Older cross-sectional studies suggested higher transmission efficiency from males-to-females,[281,362,428] but more recent cohort studies have shown no significant difference in male-to-female transmission in contrast to female-to-male transmission.[448] Nonetheless, HTLV-1 rates in settings worldwide are higher in females than in males, as are rates in older cohorts than in younger ones, suggesting a cumulative risk over time.[470,494] The infected individual's proviral load is likely an important factor in risk for transmission between discordant partners. In the Retrovirus Epidemiology Donation Study, which has enrolled volunteer blood donors from five participating blood centers since 1988, HTLV-transmitting men had proviral loads higher than those of nontransmitters.[252] Transmitting men also tended to have higher antibody titers against various viral proteins than did nontransmitters; in general, antibody titers correlated highly with proviral load.[252]

Similar to other sexually transmitted infections, HTLV-1 seropositivity is associated with higher numbers of lifetime sexual partners, presence of genital sores, paying for or receiving money for sex, and anoreceptive intercourse amongst men who have sex with men.[27,39,428,479,562] In studies from several different countries, prevalence of HTLV-1 has been found to be elevated in female sex workers.[124,187,566] In these studies, the lack of consistent use of condoms, the duration of prostitution, older age, infection with *Chlamydia trachomatis* or syphilis, antibody to herpes simplex virus type 1, and concomitant HIV-1 infection were associated with elevated HTLV-1 prevalence.[187,371] Among sex workers in Peru, condom use has been shown to protect against HTLV-1c infection.[187,520] An increased risk for HTLV-1 seropositivity has been observed in persons attending clinics that treat sexually transmitted diseases.[151,260,371,562] In such clinics in the United States, HTLV seropositivity ranges from 0.18 to 2.0 percent; in the United Kingdom, rates in similar clinics were between 0.5 to 1.0 percent,[101,133] well higher than rates in the general population of less than 0.01 percent in both settings.

Mother-to-Child Transmission

Occurring in 15 to 25 percent of infants born to HTLV-1–positive women,[438] mother-to-child ("vertical") HTLV-1

transmission is of great importance, because HTLV-1 infection in early life is associated with the development of adult T-cell leukemia/lymphoma (ATL), a key HTLV-I–associated pathology, later in life.[500] Vertical transmission of HTLV-1 infection is the principal mode of infection in children, with more than 90 percent of HTLV-1 seropositive Japanese children having seropositive mothers and the vast majority of perinatal HTLV-1 transmission occurring through breast-feeding.[364] Transplacental transmission is believed to be unlikely, given the near absence of proviral HTLV DNA in umbilical cord lymphocytes of HTLV-infected mothers.[452,532] Indeed, prenatal and perinatal transmission are likely minor contributors to the entirety of vertical HTLV-1 transmission, with evidence implicating breast-feeding, particularly prolonged breast-feeding, as the major source of perinatal transmissions.[364,500] In a sample from a neonatal screening program in Brazil of 42 infants born to HTLV-positive mothers, none were PCR positive for HTLV-1 or HTLV-2 at birth.[438] Globally, fewer than 3 percent of HTLV-1–exposed infants who are fed formula are subsequently diagnosed with HTLV-1 infection.[17] For breast-feeding infants, duration of breast-feeding, as well as the provirus load in breast milk and HLA class I concordance between mother and child,[47] are associated with increased transmission risk.[47,293] Approximately 5 percent of breast-feeding infants becoming infected after 3 months of breast-feeding, whereas up to 40 percent of infants become infected when breast-feeding is prolonged.[17] The median time to infection in a Jamaican cohort was 11.9 months,[159] and the passive transfer of maternal HTLV-1 is believed to be protective against infection.[17,438,500] Other associations with risk for vertical transmission of HTLV-1 include female gender of the child, older maternal age, long duration of membrane rupture (>4 hours), and lower maternal income.*

HTLV-1 transmission appears to require cell-to-cell contact,[125,500] because cells naturally infected with HTLV-1 produce very little non–cell-associated virion.[21] Although it is historically assumed that $CD4^+$ T cells were the source of HTLV-1 infection in breast milk,[263,580] the majority of cells in breast milk, especially in early colostrum, are $CD14^+$ macrophages, not T cells,[458] and in some HTLV-1–infected women HTLV-1 antigen–positive T cells are not detected at all.[580] The properties of breast milk macrophages (BMMs) make them a likely source of host T-cell infection through breast-feeding.[500] BMMs stimulated with IL-4 have been shown to have strong transmission capacity for HIV-1,[458] and HTLV-infected BMMs have been shown to stimulate T cells,[500] important in that stimulated T cells are highly susceptible to viral infections, including with HTLV-1.[500] Indeed, in vitro contact between HTLV-1–infected BMMs and stimulated T cells has been shown to yield HTLV-1 transmission to the T cells, establishing BMMs as both a reservoir for and transmitter of HTLV-1.[500] As well, breast-derived luminal epithelial cells present in human milk during early and long-term lactation, can be infected persistently with HTLV-1 and undergo transformation in vitro. These cells can transmit HTLV-1 infection to epithelial cells in breast milk or the intestines or to T cells derived from milk or blood.[482]

In some countries where HTLV-1 is endemic, including Japan and Brazil, HTLV-1 is screened for either in pregnancy or in neonates.[438] Policy in both countries and in other similarly well-resourced settings is for HTLV-infected mothers to completely avoid breast-feeding and for formula to be provided for HTLV-1–exposed infants. If breast-feeding is unavoidable, weaning before 6 months will prevent the vast majority of HTLV-1 infections. Indeed, where this approach has been put into practice, HTLV prevalence has dropped sharply.[215,253]

Yet, although complete cessation or limiting breast-feeding has clear advantages for preventing perinatal transmission of HTLV-1, no international guidelines (World Health Organization, United Nations Children's Fund, Centers for Disease Control and Prevention [CDC], or similar) exist for breast-feeding and HTLV-1 prevention in resource-poor settings,[542] which include most developing countries and most HTLV-1–endemic settings. Although clear risks exist in HTLV-1 infection, including severe disease (e.g., ATL or HAM/TSP) in approximately 5 to 10 percent of infected persons and many other morbidities of variable impact, well-defined and substantial risks exist to both early weaning and formula-feeding,[542] particularly in resource-poor countries, yet high HTLV-1 prevalence is found in pregnancy in many settings.[17,146,542] Southeast Gabon, for example, has HTLV-1 prevalence in pregnancy in excess of 5 percent, one of the highest rates in the world.[146]

Complicating issues for HTLV-1 transmission prevention in resource-poor settings is the recognition of breast-feeding as one of the most powerful child survival interventions, a cornerstone of efforts to reduce mortality worldwide in children younger than 5 years of age,[245] although African studies on prevention of perinatal HIV transmission have shown serious morbidity and mortality risks to be associated with formula-feeding. In Botswana's Mashi trial, cumulative mortality from all causes at 7 months was significantly higher (9.3% vs. 4.9%) in infants randomly assigned to formula-feeding versus those assigned to breast-feeding plus zidovudine (ZDV). HIV-free survival at 18 months was equivalent between the two groups, showing that the early mortality increase seen with formula-feeding negates the benefits of reduced HIV transmission.[512] Work in Guinea-Bissau, where HTLV-1 prevalence is 5 percent and socioeconomic indicators among the most austere in Africa, has shown increased risk for diarrheal diseases and mortality among children weaned before 12 months of age.[542,543] Additionally, during an outbreak of diarrhea in Botswana in early 2006, more than 22,000 Botswanan infants experienced diarrhea (in contrast to just over 9000 over the same period in 2005) and the number of deaths in children younger than 5 years of age increased by 20-fold.[104] Virtually all deaths in the diarrheal cohort were in formula-fed infants, suggesting a lack of protective immunity in formula-fed versus breast-fed infants, a finding consistent with the long-appreciated immunologic benefits of breast-feeding. Malnutrition and growth failure are also more common in formula-fed than breast-fed HIV-exposed infants.[270]

*References 216-218, 253, 495, 532, 564, 565.

It is unclear to what extent cesarean section helps prevent perinatal HTLV-1 transmission. In a Brazilian study, none of the HTLV-1–exposed infants subsequently diagnosed with HTLV-1, including both those born vaginally and by cesarean section, were PCR positive for HTLV-1 shortly after birth, yet all had breast-fed, supporting perinatal HTLV-1 transmission as postnatal via breast-feeding.[438] Yet the finding that the duration of rupture of the membrane is associated with transmission of HTLV-1 suggests that some percentage of transmissions may occur intrapartum, and some HTLV-1–infected infants by maternal report did not breast-feed. The utility of cesarean section in preventing perinatal HIV transmission is chiefly in mothers with a detectable viral load at term, especially when viral load is high.[400] Currently, in most settings globally, HTLV-1 proviral load testing is not readily available for incorporation into decisions on mode of delivery nor is formal guidance accessible on the use of either HTLV-1 proviral load testing or cesarean section in the management of perinatal HTLV-1 prevention.

Parenteral Transmission

Parenteral transmission by transfusion and intravenous drug use is well documented and appears to be the most efficient mode of transmission, with a 40 to 60 percent chance of infection occurring in those receiving HTLV-1–infected cellular products.[388,428,441] Comparative rates of transmission of HTLV-1, HTLV-2, and HIV-1 were evaluated retrospectively in a large repository of U.S. blood donor serum from the Transfusion Safety Study.[129] Consistent with a requirement for cell–cell contact for transmission of HTLV and in contrast to HIV-1, transmission of HTLV-1 and HTLV-2 was observed only with the transfusion of cellular blood components. Infectivity appeared to decrease with an increasing period of blood storage; no apparent transmission occurred after the transfusion of components stored more than 10 days, a finding suggesting that the known decrease in the ability of donor lymphocytes to be activated or to proliferate with storage renders the cells noninfectious. In this study, rates of transfusion-related transmission of HTLV-1 and HTLV-2 were similar; approximately 27 percent of recipients of blood components from seropositive donors became infected. In contrast, 89 percent of the recipients of HIV-1–positive blood became infected, regardless of the blood product component type, and no effect of storage on transmission risk was seen. The median time to seroconversion after the transfusion of HTLV-1–contaminated blood products is 51 to 65 days.[428]

Over the past two decades in Japan, Brazil, the United States, Canada, and much of western Europe, screening of blood donors for HTLV-1 and HTLV-2 has dramatically reduced the number of transfusion-associated HTLV infections and contributed to reductions in HTLV prevalence in the general population.[133,428,547] Because many blood donors are repeat donors, exclusion of HTLV-infected donors from the eligible pool yields a resource-usage benefit over time for blood banks, with fewer HTLV-positive tests seen year to year after instituting screening.[133] In the United Kingdom, where screening was instituted in 2002, by 2004 the number of HTLV-positive screens had fallen by more than 80 percent.[133]

However, in countries in which screening of blood for HTLV-1 and HTLV-2 is not performed, transfusions continue to provide an important source of infection. Patients with transfusion-dependent conditions, such as sickle-cell disease, are especially at risk. In a study of hospitalized children in Gabon in Africa, multiple blood transfusions secondary to complications of sickle-cell disease were as predominant a mode of transmission of HTLV-1 as perinatal transmission.[125] Similarly, in Martinique, HTLV-1 seroprevalence in patients with sickle-cell anemia was 10 percent versus 1 to 3 percent in blood donors, and HTLV-1–seropositive patients had received more transfusions than had seronegative persons.[455]

Intravenous drug use is a notable source of other parentally transmitted infections, such as HIV. Somewhat paradoxically, however, HTLV-1 is rarely detected in injection drug users,[117,428] especially younger cohorts.[117] A Spanish study of young injection heroin users showed no HTLV-1 in 981 injection drug users screened, whereas 27 individuals were HTLV-2 positive.[117] This study's HTLV-2 findings are not novel, because HTLV-2 is considerably more commonly detected in injection drug users than HTLV-1,[428,547] and in Spain, almost all HTLV-2 infections are diagnosed in such users.[39,302,515] Some cohorts, however, defy this trend, with HTLV-1 prevalent among injection drug users in New York and Brazil.[147,547]

Descriptive studies have suggested that ecologic factors may influence the rate of seropositivity in a population; for example, residence in a lower-altitude, tropical environment was associated with higher rates of seropositivity in some reports.[312,335] A role for insect vectors, such as mosquitoes, was postulated. However, no evidence has shown that retroviruses can replicate in arthropods, and any hypothesized insect-borne transmission would need to occur mechanically by the mouth parts of biting insects that were contaminated with a significant amount of infected and infectious lymphocytes; such transmission, if it occurred, would be expected to be very unusual.[152] However, a very strong epidemiologic association exists between infection with *Strongyloides* and HTLV. A case-control study from Guadeloupe ties together *Strongyloides* infection and the socioeconomic and environmental risk factors (i.e., low socioeconomic status, low educational level, and agricultural activity) associated with many HTLV clusters noted in diverse tropical settings worldwide, suggesting *Strongyloides* infection may increase susceptibility to HTLV infection and be responsible in some settings for both the notable clustering of HTLV within endemic areas and the decrease in HTLV prevalence noted in migrant communities from endemic areas over time.[102]

Disease Associations

In HTLV-1–infected individuals, persistent viral infection and antigen production elicit a strong humoral and cellular immune response that results in high antibody titers to HTLV-1 structural and regulatory proteins and

increased circulating activated HTLV-1 Tax protein–specific cytotoxic T lymphocytes.[107,233] In addition, even in asymptomatic patients, spontaneous in vitro proliferation of lymphocytes is observed.[268]

Early in the course of primary infection, the HTLV-1 proviral load is high but becomes controlled rapidly. Within 90 days of primary infection, a narrow range of proviral load is observed in an individual that generally stays relatively constant over the course of time.[314,386,508] Antibody titer is highly correlated with proviral load after the initial set point is reached. The HTLV-1 proviral load in asymptomatic HTLV-1 carriers can be as high as 5 percent of all peripheral blood mononuclear cells, and it is particularly high in individuals with HAM/TSP, in whom as many as 20 percent of peripheral blood mononuclear cells can be infected.[314,353] A high peripheral blood proviral load appears to predate the development of neurologic and ophthalmologic HTLV-1–associated disease entities.

Although the majority of individuals infected with HTLV-1 will remain asymptomatic lifelong, regardless of age at time of infection or infection route, between 5 and 10 percent of infected individuals will develop HTLV-associated illness, which can be, especially in the case of ATL and HAM/TSP, extraordinarily severe. Indeed, HTLV-1 may be a prototype for other retroviruses yet to be discovered that predispose to the development of active diseases with long latency after exposure at birth or early in life.

A variety of less intense clinical manifestations have been noted in HTLV-infected individuals, including uveitis, other rheumatic and neurologic conditions, pulmonary disease, other coinfections, and, especially in children, infective dermatitis.[23,76,413,428,547] Some manifestations of HTLV-associated disease are different in children and adolescents than in adults; HAM/TSP in children, for example, while rare, usually is associated with infective dermatitis and, unlike most cases in adults, rapidly progressive.[23] Studies from Brazil of recently diagnosed individuals indicate neurologic, ocular, and rheumatologic symptoms may be the first manifestations of HTLV-1 infection,[413] and in Brazilian cohorts with longer-standing HTLV-1 infection, HTLV-1–infected individuals were more likely than controls to report extremity weakness or hand or foot numbness, arthralgia, nocturia, and oral findings such as gingivitis, periodontitis, or dry mouth.[76]

Adult T-Cell Leukemia and Other Malignancies

First described in Japan by Takatsuki in 1977,[528] and the first disease to be causally linked to HTLV-1 infection, ATLL is a malignancy of mature peripheral T lymphocytes.[235] Approximately 4 percent of HTLV-I carriers will ultimately develop ATLL,[529] and ATLL is much more common in individuals who are thought to have acquired HTLV-1 infection perinatally.[529] After infection, HTLV-1 randomly integrates as a provirus into the genome of host T cells, then drives proliferation of T cells to clonal expansion of HTLV-1–infected cells (L2-14 to -16) in a proportion of asymptomatic carriers who then manifest high proviral loads,[499,529] a risk factor for developing ATLL.[238,387]

The distribution of ATLL follows that of HTLV-1, with most cases in southwestern Japan, tropical Africa, the Caribbean, Middle East, Irian Jaya, Papua New Guinea, and parts of South America.[528] In hyperendemic southwestern Japan as many as 9.3 percent of patients newly diagnosed with hematologic disorders have ATLL.[235] The annual incidence of ATLL in Japan is approximately 700 cases, with substantial increase with age and a mean age of ATLL onset of 57 years.[306] With a total number of HTLV-1 infected persons in Japan of 1.2 million, the yearly incidence per 1000 carriers over 40 years of age is 0.6 to 1.5,[306] and the additive lifetime risk is 1 to 5 percent among HTLV-1 carriers.[511] ATLL epidemiology is similar in endemic areas of the Caribbean such as Jamaica and French Guiana,[358] as well as in Brazil and Iran,[306] although average age of onset in Africa, Brazil, and the Caribbean is lower (42 to 49 years) and in both regions ATLL is more common in women than in Japan,[22,53,416,574] where the male-to-female ratio is 1.4:1.[306] One hypothesis for the younger age of ATLL onset in the tropics is the presence of *Strongyloides* coinfection. *Strongyloides stercoralis* stimulates oligoclonal proliferation of HTLV-1–infected cells in asymptomatic carriers, potentially serving as a cofactor for ATLL development.[165]

Outside endemic areas, ATLL is most commonly seen in immigrants from endemic areas, most of whom are assumed to have obtained their HTLV-1 infection perinatally.[329] In Great Britain and France, almost all ATLL cases are seen in first- or second-generation immigrants of African or Afro-Caribbean descent.[306,329] In the United States, the incidence of ATLL is low; cases are seen primarily in immigrants from HTLV-1–endemic areas or in blacks in the southeastern United States, in whom endemic HTLV-1 infection has been documented.[432] In a study in central Brooklyn, which has a large Caribbean migrant population, the annual incidence of ATLL in African-Americans of all geographic backgrounds was approximately 3.1/100,000 person-years.[286]

Given the long incubation period generally associated with ATLL, the condition in children is quite rare. Most cases of ATLL in childhood occur in tropical countries; nearly half of those reported have been from Brazil.[53,98] Mean age of diagnosis in case reports in the literature is 15 years,[53,305,415] although cases have been diagnosed in children as young as 2 years of age.[415] In one case series, maternal HTLV-1 serology was positive in 16/19 cases where it was available,[53] with 1 of the 3 cases with maternal serology having received a blood transfusion and the other 2 having been breast-fed by women of unknown serologic status.[291,415] Most cases in children present similarly to cases in adults and, like adult cases, have poor outcomes.[53]

Given substantial diversity in how ATLL presents and evolves clinically, diagnostic criteria developed in Japan are used for classifying the malignancy into four major subtypes.[306,475] For diagnosis, histologically or cytologically proved lymphoid malignancy must be found, with expression of specific T-cell surface antigens; abnormal

TABLE 191A-2 Requirements for Diagnosis of Adult T-Cell Leukemia/Lymphoma

1. HTLV-1 seropositivity
2. Histologic or cytologic proved lymphoid malignancy with T-cell surface antigens present
3. Abnormal T lymphocytes ("flower cells") consistently present in peripheral blood (except for lymphoma subtype of ATLL)
4. Demonstration of clonality of proviral DNA as well as clonal integration of proviral DNA

ATLL, Adult T-cell leukemia/lymphoma; *HTLV-1,* human T-cell lymphotropic virus type 1.

$CD4^+$ and $CD25^+$ T lymphocytes[33] with characteristic lobulated nuclei (so-called flower cells, containing monoclonal or oligoclonal HTLV-1 provirus integrated into the cell genome) in peripheral blood (except in the ATLL type), with many asymptomatic HTLV-1 carriers expressing a persistent leukocytosis; and positive serum antibodies against HTLV-1 at the time of diagnosis.[306] These criteria are summarized in Table 191A-2. Monoclonal expansion of HTLV-I–infected cells in carriers is directly associated with ATLL onset.[306] A method for detecting a persistently infected HTLV-1 monoclonal cell population could serve as a monitoring approach, especially for perinatally infected individuals at highest risk for developing ATLL.[306]

Why ATLL develops in only a small percentage of HTLV-1–infected individuals, including those infected perinatally and at greatest risk for ATLL, is not completely clear. Although ATLL is clearly linked to the integration of HTLV-1 into every malignant cell,[329] ex vivo studies of ATLL cells indicate lack of expression of viral proteins or RNA, and occasionally defective viral genomes are detected.[102,329] Because malignant transformation probably is a multistep process, intermediate factors such as the host immune response, oncogenic environmental stimuli, or both, may be involved.[228] Familial ATLL has been documented in several countries, including Japan, the United States, and the United Kingdom,[330,576] yet it is not known whether genetic predisposition is involved in the development of ATLL in such cases.[329]

Monoclonal HTLV-1 provirus is found integrated into the DNA of ATLL malignant cells. HTLV-1 is known to transform normal $CD4^+$ lymphocytes in vitro and results in immortalization, high levels of IL-2 expression, and increased expression of the IL-2 α-chain receptor on the cell surface. The presence of excessive receptors for IL-2, a known growth factor for T cells, may be linked to development of the proliferative leukemic process of ATLL.[574] Additionally, the HTLV-1 Tax protein activates the promoters of many genes involved in cell growth and differentiation and interferes with DNA repair functions.[354] Indeed, studies of transgenic mice and human $CD34^+$ stem cells suggest Tax initiates ATLL, because leukemia with ATLL features has been induced by Tax expression.[20,205,419]

One hypothesis is that the continuous proliferation observed in HTLV-1–infected cells may render them more susceptible to spontaneous mutagenesis, as well as to the effect of external carcinogens.[2] The finding that persistent clonal expansion of HTLV-1–infected cells precedes the development of ATLL suggests that tumor cells may originate in clonally expanding nonmalignant cells through a multistep process that may include the mutation or deletion of tumor suppressor genes such as *p53* or $p15^{INK4B}/p16^{INK4A}$, which are detected in 30 to 50 percent of ATLL cases and associated with clinical subtypes and prognosis.[38,164] Along with affecting tumor suppression genes and reducing DNA repair, Tax downregulates DNA polymerase B, affecting base excision repair[38] and suppressing nucleotide excision repair.[116,250] Tax also reduces the activity of telemerase, contributing to changes in chromosome number[38,164,250]; ATLL cells are usually aneuploid.[322] Accordingly, chromosomal fusions and shortened telomeres are also regularly detected in ATLL cells, perhaps reflecting this decreased human telomerase expression in early transformation stages.[38,164]

The host immune response also may be involved in malignant transformation, because cells may escape immune surveillance by deleting Tax,[33] the major target of cytotoxic T cells in HTLV-1 infection.[220] Indeed, HTLV-1–infected patients with ATLL have poorly detectable Tax-specific cytotoxic T-cell activity,[249,384] which could permit more unrestrained proliferation of infected cells and lead to increased HTLV-1 viral load, higher HTLV-1 antibody levels to non-Tax viral proteins, and, eventually, malignant transformation. HTLV-1 bZIP (HBZ) factor gene has growth-promoting activity and is expressed in all ATLL cells,[451] suggesting HBZ also may play a role in cellular transformation and leukemogenisis.[33,327] HBZ expression correlates with provirus load,[33] which itself correlates with risk for developing ATLL.[256]

The Japanese Lymphoma Study Group has defined ATLL in four major subtypes, based on how the condition presents[475] and for which there are clinical and laboratory criteria (Table 191A-3). The distribution of clinical ATLL subtypes varies geographically. In Japan, most cases are acute ATLL with leukemic manifestations; however, in the Caribbean, most cases are initially of the lymphoma subtype.[286,287] ATLL frequently presents aggressively, either as a leukemia or with lymph node enlargement without peripheral blood involvement (<1% leukemic cells in peripheral blood).[33] Most commonly globally (about 60% of cases),[33] ATLL presents acutely as leukemia along with systemic symptoms, organomegaly, and prominent lymphadenopathy.[329] The lymphoma subtype represents 20 percent of global ATLL cases.[33] In both aggressive subtypes, massive lymphadenopathy usually is present, although sparing the mediastinum,[33] as are hepatosplenomegaly and multiple visceral lesions, including skin, lung, and gastrointestinal infiltration.[33] ATLL cells in vitro express receptor activator of nuclear factor κ-β ligand on their surface and, along with elevated levels of mononuclear phagocyte colony-stimulating factor, induce the differentiation of hematopoietic precursor cells to osteoclasts.[306] The resultant acceleration in bone resorption leads to lytic bone lesions in some cases,[306,329] and, with or without lytic bone lesions, to the

TABLE 191A-3 **Clinical Subtypes of Adult T-Cell Leukemia/Lymphoma: Clinical and Laboratory Findings**

	Adult T-Cell Leukemia/Lymphoma Clinical Subtype			
	Acute	**Chronic**	**Lymphoma**	**Smoldering**
Percentage with Subtype (%)	**55**	**20**	**20**	**5**
Survival				
Median	6 mo	24 mo	10 mo	>24 mo
Four-year survival rate (%)	5	27	6	66
Clinical Findings				
Lymphadenopathy	±	±	Yes	No
Hepatomegaly/splenomegaly	±	±	±	No
Skin involvement	±	±	±	±
Bone lesions	±	No	±	No
Bone marrow involvement	±	No	±	No
Laboratory Findings				
Absolute lymphocyte count ($\times 10^9$)	Not definitive	≥4	<4	<4
Abnormal circulating lymphocytes (%)	≥5*	≥5*	≤1	≥5*
Polylobulated lymphocytes ("flower cells")	Yes	Occasionally	No	Occasionally
Calcium	Normal or elevated	Normal	Normal or elevated	Normal
Lactate dehydrogenase	Normal or elevated	<2 times normal	Normal or elevated	<1.5 times normal

Modified from Verdonck K, Gonzalez E, Van Dooren S, et al. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* 2007;7:266-81.

*If <5% of circulating abnormal lymphocytes are present, at least one histologically proved tumor lesion from the lungs, skin, or elsewhere is required for diagnosis.

prominent hypercalcemia characteristic of acute ATLL.[306] Of acute ATLL cases, 20 to 30 percent present with hypercalcemia at diagnosis and more than 70 percent manifest hypercalcemia at some point in the illness course.[306,329,505] ATLL cells also have been found to secrete excessive amounts of parathyroid hormone–related peptide, probably because of Tax-mediated transactivation of the gene for this protein, which also contributes to hypercalcemia.[139] Skin lesions also are common in acute ATLL,[329] caused by dense skin infiltration by clonal HTLV-1 tumoral cells.[329,575] Multiple types of skin lesions have been described, from papules and nodules to erythroderma and ulcerative lesions.[306] In some acute ATLL presentations, cutaneous findings dominate the clinical picture; so-called cutaneous ATLL can be challenging to differentiate from mycosis fungoides and Sézary syndrome.[120,306]

The chronic form of ATLL manifests as lymphocytosis with ATLL cells, often stable for months to years, skin findings, low-grade lymphadenopathy and hepatosplenomegaly, occasional lung involvement and the absence of systemic symptoms, hypercalcemia, and lactate dehydrogenase (LDH) either normal or only slightly raised.* The central nervous system (CNS) is not involved in chronic ATLL,[33] which is separated into favorable and unfavorable subgroups, with the worse prognosis defined by low serum albumin, high LDH or high urea levels, and an elevated expression of Ki-67 antigen.[572] In smoldering ATLL, the least common ATLL presentation, affected individuals are usually asymptomatic, but may have skin rash pulmonary infiltrates, with absence of other visceral involvement.[33,234,329] Cutaneous manifestations of smoldering ATLL often respond to topical steroids, and pulmonary findings may be misdiagnosed as infectious or other processes.[329] Key to the diagnosis of smoldering ATLL versus the chronic form of ATLL is a normal leukocyte count,[329] of which abnormal lymphocytes make up 1 to 5 percent.[33] Although not common, cases have been reported of disease progression from chronic or smoldering ATLL to acute, leukemic manifestations.[329] Table 191A-4 details clinical findings in ATLL presentations.

TABLE 191A-4 **Clinical Findings in Adult T-Cell Leukemia/Lymphoma**

Median age of onset	40-60 yr
Gender	Caribbean, Africa: Females > males Japan: Males slightly > females (1.4:1)
Clinical and Laboratory Findings	
Generalized lymphadenopathy (%)	50-80
Hepatosplenomegaly (%)	25-67
Skin involvement (%)	40-60
Elevated white blood cell count (%)	60-66
Hypercalcemia (%)	32-63
Pulmonary involvement (%)	14
Lytic bone lesions (%)	2-10
Central nervous system involvement (%)	2-10

*References 33, 102, 106, 107, 329, 476.

Although the described classification scheme is used globally as the standard for describing ATLL and its prognosis and treatment, gaps exist in its definitions.[33] Given the definition of lymphoma subtype by the presence of fewer than 1 percent circulating abnormal lymphocytes, patients with aggressive presentations and more than 1 percent circulating abnormal lymphocytes are classified as having acute ATLL, even when bulky lymphadenopathy or visceral involvement is present. Current data suggest such patients with substantial lymph node or organ involvement do not respond to antiviral therapy alone, as do leukemic ATLL patients with less prominent lymph node and organ involvement,[33] suggesting, from a treatment perspective, a third aggressive category may exist and be in need of description. As well, debate is ongoing over how to classify patients with isolated circulating abnormal lymphocytes (>5%). In the current approach, they are classified as having smoldering ATLL, although data suggest this group of patients has outstanding long-term survival,[227] in contrast to patients with smoldering ATLL with infiltration of skin or lung.[498]

Individuals with ATLL are immunosuppressed, with severely impaired T cell–mediated immunity.[329,560] Opportunistic infections are common, as well as a common cause of mortality.[306,329] *S. stercoralis* infection is commonly present, and a hyperinfection syndrome may develop, accompanied by gram-negative sepsis, with a mortality rate of greater than 70 percent. As mentioned earlier, evidence suggests that *S. stercoralis* infection may be a cofactor for HTLV-1–induced leukemogenesis.[306] Other common opportunistic infections include bacterial infections, *Pneumocystis* pneumonia, and serious fungal infections. Findings indicative of poor survival include age older than 40 years, increased tumor bulk, poor performance status, high LDH level, hypercalcemia, and the presence of clones of cells containing multiple or defective copies of the HTLV-1 provirus.[11,438] The CNS, which is affected in 2.5 to 10 percent of patients, can act as a sanctuary and has been found to be an important site for relapse when chemotherapy is given to treat ATLL.[404]

The prognosis and treatment of ATLL depends on the subtype. Whereas a prognostic index developed in Japan associates high risk with age older than 70 years, serum albumin less than 3.5 g/dL, and serum IL-2 over 20,000 U/mL,[256] older survival data from Japan indicated a median survival time of 9 months, with a 2-year survival rate of 27 percent and a 4-year survival rate of 10 percent.[306] In these data the rate varied markedly by subtype, with a 4-year survival of 66 percent for smoldering ATLL, 27 percent for the chronic form of ATLL, and 5 to 6 percent for acute and lymphoma types. Aggressive ATLL presentations (acute and lymphoma) are among the most severe lymphoproliferations known to medicine[29] and have very poor prognoses for multiple reasons, including large tumor burden, hypercalcemia, liver or kidney dysfunction, and substantial immunodeficiency resulting in frequent infectious complications.[29,33,306] Importantly, as well, ATLL cells possess intrinsic chemoresistance resulting from elevated expression of the multidrug resistance gene[282] and mutations in the *p53* gene.[453] Complete remission in ATLL is defined by normalization of the complete blood count and resolution of all tumor masses, lasting for at least 1 month,[33] although patients with these factors but maintaining fewer than 5 percent atypical lymphocytes are also considered in complete remission, because this situation may be noted in HTLV-1 carriers without malignant disease ("pre-ATL," which may either disappear or progress to clinical ATLL).[33,556] Different chemotherapy combinations and regimens have been investigated over the years,[33] particularly in Japan, where ATLL is common and the resources to intensively address it exist, unlike in most HTLV-1–endemic settings. First- and second-generation Japanese protocols with the regimen of cyclophosphamide, hydroxydaunomycin, Oncovin, and prednisone (CHOP) and other combined chemotherapies obtained complete remission of up to 42 percent, but with early relapses and 4-year survivals less than 10 percent for aggressive subtypes.[33] Similar results were seen historically from outside Japan.[440] Intensive induction therapy supported by granulocyte colony-stimulating factor yielded complete remission rates as high as 67 percent for lymphoma subtype, but still poor long-term survival.[572] New-generation cytotoxic agents, including deoxycoformycin (a nucleoside analog),[522] irinotecan (a topoisomerase I inhibitor),[521] and MST-16 (a topoisomerase II inhibitor) have been used in pilot phase II studies of relapsed ATLL patients,[502] but with poor outcomes, as with earlier chemotherapy approaches.[33]

Given ATLL's intrinsic chemotherapy resistance, alternative treatment approaches have been explored. Noting ATLL cells' ubiquitous surface expression of IL-2 receptors, monoclonal antibodies against the IL-2 receptor have been used in trial in patients with relapsed or refractory ATLL.[33,107] Good responses are uncommon.[33] Case reports indicate some initial success with alemtuzumab anti-CD52 antibody, but duration of response is short and substantial risk for opportunistic infections exists, especially cytomegalovirus reactivation.[343,433] T-helper type 2 and regulatory T cells express CC chemokine receptor 4 (CCR4), and a next-generation humanized anti-CCR4 antibody, KW-0761, has demonstrated possible benefit versus relapsed ATLL expressing the CCR4 phenotype.[578] In chronic and smoldering ATLL subtypes, monoclonal antibodies can be used as single agents, although clinical trials of monoclonal antibodies with chemotherapy in the lymphoma subtype and antiretroviral therapy in the acute subtype are needed to better define their ultimate utility in treating ATLL.[33]

In Japan, allogenic hematopoietic stem cell transplantation (allo-HSCT) has improved ATLL prognosis, with several studies showing that discontinuation of immunosuppressant therapy induces sustained remission in substantial numbers of posttransplant relapsed patients, suggesting that ATLL may be susceptible to a graft-versus-leukemia effect.[247] In a large Japanese review, outcomes were evaluated for 386 patients who had undergone allo-HSCT.[221] At a median follow-up of 41 months, 3-year overall survival was 33 percent.[221] Donor lymphocyte infusions have been used in Japan for relapse after allo-HSCT, with sustained complete remission of more than 8 years in case reports.[247] Allo-HSCT, while promising in some cases, has limited utility for ATLL presenters as a whole, given limits to eligibility for allo-HSCT based

on severe immunosuppression at presentation, older age, and poor overall health status.[33] Especially in acute ATLL, complete remission remains low even with allo-HSCT.[33]

Antiviral therapy is an important aspect of ATLL treatment,* especially for acute ATLL, and even more so for acute ATLL that has not been previously treated.[560] ZDV, also known as AZT, plus interferon (IFN)-α (AZT/IFN), yields a high response rate, especially in previously untreated ATLL.[33,211,560] Studies in the United States showed lower response rates when AZT/IFN was used in heavily pretreated patients,[560] but U.K. and French studies of AZT/IFN in newly diagnosed ATLL patients have confirmed the regimen's efficacy.[211,331] A meta-analysis of ATL survival since 1995 compared different treatment strategies for ATLL—antiviral therapy alone, chemotherapy alone, and chemotherapy followed by maintenance antiviral therapy. The 5-year overall survival rates were 46 percent for 75 patients who received first-line antiviral therapy, 20 percent for 77 patients who received first-line chemotherapy, and 12 percent for patients who received first-line chemotherapy followed by antiviral therapy.[31] Patients with acute, chronic, and smoldering ATLL substantially benefitted from first-line antiviral therapy, and ATLL lymphoma patients did better with chemotherapy.[33] Acute ATLL patients achieved a 5-year survival of 28 percent in contrast to 10 percent 5-year survival with chemotherapy with or without maintenance antiviral therapy.[33] Achievement of complete remission with antiviral therapy was important, with 82 percent of this subgroup surviving at 5 years.[33] Antiviral therapy resulted in 100 percent 5-year survival in chronic and smoldering forms, yet in ATLL lymphoma a survival disadvantage (median survival, 7 month; 5-year survival of 0) was seen in contrast to first-line chemotherapy with or without maintenance antiviral therapy.[31] Other antiretroviral agents, including lamivudine (3TC), have been evaluated in ATLL, but without benefit being shown.[33]

Standard therapy for acute ATLL is now AZT/IFN, with patients possessing wild-type *p53* at substantial survival benefit over those with mutated *p53*.[112] Long-term therapy is required, because relapse will occur if treatment is stopped, and eventually, even though survival is prolonged, patients with acute ATLL usually relapse eventually and die.[33] For young patients with acute ATLL and a matched donor, allo-HSCT is recommended.[33]

Arsenic trioxide synergizes with IFN to induce cell-cycle arrest and apoptosis in HTLV-1–infected ATLL leukemia cells,[30] eradicating leukemia-initiating cell activity.[140] Trials are currently ongoing to assess the role of arsenic/IFN as consolidation therapy for patients achieving satisfactory responses to induction therapy.[33] Particularly with chronic and smoldering subtypes, suggestions have been made that arsenic may have a role in achieving cure by eliminating leukemia-initiating cell,[30,140] and arsenic/IFM maintenance therapy is being investigated in acute ATLL, as well.[33]

Supportive therapy is necessary in managing ATLL, including treatment of hypercalcemia and prophylaxis against *Pneumocystis jiroveci* pneumonia, viral infections, and fungal infections.[523] In patients with potential past or present *Strongyloides* exposure, prophylaxis with ivermectin or albendazole should be given.[33] Patients with aggressive ATLL should be considered for intrathecal prophylaxis, because more than half of relapses at new sites after chemotherapy are in the CNS.[33]

Other malignant diseases have been associated with HTLV-1 infection, although the supporting evidence for these associations is less clear than that for ATLL. Cutaneous T-cell lymphomas, such as mycosis fungoides and Sézary syndrome, have been described in adults and a child with HTLV-1 infection.[32,198,553,586] In one small study of eight patients with Sézary syndrome, HTLV-1 mRNA expression was found in four patients,[179] and HTLV-1–like sequences have been found in patients with mycosis fungoides.[61] Multiple myeloma, B-cell chronic lymphocytic leukemia,[24] acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) also have been described in patients infected with HTLV-1.[24] These malignancies may arise as a result of chronic antigenic stimulation of B cells by HTLV-1–infected T cells, with such stimulation leading to uncontrolled B-cell expansion. In the Caribbean and Chile, HTLV-1 has been associated with the development of T-cell non-Hodgkin lymphoma.[24,313] Although some cases of adult HTLV-1–associated lymphomas are similar in character to anaplastic large cell lymphoma (ALCL), in a series of 33 cases of pediatric ALCL from Brazil, where HTLV-1 is endemic, no cases demonstrated proviral HTLV-1 DNA, suggesting no relationship between HTLV-1 and ALCL in children.[194] A case of primary brain T-cell lymphoma in a young HTLV-1–positive adult has been reported,[297] as has a case of small cell cancer of the lung with monoclonally integrated HTLV-1. A pulmonary infiltrative syndrome resembling lymphoid pulmonary hyperplasia seen in HIV-1–infected children has been reported in patients with HTLV-1–associated myelopathy.[485]

Human T-Cell Lymphotropic Virus Type 1–Associated Myelopathy

In the 1880s, a "multiple neuritis" syndrome with a predominantly ataxic motor neuropathy was reported in Jamaica. In subsequent years, similar syndromes of unknown origin were described in other geographic locales and given different names, including *Strachan disease*, *central neuritis*, *Jamaican neuropathy*, *tropical spastic paraplegia*, and *tropical spastic paraparesis*.[587] The identification of HTLV-1 in 1980, the development of antibody tests to diagnose HTLV-1 infection, and the recognition that HTLV-1 and TSP were endemic in the same geographic locations led to the hypothesis that these entities could be associated. In 1985, Gessain and colleagues[177] performed a case-control study in Martinique in which patients with TSP of unknown cause were compared with a control group of nurses and blood donors without neurologic disease. Sixty percent of the patients with TSP versus only 4 percent of controls had HTLV-1 serum antibodies detected.[157,177] In the same year, a report was published on the detection of HTLV-1 antibodies in the cerebrospinal fluid (CSF) and serum of individuals afflicted with TSP.[445] In 1986, a series of Japanese patients

*References 31, 33, 211, 259, 331, 560.

with a similar clinical syndrome who also had HTLV-1 antibodies in their CSF were described, and these investigators proposed that the syndrome be called *HTLV-1–associated myelopathy*.[393] At a 1988 meeting of the World Health Organization's (WHO's) Scientific Group on HTLV-1 Infections and Its Associated Disease, researchers proposed that HAM and HTLV-1–associated TSP were clinically and pathologically identical and recommended that the disorder be known by the acronym *HAM/TSP*.[570]

Diagnostic criteria developed at this meeting, and revised in 1989, base diagnoses of HAM/TSP on the presence of a variety of clinical and laboratory criteria,[191] including anti–HTLV-1 antibodies in serum and CSF.[53,427] With advances in diagnostic technology, particularly molecular techniques and HTLV-1 proviral load testing, in 2006 De Castro-Costa and colleagues proposed simplified diagnostic criteria for HAM/TSP that may allow diagnosis at earlier stages, when treatment may be more successful. Widely adopted in Brazil, De Castro-Costa criteria[114] use myelopathic symptoms, serologic findings or detection of HTLV-1 DNA, the exclusion of other disorders, and the results of HTLV-1 proviral load testing to ascertain one of three categories of HAM/TSP: possible, probable, and definite. HTLV-1 proviral load progressively increases by De Castro-Costa ascertainment category,[13,191] with a level higher than 50,000 HTLV-1 copies/10^6 peripheral blood mononuclear cells (PBMCs) an additional criterion for differentiating definite from probable HAM/TSP cases (Table 191A-5).[191]

The disease usually occurs in HTLV-1–endemic areas, including the Caribbean, southern Japan, equatorial Africa, Central and South America, Melanesia, and southern Africa. Sporadic cases have been described in nonendemic areas, usually in immigrants from endemic areas or their sexual contacts or in recipients of blood transfusions (before the advent of HTLV blood screening). Incidence and prevalence estimates are unreliable because of the insidious nature of the disease and the lack of recognition of early symptoms by clinicians, but between 2 and 5 percent of HTLV-1–infected persons will develop neurologic disease, the most common of which is the chronic encephalomyelopathy of HAM/TSP,[13,294] and women are more commonly affected than men.[310] In HTLV-1–seropositive persons in Japan, the incidence of HAM/TSP is 3.1/100,000 HTLV-1–infected persons per year. In contrast, the annual incidence of HAM/TSP in HTLV-1–infected persons in Jamaica and Trinidad was 22.1/100,000 persons.[310] This difference in incidence is hypothesized to reflect an association between the time that HTLV-1 infection is acquired and the type of disease manifestation that occurs. In the Caribbean, most HTLV-1 infection acquired sexually during adult life is more common than in Japan and HAM/TSP is the most common clinical manifestation of the disease. In Japan, sexual transmission is less common, most infection is acquired by maternal-to-child transmission during infancy, and ATLL is the most common disease manifestation. Case reports of HAM/TSP occurring after receipt of blood transfusions and the finding in Japan and Martinique that 13 to 20 percent of patients with HAM/TSP had a history of receiving blood transfusions accelerated the decision of blood banks in the United States to screen for HTLV-1 antibodies. In Japan, a 16 percent decrease in the number of HAM/TSP cases occurred in the 2 years after initiation of blood screening.[167,392]

Although the mean onset of disease is in the fourth decade of life, incubation of this disease appears to be shorter than that of ATLL. In patients with HTLV-1 infection acquired by transfusion in whom HAM/TSP subsequently developed, the median time to development

TABLE 191A-5 Diagnostic Criteria for Human T-Cell Lymphotropic Virus Type 1–Associated Myelopathy/Tropical Spastic Paraparesis: Levels of Ascertainment

Definite

1. A nonremitting progressive spastic paraparesis with sufficiently impaired gait to be perceived by the patient. Sensory symptoms or signs may or may not be present. When present, they remain subtle and without a clear-cut sensory level. Anal and urinary sphincter signs and symptoms may or may not be present.
2. Presence of HTLV-1 antibodies in serum and CSF confirmed by western blot and/or a positive PCR for HTLV-1 in blood and/or CSF.
3. Exclusion of other disorders that can mimic HSM/TSP.*

Probable

1. Monosymptomatic presentation: Spasticity or hyperreflexia in the lower limbs or isolated Babinski sign with or without subtle sensory signs or symptoms, or neurogenic bladder only confirmed by urodynamic testing.
2. Presence of HTLV-1 antibodies in serum and/or CSF confirmed by western blot and/or a positive PCR for HTLV-1 in blood and/or CSF.
3. Exclusion of other disorders that can mimic HSM/TSP.*

Possible

1. Complete or incomplete clinical presentation.
2. Presence of HTLV-1 antibodies in serum and/or CSF confirmed by western blot and/or a positive PCR for HTLV-1 in blood and/or CSF.
3. Disorders that can mimic TSP/HSM have not been excluded.*

Modified from De Castro-Costa CM, Araujo AQ, Barreto MM, et al: Proposal for diagnostic criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). AIDS Res Hum Retroviruses 22: 931-935, 2006.

*The following conditions should be excluded by appropriate laboratory and clinical evaluation, to minimize risk for misdiagnosis of HAM/TSP: multiple sclerosis; carcinomatous meningitis; familial spastic paraparesis; transverse myelitis; primary lateral sclerosis; paraneoplastic syndromes; syringomyelia; Lyme disease; B_{12} and folate deficiency; Behçet disease; neurosyphilis; neurotuberculosis; sarcoidosis; HIV vacuolar myelopathy; collagen vascular diseases; autoimmune myelopathies; Sjögren syndrome; toxic myelopathies; amyotrophic lateral sclerosis; fungal myelopathy; spinal arteriovenous fistula; hepatic myelopathy; parasitic myelopathy (visceral larva migrans of *Toxocara canis* and *Ascaris suum*); spinal cord compression (spinal tumor, cervical spondylosis, brain parasagittal tumor, etc.); endemic regional myelopathies with similar clinical manifestations (including schistosomiasis and neurocysticercosis).

CSF, Cerebrospinal fluid; *HSM/TSP,* HTLV-1–associated myelopathy/tropical spastic paraparesis; *HTLV,* human T-cell lymphotropic virus; *PCR,* polymerase chain reaction.

of symptoms after receiving a transfusion was 3.3 years, and in one report, HAM/TSP occurred 18 weeks after blood transfusion.[189,392] Onset of symptoms is uncommon in persons younger than 20 or older than 70 years of age, and incidence increases from age 20 through age 50 years and then declines.[183] However, HAM/TSP has been reported in children and adolescents, the majority of cases from Brazil,[53,118,199,427,430] with a predominance in girls.[53] Most pediatric and adolescent cases have been in vertically infected children,[199,427] although HAM/TSP has been described in a 6-year-old child after transfusion-acquired HTLV-1 infection.[392] The vast majority of HAM/TSP cases in children and adolescents occur in patients with active, or a history of, HTLV-1–associated infective dermatitis.[427] In one study from Brazil, 60 percent of pediatric and adolescent infective dermatitis cases had neurologic symptoms and one third had frank HAM/TSP.[53,427]

Clinical features of HAM/TSP in children and adolescents are similar to those in adults,[53] particularly involving the pyramidal tracts and including chronic progressive spastic paraparesis and weakness of the limbs, particularly the legs, that results in an insidious onset of gait disturbance (see Table 191A-5). Mild sensory loss and painful paresthesias may develop and result in complaints of extremity numbness or dysesthesia and low back pain. Vesical dysfunction is particularly characteristic of pediatric disease and is characterized by urinary urgency, difficulty emptying the bladder, and incontinence.[427] Urodynamic studies are important in children, for diagnosis as well as for follow-up treatment of vesical dysfunction.[53] Bowel issues in adults include constipation and incontinence. Erectile dysfunction may be seen. Neurologic examination shows hyperactive deep tendon reflexes, clonus, extensor plantar reflexes, proximal muscle wasting, and a spastic paraparesis with a slow, scissoring gait; mild sensory changes may be observed. Cognitive function and the cranial nerves usually are spared. Systemic non-neurologic symptoms suggestive of an autoimmune process, such as pulmonary alveolitis, uveitis, arthropathy, Sjögren syndrome, and vasculitis, also may be noted.[228,230,486] Progression is variable—10 years after onset, 30 percent of patients are bedridden and 45 percent require crutches to walk.[181]

In endemic areas, patients, particularly children and adolescents, without previously known HTLV-1 infection, with symptoms of myelopathy should be serologically screened for HTLV-1.[53,427] Even when HTLV-1 infection status is known, the differential diagnosis of the symptoms described earlier is broad. Of particular importance are CSF studies to rule out other infectious diseases that may involve the spinal cord, including tuberculosis, syphilis, and toxoplasmosis, and, in at-risk geographic areas, cysticercosis and schistosomiasis.[427]

Helminthic coinfections are common in HTLV-1–infected persons,[420,421,490] including helminths other than *Strongyloides*, such as *Ascaris* and *Schistosoma* spp.[490] HTLV-1 infection is associated with a high Th1 response, although helminthic infections have been shown in some studies to downregulate Th1 responses and attenuate chronic inflammatory diseases.[72,333,398] It has been suggested that treatment of helminths in HTLV-1–infected persons may raise the risk for subsequent development of HAM/TSP, yet data to date suggest that treatment of helminthic coinfections do not affect the risk for developing neurologic disease in HTLV-1 infection or otherwise adversely affect HTLV-1–infected persons.[490]

The pediatric form of HAM/TSP has been associated with multiple comorbidities, including short stature, hypocalcemia, and increases in urinary phosphorus and cyclic adenosine monophosphate after parathyroid hormone administration, raising the question of whether HTLV-1 may induce pseudohypoparathyroidism (PHP) in some children.[584] Current thinking is that HTLV-1 does not induce PHP, rather PHP may be a risk factor for developing HAM/TSP in HTLV-1 carriers.[301] Unlike in most adult cases, in which progression is insidious, pediatric HAM/TSP is generally rapidly progressive, in some cases with substantial disability within weeks to months of first symptoms.[427] Some adult populations, such as persons older than 60 years of age acquiring HTLV-1 via blood transfusion, are more likely to experience rapid progression.[294] Subacute HAM/TSP progression (requiring wheelchair use during first 2 years of symptoms[294]) is also uncommon (<10% of cases), with almost all cases in women, the majority of whom acquired their HTLV-1 infection via sexual contact.[294] Both rapidly progressive and subacute HAM/TSP may be more amenable to improvements with aggressive immunosuppressive treatment, and attempts to risk-stratify HAM/TSP presentations for projected chronicity of symptoms have been made.[294] Studies to date have not shown an association between HTLV-1 proviral load and risk for more rapid progression, suggesting factors other than proviral load are involved in this determination.[294]

Nonspecific lesions of the brain are observed with magnetic resonance imaging (MRI) in as many as 75 percent of patients, but no clear correlation has been established between the lesions and symptoms. Atrophy of the spinal cord may occur, usually in the thoracic region, as may discrete lesions visible on MRI.[294] Multiple foci of increased T2 signal intensity are found in the periventricular white matter, similar to the findings observed in patients with multiple sclerosis. However, cognitive impairment may be noted in multiple sclerosis but is not found in HAM/TSP and HTLV-1 sequences have not been detected in the peripheral blood or CNS of patients with multiple sclerosis.[389]

High HTLV-1 antibody levels are found in both peripheral blood and CSF. In CSF, mild to moderate pleocytosis, increased protein, oligoclonal immunoglobulin bands, or any combination of these findings may be observed. Elevated CSF neopterin, an indicator of cellular immune activation, may be present, and its CSF concentration correlates with disease severity.[451] In approximately 50 percent of patients, atypical flower lymphocytes are observed and account for approximately 1 to 15 percent of peripheral blood lymphocytes; these cells also may be seen in CSF.[230] Unlike the monoclonal integration observed in ATLL, polyclonal integration of HTLV-1 is noted in cells from patients with HAM/TSP.

Pathologically, HAM/TSP is characterized by perivascular demyelination and neuronal lesions. Macroscopic atrophy of the spinal cord occurs, with changes consistent

with a chronic inflammatory process characterized by perivascular cuffing of mononuclear cells and lymphocytic infiltration of the brain and spinal cord. Early in the disease, these lymphocytes consist of both $CD8^+$ and $CD4^+$ T cells, along with B lymphocytes and macrophages in areas of parenchymal damage.[240] HTLV-1 proviral DNA can be demonstrated in $CD4^+$ cells in the infiltrates by in situ PCR. Evidence suggests that these HTLV-1–infected $CD4^+$ cells migrate from the peripheral blood and cross the blood-brain barrier to enter the nervous system.[13,83] Later in the disease, the inflammatory cells are fewer in number and consist primarily of $CD8^+$ cytotoxic T cells.[13] Marked myelin and axonal destruction and astrocytic gliosis are prominent. The lower thoracic spinal cord is particularly affected, and parenchymal damage of both white and gray matter of the cord may be present. In the brain, although perivascular mononuclear cell infiltration may be seen, parenchymal damage is an unusual finding. However, rare patients may have white matter lesions, cerebellar symptoms, amyotrophic lateral sclerosis–like symptoms, or neuropathy.[230]

The pathogenesis of HAM/TSP is not fully understood, but it is believed to be related to a high HTLV-1 provirus burden and an exaggerated proinflammatory cellular immune response,[13,551] with the risk for HAM/TSP increasing exponentially as proviral load exceeds $10{,}000/10^6$ peripheral blood mononuclear cells.[13] In a large Brazilian cohort, a proviral load cutoff of $11{,}400/10^6$ has a 78.2 percent sensitivity for identifying HAM/TSP diagnoses over a 14-year follow-up.[160] When compared with asymptomatic HTLV-1–seropositive individuals or those with ATLL, patients with HAM/TSP have higher HTLV-1 antibody titers, a higher proviral load, and elevated levels of spontaneous lymphocyte proliferation and proinflammatory cytokines, including IL-1, IFN-α, and TFN-α.[314,365,366]

One postulated mechanism for nervous system damage is direct infection of CNS glial cells by HTLV-1; a direct cytotoxic immune response to the glial cell is generated and results in demyelination.[228] However, HTLV-1 expression appears to be localized to infiltrating $CD4^+$ lymphocytes within the spinal cord lesions rather than nervous system parenchymal cells, and no clear evidence has established that HTLV-1 infects CNS cells.[368] Alternatively, the heightened HTLV-1–specific immune response in patients with HAM/TSP and neuropathologic findings suggests that immune-mediated mechanisms may have a role in the pathogenesis of disease.[285]

Tax has a role in inducing HAM/TSP.[451] HTLV-1 tax mRNA expression is significantly higher in HAM/TSP patients than in asymptomatic HTLV-1 carriers, and this correlates with HTLV-1 proviral load, Tax-specific $CD8^+$ T-cell frequency, and disease severity.[174,579] Mononuclear cells infiltrating the spinal cord overexpress several cytokines, chemokines, and matrix metalloproteinases (MMPs) transactivated by Tax protein*[118,451] and that may induce demyelination, as well as increase the transmigration of additional HTLV-1–infected lymphocytes to the inflammatory lesion.[228,365,366] These include TNF-α,[530] monocyte chemoattractant protein-1 (MCP-1)[531] MMP-9. Expression of the HBZ gene also correlates with CSF neopterin concentration and disease severity, as well as decreases after successful immunomodulatory treatment for HAM/TSP, suggesting a role for HBZ in HAM/TSP pathogenesis.[451] HTLV-1–specific $CD8^+$ cytotoxic T cells can be found in the CSF as well as the peripheral blood of patients with HAM/TSP, and a significant reduction in the naïve T-cell population occurs with a concomitant increase in the memory/effector $CD8^+$ cell population.[241,242,365] Examination of the T-cell receptor repertoire shows significant expansion of the $CD8^+$ T-cell population in patients with HAM/TSP as opposed to asymptomatic carriers. Many of these $CD8^+$ cells correspond to cytotoxic T lymphocytes directed against epitopes of the immunodominant Tax protein of HTLV-1.[532]

Specific characteristics of the virus also may influence disease manifestations. In one study, a specific tax gene phylogenetic subgroup, *tax A*, was found to occur more commonly in patients with HAM/TSP than in healthy HTLV-1 carriers, thus suggesting that functional or immunogenic differences in the transactivating Tax protein among HTLV-1 viral types may play a role in causing disease.[161] Another hypothesized mechanism is more indirect and involves an HTLV-1–associated activation of autoreactive cells that could lead to an autoimmune process inducing myelin destruction. The finding that numerous autoimmune-like diseases may occur in HTLV-1–infected patients and may coexist with HAM/TSP is consistent with the latter hypothesis.[230]

A genetic susceptibility to the development of HAM/TSP also may exist. Clustering of HAM/TSP cases between siblings or between mother and child has been observed.[429] In a study of HTLV-1–infected Japanese adults, patients with the class I allele HLA-A*02 had a proviral load one-third less than that of HTLV-1 carriers who lack this allele, and they had half the odds for development of HAM/TSP.[244] Yet HLA-DRB1*0101 is associated with an increased risk for HAM/TSP.[534] Because the risk for HAM/TSP is related to proviral load, this relationship may be the result of a more or less efficient antiviral cellular immune response in individuals with a particular class I HLA allele.

Despite extensive effort over the nearly 30 years since its definition, HAM/TSP still has no specific treatment[324] and current approaches do not cure HAM/TSP.[180,324] Mean survival after the onset of symptoms is 10 years, although prolonged survival may be seen, and the major causes of death are infection and cancer.[528] Although no data from placebo-controlled trials exist,[324] immunomodulatory and anti-inflammatory drugs have been used, such as gammaglobulin, IFN-α and IFN-β, danazol, and corticosteroids.[105,239,294,324,367] Case reports suggest that particularly in early phases of HAM/TSP, when substantial parenchymal inflammation is present, responses to such therapies are more likely, whereas later in the disease course degeneration of the white matter and gliomesenchymal tissue reactions dampen the odds for success.[294] In Brazilian pediatric cases treated with intravenous methylprednisolone early in disease course, vesical status improved in most cases but motor responses were generally poor.[428] In most reports, corticosteroids and IFN treatments are of only transient benefit and

often associated with serious side effects.[324] Patients with HAM/TSP, particularly children and adolescents, are often highly affected by the psychological and social aspects of disability, and care is best provided by a multidisciplinary team including psychologist, physical and occupational therapists, physicians, and nurses.[53] Depression and anxiety substantial enough to require specific treatment are common.[170] Symptomatic treatments with agents such as baclofen and anticonvulsants to relieve spasticity and neuropathic pain are of benefit in many patients,[53] as are oxybutynin for urinary incontinence and bethanechol for urinary retention, along with high doses of vitamin C (2 g/day) to acidify the urine and daily oral nitrofurantoin when recurrent urinary tract infection is an issue.[13] Valproic acid may prove dually useful, for both symptoms and disease activity, because it induces reductions in proviral load by activating viral gene expression and exposing HTLV-1–infected cells to the immune system.[292]

Antiretroviral drugs have been tried in treating HAM/TSP, although all studies of antiretroviral drugs have been small, open, and uncontrolled.[13] Clinical trials of ZVD have shown variable results, with no clinical benefit in one[190] and a small mobility improvement in another.[472] Clinical improvement in one HAM/TSP patient was seen with 3TC.[507] Sixteen patients were randomized to ZDV (300 mg) plus 3TC (150 mg) twice daily or placebo for 24 weeks, but no differences were noted between the two arms in proviral load, markers of T-cell activation or proliferation, or in clinical features such as disability score, pain, gait, or bladder function.[506] Raltegravir has been studied in both asymptomatic HTLV-1–infected patients and patients with HAM/TSP.[519] Although patients with HAM/TSP experienced reduction in proviral load, this was transient, proviral loads having returned to baseline at 6 months, and no effect on proviral load was seen in asymptomatic individuals.[519] HAM/TSP symptoms or progression were not affected by raltegravir.[519] The general lack of effect of antiretrovirals in HAM/TSP is likely due at least in part to the lack of continuous HTLV-1 viral replication cycles in the pathogenetic process.[519]

Given the implication of activated T cells and mononuclear phagocytes, along with their associated cytokines, in the pathogenesis of HAM/TSP, there is interest in assessing whether inhibition of T-cell and/or mononuclear phagocyte activation is of benefit in patients with early or clinically progressing HAM/TSP.[324] An open-label, proof-of-concept, 48-week pilot study enrolled onto cyclosporine A (an immunosuppressive inhibitor of activated T cells) therapy seven patients older than 16 years of age with early (<2 years) or progressive (>50% deterioration in timed walk during the preceding 3 months) HAM/TSP.[324] Five of the seven patients showed objective clinical improvement after 3 months of treatment with cyclosporine A, and the two who did not show improvement experienced clinical deterioration and stopped therapy.[324] Overall pain, mobility, spasticity, and bladder function improved by 48 weeks, and larger, randomized controlled studies are planned.[324] Minocycline is known to inhibit activated mononuclear phagocytes,[143] and recent in vitro work has shown minocycline's ability to inhibit TNF-α and IL-1β expression in cultured $CD14^+$ cells of patients with HAM/TSP, while also inhibiting IFN-γ expression in $CD8^+$ T-cells of patients with HAM/TSP, and suggesting minocycline may offer a novel treatment strategy directed at suppression of $CD8^+$ T-cell activation in patients with HAM/TSP and other HTLV-1–associated neurologic disease.[143]

Human T-Cell Lymphotropic Virus Type 1–Associated Uveitis

Defined as uveitis of unknown etiology in an HTLV-1–infected individual, HTLV-1 uveitis was first described in Japan in 1992 based on studies showing higher incidence of HTLV-1 seropositivity in individuals with uveitis of unknown etiology in contrast to uveitis of known etiology.[69] In HTLV-1–endemic areas in Japan, 38 percent of patients with idiopathic uveitis were infected with HTLV-1 in contrast to 19 percent of patients with nonuveitic ocular disease and 10 percent with uveitis of known origin.[341] In the younger age group (20 to 49 years) with idiopathic uveitis, HTLV-1 seroprevalence was 49 percent. Similarly, in Brazil, an area of lower HTLV-1 endemicity, HTLV-1 seroprevalence was elevated in patients with idiopathic uveitis; 1.8 percent of patients with idiopathic uveitis were HTLV-1–seropositive versus none of those with uveitis of known origin.[577] Along with epidemiologic associations, the causality of HTLV-1 in HTLV-1 uveitis was suggested. HTLV-1 antibody was detected in the aqueous humor of patients with HTLV-1 uveitis,[341] as was the presence of HTLV-1 virus itself and HTLV-1–infected cells in ocular fluid from HTLV-1 uveitis cases,[339] including more than 50 percent of Japanese patients with HTLV-1 uveitis. In Japan, HTLV-1 uveitis prevalence in the general population is 0.112 percent[496]; in a survey of 105 asymptomatic Brazilian HTLV-1 carriers, uveitis was found in 2.8 percent of infected persons. Women are almost twice as likely to be affected as men in Japan, yet mean age of onset is earlier in men (34.8 years vs. 48.1 years).[370] As noted for HAM/TSP, genetic factors also may be associated with a susceptibility to, or the severity of, HTLV-1–associated uveitis.[467]

HTLV-1 uveitis is likely the result of HTLV-1–infected T cells infiltrating the eye and producing inflammatory cytokines.[337,341,450,467] The long-term follow-up of Japanese patients informs HTLV-1 uveitis presentation and clinical course.[370] Clinically, HTLV-1 uveitis presents as an acute, moderate to severe vitreitis, along with mild iritis and mild retinal vasculitis, in what typically are healthy, asymptomatic HTLV-1 carriers.[69,338,340,369,474] Uveitis is unilateral in 60 percent of cases, with blurred vision and floaters the most common symptoms. Onset is typically abrupt. Characteristic findings on examination are mild anterior segment inflammation, nongranulomatous in more than 80 percent of cases, as well as vitreitis with fine granular and lacework-like membranous vitreous opacities. Retinal vasculitis, exudative retinal lesions, and optic disc abnormalities are common, occurring in 60, 25, and 20 percent of cases, respectively. HTLV-1 uveitis anatomic characterization is intermediate in more than 75 percent of cases. Five percent of

HTLV-1 uveitis cases have iritis alone.[341] The uveitis is generally mild to moderate, and visual acuity is only mildly affected in most patients. The disease usually responds to topical, periocular, or systemic steroids, with complete resolution in 4 to 8 weeks, although recurrence is seen in 40 to 50 percent of cases after steroid therapy is discontinued.[341,369] Approximately 33 percent of cases are associated with complications, including cataract, glaucoma, epiretinal membranes, persistent vitreous opacities, retinochoroidal degeneration, retinal vascular occlusion, and optic atrophy,[69,192] and poor visual outcomes may be seen in 4 to 10 percent of cases.[69] Clinical studies suggest that retinal vasculitis unresponsive to corticosteroid therapy may be a poor prognostic sign.[370] In Japanese patients, 15 to 20 percent are diagnosed with Graves disease before onset of HTLV-1 uveitis, and approximately 7 percent have concomitant HAM/TSP before or after onset of HTLV-1 uveitis.[69,192] A study of the association between Graves disease and HTLV-1 uveitis found that methimazole was administered in all cases before HTLV-1 uveitis onset.[457] ATLL has not been associated with HTLV-1 uveitis, although rare cases of ATLL can present with ocular findings that mimic severe HTLV-1 uveitis, principally a necrotizing retinal vasculitis.[289] A study of 105 asymptomatic Brazilian HTLV-1–seropositive adults found abnormal results in at least one lacrimal film evaluation test in 40 percent versus 23 percent of uninfected controls,[577] a finding thus suggesting that abnormal early ocular abnormalities may be present in asymptomatic HTLV-1 carriers.

Non-Japanese HTLV-1 uveitis cases may be associated with findings not typically described in Japan, such as corneal pathology in Brazilian patients and patients from the Caribbean.[69] HTLV-1 uveitis in children and adolescents is rare, with cases reported as young as age 3 years; girls more commonly affected than boys; and presenting symptoms and ocular examination findings similar to those in adults.[255,262] As in adults, topical or systemic steroids are usually effective in pediatric HTLV-1 uveitis cases, and topical steroids may need to be continued as maintenance therapy to prevent relapses.[262] As in adults, the presence of retinal vasculitis predicts long-term visual compromise, usually with slow progression poorly responsive to corticosteroids and resulting in diffuse chorioretinal degeneration.[53]

Pediatric Manifestations

Infective Dermatitis

A chronic recurrent eczema of childhood called *infective dermatitis* was first described in 1966 as a series of 17 cases in Jamaican children.[491] These cases were reported as presenting with severe, infected lesions involving the ears, face, scalp, shoulders, and neck, with onset of illness at approximately 2 years of age and rarely before 18 months of age.[119,491] In 1967, researchers observed that cultures of the nares or skin lesions in children with infective dermatitis often were positive for *Staphylococcus aureus* or β-hemolytic streptococci.[554] It is now recognized that infective dermatitis is always associated with either or both of these organisms, typically with nonvirulent strains.[52] Patients with these infections responded well to antibiotic therapy but relapsed once therapy was withdrawn. The refractory nature of the disorder with frequent exacerbations, infections with bacteria that usually were nonvirulent, and resistance to treatment suggested an association with immune dysfunction. After HTLV-1 was identified in 1980, epidemiologic studies demonstrated that HTLV-1 was endemic in Jamaica. Because HTLV-1 infection was known to be associated with immune dysfunction and enhanced susceptibility to infections,[323] an association between infective dermatitis and HTLV-1 infection was hypothesized. Approximately 10 percent of childhood eczema cases in Jamaica are infective dermatitis,[272] and the probability of developing infective dermatitis by age in Jamaican children after perinatal HTLV-1 infection is estimated at 2 percent.[311]

In 1990, La Grenade and colleagues[273] first linked infective dermatitis to HTLV-1 infection in Jamaican children, after observing that of 14 children with typical infective dermatitis and 11 with atopic dermatitis, all children with infective dermatitis were HTLV-1 seropositive versus none of those with atopic dermatitis. A subsequent report described 50 children with infective dermatitis, all of whom were HTLV-1 seropositive, as opposed to only 14 percent of 35 with atopic dermatitis.[268] Subsequently, cases of infective dermatitis were reported in HTLV-1–infected children in Brazil,[52,119,390] Trinidad and Tobago,[487] French Guiana,[372] Colombia, Peru,[12] Senegal,[12] and Japan.[59,524] In Japan, however, infective dermatitis is very rare,[53] despite high HTLV-1 prevalence, suggesting genetic and environmental factors may be involved in its development.[276,308] In 1996, infective dermatitis began to be referred to as infective dermatitis associated with HTLV-1 (IDH),[271] and criteria for its diagnosis were established in 1998.[274] In 2010, revised criteria for the diagnosis of IDH were suggested,[52] and these are detailed in Table 191A-6. Given the diagnosis of HTLV-1's mandatory nature in the diagnosis of IDH, it is advised that in endemic areas serology for HTLV-1 be performed in all cases of severe eczema in children and adolescents.[52]

TABLE 191A-6 Major Criteria for Diagnosis of Infective Dermatitis Associated with Human T-cell Lymphotropic Virus Type 1

1. Lesions of the scalp, ears, retroauricular areas, paranasal and periroral areas, neck, axillae, thorax, abdomen, groin, and other sites. Lesions may be erythematous/scaly, exudative and/or crusted
2. Crusting of nostrils
3. Chronic, relapsing condition with prompt response to specific appropriate therapy, but recurrence promptly upon discontinuation of antibiotics
4. Serologic or molecular evidence of HTLV-1 infection

Fulfillment of criterion 1 requires involvement of three or more areas, including scalp and retroauricular areas. Of the 4 major criteria, 3 are required for diagnosis; 1, 3, and 4 must be present.

Modified from Bittencourt AL, de Oliveira Mde F. Cutaneous manifestations associated with HTLV-1 infection. *Int J Dermatol* 2010;49:1099-110.

A recently reported study of 42 IDH cases from Bahia, Brazil details the epidemiologic and dermatologic characteristics of the condition.[119] In this series, approximately two thirds of the patients are females and all are of African descent and from underprivileged backgrounds. Of the 42 patients, 41 were perinatally infected with HTLV-1; one with an HTLV-1–seronegative mother had received a blood transfusion early in life. Mean age at onset of IDH was 2.6 years (range, 2 months to 11 years), and in 37 percent of children skin lesions first appeared before 12 months of life. All patients were symptomatic, with pruritus of mild to moderate intensity. Dermatologically, all patients had lesions on the scalp and retroauricular area; crusting of the nares was found in only 64 percent of children, and in two children was seen only during relapses. Antecubital and popliteal fossae were involved in 57 percent, and 83 percent had disseminated IDH (i.e., simultaneous involvement of scalp, neck, trunk, and limbs). Lesions were generally severe and exudative, associated with erythematous scale, and grossly infected. Other lesions noted in fewer patients included erythematous scaly papules, follicular papules (always disseminated), retroauricular fissures, and blepharoconjunctivitis. *S. aureus* was isolated from the skin lesions of 97 percent of children evaluated bacteriologically; in fewer than 10 percent, *Streptococcus pyogenes* was detected.

Immunohistologic findings were similar to those from other studies,[56] in which inflammatory infiltrates in IDH comprise mostly $CD8^+$ T-cells, as opposed to atopic dermatitis and seborrheic dermatitis, in which $CD4^+$ lymphocytes predominate.[56] The HTLV-1 genome has been detected by PCR in lymphocytes cultured from biopsy specimens of skin lesions in patients with infectious dermatitis, although cultured fibroblasts were negative, thus suggesting that HTLV-1–infected lymphocytes had infiltrated the skin.[271] Cultured keratinocytes from children with infectious dermatitis have been shown to exhibit overexpression of proinflammatory and anti-inflammatory cytokines that could be induced directly or indirectly by HTLV-1 infection.[546] Secretion of cytokines by HTLV-1–infected cells may amplify or maintain a persistent inflammatory reaction in the skin and, when combined with the enhanced susceptibility to infection induced by HTLV-1–associated immunodysfunction, could result in the clinical manifestations of infective dermatitis. While likely secreting cytokines contributing to the disease process, in IDH, infiltrating $CD8^+$ lymphocytes are typically not activated with cytotoxic granulations, whereas in atopic dermatitis, lymphocytes are activated, expressing perforin and granzyme-B and contributing to the inflammatory process.[56] This distinction, along with the difference in $CD4^+/CD8^+$ ratios amongst infiltrating cells may present a distinguishing feature in the differential diagnosis between atopic dermatitis and IDH.[52,56]

In the Bahia series, all patients responded to antibiotic treatment; trimethoprim-sulfamethoxazole is commonly used. Yet relapse was very common after discontinuation of antibiotics. Although active disease continues in approximately two thirds of patients, persistent disseminated disease is rare (a single child), indicating long-term improvements over time with treatment. In this series, in which cases have been followed for up to 12 years, the mean age at which IDH disappeared (one third of patients) was 15 years (range, 10 to 20 years).

Not infrequently, IDH progresses to HAM/TSP and rarely to ATLL.[52,119,372] In the Bahia case series, 47 percent of children with IDH progressed to HAM/TSP in childhood or adolescence, with more than 80 percent of HAM/TSP cases defined as definite. An association with development of HAM/TSP has been reported from other settings, as well.[52,372] IDH has similar virologic and immunologic features to HAM/TSP, with high proviral load and high levels of TNF-α and IFN-γ[372]; in a Brazilian study, no differences were found between IDH and HAM/TSP patients in the latter two markers. It is suggested, then, that IDH is a risk factor for the development of HAM/TSP.[372] IDH may also be a risk factor for progression to ATLL.[52,53] Progression to ATLL has been seen in the Bahia cohort and others, though much less commonly than progression to HAM/TSP.[52,119,372] Of 52 cases of ATLL with skin involvement observed in Bahia, 37 percent of patients reported a history of severe childhood eczema suggestive of IDH,[54] and flower cells were observed in 17 percent of a 30-patient IDH cohort in childhood or puberty, possibly representing a prodromal stage of ATLL.

In addition to the risk for subsequent TAM/HSP and ATLL in children with IDH, warranting periodic neurologic examinations as part of follow-up, IDH's other complications, occurring in 30 to 35 percent of patients, also should be appreciated. *Strongyloides* is a common coinfection in IDH cases. Given its putative role in the pathogenesis of ATLL via stimulating clonal expansion of lymphocytes and its generally asymptomatic nature, *Strongyloides* should be actively sought for in all IDH cases and treated when present, because appropriate *Strongyloides* treatment can reverse clonal expansion and may reduce the risk for ATLL developing.[163] Other reported complications include crusted (Norwegian) scabies, chronic bronchiectasis, lymphocytic interstitial pneumonitis, and glomerulonephritis, which reflect the systemic complications of HTLV-1 infection. Early death caused by severe bacterial infections with sepsis may occur, and rheumatic heart disease and kidney failure have been reported as causes of death in adolescents with IDH.[119]

Other Disorders in Adults and Children

Numerous autoimmune disorders have been associated with HTLV-1. In a study of 113 HTLV-1–infected patients in southern Florida, rheumatologic or autoimmune diseases were not uncommon manifestations.[204] In two settings, HTLV-1 infection is associated with arthritis[332]: in patients with ATLL and in those who are HTLV-1 seropositive but do not have ATLL or HAM/TSP.[332,375] Polyarthritis is a common presenting feature of ATLL, with some patients manifesting arthritis for as much as 5 years before ATLL diagnosis. Most patients with ATLL-associated arthritis are negative for rheumatoid factor. In patients with HTLV-1–associated arthritis (HAA) without ATLL, onset is typically after 50 years of age, and presentation is a chronic oligoarthritis with large

joints (shoulders, knees, and wrists) most commonly affected. As with HAA in ATLL, rheumatoid factor is typically negative. Strong suspicion exists that HAA represents another disease caused by HTLV-1, as opposed to simply HTLV-1 associated, because synovial fluid and tissue in HAA cases contain high proportions of ATLL-like cells, along with HTLV-1 proviral DNA. As well, HAA occurs in HTLV-1 *tax*-transgenic mice,[236] and there are suggestions that HTLV-1 Tax protein may be associated with proliferation of synovial cells, leading to erosion of cartilage and bone.

HTLV-1–associated polymyositis has been well described in patients with HAM/TSP.[346,396,574] Muscle biopsy is consistent with myositis with mononuclear interstitial infiltrates, necrosis, and regeneration. HTLV-1 provirus has been identified by in situ hybridization in $CD4^+$ cells in the inflammatory cell infiltrate.[212] The mechanism by which HTLV-1 produces disease is unknown. It is probably not a direct viral effect because HTLV-1 does not appear to infect muscle cells.[346] As hypothesized for HAM/TSP, the pathologic process could be the result of an autoimmune response, or it could be caused by the production of cytokines in focal inflammatory infiltrates in muscle by activated HTLV-1–infected $CD4^+$ cells and subsequent bystander damage to the myofibers. Cases complicating TSP/HSM may be severe, with diaphragmatic involvement and respiratory failure.[296] As well, non-HAM/TSP associated polymyositis has been noted in HTLV-1 infected carriers without HAM/TSP, with predominance in children.[149] Muscular dystrophy–like presentations have been noted,[149] with onset of muscular weakness in early childhood, initially in the lower limbs and progressing to upper limbs, in the absence of bladder dysfunction or signs of spinal cord or other CNS dysfunction, markedly elevated muscle enzymes, myopathic pattern on electroneuromyography, and diffuse muscular atrophy on MRI.[149] Polymyositis may be associated with uveitis.[261] In some HTLV-1–infected patients coinfected with HIV, inclusion-body myositis has been noted.[109] Therapy includes immunosuppressants and immunomodulators, particularly corticosteroids.[80,149]

Asymptomatic, subclinical lymphocytic pneumonitis has been described in patients with HAM/TSP, and radiologic studies have shown higher rates of pulmonary involvement in HTLV-1 carriers than in individuals not infected with HTLV-1.[385] HTLV-1 carriers have increased risk for pulmonary tuberculosis, cryptococcosis, and community-acquired pneumonia.[19,266,318] The innate immunosuppression induced by HTLV-1 infection contributes to this and can result in frankly immunosuppression-associated infections such as *P. jiroveci* pneumonia. Pulmonary manifestations vary in HTLV-1–associated lung disease, with bronchiolitis, including diffuse panbronchiolitis, alveolitis, and interstitial pneumonia having been noted.[257] HTLV-1 proviral load in peripheral blood mononuclear monocytes corresponds to the degree of bronchoalveolar lymphocytosis,[347] and HTLV-1 mRNA appears to be upregulated in bronchoalveolar lavage fluid cells in contrast to peripheral blood mononuclear monocytes.[257] Bronchoalveolar lavage has shown the presence of a T-lymphocyte alveolitis, with highly soluble IL-2 receptor levels, increased mRNA levels of cytokines and chemokines, and high proportions of HTLV-1–specific $CD8^+$ cells also found in lavage fluid from these patients, suggesting HTLV-1 induces pulmonary inflammation.[257,485,486,581] On lung biopsy, marked lymphocytic infiltration of the lung is seen.

Destructive lung disease associated with HTLV-1 has been noted in some populations. HTLV-1 has been shown to be a risk factor for the development of bronchiectasis in some endemic areas, especially in the indigenous central Australian population, where HTLV-1 is prevalent (7.2% to 13.9%, although rare elsewhere in Australia) and where bronchiectasis exacts a heavy toll in morbidity and mortality.[137] Annually, 1.5 percent of indigenous children in central Australia require admission for bronchiectasis[89]; in a recent study 13 percent of indigenous Australian adults with bronchiectasis died during a single year of follow-up.[483]

HTLV-1 also has been associated with Sjögren syndrome, a chronic exocrinopathy causing keratoconjunctivitis sicca, xerostomia, and sialadenitis and characterized by a lymphocytic infiltration of the lacrimal and salivary glands; the origin has been hypothesized to be autoimmune. In a study in Japan, HTLV-1 seroprevalence in patients with Sjögren syndrome was 23 percent, significantly higher than the 3 percent HTLV-1 seroprevalence in blood donors.[509] HTLV-1 antibody titers in HTLV-1–seropositive patients with Sjögren syndrome were significantly higher than those in asymptomatic HTLV-1 carriers and similar to those seen in patients with HAM/TSP. However, in contrast to that in patients with HAM/TSP, the HTLV-1 proviral load in peripheral blood was not consistently high. In a study of HTLV-1–infected patients from Guadeloupe, French West Indies, a sicca-like syndrome was found in almost 80 percent of patients, approximately half of whom also had neurologic findings.[34] HTLV-1 provirus has been identified by PCR in acini cells and inflammatory infiltrates in the labial salivary glands of patients with HAM/TSP, as well as in healthy HTLV-1 carriers with the sicca syndrome.[504] In addition, one of the symptoms of Sjögren syndrome is impaired sweating, and HTLV-1 pX sequences have been identified in samples of eccrine sweat gland epithelia from HTLV-1–infected individuals.[469] Transgenic animal models have indicated that HTLV-1 is tropic for ductal epithelium of the salivary and lacrimal glands.[192] Human studies also suggest this, and add to the suggestion that HTLV-1 may be causal in some patients with Sjögren syndrome, because expression of *tax* but not other HTLV-1 genes has been reported in the salivary glands of 29 percent of patients with Sjögren syndrome from areas of Japan endemic for HTLV-1[489] and in 23 percent of Sjögren syndrome from French regions not endemic for HTLV-1.[317]

As mentioned earlier, the immunosuppression induced by HTLV-1 is associated with a variety of infectious complications in HTLV-1–infected patients, including higher rates of both leprosy cases and leprosy treatment failures in areas of Africa where both conditions are endemic, as well as a generally increased susceptibility to parasitic diseases. Although malaria is common in many

HTLV-1–endemic areas, there does not appear to be an association between HTLV-1 and risk for severe malaria. The association between *Strongyloides* and HTLV-1 infection has been discussed previously, including the possible role of *Strongyloides* in the pathogenesis of HTLV-1–related disease.[75,431] As well, HTLV-1 infection is a strong risk factor for the development of *Strongyloides* hyperinfection,[75,219,344] which is often fatal,[75] as well as for refractory *Strongyloides* and the development of gastrointestinal lymphoma in coinfected patients.[92]

Dual Infection with Human Immunodeficiency Virus Type 1

HIV-1 coinfection in HTLV-1 and HTLV-2 carriers occurs with highest prevalence in large metropolitan areas in Europe, the Americas and Africa, and are probably more frequent than clinicians generally realize, because HTLV-1 and HTLV-2 testing is not routine in most HIV programs.[85] Estimates are that HTLV-1 and HTLV-2 rates in HIV-1 infected persons are 100 to 500 times greater than in the general population.[35] In some endemic settings,[26,27,147] and in at-risk populations in nonendemic settings, such as intravenous drug users in the United States, 5 to 10 percent of HIV-1–infected persons may be coinfected with HTLV-1 or HTLV-2.[46,65]

The natural history of HIV-1/HTLV-1 and HIV-1/HTLV-2 coinfections appears to be different. During the era before highly active antiretroviral therapy (HAART) (mid-1980s), in a cohort of HIV-1–infected men who have sex with men in Trinidad, progression to acquired immunodeficiency syndrome (AIDS) within 48 months of observation occurred in 50 percent of HTLV-1–coinfected men, in contrast to less than 10 percent of men without HTLV-1 coinfection.[26] Additional studies in the 1980s also observed accelerated progression to AIDS in HTLV-1–coinfected individuals.[202,206,473] HTLV-2 coinfection, in contrast to that with HTLV-1, was rarely implicated with HIV-1 disease progression. Multiple studies in adults of HIV-1/HTLV-1 and HIV-1/HTLV-2 coinfections have been carried out since the advent of HAART in the 1990s,[28,66,67,214,527] with consistent findings that HIV-1/HTLV-1 coinfection is associated with shortened survival and a possible increase in risk for HAM/TSP, other neurologic conditions, leukemia, and lymphoma,[36,66,79] including the otherwise unusual presentation of these conditions in patients with high CD4 counts. On the other hand, HIV-1/HTLV-2 coinfection is associated with delayed progression to both AIDS and death[35,37] and is associated in some cases with long-term nonprogression of HIV-1.[166] Pediatric data suggest the findings in HIV-1/HTLV-1–coinfected adults are also seen in children, with HIV-1/HTLV-1–coinfected children more likely to present with HIV disease signs and symptoms, and to die, than HIV-1–monoinfected children, despite higher CD4 cell counts at presentation in the coinfected cohort.[405] In both adults and children, CD4 levels may be a less reliable predictor of immunodeficiency in HIV-1/HTLV-1–coinfected patients, as HTLV-1 may induce elevated CD4 levels through enhancing lymphocyte proliferation, but the function of these cells may be abnormal.[78]

Why the clinical differences between HTLV-1 and HTLV-2 coinfection with HIV-1 exist is of substantial interest. Both HTLV-1 and HTLV-2 *tax* genes (*tax-1* for HTLV-1, *tax-2* for HTLV-2) are overexpressed with HIV-1 coinfection,[35] yet Tax-1 and Tax-2 proteins are structurally and functionally distinct,[45,213] with Tax-2 less able to induce cellular transformation and cytokine gene expression than Tax-1.[213,579] In HTLV-2–coinfected individuals, CC-chemokines are produced, with a beneficial role on both innate and acquired immune system function, as well as a suppressive effect on HIV-1 replication, possibly via preventing interaction of the HIV-1 env/CD4 complex with the CCR5 receptor,[85] assisting in the control of HIV infection.[96,97,169] Although both Tax1 and Tax2 have been shown to downregulate CCR5 receptor expression on lymphocytes, Tax1's potent activation of other cellular genes likely counters the beneficial nature of this effect in HTLV-1 coinfected patients.[25]

HUMAN T-CELL LYMPHOTROPIC VIRUS TYPE 2

First reported in 1982 in association with a T-cell variant of hairy cell leukemia,[246] HTLV-2 is closely related to HTLV-1,[28] with approximately 60 percent homology in nucleotide sequences[269] and sharing a tropism for T lymphocytes[166] and similar mechanisms of transmission.[28] HTLV-2 infection is endemic to central Africa and the Americas,[304] and epidemic among users of intravenous drugs in urban North and South America, Asia, and Europe.[304,359] Whereas most HTLV-2 infections in urban North and South America, Asia, and Europe are associated with sharing of HTLV-2–infected needles among intravenous drug users, in central African and indigenous American populations, HTLV-2 is probably an ancient infection that has been maintained in the population by sexual transmission and mother-to-child transmission through breast-feeding.[192,278,280] HTLV-2's role in disease causation is less clear than that of HTLV-1,[131] but a possible association with lymphoproliferative and neurologic disorders and increased rates of infectious diseases has been suggested.[49,81,476]

Viral Pathogenesis and Molecular Biology

Characterization at the molecular level of HTLV-2's envelope gene *(env)*, LTR genomic region and *Tax* gene have recognized three HTLV-2 subtypes: HTLV-2a, HTLV-2b, and HTLV-2d. A unique HTLV-2 subtype discovered in the Brazilian Amazon region originally was christened HTLV-2c,[232] but subsequent complete sequencing of this strain demonstrated it to be a molecular variant of HTLV-2a.[304]

Like HTLV-1, HTLV-2 can transform cells in vitro, with experimental evidence suggesting Tax-2 is able to immortalize $CD4^+$ T lymphocytes in vitro by oncogenic activation, as well as by promoting autophagy, resulting in increased survival and proliferation of immortalized T cells.[436] However, in vivo, HTLV-2 displays a preferential tropism for and induces clonal expansion of cells of

the $CD8^+$ T-lymphocyte phenotype,[131] whereas HTLV-1 provirus is chiefly found in $CD4^+$ T lymphocytes. Whether the difference in cell tropism between HTLV-1 and HTLV-2 may explain the differential pathogenicity between the two viruses is unknown. Differences in the two viruses' *Tax* gene expression and Tax protein itself have been explored. *Tax-1* possesses a PDZ binding motif in its sequence, a motif which is crucial for *Tax-1's* cell-transforming activity and absent from *Tax-2*.[91] The results of other gene expressions are also likely involved in the two viruses' divergent virulence. HTLV-1 and HTLV-2 encode accessory proteins p30 and p28, respectively, which possess some homology in amino acid sequence and are required for in vivo viral persistence.[131] Yet p30 and p28 have distinct host protein interaction profiles, resulting in differential expression of a variety of HTLV-1 and HTLV-2 viral genes.[131]

Some molecular differences exist between gene products of the various HTLV-2 subtypes, the clinical importance of which is not clear. Of note, Tax2 protein appears to differ in length between the HTLV-2a and HTLV-2b subtypes, with the HTLV-2b Tax protein being 25 amino acids longer than the HTLV-2a Tax protein.[401] Functionally, the HTLV-2a Tax protein is a weaker inactivator of the *p53* tumor suppressor gene and a less potent transactivator of the viral LTR in vitro than is the Tax protein from HTLV-1 or HTLV-2b, and limited data suggest that the proviral load may be lower in HTLV-2a–infected than HTLV-2b–infected individuals.[138,308] To date, nonetheless, no indications suggestion that disease associations differ in magnitude or effect between HTLV-2 subtypes.

Epidemiology

It is likely that HTLV-2's ancient origins were, like those of HTLV-1, in central Africa, where STLV-2 has been isolated alongside HTLV-2.[448] HTLV-2–infected migrants likely brought the virus to the Americas 15,000 to 35,000 years ago across the land bridge once connecting Asia and North America.[48,277] In the past several decades, HTLV-2 has passed from indigenous Americans to intravenous drug users by sharing of contaminated needles,[544] resulting in an urban HTLV-2 epidemic among this group in Europe, Asia, and the Americas that is well documented.[28,81,304,448]

HTLV-2 prevalence is highest among indigenous communities in the Americas, particularly in the Amazon region, where prevalence as high as 58 percent has been reported in some tribal groups. Rates of 8 to 10 percent have been noted among the Guaymi of Panama and Costa Rica[548]; in North American indigenous groups, fewer than 1 percent of Mayans in the Yucatan are HTLV-2 infected,[182] with higher rates in Pueblo, New Mexico (2% to 3%),[224] Seminole, Florida (13.2%),[299] and Nuu-chah-nulth Amerindians in British Columbia (1.6%).[411] HTLV-2 seroprevalence is much higher in Amerindian blood donors in New Mexico than non-Hispanic white donors (1.0% to 1.6% vs. 0.009% to 0.06%, respectively).[224] HTLV-2 prevalence in nonindigenous, non–intravenous drug user populations in South America is low, with 0.1 percent HTLV-2 prevalence noted in an urban Brazilian cohort of pregnant women[195] and less than 0.1 percent in Brazilian and Venezuelan blood donors.[99,284]

Although HTLV-2 is endemic in Africa, HTLV-2 seroprevalence appears to be relatively low. Cases are reported sporadically in west and central Africa,[178,526] and serosurveys suggest low prevalence.[63,146] Only 1/907 Gabonese pregnant women screened for HTLV-1 and HTLV-2 was HTLV-2 positive, and HTLV-1 prevalence in the same cohort was 2.1 percent.[146] HTLV-2 prevalence of 0.8 percent has been noted in Ivory Coast, 0.05 percent in Guinea, and 0.02 percent in Senegal.[63] In higher risk groups in Africa, however, HTLV-2 prevalence may be higher; 2.1 percent of female sex workers in Eritrea were noted to be HTLV-2 seropositive.[9]

In the United States, Brazil, Europe, and Southeast Asia, HTLV-2 infection is epidemic among intravenous drug users. Globally, the highest rates among intravenous drug users are seen in southern Vietnam, where more than 60 percent are HTLV-2 infected,[159] with HTLV-2 most likely introduced during the Vietnam War by U.S. military personnel.[448] High rates are also noted in intravenous drug users in south and southeast Brazil[304,348]; in the United States, rates as high as 20 percent have been noted in some urban populations.[448] In the United States, HTLV-2 accounts for most of the HTLV infections in intravenous drug users.[85] In a study of HTLV-2 and HIV seroprevalence in drug users from eight metropolitan areas in the United States, the overall prevalence of HTLV-2 alone was 15.1 percent, the prevalence of HIV-1 alone was 9.9 percent, and that of dual HIV-1/HTLV-2 infection was 3.3 percent. HTLV-2 prevalence was higher in the Southwest and Midwest than the Northeast, whereas HIV-1 prevalence was highest in the Northeast.[27] Rates of HTLV-2 infection in European intravenous drug users are also high, from 1.6 to 8 percent in Italy,[180] 0.4 to 11.5 percent in Spain,[117,447] and 15 percent in an Irish cohort.[136]

In a study involving more than 1.7 million blood donors in the United States between 1991 and 1995, HTLV-2 prevalence was 0.02 percent and HTLV-2 prevalence was significantly higher in blacks, Hispanics, and females, as well as those with only a high school or lower education.[361] Peak HTLV-2 prevalence was noted among middle-aged donors, suggesting an age-cohort effect linked to an epidemic of intravenous drug use (including sex with an intravenous drug user) in the 1960s and 1970s.[448] Much lower HTLV-2 rates have been seen in Danish, Swiss, and French blood donors.[60,128,412]

The geographic distribution of HTLV-2 subtypes varies.[196,448] HTLV-2a is the predominant infection in intravenous drug users in North America, the British Isles, northern Europe, and southeast Asia,[136,448] while also being seen in Brazilian Amerindians and intravenous drug users.[6,304] HTLV-2b is endemic in Central and South American indigenous groups, as well as more common among intravenous drug users in southern Europe.[6,136,304,448] It is also the most common strain detected in central and west Africa,[448] although HTLV-2d is also found in central African pygmy populations.[448]

Modes of Transmission

HTLV-2, like HTLV-1, is transmitted by transfusion of contaminated cellular blood products, sharing of contaminated needles during injection drug use, from mother-to-child, and sexually. Rates of perinatal transmission appear to be somewhat lower for HTLV-2 in contrast to HTLV-1, and transmission from HTLV-positive American blood donors to seronegative spouses did not differ statistically between HTLV-2 (incidence 0.5/100 person-years) and HTLV-1.[449]

Sexual transmission is an important mode of HTLV-2 acquisition. Although data are more limited for HTLV-2 than for HTLV-1, sexual transmission of HTLV-2 appears to be an important infection route in multiple populations,[448] including prostitutes and patients attending sexually transmitted diseases (STDs) clinics,[40,448] where two thirds of HTLV infections diagnosed are HTLV-2.[260] This is especially the case for individuals with both an injection drug use history and a history of STDs.[65,465,550] High HTLV-2 prevalence is also noted in non–intravenous drug using patients who have STDs.[463] In Amerindian populations in whom HTLV-2 is common, and injection drug use rare,[448,548] HTLV-2 prevalence is high among both men and women, and there is a strong association of HTLV-2 infection between spouses.[309,548] Seropositivity in Guaymi women is associated with an early age at first sexual intercourse, the number of lifetime sexual partners, and the number of long-term sexual relationships; in men, sexual intercourse with prostitutes is associated with seropositivity.[309] Preferential male-to-female transmission has been suggested by cross-sectional data, as with HTLV-1; the female preponderance of HTLV-2 infection may indicate more efficient sexual transmission of HTLV-2 from men to women than vice-versa, and higher HTLV-2 proviral loads in seropositive males are associated with higher rates of HTLV-2 transmission to their partners.[364] Prospective incidence data are sparse; the U.S. study referenced earlier showed equivalent proportions of female-to-male and male-to-female transmissions.[449] In HTLV-2–positive blood donors, a history of sexual contact with an intravenous drug user or an HTLV-2–infected sexual partner was associated with increased risk for HTLV-2 seropositivity.[464]

Injecting drug use is the primary mode of HTLV-2 acquisition in North America, Europe, Asia, and parts of Brazil, where it is endemic amongst injection drug users.[81,448] In a study of HTLV-infected blood donors (the Retrovirus Epidemiology Donor Study), HTLV-2 infection was associated significantly with injection drug use or sexual contact with an injection drug user, whereas HTLV-1 infection was not.[464] Other risk factors, such as seven or more sexual partners, were common to both HTLV-1 and HTLV-2. Sex with an injection drug user was a particular risk factor for women; 65 percent of HTLV-2–infected women reported that they had a sexual partner who used injection drugs, and 20 percent of women reported that they also injected drugs themselves. HTLV-2 infection in drug users has been associated with nonwhite race, older age, markers of previous hepatitis B virus infection, the use of a specific needle-sharing practice called backloading, a history of herpes simplex virus type 2 infection, and a history of receiving money for sex.[72,466,549] In a Spanish study of young injection and noninjection heroin users, approximately 3 percent of the cohort was HTLV infected, with all HTLV infections being HTLV-2.[117] Indeed, almost all cases of HTLV-2 reported from Spain have been in injection drug users and the prevalence in Spain in this group is one of the highest in Europe.[39,302,515] Among injection drug users in Spanish prisons, HTLV-2 prevalence is 18 percent, whereas outside prisons, HTLV-2 rates countrywide in Spain average 5 percent.[78] In an Italian study, seroprevalence among injection drug users for HTLV-2 was 8.2 percent versus 2.1 percent for HTLV-1.

HTLV-2 also has been detected in the breast milk of carrier mothers,[448] and mother-to-child transmission has been described in those who breast-feed.[210,273,275,533] Prolonged breast-feeding is common in Amerindian populations, and high prevalence rates (12% to 16%) have been documented in children under 15 years of age in several Central and South American indigenous populations.[58,150,548] Like HTLV-1, HTLV-2 appears to be transmitted infrequently in the absence of breast-feeding,[167,197,251] and breast-feeding interruption has been shown to prevent perinatal transmission of HTLV-2.[438] Passive immunization with HTLV-2 hyperimmunoglobulin in rabbits prevented blood-borne transmission of HTLV-2–infected cells.[349] Only HTLV-2, and not HTLV-1, immunoglobulin was effective in preventing HTLV-2 transmission, a finding suggesting that despite some cross reactivity on conventional ELISAs, cross neutralization between the viruses is minimal or nonexistent.[349]

Clinical Disease

Whereas HTLV-1 has been causally linked to diseases such as ATLL and HAM/TSP, as detailed earlier, to date HTLV-2 has not, although a strong association exists between HTLV-2 and HAM/TSP, especially in HIV-coinfected individuals.[422] In otherwise healthy individuals, HTLV-2 has been associated with increased mortality from a variety of causes.[51,391] In a prospective cohort study of non–HIV-infected American blood donors, controlling for intravenous drug use, all-causes mortality was increased among HTLV-2–infected patients, although no single cause of death could be identified as responsible for the effect.[391] Another prospective study noted a similar effect of HTLV-2 on all-causes mortality, tying the effect to an increase in cancer mortality.[51] Specific cancers noted in excess included lung, cervical, and colorectal cancer; hepatocellular carcinoma; leukemia; lymphoma; and melanoma,[51] suggesting that HTLV-2 may have a facilitating role in cancer development similar to that in HIV, mediated by reduced immunosurveillance of malignant cells in emerging neoplasms.[51]

Although HTLV-2–infected patients commonly have hematologic abnormalities, and cases have been reported of hairy cell leukemia, large granulocytic leukemia, T-prolymphocytic leukemia, and cutaneous lymphoma, including mycosis fungoides, noted in HTLV-2–infected individuals, in larger studies, HTLV-2 has not been

proved a cause of leukemia/lymphoma, as has HTLV-1.[277,448] Importantly, HTLV-2 integration in transformed cell or tumor samples has not been detected.[153,307] Yet, although not demonstrating a causal role in leukemia/lymphoma, HIV-coinfected individuals may be at higher risk for T-cell leukemia/lymphoma, suggesting that the expansion of HTLV-2–transformed lymphocytes may be prone to dysregulation in the presence of HIV-induced immune system dysfunction and highlighting the oncologic risks for HTLV-2/HIV–coinfected patients.[448]

Evidence suggests that HTLV-2 may be associated with neurologic disorders ranging from a spastic paraparesis-myelopathy similar to HAM/TSP to more widespread involvement of the CNS.[356,422] Myeloneuropathies indistinguishable from HAM/TSP have been reported in patients infected with HTLV-2 alone or coinfected with HIV-1.[41,223,356,361] In a prospective Brazilian cohort, 10 percent of HTLV-2/HIV–coinfected patients developed HAM/TSP over a 7-year observational period, whereas no patients infected with only HTLV-2 did, suggesting HIV coinfection is a substantial risk factor for developing neurologic disease when infected with HTLV-2.[422] In addition, sensory neuropathies have been noted in patients with HTLV-2 alone,[50] as well as with HIV coinfection,[585] and a chronic neurodegenerative disorder with ataxia as a prominent feature has been reported in HTLV-2–infected individuals.[203,472]

An increased risk for other infections in HTLV-2–infected patients has been suggested, including serious infections such as pneumonia and pyleonephritis.[448] An initial report from San Francisco described an association of HTLV-2 seropositivity and bacterial infections, particularly skin and soft tissue infections and bacterial pneumonia, but the results were confounded by intravenous drug use, which in itself could increase the risk for infections, in nearly all HTLV-2–seropositive patients.[342] In a case-control study of intravenous drug users from Baltimore with an overall HTLV-2 seroprevalence of 7 percent, no significant association between HTLV-2 seropositivity and the development of bacterial pneumonia, infective endocarditis, and skin abscess was found.[448] However, a prospective study of HIV-negative, HTLV-1–infected and HTLV-2–infected, and uninfected individuals from several large American cities found HTLV-2–infected persons were more likely to develop asthma, acute bronchitis, bladder or kidney infection, arthritis, and pneumonia than HTLV-2–uninfected persons and HTLV-1 infection was associated with only bladder or kidney infection or arthritis.[360] Although suggesting that HTLV-2 may inhibit immunologic responses to bacterial infections,[360] the natural history and clinical manifestations of HTLV-2 need further delineation in the context of ongoing prospective studies.

OTHER FORMS OF HUMAN T-CELL LYMPHOTROPIC VIRUS

Additional forms of HTLV that are distinctly different from HTLV-1 and HTLV-2 have emerged.[135,307,410,492] The identification of HTLV-3 and HTLV-4 among bushmeat hunters in Cameroon demonstrates greater HTLV diversity than was recognized previously. Preliminary data suggest that HTLV-3 comes from a simian origin, whereas HTLV-4 does not appear to have a known simian counterpart, despite extensive screening of monkeys in Cameroon.[307] Both of these newer forms of HTLV cross react with current HTLV-1 and HTLV-2 assays, giving positive or indeterminate results and highlighting the need for more specific subtype testing. Currently PCR assays specific for PTLV-3 and PTLV-4 are required for HTLV-3 or HTLV-4 detection.[410] Although both HTLV-3 and HTLV-4 contain sequences encoding for some of the proteins believed involved in the pathogenesis of HTLV-associated T-cell malignancies,[410] no clinical maladies in either humans or simians have been attributed to HTLV-3 or HTLV-4 to date[135,307,410,492] and concern exists over the spread of infection by blood donations from infected persons or by those who participate in primate hunting. A 2010 sample from New York State of more than 1000 HTLV-infected patients, HTLV-indeterminate blood donors, and other subjects with epidemiologic or clinical features suggesting risk for HTLV-associated disease was negative for HTLV-3 and HTLV-4.[410] Molecular studies have established that in terms of transcriptional activation Tax-3 is a functional analogue of Tax-1, and distinctly different from Tax-2, suggesting HTLV-3 may have pathogenic features in common with HTLV-1.[91] HTLV-4's Tax protein has similarities to that of HTLV-2, missing the PDZ motif present in Tax-1 and Tax-3 important for cellular signal transduction and transformation as Tax-2 is.[492] Further study is required to understand more clearly what implications these newer forms of HTLV have on human disease.

LENTIVIRUSES: HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

First identified in 1986, HIV-2 has a morphology and life cycle similar to those of HIV-1 but with significant antigenic, biologic, epidemiologic, and clinical differences (Table 191A-7). The viruses have similar modes of transmission and can result in immune depletion and AIDS. However, whereas HIV-1 has a global distribution, HIV-2 is confined primarily to West Africa and is found only sporadically in Europe, the United States, and other countries. In all settings over the last two decades, the incidence and prevalence of HIV-2 has declined and

TABLE 191A-7 **Comparison of Human Immunodeficiency Virus Types 1 and 2**

Characteristic	HIV-2	HIV-1
Viral genome	*vpx* gene, no *vpu* gene	*vpu* gene, no *vpx* gene
	nef gene longer in HIV-2, defective *nef* gene in 10%	Defective *nef* gene in <1%
	Difference in number and identity of transcription enhancers for LTR (less responsive to stimulation?)	Greater responsiveness of LTR to transcription enhancers?
Geographic distribution	West Africa	Global
	Restricted distribution outside Africa, primarily in countries with ties to West Africa	
	Europe: Portugal, France, Germany, Spain	
	South America: Brazil	
	India	
Modes of transmission	Sexual, blood-borne, mother-to-child	Sexual, blood-borne, mother-to-child
Risk of sexual transmission	Three-fold lower than HIV-1	
Seroconversion in commercial sex workers in Senegal[248,320]	Incidence of seroconversion in commercial sex workers in Senegal: 1% per year	Incidence of seroconversion in commercial sex workers in Senegal: 2%-3% per year
Risk of mother-to-child transmission (without antiretroviral prophylaxis)	0%-4%	25%-35%
Viral load (without therapy)	28- to 30-fold lower than HIV-1	
Viral set-point		
Seroconversion in Guinea-Bissau[10]	Median RNA, 2500 copies/mL	Median RNA, 70,000 copies/mL
Commercial sex workers in Senegal[417,418]	Median RNA, 263 copies/mL	Median RNA, 7182 copies/mL
Pregnant women in the Gambia[383]	Geometric mean RNA, 410 copies/mL	Geometric mean RNA, 15,100 copies/mL
Rate of $CD4^+$ T-cell decline (%)	1/yr	10/yr
Time to development of AIDS (%)	<0.5 per year	3-5 per year
Increase in mortality above that of HIV-seronegative Individuals	2- to 4-fold increase	10-fold increase

AIDS, Acquired immunodeficiency virus; *HIV,* human immunodeficiency virus; *LTR,* long terminal repeat.

HIV-1 infection has become more likely. The clinical course of HIV-2 infection is generally more indolent than that of HIV-1; the clinical latency period is longer, progression to AIDS is less common, and rates of perinatal and sexual transmission are lower. The basis of such differences in natural history between these two lentiviruses is unknown and may result from characteristics of the virus, the host, or both.

VIRAL GENOME AND PATHOGENESIS

Like HIV-1, HIV-2 is thought to have entered human populations through cross-species transmission. HIV-2 is closely related to simian immunodeficiency virus from the sooty mangabe (SIV_{SM}) indigenous to west Africa. Eight genetic groups of HIV-2 have been identified. However, current data suggest that only HIV-2 groups A and B are established in significant amounts in the human population,[43] with the remaining groups being identified in single individuals. Intergroup recombinant forms of HIV-2 involving groups A and B have been identified.[229,573] No significant differences in transmission, pathogenicity, or disease progression have been observed between the HIV-2 groups.

HIV-1 and HIV-2 differ in the number and size of their accessory genes. HIV-2 lacks the genetic equivalent of the HIV-1 *vpu* gene. The HIV-1 vpu protein plays an important role in intracellular processing of $CD4^+$, specifically the downregulation of $CD4^+$ receptor expression on HIV-1–infected cells. This characteristic could enhance the ability of cytoplasmic HIV-1 envelope proteins to reach the cell membrane and also enhance release of budding virions; no equivalent functional protein exists in HIV-2. HIV-2 contains the *vpx* gene, which is not found in HIV-1 but shares homology with the HIV-1 *vpr* gene and may affect early events in the replication cycle. Additionally, the *nef* ORF in HIV-1 is shorter than that in HIV-2 and the prevalence of defective *nef* genes in patients with HIV-2 infection (~10%) is significantly higher than previously seen in HIV-1–infected individuals (<1%).[493] In HIV-1, deletions in the *nef* gene have been described in patients with long-term nonprogression.

Both HIV-1 and HIV-2 bind to CD4 receptors on the surface of susceptible cells. In addition to CD4, however, binding to a chemokine coreceptor is required for effective infection of target cells. The seven-transmembrane G-protein–coupled chemokine receptors CCR5 and CXCR4 are the major coreceptors used for HIV-1 and HIV-2 infection in vivo. Chemokines act as potent chemoattractants of a large variety of mononuclear cells to sites of inflammation or secondary lymphoid organs by interacting with chemokine receptors. Binding of HIV to

chemokine receptors together with CD4 molecules permits close interaction between or fusion of the virus and cell membranes to occur and ultimately viral entry into target cells. Cell tropism of HIV is determined by the expression of $CD4^+$ and these coreceptors. Most transmitted HIV-1 viruses are R5 tropic, highlighted by the substantial protection from infection observed in individuals homozygous for a 32-bp deletion in CCR5. CXCR4-binding viruses emerge late in disease in as many as 50 percent of patients with AIDS. The presence of X4 virus as well as a switch from CCR5 to CXCR4 usage is associated with accelerated rate of disease progression, although it is not a prerequisite because not all infected individuals demonstrate this coreceptor switch. X4 strains of HIV-1 viruses primarily target populations of T cells that express CXCR4 but not CCR5.

The CCR5 receptor is used by the majority of HIV-1 isolates (R5 viruses), but some isolates from individuals with more advanced immunodeficiency use CXCR4 (X4 viruses) instead of or in addition to CCR5. Whereas both HIV-1 and HIV-2 infect $CD4^+$ T lymphocytes and use CXCR4 or CCR5 coreceptors for cell entry, primary HIV-2 isolates can use multiple additional coreceptors to infect indicator cell lines in vitro.[351] They include CCR3, CCR8, GPR1, GPR15, CXCR6, RDC1, and apj.

The cytopathicity of HIV-2 appears similar to that of HIV-1 and is determined by the type of coreceptor used for cell entry. In an in vitro study comparing HIV-1 and HIV-2 coreceptor use and cytopathicity in human lymphoid cells, HIV-2 specificity for the CCR5 coreceptor alone or in combination with other coreceptors was associated with restricted cytopathicity, whereas specificity for CXCR4 was linked to a more virulent phenotype, as observed for HIV-1.[463] More recently, coreceptor use of primary HIV variants isolated from individuals who had undetectable viral loads, preserved $CD4^+$ cell counts, and a nonprogressive clinical course was compared with those from individuals with more progressive disease. In this comparison, HIV-2 variants were found to use CCR5, GPR15, and CXCR6 with high efficiency regardless of the clinical course. In contrast and consistent with prior studies, only HIV-2 variants isolated from individuals with viremia or overt progressive disease could use CXCR4. X4 variants were not observed in all individuals with progressive infection, a finding indicating that, although the capacity to use CXCR4 is likely a determinant of HIV-2 virulence, it is not the only one. Studies have shown that the use of CCR1, CCR2b, and CCR3 coreceptors is rare.[57] Thus, the lesser virulence of HIV-2 in contrast to that of HIV-1 does not appear to be the result of a restriction in coreceptor use or lower intrinsic cytopathic potential. In addition, the broadening of the coreceptor usage did not necessarily increase the pathogenic potential of HIV 2 strains.[535] In a study of seroincident cases of HIV-1 and HIV-2 infection in Guinea-Bissau, the viral set point after seroconversion was 28-fold lower in HIV-2 than in HIV-1 recent seroconverters.[10,379] Other studies have shown a broader-based neutralizing antibody response and higher antibody levels in HIV-2–infected than HIV-1–infected individuals.[320,501] These findings suggest that HIV-2 may have low rates of viral replication, a reduced ability to mutate and escape the host immune response, or both.

Productive lentiviral infection depends on continued activation of target cells. The lower rate of viral production in HIV-2–infected cells also could reflect a lesser ability of infected cells to respond to activation or could indicate that the cells themselves have lower activation states. The transcriptional enhancer/promoter region of the HIV-2 LTR differs from that of the HIV-1 LTR in the number and identity of enhancer elements, and transcriptional upregulation of viral production may be disrupted more easily than for HIV-1.[319,417] This factor could result in lower responsiveness of the HIV-2 LTR to transcription factors in activated T cells. For example, the HIV-2 LTR has been shown to be less responsive to stimulation of viral gene expression by TNF-α than is the HIV-1 LTR.[200]

Lentiviral envelope glycoproteins can induce production of cytokines and other immunologic disturbances; differences in the effect of the HIV-1 and HIV-2 envelope glycoprotein also could contribute to differences in the pathogenicity of the viruses. Recombinant HIV-2 envelope glycoprotein is superior to the HIV-1 envelope in stimulating the production of IFN-γ and IL-16, both of which inhibit viral replication, and less effective in producing expression of IL-4, which stimulates viral replication.[374,468] The HIV-2 envelope glycoprotein inhibits T-cell proliferation more than does the glycoprotein of HIV-1 in vitro and also was found to inhibit expression of cell surface activation markers.[82] The presence of an HIV-2 protein that reduces immune system stimulation may decrease replication, result in lower levels of HIV-2 viremia, and decrease $CD4^+$ cell apoptosis. The rate of total lymphocyte apoptosis has been found to be lower in HIV-2 than in HIV-1 infection.[334] In one study, 50 percent of peripheral blood mononuclear cells from HIV-2–infected commercial sex workers resisted in vitro challenge with CCR5-dependent HIV-1 but not CXCR4-dependent HIV-1.[267] Additionally, high levels of β-chemokines RANTES (regulated on activation, normal T-cell expressed and secreted), macrophage inflammatory protein (MIP)-1α, and MIP-1 β, the natural ligands of the CCR5 receptor, were secreted when these resistant peripheral blood mononuclear cells from HIV-2–infected individuals underwent stimulation. These investigators hypothesized that β-chemokine–mediated resistance could play a role in the potential protection of some HIV-2–infected individuals from secondary HIV-1 infection.

EPIDEMIOLOGY

HIV-2 is endemic in the former Portuguese and French colonies in West and South Central Africa, including Guinea-Bissau, Burkina Faso, the Gambia, Cape Verde, Senegal, and the Ivory Coast, as well as in Angola and Mozambique.[319] Transmission occurs primarily through heterosexual contact. The overall prevalence of HIV-2 in these areas is approximately 1 to 2 percent, but in the 1990s seroprevalence rates as high as 8 percent in pregnant women in Guinea-Bissau were observed.[222] In

high-risk groups such as urban commercial sex workers, HIV-2 prevalence rates of 15 to 64 percent have been reported.[319]

HIV-2 group A infections have been identified predominantly in the western part of West Africa, mainly Guinea-Bissau, Senegal, the Gambia, and Mali.[42] HIV-2 group B infections have been found in central and eastern West African countries such as the Ivory Coast, Ghana, and Nigeria. In all settings, the prevalence of HIV-2 has declined while that of HIV-1 has become much more likely. Guinea-Bissau is considered to be the country most affected by HIV-2 infection. In the 1980s, Guinea-Bissau had the highest recorded adult prevalence of 8 percent, with up to 20 percent of individuals 40 years of age and older infected.[122,283] In the decade between 1996 and 2006, Guinea-Bissau had a reduction in the prevalence of HIV-2 even as that of HIV-1 increased rapidly. The prevalence of HIV-2 decreased from 7.4 percent in 1996 to 4.4 percent in 2006, particularly among men and women below 45 years of age. For women older than 45 years, the prevalence of HIV-2 was as high as 17.6 percent in 2006. Meanwhile, the overall prevalence of HIV-1 increased from 2.3 to 4.6 percent, but among women 25 to 34 years and men 35 to 44 years of age, the prevalence approached 9 percent.[107] There also has been a significant decline in the rate of new HIV-2 infections in Guinea-Bissau, from 0.53/100 PYO between 1987 and 1996 to 0.24/100 PYO between 1996 and 2006. Similar patterns of increasing prevalence rates of HIV-1 and declining prevalence rates of HIV-2 were reported among a cohort of policemen from Guinea-Bissau.[316]

HIV-2 has only limited distribution outside West Africa and has been described primarily in regions in Europe, South America, and India with historical socioeconomic ties to West Africa. In Europe, the greatest numbers of HIV-2 cases have been reported in Portugal, France, and Germany. The cultural and economic ties of Portugal to its former colonies in West Africa probably facilitated the spread of HIV-2 to Europe and possibly Brazil, also a former Portuguese colony. Portugal has experienced great changes in the epidemiology of HIV-2 infections over time. By the end of 2008, the cumulative number of notified HIV-2 infections in Portugal was 1813. In Portugal, until 2000, the majority of HIV-2 infected individuals were Portuguese-born males living in the north of the country, many with direct or indirect relationships with Africa. In contrast, from 2000 to 2007, most of the patients diagnosed with HIV-2 infection were females of West African origin and were living in the capital city of Lisbon.[74] These shifts in infection trends have been attributed to sociopolitical events in West African countries and population migration both to and within Portugal.

In France, 1 to 2 percent of all pediatric HIV infections are caused by HIV-2.[148] HIV-2–infected individuals have been identified in England, Wales, and Northern Ireland in increasing numbers. The prevalence of HIV-2 infection among clinic attendees with STDs was 0.006 percent (6/104,006) in 2002. Most of these patients likely were infected through heterosexual intercourse in West Africa, including Nigeria (40%), Ghana (24%), Ivory Coast (17%), Sierra Leone (5%), and the Gambia (5%). Of 1324 HIV-infected adults, 917 (69%) were HIV-1 infected and 52 (6%) were HIV-2 or HIV-1/HIV-2 coinfected; the HIV type was not reported in the remaining 355 (27%). The ratio of HIV-2 and HIV-1/HIV-2 dual infections to HIV-1 infections varied greatly by country of infection, with the Gambia (11.7% to 15.2%) and Ivory Coast (7.2% to 9.8%) having a high proportion of HIV-2 infections and Nigeria (0.7% to 1.0%) a low proportion.[132] However, indigenous HIV-2 transmission also may occur, inasmuch as HIV-2 infections not directly linked to West Africa have been reported in Portugal, France, and Spain.

From 1988 to June 2010 in the United States, 166 cases met the CDC working case definition for HIV-2 infection. Most cases (66%) were concentrated in the northeastern United States, particularly New York City, and 81 percent occurred among individuals born in West Africa.[513] Between 2000 and 2008 in New York City alone, 62 HIV-2–infected individuals were identified, of whom 82.7 percent (48/62) were born in West African countries.[513] Researchers estimated that the likelihood of sexual transmission of HIV-2 per sexual exposure is threefold less than with HIV-1. The pandemic spread of HIV-1 in contrast to the more limited geographic spread of HIV-2 is likely the result of different rates of transmission of these two viruses. This difference in transmission of the two HIV viruses is reflected in the declining prevalence rates of HIV-2 in endemic areas over time.

DUAL INFECTIONS WITH HUMAN IMMUNODEFICIENCY VIRUS TYPES 1 AND 2

Dual infection with HIV-1 and HIV-2, confirmed by PCR and culture, has been observed in areas that are endemic for both viruses.[122,173,406] Although HIV-1 infection predominated among a sampling of women living in Dakar, Senegal, HIV-2 and HIV-1 and HIV-2 dual infections occurred more commonly among female sex workers in contrast to other women.[466] In a cross-sectional survey of Guinea-Bissau households, the prevalence of HIV-1 and HIV-2 are similar, 4.6 and 4.4 percent, respectively, but HIV-1 and HIV-2 dual infections remained rare (0.5%).[121] In the United States, 11 percent (19/166) of HIV-2 cases were confirmed to be HIV-1 and HIV-2 coinfections.[513]

Although some reports suggested that infection with HIV-2 may be protective against subsequent acquisition of HIV-1 infection, that suggestion was not confirmed in other studies.[16,377,517,518,563] In two studies, HIV-2–positive subjects actually had a tendency toward a higher risk for acquiring HIV-1 infection than did seronegative individuals.[378,566] Susceptibility to dual HIV-1 and HIV-2 infection was studied in a macaque model of HIV infection.[395,429] The timing of secondary virus exposure was found to be a critical factor in the risk for acquiring infection. Productive mixed infections were established with simultaneous exposure or when viral challenge occurred

within 4 weeks after primary infection developed. However, animals exposed at 8 weeks or more after primary inoculation were resistant to secondary infection. The mechanism of protection is not known. These studies have shown inhibition against both immunosuppression and disease induced by infection with SIDV as a result of contemporaneous HIV-2 infection.[395,429]

A recent retrospective examination of data from long-term follow-up of a cohort composed of members of the Guinea-Bissau police force suggests that the progression of HIV-1 infection is inhibited by concomitant HIV-2 infection, particularly when HIV-2 infection occurred before HIV-1 infection. Differences were found in progression of infection, with the median time to developing AIDS being 68 months for those with HIV-1 infection alone in contrast to 104 months for individuals with HIV-1 and HIV-2 dual infection. When those with dual infection were stratified by whether HIV-2 infection occurred before or simultaneously with HIV-1, the median time to AIDS was 129 months and 88 months, respectively.[144]

MODE OF TRANSMISSION

The modes of acquisition of HIV-2 appear to be identical to those of HIV-1—heterosexual and homosexual intercourse, intravenous drug use, receipt of contaminated blood products, and transmission from mother to child. The infectivity of HIV-2 is lower than that of HIV-1, and transmission of HIV-2 by sexual intercourse appears to be less frequent than that observed with HIV-1.[248]

Sexual Transmission

Like other sexually transmitted infections, the likelihood of transmission of HIV depends in part on the quantity of HIV in genital tract secretions. HIV-1 is detected more commonly in semen than is HIV-2 and viral loads in semen correlate with the plasma viral loads. In one report, HIV-2–infected subjects had a 0.7 $\log_{10}$ lower HIV semen viral load than those with HIV-1, even after adjusting for plasma viral load.[185]

Female genital shedding of HIV is important in both heterosexual and mother-to-child transmission of the virus. In a cross-sectional sampling of women living in Dakar, Senegal, HIV-1 shedding occurred more commonly than did shedding of HIV-2. HIV-2 shedding was associated with older age, severe vitamin A deficiency, advanced HIV disease, and $CD4^+$ cell counts lower than 200 cells/mm^3. Viral shedding was not detected in any women with dual HIV-1 and HIV-2 infection.[466] More recently, HIV shedding again was examined in HIV-infected women in Senegal. In this study, HIV-1–infected women were more likely to shed virus and to have high levels of virus in the genital tract in contrast to those infected with HIV-2, 78 versus 58 percent, respectively. Viral shedding correlated more with plasma viral load than HIV type or CD4 count. HIV-1 and HIV-2 shedding was always detected in women with genital or cervical ulcerative disease. Women with dual HIV-1 and HIV-2 infections were not included in this study.[207]

Perinatal Transmission

Data from most studies of mother–infant pairs indicate that perinatal transmission of HIV-2 appears to be a rare occurrence, with mother-to-child transmission rates ranging from 0 to 4 percent.[4,382,399] In a large prospective study from the Ivory Coast, the risk for perinatal transmission from HIV-1–infected mothers was 21-fold greater than from HIV-2–infected mothers; transmission rates were 1.2 percent from HIV-2–infected mothers versus 24.7 percent from HIV-1–infected mothers.[4] In another large study in the Gambia, the estimated rate of mother-to-child transmission of HIV-2 was 4.0 percent versus 24.4 percent for HIV-1. In this study, three of seven HIV-2–infected infants were infected after reaching 2 months of age, a finding suggesting that HIV-2, like HIV-1, can be transmitted postnatally through breast milk.[383]

In a large French cohort of 8660 HIV-infected mothers who delivered babies between 1994 and 2007, 2.6 percent of the women were HIV-2 monoinfected. The mother-to-child transmission rate was much lower for HIV-2–infected women in contrast to those infected with HIV-1 (0.6% vs. 5.2%) and was most pronounced in those women not receiving antiretroviral therapy (ART) and those receiving nucleoside reverse transcriptase inhibitor (NRTI) monotherapy. The mother-to-child transmission among women with CD4 cell counts greater than 350 cells/mm^3 was significantly lower in HIV-2–infected mothers than in HIV-1–infected mothers. For those women with severe immunosuppression (CD4 cell counts <200 cells/mm^3), however, the risk for transmission was more similar between the groups, 5.3 and 9.0, respectively, for women infected with HIV-2 and HIV-1.[70] In this study, fewer cases of vertical HIV-1 infections occurred in the HAART era than when the standard of care was to use no ART prophylaxis or prophylaxis with ZDV monotherapy. This change is due to the reduction in maternal viral load at the time of delivery with the use of more effective ART treatment. The rate of HIV-2 vertical transmission of HIV-2 did not change significantly between the pre-HAART and the HAART eras. Viral RNA concentrations at delivery were lower in HIV-2–infected mothers in contrast to those infected with HIV-1 for women receiving no ART or treated with mono-NRTI or dual-NRTI medications. Viral loads were similar in those receiving HAART.[70]

Maternal plasma viral load at delivery has been demonstrated to be a significant risk factor for perinatal transmission of both HIV-1 and HIV-2 infection. Among mothers in the Gambia, for every 1 $\log_{10}$ increase in plasma RNA, the odds of transmission were 2.7 for HIV-1 and 2.8 for HIV-2.[383] After adjusting for viral load, the odds for transmitting HIV-1 were similar to those for HIV-2; this finding suggests that the level of viremia, as opposed to the type of virus, is the major determinant of the difference in mother-to-child transmission rates between HIV-1 and HIV-2.

Transmission from women who are dually seropositive for HIV-1 and HIV-2 has been described; HIV-1 appeared to be transmitted more efficiently than does HIV-2, and HIV-1 transmission rates from dually infected women

were similar to those from women infected with HIV-1 alone. Transmission of dual infection to the infant, although a rare occurrence, has been described. In the Ivory Coast cohort, 19.0 percent of women with dual HIV-1 and HIV-2 infection transmitted HIV to their infants; of 11 infected infants, 10 were infected with HIV-1 alone and 1 was dually infected.[4]

DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 2

The diagnosis of HIV-2 can be accomplished by culture of HIV-2 from PBMCs, measurement of HIV-2 proviral DNA, detection of HIV-2 RNA in peripheral blood, or detection of antibodies to HIV-2. The CDC does not recommend routine screening for HIV-2 except in areas where HIV-2 infections have been identified or in certain populations in which potential risk for HIV-2 infection is higher. Risk for HIV-2 infection is based on exposure history, clinical signs and symptoms suggestive of HIV but with an HIV-1 test result that is not positive, and having an unusual HIV-1 western blot pattern.

The standard algorithm for HIV diagnosis recommended the use of a screening EIA to detect antibodies to HIV-1. Specimens with repeatedly reactive EIA results then undergo confirmatory testing with a more specific supplemental test such as a western blot. Positive results to the confirmatory test are necessary to diagnose HIV-1 infection. Additional testing for HIV-2 or non-B subtypes of HIV-1 were to be considered when the confirmatory test yielded a negative or indeterminate result and the sample came from an individual with clinical, epidemiologic, or laboratory history that suggested HIV.[87] This conventional testing algorithm will detect anti-HIV antibody present in a sample but cannot detect acute infections that have been associated with a higher probability of disease transmission. The HIV-1 western blot also misclassifies many HIV-2 infections as HIV-1, which is problematic because HIV-2 infections do not respond to many common antiretroviral agents.

Recently, an alternative testing algorithm was proposed that will provide results rapidly at the point of care, identify acute infections, and differentiate between HIV-1 and HIV-2.[64] This algorithm uses a third- or fourth-generation immunoassay to screen specimens.[373,514,559] Specimens reactive to the screening IA would then be tested with an antibody assay that differentiates between HIV-1 and HIV-2. If the differentiation test is reactive, the result is positive for HIV-1, HIV-2, or both. Specimens reactive by immunoassay but negative for antibody by the type-differentiating immunoassay would proceed to HIV-1 nucleic acid testing for resolution of infection status. Individuals with a positive nucleic acid test and a negative differentiation test are considered to have acute HIV-1. In contrast to the standard protocol of EIA followed by western blot, this algorithm has been shown to be equally sensitive for detecting HIV-1. Use of the type differentiating assay had the added advantage of detecting HIV-2 infections that had been classified as HIV-1 indeterminate and differentiating HIV-2 specimens that had been classified as HIV-1 positive.[373,514,559]

Natural History

Generally, the course of HIV-2 infection has been found to be more indolent than that of HIV-1 infection, characterized by a longer asymptomatic phase and slower CD4 cell depletion and progression to AIDS.[303,321,418] HIV-2 is fully pathogenic in humans. As in HIV-1, HIV-2 disease progression is characterized by the depletion of $CD4^+$ T lymphocytes and the development of AIDS that is indistinguishable from HIV-1–associated AIDS.[325,326] Early studies showed that more favorable survival is observed with HIV-2[243,254,321,561]; nonetheless, the mortality rate is increased by 2-fold to 4-fold in contrast to a 10-fold increase with HIV-1 infection compared with that in seronegative persons.[44,424,439] HIV-2 plasma viral load significantly correlates with progression of disease as measured by CD4 cell decline or mortality, and differences in viral load throughout most of the natural history of HIV-1 and HIV-2 infection probably account for the differences in progression of disease and transmissibility reported between the two viruses.[10,15,418,481]

Just as for HIV-1 infection, the clinical course for those infected with HIV-2 can be heterogeneous. Most HIV-2–infected individuals experience little or no disease progression, whereas a minority experience disease progression to AIDS. For asymptomatic HIV-infected individuals, CD4 cell counts are higher in those infected with HIV-2 in contrast to those infected with HIV-1 (946 cells/μL and 560 cells/μL, respectively).[303] The most common pre-AIDS clinical findings among a community-based cohort in Guinea-Bissau included peripheral generalized lymphadenopathy, mucocutaneous disorders, herpes zoster, pulmonary tuberculosis, and severe weight loss.[188]

Among individuals enrolled in the French HIV-2 cohort, immunosuppression as indicated by CD4 cell depletion was associated with clinical progression of disease, with CD4 cell counts of 482 cells/μL, 293 cells/μL, and 81 cells/μL for individuals at CDC stage A, B, and C respectively.[326] In this cohort, disease progression occurred infrequently, with the probability of remaining AIDS-free at 1 and 3 years being 96.6 and 94.5 percent, respectively. Only 19 of the 152 (12.7%) patients classified as CDC group A or B at enrollment experienced at least one group B or C event. Only 9 of those who progressed developed AIDS. The most common AIDS-defining events were tuberculosis and wasting syndrome. Overall survival within the cohort was 97 and 93.4 percent at 1 and 3 years, respectively.[326]

In a clinic-based cohort of enrollees infected with HIV-1 and HIV-2 in Gambia, fewer individuals with HIV-2 than with HIV-1 developed AIDS (36.4% vs. 27.6%).[325] In this cohort, among those individuals with AIDS, CD4 cell counts were higher in those with HIV-2 in contrast to HIV-1, (176 cells/μL vs. 109 cells/μL). More individuals with HIV-2 than with HIV-1 had CD4 cell counts greater than 200 cells/μL (44% and 33%, respectively). Overall, similar to the French cohort, the most common AIDS-defining conditions included wasting syndrome and pulmonary tuberculosis. The pattern was similar between patients infected with HIV-1 and HIV-2, with two exceptions: in contrast to those

infected with HIV-1, individuals with HIV-2 had significantly more wasting syndrome and significantly less Kaposi sarcoma.[325] After the diagnosis of AIDS, individuals with HIV-2 survived twice as long as those with HIV-1, with median length of survival after AIDS diagnosis of 6.3 months and 12.6 months, respectively. Mortality rates were 52/PYO for HIV-2–infected individuals and 87/PYO for those with HIV-1. At the time of death, CD4 cell counts remained higher for those with HIV-2 in contrast to those with HIV-1 (120 cells/μL and 62 cells/μL, respectively).[325]

HIV-2 viremia has been associated with more pronounced immunosuppression, more rapid disease progression, and a greater likelihood for death. In a comparison of HIV-1–infected and HIV-2–infected persons with similar CD4 cell counts, the quantitative viral load was significantly lower in those infected with HIV-2.[471] Plasma viral load is inversely related to CD4 counts, with each decline in CD4 cell count by 100 cells/μL associated with an increase in plasma viral load of 0.07 $\log_{10}$ copies/mL.[326] In this study the risk for disease progression was higher in individuals with plasma viral loads of 1000 copies/mL or greater in contrast to those with plasma viral loads less than 1000 copies/mL—26 percent compared to 6 percent. In patients with no immunosuppression ($CD4^+$ cell count >500/mm^3), none of the HIV-2–infected individuals had a detectable plasma viral load, in contrast to 52 percent of HIV-1–infected persons, and had a significantly lower mortality rate than that of HIV-1–infected patients or those dually infected with HIV-1 and HIV-2.[461] Among HIV-infected patients with advanced disease ($CD4^+$ counts <200 cells/μL), all had a poor prognosis and no difference in mortality rates occurred among HIV-1, HIV-2, or dually infected patients.[461] In fact, the mortality rates of HIV-2–infected patients with undetectable plasma viral loads was similar to that of uninfected individuals, whereas HIV-2–infected patients with plasma viral loads of 10,000 copies/mL or greater, had mortality rates similar to those observed with HIV-1.[538]

The lower plasma viral load seen with HIV-2 infection could possibly be due to lower viral replication, enhanced host immune control, or a combination of the two factors. HIV-2 and HIV-1 have comparable cytopathicity. It is clear, however, that the two viruses have different dynamics in vivo with plasma viral loads set points and plasma RNA levels being approximately 30-fold lower than with HIV-1.[10,418] At corresponding levels of CD4 cell depletion, despite greatly disparate plasma viral loads, levels of integrated proviral HIV-2 DNA in peripheral blood mononuclear cells are similar to those in comparable groups of HIV-1–infected individuals,[14,237,418] indicating that HIV-2 is able to establish provirus. HIV-2 viral mRNA accumulates at much lower amount, indicating that HIV-2 has tighter transcriptional control.[303,479]

Similar to the clinical outcome of HIV-2 infection in adults, slower rates of disease progression and better survival rates have been observed in HIV-2–infected versus HIV-1–infected or dually infected children.[4,116,379,394,426] In a prospective study in the Gambia, the 18-month mortality rate of children born to HIV-2–infected mothers did not differ significantly from that of children born to HIV-seronegative women (7% vs. 6%, respectively). In contrast, the relative risk for death in children born to HIV-1–infected versus HIV-2–infected or seronegative mothers was increased by 2.3-fold- and 2.6-fold, respectively.[394]

The excess number of deaths in children of HIV-1–infected mothers was caused primarily by HIV-1 infection in the child; the mortality rate was 35 percent in HIV-1–infected children, as opposed to 9 percent in uninfected children born to HIV-1–infected mothers. In contrast, no deaths occurred in HIV-2–infected children. In Guinea-Bissau, the overall child mortality rates were similar in children born to HIV-2–seropositive and HIV-seronegative women (16.3% vs. 14.6%, respectively).[379] However, despite generally slower progression in HIV-2–infected children, severe immunodeficiency and AIDS occurring early in life rarely have been reported with HIV-2 infection.[172,345] Mortality in infants born to HIV-1–infected or dually infected mothers was 2.6 to 4.2 times higher than that in infants born to HIV-2–infected mothers (mortality rates of 133, 82, and 32/1000, respectively).[115] This finding is similar to that observed in the Gambia, where the childhood mortality rate was 15 percent for children born to HIV-1–infected mothers, compared with 7 percent for those born to HIV-2–positive mothers and 6 percent for those born to HIV-seronegative mothers.[394] In a small cohort study investigating the survival of children born to HIV-1–positive and HIV-2–positive mothers, the mortality rates for infected children were significantly higher than those of uninfected children.[460]

The mortality hazards ratio of HIV-1–infected children was 3.1 times that of HIV-2–infected children but was not statistically significant.

The overall mortality of HIV-uninfected children of HIV-1–seropositive and HIV-2–seropositive mothers was not significantly different from that in children of HIV-uninfected children.[462]

TREATMENT

In contrast to the vast data available to guide the treatment of HIV-1, very little information has been gathered on the treatment of HIV-2 infection, a situation that could be improved by the conduct of randomized clinical trials of ART for HIV-2.[184] Observational cohort studies have shown variable but largely poor outcomes for ART in HIV-2 infection.[94,183,326,443] Recently an international consortium of HIV-2 cohorts (A Collaboration on HIV-2 Infection [$ACHIEV_2E$]) was established to better study HIV-2 infection and standardize its management.[110] Typically, combination antiretroviral therapy is recommended for the treatment of HIV-2, as it is for HIV-1. Significant differences exist in the effectiveness of specific ART medications in treatment of HIV types 1 and 2 that must be considered in establishing an effective therapeutic regimen.

HIV-1 and HIV-2 reverse transcriptase enzyme regulates the formation of double-stranded viral DNA that is to be integrated into the host-cell genome. Reverse transcriptase catalyzes the incorporation of deoxynucleoside

triphosphates (dNTPs) forming a negative-sense DNA strand by using HIV-positive sense RNA as a template. The reverse transcriptase RNase activity catalyzes the degradation of the positive-sense RNA from the negative-sense DNA. The RT DNA polymerase then uses the negative-sense DNA as a template to form double-stranded proviral DNA. NRTIs resemble endogenous DNA triphosphates (dNTPs). These agents inhibit the formation of viral DNA by competitively inhibiting dNTPs for the reverse transcriptase enzyme and then, once incorporated into the HIV DNA strand, terminating the chain by preventing formation of 3′-5′-phosphodiester bonds. In vitro studies of antiretroviral activity against HIV-2 show NRTIs to have inhibitory effects against wild-type and mutant HIV-2 clones.[409]

The effective concentrations of (3TC), didanosine (ddI), zalcitabine (ddC), and stavudine (d4T) needed to inhibit wild-type HIV-2 are very similar to what is effective against wild-type HIV-1. Researchers initially thought that HIV-2 was inhibited by ZDV (AZT), but more recent studies suggested that wild-type HIV-2 harbors polymorphisms that confer resistance to ZDV and significantly reduces its susceptibility to this agent.[434]

HIV-1 NRTI resistance results from base changes within the reverse transcriptase genome, provoking amino acid substitutions in the transcribed enzyme that, in turn, confer structural changes at the enzyme active site or at associated functional areas. In HIV-1, each NRTI induces a predictable set of genetic alterations in a stepwise fashion. Primary mutations generally arise first, with secondary mutations developing during continued therapy. HIV-2 reverse transcriptase resistance can be conferred by mutations at sites homologous to those from HIV-1 reverse transcriptase NRTI resistance. In a cell-free system, site-directed mutagenesis of HIV-2 reverse transcriptase amino acid residues homologous to residues in HIV-1 reverse transcriptase reduces the sensitivity of mutated HIV-2 to NRTIs. HIV-1 reverse transcriptase mutation T215Y confers resistance to AZT. Clinical studies of HIV-2 clones taken from patients receiving NRTI-containing ART confirmed that phenotypic resistance is associated with genotypic changes. Amino acid changes associated with HIV-1 NRTI resistance at M184 and Q151M also seem to occur in HIV-2 and have been associated with NRTI treatment failure.[536] The significance of other mutations in the HIV-2 reverse transcriptase gene after exposure to NRTI is unknown; also not known is whether mutations in the NRTI gene accumulate in a stepwise fashion conferring increasing resistance to NRTI therapy.

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) bind to a hydrophobic pocket near the polymerase site of HIV-1 reverse transcriptase. HIV-2 appears to be intrinsically resistant to NNRTIs because of the Y188L polymorphism that occurs naturally in all HIV-2 isolates.[525] A change in amino acid at this site to Y188 renders HIV-2 reverse transcriptase sensitive to efavirenz and delavirdine but not to nevirapine.[231] Newer NNRTIs inhibit HIV-2 at effective concentrations at least 50 times higher than those that inhibit HIV-1. These concentrations limit the clinical utility of these agents against HIV-2. NNRTIs generally show little to no inhibition of HIV-2 in vitro at nontoxic levels.[568]

Studies indicated that some HIV-1 protease inhibitors also may inhibit HIV-2.[320] HIV-1, HIV-2, and SIV proteases belong to the aspartyl protease family. They are necessary to produce a mature retrovirus and are responsible for the posttranslational processing and cleavage of the polyprotein products Pr GAG and PrGAG-POL into functional core proteins and essential enzymes, including reverse transcriptase, integrase, and protease. Protease inhibitors competitively bind to the protease substrate site, with resulting production of immature noninfectious particles. Protease inhibitors are effective against HIV-2 protease, but have variable activity and accelerated genotypic resistance.[381] HIV-2 expresses natural polymorphisms in the protease gene that may be implicated in emergent drug resistance and accelerate time to development of protease inhibitor (PI) resistance. The pathways for HIV-2 protease drug resistance may differ from those for HIV-1[382]; they bind with a 10 to 100 times weaker affinity to HIV-2 protease, depending on the inhibitor.

Studies of individual protease inhibitors showed saquinavir, lopinavir, and darunavir to exert the same inhibitory activity against HIV-2 as against HIV-1,[40,68,126] whereas atazanavir, nelfinavir, ritonavir, and indinavir are less active against HIV-2 than HIV-1. Currently, the activity of tipranavir against HIV-2 is less well defined.

ART against HIV-2 with dual protease inhibitor or triple NRTI and protease–based therapy results in decreased HIV-2 viral load, increased $CD4^+$ T-cell count, and an improvement in AIDS–related symptoms. Clinical studies examining protease inhibitor susceptibility of HIV-2 clones from individuals both before and after receiving ART showed that indinavir, saquinavir, lopinavir, and tipranavir had full activity against wild-type HIV-2. Nelfinavir and amprenavir were less active against these clones. During ART with protease inhibitors, HIV-2 mutations were present, including in a patient who had been treated with lopinavir and who developed the proV47A, which translated into high-level resistance to indinavir, lopinavir, and amprenavir and hypersusceptibility to saquinavir. In isolates from the second patient, several different mutations resulted in decreased susceptibility to all protease inhibitors tested except saquinavir.[444] Naturally occurring polymorphisms or secondary mutations may decrease the activity of nelfinavir and amprenavir. On selection of primary resistance, mutations and preexisting secondary changes may play a role in the acquisition of a multi–protease inhibitor resistance phenotype in HIV-2. Although protease inhibitor resistance to HIV-1 is not completely understood, the HIV-2 protease active sites appear to differ, and this may confer natural HIV-2 resistance to ritonavir and indinavir. The D30N mutation as a natural polymorphism in HIV-2 reduces the efficacy of nelfinavir against HIV-2. A genotypic analysis of four HIV-2–infected individuals demonstrated the M46I mutation in all four individuals. V82F in one patient was associated with progressive decline in $CD4^+$ lymphocytes, despite treatment with indinavir. In a case report of two patients infected with both HIV-1 and HIV2, ART successfully suppressed HIV-1 but failed

to suppress HIV-2. Treatment-experienced HIV-2–infected patients achieved HIV-2 viral suppression on a ritonavir-boosted indinavir or lopinavir HAART regimen.[537]

In a cohort study on patients in Abidjan, Cote d'Ivoire, HIV-2–infected patients treated with dual ART or nelfinavir-containing regimens had incomplete HIV-2 viral suppression, whereas those treated with indinavir-containing regimens had complete and sustained viral suppression.[3] HIV-2 protease is 50 percent homologous to that of HIV-1. Researchers showed that mutations that confer resistance to protease inhibitors in HIV-1 were found in homologous positions in the HIV-2 protease. Natural polymorphism patterns were different in HIV-1 and HIV-2 proteases.

In particular, highly polymorphic positions of HIV-2 (positions 14, 40, 43, 46, 65, and 70) from ART-naïve patients were highly conserved in HIV-1 strains. The highest levels of natural polymorphism in HIV-1 were observed at positions 35, 37, 41, 62, 63, 64, 77, and 93. Three regions of both HIV-1 and HIV-2 protease were highly conserved: positions 25 through 32, which includes the protease active site, the flap region, and positions 78 through 87. In protease sequences from ART-naïve HIV-2–infected individuals, HIV-1 drug resistance–associated codons were identified in most cases (amino acids 36I, 46I, and 71V) or in all cases (amino acids 10V, 32I, 47V, and 73A). Fifty-nine percent of sequences from untreated HIV-2 patients harbored 46I. This finding corresponds in HIV-1 to a major drug resistance mutation to indinavir and to a minor drug resistance mutation to ritonavir, nelfinavir, and amprenavir, lopinavir/ritonavir, and atazanavir. These findings suggest a naturally reduced susceptibility of HIV-2 to these agents, even in an untreated individual.[100]

In vitro studies using serial cultured cells passaged in the presence of serially diluted protease inhibitors documented the emergence of drug resistance over time. At baseline, HIV-2 was found to express natural protease polymorphisms at several positions that could be implicated in emergent drug resistance. Certain mutations were selected with different protease inhibitors. Whereas treatment-associated mutations all were associated with homologous mutations in the HIV-1 protease, mutations in the HIV-2 sequence were identified that had not been associated previously with drug resistance in either HIV-1 or HIV-2.[381]

Integrase inhibitors raltegravir and elvitegravir have demonstrated activity against HIV-2 in vitro. Clinical response to raltegravir was reported in a patient with highly treatment-exposed HIV-2 infection.[168,571] CCR5 coreceptor antagonists are less effective in HIV-2 infection because of the ability of HIV-2 to use a broad range of coreceptors, including CXCR4, CCR5, CCT-5, GPR15, and CXCR6.[167,446,571] HIV-2 is naturally resistant to the action of fusion inhibitors.[425,567,571]

DISEASE MONITORING

Progression of disease and response to therapy are usually assessed by considering both immunologic and virologic criteria. Assays for the detection of HIV-2 RNA are not currently available commercially. However, in multiple studies examining HIV-2 viral load, an inverse relationship was found between $CD4^+$ lymphocyte counts and plasma HIV-1 and HIV-2 viral loads in singly infected individuals. Plasma HIV-1 levels were approximately 10 times higher than plasma HIV-2 levels throughout the comparable span of $CD4^+$ lymphocyte counts, and HIV-1 and HIV-2 regression lines had very similar slopes. Plasma HIV RNA levels were influenced independently by the type of HIV and $CD4^+$ lymphocyte counts, but not by sex or age. Among dually infected individuals, no correlation was found between $CD4^+$ counts and either plasma HIV-1 or HIV-2 levels.

The viral set point is significantly lower in HIV-2 infection than in HIV-1 infection. Recent HIV-2 seroconverters have plasma HIV RNA levels 28 times lower than those of recent HIV-1 seroconverters. For HIV-1, the viral set point has been demonstrated to be a very good predictor of outcome of disease. Differences between HIV-1 and HIV-2 in natural course, transmissibility, and epidemiologic features result, in large part, from lower viral levels in plasma.

HIV-2 infection may resemble a slowly progressive or nonprogressive HIV-1 infection with 86 to 95 percent of people infected with HIV-2 being long-term nonprogressors.[538] Survival of individuals with an undetectable HIV-2 viral load is similar to that of the general population. HIV-2 can cause immunosuppression leading to AIDS that is clinically indistinguishable from that caused by HIV-1. AIDS caused by HIV-2 often may be associated with lower viral load levels than HIV-1 (>10,000 copies/mL in HIV-2 in contrast to sometimes millions of copies/mL in HIV-1).[538] The finding that HIV-2 is contained to a higher degree than HIV-1 may reflect differences in the interaction between the immune system and the two HIV types or inherent differences in the biologic characteristics of the two viruses. Autologous neutralizing antibodies more frequently are found in HIV-2–infected individuals than in HIV-1–infected individuals and also may contribute to the slower disease progression in HIV-2. In a study in Guinea-Bissau of individuals dually infected with HIV-1 and HIV-2, the interaction between the two viruses and the host defense mechanisms was less predictable. Although an inverse relationship existed between levels of $CD4^+$ lymphocytes and proviral HIV-2 DNA in contrast to singly infected individuals, no correlation was found between levels of $CD4^+$ lymphocytes and plasma RNA or between HIV-1 and HIV-2 RNA load.[10]

A different study compared the $CD4^+$ cell counts and viral loads of individuals infected with HIV-1, HIV-2, and both HIV-1 and HIV-2.

$CD4^+$ cell counts were significantly higher in patients infected with HIV-2 in contrast to dually infected and HIV-1–infected individuals (582×10^6 cells/L, 165×10^6 cells/L, and 107×10^6 cells/L, respectively). HIV-1 viral loads were similar between HIV-1–infected and dually infected subjects, as were HIV-2 viral loads in HIV-2–infected and dually infected subjects. The HIV-1 viral loads were two times higher than HIV-2 viral loads.[265]

NEW REFERENCES SINCE THE SIXTH EDITION

13. Araujo A, Lima MA, Silva MT. Human T-lymphotropic virus 1 neurologic disease. *Curr Treat Options Neurol* 2008;**10**:193–200.
19. Atsumi E, Yara S, Higa F, et al. Influence of human T lymphotropic virus type I infection on the etiology of community-acquired pneumonia. *Intern Med* 2009;**48**:959–65.
24. Barrientos A, Lopez M, Sotomayor C, et al. Prevalence of human T-cell lymphotropic virus type 1 and 2 among patients with malignant hematological diseases in south Chile. *J Med Virol* 2011;**83**: 745–8.
25. Barrios CS, Abuerreish M, Lairmore MD, et al. Recombinant human T-cell leukemia virus types 1 and 2 Tax proteins induce high levels of CC-chemokines and downregulate CCR5 in human peripheral blood mononuclear cells. *Viral Immunol* 2011;**24**: 429–39.
31. Bazarbachi A, Plumelle Y, Carlos Ramos J, et al. Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes. *J Clin Oncol* 2010;**28**:4177–83.
33. Bazarbachi A, Suarez F, Fields P, et al. How I treat adult T-cell leukemia/lymphoma. *Blood* 2011;**118**:1736–45.
35. Beilke MA. Retroviral coinfections: HIV and HTLV—taking stock of more than a quarter century of research. *AIDS Res Hum Retroviruses* 2012;**28**:139–47.
40. Benard A, Damond F, Campa P, et al. Good response to lopinavir/ritonavir-containing antiretroviral regimens in antiretroviral-naive HIV-2-infected patients. *AIDS* 2009;**23**:1171–3.
45. Bertazzoni U, Turci M, Avesani F, et al. Intracellular localization and cellular factors interaction of HTLV-1 and HTLV-2 Tax proteins: similarities and functional differences. *Viruses* 2011;**3**: 541–60.
50. Biswas HH, Engstrom JW, Kaidarova Z, et al. Neurologic abnormalities in HTLV-I- and HTLV-II-infected individuals without overt myelopathy. *Neurology* 2009;**73**:781–9.
51. Biswas HH, Kaidarova Z, Garratty G, et al. Increased all-cause and cancer mortality in HTLV-II infection. *J Acquir Immune Defic Syndr* 2010;**54**:290–6.
52. Bittencourt AL, de Oliveira Mde F. Cutaneous manifestations associated with HTLV-1 infection. *Int J Dermatol* 2010;**49**: 1099–110.
54. Bittencourt AL, Barbosa HS, Vieira MD, et al. Adult T-cell leukemia/lymphoma (ATL) presenting in the skin: clinical, histological and immunohistochemical features of 52 cases. *Acta Oncol* 2009;**48**:598–604.
61. Bonin S, Tothova SM, Barbazza R, et al. Evidence of multiple infectious agents in mycosis fungoides lesions. *Exp Mol Pathol* 2010;**89**:46–50.
64. Branson BM. The future of HIV testing. *J Acquir Immune Defic Syndr* 2010;**55**(Suppl. 2):S102–5.
66. Brites C, Sampalo J, Oliveira A. HIV/human T-cell lymphotropic virus coinfection revisited: impact on AIDS progression. *AIDS Rev* 2009;**11**:8–16.
68. Brower ET, Bacha UM, Kawasaki Y, et al. Inhibition of HIV-2 protease by HIV-1 protease inhibitors in clinical use. *Chem Biol Drug Des* 2008;**71**:298–305.
70. Burgard M, Jasseron C, Matheron S, et al. Mother-to-child transmission of HIV-2 infection from 1986 to 2007 in the ANRS French Perinatal Cohort EPF-CO1. *Clin Infect Dis* 2010;**51**: 833–43.
72. Cardoso LS, Oliveira SC, Goes AM, et al. *Schistosoma mansoni* antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. *Clin Exp Immunol* 2010;**160**:266–74.
74. Carvalho AC, Valadas E, Franca L, et al. Population mobility and the changing epidemics of HIV-2 in Portugal. *HIV Med* 2011;**13**:219–25.
91. Chevalier SA, Durand S, Dasgupta A, et al. The transcription profile of Tax-3 is more similar to Tax-1 than Tax-2: insights into HTLV-3 potential leukemogenic properties. *PLoS One* 2012;**7**: e41003.
92. Choksi M, Joseph AJ, Simon E, et al. The association of HTLV-1 infection, persistent intestinal infection with *Strongyloides stercoralis* and gastrointestinal lymphoma. *BMJ Case Rep* 2009. pii: bcr07.2008.0373. doi: 10.1136/bcr.07.2008.0373. (Epub ahead of print).
104. Creek TL, Kim A, Lu L, et al. Hospitalization and mortality among primarily nonbreastfed children during a large outbreak of diarrhea and malnutrition in Botswana, 2006. *J Acquir Immune Defic Syndr* 2009;**53**:14–19.
105. Croda MG, de Oliveira AC, Vergara MP, et al. Corticosteroid therapy in TSP/HAM patients: the results from a 10 years open cohort. *J Neurol Sci* 2008;**269**:133–7.
107. da Silva ZJ, Oliveira I, Andersen A, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *AIDS* 2008;**22**: 1195–202.
110. Damond F, Benard A, Balotta C, et al. An international collaboration to standardize HIV-2 viral load assays: results from the 2009 ACHI(E)V(2E) quality control study. *J Clin Microbiol* 2011;**49**: 3491–7.
119. de Oliveira Mde F, Fatal PL, Primo JR, et al. Infective dermatitis associated with human T-cell lymphotropic virus type 1: evaluation of 42 cases observed in Bahia, Brazil. *Clin Infect Dis* 2012;**54**: 1714–19.
121. de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. *Trends Microbiol* 2008;**16**:588–95.
122. de Silva TI, van Tienen C, Onyango C, et al. Population dynamics of HIV-2 in rural Guinea-Bissau: comparison with HIV-1 and ongoing transmission at the heart of the epidemic. *AIDS* 2013; **27**:124–34.
126. Desbois D, Roquebert B, Peytavin G, et al. In vitro phenotypic susceptibility of human immunodeficiency virus type 2 clinical isolates to protease inhibitors. *Antimicrob Agents Chemother* 2008;**52**:1545–8.
131. Doueiri R, Anupam R, Kvaratskhelia M, et al. Comparative host protein interactions with HTLV-1 p30 and HTLV-2 p28: insights into difference in pathobiology of human retroviruses. *Retrovirology* 2012;**9**:64.
135. Duong YT, Jia H, Lust JA, et al. Short communication: absence of evidence of HTLV-3 and HTLV-4 in patients with large granular lymphocyte (LGL) leukemia. *AIDS Res Hum Retroviruses* 2008;**24**:1503–5.
137. Einsiedel L, Fernandes L, Spelman T, et al. Bronchiectasis is associated with human T-lymphotropic virus 1 infection in an Indigenous Australian population. *Clin Infect Dis* 2011;**54**:43–50.
140. El Hajj H, El-Sabban M, Hasegawa H, et al. Therapy-induced selective loss of leukemia-initiating activity in murine adult T cell leukemia. *J Exp Med* 2010;**207**:2785–92.
143. Enose-Akahata Y, Matsuura E, Tanaka Y, et al. Minocycline modulates antigen-specific CTL activity through inactivation of mononuclear phagocytes in patients with HTLV-I associated neurologic disease. *Retrovirology* 2012;**9**:16.
144. Esbjornsson J, Mansson F, Kvist A, et al. Inhibition of HIV-1 disease progression by contemporaneous HIV-2 infection. *N Engl J Med* 2012;**367**:224–32.
145. Eshima N, Iwata O, Iwata S, et al. Age and gender specific prevalence of HTLV-1. *J Clin Virol* 2009;**45**:135–8.
146. Etenna SL, Caron M, Besson G, et al. New insights into prevalence, genetic diversity, and proviral load of human T-cell leukemia virus types 1 and 2 in pregnant women in Gabon in equatorial central Africa. *J Clin Microbiol* 2008;**46**:3607–14.
149. Ferreira AS, Costa CM, Dantas IK, et al. Polymyositis in childhood as clinical manifestation associated with HTLV-1. *Arq Neuropsiquiatr* 2011;**68**:962–4.
160. Furtado Mdos S, Andrade RG, et al. Monitoring the HTLV-1 proviral load in the peripheral blood of asymptomatic carriers and patients with HTLV-associated myelopathy/tropical spastic paraparesis from a Brazilian cohort: ROC curve analysis to establish the threshold for risk disease. *J Med Virol* 2012;**84**:664–71.
168. Garrett N, Xu L, Smit E, et al. Raltegravir treatment response in an HIV-2 infected patient: a case report. *AIDS* 2008;**22**:1091–2.
170. Gascon MR, Capitao CG, Casseb J, et al. Prevalence of anxiety, depression and quality of life in HTLV-1 infected patients. *Braz J Infect Dis* 2012;**15**:578–82.
183. Gottlieb GS, Badiane NM, Hawes SE, et al. Emergence of multiclass drug-resistance in HIV-2 in antiretroviral-treated individuals in Senegal: implications for HIV-2 treatment in resource-limited West Africa. *Clin Infect Dis* 2009;**48**:476–83.
184. Gottlieb GS, Eholie SP, Nkengasong JN, et al. A call for randomized controlled trials of antiretroviral therapy for HIV-2 infection in West Africa. *AIDS* 2008;**22**:2069–72; discussion 2073–2064.

188. Gourlay AJ, van Tienen C, Dave SS, et al. Clinical predictors cannot replace biological predictors in HIV-2 infection in a community setting in West Africa. *Int J Infect Dis* 2012;**16**:e337–43.
191. Grassi MF, Olavarria VN, Kruschewsky Rde A, et al. Human T cell lymphotropic virus type 1 (HTLV-1) proviral load of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients according to new diagnostic criteria of HAM/TSP. *J Med Virol* 2011;**83**:1269–74.
194. Gualco G, Chioato L, Weiss LM, et al. Analysis of human T-cell lymphotropic virus in CD25$^+$ anaplastic large cell lymphoma in children. *Am J Clin Pathol* 2009;**132**:28–33.
195. Guimaraes de Souza V, Lobato Martins M, Carneiro-Proietti AB, et al. High prevalence of HTLV-1 and 2 viruses in pregnant women in Sao Luis, state of Maranhao, Brazil. *Rev Soc Bras Med Trop* 2012;**45**:159–62.
207. Hawes SE, Sow PS, Stern JE, et al. Lower levels of HIV-2 than HIV-1 in the female genital tract: correlates and longitudinal assessment of viral shedding. *AIDS* 2008;**22**:2517–25.
213. Higuchi M, Fujii M. Distinct functions of HTLV-1 Tax1 from HTLV-2 Tax2 contribute key roles to viral pathogenesis. *Retrovirology* 2009;**6**:117.
221. Hishizawa M, Kanda J, Utsunomiya A, et al. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood* 2010;**116**:1369–76.
227. Hodson A, Bain B, Fields P, et al. Smouldering adult T-cell leukaemia/lymphoma (ATLL) is not associated with an increased risk of aggressive ATLL in HTLV-1 positive patients. Fifty-second annual meeting of the American Society of Hematology, Orlando, Fla, December 4-7, 2010.
229. Ibe S, Yokomaku Y, Shiino T, et al. HIV-2 CRF01_AB: first circulating recombinant form of HIV-2. *J Acquir Immune Defic Syndr* 2010;**54**:241–7.
234. Ishitsuka K, Ikeda S, Utsunomiya A, et al. Smouldering adult T-cell leukaemia/lymphoma: a follow-up study in Kyushu. *Br J Haematol* 2008;**143**:442–4.
235. Ishitsuka K, Tamura K. Treatment of adult T-cell leukemia/lymphoma: past, present, and future. *Eur J Haematol* 2008;**80**:185–96.
238. Iwanaga M, Watanabe T, Utsunomiya A, et al. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. *Blood* 2010;**116**:1211–19.
247. Kamimura T, Miyamoto T, Kawano N, et al. Successful treatment by donor lymphocyte infusion of adult T-cell leukemia/lymphoma relapse following allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2012;**95**:725–30.
256. Katsuya H, Yamanaka T, Ishitsuka K, et al. Prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma. *J Clin Oncol* 2012;**30**:1635–40.
257. Kawabata T, Higashimoto I, Takashima H, et al. Human T-lymphotropic virus type I (HTLV-I)-specific CD8$^+$ cells accumulate in the lungs of patients infected with HTLV-I with pulmonary involvement. *J Med Virol* 2012;**84**:1120–7.
259. Kchour G, Tarhini M, Kooshyar MM, et al. Phase 2 study of the efficacy and safety of the combination of arsenic trioxide, interferon alpha, and zidovudine in newly diagnosed chronic adult T-cell leukemia/lymphoma (ATL). *Blood* 2009;**113**:6528–32.
270. Kuhn L, Aldrovandi G. Survival and health benefits of breastfeeding versus artificial feeding in infants of HIV-infected women: developing versus developed world. *Clin Perinatol* 2010;**37**:843–62, x.
297. Lotan I, Khlebtovsky A, Inbar E, et al. Primary brain T-cell lymphoma in an HTLV-1 serologically positive male. *J Neurol Sci* 2012;**314**:163–5.
304. Magri MC, Morimoto HK, de Macedo Brigido LF, et al. Long terminal repeat sequence analysis of HTLV-2 molecular variants identified in Southern Brazil. *AIDS Res Hum Retroviruses* 2010;**26**:1327–31.
307. Mahieux R, Gessain A. HTLV-3/STLV-3 and HTLV-4 viruses: discovery, epidemiology, serology and molecular aspects. *Viruses* 2011;**3**:1074–90.
316. Mansson F, Biague A, da Silva ZJ, et al. Prevalence and incidence of HIV-1 and HIV-2 before, during and after a civil war in an occupational cohort in Guinea-Bissau, West Africa. *AIDS* 2009;**23**:1575–82.
324. Martin F, Castro H, Gabriel C, et al. Ciclosporin A proof of concept study in patients with active, progressive HTLV-1 associated myelopathy/tropical spastic paraparesis. *PLoS Negl Trop Dis* 2012;**6**:e1675.
327. Matsuoka M, Green PL. The HBZ gene, a key player in HTLV-1 pathogenesis. *Retrovirology* 2009;**6**:71.
344. Montes M, Sanchez C, Verdonck K, et al. Regulatory T cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to *Strongyloides stercoralis* antigen. *PLoS Negl Trop Dis* 2009;**3**:e456.
364. Mylonas I, Bruning A, Kainer F, et al. HTLV infection and its implication in gynaecology and obstetrics. *Arch Gynecol Obstet* 2010;**282**:493–501.
372. Nascimento MC, Primo J, Bittencourt A, et al. Infective dermatitis has similar immunological features to human T lymphotropic virus-type 1-associated myelopathy/tropical spastic paraparesis. *Clin Exp Immunol* 2009;**156**:455–62.
373. Nasrullah M, Wesolowski LG, Meyer WA 3rd, et al. Performance of a fourth-generation HIV screening assay and an alternative HIV diagnostic testing algorithm. *AIDS* 2012 (Epub ahead of print).
382. Ntemgwa ML, d'Aquin Toni T, Brenner BG, et al. Antiretroviral drug resistance in human immunodeficiency virus type 2. *Antimicrob Agents Chemother* 2009;**53**:3611–19.
398. Pacifico LG, Marinho FA, Fonseca CT, et al. *Schistosoma mansoni* antigens modulate experimental allergic asthma in a murine model: a major role for CD4+ CD25+ Foxp3+ T cells independent of interleukin-10. *Infect Immun* 2009;**77**:98–107.
399. Padua E, Almeida C, Nunes B, et al. Assessment of mother-to-child HIV-1 and HIV-2 transmission: an AIDS reference laboratory collaborative study. *HIV Med* 2009;**10**:182–90.
400. Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. *Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States*. 2012, U.S. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/Ivguidelines/PerinatalGL.pdf.
405. Pedroso C, Netto EM, Weyll N, et al. Coinfection by HIV-1 and human lymphotropic virus type 1 in Brazilian children is strongly associated with a shorter survival time. *J Acquir Immune Defic Syndr* 2011;**57**(Suppl. 3):S208–11.
407. Pepin J, Labbe AC. Noble goals, unforeseen consequences: control of tropical diseases in colonial Central Africa and the iatrogenic transmission of blood-borne viruses. *Trop Med Int Health* 2008;**13**:744–53.
408. Pepin J, Labbe AC, Mamadou-Yaya F, et al. Iatrogenic transmission of human T cell lymphotropic virus type 1 and hepatitis C virus through parenteral treatment and chemoprophylaxis of sleeping sickness in colonial Equatorial Africa. *Clin Infect Dis* 2010;**51**:777–84.
410. Perzova R, Benz P, Abbott L, et al. Short communication: no evidence of HTLV-3 and HTLV-4 infection in New York State subjects at risk for retroviral infection. *AIDS Res Hum Retroviruses* 2010;**26**:1229–31.
413. Poetker SK, Porto AF, Giozza SP, et al. Clinical manifestations in individuals with recent diagnosis of HTLV type I infection. *J Clin Virol* 2011;**51**:54–8.
436. Ren T, Dong W, Takahashi Y, et al. HTLV-2 Tax immortalizes human CD4+ memory T lymphocytes by oncogenic activation and dysregulation of autophagy. *J Biol Chem* 2012;**287**:34683–93.
438. Ribeiro MA, Martins ML, Teixeira C, et al. Blocking vertical transmission of human T cell lymphotropic virus type 1 and 2 through breastfeeding interruption. *Pediatr Infect Dis J* 2012;**31**:1139–43.
447. Roquebert B, Damond F, Collin G, et al. HIV-2 integrase gene polymorphism and phenotypic susceptibility of HIV-2 clinical isolates to the integrase inhibitors raltegravir and elvitegravir in vitro. *J Antimicrob Chemother* 2008;**62**:914–20.
452. Saito M, Matsuzaki T, Satou Y, et al. In vivo expression of the HBZ gene of HTLV-1 correlates with proviral load, inflammatory markers and disease severity in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). *Retrovirology* 2009;**6**:19.

479. Soares RS, Tendeiro R, Foxall RB, et al. Cell-associated viral burden provides evidence of ongoing viral replication in aviremic HIV-2-infected patients. *J Virol* 2010;**85**:2429–38.
483. Steinfort DP, Brady S, Weisinger HS, et al. Bronchiectasis in Central Australia: a young face to an old disease. *Respir Med* 2008;**102**:574–8.
490. Sundberg MA, Costa D, Orge G, et al. Helminthic infection and the risk of neurologic disease progression in HTLV-1. *J Clin Virol* 2012;**53**:251–5.
492. Switzer WM, Salemi M, Qari SH, et al. Ancient, independent evolution and distinct molecular features of the novel human T-lymphotropic virus type 4. *Retrovirology* 2009;**6**:9.
498. Takasaki Y, Iwanaga M, Imaizumi Y, et al. Long-term study of indolent adult T-cell leukemia-lymphoma. *Blood* 2010;**115**:4337–43.
499. Takenouchi H, Umeki K, Sasaki D, et al. Defective human T-lymphotropic virus type 1 provirus in asymptomatic carriers. *Int J Cancer* 2010;**128**:1335–43.
500. Takeuchi H, Takahashi M, Norose Y, et al. Transformation of breast milk macrophages by HTLV-I: implications for HTLV-I transmission via breastfeeding. *Biomed Res* 2010;**31**:53–61.
513. Torian LV, Eavey JJ, Punsalang AP, et al. HIV type 2 in New York City, 2000-2008. *Clin Infect Dis* 2010;**51**:1334–42.
514. Torian LV, Forgione LA, Punsalang AE, et al. Comparison of multispot EIA with western blot for confirmatory serodiagnosis of HIV. *J Clin Virol* 2011;**52**(Suppl. 1):S41–4.
519. Trevino A, Parra P, Bar-Magen T, et al. Antiviral effect of raltegravir on HTLV-1 carriers. *J Antimicrob Chemother* 2012;**67**:218–21.
529. Ueno S, Umeki K, Takajo I, et al. Proviral loads of human T-lymphotropic virus type 1 in asymptomatic carriers with different infection routes. *Int J Cancer* 2011;**130**:2318–26.
538. van der Loeff MF, Larke N, Kaye S, et al. Undetectable plasma viral load predicts normal survival in HIV-2-infected people in a West African village. *Retrovirology* 2010;**7**:46.
541. van Dooren S, Salemi M, Vandamme AM. Dating the origin of the African human T-cell lymphotropic virus type-i (HTLV-I) subtypes. *Mol Biol Evol* 2001;**18**:661–71.
542. van Tienen C, Jakobsen M, Schim van der Loeff M. Stopping breastfeeding to prevent vertical transmission of HTLV-1 in resource-poor settings: beneficial or harmful? *Arch Gynecol Obstet* 2012;**286**:255–6.
541. van Tienen C, van der Loeff MF, Peterson I, et al. HTLV-1 in rural Guinea-Bissau: prevalence, incidence and a continued association with HIV between 1990 and 2007. *Retrovirology* 2010;**7**:50.
551. von Glehn F, Jarius S, Penalva de Oliveira AC, et al. Aquaporin-4 antibodies are not related to HTLV-1 associated myelopathy. *PLoS One* 2012;**7**:e39372.
571. Xu L, Anderson J, Garrett N, et al. Dynamics of raltegravir resistance profile in an HIV type 2-infected patient. *AIDS Res Hum Retroviruses* 2009;**25**:843–7.
573. Yamaguchi J, Vallari A, Ndembi N, et al. HIV type 2 intergroup recombinant identified in Cameroon. *AIDS Res Hum Retroviruses* 2008;**24**:86–91.
580. Yamamoto K, Utsunomiya A, Tobinai K, et al. Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *J Clin Oncol* 2010;**28**:1591–8.

The full reference list for this chapter is available at expertconsult.com.

191B ■ HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME

Filiz O. Seeborg • Mary E. Paul • William T. Shearer

Human immunodeficiency virus (HIV) infection continues to have a significant clinical impact on our world.[249] According to the Joint United Nations Programme on HIV/AIDS (UNAIDS), 33 million people were estimated to be living with HIV or acquired immunodeficiency syndrome (AIDS) at the end of 2009 (Table 191B-1).[127] Around the world, 2.6 million people were newly infected with HIV in 2009. Of these newly infected individuals, approximately 370,000 cases (12%) were children younger than 15 years of age. The number of newly diagnosed HIV infections in the United States has remained unchanged from 2006 to 2009 at 40,000 to 50,000 per year. In 2009, young people aged 13 to 29 years accounted for 39 percent of all new HIV infections.[47] Racial and ethnic disparities among newly HIV-infected individuals continue to be observed worldwide, including in developed nations such as the United States. Blacks had 44 percent of all new HIV infections in 2009, with the HIV infection rate among blacks in 2009 almost eight times as high as that of whites (69.9 vs. 9.1 per 100,000). Hispanics had 20 percent of all new HIV infections in 2009 despite representing only 16 percent of the total U.S. population.[44] Between 2006 and 2009, each year the largest number of new HIV infections was among white men who have sex with men (MSM), followed closely by black MSM and Hispanic MSM.[44,47] Despite the advances that have been made in care during the decades since the HIV infection first was described, HIV/AIDS continues its worldwide spread.[127] Research shows that racial and ethnic disparities in health are often a marker of a range of broader social and economic challenges. Eradicating racial disparities and preventing HIV infections in the changing face of the U.S. and global epidemic will be the medical and public health challenges for this century.[45]

DEFINITION AND STAGING

Pediatric AIDS was reported to the Centers for Disease Control and Prevention (CDC) first in 1982; AIDS was first reported in adults in 1981.[40] The first definitions of pediatric AIDS were published in 1985. In 1994, and then in 2008, the classification system again was revised to include more advanced viral diagnostic technology and a system for staging pediatric HIV disease that involved both clinical and immunologic axes.[36,180] Table 191B-2 outlines the clinical axis of the staging system, which is

REFERENCES

1. Abbaszadegan MR, Gholamin M, Tabatabaee A, et al. Prevalence of human T-lymphotropic virus type 1 among blood donors from Mashhad, Iran. *J Clin Microbiol* 2003;**41**:2593–5.
2. Aboud M, Rosner M, Dombrovsky A, et al. Interactions between retroviruses and environmental carcinogens and their role in animal and human leukemogenesis. *Leuk Res* 1992;**16**:1061–9.
3. Adje-Toure CA, Cheingsong R, Garcia-Lerma JG, et al. Antiretroviral therapy in HIV-2-infected patients: changes in plasma viral load, $CD4^+$ cell counts, and drug resistance profiles of patients treated in Abidjan, Cote d'Ivoire. *AIDS* 2003;**17**(Suppl. 3): S49–54.
4. Adjorlolo-Johnson G, De Cock KM, Ekpini E, et al. Prospective comparison of mother-to-child transmission of HIV-1 and HIV-2 in Abidjan, Ivory Coast. *JAMA* 1994;**272**:462–6.
5. Albrecht B, Collins ND, Burniston MT, et al. Human T-lymphotropic virus type 1 open reading frame I p12(I) is required for efficient viral infectivity in primary lymphocytes. *J Virol* 2000;**74**:9828–35.
6. Alcantara LC, Shindo N, Van Dooren S, et al. Brazilian HTLV type 2a strains from intravenous drug users (IDUs) appear to have originated from two sources: Brazilian Amerindians and European/North American IDUs. *AIDS Res Hum Retroviruses* 2003;**19**: 519–23.
7. Andersson S, Dias F, Mendez PJ, et al. HTLV-I and -II infections in a nationwide survey of pregnant women in Guinea-Bissau, West Africa. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**15**: 320–2.
8. Anderson S, Shugars DC, Swanstrom R, et al. Nef from primary isolates of human immunodeficiency virus type 1 suppresses surface CD4 expression in human and mouse T cells. *J Virol* 1993;**67**:4923–31.
9. Andersson S, Tessema HG, Wahren B. Is there a focus of HTLV-II infections in the Horn of Africa? *J Acquir Immune Defic Syndr* 1999;**21**:353–4.
10. Andersson S, Norrgren H, da Silva Z, et al. Plasma viral load in HIV-1 and HIV-2 singly and dually infected individuals in Guinea-Bissau, West Africa: significantly lower plasma virus set point in HIV-2 infection than in HIV-1 infection. *Arch Intern Med* 2000;**160**:3286–93.
11. Anonymous. Major prognostic factors of patients with adult T-cell leukemia-lymphoma: a cooperative study. Lymphoma Study Group (1984-1987). *Leuk Res* 1991;**15**:81–90.
12. Aquije M, Ballona R. [Dermatitis infectiva asociada a HTLV-1 en el Servicio de Dermatologia del Instituto de Salud del Nino]. *Folia Dermatol Peruana* 2005;**13**:27–31.
13. Araujo A, Lima MA, Silva MT. Human T-lymphotropic virus 1 neurologic disease. *Curr Treat Options Neurol* 2008;**10**:193–200.
14. Ariyoshi K, Berry N, Wilkins A, et al. A community-based study of human immunodeficiency virus type 2 provirus load in rural village in West Africa. *J Infect Dis* 1996;**173**:245–8.
15. Ariyoshi K, Jaffar S, Alabi AS, et al. Plasma RNA viral load predicts the rate of CD4 T cell decline and death in HIV-2-infected patients in West Africa. *AIDS* 2000;**14**:339–44.
16. Ariyoshi K, Schim van der Loeff M, Sabally S, et al. Does HIV-2 infection provide cross-protection against HIV-1 infection? *AIDS* 1997;**11**:1053–4.
17. Armah HB, Narter-Olaga EG, Adjei AA, et al. Seroprevalence of human T-cell lymphotropic virus type I among pregnant women in Accra, Ghana. *J Med Microbiol* 2006;**55**:765–70.
18. Asante-Appiah E, Skalka AM. Molecular mechanisms in retrovirus DNA integration. *Antiviral Res* 1997;**36**:139–56.
19. Atsumi E, Yara S, Higa F, et al. Influence of human T lymphotropic virus type I infection on the etiology of community-acquired pneumonia. *Intern Med* 2009;**48**:959–65.
20. Banerjee P, Rochford R, Antel J, et al. Proinflammatory cytokine gene induction by human T-cell leukemia virus type 1 (HTLV-1) and HTLV-2 Tax in primary human glial cells. *J Virol* 2007;**81**: 1690–700.
21. Bangham CR. The immune control and cell-to-cell spread of human T-lymphotropic virus type 1. *J Gen Virol* 2003;**84**: 3177–89.
22. Barbosa HS, Bittencourt AL, Barreto de Araujo I, et al. Adult T-cell leukemia/lymphoma in northeastern Brazil: a clinical, histopathologic, and molecular study. *J Acquir Immune Defic Syndr* 1999;**21**:65–71.
23. Barmak K, Harhaj E, Grant C, et al. Human T cell leukemia virus type I-induced disease: pathways to cancer and neurodegeneration. *Virology* 2003;**308**:1–12.
24. Barrientos A, Lopez M, Sotomayor C, et al. Prevalence of human T-cell lymphotropic virus type 1 and 2 among patients with malignant hematological diseases in south Chile. *J Med Virol* 2011;**83**: 745–8.
25. Barrios CS, Abuerreish M, Lairmore MD, et al. Recombinant human T-cell leukemia virus types 1 and 2 Tax proteins induce high levels of CC-chemokines and downregulate CCR5 in human peripheral blood mononuclear cells. *Viral Immunol* 2011;**24**: 429–39.
26. Bartholomew C, Blattner W, Cleghorn F. Progression to AIDS in homosexual men co-infected with HIV and HTLV-I in Trinidad. *Lancet* 1987;**2**:1469.
27. Bartholomew C, Saxinger WC, Clark JW, et al. Transmission of HTLV-I and HIV among homosexual men in Trinidad. *JAMA* 1987;**257**:2604–8.
28. Bassani S, Lopez M, Toro C, et al. Influence of human T cell lymphotropic virus type 2 coinfection on virological and immunological parameters in HIV type 1-infected patients. *Clin Infect Dis* 2007;**44**:105–10.
29. Bazarbachi A, Ghez D, Lepelletier Y, et al. New therapeutic approaches for adult T-cell leukaemia. *Lancet Oncol* 2004;**5**: 664–72.
30. Bazarbachi A, El-Sabban ME, Nasr R, et al. Arsenic trioxide and interferon-alpha synergize to induce cell cycle arrest and apoptosis in human T-cell lymphotropic virus type I-transformed cells. *Blood* 1999;**93**:278–83.
31. Bazarbachi A, Plumelle Y, Carlos Ramos J, et al. Meta-analysis on the use of zidovudine and interferon-alfa in adult T-cell leukemia/lymphoma showing improved survival in the leukemic subtypes. *J Clin Oncol* 2010;**28**:4177–83.
32. Bazarbachi A, Saal F, Laroche L, et al. HTLV-1 provirus and mycosis fungoides. *Science* 1993;**259**:1470–1.
33. Bazarbachi A, Suarez F, Fields P, et al. How I treat adult T-cell leukemia/lymphoma. *Blood* 2011;**118**:1736–45.
34. Beby-Defaux A, Frugier F, Bourgoin A, et al. Nucleotide sequence analysis of human T-cell lymphotropic virus type I pX and LTR regions from patients with sicca syndrome. *J Med Virol* 1999;**59**: 245–55.
35. Beilke MA. Retroviral coinfections: HIV and HTLV—taking stock of more than a quarter century of research. *AIDS Res Hum Retroviruses* 2012;**28**:139–47.
36. Beilke MA, Japa S, Moeller-Hadi C, et al. Tropical spastic paraparesis/human T leukemia virus type 1-associated myelopathy in HIV type 1-coinfected patients. *Clin Infect Dis* 2005;**41**: e57–63.
37. Beilke MA, Theall KP, O'Brien M, et al. Clinical outcomes and disease progression among patients coinfected with HIV and human T lymphotropic virus types 1 and 2. *Clin Infect Dis* 2004;**39**: 256–63.
38. Bellon M, Datta A, Brown M, et al. Increased expression of telomere length regulating factors TRF1, TRF2 and TIN2 in patients with adult T-cell leukemia. *Int J Cancer* 2006;**119**:2090–7.
39. Belza MJ. Prevalence of HIV, HTLV-I and HTLV-II among female sex workers in Spain, 2000-2001. *Eur J Epidemiol* 2004;**19**: 279–82.
40. Benard A, Damond F, Campa P, et al. Good response to lopinavir/ritonavir-containing antiretroviral regimens in antiretroviral-naive HIV-2-infected patients. *AIDS* 2009;**23**:1171–3.
41. Berger JR, Svenningsson A, Raffanti S, et al. Tropical spastic paraparesis-like illness occurring in a patient dually infected with HIV-1 and HTLV-II. *Neurology* 1991;**41**:85–7.
42. Berneman ZN. HTLV Molecular biology and pathogenesis: Airline Center, Warrenton, VA, USA. 17-19 March 2000. *Leukemia* 2001;**15**:647–54.
43. Berry N, Ariyoshi K, Balfe P, et al. Sequence specificity of the human immunodeficiency virus type 2 (hiv-2) long terminal repeat u3 region in vivo allows subtyping of the principal hiv-2 viral subtypes a and b. *AIDS Res Hum Retroviruses* 2001;**17**:263–7.
44. Berry N, Jaffar S, Schim van der Loeff M, et al. Low level viremia and high CD4% predict normal survival in a cohort of HIV

type-2-infected villagers. *AIDS Res Hum Retroviruses* 2002;**18**: 1167–73.
45. Bertazzoni U, Turci M, Avesani F, et al. Intracellular localization and cellular factors interaction of HTLV-1 and HTLV-2 Tax proteins: similarities and functional differences. *Viruses* 2011;**3**: 541–60.
46. Bessinger R, Beilke M, Kissinger P, et al. Retroviral coinfections at a New Orleans HIV outpatient clinic. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**14**:67–71.
47. Biggar RJ, Ng J, Kim N, et al. Human leukocyte antigen concordance and the transmission risk via breast-feeding of human T cell lymphotropic virus type I. *J Infect Dis* 2006;**193**:277–82.
48. Biggar RJ, Taylor ME, Neel JV, et al. Genetic variants of human T-lymphotrophic virus type II in American Indian groups. *Virology* 1996;**216**:165–73.
49. Biglione MM, Pizarro M, Salomon HE, et al. A possible case of myelopathy/tropical spastic paraparesis in an Argentinian woman with human T lymphocyte virus type II. *Clin Infect Dis* 2003; **37**:456–8.
50. Biswas HH, Engstrom JW, Kaidarova Z, et al. Neurologic abnormalities in HTLV-I- and HTLV-II-infected individuals without overt myelopathy. *Neurology* 2009;**73**:781–9.
51. Biswas HH, Kaidarova Z, Garratty G, et al. Increased all-cause and cancer mortality in HTLV-II infection. *J Acquir Immune Defic Syndr* 2010;**54**:290–6.
52. Bittencourt AL, de Oliveira Mde F. Cutaneous manifestations associated with HTLV-1 infection. *Int J Dermatol* 2010;**49**: 1099–110.
53. Bittencourt AL, Primo J, Oliveira MF. Manifestations of the human T-cell lymphotropic virus type I infection in childhood and adolescence. *J Pediatr (Rio J)* 2006;**82**:411–20.
54. Bittencourt AL, Barbosa HS, Vieira MD, et al. Adult T-cell leukemia/lymphoma (ATL) presenting in the skin: clinical, histological and immunohistochemical features of 52 cases. *Acta Oncol* 2009;**48**:598–604.
55. Bittencourt AL, Dourado I, Filho PB, et al. Human T-cell lymphotropic virus type 1 infection among pregnant women in northeastern Brazil. *J Acquir Immune Defic Syndr* 2001;**26**:490–4.
56. Bittencourt AL, Oliveira Mde F, Brites C, et al. Histopathological and immunohistochemical studies of infective dermatitis associated with HTLV-I. *Eur J Dermatol* 2005;**15**:26–30.
57. Blaak H, Boers PH, Gruters RA, et al. CCR5, GPR15, and CXCR6 are major coreceptors of human immunodeficiency virus type 2 variants isolated from individuals with and without plasma viremia. *J Virol* 2005;**79**:1686–700.
58. Black FL, Biggar RJ, Neel JV, et al. Endemic transmission of HTLV type II among Kayapo Indians of Brazil. *AIDS Res Hum Retroviruses* 1994;**10**:1165–71.
59. Blank A, Herrera M, Lourido MA, et al. Infective dermatitis in Colombia. *Lancet* 1995;**346**:710.
60. Boni J, Bisset LR, Burckhardt JJ, et al. Prevalence of human T-cell leukemia virus types I and II in Switzerland. *J Med Virol* 2004;**72**: 328–37.
61. Bonin S, Tothova SM, Barbazza R, et al. Evidence of multiple infectious agents in mycosis fungoides lesions. *Exp Mol Pathol* 2010;**89**:46–50.
62. Bonis J, Baillou A, Barin F, et al. Discrimination between human T-cell lymphotropic virus type I and II (HTLV-I and HTLV-II) infections by using synthetic peptides representing an immunodominant region of the core protein (p19) of HTLV-I and HTLV-II. *J Clin Microbiol* 1993;**31**:1481–5.
63. Bonis J, Verdier M, Dumas M, et al. Low human T cell leukemia virus type II seroprevalence in Africa. *J Infect Dis* 1994;**169**: 225–7.
64. Branson BM. The future of HIV testing. *J Acquir Immune Defic Syndr* 2010;**55**(Suppl. 2):S102–5.
65. Briggs NC, Battjes RJ, Cantor KP, et al. Seroprevalence of human T cell lymphotropic virus type II infection, with or without human immunodeficiency virus type 1 coinfection, among US intravenous drug users. *J Infect Dis* 1995;**172**:51–8.
66. Brites C, Sampalo J, Oliveira A. HIV/human T-cell lymphotropic virus coinfection revisited: impact on AIDS progression. *AIDS Rev* 2009;**11**:8–16.
67. Brites C, Alencar R, Gusmao R, et al. Co-infection with HTLV-1 is associated with a shorter survival time for HIV-1-infected patients in Bahia, Brazil. *AIDS* 2001;**15**:2053–5.
68. Brower ET, Bacha UM, Kawasaki Y, et al. Inhibition of HIV-2 protease by HIV-1 protease inhibitors in clinical use. *Chem Biol Drug Des* 2008;**71**:298–305.
69. Buggage RR. Ocular manifestations of human T-cell lymphotropic virus type 1 infection. *Curr Opin Ophthalmol* 2003;**14**: 420–5.
70. Burgard M, Jasseron C, Matheron S, et al. Mother-to-child transmission of HIV-2 infection from 1986 to 2007 in the ANRS French Perinatal Cohort EPF-CO1. *Clin Infect Dis* 2010;**51**: 833–43.
71. Caliendo, A. Laboratory methods for quantifying HIV RNA. *AIDS Clin Care* 1995;**7**:89–96.
72. Cardoso LS, Oliveira SC, Goes AM, et al. Schistosoma mansoni antigens modulate the allergic response in a murine model of ovalbumin-induced airway inflammation. *Clin Exp Immunol* 2010;**160**:266–74.
73. Carneiro-Proietti AB, Catalan-Soares BC, Castro-Costa CM, et al. HTLV in the Americas: challenges and perspectives. *Rev Panam Salud Publica* 2006;**19**:44–53.
74. Carvalho AC, Valadas E, Franca L, et al. Population mobility and the changing epidemics of HIV-2 in Portugal. *HIV Med* 2011;**13**: 219–25.
75. Carvalho EM, Da Fonseca Porto A. Epidemiological and clinical interaction between HTLV-1 and *Strongyloides stercoralis*. *Parasite Immunol* 2004;**26**:487–97.
76. Caskey MF, Morgan DJ, Porto AF, et al. Clinical manifestations associated with HTLV type I infection: a cross-sectional study. *AIDS Res Hum Retroviruses* 2007;**23**:365–71.
77. Cassar O, Capuano C, Meertens L, et al. Human T-cell leukemia virus type 1 molecular variants, Vanuatu, Melanesia. *Emerg Infect Dis* 2005;**11**:706–10.
78. Casseb J. Is human T-cell lymphotropic virus type I more clever than human immunodeficiency virus type 1? *Clin Infect Dis* 1998;**27**:1309–10.
79. Casseb J, Posada-Vergara MP, Montanheiro P, et al. T CD4+ cells count among patients co-infected with human immunodeficiency virus type 1 (HIV-1) and human T-cell leukemia virus type 1 (HTLV-1): high prevalence of tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM). *Rev Inst Med Trop Sao Paulo* 2007;**49**:231–3.
80. Castro-Costa CM, Araujo AQ, Menna-Barreto M, et al. [Guide of clinical management of HTLV patient: neurological aspects]. *Arq Neuropsiquiatr* 2005;**63**:548–51.
81. Catalan-Soares B, Barbosa-Stancioli EF, Alcantara LC, et al. HTLV-2 horizontal and vertical transmission in a family from a Brazilian urban area: seroepidemiological, clinical and molecular study. *AIDS Res Hum Retroviruses* 2005;**21**:521–6.
82. Cavaleiro R, Sousa AE, Loureiro A, et al. Marked immunosuppressive effects of the HIV-2 envelope protein in spite of the lower HIV-2 pathogenicity. *AIDS* 2000;**14**:2679–86.
83. Cavrois M, Gessain A, Gout O, et al. Common human T cell leukemia virus type 1 (HTLV-1) integration sites in cerebrospinal fluid and blood lymphocytes of patients with HTLV-1-associated myelopathy/tropical spastic paraparesis indicate that HTLV-1 crosses the blood-brain barrier via clonal HTLV-1-infected cells. *J Infect Dis* 2000;**182**:1044–50.
84. Centers for Disease Control and Prevention. Human T-lymphotropic virus type I screening in volunteer blood donors: United States, 1989. *MMWR Morb Mortal Wkly Rep* 1990;**39**:915, 914–21.
85. Centers for Disease Control and Prevention and the U.S.P.H.S. Working Group. Guidelines for counseling persons infected with human T-lymphotropic virus type I (HTLV-I) and type II (HTLV-II). *Ann Intern Med* 1993;**118**:448–54.
86. Centers for Disease Control and Prevention. Update: HIV-2 infection among blood and plasma donors—United States, June 1992-June 1995. *JAMA* 1995;**274**:1007–8.
87. Centers for Disease Control and Prevention. Revised guidelines for HIV counseling, testing, and referral. *MMWR Recomm Rep* 2001;**50**:1–57; quiz CE51-19a51-CE56-19a51.
88. Cereseto A, Mulloy JC, Franchini G. Insights on the pathogenicity of human T-lymphotropic/leukemia virus types I and II. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1): S69–75.
89. Chang AB, Masel JP, Boyce NC, et al. Non-CF bronchiectasis: clinical and HRCT evaluation. *Pediatr Pulmonol* 2003;**35**:477–83.

90. Chen MY, Maldarelli F, Karczewski MK, et al. Human immunodeficiency virus type 1 Vpu protein induces degradation of CD4 in vitro: the cytoplasmic domain of CD4 contributes to Vpu sensitivity. *J Virol* 1993;**67**:3877–84.
91. Chevalier SA, Durand S, Dasgupta A, et al. The transcription profile of Tax-3 is more similar to Tax-1 than Tax-2: insights into HTLV-3 potential leukemogenic properties. *PLoS One* 2012;**7**: e41003.
92. Choksi M, Joseph AJ, Simon E, et al. The association of HTLV-1 infection, persistent intestinal infection with *Strongyloides stercoralis* and gastrointestinal lymphoma. *BMJ Case Rep* 2009; **2009**.
93. Cimarelli A, Sandin S, Hoglund S, et al. Basic residues in human immunodeficiency virus type 1 nucleocapsid promote virion assembly via interaction with RNA. *J Virol* 2000;**74**: 3046–57.
94. Clark NM, Dieng Sarr A, Sankalé JL, et al. Immunologic and virologic response of HIV-2 infection to antiretroviral therapy. *AIDS* 1998;**12**:2506–7.
95. Clements JE, Zink MC. Molecular biology and pathogenesis of animal lentivirus infections. *Clin Microbiol Rev* 1996;**9**:100–17.
96. Cocchi F, DeVico AL, Garzino-Demo A, et al. Identification of RANTES, MIP-1 alpha, and MIP-1 beta as the major HIV-suppressive factors produced by $CD8^+$ T cells. *Science* 1995;**270**: 1811–15.
97. Cocchi F, DeVico AL, Yarchoan R, et al. Higher macrophage inflammatory protein (MIP)-1alpha and MIP-1beta levels from $CD8^+$ T cells are associated with asymptomatic HIV-1 infection. *Proc Natl Acad Sci U S A* 2000;**97**:13812–17.
98. Coffin JM, Rosenberg N. Retroviruses: closing the joint. *Nature* 1999;**399**:413, 415–16.
99. Colin DD, Alcântara Júnior LC, Santos FL, et al. (Seroprevalence of human T cell lymphotropic virus infection and associated factors of risk in blood donors of Rio Branco City, AC, Brazil [1998-2001]). *Rev Soc Bras Med Trop* 2003;**36**:677–83.
100. Colson P, Henry M, Tivoli N, et al. Polymorphism and drug-selected mutations in the reverse transcriptase gene of HIV-2 from patients living in southeastern France. *J Med Virol* 2005;**75**: 381–90.
101. Cooke FJ, Geretti AM, Zuckerman M. Human T-cell lymphotropic virus antibody prevalence in HIV-1-infected individuals attending a sexual health clinic in South-East London. *J Med Virol* 2005;**76**:143–5.
102. Courouble G, Rouet F, Herrmann-Storck C, et al. Epidemiologic study of the association between human T-cell lymphotropic virus type 1 and *Strongyloides stercoralis* infection in female blood donors (Guadeloupe, French West Indies). *West Indian Med J* 2004;**53**: 3–6.
103. Courouce AM, Pillonel J, Lemaire JM, et al. Seroepidemiology of HTLV-I/II in universal screening of blood donations in France. *AIDS* 1993;**7**:841–7.
104. Creek TL, Kim A, Lu L, et al. Hospitalization and mortality among primarily nonbreastfed children during a large outbreak of diarrhea and malnutrition in Botswana, 2006. *J Acquir Immune Defic Syndr* 2009;**53**:14–19.
105. Croda MG, de Oliveira AC, Vergara MP, et al. Corticosteroid therapy in TSP/HAM patients: the results from a 10 years open cohort. *J Neurol Sci* 2008;**269**:133–7.
106. Cullen BR. Retroviruses as model systems for the study of nuclear RNA export pathways. *Virology* 1998;**249**:203–10.
107. da Silva ZJ, Oliveira I, Andersen A, et al. Changes in prevalence and incidence of HIV-1, HIV-2 and dual infections in urban areas of Bissau, Guinea-Bissau: is HIV-2 disappearing? *AIDS* 2008;**22**: 1195–202.
108. Reference deleted in proofs.
109. Dalakas MC. Inflammatory, immune, and viral aspects of inclusion-body myositis. *Neurology* 2006;**66**:S33–8.
110. Damond F, Benard A, Balotta C, et al. An international collaboration to standardize HIV-2 viral load assays: results from the 2009 ACHI(E)V(2E) quality control study. *J Clin Microbiol* 2011;**49**: 3491–7.
111. Daniel R, Katz RA, Skalka AM. A role for DNA-PK in retroviral DNA integration. *Science* 1999;**284**:644–7.
112. Datta A, Bellon M, Sinha-Datta U, et al. Persistent inhibition of telomerase reprograms adult T-cell leukemia to p53-dependent senescence. *Blood* 2006;**108**:1021–9.
113. Davidson F, Lycett C, Jarvis LM, et al. Detection of HTLV-I and -II in Scottish blood donor samples and archive donations. *Vox Sang* 2006;**91**:231–6.
114. De Castro-Costa CM, Araujo AQ, Barreto MM, et al. Proposal for diagnostic criteria of tropical spastic paraparesis/HTLV-I-associated myelopathy (TSP/HAM). *AIDS Res Hum Retroviruses* 2006;**22**:931–5.
115. De Cock KM, Adjorlolo G, Ekpini E, et al. Epidemiology and transmission of HIV-2: why there is no HIV-2 pandemic. *JAMA* 1993;**270**:2083–6.
116. De Cock KM, Zadi F, Adjorlolo G, et al. Retrospective study of maternal HIV-1 and HIV-2 infections and child survival in Abidjan, Cote d'Ivoire. *BMJ* 1994;**308**:441–3.
117. de la Fuente L, Toro C, Soriano V, et al. HTLV infection among young injection and non-injection heroin users in Spain: prevalence and correlates. *J Clin Virol* 2006;**35**:244–9.
118. de Oliveira Mde F, Bittencourt AL, Brites C, et al. HTLV-I associated myelopathy/tropical spastic paraparesis in a 7-year-old boy associated with infective dermatitis. *J Neurol Sci* 2004;**222**: 35–8.
119. de Oliveira Mde F, Fatal PL, Primo JR, et al. Infective dermatitis associated with human T-cell lymphotropic virus type 1: evaluation of 42 cases observed in Bahia, Brazil. *Clin Infect Dis* 2012;**54**:1714–19.
120. de Oliveira MS, Matutes E, Famadas LC, et al. Adult T-cell leukaemia/lymphoma in Brazil and its relation to HTLV-I. *Lancet* 1990;**336**:987–90.
121. de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. *Trends Microbiol* 2008;**16**:588–95.
122. de Silva TI, van Tienen C, Onyango C, et al. Population dynamics of HIV-2 in rural Guinea-Bissau: comparison with HIV-1 and ongoing transmission at the heart of the epidemic. *AIDS* 2013;**27**: 125–34.
123. Delamarre L, Rosenberg AR, Pique C, et al. The HTLV-I envelope glycoproteins: structure and functions. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1):S85–91.
124. Delaporte E, Buve A, Nzila N, et al. HTLV-I infection among prostitutes and pregnant women in Kinshasa, Zaire: how important is high-risk sexual behavior? *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;**8**:511–15.
125. Delaporte E, Peeters M, Bardy JL, et al. Blood transfusion as a major risk factor for HTLV-I infection among hospitalized children in Gabon (Equatorial Africa). *J Acquir Immune Defic Syndr* 1993;**6**:424–8.
126. Desbois D, Roquebert B, Peytavin G, et al. In vitro phenotypic susceptibility of human immunodeficiency virus type 2 clinical isolates to protease inhibitors. *Antimicrob Agents Chemother* 2008;**52**:1545–8.
127. deShazo RD, Chadha N, Morgan JE, et al. Immunologic assessment of a cluster of asymptomatic HTLV-I-infected individuals in New Orleans. *Am J Med* 1989;**86**:65–70.
128. Dickmeiss E, Christiansen AH, Smith E. [Screening of donor blood for viral markers. Anti-HIV, HBsAg and anti-HTLV-I/II in Denmark 1990-1999]. *Ugeskr Laeger* 2001;**163**:2623–8.
129. Donegan E, Lee H, Operskalski EA, et al. Transfusion transmission of retroviruses: human T-lymphotropic virus types I and II compared with human immunodeficiency virus type 1. *Transfusion* 1994;**34**:478–83.
130. Dosik H, Goldstein MF, Poiesz BJ, et al. Seroprevalence of human T-lymphotropic virus in blacks from a selected central Brooklyn population. *Cancer Invest* 1994;**12**:289–95.
131. Doueiri R, Anupam R, Kvaratskhelia M, et al. Comparative host protein interactions with HTLV-1 p30 and HTLV-2 p28: insights into difference in pathobiology of human retroviruses. *Retrovirology* 2012;**9**:64.
132. Dougan S, Patel B, Tosswill JH, et al. Diagnoses of HIV-1 and HIV-2 in England, Wales, and Northern Ireland associated with west Africa. *Sex Transm Infect* 2005;**81**:338–41.
133. Dougan S, Smith A, Tosswill JC, et al. New diagnoses of HTLV infection in England and Wales: 2002-2004. *Euro Surveill* 2005;**10**: 232–5.
134. Dumas M, Houinato D, Verdier M, et al. Seroepidemiology of human T-cell lymphotropic virus type I/II in Benin (West Africa). *AIDS Res Hum Retroviruses* 1991;**7**:447–51.
135. Duong YT, Jia H, Lust JA, et al. Absence of evidence of HTLV-3 and HTLV-4 in patients with large granular lymphocyte (LGL)

leukemia (Short communication). *AIDS Res Hum Retroviruses* 2008;**24**:1503–5.
136. Egan JF, O'Leary B, Lewis MJ, et al. High rate of human T lymphotropic virus type IIa infection in HIV type 1-infected intravenous drug abusers in Ireland. *AIDS Res Hum Retroviruses* 1999;**15**:699–705.
137. Einsiedel L, Fernandes L, Spelman T, et al. Bronchiectasis is associated with human T-lymphotropic virus 1 infection in an Indigenous Australian population. *Clin Infect Dis* 2011;**54**: 43–50.
138. Eiraku N, Novoa P, da Costa Ferreira M, et al. Identification and characterization of a new and distinct molecular subtype of human T-cell lymphotropic virus type 2. *J Virol* 1996;**70**:1481–92.
139. Ejima E, Rosenblatt JD, Massari M, et al. Cell-type-specific transactivation of the parathyroid hormone-related protein gene promoter by the human T-cell leukemia virus type I (HTLV-I) tax and HTLV-II tax proteins. *Blood* 1993;**81**:1017–24.
140. El Hajj H, El-Sabban M, Hasegawa H, et al. Therapy-induced selective loss of leukemia-initiating activity in murine adult T cell leukemia. *J Exp Med* 2010;**207**:2785–92.
141. Emerman M, Malim MH. HIV-1 regulatory/accessory genes: keys to unraveling viral and host cell biology. *Science* 1998;**280**: 1880–4.
142. Endres MJ, Clapham PR, Marsh M, et al. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell* 1996;**87**: 745–56.
143. Enose-Akahata Y, Matsuura E, Tanaka Y, et al. Minocycline modulates antigen-specific CTL activity through inactivation of mononuclear phagocytes in patients with HTLV-I associated neurologic disease. *Retrovirology* 2012;**9**:16.
144. Esbjornsson J, Mansson F, Kvist A, et al. Inhibition of HIV-1 disease progression by contemporaneous HIV-2 infection. *N Engl J Med* 2012;**367**:224–32.
145. Eshima N, Iwata O, Iwata S, et al. Age and gender specific prevalence of HTLV-1. *J Clin Virol* 2009;**45**:135–8.
146. Etenna SL, Caron M, Besson G, et al. New insights into prevalence, genetic diversity, and proviral load of human T-cell leukemia virus types 1 and 2 in pregnant women in Gabon in equatorial central Africa. *J Clin Microbiol* 2008;**46**:3607–14.
147. Etzel A, Shibata GY, Rozman M, et al. HTLV-1 and HTLV-2 infections in HIV-infected individuals from Santos, Brazil: seroprevalence and risk factors. *J Acquir Immune Defic Syndr* 2001;**26**: 185–90.
148. Faye A, Burgard M, Crosnier H, et al. Human immunodeficiency virus type 2 infection in children. *J Pediatr* 1997;**130**:994–7.
149. Ferreira AS, Costa CM, Dantas IK, et al. Polymyositis in childhood as clinical manifestation associated with HTLV-1. *Arq Neuropsiquiatr* 2011;**68**:962–4.
150. Ferrer JF, Esteban E, Dube S, et al. Endemic infection with human T cell leukemia/lymphoma virus type IIB in Argentinean and Paraguayan Indians: epidemiology and molecular characterization. *J Infect Dis* 1996;**174**:944–53.
151. Figueroa JP, Morris J, Brathwaite A, et al. Risk factors for HTLV-I among heterosexual STD clinic attenders. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;**9**:81–8.
152. Foil LD, Issel CJ. Transmission of retroviruses by arthropods. *Annu Rev Entomol* 1991;**36**:355–81.
153. Fouchard N, Flageul B, Bagot M, et al. Lack of evidence of HTLV-I/II infection in T CD8 malignant or reactive lymphoproliferative disorders in France: a serological and/or molecular study of 169 cases. *Leukemia* 1995;**9**:2087–92.
154. Franke EK, Yuan HE, Luban J. Specific incorporation of cyclophilin A into HIV-1 virions. *Nature* 1994;**372**:359–62.
155. Freed EO. HIV-1 gag proteins: diverse functions in the virus life cycle. *Virology* 1998;**251**:1–15.
156. Freed EO, Martin MA. Virion incorporation of envelope glycoproteins with long but not short cytoplasmic tails is blocked by specific, single amino acid substitutions in the human immunodeficiency virus type 1 matrix. *J Virol* 1995;**69**:1984–9.
157. Fujino T, Fujiyoshi T, Yashiki S, et al. HTLV-I transmission from mother to fetus via placenta. *Lancet* 1992;**340**:1157.
158. Reference deleted in proofs.
159. Furnia A, Lal R, Maloney E, et al. Estimating the time of HTLV-I infection following mother-to-child transmission in a breastfeeding population in Jamaica. *J Med Virol* 1999;**59**:541–6.
160. Furtado Mdos S, Andrade RG, Romanelli LC, et al. Monitoring the HTLV-1 proviral load in the peripheral blood of asymptomatic carriers and patients with HTLV-associated myelopathy/tropical spastic paraparesis from a Brazilian cohort: ROC curve analysis to establish the threshold for risk disease. *J Med Virol* 2012;**84**:664–71.
161. Furukawa Y, Yamashita M, Usuku K, et al. Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type I in the tax gene and their association with different risks for HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Infect Dis* 2000;**182**:1343–9.
162. Furusyo N, Hayashi J, Kakuda K, et al. Markedly high seroprevalence of hepatitis B virus infection in comparison to hepatitis C virus and human T lymphotropic virus type-1 infections in selected Solomon Islands populations. *Am J Trop Med Hyg* 1999;**61**:85–91.
163. Gabet AS, Kazanji M, Couppie P, et al. Adult T-cell leukaemia/lymphoma-like human T-cell leukaemia virus-1 replication in infective dermatitis. *Br J Haematol* 2003;**123**:406–12.
164. Gabet AS, Mortreux F, Charneau P, et al. Inactivation of hTERT transcription by Tax. *Oncogene* 2003;**22**:3734–41.
165. Gabet AS, Mortreux F, Talarmin A, et al. High circulating proviral load with oligoclonal expansion of HTLV-1 bearing T cells in HTLV-1 carriers with strongyloidiasis. *Oncogene* 2000;**19**: 4954–60.
166. Gallo RC. Human retroviruses after 20 years: a perspective from the past and prospects for their future control. *Immunol Rev* 2002;**185**:236–65.
167. Gallo RC, Sliski A, Wong-Staal F. Origin of human T-cell leukaemia-lymphoma virus. *Lancet* 1983;**2**:962–3.
168. Garrett N, Xu L, Smit E, et al. Raltegravir treatment response in an HIV-2 infected patient: a case report. *AIDS* 2008;**22**: 1091–2.
169. Garzino-Demo A, Moss RB, Margolick JB, et al. Spontaneous and antigen-induced production of HIV-inhibitory beta-chemokines are associated with AIDS-free status. *Proc Natl Acad Sci U S A* 1999;**96**:11986–91.
170. Gascon MR, Capitao CG, Casseb J, et al. Prevalence of anxiety, depression and quality of life in HTLV-1 infected patients. *Braz J Infect Dis* 2012;**15**:578–82.
171. Gastaldello R, Hall WW, Gallego S. Seroepidemiology of HTLV-I/II in Argentina: an overview. *J Acquir Immune Defic Syndr* 2004;**35**:301–8.
172. Gayle HD, Gnaore E, Adjorlolo G, et al. HIV-1 and HIV-2 infection in children in Abidjan, Cote d'Ivoire. *J Acquir Immune Defic Syndr* 1992;**5**:513–17.
173. George JR, Ou CY, Parekh B, et al. Prevalence of HIV-1 and HIV-2 mixed infections in Cote d'Ivoire. *Lancet* 1992;**340**: 337–9.
174. Geraghty RJ, Panganiban AT. Human immunodeficiency virus type 1 Vpu has a CD4- and an envelope glycoprotein-independent function. *J Virol* 1993;**67**:4190–4.
175. Gessain A, de The G. What is the situation of human T cell lymphotropic virus type II (HTLV-II) in Africa? Origin and dissemination of genomic subtypes. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1):S228–35.
176. Gessain A, Gallo RC, Franchini G. Low degree of human T-cell leukemia/lymphoma virus type I genetic drift in vivo as a means of monitoring viral transmission and movement of ancient human populations. *J Virol* 1992;**66**:2288–95.
177. Gessain A, Barin F, Vernant JC, et al. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. *Lancet* 1985;**2**:407–10.
178. Gessain A, Mauclere P, Froment A, et al. Isolation and molecular characterization of a human T-cell lymphotropic virus type II (HTLV-II), subtype B, from a healthy Pygmy living in a remote area of Cameroon: an ancient origin for HTLV-II in Africa. *Proc Natl Acad Sci U S A* 1995;**92**:4041–5.
179. Ghosh SK, Abrams JT, Terunuma H, et al. Human T-cell leukemia virus type I tax/rex DNA and RNA in cutaneous T-cell lymphoma. *Blood* 1994;**84**:2663–71.
180. Giacomo M, Franco EG, Claudio C, et al. Human T-cell leukemia virus type II infection among high risk groups and its influence on HIV-1 disease progression. *Eur J Epidemiol* 1995;**11**: 527–33.

181. Giuliani M, Rezza G, Lepri AC, et al. Risk factors for HTLV-I and II in individuals attending a clinic for sexually transmitted diseases. *Sex Transm Dis* 2000;**27**:87–92.
182. Gongora-Biachi RA, Lal RB, Rudolph DL, et al. Low prevalence of HTLV-II in Mayan Indians in the Yucatan Peninsula, Mexico. *Arch Med Res* 1997;**28**:555–8.
183. Gottlieb GS, Badiane NM, Hawes SE, et al. Emergence of multiclass drug-resistance in HIV-2 in antiretroviral-treated individuals in Senegal: implications for HIV-2 treatment in resource-limited West Africa. *Clin Infect Dis* 2009;**48**:476–83.
184. Gottlieb GS, Eholie SP, Nkengasong JN, et al. A call for randomized controlled trials of antiretroviral therapy for HIV-2 infection in West Africa. *AIDS* 2008;**22**:2069–72; discussion 2073–2064.
185. Gottlieb GS, Hawes SE, Agne HD, et al. Lower levels of HIV RNA in semen in HIV-2 compared with HIV-1 infection: implications for differences in transmission. *AIDS* 2006;**20**: 895–900.
186. Gotuzzo E, Arango C, de Queiroz-Campos A, et al. Human T-cell lymphotropic virus-I in Latin America. *Infect Dis Clin North Am* 2000;**14**:211–39, x–xi.
187. Gotuzzo E, Sanchez J, Escamilla J, et al. Human T cell lymphotropic virus type I infection among female sex workers in Peru. *J Infect Dis* 1994;**169**:754–9.
188. Gourlay AJ, van Tienen C, Dave SS, et al. Clinical predictors cannot replace biological predictors in HIV-2 infection in a community setting in West Africa. *Int J Infect Dis* 2012;**16**: e337–43.
189. Gout O, Baulac M, Gessain A, et al. Rapid development of myelopathy after HTLV-I infection acquired by transfusion during cardiac transplantation. *N Engl J Med* 1990;**322**:383–8.
190. Gout O, Gessain A, Iba-Zizen M, et al. The effect of zidovudine on chronic myelopathy associated with HTLV-1. *J Neurol* 1991;**238**:108–9.
191. Grassi MF, Olavarria VN, Kruschewsky Rde A, et al. Human T cell lymphotropic virus type 1 (HTLV-1) proviral load of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients according to new diagnostic criteria of HAM/TSP. *J Med Virol* 2011;**83**:1269–74.
192. Green JE, Hinrichs SH, Vogel J, et al. Exocrinopathy resembling Sjögren's syndrome in HTLV-1 tax transgenic mice. *Nature* 1989;**341**:72–4.
193. Greene WC. The molecular biology of human immunodeficiency virus type 1 infection. *N Engl J Med* 1991;**324**:308–17.
194. Gualco G, Chioato L, Weiss LM, et al. Analysis of human T-cell lymphotropic virus in $CD25^+$ anaplastic large cell lymphoma in children. *Am J Clin Pathol* 2009;**132**:28–33.
195. Guimaraes de Souza V, Lobato Martins M, Carneiro-Proietti AB, et al. High prevalence of HTLV-1 and 2 viruses in pregnant women in Sao Luis, state of Maranhao, Brazil. *Rev Soc Bras Med Trop* 2012;**45**:159–62.
196. Gummuluru S, Emerman M. Advances in HIV molecular biology. *AIDS* 2002;**16**(Suppl. 4):S17–23.
197. Hall WW, Ishak R, Zhu SW, et al. Human T lymphotropic virus type II (HTLV-II): epidemiology, molecular properties, and clinical features of infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1):S204–14.
198. Hall WW, Liu CR, Schneewind O, et al. Deleted HTLV-I provirus in blood and cutaneous lesions of patients with mycosis fungoides. *Science* 1991;**253**:317–20.
199. Hanchard B. Outcomes of early life exposure to human T cell lymphotropic virus type 1. *Clin Infect Dis* 2005;**41**:542–3.
200. Hannibal MC, Markovitz DM, Clark N, et al. Differential activation of human immunodeficiency virus type 1 and 2 transcription by specific T-cell activation signals. *J Virol* 1993;**67**: 5035–40.
201. Harouse JM, Bhat S, Spitalnik SL, et al. Inhibition of entry of HIV-1 in neural cell lines by antibodies against galactosyl ceramide. *Science* 1991;**253**:320–3.
202. Harper ME, Kaplan MH, Marselle LM, et al. Concomitant infection with HTLV-I and HTLV-III in a patient with T8 lymphoproliferative disease. *N Engl J Med* 1986;**315**:1073–8.
203. Harrington WJ Jr, Sheremata W, Hjelle B, et al. Spastic ataxia associated with human T-cell lymphotropic virus type II infection. *Ann Neurol* 1993;**33**:411–14.
204. Harrington WJ Jr, Ucar A, Gill P, et al. Clinical spectrum of HTLV-I in south Florida. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;**8**:466–73.
205. Hasegawa H, Sawa H, Lewis MJ, et al. Thymus-derived leukemia-lymphoma in mice transgenic for the Tax gene of human T-lymphotropic virus type I. *Nat Med* 2006;**12**:466–72.
206. Hattori T, Koito A, Takatsuki K, et al. Frequent infection with human T-cell lymphotropic virus type I in patients with AIDS but not in carriers of human immunodeficiency virus type 1. *J Acquir Immune Defic Syndr* 1989;**2**:272–6.
207. Hawes SE, Sow PS, Stern JE, et al. Lower levels of HIV-2 than HIV-1 in the female genital tract: correlates and longitudinal assessment of viral shedding. *AIDS* 2008;**22**:2517–25.
208. Heinzinger NK, Bukinsky MI, Haggerty SA, et al. The Vpr protein of human immunodeficiency virus type 1 influences nuclear localization of viral nucleic acids in nondividing host cells. *Proc Natl Acad Sci U S A* 1994;**91**:7311–15.
209. Heneine W, Kuehnert MJ. Preserving blood safety against emerging retroviruses. *Transfusion* 2006;**46**:1276–8.
210. Heneine W, Woods T, Green D, et al. Detection of HTLV-II in breastmilk of HTLV-II infected mothers. *Lancet* 1992;**340**: 1157–8.
211. Hermine O, Allard I, Levy V, et al. A prospective phase II clinical trial with the use of zidovudine and interferon-alpha in the acute and lymphoma forms of adult T-cell leukemia/lymphoma. *Hematol J* 2002;**3**:276–82.
212. Higuchi I, Hashimoto K, Matsuoka E, et al. The main HTLV-I-harboring cells in the muscles of viral carriers with polymyositis are not macrophages but $CD4^+$ lymphocytes. *Acta Neuropathol* 1996;**92**:358–61.
213. Higuchi M, Fujii M. Distinct functions of HTLV-1 Tax1 from HTLV-2 Tax2 contribute key roles to viral pathogenesis. *Retrovirology* 2009;**6**:117.
214. Hill SA, Lloyd PA, McDonald S, et al. Susceptibility of human T cell leukemia virus type I to nucleoside reverse transcriptase inhibitors. *J Infect Dis* 2003;**188**:424–7.
215. Hino S, Katamine S, Kawase K, et al. Intervention of maternal transmission of HTLV-1 in Nagasaki, Japan. *Leukemia* 1994; **8**(Suppl. 1):S68–70.
216. Hino S, Katamine S, Miyamoto T, et al. Association between maternal antibodies to the external envelope glycoprotein and vertical transmission of human T-lymphotropic virus type I. Maternal anti-env antibodies correlate with protection in non-breast-fed children. *J Clin Invest* 1995;**95**:2920–5.
217. Hino S, Katamine S, Miyata H, et al. Primary prevention of HTLV-I in Japan. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1):S199–203.
218. Hirata M, Hayashi J, Noguchi A, et al. The effects of breastfeeding and presence of antibody to p40tax protein of human T cell lymphotropic virus type-I on mother to child transmission. *Int J Epidemiol* 1992;**21**:989–94.
219. Hirata T, Uchima N, Kishimoto K, et al. Impairment of host immune response against *Strongyloides stercoralis* by human T cell lymphotropic virus type 1 infection. *Am J Trop Med Hyg* 2006; **74**:246–9.
220. Hisada M, Okayama A, Shioiri S, et al. Risk factors for adult T-cell leukemia among carriers of human T-lymphotropic virus type I. *Blood* 1998;**92**:3557–61.
221. Hishizawa M, Kanda J, Utsunomiya A, et al. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood* 2010;**116**:1369–76.
222. HIV Infection in Newborns French Collaborative Study Group. Comparison of vertical human immunodeficiency virus type 2 and human immunodeficiency virus type 1 transmission in the French prospective cohort. *Pediatr Infect Dis J* 1994;**13**:502–6.
223. Hjelle B, Appenzeller O, Mills R, et al. Chronic neurodegenerative disease associated with HTLV-II infection. *Lancet* 1992;**339**: 645–6.
224. Hjelle B, Mills R, Swenson S, et al. Incidence of hairy cell leukemia, mycosis fungoides, and chronic lymphocytic leukemia in first known HTLV-II-endemic population. *J Infect Dis* 1991;**163**: 435–40.
225. Ho DD, Neumann AU, Perelson AS, et al. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* 1995;**373**:123–6.

226. Ho GY, Nomura AM, Nelson K, et al. Declining seroprevalence and transmission of HTLV-I in Japanese families who immigrated to Hawaii. *Am J Epidemiol* 1991;**134**:981–7.
227. Hodson A, Bain B, Fields P, et al. Smouldering adult T-cell leukaemia/lymphoma (ATLL) is not associated with an increased risk of aggressive ATLL in HTLV-1 positive patients. Fifty-second annual meeting of the American Society of Hematology, Orlando, Fla, December 4-7, 2010.
228. Hollsberg P, Hafler DA. Seminars in medicine of the Beth Israel Hospital, Boston: pathogenesis of diseases induced by human lymphotropic virus type I infection. *N Engl J Med* 1993;**328**: 1173–82.
229. Ibe S, Yokomaku Y, Shiino T, et al. HIV-2 CRF01_AB: first circulating recombinant form of HIV-2. *J Acquir Immune Defic Syndr* 2010;**54**:241–7.
230. Ijichi S, Osame M. Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP): recent perspectives. *Intern Med* 1995;**34**:713–21.
231. Isaka Y, Miki S, Kawauchi S, et al. A single amino acid change at Leu-188 in the reverse transcriptase of HIV-2 and SIV renders them sensitive to non-nucleoside reverse transcriptase inhibitors. *Arch Virol* 2001;**146**:743–55.
232. Ishak R, Vallinoto AC, Azevedo VN, et al. Epidemiological aspects of retrovirus (HTLV) infection among Indian populations in the Amazon Region of Brazil. *Cad Saude Publica* 2003;**19**: 901–14.
233. Ishihara S, Okayama A, Stuver S, et al. Association of HTLV-I antibody profile of asymptomatic carriers with proviral DNA levels of peripheral blood mononuclear cells. *J Acquir Immune Defic Syndr* 1994;**7**:199–203.
234. Ishitsuka K, Ikeda S, Utsunomiya A, et al. Smouldering adult T-cell leukaemia/lymphoma: a follow-up study in Kyushu. *Br J Haematol* 2008;**143**:442–4.
235. Ishitsuka K, Tamura K. Treatment of adult T-cell leukemia/lymphoma: past, present, and future. *Eur J Haematol* 2008;**80**: 185–96.
236. Iwakura Y, Saijo S, Kioka Y, et al. Autoimmunity induction by human T cell leukemia virus type 1 in transgenic mice that develop chronic inflammatory arthropathy resembling rheumatoid arthritis in humans. *J Immunol* 1995;**155**:1588–98.
237. Iwakura Y, Tosu M, Yoshida E, et al. Induction of inflammatory arthropathy resembling rheumatoid arthritis in mice transgenic for HTLV-I. *Science* 1991;**253**:1026–8.
238. Iwanaga M, Watanabe T, Utsunomiya A, et al. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. *Blood* 2010;**116**:1211–19.
239. Izumo S, Goto I, Itoyama Y, et al. Interferon-alpha is effective in HTLV-I-associated myelopathy: a multicenter, randomized, double-blind, controlled trial. *Neurology* 1996;**46**:1016–21.
240. Izumo S, Umehara F, Kashio N, et al. Neuropathology of HTLV-1-associated myelopathy (HAM/TSP). *Leukemia* 1997; **11**(Suppl. 3):82–4.
241. Jacobson S, McFarlin DE, Robinson S, et al. HTLV-I-specific cytotoxic T lymphocytes in the cerebrospinal fluid of patients with HTLV-I-associated neurological disease. *Ann Neurol* 1992;**32**: 651–7.
242. Jacobson S, Shida H, McFarlin DE, et al. Circulating $CD8^+$ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature* 1990;**348**: 245–8.
243. Jaffar S, Wilkins A, Ngom PT, et al. Rate of decline of percentage CD4+ cells is faster in HIV-1 than in HIV-2 infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**16**:327–32.
244. Jeffery KJ, Usuku K, Hall SE, et al. HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. *Proc Natl Acad Sci U S A* 1999; **96**:3848–53.
245. Jones G, Steketee RW, Black RE, et al. How many child deaths can we prevent this year? *Lancet* 2003;**362**:65–71.
246. Kalyanaraman VS, Sarngadharan MG, Robert-Guroff M, et al. A new subtype of human T-cell leukemia virus (HTLV-II) associated with a T-cell variant of hairy cell leukemia. *Science* 1982;**218**: 571–3.
247. Kamimura T, Miyamoto T, Kawano N, et al. Successful treatment by donor lymphocyte infusion of adult T-cell leukemia/lymphoma relapse following allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2012;**95**:725–30.
248. Kanki PJ, Travers KU, MBoup S, et al. Slower heterosexual spread of HIV-2 than HIV-1. *Lancet* 1994;**343**:943–6.
249. Kannagi M, Matsushita S, Shida H, et al. Cytotoxic T cell response and expression of the target antigen in HTLV-I infection. *Leukemia* 1994;**8**(Suppl. 1):S54–9.
250. Kao SY, Lemoine FJ, Marriott SJ. Suppression of DNA repair by human T cell leukemia virus type 1 Tax is rescued by a functional p53 signaling pathway. *J Biol Chem* 2000;**275**:35926–31.
251. Kaplan JE, Abrams E, Shaffer N, et al. Low risk of mother-to-child transmission of human T lymphotropic virus type II in non-breast-fed infants. The NYC Perinatal HIV Transmission Collaborative Study. *J Infect Dis* 1992;**166**:892–5.
252. Kaplan JE, Khabbaz RF, Murphy EL, et al. Male-to-female transmission of human T-cell lymphotropic virus types I and II: association with viral load. The Retrovirus Epidemiology Donor Study Group. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**12**:193–201.
253. Kashiwagi K, Furusyo N, Nakashima H, et al. A decrease in mother-to-child transmission of human T lymphotropic virus type I (HTLV-I) in Okinawa, Japan. *Am J Trop Med Hyg* 2004;**70**:158–63.
254. Kassim S, Sassan-Morokro M, Ackah A, et al. Two-year follow-up of persons with HIV-1- and HIV-2-associated pulmonary tuberculosis treated with short-course chemotherapy in West Africa. *AIDS* 1995;**9**:1185–91.
255. Katakura S, Iijima Y, Imagawa T, et al. Human T cell lymphotropic virus type I associated uveitis in a child. *Br J Ophthalmol* 1997;**81**:1016.
256. Katsuya H, Yamanaka T, Ishitsuka K, et al. Prognostic index for acute- and lymphoma-type adult T-cell leukemia/lymphoma. *J Clin Oncol* 2012;**30**:1635–40.
257. Kawabata T, Higashimoto I, Takashima H, et al. Human T-lymphotropic virus type I (HTLV-I)-specific $CD8^+$ cells accumulate in the lungs of patients infected with HTLV-I with pulmonary involvement. *J Med Virol* 2012;**84**:1120–7.
258. Kazanji M, Gessain A. Human T-cell Lymphotropic virus types I and II (HTLV-I/II) in French Guiana: clinical and molecular epidemiology. *Cad Saude Publica* 2003;**19**:1227–40.
259. Kchour G, Tarhini M, Kooshyar MM, et al. Phase 2 study of the efficacy and safety of the combination of arsenic trioxide, interferon alpha, and zidovudine in newly diagnosed chronic adult T-cell leukemia/lymphoma (ATL). *Blood* 2009;**113**: 6528–32.
260. Khabbaz RF, Onorato IM, Cannon RO, et al. Seroprevalence of HTLV-1 and HTLV-2 among intravenous drug users and persons in clinics for sexually transmitted diseases. *N Engl J Med* 1992; **326**:375–80.
261. Khan RB, Bertorini TE, Levin MC. HTLV-1 and its neurological complications. *Neurologist* 2001;**7**:271–8.
262. Kihara K, Tsuruda M, Ono A, et al. [Human T-lymphotropic virus type 1 uveitis in children]. *Nihon Ganka Gakkai Zasshi* 1997; **101**:538–43.
263. Kinoshita K, Yamanouchi K, Ikeda S, et al. Oral infection of a common marmoset with human T-cell leukemia virus type-I (HTLV-I) by inoculating fresh human milk of HTLV-I carrier mothers. *Jpn J Cancer Res* 1985;**76**:1147–53.
264. Kishihara Y, Furusyo N, Kashiwagi K, et al. Human T lymphotropic virus type 1 infection influences hepatitis C virus clearance. *J Infect Dis* 2001;**184**:1114–19.
265. Koblavi-Deme S, Kestens L, Hanson D, et al. Differences in HIV-2 plasma viral load and immune activation in HIV-1 and HIV-2 dually infected persons and those infected with HIV-2 only in Abidjan, Cote D'Ivoire. *AIDS* 2004;**18**:413–19.
266. Kohno S, Koga H, Kaku M, et al. Prevalence of HTLV-I antibody in pulmonary cryptococcosis. *Tohoku J Exp Med* 1992;**167**: 13–18.
267. Kokkotou EG, Sankale JL, Mani I, et al. In vitro correlates of HIV-2-mediated HIV-1 protection. *Proc Natl Acad Sci U S A* 2000;**97**:6797–802.
268. Kramer A, Jacobson S, Reuben JF, et al. Spontaneous lymphocyte proliferation in symptom-free HTLV-I positive Jamaicans. *Lancet* 1989;**2**:923–4.
269. Kubota T, Morishita N, Tanaka Y, et al. Establishment of novel lymphoid cell lines dually infected with human T cell

lymphotropic viruses types I and II. *J Infect Dis* 1995;**172**: 220–4.
270. Kuhn L, Aldrovandi G. Survival and health benefits of breastfeeding versus artificial feeding in infants of HIV-infected women: developing versus developed world. *Clin Perinatol* 2010;**37**:843–62, x.
271. La Grenade L. HTLV-I-associated infective dermatitis: past, present, and future. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1):S46–9.
272. La Grenade L, Schwartz RA, Janniger CK. Childhood dermatitis in the tropics: with special emphasis on infective dermatitis, a marker for infection with human T-cell leukemia virus-I. *Cutis* 1996;**58**:115–18.
273. La Grenade L, Hanchard B, Fletcher V, et al. Infective dermatitis of Jamaican children: a marker for HTLV-I infection. *Lancet* 1990;**336**:1345–7.
274. La Grenade L, Manns A, Fletcher V, et al. Clinical, pathologic, and immunologic features of human T-lymphotrophic virus type I-associated infective dermatitis in children. *Arch Dermatol* 1998; **134**:439–44.
275. LaGrenade L, Morgan C, Carberry C, et al. Tropical spastic paraparesis occurring in HTLV-1 associated infective dermatitis: report of two cases. *West Indian Med J* 1995;**44**:34–5.
276. LaGrenade L, Sonoda S, Miller W, et al. HLA DRB1*DQB1* haplotype in HTLV-I-associated familial infective dermatitis may predict development of HTLV-I-associated myelopathy/tropical spastic paraparesis. *Am J Med Genet* 1996;**61**:37–41.
277. Lairmore MD, Jacobson S, Gracia F, et al. Isolation of human T-cell lymphotropic virus type 2 from Guaymi Indians in Panama. *Proc Natl Acad Sci U S A* 1990;**87**:8840–4.
278. Lal RB, Gongora-Biachi RA, Pardi D, et al. Evidence for mother-to-child transmission of human T lymphotropic virus type II. *J Infect Dis* 1993;**168**:586–91.
279. Lal RB, Owen SM, Rudolph DL, et al. In vivo cellular tropism of human T-lymphotropic virus type II is not restricted to $CD8^+$ cells. *Virology* 1995;**210**:441–7.
280. Lal RB, Owen SM, Segurado AA, et al. Mother-to-child transmission of human T-lymphotropic virus type II (HTLV-II). *Ann Intern Med* 1994;**120**:300–1.
281. Larsen O, Andersson S, da Silva Z, et al. Prevalences of HTLV-1 infection and associated risk determinants in an urban population in Guinea-Bissau, West Africa. *J Acquir Immune Defic Syndr* 2000;**25**:157–63.
282. Lau A, Nightingale S, Taylor GP, et al. Enhanced MDR1 gene expression in human T-cell leukemia virus-I-infected patients offers new prospects for therapy. *Blood* 1998;**91**:2467–74.
283. Lemey P, Pybus OG, Wang B, et al. Tracing the origin and history of the HIV-2 epidemic. *Proc Natl Acad Sci U S A* 2003;**100**: 6588–92.
284. Leon G, Quiros AM, Lopez JL, et al. [Seropositivity for human T-lymphotropic virus types I and II among donors at the Municipal Blood Bank of Caracas and associated risk factors]. *Rev Panam Salud Publica* 2003;**13**:117–23.
285. Levin MC, Lehky TJ, Flerlage AN, et al. Immunologic analysis of a spinal cord-biopsy specimen from a patient with human T-cell lymphotropic virus type I-associated neurologic disease. *N Engl J Med* 1997;**336**:839–45.
286. Levine PH, Cleghorn F, Manns A, et al. Adult T-cell leukemia/lymphoma: a working point-score classification for epidemiological studies. *Int J Cancer* 1994;**59**:491–3.
287. Levine PH, Dosik H, Joseph EM, et al. A study of adult T-cell leukemia/lymphoma incidence in central Brooklyn. *Int J Cancer* 1999;**80**:662–6.
288. Levy DN, Fernandes LS, Williams WV, et al. Induction of cell differentiation by human immunodeficiency virus 1 vpr. *Cell* 1993;**72**:541–50.
289. Levy-Clarke GA, Buggage RR, Shen D, et al. Human T-cell lymphotropic virus type-1 associated T-cell leukemia/lymphoma masquerading as necrotizing retinal vasculitis. *Ophthalmology* 2002;**109**:1717–22.
290. Lewinski MK, Yamashita M, Emerman M, et al. Retroviral DNA integration: viral and cellular determinants of target-site selection. *PLoS Pathog* 2006;**2**:e60.
291. Lewis JM, Vasef MA, Seabury Stone M. HTLV-I-associated granulomatous T-cell lymphoma in a child. *J Am Acad Dermatol* 2001;**44**:525–9.
292. Lezin A, Gillet N, Olindo S, et al. Histone deacetylase mediated transcriptional activation reduces proviral loads in HTLV-1 associated myelopathy/tropical spastic paraparesis patients. *Blood* 2007;**110**:3722–8.
293. Li HC, Biggar RJ, Miley WJ, et al. Provirus load in breast milk and risk of mother-to-child transmission of human T lymphotropic virus type I. *J Infect Dis* 2004;**190**:1275–8.
294. Lima MA, Harab RC, Schor D, et al. Subacute progression of human T-lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis. *J Neurovirol* 2007;**13**:468–73.
295. Lin TY, Emerman M. Cyclophilin A interacts with diverse lentiviral capsids. *Retrovirology* 2006;**3**:70.
296. Littleton ET, Man WD, Holton JL, et al. Human T cell leukaemia virus type I associated neuromuscular disease causing respiratory failure. *J Neurol Neurosurg Psychiatry* 2002;**72**:650–2.
297. Lotan I, Khlebtovsky A, Inbar E, et al. Primary brain T-cell lymphoma in an HTLV-1 serologically positive male. *J Neurol Sci* 2012;**314**:163–5.
298. Louis J, Gardon J, Trebucq A, et al. [Epidemiological features of retroviral infection by HTLV-1 in central Africa]. *Bull Soc Pathol Exot* 1993;**86**:163–8.
299. Lowis GW, Sheremata WA, Wickman PR, et al. HTLV-II risk factors in Native Americans in Florida. *Neuroepidemiology* 1999;**18**: 37–47.
300. Lu SC, Chen BH. Seroindeterminate HTLV-1 prevalence and characteristics in blood donors in Taiwan. *Int J Hematol* 2003; **77**:412–13.
301. Machigashira N, Yoshida Y, Wang S, et al. HTLV-1-associated myelopathy/tropical spastic paraparesis with pseudohypoparathyroidism. *Neurology* 2001;**56**:104–6.
302. Machuca A, Tuset C, Soriano V, et al. Prevalence of HTLV infection in pregnant women in Spain. *Sex Transm Infect* 2000;**76**:366–70.
303. MacNeil A, Sarr AD, Sankale JL, et al. Direct evidence of lower viral replication rates in vivo in human immunodeficiency virus type 2 (HIV-2) infection than in HIV-1 infection. *J Virol* 2007; **81**:5325–30.
304. Magri MC, Morimoto HK, de Macedo Brigido LF, et al. Long terminal repeat sequence analysis of HTLV-2 molecular variants identified in Southern Brazil. *AIDS Res Hum Retroviruses* 2010;**26**:1327–31.
305. Mahé A, Meertens L, Ly F, et al. Human T-cell leukaemia/lymphoma virus type 1-associated infective dermatitis in Africa: a report of five cases from Senegal. Human T-cell leukaemia/lymphoma virus type 1-associated infective dermatitis in Africa: a report of five cases from Senegal. *Br J Dermatol* 2004;**150**: 958–65.
306. Mahieux R, Gessain A. Adult T-cell leukemia/lymphoma and HTLV-1. *Curr Hematol Malig Rep* 2007;**2**:257–64.
307. Mahieux R, Gessain A. HTLV-3/STLV-3 and HTLV-4 viruses: discovery, epidemiology, serology and molecular aspects. *Viruses* 2011;**3**:1074–90.
308. Mahieux R, Pise-Masison CA, Lambert PF, et al. Differences in the ability of human T-cell lymphotropic virus type 1 (HTLV-1) and HTLV-2 tax to inhibit p53 function. *J Virol* 2000;**74**:6866–74.
309. Maloney EM, Armien B, Gracia F, et al. Risk factors for human T cell lymphotropic virus type II infection among the Guaymi Indians of Panama. *J Infect Dis* 1999;**180**:876–9.
310. Maloney EM, Cleghorn FR, Morgan OS, et al. Incidence of HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in Jamaica and Trinidad. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;**17**:167–70.
311. Maloney EM, Hisada M, Palmer P, et al. Human T cell lymphotropic virus type I-associated infective dermatitis in Jamaica: a case report of clinical and biologic correlates. *Pediatr Infect Dis J* 2000;**19**:560–5.
312. Maloney EM, Murphy EL, Figueroa JP, et al. Human T-lymphotropic virus type I (HTLV-I) seroprevalence in Jamaica. II. Geographic and ecologic determinants. *Am J Epidemiol* 1991; **133**:1125–34.
313. Manns A, Cleghorn FR, Falk RT, et al. Role of HTLV-I in development of non-Hodgkin lymphoma in Jamaica and Trinidad and Tobago. The HTLV Lymphoma Study Group. *Lancet* 1993; **342**:1447–50.
314. Manns A, Miley WJ, Wilks RJ, et al. Quantitative proviral DNA and antibody levels in the natural history of HTLV-I infection. *J Infect Dis* 1999;**180**:1487–93.

315. Mansky LM. In vivo analysis of human T-cell leukemia virus type 1 reverse transcription accuracy. *J Virol* 2000;**74**:9525–31.
316. Mansson F, Biague A, da Silva ZJ, et al. Prevalence and incidence of HIV-1 and HIV-2 before, during and after a civil war in an occupational cohort in Guinea-Bissau, West Africa. *AIDS* 2009;**23**:1575–82.
317. Mariette X, Cherot P, Cazals D, et al. Antibodies to HTLV-I in Sjögren's syndrome. *Lancet* 1995;**345**:71.
318. Marinho J, Galvao-Castro B, Rodrigues LC, et al. Increased risk of tuberculosis with human T-lymphotropic virus-1 infection: a case-control study. *J Acquir Immune Defic Syndr* 2005;**40**:625–8.
319. Markovitz DM. Infection with the human immunodeficiency virus type 2. *Ann Intern Med* 1993;**118**:211–18.
320. Marlink R. Lessons from the second AIDS virus, HIV-2. *AIDS* 1996;**10**:689–99.
321. Marlink R, Kanki P, Thior I, et al. Reduced rate of disease development after HIV-2 infection as compared to HIV-1. *Science* 1994;**265**:1587–90.
322. Marriott SJ, Lemoine FJ, Jeang KT. Damaged DNA and miscounted chromosomes: human T cell leukemia virus type I tax oncoprotein and genetic lesions in transformed cells. *J Biomed Sci* 2002;**9**:292–8.
323. Marsh BJ. Infectious complications of human T cell leukemia/lymphoma virus type I infection. *Clin Infect Dis* 1996;**23**:138–45.
324. Martin F, Castro H, Gabriel C, et al. Ciclosporin A proof of concept study in patients with active, progressive HTLV-1 associated myelopathy/tropical spastic paraparesis. *PLoS Negl Trop Dis* 2012;**6**:e1675.
325. Martinez-Steele E, Awasana AA, Corrah T, et al. Is HIV-2-induced AIDS different from HIV-1-associated AIDS? Data from a West African clinic. *AIDS* 2007;**21**:317–24.
326. Matheron S, Pueyo S, Damond F, et al. Factors associated with clinical progression in HIV-2 infected-patients: the French ANRS cohort. *AIDS* 2003;**17**:2593–601.
327. Matsuoka M, Green PL. The HBZ gene, a key player in HTLV-1 pathogenesis. *Retrovirology* 2009;**6**:71.
328. Matsuzaki T, Otose H, Hashimoto K, et al. Diseases among men living in human T-lymphotropic virus type I endemic areas in Japan. *Intern Med* 1993;**32**:623–8.
329. Matutes E. Adult T-cell leukaemia/lymphoma. *J Clin Pathol* 2007;**60**:1373–7.
330. Matutes E, Spittle MF, Smith NP, et al. The first report of familial adult T-cell leukaemia lymphoma in the United Kingdom. *Br J Haematol* 1995;**89**:615–19.
331. Matutes E, Taylor GP, Cavenagh J, et al. Interferon alpha and zidovudine therapy in adult T-cell leukaemia lymphoma: response and outcome in 15 patients. *Br J Haematol* 2001;**113**:779–84.
332. McCallum RM, Patel DD, Moore JO, et al. Arthritis syndromes associated with human T cell lymphotropic virus type I infection. *Med Clin North Am* 1997;**81**:261–76.
333. McKee AS, Pearce EJ. $CD25^+CD4^+$ cells contribute to Th2 polarization during helminth infection by suppressing Th1 response development. *J Immunol* 2004;**173**:1224–31.
334. Michel P, Balde AT, Roussilhon C, et al. Reduced immune activation and T cell apoptosis in human immunodeficiency virus type 2 compared with type 1: correlation of T cell apoptosis with beta2 microglobulin concentration and disease evolution. *J Infect Dis* 2000;**181**:64–75.
335. Miller GJ, Lewis LL, Colman SM, et al. Clustering of human T lymphotropic virus type I seropositive in Montserrat, West Indies: evidence for an environmental factor in transmission of the virus. *J Infect Dis* 1994;**170**:44–50.
336. Miller RJ, Cairns JS, Bridges S, et al. Human immunodeficiency virus and AIDS: insights from animal lentiviruses. *J Virol* 2000;**74**:7187–95.
337. Mochizuki M, Sugita S, Ishikawa N, et al. Immunoregulation by aqueous humor. *Cornea* 2000;**19**:S24–5.
338. Mochizuki M, Tajima K, Watanabe T, et al. Human T lymphotropic virus type 1 uveitis. *Br J Ophthalmol* 1994;**78**:149–54.
339. Mochizuki M, Watanabe T, Yamaguchi K, et al. HTLV-I uveitis: a distinct clinical entity caused by HTLV-I. *Jpn J Cancer Res* 1992;**83**:236–9.
340. Mochizuki M, Watanabe T, Yamaguchi K, et al. Uveitis associated with human T-cell lymphotropic virus type I. *Am J Ophthalmol* 1992;**114**:123–9.
341. Mochizuki M, Watanabe T, Yamaguchi K, et al. Uveitis associated with human T lymphotropic virus type I: seroepidemiologic, clinical, and virologic studies. *J Infect Dis* 1992;**166**:943–4.
342. Modahl LE, Young KC, Varney KF, et al. Are HTLV-II-seropositive injection drug users at increased risk of bacterial pneumonia, abscess, and lymphadenopathy? *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**16**:169–75.
343. Mone A, Puhalla S, Whitman S, et al. Durable hematologic complete response and suppression of HTLV-1 viral load following alemtuzumab in zidovudine/IFN-α-refractory adult T-cell leukemia. *Blood* 2005;**106**:3380–2.
344. Montes M, Sanchez C, Verdonck K, et al. Regulatory T cell expansion in HTLV-1 and strongyloidiasis co-infection is associated with reduced IL-5 responses to *Strongyloides stercoralis* antigen. *PLoS Negl Trop Dis* 2009;**3**:e456.
345. Morgan G, Wilkins HA, Pepin J, et al. AIDS following mother-to-child transmission of HIV-2. *AIDS* 1990;**4**:879–82.
346. Morgan OS, Rodgers-Johnson P, Mora C, et al. HTLV-1 and polymyositis in Jamaica. *Lancet* 1989;**2**:1184–7.
347. Mori S, Mizoguchi A, Kawabata M, et al. Bronchoalveolar lymphocytosis correlates with human T lymphotropic virus type I (HTLV-I) proviral DNA load in HTLV-I carriers. *Thorax* 2005;**60**:138–43.
348. Morimoto HK, Caterino-De-Araujo A, Morimoto AA, et al. Seroprevalence and risk factors for human T cell lymphotropic virus type 1 and 2 infection in human immunodeficiency virus-infected patients attending AIDS referral center health units in Londrina and other communities in Parana, Brazil. *AIDS Res Hum Retroviruses* 2005;**21**:256–62.
349. Morishita N, Ishii K, Tanaka Y, et al. Immunoglobulin prophylaxis against human T cell lymphotropic virus type II in rabbits. *J Infect Dis* 1994;**169**:620–3.
350. Moriuchi M, Inoue H, Moriuchi H. Reciprocal interactions between human T-lymphotropic virus type 1 and prostaglandins: implications for viral transmission. *J Virol* 2001;**75**:192–8.
351. Mörner A, Bjorndal A, Albert J, et al. Primary human immunodeficiency virus type 2 (HIV-2) isolates, like HIV-1 isolates, frequently use CCR5 but show promiscuity in coreceptor usage. *J Virol* 1999;**73**:2343–9.
352. Morofuji-Hirata M, Kajiyama W, Nakashima K, et al. Prevalence of antibody to human T-cell lymphotropic virus type I in Okinawa, Japan, after an interval of 9 years. *Am J Epidemiol* 1993;**137**:43–8.
353. Mortreux F, Kazanji M, Gabet AS, et al. Two-step nature of human T-cell leukemia virus type 1 replication in experimentally infected squirrel monkeys *(Saimiri sciureus)*. *J Virol* 2001;**75**:1083–9.
354. Mortreux F, Leclercq I, Gabet AS, et al. Somatic mutation in human T-cell leukemia virus type 1 provirus and flanking cellular sequences during clonal expansion in vivo. *J Natl Cancer Inst* 2001;**93**:367–77.
355. Mueller N, Okayama A, Stuver S, et al. Findings from the Miyazaki Cohort Study. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1):S2–7.
356. Murphy EL, Engstrom JW, Miller K, et al. HTLV-II associated myelopathy in 43-year-old woman. REDS Investigators. *Lancet* 1993;**341**:757–8.
357. Murphy EL, Figueroa JP, Gibbs WN, et al. Human T-lymphotropic virus type I (HTLV-I) seroprevalence in Jamaica. I. Demographic determinants. *Am J Epidemiol* 1991;**133**:1114–24.
358. Murphy EL, Hanchard B, Figueroa JP, et al. Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. *Int J Cancer* 1989;**43**:250–3.
359. Murphy EL, Mahieux R, de The G, et al. Molecular epidemiology of HTLV-II among United States blood donors and intravenous drug users: an age-cohort effect for HTLV-II RFLP type aO. *Virology* 1998;**242**:425–34.
360. Murphy EL, Wang B, Sacher RA, et al. Respiratory and urinary tract infections, arthritis, and asthma associated with HTLV-I and HTLV-II infection. *Emerg Infect Dis* 2004;**10**:109–16.
361. Murphy EL, Watanabe K, Nass CC, et al. Evidence among blood donors for a 30-year-old epidemic of human T lymphotropic virus type II infection in the United States. *J Infect Dis* 1999;**180**:1777–83.

362. Murphy EL, Wilks R, Hanchard B, et al. A case-control study of risk factors for seropositivity to human T-lymphotropic virus type I (HTLV-I) in Jamaica. *Int J Epidemiol* 1996;**25**: 1083–9.
363. Murray SM, Linial ML. Foamy virus infection in primates. *J Med Primatol* 2006;**35**:225–35.
364. Mylonas I, Bruning A, Kainer F, et al. HTLV infection and its implication in gynaecology and obstetrics. *Arch Gynecol Obstet* 2010;**282**:493–501.
365. Nagai M, Kubota R, Greten TF, et al. Increased activated human T cell lymphotropic virus type I (HTLV-I) Tax11-19-specific memory and effector $CD8^+$ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. *J Infect Dis* 2001;**183**:197–205.
366. Nagai M, Usuku K, Matsumoto W, et al. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *J Neurovirol* 1998;**4**:586–93.
367. Nakagawa M, Nakahara K, Maruyama Y, et al. Therapeutic trials in 200 patients with HTLV-I-associated myelopathy/ tropical spastic paraparesis. *J Neurovirol* 1996;**2**:345–55.
368. Nakamura T. Immunopathogenesis of HTLV-I-associated myelopathy/tropical spastic paraparesis. *Ann Med* 2000;**32**: 600–7.
369. Nakao K, Ohba N. Clinical features of HTLV-I associated uveitis. *Br J Ophthalmol* 1993;**77**:274–9.
370. Nakao K, Ohba N. Human T-cell lymphotropic virus type 1-associated retinal vasculitis in children. *Retina* 2003;**23**: 197–201.
371. Nakashima K, Kashiwagi S, Kajiyama W, et al. Sexual transmission of human T-lymphotropic virus type I among female prostitutes and among patients with sexually transmitted diseases in Fukuoka, Kyushu, Japan. *Am J Epidemiol* 1995;**141**:305–11.
372. Nascimento MC, Primo J, Bittencourt A, et al. Infective dermatitis has similar immunological features to human T lymphotropic virus-type 1-associated myelopathy/tropical spastic paraparesis. *Clin Exp Immunol* 2009;**156**:455–62.
373. Nasrullah M, Wesolowski LG, Meyer WA 3rd, et al. Performance of a fourth-generation HIV screening assay and an alternative HIV diagnostic testing algorithm. *AIDS* 2012. (Epub ahead of print).
374. Neoh LP, Akimoto H, Kaneko H, et al. The production of beta-chemokines induced by HIV-2 envelope glycoprotein. *AIDS* 1997;**11**:1062–3.
375. Nishioka K, Nakajima T, Hasunuma T, et al. Rheumatic manifestation of human leukemia virus infection. *Rheum Dis Clin North Am* 1993;**19**:489–503.
376. Norkin LC. Virus receptors: implications for pathogenesis and the design of antiviral agents. *Clin Microbiol Rev* 1995;**8**: 293–315.
377. Norrgren H, Andersson S, Biague AJ, et al. Trends and interaction of HIV-1 and HIV-2 in Guinea-Bissau, west Africa: no protection of HIV-2 against HIV-1 infection. *AIDS* 1999;**13**:701–7.
378. Norrgren H, Fonseca A, Andersson S, et al. Child survival in children born to HIV-2 infected women in Guinea-Bissau, West Africa. *Acta Trop* 1999;**72**:309–15.
379. Norrgren H, Marquina S, Leitner T, et al. HIV-2 genetic variation and DNA load in asymptomatic carriers and AIDS cases in Guinea-Bissau. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**16**:31–8.
380. Nowak MA. AIDS pathogenesis: from models to viral dynamics in patients. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995; **10**(Suppl. 1):S1–5.
381. Ntemgwa M, Brenner BG, Oliveira M, et al. Natural polymorphisms in the human immunodeficiency virus type 2 protease can accelerate time to development of resistance to protease inhibitors. *Antimicrob Agents Chemother* 2007;**51**:604–10.
382. Ntemgwa ML, d'Aquin Toni T, Brenner BG, et al. Antiretroviral drug resistance in human immunodeficiency virus type 2. *Antimicrob Agents Chemother* 2009;**53**:3611–19.
383. O'Donovan D, Ariyoshi K, Milligan P, et al. Maternal plasma viral RNA levels determine marked differences in mother-to-child transmission rates of HIV-1 and HIV-2 in The Gambia. MRC/Gambia Government/University College London Medical School working group on mother-child transmission of HIV. *AIDS* 2000;**14**:441–8.
384. Ohashi T, Hanabuchi S, Kato H, et al. Prevention of adult T-cell leukemia-like lymphoproliferative disease in rats by adoptively transferred T cells from a donor immunized with human T-cell leukemia virus type 1 Tax-coding DNA vaccine. *J Virol* 2000;**74**:9610–16.
385. Okada F, Ando Y, Yoshitake S, et al. Pulmonary CT findings in 320 carriers of human T-lymphotropic virus type 1. *Radiology* 2006;**240**:559–64.
386. Okayama A, Stuver S, Iga M, et al. Sequential change of virus markers in seroconverters with community-acquired infection of human T lymphotropic virus type I. *J Infect Dis* 2001;**183**: 1031–7.
387. Okayama A, Stuver S, Matsuoka M, et al. Role of HTLV-1 proviral DNA load and clonality in the development of adult T-cell leukemia/lymphoma in asymptomatic carriers. *Int J Cancer* 2004;**110**:621–5.
388. Okochi K, Sato H, Hinuma Y. A retrospective study on transmission of adult T cell leukemia virus by blood transfusion: seroconversion in recipients. *Vox Sang* 1984;**46**:245–53.
389. Oksenberg JR, Mantegazza R, Sakai K, et al. HTLV-I sequences are not detected in peripheral blood genomic DNA or in brain cDNA of multiple sclerosis patients. *Ann Neurol* 1990;**28**: 574–7.
390. Oliveira Mde F, Brites C, Ferraz N, et al. Infective dermatitis associated with the human T cell lymphotropic virus type I in Salvador, Bahia, Brazil. *Clin Infect Dis* 2005;**40**:e90–6.
391. Orland JR, Wang B, Wright DJ, et al. Increased mortality associated with HTLV-II infection in blood donors: a prospective cohort study. *Retrovirology* 2004;**1**:4.
392. Osame M, Janssen R, Kubota H, et al. Nationwide survey of HTLV-I-associated myelopathy in Japan: association with blood transfusion. *Ann Neurol* 1990;**28**:50–6.
393. Osame M, Usuku K, Izumo S, et al. HTLV-I associated myelopathy, a new clinical entity. *Lancet* 1986;**1**:1031–2.
394. Ota MO, O'Donovan D, Alabi AS, et al. Maternal HIV-1 and HIV-2 infection and child survival in The Gambia. *AIDS* 2000;**14**:435–9.
395. Otten RA, Ellenberger DL, Adams DR, et al. Identification of a window period for susceptibility to dual infection with two distinct human immunodeficiency virus type 2 isolates in a *Macaca nemestrina* (pig-tailed macaque) model. *J Infect Dis* 1999;**180**: 673–84.
396. Ozden S, Gessain A, Gout O, et al. Sporadic inclusion body myositis in a patient with human T cell leukemia virus type 1-associated myelopathy. *Clin Infect Dis* 2001;**32**:510–14.
397. Pachl C, Todd JA, Kern DG, et al. Rapid and precise quantification of HIV-1 RNA in plasma using a branched DNA signal amplification assay. *J Acquir Immune Defic Syndr Hum Retroviral* 1995;**8**:446–54.
398. Pacifico LG, Marinho FA, Fonseca CT, et al. Schistosoma mansoni antigens modulate experimental allergic asthma in a murine model: a major role for $CD4^+$ $CD25^+$ $Foxp3^+$ T cells independent of interleukin-10. *Infect Immun* 2009;**77**:98–107.
399. Padua E, Almeida C, Nunes B, et al. Assessment of mother-to-child HIV-1 and HIV-2 transmission: an AIDS reference laboratory collaborative study. *HIV Med* 2009;**10**:182–90.
400. Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. *Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States*. 2012, U.S. Department of Health and Human Services. Available at http://aidsinfo.nih.gov/contentfiles/Ivguidelines/PerinatalGL.pdf.
401. Pardi D, Kaplan JE, Coligan JE, et al. Identification and characterization of an extended Tax protein in human T-cell lymphotropic virus type II subtype b isolates. *J Virol* 1993;**67**:7663–7.
402. Patnaik A, Chau V, Wills JW. Ubiquitin is part of the retrovirus budding machinery. *Proc Natl Acad Sci U S A* 2000;**97**:13069–74.
403. Paun L, Ispas O, Del Mistro A, et al. HTLV-I in Romania. *Eur J Haematol* 1994;**52**:117–18.
404. Pawson R, Mufti GJ, Pagliuca A. Management of adult T-cell leukaemia/lymphoma. *Br J Haematol* 1998;**100**:453–8.
405. Pedroso C, Netto EM, Weyll N, et al. Coinfection by HIV-1 and human lymphotropic virus type 1 in Brazilian children is strongly associated with a shorter survival time. *J Acquir Immune Defic Syndr* 2011;**57**(Suppl. 3):S208–11.

406. Peeters M, Gershy-Damet GM, Fransen K, et al. Virological and polymerase chain reaction studies of HIV-1/HIV-2 dual infection in Cote d'Ivoire. *Lancet* 1992;**340**:339–40.
407. Pepin J, Labbe AC. Noble goals, unforeseen consequences: control of tropical diseases in colonial Central Africa and the iatrogenic transmission of blood-borne viruses. *Trop Med Int Health* 2008;**13**:744–53.
408. Pepin J, Labbe AC, Mamadou-Yaya F, et al. Iatrogenic transmission of human T cell lymphotropic virus type 1 and hepatitis C virus through parenteral treatment and chemoprophylaxis of sleeping sickness in colonial Equatorial Africa. *Clin Infect Dis* 2010;**51**:777–84.
409. Perach M, Rubinek T, Hizi A. Resistance to nucleoside analogs of selective mutants of human immunodeficiency virus type 2 reverse transcriptase. *J Virol* 1995;**69**:509–12.
410. Perzova R, Benz P, Abbott L, et al. Short communication: no evidence of HTLV-3 and HTLV-4 infection in New York State subjects at risk for retroviral infection. *AIDS Res Hum Retroviruses* 2010;**26**:1229–31.
411. Peters AA, Coulthart MB, Oger JJ, et al. HTLV type I/II in British Columbia Amerindians: a seroprevalence study and sequence characterization of an HTLV type IIa isolate. *AIDS Res Hum Retroviruses* 2000;**16**:883–92.
412. Pillonel J, David D, Pinget R, et al. [Prevalence of HBV, HCV, HIV and HTLV in autologous blood donors in France between 1993 and 2000]. *Transfus Clin Biol* 2002;**9**:289–96.
413. Poetker SK, Porto AF, Giozza SP, et al. Clinical manifestations in individuals with recent diagnosis of HTLV type I infection. *J Clin Virol* 2011;**51**:54–8.
414. Poiesz BJ, Ruscetti FW, Gazdar AF, et al. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc Natl Acad Sci U S A* 1980;**77**:7415–19.
415. Pombo-de-Oliveira MS, Dobbin JA, Loureiro P, et al. Genetic mutation and early onset of T-cell leukemia in pediatric patients infected at birth with HTLV-I. *Leuk Res* 2002;**26**:155–61.
416. Pombo De Oliveira MS, Loureiro P, Bittencourt A, et al. Geographic diversity of adult T-cell leukemia/lymphoma in Brazil. The Brazilian ATLL Study Group. *Int J Cancer* 1999;**83**: 291–8.
417. Popper SJ, Sarr AD, Gueye-Ndiaye A, et al. Low plasma human immunodeficiency virus type 2 viral load is independent of proviral load: low virus production in vivo. *J Virol* 2000;**74**:1554–7.
418. Popper SJ, Sarr AD, Travers KU, et al. Lower human immunodeficiency virus (HIV) type 2 viral load reflects the difference in pathogenicity of HIV-1 and HIV-2. *J Infect Dis* 1999;**180**: 1116–21.
419. Portis T, Harding JC, Ratner L. The contribution of NF-kappa B activity to spontaneous proliferation and resistance to apoptosis in human T-cell leukemia virus type 1 Tax-induced tumors. *Blood* 2001;**98**:1200–8.
420. Porto AF, Santos SB, Alcantara L, et al. HTLV-1 modifies the clinical and immunological response to schistosomiasis. *Clin Exp Immunol* 2004;**137**:424–9.
421. Porto AF, Santos SB, Muniz AL, et al. Helminthic infection down-regulates type 1 immune responses in human T cell lymphotropic virus type 1 (HTLV-1) carriers and is more prevalent in HTLV-1 carriers than in patients with HTLV-1-associated myelopathy/tropical spastic paraparesis. *J Infect Dis* 2005;**191**:612–18.
422. Posada-Vergara MP, Montanheiro P, Fukumori LM, et al. Clinical and epidemiological aspects of HTLV-II infection in Sao Paulo, Brazil: presence of tropical spastic paraparesis/HTLV-associated myelopathy (TSP/HAM) simile diagnosis in HIV-1-co-infected subjects. *Rev Inst Med Trop Sao Paulo* 2006;**48**:207–10.
423. Pouliquen JF, Hardy L, Lavergne A, et al. High seroprevalence of human T-cell lymphotropic virus type 1 in blood donors in Guyana and molecular and phylogenetic analysis of new strains in the Guyana shelf (Guyana, Suriname, and French Guiana). *J Clin Microbiol* 2004;**42**:2020–6.
424. Poulsen AG, Aaby P, Larsen O, et al. 9-year HIV-2-associated mortality in an urban community in Bissau, west Africa. *Lancet* 1997;**349**:911–14.
425. Poveda E, Rodes B, Labernardiere JL, et al. Evolution of genotypic and phenotypic resistance to enfuvirtide in HIV-infected patients experiencing prolonged virologic failure. *J Med Virol* 2004;**74**:21–8.
426. Prazuck T, Yameogo JM, Heylinck B, et al. Mother-to-child transmission of human immunodeficiency virus type 1 and type 2 and dual infection: a cohort study in Banfora, Burkina Faso. *Pediatr Infect Dis J* 1995;**14**:940–7.
427. Primo JR, Brites C, Oliveira Mde F, et al. Infective dermatitis and human T cell lymphotropic virus type 1-associated myelopathy/tropical spastic paraparesis in childhood and adolescence. *Clin Infect Dis* 2005;**41**:535–41.
428. Proietti FA, Carneiro-Proietti AB, Catalan-Soares BC, et al. Global epidemiology of HTLV-I infection and associated diseases. *Oncogene* 2005;**24**:6058–68.
429. Putkonen P, Thorstensson R, Albert J, et al. Infection of cynomolgus monkeys with HIV-2 protects against pathogenic consequences of a subsequent simian immunodeficiency virus infection. *AIDS* 1990;**4**:783–9.
430. Quintas S, Moreno T, Lobo-Antunes N, et al. [Tropical spastic paraparesis and HTLV-I associated myelopathy in infancy: a case report and review of the literature]. *Rev Neurol* 2004;**39**: 1133–6.
431. Ratner L, Poiesz BJ. Leukemias associated with human T-cell lymphotropic virus type I in a non-endemic region. *Medicine (Baltimore)* 1988;**67**:401–22.
432. Ratner L, Grant C, Zimmerman B, et al. Effect of treatment of Strongyloides infection on HTLV-1 expression in a patient with adult T-cell leukemia. *Am J Hematol* 2007;**82**:929–31.
433. Ravandi F, Faderl S. Complete response in a patient with adult T-cell leukemia (ATL) treated with combination of alemtuzumab and pentostatin. *Leuk Res* 2006;**30**:103–5.
434. Reid P, MacInnes H, Cong ME, et al. Natural resistance of human immunodeficiency virus type 2 to zidovudine. *Virology* 2005;**336**: 251–64.
435. Reference deleted in proofs.
436. Ren T, Dong W, Takahashi Y, et al. HTLV-2 Tax Immortalizes Human $CD4^+$ memory T lymphocytes by oncogenic activation and dysregulation of autophagy. *J Biol Chem* 2012;**287**: 34683–93.
437. Reference deleted in proofs.
438. Ribeiro MA, Martins ML, Teixeira C, et al. Blocking vertical transmission of human T cell lymphotropic virus type 1 and 2 through breastfeeding interruption. *Pediatr Infect Dis J* 2012;**31**: 1139–43.
439. Ricard D, Wilkins A, N'Gum PT, et al. The effects of HIV-2 infection in a rural area of Guinea-Bissau. *AIDS* 1994;**8**: 977–82.
440. Rio B, Louvet C, Gessain A, et al. [Adult T-cell leukemia and non-malignant adenopathies associated with HTLV I virus: apropos of 17 patients born in the Caribbean region and Africa]. *Presse Med* 1990;**19**:746–51.
441. Rios M, Khabbaz RF, Kaplan JE, et al. Transmission of human T cell lymphotropic virus (HTLV) type II by transfusion of HTLV-I-screened blood products. *J Infect Dis* 1994;**170**: 206–10.
442. Roberts BD, Foung SK, Lipka JJ, et al. Evaluation of an immunoblot assay for serological confirmation and differentiation of human T-cell lymphotropic virus types I and II. *J Clin Microbiol* 1993;**31**:260–4.
443. Rodes B, Holguin A, Soriano V, et al. Emergence of drug resistance mutations in human immunodeficiency virus type 2-infected subjects undergoing antiretroviral therapy. *J Clin Microbiol* 2000;**38**:1370–4.
444. Rodes B, Sheldon J, Toro C, et al. Susceptibility to protease inhibitors in HIV-2 primary isolates from patients failing antiretroviral therapy. *J Antimicrob Chemother* 2006;**57**:709–13.
445. Rodgers-Johnson P, Gajdusek DC, Morgan OS, et al. HTLV-I and HTLV-III antibodies and tropical spastic paraparesis. *Lancet* 1985;**2**:1247–8.
446. Roquebert B, Damond F, Collin G, et al. HIV-2 integrase gene polymorphism and phenotypic susceptibility of HIV-2 clinical isolates to the integrase inhibitors raltegravir and elvitegravir in vitro. *J Antimicrob Chemother* 2008;**62**:914–20.
447. Roucoux DF, Murphy EL. The epidemiology and disease outcomes of human T-lymphotropic virus type II. *AIDS Rev* 2004;**6**:144–54.
448. Roucoux DF, Wang B, Smith D, et al. A prospective study of sexual transmission of human T lymphotropic virus (HTLV)-I and HTLV-II. *J Infect Dis* 2005;**191**:1490–7.

449. Safaeian M, Wilson LE, Taylor E, et al. HTLV-II and bacterial infections among injection drug users. *J Acquir Immune Defic Syndr* 2000;**24**:483–7.
450. Sagawa K, Mochizuki M, Masuoka K, et al. Immunopathological mechanisms of human T cell lymphotropic virus type 1 (HTLV-I) uveitis: detection of HTLV-I-infected T cells in the eye and their constitutive cytokine production. *J Clin Invest* 1995;**95**:852–8.
451. Saito M, Matsuzaki T, Satou Y, et al. In vivo expression of the HBZ gene of HTLV-1 correlates with proviral load, inflammatory markers and disease severity in HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP). *Retrovirology* 2009;**6**:19.
452. Saji F, Tokugawa Y, Kamiura S, et al. Vertical transmission of human T-cell leukemia virus type I (HTLV-I): detection of proviral DNA in HTLV-I carrier gravida. *J Clin Immunol* 1989;**9**:409–14.
453. Sakashita A, Hattori T, Miller CW, et al. Mutations of the p53 gene in adult T-cell leukemia. *Blood* 1992;**79**:477–80.
454. Reference deleted in proofs.
455. Sanhadji K, Gessain A, Chout R, et al. HTLV-I antibody and cell-mediated immunity status in sickle cell anemia. *Clin Immunol Immunopathol* 1987;**43**:140–4.
456. Sarkodie F, Adarkwa M, Adu-Sarkodie Y, et al. Screening for viral markers in volunteer and replacement blood donors in West Africa. *Vox Sang* 2001;**80**:142–7.
457. Sarui H, Sano A, Maruyama T, et al. Severe graves ophthalmopathy accompanied by HTLV-1-associated uveitis (HAU) and antineutrophil cytoplasmic antibody-related vasculitis: a case report and a review of the literature of HAU with Graves disease. *Am J Med Sci* 2002;**324**:109–14.
458. Satomi M, Shimizu M, Shinya E, et al. Transmission of macrophage-tropic HIV-1 by breast-milk macrophages via DC-SIGN. *J Infect Dis* 2005;**191**:174–81.
459. Schaeffer E, Geleziunas R, Greene WC. Human immunodeficiency virus type 1 Nef functions at the level of virus entry by enhancing cytoplasmic delivery of virions. *J Virol* 2001;**75**:2993–3000.
460. Schim van der Loeff MF, Hansmann A, Awasana AA, et al. Survival of HIV-1 and HIV-2 perinatally infected children in The Gambia. *AIDS* 2003;**17**:2389–94.
461. Schim van der Loeff MF, Jaffar S, Aveika AA, et al. Mortality of HIV-1, HIV-2 and HIV-1/HIV-2 dually infected patients in a clinic-based cohort in The Gambia. *AIDS* 2002;**16**:1775–83.
462. Schramm B, Penn ML, Palacios EH, et al. Cytopathicity of human immunodeficiency virus type 2 (HIV-2) in human lymphoid tissue is coreceptor dependent and comparable to that of HIV-1. *J Virol* 2000;**74**:9594–600.
463. Schreiber GB, Murphy EL, Horton JA, et al. Risk factors for human T-cell lymphotropic virus types I and II (HTLV-I and -II) in blood donors: the Retrovirus Epidemiology Donor Study. NHLBI Retrovirus Epidemiology Donor Study. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**14**:263–71.
464. Schubert U, Ott DE, Chertova EN, et al. Proteasome inhibition interferes with gag polyprotein processing, release, and maturation of HIV-1 and HIV-2. *Proc Natl Acad Sci U S A* 2000;**97**:13057–62.
465. Schwebke J, Calsyn D, Shriver K, et al. Prevalence and epidemiologic correlates of human T cell lymphotropic virus infection among intravenous drug users. *J Infect Dis* 1994;**169**:962–7.
466. Seck K, Samb N, Tempesta S, et al. Prevalence and risk factors of cervicovaginal HIV shedding among HIV-1 and HIV-2 infected women in Dakar, Senegal. *Sex Transm Infect* 2001;**77**:190–3.
467. Seki N, Yamaguchi K, Yamada A, et al. Polymorphism of the 5′-flanking region of the tumor necrosis factor (TNF)-alpha gene and susceptibility to human T-cell lymphotropic virus type I (HTLV-I) uveitis. *J Infect Dis* 1999;**180**:880–3.
468. Sekigawa I, Kaneko H, Neoh LP, et al. Differences of HIV envelope protein between HIV-1 and HIV-2: possible relation to the lower virulence of HIV-2. *Viral Immunol* 1998;**11**:1–8.
469. Setoyama M, Mizoguchi S, Eizuru Y. Human T-cell lymphotropic virus type I infects eccrine sweat gland epithelia. *Int J Cancer* 1999;**80**:652–5.
470. Seydel J, Kramer A. Transmission and population dynamics of HTLV-I infection. *Int J Cancer* 1996;**66**:197–200.
471. Shanmugam V, Switzer WM, Nkengasong JN, et al. Lower HIV-2 plasma viral loads may explain differences between the natural histories of HIV-1 and HIV-2 infections. *J Acquir Immune Defic Syndr* 2000;**24**:257–63.
472. Sheremata WA, Benedict D, Squilacote DC, et al. High-dose zidovudine induction in HTLV-I-associated myelopathy: safety and possible efficacy. *Neurology* 1993;**43**:2125–9.
473. Shibata D, Brynes RK, Rabinowitz A, et al. Human T-cell lymphotropic virus type I (HTLV-I)-associated adult T-cell leukemia-lymphoma in a patient infected with human immunodeficiency virus type 1 (HIV-1). *Ann Intern Med* 1989;**111**:871–5.
474. Shibata K, Shimamoto Y, Nishimura T, et al. M: Ocular manifestations in adult T-cell leukemia/lymphoma. *Ann Hematol* 1997;**74**:163–8.
475. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma: a report from the Lymphoma Study Group (1984-87). *Br J Haematol* 1991;**79**:428–37.
476. Silva EA, Otsuki K, Leite AC, et al. HTLV-II infection associated with a chronic neurodegenerative disease: clinical and molecular analysis. *J Med Virol* 2002;**66**:253–7.
477. Sison AV, Campos JM. Laboratory methods for early detection of human immunodefieciency virus type 1 in newborns and infants. *Clin Microbiol Rev* 1992;**5**:238–47.
478. Deleted in proofs.
479. Soares RS, Tendeiro R, Foxall RB, et al. Cell-associated viral burden provides evidence of ongoing viral replication in aviremic HIV-2-infected patients. *J Virol* 2010;**85**:2429–38.
480. Song KJ, Nerurkar VR, Saitou N, et al. Genetic analysis and molecular phylogeny of simian T-cell lymphotropic virus type I: evidence for independent virus evolution in Asia and Africa. *Virology* 1994;**199**:56–66.
481. Soriano V, Gomes P, Heneine W, et al. Human immunodeficiency virus type 2 (HIV-2) in Portugal: clinical spectrum, circulating subtypes, virus isolation, and plasma viral load. *J Med Virol* 2000;**61**:111–16.
482. Southern SO, Southern PJ. Persistent HTLV-I infection of breast luminal epithelial cells: a role in HTLV transmission? *Virology* 1998;**241**:200–14.
483. Steinfort DP, Brady S, Weisinger HS, et al. Bronchiectasis in Central Australia: a young face to an old disease. *Respir Med* 2008;**102**:574–8.
484. Strack B, Calistri A, Accola MA, et al. A role for ubiquitin ligase recruitment in retrovirus release. *Proc Natl Acad Sci U S A* 2000;**97**:13063–8.
485. Sugimoto M, Nakashima H, Matsumoto M, et al. Pulmonary involvement in patients with HTLV-I-associated myelopathy: increased soluble IL-2 receptors in bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1989;**139**:1329–35.
486. Sugimoto M, Nakashima H, Watanabe S, et al. T-lymphocyte alveolitis in HTLV-I-associated myelopathy. *Lancet* 1987;**2**:1220.
487. Suite M, Jack N, Basedo-Maharaj K, et al. Infective dermatitis in Trinidad and Tobago. *AIDS Res Hum Retroviruses* 1994;**10**:447.
488. Sullivan N, Sun Y, Sattentau Q, et al. CD4-Induced conformational changes in the human immunodeficiency virus type 1 gp120 glycoprotein: consequences for virus entry and neutralization. *J Virol* 1998;**72**:4694–703.
489. Sumida T, Yonaha F, Maeda T, et al. Expression of sequences homologous to HTLV-I tax gene in the labial salivary glands of Japanese patients with Sjögren's syndrome. *Arthritis Rheum* 1994;**37**:545–50.
490. Sundberg MA, Costa D, Orge G, et al. Helminthic infection and the risk of neurologic disease progression in HTLV-1. *J Clin Virol* 2012;**53**:251–5.
491. Sweet RD. A pattern of eczema in Jamaica. *Br J Dermatol* 1966;**78**:93–100.
492. Switzer WM, Salemi M, Qari SH, et al. Ancient, independent evolution and distinct molecular features of the novel human T-lymphotropic virus type 4. *Retrovirology* 2009;**6**:9.
493. Switzer WM, Wiktor S, Soriano V, et al. Evidence of Nef truncation in human immunodeficiency virus type 2 infection. *J Infect Dis* 1998;**177**:65–71.
494. Tajima K. The 4th nation-wide study of adult T-cell leukemia/lymphoma (ATL) in Japan: estimates of risk of ATL and its geographical and clinical features. The T- and B-cell Malignancy Study Group. *Int J Cancer* 1990;**45**:237–43.

495. Takahashi K, Takezaki T, Oki T, et al. Inhibitory effect of maternal antibody on mother-to-child transmission of human T-lymphotropic virus type I. The Mother-to-Child Transmission Study Group. *Int J Cancer* 1991;**49**:673–7.
496. Takahashi T, Takase H, Urano T, et al. Clinical features of human T-lymphotropic virus type 1 uveitis: a long-term follow-up. *Ocul Immunol Inflamm* 2000;**8**:235–41.
497. Takao S, Ishida T, Bhatia KK, et al. Seroprevalence of human T-lymphotropic virus type 1 in Papua New Guinea and Irian Jaya measured using different western blot criteria. *J Clin Virol* 2000;**16**:129–33.
498. Takasaki Y, Iwanaga M, Imaizumi Y, et al. Long-term study of indolent adult T-cell leukemia-lymphoma. *Blood* 2010;**115**: 4337–43.
499. Takenouchi H, Umeki K, Sasaki D, et al. Defective human T-lymphotropic virus type 1 provirus in asymptomatic carriers. *Int J Cancer* 2010;**128**:1335–43.
500. Takeuchi H, Takahashi M, Norose Y, et al. Transformation of breast milk macrophages by HTLV-I: implications for HTLV-I transmission via breastfeeding. *Biomed Res* 2010;**31**:53–61.
501. Tamalet C, Simon F, Dhiver C, et al. Autologous neutralizing antibodies and viral load in HIV-2-infected individuals. *AIDS* 1995;**9**:90–1.
502. Tanabe K, Ikegami Y, Ishida R, et al. Inhibition of topoisomerase II by antitumor agents bis(2,6-dioxopiperazine) derivatives. *Cancer Res* 1991;**51**:4903–8.
503. Tang H, Kuhen KL, Wong-Staal F. Lentivirus replication and regulation. *Annu Rev Genet* 1999;**33**:133–70.
504. Tangy F, Ossondo M, Vernant JC, et al. Human T cell leukemia virus type I expression in salivary glands of infected patients. *J Infect Dis* 1999;**179**:497–502.
505. Tannir N, Riggs S, Velasquez W, et al. Hypercalcemia, unusual bone lesions, and human T-cell leukemia-lymphoma virus in adult T-cell lymphoma. *Cancer* 1985;**55**:615–19.
506. Taylor GP, Goon P, Furukawa Y, et al. Zidovudine plus lamivudine in human T-lymphotropic virus type-I-associated myelopathy: a randomised trial. *Retrovirology* 2006;**3**:63.
507. Taylor GP, Hall SE, Navarrete S, et al. Effect of lamivudine on human T-cell leukemia virus type 1 (HTLV-1) DNA copy number, T-cell phenotype, and anti-tax cytotoxic T-cell frequency in patients with HTLV-1-associated myelopathy. *J Virol* 1999;**73**: 10289–95.
508. Taylor GP, Tosswill JH, Matutes E, et al. Prospective study of HTLV-I infection in an initially asymptomatic cohort. *J Acquir Immune Defic Syndr* 1999;**22**:92–100.
509. Terada K, Katamine S, Eguchi K, et al. Prevalence of serum and salivary antibodies to HTLV-1 in Sjögren's syndrome. *Lancet* 1994;**344**:1116–19.
510. Thali M, Bukovsky A, Kondo E, et al. Functional association of cyclophilin A with HIV-1 virions. *Nature* 1994;**372**:363–5.
511. The T- and B-cell Malignancy Study Group. The third nation-wide study on adult T-cell leukemia/lymphoma (ATL) in Japan: characteristic patterns of HLA antigen and HTLV-I infection in ATL patients and their relatives. *Int J Cancer* 1988;**41**: 505–12.
512. Thior I, Lockman S, Smeaton LM, et al. Breastfeeding plus infant zidovudine prophylaxis for 6 months vs formula feeding plus infant zidovudine for 1 month to reduce mother-to-child HIV transmission in Botswana: a randomized trial: the Mashi Study. *JAMA* 2006;**296**:794–805.
513. Torian LV, Eavey JJ, Punsalang AP, et al. HIV type 2 in New York City, 2000-2008. *Clin Infect Dis* 2010;**51**:1334–42.
514. Torian LV, Forgione LA, Punsalang AE, et al. Comparison of Multispot EIA with Western blot for confirmatory serodiagnosis of HIV. *J Clin Virol* 2011;**52**(Suppl. 1):S41–4.
515. Toro C, Rodes B, Aguilera A, et al. [HIV-2 and HTLV-I/II infections in Spain]. *Enferm Infecc Microbiol Clin* 2004;**22**: 177–82.
516. Tortevoye P, Tuppin P, Carles G, et al. Comparative trends of seroprevalence and seroincidence rates of human T cell lymphotropic virus type I and human immunodeficiency virus 1 in pregnant women of various ethnic groups sharing the same environment in French Guiana. *Am J Trop Med Hyg* 2005;**73**:560–5.
517. Travers K, Mboup S, Marlink R, et al. Natural protection against HIV-1 infection provided by HIV-2. *Science* 1995;**268**: 1612–15.
518. Travers KU, Eisen GE, Marlink RG, et al. Protection from HIV-1 infection by HIV-2. *AIDS* 1998;**12**:224–5.
519. Trevino A, Parra P, Bar-Magen T, et al. Antiviral effect of raltegravir on HTLV-1 carriers. *J Antimicrob Chemother* 2012;**67**: 218–21.
520. Trujillo L, Munoz D, Gotuzzo E, et al. Sexual practices and prevalence of HIV, HTLV-I/II, and *Treponema pallidum* among clandestine female sex workers in Lima, Peru. *Sex Transm Dis* 1999;**26**:115–18.
521. Tsuda H, Takatsuki K, Ohno R, et al. Treatment of adult T-cell leukaemia-lymphoma with irinotecan hydrochloride (CPT-11). CPT-11 Study Group on Hematological Malignancy. *Br J Cancer* 1994;**70**:771–4.
522. Tsukasaki K, Tobinai K, Shimoyama M, et al. Deoxycoformycin-containing combination chemotherapy for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study (JCOG9109). *Int J Hematol* 2003;**77**:164–70.
523. Tsukasaki K, Utsunomiya A, Fukuda H, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *J Clin Oncol* 2007;**25**:5458–64.
524. Tsukasaki K, Yamada Y, Ikeda S, et al. Infective dermatitis among patients with ATL in Japan. *Int J Cancer* 1994;**57**:293.
525. Tuillon E, Gueudin M, Lemee V, et al. Phenotypic susceptibility to nonucleoside inhibitors of virion-associated reverse transcriptase from different HIV types and groups. *J Acquir Immune Defic Syndr* 2004;**37**:1543–9.
526. Tuppin P, Gessain A, Kazanji M, et al. Evidence in Gabon for an intrafamilial clustering with mother-to-child and sexual transmission of a new molecular variant of human T-lymphotropic virus type-II subtype B. *J Med Virol* 1996;**48**:22–32.
527. Turci M, Pilotti E, Ronzi P, et al. Coinfection with HIV-1 and human T-Cell lymphotropic virus type II in intravenous drug users is associated with delayed progression to AIDS. *J Acquir Immune Defic Syndr* 2006;**41**:100–6.
528. Uchiyama T, Yodoi J, Sagawa K, et al. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood* 1977;**50**: 481–92.
529. Ueno S, Umeki K, Takajo I, et al. Proviral loads of human T-lymphotropic virus Type 1 in asymptomatic carriers with different infection routes. *Int J Cancer* 2011;**130**:2318–26.
530. Umehara F, Izumo S, Ronquillo AT, et al. Cytokine expression in the spinal cord lesions in HTLV-I-associated myelopathy. *J Neuropathol Exp Neurol* 1994;**53**:72–7.
531. Umehara F, Izumo S, Takeya M, et al. Expression of adhesion molecules and monocyte chemoattractant protein-1 (MCP-1) in the spinal cord lesions in HTLV-I-associated myelopathy. *Acta Neuropathol* 1996;**91**:343–50.
532. Ureta-Vidal A, Angelin-Duclos C, Tortevoye P, et al. Mother-to-child transmission of human T-cell-leukemia/lymphoma virus type I: implication of high antiviral antibody titer and high proviral load in carrier mothers. *Int J Cancer* 1999;**82**:832–6.
533. Urnovitz HB, Murphy WH. Human endogenous retroviruses: nature, occurrence, and clinical implications in human disease. *Clin Microbiol Rev* 1996;**9**:72–99.
534. Usuku K, Sonoda S, Osame M, et al. HLA haplotype-linked high immune responsiveness against HTLV-I in HTLV-I-associated myelopathy: comparison with adult T-cell leukemia/lymphoma. *Ann Neurol* 1988;**23**(Suppl):S143–50.
535. van der Ende ME, Guillon C, Boers PH, et al. Antiviral resistance of biologic HIV-2 clones obtained from individuals on nucleoside reverse transcriptase inhibitor therapy. *J Acquir Immune Defic Syndr* 2000;**25**:11–18.
536. van der Ende ME, Guillon C, Boers PH, et al. Broadening of coreceptor usage by human immunodeficiency virus type 2 does not correlate with increased pathogenicity in an in vivo model. *J Gen Virol* 2000;**81**:507–13.
537. van der Ende ME, Prins JM, Brinkman K, et al. Clinical, immunological and virological response to different antiretroviral regimens in a cohort of HIV-2-infected patients. *AIDS* 2003;**17** (Suppl. 3):S55–61.
538. van der Loeff MF, Larke N, Kaye S, et al. Undetectable plasma viral load predicts normal survival in HIV-2-infected people in a West African village. *Retrovirology* 2010;**7**:46.
539. van Dooren S. Central Africa: cradle of divergent PTLV types. *AIDS Reviews* 2005;**7**:126–7.

540. van Dooren S, Salemi M, Vandamme AM. Dating the origin of the African human T-cell lymphotropic virus type-i (HTLV-I) subtypes. *Mol Biol Evol* 2001;**18**:661–71.
541. van Dooren S, Gotuzzo E, Salemi M, et al. Evidence for a post-Columbian introduction of human T-cell lymphotropic virus [type I] [corrected] in Latin America. *J Gen Virol* 1998;**79**(Pt 11): 2695–708.
542. van Tienen C, Jakobsen M, Schim van der Loeff M. Stopping breastfeeding to prevent vertical transmission of HTLV-1 in resource-poor settings: beneficial or harmful? *Arch Gynecol Obstet* 2012;**286**:255–6.
543. van Tienen C, van der Loeff MF, Peterson I, et al. HTLV-1 in rural Guinea-Bissau: prevalence, incidence and a continued association with HIV between 1990 and 2007. *Retrovirology* 2010; **7**:50.
544. Vandamme AM, Bertazzoni U, Salemi M. Evolutionary strategies of human T-cell lymphotropic virus type II. *Gene* 2000;**261**: 171–80.
545. Vandamme AM, Salemi M, Desmyter J. The simian origins of the pathogenic human T-cell lymphotropic virus type I. *Trends Microbiol* 1998;**6**:477–83.
546. Venkateshan CN, La Grenade L, De SK. Overexpression of pro- and anti-inflammatory cytokines by cultured keratinocytes from HTLV-I associated familial infective dermatitis. *AIDS Res Hum Retroviruses* 1995;**11**:143.
547. Verdonck K, Gonzalez E, Van Dooren S, et al. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis* 2007;**7**:266–81.
548. Vitek CR, Gracia FI, Giusti R, et al. Evidence for sexual and mother-to-child transmission of human T lymphotropic virus type II among Guaymi Indians, Panama. *J Infect Dis* 1995;**171**: 1022–6.
549. Vlahov D, Khabbaz RF, Cohn S, et al. Incidence and risk factors for human T-lymphotropic virus type II seroconversion among injecting drug users in Baltimore, Maryland, U.S.A. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;**9**:89–96.
550. Vogt VM. Ubiquitin in retrovirus assembly: actor or bystander? *Proc Natl Acad Sci U S A* 2000;**97**:12945–7.
551. von Glehn F, Jarius S, Penalva de Oliveira AC, et al. Aquaporin-4 antibodies are not related to HTLV-1 associated myelopathy. *PLoS One* 2012;**7**:e39372.
552. von Schwedler U, Song J, Aiken C, et al. Vif is crucial for human immunodeficiency virus type 1 proviral DNA synthesis in infected cells. *J Virol* 1993;**67**:4945–55.
553. Wagner M, Rose VA, Linder R, et al. Human pathogenic virus-associated pseudolymphomas and lymphomas with primary cutaneous manifestation in humans and animals. *Clin Infect Dis* 1998;**27**:1299–308.
554. Walshe MM. Infective dermatitis in Jamaican children. *Br J Dermatol* 1967;**79**:229–36.
555. Wang Y, Li X, Song A, et al. Prevalence and partial sequence analysis of human T cell lymphotropic virus type I in China. *J Med Virol* 2005;**76**:613–18.
556. Wattel E, Vartanian JP, Pannetier C, et al. Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. *J Virol* 1995;**69**: 2863–8.
557. Wei X, Ghosh SK, Taylor ME, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* 1995;**373**: 117–22.
558. Weinberg JB, Spiegel RA, Blazey DL, et al. Human T-cell lymphotropic virus I and adult T-cell leukemia: report of a cluster in North Carolina. *Am J Med* 1988;**85**:51–8.
559. Wesolowski LG, Delaney KP, Hart C, et al. Performance of an alternative laboratory-based algorithm for diagnosis of HIV infection utilizing a third generation immunoassay, a rapid HIV-1/HIV-2 differentiation test and a DNA or RNA-based nucleic acid amplification test in persons with established HIV-1 infection and blood donors. *J Clin Virol* 2011;**52**(Suppl. 1):S45–9.
560. White JD, Wharfe G, Stewart DM, et al. The combination of zidovudine and interferon alpha-2B in the treatment of adult T-cell leukemia/lymphoma. *Leuk Lymphoma* 2001;**40**: 287–94.
561. Whittle H, Morris J, Todd J, et al. HIV-2-infected patients survive longer than HIV-1-infected patients. *AIDS* 1994;**8**: 1617–20.
562. Wiktor SZ, Cannon RO, Atkinson WL, et al. Infection with human T lymphotropic virus types I and II in sexually transmitted disease clinics in Baltimore and New Orleans. *J Infect Dis* 1992;**165**:920–4.
563. Wiktor SZ, Nkengasong JN, Ekpini ER, et al. Lack of protection against HIV-1 infection among women with HIV-2 infection. *AIDS* 1999;**13**:695–9.
564. Wiktor SZ, Pate EJ, Murphy EL, et al. Mother-to-child transmission of human T-cell lymphotropic virus type I (HTLV-I) in Jamaica: association with antibodies to envelope glycoprotein (gp46) epitopes. *J Acquir Immune Defic Syndr* 1993;**6**: 1162–7.
565. Wiktor SZ, Pate EJ, Rosenberg PS, et al. Mother-to-child transmission of human T-cell lymphotropic virus type I associated with prolonged breast-feeding. *J Hum Virol* 1997;**1**:37–44.
566. Wiktor SZ, Piot P, Mann JM, et al. Human T cell lymphotropic virus type I (HTLV-I) among female prostitutes in Kinshasa, Zaire. *J Infect Dis* 1990;**161**:1073–7.
567. Witvrouw M, Pannecouque C, Switzer WM, et al. Susceptibility of HIV-2, SIV and SHIV to various anti-HIV-1 compounds: implications for treatment and postexposure prophylaxis. *Antivir Ther* 2004;**9**:57–65.
568. Witvrouw M, Pannecouque C, Van Laethem K, et al. Activity of non-nucleoside reverse transcriptase inhibitors against HIV-2 and SIV. *AIDS* 1999;**13**:1477–83.
569. Wolfe ND, Switzer WM, Carr JK, et al. Naturally acquired simian retrovirus infections in central African hunters. *Lancet* 2004;**363**: 932–7.
570. World Health Organization. Report of the scientific group on HTLV-I and associated disease. Kagoshima, Japan, December 1988: Virus disease human T lymphotropic virus type I (HTLV-I). *Wkly Epidem Rec* 1989;**49**:382–3.
571. Xu L, Anderson J, Garrett N, et al. Dynamics of raltegravir resistance profile in an HIV type 2-infected patient. *AIDS Res Hum Retroviruses* 2009;**25**:843–7.
572. Yamada Y, Tomonaga M, Fukuda H, et al. A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. *Br J Haematol* 2001;**113**:375–82.
573. Yamaguchi J, Vallari A, Ndembi N, et al. HIV type 2 intergroup recombinant identified in Cameroon. *AIDS Res Hum Retroviruses* 2008;**24**:86–91.
574. Yamaguchi K. Human T-lymphotropic virus type I in Japan. *Lancet* 1994;**343**:213–16.
575. Yamaguchi K, Nishimura H, Kohrogi H, et al. A proposal for smoldering adult T-cell leukemia: a clinicopathologic study of five cases. *Blood* 1983;**62**:758–66.
576. Yamaguchi K, Yul LS, Shimizu T, et al. Concurrence of lymphoma type adult T-cell leukemia in three sisters. *Cancer* 1985;**56**: 1688–90.
577. Yamamoto JH, Segurado AA, Hirata CE, et al. Human T-cell lymphotropic virus type 1 infection and ocular manifestations in Sao Paulo, Brazil. *Arch Ophthalmol* 1999;**117**:513–17.
578. Yamamoto K, Utsunomiya A, Tobinai K, et al. Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *J Clin Oncol* 2010;**28**: 1591–8.
579. Yamano Y, Nagai M, Brennan M, et al. Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific CD8(+) T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). *Blood* 2002;**99**: 88–94.
580. Yamanouchi K, Kinoshita K, Moriuchi R, et al. Oral transmission of human T-cell leukemia virus type-I into a common marmoset *(Callithrix jacchus)* as an experimental model for milk-borne transmission. *Jpn J Cancer Res* 1985;**76**:481–7.
581. Yamazato Y, Miyazato A, Kawakami K, et al. High expression of p40(tax) and pro-inflammatory cytokines and chemokines in the lungs of human T-lymphotropic virus type 1-related bronchopulmonary disorders. *Chest* 2003;**124**:2283–92.
582. Yoshida M. Molecular biology of HTLV-I: recent progress. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**(Suppl. 1): S63–8.
583. Yoshida M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu Rev Immunol* 2001;**19**:475–96.

584. Yoshida Y, Sakamoto Y, Yoshimine A, et al. Three cases of juvenile onset HTLV-I-associated myelopathy with pseudohypoparathyroidism. *J Neurol Sci* 1993;**118**:145–9.
585. Zehender G, Colasante C, Santambrogio S, et al. Increased risk of developing peripheral neuropathy in patients coinfected with HIV-1 and HTLV-2. *J Acquir Immune Defic Syndr* 2002;**31**: 440–7.
586. Zucker-Franklin D, Kosann MK, Pancake BA, et al. Hypopigmented mycosis fungoides associated with human T cell lymphotropic virus type I tax in a pediatric patient. *Pediatrics* 1999; **103**:1039–45.
587. Zunt J. Tropical spastic paraparesis: an old disease with a new name. *Arch Neurol* 2001;**58**:122–4.
588. Zurita S, Costa C, Watts D, et al. Prevalence of human retroviral infection in Quillabamba and Cuzco, Peru: a new endemic area for human T cell lymphotropic virus type 1. *Am J Trop Med Hyg* 1997;**56**:561–5.

composed of four clinical categories (N, A, B, C).[180] The clinical axis is intended for unidirectional use in any individual child; that is, severity of disease proceeds from N (asymptomatic), to A (mildly symptomatic), to B (moderately symptomatic), to C (severely symptomatic), and then to HIV-associated death. Clinical category C describes children with AIDS-defining characteristics, namely, wasting, encephalopathy, opportunistic infections, and malignancies, but it excludes lymphoid interstitial pneumonitis/pulmonary lymphoid hyperplasia (LIP/PLH). Clinical category B describes children with other specific HIV-associated illnesses (e.g., single episodes of bacteremia, leiomyosarcomas, lymphoproliferative lung disorders, anemia, thrombocytopenia) and excludes children with a clinical category C event. Clinical category A describes children with two or more specific HIV-associated illnesses, namely, lymphadenopathy, hepatomegaly, splenomegaly, upper respiratory tract infections/sinusitis/otitis media, parotitis, and dermatitis, and excludes children with a clinical category C or B event. Clinical category N describes children without clinical category C, B, or A events; these children may or may not be completely asymptomatic (single category A events, such as lymphadenopathy, may exist).

Table 191B-3 provides an expanded description of the immunologic axis of the staging system, which includes categories 1 (no evidence of immune suppression), 2 (moderate immunosuppression), and 3 (severe immunosuppression).[180]

TABLE 191B-1 **Estimated Number of Adults and Children Newly Infected with Human Immunodeficiency Virus During 2011**

Region	No. of Infected Adults/Children
African Continent	1.8 million (1.6-2.0 million)
South/Southeast Asia	280,000 (170,000-370,000)
Eastern Europe/Central Asia	140,000 (91,000-210,000)
Latin America	83,000 (51,000-140,000)
North America	51,000 (19,000-120,000)
Caribbean	13,000 (9,600-16,000)
Western/Central Europe	30,000 (21,000-40,000)
Oceania	2,900 (2,200-3,800)
Total	2.5 million (2.2-2.8 million)

Modified from Joint United Nations Programme on HIV/AIDS (UNAIDS). *Global report: UNAIDS report on the global AIDS epidemic: 2012*. Publication No. UNAIDS/JC24117E. New York: UNAIDS; 2012, p. 14.

EPIDEMIOLOGY AND TRANSMISSION AND PREVENTION

Transmission by Blood Products

Pediatric HIV transmission has been documented by blood and blood products, by perinatal transmission from mothers with identified risk factors, by breast milk,[75] and by sexual transmission to children and adolescents,[96] a development that may become epidemic. Since HIV screening techniques, including nucleic acid amplification testing to pooled blood products, were adapted and

TABLE 191B-2 **Pediatric Human Immunodeficiency Virus Classification: Clinical Categories**

Immunologic Categories	N: No Signs/ Symptoms	A: Mild Signs/ Symptoms	B: Moderate Signs/ Symptoms	C: Severe Signs/ Symptoms
1. No evidence of suppression	N1	A1	B1	C1
2. Evidence of moderate suppression	N2	A2	B2	C2
3. Severe suppression	N3	A3	B3	C3

Modified from Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;***58***(RR-11):1-166.

TABLE 191B-3 **Immunologic Categories Based on Age-Specific CD4⁺ T-Lymphocyte Counts and Percentage of Total Lymphocytes**

	Age of Child					
	<12 mo		1-5 yr		6-12 yr	
Immunologic Category	μL	%	μL	%	μL	%
1. No evidence of suppression	≥1500	≥25	≥1000	≥25	≥500	≥25
2. Evidence of moderate suppression	750-1499	15-24	500-999	15-24	200-499	15-24
3. Severe suppression	<750	<15	<500	<15	<200	<15

Modified from Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;***58***(RR-11):1-166.

recombinant factor products were used increasingly, transmission of HIV by blood products rarely has been reported in the United States.[70]

Perinatal Transmission

In the United States, the total annual number of AIDS cases resulting from perinatal transmission peaked in 1992, with just fewer than 1000 newly affected children reported.[19] Predominance of perinatal transmission is found worldwide, especially in countries where adult heterosexual transmission is uniquely prevalent (e.g., in Africa).[45,223] Before the use of perinatal antiretroviral interventions, the transmission rate for perinatally acquired HIV infection was reported to be between 12 and 25 percent in the United States and Europe and is probably higher in Africa.[61,257]

Maternal risk factors associated with enhanced perinatal HIV transmission and identified through national surveillance in reported AIDS cases include drug abuse, heterosexual infection by sexual partners with risk factors for acquiring HIV disease, and maternal transfusion before 1985. Prospective and retrospective evaluations of maternal predictors for perinatal HIV transmission have been the focus of multiple studies. Maternal transmission predictors identified to date include maternal viremia (measured as quantitative HIV RNA polymerase chain reaction [PCR] or viral load),[89,162] maternal immunosuppression, or an inadequate immune response (measured by the $CD4^+$ T-cell count, neutralizing antibody production)[162] and viral characteristics (chemokine receptor tropism, resistance patterns of maternal virus at delivery or infant virus at birth).[227] Pregnancy and placental variables (delivery mode, duration of rupture of membranes, chorioamnionitis) also may influence the risk for perinatal transmission of HIV.[187,247,256] Infant variables evaluated as predictors of transmission of HIV include specific human leukocyte antigen (HLA) markers and the infant cellular immune response (cytokine production, activated T-cell function).[78,161,162,207] In addition, immunogenetic factors (chemokine coreceptor expression) have been suggested to confer protection against progression of disease.[232]

Perinatal transmission of HIV has been proposed to take place in the antepartum, peripartum, and postpartum periods. The distribution of transmission across these timing periods has not been defined precisely, but in non–breast-feeding populations, more than 50 percent of transmission is estimated to occur at the end of pregnancy.[110,146] The timing of the first positive culture in perinatally infected infants—that is, early (the first 7 days of life) versus late (second week of life or later)—has been used epidemiologically to differentiate children potentially infected in utero from those with intrapartum infection.[27] Such distinctions and a need for more precise definition of the timing of distribution of perinatal transmission are of paramount importance in defining interventions for interruption of transmission. Such interruption is especially important because investigators have suggested that early HIV-1 infection can have an impact on the rate of neurodevelopmental dysfunction within the first 2 years of life.[2,244]

In an African study, evaluation of gender-specific risks for mother-to-child transmission of HIV showed preference for intrapartum transmission to female infants.[254] In this cohort, the overall transmission rate at birth was 9.5 percent. HIV-exposed male infants (n = 966) had a 6.3 percent transmission rate as compared with 998 HIV-exposed female infants, who had a 12.6 percent transmission rate. Attempts to assess progression of disease have led to descriptions of subpopulations of HIV-infected children: rapid, usual, and slow progressors.[125,139] Standardized definitions for rapid or usual progressors are lacking. A stringent definition of slow progressors has been proposed (8 years of age without evidence of clinical or immunologic decline).[194] In a multicenter, longitudinal U.S. study of 360 HIV-infected children, maternal advanced disease (clinical class C symptoms, lowered $CD4^+$ T-cell count, and increased viral load) was associated with faster progression of disease in infected infants.[3]

Advances in perinatal primary antiretroviral therapy—antepartum, peripartum, and postpartum delivery of zidovudine in non–breast-feeding HIV-infected women without severe immunosuppression in the United States and developing nations—led to a decrease in perinatal transmission rates. The CDC estimates that the number of infants born annually with HIV in the United States decreased from 1650 in 1991 to 100 to 200 in 2004. At the same time, new pediatric AIDS cases and AIDS–related deaths also decreased dramatically owing to powerful combinations of antiretroviral drugs. In 1994, the Pediatric AIDS Clinical Trials Group (PACTG) protocol 076 documented the remarkable effectiveness of zidovudine in reducing the maternal-infant transmission of HIV-1 by 66 percent.[61] The three-part regimen consisted of the following: (1) zidovudine initiated during 14 to 34 weeks' gestation (100 mg orally five times daily) and continued throughout pregnancy, (2) intrapartum zidovudine (intravenous loading dose of 2 mg/kg followed by a continuous infusion of 1 mg/kg until delivery), and (3) oral zidovudine for the neonate (2 mg/kg per dose every 6 hours orally for 6 weeks) or intravenous dosing for infants unable to tolerate oral administration (1.5 mg/kg per dose every 6 hours). The new Perinatal Guidelines for HIV prevention recommend full-term neonates receive 4 mg/kg of zidovudine orally twice daily for the first 6 weeks of life, beginning as soon after birth as possible and preferably within 6 to 12 hours of delivery.[199*] Inclusion of highly active antiretroviral therapy (HAART) and zidovudine to suppress HIV viral load and restore or preserve immune function in pregnant HIV-infected women resulted in a further reduction in transmission rates in the United States, now reported as less than 2 percent.[63] Guidelines to prevent perinatal transmission include promotion of early maternal and infant testing, prevention counseling for women of child-bearing age, and treatment intervention in women of child-bearing age with newly identified HIV infection.[199] The selection of antiretroviral therapy to reduce perinatal HIV transmission must include consideration of optimal treatment for the infected pregnant women and balance potential for fetal harm. Maternal factors that should be considered include

*AIDS*info:* http://AIDSinfo.nih.gov.

the mother's viral load, degree of immunosuppression, medications for use in treatment of comorbid conditions (e.g., tuberculosis, hepatitis C virus), and the potential for inducing viral resistance. Transmission of resistant HIV to infants has been described in the literature, but it is an infrequent event.

Other considerations to prevent perinatal transmission of HIV include scheduled delivery to minimize prolonged rupture of membranes, including a recommendation from the American College of Obstetrics for scheduled cesarean section for women with viral loads exceeding HIV RNA levels of 1000 copies/mL.[60] In a meta-analysis of 15 North American and European cohorts of HIV-infected pregnant women (n = 100 women), vertical transmission rates differed by 5 percent for those with scheduled delivery by cesarean section (transmission rate, 2%) as compared with those with other delivery modes (transmission rate, 7.3%).[256] Evaluation of the morbidity and mortality related to such scheduled cesarean sections suggests that HIV-infected pregnant women may have a higher incidence of postpartum hemorrhage with resultant transfusion, sepsis, pneumonia, and death than do their noninfected pregnant peers undergoing the same scheduled procedure (odds ratio, 1.6).[159] HIV-infected pregnant women without detectable viral loads should be informed preoperatively of their risk for having morbidity related to cesarean section performed to prevent perinatal HIV transmission.

If the maternal HIV status is unknown at the time of delivery, options for perinatal transmission prophylaxis may not be available for use in the antepartum or postpartum period. Early and rapid HIV testing is being promoted as a public health measure to prevent perinatal HIV transmission. In the multicenter Mother-Infant Rapid Intervention at Delivery (MIRIAD) study, voluntary rapid HIV testing and 24-hour counseling were conducted at 16 U.S. hospitals, with mean turn-around time of 66 minutes for test results.[28] The sensitivity and specificity of the rapid test performed on whole blood were 100 and 99 percent, respectively, and the positive predictive value of the testing compared favorably with the standard of enzyme immunoassay (EIA).

Sexual Transmission: Second Wave of Pediatric Acquired Immunodeficiency Syndrome

In the adult, young adult and adolescent populations, sexual acquisition of HIV infection is the predominant transmission pattern. The intersection of pediatric sexual abuse and transmission of HIV has been reported. Sexual abuse in young children and infants may be associated with the transmission of sexual disease (e.g., syphilis, gonorrhea, human papillomavirus). HIV infection should be considered in the differential diagnosis of possible sexually transmitted diseases in children assessed for sexual assault. In contrast to the infrequent reports of sexual transmission in infants and children younger than 13 years of age, adolescents, who account for 1 percent of AIDS cases in the United States, are infected frequently by sexual transmission. In an inner city urban U.S. community, 41 percent of sexually active female youth described having vaginal intercourse and receptive anal intercourse in casual relationships.[120] Use of condoms was reported in 64 percent of youth, but receptive anal intercourse was used by some as a form of contraception, and condom use in this setting was reported in only 41 percent of these encounters. The American Academy of Pediatrics (AAP) stressed the importance of preadolescent and adolescent care and encouraged performing examinations in patients who are 11 to 14 years of age. Health care providers can take advantage of these preteen/teen visits as an opportunity to explore sexual exposures of youth in their care and to educate sexually active youth on options for contraception.

Because the symptom-free period for clinical expression of HIV infection in adolescents may approach adult standards (i.e., >10 years), many young adults (20 to 29 years of age) with AIDS probably became infected with HIV during adolescence. Adolescents with AIDS, like affected infants and children, are more likely to be poor and black or Hispanic. Reported risks for transmission in adolescents vary by age, sex, and race or ethnicity. Behavioral transmission (men who have sex with men and heterosexual contact) and transmission of unknown risk predominate as reported risk behavior in U.S. youth.[278] The category of heterosexual contact accounts for the largest proportional increase in the reported risk for transmission in both female and male adolescent cases of AIDS. The nature of youth (experimentation, search for self and sexual identity, and inability to access health care easily) places adolescents at risk for heterosexual transmission of HIV infection.[274]

Recommendations for postexposure prophylaxis for nonoccupational exposure to blood, genital, or other body fluids were updated by the CDC in 2005.[243] With exposure to these fluids from an HIV-infected individual and with determination of significant exposure risk, a 28-day regimen of HAART is recommended if administration can begin within 72 hours of exposure. For these exposures, risk is defined as 1 per 10,000 exposures to the infected source. Other risk assignments are blood transfusions (9,000 per 10,000 exposures), percutaneous needle stick injuries (30 per 10,000 exposures), and sexual exposure risk ranging from 50 (anal receptive intercourse) to 0.5 (oral intercourse). Merchant and colleagues[173] published a stratified regimen for postexposure prophylaxis in children and youth that includes modification of the dosing regimen, evaluates adverse events commonly associated with postexposure prophylaxis, and recommends institution of antiretroviral agents within 1 hour of exposure when possible.

Other Modes of Transmission

Less frequently reported transmission routes of HIV infection are important for pediatric health care providers to acknowledge and target. The risk for transmission of HIV through breast-feeding is reported infrequently in the United States. In a study of HIV-infected, breast-feeding mothers and their exposed infants, Dunn and associates[67] documented a 14 percent risk for HIV

transmission through breast-feeding above and beyond the established perinatal transmission risk. These studies prompted the adoption of guidelines for preventing transmission by this route by avoiding breast-feeding in HIV-seropositive postpartum mothers in the United States. Transmission of HIV to infants by breast-feeding represents approximately half of all perinatal transmission worldwide, and the risk for transmission continues with prolonged breast-feeding after the first year of life.[145]

Children may be prone to needle stick or sharps injuries that occur in a nonhospital, nonmedical setting (e.g., needle stick injury associated with inappropriately discarded needles in public places such as public parks, bathrooms). Recommendations for psychological support, discussion of adherence to prescribed medications, and toxicity monitoring are provided by the AAP Committee on Pediatric AIDS.[115]

An estimated 13 percent of 159 diagnoses of HIV and AIDS among children aged younger than 13 years were attributed to modes other than perinatal transmission. In 2008, a case series of three pediatric HIV cases concluded that premastication was the likely mode of transmission for these children, as a route of HIV transmission through blood in saliva.[95] A cross-sectional survey conducted by the CDC in nine pediatric HIV clinics in the United States indicated that among primary caregivers of children aged 6 months or older, 31 percent of 154 reported the children received premasticated food from themselves or someone else. Although data on the risk for HIV transmission via premastication are limited, the CDC recommends that HIV-infected caregivers not premasticate food for HIV-uninfected children.[46] Health care providers should routinely inquire about premastication of foods fed to infants, instruct HIV-infected caregivers to avoid this practice, and advise on safer feeding options. To date, no data suggest that HIV infection is transmitted casually from HIV-infected children to siblings, playmates, or caregivers.[240] Documentation of transmission routes and lack of casual transmission have prompted defining appropriate measures for "mainstreaming" of HIV-infected children and families.[72] This measure includes school, daycare, and foster care and adoption placement for HIV-infected children and promotes social incorporation of these children.

ETIOLOGY

Identification of the origin of AIDS followed closely behind the clinical description of this complex immunodeficiency. In 1983, investigators worldwide identified a viral cause for AIDS and described human lymphotropic virus III, lymphadenopathy-associated virus, and a virus associated with persistent generalized lymphadenopathy.[58,93] These viruses subsequently were identified as similar and now are called HIV-1, or simply HIV (Fig. 191B-1).[66] Two species of HIV are recognized: HIV-1 and HIV-2. HIV-1 has been the more prevalent pathogenic species and, especially in the United States, almost uniformly has been associated with reported AIDS cases. Viral divergence is not uncommon, and HIV-1 can be divided into three groups: M, major; O, outliers; and N, non-M/non-O; the M group represents the major group with subtypes (clades) showing significant symmetry.[116] The major subtypes have been identified, and data from the WHO HIV Vaccine Initiative identified clade C in 50 percent of global infections in 2004.[116] Clade B is identified most frequently in industrialized countries, including the United States. In 2004, recombinant subtypes were responsible for 18 percent of worldwide infections; continued collection of such data will be important in the successful development of a vaccine that can be applied universally to stem transmission of HIV. A detailed description of these RNA viruses, their method of viral introduction into the affected host, and their impact on critical immune components is provided in Chapter 191A.

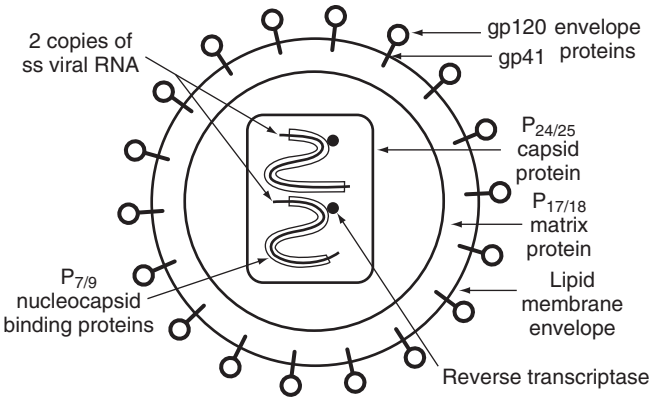

FIGURE 191B-1 ■ Diagram of the virion of human immunodeficiency virus type 1 (HIV-1). (From Demmler GJ, Taber LH. Virology of HIV-1. *Semin Pediatr Infect Dis* 1990;1:17-20.)

PATHOGENESIS

The Developing Immune System

In perinatally infected infants, the disruption that HIV creates in the fetal/neonatal immune system is envisioned best by considering the intricate normal differentiation pathways of the bone marrow stem cell.[269] T- and B-lymphocyte differentiation pathways are presented in Figure 191B-2 to illustrate points where genetic defects can occur and then cause congenital immunodeficiency. In many regards, the immunodeficiency of neonatal HIV infection parallels that of genetically inherited immune disorders.

HIV affects almost all immune cell types and alters their ability to produce important chemokines and interleukins that are integral to immunologic homeostasis. Independent of cell damage, host response also can vary according to individual genetic HLA representation and predict either probability of infection occurring after exposure or progression of disease.[80] $CD4^+$ cells bear a receptor (the $CD4^+$ molecule itself) for the gp120 component of the HIV virion coat, and through this mechanism viral entry into the host cell is allowed. This entry does not occur in isolation, and host expression of a

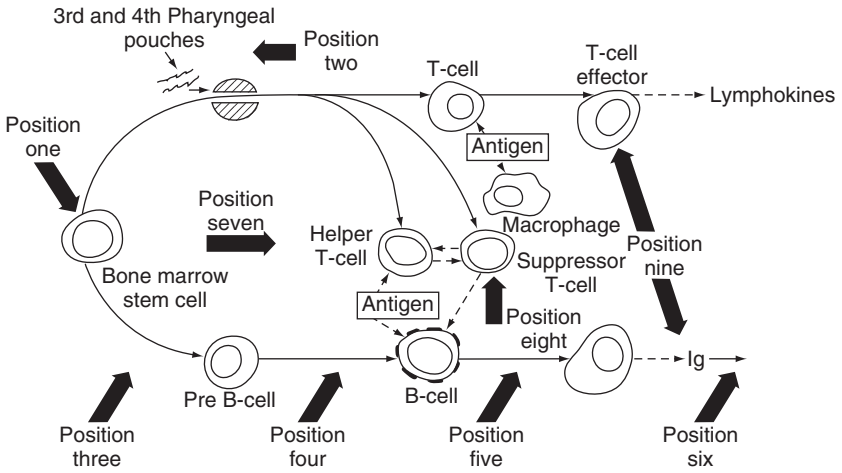

FIGURE 191B-2 ■ Model of the events of cellular maturation, cellular interaction, and cellular biosynthesis required for a normal immune response. The *arrows* indicate presumed defects in various immunodeficiency states. *Position 1,* Failure of B- and T-cell development (e.g., severe combined immunodeficiency disease). *Position 2,* Failure of development of the thymus (e.g., DiGeorge syndrome). *Position 3,* Failure of maturation of stem cells into pre-B cells (e.g., thymoma, hypogammaglobulinemia). *Position 4,* Failure of maturation of pre-B cells into B cells (e.g., X-linked hypogammaglobulinemia). *Position 5,* Failure of maturation of B cells into plasma cells (e.g., common variable hypogammaglobulinemia). *Position 6,* Hypercatabolism of immunoglobulin (e.g., myotonic dystrophy). *Position 7,* Reduced helper T cells (e.g., subset of common variable hypogammaglobulinemia). *Position 8,* Increase in suppressor T-cell activity (e.g., subset of common variable hypogammaglobulinemia). *Position 9,* Excessive loss of immunoglobulins and lymphocytes (e.g., intestinal lymphangiectasia). (From Waldmann TA. Immunodeficiency diseases: primary and acquired. In: Samter M, Talmage DW, Frank MM, et al., editors. *Immunological diseases.* 4th ed. Boston: Little, Brown; 1988, p. 411-65.)

chemokine receptor (CXCR4 for T-lymphotropic HIV strains; CCR5 for monocyte tropic HIV strains) interacts in promoting viral particle binding to the host cell membrane and release of viral particles into the cytoplasm.[55] Data suggest that heterozygous allele expression of these coreceptors may affect expression and progression of HIV disease. Density expression of CCR5 molecules in infants has been correlated with progression of disease even in the presence of antiretroviral therapy and with the slope of $CD4^+$ cell decline from birth.[49]

Once infected, the infant with perinatal transmission exhibits a quick rise in detectable HIV viral load that can exceed 750,000 copies/mL in the first weeks or months of life and persists unchecked without institution of antiviral therapy through the first years of life.[3] This persistent and lengthy viremia in early perinatal HIV infection differs from acute HIV-1 infection in adults, in which viremia initially may be equally elevated but abates with host immunologic response (albeit without complete elimination) over the course of a shorter time frame (weeks to months). These distinctions in host response reflect the more ineffective infant immunologic host response to HIV infection. Proposed mechanisms of immune dysfunction associated with continued viremia in infants include thymic damage with thymocyte depletion, disruption of thymic architecture, early $CD4^+$ cell depletion that affects $CD8^+$ cell response to HIV antigens, early lymphocyte activation as measured by $CD8^+DR^+CD38^+$ T cells that impede effective host T-cell response, and genetic predisposition to allow enhanced HIV entry into host cells.[226,280] Most clinicians opt for early treatment of HIV-infected infants younger than 1 year of age, independent of the clinical or immunologic ($CD4^+$ cell count) presentation. Studies have documented that early therapy slows immune system destruction and preserves immune function, preventing clinical disease progression.[53]

Immune Dysfunction

Abnormalities in adult HIV infection include leukopenia, lymphopenia, and decreased $CD4^+$ helper-inducer cells with an expanded $CD8^+$ cell population, which results in an inverted CD4/CD8 ratio, typically less than 1.0. Early in the course of infection, T-lymphocyte function has been noted to be diminished (<50%), with decreased in vitro responses to soluble antigens preceding $CD4^+$ cell depletion. Although in vitro responses to plant lectins, such as phytohemagglutinin, concanavalin A, and pokeweed, appear to be normal early in the course of HIV infection, these responses begin to decrease with clinical decline; characteristically, mitogen responses to pokeweed disappear first. Anergy is not unusual to commonly tested antigens (tetanus, *Candida*). Levels of proinflammatory cytokines (interleukin [IL]-1, IL-6, IL-8) increase whereas levels of T-helper type 1 (Th1) cytokines (IL-2, tumor necrosis factor-α, interferon-γ, IL-12) decrease. Proinflammatory cytokines appear to induce viral replication by activating the host cell.[59] In addition, HIV-infected $CD4^+$ cells release soluble IL-2 receptors. These elevated serum IL-2 receptor levels produce a blockade of cell-bound IL-2 receptors by competition for IL-2. $CD8^+$ cell response to HIV-specific antigens is impaired.[197] Children with HIV infection seldom are lymphopenic in terms of values observed in adults. If lymphopenia is observed in children with HIV infection, it usually is seen in older children or in children who have progressed to end-stage HIV disease. In fact, $CD4^+$ cell counts in HIV-infected children commonly exceed 400 cells/mm^3. Moreover, Denny and associates[68] showed that in infants

aged 12 to 24 months, a $CD4^+$ cell value of less than 1000 cells/mm^3 is significant for selective HIV-related $CD4^+$ depletion. In infants younger than 12 months, a value of less than 1500 cells/mm^3 similarly applies. Lymphoproliferative responses to recall antigens have been documented as relatively normal findings early in the course of infection; decline is correlated with the onset of clinical symptoms. As described earlier in discussions of pathogenesis, HIV-1-specific $CD8^+$ cytotoxic-suppressor cell function has been documented as being ineffective in infants with primary infection (early perinatal infection).

After administration of HAART, T-cell function against specific HIV antigens continues to be imperfect. Despite lowering of viral load, $CD4^+$ cell depletion may not be completely restored for many weeks. Differential restoration of subpopulations of $CD4^+$ cells (effector memory cells more commonly encountered than naïve cells) may occur. In 643 children with significant $CD4^+$ depletion ($CD4^+$ cells <250 cells/mm^3) and viremia, HAART resulted in a median rise of $CD4^+$ cell count of 100 cells/mm^3 over the course of 2 years, but anergy was reported in 80 percent, lack of proliferative response to the specific antigen of tetanus in 73 percent, and impaired serologic response to hepatitis A virus vaccination in 54 percent.[153] Hence, despite administration of antiretroviral therapy, T-cell immune restoration may not be complete in all affected children.

B-cell dysfunction is identified similarly in HIV-infected adults and children and manifests as hypergammaglobulinemia. HIV-infected adults also demonstrate circulating immune complexes and production of autoantibodies secondary to polyclonal activation of B cells by HIV itself or concomitant viral infection with cytomegalovirus (CMV) or Epstein-Barr virus (EBV).[150] In particular, immunoglobulin G (IgG) levels may rise to 2 to 3 standard deviations above the mean of normal values, and serum IgA, IgM, and IgE levels may be elevated as well. This extreme hypergammaglobulinemia in children may be caused by polyclonal stimulation of B cells by HIV or coinfection with CMV or EBV, or it may be caused by the absence of normal $CD4^+$ immunoregulatory cells. Specific antibody functions measured as childhood vaccine responses often are abnormal and are not always completely restored in the most severely immunocompromised individuals after administration of HAART therapy.[153]

Other host defense cells affected by HIV infection include natural killer (NK) cells. In adults with chronic HIV infection, reported NK cell abnormalities include differential representation of certain NK cell subpopulations, downregulation of NK ligand expression that affects cytotoxicity of HIV-infected cells, and NK cell/dendritic cell (DC) interactions. Human DCs contain two subsets: myeloid and plasmacytoid DCs. Both subsets have additional roles in HIV infection that lead to viral elimination or control.[6] Activated myeloid DCs produce cytokines such as IL-12, IL-15, and IL-18. IL-12 is critical for myeloid DCs to induce Th1 cell responses, which subsequently promote potent cytotoxic T-lymphocyte responses that are necessary for clearing virus-infected cells.[151] Plasmacytoid DCs produce more type I interferons (IFNs) in response to HIV than any other cell in the body and stimulate myeloid DCs in a bystander fashion as well as directly activating NK cells.[218] The implications of these events underlie the need to develop vaccine strategies that enhance the ability of DCs to prime potent T-cell responses that can subvert the potential inhibitory effects of HIV on the immune system. Monocytes and macrophages play important roles in the pathogenesis of HIV infection by serving as reservoirs of HIV. Defective chemotaxis and bacterial killing have been observed in HIV-infected monocytes and macrophages, as has defective induction of IL-1, which possibly accounts for the decreased IL-2 response in $CD4^+$ cells.[279] The immune system also seems to contribute directly to HIV-induced encephalopathy (Fig. 191B-3).[26,118] HIV-induced encephalopathy is particularly devastating in young infants, who fail to reach early motor milestones or, worse, regress from acquired early development. The incomplete state of myelination of central nervous system (CNS) tissue may account for susceptibility to the

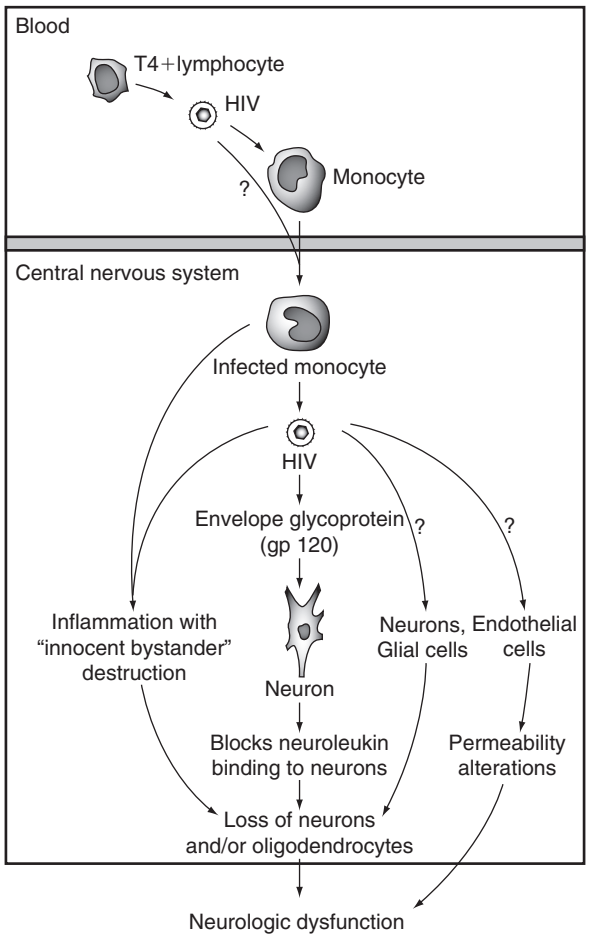

FIGURE 191B-3 ■ Role of the immune system in pediatric human immunodeficiency virus (HIV)–induced encephalopathy. HIV-infected $CD4^+$ lymphocytes and macrophages enter the central nervous system. HIV may escape from these transport cells and infect nerve cells directly or cause indirect damage to neurons by the release of cytokines such as tumor necrosis factor and interleukin-2. (Reprinted by permission from Ho D, Pomerantz RJ, Kaplan JC. Pathogenesis of infection with human immunodeficiency virus. *N Engl J Med* 1987;317:278-86.)

neurologic effects of HIV infection. In adults with progression of disease, depletion of $CD4^+$ cells has been linked to shifts in coreceptor use (from CXCR4 to CCR5), and these individuals with more severe disease appear to harbor predominantly CCR5-dependent (R5) viral strains.[99] HIV-containing monocytes are thought to transport HIV into the CNS and infect microglial cells including astrocytes.[56] Also implicated in HIV-associated neurologic complications are glial soluble factors that may have a direct neurotoxic effect.[149] Cytokines and reactive oxygen species released by HIV-infected lymphocytes and macrophages in the CNS may damage exposed nerve tissue.[268] The normal CNS immune defense system can attempt to enhance neuronal survival.

Origin of Immunodeficiency

The use of HAART has provided important opportunities to evaluate immune restoration in infected hosts. $CD4^+$ cell-depletion hypotheses of programmed cell death (apoptosis), depletion of tissue-associated $CD4^+CD45^+$ effector memory cells during acute infection, chronic ineffective immune activation (cellular and humoral), and switch from CXCR4-dependent virus (X4 virus) in acute infection to CCR5-dependent virus (R5 virus) in chronic infection all have been implicated as causes of HIV-induced immunodeficiency. In a recent study, using ligands to Toll-like receptors (TLRs) 5 and 9, the effect of TLRs on HIV-1 replication in lymphoid tissue was evaluated. Although flagellin (TLR5 agonist) treatment enhanced replication of CCR5-tropic and CXCR4-tropic HIV-1, treatment with oligodeoxynucleotide (ODN) M362 (TLR9 agonist) suppressed both viral variants. The differential effects of these TLR ligands on HIV-1 replication correlated with changes in production of CC chemokines CCL3, CCL4, CCL5 and of CXC chemokines CXCL10 and CXCL12 in the ligand-treated HIV-1–infected tissues.[20] These results suggest that a particular pattern recognition receptor engaged by a microbe may account for various microbial effects on HIV-1 replication in coinfected tissues and eventually affect the course of HIV-1 disease.

Ligands that induce apoptosis have been studied in HIV-infected individuals, and tumor necrosis factor–related apoptosis inducing ligand (TRAIL) has been documented to result in neuronal death.[178] TRAIL-associated apoptosis appears to require CXCR4 coreceptor expression but not CCR5 or CD4 receptor expression.[160]

Investigators hypothesize that HIV first depletes mucosal $CCR5^+CD4^+$ effector memory cells, thus leaving short-lived central memory or naïve $CD4^+$ cells and other immunoregulatory cells in a chronic state of activation that leads to an ineffective host response.[69,108] Infection of $CCR5^+CD4^+$ effector memory cells also is associated with higher expression of CTLA-4, a potent negative regulator for $CD4^+$ cells.[282] High expression of CTLA-4 may interrupt IL-2 production by $CD4^+$ cells, thereby impeding the ability of these cells to proliferate and hence depleting the population. The resultant chronic immune activation may lead to disorganization of the host immune response and further deplete $CD4^+$ cell regeneration. For patients with the most profound $CD4^+$ T-cell depletion, reduction of viral load with antiviral agents does not immediately result in correction of their immune defects.[99] The study of immune reconstitution with HAART may illuminate the immunopathogenesis of HIV infection. For example, after administration of HAART, $CD4^+$ NK T cells that play an important part in regulatory immune response to viral infections are restored rapidly. Restoration of such $CD4^+$ cell populations after administration of HAART and their important immune function may explain why, in the presence of persistently skewed $CD4^+$ cell expression in peripheral blood, clinical disease appears to improve dramatically.[205,264]

In perinatal HIV infection, the role of the placenta in affording protection or permitting infection continues to be defined, and potential perinatal cofactors are beginning to be understood more clearly. Thus, HIV may be fetotoxic, with an impact most notably reported in the thymus, where precocious involution, epithelial injury, and, occasionally, severe thymitis are described. Loss of lymphocytes at the corticomedullary junction of the thymus implies a defect induced by HIV in the immunologic selection process.

CLINICAL MANIFESTATIONS

In children and adults, HIV infection causes a spectrum of clinical abnormalities that affect multiple organ systems and include the symptom constellation of AIDS. Table 191B-4 lists the pre-HAART frequency of AIDS-defining events per 100 child-years.[181]

Infection with HIV, independent of a diagnosis of AIDS, may be attended by nonspecific clinical findings, including mild failure to thrive, hepatosplenomegaly,

TABLE 191B-4 **Frequency of Opportunistic Infections in HIV-Infected Children Before Widespread Use of Highly Active Antiretroviral Therapy (HAART)**

Pre-HAART Era (Event Rates >1/100 Child-Years)
Candidiasis
Disseminated *Mycobacterium avium* complex
Serious bacterial infections (pneumonia)
Herpes zoster
Pneumocystis jiroveci (formerly *P. carinii*) pneumonia
Serious bacterial infections (pneumonia)
Pre-HAART Era (Event Rates <1/100 Child-Years)
Cryptosporidiosis
Cytomegalovirus disease
Systemic fungal disease
Tuberculosis
Toxoplasmosis

Modified from Mofenson LM, Oleske J, Serchuck L, et al. Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. *MMWR Recomm Rep* 2004;***53***:1-92.

acquired microcephaly, parotitis, generalized lymphadenopathy, nonspecific intermittent diarrhea, intermittent fever, and chronic skin disease. These clinical symptoms are shared by other pediatric disease processes and can, when manifested singly in an HIV-infected host, cause a delay in establishing the diagnosis. However, a careful and detailed history should provide helpful insight into potential HIV risk factors and should prompt inclusion of HIV infection in the differential diagnosis. With the use of HAART, HIV infection has become a chronic disease and new clinical disease manifestations (e.g., lipodystrophy, hyperlipidemia), most closely related to chronic antiviral use, have been described. In general, the health of HIV-infected children has improved during the past decade. Kourtis and associates reported a dramatic decrease (71%) in the number of hospitalizations among children with HIV infection who were 18 years and younger between 1994 (pre-HAART) and 2004 (post-HAART).[144]

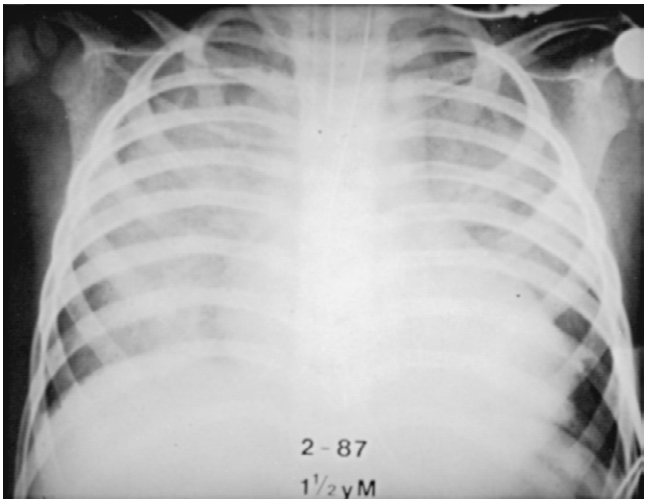

FIGURE 191B-4 ■ Classic diffuse interstitial infiltrates of acute and fatal pneumonia due to infection with *Pneumocystis jiroveci* in a 15-month-old child with perinatal acquired immunodeficiency syndrome. (From Hanson IC. Respiratory infections in HIV-infected children. Immunol Allergy Clin North Am 1993;13:205-17.)

Opportunistic Infections

Opportunistic infections plague HIV-infected individuals and are the most prominent cause of morbidity and mortality in this cohort. Before the use of HAART intervention, opportunistic infections in children more often were primary infections as compared with the case in adults, who had reactivation of infection.[181] The introduction of effective HAART with associated host immune restoration significantly modified the expression of certain opportunistic infections in adults and children.[252] In children, the overall incidence of these infections declined from 14.4 cases per 100 patient-years before 1997 to 1.1 cases per 100 patient-years.[190] Additionally, in pre-HAART years, the age at clinical presentation was much younger and age younger than 3 years was associated with very rapid $CD4^+$ cell decline. Although the number of these opportunistic diseases has diminished, their prevalence appears to be unaffected. The administration of combination HIV therapy, including protease inhibitors (PIs) in adults and children, has improved the survival of perinatally infected children.[100]

Pneumocystis Pneumonia

P. jiroveci (formerly *P. carinii*) pneumonia (PCP) is the opportunistic infection most commonly reported in HIV-infected children. Declines in the incidence of PCP have been documented, with rates of 5.8 cases per 100 patient-years reported before 1997 and 0.3 cases per 100 patient-years reported after 1997.[190] PCP has a fulminant course in the pediatric population, with the highest mortality rates occurring in affected children younger than 1 year old. In a study of 172 children with perinatal HIV infection without HIV-specific intervention, 9 percent had PCP when they were younger than 1 year, with a median survival of 1 month. Clinical expression of pediatric PCP often can be distinguished from other pulmonary diseases by the severity of the hypoxemia (higher alveolar-arterial oxygen gradients), elevated serum lactate dehydrogenase levels, the rapidity of progression of disease with tachypnea and fever, characteristic diffuse interstitial infiltrates on radiography, and the usual lack of digital clubbing.[113] The more insidious manifestations of PCP characteristic of HIV-infected adults, namely, prolonged fever (>7 weeks), cough, and dyspnea (averaging 3 weeks), are appreciated less commonly in infants and children. Radiographically, pediatric PCP is associated with diffuse interstitial markings progressing to the "white out" picture of adult respiratory distress syndrome (Fig. 191B-4).[114] However, PCP can be seen initially as a unilateral streaky pneumonic infiltrate, as lobar consolidation, or with accompanying pleural effusions.

Aggressive diagnostic measures may be indicated to establish a diagnosis of PCP. Bronchoscopic alveolar lavage (BAL) is the preferred diagnostic tool in adults and children and should be considered a first choice for presumed PCP in children who cannot provide sputum specimens for evaluation. In older children and adults, evaluation of sputum for the presence of *Pneumocystis* with appropriate stains or monoclonal antibodies may preempt the need for using invasive diagnostic measures. Polymerase chain reaction (PCR) assays have also been developed for diagnostic evaluation of PCP that are usually more sensitive but less specific than microscopic methods.[121] Lung biopsy carries the highest sensitivity for identifying the organism but has attendant morbidity related to thoracotomy or video-assisted thoracoscopy (VATS) and placement of a chest tube.

Acute PCP is treated with parenteral trimethoprim-sulfamethoxazole (TMP-SMX) or pentamidine or atovaquone for patients with TMP-SMX or pentamidine hypersensitivity. TMP-SMX treatment consists of 15 to 20 mg/kg/day of the TMP component delivered intravenously three to four times daily. Adjunctive corticosteroid therapy given early in moderate to severe PCP provides significant benefit with only limited evidence of concomitant immune suppression and attendant infectious complications.[91,242] Concomitant CMV and PCP has been documented in HIV-infected children. In this clinical

TABLE 191B-5 Recommendations for *Pneumocystis* Pneumonia Prophylaxis and CD4⁺ Monitoring for Infants Exposed to and Children Infected with HIV by Age and HIV Infection Status

Age/HIV Infection Status	Prophylaxis	CD4⁺ Monitoring
Birth to 4-6 wk, HIV exposed	No prophylaxis	
4-6 wk to 12 mo, HIV exposed	No prophylaxis*	
<12 mo, HIV infected or indeterminate	Prophylaxis	Every 3-4 mo
1-5 yr, HIV infected	Prophylaxis *if* CD4⁺ count is <500 cells/μL or CD4⁺ percentage is <15%	Every 3-4 mo
6-12 yr, HIV infected	Prophylaxis *if* CD4⁺ count is <200 cells/μL or CD4⁺ percentage is <15%	Every 3-4 mo

*Until HIV infection is either presumptively excluded (by documentation of two negative virologic test results, one at ≥2 wk of age and one at ≥4 wk of age) or definitively excluded (by documentation of two negative virologic test results, one at ≥1 mo of age and one at ≥4 mo of age). For these definitions of HIV exclusion to be valid, the infant must not be breast-feeding.

Modified from Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;***58***(RR-11):1-166.

situation of concomitant viral infection, no existing data recommend delay of corticosteroid use. Treatment of PCP should not be altered. Dosing regimens for corticosteroids are published and include options for use of oral prednisone or oral methylprednisolone or intravenous methylprednisolone.[39]

Following the occurrence of PCP infection, secondary PCP prophylaxis (therapy initiated after acute disease treatment and resolution) is recommended (see Table 191B-5). Proposed pediatric regimens include TMP-SMX at 150 mg/m²/day and 750 mg/m²/day, respectively, in two divided doses on 3 consecutive days/week (acceptable alternate regimens include a single dose on 3 consecutive days/week, two divided doses on 3 alternate days a week, two divided doses daily), dapsone for children 1 month or older at 2 mg/kg daily (single dosing not to exceed 100 mg/day) or 4 mg/kg weekly (single dosing not to exceed 200 mg), for children 5 years or older, pentamidine (aerosolized [300 mg by Respirgard II inhaler monthly]), and atovaquone (30 mg/kg daily for children 1 to 3 months and >24 months and 45 mg/kg daily for children 4 to 24 months).[180] In adults and adolescents, recommendations to discontinue secondary PCP prophylaxis are proposed once there is documentation of immune restoration (CD4⁺ cell counts that have increased to >200 cells/mm³ for ≥3 months as a result of HAART).[39] Data from the European PCP-Withdrawal Study Group suggest that with immunorestoration of HAART the risk for PCP development is low and that withdrawal of PCP prophylaxis as recommended in the adult host appears to be a reasonable risk.[263] U.S. guidelines continue to recommend lifelong primary PCP prophylaxis in individuals who acquired their PCP before reaching 13 years of age.[181]

Primary PCP prophylaxis (therapy initiated to prevent primary infection) for adults and children is accepted as standard care.[39] Pediatric regimens and dosing for secondary PCP prophylaxis do not differ from those outlined earlier. In 1993, the CDC published the first PCP prophylaxis guidelines for HIV-infected children; these guidelines linked the institution of therapy to the level of immunosuppression as measured by the CD4⁺ cell count or percentage.[34] Guidelines for PCP prophylaxis were modified in 2009.[180] The most significant change in the revised guidelines was concerning diagnosis of HIV infection and presumptively excluding HIV infection in infants that affect the need for initiation of prophylaxis to prevent PCP in neonates. Based on this revision, prophylaxis is not recommended for infants who meet criteria for being definitively or presumptively not HIV infected. In non–breast-feeding infants with no positive HIV virologic test results, presumptive exclusion of HIV infection can be based on two negative virologic test results (one obtained at 2 weeks or older and one obtained at 4 weeks of age), on one negative virologic test result obtained at 8 weeks of age or older, or on one negative HIV-antibody test result obtained at 6 months of age or older. Definitive exclusion of HIV infection is based on two negative virologic test results (one obtained at 1 month of age or older and one obtained at 4 months of age or older) or on two negative HIV-antibody test results from separate specimens obtained at 6 months of age or older. For both presumptive and definitive exclusion of infection, the child should have no other laboratory or clinical evidence of HIV infection. Infants born to HIV-infected mothers should be considered for prophylaxis beginning at 4 to 6 weeks of age. HIV-infected infants should be administered prophylaxis until 1 year of age, at which time they should be reassessed on the basis of the age-specific CD4⁺ T-cell count or percentage thresholds. Infants with indeterminate HIV infection status should receive prophylaxis until they are determined to be HIV-uninfected or presumptively uninfected with HIV. Data from Pediatric AIDS Clinical Trials Group protocol 1088 documented that primary PCP prophylaxis in HIV-infected children could be discontinued safely with immune restoration on stable antiretroviral therapy.[189]

Infection with *Mycobacterium avium–intracellulare* Complex

The *M. avium–intracellulare* complex (MAC), which caused disseminated infections in both adults and children with HIV infection in the early 1980s and 1990s, waned significantly in incidence with the increasing and

successful use of HAART. In a 1990 review of opportunistic infections in pediatric patients with AIDS, 43 of 552 (7.8 percent) children from birth to 9 years of age were found to have disseminated MAC infection.[113] The incidence of pediatric MAC infection in HIV-infected children dropped from 1.3 cases per 100 patient-years reported before 1997 to 0.2 cases per 100 patient-years after 1997.[190] Epidemiologically, MAC has been linked to significant immunosuppression ($CD4^+$ T cell counts lower than 50 cells/mm^3) in adults.[39,182] Researchers noted that the development of MAC infection in children may be associated with $CD4^+$ T cell counts greater than 50 cells/mm^3, especially in children younger than 6 years old.[222] Symptoms of MAC infection include fever, malaise, weight loss, anorexia, and night sweats. Gastrointestinal manifestations are not uncommon and have included abdominal pain, diarrhea, malabsorption, and intestinal perforation. Rarely, MAC has been reported with extrabiliary obstructive jaundice (presumed to be secondary to lymphadenopathy) and endobronchial masses. The diagnosis of disseminated MAC infection relies on identification of these microorganisms from the blood, lymph tissue, bone marrow, liver, lungs, and gastrointestinal tract. Therapy for disseminated MAC infection in the pediatric population includes some combination of clarithromycin or azithromycin (maximal dosing of 500 mg and 250 mg, respectively) and/or rifabutin (maximal dosing of 300 mg). The epidemiologic link with severe immunosuppression and the advent of therapeutic prophylactic interventions (clarithromycin or azithromycin and/or rifabutin) prompted the implementation of MAC prophylaxis guidelines for adults, adolescents, and older children (≥6 years) with $CD4^+$ T cell counts lower than 50 cells/mm^3.[35] Primary MAC prophylaxis can be discontinued in HIV-infected children older than 2 years of age receiving stable HAART for 6 months or more and sustaining (>3 months) $CD4^+$ T cell recovery.[189]

An unusual immune restoration inflammatory syndrome (IRIS) has been described as a paradoxical event after immune recovery has been achieved in adults and adolescents receiving HAART.[248] IRIS is associated with reactivation of opportunistic infections such as MAC. Activation of other latent complications, including carcinoma and neurologic symptoms, has been described.[261] Recent data indicate that MAC prophylaxis with azithromycin did not prevent IRIS.[203] In children with IRIS, treatment of the reactivated opportunistic infection may include administration of anti-inflammatory agents including systemic corticosteroids.

Tuberculosis

Overall case rates of tuberculosis (TB) in the United States are declining, with 4.4 new cases of TB per 100,000 population (a total of 13,299 cases) reported in 2007. The percentage of patients with TB and with known HIV infection also decreased from 15.0 percent in 2003 to 12.4 percent in 2006.[43] Primary disease accounts for one third or more of cases of TB in HIV-infected populations.[277] In a cohort of pregnant and nonpregnant HIV-infected women monitored in a multicenter longitudinal study, the prevalence of tuberculosis determined by medical history or positive skin tests was 14 percent.[181] Children most commonly are infected with *Mycobacterium tuberculosis* from exposure in their immediate environment, usually the household. In a study of 60 HIV-infected families, the incidence of tuberculosis was approximately 6 percent, with both HIV-infected and HIV-uninfected children affected. The vector for transmission was identified as an infected family member or caregiver.[9] Tuberculosis should be considered in the differential diagnosis of pulmonary disease in HIV-exposed children. The principles for treating TB in the HIV-infected child are the same as for the HIV-uninfected child and should include at least a four-drug regimen. However, treating TB in an HIV-infected child is complicated by antiretroviral drug interactions with the rifamycins and overlapping toxicities caused by antiretroviral agents and TB medications. The duration of treatment is 9 to 12 months, with the longest duration being in children with extrapulmonary disease.[38]

Secondary prophylaxis is not recommended for children who have had a prior episode of TB.

Cytomegalovirus

In the pre-antiretroviral era, CMV caused 8 to 10 percent of pediatric AIDS-defining illness.[136] The adult spectrum of CMV disease, including retinitis, pneumonitis, esophagitis, gastritis, colitis, hepatitis, cholangitis, and encephalitis, is not reported uniformly in the pediatric AIDS literature. CMV clearly can cause primary pneumonitis in children or can be found in association with other pulmonary pathogens, especially *Pneumocystis*. Unusual gastrointestinal manifestations have included pyloric obstruction, enterocolitis, and oral and esophageal ulcers. CMV retinitis is the most frequent severe manifestation of CMV disease among HIV-infected children, accounting for approximately 25 percent of CMV AIDS-defining illnesses.[52] However, it appears to be decreasing in incidence with the wider use of HAART.[190] Annual CMV antibody testing is recommended beginning at 1 year of age for CMV-seronegative HIV-infected infants and children who are severely immunosuppressed.

Treatment of CMV disease-associated with HIV infection focuses on progression of disease and not on a curative outcome. Therapeutic intervention includes the use of ganciclovir, valganciclovir, foscarnet, or cidofovir. These drugs have significant side effects, including bone marrow suppression and renal toxicity. Administration of these agents is divided into induction and maintenance dosing. The optimal interval for efficacy has not been determined and often is selected on an individual basis. Although many adults report experiencing subjective and objective improvement while receiving therapy, discontinuation of therapy is associated with high relapse rates, independent of the affected site. Careful monitoring of children receiving treatment for CMV and antiretroviral therapy is imperative because the use of both classes of agents may depress bone marrow function. CMV end-organ disease is best prevented by antiretroviral therapy to maintain CD4 count greater than 100 cells/mm^3. If this is not possible, prophylaxis with valganciclovir can be considered for HIV-infected older children who are

CMV seropositive, with severe immune suppression ($CD4^+$ T cell count <50 cells/mm^3), at a dose of 15 mg/kg/dose every 12 hours, using a 50-mg/mL pediatric solution that is now available, or using tablets if the child weighs enough to receive adult doses of valganciclovir. Prevention of complications of CMV in HIV-infected children with severe immunosuppression should include careful and regular (every 4 to 6 months) retinal monitoring for evidence of eye disease. Once secondary prophylaxis for disseminated CMV disease is initiated, its discontinuation should be considered on a case-by-case basis because current data to support discontinuation are limited.[272] As reported with MAC, reactivation of CMV disease in the IRIS has been described.

Other Herpesvirus Infections

Infections with the herpesvirus family are not uncommon events in HIV-infected children. In a study in the United States during the HAART era, the incidence of systemic HSV infection was 0.9 per 100 child-years and was most common among children with reduced CD4 percentage (<25%).[98] In general, HSV infection in pediatric AIDS has been limited to mild to severe localized infections without reports of dissemination. Varicella-zoster virus (VZV) and herpes-zoster virus (HZV) infections have contributed significant morbidity to HIV-infected persons. Disseminated and chronic HZV infections are reported in pediatric patients with AIDS. Because episodes of HSV disease can be treated successfully, chronic therapy with acyclovir is not required after lesions resolve. However, children who have frequent or severe recurrences can be administered daily suppressive therapy with oral acyclovir.[77] Prevention of herpes infections in school-aged children is especially important because exposure to varicella may be considerable. Administering varicella-zoster immune globulin, if available, should be considered for susceptible HIV-infected children with exposure to VZV. Because the varicella vaccine is an attenuated live viral vaccine, its use in HIV-infected children is limited currently to children with asymptomatic or mildly symptomatic HIV disease and no evidence of immune suppression.[42]

Fungal Diseases

The most common fungal infections among HIV-infected children are caused by *Candida* spp. Oral thrush and diaper dermatitis occur among 50 to 85 percent of HIV-infected children.[98] Affected mucous membranes or skin often does not respond well to treatment with topical antifungal agents. Systemic therapy with one of the oral azoles including fluconazole, ketoconazole, or itraconazole is effective in oropharyngeal or esophageal candidiasis. In severe fungal infections, amphotericin B or other agents effective against the *Candida* spp. should be administered intravenously. Because of limited experience in children and no data in HIV-infected children, data are insufficient to recommend echinocandin class antifungal medications, including caspofungin, micafungin, and anidulafungin, as first-line agents for invasive candidiasis in children.[233] Prophylaxis for candidal infections is not recommended because of concern for development of resistance to azoles such as fluconazole, which could limit the use of these agents in the care of disseminated or mucosal disease.[39]

Other disseminated fungal diseases, such as histoplasmosis and cryptococcosis, less commonly affect pediatric patients with AIDS. Histoplasmosis may be prevalent in HIV-infected children who reside in parts of the United States where *Histoplasma capsulatum* is endemic. Symptoms of histoplasmosis include fever, rash, cough, lymphadenopathy, splenomegaly, thrombocytopenia, low-grade disseminated intravascular coagulopathy, adult respiratory distress syndrome, meningoencephalitis, and neurologic abnormalities consistent with intracranial mass lesions. Symptoms of cryptococcosis include fever, headache, pulmonary involvement, and subacute meningitis or meningoencephalitis.[177] Because of the low incidence of histoplasmosis in pediatric HIV-infected patients, possibility of drug interactions, potential antifungal drug resistance, and cost, the routine use of antifungal medications for primary prophylaxis of *Histoplasma* infections in children is not recommended. However, primary prophylaxis for adults and adolescents is suggested for those with compromised immune systems. Specifically, fluconazole is suggested as primary cryptococcosis prophylaxis for adults and adolescents with $CD4^+$ T cell counts of less than 50 cells/mm^3 and itraconazole is advised for histoplasmosis prophylaxis in adults and adolescents with $CD4^+$ cell counts of less than 150 cells/mm^3. The safety of discontinuing secondary prophylaxis for cryptococcosis after immune reconstitution with HAART has not been studied in children, and decisions in this regard should be made on a case-by-case basis. Recommendations to discontinue prophylaxis as a result of HAART-related improved immune function have been developed for cryptococcosis and include sustained (>6 months) $CD4^+$ T cell counts higher than 200 cells/mm^3.[134] In contrast, no such recommendations exist for discontinuation of histoplasmosis prophylaxis in HIV-infected adults or adolescents with evidence of HAART-related reconstitution of immunity.

Bacterial Diseases

Pediatric patients have an increased incidence of severe bacterial infections, including infections with *Streptococcus pneumoniae*, *Staphylococcus aureus*, and various gram-negative organisms. One of the most common first-time infections in HIV-infected children is bacterial infections (bacterial pneumonia).[98] In a study of 372 HIV-infected children monitored for a median of 17 months, 14 percent experienced one or more laboratory-proven serious bacterial infections. More recent findings suggested that the incidence of bacterial disease dropped from rates of 4.7 cases per 100 patient-years before 1997 to 0.2 cases per 100 patient-years after 1997 (HAART era).[190] Reported clinical infections included bacteremia, pneumonia, osteomyelitis, meningitis, and sinusitis. Therapy is directed against the specific isolated bacterial pathogen, and the dosing and duration of therapy depend on the affected site. Prophylaxis against bacterial infections initially should include vaccination with appropriate

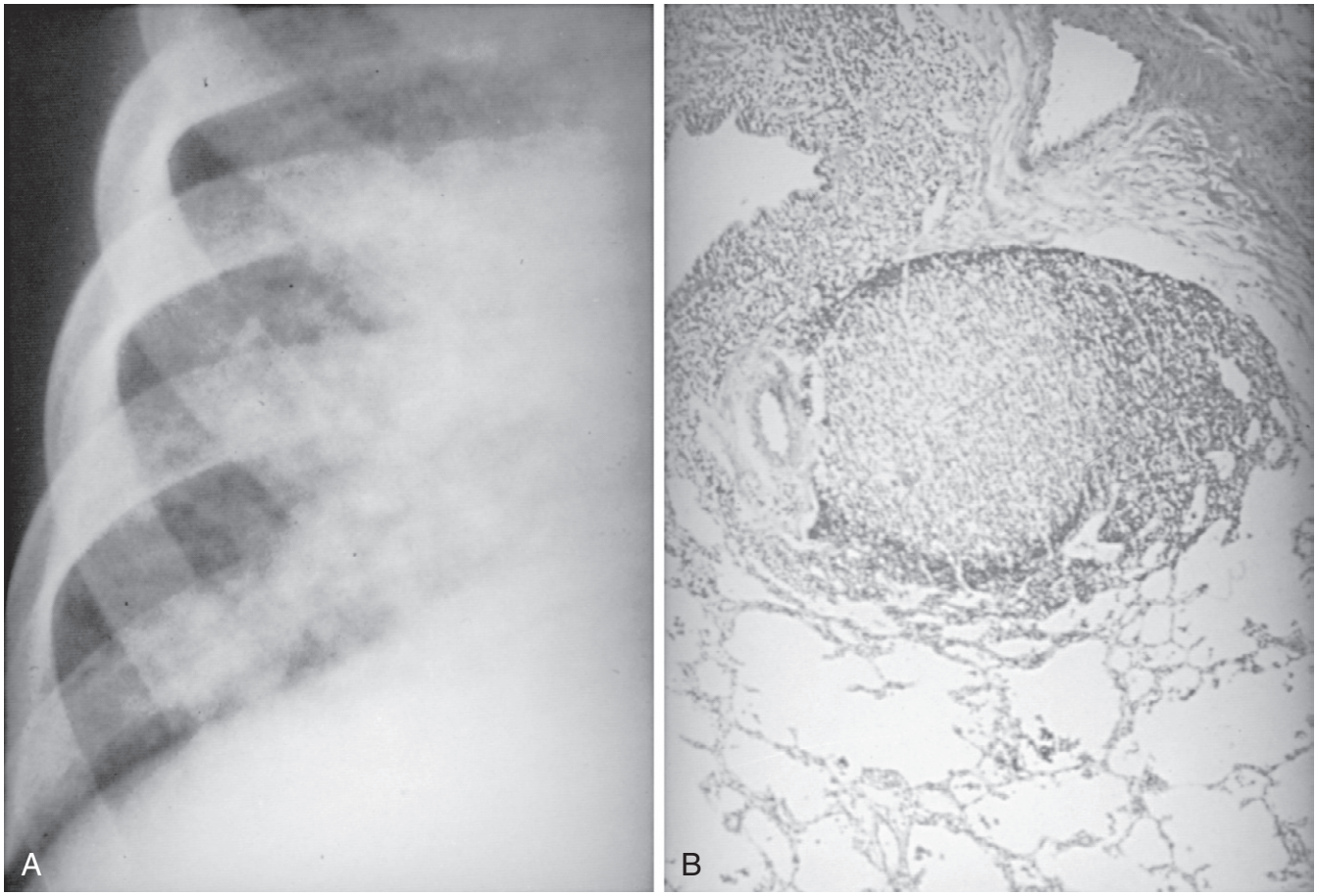

FIGURE 191B-5 ■ **A,** Chest radiograph documenting parenchymal nodularity in a 3-year-old child with perinatal AIDS. **B,** Pulmonary lymphoid hyperplasia in the child showing histologic changes, including interstitial widening and prominent peribronchial lung nodes.

childhood vaccines, including *Haemophilus influenzae* type b, pneumococcal conjugate vaccines, meningococcal conjugate vaccines, and inflluenza.[193] Other medications available for bacterial prophylaxis include intravenous immune globulin (IVIG), TMP-SMX, atovaquone, and azithromycin. Of note, IVIG is no longer recommended for primary prevention of serious bacterial infections in HIV-infected children unless hypogammaglobulinemia is present or functional antibody deficiency is demonstrated.

COMPLICATIONS

Pulmonary Complications

In addition to PCP and chronic sinopulmonary infection, noninfectious pulmonary complications of pediatric AIDS are associated with morbidity. Chronic lung disease is common in HIV-infected children with increasing age. A longitudinal birth cohort study reported a cumulative incidence of chronic radiographic lung changes in HIV-infected children of 33 percent by 4 years of age.[192] The spectrum of chronic HIV-associated lung disease includes lymphocytic interstitial pneumonia (LIP)/pulmonary lymphoid hyperplasia (PLH), chronic infections, IRIS, bronchiectasis, and malignancies.[281] Lymphoid interstitial pneumonitis and *P. jiroveci* pneumonitis have largely disappeared in developed countries but remain a significant problem in developing countries.[281] The incidence of *P. jiroveci* pneumonitis (previously 1.3 per 100 child-years) is now 0.13 per 100 child-years. LIP is similarly affected by HAART. Bacterial pneumonia remains relatively high (2.2 per child 100 years) although markedly reduced from the time period prior to HAART when the incidence was fivefold higher.[281] Bronchiectasis may reappear as a serious complication of HIV infection if chronic inflammation of the lung (generally controlled by HAART therapy) takes its toll over the years, given that the oldest surviving HIV-infected children are now approaching 30 years of age.

Histopathologically, LIP and PLH appear to be distinct entities, although whether these disorders represent a continuum of reactive hyperplasia of lymphoid tissue is somewhat controversial. In LIP, small lymphoid infiltrates are dispersed throughout parenchymal lung tissue and often are accompanied by alveolar epithelial hyperplasia and interstitial widening. PLH describes larger, dense nodular aggregates of lymphoid tissue both in distal parenchymal tissue and in the walls of bronchi and bronchioles (Fig. 191B-5A). Compression of blood and lymphatic vessels by these nodules may contribute to the

accompanying clinical interstitial widening. The cause of LIP/PLH has not been defined. Simultaneous infection with EBV and HIV may amplify the risk for development of LIP. B lymphocytes infected with EBV are very susceptible to HIV infection in vitro and could enhance intrapulmonary replication of HIV.[259] Clinically, LIP/PLH is characterized by a nonproductive cough and the insidious onset of progressive hypoxia. The hypoxemia may be subtle and best appreciated during febrile, upper respiratory tract illnesses. Digital clubbing, generalized lymphadenopathy, chronic parotitis, or failure to thrive may accompany LIP/PLH[201,253] Radiographically, LIP/PLH often demonstrates characteristic interstitial infiltrates with a nodular pattern (see Fig. 191B-5B). This radiographic picture frequently mimics that of miliary tuberculosis and warrants exclusion of this pulmonary infection. In an immunocompromised, anergic, HIV-positive child, simple delayed hypersensitivity skin testing for exclusion of tuberculosis may not suffice and BAL or gastric aspiration for detection of acid-fast microorganisms may be necessary. Lung biopsy is the definitive diagnostic procedure for LIP/PLH. However, in HIV-infected children, a presumed diagnosis of LIP/PLH by the less invasive exclusion of infectious pathogens is preferred.

Therapy for LIP/PLH is not defined clearly because the clinical outcome is variable. HIV-infected children with LIP/PLH have been noted to have spontaneous remissions without therapeutic intervention, whereas other affected children progress to respiratory insufficiency and failure. Therapeutic interventions have included IVIG supplementation, antiretroviral therapy, corticosteroids (daily or alternate-day dosing ranging from 0.5 to 2.0 mg/kg/day), and observation. No significant associated infectious sequelae (bacteremia, fungemia) of corticosteroid use were reported in treated children. Supportive therapy consisting of oxygen supplementation, chest physical therapy, and attention given to adequate nutritional intake is helpful.[103,201]

Other noninfectious pulmonary complications have been reported less frequently in the pediatric HIV-infected population. Evidence of an increased incidence of asthma in HIV-infected children, adolescents, and young adults has been shown in a series of peer-reviewed publications.[86-88,111,238] In the most recent of these, both physician-diagnosed asthma and asthma medication use demonstrated at least a 30 percent increase in the incidence of asthma (HIV-infected vs. HIV-exposed but not infected patients) and offered compelling evidence that more than one manifestation of allergic disease appeared in these patients.[238] Atopic dermatitis was also increased in HIV-infected patients and appeared shortly after birth and before the asthmatic child was diagnosed, very much resembling the order that appears in the general pediatric population.

So far, two potential mechanisms have been discovered that may underlie the increased incidence of asthma in HIV-infected youth. The first of these is genetic inheritance of human leukocyte antigens (HLA)-class I genes that are associated with new disease appearance after patients with HIV infection are immune reconstituted with HAART.[86] The second mechanism that may be applicable to the appearance of new disease in immunoreconstituted patients infected with HIV is a sudden return of cellular immunity (as measured by $CD4^+$ T-cell increases) that may precipitate a dysregulated attack on HIV in the airways, as well as commensal fungi that colonize and/or infect the airways of HIV-infected patients.[13] In experimental animal models, this sudden burst of immune stimulation precipitates an asthma attack.[209,210]

Central Nervous System Complications

Neurologic abnormalities have been documented in persons with HIV and can be attributed to opportunistic infections, adverse events of primary treatment, or primary infection with HIV, especially in light of HIV's described tropism for monocytes. Diverse neurologic and neurobehavioral deficits are common in children with HIV-1 infection and include abnormal neurologic examinations (e.g., global pyramidal tract signs, hemiparesis, peripheral neuropathy, visual, and hearing impairment), neurobehavioral problems, encephalopathy, and epilepsy.[11,101,129] Children with severe immunosuppression, with immune activation not receiving antiretroviral therapy, and who are growth impaired and younger than 1 year of age are at greatest risk for developing neurologic complications.[148,172] In 1994, the definition of HIV encephalopathy was adapted directly to children as a criteria for the diagnosis of AIDS and consists of at least one of the following features present for at least 2 months: (1) failure to attain or loss of developmental milestones or loss of intellectual ability, verified by a standard developmental scale or neuropsychological tests; (2) impaired brain growth or acquired microcephaly demonstrated by head circumference measurements or brain atrophy identified by computed tomography (CT) or magnetic resonance imaging (MRI) (serial imaging is required for children younger than 2 years old); and (3) acquired symmetric motor deficit manifested by two or more of the following: paresis, pathologic reflexes, ataxia, or gait disturbance.[36]

The precise pathogenesis of the encephalopathic changes associated with HIV infection remains elusive, although cellular tropism of HIV has been implicated.[143] Histopathologically, most children with involvement of the CNS show significant brain atrophy. Inflammatory lesions usually are sparse and alone cannot account for the significant amount of atrophy observed. Other purported factors potentially contributing to diminished brain size have included the following: (1) direct or indirect interference of HIV with brain growth (HIV toxic effect versus competition with brain growth factors such as neuroleukin), (2) severe malnutrition, (3) severe hypoxia from cardiac and pulmonary compromise, and (4) therapeutic regimens for infectious or noninfectious processes that mandate the prolonged use of medications that may inhibit brain growth.[157,231] A prospective study of a cohort of 319 perinatally HIV-infected children aged 6 to 17 years observed over a 2-year period indicated associations of past and current CDC class C designation with less severe attention-deficit/hyperactivity disorder inattention symptoms, older age at nadir $CD4^+$ T cell percentage and lower $CD4^+$ T cell percentage at study

entry with more severe conduct disorder symptoms, higher RNA viral load at study entry with more severe depression symptoms, and lower $CD4^+$ T cell percentage at study entry with less severe symptoms of depression. There was little evidence of an association between specific antiretroviral therapy and severity of psychiatric symptoms. A lower nadir $CD4^+$ T cell percentage was associated with lower quality of life, worse Wechsler Intelligence Scale for Children Coding Recall scores, and worse social functioning.[188] In a cohort of perinatally HIV-infected children with severe disease, Kapetanovic and colleagues concluded that higher $CD4^+CD38^+HLADR^+$ percentage may be associated with a neuroprotective effect and higher percentage of $CD4^+CD38^+$ but $HLADR^-$ T cells may be deleterious.[130] Attention-deficit hyperactivity disorder, learning disability, and graphomotor weakness have been described in higher proportions in HIV-infected children with transfusion-acquired infection.

Behavioral, neurodevelopmental, and, occasionally, imaging improvements have been documented with nucleoside inhibitor intervention. In a study of 146 perinatally HIV-infected children born between 1990 and 2003, the prevalence of encephalopathy (progressive or static) decreased from 40.7 percent before 1996 (limited combination antiviral therapy) to 18.2 percent after that year.[234] In this cohort, neurocognitive scores remained stable over the course of a 6-month period in children receiving combined antiviral therapy and only a weak association with lowered viral load could be found. Clearly, antiviral therapy not only must achieve effective serum and plasma concentrations but also must be able to cross the blood-brain barrier and affect HIV-infected CNS monocytes. On the contrary, the results of a longitudinal cohort study involving HIV-infected and HIV-exposed but uninfected infants and children reported effects of PI-based HAART showing a positive but limited impact on neurodevelopmental trajectories and the rate of significant cognitive and motor impairment in HIV-infected infants and young children during the first 3 years of life.[156] In this study, infants with immune suppression ($CD4^+$ T cell percentages <25%) seemed to be most vulnerable with respect to neurodevelopmental functioning, demonstrating mental and motor scores lower than those of the HIV-negative infants, as well as HIV-infected infants with less immune suppression.

Sleep disturbances, neurocognitive deficits, and abnormal psychosocial function are associated with abnormal cytokine levels in children with HIV infection.[17,195,229,276] A National Heart, Lung and Blood Institute (NHLBI)–funded study attempted to discern a molecular pathway of these complications of pediatric HIV infection by assessing proinflammatory cytokine production in $CD4^+$ and $CD8^+$ T-cells, disturbed sleep patterns, and results of neurodevelopmental testing in HIV-infected versus HIV-exposed children.[85] Compared with HIV-exposed children, there were significant associations in HIV-infected children between changes in intracellular proinflammatory cytokines (TNF-α, IFN-γ, IL-12), sleep patterns (particularly increased daytime sleep), and lower neurocognitive scores on socioemotional, behavioral, and executive function: working memory and mental fatigue; verbal memory; and sustained concentrations and vigilance. The authors of this sleep study in HIV-infected children proposed that therapies that reduced immune-mediated inflammation and prescribed sleeping habits might improve the neurocognitive and psychosocial problems.[85]

Children with neurologic or behavioral deficits and HIV infection should be evaluated by a pediatric neurologist, and developmental, audiologic, and ophthalmologic assessments are warranted. These children may need support systems including physical therapy, medications to assist with seizure control or contractures, specialized equipment (e.g., braces, splints, wheelchairs), and, as needed, medications or devices to assist with swallowing or food ingestion.

In pediatric AIDS, bacterial pathogens predominate as the most common cause of meningitis.[193] HIV rarely is isolated from cerebrospinal fluid. Other pathogens reported in the pediatric HIV-infected population include *Candida* spp., CMV, *M. tuberculosis*, *Toxoplasma*, and *Cryptococcus*. In the reported adult cases of IRIS-associated progressive multifocal leukoencephalopathy, brain biopsy lesions reveal severe demyelination and perivascular inflammatory response of monocytes and T lymphocytes.[105]

Gastrointestinal Complications

Gastrointestinal complications of HIV disease are encountered frequently in both children and adults. Before HAART, malnutrition was one of the most frequent and devastating complications of pediatric HIV, and it predicted both morbidity and mortality. Malnutrition in HIV-infected children has several causes, including reduced oral intake (HIV-induced primary cachexia, opportunistic infections of the upper gastrointestinal tract, and medication side effects), malabsorption (HIV alone or infections of the gastrointestinal tract), altered metabolic states (proinflammatory effects of chronic HIV infection), and socioeconomic factors.[174] Wasting has been revised from the 1987 definition to its present definition (1994): (1) persistent weight loss greater than 10 percent of baseline, (2) downward crossing of at least two of the percentile lines on the weight-for-age chart in a child 1 year or older, or (3) less than the 5th percentile on a weight-for-height chart on two consecutive measurements at least 30 days apart, plus (a) chronic diarrhea (at least two loose stools per day for ≥30 days) or (b) documented fever (≥30 days, intermittent or constant).[36] Although the prevalence of AIDS-associated wasting has declined in the developed world because of HAART, it is still a concern for children in both rich and poor nations. HIV and its complications also have been associated with specific nutritional disorders. Higher HIV viral load, lower $CD4^+$ T-lymphocyte counts, infections, maternal drug use, and zidovudine have been associated with growth problems.[176] The intestine is a primary target organ for HIV and also is important in the pathogenesis of HIV infection. In untreated HIV-infected children, as many as 80 percent will have one or more intestinal abnormalities at a given time. The pattern of intestinal dysfunction may change with time and can

occur without evident cause or change in clinical HIV stage or immunologic status.[65,109] The transactivator transfer factor, Tat, may be involved in intestinal dysfunction (HIV enteropathy) because it can impair enterocyte proliferation by targeting its L-type calcium sodium-glucose symporter. This pathway may explain many features of intestinal dysfunction, such as intestinal atrophy, carbohydrate malabsorption, and diarrhea, that are frequently observed.[65] Associated protein and micronutrient (zinc, selenium) deficiencies have been documented in HIV-infected adults and children.[51] Despite use of HAART, gastrointestinal conditions, nutritional deficiencies, and fluid and electrolyte disorders continue to be the most frequently described diagnoses for hospitalized HIV-infected children.[147]

More widespread use of maternal antiretroviral therapy to prevent HIV transmission has resulted in a decline in the percentage of HIV-exposed infants with low birth weight.[230] A study of more than 11,000 HIV-exposed or infected infants observed between 1989 and 2004 showed a 14 percent decline in low birth weight, from 35 to 21 percent, and this decline occurred independent of race or ethnicity. In contrast, growth and metabolic abnormalities persist in HIV-infected children either as a direct consequence of HIV infection or as an adverse outcome of antiviral therapy. Wasting and weight loss in HIV-infected individuals appear to be independent predictors of mortality and are associated with lower $CD4^+$ cell counts.[81,164] These studies have prompted careful attention to the nutritional needs of HIV-infected children. Specialized nutritional supplements such as nucleotides, polyamines, probiotics, and zinc can improve gastrointestinal absorption. Zinc may control diarrhea in HIV-infected children because it specifically counteracts Tat-induced intestinal ion secretion.[32] The polyamines, such as spermine, spermidine, and putrescine, can modulate intestinal maturation. Probiotics, including *Saccharomyces boulardii*, target the gastrointestinal tract and have antioxidant, antibacterial, enzymatic, anti-infective, metabolic, and anti-inflammatory activity. However, their effectiveness is not well established.[71,94]

The use of more potent antiretroviral therapy regimens, particularly those including PIs, has resulted in unwanted biochemical lipid abnormalities and changes in body composition for some HIV-infected adults and children. In a 2004 study of 94 infected children receiving long-term PI-containing antiviral regimens, 10 percent developed redistribution of fat, 52 percent developed dyslipidemia without associated somatic changes, and 38 percent had no associated findings.[255] In this cohort, changes in redistribution of fat and dyslipidemia were most likely to occur near ages consistent with the start of puberty (10 to 15 years). In a multicenter U.S. Perinatal AIDS Collaboration Transmission Study, 178 HIV-infected children were evaluated for hypercholesterolemia or hypertriglyceridemia and 47 and 67 percent, respectively, met criteria for these diagnoses at least once during their follow-up.[33] In this cohort, hypercholesterolemia was associated with multiple PI-containing regimens and undetectable HIV viral load as measured by RNA PCR. Hypertriglyceridemia was predicted by elevated body mass index and use of the PI ritonavir (RTV). These clinical findings of lipodystrophy and dyslipidemia have been treated with some success with diet, exercise, or lipid-lowering medications, although the lipid-lowering medications may result in drug-drug interactions when they are combined with antiviral agents.

Nutritional goals for HIV-infected children should include preservation of normal growth and development, provision of adequate amounts of all nutrients, and prevention of malabsorption and deficiencies of nutrients that alter immunologic function, all while improving quality of life.[175]

Malignancy

Human immunodeficiency virus infection has been associated with an increased risk for malignancy, both AIDS defining and non–AIDS defining.[76] Initiation of HAART in the mid 1990s has resulted in a significant decrease in AIDS-defining neoplasms such as Kaposi sarcoma and certain lymphomas, particularly primary CNS lymphoma and high-grade B-cell non-Hodgkin lymphoma, but malignancy continues to be reported.[57,76,104,128] The majority of malignancies that affect HIV-infected children are non-Hodgkin lymphoma.[104,133,141] In a retrospective study of 4954 children with AIDS in the United States (1978 to 1996), approximately 2.5 percent had documented malignant diseases: non-Hodgkin lymphoma (n = 100), Kaposi sarcoma (n = 8), leiomyosarcoma (n = 4), and Hodgkin disease (n = 2); 10 others had unspecified cancers.[12] Associated in situ infection with HIV, EBV, or both, has been documented in pediatric malignancies, although the precise role in pathogenesis is not defined. In a case-control study, a high viral burden of EBV was associated with the development of malignancy, but only in children with $CD4^+$ T cell counts >200/μL.[206] Early chemotherapeutic intervention has enhanced the quality of life and longevity and often has been provided in conjunction with antiretroviral therapy.

Kaposi sarcoma clearly occurs much less commonly in HIV-infected children. The pathogenesis of Kaposi sarcoma, both HIV and non–HIV related, has been elucidated, and human herpesvirus–8 has been documented in lesions.[117,152] Pathogenesis has been linked to oncogenesis and cytokine-induced growth. The clinical manifestations of Kaposi sarcoma associated with AIDS affect multiple organs. The skin, gastrointestinal tract, lungs, and heart have been affected by Kaposi sarcoma.[7] Management of Kaposi sarcoma has improved with increasing use of HAART to restore immune function in HIV-infected individuals.[200]

Leiomyomas and leiomyosarcomas are the second most common malignancies observed in HIV-infected children. Although this cancer is usually seen in the gastrointestinal tract, it may also involve the lungs or spleen. The pulmonary lesions are usually visible as nodules on the chest CT, whereas the gastrointestinal tumors present as evidence of obstruction, abdominal pain, and bloody diarrhea.[104] Since the advent of HAART, HIV-related malignant disease in the United States is reported less frequently. In a study of 2,969 HIV-infected children observed in the Pediatric AIDS Clinical Trials Group 219/219C from 1993 to 2003, only 37 cases of malignant

disease were reported, representing a prevalence of 0.6 percent malignancies and an incidence of 1.56 per 1,000 person-years.[133] In this analysis, the standardized incidence cancer rate for infected children was 10.08 as compared with uninfected exposed controls. The incidence of cancer was highest in children with severe immunocompromise and in those who had received HAART for less than 2 years. In contrast, developing nations continue to report high incidences of malignant disease.[241]

Other Complications

Cardiac Abnormalities

Cardiac manifestations of HIV infection in children include left ventricular dysfunction, increased left ventricular mass, myocarditis, and pericardial effusion.[266] Clinically, HIV-infected children have been described with congestive heart failure and cardiomegaly, cardiac tamponade, nonbacterial thrombotic endocarditis, conduction disturbances, and sudden death, presumably secondary to primary ventricular arrhythmia associated with severe cardiomyopathy. In a postmortem analysis of 30 HIV-infected children followed longitudinally in the prospective P2C2 HIV Multicenter Study, 50 percent had cardiomegaly with associated clinical findings of increased heart rate, increased left ventricular mass, and chronic heart disease.[131]

Asymptomatic children with HIV infection additionally have been documented to exhibit cardiac abnormalities. In a cross-sectional study of 49 HIV-infected children, carotid artery wall stiffness was larger and systolic-diastolic variations in diameter of the carotid artery were significantly lower compared with controls in the absence of cardiovascular risk factors.[16] Other investigators reported higher carotid intima-media thickness in HIV-infected subjects than in healthy controls, suggesting early atherosclerosis; duration of antiretroviral therapy and $CD4^+$ T-cell percentage were associated with these changes.[220]

A recent prospective cohort study of HIV-exposed infants suggested associations of in utero antiretroviral therapy exposure with reduced left ventricular mass and dimension and septal wall thickness z-scores and increased left ventricular fractional shortening and contractility up to 2 years of life.[154]

No evidence suggests that HIV is directly cardiotoxic, although HIV has been documented within myocardial cells by in situ hybridization.[82] Other factors may have an impact on the development of cardiac abnormalities and include malnutrition and infectious agents such as CMV and EBV.

The increasing use of HAART and, specifically, PIs in the pediatric HIV-infected population prompted careful management of associated hypercholesterolemia and hyperlipidemia. In univariate analysis, hypercholesterolemia in children with HIV infection treated with a PI regimen was associated with elevated systolic blood pressure but not with body mass.[79] Careful follow-up of lipid profiles should be evaluated routinely in HIV-infected children receiving antiretroviral therapy.

Renal Dysfunction

Renal disease is a well-known complication of HIV infection. HIV-1–associated nephropathy (HIVAN) is a clinical and renal histologic disease characterized by the presence of heavy proteinuria and nephrotic syndrome leading to a rapid progression to chronic renal failure. In a retrospective evaluation of 155 pediatric patients with AIDS, 12 were noted to have significant proteinuria.[250] The renal abnormalities described in HIVAN included nephritis (focal and segmental glomerulosclerosis and mesangial hyperplasia) and nephrosis.[169,251] The immunopathologic characteristics of HIVAN include inflammatory infiltrations predominantly composed of activated T cells ($CD4^+$ T cells usually exceeding $CD8^+$ T cells).[216]

Persistent metabolic acidosis associated with renal tubular disease and high anion gap acidosis has been reported in pediatric HIV infection.[216] Renal disease in HIV-infected children often appears in concert with profound immunodeficiency and end-stage HIV disease. Because most pediatric patients with AIDS and renal disease have perinatally acquired HIV infection, the importance of congenital or early concomitant infections (e.g., CMV) has been postulated to affect pathogenesis. Recent studies show that host genetic factors also play an essential role in HIVAN.[211]

The nephrosis of HIV disease can be particularly difficult to treat in an already malnourished, hypoproteinemic, HIV-infected child. HAART is considered the best treatment for HIVAN. Corticosteroids in combination with HAART may be attempted. However, the risks associated with their long-term use in HIV-infected children should be balanced against their potential benefits.[212] Many of the reported children with renal disease have associated growth failure. Nutritional supplementation and dietary restriction may be supportive adjunctive therapy.

Bone Marrow Suppression

Hematologic abnormalities associated with HIV infection in children include leukopenia, anemia, and thrombocytopenia. The etiology of neutropenia is often multifactorial in HIV-infected patients, representing the combined effects of systemic infection, medications, and HIV itself.[8] Neutropenia often has been described in association with circulating antineutrophil antibodies and may respond to blockade therapy with IVIG. Granulocyte colony-stimulating factor has been used successfully in neutropenia, both drug induced and HIV associated.[106,107,186]

The anemia of HIV infection may be microcytic, hypochromic as seen in chronic infection, autoimmune with positive Coombs testing, or typical of nutritional deprivation (iron or vitamin B_{12} deficiency). The origin of anemia in AIDS is difficult to sort out and is confounded by multiple cofactors that affect red blood cell counts, that is, poor nutritional status and concomitant use of toxic therapeutic agents (zidovudine). In adults and children, recombinant erythropoietin has been beneficial in treating anemia associated with antiviral therapy.[21] In

developing nations, infant anemia of HIV exposure or infection coupled with acute local parasitic infections (malaria) may significantly affect infant morbidity. The risk for developing severe and potentially fatal malarial anemia is increased for HIV-exposed or infected children with concomitant acute parasitemia, as compared with HIV-negative controls.[196]

Immune thrombocytopenia has been reported in 13 percent of children with symptomatic HIV infection with an onset as early as the first year of life.[97,165] Thrombocytopenia in HIV-1–infected patients is multifactorial, including a direct effect of HIV-1 with a depressed platelet production due to HIV infection of megakaryocytes, and peripheral destruction due to platelet-associated autoantibodies triggered by molecular mimicry between some HIV-1 peptides and membrane-associated platelet glycoproteins.[155] Therapy for thrombocytopenia in children and adults has included no intervention, platelet transfusions, systemic corticosteroids, IVIG, antiretroviral therapy, and, most recently in adults, IFN-α. Immune thrombocytopenia has resolved spontaneously in some children with simple supportive measures.

DIAGNOSIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION IN INFANTS AND CHILDREN

Early Transmission to Fetuses

The human placenta plays a role in prenatal transmission of HIV infection from mother to fetus. Studies suggested that fetal infection can take place as early as 9 to 11 weeks of gestational age.[25,167] Preliminary examination of birth placental tissue from HIV-infected mothers in another study indicated that HIV core antigens could be detected in approximately 50 percent (23 of 51) of the cases studied; furthermore, HIV antigens were localized in the Hofbauer cells.[262] Finally, investigators documented that placental trophoblasts can be infected by CD4-independent isolates of HIV-1 in vitro.[4] Placental cytokines and chemokines influence HIV replication in trophoblasts. Genetic analysis of HIV-1 sequences verify the interaction of HIV-1 and placental tissue. The vertical transmission of HIV-1 is characterized by selection of genotype variants that escape the mother's immune system.[5]

An increase in the incidence of intrauterine fetal demise was demonstrated in the literature in HIV-infected pregnant women.[74] In HIV-negative fetuses, placental lesions known to be associated with fetal demise were identified: abruption, infarction, and other infections (e.g., CMV infection). In HIV-positive fetuses, similar placental lesions could not be identified and death was attributed to HIV infection of fetal tissue as detected by in situ hybridization. Two hypotheses for transmission are proposed: (1) direct cell-to-cell spread of HIV from infected maternal mononuclear cells through the placental cells and eventually to fetal tissue itself and (2) infected maternal cells that gain access to the fetal circulation.

TABLE 191B-6 **Guidelines for the Diagnosis of HIV Infection in Infants Born to HIV-Infected Mothers**

Diagnosis in Infants Aged <18 mo

Virologic assays that directly detect HIV, preferably HIV RNA assays or the HIV DNA polymerase chain reaction, must be used to diagnose HIV infection in infants aged <18 mo. A positive virologic test should be confirmed by a repeat virologic test on a second specimen.

Diagnosis in Infants Aged ≥18 mo

A positive HIV antibody test with confirmatory Western blot or immunofluorescent antibody (IFA) assay or virologic assay is used for diagnosis of infants aged ≥18 mo.

Exclusion of HIV Infection in infants

Definitive exclusion of HIV infection in non–breast-fed infants is based on two or more negative virologic tests, with one obtained at age ≥1 mo and one at age ≥4 mo, or on two negative HIV antibody tests from separate specimens obtained at age ≥6 mo.

Modified from Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. Accessed November 25, 2012.

Methods Used to Diagnose Infection in Children Older Than 18 Months

In children older than 18 months in whom the presence of maternal anti-HIV antibody no longer is a confounding variable, the conventional tests used to diagnose HIV infection in adults are applicable (Table 191B-6). Thus, antibody detection tests including the HIV rapid test, enzyme immunoassays (EIAs), Western blot analysis, indirect fluorescent antibody assay, and p24 HIV antigen analysis (p24Ag), HIV culture, and HIV PCR assay may be used. The period between infection and detection of HIV antibody by a particular test is referred to as the "window period" for that assay. The window period for detection of HIV has been progressively shortened as EIA techniques have improved. Various generations of screening EIAs begin to detect anti-HIV antibody 2 to 6 weeks after HIV RNA is detected. EIAs that test for both HIV-1 and HIV-2 are used for screening. Western blots, which have been used for confirmation of positive screening EIAs, become diagnostically reactive 5 to 6 weeks after infection. Aside from the HIV-1 Western blot, confirmation of HIV-1 infection can be achieved with HIV RNA PCR (qualitative or quantitative) or HIV DNA PCR analysis. In addition, combined fourth-generation tests that detect both HIV p24 antigen and HIV antibody have been utilized for initial screening with confirmation of infection provided by HIV RNA PCR testing to reduce the window period for diagnosis in newer diagnostic algorithms.[18]

Methods Used to Make an Early Diagnosis of Infection in Infants and Children Younger Than 18 Months

The clinical manifestations of HIV infection in children are varied and nonspecific and include chronic pneumonitis, failure to thrive, hepatosplenomegaly, thrombocytopenia, and chronic diarrhea. In children younger than 18 months, a positive serologic determination of HIV is not accepted as indicative of the presence of HIV infection because of passive maternal antibody. Documentation of HIV infection in children younger than 18 months requires identification of viral components from blood. The most commonly used virologic assay in the United States is HIV PCR (DNA or RNA).[199] In resource-poor countries where virologic laboratories are not available, the CD4/CD8 ratio may be used to diagnose infants with HIV infection.[187]

Table 191B-6 outlines the current guidelines for determining positive HIV infection status in infants younger than 18 months who are born to HIV-infected mothers. Cord blood determinations for HIV are problematic because of contamination by maternal cells. Experience with the use of PCR in neonates born to HIV-infected women has documented HIV infection as early as the first days or month of life.[179] The estimated sensitivity of HIV DNA PCR assay, which can be used to measure integrated proviral HIV DNA, is 38 percent from birth to 48 hours of life, 93 percent by 14 days of age, and 96 percent by 28 days of age. HIV-1 RNA PCR measures extracellular viral RNA and has a sensitivity of 25 to 40 percent in the first weeks of life and 90 to 100 percent by the time the child reaches 2 to 3 months of age. The newer qualitative HIV RNA PCR assay has sensitivities similar to that of the HIV DNA PCR assay.

Current pediatric PCP prophylaxis guidelines suggest that infants with two negative virologic assays, both performed at 1 month or older and at 4 months or older, are not infected with HIV, and interruption of the therapeutic intervention is warranted.[37] Hence, early diagnosis in HIV-exposed infants has been improved both for identification of HIV-infected children (as early as the first month of life) and for uninfected children labeled as seroreverters (as early as the fourth month of life). Schedules for testing of HIV-exposed infants vary nationally. However, advances in diagnostic technology allow for early testing of HIV-exposed infants, including shortly after birth through 14 days of life, at 1 to 2 months of age, and then again at 3 to 6 months. Confirmation of negative virologic assays (HIV PCR) can be performed with typical antibody testing when the child is 18 months of age.

The assays outlined earlier easily detect HIV-1 subtype B HIV commonly found in developed nations. To detect non-B HIV infection in individuals from or in developing nations, HIV RNA PCR may be preferred, because it has a lower incidence of false-negative assays as compared with HIV DNA PCR. If non–subtype B HIV is suspected, then use of HIV RNA testing that is sensitive for these subtypes (e.g., more sensitive RNA PCR assays or branched DNA assays that are commercially available in the United States) should be considered.

IMMUNOLOGIC AND CLINICAL MONITORING OF HUMAN IMMUNODEFICIENCY VIRUS–INFECTED CHILDREN

Laboratory monitoring of HIV-infected children is linked to those variables that predict morbidity and mortality, namely, $CD4^+$ T-cell percentage and HIV RNA PCR assay results. Clinical evaluations should occur at a minimum of every 3 to 6 months, or sooner if the patient has clinical signs or AIDS-defining events, and measures of $CD4^+$ T-cell and HIV RNA PCR values should be performed. Because of variability across commercially available RNA PCR assays that may be as great as twofold, the individual HIV-infected patient should, when possible, be tested with a single assay type. Recommendations for changes in treatment based on changes in $CD4^+$ T-cell percentages or HIV RNA PCR should be undertaken in consultation with a specialist who routinely provides care to HIV-infected individuals. Evaluation of the individual HIV-infected child for viral resistance can be accomplished with use of commercially available phenotyping or genotyping assays. Such studies should be performed in consultation with an HIV specialist. Depending on treatment regimens, most children also should have laboratory studies performed to assess their potential for developing treatment-induced toxicities including bone marrow suppression, metabolic acidosis, dyslipidemia disorders, and pancreatitis. It is recommended that this cohort annually receive dental and ophthalmologic assessments as well as tuberculosis screening. Routine recommendations for childhood vaccine delivery are outlined later.

TREATMENT

Primary Anti–Human Immunodeficiency Virus Infection Treatment

Implementation of combination antiretroviral therapy consisting of at least three antiviral drugs has enhanced survival rates, improved immune status, and reduced opportunistic infections, as previously outlined.[24,98,168,215] Increased survival of HIV-infected children brings challenges in selecting successive new antiretroviral therapy regimens and specific issues to address include short- and long-term toxicities related to therapy and development of drug resistance.

In general, the principles of therapy are similar in all affected age groups. Treatment should be aggressive and use multiple drugs as early in the course of the infection as possible to suppress viral replication fully, reduce development of resistant viral strains, minimize drug-related toxicity, and preserve immune function. Considerations specific to the pediatric host include the following: (1) early clinical severity of HIV disease, viral load, and level of immune suppression; (2) availability of appropriate drug formulations and dosing; (3) unique pharmacokinetics (body composition, renal excretion, liver metabolism, enzyme maturation); (4) regimen

potency, complexity, short- and long-term toxicity potential; (5) impact of changing therapy; (6) comorbidities that affect drug choice; (7) drug interactions; and (8) the ability of the parent or child to adhere to a prescribed regimen.

Guidelines for initiation of therapy differ somewhat for adults and children. Current pediatric recommendations include initiation of therapy for all HIV-infected children, with the strongest support for the recommendation in symptomatic and immunosuppressed HIV-infected children and in children aged younger than 12 months regardless of clinical, immunologic, or virologic status (Table 191B-7). Current antiretroviral therapy is designed to target different steps in the replication cycle of HIV (Fig. 191B-6).[138] The Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children currently recommends aggressive combination of at least three medications from at least two drug classes for initial treatment of HIV-infected infants, children, and adolescents. With improvements in treatment formulations, dosing recommendations for antiviral agents can alter over time. Hence, for updated specific dosing and drug-drug interactions of antiviral agents, the reader is referred to guidelines from the Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children.*

Inhibitors of Human Immunodeficiency Virus Cell Entry

Entry of HIV-1 into target cells begins with interactions of the viral envelope protein gp120 with CD4 and a chemokine coreceptor, usually CCR5 or CXCR4. Both cellular receptors and structures in envelope proteins associated with membrane attachment and fusion are targets for therapeutic intervention.

Blockade of Human Immunodeficiency Virus by CD4-Receptor Inhibitors

CD4-based molecules can neutralize HIV by several mechanisms. Initial studies demonstrated the efficacy of soluble preparations of CD4 (sCD4) against HIV in vitro.[122,228,266] A chimeric molecule consisting of recombinant CD4 and immunoglobulin G (rCD4-IgG) was developed with an extended half-life and enhanced efficacy. Shearer and colleagues[237] documented safety, evidence of placental transfer, and appropriate elimination after intravenous administration of rCD4-IgG2 in pregnant women. In a phase I/II study, 18 children infected with HIV-1 received single or multiple intravenous doses of rCD4-IgG2 and demonstrated evidence of antiviral activity and tolerance.[236] Certain small molecules may competitively inhibit viral entry by binding gp120, thus blocking the gp120-CD4 interaction. It is generally considered that these small molecules in development as antiretroviral medications interfere with the gp120-CD4 interaction through altering the conformation of gp120

*Working Group on Antiretroviral Therapy and Medical Management of HIV-Infected Children guidelines: Available at http://AIDSinfo.nih.gov.

TABLE 191B-7 Treatment Recommendations for Initiation of Therapy in Antiretroviral-Naïve HIV-Infected Infants and Children

- Antiretroviral therapy should be initiated in all children with AIDS or significant symptoms (Clinical Category C or most Clinical Category B conditions) (AI*).
- Antiretroviral therapy should be initiated in HIV-infected infants <12 months of age regardless of clinical status, CD4 percentage or viral load (AI for infants <12 wk of age and AII for infants ≥12 wk to 12 mo).
- Antiretroviral therapy should be initiated in HIV-infected children ≥1 yr who are asymptomatic or have mild symptoms with the following CD4 values:
  - Age 1 to <3 yr
    - With CD4 T lymphocyte (CD4 cell) count <1000 cells/mm^3 or CD4 percentage <25% (AII)
  - Age 3 to <5 yr
    - With CD4 cell count <750 cells/mm^3 or CD4 percentage <25% (AII)
  - Age ≥5 yr
    - With CD4 cell count <350 cells/mm^3 (AI*)
    - With CD4 cell count 350-500 cells/mm^3 (BII*)
- Antiretroviral therapy should be considered for HIV-infected children ≥1 yr who are asymptomatic or have mild symptoms with the following CD4 values:
  - Age 1 to <3 yr
    - With CD4 cell count ≥1000 cells/mm^3 or CD4 percentage ≥25% (BIII)
  - Age 3 to <5 yr
    - With CD4 cell count ≥750 cells/mm^3 or CD4 percentage ≥25% (BIII)
  - Age ≥5 yr
    - With CD4 cell count >500 cells/mm^3 (BIII)
- In children with lower-strength (B level) recommendations for treatment, plasma HIV RNA levels >100,000 copies/mL provide stronger evidence for initiation of treatment (BII).
- Issues associated with adherence should be assessed and discussed with an HIV-infected child's caregivers before initiation of therapy (AIII). Patients/caregivers may choose to postpone therapy and, on a case-by-case basis, providers may elect to defer therapy based on clinical and/or psychosocial factors.

Rating of Recommendations: A = Strong; B = Moderate; C = Optional.

Rating of Evidence: I = One or more randomized trials in children† with clinical outcomes and/or validated end points; I* = One or more randomized trials in adults with clinical outcomes and/or validated laboratory end points with accompanying data in children† from one or more well-designed, nonrandomized trials or observational cohort studies with long-term clinical outcomes; II = One or more well-designed, nonrandomized trials or observational cohort studies in children† with long-term outcomes; II* = One or more well-designed, nonrandomized trials or observational studies in adults with long-term clinical outcomes with accompanying data in children† from one or more similar nonrandomized trials or cohort studies with clinical outcome data; III = expert opinion.

†Studies that include children or children and adolescents but not studies limited to postpubertal adolescents.

Modified from Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. Guidelines for the use of antiretroviral agents in pediatric HIV infection. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. Accessed November 25, 2012.

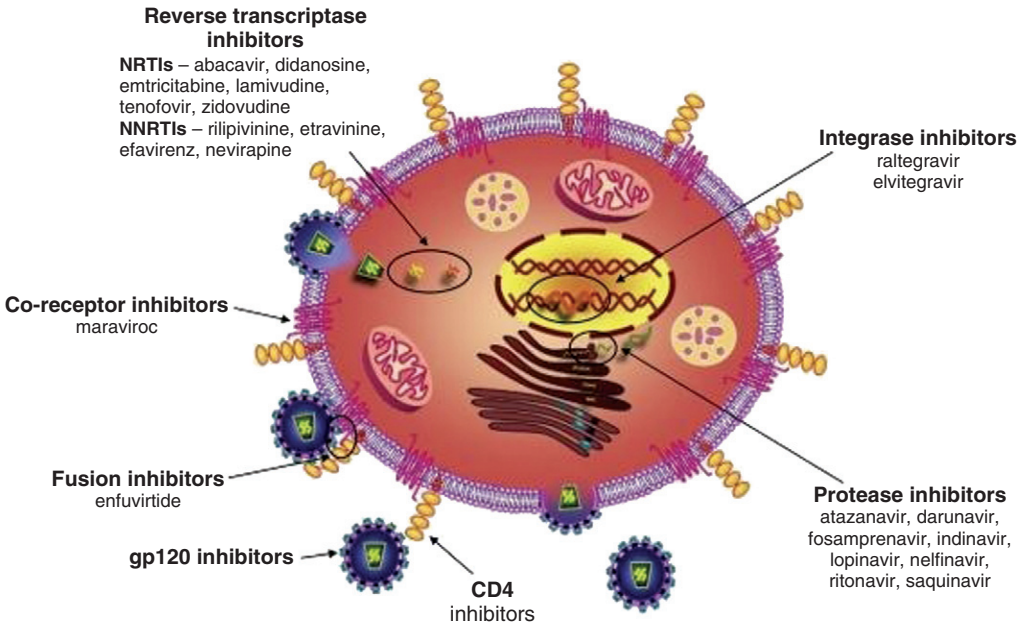

FIGURE 191B-6 ■ Inhibition of HIV-1 replication can be accomplished at different steps in the viral life cycle. (See companion Expert Consult website for color version.) (Modified from Pirrone V, Thakkar N, Jacobson JM, et al. Combinatorial approaches to the prevention and treatment of HIV infection. *Antimicrob Agents Chemother* 2011;**55**:1833.)

induced by CD4 instead of directly blocking CD4 binding.[64]

Blockade of Human Immunodeficiency Virus by Chemokine (Coreceptor) Inhibitors

Agents that target blockade of CXCR4 and CCR5 are being evaluated in clinical trials. Concerns about the use of coreceptor inhibitors include potential for unintended immune modulation and for viral escape to occur from tropism selection with resultant disease progression.[185] Maraviroc, a first-in-its-class selective CCR5 coreceptor antagonist, received approval by the U.S. Food and Drug Administration (FDA) for use in adults in August 2007. It is recommended as adjunct therapy to combination antiviral regimens and targets treatment of CCR5-tropic HIV-1 (R5 virus). The most common adverse effects are cough, fever, upper respiratory tract infections, rash, musculoskeletal symptoms, abdominal pain, and dizziness.

Blockade of Human Immunodeficiency Virus by Interference with gp120-CD4 Interactions

Polyanionic compounds such as dextran sulfate and heparin inhibit binding of HIV to the cell membrane, targeting the V3 loop of gp120.[184] Because of their systemic toxicity, most of these compounds have been evaluated in clinical trials as topical microbicides to prevent sexual transmission of HIV in humans. Unfortunately, clinical trials involving polyanion-containing microbicide formulations demonstrated that these products were ineffective and may have, in some circumstances, increased the risk for HIV-1 infection.[204] An additional substance, the green tea flavonoid epigallocatechin gallate (EGCG), binds to the CD4 molecule at the gp120 binding site, thus inhibiting gp120-CD4 interaction in a dose-dependent manner.[273] These adjunctive mechanisms to restrict HIV infection remain under development.

Blockade of Human Immunodeficiency Virus by Fusion Inhibitors

As previously described, fusion of the HIV envelope (glycoprotein gp41) with the target cell membrane is an important step in host cell infection. During fusion, FP of gp41 inserts into the target cell membrane and gp41 heptad repeats (HR1 and HR2) alter their conformation to form a six-helix bundle. This process results in the formation of a fusion pore through which the HIV capsid passes into the target cell. Fusion inhibitors represent the first class of entry inhibitors for which there is a licensed drug. Enfuvirtide (T-20) is a synthetic peptide that binds to the HR1 domain of gp41, prevents six-helix formation, and thus inhibits fusion.[50,214] Enfuvirtide is approved for use in HIV-infected adults and children aged 6 years or older whose HIV infection has not been controlled by other anti-HIV drugs. Addition of enfuvirtide to an optimized background regimen of antiretroviral therapy agents has demonstrated modest decreases of HIV viral load for long periods.[275] Although enfuvirtide is FDA approved for use in children, it is not commonly used because of its high cost, need for twice-daily subcutaneous injections, and high rate of injection-site reactions. The most common adverse effects include mild to moderate erythema, induration, pain, lymph node swelling, itching, and tenderness at the injection site. Other side effects reported are diarrhea, nausea, fatigue, headache, dizziness, peripheral neuropathy, and hypersensitivity reactions.[67] In the absence of a fully suppressive antiretroviral therapy regimen, mutations associated with resistance to enfuvirtide are demonstrated to be rapid.

Nucleoside Reverse Transcriptase Inhibitors

Nucleoside reverse transcriptase inhibitors (NRTIs) have no direct effect on HIV reverse transcriptase. After phosphorylation by the host cell kinases, they inhibit viral reverse transcriptase by competing with the natural substrate at the same binding site on the enzyme. With their incorporation into the newly forming proviral DNA, further viral transcription is prematurely terminated. Six NRTIs are licensed and currently available in the United States: zidovudine (ZDV), didanosine (ddI), stavudine (d4T), lamivudine (3TC), abacavir (ABC), and emtricitabine (FTC).

Zidovudine

Early pediatric studies demonstrated clinical, immunologic, and virologic improvements after intravenous and oral administrations of ZDV.[170] However, sustained $CD4^+$ cell restoration with ZDV monotherapy could not be achieved.[171]

ZDV is available in capsules, tablets, an oral solution, and an intravenous infusion. ZDV has good oral bioavailability (65%) and CNS penetration, with a mean serum half-life of 0.5 to 3 hours. It is rapidly metabolized in the liver by glucuronidation and is eliminated primarily by glomerular filtration and active tubular secretion. The most frequent adverse effects of ZDV are granulocytopenia and macrocytic anemia, often requiring limitations on doses. Some clinicians administer growth factors to counteract these adverse effects.[107] Other toxic effects include symptomatic myopathy, lactic acidosis, and hepatosteatosis.

In 1994, PACTG protocol 076 documented the remarkable effectiveness of ZDV in reducing the rate of maternal-infant transmission of HIV-1 by 66 percent (Fig. 191B-7).[61] The short-term toxicity most frequently described among infants was mild and reversible anemia. Most clinicians continue to use ZDV as part of their regimen to prevent maternal transmission, but maternal dosing includes additional antiviral medications to maximally suppress maternal viral load, and the administration of ZDV for maternal to child prevention of transmission is typically twice daily rather than the four times daily dosing used in the PACTG 076 trial.

ZDV and other NRTIs are suspected of inducing mitochondrial dysfunction secondary to their affinity for mitochondrial gamma DNA polymerase. This effect has been a source of particular concern for the ZDV- and HIV-exposed fetus and infant. Adding to this concern was a report from a large French study that 12 of 2644 NRTI- (predominantly ZDV) and HIV-exposed infants had evidence of mitochondrial dysfunction.[10] An additional 14 children in this cohort had unexplained symptoms that could be attributed to mitochondrial dysfunction. In response to this and other smaller cohort publications, the Perinatal Safety Review Working Group reviewed deaths in five large HIV-exposed perinatal cohorts (total of >11,000 NRTI- and HIV-exposed infants and children) in the United States to determine whether similar cases of severe mitochondrial toxicity could be documented.[73] Prophylaxis with ZDV was not associated with findings consistent with mitochondrial dysfunction, including a careful review of the cohort's 85 deaths. In early treatment studies, low birth weight and premature delivery were ascribed to NRTI exposure in small study cohorts.[158] With further treatment including HAART and specifically ZDV, low birth weight appears to be reduced in incidence.[231] Concerns about NRTI therapy in HIV-exposed but uninfected infants led to other large-scale studies that documented safety of the maternal-infant ZDV regimen through age 18 to 38 months.[112,245] Resistance to ZDV has been described, and the characteristic finding is a mutation at codon 215 of the reverse transcriptase.[124]

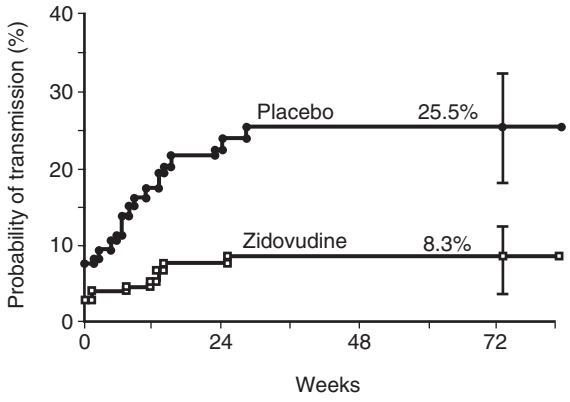

Placebo	183	84	42	37
Zidovudine	180	105	51	43

FIGURE 191B-7 ■ Analysis demonstrating a 66 percent reduction in perinatal transmission rates from 25 percent to 8 percent with antepartum, peripartum, and postpartum zidovudine therapy in HIV-infected pregnant women and their infants. (Reprinted by permission from Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med* 1994;**331**: 1173-80.)

Didanosine

In early studies, ddI was well tolerated and improvements in clinical and immunologic measures were documented in previously untreated HIV-infected children and those who had received prior ZDV treatment.[15,123,124] Didanosine is formulated as an enteric capsule with fewer gastrointestinal side effects and should be administered on an empty stomach. Common toxicities are diarrhea, abdominal pain, and nausea. The frequency of severe clinical toxicities, pancreatitis, and peripheral neuropathy is dose related. Lactic acidosis, severe hepatomegaly with steatosis, and retinal and visual changes also have been reported in patients treated with ddI. This drug may interact with other antiretroviral agents and produce toxicity or unwanted clinical outcomes. For example, ddI should be used cautiously in combination with tenofovir, a nucleotide inhibitor, because of increasing plasma levels of ddI and decreasing $CD4^+$ T-cell counts.[271]

Stavudine

Like ZDV and ddI, d4T is a potent inhibitor of in vitro HIV replication. In a phase I/II clinical trial of d4T in HIV-infected children, d4T was well tolerated without dose-related clinical or laboratory adverse events.[140] The oral bioavailability ranges from 61 to 78 percent, and the cerebrospinal fluid concentrations range from 16 to 97 percent 2 to 3 hours after oral administration.[139] Compared with ZDV, d4T is associated with better preservation of $CD4^+$ T cell counts.[137] d4T is available in capsules and as an oral solution. Reported severe toxicities include peripheral neuropathy, pancreatitis, lipodystrophy, and lactic acidosis. d4T and ddI should not be combined in the same regimen because of in vitro antagonism. d4T is no longer recommended for long-term use in pediatrics owing to concerns for toxicity, especially lipodystrophy.

Lamivudine

3TC has good antiviral potency to both HIV-1 and hepatitis B virus (HBV), and the drug is well tolerated orally and typically dosed twice daily.[203] 3TC is available in a tablet and a solution; its bioavailability is not affected by food. A combined product (Combivir) provides a preformed tablet formulation of ZDV (300 mg) and 3TC (150 mg). Toxicities are rare but include pancreatitis and lactic acidosis.[202]

Abacavir

ABC is approved for use in combination with other antiretroviral agents in children and adults. It is well absorbed, is unaffected by food, is well tolerated, and has good in vivo and in vitro potency in reducing HIV viral load when combined with other antiretroviral agents.[225] An important clinical side effect of this drug is ABC hypersensitivity reaction that occurs in approximately 5 percent of patients at a mean of 11 days after initiation of this agent.[67] Symptoms include fever, rash, malaise or fatigue, and gastrointestinal symptoms such as nausea and vomiting. HLA-B*5701 is documented to be a risk factor for development of hypersensitivity to ABC; U.S. guidelines recommend that ABC use is avoided in patients who are HLA-B*5701 positive.[246] Hypersensitivity to ABC requires permanent discontinuation of this agent because hypotension and death have occurred on repeat challenge. ABC is available in a tablet and an oral solution. A combined product, Trizivir, provides a co-formulated tablet of ZDV (300 mg), 3TC (150 mg), and ABC (300 mg). Epzicom is a co-formulated tablet containing 3TC (300 mg) and ABC (600 mg).

Emtricitabine

FTC was approved for use in combination with other anti-HIV medications. It is similar in many aspects to lamivudine and, like that drug, is active against both HIV-1 and HBV.[224] FTC has potent in vivo antiretroviral activity, has 4 to 10 times more potency than 3TC in vitro, is dosed once daily, and is well tolerated.[270] Because of cross resistance of FTC to the M184V resistance, mutations commonly observed in patients in whom a 3TC-containing regimen has failed, this agent has little utility in 3TC-experienced patients. It is available as a hard gelatin capsule and as an oral solution. Skin discoloration that manifests as hyperpigmentation on the palms and soles is a unique toxicity of FTC therapy and is usually mild.

Nucleotide Reverse Transcriptase Inhibitors

Nucleotide reverse transcriptase inhibitors (NtRTIs) function like previously described NRTIs, but they do not require phosphorylation. Tenofovir disoproxil fumarate (tenofovir DF, TDF), is an orally bioavailable prodrug of tenofovir. Tenofovir has good in vivo potency against both HIV-1 and HBV.[92] Tenofovir has long serum and intracellular half-lives that allow once-daily dosing. High-dose tenofovir treatment has been associated with skeletal abnormalities in young animals[265] and a significant decrease in absolute bone mineral density in HIV-infected children.[90] These changes in bone mineral density seemed to stabilize by 24 weeks of the treatment, and none of the patients experienced fractures during the study period. U.S. pediatric guidelines suggest judicious use in children and young adolescents because of unfavorable bone mineral density changes at a time when bone growth is so important. In addition, new onset or worsening of renal impairment has been reported in adults and children receiving tenofovir and may be more common in those with higher tenofovir trough plasma concentrations.[217] A combined product, Truvada, provides a preformed tablet formulation of FTC (200 mg) and TDF (300 mg) available for use once daily.

Non-nucleoside Reverse Transcriptase Inhibitors

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) work by binding directly to the reverse-transcriptase enzyme, thus rendering it nonfunctional. Five available drugs in this class are approved for use in the United States: nevirapine (NVP), delavirdine, which is not recommended in pediatrics and will not be discussed further, efavirenz (EFV), etravirine (ETR), and rilpivirine (RPV). RPV, co-formulated with tenofovir and emtricitabine, has not currently been studied in pediatrics. The major disadvantage of the two drugs widely used in pediatrics in this category, NVP and EFV, is the rapid development of resistance with resultant cross resistance between the two drugs. A newer NNRTI, ETR, retains activity against NVP- or EFV-resistant viruses when used in a regimen that also contains darunavir/ritonavir and if the number of NNRTI resistance-associated mutations is limited.[23] Additional NNRTIs are in development.[267]

Nevirapine

NVP has been shown to have antiretroviral efficacy in a number of different combination regimens in children. A mainstay of therapy for infants and children in resource

limited settings, NVP also is used to reduce perinatal HIV transmission. Guidelines describing data supporting the use of NVP in perinatal HIV prevention are available through the Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission.[199]* NVP in three doses over the first several weeks of life has been shown to further reduce transmission of HIV when used in combination with the standard ZDV regimen for infants born to HIV-infected women who did not received antepartum antiretroviral drugs.[191] NVP is available in both tablet form, including a long-acting once-a-day formulation that is currently FDA approved for children 6 years old and older, and as an oral solution. Severe hepatotoxicity, hepatic necrosis, and hepatic failure have been reported in children typically occurring in the first 12 weeks of therapy. NVP should be discontinued permanently in children or adults who develop severe rash or rash with constitutional symptoms and hepatotoxicity. Women with $CD4^+$ T-cell counts greater than 250 cells/mm^3 have particularly increased susceptibility to nevirapine toxicity.

Efavirenz

EFV is approved as a single-daily dosing agent and for that reason has been attractive for use in adolescents and older children. It is available in capsule and tablet forms only. A fixed-dose tablet formulation, Atripla, provides a combination of FTC (200 mg), TDF (300 mg), and EFV (600 mg) available for use once daily in adolescents and adults. In addition to toxicities of rash and elevated aminotransferase levels, CNS abnormalities including somnolence, insomnia, bad dreams, irritability, and depression have been reported within the first 2 weeks of treatment. EFV has been reclassified as FDA pregnancy category D because in animal models EFV was associated with cleft palate and anencephaly.[31] Use in women of child-bearing age should include intermittent pregnancy testing and counseling for pregnancy prevention.

Etravirine

ETR is FDA approved for antiretroviral-experienced children and adolescents aged 6 to 18 years and is under study for use in younger children. It is available in tablet form and can be dispersed in liquids for those patients who cannot swallow tablets. ETR is associated with multiple drug interactions, which limits its use. ETR should not be coadministered with the following antiretroviral drugs: tipranavir/ritonavir, fosamprenavir/ritonavir, atazanavir/ritonavir, and unboosted protease inhibitors. It should not be administered with other NNRTIs (nevirapine, efavirenz, or rilpivirine). Limited data in adults suggest that ETR may reduce the trough concentration of raltegravir, but no dose adjustment is currently recommended when ETR and raltegravir are used together. ETR is often combined with ritonavir-boosted darunavir for treatment of HIV-infected adults with prior virologic failure, and this combination has been effective and well tolerated in small studies of HIV-infected adolescents.[142,258] More study is needed in pediatrics to determine optimal dosing.

Protease Inhibitors

PIs are relatively small peptides that share structural features with the substrates for viral protease. These molecules bind to and inhibit viral protease, thereby preventing the virus from maturing into an infectious virion. These drugs are extremely potent in reducing plasma RNA viral loads. They exhibit a high barrier for development of drug resistance. As a class, they interact with the hepatic cytochrome P-450 enzyme system, and many induce their own metabolism. Thus, the potential for significant drug-drug interactions is considerable. Examples of such interactions include those with oral contraceptives, antituberculous drugs, antiepileptic drugs, some antihistamines, and methadone. Adverse events of use of PI include previously described metabolic complications (dyslipidemia, lipodystrophy, insulin resistance, gastrointestinal effects of nausea or diarrhea), higher pill burden than with NRTI- or NNRTI-based regimens, and poor palatability of liquid preparations. Current PIs approved for treatment of HIV infection include saquinavir, RTV, lopinavir (LPV)/RTV, indinavir, nelfinavir (NFV), atazanavir (ATV), fosamprenavir (FPV), tipranavir (TPV), and darunavir (DRV). Previously used amprenavir is no longer manufactured in the United States. Saquinavir (high pill burden, must be boosted with another PI owing to low bioavailability, limited pediatric pharmacokinetics data) and indinavir (unfavorable toxicity profile and limited pediatric pharmacokinetics data) are not generally used in pediatrics and will not be further discussed.

Ritonavir

Ritonavir is a potent inhibitor of the hepatic cytochrome P-450 3A4 isoenzyme and therefore can improve the bioavailability and prolong the elimination half-life of most coadministered PIs.[62] This PI has been used as a pharmacokinetic booster in low doses combined with indinavir, LPV, saquinavir, FPV, ATV, TPV, DRV, and TPV to enhance these PI serum levels. It is available in capsule form and as an oral solution. When RTV is used at lower doses as a pharmacokinetic booster of other PIs in adults, it is used as 100 mg twice daily or 200 mg once daily. Side effects include nausea and vomiting, paresthesias, hyperlipidemia, and lipodystrophy.

Atazanavir

Atazanavir is FDA approved for use in children age 6 and older and adolescents, is available in capsule form, and is recommended in the U.S. pediatric treatment guidelines for both treatment-naïve and treatment-experienced children and youth whose HIV virus is sensitive to ATV.[198] RTV-boosted ATV is generally preferred over unboosted ATV and is used once daily in combination with NRTIs. The safety profile is notable for less gastrointestinal upset and fewer lipid perturbations than other

*Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission: http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.

PIs, although lipid levels are higher when boosted with RTV. The most common toxicity of ATV is asymptomatic accumulation of indirect bilirubin due to inhibition of uridine glucuronosyl transferase 1A1; thus, ATV is not recommended for use in neonates. Efficacy when used in combination with NRTIs has been established in adolescents and in children.[22,135]

Darunavir

Darunavir boosted with RTV is FDA approved for use twice daily in combination with RTV in children aged 3 years and older as part of combination antiretroviral therapy. Several studies of treatment-experienced children given twice-daily DRV/RTV-based therapy has been shown to be well tolerated and efficacious.[14,126] Studies in treatment-naïve pediatric patients are ongoing. Diarrhea, vomiting, and rash are the most common side effects. The taste of the DRV liquid is better than the poor taste of the RTV needed for pharmacokinetic boosting; RTV liquid limits the palatability of this combination regimen.

Fosamprenavir

Fosamprenavir, the prodrug of amprenavir which is no longer manufactured, is approved and available in a pediatric liquid formulation and a tablet formulation, which must be given with food. FPV suspension is FDA approved for use in PI-naïve children as young as 4 weeks who were born at 38 weeks' gestation or later and have attained a postnatal age of 28 days and for use in pediatric patients aged 2 years or older.[84] The approval was based on two open-label studies in pediatric patients aged 2 to 18 years.[83] Overall, FPV is well tolerated and effective in suppressing viral load and increasing CD4 cell count; however, there is less pediatric experience with FPV than with LPV/RTV. The U.S. pediatric guideline recommends use only for children aged 6 months or older with RTV boost because unboosted FPV may result in low exposures and may select for mutations associated with resistance to darunavir.[198] FPV contains a sulfa moiety; rash is a side effect. Potential for cross sensitivity between FPV and other drugs in sulfonamide class is unknown.

Lopinavir/Ritonavir

Lopinavir/ritonavir (LPV/r) is a co-formulation of LPV and RTV. RTV acts as a pharmacologic enhancer rather than as an antiretroviral agent by inhibiting the metabolism of LPV and increasing LPV plasma concentration. It is available in tablet form (100 mg LPV/25 mg RTV; 200 mg LPV/50 mg RTV) and as an oral solution (80 mg LPV/20 mg RTV per milliliter; contains 42.4% alcohol by volume). LPV/r is widely used in pediatrics owing to efficacy, high genetic barrier to development of resistance, extensive experience in use in pediatrics, and availability in a liquid formulation. Currently, it is not recommended for use in neonates younger than 14 days of age or in premature infants younger than 42 weeks gestational age. Toxicities described in preterm infants include cardiac arrhythmias and dysfunction, lactic acidosis, acute renal failure, central nervous system depression, respiratory depression, and transient adrenal insufficiency.[119,239] Major toxicities are diarrhea, nausea, vomiting, headache, asthenia, and lipid abnormalities. The 42.4 percent alcohol content in this oral formulation leads to almost uniform symptoms of nausea or vomiting and often can be overcome with concomitant delivery of foods with strong tastes. Dosing compliance may be affected by taste and a caregiver's discomfort in delivery of this formulation.

Nelfinavir

Nelfinavir has been extensively studied in pediatric populations but, owing to lower potency, it is no longer widely used. NFV is not recommended for treatment in children aged younger than 2 years. It is available in tablet form. The most common side effects of NFV are diarrhea and lipodystrophy.

Tipranavir

Tipranavir is FDA approved for use as part of an RTV-boosted combination antiretroviral regimen in treatment-experienced children and adolescents age 2 to 18 years infected with HIV strains resistant to more than one PI. TPV is available as a capsule and as an oral solution. The boosting dose of RTV is higher than that for most PIs and is associated with more toxicity when used in combination, including nausea, diarrhea, vomiting, and hyperlipidemia. Also, TPV is associated with additional serious toxicity that limits its use, including hepatotoxicity, clinical hepatitis, and hepatic decompensation, including some fatalities; spontaneous bleeding in hemophiliacs; and increased risk for intracranial hemorrhage. TPV should be used with caution in patients who may be at risk for increased bleeding from trauma, surgery, or other medical conditions. TPV is associated with rash and contains a sulfa moiety.

Integrase Inhibitors

Joining of the viral genome and the host's chromosomal DNA is the hallmark of a retroviral infection. Integrase, the enzyme that catalyzes this step of the HIV life cycle, is an effective therapeutic target. On binding to specific sequences in viral DNA, HIV-1 integrase catalyzes two consecutive steps during integration: 3′ processing, endonucleolytic cleavage of the 3′ ends of the viral cDNA, and strand transfer, ligation of the viral 3′-OH cDNA ends to the 5′-DNA phosphate of an acceptor DNA (host chromosome).[208] Several compounds have been developed to block integration at different steps. Compounds that inhibit integrase at its catalytic site with an effect on strand transfer, known as strand-transfer inhibitors—raltegravir (RAL) and elvitegravir—are approved for use, and second-generation drugs are under development. Elvitegravir is available in a combination "quad" pill containing elvitegravir, a novel boosting agent with no antiviral activity named cobicistat, tenofovir DF, and emtricitabine. Elvitegravir has not been studied in pediatrics. Allosteric integrase inhibitors intended to interfere

with the integrase-LEDGF/p75 interaction are being designed; these new inhibitors, called LEDGINs, have an effect on 3′ processing and strand transfer.[163]

Raltegravir

Raltegravir is a first-in-its-class oral integrase inhibitor. RAL is available as a film-coated tablet or chewable tablet that are not interchangeable and is approved for use in 2- to 18-year-old patients. It is currently used as a preferred treatment in adults and for treatment-experienced pediatric patients. IMPAACT P1066 is a phase I/II open label multicenter trial to evaluate the pharmacokinetic profile, safety, tolerability, and efficacy of RAL in HIV-infected children. This study enrolled 126 treatment-experienced children and adolescents 2 to 18 years of age. Subjects received either the 400-mg film-coated tablet formulation (ages 6 to 18 years) or the chewable tablet formulation (ages 2 to <12 years). Of the 96 subjects studied at optimal dosing at week 24, 54 percent achieved HIV RNA less than 50 copies/mL; 72 percent achieved HIV RNA less than 400 copies/mL or 1 $\log_{10}$ or more HIV RNA drop from baseline. The mean CD4 count (percent) increase from baseline to week 24 was 119 cells/mm^3 (3.8%).[84] Side effects include rash, including Stevens-Johnson syndrome, hypersensitivity reaction, and toxic epidermal necrolysis; nausea; diarrhea; headache; fever; creatine phosphokinase elevation; muscle weakness; and rhabdomyolysis.

Supportive Treatment

Preventive measures applicable to immunodeficient individuals should be considered for the HIV-infected child. Examples include measles, influenza, and varicella prophylaxis after exposure. Additionally, routine childhood health care preventive measures, including adequate nutritional support, age and developmentally appropriate stimulation, dental and skin hygiene, and immunizations, should be offered to children with HIV infection or AIDS. The live viral measles-mumps-rubella vaccine is recommended for all HIV-infected children except those who are severely immunosuppressed ($CD4^+$ T-lymphocyte percentages <15 percent). The first dose is recommended as soon as possible after the first birthday, and the second dose should be given as soon as 1 month after the first dose to ensure early seroconversion. Varicella vaccine is recommended for HIV-infected children without significant immune compromise as well. Eligible children should receive two doses 3 months apart, with the first dose administered as soon as possible after the first birthday. Immunization for pneumococcus includes routine immunization with the conjugate pneumococcal vaccine. In addition, children aged 2 years or older also should receive the 23-valent pneumococcal polysaccharide vaccine 2 or more months after their last PCV dose. Influenza vaccine should be administered yearly starting at age 6 months. Patients with HIV infection are at increased risk for acquiring meningococcal disease. Meningococcal conjugate vaccine (MCV4) is preferred in patients aged 11 to 55 years and now is approved for use in 2- to 10-year-old children.[41]

The newer quadrivalent human papillomavirus (HPV) vaccine, Gardasil, is a vaccine consisting of noninfectious HPV-like particles. It is now recommended in a three-dose schedule for girls and boys aged 11 to 12 years, although it may be administered from as young as 9 years to 26 years.[41,48,213]

Immune-Based Therapies

The cardinal immune manifestation of HIV infection is the development of progressive $CD4^+$ lymphopenia with resultant clinical disease associated with severe immunodeficiency. Therefore, replacement of the $CD4^+$ T-lymphocyte pool is a logical approach for immune-based therapies.[235] Immune modulators have been assessed as single therapeutic agents or in combination regimens in adult clinical trials. An immune-based therapy, IL-2, demonstrated $CD4^+$ T-cell count increases but no clinical benefit in two large randomized studies.[1] Currently, other immune-based therapies (e.g., gene therapies, growth hormone, IL-7) are under investigation and should only be used in the treatment of HIV in the context of a clinical trial. IFN-α initially was studied as a therapeutic agent for Kaposi sarcoma with good response rates, but significant side effects limit its use in HIV infection. The utility of immune-based therapies in pediatric or adolescent HIV infection may include their use in treatment of concomitant chronic hepatitis B and C virus infection or HIV-associated immune thrombocytopenia.[54]

Chronic immune activation has been associated with poor outcomes—both AIDS-defining and non–AIDS-defining clinical events—in addition to persistent $CD4^+$ T-cell depletion. The cause of chronic immune activation in well-controlled HIV infection in the era of combined antiretroviral therapy, aside from coinfecting pathogens, may include low-level viral replication and microbial translocation. Therapeutic interventions that target immune activation including interfering directly with activation and inflammatory pathways are being developed.[30]

Vaccines

Goals for the development of an HIV vaccine include prevention of persistent HIV infection, prevention of severe clinical disease (AIDS), and therapeutic immunization to control HIV infection. The first candidate preventive vaccines tested in human trials in the late 1980s were based on the HIV-1 envelope glycoproteins gp120 or gp160, an approach aiming to induce neutralizing antibodies. The major limitations of this approach were lack of neutralizing antibody activity against a diversity of primary patient isolates of HIV-1 because of genetic variability and structural complexity of the HIV-1 envelope glycoproteins and absence of envelope-specific cytotoxic T-lymphocyte responses.[102,132,166] Currently, more than 30 HIV vaccines have been tested in human trials.[220,231] The vaccines had produced disappointing results until a recently completed HIV-1 vaccine efficacy trial in Thailand (RV144) showed that priming with a recombinant canarypox vector (vCP1521) and boosting with this

vector plus bivalent gp120 protein (AIDSVAX B/E) provided partial protection against HIV infection in heterosexual men and women.[219] Subsequent study has shown that the peak neutralizing antibody response (Nab) in RV144 was substantially weaker than the peak Nab response in a study of AIDSVAX B/E in injection drug users that showed no efficacy.[183] The results suggest either that weak neutralizing antibody responses can be partially protective against HIV in low-risk heterosexual populations or that the efficacy seen in RV144 was mediated by other immune responses, either alone or in combination with neutralizing antibodies.

Antigenically diverse viruses including HIV do contain highly conserved exposed sites, usually associated with function, which can be targeted by broadly neutralizing antibodies, and advances in the field have enabled increasing numbers to be identified.[29] Molecular characterizations of the antibodies and of the HIV antigens that they recognize offer new hope for the discovery of new vaccines and drugs.

PROGNOSIS

Mortality and morbidity rates for children and adolescents with HIV infection have declined dramatically. Effective efforts directed toward early diagnosis have allowed for earlier administration of primary viral therapy to prevent HIV transmission and prompt treatment and prevention of opportunistic infections. However, owing to untreated HIV disease, transmission of HIV is ongoing and opportunistic disease and death still affect HIV-infected pediatric populations worldwide. Public health strategies to slow these outcomes are continuing. Pediatric research should continue to address therapeutic interventions that allow for care of now chronically infected children and adolescents who are surviving and striving to contribute to their world. In addition, careful follow-up of HIV-exposed and neonatal antiviral-exposed individuals should be a priority in an effort to investigate long-term outcomes in individuals exposed to mother-to-child prevention regimens.

For developing nations, resources and research to stem the continued tide of HIV infection are of paramount importance. Efforts must be made to reduce global perinatal transmission rates further and to define HIV detection methods that allow earliest intervention in affected children. Effective vaccine development clearly would have a significant and worldwide impact on adults and children exposed to HIV.

Research and prevention initiatives must now be coupled with socioeconomic and political efforts to find the will to sustain a global impact on HIV transmission and treatment. Duplication of the achieved successes in preventing perinatal transmission in industrialized countries is needed desperately in sub-Saharan Africa, East Asia, and Central America, where competing medical needs limit available local funding for prevention of HIV transmission and for HIV treatment. When viewed from this perspective, placing the highest priority on global confrontation of this epidemic that threatens the future of our children is imperative.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abrams D, Levy Y, Losso MH, et al. Interleukin-2 therapy in patients with HIV infection. *N Engl J Med* 2009;**361**:1548–59.
5. Al-Husaini AM. Role of placenta in the vertical transmission of human immunodeficiency virus. *J Perinatol* 2008;**29**:331–6.
6. Altfeld M, Fadda L, Frleta D, et al. DCs and NK cells: critical effectors in the immune response to HIV-1. *Nat Rev Immunol* 2011;**11**:176–86.
7. Arkin LM, Cox CM, Kovarik CL. Kaposi's sarcoma in the pediatric population: the critical need for a tissue diagnosis. *Pediatr Infect Dis J* 2009;**28**:426–8.
8. Bae WH, Wester C, Smeaton LM, et al. Hematologic and hepatic toxicities associated with antenatal and postnatal exposure to maternal highly active antiretroviral therapy among infants. *AIDS* 2008;**22**:1633.
14. Blanche S, Bologna R, Cahn P, et al. Pharmacokinetics, safety and efficacy of darunavir/ritonavir in treatment-experienced children and adolescents. *AIDS* 2009;**23**:2005–13.
17. Borges-Almeida E, Milanez HM, Vilela MM, et al. The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected newborns. *BMC Infect Dis* 2011;**11**:38.
18. Branson B. The future of HIV testing. *J Acquir Immune Defic Syndr* 2010;**55**:S102–5.
20. Brichacek B, Vanpouille C, Kiselyeva Y, et al. Contrasting roles for TLR ligands in HIV-1 pathogenesis. *PloS One* 2010;**5**:e12831.
22. Bristol-Myers Squibb. *Reyataz Package Insert*. 2012; Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021567s028lbl.pdf.
23. Briz V, Palladino C, Navarro M, et al. Etravirine-based highly active antiretroviral therapy in HIV-1-infected paediatric patients. *HIV Med* 2011;**12**:442–6.
29. Burton DR, Poignard P, Stanfield RL, et al. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. *Science* 2012;**6091**:183–6.
30. Butler SL, Valdez, H, Westby M, et al. Disease-modifying therapeutic concepts for HIV in the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2011;**58**:297–303.
44. Centers for Disease Control and Prevention. *Fact sheet: estimates of new HIV infections in the United States, 2006-2009*. Atlanta: Centers for Disease Control and Prevention; August 2011.
45. Centers for Disease Control and Prevention. *High impact HIV prevention: CDC's approach to reducing HIV infections in the United States*. Atlanta: Centers for Disease Control and Prevention; August 2011.
46. Centers for Disease Control and Prevention. Premastication of food by caregivers of HIV-exposed children: nine U.S. sites, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:273–5.
47. Centers for Disease Control and Prevention. *HIV surveillance report*, 2009. Available at http://www.cdc.gov/hiv/topics/surveillance/resources/reports/. Published February 2011. Accessed October 28, 2012.
48. Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 0-18 years—United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**:1–4.
54. Chiappini E, Galli L, Tovo PA, et al. Five-year follow-up of children with perinatal HIV-1 infection receiving early highly active antiretroviral therapy. *BMC Infect Dis* 2009;**9**:140.
57. Cinti SK, Gandhi T, Riddell J. Non-AIDS-defining cancers: should antiretroviral therapy be initiated earlier? *AIDS Read* 2008;**18**:18–20.
64. Da LT, Quan JM, Wu YD. Understanding the binding mode and function of BMS-488043 against HIV-1 viral entry. *Proteins* 2011;**79**:1810–19.
77. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. *Int J Cancer* 2008;**123**:187–94.
83. Food and Drug Administration. *Lexiva FDA Label*, 2009. Available at www.accessdata.fda.gov/drugsatfda_docs/label/2009/022145s001lbl.pdf.
84. Food and Drug Administration. *Isentress FDA Label*, 2012. Available at www.accessdata.fda.gov/drugsatfda_docs/label/2012/021548s028,022116s012lbl.pdf.

85. Foster SB, Lu M, Glaze DG, et al. Associations of cytokines, sleep patterns, and neurocognitive function in youth with HIV infection. *Clin Immunol* 2012;**144**:13–23.
86. Foster SB, Lu M, Thompson B, et al. Association between HLA inheritance and asthma medication use in HIV-positive children. *AIDS* 2010;**24**:2133–35.
87. Foster SB, McIntosh K, Thompson B, et al. Increased incidence of asthma in HIV-infected children treated with highly active antiretroviral therapy in the National Institutes of Health Women and Infants Transmission Study. *J Allergy Clin Immunol* 2008;**122**:159–65.
95. Gaur AH, Dominguez KL, Kalish ML, et al. Practice of feeding premasticated food to infants: a potential risk factor for HIV transmission. *Pediatrics* 2009;**124**:658–66.
97. Ghosn J, Deveau C, Chaix ML, et al. Despite being highly diverse, immunovirological status strongly correlates with clinical symptoms during primary HIV-1 infection: a cross-sectional study based on 674 patients enrolled in the ANRS CO 06 PRIMO cohort. *J Antimicrob Chemother* 2010;**65**:741–8.
101. Govender R, Eley B, Walker K, et al. Neurologic and neurobehavioral sequelae in children with HIV-1 infection. *J Child Neurol* 2011;**26**:1355–64.
111. Gutin F, Butt A,Alame W, et al. Asthma in immune-competent children with human immunodeficiency virus. *Ann Allergy Asthma Immunol* 2009;**102**:438.
116. Hemelaar J, Gouws E, Ghys P, et al. WHO-UNAIDS. Network for HIV isolation and characterization. Global trends in molecular epidemiology of HIV during 2000-2007. *AIDS* 2011;**25**:678–89.
119. Holgate SL, Rabie H, Smith P, et al. Trough lopinavir concentrations in preterm HIV-infected infants. *Pediatr Infect Dis J* 2012;**31**:602–4.
126. Janssen Pharmaceuticals. *Prezista Package Insert*, 2012. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021976s022lbl.pdf.
127. Joint United Nations Programme on HIV/AIDS (UNAIDS). Global report: UNAIDS report on the global AIDS epidemic. 2012.
129. Kanjanasut N, Phanthumchinda K, Bhidayasiri R. HIV-related opsoclonus-myoclonus-ataxia syndrome: report on two cases. *Clin Neurol Neurosurg* 2010;**112**:572–4.
130. Kapetanovic S, Aaron L, Montepiedra G, et al. T-cell activation and neurodevelopmental outcomes in perinatally HIV-infected children. *AIDS* 2012;**26**:959–69.
135. Kiser JJ, Rutstein RM, Samson P, et al. Atazanavir and atazanavir/ritonavir pharmacokinetics in HIV-infected infants, children, and adolescents. *AIDS* 2011;**25**:1489–96.
142. Konigs C, Feiterna-Sperling C, Esposito S, et al. Pharmacokinetics and short-term safety and tolerability of etravirine in treatment-experienced HIV-1-infected children and adolescents. *AIDS* 2012;**26**:447–55.
143. Koppensteiner H, Brack-Werner R, Schindler M. Macrophages and their relevance in human immunodeficiency virus type 1 infection. *Retrovirology* 2012;**9**:82.
148. Kovacs A. Early immune activation predicts central nervous system disease in HIV-infected infants: implications for early treatment. *Clin Infect Dis* 2009;**48**:347–9.
152. Laurent C, Meggetto F, Brousset P. Human herpesvirus 8 infections in patients with immunodeficiencies. *Hum Pathol* 2008;**39**:983–93.
156. Lipshultz SE, Shearer WT, Thompson B, et al. Cardiac effects of antiretroviral therapy in HIV-negative infants born to HIV-positive mothers: NHLBI CHAART-1 (National Heart, Lung, and Blood Institute Cardiovascular Status of HAART therapy in HIV-exposed infants and children cohort study). *J Am Coll Cardiol* 2011;**57**:76–85.
163. Malet I, Calvez V, Marcelin AG. The future of integrase inhibitors of HIV-1. *Curr Opin Virol* 2012;**2**:580–7.
169. McCulloch MI, Ray PE. Kidney disease in HIV-positive children. *Semin Nephrol* 2008;**28**:585–94.
172. Mekmullica J, Brouwers P, Charurat M, et al. Early immunological predictors of neurodevelopmental outcomes in HIV-infected children. *Clin Infect Dis* 2009;**48**:338–46.
175. Miller TL, Agostoni C, Duggan C, et al. Gastrointestinal and nutritional complications of human immunodeficiency virus infection. *J Pediatr Gastroenterol Nutr* 2008;**47**:247–53.
180. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**(RR-11):1–166.
183. Montefiori DC, Karnasuta C, Huang Y. Magnitude and breadth of neutralizing antibody response in the RV 144 and Vax003 HIV-1 vaccine efficacy trials. *J Infect Dis* 2012;**206**:431–41.
188. Nachman S, Chernoff M, Williams P, et al. Human immunodeficiency virus disease severity, psychiatric symptoms, and functional outcomes in perinatally infected youth. *Arch Pediatr Adolesc Med* 2012;**166**:528–35.
191. Nielsen-Saines K, Watts DH, Veloso VG, et al. Three postpartum antiretroviral regimens to prevent intrapartum HIV infection. *N Engl J Med* 2012;**366**:2368–79. Available at http://www.ncbi.nlm.nih.gov/pubmed/22716975.
193. Nysasulo P, Cohen C, De Gouveia L, et al. *Pediatr Infect Dis J* 2011;**30**:1075–80.
195. Omonuwa TS, Goforth HW, Preud'homme X, et al. The pharmacologic management of insomnia in patients with HIV. *J Clin Sleep Med* 2009;**5**:251–62.
198. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. *Guidelines for the use of antiretroviral agents in Pediatric HIV infection*. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. Accessed November 25, 2012.
199. Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. *Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States*. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf. Accessed November 11, 2012.
204. Pirrone V, Passic S, Wigdahl B, et al. Application and removal of polyanionic microbicide compounds enhances subsequent infection by HIV-1. *Virol J* 2012;**9**:33.
209. Porter PC, Roberts L, Fields A, et al. Necessary and sufficient role for T helper cells to prevent fungal dissemination in allergic lung disease. *Infect Immun* 2011;**79**:4459–71.
210. Porter PC, Susarla SC, Polikepahad S, et al. Link between allergic asthma and airway mucosal infection suggested by proteinase-secreting household fungi. *Mucosal Immun* 2009;**2**:504–17.
211. Ray PE, Hu CA. Advances in our understanding of the pathogenesis of HIV-1 associated nephropathy in children. *Future Virol* 2011;**6**:883–94.
213. Recommendations on the use of quadrivalent human papillomavirus vaccine in males—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1705–8.
215. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 2009;**361**:2209–20.
218. Rodriguez-Novoa S, Labarga P, D'Avolio A, et al. Impairment in kidney tubular function in patients receiving tenofovir is associated with higher tenofovir plasma concentrations. *AIDS* 2010;**24**:1064–6.
220. Ross A, Bråve A, Scarlatti G, et al. Progress towards development of an HIV vaccine: report of the AIDS Vaccine 2009 Conference. *Lancet Infect Dis* 2010;**10**:305–16.
221. Ross AC, Storer N, O'Riordan MA, et al. Longitudinal changes in carotid intima-media thickness and cardiovascular risk factors in HIV-infected children and young adults compared with healthy controls. *Pediatr Infect Dis J* 2010;**29**:634–8.
229. Schouten J, Cinque P,Gisslen M, et al. HIV-1 infection and cognitive impairment in the cART era: a review. *AIDS* 2011;**25**:561–75.
238. Siberry GK, Leister E, Jacobson DL, et al. Increased risk of asthma and atopic dermatitis in perinatally HIV-infected children and adolescents. *Clin Immunol* 2012;**142**:201–8.
239. Simon A, Warszawski J, Kariyawasam D, et al. Association of prenatal and postnatal exposure to lopinavir-ritonavir and adrenal dysfunction among uninfected infants of HIV-infected mothers. *JAMA* 2011;**306**:70–7.

258. Thuret I, Chaix ML, Tamalet C, et al. Raltegravir, etravirine and r-darunavir combination in adolescents with multidrug-resistant virus. *AIDS* 2009;**23**:2364–6.
259. Toro AA, Altemani AM, da Silva MT, et al. Epstein-Barr virus (EBV) gene expression in interstitial pneumonitis in Brazilian human immunodeficiency virus-1–infected children: is EBV associated or not? *Pediatr Dev Pathol* 2010;**13**:184–91.
267. Vernazza P, Wang C, Pozniak A, et al. Efficacy and safety of lersivirine (UK-453,061) versus efavirenz in antiretroviral treatment-naïve HIV-1-infected patients: week 48 primary analysis results from an ongoing, multicenter, randomized, double-blind, phase IIb trial. *J Acquir Immune Defic Syndr* 2013;**62**:171–9.
274. Wilson CM, Wright PF, Safrit JT, et al. Epidemiology of HIV infection and risk in adolescents and youth. *J Acquir Immune Defic Syndr* 2010;**54**(Suppl. 1):S5–6.
276. Wood SM, Shah SS, Steenhoff AP, et al. The impact of AIDS diagnoses on long-term neurocognitive and psychiatric outcomes of surviving adolescents with perinatally acquired HIV. *AIDS* 2009;**23**:1859–65.
278. World Health Organization. AIDS epidemic update: 2009. Available at http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf. Accessed November 1, 2012.
279. Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. *J Neuroimune Pharmacol* 2009;**4**:430–47.
281. Zar HJ. Chronic lung disease in human immunodeficiency virus infected children. *Pediatr Pulmonol* 2008;**43**:1–10.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 13
PRION-RELATED DISEASES

CHAPTER 192

Transmissible Spongiform Encephalopathies (Creutzfeldt-Jakob Disease, Gerstmann-Sträussler-Scheinker Disease, Kuru, Fatal Familial Insomnia, New Variant Creutzfeldt-Jakob Disease, Sporadic Fatal Insomnia)

William J. Britt • Gail J. Harrison

The group of neurodegenerative diseases classified as transmissible spongiform encephalopathies (TSE) represents a subset within a group of diseases that also have been termed *infectious amyloidoses* or *cerebral proteopathies*.[196,246] Although it has been speculated that all neurodegenerative diseases may be prion diseases, the TSE[93,179] are characterized by progressive cortical dysfunction linked to the accumulation of insoluble, proteinaceous aggregates in the central nervous system (CNS) (Table 192-1). The protein aggregates characteristic of

REFERENCES

1. Abrams D, Levy Y, Losso MH, et al. Interleukin-2 therapy in patients with HIV infection. *N Engl J Med* 2009;**361**:1548–59.
2. Abrams EJ, Weedon J, Steketee RW, et al. Association of human immunodeficiency virus (HIV) load early in life with disease progression among HIV-infected infants: New York City Perinatal HIV Transmission Collaborative Study Group. *J Infect Dis* 1998;**178**:101–8.
3. Abrams EJ, Wiener J, Carter R, et al. Maternal health factors and early pediatric antiretroviral therapy influence the rate of perinatal HIV-1 disease progression in children. *AIDS* 2003;**17**:867–77.
4. Al-Harthi L, Guilbert LJ, Hoxie JA, et al. Trophoblasts are productively infected by CD4-independent isolate of HIV type 1. *AIDS Res Hum Retroviruses* 2002;**18**:13–17.
5. Al-Husaini AM. Role of placenta in the vertical transmission of human immunodeficiency virus. *J Perinatol* 2008;**29**:331–6.
6. Altfeld M, Fadda L, Frleta D, et al. DCs and NK cells: critical effectors in the immune response to HIV-1. *Nat Rev Immunol* 2011;**11**:176–86.
7. Arkin LM, Cox CM, Kovarik CL. Kaposi's sarcoma in the pediatric population: the critical need for a tissue diagnosis. *Pediatr Infect Dis J* 2009;**28**:426–8.
8. Bae WH, Wester C, Smeaton LM, et al. Hematologic and hepatic toxicities associated with antenatal and postnatal exposure to maternal highly active antiretroviral therapy among infants. *AIDS* 2008;**22**:1633.
9. Bakshi SS, Alvarez D, Hilfer CL, et al. Tuberculosis in human immunodeficiency virus–infected children: a family infection. *Am J Dis Child* 1993;**147**:320–4.
10. Barret B, Tardieu M, Rustin P, et al. Persistent mitochondrial dysfunction on HIV-1–exposed but uninfected infants: clinical screening in a large prospective cohort. *AIDS* 2003;**17**:1769–85.
11. Belman AL, Diamond G, Dickson D, et al. Pediatric acquired immunodeficiency syndrome: neurologic syndromes. *Am J Dis Child* 1988;**142**:29–35.
12. Biggar RJ, Frisch M, Goedert JJ. Risk of cancer in children with AIDS: AIDS-Cancer Match Registry Study Group. *JAMA* 2000;**284**:2593–4.
13. Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med* 2007;**357**:1487–95.
14. Blanche S, Bologna R, Cahn P, et al. Pharmacokinetics, safety and efficacy of darunavir/ritonavir in treatment-experienced children and adolescents. *AIDS* 2009;**23**:2005–13.
15. Blanche S, Calvez T, Rouzioux C, et al. Randomized study of two doses of didanosine in children infected with human immunodeficiency virus. *J Pediatr* 1993;**122**:966–73.
16. Bonnet D, Aggoun Y, Szezepanski I, et al. Arterial stiffness and endothelial dysfunction in HIV-infected children. *AIDS* 2004; **18**:1037–41.
17. Borges-Almeida E, Milanez HM, Vilela MM, et al. The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected newborns. *BMC Infect Dis* 2011;**11**:38.
18. Branson B. The future of HIV testing. *J Acquir Immune Defic Syndr* 2010;**55**:S102–5.
19. Branson RM, Handsfield HH, Lampe JA, et al. Revised recommendations for HIV testing of adults, adolescents, and pregnant women in health-care settings. *MMWR Recomm Rep* 2006; **55**:1–17.
20. Brichacek B, Vanpouille C, Kiselyeva Y, et al. Contrasting roles for TLR ligands in HIV-1 pathogenesis. *PloS One* 2010;**5**:e12831.
21. Brigitta UM, Jacobsen F, Butler KM, et al. Combination treatment with azidothymidine and granulocyte colony-stimulating factor in children with human immunodeficiency virus infection. *J Pediatr* 1992;**121**:797–802.
22. Bristol-Myers Squibb. *Reyataz Package Insert*, 2012. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021567s028lbl.pdf.
23. Briz V, Palladino C, Navarro M, et al. Etravirine-based highly active antiretroviral therapy in HIV-1-infected paediatric patients. *HIV Med* 2011;**12**:442–6.
24. Brogly S, Williams P, Seage GR, et al. Antiretroviral treatment in pediatric HIV infection in the United States. *JAMA* 2005; **293**:2213–20.
25. Brossard Y, Aubin JT, Madnelbrot L, et al. Frequency of early in utero HIV-1 infection: a blind DNA polymerase chain reaction study on 100 fetal thymuses. *AIDS* 1995;**9**:359–66.
26. Brouwers P, Belman A, Epstein LG. Central nervous system involvement: manifestations, evaluation and pathogenesis. In: Pizzo PA, Wilfert CM, editors. *Pediatric AIDS: the challenge of HIV infection in infants, children, and adolescents*. Baltimore: Williams & Wilkins; 1994. p. 433–55.
27. Bryson YJ, Luzuriaga K, Sullivan JL, et al. Proposed definitions for in utero versus intrapartum transmission of HIV-1. *N Engl J Med* 1992;**327**:1246–7.
28. Bulterys M, Jamieson DJ, O'Sullivan MJ, et al. Rapid HIV-1 testing during labor: a multicenter study. *JAMA* 2004;**292**:219–23.
29. Burton DR, Poignard P, Stanfield RL, et al. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. *Science* 2012;**6091**:183–6.
30. Butler SL, Valdez, H, Westby M, et al. Disease-modifying therapeutic concepts for HIV in the era of highly active antiretroviral therapy. *J Acquir Immune Defic Syndr* 2011;**58**:297–303.
31. Cadman J. Efavirenz pregnancy warning. *GMHC Treat Issues* 1998;**12**:12.
32. Canani RB, Ruotolo S, Buccigrossi V, et al. Zinc fights diarrhoea in HIV-1 infected children: in-vitro evidence to link clinical data and pathophysiological mechanisms. *AIDS* 2007;**21**:108–10.
33. Carter RJ, Weiner J, Abrams EJ, et al. Dyslipidemia among perinatally HIV-infected children enrolled in the PACTS-HOPE cohort, 1999-2004: a longitudinal analysis. *J Acquir Immune Defic Syndr* 2006;**41**:453–60.
34. Centers for Disease Control and Prevention. Guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for children infected with human immunodeficiency virus. *MMWR Recomm Rep* 1991;**40**(RR-2):1–13.
35. Centers for Disease Control and Prevention. Recommendations on prophylaxis and therapy for disseminated *Mycobacterium avium* complex for adults and adolescents infected with human immunodeficiency virus. U.S. Public Health Service Task Force on prophylaxis and therapy for *Mycobacterium avium* complex. *MMWR Recomm Rep* 1993;**42**(RR-9):14–20.
36. Centers for Disease Control and Prevention. 1994 Revised classification system for human immunodeficiency virus infection in children less than 13 years of age. *MMWR Morb Mortal Wkly Rep* 1994;**43**:1–17.
37. Centers for Disease Control and Prevention. 1995 revised guidelines for prophylaxis against *Pneumocystis carinii* pneumonia for children infected with or perinatally exposed to human immunodeficiency virus: National Pediatric and Family HIV Resource Center and National Center for Infectious Diseases, Centers for Disease Control and Prevention. *MMWR Recomm Rep* 1995;**44**:1–11.
38. Centers for Disease Control and Prevention. Acquired rifamycin resistance in persons with advanced HIV disease being treated for active tuberculosis with intermittent rifamycin based regimens. *MMWR Morb Mortal Wkly Rep* 2002;**51**:214–15.
39. Centers for Disease Control and Prevention. Guidelines for preventing opportunistic infections among HIV-infected persons–2002: Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *MMWR Recomm Rep* 2002;**51**(RR-8):1–52.
40. Centers for Disease Control and Prevention. New strategies for a changing epidemic: United States, 2003. *MMWR Morb Mortal Wkly Rep* 2003;**52**:329–32.
41. Centers for Disease Control and Prevention. Notice to Readers. Revised recommendations of the Advisory Committee on Immunization Practices to vaccinate all persons aged 11-18 years with meningococcal conjugate vaccine. *MMWR Morb Mortal Wkly Rep* 2007;**56**:794–5.
42. Centers for Disease Control and Prevention. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007;**56**:1–40.
43. Centers for Disease Control and Prevention. Trends in tuberculosis incidence—United States. *MMWR Morb Mortal Wkly Rep* 2007;**57**:281–5.
44. Centers for Disease Control and Prevention. *Fact Sheet: estimates of new HIV infections in the United States, 2006-2009*. Atlanta: Centers for Disease Control and Prevention; August 2011.

45. Centers for Disease Control and Prevention. *High impact HIV prevention: CDC's approach to reducing HIV infections in the United States*. Atlanta: Centers for Disease Control and Prevention; August 2011.
46. Centers for Disease Control and Prevention. Premastication of food by caregivers of HIV-exposed children: nine U.S. sites, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:273–5.
47. Centers for Disease Control and Prevention. *HIV surveillance report*, 2009. Available at hhtp://www.cdc.gov/hiv/topics/surveillance/resources/reports/. Published February 2011. Accessed October 28, 2012.
48. Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 0-18 years—United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**:1–4.
49. Cervaix A, Nicolas J, Portales P, et al. Response to treatment and disease progression linked to $CD4^+$ T cell surface CC chemokine receptor 5 density in human immunodeficiency virus type 1 vertical infection. *J Infect Dis* 2002;**185**:1055–61.
50. Cervia JS, Smith MA. Enfuvirtide (T-20): a novel human immunodeficiency virus type 1 fusion inhibitor. *Clin Infect Dis* 2003;**37**:1102–6.
51. Chakraborty R, Uy CS, Oleske JM, et al. Persistent non-gastrointestinal metabolic acidosis in pediatric HIV-1 infection. *AIDS* 2003;**17**:673–7.
52. Chandwani S, Kaul A, Bebenroth D, et al. Cytomegalovirus infection in human immunodeficiency virus type 1–infected children. *Pediatr Infect Dis J* 1996;**15**:310–14.
53. Chen K, Huang J, Zhang C, et al. Alpha interferon potently enhances the anti-human immunodeficiency virus type-1 activity of APOBEC3G in resting primary CD4 T cells. *J Virol* 2006;**80**:7645–57.
54. Chiappini E, Galli L, Tovo PA, et al. Five-year follow-up of children with perinatal HIV-1 infection receiving early highly active antiretroviral therapy. *BMC Infect Dis* 2009;**9**:140.
55. Chinen J, Shearer WT. Molecular virology and immunology of HIV infection. *J Allergy Clin Immunol* 2002;**110**:189–98.
56. Churchill MF, Gorry PR, Cowley D, et al. Use of laser capture microdissection to detect integrated HIV-1 DNA in macrophages and astrocytes from autopsy brain tissues. *J Neurovirol* 2006;**12**:146–52.
57. Cinti SK, Gandhi T, Riddell J. Non–AIDS-defining cancers: should antiretroviral therapy be initiated earlier? *AIDS Read* 2008;**18**:18–20.
58. Clavel F, Guetard D, Brun-Vezinet F, et al. Isolation of a new human retrovirus from West African patients with AIDS. *Science* 1986;**233**:343–6.
59. Clerici M, Galli M, Bosis S, et al. Immunoendocrinologic abnormalities in human immunodeficiency virus infection. *Ann N Y Acad Sci* 2000;**917**:956–61.
60. Committee on Obstetric Practice. ACOG committee opinion scheduled cesarean delivery and the prevention of vertical transmission of HIV infection: Number 234, May 2000 (replaces number 219, August 1999). *Int J Gynaecol Obstet* 2001;**73**:279–81.
61. Connor EM, Sperling RS, Gelber R, et al. Reduction of maternal-infant transmission of human immunodeficiency virus type 1 with zidovudine treatment. Pediatric AIDS Clinical Trials Group Protocol 076 Study Group. *N Engl J Med* 1994;**331**:1173–80.
62. Cooper CL, van Heeswijk RPG, Gallicano K, et al. A review of low-dose ritonavir in protease inhibitor combination therapy. *Clin Infect Dis* 2003;**36**:1585–92.
63. Cooper ER, Charurat M, Mofenson L, et al. Combination antiretroviral strategies for the treatment of pregnant HIV-1–infected women and prevention of perinatal HIV-1 transmission. *J Acquir Immune Defic Syndr* 2002;**29**:484–94.
64. Da LT, Quan JM, Wu YD. Understanding the binding mode and function of BMS-488043 against HIV-1 viral entry. *Proteins* 2011;**79**:1810–19.
65. Davis C, Shearer WT. Diagnosis and management of HIV drug hypersensitivity. *J Allergy Clin Immunol* 2008;**121**:826–32.
66. Dandekar S. Pathogenesis of HIV in the gastrointestinal tract. *Curr HIV/AIDS Rep* 2007;**4**:10–15.
67. Demmler GJ, Taber LH. Virology of HIV-1. *Semin Pediatr Infect Dis* 1990;**1**:17–20.
68. Denny TN, Yogev R, Gelman R, et al. Lymphocyte subsets in healthy children during the first 5 years of life. *JAMA* 1992;**267**:1484–8.
69. Derdeyn CA, Silvestri G. Viral and host factors in the pathogenesis of HIV infection. *Curr Opin Immunol* 2005;**17**:366–73.
70. Dodd RY, Notari EP 4th, Stramer SL. Current prevalence and incidence of infectious disease markers and estimated window-period risk in the American Red Cross blood donor population. *Transfusion* 2002;**42**:975–9.
71. Doherty T, Chopra M, Jackson D, et al. Effectiveness of the WHO/UNICEF guidelines on infant feeding for HIV-positive women: results from a prospective cohort study in South Africa. *AIDS* 2007;**21**:1791–7.
72. Dominguez K. Management of HIV infected children in the home and institutional settings: care of children and infections control in schools, day care, hospital settings, home, foster care and adoption. *Pediatr Clin North Am* 2000;**47**:203–9.
73. Dominguez K, Bertolli J, Fowler M, et al. Lack of definitive severe mitochondrial signs and symptoms among deceased HIV-uninfected and HIV-indeterminate children ≤5 years of age, Pediatric Spectrum of HIV Disease Project (PSD), USA. *Ann N Y Acad Sci* 2000;**918**:236–46.
74. D'Ubalo C, Pezzotti P, Rezza G, et al. Association between HIV-1 infection and miscarriage: a retrospective study. DIANAIDS (Diagnosi Iniziale Anomalie Neoplastiche AIDS) Collaborative Study Group. *AIDS* 1998;**12**:1087–93.
75. Dunn DT, Newell ML, Ades AE, et al. Risk of human immunodeficiency virus type 1 transmission through breastfeeding. *Lancet* 1992;**340**:585–8.
76. Eksborg S. The pharmacokinetics of antiviral therapy in pediatric patients. *Herpes* 2003;**10**:66–71.
77. Engels EA, Biggar RJ, Hall HI, et al. Cancer risk in people infected with human immunodeficiency virus in the United States. *Int J Cancer* 2008;**123**:187–94.
78. European Collaborative Study. Children born to women with HIV-1 infection: natural history and transmission. *Lancet* 1991;**337**:253–60.
79. Farley J, Gona P, Crain M, et al. Prevalence of elevated cholesterol and associated risk factors among perinatally HIV-infected children (4-19 years old) in Pediatric AIDS Clinical Trials Group 219C. *J Acquir Immune Defic Syndr* 2005;**38**:480–7.
80. Fellary J, Shianna KV, Ge D, et al. A whole-genome association study of major determinants for host control of HIV-1. *Science* 2007;**317**:944–77.
81. Filippini P, Scolastico C, Battaglia M, et al. Lipodystrophy and serum lipid abnormalities in HIV-positive sub-Saharan population on ART. *J Infect* 2006;**53**:e29–33.
82. Fisher SD, Lipshultz SE. Epidemiology of cardiovascular involvement in HIV disease and AIDS. *Ann N Y Acad Sci* 2002;**946**:13–22.
83. Food and Drug Administration. *Lexiva FDA Label*, 2009. Available at www.accessdata.fda.gov/drugsatfda_docs/label/2009/022145s001lbl.pdf.
84. Food and Drug Administration. *Isentress FDA Label*, 2012. Available at www.accessdata.fda.gov/drugsatfda_docs/label/2012/021548s028,022116s012lbl.pdf.
85. Foster SB, Lu M, Glaze DG, et al. Associations of cytokines, sleep patterns, and neurocognitive function in youth with HIV infection. *Clin Immunol* 2012;**144**:13–23.
86. Foster SB, Lu M, Thompson B, et al. Association between HLA inheritance and asthma medication use in HIV-positive children. *AIDS* 2010;**24**:2133–5.
87. Foster SB, McIntosh K, Thompson B, et al. Increased incidence of asthma in HIV-infected children treated with highly active antiretroviral therapy in the National Institutes of Health Women and Infants Transmission Study. *J Allergy Clin Immunol* 2008;**122**:159–65.
88. Foster SB, Paul ME, Kozinetz CA, et al. Prevalence of asthma in children and young adults with HIV infection. *J Allergy Clin Immunol* 2007;**119**:750–2.
89. Gabiano C, Tovo P, de Martino M, et al. Mother-to-child transmission of human immunodeficiency virus type-1: risk of infection and correlates of transmission. *Pediatrics* 1992;**90**:369–74.
90. Gafni RI, Hazra R, Reynolds JC, et al. Tenofovir disoproxil fumarate and an optimized background regimen of antiretroviral agents as salvage therapy: impact on bone mineral density in HIV-infected children. *Pediatrics* 2006;**118**:e711–18.
91. Gagnon S, Boota AM, Fischl MA, et al. Corticosteroids as adjunctive therapy for severe *Pneumocystis carinii* pneumonia in the

acquired immunodeficiency syndrome: a double-blind, placebo-controlled trial. *N Engl J Med* 1990;**323**:1444–50.
92. Gallant JE, Deresinski S. Tenofovir disoproxil fumarate. *Clin Infect Dis* 2003;**34**:944–50.
93. Gallo RC, Salahuddin SZ, Shearer GM, et al. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 1984;**224**:500–3.
94. Galpin L, Manary MJ, Fleming K, et al. Effect of Lactobacillus GG on intestinal integrity in Malawian children at risk of tropical enteropathy. *Am J Clin Nutr* 2005;**82**:1040–5.
95. Gaur AH, Dominguez KL, Kalish ML, et al. Practice of feeding premasticated food to infants: a potential risk factor for HIV transmission. *Pediatrics* 2009;**124**:658–66.
96. Gellert GA, Durfee MJ, Berkowitz CD, et al. Situational and sociodemographic characteristics of children infected with human immunodeficiency virus from pediatric sexual abuse. *Pediatrics* 1993;**91**:39–44.
97. Ghosn J, Deveau C, Chaix ML, et al. Despite being highly diverse, immunovirological status strongly correlates with clinical symptoms during primary HIV-1 infection: a cross-sectional study based on 674 patients enrolled in the ANRS CO 06 PRIMO cohort. *J Antimicrob Chemother* 2010;**65**:741–8.
98. Gona P, Van Dyke RB, Williams PL, et al. Incidence of opportunistic and other infections in HIV-infected children in the HAART era. *JAMA* 2006;**296**:292–300.
99. Gorry PR, Sterjovski J, Churchill M, et al. The role of viral co-receptors and enhanced macrophage tropism in human immunodeficiency virus type 1 disease progression. *Sex Health* 2004;**1**:23–4.
100. Gortmaker SL, Hughes M, Cervia J, et al. Effect of combination therapy including protease inhibitors on mortality among children and adolescents infected with HIV-1. *N Engl J Med* 2001;**345**:1522–8.
101. Govender R, Eley B, Walker K, et al. Neurologic and neurobehavioral sequelae in children with HIV-1 infection. *J Child Neurol* 2011;**26**:1355–64.
102. Graham BS, Keefer MC, McElrath JM, et al. Safety and immunogenicity of a candidate HIV-1 vaccine in healthy adults: recombinant glycoprotein (rgp) 120. A randomized, double-blind trial. *Ann Intern Med* 1996;**125**:270–9.
103. Graham SM. Non-tuberculosis opportunistic infections and other lung diseases in HIV-infected infants and children. *Int J Tuberc Lung Dis* 2005;**9**:592–602.
104. Granovsky MO, Mueller BU, Nicholson HS, et al. Cancer in human immunodeficiency virus infected children: a case series from the Children's Cancer Group and the National Cancer Institute. *J Clin Oncol* 1998;**16**:1729–35.
105. Gray F,Bazille C, Adle-Biassette H, et al. Central nervous system immune reconstitution disease in acquired immunodeficiency syndrome patients receiving highly active antiretroviral treatment. *J Neurovirol* 2005;**11**:16–22.
106. Groopman JE, Feder D. Hematopoietic growth factors in AIDS. *Semin Oncol* 1992;**19**:408–14.
107. Groopman JE, Mitsuyasu RT, Delco MJ, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. *N Engl J Med* 1987;**317**:593–8.
108. Grossman Z, Meier-Schellersheim M, Paul WE, et al. Pathogenesis of HIV infection: what the virus spares is as important as what it destroys. *Nat Med* 2006;**12**:289–95.
109. Guarino A, Bruzzese E, De Marco G, et al. Management of gastrointestinal disorders in children with HIV infection. *Pediatr Drugs* 2004;**6**:347–62.
110. Guay LA, Musoke P, Fleming T, et al. Intrapartum and neonatal single-dose nevirapine compared with zidovudine for prevention of mother-to-child transmission of HIV-1 in Kampala, Uganda: HIVNET 012 randomised trial. *Lancet* 1999;**354**:795–802.
111. Gutin F, Butt A, Alame W, et al. Asthma in immune-competent children with human immunodeficiency virus. *Ann Allergy Asthma Immunol* 2009;**102**:438.
112. Hanson IC. Respiratory infections in HIV-infected children. *Immunol Allergy Clin North Am* 1996;**13**:205–15.
113. Hanson IC, Antonelli TA, Sperling RS, et al. Lack of tumors in infants with perinatal HIV-1 exposure and fetal/neonatal exposure to zidovudine. *J Acquir Immune Defic Syndr Hum Retrovirol* 1999;**20**:463–7.
114. Hanson IC, Kaplan SL. Opportunistic infections. *Semin Pediatr Infect Dis* 1990;**1**:31–9.
115. Havens PL; the American Academy of Pediatrics Committee on Pediatric AIDS. Postexposure prophylaxis in children and adolescents for non-occupational exposure to human immunodeficiency virus. *Pediatrics* 2003;**111**:1475–89.
116. Hemelaar J, Gouws E, Ghys P, et al; WHO-UNAIDS. Network for HIV isolation and characterization. Global trends in molecular epidemiology of HIV during 2000-2007. *AIDS* 2011;**25**:678–89.
117. Hengge UR, Ruzicka T, Tyring SK, et al. Update on Kaposi's sarcoma and other HHV8 associated diseases, part II: pathogenesis, Castleman's disease, and pleural effusion lymphoma. *Lancet Infect Dis* 2002;**2**:344–52.
118. Ho D, Pomerantz RJ, Kaplan JC. Pathogenesis of infection with human immunodeficiency virus. *N Engl J Med* 1987;**317**:278–86.
119. Holgate SL, Rabie H, Smith P, et al. Trough lopinavir concentrations in preterm HIV-infected infants. *Pediatr Infect Dis J* 2012;**31**:602–4.
120. Houston AM, Fang J, Husman C, et al. More than just vaginal intercourse: anal intercourse and condom use patterns in the context of "main" and "casual" sexual relationships among urban minority adolescent females. *J Pediatr Gynecol* 2007;**20**:299–304.
121. Huang L, Morris A, Limper A, et al. An official ATS workshop summary: recent advances and future directions in *Pneumocystis* pneumonia (PCP). *Proc Am Thorac Soc* 2006;**3**:655–64.
122. Hussey RE, Richardson NE, Kowalski M, et al. A soluble CD4 protein selectively inhibits HIV replication and syncytium formation. *Nature* 1988;**331**:78–81.
123. Husson RN, Mueller BU, Farley M, et al. Zidovudine and didanosine combination therapy in children with human immunodeficiency virus infection. *Pediatrics* 1994;**93**:316–22.
124. Husson RN, Shirasaka T, Butler KM, et al. High-level resistance to zidovudine but not to zalcitabine or didanosine in human immunodeficiency virus from children receiving antiretroviral therapy. *J Pediatr* 1993;**123**:9–16.
125. Italian Register for HIV Infection in Children. Features of children perinatally infected with HIV-1 surviving longer than 5 years. *Lancet* 1994;**343**:191–5.
126. Janssen Pharmaceuticals. *Prezista Package Insert*, 2012. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021976s022lbl.pdf.
127. Joint United Nations Programme on HIV/AIDS (UNAIDS). *Global report: UNAIDS report on the global AIDS epidemic: 2012*. Publication No. UNAIDS/JC24117E. New York: UNAIDS; 2012. p. 14.
128. Kadan-Lottick NS, Skluzacek MD, Gurney JG. Decreasing incidence rates of primary central nervous system lymphoma. *Cancer* 2002;**95**:193–202.
129. Kanjanasut N, Phanthumchinda K, Bhidayasiri R. HIV-related opsoclonus-myoclonus-ataxia syndrome: report on two cases. *Clin Neurol Neurosurg* 2010;**112**:572–4.
130. Kapetanovic S, Aaron L, Montepiedra G, et al. T-cell activation and neurodevelopmental outcomes in perinatally HIV-infected children. *AIDS* 2012;**26**:959–69.
131. Kearney DL, Perez-Atayde AR, Easley KA, et al. Postmortem cardiomegaly and echocardiographic measurements of left ventricular size and function in children infected with the human immunodeficiency virus: the Prospective P2C2 HIV Multicenter Study. *Cardiovasc Pathol* 2003;**12**:140–8.
132. Keefer MC, Graham BS, Belshe RB, et al. Studies of high doses of a human immunodeficiency virus type 1 recombinant glycoprotein 160 candidate vaccine in HIV type-1–seronegative humans: the AIDS Vaccine Clinical Trials Network. *AIDS Res Hum Retroviruses* 1994;**10**:1713–23.
133. Kest H, Brogly S, McSherry G, et al. Malignancy in perinatally human immunodeficiency virus–infected children in the United States. *Pediatr Infect Dis J* 2005;**24**:237–42.
134. Kirk O, Reiss P, Uberti-Foppa C, et al. Safe interruption of maintenance therapy against previous infection with four common HIV-associated opportunistic pathogens during potent antiretroviral therapy. *Ann Intern Med* 2002;**137**:239–50.
135. Kiser JJ, Rutstein RM, Samson P, et al. Atazanavir and atazanavir/ritonavir pharmacokinetics in HIV-infected infants, children, and adolescents. *AIDS* 2011;**25**:1489–96.

136. Kitchen BJ, Engler HD, Gill VJ, et al. Cytomegalovirus infection in children with human immunodeficiency virus infection. *Pediatr Infect Dis J* 1997;**16**:358–63.
137. Kline MW, Dunkle LM, Church JA, et al. A phase I/II evaluation of stavudine (d4T) in children with human immunodeficiency virus infection. *Pediatrics* 1995;**96**:247–52.
138. Kline MW, Shearer WT. HIV infection and AIDS in children. In: Rich RR, Fleisher TA, Schwartz WT, et al, editors. *Clinical immunology*. St. Louis: Mosby; 1996. p. 739–50.
139. Kline MW, Paul ME, Bohannon B, et al. Characteristics of children surviving to five years of age or older with vertically acquired human immunodeficiency. *Pediatr AIDS* 1995;**6**:350–3.
140. Kline MW, Van Dyke RB, Lindsey JC, et al. A randomized comparative trial of stavudine (d4T) versus zidovudine (ZDV, AZT) in children with human immunodeficiency virus infection. *Pediatrics* 1998;**101**:214–20.
141. Knowles DM. Etiology and pathogenesis of AIDS-related non-Hodgkin's lymphoma. *Hematol Oncol Clin North Am* 2003;**17**: 785–820.
142. Konigs C, Feiterna-Sperling C, Esposito S, et al. Pharmacokinetics and short-term safety and tolerability of etravirine in treatment-experienced HIV-1-infected children and adolescents. *AIDS* 2012;**26**:447–55.
143. Koppensteiner H, Brack-Werner R, Schindler M. Macrophages and their relevance in human immunodeficiency virus type 1 infection. *Retrovirology* 2012;**9**:82.
144. Kourtis AP, Bansil P, Posner SF, et al. Trends in hospitalizations of HIV-infected children and adolescents in the United States: analysis of data from the 1994-2003 nationwide inpatient sample. *Pediatrics* 2007;**120**:e236–43.
145. Kourtis AP, Jamieson DJ, deVincenzi I, et al. Prevention of human immunodeficiency virus–1 transmission to the infant through breastfeeding: new developments. *Am J Obstet Gynecol* 2007;**197** (Suppl):S113–22.
146. Kourtis AP, Lee FK, Abrams EJ, et al. Mother-to-child transmission of HIV-1: timing and implications for prevention. *Lancet Infect Dis* 2006;**6**:726–32.
147. Kourtis AP, Paramsothy P, Posner SF, et al. National estimates of hospital use by children with HIV infection in the United States: analysis of data from the 2000 KIDS Inpatient Database. *Pediatrics* 2006;**118**:e167–73.
148. Kovacs A. Early immune activation predicts central nervous system disease in HIV-infected infants: implications for early treatment. *Clin Infect Dis* 2009;**48**:347–9.
149. Kramer-Hammerle S, Rothenaigner I, Wolff H, et al. Cells of the central nervous system as targets and reservoirs of the human immunodeficiency virus. *Virus Res* 2005;**111**:194–213.
150. Lane HC, Depper JM, Green WC, et al. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome: evidence for a selective defect in soluble antigen recognition. *N Engl J Med* 1985;**313**:79–84.
151. Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. *Cell* 2001;**106**:263–6.
152. Laurent C, Meggetto F, Brousset P. Human herpesvirus 8 infections in patients with immunodeficiencies. *Hum Pathol* 2008;**39**: 983–93.
153. Lederman HM, Williams PL, Wu JW, et al. Incomplete immune reconstitution after initiation of highly active antiretroviral therapy in human immunodeficiency virus–infected patients with severe $CD4^+$ T cell depletion. *J Infect Dis* 2003;**188**:1799–803.
154. Li Z, Nardi MA, Karpatkin S. Role of molecular mimicry to HIV-1 peptides in HIV-1–related immunologic thrombocytopenia. *Blood* 2005;**106**:572–6.
155. Lindsey JC, Malee KM, Brouwers P, et al. Neurodevelopmental functioning in HIV-infected infants and young children before and after the introduction of protease-inhibitor-based highly active antiretroviral therapy. *Pediatrics* 2007;**119**:e681–93.
156. Lipshultz SE, Shearer WT, Thompson B, et al. Cardiac effects of antiretroviral therapy in HIV-negative infants born to HIV-positive mothers: NHLBI CHAART-1 (National Heart, Lung, and Blood Institute Cardiovascular Status of HAART therapy in HIV-exposed infants and children cohort study). *J Am Coll Cardiol* 2011;**57**:76–85.
157. Llorente A, Brouwers P, Thompson B, et al. Effects of polymorphisms of chemokine reseptors on neurodevelopmental and the onset of encephalopathy in children with perinatal HIV-1 infection. *Appl Neuropsychol* 2006;**13**:180–9.
158. Lorenzi P, Spicher VM, Laubereau B, et al. Antiretroviral therapies in pregnancy: maternal, fetal and neonatal effects. Swiss HIV Cohort study, the Swiss Collaborative HIV and Pregnancy Study, and the Swiss Neonatal HIV Study. *AIDS* 1998;**12**: F241–7.
159. Louis J, Landon MB, Gersnoviez RJ, et al. Perioperative morbidity and mortality among human immunodeficiency virus infected women undergoing cesarean delivery. *Obstet Gynecol* 2007;**10**: 385–90.
160. Lum JJ, Schnepple DJ, Badley AD. Acquired T-cell sensitivity to TRAIL mediated killing during HIV infections regulated by CXCR4-gp120 interactions. *AIDS* 2005;**19**:1125–33.
161. Luzuriaga K, Koup RA, Pikora CA, et al. Deficient human immunodeficiency virus type–1 specific cytotoxic T-cell responses in vertically infected children. *Pediatrics* 1991;**119**:230–6.
162. Magder LS, Mofenson L, Paul ME, et al. Risk factors for in utero and intrapartum transmission of HIV. *J Acquir Immune Defic Syndr* 2005;**38**:87–95.
163. Malet I, Calvez V, Marcelin AG. The future of integrase inhibitors of HIV-1. *Curr Opin Virol* 2012;**2**:580–7.
164. Mangili A, Murman DH, Zampini AM, et al. Nutrition and HIV infection: review of weight loss and wasting in the era of highly active antiretroviral therapy from the nutrition for healthy living cohort. *Clin Infect Dis* 2006;**42**:836–42.
165. Marroni M, Gresele P, Landonio G, et al. Interferon-alpha is effective in the treatment of HIV-related, severe, zidovudine-resistant thrombocytopenia: a prospective, placebo-controlled, double-blind trial. *Ann Intern Med* 1994;**121**:423–9.
166. Mascola JR, Snyder SW, Weislow OS, et al. Immunization with envelope subunit vaccine products elicits neutralizing antibodies against laboratory-adapted but not primary isolates of human immunodeficiency virus type 1: the National Institute of Allergy and Infectious Diseases AIDS Vaccine Evaluation Group. *J Infect Dis* 1996;**173**:340–8.
167. Maury W, Potts BJ, Rabson AB. HIV-1 infection of first trimester and term human placental tissue: a possible mode of maternal-fetal transmission. *J Infect Dis* 1989;**160**:583–8.
168. McConnell MS, Byers RH, Frederick T, et al. Trends in antiretroviral therapy use and survival rates for a large cohort of HIV-infected children and adolescents in the United States, 1989-2001. *J Acquir Immune Defic Syndr* 2005;**38**:488–94.
169. McCulloch MI, Ray PE. Kidney disease in HIV-positive children. *Semin Nephrol* 2008;**28**:585–94.
170. McKinney RA Jr, Maha MA, Connor EM, et al. A multicenter trial of oral zidovudine in children with advanced human immunodeficiency virus disease: The Protocol 043 Study Group. *N Engl J Med* 1991;**324**:1018–25.
171. McKinney RE, Pizzo PA, Scott GB, et al. Safety and tolerance of intermittent intravenous and oral zidovudine therapy in human immunodeficiency virus–infected pediatric patients. *J Pediatr* 1990;**116**:640–7.
172. Mekmullica J, Brouwers P, Charurat M, et al. Early immunological predictors of neurodevelopmental outcomes in HIV-infected children. *Clin Infect Dis* 2009;**48**:338–46.
173. Merchant RC, Keshavarz R. Human immunodeficiency virus postexposure prophylaxis for adolescents and children. *Pediatrics* 2001;**108**:e38.
174. Miller TL. Nutritional aspects of HIV-infected children receiving highly active antiretroviral therapy. *AIDS* 2003;**17**(Suppl. 1): S130–40.
175. Miller TL, Agostoni C, Duggan C, et al. *J Pediatr Gastroenterol Nutr* 2008;**47**:247–53.
176. Miller TL, Easley KA, Zhang W, et al. Maternal and infant factors associated with failure to thrive in children with vertically transmitted human immunodeficiency virus–1 infection: the prospective, P2C2 human immunodeficiency virus multicenter study. *Pediatrics* 2001;**108**:1287–96.
177. Mirza S, Phelan M, Rimland D, et al. The changing epidemiology of cryptococcosis: an update from population-based active surveillance in 2 large metropolitan areas, 1992-2000. *Clin Infect Dis* 2003;**36**:789–94.
178. Miura Y, Koyanagi Y. Death ligand-mediated apoptosis in HIV infection. *Rev Med Virol* 2005;**15**:169–78.

179. Mofenson LM, Committee on Pediatric AIDS. Technical report: perinatal human immunodeficiency virus testing and prevention of transmission. *Pediatrics* 2000;**106**:e88.
180. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**(RR-11):1–166.
181. Mofenson LM, Oleske J, Serchuck L, et al. Treating opportunistic infections among HIV-exposed and infected children: recommendations from CDC, the National Institutes of Health, and the Infectious Diseases Society of America. *MMWR Recomm Rep* 2004;**53**:1–92.
182. Mofenson LM, Rodriguez EM, Hershow R, et al. *Mycobacterium tuberculosis* infection in pregnant and nonpregnant women infected with HIV in the Women and Infants Transmission Study. *Arch Intern Med* 1995;**155**:1066–72.
183. Montefiori DC, Arnasuta KC, Huang Y. Magnitude and breadth of neutralizing antibody response in the RV 144 and Vax003 HIV-1 vaccine efficacy trials. *J Infect Dis* 2012;**206**:431–41.
184. Moulard M, Lortat-Jacob H, Mondor I, et al. Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. *J Virol* 2000;**74**:1948–60.
185. Moyle GJ, Wildfire A, Mandalia S, et al. Epidemiology and predictive factors for chemokine receptor use in HIV-1 infection. *J Infect Dis* 2005;**191**:866–72.
186. Mueller BU, Jacobsen F, Butler KM, et al. Combination treatment with azidothymidine and granulocyte colony-stimulating factor in children with human immunodeficiency virus infection. *J Pediatr* 1992;**121**:797–802.
187. Mwanyumba F, Gaillard P, Inion I, et al. Placental inflammation and perinatal transmission of HIV-1. *J Acquir Immune Defic Syndr* 2002;**1**:29.
188. Nachman S, Chernoff M, Williams P, et al. Human immunodeficiency virus disease severity, psychiatric symptoms, and functional outcomes in perinatally infected youth. *Arch Pediatr Adolesc Med* 2012;**166**:528–35.
189. Nachman S, Gona P, Dankner W, et al. The rate of serious bacterial infections among HIV-infected children with immune reconstitution who have discontinued opportunistic infection prophylaxis. *Pediatrics* 2005;**115**:e488–94.
190. Nesheim SR, Kapogiannis BG, Soe MM, et al. Trends in opportunistic infections in the pre-and post-highly active antiretroviral therapy eras among HIV-infected children in the Perinatal AIDS Collaborative Transmission Study, 1986-2004. *Pediatrics* 2007;**120**:100–9.
191. Nielsen-Saines K, Watts DH, Veloso VG, et al. Three postpartum antiretroviral regimens to prevent intrapartum HIVinfection. *N Engl J Med* 2012;**366**:2368–79. Available at http://www.ncbi.nlm.nih.gov/pubmed/22716975.
192. Norton KI, Kattan M, Rao JS, et al. Chronic radiographic lung changes in children with vertically transmitted HIV-1 infection. *AJR Am J Roentgenol* 2001;**176**:1553–8.
193. Nyasulo P. Increased risk of death in human immunodeficiency virus-infected children with pneumococcal meningitis in South Africa.. *Pediatr Infect Dis J* 2011;**30**:1075–80.
194. Ofori-Mante JA, Kaul A, Rigaud M, et al. Natural history of HIV-infected pediatric long-term or slow progressor population after the first decade of life. *Pediatr Infect Dis J* 2007;**26**:217–20.
195. Omonuwa TS, Goforth HW, Preud'homme X, et al. The pharmacologic management of insomnia in patients with HIV. *J Clin Sleep Med* 2009;**5**:251–62.
196. Otieno RO, Ouma C, Ong'echa JM, et al. Increased severe anemia in HIV-1 exposed and HIV-1-positive infants and children during acute malaria. *AIDS* 2006;**20**:275–80.
197. Pahwa R, McCloskey TW, Aroniadis OC, et al. $CD8^+$ T cells in HIV disease exhibit cytokine receptor perturbation and poor T cell receptor activation but are responsive to gamma-chain cytokine-driven proliferation. *J Infect Dis* 2006;**193**:879–87.
198. Panel on Antiretroviral Therapy and Medical Management of HIV-Infected Children. *Guidelines for the use of antiretroviral agents in pediatric HIV infection*. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/pediatricguidelines.pdf. Accessed November 25, 2012.
199. Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. *Recommendations for use of antiretroviral drugs in pregnant HIV-1–infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States*. Available at http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf. Accessed November 11, 2012.
200. Pantanowitz L, Dezube BJ. Advances in the pathobiology and treatment of Kaposi sarcoma. *Curr Opin Oncol* 2004;**16**:443–9.
201. Perez MS, Van Dyke RB. Pulmonary infections in children with HIV infection. *Semin Respir Infect* 2002;**17**:33–46.
202. Perry CM, Faulds D. Lamivudine: a review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in the management of HIV infection. *Drugs* 1997;**53**:657–80.
203. Phillips P, Chan K, Hogg R, et al. Azithromycin prophylaxis for *Mycobacterium avium* complex during the era of highly active antiretroviral therapy: evaluation of a provincial program. *Clin Infect Dis* 2002;**34**:371–8.
204. Pirrone V, Passic S, Wigdahl B, et al. Application and removal of polyanionic microbicide compounds enhances subsequent infection by HIV-1. *Virol J* 2012;**9**:33.
205. Poli G, Orenstein JM, Kinter A, et al. Interferon-α but not AZT suppresses HIV expression in chronically infected cell lines. *Science* 1989;**244**:575–7.
206. Pollock B, Jenson H, Leach C, et al. Risk factors for pediatric human immunodeficiency virus-related malignancy. *JAMA* 2003;**289**:2393–9.
207. Polycarpou A, Ntais C, Korber BT, et al. Association between maternal and infant class I and II HLA alleles and of their concordance with the risk of perinatal HIV type 1 transmission. *AIDS Res Hum Retroviruses* 2002;**18**:741–6.
208. Pommier Y, Johnson AA, Marchand C. Integrase inhibitors to treat HIV/AIDS. *Nat Rev Drug Discov* 2005;**4**:236–48.
209. Porter PC, Roberts L, Fields A, et al. Necessary and sufficient role for T helper cells to prevent fungal dissemination in allergic lung disease. *Infect Immun* 2011;**79**:4459–71.
210. Porter PC, Susarla SC, Polikepahad S, et al. Link between allergic asthma and airway mucosal infection suggested by proteinase-secreting household fungi. *Mucosal Immun* 2009;**2**:504–17.
211. Ray PE, Hu CA. Advances in our understanding of the pathogenesis of HIV-1 associated nephropathy in children. *Future Virol* 2011;**6**:883–94.
212. Ray PE, Xu L, Rakusan T, et al. A 20-year history of childhood HIV-associated nephropathy. *Pediatr Nephrol* 2004;**19**:1075–92.
213. Recommendations on the use of quadrivalent human papillomavirus vaccine in males—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR* 2011;**60**:1705–8.
214. Reeves JD, Gallo SA, Ahmad N, et al. Sensitivity of HIV-1 to entry inhibitors correlates with envelope/co-receptor affinity, receptor density, and fusion kinetics. *Proc Natl Acad Sci U S A* 2002;**99**:16249–54.
215. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, et al. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. *N Engl J Med* 2009;**361**:2209–20.
216. Resino S, Resino R, Bellon JM, et al. Clinical outcomes improve with highly active antiretroviral therapy in vertically HIV type-1–infected children. *Clin Infect Dis* 2006;**43**:243–52.
217. Rey L, Viciana A, Ruiz P. Immunopathological characteristics of in situ T-cell subpopulations in human immunodeficiency virus–associated nephropathy. *Hum Pathol* 1995;**26**:408–15.
218. Rodriguez-Novoa S, Labarga P, D'Avolio A, et al. Impairment in kidney tubular function in patients receiving tenofovir is associated with higher tenofovir plasma concentrations. *AIDS* 2010;**24**:1064–6.
219. Romagnani C, Della Chiesa M, Kohler S, et al. Activation of human NK cells by plasmacytoid dendritic cells and its modulation by $CD4^+$ T helper cells and $CD4^+$ CD25hi T regulatory cells. *Eur J Immunol* 2005;**35**:2452–8.
220. Ross A, Bråve A, Scarlatti G, et al. Progress towards development of an HIV vaccine: report of the AIDS Vaccine 2009 Conference. *Lancet Infect Dis* 2010;**10**:305–16.
221. Ross AC, Storer N, O'Riordan MA, et al. Longitudinal changes in carotid intima-media thickness and cardiovascular risk factors

in HIV-infected children and young adults compared with healthy controls. *Pediatr Infect Dis J* 2010;**29**:634–8.
222. Rutstein R, Cobb P, McGowan K, et al. *Mycobacterium avium-intracellulare* complex in HIV-infected children. *AIDS* 1993;**7**: 507–12.
223. Ryder RW, Nsa W, Hassig SE, et al. Perinatal transmission of the human immunodeficiency virus type 1 to infants of seropositive women in Zaire. *N Engl J Med* 1989;**320**:1637–42.
224. Saag MS. Emtricitabine, a new antiretroviral agent with activity against HIV and hepatitis B virus. *Clin Infect Dis* 2006;**42**: 126–31.
225. Saag MS, Sonnerborg A, Torres RA, et al. Antiretroviral effect and safety of abacavir alone and in combination with zidovudine in HIV-infected adults. *AIDS* 1998;**12**:F203–9.
226. Sandberg JK, Fast NM, Jordan KA, et al. HIV-specific $CD8^+$ T cell function in children with vertically acquired HIV-1 infection is critically influenced by the state of the $CD4^+$ T cell compartment. *J Immunol* 2003;**170**:4403–10.
227. Scarlatti G. Mother-to-child transmission of HIV-1: advances and controversies of the twentieth centuries. *AIDS Rev* 2004;**6**: 67–78.
228. Schooley RT, Merigan TC, Gaut P, et al. Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex: a phase I-II escalating dosage trial. *Ann Intern Med* 1990;**115**:247–53.
229. Schouten J, Cinque P, Gisslen M, et al. HIV-1 infection and cognitive impairment in the cART era: a review. *AIDS* 2011;**25**: 561–75.
230. Schulte J, Dominguez K, Sukalac T, et al. Declines in low birth weight and preterm birth among infants who were born to HIV-infected women during an era of increased use of maternal antiretroviral drugs: Pediatric spectrum of disease, 1989-2004. *Pediatrics* 2007;**119**:e900–6.
231. Schwartz L, Major EO. Neural progenitors and HIV-1-associated central nervous system disease in adults and children. *Curr HIV Res* 2006;**4**:319–27.
232. Sei S, Boler AM, Nguyen GT, et al. Protective effect of CCR5 delta 32 heterozygosity is restricted by SDF-1 genotype in children with HIV-1 infection. *AIDS* 2002;**27**:1343–52.
233. Seibel NL, Schwartz C, Arrieta A, et al. Safety, tolerability, and pharmacokinetics of micafungin (FK463) in febrile neutropenic pediatric patients. *Antimicrob Agents Chemother* 2005;**49**: 3317–24.
234. Shanbhag MC, Rutstein RM, Zaoutis T, et al. Neurocognitive functioning in pediatric human immunodeficiency virus infection: effects of combined therapy. *Arch Pediatr Adolesc Med* 2005;**159**: 651–6.
235. Shearer WT, Duliege AM, Kline MW, et al. Transport of recombinant human CD4-immumoglobulin G across the human placenta: pharmacokinetics and safety in six mother-infant pairs in AIDS Clinical Trial Protocol 146. *Clin Diagn Lab Immunol* 1995;**2**:281–5.
236. Shearer WT, Israel RJ, Starr S, et al. Recombinant CD4-IgG2 in human immunodeficiency virus type 1–infected children: phase 1/2 study. *J Infect Dis* 2000;**182**:1774–9.
237. Shearer WT, Pahwa S, Read JS, et al. CD4/CD8 T cell ratio predicts HIV infection in infants: the National Heart, Lung, and Blood P(2)C(2) Study. *J Allergy Clin Immunol* 2007;**120**: 1449–56.
238. Siberry GK, Leister E, Jacobson DL, et al. Increased risk of asthma and atopic dermatitis in perinatally HIV-infected children and adolescents. *Clin Immunol* 2012;**142**:201–8.
239. Simon A, Warszawski J, Kariyawasam D, et al. Association of prenatal and postnatal exposure to lopinavir-ritonavir and adrenal dysfunction among uninfected infants of HIV-infected mothers. *JAMA* 2011;**306**:70–7.
240. Simonds RJ, Chanock S. Medical issues related to caring for human immunodeficiency virus–infected children in and out of the home. *Pediatr Infect Dis J* 1993;**12**:845–52.
241. Sinfield RL, Molyneux EM, Banda K, et al. Spectrum and presentation of pediatric malignancies in the HIV era: experience from Blantryre, Malawi, 2998-2003. *Pediatr Blood Cancer* 2007;**48**: 515–20.
242. Sleasman JW, Hemenway C, Klein AS, et al. Corticosteroids improve survival of children with AIDS and *Pneumocystis carinii* pneumonia. *Am J Dis Child* 1993;**147**:30–4.
243. Smith DK, Grohskopf LA, Black RJ, et al. Antiretrrviral postexposure prophylaxis after sexual, infection-drug use, or other non-occupational exposure to HIV in the United States. *MMWR Recomm Rep* 2005;**54**:1–20.
244. Smith R, Malee K, Charurat M, et al. Timing of perinatal human immunodeficiency virus type 1 infection and rate of neurodevelopment: the Women and Infant Transmission Study Group. *Pediatr Infect Dis* 2000;**19**:862–71.
245. Sperling RS, Shapiro DE, McSherry GD, et al. Safety of the maternal-infant zidovudine regimen utilized in the Pediatric AIDS Clinical Trial Group 076 study. *AIDS* 1998;**12**:1805–13.
246. Stekler J, Maenza J, Stevens C, et al. Abacavir hypersensitivity reaction in primary HIV infection. *AIDS* 2006;**20**:1269–74.
247. St. Louis ME, Kamenga M, Brown C, et al. Risk of perinatal transmission according to maternal immunologic, virologic, and placental factors. *JAMA* 1993;**269**:2853–9.
248. Stoll M, Schmidt RE. Immune restoration inflammatory syndromes: apparently paradoxical clinical events after the initiation of HAART. *Curr HIV/AIDS Rep* 2004;**1**:122–7.
249. Stoneburner R, Sato P, Burton A, et al. The global HIV pandemic. *Acta Paediatr* 1994;**400**(Suppl.):1–4.
250. Strauss J, Abitbol C, Zilleruelo G, et al. Renal disease in children with the acquired immunodeficiency syndrome. *N Engl J Med* 1989;**321**:625–30.
251. Strauss J, Zilleruelo G, Abitbol C, et al. Human immunodeficiency virus nephropathy. *Pediatr Nephrol* 1993;**7**:220–5.
252. Sullivan JL, Luzuriaga K. The changing face of pediatric HIV-1 infection. *N Engl J Med* 2001;**345**:1568–9.
253. Swigris JJ, Berry GJ, Raffin TA, et al. Lymphoid interstitial pneumonia: a narrative review. *Chest* 2002;**122**:2150–64.
254. Taha TE, Nour S, Kumwenda NI, et al. Gender differences in perinatal HIV acquisition among African infants. *Pediatrics* 2005;**115**:e167–72.
255. Taylor P, Worrell C, Stienberg SM, et al. Natural history of lipid abnormalities and fat redistribution among human immunodeficiency virus–infected children receiving long-term protease inhibitor–containing, highly active antiretroviral therapy regimens. *Pediatrics* 2004;**114**:e235–42.
256. The International Perinatal HIV Group. The mode of delivery and the risk of vertical transmission of human immunodeficiency virus type 1: a meta-analysis of 15 prospective cohort studies. *N Engl J Med* 1999;**340**:977–87.
257. The Working Group on Mother-to-Child Transmission of HIV. Rates of mother-to-child transmission of HIV-1 in Africa, America, and Europe: results from 13 perinatal studies. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;**8**:506–10.
258. Thuret I, Chaix ML, Tamalet C, et al. Raltegravir, etravirine and r-darunavir combination in adolescents with multidrug-resistant virus. *AIDS* 2009;**23**:2364–6.
259. Toro AA, Altemani AM, da Silva MT, et al. Epstein-Barr virus (EBV) gene expression in interstitial pneumonitis in Brazilian human immunodeficiency virus-1–infected children: is EBV associated or not? *Pediatr Dev Pathol* 2010;**13**:184–91.
260. Deleted in proofs.
261. Tuon FF, Mulatti GC, Pinto WP, et al. Immune reconstitution inflammatory syndrome associated with disseminated mycobacterial infection in patients with AIDS. *AIDS Patient Care STDS* 2007;**21**:527–32.
262. Unger M, Jimenez E, Backe E, et al. Allantoic vasculopathy of the placenta in HIV-exposed pregnancies: correlation to clinical findings. Abstract FB452. Presented at the Sixth International Conference on AIDS, San Francisco, June 1990. p. 191.
263. Urschel S, Ramos J, Mellado M, et al. Withdrawal of *Pneumocystis jirovecii* prophylaxis in HIV-infected children under highly active antiretroviral therapy. *AIDS* 2005;**19**:2103–8.
264. van der Vilet HJ, van Vonderen MD, Molling JW, et al. Cutting edge: rapid recovery of NKT cells upon institution of highly active antiretroviral therapy for HIV-1 infection. *J Immunol* 2006;**177**: 5775–8.
265. Van Rompay KKA, Brignolo LL, Meyer DJ, et al. Biological effects of short-term or prolonged administration of 9-[2-(phosphonomethoxy) propyl] adenine (tenofovir) to newborn and infant rhesus macaques. *Antimicrob Agents Chemother* 2004;**48**:1469–87.
266. Velasquez EM, Glancy DL, Helmcke F, et al. Echocardiographic findings in HIV disease and AIDS. *Echocardiography* 2005;**22**: 861–6.

267. Vernazza P, Wang C, Pozniak A, et al. Efficacy and safety of lersivirine (UK-453,061) versus efavirenz in antiretroviral treatment-naïve HIV-1-infected patients: week 48 primary analysis results from an ongoing, multicenter, randomized, double-blind, phase IIb trial. *J Acquir Immune Defic Syndr* 2013;**62**:171–9.
268. Vivani B, Corsini E, Binaglia M, et al. Reactive oxygen species generated by glia are responsible for neuron death induced by human immunodeficiency virus glycoprotein 120 in vitro. *Neuroscience* 2001;**107**:51–8.
269. Waldmann TA. Immunodeficiency diseases: primary and acquired. In: Samter M, Talmage DW, Frank MM, et al, editors. *Immunological diseases*. 4th ed. Boston: Little, Brown; 1988. p. 411–65.
270. Wang LH, Wiznia AA, Rathore MH, et al. Pharmacokinetics and safety of single oral doses of emtricitabine in human immunodeficiency virus–infected children. *Antimicrob Agents Chemother* 2004;**48**:183–91.
271. Waters L, Maitland D, Moyle GJ. Tenofovir and didanosine: a dangerous liaison. *AIDS Read* 2005;**15**:403–6.
272. Weinberg A, Wiznia AA, Lafleur BJ, et al. Cytomegalovirus-specific cell-mediated immunity in HIV-infected children on HAART. *AIDS Res Hum Retroviruses* 2006;**22**:283–8.
273. Williamson MP, McCormick TG, Nance CL, Shearer WT. Epigallocatechin gallate, the main polyphenol in green tea, binds to the T-cell receptor, CD4: Potential for HIV-1 therapy. *J Allergy Clin Immunol* 2006;**118**:1369–3174.
274. Wilson CM, Wright PF, Safrit JT, et al. Epidemiology of HIV infection and risk in adolescents and youth. *J Acquir Immune Defic Syndr* 2010;**54**(Suppl. 1):S5–6.
275. Wiznia A, Church J, Emmanuel P, et al. Safety and efficacy of enfuvirtide for 48 weeks as part of an optimized antiretroviral regimen in pediatric human immunodeficiency virus 1-infected patients. *Pediatr Infect Dis J* 2007;**26**:799–805.
276. Wood SM, Shah SS, Steenhoff AP, et al. The impact of AIDS diagnoses on long-term neurocognitive and psychiatric outcomes of surviving adolescents with perinatally acquired HIV. *AIDS* 2009;**23**:1859–65.
277. World Health Organization. *Global tuberculosis control—surveillance, planning, financing*. Geneva: WHO Report; 2007.
278. World Health Organization. AIDS epidemic update: 2009. Available at http://data.unaids.org/pub/Report/2009/JC1700_Epi_Update_2009_en.pdf. Accessed November 1, 2012.
279. Yadav A, Collman RG. CNS inflammation and macrophage/microglial biology associated with HIV-1 infection. *J Neuroimune Pharmacol* 2009;**4**:430–47.
280. Ye P, Kirschner DE, Kourtis AP. The thymus during HIV disease: role in pathogenesis and in immune recovery. *Curr HIV Res* 2004;**2**:177–83.
281. Zar HJ. Chronic lung disease in human immunodeficiency virus infected children. *Pediatr Pulmonol* 2008;**43**:1–10.
282. Zaunders JJ, Ip S, Munier ML, et al. Infections of $CD127^+$ (interleukin-7 $receptor^+$) $CD4^+$ cells and overexpression of CTLA-4 are linked to loss of antigen-specific CD4 T cells during primary human immunodeficiency virus type 1 infection. *J Virol* 2006;**80**:10162–72.

TABLE 192-1 Naturally Occurring Transmissible Spongiform Encephalopathies

Host	Disease
Human	Kuru
	Creutzfeldt-Jakob disease
	Sporadic
	Familial
	Infectious
	Variant Creutzfeldt-Jakob disease
	Gerstmann-Sträussler-Scheinker syndrome
	Fatal familial insomnia, sporadic fatal insomnia
Sheep, goats	Scrapie
Mink	Transmissible mink encephalopathy
Deer, elk	Chronic wasting disease
Cattle	Bovine spongiform encephalopathy, bovine amyloidotic spongiform encephalopathy
Cats	Feline spongiform encephalopathy

these diseases are composed of polymeric fibrils of a host-encoded protein deposited in several different regions of the CNS and within other organ systems, particularly in lymphoid organs. Amyloid may be present in TSE, and its role in the pathogenesis of these diseases is uncertain. However, the symptoms associated with the spongiform encephalopathies are thought to arise from cellular and end organ dysfunction associated with the abnormal deposition of an isoform of a normal cellular protein, PrP^c, encoded by the *PRNP* gene.[12,37,73,196] The physiologic function of PrP^c remains incompletely defined, and no null alleles of the *PRNP* gene have been identified, raising the possibility that some of the symptoms associated with these diseases are caused by a loss of normal PrP^c function.[175] Although the biologic determinants of the phenotypic expression and variation of Creutzfeldt-Jakob disease (CJD) are not clear, there is a suggestion that prions may exist as multiple prion strains or genotypes that may influence disease expression and the prion propagation may also be associated with production of toxic species, which cause disease.[74,99,138] Six subgroups, (MM1, MM2, MV1, MV2, W1, and VV), based on prion protein codon 129 genotype and biochemical characteristics of PrP have been proposed to correlate with transmissibility and with the clinical and pathologic features of CJD.[20,106,211] The TSE share several clinical features with other cerebral amyloidoses, and the insoluble protein aggregates exhibit biophysical properties similar to plaques found in Alzheimer disease, currently the most common cerebral proteopathy.[93] In contrast to these diseases, the TSE can by definition be transmitted by parenteral inoculation or by other less efficient routes, such as ingestion of contaminated tissue from the nervous system.

TSE were diseases recognized only in domestic livestock in northern Europe until late in the 20th century. Scrapie, a disease of sheep, was described first in the mid 1700s.[37,218] Farmers realized early on that the agent responsible for scrapie is communicable and instituted control measures that included isolation and destruction of affected animals and herds.[37] Evidence has suggested that the scrapie agent can persist in contaminated soil for at least 16 years, a finding that explains the reported recurrences of this disease in flocks that had been culled decades earlier.[101] Although farmers were aware of the communicability of scrapie between herds of sheep and had documented its spread among sheep, its transmissibility was not formally demonstrated until 1936.[82] Shortly thereafter, scrapie developed in a flock of sheep that were inoculated with a vaccine for louping ill prepared from CNS tissue derived from sheep that had been exposed to scrapie.[37,218] Together, these reports provided definitive evidence of the transmissible nature of scrapie. The term *slow virus disease* was initially coined by veterinarians to describe the natural history of this curious group of diseases in domestic animals that included a prolonged incubation period measured in years and a relentlessly progressive clinical deterioration once symptoms appeared.[220] This term was used to describe this group of diseases until the 1980s, when investigators began to consider that although the agent responsible for the spongiform changes in scrapie-infected mice is transmissible, it cannot be classified as a conventional virus. A decade earlier, the observational and laboratory studies of kuru, a progressive cerebellar ataxia and dementia that occurred predominantly in adolescent and young adults of the Fore tribe in Papua New Guinea, provided an important piece of the puzzle of the etiology of this group of diseases.[3,105] The natural history of kuru was described initially by Vincent Zigas and Carleton Gajdusek and was noted to be associated with the ritual cannibalism practiced by this tribe. In 1959, an astute veterinary neuropathologist, William Hadlow, observed a striking similarity of the spongiform changes in the brains of patients with kuru to the brains of sheep with scrapie and suggested that these two diseases could be caused by similar mechanisms, possibly a transmissible agent.[114] Considering the possibility that kuru is caused by a transmissible agent, Gajdusek and coworkers[95-97] eventually demonstrated that the clinical and histopathologic findings of kuru can be transmitted to chimpanzees and other nonhuman primates by inoculation of brain tissue from patients with kuru. Interestingly, Gajdusek and coworkers initially thought that the experiment was a failure because the animals did not become symptomatic until nearly 2 years (18 to 21 months) after intracranial inoculation was performed, and later reports from this same laboratory indicated that nearly 20 years elapsed between the inoculation and the development of disease in some chimpanzees.[14,37] Subsequent studies by other investigators have demonstrated that brain homogenates from patients with CJD, Gerstmann-Sträussler-Scheinker (GSS) disease, fatal familial insomnia (FFI), and, most recently, variant CJD (vCJD) can transmit spongiform changes to nonhuman primates.[22,26,194] A variant of FFI, sporadic fatal insomnia, has been identified and added to this group of transmissible neurodegenerative diseases.[172,188] The hypothesis that transmissible neurodegenerative diseases are caused by a protein was suggested first by Griffith[113] in 1967 and later more extensively developed by Prusiner[194] and coworkers. The claim that the etiologic agent of scrapie is an isoform of a normal

host protein, PrP^c (alternatively referred to as prion protein), that can transmit disease in the absence of nucleic acid was met initially with great skepticism and even today continues to evoke debate.[64,65,168,194] During the ensuing decade, the hypothesis that a protein can promote the formation of a polymeric fibrillary, insoluble plaque without the requirement of nucleic acid encoding a protein of an infectious agent has been supported by studies from several laboratories. This hypothesis represented a paradigm shift in biology, and Stanley Prusiner was awarded a Nobel Prize in 1997 for his groundbreaking studies of prion diseases. Interestingly, approximately 20 years earlier, Gajdusek also was awarded a Nobel Prize for his description of the transmissible nature of kuru and similar diseases of the CNS.

An important chapter in the TSE saga is the appearance of mad cow disease (bovine spongiform encephalopathy [BSE]) in Great Britain in the mid 1980s; and by the late 1990s, nearly 200,000 diseased animals had been destroyed.[49] Mad cow disease has been discovered in cattle in several countries within Europe and most recently in the United States. Of greatest concern has been the transmission of BSE to more than 100 individuals in Great Britain and the report of at least three cases in France.[49] BSE has been reported in the United States, and a significant increase in the incidence of TSE of elk and deer (chronic wasting disease) has been reported in wild game farms in the western United States as well as in wild deer herds in the midwestern United States.[173,247] Because of the concern that large numbers of human cases could eventually develop secondary to contamination of food products and pharmaceutical reagents with BSE or through contact with contaminated tissue from deer or elk, substantial interest has developed in the study of TSE.

EPIDEMIOLOGY

The natural history of human TSE usually is described in the context of the epidemiology of CJD. Three different modes of acquisition of disease have been described: (1) sporadic, (2) inherited mutations in the cellular gene encoding PrP^c (genetic), and (3) acquired or infectious following exogenous exposure to prion-contaminated material, such as after neurologic procedures (infectious) (see Table 192-1). Little is known about genetic or environmental factors that contribute to the development of sporadic forms of these diseases, although numerous epidemiologic studies have claimed associations ranging from ingestion of mutton to exposure to blood products after surgery.[4,41,80,171] Common migratory scavenger birds, such as crows, also may play a role in the geographic spread of TSE diseases.[241]

Studies in cases of CJD have revealed numerous genetic mutations in the cellular gene encoding the normal cellular prion protein, PrP^c, associated with the development of disease, and they have been defined as genetic causes of CJD and other TSE (see following discussion).[140] Polymorphism in the methionine at position 129 in the coding sequence of PrP^c is the genetic polymorphism most commonly recognized as being associated with sporadic CJD.[187] Homozygosity at methionine 129 of PrP^c is seen in 64 to 81 percent of patients with sporadic CJD yet is present in only approximately 39 percent of the general population and is thought to predispose an individual to both iatrogenic and sporadic CJD.[2,187,255] Interestingly, valine homozygosity at this position was associated more frequently with cases of CJD with onset in those younger than 40 years.[2] Furthermore, a retrospective analysis of a small number of patients with kuru revealed that all were homozygous for methionine at position 129 and all had more rapidly progressive disease with a shorter incubation period than did other patients with kuru from this cohort.[157] In Papua New Guinea, Fore women surviving exposure to contaminated material during the ritual cannibalistic feasts were shown to be more likely to be heterozygous at methionine 129 (76%) than were similarly aged Fore women who had not been exposed to the cannibalistic feasts.[175] This result suggested that heterozygosity at methionine 129 provided a survival advantage. Population geneticists have argued that haplotype diversity at codon 129 of the PRNP may have been in response to an epidemic of prion disease. If so, this response would represent a recent balancing selection in humans.[175a,175b] The codon at position 129 also appears to modulate the clinical course of inherited TSE such as FFI, and patients homozygous for methionine at position 129 have a more rapid clinical course than do patients heterozygous at this codon.[181] Polymorphisms in the PrP^c, including those that occur at position 129, have not been associated with the development of vCJD after exposure to BSE-contaminated material; in fact, all human cases in Great Britain have been homozygous for methionine at position 129.[135] Heterozygosity at methionine 129 is thought to confer resistance by inhibiting PrP^c protein:protein interactions.[187] Interestingly, sequence analysis of the *PRNP* gene from populations throughout the world suggested that the observed increased frequency of heterozygosity at position 129 could be explained by widespread cannibalism among primitive humans and selection of methionine 129 heterozygotes secondary to increased disease penetrance associated with exposure of susceptible methionine 129 homozygotes to prion-contaminated tissue.[175] This conclusion has been challenged by other investigators.[222]

Sporadic CJD is the most common TSE in humans and accounts for approximately 85 percent of all cases of CJD.[41,140] It occurs worldwide at a rate of approximately $1/10^6$ population.[41] CJD is a disease of late middle age, with a mean age at onset of 55 to 65 years. Cases of CJD range from 20 to 84 years, with the youngest cases being described in patients 16 to 18 years old.[40,90,180] Little evidence exists for case-to-case transmission of CJD, with the obvious exception of cases resulting from iatrogenic transmission of CJD.[62] Vertical transmission of CJD has not been reported in studies of human CJD. Anecdotal reports of occurrence of CJD in the spouses of individuals with CJD raise the possibility of horizontal transmission.[43] Alternatively, disease in spouses could result from shared environmental exposures. Sporadic cases of non-CJD TSE previously thought to be caused solely by genetic mutations have been reported, thus extending the

spectrum of TSE in humans.[158,172] With the exception of cases that can be linked to an inherited genetic mutation, no epidemics of CJD have been reported, an observation that led British epidemiologists to suspect that a link exists between what was initially thought to be an increased frequency of CJD in young adults and the outbreak of BSE in the British Isles.*

Genetic mutations in the cellular prion gene, *PRNP*, can result in a variety of inherited TSE and are likely to account for the reported outbreaks of TSE in isolated populations. Because of the relatively late onset of disease even in patients with well-characterized mutations in *PRNP*, the possibility that TSE are inherited diseases of the CNS was not considered until more recent studies of the host cell origin of prions and the discovery that a cellular gene encoded the pathogenic protein associated with TSE. Estimates from various studies suggest that between 5 and 15 percent of cases of CJD may arise from mutations in the cellular *PRNP* gene.[40] Genetic linkage studies have demonstrated that TSE are inherited as an autosomal dominant trait, an observation consistent with several studies in families with FFI and GSS.[21,131,176] In addition, defined mutations in *PRNP* have been shown to account for the increased rate of TSE in either geographically or ethnically isolated populations, such as Libyan-born Jews, an ethnic group with an incidence of TSE nearly 40 times greater than that observed in other populations.† A large number (>20) of mutations in *PRNP* have been described and appeared to be scattered throughout the gene. Reported mutations include both point mutations in the coding sequence changes, resulting in amino acid substitutions, and octapeptide insertions.‡ Because mutations in *PRNP* by definition are not present in cases of sporadic CJD, mutations of this cellular gene are not thought to play a direct role in the development of CJD in these individuals. Thus, the mode of acquisition of the TSE agent in these individuals has not been defined. In sporadic cases of CJD, the proposal has been made that spontaneous generation of the TSE agent could be occurring extremely infrequently and result in CJD. However, when scrapie was eliminated in sheep in New Zealand and Australia by culling diseased herds, spontaneous development of scrapie has not been described in these herds, suggesting that spontaneous development of the agent of TSE leading to scrapie in sheep is almost nonexistent.[65] In most cases, disease secondary to a genetic mutation in *PRNP* develops in patients of middle age, suggesting that although these diseases exhibit an autosomal dominance inheritance, the genetic background of the host contributes significantly to the observed phenotypes.

Although farmers and neuropathologists were well aware that scrapie is readily transmissible, the possibility that human TSE also are transmissible was not demonstrated until Gajdusek and coworkers defined the natural history of kuru in the Fore tribe in Papua New Guinea.[3] Kuru was seen most commonly in adolescent and young adults of both sexes but only in older women. The disease was common, with an incidence of 1 percent in this tribe, and was a significant cause of death in this population.[3] Gajdusek carried out a detailed observational study of the Fore people, including their ritualistic cannibalism of the dead. This ritual included homogenization of the brains of dead relatives in bamboo cylinders with use of crude hand tools. The brain was reportedly ingested as well as smeared over the body by relatives and other members of the tribe. During most of their childhood, the Fore children remained with their mothers and other women of the village, including the time spent preparing the brains of dead relatives for ritual cannibalism. It is during this time that the exposure to the agent responsible for kuru was thought to occur, either by ingestion of the contaminated brain material or through skin abrasions that were present secondary to the nearly universal scabies infestation. Gajdusek and coworkers hypothesized that kuru is caused by an infectious agent and injected homogenates prepared from the brain of a deceased kuru patient into nonhuman primates; following an incubation period of more than 2 years, a disease similar to kuru was observed in the experimental primates.[14] After the transmissibility of kuru was demonstrated in experimental animals, several other TSE were shown to be transmissible in nonhuman primates and other experimental animals.[14,45,76,230,232] Subsequent studies demonstrated that kuru could be transmitted to nonhuman primates by oral ingestion of contaminated tissue.[103] Although the origin of the TSE agent responsible for kuru in the Fore tribe is unknown, the most plausible explanation is that a spontaneous mutation in the cellular PrP^c gene resulted in a case of TSE in the Fore tribe that then was introduced and propagated in the population by cannibalism. After ritual cannibalism was halted by the government of these districts in Papua New Guinea, new cases of the disease disappeared, and the last case of kuru in an individual younger than 30 years was reported in 1985.[3] Cases of kuru have developed some 40 to 50 years after exposure to infectious material, illustrating the prolonged incubation period of these diseases.[77,78,148]

Before the 1980s, cases of TSE occurring in children and adolescents, with the exception of kuru, were either extremely rare or not recognized. In the late 1980s, cases of TSE resembling CJD were reported in young adults who as young children had received injections of human growth hormone prepared from cadaveric pituitary glands.[36,45,47] The thought was that the practice of pooling pituitary glands from a large number of cadavers increased the risk of contamination of the preparation with prions from subclinical cases of TSE. More than 100 cases of TSE occurring after patients were injected with contaminated growth hormone preparations have been reported.[61] Whether new cases of TSE will develop in adults given cadaveric growth hormone before the mid 1980s remains uncertain, but with a mean incubation period of 12 years in documented cases, it is unlikely that significant numbers of new cases will be reported.[38,47] Other sources of TSE include dura grafts, corneal transplants, contaminated stereotaxic neurosurgical equipment, and, more recently, blood transfusions.* An alarming case of TSE

*References 6, 13, 81, 108, 240, 253.
†References 62, 84, 107, 132, 133, 135, 224.
‡References 56, 62, 105, 133, 191, 199.

*References 7, 38, 42, 85, 112, 115, 128, 184, 191, 186.

occurred in a chimpanzee after the use of a stereotaxic electrode that 2 years previously had been used in a procedure involving a patient with CJD. Because this electrode had been decontaminated numerous times with conventional sterilization methods before its use in this experimental animal, this case illustrated the potential for the occurrence of iatrogenic transmission of TSE to humans undergoing neurosurgical procedures.[104] Two confirmed and two probable cases of vCJD occurring after receipt of blood transfusions have been described in Great Britain.[115] Studies of experimental models of TSE, primarily scrapie in mice, have shown that the scrapie agent initially amplifies in titer in spleen and regional lymph nodes and enters the CNS by a bloodborne route or by infection of the peripheral nervous system.* In addition, studies in mice have demonstrated that brain tissue from animals injected with hamster scrapie can harbor the infectious agent for a prolonged time without evidence of disease.[123,202] Even during a period of inactive persistence and replication, the scrapie prion could be detected, suggesting that transmission of prion disease by contaminated blood products or surgical instruments may occur even if screening assays for the detection of prions are mandated.[189] To further cloud the issue, other investigators have postulated that less virulent strains of prions can attenuate the virulence of other strains of prions, which then can be transmitted readily during asymptomatic periods.[170,243] After transmission to a secondary host has occurred, the phenotype of the more virulent strain could be expressed. These findings are particularly worrisome because of the possibility that food products from cattle with subclinical BSE could continue to be consumed. Similarly, the possibility exists that the low incidence of vCJD in Great Britain, in spite of what most authorities consider was a much larger exposure to BSE before eradication of BSE-containing herds, could be explained by persistence of infectious agents in genetically resistant individuals or possibly be secondary to persistence in non-CNS organ systems.[29,123] Previously, these studies raised the possibility that TSE could be acquired by transfusion of blood and blood products obtained from infected hosts.[156]

Considerable controversy existed about the importance of transmissibility of TSE by blood and blood products.[120] Investigators argued that the risk is exceedingly small on the basis of experimental studies in rodents and epidemiologic studies involving patients receiving quantities of blood products, such as hemophiliacs. However, at least four patients are believed to have contracted CJD from blood transfusions and at least one from human plasma–derived clotting factor.† However, BSE has been transmitted to sheep by blood transfusions, and buffy coat preparations have been shown to transmit TSE to experimental primates.[25,130] The U.S. Food and Drug Administration has recommended a very conservative policy for blood and blood products because of these theoretical concerns, and, more recently, this policy has been reinforced by the reports of blood transfusion–acquired vCJD.[119,159,259] In addition to the exclusion of donors with symptoms consistent with TSE, individuals who have lived in or visited Great Britain and other countries with BSE for a cumulative period of 3 months during the interval of 1980 to 1996 are also excluded as donors.* With the possible exception of kuru, little evidence was available previously to suggest that TSE such as CJD could be acquired by ingestion of contaminated tissue.[41] This claim was challenged in the early 1990s by the reports of several young adults with CJD in Great Britain.[81,253] Inspection of the rates of CJD in England revealed that the incidence of CJD was approximately 15-fold higher than earlier, based on surveillance studies from previous decades.[36] In the years preceding the startling increase in rate of CJD, the cattle industry in Great Britain was being devastated by the spreading epidemic of BSE, a disease ultimately shown to be a TSE.[117,221,251] A similar disease had been noted in domestic cats as well as in animals housed in zoos, where they had been fed British beef and beef byproducts.[26,77,221] The first case of human disease thought to be associated with BSE was termed *vCJD* and was described in 1995, about 10 years after the first cases of BSE were reported in cows, although more careful analysis suggested that the first cases of BSE probably occurred in the 1970s.[221,253] During the ensuing years, investigators have closed the circle of evidence, showing that vCJD represents the transmission of BSE to humans, presumably by ingestion of contaminated beef or byproducts from BSE-infected cattle.[72,125,220,223] These byproducts include many common household and medical products, such as gelatin in food products and in pharmaceutical capsules, bouillon cubes used for food preparation, and a wide variety of foodstuffs containing beef.

Although the origin of BSE is far from settled, most investigators concur that changes in the rendering of beef and sheep carcasses to remove fat for the preparation of meat and bone meal for use as a protein supplement in animal feeds led to the epidemic. Before the late 1970s, beef carcasses were heated and extracted with hydrocarbons such as chloroform to delipidate the homogenate.[33,83,231,251,253] Omitting the hydrocarbon-extraction step apparently decreased inactivation of bovine prions, thus allowing introduction of this agent into the food products of British cattle. The epidemic began slowly in cattle, with the first case documented in 1986; by the mid 1990s, more than 3,500 new cases of BSE were being reported each year. As a result of the BSE epidemic, 200,000 diseased cattle died as a result of BSE and nearly 4.5 million cattle were slaughtered preemptively, at a cost of approximately 4 billion English pounds, to curtail the epidemic.[5,49,221,258] BSE has been documented in U.S. herds, resulting in a ban of exportation of beef to several economically important markets.[206] As noted earlier, transmission to humans was suggested first in 1995 after the findings of CJD in three young adults were reported; and by 1996, additional cases of CJD in young adults were thought to be secondary to transmission from exposure to beef from BSE-infected cows.[72] Of the initial 20 human cases, only one individual was older than

*References 1, 142, 144, 146, 155, 166.
†References 1, 32, 35, 87, 120, 237, 239, 254.

*American Red Cross website: http://www.redcross.org/services/biomed/0,1082,0_557_,00.html.

40 years, and the mean age of infected humans was 28 years.[6,195,240,243,260] Additional evidence from in vitro studies of the protein agents responsible for BSE and vCJD and from animal inoculation indicated that vCJD represented a human infection with the agent responsible for BSE.[52,76,124,154] Because the BSE epidemic was recognized first in 1985, researchers argued that the incubation period is approximately 10 years. On the basis of studies in kuru and experimental animal models of TSE, this estimate was determined to be dependent on exposure, genetic susceptibility, and as yet other unrecognized risk factors.[61,160] For this and other reasons, mathematical projections of the extent of the human epidemic have been based on theoretical estimates of exposure rates, possible dose-dependent incubation periods, and as yet undefined host genes that may alter susceptibility to TSE. Even though most of these variables cannot be quantified, arguments have been put forth that the epidemic in humans has peaked and the 120 or so cases of vCJD that have resulted from contaminated beef will be the extent of human disease associated with BSE.[102,243] In contrast, other models of the current data suggest that as many as 100,000 people may be affected. Perhaps the most compelling argument against such a large number of human cases has been the slowly evolving epidemic of vCJD in humans, with the number of new cases declining, although several investigators have contested this hypothesis.[70,102,243,252]

PATHOLOGY AND PATHOGENESIS

Although the histopathologic features of TSE have been described well, the pathogenesis of these diseases remains unknown and the nature of the etiologic agent continues to evoke controversy. The initial claim that TSE resulted from a transmissible protein was met with considerable skepticism both because a paradigm of biology was challenged and because key elements of the supporting scientific data were incomplete. The vast majority of experimental studies have been performed with the agent responsible for scrapie in sheep, which had been adapted for growth in mice and other small animals. Early studies that argued that the scrapie agent did not contain a nucleic acid were based on studies with ultraviolet radiation inactivation of the agent. An important note is that these studies did not exclude the possibility that the scrapie agent contained nucleic acid but instead argued that if it contained nucleic acids, the coding sequence was so limited that it could not encode any replicative functions.[16,17,194] In fact, later calculations based on inactivation by gamma rays suggest that the scrapie agent could contain between 2- and 4-kilobase pairs of DNA.[208] Other experiments demonstrated that the infectivity of the scrapie agent could be eliminated or significantly reduced by treatment with agents that denatured proteins, such as guanidine hydrochloride, sodium hypochlorite (bleach), and proteases.[194] Together, these data led several groups of investigators to postulate that the agent is an infectious protein.[24,113,197] Prusiner and coworkers[187] isolated an infectious fraction from a scrapie-infected hamster brain homogenate and obtained partial amino acid sequence. This fundamental finding led to the identification of the prion (PrP^c) and eventually the finding that a cellular gene, *PRNP*, present on chromosome 20, encoded the transmissible agent responsible for the development of scrapie in mice and hamsters.[13,66] Not all investigators have been convinced that the scrapie agent is an infectious protein, and recently published data have been cited as evidence that it is a small virus.[168,169] Once the protein encoded by the *PRNP* gene had been identified and definitively linked to scrapie and other TSE, many investigators thought that the pathogenesis of these diseases would be elucidated quickly. However, the pathogenesis of these diseases remains uncertain, perhaps because of the inability to assign a function to the normal protein product, PrP^c. The conformation of the normal cellular form of PrP^c is primarily α-helical and is susceptible to protease digestion. Various functions have been proposed for the normal nonpathogenic isoform of the cellular PrP^c, yet transgenic mice lacking the gene encoding PrP^c have a normal life span and exhibit only minor variations in normal sleep patterns.[54,55,79,198] Most interestingly, these mice are completely resistant to prion disease regardless of the source of exposure, a finding that is consistent with the hypothesis that PrP^c is necessary for the disease process associated with scrapie and other TSE.[1,54] In contrast to the normal cellular form of PrP^c that is composed of four α-helical domains, the pathogenic form of PrP^{sc} (scrapie prion protein) exists in an extended beta sheet, is insoluble, and is partially resistant to protease digestion (PrP protease resistant [PrP^{res}]). The partial resistance of PrP^{res} to proteinase K digestion is a characteristic that allows pathogenic prions to be detected in tissue specimens and has been widely used to identify products of the conversion reaction of PrP^c to PrP^{res}.[174,213] The conversion of PrP^c to PrP^{res} represents a central paradigm of the prion hypothesis and is thought to occur either as the result of a process similar to the nucleation of crystal formation by seeding the normal cellular pool of PrP^c with PrP^{res} or, alternatively, as the result of a template-directed misfolding of a normal cellular protein. Thus, PrP^{res} can be viewed as a transmissible, infectious agent that catalyzes the conversion of the normal cellular protein to a form associated with protein deposition in the CNS, cell death, and, eventually, clinical disease in the host.

The normal cellular protein, PrP^c, is a small membrane glycoprotein that is covalently linked to cellular membranes, including the plasma membrane, by a glycosyl phosphoinositol (GPI) linkage.[225-227] This cell surface form can be released by treating cellular membranes with phospholipases.[225] In addition, a secreted form of the protein also is expressed. Genetic mutations in the cellular PrP^c gene appear to predispose the protein to assume other topologies, including a transmembrane form.[116,236] Membrane-associated forms are postulated to play an important role in the neurodegeneration associated with the accumulation of the misfolded forms of PrP^c, and secreted forms can accelerate disease in a genetically susceptible animal.[116,200] Chesebro and coworkers[65a,67,228a] demonstrated that transgenic mice encoding a GPI-anchorless form of PrP^c could generate an abnormal protease-resistant PrP^{res} that could be

transmitted to other animals and induce formation of amyloid plaques but failed to cause clinical spongiform encephalopathy (scrapie equivalent in mice) in experimental animals. Interestingly, when the anchorless form was coexpressed with the wild-type form of PrP^c, accelerated disease was seen. Thus, it appears that a cell surface–linked PrP^c is required for induction of disease yet that amyloid-like deposits can be formed from the secreted form of PrP^c and disease can be induced when both secreted and wild-type PrP^c are expressed.[67] Consistent with this observation has been the description of a transmissible PrP^c amyloid disease without spongiform encephalopathy after inoculation of tissue containing amyloid deposits from a patient with GSS without CNS spongiform degeneration.[193,200] Together, these studies argue that PrP^c misfolding, protease resistance, and amyloid formation are sufficient to confer transmissibility to the PrP^{res} but that these characteristics in themselves may not be directly responsible for transmissible disease. The challenge to existing paradigms of molecular biology and genetics, including the self-replication of a protein, were quickly noted by investigators in this field. Importantly, several investigators previously demonstrated that distinctive strains of the scrapie agent exist and that these strains induce definable and reproducible phenotypes of disease in experimental animals.[18,28,50,51,143] These well-accepted and reproducible studies strongly argued for the presence of a genetic program in the scrapie agent that was most consistent with a nucleic acid–containing agent. More recently, several studies have suggested that the phenotypic behavior of scrapie strains may be entirely dependent on the pathogenic conformation that the PrP^c assumes when it is exposed to pathogenic forms of PrP^c, which in turn is dependent on its posttranslational modifications.* Thus, researchers argued that the phenotypes of scrapie strains are entirely dependent on the protein structure of PrP^c and independent of any nucleic acid–encoded genetic trait. A second characteristic of etiologic agents of TSE that was difficult to ascribe to an infectious protein is the species barrier for transmission of prions between species. This phenotypic characteristic was noted when the scrapie agent from sheep was first adapted to mice and subsequently to other species, including hamsters. Other examples of a species barrier include BSE, in which 1000 lethal doses (as defined in cattle) must be given to a mouse to induce disease.[249] Interestingly, the species barrier not only contributes to the absolute resistance of species to infection with prions derived from other species but also can lengthen the mean incubation period in a population. The species barrier often is invoked to explain the rarity of TSE, even in populations repeatedly exposed to TSE in other species (e.g., humans exposed to scrapie-infected sheep), and possibly the relatively low number of human cases of vCJD that develop after exposure to beef contaminated with BSE.[72] A more worrisome aspect of the phenomenon of species barrier is that subclinical infections could result from exposure to BSE and subsequent transmission, leading to secondary cases with clinical symptoms.[123,243] A unifying explanation for the restriction of the transmission of prion disease between species that has been proposed argues that a species restriction is related to the efficiency with which exogenous prions interact with endogenous host-derived PrP^c to form PrP^{res}.* Although considerable gaps in the understanding of the infectious agent responsible for TSE persist and at times have contributed to the controversy that surrounds the infectious protein hypothesis, more recent in vitro studies have shifted the view of the field toward the infectious protein hypothesis. These studies include the findings that protease-resistant prions (PrP^{res}) that appear identical biochemically to PrP^{res} derived from infected brain can be produced in cell-free systems in vitro.[1,59,149] However, until recently, these preparations could not transmit disease to uninfected animals unless they were supplemented with brain tissue derived from diseased animals, presumably because their titer was too low.[121] A more recent study using a system to amplify prion production through sequential folding, sonication, and feeding of new substrate produced titers of in vitro–derived prions that could transmit TSE to susceptible animals and accelerate the development of symptoms in infected animals.[58] The results from this in vitro system have provided compelling evidence for a mechanism of recruitment of cellular proteins by a misfolded protein and subsequent protein deposition as an etiology of diseases classified as TSE.

Although several mechanisms can be envisioned to explain how a misfolded host protein can lead to the recruitment and misfolding of additional copies of the protein, how the deposition of PrP^{res} can lead to disease remains unclear. Based on solid-state nuclear magnetic resonance and x-ray crystallography, the underlying structure of prion amyloids appear to share common structural features and single molecular methods will continue to advance our understanding of misfolding and aggregation.[153] The loss of normal protein function and the development of disease in humans have several well-studied examples, such as cystic fibrosis.[111,256] These diseases are associated with loss of function of an essential cellular protein. In contrast, the accumulation of cellular proteins, such as amyloid secondary to overproduction of a normal protein, can lead to disease and usually is associated with deposition and loss of organ function. In the case of TSE, the proposed mechanism for the generation of pathogenic prions appears fundamentally different in that the conformational change from a predominance of α-helical structure of PrP^c to molecules containing extensive amounts of an extended β sheet, PrP^{res}, results in the accumulation of insoluble, protease-resistant proteins that can recruit additional normal cellular proteins into this conformation. In vitro cell-free systems and studies from kindred with inherited TSE such as GSS and FFI have shown that exposure to the misfolded prion protein such as PrP^{res} can result in this conversion.† In genetic TSE such as GSS and FFI, the genetic mutation is inherited as an autosomal dominant trait, and, interestingly, the PrP^{res} pathogenic form of the prion protein has been shown to be derived from the mutant allele individual

*References 19, 59, 60, 229, 243, 244, 248.

*References 1, 59, 60, 72, 129, 150, 203, 229, 242.
†References 1, 59, 60, 63, 94, 207.

heterozygous for this allele.[1,59,63] The energetics required for the conversion in conformation of PrP^{c} to PrP^{res} are thought to be unfavorable and could require a mutant template or perhaps a chaperone protein (protein X).[59,141,149,233] Because the conversion reaction is essentially irreversible, this change in protein conformation results in the accumulation of the insoluble and protease-resistant PrP^{res} protein. Arguments have been put forth that certain conformations of PrP^{c} favor the conversion to pathogenic forms of PrP^{res}, thus providing additional constraints on the expression of disease that could explain the non-PrP^{c} genetic contributions to disease susceptibility and also provide a final common pathway for genetic and infectious forms of the disease.[1,236] Interestingly, several studies have argued that different protease fragments that are generated after PrP^{res} treatment with protease K may be associated with different patterns of disease in the CNS and thus provide additional diversity in disease phenotype attributed to this single, small protein.[189,190,243] Once the pathogenic PrP^{res} accumulates, additional newly synthesized molecules are converted into PrP^{res}, and clinical symptoms develop, depending on the site of cell loss in the CNS. Accumulation of the PrP^{res} isoform probably involves a combination of new synthesis of PrP^{res} and the decreased clearance of the misfolded aggregate. The failure in the clearance of the misfolded pathogenic isoform may ultimately be the major pathway leading to accumulation of PrP^{res} and disease.[1,212] Finally, studies have linked copper binding to the product of the PrP^{c} and oxidation of the prion protein.[1,30,204,216,257] Alternatively, a copper-containing prion complex localized to the synaptic junction could protect normal synapses from oxidants or perhaps play a direct role in copper metabolism or transport.[137,216] Loss of normal prion function by misfolding could then lead to neuronal loss. The importance of these newly described characteristics of PrP^{c} remains incompletely defined at this time.

Although inoculation of a susceptible host with prion-contaminated cadaveric growth hormone or the use of contaminated instruments during neurosurgical procedures clearly can transmit CJD, the route of infection leading to vCJD is only assumed to be through oral ingestion of contaminated beef, based on studies in non-human primates.[26,243] As noted earlier, studies have demonstrated that other TSE, including kuru, can be transmitted by oral ingestion of infectious material, but only ingestion of large inoculums and often requiring extended incubation periods.[103] In studies in mice, researchers have shown that 10 g of BSE-infected cow brain can infect and kill most mice.[11] Even though extrapolating from these data a comparable dose required for human infection will be difficult, these findings demonstrate that the BSE agent can be transmitted by oral ingestion.

The pathway leading from ingestion of PrP^{res} tissue to deposition in the CNS has been shown to require local replication in lymphoid tissue, which is thought to be followed by bloodborne dissemination to the CNS.[1,118,144,146] High titers of infectious prions can be found in the liver and the spleen as well as in lymphoid tissue such as the tonsils and other lymphoid tissue of the oropharynx and gut, suggesting that the titer of the infecting PrP^{res} is amplified in these tissues before spread to the CNS. In vCJD, PrP^{res} can be found in the tonsils at levels as high as 10 percent of the infected brain.[121,245] However, these findings were reported to be restricted to vCJD, reflecting a characteristic of the PrP^{res} of vCJD or possibly secondary to the route of inoculation. Studies in mice have demonstrated that performing splenectomy before inoculating with the scrapie agent can prolong the disease course, and subsequent studies have demonstrated the importance of replication in cells derived from bone marrow in neuroinvasion.[69,139,151] Other studies have demonstrated that expression of PrP^{res} in the peripheral nervous system is sufficient to transmit disease, suggesting that direct hematogenous dissemination to the brain is not necessary for transmission of TSE.[201] Prions are thought to replicate in these tissues before spreading to the CNS, and a study documented the presence of PrP^{res} in the appendix of an individual that was removed 8 months before the onset of TSE.[121] Investigators once thought the B lymphocytes represented a likely candidate for the cell transmitting the PrP^{res} to the CNS; however, studies have provided data inconsistent with this mechanism of transmission.* Studies have provided evidence for the role of follicular dendritic cells in lymphoid tissue as sites of prion replication and may direct the prion to the CNS.[139,151] Other investigators have suggested that host cell molecules as yet unidentified may provide the necessary interactions with the prion molecule, which permits their trafficking with migratory myeloid cells and, ultimately, entry into the CNS.[162a] Candidate molecules included components of the complement system based on the study of PrP^{res} in transgenic mice lacking specific components of the complement system, perhaps secondary to the expression of Fcγ and complement receptors CD21/CD35 by follicular dendritic cells.[21,147,162]

The pathologic findings of TSE include the hallmark of a triad of histologic findings: (1) neuronal loss, (2) proliferation of reactive astrocytes, and (3) status spongiosis.[15] *Status spongiosis* is a descriptive term of degeneration of neurons and collapse of the cortical cytoarchitecture leading to vacuolation of the neuropil.[15] Amyloid plaques also are observed in some patients, and in some cases PrP^{res} is present in these plaques.[15,53,145] Whether the distribution of spongiform lesions is related to the pathogenesis of these disorders or merely reflects the duration of disease before the onset of clinical symptoms is unknown; however, some TSE, such as FFI, exhibit a preponderance of histopathologic changes in specific locations, such as the thalamus.[15,53,165,188,234] Recent descriptions of the neuropathologic process of vCJD have suggested that distinctive histopathologic changes may be present in the cases thus far studied[243] and include a distinctive plaque containing a central amyloid core and fibrillary periphery with extensive spongiform changes in the immediate periphery of the plaque.[91,135] The surrounding spongiform changes are proximal to the plaque, and the remaining neuropil is relatively intact.[53,135] These findings are unique to vCJD cases and are not seen in sporadic CJD cases from outside Great Britain.[52,136] Histopathologic lesions in the cerebral and cerebellar

*References 9, 31, 75, 146, 182, 183, 217.

TABLE 192-2 **Human Transmissible Spongiform Encephalopathies**

Disease	Acquisition	Clinical Features
Kuru	Ingestion of or percutaneous inoculation with contaminated central nervous system tissue during ritual cannibalism in Fore tribe in New Guinea	Bimodal age distribution with disease in adolescents and in older adults Adolescents presented with ataxia and dementia with prominent cerebellar involvement
	Last case reported in 1985	Rapidly progressive disease
Creutzfeldt-Jakob disease (CJD)	Sporadic cases account for 85% of reported cases of CJD; approximately 15% are secondary to defined mutation in PrP^c, and an unknown number are secondary to infection	Classic presentation of dementia and sensory abnormalities followed by loss of motor functions
	Well-documented cases after neurosurgical procedures and injection of cadaveric growth hormone	Rapid progression of symptoms with death usually occurring <1 year of onset Disease of late middle age except in cases acquired after iatrogenic transmission
Variant Creutzfeldt-Jakob disease (vCJD)	Acquired through exposure to beef or beef byproducts contaminated with bovine spongiform encephalopathy	Presentation similar to CJD but disease progresses more slowly, with death occurring >12 months after symptoms develop
	Route of acquisition unknown but presumed to be oral	Mean age of cases approximately 28 years, with documented cases in adolescents
Gerstmann-Sträussler-Scheinker syndrome (GSS)	Autosomal dominant inheritance with documented point mutations in PrP^c gene	Disease of middle age, with mean age at onset of 45 years
		Motor abnormalities and progressive dementia
Familial fatal insomnia (FFI)	Autosomal dominant inheritance with point mutations in PrP^c gene (missense mutation codon 178)	Disease of middle age Presentation includes insomnia, dysautonomia, and motor dysfunction
Sporadic fatal insomnia	No genetic mutations identified, but homozygosity at methionine 129 characteristic	Presentation similar to FFI and neuropathologic changes similar

cortices, basal ganglia, thalamus, and brain stem also have been described.[136] Involvement of the cerebellum appears to be more common in vCJD than in reported cases of sporadic CJD, but this finding may be related to the differences in the age at onset of disease in these two populations, because recent findings from young patients with sporadic CJD have suggested that these patients have a syndrome more closely resembling vCJD than sporadic CJD in older patients.[23] Extensive widespread CJD findings that included involvement of cerebellar white matter, corticospinal degeneration, and ballooned neurons, were also reported in an 11-year old who died of CJD after treatment with native human growth hormone.[178] Last, electron microscopic studies of plaques from vCJD brain tissue have suggested subtle but recognizable differences between plaques obtained from patients with vCJD and CJD.[91,94]

CLINICAL MANIFESTATIONS

Only the clinical manifestations of sporadic CJD and vCJD are discussed; other TSE, such as GSS and FFI, are diseases that are unlikely to be encountered in children (Table 192-2). The interested reader is referred to discussions of these diseases in adults.* Clinical signs and symptoms during the early stages of CJD are subtle and often complex and include both motor and sensory dysfunction.[126] Abnormalities in gait and vision and complaints of headache, dizziness, and paresthesias often are noted.[39,48,185,209] Intellectual dysfunction, including loss of memory, speech abnormalities, anxiety, and depression, also are often presenting complaints of patients with early stages of CJD.[23,39,48] Neurologic abnormalities may include corticospinal tract dysfunction as manifested by hyperreflexia, spasticity, and extensor plantar reflexes. Visual disturbances include visual field abnormalities and, in some cases, cortical blindness. Seizures are not a frequent clinical symptom of patients with CJD. Other less frequent findings include evidence of autonomic system dysfunction and, rarely, lower motor neuron disease.[185,214] Although death secondary to complications associated with the vegetative state usually occurs within 1 year of onset of symptoms, a small percentage of patients may exhibit a prolonged disease course.[48]

The clinical symptoms of vCJD are distinct from those of sporadic CJD. This disease has been reported in an 11-year old child with neonatal growth hormone deficiency treated since infancy with injections of native human growth hormone, as well as an adolescent; therefore, CJD or vCJD could potentially be encountered by pediatricians.[178] The age range for reported cases of vCJD is 11 to 48 years, a distribution clearly outside that of sporadic CJD. Symptoms have included psychiatric and sensory disturbances, such as dysesthesias and paresthesias with electromyographic evidence of denervation. Psychiatric symptoms included anxiety, depression, anorexia, social withdrawal, and other nonspecific complaints. As the disease progressed, more familiar findings of TSE, including pyramidal tract dysfunction, rigidity, cerebellar dysfunction, and myoclonus, developed. Visual disturbances also were noted late in the disease. The duration of illness has exceeded 1 year in most patients with vCJD and follows complications associated with the

*References 39, 88, 98, 126, 161, 165.

vegetative state. As noted before, the clinical presentation of sporadic CJD in young individuals more closely resembles that of vCJD than of sporadic CJD in older individuals.[23]

DIAGNOSIS

The diagnoses of CJD and vCJD require a high index of suspicion and, in the case of vCJD, a history compatible with exposure to contaminated beef or beef byproducts or, possibly, a previous neurosurgical procedure, growth hormone injection, or corneal transplantation. Routine laboratory findings are nonspecific and not helpful. No consistent reports demonstrate systemic or CNS inflammation; thus, routine laboratory analysis of cerebrospinal fluid often is nondiagnostic.

Assays for specific cerebrospinal fluid proteins, such as the 14-3-3 protein, tau, or neuron-specific enolase, may provide helpful laboratory evidence of CJD or vCJD.* Definitive premortem diagnosis requires biopsy tissue, and histopathologic diagnosis can be facilitated by use of PrP^{res}-specific antibodies in both immunocytochemistry and Western blot assays.[8,53,243,245] The use of monoclonal antibodies specific for PrP^{res} has permitted a more thorough understanding of the distribution of prions in CNS and non-CNS tissue in patients with TSE. Hill and coworkers have reported that tonsillar biopsy followed by detection of PrP^{res} by both Western blot and immunocytochemistry or *PRNP* sequencing is useful for establishing the diagnosis of vCJD disease.[92,122] This approach has been particularly valuable for defining the incidence of vCJD infection in populations such as has been carried out in studies in Great Britain.[92,127] The National Prion Disease Pathology Surveillance Center† offers diagnostic testing assays for 14-3-3 and tau proteins in CSF, *PRNP* sequencing, Western blot analysis to identify and characterize PrP, as well as histologic processing of brain tissues, and expert neuropathologic consultation in patients suspected of having CJD.[99] More recently, in-vitro prion formation using CSF has been shown to be very sensitive and specific for the diagnosis of prion disease.[8a,173a,188a]

Imaging of patients with suspected prion disease has been extremely helpful in establishing the diagnosis of these rare diseases. Although computed tomography may reveal a variety of structural abnormalities, none is sufficiently specific to be considered diagnostic. In contrast, magnetic resonance imaging findings of increased T2 signals from the striatum and thalamus are specific for prion diseases, especially vCJD.[10,89] Abnormal signals originating from the pulvinar in the posterior thalamus ("pulvinar sign") are distinctive for vCJD and should be considered highly predictive for the disease.[10,71,164] Other studies have shown lesions in the cortex and basal ganglia, without involvement of the thalamus, in patients with biopsy-confirmed CJD.[177,235] Therefore, cortical signal increases, as well as hyperintensities in the basal ganglia and the thalamus, may all occur in patients with CJD.

Electroencephalographic tracings from patients with CJD and vCJD are characteristic but not diagnostic. The classic findings are synchronous, bilateral biphasic or triphasic periodic sharp waves of 1-cycle to 2-cycles per second on a background of generalized slowing. Repeat or serial tracings may be necessary to document these changes.[27,68,228]

TREATMENT

No known treatments of TSE are licensed for medical practice. In vitro models of PrP^{c} folding have suggested that a variety of agents may limit production of pathogenic forms of PrP^{res} in neuroblastoma cells in vitro. Comprehensive reviews of treatment modalities have been published, and the interested reader is referred to these publications.[57,238] Derivatives of Congo red, polyene antibiotics related to amphotericin B, branched polyamines, and phenothiazines all have been reported to exhibit some activity and to delay onset of disease in experimental animals.[238] To date, no evidence that any of these proposed therapies would be effective in human TSE has been reported. Interestingly, at least two reports have suggested that anti-prion antibodies may offer a therapeutic approach to TSE. These reports suggested that anti-prion antibodies directed at cell surface PrP^{c} prevented infection of susceptible cells and also eliminated infection in a chronically infected cell line.[86,192,250] These findings argued that impaired clearance of misfolded PrP^{c} contributed to disease in animals infected with PrP^{res} and that passive antibody therapy could potentially cure similar infections in vivo. Treatment of mice with soluble lymphotoxin-β blocked follicular dendritic cell maturation and inhibited the development of scrapie in inoculated, susceptible mice, suggesting that interventions with agents that can block neuroinvasion may be efficacious.[163,183] Small molecules that target protein misfolding are in preclinical development.[100,219]

PREVENTION

Because effective treatments of TSE remain as yet undefined, prevention of disease remains the goal of current medical practice. Infection that occurs after exposure during neurosurgical procedures (including dural grafts and stereotaxic electrodes), exposure to contaminated CNS tissue in the form of growth hormone or gonadotropic hormone preparations, and corneal grafts are well-known modes of transmission of TSE that are preventable in most cases. Because transmissions by blood and blood products have been reported, the American Red Cross has placed restrictions on donors to exclude those who may have been exposed to agents associated with TSE (see earlier discussion). Cases of TSE that follow exposure to human feces, urine, or mucosal secretions have not been reported; therefore, current universal precautions employed in health care settings should suffice to prevent health care–associated transmission during routine care of the patient with TSE. The inactivation of tissue and fluid suspected of being contaminated with

*References 109, 110, 134, 152, 215, 261.

†National Prion Disease Pathology Surveillance Center website: http://www.cjdsurveillance.com.

prions is problematic. Early studies indicated that subjecting prion-containing brain tissue to temperatures in excess of 360°C resulted in only approximately a 90 percent reduction in infectivity.[34,46] In addition, CJD has been transmitted from paraffin-embedded tissue sections.[43,44] Several different approaches for decontamination of prion-contaminated tissue and instruments have been proposed.[167,205,210] Tissues, such as brain, eyes, and spinal cords from infected patients, may have high levels of infectivity. Therefore the tissues and instruments in contact with these tissues should be handled as biohazardous materials. Instruments should be cleaned with high concentrations of sodium hydroxide or undiluted sodium hypochlorite and then autoclaved at high temperatures for prolonged periods of time, or, if practical, incinerated. Suffice it to say that the concentration of prions in lymphoid and nervous tissue should be emphasized to anyone in contact with patients with TSE, and material that may be contaminated with prions should be handled in a rigorous manner to ensure safe disposal. To date, no cases of transmission after routine dental procedures have been reported. Genetic counseling may be helpful in familial disease in families with a *PRNP* mutation.[140] There is no vaccine available against prion disease.

NEW REFERENCES SINCE THE SIXTH EDITION

8a. Atarashi R, Satoh K, Sano K, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011;**17**:175–8.

20. Bishop M, Will R, Manson J. Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties. *Proc Natl Acad Sci U S A* 2010;**207**:12005–10.

65a. Chesebro B, Race B, Meade-White K, et al. Fatal transmissible amyloid encephalopathy: a new type of prion disease associated with lack of prion protein membrane anchoring. *PLoS Pathog* 2010;**6**:e1000800.

74. Collinge J, Clarke A. A general model of prion strains and their pathogenicity. *Science* 2007;**318**:930–6.

93. Frost B, Diamond M. The expanding realm of prion phenomena in neurodegenerative disease. *Prion* 2009;**3**:74–7.

99. Gambetti P, Puoti G, Zou W. Variably protease-sensitive prionopathy: a novel disease of the prion protein. *J Mol Neurosci* 2011;**45**:422–4.

100. Gavrin L, Denny R, Saiah E. Small molecules that target protein misfolding. *J Med Chem* 2012;**55**:10823–43.

138. Jansen C, Parchi P, Capellari S, et al. Human prion diseases in the Netherlands (1998-2009): clinical, genetic and molecular aspects. *PLoS One* 2012;**7**:e36333.

153. Krasnoslobodtsev A, Portillo A, Deckert-Gaudig T, et al. *Prion* 2010;**4**:265–74.

162a. Mabbott NA. Prion pathogenesis and secondary lymphoid organs (SLO): tracking the SLO spread of prions to the brain. *Prion* 2012;**6**:322–33.

173a. McGuire LI, Peden AH, Orru CD, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012;**72**:278–85.

175a. Mead S, Whitfield J, Poulter M, et al. A novel protective prion protein variant that colocalizes with kuru exposure. *N Engl J Med* 2009;**361**:2056–65.

175b. Mead S, Whitfield J, Poulter M, et al. Genetic susceptibility, evolution and the kuru epidemic. *Philos Trans R Soc Lond B Biol Sci* 2008;**363**:3741–6.

177. Meissner B, Kallenberg K, Sanchez-Juan P, et al. MRI lesion profiles in sporadic Creutzfeldt-Jakob disease. *Neurology* 2009;**72**:1994–2001.

178. Mikol J, Deslys J, Zou W, et al. Creutzfeldt-Jakob disease with unusually extensive neuropathology in a child treated with native human growth hormone. *Clin Neuropathol* 2012;**31**:127–34.

179. Miller G. Neurodegeneration: could they all be prion disease? *Science* 2009;**326**:1337–9.

186. Nozaki I, Hamaguchi T, Sanjo N. Prospective 10-year surveillance of human prion disease in Japan. *Brain* 2010;**133**:3043–57.

188a. Parchi P, Capellari S. Prion disease: diagnostic value of cerebrospinal fluid markers. *Nat Rev Neurol* 2013;**9**:10–11.

211. Safar J. Molecular pathogenesis of sporadic prion diseases in man. *Prion* 2012;**6**:108–15.

219. Silva J, Vieira T, Gomes M, et al. Ligand binding and hydration in protein misfolding: insights from studies of prion and p53 tumor suppressor proteins. *Acc Chem Res* 2010;**43**:271–9.

228a. Stohr J, Watts JC, Legname G, et al. Spontaneous generation of anchorless prions in transgenic mice. *Proc Natl Acad Sci U S A* 2011;**108**:21223–8.

235. Tian H, Zhang J, Lang S, et al. MRI sequence findings in sporadic Creutzfeldt-Jakob disease. *J Clin Neurosci* 2010;**17**:1378–80.

241. VerCauteren K, Pilon J, Nash P, et al. Prion remains infectious after passage through digestive system of American crows *(Corvus brachyrhynchos)*. *PLoS One* 2012;**7**:e45774.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aguzzi A, Heikenwalder M. Pathogenesis of prion diseases: current status and future outlook. *Nat Rev Microbiol* 2006;**4**:765.
2. Alperovitch A, Zerr I, Pocchiari M, et al. Codon 129 prion protein genotype and sporadic Creutzfeldt-Jakob disease. *Lancet* 1999; **353**:1673.
3. Alpers MP. Epidemiology and clinical aspects of kuru. In: Prusiner SB, McKinley M, editors. *Prions: novel infectious pathogens causing scrapie and Creutzfeldt-Jakob disease*. San Diego: Academic Press; 1987.
4. Alter M, Hoenig E, Pratzon G. Creutzfeldt-Jakob disease: possible association with eating brains. *N Engl J Med* 1977;**296**:820.
5. Anderson RM, Donnelly CA, Ferguson NM, et al. Transmission dynamics and epidemiology of BSE in British cattle. *Nature* 1996;**382**:779.
6. Andrews NJ, Farrington CP, Cousens SN, et al. Incidence of variant Creutzfeldt-Jakob disease in the UK. *Lancet* 2000; **356**:481.
7. Anonymous. MSL-109 adjuvant therapy for cytomegalovirus retinitis in patients with acquired immunodeficiency syndrome: The Monoclonal Antibody Cytomegalovirus Retinitis Trial. The Studies of Ocular Complications of AIDS Research Group. AIDS Clinical Trials Group. *Arch Ophthalmol* 1997;**115**:1528.
8. Armstrong RA, Cairns NJ, Lantos PL. Spatial pattern of prion protein deposits in patients with sporadic Creutzfeldt-Jakob disease. *Neuropathology* 2001;**21**:19.

8a. Atarashi R, Satoh K, Sano K, et al. Ultrasensitive human prion detection in cerebrospinal fluid by real-time quaking-induced conversion. *Nat Med* 2011;**17**:175–8.

9. Aucouturier P, Geissmann F, Damotte D, et al. Infected splenic dendritic cells are sufficient for prion transmission to the CNS in mouse scrapie. *J Clin Invest* 2001;**108**:703.
10. Bahn MM, Kido DK, Lin W, et al. Brain magnetic resonance diffusion abnormalities in Creutzfeldt-Jakob disease. *Arch Neurol* 1997;**54**:1411.
11. Barlow RM, Middleton DJ. Dietary transmission of bovine spongiform encephalopathy to mice. *Vet Rec* 1990;**126**:111.
12. Basler K, Oesch B, Scott M, et al. Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* 1986;**46**:417.
13. Bateman D, Hilton D, Love S, et al. Sporadic Creutzfeldt-Jakob disease in a 18-year-old in the UK. *Lancet* 1995;**346**:1155.
14. Beck E, Daniel PM, Alpers M, et al. Experimental "kuru" in chimpanzees: a pathological report. *Lancet* 1966;**2**:1056.
15. Bell JE, Ironside JW. Neuropathology of spongiform encephalopathies in humans. *Br Med Bull* 1993;**49**:738.
16. Bellinger-Kawahara C, Cleaver JE, Diener TO, et al. Purified scrapie prions resist inactivation by UV irradiation. *J Virol* 1987;**61**:159.
17. Bellinger-Kawahara C, Diener TO, McKinley MP, et al. Purified scrapie prions resist inactivation by procedures that hydrolyze, modify, or shear nucleic acids. *Virology* 1987;**160**:271.
18. Belt PB, Muileman IH, Schreuder BE, et al. Identification of five allelic variants of the sheep *PrP* gene and their association with natural scrapie. *J Gen Virol* 1995;**76**:509.
19. Bessen RA, Kocisko DA, Raymond GJ, et al. Non-genetic propagation of strain-specific properties of scrapie prion protein. *Nature* 1995;**375**:698.
20. Bishop M, Will R, Manson J. Defining sporadic Creutzfeldt-Jakob disease strains and their transmission properties. *Proc Natl Acad Sci U S A* 2010;**207**:12005–10.
21. Blanquet-Grossard F, Thielens NM, Vendrely C, et al. Complement protein C1q recognizes a conformationally modified form of the prion protein. *Biochemistry* 2005;**44**:4349.
22. Boellaard JW, Brown P, Tateishi J. Gerstmann-Sträussler-Scheinker disease—the dilemma of molecular and clinical correlations. *Clin Neuropathol* 1999;**18**:271.
23. Boesenberg C, Schulz-Schaeffer WJ, Meissner B, et al. Clinical course in young patients with sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2005;**58**:533.
24. Bolton DC, McKinley MP, Prusiner SB. Identification of a protein that purifies with the scrapie prion. *Science* 1982;**218**:1309.
25. Bons N, Lehmann S, Mestre-Frances N, et al. Brain and buffy coat transmission of bovine spongiform encephalopathy to the primate *Microcebus murinus*. *Transfusion* 2002;**42**:513.
26. Bons N, Mestre-Frances N, Belli P, et al. Natural and experimental oral infection of nonhuman primates by bovine spongiform encephalopathy agents. *Proc Natl Acad Sci U S A* 1999;**96**:4046.
27. Bortone E, Bettoni L, Giorgi C, et al. Reliability of EEG in the diagnosis of Creutzfeldt-Jakob disease. *Electroencephalogr Clin Neurophysiol* 1994;**90**:323.
28. Bossers A, Schreuder BE, Muileman IH, et al. PrP genotype contributes to determining survival times of sheep with natural scrapie. *J Gen Virol* 1996;**77**:2669.
29. Brandner S. CNS pathogenesis of prion diseases. *Br Med Bull* 2003;**66**:131.
30. Brown DR. Copper and prion disease. *Brain Res Bull* 2001;**55**:165.
31. Brown KL, Stewart K, Ritchie D, et al. Follicular dendritic cells in scrapie pathogenesis. *Arch Virol* 2000;(Suppl. 13).
32. Brown P. Can Creutzfeldt-Jakob disease be transmitted by transfusion? *Curr Opin Hematol* 1995;**2**:472.
33. Brown P. On the origins of BSE. *Lancet* 1998;**352**:252.
34. Brown P. Transmission of spongiform encephalopathy through biological products. *Dev Biol Stand* 1998;**93**:73.
35. Brown P. The risk of blood-borne Creutzfeldt-Jakob disease. *Dev Biol (Basel)* 2000;**102**:53.
36. Brown P. Afterthoughts about bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease. *Emerg Infect Dis* 2001;**7**:598.
37. Brown P, Bradley R. 1755 and all that: a historical primer of transmissible spongiform encephalopathy. *BMJ* 1998;**317**:1688.
38. Brown P, Brandel JP, Preece M, et al. Iatrogenic Creutzfeldt-Jakob disease: the waning of an era. *Neurology* 2006;**67**:389.
39. Brown P, Cathala F, Castaigne P, et al. Creutzfeldt-Jakob disease: clinical analysis of a consecutive series of 230 neuropathologically verified cases. Ann. *Neurol* 1986;**20**:597.
40. Brown P, Cathala F, Labauge R, et al. Epidemiologic implications of Creutzfeldt-Jakob disease in a 19 year-old girl. *Eur J Epidemiol* 1985;**1**:42.
41. Brown P, Cathala F, Raubertas RF, et al. The epidemiology of Creutzfeldt-Jakob disease: conclusion of a 15-year investigation in France and review of the world literature. *Neurology* 1987; **37**:895.
42. Brown P, Cervenakova L, Goldfarb LG, et al. Iatrogenic Creutzfeldt-Jakob disease: an example of the interplay between ancient genes and modern medicine. *Neurology* 1994;**44**:291.
43. Brown P, Cervenakova L, McShane L, et al. Creutzfeldt-Jakob disease in a husband and wife. *Neurology* 1998;**50**:684.
44. Brown P, Gibbs CJ Jr, Gajdusek DC, et al. Transmission of Creutzfeldt-Jakob disease from formalin-fixed, paraffin-embedded human brain tissue. *N Engl J Med* 1986;**315**:1614.
45. Brown P, Gibbs CJ Jr, Rodgers-Johnson P, et al. Human spongiform encephalopathy: the National Institutes of Health series of 300 cases of experimentally transmitted disease. *Ann Neurol* 1994;**35**:513.
46. Brown P, Liberski PP, Wolff A, et al. Resistance of scrapie infectivity to steam autoclaving after formaldehyde fixation and limited survival after ashing at 360 degrees C: practical and theoretical implications. *J Infect Dis* 1990;**161**:467.
47. Brown P, Preece M, Brandel JP, et al. Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 2000;**55**:1075.
48. Brown P, Rodgers-Johnson P, Cathala F, et al. Creutzfeldt-Jakob disease of long duration: clinicopathological characteristics, transmissibility, and differential diagnosis. *Ann Neurol* 1984;**16**:295.
49. Brown P, Will RG, Bradley R, et al. Bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: background, evolution, and current concerns. *Emerg Infect Dis* 2001;**7**:6.
50. Bruce ME, Dickinson AG. Biological evidence that scrapie agent has an independent genome. *J Gen Virol* 1987;**68**:79.
51. Bruce ME, McConnell I, Fraser H, et al. The disease characteristics of different strains of scrapie in Sinc congenic mouse lines: implications for the nature of the agent and host control of pathogenesis. *J Gen Virol* 1991;**72**:595.
52. Bruce ME, Will RG, Ironside JW, et al. Transmissions to mice indicate that "new variant" CJD is caused by the BSE agent. *Nature* 1997;**389**:498.
53. Budka H, Aguzzi A, Brown P, et al. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). *Brain Pathol* 1995;**5**:459.
54. Bueler H, Aguzzi A, Sailer A, et al. Mice devoid of PrP are resistant to scrapie. *Cell* 1993;**73**:1339.

55. Bueler H, Fischer M, Lang Y, et al. Normal development and behaviour of mice lacking the neuronal cell-surface PrP protein. *Nature* 1992;**356**:577.
56. Carlson GA, Hsiao K, Oesch B, et al. Genetics of prion infections. *Trends Genet* 1991;**7**:61.
57. Cashman NR, Caughey B. Prion diseases—close to effective therapy? *Nat Rev Drug Discov* 2004;**3**:874.
58. Castilla J, Saa P, Hetz C, et al. In vitro generation of infectious scrapie prions. *Cell* 2005;**121**:195.
59. Caughey B, Baron GS. Prions and their partners in crime. *Nature* 2006;**443**:803.
60. Caughey B, Chesebro B. Transmissible spongiform encephalopathies and prion protein interconversions. *Adv Virus Res* 2001;**56**: 277.
61. Cervenakova L, Goldfarb LG, Garruto R, et al. Phenotype-genotype studies in kuru: implications for new variant Creutzfeldt-Jakob disease. *Proc Natl Acad Sci U S A* 1998;**95**:13239.
62. Chapman J, Brown P, Rabey JM, et al. Transmission of spongiform encephalopathy from a familial Creutzfeldt-Jakob disease patient of Jewish Libyan origin carrying the *PRNP* codon 200 mutation. *Neurology* 1992;**42**:1249.
63. Chen SG, Parchi P, Brown P, et al. Allelic origin of the abnormal prion protein isoform in familial prion diseases. *Nat Med* 1997; **3**:1009.
64. Chesebro B. BSE and prions: Uncertainties about the agent. *Science* 1998;**279**:42.
65. Chesebro B. Introduction to the transmissible spongiform encephalopathies or prion diseases. *Br Med Bull* 2003;**66**:1.

65a. Chesebro B, Race B, Meade-White K, et al. Fatal transmissible amyloid encephalopathy: a new type of prion disease associated with lack of prion protein membrane anchoring. *PLoS Pathog* 2010;**6**:e1000800.

66. Chesebro B, Race R, Wehrly K, et al. Identification of scrapie prion protein-specific MRNA in scrapie-infected and uninfected brain. *Nature* 1985;**315**:331.
67. Chesebro B, Trifilo M, Race R, et al. Anchorless prion protein results in infectious amyloid disease without clinical scrapie. *Science* 2005;**308**:1435.
68. Chiofalo N, Fuentes AL, Gálvez S. Serial EEG findings in 27 cases of Creutzfeldt-Jakob disease. *Arch Neurol* 1980;**37**:143.
69. Clarke MC, Haig DA. Multiplication of scrapie agent in mouse spleen. *Res Vet Sci* 1971;**12**:195.
70. Clarke P, Will RG, Ghani AC. Is there the potential for an epidemic of variant Creutzfeldt-Jakob disease via blood transfusion in the UK? *J R Soc Interface* 2007;**4**:675.
71. Collie DA, Summers DM, Sellar RJ, et al. Diagnosing variant Creutzfeldt-Jakob disease with the pulvinar sign: MR imaging findings in 86 neuropathologically confirmed cases. *AJNR Am J Neuroradiol* 2003;**24**:1560.
72. Collinge J. Variant Creutzfeldt-Jakob disease. *Lancet* 1999;**354**: 317.
73. Collinge J. Prion diseases of humans and animals: their causes and molecular basis. *Ann Rev Neurosci* 2001;**24**:519.
74. Collinge J, Clarke A. A general model of prion strains and their pathogenicity. *Science* 2007;**318**:930–6.
75. Collinge J, Hawke S. B lymphocytes in prion neuroinvasion: central or peripheral players? [Letter; comment.] *Nat Med* 1998;**4**:1369.
76. Collinge J, Palmer MS, Sidle KC, et al. Transmission of fatal familial insomnia to laboratory animals. *Lancet* 1995;**346**:569.
77. Collinge J, Sidle KC, Meads J, et al. Molecular analysis of prion strain variation and the aetiology of "new variant" CJD. *Nature* 1996;**383**:685.
78. Collinge J, Whitfield J, McKintosh E, et al. Kuru in the 21st century—an acquired human prion disease with very long incubation periods. *Lancet* 2006;**367**:2068.
79. Collinge J, Whittington MA, Sidle KC, et al. Prion protein is necessary for normal synaptic function. *Nature* 1994;**370**:295.
80. Collins S, Law MG, Fletcher A, et al. Surgical treatment and risk of sporadic Creutzfeldt-Jakob disease: a case-control study. *Lancet* 1999;**353**:693.
81. Cousens SN, Zeidler M, Esmonde TF, et al. Sporadic Creutzfeldt-Jakob disease in the United Kingdom: analysis of epidemiological surveillance data for 1970-96. *BMJ* 1997;**315**:389.
82. Cuille J, Chelle PL. Pathologie animale la maladie dile tremblante du mouton est-elle inoculable. *C R Acad Sci* 1936;**203**:1552.
83. Di Martino A, Safar J, Gibbs CJ. The consistent use of organic solvents for purification of phospholipids from brain tissue effectively removes scrapie infectivity. *Biologicals* 1994;**22**:221.
84. DiProspero NA, Meiners S, Geller HM. Inflammatory cytokines interact to modulate extracellular matrix and astrocytic support of neurite outgrowth. *Exp Neurol* 1997;**148**:628.
85. Duffy P, Wolf J, Collins G, et al. Possible person-to-person transmission of Creutzfeldt-Jakob disease. *N Engl J Med* 1974;**290**:692.
86. Enari M, Flechsig E, Weissmann C. Scrapie prion protein accumulation by scrapie-infected neuroblastoma cells abrogated by exposure to a prion protein antibody. *Proc Natl Acad Sci U S A* 2001;**98**:9295.
87. Evatt B, Austin H, Barnhart E, et al. Surveillance for Creutzfeldt-Jakob disease among persons with hemophilia. *Transfusion* 1998; **38**:817.
88. Farlow MR, Yee RD, Dlouhy SR, et al. Gerstmann-Sträussler-Scheinker disease, part I: extending the clinical spectrum. *Neurology* 1989;**39**:1446.
89. Finkenstaedt M, Szudra A, Zerr I, et al. MR imaging of Creutzfeldt-Jakob disease. *Radiology* 1996;**199**:793.
90. Fong IW, Britton CB, Luinstra KE, et al. Diagnostic value of detecting JC virus DNA in cerebrospinal fluid of patients with progressive multifocal leukoencephalopathy. *J Clin Microbiol* 1995;**33**:484.
91. Fournier JG, Kopp N, Streichenberger N, et al. Electron microscopy of brain amyloid plaques from a patient with new variant Creutzfeldt-Jakob disease. *Acta Neuropathol* 2000;**99**:637.
92. Frosh A, Smith LC, Jackson CJ, et al. Analysis of 2000 consecutive UK tonsillectomy specimens for disease-related prion protein. *Lancet* 2004;**364**:1260.
93. Frost B, Diamond M. The expanding realm of prion phenomena in neurodegenerative disease. *Prion* 2009;**3**:74–7.
94. Gabizon R, Telling G, Meiner Z, et al. Insoluble wild-type and protease-resistant mutant prion protein in brains of patients with inherited prion disease. *Nat Med* 1996;**2**:59.
95. Gajdusek DC, Gibbs CJ Jr. Transmission of two subacute spongiform encephalopathies of man (kuru and Creutzfeldt-Jakob disease) to new world monkeys. *Nature* 1971;**230**:588.
96. Gajdusek DC, Gibbs CJ Jr. Transmission of kuru from man to rhesus monkey *(Macaca mulatta)* 8 and one-half years after inoculation. *Nature* 1972;**240**:351.
97. Gajdusek DC, Gibbs CJ Jr, Alpers M. Transmission and passage of experimental "kuru" to chimpanzees. *Science* 1967;**155**:212.
98. Gallassi R, Morreale A, Montagna P, et al. Fatal familial insomnia: behavioral and cognitive features. *Neurology* 1996;**46**:935.
99. Gambetti P, Puoti G, Zou W. Variably protease-sensitive prionopathy: a novel disease of the prion protein. *J Mol Neurosci* 2011;**45**:422–4.
100. Gavrin L, Denny R, Saiah E. Small molecules that target protein misfolding. *J Med Chem* 2012;**55**:10823–43.
101. Georgsson G, Sigurdarson S, Brown P. Infectious agent of sheep scrapie may persist in the environment for at least 16 years. *J Gen Virol* 2006;**87**:3737.
102. Ghani AC, Ferguson NM, Donnelly CA, et al. Predicted vCJD mortality in Great Britain. *Nature* 2000;**406**:583.
103. Gibbs CJ. Virus-induced subacute degenerative diseases of the central nervous system. *Ophthalmology* 1980;**87**:1208.
104. Gibbs CJ, Asher DM, Kobrine A, et al. Transmission of Creutzfeldt-Jakob disease to a chimpanzee by electrodes contaminated during neurosurgery. *J Neurol Neurosurg Psychiatry* 1994; **57**:757.
105. Goldfarb LG. Kuru: the old epidemic in a new mirror. *Microbes Infect* 2002;**4**:875.
106. Goldfarb LG, Brown P, McCombie WR, et al. Transmissible familial Creutzfeldt-Jakob disease associated with five, seven, and eight extra octapeptide coding repeats in the PRNP gene. *Proc Natl Acad Sci U S A* 1991;**88**:10926.
107. Goldfarb LG, Korczyn AD, Brown P, et al. Mutation in codon 200 of scrapie amyloid precursor gene linked to Creutzfeldt-Jakob disease in Sephardic Jews of Libyan and non-Libyan origin. *Lancet* 1990;**336**:637.
108. Gore SM. Commentary: age-related exposure of patients to the agent of BSE should not be downplayed. *BMJ* 1997;**315**:395.
109. Green A, Sanchez-Juan P, Ladogana A, et al. CSF analysis in patients with sporadic CJD and other transmissible spongiform encephalopathies. *Eur J Neurol* 2007;**14**:121.

110. Green AJ, Thompson EJ, Stewart GE, et al. Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt-Jakob disease. *J Neurol Neurosurg Psychiatry* 2001; **70**:744.
111. Gregersen N, Bross P, Jorgensen MM, et al. Defective folding and rapid degradation of mutant proteins is a common disease mechanism in genetic disorders. *J Inherit Metab Dis* 2000;**23**:441.
112. Griffin DE, Ubol S, Despres P, et al. Role of antibodies in controlling alphavirus infection of neurons. *Curr Top Microbiol Immunol* 2001;**260**:191.
113. Griffith JS. Self-replication and scrapie. *Nature* 1967;**215**:1043.
114. Hadlow WJ. Scrapie and kuru. *Lancet* 1959;**2**:289.
115. Heckmann JG, Lang CJ, Petruch F, et al. Transmission of Creutzfeldt-Jakob disease via a corneal transplant. *J Neurol Neurosurg Psychiatry* 1997;**63**:388.
116. Hegde RS, Mastrianni JA, Scott MR, et al. A transmembrane form of the prion protein in neurodegenerative disease. *Science* 1998;**279**:827.
117. Heim D, Wilesmith JW. Surveillance of BSE. *Arch Virol* 2000;**16**:127.
118. Heppner FL, Christ AD, Klein MA, et al. Transepithelial prion transport by M cells. *Nat Med* 2001;**7**:976.
119. Hewitt PE, Llewelyn CA, Mackenzie J, et al. Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study. *Vox Sang* 2006;**91**:221.
120. Hewitt PE, Llewelyn CA, Mackenzie J, et al. Three reported cases of variant Creutzfeldt-Jakob disease transmission following transfusion of labile blood components. *Vox Sang* 2006;**91**:348.
121. Hill AF, Antoniou M, Collinge J. Protease-resistant prion protein produced in vitro lacks detectable infectivity. *J Gen Virol* 1999;**80**:11,
122. Hill AF, Butterworth RJ, Joiner S, et al. Investigation of variant Creutzfeldt-Jakob disease and other human prion diseases with tonsil biopsy samples. *Lancet* 1999;**353**:183.
123. Hill AF, Collinge J. Subclinical prion infection. *Trends Microbiol* 2003;**11**:578.
124. Hill AF, Desbruslais M, Joiner S, et al. The same prion strain causes vCJD and BSE. *Nature* 1997;**389**:448.
125. Hilton DA. Pathogenesis and prevalence of variant Creutzfeldt-Jakob disease. *J Pathol* 2006;**208**:134.
126. Hilton DA, Fathers E, Edwards P, et al. Prion immunoreactivity in appendix before clinical onset of variant Creutzfeldt-Jakob disease. *Lancet* 1998;**352**:703.
127. Hilton DA, Sutak J, Smith ME, et al. Specificity of lymphoreticular accumulation of prion protein for variant Creutzfeldt-Jakob disease. *J Clin Pathol* 2004;**57**:300.
128. Hogan RN, Brown P, Heck E, et al. Risk of prion disease transmission from ocular donor tissue transplantation. *Cornea* 1999; **18**:2.
129. Horiuchi M, Priola SA, Chabry J, et al. Interactions between heterologous forms of prion protein: binding, inhibition of conversion, and species barriers. *Proc Natl Acad Sci U S A* 2000;**97**: 5836.
130. Houston F, Foster JD, Chong A, et al. Transmission of BSE by blood transfusion in sheep. *Lancet* 2000;**356**:999.
131. Hsiao K, Dlouhy SR, Farlow MR, et al. Mutant prion proteins in Gerstmann-Sträussler-Scheinker disease with neurofibrillary tangles. *Nat Genet* 1992;**1**:68.
132. Hsiao K, Meiner Z, Kahana E, et al. Mutation of the prion protein in Libyan Jews with Creutzfeldt-Jakob disease. *N Engl J Med* 1991;**324**:1091.
133. Hsiao K, Prusiner SB. Inherited human prion diseases. *Neurology* 1990;**40**:1820.
134. Hsich G, Kenney K, Gibbs CJ, et al. The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. *N Engl J Med* 1996;**335**:924.
135. Ironside JW. Pathology of variant Creutzfeldt-Jakob disease. *Arch Virol* 2000;(Suppl. 143).
136. Ironside JW, Bell JE. Florid plaques and new variant Creutzfeldt-Jakob disease. *Lancet* 1997;**350**:1475.
137. Jackson GS, Murray I, Hosszu LL, et al. Location and properties of metal-binding sites on the human prion protein. *Proc Natl Acad Sci U S A* 2001;**98**:8531.
138. Jansen C, Parchi P, Capellari S, et al. Human prion diseases in the Netherlands (1998-2009): clinical, genetic and molecular aspects. *PLoS One* 2012;**7**:e36333.
139. Kaeser PS, Klein MA, Schwarz P, et al. Efficient lymphoreticular prion propagation requires PrP^c in stromal and hematopoietic cells. *J Virol* 2001;**75**:7097.
140. Kahana E, Alter M, Braham J, et al. Creutzfeldt-Jakob disease: focus among Libyan Jews in Israel. *Science* 1974;**183**:90.
141. Kaneko K, Zulianello L, Scott M, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. *Proc Natl Acad Sci U S A* 1997;**94**:10069.
142. Kimberlin RH, Walker CA. Intraperitoneal infection with scrapie is established within minutes of injection and is non-specifically enhanced by a variety of different drugs. *Arch Virol* 1990;**112**:103.
143. Kimberlin RH, Walker CA, Fraser H. The genomic identity of different strains of mouse scrapie is expressed in hamsters and preserved on reisolation in mice. *J Gen Virol* 1989;**70**:2017.
144. Kitamoto T, Muramoto T, Mohri S, et al. Abnormal isoform of prion protein accumulates in follicular dendritic cells in mice with Creutzfeldt-Jakob disease. *J Virol* 1991;**65**:6292.
145. Kitamoto T, Tateishi J, Tashima T, et al. Amyloid plaques in Creutzfeldt-Jakob disease stain with prion protein antibodies. *Ann Neurol* 1986;**20**:204.
146. Klein M, Mach M. The neutralizing antibody response against HCMV is strain specific. 6th International Cytomegalovirus Workshop, Orange Beach, Alabama.
147. Klein MA, Kaeser PS, Schwarz P, et al. Complement facilitates early prion pathogenesis. *Nat Med* 2001;**7**:488.
148. Klitzman RL, Alpers MP, Gajdusek C. The natural incubation period of kuru and the episodes of transmission in three clusters of patients. *Neuroepidemiology* 1984;**3**:3.
149. Kocisko DA, Come JH, Priola SA, et al. Cell-free formation of protease-resistant prion protein. *Nature* 1994;**370**:471.
150. Kocisko DA, Priola SA, Raymond GJ, et al. Species specificity in the cell-free conversion of prion protein to protease-resistant forms: a model for the scrapie species barrier. *Proc Natl Acad Sci U S A* 1995;**92**:3923.
151. Kosco-Vilbois MH. Follicular dendritic cells: a license to tangle with scrapie. *Immunol Today* 2000;**21**:468.
152. Krasnianski A, Meissner B, Heinemann U, et al. Clinical findings and diagnostic tests in Creutzfeldt-Jakob disease and variant Creutzfeldt-Jakob disease. *Folia Neuropathol* 2004;**42**(Suppl. B):24.
153. Krasnoslobodtsev A, Portillo A, Deckert-Gaudig T, et al. *Prion* 2010;**4**(4):265–74.
154. Lasmezas CI, Fournier JG, Nouvel V, et al. Adaptation of the bovine spongiform encephalopathy agent to primates and comparison with Creutzfeldt-Jakob disease: implications for human health. *Proc Natl Acad Sci U S A* 2001;**98**:4142.
155. Lavelle GC, Sturman L, Hadlow WJ. Isolation from mouse spleen of cell populations with high specific infectivity for scrapie virus. *Infect Immun* 1972;**5**:319.
156. Lee DC, Stenland CJ, Miller JL, et al. A direct relationship between the partitioning of the pathogenic prion protein and transmissible spongiform encephalopathy infectivity during the purification of plasma proteins. *Transfusion* 2001;**41**:449.
157. Lee HS, Brown P, Cervenakova L, et al. Increased susceptibility to kuru of carriers of the PRNP 129 methionine/methionine genotype. *J Infect Dis* 2001;**183**:192.
158. Liberski PP, Barcikowska M, Cervenakova L, et al. A case of sporadic Creutzfeldt-Jakob disease with a Gerstmann-Sträussler-Scheinker phenotype but no alterations in the PRNP gene. *Acta Neuropathol* 1998;**96**:425.
159. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. *Lancet* 2004;**363**:417.
160. Lloyd SE, Onwuazor ON, Beck JA, et al. Identification of multiple quantitative trait loci linked to prion disease incubation period in mice. *Proc Natl Acad Sci U S A* 2001;**98**:6279.
161. Lugaresi E, Medori R, Montagna P, et al. Fatal familial insomnia and dysautonomia with selective degeneration of thalamic nuclei. *N Engl J Med* 1986;**315**:997.
162. Mabbott NA, Bruce ME, Botto M, et al. Temporary depletion of complement component C3 or genetic deficiency of C1q significantly delays onset of scrapie. *Nat Med* 2001;**7**:485.

162a. Mabbott NA. Prion pathogenesis and secondary lymphoid organs (SLO): tracking the SLO spread of prions to the brain. *Prion* 2012;**6**:322–33.

163. Mabbott NA, Young J, McConnell I, et al. Follicular dendritic cell dedifferentiation by treatment with an inhibitor of the lymphotoxin pathway dramatically reduces scrapie susceptibility. *J Virol* 2003;**77**:6845.
164. Macfarlane RG, Wroe SJ, Collinge J, et al. Neuroimaging findings in human prion disease. *J Neurol Neurosurg Psychiatry* 2007;**78**:664.
165. Manetto V, Medori R, Cortelli P, et al. Fatal familial insomnia: clinical and pathologic study of five new cases. *Neurology* 1992;**42**:312.
166. Manuelidis EE, Gorgacs EJ, Manuelidis L. Viremia in experimental Creutzfeldt-Jakob disease. *Science* 1978;**200**:1069.
167. Manuelidis L. Decontamination of Creutzfeldt-Jakob disease and other transmissible agents. *J Neurovirol* 1997;**3**:62.
168. Manuelidis L. A 25-nm virion is the likely cause of transmissible spongiform encephalopathies. *J Cell Biochem* 2007;**100**:897.
169. Manuelidis L, Yu ZX, Banquero N, et al. Cells infected with scrapie and Creutzfeldt-Jakob disease agents produce intracellular 25-nm virus-like particles. *Proc Natl Acad Sci U S A* 2007;**104**:1965.
170. Manuelidis L, Yun Lu Z. Attenuated Creutzfeldt-Jakob disease agents can hide more virulent infections. *Neurosci Lett* 2000; **293**:163.
171. Masters CL, Harris JO, Gajdusek DC, et al. Creutzfeldt-Jakob disease: patterns of worldwide occurrence and the significance of familial and sporadic clustering. *Ann Neurol* 1979;**5**:177.
172. Mastrianni JA, Nixon R, Layzer R, et al. Prion protein conformation in a patient with sporadic fatal insomnia. *N Engl J Med* 1999;**340**:1630.
173. Mawhinney S, Pape WJ, Forster JE, et al. Human prion disease and relative risk associated with chronic wasting disease. *Emerg Infect Dis* 2006;**12**:1527.
173a. McGuire LI, Peden AH, Orru CD, et al. Real time quaking-induced conversion analysis of cerebrospinal fluid in sporadic Creutzfeldt-Jakob disease. *Ann Neurol* 2012;**72**:278–85.
174. McKinley MP, Bolton DC, Prusiner SB. A protease-resistant protein is a structural component of the scrapie prion. *Cell* 1983;**35**:57.
175. Mead S, Stumpf MP, Whitfield J, et al. Balancing selection at the prion protein gene consistent with prehistoric kurulike epidemics. *Science* 2003;**300**:640.
175a. Mead S, Whitfield J, Poulter M, et al. A novel protective prion protein variant that colocalizes with kuru exposure. *N Engl J Med* 2009;**361**:2056–65.
175b. Mead S, Whitfield J, Poulter M, et al. Genetic susceptibility, evolution and the kuru epidemic. *Philos Trans R Soc Lond B Biol Sci* 2008;**363**:3741–6.
176. Medori R, Tritschler HJ, LeBlanc A, et al. Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N Engl J Med* 1992;**326**:444.
177. Meissner B, Kallenberg K, Sanchez-Juan P, et al. MRI lesion profiles in sporadic Creutzfeldt-Jakob disease. *Neurology* 2009;**72**: 1994–2001.
178. Mikol J, Deslys J, Zou W, et al. Creutzfeldt-Jakob disease with unusually extensive neuropathology in a child treated with native human growth hormone. *Clin Neuropathol* 2012;**31**:127–34.
179. Miller G. Neurodegeneration: could they all be prion disease? *Science* 2009;**326**:1337–9.
180. Monreal J, Collins GH, Masters CL, et al. Creutzfeldt-Jakob disease in an adolescent. *J Neurol Sci* 1981;**52**:341.
181. Montagna P, Cortelli P, Avoni P, et al. Clinical features of fatal familial insomnia: phenotypic variability in relation to a polymorphism at codon 129 of the prion protein gene. *Brain Pathol* 1998;**8**:515.
182. Montrasio F, Cozzio A, Flechsig E, et al. B lymphocyte–restricted expression of prion protein does not enable prion replication in prion protein knockout mice. *Proc Natl Acad Sci U S A* 2001;**98**:4034.
183. Montrasio F, Frigg R, Glatzel M, et al. Impaired prion replication in spleens of mice lacking functional follicular dendritic cells. *Science* 2000;**288**:1257.
184. Nakamura Y, Yanagawa H, Kitamoto T, et al. Epidemiologic features of 65 Creutzfeldt-Jakob disease patients with a history of cadaveric dura mater transplantation in Japan. *Epidemiol Infect* 2000;**125**:201.
185. Nomura E, Harada T, Kurokawa K, et al. Creutzfeldt-Jakob disease associated with autonomic nervous system dysfunction in the early stage. *Intern Med* 1997;**36**:492.
186. Nozaki I, Hamaguchi T, Sanjo N. Prospective 10-year surveillance of human prion disease in Japan. *Brain* 2010;**133**: 3043–57.
187. Palmer MS, Dryden AJ, Hughes JT, et al. Homozygous prion protein genotype predisposes to sporadic Creutzfeldt-Jakob disease. *Nature* 1991;**352**:340.
188. Parchi P, Capellari S, Chin S, et al. A subtype of sporadic prion disease mimicking fatal familial insomnia. *Neurology* 1999;**52**:1757.
188a. Parchi P, Capellari S. Prion disease: diagnostic value of cerebrospinal fluid markers. *Nat Rev Neurol* 2013;**9**:10–11.
189. Parchi P, Castellani R, Cortelli P, et al. Regional distribution of protease-resistant prion protein in fatal familial insomnia. *Ann Neurol* 1995;**38**:21.
190. Parchi P, Chen SG, Brown P, et al. Different patterns of truncated prion protein fragments correlate with distinct phenotypes in P102L Gerstmann-Sträussler-Scheinker disease. *Proc Natl Acad Sci U S A* 1998;**95**:8322.
191. Parker CA, Weber JM. An enzyme-linked immunosorbent assay for the detection of IgG and IgM antibodies to human herpesvirus type 6. *J Virol Methods* 1993;**41**:265.
192. Peretz D, Williamson RA, Kaneko K, et al. Antibodies inhibit prion propagation and clear cell cultures of prion infectivity. *Nature* 2001;**412**:739.
193. Piccardo P, Manson JC, King D, et al. Accumulation of prion protein in the brain that is not associated with transmissible disease. *Proc Natl Acad Sci U S A* 2007;**104**:4712.
194. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. *Science* 1982;**216**:136.
195. Prusiner SB. Prion diseases and the BSE crisis. *Science* 1997; **278**:245.
196. Prusiner SB. Shattuck lecture—neurodegenerative diseases and prions. *N Engl J Med* 2001;**344**:1516.
197. Prusiner SB, Groth DF, Bolton DC, et al. Purification and structural studies of a major scrapie prion protein. *Cell* 1984;**38**:127.
198. Prusiner SB, Groth D, Serban A, et al. Ablation of the prion protein (PrP) gene in mice prevents scrapie and facilitates production of anti-PrP antibodies. *Proc Natl Acad Sci U S A* 1993;**90**: 10608.
199. Prusiner SB, Scott MR. Genetics of prions. *Annu Rev Genet* 1997;**31**:139.
200. Race R, Meade-White K, Raines A, et al. Subclinical scrapie infection in a resistant species: persistence, replication, and adaptation of infectivity during four passages. *J Infect Dis* 2002;**186**(Suppl. 2):S166.
201. Race R, Oldstone M, Chesebro B. Entry versus blockade of brain infection following oral or intraperitoneal scrapie administration: role of prion protein expression in peripheral nerves and spleen. *J Virol* 2000;**74**:828.
202. Race R, Raines A, Raymond GJ, et al. Long-term subclinical carrier state precedes scrapie replication and adaptation in a resistant species: analogies to bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease in humans. *J Virol* 2001;**75**:10106.
203. Raymond GJ, Hope J, Kocisko DA, et al. Molecular assessment of the potential transmissibilities of BSE and scrapie to humans. *Nature* 1997;**388**:285.
204. Requena JR, Groth D, Legname G, et al. Copper-catalyzed oxidation of the recombinant SHa(29-231) prion protein. *Proc Natl Acad Sci U S A* 2001;**98**:7170.
205. Richard M, Biacabe AG, Perret-Liaudet A, et al. Protection of personnel and environment against Creutzfeldt-Jakob disease in pathology laboratories. *Clin Exp Pathol* 1999;**47**:192.
206. Richt JA, Kunkle RA, Alt D, et al. Identification and characterization of two bovine spongiform encephalopathy cases diagnosed in the United States. *J Vet Diagn Invest* 2007;**19**:142.
207. Rideout WM 3rd, Wakayama T, Wutz A, et al. Generation of mice from wild-type and targeted ES cells by nuclear cloning. *Nat Genet* 2000;**24**:109.
208. Rohwer RG. The scrapie agent: "a virus by any other name." *Curr Top Microbiol Immunol* 1991;**172**:195.
209. Roose R, Gajdusek DC, Gibbs CJ. The clinical characteristics of transmissible Creutzfeldt-Jakob disease. *Brain* 1973;**96**:1.
210. Rutala WA, Weber DJ. Creutzfeldt-Jakob disease: recommendations for disinfection and sterilization. *Clin Infect Dis* 2001;**32**: 1348.
211. Safar J. Molecular pathogenesis of sporadic prion diseases in man. *Prion* 2012;**6**:108–15.

212. Safar J, Cohen FE, Prusiner SB. Quantitative traits of prion strains are enciphered in the conformation of the prion protein. *Arch Virol* 2000;(Suppl. 227).
213. Safar J, Wille H, Itri V, et al. Eight prion strains have PrP^{sc} molecules with different conformations. *Nat Med* 1998;**4**:1157.
214. Salazar AM, Masters CL, Gajdusek DC, et al. Syndromes of amyotrophic lateral sclerosis and dementia: relation to transmissible Creutzfeldt-Jakob disease. *Ann Neurol* 1983;**14**:17.
215. Sanchez-Juan P, Green A, Ladogana A, et al. CSF tests in the differential diagnosis of Creutzfeldt-Jakob disease. *Neurology* 2006;**67**:637.
216. Shaked Y, Rosenmann H, Hijazi N, et al. Copper binding to the PrP isoforms: a putative marker of their conformation and function. *J Virol* 2001;**75**:7872.
217. Shlomchik MJ, Radebold K, Duclos N, et al. Neuroinvasion by a Creutzfeldt-Jakob disease agent in the absence of B cells and follicular dendritic cells. *Proc Natl Acad Sci U S A* 2001;**98**:9289.
218. Sigurdsson B. Rida, a chronic encephalitis of sheep, with general remarks on infections which develop slowly and some of their special characteristics. *Br Vet J* 1954;**110**:341.
219. Silva J, Vieira T, Gomes M, et al. Ligand binding and hydration in protein misfolding: insights from studies of prion and p53 tumor suppressor proteins. *Acc Chem Res* 2010;**43**:271–9.
220. Smith PG. The epidemics of bovine spongiform encephalopathy and variant Creutzfeldt-Jakob disease: current status and future prospects. *Bull World Health Organ* 2003;**81**:123.
221. Smith PG, Bradley R. Bovine spongiform encephalopathy (BSE) and its epidemiology. *Br Med Bull* 2003;**66**:185.
222. Soldevila M, Andres AM, Ramirez-Soriano A, et al. The prion protein gene in humans revisited: lessons from a worldwide resequencing study. *Genome Res* 2006;**16**:231.
223. Spencer JV, Lockridge KM, Barry PA, et al. Potent immunosuppressive activities of cytomegalovirus-encoded interleukin-10. *J Virol* 2002;**76**:1285.
224. Spudich S, Mastrianni JA, Wrensch M, et al. Complete penetrance of Creutzfeldt-Jakob disease in Libyan Jews carrying the E200K mutation in the prion protein gene. *Mol Med* 1995;**1**:607.
225. Stahl N, Baldwin MA, Burlingame AL, et al. Identification of glycoinositol phospholipid linked and truncated forms of the scrapie prion protein. *Biochemistry* 1990;**29**:8879.
226. Stahl N, Borchelt DR, Hsiao K, et al. Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* 1987;**51**:229.
227. Stahl N, Borchelt DR, Prusiner SB. Differential release of cellular and scrapie prion proteins from cellular membranes by phosphatidylinositol-specific phospholipase C. *Biochemistry* 1990; **29**:5405.
228. Steinhoff BJ, Racker S, Herrendorf G, et al. Accuracy and reliability of periodic sharp wave complexes in Creutzfeldt-Jakob disease. *Arch Neurol* 1996;**53**:162.
228a. Stohr J, Watts JC, Legname G, et al. Spontaneous generation of anchorless prions in transgenic mice. *Proc Natl Acad Sci U S A* 2011;**108**:21223–8.
229. Tanaka M, Chien P, Yonekura K, et al. Mechanism of cross-species prion transmission: an infectious conformation compatible with two highly divergent yeast prion proteins. *Cell* 2005; **121**:49.
230. Tateishi J, Brown P, Kitamoto T, et al. First experimental transmission of fatal familial insomnia. *Nature* 1995;**376**:434.
231. Taylor DM, Fernie K, McConnell I, et al. Solvent extraction as an adjunct to rendering: the effect on BSE and scrapie agents of hot solvents followed by dry heat and steam. *Vet Rec* 1998; **143**:6.
232. Telling GC, Scott M, Hsiao KK, et al. Transmission of Creutzfeldt-Jakob disease from humans to transgenic mice expressing chimeric human-mouse prion protein. *Proc Natl Acad Sci U S A* 1994;**91**:9936.
233. Telling GC, Scott M, Mastrianni J, et al. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. *Cell* 1995;**83**:79.
234. Thurnher MM, Thurnher SA, Muhlbauer B, et al. Progressive multifocal leukoencephalopathy in AIDS: initial and follow-up CT and MRI. *Neuroradiology* 1997;**39**:611.
235. Tian H, Zhang J, Lang S, Wang X. MRI sequence findings in sporadic Creutzfeldt-Jakob disease. *J Clin Neurosci* 2010;**17**: 1378–80.
236. Tomazin R, Boname J, Hegde NR, et al. Cytomegalovirus US2 destroys two components of the MHC class II pathway, preventing recognition by $CD4^+$ T cells. *Nat Med* 1999;**5**:1039.
237. Trejo SR, Hotta JA, Lebing W, et al. Evaluation of virus and prion reduction in a new intravenous immunoglobulin manufacturing process. *Vox Sang* 2003;**84**:176.
238. Trevitt CR, Collinge J. A systematic review of prion therapeutics in experimental models. *Brain* 2006;**129**:2241.
239. Turner A, Bruun B, Minson T, et al. Glycoproteins gB, gD, and gHgL of herpes simplex virus type 1 are necessary and sufficient to mediate membrane fusion in a Cos cell transfection system. *J Virol* 1998;**72**:873.
240. Verity CM, Nicoll A, Will RG, et al. Variant Creutzfeldt-Jakob disease in UK children: a national surveillance study. *Lancet* 2000;**356**:1224.
241. VerCauteren K, Pilon J, Nash P, et al. Prion remains infectious after passage through digestive system of American crows *(Corvus brachyrhynchos)*. *PLoS One* 2012;**7**(10):e45774.
242. Wadsworth JD, Asante EA, Desbruslais M, et al. Human prion protein with valine 129 prevents expression of variant CJD phenotype. *Science* 2004;**306**:1793.
243. Wadsworth JD, Collinge J. Update on human prion disease. *Biochim Biophys Acta* 2007;**1772**:598.
244. Wadsworth JD, Jackson GS, Hill AF, et al. Molecular biology of prion propagation. *Curr Opin Genet Dev* 1999;**9**:338.
245. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001;**358**:171.
246. Walker LC, LeVine H. The cerebral proteopathies: neurodegenerative disorders of protein conformation and assembly. *Mol Neurobiol* 2000;**21**:83.
247. Watts JC, Balachandran A, Westaway D. The expanding universe of prion diseases. *PLOS Pathog* 2006;**2**:e26.
248. Weissmann C. The state of the prion. *Nat Rev Microbiol* 2004;**2**:861.
249. Wells GA, Hawkins SA, Green RB, et al. Preliminary observations on the pathogenesis of experimental bovine spongiform encephalopathy (BSE): an update. *Vet Rec* 1998;**142**:103.
250. White AR, Enever P, Tayebi M, et al. Monoclonal antibodies inhibit prion replication and delay the development of prion disease. *Nature* 2003;**422**:80.
251. Wilesmith JW. Bovine spongiform encephalopathy. *Vet Rec* 1988;**122**:614.
252. Will RG, Cousens SN, Farrington CP, et al. Deaths from variant Creutzfeldt-Jakob disease. *Lancet* 1999;**353**:979.
253. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. *Lancet* 1996;**347**:921.
254. Will RG, Kimberlin RH. Creutzfeldt-Jakob disease and the risk from blood or blood products. *Vox Sang* 1998;**75**:178.
255. Windl O, Dempster M, Estibeiro JP, et al. Genetic basis of Creutzfeldt-Jakob disease in the United Kingdom: a systematic analysis of predisposing mutations and allelic variation in the *PRNP* gene. *Hum Genet* 1996;**98**:259.
256. Wine JJ. The genesis of cystic fibrosis lung disease. *J Clin Invest* 1999;**103**:309.
257. Wong BS, Chen SG, Colucci M, et al. Aberrant metal binding by prion protein in human prion disease. *J Neurochem* 2001;**78**:1400.
258. Woolhouse ME, Anderson RM. Understanding the epidemiology of BSE. *Trends Microbiol* 1997;**5**:421.
259. Wroe SJ, Pal S, Siddique D, et al. Clinical presentation and premortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report. *Lancet* 2006;**368**:2061.
260. Zeidler M, Johnstone EC, Bamber RW, et al. New variant Creutzfeldt-Jakob disease: psychiatric features. *Lancet* 1997; **350**:908.
261. Zerr I, Bodemer M, Gefeller O, et al. Detection of 14-3-3 protein in the cerebrospinal fluid supports the diagnosis of Creutzfeldt-Jakob disease. *Ann Neurol* 1998;**43**:32.

SECTION **XVIII**

CHLAMYDIA

CHAPTER 193

Chlamydia Infections

Margaret R. Hammerschlag • Stephan A. Kohlhoff

Chlamydiae are obligate intracellular pathogens that have established a unique niche within the host cell. They cause a variety of diseases in animal species at virtually all phylogenic levels. In 1999, Everett and colleagues[40,46] reported a taxonomic analysis involving the 16S and 23S rRNA genes that found that the order Chlamydiales contained at least four distinct groups at the family level and that within the family Chlamydiaceae there were two distinct lineages; they suggested splitting of the genus *Chlamydia* into two genera, *Chlamydia* and *Chlamydophila*. This classification was not universally accepted, and recently it was agreed that the family Chlamydiaceae contains a single genus, *Chlamydia*.[58] This position has been supported by additional data on *Chlamydia* genome sequences. The genus *Chlamydia* now contains nine recognized species: *C. trachomatis*, *C. psittaci*, *C. pneumoniae*, *C. pecorum* (infects cattle, other ruminants, and koalas),[40,46] *C. muridarum* (formerly the agent of mouse pneumonitis), *C. suis* (an important pathogen of swine), *C. abortus* (causes abortion in cattle and sheep; rarely has caused abortion in humans), *C. caviae* (formerly *C. psittaci* guinea pig conjunctivitis strain), and *C. felis* (causes epidemic keratoconjunctivitis in cats). *C. trachomatis* and *C. pneumoniae* are the most significant human pathogens, and *C. psittaci* is an important zoonosis.

Recently, several chlamydia-like organisms that are endosymbionts of free-living amoebae have been identified.[59] These organisms, which include *Parachlamydia acanthamoebae*, *Simkania negavensis*, and *Neochlamydia hartmannellae*, have been termed *environmental chlamydiae*.[59,100] Analyses of nearly full-length 16S rRNA gene sequences of these isolates showed that they clustered with other members of the order Chlamydiales but in a lineage separate from those of *Chlamydia* (16S rRNA sequence similarities >88%). Data on the potential role of these organisms in human disease are limited. This bacteria–protozoa interaction might have been a driving force for the development of effective mechanisms by bacteria to survive phagocytosis by unicellular eukaryotes, which in turn may have been a first step in the evolution of intracellular bacterial pathogens of higher organisms.[32,123]

Chlamydiae are characterized by a unique developmental cycle with morphologically distinct infectious and reproductive forms—the elementary body and the reticulate body. They have a gram-negative envelope without detectable peptidoglycan, although recent genomic analysis has revealed that both *C. trachomatis* and *C. pneumoniae* encode proteins forming a nearly complete pathway for the synthesis of peptidoglycan, including penicillin-binding proteins.[85,122,124,136] This finding is the basis for the so-called chlamydial peptidoglycan paradox, given that it has been known for decades that chlamydial development is sensitive to β-lactam antibiotics.[122] Chlamydiae also share a group-specific lipopolysaccharide (LPS) antigen and use host adenosine triphosphate (ATP) for the synthesis of chlamydial protein.[136] Although chlamydiae are auxotrophic for three of four nucleoside triphosphates, they encode functional glucose-catabolizing enzymes that can be used for the generation of ATP. As with peptidoglycan synthesis, for some reason these genes are turned off.[136] All chlamydiae also encode an abundant protein, the major outer membrane protein (MOMP or OmpA), that is surface exposed in *C. trachomatis* and *C. psittaci* but apparently not in *C. pneumoniae*.[136] MOMP is the major determinant of the serologic classification of *C. trachomatis* and *C. psittaci* isolates.

After infection develops, the infectious elementary bodies, which are 200 to 400 μm in diameter, attach to the host cell by a process of electrostatic binding and are taken into the cell by endocytosis that does not depend on the microtubule system. Within the host cell, the elementary body remains within a membrane-lined phagosome. The phagosome does not fuse with the host cell lysosome. The inclusion membrane is devoid of host cell markers, but lipid markers traffic to the inclusion, which suggests functional interaction with the Golgi apparatus. The elementary bodies then differentiate into reticulate bodies that undergo binary fission. After approximately 36 hours, reticulate bodies differentiate into elementary bodies. At approximately 48 hours, release may occur by cytolysis or by a process of exocytosis or extrusion of the whole inclusion, with the host cell left intact. Chlamydiae also may enter a persistent state after treatment with certain cytokines such as interferon-γ, treatment with antibiotics, or restriction of certain nutrients. In the persistent state, metabolic activity is reduced. The ability to cause prolonged, often subclinical infection is one of the major characteristics of chlamydiae.[70]

INFECTIONS CAUSED BY CHLAMYDIA TRACHOMATIS

Epidemiology

C. trachomatis infection is the most prevalent sexually transmitted infection (STI) and infectious disease in the

United States today.[26-28] In 2010, over 1.3 million were reported to the Centers for Disease Control and Prevention (CDC).[27,28] The prevalence of chlamydial infection is associated less with socioeconomic status, urban or rural residence, and race or ethnicity than is the case with gonorrhea and syphilis. Infection with *C. trachomatis* tends to be asymptomatic and of long duration. If a pregnant woman has active infection during delivery, the infant may acquire the infection and, as a consequence, either conjunctivitis or pneumonia.[26,44,71,73,141] Children also may acquire chlamydial infection as a result of sexual abuse.

Chlamydia Trachomatis Infections in the Neonate

History

At the beginning of the 20th century, before expectant mothers began being screened for sexually transmitted diseases (STDs), the term *ophthalmia neonatorum* was, for all practical purposes, synonymous with gonococcal conjunctivitis. As neonatal conjunctivitis came under control with silver nitrate prophylaxis, the importance of another form of ophthalmia neonatorum, *inclusion blennorrhea*, was noted. The relationship between maternal genital infection and conjunctivitis of the newborn associated with inclusion bodies within epithelial cells was established by Thygeson and Stone in 1942.[150] Respiratory infection in infants caused by *C. trachomatis* probably was reported first in 1941 by Botsztejn,[21] who described an entity that he called *pertussoid eosinophilic pneumonia*.

Epidemiology

The rate of cervical infection with *C. trachomatis* during pregnancy varies from 1 to 37 percent, with the highest rates occurring in women 25 years of age and younger. *C. trachomatis* infection is acquired by the infant from the mother during parturition, as demonstrated in multiple well-controlled prospective studies conducted in the 1970s and 1980s of maternal–infant infection in which infection occurred only in infants born to infected mothers.[26,44,71,63,141] No convincing evidence demonstrates horizontal transmission from mother to infant, from other family members to the infant, or from infant to infant after delivery. Infection after cesarean delivery, usually associated with rupture of the membranes, or infection through intact membranes is a rare event but may occur.[12] The overall risk for an infant born to a mother with active chlamydial infection becoming infected at any anatomic site has been reported to be approximately 50 to 75 percent in various studies (Table 193-1).[44,73,141] Infants can be infected at more than one site, including the conjunctiva, nasopharynx, rectum, and vagina. The most frequent clinical manifestation of neonatal chlamydial infection, inclusion conjunctivitis, has been reported to occur in 15 to 37 percent of infants born to mothers with untreated cervical chlamydial infection.[44,73,141] The most frequent site of infection, however, is the nasopharynx, with 78 percent of infected infants having positive nasopharyngeal cultures in one study.[61] Approximately half of infants with inclusion conjunctivitis also will be infected in the nasopharynx. In only a minority of infants with nasopharyngeal infection does chlamydial pneumonia eventually develop; Hammerschlag and colleagues[71] found that pneumonia subsequently developed in only 4/12 (33%) infants with isolated nasopharyngeal infection. The overall risk for pneumonia developing in infants born to chlamydia-positive mothers has been reported to range from 1 to 22 percent.[44,73,141]

Data on the risk for acquiring rectal or vaginal infection are more limited. Bell and colleagues[13] demonstrated that perinatally acquired *C. trachomatis* infection may persist for months to more than 2 years. Twenty-two infants born to women with culture-documented chlamydial infection were monitored, and positive cultures from the nasopharynx and oropharynx in the infants were detected as late as 28.5 months after birth. Rectal and vaginal infections were asymptomatic and persisted for at least 1 year, which can become an important confounding

TABLE 193-1 **Selected Studies of Perinatal Chlamydial Infection**

	Prevalence of Maternal Genital Infection		*Proportion of Infants with Chlamydial Infection Born to Infected Mothers*				
Author, Year, City	**Total**	**No. Infected (%)**	**Total**	**Conjunctivitis (%)**	**Pneumonia (%)**	**NP (%)**	**Rectum/Vagina (%)**
Frommell et al., 1979, Denver[44]	340	30 (8.8)	67	39	11	6	NS
Schachter et al., 1986, San Francisco[141,142]	5531	262 (4.7)	131	17.6	16	11.5	14
Hammerschlag et al., 1989, Brooklyn[73]	4357	341 (7.8)	45	15	1	4	NS

NP, Nasopharynx; *NS,* not studied.

variable when young children are tested for the presence of *C. trachomatis* during evaluation for suspected sexual abuse.

Conjunctivitis. *C. trachomatis* was the most frequent identifiable infectious cause of neonatal conjunctivitis, and conjunctivitis was the major clinical manifestation of neonatal chlamydial infection in the United States in the 1990s. The introduction of systematic screening and treatment of pregnant women has resulted in a dramatic decrease in the number of perinatal chlamydial infections, including conjunctivitis, in the United States.[1,62] However, in countries in which pregnant women are not screened routinely, including many developing countries, *C. trachomatis* remains the most frequent cause of neonatal conjunctivitis.* The incubation period of *C. trachomatis* conjunctivitis is 5 to 14 days after delivery or earlier if premature rupture of membranes has occurred. At least 50 percent of infants with chlamydial conjunctivitis also will have nasopharyngeal infection. The manifestation is extremely variable and ranges from mild conjunctivitis with scant mucoid discharge to severe conjunctivitis with copious purulent discharge, chemosis, and pseudomembrane formation. The conjunctiva can be very friable and may bleed when stroked with a swab. Eyelid erythema and edema frequently are present. A Gram-stained conjunctival smear initially may reveal a predominance of polymorphonuclear leukocytes. Chlamydial conjunctivitis needs to be differentiated from gonococcal ophthalmia in some infants, especially those born to mothers who did not receive any prenatal care, had gonorrhea during pregnancy, or abused drugs. An overlap in both incubation periods and clinical findings can occur. Bilateral infections are present in two thirds of cases. A follicular reaction is not seen because infants younger than 3 months of age do not have the requisite lymphoid tissue present in the conjunctiva.

Pneumonia. As stated previously, approximately 70 percent of infected infants will have positive nasopharyngeal cultures, but the majority of these infections are asymptomatic.[71,138] Chlamydial pneumonia develops in only approximately 30 percent of infants with nasopharyngeal infection.[71] In infants in whom pneumonia does develop, the manifestations and clinical findings are very characteristic.[10,77,151] The children usually are seen initially when they are between the ages of 4 and 12 weeks. A few cases have been reported in infants as young as 2 weeks of age, but no cases have been seen in infants older than 4 months. The infants frequently have a history of cough and congestion, with an absence of fever. On physical examination, the infant is tachypneic, and rales are heard on auscultation of the chest; wheezing is a distinctly uncommon finding.[10,77,151] No specific radiographic findings except hyperinflation are found (Fig. 193-1). Significant laboratory findings include peripheral eosinophilia (>300 cells/cm^3) and elevated serum immunoglobulins. If cultures are performed, infants with *C. trachomatis* pneumonia may remain symptomatic and shed the organism from the nasopharynx for protracted periods.[13,62] Generally, infantile pneumonia caused by *C. trachomatis* appears to be self-limited. Most infants can be managed as outpatients, although a few cases of severe disease requiring hospitalization and assisted ventilation have been reported. *C. trachomatis* pneumonia in infants also appears to be associated with few sequelae, although data are limited. Rarely, infants with *C. trachomatis* pneumonia may have concomitant otitis media.[151]

FIGURE 193-1 ■ Anteroposterior chest radiograph of a severely ill 1-month-old male infant with *Chlamydia trachomatis* pneumonitis. Diffuse interstitial infiltrates and hyperaeration with a flattened diaphragm are prominent.

Diagnosis of *Chlamydia trachomatis* Infection in Infants

The diagnosis of neonatal conjunctivitis cannot be made on clinical grounds alone. Significant overlap in both incubation period and clinical findings occurs with infections by other organisms, especially *Neisseria gonorrhoeae*. In a high-risk population, particularly infants born to women with no prenatal care, gonococcal ophthalmia must be considered seriously.[73] The incubation period for gonococcal conjunctivitis is usually 3 to 5 days, but it can be longer. The incubation period for chlamydial conjunctivitis is approximately 5 to 14 days. Most cases will be evident by the time the infant is 2 weeks of age, which is after the infant leaves the hospital. Epidemiologic clues can help the physician decide whether gonococcal ophthalmia needs to be considered. Hammerschlag and

*References 116, 132, 137, 156, 160, 161.

colleagues[63,73] noted that seven of the eight infants with gonococcal conjunctivitis were born to mothers who had not received prenatal care. Five of these women were abusers of crack cocaine. Another epidemiologic clue is a history of gonorrhea or other STD during pregnancy. The clinical manifestation of *C. trachomatis* pneumonitis in infants is fairly characteristic, and clinical diagnosis may be established with a degree of certainty.

The gold standard for diagnosis of *C. trachomatis* infection in infants and children remains isolation of *C. trachomatis* by culture from the conjunctiva, nasopharynx, vagina, or rectum. There are currently three U.S. Food and Drug Administration (FDA)-approved, commercially available nucleic acid amplification tests (NAATs) for the diagnosis of *C. trachomatis* infection (described in detail in the discussion on infection in older children and adolescents). None are currently approved for use in children. However, available data suggest that NAATs are equivalent to culture for detection of *C. trachomatis* in the conjunctiva and nasopharynx of infants with conjunctivitis.[75,161]

Treatment of Chlamydial Conjunctivitis and Pneumonia in Infants

Oral erythromycin suspension (ethylsuccinate or stearate) 50 mg/kg/day for 14 days is the therapy of choice for the treatment of chlamydial conjunctivitis and pneumonia in infants.[11,26,62] It provides better and faster resolution of the conjunctivitis, in addition to treating any concurrent nasopharyngeal infection, which prevents the development of pneumonia. Additional topical therapy is not needed. The efficacy of this regimen has been reported to range from 80 to 90 percent; however, as many as 20 percent of infants may require another course of therapy.[11,62] Erythromycin given at the same dose for 2 weeks is the treatment of choice for pneumonia and results in clinical improvement and elimination of the organism from the respiratory tract.

Treatment with oral erythromycin has been associated with hypertrophic pyloric stenosis in infants younger than 6 weeks of age who were given the drug for prophylaxis after nursery exposure to pertussis.[33,81] Erythromycin is a motilin receptor agonist. Data on the use of other macrolides, including azithromycin or clarithromycin, for the treatment of neonatal chlamydial infection are limited. No studies of clarithromycin have been published; only one small study evaluated azithromycin and found that a short course of azithromycin suspension 20 mg/kg/day orally, one dose daily for 3 days, was as effective as was 2 weeks of erythromycin for eradication of *C. trachomatis* from the conjunctivae and nasopharynx of infants with conjunctivitis.[66,74]

Prevention and Control Strategies

Because *C. trachomatis* infections are transmitted vertically from mother to infant during delivery, several possible options for intervention exist. Prospective studies of mother-to-infant transmission of *C. trachomatis* have demonstrated that neonatal ocular prophylaxis with silver nitrate, erythromycin, or tetracycline ophthalmic ointment does not prevent the development of chlamydial conjunctivitis.[29,73] The most effective method of control of perinatal *C. trachomatis* infection is screening and treatment of pregnant women.[62,65,114] In the 1980s, McMillan and colleagues[111] and Schachter and coworkers[142] reported that treatment of pregnant women infected with *C. trachomatis* with erythromycin resulted in a dramatic decrease in chlamydial infection (i.e., conjunctivitis and nasopharyngeal infection) in their infants in contrast to infants born to untreated infected women. The introduction of NAATs for diagnosis and the use of well-tolerated antibiotic regimens, including amoxicillin and single-dose azithromycin, have increased the efficacy of prenatal screening and treatment.[26] This approach has been validated by a dramatic decrease in perinatal chlamydial infection in the United States[62,72] and persistence of these infections in countries in which screening and treatment of pregnant women are not standard practice, such as the Netherlands, Ireland, India, and China.[116,132,137,156,160] Rours and colleagues[137] reported that from 1996 through 2001, *C. trachomatis* was responsible for 61 to 64 percent of cases of neonatal conjunctivitis seen in a large university-affiliated hospital in Rotterdam. Even though the rate of *C. trachomatis* infection among pregnant women in Rotterdam exceeded 5 percent, prenatal screening and treatment are not part of routine prenatal care in the Netherlands.

Reasons for failure of maternal treatment to prevent infantile chlamydial infection include poor compliance and reinfection from untreated or new sexual partners. Even with effective screening, some infected women will be missed, depending on the methods used. Additionally, some women do not seek prenatal care.

Infections in Older Children

C. trachomatis has not been associated with any specific clinical syndrome in older infants and children. Most attention given to *C. trachomatis* infection in these children has concentrated on its relationship to sexual abuse. Isolation of *C. trachomatis* from a rectal or genital site in children without previous sexual activity may be a marker of sexual abuse; moreover, evidence for other modes of spread, such as through fomites, is lacking for this organism.[26,63,65,83-86] Perinatal maternal-to-infant transmission resulting in vaginal or rectal infection has been documented, with prolonged infection lasting for periods up to 3 years.[13,139,141] This duration can be an important confounding variable in evaluation of possible sexual abuse in a young child.

Four studies examining the epidemiology of STIs in children and adolescents being evaluated for suspected sexual abuse have been published since 2005. Three were retrospective chart reviews, including one each from Vienna, Austria,[93,95] Auckland, New Zealand[90,92] and Miami, Florida.[143] Despite differences in population and methodologies,[143,145] the results of the retrospective studies were fairly consistent. Charts were examined from a total of 4350 children, who were seen over periods

ranging from 4 to 7 years. The ages ranged from 0 to 17 years, and the overwhelming majority were girls. The prevalence of STIs, specifically gonorrhea and *C. trachomatis*, was low, ranging from 0.4 to 1.8 percent. These findings are consistent with earlier published studies.[37,83-86] Because these were retrospective chart reviews, they had some major limitations. Inclusion criteria varied, and not every child or adolescent was tested for every STI. In the one prospective study, Giradet and colleagues[51,52] examined children 0 to 13 years of age being evaluated for suspected sexual abuse or assault at seven tertiary care centers in the United States (in Houston, Tex, Atlanta, Ga, Harrisburg, Pa, and New York City). All children were tested at multiple sites for *N. gonorrhoeae* and *C. trachomatis* by culture. Vaginal and urethral swabs and urine were also tested using two NAATs. A total of 536 children were enrolled, 485 (90.5%) were girls. None of the 51 boys enrolled were positive for an STI. Overall, 40 (8.2%) of the girls were found to have one or more STIs. *C. trachomatis* was detected by culture and/or NAAT in 15 (3.1%) of the girls enrolled. Sixty percent of the children who were positive for *C. trachomatis* gave a clear, credible, detailed description of molestation.

In the setting of repeated abuse by a family member over a long period, the development of infection would be difficult to demonstrate. The 2010 CDC STD Treatment Guidelines do not recommend that samples for culture of *C. trachomatis* be obtained routinely from the pharynx and urethra in children who are suspected victims of sexual abuse.[26] The major reasons were the low yield from the urethra, the tendency for longer persistence of perinatally acquired pharyngeal infection, and the potential confusion with *C. pneumoniae*.[8]

Genital *Chlamydia trachomatis* Infections in Adolescents

As stated previously, *C. trachomatis* is the most prevalent STI and infectious disease in the United States. At least 70 percent (930,000) of cases were among men and women between 15 and 24 years of age, with the highest rates in women 15 to 19 years of age.[28,43] In sexually active adolescents, the prevalence commonly exceeds 10 percent and may exceed 20 percent.[43] The case rate of infection in females 15 to 19 years of age was 3378.2/100,000, more than 4 times the rate in males the same age (774.3/100,000).[28] The reasons for this sex discrepancy, which is seen in almost every age bracket, are not clear but may include better screening of female adolescents and increased susceptibility to infection in women. More than 70 percent of infections in women are asymptomatic. Reinfection is very common, with as many as 40 percent of adolescent women being reinfected, usually from an untreated partner, within 9 months.[7] Women sustain a disproportionate share of the morbidity from these *C. trachomatis* infections through their well-known complications of pelvic inflammatory disease, ectopic pregnancy, and tubal factor infertility.[26] Although *C. trachomatis* infection can be treated effectively with antibiotics, infections may result in tissue damage even with appropriate antimicrobial therapy. Although most infections are asymptomatic, in men they can cause urethritis and *C. trachomatis* infection is the most frequent cause of epididymitis in men younger than 30 years of age.[26] The symptoms of chlamydial genital tract infections are less acute than those of gonorrhea, consisting of a discharge that is usually mucoid rather than purulent.

Lymphogranuloma Venereum

Lymphogranuloma venereum (LGV) is an STD caused by the L1-3 serovars of *C. trachomatis* that are endemic in tropical and subtropical areas (e.g., parts of Southeast Asia, Africa, South America, India, and the Caribbean) but rare in the United States.[14,26] About 20 cases of LGV have been reported in children in the literature. Fewer than 1000 cases are reported in adults in the United States annually. Recently a resurgence of LGV infections has occurred among men who have sex with men (MSM) in Europe and the United States. Some common characteristics of these outbreaks are (1) it affected predominantly white MSM; (2) it presented mostly as proctocolitis (with no inguinal syndrome); (3) individuals affected had very high human immunodeficiency virus (HIV) coinfection rates (~70%); (4) patients often reported multiple sexual contacts met at casual sex gatherings: (5) 71 percent met partners at sex-on-premises venues or sex parties; and (6) 23 percent met partners via the Internet.[5]

LGV is a disease of the lymphatic tissue that has acute and chronic manifestations. Unlike other anogenital *C. trachomatis* infections, in which replication is confined to mucosal epithelial cells, LGV can replicate in macrophages and produce chronic invasive infection. Progression follows several defined stages, similar to syphilitic infection. The primary lesion of LGV is a small, inconspicuous, asymptomatic genital papule or ulcer that heals without a scar. Days to weeks later, unilateral acute lymphadenitis with bubo formation occurs at the site of lymphatic drainage of the primary lesion; acute hemorrhagic proctitis develops after a rectal lesion. Systemic symptoms can include fever, myalgia, or headache. Approximately one third of inguinal buboes become fluctuant and rupture; the remainder involute slowly. Although most patients recover from LGV after this secondary stage, a small number develop a chronic inflammatory response with fibrosis (as a result of the persistence of *Chlamydia* organisms in anogenital tissues) that can result in chronic genital ulcers or fistulas, rectal strictures, or genital elephantiasis. In females, the vulvar lymph drains to the retroperitoneal nodes; thus, they may not have inguinal lymphadenopathy but rather the genitoanorectal syndrome with rectovaginal fistulas and rectal strictures.

To our knowledge, cases in the pediatric population have not been reported since the emergence of the new clusters of cases in 2003. We reported a case of a 16-year-old boy with LGV proctocolitis after having receptive unprotected anal intercourse with a 30-year-old man he met on the Internet.[5] This history was obtained after the boy was found to be HIV positive. The diagnosis of LGV,

in particular when it presents with proctocolitis, relies on a high index of suspicion that would lead to emphasizing certain aspects of the history and ordering the pertinent diagnostic tests. Many pediatricians and pediatric gastroenterologists might not be familiar with the entity and might not entertain it as a diagnostic consideration in pediatric patients. The diagnosis can be further suggested by *C. trachomatis* testing—culturing the organism or, more commonly, by NAATs. Currently available NAATs will not differentiate LGV from other *C. trachomatis* serovars.[26] NAATs for *C. trachomatis* are also not FDA-cleared for testing rectal specimens. Trying to ascertain the *C. trachomatis* serovar for confirmation of LGV has therapeutic implications, because a single dose of azithromycin is unlikely to eradicate the infection and a 3-week course of doxycycline is the preferred treatment.[26]

Trachoma

Trachoma, the most important preventable cause of blindness in the world, is caused primarily by the A, B, Ba, and C serotypes of *C. trachomatis*.[15] It is endemic in the Middle East and Southeast Asia. The disease is spread from eye to eye via infected secretions. Flies are a frequent vector.

Trachoma begins as a follicular conjunctivitis, usually in early childhood. The follicles heal, leading to conjunctival scarring that may result in entropion, in which the eyelid turns inward so that the lashes abrade the cornea. It is the corneal ulceration secondary to the constant trauma that leads to scarring and blindness. Bacterial superinfection also may contribute to scarring. Blindness occurs years after the active disease.

Trachoma can be diagnosed clinically. The World Health Organization (WHO) suggests that at least 2/4 criteria must be present for a diagnosis of trachoma: (1) lymphoid follicles on the upper tarsal conjunctivae, (2) typical conjunctival scarring, (3) vascular pannus, and (4) limbal follicles.[15] The diagnosis is confirmed by culture, microscopy, or NAAT for *C. trachomatis* performed during the active stage of disease. Serologic tests are not helpful clinically because of the long duration of the disease and the high seroprevalence in endemic populations.

Poverty and lack of sanitation are important factors in the spread of trachoma. As socioeconomic conditions improve, the incidence of the disease decreases substantially. The WHO recommends single-dose azithromycin 20 mg/kg, maximum 1 g, for the treatment of trachoma in children. Numerous studies have demonstrated that mass community treatment with a single dose of azithromycin to all residents of a village dramatically reduced the prevalence and intensity of infection.[15] This effect continued for 2 years after treatment, probably by interrupting the transmission of ocular *C. trachomatis* infection.

Diagnosis of *Chlamydia trachomatis* Infections

Culture remains the gold standard of diagnosis for detection of *C. trachomatis* from the vagina or rectum in prepubertal children. *Chlamydia* culture has been defined further by the CDC as isolation of the organism in tissue culture and confirmation by microscopic identification of the characteristic inclusions by fluorescent antibody staining using a species-specific conjugated monoclonal antibody.[26]

The major advance in the diagnosis of *C. trachomatis* infection during the past decade has been the introduction of NAATs. These tests have high sensitivity, perhaps even detecting 10 to 20 percent more cases than is possible with culture, while retaining high specificity.[26] Currently, three commercially available NAATs are FDA approved and available in the United States: polymerase chain reaction (PCR), transcription-mediated amplification, and strand displacement amplification.[64,75] PCR and strand displacement amplification are DNA amplification tests; both use primers that target gene sequences on the cryptogenic *C. trachomatis* plasmid, which has approximately 10 copies per cell. Transcription-mediated amplification is an RNA amplification assay. NAATs that are currently commercially available have FDA approval for cervical and vaginal swabs from women, urethral swabs from men, and urine samples from men and women. Several studies have demonstrated that self-collected vaginal swabs also are suitable specimens.[26,64,75] *C. trachomatis* DNA and RNA may persist for 9 or more days after treatment.[47]

Even though culture is considered the gold standard for detection of *C. trachomatis* in children being evaluated for suspected sexual abuse, *C. trachomatis* culture is not standardized or regulated in any way. Sensitivity may vary significantly across laboratories. Enzyme immunoassays (EIAs) are not acceptable as confirmatory tests and have been associated with false positive results, especially when used with vaginal and rectal specimens.[65,69,130] The methods used for culture confirmation became an issue when several large commercial laboratories started using an EIA instead of fluorescent antibody staining and visual identification of inclusions for culture confirmation. This has resulted in at least one "outbreak" of *C. trachomatis* infection in the evaluation of suspected sexual abuse among residents and staff of an institution for those with intellectual disabilities in Ohio in 1990.[65,71] All of the "positive" cultures, mostly rectal specimens, were subsequently determined to be false-positives resulting from carryover of fecal material and bacteria in the culture specimens. The major advantage of culture is that it is 100 percent specific; however, because confirmation depends on visual identification of inclusions, a subjective component exists that could lead to misidentification of artifacts as chlamydial inclusions. This appears to have happened in two pseudo-outbreaks in other states, one in a residential institution[60] and one among children being evaluated for suspected sexual abuse.[144] In the latter, specimens for culture were outsourced to a commercial laboratory.

NAATs are not currently approved for the detection of *C. trachomatis* in rectogenital specimens or urine from prepubertal children and rectal specimens in adults.[26,64] Several studies, predominantly from MSM, found NAATs to be sensitive and specific for detection of rectal chlamydial infection. Data on the use of NAATs

for vaginal specimens or urine from children are very limited.[26,64,70] To date, four studies have been published comparing NAATs to *C. trachomatis* culture in children being evaluated for suspected sexual abuse.[16,51,52,91,109] They include the recent multicenter study by Black and colleagues[16,17] that evaluated SDA and TMA. Girardet and colleagues[51] and Kellogg and associates[91] primarily evaluated girls, most of whom were postpubertal. Both studies used ligase chain reaction, which is no longer manufactured. Kellogg and associates[91] also evaluated PCR in addition to LCR. These studies had major limitations—failure to use an independent reference standard in estimating test performance, failure to separately analyze test performance by age and gender, and the majority of girls were postpubertal and many were sexually active. The ages of the children ranged from 1 month to 17 years, with a mean age of 8.6 years, and 82.4 percent were female. Anatomic sites tested included vagina, cervix, rectum, pharynx, male urethra, and urine. The overall prevalence of *C. trachomatis* infection in these studies ranged from 6.6 to 8.3 percent by LCR and/or PCR and 0.8 to 5.3 percent by culture. Many discrepant results were found, especially between culture and NAATs, which were more frequent with rectal specimens, but the numbers of specimens from nongenital sites was too small to accurately assess the performance of the assays at these sites.

The recent CDC-sponsored multicenter study also evaluated the use of SDA and TMA using urine and genital swabs (vagina and urethra) in contrast to culture for diagnosis of *C. trachomatis* in children birth to 13 years of age.[17] Cultures for *C. trachomatis* were performed at the laboratories of each center, according to their own protocols. Culture protocols at all sites included the isolation of *C. trachomatis* in cycloheximide-pretreated McCoy cells, in shell vials or 24-well or 96-well tissue culture plates. The fixed monolayers were stained to detect chlamydial inclusions with fluorescein-conjugated *Chlamydia* genus–specific or *C. trachomatis* species–specific monoclonal antibodies. All samples were processed and tested according to manufacturers' protocols, except for the TMA tests, which were performed on previously frozen urine or swabs collected in BD ProbeTec sample collection medium. Test results that were positive by SDA for *C. trachomatis* were confirmed using in-house PCR targeting the *ompA* gene performed at the CDC. Of 485 female participants, 15 (3.1%) had a positive result for *C. trachomatis* by any test (1.4% by culture; 2.3% by vaginal NAAT; 2.7% by urine NAAT). All participants who had a positive vaginal culture for *C. trachomatis* also had positive urine NAAT. Two girls had positive *C. trachomatis* cultures from rectal swab specimens, but negative vaginal swab specimens by both culture and NAATs and negative urine NAATs. No discrepant results were found in any of the participants tested by SDA and TMA for *C. trachomatis*. All *C. trachomatis*–positive results were confirmed by DNA sequence genotyping. The sensitivity of vaginal culture for *C. trachomatis* was 39 percent in all girls studied. In contrast, the sensitivities of urine and vaginal swab NAATs were 100 and 85 percent in all female children, respectively, for detection of *C. trachomatis*. Based on the results of this study, the 2010 CDC STD Treatment Guidelines now recommend that NAATs can be used for detection of *C. trachomatis* in girls being evaluated for suspected sexual abuse, with important limitations, as follows[26]:

1. Because the prevalence of *C. trachomatis* in this population is low, additional testing is probably necessary.
2. Extrapolation cannot be made from these results to other NAATs, specifically PCR, or used in specimens other than vagina and urine in girls.
3. Recommendations cannot be made on the use of these assays in prepubertal boys.

Some of the more recently available commercial NAATs, such as TMA (Aptima 2), offer an alternative target confirmation method that can be used on the same testing platform; however, no data exist on the use of this confirmatory test in this setting. Additional options include sending blinded specimens to an independent or reference laboratory for confirmation testing, confirming a NAAT-positive result by culture test (requires a separate, invasive specimen), or use of a second, alternative technology commercial NAAT. Specimens collected from children for forensic applications should be retained in the laboratory for purposes of additional testing, in accordance with local policies and procedures. A new generation of NAATs, including highly sensitive point-of-care tests, will be coming to market.[64,82] These assays will need to be evaluated in children, because extrapolation cannot be made from their performance in adults or adolescents.

Treatment

Chlamydial infections in older children may be treated with oral erythromycin 50 mg/kg/day orally four times daily, with a maximum of 2 g/day for 7 to 14 days.[26] Children older than 8 years of age may be treated with tetracycline 25 to 50 mg/kg/day orally four times daily for 7 days. Azithromycin 1 g orally as a single dose also may be used in children who weigh at least 45 kg, are 8 years of age or older, or both.[26]

The first-line treatment regimen recommended by the CDC for uncomplicated *C. trachomatis* genital infection in men and nonpregnant women is azithromycin 1 g orally as a single dose or doxycycline 100 mg orally twice daily for 7 days. Alternative regimens are erythromycin base 500 mg orally four times daily for 7 days, erythromycin ethylsuccinate 800 mg orally four times daily for 7 days), levofloxacin 500 mg orally once daily for 7 days. The high erythromycin dosages may not be well tolerated. Doxycycline and ofloxacin or levofloxacin are contraindicated in pregnant women; quinolones are contraindicated in persons younger than 18 years of age. However, use of levofloxacin offers no advantages over doxycycline. For pregnant women, the recommended treatment regimen is also single-dose azithromycin. Alternative regimens are erythromycin base 500 mg orally twice daily for 7 days, amoxicillin 500 mg orally three times daily for 7 days, erythromycin base 250 mg orally four times daily for 14 days, and erythromycin ethylsuccinate 800 mg orally four times daily for 7 days

or 400 mg orally four times daily for 14 days. Amoxicillin at this dosage is as effective as azithromycin or any of the erythromycin regimens and is much better tolerated.

INFECTION CAUSED BY *CHLAMYDIA PSITTACI*

Human infection with *C. psittaci* was described first probably by Juergensen in 1874 or Ritter in 1876. Ritter described seven cases of an unusual pneumonia that appeared to be caused by parrots and finches that were caged in the study of his brother's home in Switzerland. After these reports, several outbreaks of a similar disease in Europe established the association with exposure to birds. The term *psittacosis* was coined by Morange in 1892 from the Greek word for parrots, *psittakos*.

The Organism

C. psittaci is a diverse species that affects psittacine and nonpsittacine birds.[1] The known host range includes 259 avian species.[25,28,79,126] Birds that have been reported to have *C. psittaci* infection contain six major domestic species: chickens, turkeys, Pekin ducks, Muscovy ducks, geese, and pigeons. Infections in these species have been associated with significant outbreaks in humans.[24,28,146] In the past, chlamydial infection in psittacine birds was termed *psittacosis*, whereas disease in wild and domestic fowl was called *ornithosis*. However, because *C. psittaci* strains isolated from psittacine or nonpsittacine species have been shown to produce identical disease in birds of either grouping, this distinction is artificial. The more universally applicable term *chlamydiosis* can be usefully employed to describe chlamydial infections of all animal species.

Epidemiology

Chlamydial infections in birds are important because they represent a biologic hazard to human health and economic loss to the poultry industry.[25,28,146] *C. psittaci* has been recovered from symptomatic and apparently healthy birds. Infection principally involves the gastrointestinal tract, and the chlamydiae are shed in feces or through infectious respiratory tract discharges.[154] Clinically inapparent, latent infections may be the predominant state. Overt clinical disease may be activated by stress factors, including overcrowding, poor nutrition, other bacterial and viral infections, and transportation.

Apparently healthy birds shedding chlamydiae can infect other birds or humans through contact. Infectious chlamydiae in respiratory secretions or feces may remain viable for several months. Transmission of disease is mainly through aerosols of fecal or feather dust, but oral infection is an alternative route. Transmission through eggs has been shown in chickens, ducks, seagulls, and psittacine birds.[154] However, most infected eggs do not hatch. In the nest, parent birds may infect their young, which may carry the infectious agent for many years. Young birds are more susceptible to infection than are older birds, and some species seem to be more susceptible than others. Often, disease carriers may be identified by the transmission of disease to other susceptible birds or by the sudden death of young nestlings with apparently healthy parents.[137]

From 2003 through 2010, 94 human cases of psittacosis were reported to the CDC, a dramatic decrease from 935 cases reported from 1998 to 2003.[28] Eighty-five percent of cases of psittacosis in the United States were associated with exposure to birds; 70 percent of these reported cases were the result of exposure to caged pet birds.[146] Individuals at highest risk for acquiring psittacosis included bird owners or fanciers and pet shop employees. Since 1984, several major outbreaks of psittacosis have occurred in the United States in turkey-processing plants, in which approximately 300 persons were infected. Workers exposed to turkey viscera were at the highest risk for acquiring infection.[25,79] In 1995, the CDC investigated an outbreak of avian chlamydiosis in a shipment of more than 700 pet birds from a Florida bird distributor to the Atlanta area.[119] Affected birds included parrots, parakeets, finches, lovebirds, cockatiels, conures, and canaries. Clinical psittacosis or serologic evidence of *C. psittaci* infection was found in 30.7 percent of households with birds from the infected flock. An average of 21 days (range, 1 to 47 days) elapsed between purchase of the bird and the onset of symptoms. Most of the infected individuals had mild or asymptomatic illnesses. Among persons in exposed households, illness occurred more frequently if the recently purchased bird had become sick or had died. Kissing or nuzzling, handling, and feeding the bird all were significantly associated with the development of clinical psittacosis, but in contrast to earlier studies, cleaning the bird's cage was not. The risk for acquiring clinical psittacosis varied significantly by type of bird to which the individual was exposed. The attack rate was highest for individuals exposed to parrots.

Inhalation of infectious aerosols derived from the feces, fecal dust, or secretions of *C. psittaci*–infected animals is thought to be the primary route of infection. The source birds can be infected asymptomatically or can show signs of infection, such as anorexia, ruffled feathers, depression, and watery green droppings. Psittacosis frequently is a systemic infection in birds, and the turkey strains can induce severe pericarditis.[154]

Psittacosis is an uncommon occurrence in children. In a series of 135 cases from Australia observed during a 15-year period, the youngest patient was 17 years of age.[162] Children may be less likely to be exposed to birds. Bird-keeping is more commonly a hobby of adults, and the parents are the ones who usually clean the cage of the family's pet bird. An outbreak of psittacosis involving two adolescents was reported from a small village in Scotland. The source of the infection appeared to be the local pet shop, which had taken delivery of four lovebirds, two of which died shortly after arrival.[120]

Clinical Manifestations

Infection with *C. psittaci* in humans may range from clinically inapparent to severe infection involving multiple

organ systems, as well as pneumonia.[9,45,119,162] Symptomatic *C. psittaci* infection in humans may present as two forms: a severe atypical pneumonia or pneumonitis and a systemic toxic or septic form without respiratory involvement. In both cases, fever, chills, muscle aches and pains, and severe headache are typical. Other manifestations may be diarrhea, nausea, and vomiting. The mean incubation period is 15 days after exposure (range, 5 to 21 days). The onset usually is abrupt, with complaints of fever, cough, and headache. The fever is high and frequently associated with rigors and sweats. The headache can be so severe that meningitis can be considered a possibility; 33 percent of patients in the Australian series underwent lumbar puncture.[162] The cough usually is nonproductive, and rales may be heard on auscultation. Chest radiographs generally are abnormal, with variable infiltrates. Pleural effusions also may be present. In contrast, most of the individuals in the Atlanta outbreak had very mild disease characterized by fever, headache, and cough.[119]

Laboratory Findings

The white blood cell count usually is not elevated, but mild leukocytosis may be present. Almost 50 percent of patients in the Australian series had abnormal liver function test results, including elevated levels of aspartate aminotransferase, alkaline phosphatase, and bilirubin.[162]

Diagnosis

The CDC and the Council of State and Territorial Epidemiologists have established national case definitions for epidemiologic surveillance of psittacosis. The updated case definitions were published in 2010.[146] A patient is considered to have a confirmed case of psittacosis if clinical illness is compatible with psittacosis and the case is laboratory confirmed by one of two methods: isolation of *C. psittaci* from respiratory specimens (e.g., sputum, pleural fluid, or tissue) or blood or fourfold or greater increase in antibody (immunoglobulin [Ig]G) against *C. psittaci* by complement fixation or microimmunofluorescence (MIF) between paired acute- and convalescent-phase serum specimens obtained at least 2 to 4 weeks apart. A patient is considered to have a probable case of psittacosis if the clinical illness is compatible with psittacosis and one of the two following laboratory results is present: supportive serology (e.g., *C. psittaci* antibody titer [IgM] of 32 or greater in at least one serum specimen obtained after onset of symptoms) or detection of *C. psittaci* DNA in a respiratory specimen (e.g., sputum, pleural fluid, or tissue) via amplification of a specific target by PCR assay.

Most diagnoses are established by clinical presentation and positive antibodies against *C. psittaci* in paired sera using MIF methods. The MIF is more sensitive and specific than the previously used complement fixation tests; however, some cross reactivity occurs with other chlamydiae (i.e., *C. pneumoniae*, *C. trachomatis*, and *C. felis)*, so a titer result less than 1:128 should be interpreted with caution.[155] Acute-phase serum specimens should be obtained as soon as possible after the onset of symptoms, and convalescent-phase serum specimens should be obtained at least 2 weeks after the first specimen. Because antimicrobial treatment can delay or diminish the antibody response, a third serum sample 4 to 6 weeks after the acute sample might help confirm the diagnosis. To increase the reliability of serologic results, acute and convalescent sera should be analyzed simultaneously at the same laboratory.

C. psittaci also can be isolated from the patient's sputum, pleural fluid, or clotted blood during acute illness and before treatment with antimicrobial agents. Although *C. psittaci* will grow in the same culture systems used for the isolation of *C. trachomatis* and *C. pneumoniae*, very few laboratories culture for *C. psittaci*, mainly because of the potential biohazard.

Real-time PCR (rt-PCR) assays have been developed for use in the detection of *C. psittaci* in respiratory specimens.[9,112] These assays can distinguish *C. psittaci* from other chlamydial species and identify different *C. psittaci* genotypes. While the assays appear to be highly sensitive and specific in avian samples, they have not yet been validated for use in human samples. Because proper sample collection techniques and handling are critical to obtain accurate test results, clinical laboratories performing these tests should be contacted directly for specifics on specimen submission.

Information about laboratory testing is available from state public health departments. Few commercial laboratories provide MIF testing, and manufacturing problems in 2009 resulted in limited availability of the MIF testing kits. Certain laboratories accept human specimens to confirm *C. psittaci* infection through culture, MIF, or PCR (Table 193-2).

Treatment

The recommended treatment of psittacosis in adults and children older than 8 years of age is tetracycline 500 mg orally four times daily for 7 to 10 days.[148] Erythromycin 50 mg/kg/day, up to 2 g/day, for 7 to 10 days also can be used. The experience in the Australian series[162] and anecdotal reports suggest that tetracycline may be more effective than erythromycin. The initial infection does not appear to be followed by long-term immunity. Reinfection and clinical disease can develop within 2 months of treatment; two well-documented cases of reinfection are reported in the literature. A pet shop employee had two episodes of psittacosis 11 months apart. Each episode met the CDC's confirmed case definition.[24]

INFECTION CAUSED BY *CHLAMYDIA PNEUMONIAE*

The Organism

The first isolates of *C. pneumoniae* were obtained serendipitously during trachoma studies in the 1960s.[55] After the recovery of a similar isolate from the respiratory tract of a college[57,149] applied the designation TWAR after

TABLE 193-2 **Laboratories That Test Human Specimens for *Chlamydia psittaci*[146]**

Laboratory	Tests Performed	Telephone Number and Website
Focus Diagnostics Inc. (Quest subsidiary), Cypress, CA	Culture, MIF (IgM, IgA, IgG)	800-445-4032 http://www.focusdx.com
Laboratory Corporation of America, Burlington, NC	Culture, MIF (IgM, IgG)	800-222-7566 http://www.labcorp.com
Quest Diagnostics, Valencia, CA	MIF (IgM, IgG, IgA)	800-421-7110 http://www.specialtylabs.com
ViroMed Laboratories Minnetonka, MN	Culture, MIF (IgG, IgM)	800-582-0077 http://www.viromed.com
Response and Surveillance Laboratory, Respiratory Diseases Branch, CDC Atlanta, GA*	MIF (requires paired sera), PCR, culture, genotyping (multiple specimen types)	404-639-4921

*CDC is a reference laboratory and samples must be submitted through State Health Departments
MIF, Microimmunofluorescence; *PCR,* polymerase chain reaction.

their first two isolates, TW-183 and AR-39. On the basis of inclusion morphology and staining characteristics in cell culture, *C. pneumoniae* initially was considered a *C. psittaci* strain. Subsequent analysis, however, has demonstrated that this organism is distinct from both *C. psittaci* and *C. trachomatis.*[22,56] Sequencing has revealed that *C. pneumoniae* differs significantly from *C. trachomatis* in several areas. *C. pneumoniae* encodes 21 polymorphic membrane proteins versus 9 in *C. trachomatis*. Polymorphic membrane proteins may be surface exposed in *C. pneumoniae.*[88,136] Ultrastructural studies have demonstrated an elementary body morphology distinct from that of *C. trachomatis* and *C. psittaci* (Fig. 193-2).[30] However, some isolates of *C. pneumoniae*, including IOL-207, have been found to have round elementary bodies.[23,127,129] Thus, it may not be a consistent species characteristic. At this point, a strain typing system for *C. pneumoniae* has not been developed. A plasmid has been detected in a single isolate obtained from a horse but not from any human isolate of *C. pneumoniae*.

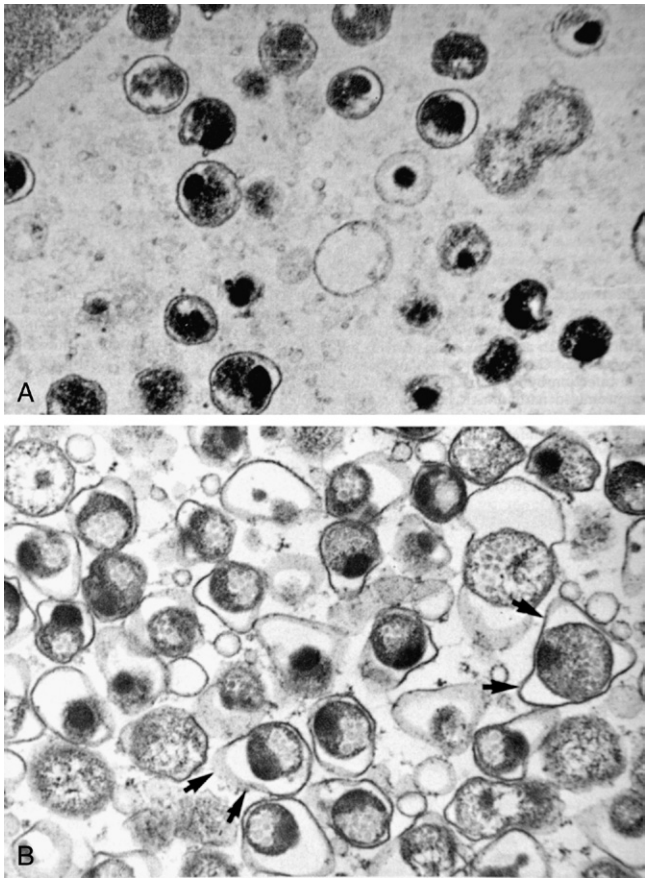

FIGURE 193-2 ■ Electron micrograph of *Chlamydia trachomatis* **(A)** and *Chlamydia pneumoniae* **(B)** inclusions demonstrating the morphology of the elementary body. The elementary bodies of *C. pneumoniae* have a pear shape because of a loose periplasmic membrane *(arrow),* unlike the typically round elementary bodies of *C. trachomatis* and *C. psittaci.*

Epidemiology

C. pneumoniae appears to be a common human respiratory pathogen. The organism also has been isolated from nonhuman mammalian species, including a horse, koalas, bandicoots, and reptiles and amphibians, although the role these infections may play in human disease is unknown.[20,40,86,88,98,117,131] The animal isolates are diverse and have DNA sequences that differ slightly from human *C. pneumoniae* isolates.[115] It has been hypothesized that human *C. pneumoniae* may be zoonotic in origin.[115,117,121,123] Human isolates have been found to be relatively uniform.[17] Currently no strain typing system has been developed for *C. pneumoniae.* The mode of transmission remains unclear but probably involves infected respiratory tract secretions. Acquisition of infection by droplet aerosol was described during a laboratory accident.[81] *C. pneumoniae* can remain viable on Formica countertops for 30 hours and can survive small-particle aerosolization.[41,146] Spread of *C. pneumoniae* within families and enclosed populations such as military recruits has been described.[92,94,158] The proportion of community-acquired pneumonia associated with *C. pneumoniae* infection has ranged from 0 to 19 percent, varying with the geographic location, the age group examined, and the diagnostic methods used.*

Studies of the role of *C. pneumoniae* in lower respiratory tract infections in pediatric populations have found evidence of infection in 0.3 to more than 44 percent

*References 6, 19, 42, 64, 99, 102-104, 111, 115, 129, 137, 139, 149, 150, 152, 157, 159.

TABLE 193-3 **Selected Studies of *Chlamydia pneumoniae* Lower Respiratory Tract Infection in Children**

				No. Tested/No. Positive Results (%)			
Study	**Year**	**Country**	**Age**	**No.**	**Culture**	**PCR**	**Serology**
Saikku et al.[140]	1988	Philippines	<5 yr	220	ND	ND	14 (6.4)
Forgie et al.[42]	1991	Gambia	1-9 yr	74	ND	ND	9 (12.1)
Jantos et al.[89]	1995	Germany	2 d-15 yr	290	1 (0.3)	2/290 (0.7)	2/101 (2)
Block et al.[19]	1995	United States	3-12 yr	260	34 (13)	ND	48 (18.5)
Harris et al.[76,77]	1998	United States	6 mo-16 yr	456	31 (7.3)	ND	37 (8.8)
Principi et al.[131]	2001	Italy	2-14 yr	613	ND	48 (7.8)	52 (11.4)
Baer et al.[6]	2003	Switzerland	1-18 yr	50	1 (2)	1 (2)	2 (4)
Likitnukul et al.[104]	2003	Thailand	1 mo-18 yr	333	ND	ND	149 (44.7)
Tsolia et al.[152]	2004	Greece	5-14 yr	75	ND	0	2 (3)
Michelow et al.[113]	2004	United States	6 wk-18 yr	154	ND	ND	14 (9)
Liu et al.[106]	2005	China	≤5 yr	85	ND	4 (2.2)	ND

ND, Not done; *PCR,* polymerase chain reaction.

(Table 193-3). Most of these studies relied entirely on serology for diagnosis. Early studies that relied on serology suggested that infection in children younger than 5 years of age was rare[100,157]; however, in a study of Filipino children younger than 5 years with lower respiratory tract infection, nearly 10 percent had either acute or chronic antibody to *C. pneumoniae*.[137] In Brooklyn, the proportion of lower respiratory tract infections associated with *C. pneumoniae*, as determined by culture, increased from 9 percent in children younger than 5 years to 19 percent in children and adolescents 5 to 16 years of age.[31]

Studies that have used culture or PCR have found a poor correlation with serology, especially in children.* Although 7 to 13 percent of children 6 months to 16 years of age enrolled in two multicenter pneumonia treatment studies were culture positive and 7 to 18 percent met the serologic criteria for acute infection with the MIF test, they were not the same patients.[19,75] Only 1 to 3 percent of the culture-positive children met the serologic criteria, and approximately 70 percent were seronegative.

In studies to date, acute infection with *C. pneumoniae* does not appear to vary by season. Recent studies have found much lower prevalence of infection than reported in the 1990s, suggesting that cycling over the course of time may occur.[76,84,96,100,104,106]

Prolonged culture positivity lasting several weeks to several years after acute infection has been reported.[31,38,72] Asymptomatic nasopharyngeal carriage also occurs in 2 to 5 percent of adults and children.[18,38,53,82,83,96] The role that asymptomatic carriage plays in the epidemiology of *C. pneumoniae* is not known, but possibly these persons could serve as a reservoir for spread of infection.

Clinical Manifestations

Most *C. pneumoniae* respiratory infections are mild or asymptomatic. Longitudinal serologic data obtained during an epidemic among military recruits in Finland suggest that only approximately 10 percent of infections result in clinically apparent pneumonia.[92,94] Initial reports emphasized mild atypical pneumonia clinically resembling that associated with *Mycoplasma pneumoniae*.[54,55,138,140] In several subsequent studies, however, pneumonia associated with *C. pneumoniae* was clinically indistinguishable from other pneumonias.[19,75,77] Coinfection with other pathogens, especially *M. pneumoniae*, *Streptococcus pneumoniae*, and viruses are frequent.[19,75,77,147,149] In one multicenter pneumonia treatment study, 20 percent of the children with positive *C. pneumoniae* cultures were coinfected with *M. pneumoniae;* they could not be distinguished from children who were infected with either organism alone.[19] *C. pneumoniae* has been associated with severe illness and even death, although the role of preexisting chronic conditions as contributing factors in many of these patients is difficult to assess. In some cases, however, *C. pneumoniae* clearly appears to be implicated as a serious pathogen, even in the absence of underlying disease. *C. pneumoniae* was isolated from the respiratory tract and the pleural fluid of a previously healthy adolescent boy with severe pneumonia complicated by respiratory tract failure and pleural effusions (Fig. 193-3).[4]

C. pneumoniae appeared to be responsible for 14 to 19 percent of episodes of acute chest syndrome in children with sickle-cell disease.[35,99,113,114] *C. pneumoniae* infection in these patients appeared to be associated with more severe hypoxia than was infection with *M. pneumoniae*.

C. pneumoniae may act as an inflammatory trigger for asthma. Several cases of culture-documented *C. pneumoniae* infection in patients with significant bronchospasm have been reported.[61,72] Asthmatic bronchitis was diagnosed in one patient, and she was receiving systemic and inhaled steroids.[72] This patient did not improve until her chlamydial infection was treated. Hahn and associates[61] reported an association between serologic evidence of acute *C. pneumoniae* infection and wheezing in adults

*References 19, 38, 48, 49, 75, 99, 101.

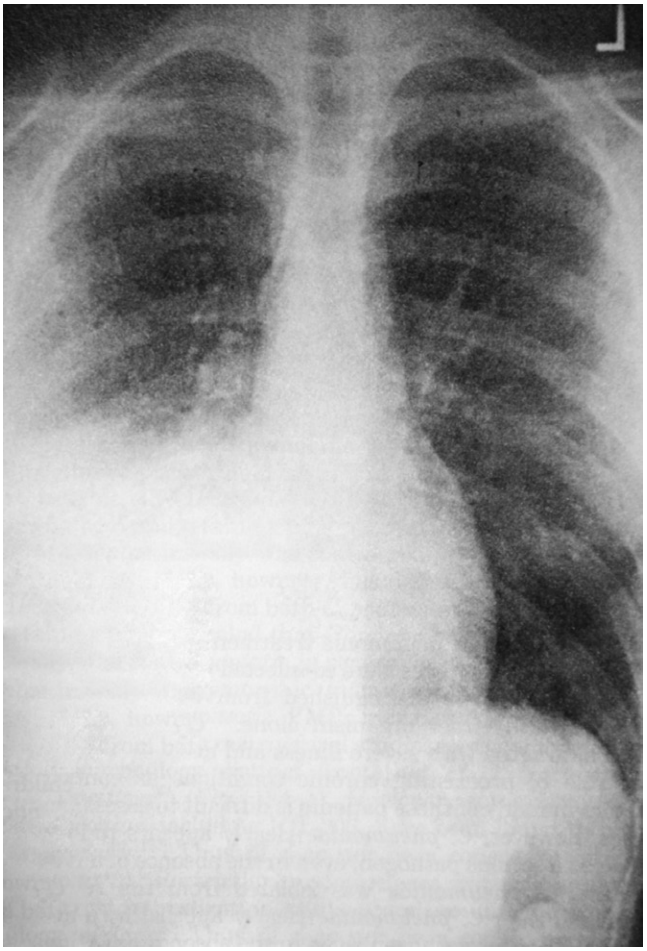

FIGURE 193-3 ■ Chest radiograph of a 19-year-old man with *Chlamydia pneumoniae* pneumonia demonstrating pleural effusion in the right lung. *C. pneumoniae* was isolated from the pleural fluid.

seen for lower respiratory tract illness. However, they were able to isolate the organism from only 1/365 patients. As part of a study in children, *C. pneumoniae* was isolated from 13/118 children (11%) 5 to 15 years of age who were evaluated initially for either new or acute exacerbations of asthma.[38] Treatment of the infection appeared to result in both clinical improvement and improvement in pulmonary function test scores. Only 5 of the children with confirmed infection had detectable IgG antibody to *C. pneumoniae*. One child who did not comply with his antibiotic therapy was culture positive on five occasions during a 3-month period. Anti–*C. pneumoniae* antibody as determined by MIF was never detected. Specific anti–*C. pneumoniae* IgE was, however, detected in 85.7 percent of the culture-positive asthmatics in contrast to 9 percent of the children with *C. pneumoniae* pneumonia who were not wheezing.[39] This finding suggests that the bronchial reactivity seen with *C. pneumoniae* infection may be IgE mediated. The potential prolonged, persistent *C. pneumoniae* infection may produce chronic inflammation and trigger bronchospasm in susceptible persons. *C. pneumoniae* has been demonstrated to induce in vitro ciliostasis in ciliated bronchial epithelial cells.[141,143] Animal studies also suggest that steroids can reactivate *C. pneumoniae* lung infection in mice.[106] In addition, immune-mediated phenomena, including erythema nodosum and iritis, have been described as complicating *C. pneumoniae* infection.[145,147,155,157]

The role of *C. pneumoniae* in upper respiratory tract infections is not as well defined. *C. pneumoniae* has been isolated from the middle ear fluid of children and adults with otitis media.[18,123-125] Block and colleagues[18] recovered *C. pneumoniae* from the middle ear fluid of eight children, 3 months to 14 years of age, with acute otitis media. *C. pneumoniae* was the sole pathogen isolated in two patients. Co-pathogens in the remaining six patients included β-lactamase–positive *Haemophilus influenzae* and *Moraxella catarrhalis*, along with penicillin-resistant and penicillin-sensitive *S. pneumoniae*. Five of the children who were positive for *C. pneumoniae* responded favorably despite not being treated with antibiotics active against the organism, either a course of an oral β-lactam or single-dose intramuscular ceftriaxone. Symptoms suggestive of sinus involvement are not uncommon in patients with upper respiratory tract infection associated with *C. pneumoniae*, but only one case of isolation of the organism, from a 47-year-old man with sinusitis, has been reported.[77,78] Cultrara and colleagues[34] examined nasopharyngeal swabs, ethmoid and maxillary sinus biopsy samples, and adenoids from 20 children, 3 to 16 years of age, with chronic sinusitis. *C. pneumoniae* was not isolated from any sinus tissue but was isolated from the nasopharyngeal swab and adenoid tissue of a 6-year-old child. The results of this study suggest that *C. pneumoniae* does not play a significant role in chronic sinusitis in children.

Diagnosis

A specific laboratory diagnosis of *C. pneumoniae* infection can be made by isolation of the organism from nasopharyngeal or throat swabs, sputum, or pleural fluid, if present. The nasopharynx appears to be the optimal site for isolation of the organism.[19] *C. pneumoniae* grows readily in cell lines derived from respiratory tract tissue, specifically, HEp-2 and HL cells.[132,135] Culture with an initial inoculation and one passage can take 4 to 7 days.

Nasopharyngeal cultures can be obtained with Dacron-tipped, wire-shafted swabs. Each lot of swabs should be treated in a mock infection system to ensure that no inhibitory effects occur on either the viability of cells or recovery of chlamydiae. Specimens for culture should be placed in appropriate transport media, usually a sucrose phosphate buffer with antibiotics and fetal calf serum, and stored immediately at 4°C (39.2°F) for no longer than 24 hours. Viability decreases if specimens are held at room temperature. If the specimen cannot be processed within 24 hours, it should be frozen at –70°C (–94°F) until culture can be performed. After 72 hours of incubation, culture confirmation can be performed by staining with either a *C. pneumoniae* species–specific

or a *Chlamydia* genus–specific (anti-LPS) fluorescein-conjugated monoclonal antibody.[116-118,132,135] Unfortunately, no commercially produced *C. pneumoniae*–specific culture-confirmation reagents are available. If a genus-specific antibody is used, *C. pneumoniae* should be confirmed by differential staining with a specific *C. trachomatis* antibody; if that result is negative, the isolate is either *C. pneumoniae* or *C. psittaci*. If the patient has not had avian exposure, psittacosis would be highly unlikely.

The most common method still used by investigators for the diagnosis of *C. pneumoniae* infection is serologic testing. Assays available for detection of *C. pneumoniae*–specific antibodies include MIF tests, enzyme-linked immunosorbent assays (ELISAs), and EIAs, each of which exists in a variety of in-house and commercial versions.[151] MIF is considered by the CDC to be the only currently acceptable serologic test, and, therefore, it is considered to be the reference standard for serodiagnosis, despite significant limitations.[36] The recommendations proposed by the CDC[36] in 2001 defined the criteria for serologic diagnosis of acute *C. pneumoniae* infection as a single IgM titer of 1:16 or greater or a fourfold increase in the IgG titer, as determined by MIF testing. The use of single IgG titers was discouraged. However, not only is the literature replete with studies that deviate widely from the proposed guidelines[100] but also several limitations exist that are inherent for serodiagnosis of *C. pneumoniae* infection, even when these guidelines are observed. The MIF assay has been repeatedly and conclusively demonstrated to be insensitive and have poor correlation with the detection of the organism by culture or PCR assay, particularly for children.[49,100] Seventy percent of children 6 months to 16 years of age in two multicenter pneumonia treatment studies and 7/12 (58.3%) children 5 to 16 years of age with acute wheezing and who had culture-documented infection had seronegative results by MIF test.[19,38,75,76] Only 1 to 3 percent of culture-positive children in the former study and 25 percent in the latter met the serologic criteria for acute infection. The majority of the children remained seronegative, even after 3 months of follow-up.[38]

Background rates of seropositivity also can be high in some populations. Hyman and associates,[82,84] as part of a study of asymptomatic *C. pneumoniae* infection in subjectively healthy adults in Brooklyn, found 81 percent to have IgG or IgM titers of 16 or higher. Seventeen percent had evidence of "acute infection" (i.e., IgG titer ≥512, IgM titer ≥16, or both). However, none of these persons were culture or PCR positive. Similar results were reported by Kern and associates[93] in healthy firefighters and policemen in Rhode Island. Some IgG antibody may result from a heterotypic response to other chlamydial species because cross reactions with MOMP occur among the three species, as do cross reactions caused by the genus LPS antigen. Moss and colleagues[121] reported that antibodies to *C. pneumoniae* and *C. psittaci* were found in as many as half of all chlamydial IgG–positive persons attending an STD clinic. This point is reinforced by the observation that studies from the early 1980s suggesting *C. trachomatis* as a cause of community-acquired pneumonia and pharyngitis in adults and children were probably detecting antibody to *C. pneumoniae* rather than *C. trachomatis*[50,94,95,97,128] or, in one outbreak in a boys school, *C. psittaci*.[126] Other organisms that have been reported as possibly causing cross-reactions on MIF are *Bartonella*[108,110] and *Bordetella pertussis*.[85,87] The latter could be significant because older children and adolescents with pertussis frequently have a chronic cough or severe bronchitis, which is a clinical feature often ascribed to *C. pneumoniae*. Other studies have found significant homology between human and *C. pneumoniae* heat shock protein 60 (HSP-60) and *Escherichia coli* GroEL.[122,124]

Moreover, the MIF test is not standardized, and reading of the slides has a large subjective component and requires an experienced microscopist. Littman and associates[105] evaluated the interlaboratory reliability of the MIF test for measurement of *C. pneumoniae*–specific IgA and IgG titers for 392 serum samples, using reagents and antigens obtained from a common source. The investigators reported the exact agreement of IgA and IgG titers to be 55 and 38 percent, respectively, and the agreement within a twofold dilution to be 75 and 66 percent, respectively. EIAs may overcome some of the limitations of the MIF test by being objective and less technically complex. However, the CDC did not endorse any EIA, pending demonstration of adequate sensitivity and specificity in contrast to the MIF test. None of the currently available EIAs have FDA approval. Hermann and colleagues[80] compared seven commercial EIAs or ELISAs (one of which was genus-specific) with four MIF assays for detection of specific IgG antibodies, using serum samples from 80 healthy subjects; they reported sensitivities and specificities ranging from 88 to 100 and 42 to 100 percent, respectively. Similar results have been reported by others with the same assays in different populations, including children.[99,107,125,150,151,153]

The number of *C. pneumoniae* organisms present in the respiratory tract of persons with pneumonia or other respiratory tract diseases is smaller than the number found in genital *C. trachomatis* infection. PCR appears to be the most promising technology in the development of a rapid, nonculture method for detection of *C. pneumoniae*. More than 25 in-house PCR assays for detection of *C. pneumoniae* in clinical specimens have been reported in the literature.[100,107] None of these assays is standardized or extensively validated in contrast to culture for detection of *C. pneumoniae* in respiratory specimens. None is commercially available or has FDA approval. Major variations in these methods include collection and processing of specimens, primer design, nucleic acid extraction, detection and identification of amplification products, and ways to prevent possible false-positive and inhibitory reactions. The primers used most frequently have been those based on the *omp1* gene, the 16S rRNA gene, the 16S and 16S-23S spacer rRNA genes, and a *C. pneumoniae*–specific cloned *Pst*I fragment.[99,100] Some assays have used single amplification, and some have been nested. Methods for detecting the amplification product include agarose gel electrophoresis, Southern blot, EIA, and polyacrylamide gel electrophoresis.[100] Studies suggest significant interlaboratory variation in performance of PCR for *C. pneumoniae*.[2,21,104] Use of nested PCRs has been associated with a high risk for contamination because of amplicon

carryover.[2] Real-time PCR appears to be the method of choice, preferably using MOMP-based primers; several assays have been reported in the literature, but none has been extensively validated in contrast to culture.[2,3,101]

Treatment

Chlamydia spp. are susceptible to tetracyclines, macrolides, and quinolones.[63,66] *C. pneumoniae*, like *C. psittaci*, is resistant to sulfonamides.[63,66] To date, few published data have described the response of *C. pneumoniae* to antimicrobial therapy. Most of the treatment studies of pneumonia caused by *C. pneumoniae* published thus far have relied entirely on diagnosis by serology; consequently, microbiologic efficacy could not be assessed. Anecdotal reports have suggested that prolonged courses, up to 3 weeks, of either tetracyclines or erythromycin may be needed to eradicate *C. pneumoniae* from the nasopharynx of adults with influenza-like illness and pharyngitis.[72] The results of two pediatric multicenter pneumonia treatment studies found that 10-day courses of erythromycin and clarithromycin and 5 days of azithromycin suspension were equally efficacious; they eradicated the organism in 79 to 86 percent of children.[19,75,77,133] Quinolones, including levofloxacin and moxifloxacin, also have been demonstrated to have 70 to 80 percent efficacy in eradicating *C. pneumoniae* from adults with community-acquired pneumonia.[67-69] Most patients improved clinically despite persistence of the organism. Persistence does not appear to be secondary to the development of antibiotic resistance, because the MICs of the isolates obtained after treatment did not change.[63,66-69,76,133,134]

On the basis of these limited data, the following regimens for respiratory tract infection caused by *C. pneumoniae* can be suggested: in adults, doxycycline 100 mg twice daily for 14 to 21 days; tetracycline 250 mg four times daily for 14 to 21 days; azithromycin 1.5 g for 5 days; levofloxacin 500 mg/day orally or intravenously for 7 to 14 days; and moxifloxacin 400 mg/day orally for 10 days. For children, suggested regimens include erythromycin suspension 50 mg/kg/day for 10 to 14 days; clarithromycin suspension 15 mg/kg/day for 10 days; and azithromycin suspension 10 mg/kg on day 1 followed by 5 mg/kg/day once daily on days 2 to 5. Some patients may require retreatment.

NEW REFERENCES SINCE THE SIXTH EDITION

5. Bäcker M, Hammerschlag MR. *Case 09011: a teenager with constipation and weight loss [Internet]*. Partners Infectious Disease Images. Available from: http://www.idimages.org/idreview/case/caseid=157.
7. Batteiger BE, Tu W, Ofiner S, et al. Repeated *Chlamydia trachomatis* genital infections in adolescent women. *J Infect Dis* 2009; **201**:42–51.
9. Beeckman DSA, Vanrompay DCG. Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. *Clin Microbiol Infect* 2009;**15**:11–17.
15. Bhosai SJ, Bailey RL, Gaynor BD, et al. Trachoma: an update on prevention, diagnosis and treatment. *Curr Opin Ophthalmol* 2012; **23**:288–95.
16. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
26. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010; **59**(RR-12):1–116.
27. Centers for Disease Control and Prevention. Compendium of measures to prevent disease associated with animals in public settings, 2011. *MMWR Recomm Rep* 2011;**60**(RR-4):1–28.
28. Centers for Disease Control and Prevention. Summary of notifiable diseases, 2010. *MMWR 2010* 2012;**59**:1–116.
32. Clarke IN. Evolution of *Chlamydia trachomatis*. *Ann N Y Acad Sci* 2011;**1230**:E11–18.
43. Forhan SE, Gottlieb SL, Sternberg MR, et al. Prevalence of sexually transmitted infections among female adolescents aged 14 to 19 in the United States. *Pediatrics* 2009;**124**:1505–12.
52. Girardet RG, Lahoti S, Howard LA, et al. The epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 2009;**124**:79–86.
58. Greub G. International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Chlamydiae*. Minutes of the closed meeting, 21 June 2010, Hof bei Salzburg, Austria. *Int J Syst Evol Microbiol* 2010;**60**:2694.
60. Gust DA, Wang SA, Black CM, et al. A pseudo-outbreak of *Chlamydia trachomatis* in a state residential facility: implications for diagnostic testing. *J Infect Dis* 2002;**185**:841–4.
62. Hammerschlag MR. Chlamydial and gonococcal infections in infants and children. *Clin Infect Dis* 2011;**53**(Suppl. 3):S99–102.
63. Hammerschlag MR. Sexual assault and abuse in children. *Clin Infect Dis* 2011;**53**(Suppl. 3):S102–9.
65. Hammerschlag MR, Guillén CD. Medical and legal implications of testing for sexually transmitted infections in children. *Clin Microbiol Rev* 2010;**23**:493–506.
66. Hammerschlag MR, Kohlhoff SA. Treatment of chlamydial infections. *Expert Opin Pharmacother* 2012;**13**:545–52.
70. Hammerschlag MR, Kohlhoff SA, Darville T. *Chlamydia pneumoniae* and *Chlamydia trachomatis*. In: Fratamico PM, Smith JL, Brogden KA, editors. *Sequelae and long-term consequences of infectious diseases*. Washington, DC: American Society for Microbiology; 2009. p. 27–52.
82. Huang W, Gaydos CA, Barnes MR, et al. Comparative effectiveness of a rapid point of care test for detection of *Chlamydia trachomatis* among women in a clinical setting. *Sex Transm Dis* 2013; **89**:108–14.
92. Kelly P, Koh J. Sexually transmitted infections in alleged sexual abuse of children and adolescents. *J Paediatr Child Health* 2006; **42**:434–40.
95. Kohlberger P, Bancher-Todesca D. Bacterial colonization in suspected sexually abused children. *J Pediatr Adolesc Gynecol* 2007;**20**: 289–92.
105. Littman AJ, Jackson LA, White E, et al. Interlaboratory reliability of microimmunofluorescence test for measurement of *Chlamydia pneumoniae*-specific immunoglobulin A and G antibody titers. *Clin Diagn Lab Immunol* 2004;**11**:615–17.
117. Mitchell CM, Hutton S, Myers GS, et al. *Chlamydia pneumoniae* is genetically diverse in animals and appears to have crossed the host barrier to humans on (at least) two occasions. *PLoS Pathog* 2010;**6**:e1000903.
123. Myers GS, Matthews SA, Eppinger M, et al. Evidence that *Chlamydia pneumoniae* was zoonotically acquired. *J Bacteriol* 2009;**23**:7225–33.
126. Pannekoek Y, Dickx V, Beeckman S, et al. Multi-locus sequence typing of *Chlamydia* reveals an association between *Chlamydia psittaci* genotypes and host species. *PLoS One* 2010;**5**:e14179.
132. Quirke M, Cullinane A. Recent trends in chlamydial and gonococcal conjunctivitis among neonates and adults in an Irish hospital. *Int J Infect Dis* 2008;**12**:371–3.
137. Rours GI, Hammerschlag MR, de Faber JTHN, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. *Pediatrics* 2008;**121**:e321–6.
138. Rours GI, Hammerschlag MR, Van Doornum GJJ, et al. *Chlamydia trachomatis* respiratory infection in Dutch infants. *Arch Dis Child* 2009;**94**:705–7.
144. Siffermann E, Lorand M, Beavis KG, et al. A pseudo-outbreak of *Chlamydia trachomatis* infection among children being evaluated for suspected sexual abuse. In: Ouburg S, Morré SA, editors. *Proceedings of the 7th Meeting of the European Society for Chlamydia Research*. Amsterdam, the Netherlands: VU University; 2012. p. 229–30.

145. Simmons KJ, Hicks DJ. Child sexual abuse examination: is there a need for routine screening for *N. gonorrhoeae* and *C. trachomatis*. *J Pediatr Adolesc Gynecol* 2005;**18**:343–5.
146. Smith KA, Campbell CT, Murphy J, et al. *Compendium of measures to control* Chlamydophila psittaci *infection among humans (psittacosis) and pet birds (avian chlamydiosis)*, 2010. Available at http://www.nasphv.org/Documents/Psittacosis.pdf.
160. Yip TPP, Chan WH, Yip KT, et al. Incidence of neonatal chlamydial conjunctivitis and its association with nasopharyngeal colonization in a Hong Kong hospital assessed by polymerase chain reaction. *Hong Kong Med J* 2007;**13**:22–6.
161. Yip PP, Chan WH, Yip KT, et al. The use of polymerase chain reaction assay versus conventional methods in detecting neonatal chlamydial conjunctivitis. *J Pediatr Ophthalmol Strabis* 2008;**45**:234–9.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Anderson AA. Serotyping of US isolates of *Chlamydophila psittaci* from domestic and wild birds. *J Vet Diagn Invest* 2005;**17**:479–82.
2. Apfalter P, Reischl U, Hammerschlag MR. In-house nucleic acid amplification assays in research: How much quality control is needed before one can rely upon the results? *J Clin Microbiol* 2005;**43**:5835–41.
3. Apfalter P, Barousch W, Nehr M, et al. Comparison of a new quantitative *ompA*-based real-time PCR TaqMan assay for detection of *Chlamydia pneumoniae* DNA in respiratory specimens with four conventional PCR assays. *J Clin Microbiol* 2003;**41**:592–600.
4. Augenbraun MH, Roblin PM, Mandel LJ, et al. *Chlamydia pneumoniae* pneumonia with pleural effusion: diagnosis by culture. *Am J Med* 1991;**91**:437–8.
5. Bäcker M, Hammerschlag MR. *Case 09011: a teenager with constipation and weight loss [Internet]*. Partners Infectious Disease Images. Available from: http://www.idimages.org/idreview/case/caseid=157.
6. Baer G, Engelcke G, Abele-Horn M, et al. Role of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* as causative agents of community-acquired pneumonia in hospitalised children and adolescents. *Eur J Clin Microbiol Infect Dis* 2003;**22**:742–5.
7. Batteiger BE, Tu W, Ofner S, et al. Repeated *Chlamydia trachomatis* genital infections in adolescent women. *J Infect Dis* 2009;**201**:42–51.
8. Bauwens JE, Gibbons MS, Hubbard MM, et al. *Chlamydia pneumoniae* (strain TWAR) isolated from two symptom-free children during evaluation for possible sexual assault. *J Pediatr* 1991;**119**:591–3.
9. Beeckman DSA, Vanrompay DCG. Zoonotic *Chlamydophila psittaci* infections from a clinical perspective. *Clin Microbiol Infect* 2009;**15**:11–17.
10. Beem MO, Saxon EM. Respiratory-tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N Engl J Med* 1977;**296**:306–10.
11. Beem MO, Saxon E, Tipple MA. Treatment of *Chlamydia* pneumonia of infancy. *Pediatrics* 1979;**63**:198–203.
12. Bell TA, Stamm WE, Kuo CC, et al. Risk of perinatal transmission of *Chlamydia trachomatis* by mode of delivery. *J Infect* 1994;**29**:165.
13. Bell TA, Stamm WE, Wang SP, et al. Chronic *Chlamydia trachomatis* infections in infants. *JAMA* 1992;**267**:400–2.
14. Bernstein DI, Hubbard T, Wenman WM, et al. Mediastinal and supraclavicular lymphadenitis and pneumonitis due to *Chlamydia trachomatis* serovars L_1 and L_2. *N Engl J Med* 1984;**311**:1543–6.
15. Bhosai SJ, Bailey RL, Gaynor BD, et al. Trachoma: an update on prevention, diagnosis and treatment. *Curr Opin Ophthalmol* 2012;**23**:288–95.
16. Black CM, Driebe EM, Howard LA, et al. Multicenter study of nucleic acid amplification tests for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in children being evaluated for sexual abuse. *Pediatr Infect Dis J* 2009;**28**:608–13.
17. Black CM, Johnson JE, Farshy CE, et al. Antigenic variation among strains of *Chlamydia pneumoniae*. *J Clin Microbiol* 1991;**29**:1312–16.
18. Block S, Hammerschlag MR, Hedrick J, et al. *Chlamydia pneumoniae* in acute otitis media. *Pediatr Infect Dis J* 1997;**16**:858–62.
19. Block S, Hedrick J, Hammerschlag MR, et al. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. *J Pediatr Infect Dis* 1995;**14**:471–7.
20. Bodetti TJ, Viggers K, Warren K, et al. Wide range of Chlamydiales types detected in native Australian mammals. *Vet Microbiol* 2003;**96**:177–87.
21. Botsztejn A. Die pertossoide eosinophile Pneumoniae des Säuglings. *Ann Paediatr (Basel)* 1941;**157**:28–46.
22. Campbell LA, Kuo CC, Grayston JT. Characterization of the new *Chlamydia* agent, TWAR, as a unique organism by restriction endonuclease analysis and DNA-DNA hybridization. *J Clin Microbiol* 1987;**25**:1911–16.
23. Carter MW, Sah AM, Treharne JD, et al. Nucleotide sequence and taxonomic value of the major outer membrane protein gene of *Chlamydia pneumoniae* IOL-207. *J Gen Microbiol* 1991;**137**:465–75.
24. Cartwright KA, Caul EO, Lamb RW. Symptomatic *Chlamydia psittaci* reinfection. *Lancet* 1988;**1**:1004.
25. Centers for Disease Control and Prevention. Psittacosis at a turkey processing plant: North Carolina, 1989. *MMWR Morb Mortal Wkly Rep* 1990;**39**:460–9.
26. Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):1–116.
27. Centers for Disease Control and Prevention. Compendium of measures to prevent disease associated with animals in public settings, 2011. *MMWR* 2011;**60**(RR-4):1–28.
28. Centers for Disease Control and Prevention. Summary of notifiable diseases, 2010. *MMWR Morb Mortal Wkly Rep 2010* 2012;**59**:1–116.
29. Chen JY. Prophylaxis of ophthalmia neonatorum: comparison of silver nitrate, tetracycline, erythromycin and no prophylaxis. *Pediatr Infect Dis J* 1992;**12**:1026–30.
30. Chi EY, Kuo CC, Grayston JT. Unique ultrastructure in the elementary body of *Chlamydia* sp. strain TWAR. *J Bacteriol* 1987;**169**:3757–63.
31. Chirgwin K, Roblin PM, Gelling M, et al. Infection with *Chlamydia pneumoniae* in Brooklyn. *J Infect Dis* 1991;**163**:757–61.
32. Clarke IN. Evolution of *Chlamydia trachomatis*. *Ann N Y Acad Sci* 2011;**1230**:E11–18.
33. Copper WO, Griffin MR, Arbogast P, et al. Very early exposure to erythromycin and infantile hypertrophic pyloric stenosis. *Arch Pediatr Adolesc Med* 2002;**156**:64–50.
34. Cultrara A, Goldstein N, Ovchinsky A, et al. The role of *Chlamydia pneumoniae* in children with chronic sinusitis. *Arch Otolaryngol* 2003;**129**:1094–7.
35. Dean D, Neumayr L, Kelly DM, et al. *Chlamydia pneumoniae* and acute chest syndrome in patients with sickle cell disease. *J Pediatr Hematol Oncol* 2003;**25**:46–55.
36. Dowell SF, Peeling RW, Boman J, et al. Standardizing *Chlamydia pneumoniae* assays: recommendations from the Centers for Disease Control and Prevention (USA) and the Laboratory Centre for Disease Control (Canada). *Clin Infect Dis* 2001;**33**:492–503.
37. Embree JE, Lindsay D, Williams T, et al. Acceptability and usefulness of vaginal washes in premenarcheal girls as a diagnostic procedure for sexually transmitted diseases. *Pediatr Infect Dis J* 1996;**8**:662–7.
38. Emre U, Roblin PM, Gelling M, et al. The association of *Chlamydia pneumoniae* infection and reactive airway disease in children. *Arch Pediatr Adolesc Med* 1994;**148**:727–32.
39. Emre U, Sokolovskaya N, Roblin PM, et al. Detection of anti–*Chlamydia pneumoniae* IgE in children with reactive airway disease. *J Infect Dis* 1995;**172**:265–7.
40. Everett KDE, Bush RM, Anderson AA. Emended description of the order Chlamydiales, proposal of Parachlamydiaceae fam. nov. and Simkaniaceae fam. nov., each containing one monotypic genus, revised taxonomy of the family Chlamydiaceae, including a new genus and five new species, and standards for identification of organisms. *Int J Syst Bacteriol* 1999;**49**:425–40.
41. Falsey AR, Walsh EE. Transmission of *Chlamydia pneumoniae*. *J Infect Dis* 1993;**168**:493–6.
42. Forgie IM, O'Neill KP, Lloyd-Evans N, et al. Etiology of acute lower respiratory tract infections in Gambian children. II. Acute lower respiratory tract infection in children ages one to nine years presenting at the hospital. *J Pediatr Infect Dis* 1991;**10**:42–7.
43. Forhan SE, Gottlieb SL, Sternberg MR, et al. Prevalence of sexually transmitted infections among female adolescents aged 14 to 19 in the United States. *Pediatrics* 2009;**124**:1505–12.
44. Frommell GT, Rothenberg R, Wang SP, et al. Chlamydial infection of mothers and their infants. *J Pediatr* 1979;**95**:28–32.
45. Fryden A, Kihlstrom E, Maller R, et al. A clinical and epidemiological study of "ornithosis" caused by *Chlamydia psittaci* and *Chlamydia pneumoniae* (strain TWAR). *Scand J Infect Dis* 1989;**21**:681–91.
46. Fukushi H, Hirai K. *Chlamydia pecorum*: the fourth species of genus *Chlamydia*. *Microbiol Immunol* 1993;**37**:516–22.
47. Gaydos CA, Crotchfelt KA, Howell MR, et al. Molecular amplification assays to detect chlamydial infections from high school female students and to monitor the persistence of chlamydia DNA after therapy. *J Infect Dis* 1998;**177**:417–24.
48. Gaydos CA, Eiden JJ, Oldach D, et al. Diagnosis of *Chlamydia pneumoniae* infection in patients with community-acquired pneumonia by polymerase chain reaction enzyme immunoassay. *Clin Infect Dis* 1994;**19**:157–60.

49. Gaydos CA, Roblin PM, Hammerschlag MR, et al. Diagnostic utility of PCR-enzyme immunoassay, culture and serology for detection of *Chlamydia pneumoniae* in symptomatic and asymptomatic patients. *J Clin Microbiol* 1994;**32**:903–5.
50. Gerber MA, Ryan RW, Tilton RC, et al. Role of *Chlamydia trachomatis* in acute pharyngitis in young adults. *J Clin Microbiol* 1984;**20**:993–4.
51. Girardet RG, Lahoti S, Howard LA, et al. The epidemiology of sexually transmitted infections in suspected child victims of sexual assault. *Pediatrics* 2009;**124**:79–86.
52. Giradet RG, McClain N, Lahoti S, et al. Comparison of the urine-based ligase chain reaction test to culture for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in pediatric sexual abuse victims. *Pediatr Infect Dis J* 2001;**20**:144–7.
53. Gnarpe J, Gnarpe H, Sundelof B. Endemic prevalence of *Chlamydia pneumoniae* in subjectively healthy persons. *Scand J Infect Dis* 1991;**23**:387–8.
54. Grayston JT, Aldous MB, Easton A, et al. Evidence that *Chlamydia pneumoniae* causes pneumonia and bronchitis. *J Infect Dis* 1993; **168**:1231–5.
55. Grayston JT, Campbell LA, Kuo CC, et al. A new respiratory tract pathogen: *Chlamydia pneumoniae* strain TWAR. *J Infect Dis* 1990;**161**:618–25.
56. Grayston JT, Kuo CC, Campbell LA, et al. *Chlamydia pneumoniae sp. nov. for Chlamydia* sp. strain TWAR. *Int J Syst Bacteriol* 1989; **39**:88–90.
57. Grayston JT, Kuo CC, Wang SP, et al. A new *Chlamydia psittaci* strain, TWAR, isolated in acute respiratory tract infections. *N Engl J Med* 1986;**315**:161–8.
58. Greub G. International Committee on Systematics of Prokaryotes. Subcommittee on the taxonomy of *Chlamydiae*. Minutes of the closed meeting, 21 June 2010, Hof bei Salzburg, Austria. *Int J Syst Evol Microbiol* 2010;**60**:2694.
59. Greub G, Raoult D. Parachlamydiaceae: Potential emerging pathogens. *Emerg Infect Dis* 2002;**8**:625–30.
60. Gust DA, Wang SA, Black CM, et al. A pseudo-outbreak of *Chlamydia trachomatis* in a state residential facility: implications for diagnostic testing. *J Infect Dis* 2002;**185**:841–4.
61. Hahn DL, Dodge RW, Golubjatnikov R. Association of *Chlamydia pneumoniae* (strain TWAR) infection with wheezing, asthmatic bronchitis, and adult-onset asthma. *JAMA* 1991;**266**:225–30.
62. Hammerschlag MR. Chlamydial and gonococcal infections in infants and children. *Clin Infect Dis* 2011;**53**(Suppl. 3):S99–102.
63. Hammerschlag MR. Sexual assault and abuse in children. *Clin Infect Dis* 2011;**53**(Suppl. 3):S102–9.
64. Hammerschlag MR, Gaydos CA. Guidelines for the use of molecular biological methods to detect sexually transmitted pathogens in cases of suspected sexual abuse in children. *Methods Mol Biol* 2012;**903**:307–17.
65. Hammerschlag MR, Guillén CD. Medical and legal implications of testing for sexually transmitted infections in children. *Clin Microbiol Rev* 2010;**23**:493–506.
66. Hammerschlag MR, Kohlhoff SA. Treatment of chlamydial infections. *Expert Opin Pharmacother* 2012;**13**:545–52.
67. Hammerschlag MR, Roblin PM. Microbiologic efficacy of levofloxacin for the treatment of community-acquired pneumonia due to *Chlamydia pneumoniae*. *Antimicrob Agents Chemother* 2000;**44**: 1409.
68. Hammerschlag MR, Roblin PM. Microbiologic efficacy of moxifloxacin for the treatment of community-acquired pneumonia due to *Chlamydia pneumoniae*. *Int J Antimicrob Agents* 2000;**15**:149–52.
69. Hammerschlag MR, Ajl S, Laraque D. Inappropriate use of nonculture tests for the detection of *Chlamydia trachomatis* in suspected victims of child sexual abuse: a continuing problem. *Pediatrics* 1999;**104**:1137–9.
70. Hammerschlag MR, Kohlhoff SA, Darville T. *Chlamydia pneumoniae* and *Chlamydia trachomatis*. In: Fratamico PM, Smith JL, Brogden KA, editors. *Sequelae and long-term consequences of infectious diseases*. Washington, DC: American Society for Microbiology; 2009. p. 27–52.
71. Hammerschlag MR, Chandler JW, Alexander ER, et al. Longitudinal studies of chlamydial infection in the first year of life. *Pediatr Infect Dis* 1982;**1**:395–401.
72. Hammerschlag MR, Chirgwin K, Roblin PM, et al. Persistent infection with *Chlamydia pneumoniae* following acute respiratory illness. *Clin Infect Dis* 1992;**14**:178–82.
73. Hammerschlag MR, Cummings CC, Roblin PM, et al. Efficacy of neonatal ocular prophylaxis for the prevention of chlamydia and gonococcal conjunctivitis. *N Engl J Med* 1989;**320**:769–72.
74. Hammerschlag MR, Gelling M, Roblin PM, et al. Treatment of neonatal chlamydial conjunctivitis with azithromycin. *Pediatr Infect Dis J* 1998;**17**:1049–50.
75. Hammerschlag MR, Roblin PM, Gelling M, et al. Use of polymerase chain reaction for the detection of *Chlamydia trachomatis* in ocular and nasopharyngeal specimens from infants with conjunctivitis. *Pediatr Infect Dis J* 1997;**16**:293–7.
76. Harris JA, Kolokathis A, Campbell M, et al. Safety and efficacy of azithromycin in the treatment of community acquired pneumonia in children. *Pediatr Infect Dis J* 1998;**17**:865–71.
77. Harrison HR, English MG, Lee CK, et al. *Chlamydia trachomatis* infant pneumonitis: comparison with matched controls and other infant pneumonitis. *N Engl J Med* 1978;**298**:702–8.
78. Hashigucci K. Isolation of *Chlamydia pneumoniae* from the maxillary sinus of a patient with purulent sinusitis. *Clin Infect Dis* 1992; **15**:570–1.
79. Hedberg K, White KE, Forfang JC, et al. An outbreak of psittacosis in Minnesota turkey industry workers: Implications for modes of transmission and control. *Am J Epidemiol* 1989;**130**: 569–77.
80. Herrmann SB, Salih MAM, Yousif BE, et al. Chlamydial etiology of acute lower respiratory tract infections in children in the Sudan. *Acta Paediatr* 1994;**83**:169–72.
81. Honein MA, Paulozzi LJ, Himelright IM, et al. Infantile hypertrophic pyloric stenosis after pertussis prophylaxis with erythromycin: a case review and cohort study. *Lancet* 1999;**354**:2101–5.
82. Huang W, Gaydos CA, Barnes MR, et al. Comparative effectiveness of a rapid point of care test for detection of *Chlamydia trachomatis* among women in a clinical setting. *Sex Transm Dis* 2013;**89**:108–14.
83. Hyman CL, Augenbraun MH, Roblin PM, et al. Asymptomatic respiratory tract infection with *Chlamydia pneumoniae*. *J Clin Microbiol* 1991;**29**:2082–3.
84. Hyman CL, Roblin PM, Gaydos CA, et al. Prevalence of asymptomatic nasopharyngeal carriage of *Chlamydia pneumoniae* in subjectively healthy adults: assessment by polymerase chain reaction–enzyme immunoassay and culture. *Clin Infect Dis* 1995; **20**:1174–8.
85. Ingram DL, Runyan DK, Collins AD, et al. Vaginal *Chlamydia trachomatis* infection in children with sexual contact. *Pediatr Infect Dis* 1984;**3**:97–9.
86. Ingram DL, White ST, Occhiuti AR, et al. Childhood vaginal infections: association of *Chlamydia trachomatis* with sexual contact. *Pediatr Infect Dis* 1986;**5**:226–9.
87. Jackson LA, Cherry JD, Wang SP, et al. Frequency of serological evidence of *Bordetella* infections and mixed infections with other respiratory pathogens in university students with cough illnesses. *Clin Infect Dis* 2000;**31**:3–6.
88. Jackson M, White N, Giffard P, et al. Epizootiology of *Chlamydia* infections in two free-range koala populations. *Vet Microbiol* 1999;**65**:255–64.
89. Jantos CA, Wienpahl B, Schiefer HG, et al. Infection with *Chlamydia pneumoniae* in infants and children with acute lower respiratory tract disease. *Pediatr Infect Dis J* 1995;**14**:117–22.
90. Kalman S, Mitchell W, Marathe R, et al. Comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*. *Nat Genet* 1999;**21**: 385–9.
91. Kellogg ND, Baillargeon J, Lukefahr JL, et al. Comparison of nucleic acid amplification tests and culture techniques for the detection of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* in victims of suspected child sexual abuse. *J Pediatr Adolesc Gynecol* 2004;**17**:331–9.
92. Kelly P, Koh J. Sexually transmitted infections in alleged sexual abuse of children and adolescents. *J Paediatr Child Health* 2006; **42**:434–40.
93. Kern DG, Neill MA, Schachter J. A seroepidemiologic study of *Chlamydia pneumoniae* in Rhode Island. *Chest* 1993;**104**:208–13.
94. Kleemola M, Saikku P, Visakorpi R, et al. Epidemics of pneumonia caused by TWAR, a new *Chlamydia* organism, in military trainees in Finland. *J Infect Dis* 1988;**157**:230–6.
95. Kohlberger P, Bancher-Todesca D. Bacterial colonization in suspected sexually abused children. *J Pediatr Adolesc Gynecol* 2007; **20**:289–92.

96. Komaroff AL, Aronson MD, Schachter J. *Chlamydia trachomatis* infection in adults with community-acquired pneumonia. *JAMA* 1981;**245**:1319–22.
97. Komaroff AL, Aronson MD, Pass CT, et al. Serologic evidence of chlamydial and mycoplasmal pharyngitis in adults. *Science* 1983; **222**:927–8.
98. Kroon FP, van't Wout JW, Weiland HT, et al. *Chlamydia trachomatis* pneumonia in an HIV-seropositive patient. *N Engl J Med* 1989;**320**:806–7.
99. Kumar S, Hammerschlag MR. Acute respiratory infection due to *Chlamydia pneumoniae*: current status of diagnostic methods. *Clin Infect Dis* 2007;**44**:568–76.
100. Kumar S, Kohlhoff S, Gelling M, et al. Infection with *Simkania negevensis* in Brooklyn, New York. *Pediatr Infect Dis J* 2005;**24**: 989–92.
101. Kumar S, Kutlin S, Roblin P, et al. Isolation and antimicrobial susceptibilities of chlamydial isolates from Western barred bandicoots. *J Clin Microbiol* 2007;**45**:392–4.
102. Kutlin A, Roblin PM, Hammerschlag MR. Antibody response to *Chlamydia pneumoniae* infection in children with respiratory illness. *J Infect Dis* 1998;**177**:720–4.
103. Kutlin A, Tsumura N, Emre U, et al. Evaluation of *Chlamydia* immunoglobulin M (IgM), IgG, and IgA rELISAs Medac for diagnosis of *Chlamydia pneumoniae* infection. *Clin Diagn Lab Immunol* 1997;**4**:213–16.
104. Likitnukul S, Nunthapisud P, Prapphal N. Prevalence of *Chlamydia pneumoniae* infection in Thai children with community-acquired pneumonia. *Pediatr Infect Dis J* 2003;**22**:749–50.
105. Littman AJ, Jackson LA, White E, et al. Interlaboratory reliability of microimmunofluorescence test for measurement of *Chlamydia pneumoniae*-specific immunoglobulin A and G antibody titers. *Clin Diagn Lab Immunol* 2004;**11**:615–17.
106. Liu G, Talkington DF, Fields BS, et al. *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in young children from China with community-acquired pneumonia. *Diagn Microbiol Infect Dis* 2005; **52**:7–14.
107. Loens K, Beck T, Ursi D, et al. Two quality control exercises involving nucleic acid amplification methods for detection of *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* and carried out 2 years apart (in 2002 and 2004). *J Clin Microbiol* 2006;**44**: 899–0.
108. Malinverni R, Kuo CC, Campbell LA, et al. Reactivation of *Chlamydia pneumoniae* lung infection in mice by cortisone. *J Infect Dis* 1995;**172**:593–5.
109. Mathews-Greer J, Sloop G, Springer A, et al. Comparison of detection methods for *Chlamydia trachomatis* in specimens obtained from pediatric victims of suspected sexual abuse. *Pediatr Infect Dis J* 1999;**18**:165–7.
110. Maurin M, Eb F, Etienne J, Raoult D. Serologic cross-reactions between *Bartonella* and *Chlamydia* species: implications for diagnosis. *J Clin Microbiol* 1997;**35**:2283–7.
111. McMillan JA, Weiner LB, Lamberson HV, et al. Efficacy of maternal screening and therapy in the prevention of chlamydia infection in the newborn. *Infection* 1985;**13**:263–6.
112. Messmer TO, Skelton SK, Moroney JF, et al. Application of a nested, multiplex PCR to psittacosis outbreaks. *J Clin Microbiol* 1997;**35**:2043–6.
113. Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 2004;**113**:701–7.
114. Miller JM, Martin DH. Treatment of *Chlamydia trachomatis* infections in pregnant women. *Drugs* 2000;**60**:597–605.
115. Miller ST, Hammerschlag MR, Chirgwin K, et al. The role of *Chlamydia pneumoniae* in acute chest syndrome of sickle cell disease. *J Pediatr* 1991;**118**:30–3.
116. Mohile M, Dorari AK, Satpathy G, et al. Microbiologic study of neonatal conjunctivitis with special reference to *Chlamydia trachomatis*. *Indian J Ophthalmol* 2002;**50**:295–9.
117. Mitchell CM, Hutton S, Myers GS, et al. *Chlamydia pneumoniae* is genetically diverse in animals and appears to have crossed the host barrier to humans on (at least) two occasions. *PLoS Pathog* 2010;**6**:e1000903.
118. Montalban GS, Roblin PM, Hammerschlag MR. Performance of three commercially available monoclonal reagents for confirmation of *Chlamydia pneumoniae* in cell culture. *J Clin Microbiol* 1994;**32**:1406–7.
119. Moroney JF, Guevara R, Iverson C, et al. Detection of chlamydiosis in a shipment of pet birds, leading to recognition of an outbreak of clinically mild psittacosis in humans. *Clin Infect Dis* 1998;**26**: 1425–9.
120. Morrison WM, Hutchinson RB, Thomason J, et al. An outbreak of psittacosis. *Br Soc Study Infect* 1991;**91**:71–5.
121. Moss TR, Darougar S, Woodland RM, et al. Antibodies to chlamydia species in patients attending a genitourinary clinic and the impact of antibodies to *Chlamydia pneumoniae* and *Chlamydia psittaci* on the sensitivity and the specificity of *Chlamydia trachomatis* serology tests. *Sex Transm Dis* 1993;**20**:61–5.
122. Moulder JW. Interaction of chlamydiae and host cells in vitro. *Rev Clin Microbiol* 1991;**55**:143–90.
123. Myers GS, Matthews SA, Eppinger M, et al. Evidence that *Chlamydia pneumoniae* was zoonotically acquired. *J Bacteriol* 2009;**23**:7225–33.
124. Ochiai Y, Fukushi H, Yan C, et al. Comparative analysis of the putative amino acid sequences of chlamydial heat shock protein 60 and *Escherichia coli* GroEL. *J Vet Med Sci* 2000;**62**:941–5.
125. Ogawa H, Hashiguchi K, Kazuyama Y. Recovery of *Chlamydia pneumoniae* in six patients with otitis media with effusion. *J Laryngol Otol* 1992;**106**:490–2.
126. Pannekoek Y, Dickx V, Beeckman S, et al. Multi-locus sequence typing of *Chlamydia* reveals an association between *Chlamydia psittaci* genotypes and host species. *PLoS One* 2010;**5**:e14179.
127. Persson K, Haidl S. Evaluation of a commercial test for antibodies to the chlamydial lipopolysaccharide (Medac) for serodiagnosis of acute infections by *Chlamydia pneumoniae* (TWAR) and *Chlamydia psittaci*. *APMIS* 2000;**108**:131–8.
128. Pether JVS, Wang SP, Grayston JT. *Chlamydia pneumoniae*, strain TWAR, as the cause of an outbreak in a boys' school previously called psittacosis. *Epidemiol Infect* 1989;**103**:395–400.
129. Popov VL, Shatkin AA, Pankratova VN, et al. Ultrastructure of *Chlamydia pneumoniae* in cell culture. *FEMS Microbiol Lett* 1991; **84**:129–34.
130. Porder K, Sanchez N, Roblin PM, et al. Lack of specificity of Chlamydiazyme for detection of vaginal chlamydial infection in prepubertal girls. *Pediatr Infect Dis J* 1989;**8**:358–60.
131. Principi N, Esposito S, Blasi F, et al. Mowgli Study Group. Role of *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in children with community-acquired lower respiratory tract infections. *Clin Infect Dis* 2001;**32**:1281–9.
132. Quirke M, Cullinane A. Recent trends in chlamydial and gonococcal conjunctivitis among neonates and adults in an Irish hospital. *Int J Infect Dis* 2008;**12**:371–3.
133. Reed KD, Ruth GR, Meyer JA, et al. *Chlamydia pneumoniae* infection in a breeding colony of African clawed frogs *(Xenopus tropicalis)*. *Emerg Infect Dis* 2000;**6**:196–9.
134. Roblin PM, Hammerschlag MR. Microbiologic efficacy of azithromycin and susceptibility to azithromycin of isolates of *Chlamydia pneumoniae* from adults and children with community acquired pneumonia. *Antimicrob Agents Chemother* 1998;**42**: 194–6.
135. Roblin PM, Dumornay W, Hammerschlag MR. Use of HEp-2 cells for improved isolation and passage of *Chlamydia pneumoniae*. *J Clin Microbiol* 1992;**30**:1968–71.
136. Rockey DD, Lenart J, Stephens RS. Genome sequencing and our understanding of chlamydiae. *Infect Immun* 2000;**68**:5473–9.
137. Rours GI, Hammerschlag MR, de Faber JTHN, et al. *Chlamydia trachomatis* as a cause of neonatal conjunctivitis in Dutch infants. *Pediatrics* 2008;**121**:e321–6.
138. Rours GI, Hammerschlag MR, Van Doornum GJJ, et al. *Chlamydia trachomatis* respiratory infection in Dutch infants. *Arch Dis Child* 2009;**94**:705–7.
139. Saikku P, Ruutu P, Leinonen M, et al. Acute lower-respiratory-tract infection associated with chlamydial TWAR antibody in Filipino children. *J Infect Dis* 1988;**158**:1095–7.
140. Saikku P, Wang SP, Kleemola M, et al. An epidemic of mild pneumonia due to an unusual *Chlamydia psittaci* strain. *J Infect Dis* 1985; **151**:832–9.
141. Schachter J, Grossman M, Sweet RL, et al. Prospective study of perinatal transmission of *Chlamydia trachomatis*. *JAMA* 1986; **255**:3374–7.
142. Schachter J, Sweet RL, Grossman M, et al. Experience with the routine use of erythromycin for chlamydia infections in pregnancy. *N Engl J Med* 1986;**314**:276–9.

143. Shemer AY, Lieberman D. *Chlamydia pneumoniae:* induced ciliostasis in ciliated bronchial epithelial cells. *J Infect Dis* 1995;**171**: 1274–8.
144. Siffermann E, Lorand M, Beavis KG, et al. A pseudo-outbreak of *Chlamydia trachomatis* infection among children being evaluated for suspected sexual abuse. In: Ouburg S, Morré SA, editors. *Proceedings of the 7th Meeting of the European Society for Chlamydia Research*. Amsterdam, the Netherlands: VU University; 2012. p. 229–30.
145. Simmons KJ, Hicks DJ. Child sexual abuse examination: is there a need for routine screening for *N. gonorrhoeae* and *C. trachomatis*. *J Pediatr Adolesc Gynecol* 2005;**18**:343–5.
146. Smith KA, Campbell CT, Murphy J, et al. *Compendium of measures to control* Chlamydophila psittaci *infection among humans (psittacosis) and pet birds (avian chlamydiosis)*, 2010. Available at http://www.nasphv.org/Documents/Psittacosis.pdf.
147. Sundelof B, Gnarpe H, Gnarpe J. An unusual manifestation of *Chlamydia pneumoniae* infection: meningitis, hepatitis, iritis and atypical erythema nodosum. *Scand J Infect Dis* 1993;**25**:259–61.
148. Theunissen HJH, Toom NAL, Burggraaf A, et al. Influence of temperature and relative humidity on the survival of *Chlamydia pneumoniae* in aerosols. *Appl Environ Microbiol* 1993;**59**:2589–93.
149. Thom DH, Grayston JT, Wang SP, et al. *Chlamydia pneumoniae* strain TWAR, *Mycoplasma pneumoniae* and viral infections in acute respiratory disease in a university study health clinic population. *Am J Epidemiol* 1990;**132**:248–56.
150. Thygeson P, Stone W. Epidemiology of inclusion conjunctivitis. *Arch Ophthalmol* 1942;**27**:91–122.
151. Tipple MA, Beem MO, Saxon EM. Clinical characteristics of the afebrile pneumonia associated with *Chlamydia trachomatis* infection in infants less than 6 months of age. *Pediatrics* 1979;**63**: 192–7.
152. Tsolia MN, Psarras S, Bossios A, et al. Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 2004;**39**: 681–6.
153. Tuuminen T, Palomaki P, Paavonen J. The use of serologic tests for the diagnosis of chlamydial infections. *J Microbiol Methods* 2000;**42**:265–79.
154. Van Buuren CE, Dorrestein GM, Van Dijk JE. *Chlamydia psittaci* infections in birds: a review on the pathogenesis and histopathological features. *Vet Q* 1994;**16**:38–41.
155. Wong KH, Skelton SK, Daugharty H. Utility of complement fixation and microimmunofluorescence assays for detecting serologic responses in patients with clinically diagnosed psittacosis. *J Clin Microbiol* 1994;**32**:2417–21.
156. Wu SX, Yang J, Liu G. A clinical study in China of neonatal conjunctivitis caused by *Chlamydia trachomatis*. *Clin Pediatr (Phila)* 2003;**42**:83.
157. Yamada S, Tsumura N, Nagai K, et al. A child with iritis due to *Chlamydia pneumoniae* infection. *J Jpn Assoc Infect Dis* 1994;**68**: 1543–7.
158. Yamazaki T, Nakada H, Sakurai N, et al. Transmission of *Chlamydia pneumoniae* in young children in a Japanese family. *J Infect Dis* 1990;**162**:1390–2.
159. Yeung SM, McLeod K, Wang SP, et al. Lack of evidence of *Chlamydia pneumoniae* infection in infants with acute lower respiratory tract disease. *Eur J Clin Microbiol Infect Dis* 1993;**12**: 850–3.
160. Yip TPP, Chan WH, Yip KT, et al. Incidence of neonatal chlamydial conjunctivitis and its association with nasopharyngeal colonization in a Hong Kong hospital assesses by polymerase chain reaction. *Hong Kong Med J* 2007;**13**:22–6.
161. Yip PP, Chan WH, Yip KT, et al. The use of polymerase chain reaction assay versus conventional methods in detecting neonatal chlamydial conjunctivitis. *J Pediatr Ophthalmol Strabis* 2008;**45**: 234–9.
162. Yung AP, Grayson ML. Psittacosis: a review of 135 cases. *Med J Aust* 1988;**148**:228–33.

SECTION **XIX**

RICKETTSIAL DISEASES

CHAPTER 194

Rickettsial and Ehrlichial Diseases

Paul M. Lantos • Ross McKinney Jr.

The Rickettsiaceae are a family of organisms that share a fascinating history and common biology. The critical steps in the epidemiology were first characterized by Howard T. Ricketts in the early 20th century. He was doing field work in the Bitterroot Valley in Montana and described that Rocky Mountain spotted fever (RMSF) was transmitted by the Rocky Mountain wood tick. Interestingly, before he did his work demonstrating tick-dependent transmission using guinea pigs, the fact that human transmission was through a tick vector had already been proved. In the *Transactions of the Chicago Pathological Society*, Dr. Ricketts reported:

> At the time this work was done I had no knowledge of previous experiments which demonstrated the ability of the "wood tick" to transfer spotted fever. Recently I have had authoritative information that Dr. L. P. McCalla and Dr. H. A. Brereton, of Boise City, Idaho, transmitted the disease from man to man in two experiments. The tick was obtained "from the chest of a man very ill with spotted fever," and "applied to the arm of a man who had been in the hospital for two months and a half, and had lost both feet from gangrene due to freezing." On the eighth day the patient became ill and passed through a mild course of spotted fever, leaving a characteristic eruption. The experiment was repeated by placing the tick on a woman's leg, and she likewise was infected with spotted fever. Although these results received no publicity other than that given in reports to local societies, I take pleasure in according to Dr. McCalla and to his colleague, Dr. Brereton, the credit of having first shown that the tick may act as a carrier of spotted fever. Their experiments preceded by more than a year those of Dr. King and of myself, and they were performed with the full consent of the patients concerned.[148]

The study was more fully described by Dr. McCalla in 1908.[115]

Sadly, funding for Dr. Ricketts' RMSF research dried up, and he moved to Mexico to investigate an outbreak of tabardillo, a Mexican typhus fever, which he suspected was similar in biology to RMSF. He identified lice as the vector for the disease, but in his attempt to isolate the causative organism, he contracted typhus and died. Shortly before his death, he learned that the state of Montana was ready to resume funding of his research on RMSF. (This is, of course, a cautionary tale to all academics with insecure funding.)

Rickettsiae are small coccobacilli in the class Alphaproteobacteria,[69] visible by light microscopy, that cause their disease intracellularly. The challenging nature of their physical identification was borne out by the fact that Howard Ricketts thought the cause of RMSF was a viral infection. The organism eventually named *Rickettsia rickettsii* was first described by Simeon Burt Wohlbach in 1916, although it took several years for his identification of a 0.2- to 0.5-μm gram-negative organism to be confirmed.[76,193] Rickettsiae cause disease by multiplying in the vascular endothelium, producing a generalized vasculitis involving mostly small blood vessels and symptoms that can involve nearly the entire body.

The human rickettsiae are in the family Rickettsiaceae, which is divided into two genera, *Rickettsia* and *Orientia*.[35] The rickettsiae are the more important pathogens and consist of the spotted fever group and the typhus group.[165] *Orientia* includes only one pathogen, *Orientia tsutsugamushi*, the cause of scrub typhus. The Anaplasmataceae family is closely related and is divided into two genera, *Anaplasma* and *Ehrlichia*.[145]

All of the infections discussed in this chapter are transmitted by arthropod vectors. The spotted fever group of rickettsiae is predominantly transmitted by ticks, although rickettsialpox is spread by mites. Scrub typhus is spread by human body lice as well as by ticks, and murine typhus is spread by fleas (Table 194-1).

Rickettsiae are important causes of human disease and have, in fact, played a profound effect on human history.[202] This chapter reviews the pediatric manifestations of diseases caused by the spotted fever and typhus groups of *Rickettsia*, *Ehrlichia*, and Anaplasmataceae.

THE SPOTTED FEVERS

The spotted fever group of the genus *Rickettsia* is composed of pathogens that are arthropod borne, mostly by ticks. The exception is rickettsialpox, caused by *Rickettsia akari*, which is a mite-borne infection. Disease produced by *R. rickettsii*, of which RMSF is the prototype, occurs throughout the Americas. There are a variety of other spotted fever illnesses seen around the world, most of which are less clinically severe. These diseases are distinguished by their geography and by variations in the causative organism, but they share their sensitivity to tetracyclines and their obligately intracellular life cycle.

Rocky Mountain Spotted Fever

Historical Aspects. RMSF has an unusually fascinating history. It was originally recognized in the Bitterroot Valley of western Montana, nestled against the Idaho border. According to Howard Ricketts, the first description of "the so-called spotted fever of Idaho" was made

TABLE 194-1 **Classification, Primary Vector, and Reservoir Occurrence of Rickettsiae Known to Cause Disease in Humans**

Antigenic Group	Disease	Species	Vector	Animal Reservoirs	Geographic Distribution
Anaplasma	Human granulocytic anaplasmosis	*Anaplasma phagocytophilum*	Tick	Deer, elk, small mammals, and rodents	Worldwide
Ehrlichia	Human monocytic ehrlichiosis	*Ehrlichia chaffeensis*	Tick	Deer, wild and domestic dogs, domestic ruminants, and rodents	Worldwide
	Ehrlichiosis	*E. muris*	Tick	Deer and rodents	Western United States, Russia, Japan
	Ehrlichiosis	*E. ewingii*	Tick	Deer, wild and domestic dogs, and rodents	North America
Neorickettsia	Sennetsu fever	*Neorickettsia sennetsu*	Trematode	Fish	Japan, Malaysia, possibly other parts of Asia
Scrub typhus	Scrub typhus	*Orientia tsutsugamushi*	Larval mite (chigger)	Rodents	Asia-Pacific region from maritime Russia and China to Indonesia and North Australia to Afghanistan
Spotted fever	Rickettsiosis	*Rickettsia aeschlimannii*	Tick	Unknown	South Africa, Morocco, Mediterranean littoral
	African tick bite fever	*R. africae*	Tick	Ruminants	Sub-Saharan Africa, West Indies
	Rickettsialpox	*R. akari*	Mite	House mice, wild rodents	Countries of the former Soviet Union, South Africa, Korea, Turkey, Balkan countries, North and South America
	Queensland tick typhus	*R. australis*	Tick	Rodents	Australia, Tasmania
	Mediterranean spotted fever or boutonneuse fever	*R. conorii**	Tick	Dogs, rodents	Southern Europe, southern and western Asia, Africa, India
	Cat flea rickettsiosis	*R. felis*	Flea	Domestic cats, rodents, opossums	Europe, North and South America, Africa, Asia
	Far Eastern spotted fever	*R. heilongjiangensis*	Tick	Rodents	Far East of Russia, Northern China, eastern Asia
	Aneruptive fever	*R. helvetica*	Tick	Rodents	Central and northern Europe, Asia
	Flinders Island spotted fever, Thai tick typhus	*R. honei*	Tick	Unknown	Australia, Thailand
	Japanese spotted fever	*R. japonica*	Tick	Rodents	Japan
	Australian spotted fever	*R. marmionii*	Tick	Rodents, reptiles	Australia

	Mediterranean spotted fever–like disease	*R. massiliae*	Tick	Unknown	France, Greece, Spain, Portugal, Switzerland, Sicily, central Africa, and Mali
	Maculatum infection	*R. parkeri*	Tick	Rodents	North and South America
	Rocky Mountain spotted fever, febre maculosa, São Paulo exanthematic typhus, Minas Gerais exanthematic typhus, Brazilian spotted fever	*R. rickettsii*	Tick	Rodents	North, Central, and South America
	North Asian tick typhus, Siberian tick typhus	*R. sibirica*	Tick	Rodents	Russia, China, Mongolia
	Lymphangitis-associated rickettsiosis	*R. sibirica mongolotimonae*	Tick	Rodents	Southern France, Portugal, China, sub-Saharan Africa
	Tick-borne lymphadenopathy (TIBOLA), *Dermacentor*-borne necrosis and lymphadenopathy (DEBONEL)	*R. slovaca*	Tick	Lagomorphs, rodents	Southern and eastern Europe, Asia
Typhus fever	Epidemic typhus, sylvatic typhus	*R. prowazekii*	Human body louse, flying squirrel ectoparasites, *Amblyomma* ticks	Humans, flying squirrels	Central Africa; Asia; Central, North, and South America
	Murine typhus	*R. typhi*	Flea	Rodents	Tropical and subtropical areas worldwide

*Includes four different subspecies that can be distinguished serologically and by PCR assay and respectively are the etiologic agents of boutonneuse fever and Mediterranean tick fever in southern Europe and Africa (*R. conorii* subsp. *conorii*), Indian tick typhus in south Asia (*R. conorii* subsp. *indica*), Israeli tick typhus in southern Europe and Middle East (*R. conorii* subsp. *israelensis*), and Astrakhan spotted fever in the North Caspian region of Russia (*R. conorii* subsp. *caspiae*).

From Eremeeva ME, Dasch GA. Rickettsial (spotted & typhus fevers) & related infections (anaplasmosis & ehrlichiosis). In: *CDC Yellow Book Online*. Available at http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/rickettsial-spotted-and-typhus-fevers-and-related-infections-anaplasmosis-and-ehrlichiosis.htm.

by Edward E. Maxey of Boise City, Idaho, in 1899.[148] The description of the illness, like many clinical characterizations of disease in the pre-laboratory era, was surprisingly consistent with our view today, "an acute, endemic, noncontagious, but probably infectious, febrile disease, characterized clinically by a continuous moderately high fever, severe arthritic and muscular pains, and a profuse petechial or purpuric eruption in the skin, appearing first on the ankles, wrists, and forehead, but rapidly spreading to all parts of the body."[148] The disease was soon recognized throughout the Rocky Mountain states. Whereas there were speculations that ticks were the vectors of RMSF, it was Ricketts who unequivocally proved the association through carefully performed experiments with a guinea pig model of disease. As noted earlier, he died before he was able to identify the causative organism of RMSF with certainty, a step performed by Simeon Burt Wohlbach in 1916. Wohlbach noted that the organism occurred in large numbers in the smooth muscle cells of arteries and veins. There were disputes between Wohlbach and researchers at the Rockefeller Institute in New York as to whether this new small organism was in fact the real pathogen. The dispute was sadly resolved when a laboratory technician working with RMSF at the Rockefeller Institute developed a fever, perhaps after a needle scratch, and died. The technician's blood was transported to Wohlbach's laboratory, where he inoculated guinea pigs and they became symptomatic with characteristic RMSF (in a guinea pig, fever, rash, and vasculitis). Wohlbach chose to name the organism after Howard Ricketts, whom he believed had, in fact, first seen the organism.

Another challenge was to grow the organism, then known as *Dermacentroxenus rickettsii*. It was originally thought that the organism could be grown only in cell cultures,[194] which led to confused debates as to whether the organism was an intracellular bacterium or a virus. The answers came as Herald R. Cox discovered that rickettsiae could be grown in larger numbers in the yolk sacs of fertilized chicken eggs.[39]

Many mysteries remain about certain elements in the natural history of RMSF. For example, the pretreatment mortality rate of RMSF in Idaho was 5 to 7 percent, whereas in the Bitterroot Valley of Montana it was 70 percent.[88] The molecular basis for this difference in virulence remains unclear even today.[35] The epidemiology of RMSF has evolved during the 20th century from being a disease predominantly recognized in the northern Rocky Mountains to a disease most commonly found in a band of contiguous states from Oklahoma to the Carolinas. North Carolina, Tennessee, Arkansas, Missouri, and Oklahoma account for 60 percent of the reported RMSF cases in the United States.[32] The incidence of RMSF has been generally rising, whereas the case-fatality rate has been falling (Fig. 194-1). In 2008, there were roughly 2500 cases of RMSF in the United States, the largest number on record.[32]

Etiology, Morphology, Growth, and Metabolism. *R. rickettsii*, the etiologic agent of RMSF, is a small, nonmotile, pleomorphic, weakly gram-negative coccobacillus measuring 0.3 to 0.5 μm in diameter and 0.3 to 4 μm in length. The cell wall and cytoplasmic membrane are fairly typical for gram-negative bacteria. Rickettsiae must penetrate into living cells to grow and multiply (i.e., they are obligately intracellular). They are grown most readily in the yolk sacs of embryonated eggs; but under special conditions, they also grow well in certain tissue culture

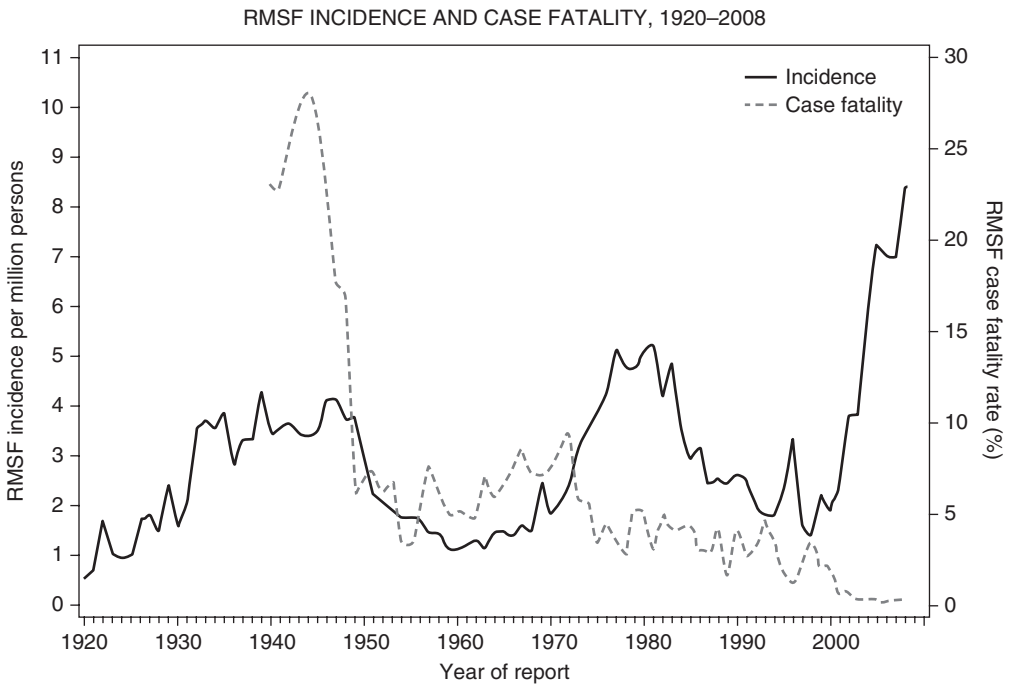

FIGURE 194-1 ■ Rocky Mountain spotted fever incidence and case fatality, 1920-2008. (From Centers for Disease Control and Prevention. Statistics and epidemiology: annual cases of RMSF in the United States. Available at http://www.cdc.gov/rmsf/stats/.)

cells. Once inside a cell, rickettsial cells multiply by transverse binary fission.[78] The organisms lack the intrinsic ability to metabolize carbohydrates and amino acid substrates and derive energy from host adenosine triphosphate (ATP).[35]

Rickettsiae take on a characteristic red color when stained with Gimenez stain. *R. rickettsii* possess a soluble antigenic moiety that is shared with antigenic variants in the spotted fever group and rickettsialpox.

Epidemiology and Transmission. Because *R. rickettsii* is transmitted to people through adult tick vectors, understanding of tick ecology explains much of the epidemiology of RMSF. The vectors for RMSF are *Dermacentor variabilis* (the American dog tick) in the eastern and central United States and *Dermacentor andersoni* (the Rocky Mountain wood tick) in the Rocky Mountain states and West. RMSF has also been reported recently in an area in eastern Arizona where the vector is *Rhipicephalus sanguineus* (the brown dog tick).[32] These ticks prefer woodlands where small rodents share habitats with large domestic and wild animals.[22] RMSF is most common in spring and summer months when ticks are most active.

Regionally, RMSF is concentrated. Fifty-six percent of cases come from five states (North Carolina, South Carolina, Tennessee, Oklahoma, and Arkansas). However, it is widely distributed in North America, and cases have been reported from 46 of the 48 contiguous United States (Maine and Vermont are the exceptions).[34] It tends to occur in hyperendemic foci, and there are reports of family clusters of disease.[28] *R. rickettsii* infection occurs throughout Central and South America via several different tick species; there are a variety of regional names for the infection, such as Tobia fever (Colombia) and São Paolo fever (Brazil).

The life cycle of *Dermacentor andersoni* ticks requires roughly 2 years. In the spring and early summer, adult ticks seek out hosts for a blood meal. Ticks that attach feed for 7 to 9 days, then mate. The engorged females drop off their hosts, laying 5000 to 7000 eggs each. After 35 days, the eggs hatch and the larvae seek out small mammals to which to attach. After 2 to 6 days of feeding, the larvae drop off, mature into nymphs, and become inactive for the winter. In the spring, the nymphs attach to small mammals, feed for 7 days, drop off, hide, and mature into adults. They generally hide for the duration of the summer, fall, and winter and in the spring seek out their adult blood meal.[22]

D. variabilis is similar to the Rocky Mountain wood tick but adapted to the climate of the central and eastern United States. The larvae and nymphs have a narrower host range and show a preference for meadow and white-footed mice. Dogs are the primary hosts for the adult ticks, but the adult ticks are willing to bite a wide range of large mammals, including humans, who are thus an incidental victim. Larvae are most active in the early spring (March and April), then become active again in the late summer (August and September) if they did not successfully feed in the spring. Nymphs are also active in spring and late summer, with the spring nymphs having overwintered and the late summer nymphs a product of spring larvae. Adults also most actively seek blood meals from April to September, although they can become active in any month if the weather is warm enough.[22]

Rickettsiae multiply in ticks and small mammals. The organisms are found in the salivary gland, and during a blood meal the organisms are activated and become more pathogenic. They are ultimately injected into the host by the tick during the course of the blood meal, although ticks can also transmit as they are squeezed during the process of tick removal.[35] Rickettsiae can be passed from one generation of tick to the next transovarially and can persist from hatching into the adult tick. This is particularly impressive because adult ticks can wait up to 4 years for a blood meal. However, infected ticks seem to have a biologic disadvantage, and relatively few *R. rickettsii*–infected larvae ultimately mature into adults.[49] The organisms are found in the salivary glands and the gastrointestinal tract of the ticks. Interestingly, RMSF is also a sexually transmitted disease; it can be spread from infected male ticks to uninfected females through infected spermatozoa.[79,133]

Although it is uncommon, RMSF can be spread through aerosolization in the laboratory, so care should be taken (it requires a biosafety level 3 facility). In addition, there have been uncommon transmissions by transfusion[187] and needle stick accidents.[153]

Seroprevalence studies have demonstrated that subclinical disease may be more common than symptomatic, although it is difficult to know whether the results reflect antibodies to rickettsiae other than *R. rickettsii* or perhaps cross-reactive antigens from other organisms.[191] A large seroprevalence survey of children at sites in the southeastern United States found *R. rickettsii* seropositivity rates that varied from 3.5 percent in Louisville, Kentucky, to 21.9 percent in Little Rock, Arkansas. These are rates that seem high relative to the actual incidence of RMSF. Also noteworthy was the large jump in overall seroprevalence from the 1- to 6-year-old age group (4.2%) to the 7- to 12-year-olds (13.9%), suggesting a higher rate of exposure in early school-age children.[112]

Perhaps because *R. rickettsii* is biologically disadvantageous for ticks, it is relatively uncommon even in highly endemic areas. The tick prevalence of *R. rickettsii* infection is less than one RMSF infection per 1000 ticks, whereas many ticks have nonpathogenic rickettsiae like *Rickettsia montana* or *Rickettsia rhipicephali*.[176] This low prevalence is one of the primary reasons that there is no role for doxycycline prophylaxis after removal of an engorged tick.

Pathogenesis. Vasculitis is the fundamental disease process in RMSF. Rickettsiae infect the endothelial cells lining blood vessels, particularly of small and medium-sized blood vessels, and the resultant cellular damage creates vascular lesions that can involve almost the entire vascular bed. The process begins when the tick bites an individual and begins its blood meal. Rickettsiae are introduced from the salivary glands of the tick, and the infectious cycle begins.

At a cellular level, rickettsiae are internalized by endocytosis into vacuoles. They are initially enclosed in a

vacuolar membrane but are able to degrade the membrane and escape into the cytosol. Rickettsiae are obligate intracellular pathogens, and they require access to the cytoplasm to grow. They lack the ability to metabolize carbohydrates and amino acids and thus obtain their energy from host ATP molecules.[35] Once in the cytoplasm, the organisms replicate by binary fission. They recruit host cell actin molecules, and they use the actin to spread from cell to cell, transported through cell membranes without producing damage. They spread rapidly among the endothelial cells lining the vascular and lymphatic systems. As the cells become injured by the rickettsiae, they lose their attachment to adjacent cells, and the smooth vascular surface becomes increasingly rough and leaky.

The immune response to *R. rickettsia* involves perivascular lymphocytic infiltrates. As the endothelial cells fail, small leaks in the capillary bed produce increased vascular permeability, microhemorrhages, localized edema, and local inflammation. With time, the problem becomes more generalized, involving a wide variety of organs, including the skin, brain, lungs, kidneys, and liver. Eventually the combination of leakage, edema, and inflammation can produce organ failure. On pathologic examination in affected patients, inflammation can be seen in the affected tissues.

Clearance of *R. rickettsii* is predominantly by cellular immunity, including cytotoxic T lymphocytes and interferon-γ. The immune response lyses infected cells, an action that may be the primary means by which rickettsial infection causes cell injury.[176] A broad range of antibodies are produced against *R. rickettsii*, and these antibodies appear to be the host's primary defense against reinfection. Although it is not absolutely certain, it is generally believed that people are not infected by *R. rickettsii* more than once.

The laboratory hallmarks of RMSF are also a product of this diffuse vascular injury, with often profound thrombocytopenia and mild to moderate hyponatremia. The thrombocytopenia is a result of platelet consumption along damaged vascular surfaces; the hyponatremia is probably a combination of shifting of electrolytes from the intracellular to the extracellular space, loss of sodium through excretion, exchange of sodium for potassium at the cellular level, and the syndrome of inappropriate antidiuretic hormone secretion.[92]

The extent of disease is dependent on several factors in addition to the timing of treatment initiation. For example, there is the regional variation noted earlier (Montana vs. Idaho). The inoculum size is almost certainly important and may relate to the duration of tick attachment. Most of the time, *R. rickettsii* in tick salivary glands are in a dormant, noncontagious state. During the blood meal, the rickettsiae become active and virulent, a process that may take as long as 4 to 6 hours. As a result, that duration is thought to be the necessary length of attachment for transmission to occur. Host factors that make RMSF potentially more severe include glucose-6-phosphate dehydrogenase (G6PD) deficiency (a sex-linked condition found in 12% of African American males), age (adults are at higher risk for severe disease), and gender (males more than females).[176]

Clinical Manifestations. The classic manifestations of RMSF are fever, headache, myalgias, severe malaise, and rash. The illness is seldom subtle, and most children infected with *R. rickettsii* appear quite ill. Temperatures in the range of 40°C (104°F) are typical. In the early stages, rash may not be present or may be only pale, erythematous macules. In dark-skinned patients, the early rash may not be apparent. As the illness progresses, the fever persists, the degree of malaise becomes complete prostration, and the rash evolves from macules to petechiae. Eventually, the child can become hypotensive and obtunded and may die if not appropriately treated.

RMSF is transmitted by tick bite, but surprisingly only half of patients can recall being bitten by a tick.[20] This probably reflects the facts that ticks are stealthy and generally obtain their blood meal efficiently, without pain. They may attach in areas that are not easily seen. The consequence for physicians is that the historical review should focus not only on tick bites but also on behaviors that put someone at risk for tick exposure. These behaviors include walking in woods or high grass in the spring or summer or during a sustained warm spell. In terms of RMSF, the degree of regional incidence of *R. rickettsii* infection should also be considered; the southeastern and south-central United States have the highest incidence.

The incubation period for RMSF after the tick bite is 48 hours to 14 days, with a median of 4 days.[20,35] The first symptoms are usually fever, headache, myalgias, and malaise. These are obviously nonspecific findings, making the diagnosis of RMSF difficult. Fever is virtually a sine qua non, occurring in 98 percent of pediatric RMSF cases.[20] Profound malaise is also present from early in the illness, marking RMSF as more than the typical nonspecific virus infection. Sixty percent of children develop a headache, which is often severe. Lumbar punctures typically have a mild leukocytosis, with a median of 25 cells/mm^3.[20]

Rash is not reliably present in the early stages of disease, unfortunately. The rash typically appears in the second to fourth day of illness, which is generally after patients have first sought medical care.[34] Eventually 90 percent or more of children have rash. The early rash begins as faint macules, often on the arms and lower legs. At this stage, the lesions still blanch on pressure. Petechiae evolve during several days. Classically the rash evolves from the periphery toward the trunk and involves the palms and soles. However, neither the centripetal nature nor palm and sole involvement is a definitive or reliable finding. Generalized petechiae are seen around the fifth or sixth day of illness, at which point patients are severely ill. They are likely to have organ system dysfunction, and recovery will be slow even with treatment.[34]

Other common symptoms are myalgia (45% in the Buckingham series[20]), abdominal pain that can mimic appendicitis (36%), diarrhea (34%), conjunctival injection (33%), altered mental status (33%), lymphadenopathy (29%), and peripheral edema (25%). Less common findings include hepatomegaly, photophobia, seizures, meningismus, splenomegaly, periorbital edema, and coma. Of these symptoms, the conjunctival injection and peripheral edema are most likely to point in the direction

of RMSF, although both usually occur in patients who already have extensive rash.

The laboratory findings are also only moderately helpful. As noted in the section on diagnosis, the only true acute diagnostic test is biopsy of petechial or macular lesions and demonstration of the organism by immunohistochemical staining or polymerase chain reaction (PCR) testing. Antibody tests have almost no utility acutely because the immunofluorescent antibody (IFA) assay titers will not rise into a diagnostic range until 10 days or more into the illness, days after treatment should have been started. The two most useful laboratory findings are thrombocytopenia and hyponatremia. Sixty percent of children have a platelet count below 150,000 cells/mm^3 at their initial presentation, and the median platelet count is 128,000 cells/mm^3. In regard to serum sodium, 52 percent have sodium concentrations below 135 mEq/L, with a median concentration of 133 mEq/L. Other laboratory values that can be abnormal include hepatic enzymes (alanine aminotransferase, aspartate aminotransferase), serum bilirubin, hemoglobin, cerebrospinal fluid cell count, glucose, and protein.

Some patients with RMSF have focal cardiac disease, in particular myocarditis, and rare patients have evidence of pneumonia.

Even with treatment, RMSF can leave patients with residual neurologic damage, as described later in the prognosis section. Conditions associated with more severe RMSF, in addition to delayed treatment, include G6PD deficiency and early treatment with sulfa drugs.

Diagnosis. Because the early manifestations of *R. rickettsii* infection are nonspecific, the problem is to diagnose RMSF effectively and to avoid overtreating a multitude of children. In the right season (summer) in an endemic region, this can be a significant challenge. If an error is made, it should be in overtreatment of patients in whom infection is suspected, given the relative risks of undertreatment of RMSF versus the potential for unnecessary exposure to doxycycline.

For confirmation of a diagnosis of *R. rickettsii* infection, the most widely used test is the indirect IFA assay. The sensitivity of this assay is inadequate to be useful in the first 10 to 12 days of illness. There is essentially no value in a negative test result; it does not rule out RMSF and should not be used for that purpose. The value of IFA assay comes to confirm whether an illness was RMSF ex post facto. The test is 94 percent sensitive by 14 to 21 days.[91] The diagnosis requires a fourfold rise in titer between acute and convalescent sera. A "probable" diagnosis is generally made on the basis of a single convalescent IFA titer of 1:64 or greater.

To make a diagnosis of RMSF more acutely, if a patient has a rash, a skin biopsy can be performed to sample petechial lesions. The lesion can be sent for either immunohistochemical staining or PCR testing, depending on availability. Immunohistochemical staining of biopsy material has been found to be 100 percent specific and 70 percent sensitive.[177] It is most useful either before or very early in treatment. PCR is less established as a diagnostic method but can be performed on either tissue or blood.

The other important but nonspecific diagnostic clues pointing toward RMSF are thrombocytopenia, hyponatremia, and the rash. One case series found the average platelet count at presentation to be 128,000 cells/mm^3; forty-one percent had fewer than 100,000 platelets at initial presentation.[20] In terms of sodium, the median sodium concentration at presentation was 133 mEq/L, and 52 percent of children had a serum sodium concentration below 135 mEq/L. The rash, when present, is an important clue, but the absence of rash does not exclude RMSF. As noted before, the rash begins as faint macules, which become petechial as the disease progresses. They may be found on the palms and soles, but rashes in those locations are seen in only two thirds of children.[20]

Differential Diagnosis. Perhaps the greatest challenge to RMSF is making a clinical diagnosis in time for effective treatment. The characteristics that define early RMSF are fairly nonspecific: fever, headache, some photophobia, and severe malaise. The first critical clue is whether the patient has been in an endemic area, although RMSF has occurred in all of the lower 48 states except Maine and Vermont, and whether the season is right (spring, summer, or a recent warm spell). A rash usually appears somewhere between the third and fifth day and can be a helpful clue, but roughly 10 percent of patients with confirmed RMSF never have a rash. Because of the lack of specificity to the symptoms, clinicians must begin with a high level of suspicion. Enteroviral infections (echovirus, coxsackievirus) appear similar to RMSF, have the same summer seasonality, and can produce a similar rash. In endemic regions, the practical reality is that quite a few enteroviral infections are "cured" with doxycycline.

A more problematic overlap is with *Neisseria meningitidis*. The early symptoms of meningococcemia are again similar to RMSF, with fever, headache, and malaise. Laboratory findings may also be similar, with thrombocytopenia and a leukocyte count that reflects a left shift. Because neither RMSF nor meningococcemia can be quickly confirmed, and because both require a rapid initiation of treatment, it is reasonable and appropriate to begin both doxycycline and penicillin (or some other appropriate β-lactam or cephalosporin) until the situation can be sorted out (primarily by blood culture).

The full differential diagnosis for RMSF includes enteroviral infections, adenoviral infections, meningococcemia, influenza, gram-negative bacterial sepsis, toxic shock syndrome, measles, rubella, secondary syphilis, leptospirosis, typhoid fever, disseminated gonococcal infection, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, immune complex vasculitis (e.g., systemic lupus erythematosus), infectious mononucleosis, hypersensitivity reaction to drugs, murine typhus, rickettsialpox, recrudescent typhus, and sylvatic *Rickettsia prowazekii* infection that is enzootic in flying squirrels.[176]

During the summer in endemic regions, it is fairly common practice to see patients early in the course of a febrile illness. In the first 2 days of fever, practitioners often obtain screening laboratory tests (a complete blood count and chemistry) and, if the patient does not have severe malaise, opt to watch. If the fever persists into the

third day, if the screening laboratory test results appear suggestive, or if the patient appears to be more ill than is usually the case with a viral illness, treatment with doxycycline is initiated. The worst outcomes with RMSF occur when a physician dogmatically holds to a preliminary diagnosis in the face of persisting illness, often because of the absence of a rash or laboratory test result pointing toward RMSF.

Treatment. RMSF is an easily treatable disease with a good prognosis if treatment is begun suitably early. However, therapeutic failures occur every summer because the diagnosis is not considered. The symptoms can mimic other diseases (e.g., enterovirus infection, meningococcemia, infectious mononucleosis, appendicitis, pharyngitis), prompting inappropriate treatment decisions. If doxycycline is initiated within the first 5 days of fever, the prognosis is excellent. The problem is deciding which patients to treat, which to observe. Even in areas where RMSF is endemic, 60 to 75 percent of patients with RMSF are given an alternative diagnosis at their first clinical visit. The probabilities of morbidity and mortality escalate rapidly with each day of treatment delay.

The other important challenge to treating RMSF is the need to begin treatment before the standard diagnostic test results become definitively positive. At the onset of fever, antibody titers against *R. rickettsii* will almost always be negative. In most cases, the IFA test result becomes positive between 7 and 10 days, and by 14 to 21 days, IFA titers are 94 percent sensitive. However, during the first week, when treatment needs to be initiated, antibody titers will be negative. Even defining who is at risk can be difficult. Because ticks are very good at their profession (blood sucking), in only 50 percent of cases do patients report a time-appropriate tick exposure.[20]

Doxycycline is the primary antibiotic used to treat RMSF. Historically, chloramphenicol was demonstrated to be equally effective, and it remains the only documented alternative treatment, but its side effect profile, particularly in causing aplastic anemia, has made oral chloramphenicol unavailable in the United States. Doxycycline is dosed at 2.2 mg/kg/dose every 12 hours (maximum, 100 mg every 12 hours) for the duration of fever and an additional 3 days (at least 5 days, and typically 7 to 10 days). Once the patient is improving, relapse is highly unlikely, and there is no chronic form of *R. rickettsii* infection. Doxycycline may be given intravenously or orally, depending on the degree of illness. Whereas tetracyclines have been associated with dental staining, doxycycline does not pose a significant risk (particularly in comparison to untreated RMSF).

Severe RMSF can require hospitalization and, at times, advanced life support: intravenous fluids, pressors, and assisted ventilation. In its severe form, RMSF includes a profound vasculitis that can mimic disseminated intravascular coagulopathy, with thrombocytopenia, generalized petechiae, hemorrhages, and shock. In many ways, it clinically resembles meningococcemia.

Whereas some children with RMSF are empirically treated with antibiotics other than doxycycline during the early phases of management, given the already noted similarity to meningococcemia, most of those antibiotics will have no effect on the course of RMSF. The one exception is sulfa drugs, including trimethoprim-sulfamethoxazole, which can make RMSF more severe. The mechanism for this observation is still unclear.[34]

Prognosis. The mortality rate in the modern era for untreated RMSF is 20 percent; for treated patients, it is roughly 5 percent.[34] Interestingly, in the preantibiotic era, the mortality rate for RMSF was highly variable. For example, RMSF in the Bitterroot Valley of Montana had a mortality rate of 75 percent, whereas in the Snake River Valley in southern Idaho it was 3 percent.[3] The reasons for differential rates like these have never been explained.[35] Most of the current mortality is in patients with delayed initiation of doxycycline. For patients treated with doxycycline in the first 5 days of symptoms, the prognosis is excellent.

In children who have had severe RMSF, there is a significant rate of residual problems. In one series, 13 of 89 surviving children were discharged from the hospital with neurologic deficits, including speech and swallowing dysfunction, global encephalopathy, ataxia or gait disturbances, and cortical blindness.[20] Many of the children with long-term follow-up continued to have neurologic problems. Two of the 89 children had loss of digits due to autoamputation.

Prevention. The most effective measures for control of RMSF are personal steps to avoid ticks. Wearing pants tucked into boots or socks is not a fashion statement, but it can be useful in tick avoidance. Permethrin-based insect repellants can be effective, although they should be applied to clothes rather than directly on skin (a caution for which users will be grateful after smelling permethrin-based repellants). Insect repellants containing 20 to 50 percent DEET applied to skin may have some effect, although they are probably more effective in deterring mosquitoes than ticks. Adults should perform regular tick inspections on themselves and on children for whom they are responsible because ticks require 4 to 6 hours of attachment to transmit *R. rickettsii*. Ticks are fond of high grasses, brushy areas, and mixed forests, so care should be taken to stay in the center of well-trodden trails. Pets can be protected with medications or flea and tick collars.[29]

Patients should be taught the art of removing ticks from skin. There are many old wives' tales about how to remove ticks: touching their backs with an extinguished match, applying clear nail polish to suffocate them, applying petroleum jelly. Given the effectiveness of these strategies, the surprise is that there are any old wives left to tell tales. To remove a tick, use tweezers, grasp the tick's head as close to the skin as possible, and twist out. The area should be scrubbed with soap or alcohol because any residual tick spit or fecal material has the potential to be infectious and to contaminate the bite wound. Incinerating a tick after removal is, however, perfectly appropriate, as is the more usual approach of flushing it into the sewer system.

Whereas vaccines against RMSF were in use for many years, they were probably marginally effective, if at all, and are no longer available.

Mediterranean Spotted Fever

Overview. Mediterranean spotted fever (MSF) is a tick-borne infection caused by *Rickettsia conorii*. The disease was first described by Conor and Bruch in 1910,[38] and the name "Mediterranean spotted fever" was adopted in 1932. In the Mediterranean region, this infection is also known as boutonneuse fever and Marseilles fever. Whereas the infection has been recognized for a century, until recently it was regarded as a benign disease. In 1982 the first case report of fatal MSF was published.[140] Its potential lethality has been demonstrated in numerous other studies, including one in which 32.3 percent of hospitalized patients died.[43] It is now regarded as comparable to RMSF in its potential for mortality.

Epidemiology. *R. conorii* infection occurs across a wide geographic swath that includes not only the Mediterranean region but also parts of central and southern Africa, the Middle East, and central and south Asia. Locale-specific names given to this infection include Astrakhan spotted fever, Kenya tick bite fever, African tick typhus, India tick typhus, and Israeli spotted fever. The geographic diversity of MSF is matched by its genetic diversity; there are numerous regional subspecies of *R. conorii* capable of causing human disease.[201] The only known vector is the brown dog tick, *Rhipicephalus sanguineus*. The tick is the only known reservoir as well as the vector for *R. conorii*.

MSF occurs in all age groups of both sexes. The epidemiologic pattern is determined by the biology of the tick; consequently, a consistent seasonal peak occurs from June to October. The incidence of the disease appears to rise and fall periodically. The most recent decades have seen an increase in cases in a number of countries, including Portugal, Italy, France, Spain, Bulgaria, and Algeria.[151]

Clinical Manifestations. The infecting bite is unnoticed in most cases. Conjunctival inoculation occurs occasionally. The incubation period ranges from 6 to 10 days. The primary lesion, tache noire (black spot), described and named by Pieri in the 1920s, develops at the site of the tick bite, is not painful, and is rarely pruritic.[178] It occurs most often on the head in children and on the legs in adults. The lesion develops central necrosis, then an eschar, and regional lymph nodes enlarge. The initial lesion heals slowly and resolves after 10 to 20 days without scarring, although residual pigmentation can persist indefinitely.

Tache noire is pathognomonic but is evident in only 30 to 90 percent of patients. Multiple lesions occur occasionally. Formation of the lesion requires a substance secreted by the tick and another of rickettsial origin; it is not reproduced by a separate bite of an uninfected tick or by experimental inoculation of *R. conorii*. An inflammatory infiltrate, predominantly mononuclear, accumulates at the tache noire site, suggesting the importance of T cell–mediated immunity in local host defense.

The onset of disease usually is abrupt, with malaise and fever that reaches temperatures of 39° C to 40° C (102.2° F to 104° F) within the first 2 or 3 days. The fever continues for 6 to 12 days, but antibiotics can shorten the febrile period. Severe and unremitting headache is a typical feature of the disease in adults.[5] On the other hand, headache occurs in less than one third of pediatric patients.[37] Arthralgia and myalgia, especially of the leg muscles, are prominent features in adults; in children, they are rarely of sufficient severity to restrict mobility.

A maculopapular exanthem that develops on the third to the fifth febrile day is almost universally present. Lesions are present initially on the extremities, spreading within a day or so to the trunk, neck, face, buttocks, palms, and soles. The initial lesions are macular, pink, and irregularly defined but become maculopapular within hours. They generally measure 1 to 4 mm in diameter. The rash persists for 10 to 20 days after the remission of clinical symptoms. It can become purpuric or intensely pruritic. Atypical cutaneous manifestations, such as petechial, purpuric, or papulovesicular rashes, occur occasionally.[37,61]

The cutaneous manifestations of MSF are caused by involvement of the vascular structures of the dermis. Rickettsemia during the incubation period probably seeds the endothelial cells of the capillaries, arterioles, and venules. The vasculitis produced is much like that seen in RMSF. It gradually disappears during convalescence, and as the maculopapules fade, a brown discoloration of the skin may be noticed.

Cardiovascular and respiratory changes are transient and nonspecific. Bradycardia is the most consistent finding, but other dysrhythmias can occur. In more seriously ill patients, pericarditis, heart failure, and myocarditis have developed. Phlebitis of the lower limbs is the main vascular complication. Venous thrombosis is a recognized complication, and pregnant patients are particularly prone to venous thrombosis. Pneumonitis, pleuritis, pleuropericarditis, and adult respiratory distress syndrome have been described in association with MSF.

In addition to the headache that is characteristic of rickettsial illnesses, varying degrees of impaired consciousness can occur. Rarely, stupor, delirium, convulsions, and transient hypoacusis may be noted. Neurologic sequelae have been observed after rickettsial encephalitis.

Renal function is not altered in most cases, although nephritis with acute renal failure has occurred occasionally. The liver is palpable in a third of patients, and the spleen can be enlarged in 20 percent of children. Tests of hepatic function reveal an increase in serum transaminase levels in one fourth to one half of pediatric cases. Alkaline phosphatase levels are elevated in one third of patients. Needle biopsy of the liver reveals foci of hepatocellular necrosis and a predominantly mononuclear reaction to the necrosis at sites of infection by *R. conorii*. The lesion differs from a true granuloma in that it is not an aggregate of epithelioid macrophages.[179]

A variety of other systemic symptoms can occur. Photophobia and bilateral conjunctivitis have been reported. Severe unilateral conjunctivitis suggests that transmission of the disease occurs by the conjunctival route. Uveitis, choroiditis, retinal artery occlusion, and neuroretinitis are uncommon ocular disturbances.[2,46]

Hematologic abnormalities include isolated cases of autoimmune anemia and mixed cryoglobulinemia associated with MSF. Some studies report a high incidence of

hypoproteinemia. Immune complex–mediated vasculitis associated with MSF has been described.[41]

On occasion, MSF can have a malignant, rapidly fatal course, even in previously healthy children. The illness is consistent with a widespread vasculitis characterized by irreversible shock, encephalopathy, disseminated intravascular coagulopathy, and renal failure.[199]

Diagnosis. Rickettsial organisms can be detected by immunofluorescence if biopsy is performed early in the course of disease or by restriction fragment length polymorphism analysis of a PCR product from the tache noire.[118,192] *R. conorii* cannot be isolated from blood cultures by routine laboratory procedures. The clinical findings, geographic location, and epidemiologic considerations help establish the diagnosis.

The diagnosis can be established by one of multiple serologic tests that include complement fixation, latex agglutination, microagglutination, Western blot, and indirect IFA assays.[61,82,169] An IFA assay for *R. conorii* is commercially available. Identification of specific IgM by immunofluorescence can identify acute infection, although some patients with proven MSF treated early with antibiotics have normal antirickettsial IgM levels.

Differential Diagnosis. Before the rash appears, differentiation of MSF from other acute infections is difficult. Even after appearance of the rash, the disease can be confused with measles, meningococcemia, and secondary syphilis. Other rickettsial diseases should be considered, especially in the absence of a tache noire. Cross-reactions among rickettsiae occur with indirect immunofluorescence.

Treatment and Prevention. MSF generally has a benign course, and fatalities rarely occur. Doxycycline is the drug of choice. An alternative regimen is clarithromycin or azithromycin administered orally at a dosage of 15 mg/kg/day in two doses for 7 days and 10 mg/kg once daily for 3 days.[25,37] Use of chloramphenicol or ciprofloxacin also is effective therapy. The optimal duration of specific therapy has not been definitively established, and regimens ranging from single doses to treatment for up to 15 days have been reported.[74]

The major effective methods of control involve avoidance of tick bites. Natural immunity occurs after infection, and antibodies persist for as long as 4 years after acute illness. Effective vaccines are not available.[111]

Rickettsialpox

Historical Aspects. First described in New York City in 1946 by Sussman as "Kew Gardens spotted fever," rickettsialpox is a benign spotted fever group rickettsial disease caused by *Rickettsia akari*.[85,87,163] Reporting of rickettsialpox declined in the latter decades of the 20th century; fewer than 50 cases were reported by the New York City Department of Health between 1980 and 1999. At least 34 cases were confirmed in 2001 and 2002, possibly as the result of increased awareness and detection of the disease after the bioterrorism anthrax attacks of 2001.[127]

The Organism. The etiologic agent, *R. akari*, is an obligate intracellular gram-negative coccobacillus that is morphologically identical to *R. rickettsii*. The principal vector is the house mouse mite, *Liponyssoides sanguineus*. Rodents, particularly the house mouse, *Mus musculus*, serve as a reservoir for *R. akari*.

Epidemiology and Transmission. Most rickettsialpox in the United States has been reported from New York City, but cases also have occurred in many other cities in the United States and worldwide in Russia, Korea, and South Africa. Whereas house mice are the natural hosts of the mite transmitting rickettsialpox in the United States, rats have been shown to host the mite in Russia, and wild rodents are suspected of carrying the disease in South Africa. The reservoir for rickettsialpox may extend to additional, as yet unidentified, wild or domestic animals.

The disease has a natural cycle between the mite vector and the house mouse.[86] The mite passes the disease transovarially, so it is both a reservoir and vector. Humans acquire infection when depletion of mouse hosts caused by reduced availability of food, poison, disease, or trapping forces infected mites to seek an alternative host, namely, people. The disease affects persons of all ages. Males and females are equally susceptible.[19,73]

Pathology. Biopsy specimens from eschars are characterized by variable degrees of epidermal and dermal necrosis. Ulcerated lesions typically have neutrophils and thrombosed vessels in the ulcer base. The primary pattern of inflammation is a deep perivascular mononuclear cell infiltrate. Immunohistochemical staining reveals spotted fever group rickettsiae predominantly within the macrophages or mononuclear cells of the perivascular infiltrates associated with eschars or papules.[99]

Clinical Manifestations. The incubation period of rickettsialpox is 9 to 14 days after the usually painless mite bite occurs.[19,93] The primary lesion is a papule that develops at the site of the mite bite. This lesion progresses through a papulonodular stage to a tense 0.5- to 2-cm vesicle that ruptures to form an eschar with surrounding induration, which is the hallmark of the disease.[152] Two eschars may be seen. Regional lymph nodes related to the primary eschar are almost invariably enlarged.

Constitutional symptoms develop approximately a week after the mite bite. Fever and malaise are commonly present. The fever is irregular, with temperature fluctuating between 37.8°C and 39.5°C (100°F and 103°F), and rarely lasts longer than 6 or 7 days. It usually is accompanied by the frontal headache characteristic of rickettsial diseases. Rhinorrhea, cough, sore throat, nausea, vomiting, and abdominal pain are occasional findings.[72]

The rash is the most remarkable aspect of the disease. It usually develops within several days of the onset of fever as scattered nonpruritic macules, which rapidly become firm maculopapules. Within a day or two, vesicles develop on the summits of the papules. The lesions usually appear on the face, trunk, and extremities, with

sparing of the palms and soles.[18] The number of lesions ranges from 5 or 6 to more than 100.

Diagnosis. The diagnosis can be made by immunohistochemical staining of skin biopsy specimens, by PCR analysis of amplified gene products from eschars or culture of *R. akari* from eschars, and by serologic assays. These tests, used singly or in combination, to establish the diagnosis are available through the Centers for Disease Control and Prevention (CDC). A fourfold increase in serum antibodies to *R. akari* between acute and convalescent sera or a single titer of more than a 1:64 dilution is considered diagnostic. Cross-reaction is seen between antibodies to *R. akari* and *R. rickettsii*, but titers are greater with *R. akari* than with *R. rickettsii* antigen.[127]

Differential Diagnosis. Anthrax is a differential consideration. Rickettsialpox lacks the striking brawny, nonpitting edema surrounding the eschar that is characteristic of anthrax. Anthrax is not associated with the characteristic papulovesicular rash of rickettsialpox. The haphazard distribution of the characteristic papulovesicles is similar in appearance to chickenpox rash in an adult. Infectious mononucleosis, gonococcemia, and infection with echovirus or coxsackieviruses also should be considered.[106] Patients often give a history of having worked in basements, around incinerators, or in similar areas that might be infested by house mice and their mites.

Treatment. Rickettsialpox is a self-limited nonfatal disease that resolves within 7 to 10 days in untreated patients. Treatment shortens the duration of symptoms, usually to 24 to 48 hours.[19] Doxycycline is the drug of choice. A treatment course of 3 to 5 days is sufficient.

Other Spotted Fever Group Rickettsioses

A number of antigenically distinct *Rickettsia* spp. cause disease in humans. Among these, *Rickettsia parkeri* is perhaps the most important emerging rickettsial disease in the United States. This organism produces a mild spotted fever illness that may be difficult to clinically distinguish from mild RMSF.[126] In contrast with RMSF, an eschar is an important feature of *R. parkeri* infection. The vector for *R. parkeri* is the Gulf Coast tick, *Amblyomma maculatum*, which is most heavily concentrated in Gulf Coast and southeastern states. Whereas most cases occur in the Deep South, both infected *A. maculatum* ticks and at least one human case of *R. parkeri* infection have been reported from Virginia.[62,189,198] Serologic assays in clinical use cannot distinguish *R. parkeri* from *R. rickettsii*. Whereas *R. parkeri* infection may be self-limited, it would be prudent to treat all suspected *R. parkeri* cases as if they are RMSF.

Another notable member of the spotted fever group is *Rickettsia africae*, the cause of African tick bite fever (ATBF). Evidence suggests that this is one of the most common acute febrile illnesses experienced by travelers to Africa.[90] This disease has primarily been associated with travel to southern Africa and in particular travel in the game parks in this part of the continent.[63,139] Its known range is broader, however, and includes much of sub-Saharan Africa. It is also endemic on some eastern Caribbean islands. Cattle ticks from the genus *Amblyomma* are responsible for transmission of the organism. The clinical hallmarks of ATBF are fever, headache, neck muscle myalgias, and an inoculation eschar with regional lymphadenopathy. Up to 50 percent of patients have multiple eschars that ascend along the lymphatic drainage.[105] Rash is unusual in ATBF. This is a benign infection and is probably self-limited in most cases. Doxycycline has been associated with rapid resolution.

Many other *Rickettsia* species are known to infect humans. *Rickettsia japonica* is the etiologic agent of Japanese spotted fever, *Rickettsia sibirica* of Siberian tick typhus, and *Rickettsia australis* of Queensland tick typhus. Dogs are the principal mammalian reservoir; ticks also act as reservoirs by virtue of transovarial transmission. Other newly recognized tick-borne diseases, such as *Rickettsia slovaca* infection, for which the wild boar is the main host, have been described in Europe.[15] *Rickettsia felis* is transmitted by fleas, and its natural reservoirs are the opossum and the cat.[17] The diseases caused by these and other rickettsial species have similar clinical, pathologic, and epidemiologic patterns. As with other rickettsioses, doxycycline is the preferred therapy.

TYPHUS

The rather imprecise term *typhus* is most commonly used to refer to three rickettsial diseases: epidemic typhus, murine or endemic typhus, and scrub typhus. Whereas the respective agents of epidemic and murine typhus, *Rickettsia prowazekii* and *Rickettsia typhi*, are phylogenetically related, scrub typhus is caused by *Orientia tsutsugamushi*, which is not even a member of the genus *Rickettsia*. To make matters more confusing, various spotted fevers are referred to as tick typhus (e.g., Queensland tick typhus, Siberian tick typhus).

Rickettsia prowazekii Infection (Epidemic Typhus)

Overview. Epidemic typhus is a louse-borne infection caused by *R. prowazekii*. Conditions of squalor and overcrowding have resulted in explosive epidemics of typhus in modernity. Typhus has been the suspected cause of some human epidemics dating back to the Dark Ages, but the first known epidemic occurred in 1489 during the Spanish siege of Moorish Granada. In subsequent centuries, most outbreaks have struck during times of war. Outbreaks of typhus occurred throughout the world during the 19th century, famously ravaging Napoleon's army as it retreated from Moscow. Typhus afflicted thousands of soldiers during World War I, particularly on the eastern front. A devastating outbreak struck Russia during its civil war between 1917 and 1925, afflicting an estimated 25 million people and killing 3 million. During World War II, typhus killed tens of thousands as a result of the deplorable conditions in the Nazi ghettos and concentration camps; typhus also struck

soldiers in the North African and Eastern European theaters of war.

The use of dichlorodiphenyltrichloroethane (DDT) in the 1950s resulted in a dramatic worldwide decline in typhus. Since then, it has reemerged predominantly in highland regions of sub-Saharan Africa and to a lesser extent Central and South America. Rwanda, Burundi, and Ethiopia have been particularly affected, certainly as a result of the coexistence of poverty and war.[130] In 1997 approximately 100,000 persons in Burundi were affected and as many as 15 percent died.[13,142,144] A smaller outbreak occurred in Russia in 1997.[167] Although autochthonous cases in the United States are rare, there have been at least 30 cases since 1976. These are most likely due to exposure to the flying squirrel (*Glaucomys volans*), which is a sylvatic reservoir for *R. prowazekii*.[48,116,146]

Epidemiology and Transmission. Humans are the primary reservoir for *R. prowazekii*. Persons who recover from typhus can become a reservoir of *R. prowazekii* in interepidemic periods. This is particularly true in persons with Brill-Zinsser disease, a form of relapsed typhus that can occur many years after the original infection.[68] The best established natural reservoir for *R. prowazekii* is the flying squirrel, *Glaucomys volans*. Lice that infest these animals will not bite humans, suggesting that human infection results from direct exposure to aerosolized louse feces.

The human body louse, *Pediculus humanus corporis*, is the vector for epidemic typhus. The human body louse inhabits clothing, from which it will take blood meals from its host. It is sensitive to dehydration, and frequent blood meals are required. It is also sensitive to both high and low deviations from normal human body temperature. Cold conditions, in which humans have more clothing and blankets, will increase louse habitat. Lice will not survive for long away from a suitable host, so impoverished persons who do not have changes of clothing will be most heavily parasitized. Lice will proliferate under these conditions, and a single person may be infested by thousands of lice. Under crowded and unhygienic conditions, lice will readily pass between people. A heavily infested person who develops typhus can infect thousands of lice, which will promptly seek a new host once fever develops. Because each infected person can secondarily infect many others, there can be explosive epidemic potential under conditions of both high human density and high louse density.

R. prowazekii incubates in the louse for 5 to 10 days after it bites an infected person. Great numbers of rickettsiae are then shed in louse feces.[143] Because the louse defecates as it feeds, infectious feces can be rubbed into a louse bite wound. The bodies of crushed lice are also infectious. Dried louse feces can be aerosolized and cause infection through the eye or the respiratory tract.

Pathogenesis. The organism initially spreads through lymphatics and hematogenously to infect endothelial cells of small blood vessels in a wide variety of target organs.[9] Intracellular proliferation of the organism results in mechanical cell lysis, producing widespread vasculitis and capillary leak.

Clinical Manifestations. Illness begins after a 10- to 14-day incubation period. An initial period of malaise is followed by high fever, headache, and rash.[9] Body temperature generally rises rapidly to 40°C (104°F) or higher. In untreated patients, it remains at this level with minor fluctuations until death or recovery occurs. Headache is common and may be severe or intractable. Cough is also a frequent complaint. The rash usually appears on the trunk by the fourth to seventh day; it spreads peripherally to the extremities and typically spares the face, palms, and soles. Initially, the rash consists of macules that fade on pressure; they soon become fixed as maculopapules and later become petechial or hemorrhagic. Rash is less frequently recognized in individuals with dark skin. Central nervous system manifestations are described in up to 80 percent of patients, including delirium, coma, seizures, and hearing loss.[65] Other complications include parotitis, otitis media, pericarditis, myocarditis, pericardial effusion, pleural effusion, and pneumonia.[45,195,196] Severe cases of typhus can be manifested with shock or with tissue ischemia, including gangrene. Mortality was as high as 60 percent in the preantibiotic era and is higher in malnourished populations and the elderly. With appropriate antibiotics, the mortality is approximately 4 percent. Recrudescent infection (Brill-Zinsser disease) can occur years after the primary infection if it is not eradicated. Partial immunity attenuates the severity of Brill-Zinsser disease.

Diagnosis. Typhus is diagnosed by evidence of seroconversion. A fourfold rise in IgG antibody titer is considered diagnostic. This requires paired acute and convalescent sera. Several testing methods are available, but the preferred method is indirect IFA assay.[102,124] IFA assay will not discriminate epidemic from endemic typhus; discrimination between the two infections requires Western blotting with cross-adsorption.[103] Shell vial culture can detect *R. prowazekii* from blood specimens.[12] The Weil-Felix agglutination test is no longer recommended because of lack of specificity (due to cross-reactivity) and poor sensitivity in Brill-Zinsser disease.

Differential Diagnosis. Typhus may be difficult to distinguish from other acute febrile illnesses, especially early in the course of disease. Depending on the local epidemiology, these can include typhoid fever, dengue, malaria, influenza, leptospirosis, and other rickettsial illnesses. The rash of louse-borne typhus begins centrally on the trunk and spreads peripherally to the extremities, whereas the reverse is true for RMSF. Moreover, a rash on the palms and soles, a common event in RMSF, rarely is observed in typhus.

Treatment. Doxycycline is the treatment of choice for epidemic typhus.[100] Therapy is given until the patient is afebrile for at least 3 days and clinical improvement is evident; the usual duration is 7 to 10 days. Lack of defervescence after 3 days suggests an alternative diagnosis.

Single-dose therapy with doxycycline 200 mg has been effective in epidemic settings.[131] Chloramphenicol is an alternative agent for those with a serious contraindication to doxycycline. As with other rickettsial infections, the risk of tooth staining is negligible for young children receiving this degree of doxycycline exposure; moreover, the potential lethality of typhus justifies the use of doxycycline in children in whom the diagnosis is plausible. Whereas fluoroquinolones have in vitro activity, there has been a treatment failure with ciprofloxacin resulting in death.[200]

Prevention. DDT was effective at limiting the burden of typhus in the 1950s. Its use has been long banned by many countries. Along with DDT, malathion and permethrin are also effective at eradicating lice from infested clothing and bedding. Lice will die within days if they do not have access to a host, so removal of infested clothing for 1 week may suffice.[143] Relief workers assisting persons at risk for typhus can pretreat their clothing with permethrin. Doxycycline prophylaxis appears to be effective in select populations at risk for scrub typhus; whether this is true for epidemic typhus remains unknown.

Rickettsia typhi Infection (Murine Typhus)

Overview. Murine typhus, also known as flea-borne or endemic typhus, is a flea-borne zoonosis primarily of rats, opossums, and cats. The disease occurs worldwide, especially in warm climates where rats or opossums are abundant. During the early 20th century, there were thousands of annual cases of murine typhus in the United States, particularly along the Gulf Coast and the southern Atlantic seaboard. In recent years, 60 to 80 cases have been reported annually, most of which are from southern California, the southeastern Gulf Coast including Texas, and Hawaii.[8,58] The actual incidence in the United States is unknown. Approximately 0.6 percent of children in south-central states have an *R. typhi* titer of greater than 1:64.[113] The seroprevalence in southeastern Texas may be as high as 15 percent. These figures suggest that murine typhus is considerably underrecognized in certain regions.[58,137,190]

Epidemiology and Transmission. *R. typhi* infection is a vector-borne zoonosis that persists in a flea-mammal cycle in nature. The classically described cycle involves the Oriental rat flea, *Xenopsylla cheopis*, and peridomestic rats. Rat infestations, especially near granaries and ports, pose a risk for human contact. A more recently described transmission cycle involves the cat flea, *Ctenocephalides felis*, and opossums or domesticated cats as their primary mammalian host. This cycle seems primarily responsible for ongoing transmission of both *R. typhi* and *R. felis* in suburban regions of southern California and south Texas.[1,16,157] Uninfected fleas acquire the organism from infected mammals and can transmit it to new hosts, including humans; in turn, an infected mammal can serve as a reservoir for many fleas. Rickettsiae multiply and reside in both the digestive and reproductive tracts of the flea, which is unaffected by the organism. The flea goes on to produce highly infectious feces for the rest of its life. It can also pass the organism transovarially to its offspring. Humans are not the preferred hosts for fleas, but the cat flea in particular will readily bite humans. If the flea bites a person, infection typically occurs when flea feces is rubbed into the bite wound; it can also be inoculated by direct contact or by aerosol to mucous membranes.

Clinical Manifestations. Murine typhus is clinically similar to epidemic typhus (*R. prowazekii* infection), but it is milder, of shorter duration, and less likely to be severe or fatal. Symptoms develop after a 5- to 10-day incubation period, although an antecedent flea bite is not always reported. All affected patients develop fever, and the majority have headache or rash.[188] The classic triad of fever, headache, and rash occurs in half of patients. This may be because rash typically appears between days 3 and 10 of illness and may not be present when patients first seek medical attention. Other common symptoms include malaise, myalgias, and vomiting. Rashes are typically macular or maculopapular; only in rare cases have they been described as petechial. Laboratory abnormalities are inconsistently reported, but the most common include elevated hepatic transaminases and mild hyponatremia.[58,157] Children who receive appropriate antibiotics undergo defervescence in 1 to 3 days. Even without appropriate treatment, murine typhus is generally self-limited, with recovery in 1 to 2 weeks and a very low rate of complications.[58,157]

Diagnosis. A fourfold change in titer between acute and convalescent sera by indirect IFA assay, latex agglutination, complement fixation, or enzyme immunoassay is diagnostic. Differentiation among antibodies produced in response to epidemic typhus is difficult but may be accomplished by use of an IgM-specific enzyme immunoassay, if needed. Immunohistochemical staining or PCR analysis of amplified gene product of tissues and culture, available through the CDC, also can be used for confirmation.

Differential Diagnosis. Murine typhus is an undifferentiated acute febrile illness and resembles numerous other infections. In the United States, these include but are not limited to enteroviral disease, other viral exanthems, other rickettsial diseases, and group A streptococcal infection. A history of recent travel to tropical or developing countries could expand the differential diagnosis to include other febrile illnesses, such as malaria, dengue, typhoid fever, and leptospirosis.

Treatment. Doxycycline is the treatment of choice for murine typhus. Chloramphenicol is an alternative in patients with an important contraindication to tetracyclines. Whereas fluoroquinolones have been used successfully to treat *R. typhi* infection, at least one treatment failure has been documented.[104,161] Treatment should be continued for at least 3 days after defervescence and evidence of clinical improvement; this is typically 3 to 7 days.

Prevention. Rat control measures can lower the likelihood of human disease in areas where *R. typhi* is transmitted.

Scrub Typhus

Overview. Scrub typhus is a chigger-borne rickettsial disease caused by *Orientia tsutsugamushi* that exclusively occurs in the Asia-Pacific region. This infection may have been recognized as early as the 3rd century AD and was the subject of non-Western medical writing in Japan during the 19th century.[120] Scrub typhus affected more than 16,000 Allied soldiers in the South Pacific theater of World War II and was second only to malaria as a cause of fever.[120,134] It was also an important pathogen among febrile American soldiers in the Vietnam conflict.[10,80]

The Organism. The causative organism, *O. tsutsugamushi*, was formerly a member of the genus *Rickettsia*. In 1995 it was reclassified to the novel genus *Orientia* on the basis of distinctive phenotypic and genetic features.[165] *O. tsutsugamushi* is distinguished by remarkable antigenic heterogeneity. Strain differences also appear to contribute to striking differences in the severity of scrub typhus.

Epidemiology and Transmission. Scrub typhus is widely distributed throughout central, eastern, and southern Asia. It is transmitted as far north as Kamchatka in eastern Russia and as far west as Pakistan and Afghanistan. Its southern range includes the islands of Indonesia and Oceania as well as northern Australia.[94] The disease is distributed according to the habitat of its vectors, which in turn are distributed in widely diverse habitats. Scrub typhus has been described from sea level to alpine meadows above 3000 meters in elevation, in both arid and humid environments, in a variety of agricultural regions, and more recently in cities.[154,162,173,174] Humans are most likely to encounter chiggers in undergrowth in rural areas. Travelers engaged in outdoor activities, such as camping and trekking, are at risk for acquiring scrub typhus; some of these individuals do not become clinically ill until after return to their home country.[83,154]

Trombiculid mites from the genus *Leptotrombidium* are the vectors for scrub typhus. Only the six-legged larvae, known colloquially as chiggers, will feed on humans. The eight-legged nymphs and adults feed on plant matter and do not parasitize mammals. Wild rodents, particularly rats, are the preferred host for chiggers and are important in the ecology of scrub typhus. The rodents, however, are not thought to be a natural reservoir for *O. tsutsugamushi*, and in fact each mite feeds on a mammalian host only once in its life. Rather, the microorganism persists transstadially (across life cycle stages) within the mite and is passed transovarially to subsequent generations.[64,164,180] Thus, the mite itself is the natural reservoir for this infection.

Pathogenesis. *O. tsutsugamushi* primarily infects endothelial cells and mononuclear leukocytes. Human autopsy specimens have shown endothelial cells infected with microorganisms in numerous organs, including brain, heart, kidney, and lung.[119] They were also found in both macrophages and cardiac muscle cells. The importance of mononuclear leukocytes in scrub typhus has been elucidated in recent years. Dissemination from the inoculation site to target organs may occur through infected peripheral blood mononuclear cells.[182] Infection of monocytes also induces a robust inflammatory cascade that may be responsible for tissue injury and both systemic and organ-specific disease manifestations.[98,166] Marked differences in disease severity and mortality have been noted, and these are attributed to strain variation within *O. tsutsugamushi*. The phenotypic factors responsible for this variability are poorly understood.

Clinical Manifestations. In half to two thirds of cases, the initial mite bite lesion develops into a necrotic eschar. The preferential site for eschar distribution in adults is truncal[96]; in children, eschars are seen commonly in moist intertriginous areas such as the genitalia and perineum.[156] The incubation period is approximately 1 to 2 weeks, and characteristic features of the disease develop at approximately the same time that the eschar is noted. After regression of the eschar, a scar often remains and has been shown to persist for up to 25 years.[186]

Aside from fever, the most consistently described signs in scrub typhus are hepatomegaly, respiratory distress, and lymphadenopathy. In a contemporary prospective report of scrub typhus in 30 children presenting with febrile illness of at least 5 days' duration in Thailand, common physical signs included lymphadenopathy (93%), hepatomegaly (73%), eschar (68%), conjunctival hyperemia (33%), maculopapular rash (30%), interstitial pneumonia (30%), and splenomegaly (23%).[156] In a separate series of 60 Sri Lankan children, the most common findings in patients with confirmed scrub typhus were hepatomegaly (95%), lymphadenopathy (60%), respiratory distress (45%), and splenomegaly (35%).[42] Hepatomegaly was observed in 80 percent and cough in 73 percent of Indian children with scrub typhus.[128] The lymphadenopathy is generalized and is especially prominent in the axilla, neck, and inguinal areas. Myocarditis, disseminated intravascular coagulation, and the acute respiratory distress syndrome are among the complications that can be seen.[121,128] Nervous system disease can be manifested as aseptic meningitis, meningoencephalitis, or cranial or peripheral neuropathies. The severity of the clinical manifestations varies widely, and the lethality of scrub typhus varied even in the preantibiotic era.

Diagnosis. The mainstay of scrub typhus diagnosis is serologic testing. The "gold standard" is the indirect IFA assay; however, there is little consensus in IFA methodology and positivity cutoff limits. Blacksell and colleagues reviewed the evidence base for various methodologies and the criteria for positive IFA assay results and concluded that the diagnosis should be based on a fourfold or greater increase in the IFA titer in paired serum specimens and on a single sample only when there is an adequate local evidence base.[14] The limited number of source strains used for IFA assay antigen may be inadequate given the enormous antigenic diversity and geographic heterogeneity of *O. tsutsugamushi*.[94]

Other tests, such as enzyme-linked immunosorbent assay, dot immunoassay, indirect immunoperoxidase, and Weil-Felix OX-K assay, are also used.[89,122] The last is the most commonly used diagnostic modality in developing countries; however, this assay lacks both sensitivity and specificity. OX-K agglutinins develop in only a little more than 50 percent of scrub typhus patients. Moreover, OX-K agglutinins also are produced in relapsing fever.

Identification of the rickettsial strain in infected patients by use of strain-specific monoclonal antibodies in an inhibition enzyme-linked immunosorbent assay or PCR with strain-specific primers offers potential to establish the diagnosis in the acute stage of the illness.[66,67] An eschar PCR assay, evaluated prospectively, demonstrated high sensitivity and specificity compared with IFA assay.[95] Cell culture and xenodiagnosis-based assays are expensive and laborious and are not available clinically.

Differential Diagnosis. Scrub typhus can be suspected when a patient gives a history of recent exposure in a geographic area where scrub typhus occurs. If, in addition, a local eschar, evanescent rash, and general and regional lymphadenopathy along with fever, headache, and conjunctival suffusion are present, the physician should be alerted to suspect scrub typhus; however, it cannot be differentiated with certainty from other febrile illnesses that occur in the region. These include but are not limited to dengue, chikungunya, leptospirosis, malaria, typhoid fever, and other rickettsioses such as murine typhus.

Treatment. Doxycycline is the treatment of choice. Children have responded well to treatment for 4 to 7 days.[156] Doxycycline is rickettsiostatic, and patients with scrub typhus who are treated in the first week of illness can require sporadic short courses of antibiotic therapy for prevention of relapse.[175] Chloramphenicol, azithromycin, and rifampin are alternatives.[97,129,135] Strains with reduced susceptibility to antibiotics have been observed in northern Thailand.[183] In one study from this region, rifampin was found to produce a significantly faster clinical response than did doxycycline.[184]

Prognosis. The prognosis varies widely because of significant differences in the severity of disease caused by different strains in different populations and in various geographic areas. Mortality rates in the preantibiotic era ranged from 1 to 60 percent. With the use of antimicrobials, fatalities rarely occur. When treatment is begun early in the course of scrub typhus, relapses as well as definite second attacks of the disease can occur. The heterogeneity of scrub typhus strains probably accounts for reinfections.

Prevention. Vector control involves impregnating clothing and smearing exposed skin surfaces with dimethyl or dibutyl phthalate. Short-term vector control of camping grounds can be accomplished by cutting, burning, or bulldozing vegetation, along with heavy spraying with insecticides such as lindane. Chemoprophylaxis is feasible for persons with high-risk exposure for short periods. Doxycycline given once a week provides effective chemoprophylaxis against naturally transmitted scrub typhus if the prophylaxis is started before exposure to infection and continued for 6 weeks after exposure.[175] No satisfactory vaccine has been produced.

EHRLICHIOSIS AND ANAPLASMOSIS

Overview. Ehrlichiosis and anaplasmosis are tick-borne infections caused by rickettsial organisms from the genera *Ehrlichia* and *Anaplasma*. Strictly speaking, they are best thought of as anaplasmoses because both genera are members of the family Anaplasmataceae of the order Rickettsiales. Six members of this family are known to infect humans: *Ehrlichia chaffeensis*, *Ehrlichia ewingii*, *Ehrlichia canis*, *Ehrlichia muris*–like, *Anaplasma phagocytophilum*, and *Neorickettsia sennetsu*. The last two were formerly members of the genus *Ehrlichia*; *A. phagocytophilum* was reclassified to its own genus in 2001.[51]

Unlike many rickettsial infections, which have been recognized for decades if not centuries, ehrlichiosis and anaplasmosis are emerging diseases that have been only recently identified. From 1953 until the late 1980s, the only known human infection from this group was Sennetsu fever, a mild febrile illness in East Asia caused by *Neorickettsia* (formerly *Ehrlichia*) *sennetsu*. The disease that ultimately became known as human monocytic ehrlichiosis (HME) was first described in humans in 1986, and it was suspected that this was due to *E. canis*, a known veterinary pathogen.[55,109] It was not until 1991 that its primary cause, *E. chaffeensis*, was identified.[4] Human ehrlichiosis can also be caused by *E. ewingii*, *E. canis*, and an *E. muris*–like organism.[21,136] In the early 1990s, the syndrome now known as human granulocytic anaplasmosis (HGA) was identified in the upper Midwest; its cause, *A. phagocytophilum*, was recognized in 1994.[36]

The Organisms. *Ehrlichia* and *Anaplasma* are small gram-negative obligately intracellular bacteria. *E. chaffeensis* propagates in macrophages; *A. phagocytophilum* and *E. ewingii* multiply in neutrophils.

Epidemiology and Transmission. The incidence for both HME and HGA has increased substantially during the last decade, although this may be partly a function of increased recognition. These infections have also been reportable only since the late 1990s, which suggests that some of the increased incidence is a function of more complete reporting and case ascertainment practices. There is also annual variation in the incidence of both diseases, which lacks a satisfactory explanation. Nonetheless, hundreds of persons are affected annually. In 2009 there were 944 cases of HME and 1161 cases of HGA.[33] In 2010 there were 740 cases of HME and 1761 cases of HGA. The prevalence of *E. chaffeensis* titers of 1:160 or higher was 3 percent among children residing in the southeast and south-central regions of the United States, suggesting that exposure is far more common than is the incidence of recognized disease.[114]

The geographic distributions of both HME and HGA are tied to the distribution of their respective tick vectors.

The lone star tick, *Amblyomma americanum*, is the principal vector of HME and *E. ewingii* ehrlichiosis. This aggressive tick is by far the most common "pest" tick in the southern and southeastern United States.[23,56,70,117] In low numbers, its range extends as far north as New England, but it is particularly abundant in Missouri, North Carolina, and states farther south. All active stages of the tick will readily bite humans. Individuals can become multiply parasitized by larval ticks (known colloquially as seed ticks). The adult female tick is easily recognized by a conspicuous light-colored spot, but this marking is not present on males or on immature ticks. HME is transmitted in the eastern half of the United States, from the Great Plains to the Atlantic Coast. Oklahoma, Arkansas, and Missouri together account for 35 percent of all cases.[27] Southeastern and mid-Atlantic states account for the majority of the remainder, but HME acquisition is well documented in Delaware and New Jersey and occasionally occurs even in coastal New England. There is also serologic evidence of HME transmission in Central and South America. The white-tailed deer is the most likely natural reservoir for *E. chaffeensis*.[107]

HGA, by contrast, is transmitted in North America, Europe, and Asia. In the United States, it primarily occurs in northeastern states, from New Jersey through northern New England, as well as in Minnesota and Wisconsin in the upper Midwest.[27] The black-legged or deer tick, *Ixodes scapularis*, is the vector for HGA. *A. phagocytophilum* is maintained in nature in a tick-mammal cycle that primarily involves small rodents.[26,168] Whereas adult *I. scapularis* ticks will occasionally feed on humans, it is primarily the nymph whose feeding habits make it a vector for human infections. The nymph is most active in late spring and early summer. Because this immature stage of the tick is quite small, a tick bite often will go unrecognized. HGA shares a vector with Lyme disease, and there is evidence that the endemic range for Lyme disease is expanding; this may portend further expansion of the anaplasmosis-endemic zone as well. HGA is rarely acquired in California, where the Western black-legged tick (*Ixodes pacificus*) is the responsible vector. In Europe, it is transmitted by *Ixodes ricinus*; in Asia, its vector is *Ixodes persulcatus*.

Both anaplasmosis and ehrlichiosis are predominantly transmitted from May through October, in conjunction with the peak activity of their tick vectors. In contrast to other tick-borne diseases such as Lyme disease and RMSF, in which children have among the highest incidence rates, it is older adults who suffer the highest incidence of HGA and HME.

Pathogenesis and Pathology. The pathogenesis of ehrlichiosis is incompletely elucidated. Although it is capable of establishing infection in numerous organs and tissues, the primary target cell for HGA is the granulocyte; for HME, it is the macrophage. Granulomas of the bone marrow occur frequently in HME, suggesting that involvement of the reticuloendothelial system may be important in pathogenesis.[52]

Organisms enter the cytoplasm of host cells and proliferate in phagosomes into elementary bodies. These individual organisms multiply by binary fission into immature inclusions called initial bodies. Mature groups of elementary bodies form morulae, which are released by rupture of the cell to reinitiate the infecting process.[109]

Clinical Manifestations. Most patients become clinically ill within 2 weeks of exposure. HME and HGA are acute febrile illnesses in which fever and malaise are universal symptoms.[50,53,60,132] Headache, myalgia, arthralgias, and nausea are also common. Confusion occurs in about 20 percent of patients with HME, primarily among older adults; this symptom is less common in HGA. Rash is more common in ehrlichiosis than in anaplasmosis and primarily occurs in children. Rashes can be macular, maculopapular, or petechial and seldom develop in adult infections. Rashes are reported in about 30 percent of cases of HME, compared with 10 percent or less in HGA.

Meningitis as a manifestation of HME has been reported in children. Symptoms can range from irritability and meningismus to obtundation with response only to painful stimuli.[11,47,71] Initial examination of cerebrospinal fluid reveals pleocytosis ranging from approximately 50 to 1400 white blood cells, usually with a predominance of mononuclear cells; a range of 5 to 40 red blood cells; mildly elevated protein concentration; and a normal to slightly low glucose value. Complete recovery is the rule. Central nervous system involvement is much less common in HGA.

The most common laboratory findings in both infections are hematologic abnormalities and elevated hepatic transaminases.[50] Thrombocytopenia is seen in three quarters of patients, leukopenia in two thirds, and anemia in half. In HME, the leukocyte abnormalities are primarily lymphopenia and monocytopenia. In HGA, neutropenia is more common, especially with illness of longer duration. Elevations of hepatic transaminases are seen up to 80 percent of the time in both HGA and HME.

A variety of complications may be seen in both infections. These include protracted fever, renal dysfunction occasionally of sufficient severity to require dialysis, acute respiratory distress syndrome, myocarditis, neuropathies and plexopathies, opportunistic infections, and a toxic shock–like syndrome.[50,59,150] Whereas persistent infection is much better documented in reservoir hosts, there has been a case report of fatal, treatment-refractory persistent infection in an older adult, although relapse after doxycycline therapy is thought to be otherwise unheard of.[54] There is one case report of fatal seronegative HME in a patient with advanced human immunodeficiency virus (HIV) infection.[125] Up to half of patients with HME or HGA require hospitalization. The mortality rate is 3 percent for ehrlichiosis and 0.5 percent for anaplasmosis.

Diagnosis. In contrast to RMSF and other infections with agents in the genus *Rickettsia*, anaplasmosis and ehrlichiosis are primarily infections of circulating leukocytes. This potentially allows the diagnosis to be made at the point of care or during the patient's clinical illness. Peripheral blood smear examination will reveal morulae in neutrophils in 25 to 75 percent of patients with HGA;

intramonocytic morulae are seen in 10 percent or less of patients with HME.[6,7,159] Whereas the sensitivity of this examination is low, a positive blood smear is pathognomonic and will allow a real-time diagnosis. PCR amplification of a peripheral blood specimen is a more sensitive alternative for both anaplasmosis and ehrlichiosis; it is also the only modality available for the specific diagnosis of *E. ewingii* infection. Reported sensitivities range from 60 to 85 percent for HME and 67 to 90 percent for HGA.[6,7,84,123] These assays are available from some clinical laboratories. The sensitivity of both blood smear examination and PCR is abrogated by antecedent doxycycline treatment.

Serology by indirect IFA assay remains the most sensitive and widely used means to diagnose ehrlichiosis and anaplasmosis. Paired acute and convalescent sera must be obtained, and a fourfold rise in antibody titer is considered diagnostic. The reported sensitivity is 88 to 90 percent for HME and 82 to 100 percent for HGA.[40,123,181] Antibodies to *Ehrlichia* and *Anaplasma* may be crossreactive, so simultaneous serologic testing for both infections is preferred. The use of paired acute and convalescent sera must be emphasized for two reasons: (1) a single acute serum specimen will yield a positive result in only 3 percent of cases; and (2) a positive titer at a single time point may be due to prior infection, asymptomatic seropositivity, or crossreactivity to other infectious agents.[8,123,181]

Differential Diagnosis. A variety of febrile illnesses can be indistinguishable from ehrlichiosis and anaplasmosis, especially early in infection. The most common symptoms, fever, headache, and malaise, are generic. A careful history, exposure assessment, and physical examination can help limit the differential diagnosis. Differential considerations include but are not limited to viral infections (e.g., enteroviruses, adenoviruses) and bacterial infections (including septic and toxic shock syndromes and focal bacterial infections such as pneumonia that have not yet manifested focally). A number of tropical infectious diseases can have similar clinical presentations and laboratory findings; these include dengue virus infection, typhoid fever, malaria, and leptospirosis.

HME and HGA must be distinguished from other tick-borne diseases, in particular RMSF. Compared with RMSF, these infections are less likely to be accompanied by rash and more often are associated with leukopenia or pancytopenia. The similarity of ehrlichiosis and RMSF is emphasized by two retrospective serosurveys in which approximately 10 percent of specimens from patients lacking the serologic criteria for a diagnosis of RMSF fulfilled diagnostic criteria for ehrlichiosis.[77,149] Other tick-borne illnesses, such as babesiosis, Colorado tick fever, relapsing fever, and tularemia, should be included in the differential diagnosis. In fact, the only major tick-borne infection in North America that is unlikely to be confused for HGA or HME is Lyme disease as high fever and systemic toxicity would be unusual for this infection.

Treatment. The drug of choice for treatment of human ehrlichiosis and anaplasmosis is doxycycline. The recommended dosage is 4 mg/kg/day in two divided doses (maximum adult dose, 100 mg twice daily). Treatment should continue for at least 3 days after defervescence for a total course of 5 to 14 days.[170,197] Because both infections may be severe or fatal, doxycycline treatment should be initiated when the diagnosis is suspected, without regard for the age of the child. Severe cases can require a longer course of treatment. Rifampin is an alternative that can be used in pregnancy and in early childhood, particularly when the index of suspicion for anaplasmosis or ehrlichiosis is low. There is contradictory in vitro evidence about fluoroquinolones, and these are not recommended for the treatment of either infection. Prophylactic treatment after tick bites is discouraged for the prevention of HGA, HME, or other tick-borne rickettsial illnesses.

Coinfection. HGA shares the same tick vector and geographic distribution as *Borrelia burgdorferi* and *Babesia microti*, the agents of Lyme disease and human babesiosis. Simultaneous coinfection with two or even three of these pathogens occasionally occurs and is well described. As Lyme disease is by far the most common of these three, one should consider coinfection in patients who have Lyme disease that is accompanied by atypically high fever, systemic toxicity, uncharacteristic laboratory abnormalities, or poor response to therapy. Doxycycline therapy for anaplasmosis will be active against *B. burgdorferi*, but the duration of therapy may be shorter than that recommended for early Lyme disease (typically 10 to 14 days). Treatment of babesiosis requires either clindamycin with quinine or atovaquone plus azithromycin.

Q FEVER

Historical Aspects. Q fever (the "Q" is for "query") was first described by Edward Derrick as part of an investigation of an apparently infectious febrile illness in 1937.[44] The cause of Q fever was discovered in the late 1930s independently by Burnet and Freeman in Australia and by Cox in the United States. The organism was named *Coxiella burnetii* in honor of two of the discoverers. Infection with *C. burnetii* is worldwide in distribution and is often asymptomatic. When it causes symptoms, the disease is Q fever; it is typically an influenza-like acute illness characterized by fever, headache, fatigue, and myalgias.[172] It can also cause a difficult to diagnose chronic infection, particularly in patients with preexisting valvular heart disease.

The Organism. *C. burnetii* is a small gram-negative coccobacillus that looks like most rickettsiae. However, it has recently been reclassified in the Gammaproteobacteria on the basis of 16s rDNA studies (Order: Legionellales) and is now understood to be relatively closely related to *Legionella pneumophila*.[101,160] *C. burnetii* is an intracellular pathogen that multiplies within phagosomes in the cells it infects, in contrast to the rickettsiae, which can live independently in the cytoplasm or nucleus of infected cells. It is believed that *C. burnetii* is the only intracellular

pathogen that is able to neutralize the acidic, degradative environment of eukaryotic polyphagolysosomes.[81] Its life cycle is distinctive in that it produces endospores that are similar to those seen in some gram-positive organisms.[155] These small cell variants protect the organism in the external environment, where it can survive for long periods.[172]

C. burnetii is also distinctive because it exhibits antigenic phase variation. Phase I variants are associated with mammalian infection and are highly contagious; phase II variants, of reduced virulence, follow propagation and are associated with a chromosomal deletion that results in a partial loss of lipopolysaccharide that makes the organism noninfectious.[141]

Epidemiology and Transmission. Q fever is distinct from human rickettsial infections in that it is primarily a disease of animals that is transmitted to humans by contact with infected animals rather than by an arthropod bite. It infects a broad range of animals including mammals, birds, and arthropods (mostly ticks). The most common sources of human infection are cattle, goats, and sheep, but pets (including dogs, cats, rabbits, and pigeons) can be vectors.[172] *C. burnetii* organisms are found in very high concentration in the placentas of infected parturient animals and are shed into the environment after labor or abortion.[101] Transmission of infection to humans is through aerosols. Unfortunately, the organisms can be wafted by the wind and infect people with no known exposure.[171] Epidemics have occurred in a variety of situations like abattoirs, tanneries, and shearing camps in rural areas. Cases have been reported of schoolchildren who went to farms on field trips.[110] Laboratory outbreaks are also a concern,[75] and the organism should be cultivated only in a biosafety level 3 laboratory.[101]

Outbreaks of Q fever can occur and cause massive public health ramifications. There was, for example, an epidemic of more than 2200 cases in the Netherlands in 2009 that resulted in the prophylactic slaughter of 50,000 dairy goats in an attempt to end the outbreak.[108]

Pregnant women can transmit the infection to their infants, although the risk of abortion with Q fever is considerable. Those infants not aborted are likely to be delivered prematurely or to have low birth weight.[138]

The prevalence of human Q fever is unknown. The diagnosis is difficult to make, and systematic epidemiologic evaluations are few and overreliant on antibody testing in subjects with variable suspicion of disease. The number of reported cases (an average of 50 to 60 cases per year to the CDC) almost certainly substantially underestimates the actual incidence.[31] The disease has been diagnosed in children younger than 3 years and should be considered during an evaluation for fever of unknown origin.[147]

Pathology. Mortality from Q fever is rare, so autopsy specimens are relatively uncommon. Pathologic series obtained during outbreaks described liver and bone marrow disease, in which distinctive fibrin-ring and lipid granulomas were seen. Granulomas have also been seen in the lungs at autopsy. The cellular response was thought to be primarily histiocytic.[158] Biologic control of *C. burnetii* requires a T-cell response, and patients with impaired cellular immunity (cancer patients, HIV-infected people) are at high risk for chronic infection.[101]

Clinical Manifestations. Q fever is manifested in both acute and chronic forms. The most common outcome of *C. burnetii* infection is an asymptomatic seroconversion. Symptomatic Q fever is particularly uncommon in children; infected adults are more likely to be symptomatic than children.[172] Using standard diagnostic criteria, in 2002, Maltezou and Raoult could identify 46 published pediatric cases (many patients previously described did not meet current standards for diagnosis). The most common manifestations in children were fever and pneumonia. Other common manifestations were febrile seizures, meningeal irritation, malaise, lymphadenitis, and anorexia. The fever was typically sustained for 5 to 10 days.[110] Q fever myocarditis has been diagnosed in three pediatric patients.

In adults, acute Q fever is manifested as an influenza-like syndrome with the abrupt onset of high fever, fatigue, headaches, and myalgias. Atypical pneumonias can occur, but the chest radiograph is usually normal and the primary manifestation is a nonproductive cough. Hepatitis is common, although it appears to be regionally distributed (some areas have more pneumonia, some more hepatitis). Maculopapular and purpuric rashes are seen in 10 percent, and myocarditis (although rare) can be potentially fatal.[172]

Chronic Q fever most often occurs in patients with some form of immune compromise. In particular, patients with T-cell defects, pregnant women, and patients with abnormal heart valves are at risk for chronic Q fever. It occurs in 1 to 5 percent of *C. burnetii*–infected patients, symptomatic and not, and may develop months or years after the primary infection.[172] Q fever endocarditis is manifested with low-grade fever, nonspecific cardiac findings, and a positive antibody test response for *C. burnetii*. It occurs primarily in patients with preexisting valvular disease. A 2002 review article found five cases of pediatric Q fever endocarditis, all of which had vegetations on the child's heart valves. The diagnosis is made serologically. *C. burnetii* was demonstrated on the valves from two of the cases. Q fever osteomyelitis has also been rarely seen in normal children.

Q fever can be severe during pregnancy; 33 confirmed cases were investigated, and in only 5 cases was there a normal pregnancy and healthy term delivery.[110] A subsequent study identified 53 pregnant women with Q fever. Eighty-one percent of patients who were not treated with long-term trimethoprim-sulfamethoxazole therapy had complications: 5 had spontaneous abortions, 10 experienced intrauterine fetal deaths, 10 had fetal intrauterine growth retardation, and 10 were delivered prematurely. Patients treated with long-term trimethoprim-sulfamethoxazole therapy were protected from chronic maternal Q fever, placental infections, and obstetric complications.[24]

Diagnosis. *C. burnetii* can be diagnosed by several techniques, but for practical reasons the primary diagnostic modality is serology. The most commonly used test is an

IFA assay. The test is actually directed at detection of antibodies against phase II antigens, the nonpathogenic form of the organism. Antibodies peak 4 to 8 weeks after the onset of disease. Detection of anti–phase I antigens is used in the diagnosis of chronic Q fever (e.g., endocarditis, osteomyelitis) because in chronic Q fever, the level of anti–phase I antibodies will exceed anti–phase II antibodies.[101] Culture of *Coxiella* is possible, but because of the high degree of transmissibility, culture should be undertaken only by reference laboratories with biosafety level 3 facilities. If culture is used, either blood or operative specimens of vegetations are acceptable, although the latter will be more sensitive.[185] PCR is also available from reference laboratories; tests on samples from vegetations will be more sensitive than those from blood. A variety of staining techniques can also be used to demonstrate *C. burnetii* in infected tissues.[101]

Differential Diagnosis. Because Q fever is not common, routinely searching for it seems impractical, but it is almost certainly underdiagnosed. The acute form of Q fever is essentially self-resolving, and antibody titers do not peak for several weeks after the onset of disease, so diagnostic testing is generally unnecessary. In the correct situation, where there are outbreaks (as occurred in the Netherlands), or for individuals with animal exposure or living in rural areas (particularly with heavy sheep, goat, and cattle farming), *C. burnetii* infection should certainly be considered in the differential. Where the diagnosis becomes critical is in patients with classic fever of unknown origin, for which chronic Q fever should be strongly considered. For patients with known valvular disease or immune compromise, including pregnancy, the possibility of Q fever should be evaluated through the use of serologic screening.

Treatment. Because acute Q fever is generally self-resolving, particularly in children, treatment is generally not necessary. The classic therapy for adults is doxycycline, and alternatives include trimethoprim-sulfamethoxazole, fluoroquinolones, and clarithromycin.[110] The important exception in regard to treatment is the situation of valvular heart disease because 39 percent of cases of acute Q fever will result in endocarditis in patients with preexisting valvular disease.[57] In those cases, the acute treatment should include both doxycycline and hydroxychloroquine.

In contrast to acute disease, chronic Q fever requires prolonged treatment. Eighteen to 36 months of both doxycycline and hydroxychloroquine is recommended.[110] Valve replacement may unfortunately be necessary for cure in cases of endocarditis. Pregnant women should be treated throughout pregnancy with trimethoprim-sulfamethoxazole to prevent transmission and abortion or premature delivery.[138]

Prevention. A vaccine for Q fever was developed in Australia for use in people with occupational risks, but it is not available in the United States. Most of the preventive strategies involve minimizing contact with infected animals and post-delivery materials through the rapid and hygienic disposal of placentas and aborted fetuses.[30]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Adams, WH, Emmons RW, Brooks JE. The changing ecology of murine (endemic) typhus in Southern California. *Am J Trop Med Hyg* 1970;**19**:311–18.
2. Adan A, Lopez-Soto A, Moser C, Coca A. Use of steroids and heparin to treat retinal arterial occlusion in Mediterranean spotted fever. *J Infect Dis* 1988;**158**:1139–40.
3. Aikawa J. *Rocky Mountain spotted fever*. Springfield, Ill: Charles C Thomas; 1966.
4. Anderson BE, Dawson JE, Jones DC, Wilson KH. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *J Clin Microbiol* 1991;**29**:2838–42.
5. Anton E, Font B, Munoz T, et al. Clinical and laboratory characteristics of 144 patients with Mediterranean spotted fever. *Eur J Clin Microbiol Infect Dis* 2003;**22**:126–8.
6. Bakken JS, Aguero-Rosenfeld ME, Tilden RL, et al. Serial measurements of hematologic counts during the active phase of human granulocytic ehrlichiosis. *Clin Infect Dis* 2001;**32**:862–70.
7. Bakken JS, Dumler JS. Human granulocytic ehrlichiosis. *Clin Infect Dis* 2000;**31**:554–60.
8. Bakken JS, Haller I, Riddell D, et al. The serological response of patients infected with the agent of human granulocytic ehrlichiosis. *Clin Infect Dis* 2002;**34**:22–7.
9. Bechah Y, Capo C, Mege JL, Raoult D. Epidemic typhus. *Lancet Infect Dis* 2008;**8**:417–26.
10. Berman SJ, Irving GS, Kundin WD, et al. Epidemiology of the acute fevers of unknown origin in South Vietnam: effect of laboratory support upon clinical diagnosis. *Am J Trop Med Hyg* 1973;**22**:796–801.
11. Berry DS, Miller RS, Hooke JA, et al. Ehrlichial meningitis with cerebrospinal fluid morulae. *Pediatr Infect Dis J* 1999;**18**:552–5.
12. Birg ML, La Scola B, Roux V, et al. Isolation of *Rickettsia prowazekii* from blood by shell vial cell culture. *J Clin Microbiol* 1999;**37**: 3722–4.
13. Bise G, Coninx R. Epidemic typhus in a prison in Burundi. *Trans R Soc Trop Med Hyg* 1997;**91**:133–4.
14. Blacksell SD, Bryant NJ, Paris DH, et al. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion. *Clin Infect Dis* 2007;**44**:391–401.
15. Blanco JR, Oteo JA. Rickettsiosis in Europe. *Ann N Y Acad Sci* 2006;**1078**:26–33.
16. Boostrom A, Beier MS, Macaluso JA, et al. Geographic association of *Rickettsia felis*–infected opossums with human murine typhus, Texas. *Emerg Infect Dis* 2002;**8**:549–54.
17. Bouyer DH, Stenos J, Crocquet-Valdes P, et al. *Rickettsia felis*: molecular characterization of a new member of the spotted fever group. *Int J Syst Evol Microbiol* 2001;**51**:339–47.
18. Boyd AS. Rickettsialpox. *Dermatol Clin* 1997;**15**:313–18.
19. Brettman LR, Lewin S, Holzman RS, et al. Rickettsialpox: report of an outbreak and a contemporary review. *Medicine (Baltimore)* 1981;**60**:363–72.
20. Buckingham S, Marshall G, Schutze G, et al. Tick-borne Infections in Children Study Group. Clinical and laboratory features, hospital course, and outcome of Rocky Mountain spotted fever in children. *J Pediatr* 2007;**150**:180–4.
21. Buller RS, Arens M, Hmiel SP, et al. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. *N Engl J Med* 1999; **341**:148–55.
22. Burgdorfer W. Ecology of tick vectors of American spotted fevers. *Bull World Health Organ* 1969;**40**:375–81.
23. Campbell BS, Bowles DE. Human tick bite records in a United States Air Force population, 1989-1992: implications for tick-borne disease risk. *J Wilderness Med* 1994;**5**:405–12.
24. Carcopino X, Raoult D, Bretelle FL, et al. Managing Q fever during pregnancy: the benefits of long-term cotrimoxasole therapy. *Clin Infect Dis* 2007;**45**:548–55.
25. Cascio A, Colomba C, Di Rosa D, et al. Efficacy and safety of clarithromycin as treatment for Mediterranean spotted fever in children: a randomized controlled trial. *Clin Infect Dis* 2001;**33**: 409–11.
26. Castro MB, Nicholson WL, Kramer VL, Childs JE. Persistent infection in *Neotoma fuscipes* (Muridae: Sigmodontinae) with *Ehrlichia phagocytophila* sensu lato. *Am J Trop Med Hyg* 2001;**65**: 261–7.

27. Centers for Disease Control and Prevention. *Ehrlichiosis: statistics and epidemiology*. Available at http://www.cdc.gov/Ehrlichiosis/stats/. Accessed November 19, 2012.
28. Centers for Disease Control and Prevention. Fatal cases of Rocky Mountain spotted fever in family clusters—three states, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:407–10.
29. Centers for Disease Control and Prevention. *Preventing tick bites*. Available at http://www.cdc.gov/ticks/avoid/on_people.html. Accessed September 16, 2012.
30. Centers for Disease Control and Prevention. *Q fever*. Available at http://www.cdc.gov/qfever/prevention/index.html. Accessed September 8, 2012.
31. Centers for Disease Control and Prevention. *Q fever: epidemiologic overview for clinicians*. Available at http://www.bt.cdc.gov/agent/qfever/clinicians/epidemiology.asp. Accessed September 5, 2012.
32. Centers for Disease Control and Prevention. *Statistics and epidemiology: annual cases of RMSF in the United States*. Available at http://www.cdc.gov/rmsf/stats/. Accessed August 29, 2012.
33. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
34. Chapman A, Bakken JS, Folk SM, et al. Tickborne Rickettsial Diseases Working Group; CDC. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: practical guide for physicians and other health-care and public health professionals. *MMWR Recomm Rep* 2006;**55**(RR-4):1–27.
35. Chen LF, Sexton DJ. What's new in Rocky Mountain spotted fever? *Infect Dis Clin North Am* 2008;**22**:415–32.
36. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 1994;**32**:589–95.
37. Colomba C, Saporito L, Polara VF, et al. Mediterranean spotted fever: clinical and laboratory characteristics of 415 Sicilian children. *BMC Infect Dis* 2006;**6**:60.
38. Conor A, Bruch A. Une fièvre éruptive observèe en Tunisie. *Bull Soc Pathol Exot Filial* 1910;**8**:492–6.
39. Cox H. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups. *Public Health Res* 1938;**53**:2241–47.
40. Dawson JE, Fishbein DB, Eng TR, et al. Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: kinetics and specificity. *J Infect Dis* 1990;**162**:91–5.
41. De Micco C, Raoult D, Benderitter T, et al. Immune complex vasculitis associated with Mediterranean spotted fever. *J Infect* 1987;**14**:163–5.
42. De Silva N, Wijesundara S, Liyanapathirana V, et al. Scrub typhus among pediatric patients in Dambadeniya: a base hospital in Sri Lanka. *Am J Trop Med Hyg* 2012;**87**:342–4.
43. de Sousa R, Nobrega SD, Bacellar F, Torgal J. Mediterranean spotted fever in Portugal: risk factors for fatal outcome in 105 hospitalized patients. *Ann N Y Acad Sci* 2003;**990**:285–94.
44. Derrick E. The query fever—the Elkington oration. *Queensland Health* 1964;**1**:1–20.
45. Diab SM, Araj GF, Fenech FF. Cardiovascular and pulmonary complications of epidemic typhus. *Trop Geogr Med* 1989;**41**:76–9.
46. Diez Ruiz A, Ramos Jimenez A, Lopez Ruz MA, Gil Extremera B. Boutonneuse fever transmitted by conjunctival inoculation. *Klin Wochenschr* 1988;**66**:1212–13.
47. Dimmitt DC, Fishbein DB, Dawson JE. Human ehrlichiosis associated with cerebrospinal fluid pleocytosis: a case report. *Am J Med* 1989;**87**:677–8.
48. Duma RJ, Sonenshine DE, Bozeman FM, et al. Epidemic typhus in the United States associated with flying squirrels. *JAMA* 1981;**245**:2318–23.
49. Dumler J, Walker D. Rocky mountain spotted fever—changing ecology and persisting virulence. *N Engl J Med* 2005;**353**:551–3.
50. Dumler JS. *Anaplasma* and *Ehrlichia* infection. *Ann N Y Acad Sci* 2005;**1063**:361–73.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adams WH, Emmons RW, Brooks JE. The changing ecology of murine (endemic) typhus in Southern California. *Am J Trop Med Hyg* 1970;**19**:311–18.
2. Adan A, Lopez-Soto A, Moser C, Coca A. Use of steroids and heparin to treat retinal arterial occlusion in Mediterranean spotted fever. *J Infect Dis* 1988;**158**:1139–40.
3. Aikawa J. *Rocky Mountain spotted fever*. Springfield, Ill: Charles C Thomas; 1966.
4. Anderson BE, Dawson JE, Jones DC, Wilson KH. *Ehrlichia chaffeensis*, a new species associated with human ehrlichiosis. *J Clin Microbiol* 1991;**29**:2838–42.
5. Anton E, Font B, Munoz T, et al. Clinical and laboratory characteristics of 144 patients with Mediterranean spotted fever. *Eur J Clin Microbiol Infect Dis* 2003;**22**:126–8.
6. Bakken JS, Aguero-Rosenfeld ME, Tilden RL, et al. Serial measurements of hematologic counts during the active phase of human granulocytic ehrlichiosis. *Clin Infect Dis* 2001;**32**:862–70.
7. Bakken JS, Dumler JS. Human granulocytic ehrlichiosis. *Clin Infect Dis* 2000;**31**:554–60.
8. Bakken JS, Haller I, Riddell D, et al. The serological response of patients infected with the agent of human granulocytic ehrlichiosis. *Clin Infect Dis* 2002;**34**:22–7.
9. Bechah Y, Capo C, Mege JL, Raoult D. Epidemic typhus. *Lancet Infect Dis* 2008;**8**:417–26.
10. Berman SJ, Irving GS, Kundin WD, et al. Epidemiology of the acute fevers of unknown origin in South Vietnam: effect of laboratory support upon clinical diagnosis. *Am J Trop Med Hyg* 1973; **22**:796–801.
11. Berry DS, Miller RS, Hooke JA, et al. Ehrlichial meningitis with cerebrospinal fluid morulae. *Pediatr Infect Dis J* 1999;**18**:552–5.
12. Birg ML, La Scola B, Roux V, et al. Isolation of *Rickettsia prowazekii* from blood by shell vial cell culture. *J Clin Microbiol* 1999; **37**:3722–4.
13. Bise G, Coninx R. Epidemic typhus in a prison in Burundi. *Trans R Soc Trop Med Hyg* 1997;**91**:133–4.
14. Blacksell SD, Bryant NJ, Paris DH, et al. Scrub typhus serologic testing with the indirect immunofluorescence method as a diagnostic gold standard: a lack of consensus leads to a lot of confusion. *Clin Infect Dis* 2007;**44**:391–401.
15. Blanco JR, Oteo JA. Rickettsiosis in Europe. *Ann N Y Acad Sci* 2006;**1078**:26–33.
16. Boostrom A, Beier MS, Macaluso JA, et al. Geographic association of *Rickettsia felis*–infected opossums with human murine typhus, Texas. *Emerg Infect Dis* 2002;**8**:549–54.
17. Bouyer DH, Stenos J, Crocquet-Valdes P, et al. *Rickettsia felis*: molecular characterization of a new member of the spotted fever group. *Int J Syst Evol Microbiol* 2001;**51**:339–47.
18. Boyd AS. Rickettsialpox. *Dermatol Clin* 1997;**15**:313–18.
19. Brettman LR, Lewin S, Holzman RS, et al. Rickettsialpox: report of an outbreak and a contemporary review. *Medicine (Baltimore)* 1981;**60**:363–72.
20. Buckingham S, Marshall G, Schutze G, et al. Tick-borne Infections in Children Study Group. Clinical and laboratory features, hospital course, and outcome of Rocky Mountain spotted fever in children. *J Pediatr* 2007;**150**:180–4.
21. Buller RS, Arens M, Hmiel SP, et al. *Ehrlichia ewingii*, a newly recognized agent of human ehrlichiosis. *N Engl J Med* 1999; **341**:148–55.
22. Burgdorfer W. Ecology of tick vectors of American spotted fevers. *Bull World Health Organ* 1969;**40**:375–81.
23. Campbell BS, Bowles DE. Human tick bite records in a United States Air Force population, 1989-1992: implications for tick-borne disease risk. *J Wilderness Med* 1994;**5**:405–12.
24. Carcopino X, Raoult D, Bretelle F, et al. Managing Q Fever during pregnancy: the benefits of long-term cotrimoxasole therapy. *Clin Infect Dis* 2007;**45**:548–55.
25. Cascio A, Colomba C, Di Rosa D, et al. Efficacy and safety of clarithromycin as treatment for Mediterranean spotted fever in children: a randomized controlled trial. *Clin Infect Dis* 2001;**33**: 409–11.
26. Castro MB, Nicholson WL, Kramer VL, Childs JE. Persistent infection in *Neotoma fuscipes* (Muridae: Sigmodontinae) with *Ehrlichia phagocytophila* sensu lato. *Am J Trop Med Hyg* 2001;**65**: 261–7.
27. Centers for Disease Control and Prevention. *Ehrlichiosis: statistics and epidemiology*. Available at http://www.cdc.gov/Ehrlichiosis/stats/. Accessed November 19, 2012.
28. Centers for Disease Control and Prevention. Fatal cases of Rocky Mountain spotted fever in family clusters—three states, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:407–10.
29. Centers for Disease Control and Prevention. *Preventing tick bites*. Available at http://www.cdc.gov/ticks/avoid/on_people.html. Accessed September 16, 2012.
30. Centers for Disease Control and Prevention. *Q fever*. Available at http://www.cdc.gov/qfever/prevention/index.html. Accessed September 8, 2012.
31. Centers for Disease Control and Prevention. *Q fever: epidemiologic overview for clinicians*. Available at http://www.bt.cdc.gov/agent/qfever/clinicians/epidemiology.asp. Accessed September 5, 2012.
32. Centers for Disease Control and Prevention. *Statistics and epidemiology: annual cases of RMSF in the United States*. Available at http://www.cdc.gov/rmsf/stats/. Accessed August 29, 2012.
33. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111.
34. Chapman A, Bakken JS, Folk SM, et al. Tickborne Rickettsial Diseases Working Group; CDC. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis—United States: practical guide for physicians and other health-care and public health professionals. *MMWR Recomm Rep* 2006;**55**(RR-4):1–27.
35. Chen LF, Sexton DJ. What's new in Rocky Mountain spotted fever? *Infect Dis Clin North Am* 2008;**22**:415–32.
36. Chen SM, Dumler JS, Bakken JS, Walker DH. Identification of a granulocytotropic *Ehrlichia* species as the etiologic agent of human disease. *J Clin Microbiol* 1994;**32**:589–95.
37. Colomba C, Saporito L, Polara VF, et al. Mediterranean spotted fever: clinical and laboratory characteristics of 415 Sicilian children. *BMC Infect Dis* 2006;**6**:60.
38. Conor A, Bruch A. Une fièvre éruptive observèe en Tunisie. *Bull Soc Pathol Exot Filial* 1910;**8**:492–6.
39. Cox H. Use of yolk sac of developing chick embryo as medium for growing rickettsiae of Rocky Mountain spotted fever and typhus groups. *Public Health Res* 1938;**53**:2241–47.
40. Dawson JE, Fishbein DB, Eng TR, et al. Diagnosis of human ehrlichiosis with the indirect fluorescent antibody test: kinetics and specificity. *J Infect Dis* 1990;**162**:91–5.
41. De Micco C, Raoult D, Benderitter T, et al. Immune complex vasculitis associated with Mediterranean spotted fever. *J Infect* 1987;**14**:163–5.
42. De Silva N, Wijesundara S, Liyanapathirana V, et al. Scrub typhus among pediatric patients in Dambadeniya: a base hospital in Sri Lanka. *Am J Trop Med Hyg* 2012;**87**:342–4.
43. de Sousa R, Nobrega SD, Bacellar F, Torgal J. Mediterranean spotted fever in Portugal: risk factors for fatal outcome in 105 hospitalized patients. *Ann N Y Acad Sci* 2003;**990**:285–94.
44. Derrick E. The query fever—the Elkington oration. *Queensland Health* 1964;**1**:1–20.
45. Diab SM, Araj GF, Fenech FF. Cardiovascular and pulmonary complications of epidemic typhus. *Trop Geogr Med* 1989;**41**: 76–9.
46. Diez Ruiz A, Ramos Jimenez A, Lopez Ruz MA, Gil Extremera B. Boutonneuse fever transmitted by conjunctival inoculation. *Klin Wochenschr* 1988;**66**:1212–13.
47. Dimmitt DC, Fishbein DB, Dawson JE. Human ehrlichiosis associated with cerebrospinal fluid pleocytosis: a case report. *Am J Med* 1989;**87**:677–8.
48. Duma RJ, Sonenshine DE, Bozeman FM, et al. Epidemic typhus in the United States associated with flying squirrels. *JAMA* 1981; **245**:2318–23.
49. Dumler J, Walker D. Rocky Mountain spotted fever—changing ecology and persisting virulence. *N Engl J Med* 2005;**353**: 551–3.
50. Dumler JS. *Anaplasma* and *Ehrlichia* infection. *Ann N Y Acad Sci* 2005;**1063**:361–73.
51. Dumler JS, Barbet AF, Bekker CP, et al. Reorganization of genera in the families Rickettsiaceae and Anaplasmataceae in the order Rickettsiales: unification of some species of *Ehrlichia* with *Anaplasma*, *Cowdria* with *Ehrlichia* and *Ehrlichia* with *Neorickettsia*, descriptions of six new species combinations and designation of

Ehrlichia equi and "HGE agent" as subjective synonyms of *Ehrlichia phagocytophila*. *Int J Syst Evol Microbiol* 2001;**51**:2145–65.
52. Dumler JS, Dawson JE, Walker DH. Human ehrlichiosis: hematopathology and immunohistologic detection of *Ehrlichia chaffeensis*. *Hum Pathol* 1993;**24**:391–6.
53. Dumler JS, Madigan JE, Pusterla N, Bakken JS. Ehrlichioses in humans: epidemiology, clinical presentation, diagnosis, and treatment. *Clin Infect Dis* 2007;**45**(Suppl. 1):S45–51.
54. Dumler JS, Sutker WL, Walker DH. Persistent infection with *Ehrlichia chaffeensis*. *Clin Infect Dis* 1993;**17**:903–5.
55. Edwards MS, Jones JE, Leass DL, et al. Childhood infection caused by *Ehrlichia canis* or a closely related organism. *Pediatr Infect Dis J* 1988;**7**:651–4.
56. Felz MW, Durden LA, Oliver JH Jr. Ticks parasitizing humans in Georgia and South Carolina. *J Parasitol* 1996;**82**:505–8.
57. Fenollar F, Fournier P-E, Carrieri M, et al. Risk factors and prevention of Q Fever endocarditis. *Clin Infect Dis* 2001;**33**:312–16.
58. Fergie JE, Purcell K, Wanat D. Murine typhus in South Texas children. *Pediatr Infect Dis J* 2000;**19**:535–8.
59. Fichtenbaum CJ, Peterson LR, Weil GJ. Ehrlichiosis presenting as a life-threatening illness with features of the toxic shock syndrome. *Am J Med* 1993;**95**:351–7.
60. Fishbein DB, Sawyer LA, Holland CJ, et al. Unexplained febrile illnesses after exposure to ticks. Infection with an Ehrlichia? *JAMA* 1987;**257**:3100–4.
61. Font-Creus B, Bella-Cueto F, Espejo-Arenas E, et al. Mediterranean spotted fever: a cooperative study of 227 cases. *Rev Infect Dis* 1985;**7**:635–42.
62. Fornadel CM, Zhang X, Smith JD, et al. High rates of *Rickettsia parkeri* infection in Gulf Coast ticks (*Amblyomma maculatum*) and identification of "*Candidatus* Rickettsia andeanae" from Fairfax County, Virginia. *Vector Borne Zoonotic Dis* 2011;**11**:1535–9.
63. Fournier PE, Roux V, Caumes E, et al. Outbreak of *Rickettsia africae* infections in participants of an adventure race in South Africa. *Clin Infect Dis* 1998;**27**:316–23.
64. Frances SP, Watcharapichat P, Phulsuksombati D. Vertical transmission of *Orientia tsutsugamushi* in two lines of naturally infected *Leptotrombidium deliense* (Acari: Trombiculidae). *J Med Entomol* 2001;**38**:17–21.
65. Friedmann I, Frohlich A, Wright A. Epidemic typhus fever and hearing loss: a histological study (Hallpike collection of temporal bone sections). *J Laryngol Otol* 1993;**107**:275–83.
66. Furuya Y, Yamamoto S, Otu M, et al. Use of monoclonal antibodies against *Rickettsia tsutsugamushi* Kawasaki for serodiagnosis by enzyme-linked immunosorbent assay. *J Clin Microbiol* 1991;**29**:340–5.
67. Furuya Y, Yoshida Y, Katayama T, et al. Serotype-specific amplification of *Rickettsia tsutsugamushi* DNA by nested polymerase chain reaction. *J Clin Microbiol* 1993;**31**:1637–40.
68. Gaon JA, Murray ES. The natural history of recrudescent typhus (Brill-Zinsser disease) in Bosnia. *Bull World Health Organ* 1966;**35**:133–41.
69. Gillespie J, Williams K, Shukla M, et al. Rickettsia phylogenomics: unwinding the intricacies of obligate intracellular life. *PLoS ONE* 2008;**3**:e2018. doi:10.1371/journal.pone.0002018.
70. Goddard J, McHugh CP. Impact of a severe tick infestation at Little Rock AFB, Arkansas on volant scorpion military training. *Mil Med* 1990;**155**:277–80.
71. Golden SE. Aseptic meningitis associated with *Ehrlichia canis* infection. *Pediatr Infect Dis J* 1989;**8**:335–7.
72. Greenberg M, Pellitteri O. Rickettsialpox; a newly recognized rickettsial disease; clinical observations. *JAMA* 1947;**133**:901–6.
73. Greenberg M, Pellitteri OJ, Jellison WL. Rickettsialpox—a newly recognized rickettsial disease: III. Epidemiology. *Am J Public Health Nations Health* 1947;**37**:860–8.
74. Gudiol F, Pallares R, Carratala J, et al. Randomized double-blind evaluation of ciprofloxacin and doxycycline for Mediterranean spotted fever. *Antimicrob Agents Chemother* 1989;**33**:987–8.
75. Hall C, Richmond S, Caul E, et al. Laboratory outbreak of Q fever acquired from sheep. *Lancet* 1982;**1**:1004–6.
76. Harden V. *Rocky Mountain spotted fever: history of a twentieth-century disease*. Baltimore: Johns Hopkins University Press; 1990.
77. Harkess JR, Ewing SA, Crutcher JM, et al. Human ehrlichiosis in Oklahoma. *J Infect Dis* 1989;**159**:576–9.
78. Hase T. Developmental sequence and surface membrane assembly of rickettsiae. *Annu Rev Microbiol* 1985;**39**:69–88.
79. Hayes S, Burgdorfer W, Aeschlimann A. Sexual transmission of spotted fever group rickettsiae by infected male ticks: detection of rickettsiae in immature spermatozoa of *Ixodes ricinus*. *Infect Immun* 1980;**27**:638–42.
80. Hazlett DR. Scrub typhus in Vietnam: experience at the 8th Field Hospital. *Mil Med* 1970;**135**:31–4.
81. Hechemy K. History and prospects of *Coxiella burnetti* reserach. *Adv Exp Med Biol* 2012;**984**:1–11.
82. Hechemy KE, Raoult D, Eisemann C, et al. Detection of antibodies to *Rickettsia conorii* with a latex agglutination test in patients with Mediterranean spotted fever. *J Infect Dis* 1986;**153**:132–5.
83. Hendershot EF, Sexton DJ. Scrub typhus and rickettsial diseases in international travelers: a review. *Curr Infect Dis Rep* 2009;**11**:66–72.
84. Horowitz HW, Aguero-Rosenfeld ME, McKenna D, et al. Clinical and laboratory spectrum of culture-proven human granulocytic ehrlichiosis: comparison with culture-negative cases. *Clin Infect Dis* 1998;**27**:1314–17.
85. Huebner RJ, Jellison WL, Armstrong C. Rickettsialpox; a newly recognized rickettsial disease; recovery of *Rickettsia akari* from a house mouse (*Mus musculus*). *Public Health Rep* 1947;**62**:777–80.
86. Huebner RJ, Jellison WL, Pomerantz C. Rickettsialpox, a newly recognized rickettsial disease; isolation of a rickettsia apparently identical with the causative agent of rickettsialpox from *Allodermanyssus sanguineus*, a rodent mite. *Public Health Rep* 1946;**61**:1677–82.
87. Huebner RJ, Stamps P, Armstrong C. Rickettsialpox; a newly recognized rickettsial disease; isolation of the etiological agent. *Public Health Rep* 1946;**61**:1605–14.
88. Hunter WD, Bishopp FC. *The Rocky Mountain spotted fever tick. With special reference to the problem of its control in the Bitter Root Valley in Montana.* Bulletin 105. Washington, DC: U.S. Department of Agriculture; 1911.
89. Isaac R, Varghese GM, Mathai E, et al. Scrub typhus: prevalence and diagnostic issues in rural Southern India. *Clin Infect Dis* 2004;**39**:1395–6.
90. Jensenius M, Fournier PE, Vene S, et al. Norwegian African Tick Bite Fever Study. African tick bite fever in travelers to rural sub-Equatorial Africa. *Clin Infect Dis* 2003;**36**:1411–17.
91. Kaplan J, Schonberger L. The sensitivity of various serologic tests in the diagnosis of Rocky Mountain spotted fever. *Am J Trop Med Hyg* 1986;**35**:840–4.
92. Kaplowitz L, Robertson G. Hyponatremia in Rocky Mountain spotted fever: role of antidiuretic hormone. *Ann Intern Med* 1983;**98**:334–5.
93. Kass EM, Szaniawski WK, Levy H, et al. Rickettsialpox in a New York City hospital, 1980 to 1989. *N Engl J Med* 1994;**331**:1612–17.
94. Kelly DJ, Fuerst PA, Ching WM, Richards AL. Scrub typhus: the geographic distribution of phenotypic and genotypic variants of *Orientia tsutsugamushi*. *Clin Infect Dis* 2009;**48**(Suppl. 3):S203–30.
95. Kim DM, Kim HL, Park CY, et al. Clinical usefulness of eschar polymerase chain reaction for the diagnosis of scrub typhus: a prospective study. *Clin Infect Dis* 2006;**43**:1296–300.
96. Kim DM, Won KJ, Park CY, et al. Distribution of eschars on the body of scrub typhus patients: a prospective study. *Am J Trop Med Hyg* 2007;**76**:806–9.
97. Kim YS, Yun HJ, Shim SK, et al. A comparative trial of a single dose of azithromycin versus doxycycline for the treatment of mild scrub typhus. *Clin Infect Dis* 2004;**39**:1329–35.
98. Koo JE, Hong HJ, Dearth A, et al. Intracellular invasion of *Orientia tsutsugamushi* activates inflammasome in ASC-dependent manner. *PLoS One* 2012;**7**:e39042.
99. Koss T, Carter EL, Grossman ME, et al. Increased detection of rickettsialpox in a New York City hospital following the anthrax outbreak of 2001: use of immunohistochemistry for the rapid confirmation of cases in an era of bioterrorism. *Arch Dermatol* 2003;**139**:1545–52.
100. Krause DW, Perine PL, McDade JE, Awoke S. Treatment of louse-borne typhus fever with chloramphenicol, tetracycline or doxycycline. *East Afr Med J* 1975;**52**:421–7.
101. La Scola B. Current laboratory diagnosis of Q fever. *Semin Pediatr Infect Dis* 2002;**13**:257–62.
102. La Scola B, Raoult D. Laboratory diagnosis of rickettsioses: current approaches to diagnosis of old and new rickettsial diseases. *J Clin Microbiol* 1997;**35**:2715–27.

103. La Scola B, Rydkina L, Ndihokubwayo JB, et al. Serological differentiation of murine typhus and epidemic typhus using cross-adsorption and Western blotting. *Clin Diagn Lab Immunol* 2000;**7**: 612–16.
104. Laferl H, Fournier PE, Seiberl G, et al. Murine typhus poorly responsive to ciprofloxacin: a case report. *J Travel Med* 2002; **9**:103–4.
105. Lepidi H, Fournier PE, Raoult D. Histologic features and immunodetection of African tick-bite fever eschar. *Emerg Infect Dis* 2006;**12**:1332–7.
106. Lerner AM, Klein JO, Cherry JD, Finland M. New viral exanthems. *N Engl J Med* 1963;**269**:678–85.
107. Lockhart JM, Davidson WR, Stallknecht DE, et al. Isolation of *Ehrlichia chaffeensis* from wild white-tailed deer (*Odocoileus virginianus*) confirms their role as natural reservoir hosts. *J Clin Microbiol* 1997;**35**:1681–6.
108. Lubick N. Tough lessons from Dutch Q fever outbreak. *Nature* 2010;March 3. doi:10.1038/news.2010.102.
109. Maeda K, Markowitz N, Hawley RC, et al. Human infection with *Ehrlichia canis*, a leukocytic rickettsia. *N Engl J Med* 1987;**316**: 853–6.
110. Maltezou H, Raoult D. Q Fever in children. *Lancet Infect Dis* 2002;**2**:686–91.
111. Mansueto S, Vitale G, Bentivegna M, et al. Persistence of antibodies to *Rickettsia conorii* after an acute attack of boutonneuse fever. *J Infect Dis* 1985;**151**:377.
112. Marshall G, Stout G, Jacobs R, et al. Tick-Borne Infections in Children Study Group. Antibodies reactive to *Rickettsia rickettsii* among children living in the southeast and south central regions of the United States. *Arch Pediatr Adolesc Med* 2003;**157**:443–8.
113. Marshall GS. *Rickettsia typhi* seroprevalence among children in the Southeast United States. Tick-Borne Infections in Children Study (TICKS) Group. *Pediatr Infect Dis J* 2000;**19**:1103–4.
114. Marshall GS, Jacobs RF, Schutze GE, et al. *Ehrlichia chaffeensis* seroprevalence among children in the southeast and south-central regions of the United States. *Arch Pediatr Adolesc Med* 2002;**156**: 166–70.
115. McCalla L. Direct transmission from man to man of the Rocky Mountain spotted (tick) fever. *Medical Sentinal* 1908;**16**:87–8.
116. McDade JE, Shepard CC, Redus MA, et al. Evidence of *Rickettsia prowazekii* infections in the United States. *Am J Trop Med Hyg* 1980;**29**:277–84.
117. Merten HA, Durden LA. A state-by-state survey of ticks recorded from humans in the United States. *J Vector Ecol* 2000;**25**:102–13.
118. Montenegro MR, Mansueto S, Hegarty BC, Walker DH. The histology of "taches noires" of boutonneuse fever and demonstration of *Rickettsia conorii* in them by immunofluorescence. *Virchows Arch A Pathol Anat Histopathol* 1983;**400**:309–17.
119. Moron CG, Popov VL, Feng HM, et al. Identification of the target cells of *Orientia tsutsugamushi* in human cases of scrub typhus. *Mod Pathol* 2001;**14**:752–9.
120. Oaks SC Jr, Ridgway RL, Shirai A, Twartz JC. Scrub typhus. *Inst Med Res (Malaysia) Bull* 1983;**21**:1–98.
121. Ognibene AJ, O'Leary DS, Czarnecki SW, et al. Myocarditis and disseminated intravascular coagulation in scrub typhus. *Am J Med Sci* 1971;**262**:233–9.
122. Ohashi N, Tamura A, Suto T. Immunoblotting analysis of antirickettsial antibodies produced in patients of tsutsugamushi disease. *Microbiol Immunol* 1988;**32**:1085–92.
123. Olano JP, Masters E, Hogrefe W, Walker DH. Human monocytotropic ehrlichiosis, Missouri. *Emerg Infect Dis* 2003;**9**:1579–86.
124. Ormsbee R, Peacock M, Philip R, et al. Serologic diagnosis of epidemic typhus fever. *Am J Epidemiol* 1977;**105**:261–71.
125. Paddock CD, Suchard DP, Grumbach KL, et al. Brief report: fatal seronegative ehrlichiosis in a patient with HIV infection. *N Engl J Med* 1993;**329**:1164–7.
126. Paddock CD, Sumner JW, Comer JA, et al. *Rickettsia parkeri*: a newly recognized cause of spotted fever rickettsiosis in the United States. *Clin Infect Dis* 2004;**38**:805–11.
127. Paddock CD, Zaki SR, Koss T, et al. Rickettsialpox in New York City: a persistent urban zoonosis. *Ann N Y Acad Sci* 2003;**990**: 36–44.
128. Palanivel S, Nedunchelian K, Poovazhagi V, et al. Clinical profile of scrub typhus in children. *Indian J Pediatr* 2012;**79**:1459–62.
129. Panpanich R, Garner P. Antibiotics for treating scrub typhus. *Cochrane Database Syst Rev* 2002;(3):CD002150.
130. Perine PL, Chandler BP, Krause DK, et al. A clinico-epidemiological study of epidemic typhus in Africa. *Clin Infect Dis* 1992;**14**:1149–58.
131. Perine PL, Krause DW, Awoke S, McDade JE. Single-dose doxycycline treatment of louse-borne relapsing fever and epidemic typhus. *Lancet* 1974;**2**:742–4.
132. Petersen LR, Sawyer LA, Fishbein DB, et al. An outbreak of ehrlichiosis in members of an Army Reserve unit exposed to ticks. *J Infect Dis* 1989;**159**:562–8.
133. Philip C, Parker R. Rocky Mountain spotted fever: investigation of sexual transmission in the wood tick *Dermacenter andersoni*. *Public Health Rep* 1933;**48**:266–72.
134. Philip CB. Tsutsugamushi disease in World War II. *J Parasitol* 1948;**34**:169–91.
135. Phimda K, Hoontrakul S, Suttinont C, et al. Doxycycline versus azithromycin for treatment of leptospirosis and scrub typhus. *Antimicrob Agents Chemother* 2007;**51**:3259–63.
136. Pritt BS, Sloan LM, Johnson DK, et al. Emergence of a new pathogenic *Ehrlichia* species, Wisconsin and Minnesota, 2009. *N Engl J Med* 2011;**365**:422–9.
137. Purcell K, Fergie J, Richman K, Rocha L. Murine typhus in children, South Texas. *Emerg Infect Dis* 2007;**13**:926–7.
138. Raoult D, Fenollar F, Stein A. Q Fever during pregnancy. *Arch Intern Med* 2002;**162**:701–4.
139. Raoult D, Fournier PE, Fenollar F, et al. *Rickettsia africae*, a tick-borne pathogen in travelers to sub-Saharan Africa. *N Engl J Med* 2001;**344**:1504–10.
140. Raoult D, Kohler JL, Gallais H, et al. Fatal rickettsiosis [in French]. *Nouv Presse Med* 1982;**11**:607.
141. Raoult D, Marrie T, Mege J. Natural history and pathophysiology of Q fever. *Lancet Infect Dis* 2005;**5**:219–26.
142. Raoult D, Ndihokubwayo JB, Tissot-Dupont H, et al. Outbreak of epidemic typhus associated with trench fever in Burundi. *Lancet* 1998;**352**:353–8.
143. Raoult D, Roux V. The body louse as a vector of reemerging human diseases. *Clin Infect Dis* 1999;**29**:888–911.
144. Raoult D, Roux V, Ndihokubwayo JB, et al. Jail fever (epidemic typhus) outbreak in Burundi. *Emerg Infect Dis* 1997;**3**:357–60.
145. Rar V, Golovljova I. *Anaplasma*, *Ehrlichia*, and "*Candidatus* Neoehrlichia" bacteria: pathogenicity, biodiversity, and molecular genetic characteristics, a review. *Infect Genet Evol* 2011;**11**:1842–61.
146. Reynolds MG, Krebs JS, Comer JA, et al. Flying squirrel–associated typhus, United States. *Emerg Infect Dis* 2003;**9**:1341–3.
147. Richardus J, Dumas A, Husiman J, Schaap G. Q fever in infancy: a review of 18 cases. *Pediatr Infect Dis J* 1985;**4**:369–73.
148. Ricketts H. A summary of investigations of the nature and means of transmission of Rocky Mountain spotted fever. *Transactions of the Chicago Pathological Society* 1907;73–82.
149. Rohrbach BW, Harkess JR, Ewing SA, et al. Epidemiologic and clinical characteristics of persons with serologic evidence of *E. canis* infection. *Am J Public Health* 1990;**80**:442–5.
150. Roland WE, McDonald G, Caldwell CW, Everett ED. Ehrlichiosis—a cause of prolonged fever. *Clin Infect Dis* 1995;**20**: 821–5.
151. Rovery C, Brouqui P, Raoult D. Questions on Mediterranean spotted fever a century after its discovery. *Emerg Infect Dis* 2008; **14**:1360–7.
152. Saini R, Pui JC, Burgin S. Rickettsialpox: report of three cases and a review. *J Am Acad Dermatol* 2004;**51**:S137–42.
153. Sexton D, Gallis H, McRae J, Cate T. Possible needle-associated Rocky Mountain spotted fever [Letter]. *N Engl J Med* 1975;**292**:645.
154. Silpapojakul K. Scrub typhus in the Western Pacific region. *Ann Acad Med Singapore* 1997;**26**:794–800.
155. Silverman D. Some contributions of electron microscopy to the study of the rickettsiae. *Eur J Epidemiol* 1991;**7**:200–6.
156. Sirisanthana V, Puthanakit T, Sirisanthana T. Epidemiologic, clinical and laboratory features of scrub typhus in thirty Thai children. *Pediatr Infect Dis J* 2003;**22**:341–5.
157. Sorvillo FJ, Gondo B, Emmons R, et al. A suburban focus of endemic typhus in Los Angeles County: association with seropositive domestic cats and opossums. *Am J Trop Med Hyg* 1993;**48**: 269–73.
158. Srigley J, Geddie W, Vellend H, et al. Q fever: the liver and bone marrow pathology. *Am J Surg Pathol* 1985;**9**:752–8.

159. Standaert SM, Yu T, Scott MA, et al. Primary isolation of *Ehrlichia chaffeensis* from patients with febrile illnesses: clinical and molecular characteristics. *J Infect Dis* 2000;**181**:1082–8.
160. Stein A, Saunders I, Taylor A, Raoult D. Phylogenomic homogeneity of *Coxiella burnetii* strains as determined by 16s ribosomal RNA sequencing. *FEMS Microbiol Lett* 1993;**113**:339–44.
161. Strand O, Stromberg A. Ciprofloxacin treatment of murine typhus. *Scand J Infect Dis* 1990;**22**:503–4.
162. Strickman D, Tanskul P, Eamsila C, Kelly DJ. Prevalence of antibodies to rickettsiae in the human population of suburban Bangkok. *Am J Trop Med Hyg* 1994;**51**:149–53.
163. Sussman LN. Kew Gardens spotted fever. *N Y Med* 1946;**2**: 27–8.
164. Takahashi M, Murata M, Nogami S, et al. Transovarial transmission of *Rickettsia tsutsugamushi* in *Leptotrombidium pallidum* successively reared in the laboratory. *Jpn J Exp Med* 1988;**58**:213–18.
165. Tamura A, Ohashi N, Urakami H, Miyamura S. Classification of *Rickettsia tsutsugamushi* in a new genus, *Orientia* gen. nov., as *Orientia tsutsugamushi* comb. nov. *Int J Syst Bacteriol* 1995;**45**: 589–91.
166. Tantibhedhyangkul W, Ben Amara A, Textoris J, et al. *Orientia tsutsugamushi*, the causative agent of scrub typhus, induces an inflammatory program in human macrophages. *Microb Pathog* 2013;**55**:55–63.
167. Tarasevich I, Rydkina E, Raoult D. Outbreak of epidemic typhus in Russia. *Lancet* 1998;**352**:1151.
168. Telford SR 3rd, Dawson JE, Katavolos P, et al. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick–rodent cycle. *Proc Natl Acad Sci U S A* 1996;**93**:6209–14.
169. Teysseire N, Raoult D. Comparison of Western immunoblotting and microimmunofluorescence for diagnosis of Mediterranean spotted fever. *J Clin Microbiol* 1992;**30**:455–60.
170. Thomas RJ, Dumler JS, Carlyon JA. Current management of human granulocytic anaplasmosis, human monocytic ehrlichiosis and *Ehrlichia ewingii* ehrlichiosis. *Expert Rev Anti Infect Ther* 2009;**7**:709–22.
171. Tissot-Dupont H, Amadei A-M, Nezri M, Raoult D. Wind in November, Q fever in December. *Emerg Infect Dis* 2004;**10**: 1264–9.
172. Tissot-Dupont H, Raoult D. Q fever. *Infect Dis Clin North Am* 2008;**22**:505–14.
173. Traub R, Wisseman CL Jr. Current concepts of the ecology of chigger-borne rickettsiosis (scrub typhus). *Jpn J Med Sci Biol* 1974;**27**:1–5.
174. Traub R, Wisseman CL Jr. The ecology of chigger-borne rickettsiosis (scrub typhus). *J Med Entomol* 1974;**11**:237–303.
175. Twartz JC, Shirai A, Selvaraju G, et al. Doxycycline prophylaxis for human scrub typhus. *J Infect Dis* 1982;**146**:811–18.
176. Walker D. Rocky Mountain spotted fever: a disease in need of microbiological concern. *Clin Microbiol Rev* 1989;**2**:227–40.
177. Walker D. Rocky Mountain spotted fever: a seasonal alert. *Clin Infect Dis* 1995;**20**:1111–17.
178. Walker DH, Occhino C, Tringali GR, et al. Pathogenesis of rickettsial eschars: the tache noire of boutonneuse fever. *Hum Pathol* 1988;**19**:1449–54.
179. Walker DH, Staiti A, Mansueto S, Tringali G. Frequent occurrence of hepatic lesions in boutonneuse fever. *Acta Trop* 1986;**43**: 175–81.
180. Walker JS, Chan CT, Manikumaran C, Elisberg BL. Attempts to infect and demonstrate transovarial transmission of *R. tsutsugamushi* in three species of Leptotrombidium mites. *Ann N Y Acad Sci* 1975;**266**:80–90.
181. Walls JJ, Aguero-Rosenfeld M, Bakken JS, et al. Inter- and intralaboratory comparison of *Ehrlichia equi* and human granulocytic ehrlichiosis (HGE) agent strains for serodiagnosis of HGE by the immunofluorescent-antibody test. *J Clin Microbiol* 1999;**37**: 2968–73.
182. Walsh DS, Myint KS, Kantipong P, et al. *Orientia tsutsugamushi* in peripheral white blood cells of patients with acute scrub typhus. *Am J Trop Med Hyg* 2001;**65**:899–901.
183. Watt G, Chouriyagune C, Ruangweerayud R, et al. Scrub typhus infections poorly responsive to antibiotics in northern Thailand. *Lancet* 1996;**348**:86–9.
184. Watt G, Kantipong P, Jongsakul K, et al. Doxycycline and rifampicin for mild scrub-typhus infections in northern Thailand: a randomised trial. *Lancet* 2000;**356**:1057–61.
185. Wegdam-Blans M, Kampschreur L, Delsing C, et al. Dutch Q fever Consensus Group. Chronic Q fever: review of the literature and a proposal of new diagnostic criteria. *J Infect* 2012;**64**:247–59.
186. Wei JJ, Tong GZ, Shi SF. Study on *Leptotrombidium gaohuensis* sp. nov., newly discovered vector of tsutsugamushi disease. *Chin Med J (Engl)* 1987;**100**:590–4.
187. Wells G, Woodward T, Fiset P, Hornick R. Rocky Mountain spotted fever caused by blood transfusion. *JAMA* 1978;**239**: 2763–5.
188. Whiteford SF, Taylor JP, Dumler JS. Clinical, laboratory, and epidemiologic features of murine typhus in 97 Texas children. *Arch Pediatr Adolesc Med* 2001;**155**:396–400.
189. Whitman TJ, Richards AL, Paddock CD, et al. *Rickettsia parkeri* infection after tick bite, Virginia. *Emerg Infect Dis* 2007;**13**: 334–6.
190. Wiggers RJ, Stewart RS. Ownership of cats or dogs does not increase exposure to *Rickettsia typhi*. *Tex Med* 2002;**98**:56–7.
191. Wilfert C, MacCormack J, Kleeman K, et al. The prevalence of antibodies to *Rickettsia rickettsii* in an area endemic for Rocky Mountain spotted fever. *J Infect Dis* 1985;**151**:823–31.
192. Williams WJ, Radulovic S, Dasch GA, et al. Identification of *Rickettsia conorii* infection by polymerase chain reaction in a soldier returning from Somalia. *Clin Infect Dis* 1994;**19**:93–9.
193. Wohlbach S. The etiology of Rocky Mountain spotted fever (a preliminary report). *J Med Res* 1916;**34**:121–5.
194. Wohlbach SB, Schlesinger MJ. The cultivation of the microorganisms of Rocky Mountain spotted fever (*Dermacentroxenus rickettsii*) and of typhus (*Rickettsia prowazeki*) in tissue plasma cultures. *J Med Res* 1923;**44**:231–56.
195. Wolfman DE, Fenton W Jr, Donald PJ. Typhus-induced facial necrosis. *Otolaryngol Head Neck Surg* 1986;**94**:390–3.
196. Woo ML, Leung JWC, French GL. Rickettsial infection presenting as culture-negative meningitis. *Postgrad Med J* 1988;**64**: 614–16.
197. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006;**43**:1089–134.
198. Wright CL, Nadolny RM, Jiang J, et al. *Rickettsia parkeri* in Gulf Coast ticks, southeastern Virginia, USA. *Emerg Infect Dis* 2011;**17**: 896–8.
199. Yagupsky P, Wolach B. Fatal Israeli spotted fever in children. *Clin Infect Dis* 1993;**17**:850–3.
200. Zanetti G, Francioli P, Tagan D, et al. Imported epidemic typhus. *Lancet* 1998;**352**:1709.
201. Zhu Y, Fournier PE, Eremeeva M, Raoult D. Proposal to create subspecies of *Rickettsia conorii* based on multi-locus sequence typing and an emended description of *Rickettsia conorii*. *BMC Microbiol* 2005;**5**:11.
202. Zinnser H. *Rats, lice and history*. Boston: Little, Brown; 1934.

SECTION **XX**

MYCOPLASMA

CHAPTER 195

Mycoplasma and Ureaplasma Infections

James D. Cherry • Natalie M. Quanquin

Mycoplasmas and ureaplasmas, the smallest free-living microorganisms, are ubiquitous in nature. More than 100 species have been recovered from many animals, including human beings.[811,978,1012,1020] Of this group, 17 have been identified as human pathogens or as part of the normal human flora, with *Mycoplasma pneumoniae* causing respiratory disease as well as a protean array of other clinical manifestations and the genital mycoplasmas (*Mycoplasma hominis*, *Mycoplasma genitalium*, *Ureaplasma urealyticum*, and *Ureaplasma parvum*) having clinical significance in neonates and as sexually transmitted infections (STIs).

These organisms lack a cell wall, giving them the class name Mollicutes, which means "soft skin."[438] The genera name *Mycoplasma* is derived from Greek and Latin. *Myco* refers to the mycelial, or filamentous, characteristic, and *plasma* indicates the plasticity and pleomorphism of the organism.[951] *Urea* in *Ureaplasma* indicates the presence of urease,[327] and although this group is in its own genus, because of its shared history it is commonly included as a "mycoplasma" in the literature.

HISTORY

In 1898 Nocard and Roux[743] recovered the first *Mycoplasma* organism from cattle with contagious pleuropneumonia. Shortly after the original discovery, many other mycoplasmas were recovered from several different animals.[397] These other mycoplasmas, which frequently were not associated with disease, originally were called *pleuropneumonia-like organisms;* this designation was abbreviated to PPLO. The term PPLO enjoyed general use until the early 1960s.

The first isolation of a mycoplasma from a human was reported in 1937 by Dienes and Edsall.[234] This organism, now recognized as *M. hominis*, was recovered from an abscessed Bartholin gland. In 1944, Eaton and colleagues[257] reported the recovery of an organism, originally called the Eaton agent, from persons ill with primary atypical pneumonia. For many years, the Eaton agent was considered to be a virus, even though it was inhibited by streptomycin and chlortetracycline.[255,256] In 1961, Marmion and Goodburn[633] noted that the Eaton agent was morphologically similar to PPLOs. In 1962, the organism now known as *M. pneumoniae* was cultivated on a cell-free agar medium and was shown in human volunteer studies to be the etiologic agent of primary atypical pneumonia.[146,150,397]

In 1954, Shepard[879] reported the recovery of PPLOs with a distinctive small-colony characteristic from men with and without nongonococcal urethritis. These T strains (T for tiny), as they were called, now are classified as *Ureaplasma*. Early literature referred to both ureaplasmas as *U. urealyticum;* however, sequencing techniques have shown sufficient differences that they are now recognized as a separate species—*U. parvum* (formerly biovar 1) and *U. urealyticum* (formerly biovar 2).[115,152,154] One center that rescreened respiratory and genital samples sent to them over the previous 10 years found that the majority of mollicutes isolated were *Ureaplasma* spp. and that the majority of those sequenced (67%) were actually *U. parvum*.[875]

The observation in the 1970s that some men with nongonococcal urethritis and negative cultures still responded to tetracycline led to the closer examination of urethral smears and the discovery of small motile spiral forms that resembled the insect and plant pathogens of the genus *Spiroplasma*. In 1981, this new microbe was classified as *M. genitalium*, and with a 580-kb genome, it has been considered the smallest known organism capable of independent growth and reproduction.[956,958,978,981,982] It served as a guide for the minimal requirements needed to create a synthetic genome, achieved by John Craig Venter and his team in 2010.[344]

CLASSIFICATION

Mycoplasma, *Ureaplasma*, and *Acholeplasma* are genera of the family Mycoplasmataceae, in the order Mycoplasmatales, which belongs to the class Mollicutes.[326,811,1008,1012] Both *Mycoplasma* and *Ureaplasma* require cholesterol for growth, have a genome with a molecular weight of approximately 4.5×10^8, and have nicotinamide adenine dinucleotide (NADH) oxidase localized in the cytoplasm. The limited size of their genomes prevents them from encoding many of the enzymes necessary for amino acid synthesis and energy production, making them slow-growing, fastidious organisms that are difficult to isolate in culture. They generate adenosine triphosphate (ATP) through carbohydrate fermentation (glycolysis), lactate oxidation to pyruvate, and/or arginine hydrolysis, depending on the species.[491] Members of the genus *Ureaplasma* are also able to hydrolyze urea.[881,882,955]

The normal human mollicate flora includes 14 *Mycoplasma*, 2 *Ureaplasma*, and 1 *Acholeplasma* spp.* These

*References 138, 264, 327, 579, 970, 978.

TABLE 195-1 **Mollicutes Flora of Humans Listed by Site of Most Common Isolation and Prevalence**

Organism	Prevalence
Respiratory Tract	
Mycoplasma salivarium	Very common
Mycoplasma orale	Very common
Mycoplasma buccale	Rare
Mycoplasma faucium	Rare
Mycoplasma lipophilum	Rare
Mycoplasma pneumoniae	Common
Mycoplasma amphoriforme	Rare
Acholeplasma laidlawii	Rare
Genitourinary Tract	
Mycoplasma hominis	Very common
Mycoplasma genitalium	Rare
Mycoplasma fermentans	Rare
Mycoplasma primatum	Rare
Mycoplasma spermatophilum	Rare
Mycoplasma penetrans	Rare
Ureaplasma urealyticum	Very common
Ureaplasma parvum	Very common
Blood	
Mycoplasma pirum	Rare

Data from References 579, 630, 912, 970, 978, 981, 1016, 1020.

organisms are listed by site of most common isolation and frequency of occurrence in Table 195-1. Most species do not invade submucosal tissues and do not cause disease in immunocompetent hosts unless this protective barrier is breached through surgery or trauma. *Mycoplasma salivarium* and *Mycoplasma orale* are commonly part of the normal respiratory flora and have not been associated with illness in nonimmunocompromised persons. *M. hominis*, *U. urealyticum*, and *U. parvum* also are recovered commonly from human genital tracts, and although they are considered normal flora they can trigger inflammatory processes in pregnant women that have been linked to preterm labor. *M. genitalium* is also found in the genital tracts of sexually active individuals and is a cause of nongonococcal urethritis. Inflammatory processes resulting from the presence of *M. genitalium* and *Mycoplasma fermentens*, another colonizer of the genital tract, are possible; however, a link with adverse pregnancy outcomes has not been established (Tully J G. Personal communication. December 1, 1991).[550,952,1012,1019] Persistent infections in blood, bones, joints, and kidneys with *Mycoplasma penetrans*, *Mycoplasma pirum*, *M. fermentans*, *M. hominis*, and *U. urealyticum* have occurred in patients with immunodeficiencies (Tully J G. Personal communication. December 1, 1991).[55,688] *Mycoplasma buccale*, *Mycoplasma faucium*, *Mycoplasma amphoriforme*, *Mycoplasma primatum*, *Acholeplasma laidlawii*, and *Mycoplasma lipophilum* rarely are isolated and are not thought to cause disease in nonimmunocompromised humans. *M. pneumoniae* is a common cause of respiratory and other human illness.

MYCOPLASMA PNEUMONIAE

Properties

Morphology

Because mycoplasmas lack a cell wall, they all tend to be pleomorphic. Kammer and colleagues[480] studied the morphologic characteristics of *M. pneumoniae* organisms grown in broth medium by scanning electron microscopy and grouped their observations by days of incubation. From 0.3 to 2 days, the predominant morphologic feature consisted of 0.51 ± 0.011-μm symmetric round forms in tightly packed clusters. During the interval from day 2 to day 6, branched and straight filaments and bulbs were the predominant forms. The bulbous elements had a diameter of 0.25 ± 0.006 μm, and the filamentous forms were 0.19 ± 0.005 μm in diameter. The filaments were intertwined, and occasional round forms were observed. From day 6 to day 10, the organisms had a rounded shape but were asymmetric. Their diameter was 0.72 ± 0.027 μm, and they occurred in groups of three or four cells. Biberfeld and Biberfeld[71] noted that *M. pneumoniae* filamentous forms varied in length from 1 to 5 μm.

The ultrastructure of *M. pneumoniae*, as well as that of all members of the family Mycoplasmataceae, is relatively simple and consists of a cell membrane and cytoplasm.[327,977] In 7-day *M. pneumoniae* cultures, Domermuth and associates[237] noted the following characteristics: elementary bodies 105 to 120 nm in diameter, mature cells with a maximal diameter of 690 to 750 nm and an average diameter of 440 to 590 nm, asymmetry of the limiting membrane, electron-dense lines outside the limiting membrane, and dense bodies as cytoplasmic inclusions.

Wilson and Collier[1048] studied the ultrastructure of *M. pneumoniae* in hamster tracheal organ culture and noted filamentous organisms with three-layered membranes; the cells were polymorphic, but each had a specialized terminal structure at the site of attachment to the organ culture. This terminal structure had a dense central core containing a denser central filament. Between the organism and the organ culture cell, fusion was not detected, but a loose network of fibrils was noted between the two surfaces. The bodies of the mycoplasmas contained densely staining fibrillar material and cytoplasmic granules, both of which contained nucleic acid.

Motility and Multiplication

Bredt[101] studied *M. pneumoniae* motility and multiplication on a glass surface in liquid medium by phase-contrast microscopy. The organisms were observed to multiply by binary fission; first, short filamentous structures were formed, which then separated into two cells. A growth cycle between two separations lasted approximately 3 hours. After division, the new cells moved by means of a gliding motion. The gliding speed has been noted to be approximately 0.2 to 0.5 μm/sec, but maximal speeds of 1.5 to 2.0 μm/sec have been observed.[804]

Composition

Mycoplasmas are composed of approximately 40 to 60 percent protein, 10 to 20 percent lipid, and a variable amount of carbohydrate. The *M. pneumoniae* genome is circular, double-stranded DNA with a contour length of 4.8×10^8 D and a size of 816 kb.[695] The guanosine plus cytosine content of the DNA is 38.6 to 40.8 percent.[93,729,811,954]

Growth Characteristics and Physical Properties[145]

M. pneumoniae grows in *Mycoplasma* broth medium and on agar enriched with yeast extract and animal serum. *M. pneumoniae* ferments carbohydrates and requires sterol for growth. It grows under both anaerobic and aerobic conditions, but growth is more consistent when it is incubated in nitrogen and 5 percent carbon dioxide. When compared with other mycoplasmas isolated from humans, *M. pneumoniae* grows relatively slowly, with visible formation of colonies rarely occurring in less than 1 week and possibly taking 3 weeks or more. Repeated agar passage results in more rapid growth, and laboratory strains thus treated produce colonies in 3 days.

M. pneumoniae colonies on agar generally appear different from the classic *Mycoplasma* "fried egg" look noted with other types recovered from humans. The *M. pneumoniae* colony is spherical and dense, with a rough ("mulberry") surface.

M. pneumoniae has the following enzyme systems: $NADH_2$ oxidase, nicotinamide adenine dinucleotide phosphate ($NADPH_2$) oxidase, lactate dehydrogenase, probably succinic dehydrogenase, and diaphorases.[397] In liquid medium the following can be noted: acid color change in medium with added glucose and phenol red as a result of glucose metabolism, reduction of methylene blue in medium as a result of dehydrogenase activity, and reduction of 2-3-5 tetrazolium chloride to red formazan by dehydrogenase activity.[951]

On agar, *M. pneumoniae* hemolyzes erythrocytes in an agar overlay by liberation of peroxide. Erythrocytes and other cells adsorb to *M. pneumoniae* colonies, and organisms in suspension cause hemagglutination. *M. pneumoniae* has also been found to create biolfims on abiotic surfaces,[524] which may help it survive in the environment.

Mycoplasmas, including *M. pneumoniae*, are heat sensitive. They have a half-life of less than 2 minutes at 50° C (122° F) and lose viability within 1 week at room temperature.[977] They can be stored for several years at –20° C (–4°F), but –70° C (–94° F) is optimal for long-term storage.

Mycoplasmas are resistant to osmotic environmental changes but are sensitive to detergents. They are inhibited by gold salts and antibiotics not directed against cell wall synthesis.

Antigenic Composition

The following immunologic reactions have been noted in association with *M. pneumoniae*–host serum interactions: specific complement fixation; precipitation in gel; growth inhibition; indirect hemagglutination; metabolic inhibition; antigen-antibody union identified by immunofluorescence, enzyme-linked immunosorbent assay (ELISA), and radioimmunoassay; adherence inhibition assay; and nonspecific complement fixation (i.e., positive serologic test result for syphilis) and agglutination (i.e., cold and *Streptococcus* MG agglutinins).* *M. pneumoniae* organisms have both membrane and cytoplasmic antigens.[291,497] Two membrane antigens can be identified by immunodiffusion,[789] with the major membrane antigen being found in the lipid fraction of the organism.[497] The antigens are glycolipids and are of major importance in complement fixation, metabolic inhibition, and mycoplasmacidal reactions.[125] The cytoplasmic (soluble) antigen, which also contains lipid, can be identified by complement fixation when the antigen is prepared by phenol extraction. Many surface lipoproteins are also differentially expressed on contact with epithelial cells[385] and are thought to play a role in both activating and evading the innate immune system.[109,439]

Humans have an immunoglobulin (Ig)G immune response to seven principal protein antigens.† These seven polypeptides have the following molecular masses: 170, 130, 90, 68, 45, 35, and 30 kDa. The 170-kDa antigen is the P1 protein. This protein is localized at the surface of the terminal organelle (terminal structure), is the major adhesin responsible for attachment, and is the cause of the gliding motility of the organism. Antibodies to P1 protein inhibit hemadsorption and adherence to respiratory epithelium.‡ Another important adhesin is the 30-kDa protein (P30 adhesin). In addition to the P1 and P30 proteins, multiple accessory proteins are involved in the adherence of *M. pneumoniae* to host cells,[810] including P65, HMW1, HMW2, HMW3, and A, B, and C.[46,393]

The 68-kDa community-acquired respiratory distress syndrome (CARDS) toxin is a recently identified protein unique to *M. pneumoniae* but that shares some homology with pertussis toxin.[482,662] It possesses adenosine diphosphate (ADP) ribosyltransferase (ART) activity, is highly immunogenic, and has been associated with airway cellular damage.[662] Diagnostic assays based on this protein have recently been developed.[702]

M. pneumoniae strains can be divided into two types on the basis of the sequence variation in the P1 gene.[242,243,251] Five subtypes of the type 1 strain and three subtypes of the type 2 strain have been noted by restriction fragment length polymorphism (RFLP).

Membrane determinants of *M. pneumoniae* cross react with the erythrocyte glycoprotein containing the I antigen and the related sugar chain (F1)[404,454]; with pneumococcal serotypes 23 and 32[17]; with the glycolipids of spinach, parsnips, and carrots; with selected strains of *Staphylococcus aureus* and group A streptococci; and perhaps with the filamentous hemagglutinin of *Bordetella pertussis*.[369,446,498,999]

*References 119, 428, 449, 579, 798, 951.
†References 15, 93, 152, 218, 219, 325, 533, 541, 1006.
‡References 425, 426, 427, 448, 478, 534, 932.

Animal Susceptibility

M. pneumoniae grows and causes pneumonia in hamsters and cotton rats and causes inapparent infection of the bronchial epithelium of chicken embryos.[798]

Epidemiology

Epidemic Pattern

In large urban areas, *M. pneumoniae* is endemic; infection and disease occur throughout the year. Foy and associates[309,314] noted cultural or serologic evidence of *M. pneumoniae* infection in a Seattle, Washington prepaid medical care group during all seasons throughout an 11-year period. Similar endemicity has been reported in other studies.[686,692,742] Wan and colleagues[1023] tested air filters at a hospital in Taiwan and detected seasonal peaks of *M. pneumoniae* in the pediatric emergency department (July) and outpatient areas (August to January). In addition to the background endemic pattern, *M. pneumoniae* enjoys a cyclic epidemic pattern that is specific for a particular urban community. Epidemics have occurred at 3- to 7-year intervals,[276,314,475,582,742] most recently in Europe and Jerusalem from 2010 to 2012.[141,261,569,738,986] Epidemics, which develop slowly, usually start in the fall and persist in the community for 12 to 30 months.

As part of an influenza surveillance program, Layani-Milon and associates[555] studied acute respiratory infections in outpatients for six winters in the Rhône-Alpes region of France by polymerase chain reaction (PCR) plus a hybridization-based detection system. Each year from 1992 to 1997, at least one peak of *M. pneumoniae* infection was noted in the late fall. Overall, they studied 3897 children and adults with acute respiratory illnesses, and 7.3 percent were found to be infected with *M. pneumoniae*. The yearly rate of *M. pneumoniae* cases fell from 10.1 percent in 1992 to 1993 to 2.0 percent in 1995 to 1996; in the subsequent year (1996 to 1997), it rose to 4.2 percent.

Incidence of Infection and Disease

In the past, most pediatricians and other physicians considered *M. pneumoniae* illness to be uncommon in the general population. The initial epidemiologic studies were concerned mainly with the occurrence of pneumonia in closed populations such as the military and boarding schools.* During the past 49 years, however, many large studies in civilian populations, coupled with more sensitive diagnostic techniques, have indicated that both infection and disease with *M. pneumoniae* occur commonly.†

Hornsleth[418] examined 367 serum samples collected from children hospitalized in Copenhagen from September 1963 to May 1965 for complement-fixing antibodies. He noted that 42 percent of infants 6 to 11 months of age had demonstrable antibody; from 1 to 9 years of age, more than two thirds of the children had antibody. Suhs and Feldman[936] found a similar high prevalence of hemagglutination-inhibiting antibody in the sera of residents of Point Barrow, Alaska, but only 5 percent of the serum samples from a children's home in Syracuse had measurable antibody. A study of 319 pediatric patients with chronic respiratory disease found that 32.6 percent had bronchoalveolar lavage fluid positive for mycoplasmas, of which only 31 percent represented *M. pneumonia*.[778] Brunner and associates[114] detected serum antibody by the sensitive radioimmunoprecipitation test in 28 percent of 7- to 12-month-old infants, 55 percent of 13- to 24-month-old children, 67 percent of 25- to 60-month-old children, and 97 percent of persons older than 17 years. The high antibody prevalence noted in this study, as well as the findings in Copenhagen and Alaska, suggests an infection incidence rate of approximately 20 to 30 percent per year in a susceptible population of young children. The prevalent antibody noted at an early age in these studies possibly is not caused specifically by *M. pneumoniae* infection but instead could be the result of exposure to the many cross-reacting antigens in nature.[17,342,498] However, studies by Fernald and colleagues,[294] in which infants and children in a daycare center were monitored systematically, indicated a yearly infection rate of approximately 12 percent.

Monto and colleagues,[692] in a large study involving 3243 persons, investigated the incidence of infection in six yearly cohort groups of children and adults. Infection was determined by significant rises in titer of complement-fixing antibody on three serum specimens collected during a 1-year period from each subject. The overall yearly infection rate was found to be 5.3 percent. The highest rate (8.8%) occurred in the 5- to 9-year-old group. Infants younger than 1 year had a rate of 2.8 percent.

Brunner and associates[114] noted that geometric mean antibody titers tended to increase with increasing age, which suggested that the older children were being reinfected. Fernald and colleagues[294] reported that 5/22 children infected with *M. pneumoniae* in their investigation experienced reinfections during the 5-year observation period. In the study by Monto and associates,[692] 24.4 percent of the 172 infections detected in subjects of all ages were re-infections. During a 12-year serologic surveillance period, Foy and coworkers[314] noted great variation in the incidence of *M. pneumoniae* infection; in the period from October 1965 to May 1966, only 0.2 percent of 398 children had fourfold rises in complement-fixation antibody titer, whereas during the May 1973 to May 1974 period, 35 percent of 246 subjects 10 to 20 years of age had serologic evidence of infection.

The incidence of disease caused by *M. pneumoniae* depends on the endemic or epidemic prevalence of the organism in the community and is age related. The incidence of *M. pneumoniae* by age for two epidemics and the surrounding endemic periods in Seattle was studied by Foy and associates.[314] The highest epidemic attack rate was 14/1000 children 5 to 9 years of age, and the highest endemic attack rate (4/1000) occurred in the same age group. Children 10 to 14 years of age had the second highest attack rate during both epidemic and endemic

*References 149, 686, 838, 928, 930, 993.
†References 39, 40, 73, 114, 130, 200, 231, 239, 248, 264, 272, 294, 307, 311-314, 316, 318, 353, 356, 367, 418, 457, 466, 475, 582, 591, 597, 671, 691, 692, 736, 742, 766, 878, 936, 976.

periods. The attack rate in children younger than 5 years was about twice that observed in young adults.

The ratio of symptomatic to asymptomatic infection has varied across studies. In family studies, both Balassanian and Robbins[45] and Foy and associates[307] noted that only 15 percent of infections were asymptomatic, whereas Saliba and associates[838] noted that 55 percent of the residents of a boys' home had asymptomatic infection. Chanock and colleagues[149] observed that only 1/30 infections in Marine recruits manifested as clinically apparent pneumonia.

Incubation Period

The reported incubation period has varied from a mean of approximately 1 week in volunteer studies and point-source epidemics to 3 weeks in community outbreaks.* In a volunteer study, Rifkind and colleagues[816] administered tissue culture–grown *M. pneumoniae* in a concentration of 320 to 1280 EID_{50} (mean egg-infective dose) into the nose and posterior pharyngeal area of 27 men with no demonstrable antibody. In this study, pneumonia occurred 9 to 12 days after inoculation, following 1 to 3 days of upper respiratory tract illness. In six volunteers, only upper respiratory tract illness was noted, and the incubation period varied from 4 to 9 days. In a similar study in which volunteers received 10^6 to 10^7 broth-grown organisms, the incubation period was 8 to 10 days.[900]

In an interesting common-source outbreak caused by an intense 8-hour exposure at a party, the peak incubation period was 13 days and most of the cases occurred between days 11 and 14.[278] In another probable point-source outbreak, which may have resulted from a room aerosol, the incubation period varied from 4 to 9 days.[846] In studies of case-to-case intervals in families, Foy and associates[307] reported a median incubation time of 23 days, with most of the cases occurring between days 16 and 25. In similar studies, Copps and colleagues[200] noted an average interval of 21 days, and Biberfeld and Sterner[73] found a modal value of 20 days. A point-source outbreak in a family unit was described in which all seven family members became ill 10 to 16 days after the onset of symptoms in the index case.[503]

The longer incubation period in the family situation than in the volunteer studies and the point-source outbreaks may be the result of larger inocula in the last instances. In contrast to other respiratory illnesses such as measles and influenza, the spread of disease caused by *M. pneumoniae* in both closed populations, such as military training units and boarding schools, and open communities is usually slow. For example, the introduction of influenza or measles into a family most often results in infection of all susceptible persons from the primary case. In contrast, the spread of *M. pneumoniae* through a family of six people probably would require three or four passages. Foy and associates[307] noted secondary attack rates in families of 64 percent for children and 17 percent for adults. Biberfeld and Sterner[73] reported secondary infection rates of 41 and 84 percent, respectively, for adults and children in families. In contrast to family groups, the spread of *M. pneumoniae* in schools and other situations of brief exposure is low.[311,317] In one Seattle elementary school, the infection rate was 18 percent. Foy and Alexander[309] believe that neighborhood spread among playmates is more important than school exposure in community transmission of *M. pneumoniae*.

Transmission in families occurs during the acute phase of illness, and transmission by persons with asymptomatic infection has not been documented.[311]

Geography

The endemic and epidemic presence of *M. pneumoniae* has been demonstrated in the urban areas of developed countries with temperate climates throughout the world.* Serologic investigations in more remote areas, including both arctic and tropical zones, also indicate the presence of *M. pneumoniae* infection. Suhs and Feldman[936] found measurable antibody in the serum of 68 percent of 169 persons in Point Barrow, and Golubjatnikov and associates,[356] in a study of children in a remote Mexican highland community, detected seropositivity in 16 percent of 637 children. Serologic evidence of infection also has been noted in Cairo, Singapore, Hong Kong, the West Indies, and southern Africa.[144,475] Incidence studies have not been performed in rural areas, but patterns of infection probably would be characterized by epidemic periods of a year or so and then complete absence of *M. pneumoniae* circulation for several years.

Sex

Although the results of studies have varied, the difference in the incidence of *M. pneumoniae* disease by sex is small. During the 11 years of study in Seattle, Foy and associates[314] noted that the rate of *M. pneumoniae* pneumonia was higher in women than in men in the 30- to 39-year age group (1.8 vs. 1.2/1000); in infants, boys were afflicted more often than girls, but otherwise the rates for children by sex were virtually identical. Jensen and associates[466] found that pneumonia, otitis media, and nasopharyngitis in various combinations occurred more commonly in boys than in girls. Monto and colleagues[692] noted that boys younger than 5 years of age had more infections, but that the reverse was true for children 5 to 14 years of age. In a review of Japan's National Surveillance Data, Eshima and colleagues[271] found a slightly greater female preponderance in symptomatic infection at all ages except those over 70 years old. In other studies involving all age groups, males have shown a slightly greater frequency of illness than females.[632,742] In the Seattle family studies, symptoms were more severe in boys than in girls.

Pathogenesis and Pathology

Sequence of Events in Infection

M. pneumoniae infection is acquired via the respiratory route from the respiratory secretions of an ill person infected with this agent. Spread can be accomplished by

*References 73, 200, 278, 307, 311, 315, 816, 846, 901, 978.

*References 73, 231, 286, 311, 314, 457, 582, 612, 632, 716, 742, 921.

small-particle aerosols or large droplets of secretions coming in contact with the epithelial surface of the nasopharynx and perhaps the surfaces of the lower respiratory tract (i.e., trachea, bronchi, and bronchioles), as well. In volunteer studies, Couch[205] observed that the 50 percent human infectious dose by small-particle aerosol was 1 colony-forming unit (nasal instillation required a dose that was 100 times greater).

Because epidemiologic data indicate the need for close and perhaps prolonged personal contact for transmission of infection, transmission by small-particle aerosol probably occurs rarely under natural conditions. After acquisition of the infectious agent, multiplication occurs extracellularly on mucous membrane surfaces. The incubation period, which varies from 4 days to more than 3 weeks, probably depends strongly on the size of the original inoculum. The extent of the respiratory infection increases during the incubation period, and shedding of organisms in respiratory secretions can be observed 2 to 8 days before clinical illness.[205] Initial symptoms of infection include headache, malaise, fever, sore throat, and cough; evidence of lower respiratory tract disease is present within the succeeding 3 days.[231,816] The method of extension of infection within the respiratory tract is unknown. The extent of disease possibly depends totally on the initial distribution of the infectious agent at the time of acquisition rather than on spread of infection from a primary upper respiratory site.

After the onset of clinical symptoms, the concentration of *M. pneumoniae* in respiratory secretions peaks, remains high for approximately 1 week, and then persists for 4 to 6 weeks or longer.[205,231] The associated symptoms and signs in disease caused by *M. pneumoniae* (i.e., meningitis, arthritis, hemolytic anemia, rash, and pericarditis) suggest the possibility of frequent dissemination of the organism from the respiratory tract. Some reviews on the subject tend to discount the possibility of generalized *M. pneumoniae* infection and attribute the associated systemic clinical findings to immunologic events related to respiratory infection.[205,231] However, little published evidence indicates that the organism has been sought carefully in the blood and other sites of dissemination. In many instances *M. pneumoniae* has been recovered from or identified by PCR in the blood, pericardial fluid, middle ear fluid, vesicular skin lesions, pleural fluid, kidneys, brain, and cerebrospinal fluid (CSF).* In addition, the observation of low cerebrospinal fluid glucose in *M. pneumoniae* meningoencephalitis suggests direct involvement by the organism.[515]

Pathology

Pathologic findings in *M. pneumoniae* disease in children have not been reported, and only minimal data from adults are available. However, a reasonable understanding of the pathologic process of *M. pneumoniae* disease can be constructed from studies in the hamster, various tracheal organ cultures, and human biopsy and postmortem material.† The primary damage in *M. pneumoniae* infection is to the epithelial lining of the mucosal surfaces of the respiratory tract. This damage has been observed on the surface of bronchi, bronchioles, and alveoli, and clinical symptoms in children suggest that similar pathologic changes occur in the trachea and upper respiratory tract, as well. Specifically conspicuous is destruction of the ciliated epithelium of the bronchi and bronchioles. Because of mucosal desquamation and ulceration, the lumina contain considerable debris; added to this debris is an inflammatory exudate consisting of fibrin, mononuclear cells, and neutrophils. The alveolar spaces contain similar exudate and edema fluid.

The walls of the bronchi and bronchioles are thickened by edema and contain an infiltrate of macrophages, lymphocytes, and plasma cells. The alveolar walls are also thickened and contain lymphocytes, mononuclear cells, and erythrocytes. Dilation of the septal capillaries occurs. Edema and cellular infiltration extend into the interstitial spaces. Gross examination of the lungs reveals areas of hemorrhage and congestion. The pleura may contain patches of fibrinous exudate, and pleural fluid may be present. The pneumonic areas may be discrete or widespread.

A biopsy specimen of a vesiculopustular skin lesion revealed an epidermis with mild acanthosis and marked edema that primarily was intracellular.[962] The papillary and upper reticular dermis contained neutrophils and round cells, and hemorrhagic foci were present within the upper corium and epidermis. The blister fluid contained plasma protein and neutrophils. Findings in other organs include mesenteric lymphadenitis, focal hepatic necrosis, follicular splenitis, acute myocarditis, and hemorrhagic encephalitis.

Immunologic Events

Specific Antibody. A specific serum antibody response usually occurs after infection with *M. pneumoniae*, and it can be measured by many different serologic techniques: immunofluorescence, complement fixation, indirect hemagglutination, precipitation, growth inhibition, mycoplasmacidal antibody test, ELISA, radioimmunoassay, adherence inhibition assay, and radioimmunoprecipitation test.*

Complement-fixing antibodies occur early in *M. pneumoniae* disease, reach a peak titer in approximately 1 month, and then decline slowly over a variable period. Fluorescent-staining antibodies and antibody determined by ELISA have temporal patterns similar to those of complement-fixing antibodies. Growth-inhibiting antibodies appear later (i.e., 2 to 3 weeks after the onset of illness), peak later, and persist longer than do complement-fixing antibodies. The initial serum immune response includes specific IgM, IgG, and IgA antibodies. After clinical illness and convalescence, specific antibody is located mainly in the IgG serum fraction. Occasionally, significant levels of IgM antibody persist for several months or years after infection.[70,115,267,472] Antibody titer responses in infected children are generally of a lesser

*References 2, 56, 63, 80, 226, 303, 434, 484, 519, 553, 600, 711, 712, 725, 726, 725, 726, 811, 907, 909, 941, 973.

†References 241, 434, 476, 519, 553, 600, 616, 673, 774, 962, 1072.

*References 111, 114, 119, 247, 293, 398, 428, 449, 579, 590, 798, 862, 959.

magnitude than those in adults.[248] Asymptomatic infections in children may not be associated with a measurable serum antibody response. *M. pneumoniae*–specific IgE antibodies have been detected in the sera of patients with asthma, atopic dermatitis, or both conditions.[972]

After infection, specific antibody is also present in nasal secretions and sputum.[69,113] In volunteer studies, Brunner and colleagues[113] noted that 42 and 73 percent of subjects had IgA nasal and sputum responses, respectively. Biberfeld and Sterner[74] found specific antibody in 44/55 sputum specimens from patients with *M. pneumoniae* infection of the lower respiratory tract. They noted IgA antibody in all specimens tested, IgG antibody in 24/31 specimens, and IgM antibody in 13/27 specimens.

Specific Cell-Mediated Immunity. Fernald and coworkers[289,290,291] demonstrated that lymphocytes from adults previously infected with *M. pneumoniae* undergo blast transformation when cultured in vitro in the presence of *M. pneumoniae* organisms. In age-related studies, researchers noted that only one of nine children younger than 4 years of age with documented previous infection had evidence of specific cell-mediated immunity as measured by lymphocyte stimulation.[294] In contrast, 7/12 children older than 4 years and 87 percent of an adult group demonstrated specific lymphocyte stimulation. This study suggests that specific cell-mediated immunity increases as a function of age and depends on repeated infection. Koh and colleagues[517] noted high levels of interleukin (IL)-4 and a high IL-4/interferon (IFN)-γ ratio in the bronchoalveolar lavage fluid of children with *M. pneumoniae* pneumonia, thus suggesting a predominant T_H2-like cytokine response.

Martin and colleagues[635] found that leukocytes from volunteers with *M. pneumoniae* infection demonstrated chemotaxis in the presence of the organism, whereas leukocytes collected before infection did not. Patients infected with *M. pneumoniae* also respond with IFN-γ in their blood and nasopharyngeal secretions early in infection and with IFN-γ during convalescence.[717,718]

Nonspecific Responses. Antibodies to several diverse antigens develop during human infection with *M. pneumoniae*. The best known of these antibodies are cold agglutinins, and they are useful in the diagnosis of *M. pneumoniae* pneumonia.[203,284,398,457,902] Cold agglutinins are directed against the I antigen of erythrocytes.[454,583,902] Most pneumonias in which serum cold agglutinins are noted are caused by *M. pneumoniae*. Cold agglutinins are detected in the serum of approximately 75 percent of patients with *M. pneumoniae* pneumonia; they are less common in *M. pneumoniae* infection without pneumonia.[145]

Patients with *M. pneumoniae* also frequently develop antibodies to the MG strain of nonhemolytic streptococci[581] and occasionally to *M. genitalium*,[585] *M. hominis*,[849] *M. salivarium*, *Mycoplasma hyorhinis*, *Mycoplasma orale*, *Mycoplasma pulmonis*, and *Mycoplasma mycoides* variety mycoides, the etiologic agent of contagious pleuropneumonia of cattle.[568] Cross-reacting antibodies to filamentous hemagglutinin and pertactin of *B. pertussis* also occur.[999] Other heterologous antibodies found in the serum of patients with *M. pneumoniae* infection include those to smooth muscle, the mitotic spindle apparatus, brain, lung, liver, and Wasserman (WR) cardiolipin antigen.[69,75,584] In addition to these findings, Biberfeld and Norberg[72] detected immune complexes by the platelet aggregation technique in the sera of 16/39 patients with acute respiratory illness caused by *M. pneumoniae*. Mizutani[682] demonstrated the presence of circulating immune complexes in most patients with pneumonia caused by *M. pneumoniae*. The same investigator also detected rheumatoid factor in the sera of patients with *M. pneumoniae* disease.[683]

Possible Mechanisms of Disease Production

Numerous studies have been performed in an attempt to understand the pathogenesis of respiratory disease caused by mycoplasmas.* Of particular interest in human *M. pneumoniae* infection is the apparent high prevalence of infection in infants, children, adolescents, and young adults, but the frequently mild nature of disease in infants and young children in comparison with older patients. Some studies suggest that the more severe disease in older patients is associated with reinfection and is mediated somewhat by immunologic responses.

Organ culture and animal studies indicate that damage at the site of primary infection—the respiratory epithelium—is the result of close organism–cell attachment.† This attachment of organism to cell uses neuraminic acid receptors on the cell.[908] In an organ culture system with *M. mycoides* variety capri, ciliary damage was decreased when the cellular receptor sites were treated with receptor-destroying enzyme.[162] Lipman and associates[587,588] noted that one attenuated *M. pneumoniae* strain had lost its ability to cytadsorb. The close association of organism and cell allows the transport of specific damaging material to the cell. Although the precise nature of this substance is not known, the data available suggest that it might be hydrogen peroxide. It is liberated by *M. pneumoniae* in vitro; in organ culture studies, hydrogen peroxide has been shown to be the damaging factor in another *Mycoplasma* infection.[166,588,589] In cell culture, *M. pneumoniae* inhibits host–cell catalase activity.[19] This inhibition of catalase activity enhances the toxicity of the hydrogen peroxide generated by the microorganism. *M. pneumoniae* also enters host cells and persists intracellularly for at least 7 days.[53]

Damage to ciliated respiratory epithelial cells also could be caused by CARDS toxin.[482,662] Another possible important component of *M. pneumoniae* pathogenicity is the induction of proinflammatory and other cytokines during infection.[1057]

In addition to direct damage from inflammatory cytokines and bacterial cytotoxins, additional mechanisms have been proposed in the development of extrapulmonary findings in *M. pneumoniae* infection.[722,1009] Autoantibodies are often detected in these cases, most likely as a result of cross reactivity between host cell proteins and

*References 114, 162-164, 166, 185-187, 193, 195-198.
†References 166, 196, 198, 424, 587, 588, 708, 795, 985.

Mycoplasma membrane antigens. Autoimmune mechanisms have been linked to findings as varied as arthritis, coagulopathies, and encephalitis in *M. pneumoniae* infection. Another mechanism of pathology commonly seen is vascular occlusion, caused by either direct inflammation of the vessels or various coagulopathies associated with *M. pneumoniae* infection. This led to associated findings such as stroke, pulmonary emboli, and coronary artery disease. Although not directly proved, the ability of *Mycoplasma* to bind to erythrocytes and disseminate through the bloodstream may contribute to the high frequency of extrapulmonary findings seen with these infections.[730]

Fernald and associates[292] noted that specific cell-mediated immunity to *M. pneumoniae* as measured by lymphocyte transformation becomes more prevalent with increasing age, as does specific antibody. Their studies suggest that more than one exposure to antigen may be necessary to elicit both humoral and cellular responses. Fernald and Glezen,[292] in an inactivated *M. pneumoniae* vaccine trial in children, noted that lymphocyte sensitivity developed in many recipients but not a humoral antibody response. In a trial of an inactivated vaccine in adults, Smith and associates[900] found that on challenge infection, an exaggerated illness occurred in vaccinees who failed to form humoral antibodies after immunization. These findings have led to the consideration that persistent specific cell-mediated responsiveness might contribute to the pulmonary process in *M. pneumoniae* infection. However, the incubation period of illness in adults and children is similar, which argues against the sensitization theory.[182]

Foy and associates[311] noted that complement-fixing antibodies remained elevated for 2 to 9 years after infection with pneumonia but fell quickly after the second year in persons with mild illness. Protection against reinfection was better in those who previously had pneumonia than in those with mild symptoms.

Clinical Manifestations

Pneumonia

Pneumonia is the most important clinical manifestation of *M. pneumoniae* infection, and this agent is responsible for 10 to 20 percent of all cases of pneumonia.* The highest incidence of pneumonia caused by *M. pneumoniae* in Seattle occurred in children 5 to 14 years of age.[314] In a study in Chiba Prefecture, Japan, researchers noted that the peak age of lower respiratory tract illness caused by *M. pneumoniae* was 4 years.[713] In a prospective study of community-acquired pneumonia in Finland, 11 percent of the children with *M. pneumoniae* infection were younger than 5 years of age, 32 percent were between 5 and 9 years of age, and 57 percent were 10 to 14 years of age.[402] Although researchers frequently state that *M. pneumoniae* pneumonia rarely occurs in children younger than 5 years of age, in actuality, the incidence in this group was found to be about twice that noted in young adults in Seattle.[314]

*References 189, 231, 317, 318, 324, 402, 421, 591, 755, 229, 657, 672, 736, 766, 976, 1063, 1064.

Pneumonia caused by *M. pneumoniae* occurs less commonly in children younger than 2 years of age and rarely in infants younger than 6 months of age. The apparent frequency of *M. pneumoniae* pneumonia is influenced by the relative occurrence of pneumonia caused by other pathogens. During the child's first 5 years of life, *M. pneumoniae* is only one of many agents (e.g., respiratory syncytial virus, adenoviruses, parainfluenza viruses, influenza viruses, *Streptococcus pneumoniae*, and *Haemophilus influenzae*) that cause pneumonia. During later childhood and adolescence, pneumonia as a consequence of infection with these other agents is a rare event; therefore *M. pneumoniae* is the leading cause of pneumonia in these persons.

Because isolation rates of *M. pneumoniae* during both endemic and epidemic periods do not vary greatly by season, as do those of common respiratory viruses, the proportion of patients with *M. pneumoniae* pneumonia increases during the summer months.

Symptoms and Signs. Since 1961, a large number of studies have reported the frequency of signs and symptoms in *M. pneumoniae* infection.* Unfortunately, many studies have included only special populations such as the military and, with few exceptions, community investigations have failed to indicate differences by age. In only a handful of investigations have data regarding children been itemized separately.[107,229,320,931,1064] Table 195-2 presents the relative frequency of symptoms and signs as compiled from eight studies in which both children and adults were included. The hallmark of pneumonia caused by *M. pneumoniae* is fever and cough. The onset of illness usually cannot be demarcated clearly, but malaise, fever, and headache are early complaints. Cough has an onset 3 to 5 days after the beginning of illness and is initially nonproductive. Foy and colleagues[317] and Biberfeld and coworkers[77] noted that 77 and 100 percent, respectively, of the patients that they studied had maximal temperatures greater than 38.9° C (102° F). Copps and associates[200] found that 58 percent of the group that they evaluated had temperatures higher than 39.4° C (103° F) and 4 percent had temperatures higher than 40.6° C (105° F).

The reporting of headache in association with *M. pneumoniae* pneumonia has varied considerably. Nakao and associates[716] noted this report in only 8 percent of subjects, whereas Biberfeld and associates[77] and Foy and colleagues[317] reported it in two thirds of those studied. Chills and the production of sputum are present in about 50 percent of ill patients. Again, great differences among investigations are noted, and they probably are related to the relative ages of the patients.

Coryza is unusual in *M. pneumoniae* pneumonia; therefore, its occurrence should suggest another etiologic agent for illness in a specific patient. In a study involving

*References 2, 8-11, 13, 25, 27, 30, 33, 37, 39, 56, 62, 63, 73, 76, 80, 110, 124, 176, 188, 192, 225, 229, 241, 249, 277, 285, 295, 302, 324, 355, 410, 411, 421, 445, 484, 493, 504, 509, 515, 520, 522, 523, 527, 537, 551, 565, 571, 586, 606, 636, 674, 698, 703, 723, 735, 740, 742, 746, 750, 762, 772, 792, 828, 837, 860, 883, 897, 909, 922, 926, 931, 949, 960, 965, 973, 975, 985, 1005, 1031, 1040, 1064.

TABLE 195-2 **Frequency of Clinical Findings in Children and Adults with *Mycoplasma Pneumoniae* Pneumonia**

Finding	Frequency
Symptoms	
Fever	++++
Cough	++++
Malaise	+++
Headache	++
Sputum	++
Chills	++
Hoarseness	+
Earache	+
Coryza	+
Sore throat	+
Diarrhea	+
Nausea and/or vomiting	+
Chest pain	+
Signs	
Rales	+++
Pharyngitis	++
Lymphadenopathy	+
Conjunctivitis	±
Rash	±
Otitis media	±

Compiled from eight studies in which both children and adults were included: references 72, 74, 200, 317, 324, 458, 625, 716.
++++, close to 100%; +++, 75%; ++, 50%; +, 25 percent; ±, 0% to 10%.

children exclusively, Stevens and colleagues[931] noted that coryza occurred more commonly in young children; as might be expected, they found productive cough more frequently in their older patients. Hoarseness, earache, sore throat, gastrointestinal complaints, and chest pain occur in approximately 25 percent of patients.

On physical examination, approximately 75 percent of patients have auscultatory evidence of pneumonia and about half have pharyngitis. Remarkable lymphadenopathy, particularly with cervical involvement, is noted in approximately 25 percent of patients. Twenty-one percent of the patients studied by Foy and associates[317] had otitis media. In other studies, this manifestation was noted in about 5 to 10 percent of cases. Conjunctivitis was reported in almost half the patients reported by Fransen and associates.[323] In contrast, except for Jansson and colleagues,[458] who noted conjunctivitis in 3 percent of their study group, this finding was not mentioned in the other reports. Similarly, rash was reported in 6, 11, and 17 percent, respectively, in studies in Minnesota, Wisconsin, and Seattle,[200,318,625] but it was not mentioned in the other studies.

The most common finding on chest auscultation is dry rales, but musical rales with expiration are noted occasionally. Rales usually persist for 2 weeks, and hearing them a month or more after the onset of disease is not unusual. Occasionally, patients have no auscultatory evidence of pulmonary disease throughout their illness, in spite of the presence of abnormalities on chest radiographs. During illness, cough becomes increasingly prominent; initially it is nonproductive, but later it may produce a frothy white sputum in older children and adolescents. The sputum also may appear purulent and contain blood. Cough persists for 3 to 4 weeks and longer after nonrespiratory symptoms such as fever and headache have subsided.

In a study of 44 children with lower respiratory tract illness caused by *M. pneumoniae*, Stevens and associates[931] noted the following frequencies of symptoms and signs: cough, 97 percent; malaise, 82 percent; vomiting, 40 percent; abdominal pain, 35 percent; headache, 32 percent; rash, 20 percent; fever higher than 38° C (>100.4° F), 78 percent; rales, 78 percent; pharyngitis, 32 percent; rhonchi, 30 percent; bronchial breathing, 27 percent; and otitis media, 27 percent. Foy and coworkers[320] found that chills and productive cough were more common in adults than children and that temperatures tended to be higher in children.

In a large study involving 108 children with *M. pneumoniae* infection, wheezing occurred with the acute illness in 40 percent.[834] When the children in this study were evaluated 3 years after their acute illness, they were found to have three indicators of lung function that had mean values significantly lower than those in control children.

Few reports specifically describe pneumonia caused by *M. pneumoniae* in young children.* However, a review of the case descriptions available indicates that illness, when it occurs, can be severe and relatively prolonged in contrast to common viral and bacterial infections. Singer and DeVoe[896] reported a 3-year-old severely ill child who had a temperature of 39.4° C (102.9° F), a pulse of 150 beats/min, and a respiratory rate of 40 breaths/min. Diffuse pulmonary involvement of the right upper lobe and lingular segments of the left lower lobe was observed by radiography, although rales and altered breath sounds could not be heard. The patient's condition worsened over a 6-day period. At this time, specific therapy with erythromycin was instituted, and slow recovery followed. The child had a normal white blood cell count, transiently elevated serum aspartate aminotransferase and alanine aminotransferase values, and microscopic hematuria. Grix and Giammona[374] observed two 5-year-old children with extensive pneumonia, pleural effusions, and febrile periods of 10 and 16 days. Stevens and associates[931] reported a 5-year-old boy with pulmonary consolidation and aseptic meningitis, and Clyde and Denny[190] described an asymptomatic 3-year-old boy with a "feathery infiltrate" in the right upper lung field. In a family study, Foy and colleagues[307] investigated four children younger than 6 years. In a 4-year-old child, the pneumonia persisted for more than 1 month, and in this child's brother, the illness lasted approximately 2 weeks. I have seen a 4½-year-old girl with scattered infiltrates throughout both lung fields and febrile illness of 14 days' duration.[159]

*References 39, 158, 190, 229, 307, 374, 713, 896, 931, 1064.

Severe and extensive pulmonary disease occurs occasionally in *M. pneumoniae* infection.* Massive lobar pneumonia is noted on occasion, and pleural effusions are fairly common findings.† A number of cases of necrotizing pneumonitis with massive pleural effusions have been described.[50,172,1027] All of these children had protracted periods of fever and respiratory distress. Acute respiratory distress syndrome has been observed, and illness has suggested pulmonary embolism with infarction.‡ Chronic intestinal pulmonary fibrosis and fulminant fatal diffuse interstitial fibrosis has been noted in two adults with *M. pneumoniae* pneumonia, and localized bronchiectasis developed in a 20-year-old man at the site of previous acute lung infection.[171,359,478,942] Children who had a delay in onset or inadequate duration of macrolide treatment were found by Marc and associates[630a] to have reduced lung diffusion capacity after contracting *M. pneumoniae* pneumonia. Barreira and associates[50] describe a previously health 8-year-old boy who developed necrotizing pneumonitis, septic shock, and meningoencephalitis from *M. pneumoniae*. A 5-year-old girl had severe necrotizing pneumonitis, and an adult had bronchiolitis obliterans–organizing pneumonia.[592,750] *M. pneumoniae* pneumonia is generally more severe in patients with preexisting cardiorespiratory problems, immunodeficiencies, and sickle-cell disease.§

Five patients—two children, one adolescent, and two adults—were found to have lung abscesses in association with *M. pneumoniae* infection.[171,570,576,891] The illness in the adolescents and adults was characterized by productive cough and chest pain for 2 to 4 weeks. In one patient, clinical recovery and clearance of the pulmonary lesion were dramatic with tetracycline therapy; two other patients received suboptimal therapy but eventually recovered. One of the child cases was a 6-year-old girl who had a 7-day history of cough and a 4-day history of fever.[171] In addition to the abscess, she had pleural effusion, thrombocytopenia, and disseminated intravascular coagulation. The other child was a 10-year-old boy with fever and cough for 15 days refractory to treatment with amoxicillin and ceftriaxone, but with rapid improvement on clarithromycin.[570] One 18-year-old man with extensive consolidation of the right lower lung field had residual pleural scarring 8 months after the acute illness.[701]

Newborns who contracted congenital pneumonia, probably via vertical transmission of *M. pneumoniae*, have been described.[916,989]

Clyde[189] reported factors that correlated with *M. pneumoniae* pneumonia in a study of 1139 subjects with community-acquired pneumonia. Positive factors were sore throat, headache, fever of 38.9° C (102° F) or higher, exanthem, family size of four or more, and ear infection. In the same study, pneumonia did not correlate with coryza, leukocytosis (15×10^9/L or more and 10×10^9/L or more), preexisting disease, recurrent pneumonia, hospitalization for treatment, or cigarette smoking.

Although recovery from *M. pneumoniae* pneumonia is usually complete, two studies suggest that persistent abnormalities in lung function can occur after illness.[689,834]

Radiography. Because the classic clinical entity primary atypical pneumonia has numerous causes but often is used as a synonym for *M. pneumoniae* pneumonia, much confusion has ensued about the spectrum of the radiographic appearance of the specific mycoplasmal infection. The radiographic pattern of primary atypical pneumonia is varied, but bilateral, diffuse, reticular infiltrates are common components.[599,863,899] Subsequent study has indicated that the diffuse interstitial pattern is an uncommon finding in *M. pneumoniae* infection and more often is the result of infection with other agents such as viruses, fungi, and *Chlamydia*.[106,341,727,800,983]

Brolin and Wernstedt[106] carefully evaluated the radiographic findings in 56 patients with significant *M. pneumoniae* pneumonia; 21 of the patients were younger than 20 years. They noted the following distribution of different patterns: typical lobar pneumonia, 8 patients; predominantly alveolar but not total consolidation, 13; interstitial (either reticular or noduloreticular), 20; a combination of lobar pneumonia and other alveolar involvement without total consolidation, 2; a combination of lobar involvement and interstitial, 10; and a combination of alveolar involvement without total consolidation and interstitial, 3. Alveolar patterns were more common in females; interstitial involvement was found more frequently in males. Twenty-two percent of patients had enlargement of the hilar or paratracheal lymph nodes, and 14 percent had pleural effusion.

The persistence of radiographic changes is variable. Brolin and Wernstedt[106] noted that 13 percent of their patients who underwent follow-up studies had abnormal findings more than 4 weeks after the initial study. They observed that persistence tended to be longer in patients with alveolar disease than in those with interstitial patterns. The degree of clinical symptoms and pulmonary physical findings frequently correlates poorly with the apparent degree of involvement noted on radiographs. In many patients with significant symptoms, only minimal interstitial changes are observed. In other instances, patients with lobar pneumonia often have few clinical findings indicating pulmonary disease.

Lee and colleagues[558] reported the chest computed tomography (CT) features in 11 children and 5 adults with *M. pneumoniae* pneumonia. The children had the following findings: lobar or segmental consolidation, 100 percent; pleural effusion, 82 percent; lymphadenopathy, 82 percent; and volume decrease of the involved lobe, 73 percent. None of the children had diffuse nodules of ground-glass attenuation, septal thickening, or bronchial wall thickening. In contrast with the children, the adults had diffuse nodules of ground-glass attenuation, 80 percent; septal thickening, 40 percent; lymphadenopathy, 40 percent; bronchial wall thickening, 40 percent; and volume decrease of the involved lobe, 20 percent. None of the adults had lobar or segmental consolidation and pleural effusion. In a retrospective analysis of 64 adult

*References 50, 160, 167, 171, 172, 180, 199, 298, 301, 311, 374, 379, 437, 489, 576, 592, 618, 669, 700, 715, 744, 750, 774, 805, 817, 887, 891, 893, 896, 911, 920, 997, 1027.
†References 40, 167, 169, 172, 191, 228, 298, 301, 374, 596, 600, 701, 712, 715, 744, 896, 920, 996, 1027.
‡References 50, 153, 301, 805, 893, 991, 992.
§References 64, 180, 322, 337, 461, 669, 785, 887, 911.

cases of *M. pneumoniae* pneumonia in Japan, the most common CT findings were bronchial wall thickening (81%), followed by centrilobular nodules (78%), ground-glass attenuation (78%), and consolidation (61%).[681]

Kim and associates[505] performed high-resolution CT on 38 children who had been hospitalized with *M. pneumoniae* pneumonia 1 to 2.2 years previously. Abnormalities in two or more lobes, which corresponded to the initial chest radiographic infiltrates, were found in 37 percent of the children. Young age (<8 years) at the time of hospitalization and high *M. pneumoniae* antibody titer were found to be risk factors for the subsequent abnormalities.

Nonspecific Laboratory Data. The total leukocyte count in patients with pneumonia caused by *M. pneumoniae* is most often normal, but variation is considerable.* In a group of more than 250 children younger than 15 years, Foy and associates[320] noted that 30 percent and 6 percent had total leukocyte counts greater than 10,000 and 15,000 cells/mm^3, respectively. In a group of 45 children, Stevens and colleagues[931] observed leukocytosis in 33 percent of patients and leukopenia in one patient. Sixty-seven percent of the children had neutrophilia, and one patient had neutropenia. An increased percentage of band form neutrophils in *M. pneumoniae* pneumonia is an unusual finding.

The erythrocyte sedimentation rate is elevated in all cases,[76,200,457] and this elevation is usually marked. Biberfeld and colleagues[76] noted that 16/37 patients had erythrocyte sedimentation rates of 50 mm/hr. Serologic tests for syphilis are found to be falsely positive on occasion, and serum cold agglutinins and antibodies to *Streptococcus* MG antigen are common findings.[70,144,149,231] Results of the direct Coombs test are frequently positive, and elevated levels of serum IgM are noted.[70,284] Urinalysis results are generally normal.

Respiratory Disease Other Than Pneumonia

Common Cold and Unspecified Upper Respiratory Illness. By strict definition (i.e., significant nasal symptoms, no pharyngitis, and minimal fever), *M. pneumoniae* rarely causes the common cold. However, mild upper respiratory tract illness is noted frequently as the only manifestation of *M. pneumoniae* infection in children, adolescents, and young adults.† The frequency of unspecified upper respiratory tract illness as a manifestation of *M. pneumoniae* infection, in contrast to other manifestations resulting from infection with this agent, varies considerably among studies. Feizi[285] studied patients from a country practice in England and noted that 50 percent of the patients had upper respiratory tract symptoms. Illness in these persons often was prolonged, however, and lasted as long as 7 to 10 weeks. In a review in Scotland, only 3 percent of 596 *M. pneumoniae* infections were classified as upper respiratory tract illness. In studies of common respiratory tract illnesses in children in which viruses and other agents were sought, *M. pneumoniae* was noted in 2 to 5 percent of patients with upper respiratory tract illness.[147,231,597]

Pharyngitis and Nasopharyngitis. As noted in Table 195-2, pharyngitis is observed in approximately half of all patients with *M. pneumoniae* pneumonia. However, pharyngitis as the major manifestation of *M. pneumoniae* infection occurs less commonly. Parrott[776] found that 12 percent of children admitted to the hospital with severe "bronchitis-pharyngitis" had *M. pneumoniae* infection. Jensen and associates[466] noted the frequent occurrence of pharyngitis and otitis media in children infected with *M. pneumoniae*. In a study of 715 children and adolescents with pharyngitis, Glezen and colleagues[352] reported that 36.8 percent had group A streptococcal infection and 3.1 percent were infected with *M. pneumoniae*. When the *M. pneumoniae* infections were grouped by age, the peak (11.4%) occurred in the 12- to 14-year-old group and none were observed in children younger than 6 years. Five patients with *M. pneumoniae* infection had concomitant group A streptococcal infection, but the illnesses in these cases could not be distinguished clinically from those caused by either agent alone. Cervical lymphadenopathy occurred in approximately 50 percent of those infected, and the pharyngeal lesion was exudative in 43 percent. In a study of 127 children with acute pharyngitis, 25 (20%) were found to have serologic evidence of *M. pneumoniae* infection.[272] Seven (28%) of these children had pharyngeal exudates and 10 (40%) had cervical lymphadenopathy.

In a study involving 131 adult patients with pharyngitis, 10.6 percent were found to have serologic evidence of *M. pneumoniae* infection.[521]

Otitis Media and Bullous Hemorrhagic Myringitis. Although the incidence has varied across studies, otitis media is noted in approximately 5 percent of children and adolescents with *M. pneumoniae* pneumonia. The role of *M. pneumoniae* as an etiologic agent in common acute otitis media in children is unclear. Halsted and associates[386] noted that 12 percent of children with otitis media had serologic evidence of *M. pneumoniae* infection, but they were unable to recover the agent from middle ear fluid. In a study in which children were selected because of *M. pneumoniae* infection in a family member, 47 of 49 children with otitis media had *M. pneumoniae* infection.[466] Räty and Kleemola[808] used PCR to detect *M. pneumoniae* in 16/380 (4%) middle ear fluid samples from 138 children with acute otitis media.

In a volunteer study, myringitis developed in 13/52 subjects.[816] Findings were usually bilateral and associated with throbbing pain. The appearance of the tympanic membrane varied from mild injection to severe inflammation with edema. Hemorrhagic areas on the drum were noted in five subjects, and serous-appearing blebs containing blood were observed in two. Bullous myringitis also has been noted only occasionally with natural *M. pneumoniae* infection.* In one study of 148 children and adults with *M. pneumoniae* pneumonia, 27 (18%) were found to have bullous myringitis.[625] Using PCR, Kotikoski and associates[528] studied middle ear fluid from

*References 76, 200, 276, 317, 320, 324, 457, 686, 931, 983.

†References 147, 202, 231, 248, 282, 285, 286, 294, 307, 317, 417, 555, 597, 686, 716, 735, 838.

*References 107, 190, 307, 317, 625, 907.

30 children and blister fluid from 12 children with acute meningitis and found no evidence of *M. pneumoniae* DNA.

Sinusitis. Although clinically recognized sinusitis has been reported rarely in patients with *M. pneumoniae* infection, Griffin and Klein[372] found radiographic evidence of sinusitis in approximately two thirds of a group of Navy recruits with *M. pneumoniae* pneumonia. In general, the patients with sinusitis had more prolonged illness than did recruits without sinusitis. Savolainen and colleagues[853] noted that 11/310 patients with acute maxillary sinusitis had fourfold or greater rises in complement-fixing antibody to *M. pneumoniae*. In chronic suppurative maxillary sinusitis, cultures for *M. pneumoniae* have been performed but no organisms were isolated.[68,915]

Acute Bronchitis. Acute bronchitis characterized by fever, cough, and rhonchi, with or without associated pharyngitis, is a frequent manifestation of *M. pneumoniae* infection.* Of 40 patients with *M. pneumoniae* infection, Feizi[285] reported that 6 had bronchitis, 3 had upper respiratory tract illness plus bronchitis, and 1 had sinusitis plus bronchitis. In contrast to these findings, Hornsleth[418] noted that only 1/25 patients with *M. pneumoniae* infection had acute bronchitis. In the differential diagnosis of acute bronchitis, *M. pneumoniae* infection accounts for 10 to 20 percent of cases.[145,148,231,276,789]

Croup. *M. pneumoniae* infection has been associated only occasionally with croup. Parrott[776] found no instances of *M. pneumoniae* infection in a large number of children with croup, and Chanock and Parrott[145] did not list this agent as an etiologic consideration in croup. In contrast, extensive studies in both Seattle and Chapel Hill, North Carolina, have revealed that approximately 2 percent of croup cases are associated with *M. pneumoniae* infection.[231,312,353,596] Because no descriptions of clinical illness are available, a reasonable assumption is that croup caused by *M. pneumoniae* infection is generally mild and without distinguishing characteristics.

Bronchiolitis and Infectious Asthma. The presence of *M. pneumoniae* in the lower airways can trigger a proinflammatory cytokine response similar to that seen in asthmatic and allergic patients.[392,662,666] *M. pneumoniae* pneumonia itself can cause bronchiolitis and wheezing[40,834]; however, it is also commonly found in asthmatics and is a cause of their symptomatic exacerbations.[67,430,703,766,878] Approximately 5 percent of cases of bronchiolitis are caused by infection with *M. pneumoniae*, but the percentage varies across studies.† In two large studies, no instances of *M. pneumoniae*–associated bronchiolitis were reported.[417,418] Horn and associates[703] noted that *M. pneumoniae* was isolated from 6.6 percent of children with wheezy bronchitis, and Berkovich and associates[67] found *M. pneumoniae* infection in 7/33 episodes of wheezing in asthmatic children. Biscardi and coworkers[79] studied children 2 to 15 years of age hospitalized for severe asthma. Of 119 children with previously diagnosed asthma, 24 (20%) had *M. pneumoniae* infections during the exacerbation that caused their hospital admission. In 51 children with first asthma episodes, *M. pneumoniae* infection was demonstrated in 26 (50%). Children with a first attack of asthma associated with *M. pneumonia* infection were significantly more likely to have asthma recurrences than children whose primary episodes were not related to *M. pneumoniae* infection. Lehtinen and colleagues[564] studied 220 children with wheezing associated with viral infection and found that 5 percent were coinfected with *M. pneumoniae*. Freymuth and colleagues[327] identified *M. pneumoniae* in nasal aspirate samples by PCR in 3/132 children (2%) with acute exacerbations of asthma.

Although a significant number of asthma exacerbations can be linked to *M. pneumoniae* infection, there is still conflicting evidence on the benefit of prophylactic or empirical antibiotic therapy in asthmatics.[525] Macrolides are sometimes advocated not only as an antibiotic but also for their anti-inflammatory properties.[357]

Other. Exacerbations of chronic obstructive pulmonary disease have been associated with *M. pneumoniae* infection.[168,545,661,903,1041] By culture, Cherry and associates[168] did not obtain any *M. pneumoniae* isolates from the bronchial specimens of adults with chronic bronchitis. However, more recently, Kraft and colleagues,[531] using PCR, detected *M. pneumoniae* in the bronchoalveolar lavage or bronchial biopsy specimens of 9/18 adults with chronic asthma. Smith and associates[902] were unable to show that patients with chronic obstructive pulmonary disease had increased susceptibility to infection in contrast to normal subjects. Illness suggestive of pertussis has been described in three children.[547,931] Teig and associates[961] studied nasal brush specimens and induced sputum from 38 children with stable chronic lung disease and 42 healthy controls for the presence of *M. pneumoniae* DNA by PCR. Specimens from 4 (10.5%) of the children with chronic lung disease had *M. pneumoniae* DNA, whereas none of the specimens from control patients were positive. In two provocative studies, Esposito and coworkers[273,274] found that *M. pneumoniae* infections may play a role in recurrent respiratory infections in children. In their studies, they found that children with tonsillopharyngitis or other respiratory infections caused by *M. pneumonia* who were treated with azithromycin 10 mg/kg/day for 3 days weekly for 3 weeks were significantly less likely to have recurrent respiratory tract infections than children with similar *M. pneumoniae* infections who did not receive azithromycin treatment.

Exanthem and Enanthem

Exanthem as a manifestation of *M. pneumoniae* infection is a common occurrence, but its incidence has varied considerably among different studies.* In large studies involving children in which *M. pneumoniae* infection was evaluated in a specific geographic area, the incidence of exanthem has varied from 3 to 33 percent.† Foy and

*References 145, 147, 148, 275, 285, 417, 418, 716, 735, 776, 838.
†References 145, 147, 148, 231, 248, 312, 353, 596, 776.

*References 12, 125, 158, 167, 180, 199, 200, 276, 286, 304, 307, 310, 315, 317, 358, 367, 379, 417, 457, 488, 547, 552, 556, 604, 607, 612, 625, 715, 716, 735, 810, 830, 847, 856, 922, 931, 935, 962, 983.
†References 200, 286, 307, 317, 417, 457, 547, 612, 625, 716, 735, 922, 931.

associates[317] noted rash in 17 percent of 319 patients with *M. pneumoniae* pneumonia during a 5-year surveillance period. In a study involving only children, Stevens and colleagues[931] noted exanthem in 9 percent of their patients. Copps and coworkers,[200] in a community outbreak in La Crosse, Wisconsin, found that 11 percent of their patients with pneumonia also had rash.

The cutaneous manifestations in *M. pneumoniae* infection are protean. Most common is an erythematous maculopapular rash that is most prominent on the trunk and back; the lesions may be discrete (rubelliform) or confluent (morbilliform). Although not the most common cutaneous manifestations of *M. pneumoniae* infection, erythema multiforme and Stevens-Johnson syndrome are the most often reported and the most serious.* In Table 195-3, the clinical findings in 29 well-documented cases of *M. pneumoniae* infection with exanthem are presented. Of the total group, all but 8 were males, 24/29 were younger than 20 years, and 12/20 were younger than 11 years. The duration of exanthem was longer than 7 days in all but 2 patients; all patients were febrile, and in 17 cases, the rash occurred during fever.

Enanthems are also common with *M. pneumoniae* and have been given their own name—*Mycoplasma pneumoniae*–associated mucositis (MPAM).[852] Oral lesions appear most frequently, followed by ocular and urogenital findings. Because cutaneous involvement is not always concurrently seen, some also to refer to this syndrome as an "atypical Stevens-Johnson" or "Fuchs syndrome."[654,857,668,806] In Table 195-4 the specific mucocutaneous findings in 20/29 patients are itemized. Of these, 14 patients had generalized ulcerative stomatitis and 7 had tonsillitis or pharyngitis. Severe conjunctivitis was observed in 8 patients, and this manifestation was seen only in those with vesicular or bullous cutaneous lesions. All 8 patients with severe conjunctivitis also had generalized ulcerative stomatitis. Surprisingly, vesicular or bullous exanthems with oral and eye lesions (i.e., Stevens-Johnson syndrome) rarely occur in females.[40,641,931] Mucocutaneous findings also are present in vasculitic disorders such as Kawasaki disease and Behçet syndrome, and both of these have also been associated with *Mycoplasma* infection.[258,560,1002,1074] As noted in Table 195-3, 25/29 patients had pneumonia. The occurrence of rash as the major manifestation of *M. pneumoniae* infection is probably rare. In a study of 112 patients who had suspected infectious exanthems without pneumonia, Cherry and associates[166] could find none with *M. pneumoniae* infection. Foy and colleagues[307] noted a 2-year-old child with only rash, Stutman[935] reported a 15-year-old boy with Stevens-Johnson syndrome but not pneumonia, from whom the organism was recovered from a vesicular lesion. Ruhrmann and Holthusen[830] observed frequent cases of mild erythema multiforme without pneumonia.

In an analysis of 42 cases of erythema multiforme seen during a 20-year period, Villiger and associates[998] noted a presumptive or definite diagnosis of *M. pneumoniae* infection in 14 children. Nine of the children were boys (64%), and eight of the illnesses had mucous membrane involvement.

Many patients with *M. pneumoniae* infection and exanthem have a history of antibiotic administration before development of the rash; this observation suggests the possibility that the rash is drug induced rather than a

*References 40, 125, 167, 199, 285, 286, 310, 479, 488, 540, 604, 607, 627, 641, 735, 810, 812, 847, 858, 865, 890, 931, 935, 983, 998.

TABLE 195-3 **Selected Clinical Findings in 29 Patients with *Mycoplasma Pneumoniae* Infection and Exanthem**

Clinical Findings	No. Patients
Predominant Components of Exanthem	
Erythematous macular	4
Erythematous maculopapular	14
Vesicular	14
Bullous	6
Petechial	1
Urticarial	2
Discrete lesions	11
Confluent lesions	7
Pruritic	6
Predominant Distribution of Exanthem	
Hands	9
Arms	20
Feet	8
Legs	19
Trunk	19
Face	11
Buttocks	9
Genitals	8
Duration of Exanthem (Days)	
<7	2
7-14	11
>14	10
Time of Onset of Exanthem	
Before fever	2
With fever	4
During fever	17
After fever	1
Antibiotics Administered Before Exanthem	
Yes	17
No	10
Enanthem	
Generalized ulcerative stomatitis	14
Tonsillitis or pharyngitis	7
Conjunctivitis	
Severe	8
Mild	3
Pneumonia	
Yes	25
No	4

From Cherry JD. Anemia and mucocutaneous lesions due to Mycoplasma pneumoniae *infections.* Clin Infect Dis *1993;**17**(suppl 1):47-51.*

TABLE 195-4 **Mucocutaneous Findings in 20 Patients with Mycoplasma Pneumoniae Infections and Exanthem**

Case	Reference	Age (yr)	Sex	Distinguishing Characteristic of Exanthem	Generalized Ulcerative Stomatitis	Conjunctivitis
1	167	16	M	Fiery-red confluent maculopapular	0	0
2	167	17	F	Blotchy erythematous	0	0
3	158	4.5	F	Morbilliform	0	0
4	303	9	M	Erythematous maculopapular	+	0
5	303	8	M	Papulovesicular; "target" appearance	+	0
6	314	14	M	Symmetric macular and bullous	+	+
7	488	19	M	Erythematous maculopapular, vesicles; "iris" lesions	+	0
8	547	10	F	Macular and petechial	0	0
9	552	16	M	Varicella-like	+	+
10	983	8	M	Macular	+	0
11	983	6	M	Diagnosed as measles	0	0
12	604	16	M	Scattered vesicular; generalized	+	+
13	604	6	M	Vesicular; generalized	+	0
14	715	7	M	Urticarial	0	0
15	715	9	M	Maculopapular	0	0
16	847	10	M	Vesiculobullous and maculopapular	+	+
17	962	27	M	Vesiculopustular to papular; "pityriasis-like"	0	0
18	309	10	M	Vesiculobullous; generalized	+	0
19	556	5	M	Maculopapular	0	0
20	612	11	F	Papular; most marked on hands and feet	0	0

Modified from Cherry JD, Hurwitz ES, Welliver RC. Mycoplasma pneumoniae *infections and exanthems. J Pediatr 1975;**87**:369-373.*

result of the infectious process. As noted in Table 195-3, 17 patients had received antibiotics before the rash appeared, and the exanthem was present before the administration of antibiotic therapy in 10. Although these data incriminate the infection as a cause of exanthem, the large number of cases in association with the administration of antibiotic raises the possibility that the antibiotic intensifies the dermosensitive potential of the infectious agent in a manner similar to that noted between Epstein-Barr virus and ampicillin in infectious mononucleosis. *M. pneumoniae* has been recovered from the blister fluid of two patients with erythema multiforme.[607]

Other unusual cutaneous manifestations include erythema nodosum, pityriasis rosea, varicella-like urticaria, papular purpuric gloves and socks syndrome, and Cockade purpura.*

Cardiac Manifestations

Cardiac involvement during *M. pneumoniae* infection generally is considered to be unusual.† However, studies of Pönkä[791] and Sands and associates[848] and survey data of Noah[742] and Assaad and Borecka[33] indicate that *M. pneumoniae* myocarditis and pericarditis are important causes of both morbidity and mortality. In a study of fatal viral and mycoplasmal infections, Assaad and Borecka[33] noted six cardiovascular deaths related to *M. pneumoniae* infection during a 9-year period. Noah[742] found that 1 percent of 700 patients with *M. pneumoniae* infection had cardiac manifestations as the main clinical feature. Pönkä[791] published findings of a 7-year study involving 560 patients with serologic evidence of *M. pneumoniae* infection. In this group, 69 patients with cardiac manifestations were detected; of these 69 patients, 25 had carditis for which no causal agent other than *M. pneumoniae* could be incriminated. Pönkä[791] also reviewed the world literature and found a total of 33 other cases of carditis.

Of 25 carefully studied patients, 17 had respiratory symptoms before the diagnosis of carditis and 10 had radiologically confirmed pneumonia. All but four patients had fever. Of the 25 patients, 2 were younger than 10 years and 2 were in the 10- to 19-year-old age group. Of the 33 cases in the literature, 7 were 20 years or younger. Of this survey group, 25/33 had respiratory illness and in 19 it was recorded as pneumonia.

Of the 25 patients in Finland reviewed by Pönkä, 6 had pericarditis and the remainder had perimyocarditis. Antibiotic therapy in 11 patients did not appear to shorten the duration of illness or diminish the number of cardiac sequelae. At a 16-month follow-up, 11 patients had persistent cardiac damage. Another interesting aspect of this study was the finding of rises in *M. pneumoniae* complement-fixing antibody titer in 5 adults with myocardial infarcts.

Chergui and associates[157] described a 45-year-old man with bilateral interstitial infiltrates and second-degree heart block. He was treated with erythromycin, and the heart block, fever, and respiratory distress cleared on hospital day 5.

Mycoplasma may also affect the heart through vasculitis, valvular disease, atherosclerosis, and coronary artery

*References 163, 250, 358, 367, 483, 552, 715, 781, 830, 867, 962, 1051.
†References 161, 199, 224, 283, 284, 297, 305, 396, 421, 502, 616, 819, 869, 854, 894, 931, 950.

disease, although there are conflicting data on the subject.[58,223,377,615]

Hematologic Manifestations

Severe hemolytic anemia has been reported on several occasions in patients with *M. pneumoniae* infection.* Most cases of *M. pneumoniae* hemolytic anemia have been associated with marked pulmonary involvement. Stevens-Johnson syndrome is a common associated finding, and myocarditis has been noted on two occasions.[199,283,285,502,931] In general, severity of illness correlates with high titers of cold agglutinins. One unusual case of a 2-year-old boy with severe *M. pneumoniae* IgM-mediated hemolytic anemia without pulmonary symptoms was described.[529]

The hemolysis may be severe and acute, often with a 50 percent reduction in hemoglobin concentration. In contrast to uncomplicated pulmonary disease, the leukocyte count in patients with hemolytic anemia frequently is elevated markedly with a predominance of neutrophils. Results of the direct Coombs test are usually positive. Clinical experience suggests that administration of steroids in conjunction with proper antibiotic therapy may be beneficial in this illness. Boccardi and associates[91] observed a 7-year-old boy with hemolytic anemia and transitory paroxysmal cold hemoglobinuria.

Feizi[284] has demonstrated that clinically inapparent compensated hemolysis frequently is associated with *M. pneumoniae* pulmonary infection. Fiala and associates[297] noted that bone marrow suppression also contributed to the anemia in a patient whom they studied, and pancytopenia has also rarely been described.[381] Ruhrmann and Holthusen[830] reported a hemorrhagic variant of erythema multiforme (Cockade purpura) similar to Henoch-Schönlein disease in *M. pneumoniae* infection; they also noted severe thrombocytopenia not associated with hemolytic anemia. Gill and Marrie[347] reported a 27-year-old man who had hemophagocytosis with an *M. pneumoniae* infection. Two children with hemophagocytic lymphohistiocytosis in association with *M. pneumoniae* infections have been reported.[442] Also noted has been a 4-year-old girl with neutropenia, thrombocytopenia, and acute hepatitis.[154] Severe *M. pneumoniae* infection with pneumonia, thrombocytopenia, and disseminated intravascular coagulation (DIC) has been described.[171,177] Hypercoagulability resulting from *M. pneumoniae* infection with evidence of autoimmune antibodies has manifested as venous thrombosis and pulmonary embolisms in several cases.[31,36,44,108,365] Aviner and colleagues[36] also describe eight cases of *M. pneumoniae* infection with associated thrombocytopenia not related to DIC.

Gastrointestinal Findings

Nonspecific Findings. Approximately 25 percent of patients with *M. pneumoniae* pneumonia have nausea, vomiting, diarrhea, or some combination thereof (see Table 195-2). Aside from these complaints, gastrointestinal problems in association with *M. pneumoniae* infection are rare events. Stevens and associates[931] found that 15 percent of a group of 44 children with infection had notable abdominal pain. Referred abdominal pain with pneumonia also has been observed.[285]

Liver Involvement. Liver involvement in *M. pneumoniae* infection is a surprisingly rare event. Levine and Lerner[574] reported that mild increases in transaminases occur and that acute and chronic active hepatitis has been noted with respiratory symptoms and proved *M. pneumoniae* infection, but they provided no further information. MacLean[612] reported a 13-year-old girl who initially had a sore throat and 9 days later had clinical and laboratory evidence of hepatitis. Murray and associates[706] presented a case report of an adult with typical *M. pneumoniae* pneumonia in whom liver function and enzyme studies indicated hepatitis. Helms and colleagues[403] noted that 6/17 patients with *M. pneumoniae* pneumonia had elevated aspartate aminotransferase values. Enzyme changes have been observed in other case evaluations,[576,625,896] some with coagulopathy,[142] and hepatic necrosis has occurred.[616,704]

Simonian and Janner[894] reported a 12-year-old boy who had pleural effusion, hepatitis, and hemolytic anemia. Narita and associates[726] noted two children with lymphadenopathy and liver dysfunction. These two patients are of interest because both had mycoplasmemia and neither had pneumonia. Elevated liver enzymes without respiratory symptoms also have been found in young adults with elevated *M. pneumoniae* serologies.[560,803,825]

Splenic Infarct. Two children (a 13-year-old girl and a 10-year-old boy) with pneumonia resulting from *M. pneumoniae* infection had splenic infarcts and transient antiphospholipid antibodies.[1049] A 24-year-old man with an elevated *M. pneumoniae* IgM titer developed hepatosplenomegally and splenic infact.[773]

Pancreatitis. In 1974, Märdh and Ursing[630] reported six patients with respiratory tract illnesses, serologic evidence of *M. pneumoniae* infection, and pancreatitis. In four patients, pancreatic symptoms began 1 to 2 weeks after the onset of respiratory tract illness; in the other two patients, the pancreatitis was subclinical. Diabetes developed in two patients, one of whom died. At postmortem examination, pneumonitis and pancreatitis were confirmed. Nakagawa[714] and colleagues report on an 8-year-old girl who developed necrotizing pancreatitis 10 days into her treatment for *M. pneumoniae* pneumonia. In a study of pancreatitis, Leinikki and Pantzar[566] noted that sera from 18/56 patients had rises in complement-fixing antibody titer to *M. pneumoniae*. Because none of the patients in this study had respiratory tract illness suggestive of *M. pneumoniae* infection, the investigators suggested that perhaps the antibody responses were not specific for *M. pneumoniae* infection but were caused by a cross-reacting infection or were the result of autoantigens from pancreatic damage. Leinikki and associates[567] have conducted further studies; their thought is that the antibody response is nonspecific, but their data neither confirm nor refute this assumption. In another study, Freeman and McMahon[325] also noted serologic evidence

*References 155, 157, 165, 279, 283, 331, 367, 496, 501, 502, 529, 546, 575, 598, 711, 741, 770, 941, 1051.

of *M. pneumoniae* infection in 33 percent of patients with pancreatitis. Oderda and Kraut[749] reported a 22-month-old girl with pancreatitis and a rise in complement-fixing antibody titer to *M. pneumoniae*. This child had no respiratory symptoms.

Arthritis

Mycoplasmas are a common cause of arthritis in animals other than human beings.[194,456] Likewise, *M. pneumoniae* infection is associated occasionally with joint manifestations.* In a review of 1259 patients with *M. pneumoniae* infection, Pönkä[790] noted transient arthritis in 0.9 percent. Two patients were found to have Reiter syndrome. Hernandez and colleagues[407] reported seven instances of arthritis in 38 persons with *M. pneumoniae* respiratory disease. In one patient, the illness lasted 18 months and was associated with the development of rheumatoid factor.

Twenty-one instances of illness suggestive of rheumatic fever have been described.† In all 21 patients, large joints were involved; 18 patients had joint swelling or effusion, whereas 3 had only pain. Most patients had a history of preceding respiratory illness with sore throat, and 10/21 had radiographic evidence of pneumonia. The sedimentation rate was elevated in all patients in whom the test was performed. One child had an erythema marginatum–like rash, as well as polyarthritis and fever.[693] Poggio and colleagues[788] reported a child with reactive arthritis 3 weeks after the onset of diarrhea and a respiratory tract infection. Leukocytoclastic vasculitis plus polyarthritis associated with *M. pneumoniae* infection has been described in a young adult.[784]

Muscular Disease

Berger and Wadowksy[66] reported a 15-year-old girl with right lower lobe pneumonia, hepatitis, and rhabdomyolysis associated with *M. pneumoniae* infection. Minami and associates[675] reported a 4-year-old boy with pneumonia and a rhabdomyolysis associated with a marked titer rise to *M. pneumoniae* using a passive agglutination assay. Oishi and associates[754] described a case of multidrug resistant *M. pneumoniae* causing rhabdomyolysis in a 7-year-old girl. Polymyositis has been noted in association with *M. pneumoniae* infection on six occasions.[783]

Neurologic Disease

Several large community and military studies of *M. pneumoniae* pneumonia and other respiratory tract illnesses are notable in that neurologic disease is not described.‡ However, other studies, particularly those performed more recently, indicate a surprising spectrum of neurologic illness associated with *M. pneumoniae* infection.§ The failure to find neurologic disease in the initial large studies mentioned was probably due not to its absence but to the orientation of the investigators; neurologic disease was not studied under the respiratory investigation protocols. In three large studies of *M. pneumoniae* illness involving 1856 cases, 2.6 to 4.8 percent had neurologic illness.[735,742,793] Assaad and Borecka[33] noted five fatal *M. pneumoniae* infections in which central nervous system (CNS) findings were the major clinical manifestations.

In 1973, Lerer and Kalavsky[573] reported 5 cases of neurologic disease associated with *M. pneumoniae* infection and analyzed 45 cases from the literature. They noted the following frequencies of specific clinical involvement: generalized encephalitis, 30 percent; spinal nerve roots, 30 percent; meningitis, 20 percent; cranial nerves, 20 percent; focal encephalitis, 16 percent; cerebellum, 14 percent; psychosis, 8 percent; and spinal cord, 2 percent. Combined involvement was noted in 36 percent of cases; in 79 percent, a history of antecedent respiratory illness was elicited. Of the patients, 53 percent were 20 years or younger, and 15 percent were younger than 10 years. Eighty percent of the children and adolescents were males. The onset of neurologic disease occurred 3 to 23 days after the onset of respiratory illness, with a mean value of 10 days. Five deaths were noted, and 22 percent of the survivors had residual neurologic deficits.

In a review of 61 patients with *M. pneumoniae*–associated neurologic disease during a 24-year period in Helsinki, Finland, Koskiniemi[527] noted that 45 of the patients were children and that all these children had encephalitis. Of the total group, 5 patients (8%) died and 14 (23%) had severe sequelae.

In a study of acute childhood encephalitis in Toronto, Canada, 9/50 children (18%) with adequate microbiologic study had evidence of *M. pneumoniae* infection.[520] Interestingly, 4 of the 9 children also had evidence of concomitant viral infection. In a further study of encephalitis at the same center during a 5-year period, researchers noted that 50/159 children (31%) with encephalitis had evidence of *M. pneumoniae* infection[81]; in this analysis, 30 of the 50 cases (60%) had microbiologic evidence of other concomitant infections. Respiratory prodromal symptoms preceded the encephalitis in approximately 67 percent of the patients in whom the diagnosis was based on culture, PCR, or both. Two children had acute demyelinating encephalomyelitis. Long-term neurologic sequelae occurred in 48 to 64 percent of cases. From 1998 to 2006, 1988 patients with encephalitis of unclear etiology were referred to the California Encephalitis Project (CEP).[176] *M. pneumoniae* was the most common agent implicated; 111 patients had evidence of *M. pneumoniae* infection, and 84 (76%) of these patients were children. In recent years, the CEP began to detect an increasing number of cases of anti–*N*-methyl-D-aspartate receptor (anti-NMDAR) encephalitis among children,[329] an autoimmune process that in some cases has been linked to *M. pneumoniae* infection.[328]

In other studies, aseptic meningitis is reported more frequently; other less common findings include poliomyelitis-like syndrome, bilateral sensorineural

*References 41, 65, 222, 337, 342, 407, 469, 473, 546, 547, 630, 708, 721, 790, 832, 1038.
†References 65, 183, 473, 546, 693, 711, 721, 868, 1038.
‡References 150, 200, 275, 317, 457, 686, 700.
§References 2, 13, 25, 27, 35, 37, 47, 54, 62, 80, 170, 192, 241, 249, 268, 355, 395, 411, 445, 504, 509, 515, 523, 551, 571, 586, 636, 698, 703, 723, 740, 745, 748, 772, 775, 796, 828, 837, 860, 883, 895, 922, 931, 949, 965, 975, 985, 1005, 1031, 1040, 1059.

deafness, Reye syndrome, cerebral infarction, optic disk swelling, opsoclonus-myoclonus syndrome, brain stem syndrome, transverse myelitis, psychosis, cerebellar syndrome, radiculopathy, brachial plexus neuropathy, demyelinating polyneuropathy with syndrome of inappropriate secretion of antidiuretic hormone (SIADH), acute disseminated encephalomyelitis (ADEM), bilateral striatal necrosis, Kluver-Bucy syndrome, Tourette syndrome, obsessive-compulsive disorder, hydrocephalus and intracranial hypertension, Bell palsy, and Guillain-Barré syndrome.

Arthur and Margolis[30] noted the appearance of *Mycoplasma*-like structures in granulomatous angiitis of the CNS at postmortem examination of a 35-year-old man.

Mixed Infections

In many studies of *M. pneumoniae* infection, cultural or serologic evidence of concomitant or sequential infection with other infectious agents has been noted.* In a large study of patients hospitalized with acute respiratory illness, Fransen and associates[323] found that 64 percent of patients with rises in complement-fixing antibody titer to *M. pneumoniae* also had antibody titer rises to viral, chlamydial, or bacterial agents. In this group, the most common concomitant infections were with parainfluenza viruses. The occurrence of mixed infection did not appear to have a pronounced effect on clinical manifestations; the only significant difference between patients with mixed infection and those with single *M. pneumoniae* infection was the more common finding of a high erythrocyte sedimentation rate in the former group. In several other large studies of disease caused by *M. pneumoniae*, concomitant infections were common, but no evidence of synergistic or antagonistic roles of one agent for another was noted.[286,352,686,921,922] By contrast, Johansson and associates[467] did observe more severe disease in patients with mixed viral and bacterial infections, including *M. pneumoniae*, than those with bacteria alone.

Renner and associates[814] found a lower than expected frequency of seropositivity to *M. pneumoniae* in 91 serum pairs with seroconversion to influenza A virus.

The high rate of possible mixed infections in the large encephalitis study in Toronto is of particular interest.[81] In addition to common respiratory viruses and enteroviruses, they noted herpes group viruses, *Bartonella henselae*, and *Mycobacterium tuberculosis*. Similar to *M. pneumoniae*, *M. tuberculosis* is also able to induce cold-agglutinins and hemolytic anemia, and other cases of dual infection with both organisms have been described.[1050] In studies of *Bordetella pertussis* infection, evidence of mixed infection with *M. pneumoniae* or perhaps serologic cross reactivity has been found repeatedly.[446,999,1073] Vervloet and colleagues[996] found that of 44 cases of *M. pneumoniae* pneumonia, 9 had concomitant *S. pneumoniae* infection.

The observation by Grady and Gilfillan[361] that 81 percent of patients with legionnaires' disease also had serologic evidence of *M. pneumoniae* infection is interesting. In the same study, 29 percent of all patients seropositive for *M. pneumoniae* also were seropositive for the legionnaires' disease antigen. Comparable studies performed by the Centers for Disease Control and Prevention failed to find a similar rate of high co-positivity in sera obtained in other legionnaires' disease epidemics. Another study found no serologic relationship between *M. pneumoniae* and *Legionella pneumophila*.[813] Severe bacterial disease after *M. pneumoniae* infection has been reported occasionally. Stadel and colleagues[917] noted *Hemophilus influenzae* pneumonia and bacteremia after a mild *M. pneumoniae* illness; Biberfeld and colleagues[77] reported staphylococcal septicemia in two cases of *M. pneumoniae* pneumonia, and Rykner and associates[832] recovered pneumococci from the pleural exudate of a patient with *M. pneumoniae* pneumonia.

Kleemola and Kayhty[511] found fourfold or greater increases in *M. pneumoniae* complement-fixing antibody titer in 40.7 percent of 54 patients with bacterial meningitis. However, they thought that this antibody response was not caused by specific *M. pneumoniae* infection but by cross-reactive glycolipids resulting from the bacterial infection.[511,512]

Lind and associates[586] reported that 4/19 patients with neurologic disease and *M. pneumoniae* infection had serologic evidence of concomitant viral infection. Wang and colleagues[1026] discovered that 3/22 children with *M. pneumoniae*–positive PCR assay also had a positive PCR for respiratory viruses.

Other Disease Associations

Foy and colleagues[307] noted that both ear involvement and pneumonia as manifestations of *M. pneumoniae* infection occurred more commonly in children with previous tonsillectomy. Putman and associates[799] found that in all but 3/31 patients with sarcoidosis, the serum complement-fixing antibody titer to *M. pneumoniae* was 1:32 or higher, whereas in a similar-sized control group without sarcoidosis, only 2 persons had titers of 1:32 and none had higher titers. Other interesting observations include multiple birth defects in a newborn exposed to *M. pneumoniae* in utero,[100] a tubo-ovarian abscess in a young woman from whom *M. pneumoniae* was isolated in pure culture,[969] fever of unknown origin in a 32-year-old man,[538] glomerulonephritis in a few patients with pneumonia,[9,793] SIADH in a 6-year-old boy,[589] acute IgA nephropathy,[938] and optic disk swelling and iritis.[840]

Recurrent Disease

The results of several investigations suggest that recurrent *M. pneumoniae* infection is a frequent finding.[113,295,928] Repeat infections can be associated with severe disease such as pneumonia. In the Seattle studies, second attacks of pneumonia have been documented, and similar findings have been observed in England.[309,319,321,417] Recurrent *M. pneumoniae* infections also occurred throughout the lifetime of a child who was immunocompromised from both poorly controlled human immunodeficiency virus (HIV) and B cell lymphoma therapy, and died at 8 years of age.[1035]

*References 12, 40, 39, 263, 286, 287, 324, 352, 361, 370, 383, 446, 447, 514, 520, 572, 583, 626, 634, 686, 723, 814, 917, 921, 922, 996, 997, 999, 1026, 1037, 1073.

Disease in the Neonate

M. pneumoniae is a common cause of respiratory infection beyond the neonatal period; however, rare cases of intrauterine infection leading to pneumonia in infancy have been found.[916,990]

Diagnosis

Differential Diagnosis

Because the clinical manifestations of *M. pneumoniae* infection are protean and because infections occur commonly in children and adolescents, this agent should be considered in the differential diagnosis of most infectious illnesses. Most important is its consideration in patients with pulmonary disease, in whom illnesses caused by viruses (particularly adenoviruses, parainfluenza viruses, and influenza viruses), *Chlamydia psittaci*, *Chlamydia pneumoniae*, *Coxiella burnetii*, bacteria (particularly *Streptococcus pneumoniae*, *B. pertussis*, *H. influenzae*, and *M. tuberculosis*); and fungi (particularly *Histoplasma capsulatum* and *Coccidioides immitis*) are the main differential possibilities. Because the clinical manifestations, including the radiographic appearance of the lungs, are frequently similar in the various differential possibilities, the following other factors are important: status of the host (i.e., normal or immunologically compromised), the environment (i.e., human, animal, or inanimate source), the age of the patient, the incubation period, and the season.

In otherwise healthy children, *M. pneumoniae* is a common cause of pneumonia in those older than 3 years and is the leading cause of pneumonia in older children and adolescents. The lack of coryza is sometimes useful in differentiating *M. pneumoniae* pneumonia from that caused by common viral agents, and elevation of the white blood cell count along with an increase in band form neutrophils is evidence against a mycoplasmal etiologic agent, except in patients with concomitant hemolytic anemia. The occurrence of exanthem and, in particular, Stevens-Johnson syndrome should lead the physician to suspect *M. pneumoniae;* similarly, the occurrence of hemolytic anemia, joint manifestations, or neurologic signs and symptoms with pneumonia should lead the physician to strongly suspect *M. pneumoniae* as the etiologic agent. Because the pulmonary manifestations of *M. pneumoniae* infection are not always clinically apparent, a physician investigating an unusual acute or subacute case (i.e., aseptic meningitis or other neurologic illness; exanthem; enanthem; hepatitis; pancreatitis; pericarditis, myocarditis, or both; and arthritis) would be wise to consider the possibility of *M. pneumoniae* as the etiologic agent and to obtain appropriate chest radiographs, as well as definitive cultures and serologic studies.

Specific Diagnosis

Serum Cold Agglutinins. Despite the considerable confusion in the literature and by physicians in general regarding the diagnostic value of the serum cold agglutination test for *M. pneumoniae* infection, my opinion is that when it is used appropriately, the test is a simple and useful procedure. One cause for confusion was a report in 1966 in which only 1/28 children with positive cold agglutination titers actually had serologic evidence of *M. pneumoniae* infection by complement fixation.[937] However, this report can be criticized because the study population consisted of 444 children younger than 4 years of age and only 170 of this group had pneumonia. Because cold agglutinins are noted occasionally in the sera of patients of all ages with a variety of illnesses, for useful results, their study should have been restricted to patients likely to have *M. pneumoniae* lower respiratory tract disease.[167,299,342,582] In various studies of pneumonia, serum cold agglutinins at a titer of 1:32 or higher were found in 50 to 90 percent of patients with *M. pneumoniae* infection.* In general, the cold agglutinin response correlates directly with the severity of pulmonary involvement; patients with extensive lobar involvement nearly always have positive titers[8] (1:32), whereas those with only minimal findings on radiographic study frequently have equivocal or negative titers. Positive cold agglutination titers have been observed in 18 percent of adenoviral pneumonias in a study involving a military population.[342] In general, the higher the cold agglutinin titer, the more likely that a particular illness is caused by *M. pneumoniae*.

A rapid screening test for cold agglutinins is available and useful.[337,371] This test is performed by adding 4 drops of blood to a tube containing sodium citrate or another anticoagulant. The tube is placed in ice water (0° to 4° C [32° to 39.2° F]) in a freezer for approximately 30 seconds and then examined immediately for coarse agglutination by tilting the tube on its side. When the tube is warmed, the agglutination should resolve, and it can be reproduced again by repeating the ice water cooling procedure. A modified version of this rapid test was used in 126 children in an emergency department who had asthma exacerbations.[143] The test had a sensitivity of 78.3 percent and a specificity of 41.3 percent in contrast to an IgM ELISA.

Specific Antibody Determinations. Several specific antibody tests (i.e., growth inhibition, immunofluorescence, indirect hemagglutination, precipitation, mycoplasmacidal antibody, complement fixation, ELISA, adherence inhibition assay, radioimmunoassay, and radioimmunoprecipitation) can be used to measure serum antibodies to *M. pneumoniae*. In the past only the complement-fixation test was available routinely. A fourfold rise in complement-fixation antibody titer indicates acute *M. pneumoniae* infection. Because complement-fixation antibody in *M. pneumoniae* infection is of relatively short duration, the observation of a fourfold fall in titer also can be useful on occasion in assigning etiologic significance in a particular illness. High single titers (≥1:256) usually indicate recent infection but rarely can be used to relate the cause of an illness specifically to *M. pneumoniae*. Because *M. pneumoniae* infection is associated with a relatively long incubation period, the development of antibodies is occurring at the time of acute disease. As a consequence, fourfold changes in titer can occur in a short interval (5 days), and collection of paired sera 5 to 7 days apart usually reveals a significant rise in complement-fixation antibody titer.

*References 73, 144, 149, 324, 342, 457, 700, 931, 993.

Most diagnostic laboratories have replaced the complement-fixation test with commercial immunofluorescence or ELISA for demonstration of antibodies to *M. pneumoniae* antigens.* These tests, in addition to demonstrating rises in antibody values in paired sera, can identify specific IgM and IgA antibodies in single serum samples. In general, when used by experienced laboratory personnel, both immunofluorescence and ELISA have sensitivities and specificities similar to those of the complement-fixation test for determining significant increases in antibody values in paired serum specimens. In addition, demonstration of specific IgM or IgA antibody in a single serum sample suggests a recent infection. Today the most common method used for diagnosing *M. pneumoniae* infection in clinical practice in the United States is the single serum IgM assay (although PCR assay is becoming more frequent in the hospital setting). It should be pointed out that most available serologic tests have low specificity and that overdiagnosis is common[61,213,253,739,946]; therefore, new panels of antigens are being developed.[252] Furthermore, the specific IgM and IgA responses after infection may last for several months; therefore, demonstration of these antibodies in a single serum sample may be misleading with regard to the diagnosis of specific illness. Hence, in most instances, paired sera (i.e., 5 to 14 days apart) should be examined to confirm a clinical diagnosis. Also useful is the determination of cold agglutinin titers at the time of IgM ELISA. If an ELISA IgM test is positive and a cold agglutinin test is negative, the physician should be skeptical of the former value.

Culture. With proper media, experienced personnel have little difficulty isolating *M. pneumoniae* from throat swabs of infected patients.[18,980] However, because *M. pneumoniae* is relatively slow growing, in most instances requiring more than 1 week of incubation, culture is of less use for diagnosis of routine cases than is serologic study. Cultures should be performed in all unusual situations; specifically, joint fluid, CSF, pericardial fluid, and biopsy material should be cultured. The modified SP-4 medium, which is more sensitive than conventional *Mycoplasma* culture media, coupled with the agar plate immunofluorescence identification procedure, may assist the cultural diagnosis of *M. pneumoniae*.[980] However, low yield and sensitivity have caused some to recommend abandoning this diagnostic method.[876]

Detection by Polymerase Chain Reaction. A large number of studies have indicated the usefulness of PCR for demonstration of specific *M. pneumoniae* DNA in sputum, throat, nasopharyngeal, blood, CSF, urine, and tissue specimens.† Numerous different primers have been used to identify gene sequences of the P1 cytoadhesin protein, ATPase operon, 16S rRNA gene, and 16S-23 S rRNA spacer, p1 adhesin, tuf, parE, dnaK, pdhA, repMp1 noncoding element, and the CARDS toxin gene sequences.[1007]

Using culture plus serologic criteria as the comparative standard, several studies have shown excellent sensitivity and specificity. However, because both serology and PCR will occasionally give nonoverlapping false-negative results, a combination of these two diagnostic methods would be optimal.[485]

Treatment

Antimicrobial Therapy

M. pneumoniae is sensitive in vitro to erythromycin, tetracyclines, chloramphenicol, clarithromycin, azithromycin, several aminoglycosides, and quinolones.* It is resistant to all penicillins and for practical purposes to the cephalosporins. In spite of this demonstrated in vitro sensitivity of the organism, plus several studies that have shown clinical therapeutic effectiveness,† a common misconception of many physicians is that antibiotic therapy is of little value in the treatment of illness caused by *M. pneumoniae.* This idea had its origin before the present era, when many patients with viral pneumonia were given a diagnosis of primary atypical pneumonia and treated unsuccessfully with antibiotics.

In 1961, Kingston and associates[507] demonstrated the therapeutic effectiveness of demethylchlortetracycline for pneumonia caused by *M. pneumoniae*. Since then, several other antibiotics have been studied carefully and also have been found to be effective against *M. pneumoniae* pneumonia.‡ The drugs of choice for pneumonia caused by *M. pneumoniae* are either erythromycins or tetracyclines. Because of the adverse effects of tetracyclines on teeth, a macrolide is the drug of choice in children. In *M. pneumoniae* pneumonia, the dose of erythromycin for children is 40 to 50 mg/kg every 24 hours administered every 6 hours for a minimum of 10 days. For adolescents and adults, the dosage of erythromycin or tetracycline is 2 g every 24 hours administered every 6 hours. In general, the effectiveness of antibiotic therapy correlates directly with the severity of pneumonia and the elapsed time of illness before the initiation of therapy.

Azithromycin and clarithromycin both are approved for the treatment of community-acquired pneumonia in children.[89,390] Their advantage is less frequent dosing, shorter duration of therapy, and less gastrointestinal disturbance in older patients. The azithromycin dosing schedule is 10 mg/kg/day (maximal dose, 1 g/day) on day 1, followed by 5 mg/kg/day (maximal dose, 500 mg/day) for 4 days. The clarithromycin dose is 15 mg/kg/day administered every 12 hours for 10 days (maximal dose, 1 g/day). A growing number of macrolide-resistant strains of *M. pneumoniae* from clinical isolates

*References 15 , 61, 127, 213, 252, 253, 259, 281, 300, 419, 518, 562, 699, 739, 807, 892, 904, 946, 966, 967, 987, 1030.
†References 2, 81, 88, 122, 227, 244, 326, 348, 415, 435, 464, 485, 617, 653, 672, 680, 697, 710, 724, 786, 809, 815, 864, 872, 909, 947, 1047, 1055, 1066.

*References 59, 231, 440, 441, 459, 628, 737, 898.
†References 89, 144, 231, 307, 317, 342, 457, 507, 830, 866, 871, 898, 901.
‡References 144, 231, 317, 342, 457, 830, 871, 898, 901.

have been noted in Japan since 2000, associated with mutations in the 23S rRNA gene.[638,696] These strains have since spread worldwide, with a rate of 8.2 percent resistance found in the United States between 2007 to 2010.[126,1053] In such cases, minocycline or levofloxacin have become preferred choices of therapy.[490] Ciprofloxacin also has been used successfully,[603] although it carries a small risk for tendonitis.

In all other clinical manifestations of *M. pneumoniae* infection except pneumonia (e.g., nonpulmonary respiratory infection, neurologic disease, and Stevens-Johnson syndrome), antibiotic therapy has not been evaluated adequately. In general, otitis media, pharyngitis, croup, and bronchiolitis appear to be mild, self-limited illnesses that require no therapy. In more serious illness, such as Stevens-Johnson syndrome and neurologic disease, individual case studies have indicated little evidence of therapeutic benefit with either erythromycin or tetracycline therapy. However, my opinion is that when diagnosed, most *M. pneumoniae* infections should be treated because there is little to lose and in vitro data suggest the possibility of efficacy. Jensen and colleagues[466] noted that prophylactic administration of oxytetracycline to family contacts prevented disease but not infection. Azithromycin prophylaxis has been used successfully in two hospital outbreaks of *M. pneumoniae* pneumonia.[433,510]

Corticosteroid Therapy

Steroids have been used in the management of severe pulmonary disease, Stevens-Johnson syndrome, encephalitis, and hemolytic anemia. Although definitive data are lacking, several case studies suggest associated clinical benefit; steroids seem to be particularly useful in treating severe hemolytic anemia.

Two groups[559,948] reported on the use of prednisolone or methylprednisolone in the treatment of children with *M. pneumoniae* pneumonia in whom the clinical course worsened while receiving appropriate macrolide therapy. Corticosteroid treatment in these children was related to clinical and radiologic improvement. Cimolai[181] noted dramatic improvement in a 7-year-old girl treated with methylprednisolone, and in 1964 Copps[199] noted dramatic improvement in an adolescent with persistent fever, severe pneumonia, and hemolytic anemia when treated with a corticosteroid and erythromycin. Lu and accociates[603] successfully treated six cases of macrolide-resistant *M. pneumoniae* infection with ciprofloxacin and corticosteroids.

Intravenous Immunoglobulin

Intravenous immunoglobulin (IVIG) therapy has been used successfully in certain cases of severe refractory *M. pneumoniae* infection, especially when there is suspicion of an autoimmune process.[34,105,877,1063] It is also used in cases of *Mycoplasma*-induced Kawasaki disease.

A 6-year-old girl with brain stem and striatal encephalitis complicating *M. pneumoniae* pneumonia experienced neurologic improvement within 48 hours of administration of intravenous immunoglobulin.[837]

Plasmapheresis

Plasmapheresis has been used rarely to treat severe manifestations of *M. pneumoniae* disease, including transverse myelitis with psychosis[204] and glomerulonephritis.[9]

General Management

Children and adolescents with *M. pneumoniae* pneumonia should be discouraged from engaging in excessive physical activity during the acute illness and for a 2-week period during convalescence because clearance, as observed by radiography, is slow and lags behind apparent clinical well-being. Older children and adolescents should be advised of their contagiousness to others; this risk period exists as long as cough persists, even with successful antibiotic therapy.

Prevention

Because of the marked and prolonged morbidity associated with *M. pneumoniae* infection, which has been particularly troublesome in the military, much effort was directed toward the development of vaccines. In 1965 Jensen and associates[465] reported encouraging initial trials with an inactivated vaccine. In this study, significant rises in *M. pneumoniae* growth-inhibiting antibody titer developed in 25 of 30 volunteers. Later challenge studies with the same vaccine indicated that 9/10 volunteers with serum antibody were protected, but illness more severe than that in the unvaccinated control group occurred in vaccinees who did not have an antibody response after initial immunization.[900] This altered reactivity on challenge suggested a sensitization process perhaps similar to that observed with other inactivated antigen vaccines and indicated the need for caution in further trials.[208,295] Additionally, because of the large incidence of autoantibody formation in *M. pneumoniae* infection, autoimmune sequelae could potentially be a significant adverse effect with these vaccines.[468] Other trials of inactivated vaccines in both adults and children have had varying degrees of success.[580,667,687,1039]

A trial with a live attenuated vaccine (i.e., a temperature-sensitive mutant) gave encouraging results.[368] However, because further study of natural *M. pneumoniae* disease indicated that reinfection occurs commonly and because sensitization may play a role in pathogenesis, proceeding slowly in conducting further vaccine trials in children seems prudent.[294]

DNA vaccines encoding a fusion of the *M. pneumoniae* P1 protein with an *Escherichia coli* toxin were recently found to confer protection in mice.[1070,1071]

The degree of contagion of *M. pneumoniae* is relatively low, so isolation methods should be effective in preventing spread of disease. The studies of Jensen and associates[466] and the hospital azithromycin trials[433,510] indicate that in certain circumstances (in particular, high-risk subjects such as patients with sickle-cell disease and high-risk populations), prophylactic administration of antibiotics may be justified.

UREAPLASMA

Properties

Ureaplasmas (formerly T-strain mycoplasmas) are distinguished from all other members of the order Mycoplasmatales by their production of urease and their ability to hydrolyze urea.[765,881,882,955] The morphologic characteristics of *Ureaplasma* spp. in young liquid medium culture are similar to those of other mycoplasmas. Round-ovoid elements approximately 330 nm in diameter with a range of 100 to 850 nm are found; rod-shaped and filamentous structures also occur, and the latter have a length of 2 µm and a width of 50 to 300 nm.[84,881,1044] In clinical material, short, bacillary forms with monopointed ends are common findings. Organisms are surrounded by a single trilaminar membrane approximately 10 nm thick with pilus-like structures radiating from the surface. Multiplication occurs by a simple budding process and perhaps by binary fission.

On unbuffered standard *Mycoplasma* agar with a pH of 6.0, *Ureaplasma* spp. colonies are small (20 to 30 µm) and circular, with irregular borders, and grow downward into the agar.[881] On buffered agar, *Ureaplasma* spp. colonies are bigger and often have the "fried egg" appearance of typical large colony-forming mycoplasmas.[618]

Isolation of *Ureaplasma* spp. from clinical material is assisted by the demonstration of urease activity.[296,880,957] In liquid medium containing urea and phenol red, growth of *Ureaplasma* spp. results in the production of ammonia, with a resultant increase in pH and a change in color. Subculture from broth to agar medium that contains urea and manganese sulfate yields dark brown ureaplasmal colonies.

Epidemiology

The main reservoirs of human strains of Ureaplasma spp. are the genital tracts of adult men and women.* *Ureaplasma* spp. are sexually transmitted organisms that colonize the urogenital tract of women at rates of 30 to 80 percent.[99,262,649,957] One study found that pregnancy was associated with a higher rate of *U. urealyticum* carriage in the cervix in contrast to rates in nonpregnant women (26% vs. 15.3%).[38] High rates of colonization have been associated with younger age, lower socioeconomic status, sexual activity with multiple partners, black ethnicity, and use of oral contraceptives, although not with HIV status.[360]

In general, cervicovaginal colonization with *Ureaplasma* spp. and *M. hominis* during pregnancy is not predictive of adverse outcomes such as premature delivery, low-birth-weight infants, and spontaneous abortion.[137,173,270,360,1012] However, preterm labor has been associated with the altered microbial environment found in bacterial vaginosis, although there have been conflicting reports on whether genital mycoplasmas may play a role as well.[240,561] High-density genital ureaplasmal colonization has been associated with chorioamnionitis and preterm delivery.[5,477,761] It is capable of invading the upper genital tract in a subpopulation of women, as evidenced by their isolation from the endometrium,[548] placenta,[266,399,538] and amniotic fluid.* These organisms also have immunogenic surface lipoproteins that may trigger inflammatory reactions that lead to preterm premature rupture of membranes and/or preterm labor.[389,477,550,747,780]

Ureaplasma spp. have been associated strongly with histologic chorioamnionitis, postpartum fever, and endometritis.†

Infants become colonized during passage through the birth canal of an infected woman.[320,513,563,940,1012] With ruptured membranes, the infant can be infected in utero.[513] *Ureaplasma* spp. have been recovered from the following sites in newborn infants: throat, nose, genitourinary tract of girls, urine of boys, external auditory canal, umbilicus, and perineum. Not all infants of infected women become colonized, and neonatal colonization tends to not persist. In one study in which *Ureaplasma* spp. were recovered from 38 percent of girls and 6 percent of boys at birth, follow-up during a 2-year period revealed a decreasing prevalence of colonization; at 2 years, none of the children had positive cultures.[316] However, in one large study of pediatric patients with chronic respiratory disease, 8.8 percent carried *U. parvum* and 2.8 percent carried *U. urealyticum* in their respiratory tract.[778]

During prepubertal childhood, *Ureaplasma* spp. are recovered only rarely from urine or genital specimens.[314,563] After puberty, colonization is a common occurrence and is primarily the result of sexual contact.[643,645,647] Colonization in adults is related directly to sexual activity. In population studies, *Ureaplasma* spp. rarely are isolated from persons with no sexual experience, but they occur in approximately 50 percent of men and 75 percent of women for whom sexual intercourse with three or more partners is reported.

Clinical Manifestations

Because *Ureaplasma* can be recovered with considerable frequency from the throat, eyes, and genitourinary tract of babies and from the genitourinary tract of postpubertal males and females who are well, establishing cause-and-effect relationships in disease frequently has been difficult. Studies suggest the following disease associations with *Ureaplasma* in human genitourinary and reproductive disease: good to strong association with nongonococcal urethritis, prostatitis, and urethral syndrome; moderate association with epididymitis, involuntary infertility, repeated spontaneous abortion and stillbirth, chorioamnionitis, and low birth-weight; weak association with urinary calculi, pyelonephritis, Reiter disease, and pelvic inflammatory disease; and no association with Bartholin gland abscess, vaginitis, cervicitis, postabortal fever, and postpartum fever.‡

*References 28, 316, 645, 646, 647, 648, 957, 978, 985, 1012.

*References 128, 134, 136, 306, 366, 420, 971, 1060, 1061.
†References 23, 24, 129, 136, 137, 399, 416, 913.
‡References 112, 134, 138, 266, 539, 544, 652, 924, 934, 957, 974, 982.

Adverse Pregnancy Outcomes and Infertility

There has been much debate regarding genital mycoplasmas and whether they play a role in spontaneous abortion and premature labor, although supporting evidence is growing.[269,1014] The Alabama Preterm Birth Study in 2008[354] examined umbilical cord blood cultures for genital mycoplasmas in very preterm newborn neonates (i.e., 23 to 32 weeks' gestation). They found *U. urealyticum*, *M. hominis*, or both in 23 percent of the samples and more commonly in infants of nonwhite mothers (27.9% vs. 16.8%, $p = .016$), mothers younger than 20 years of age, those having a spontaneous rather than an indicated preterm delivery (34.7% vs. 3.2%, $p = .0001$), and those delivering at an earlier gestational age. Infants with positive cultures were more likely to have had intrauterine infection and inflammation, as shown by positive placental cultures, elevated cord blood IL-6 levels, and placental histology. Positive growth for these genital mycobacteria in cord blood also was associated with systemic inflammatory response syndrome (41.3% vs. 25.7%, $p = .007$). No significant difference between groups was found for intraventricular hemorrhage or death. Romero and Garite[824] commented on particularly significant findings from this study, including that genital mycoplasmas caused bacteremia in 1/4 of very preterm newborns, most commonly *U. urealyticum*, although a large number (i.e., 22% of those with positive umbilical blood cultures) had simultaneous colonization with *M. hominis*. They raise the question of whether these organisms should be screened for in routine prenatal and perinatal care, and whether standard neonatal sepsis antibiotic regimens should be adjusted to cover them. Waites and associates[1019] wrote a comprehensive review of recent literature trying to support a link between genital mycoplasmas and adverse outcomes. Comparisons of recent findings in prophylactic antibiotic use to forestall these outcomes, however, were inconclusive, possibly as a result of the varied methods used.

Genital mycoplasmas can invade the upper genital tract and cause inflammation, and this has been proposed as a potential cause of infertility.[380,436] In addition, some *Mycoplasma* spp. can bind and affect sperm; infertility in men has been associated with these infections.[232,339,839] These topics will not be discussed further because they are outside the scope of this text's pediatric focus.

Nongonococcal Urethritis

Nongonococcal urethritis occurs more commonly than gonococcal urethritis in men in most developed countries (Tully J G. Personal communication. December 1, 1991).[391,451,642] Approximately 40 percent of cases of nongonococcal urethritis are caused by *Chlamydia trachomatis*, and 20 to 30 percent are the result of *Ureaplasma* infection.* Clinical differentiation of disease caused by *C. trachomatis* and *Ureaplasma* has not been studied, but nongonococcal and gonococcal urethritis have been evaluated comparatively.[388,451,563,1004]

*References 94, 95, 206, 413, 648, 855.

The incubation period in nongonococcal urethritis is relatively long, with most cases occurring 10 to 20 days after exposure, whereas with gonorrhea the period is shorter, usually less than 1 week.[388] The onset of symptoms in nongonococcal urethritis is generally more gradual than that associated with gonorrhea. Virtually all men with gonorrhea have a urethral discharge, and most have both discharge and dysuria. In contrast, Jacobs and Kraus[451] found that only 38 percent of men with nongonococcal urethritis had both dysuria and discharge. In the same study, 15 percent of patients with nongonococcal urethritis had only dysuria, whereas only 2 percent of those with gonococcal urethritis had a similar problem. On examination, Handsfield[388] found the discharge in nongonococcal urethritis to be purulent in 36 percent of his cases, nonpurulent in 9 percent, and of an intermediate character in the remaining 55 percent. In contrast, 73 percent of patients with gonorrhea had a purulent discharge, 27 percent had an intermediate discharge, and none had a nonpurulent discharge. Because of the more gradual onset and the usually less severe symptoms, patients with nongonococcal urethritis are less prompt in seeking medical care than those with gonorrhea. Jacobs and Kraus[451] found that 76 percent of patients with discharge and gonococcal infection came to the clinic within 4 days of onset, whereas only 43 percent of patients with similar nongonococcal urethritis visited the clinic within 4 days of disease onset. Without treatment, nongonococcal urethritis symptoms subside gradually in some patients during a 1- to 3-month period.[763]

After penicillin, ampicillin, or spectinomycin treatment of men with urethral gonorrhea, urethritis recurs (postgonococcal urethritis) in many patients.[414] Studies of postgonococcal urethritis indicate an etiologic role for *C. trachomatis;* genital mycoplasmas are probably responsible for some cases,[764] especially as persistent urethritis after doxycycline treatment also has been reported.[1045]

A 7½-year-old sexually inactive boy with recurrent urethritis associated with *U. urealyticum* infection has been described.[874]

Other Infections

Ureaplasma has also been associated with postoperative mediastinitis in adults.[335,340,386]

Disease in the Neonate

Transmission. Vertical transmission of *Ureaplasma* spp. and *M. hominis* from a colonized mother to her newborn occurs in utero or during delivery.[6,16,362,842,1014] The relative frequency of occurrence at each time point is not fully known. Acquisition of mycoplasmas by newborns can occur at the time of delivery through contact with a colonized birth canal, but they also have been found on the mucosal surfaces of newborns delivered by cesarean section performed before the onset of labor and rupture of amniotic membranes.[844,940] In utero transmission occurs either transplacentally by the hematogenous route or via an ascending intrauterine infection from a colonized maternal genital tract. Mycoplasmas have been isolated from maternal blood at the time of delivery and

from umbilical cord blood, amniotic fluid, endometrium, chorioamnion, placenta, and aborted fetal tissue. Specific IgM antibody responses have been detected in neonatal serum. Postpartum or nosocomial transmission probably occurs, but definitive proof is lacking. The idea was suggested by the finding of ureaplasmal colonization in infants at 3 to 4 weeks of age who were not previously shown to be colonized with *Ureaplasma* spp. while in a neonatal intensive care unit (NICU).[844]

The rate of vertical transmission of *Ureaplasma* spp. ranges from 0.9 to 55 percent in full-term infants and 8.5 to 58 percent in preterm infants, depending on the number and type of mucosal surfaces sampled.[16,179,751,842-844,940] *M. hominis* has been isolated from the nasopharyngeal aspirates and gastric secretions of 30 to 42 percent[178,179] and 8 percent,[362] respectively, of infants born to colonized mothers. The rate of vertical transmission of *Ureaplasma* spp. is not affected by the method of delivery or the duration of time after rupture of membranes. Vertical transmission is increased significantly in the presence of chorioamnionitis and intraamniotic infection.[235,888] Colonization of newborn infants increases with decreasing gestational age and birth-weight[10] and is highest in infants weighing less than 1000 g at birth.[844] Female newborns are more likely than males to be colonized with *Ureaplasma* because the vagina is a common site of colonization.[315,843]

Colonization with *Ureaplasma* persists through early infancy; 68, 33, and 37 percent of full-term newborns colonized in the throat, eye, and vagina, respectively, are still colonized at 3 months of age.[940] However, most of them lose colonization by the time they reach 2 years of age.[315] Among preterm infants, 65 percent remain colonized at the time of discharge from the NICU or at 28 days of life.[844] Overall, the prevalence of ureaplasmal colonization varies from 2 to 86 percent among infants admitted to NICUs, and as many as 14 to 41 percent of infants have endotracheal aspirate cultures positive for *Ureaplasma* spp.*

Clinical Manifestations

The role of *Ureaplasma* spp. in neonatal disease continues to be investigated and defined. *M. hominis* and *Ureaplasma* spp. have been recovered from the lungs, brain, heart, and viscera of aborted fetuses and stillborn infants, with histologic findings of bronchopneumonia present in the lungs.[614,943] *Ureaplasma* spp. were isolated from blood in as many as 34 percent of infants younger than 34 weeks' gestational age.[758] Twenty-six percent of preterm infants had positive endotracheal aspirate cultures for *Ureaplasma* spp. in one study.[135] Genital mycoplasmas also have been isolated from the blood, urine, CSF, and lung tissue of infants with clinical signs of infection. These organisms frequently colonize the mucosal surfaces of newborns,[513,844] and ascribing disease often is difficult. However, isolation from normally sterile body fluids in symptomatic infants has led to the recognition that these organisms are neonatal pathogens.

*References 217, 254, 400, 444, 759, 842.

Chorioamnionitis

In a study of 249 puerperal women and their babies, Shurin and colleagues[888] noted on histologic examination of the placentas that *Ureaplasma* spp. were recovered from 37.5 percent of babies whose placentas showed chorioamnionitis and from only 19 percent of those with normal placentas. In this study, no adverse effects could be attributed to either the placental lesions or colonization of the babies. Another group found that *U. urealyticum* and *M. hominis* in the umbilical cord was associated with funisitis, but the presence of these organisms in the placenta, tracheal aspirate, or gastric fluid of preterm infants did not show a significant difference in the presence of chorioamnionitis in the mother or chronic lung disease in the baby.[260] Caspi and associates[128] reported a 32-year-old woman with amnionitis in whom *Ureaplasma* was recovered from blood. After delivery, the same organism was recovered from the blood of one of the twin infants.

Pneumonia

Fatal neonatal pneumonia in a term infant was documented by isolation of *Ureaplasma* from lung tissue at autopsy, with demonstrated elevated serum IgG and IgM titers to the organism.[801] Afebrile pneumonitis was reported in infants younger than 3 months of age.[918] Pneumonia and persistent pulmonary hypertension were documented in three infants from whom *Ureaplasma* spp. were isolated from blood, endotracheal aspirates, pleural fluid, or lung tissue (or any combination of these sites) at autopsy.[1014] Cultrera and colleagues[214] reported finding an association between colonization of the lower respiratory tract by *Ureaplasma* spp., particularly by *U. parvum* in preterm newborns, and respiratory distress syndrome. The potential role of *Ureaplasma* in neonatal pneumonia[116,332,350,769] has been strengthened by demonstration of histologic evidence of pneumonia in the lungs of newborn mice and premature baboons; ureaplasmal isolates were obtained from the pleural fluid, lung biopsy specimens, and lung tissue of these experimental pneumonia models.[132,829,1021] Crouse and associates[209] demonstrated that pneumonia is produced in newborn mice, but significantly less often in mice older than 14 days, and is potentiated by oxygen therapy. *Ureaplasma* spp. have been shown to induce ciliostasis and mucosal lesions in human fetal tracheal organ cultures.[132] In addition, *Ureaplasma* can induce the production of alveolar macrophage proinflammatory cytokines in vitro,[577] and both *Ureaplasma* spp. and *M. hominis* stimulate the production of tumor necrosis factor (TNF)-α and inducible nitric oxide synthase from murine macrophages.[210] Viscardi and colleagues[1001] developed the first juvenile mouse model of *Ureaplasma* pneumonia. Their data suggest that *Ureaplasma* alone may cause limited inflammation and minimal tissue injury in the early phase of infection but may promote a mild chronic inflammatory response in the later phase of infection (days 14 to 28), similar to the process that occurs in human newborns.

Chronic Lung Disease

Isolation of *Ureaplasma* spp. from endotracheal secretions, the nasopharynx, the throat, or gastric aspirates (or any combination of these sites) has been associated with chronic lung disease of prematurity. Development of chronic lung disease was described in low-birth-weight infants whose respiratory tracts were colonized with *Ureaplasma* spp. in the first week of life.*

The results from a meta-analysis performed by Wang and colleagues[1024] involving 17 publications supported a significant association between ureaplasmal colonization and the subsequent development of chronic lung disease. Crouse and associates[211] noted that infants who weighed 1250 g or less at birth, had respiratory tract disease, and were colonized with *Ureaplasma* spp. in their tracheal secretions were more likely to have radiographic evidence of more severe pulmonary disease than were infants who were not colonized. Similar findings were made by Honma and coworkers,[416] who noted that infants younger than 32 weeks' gestational age with elevated white blood cell counts and *U. urealyticum* colonization were more likely to develop infection-related chronic lung disease. These findings were not supported by studies by Cordero and colleagues,[201] who did not detect any specific radiographic abnormalities in 183 infants with a birth-weight of 1250 g or less and who had endotracheal colonization with *Ureaplasma* spp., gram-negative cocci, or gram-negative bacilli. However, Ollikainen and associates[755] found that preterm infants colonized with Ureaplasma spp. had higher leukocyte counts on the first 2 days of life and that they required high-frequency oscillatory ventilation more often than those not colonized. In addition, the isolation by Walsh and colleagues[1022] of *Ureaplasma* spp. from the lungs of infants with chronic lung disease implies an invasive bacterial process as part of the pathogenesis of lung injury. In a retrospective study of 60 ventilated babies born at less than 30 weeks' gestation, 25 of whom were *Ureaplasma* culture–positive, Theilen and associates[968] found that the ventilated babies with *Ureaplasma* in their tracheal secretions had a clinical and radiologic course different from that of the infants who were culture-negative. Specifically, they had less acute lung disease but an earlier onset of chronic lung disease.

In contrast to these studies, Heggie and colleagues,[400] Da Silva and associates,[217] and Couroucli and colleagues[207] found no association between *Ureaplasma* and the development of bronchopulmonary dysplasia (BPD). Bowman and colleagues[96] used erythromycin to treat infants with extremely low birth-weight infants colonized with *Ureaplasma* and found no association between the initial colonization and the development of chronic lung disease. They also called attention to the fact that in the study by Heggie and coworkers,[400] a substantial proportion of the colonized infants were treated with erythromycin, which might explain the lack of an association between colonization and subsequent chronic lung disease. In 1997, Van Waarde and associates[993] noted that preterm infants who were at the highest risk for the development of chronic lung disease (i.e., those with the lowest gestational age and birth-weight) also were the ones most likely to be colonized with *Ureaplasma* spp. Castro-Alcaraz and coworkers[139] found that a persistently positive colonization pattern, which accounted for 45 percent of the *Ureaplasma*-positive infants, was associated with a significantly increased risk for the development of chronic lung disease.

One theory suggests that *Ureaplasma* is not the primary cause of BPD but might be the cause of undetected pneumonia.[133] This pneumonia results in an increased requirement for supplemental oxygen, and BPD is the result of oxygen toxicity caused by the supplemental oxygen therapy. Viscardi and associates,[1000] using the preterm baboon model, suggested that a prolonged proinflammatory response initiated by intrauterine *Ureaplasma* infection contributed to the early development of fibrosis and altered developmental signaling in the immature lung.

The possibility that ureaplasmal colonization of the respiratory tract induces an inflammatory response without direct pulmonary invasion and infection cannot be excluded.[375,608] It has been supported by the finding of elevated levels of TNF-α, IL-6, IL-8, and monocyte chemoattractant protein–1 (MCP-1) in the tracheal secretions of colonized infants,[42,132,376,624,925] as well as by elevated white blood cell counts and eosinophilia in infants whose respiratory tract is colonized by *Ureaplasma* spp.[753,755,771] The contribution of *U. urealyticum* and *U. parvum* was evaluated by Heggie and colleagues.[400] Both species colonized preterm infants with a birth-weight of less than 1500 g; however, no association was seen between endotracheal colonization with either species and the development of chronic lung disease. In a study performed by Katz and colleagues,[487] *U. parvum* was detected more often than *U. urealyticum*, but the researchers found no significant difference or trend in the prevalence of either species between infants with or without BPD. Similarly, the Alabama Preterm Birth Study showed no association between *Ureaplasma* infection and respiratory distress syndrome (RDS).[354] However, Cultrera and associates[214] did find a strong association in preterm infants of RDS development and colonization, especially with *U. parvum*.

In a meta-analysis, Schelonka and colleagues[859] reviewed 36 articles in which cohorts of neonates were screened for the presence of *Ureaplasma* by culture with or without PCR and monitored prospectively for the development of BPD. They concluded that colonization with *Ureaplasma* was associated with higher reported rates of BPD. However, the greatest reported effect was seen in the small studies, and, therefore, reporting bias may be partially responsible for the higher rates of BPD in colonized infants. The Alabama study could also find no significant association with BPD when adjusted for maternal race, infant gestational age, and gender.[354]

Central Nervous System Infections

Both *Ureaplasma* spp. and *M. hominis* have been isolated from the CSF of both full-term and preterm infants.*

*References 3, 15, 17, 27, 45, 56, 94, 114, 122, 127, 133, 138, 155, 188, 190, 191, 202, 220, 259, 264, 416, 532, 779.

*References 20, 119, 336, 346, 394, 409, 508, 629, 651, 728, 873, 889, 919, 990, 1016, 1018.

The repeated isolation of these organisms from the CSF of predominantly preterm infants with suspected meningitis and their association with a CSF pleocytosis consisting of polymorphonuclear or mononuclear cells, hypoglycorrhachia, and elevated protein content support their role in causing neonatal meningitis. Waites and colleagues[1015] noted hemiplegia, hydrocephalus, and developmental delay in survivors.

Isolation of *Ureaplasma* spp. from the CSF of preterm infants also has been associated with severe intraventricular hemorrhage.[4,757,1017] However, Likitnukul and associates[578] cultured the CSF and blood of 203 infants with suspected sepsis and failed to isolate *Ureaplasma* spp. Primarily in full-term infants, isolation of *Ureaplasma* spp. and *M. hominis* often has been associated with minimal if any CSF abnormalities, and these infants have done well without receiving specific antimicrobial therapy.[401,728,859,990,1016] In such instances, the presence of these organisms remains of unclear clinical significance, and their role in producing disease is questionable.

Other Infections

Nonimmune hydrops fetalis was reported in a newborn at 32 weeks' gestation in whom *U. urealyticum* was isolated from bronchial secretions and lung and brain tissue at autopsy.[756] Osteomyelitis of the femur was associated with isolation of *Ureaplasma* from the blood of a preterm infant.[349] *Ureaplasma* was recovered from a scalp abscess at the site of an internal fetal electrode monitor.[387]

Differential Diagnosis

In clinical practice the most important differential consideration is between gonococcal infection and nongonococcal urethritis. Although the symptoms of the two illnesses are frequently different, sufficient overlap exists to render arriving at a specific diagnosis without laboratory aid hazardous. Microscopic examination of a urethral specimen is essential. In most instances the observation of gram-negative cell-associated diplococci on Gram stain is sufficient for a diagnosis of *Neisseria gonorrhoeae* infection. When smears reveal polymorphonuclear neutrophils without organisms suggestive of gonococci, a specific bacterial culture should be performed. Because infection with multiple agents occurs commonly and postgonococcal urethritis is a frequent problem, thorough investigation of initial illnesses in adolescents with cultures for bacteria, *Chlamydia*, and *Ureaplasma* is advised.

Because of the frequent colonization of newborn infants with mycoplasmas, an etiologic role for these agents cannot be supported by detection of organisms on mucosal surfaces only. However, isolation from normally sterile sites would support further workup and possibly treatment.

MYCOPLASMA HOMINIS

Properties

Three basic morphologic forms of *M. hominis* have been observed by phase-contrast microscopy: coccoidal cells 30 to 80 nm in diameter, diploforms and filamentous forms with a thickness of 30 to 40 nm, and forms with variable lengths reaching 40 μm or more.[90,102,104,237] Bredt[103] studied newly isolated strains and noted that coccoid forms and ring- or disk-shaped cells were predominant; with some strains, filamentous forms of variable length also were noted. Multiplication occurs by binary fission, by fragmentation of filaments and rings, and by budding.[90,820]

Anderson and Barile[22] studied the ultrastructure of *M. hominis* and noted considerable variability in internal components. In some cells, ribosome-like granules in the cytoplasm and a more central area of netlike strands were present, suggestive of a nucleus. Other cells had only irregular densities within the cytoplasm. In some instances, dense cytoplasmic bodies were observed; in other cells, vacuoles were seen.

On *Mycoplasma* agar, M. *hominis* colonies are approximately 200 to 300 μm in diameter and have the typical mycoplasmal "fried egg" appearance.[958] *M. hominis* grows on ordinary blood agar and produces pinpoint nonhemolytic colonies. It metabolizes arginine to ammonia, so arginine-supplemented liquid medium with a pH indicator (phenol red) can be used for primary isolation. *M. hominis* can be identified specifically and differentiated from other human mycoplasmas that metabolize arginine by growth inhibition by specific antibody.

M. hominis has two cytoadhesins that are membrane proteins; they allow attachment to cells of the urogenital tract and to trichomonas.[405] Attachment is to sulfated glycolipids of the host cells.[760]

Epidemiology

M. hominis colonizes the urogenital tracts of sexually active adults,* with the rate of carriage in women estimated to be 20 to 50 percent.[99,649,957] One study study that found an association between pregnancy and *Ureaplasma* colonization noted only minimal rates of *M. hominis* carriage and no significant difference in rate in pregnancy.[38] The rest of its epidemiology follows the same characteristics as those listed for the *Ureaplasma* spp. Similarly, the simple presence of *M. hominis* in the lower genital tract during pregnancy is not predictive of adverse outcomes[137,270,1013] and has a controversial role in causing spontaneous abortion and preterm delivery.[269,1012] Like the *Ureaplasma* spp., it can invade the upper genital tract. *M. hominis* has been shown to be a cause of pelvic inflammatory disease, postpartum septicemia, and endometritis,[132,494,650,732,787] and it has been associated with surgical wound infection after cesarean delivery.[526,611,819] It can be recovered from the oral cavity of 1 to 5 percent of normal adults.[912] In one large study it was found in the respiratory tract of 4.7 percent of pediatric patients with chronic respiratory tract disease.[778]

M. hominis has been identified inside *Trichomonas vaginalis*, where it is believed to play a symbiotic role.[233,694] *T. vaginalis* strains harboring *M. hominis* were found to have increased virulence behavior.[994] Conflicting reports exist of metronidazole resistance being conferred by *M. hominis*.[120,1052]

*References 320, 643, 645, 647-649, 957, 1012.

Clinical Manifestations

Studies suggest the following disease associations with *M. hominis* in human genitourinary and reproductive diseases: good to strong association with pyelonephritis, pelvic inflammatory disease, postabortal fever, and postpartum fever; moderate association with prostatitis, vaginitis, and cervicitis; weak association with Bartholin's gland abscess and involuntary infertility[339,506]; and no association with nongonococcal urethritis epididymitis, urinary calculi, Reiter disease, urethral syndrome, repeated spontaneous abortion and stillbirth, and chorioamnionitis.* With the exception of pelvic inflammatory disease and complications of pregnancy, which occur in adolescents, the other disease associations reported do not involve pediatric patients.

M. hominis has been recovered on two occasions from the CSF of a 2½-year-old girl with a ventriculoperitoneal shunt.[1015] Because she had no complications from the infection and only minimal CSF inflammation, no treatment was initiated. Three months later, the organism could not be isolated from CSF and the child was doing well.

In volunteer studies in adults, researchers found that *M. hominis* could produce exudative pharyngitis.[149] Moffet and associates[685] isolated *M. hominis* from the throat of 1/174 infants and children with pharyngitis but made no similar isolation from a control group of children without pharyngitis. Neu and Ellner[733] recovered *M. hominis* from the throat of 1 child in a group of 56 with exudative pharyngitis. Other *M. hominis* infections include septicemia in a 10-month-old burned infant,[221] chronic multifocal osteomyelitis in an 8-year-old child,[432] septicemia after heart surgery in a 5-year-old girl,[220] severe pneumonia and pericarditis in a healthy 15-year-old female,[777] and exudative vaginitis in a 10-year-old girl.[1015] In one study, 10 patients with sickle-cell disease were found to have acute chest syndrome in association with *M. hominis* recovery from sputum or bronchoscopy specimens.[734] A 4-year-old girl was found to have *M. hominis* endocarditis after biventricular repair of her congenital heart defect.[238] Surgical wound infections have been due to *M. hominis* infections.[536,818]

In adult patients, the following clinical manifestations have been caused by *M. hominis:* mediastinitis,[335,639,709] endocarditis,[288,422,452] isolated pericarditis in a lung transplant recipient,[679] pneumonia in a previously healthy man,[836] ICU-acquired infection in seven critically ill men,[333] soft tissue and cavity infections at surgical sites,[123,526,676,1054] parapharyngeal abscess after Epstein-Barr virus infection,[495] septic arthritis in a patient with hypogammaglobulinemia,[851] brain abscess,[14,423,541,542,1068] and bacteremia in a patient with multiple injuries.[378]

Disease in the Neonate

Infants become colonized during passage through the birth canal, but such colonization tends to not persist. In a recent study of 208 women at delivery, *M. hominis* was recovered from cervicovaginal specimens in 11 percent and the gastric secretions of 1 percent of newborns.[362] In prepubertal children, *M. hominis* only rarely is recovered from urine or genital specimens.

M. hominis also has been associated with neonatal pneumonia,[988] septicemia,[132] and meningitis in premature infants.[399,1036] A review by Hata and colleagues[394] associated poor outcomes with delayed or infective initial therapy of *M. hominis* meningitis, and presented a case in a premature infant that was successfully treated with moxifloxacin, promoting fourth-generation fluoroquinolones as an alternative treatment. A case was found of an infant sustaining birth-related eye injury who subsequently developed multiple subdural empyemas caused by *M. hominis.*[174] Other systemic presentations include brain and scalp abscesses, ventriculitis, submandibular adenitis, abscesses of subcutaneous tissue, pericarditis, and conjunctivitis.[1,351,471,673,794,835]

Differential Diagnosis

Illness caused by *M. hominis* infection rarely occurs in children. This organism should be considered an etiologic possibility in wound infections and a variety of illnesses in which routine cultures are negative. The possibility of *M. hominis* as an etiologic agent also should be considered in adolescent girls with urethritis or pelvic inflammatory disease.

MYCOPLASMA GENITALIUM

Properties

A thorough review by Taylor-Robinson and Jensen[956] summarizes the recent characterization of *M. genitalium*. It is a pleomorphic organism most often found with a tapered end, giving it a flask or bottle shape. It has a length of 0.6 to 0.7 μm and a diameter of 0.3 to 0.4 μm at the broadest part and 0.06 to 0.07 μm at the tip. As in other mycoplasmas, the terminal rodlike structure is believed to play a role in motility, cytoadherence, and binary fission.

Colonies have the typical "fried egg" morphology on solid media. Growth is suppressed on *Mycoplasma* media containing thallous acetate, which is normally used to prevent bacterial contamination. In media containing phenol red and glucose, *M. genitalium* metabolism can be detected by a color change from red to yellow. *M. genitalium* does not metabolize arginine or urea.

M. pneumoniae is closely related to *M. genitalium* and is believed to contain all of the latter's open reading frames within its genome.[408] Because of similarities in their antigens, cross reactivity between the two can occur in serologic testing. The genome of *M. genitalium* (580 kb) is much smaller, however, which necessitates efficiency. Rather than encode gene regulators, it is believed to rely on DNA supercoiling,[245] antisense RNA,[593] and environmental signals such as osmotic stress[1067] and temperature[707] to modify transciption. It is also able to produce unlimited variants of its genes through homologous recombination within its own

*References 87, 112, 134, 265, 266, 343, 379, 543, 767, 768, 827, 884, 924, 958, 981, 982.

genome and through gene exchange between different clones.[443,610]

Epidemiology

Over recent years, *M. genitalium* has become recognized as an STI that causes nongonococcal urethritis in men and is strongly implicated in several inflammatory reproductive tract syndromes in women, including cervicitis, endometritis, pelvic inflammatory disease, and infertility in both men and women.[82,339,382,620,655] A comprehensive review of the literature by McGowin and associates[656] found a worldwide incidence of 7.3 percent and 2.0 percent, in high- and low-risk female populations, respectively. Rates of infection are not associated with ethnicity, gender, isolation site, or previous pregnancies in women,[554,826] but have occasionally been shown to be associated with younger age, thought to be related to sexual promiscuity and risky behavior.[470,684] *M. genitalium* infection was shown to be significantly reduced in circumcised versus uncircumcised men (8.2% and 13.4%, respectively).[663] Studies in the adult general population abroad have shown an asymptomatic carrier rate of 0.3 percent in Korea,[506] 0.6 percent in the United Kingdom,[826] and 1 percent in Japan,[945] whereas in a study by the University of Illinois, investigators reported that of the 52 male patients with *M. genitalium* in their patient population in Kenya (9.9% prevalence), all but one were asymptomatic.[663]

In addition, multiple studies have shown elevated coinfection rates of *M. genitalium* with HIV.[338,470,640,720] It is unclear whether this relationship is pathologically significant or merely represents the elevated risk for multiple infections in people with high-risk sexual activity.[619,956] Reports are conflicting about whether infection with *M. genitalium* is associated with increased vaginal HIV shedding.[338,623,656]

The organism also has been recovered from the respiratory tract of patients with pneumonia who were participating in an *M. pneumoniae* vaccine trial.[52] Patel and colleagues[778] found colonies in the lower respiratory tract of a large pediatric population with chronic respiratory disease to be 9.8 percent.

Clinical Manifestations

The role of *M. genitalium* as a sexually transmitted pathogen was initially unclear and required careful characterization, including studies of disease in experimentally infected chimpanzees.[958,978,982] With the use of PCR, Jensen and associates[463] presented evidence suggesting a causative role in some cases of nongonococcal urethritis. *M. genitalium* is an emerging STI[622] and a cause of mucopurulent cervicitis in women[621] and nongonococcal urethritis in men.[664]

M. genitalium has been recovered from pediatric patients with pneumonia and chronic respiratory disease.[52,778] Respiratory colonization has been shown to occur during neonatal passage though the birth canal for other mycoplasmas. Hematogenous spread of *M. genitalium* is also possible and has been postulated as the mechanism for cases of arthritis with this organism.[956,979]

Differential Diagnosis

M. genitalium presents most commonly as an STI causing pathology in the lower genital tracts of both men and women and a possible role in upper tract inflammation. It should be considered in the workup of adolescents with urethritis or pelvic inflammatory disease.

MYCOPLASMA FERMENTANS

M. fermentans originally was isolated from the genital tract of men and women over 50 years ago, but it has not been established as a cause of genitourinary disease.[831,953,978] This organism has been isolated from the blood of leukemia patients, from the joint fluid of patients with arthritis, and from the blood and urine of patients with acquired immunodeficiency syndrome (AIDS).* *M. fermentans* has been identified by PCR in the peripheral blood mononuclear cells and lymph nodes of HIV-infected patients.[395,486,533,850] The organism also has been recovered from the blood of homosexual men without HIV infection.[486,533,613] *M. fermentans* has been identified in the blood and synovial fluid of patients with inflammatory arthritic diseases, including rheumatoid arthritis.[345,469,910]

MYCOPLASMA PENETRANS

M. penetrans is a relatively newly recognized species isolated from the urogenital tract of patients with AIDS.[594,595] In a seroprevalence study, Wang and associates[1028] found that 35.4 percent of HIV-infected patients had antibody versus only 0.4 percent of HIV-seronegative subjects. They subsequently noted a high prevalence of antibody to *M. penetrans* in the sera of homosexual men but not in the sera of other HIV transmission groups.[1029] In a more recent study, Grau and colleagues[364] found that 18.2 percent of HIV-infected patients had antibody to *M. penetrans*, whereas only 1.3 percent of HIV-seronegative persons had antibody. *M. penetrans* antibody seroprevalence increased with progression of HIV-associated disease, and it was associated predominantly with homosexual practices in the HIV-infected patients. No pediatric data relating to *M. penetrans* seroprevalence are available.

DIAGNOSIS OF CONGENITAL MYCOPLASMAS

M. hominis, *U. urealyticum*, and *U. parvum* are ubiquitous in the genital tracts of normal persons and frequent colonizers of mucosal surfaces in infants; therefore, assigning causation of disease based on isolation is difficult. *M. genitalium* is becoming more widely recognized as an STI, and its identification in cases of urethritis carries more suspicion. Culture or PCR for mycoplasmas may be warranted in nongonoccocal urethritis when smears reveal polymorphonuclear neutrophils without

*References 53, 85, 630, 690, 705, 1034, 1046.

organisms suggestive of gonococci. Isolation of these species from a normally sterile site or suppurative focus warrants further investigation, especially in newborns and others with weakened immune systems. The clinical significance of isolation of genital mycoplasmas from urine obtained by suprapubic bladder aspiration in infants remains to be determined.[579] In such instances, analysis of urinary sediment has been normal.

Because of their fastidious growth, these species cannot be identified by routine bacteriologic culture methods, and therefore specific culture for *Mycoplasma* must be requested. Genital mycoplasmas may be isolated on special broth and solid media that are available commercially. Shepard 10B broth and A8 agar have been used successfully for cultivation.[132] A recent study comparing different culture specimens found that urethral swabs were more accurate (specificity, 99.9%; positive predictive value [PPV], 99.6%) than vaginal swabs (specificity 95.1%; PPV, 67%) or urine cultures (specificity, 96%; PPV, 75%) for detecting *M. hominis* in women with lower urinary tract symptoms, with similar numbers for *U. urealyticum*.[431]

Cassell and colleagues[131,132] recommended that mucosal specimens be obtained with a Dacron or calcium alginate swab and be placed in a specific mycoplasmal transport medium such as Shepard 10B broth. Specimens should be refrigerated at 4° C (39.2° F) until they are transported to the laboratory and should be protected from drying. Alternatively, specimens in appropriate transport media can be frozen at −70° C (−94° F) because mycoplasmas are stable for long periods under these conditions. Specimens should be diluted serially in 10B broth to at least 10^{-3} (preferably to 10^{-5}) to overcome any potential inhibitory substances or metabolites, and an aliquot of the original sample and dilution should be plated directly onto A8 agar. Body fluids (e.g., blood, CSF, and pleural fluid) should be inoculated into 10B broth in an approximate 1 : 10 ratio (usually 0.1 mL fluid/0.9 mL 10B broth). Blood should be collected free of anticoagulants. Broth cultures and agar plates are incubated under 95 percent nitrogen and 5 percent carbon dioxide. Cultures generally become positive within 2 to 5 days. The presence of mycoplasmal growth in 10B medium is indicated by a change in color from yellow to pink, which is caused by an alkaline shift in the media as a result of either the urease activity of ureaplasmas or arginine hydrolysis by other *Mycoplasma* spp. Growth of mycoplasmas in broth culture, as indicated by a change in color, should be confirmed by inoculation of a broth specimen onto A8 agar. Characteristic colonies can be identified readily on A8 agar after 24 to 72 hours of incubation.

Serologic tests have been used to measure antibody to genital mycoplasmas. Such tests include the metabolic inhibition assay, enzyme-linked immunosorbent assay, mycoplasmacidal test, indirect hemagglutination, indirect immunofluorescence, and IgG and IgM immunoblotting.[132,236,330,802] Because of the presence of maternal antibodies, as well as their own poorly functional immune system, use of these tests for establishing the diagnosis of mycoplasmal infection in infants remains problematic and is not well established. In these cases, diagnosis should rely on culture results and/or PCR.

PCR assays have become commercially available over recent years for detecting genital mycoplasmas.[86,821,929,1063] Comparison studies show that PCR is a more rapid and sensitive method of detection for genital mycoplasmas than culture. On endotracheal secretions, Blanchard and associates[86] reported finding a sensitivity of 100 percent and a specificity of 99 percent for *Ureaplasma* spp.

PCR assays involving the urease gene, 16S RNA genes, and multiple-banded antigen genes have been used to detect *Ureaplasma* spp.* PCR detection of 16S rRNA and ribosomal DNA targets of *M. hominis*[6,363,605] and the 23S rRNA[460] and 16S rRNA[678] from *M. genitalium* are available. Assays now exist that can screen for multiple *Mycoplasma*-related species simultaneously, using fluorescence polarization,[48] PCR-RFLPs,[678] multiplex PCR[658-660,841] and oligonucleotide microarrays.[453] Ultimately, these methods may aid in identifying colonized or infected infants more readily (results available in 1 day) and reliably, given the fastidious nature of these organisms and the scarcity of microbiology laboratories that routinely perform mycoplasmal culture.

OTHER *MYCOPLASMA* SPECIES AND *MYCOPLASMA* RELATIONSHIPS

Mycoplasma Salivarium

M. salivarium is part of the normal human oral flora and has been thought to occasionally play a role in oral and periodontal infections[1032]; however, it has rarely been associated with disease in other sites of the body. Examples include arthritic joints such as the knee of a 39-year-old-woman with hypogammaglobulinemia,[906] the knees of rheumatoid arthritis patients,[469] the synovial fluid of patients (including a 15- and 16-year-old girl) with temperomandibular joint disorders,[1032] a submasseteric abscess in an 84-year-old woman,[373] and an empyema in a 39-year-old man with laryngeal cancer.[49] The organism was also found in the polymicrobial biofilm of an occluded biliary stent in a 71-year-old man with liver cirrhosis.[406]

Mycoplasma Pirum

M. pirum originally was recovered from eukaryotic cell cultures, and its origin was traced to a human tumor cell line.[15,230,557] It has been recovered more recently from primary lymphocyte cells in patients with AIDS.[85]

Zoonotic *Mycoplasma*

Mycoplasma spp. have been identified that infect a wide variety of birds, reptiles, fish, and mammals. Human infection with these species is rare but can occur when there is close contact, such as in veterinary medicine, animal training, hunting, fishing, and agriculture. There have been reports of fishermen with open hand wounds or bites catching *Mycoplasma phocacerebrale* from seals. Commonly referred to as "seal finger," this disease

*References 6, 7, 21, 86, 156, 173, 216, 605, 608, 731, 821, 861, 963, 1062.

involves cellulitis of the affected area with eventual joint involvement if not treated.[43,455,1043] Hemotropic mycoplasmas (also known as "hemoplasmas") cause hemolytic anemia in many domestic and farm animals, and cases have been described of humans being infected through arthropod vectors associated with pigs, sheep, cattle, dogs, and cats,[92,246,429,939,1034,1065] and in one case a novel hemoplasma with no identified origin was found in a 60-year-old woman.[927] Coinfections with *Bartonella* spp. are not uncommon. Outbreaks of hemoplasmas in China have been described, including a high prevalence of congenital infection with severe disease.[429,1056]

Acquired Immunodeficiency Syndrome–Associated Mycoplasmal Infections

The frequent identification of *M. fermentans*, *M. pirum*, and *M. penetrans* infections in HIV-infected patients led to the consideration that they may function as cofactors in the progression of HIV infection and immunodeficiency.[85,530,419,690] Although these mycoplasmas have the capacity to invade cells and to be potent immunomodulators, their pathogenic role, if any, in association with HIV has not yet been determined. Studies on *U. urealyticum*, *M. hominis*, *M. salivarium*, and *M. orale* infection rates in HIV-positive and HIV-negative subjects showed no significant differences[151,549]

Mycoplasma and *Ureaplasma* Infections in Immunocompromised Patients

Patients with hypogammaglobulinemia are susceptible to severe persistent infection with *Ureaplasma* spp., *M. hominis*, *M. amphoriforme*, *M. pneumoniae*, *M. salivarium*, and *M. orale*.* Clinical manifestations include osteomyelitis, arthritis, cellulitis, and chronic respiratory tract illness. Patients need to be treated for prolonged periods with high-dose intravenous immunoglobulin and antibiotics to which the specific agents are susceptible. Severe and persistent infections also have occurred in liver, kidney, and bone marrow transplant recipients, as well as other immunocompromised patients.†

Yechouron and associates[1058] reported a 64-year-old man with Hodgkin lymphoma who died of septicemia caused by *Mycoplasma arginini*, an animal pathogen. A similar case was described by Watanabe and Colleges.[1033] Three children with cancer had pneumonia in which bronchoalveolar lavage fluid specimens yielded *U. urealyticum*.[117] Two of the patients died, and the survivor's improvement coincided with erythromycin treatment. A 9-year-old boy had an abdominal infection with *M. hominis* after liver transplantation,[602] and a 15-year-old girl had septic arthritis with *M. hominis* 2 months after renal transplantation.[670]

Mycoplasma and Cancer

Mycoplasma invasion into human cells and subsequent chronic inflammatory processes have been proposed as potential mechanisms of tumorigenesis, alone or in conjunction with other oncogenic infections. Studies have attempted to link *Mycoplasma* infections with prostate cancer and lung cancer.[822] Charles Rosser's laboratory has been able to induce malignant transformation of human prostate cells in vitro using *Mycoplasma*.[719] Studies have also found an increased rate of *M. hominis* infection in patients with prostate cancer.[51] Studies in women with high-grade cervical dysplasia found that a majority were also infected with *Ureaplasma* spp. with or without *M. hominis*.[262] Genital mycoplasmas have been considered an important cofactor for human papillomavirus infection.[78,262]

Mycoplasma as a Cell Culture Contaminant

When *Mycoplasma* spp. were still first being recognized as human pathogens, they were also being studied as a prevalent and serious contaminant in the research and biopharmaceutical industries.[29] Because they are slow-growing, pleomorphic, and resistant to β-lactams and other antibiotics frequently used to eliminate bacteria from cell culture media, contamination is often difficult to identify. Possible sources of *Mycoplasma* include animal- and plant-derived raw materials in culture media and the skin from workers with poor aseptic technique. Fortunately, with better antibiotic options and rapid detection by PCR, these issues are resolving.[631,870]

TREATMENT OF GENITAL MYCOPLASMAS

Having no cell wall, mycoplasmas are naturally resistant to β-lactam antibiotics (i.e., penicillins, cephalosporins, monobactams, and carbapenems), polymyxins, sulfonamides, and vancomycin.[98,1010,1011,1015] Although they may have moderate sensitivity to the aminoglycosides, the minimal inhibitory concentrations (MICs) of these agents for genital mycoplasmas usually are too high for therapeutic use. Tetracyclines and macrolides have been the drugs of choice, although because of concern for tetracycline toxicities and the comparative ease of taking a short course of azithromycin, the latter has come more into favor. Azithromycin is also seen as having the added benefit of treating both gonococcal and chlamydial genital infections when the etiology is not yet identified or could be polymicrobial. However, antibiotic resistance is growing, and some *Mycoplasma* spp. now require alternative regimens. Newer studies show that ofloxacin, moxifloxacin, and levofloxacin have retained good activity against all genital mycoplasmas and ureaplasmas.[60,535,944] When possible, antibiotic susceptibility testing should be performed on all clinically significant isolates. The discovery that genital mycoplasmas may also create biofilms, protecting them from antibiotic activity, would further complicate therapy.[334]

Ureaplasma spp. have retained susceptibility to macrolides, doxycycline, tetracycline, and chloramphenicol. Resistance to ciprofloxacin is developing; however, oxifloxacin and moxifloxacin are still effective and better than levofloxacin.[535] Doxycycline has been recognized as a top choice for ureaplasmal infections while also

*References 32, 308, 323, 688, 797, 823, 906.
†References 184, 450, 481, 609, 677, 785, 914, 1003.

being effective against coinfection with *M. hominis* or gonorrhea.* Erythromycin is also commonly used, although it is less favored as a result of growing resistance[535] and adverse effects. Cardiac toxicity consisting of acute cardiorespiratory deterioration possibly secondary to cardiac arrhythmias has been reported in neonates treated with intravenous erythromycin lactobionate for presumed ureaplasmal pneumonia.[280] Ototoxicity has been seen in adults but not in neonates.[212] Oral erythromycin has been associated with hypertrophic pyloric stenosis in infants younger than 6 weeks.[140] Azithromycin and clarithromycin are active in vitro against *Ureaplasma* spp.,[492,833,905,1013] but their use in neonates has not been evaluated thoroughly. When given orally to infants with very low birthweight colonized with *Ureaplasma* spp., serum levels of clarithromycin at a dose of 7.5 mg/kg every 12 hours were subtherapeutic.[845] For this reason, a dose of 15 mg/kg every 12 hours is recommended. Although the exact duration of therapy is not known, a 10- to 14-day course seems reasonable when clinical improvement and microbiologic eradication are observed during that period.

M. hominis has developed marked resistance to common macrolides, and they are no longer recommended for treatment.[833,1013] By contrast, 16-membered ring macrolides such as josamycin and related lincosamides have shown effectiveness against *M. hominis;* however, they have not been extensively studied and are not FDA approved in the United States.[492,535,782,1069] Other therapies include chloramphenicol, clindamycin, rifampicin, quinolones (i.e., ofloxacin and moxifloxacin), and tetracyclines (doxycycline especially).[60,516,1010] *M. hominis* is usually sensitive to tetracyclines, and these antibiotics should be used if the benefits outweigh the risk for toxicity.[98,121,933,951,1042] During the past decade, resistance of *M. hominis* to tetracycline itself has increased; however, doxycycline remains effective.[215,535,644]

M. genitalium remains sensitive to macrolides and some quinolones such as moxifloxacin.[384,923,944,964] Azithromycin has been a favored choice because it also treats both gonococcal and chlamydial genital infections when the etiology is not yet identified or could be polymicrobial. However, resistance of *M. genitalium* to azithromycin is beginning to appear; therefore, 5-day dosing is being recommended over single-dose treatment.[97,175,462,886] Extended dosing with azithromycin has been found to be significantly more effective than even multidose doxyclycline.[83,665]

Mycoplasmas are not susceptible to the antimicrobial agents routinely used to treat neonatal infections. The decision to cover for possible mycoplasmal infection should be based on the clinical picture and culture results. In general, isolation of mycoplasmas from a normally sterile site in an ill neonate is an indication for consideration of treatment. The possibility of *Mycoplasma* infection also should be suspected when a child appears septic and is not responding to standard antibacterial or antiviral therapy, and appropriate samples for *Mycoplasma* culture or PCR assay should be obtained. However, no large randomized clinical trials have determined the efficacy of treatment in neonates. Experience on which to base treatment decisions, the choice of drug, and the duration of therapy is very limited.[1025]

*References 57, 516, 535, 637, 995, 1010, 1020, 1069.

Isolation of mycoplasmas from endotracheal secretions is not diagnostic of pneumonia, and most of these infants do not require any antimycoplasmal therapy. However, if pneumonia is suspected and the infant's clinical condition is deteriorating, a trial of therapy may be indicated despite the unknown efficacy of treatment. Azithromycin has been used for its anti-inflammatory properties to protect infants from BPD. However, the safety and efficacy of azithromycin in infants with very low birth-weight has not yet been studied.

Mutations in antibiotic targets that confer resistance have been identified, as well as resistance genes carried on plasmids or transposable genetic elements, sometimes conferring resistance to multiple groups of antibiotics.* Recently a group has shown that if the site of the mutation is known, high-resolution melt analysis can be used to rapidly identify antibiotic resistance patterns at the time of the initial diagnosis and eliminate the risk for treatment failure.[984]

PREVENTION OF GENITAL MYCOPLASMAS

Erythromycin administered between 26 and 35 weeks' gestation to pregnant women colonized with *Ureaplasma* spp. was not effective in reducing adverse outcomes such as preterm delivery, low birth-weight, or premature rupture of membranes.[270] In the ORACLE I study,[499] in which the use of broad-spectrum antibiotics was evaluated in mothers with premature rupture of membranes, use of erythromycin was associated with prolongation of pregnancy, reduction in neonatal treatment with surfactant, decrease in oxygen dependence at 28 days of age and older, fewer major cerebral abnormalities on ultrasonography before discharge, and fewer positive blood cultures. The ORACLE II study, which consisted of 6295 women with spontaneous preterm labor, intact membranes, and no evidence of clinical infection, showed that none of the trial antibiotics were associated with a lower rate of the composite primary outcome events than occurred in placebo recipients.[500] Because erythromycin therapy does not eliminate *Ureaplasma* spp. from the lower genital tract, it most likely also does not prevent neonatal ureaplasmal colonization.[752] Smorgick and associates[905] reported on a woman with preterm labor at 27 weeks' gestation and amniotic colonization with *U. urealyticum*, treated with 3 days of ciprofloxacin that was changed to 7 additional days of erythromycin after developing an allergic pruritic reaction. This regimen failed to eradicate the organism, and she was switched to 10 days of intravenous clindamycin and oral levofloxacin. Although there was no further testing of amniotic fluid for infection, the patient was able to delay delivery for 6 weeks and the infant was healthy despite placental pathology revealing chorioamnionitis with acute funisitis. Smorgick and associates[905] support that in cases of *Mycoplasma* spp. intra-amniotic colonization remote from term, expectant management is possible and that fluoroquinolones, clindamycin, or both may be more effective than erythromycin.

*References 460, 461, 535, 782, 885, 886.

The effect on the effect of erythromycin on prevention of neonatal disease has not been evaluated fully.[26] Administration of erythromycin to preterm infants whose mothers had *Ureaplasma* spp. in their lower genital tract reduced respiratory tract colonization but did not decrease the duration of supplemental oxygen therapy[474] or chronic lung disease.[96,608] Treating infants with very low birthweight who have respiratory tract colonization with *Ureaplasma* spp. to prevent chronic lung disease cannot be recommended at present.[118]

Patients with nongonococcal urethritis should be treated with tetracycline 40 mg/kg/day every 6 hours; persons weighing more than 50 kg should receive 500 mg every 6 hours for 10 days.[388,412] *Chlamydia* and *Ureaplasma* are also sensitive to erythromycin, so this antibiotic is a useful alternative for patients in whom tetracycline is contraindicated. With adolescent patients, it is prudent to seek out and treat the sex partners whenever possible.

NEW REFERENCES SINCE THE SIXTH EDITION

14. Al Masalma M, Drancourt M, Dufour H, et al. *Mycoplasma hominis* brain abscess following uterus curettage: a case report. *J Med Case Rep* 2011;**5**:278.
31. Ascer E, Marques M, Gidlund M. *M pneumoniae* infection, pulmonary thromboembolism and antiphospholipid antibodies. *BMJ Case Rep* 2011:2011.
36. Aviner S, Miskin H, London D, et al. *Mycoplasma pneumonia* infection: a possible trigger for immune thrombocytopenia. *Indian J Hematol Blood Transfus* 2011;**27**:46–50.
49. Baracaldo R, Foltzer M, Patel R, et al. Empyema caused by *Mycoplasma salivarium*. *J Clin Microbiol* 2012;**50**:1805–6.
51. Barykova YA, Logunov DY, Shmarov MM, et al. Association of *Mycoplasma hominis* infection with prostate cancer. *Oncotarget* 2011;**2**:289–97.
58. Bayram A, Erdogan MB, Eksi F, et al. Demonstration of *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, Cytomegalovirus, and Epstein-Barr virus in atherosclerotic coronary arteries, nonrheumatic calcific aortic and rheumatic stenotic mitral valves by polymerase chain reaction. *Anadolu Kardiyol Derg* 2011;**11**:237–43.
78. Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, et al. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. *Adv Med Sci* 2011;**56**:299–303.
82. Bjartling C, Osser S, Persson K. *Mycoplasma genitalium* in cervicitis and pelvic inflammatory disease among women at a gynecologic outpatient service. *Am J Obstet Gynecol* 2012;**206**:476.e471–8.
88. Blanco S, Fuenzalida L, Bas A, et al. Comparison of 2 molecular assays and a serologic test in diagnosing *Mycoplasma pneumoniae* infection in paediatrics patients. *Diagn Microbiol Infect Dis* 2011;**71**:463–6.
105. Bressan S, Mion T, Andreola B, et al. Severe *Mycoplasma pneumoniae*-associated mucositis treated with immunoglobulins. *Acta Paediatr* 2011;**100**:e238–40.
109. Browning GF, Marenda MS, Noormohammadi AH, et al. The central role of lipoproteins in the pathogenesis of mycoplasmoses. *Vet Microbiol* 2011;**153**:44–50.
126. Cardinale F, Chironna M, Dumke R, et al. Macrolide-resistant *Mycoplasma pneumoniae* in paediatric pneumonia. *Eur Respir J* 2011;**37**:1522–4.
141. Chalker V, Stocki T, Litt D, et al. Increased detection of *Mycoplasma pneumoniae* infection in children in England and Wales, October 2011 to January 2012. *Euro Surveill* 2012:**17**.
156. Cheong KA, Agrawal SR, Lee AY. Validation of nested PCR and a selective biochemical method as alternatives for mycoplasma detection. *J Basic Microbiol* 2011;**51**:215–19.
170. Chiang CH, Huang CC, Chan WL, et al. Association between *Mycoplasma pneumonia* and increased risk of ischemic stroke: a nationwide study. *Stroke* 2011;**42**:2940–3.
173. Choi SJ, Park SD, Jang IH, et al. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. *Ann Lab Med* 2012;**32**:194–200.
175. Chrisment D, Charron A, Cazanave C, et al. Detection of macrolide resistance in *Mycoplasma genitalium* in France. *J Antimicrob Chemother* 2012;**67**:2598–601.
223. Daxbock F, Assadian A, Watkins-Riedel T, et al. Persistently elevated IgA antibodies to *Mycoplasma pneumoniae* in patients with internal carotid artery stenosis. *GMS Krankenhhyg Interdiszip* 2011;**6**:Doc04.
245. Dorman CJ. Regulation of transcription by DNA supercoiling in *Mycoplasma genitalium:* global control in the smallest known self-replicating genome. *Mol Microbiol* 2011;**81**:302–4.
250. Dua J, Nandagudi A, Sutcliffe N. *Mycoplasma pneumoniae* infection associated with urticarial vasculitis mimicking adult-onset Still's disease. *Rheumatol Int* 2012;**32**:4053–6.
252. Dumke R, Strubel A, Cyncynatus C, et al. Optimized serodiagnosis of *Mycoplasma pneumoniae* infections. *Diagn Microbiol Infect Dis* 2012;**73**:200–3.
258. Ebrahim M, Gabay M, Rivas-Chacon RF. Evidence of acute *Mycoplasma* infection in a patient with incomplete and atypical kawasaki disease: a case report. *Case Report Med* 2011:606920.
261. Eibach D, Casalegno JS, Escuret V, et al. Increased detection of *Mycoplasma pneumoniae* infection in children, Lyon, France, 2010 to 2011. *Euro Surveill* 2012:**17**.
271. Shima N, Tokumaru O, Hara S, et al. Age-specific sex-related differences in infections: a statistical analysis of national surveillance data in Japan. *PLoS One* 2012;**7**:e42261.
329. Gable MS, Sheriff H, Dalmau J, et al. The frequency of autoimmune N-methyl-D-aspartate receptor encephalitis surpasses that of individual viral etiologies in young individuals enrolled in the California Encephalitis Project. *Clin Infect Dis* 2012;**54**:899–904.
338. Gatski M, Martin DH, Theall K, et al. *Mycoplasma genitalium* infection among HIV-positive women: prevalence, risk factors and association with vaginal shedding. *Int J STD AIDS* 2011;**22**:155–9.
357. Good JT Jr, Rollins DR, Martin RJ. Macrolides in the treatment of asthma. *Curr Opin Pulm Med* 2012;**18**:76–84.
377. Grub C, Brunborg C, Hasseltvedt V, et al. Antibodies to common infectious agents in coronary artery disease patients with and without rheumatic conditions. *Rheumatology (Oxford)* 2012;**51**:679–85.
382. Haggerty CL, Taylor BD. *Mycoplasma genitalium:* an emerging cause of pelvic inflammatory disease. *Infect Dis Obstet Gynecol* 2011;**2011**:959816.
384. Hagiwara N, Yasuda M, Maeda S, et al. In vitro activity of azithromycin against *Mycoplasma genitalium* and its efficacy in the treatment of male *Mycoplasma genitalium*-positive nongonococcal urethritis. *J Infect Chemother* 2011;**17**:821–4.
393. Hasselbring BM, Sheppard ES, Krause DC. P65 truncation impacts P30 dynamics during *Mycoplasma pneumoniae* gliding. *J Bacteriol* 2012;**194**:3000–7.
396. Hawkins S, Rausch CM, McCanta AC. Constrictive pericarditis secondary to infection with *Mycoplasma pneumoniae*. *Curr Opin Pediatr* 2011;**23**:126–9.
431. Humburg J, Frei R, Wight E, et al. Accuracy of urethral swab and urine analysis for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in women with lower urinary tract symptoms. *Arch Gynecol Obstet* 2012;**285**:1049–53.
452. Jamil HA, Sandoe JA, Gascoyne-Binzi D, et al. Late-onset prosthetic valve endocarditis caused *by Mycoplasma hominis*, diagnosed using broad-range bacterial PCR. *J Med Microbiol* 2012;**61**:300–1.
455. Jansen LC, Justesen US, Roos SM, et al. [Seal finger in Denmark diagnosed by PCR-technique]. *Ugeskr Laeger* 2012;**174**:426–7.
460. Jensen JS. Protocol for the detection of *Mycoplasma genitalium* by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. *Methods Mol Biol* 2012;**903**:129–39.
467. Johansson N, Kalin M, Hedlund J. Clinical impact of combined viral and bacterial infection in patients with community-acquired pneumonia. *Scand J Infect Dis* 2011;**43**:609–15.
470. Johnston LG, Paz-Bailey G, Morales-Miranda S, et al. High prevalence of *Mycoplasma genitalium* among female sex workers

in Honduras: implications for the spread of HIV and other sexually transmitted infections. *Int J STD AIDS* 2012;**23**:5–11.
490. Kawai Y, Miyashita N, Yamaguchi T, et al. Clinical efficacy of macrolide antibiotics against genetically determined macrolide-resistant *Mycoplasma pneumoniae* pneumonia in paediatric patients. *Respirology* 2012;**17**:354–62.
506. Kim SJ, Lee DS, Lee SJ. The prevalence and clinical significance of urethritis and cervicitis in asymptomatic people by use of multiplex polymerase chain reaction. *Korean J Urol* 2011;**52**: 703–8.
514. Klein NC, Petelin A, Cunha BA. *Mycoplasma pneumoniae* preceding Lemierre's syndrome due to *Fusobacterium nucleatum* complicated by acute Epstein-Barr virus (EBV) infectious mononucleosis in an immunocompetent host. *Heart Lung* 2013;**42**:74–6.
524. Kornspan JD, Tarshis M, Rottem S. Adhesion and biofilm formation of *Mycoplasma pneumoniae* on an abiotic surface. *Arch Microbiol* 2011;**193**:833–6.
526. Koshiba H, Koshiba A, Daimon Y, et al. Hematoma and abscess formation caused by *Mycoplasma hominis* following cesarean section. *Int J Womens Health* 2011;**3**:15–18.
540. Kunimi Y, Hirata Y, Aihara M, et al. Statistical analysis of Stevens-Johnson syndrome caused by *Mycoplasma pneumonia* infection in Japan. *Allergol Int* 2011;**60**:525–32.
560. Lee MN, Cha JH, Ahn HM, et al. *Mycoplasma pneumoniae* infection in patients with Kawasaki disease. *Korean J Pediatr* 2011; **54**:123–7.
569. Lenglet A, Herrador Z, Magiorakos AP, et al. Surveillance status and recent data for *Mycoplasma pneumoniae* infections in the European Union and European Economic Area, January 2012. *Euro Surveill* 2012:**17**.
603. Lu A, Wang L, Zhang X, et al. Combined treatment for child refractory *Mycoplasma pneumoniae* pneumonia with ciprofloxacin and glucocorticoid. *Pediatr Pulmonol* 2011;**46**:1093–7.
619. Manhart LE. Another STI associated with HIV-1 acquisition: now what? *AIDS* 2012;**26**:635–7.
627. Manwani NS, Balasubramanian S, Dhanalakshmi K, et al. Stevens johnson syndrome in association with *Mycoplasma pneumonia*. *Indian J Pediatr* 2012;**79**:1097–9.
630a. Marc E, Chaussain M, Moulin F, et al. Reduced lung diffusion capacity after Mycoplasma pneumoniae pneumonia. *Pediatr Infect Dis J* 2000;**19**:706–10.
631. Mariotti E, D'Alessio F, Mirabelli P, et al. Mollicutes contamination: a new strategy for an effective rescue of cancer cell lines. *Biologicals* 2012;**40**:88–91.
640. Mavedzenge SN, Van Der Pol B, Weiss HA, et al. The association between *Mycoplasma genitalium* and HIV-1 acquisition in African women. *AIDS* 2012;**26**:617–24.
654. McGouran DC, Petterson T, McLaren JM, et al. Mucositis, conjunctivitis but no rash: the "atypical Stevens-Johnson syndrome." *Acute Med* 2011;**10**:81–2.
655. McGowin CL, Anderson-Smits C. *Mycoplasma genitalium:* an emerging cause of sexually transmitted disease in women. *PLoS Pathog* 2011;**7**:e1001324.
660. McKechnie ML, Hillman RJ, Jones R, et al. The prevalence of urogenital micro-organisms detected by a multiplex PCR-reverse line blot assay in women attending three sexual health clinics in Sydney, Australia. *J Med Microbiol* 2011;**60**:1010–16.
662. Medina JL, Coalson JJ, Brooks EG, et al. *Mycoplasma pneumoniae* CARDS toxin induces pulmonary eosinophilic and lymphocytic inflammation. *Am J Respir Cell Mol Biol* 2012;**46**:815–22.
663. Mehta SD, Gaydos C, Maclean I, et al. The effect of medical male circumcision on urogenital *Mycoplasma genitalium* among men in Kisumu, Kenya. *Sex Transm Dis* 2012;**39**:276–80.
668. Meyer Sauteur PM, Gansser-Kalin U, Lautenschlager S, et al. Fuchs syndrome associated with *Mycoplasma pneumoniae* (Stevens-Johnson syndrome without skin lesions). *Pediatr Dermatol* 2011;**28**:474–6.
678. Mirnejad R, Amirmozafari N, Kazemi B. Simultaneous and rapid differential diagnosis of *Mycoplasma genitalium* and *Ureaplasma urealyticum* based on a polymerase chain reaction-restriction fragment length polymorphism. *Indian J Med Microbiol* 2011; **29**:33–6.
684. Mobley VL, Hobbs MM, Lau K, et al. *Mycoplasma genitalium* infection in women attending a sexually transmitted infection clinic: diagnostic specimen type, coinfections, and predictors. *Sex Transm Dis* 2012;**39**:706–9.
702. Muir MT, Cohn SM, Louden C, et al. Novel toxin assays implicate *Mycoplasma pneumoniae* in prolonged ventilator course and hypoxemia. *Chest* 2011;**139**:305–10.
738. Nir-Paz R, Abutbul A, Moses AE, et al. Ongoing epidemic of *Mycoplasma pneumoniae* infection in Jerusalem, Israel, 2010 to 2012. *Euro Surveill* 2012:**17**.
748. Nunes JC, Bruscato AM, Walz R, et al. Opsoclonus-myoclonus syndrome associated with *Mycoplasma pneumoniae* infection in an elderly patient. *J Neurol Sci* 2011;**305**:147–8.
754. Oishi T, Narita M, Ohya H, et al. Rhabdomyolysis associated with antimicrobial drug-resistant *Mycoplasma pneumoniae*. *Emerg Infect Dis* 2012;**18**:849–51.
770. Pana Z, Hatzipantelis E, Douma S, et al. An unusual case of *Mycoplasma pneumoniae*-associated autoimmune haemolytic anaemia in a 10-year-old female child. *J Paediatr Child Health* 2012;**48**:621–2.
773. Park SJ, Lee YM, Lee CH, et al. A case of splenic infarction possibly attributable to *Mycoplasma pneumoniae* infection without accompanying pneumonia. *J Infect Chemother* 2012.
778. Patel KK, Salva PS, Webley WC. Colonization of paediatric lower respiratory tract with genital *Mycoplasma species*. *Respirology* 2011;**16**:1081–7.
781. Pemira SM, Tolan RW Jr. *Mycoplasma pneumoniae* infection presenting as bullous papular purpuric gloves and socks syndrome: novel association and review of the literature. *Clin Pediatr (Phila)* 2011;**50**:1140–3.
796. Powers JM, Johnson MD. Mycoplasmal panencephalitis: a neuropathologic documentation. *Acta Neuropathol* 2012;**124**: 143–8.
806. Ramasamy A, Patel C, Conlon C. Incomplete Stevens-Johnson syndrome secondary to atypical pneumonia. *BMJ Case Rep* 2011.
822. Rogers MB. *Mycoplasma* and cancer: in search of the link. *Oncotarget* 2011;**2**:271–3.
839. Salmeri M, Valenti D, La Vignera S, et al. Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* infection in unselected infertile men. *J Chemother* 2012;**24**:81–6.
841. Samra Z, Rosenberg S, Madar-Shapiro L. Direct simultaneous detection of 6 sexually transmitted pathogens from clinical specimens by multiplex polymerase chain reaction and auto-capillary electrophoresis. *Diagn Microbiol Infect Dis* 2011;**70**:17–21.
851. Sato H, Iino N, Ohashi R, et al. Hypogammaglobulinemic patient with polyarthritis mimicking rheumatoid arthritis finally diagnosed as septic arthritis caused by *Mycoplasma hominis*. *Intern Med* 2012;**51**:425–9.
852. Sauteur PM, Goetschel P, Lautenschlager S. *Mycoplasma pneumoniae* and mucositis: part of the Stevens-Johnson syndrome spectrum. *J Dtsch Dermatol Ges* 2012;**10**:740–6.
857. Schalock PC, Thyssen JP, Dinulos JG. *Mycoplasma pneumoniae*-associated mucositis: by any other name is not so sweet. *Pediatr Dermatol* 2012;**29**:392.
865. Schomburg J, Vogel M. A 12-year-old boy with severe mucositis: extrapulmonary manifestation of *Mycoplasma pneumoniae* infection. *Klin Padiatr* 2012;**224**:94–5.
877. Shen Y, Zhang J, Hu Y, et al. Combination therapy with immunemodulators and moxifloxacin on fulminant macrolide-resistant *Mycoplasma pneumoniae* infection: a case report. *Pediatr Pulmonol* 2012. Epub ahead of print.
886. Shimada Y, Deguchi T, Nakane K, et al. Macrolide resistance-associated 23S rRNA mutation in *Mycoplasma genitalium*, Japan. *Emerg Infect Dis* 2011;**17**:1148–50.
895. Simpkins A, Strickland SM, Oliver J, et al. Complete resolution of advanced *Mycoplasma pneumoniae* encephalitis mimicking brain mass lesions: report of two pediatric cases and review of literature. *Neuropathology* 2012;**32**:91–9.
927. Steer JA, Tasker S, Barker EN, et al. A novel hemotropic *Mycoplasma* (hemoplasma) in a patient with hemolytic anemia and pyrexia. *Clin Infect Dis* 2011;**53**:e147–51.
944. Takahashi S, Ichihara K, Hashimoto J, et al. Clinical efficacy of levofloxacin 500 mg once daily for 7 days for patients with non-gonococcal urethritis. *J Infect Chemother* 2011;**17**:392–6.
957. Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium:* from chrysalis to multicolored butterfly. *Clin Microbiol Rev* 2011;**24**: 498–514.
964. Terada M, Izumi K, Ohki E, et al. Antimicrobial efficacies of several antibiotics against uterine cervicitis caused by *Mycoplasma genitalium*. *J Infect Chemother* 2012;**18**:313–17.

984. Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant *Mycoplasma genitalium* infections detected by rapid high resolution melt analysis. *PLoS One* 2012;**7**:e35593.
986. Uldum SA, Bangsborg JM, Gahrn-Hansen B, et al. Epidemic of *Mycoplasma pneumoniae* infection in Denmark, 2010 and 2011. *Euro Surveill* 2012:**17**.
995. Ventolini G, Lee M. Clinical antibiotic response of mycoplasma and ureaplasma in patients with symptomatic recurrent vulvovaginal colonization. *J Reprod Med* 2011;**56**:138–41.
996. Vervloet LA, Vervloet VE, Tironi Junior M, et al. *Mycoplasma pneumoniae*-related community-acquired pneumonia and parapneumonic pleural effusion in children and adolescents. *J Bras Pneumol* 2012;**38**:226–36.
1007. Waites KB. What's new in diagnostic testing and treatment approaches for *Mycoplasma pneumoniae* infections in children? *Adv Exp Med Biol* 2011;**719**:47–57.
1023. Wan GH, Huang CG, Huang YC, et al. Surveillance of airborne adenovirus and *Mycoplasma pneumoniae* in a hospital pediatric department. *PLoS One* 2012;**7**:e33974.
1026. Wang K, Chalker V, Bermingham A, et al. *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough in England: a retrospective analysis. *Pediatr Infect Dis J* 2011;**30**:1047–51.
1032. Watanabe M, Hitomi S, Goto M, et al. Bloodstream infection due to *Mycoplasma arginini* in an immunocompromised patient. *J Clin Microbiol* 2012;**50**:133–5.
1036. Watt KM, Massaro MM, Smith B, et al. Pharmacokinetics of moxifloxacin in an infant with *Mycoplasma hominis* meningitis. *Pediatr Infect Dis J* 2012;**31**:197–9.
1050. Wu B, Rong R. Cold agglutinin syndrome with severe haemolytic anaemia in a patient diagnosed of disseminated tuberculosis and concomitant *Mycoplasma pneumoniae* infection. *Transfus Med* 2012;**22**:151–2.
1053. Yamada M, Buller R, Bledsoe S, et al. Rising rates of macrolide-resistant *Mycoplasma pneumoniae* in the central United States. *Pediatr Infect Dis J* 2012;**31**:409.
1063. Youn YS, Lee KY. *Mycoplasma pneumoniae* pneumonia in children. *Korean J Pediatr* 2012;**55**:42–7.
1066. Zhang L, Zong ZY, Liu YB, et al. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review & meta-analysis. *Indian J Med Res* 2011;**134**:270–80.
1067. Zhang W, Baseman JB. Transcriptional response of *Mycoplasma genitalium* to osmotic stress. *Microbiology* 2011;**157**:548–56.
1069. Zhu C, Liu J, Ling Y, et al. Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in Chinese women with genital infectious diseases. *Indian J Dermatol Venereol Leprol* 2012;**78**:406–7.
1070. Zhu C, Wang S, Hu S, et al. Protective efficacy of a *Mycoplasma pneumoniae* P1C DNA vaccine fused with the B subunit of *Escherichia coli* heat-labile enterotoxin. *Can J Microbiol* 2012;**58**:802–10.
1071. Zhu C, Wu Y, Chen S, et al. Protective immune responses in mice induced by intramuscular and intranasal immunization with a *Mycoplasma pneumoniae* P1C DNA vaccine. *Can J Microbiol* 2012;**58**:644–52.
1073. Zouari A, Touati A, Smaoui H, et al. Dual infection with *Bordetella pertussis* and *Mycoplasma pneumoniae* in three infants: case reports. *Infection* 2012;**40**:213–17.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdel-Haq N, Asmar B, Brown W. *Mycoplasma hominis* scalp abscess in the newborn. *Pediatr Infect Dis J* 2002;**21**:1171–3.
2. Abele-Horn M, Franck W, Busch U, et al. Transverse myelitis associated with *Mycoplasma pneumoniae* infection. *Clin Infect Dis* 1998;**26**:909–12.
3. Abele-Horn M, Genzel-Boroviczeny O, Uhlig T, et al. *Ureaplasma urealyticum* colonization and bronchopulmonary dysplasia: a comparative prospective multicentre study. *Eur J Pediatr* 1998;**157**:1004–11.
4. Abele-Horn M, Peters J, Genzel-Boroviczeny O, et al. Vaginal *Ureaplasma urealyticum* colonization: influence on pregnancy outcome and neonatal morbidity. *Infection* 1997;**25**:286–91.
5. Abele-Horn M, Scholz M, Wolff C, et al. High-density vaginal *Ureaplasma urealyticum* colonization as a risk factor for chorioamnionitis and preterm delivery. *Acta Obstet Gynecol Scand* 2000; **79**:973–8.
6. Abele-Horn M, Wolff C, Dressel P, et al. Polymerase chain reaction versus culture for detection of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the urogenital tract of adults and the respiratory tract of newborns. *Eur J Clin Microbiol Infect Dis* 1996; **15**:595–8.
7. Abele-Horn M, Wolff C, Dressel P, et al. Association of *Ureaplasma urealyticum* biovars with clinical outcome for neonates, obstetric patients, and gynecological patients with pelvic inflammatory disease. *J Clin Microbiol* 1997;**35**:1199–202.
8. Abramovitz P, Schvartzman P, Harel D, et al. Direct invasion of the central nervous system by *Mycoplasma pneumoniae:* a report of two cases. *J Infect Dis* 1987;**155**:482–7.
9. Adra AL, Vigue MG, Dalla Vale F, et al. Favorable outcome in a case of *Mycoplasma pneumoniae*-associated crescentic glomerulonephritis. *Pediatr Nephrol* 2010;**25**:1765–9.
10. Agarwal P, Rajadurai VS, Pradeepkumar VK, et al. *Ureaplasma urealyticum* and its association with chronic lung disease in Asian neonates. *J Paediatr Child Health* 2000;**36**:487–90.
11. Agustin ET, Gill V, Cunha BA. *Mycoplasma pneumoniae* meningoencephalitis complicated by diplopia. *Heart Lung* 1994;**23**: 436–7.
12. Aiello LF, Luby JP. Concomitant *Mycoplasma* and adenovirus infection in a family. *Am J Dis Child* 1974;**128**:874–7.
13. Al-Mateen M, Gibbs M, Dietrich R, et al. Encephalitis lethargica-like illness in a girl with mycoplasma infection. *Neurology* 1988; **38**:1155–8.
14. Al Masalma M, Drancourt M, Dufour H, et al. *Mycoplasma hominis* brain abscess following uterus curettage: a case report. *J Med Case Rep* 2011;**5**:278.
15. Alexander TS, Gray LD, Kraft JA, et al. Performance of Meridian ImmunoCard *Mycoplasma* test in a multicenter clinical trial. *J Clin Microbiol* 1996;**34**:1180–3.
16. Alfa MJ, Embree JE, Degagne P, et al. Transmission of *Ureaplasma urealyticum* from mothers to full and preterm infants. *Pediatr Infect Dis J* 1995;**14**:341–5.
17. Allen PZ, Prescott B. Immunochemical studies on a *Mycoplasma pneumoniae* polysaccharide fraction: cross-reactions with type 23 and 32 antipneumococcal rabbit sera. *Infect Immun* 1978;**20**: 421–9.
18. Allen V, Sueltmann S, Lawson C. Laboratory diagnosis of *Mycoplasma pneumonia* in a public health laboratory. *Health Lab Sci* 1967;**4**:90–5.
19. Almagor M, Yatziv S, Kahane I. Inhibition of host cell catalase by *Mycoplasma pneumoniae:* a possible mechanism for cell injury. *Infect Immun* 1983;**41**:251–6.
20. Alonso-Vega C, Wauters N, Vermeylen D, et al. A fatal case of *Mycoplasma hominis* meningoencephalitis in a full-term newborn. *J Clin Microbiol* 1997;**35**:286–7.
21. Amirmozafari N, Mirnejad R, Kazemi B, et al. Comparison of polymerase chain reaction and culture for detection of genital mycoplasma in clinical samples from patients with genital infections. *Saudi Med J* 2009;**30**:1401–5.
22. Anderson DR, Barile MF. Ultrastructure of *Mycoplasma hominis*. *J Bacteriol* 1965;**90**:180–92.
23. Andrews WW, Hauth JC, Cliver SP, et al. Randomized clinical trial of extended spectrum antibiotic prophylaxis with coverage for *Ureaplasma urealyticum* to reduce post-cesarean delivery endometritis. *Obstet Gynecol* 2003;**101**:1183–9.
24. Andrews WW, Shah SR, Goldenberg RL, et al. Association of post-cesarean delivery endometritis with colonization of the chorioamnion by *Ureaplasma urealyticum*. *Obstet Gynecol* 1995;**85**: 509–14.
25. Anikster Y, Glustein JZ, Weill M, et al. Extrapulmonary manifestations of *Mycoplasma pneumoniae* infections. *Isr J Med Sci* 1994;**30**:412–13.
26. Antsaklis A, Daskalakis G, Michalas S, et al. Erythromycin treatment for subclinical *Ureaplasma urealyticum* infection in preterm labor. *Fetal Diagn Ther* 1997;**12**:89–92.
27. Arakawa H, Yuhara Y, Todokoro M, et al. Immunoadsorption therapy for a child with Guillain-Barré syndrome subsequent to *Mycoplasma* infection: a case study. *Brain Dev* 2005;**27**:431–3.
28. Archer JF. "T" strain mycoplasma in the female uro-genital tract. *Br J Vener Dis* 1968;**44**:232–4.
29. Armstrong SE, Mariano JA, Lundin DJ. The scope of mycoplasma contamination within the biopharmaceutical industry. *Biologicals* 2010;**38**:211–13.
30. Arthur G, Margolis G. *Mycoplasma*-like structures in granulomatous angiitis of the central nervous system: case reports with light and electron microscopic studies. *Arch Pathol Lab Med* 1977; **101**:382–7.
31. Ascer E, Marques M, Gidlund M. M pneumoniae infection, pulmonary thromboembolism and antiphospholipid antibodies. *BMJ Case Rep* 2011;**2011**.
32. Asmar BI, Andresen J, Brown WJ. *Ureaplasma urealyticum* arthritis and bacteremia in agammaglobulinemia. *Pediatr Infect Dis J* 1998;**17**:73–6.
33. Assaad F, Borecka I. Nine-year study of WHO virus reports on fatal viral infections. *Bull World Health Organ* 1977;**55**:445–53.
34. Attilakos A, Palaiologou P, Lagona E, et al. *Mycoplasma pneumoniae* encephalopathy: recovery after intravenous immunoglobulin. *Pediatr Neurol* 2008;**38**:357–9.
35. Auvichayapat N, Auvichayapat P, Watanatorn J, et al. Kluver-Bucy syndrome after mycoplasmal bronchitis. *Epilepsy Behav* 2006;**8**:320–2.
36. Aviner S, Miskin H, London D, et al. *Mycoplasma pneumonia* infection: a possible trigger for immune thrombocytopenia. *Indian J Hematol Blood Transfus* 2011;**27**:46–50.
37. Aydin A, Atasever S, Cakmakci H. Acute disseminated encephalomyelitis presenting with bilateral transient amaurosis. *Pediatr Neurol* 2005;**32**:60–3.
38. Aydin Y, Atis A, Ocer F, et al. Association of cervical infection of *Chlamydia trachomatis*, *Ureaplasma urealyticum* and *Mycoplasma hominis* with peritoneum colonisation in pregnancy. *J Obstet Gynaecol* 2010;**30**:809–12.
39. Azimi PH, Koranyi KI. *Mycoplasma pneumoniae* infections in a family: a description, with comments. *Clin Pediatr (Phila)* 1977; **16**:1138–9.
40. Azimi PH, Chase PA, Petru AM. Mycoplasmas: their role in pediatric disease. *Curr Probl Pediatr* 1984;**14**:1–46.
41. Azumagawa K, Kambara Y, Murata T, et al. Four cases of arthritis associated with *Mycoplasma pneumoniae* infection. *Pediatr Int* 2008;**50**:511–13.
42. Baier RJ, Loggins J, Kruger TE. Monocyte chemoattractant protein-1 and interleukin-8 are increased in bronchopulmonary dysplasia: relation to isolation of *Ureaplasma urealyticum*. *J Investig Med* 2001;**49**:362–9.
43. Baker AS, Ruoff KL, Madoff S. Isolation of *Mycoplasma* species from a patient with seal finger. *Clin Infect Dis* 1998; **27**:1168–70.
44. Bakshi M, Khemani C, Vishwanathan V, et al. *Mycoplasma pneumonia* with antiphospholipid antibodies and a cardiac thrombus. *Lupus* 2006;**15**:105–6.
45. Balassanian N, Robbins FC. *Mycoplasma pneumoniae* infection in families. *N Engl J Med* 1967;**277**:719–25.
46. Balish MF, Krause DC. Mycoplasmas: a distinct cytoskeleton for wall-less bacteria. *J Mol Microbiol Biotechnol* 2006;**11**:244–55.
47. Banerjee B, Petersen K. Psychosis following mycoplasma pneumonia. *Mil Med* 2009;**174**:1001–4.
48. Bao T, Chen R, Zhang J, et al. Simultaneous detection of *Ureaplasma parvum*, *Ureaplasma urealyticum*, *Mycoplasma genitalium* and *Mycoplasma hominis* by fluorescence polarization. *J Biotechnol* 2010;**150**:41–3.
49. Baracaldo R, Foltzer M, Patel R, et al. Empyema caused by *Mycoplasma salivarium*. *J Clin Microbiol* 2012;**50**:1805–6.

50. Barreira ER, Souza DC, Goes PF, et al. Septic shock, necrotizing pneumonitis, and meningoencephalitis caused by *Mycoplasma pneumoniae* in a child: a case report. *Clin Pediatr (Phila)* 2009; **48**:320–2.
51. Barykova YA, Logunov DY, Shmarov MM, et al. Association of *Mycoplasma hominis* infection with prostate cancer. *Oncotarget* 2011;**2**:289–97.
52. Baseman JB, Dallo SF, Tully JG, et al. Isolation and characterization of *Mycoplasma genitalium* strains from the human respiratory tract. *J Clin Microbiol* 1988;**26**:2266–9.
53. Baseman JB, Lange M, Criscimagna NL, et al. Interplay between mycoplasmas and host target cells. *Microb Pathog* 1995;**19**: 105–16.
54. Bauer DF, Tubbs RS, Acakpo-Satchivi L. Mycoplasma meningitis resulting in increased production of cerebrospinal fluid: case report and review of the literature. *Childs Nerv Syst* 2008;**24**: 859–62.
55. Bauer FA, Wear DJ, Angritt P, et al. *Mycoplasma fermentans* (incognitus strain) infection in the kidneys of patients with acquired immunodeficiency syndrome and associated nephropathy: a light microscopic, immunohistochemical, and ultrastructural study. *Hum Pathol* 1991;**22**:63–9.
56. Bayer AS, Galpin JE, Theofilopoulos AN, et al. Neurological disease associated with *Mycoplasma pneumoniae* pneumonitis: demonstration of viable *Mycoplasma pneumoniae* in cerebrospinal fluid and blood by radioisotopic and immunofluorescent tissue culture techniques. *Ann Intern Med* 1981;**94**:15–20.
57. Bayraktar MR, Ozerol IH, Gucluer N, et al. Prevalence and antibiotic susceptibility of *Mycoplasma hominis* and *Ureaplasma urealyticum* in pregnant women. *Int J Infect Dis* 2010;**14**:e90–5.
58. Bayram A, Erdogan MB, Eksi F, et al. Demonstration of *Chlamydophila pneumoniae*, *Mycoplasma pneumoniae*, Cytomegalovirus, and Epstein-Barr virus in atherosclerotic coronary arteries, nonrheumatic calcific aortic and rheumatic stenotic mitral valves by polymerase chain reaction. *Anadolu Kardiyol Derg* 2011;**11**: 237–43.
59. Bebear CM, de Barbeyrac B, Pereyre S, et al. Activity of moxifloxacin against the urogenital mycoplasmas *Ureaplasma* spp., *Mycoplasma hominis* and *Mycoplasma genitalium* and *Chlamydia trachomatis*. *Clin Microbiol Infect* 2008;**14**:801–5.
60. Bébéar C, Dupon M, Renaudin H, et al. Potential improvements in therapeutic options for mycoplasmal respiratory infections. *Clin Infect Dis* 1993;**17**:202–7.
61. Beersma MF, Dirven K, van Dam AP, et al. Evaluation of 12 commercial tests and the complement fixation test for *Mycoplasma pneumoniae*-specific immunoglobulin G (IgG) and IgM antibodies, with PCR used as the "gold standard." *J Clin Microbiol* 2005;**43**:2277–85.
62. Beirne P, Taylor P, Choudhury RP, et al. Cerebellar syndrome complicating *Mycoplasma pneumoniae* pneumonia. *J R Soc Med* 2000;**93**:28–9.
63. Bencina D, Dovc P, Mueller-Premru M, et al. Intrathecal synthesis of specific antibodies in patients with invasion of the central nervous system by *Mycoplasma pneumoniae*. *Eur J Clin Microbiol Infect Dis* 2000;**19**:521–30.
64. Benisch BM, Fayemi A, Gerber MA, et al. Mycoplasmal pneumonia in a patient with rheumatic heart disease. *Am J Clin Pathol* 1972;**58**:343–8.
65. Berant M, Cohen N, Wagner Y. *Mycoplasma pneumoniae* infection presenting as acute rheumatic fever. *Helv Paediatr Acta* 1981;**36**: 567–72.
66. Berger RP, Wadowksy RM. Rhabdomyolysis associated with infection by *Mycoplasma pneumoniae:* a case report. *Pediatrics* 2000;**105**:433–6.
67. Berkovich S, Millian SJ, Snyder RD. The association of viral and mycoplasma infections with recurrence of wheezing in the asthmatic child. *Ann Allergy* 1970;**28**:43–9.
68. Bhattacharyya TK, Mehra YN, Agarwal SC. Incidence of bacteria, L-form and mycoplasma in chronic sinusitis. *Acta Otolaryngol* 1972;**74**:293–6.
69. Biberfeld G. Antibodies to brain and other tissues in cases of *Mycoplasma pneumoniae* infection. *Clin Exp Immunol* 1971;**8**: 319–33.
70. Biberfeld G. Antibody responses in *Mycoplasma pneumoniae* infection in relation to serum immunoglobulins, especially IgM. *Acta Pathol Microbiol Scand [B]* 1971;**79**:620–34.
71. Biberfeld G, Biberfeld P. Ultrastructural features of *Mycoplasma pneumoniae*. *J Bacteriol* 1970;**102**:855–61.
72. Biberfeld G, Norberg R. Circulating immune complexes in *Mycoplasma pneumoniae* infection. *J Immunol* 1974;**112**:413–15.
73. Biberfeld G, Sterner G. A study of *Mycoplasma pneumoniae* infections in families. *Scand J Infect Dis* 1969;**1**:39–46.
74. Biberfeld G, Sterner G. Antibodies in bronchial secretions following natural infection with *Mycoplasma pneumoniae*. *Acta Pathol Microbiol Scand B Microbiol Immunol* 1971;**79**:599–605.
75. Biberfeld G, Sterner G. Smooth muscle antibodies in *Mycoplasma pneumoniae* infection. *Clin Exp Immunol* 1976;**24**:287–91.
76. Biberfeld G, Johnsson T, Jonsson J. Studies on *Mycoplasma pneumoniae* infection in Sweden. *Acta Pathol Microbiol Scand* 1965; **63**:469–75.
77. Biberfeld G, Stenbeck J, Johnsson T. *Mycoplasma pneumoniae* infection in hospitalized patients with acute respiratory illness. *Acta Pathol Microbiol Scand* 1968;**74**:287–300.
78. Biernat-Sudolska M, Szostek S, Rojek-Zakrzewska D, et al. Concomitant infections with human papillomavirus and various mycoplasma and ureaplasma species in women with abnormal cervical cytology. *Adv Med Sci* 2011;**56**:299–303.
79. Biscardi S, Lorrot M, Marc E, et al. *Mycoplasma pneumoniae* and asthma in children. *Clin Infect Dis* 2004;**38**:1341–6.
80. Bitnun A, Richardson SE. *Mycoplasma pneumoniae:* innocent bystander or a true cause of central nervous system disease? *Curr Infect Dis Rep* 2010;**12**:282–90.
81. Bitnun A, Ford-Jones EL, Petric M, et al. Acute childhood encephalitis and *Mycoplasma pneumoniae*. *Clin Infect Dis* 2001;**32**: 1674–84.
82. Bjartling C, Osser S, Persson K. *Mycoplasma genitalium* in cervicitis and pelvic inflammatory disease among women at a gynecologic outpatient service. *Am J Obstet Gynecol* 2012;**206**: 476.e471–8.
83. Bjornelius E, Anagrius C, Bojs G, et al. Antibiotic treatment of symptomatic *Mycoplasma genitalium* infection in Scandinavia: a controlled clinical trial. *Sex Transm Infect* 2008;**84**:72–6.
84. Black FT, Birch-Andersen A, Freundt EA. Morphology and ultrastructure of human T-mycoplasmas. *J Bacteriol* 1972;**111**: 254–9.
85. Blanchard A, Montagnier L. AIDS-associated mycoplasmas. *Annu Rev Microbiol* 1994;**48**:687–712.
86. Blanchard A, Hentschel J, Duffy L, et al. Detection of *Ureaplasma urealyticum* by polymerase chain reaction in the urogenital tract of adults, in amniotic fluid, and in the respiratory tract of newborns. *Clin Infect Dis* 1993;**17**(Suppl. 1):S148–53.
87. Blanco JD, Gibbs RS, Malherbe H, et al. A controlled study of genital mycoplasmas in amniotic fluid from patients with intraamniotic infection. *J Infect Dis* 1983;**147**:650–3.
88. Blanco S, Fuenzalida L, Bas A, et al. Comparison of 2 molecular assays and a serologic test in diagnosing *Mycoplasma pneumoniae* infection in paediatrics patients. *Diagn Microbiol Infect Dis* 2011; **71**:463–6.
89. Block S, Hedrick J, Hammerschlag MR, et al. *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin ethylsuccinate. *Pediatr Infect Dis J* 1995;**14**:471–7.
90. Boatman ES. Morphology and ultrastructure of the Mycoplasmatales. In: Barile MF, Razin S, editors. *The mycoplasmas*, vol. 1. New York: Academic Press; 1979. p. 63–102.
91. Boccardi V, D'Annibali S, Di Natale G, et al. *Mycoplasma pneumoniae* infection complicated by paroxysmal cold hemoglobinuria with anti-P specificity of biphasic hemolysin. *Blut* 1977;**34**: 211–14.
92. Bosnic D, Baresic M, Anic B, et al. Rare zoonosis (hemotrophic mycoplasma infection) in a newly diagnosed systemic lupus erythematosus patient followed by a *Nocardia asteroides* pneumonia. *Braz J Infect Dis* 2010;**14**:92–5.
93. Bové JM. Molecular features of mollicutes. *Clin Infect Dis* 1993; **17**:10–31.
94. Bowie WR, Floyd JF, Miller Y, et al. Differential response of chlamydial and ureaplasma-associated urethritis to sulphafurazole (sulfisoxazole) and aminocyclitols. *Lancet* 1976;**2**:1276–8.
95. Bowie WR, Wang SP, Alexander ER, et al. Etiology of nongonococcal urethritis: evidence for *Chlamydia trachomatis* and *Ureaplasma urealyticum*. *J Clin Invest* 1977;**59**:735–42.

96. Bowman ED, Dharmalingam A, Fan WQ, et al. Impact of erythromycin on respiratory colonization of *Ureaplasma urealyticum* and the development of chronic lung disease in extremely low birth weight infants. *Pediatr Infect Dis J* 1998;**17**:615–20.
97. Bradshaw CS, Jensen JS, Tabrizi SN, et al. Azithromycin failure in *Mycoplasma genitalium* urethritis. *Emerg Infect Dis* 2006;**12**: 1149–52.
98. Braun P, Klein JO, Kass EH. Susceptibility of genital mycoplasmas to antimicrobial agents. *Appl Microbiol* 1970;**19**:62–70.
99. Braun P, Klein JO, Lee YH, et al. Methodologic investigations and prevalence of genital mycoplasmas in pregnancy. *J Infect Dis* 1970;**121**:391–400.
100. Bray PF, Hackett TN. Multiple birth defects in a newborn exposed to *Mycoplasma pneumoniae* in utero. *Am J Dis Child* 1976;**130**:312–14.
101. Bredt W. Motility and multiplication of *Mycoplasma pneumoniae:* a phase contrast study. *Pathol Microbiol (Basel)* 1968;**32**: 321–6.
102. Bredt W. Filamentous growth of some *Mycoplasma* species of man. *Experientia* 1969;**25**:1118–19.
103. Bredt W. Celular morphology of newly isolated *Mycoplasma hominis* strains. *J Bacteriol* 1971;**105**:449–50.
104. Bredt W, Heunert HH, Hofling KH, et al. Microcinematographic studies of *Mycoplasma hominis* cells. *J Bacteriol* 1973;**113**: 1223–7.
105. Bressan S, Mion T, Andreola B, et al. Severe *Mycoplasma pneumoniae*-associated mucositis treated with immunoglobulins. *Acta Paediatr* 2011;**100**:e238–40.
106. Brolin I, Wernstedt L. Radiographic appearance of mycoplasmal pneumonai. *Scand J Respir Dis* 1978;**59**:179–89.
107. Broome CV, LaVenture M, Kaye HS, et al. An explosive outbreak of *Mycoplasma pneumoniae* infection in a summer camp. *Pediatrics* 1980;**66**:884–8.
108. Brown SM, Padley S, Bush A, et al. *Mycoplasma pneumonia* and pulmonary embolism in a child due to acquired prothrombotic factors. *Pediatr Pulmonol* 2008;**43**:200–2.
109. Browning GF, Marenda MS, Noormohammadi AH, et al. The central role of lipoproteins in the pathogenesis of mycoplasmoses. *Vet Microbiol* 2011;**153**:44–50.
110. Bruch LA, Jefferson RJ, Pike MG, et al. *Mycoplasma pneumoniae* infection, meningoencephalitis, and hemophagocytosis. *Pediatr Neurol* 2001;**25**:67–70.
111. Brunner H, Horswood RL, Chanock RM. More sensitive methods for detection of antibody to *Mycoplasma pneumoniae*. *J Infect Dis* 1973;**127**:52–5.
112. Brunner H, Weidner W, Schiefer HG. Studies on the role of *Ureaplasma urealyticum* and *Mycoplasma hominis* in prostatitis. *J Infect Dis* 1983;**147**:807–13.
113. Brunner H, Greenberg HB, James WD, et al. Antibody to *Mycoplasma pneumoniae* in nasal secretions and sputa of experimentally infected human volunteers. *Infect Immun* 1973;**8**: 612–20.
114. Brunner H, Prescott B, Greenberg H, et al. Unexpectedly high frequency of antibody to *Mycoplasma pneumoniae* in human sera as measured by sensitive techniques. *J Infect Dis* 1977;**135**: 524–30.
115. Brunner H, Schaeg W, Bruck U, et al. Determination of IgG, IgM, and IgA antibodies to *Mycoplasma pneumoniae* by an indirect staphylococcal radioimmunoassy. *Med Microbiol Immunol* 1978; **165**:29–41.
116. Brus F, van Waarde WM, Schoots C, et al. Fatal ureaplasmal pneumonia and sepsis in a newborn infant. *Eur J Pediatr* 1991; **150**:782–3.
117. Buckingham SC, Crouse DT, Knapp KM, et al. Pneumonitis associated with *Ureaplasma urealyticum* in children with cancer. *Clin Infect Dis* 2003;**36**:225–8.
118. Buhrer C, Hoehn T, Hentschel J. Role of erythromycin for treatment of incipient chronic lung disease in preterm infants colonised with *Ureaplasma urealyticum*. *Drugs* 2001;**61**:1893–9.
119. Busolo F, Tonin E, Meloni GA. Enzyme-linked immunosorbent assay for serodiagnosis of *Mycoplasma pneumoniae* infections. *J Clin Microbiol* 1983;**18**:432–5.
120. Butler SE, Augostini P, Secor WE. *Mycoplasma hominis* infection of *Trichomonas vaginalis* is not associated with metronidazole-resistant trichomoniasis in clinical isolates from the United States. *Parasitol Res* 2010;**107**:1023–7.
121. Bygdeman SM, Mardh PA. Antimicrobial susceptibility and susceptibility testing of *Mycoplasma hominis:* a review. *Sex Transm Dis* 1983;**10**:366–70.
122. Cadieux N, Lebel P, Brousseau R. Use of a triplex polymerase chain reaction for the detection and differentiation of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* in the presence of human DNA. *J Gen Microbiol* 1993;**139**:2431–7.
123. Camara B, Mouzin M, Ribes D, et al. Perihepatitis and perinephric abscess due to *Mycoplasma hominis* in a kidney transplant patient. *Exp Clin Transplant* 2007;**5**:708–9.
124. Candler PM, Dale RC. Three cases of central nervous system complications associated with *Mycoplasma pneumoniae*. *Pediatr Neurol* 2004;**31**:133–8.
125. Cannell H, Churcher GM, Milton-Thompson GJ. Stevens-Johnson syndrome associated with *Mycoplasma pneumoniae* infection. *Br J Dermatol* 1969;**81**:196–9.
126. Cardinale F, Chironna M, Dumke R, et al. Macrolide-resistant *Mycoplasma pneumoniae* in paediatric pneumonia. *Eur Respir J* 2011;**37**:1522–4.
127. Carter JB. Serologic diagnosis of *Mycoplasma pneumoniae* infection: Introduction of an indirect fluorescent antibody (IFA) procedure. *Immunopathology* 1984;**8**:1–7.
128. Caspi E, Herczeg E, Solomon F, et al. Amnionitis and T strain mycoplasmemia. *Am J Obstet Gynecol* 1971;**111**:1102–6.
129. Cassell GH. Ureaplasmas of human: with emphasis upon maternal and neonatal infections: future considerations—maternal and neonatal aspects. *Pediatr Infect Dis* 1986;**5**:S341–4.
130. Cassell GH, Cole BC. Mycoplasmas as agents of human disease. *N Engl J Med* 1981;**304**:80–9.
131. Cassell GH, Blanchard A, Duffy L. Mycoplasmas. In: Howard BJ, Klaas J III, Rubin SJ, et al, editors. *Clinical and pathogenic microbiology*. St. Louis: Mosby; 1994. p. 491–502.
132. Cassell GH, Waites KB, Crouse DT. Mycoplasmal infections. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 5th ed. Philadelphia: W. B. Saunders; 2001. p. 733–67.
133. Cassell GH, Crouse DT, Waites KB, et al. Does *Ureaplasma urealyticum* cause respiratory disease in newborns? *Pediatr Infect Dis J* 1988;**7**:535–41.
134. Cassell GH, Davis RO, Waites KB, et al. Isolation of *Mycoplasma hominis* and *Ureaplasma urealyticum* from amniotic fluid at 16-20 weeks of gestation: potential effect on outcome of pregnancy. *Sex Transm Dis* 1983;**10**:294–302.
135. Cassell GH, Waites KB, Crouse DT, et al. Association of *Ureaplasma urealyticum* infection of the lower respiratory tract with chronic lung disease and death in very-low-birth-weight infants. *Lancet* 1988;**2**:240–5.
136. Cassell GH, Waites KB, Gibbs RS, et al. Role of *Ureaplasma urealyticum* in amnionitis. *Pediatr Infect Dis* 1986;**5**: S247–52.
137. Cassell GH, Waites KB, Watson HL, et al. *Ureaplasma urealyticum* intrauterine infection: role in prematurity and disease in newborns. *Clin Microbiol Rev* 1993;**6**:69–87.
138. Cassell GH, Younger JB, Brown MB, et al. Microbiologic study of infertile women at the time of diagnostic laparoscopy: association of *Ureaplasma urealyticum* with a defined subpopulation. *N Engl J Med* 1983;**308**:502–5.
139. Castro-Alcaraz S, Greenberg EM, Bateman DA, et al. Patterns of colonization with *Ureaplasma urealyticum* during neonatal intensive care unit hospitalizations of very low birth weight infants and the development of chronic lung disease. *Pediatrics* 2002;**110**:e45.
140. Centers for Disease C Prevention. Hypertrophic pyloric stenosis in infants following pertussis prophylaxis with erythromycin: Knoxville, Tennessee, 1999. *MMWR Morb Mortal Wkly Rep* 1999;**48**:1117–20.
141. Chalker V, Stocki T, Litt D, et al. Increased detection of *Mycoplasma pneumoniae* infection in children in England and Wales, October 2011 to January 2012. *Euro Surveill* 2012;**17**.
142. Chang JH, Kwon YS, Kim BK, et al. A case of acute hepatitis with *Mycoplasma pneumoniae* infection and transient depression of multiple coagulation factors. *Yonsei Med J* 2008;**49**:1055–9.
143. Chang YT, Yang YH, Chiang BL. The significance of a rapid cold hemagglutination test for detecting mycoplasma infections in children with asthma exacerbation. *J Microbiol Immunol Infect* 2006;**39**:28–32.

144. Chanock RM. Mycoplasma infections of man. *N Engl J Med* 1965;**273**:1257–64.
145. Chanock RM, Parrott RH. Acute respiratory disease in infancy and childhood: present understanding and prospects for prevention. *Pediatrics* 1965;**36**:21–39.
146. Chanock RM, Hayflick L, Barile MF. Growth on artificial medium of an agent associated with atypical pneumonia and its identification as a PPLO. *Proc Natl Acad Sci U S A* 1962; **48**:41–9.
147. Chanock R, Chambon L, Chang W, et al. WHO respiratory disease survey in children: a serological study. *Bull World Health Organ* 1967;**37**:363–9.
148. Chanock RM, Cook MK, Fox HH, et al. Serologic evidence of infection with Eaton agent in lower respiratory illness in childhood. *N Engl J Med* 1960;**262**:648–54.
149. Chanock RM, Mufson MA, Bloom HH, et al. Eaton agent pneumonia. *JAMA* 1961;**175**:213–20.
150. Chanock RM, Mufson MA, Somerson NL, et al. Role of *Mycoplasma* (Pplo) in human respiratory disease. *Am Rev Respir Dis* 1963;**88**(Suppl):218–39.
151. Chattin-Kacouris BR, Ishihara K, Miura T, et al. Heat shock protein of *Mycoplasma salivarium* and *Mycoplasma orale* strains isolated from HIV-seropositive patients. *Bull Tokyo Dent Coll* 2002;**43**:231–6.
152. Chaudhry R, Nisar N, Hora B, et al. Expression and immunological characterization of the carboxy-terminal region of the P1 adhesin protein of *Mycoplasma pneumoniae*. *J Clin Microbiol* 2005; **43**:321–5.
153. Chaudhry R, Tabassum I, Kapoor L, et al. A fulminant case of acute respiratory distress syndrome associated with *Mycoplasma pneumoniae* infection. *Indian J Pathol Microbiol* 2010;**53**: 555–7.
154. Chen CJ, Juan CJ, Hsu ML, et al. *Mycoplasma pneumoniae* infection presenting as neutropenia, thrombocytopenia, and acute hepatitis in a child. *J Microbiol Immunol Infect* 2004;**37**:128–30.
155. Chen SC, Tsai CC, Nouri S. Carditis associated with *Mycoplasma pneumoniae* infection. *Am J Dis Child* 1986;**140**:471–2.
156. Cheong KA, Agrawal SR, Lee AY. Validation of nested PCR and a selective biochemical method as alternatives for mycoplasma detection. *J Basic Microbiol* 2011;**51**:215–19.
157. Chergui K, Fourme T, Veillard-Baron A, et al. *Mycoplasma pneumoniae* and second-degree heart block. *Clin Infect Dis* 1998;**27**: 1534–5.
158. Cherry JD. Newer viral exanthems. *Adv Pediatr* 1969;**16**: 233–86.
159. Cherry JD. Newer respiratory viruses: their role in respiratory illness of children. *Adv Pediatr* 1973;**20**:225–90.
160. Cherry JD. Mycoplasma infections. In: Shen JTY, editor. *The clinical practice of adolescent medicine*. New York: Appleton-Century-Crofts; 1980. p. 80–93.
161. Cherry JD. Anemia and mucocutaneous lesions due to *Mycoplasma pneumoniae* infections. *Clin Infect Dis* 1993;**17**: 47–51.
162. Cherry JD, Taylor-Robinson D. Peroxide production by mycoplasmas in chicken tracheal organ cultures. *Nature* 1970;**228**: 1099–100.
163. Cherry JD, Taylor-Robinson D. Growth and pathogenicity studies of *Mycoplasma gallisepticum* in chicken tracheal organ cultures. *J Med Microbiol* 1971;**4**:441–9.
164. Cherry JD, Taylor-Robinson D. *Mycoplasma* pathogenicity studies in organ cultures. *Ann N Y Acad Sci* 1973;**225**:290–303.
165. Cherry JD, Welliver RC. *Mycoplasma pneumoniae* infections of adults and children. *West J Med* 1976;**125**:47–55.
166. Cherry JD, Allen VD, Sueltmann S. Search for *Mycoplasma pneumoniae* infection in patients with exanthem but without pneumonia. *Arch Environ Health* 1968;**16**:911–12.
167. Cherry JD, Hurwitz ES, Welliver RC. *Mycoplasma pneumoniae* infections and exanthems. *J Pediatr* 1975;**87**:369–73.
168. Cherry JD, Taylor-Robinson D, Willers H, et al. A search for mycoplasma infections in patients with chronic bronchitis. *Thorax* 1971;**26**:62–7.
169. Chester A, Kane J, Garagusi V. *Mycoplasma* pneumonia with bilateral pleural effusions. *Am Rev Respir Dis* 1975;**112**:451–6.
170. Chiang CH, Huang CC, Chan WL, et al. Association between *Mycoplasma* pneumonia and increased risk of ischemic stroke: a nationwide study. *Stroke* 2011;**42**:2940–3.
171. Chiou CC, Liu YC, Lin HH, et al. *Mycoplasma pneumoniae* infection complicated by lung abscess, pleural effusion, thrombocytopenia and disseminated intravascular coagulation. *Pediatr Infect Dis J* 1997;**16**:327–9.
172. Chiu CY, Chiang LM, Chen TP. *Mycoplasma pneumoniae* infection complicated by necrotizing pneumonitis with massive pleural effusion. *Eur J Pediatr* 2006;**165**:275–7.
173. Choi SJ, Park SD, Jang IH, et al. The prevalence of vaginal microorganisms in pregnant women with preterm labor and preterm birth. *Ann Lab Med* 2012;**32**:194–200.
174. Chong JS, Tseung SY, Lam HS, et al. Successful treatment of multiple subdural empyemata caused by *Mycoplasma hominis* in a newborn. *Neonatology* 2009;**95**:179–82.
175. Chrisment D, Charron A, Cazanave C, et al. Detection of macrolide resistance in *Mycoplasma genitalium* in France. *J Antimicrob Chemother* 2012;**67**:2598–601.
176. Christie LJ, Honarmand S, Talkington DF, et al. Pediatric encephalitis: what is the role of *Mycoplasma pneumoniae*? *Pediatrics* 2007;**120**:305–13.
177. Chryssanthopoulos C, Eboriadou M, Monti K, et al. Fatal disseminated intravascular coagulation caused by *Mycoplasma pneumoniae*. *Pediatr Infect Dis J* 2001;**20**:634–5.
178. Chua KB, Ngeow YF, Ng KB, et al. *Ureaplasma urealyticum* and *Mycoplasma hominis* isolation from cervical secretions of pregnant women and nasopharyngeal secretions of their babies at delivery. *Singapore Med J* 1998;**39**:300–2.
179. Chua KB, Ngeow YF, Lim CT, et al. Colonization and transmission of *Ureaplasma urealyticum* and *Mycoplasma hominis* from mothers to full and preterm babies by normal vaginal delivery. *Med J Malaysia* 1999;**54**:242–6.
180. Chusid MJ, Lachman BS, Lazerson J. Severe *Mycoplasma pneumonia* and vesicular eruption in SC hemoglobinopathy. *J Pediatr* 1978;**93**:449–51.
181. Cimolai N. Corticosteroids and complicated *Mycoplasma pneumoniae* infection. *Pediatr Pulmonol* 2006;**41**:1008–9; author reply 1010.
182. Cimolai N, Mah DG, Taylor GP, et al. Bases for the early immune response after rechallenge or component vaccination in an animal model of acute *Mycoplasma pneumoniae* pneumonitis. *Vaccine* 1995;**13**:305–9.
183. Cimolai N, Malleson P, Thomas E, et al. *Mycoplasma pneumoniae* associated arthropathy: confirmation of the association by determination of the antipolypeptide IgM response. *J Rheumatol* 1989;**16**:1150–2.
184. Clough W, Cassell GH, Duffy LB, et al. Septic arthritis and bacteremia due to *Mycoplasma* resistant to antimicrobial therapy in a patient with systemic lupus erythematosus. *Clin Infect Dis* 1992;**15**:402–7.
185. Clyde WA. *Mycoplasma pneumoniae* infections of man. In: Tully JG, Whitcomb RF, editors. *The mycoplasmas*, vol. II. New York: Academic Press; 1979. p. 275–306.
186. Clyde WA Jr. An experimental model for human mycoplasma disease. *Yale J Biol Med* 1968;**40**:436–43.
187. Clyde WA Jr. Immunopathology of experimental *Mycoplasma pneumoniae* disease. *Infect Immun* 1971;**4**:757–63.
188. Clyde WA Jr. Neurological syndromes and mycoplasmal infections. *Arch Neurol* 1980;**37**:65–6.
189. Clyde WA Jr. Clinical overview of typical *Mycoplasma pneumoniae* infections. *Clin Infect Dis* 1993;**17**:32–6.
190. Clyde WA Jr, Denny FW. *Mycoplasma* infections in childhood. *Pediatrics* 1967;**40**:669–84.
191. Cockcroft DW, Stilwell GA. Lobar pneumonia caused by *Mycoplasma pneumoniae*. *Can Med Assoc J* 1981;**124**:1463–8.
192. Coelho M, Leite A, Reves A, et al. *Mycoplasma pneumoniae* causing nervous system lesion and SIADH in the absence of pneumonia. *Clin Neurol Neurosurg* 2004;**106**:129–31.
193. Cohen G, Somerson NL. Glucose-dependent secretion and destruction of hydrogen peroxide by *Mycoplasma pneumoniae*. *J Bacteriol* 1969;**98**:547–51.
194. Cole BC, Cassell GH. *Mycoplasma* infections as models of chronic joint inflammation. *Arthritis Rheum* 1979;**22**:1375–81.
195. Collier AM, Baseman JB. Organ culture techniques with mycoplasmas. *Ann N Y Acad Sci* 1973;**225**:277–89.
196. Collier AM, Clyde WA. Relationships between *Mycoplasma pneumoniae* and human respiratory epithelium. *Infect Immun* 1971;**3**: 694–701.

197. Collier AM, Clyde WA Jr, Denny FW. Biologic effects of *Mycoplasma pneumoniae* and other mycoplasmas from man on hamster tracheal organ culture. *Proc Soc Exp Biol Med* 1969; **132**:1153–8.
198. Collier AM, Clyde WA Jr, Denny FW. *Mycoplasma pneumoniae* in hamster tracheal organ culture: immunofluorescent and electron microscopic studies. *Proc Soc Exp Biol Med* 1971;**136**:569–73.
199. Copps SC. Primary atypical pneumonia with hemolytic anemia and erythema multiforme. *Clin Pediatr (Phila)* 1964;**3**:491–5.
200. Copps SC, Allen VD, Sueltmann S, et al. A community outbreak of *Mycoplasma pneumonia*. *JAMA* 1968;**204**:123–8.
201. Cordero L, Coley BD, Miller RL, et al. Bacterial and *Ureaplasma* colonization of the airway: radiologic findings in infants with bronchopulmonary dysplasia. *J Perinatol* 1997;**17**:428–33.
202. Cordero L, Cuadrado R, Hall CB, et al. Primary atypical pneumonia: an epidemic caused by *Mycoplasma pneumoniae*. *J Pediatr* 1967;**71**:1–12.
203. Costea N, Yakulis VJ, Heller P. Inhibition of cold agglutinins (anti-I) by M. pneumoniae antigens. *Proc Soc Exp Biol Med* 1972;**139**:476–9.
204. Cotter FE, Bainbridge D, Newland AC. Neurological deficit associated with *Mycoplasma pneumoniae* reversed by plasma exchange. *Br Med J (Clin Res Ed)* 1983;**286**:22.
205. Couch RB. *Mycoplasma pneumoniae* (primary atypical pneumonia). In: Mandell GL, Douglas RG Jr, Bennett JE, editors. *Principles and practice of infectious diseases*. New York: John Wiley & Sons; 1980. p. 1484–98.
206. Coufalik ED, Taylor-Robinson D, Csonka GW. Treatment of nongonococcal urethritis with rifampicin as a means of defining the role of *Ureaplasma urealyticum*. *Br J Vener Dis* 1979;**55**: 36–43.
207. Couroucli XI, Welty SE, Ramsay PL, et al. Detection of microorganisms in the tracheal aspirates of preterm infants by polymerase chain reaction: association of adenovirus infection with bronchopulmonary dysplasia. *Pediatr Res* 2000;**47**:225–32.
208. Craighead JE. Report of a workshop: disease accentuation after immunization with inactivated microbial vaccines. *J Infect Dis* 1975;**131**:749–54.
209. Crouse DT, Cassell GH, Waites KB, et al. Hyperoxia potentiates *Ureaplasma urealyticum* pneumonia in newborn mice. *Infect Immun* 1990;**58**:3487–93.
210. Crouse DT, English BK, Livingston L, et al. Genital mycoplasmas stimulate tumor necrosis factor-alpha and inducible nitric oxide synthase production from a murine macrophage cell line. *Pediatr Res* 1998;**44**:785–90.
211. Crouse DT, Odrezin GT, Cutter GR, et al. Radiographic changes associated with tracheal isolation of *Ureaplasma urealyticum* from neonates. *Clin Infect Dis* 1993;**17**(Suppl. 1):S122–30.
212. Crouse DT, Waites KR, Geerts MH, et al. Parenteral erythromycin is not associated with hearing loss in preterm infants (abstract). *Clin Res* 1991;**39**:832.
213. Csángó PA, Pedersen JE, Hess RD. Comparison of four *Mycoplasma pneumoniae* IgM-, IgG- and IgA-specific enzyme immunoassays in blood donors and patients. *Clin Microbiol Infect* 2004;**10**:1089–104.
214. Cultrera R, Seraceni S, Germani R, et al. Molecular evidence of *Ureaplasma urealyticum* and *Ureaplasma parvum* colonization in preterm infants during respiratory distress syndrome. *BMC Infect Dis* 2006;**6**:166.
215. Cummings MC, McCormack WM. Increase in resistance of *Mycoplasma hominis* to tetracyclines. *Antimicrob Agents Chemother* 1990;**34**:2297–9.
216. Cunliffe NA, Fergusson S, Davidson F, et al. Comparison of culture with the polymerase chain reaction for detection of *Ureaplasma urealyticum* in endotracheal aspirates of preterm infants. *J Med Microbiol* 1996;**45**:27–30.
217. Da Silva O, Gregson D, Hammerberg O. Role of *Ureaplasma urealyticum* and *Chlamydia trachomatis* in development of bronchopulmonary dysplasia in very low birth weight infants. *Pediatr Infect Dis J* 1997;**16**:364–9.
218. Dallo SF, Chavoya A, Baseman JB. Characterization of the gene for a 30-kilodalton adhesion-related protein of *Mycoplasma pneumoniae*. *Infect Immun* 1990;**58**:4163–5.
219. Dallo SF, Lazzell AL, Chavoya A, et al. Biofunctional domains of the *Mycoplasma pneumoniae* P30 adhesin. *Infect Immun* 1996;**64**: 2595–601.
220. Dan M, Robertson J. *Mycoplasma hominis* septicemia after heart surgery. *Am J Med* 1988;**84**:976–7.
221. Dan M, Tyrrell DL, Stemke GW, et al. *Mycoplasma hominis* septicemia in a burned infant. *J Pediatr* 1981;**99**:743–5.
222. Davis CP, Cochran S, Lisse J, et al. Isolation of *Mycoplasma pneumoniae* from synovial fluid samples in a patient with pneumonia and polyarthritis. *Arch Intern Med* 1988;**148**: 969–70.
223. Daxböck F, Assadian A, Watkins-Riedel T, et al. Persistently elevated IgA antibodies to *Mycoplasma pneumoniae* in patients with internal carotid artery stenosis. *GMS Krankenhhyg Interdiszip* 2011;**6**:Doc04.
224. Daxböck F, Zedtwitz-Liebenstein K, Burgmann H, et al. Severe hemolytic anemia and excessive leukocytosis masking mycoplasma pneumonia. *Ann Hematol* 2001;**80**:180–2.
225. Daxboeck F, Blacky A, Seidl R, et al. Diagnosis, treatment, and prognosis of *Mycoplasma pneumoniae* childhood encephalitis: systematic review of 58 cases. *J Child Neurol* 2004;**19**:865–71.
226. Daxboeck F, Khanakah G, Bauer C, et al. Detection of *Mycoplasma pneumoniae* in serum specimens from patients with mycoplasma pneumonia by PCR. *Int J Med Microbiol* 2005;**295**: 279–85.
227. deBarbeyrac B, Bernet-Poggi C, Febrer F, et al. Detection of *Mycoplasma pneumoniae* and *Mycoplasma genitalium* in clinical samples by polymerase chain reaction. *Clin Infect Dis* 1993; **17**:83–9.
228. Decancq HG Jr, Lee FA. *Mycoplasma pneumoniae* pneumonia: massive pulmonary involvement and pleural effusion. *JAMA* 1965;**194**:1010–11.
229. Defilippi A, Silvestri M, Tacchella A, et al. Epidemiology and clinical features of *Mycoplasma pneumoniae* infection in children. *Respir Med* 2008;**102**:1762–8.
230. Del Giudice RA, Tully JG, Rose DL, et al. *Mycoplasma pirum* sp. nov.: a terminal structured mollicute from cell cultures. *Int J Syst Bacteriol* 1985;**35**:285–91.
231. Denny FW, Clyde WA Jr, Glezen WP. *Mycoplasma pneumoniae* disease: clinical spectrum, pathophysiology, epidemiology, and control. *J Infect Dis* 1971;**123**:74–92.
232. Diaz-Garcia FJ, Herrera-Mendoza AP, Giono-Cerezo S, et al. *Mycoplasma hominis* attaches to and locates intracellularly in human spermatozoa. *Hum Reprod* 2006;**21**:1591–8.
233. Diaz N, Dessi D, Dessole S, et al. Rapid detection of coinfections by *Trichomonas vaginalis*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* by a new multiplex polymerase chain reaction. *Diagn Microbiol Infect Dis* 2010;**67**:30–6.
234. Dienes L, Edsall J. Observations on L-organisms of Klieneberger. *Proc Soc Exp Biol Med* 1937;**36**:740–4.
235. Dinsmoor MJ, Ramamurthy RS, Gibbs RS. Transmission of genital mycoplasmas from mother to neonate in women with prolonged membrane rupture. *Pediatr Infect Dis J* 1989;**8**: 483–7.
236. Dinsmoor MJ, Ramamurthy RS, Cassell GH, et al. Neonatal serologic response at term to the genital mycoplasmas. *Pediatr Infect Dis J* 1989;**8**:487–91.
237. Domermuth CH, Nielsen MH, Freundt EA, et al. Ultrastructure of *Mycoplasma* species. *J Bacteriol* 1964;**88**:727–44.
238. Dominguez SR, Littlehorn C, Nyquist AC. Mycoplasma hominis endocarditis in a child with a complex congenital heart defect. *Pediatr Infect Dis J* 2006;**25**:851–2.
239. Don M, Fasoli L, Paldanius M, et al. Aetiology of community-acquired pneumonia: serological results of a paediatric survey. *Scand J Infect Dis* 2005;**37**:806–12.
240. Donders GG, Van Calsteren K, Bellen G, et al. Predictive value for preterm birth of abnormal vaginal flora, bacterial vaginosis and aerobic vaginitis during the first trimester of pregnancy. *BJOG* 2009;**116**:1315–24.
241. Dorff B, Lind K. Two fatal cases of meningoencephalitis associated with *Mycoplasma pneumoniae* infection. *Scand J Infect Dis* 1976;**8**:49–51.
242. Dorigo-Zetsma JW, Dankert J, Zaat SA. Genotyping of *Mycoplasma pneumoniae* clinical isolates reveals eight P1 subtypes within two genomic groups. *J Clin Microbiol* 2000;**38**:965–70.
243. Dorigo-Zetsma JW, Wilbrink B, Dankert J, et al. *Mycoplasma pneumoniae* P1 type 1- and type 2-specific sequences within the P1 cytadhesin gene of individual strains. *Infect Immun* 2001;**69**: 5612–18.

244. Dorigo-Zetsma JW, Zaat SA, Vriesema AJ, et al. Demonstration by a nested PCR for *Mycoplasma pneumoniae* that *M. pneumoniae* load in the throat is higher in patients hospitalised for *M. pneumoniae* infection than in non-hospitalised subjects. *J Med Microbiol* 1999;**48**:1115–22.
245. Dorman CJ. Regulation of transcription by DNA supercoiling in *Mycoplasma genitalium:* global control in the smallest known self-replicating genome. *Mol Microbiol* 2011;**81**:302–4.
246. dos Santos AP, dos Santos RP, Biondo AW, et al. Hemoplasma infection in HIV-positive patient, Brazil. *Emerg Infect Dis* 2008;**14**:1922–4.
247. Dowdle WR, Robinson RQ. An indirect hemagglutination test for diagnosis of mycoplasma pneumoniae infections. *Proc Soc Exp Biol Med* 1964;**116**:947–50.
248. Dowdle WR, Stewart JA, Heyward JT, et al. *Mycoplasma pneumoniae* infections in a children's population: a five-year study. *Am J Epidemiol* 1967;**85**:137–46.
249. Dowling PC, Cook SD. Role of infection in Guillain-Barre syndrome: laboratory confirmation of herpesviruses in 41 cases. *Ann Neurol* 1981;**9**(Suppl.):44–55.
250. Dua J, Nandagudi A, Sutcliffe N. *Mycoplasma pneumoniae* infection associated with urticarial vasculitis mimicking adult-onset Still's disease. *Rheumatol Int* 2012;**32**:4053–6.
251. Dumbke R, Lück PC, Noppen C, et al. Culture-independent molecular subtyping of *Mycoplasma pneumoniae* in clinical samples. *J Clin Microbiol* 2006;**44**:2567–70.
252. Dumke R, Strubel A, Cyncynatus C, et al. Optimized serodiagnosis of *Mycoplasma pneumoniae* infections. *Diagn Microbiol Infect Dis* 2012;**73**:200–3.
253. Dunn JJ, Malan AK, Evans J, et al. Rapid detection of *Mycoplasma pneumoniae* IgM antibodies in pediatric patients using Immuno-Card *Mycoplasma* compared to conventional enzyme immunoassays. *Eur J Clin Microbiol Infect Dis* 2003;**23**:412–14.
254. Dyke MP, Grauaug A, Kohan R, et al. *Ureaplasma urealyticum* in a neonatal intensive care population. *J Paediatr Child Health* 1993;**29**:295–7.
255. Eaton MD. Action of aureomycin and chloromycetin on the virus of primary atypical pneumonia. *Proc Soc Exp Biol Med* 1950;**73**:24–9.
256. Eaton MD, Liu C. Studies on sensitivity to streptomycin of the atypical pneumonia agent. *J Bacteriol* 1957;**74**:784–7.
257. Eaton MD, Meiklejohn G, van Herick W. Studies on the etiology of primary atypical pneumonia : a filterable agent transmissible to cotton rats, hamsters, and chick embryos. *J Exp Med* 1944;**79**:649–68.
258. Ebrahim M, Gabay M, Rivas-Chacon RF. Evidence of acute *Mycoplasma* infection in a patient with incomplete and atypical Kawasaki disease: a case report. *Case Report Med* 2011;**2011**:606920.
259. Echevarria JM, Leon P, Balfagon P, et al. Diagnosis of *Mycoplasma pneumoniae* infection by microparticle agglutination and antibody-capture enzyme-immunoassay. *Eur J Clin Microbiol Infect Dis* 1990;**9**:217–20.
260. Egawa T, Morioka I, Morisawa T, et al. *Ureaplasma urealyticum* and *Mycoplasma hominis* presence in umbilical cord is associated with pathogenesis of funisitis. *Kobe J Med Sci* 2007;**53**:241–9.
261. Eibach D, Casalegno JS, Escuret V, et al. Increased detection of *Mycoplasma pneumoniae* infection in children, Lyon, France, 2010 to 2011. *Euro Surveill* 2012;**17**.
262. Ekiel AM, Friedek DA, Romanik MK, et al. Occurrence of *Ureaplasma parvum* and *Ureaplasma urealyticum* in women with cervical dysplasia in Katowice, Poland. *J Korean Med Sci* 2009;**24**:1177–81.
263. Elbers JM, Bitnun A, Richardson SE, et al. A 12-year prospective study of childhood herpes simplex encephalitis: is there a broader spectrum of disease? *Pediatrics* 2007;**119**:e399–407.
264. Embree JE, Embil JA. Mycoplasmas in diseases of humans. *Can Med Assoc J* 1980;**123**:105–11.
265. Embree JE, Krause VW, Embil JA. *Mycoplasma hominis:* a placental pathogen? *Sex Transm Dis* 1983;**10**:307–10.
266. Embree JE, Krause VW, Embil JA, et al. Placental infection with *Mycoplasma homonis* and *Ureaplasma urealyticum:* clinical correlation. *Obstet Gynecol* 1980;**56**:475–81.
267. Emmons J, Schluederberg A, Cordero L. An aid to the rapid diagnosis of *Mycoplasma pneumoniae* infections. *J Infect Dis* 1969;**119**:650–3.
268. Ercan TE, Ercan G, Severge B, et al. *Mycoplasma pneumoniae* infection and obsessive-compulsive disease: a case report. *J Child Neurol* 2008;**23**:338–40.
269. Eschenbach DA. *Ureaplasma urealyticum* as a cause of postpartum fever. *Pediatr Infect Dis* 1986;**5**:S258–61.
270. Eschenbach DA, Nugent RP, Rao AV, et al. A randomized placebo-controlled trial of erythromycin for the treatment of *Ureaplasma urealyticum* to prevent premature delivery. The Vaginal Infections and Prematurity Study Group. *Am J Obstet Gynecol* 1991;**164**:734–42.
271. Eshima N, Tokumaru O, Hara S, et al. Age-specific sex-related differences in infections: a statistical analysis of national surveillance data in Japan. *PLoS One* 2012;**7**:e42261.
272. Esposito S, Blasi F, Bosis S, et al. Aetiology of acute pharyngitis: the role of atypical bacteria. *J Med Microbiol* 2004;**53**:645–51.
273. Esposito S, Bosis S, Begliatti E, et al. Acute tonsillopharyngitis associated with atypical bacterial infection in children: natural history and impact of macrolide therapy. *Clin Infect Dis* 2006;**43**:206–9.
274. Esposito S, Bosis S, Faelli N, et al. Role of atypical bacteria and azithromycin therapy for children with recurrent respiratory tract infections. *Pediatr Infect Dis J* 2005;**24**:438–44.
275. Evans AS, Brobst M. Bronchitis, pneumonitis and pneumonia in University of Wisconsin students. *N Engl J Med* 1961;**265**:401–9.
276. Evans AS, Allen V, Sueltmann S. *Mycoplasma pneumoniae* infections in University of Wisconsin students. *Am Rev Respir Dis* 1967;**96**:237–44.
277. Evans MR, Marshall AJ. Recovery from mycoplasma meningoencephalitis, credited to penicillin allergy. *Lancet* 1990;**335**:1100.
278. Evatt BL, Dowdle WR, Johnson M Jr, et al. Epidemic mycoplasma pneumonia. *N Engl J Med* 1971;**285**:374–8.
279. Farraj RS, McCully RB, Oh JK, et al. *Mycoplasma*-associated pericarditis. *Mayo Clin Proc* 1997;**72**:33–6.
280. Farrar HC, Walsh-Sukys MC, Kyllonen K, et al. Cardiac toxicity associated with intravenous erythromycin lactobionate: two case reports and a review of the literature. *Pediatr Infect Dis J* 1993;**12**:688–91.
281. Fedorko DP, Emery DD, Franklin SM, et al. Evaluation of a rapid enzyme immunoassay system for serologic diagnosis of *Mycoplasma pneumoniae* infection. *Diagn Microbiol Infect Dis* 1995;**23**:85–8.
282. Feikin DR, Moroney JF, Talkington DF, et al. An outbreak of acute respiratory disease caused by *Mycoplasma pneumoniae* and adenovirus at a federal service training academy: new implications from an old scenario. *Clin Infect Dis* 1999;**29**:1545–50.
283. Feizi O, Grubb C, Skinner JI, et al. Primary atypical pneumonia due to *Mycoplasma pneumoniae* complicated by haemorrhagic pleural effusion, haemolytic anaemia and myocarditis. *Br J Clin Pract* 1973;**27**:99–101.
284. Feizi T. Cold agglutinins, the direct coombs' test and serum immunoglobulins in *Mycoplasma pneumoniae* infection. *Ann N Y Acad Sci* 1967;**143**:801–12.
285. Feizi T. Syndromes associated with mycoplasmas. *Postgrad Med J* 1967;**43**(Suppl. 43):106–8.
286. Feizi T, Maclean H, Sommerville RG, et al. Studies on an epidemic of respiratory disease caused by *Mycoplasma pneumoniae*. *Br Med J* 1967;**1**:457–60.
287. Fekety FR Jr, Caldwell J, Gump D, et al. Bacteria, viruses, and mycoplasmas in acute pneumonia in adults. *Am Rev Respir Dis* 1971;**104**:499–507.
288. Fenollar F, Gauduchon V, Casalta JP, et al. *Mycoplasma* endocarditis: two case reports and a review. *Clin Infect Dis* 2004;**38**:e21–4.
289. Fernald GW. In vitro response of human lymphocytes to *Mycoplasma pneumoniae*. *Infect Immun* 1972;**5**:552–8.
290. Fernald GW. Role of host response in *Mycoplasma pneumoniae* disease. *J Infect Dis* 1973;**127**:55–8.
291. Fernald GW. Humoral and cellular immune responses to mycoplasmas. In: Tully JG, Whitcomb RF, editors. *The mycoplasmas*, vol. II. New York: Academic Press; 1979. p. 399–423.
292. Fernald GW, Glezen WP. Humoral and cellular immune responses to an inactivated *Mycoplasma pneumoniae* vaccine in children. *J Infect Dis* 1973;**127**:498–504.

293. Fernald GW, Clyde WA Jr, Denny FW. Nature of the immune response to *Mycoplasma pneumoniae*. *J Immunol* 1967;**98**: 1028–38.
294. Fernald GW, Collier AM, Clyde WA Jr. Respiratory infections due to *Mycoplasma pneumoniae* in infants and children. *Pediatrics* 1975;**55**:327–35.
295. Fernandez CV, Bortolussi R, Gordon K, et al. *Mycoplasma pneumoniae* infection associated with central nervous system complications. *J Child Neurol* 1993;**8**:27–31.
296. Fiacco V, Miller MJ, Carney E, et al. Comparison of media for isolation of *Ureaplasma urealyticum* and *genital Mycoplasm*a species. *J Clin Microbiol* 1984;**20**:862–5.
297. Fiala M, Myhre BA, Chinh LT, et al. Pathogenesis of anemia associated with *Mycoplasma pneumoniae*. *Acta Haematol* 1974;**51**: 297–301.
298. Fine NL, Smith LR, Sheedy PF. Frequency of pleural effusions in mycoplasma and viral pneumonias. *N Engl J Med* 1970;**283**: 790–3.
299. Finland M, Peterson OL, Allen HE, et al. Cold agglutinins. I. Occurrence of cold isohemagglutinins in various conditions. *J Clin Invest* 1945;**24**:451–7.
300. Fischer GS, Sweimler WI, Kleger B. Comparison of MYCOPLASMELISA with complement fixation test for measurement of antibodies to *Mycoplasma pneumoniae*. *Diagn Microbiol Infect Dis* 1986;**4**:139–45.
301. Fischman RA, Marschall KE, Kislak JW, et al. Adult respiratory distress syndrome caused by *Mycoplasma pneumoniae*. *Chest* 1978;**74**:471–3.
302. Fisher RS, Clark AW, Wolinsky JS, et al. Postinfectious leukoencephalitis complicating *Mycoplasma pneumoniae* infection. *Arch Neurol* 1983;**40**:109–13.
303. Fleming PC, Krieger E, Turner JA, et al. Febrile mucocutaneous syndrome with respiratory involvement, associated with isolation of *Mycoplasma pneumoniae*. *Can Med Assoc J* 1967;**97**: 1458–9.
304. Forgacs P, Kundsin RB, Margles SW, et al. A case of *Ureaplasma urealyticum* septic arthritis in a patient with hypogammaglobulinemia. *Clin Infect Dis* 1993;**16**:293–4.
305. Formosa GM, Bailey M, Barbara C, et al. *Mycoplasma pneumonia:* an unusual cause of acute myocarditis in childhood. *Images Paediatr Cardiol* 2006;**8**:7–10.
306. Foulon W, Naessens A, Dewaele M, et al. Chronic *Ureaplasma urealyticum* amnionitis associated with abruptio placentae. *Obstet Gynecol* 1986;**68**:280–2.
307. Foy H, Kenny G, Bor E, et al. Prevalence of *Mycoplasma hominis* and *Ureaplasma urealyticum* (T strains) in urine of adolescents. *J Clin Microbiol* 1975;**2**:226–30.
308. Foy HM. Infections caused by *Mycoplasma pneumoniae* and possible carrier state in different populations of patients. *Clin Infect Dis* 1993;**17**:37–46.
309. Foy HM, Alexander ER. *Mycoplasma pneumoniae* infections in childhood. *Adv Pediatr* 1969;**16**:301–23.
310. Foy HM, Kenny GE, Koler J. Mycoplasma pneumoniae in Stevens-Johnson's syndrome. *Lancet* 1966;**2**:550–1.
311. Foy HM, Cooney MK, Maletzky AJ, et al. Incidence and etiology of pneumonia, croup and bronchiolitis in preschool children belonging to a prepaid medical care group over a four-year period. *Am J Epidemiol* 1973;**97**:80–92.
312. Foy HM, Cooney MK, McMahan R, et al. Viral and mycoplasmal pneumonia in a prepaid medical care group during an eight-year period. *Am J Epidemiol* 1973;**97**:93–102.
313. Foy HM, Grayston JT, Kenny GE, et al. Epidemiology of *Mycoplasma pneumoniae* infection in families. *JAMA* 1966;**197**: 859–66.
314. Foy HM, Kenny GE, Cooney MK, et al. Naturally acquired immunity to pneumonia due to *Mycoplasma pneumoniae*. *J Infect Dis* 1983;**147**:967–73.
315. Foy HM, Kenny GE, Levinsohn EM, et al. Acquisition of mycoplasmata and T-strains during infancy. *J Infect Dis* 1970;**121**: 579–87.
316. Foy HM, Kenny GE, McMahan R, et al. *Mycoplasma pneumoniae* in the community. *Am J Epidemiol* 1970;**93**:55–67.
317. Foy HM, Kenny GE, McMahan R, et al. *Mycoplasma pneumoniae* pneumonia in an urban area: five years of surveillance. *JAMA* 1970;**214**:1666–72.
318. Foy HM, Kenny GE, Sefi R, et al. Second attacks of pneumonia due to *Mycoplasma pneumoniae*. *J Infect Dis* 1977;**135**:673–7.
319. Foy HM, Kenny GE, Wentworth BB, et al. Isolation of *Mycoplasma hominis*, T-strains, and cytomegalovirus from the cervix of pregnant women. *Am J Obstet Gynecol* 1970;**106**:635–43.
320. Foy HM, Nugent CG, Kenny GE, et al. Repeated *Mycoplasma pneumoniae* pneumonia after 4 and one-half years. *JAMA* 1971;**216**:671–2.
321. Foy HM, Ochs H, Davis SD, et al. *Mycoplasma pneumoniae* infections in patients with immunodeficiency syndromes: report of four cases. *J Infect Dis* 1973;**127**:388–93.
322. Frangogiannis NG, Cate TR. Endocarditis and *Ureaplasma urealyticum* osteomyelitis in a hypogammaglobulinemic patient: a case report and review of the literature. *J Infect* 1998;**37**: 181–4.
323. Fransen H, Forsgren M, Heigl Z, et al. Studies on *Mycoplasma pneumoniae* in patients hospitalized with acute respiratory illness. *Scand J Infect Dis* 1969;**1**:91–8.
324. Franzoso G, Hu PC, Meloni GA, et al. The immunodominant 90-kilodalton protein is localized on the terminal tip structure of *Mycoplasma pneumoniae*. *Infect Immun* 1993;**61**:1523–30.
325. Freeman R, McMahon MJ. Acute pancreatitis and serological evidence of infection with *Mycoplasma pneumoniae*. *Gut* 1978;**19**:367–70.
326. Freundt EA, Edward DG. Classification and taxonomy. In: Barile MF, Razin S, editors. *The mycoplasmas*, vol. I. New York: Academic Press; 1979. p. 1–41.
327. Freymuth F, Vabret A, Brouard J, et al. Detection of viral, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* infections in exacerbations of asthma in children. *J Clin Virol* 1999;**13**: 131–9.
328. Gable MS, Gavali S, Radner A, et al. Anti-NMDA receptor encephalitis: report of ten cases and comparison with viral encephalitis. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1421–9.
329. Gable MS, Sheriff H, Dalmau J, et al. The frequency of autoimmune N-methyl-D-aspartate receptor encephalitis surpasses that of individual viral etiologies in young individuals enrolled in the California Encephalitis Project. *Clin Infect Dis* 2012;**54**: 899–904.
330. Gallo D, Dupuis KW, Schmidt NJ, et al. Broadly reactive immunofluorescence test for measurement of immunoglobulin M and G antibodies to *Ureaplasma urealyticum* in infant and adult sera. *J Clin Microbiol* 1983;**17**:614–18.
331. Ganick DJ, Wolfson J, Gilbert EF, et al. *Mycoplasma* infection in the immunosuppressed leukemic patient. *Arch Pathol Lab Med* 1980;**104**:535–6.
332. Gannon H. *Ureaplasma urealyticum* and its role in neonatal lung disease. *Neonatal Netw* 1993;**12**:13–18.
333. Garcia C, Ugalde E, Monteagudo I, et al. Isolation of *Mycoplasma hominis* in critically ill patients with pulmonary infections: clinical and microbiological analysis in an intensive care unit. *Intensive Care Med* 2007;**33**:143–7.
334. Garcia-Castillo M, Morosini MI, Galvez M, et al. Differences in biofilm development and antibiotic susceptibility among clinical *Ureaplasma urealyticum* and *Ureaplasma parvum* isolates. *J Antimicrob Chemother* 2008;**62**:1027–30.
335. Garcia-de-la-Fuente C, Minambres E, Ugalde E, et al. Postoperative mediastinitis, pleuritis and pericarditis due to *Mycoplasma hominis* and *Ureaplasma urealyticum* with a fatal outcome. *J Med Microbiol* 2008;**57**:656–7.
336. Garland SM, Murton LJ. Neonatal meningitis caused *by Ureaplasma urealyticum*. *Pediatr Infect Dis J* 1987;**6**:868–70.
337. Garrow DH. A rapid test for the presence of increased cold agglutinins. *Br Med J* 1958;**2**:206–8.
338. Gatski M, Martin DH, Theall K, et al. *Mycoplasma genitalium* infection among HIV-positive women: prevalence, risk factors and association with vaginal shedding. *Int J STD AIDS* 2011;**22**:155–9.
339. Gdoura R, Kchaou W, Chaari C, et al. *Ureaplasma urealyticum*, *Ureaplasma parvum*, *Mycoplasma hominis* and *Mycoplasma genitalium* infections and semen quality of infertile men. *BMC Infect Dis* 2007;**7**:129.
340. Geers TA, Taege AJ, Longworth DL, et al. *Ureaplasma urealyticum:* unusual cause of culture-negative mediastinitis. *Clin Infect Dis* 1999;**29**:949–50.

341. George RB, Weill H, Rasch JR, et al. Roentgenographic appearance of viral and mycoplasmal pneumonias. *Am Rev Respir Dis* 1967;**96**:1144–50.
342. George RB, Ziskind MM, Rasch JR, et al. *Mycoplasma* and adenovirus pneumonias: comparison with other atypical pneumonias in a military population. *Ann Intern Med* 1966;**65**:931–42.
343. Gibbs RS, Blanco JD, St Clair PJ, et al. *Mycoplasma hominis* and intrauterine infection in late pregnancy. *Sex Transm Dis* 1983; **10**:303–6.
344. Gibson DG, Glass JI, Lartigue C, et al. Creation of a bacterial cell controlled by a chemically synthesized genome. *Science* 2010;**329**:52–6.
345. Gil C, Rivera A, Banuelos D, et al. Presence of *Mycoplasma fermentans* in the bloodstream of Mexican patients with rheumatoid arthritis and IgM and IgG antibodies against whole microorganism. *BMC Musculoskelet Disord* 2009;**10**:97.
346. Gilbert GL, Law F, Macinnes SJ, et al. Chronic *Mycoplasma hominis* infection complicating severe intraventricular hemorrhage, in a premature neonate. *Pediatr Infect Dis J* 1988;**7**: 817–18.
347. Gill K, Marrie TJ. Hemophagocytosis secondary to *Mycoplasma pneumoniae* infection. *Am J Med* 1987;**82**:668–70.
348. Ginevra C, Barranger C, Ros A, et al. Development and evaluation of Chlamylege, a new commercial test allowing simultaneous detection and identification of *Legionella*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae* in clinical respiratory specimens by multiplex PCR. *J Clin Microbiol* 2005;**43**: 3247–54.
349. Gjuric G, Prislin-Muskic M, Nikolic E, et al. *Ureaplasma urealyticum* osteomyelitis in a very low birth weight infant. *J Perinat Med* 1994;**22**:79–81.
350. Gjuric G, Prislin-Muskic M, Zurga B, et al. *Ureaplasma urealyticum* infection in newborns: three case reports. *Eur J Pediatr* 1993;**152**:599–600.
351. Glaser JB, Engelberg M, Hammerschlag M. Scalp abscess associated with *Mycoplasma hominis* infection complicating intrapartum monitoring. *Pediatr Infect Dis* 1983;**2**:468–70.
352. Glezen WP, Clyde WA Jr, Senior RJ, et al. Group A streptococci, mycoplasmas, and viruses associated with acute pharyngitis. *JAMA* 1967;**202**:455–60.
353. Glezen WP, Loda FA, Clyde WA Jr, et al. Epidemiologic patterns of acute lower respiratory disease of children in a pediatric group practice. *J Pediatr* 1971;**78**:397–406.
354. Goldenberg RL, Andrews WW, Goepfert AR, et al. The Alabama Preterm Birth Study: umbilical cord blood *Ureaplasma urealyticum* and *Mycoplasma hominis* cultures in very preterm newborn infants. *Am J Obstet Gynecol* 2008;**198**:43.e41–5.
355. Goldschmidt B, Menonna J, Fortunato J, et al. *Mycoplasma* antibody in Guillain-Barré syndrome and other neurological disorders. *Ann Neurol* 1980;**7**:108–12.
356. Golubjatnikov R, Allen VD, Olmos-Blancarte MP, et al. Serologic profile of children in a Mexican highland community: prevalence of complement-fixing antibodies to *Mycoplasma pneumoniae*, respiratory syncytial virus and parainfluenza viruses. *Am J Epidemiol* 1975;**101**:458–64.
357. Good JT Jr, Rollins DR, Martin RJ. Macrolides in the treatment of asthma. *Curr Opin Pulm Med* 2012;**18**:76–84.
358. Goodburn GM, Marmion BP, Kendall EJ. Infection with Eaton's primary atypical pneumonia agent in England. *Br Med J* 1963;**1**:1266–70.
359. Goudie BM, Kerr MR, Johnson RN. *Mycoplasma pneumonia* complicated by bronchiectasis. *J Infect* 1983;**7**:151–2.
360. Govender S, Theron GB, Odendaal HJ, et al. Prevalence of genital mycoplasmas, ureaplasmas and chlamydia in pregnancy. *J Obstet Gynaecol* 2009;**29**:698–701.
361. Grady GF, Gilfillan RF. Relation of *Mycoplasma pneumoniae* seroreactivity, immunosuppression, and chronic disease to Legionnaires' disease: a twelve-month prospective study of sporadic cases in Massachusetts. *Ann Intern Med* 1979;**90**:607–10.
362. Grattard F, Soleihac B, De Barbeyrac B, et al. Epidemiologic and molecular investigations of genital mycoplasmas from women and neonates at delivery. *Pediatr Infect Dis J* 1995;**14**:853–8.
363. Grau O, Kovacic R, Griffais R, et al. Development of PCR-based assays for the detection of two human mollicute species, *Mycoplasma penetrans* and *M. hominis*. *Mol Cell Probes* 1994;**8**:139–47.
364. Grau O, Slizewicz B, Tuppin P, et al. Association of *Mycoplasma penetrans* with human immunodeficiency virus infection. *J Infect Dis* 1995;**172**:672–81.
365. Graw-Panzer KD, Verma S, Rao S, et al. Venous thrombosis and pulmonary embolism in a child with pneumonia due to *Mycoplasma pneumoniae*. *J Natl Med Assoc* 2009;**101**:956–8.
366. Gray DJ, Robinson HB, Malone J, et al. Adverse outcome in pregnancy following amniotic fluid isolation of *Ureaplasma urealyticum*. *Prenat Diagn* 1992;**12**:111–17.
367. Grayston JT, Alexander ER, Kenny GE, et al. *Mycoplasma pneumoniae* infections: clinical and epidemiologic studies. *JAMA* 1965;**191**:369–74.
368. Greenberg H, Helms CM, Brunner H, et al. Asymptomatic infection of adult volunteers with a temperature sensitive mutant of *Mycoplasma pneumoniae*. *Proc Natl Acad Sci U S A* 1974; **71**:4015–19.
369. Greenberg H, Prescott B, Brunner H, et al. Sharing of glycolipid antigenic determinants by *Mycoplasma pneumoniae* vegetables and certain bacteria. In: Proceedings of the Symposium on New Approaches for Inducing Natural Immunity to Pyogenic Organisms, Pub. no. (NIH) 74-553, 1973. Winter Park, Florida: Washington, DC, U.S. Department of Health, Education and Welfare; 1973. p. 151–6.
370. Greenstone G. Infectious mononucleosis complicated by *Mycoplasma pneumonias:* report of two cases. *J Pediatr* 1977;**90**:492.
371. Griffin JP. Rapid screening for cold agglutinins in pneumonia. *Ann Intern Med* 1969;**70**:701–5.
372. Griffin JP, Klein EW. Role of sinusitis in primary atypical pneumonia. *Clin Med* 1971;**78**:23–7.
373. Grisold AJ, Hoenigl M, Leitner E, et al. Submasseteric abscess caused by Mycoplasma salivarium infection. *J Clin Microbiol* 2008;**46**:3860–2.
374. Grix A, Giammona ST. Pneumonitis with pleural effusion in children due to *Mycoplasma pneumoniae*. *Am Rev Respir Dis* 1974;**109**:665–71.
375. Groneck P, Goetze-Speer B, Speer CP. Inflammatory bronchopulmonary response of preterm infants with microbial colonisation of the airways at birth. *Arch Dis Child Fetal Neonatal Ed* 1996;**74**:F51–5.
376. Groneck P, Schmale J, Soditt V, et al. Bronchoalveolar inflammation following airway infection in preterm infants with chronic lung disease. *Pediatr Pulmonol* 2001;**31**:331–8.
377. Grub C, Brunborg C, Hasseltvedt V, et al. Antibodies to common infectious agents in coronary artery disease patients with and without rheumatic conditions. *Rheumatology (Oxford)* 2012;**51**: 679–85.
378. Guerrero MLF, Manuel Ramos J, Soriano F. *Mycoplasma hominis* bacteraemia not associated with genital infections. *J Infect Dis* 1999;**39**:91–4.
379. Gump DW, Hawley HB. Severe *Mycoplasma pneumoniae* pneumonia. *Respiration* 1976;**33**:475–86.
380. Gupta A, Gupta S, Mittal A, et al. Correlation of mycoplasma with unexplained infertility. *Arch Gynecol Obstet* 2009;**280**: 981–5.
381. Gursel O, Altun D, Atay AA, et al. *Mycoplasma pneumoniae* infection associated with pancytopenia: a case report. *J Pediatr Hematol Oncol* 2009;**31**:760–2.
382. Haggerty CL, Taylor BD. Mycoplasma genitalium: an emerging cause of pelvic inflammatory disease. *Infect Dis Obstet Gynecol* 2011;**2011**:959816.
383. Hagiwara H, Sakamoto S, Katsumata T, et al. Acute disseminated encephalomyelitis developed after *Mycoplasma pneumoniae* infection complicating subclinical measles infection. *Intern Med* 2009;**48**:479–83.
384. Hagiwara N, Yasuda M, Maeda S, et al. In vitro activity of azithromycin against *Mycoplasma genitalium* and its efficacy in the treatment of male *Mycoplasma genitalium*-positive nongonococcal urethritis. *J Infect Chemother* 2011;**17**:821–4.
385. Hallamaa KM, Tang SL, Ficorilli N, et al. Differential expression of lipoprotein genes in *Mycoplasma pneumoniae* after contact with human lung epithelial cells, and under oxidative and acidic stress. *BMC Microbiol* 2008;**8**:124.
386. Halsted C, Lepow ML, Balassanian N, et al. Otitis media: clinical observations, microbiology, and evaluation of therapy. *Am J Dis Child* 1968;**115**:542–51.

387. Hamrick HJ, Mangum ME, Katz VL. *Ureaplasma urealyticum* abscess at site of an internal fetal heart rate monitor. *Pediatr Infect Dis J* 1993;**12**:410–11.
388. Handsfield HH. Gonorrhea and nongonococcal urethritis: recent advances. *Med Clin North Am* 1978;**62**:925–43.
389. Harada K, Tanaka H, Komori S, et al. Vaginal infection with *Ureaplasma urealyticum* accounts for preterm delivery via induction of inflammatory responses. *Microbiol Immunol* 2008;**52**: 297–304.
390. Harris JA, Kolokathis A, Campbell M, et al. Safety and efficacy of azithromycin in the treatment of community-acquired pneumonia in children. *Pediatr Infect Dis J* 1998;**17**:865–71.
391. Hart G. Sexually transmitted diseases. In: Shen JTY, editors. *The clinical practice of adolescent medicine*. New York; Appleton-Century-Crofts; 1980. p. 101–10.
392. Hassan J, Irwin F, Dooley S, et al. *Mycoplasma pneumoniae* infection in a pediatric population: analysis of soluble immune markers as risk factors for asthma. *Hum Immunol* 2008;**69**:851–5.
393. Hasselbring BM, Sheppard ES, Krause DC. P65 truncation impacts P30 dynamics during *Mycoplasma pneumoniae* gliding. *J Bacteriol* 2012;**194**:3000–7.
394. Hata A, Honda Y, Asada K, et al. *Mycoplasma hominis* meningitis in a neonate: case report and review. *J Infect* 2008;**57**:338–43.
395. Hawkins RE, Rickman LS, Vermund SH, et al. Association of mycoplasma and human immunodeficiency virus infection: detection of amplified *Mycoplasma fermentans* DNA in blood. *J Infect Dis* 1992;**165**:581–5.
396. Hawkins S, Rausch CM, McCanta AC. Constrictive pericarditis secondary to infection with *Mycoplasma pneumoniae*. *Curr Opin Pediatr* 2011;**23**:126–9.
397. Hayflick L. Fundamental biology of the class Mollicutes, order Mycoplasmatales. In: Hayflick L, editor. *The Mycoplasmatales and the l-phase of bacteria*. New York: Appleton-Century-Crofts; 1969. p. 15–47.
398. Hayflick L, Chanock RM. *Mycoplasma* species of man. *Bacteriol Rev* 1965;**29**:185–221.
399. Hecht JL, Onderdonk A, Delaney M, et al. Characterization of chorioamnionitis in 2nd-trimester C-section placentas and correlation with microorganism recovery from subamniotic tissues. *Pediatr Dev Pathol* 2008;**11**:15–22.
400. Heggie AD, Bar-Shain D, Boxerbaum B, et al. Identification and quantification of ureaplasmas colonizing the respiratory tract and assessment of their role in the development of chronic lung disease in preterm infants. *Pediatr Infect Dis J* 2001;**20**:854–9.
401. Heggie AD, Jacobs MR, Butler VT, et al. Frequency and significance of isolation of *Ureaplasma urealyticum* and *Mycoplasma hominis* from cerebrospinal fluid and tracheal aspirate specimens from low birth weight infants. *J Pediatr* 1994;**124**:956–61.
402. Heiskanen-Kosma T, Korppi M, Jokinen C, et al. Etiology of childhood pneumonia: serologic results of a prospective, population-based study. *Pediatr Infect Dis J* 1998;**17**:986–91.
403. Helms CM, Viner JP, Sturm RH, et al. Comparative features of pneumococcal, mycoplasmal, and Legionnaires' disease pneumonias. *Ann Intern Med* 1979;**90**:543–7.
404. Hengge UR, Kirschfink M, Konig AL, et al. Characterization of I/F1 glycoprotein as a receptor for *Mycoplasma pneumoniae*. *Infect Immun* 1992;**60**:79–83.
405. Henrich B, Feldmann RC, Hadding U. Cytoadhesins of *Mycoplasma hominis*. *Infect Immun* 1993;**61**:2945–51.
406. Henrich B, Schmitt M, Bergmann N, et al. *Mycoplasma salivarium* detected in a microbial community with *Candida glabrata* in the biofilm of an occluded biliary stent. *J Med Microbiol* 2010;**59**: 239–41.
407. Hernandez LA, Urquhart GE, Dick WC. *Mycoplasma pneumoniae* infection and arthritis in man. *Br Med J* 1977;**2**:14–16.
408. Himmelreich R, Plagens H, Hilbert H, et al. Comparative analysis of the genomes of the bacteria *Mycoplasma pneumoniae* and *Mycoplasma genitalium*. *Nucleic Acids Res* 1997;**25**:701–12.
409. Hjelm E, Jonsell G, Linglof T, et al. Meningitis in a newborn infant caused by *Mycoplasma hominis*. *Acta Paediatr Scand* 1980; **69**:415–18.
410. Hodges GR, Perkins RL. Landry-Guillain-Barré syndrome associated with *Mycoplasma pneumoniae* infection. *JAMA* 1969;**210**: 2088–90.
411. Hodges GR, Fass RJ, Saslaw S. Central nervous system disease associated with *Mycoplasma pneumoniae* infection. *Arch Intern Med* 1972;**130**:277–82.
412. Holmes KK, Johnson DW, Floyd TM. Studies of venereal disease. III. Double-blind comparison of tetracycline hydrochloride and placebo in treatment of nongonococcal urethritis. *JAMA* 1967;**202**:138–40.
413. Holmes KK, Handsfield HH, Wang SP, et al. Etiology of nongonococcal urethritis. *N Engl J Med* 1975;**292**:1199–205.
414. Holmes KK, Johnson DW, Floyd TM, et al. Studies of venereal disease. II. Observations on the incidence, etiology, and treatment of the postgonococcal urethritis syndrome. *JAMA* 1967;**202**:467–73.
415. Honda J, Yano T, Kusaba M, et al. Clinical use of capillary PCR to diagnose *Mycoplasma pneumonia*. *J Clin Microbiol* 2000;**38**: 1382–4.
416. Honma Y, Yada Y, Takahashi N, et al. Certain type of chronic lung disease of newborns is associated with *Ureaplasma urealyticum* infection in utero. *Pediatr Int* 2007;**49**:479–84.
417. Horn ME, Brain E, Gregg I, et al. Respiratory viral infection in childhood: a survey in general practice, Roehampton 1967-1972. *J Hyg (Lond)* 1975;**74**:157–68.
418. Hornsleth A. *Mycoplasma pneumoniae* infection in infants and children in Copenhagen 1963-65: incidence of complement-fixing antibodies in age groups 0-9 years. *Acta Pathol Microbiol Scand [B]* 1967;**69**:304–13.
419. Horowitz S, Horowitz J, Hou L, et al. Antibodies to mycoplasma fermentans in HIV-positive heterosexual patients: seroprevalence and association with AIDS. *J Infect* 1998;**36**:79–84.
420. Horowitz S, Mazor M, Romero R, et al. Infection of the amniotic cavity with *Ureaplasma urealyticum* in the midtrimester of pregnancy. *J Reprod Med* 1995;**40**:375–9.
421. Hosker HS, Tam JS, Chain CH, et al. *Mycoplasma pneumoniae* infection in Hong Kong: clinical and epidemiological features during an epidemic. *Respiration* 1993;**60**:237–40.
422. Houpikian P, Raoult D. Blood culture-negative endocarditis in a reference center: etiologic diagnosis of 348 cases. *Medicine (Baltimore)* 2005;**84**:162–73.
423. House P, Dunn J, Carroll K, et al. Seeding of a cavernous angioma with *Mycoplasma hominis:* case report. *Neurosurgery* 2003;**53**:749–52; discussion 752–43.
424. Hu PC, Collier AM, Baseman JB. Interaction of virulent *Mycoplasma pneumoniae* with hamster tracheal organ cultures. *Infect Immun* 1976;**14**:217–24.
425. Hu PC, Cole RM, Huang YS, et al. *Mycoplasma pneumoniae* infection: role of a surface protein in the attachment organelle. *Science* 1982;**216**:313–15.
426. Hu PC, Huang CH, Collier AM, et al. Demonstration of antibodies to *Mycoplasma pneumoniae* attachment protein in human sera and respiratory secretions. *Infect Immun* 1983;**41**: 437–9.
427. Hu PC, Huang CH, Huang YS, et al. Demonstration of multiple antigenic determinants on *Mycoplasma pneumoniae* attachment protein by monoclonal antibodies. *Infect Immun* 1985;**50**:292–6.
428. Hu PC, Powell DA, Albright F, et al. A solid-phase radioimmunoassay for detection of antibodies against *Mycoplasma pneumoniae*. *J Clin Lab Immunol* 1983;**11**:209–13.
429. Hu Z, Yin J, Shen K, et al. Outbreaks of hemotrophic mycoplasma infections in China. *Emerg Infect Dis* 2009;**15**:1139–40.
430. Huhti E, Mokka T, Nikoskelainen J, et al. Association of viral and mycoplasma infections with exacerbations of asthma. *Ann Allergy* 1974;**33**:145–9.
431. Humburg J, Frei R, Wight E, et al. Accuracy of urethral swab and urine analysis for the detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in women with lower urinary tract symptoms. *Arch Gynecol Obstet* 2012;**285**:1049–53.
432. Hummell DS, Anderson SJ, Wright PF, et al. Chronic recurrent multifocal osteomyelitis: are mycoplasmas involved? *N Engl J Med* 1987;**317**:510–11.
433. Hyde TB, Gilbert M, Schwartz SB, et al. Azithromycin prophylaxis during a hospital outbreak of *Mycoplasma pneumoniae* pneumonia. *J Infect Dis* 2001;**183**:907–12.
434. Ieven M, Demey H, Ursi D, et al. Fatal encephalitis caused by *Mycoplasma pneumoniae* diagnosed by the polymerase chain reaction. *Clin Infect Dis* 1998;**27**:1552–3.

435. Ieven M, Ursi D, Van Bever H, et al. Detection of *Mycoplasma pneumoniae* by two polymerase chain reactions and role of *M. pneumoniae* in acute respiratory tract infections in pediatric patients. *J Infect Dis* 1996;**173**:1445–52.
436. Imudia AN, Detti L, Puscheck EE, et al. The prevalence of *Ureaplasma urealyticum*, *Mycoplasma hominis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections, and the rubella status of patients undergoing an initial infertility evaluation. *J Assist Reprod Genet* 2008;**25**:43–6.
437. Inamo Y, Ishizuka Y, Hashimoto K, et al. A 7-year-old girl with subcutaneous emphysema, pneumomediastinum, pneumothorax, and pneumoretroperitoneum caused by *Mycoplasma pneumoniae* pneumonia. *J Infect Chemother* 2012;**18**:247–50.
438. International Committee on Systematic Bacteriology. Proposal of minimal standards for descriptions of new species of the class Mollicutes. *Int J Syst Bacteriol* 1979;**29**:172–80.
439. Into T, Dohkan J, Inomata M, et al. Synthesis and characterization of a dipalmitoylated lipopeptide derived from paralogous lipoproteins of *Mycoplasma pneumoniae*. *Infect Immun* 2007;**75**: 2253–9.
440. Ishida K, Kaku M, Irifune K, et al. In vitro and in vivo activities of macrolides against *Mycoplasma pneumoniae*. *Antimicrob Agents Chemother* 1994;**38**:790–8.
441. Ishida K, Kaku M, Irifune K, et al. In-vitro and in-vivo activity of a new quinolone AM-1155 against *Mycoplasma pneumoniae*. *J Antimicrob Chemother* 1994;**34**:875–83.
442. Ishida Y, Hiroi K, Tauchi H, et al. Hemophagocytic lymphohistiocytosis secondary to *Mycoplasma pneumoniae* infection. *Pediatr Int* 2004;**46**:174–7.
443. Iverson-Cabral SL, Astete SG, Cohen CR, et al. mgpB and mgpC sequence diversity in *Mycoplasma genitalium* is generated by segmental reciprocal recombination with repetitive chromosomal sequences. *Mol Microbiol* 2007;**66**:55–73.
444. Izraeli S, Samra Z, Sirota L, et al. Genital mycoplasmas in preterm infants: prevalence and clinical significance. *Eur J Pediatr* 1991;**150**:804–7.
445. Jachuck SJ, Gardner-Thorpe C, Clark F, et al. A brainstem syndrome associated with *Mycoplasma pneumoniae* infection: a report of two cases. *Postgrad Med J* 1975;**51**:475–7.
446. Jackson LA, Cherry JD, Wang SP, et al. Frequency of serological evidence of *Bordetella* infections and mixed infections with other respiratory pathogens in university students with cough illnesses. *Clin Infect Dis* 2000;**31**:3–6.
447. Jacobi CA, Riessen R, Schumacher U, et al. Life-threatening pneumonia caused by human cytomegalovirus and *Mycoplasma pneumoniae* coinfection in a young, immunocompetent patient. *J Med Microbiol* 2010;**59**:980–3.
448. Jacobs E, Rock R, Dalehite L. A B cell-, T cell-linked epitope located on the adhesin of *Mycoplasma pneumoniae*. *Infect Immun* 1990;**58**:2464–9.
449. Jacobs E, Schopperle K, Bredt W. Adherence inhibition assay: a specific serological test for detection of antibodies to *Mycoplasma pneumoniae*. *Eur J Clin Microbiol* 1985;**4**:113–18.
450. Jacobs F, Van de Stadt J, Gelin M, et al. *Mycoplasma hominis* infection of perihepatic hematomas in a liver transplant recipient. *Surgery* 1992;**111**:98–100.
451. Jacobs NF, Kraus SJ. Gonococcal and nongonococcal urethritis in men: clinical and laboratory differentiation. *Ann Intern Med* 1975;**82**:7–12.
452. Jamil HA, Sandoe JA, Gascoyne-Binzi D, et al. Late-onset prosthetic valve endocarditis caused by *Mycoplasma hominis*, diagnosed using broad-range bacterial PCR. *J Med Microbiol* 2012;**61**: 300–1.
453. Jang H, Kim H, Kang B, et al. Oligonucleotide array-based detection and genotyping of mollicutes (*Acholeplasma*, *Mycoplasma*, and *Ureaplasma*). *J Microbiol Biotechnol* 2009;**19**:265–70.
454. Janney FA, Lee LT, Howe C. Cold hemagglutinin cross-reactivity with *Mycoplasma pneumoniae*. *Infect Immun* 1978;**22**:29–33.
455. Jansen LC, Justesen US, Roos SM, et al. [Seal finger in Denmark diagnosed by PCR-technique]. *Ugeskr Laeger* 2012;**174**:426–7.
456. Jansson E. Mycoplasmas and arthritis. *Scand J Rheumatol* 1975;**4**:39–42.
457. Jansson E, von Essen R, Tuuri S. *Mycoplasma pneumoniae* pneumonia in Helsinki 1962-1970: epidemic pattern and autoimmune manifestations. *Scand J Infect Dis* 1971;**3**:51–4.
458. Jansson E, Wager O, Stenstroem R, et al. Studies on Eaton Pplo pneumonia. *Br Med J* 1964;**1**:142–5.
459. Jao RL. Susceptibility of *Mycoplasma pneumoniae* to 21 antibiotics in vitro. *Am J Med Sci* 1967;**253**:639–50.
460. Jensen JS. Protocol for the detection of *Mycoplasma genitalium* by PCR from clinical specimens and subsequent detection of macrolide resistance-mediating mutations in region V of the 23S rRNA gene. *Methods Mol Biol* 2012;**903**:129–39.
461. Jensen JS, Heilmann C, Valerius NH. *Mycoplasma pneumoniae* infection in a child with AIDS. *Clin Infect Dis* 1994;**19**:207.
462. Jensen JS, Bradshaw CS, Tabrizi SN, et al. Azithromycin treatment failure in *Mycoplasma genitaliu*m-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis* 2008;**47**:1546–53.
463. Jensen JS, Orsum R, Dohn B, et al. *Mycoplasma genitalium:* a cause of male urethritis? *Genitourin Med* 1993;**69**:265–9.
464. Jensen JS, Sondergard-Andersen J, Uldum SA, et al. Detection of *Mycoplasma pneumoniae* in simulated clinical samples by polymerase chain reaction: brief report. *APMIS* 1989;**97**: 1046–8.
465. Jensen KE, Senterfit LB, Chanock RM, et al. An inactivated *Mycoplasma pneumoniae* vaccine. *JAMA* 1965;**194**:248–52.
466. Jensen KJ, Senterfit LB, Scully WE, et al. *Mycoplasma pneumoniae* infections in children: an epidemiologic appraisal in families treated with oxytetracycline. *Am J Epidemiol* 1967;**86**:419–32.
467. Johansson N, Kalin M, Hedlund J. Clinical impact of combined viral and bacterial infection in patients with community-acquired pneumonia. *Scand J Infect Dis* 2011;**43**:609–15.
468. Johnson S. Possibly autoantibody complications in *Mycoplasma pneumoniae* infection. *Clin Infect Dis* 2006;**43**:1246.
469. Johnson SM, Bruckner F, Collins D. Distribution of *Mycoplasma pneumoniae* and *Mycoplasma salivarium* in the synovial fluid of arthritis patients. *J Clin Microbiol* 2007;**45**:953–7.
470. Johnston LG, Paz-Bailey G, Morales-Miranda S, et al. High prevalence of *Mycoplasma genitalium* among female sex workers in Honduras: implications for the spread of HIV and other sexually transmitted infections. *Int J STD AIDS* 2012;**23**:5–11.
471. Jones DM, Tobin B. Neonatal eye infections due to *Mycoplasma hominis*. *Br Med J* 1968;**3**:467–8.
472. Jones GR, Stewart SM. A prospective study of the persistence of *Mycoplasma pneumoniae* antibody levels. *Scott Med J* 1974;**19**: 129–33.
473. Jones MC. Aethritis and arthralgia in infection with *Mycoplasma pneumoniae*. *Thorax* 1970;**25**:748–50.
474. Jonsson B, Rylander M, Faxelius G. *Ureaplasma urealyticum*, erythromycin and respiratory morbidity in high-risk preterm neonates. *Acta Paediatr* 1998;**87**:1079–84.
475. Joosting AC, Harwin RM, Coppin A, et al. A serological investigation of *Mycoplasma pneumoniae* infection on the Witwatersrand. *S Afr Med J* 1976;**50**:2134–5.
476. Jordan WS Jr, Dingle JH. *Mycoplasma pneumoniae* infections. In: Dubos RJ, Hirsch JG, editors. *Bacterial and mycotic infections of man*. Philadelphia: J. B. Lippincott; 1965. p. 810–24.
477. Kacerovsky M, Pavlovsky M, Tosner J. Preterm premature rupture of the membranes and genital mycoplasmas. *Acta Medica (Hradec Kralove)* 2009;**52**:117–20.
478. Kahane I, Tucker S, Leith DK, et al. Detection of the major adhesin P1 in triton shells of virulent *Mycoplasma pneumoniae*. *Infect Immun* 1985;**50**:944–6.
479. Kalb RE, Grossman ME, Neu HC. Stevens-Johnson syndrome due to *Mycoplasma pneumoniae* in an adult. *Am J Med* 1985;**79**: 541–4.
480. Kammer GM, Pollack JD, Klainer AS. Scanning-beam electron microscopy of *Mycoplasma pneumoniae*. *J Bacteriol* 1970;**104**: 499–502.
481. Kane JR, Shenep JL, Krance RA, et al. Diffuse alveolar hemorrhage associated with *Mycoplasma hominis* respiratory tract infection in a bone marrow transplant recipient. *Chest* 1994;**105**: 1891–2.
482. Kannan TR, Baseman JB. ADP-ribosylating and vacuolating cytotoxin of *Mycoplasma pneumoniae* represents unique virulence determinant among bacterial pathogens. *Proc Natl Acad Sci U S A* 2006;**103**:6724–9.
483. Kano Y, Mitsuyama Y, Hirahara K, et al. *Mycoplasma pneumoniae* infection-induced erythema nodosum, anaphylactoid purpura,

and acute urticaria in 3 people in a single family. *J Am Acad Dermatol* 2007;**57**:S33–5.
484. Kasahara I, Otsubo Y, Yanase T, et al. Isolation and characterization of *Mycoplasma pneumoniae* from cerebrospinal fluid of a patient with pneumonia and meningoencephalitis. *J Infect Dis* 1985;**152**:823–5.
485. Kashyap B, Kumar S, Sethi GR, et al. Comparison of PCR, culture & serological tests for the diagnosis of *Mycoplasma pneumoniae* in community-acquired lower respiratory tract infections in children. *Indian J Med Res* 2008;**128**:134–9.
486. Katseni VL, Gilroy CB, Ryait BK, et al. *Mycoplasma fermentans* in individuals seropositive and seronegative for HIV-1. *Lancet* 1993;**341**:271–3.
487. Katz B, Patel P, Duffy L, et al. Characterization of ureaplasmas isolated from preterm infants with and without bronchopulmonary dysplasia. *J Clin Microbiol* 2005;**43**:4852–4.
488. Katz HI, Wooten JW, Davis RG, et al. Stevens-Johnson syndrome: report of a case associated with culturally proven *Mycoplasma pneumoniae* infection. *JAMA* 1967;**199**:504–6.
489. Kaufman JM, Cuvelier CA, Van Der Straeten M. *Mycoplasma pneumonia* with fulminant evolution into diffuse interstitial fibrosis. *Thorax* 1980;**35**:140–4.
490. Kawai Y, Miyashita N, Yamaguchi T, et al. Clinical efficacy of macrolide antibiotics against genetically determined macrolide-resistant *Mycoplasma pneumoniae* pneumonia in paediatric patients. *Respirology* 2012;**17**:354–62.
491. Keceli SA, Miles RJ. Differential inhibition of mollicute growth: an approach to development of selective media for specific mollicutes. *Appl Environ Microbiol* 2002;**68**:5012–16.
492. Kechagia N, Bersimis S, Chatzipanagiotou S. Incidence and antimicrobial susceptibilities of genital mycoplasmas in outpatient women with clinical vaginitis in Athens, Greece. *J Antimicrob Chemother* 2008;**62**:122–5.
493. Keegan BM, Lowry NJ, Yager JY. *Mycoplasma pneumoniae:* a cause of coma in the absence of meningoencephalitis. *Pediatr Neurol* 1999;**21**:822–5.
494. Kelly VN, Garland SM, Gilbert GL. Isolation of genital mycoplasmas from the blood of neonates and women with pelvic infection using conventional SPS-free blood culture media. *Pathology* 1987;**19**:277–80.
495. Kennedy KJ, Prince S, Makeham T. *Mycoplasma hominis*-associated parapharyngeal abscess following acute Epstein-Barr virus infection in a previously immunocompetent adult. *J Clin Microbiol* 2009;**47**:3050–2.
496. Kenney RT, Li JS, Clyde WA Jr, et al. Mycoplasmal pericarditis: evidence of invasive disease. *Clin Infect Dis* 1993;**17**(Suppl. 1):S58–62.
497. Kenny GE. Antigenic determinants. In: Barile MF, Razin S, editors. *The mycoplasmas*, vol. I. New York: Academic Press; 1979. p. 351–84.
498. Kenny GE, Newton RM. Close serological relationship between glycolipids of *Mycoplasma pneumoniae* and glycolipids of spinach. *Ann N Y Acad Sci* 1973;**225**:54–61.
499. Kenyon SL, Taylor DJ, Tarnow-Mordi W, et al. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: the ORACLE I randomised trial. ORACLE Collaborative Group. *Lancet* 2001;**357**:979–88.
500. Kenyon SL, Taylor DJ, Tarnow-Mordi W, et al. Broad-spectrum antibiotics for spontaneous preterm labour: the ORACLE II randomised trial. ORACLE Collaborative Group. *Lancet* 2001;**357**:989–94.
501. Khan FY, Ayassin M. *Mycoplasma pneumoniae* associated with severe autoimmune hemolytic anemia: case report and literature review. *Braz J Infect Dis* 2009;**13**:77–9.
502. Khatib M, Lerner AM. Myocarditis in *Mycoplasma pneumoniae* pneumonia: occurrence with hemolytic anemia and extraordinary titers of cold isohemagglutinins. *JAMA* 1975;**231**:493–4.
503. Khatib R, Schnarr D. Point-source outbreak of *Mycoplasma pneumoniae* infection in a family unit. *J Infect Dis* 1985;**151**:186–7.
504. Kidron D, Barron SA, Mazliah J. Mononeuritis multiplex with brachial plexus neuropathy coincident with *Mycoplasma pneumoniae* infection. *Eur Neurol* 1989;**29**:90–2.
505. Kim CK, Chung CY, Kim JS, et al. Late abnormal findings on high-resolution computed tomography after *Mycoplasma pneumonia*. *Pediatrics* 2000;**105**:372–8.
506. Kim SJ, Lee DS, Lee SJ. The prevalence and clinical significance of urethritis and cervicitis in asymptomatic people by use of multiplex polymerase chain reaction. *Korean J Urol* 2011;**52**:703–8.
507. Kingston JR, Chanock RM, Mufson MA, et al. Eaton agent pneumonia. *JAMA* 1961;**176**:118–23.
508. Kirk N, Kovar I. *Mycoplasma hominis* meningitis in a preterm infant. *J Infect* 1987;**15**:109–10.
509. Klar A, Gross-Kieselstein E, Hurvitz H, et al. Bilateral Bell's palsy due to *Mycoplasma pneumoniae* infection. *Isr J Med Sci* 1985;**21**:692–4.
510. Klausner JD, Passaro D, Rosenberg J, et al. Enhanced control of an outbreak of *Mycoplasma pneumoniae* pneumonia with azithromycin prophylaxis. *J Infect Dis* 1998;**177**:161–6.
511. Kleemola M, Kayhty H. Increase in titers of antibodies to *Mycoplasma pneumoniae in patients with purulent meningitis*. *J Infect Dis* 1982;**146**:284–8.
512. Kleemola M, Kayhty H, Raty R. Presence of antibodies to *Mycoplasma pneumoniae* in patients with bacterial meningitis: Reply. *J Infect Dis* 1983;**148**:363–5.
513. Klein JO, Buckland D, Finland M. Colonization of newborn infants by mycoplasmas. *N Engl J Med* 1969;**280**:1025–30.
514. Klein NC, Petelin A, Cunha BA. Mycoplasma pneumoniae preceding Lemierre's syndrome due to *Fusobacterium nucleatum* complicated by acute Epstein-Barr virus (EBV) infectious mononucleosis in an immunocompetent host. *Heart Lung* 2013;**42**:74–6.
515. Klimek JJ, Russman BS, Quintiliani R. *Mycoplasma pneumoniae* meningoencephalitis and transverse myelitis in association with low cerebrospinal fluid glucose. *Pediatrics* 1976;**58**:133–5.
516. Knausz M, Niederland T, Dosa E, et al. Meningo-encephalitis in a neonate caused by maternal *Mycoplasma hominis* treated successfully with chloramphenicol. *J Med Microbiol* 2002;**51**:187–8.
517. Koh YY, Park Y, Lee HJ, et al. Levels of interleukin-2, interferon-gamma, and interleukin-4 in bronchoalveolar lavage fluid from patients with *Mycoplasma pneumonia:* implication of tendency toward increased immunoglobulin E production. *Pediatrics* 2001;**107**:E39.
518. Kok TW, Marmion BP, Varkanis G, et al. Laboratory diagnosis of *Mycoplasma pneumoniae* infection. III. Detection of IgM antibodies to *M. pneumoniae* by a modified indirect haemagglutination test. *Epidemiol Infect* 1989;**103**:613–23.
519. Koletsky RJ, Weinstein AJ. Fulminant *Mycoplasma pneumoniae* infection: report of a fatal case, and a review of the literature. *Am Rev Respir Dis* 1980;**122**:491–6.
520. Kolski H, Ford-Jones EL, Richardson S, et al. Etiology of acute childhood encephalitis at the hospital for sick children, Toronto, 1994-1995. *Clin Infect Dis* 1998;**26**:398–409.
521. Komaroff AL, Aronson MD, Pass TM. Serologic evidence of chlamydial and mycoplasmal pharyngitis in adults. *Science* 1983;**222**:927–9.
522. Komatsu H, Kuroki S, Shimizu Y, et al. *Mycoplasma pneu*moniae meningoencephalitis and cerebellitis with antiganglioside antibodies. *Pediatr Neurol* 1998;**18**:160–4.
523. Kopelman P. Raised mean cell volume and meningoencephalitis associated with *Mycoplasma pneumoniae* infection. *Br Med J* 1977;**1**:881–2.
524. Kornspan JD, Tarshis M, Rottem S. Adhesion and biofilm formation of *Mycoplasma pneumoniae* on an abiotic surface. *Arch Microbiol* 2011;**193**:833–6.
525. Korppi M. Bacterial infections and pediatric asthma. *Immunol Allergy Clin North Am* 2010;**30**:565–74, vii.
526. Koshiba H, Koshiba A, Daimon Y, et al. Hematoma and abscess formation caused by *Mycoplasma hominis* following cesarean section. *Int J Womens Health* 2011;**3**:15–18.
527. Koskiniemi M. CNS manifestations associated with *Mycoplasma pneumoniae* infections: summary of cases at the University of Helsinki and review. *Clin Infect Dis* 1993;**17**:S52–7.
528. Kotikoski MJ, Kleemola M, Palmu AAI. No evidence of *Mycoplasma pneumoniae* in acute myringitis. *Pediatr Infect Dis J* 2004;**23**:465–6.
529. Kottayam R, Rozenberg G, Cohn RJ. Unusual haematologic manifestations of *Mycoplasma pneumoniae* infection. *J Paediatr Child Health* 2007;**43**:80–2.

530. Kovacic R, Launay V, Tuppin P, et al. Search for the presence of six *Mycoplasma* species in peripheral blood mononuclear cells of subjects seropositive and seronegative for human immunodeficiency virus. *J Clin Microbiol* 1996;**34**:1808–10.
531. Kraft M, Cassell GH, Henson JE, et al. Detection of *Mycoplasma pneumoniae* in the airways of adults with chronic asthma. *Am J Respir Crit Care Med* 1998;**158**:998–1001.
532. Kramer BW, Kallapur S, Newnham J, et al. Prenatal inflammation and lung development. *Semin Fetal Neonatal Med* 2009;**14**: 2–7.
533. Krause DC. *Mycoplasma pneumoniae* cytadherence: unravelling the tie that binds. *Mol Microbiol* 1996;**20**:247–53.
534. Krause DC, Baseman JB. Inhibition of *Mycoplasma pneumoniae* hemadsorption and adherence to respiratory epithelium by antibodies to a membrane protein. *Infect Immun* 1983;**39**:1180–6.
535. Krausse R, Schubert S. In-vitro activities of tetracyclines, macrolides, fluoroquinolones and clindamycin against *Mycoplasma hominis* and *Ureaplasma* ssp. isolated in Germany over 20 years. *Clin Microbiol Infect* 2010;**16**:1649–55.
536. Krijnen MR, Hekker T, Algra J, et al. *Mycoplasma hominis* deep wound infection after neuromuscular scoliosis surgery: the use of real-time polymerase chain reaction (PCR). *Eur Spine J* 2006; **15**:599–603.
537. Kumada S, Kusaka H, Okaniwa M, et al. Encephalomyelitis subsequent to mycoplasma infection with elevated serum anti-Gal C antibody. *Pediatr Neurol* 1997;**16**:241–4.
538. Kundsin RB, Driscoll SG, Monson RR, et al. Association of *Ureaplasma urealyticum* in the placenta with perinatal morbidity and mortality. *N Engl J Med* 1984;**310**:941–5.
539. Kundsin RB, Driscoll SG, Pelletier PA. *Ureaplasma urealyticum* incriminated in perinatal morbidity and mortality. *Science* 1981; **213**:474–5.
540. Kunimi Y, Hirata Y, Aihara M, et al. Statistical analysis of Stevens-Johnson syndrome caused by *Mycoplasma pneumonia* infection in Japan. *Allergol Int* 2011;**60**:525–32.
541. Kupila L, Rantakokko-Jalava K, Jalava J, et al. Aetiological diagnosis of brain abscesses and spinal infections: application of broad range bacterial polymerase chain reaction analysis. *J Neurol Neurosurg Psychiatry* 2003;**74**:728–33.
542. Kupila L, Rantakokko-Jalava K, Jalava J, et al. Brain abscess caused by *Mycoplasma hominis:* a clinically recognizable entity? *Eur J Neurol* 2006;**13**:550–1.
543. Ladefoged SA. Molecular dissection of *Mycoplasma hominis*. *APMIS Suppl* 2000;**97**:1–45.
544. Lam K, Bayer AS. *Mycoplasma pneumoniae* as a cause of the 'fever of unknown origin' syndrome. *Arch Intern Med* 1982;**142**: 2312–13.
545. Lambert HP. Antibody to *Mycoplasma pneumoniae* in normal subjects and in patients with chronic bronchitis. *J Hyg (Lond)* 1968;**66**:185–9.
546. Lambert HP. Syndrome with joint manifestations in association with *Mycoplasma pneumoniae* infection. *Br Med J* 1968;**3**:156–7.
547. Lambert HP. Infections caused by *Mycoplasma pneumoniae*. *Br J Dis Chest* 1969;**63**:71–82.
548. Lamey JR, Foy HM, Kenny GE. Infection with *Mycoplasma hominis* and T-strains in the female genital tract. *Obstet Gynecol* 1974;**44**:703–8.
549. Lanzafame M, Delama A, Lattuada E, et al. Prevalence and clinical significance of *Ureaplasma urealyticum* and *Mycoplasma hominis* in the lower genital tract of HIV-1-infected women. *Infez Med* 2006;**14**:213–15.
550. Larsen B, Hwang J. *Mycoplasma*, *Ureaplasma*, and adverse pregnancy outcomes: a fresh look. *Infect Dis Obstet Gynecol* 2010, doi: 10.1155/2010/521921. Epub ahead of print.
551. Larsen PD, Crisp D. Acute bilateral striatal necrosis associated with *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis J* 1996; **15**:1124–6.
552. Lascari AD, Garfunkel JM, Mauro DJ. Varicella-like rash associated with *Mycoplasma* infection. *Am J Dis Child* 1974;**128**: 254–5.
553. Laso M, Cadario ME, Haymes L, et al. *Mycoplasma pneumoniae* detection with PCR in renal tissue of a patient with acute glomerulonephritis. *Pediatr Nephrol* 2006;**21**:1483–6.
554. Lawton BA, Rose SB, Bromhead C, et al. High prevalence of *Mycoplasma genitalium* in women presenting for termination of pregnancy. *Contraception* 2008;**77**:294–8.
555. Layani-Milon MP, Gras I, Valette M, et al. Incidence of upper respiratory tract *Mycoplasma pneumoniae* infections among outpatients in Rhone-Alpes, France, during five successive winter periods. *J Clin Microbiol* 1999;**37**:1721–6.
556. Leach A, Lewis BW. Unusual *Mycoplasma pneumoniae*. *Br Med J* 1969;**1**:185.
557. Leach RH, Hales A, Furr PM, et al. Problems in the identification of *Mycoplasma pirum* isolated from human lymphoblastoid cell cultures. *FEMS Microbiol Lett* 1987;**44**:293–7.
558. Lee I, Kim TS, Yoon HK. Mycoplasma pneumoniae pneumonia: CT features in 16 patients. *Eur Radiol* 2006;**16**:719–25.
559. Lee KY, Lee HS, Hong JH, et al. Role of prednisolone treatment in severe *Mycoplasma pneumoniae* pneumonia in children. *Pediatr Pulmonol* 2006;**41**:263–8.
560. Lee MN, Cha JH, Ahn HM, et al. *Mycoplasma pneumoniae* infection in patients with Kawasaki disease. *Korean J Pediatr* 2011; **54**:123–7.
561. Lee SE, Romero R, Kim EC, et al. A high Nugent score but not a positive culture for genital mycoplasmas is a risk factor for spontaneous preterm birth. *J Matern Fetal Neonatal Med* 2009;**22**:212–17.
562. Lee SH, Charoenying S, Brennan T, et al. Comparative studies of three serologic methods for the measurement of Mycoplasma *pneumoniae* antibodies. *Am J Clin Pathol* 1989;**92**:342–7.
563. Lee YH, Rosner B, Alpert S, et al. Clinical and microbiological investigation of men with urethritis. *J Infect Dis* 1978;**138**: 798–803.
564. Lehtinen P, Jartti T, Virkki R, et al. Bacterial coinfections in children with viral wheezing. *Eur J Clin Microbiol Infect Dis* 2006;**25**:463–9.
565. Lehtokoski-Lehtiniemi E, Koskiniemi ML. *Mycoplasma pneumoniae* encephalitis: a severe entity in children. *Pediatr Infect Dis J* 1989;**8**:651–3.
566. Leinikki P, Pantzar P. Acute pancreatitis in mycoplasma pneumoniae infections. *Br Med J* 1973;**2**:554.
567. Leinikki PO, Panzar P, Tykka H. Immunoglobulin M antibody response against *Mycoplasma pneumoniae* lipid antigen in patients with acute pancreatitis. *J Clin Microbiol* 1978;**8**:113–18.
568. Lemcke RM, Shaw EJ, Marmion BP. Related antigens in *Mycoplasma pneumoniae* and *Mycoplasma mycoides* var. mycoides. *Aust J Exp Biol Med Sci* 1965;**43**:761–70.
569. Lenglet A, Herrador Z, Magiorakos AP, et al. Surveillance status and recent data for *Mycoplasma pneumoniae* infections in the European Union and European Economic Area, January 2012. *Euro Surveill* 2012;**17**.
570. Leonardi S, del Giudice MM, Spicuzza L, et al. Lung abscess in a child with *Mycoplasma pneumoniae* infection. *Eur J Pediatr* 2010;**169**:1413–15.
571. Leonardi S, Pavone P, Rotolo N, et al. Stroke in two children with *Mycoplasma pneumoniae* infection: a causal or casual relationship? *Pediatr Infect Dis J* 2005;**24**:843–5.
572. Lepow ML, Balassanian N, Emmerich J, et al. Interrelationships of viral, mycoplasmal, and bacterial agents in uncomplicated pneumonia. *Am Rev Respir Dis* 1968;**97**:533–45.
573. Lerer RJ, Kalavsky SM. Central nervous system disease associated with *Mycoplasma pneumoniae* infection: report of five cases and review of the literature. *Pediatrics* 1973;**52**: 658–68.
574. Levine DP, Lerner AM. The clinical spectrum of *Mycoplasma pneumoniae* infections. *Med Clin North Am* 1978;**62**:961–78.
575. Lewes D, Rainford DJ, Lane WF. Symptomless myocarditis and myalgia in viral and *Mycoplasma pneumoniae* infections. *Br Heart J* 1974;**36**:924–32.
576. Lewis JE, Sheptin C. Mycoplasmal pneumonia associated with abscess of the lung. *Calif Med* 1972;**117**:69–72.
577. Li YH, Brauner A, Jonsson B, et al. *Ureaplasma urealyticum*-induced production of proinflammatory cytokines by macrophages. *Pediatr Res* 2000;**48**:114–19.
578. Likitnukul S, Kusmiesz H, Nelson JD, et al. Role of genital mycoplasmas in young infants with suspected sepsis. *J Pediatr* 1986;**109**:971–4.
579. Lin JS. Human mycoplasmal infections: serologic observations. *Rev Infect Dis* 1985;**7**:216–31.
580. Linchevski I, Klement E, Nir-Paz R. *Mycoplasma pneumoniae* vaccine protective efficacy and adverse reactions: systematic review and meta-analysis. *Vaccine* 2009;**27**:2437–46.

581. Lind K. Immunological relationships between *Mycoplasma pneumoniae* and *Streptococcus* MG. *Acta Pathol Microbiol Scand* 1968;**73**:237–44.
582. Lind K. Incidence of *Mycoplasma pneumoniae* infection in Denmark from 1958 to 1969. *Acta Pathol Microbiol Scand B Microbiol Immunol* 1971;**79**:239–47.
583. Lind K. Production of cold agglutinins in rabbits induced by *Mycoplasma pneumoniae*, *Listeria monocytogenes* or *Streptococcus* MG. *Acta Pathol Microbiol Scand B Microbiol Immunol* 1973;**81**: 487–96.
584. Lind K, Hoier-Madsen M, Wiik A. Autoantibodies to the mitotic spindle apparatus in *Mycoplasma pneumoniae* disease. *Infect Immun* 1988;**56**:714–15.
585. Lind K, Lindhardt BO, Schutten HJ. Serological cross-reactions between *Mycoplasma genitalium* and *Mycoplasma pneumoniae*. *J Clin Microbiol* 1984;**20**:1036–43.
586. Lind K, Zoffmann H, Larsen SO, et al. *Mycoplasma pneumoniae* infection associated with affection of the central nervous system. *Acta Med Scand* 1979;**205**:325–32.
587. Lipman RP, Clyde WA Jr. The interrelationship of virulence, cytadsorption, and peroxide formation in *Mycoplasma pneumoniae*. *Proc Soc Exp Biol Med* 1969;**131**:1163–7.
588. Lipman RP, Clyde WA Jr, Denny FW. Characteristics of virulent, attenuated, and avirulent *Mycoplasma pneumoniae* strains. *J Bacteriol* 1969;**100**:1037–43.
589. Little TM, Dowdle RH. *Mycoplasma pneumonia* with inappropriate secretion of antidiuretic hormone (Letter). *Br Med J* 1975;**1**:517.
590. Liu C, Eaton MD, Heyl JT. Studies on primary atypical pneumonia. II. Observations concerning the development and immunological characteristics of antibody in patients. *J Exp Med* 1959;**109**:545–56.
591. Liu G, Talkington DF, Fields BS, et al. *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in young children from China with community-acquired pneumonia. *Diagn Microbiol Infect Dis* 2005;**52**:7–14.
592. Llibre JM, Urban A, Garcia E, et al. Bronchiolitis obliterans organizing pneumonia associated with acute *Mycoplasma pneumoniae* infection. *Clin Infect Dis* 1997;**25**:1340–2.
593. Lluch-Senar M, Vallmitjana M, Querol E, et al. A new promoterless reporter vector reveals antisense transcription in *Mycoplasma genitalium*. *Microbiology* 2007;**153**:2743–52.
594. Lo SC, Hayes MM, Tully JG, et al. *Mycoplasma penetrans* sp. nov., from the urogenital tract of patients with AIDS. *Int J Syst Bacteriol* 1992;**42**:357–64.
595. Lo SC, Hayes MM, Wang RY, et al. Newly discovered mycoplasma isolated from patients infected with HIV. *Lancet* 1991;**338**:1415–18.
596. Loda FA, Clyde WA Jr, Glezen WP, et al. Studies on the role of viruses, bacteria, and *M. pneumoniae* as causes of lower respiratory tract infections in children. *J Pediatr* 1968;**72**:161–76.
597. Loda FA, Glezen WP, Clyde WA Jr. Respiratory disease in group day care. *Pediatrics* 1972;**49**:428–37.
598. Lodi G, Resca D, Reverberi R. Fatal cold agglutinin-induced haemolytic anaemia: a case report. *J Med Case Rep* 2010;**4**:252.
599. Longcope WT. Bronchopneumonia of an unknown etiology (variety X). *Bull Johns Hopkins Hosp* 1940;**67**:268.
600. Loo VG, Richardson S, Quinn P. Isolation of *Mycoplasma pneumoniae* from pleural fluid. *Diagn Microbiol Infect Dis* 1991;**14**: 443–5.
601. Lorber J, Kalhan SC, Mahgrefte B. Treatment of ventriculitis with gentamicin and cloxacillin in infants born with spina bifida. *Arch Dis Child* 1970;**45**:178–85.
602. Losada I, Canle D, Moure R, et al. *Mycoplasma hominis* infection after liver transplantation in a child. *Transplantation* 2004;**77**: 1624–5.
603. Lu A, Wang L, Zhang X, et al. Combined treatment for child refractory *Mycoplasma pneumoniae* pneumonia with ciprofloxacin and glucocorticoid. *Pediatr Pulmonol* 2011;**46**:1093–7.
604. Ludlam GB, Bridges JB. Association of Stevens-Johnson syndrome with antibody for *Mycoplasma pneumoniae*. *Lancet* 1964;**1**:958–9.
605. Luki N, Lebel P, Boucher M, et al. Comparison of polymerase chain reaction assay with culture for detection of genital mycoplasmas in perinatal infections. *Eur J Clin Microbiol Infect Dis* 1998;**17**:255–63.
606. Lum G, Kulhanjian J. Severe encephalitis in a three-year-old girl. *Pediatr Infect Dis J* 1996;**15**:181, 184–6.
607. Lyell A, Gordon AM, Dick HM, et al. Mycoplasmas and erythema multiforme. *Lancet* 1967;**2**:1116–18.
608. Lyon AJ, McColm J, Middlemist L, et al. Randomised trial of erythromycin on the development of chronic lung disease in preterm infants. *Arch Dis Child Fetal Neonatal Ed* 1998;**78**: F10–14.
609. Lyon GM, Alspaugh JA, Meredith FT, et al. *Mycoplasma hominis* pneumonia complicating bilateral lung transplantation: case report and review of the literature. *Chest* 1997;**112**:1428–32.
610. Ma L, Jensen JS, Myers L, et al. *Mycoplasma genitalium:* an efficient strategy to generate genetic variation from a minimal genome. *Mol Microbiol* 2007;**66**:220–36.
611. Maccato M, Faro S, Summers KL. Wound infections after cesarean section with *Mycoplasma hominis* and *Ureaplasma urealyticum:* a report of three cases. *Diagn Microbiol Infect Dis* 1990;**13**: 363–5.
612. MacLean DW. *Mycoplasma pneumoniae:* one year's experience in an urban practice. *Scott Med J* 1969;**14**:312–20.
613. Macon WR, Lo SC, Poiesz BJ, et al. Acquired immunodeficiency syndrome-like illness associated with systemic *Mycoplasma fermentans* infection in a human immunodeficiency virus-negative homosexual man. *Hum Pathol* 1993;**24**:554–8.
614. Madan E, Meyer MP, Amortegui AJ. Isolation of genital mycoplasmas and *Chlamydia trachomatis* in stillborn and neonatal autopsy material. *Arch Pathol Lab Med* 1988;**112**:749–51.
615. Maia IL, Nicolau JC, Machado MN, et al. Prevalence of *Chlamydia pneumoniae* and *Mycoplasma pneumoniae* in different forms of coronary disease. *Arq Bras Cardiol* 2009;**92**:405–11, 422–8, 439–45.
616. Maisel JC, Babbitt LH, John TJ. Fatal *Mycoplasma pneumoniae* infection with isolation of organisms from lung. *JAMA* 1967;**202**:287–90.
617. Maltezou HC, La-Scola B, Astra H, et al. *Mycoplasma pneumoniae* and *Legionella pneumophila* in community-acquired lower respiratory tract infections among hospitalized children: diagnosis by real time PCR. *Scand J Infect Dis* 2004;**36**:639–42.
618. Manchee RJ, Taylor-Robinson D. Enhanced growth of T-strain mycoplasmas with N-2-hydroxyethylpiperazone-N'-2-ethanesulfonic acid buffer. *J Bacteriol* 1969;**100**:78–85.
619. Manhart LE. Another STI associated with HIV-1 acquisition: now what? *AIDS* 2012;**26**:635–7.
620. Manhart LE, Kay N. Mycoplasma genitalium: is it a sexually transmitted pathogen? *Curr Infect Dis Rep* 2010;**12**:306–13.
621. Manhart LE, Critchlow CW, Holmes KK, et al. Mucopurulent cervicitis and *Mycoplasma genitalium*. *J Infect Dis* 2003;**187**: 650–7.
622. Manhart LE, Holmes KK, Hughes JP, et al. *Mycoplasma genitalium* among young adults in the United States: an emerging sexually transmitted infection. *Am J Public Health* 2007;**97**: 1118–25.
623. Manhart LE, Mostad SB, Baeten JM, et al. High *Mycoplasma genitalium* organism burden is associated with shedding of HIV-1 DNA from the cervix. *J Infect Dis* 2008;**197**:733–6.
624. Manimtim WM, Hasday JD, Hester L, et al. *Ureaplasma urealyticum* modulates endotoxin-induced cytokine release by human monocytes derived from preterm and term newborns and adults. *Infect Immun* 2001;**69**:3906–15.
625. Mansel JK, Rosenow EC 3rd, Smith TF, et al. *Mycoplasma pneumoniae* pneumonia. *Chest* 1989;**95**:639–46.
626. Manteau C, Liet JM, Caillon J, et al. Acute severe spinal cord dysfunction in a child with meningitis: *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* co-infection. *Acta Paediatr* 2005;**94**: 1339–41.
627. Manwani NS, Balasubramanian S, Dhanalakshmi K, et al. Stevens Johnson syndrome in association with *Mycoplasma pneumonia*. *Indian J Pediatr* 2012;**79**:1097–9.
628. Mardh PA. Human respiratory tract infections with mycoplasmas and their in vitro susceptibility to tetracyclines and some other antibiotics. *Chemotherapy* 1975;**21**:47–57.
629. Mardh PA. *Mycoplasma hominis* infection of the central nervous system in newborn infants. *Sex Transm Dis* 1983;**10**:331–4.
630. Märdh PA, Ursing B. The occurrence of acute pancreatitis in *Mycoplasma pneumoniae* infection. *Scand J Infect Dis* 1974;**6**: 167–71.

630a. Marc E, Chaussain M, Moulin F, et al. Reduced lung diffusion capacity after *Mycoplasma pneumoniae* pneumonia. *Pediatr Infect Dis J* 2000;**19**:706–10.

631. Mariotti E, D'Alessio F, Mirabelli P, et al. Mollicutes contamination: a new strategy for an effective rescue of cancer cell lines. *Biologicals* 2012;**40**:88–91.

632. Markham JG. *Mycoplasma pneumoniae* infection in 1978. *N Z Med J* 1979;**90**:473–4.

633. Marmion BP, Goodburn GM. Effect of an organic gold salt on Eaton's primary atypical pneumonia agent and other observations. *Nature* 1961;**189**:247–8.

634. Martelli A. Sulla reazione di fissazione del complemento per *Mycoplasma pneumoniae* nella diagnosi sierologica di affezioni respiratorie. *Giornale di batteriologia, virologia, ed immunologia ed annali dell'Ospedale Maria Vittoria di Torino* 1972:**65**.

635. Martin RR, Warr G, Couch R, et al. Chemotaxis of human leukocytes: responsiveness to *Mycoplasma pneumoniae*. *J Lab Clin Med* 1973;**81**:520–9.

636. Masutani S, Takayama R, Tsugawa T, et al. Intracranial hypertension in a boy with *Mycoplasma pneumoniae* infection: the first report. *Scand J Infect Dis* 2004;**36**:700–1.

637. Matlow A, Th'ng C, Kovach D, et al. Susceptibilities of neonatal respiratory isolates of *Ureaplasma urealyticum* to antimicrobial agents. *Antimicrob Agents Chemother* 1998;**42**:1290–2.

638. Matsuoka M, Narita M, Okazaki N, et al. Characterization and molecular analysis of macrolide-resistant *Mycoplasma pneumoniae* clinical isolates obtained in Japan. *Antimicrob Agents Chemother* 2004;**48**:4624–30.

639. Mattila PS, Carlson P, Sivonen A, et al. Life-threatening *Mycoplasma hominis* mediastinitis. *Clin Infect Dis* 1999;**29**:1529–37.

640. Mavedzenge SN, Van Der Pol B, Weiss HA, et al. The association between *Mycoplasma genitalium* and HIV-1 acquisition in African women. *AIDS* 2012;**26**:617–24.

641. McCormack JG. *Mycoplasma pneumoniae* and the erythema multiforme: Stevens Johnson syndrome. *J Infect* 1981;**3**:32–6.

642. McCormack WM. Common sexually transmitted diseases and their treatment. *Bull N Y Acad Med* 1978;**54**:216–22.

643. McCormack WM. Epidemiology of *Mycoplasma hominis*. *Sex Transm Dis* 1983;**10**:261–2.

644. McCormack WM. Susceptibility of mycoplasmas to antimicrobial agents: clinical implications. *Clin Infect Dis* 1993;**17**(Suppl. 1):S200–1.

645. McCormack WM, Lee YH, Zinner SH. Sexual experience and urethral colonization with genital mycoplasmas: a study in normal men. *Ann Intern Med* 1973;**78**:696–8.

646. McCormack WM, Rosner B, Lee YH. Colonization with genital mycoplasmas in women. *Am J Epidemiol* 1973;**97**:240–5.

647. McCormack WM, Almeida PC, Bailey PE, et al. Sexual activity and vaginal colonization with genital mycoplasmas. *JAMA* 1972;**221**:1375–7.

648. McCormack WM, Braun P, Lee YH, et al. The genital mycoplasmas. *N Engl J Med* 1973;**288**:78–89.

649. McCormack WM, Rosner B, Alpert S, et al. Vaginal colonization with *Mycoplasma hominis and Ureaplasma urealyticum*. *Sex Transm Dis* 1986;**13**:67–70.

650. McCormack WM, Rosner B, Lee YH, et al. Isolation of genital mycoplasmas from blood obtained shortly after vaginal delivery. *Lancet* 1975;**1**:596–9.

651. McDonald JC, Moore DL. *Mycoplasma hominis* meningitis in a premature infant. *Pediatr Infect Dis J* 1988;**7**:795–8.

652. McDonald MI, Lam MH, Birch DF, et al. *Ureaplasma urealyticum* in patients with acute symptoms of urinary tract infection. *J Urol* 1982;**128**:517–19.

653. McDonough EA, Barrozo CP, Russell KL, et al. A multiplex PCR for detection of *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Bordetella pertussis* in clinical specimens. *Mol Cell Probes* 2005;**19**:314–22.

654. McGouran DC, Petterson T, McLaren JM, et al. Mucositis, conjunctivitis but no rash: the "atypical Stevens-Johnson syndrome." *Acute Med* 2011;**10**:81–2.

655. McGowin CL, Anderson-Smits C. *Mycoplasma genitalium:* an emerging cause of sexually transmitted disease in women. *PLoS Pathog* 2011;**7**:e1001324.

656. McGowin CL, Popov VL, Pyles RB. Intracellular *Mycoplasma genitalium* infection of human vaginal and cervical epithelial cells elicits distinct patterns of inflammatory cytokine secretion and provides a possible survival niche against macrophage-mediated killing. *BMC Microbiol* 2009;**9**:139.

657. McIntosh JC, Gutierrez HH. Mycoplasmal infections: epidemiology, immunology, diagnostic techniques, and therapeutic strategies. *Immunol Allergy Clin North Am* 1993;**13**:43–57.

658. McIver CJ, Rismanto N, Smith C, et al. Multiplex PCR testing detection of higher-than-expected rates of cervical mycoplasma, ureaplasma, and trichomonas and viral agent infections in sexually active australian women. *J Clin Microbiol* 2009;**47**: 1358–63.

659. McKechnie ML, Hillman R, Couldwell D, et al. Simultaneous identification of 14 genital microorganisms in urine by use of a multiplex PCR-based reverse line blot assay. *J Clin Microbiol* 2009;**47**:1871–7.

660. McKechnie ML, Hillman RJ, Jones R, et al. The prevalence of urogenital micro-organisms detected by a multiplex PCR-reverse line blot assay in women attending three sexual health clinics in Sydney, Australia. *J Med Microbiol* 2011;**60**:1010–16.

661. McNamara MJ, Phillips IA, Williams OB. Viral and *Mycoplasma pneumoniae* infections in exacerbations of chronic lung disease. *Am Rev Respir Dis* 1969;**100**:19–24.

662. Medina JL, Coalson JJ, Brooks EG, et al. *Mycoplasma pneumoniae* CARDS toxin induces pulmonary eosinophilic and lymphocytic inflammation. *Am J Respir Cell Mol Biol* 2012;**46**:815–22.

663. Mehta SD, Gaydos C, Maclean I, et al. The effect of medical male circumcision on urogenital *Mycoplasma genitalium* among men in Kisumu, Kenya. *Sex Transm Dis* 2012;**39**:276–80.

664. Mena L, Wang X, Mroczkowski TF, et al. *Mycoplasma genitalium* infections in asymptomatic men and men with urethritis attending a sexually transmitted diseases clinic in New Orleans. *Clin Infect Dis* 2002;**35**:1167–73.

665. Mena LA, Mroczkowski TF, Nsuami M, et al. A randomized comparison of azithromycin and doxycycline for the treatment of *Mycoplasma genitalium*-positive urethritis in men. *Clin Infect Dis* 2009;**48**:1649–54.

666. Metz G, Kraft M. Effects of atypical infections with *Mycoplasma* and *Chlamydia* on asthma. *Immunol Allergy Clin North Am* 2010;**30**:575–85, vii–viii.

667. Metzgar DP, Woodhour AF, Vella PP, et al. Respiratory virus vaccines. II. *Mycoplasma pneumoniae* (Eaton agent) vaccines. *Am Rev Respir Dis* 1966;**94**:1–9.

668. Meyer Sauteur PM, Gansser-Kalin U, Lautenschlager S, et al. Fuchs syndrome associated with *Mycoplasma pneumoniae* (Stevens-Johnson syndrome without skin lesions). *Pediatr Dermatol* 2011;**28**:474–6.

669. Meyers BR, Hirschman SZ. Fatal infections associated with *Mycoplasma pneumoniae:* discussion of three cases with necropsy findings. *Mt Sinai J Med* 1972;**39**:258–64.

670. Mian AN, Farney AC, Mendley SR. *Mycoplasma hominis* septic arthritis in a pediatric renal transplant recipient: case report and review of the literature. *Am J Transplant* 2005;**5**:183–8.

671. Michelow IC, Olsen K, Lozano J, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. *Pediatrics* 2004;**113**:701–7.

672. Michelow IC, Olsen K, Lozano J, et al. Diagnostic utility and clinical significance of naso- and oropharyngeal samples used in a PCR assay to diagnose infection in children with community-acquired pneumonia. *J Clin Microbiol* 2004;**42**:3339–41.

673. Miller TC, Sudhaker IB, Albers WH. Massive pericardial effusion due to *Mycoplasma hominis* in a newborn. *Am J Dis Child* 1982;**136**:271–2.

674. Mills RW, Schoolfield L. Acute transverse myelitis associated with *Mycoplasma pneumoniae* infection: a case report and review of the literature. *Pediatr Infect Dis J* 1992;**11**:228–31.

675. Minami K, Maeda H, Yanagawa T, et al. Rhabdomyolysis associated with *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis J* 2003;**22**:291–3.

676. Miranda C, Camacho E, Reina G, et al. Isolation of *Mycoplasma hominis* from extragenital cultures. *Eur J Clin Microbiol Infect Dis* 2005;**24**:334–7.

677. Miranda C, Carazo C, Banon R, et al. *Mycoplasma hominis* infection in three renal transplant patients. *Diagn Microbiol Infect Dis* 1990;**13**:329–31.

678. Mirnejad R, Amirmozafari N, Kazemi B. Simultaneous and rapid differential diagnosis of *Mycoplasma genitalium* and *Ureaplasma urealyticum* based on a polymerase chain reaction-restriction

fragment length polymorphism. *Indian J Med Microbiol* 2011;**29**: 33–6.
679. Mitsani D, Nguyen MH, Silveira FP, et al. *Mycoplasma hominis* pericarditis in a lung transplant recipient: review of the literature about an uncommon but important cardiothoracic pathogen. *Transpl Infect Dis* 2010;**12**:146–50.
680. Miyashita N, Saito A, Kohno S, et al. Multiplex PCR for the simultaneous detection of *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella pneumophila* in community-acquired pneumonia. *Respir Med* 2004;**98**:542–50.
681. Miyashita N, Sugiu T, Kawai Y, et al. Radiographic features of *Mycoplasma pneumoniae* pneumonia: differential diagnosis and performance timing. *BMC Med Imaging* 2009;**9**:7.
682. Mizutani H. Immunologic responses in patients with *Mycoplasma pneumoniae* infections. *Am Rev Respir Dis* 1983;**127**:175–9.
683. Mizutani H. Circulating immune complexes in patients with mycoplasmal pneumonia. *Am Rev Respir Dis* 1984;**130**: 627–9.
684. Mobley VL, Hobbs MM, Lau K, et al. *Mycoplasma genitalium* infection in women attending a sexually transmitted infection clinic: diagnostic specimen type, coinfections, and predictors. *Sex Transm Dis* 2012;**39**:706–9.
685. Moffet HL, Siegel AC, Doyle HK. Nonstreptococcal pharyngitis. *J Pediatr* 1968;**73**:51–60.
686. Mogabgab WJ. Beta-hemolytic streptococcal and concurrent infections in adults and children with respiratory disease, 1958 to 1969. *Am Rev Respir Dis* 1970;**102**:23–34.
687. Mogabgab WJ. Protective efficacy of killed *Mycoplasma pneumoniae* vaccine measured in large-scale studies in a military population. *Am Rev Respir Dis* 1973;**108**:899–908.
688. Mohiuddin AA, Corren J, Harbeck RJ, et al. *Ureaplasma urealyticum* chronic osteomyelitis in a patient with hypogammaglobulinemia. *J Allergy Clin Immunol* 1991;**87**:104–7.
689. Mok JYQ, Waugh PR, Simpson H. *Mycoplasma pneumoniae* infection: a follow-up study of 50 children with respiratory illness. *Arch Dis Child* 1979;**54**:506–11.
690. Montagnier L, Blanchard A. Mycoplasmas as cofactors in infection due to the human immunodeficiency virus. *Clin Infect Dis* 1993;**17**:S309–15.
691. Monto AS, Lim SK. The tecumseh study of respiratory illness: III. Incidence and periodicity of respiratory syncytial virus and *Mycoplasma pneumoniae* infections. *Am J Epidemiol* 1971;**94**: 290–301.
692. Monto AS, Bryan ER, Rhodes LM. The Tecumseh study of respiratory illness. VII. Further observations on the occurrence of respiratory syncytial virus and *Mycoplasma pneumoniae* infections. *Am J Epidemiol* 1974;**100**:458–68.
693. Moore P, Martland T. *Mycoplasma pneumoniae* infection mimicking acute rheumatic fever. *Pediatr Infect Dis J* 1994;**13**:81–2.
694. Morada M, Manzur M, Lam B, et al. Arginine metabolism in *Trichomonas vaginalis* infected with *Mycoplasma hominis*. *Microbiology* 2010;**156**:3734–43.
695. Morowitz HJ, Wallace DC. Genome size and life cycle of the mycoplasma. *Ann N Y Acad Sci* 1973;**225**:62–73.
696. Morozumi M, Takahashi T, Ubukata K. Macrolide-resistant *Mycoplasma pneumoniae:* characteristics of isolates and clinical aspects of community-acquired pneumonia. *J Infect Chemother* 2010;**16**:78–86.
697. Morozumi M, Ito A, Murayama SY, et al. Assessment of real-time PCR for diagnosis of *Mycoplasma pneumoniae* pneumonia in pediatric patients. *Can J Microbiol* 2006;**52**:125–9.
698. Moskal MJ, Kaylarian VH, Doro JM. Psychosis complicating *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis* 1984;**3**: 63–6.
699. Moule JH, Caul EO, Wreghitt TG. The specific IgM response to *Mycoplasma pneumoniae* infection: interpretation and application to early diagnosis. *Epidemiol Infect* 1987;**99**:685–92.
700. Mufson MA, Manko MA, Kingston JR, et al. Eaton agent pneumonia-clinical features. *JAMA* 1961;**178**:369–74.
701. Mufson MA, Sanders V, Wood SC, et al. Primary atypical pneumonia due to *Mycoplasma pneumoniae* (Eaton agent): report of a case with a residual pleural abnormality. *N Engl J Med* 1963;**268**:1109–11.
702. Muir MT, Cohn SM, Louden C, et al. Novel toxin assays implicate *Mycoplasma pneumoniae* in prolonged ventilator course and hypoxemia. *Chest* 2011;**139**:305–10.
703. Muller N, Riedel M, Blendinger C, et al. *Mycoplasma pneumoniae* infection and Tourette's syndrome. *Psychiatry Res* 2004;**129**: 119–25.
704. Murayama A, Abukawa D, Watanabe K, et al. Severe liver dysfunction in patients with *Mycoplasma pneumoniae* infection. *Pediatr Int* 2010;**52**:e105–7.
705. Murphy WH, Bullis C, Dabich L, et al. Isolation of mycoplasma from leukemic and nonleukemic patients. *J Natl Cancer Inst* 1970;**45**:243–51.
706. Murray HW, Masur H, Senterfit LB, et al. The protean manifestations of *Mycoplasma pneumoniae* infection in adults. *Am J Med* 1975;**58**:229–42.
707. Musatovova O, Dhandayuthapani S, Baseman JB. Transcriptional heat shock response in the smallest known self-replicating cell, *Mycoplasma genitalium*. *J Bacteriol* 2006;**188**: 2845–55.
708. Muse KE, Powell DA, Collier AM. *Mycoplasma pneumoniae* in hamster tracheal organ culture studied by scanning electron microscopy. *Infect Immun* 1976;**13**:229–37.
709. Myers PO, Khabiri E, Greub G, et al. *Mycoplasma hominis* mediastinitis after acute aortic dissection repair. *Interact Cardiovasc Thorac Surg* 2010;**11**:857–8.
710. Nadala D, Bossart W, Zucol F, et al. Community-acquired pneumonia in children due to *Mycoplasma pneumoniae:* diagnostic performance of a seminested 16S rDNA-PCR. *Diagn Microbiol Infect Dis* 2001;**39**:15–19.
711. Naftalin JM, Wellisch G, Kahana Z, et al. Mycoplasma pneumoniae septicemia (Letter). *JAMA* 1974;**228**:565.
712. Nagayama Y, Sakurai N, Tamai K, et al. Isolation of *Mycoplasma pneumoniae* from pleural fluid and/or cerebrospinal fluid: report of four cases. *Scand J Infect Dis* 1987;**19**: 521–4.
713. Nagayama Y, Sakurai N, Yamamoto K, et al. Isolation of *Mycoplasma pneumoniae* from children with lower-respiratory-tract infections. *J Infect Dis* 1988;**157**:911–17.
714. Nakagawa M, Ogino H, Shimohira M, et al. Continuous regional arterial infusion therapy for acute necrotizing pancreatitis due to *Mycoplasma pneumoniae* infection in a child. *Cardiovasc Intervent Radiol* 2009;**32**:581–4.
715. Nakao T, Orii T, Umetsu M. *Mycoplasma pneumoniae* pneumonia with pleural effusion, with special reference to isolation of *Mycoplasma pneumoniae* from pleural fluid. *Tohoku J Exp Med* 1971;**104**:13–18.
716. Nakao T, Umetsu M, Watanabe N, et al. An outbreak of *Mycoplasma pneumoniae* infection in a community. *Tohoku J Exp Med* 1970;**102**:23–31.
717. Nakayama T, Sonoda S, Urano T, et al. Interferon production during the course of *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis J* 1992;**11**:72–7.
718. Nakayama T, Urano T, Osano M, et al. Alpha interferon in the sera of patients infected with Mycoplasma pneumoniae. *J Infect Dis* 1986;**154**:904–6.
719. Namiki K, Goodison S, Porvasnik S, et al. Persistent exposure to *Mycoplasma* induces malignant transformation of human prostate cells. *PLoS One* 2009;**4**:e6872.
720. Napierala Mavedzenge S, Weiss HA. Association of *Mycoplasma genitalium* and HIV infection: a systematic review and meta-analysis. *AIDS* 2009;**23**:611–20.
721. Naraqi S, Kabins SA. *Mycoplasma pneumoniae* monoarticular arthritis. *J Pediatr* 1973;**83**:621–3.
722. Narita M. Pathogenesis of extrapulmonary manifestations of *Mycoplasma pneumoni*ae infection with special reference to pneumonia. *J Infect Chemother* 2010;**16**:162–9.
723. Narita M, Itakura O, Matsuzono Y, et al. Analysis of mycoplasmal central nervous system involvement by polymerase chain reaction. *Pediatr Infect Dis J* 1995;**14**:236–7.
724. Narita M, Matsuzono Y, Itakura O, et al. Survey of mycoplasmal bacteremia detected in children by polymerase chain reaction. *Clin Infect Dis* 1996;**23**:522–5.
725. Narita M, Matsuzono Y, Itakura O, et al. Analysis of mycoplasmal pleural effusion by the polymerase chain reaction. *Arch Dis Child* 1998;**78**:67–9.
726. Narita M, Yamada S, Nakayama T, et al. Two cases of lymphadenopathy with liver dysfunction due to *Mycoplasma pneumoniae* infection with mycoplasmal bacteraemia without pneumonia. *J Infect* 2001;**42**:154–6.

727. Nastro JA, Littner MR, Tashkin DP, et al. Diffuse, pulmonary, interstitial infiltrate and mycoplasmal pneumonia. *Am Rev Respir Dis* 1974;**110**:659–62.
728. Neal TJ, Roe MF, Shaw NJ. Spontaneously resolving *Ureaplasma urealyticum* meningitis. *Eur J Pediatr* 1994;**153**:342–3.
729. Neimark HC. Division of mycoplasmas into subgroups. *J Gen Microbiol* 1970;**63**:249–63.
730. Neimark H, Gesner M. Is *Mycoplasma pneumoniae* adherence to erythrocytes a factor in extrapulmonary dissemination? *PLoS Pathog* 2010;**6**:e1001219.
731. Nelson S, Matlow A, Johnson G, et al. Detection of *Ureaplasma urealyticum* in endotracheal tube aspirates from neonates by PCR. *J Clin Microbiol* 1998;**36**:1236–9.
732. Neman-Simha V, Renaudin H, de Barbeyrac B, et al. Isolation of genital mycoplasmas from blood of febrile obstetrical-gynecologic patients and neonates. *Scand J Infect Dis* 1992;**24**: 317–21.
733. Neu H, Ellner PD. Role of mycoplasma in exudative pharyngitis. *N Y State J Med* 1969;**69**:3026–8.
734. Neumayr L, Lennette E, Kelly D, et al. *Mycoplasma* disease and acute chest syndrome in sickle cell disease. *Pediatrics* 2003;**112**: 87–95.
735. News and Notes. Epidemiology: *Mycoplasma pneumoniae* 1977. *Br Med J* 1978;**1**:726–32.
736. Ngeow YF, Suwanjutha S, Chantarojanasriri T, et al. An Asian study on the prevalence of atypical respiratory pathogens in community-acquired pneumonia. *Int J Infect Dis* 2005;**9**: 144–53.
737. Niitu Y, Hasegawa S, Suetake T, et al. Resistance of *Mycoplasma pneumoniae* to erythromycin and other antibiotics. *J Pediatr* 1970;**76**:438–43.
738. Nir-Paz R, Abutbul A, Moses AE, et al. Ongoing epidemic of *Mycoplasma pneumoniae* infection in Jerusalem, Israel, 2010 to 2012. *Euro Surveill* 2012:**17**.
739. Nir-Paz R, Michael-Gayego A, Ron M, et al. Evaluation of eight commercial tests for *Mycoplasma pneumoniae* antibodies in the absence of acute infection. *Clin Microbiol Infect* 2006;**12**:685–8.
740. Nishioka K, Fujimoto M, Date R, et al. Bilateral sensorineural deafness associated with *Mycoplasma pneumoniae* infection: the first case report. *Hiroshima J Med Sci* 1984;**33**:585–9.
741. Nissen M, McEniery J, Delbridge G, et al. Acute hypertrophic cardiomyopathy possibly associated with *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis J* 1995;**14**:74–6.
742. Noah ND. *Mycoplasma pneumoniae* infection in the United Kingdom: 1967-73. *Br Med J* 1974;**2**:544–6.
743. Nocard E, Roux ER. Le microbe de la pleuro-pneumoniae. *Ann Inst Pasteur (Paris)* 1898;**12**:240–62.
744. Noriega ER, Simberkoff MS, Gilroy FJ, et al. Life-threatening *Mycoplasma pneumoniae* pneumonia. *JAMA* 1974;**229**:1471–2.
745. Novelli VM, Marshall WC. Optic disc swelling and *Mycoplasma pneumoniae*. *Pediatr Infect Dis* 1984;**3**:597.
746. Novelli VM, Matthew DJ, Dinwiddie RD. Acute fulminant toxic encephalopathy associated with *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis* 1985;**4**:413–15.
747. Novy MJ, Duffy L, Axthelm MK, et al. *Ureaplasma parvum* or *Mycoplasma hominis* as sole pathogens cause chorioamnionitis, preterm delivery, and fetal pneumonia in rhesus macaques. *Reprod Sci* 2009;**16**:56–70.
748. Nunes JC, Bruscato AM, Walz R, et al. Opsoclonus-myoclonus syndrome associated with *Mycoplasma pneumoniae* infection in an elderly patient. *J Neurol Sci* 2011;**305**:147–8.
749. Oderda G, Kraut JR. Rising antibody titer to *Mycoplasma pneumoniae* in acute pancreatitis. *Pediatrics* 1980;**66**:305–6.
750. Oermann C, Sockrider MM, Langston C. Severe necrotizing pneumonitis in a child with *Mycoplasma pneumoniae* infection. *Pediatr Pulmonol* 1997;**24**:61–5.
751. Ogasawara KK, Goodwin TM. The efficacy of prophylactic erythromycin in preventing vertical transmission of *Ureaplasma urealyticum*. *Am J Perinatol* 1997;**14**:233–7.
752. Ogasawara KK, Goodwin TM. Efficacy of azithromycin in reducing lower genital *Ureaplasma urealyticum* colonization in women at risk for preterm delivery. *J Matern Fetal Med* 1999;**8**:12–16.
753. Ohlsson A, Wang E, Vearncombe M. Leukocyte counts and colonization with *Ureaplasma urealyticum* in preterm neonates. *Clin Infect Dis* 1993;**17**(Suppl. 1):S144–7.
754. Oishi T, Narita M, Ohya H, et al. Rhabdomyolysis associated with antimicrobial drug-resistant *Mycoplasma pneumoniae*. *Emerg Infect Dis* 2012;**18**:849–51.
755. Ollikainen J, Heiskanen-Kosma T, Korppi M, et al. Clinical relevance of *Ureaplasma urealyticum* colonization in preterm infants. *Acta Paediatr* 1998;**87**:1075–8.
756. Ollikainen J, Hiekkaniemi H, Korppi M, et al. Hydrops fetalis associated with *Ureaplasma urealyticum*. *Acta Paediatr* 1992;**81**: 851–2.
757. Ollikainen J, Hiekkaniemi H, Korppi M, et al. *Ureaplasma urealyticum* cultured from brain tissue of preterm twins who died of intraventricular hemorrhage. *Scand J Infect Dis* 1993;**25**: 529–31.
758. Ollikainen J, Hiekkaniemi H, Korppi M, et al. *Ureaplasma urealyticum* infection associated with acute respiratory insufficiency and death in premature infants. *J Pediatr* 1993;**122**:756–60.
759. Ollikainen J, Korppi M, Heiskanen-Kosma T, et al. Chronic lung disease of the new. born is not associated with *Ureaplasma urealyticum*. *Pediatr Pulmonol* 2001;**32**:303–7.
760. Olson LD, Gilbert AA. Characteristics of *Mycoplasma hominis* adhesion. *J Bacteriol* 1993;**175**:3224–7.
761. Onderdonk AB, Delaney ML, DuBois AM, et al. Detection of bacteria in placental tissues obtained from extremely low gestational age neonates. *Am J Obstet Gynecol* 2008;**198**: 110.e111–17.
762. Ong EL, Ellis ME, Yuill GM. Neurological complication of *Mycoplasma pneumoniae* infection. *Respir Med* 1989;**83**:441–2.
763. Oriel JD. Treatment of nongonococcal urethritis. In: Hobson D, Holmes KK, editors. *Nongonococcal urethritis and related infections*. Washington, D.C.: American Society for Microbiology; 1977. p. 38–42.
764. Oriel JD, Ridgway GL, Reeve P, et al. The lack of effect of ampicillin plus probenecid given for genital infections with *Neisseria gonorrhoeae* on associated infections with *Chlamydia trachomatis*. *J Infect Dis* 1976;**133**:568–71.
765. Oshima K, Nishida H. Detection of the genes evolving under *Ureaplasma*-specific selection. *J Mol Evol* 2008;**66**:529–32.
766. Othman N, Isaacs D, Kesson A. *Mycoplasma pneumoniae* infections in Australian children. *J Paediatr Child Health* 2005;**41**: 671–6.
767. Paavonen J, Miettinen A, Stevens CE, et al. *Mycoplasma hominis* in cervicitis and endometritis. *Sex Transm Dis* 1983;**10**:276–80.
768. Paavonen J, Miettinen A, Stevens CE, et al. *Mycoplasma hominis* in nonspecific vaginitis. *Sex Transm Dis* 1983;**10**:271–5.
769. Pacifico L, Panero A, Roggini M, et al. *Ureaplasma urealyticum* and pulmonary outcome in a neonatal intensive care population. *Pediatr Infect Dis J* 1997;**16**:579–86.
770. Pana Z, Hatzipantelis E, Douma S, et al. An unusual case of *Mycoplasma pneumoniae*-associated autoimmune haemolytic anaemia in a 10-year-old female child. *J Paediatr Child Health* 2012;**48**:621–2.
771. Panero A, Pacifico L, Rossi N, et al. *Ureaplasma urealyticum* as a cause of pneumonia in preterm infants: analysis of the white cell response. *Arch Dis Child Fetal Neonatal Ed* 1995;**73**:F37–40.
772. Papaevangelou V, Falaina V, Syriopoulou V, et al. Bell's palsy associated with *Mycoplasma pneumoniae* infection. *Pediatr Infect Dis J* 1999;**18**:1024–6.
773. Park SJ, Lee YM, Lee CH, et al. A case of splenic infarction possibly attributable to *Mycoplasma pneumoniae* infection without accompanying pneumonia. *J Infect Chemother* 2012.
774. Parker F Jr, Jolliffe LS, Finland M. Primary atypical pneumonia; report of eight cases with autopsies. *Arch Pathol (Chic)* 1947;**44**: 581–608.
775. Parker P, Puck J, Fernandez F. Cerebral infarction associated with *Mycoplasma pneumoniae*. *Pediatrics* 1981;**67**:373–5.
776. Parrott RH. Viral respiratory tract illnesses in children. *Bull N Y Acad Med* 1963;**39**:629–48.
777. Pascual A, Perez MH, Jaton K, et al. *Mycoplasma hominis* necrotizing pleuropneumonia in a previously healthy adolescent. *BMC Infect Dis* 2010;**10**:335.
778. Patel KK, Salva PS, Webley WC. Colonization of paediatric lower respiratory tract with genital *Mycoplasma* species. *Respirology* 2011;**16**:1081–7.
779. Payne MS, Goss KC, Connett GJ, et al. Molecular microbiological characterization of preterm neonates at risk of bronchopulmonary dysplasia. *Pediatr Res* 2010;**67**:412–18.

780. Peltier MR, Freeman AJ, Mu HH, et al. Characterization of the macrophage-stimulating activity from *Ureaplasma urealyticum*. *Am J Reprod Immunol* 2007;**57**:186–92.
781. Pemira SM, Tolan RW Jr. *Mycoplasma pneumoniae* infection presenting as bullous papular purpuric gloves and socks syndrome: novel association and review of the literature. *Clin Pediatr (Phila)* 2011;**50**:1140–3.
782. Pereyre S, Renaudin H, Charron A, et al. Emergence of a 23S rRNA mutation in *Mycoplasma hominis* associated with a loss of the intrinsic resistance to erythromycin and azithromycin. *J Antimicrob Chemother* 2006;**57**:753–6.
783. Perez C, Gurtubay I, Martinez-Ibanez F, et al. More on polymyositis associated with *Mycoplasma pneumoniae* infection. *Scand J Rheumatol* 1999;**28**:125.
784. Perez C, Mendoza H, Hernandez R, et al. Leukocytoclastic vasculitis and polyarthritis associated with *Mycoplasma pneumoniae* infection. *Clin Infect Dis* 1997;**25**:154–5.
785. Perez CR, Leigh MW. *Mycoplasma pneumoniae* as the causative agent for pneumonia in the immunocompromised host. *Chest* 1991;**100**:860–1.
786. Pitcher D, Chalker VJ, Sheppard C, et al. Real-time detection of *Mycoplasma pneumoniae* in respiratory samples with an internal processing control. *J Med Microbiol* 2006;**55**:149–55.
787. Platt R, Lin JS, Warren JW, et al. Infection with *Mycoplasma hominis* in postpartum fever. *Lancet* 1980;**2**:1217–21.
788. Poggio TV, Orlando N, Galanternik L, et al. Microbiology of acute arthropathies among children in Argentina: *Mycoplasma pneumoniae* and *hominis* and *Ureaplasma urealyticum*. *Pediatric Infectious Disease Journal* 1998;**17**:304–8.
789. Pollack JD, Somerson NL, Senterfit LB. Isolation, characterization, and immunogenicity of *Mycoplasma pneumoniae* membranes. *Infect Immun* 1970;**2**:326–39.
790. Pönkä A. Arthritis associated with *Mycoplasma pneumoniae* infection. *Scand J Rheumatol* 1979;**8**:27–32.
791. Pönkä A. Carditis associated with *Mycoplasma pneumoniae* infection. *Acta Med Scand* 1979;**206**:77–86.
792. Pönkä. The occurrence and clinical picture of serologically verified n*Mycoplasma pneumoniae* infections with emphasis on central nervous system, cardiac and joint manifestations. *Ann Clin Res* 1979;**11**(Suppl. 24):1–60.
793. Pönkä A. Central nervous system manifestations associated with serologically verified *Mycoplasma pneumoniae* infection. *Scand J Infect Dis* 1980;**12**:175–84.
794. Powell DA, Miller K, Clyde WA Jr. Submandibular adenitis in a newborn caused by *Mycoplasma hominis*. *Pediatrics* 1979;**63**: 798–9.
795. Powell DA, Hu PC, Wilson M, et al. Attachment of *Mycoplasma pneumoniae* to respiratory epithelium. *Infect Immun* 1976;**13**: 959–66.
796. Powers JM, Johnson MD. Mycoplasmal panencephalitis: a neuropathologic documentation. *Acta Neuropathol* 2012;**124**:143–8.
797. Puechal X, Hilliquin P, Renoux M, et al. *Ureaplasma urealyticum* destructive septic polyarthritis revealing a common variable immunodeficiency. *Arthritis Rheum* 1995;**38**:1524–6.
798. Purcell RH, Chanock RM. Mycoplasmas of human origin. In: Lennette EH, editor: *Diagnostic procedures for viral and rickettsial infections*. New York: American Public Health Association; 1969. p. 786–825.
799. Putman CE, Baumgarten A, Gee JB. The prevalence of mycoplasmal complement-fixing antibodies in sarcoidosis. *Am Rev Respir Dis* 1975;**111**:364–5.
800. Putman CE, Curtis AM, Simeone JF, et al. *Mycoplasma pneumonia:* clinical and roentgenographic patterns. *Am J Roentgenol Radium Ther Nucl Med* 1975;**124**:417–22.
801. Quinn PA, Gillan JE, Markestad T, et al. Intrauterine infection with *Ureaplasma urealyticum* as a cause of fatal neonatal pneumonia. *Pediatr Infect Dis* 1985;**4**:538–43.
802. Quinn PA, Li HC, Th'ng C, et al. Serological response to *Ureaplasma urealyticum* in the neonate. *Clin Infect Dis* 1993;**17**(Suppl. 1):S136–43.
803. Quioc JJ, Trabut JB, Drouhin F, et al. Acute cholestatic hepatitis revealing *Mycoplasma pneumoniae* infection without lung involvement in an adult patient. *Eur J Gastroenterol Hepatol* 2009;**21**: 220–1.
804. Radestock U, Bredt W. Motility of Mycoplasma pneumoniae. *J Bacteriol* 1977;**129**:1495–501.
805. Radisic M, Torn A, Gutierrez P, et al. Severe acute lung injury caused by *Mycoplasma pneumoniae:* potential role for steroid pulses in treatment. *Clin Infect Dis* 2000;**31**:1507–11.
806. Ramasamy A, Patel C, Conlon C. Incomplete Stevens-Johnson syndrome secondary to atypical pneumonia. *BMJ Case Rep* 2011. doi: 10.1136/bcr.08.2011.4568.
807. Rastawicki W, Jagielski M. Enzyme-linked immunosorbent assay, complement fixation test and immunoelectroprecipitation test in the diagnosis of *Mycoplasma pneumoniae* infections: comparative analysis. *Zentralbl Bakteriol* 1996;**283**:477–84.
808. Raty R, Kleemola M. Detection of *Mycoplasma pneumoniae* by polymerase chain reaction in middle ear fluids from infants with acute otitis media. *Pediatr Infect Dis J* 2000;**19**:666–8.
809. Räty R, Rönkkö E, Kleemola M. Sample type is crucial to the diagnosis of *Mycoplasma pneumoniae* pneumonia by PCR. *J Med Microbiol* 2005;**54**:287–91.
810. Ravin KA, Rappaport LD, Zuckerbraun NS, et al. *Mycoplasma pneumoniae* and atypical Stevens-Johnson syndrome: a case series. *Pediatrics* 2007;**119**:e1002–5.
811. Razin S, Yogev D, Naot Y. Molecular biology and pathogenicity of mycoplasmas. *Microbiol Mol Biol Rev* 1998;**62**:1094–156.
812. Reichert-Penetrat S, Barbaud A, Antunes A, et al. An unusual form of Stevens-Johnson syndrome with subcorneal pustules associated with *Mycoplasma pneumoniae* infection. *Pediatr Dermatol* 2000;**17**:202–4.
813. Renner ED, Helms CM, Hall NH, et al. Seroreactivity to *Mycoplasma pneumoniae* and *Legionella pneumophila:* lack of a statistically significant relationship. *J Clin Microbiol* 1981;**13**: 1096–8.
814. Renner ED, Helms CM, Johnson W, et al. Coinfections of *Mycoplasma pneumoniae* and *Legionella pneumophila* with influenza A virus. *J Clin Microbiol* 1983;**17**:146–8.
815. Reznikov M, Blackmore TK, Finlay-Jones JJ, et al. Comparison of nasopharyngeal aspirates and throat swab specimens in a polymerase chain reaction-based test for *Mycoplasma pneumoniae*. *Eur J Clin Microbiol Infect Dis* 1995;**14**:58–61.
816. Rifkind D, Chanock R, Kravetz H, et al. Ear involvement (myringitis) and primary atypical pneumonia following inoculation of volunteers with Eaton agent. *Am Rev Respir Dis* 1962;**85**: 479–89.
817. Roberts JE, Isaacs D. Neurological and pulmonary complications of *Mycoplasma pneumoniae* infection in a pre-school child. *J Infect* 1986;**12**:251–2.
818. Roberts S, Maccato M, Faro S, et al. The microbiology of postcesarean wound morbidity. *Obstet Gynecol* 1993;**81**:383–6.
819. Roberts-Thomson IC, Cottew GS, Fraser JR. *Mycoplasma pneumonia* with severe haemolytic anaemia. *Med J Aust* 1973;**2**: 1046–7.
820. Robertson J, Gomersall M, Gill P. *Mycoplasma hominis:* growth, reproduction, and isolation of small viable cells. *J Bacteriol* 1975;**124**:1007–18.
821. Robertson JA, Vekris A, Bebear C, et al. Polymerase chain reaction using 16S rRNA gene sequences distinguishes the two biovars of *Ureaplasma urealyticum*. *J Clin Microbiol* 1993;**31**: 824–30.
822. Rogers MB. *Mycoplasma* and cancer: in search of the link. *Oncotarget* 2011;**2**:271–3.
823. Roifman CM, Rao CP, Lederman HM, et al. Increased susceptibility to *Mycoplasma* infection in patients with hypogammaglobulinemia. *Am J Med* 1986;**80**:590–4.
824. Romero R, Garite TJ. Twenty percent of very preterm neonates (23-32 weeks of gestation) are born with bacteremia caused by genital mycoplasmas. *Am J Obstet Gynecol* 2008;**198**:1–3.
825. Romero-Gomez M, Otero MA, Sanchez-Munoz D, et al. Acute hepatitis due to *Mycoplasma pneumoniae* infection without lung involvement in adult patients. *J Hepatol* 2006;**44**:827–8.
826. Ross JD, Brown L, Saunders P, et al. *Mycoplasma genitalium* in asymptomatic patients: implications for screening. *Sex Transm Infect* 2009;**85**:436–7.
827. Ross JM, Furr PM, Taylor-Robinson D, et al. The effect of genital mycoplasmas on human fetal growth. *Br J Obstet Gynaecol* 1981;**88**:749–55.
828. Rothstein TL, Kenny GE. Cranial neuropathy, myeloradiculopathy, and myositis: complications of *Mycoplasma pneumoniae* infection. *Arch Neurol* 1979;**36**:476–7.

829. Rudd PT, Cassell GH, Waites KB, et al. *Ureaplasma urealyticum* pneumonia: experimental production and demonstration of age-related susceptibility. *Infect Immun* 1989;**57**:918–25.
830. Ruhrmann G, Holthusen W. *Mycoplasma* infection and erythromycin therapy in childhood. *Scott Med J* 1977;**22**:401–3.
831. Ruiter M, Wentholt HM. A pleuropneumonia-like organism in primary fusospirochetal gangrene of the penis. *J Invest Dermatol* 1950;**15**:301–4.
832. Rykner G, Bonnafous J, Manigand G. [Serologic study of *Mycoplasma pneumoniae* infections by metabolic inhibition test: study of 114 pairs of serums from patients with pneumonia]. *Sem Hop* 1970;**46**:1498–502.
833. Rylander M, Hallander HO. In vitro comparison of the activity of doxycycline, tetracycline, erythromycin and a new macrolide, CP 62993, against *Mycoplasma pneumoniae*, *Mycoplasma hominis* and *Ureaplasma urealyticum*. *Scand J Infect Dis Suppl* 1988;**53**:12–17.
834. Sabato AR, Martin AJ, Marmion BP, et al. *Mycoplasma pneumoniae:* acute illness, antibiotics, and subsequent pulmonary function. *Arch Dis Child* 1984;**59**:1034–7.
835. Sacker I, Walker M, Brunell PA. Abscess in newborn infants caused by *Mycoplasma*. *Pediatrics* 1970;**46**:303–4.
836. Saez A, Monteagudo I, Minambres E, et al. Pneumonia due to *Mycoplasma hominis* in a healthy adult. *Scand J Infect Dis* 2003; **35**:282–4.
837. Sakoulas G. Brainstem and striatal encephalitis complicating *Mycoplasma pneumoniae* pneumonia: possible benefit of intravenous immunoglobulin. *Pediatr Infect Dis J* 2001;**20**:543–5.
838. Saliba GS, Glezen WP, Chin TD. *Mycoplasma pneumoniae* infection in a resident boys' home. *Am J Epidemiol* 1967;**86**: 408–18.
839. Salmeri M, Valenti D, La Vignera S, et al. Prevalence of *Ureaplasma urealyticum* and *Mycoplasma hominis* infection in unselected infertile men. *J Chemother* 2012;**24**:81–6.
840. Salzman MB, Sood SK, Slavin ML, et al. Ocular manifestations of *Mycoplasma pneumoniae* infection. *Clin Infect Dis* 1992;**14**: 1137–9.
841. Samra Z, Rosenberg S, Madar-Shapiro L. Direct simultaneous detection of 6 sexually transmitted pathogens from clinical specimens by multiplex polymerase chain reaction and auto-capillary electrophoresis. *Diagn Microbiol Infect Dis* 2011;**70**:17–21.
842. Sanchez PJ. Perinatal transmission of *Ureaplasma urealyticum:* current concepts based on review of the literature. *Clin Infect Dis* 1993;**17**(Suppl. 1):S107–11.
843. Sanchez PJ, Regan JA. Vertical transmission of *Ureaplasma urealyticum* in full term infants. *Pediatr Infect Dis J* 1987;**6**:825–8.
844. Sanchez PJ, Regan JA. Vertical transmission of *Ureaplasma urealyticum* from mothers to preterm infants. *Pediatr Infect Dis J* 1990;**9**:398–401.
845. Sanchez PJ, Zeray F, Priest C, et al. Abstract 110. Pharmacokinetic analysis of clarithromycin in very-low-birth-weight infants colonized with *Ureaplasma urealyticum*. Paper presented at: 37th Interscience Conference on Antimicrobial Agents and Chemotherapy; Toronto, Canada. September 28-October 1, 1997.
846. Sande MA, Gadot F, Wenzel RP. Point source epidemic of *Mycoplasma pneumoniae* infection in a prosthodontics laboratory. *Am Rev Respir Dis* 1975;**112**:213–17.
847. Sanders DY, Johnson HW. Stevens-Johnson syndrome associated with *Mycoplasma pneumoniae* infection. *Am J Dis Child* 1971;**121**:243–5.
848. Sands MJ Jr, Satz JE, Turner WE Jr, et al. Pericarditis and perimyocarditis associated with active *Mycoplasma pneumoniae* infection. *Ann Intern Med* 1977;**86**:544–8.
849. Sasaki T, Bonissol C, Stoiljkovic B. Cross-reactive antibodies to mycoplasmas found in human sera by the enzyme-linked immunosorbent assay (ELISA). *Microbiol Immunol* 1987;**31**:521–30.
850. Sasaki Y, Honda M, Naitou M, et al. Detection of *Mycoplasma fermentans* DNA from lymph nodes of acquired immunodeficiency syndrome patients. *Microb Pathog* 1994;**17**:131–5.
851. Sato H, Iino N, Ohashi R, et al. Hypogammaglobulinemic patient with polyarthritis mimicking rheumatoid arthritis finally diagnosed as septic arthritis caused by *Mycoplasma hominis*. *Intern Med* 2012;**51**:425–9.
852. Sauteur PM, Goetschel P, Lautenschlager S. *Mycoplasma pneumoniae* and mucositis—part of the Stevens-Johnson syndrome spectrum. *J Dtsch Dermatol Ges* 2012;**10**;740–6.
853. Savolainen S, Jousimies-Somer H, Kleemola M, et al. Serological evidence of viral or *Mycoplasma pneumoniae* infection in acute maxillary sinusitis. *Eur J Clin Microbiol Infect Dis* 1989;**8**:131–5.
854. Scapini JP, Flynn LP, Sciacaluga S, et al. Confirmed *Mycoplasma pneumoniae* endocarditis. *Emerg Infect Dis* 2008;**14**:1664–5.
855. Schachter J, Hanna L, Hill EC, et al. Are chlamydial infections the most prevalent venereal disease? *JAMA* 1975;**231**:1252–5.
856. Schalock PC, Dinulos JG. *Mycoplasma pneumoniae*-induced cutaneous disease. *Int J Dermatol* 2009;**48**:673–80; quiz 680–71.
857. Schalock PC, Thyssen JP, Dinulos JG. *Mycoplasma pneumoniae*-associated mucositis: by any other name is not so sweet. *Pediatr Dermatol* 2012;**29**:392.
858. Schamberger MS, Goel J, Braddock SR, et al. Stevens-Johnson syndrome and respiratory failure in a 9-year-old boy. *South Med J* 1997;**90**:755–7.
859. Schelonka RL, Katz B, Waites KB, et al. Critical appraisal of the role of *Ureaplasma* in the development of bronchopulmonary dysplasia with metaanalytic techniques. *Pediatr Infect Dis J* 2005;**24**:1033–9.
860. Schessl J, Luther B, Kirschner J, et al. Infections and vaccinations preceding childhood Guillain-Barré syndrome: a prospective study. *Eur J Pediatr* 2006;**165**:605–12.
861. Scheurlen W, Frauendienst G, Schrod L, et al. Polymerase chain reaction-amplification of urease genes: rapid screening for *Ureaplasma urealyticum* infection in endotracheal aspirates of ventilated newborns. *Eur J Pediatr* 1992;**151**:740–2.
862. Schmidt NJ, Lennette EH, Dennis J, et al. On the nature of complement-fixing antibodies to *Mycoplasma pneumoniae*. *J Immunol* 1966;**97**:95–9.
863. Schmitz RC. Primary atypical pneumonia of unknown cause. *Arch Intern Med* 1945;**75**:222–32.
864. Schneeberger PM, Dorigo-Zetsma JW, van der Zee A, et al. Diagnosis of atypical pathogens in patients hospitalized with community-acquired respiratory infection. *Scand J Infect Dis* 2004;**36**:269–73.
865. Schomburg J, Vogel M. A 12-year-old boy with severe mucositis: extrapulmonary manifestation of *Mycoplasma pneumoniae* infection. *Klin Padiatr* 2012;**224**:94–5.
866. Schonwald S, Barsic B, Klinar I, et al. Three-day azithromycin compared with ten-day roxithromycin treatment of atypical pneumonia. *Scand J Infect Dis* 1994;**26**:706–10.
867. Schulman P, Piemonte TC, Singh B. Acute renal failure, hemolytic anemia, and *Mycoplasma pneumoniae*. *JAMA* 1980;**244**: 1823–4.
868. Schwartz R, Garty BZ. Variability of arthritis associated with *Mycoplasma pneumoniae* infection in children. *Clin Pediatr (Phila)* 2005;**44**:633–6.
869. Sequeira W, Jones E, Bronson DM. *Mycoplasma pneumoniae* infection with arthritis and a varicella-like eruption. *JAMA* 1981;**246**:1936–7.
870. Shahhosseiny MH, Hosseiny Z, Khoramkhorshid HR, et al. Rapid and sensitive detection of Mollicutes in cell culture by polymerase chain reaction. *J Basic Microbiol* 2010;**50**:171–8.
871. Shames JM, George RB, Holliday WB, et al. Comparison of antibiotics in the treatment of mycoplasmal pneumonia. *Arch Intern Med* 1970;**125**:680–4.
872. Sharma S, Brousseau R, Kasatiya S. Detection and confirmation of *Mycoplasma pneumoniae* in urogenital specimens by PCR. *J Clin Microbiol* 1998;**36**:277–80.
873. Shaw NJ, Pratt BC, Weindling AM. *Ureaplasma* and *Mycoplasma* infections of the central nervous system in preterm infants. *Lancet* 1989;**2**:1530–1.
874. Shawn DH, Quinn PA, Prober C, et al. Recurrent urethritis associated with *Ureaplasma urealyticum* in a prepubertal boy. *Pediatr Infect Dis J* 1987;**6**:687–8.
875. She RC, Simmon KE, Bender JM, et al. Mollicute infections in neonates. *Pediatr Infect Dis J* 2009;**28**:248–50.
876. She RC, Thurber A, Hymas WC, et al. Limited utility of culture for *Mycoplasma pneumoniae* and *Chlamydophila pneumoniae* for diagnosis of respiratory tract infections. *J Clin Microbiol* 2010; **48**:3380–2.
877. Shen Y, Zhang J, Hu Y, et al. Combination therapy with immunemodulators and moxifloxacin on fulminant macrolide-resistant *Mycoplasma pneumoniae* infection: a case report. *Pediatr Pulmonol* 2012.

878. Shenoy VD, Upadhyaya SA, Rao SP, et al. *Mycoplasma pneumoniae* infection in children with acute respiratory infection. *J Trop Pediatr* 2005;**51**:232–5.
879. Shepard MC. The recovery of pleuropneumonia-like organisms from Negro men with and without nongonococcal urethritis. *Am J Syph Gonorrhea Vener Dis* 1954;**38**:113–24.
880. Shepard MC, Lunceford CD. Differential agar medium (A7) for identification of *Ureaplasma urealyticum* (human T mycoplasmas) in primary cultures of clinical material. *J Clin Microbiol* 1976;**3**:613–25.
881. Shepard MC, Masover GK. Special features of ureaplasmas. In: Barile MF, Razins S, editors. *The mycoplasmas*, vol. I. New York: Academic Press; 1979. p. 451–94.
882. Shepard MC, Lunceford CD, Ford DK, et al. *Ureaplasma urealyticum* gen. nov. sp. nov.: proposed nomenclature for the human T (T-strain) mycoplasmas. *Int J Syst Bacteriol* 1974;**24**:160–71.
883. Sheth RD, Goulden KJ, Pryse-Phillips WE. The focal encephalopathies associated with *Mycoplasma pneumoniae*. *Can J Neurol Sci* 1993;**20**:319–23.
884. Shimada M, Kotani T, Sameshima H, et al. Two patients with premature labour associated with *Mycoplasma hominis* infection. *J Med Microbiol* 1998;**47**:179–82.
885. Shimada Y, Deguchi T, Nakane K, et al. Emergence of clinical strains of *Mycoplasma genitalium* harbouring alterations in ParC associated with fluoroquinolone resistance. *Int J Antimicrob Agents* 2010;**36**:255–8.
886. Shimada Y, Deguchi T, Nakane K, et al. Macrolide resistance-associated 23S rRNA mutation in *Mycoplasma genitalium:* Japan. *Emerg Infect Dis* 2011;**17**:1148–50.
887. Shulman ST, Bartlett J, Clyde WA Jr, et al. The unusual severity of mycoplasmal pneumonia in children with sickle-cell disease. *N Engl J Med* 1972;**287**:164–7.
888. Shurin PA, Alpert S, Bernard Rosner BA, et al. Chorioamnionitis and colonization of the newborn infant with genital mycoplasmas. *N Engl J Med* 1975;**293**:5–8.
889. Siber GR, Alpert S, Smith AL, et al. Neonatal central nervous system infection due to. *J Pediatr* 1977;**90**:625–7.
890. Sieber OF Jr, John TJ, Fulginiti VA, et al. Stevens-Johnson syndrome associated with *Mycoplasma pneumoniae* infection. *JAMA* 1967;**200**:79–81.
891. Siegler DI. Lung abscess associated with *Mycoplasma pneumoniae* infection. *Br J Dis Chest* 1973;**67**:123–7.
892. Sillis M. The limitations of IgM assays in the serological diagnosis of *Mycoplasma pneumoniae* infections. *J Med Microbiol* 1990;**33**:253–8.
893. Simmons BP, Aber RC. *Mycoplasma pneumoniae* pneumonia: symptoms mimicking pulmonary embolism with infarction. *JAMA* 1979;**241**:1268–9.
894. Simonian N, Janner D. Pleural effusion, hepatitis and hemolytic anemia in a twelve-year-old male child. *Pediatr Infect Dis J* 1998;**17**:173–4, 176–7.
895. Simpkins A, Strickland SM, Oliver J, et al. Complete resolution of advanced *Mycoplasma pneumoniae* encephalitis mimicking brain mass lesions: report of two pediatric cases and review of literature. *Neuropathology* 2012;**32**:91–9.
896. Singer JI, DeVoe WM. Severe *Mycoplasma pneumoniae* infection in otherwise healthy siblings. *J Pediatr* 1979;**95**:999–1001.
897. Skoldenberg B. Aseptic meningitis and meningoencephalitis in cold-agglutinin–positive infections. *B M J* 1965;**1**:100–2.
898. Slotkin RI, Clyde WA Jr, Denny FW. The effect of antibiotics on *Mycoplasma pneumoniae* in vitro and in vivo. *Am J Epidemiol* 1967;**86**:225–37.
899. Smiley DF, Showacre E, Lee W, et al. Acute interstitial pneumonitis: a new disease entity. *J A M A* 1939;**112**:1901.
900. Smith CB, Friedewald WT, Chanock RM. Inactivated *Mycoplasma pneumoniae* vaccine: evaluation in volunteers. *JAMA* 1967;**199**:353–8.
901. Smith CB, Friedewald WT, Chanock RM. Shedding of *Mycoplasma pneumoniae* after tetracycline and erythromycin therapy. *N Engl J Med* 1967;**276**:1172–5.
902. Smith CB, McGinniss MH, Schmidt PJ. Changes in erythrocyte I agglutinogen and anti-I-agglutinins during *Mycoplasma pneumoniae* infection in man. *J Immunol* 1967;**99**:333–9.
903. Smith CB, Golden CA, Kanner RE, et al. Association of viral and *Mycoplasma pneumoniae* infections with acute respiratory illness in patients with chronic obstructive pulmonary diseases. *Am Rev Respir Dis* 1980;**121**:225–32.
904. Smith TF. *Mycoplasma pneumoniae* infections: diagnosis based on immunofluorescence titer of IgG and IgM antibodies. *Mayo Clin Proc* 1986;**61**:830–1.
905. Smorgick N, Frenkel E, Zaidenstein R, et al. Antibiotic treatment of intra-amniotic infection with *Ureaplasma urealyticum:* a case report and literature review. *Fetal Diagn Ther* 2007;**22**:90–3.
906. So AK, Furr PM, Taylor-Robinson D, et al. Arthritis caused by *Mycoplasma salivarium* in hypogammaglobulinaemia. *Br Med J (Clin Res Ed)* 1983;**286**:762–3.
907. Sobeslavsky O, Abrahamovic M. The etiological role of *Mycoplasma pneumoniae* in otitis media in children. *Pediatrics* 1965;**35**:652–7.
908. Sobeslavsky O, Prescott B, Chanock RM. Adsorption of *Mycoplasma pneumoniae* to neuraminic acid receptors of various cells and possible role in virulence. *J Bacteriol* 1968;**96**:695–705.
909. Socan M, Ravnik I, Bencina D, et al. Neurological symptoms in patients whose cerebrospinal fluid is culture- and/or polymerase chain reaction-positive for *Mycoplasma pneumoniae*. *Clin Infect Dis* 2001;**32**:E31–5.
910. Sohaeverbeke T, Gilroy CB, Bebear C, et al. *Mycoplasma fermentans* in joints of patients with rheumatoid arthritis and other joint disorders. *Lancet* 1996;**347**:1418.
911. Solanki DL, Berdoff RL. Severe mycoplasma pneumonia with pleural effusions in a patient with sickle cell-hemoglobin C(SC) disease: case report and review of the literature. *Am J Med* 1979;**66**:707–10.
912. Somerson NL, Cole BC. The *Mycoplasma* flora of human and nonhuman primates. In: Tully JG, Whitcomb RF, editors. *The mycoplasmas*, vol. II. New York: Academic Press; 1979. p. 191–216.
913. Sompolinsky D, Solomon F, Leiba H, et al. Puerperal sepsis due to T-strain *Mycoplasma*. *Isr J Med Sci* 1971;**7**:745–8.
914. Souweine B, Mathevon T, Bret L, et al. Successful treatment of infection due to *Mycoplasma hominis* with streptogramins in a renal transplant patient: case report and review. *Clin Infect Dis* 1998;**26**:1233–4.
915. Sprinkle PM. Current status of mycoplasmatales and bacterial variants in chronic otolaryngic disease. *Laryngoscope* 1972;**82**:737–47.
916. Srinivasjois RM, Kohan R, Keil AD, et al. Congenital *Mycoplasma pneumoniae* pneumonia in a neonate. *Pediatr Infect Dis J* 2008;**27**:474–5.
917. Stadel BV, Foy HM, Nuckolls JW, et al. *Mycoplasma pneumoniae* infection followed by *Haemophilus influenzae* pneumonia and bacteremia. *Am Rev Respir Dis* 1975;**112**:131–3.
918. Stagno S, Brasfield DM, Brown MB, et al. Infant pneumonitis associated with cytomegalovirus, *Chlamydia*, *Pneumocystis*, and *Ureaplasma:* a prospective study. *Pediatrics* 1981;**68**:322–9.
919. Stahelin-Massik J, Levy F, Friderich P, et al. Meningitis caused by *Ureaplasma urealyticum* in a full term neonate. *Pediatr Infect Dis J* 1994;**13**:419–21.
920. Stallings MW, Archer SB. Atypical *Mycoplasma* pneumonia. *Am J Dis Child* 1973;**126**:837–8.
921. Stallman ND, Allan BC. A survey of antibodies to *Mycoplasma pneumoniae* in Queensland. *Med J Aust* 1970;**1**:800–2.
922. Stallman ND, Allan BC, Wiemers MA. Infection with *Mycoplasma pneumoniae:* clinical and serological data on 286 patients. *Med J Aust* 1976;**1**:340–3.
923. Stamm WE, Batteiger BE, McCormack WM, et al. A randomized, double-blind study comparing single-dose rifalazil with single-dose azithromycin for the empirical treatment of nongonococcal urethritis in men. *Sex Transm Dis* 2007;**34**:545–52.
924. Stamm WE, Running K, Hale J, et al. Etiologic role of *Mycoplasma hominis* and *Ureaplasma urealyticum* in women with the acute urethral syndrome. *Sex Transm Dis* 1983;**10**:318–22.
925. Stancombe BB, Walsh WF, Derdak S, et al. Induction of human neonatal pulmonary fibroblast cytokines by hyperoxia and *Ureaplasma urealyticum*. *Clin Infect Dis* 1993;**17**(Suppl. 1):S154–7.
926. Steele JC, Thanasophon S, Gladstone RM, et al. *Mycoplasma pneumoniae* as a determinant of the Guillain-Barré syndrome. *Lancet* 1969;**2**:710–14.

927. Steer JA, Tasker S, Barker EN, et al. A novel hemotropic *Mycoplasma* (hemoplasma) in a patient with hemolytic anemia and pyrexia. *Clin Infect Dis* 2011;**53**:e147–51.
928. Steinberg P, White RJ, Fuld SL, et al. Ecology of *Mycoplasma pneumoniae* infections in marine recruits at Parris Island, South Carolina. *Am J Epidemiol* 1969;**89**:62–73.
929. Stellrecht KA, Woron AM, Mishrik NG, et al. Comparison of multiplex PCR assay with culture for detection of genital mycoplasmas. *J Clin Microbiol* 2004;**42**:1528–33.
930. Sterner G, DeHevesy G, Tunevall G, et al. Acute respiratory illness with *Mycoplasma pneumoniae:* an outbreak in a home for children. *Acta Paediatr Scand* 1966;**55**:280–6.
931. Stevens D, Swift PG, Johnston PG, et al. *Mycoplasma pneumoniae* infections in children. *Arch Dis Child* 1978;**53**:38–42.
932. Stevens MK, Krause DC. Disulfide-linked protein associated with *Mycoplasma pneumoniae* cytadherence phase variation. *Infect Immun* 1990;**58**:3430–3.
933. Stewart SM, Burnet ME, Young JE. In-vitro sensitivity of strains of mycoplasmas from human sources to antibiotics and to sodium aurothiomalate and tylosin tartrate. *J Med Microbiol* 1969;**2**: 287–92.
934. Stray-Pedersen B, Bruu AL, Molne K. Infertility and uterine colonization with *Ureaplasma urealyticum*. *Acta Obstet Gynecol Scand* 1982;**61**:21–4.
935. Stutman HR. Stevens-Johnson syndrome and *Mycoplasma pneumoniae:* evidence for cutaneous infection. *J Pediatr* 1987;**111**: 845–7.
936. Suhs RH, Feldman HA. Serologic epidemiologic studies with *M. pneumoniae*. II. Prevalence of antibodies in several populations. *Am J Epidemiol* 1966;**83**:357–65.
937. Sussman SJ, Magoffin RL, Lennette EH, et al. Cold agglutinins, Eaton agent, and respiratory infections of children. *Pediatrics* 1966;**38**:571–7.
938. Suzuki K, Hirano K, Onodera N, et al. Acute IgA nephropathy associated with *Mycoplasma pneumoniae* infection. *Pediatr Int* 2005;**47**:583–5.
939. Sykes JE, Lindsay LL, Maggi RG, et al. Human coinfection with *Bartonella henselae* and two hemotropic mycoplasma variants resembling *Mycoplasma ovis*. *J Clin Microbiol* 2010;**48**:3782–5.
940. Syrogiannopoulos GA, Kapatais-Zoumbos K, Decavalas GO, et al. *Ureaplasma urealyticum* colonization of full term infants: perinatal acquisition and persistence during early infancy. *Pediatr Infect Dis J* 1990;**9**:236–40.
941. Szymanski M, Petric M, Saunders FE, et al. *Mycoplasma pneumoniae* pericarditis demonstrated by polymerase chain reaction and electron microscopy. *Clin Infect Dis* 2002;**34**:E16–17.
942. Tablan OC, Reyes MP. Chronic interstitial pulmonary fibrosis following *Mycoplasma pneumoniae* pneumonia. *Am J Med* 1985; **79**:268–70.
943. Tafari N, Ross S, Naeye RL, et al. *Mycoplasma* T strains and perinatal death. *Lancet* 1976;**1**:108–9.
944. Takahashi S, Ichihara K, Hashimoto J, et al. Clinical efficacy of levofloxacin 500 mg once daily for 7 days for patients with non-gonococcal urethritis. *J Infect Chemother* 2011;**17**:392–6.
945. Takahashi S, Takeyama K, Miyamoto S, et al. Detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum* DNAs in urine from asymptomatic healthy young Japanese men. *J Infect Chemother* 2006;**12**: 269–71.
946. Talkington DF, Shott S, Fallon MT, et al. Analysis of eight commercial enzyme immunoassay tests for detection of antibodies to *Mycoplasma pneumoniae* in human serum. *Clin Diagn Lab Immunol* 2004;**11**:862–7.
947. Talkington DF, Thacker WL, Keller DW, et al. Diagnosis of *Mycoplasma pneumoniae* infection in autopsy and open-lung biopsy tissues by nested PCR. *J Clin Microbiol* 1998;**36**:1151–3.
948. Tamura A, Matsubara K, Tanaka T, et al. Methylprednisolone pulse therapy for refractory *Mycoplasma pneumoniae* pneumonia in children. *J Infect* 2008;**57**:223–8.
949. Tanir G, Aydemir C, Yilmaz D, et al. Internal carotid artery occlusion associated with *Mycoplasma pneumoniae* infection in a child. *Turk J Pediatr* 2006;**48**:166–71.
950. Tanowitz HB, Robbins N, Leidich N. Hemolytic anemia associated with severe *Mycoplasma pneumoniae* pneumonia. *N Y State J Med* 1978;**78**:2231–2.
951. Taylor-Robinson D. The biology of mycoplasmas. *J Clin Pathol Suppl Coll Pathol* 1968;**2**:38–51.
952. Taylor-Robinson D. *Mycoplasma* infections of the human urogenital tract with particular reference to non-gonococcal urethritis. *Ann Microbiol (Paris)* 1984;**135A**:129–34.
953. Taylor-Robinson D. Genital mycoplasma infections. *Clin Lab Med* 1989;**9**:501–23.
954. Taylor-Robinson D. The Mycoplasmatales: *Mycoplasma*, *Ureaplasma*, *Acholeplasma*, *Spiroplasma* and *Anaeroplasma*. In: Parker MT, Duerden BI, editors. *Principles of bacteriology, virology and immunity*, vol. 2. Systemic Bacteriology. Philadelphia: B. C. Decker; 1990. p. 664–81.
955. Taylor-Robinson D, Furr PM. The distribution of T-mycoplasmas within and among various animal species. *Ann N Y Acad Sci* 1973;**225**:108–17.
956. Taylor-Robinson D, Jensen JS. Mycoplasma genitalium: from chrysalis to multicolored butterfly. *Clin Microbiol Rev* 2011;**24**: 498–514.
957. Taylor-Robinson D, McCormack WM. The genital mycoplasmas (second of two parts). *N Engl J Med* 1980;**302**:1063–7.
958. Taylor-Robinson D, Gilroy CB, Hay PE. Occurrence of *Mycoplasma genitalium* in different populations and its clinical significance. *Clin Infect Dis* 1993;**17**:66–8.
959. Taylor-Robinson D, Shirai A, Sobeslavsky O, et al. Serologic response to *Myocplasma pneumoniae infection.* II. Significance of antibody measured by different techniques. *Am J Epidemiol* 1966;**84**:301–13.
960. Taylor MJ, Burrow GN, Strauch B, et al. Meningoencephalitis associated with pneumonitis due to *Mycoplasma pneumoniae*. *JAMA* 1967;**199**:813–16.
961. Teig N, Anders A, Schmidt C, et al. *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* in respiratory specimens of children with chronic lung diseases. *Thorax* 2005;**60**:962–6.
962. Teisch JA, Shapiro L, Walzer RA. Vesiculopustular eruption with mycoplasma infection. *JAMA* 1970;**211**:1694–7.
963. Teng LJ, Zheng X, Glass JI, et al. *Ureaplasma urealyticum* biovar specificity and diversity are encoded in multiple-banded antigen gene. *J Clin Microbiol* 1994;**32**:1464–9.
964. Terada M, Izumi K, Ohki E, et al. Antimicrobial efficacies of several antibiotics against uterine cervicitis caused by *Mycoplasma genitalium*. *J Infect Chemother* 2012;**18**:313–17.
965. Termine C, Uggetti C, Veggiotti P, et al. Long-term follow-up of an adolescent who had bilateral striatal necrosis secondary to *Mycoplasma pneumoniae* infection. *Brain Dev* 2005;**27**: 62–5.
966. Thacker WL, Talkington DF. Comparison of two rapid commercial tests with complement fixation for serologic diagnosis of *Mycoplasma pneumoniae* infections. *J Clin Microbiol* 1995;**33**: 1212–14.
967. Thacker WL, Talkington DF. Analysis of complement fixation and commercial enzyme immunoassays for detection of antibodies to Mycoplasma pneumoniae in human serum. *Clin Diagn Lab Immunol* 2000;**7**:778–80.
968. Theilen U, Lyon AJ, Fitzgerald T, et al. Infection with *Ureaplasma urealyticum:* is there a specific clinical and radiological course in the preterm infant? *Arch Dis Child Fetal Neonatal Ed* 2004;**89**:F163–7.
969. Thomas M, Jones M, Ray S, et al. Letter: *Mycoplasma pneumoiae* in a tubo-ovarian abscess. *Lancet* 1975;**2**:774–5.
970. Thomsen AC. Occurrence and pathogenicity of *Mycoplasma hominis* in the upper urinary tract: a review. *Sex Transm Dis* 1983;**10**:323–6.
971. Thomsen AC, Taylor-Robinson D, Brogaard Hansen K, et al. The infrequent occurrence of mycoplasmas in amniotic fluid from women with intact fetal membranes. *Acta Obstet Gynecol Scand* 1984;**63**:425–9.
972. Tipirneni P, Moore BS, Hyde JS, et al. IgE antibodies to *Mycoplasma pneumoniae* in asthma and other atopic diseases. *Ann Allergy* 1980;**45**:1–7.
973. Tjhie JH, van de Putte EM, Haasnoot K, et al. Fatal encephalitis caused by *Mycoplasma pneumoniae* in a 9-year-old girl. *Scand J Infect Dis* 1997;**29**:424–5.
974. Toth A, Lesser ML, Brooks C, et al. Subsequent pregnancies among 161 couples treated for T-mycoplasma genital-tract infection. *N Engl J Med* 1983;**308**:505–7.

975. Tsiodras S, Kelesidis T, Kelesidis I, et al. *Mycoplasma pneumoniae*-associated myelitis: a comprehensive review. *Eur J Neurol* 2006;**13**:112–24.
976. Tsolia MN, Psarras S, Bossios A, et al. Etiology of community-acquired pneumonia in hospitalized school-age children: evidence for high prevalence of viral infections. *Clin Infect Dis* 2004;**39**:681–6.
977. Tully JG. Biology of the mycoplasmas. In: McGarrity GJ, Murphy DG, Nichols WW, editors. *Mycoplasma infection of cell cultures*. New York: Plenum; 1978. p. 1–33.
978. Tully JG. Current status of the mollicute flora of humans. *Clin Infect Dis* 1993;**17**:2–9.
979. Tully JG, Rose DL, Baseman JB, et al. *Mycoplasma pneumoniae* and *Mycoplasma genitalium* mixture in synovial fluid isolate. *J Clin Microbiol* 1995;**33**:1851–5.
980. Tully JG, Rose DL, Whitcomb RF, et al. Enhanced isolation of *Mycoplasma pneumoniae* from throat washings with a newly-modified culture medium. *J Infect Dis* 1979;**139**: 478–82.
981. Tully JG, Taylor-Robinson D, Rose DL, et al. *Mycoplasma genitalium*, a new species from the human urogenital tract. *Int J Syst Bacteriol* 1983;**33**:387–96.
982. Tully JG, Taylor-Robinson D, Rose DL, et al. Urogenital challenge of primate species with *Mycoplasma genitalium* and characteristics of infection induced in chimpanzees. *J Infect Dis* 1986;**153**:1046–54.
983. Turner JA, Burchak EC, Bannatyne RM, et al. The protean manifestattions of mycoplasma infections in childhood. *Can Med Assoc J* 1968;**99**:633–7.
984. Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant *Mycoplasma genitalium* infections detected by rapid high resolution melt analysis. *PLoS One* 2012;**7**:e35593.
985. Twomey JA, Espir ML. Neurological manifestations and *Mycoplasma pneumoniae* infection. *Br Med J* 1979;**2**:832–3.
986. Uldum SA, Bangsborg JM, Gahrn-Hansen B, et al. Epidemic of *Mycoplasma pneumoniae* infection in Denmark, 2010 and 2011. *Euro Surveill* 2012;**17**.
987. Uldum SA, Jensen JS, Sondergard-Andersen J, et al. Enzyme immunoassay for detection of immunoglobulin M (IgM) and IgG antibodies to *Mycoplasma pneumoniae*. *J Clin Microbiol* 1992;**30**: 1198–204.
988. Unsworth PF, Taylor-Robinson D, Shoo EE, et al. Neonatal mycoplasmaemia: *Mycoplasma hominis* as a significant cause of disease? *J Infect* 1985;**10**:163–8.
989. Ursi D, Ursi JP, Ieven M, et al. Congenital pneumonia due to *Mycoplasma pneumoniae*. *Arch Dis Child Fetal Neonatal Ed* 1995; **72**:F118–20.
990. Valencia GB, Banzon F, Cummings M, et al. *Mycoplasma hominis* and *Ureaplasma urealyticum* in neonates with suspected infection. *Pediatr Infect Dis J* 1993;**12**:571–3.
991. Van Bever HP, Van Doorn JW, Demey HE. Adult respiratory distress syndrome associated with *Mycoplasma pneumoniae* infection. *Eur J Pediatr* 1992;**151**:227–8.
992. Van der Veen J, Van Nunen CJ. Role of *Mycoplasma pneumoniae* in acute respiratory disease in a military population. *Am J Hyg* 1963;**78**:293–301.
993. van Waarde WM, Brus F, Okken A, et al. *Ureaplasma urealyticum* colonization, prematurity and bronchopulmonary dysplasia. *Eur Respir J* 1997;**10**:886–90.
994. Vancini RG, Pereira-Neves A, Borojevic R, et al. Trichomonas vaginalis harboring *Mycoplasma hominis* increases cytopathogenicity in vitro. *Eur J Clin Microbiol Infect Dis* 2008;**27**: 259–67.
995. Ventolini G, Lee M. Clinical antibiotic response of mycoplasma and ureaplasma in patients with symptomatic recurrent vulvovaginal colonization. *J Reprod Med* 2011;**56**:138–41.
996. Vervloet LA, Vervloet VE, Tironi Junior M, et al. *Mycoplasma pneumoniae*-related community-acquired pneumonia and parapneumonic pleural effusion in children and adolescents. *J Bras Pneumol* 2012;**38**:226–36.
997. Vianello F, Barbaro F, Cogo P, et al. Co-infection with *Mycoplasma pneumon*iae and human herpesvirus 6 (HHV-6) in an immunocompetent child with meningoencephalitis: a random association? *Infection* 2008;**36**:174–6.
998. Villiger RM, von Vigier RO, Ramelli GP, et al. Precipitants in 42 cases of erythema multiforme. *Eur J Pediatr* 1999;**158**: 929–32.
999. Vincent JM, Cherry JD, Nauschuetz WF, et al. Prolonged afebrile nonproductive cough illnesses in American soldiers in Korea: a serological search for causation. *Clin Infect Dis* 2000;**30**: 534–9.
1000. Viscardi RM, Atamas SP, Luzina IG, et al. Antenatal *Ureaplasma urealyticum* respiratory tract infection stimulates proinflammatory, profibrotic responses in the preterm baboon lung. *Pediatr Res* 2006;**60**:141–6.
1001. Viscardi RM, Kaplan J, Lovchik JC, et al. Characterization of a murine model of *Ureaplasma urealyticum* pneumonia. *Infect Immun* 2002;**70**:5721–9.
1002. Vitale EA, La Torre F, Calcagno G, et al. *Mycoplasma pneumoniae:* a possible trigger of Kawasaki disease or a mere coincidental association? Report of the first four Italian cases. *Minerva Pediatr* 2010;**62**:605–7.
1003. Vogel U, Luneberg E, Kuse ER, et al. Extragenital *Mycoplasma hominis* infection in two liver transplant recipients. *Clin Infect Dis* 1997;**24**:512–13.
1004. Volk J, Kraus SJ. Nongonococcal urethritis: a venereal disease as prevalent as epidemic gonorrhea. *Arch Intern Med* 1974;**134**: 511–14.
1005. Volter C, Helms J, Weissbrich B, et al. Frequent detection of *Mycoplasma pneumoniae* in Bell's palsy. *Eur Arch Otorhinolaryngol* 2004;**261**:400–4.
1006. Vu AC, Foy HM, Cartwright FD, et al. The principal protein antigens of isolates of *Mycoplasma pneumoniae* as measured by levels of immunoglobulin G in human serum are stable in strains collected over a 10-year period. *Infect Immun* 1987; **55**:1830–6.
1007. Waites KB. What's new in diagnostic testing and treatment approaches for *Mycoplasma pneumoniae* infections in children? *Adv Exp Med Biol* 2011;**719**:47–57.
1008. Waites KB, Taylor-Robinson D. *Mycoplasma* and *Ureaplasma*. In: Murray PR, editor. *Manual of clinical microbiology*. Washington, DC: ASM Press; 2007. p. 1004–20.
1009. Waites KB, Balish MF, Atkinson TP. New insights into the pathogenesis and detection of *Mycoplasma pneumoniae* infections. *Future Microbiol* 2008;**3**:635–48.
1010. Waites KB, Crouse DT, Cassell GH. Antibiotic susceptibilities and therapeutic options for *Ureaplasma urealyticum* infections in neonates. *Pediatr Infect Dis J* 1992;**11**:23–9.
1011. Waites KB, Crouse DT, Cassell GH. Therapeutic considerations for *Ureaplasma urealyticum* infections in neonates. *Clin Infect Dis* 1993;**17**(Suppl. 1):S208–14.
1012. Waites KB, Katz B, Schelonka RL. Mycoplasmas and ureaplasmas as neonatal pathogens. *Clin Microbiol Rev* 2005;**18**: 757–89.
1013. Waites KB, Cassell GH, Canupp KC, et al. In vitro susceptibilities of mycoplasmas and ureaplasmas to new macrolides and aryl-fluoroquinolones. *Antimicrob Agents Chemother* 1988;**32**: 1500–2.
1014. Waites KB, Crouse DT, Philips JB 3rd, et al. Ureaplasmal pneumonia and sepsis associated with persistent pulmonary hypertension of the newborn. *Pediatrics* 1989;**83**:79–85.
1015. Waites KB, Duffy LB, Baldus K, et al. Mycoplasmal infections of cerebrospinal fluid in children undergoing neurosurgery for hydrocephalus. *Pediatr Infect Dis J* 1991;**10**:952–3.
1016. Waites KB, Duffy LB, Crouse DT, et al. Mycoplasmal infections of cerebrospinal fluid in newborn infants from a community hospital population. *Pediatr Infect Dis J* 1990;**9**:241–5.
1017. Waites KB, Figarola TA, Schmid T, et al. Comparison of agar versus broth dilution techniques for determining antibiotic susceptibilities of *Ureaplasma urealyticum*. *Diagn Microbiol Infect Dis* 1991;**14**:265–71.
1018. Waites KB, Rudd PT, Crouse DT, et al. Chronic *Ureaplasma urealyticum* and *Mycoplasma hominis* infections of central nervous system in preterm infants. *Lancet* 1988;**1**:17–21.
1019. Waites KB, Schelonka RL, Xiao L, et al. Congenital and opportunistic infections: *Ureaplasma* species and *Mycoplasma hominis*. *Semin Fetal Neonatal Med* 2009;**14**:190–9.
1020. Waites KB, Sims PJ, Crouse DT, et al. Serum concentrations of erythromycin after intravenous infusion in preterm neonates

treated for *Ureaplasma urealyticum* infection. *Pediatr Infect Dis J* 1994;**13**:287–93.
1021. Walsh WF, Butler J, Coalson J, et al. A primate model of *Ureaplasma urealyticum* infection in the premature infant with hyaline membrane disease. *Clin Infect Dis* 1993;**17**(Suppl. 1): S158–62.
1022. Walsh WF, Stanley S, Lally KP, et al. *Ureaplasma urealyticum* demonstrated by open lung biopsy in newborns with chronic lung disease. *Pediatr Infect Dis J* 1991;**10**:823–7.
1023. Wan GH, Huang CG, Huang YC, et al. Surveillance of airborne adenovirus and *Mycoplasma pneumoniae* in a hospital pediatric department. *PLoS One* 2012;**7**:e33974.
1024. Wang EE, Ohlsson A, Kellner JD. Association of *Ureaplasma urealyticum* colonization with chronic lung disease of prematurity: results of a metaanalysis. *J Pediatr* 1995;**127**:640–4.
1025. Wang EE, Matlow AG, Ohlsson A, et al. *Ureaplasma urealyticum* infections in the perinatal period. *Clin Perinatol* 1997;**24**: 91–105.
1026. Wang K, Chalker V, Bermingham A, et al. *Mycoplasma pneumoniae* and respiratory virus infections in children with persistent cough in England: a retrospective analysis. *Pediatr Infect Dis J* 2011;**30**:1047–51.
1027. Wang RS, Wang SY, Hsieh KS, et al. Necrotizing pneumonitis caused by *Mycoplasma pneumoniae* in pediatric patients: report of five cases and review of literature. *Pediatr Infect Dis J* 2004;**23**: 564–7.
1028. Wang RY, Shih JW, Grandinetti T, et al. High frequency of antibodies to *Mycoplasma penetrans* in HIV-infected patients. *Lancet* 1992;**340**:1312–16.
1029. Wang RY, Shih JW, Weiss SH, et al. *Mycoplasma penetrans* infection in male homosexuals with AIDS: high seroprevalence and association with Kaposi's sarcoma. *Clin Infect Dis* 1993;**17**: 724–9.
1030. Waris ME, Toikka P, Saarinen T, et al. Diagnosis of *Mycoplasma pneumoniae* pneumonia in children. *J Clin Microbiol* 1998;**36**: 3155–9.
1031. Warren P, Fischbein C, Mascoli N, et al. Poliomyelitis-like syndrome caused by *Mcyoplasma pneumoniae*. *J Pediatr* 1978;**93**: 451–2.
1032. Watanabe T, Matsuura M, Seto K. Enumeration, isolation, and species identification of mycoplasmas in saliva sampled from the normal and pathological human oral cavity and antibody response to an oral mycoplasma *(Mycoplasma salivarium)*. *J Clin Microbiol* 1986;**23**:1034–8.
1033. Watanabe M, Hitomi S, Goto M, et al. Bloodstream infection due to *Mycoplasma arginini* in an immunocompromised patient. *J Clin Microbiol* 2012.
1034. Watanabe T, Shibata K, Yoshikawa T, et al. Detection of *Mycoplasma salivarium* and *Mycoplasma fermentans* in synovial fluids of temporomandibular joints of patients with disorders in the joints. *FEMS Immunol Med Microbiol* 1998;**22**:241–6.
1035. Watson ME Jr, Storch GA. Recurrent *Mycoplasma pneumoniae* infection in a human immunodeficiency virus-positive child. *Pediatr Infect Dis J* 2008;**27**:1037–8.
1036. Watt KM, Massaro MM, Smith B, et al. Pharmacokinetics of moxifloxacin in an infant with *Mycoplasma hominis* meningitis. *Pediatr Infect Dis J* 2012;**31**:197–9.
1037. Weinberg A, Bloch KC, Li S, et al. Dual infections of the central nervous system with Epstein-Barr virus. *J Infect Dis* 2005;**191**: 234–7.
1038. Weinstein MP, Hall CB. *Mycoplasma pneumoniae* infection associated with migratory polyarthritis. *Am J Dis Child* 1974;**127**: 125–6.
1039. Wenzel RP, Craven RB, Davies JA, et al. Protective efficacy of an inactivated *Mycoplasma pneumoniae* vaccine. *J Infect Dis* 1977;**136**:204–7.
1040. Westenfelder GO, Akey DT, Corwin SJ, et al. Acute transverse myelitis due to *Mycoplasma pneumoniae* infection. *Arch Neurol* 1981;**38**:317–18.
1041. Westerberg SC, Smith CB, Renzetti AD. *Mycoplasma* infections in patients with chronic obstructive pulmonary disease. *J Infect Dis* 1973;**127**:491–7.
1042. Westrom L, Mardh PA. The effect of antibiotic therapy on mycoplasma in the female genital tract:i vitro and in vivo studies on the sensitivity of *Mycoplasma hominis* and T-mycoplasmas to tetracyclines and other antibiotics. *Acta Obstet Gynecol Scand* 1971;**50**:25–31.
1043. White CP, Jewer DD. Seal finger: a case report and review of the literature. *Can J Plast Surg* 2009;**17**:133–5.
1044. Whitescarver J, Furness G. T-mycoplasmas: a study of the morphology, ultrastructure and mode of division of some human strains. *J Med Microbiol* 1975;**8**:349–55.
1045. Wikstrom A, Jensen JS. *Mycoplasma genitalium:* a common cause of persistent urethritis among men treated with doxycycline. *Sex Transm Infect* 2006;**82**:276–9.
1046. Williams MH, Brostoff J, Roitt IM. Possible role of *Mycoplasma fermentans* in pathogenesis of rheumatoid arthritis. *Lancet* 1970;**2**:277–80.
1047. Williamson J, Marmion BP, Kok T, et al. Confirmation of fatal *Mycoplasma pneumoniae* infection by polymerase chain reaction detection of the adhesin gene in fixed lung tissue. *J Infect Dis* 1994;**170**:1052–3.
1048. Wilson MH, Collier AM. Ultrastructural study of *Mycoplasma pneumoniae* in organ culture. *J Bacteriol* 1976;**125**:332–9.
1049. Witmer CM, Steenhoff AP, Shah SS, et al. *Mycoplasma pneumoniae*, splenic infarct, and transient antiphospholipid antibodies: a new association? *Pediatrics* 2007;**119**:e292–5.
1050. Wu B, Rong R. Cold agglutinin syndrome with severe haemolytic anaemia in a patient diagnosed of disseminated tuberculosis and concomitant *Mycoplasma pneumoniae* infection. *Transfus Med* 2012;**22**:151–2.
1051. Wu CC, Kuo HC, Yu HR, et al. Association of acute urticaria with *Mycoplasma pneumoniae* infection in hospitalized children. *Ann Allergy Asthma Immunol* 2009;**103**:134–9.
1052. Xiao JC, Xie LF, Fang SL, et al. Symbiosis of *Mycoplasma hominis* in *Trichomonas vaginalis* may link metronidazole resistance in vitro. *Parasitol Res* 2006;**100**:123–30.
1053. Yamada M, Buller R, Bledsoe S, et al. Rising rates of macrolide-resistant *Mycoplasma pneumoniae* in the central United States. *Pediatr Infect Dis J* 2012;**31**:409–11.
1054. Yamaguchi M, Kikuchi A, Ohkusu K, et al. Abscess formation due to *Mycoplasma hominis* infection after cesarean section. *J Obstet Gynaecol Res* 2009;**35**:593–6.
1055. Yamazaki T, Narita M, Sasaki N, et al. Comparison of PCR for sputum samples obtained by induced cough and serological tests for diagnosis of *Mycoplasma pneumoniae* infection in children. *Clin Vaccine Immunol* 2006;**13**:708–10.
1056. Yang D, Tai X, Qiu Y, et al. Prevalence of *Eperythrozoon* spp. infection and congenital eperythrozoonosis in humans in Inner Mongolia, China. *Epidemiol Infect* 2000;**125**:421–6.
1057. Yang J, Hooper WC, Phillips DJ, et al. Cytokines in *Mycoplasma pneumoniae* infections. *Cytokine Growth Factor Rev* 2004;**15**: 157–68.
1058. Yechouron A, Lefebvre J, Robson HG, et al. Fatal septicemia due to *Mycoplasma arginini:* a new human zoonosis. *Clin Infect Dis* 1992;**15**:434–8.
1059. Yis U, Kurul SH, Cakmakci H, et al. *Mycoplasma pneumoniae:* nervous system complications in childhood and review of the literature. *Eur J Pediatr* 2008;**167**:973–8.
1060. Yoon BH, Romero R, Kim M, et al. Clinical implications of detection of *Ureaplasma urealyticum* in the amniotic cavity with the polymerase chain reaction. *Am J Obstet Gynecol* 2000;**183**: 1130–7.
1061. Yoon BH, Romero R, Park JS, et al. Microbial invasion of the amniotic cavity with *Ureaplasma urealyticum* is associated with a robust host response in fetal, amniotic, and maternal compartments. *Am J Obstet Gynecol* 1998;**179**:1254–60.
1062. Yoshida T, Maeda S, Deguchi T, et al. Rapid detection of *Mycoplasma genitalium*, *Mycoplasma hominis*, *Ureaplasma parvum*, and *Ureaplasma urealyticum* organisms in genitourinary samples by PCR-microtiter plate hybridization assay. *J Clin Microbiol* 2003; **41**:1850–5.
1063. Youn YS, Lee KY. *Mycoplasma pneumoniae* pneumonia in children. *Korean J Pediatr* 2012;**55**:42–7.
1064. Youn YS, Lee KY, Hwang JY, et al. Difference of clinical features in childhood *Mycoplasma pneumoniae* pneumonia. *BMC Pediatr* 2010;**10**:48.
1065. Yuan CL, Liang AB, Yao CB, et al. Prevalence of *Mycoplasma suis* (*Eperythrozoon suis)* infection in swine and swine-farm workers in Shanghai, China. *Am J Vet Res* 2009;**70**:890–4.

1066. Zhang L, Zong ZY, Liu YB, et al. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review & meta-analysis. *Indian J Med Res* 2011;**134**:270–80.
1067. Zhang W, Baseman JB. Transcriptional response of *Mycoplasma genitalium* to osmotic stress. *Microbiology* 2011;**157**:548–56.
1068. Zheng X, Olson DA, Tully JG, et al. Isolation of *Mycoplasma hominis* from a brain abscess. *J Clin Microbiol* 1997;**35**: 992–4.
1069. Zhu C, Liu J, Ling Y, et al. Prevalence and antimicrobial susceptibility of *Ureaplasma urealyticum* and *Mycoplasma hominis* in Chinese women with genital infectious diseases. *Indian J Dermatol Venereol Leprol* 2012;**78**:406–7.
1070. Zhu C, Wang S, Hu S, et al. Protective efficacy of a *Mycoplasma pneumoniae* P1C DNA vaccine fused with the B subunit of *Escherichia coli* heat-labile enterotoxin. *Can J Microbiol* 2012;**58**: 802–10.
1071. Zhu C, Wu Y, Chen S, et al. Protective immune responses in mice induced by intramuscular and intranasal immunization with a *Mycoplasma pneumoniae* P1C DNA vaccine. *Can J Microbiol* 2012;**58**:644–52.
1071. Zinserling A. Pecularities of lesions in viral and mycoplasma infections of the respiratory tract. *Virchows Arch A Pathol Pathol Anat* 1972;**356**:259–73.
1073. Zouari A, Touati A, Smaoui H, et al. Dual infection with *Bordetella pertussis* and *Mycoplasma pneumoniae* in three infants: case reports. *Infection* 2012;**40**:213–17.
1074. Zouboulis CC, Turnbull JR, Muhlradt PF. Association of *Mycoplasma fermentans* with Adamantiades-Behçet's disease. *Adv Exp Med Biol* 2003;**528**:191–4.

SECTION **XXI**

FUNGAL DISEASES

CHAPTER 196

Classification of Fungi

David A. Bruckner • Eszter Deak

Fungal infections are an important and growing cause of morbidity and mortality. This is largely due to an expanding population of immunosuppressed individuals who serve as susceptible hosts, including persons with human immunodeficiency virus (HIV) infections, recipients of solid organ or hematopoietic stem cell transplants, and patients with hematologic malignancies, burns, and indwelling medical devices. Less than 50 of the more than 100,000 currently identified species of fungi are known primary pathogens in humans, but many fungal organisms previously thought of as contaminants in clinical specimens are now recognized as etiologic agents of disease under the right circumstances. The challenging task of the clinical microbiologist is to learn to recognize and correctly identify clinically relevant fungi to guide proper patient management.

Medically important fungi belong to the kingdom Fungi.[5] These organisms are classified by their basic growth pattern as yeasts or molds. Yeasts are unicellular fungi that reproduce by budding (*Candida* spp.) or by fission *(Schizosaccharomyces pombe)*. The buds produced by infectious yeasts, termed *blastoconidia*, can form chains, or *pseudohyphae*. Molds are multicellular fungi that grow by means of filamentous cell projections, or *hyphae*. The nuclei in different cells may or may not be separated by characteristic cross walls, or septa. Many medically important fungi change from a multicellular hyphal form in the natural environment or in vitro to a budding single-celled yeast form in tissue. These pathogens are known as *dimorphic* fungi and include *Blastomyces dermatitidis*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, and *Penicillium marneffei*.[6,8]

Fungi may reproduce sexually or asexually. The sexual form of growth is the teleomorph, and the asexual form is the anamorph. Some fungi appear to have more than one name. Often this is because the anamorph and teleomorph were described and named at different times, without the connection between them being recognized. As a result, the teleomorph and the anamorph forms of the same fungus have different names. *Emericella nidulans*, for example, is the teleomorph for the anamorph *Aspergillus nidulans*. Some teleomorphic fungi can produce more than one asexual form of propagation. *Pseudallescheria boydii* is the teleomorph of two anamorphs, *Scedosporium apiospermum* and *Graphium eumorphum*. These three names all refer to the same organism. Although the sexual means of reproduction forms the basis of the taxonomy of fungi, it is the anamorph that is usually obtained in culture. For this reason, the anamorph name is usually used in reports from a clinical microbiology laboratory.

The kingdom Fungi is currently divided into seven phyla.[1,3,6,8] The medically important fungi are contained within the phylum Ascomycota, phylum Basidiomycota, and what was previously named phylum Zygomycota, for which sexual spores and their mode of production form the main basis for classification.[2,4,7] Accepted fungal scientific names are published by the International Code of Nomenclature for Algae, Fungi and Plants (ICN). The phylum names and the type of sexual spore formed by its members are as follows:

1. Zygomycota (zygospores)
2. Ascomycota (ascospores)
3. Basidiomycota (basidiospores)

The phylum Zygomycota is no longer accepted because it is not monophyletic. Organisms previously assigned to the phylum Zygomycota have been divided among subphylum Mucormycotina and subphylum Entomophthoromycotina within the phylum Glomeromycota.[1,3,6,8] Current fungal taxonomy and classification into phyla and subphyla is presently based on both the mode of sexual reproduction and concordant multilocus DNA sequences. Asexual fungi with no known teleomorph phase, previously characterized as a separate formal group known as the "Fungi Imperfecti" also have been designated to the familiar phyla based on molecular phylogenetics.

The taxonomic scheme depicted is based on various sources. The higher level classification of the kingdom Fungi was revised by Hibbett and colleagues.[3]

The basic taxonomic units are the species, and they are grouped into a hierarchical system that includes genera, families, orders, classes, and phyla. The categories may be subdivided (e.g., subphylum, subclass, and suborder) to indicate degrees of relationship. Populations within a given species that have some characteristics in common may be set apart as tribes, varieties, or some other subset designations. Delineation of the zoopathogen *Ajellomyces capsulatus*, the teleomorph of *Histoplasma capsulatum*, is as follows:

Kingdom: Fungi
Phylum: Ascomycota
Class: Ascomycetes
Order: Onygenales
Family: Onygenaceae
Genus: Ajellomyces
Species: *Ajellomyces capsulatus* (anamorph: *Histoplasma capsulatum*)
Variety: The varietal state applies to the anamorph.[5]
H. capsulatum var. capsulatum

H. capsulatum var. duboisii
H. capsulatum var. farciminosum

This chapter includes taxa known to contain medically important fungi. Classes, orders, and families not known to contain pathogens are omitted. More detailed considerations can be obtained by consulting the references.[1-8]

Kingdom: Fungi
- Phylum: Glomeromycota. The teleomorph (sexual form) consists of zygospores. The anamorph (asexual form) consists of sporangiospores within a sporangium.
  - Subphylum: Mucormycotina
    - Order: Mucorales
      - Family: Mucoraceae
        - Genus: *Apophysomyces*
          Lichtheimia
          Mucor
          Rhizomucor
          Rhizopus
      - Family: Cunninghamella
        - Genus: *Cunninghamella*
      - Family: Mortierellaceae
        - Genus: *Mortierella*
      - Family: Saksenaeaceae
        - Genus: *Saksenaea*
      - Family: Syncephalastraceae
        - Genus: *Syncephalastrum*
      - Family: Thamnidiaceae
        - Genus: *Cokeromyces*
  - Subphylum: Entomophthoromycotina
    - Order: Entomophthorales—agents of entomophthoromycoses
      - Family: Basidiobolaceae
        - Genus: *Basidiobolus*
      - Family: Ancylistaceae
        - Genus: *Conidiobolus*
- Phylum: Ascomycota. The teleomorph (sexual phase) consists of ascospores borne in an ascus. The anamorph (asexual phase) may be unicellular yeasts or multicellular molds. This group accounts for 75 percent of all described fungi. Molecular phylogeny studies have been used to define this group, even when a known teleomorph for a given anamorph has not been described.
  - Subphylum: Saccharomycotina
    - Class: Saccharomycetes
      - Order: Saccharomycetales—ascomata absent. Asci are formed singly or in chains. Yeast cells divide by budding or fission.
        - Family: Saccharomycetaceae
          - Genus: *Candida*
            Debaryomyces (teleomorph)
            Kluyveromyces (teleomorph)
            Pichia (teleomorph)
            Clavispora (teleomorph)
            Issatchenkia (teleomorph)
            Saccharomyces (teleomorph)
  - Subphylum: Pezizomycotina—over 90 percent of Ascomycota are mold species
    - Class: Eurotiomycetes
      - Order: Onygenales—ascomata are cleistothecia
        - Family: Arthrodermataceae—agents of tinea (ringworm)
          - Genus: *Arthroderma* (teleomorph)
            Microsporum (anamorph)
            Trichophyton (anamorph)
            Epidermophyton (anamorph)
            Chrysosporium (teleomorph)
        - Family: Onygenaceae—agents of endemic pulmonary mycoses
          - Genus: *Coccidioides* (anamorph)
        - Family: Ajellomycetaceae
          - Genus: *Ajellomyces* (teleomorph)
            Histoplasma (anamorph)
            Blastomyces (anamorph)
            Emmonsia (anamorph)
            Paracoccidioides (anamorph)
      - Order: Chaetothyriales
        - Family: Herpotrichiellaceae—agents of phaeohyphomycoses and chromoblastomycoses
          - Genus: *Capronia* (teleomorph)
            Cladophialophora (anamorph)
            Exophiala (anamorph)
            Fonsecaea (anamorph)
            Phialophora (anamorph)
            Ramichloridium (anamorph)
            Rhinocladiella (anamorph)
      - Order: Eurotiales—ascomata are cleisothecia
        - Family: Trichomaceae—contains the greatest number of human pathogens
          - Genus: *Aspergillus* (anamorph)
            Penicillium (anamorph)
            Talaromyces (teleomorph)
            Emericella (teleomorph)
            Eurotium (teleomorph)
            Neosartorya (teleomorph)
            Paecilomyces (anamorph)
      - Order: Ophiostomales—ascomata are perithecia with long necks
        - Family: Ophiostomaceae
          - Genus: *Ophiostoma* (teleomorph)
            Sporothrix (anamorph)
    - Class: Sordariomycetes
      - Order: Hypocreales—ascomata are mostly perithecia
        - Family: Hypocreaceae—contains agents of mycoses
          - Genus: *Gibberella* (teleomorph)
            Nectria (teleomorph)
            Acremonium (anamorph)
            Fusarium (anamorph)
            Trichoderma (anamorph)
            Verticillium (anamorph)
      - Order: Microascales—ascomata are cleistothecia or perithecia
        - Family: Microascaceae
          - Genus: *Pseudallescheria* (teleomorph)
            Scedosporium (anamorph)
            Microascus (teleomorph)
            Scopulariopsis (anamorph)
      - Order: Sordariales—ascomata are cleistothecia or perithecia; rarely pathogens

Class: Dothideomycetes
Order: Dothideales—ascomata are cleistothecia
Family: Dithoraceae
Genus: *Aureobasidium* (anamorph)
Hortaea (anamorph)
Family: Didymosphaeriaceae
Genus: *Neostudina* (teleomorph)
Family: Mycosphaerellaceae
Genus: *Cladosporium* (anamorph)
Family: Piedraiaceae
Genus: *Piedraia* (teleomorph)
Order: Pleosporales
Family: Pleosporaceae
Genus: *Cochliobolus* (teleomorph)
Bipolaris (anamorph)
Exserohilum (anamorph)
Setosphaeria (teleomorph)
Curvularia (anamorph)
Drechslera (anamorph)
Alternaria (anamorph)
Ulocladium (anamorph)
Lewia (teleomorph)
Class: Pneumocystidomycetes—found as trophozoites, cysts, and intracystic bodies; originally considered members of the kingdom Protozoa
Order: Pneumocystidales
Family: Pneumocystidaceae
Genus: *Pneumocystis*
Phylum: Basidiomycota. The teleomorph is a basidium bearing basidiospores. The anamorphs are yeasts or molds. Arthroconidia are reproductive propagules of some molds.
Class: Tremellomycetes
Order: Tremellales
Family: Filobasidiaceae
Genus: *Filobasidiella* (teleomorph)
Filobasidium (teleomorph)
Cryptococcus (anamorph)
Trichosporon (anamorph)
Class: Ustilaginomycetes
Order: Malasseziales
Family: Malasseziaceae
Genus: *Malassezia* (anamorph)
Class: Agaricomycetes
Order: Agaricales
Family: Schizophyllaceae
Genus: *Schizophyllum*

NEW REFERENCES SINCE THE SIXTH EDITION

1. Boekhout T, Gueidan C, de Hoog S, et al. Fungal taxonomy: new developments in medically important fungi. *Curr Fung Infect Rep* 2009;**3**:170–8.
3. Hibbett DS, Binder M, Bischoff JF, et al. A higher-level phylogenetic classification of the fungi. *Mycol Res* 2007;**111**:509–47.
6. Reiss E, Shadomy HJ, Lyon GM. *Fundamental medical mycology*. New Jersey: Wiley-Blackwell; 2011.
8. Versalovic J, editor. *Manual of clinical microbiology*. 10th ed. Washington DC: ASM Press; 2011.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 197

Aspergillosis

William J. Steinbach

Aspergillosis often has a high mortality, and its incidence is increasing.[10,111,149] Invasive aspergillosis (IA) is now the most common cause of mortality due to the major invasive mycoses, an occurrence that generally is a result of success with prophylactic antifungal regimens and improved diagnostic studies for *Candida* infection. IA is likely also gaining prominence because of the increasing number of severely immunosuppressed patients due to more intensive immunosuppressive therapies for graft-versus-host disease (GVHD) and rheumatologic diseases, increased use of mismatched or unrelated donor transplantations, newer preparative regimens to avoid rejection or relapse, and increase in early posttransplantation survival resulting from better control of bacterial and cytomegaloviral infections.[111,191]

THE ORGANISM

The genus *Aspergillus* contains approximately 185 species, but most human disease is primarily caused by *A. fumigatus*, *A. flavus*, *A. niger*, *A. terreus*, and *A. nidulans*.

REFERENCES

1. Boekhout T, Gueidan C, de Hoog S, et al. Fungal taxonomy: new developments in medically important fungi. *Curr Fung Infect Rep* 2009;**3**:170–8.
2. de Hoog GS, Guarro J, Gene J, et al. *Atlas of clinical fungi*. 2nd ed. Baarn and Delft, The Netherlands: Centraalbureau voor Schimmelcultures; 2000.
3. Hibbett DS, Binder M, Bischoff JF, et al. A higher-level phylogenetic classification of the fungi. *Mycol Res* 2007;**111**:509–47.
4. Kirk PM, Cannon PF, David JC, et al. *Ainsworth & Bisby's dictionary of the fungi*. 9th ed. Wallingford, UK: CAB International; 2001.
5. Kwon-Chung KJ, Bennet JE. *Medical mycology*. Philadelphia: Lea & Febiger; 1992.
6. Reiss E, Shadomy HJ, Lyon GM. *Fundamental medical mycology*. New Jersey: Wiley-Blackwell; 2011.
7. Sutton DA, Fothergill AW, Rinaldi MG. *Guide to clinically significant fungi*. Baltimore: Williams & Wilkins; 1998.
8. Versalovic J, editor. *Manual of clinical microbiology*. 10th ed. Washington DC: ASM Press; 2011.

The classification of the genus *Aspergillus* has been revised from the original taxonomy to now incorporate both phenotype and morphology as well as newer molecular approaches; consequently, the taxonomy remains dynamic.[154] For instance, newer and pathogenic species have been described, such as *Aspergillus lentulus*,[11] *Neosartorya udagawa*,[188] and *Neosartorya pseudofischeri*,[79] many of which have reduced antifungal susceptibility.[3] *A. fumigatus* causes approximately 90 percent of cases of IA[38] but is difficult to differentiate from the other closely-related species based on morphology. *A. fumigatus* is responsible for most pulmonary disease, whereas isolated sinus disease is caused by *A. niger* and *A. flavus*.[141] Specific determination of the species of *Aspergillus* is clinically important for therapeutic reasons, and a review of *Aspergillus* cultures found that *A. terreus* was seen in only 3 percent of isolates in cases of IA but found exclusively in cases of invasive disease (i.e., no cases of colonization).[141] It has also been well documented that *A. terreus* is resistant to amphotericin B.[167]

Aspergillus is a ubiquitous organism, but its ecologic niche is the soil, where it functions as a saprophytic fungus growing on organic debris and recycling carbon and nitrogen.[202] A classic sexual stage has been identified for *A. fumigatus*,[131] yet the clinical relevance is unclear. Asexual reproduction is abundant and characterized by the production of green pigmented echinulate asexual conidia (spores). The conidia are produced in chains basipetally from the phialides borne on clavate vesicles. This reproductive structure is where the genus name *Aspergillus* is derived, from the aspergillum used to sprinkle holy water in the Roman Catholic mass.

Aspergillus aerosolize conidia, which immunocompetent people asymptomatically breathe every day; it has been estimated that most people inhale several hundred *A. fumigatus* conidia per day.[60] Infection in immunocompromised patients is usually also acquired through inhalation of airborne conidia. Two lines of host defense exist against *Aspergillus*—macrophages and neutrophils—and it appears that both mechanisms of host defense are required for resistance to invasive disease. The first defense is formed by alveolar macrophages, and in vitro murine studies have suggested that resident pulmonary macrophages are responsible for eliminating inhaled *Aspergillus* conidia from the lung.[157,158] If conidia escape, they develop into invasive *Aspergillus* hyphae and become susceptible to neutrophil killing by the release of toxic reactive oxygen species. The host can develop disease through neutropenia, high challenge doses of conidia overcoming macrophages, or corticosteroid suppression of macrophage conidiacidal activity.[46]

A. fumigatus, in particular, is highly successful at causing invasive disease in immunocompromised patients owing to several characteristics, such as thermotolerance, small and abundant pigmented conidia, fast growth rate, production of toxic secondary metabolites, and numerous enzymes involved in breaking down complex polysaccharides. The ability of this fungus to grow at 37°C is crucial for the development of human disease. Although some *Aspergillus* species can grow at this high temperature, it has been reported that *A. fumigatus* can survive at temperatures up to 75°C, and this thermotolerance is likely a major factor in its ability to cause human morbidity.[14] In addition, at 37°C in a nutrient-rich environment *A. fumigatus* can grow at an extremely fast rate.

Conidia are 2.5 to 3.0 μm in diameter, and this small size allows them to remain buoyant in the air for prolonged periods of time and be inhaled deep into the lung alveoli. When inhaled by an immunocompetent person, conidia rarely have deleterious effects because they are cleared by phagocytic cells of the innate immune system. However, severe allergic disease can occur with repeated exposure to large doses of conidia or in those patients who are more atopic. Pigments found on the conidial surface are thought to play important roles in mediating the effects of damaging reactive oxygen species produced by phagocytic cells.[144] Mutants of *A. fumigatus* that lack these conidial pigments are more susceptible to killing by alveolar macrophages than their pigmented counterparts.[97] Whole-genome sequencing of several *Aspergillus* species has revealed that these fungi contain large numbers of enzymes involved in degrading complex polysaccharides.[54] Although these enzymes allow *Aspergillus* species to be enormously successful as saprophytes, it is generally also thought that these enzymes contribute to their ability to cause invasive disease.

Species of *Aspergillus* are also well known for their production of secondary metabolites. Perhaps the most well-known *Aspergillus* metabolite is the carcinogen aflatoxin produced by some strains of *A. flavus*. Although aflatoxin is extremely carcinogenic, its role in the development of human mycoses has not been thoroughly examined. Various suspected putative *Aspergillus* virulence factors have been exhaustively reviewed,[100] and earlier gene disruption studies examined proteases, toxins, hemolysins, melanin pigmentation, and other gene products with little success. It appears the true virulence of *A. fumigatus* is likely a multifactorial combination of attributes.

CLINICAL MANIFESTATIONS

Aspergillus species are relatively unique among pathogens because they are responsible for a gamut of infections extending across the clinical spectrum to include primary allergic reactions, saprophytic involvement, and invasive disease.[114] The type of *Aspergillus* infection generally depends on the immunologic background of the infected host, with immunodeficient patients developing invasive disease while immunoreactive patients have allergic disease. Most frequently encountered among the myriad of clinical presentations are features involving the lungs, such as allergic bronchopulmonary aspergillosis (ABPA) and acute or chronic invasive pulmonary aspergillosis (IPA). Other common infections caused by *Aspergillus* include invasive acute or chronic sinusitis, cutaneous aspergillosis, aspergilloma, and cerebral aspergillosis.[38,114] The clinical manifestations of these infections in immunocompromised patients can be subtle and very nonspecific and commonly occur late in the course of disease. As a result, a high index of suspicion needs to be maintained to implement treatment in the early stages of disease. Unfortunately, the most vulnerable patients against

Aspergillus infections are the least likely to display significant symptoms, adding to the difficulty of diagnosis.[38]

Invasive Pulmonary Aspergillosis

Aspergillus species are ubiquitous in the environment, and one major portal of entry is the respiratory tract. In some immunocompetent patients this inhalation could result in nonpathogenic saprophytic colonization, but in the immunocompromised patient this conidial acquisition will likely result in establishment of invasive disease.[180] IPA is the most frequently documented form of IA and contributes considerably to morbidity and mortality among high-risk patients.[40] In a systematic review of the literature regarding the case-fatality rate of aspergillosis, 70 percent of infections were found to be invasive pulmonary disease.[102] As with other forms of *Aspergillus* infections, the burden of IPA is sustained by immunocompromised patients, including those who have undergone hematopoietic stem cell transplantation (HSCT), solid-organ transplant (SOT) recipients, cancer patients, patients with various congenital immune deficiencies such as chronic granulomatous disease (CGD), and patients treated for autoimmune diseases.[150]

The clinical manifestations of IPA are heterogeneous; typically they may include fever unresponsive to broad-spectrum antibiotics, dry cough, shortness of breath, pleuritic chest pain, hemoptysis, and pulmonary infiltrates on radiography.[4] Although neutropenic patients more commonly have a fever, in about 20 percent of patients the fever and cough will not be present for the first several days of infection,[114] specifically in patients receiving high-dose corticosteroid therapy.[71] In general, disease is often bilateral diffuse pulmonary infection; and dyspnea is a more common presentation in these patients. Progression of infection is characterized by invasion of small vessels, leading to hemoptysis as a leading symptom of IPA in some neutropenic patients. Two patterns of hemorrhage may be identified: (1) hemorrhagic infarction due to vascular invasion and (2) formation of mycotic aneurysms during recovery from neutropenia that can rupture and result in fatal hemoptysis.[122]

Whereas cancer patients undergoing treatment constitute the majority of patients at risk for *Aspergillus* infections, another largely pediatric patient population deserving a separate discussion is those with underlying CGD. IPA is the leading cause of mortality in this patient population. Patients with CGD have an estimated 33 percent lifetime risk for IA, and IPA may, in fact, be the first manifestation of CGD. Although disease in most patient groups is caused by either *A. fumigatus* or *A. flavus*, most cases of *A. nidulans* infection have been reported in patients with CGD.[27] In one review, *A. nidulans* occurred in six patients with CGD but did not occur in any other patient group.[161] Although *A. fumigatus* was more common than *A. nidulans* in CGD patients, infection with *A. nidulans* was significantly more likely to be refractory to antifungal therapy and result in dissemination and death.

In addition to differences in the epidemiology of specific *Aspergillus* isolates, the clinical presentation in patients with CGD can be unique from neutropenic patients. Unlike the acute, rapidly progressive illness described for other immunocompromised patients, in patients with CGD the clinical course is characterized by an insidious onset of fatigue, fever, increased sedimentation rate, and pneumonia.[161] A patient with CGD often does not have typical clinical features (and may possibly be completely asymptomatic) or may have only an elevated erythrocyte sedimentation rate in the setting of no fever. In early disease there is an acute neutrophilic response where the neutrophils surround hyphae. However, in patients with CGD the hyphae remain intact, owing to impaired neutrophil-mediated killing of hyphae. In this setting, IPA in a patient with CGD is a chronic progressive infection that may spread locally to involve pleura, vertebrae, and the chest wall. *Aspergillus* osteomyelitis is a common presentation in patients with CGD, often involving the rib, vertebrae, or femur.[47] Specifically, thoracic wall extension of pulmonary IA has been repeatedly noted.[200] In a study of 24 cases of osteomyelitis caused by *Aspergillus* species in 22 CGD patients, 14 isolates (58%) were due to *A. nidulans* and 10 were due to *A. fumigatus*.[48]

In contrast to patients with neutropenia, hyphal angioinvasion is not a feature of disease in patients with CGD. The "halo" sign (angioinvasion with surrounding tissue ischemia), cavitated lesions, and pulmonary infarcts are not typical in patients with IA and CGD.

Invasive *Aspergillus* Sinusitis

Fungal sinusitis can manifest as allergic, saprophytic, or invasive disease. Invasive *Aspergillus* sinusitis is likely underdiagnosed owing to its variable clinical presentation and difficulty in establishing the diagnosis[38,71,114] possibly because of a decreased inflammatory response in affected patients. Patients can have nasal congestion, discharge, headache, facial pain or swelling, and abnormal findings of the nasal cavity such as pallor of the nasal septum or turbinate mucosa. Epistaxis, orbital swelling, and high fever also can be present.[124] However, the definitive diagnosis can be best established by endoscopic evaluation and selective biopsy. Common findings on endoscopy include pallor of the mucosa and discoloration or granulation of the mucosa due to ischemia as a result of angioinvasion; and, as the disease progresses, a blackened necrotic focus can be found (Figs. 197-1 and 197-2). Extension into bony structures can occur at the site of necrosis leading to spread of disease into adjacent structures, such as the orbit and the brain, which carries a high mortality rate.

Although imaging is not diagnostic, it can aid in establishing the diagnosis because it can be used as a roadmap for endoscopy by showing which sinuses are involved (Fig. 197-3). In a review of 25 patients with rhinosinusitis, 44 percent showed evidence of invasion beyond the sinus cavities on computed tomography (CT).[58] In the same study, however, 12 percent of patients had negative CT findings, again highlighting that a high index of clinical suspicion must guide establishment of the diagnosis to ensure the best outcome.

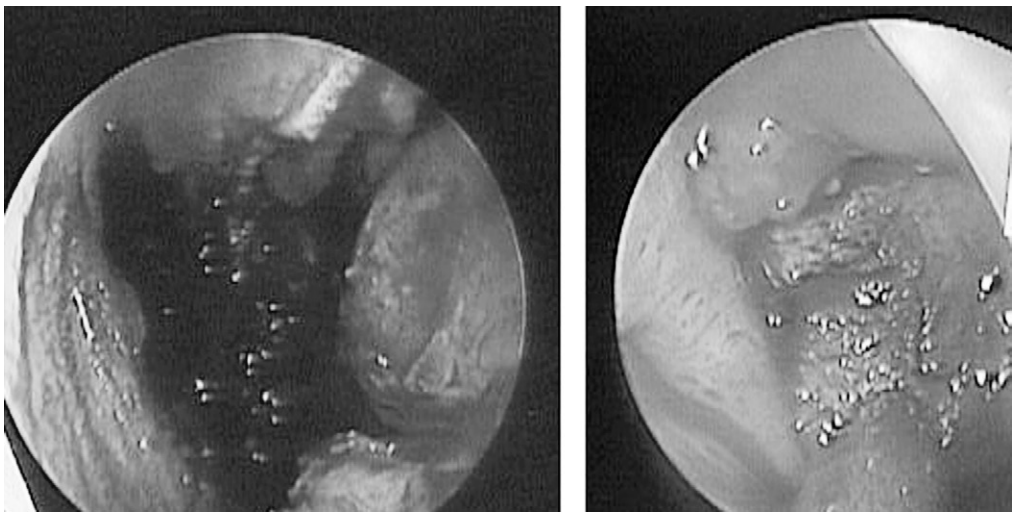

FIGURE 197-1 ■ Acute myelogenous leukemia in a 12-year old with unrelated cord blood transplant who developed *A. fumigatus* sinusitis. Endoscopy shows classic pale mucosa.

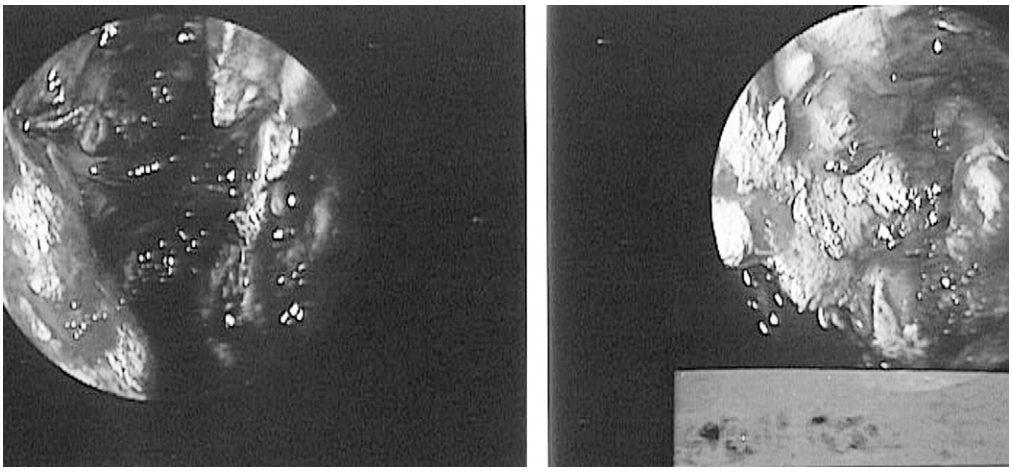

FIGURE 197-2 ■ Same patient as in Figure 197-1. Endoscopy shows necrotic mucosa at the basilar skull area.

Cerebral Aspergillosis

Aspergillus infections most commonly involve the lungs, but disease can disseminate via the bloodstream and involve distant organs. One of the most frequent sites of dissemination is the central nervous system (CNS).[63] Cerebral aspergillosis may also be a result of direct extension through the sinuses. There are studies that have estimated that CNS aspergillosis may be found in 40 to 50 percent of patients with IA and acute leukemia or allogeneic stem cell transplant.[77,156] As with other *Aspergillus* infections, *A. fumigatus* is the most frequently encountered species in cerebral aspergillosis, but other implicated species are *A. flavus*, *A. niger*, and *A. nidulans*.[78] Clinical presentation often does not include more classic symptoms for an intracranial process, such as headache, nausea, or vomiting. Instead, patients have an altered mental status, convulsions, hemiplegia or hemiparesis, ophthalmoplegia, and loss of consciousness. Severely immunocompromised patients may not display these symptoms, and their disease progresses more rapidly.

Aspergillus hyphae are angioinvasive and thrombose arteries to create hemorrhagic infarcts and, as a result, CNS aspergillosis can present as solitary or multiple abscesses and, less commonly, as mycotic aneurysms and carotid artery invasion. Cerebral aspergillosis can also appear as meningitis or granuloma.[78] Cerebral aspergillosis presents as multiple areas of low density and no enhancement even with contrast on CT, and lesions are usually located within the basal ganglia and gray-white matter junction. On magnetic resonance imaging (MRI), these same abnormalities appear as foci of intermediate T2-weighted signal surrounded by a rim of higher signal.[133,179] Aspergillosis of the CNS carries a case-fatality rate of nearly 90 percent,[102] so prompt diagnosis and treatment is key to survival. Unfortunately, definitive diagnosis requires biopsy, and typically these patients are often too coagulopathic to undergo such a procedure.

Cutaneous Aspergillosis

Neonates, burn victims, HSCT recipients, SOT recipients, and other immunocompromised patients are the

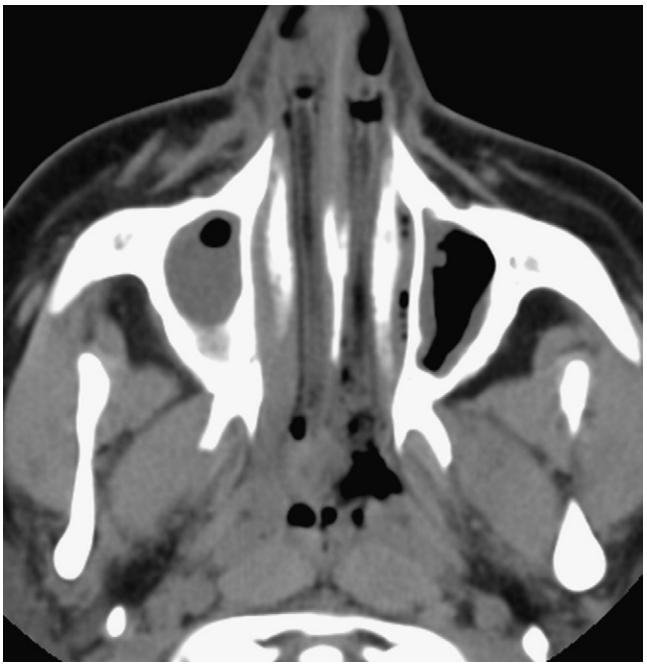

FIGURE 197-3 ■ Same patient as in Figures 197-1 and 197-2. CT shows complete opacification of the right maxillary sinus.

ones most frequently found to develop cutaneous aspergillosis. Although not the most commonly encountered *Aspergillus* infection, cutaneous infection has a reported incidence between 4 and 11 percent.[36,199] Cutaneous aspergillosis can be primary, a result of direct skin injury or traumatic inoculation, or secondary, a result of hematogenous spread or extension from infected underlying structures.[183] Primary cutaneous aspergillosis has been associated with intravenous access devices, adhesive dressings, and sites of burns or surgery. Premature infants are at particular risk because their skin is immature and most vulnerable. HSCT recipients generally develop disease as a result of hematogenous seeding from a primary source, usually the lungs. Lesions often begin as erythematous, indurated papules that progress to ulcerative, painful and necrotic lesions.[170] Treatment involves débridement and excision of necrotic tissue, which provides diagnostic material, and systemic intravenous antifungal agents as well as topical preparations. The role of topical antifungal therapy alone is unclear, because often cutaneous aspergillosis is a harbinger for underlying undiagnosed systemic disease, so relying on topical antifungal coverage alone is likely inadequate.

Chronic Aspergillosis

Chronic aspergillosis is less frequently encountered than acute disease, and the patient population affected is typically distinct as well. A degree of immune suppression does exist in these patients, and exposure to corticosteroids is common. Underlying conditions include corticosteroid use for chronic lung disease, diabetes mellitus, and alcoholism.[38] Patients often have underlying pulmonary disease, such as cavitary tuberculosis, sarcoidosis, emphysema, or fibrotic lung disease. There are three separate categories used to describe chronic aspergillosis.[42] The first is chronic cavitary pulmonary aspergillosis (CCPA), in which multiple cavities form and expand and may be occupied by fungus balls. The second is chronic fibrosing pulmonary aspergillosis, which, as the name connotes, is characterized by progression of the cavities into extensive pulmonary fibrosis. The final category has been defined as chronic necrotizing pulmonary aspergillosis (CNPA), also known as either subacute invasive or semi-invasive pulmonary aspergillosis.[42] The latter category is an indolent pulmonary infection of patients with a mild or moderate defect in immune function, such as in the setting of prolonged corticosteroid use, and may be seen after resolution of neutropenia.[114] CNPA is a slowly progressive disease, and clinical signs observed include, cough, fever, fatigue, and weight loss.

The most serious complication from chronic pulmonary aspergillosis is potentially life-threatening hemoptysis. Diagnosis of chronic disease requires biopsy, and treatment is with long-term antifungal therapy.[85] Management of CCPA can sometimes be via surgical resection, although long-term antifungal therapy, as for IA, is indicated. Management of CNPA is similar to that of IPA, although the disease is more indolent; thus, there is a greater emphasis on long-term oral therapy.

Aspergilloma

Aspergillomas consist of a mass of tangled hyphae, cellular debris, fibrin, and few inflammatory cells. They develop as a complication of existing cavitary lesions and are typically found in the upper lobes where there is poor drainage.[114,180] The cavitary lesions may result from tuberculosis, sarcoidosis, bullous emphysema, and other diseases. *A. fumigatus* typically causes pulmonary aspergilloma; and although patients may remain asymptomatic, many have recurrent hemoptysis. Perhaps the earliest radiographic sign is pleural thickening of a preexisting lung cavity. Later, as the fungus ball develops, CT or plain radiography will show a solid and round mass within the cavity separated from the thickened wall by an air crescent sign.[62] Aspergillomas can be divided into simple or complex, based on radiologic criteria, with simple aspergillomas lacking constitutional symptoms, paracystic lung opacities, cyst expansion, or progressive pleural thickening.[86] Treatment of active disease is aimed at controlling and preventing complicating symptoms such as hemoptysis, and surgical resection is the definitive treatment. However, given the high morbidity and mortality rate in patients with complex aspergilloma, surgical intervention is reserved for high-risk patients. Other treatment modalities, such as endobronchial and intracavitary instillation of antifungal agents, have been attempted with some success, given that systemic therapy is ineffective at diffusing antifungal agents into the cavity containing the fungus ball.[86,114]

Allergic Bronchopulmonary Aspergillosis

ABPA occurs in asthmatic and cystic fibrosis patients and is a pulmonary disease caused by type I and III

hypersensitivity reactions to *A. fumigatus* allergens. Exposure and inhalation of *Aspergillus* conidia results in saprophytic (noninvasive) colonization of the bronchial airways, which then triggers an IgE-mediated allergic inflammatory response. Over time, bronchial obstruction takes place and patients can have a productive cough, wheezing, and chest pain; fever and malaise are also presenting symptoms.[171]

Diagnosis of ABPA is based on eight primary criteria: (1) history of bronchial obstruction (e.g., asthma), (2) peripheral blood eosinophilia, (3) elevated serum IgE levels, (4) specific IgE and IgG to *Aspergillus* antigen in serum, (5) immediate (type I) skin reactivity to *Aspergillus* antigen, (6) precipitating antibodies to *Aspergillus*, (7) pulmonary infiltrates, and (8) central bronchiectasis. Radiologically, ABPA is characterized by bronchial wall thickening, pulmonary infiltrates, and central bronchiectasis, a result of the type III hypersensitivity reaction.[114,180] The diagnosis of ABPA in patients with cystic fibrosis is difficult because many of the diagnostic criteria overlap with common manifestations of cystic fibrosis.

Treatment depends on relieving inflammation via an extended course of systemic corticosteroids. Addition of the antifungal agent itraconazole is used to decrease the fungal burden and diminish the inciting stimulus for inflammation.[24,29,172] Because disease activity is correlated with serum IgE levels, monitoring of IgE levels can be used as a marker to define duration of therapy.

EPIDEMIOLOGY

It is critical to understand both the risk factors of the individual patient and the epidemiology of the pathogen to predict both likelihood of disease development as well as outcome. Host factors are paramount, and the clinician needs to take into consideration the underlying and concomitant diseases as well as the degree and type of immunosuppression. IA was the most common mold infection in a review of approximately 5500 patients who underwent HSCT; although more than 7 percent of HSCT recipients had mold infections, *Aspergillus* infections were the most common, followed by *Fusarium*, Zygomycetes, and *Scedosporium* infections.[111] IA is the leading cause of infectious death in HSCT recipients, and one study showed that 36 percent of all confirmed nosocomial pneumonia in these patients was caused by *Aspergillus* infection, with a crude mortality of 95 percent.[135] The incidence of *Aspergillus* infection in HSCT recipients has ranged from 3 to 7 percent,[118,203] but the true incidence is likely dependent on multiple factors. In one study of HSCT patients with IA, the risk for developing the disease was 12.8 times higher among recipients of allogeneic than autologous HSCT.[129] A retrospective review of 409 patients showed 13.1 percent had IA, which represented 17.2 percent of all isolated pathogens in allogeneic transplant recipients and 3.8 percent in autologous transplant recipients.[92]

A large prospective French study analyzed 424 cases of IA and found an incidence of 0.9 percent in autologous and 8.1 percent in allogeneic HSCT patients, respectively,[103] highlighting a consistent finding that IA occurs much more readily in the allogeneic HSCT recipient. Among SOT recipients, the highest incidence of IA was for heart transplant recipients (4.8%), followed by lung transplant recipients (4.1%), and significantly dropping for liver (0.8%) and kidney (0.3%) transplant recipients, findings that have also continued through most larger studies. The majority of patients with IA had a hematologic malignancy, and the largest group had acute leukemia (35%), but the second largest group had chronic lymphoproliferative disorders (22%). For those with acute leukemia, IA occurred in 68 percent during the induction phase of chemotherapy, when cytotoxic agents are generally the most intense, and in 27 percent during the consolidation phase. The time to developing IA after transplantation was similar in different transplantation settings, with 68 percent diagnosed more than 100 days after HSCT and the majority (18/27) of SOT recipients with IA diagnosed at least 100 days after transplant. Multivariate analysis showed factors independently associated with increased risk for death included older age, diagnosis based on positive culture with two positive galactomannan assays, and presence of pleural effusion or CNS involvement. Mortality in patients with acute leukemia was 38 percent, whereas allogeneic HSCT recipients had a staggering 56 percent overall mortality.

The Transplant Associated Infection Surveillance Network (TRANSNET) study sponsored by the Centers for Disease Control and Prevention evaluated HSCT and SOT recipients from 23 U.S. medical centers (2001-2005) and included evaluation of a total of 642 cases of IA.[9] Twelve-month cumulative incidence of IA in all HSCT recipients was 1.6 percent, compared with 0.63 percent in SOT recipients. Twelve-week all-cause mortality was 57.5 percent among HSCT recipients and 34.4 percent among SOT recipients. Multivariable analysis demonstrated that neutropenia, renal insufficiency, hepatic insufficiency, early onset (<30 days) IA, proven IA, and methylprednisolone use, often for GHVD, were independently associated with mortality. Analysis for SOT recipients revealed that hepatic insufficiency, malnutrition, and CNS disease were independently associated with increased risk for death. Among both HSCT and SOT recipients, receipt of voriconazole as part of the initial antifungal therapy was more common among survivors.

A TRANSNET focus on just the 875 HSCT recipients found IA was the most common (43% of all invasive fungal infections), followed by invasive candidiasis (28%).[91] The median age at development of IA after HSCT was 99 days. Of the 80 cases of IA in autologous HSCT, 50 percent occurred within 1 month after receipt of the transplant, whereas in allogeneic HSCT recipients only 22 percent occurred within 1 month after transplantation. Autologous HSCT recipients had all-cause mortality of 13 percent at 12 months, whereas in allogeneic recipients it was 36 percent at 12 months. The 12-month cumulative incidence for IA in all HSCT recipients was 1.6 percent, compared with 1.1 percent for invasive candidiasis, and the overall 1-year survival among HSCT recipients with IA was only 25.4 percent.

Another large database, the Prospective Antifungal Therapy (PATH) Alliance, also found IA most frequent

among HSCT recipients, and approximately 70 percent of those HSCT recipients with IA were allogeneic transplants. The median time to develop IA after HSCT was similar (82 days), with a diagnosis of a median of 51 days after autologous transplant and 83 days after allogeneic transplants.[126] Another study in HSCT recipients found prognostic factors at diagnosis strongly related to death due to IA were dissemination of IA, pleural effusion, low monocyte count, and corticosteroids within the previous 2 months as well as the receipt of a dose 2 mg/kg or more at the time of diagnosis and uncontrolled GVHD.[31]

There is a well-characterized bimodal distribution of aspergillosis in HSCT recipients that correlates with preengraftment neutropenia (median of 16 days after transplantation) and the peak of GVHD (median of 96 days after transplantation).[190] This likely relates to the two major mechanisms of protection against IA: alveolar macrophages and granulocytes. Most patients (86%) with autologous transplants were diagnosed with IA while neutropenic, whereas patients with allogeneic transplants were at greatest risk after engraftment or during impairment of cell-mediated immunity due to cytomegaloviral infection or GVHD.[190] In large reviews of patients undergoing allogeneic HSCT, IA was diagnosed at a mean of 88 to 115 days post transplant,[115,129,149,156] with mortality exceeding 80 percent. In some studies, most cases were diagnosed after day 30,[115,129] and in one study no cases of IA were diagnosed during the neutropenic period after transplantation.[76]

The TRANSNET database also analyzed all invasive fungal infections among SOT recipients and found IA (19%) was the second most common invasive fungal infection, and, unlike HSCT recipients, invasive candidiasis (53%) was more common in SOT recipients.[136] The median time to onset of IA was 184 days after SOT transplantation, with a 1-year incidence of developing IA at 0.65 percent and a 12-month survival of 59 percent. The PATH Alliance analysis in SOT again found IA (25%) second to invasive candidiasis, but IA was more common in lung transplant recipients (60%).[125] IA developed a median of 400 days after transplantation, which was a median 100 days after liver transplantation, compared with 504 days and 384 days in lung and heart transplant recipients, respectively. Most cases of IA in liver transplant recipients occurred less than 6 months post transplant, whereas 62 percent of lung transplant recipients with IA developed disease after 1 year. This contrasted to a retrospective review of 158 cases of IA in Spanish SOT recipients in which it was found that 57 percent had early-onset IA (first 3 months after transplantation) yet the mean time to develop IA was still 234 days.[55] The overall incidence of IA in their SOT recipients was 1.4 percent, including a similar distribution among specific organ transplants, with an incidence of 3 percent (lung), 2.4 percent (heart), 2 percent (liver), and 0.2 percent (kidney). Overall mortality was 77 percent with no significant differences between SOT groups. Risk factors for developing early-onset IA included a complicated postoperative period, repeated bacterial infections or cytomegaloviral infection, and renal failure.

Factors associated with attributable mortality for patients with IA include allogeneic HSCT or SOT versus hematologic malignancy, disease progression, corticosteroid use (≥0.2 mg/kg), renal insufficiency, neutropenia (<500 cells/μL), disseminated IA, diffuse pulmonary involvement, and possible IA.[130] One study found the all-cause mortality (47.8%) was similar to attributable mortality from IA (40.2%). This study again found receipt of voriconazole as initial antifungal therapy led to greater overall survival (69.4% vs. 49.0%) compared with use of amphotericin B or itraconazole as initial therapy. A different study found that 74 percent of the deaths in patients with IA occurred in the first 6 weeks after initiating therapy, and the majority (68%) of those deaths were attributable to IA, whereas most deaths after 6 weeks of therapy were judged to be due to underlying disease and its treatment and not attributable to IA.[204] Again, there was a difference between amphotericin B and voriconazole as initial therapy related to deaths due to IA, but no difference between voriconazole and amphotericin B in deaths due to all causes other than due to IA.

A unique study found that the majority (59%) of patients with IA had neutropenia as a risk factor, most of whom had hematologic or solid malignancies. Among the 41 percent of non-neutropenic patients, most had corticosteroid-treated chronic obstructive pulmonary disease, asthma, or rheumatologic disorders. Compared with neutropenic patients, the non-neutropenic patients were significantly less likely to have symptoms of IA and more likely to have frequent intercurrent pneumonia with another microorganism. The mortality rate was significantly higher among non-neutropenic (89%) compared with neutropenic patients (60%) with IA,[35] yet the sensitivity of galactomannan antigenemia was similar (approximately 65%) for neutropenic and non-neutropenic patients.

The risk for IA is calculated to increase from 1 percent per day after the first 3 weeks of neutropenia to 4 to 5 percent per day after 5 weeks,[160] with a 70 percent incidence after neutropenia exceeds 34 days.[57] Prolonged or marked macrophage dysfunctions that occur as a result of underlying disease and its treatment can also predispose patients to IA. Therefore, risk for infection is higher with advanced underlying disease, transplantation during relapse of malignancy or chemotherapeutic rescue therapy, GVHD, or concurrent infection such as from cytomegalovirus.[160] In a survey of 24 medical centers of 148 patients with IA, 30 percent also had a bacterial infection, 20 percent also had a viral infection, and 19 percent also had another fungal infection.[141]

Corticosteroids are also a well-known major risk factor for the development of IA and can suppress the ability of monocytes/macrophages to kill conidia through inhibition of nonoxidative processes and impairment of lysosomal activity. Corticosteroids also inhibit polymorphonuclear neutrophils in their chemotaxis, oxidative bursts, and activity against hyphae.[49] Generally, corticosteroids suppress macrophages whereas cytotoxic chemotherapy decreases neutrophil number and function. Corticosteroids also greatly accelerate the growth of *A. fumigatus*; in one study the doubling time increased to 48 minutes.[127]

In several HSCT patient risk factor studies, only moderate-to-severe GVHD and corticosteroid prophylaxis

for GVHD[12,115,149,206] and total-body irradiation[206] were significant variables in the multivariate analyses. In one study, several parameters were found to influence survival in the period from HSCT to diagnosis of fungal infection, all related to cumulative dose of prednisolone. In the multivariate analysis, relative risk (RR) was 8.78 of death from IA in patients with acute active GVHD (grade 2 or more) or extensive chronic GVHD combined with a cumulative total prednisolone dose of greater than 7 mg/kg dose 1 week before diagnosis.[149]

There is little specific information on the fundamental epidemiology of pediatric IA, and most epidemiologic investigations do not offer pediatric analyses.[34,53,92,206] One study found that the highest incidence of IA was seen in children who had undergone allogeneic bone marrow transplantation (4.5%) and those with acute myelogenous leukemia (4%). Specifically, the incidence of IA in patients with acute myelogenous leukemia was significantly greater than the incidence in patients with acute lymphoblastic leukemia (RR, 5.6; 95% confidence interval [CI], 4.6 to 7.0).[207]

The largest analysis was a retrospective multicenter study of 139 children with IA. *A. fumigatus* was the species most frequently recovered (52.8%), and the majority of the children had a malignancy with or without HSCT. Significant risk factors that impacted survival were immunosuppressive therapies and allogeneic stem cell transplant. Analysis did not show superiority of any one antifungal agent related to overall mortality, and 52.5 percent (73 of 139) of the children died during treatment of IA.[22]

Analysis of a large pediatric inpatient database found an annual incidence of 0.4 percent of IA among immunocompromised children, and the majority of cases included an underlying malignancy.[207] The highest incidence for IA was 4.5 percent among children who underwent allogeneic HSCT and 4 percent among patients with acute myelogenous leukemia, whereas autologous HSCT had a lower incidence of 0.3 percent. Lung SOT recipients had the greatest incidence among those pediatric SOT recipients (5%). Children with CGD had an incidence of 6.5 percent, whereas those with Wiskott-Aldrich syndrome (n = 267) had an incidence of a staggering 30 percent. Comparing mortality in pediatric patients with specific underlying diseases with and without IA revealed the relative risk for death was increased in CNS tumors (RR, 21.6), acute lymphocytic leukemia (RR, 14.9), and lymphoma (RR, 13.5), showcasing the overall good survival rates of those common pediatric malignancies and the devastating effect of adding IA to reasonably curable underlying pediatric malignancies.

Pediatric patients undergoing transplantation are generally less vulnerable to all infections than their adult counterparts,[51] and a review of 585 infections in 276 children who underwent HSCT found 38 (6%) with mold infections in the first year after transplantation, including 28 *Aspergillus* infections.[12] A larger report reviewed 510 pediatric HSCTs in 485 patients from 1990 through 1998.[12] There were 26 IA cases (4.79% of infections) in 584 culture-proven infections during the first year post transplant. This pediatric report was the first to employ tools beyond descriptive statistics to analyze pediatric IA. A multivariable analysis showed that IA was more likely to be associated with severe GVHD (RR, 7.5%; 95% CI, 3.0 to 18.4). Additional analyses revealed that in the first 30 days there were 10 cases of IA, 13 cases from day 31 to 100 post transplant, and 3 cases from day 101 to 365 post transplant.

In an excellent review of IA case-fatality rates pooled from 1941 patients from clinical trials, cohort or case-control studies, and case series of 10 or more patients with definite or probable IA from 1995 through 1999, there was some stratification of case-fatality rate by decade of life.[102] The mean age of all patients was 44.2 years (range, 3 to 91 years), and the youngest cohort (≤20 years) had a case-fatality rate of 68.2 percent (15/22). The next highest case-fatality rate was 59.3 percent in the age group from 21 to 30 years. This suggests the epidemiologic possibility that pediatric and adult IA differ in outcome.

DIAGNOSIS

The diagnosis of IA is often not straightforward. Because of the many clinical presentations, an IA diagnosis is categorized as "proven," "probable," or "possible" disease based on meeting certain clinical, microbiologic, and radiologic criteria designed and later revised by the European Organization for the Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG).[7,37] These criteria have served as a standard in clinical trials to group patients with similar disease characteristics, but it should be noted that these criteria are not perfect and the designers have specifically cautioned against their implementation in routine clinical practice. Nonetheless, these distinctions have served the community well to establish a common framework for discussion about disease in the complicated high-risk patient. Generally, "proven" and "probable" IA can be considered as one entity because numerous clinical trials have shown their general equivalency in patient outcomes. However, "possible" disease is a vague entity and should be excluded from clinical trial design and the clinical diagnosis strongly reconsidered.

Cultures

A proven diagnosis of IA requires isolation from an otherwise sterile culture and histologic demonstration (Fig. 197-4).[37] However, this "gold standard" of tissue biopsy is often considered too invasive and complicated by bleeding or secondary infection in high-risk patients. An important distinction for a positive *Aspergillus* culture has to be made between disease and colonization. A 1-year retrospective study found that only 12 percent of patients with *Aspergillus*-positive cultures met criteria for IA. Among high-risk patients, a positive culture was associated with IA in 50 to 65 percent of cases; among intermediate-risk patients, this occurred in 8 to 28 percent, whereas patients with low risk rarely had IA.[141]

In one study the predictive value of respiratory tract cultures from patients with IPA was 40 to 100 percent.[181] Even in patients with established disease the sputum

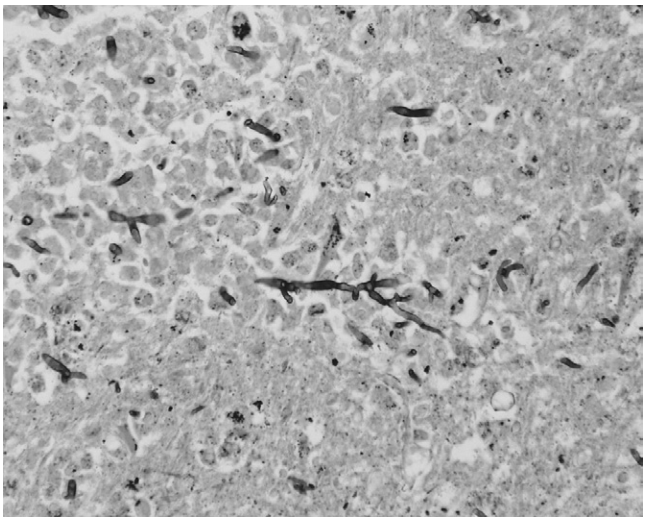

FIGURE 197-4 ■ Histopathologic findings after a lung biopsy of invasive aspergillosis show septate hyphae (Gomori methenamine silver stain). (Courtesy Dr. Thomas J. Cummings, Duke University Medical Center.)

specimens are commonly negative,[71,152] which is likely because IPA is predominantly infiltrative and does not have aerial growth in the bronchial tree.[71] Colonization with *Aspergillus* species has been a marker for reduced short-term survival, because 12 percent of colonized patients died within 3 months. IPA was diagnosed in 12 percent of patients with a positive culture, but this is likely an underestimate because culture specimens were not always obtained because diagnosis was often made by radiographic imaging.[141] In one study of heart transplant recipients, during a 10-year study period *Aspergillus* species were recovered from 30 episodes from 27 heart transplant recipients (incidence, 10.5%). The overall positive predictive value was 60 to 70 percent, but this increased to 88 to 100 percent when the organism was recovered from a respiratory specimen other than sputum and decreased to 50 to 67 percent when it was recovered from sputum. The sensitivities of fungal and conventional media for the recovery of *Aspergillus* species were 95 to 100 percent and 33 to 38 percent, respectively.[123]

Prior nasal colonization before transplant has been associated as a predictor of subsequent IPA in some studies[2] but has not been duplicated in others.[80] Prior IA does not eliminate the chance of a successful HSCT because in one study there were recurrences in nine patients with IA before transplantation, and all patients showed no signs of delayed engraftment compared with transplant recipients without a history of IA.[162]

Aspergillosis diagnosed by blood culture is rare.[59] In a study of 1477 separate positive cultures, there were more than a dozen positive blood cultures, but most were associated with pseudofungemia or terminal events noted at autopsy.[141] In general, the *Aspergillus* hyphal mass that develops in the lumen during angioinvasion remains in place until the force of blood flow causes hyphal breakage, which then allows the mass to circulate. The likelihood of a blood culture capturing these irregularly and infrequently discharged units is small. The difficulty in detecting *A. fumigatus* in blood culture stands in contrast to other angioinvasive filamentous fungi (e.g., *Fusarium* spp., *Paecilomyces lilacinus*, *Scedosporium prolificans*, *Acremonium* spp.) that have the ability to discharge a steady series of unicellular spores into the bloodstream that are more likely to be captured in a blood sample. This ability to sporulate in tissue and blood has been termed *adventitious sporulation*.[159] Because *A. terreus* also displays adventitious sporulation, histopathology and examination with potassium hydroxide of these spores also can allow rapid, presumptive identification of *A. terreus*. Therefore, a positive blood culture with *A. terreus* or another mold that demonstrates adventitious sporulation should not be ignored whereas a positive blood culture with other *Aspergillus* species should be further evaluated.

Radiology

IPA characteristically manifests on radiographs as multiple, ill-defined, 1- to 3-cm peripheral nodules that gradually coalesce into larger masses or areas of subsegmental and segmental consolidation. Lobar or diffuse pulmonary consolidation is a common finding.[28] Chest radiographs can be abnormal, but in one series they were normal in approximately 30 percent of patients in the week preceding death.[148]

There are two classic radiologic signs of IPA. The "halo" sign occurs in neutropenic patients with a hemorrhagic nodule due to angioinvasion. An early CT finding of the halo sign is a rim of ground-glass opacity surrounding the nodule. In one study, the halo sign was seen in all patients with biopsy-proven IPA, but it is so nonspecific it was also seen in patients with zygomycosis, organizing pneumonia, or pulmonary hemorrhage.[205] These early lesions subsequently change into a cavitary lesion or lesion with an "air crescent" sign 2 to 3 weeks later when neutropenia recovers.[28,93] In one study this was seen in 48 percent of patients 3 to 10 days after recovery of neutropenia.[156] Cavitation of the nodules or masses occurs in about 40 percent of patients and is characterized by an intracavitary mass composed of sloughed lung and a surrounding rim of air. A retrospective review of the CT finding for 47 autopsy-proven cases of IPA showed that nodular lesions and cavitation occurred significantly more often compared with controls.[65]

Just as the clinical presentation is nonspecific and heterogeneous, so are the findings in radiologic studies (Figs. 197-5 and 197-6). Whereas in most patients disease is seen as round infiltrates, peripheral wedge-shaped lesions, and the typical halo sign early in the course of IPA during the neutropenic period, in patients with CGD the hyphal angioinvasion is not a feature of disease and therefore the halo sign, cavitated lesions, and pulmonary infarcts are not typically found (Fig. 197-7). Instead, there are areas of tissue destruction secondary to a reactive acute and granulomatous inflammatory process.[120]

A long-term CT follow-up in 40 non–human immunodeficiency virus (HIV)-immunocompromised patients with IPA showed that formation of cavitation most strongly predicted time until radiologic remission and beneficial outcome. In that study, the natural history of early IPA lesions was evaluated and it was found that 90

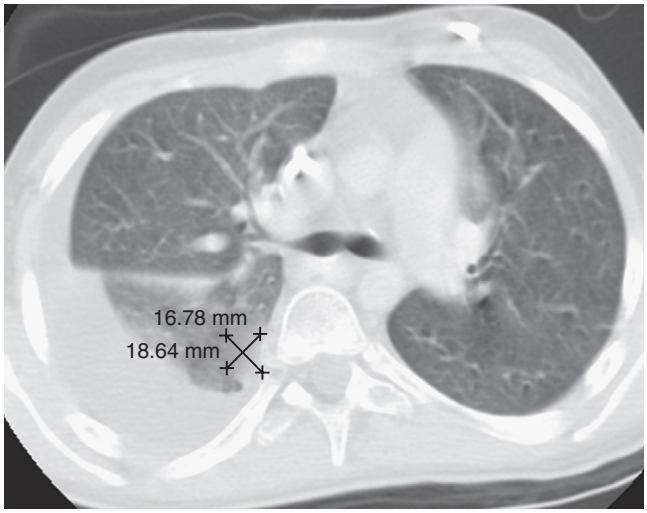

FIGURE 197-5 ■ CT scan of a 15-year old with Schwachman-Diamond syndrome and acute myelogenous leukemia shows right lower lobe pulmonary nodules and pleural effusion.

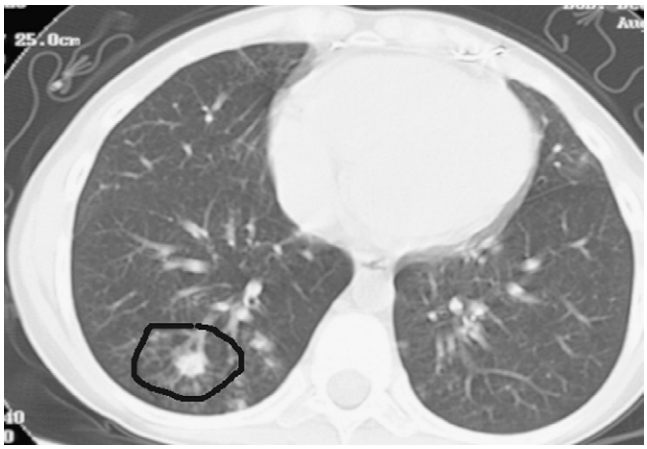

FIGURE 197-6 ■ CT scan of an 11-year old with cystic fibrosis status post bilateral lung transplant shows bibasilar nodular opacities. Encircled is a 1-cm opacity associated with some peripheral speculation.

FIGURE 197-7 ■ CT scan of a 9-year old with chronic granulomatous disease shows a soft tissue mass within the anterior mediastinum with heterogenous enhancement. The mass caused a convex deformity of the left anterior chest wall.

percent of patients experienced an increase in lesion size and number followed by a plateau in size and a decrease in number. Cavitation of the lesions developed in 55 percent of patients, and complete radiologic remission within a median 80 days was observed in 42.5 percent of patients. The number of days until remission without cavitation (50 days) was less than for those with cavitation (95 days), so formation of no cavitation was strongly predictive of radiologic remission.[18] In one study the appearance of the air crescent sign had no relationship to duration of neutropenia and showed a tendency to appear in large lesions such as consolidation or mass rather than small lesions like nodules.[88]

There may be radiologic differences for IA in adult and pediatric patients. In adult series of pulmonary aspergillosis, approximately 50 percent of cases show cavitation and 40 percent show air crescent formation.[56] In one 10-year review of pediatric patients (mean age, 5 years), there was central cavitation of small nodules in only 25 percent of children and no evidence of air crescent formation within any area of consolidation.[179] In another pediatric report there was a 22 percent (6/27) rate of cavitation on chest radiography[5] and in another there was a 43 percent (6/14) rate of cavitation on CT.[177] In these last two pediatric series the mean ages were higher than the other report of lower rates of cavitation and no air crescent formation, suggesting that there is a spectrum of radiologic disease presentation that is directly related to age, with cavitation and air crescent formation more likely in the older child and adult than in the younger child.

A review of 27 pediatric patients showed that radiographic changes that developed after HSCT but before diagnosis of pulmonary fungal infection included unilateral infiltrates (52%) slightly more often than bilateral infiltrates. At the onset the infiltrates were interstitial (41%), alveolar (41%), and mixed (18%). Hilar or mediastinal lymphadenopathy and pleural effusion/thickening were rare. By the time of diagnosis of pulmonary fungal infection the infiltrates were largely bilateral (66%) and alveolar or nodular (74%), including 22 percent of patients who had cavitary lesions.[5] The largest series of contemporary pediatric IA cases found the most common clinical site of IA was the lungs (59%), and the most frequent diagnostic radiologic finding was nodules (34.6%). Importantly, only 2.2 percent of the children showed the air crescent sign, 11 percent demonstrated the halo sign, and cavitation was seen in 24.5 percent of patients.[22]

Thoracic MRI findings are not as characteristic as chest CT findings, and the typical MRI sign is the "target" sign, a nodular lesion with a lower signal in the center compared with a higher, contrast-enhancing signal intensity in the rim on T1-weighted images. This sign is highly suggestive of late-stage disease.[15] MRI is the modality of choice for diagnosing cerebral aspergillosis and is preferred over CT for sensitivity (Fig. 197-8). Findings often show multiple lesions located in the basal ganglia that include an intermediate signal intensity, lack of contrast enhancement, and absence of mass effect.[119] CT of the head often reveals one or multiple hypodense, well-demarcated lesions. Hemorrhage and mass effect are

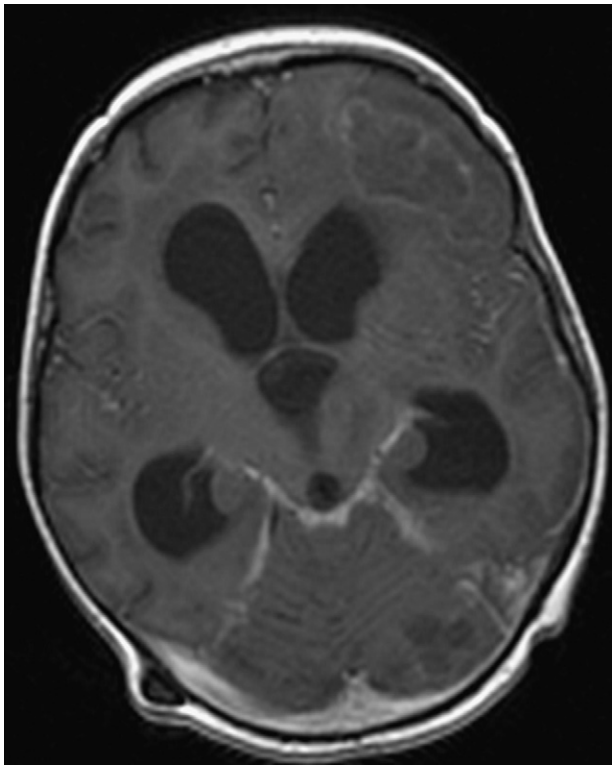

FIGURE 197-8 ■ A 3-year old with medulloblastoma after chemotherapy and irradiation had an acute change in mental status. T1-weighted axial MR image shows multiple lesions in the subcortical area.

unusual, but for patients with adequate peripheral white blood cell counts a ring enhancement and surrounding edema are more frequent.[38] T2-weighted MR images show very decreased signal intensity compared with those of bacterial sinusitis, which show increased signal intensity.[38]

Serology

Serologic testing for antibodies to *Aspergillus* antigens is helpful to diagnosis aspergilloma or ABPA in immunocompetent individuals, but unfortunately serology plays little role in the immunocompromised patient because *Aspergillus* growth does not correlate with an increase in anti-*Aspergillus* antibody titers.[100] For example, serologic examination of 18 patients with IA showed anti-*Aspergillus* antibody detection was negative in all cases, likely owing to profound immunosuppression.[156]

However, despite the historical lack of success with antibody testing, there is a movement to utilize antibody testing in patients before immunosuppression in an attempt to discern who is most likely to subsequently develop IA. In a study to evaluate antibody responses using an enzyme-linked immunosorbent assay (ELISA) format in different types of aspergillosis, patients showed that three recombinant antigens may have an important diagnostic potential.[155] The recombinant antigens with possible diagnostic utility are 18-kDa ribonuclease (RNU), 360-kDa catalase, and 88-kDa dipeptidylpeptidase V (DPPV). In aspergilloma patients, all three of the antigens were equally useful for diagnosis but only catalase was found to be useful in both immunocompetent and immunocompromised patients.

Galactomannan Antigen

Galactomannan (GM) is a major cell wall component of *Aspergillus*, and it is known that the highest concentrations of GM are released in the terminal phases of the disease.[100] An ELISA technique was introduced using a rat anti-GM monoclonal antibody, EB-A2, which recognizes the (1→5)-β-D-galactofuranoside side chains of the GM molecule.[107] A sandwich ELISA technique was introduced in 1995[173]; and by using the same antibody as both a capture and detector antibody in the sandwich ELISA (Platelia *Aspergillus*, BioRad), the threshold for detection can be lowered to 1 ng/mL. This technique is employed in the currently commercially available GM assay for diagnosis of IA.

Positive assay results are suggestive of invasive disease, and a 3-year prospective trial showed the sensitivity of serial monitoring was 90 percent, the specificity was 98 percent, and the negative predictive value was 98 percent. All 30 patients with proven IA tested positive, with no false-negative results. GM detection preceded the development of infiltrates on a chest radiograph in 68 percent of patients. The false-positive rate was 14 percent, and therefore the improved sensitivity over latex agglutination is counterbalanced by the loss of specificity and greater false-positive results.[108] Other studies have reported false-positive results of 5 to 8 percent and suggested they were due to cytotoxic agents, increased resorption of GM, or cross-reacting factors from the intestines.[185] In a meta-analysis conducted to characterize the clinical utility of GM assay, 27 studies from 1996 to February 28, 2005, were identified and overall the GM assay had a sensitivity of 71 percent and a specificity of 89 percent for proven cases of IA as defined by the specific clinical criteria.[143] Of note, there was considerable heterogeneity among the studies, and in subgroup analysis the performance of the test differed by patient population as well as by which reference diagnostic standard was utilized. In patients with hematologic malignancy or who have undergone hematopoietic transplant the assay was most useful, but in SOT recipients the sensitivity and specificity were 22 percent and 84 percent, respectively.

Serial testing at least twice a week is recommended.[39] In one study an increase in value during the first week of observation was predictive of treatment failure in allogeneic HSCT patients.[16] In a large prospective study of hematology and HSCT patients with confirmed or probable IA, GM was detected in 65 percent of patients an average of 8.4 days before positive CT scans or cultures, and it was detected in 40 percent of patients before the onset of clinical symptoms by a mean of 6.9 days. The sensitivity of GM detection in HSCT recipients was 89 percent, with a specificity of 98 percent. The test was performed easily with results available in 4 hours, and two

successive samples with elevated GM values were required for positivity.[174] One study found the sandwich ELISA had a sensitivity of 60 percent and a specificity of 82 percent.[109] Another prospective study involved 186 consecutive patients at risk for IA monitored twice weekly and yielded a sensitivity of 93 percent and specificity of 95 percent, and in most cases antigenemia was detected a median of 6 days before clinical symptoms.[107]

Unfortunately the GM assay has decreased sensitivity in the setting of a patient receiving anti-*Aspergillus* (mold-active) antifungal agents, while the specificity for detection does not change.[110] This is thought to be due to the decreased fungal burden in a patient receiving mold-active antifungal agents, and the difficulty lies in the fact that those are exactly the high-risk patients in whom the GM assay would be utilized. A subsequent study found that the GM test was best performed in patients not receiving any antifungal agents, in whom sensitivity was 89 percent versus 52 percent among patients who received empirical mold-active antifungal agents.[113]

Neutropenia also affects GM diagnosis. The GM value in patients with an absolute neutrophil count (ANC) less than 100 cells/μL and not receiving antifungal therapy was statistically higher than in those patients with an ANC greater than 100 cells/μL. However, GM values were not statistically different in patients with an ANC less than 100 cells/μL and receiving antifungal therapy versus those with an ANC greater than 100 cells/μL.[30] It is possible this neutropenia effect is due to fungal burden higher at the time of initial IA in neutropenia or that IA lesions are more extensive in the setting of neutropenia.

Although GM has been extensively validated in patients with hematologic malignancy and those who have undergone HSCT, for unclear reasons GM appears less useful in SOT recipients. A meta-analysis of serum GM studies revealed an overall sensitivity of 71 percent with a specificity of 89 percent; however, sensitivity dropped to only 22 percent in SOT recipients and rose to 82 percent in HSCT recipients.[143] In liver transplant recipients there was a high false-positivity, especially in patients with autoimmune liver disease or dialysis,[95] and in lung transplant recipients there was a greatly decreased sensitivity, perhaps owing to the pathophysiologic differences in *Aspergillus* tracheobronchitis or anastomotic disease seen in these patients.[74]

False-positive results with GM can hamper its clinical utility and are seen in patients concurrently receiving some β-lactam antibacterial agents such as piperacillin-tazobactam, amoxicillin, and amoxicillin-clavulanate. One study that evaluated these false-positive GM kinetics found a gradual increase in GM on patients receiving these β-lactam antibacterial agents and an average of 5.5 days to a negative antigen test.[8] There were numerous theories as to a supposed increased false positivity in children, ranging from *Bifidobacterium bifidum* spp. in the gut microflora that mimic the epitope recognized by the EB-A2 in the ELISA kit[117] to GM-positive infant formula used in pediatric patients.[1] We know now that false positivity is likely due to the ELISA testing itself cross reacting with specific antigens, likely not all of which have been defined, and this phenomenon appears irrespective of the patient's age. There are also questions about the GM false-negative results in some specific pediatric patients, such as those with CGD. One report details a non-neutropenic 4-year old child with CGD and IA diagnosed by lung biopsy who had persistent false-negative serum GM testing.[187]

Diagnosis of pediatric IA with a GM assay, while originally reported to be less useful due to a higher false-positive rate,[70,175] has been validated to be effective in children. A study of 64 pediatric allogeneic HSCT recipients found that, after excluding those patients receiving piperacillin-tazobactam, the false-positive rate was only 1.6 percent, leading to a specificity of 98.4 percent in this high-risk population. In that study, GM was falsely positive for the first sample obtained after initiation of piperacillin-tazobactam but the cessation of piperacillin-tazobactam resulted in a negative test in the immediate subsequent samples obtained (range, 3 to 4 days).[165] A subsequent report on 56 pediatric oncology patients found a pediatric sensitivity of 66 percent with a specificity of 87 percent, and in 6 of 7 cases of IA the GM test was positive before clinical or radiographic evidence of disease.[67] A retrospective study reported a similarly low false-positive rate (1.5%) and found that the GM test performed better in periods after aggressive chemotherapy than in those after allogeneic HSCT.[25]

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) is often useful in diagnosing IA, but a negative BAL culture does not conclusively rule out disease. In one study, the BAL fluid was analyzed in 23 consecutive patients with histologically proven IA and only 7 patients (30%) had BAL specimens diagnostic for IA. In the group in whom it was diagnostic, 71 percent had multiple changes on thoracic CT, compared with 25 percent of patients with negative BAL specimens. The diagnostic yield of BAL culture was not associated with clinical symptoms or duration of neutropenia. A thorough review of the diagnostic yield of BAL specimens in histologically proven IA patients yielded sensitivities of approximately 40 percent (range, 0 to 67%)[147]; but in one study, BAL had a sensitivity of only 50 percent even in patients with focal IA.[116] The sensitivity of respiratory tract culture specimens in general has ranged from 15 to 69 percent,[141] and in one study it increased to 50 to 70 percent in high-risk IA groups.

However, the sensitivity of BAL samples may be increased by use of the GM test, thus increasing the yield of bronchoscopy and possibly avoiding the need for further invasive procedures. Although the GM test cutoff value in sera is 0.5, it is better to use a value of 1.0 for BAL fluid GM testing. A retrospective analysis of 99 high-risk hematology patients, including 58 with IA, who underwent BAL for the diagnosis of new pulmonary infiltrates found that a BAL GM test value of 1.0 or higher yielded an increased sensitivity (91.3%) compared with BAL culture (50%) or BAL microscopy (53.3%). The combined sensitivity of all three BAL methodologies was 98.2 percent.[106] Further analyses in that study found that

the mean BAL GM result was not different in neutropenic versus non-neutropenic patients.

A meta-analysis of BAL GM studies found sensitivities to be between 59 and 100 percent and specificities to be between 76 and 100 percent and most importantly that antifungal therapy did not significantly affect sensitivity, as it does with serum GM.[64] A retrospective study in patients with hematologic malignancies and in HSCT recipients also found that the BAL GM test was more sensitive than a serum GM test for diagnosing IA and found that BAL GM was not impacted by mold-active antifungal agents, suggesting that this may be due to the levels of antifungal agents found in sera versus alveolar fluid. This study supported the use of BAL GM to diagnose IA, optimizing the positive predictive value of BAL GM, whereas serum GM might be best utilized as a screening test, with the lower cutoff value used to maximize the negative predictive value.[128] A retrospective pediatric BAL GM found an optimal BAL GM cutoff value of 0.98 to yield the best sensitivity (78%) and specificity (92%). Using a BAL GM value of 1.0 or higher and a concurrent serum GM of 0.5 or higher yielded the best sensitivity (89%) and specificity (90%).[44]

(1→3)-β-D-Glucan

(1→3)-β-D-Glucan is an integral cell wall component and, in contrast to GM, is not normally released from the fungal cell.[100] Factor G, a coagulation factor of the horseshoe crab, is a highly sensitive natural detector of (1→3)-β-D-glucan.[132] The "G test" detects (1→3)-β-D-glucan via a modified limulus endotoxin assay and detects *Aspergillus*, *Candida*, and even *Cryptococcus* but does not identify the genus of the fungi detected.[132] The G test is widely used in Japan, but these tests yield positive results only at advanced stages of infection in a majority of patients.[83] A 1-year prospective study of patients with hematologic malignancy and control subjects found the sensitivity of real-time polymerase chain reaction (PCR) assay was 79 percent, that for GM was 58 percent, and that for the G test was 67 percent, with specificities of 92 percent, 97 percent, and 84 percent, respectively.[82] Currently the (1→3)-β-D-glucan assay is available in the United States as the Fungitell assay.

In a study comparing (1→3)-β-D-glucan and GM, the sensitivity, specificity, and positive and negative predictive values for GM and (1→3)-β-D-glucan were identical. False-positive reactions occurred at a rate of 10.3 percent in tests using both substances, but the patients showing false-positive results were different in each test. Both tests anticipated the clinical diagnosis and CT abnormalities, but (1→3)-β-D-glucan tended to become positive earlier than GM. A combination of the two tests improved the specificity (to 100%) and positive predictive value (to 100%) of each individual test without affecting the sensitivity and negative predictive values.[139] Another study compared GM, PCR, and (1→3)-β-D-glucan assays on patients with hematologic disorders, and the receiver-operating characteristic (ROC) analysis showed an area under the ROC curve was greatest for the GM assay, using two consecutive positive results. This suggests that the GM test was the most sensitive at predicting the diagnosis of IA in high-risk patients with hematologic disorders.[87] A meta-analysis of cohort studies of (1→3)-β-D-glucan for IA revealed that using a single test resulted in a pooled sensitivity of 57 percent with a specificity of 97 percent.[96]

Polymerase Chain Reaction Assay

Although GM assays created a noninvasive test with improved sensitivity and specificity, more recent efforts have focused on defining an optimal primer sequence for a PCR detection method. At present, this diagnostic method is not commercially available and reports can be difficult to interpret owing to the lack of experimental standardization between centers. Because of the ubiquitous nature of the mold, it is likely the value of this test will be its high negative predictive value. Issues remaining unresolved in the use of the PCR assay are the best source of material (e.g., whole blood, serum, plasma, BAL specimens), the amplification protocol (e.g., real time, sample volume, extraction methods), and primer selection (e.g., "panfungal," 18S rRNA, 28S rRNA, mitochondrial DNA).[90]

Reported sensitivities in numerous retrospective reports of PCR are 55 to 100 percent, with negative predictive values generally around 100 percent.[146] The high negative predictive value is consistent with PCR as a sensitive marker for any colonizing or infected aspergilli, and a negative PCR assay finding in a patient with a suspected IA most likely shows that the patient does not have the organism.[145] In a 2-year prospective study of 121 patients with hematologic malignancies analyzed twice weekly by screening of whole-blood samples, the negative predictive value of two or three consecutive positive PCR results was 98 percent, with a sensitivity of 75 percent and a specificity of 96 percent. When PCR was detected only once, it was never associated with disease and resolved without antifungal treatment, thus indicating only a transient *Aspergillus* DNAemia.[98]

In another prospective PCR study in 84 allogeneic HSCT patients, all patients with proven or probable IA were PCR positive and PCR was found to be the earliest indicator for patients with new-onset IA, preceding the first clinical signs by a median of 2 days and preceding the diagnosis based on typical radiographic findings by a median of 9 days. For patients without a history of IA who tested positive PCR twice, sensitivity was 100 percent and the negative predictive value was 100 percent.[68] In another study in patients with IA diagnosed by a positive PCR assay, the positive findings preceded radiologic signs by a median of 4 days. Additionally, the disappearance of fungal DNA correlated with successful therapy and post mortem the patients remained PCR positive.[50] In one study, PCR positivity preceded radiologic diagnosis in 11 of 18 patients and an ELISA did not precede a positive PCR result on any patient. The PCR assay was more sensitive than the ELISA, although the ELISA had no false-positive results.[201] Early PCR testing in children has yielded mixed results,[6] but the future will likely hold some combined use of the GM and PCR assays.

The PCR assay has been used to detect *Aspergillus* in BAL specimens, but it does not allow distinction between colonization and invasive disease. A prospective evaluation of 197 BAL samples in febrile, neutropenic patients

with lung infiltrates showed all immunocompromised patients with a negative PCR had no evidence of fungal disease and only a few patients without immunocompromise showed positive results without evidence of disease. After further dividing the patients into a high-risk group (patients with acute leukemia undergoing chemotherapy), the negative predictive value was 100 percent.[20] A two-step PCR assay was shown to detect down to as little as 10 femtogram (fg) of *Aspergillus* DNA (approximately 5 CFU/mL) and was tested in 100 consecutive patients by BAL and 278 consecutive patient blood samples. The BAL PCR test was positive in 17 immunocompromised patients, with a specificity of 93 percent and negative predictive value of 100 percent. In comparison, in the blood samples, sensitivity was only 20 percent but negative predictive value was 100 percent.[19]

TREATMENT

Overall success treating IA is dependent on numerous factors, not simply the choice of a specific antifungal therapy. As with all immunocompromised patients, detailed knowledge of host factors, underlying disease, concomitant infections, and degree and duration of immunosuppression are key. It is well known that immune reconstitution is paramount to successful IA therapy, and continued exposure to certain immunosuppressive medications, such as corticosteroids, is known to worsen IA. Any antifungal prophylaxis used before the diagnosis of IA could also have an effect on the ultimate choice of targeted therapy. Although the diagnostic workup needs to be aggressive, it should never delay antifungal therapy in the setting of true concern for IA. As such, the cornerstone of antifungal therapy for IA is prompt and aggressive institution of antifungal therapy, based on diagnostic tools but also on clinical suggestion of infection. Antifungal resistance is slowly and steadily increasing among *Aspergillus* isolates and continues to have specific geographic trends that could influence the choice of antifungal agent. There is also the question of using antifungal monotherapy or combination antifungal therapy and, if so, which classes of agents. Finally, although immune reconstitution is paramount, the role, and real benefit, of adjunctive immunotherapy remains somewhat unclear.

Since its initial approval for use in 1958, amphotericin B deoxycholate was the first line therapy of IA and the gold standard against which other therapies were measured. However, with overall survival rates of around 35 percent among patients treated with amphotericin B for several decades, in addition to limited tolerance as a result of acute and chronic toxicities, there was a clear need to develop a better antifungal agent. Although itraconazole became available in 1990 for the treatment of IA, it was not without flaws; most prominent among these were decreased fungicidal activity compared with amphotericin B, unpredictable bioavailability in the capsular formulation in high-risk patients, and significant drug interactions.[168] The next step taken in the search of a better antifungal agent was the development of three different lipid formulations of amphotericin B whose advantages included an increased daily dose of the parent drug, high tissue concentrations with better delivery to reticuloendothelial organs, decreased infusion-related side effects, and decreased renal toxicity.[45] These three lipid formulations, amphotericin B lipid complex, amphotericin B colloidal dispersion, and liposomal amphotericin B, allowed greater safety in patients who could not tolerate conventional amphotericin B.[168] A study to determine if lipid formulations are better than conventional amphotericin B for IA, while informative, may not ever take place, owing to the introduction of the second-generation triazoles and a whole new class of antifungal agents, the echinocandins.

Voriconazole is a second-generation triazole and synthetic derivative of fluconazole that was approved for use in the United States in May 2002 for therapy for IA. The global clinical trial that led to its approval was a prospective comparative study that showed that voriconazole had a superior response rate in the primary treatment of IA compared with amphotericin B—52.8 percent versus 31.6 percent, respectively. Survival rates also increased, from 57.9 percent for amphotericin B to 70.8 percent for voriconazole.[69] Although that study was criticized for employing conventional amphotericin B as the comparator to voriconazole (owing to approval indications at the time of study design), a subsequent analysis clearly showed that primary therapy with voriconazole was superior to primary therapy with amphotericin B, regardless of subsequent switching for intolerance or refractory disease to any other licensed antifungal therapies (e.g., liposomal amphotericin B, itraconazole, other agents).[138] These and other studies led to the very clear guideline recommendations of the Infectious Diseases Society of America to use voriconazole for the primary treatment of IA in all clinical sites.[191] In antifungal parlance, primary therapy is the initial treatment for IA whereas salvage therapy is therapy begun after a failure or intolerance of the primary antifungal therapy.

More recent epidemiologic studies have also shown the benefit of voriconazole versus the decades of data with amphotericin B. Controlled clinical trials have the benefit of rigorous evaluation, but they also often suffer certain biases pertaining to a generally healthier patient enrollment. A Belgian multicenter observational study observed 113 adult patients with IA and found an overall successful response rate, generally viewed in antifungal clinical trials as the sum of both the complete response and partial response rate, of 50.4 percent with voriconazole.[75] A French epidemiology study found similar results analyzing 393 adult patients with IA, concluding that any treatment regimen containing voriconazole, alone or in an antifungal combination, was superior in terms of survival to any antifungal regimen without voriconazole.[103] This improved voriconazole response rate has also translated well to the pediatric population, as shown by a review of 42 children treated for IA with voriconazole.[195] An analysis of the compassionate open-label use of voriconazole in children for refractory IA demonstrated a 43 percent complete or partial response. Voriconazole was also shown in these studies to be better tolerated and to have less toxicity than amphotericin B in both adults and children. The main side effects of voriconazole are reversible visual disturbances in up to 40 percent of patients, elevated aminotransferase levels,

and occasional skin reactions, most likely owing to photosensitization.[153]

Another new triazole is posaconazole, a derivative of itraconazole. There are no randomized studies examining posaconazole for primary therapy for IA, but numerous in vitro and in vivo data suggest that this triazole will be as effective as voriconazole as a potential first-line agent against IA. A multicenter salvage therapy study evaluated 107 patients with IA treated with posaconazole versus 86 historic controls and found a response rate of 42 percent versus 26 percent, respectively, as well as improved survival at 30 days of 74 percent versus 49 percent, respectively.[197] This study was significant because the patients were all undergoing salvage therapy (generally after failing amphotericin B products or itraconazole), demonstrating that the newer triazoles are indeed the best treatment option for IA. Additional studies support this general argument, with voriconazole or posaconazole therapy resulting in fewer deaths compared with amphotericin B products or caspofungin in an Italian study of 140 cases of IA in acute myelogenous leukemia patients[134] and with voriconazole therapy with more survivors than caspofungin, amphotericin B formulations, or itraconazole in 642 cases of IA in HSCT and SOT recipients across the United States.[9]

Although voriconazole is the clear guideline-recommended antifungal agent for primary therapy of IA, there are recommended alternatives,[191] such as liposomal amphotericin B, amphotericin B lipid complex, caspofungin, micafungin, posaconazole, or itraconazole. Because conventional amphotericin B was shown to be inferior to voriconazole, few experts recommend conventional amphotericin B for IA management but some still advocate lipid formulation amphotericin B as a potential alternative therapy, generally in the setting of prior azole exposure and/or antifungal resistance. Amphotericin B has always been considered a concentration-dependent antifungal agent, and one clinical trial evaluated liposomal amphotericin B at either 3 mg/kg/day (n = 107 patients) versus 10 mg/kg/day (n = 94 patients).[32] The favorable overall responses for the lower dose (50%) and the higher dose (46%) were similar, with no demonstrable additional benefit to higher amphotericin B dosing and only higher rates of nephrotoxicity. However, although these response rates are generally similar to the favorable response seen earlier with voriconazole,[69] most of the amphotericin B responses were partial responses and not complete treatment responses, suggesting that, overall, the triazole was more effective in disease eradication.

The echinocandins have an entirely different mechanism of antifungal action and interfere with cell wall biosynthesis, compared with the second-generation triazoles, which work by inhibiting cytochrome P-450 enzymes responsible for conversion of lanosterol to sterol and thus compromising the fungal cell membrane.[198] Three compounds are currently approved in the United States: caspofungin, micafungin, and anidulafungin. Several in vitro antifungal susceptibility studies have shown the general equivalency of all three echinocandins against *Aspergillus* species.[142] The echinocandin activity against *Aspergillus* is fungistatic, as compared with the triazole fungicidal activity, and results in activity blunting the hyphal tip but not killing the organism[17] and subsequently the reporting of an in vitro minimal effective concentration (MEC) and not a minimal inhibitory concentration (MIC).[94] Caspofungin was first studied an open-label, noncomparative salvage therapy study in immunocompromised patients with proven or probable aspergillosis.[105] It produced a complete or partial response in 45 percent (37/83) of patients, which is again very significant because these were patients were either refractory or intolerant to previous antifungal therapy. This was validated by two subsequent clinical trials in which caspofungin resulted in complete resolution or stabilization of disease in 74 percent of patients (n = 31) in one trial; and in the other trial, caspofungin had an overall response rate of 56 percent (n = 32).[23,182] Few studies have evaluated caspofungin as primary therapy for IA. One phase 2 dose-escalation clinical trial evaluated 46 patients with caspofungin as primary therapy and found a favorable response rate of 54.3 percent, but there was no dose-dependent effect on efficacy for caspofungin.[33] A more recent observational study of caspofungin as primary or salvage therapy, and as monotherapy or in a combination approach, found an overall 56.4 percent favorable response, validating caspofungin as a viable therapeutic option.[104]

The largest study with micafungin was a noncomparative, open-label, multicenter study in adult and pediatric patients to examine the safety and efficacy of micafungin in the treatment those patients with IA who had failed to respond to prior therapy or could not tolerate other therapy. Of the 225 patients who met diagnostic criteria, a favorable response rate occurred in 35.6 percent (80/225), and of those treated with only micafungin, a favorable response was seen in 6/12 (50%) of the primary and 9/22 (40.9%) of the salvage therapy group.[41] Anidulafungin has been approved in the United States for treatment of candidemia and esophageal candidiasis; and although in vitro studies show activity against *Aspergillus* species isolates, there are no clinical studies against IA as primary or salvage monotherapy.

Although voriconazole is the guideline-recommended first-line agent for primary therapy for IA, it is not completely clear when the alternative agents, such as amphotericin B lipid formulations or echinocandins or the other triazoles (posaconazole or itraconazole), should be used in place of voriconazole. The most common reason for using an alternative agent is refractory disease after primary antifungal therapy and the need to move to salvage therapy. However, it is important to first allow the primary antifungal agent adequate time for clinical effect, without continuing the use of an ineffective agent for too long in a severely ill patient. In a clinically refractory setting, it is also paramount to confirm the correct diagnosis of IA if that has not been established. For example, because mucormycosis and aspergillosis appear radiographically similar, treatment of mucormycosis with voriconazole will not result in success. Assuming that the diagnosis of IA is correct, the first step in refractory disease should be to establish that the correct dose of voriconazole is being utilized, which is especially important in children because the fundamental

pharmacokinetics of voriconazole are different in children (linear) than in adults (nonlinear).[194] For instance, although the recommended starting dose of voriconazole in adult patients is 6 mg/kg/dose twice daily for the first day and then a maintenance dosing of 4 mg/kg/dose twice daily, the preferred pediatric dosing is substantially higher. Population pharmacokinetic analyses of voriconazole in children, adolescents, and adults reveal that, based on the area under the concentration-time curve, children should be given an intravenous 9 mg/kg/dose loading dose to be comparable to the 6 mg/kg/dose given to adults.[52] Maintenance intravenous dosing in children at 8 mg/kg/dose was comparable to 4 mg/kg/dose in adults, and the oral dosing of 9 mg/kg/dose was found to be similar to adults receiving 200 mg oral voriconazole twice daily. The majority of adolescents can be dosed as adults, but in those younger adolescents (ages 12 to 14), the analysis found that body weight was more important than age in predicting voriconazole pharmacokinetics in this age. Therefore, during this age transition period, adolescents aged 12 to 14 years old should be dosed as children if their weight is less than 50 kg and dosed as adults if their weight is 50 kg or more.[52] Additionally, the oral bioavailability of voriconazole, while believed to be greater than 95 percent in adults, is lower in children at 50 to 65 percent.[84,193] This is very important, especially for those patients receiving oral voriconazole long after a transplant and during the second bimodal peak of disease at approximately day 100 after transplantation. However, even with these dosing increases, there are still suggestions of the need for higher voriconazole doses. A population pharmacokinetic analysis in adult patients found that higher voriconazole doses were required to achieve adequate therapeutic serum levels.[137]

The triazole antifungal agents require therapeutic drug monitoring. Effective management of IA requires obtaining a voriconazole trough level, which is well known to have high interpatient variability, limiting its extrapolation to a larger population, but much lower intrapatient variability, allowing successive trough levels to be used to monitor dose adjustments in the individual patient.[164] The exact voriconazole trough level for clinical efficacy against IA is unknown, because there have been several clinical studies in which occasional individual patients have had undetectable voriconazole levels and still shown a clinical response. However, it is commonly believed that a serum trough level at least greater than the usual MIC of the infecting *Aspergillus* species would be preferable. Although individual studies have debated the exact cutoff, what is clear is that the serum level should be a true trough level and not a random drug level. Most experts today would advocate for a serum trough voriconazole level of at least greater than 1.0 μg/mL and stress the importance of individualized therapy for each patient. Inherent in the individualized treatment approach is the understanding that different populations also metabolize voriconazole uniquely, with three major genotypes due to allelic polymorphisms in the human cytochrome P-450 isoenzymes (largely CYP2C19) responsible for voriconazole metabolism.[81] In refractory disease, after optimizing the dose of voriconazole, the most-studied antifungal agent for the treatment of IA, then refractory disease can be approached with one of two main options. The first is to change the class of antifungal agent used and treat with an echinocandin or lipid amphotericin B formulation to replace the triazole already used. This is especially important in the setting of prior azole (antifungal prophylaxis prior to diagnosis) exposure because of the potential concern for development of antifungal resistance. Itraconazole resistance was first described in 1997,[43] and antifungal resistance to echinocandins is currently largely due to researchers genetically modifying the *fks1* gene (target of echinocandins) in *A. fumigatus*.[151] However, azole resistance is now increasing in clinical strains,[21] including multiple-azole or pan-azole (voriconazole, posaconazole, itraconazole)–resistant strains. Azole resistance in *A. fumigatus* has emerged over the past decade, with a prevalence as high as 5 to 13 percent in the Netherlands and Great Britain.[73,184] The most common multiple-azole resistance mechanism consists of mutations in the *cyp51A* gene involved in ergosterol biosynthesis.[186] Additionally, some *Aspergillus* species are more likely to be inherently resistant to certain antifungal agents. For years, *A. terreus* was a recalcitrant *Aspergillus* species owing to its now well-described in vitro resistance to amphotericin B,[176] which has been confirmed in animal models.[196] A review of in vitro analyses, testing in multiple animals, and previously reported clinical cases showed near uniform failure with amphotericin B against *A. terreus*.[167] A multicenter retrospective analysis of more than 80 cases demonstrated that mortality was decreased in those patients who received voriconazole instead of amphotericin B.[166] Other studies have also shown that patients with *A. terreus* disease are more likely to be neutropenic,[66] more likely to have disseminated disease, and have a decreased response to all classes of antifungal agents compared with patients infected with *A. fumigatus*.[99]

Beyond occasional enrollment into antifungal clinical trials, there has been very little investigation into the treatment practice patterns or outcomes of pediatric IA patients. The largest analysis was a retrospective multicenter study of 139 children with IA that found that after the diagnosis of IA, 57 percent were treated with a lipid formulation of amphotericin B; however, 45.8 percent received three or more concomitant antifungal agents.[22] A total of 52.5 percent (73 of 139) of the patients died during treatment for IA, and immune reconstitution, rather than a specific antifungal therapy, was found to be the best predictor of survival.

Combination Antifungal Therapy for Invasive Aspergillosis

Although no controlled clinical trial fully supports it use and the efficacy of combination therapy for IA has not been conclusively established and is not guideline recommended, clinicians are desperately seeking new strategies for improved outcome. With the surge in the development of newer antifungal agents for IA, there are now more permutations of new potential combination antifungal therapies. Drawing from other infectious diseases such as HIV infection, tuberculosis, and cryptococcal meningitis,[13] combination therapy for IA seems plausible

to optimize therapy. Unfortunately, the range of in vitro, animal model, and small clinical series data extend from synergy to antagonism and actually parallel the wide range of unproven treatment practices used by clinicians today searching for the best care for their patients.

The largest analysis of combination therapy for IA reviewed 6281 total cases of IA management from 1966 through 2001, focusing on 249 clinical combination reports in 128 clinical articles.[169] Unfortunately, three combination regimens that are not commonly used now nor currently recommended (amphotericin B + flucytosine, amphotericin B + itraconazole, and amphotericin B + rifampin) comprised the majority of reported clinical experience at that time. Although these data helped lay the foundation of combination therapy in the past, the combinations used historically are not necessarily the combinations considered by today's clinician.

There are several foreseeable advantages to combination antifungal therapy: a widened spectrum and potency of drug activity, more rapid antifungal effect, synergy, lowered dosing of toxic drugs, and a reduced risk for antifungal resistance.[101] Although each individual antifungal agent has limitations, combinations might prove more effective, as seen with the now standard highly active antiretroviral therapy (HAART) used with HIV-infected patients. Absent large-scale clinical trials, clinicians often derive treatment strategy information from experimental in vitro or in vivo data. Unfortunately, because of the extensive heterogeneity of both experimentation and interpretation, one cannot accurately draw a firm conclusion as to the clinical relevance of the experimental combination antifungal experiments. Perhaps the greatest usefulness for in vitro combination is to screen antagonistic interactions before investigating animal model or clinical studies.

Animal model testing is therefore preferred over in vitro analysis because of greater predictive value for human pharmacokinetic and pharmacodynamic effects, including tissue penetration and achievable drug levels at the foci of infection and toxicities. In vivo testing can also compare various disease location models and analyze different host immune states. In addition, histologic examination can examine fungal sterilization. Although in vitro testing might suggest an effective antifungal agent, the dose needed to achieve the desired effect might be unachievable safely in vivo or the drug may not penetrate infected tissue adequately.

In the simplest terms, for combination antifungal therapy to be viable, the combination treatment would need to be superior to any of the individual therapies. The advent of voriconazole has made this task more complicated because the agent is quite effective at killing *Aspergillus*, sterilizing infected animal tissue in numerous animal models, and clinically curing patients who were once thought hopeless. Regardless of the continuous stream of data, the in vitro and in vivo interactions must continue to be questioned because of so many confounding patient variables, leaving clinical experience still the most accurate tool. However, clinical relevance might best be related to patient factors (e.g., recovery of neutropenia, cessation of glucocorticoid therapy) and not intrinsically related to the susceptibility of the fungus itself.

Combination in vitro testing has many potential pitfalls, and the final fractional inhibitory concentration index (FICI) itself, the mathematical foundation behind the commonly used checkerboard test, can also be clinically deceiving. For example, in one study the interaction between fluconazole and terbinafine was reported to be synergistic against *A. fumigatus* strains (FICI 0.38).[121] However, individually fluconazole is well known to have zero anti-*Aspergillus* activity and terbinafine has poor tissue penetrance[72] and would be a likely poor clinical choice for IA. Not surprisingly, the individual MIC of fluconazole was 512 μg/mL and that of terbinafine was 8 μg/mL, yet both did improve when in combination to 64 μg/mL and 2 μg/mL, respectively, therefore mathematically achieving the definition of antifungal synergy. However, one could argue that this result only indicates that two already poor choices for treatment were simply made less poor in combination. It is unlikely clinicians would advocate combination therapy with fluconazole and terbinafine for their IA patients despite a final result of "synergy" based on in vitro testing.

Although in vitro combination studies have inherent experimental variations, there is likewise no standardization in animal model experimentation. It has been suggested that if a single antifungal agent offers as much as 50 percent protection or many infected control animals survive, it is difficult to show improvement with combination therapy over single agents.[61] Many variables influence outcome in animal model experiments similar to patient cases, including the degree of neutropenia, route of infection determining which target organs are infected, timing of drug delivery, and tissue processing and fungal burden measurement.

Although it is impossible to review all previous animal model studies of IA, the most often quoted in vivo combination antifungal model study examined voriconazole + caspofungin.[89] In this immunosuppressed guinea pig model of intravenously inoculated IA, treatment with amphotericin B or caspofungin 1 mg/kg/day or 2.5 mg/kg/day resulted in 30 to 50 percent mortality. However, treatment with voriconazole 5 mg/kg/day or voriconazole plus either caspofungin dose resulted in no mortality (0/12 in all experimental arms). Although this report helped ignite excitement about this particular combination, this guinea pig model actually showed no difference in mortality (0/12) or mean times of survival (8 days post infection) in the combination therapy arm compared with voriconazole monotherapy. Semi-quantitative organ cultures for fungal burden (colony-forming units per gram of tissue) from the liver, lung, kidney, and brain in the combinations were better than untreated controls ($p < .0025$), but there is no report of any difference between the combinations and the voriconazole monotherapy. Treatment with the combinations was significantly more effective than any of the other regimens in reducing the number of positive organ cultures compared with untreated controls ($p < .0025$), but again there was no report of any differences compared with voriconazole monotherapy. The voriconazole + caspofungin 1 mg/kg/day trial arm yielded 17 percent (8/48) positive cultures, whereas the voriconazole + caspofungin 2.5 mg/kg/day trial arm yielded 24 percent (11/48) positive cultures and

the voriconazole monotherapy arm yielded 52 percent (25/48) positive cultures.

In the clinical setting, the antifungal combination of voriconazole + caspofungin has the most anecdotal clinical experience. This is likely due to both approval and availability, as well as the presumed synergistic interaction due to the simultaneous inhibition of (1→3)-β-D-glucan synthesis in the fungal cell wall and ergosterol synthesis in the fungal cell membrane.[89,140] One pivotal study analyzed voriconazole + caspofungin as a retrospective review of 47 patients with proven or probable IA from 1997 through 2001 who experienced failure of primary therapy with amphotericin B formulations.[112] Salvage therapy was begun with voriconazole (n = 31) versus voriconazole + caspofungin (n = 16) after 7 or more days of failed amphotericin B therapy. The overall survival rate 3 months after the day of diagnosis of IA was slightly higher among those who received combination therapy (p = .048). In the bivariable analysis (controlling for antifungal therapy and receipt of HSCT), the combination showed an improved 3-month overall survival rate (hazard ratio, 0.27; 95% CI, 0.09 to 0.78; p = .008). An accompanying editorial to this article pointed out the preclinical data with combination therapy seem promising yet inconsistent.[189] The authors responded,[26] and in analyzing the patients at 1 year they found there was no difference in overall survival (p = .26).

Similar to the in vitro and animal models experiences, it is impossible to review all combination clinical studies (many as smaller and uncontrolled case series). All ranges of combinations have been reported, and results have again spanned the spectrum from an increased effect to a worsening of outcome. The obvious vast clinical heterogeneity of these reports limits direct cross-study comparisons. The prevailing expert opinion is that if a combination antifungal therapy approach is beneficial to treating IA, it is likely best taken with the combination of a cell membrane–active triazole with a cell wall–active echinocandin. A recently completed multi-national randomized clinical trial comparing voriconazole with and without the echinocandin anidulafungin showed no clear benefit of primary combination antifungal therapy for IA, and only statistical trends in improvement in certain groups.[114a] The results of a large clinical trial of voriconazole + anidulafungin versus voriconazole alone are pending, but it is unclear if any combination therapy will have the great advance that was achieved with voriconazole versus conventional amphotericin B, owing to the ever-increasing fragility of our most immunosuppressed patients with IA. Although the latest treatment guidelines state that primary combination antifungal therapy is not routinely recommended based on a lack of definitive clinical data, one retrospective study used voriconazole + caspofungin primary combination therapy in lung transplant recipients because of the known delay in time to achieve an adequate voriconazole serum level, thereby using the second agent only during those initial early weeks of therapy.[178] Other interventional therapies used in the treatment of IA include growth factors, such as granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor, granulocyte transfusions, interferon-γ, and surgery. Granulocyte colony-stimulating factor increases the number of circulating polymorphonuclear leukocytes and enhances phagocytic activity and oxidative burst metabolism. Interferon-γ promotes tumor necrosis factor-α production and enhances polymorphonuclear leukocytes and mononuclear cell–induced damage. It has been proven to help prevent IA in patients with CGD and is used as prophylactic therapy in these patients.[168] General expert opinion is that neutropenic patients with IA may benefit from the addition of a colony-stimulating factor, or possibly granulocyte transfusions, but there are limited data on efficacy.

Optimal duration of therapy in IA is unknown but likely depends on the immune status of the patient. Many experts believe that treatment should continue until complete clinical and radiographic resolution of disease, often with accompanying significant decrease in biomarkers (e.g., GM). If a patient remains substantially chronically immunosuppressed owing to ongoing cytotoxic chemotherapy or lack of engraftment after HSCT, it is advisable to continue the antifungal therapy for IA until there is no evidence of disease, often 3 months or longer. Surgical therapy may be useful in patients with lesions contiguous with the great vessels of pericardium to avoid fatal hemoptysis, but surgery to debulk disseminated lesions is generally not advised.

ABPA is a hypersensitivity disease, and therefore therapy against ABPA centers on corticosteroids to manage the allergic component of *Aspergillus*, coupled with adjunctive oral antifungal therapy to diminish the fungal burden. Itraconazole has been the most well-studied antifungal agents against ABPA, and clinical trials have shown a clear benefit in itraconazole against ABPA, including patients with cystic fibrosis and ABPA.[163] Single aspergillomas should be managed to prevent life-threatening hemoptysis, and surgical resection, if technically possible, is ideal. Medical therapy has limited utility in the treatment of aspergilloma yet is required in chronic cavitary pulmonary aspergillosis and often lifelong.

NEW REFERENCES SINCE SIXTH EDITION

1. Aceti A, Corvaglia L, Faldella G. Infant formulas thickened with carob bean gum causing false-positive galactomannan test reactivity. *Pediatr Infect Dis J* 2008;**27**:769.
3. Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, et al. *Aspergillus* section *Fumigati:* antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Chemother* 2008; **52**:1244–51.
6. Armenian SH, Nash KA, Kapoor N, et al. Prospective monitoring for invasive aspergillosis using galactomannan and polymerase chain reaction in high risk pediatric patients. *J Pediatr Hematol/Oncol* 2009;**31**:920–6.
9. Baddley JW, Andes DR, Marr KA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. *Clin Infect Dis* 2010;**50**:1559–67.
21. Bueid A, Howard SJ, Moore CB, et al. Azole antifungal resistance in *Aspergillus fumigatus:* 2008 and 2009. *J Antimicrob Chemother* 2010;**65**:2116–18.
22. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics* 2008;**121**:e1286–94.
25. Castagnola E, Furfaro E, Caviglia I, et al. Performance of the galactomannan antigen detection test in the diagnosis of invasive aspergillosis in children with cancer or undergoing haemopoietic stem cell transplantation. *Clin Microbiol Infect* 2010;**16**:1197–203.
30. Cordonnier C, Botterel F, Ben Amor R, et al. Correlation between galactomannan antigen levels in serum and neutrophil counts in

haematological patients with invasive aspergillosis. *Clin Microbiol Infect* 2009;**15**:81–6.
33. Cornely OA, Vehreschild JJ, Vehreschild MJ, et al. Phase II dose escalation study of caspofungin for invasive aspergillosis. *Antimicrob Agents Chemother* 2011;**55**:5798–803.
37. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**:1813–21.
44. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009;**28**:283–6.
52. Friberg LE, Ravva P, Karlsson MO, et al. Integrated population pharmacokinetic analysis of voriconazole in children, adolescents, and adults. *Antimicrob Agents Chemother* 2012;**56**: 3032–42.
67. Hayden R, Pounds S, Knapp K, et al. Galactomannan antigenemia in pediatric oncology patients with invasive aspergillosis. *Pediatr Infect Dis J* 2008;**27**:815–19.
73. Howard SJ, Cerar D, Anderson MJ, et al. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009;**15**:1068–76.
75. Jacobs F, Selleslag D, Aoun M, et al. An observational efficacy and safety analysis of the treatment of acute invasive aspergillosis using voriconazole. *Eur J Clin Microbiol Infect Dis* 2012;**31**:1173–9.
84. Karlsson MO, Lutsar I, Milligan PA. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.
91. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. *Clin Infect Dis* 2010;**50**:1091–100.
96. Lamoth F, Cruciani M, Mengoli C, et al. β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin Infect Dis* 2012;**54**: 633–43.
103. Lortholary O, Gangneux JP, Sitbon K, et al. Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005–2007). *Clin Microbiol Infect* 2011;**17**:1882–9.
104. Maertens J, Egerer G, Shin WS, et al. Caspofungin use in daily clinical practice for treatment of invasive aspergillosis: results of a prospective observational registry. *BMC Infect Dis* 2010;**10**: 182–9.
106. Maertens J, Maertens V, Theunissen K, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin Infect Dis* 2009;**49**:1688–93.
114a. Marr KA, Schlamm H, Rottinghaus ST, et al. A randomised, double-bind study of combination antifungal therapy with voriconazole and anidulafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis. *ECCMID* 2013; London.
125. Neofytos D, Fishman JA, Horn D, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. *Transplant Infectious Disease* 2010;**12**:220–9.
126. Neofytos D, Horn D, Anaissie E, et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. *Clin Infect Dis* 2009;**48**:265–73.
128. Nguyen MH, Leather H, Clancy CJ, et al. Galactomannan testing in bronchoalveolar lavage fluid facilitates the diagnosis of invasive pulmonary aspergillosis in patients with hematologic malignancies and stem cell transplant recipients. *Biol Blood Marrow Transplant* 2011;**17**:1043–50.
130. Nivoix Y, Velten M, Letscher-Bru V, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. *Clin Infect Dis* 2008;**47**:1176–84.
131. O'Gorman CM, Fuller HT, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* 2009;**457**:471–4.
134. Pagano L, Caira M, Candoni A, et al. Invasive aspergillosis in patients with acute myeloid leukemia: a SEIFEM-2008 registry study. *Haematologica* 2010;**95**:644–50.
136. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**:1101–11.
137. Pascual A, Csajka C, Buclin T, et al. Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis of adult patients with invasive fungal infections. *Clin Infect Dis* 2012;**55**: 381–90.
142. Pfaller MA, Boyken L, Hollis RJ, et al. In vitro susceptibility of clinical isolates of *Aspergillus* spp. to anidulafungin, caspofungin, and micafungin: a head-to-head comparison using the CLSI M38-A2 broth microdilution method. *J Clin Microbiol* 2009;**47**: 3323–5.
178. Thomas A, Korb V, Guillemain R, et al. Clinical outcomes of lung-transplant recipients treated by voriconazole and caspofungin combination in aspergillosis. *J Clin Pharm Ther* 2010;**35**: 49–53.
184. Van der Linden JW, Snelders E, Kampinga GA, et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. *Emerg Infect Dis* 2011;**17**:1846–54.
186. Verweij PE, Snelders E, Kema GH, et al. Azole resistance in *Aspergillus fumigatus:* a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009;**9**:789–95.
188. Vinh DC, Shea YR, Sugui JA, et al. Invasive aspergillosis due to *Neosartorya udagawae*. *Clin Infect Dis* 2009;**49**:102–11.
191. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
193. Walsh TJ, Driscoll T, Milligan PA, et al. Pharmacokinetics, safety, and tolerability of voriconazole in immunocompromised children. *Antimicrob Agents Chemother* 2010;**54**:4116–23.
204. Wingard JR, Ribaud P, Schlamm HT, et al. Changes in causes of death over time after treatment for invasive aspergillosis. *Cancer* 2008;**112**:2309–12.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Aceti A, Corvaglia L, Faldella G. Infant formulas thickened with carob bean gum causing false-positive galactomannan test reactivity. *Pediatr Infect Dis J* 2008;**27**:769.
2. Aisner J, Murillo J, Schimpff SC, et al. Invasive aspergillosis in acute leukemia: correlation with nose cultures and antibiotic use. *Ann Intern Med* 1979;**90**:4–9.
3. Alcazar-Fuoli L, Mellado E, Alastruey-Izquierdo A, et al. *Aspergillus* section *Fumigati:* antifungal susceptibility patterns and sequence-based identification. *Antimicrob Agents Chemother* 2008; **52**:1244–51.
4. Ali R, Ozkalemkas F, Ozcelik T, et al. Invasive pulmonary aspergillosis: role of early diagnosis and surgical treatment in patients with acute leukemia. *Ann Clin Microbiol Antimicrob* 2006;**5**:17.
5. Allen BT, Patton D, Ramsey NKC, et al. Pulmonary fungal infections after bone marrow transplantation. *Pediatr Radiol* 1988;**18**: 118–22.
6. Armenian SH, Nash KA, Kapoor N, et al. Prospective monitoring for invasive aspergillosis using galactomannan and polymerase chain reaction in high risk pediatric patients. *J Pediatr Hematol/ Oncol* 2009;**31**:920–6.
7. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002;**34**:7–14.
8. Aubry A, Porcher R, Bottero J, et al. Occurrence and kinetics of false-positive *Aspergillus* galactomannan test results following treatment with beta-lactam antibiotics in patients with hematological disorders. *J Clin Microbiol* 2006;**44**:389–94.
9. Baddley JW, Andes DR, Marr KA, et al. Factors associated with mortality in transplant patients with invasive aspergillosis. *Clin Infect Dis* 2010;**50**:1559–67.
10. Baddley JW, Stroud TP, Salzman D, et al. Invasive mold infections in allogeneic bone marrow transplant recipients. *Clin Infect Dis* 2001;**32**:1319–24.
11. Balajee SA, Gribskov JL, Hanley E, et al. *Aspergillus lentulus* sp. nov., a new sibling species of *A. fumigatus*. *Eukaryot Cell* 2005;**4**: 625–32.
12. Benjamin DKJ, Miller WC, Bayliff S, et al. Infections diagnosed in the first year after pediatric stem cell transplantation. *Pediatr Infect Dis J* 2002;**21**:227–34.
13. Bennett JE, Dismukes WE, Duma RJ, et al. A comparison of amphotericin B alone and combined with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med* 1979;**301**: 126–8.
14. Bhabhra R, Askew D. Thermotolerance and virulence of *Aspergillus fumigatus:* role of the fungal nucleolus. *Med Mycol* 2005;**43**(Suppl. 1):S87–93.
15. Blum U, Windfuhr M, Buitrago-Tellez C, et al. Invasive pulmonary aspergillosis MRI, CT, and plain radiographic findings and their contributions for early diagnosis. *Chest* 1994;**106**:1156–61.
16. Boutboul F, Alberti C, Leblanc T, et al. Invasive aspergillosis in allogeneic stem cell transplant recipients: increasing antigenemia is associated with progressive disease. *Clin Infect Dis* 2002;**34**: 939–43.
17. Bowman JC, Hicks S, Kurtz MB, et al. The antifungal echinocandin caspofungin acetate kills growing cells of *Aspergillus fumigatus* in vitro. *Antimicrob Agents Chemother* 2002;**46**:3001–12.
18. Brodoefel H, Vogel M, Hebart H, et al. Long-term CT follow-up in 40 non-HIV immunocompromised patients with invasive pulmonary aspergillosis: kinetics of CT morphology and correlation with clinical findings and outcome. *AJR Am J Roentgenol* 2006;**187**: 404–13.
19. Buchheidt D, Baust C, Skladny H, et al. Detection of *Aspergillus* species in blood and bronchoalveolar lavage samples from immunocompromised patients by means of 2-step polymerase chain reaction: clinical results. *Clin Infect Dis* 2001;**33**:428–35.
20. Buchheidt D, Baust C, Skladny H, et al. Clinical evaluation of a polymerase chain reaction assay to detect *Aspergillus* species in bronchoalveolar lavage samples of neutropenic patients. *Br J Haematol* 2002;**116**:803–11.
21. Bueid A, Howard SJ, Moore CB, et al. Azole antifungal resistance in *Aspergillus fumigatus:* 2008 and 2009. *J Antimicrob Chemother* 2010;**65**:2116–18.
22. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics* 2008;**121**:e1286–94.
23. Candoni A, Mestroni R, Damiani D, et al. Caspofungin as first line therapy of pulmonary invasive fungal infections in 32 immunocompromised patients with hematologic malignancies. *Eur J Haematol* 2005;**75**:227–33.
24. Caputo R. Itraconazole (Sporanox) in superficial and systemic fungal infections. *Expert Rev Anti Infect Ther* 2003;**1**:531–42.
25. Castagnola E, Furfaro E, Caviglia I, et al. Performance of the galactomannan antigen detection test in the diagnosis of invasive aspergillosis in children with cancer or undergoing haemopoietic stem cell transplantation. *Clin Microbiol Infect* 2010;**16**:1197–203.
26. Cesaro S, Visintin G. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2005;**40**:1073–6.
27. Cohen MS, Isturiz RE, Malech HL, et al. Fungal infection in chronic granulomatous disease: the importance of the phagocyte in defense against fungi. *Am J Med* 1981;**71**:59–66.
28. Connolly JE, McAdams HP, Erasmus JJ, et al. Opportunistic fungal pneumonia. *J Thorac Imaging* 1999;**14**:51–62.
29. Conway SP, Etherington C, Peckham DG, et al. Pharmacokinetics and safety of itraconazole in patients with cystic fibrosis. *J Antimicrob Chemother* 2004;**53**:841–7.
30. Cordonnier C, Botterel F, Ben Amor R, et al. Correlation between galactomannan antigen levels in serum and neutrophil counts in haematological patients with invasive aspergillosis. *Clin Microbiol Infect* 2009;**15**:81–6.
31. Cordonnier C, Ribaud P, Herbrecht R, et al. Prognostic factors for death due to invasive aspergillosis after hematopoietic stem cell transplantation: a 1-year retrospective study of consecutive patients at French transplantation centers. *Clin Infect Dis* 2006;**42**: 955–63.
32. Cornely OA, Maertens J, Bresnik M, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: A randomized trial comparing a high–loading dose regimen with standard dosing (AmBiLoad Trial). *Clin Infect Dis* 2007;**44**:1289–97.
33. Cornely OA, Vehreschild JJ, Vehreschild MJ, et al. Phase II dose escalation study of caspofungin for invasive aspergillosis. *Antimicrob Agents Chemother* 2011;**55**:5798–803.
34. Cornet M, Fleury L, Maslo C, et al. Epidemiology of invasive aspergillosis in France: a six-year multicenter survey in the greater Paris area. *J Hosp Infect* 2002;**51**:288–96.
35. Cornillet A, Camus C, Nimubona S, et al. Comparison of epidemiological, clinical, and biological features of invasive aspergillosis in neutropenic and nonneutropenic patients: a 6-year survey. *Clin Infect Dis* 2006;**43**:577–84.
36. D'Antonio D, Pagano L, Girmenia C, et al. Cutaneous aspergillosis in patients with haematological malignancies. *Eur J Clin Microbiol Infect Dis* 2000;**19**:362–5.
37. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**:1813–21.
38. Denning D. Invasive aspergillosis. *Clin Infect Dis* 1998;**26**:781–805.
39. Denning DW, Evans EG, Kibbler CC, et al. Guidelines for the investigation of invasive fungal infections in haematological malignancy and solid organ transplantation. British Society for Medical Mycology. *Eur J Clin Microbiol Infect Dis* 1997;**16**: 424–36.
40. Denning DW, Marinus A, Cohen J, et al. An EORTC multicentre prospective survey of invasive aspergillosis in haematological patients: diagnosis and therapeutic outcome. *J Infect* 1998;**37**: 173–80.
41. Denning DW, Marr KA, Lau WM, et al. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. *J Infect* 2006;**53**: 337–49.
42. Denning DW, Riniotis K, Dobrashian R, et al. Chronic cavitary and fibrosing pulmonary and pleural aspergillosis: case series, proposed nomenclature change, and review. *Clin Infect Dis* 2003; **37**(Suppl. 3):S265–80.

43. Denning DW, Venkateswarlu K, Oakley KL, et al. Itraconazole resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 1997;**41**:1364–8.
44. Desai R, Ross LA, Hoffman JA. The role of bronchoalveolar lavage galactomannan in the diagnosis of pediatric invasive aspergillosis. *Pediatr Infect Dis J* 2009;**28**:283–6.
45. Dismukes WE. Introduction to antifungal agents. *Clin Infect Dis* 2000;**30**:653–7.
46. Dixon DM, Polak A, Walsh TJ. Fungus dose-dependent primary pulmonary aspergillosis in immunosuppressed mice. *Infect Immun* 1989;**75**:1452–6.
47. Dotis J, Panagopoulou P, Filioti J, et al. Femoral osteomyelitis due to *Aspergillus nidulans* in a patient with chronic granulomatous disease. *Infection* 2003;**31**:121–4.
48. Dotis J, Roilides E. Osteomyelitis due to *Aspergillus* spp. in patients with chronic granulomatous disease: comparison of *Aspergillus nidulans* and *Aspergillus fumigatus*. *Int J Infect Dis* 2004;**8**:103–10.
49. Duong M, Ouellet N, Simard M, et al. Kinetic study of host defense and inflammatory response to *Aspergillus fumigatus* in steroid-induced immunosuppressed mice. *J Infect Dis* 1998;**178**: 1472–82.
50. Einsele H, Hebart H, Roller G, et al. Detection and identification of fungal pathogens in blood by using molecular probes. *J Clin Microbiol* 1997;**35**:1353–60.
51. Engelhard D. Bacterial and fungal infections in children undergoing bone marrow transplantation. *Bone Marrow Transplant* 1998; **21**:S78–80.
52. Friberg LE, Ravva P, Karlsson MO, et al. Integrated population pharmacokinetic analysis of voriconazole in children, adolescents, and adults. *Antimicrob Agents Chemother* 2012;**56**:3032–42.
53. Fukuda T, Boeckh M, Cater RA, et al. Risks and outcomes of invasive fungal infections in recipients of allogeneic hematopoietic stem cell transplants after nonmyeloablative conditioning. *Blood* 2003;**102**:827–33.
54. Galagan JE, Calvo SE, Cuomo C, et al. Sequencing of *Aspergillus nidulans* and comparative analysis with *A. fumigatus* and *A. oryzae*. *Nature* 2005;**438**:1105–15.
55. Gavalda J, Len O, San Juan R, et al. Risk factors for invasive aspergillosis in solid-organ transplant recipients: a case-control study. *Clin Infect Dis* 2005;**41**:52–9.
56. Gefter WB, Albelda SM, Talbot GH, et al. Invasive pulmonary aspergillosis and acute leukemia: limitations in the diagnostic utility of the air crescent sign. *Radiology* 1985;**157**:605–10.
57. Gerson SI, Talbot GH, Hurwitz S, et al. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann Intern Med* 1984;**100**:345–51.
58. Gillespie MB, O'Malley BW Jr, Francis HW. An approach to fulminant invasive fungal rhinosinusitis in the immunocompromised host. *Arch Otolaryngol Head Neck Surg* 1998;**124**:520–6.
59. Girmenia C, Nucci M, Martino P. Clinical significance of *Aspergillus* fungaemia in patients with haematological malignancies and invasive aspergillosis. *Br J Haematol* 2001;**114**:93–8.
60. Goodley J, Clayton YM, Hay RJ. Environmental sampling for aspergilli during building construction on a hospital site. *J Hosp Infect* 1994;**26**:27–35.
61. Graybill JR, Bocanegra R, Gonzalez GM, et al. Combination antifungal therapy of murine aspergillosis: liposomal amphotericin B and micafungin. *J Antimicrob Chemother* 2003;**52**:656–62.
62. Greene R. The radiological spectrum of pulmonary aspergillosis. *Med Mycol* 2005;**43**(Suppl. 1):S147–54.
63. Groll AH, Shah PM, Mentzel C, et al. Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. *J Infect* 1996;**33**:23–32.
64. Guo YL, Chen YQ, Wang K, et al. Accuracy of BAL galactomannan in diagnosing invasive aspergillosis: a bivariate metaanalysis and systematic review. *Chest* 2010;**138**:817–24.
65. Hachem R, Sumoza D, Hanna H, et al. Clinical and radiologic predictors of invasive pulmonary aspergillosis in cancer patients: should the European Organization for Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) criteria be revised? *Cancer* 2006;**106**:1581–6.
66. Hachem RY, Kontoyiannis DP, Boktour MR, et al. *Aspergillus terreus:* an emerging amphotericin B–resistant opportunistic mold in patients with hematologic malignancies. *Cancer* 2004;**101**: 1594–600.
67. Hayden R, Pounds S, Knapp K, et al. Galactomannan antigenemia in pediatric oncology patients with invasive aspergillosis. *Pediatr Infect Dis J* 2008;**27**:815–19.
68. Hebart H, Loffler J, Meisner C, et al. Early detection of *Aspergillus* infection after allogeneic stem cell transplantation by polymerase chain reaction screening. *J Infect Dis* 2000;**181**:1713–19.
69. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002;**347**:408–15.
70. Herbrecht R, Letscher-Bru V, Oprea C, et al. *Aspergillus* galactomannan detection in the diagnosis of invasive aspergillosis in cancer patients. *J Clin Oncol* 2002;**7**:1898–906.
71. Ho PL, Yuen KY. Aspergillosis in bone marrow transplant recipients. *Crit Rev Oncol/Hematol* 2000;**34**:55–69.
72. Hosseini-Yeganeh M, McLachlan AJ. Physiologically based pharmacokinetic model for terbinafine in rats and humans. *Antimicrob Agents Chemother* 2002;**46**:2219–28.
73. Howard SJ, Cerar D, Anderson MJ, et al. Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. *Emerg Infect Dis* 2009;**15**:1068–76.
74. Husain S, Kwak EJ, Obman A, et al. Prospective assessment of Platelia *Aspergillus* galactomannan antigen for the diagnosis of invasive aspergillosis in lung transplant recipients. *Am J Transplant* 2004;**4**:796–802.
75. Jacobs F, Selleslag D, Aoun M, et al. An observational efficacy and safety analysis of the treatment of acute invasive aspergillosis using voriconazole. *Eur J Clin Microbiol Infect Dis* 2012;**31**:1173–9.
76. Jantunen E, Ruutu P, Niskanen L, et al. Incidence and risk factors for invasive fungal infections in allogeneic BMT recipients. *Bone Marrow Transplant* 1997;**19**:801–8.
77. Jantunen E, Ruutu P, Piilonen A, et al. Treatment and outcome of invasive *Aspergillus* infections in allogeneic BMT recipients. *Bone Marrow Transplant* 2000;**26**:759–62.
78. Jantunen E, Volin L, Salonen O, et al. Central nervous system aspergillosis in allogeneic stem cell transplant recipients. *Bone Marrow Transplant* 2003;**31**:191–6.
79. Järv H, Lehtmaa J, Summerbell RC, et al. Isolation of *Neosartorya pseudofischeri* from blood: first hint of pulmonary aspergillosis. *J Clin Microbiol* 2004;**42**:925–8.
80. Jeffery GM, Beard ME, Ikram RB, et al. Intranasal amphotericin B reduces the frequency of invasive aspergillosis in neutropenic patients. *Am J Med* 1991;**90**:685–92.
81. Johnson LB, Kauffman CA. Voriconazole: a new triazole antifungal agent. *Clin Infect Dis* 2003;**36**:630–7.
82. Kami M, Fukui T, Ogawa S, et al. Use of real-time PCR on blood samples for diagnosis of invasive aspergillosis. *Clin Infect Dis* 2001; **33**:1504–12.
83. Kami M, Tanaka Y, Ogawa S, et al. The limitation of the circulating *Aspergillus* antigen detection methods for BMT recipients. *Bone Marrow Transplant* 1998;**22**:832–3.
84. Karlsson MO, Lutsar I, Milligan PA. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.
85. Kato T, Usami I, Morita H, et al. Chronic necrotizing pulmonary aspergillosis in pneumoconiosis: clinical and radiologic findings in 10 patients. *Chest* 2002;**121**:118–27.
86. Kawamura S, Maesaki S, Tomono K, et al. Clinical evaluation of 61 patients with pulmonary aspergilloma. *Intern Med* 2000;**39**: 209–12.
87. Kawazu M, Kanda Y, Nannya Y, et al. Prospective comparison of the diagnostic potential of real-time PCR, double-sandwich enzyme-linked immunosorbent assay for galactomannan, and a (1→3)-beta-D-glucan test in weekly screening for invasive aspergillosis in patients with hematological disorders. *J Clin Microbiol* 2004;**42**:2733–41.
88. Kim MJ, Lee KS, Kim J, et al. Crescent sign in invasive pulmonary aspergillosis: frequency and related CT and clinical factors. *J Comput Assist Tomogr* 2001;**25**:305–10.
89. Kirkpatrick WR, Perea S, Coco BJ, et al. Efficacy of caspofungin alone and in combination with voriconazole in a guinea pig model of invasive aspergillosis. *Antimicrob Agents Chemother* 2002;**46**: 2564–8.
90. Kontoyiannis DP, Bodey GP. Invasive aspergillosis in 2002: an update. *Eur J Clin Microbiol Infect Dis* 2002;**21**:161–72.
91. Kontoyiannis DP, Marr KA, Park BJ, et al. Prospective surveillance for invasive fungal infections in hematopoietic stem cell

transplant recipients, 2001–2006: overview of the Transplant-Associated Infection Surveillance Network (TRANSNET) database. *Clin Infect Dis* 2010;**50**:1091–100.
92. Kruger W, Russmann B, Kroger N, et al. Early infections in patients undergoing bone marrow or blood stem cell transplantation—a 7-year single centre investigation of 409 cases. *Bone Marrow Transplant* 1999;**23**:589–97.
93. Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. *Radiology* 1985;**157**:611–14.
94. Kurtz MB, Douglas CM. Lipopeptide inhibitors of fungal glucan synthase. *J Med Vet Mycol* 1997;**35**:79–86.
95. Kwak EJ, Husain S, Obman A, et al. Efficacy of galactomannan antigen in the Platelia *Aspergillus* enzyme immunoassay for diagnosis of invasive aspergillosis in liver transplant recipients. *J Clin Microbiol* 2004;**42**:435–8.
96. Lamoth F, Cruciani M, Mengoli C, et al. β-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin Infect Dis* 2012;**54**:633–43.
97. Langfelder K, Jahn B, Gehringer H, et al. Identification of a polyketide synthase gene (pksP) of *Aspergillus fumigatus* involved in conidial pigment biosynthesis and virulence. *Med Microbiol Immunol (Berlin)* 1998;**187**:79–89.
98. Lass-Florl C, Aigner J, Gunsilius E, et al. Screening for *Aspergillus* spp. using polymerase chain reaction of whole blood samples from patients with haematological malignancies. *Br J Haematol* 2001; **113**:180–4.
99. Lass-Florl C, Griff K, Mayr A, et al. Epidemiology and outcome of infections due to *Aspergillus terreus:* 10-year single centre experience. *Br J Haematol* 2005;**131**:201–7.
100. Latge JP. *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* 1999;**12**:310–50.
101. Lewis RE, Kontoyiannis DP. Rationale for combination antifungal therapy. *Pharmacotherapy* 2001;**21**:149S–64S.
102. Lin SJ, Schranz J, Teutsch SM. Aspergillosis case-fatality rate: systematic review of the literature. *Clin Infect Dis* 2001;**32**: 358–66.
103. Lortholary O, Gangneux JP, Sitbon K, et al. Epidemiological trends in invasive aspergillosis in France: the SAIF network (2005–2007). *Clin Microbiol Infect* 2011;**17**:1882–9.
104. Maertens J, Egerer G, Shin WS, et al. Caspofungin use in daily clinical practice for treatment of invasive aspergillosis: results of a prospective observational registry. *BMC Infect Dis* 2010;**10**: 182–9.
105. Maertens J, Raad I, Petrikkos G, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004;**39**:1563–71.
106. Maertens J, Maertens V, Theunissen K, et al. Bronchoalveolar lavage fluid galactomannan for the diagnosis of invasive pulmonary aspergillosis in patients with hematologic diseases. *Clin Infect Dis* 2009;**49**:1688–93.
107. Maertens J, Verhaegen J, Demuynck H, et al. Autopsy-controlled prospective evaluation of serial screening for circulating galactomannan by a sandwich enzyme-linked immunosorbent assay for hematological patients at risk for invasive aspergillosis. *J Clin Microbiol* 1999;**37**:3223–8.
108. Maertens J, Verhaegen J, Lagrou K, et al. Screening for circulating galactomannan as a noninvasive diagnostic tool for invasive aspergillosis in prolonged neutropenic patients and stem cell transplantation recipients: a prospective validation. *Blood* 2001;**97**: 1604–10.
109. Manchetti M, Feasi M, Mordini N, et al. Comparison of an enzyme immunoassay and a latex agglutination system for the diagnosis of invasive aspergillosis in bone marrow transplant recipients. *Bone Marrow Transplant* 1998;**21**:917–21.
110. Marr KA, Balajee SA, McLaughlin L, et al. Detection of galactomannan antigenemia by enzyme immunoassay for the diagnosis of invasive aspergillosis: variables that affect performance. *J Infect Dis* 2004;**190**:641–9.
111. Marr KA, Carter RA, Crippa F, et al. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:909–17.
112. Marr KA, Boeckh M, Carter RA, et al. Combination antifungal therapy for invasive aspergillosis. *Clin Infect Dis* 2004;**39**:797–802.
113. Marr KA, Laverdiere M, Gugel A, et al. Antifungal therapy decreases sensitivity of the *Aspergillus* galactomannan enzyme immunoassay. *Clin Infect Dis* 2005;**40**:1762–9.
114. Marr KA, Patterson T, Denning D. Aspergillosis: pathogenesis, clinical manifestations, and therapy. *Infect Dis Clin North Am* 2002;**16**:875–94, vi.
114a. Marr KA, Schlamm H, Rottinghaus ST, et al. A randomised, double-bind study of combination antifungal therapy with voriconazole and anidulafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis. *ECCMID* 2013; London.
115. Martino R, Subira M, Rovira M, et al. Invasive fungal infections after allogeneic peripheral blood stem cell transplantation: incidence and risk factors in 395 patients. *Br J Haematol* 2002;**116**: 475–82.
116. McWhinney PH, Kibbler CC, Hamon MD, et al. Progress in the diagnosis and management of aspergillosis in bone marrow transplantation: 13 years' experience. *Clin Infect Dis* 1993;**17**:397–404.
117. Mennink-Kersten MASH, Klont RR, Warris A, et al. Bifidobacterium lipoteichoic acid and false ELISA reactivity in *Aspergillus* antigen detection. *Lancet* 2004;**363**:325–7.
118. Meyers JD. Fungal infections in bone marrow transplant patients. *Semin Oncol* 1990;**3**:10–13.
119. Miaux Y, Ribaud P, Williams M, et al. MR of cerebral aspergillosis in patients who have had bone marrow transplantation. *Am J Neuroradiol* 1995;**16**:555–62.
120. Moskaluk CA, Pogrebniak HW, Pass HI, et al. Surgical pathology of the lung in chronic granulomatous disease. *Am J Clin Pathol* 1994;**102**:684–91.
121. Mosquera J, Sharp A, Moore CB, et al. In vitro interaction of terbinafine with itraconazole, fluconazole, amphotericin B and 5-flucytosine against *Aspergillus* spp. *J Antimicrobial Chemother* 2002;**50**:189–94.
122. Muller FM, Trusen A, Weig M. Clinical manifestations and diagnosis of invasive aspergillosis in immunocompromised children. *Eur J Pediatr* 2002;**161**:563–74.
123. Munoz P, Alcala L, Sanchez Conde M, et al. The isolation of *Aspergillus fumigatus* from respiratory tract specimens in heart transplant recipients is highly predictive of invasive aspergillosis. *Transplantation* 2003;**75**:326–9.
124. Myoken Y, Sugata T, Fujita Y, et al. Early diagnosis and successful management of atypical invasive *Aspergillus* sinusitis in a hematopoietic cell transplant patient: a case report. *J Oral Maxillofac Surg* 2006;**64**:860–3.
125. Neofytos D, Fishman JA, Horn D, et al. Epidemiology and outcome of invasive fungal infections in solid organ transplant recipients. *Transplant Infectious Disease* 2010;**12**:220–9.
126. Neofytos D, Horn D, Anaissie E, et al. Epidemiology and outcome of invasive fungal infection in adult hematopoietic stem cell transplant recipients: analysis of Multicenter Prospective Antifungal Therapy (PATH) Alliance registry. *Clin Infect Dis* 2009;**48**:265–73.
127. Ng TTC, Robson GD, Denning DW. Hydrocortisone-enhanced growth of *Aspergillus spp.:* implications for pathogenesis. *Microbiology* 1994;**140**:2475–9.
128. Nguyen MH, Leather H, Clancy CJ, et al. Galactomannan testing in bronchoalveolar lavage fluid facilitates the diagnosis of invasive pulmonary aspergillosis in patients with hematologic malignancies and stem cell transplant recipients. *Biol Blood Marrow Transplant* 2011;**17**:1043–50.
129. Ninin E, Milpied N, Moreau P, et al. Longitudinal study of bacterial, viral, and fungal infections in adult recipients of bone marrow transplants. *Clin Infect Dis* 2001;**33**:41–7.
130. Nivoix Y, Velten M, Letscher-Bru V, et al. Factors associated with overall and attributable mortality in invasive aspergillosis. *Clin Infect Dis* 2008;**47**:1176–84.
131. O'Gorman CM, Fuller HT, Dyer PS. Discovery of a sexual cycle in the opportunistic fungal pathogen *Aspergillus fumigatus*. *Nature* 2009;**457**:471–4.
132. Obayashi T, Yoshida M, Mori T, et al. Plasma (1–3)-β-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. *Lancet* 1995;**345**:17–20.

133. Okafuji T, Yabuuchi H, Nagatoshi Y, et al. CT and MR findings of brain aspergillosis. *Comput Med Imaging Graphics* 2003;**27**: 489–92.
134. Pagano L, Caira M, Candoni A, et al. Invasive aspergillosis in patients with acute myeloid leukemia: a SEIFEM-2008 registry study. *Haematologica* 2010;**95**:644–50.
135. Pannuti CS, Gingrich RD, Pfaller MA, et al. Nosocomial pneumonia in adult patients undergoing bone marrow transplantation: a 9-year study. *J Clin Oncol* 1991;**9**:77–84.
136. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**:1101–11.
137. Pascual A, Csajka C, Buclin T, et al. Challenging recommended oral and intravenous voriconazole doses for improved efficacy and safety: population pharmacokinetics-based analysis of adult patients with invasive fungal infections. *Clin Infect Dis* 2012;**55**: 381–90.
138. Patterson TF, Boucher HW, Herbrecht R, et al. Strategy of following voriconazole versus amphotericin B therapy with other licensed antifungal therapy for primary treatment of invasive aspergillosis: impact of other therapies on outcome. *Clin Infect Dis* 2005;**41**:1448–52.
139. Pazos C, Ponton J, Del Palacio A. Contribution of (1→3)-beta-D-glucan chromogenic assay to diagnosis and therapeutic monitoring of invasive aspergillosis in neutropenic adult patients: a comparison with serial screening for circulating galactomannan. *J Clin Microbiol* 2005;**43**:299–305.
140. Perea S, Gonzalez G, Fothergill A, et al. In vitro interaction of caspofungin acetate with voriconazole against clinical isolates of *Aspergillus* spp. *Antimicrob Agents Chemother* 2002;**4**:3039–41.
141. Perfect JR, Cox GM, Lee JY, et al. The impact of culture isolation of *Aspergillus* species: a hospital-based survey of aspergillosis. *Clin Infect Dis* 2001;**33**:1824–33.
142. Pfaller MA, Boyken L, Hollis RJ, et al. In vitro susceptibility of clinical isolates of *Aspergillus* spp. to anidulafungin, caspofungin, and micafungin: a head-to-head comparison using the CLSI M38-A2 broth microdilution method. *J Clin Microbiol* 2009;**47**: 3323–5.
143. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of invasive aspergillosis using a galactomannan assay: a meta-analysis. *Clin Infect Dis* 2006; **42**:1417–27.
144. Philippe B, Ibrahim-Granet O, Prevost MC, et al. Killing of *Aspergillus fumigatus* by alveolar macrophages is mediated by reactive oxidant intermediates. *Infect Immun* 2003;**71**:3034–42.
145. Raad I, Hanna H, Huaringa A, et al. Diagnosis of invasive pulmonary aspergillosis using polymerase chain reaction-based detection of *Aspergillus* in BAL. *Chest* 2002;**121**:1171–6.
146. Raad I, Hanna H, Sumoza D, et al. Polymerase chain reaction on blood for the diagnosis of invasive pulmonary aspergillosis in cancer patients. *Cancer* 2002;**94**:1032–6.
147. Reichenberger F, Habicht J, Matt P, et al. Diagnostic yield of bronchoscopy in histologically proven invasive pulmonary aspergillosis. *Bone Marrow Transplant* 1999;**24**:1195–9.
148. Rhame FS, Streifel AJ, Kersey JHJ, et al. Extrinsic risk factors for pneumonia in the patient at high risk of infection. *Am J Med* 1984;**76**:42–52.
149. Ribaud P, Chastang C, Latge J-P, et al. Survival and prognostic factors of invasive aspergillosis after allogeneic bone marrow transplantation. *Clin Infect Dis* 1999;**28**:322–30.
150. Richardson MD. Changing patterns and trends in systemic fungal infections. *J Antimicrob Chemother* 2005;**56**(Suppl. 1):i5–11.
151. Rocha EM, Garcia-Effron G, Park S, et al. A Ser678Pro substitution in Fks1p confers resistance to echinocandin drugs in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2007;**51**: 4174–6.
152. Ruutu P, Valtonen V, Elonen E, et al. Invasive pulmonary aspergillosis: a diagnostic and therapeutic problem. Clinical experience with eight haematologic patients. *Scand J Infect Dis* 1987;**19**: 569–75.
153. Sabo JA, Abdel-Rahman SM. Voriconazole: a new triazole antifungal. *Ann Pharmacother* 2000;**34**:1032–43.
154. Samson RA, Hong S-B, Frisvad JC. Old and new concepts of species differentiation in *Aspergillus*. *Med Mycol* 2006;**44**(Suppl. 1):S133–48.
155. Sarfati J, Monod M, Recco P, et al. Recombinant antigens as diagnostic markers for aspergillosis. *Diagn Microbiol Infect Dis* 2006;**55**:279–91.
156. Saugier-Veber P, Devergie A, Sulahian A, et al. Epidemiology and diagnosis of invasive pulmonary aspergillosis in bone marrow transplant patients: results of a 5 year retrospective study. *Bone Marrow Transplant* 1993;**12**:121–4.
157. Schaffner A. Macrophage-*Aspergillus* interactions. *Immunol Ser* 1994;**60**:545–52.
158. Schaffner A, Douglas H, Braude A. Selective protection against conidia by mononuclear and against mycelia by polymorphonuclear phagocytes in resistance to *Aspergillus:* observations on these two lines of defense in vivo and in vitro with human and mouse phagocytes. *J Clin Invest* 1982;**69**:617–31.
159. Schell WA. New aspects of emerging fungal pathogens: a multifaceted challenge. *Clin Lab Med* 1995;**15**:365–87.
160. Schwartz RS, Mackintosh FR, Schrier SL, et al. Multivariate analysis of factors associated with invasive fungal disease during remission induction therapy for acute myelogenous leukemia. *Cancer* 1984;**53**:411–19.
161. Segal BH, DeCarlo ES, Kwon-Chung KJ, et al. *Aspergillus nidulans* infection in chronic granulomatous disease. *Medicine* 1998;**77**: 345–54.
162. Sevilla J, Hernandez-Maraver D, Aguado MJ, et al. Autologous peripheral blood stem cell transplant in patients previously diagnosed with invasive aspergillosis. *Ann Hematol* 2001;**80**:456–9.
163. Skov M, Høiby N, Koch C. Itraconazole treatment of allergic bronchopulmonary aspergillosis in patients with cystic fibrosis. *Allergy* 2002;**57**:723–8.
164. Smith J, Safdar N, Knasinski V, et al. Voriconazole therapeutic drug monitoring. *Antimicrobial Agents Chemother* 2006;**50**:1570–2.
165. Steinbach WJ, Addison RM, McLaughlin L, et al. Prospective *Aspergillus* galactomannan antigen testing in pediatric hematopoietic stem cell transplant recipients. *Pediatr Infect Dis J* 2007;**26**: 558–64.
166. Steinbach WJ, Benjamin DK JR, Kontoyiannis DP, et al. Infections due to *Aspergillus terreus:* a multicenter retrospective analysis of 83 cases. *Clin Infect Dis* 2004;**39**:192–8.
167. Steinbach WJ, Perfect JR, Schell WA, et al. In vitro analyses, animal models, and 60 clinical cases of invasive *Aspergillus terreus* infection. *Antimicrob Agents Chemother* 2004;**48**:3217–25.
168. Steinbach WJ, Stevens DA. Review of newer antifungal and immunomodulatory strategies for invasive aspergillosis. *Clin Infect Dis* 2003;**37**(Suppl. 3):S157–87.
169. Steinbach WJ, Stevens DA, Denning DW. Combination and sequential antifungal therapy for invasive aspergillosis: review of published in vitro and in vivo interactions and 6281 clinical cases from 1966–2001. *Clin Infect Dis* 2003;**37**(Suppl. 3):S188–224.
170. Stevens DA, Kan VL, Judson MA, et al. Practice guidelines for diseases caused by *Aspergillus.* Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:696–709.
171. Stevens DA, Moss RB, Kurup VP, et al. Allergic bronchopulmonary aspergillosis in cystic fibrosis—state of the art: Cystic Fibrosis Foundation Consensus Conference. *Clin Infect Dis* 2003;**37**(Suppl. 3):S225–64.
172. Stevens DA, Schwartz HJ, Lee JY, et al. A randomized trial of itraconazole in allergic bronchopulmonary aspergillosis. *N Engl J Med* 2000;**342**:756–62.
173. Stynen D, Goris A, Sarfati J, et al. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. *J Clin Microbiol* 1995;**33**:497–500.
174. Sulahian A, Boutboul F, Ribaud P, et al. Value of antigen detection using an enzyme immunoassay in the diagnosis and prediction of invasive aspergillosis in two adult and pediatric hematology units during a 4-year prospective study. *Cancer* 2001;**91**:311–18.
175. Sulahian A, Tabouret M, Ribaud P, et al. Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of invasive aspergillosis. *Eur J Clin Microbiol Infect Dis* 1996;**15**:139–45.
176. Sutton DA, Sanchie SE, Revankar SG, et al. In vitro amphotericin B resistance in clinical isolates of *Aspergillus terreus*, with a head-to-head comparison to voriconazole. *J Clin Microbiol* 1999;**37**: 2343–5.

177. Taccone A, Occhi M, Garaventa A, et al. CT of invasive pulmonary aspergillosis in children with cancer. *Pediatr Radiol* 1993;**23**: 177–80.
178. Thomas A, Korb V, Guillemain R, et al. Clinical outcomes of lung-transplant recipients treated by voriconazole and caspofungin combination in aspergillosis. *J Clin Pharm Ther* 2010;**35**: 49–53.
179. Thomas KE, Owens CM, Veys PA, et al. The radiological spectrum of invasive aspergillosis in children: a 10-year review. *Pediatr Radiol* 2003;**33**:453–60.
180. Tomee JF, van der Werf TS. Pulmonary aspergillosis. *Neth J Med* 2001;**59**:244–58.
181. Treger TR, Visscher DW, Bartlett MS, et al. Diagnosis of pulmonary infection caused by *Aspergillus:* usefulness of respiratory cultures. *J Infect Dis* 1985;**152**:572–6.
182. Trenschel R, Ditschkowski M, Elmaagacli AH, et al. Caspofungin as second-line therapy for fever of unknown origin or invasive fungal infection following allogeneic stem cell transplantation. *Bone Marrow Transplant* 2005;**35**:583–6.
183. Van Burik JH, Colven R, Spach DH. Cutaneous aspergillosis. *J Clin Microbiol* 1998;**36**:3115–21.
184. Van der Linden JW, Snelders E, Kampinga GA, et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. *Emerg Infect Dis* 2011;**17**:1846–54.
185. Verweij PE, Erjavec Z, Sluiters W, et al. Detection of antigen in sera of patients with invasive aspergillosis: intra- and interlaboratory reproducibility. *J Clin Microbiol* 1998;**36**:1612–16.
186. Verweij PE, Snelders E, Kema GH, et al. Azole resistance in *Aspergillus fumigatus:* a side-effect of environmental fungicide use? *Lancet Infect Dis* 2009;**9**:789–95.
187. Verweij PE, Weemaes CM, Curfs JHAJ, et al. Failure to detect circulating *Aspergillus* markers in a patient with chronic granulomatous disease and invasive aspergillosis. *J Clin Microbiol* 2000;**38**: 3900–1.
188. Vinh DC, Shea YR, Sugui JA, et al. Invasive aspergillosis due to *Neosartorya udagawae*. *Clin Infect Dis* 2009;**49**:102–11.
189. Viscoli C. Combination therapy for invasive aspergillosis. *Clin Infect Dis* 2004;**39**:803–5.
190. Wald A, Leisenring W, van Burik JAH, et al. Epidemiology of *Aspergillus* infection in a large cohort of patients undergoing bone marrow transplantation. *J Infect Dis* 1997;**175**:1459–66.
191. Walsh TJ, Anaissie EJ, Denning DW, et al. Treatment of aspergillosis: clinical practice guidelines of the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:327–60.
192. Walsh TJ, Dixon DM. Nosocomial aspergillosis: environmental microbiology, hospital epidemiology, diagnosis, and treatment. *Eur J Epidemiol* 1989;**5**:131–42.
193. Walsh TJ, Driscoll T, Milligan PA, et al. Pharmacokinetics, safety, and tolerability of voriconazole in immunocompromised children. *Antimicrob Agents Chemother* 2010;**54**:4116–23.
194. Walsh TJ, Karlsson MO, Driscoll T, et al. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. *Antimicrob Agents Chemother* 2004; **48**:2166–72.
195. Walsh TJ, Lutsar I, Driscoll T, et al. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *Pediatr Infect Dis J* 2002;**21**:240–8.
196. Walsh TJ, Petraitis V, Petraitiene R, et al. Experimental pulmonary aspergillosis due to *Aspergillus terreus:* pathogenesis and treatment of an emerging fungal pathogen resistant to amphotericin B. *J Infect Dis* 2002;**188**:305–19.
197. Walsh TJ, Raad I, Patterson TF, et al. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: an externally controlled trial. *Clin Infect Dis* 2007;**44**:2–12.
198. Walsh TJ, Viviani MA, Arathoon E, et al. New targets and delivery systems for antifungal therapy. *Med Mycol* 2000;**38**(Suppl. 1): 335–47.
199. Watsky KL, Eisen RN, Bolognia JL. Unilateral cutaneous emboli of *Aspergillus*. *Arch Dermatol* 1990;**126**:1214–17.
200. Wilhelm L, McLeary MS, Janner D. MR diagnosis of pulmonary and chest wall aspergillosis as an initial presentation of chronic granulomatous disease in a 7-month-old male. *Pediatr Radiol* 2000;**30**:719–20.
201. Williamson ECM, Leeming JP, Palmer HM, et al. Diagnosis of invasive aspergillosis in bone marrow transplant recipients by polymerase chain reaction. *Br J Haematol* 2000;**108**:132–9.
202. Wilson D, Mubatanhema W, Jurjevic Z. Biology and ecology of mycotoxigenic *Aspergillus* species as related to economic and health concerns. *Adv Exp Med Biol* 2002;**504**:3–17.
203. Wingard JR, Beals SU, Santos GW, et al. *Aspergillus* infections in bone marrow transplant recipients. *Bone Marrow Transplant* 1987;**2**:175–81.
204. Wingard JR, Ribaud P, Schlamm HT, et al. Changes in causes of death over time after treatment for invasive aspergillosis. *Cancer* 2008;**112**:2309–12.
205. Won HJ, Lee KS, Cheon J-E, et al. Invasive pulmonary aspergillosis: prediction at thin-section CT in patients with neutropenia—a prospective study. *Radiology* 1998;**208**:777–82.
206. Yuen KY, Woo PCY, Ip MSM, et al. Stage-specific manifestations of mold infections in bone marrow transplant recipients: risk factors and clinical significance of positive concentrated smears. *Clin Infect Dis* 1997;**25**:37–42.
207. Zaoutis TE, Heydon K, Chu JH, et al. Epidemiology, outcomes, and costs of invasive aspergillosis in immunocompromised children in the United States, 2000. *Pediatrics* 2006;**117**:e711–16.

CHAPTER 198

Blastomycosis

Gregory M. Gauthier • Bruce S. Klein

Blastomyces dermatitidis, the etiologic agent of blastomycosis, belongs to a group of ascomycete fungi that exhibit thermal dimorphism. These pathogens include *Histoplasma capsulatum*, *Coccidioides immitis*, *Coccidioides posadasii*, *Paracoccidioides brasiliensis*, *Sporothrix schenckii*, and *Penicillium marneffei*. In the soil (at 22° C to 25° C), these fungi grow as filamentous mold that produce infectious conidia (asexual spores). After disruption of soil by activities such as construction, conidia and mold fragments that are aerosolized and inhaled into the lungs of the host (37° C) convert into pathogenic yeast. In the yeast form, *B. dermatitidis* can evade immune defenses by impairing the host's cytokine response. In 25 to 40 percent of patients, infection disseminates to other organs such as the skin or bone. Diagnosis requires a high degree of clinical suspicion coupled with the use of culture and nonculture diagnostic tests. Treatment recommendations, which were updated in 2008 by the Infectious Diseases Society of America, include the use of polyene or azole antifungal agents. Echinocandins are not effective. Selection of anti-infective therapy is influenced by disease severity, site of infection, and immunosuppression.

HISTORICAL PERSPECTIVE

Blastomycosis was first described as "protozoan dermatitis" by Thomas Casper Gilchrist at the 1894 American Dermatological Association meeting in Washington, D.C.[49] Two years later, Dr. Gilchrist published a detailed histologic analysis of the cutaneous findings in a manuscript titled "A case of blastomycetic dermatitis in man."[50] In 1896 and 1898, Drs. Gilchrist and W.R. Stokes described a second case of blastomycetic dermatitis and named the pathologic agent as *Blastomyces dermatitidis*.[51,52] Nearly a decade later in 1907, Walter W. Hamburger analyzed four clinical isolates and described the growth of *B. dermatitidis* as mold at room temperature and as budding yeast at 37° C.[60] During this time, heightened awareness of the disease resulted in the publication of several case reports and case series describing the clinical characteristics of blastomycosis, including invasive disease.[95,121] The majority of the clinical cases were from the Chicago area, and the disease became known as "Chicago disease" or "Gilchrist disease." In these early publications, two distinct forms of the disease were recognized: cutaneous and systemic.[95,121] In the early 1950s, Drs. Jan Schwarz and Gerald L. Baum performed a detailed clinicopathologic analysis of patients with blastomycosis and established that the primary portal of entry for *B. dermatitidis* was pulmonary and most cutaneous lesions were the result of dissemination from the lungs.[118] In the mid 1980s *B. dermatitidis* caused an outbreak in children and was successfully isolated from the soil.[74] In 2009, the genome of *B. dermatitidis* (strain SLH14081) was sequenced and annotated by the Broad Institute.[18] Genome sequencing of additional *B. dermatitidis* strains along with deep RNA sequencing to characterize the transcriptome is ongoing.

MYCOLOGY

Blastomyces dermatitidis, the etiologic agent of blastomycosis, is a haploid ascomycete fungus that exhibits thermal dimorphism by growing as yeast at 37° C and as mold at 22° to 25° C (Fig. 198-1). *B. dermatitidis* yeast forms (8 to 20 μM) are characterized by a broad-based bud (4 to 5 μM) and doubly refractile cell wall.[136] This appearance is unique among the dimorphic fungi; however, giant yeast forms (28 to 40 μM) have been reported and can potentially be confused with *Coccidioides*.[137] At environmental temperature, *B. dermatitidis* grows as septate hyphae (1 to 2 μM diameter) capable of producing conidia (asexual spores).[135,136] Conidia are small (4 to 5 μM) and are oval or pyriform. Individual conidia are attached to hyphae by a short conidiophore and have the appearance of a lollipop.[135,136] Macroscopically, yeast colonies appear off-white and colonies can have a smooth or rough appearance. Mold colonies initially appear off-white or tan.[136] The morphologic characteristics for *B. dermatitidis* mold are not pathognomonic, and definitive identification requires molecular confirmation or conversion to yeast at 37° C. The conversion between mold and yeast is reversible and can be induced in the laboratory by shifting the temperature between 22° C to 25° C and 37° C.

B. dermatitidis is considered the asexual or anamorph form. In contrast, the sexual or teleomorph form is known as *Ajellomyces dermatitidis*.[90,91] In the sexual stage, *A. dermatitidis* exhibits heterothallic mating, which means that opposite mating types (+ and –) exchange genetic material to produce progeny. During mating, *A. dermatitidis* produces cleistothecia with coiled spirals that can give rise to spores known as asci. Individual asci are capable of forming hyphae with conidia (at 25° C) or converting to virulent yeast when inoculated into mice (at 37° C).[90,91] Both sexual and asexual spores, as well as mating types (+ and –), are capable of causing infection.[88,89]

The genomes of clinical and environmental *B. dermatitidis* isolates have been sequenced, annotated, and made available to the scientific community.[18] The size of the *B.*

dermatitidis genome ranges from 66 to 75 megabases (Mb) and contains 9,522 to 10,104 genes.[18] Seventeen to 19 percent of the genome is composed of coding sequences.[18]

ECOLOGY AND EPIDEMIOLOGY

B. dermatitidis is endemic to North America and is geographically restricted to the midwestern, south-central, and southeastern regions of the United States and several Canadian provinces (Saskatchewan, Manitoba, Ontario, Quebec) (Fig. 198-2A). In endemic regions, *B. dermatitidis* is not uniformly distributed but grows in an ecologic niche characterized by forested, sandy soils of acidic pH that are located near water and contain decaying vegetation or organic matter.[74] Similar to *H. capsulatum*, a related dimorphic fungus, *B. dermatitidis* can grow in bird and animal excreta. Knowledge about the niche and conditions that promote growth of the mold form has been gleaned mostly from analysis of outbreaks of blastomycosis (Table 198-1). Occupational and recreational activities associated with infection involve disruption of soil and include construction of homes or roads, boating and canoeing, fishing, exploration of beaver dams and underground forts, and use of a community compost pile (see Table 198-1).

Outside North America, blastomycosis is a rare cause of deep fungal infection, but autochthonous cases of culture-proven infections have been reported from India and 18 countries in Africa (see Fig. 198-2).[5,87,124] *B. dermatitidis* is not considered endemic to Central America, South America, Europe, Asia, or Australia. In the developing world, the ecologic niche inhabited by *B. dermatitidis* has not been identified. *B. dermatitidis* has never been isolated from soil outside North America, nor have there been human or veterinarian outbreaks to help define the geographic distribution of this pathogen in Africa or India. When compared with North American strains, African *B. dermatitidis* isolates display unique features, including decreased ability to convert to yeast after a shift in temperature from 25°C to 37°C, unique media requirements for maintenance of yeast growth in vitro, and a less antigenically complex cell surface.[68]

Our understanding of the epidemiology of blastomycosis in North America is based on retrospective studies and passive surveillance. In the endemic zone, seven states (Wisconsin, Minnesota, Michigan, Missouri, Arkansas, Louisiana, and Mississippi) and two provinces (Ontario, Manitoba) mandate reporting of new cases of *B. dermatitidis* infection. In North America, the annual incidence of blastomycosis ranges from 0.2 to 1.94 cases per 100,000 persons (Table 198-2). Several geographic areas are considered to be hyperendemic: Kenora, Ontario (117.2 human cases/100,000 population), Vilas County, Wisconsin (40.4/100,000), Eagle River, Wisconsin (101.3/100,000), Washington Parish, Louisiana (6.8/100,000), and central and south-central Mississippi (> 5/100,000) (see Table 198-2).

The true incidence of blastomycosis is likely much greater than the reported numbers. Reliable skin and serologic tests are not available, and about 50 percent of infected persons develop subclinical or asymptomatic illness.[74] Thus, epidemiologic analysis is limited to patients with clinically apparent infection that is recognized, diagnosed, and reported to public health agencies when required by law. Most epidemiologic studies show a slight male predominance for patients diagnosed with blastomycosis (see Table 198-2), which is also observed for coccidioidomycosis and histoplasmosis. Although most patients with clinical disease have normal immune function, several medical conditions are associated with the development of severe clinical disease, including immunosuppression due to solid-organ transplantation, the acquired immunodeficiency syndrome (AIDS), and

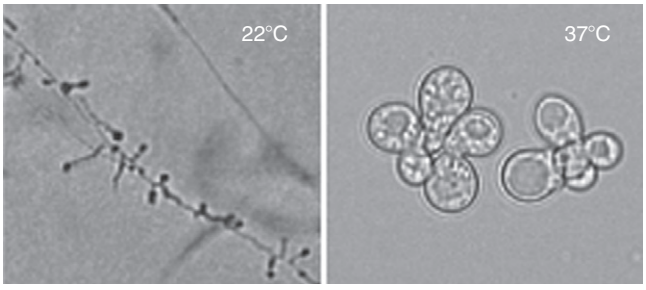

FIGURE 198-1 ■ Mold (22°C) and yeast (37°C) forms of *B. dermatitidis.*

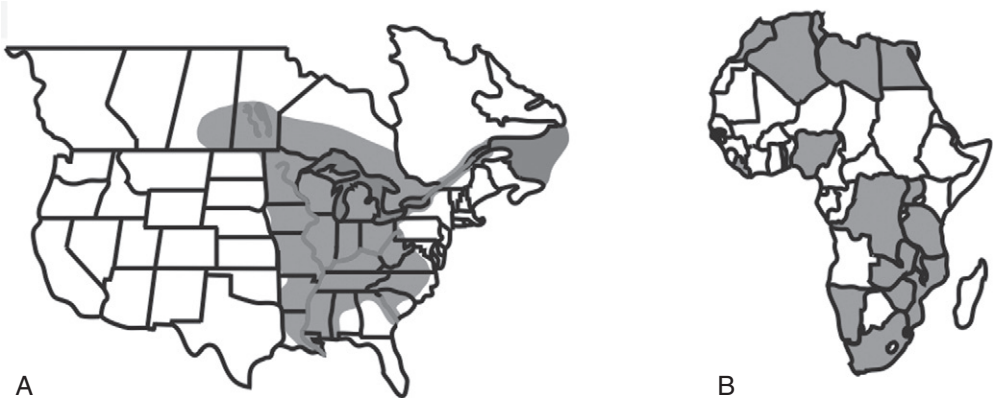

FIGURE 198-2 ■ Endemic regions of blastomycosis in **(A)** North America and **(B)** Africa.

TABLE 198-1 **Outbreaks of Human Blastomycosis in North America**

State	Year	No. Cases	Source of Outbreak	Reference
North Carolina	1953–1954	11	Not identified	119
Minnesota	1972	12	Cabin construction	127
North Carolina	1975	5	Peanut farm harvest	23
Illinois	1974–1975	5	Apartment complex construction	67
Wisconsin	1979	8	Canoeing on Namekegon River	27
Wisconsin	1984	48	Abandoned beaver lodge	74
Virginia	1984	4	Raccoon hunting	4
Wisconsin	1985	14	Underground timber fort; fishing	72
Wisconsin	1988	32	Hotel construction near Eagle River	11
Tennessee	1989	3	Construction at a rayon factory	44
Wisconsin	1989–1990	8	Not identified	107
Colorado	1998	2	Prairie dog relocation	31
Wisconsin	1998–2000	9	Likely related to construction/excavation	12
North Carolina	2001–2002	8	Likely related to construction projects	82
Wisconsin	2006	21	Community yard waste site	104

TABLE 198-2 **Incidence of Human Blastomycosis in the United States and Canada**

State or Province	Years Studied	Annual Incidence /100,000	Ratio (M:F)	Immune Suppression	Blastomycosis Attributable Mortality	Reference
Wisconsin	1973–1982	0.48	1.8:1	NR	NR	70
	1986–1995	1.4	1.5:1	NR	4.3%	24
	1984–1990	40.4 for Vilas County; 101.3 for Eagle River area	1.6:1	6.7%	2.7%	10
Illinois	1981–1989	1.94*	2.2:1	NR	NR	83
Indiana	2003–2008	0.65†	1.8:1	23.7%	1.9%	21
Kentucky	1960–1965	0.44	NR	NR	NR	45
Tennessee	1980–1995	0.77‡	2.3:1	12.5%	16.7%	129
	1996–2005	1.23‡	2.5:1	19.7%	14.8%	63
Missouri	1992–1999	0.2	2.1:1	14.8%	22%	20
Arkansas	1960–1965	0.43	NR	NR	NR	92
Mississippi	1963–1967	0.61	NR	NR	NR	45
	1979–1988	1.3	1.7:1	17.2%	9.6%	26
Louisiana	1976–1985	0.23 (6.8§)	1.1:1	NR	16.6%§	80
Ontario	1997–1999	117.2 for Kenora	1.1:1	6.5%	3.3%	37
	1994–2003	0.30	1.9:1	NR	NR	96
Manitoba	1988–1999	0.62	1.9:1	1.4%	6.3%	30

*Data collected from Rockford, Illinois.
†Data collected from Indianapolis, Indiana.
‡Data collected from northeastern Tennessee; incidence in Washington County is 3.01 to 3.5/100,000.
§Data collected from Washington Parish, Louisiana.
NR, not reported.

treatment with medications that block the activity of tumor necrosis factor–α (TNF-α). Most clinically recognized cases of blastomycosis are sporadic; however, 15 well-defined outbreaks have been reported in North America (see Table 198-1).

In the developing world, blastomycosis is found mainly in Africa and India.[5,124] In Africa, nearly 100 cases have been described from countries north and south of the equator [5,22,43] (see Fig. 198-2B). Only a few cases have been identified from western Africa and none from the island of Madagascar. This contrasts to *H. capsulatum* var. *duboisii*, which is endemic to western and central Africa as well as Madagascar. In India, fewer than a dozen cases have been reported. Limitations in public health infrastructure and diagnostic capabilities likely contribute to underestimation of the true incidence of disease. The clinical presentation of blastomycosis often mimics that of other pulmonary and cutaneous infections. Physicians in North America and the developing world confront a similar challenge in considering blastomycosis in the differential diagnosis, which is important for using special stains and culture media for identification.

B. dermatitidis causes disease in children with normal and impaired immune defenses. Blastomycosis is uncommon in children, with an estimated 2 to 13 percent of cases occurring in the pediatric population.* Few studies have focused solely on the pediatric population.[39,106,117,120] In these studies, the average age was 9.1 to 11.5 years, with a range of 18 days to 18 years old, and gender distribution usually had a slight male predominance.[39,77,106,117,120] Perinatal blastomycosis is rare, with only a few reported cases.[30,86,133,140] In two well-described cases, the mothers developed disseminated blastomycosis (during the first and third trimesters) and the neonates were admitted to the hospital for medical care at 18 and 21 days of life.[86,133,140] Both neonates died of respiratory failure from severe pulmonary blastomycosis.[86,133] Similar to the neonate population, blastomycosis in infants (1 to 12 months of age) is exceedingly uncommon.[30,39,92,96]

Although the majority of cases of blastomycosis are sporadic, outbreaks are common and often result in clinical disease in children. Of the 15 reported outbreaks (see Table 198-1), 12 involved pediatric patients. Collectively, the literature on pediatric blastomycosis suggests that risk for infection is due to environmental exposure and is not influenced significantly by age or gender.[128]

PATHOGENESIS

The ability to convert from mold to yeast is an essential event in the pathogenesis of *B. dermatitidis* and other dimorphic fungi. In the soil (at 22°C to 25°C), *B. dermatitidis* grows as a mold that produces infectious conidia. After disruption of soil, aerosolized conidia and mold fragments inhaled into the lungs of a human host (at 37°C) convert into pathogenic yeast.[46] Temperature is the primary environmental stimulus that promotes this morphologic switch. The phase transition is a complex event that involves global changes in transcription, metabolism, cell signaling, cell wall composition, and plasma membrane lipid content.[46]

Over the past 2 decades, the molecular tools available to genetically manipulate the dimorphic fungi have expanded and enabled the discovery of several *B. dermatitidis* genes that impact the phase transition, including *DRK1* (dimorphism regulating kinase-1) and *SREB* (siderophore biosynthesis repressor in *Blastomyces*). *DRK1* encodes a histidine kinase that regulates the conversion of mold to yeast, production of conidia, cell wall carbohydrate composition, and expression of BAD-1 (*Blastomyces* adhesion-1; formerly WI-1).[97] Genetic deletion of *DRK1* in *B. dermatitidis* and *H. capsulatum* resulted in hyphal growth at 37°C (and 22°C), reduced transcription of the essential virulence factor BAD-1, failure to produce conidia at 22°C, and decreased cell wall glucan.[97] Moreover, *B. dermatitidis* and *H. capsulatum* with reduced *DRK1* transcript were avirulent in a murine model of infection. These studies offered genetic proof that the conversion from mold to yeast is required for virulence of the dimorphic fungi. *SREB* encodes a GATA transcription factor that promotes the conversion from yeast to mold after a drop in temperature to 22°C and regulates genes involved with the biosynthesis and uptake of specialized iron-gathering molecules known as siderophores.[48] *SREB* null mutants fail to complete the phase transition to mold at 22°C and are unable to properly maintain iron homeostasis.[48]

B. dermatitidis is considered a primary fungal pathogen because it causes disease in persons with intact or impaired immune defenses. Features of this pathogen that promote its growth in human tissue include its ability to survive core human body temperature (37°C), convert to budding yeast, express yeast-phase–specific virulence factors, and evade killing by host immune cells.[16,17,41,42,97] In the yeast phase, *B. dermatitidis* upregulates the expression of an essential virulence factor, BAD-1, which encodes a 120-kDa protein that is secreted extracellularly and binds back to chitin on the fungal cell wall.[16,41,42] Bound and soluble BAD-1 bind macrophage receptors (CD14, CR3) to induce downregulation of TNF-α in transforming growth factor–β (TGF-β)–dependent and –independent manner, respectively.[16,41,42] TNF-α is a critical cytokine for host defense against *B. dermatitidis;* antibody-mediated neutralization of TNF-α results in progressive pulmonary infection in animal models.[42] Deletion of BAD-1 renders *B. dermatitidis* nonpathogenic in a murine model of pulmonary infection, indicating this yeast-phase–specific virulence factor is essential for virulence.[17] BAD-1 also facilitates adhesion of yeast to lung tissue,[17] binds exogenous calcium,[15] and impedes binding of complement (C3) to the yeast cell surface.[142]

Changes in cell wall carbohydrate composition associated with the phase transition may also contribute to virulence and immune evasion. During the conversion from mold to yeast, the amount of α-(1,3)-glucan increases and that of β-(1,3)-glucan decreases. *B. dermatitidis* mutants deficient in α-(1,3)-glucan have impaired virulence in a murine model of infection.[61] Similarly, reduction or complete loss of α-(1,3)-glucan synthase activity in *H. capsulatum* renders this dimorphic fungal pathogen avirulent.[110] The concentration of cell wall β-(1,3)-glucan drops from 40 to 50 percent in mycelia to less than 5 percent in yeast.[65] In addition, exposed β-(1,3)-glucan binds mannose-binding lectins (MBL-A and MBL-C).[75] Collectively, these events may block the recognition of *B. dermatitidis* β-(1,3)-glucan by dectin-1 receptors on innate immune cells. Moreover, the reduction of β-glucan in the yeast cell wall precludes the use of the (1,3)-β-D-glucan test for diagnosis and of echinocandins for treatment of blastomycosis.[25,54]

To combat *B. dermatitidis*, both the innate and adaptive immune responses are required, whereas humoral immunity is dispensable. After inhalation of aerosolized conidia, alveolar macrophages are capable of phagocytizing and killing a large percentage of conidia as well as inhibiting germination to yeast.[35,122,123] Neutrophils are also capable of ingesting and killing conidia.[35] Conidia that survive the initial assault by macrophages and neutrophils germinate into yeast. *B. dermatitidis* yeast forms are more difficult to kill because they fail to induce a robust oxidative response, are relatively resistant to reactive oxygen species, and suppress nitric oxide production in macrophages.[111] The adaptive immune response, which

*References 30, 38, 45, 79, 92, 96.

is coordinated by T lymphocytes, is important for controlling *B. dermatitidis* infection in a manner that requires T-helper 17 and T-helper 1 cytokine responses (i.e., interleukin-17 and interferon-γ and TNF-α, respectively) to activate macrophages to enhance fungicidal activity and promote clearance.[14,138] After infection, patients develop specific cellular immunity to *B. dermatitidis* that persists for at least 2 years.[69]

CLINICAL MANIFESTATIONS

The clinical manifestations of blastomycosis are heterogeneous and range from asymptomatic infection to acute pneumonia to disseminated disease. The lung is the primary portal of entry for aerosolized conidia and mycelial fragments after disruption of soil. After evasion of pulmonary host defenses, *B. dermatitidis* can remain localized in the lung or disseminate to other organs. Less common routes of transmission in children include direct cutaneous inoculation and inhalation of infected vaginal secretions at birth.[86,133,140,141] Definitive evidence for transplacental transmission to the fetus is lacking but is theoretically possible; *B. dermatitidis* has been isolated from placental tissue.[81] Transmission of *B. dermatitidis* through solid-organ transplantation (i.e., infected allograft) has not been reported, but this route of transmission has been described for *H. capsulatum* and *Coccidioides* species.[84] Onset of symptomatic disease occurs 3 weeks to 3½ months after exposure to *B. dermatitidis*.[73,74]

Pulmonary Disease

Acute pneumonia is the most common clinical manifestation of blastomycosis, and symptomatic disease develops in approximately 50 percent of infected children.[74] Clinical manifestations resemble community-acquired pneumonia and include fevers, chills, headache, cough (which is often productive), dyspnea, chest pain, and malaise.[39,106,128,139] Severity of disease ranges from mild illness that can be managed in the outpatient setting to acute respiratory distress syndrome (ARDS) requiring intubation and mechanical ventilation.[39,72,74,106] Chronic pneumonia can develop and is often associated with extrapulmonary dissemination and a delay in diagnosis.[39,117,120]

The lung is the most common organ affected by *B. dermatitidis* and is involved in 79 to 85 percent of patients with symptomatic blastomycosis.[39,120,139] Consolidation, which can be unilobar, multilobar, or diffuse, is the most frequent radiographic pattern described in children with pulmonary blastomycosis (Fig. 198-3).* Consolidation from blastomycosis is indistinguishable from bacterial pneumonia. Nodular and interstitial infiltrates are the second and third most common findings on chest radiography, respectively.[2,38,39,65] Hilar lymphadenopathy, pleural effusion, and lung abscess are infrequently encountered (Fig. 198-4).[2,39,72,106] Cavitary disease and mass lesions are rare in children and can mimic tuberculosis or neoplasm. After treatment of pulmonary blastomycosis, radiographic abnormalities can persist for several years and functional pulmonary deficits (i.e., restrictive or obstructive disease) can be slow to normalize in patients with severe disease.[1,2,106] Fortunately, a majority of patients experience a full recovery without long-term pulmonary sequelae.[1]

*References 2, 39, 72, 74, 106, 117.

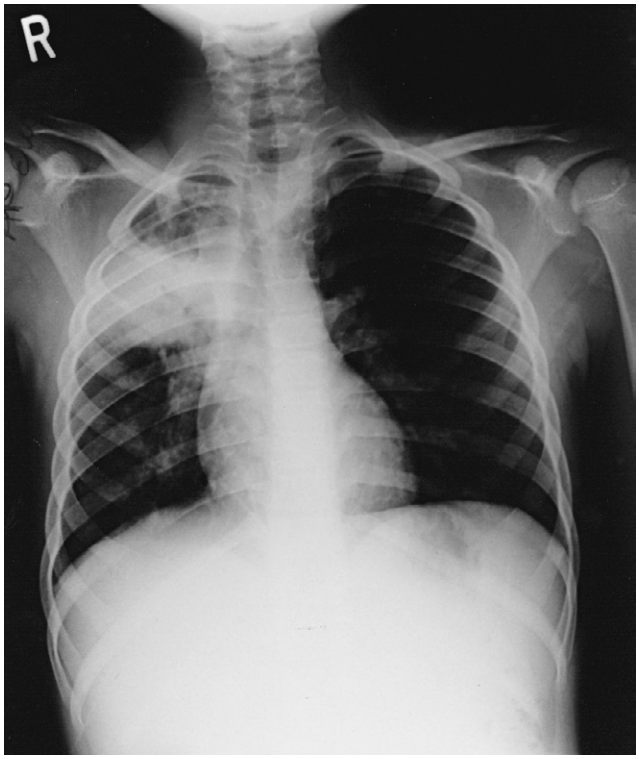

FIGURE 198-3 ■ Chest radiograph of a right upper lobe infiltrate secondary to infection with *B. dermatitidis.*

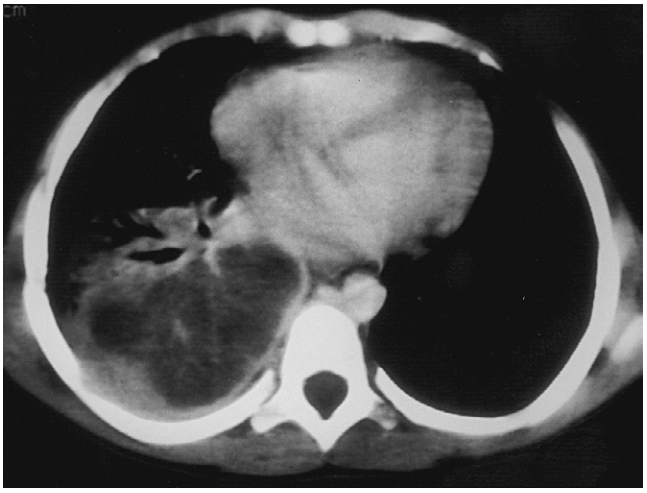

FIGURE 198-4 ■ CT scan of the chest revealing consolidation and abscess formation in the right lower lobe of the lung.

Disseminated Disease

Once infection is established in the lung, *B. dermatitidis* can disseminate to almost any organ in the body. Based

FIGURE 198-5 ■ Verrucous lesion of blastomycosis on the face of a child.

on retrospective studies, disseminated disease in children occurred in 38 to 50 percent of sporadic cases,[39,117] which is similar to adults with blastomycosis (25% to 40%).[25] In contrast, dissemination with acute pulmonary blastomycosis associated with point-source outbreaks occurred in a minority of patients.[128] The time from symptom onset to diagnosis is often delayed in patients who are diagnosed with disseminated disease.[39,117] Collectively, these data suggest that the duration of infection rather than age influences the risk for dissemination.[128]

Disseminated blastomycosis most commonly affects the skin and bone.[39,120,139] Cutaneous disease often begins as papulopustular lesions and progresses to ulcerative, verrucous, or crusted lesions (Figs. 198-5 and 198-6).[139] Erythema nodosum, which is common in patients with histoplasmosis and coccidioidomycosis, is rare in blastomycosis.[139] Skin involvement can also be associated with underlying osteomyelitis and manifests as pustules, abscesses, and ulcers (Figs. 198-7 and 198-8).[98] Invasion of *B. dermatitidis* into the bone results in lytic destruction that is often associated with a periosteal reaction and a sclerotic margin.[98] Although any bone can be infected, the most common sites include the long bones (e.g., tibia, humerus), skull, ribs, and vertebral bodies (Figs. 198-9 and 198-10).[39,98,112,120,139] Clinical manifestations of osteomyelitis include bone pain and soft tissue swelling associated with a draining sinus, ulcer, or abscess.[98] Vertebral osteomyelitis can be complicated by extension into the soft tissues resulting in paravertebral and psoas abscesses or invasion of the adjacent intervertebral discs and vertebral bodies.[112] These features can mimic *Mycobacterium* tuberculosis (Pott disease).[98] Progressive destruction of vertebral bone can result in compression fractures.[112] Similarly, osteomyelitis of the long bones can be complicated by pathologic fracture or extend into the joint space to cause septic arthritis.[77,98]

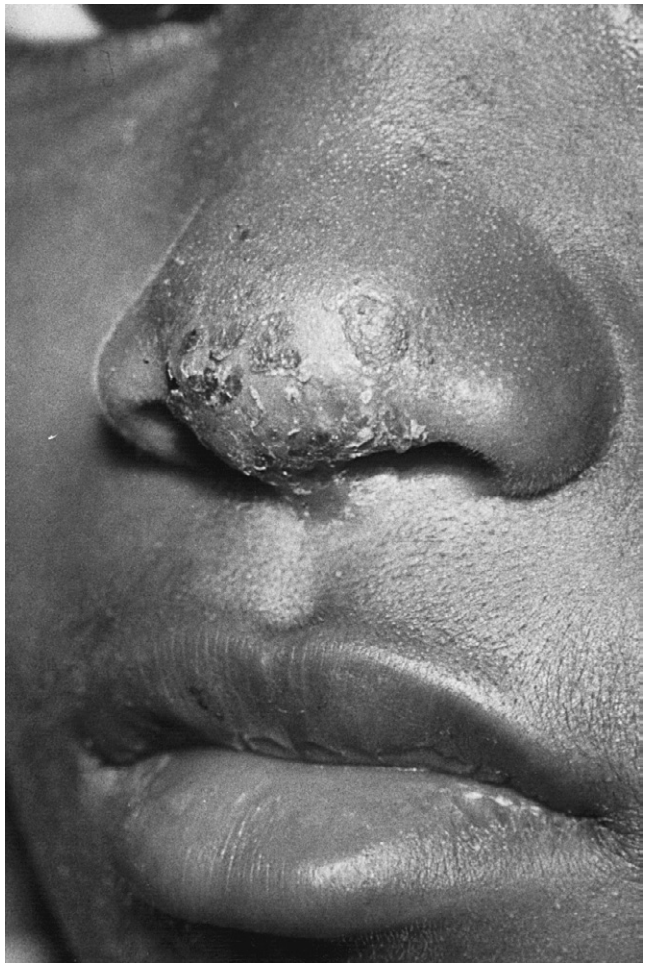

FIGURE 198-6 ■ Crusted skin lesion on the nose caused by *B. dermatitidis.*

Blastomycosis rarely disseminates to the central nervous system (CNS) or other organs such as the liver, spleen, heart, or middle ear.[62,64,139] Otitis media from blastomycosis is an uncommon complication related to contiguous extension from an extra-auditory focus into the middle ear.[40,64] CNS blastomycosis is estimated to occur in less than 5 to 10 percent of nonimmunocompromised patients and results from hematogenous spread or direct invasion from untreated infection of the scalp or skull.[39,78] CNS blastomycosis manifests as meningitis, epidural abscess, or brain abscess involving the cerebrum or cerebellum (Fig. 198-11).[9,39,78] A minority of patients can have concomitant meningitis and brain abscess.[9] Complications include hydrocephalus, mass effect from edema, cerebral herniation, infarction, seizures, panhypopituitarism, residual weakness, and impaired ability to function at school.[9,39] In patients with meningitis, cerebrospinal fluid (CSF) demonstrates a lymphocytic or neutrophilic pleocytosis associated with elevated protein and hypoglycorrhachia.[9] CSF cultures grow *B. dermatitidis* 45 percent of the time.[9]

FIGURE 198-7 ■ Purulent wound drainage overlying a region of osteomyelitis with blastomycosis.

Disease in Immunocompromised Patients

Information regarding the clinical course and treatment outcomes for blastomycosis in children with impaired immunity is limited to only a few case reports.[131,141] Clinical studies investigating blastomycosis in persons who have undergone solid-organ transplantation, developed AIDS, or been diagnosed with malignancy involve adult patients.[47,58,102,103,126] Blastomycosis is estimated to occur in less than 0.2 percent of solid-organ allograft recipients and presents 0.4 to 250 months after transplantation (near equal distribution before and after the first year of transplantation).[47,58] Infection most commonly causes pneumonia, which is often complicated by respiratory failure or ARDS.[47,58] The frequency of extrapulmonary infection (33% to 50%) is similar to that in the general population (25% to 40%).[10,47,58] Mortality ranges from 33 to 38 percent; however, it increases to 67 percent in patients with ARDS.[47,58,103]

Blastomycosis in AIDS is uncommon, and a single case series of 15 patients was published before the use of highly active antiretroviral therapy (HAART) that systemically investigated *B. dermatitidis* infection in this patient population.[102] The lung was infected in 87 percent

FIGURE 198-8 ■ Ulcerative lesion overlying a region of osteomyelitis secondary to blastomycosis.

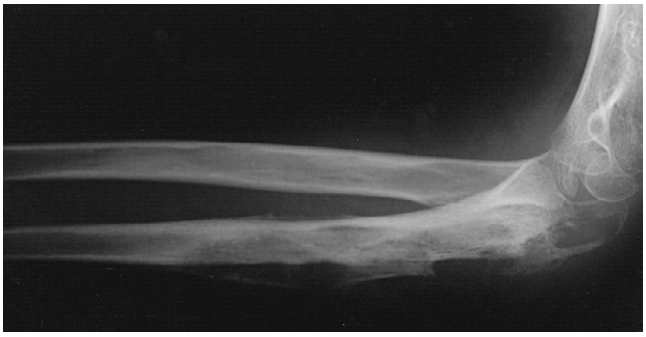

FIGURE 198-9 ■ Bone destruction of the ulna by blastomycosis.

FIGURE 198-10 ■ MR image of the spine demonstrating signal abnormalities at T11 through L3 with a severe compression deformity of the L1 vertebral body and multiple oval lytic lesions caused by blastomycosis.

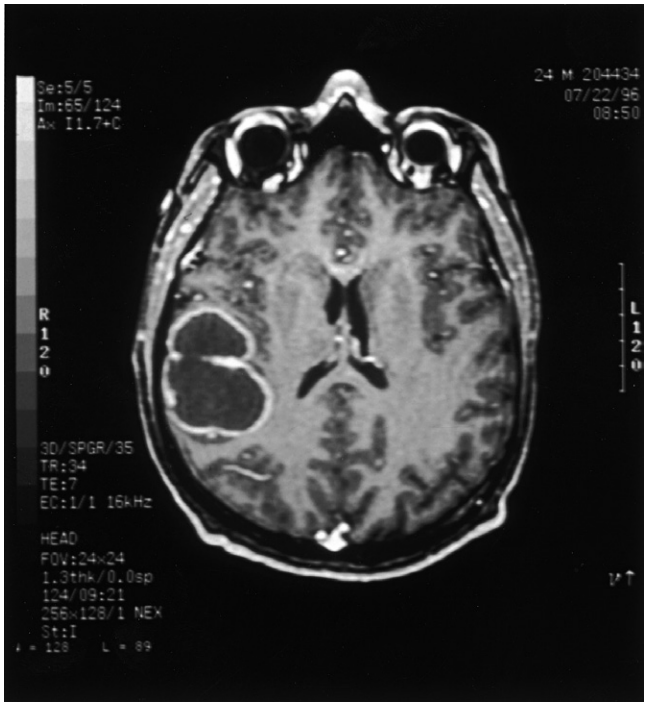

FIGURE 198-11 ■ CT scan of the brain demonstrating a multiloculated abscess in the right temporal lobe as a result of infection with *B. dermatitidis.*

of patients, and extrapulmonary disease occurred in 53 percent.[102] Invasion of *B. dermatitidis* into the CNS was common and occurred in 40 percent of patients. This is in sharp contrast to patient's immunosuppressed by transplantation or malignancy in whom the incidence of CNS blastomycosis is similar to that in the general population (<5% to 10%).[47,58,78,103] Approximately two thirds of AIDS patients with blastomycosis experienced prior opportunistic infections. The $CD4^+$ T-lymphocyte count at the time of diagnosis for blastomycosis was often less than 200 cells/mm^3.[102] Mortality attributable to blastomycosis was 53 percent.[102]

B. dermatitidis can infect patients with hematologic or solid-organ malignancies, including those with lung carcinoma, squamous cell cancers of the head and neck, breast cancer, gastrointestinal neoplasms, chronic lymphocytic leukemia, acute myelogenous leukemia, Hodgkin disease, and multiple myeloma.[103,126] Similar to allograft recipients and those with AIDS, pneumonia was the most common clinical manifestation and was complicated by ARDS in some patients.[103] Extrapulmonary disease occurred in approximately 50 percent.[103] Not surprisingly, mortality was high and was attributable to either the underlying malignancy or blastomycosis.[103]

Blastomycosis in Pregnancy and the Neonate

Blastomycosis is a rare complication of pregnancy, and clinical information is limited to sporadic case reports.[11,30,79] Women can be infected in any trimester, but the disease is most frequently diagnosed in the second or third trimester.[11,30,79] Disseminated disease is common (62%) and often involves the lungs, bone, and skin.[79] Isolated pulmonary infection occurs in less than half the patients (38%).[79] Reliable information regarding the frequency of placental involvement is lacking because examination by histology or culture has been performed in only one third of clinical cases. However, dissemination to the placenta is possible.[79,81] Histologic analysis identified *B. dermatitidis* yeast in the placenta of a 23-year-old women with

pulmonary blastomycosis complicated by ARDS during the third trimester.[81] Her infant was delivered by cesarean section and did not develop blastomycosis.[81]

Although clinical data are limited, *B. dermatitidis* infection during pregnancy does not appear to increase the risk for congenital malformations. However, based on isolation of *B. dermatitidis* from the placenta of one mother and two well-described clinical cases of neonatal blastomycosis, there is potential for transmission of blastomycosis during the peripartum period.[81,86,133] Regarding neonatal infection, both infants were admitted to the hospital for respiratory failure at 18 and 21 days of life. Infection was limited to the lungs, and both infants died of progressive pulmonary blastomycosis.[86,133] The underlying pathogenesis of neonatal blastomycosis is not well defined and may involve transplacental transmission or aspiration of infected vaginal secretions.

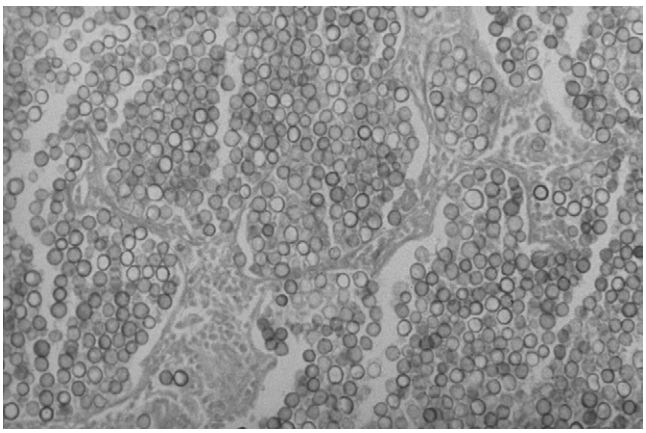

FIGURE 198-12 ■ Overwhelming infection with *B. dermatitidis* demonstrated in lung tissue with a silver stain.

DIAGNOSIS

The diagnosis of blastomycosis requires a high index of suspicion because clinical and radiographic manifestations can mimic other diseases including bacterial pneumonia, mycobacterial infections, and neoplasm. A detailed history focusing on potential exposures for the acquisition of *B. dermatitidis* can facilitate diagnosis in a timely fashion. The delay between symptom onset and diagnosis often exceeds 2 months.[39,117,120] Risks factors for endemic fungal infections include, but are not limited to, outdoor activities (i.e., canoeing, hiking, beaver dam exploration), home remodeling, construction (residential, commercial, road), playing in outdoor forts, hunting, fishing, use of a woodpile for a wood burning stove, and employment in a microbiology laboratory (see Table 198-1).[57,76] The diagnosis of blastomycosis in a household pet, frequently a dog, suggests a common source of exposure.* Thus, canine blastomycosis can serve as a harbinger of human blastomycosis.[116] Airborne transmission of *B. dermatitidis* between animals and humans does not occur; however, primary cutaneous blastomycosis has been reported from bite injuries.[55,57] Similarly, microbiology laboratory workers are at risk for cutaneous infection from injuries from sharp implements such as needlesticks.[57,76] In addition, diagnostic evaluation for blastomycosis should be considered in patients with pneumonia or cutaneous lesions that fail to respond to antibiotic therapy. The presence of pneumonia on chest radiography and cutaneous lesions on physical examination are highly suggestive of blastomycosis.

Definitive diagnosis of blastomycosis requires identification of *B. dermatitidis* in culture from sputum, skin, bone, or other clinical specimens. Commonly used culture media include Sabouraud dextrose agar and potato dextrose agar.[113] For culture of tissue specimens, supplementation of medium with brain-heart infusion agar is recommended.[113] Incubator temperatures used in most clinical laboratories (25°C to 30°C) promote the growth of *B. dermatitidis* as mold. Because several other fungal species have a similar appearance, additional confirmatory tests are required. This can include use of chemiluminescent DNA probes that target specific rRNA sequences, polymerase chain reaction assay, and conversion to yeast at 37°C.[113]

A presumptive diagnosis of blastomycosis can be made when yeast forms (8 to 20 μm) with broad-based budding and a doubly refractile cell wall are visualized by light microscopy from respiratory secretions, purulent material, or histopathologic sections. Visualization of *B. dermatitidis* requires special stains such as 10 percent potassium hydroxide, calcofluor white, Gomori methenamine silver (GMS), periodic acid–Schiff (PAS), or Papanicolaou (Fig. 198-12).[78] *B. dermatitidis* yeast can be difficult to visualize using Gram staining or hematoxylin and eosin stains. The presence of neutrophilic infiltration with noncaseating granulomas (pyogranulomas) in tissue specimens can suggest the diagnosis of blastomycosis and should prompt detailed microscopic examination for *B. dermatitidis* yeast.

For patients with pulmonary disease, specimens can be obtained from sputum, tracheal aspiration, bronchoscopy with bronchoalveolar lavage (BAL), or open-lung biopsy. Sputum cultures can be diagnostic for *B. dermatitidis* in up to 75 percent of patients[85]; however, sputum specimens tend to be more useful in older patients because collection of high-quality sputum can be difficult to obtain in young children.[117] If sputum cannot be obtained or is nondiagnostic, a tracheal aspirate or bronchoscopy with BAL can be performed. Although the diagnostic yield for BAL has been reported as 92 percent, results in the pediatric population may be suboptimal owing to technical limitations.[85,117] If specimens obtained from sputum and BAL fail to demonstrate *B. dermatitidis* and clinical suspicion remains high, then open-lung biopsy is required. In some case series,[117] but not others,[39] lung biopsy was needed for diagnosis in a majority of children.

Nonculture diagnostic techniques are often used in conjunction with fungal smear and culture to facilitate the diagnosis of blastomycosis. The development of an antigen test against a galactomannan component of the *Blastomyces* cell wall has become an important adjunctive test. Specimens for antigen testing can be collected from

*References 4, 11, 13, 19, 53, 93.

the urine, serum, BAL fluid, and CSF; however, robust clinical data exist for only urine and serum specimens.[8,29,36] Sensitivity for the urine antigen ranges from 85.1 to 92.9 percent and is influenced by the burden of infection.[8,29] In patients with localized pulmonary disease, sensitivity decreases to 50 percent, whereas in those with diffuse pulmonary disease, sensitivity increases to 87.5 percent.[9] Moreover, antigen levels tend to be higher in those with ARDS than those without.[29] Serum *Blastomyces* antigen sensitivity is 57 to 81.8 percent.[8,29] Because there is substantial cross reactivity with *H. capsulatum* (96%), *P. brasiliensis* (100%), and *P. marneffei* (70%), the specificity of the antigen test is 76.9 to 79 percent.[8,36] At times, cross reactions can cause diagnostic difficulties because *H. capsulatum* and *B. dermatitidis* exist in similar endemic regions in North America. However, this does not usually impact clinical management because treatment recommendations for blastomycosis and histoplasmosis are similar.[25,134] Diagnostic confusion related to cross reactions with *P. brasiliensis* or *P. marneffei* are uncommon, unless the patient has resided in or visited Central/South America or Southeast Asia, respectively. Urine antigen detection has been successfully used in the pediatric population for diagnosis and monitoring the response to antifungal therapy.[94]

Serologic studies such as complement fixation (CF), immunodiffusion (ID), and enzyme immunoassays (EIA) have been largely supplanted by the *Blastomyces* antigen test. Although specificity for CF and ID assays range from 97 to 100 percent, the sensitivity is 9 percent and 28 percent, respectively.[73] EIA has a sensitivity of 77 percent with a specificity of 92 percent.[73] All three serologic tests detect antibodies against the A antigen found in the *B. dermatitidis* cell wall. A radioimmunoassay against BAD-1 (formerly WI-1) has a sensitivity of 85 percent and specificity of 98 percent; however, this test is not commercially available.[71]

TREATMENT

Treatment of blastomycosis is influenced by the severity of the infection, the presence or absence of CNS dissemination, integrity of the immune system, and pregnancy (Table 198-3).[25] Antifungal therapy is recommended for all patients diagnosed with blastomycosis, including those with resolution of clinical symptoms before receiving therapy.[25] Although spontaneous resolution of symptomatic blastomycosis has been reported,[115] the frequency of this phenomenon remains unknown. Moreover, dissemination to organs such as the skin and CNS can occur after the spontaneous resolution of pulmonary disease. Because 38 and 50 percent of children with blastomycosis develop disseminated disease,[39,117] the decision to withhold therapy for pulmonary blastomycosis can be dangerous and lead to extrapulmonary spread of infection. Thus, the goal of therapy for patients with spontaneous resolution of acute pulmonary blastomycosis is to prevent extrapulmonary blastomycosis.[25]

For children with mild to moderate pulmonary blastomycosis, itraconazole 10 mg/kg/day (maximum, 400 mg/day) for 6 to 12 months of therapy is recommended (see Table 198-3).[25] For children with severe pulmonary blastomycosis including ARDS, treatment

TABLE 198-3 **Treatment of Blastomycosis in Children and Pregnant Women**

	Drug of Choice	Dose	Duration
Pulmonary Blastomycosis			
Mild to moderate	Itraconazole	10 mg/kg/day*	6–12 mo
Severe or life-threatening	Amphotericin B deoxycholate	0.7–1 mg/kg/day	1–2 wk
	Lipid amphotericin B	3–5 mg/kg/day	1–2 wk
	Itraconazole†	10 mg/kg/day*	12 mo
Extrapulmonary (non-CNS) Blastomycosis			
Mild to moderate	Itraconazole	10 mg/kg/day*	6–12 mo
Severe or life-threatening	Amphotericin B deoxycholate	0.7–1 mg/kg/day	1–2 wk
	Liposomal amphotericin B	3–5 mg/kg/day	1–2 wk
	Itraconazole†	10 mg/kg/day*	12 mo
Immunocompromised Patient	Amphotericin B deoxycholate	0.7–1 mg/kg/day	1–2 wk
	Liposomal amphotericin B	3–5 mg/kg/day	1–2 wk
	Itraconazole†	10 mg/kg/day*	12 mo
CNS Blastomycosis	Liposomal amphotericin B	5 mg/kg/day	4–6 wk
	Itraconazole‡	400–600 mg/day	12 mo
	Fluconazole‡	800 mg/day	12 mo
	Voriconazole‡	400–800 mg/day	12 mo
Pregnant Patient	Liposomal amphotericin B	3–5 mg/kg/day	
Neonate	Amphotericin B deoxycholate	1 mg/kg/day	

*Maximum dose of 400 mg/day.
†Itraconazole should be started after at least 1 to 2 weeks of therapy with amphotericin.
‡Itraconazole, fluconazole, or voriconazole should be started after at least 4 to 6 weeks of therapy with amphotericin.

consists of induction with amphotericin B for 1 to 2 weeks and consolidation with itraconazole (10 mg/kg/day; maximum, 400 mg/day) for 12 months (see Table 198-3).[25] Recommended amphotericin B formulations include amphotericin B deoxycholate (0.7 to 1 mg/kg/day) or liposomal amphotericin B (3 to 5 mg/kg/day).[25] In general, children tolerate the deoxycholate formulation better than adults.[25]

Treatment recommendations for children with impaired immunity from human immunodeficiency virus (HIV) infection or AIDS, transplantation, or malignancy involves induction therapy with amphotericin B followed by consolidation with itraconazole (see Table 198-3).[25] In some immunosuppressed patients, life-long suppression may need to be considered if immune suppression cannot be reversed.[25] Specific recommendations regarding criteria for suppressive therapy are lacking and the decision is left to the health care provider. In adults, the majority of transplant recipients adequately treated for blastomycosis do not experience relapse and life-long suppressive therapy is used only in select patients.[47,58] Whether these clinical observations in adult transplant recipients are directly applicable to immunosuppressed pediatric patients is unknown.

Children with mild to moderate disseminated blastomycosis are treated with itraconazole 10 mg/kg/day (maximum, 400 mg/day) for 6 to 12 months (see Table 198-3).[25] Patients with severe disseminated disease are treated in a similar manner as those with severe pulmonary disease. This includes induction therapy with amphotericin B for 1 to 2 weeks followed by 12 months of itraconazole.[25]

Treatment of CNS blastomycosis requires a prolonged course of liposomal amphotericin B at 5 mg/kg/day for 4 to 6 weeks followed by high-dose azole antifungal therapy for at least 12 months.[25] Liposomal amphotericin B is the preferred formulation because of its superior CNS penetration when compared with amphotericin B lipid complex (ABLC) or amphotericin B deoxycholate.[25,59] Recommended azoles include fluconazole 800 mg/day, itraconazole 200 mg two to three times a day, or voriconazole 200 to 400 mg twice a day.[25] The most effective azole for CNS disease remains to be determined. Fluconazole has excellent CSF penetration (52% to 82% of serum levels), but minimal inhibitory concentrations are higher than those with either itraconazole or voriconazole.[56,66] Itraconazole has been successfully used to treat some cases of CNS blastomycosis despite its poor penetration into the CSF at less than 10 percent.[25,66] In contrast, voriconazole levels in the CSF are 38 to 68 percent of serum and minimal inhibitory concentrations are similar to those of itraconazole.[56,66] There are several case reports of successful use of voriconazole to treat adults with CNS fungal infections including blastomycosis.[125] Surgical débridement should be considered for patients with intracranial masses, epidural abscesses, cranial osteomyelitis, and spinal osteomyelitis with instability.[132]

Pediatric patients of childbearing age with blastomycosis should undergo pregnancy testing before initiation of antifungal therapy. High-dose and prolonged treatment with triazoles increases the risk for fetal deformities and spontaneous abortion.[7,33,105,109,130] Administration of fluconazole to pregnant patients diagnosed with *Coccidioides immitis*, a dimorphic fungus related to *B. dermatitidis*, resulted in skeletal and craniofacial defects resembling Antley-Bixler syndrome.[109] Voriconazole and posaconazole can cause skeletal abnormalities in animal models.[105,130] Several human studies have demonstrated that itraconazole increases the risk for spontaneous abortion.[7,33] To minimize harm to the developing fetus, liposomal amphotericin B 3 to 5 mg/kg/day is recommended.[25,28] After delivery, the placenta should be examined histopathologically for *B. dermatitidis*. The neonate should be carefully assessed for infection and clinically monitored for the clinical signs and symptoms of blastomycosis. Amphotericin B deoxycholate 1 mg/kg/day is the recommended antifungal agent for treatment of neonatal blastomycosis.[25]

Effective clinical management of patients with blastomycosis requires efforts to minimize toxicity, avoid drug-drug interactions, optimize bioavailability, and monitor drug levels. Amphotericin B deoxycholate and its lipid formulations are fungicidal against blastomycosis and achieve high rates of clinical cure[26]; however, these agents require intravenous administration, are nephrotoxic, and promote urinary wasting of potassium, magnesium, and bicarbonate. Hydration with normal saline before and after therapy with amphotericin and use of lipid-associated formulations can minimize the risk for nephrotoxicity.[114] Replacement of potassium and magnesium losses is needed in most patients. Bicarbonate replacement is rarely required. Frequent monitoring of electrolytes and creatinine is needed for patients receiving amphotericin.[25]

In contrast to amphotericin B, azole antifungal agents are fungistatic against *B. dermatitidis* and clinical efficacy varies among the available agents. In a prospective, nonrandomized, open-label trial, itraconazole 200 to 400 mg/day resulted in a 90 percent cure rate.[34] Itraconazole is available in capsule and liquid formulations. Absorption of the capsule formulation is enhanced with gastric acidity and coadministration with food.[25,32] The liquid formulation (100 mg/10 mL) has 30 percent better bioavailability than the capsule formulation and should be administered without food.[25,32] Fluconazole is less efficacious than itraconazole and is no longer recommended by the 2008 guidelines published by the Infectious Diseases Society of America for pulmonary or non-CNS disseminated blastomycosis.[25] If fluconazole is used for treatment, 400 to 800 mg/day dosing is required to optimize clinical response. The success rate for 400 to 800 mg/day dosing was 87 percent, whereas it was 65 percent for 200 to 400 mg/day.[100,101] Approximately 50 percent of patients who received high-dose fluconazole experienced adverse events, including alopecia, xerosis, and elevated results of liver function tests.[100] Similar to fluconazole, ketoconazole is no longer recommended for the management of blastomycosis.[25] The newest triazoles, voriconazole and posaconazole, have excellent in vitro activity against *B. dermatitidis*.[56] Although clinical experience with voriconazole is limited, several case reports suggest that it can be an effective treatment option.[6,99,126] Posaconazole has demonstrated clinical efficacy in two

patients with disseminated blastomycosis.[108] To optimize bioavailability, voriconazole needs to administered in the fasting state whereas posaconazole requires coadministration with food.[105,130]

Therapeutic drug monitoring for itraconazole is recommended once steady-state levels are achieved, which is at the end of the second week of therapy. Given the long half-life of itraconazole (i.e., 24 hours), serum levels can be obtained independent of the time the drug was administered.[25] Goal therapeutic levels are more than 1 µg/mL (both itraconazole and hydroxy-itraconazole are added together).[25] Serum concentrations greater than 10 µg/mL are not needed and can increase the risk for side effects. Therapeutic drug monitoring can also be performed for voriconazole; goal serum levels are between 1.0 and 5.5 µg/mL.[3] In addition to therapeutic monitoring, liver function tests should be assessed at baseline, at 2 and 4 weeks into therapy, and then every 3 months for all azoles.[25] Moreover, careful review of all medications is needed to avoid drug-drug interactions with azoles. This is especially important for patients who are immunosuppressed by organ transplantation or receiving pharmacologic therapy for seizures.

Débridement for osteomyelitis or abscesses is not considered curative, and antifungal agents should always be administered after surgical intervention. Moreover, the duration of antifungal therapy should not be shortened.

Patients diagnosed with blastomycosis do not require isolation because person-to-person transmission through aerosols or respiratory droplets has not been reported. Standard barrier precautions (i.e., gloves) are needed in caring for patients with open wounds.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrob Agents Chemother* 2009;**53**:23–34.
8. Bariola JR, Hage CA, Durkin M, et al. Detection of *Blastomyces dermatitidis* antigen in patients with newly diagnosed blastomycosis. *Diagn Microbiol Infect Dis* 2011;**69**:187–91.
9. Bariola JR, Perry P, Pappas PG, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin Infect Dis* 2010;**50**:797–804.
18. Broad Institute Blastomyces dermatitidis database. Available at www.broadinstitute.org/annotation/genome/blastomyces_dermatitidis/MultiHome.html. Accessed August 1, 2012.
21. Carlos WG, Rose AS, Wheat LJ, et al. Blastomycosis in Indiana: digging up more cases. *Chest* 2010;**138**:1377–82.
29. Connolly P, Hage CA, Bariola JR, et al. *Blastomyces dermatitidis* antigen detection by quantitative enzyme immunoassay. *Clin Vaccine Immunol* 2012;**19**:53–6.
30. Crampton TL, Light RB, Berg GM, et al. Epidemiology and clinical spectrum of blastomycosis diagnosed at Manitoba hospitals. *Clin Infect Dis* 2002;**34**:1310–16.
31. De Groote MA, Bjerke R, Smith H, Rhodes LV III. Expanding epidemiology of blastomycosis: clinical features and investigation of 2 cases in Colorado. *Clin Infect Dis* 2000;**30**:582–4.
33. Desantis M, Di Gianantonio E, Cesari E, et al. First-trimester itraconazole exposure and pregnancy outcome: a prospective cohort study of women contacting teratology information services in Italy. *Drug Saf* 2009;**32**:239–44.
39. Fanella S, Skinner S, Trepman E, Embil JM. Blastomycosis in children and adolescents: a 30-year experience from Manitoba. *Med Mycol* 2011;**49**:627–32.
46. Gauthier GM, Klein BS. Insights into fungal morphogenesis and immune evasion. *Microbe* 2008;**3**:416–23.
48. Gauthier GM, Sullivan TD, Gallardo SS, et al. SREB, a GATA transcription factor that directs disparate fates in *Blastomyces dermatitidis* including morphogenesis and siderophore biosynthesis. *PLoS Pathog* 2010;**6**:e1000846.
58. Grim SA, Proia L, Miller R, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. *Transpl Infect Dis* 2012;**14**:17–23.
63. Hussein R, Khan S, Levy F, et al. Blastomycosis in the mountainous region of northeast Tennessee. *Chest* 2009;**135**:1019–23.
75. Koneti A, Linke MJ, Brummer E, Stevens DA. Evasion of innate immune responses: evidence for mannose binding lectin inhibition of tumor necrosis factor alpha production by macrophages in response to *Blastomyces dermatitidis*. *Infect Immun* 2008;**76**:994–1002.
84. Martín-Dávila P, Fortún J, López-Vélez R, et al. Transmission of tropical and geographically restricted infections during solid organ transplantation. *Clin Microbiol Rev* 2008;**21**:60–96.
104. Pfister JR, Archer JR, Hersil S, et al. Non-rural point source blastomycosis outbreak near a yard waste collection site. *Clin Med Res* 2011;**9**:57–65.
105. *Posaconazole [package insert]*. Kenilworth, NJ: Schering Corporation; 2008.
108. Proia LA, Harnish DO. Successful use of posaconazole for treatment of blastomycosis. *Antimicrob Agents Chemother* 2012;**56**:4029.
111. Rocco NM, Carmen JC, Klein BS. *Blastomyces dermatitidis* yeast cells inhibit nitric oxide production by alveolar macrophage inducible nitric oxide synthase. *Infect Immun* 2011;**79**:2385–95.
113. Saccente M, Woods GL. Clinical and laboratory update on blastomycosis. *Clin Microbiol Rev* 2010;**23**:367–81,
114. Saliba F, Dupont B. Renal impairment and amphotericin B formulations in patients with invasive fungal infections. *Med Mycol* 2008;**46**:97–112.
124. Shukla S, Singh S, Jain M, et al. Paediatric cutaneous blastomycosis: a rare case diagnosed on FNAC. *Diagn Cytopathol* 2009;**37**:119–21.
125. Ta M, Flowers SA, Rogers PD. The role of voriconazole in the treatment of central nervous system blastomycosis. *Ann Pharmacother* 2009;**43**:1696–700.
130. *Voriconazole [package insert]*. New York, NY: Roerig, Division of Pfizer Inc.; 2011.
138. Wuthrich M, Gern B, Hung CY, et al. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. *J Clin Invest* 2011;**121**:554–68.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alkrinawi S, Pianosi P. Pulmonary function following blastomycosis in childhood. *Clin Pediatr (Phila)* 2000;**39**:27–31.
2. Alkrinawi S, Reed MH, Pasterkamp H. Pulmonary blastomycosis in children: findings on chest radiographs. *AJR Am J Roentgenol* 1995;**165**:651–4.
3. Andes D, Pascual A, Marchetti O. Antifungal therapeutic drug monitoring: established and emerging indications. *Antimicrob Agents Chemother* 2009;**53**:23–34.
4. Armstrong CW, Jenkins SR, Kaufman L, et al. Common-source outbreak of blastomycosis in hunters and their dogs. *J Infect Dis* 1987;**155**:568–70.
5. Baily GG, Robertson VJ, Neill P, et al. Blastomycosis in Africa: clinical features, diagnosis, and treatment. *Rev Infect Dis* 1991;**13**: 1005–8.
6. Bakleh M, Aksamit AJ, Tleyjeh IM, Marshall WF. Successful treatment of cerebral blastomycosis with voriconazole. *Clin Infect Dis* 2005;**40**:e69–71.
7. Bar-Oz B, Moretti ME, Bishai R, et al. Pregnancy outcome after in utero exposure to itraconazole: a prospective cohort study. *Am J Obstet Gynecol* 2000;**183**:617–20.
8. Bariola JR, Hage CA, Durkin M, et al. Detection of *Blastomyces dermatitidis* antigen in patients with newly diagnosed blastomycosis. *Diagn Microbiol Infect Dis* 2011;**69**:187–91.
9. Bariola JR, Perry P, Pappas PG, et al. Blastomycosis of the central nervous system: a multicenter review of diagnosis and treatment in the modern era. *Clin Infect Dis* 2010;**50**:797–804.
10. Baumgardner DJ, Buggy BP, Mattson BJ, et al. Epidemiology of blastomycosis in a region of high endemicity in north central Wisconsin. *Clin Infect Dis* 1992;**15**:629–35.
11. Baumgardner DJ, Burdick JS. An outbreak of human and canine blastomycosis. *Rev Infect Dis* 1991;**13**:898–905.
12. Baumgardner DJ, Egan G, Giles S, Laundre B. An outbreak of blastomycosis on a United States Indian reservation. *Wilderness Environ Med* 2002;**13**:250–2.
13. Baumgardner DJ, Paretsky DP. The in vitro isolation of *Blastomyces dermatitidis* from a woodpile in north central Wisconsin, USA. *Med Mycol* 1999;**37**:163–68.
14. Bradsher RW, Balk RA, Jacobs RF. Growth inhibition of *Blastomyces dermatitidis* in alveolar and peripheral macrophages from patients with blastomycosis. *Am Rev Respir Dis* 1987;**135**:412–17.
15. Brandhorst TT, Gauthier GM, Stein RA, et al. Calcium binding by the essential virulence factor BAD-1 of *Blastomyces dermatitidis*. *J Biol Chem* 2005;**280**:42156–63.
16. Brandhorst TT, Wuthrich M, Finkel-Jimenez B, et al. Exploiting type 3 complement receptor for TNA-alpha suppression, immune evasion, and progressive pulmonary infection. *J Immunol* 2004; **173**:7444–53.
17. Brandhorst TT, Wuthrich M, Warner T, et al. Targeted gene disruption reveals an adhesion indispensable for pathogenicity of *Blastomyces dermatitidis*. *J Exp Med* 1999;**19**:1207–1216.
18. Broad Institute Blastomyces dermatitidis database. *Available at* www.broadinstitute.org/annotation/genome/blastomyces_dermatitidis/MultiHome.html. Accessed August 1, 2012.
19. Bromel C, Sykes JE. Epidemiology, diagnosis, and treatment of blastomycosis in dogs and cats. *Clin Tech Small Animal Pract* 2005;**20**:233–9.
20. Cano MV, Ponce-de-Leon GF, Tippen S, et al. Blastomycosis in Missouri: epidemiology and risk factors for endemic disease. *Epidemiol Infect* 2003;**131**:907–14.
21. Carlos WG, Rose AS, Wheat LJ, et al. Blastomycosis in Indiana: digging up more cases. *Chest* 2010;**138**:1377–82.
22. Carman WF, Frean JA, Crewe-Brown HH, et al. Blastomycosis in Africa: a review of known cases diagnosed between 1951 and 1987. *Mycopathologia* 1989;**107**:25–32.
23. Centers for Disease Control. Blastomycosis – North Carolina. *MMWR Morb Mortal Wkly Rep* 1976;**25**:205–6.
24. Centers for Disease Control and Prevention. Blastomycosis – Wisconsin, 1986–1995. *MMWR Morb Mortal Wkly Rep* 1996;**45**: 601–3.
25. Chapman SW, Dumukes WE, Proia LA, et al. Clinical practice guidelines for the management of blastomycosis: 2008 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2008;**46**:1801–12.
26. Chapman SW, Lin AC, Hendricks KA, et al. Endemic blastomycosis in Mississippi: epidemiologic and clinical studies. *Semin Respir Infect* 1997;**12**:219–28.
27. Cockerill FR 3rd, Roberts GD, Rosenblatt JE, et al. Epidemic of pulmonary blastomycosis (Namekagon fever) in Wisconsin canoeists. *Chest* 1984;**86**:688–92.
28. Cohen I. Absence of congenital infection and teratogenesis in three children born to mothers with blastomycosis and treated with amphotericin B during pregnancy. *Pediatr Infect Dis J* 1987; **6**:76–7.
29. Connolly P, Hage CA, Bariola JR, et al. *Blastomyces dermatitidis* antigen detection by quantitative enzyme immunoassay. *Clin Vaccine Immunol* 2012;**19**:53–6.
30. Crampton TL, Light RB, Berg GM, et al. Epidemiology and clinical spectrum of blastomycosis diagnosed at Manitoba hospitals. *Clin Infect Dis* 2002;**34**:1310–16.
31. De Groote MA, Bjerke R, Smith H, et al. Expanding epidemiology of blastomycosis: clinical features and investigation of 2 cases in Colorado. *Clin Infect Dis* 2000;**30**:582–4.
32. De Repentigny L, Ratelle J, Leclerc JM, et al. Repeated-dose pharmacokinetics of an oral solution of itraconazole in infants and children. *Antimicrob Agents Chemother* 1998;**42**:404–8.
33. Desantis M, Di Gianantonio E, Cesari E, et al. First-trimester itraconazole exposure and pregnancy outcome: a prospective cohort study of women contacting teratology information services in Italy. *Drug Saf* 2009;**32**:239–44.
34. Dismukes WE, Bradsher RW Jr, Cloud GC, et al. Itraconazole therapy for blastomycosis and histoplasmosis. *Am J Med* 1992;**93**: 489–97.
35. Drutz DJ, Frey CL. Intracellular and extracellular defenses of human phagocytes against *Blastomyces dermatitidis* conidia and yeasts. *J Lab Clin Med* 1985;**105**:737–50.
36. Durkin M, Witt J, LeMonte A, et al. Antigen assay with the potential to aid in diagnosis of blastomycosis. *J Clin Microbiol* 2004;**42**:4873–5.
37. Dwight PJ, Naus M, Sarsfield P, et al. An outbreak of human blastomycosis: the epidemiology of blastomycosis in the Kenora Catchment region of Ontario, *Canada Can Commun Dis Rep* 2000; **26**:82–92.
38. Dworkin MS, Duckro AN, Proia L, et al. The epidemiology of blastomycosis in Illinois and factors associated with death. *Clin Infect Dis* 2005;**41**:e107–111.
39. Fanella S, Skinner S, Trepman E, Embil JM. Blastomycosis in children and adolescents: a 30-year experience from Manitoba. *Med Mycol* 2011;**49**:627–32.
40. Farr RC, Gardner G, Acker JD, et al. Blastomycosis cranial osteomyelitis. *Am J Otol* 1992;**13**:582–6.
41. Finkel-Jimenez B, Wuthrich M, Brandhorst T, et al. The WI-1 adhesin blocks phagocyte TNF-α production imparting pathogenicity on *Blastomyces dermatitidis*. *J Immunol* 2001;**166**:2665–73.
42. Finkel-Jimenez B, Wuthrich M, Klein BS. BAD1, an essential virulence factor of *Blastomyces dermatitidis*, suppresses host TNF-α production through TGF-β–dependent and –independent mechanisms. *J Immunol* 2002;**168**:5746–55.
43. Frean JA, Carman WF, Crewe-Brown HH, et al. *Blastomyces dermatitidis* infections in the RSA. *S Afr Med J* 1989;**76**:13–16.
44. Frye MD, Seifer FD. An outbreak of blastomycosis in eastern Tennessee. *Mycopathologia* 1991;**116**:15–21.
45. Furcolow ML, Chick EW, Busey JF, Menges RW. Prevalence and incidence studies of human and canine blastomycosis, part I. Cases in the United States, 1885–1968. *Am Rev Respir Dis* 1970; **102**:60–7.
46. Gauthier GM, Klein BS. Insights into fungal morphogenesis and immune evasion. *Microbe* 2008;**3**:416–23.
47. Gauthier GM, Safdar N, Klein BS, et al. Blastomycosis in solid organ transplant recipients. *Transpl Infect Dis* 2007;**9**:310–17.
48. Gauthier GM, Sullivan TD, Gallardo SS, et al. SREB, a GATA transcription factor that directs disparate fates in *Blastomyces dermatitidis* including morphogenesis and siderophore biosynthesis. *PLoS Pathog* 2010;**6**:e1000846.
49. Gilchrist TC. Protozoan dermatitis. *J Cutan Genitourin Dis* 1894;**12**:496.
50. Gilchrist TC. A case of blastomycetic dermatitis in man. *Johns Hopkins Hosp Rep* 1896;**1**:269–83.

51. Gilchrist TC, Stokes WR. The presence of an Oidium in the tissues of a case of pseudo–lupus vulgaris: preliminary report. *Johns Hopkins Hosp Bull* 1896;**7**:129–33.
52. Gilchrist TC, Stokes WR. A case of pseudo-lupus vulgaris caused by a *Blastomyces*. *J Exp Med* 1898;**3**:53–78.
53. Gilor C, Graves T, Barger A, et al. Clinical aspects of natural infection with *Blastomyces dermatitidis* in cats: 8 cases (1991–2005). *J Am Vet Med Assoc* 2006;**229**:96–9.
54. Girouard G, Lachance C, Pelletier R. Observations of (1,3)-beta-D-glucan detection as a diagnostic tool in endemic mycosis caused by *Histoplasma* or *Blastomyces*. *J Med Microbiol* 2007;**56**: 1001–2.
55. Gnann JW Jr, Bressler GS, Bodet CA 3rd, et al. Human blastomycosis after a dog bite. *Ann Intern Med* 1983;**98**:48–9.
56. Gonzalez GM, Fothergill AW, Sutton DA, et al. In vitro activities of new and established triazoles against opportunistic filamentous and dimorphic fungi. *Med Mycol* 2005;**43**:281–4.
57. Gray NA, Baddour LM. Cutaneous inoculation blastomycosis. *Clin Infect Dis* 2002;**34**:e44–9.
58. Grim SA, Proia L, Miller R, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. *Transpl Infect Dis* 2012;**14**:17–23.
59. Groll AH, Giri N, Petraitis V, et al. Comparative efficacy and distribution of lipid formulations of amphotericin B in experimental *Candida albicans* infection of the central nervous system. *J Infect Dis* 2000;**182**:274–82.
60. Hamburger WW. A comparative study of four strains of organisms isolated from four cases of generalized blastomycosis. *J Infect Dis* 1907;**4**:201–9.
61. Hogan LH, Klein BS. Altered expression of surface alpha-1,3-glucan in genetically related strains of *Blastomyces dermatitidis* that differ in virulence. *Infect Immun* 1994;**62**:3543–6.
62. Hughes WT, Franco S, Oh MHK. Systemic blastomycosis in childhood: case report and review. *Clin Pediatr (Phila)* 1969;**8**: 597–601.
63. Hussein R, Khan S, Levy F, et al. Blastomycosis in the mountainous region of northeast Tennessee. *Chest* 2009;**135**:1019–23.
64. Istorico LJ, Sanders M, Jacobs RF, et al. Otitis media due to blastomycosis: report of two cases. *Clin Infect Dis* 1992;**14**: 355–8.
65. Kanetsuna F, Carbonell LM. Cell wall composition of the yeast-like and mycelial forms of *Blastomyces dermatitidis*. *J Bacteriol* 1971; **106**:946–8.
66. Kethireddy S, Andes D. CNS pharmacokinetics of antifungal agents. *Expert Opin Drug Metab Toxicol* 2007;**3**:573–81.
67. Kitchen MS, Reiber CD, Eastin GB. An urban epidemic of North American blastomycosis. *Am Rev Respir Dis* 1977;**115**:1063–6.
68. Klein BS, Aizenstein BD, Hogan LH. African strains of *Blastomyces dermatitidis* that do not express surface adhesin WI-1. *Infect Immunol* 1997;**65**:1505–9.
69. Klein BS, Bradsher RW, Vergeront JM, Davis JP. Development of long-term specific cellular immunity after acute *Blastomyces dermatitidis* infection: assessments following a large point-source outbreak in Wisconsin. *J Infect Dis* 1990;**161**:97–101.
70. Klein BS, Davis JP. A laboratory-based surveillance of human blastomycosis in Wisconsin between 1973 and 1982. *Am J Epidemiol* 1985;**122**:897–903.
71. Klein BS, Jones JM. Isolation, purification, and radiolabeling of a novel 120-kD surface protein on *Blastomyces dermatitidis* yeasts to detect antibody in infected patients. *J Clin Invest* 1990;**85**: 152–61.
72. Klein BS, Vergeront JM, Di Salvo AF, et al. Two outbreaks of blastomycosis along rivers in Wisconsin: isolation of *Blastomyces dermatitidis* from riverbank soil and evidence of its transmission along waterways. *Am Rev Respir Dis* 1987;**136**:1333–8.
73. Klein BS, Vergeront JM, Kaufman L, et al. Serological tests for blastomycosis: assessments during a large point-source outbreak in Wisconsin. *J Infect Dis* 1987;**155**:262–8.
74. Klein BS, Vergeront JM, Weeks RJ, et al. Isolation of *Blastomyces dermatitidis* in soil associated with a large outbreak of blastomycosis in Wisconsin. *N Engl J Med* 1986;**314**:529–34.
75. Koneti A, Linke MJ, Brummer E, et al. Evasion of innate immune responses: evidence for mannose binding lectin inhibition of tumor necrosis factor alpha production by macrophages in response to *Blastomyces dermatitidis*. *Infect Immun* 2008;**76**:994–1002.
76. Larson DM, Eckman MR, Alber RL et al. Primary cutaneous (inoculation) blastomycosis: an occupational hazard to pathologists. *Am J Clin Pathol* 1983;**79**:253–5.
77. Laskey WK, Sarosi GA. Blastomycosis in children. *Pediatrics* 1980;**65**:111–14.
78. Lemos LB, Guo M, Baliga M. Blastomycosis: organ involvement and etiologic diagnosis. A review of 123 patients from Mississippi. *Ann Diagn Pathol* 2000;**4**:391–406.
79. Lemos LB, Soofi M, Amir E. Blastomycosis and pregnancy. *Ann Diagn Pathol* 2002;**6**:211–15.
80. Lowry PW, Kelso KY, McFarland LM. Blastomycosis in Washington Parish, Louisiana, 1976–1985. *Am J Epidemiol* 1989; **130**:151–9.
81. MacDonald D, Alguire PC. Adult respiratory distress syndrome due to blastomycosis during pregnancy. *Chest* 1990;**98**:1527–8.
82. MacDonald PD, Langley RL, Gerkin SR, et al. Human and canine pulmonary blastomycosis, North Carolina, 2001–2002. *Emerg Infect Dis* 2006;**12**:1242–4.
83. Manetti AC. Hyperendemic urban blastomycosis. *Am J Public Health* **81**:633–6, 1991.
84. Martín-Dávila P, Fortún J, López-Vélez R, et al. Transmission of tropical and geographically restricted infections during solid organ transplantation. *Clin Microbiol Rev* 2008;**21**:60–96.
85. Marytnowicz MA, Prakash UBS. Pulmonary blastomycosis: an appraisal of diagnostic techniques. *Chest* 2002;**121**:768–73.
86. Maxson S, Miller SF, Tryka AF, et al. Perinatal blastomycosis: a review. *Pediatr Infect Dis J* 1992;**11**:760–3.
87. McCullough MJ, DiSalvo AF, Clemons KV, et al. Molecular epidemiology of *Blastomyces dermatitidis*. *Clin Infect Dis* 2000;**30**: 328–35.
88. McDonough ES, Chan DM, McNamara WJ. Dual infection by "+" and "–" mating types of *Ajellomyces (Blastomyces) dermatitidis*. *Am J Epidemiol* 1977;**106**:67–71.
89. McDonough ES, McNamara WJ, Chan DM, et al. Geographic distribution of "+" and "–" isolates of *Blastomyces dermatitidis* in North America. *Am J Epidemiol* 1973;**98**:63–7.
90. McDonough ES, Lewis AL. *Blastomyces dermatitidis:* production of the sexual stage. *Science* 1967;**156**:528–9.
91. McDonough ES, Lewis AL. The ascigerous stage of *Blastomyces dermatitidis*. *Mycologia* 1968;**60**:76–83.
92. Menges RW, Doto IL, Weeks RJ. Epidemiologic studies of blastomycosis in Arkansas. *Arch Environ Health* 1969;**18**:956–71.
93. Menges RW, Furcolow ML, Selby LA, et al. Clinical and epidemiologic studies on seventy-nine canine blastomycosis cases in Arkansas. *Am J Epidemiol* 1965;**81**:164–79.
94. Mongkolrattanothai K, Peev M, Wheat LJ, et al. Urine antigen detection of blastomycosis in pediatric patients. *Pediatr Infect Dis J* 2006;**25**:1076–8.
95. Montgomery FH, Ormsby OS. Systemic blastomycosis: its etiologic, pathologic and clinical features as established by a critical survey and summary of twenty-two cases, seven previously unpublished; the relation of blastomycosis to coccidioidal granuloma. *Arch Intern Med* 1908;**2**:1–41.
96. Morris S, Brophy J, Richardson SE, et al. Blastomycosis in Ontario, 1994–2003. *Emerg Infect Dis* 2006;**12**:274–9.
97. Nemecek JC, Wuthrich M, Klein BS. Global control of dimorphism and virulence in fungi. *Science* 2006;**312**:583–8.
98. Oppenheimer M, Embil J, Black B, et al. Blastomycosis of bones and joints. *South Med J* 2007;**100**:570–8.
99. Panicker J, Walsh T, Kamani N. Recurrent central nervous system blastomycosis in an immunocompetent child treated successfully with sequential liposomal amphotericin B and voriconazole. *Pediatr Infect Dis J* 2006;**25**:377–9.
100. Pappas PG, Bradsher RW, Chapman SW, et al. Treatment of blastomycosis with fluconazole: a pilot study. *Clin Infect Dis* 1995;**20**:267–71.
101. Pappas PG, Bradsher RW, Kauffman CA, et al. Treatment of blastomycosis with higher doses of fluconazole. *Clin Infect Dis* 1997;**25**:200–5.
102. Pappas PG, Pottage JC, Powderly WG, et al. Blastomycosis in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1992;**116**:847–53.
103. Pappas PG, Threlkeld MG, Bedsole GD, et al. Blastomycosis in immunocompromised patients. *Medicine (Baltimore)* 1993;**72**:311–25.

104. Pfister JR, Archer JR, Hersil S, et al. Non-rural point source blastomycosis outbreak near a yard waste collection site. *Clin Med Res* 2011;**9**:57–65.
105. *Posaconazole [package insert]*. Kenilworth, NJ: Schering Corporation; 2008.
106. Powell DA, Schuit KE. Acute pulmonary blastomycosis in children: clinical course and follow-up. *Pediatrics* 1979;**63**:736–40.
107. Proctor ME, Klein BS, Jones JM, Davis JP. Cluster of pulmonary blastomycosis in a rural community: evidence for multiple high-risk environmental foci following a sustained period of diminished precipitation. *Mycopathologia* 2002;**153**:113–20.
108. Proia LA, Harnish DO. Successful use of posaconazole for treatment of blastomycosis. *Antimicrob Agents Chemother* 2012;**56**:4029.
109. Pursley TJ, Blomquist IK, Abraham J, et al. Fluconazole-induced congenital abnormalities in three infants. *Clin Infect Dis* 1996;**22**: 336–40.
110. Rappleye CA, Engle JT, Goldman WE. RNA interference in *Histoplasma capsulatum* demonstrates a role for α-(1,3)-glucan in virulence. *Mol Microbiol* 2004;**53**:153–65.
111. Rocco NM, Carmen JC, Klein BS. *Blastomyces dermatitidis* yeast cells inhibit nitric oxide production by alveolar macrophage inducible nitric oxide synthase. *Infect Immun* 2011;**79**:2385–95.
112. Saccente M, Abernathy RS, Pappas PG, et al. Vertebral blastomycosis with paravertebral abscess: report of eight cases and review of the literature. *Clin Infect Dis* 1998;**26**:413–18.
113. Saccente M, Woods GL. Clinical and laboratory update on blastomycosis. *Clin Microbiol Rev* 2010;**23**:367–81.
114. Saliba F, Dupont B. Renal impairment and amphotericin B formulations in patients with invasive fungal infections. *Med Mycol* 2008;**46**:97–112.
115. Sarosi GA, Davies SF, Phillips JR. Self-limited blastomycosis: a report of 39 cases. *Semin Respir Infect* 1986;**1**:40–4.
116. Sarosi GA, Eckman MR, Davies SF, et al. Canine blastomycosis as a harbinger of human disease. *Ann Intern Med* 1979;**91**:733–5.
117. Schutze GE, Hickerson SL, Fortin EM, et al. Blastomycosis in children. *Clin Infect Dis* 1996;**22**:496–502.
118. Schwarz J, Baum GL. Blastomycosis. *Am J Clin Pathol* 1951; **21**:999–1029.
119. Smith JG Jr, Harris JS, Conant NF, et al. An epidemic of North American blastomycosis. *JAMA* 1955;**158**:641–6.
120. Steele RW, Abernathy RS. Systemic blastomycosis in children. *Pediatr Infect Dis J* 1983;**2**:304–7.
121. Stober AM. Systemic blastomycosis: a report of its pathological, bacteriological, and clinical features. *Arch Intern Med* 1914;**13**: 509–56.
122. Sugar AM, Picard M. Macrophage- and oxidant-mediated inhibition of the ability of live *Blastomyces dermatitidis* conidia to transform to the pathogenic yeast phase: implication of the pathogenesis of dimorphic fungal infections. *J Infect Dis* 1991;**163**:371–5.
123. Sugar AM, Picard M, Wagner R, Kornfeld H. Interactions between human bronchoalveolar macrophages and *Blastomyces dermatitidis* conidia: demonstration of fungicidal and fungistatic effects. *J Infect Dis* 1995;**171**:1559–62.
124. Shukla S, Singh S, Jain M, et al. Paediatric cutaneous blastomycosis: a rare case diagnosed on FNAC. *Diagn Cytopathol* 2009;**37**: 119–21.
125. Ta M, Flowers SA, Rogers PD. The role of voriconazole in the treatment of central nervous system blastomycosis. *Ann Pharmacother* 2009;**43**:1696–700.
126. Torres H, Rivero G, Kontoyiannis D. Endemic mycoses in a cancer hospital. *Medicine (Baltimore)* 2002;**81**:201–12.
127. Tosh FE, Hammerman KJ, Weeks RJ, et al. A common source epidemic of North American blastomycosis. *Am Rev Respir Dis* 1974;**109**:525–9.
128. Varkey B. Blastomycosis in children. *Semin Respir Infect* 1997;**12**: 235–42.
129. Vasquez JE, Mehta JB, Agrawal R, et al. Blastomycosis in northeast Tennessee. *Chest* 1998;**114**:436–43.
130. *Voriconazole [package insert]*. New York, NY: Roerig, Division of Pfizer Inc.; 2011.
131. Walker K, Skelton H, Smith K. Cutaneous lesions showing giant yeast forms of *Blastomyces dermatitidis*. *J Cutan Pathol* 2002;**29**: 616–18.
132. Ward BA, Parent AD, Raila F. Indications for the surgical management of central nervous system blastomycosis. *Surg Neurol* 1995;**43**:379–88.
133. Watts EA, Gard PD, Tuthill SW. First reported case of intrauterine transmission of blastomycosis. *Pediatr Infect Dis J* 1983;**2**: 308–10.
134. Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:807–25.
135. Winn W, Allen S, Janda W, et al, editors. *Mycology.* In: *Koneman's color atlas and textbook of diagnostic microbiology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006. p. 1194–8.
136. Wolf PL, Russell B, Shimoda A, editors. *Practical clinical microbiology and mycology: techniques and interpretations. Section X: Identification of dimorphic fungi causing systemic mycosis*. New York: John Wiley & Sons, 1975. p. 486–8.
137. Wu SJ, Valyi-Nagy T, Engelhard HH, et al. Secondary intracerebral blastomycosis with giant yeast forms. *Mycopathologia* 2005;**160**: 253–7.
138. Wuthrich M, Gern B, Hung CY, et al. Vaccine-induced protection against 3 systemic mycoses endemic to North America requires Th17 cells in mice. *J Clin Invest* 2011;**121**:554–68.
139. Yogev R, Davis T. Blastomycosis in children: a review of the literature. *Mycopathologia* 1979;**68**:139–43.
140. Young L, Schutze GE. Perinatal blastomycosis: the rest of the story. *Pediatr Infect Dis J* 1995;**14**:83.
141. Zampogna J, Hoy M, Ramos-Caro F. Primary cutaneous North American blastomycosis in and immunosuppressed child. *Pediatr Dermatol* 2003;**20**:128–30.
142. Zhang MX, Brandhorst TT, Kozel TR, et al. Role of gluan and surface protein BAD1 in complement activation by *Blastomyces dermatitidis* yeast. *Infect Immunol* 2001;**69**:7559–64.

CHAPTER 199

CANDIDIASIS

Grace E. Lee • David A. Kaufman • Theoklis E. Zaoutis

Over the past 3 decades, *Candida* spp. have emerged as major fungal human pathogens, largely owing to advances in health care technology and medical therapies.[26,78,340,349] Expanding use of organ transplantation, immunomodulatory therapies, and immunosuppressive chemotherapeutic regimens, as well as broad-spectrum antimicrobial therapy and invasive devices, has contributed to a growing population of immunocompromised individuals highly susceptible to invasive fungal disease. *Candida* is the most common cause of invasive fungal infection in health care settings and is associated with significant increases in health care resource utilization and attributable mortality.[84,253,286,344] A discussion is presented of the epidemiology, pathogenesis, clinical manifestations, diagnosis, and management of candidiasis in the pediatric population. Because of notable differences in the pathogenesis, presentation, and management of invasive candidiasis in neonates, neonatal candidiasis is discussed separately later in the chapter.

THE ORGANISM

Candida spp. are a heterogeneous group of eukaryotic, diploid organisms that grow predominantly as yeast morphology (i.e., unicellular). Most members are able to produce pseudohyphae; however, only *C. albicans* and *C. dublinensis* are able to produce true hyphae, making them the only polymorphic *Candida* spp. *Candida* spp. are commensal organisms, commonly found in the gastrointestinal and genitourinary tracts of healthy humans. More than 150 species have been described; of these, at least 17 different species have been found to cause invasive disease.[118,282] *C. albicans* is the most frequent species isolated from humans; other more commonly isolated pathogenic species include *C. parapsilosis*, *C. tropicalis*, *C. glabrata* (previously known as *Torulopsis glabrata*), and *C. krusei*. Less commonly, invasive *Candida* infections (ICIs) may be due to *C. guilliermondii*, *C. lusitaniae*, *C. dublinensis*, *C. pelliculosa*, *C. kefyr*, *C. rugosa*, *C. famata*, *C. norvegensis*, *C. orthopsilosis*, *C. metapsilosis*, *C. nivariensis*, and *C. inconspicua*. Previously classified as *C. albicans*, *C. dublinensis* has only more recently been appreciated as a distinct species.[301]

Candida spp. grow well on routine agar and do not require special fungal media for isolation. The color of *Candida* colonies may vary from cream to yellow, and the texture may be smooth to wrinkled and glistening or dry, depending on the species. Under optimal growth conditions, yeast grow in log phase as unicellular budding yeast forms (blastospores) that are spherical to ovoid and measure 2.5 to 6 μm in diameter.[111,282] Pseudohyphae are polarized growths that form when budding daughter cells remain attached to the parent cell wall, forming elongated, often branching forms with constricted septal junctions between cells. In contrast, true hyphae are formed from yeast cells or as branches from existing hyphae and are characterized by defined septa that divide the hyphae into separate fungal units. As previously mentioned, true hyphae are a feature of *C. albicans* and *C. dublinensis* and grow in the presence of serum at 37° C and at pH 7.[179,282,300] Germ tube formation is a precursor to hyphae and can be induced as a test for rapid, presumptive identification of *C. albicans*, although false-positive and false-negative results may occur.[125,280] In contrast, *C. glabrata* only exists as blastoconidia (yeast).

Identification of the most common *Candida* spp. is possible based on carbohydrate assimilation and fermentation testing, in addition to morphology. Commercially available kits such as API 20C AUX, the Auxacolor2, and the Uni-Yeast Tek kit utilize changes in turbidity or the production of color through enzymatic reactions to create specific biochemical profiles that permit the rapid identification of the more common *Candida* spp.[80] The advent of chromogenic agar (e.g., CHROMagar *Candida*) that incorporates substrates linked to chemical dyes in a solid medium has been particularly advantageous in allowing presumptive identification of clinically important *Candida* spp., including *C. albicans* (green smooth colonies), *C. tropicalis* (metallic blue colonies with a pink halo), *C. krusei* (pale rose flat colonies), and *C. glabrata* (white or purple smooth glistening colonies), although misidentification of less common *Candida* spp. as *C. glabrata* has been reported.[80,125,241] Chromogenic agar permits identification in as early as 24 to 48 hours and is particularly valuable in detecting coinfection with different *Candida* spp., which ordinarily may be missed during conventional subculture on a solid medium.[80,282]

EPIDEMIOLOGY

Candida is the most important cause of fungal infection in health care settings, broadly affecting a growing population of immunocompromised patients, in addition to patients with critical illness, prematurity, or complex chronic conditions. The incidence of invasive candidiasis in the United States has risen dramatically over the past 3 decades, with rates of nosocomial bloodstream infection (BSI) due to *Candida* quadrupling between 1980 and

1989.[23] More recent data demonstrated a continued 50 percent increase in candidemia-related hospitalizations in the United States between 2000 and 2005.[349] *Candida* spp. are now the third most common cause of nosocomial BSI in children, with an incidence of 43 cases per 100,000 pediatric admissions (95% confidence interval [CI], 35 to 52 per 100,000 pediatric admissions).[341,344]

The increasing burden of invasive candidiasis is especially notable given the significant morbidity and mortality associated with candidemia. Crude mortality rates in children with candidemia have been reported to range from 10.1 to 26 percent, although the attributable mortality of candidemia in children has been estimated to be 10 percent.* Evidence of dissemination has been estimated to occur in 8.3 to 17 percent of children with candidemia,[42,76,314,346] with organ involvement most commonly occurring in the lungs (45.2% to 58%), liver (23%), kidney (16%), brain (12%), heart (8% to 22.3%), spleen (8%), and eyes (3.2 to 8%).[76,346] In addition to severe clinical outcomes, candidemia in children results in a considerable increase in health care resource utilization. A retrospective cohort study using national administrative data reported a mean 21.1 day increase in total hospital length of stay (95% CI, 14.4 to 27.8 days) and a mean $92,266 increase in total charges per hospitalization (95% CI, $65,058 to $119,474).[344]

C. albicans is the predominant *Candida* spp. colonizing the gastrointestinal and genital mucosa and can be found on the mucous membranes of 30 to 60 percent of humans.[179] Not surprisingly, *C. albicans* is the most common cause of invasive fungal disease in children. A prospective (1995-1997) observational study of candidemia involving 34 U.S. tertiary-care medical centers reported *C. albicans* as the most commonly isolated species in children younger than 13 years of age, comprising 49 percent of all pediatric isolates.[225] More recently, a prospective (2007-2011) international epidemiologic study of pediatric invasive candidiasis utilizing data from 42 sites (23 in the United States, 19 in 15 other countries), recovered *C. albicans* from 44 percent of all isolates.[296] Despite its propensity to cause severe disease, *C. albicans*–specific response rates are good, with 71 percent of pediatric patients having successful outcomes (defined as microbiologic clearance and either improvement in or complete resolution of all attributable symptoms).[296]

The incidence of *C. parapsilosis* has increased in the past few decades,[236] with it becoming the second most common species isolated from children with invasive candidiasis. Two large international prospective cohort studies have reported *C. parapsilosis* as causing 22.2 to 34 percent of invasive candidiasis/candidemia in children,[225,296] which is consistent with findings from single-center pediatric studies.[75,184,294,348] In contrast to *C. albicans* (an obligate human commensal), *C. parapsilosis* can be found in a wider variety of animals, as well as the environment.[46,203,262,293,312] However, it can also be found as part of normal human skin flora. In one study examining the density of flora at various sites of the hands of 26 healthy adult volunteers, yeasts were present in 65.4 percent of samples, with *C. parapsilosis* being the most frequently isolated.[198] Not surprisingly, carriage of *C. parapsilosis* on the hands of health care workers has been associated with nosocomial outbreaks of infection with *C. parapsilosis*, with significant implications for infection prevention and control measures.[312] Experimental animal models have demonstrated *C. parapsilosis* is associated with limited virulence compared with that of *C. albicans* and *C. tropicalis*,[335] and clinical studies also have shown higher rates of treatment success in children and reduced mortality rates in adults with *C. parapsilosis* fungemia/invasive disease compared with other *Candida* spp.[225,296]

C. glabrata and *C. krusei* are less common causes of candidemia and invasive candidiasis in children. In adults, however, *C. glabrata* is the second most common cause of invasive candidiasis.[42,126,225] Importantly, *C. glabrata* and *C. krusei* are notable for their reduced susceptibility to triazole antifungal agents. *C. krusei* is intrinsically resistant to fluconazole, although most isolates are susceptible to voriconazole.[234,235] *C. glabrata* exhibits variable susceptibility to fluconazole, with cross resistance with the extended spectrum triazoles being well described.[233]

C. tropicalis is an infrequent cause of invasive candidiasis in children and predominantly occurs in oncologic populations.[169] Data from animal models indicate that *C. tropicalis* is similar in virulence to *C. albicans*, although clinically *C. tropicalis* appears to display a higher propensity for dissemination in neutropenic hosts than *C. albicans*.[165,169] *C. lusitaniae*, *C. guilliermondii*, and *C. dublinensis* are all uncommonly found in children with invasive candidiasis.

Outside the neonatal population, data on the risk factors for the development of candidemia in children remain limited. In a single-center case-control study examining risk factors for candidemia in a children's hospital, patients were more likely to have had central venous catheters, use of topical antifungal agents before candidemia, and longer exposure to hyperalimentation compared with control subjects; however, only unadjusted analysis was performed, limiting interpretation of the results.[184] A single-center, population-based, case-control study examined risk factors for candidemia in pediatric patients admitted to a pediatric intensive care unit.[348] On multivariable analysis, receipt of vancomycin or of an antimicrobial agent with anaerobic activity for 4 or more days was independently associated with a significant increased risk for candidemia (adjusted odds ratio [OR], 6.19; 95% CI, 2.40 to 15.99; and adjusted OR, 2.03; 95% CI, 1.47 to 8.38, respectively). Other significant risk factors included malignancy (OR, 4.02; 95% CI, 1.23 to 13.11) and presence of a central venous catheter (adjusted OR, 30.45; 95% CI, 7.76 to 119.49). Receipt of hyperalimentation was found to be significantly associated with candidemia on unadjusted analysis, but the association became nonsignificant after inclusion into the multivariable model. Interestingly, although hyperalimentation was not an independent risk factor for candidemia, it was found to be a significant contributor in predicting risk for candidemia. With the use of a predictive model including vancomycin, anaerobic-spectrum antibiotics, malignancy, presence of a central venous catheter, and hyperalimentation, it was found that children with three or more risk factors had between a 10 and 46 percent predicted

*References 42, 184, 225, 296, 344, 345.

probability of developing candidemia.[348] Similar risk factors of presence of central venous catheters, arterial lines, hyperalimentation, and prolonged antibiotic exposure were identified in another study performed in a population of pediatric patients in an intensive care unit.[89]

Risk factors for disseminated candidiasis in children have been examined by several investigators.[76,88,346] In a case-control study of children with candidemia, persistently positive blood cultures for *Candida* after more than 3 days with a central venous catheter in place and associated with immunosuppression (neutropenia, use of immunosuppressant medications, or history of bone marrow transplant) were found to be independent risk factors for the development of disseminated candidiasis.[346] Another case-control study of children with candidemia identified receipt of vasopressors, prolonged candidemia (≥3 days) after central venous catheter removal, and neutropenia to be independently associated with disseminated candidiasis.[76] Similarly, in an unadjusted analysis of children hospitalized with candidemia, prolonged candidemia and presence of an immunocompromising condition were found to be associated with dissemination.[88] Risk factors for mortality among pediatric patients have also been examined, with location in a pediatric intensive care unit at the time of infection, presence of an arterial catheter, tracheal intubation, and neutropenia being significantly associated with death.[225,345] With the exception of neutropenia, these risk factors are likely markers for severe illness.

PATHOGENESIS

Pathogen Factors

The virulence of a pathogen is an important determinant of the ability of a microorganism to inflict host cell damage and cause disease. Because *C. albicans* is the most common cause of invasive candidiasis and is thought to be the most pathogenic of the *Candida* spp.,[282] virulence factors have been best studied in this species. Consequently *C. albicans* will serve as the prototype for this discussion of virulence factors, with exceptions noted for other non–*C. albicans* species when relevant. The pathogenicity of *C. albicans* is mediated by several virulence factors, including adhesion, biofilm formation, secretion of hydrolytic enzymes, and morphology switching, permitting it to become the most successful opportunistic fungal pathogen in humans.

The obligatory first step in the establishment of colonization and subsequent host invasion is the adherence of *C. albicans* to host surfaces and medical devices. Adherence is essential for initiating and maintaining a commensal relationship with the host[259] and is mediated by adhesion biomolecules present at the fungal surface that permit binding to host cells and host-cell ligands. Agglutinin-like sequence proteins (Als) are the most widely expressed adhesins in *C. albicans* and encode a group of glycosyl-phosphatidyl-inositol (GPI)–anchored proteins localized on the outer cell wall layer.[313] Among all Als proteins, Als1p, Als3p, and Als5p appear to play a major role in adherence to host cells.[313] Hyphal wall protein 1 (Hwp1p) is found exclusively at the germ tube surface and is another important adhesin, mediating tight binding to buccal epithelial cells.[292] Cell wall carbohydrates such as glycosylated mannoproteins and sialic acids may also contribute to adherence.[313] Among non–*C. albicans* species, *C. tropicalis* has been shown to also produce Als proteins while *C. glabrata* has been found to produce a group of adhesins encoded by the EPA (epithelial adhesin) gene family. Cell wall adhesin-like proteins have also been discovered in *C. parapsilosis*, although further work on the virulence associated with these proteins is limited.[282]

Biofilm formation forms an important mechanism of virulence for several *Candida* spp., including *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*.[282] The capacity to establish a biofilm plays a significant role in infections involving medical devices such as central venous catheters, urinary catheters, cardiovascular devices, and prosthetic implants.[162,209] *Candida* biofilms are complex communities of yeast, pseudohyphae, and hyphae encased in a self-produced extracellular matrix. The formation, structure, and composition of the biofilm is dependent on the nature of the contact surface, environmental factors, ability to switch morphology from yeast to hyphae, and *Candida* spp.[209,282] Several studies have demonstrated that biofilm growth can dramatically enhance resistance to antifungal agents. Minimal inhibitory concentrations (MICs) of amphotericin B, flucytosine, fluconazole, itraconazole, and ketoconazole against *C. albicans* growing in biofilms have all been shown to be significantly higher (up to 2000 times higher) than the MIC in planktonic *C. albicans*.[117,170,249,307] It has been suggested that biofilm-associated *Candida* organisms acquire resistance due to low growth rate and decreased drug penetration through the matrix; however, studies have not supported these conclusions.[4,17] Instead, antifungal resistance is thought to be conferred by contact-dependent upregulation of genes encoding efflux pumps during the course of biofilm formation, although multiple mechanisms may contribute to antifungal resistance of the *Candida* biofilm.[208,248]

Production of extracellular hydrolytic enzymes is an essential aspect of the pathogenicity of *Candida* spp., facilitating tissue invasion by disruption of the host mucosal membranes and by degrading important immunologic and structural proteins, including immunoglobulin (Ig) G heavy chains, α_2-macroglobulin, β-lactoglobulin, C3 protein, lactoperoxidase, collagen, and fibronectin.[238,282] The three most important hydrolytic enzymes produced by *Candida* are the secreted aspartyl proteinases (Saps), phospholipase B enzymes, and lipases.[212,282] The Sap proteins are encoded by a family of 10 SAP genes and are considered key determinants of *Candida* virulence, with level of Sap activity in vitro appearing to correlate with the virulence of *C. albicans*, *C. tropicalis*, and *C. parapsilosis*.[212] Data are limited on the capacity of *C. glabrata* to produce proteinases.

Morphologic conversion between yeast and hyphal forms is believed to play an important role in the dissemination and tissue invasion of *C. albicans*. Initial experiments using animal models suggested that the hyphal form represented the pathogenic state of the organism,

because mutant strains lacking hyphae exhibited impaired ability to invade tissue compared with wild-type *C. albicans*.[54,182] Subsequent data demonstrating constitutively filamentous mutant strains as avirulent led to the hypothesis that it was not the morphologic form per se but the ability to undergo morphology switching that contributed to the pathogenicity of *C. albicans*.[43] More recently, a mouse model of hematogenously disseminated candidiasis using a mutant *C. albicans* strain that permitted external modulation of morphology switching demonstrated that both yeast and hyphae were important stages in the development of invasive candidiasis.[271] Seville and coworkers were able to demonstrate in a mouse model of disseminated candidiasis that mice injected with this strain under conditions permitting hyphal development succumbed to infection, whereas all those injected under conditions inhibiting the formation of hyphae survived, indicating that filamentous formation was a critical factor in pathogenicity.[271] However, fungal burdens were almost identical in animals injected with strains under hyphae-permitting and hyphae-repressing conditions, suggesting that yeast forms were important for initial extravasation and dissemination in the host. Interestingly, histopathologic examination of kidneys recovered from mice exposed to hyphae-repressing conditions revealed no significant damage despite organ invasion, with all of the invading fungal cells present only in yeast form.[271]

Host Factors

Infection due to *Candida* spp. manifests primarily as mucosal and disseminated candidiasis. The immune responses to these different compartments are distinct, as evidenced by the patterns of clinical manifestations of candidiasis in individuals with immunodeficiency affecting different aspects of the immune response. Both innate and adaptive arms of the immune system play key roles in the immune response to *Candida* and are influenced by the site of infection. A detailed discussion of the host immune response to *Candida* is beyond the scope of this chapter, but several excellent reviews are available for more in-depth coverage.[66,108,321]

The initial host response to infection with *Candida* involves recognition of pathogen- associated molecular patterns (PAMPs) present on the fungal cell wall.[321] Pattern recognition receptors (PRRs) on the surface of innate immune cells perform this function, and several classes of PRRs have been identified in the induction of innate host responses to *Candida*, including the Toll-like receptors (TLRs), the C-type lectin receptors (CLRs), and the NOD-like receptors (NLRs).[108] Recognition of yeast and hyphae morphologies is differential, with each form being recognized by different host receptors.[69,322] TLR2, TLR4, and TLR9 have all been implicated as having a key role in recognition of *Candida* and induction of proinflammatory cytokines.[321] CLRs are a large family of receptors with a shared ability to recognize microbial polysaccharides and are thought to be the most important PRR family in the recognition of fungi.[108] Within this family, dectin 1, dectin 2, macrophage mannose receptor 1, dendritic cell-specific ICAM3–grabbing non-integrin (DC-SIGN), and soluble mannose-binding lectin serve to recognize *C. albicans* and are involved in phagocytosis, production of inflammatory cytokines, neutrophil recruitment and activation, and complement activation.[108,322] Finally, NLRs located within the cytoplasm function to activate inflammasomes (protein platforms that induce the activation of interleukin [IL]-1β and IL-18 through caspase 1) and may have an important role in both T-helper cell (Th) type 1 responses and Th17-type responses against *C. albicans*, which are important for containing disseminated and mucosal infection, respectively.[320,328]

Beyond the role of PRRs in the innate immune response to *Candida*, neutrophils, macrophages, and monocytes are all critically important antifungal effector cells, as evidenced by the high risk for disseminated disease in neutropenic patients.[52,76,136,346] Phagocytes already residing in infected organs are involved in the killing of invading *Candida*, whereas neutrophils and monocytes are recruited to the site of infection. The large size of hyphae may preclude engulfment of *Candida* by these phagocytes; however, recent studies have demonstrated the important role of neutrophil extracellular traps (NETs) in the killing of both hyphal and yeast forms.[318,319]

The adaptive immune system plays a critical role in protecting against oropharyngeal candidiasis. Until recently, protective adaptive responses were thought to be mediated by $CD4^+$ Th1 effector cells. However, with the discovery in 2005 of a subset of $CD4^+$ Th17 cells,[115,173,226] it was discovered that Th17-type responses play a crucial role in controlling *C. albicans* at the mucosa.[66,108] This finding is supported by the observation that patients with chronic mucocutaneous candidiasis (CMC)—either as isolated autosomal-dominant CMC (AD-CMC) or as part of a primary immunodeficiency due to hyper-IgE syndrome or autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy (APECED)—share a common defect related to an inadequate Th17 response. The major genetic defect causing hyper-IgE syndrome is a mutation in the gene encoding STAT3 transcription factor, resulting in impaired Th17 differentiation.[66] Most patients with AD-CMC have a defect in the *STAT1* gene, which is involved in the IL-23/IL-23R pathway crucial for induction of Th17 responses. The mechanism of susceptibility to *Candida* infection in patients with APECED has only recently been elucidated, with the discovery of neutralizing autoantibodies against Th17-type cytokines in affected patients.[158,244]

With respect to hematogenously disseminated candidiasis, the portal of entry of *Candida* into the host bloodstream has been the source of much debate, with both the gastrointestinal tract and the skin being proposed as the source of acquisition. In a remarkable experiment performed in 1969, an investigator ingested 10^{12} *C. albicans* cells and underwent serial blood and urine sampling. After experiencing a transient febrile reaction with chills, *C. albicans* was isolated from the blood and urine within 3 hours of ingestion, forming the only experimental human evidence that persorption through the gut

is the source of candidemia.[168] Animal models of candidal gut colonization and subsequent development of disseminated candidiasis after immunosuppression also support the hypothesis of an intestinal source for candidemia.[211,269,317] In a systematic review of the literature investigating skin or intestinal colonization as the source of candidemia, the authors identified more compelling data in support of intestinal colonization as the primary source.[217] This conclusion is consistent with findings of anaerobic spectrum antibiotics as a significant risk factor for candidemia,[348] because antibiotics with anaerobic activity have been shown to permit a higher and more sustained increase in intestinal colonization with yeasts.[267] However, other portals of entry besides the intestine are important for the development of invasive candidiasis, as evidenced by the increased incidence of candidemia in burn patients[229] and through intravascular devices.[162]

CLINICAL MANIFESTATIONS

Infections due to *Candida* spp. have myriad presentations and depend largely on the immune status of the host as well as the existence of predisposing risk factors. The clinical manifestations of candidiasis in the neonatal population are unique and are discussed separately. Owing to the differences in pathogenesis of candidal diseases, the following section is subdivided into discussions of superficial (mucocutaneous) and invasive disease manifestations.

Superficial Candidiasis

Oropharyngeal Candidiasis

Oropharyngeal candidiasis is one of the most common manifestations of *Candida* infection. Pediatric populations in which oropharyngeal candidiasis can be seen most frequently include young infants, as well as patients with human immunodeficiency virus (HIV) infection, malignancy, or other causes of immunodeficiency, although occurrence in healthy children with other risk factors (e.g., inhaled corticosteroid exposure,[98] broad-spectrum antibiotic exposure[291]) is not uncommon.

The most common manifestation of oropharyngeal candidiasis in children is pseudomembranous candidiasis, or thrush. Pseudomembranous candidiasis is characterized by the presence of white to creamy-colored plaques on the buccal mucosa, tongue, and gums, although less frequently the soft palate, uvula, and tonsils also may be involved. These lesions may be associated with pain and dysphagia, although frequently are asymptomatic.[323] The adherent plaques may be removed by gentle scraping, revealing an erythematous base. Histologic examination of these membranes reveals epithelial cells together with yeast and filamentous forms of *Candida*.[339] Aside from pseudomembranous candidiasis, other forms include erythematous atrophic candidiasis and angular cheilitis. Erythematous atrophic candidiasis most frequently presents as painful erythematous lesions on the dorsum of the tongue and palate, although the buccal mucosa may also be involved. This form is commonly associated with prior broad-spectrum antibiotic exposure and may spontaneously resolve once antibiotics are discontinued and normal bacterial flora restored.[339] Angular cheilitis may appear alone or in conjunction with pseudomembranous or erythematous atrophic candidiasis and is characterized by painful erythema and fissuring at the angles of the mouth. *C. albicans* is the most common species isolated in children with oropharyngeal candidiasis, although *C. tropicalis*, *C. parapsilosis*, and *C. glabrata* also may be found.[100,132,199,221]

Esophageal Candidiasis

Esophageal candidiasis is most commonly seen in severely immunocompromised children and is an acquired immunodeficiency syndrome (AIDS)-defining illness in children infected with HIV. Similar to oropharyngeal candidiasis, other risk factors include immunosuppression due to malignancy, transplantation, or primary immunodeficiency, in addition to corticosteroid exposure (inhaled or systemic) and use of broad-spectrum antibiotics.* Although frequently accompanied by oropharyngeal candidiasis, the absence of oral involvement does not exclude *Candida* esophagitis. In a case series of HIV-infected children receiving highly active antiretroviral therapy, 28 percent of *Candida* esophagitis episodes were not associated with concomitant oropharyngeal candidiasis.[56] Common presenting symptoms include odynophagia, retrosternal/epigastric pain, and dysphagia, although in severely immunocompromised children fever may occasionally occur.[55,56,323]

Findings on endoscopic examination include the presence of white plaques, erythema, and edema of the esophageal mucosa, ulceration, and, in severe cases, strictures. *Candida* spp. may be cultured from endoscopic brushings, although positive isolation does not distinguish from commensal *Candida* colonization. Reliable diagnosis may be made by histologic evidence of tissue invasion in biopsy material.[323] In addition to esophageal endoscopy, barium contrast studies of the esophagus may reveal findings of shaggy mucosal irregularities and nodular filling defects suggestive of esophageal candidiasis, although other radiographic patterns can be seen.[106,176,177]

Vulvovaginal Candidiasis

Vulvovaginal candidiasis is extremely rare before menarche but increases in frequency during late adolescence and adulthood. Between 50 and 70 percent of women may be diagnosed with this condition during their lifetime.[92,290] Risk factors for the development of vulvovaginal candidiasis include diabetes mellitus, pregnancy, oral contraceptives, and use of broad-spectrum antibiotics.[2,289] Unlike other mucosal sites of *Candida* infection, HIV seropositivity is not a clear risk factor for vulvovaginal candidiasis, although rates of *Candida* vaginal colonization have been shown to be significantly higher in HIV-seropositive versus HIV-seronegative at-risk women.[2,275]

*References 13, 16, 112, 137, 160, 276.

Common symptoms include vaginal pruritus, erythema, dysuria, and an odorless, thick, white, curdlike discharge.[7] The diagnosis can be clinically supported by identification of yeast and pseudohyphae/hyphae seen with potassium hydroxide or saline on microscopy, although the sensitivity of microscopy varies between 38 and 83 percent.[7] Isolation of *Candida* from culture of vaginal secretions does not differentiate infection from colonization.

Cutaneous Candidiasis

Cutaneous candidiasis occurs in moist, occluded cutaneous sites that promote maceration of the skin and superficial invasion by *Candida*. Diaper dermatitis is a common manifestation that typically occurs in infants, with peak prevalence during the second to fourth months of life.[124] The lesions of *Candida* diaper dermatitis generally erupt in the perianal area, spreading to involve the perineum and inguinal folds, with confluent erosions, satellite pustules on an erythematous base, and marginal scaling. In infants with darker skin pigmentation, lesions may show hypopigmentation. Evidence of *Candida* colonization in the stool is present in up to 90 percent of children,[251] suggesting that the gastrointestinal tract is the most important source for *Candida* diaper dermatitis. Oropharyngeal candidiasis may be accompanied by diaper dermatitis in 34 to 52 percent of patients, indicating that diaper dermatitis may be the result of successive oral and gastrointestinal colonization.[124] Cutaneous candidiasis may also manifest as intertrigo, occurring in the inframammary, axillary, inguinal, perineal, intergluteal, and interdigital regions. Lesions may appear as pustules on an erythematous base with central confluence, as satellite lesions, and with associated pruritus and tenderness.[342] Folliculitis and balanitis are less common presentations of cutaneous infection.

Chronic Mucocutaneous Candidiasis

Chronic mucocutaneous candidiasis is a clinical syndrome characterized by persistent and/or recurrent *Candida* infections of the oropharynx, skin, nails, and, uncommonly, esophageal candidiasis and laryngitis.[157,161] In some patients, chronic localized *Candida* granulomas may occur, presenting as thick, adherent, hyperkeratotic "warty" lesions on the scalp and face.[157,342] Rather than a single entity, CMC is a manifestation of a number of heterogeneous disorders unified largely by a common downstream defect in mucosal *C. albicans*–induced Th17-type response,[108] although some exceptions exist. CMC can present as an isolated disease (autosomal-dominant chronic disseminated candidiasis [CDC]), or as part of a primary immunodeficiency disorder such as hyper-IgE syndrome, APECED, severe combined immunodeficiency (SCID), DOCK8 deficiency, and deficiencies of IL-12, IL-17, and IL-23.[326] CMC typically presents in infancy or early childhood and may be associated with recalcitrant or relapsing candidal disease despite antifungal therapy. Despite extensive cutaneous and mucosal involvement, reports in the literature of invasive *Candida* infections are rare.

Invasive Candidiasis

Candidemia

The clinical manifestations of candidemia may vary from nonspecific signs of fever and malaise to septic shock and multiple organ dysfunction syndrome.[163] Additional manifestations may be apparent as a result of dissemination through the bloodstream to distal sites such as the central nervous system, the lungs, and kidneys, with the risk for dissemination increasing with the number of days of blood culture positivity and immunosuppression.[1,346] As previously mentioned, prolonged exposure to antibiotics, placement of a central venous catheter or arterial lines, malignancy, mechanical ventilation, and hyperalimentation have all been cited as risk factors for candidemia.[89,184,348]

Acute Disseminated Candidiasis

Endovascular Candidiasis. Endovascular candidiasis presents most commonly as infective endocarditis, although septic thrombophlebitis and mycotic aneurysm are less frequently seen.[202,268] Although data are limited, the frequency of *Candida* endocarditis appears to be increasing over the past 2 decades,[258,306] mirroring the increasing incidence of candidemia reported in the literature. *Candida* may be the most common cause of health care–associated infective endocarditis in children, with one single center study reporting isolation of *Candida* spp. from 27 percent of children with nosocomial infective endocarditis.[191] Clinical manifestations are similar to those of non-*Candida* endocarditis and may include fever, onset of a new or changing murmur on auscultation, hepatosplenomegaly, and congestive heart failure in addition to the embolic and immunologic phenomenon seen with infective endocarditis. Transthoracic or transesophageal echocardiography may reveal the presence of intracardiac thrombus or valvular vegetations.

Risk factors cited for *Candida* endocarditis include prematurity, congenital or acquired heart defects or endovascular prostheses, presence of a central venous catheter, immunosuppression, broad-spectrum antibiotic exposure, and hyperalimentation.[202,306] In a literature review of pediatric *Candida* endocarditis, the frequency of involvement of right-sided intracardiac structures (e.g., right atrium, tricuspid valve) was three times that of left-sided structures for patients in whom the site of intracardiac lesion was reported,[202] likely reflecting the contributory role of central venous catheters as both a source of endothelial trauma and a hematogenous portal of entry of *Candida* in the development of this complication.

Cardiac Candidiasis. *Candida* spp. may be a rare cause of pericarditis and myocarditis in children.[331] At least six cases of pediatric *Candida* pericarditis and five cases of *Candida* myocarditis have been reported in the English literature.[70,274,331] *Candida* pericarditis and myocarditis may occur in the setting of recent cardiothoracic surgery, contiguous focus of pleural or mediastinal infection, contiguous intracardiac spread of infection (e.g., *Candida*

endocarditis), or acute hematogenous dissemination.[274,331] Clinical symptoms of *Candida* pericarditis may be subtle but include fever, chest pain, and dyspnea, with evidence of pericardial rub and cardiac tamponade on examination, enlarged cardiac silhouette on chest radiography, and ST-T wave changes on electrocardiography. Blood cultures may not be positive, complicating early recognition.[70,331] Echocardiography will reveal the presence of a pericardial effusion. Candidal invasion and necrosis of the myocardium may be extensive and produce associated signs of congestive heart failure, with electrocardiographic changes suggestive of myocarditis.[331] Mortality rates in the literature have been high, with only 1 of 11 pediatric patients reported with *Candida* pericarditis and myocarditis having survived, although this may have been due to delayed recognition, because diagnosis was made post mortem in the majority of these cases.[70,274,331]

Pulmonary Candidiasis. Three different patterns of invasive pulmonary candidiasis have been described: primary *Candida* bronchopneumonia due to tracheobronchial colonization and aspiration, secondary pneumonia in the setting of disseminated candidiasis, and secondary pneumonia in the setting of septic emboli.[114,141,227] Primary *Candida* bronchopneumonia is rare, is limited largely to neutropenic patients and neonates, and is thought to occur via aspiration of *Candida* into the upper respiratory tract. In autopsy studies of adults with invasive pulmonary disease with no other sites of dissemination or premortem candidemia, a distinct pattern of intra-alveolar hemorrhage, alveolar exudates, necrosis, and microabscesses with evidence of *Candida* may be present, with several cases also showing evidence of esophageal candidiasis.[114] A similar pattern was seen in autopsies of three infants with evidence of airspace invasive candidiasis concurrent with colonization of the upper respiratory tract and oropharynx.[141]

More commonly, invasive pulmonary candidiasis occurs as a consequence of hematogenous dissemination in a patient with candidemia or from showering of septic thrombi from an infected central venous catheter, septic thrombophlebitis, or *Candida* endocarditis. In disseminated candidiasis, pulmonary involvement is typically capillary invasive, with bilateral lung involvement. On microscopic examination, pseudohyphae are seen to invade the pulmonary capillaries, arterioles, and small arteries, thus entering the lung parenchyma. Microabscesses may be present in the lungs as well as other end-organ sites of dissemination. In embolic pulmonary candidiasis the lungs are seeded with infected emboli from an endovascular source, resulting in hemorrhagic infarcts in the periphery of the lungs and septic thrombi in the small- and medium-sized pulmonary arteries. Extrapulmonary thrombi may also be seen in other sites.[141]

The frequency of *Candida* colonization in the oropharynx and gastrointestinal tract, in addition to the frequent occurrence of tracheobronchial colonization in intubated patients in the intensive care unit, makes the use of bronchoalveolar lavage to distinguish colonization from invasive pulmonary candidiasis untenable. Histologic demonstration of invasion of *Candida* within lung tissue is the only widely accepted criterion for the definitive diagnosis of primary *Candida* pneumonia.[227] In contrast, secondary invasive pulmonary candidiasis may be suspected in the setting of candidemia and/or other sites of disseminated disease. The radiographic pattern of primary *Candida* pneumonia has been described as patchy bilateral consolidation with air bronchograms and rarely localized to one segment. In cases of disseminated candidiasis with secondary pulmonary involvement, multiple 3- to 30-mm nodules containing central necrosis with pseudohyphae and budding yeast may be seen through the lung, with consolidation found in advanced disease. Cavitation is not typically seen.[47,141,227]

Candidiasis of the Peritoneum, Gastrointestinal Tract, and Gallbladder. The most common cause of *Candida* peritonitis is peritoneal dialysis (PD)–associated peritonitis; other causes include perforation of an intra-abdominal viscus or surgery-related peritonitis due to an anastomotic leak. Fungal peritonitis has been reported to occur in 3 percent of all peritonitis episodes in children receiving PD, with *Candida* spp. comprising the vast majority of fungal peritonitis.[205,246,333] Similar to other *Candida* health care device–associated infections, colonization with subsequent biofilm formation of the PD catheter is the presumed mechanism of infection. Common presenting signs and symptoms include fever and abdominal pain; sepsis with hemodynamic instability may also be seen, although extraperitoneal dissemination is rare. An elevated leukocyte count greater than 100/mm^3 in the dialysate may be seen, whereas Gram stain may reveal budding yeast forms. Culture of the dialysate will allow identification of the organism. Recent administration of systemic antibiotics has been consistently noted to be a risk factor for the development of PD-associated fungal peritonitis.[246,333] Outcomes are generally good, with a low mortality rate in children of 0 to 6 percent compared with the 13 to 53 percent mortality seen in adults. In addition, 50 to 70 percent are able to continue PD after treatment for fungal peritonitis.[246,333]

Invasive candidiasis of the lower gastrointestinal tract is an uncommon but well-described entity in severely immunocompromised patients, although the literature has been limited to the adult population.[59,200,242,304] Clinical manifestations include invasive candidal enterocolitis with or without pneumatosis and infarction. Patients may have fever, acute abdominal pain, distention, emesis, and bloody diarrhea. Grossly, hemorrhagic ulcerations and nodules may be seen, occasionally with transmural infarction. White plaques may be seen and mistaken for pseudomembranous colitis. On histopathology, periodic acid–Schiff and Gomori methenamine silver stains may reveal budding yeast and filamentous forms invading the mucosa and submucosa.

In addition to primary and secondary *Candida* peritonitis and invasive intestinal candidiasis, *Candida* has also been described to cause acalculous candidal cholecystitis, as well as biliary obstruction due to *Candida* mycetoma.[48,200]

Candidiasis of the Urinary Tract. The presence of asymptomatic candiduria in children is common,

occurring in 8 percent of healthy males and 12 percent of healthy females. The prevalence increases with exposure to antibiotic therapy, with rates of isolation of up to 39 percent of specimens.[107] In the majority of asymptomatic individuals, the presence of yeast in the urine indicates contamination or colonization. In patients with candidemia, isolation of *Candida* in the urine may signify antegrade infection from hematogenous seeding of yeast to the renal parenchyma, with subsequent development of renal abscesses, renal mycetomas (fungus balls), collecting system obstruction, and renal failure. The clinical significance of candiduria in the setting of an indwelling urinary catheter is less clear, because *Candida* in the urine may signify a catheter-associated urinary tract infection (UTI) or simply colonization and biofilm formation of the catheter in the absence of true infection. Clinical manifestations of *Candida* UTIs may be indistinguishable from those of bacterial UTIs, with dysuria, frequency, urgency, and, in the setting of pyelonephritis, fever and flank pain. In patients with indwelling catheters, symptoms may be less recognizable, either due to a lack of dysuria or due to sedation status impeding the patient's ability to communicate symptoms in the intensive care unit, although suprapubic discomfort may be reported.[142]

In contrast to bacterial UTIs, quantification of *Candida* in the urine does not reliably predict the presence of UTI, based on pediatric and adult studies.[58,142] Although the mean colony counts of children with asymptomatic candiduria may exceed 10^3 colony-forming units (CFU)/mL,[107] biopsy-proven renal infection has been found with colony counts as low as 10^4 CFU/mL in patients without indwelling catheters, whereas in patients with indwelling catheters there may be no correlation between colony count and biopsy-proven renal infection.[142] Although pyuria may be a helpful discriminator of *Candida* UTI in noncatheterized patients, it may also be present in patients with indwelling catheters in the absence of infection.

Given the frequency of perineal *Candida* colonization, asymptomatic patients with *Candida* isolated from the urine should have a repeat midstream clean-catch urine sample sent to verify candiduria. In patients with indwelling catheters, a repeat urine specimen from a newly placed catheter will help to distinguish candiduria from colonization of the catheter. Urinalysis should be performed, because the presence of pyuria, hematuria, and/or leukocyte esterase may be useful in a noncatheterized patient. In patients who are symptomatic, imaging with renal ultrasonography is the preferred modality for identifying renal parenchymal abnormalities as well as obstruction, because it may be more sensitive for the detection of renal mycetomas than other imaging techniques.[250] Renal mycetomas may appear as hyperechoic, nonshadowing foci, whereas renal abscesses may appear as hypoechoic masses.[45,250] Other imaging modalities to identify pyelonephritis, renal abscesses, and renal mycetomas may include computed tomography (CT), magnetic resonance imaging (MRI), and renal cortical scintigraphy.

Osteoarticular Candidiasis. The frequency of *Candida* osteomyelitis appears to be increasing over the past 4 decades, commensurate with the increase in invasive candidiasis as described earlier.[99] Risk factors cited include presence of a central venous catheter, history of preceding candidemia, prior surgery, use of broad-spectrum antibiotics, and immunosuppression.[99,284] The most common sites of involvement in children (in descending order) are the femur and humerus, with ribs, vertebra, and sternum being much more infrequent. In a review of 207 cases of *Candida* osteomyelitis in children and adults, children were significantly more likely to have femur (OR, 20.5; 95% CI, 8.4 to 48.1) or humerus (OR, 46.3; 95% CI, 12.9 to 169) involvement and significantly less likely to have vertebral involvement (OR, 0.09; 95% CI, 0.04 to 0.25). Involvement of more than one site is common, with 84 percent of cases having two or more bones infected.[99] The clinical presentation of *Candida* osteomyelitis is indolent, with 90 percent reporting local pain, tenderness, erythema, and swelling lasting several weeks to months. Fever may be present in a minority of cases. Draining sinus tracts are more likely to occur with nonvertebral osteomyelitis (32%). An elevated erythrocyte sedimentation rate and C-reactive protein level are commonly seen. Imaging with radionuclide scanning may reveal multiple sites of involvement; MRI may reveal bone destruction, soft tissue extension, and abscess formation, with decreased signal intensity on T1-weighted imaging and increased signal intensity on T2-weighted imaging.[99] In the absence of candidemia, biopsy of the involved site(s) with culture and histopathology stains may be necessary to secure the diagnosis.

Up to 85 percent of cases of pediatric fungal arthritis occur in infants younger than 6 months of age.[302] Arthritis concomitant with bone involvement has been reported to occur in 11 to 80 percent of cases, with the most common synovial joints infected being the knee (11%) and hip (5%).[82,99,302] Infection originates hematogenously, with seeding of the synovium and/or metaphyseal vessels resulting in septic arthritis and osteomyelitis, respectively. *Candida* septic arthritis has been reported to manifest several months to a year after the initial episode of candidemia.[116,302] Although two thirds of patients may have acute symptoms of fever and painful swelling of the joint, *Candida* arthritis may also present indolently. Arthrocentesis of the affected joint may reveal leukocyte counts of 7,500 to 151,000/mm^3 with a neutrophilic predominance; *Candida* may be isolated from cultures of the synovial fluid. Histopathologic examination of the synovium may reveal a nonspecific mononuclear inflammatory infiltrate with thickening of the synovium. In contrast to other fungal arthritides, granuloma formation is uncommon with *Candida* arthritis.[82,302]

Central Nervous System Candidiasis. *Candida* infection of the central nervous system (CNS) can involve any part of the brain or spinal cord, manifesting as acute or chronic meningitis, ventriculitis, microabscesses, or macroabscesses. CNS involvement is most commonly a complication of hematogenous spread, occurring in 12 percent of children with candidemia. Up to 66 percent of patients with *Candida* infection of the CNS will have had a recent or concurrent positive blood culture at the

time of diagnosis.[113,197,346] Involvement of the CNS through direct inoculation at the time of surgery or through infection of an intracranial device (e.g., ventriculoperitoneal shunt, external ventricular drain) may also occur. Outside the neonatal period, patient populations most at risk for *Candida* CNS infection include patients with hematologic malignancy, hematopoietic transplant patients, and neurosurgical patients.[103,113,197]

The clinical manifestations of CNS candidiasis vary depending on the patient population and the type of involvement. Profound immunosuppression with neutropenia and consequent limited ability to mount an inflammatory response may lead to subtle symptoms in oncologic and transplant patients with candidal meningitis and brain abscesses. In a single-center case series of 12 children with leukemia who were diagnosed with candidal meningitis, 42 percent were asymptomatic with the exception of fever, which occurred in all of the patients.[197] Only 50 percent exhibited an altered level of consciousness, whereas other symptoms included seizures (33%), nuchal rigidity (25%), headache (25%), and cranial nerve abnormalities (17%). Similarly, in a case series of 19 pediatric and adult bone marrow transplant patients with *Candida* brain abscesses, 84 percent of patients had fever; other less frequent signs and symptoms included altered mental status (42%), cranial nerve abnormalities (26%), seizures (16%), and hemiplegia (11%). Headache, papilledema, nausea, and vomiting were uncommon, and nearly one third had no signs or symptoms, with diagnosis being made post mortem.[113] In both studies, findings of cerebrospinal fluid (CSF) analysis were often unremarkable, with at least half of subjects in each series lacking pleocytosis, with mildly elevated protein levels and hypoglycorrhachia in only a minority of patients. In those with CSF pleocytosis, a neutrophilic predominance is typically seen,[10,65,113,183] although a mononuclear predominance may be seen in neutropenic patients.[197] MRI appears to be more sensitive than CT in the detection of brain abscesses; however, 60 percent of patients with *Candida* brain abscesses may have normal scans, likely related to the difficulty in detecting microabscesses on imaging. *Candida* frequently produces multiple brain abscesses, with half of affected patients having three or more lesions based on autopsy evaluation.[113] Dissemination of *Candida* to other organs is frequently seen in addition to intracranial infection. Outcomes of CNS candidiasis are poor, with an associated 30 to 100 percent mortality.[103,113,131,197]

In patients with intracranial devices, isolation of *Candida* from the CSF may represent colonization of the device or true intracranial infection. In a case series of 21 neurosurgical patients from whom *Candida* was isolated from CSF, 9 patients with a single CSF culture did not receive antifungal therapy, with 4 undergoing removal of the intracranial drain as the only intervention.[103] It is worthwhile to mention that these patients also had normal CSF parameters and were clinically asymptomatic. In contrast, 10 patients had multiple positive CSF cultures and were treated for *Candida* meningitis; most were symptomatic, with lethargy, fever, meningismus, focal neurologic signs, and neutrophilic pleocytosis. Isolation of *Candida* as a contaminant may occur even in the absence of an intracranial device. In a single-center case series of 23 infants and children from a single institution with fungi isolated from the CSF, 6 patients—the majority of whom did not have an intracranial device, and of whom 5 had a negative CSF culture on repeat sampling—were considered to have *Candida* as a contaminant and did not receive antifungal therapy, with a good outcome.[10] Both of these studies highlight the importance of repeated CSF cultures as well as presence of clinical symptoms in the interpretation of *Candida* isolated from the CSF.

Ocular Candidiasis. *Candida* may involve the eye during the course of candidemia and subsequent disseminated disease, with involvement reported in up to 8 percent of children with disseminated disease.[346] Endophthalmitis may be initiated by hematogenous seeding of the choroid and retina through the small vessels, with subsequent chorioretinitis manifesting as white chorioretinal lesions, or "fluff balls," on the posterior fundus.[19] Vitritis is marked by extension of inflammation into the vitreous. Hypopyon, scleritis, and optic nerve involvement may also occur. In patients who are able to report symptoms, "floaters" and blurred vision are the most common complaint.[279]

Chronic Disseminated Candidiasis

Chronic disseminated candidiasis, also known as hepatosplenic candidiasis, is a distinct form of invasive candidiasis seen primarily in patients with leukemia and treatment-induced neutropenia. Involvement predominantly occurs in the liver, spleen, and occasionally other organs. *Candida* colonization of the gastrointestinal tract with subsequent dissemination through the portal venous circulation during periods of neutropenia is thought to be the predominant cause of this disease entity. Prolonged neutropenia is the most-cited risk factor for CDC; other reported risk factors include younger age and exposure to quinolone prophylaxis.[193,266] CDC most commonly presents in the course of neutrophil recovery and thus is thought to represent a form of immune reconstitution syndrome against invasive fungal infection. Symptoms of CDC include persistent fever (85%) and right upper quadrant pain (57%).[303] The most common laboratory finding is a persistent and significantly elevated serum alkaline phosphatase level (median duration 130 days); serum bilirubin and transaminase levels may exhibit brief elevation.[303] Blood cultures may be positive in less than 20 percent of patients with CDC, necessitating the need for biopsy and identification of *Candida* elements on histopathology or culture to confirm the diagnosis. CT has been shown to be more sensitive than ultrasonography for the detection of hepatosplenic lesions, although MRI may be superior to both imaging modalities.[193,277] Lesions are typically not apparent until neutrophil recovery and manifest as "wheel in wheel" target lesions composed of a central necrotic focus of yeast and filamentous forms with a surrounding rim of intense inflammatory infiltrate.[303] Serial imaging with CT or ultrasound is necessary to follow the resolution of lesions on antifungal therapy; lesions may decrease in size or disappear during

subsequent periods of neutropenia, underscoring the importance of follow-up imaging during neutrophil recovery.[231]

DIAGNOSIS

Culture

Isolation of *Candida* spp. from cultures of blood or other sterile sites is the gold standard of laboratory-based microbiologic confirmation of invasive candidiasis. Microbiologic confirmation is often challenging, however, owing to the low sensitivity of blood cultures in patients with invasive candidiasis, with rates of isolation ranging from 0 to 43 percent.[34,223,303] One study demonstrated that even when aerobic blood culture bottles were deliberately spiked with 10^3 *Candida* yeast cells, the yield was only 90 percent.[128]

Based on studies evaluating the yield of blood culture systems inoculated with *Candida*, the sensitivity of commercially available blood culture media to detect candidemia varies by the type of culture medium (aerobic, anaerobic, mycologic), inoculum size, and blood culture system. Depending on the blood culture system used, mycologic blood culture bottles may provide a slight advantage in the recovery of *Candida* owing to the added benefit of a lysing agent to release viable phagocytosed fungal cells, the addition of nutrients essential to fungal growth, as well as antibiotics to suppress competitive bacterial growth in case of coinfection.[127] Time to positivity is dependent on inoculum size as well as specific *Candida* spp.[104] With the use of current automated blood culture systems, most *Candida* spp. have growth detected within 24 to 36 hours, even at densities of 1 colony-forming unit/mL, although *C. glabrata* has been shown to exhibit significantly longer time to positivity.[104,127]

Although *Candida* spp. can grow on routine sheep's blood agar, use of a selective fungal medium such as Sabouraud dextrose agar or chromogenic *Candida* agar may enhance isolation of organisms from other types of body fluid and tissue specimens. However, despite the presence of visible yeast and pseudohyphae in the specimen, tissue cultures may still be negative, as evidenced by a study of 45 oncologic patients with hepatosplenic candidiasis who underwent biopsy, with negative cultures in 56.5 percent.[303] Prior antifungal exposure, nonviable organisms in the specimen, and sampling error may contribute to negative culture results.

Histology

Histopathologic examination of tissue specimens may be an important alternative means to diagnose invasive candidiasis in suspected cases when cultures are negative and may be important in differentiating tissue invasion from surface colonization. Yeast, hyphae, and pseudohyphae may be visible on hematoxylin and eosin stain. Special fungal stains such as Gomori methenamine silver and periodic acid–Schiff should be utilized to enhance identification of *Candida* structures.

Antigen

In light of the poor sensitivity of blood cultures in patients with invasive candidiasis, alternative means of establishing a rapid diagnosis have been pursued. Most promising is the use of the serum marker (1→3)-β-D-glucan (BDG), a major cell wall component of most fungi (except *Mucorales* and *Cryptococcus* spp.). BDG is released into the blood and tissues during invasive fungal infection, allowing the use of BDG antigen-based assays for the indirect diagnosis of invasive candidiasis. Commercially available BDG assays such as Fungitell, Fungitec, Wako turbidimetric assay, and Maruha colorimetric assay harness the ability of BDG to activate a coagulation cascade in amebocyte lysate from the horseshoe crab in what is called the limulus test, allowing measurement of this reaction through colorimetric or turbidimetric methods.[138] A meta-analysis of 23 cohort and case-control studies examined the performance of various BDG assays in predominantly adult populations with heterogeneous characteristics, with a pooled sensitivity of 76.8 percent and pooled specificity of 85.3 percent for proven or probable invasive fungal infection.[138] Study definitions for proven or probable invasive fungal infection were based on the 2008 revised European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions of invasive fungal disease.[72] Another meta-analysis of six cohort studies of the performance of the BDG assay in adults with hematologic malignancies found a lower pooled sensitivity of 49.6 percent but higher specificity of 98.9 percent using two consecutive results as criteria for a positive result.[172] Because of the discriminatory power of this assay, BDG assays have been incorporated into the EORTC/MSG definition of probable invasive fungal disease as indirect evidence meeting mycologic criteria.[72]

In contrast to the adult population, data on the use of BDG assays in children have been limited. Mean serum BDG levels in healthy children have been found to be higher than that in adults (68 pg/mL vs. 48 pg/mL, respectively), with lower specificities at preestablished cutoffs of 60 pg/mL and 80 pg/mL than that reported in the literature for adults.[285] In addition, a prospective study evaluating *Candida* colonization and BDG serum values in children with leukemia demonstrated that 30 percent of colonized children exceeded the assay manufacturer positive cutoff values. None of the children developed evidence of invasive fungal infection, and the authors postulated that absorption of BDG through the gut or other sources of false-positive findings may have contributed to this result.[204] Prospective studies are needed to validate the clinical utility of this test in the pediatric population.

In addition to BDG assays, the use of mannan antigen assays to diagnose invasive candidiasis has been examined. Mannan is an immunogenic polysaccharide that comprises a major component of the cell wall in *Candida* spp. and can be found in serum during infection. Different assays have been developed to detect mannan antigen or anti-mannan antibodies, with enzyme-linked

immunosorbent assays (ELISAs) being the most frequently used in Europe[201]; however, none has yet been approved for use in the United States. A meta-analysis of 14 studies evaluating the test characteristics of the mannan and anti-mannan antibody assays for proven and probable invasive candidiasis (based on EORTC/MSG criteria) demonstrated a pooled sensitivity of 58 percent and specificity of 93 percent for mannan antigen. The anti-mannan antibody assay had a pooled sensitivity of 59 percent and specificity of 83 percent, whereas the pooled sensitivity of using combined mannan antigen and anti-mannan antibody assays was 83 percent, with a specificity of 86 percent.[201] Significant heterogeneity of the studies in the meta-analysis was detected, however, and data have been extremely limited in the pediatric population. False-positive results for both mannan antigen and anti-mannan antibody in patients with *Candida* colonization have also been reported, limiting the utility of these assays.[230,278]

Molecular-Based Testing

Polymerase chain reaction (PCR)–based assays show immense potential for improving our capability for early diagnosis of invasive *Candida* infections. The ribosomal RNA gene cluster contains the 18 Svedburg (S), internal transcribed spacer (ITS) 1 and 2, 5.8S, and 28S regions and is a popular amplification target.[166] The presence of multiple copies of this ribosomal RNA cluster in fungal cells increases the sensitivity of PCR. Because blood samples for culture may contain only a small number of fungal cells (1 cell/mL),[237] PCR-based assays have a tremendous advantage, being able to detect down to 0.22 cell/mL depending on the platform.[166] In addition, DNA derived from both dead and living *Candida* cells can be amplified. Numerous DNA amplification methods have been developed, including nested PCR, multiplex PCR, and real-time (RT) PCR platforms. Most studies have reported sensitivities near or equal to 100 percent, with specificity greater than 90 percent.[166] Two studies that examined the use of PCR to detect invasive candidiasis exclusively in the pediatric population—one in the neonatal and pediatric intensive care units and the other in an oncologic population with fever and neutropenia—also demonstrated high sensitivity, although no explicit control group was included.[73,305] In a prospective study comparing *Candida* RT-PCR, BDG assay, and blood cultures in the diagnosis of invasive candidiasis in 73 hospitalized adults, RT-PCR outperformed BDG for the detection of deep-seated candidiasis (89% vs. 53%, p = .004).[214] PCR-based assays for the detection of *Candida* are a promising test modality, and further prospective validation studies as well as standardization of PCR techniques are needed to determine whether they can be incorporated into clinical practice.

ANTIFUNGAL PROPHYLAXIS

Consideration for antifungal prophylaxis has been given primarily to patients with the greatest risk factors for invasive fungal infection. Data from clinical trials on the efficacy of antifungal prophylaxis in the oncologic, liver transplant, intensive care unit, and surgical populations derive largely from studies in adults; antifungal prophylaxis in the neonatal population is a notable exception and is discussed later in the section on neonatal candidiasis.

Children undergoing chemotherapy for hematologic malignancies are at significantly high risk for developing invasive fungal infections, of which *Candida* spp. is most commonly identified. High-risk categories in this group can be refined further, with acute myeloid leukemia, relapsed acute lymphoblastic leukemia, allogeneic or autologous hematopoietic stem cell transplant, and graft-versus-host disease being associated with rates of invasive fungal infection ranging from 9 to 29 percent.[77] Severe aplastic anemia, although not an oncologic process, is also associated with a significant risk for invasive fungal infection owing to bone marrow failure and prolonged neutropenia.[310] Given the notable frequency of invasive fungal infection and high mortality rates associated with invasive *Candida* infections in these patients,[311] extrapolation from comparative data in adults with regard to the benefits of antifungal prophylaxis is reasonable. In a meta-analysis of 64 randomized, controlled trials of antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem cell transplant, antifungal prophylaxis led to a significant decrease in all-cause mortality, fungal-related death, documented invasive *Candida* infection, and occurrence of any invasive fungal infection, compared with placebo, no treatment, or nonsystemically absorbed antifungal agents. The greatest benefit was seen in allogeneic and autologous hematopoietic stem cell transplant patients.[255] A detailed discussion regarding the comparative effectiveness of different antifungal prophylaxis regimens is beyond the scope of this chapter, but with regard to the type of antifungal prophylaxis, fluconazole is currently the most commonly recommended agent in high-risk children, given its proven survival benefit, low adverse effects, and comparatively less common drug interactions compared with alternatives.[77,309] In patients for whom broader antifungal coverage may be indicated, the extended-spectrum triazoles and the echinocandins are alternative options.[77,308,311] Prophylaxis should be given during induction chemotherapy through resolution of neutropenia, during hematopoietic stem cell transplantation until engraftment, and in patients with severe graft-versus-host disease.[224]

Although pediatric liver transplant recipients have been identified as having high rates of invasive candidiasis,[49,105] pediatric studies to elucidate risk factors are limited, as well as prospective studies showing a clinical benefit from antifungal prophylaxis. Based on adult literature, current Infectious Diseases Society of America recommendations are to administer fluconazole 3 to 6 mg/kg daily or liposomal formulation amphotericin B (LFAB) 1 to 2 mg/kg daily as postoperative prophylaxis for 7 to 14 days for liver transplant recipients with at least two of the following risk factors: retransplantation, creatinine level greater than 2.0 mg/dL, choledochojejunostomy, intraoperative use of more than 40 units of blood products, prolonged intraoperative time (>1 hour), and fungal colonization detected at least 2 days prior and 3 days after transplantation.[224] Small bowel transplant recipients also appear to be at high risk for invasive

fungal infection,[91] and expert consensus recommends fluconazole 6 mg/kg/day for at least 2 weeks after transplantation.[224]

The benefit of antifungal prophylaxis in pediatric intensive care unit patients remains unclear, because more data are needed to prospectively identify those patients at highest risk for invasive candidiasis, as well as to define the benefit of targeted antifungal prophylaxis in this population. Based on data from adults, current guidelines suggest that antifungal prophylaxis may be warranted in intensive care units with very high rates of invasive candidiasis and in patients who are at highest risk (>10%) for invasive candidiasis.[224]

TREATMENT

The approach to treatment of superficial and invasive candidiasis should take into consideration the source of infection (e.g., endovascular, medical-device associated), site(s) of involvement, *Candida* spp. and susceptibility profile, prior antifungal exposure, and clinical status of the host, as well as drug-specific factors such as pharmacokinetic and pharmacodynamics properties and potential for drug interactions. This section focuses on the management of superficial and invasive candidiasis in the non-neonatal pediatric population, and relies heavily on updated clinical practice guidelines issued by the Infectious Diseases Society of America.[224] Detailed discussions of individual properties of antifungal agents are discussed in a separate chapter and will not be reviewed here. However, the choice of antifungal therapy for *Candida* infections is influenced largely by the species causing the infection, because certain *Candida* spp. exhibit characteristic antifungal susceptibility profiles. Knowledge of these susceptibility characteristics may help guide empirical therapy while awaiting antifungal susceptibility testing results. Table 199-1 illustrates the general pattern of susceptibility to antifungal agents among *Candida* spp.

Although the susceptibility of *Candida* spp. may generally be predictable, individual isolates may not necessarily follow this pattern. Although routine susceptibility testing is not typically performed for *C. albicans* owing to its low rate of resistance, expert opinion suggests that susceptibility testing should be performed for *C. glabrata* and for other *Candida* spp. when treatment failure has occurred or when azole resistance is suspected.[224] In general, the echinocandins are preferred for infection due to *C. glabrata*, although transition to fluconazole or voriconazole is possible if susceptibility of the isolate is confirmed. Fluconazole is preferred for the treatment of infections due to *C. parapsilosis*. The MIC values for echinocandins tend to be higher for *C. parapsilosis* than for other common *Candida* spp. owing to the presence of an intrinsic *FKS1* mutation encoding glucan synthase.[36] Although some treatment failures in patients with *C. parapsilosis* infections treated with echinocandins have been reported, the clinical significance of higher echinocandin MIC values in this species remains unclear.

Superficial Candidiasis

Oropharyngeal Candidiasis

Most patients with oropharyngeal candidiasis will respond to topical antifungal therapy, although in patients with moderate to severe disease, systemic antifungal therapy may be required. In children with mild cases of oropharyngeal candidiasis, 4 to 6 mL of nystatin suspension (100,000 U/mL) may be administered four times daily in a swish and swallow.[224] Effort should be made to prolong contact time with the oral mucosa as long as possible before swallowing because nystatin does not adhere well to the oral mucosa.[124] Clotrimazole 10-mg troches administered five times daily or one to two nystatin pastilles (200,000 U each) administered four times daily are alternatives.[224] For infants, 100,000 to 200,000 units can be painted onto the affected areas four times daily. When applicable, sterilization of bottle nipples and pacifiers after each use should be attempted to reduce reintroduction of *Candida* through colonized surfaces. Treatment should be administered for 7 to 14 days.

For moderate to severe disease, systemic antifungal therapy is warranted. Oral fluconazole 3 to 6 mg/kg may be administered for 7 to 14 days. For fluconazole-refractory disease, itraconazole solution 2.5 mg/kg twice daily has been shown to be effective and safe in phase 2 clinical trials in children with cancer, liver transplantation,

TABLE 199-1 **General Patterns of Susceptibility of *Candida* Species**

	Species					
Antifungal Agent	***C. albicans***	***C. tropicalis***	***C. parapsilosis***	***C. glabrata***	***C. krusei***	***C. lusitaniae***
Fluconazole	S	S	S	S-DD to R	R	S
Itraconazole	S	S	S	S-DD to R	S-DD to R	S
Voriconazole	S	S	S	S-DD to R	S	S
Posaconazole	S	S	S	S-DD to R	S	S
Flucytosine	S	S	S	S	I to R	S
Amphotericin B	S	S	S	S to I	S to I	S to R
Echinocandins	S	S	S to R*	S	S	S

*Echinocandin resistance among *C. parapsilosis* isolates uncommon.
I, intermediately susceptible; R, resistant; S, susceptible; S-DD, susceptible dose-dependent.
From Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503-35.

or HIV infection for the treatment of oropharyngeal candidiasis. Use of itraconazole in children is off label, because it has not been approved for use in children younger than 18 years of age.[110] In children 2 to 11 years of age or 12 to 14 years old and weighing less than 50 kg, voriconazole 9 mg/kg (maximum, 350 mg) orally every 12 hours may be administered, whereas in children 12 to 14 years old and weighing 50 kg or more or older than 14 years should receive 200 mg orally every 12 hours as maintenance dosing.[95,110]

In the event of treatment failure, amphotericin B deoxycholate (AmBd) given as an oral 1-mL suspension four times daily may be tried. Intravenous caspofungin at 70 mg/m^2/day on day 1 followed by 50 mg/m^2/day (maximum, 70 mg) daily on subsequent days may be given in children 3 months of age or older for refractory disease. If chronic suppressive therapy is warranted (e.g., HIV infection or severe immunosuppression), fluconazole may be administered three times weekly.[110,224]

Esophageal Candidiasis

The treatment of esophageal candidiasis requires systemic antifungal therapy. Oral fluconazole at 3 to 6 mg/kg/day for 14 to 21 days is recommended. Intravenous fluconazole at 6 mg/kg/day, AmBd at 0.3 to 0.7 mg/kg/day, or caspofungin 70 mg/m^2/day on day 1 followed by 50 mg/m^2/day (maximum, 70 mg) daily should be used for patients unable to tolerate oral therapy. For fluconazole-refractory disease, itraconazole, voriconazole, or caspofungin are suitable alternatives (see earlier discussion for pediatric dosing). As in oropharyngeal candidiasis, chronic suppressive therapy with fluconazole three times weekly may be administered.[224]

Vulvovaginal Candidiasis

The approach to the treatment of vulvovaginal candidiasis varies depending on the presence of complicated vulvovaginal disease (severe disease is suggested by four episodes of symptomatic vulvovaginal candidiasis in 1 year, non–*C. albicans* infection, or abnormal host). No data have shown the superiority of any topical regimen,[252,288] and a meta-analysis of 19 randomized, controlled trials has shown equivalent results with both oral and topical antifungal agents.[219] Uncomplicated vulvovaginal candidiasis may be treated with a single 150-mg dose of oral fluconazole. Patients with complicated vulvovaginal candidiasis are recommended to undergo induction therapy with a topical or oral azole for 10 to 14 days followed by oral fluconazole 150 mg weekly for 6 months.[224]

Cutaneous Candidiasis

Because moist, macerated skin promotes the development of cutaneous candidiasis, it is important to keep affected areas dry as much as possible; antifungal therapy alone usually does not produce a cure. For candidal diaper dermatitis, frequent diaper changes should be performed. Topical antifungal therapy with nystatin or azoles may be applied three to four times daily.

Invasive Candidiasis

Candidemia

All pediatric patients who are found to be candidemic should be treated with systemic antifungal therapy. For non-neutropenic patients who are critically ill with candidemia or who have had recent azole exposure, an echinocandin is the preferred therapy owing to its significant fungicidal activity against *Candida*. For neutropenic patients who are critically ill due to candidemia or have had recent azole exposure, either an echinocandin or LFAB (3 to 5 mg/kg daily) may be given. Fluconazole therapy may be given as first-line therapy for patients who remain hemodynamically stable and have not had recent azole exposure and should be given intravenously at 6 to 12 mg/kg/day. Step-down therapy from an echinocandin to fluconazole is recommended for patients with susceptible isolates, whereas voriconazole may be offered as step-down oral therapy for candidemia due to *C. krusei* or susceptible *C. glabrata*.[224] In children age 2 to 11 years, or 12 to 14 years and weighing less than 50 kg, voriconazole may be given as a 9 mg/kg IV loading dose every 12 hours on day 1, followed by 8 mg/kg intravenously every 12 hours as maintenance. Oral dosing may be given as 9 mg/kg (maximum, 350 mg) every 12 hours. In children 12 to 14 years of age and weighing 50 kg or more, or older than 14 years, dosing should be as in adults and may be given as a 6-mg/kg intravenous loading dose every 12 hours on day 1, followed by 4 mg/kg IV every 12 hours as maintenance dosing, whereas oral dosing may be given as 200 mg every 12 hours as maintenance dosing.[95,110,224]

A dilated funduscopic examination should be performed in all patients with candidemia within the first week after initiation of antifungal therapy, and routine blood cultures should be performed to document clearance of *Candida* from the bloodstream. In patients without evidence of disseminated disease, antifungal therapy should continue for 14 days from symptom resolution and documented bloodstream clearance of *Candida*. In patients with candidemia in the setting of neutropenia, neutrophil recovery should occur prior to completion of therapy. Current guidelines recommend that central venous catheters in non-neutropenic patients with candidemia should be removed when possible, because data from most observational studies in children and adults have shown fewer rates of complications, reduced mortality, and more rapid rates of response to antifungal therapy.[8,71,228,254] However, concerns of confounding due to the observational nature of these studies, and the possibility that patients whose central venous catheter was retained had significantly higher severity of illness compared with those whose catheters were removed (thus precluding catheter removal), have limited the strength of this recommendation.[218]

In patients who are neutropenic, the gastrointestinal tract may be a more likely source of candidemia; thus, the significance of catheter removal when candidemia is documented is less clear. In a single-center study of 404 patients with cancer and central venous catheters in place at the time of candidemia, the investigators attempted to

differentiate candidemia associated with the central venous catheter versus candidemia associated with a noncatheter source and to define the benefit of timing of catheter removal on resolution of candidemia-associated symptoms and blood culture sterilization. In patients with evidence of catheter-associated candidemia, catheter removal 72 hours or earlier from first positive blood culture was found to be significantly associated with improved response rates compared with removal after 72 hours, whereas patients with candidemia due to an alternate or undefined source did not have a statistically significant improvement in response rates with early catheter removal.[245]

Other data have shown mixed results in support of early removal of catheters in candidemic patients with cancer.[181,324] Based on expert opinion, catheter removal should be attempted for neutropenic patients with persistent candidemia when feasible.[224]

Endovascular Candidiasis

Fungicidal regimens are preferred for *Candida* endocarditis, and most experience in the literature involves the use of AmBd with or without flucytosine for treatment of *Candida* endocarditis. LFAB is associated with less toxicity and permits higher dosages; thus, LFAB 3 to 5 mg/kg with or without flucytosine 25 mg/kg four times daily is recommended for the treatment of native valve and prosthetic valve *Candida* endocarditis in the non-neonatal population. High-dose caspofungin has also been used with some success in the adult population and is considered an alternative.[224]

Based on data from case reports, case series, and a meta-analysis,[85,295] adjunctive surgery plus medical antifungal therapy appears superior to medical therapy alone in reducing the risk for death and forms the basis for recommendations that patients with *Candida* endocarditis should undergo valve replacement, with valve repair and vegetectomy being alternative approaches. Therapy duration for native valve endocarditis is at least 6 weeks from valve replacement, and possibly longer in the presence of perivalvular abscess or other complications. For prosthetic valve endocarditis, suppressive therapy with fluconazole after completion of initial therapy is recommended to prevent late recurrence.

Cardiac Candidiasis

Data are limited to guide management of *Candida* pericarditis,[274] but experience in the literature with AmBd, and the known lower toxicity profile seen with LFAB, suggests that pericarditis can be treated with LFAB at a dose of 3 to 5 mg/kg/day. AmBd at 0.6 to 1 mg/kg/day, high-dose caspofungin, and fluconazole 6 to 12 mg/kg/day are acceptable alternatives. Treatment should be continued for several months, in combination with either a pericardial window or pericardiectomy, because this appears to be the best approach for achieving cure.[224,274] Step-down therapy to fluconazole 6 to 12 mg/kg daily can be considered in patients who are clinically stable and who have shown an initial response to LFAB, AmBd, or caspofungin. Myocarditis should be treated with LFAB or AmBd with or without flucytosine for a minimum of 6 weeks; in the presence of myocardial abscesses, treatment may need to be prolonged.[224]

Peritoneal Candidiasis

Most experts agree that peritoneal catheters should be removed in patients with fungal peritonitis, based on high rates of treatment failure seen in case series of patients with retained catheters, as well as the known challenges involved in treating biofilm present on the device.[50,159,206] Recent recommendations call for prompt (<24 hours) removal of the peritoneal catheter on diagnosis of fungal peritonitis,[178] with recent data showing reduced mortality in adults with early catheter removal compared with delayed removal, although timing of catheter removal continues to be controversial.[51] Medical therapy for fungal peritonitis traditionally utilized intravenous AmBd, although with the discovery that intravenous AmBd has poor peritoneal penetration owing to its high protein binding, oral or intravenous fluconazole with or without flucytosine has been considered the drug of choice for susceptible isolates.[41,194] Fluconazole may also be given intraperitoneally as a 75-mg/L concentration added daily to the dialysate after a systemic loading dose.[205] Intraperitoneal administration of AmBd has been associated with severe peritoneal irritation as well as fibrosis and membrane loss and should be avoided.[9] The role of echinocandins in fungal peritonitis is not yet well defined.

Candidiasis of the Urinary Tract

No treatment is recommended for patients with asymptomatic candiduria. In patients with an indwelling catheter, removal of the device may be adequate to resolve the candiduria without antifungal therapy. In patients with evidence of cystitis, fluconazole is highly water soluble and is primarily excreted into the urine as active drug, and expected urinary concentrations are greater than 10-fold that in serum, exceeding the MIC even for susceptible dose-dependent *Candida* spp.[90] Fluconazole should be given at 3 mg/kg daily for 2 weeks. For fluconazole-resistant organisms, AmBd 0.3 to 0.6 mg/kg/day for 1 to 7 days and oral flucytosine 25 mg/kg dour times daily for 7 to 10 days are alternatives.[224] Continuous bladder irrigation with AmBd has also been used to treat *Candida* cystitis, although relapse rates are high.[90]

For pyelonephritis, higher doses of fluconazole may be needed for adequate tissue penetration and fluconazole 3 to 6 mg/kg daily should be administered for 2 weeks. For fluconazole-resistant strains, AmBd 0.5 to 0.7 mg/kg/day with or without flucytosine 25 mg/kg four times a day should be given.[224] LFAB should not be used to treat *Candida* UTIs because of the low drug levels achieved in the renal parenchyma and urine. Although voriconazole has been shown in an animal model of renal parenchymal candidiasis to be superior to AmBd or fluconazole, urine voriconazole levels are low, with less than 5 percent of the active drug excreted, limiting its utility in the treatment of UTIs. Likewise, the echinocandins have been shown in experimental models to clear renal parenchymal infections in the setting of disseminated

candidiasis, but very little active drug is excreted in the urine and clinical experience with treatment of UTIs is limited.[90]

Patients with mycetomas (fungus balls) in the urinary collecting system are recommended to undergo aggressive surgical débridement to relieve obstruction and to remove the bulk of the mass. Systemic treatment alone is unlikely to result in spontaneous disruption and passage of the mycetoma but should be administered due to the likelihood of coexistent disseminated candidiasis or renal parenchymal infection.[90] Fluconazole 3 to 6 mg/kg/day or AmBd 0.5 to 0.7 mg/kg/day with or without flucytosine is recommended. Irrigation of the renal collecting system with AmBd 50 mg/L in sterile water may be a possible adjunct to systemic therapy. Treatment should continue until urine cultures are negative and symptom resolution has occurred.[224]

Osteoarticular Candidiasis

The management of osteoarticular *Candida* infections is challenging, with high rates of treatment failure associated with relapsed disease despite prolonged antifungal therapy.[99] Fluconazole 6 mg/kg/day for 6 to 12 months or intravenous LFAB at 3 to 5 mg/kg/day for at least 2 weeks followed by step-down therapy to fluconazole at 6 mg/kg/day for 6 to 12 months is currently recommended. Caspofungin may be a suitable alternative to upfront parenteral LFAB administration. Adjunctive surgical débridement may be beneficial in select cases.

For septic arthritis, a minimum of 6 weeks of fluconazole 6 mg/kg/day or LFAB 3 to 6 mg/kg/day for at least 2 weeks followed by fluconazole 6 mg/kg/day is recommended. Adequate surgical drainage is a crucial aspect of the management of non-neonatal *Candida* arthritis and should be performed in all cases.[224]

Central Nervous System Candidiasis

Most experience in the literature regarding the treatment of *Candida* CNS infections has been with AmBd, with or without flucytosine.[10,103] LFAB has been preferred owing to its lower toxicity profile and known tissue penetration in the brain. The combination of LFAB and flucytosine has demonstrated synergism in vitro, and flucytosine achieves excellent CSF concentrations, making combination therapy attractive. Limited experience with the echinocandins has revealed both treatment successes and failures, in addition to breakthrough CNS infections after therapy for candidemia. The echinocandins have poor CSF penetration, with low levels compared with serum.[74] As a result, this antifungal class is not recommended for CNS candidiasis. Current recommendations for treatment of *Candida* CNS infection are to administer intravenous LFAB 3 to 5 mg/kg/day with or without flucytosine 25 mg/kg four times daily for the initial several weeks of treatment, followed by step-down therapy with fluconazole 6 to 12 mg/kg/day once treatment response to LFAB has been established. Therapy should continue until all signs and symptoms have resolved, including CSF and radiographic abnormalities. Removal of infected intracranial devices is strongly recommended.[224]

Ocular Candidiasis

No prospective studies exist for the treatment of *Candida* endophthalmitis; however, most experience has been with intravenous and/or intravitreal AmBd with or without flucytosine. Currently, AmBd 0.7 to 1 mg/kg/day combined with flucytosine 25 mg/kg four times daily is recommended for severe endophthalmitis (e.g., advancing lesions or lesions threatening the macula). For less severe cases, fluconazole 12 mg/kg on day 1 followed by 6 to 12 mg/kg/day may be administered. LFAB and voriconazole are reasonable alternatives. Early surgical intervention with partial vitrectomy may preserve vision in advanced cases. Intraocular instillation of antifungal agents is commonly practiced and may be of benefit. The optimal duration of antifungal therapy is not determined, but at least 4 to 6 weeks of system treatment is recommended, with continuation until all clinical evidence of intraocular infection has resolved.[224]

Chronic Disseminated Candidiasis

Treatment recommendations for chronic disseminated candidiasis are based on case reports and series; however, most clinical experience has been with AmBd, LFAB, and fluconazole. In clinically stable patients, fluconazole 6 mg/kg/day is recommended. LFAB 3 to 5 mg/kg daily or AmBd 0.5 to 0.7 mg/kg/day can be used to treat acutely ill patients or patients nonresponsive to fluconazole, either as a 1- to 2-week induction period before fluconazole step-down or as single therapy. Intravenous caspofungin given as a loading dose of 70 mg/m^2 on day 1 followed by 50 mg/m^2 daily are alternatives.[224] Therapy for chronic disseminated candidiasis is typically prolonged with a median duration of more than 3 to 5 months[193] and should continue until all lesions resolve or calcify. Continuation of chemotherapy has been shown to be safe in these patients, and stem cell transplantation may be performed if indicated in patients whose disease is controlled.[39,332] Adjuvant corticosteroid therapy in addition to antifungal therapy has been proposed, in light of the proposal that CDC represents a type of immune reconstitution inflammatory syndrome. Prednisone 0.5 mg/kg/day for at least 3 weeks was shown to result in rapid fever resolution in a single case series,[175] but more data are needed before corticosteroids can be recommended for this indication.

NEONATAL CANDIDIASIS

Epidemiology

Neonatal candidiasis in neonatal intensive care units (NICUs) remains a serious health problem because it is associated with substantial morbidity and mortality. Strategies for prevention and management of invasive *Candida* infections are paramount for improving outcomes for neonates.[32,33,148,298,347] The incidence of candidiasis increased in the NICU as advances in medical therapy allowed for increased survival of extremely low-birth-weight (ELBW; <1000 g at birth) and

low-gestational-age preterm neonates between the 1980s to 1990s; it then remained stable until 2001, when a decrease in the incidence was noted in infants weighing less than 1000 g owing to such factors as antifungal prophylaxis, antibiotic and medication stewardship, and central line–associated bloodstream infection (CLABSI) bundles.[57,96,164] Invasive infections occur in two main groups in the NICU—the extremely premature infant and any NICU patient with complex gastrointestinal disease (e.g., necrotizing enterocolitis [NEC], gastroschisis, and spontaneous bowel perforation).[33,87,265]

C. albicans and *C. parapsilosis* infections account for 80 to 90 percent of neonatal invasive candidiasis in centers not using antifungal prophylaxis.[33] Centers using fluconazole prophylaxis have very few infections, with the majority due to non–*C. albicans* spp. Less common *Candida* spp. include *C. glabrata* and *C. tropicalis*, and a small percentage of infections are due to *C. lusitaniae*, *C. guilliermondii*, and *C. dubliniensis*.

Invasive candidiasis in the neonate includes congenital cutaneous candidiasis and late-onset cutaneous candidiasis, BSIs, UTIs, meningitis, peritonitis, and infection of other sterile sites (e.g., bone and joint infections). These infections can be classified similar to other neonatal infections into early-onset (<72 hours after birth) and likely vertically transmitted and late-onset (≥72 hours after birth) and potentially horizontally transmitted infections.

There is variation between rates in different NICUs and incidence reported in the literature based on patient demographics, use of antifungal prophylaxis, surgical population, resuscitation of extremely preterm infants, and feeding, medication, and antibiotic practices in individual NICUs. Fridkin and associates reported the rate of *Candida* BSIs ranged from 3 percent to more than 23 percent among NICUs in infants weighing less than 1000 g, and other researchers have reported similar findings.[33,67,96] Centers that care for infants with gastroschisis, NEC, and other complex gastrointestinal diseases have an increased risk for invasive candidiasis.[87] Resuscitation practices may be the most significant area of practice variation affecting invasive candidiasis rates. NICUs in which the practice is not to resuscitate infants who are less than 25 weeks' gestational age would have a lower rate of invasive candidiasis in infants weighing less than 1000 g, compared with centers caring for 23- and 24-week gestation infants.

Studies including all invasive candidiasis in both preterm and full-term infants are rare, and most studies of incidence or risk factors focus on *Candida* BSIs. If candidemia alone is examined, the largest amount of data comes from a report from 128 United States NICUs using U.S. National Nosocomial Infections Surveillance (NNIS) system data from 1995 to 2004 (n = 130,523 neonates).[96] For infants weighing less than 1000 g, the median infection rate was 7.5 percent, whereas 25 percent of NICUs had rates of 13.5 percent or higher. The incidence decreases significantly for infants weighing more than 1000 g. Candidemia rates for infants with birthweights from 1001 to 1500 g are 1.32 percent, from 1501 to 2500 g, 0.36 percent, and more than 2501 g, 0.29 percent. The incidence increases in an inverse linear pattern, from around 3 percent at 28 weeks' gestation to 24 percent at 23 weeks' gestation.

Candida CLABSIs among NICUs from 1999 through 2009 have decreased from 0.92 to 0.2 per 1000 catheter-line days.[57] These are data from all birth weight groups in the NICU, with infants weighing less than 1000 g representing nearly 70 percent of these infections. CLABSIs due to *C. albicans* decreased from 0.53 to 0.09 and those from non–*C. albicans* infections from 0.39 to 0.1 per 1000 catheter-line days.

The incidence of invasive candidiasis in ELBW infants, not including congenital cutaneous candidiasis, is around 10 percent. The highest incidence of invasive candidiasis occurs in the most extremely preterm infants and is greater than 20 percent for infants of less than 25 weeks' gestation (Fig. 199-1).[33] *Candida* UTIs account for an additional 3 to 4 percent of invasive candidiasis in ELBW infants.[33,144,336] Two recent multicenter studies have reported that *Candida* UTIs have a high associated mortality that is similar to that for *Candida* BSIs in ELBW infants, thus confirming their invasive nature in neonates.[33,256] Finally, meningitis and peritonitis contribute

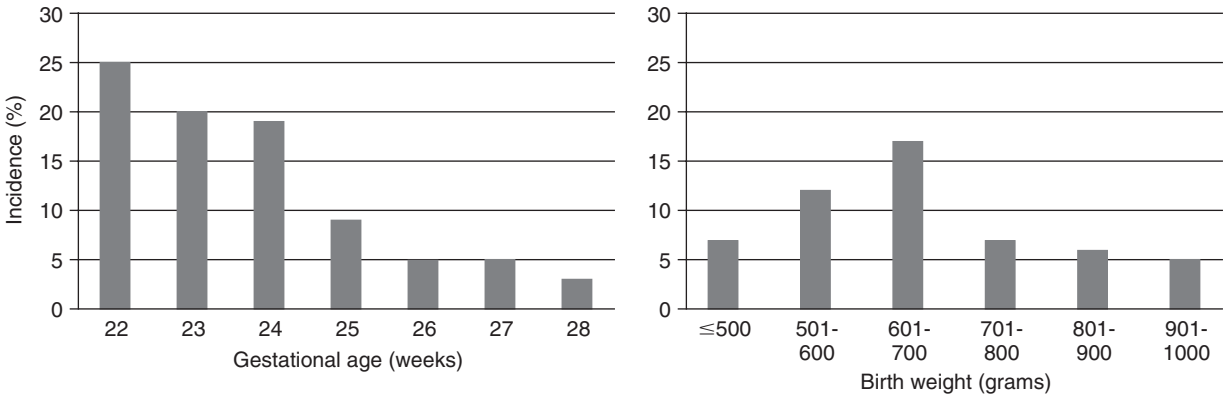

FIGURE 199-1 ■ Invasive *Candida* bloodstream infection, urinary tract infection, meningitis, or peritonitis in infants weighing less than 1000 g birth-weight not receiving antifungal prophylaxis. Gestational age has a linear relationship to invasive candidiasis compared with birth-weight and aids in defining the highest risk patients. (Data from 137 infections in 1515 infants weighing less than 1000 g from 19 centers of the National Institute of Child Health and Human Development [NICHD] Neonatal Research Network.)

an additional 1 to 2 percent of invasive candidiasis in ELBW infants.

The literature has limitations on defining incidence. In a study from the United Kingdom, a 2.1 percent incidence was reported in infants weighing less than 1000 g but antifungal prophylaxis was not ascertained. These researchers subsequently studied the use of antifungal prophylaxis and found that almost 30 percent of NICUs were using antifungal prophylaxis, which may account for the lower reported rates of infection.[60,61] Such studies illustrate how infection rates may be higher than what is reported in the literature if all data, medications, and risk factors are not captured.

Complex gastrointestinal diseases can be complicated by invasive candidiasis. Patients with NEC, focal bowel perforation, and gastroschisis have a high incidence of invasive candidiasis. Feja and colleagues found that candidemia was increased in patients with gastrointestinal pathologic processes (OR, 4.57; 95% CI, 1.62 to 12.92).[87] Gastrointestinal pathologic processes were defined in their study as tracheoesophageal fistula, gastroschisis, omphalocele, Hirschsprung disease, intestinal atresias, or episodes of NEC. Other studies have found the incidence of invasive candidiasis in patients with NEC is more than 10 percent. After combining data from studies by Noyola and associates[216] and Chapman and Faix,[53] the incidence of candidemia is 16.5 percent in patients with NEC. Focal bowel perforation, which occurs primarily in infants weighing less than 1000 g, is associated with a 50 percent incidence of invasive candidiasis, the majority of cases being *Candida* peritonitis.[62,247]

Infants with congenital heart disease are an emerging patient population who may develop invasive candidiasis. According to Ascher and associates,[11] candidemia accounts for 9 percent of BSIs in patients with congenital heart disease cared for in NICUs in the first 4 months of life; *Candida* BSI occurred at a rate of 6.3 per 1000 admissions and had an overall mortality rate of 21 percent.

Candida spp. are the second most common cause of infection in neonates on extracorporeal membrane oxygenation (ECMO).[38] Compared with the first 7 days on ECMO, infections are twofold and fourfold more common between days 8 and 14 and after 14 days on ECMO, respectively.

Risk Factors

Because neonatal candidiasis can be fatal among premature infants, an understanding of the risk factors that lead to infection is critical. Conditions that facilitate fungal colonization and proliferation and increase the risk for invasive infection include gastrointestinal dysmotility or ileus, antibiotics, acid inhibition, corticosteroids, and the immaturity of the patient's immune system. Several risk factors have been identified, with gestational age having the strongest effect. This correlates with the degree of the underdeveloped immune system, skin, respiratory, and gastrointestinal tracts in these infants.

The immature lymphocyte and antibody system predisposes preterm infants to skin and mucosal fungal colonization, whereas deficient neutrophil and innate host defense mechanisms predispose them to dissemination and overwhelming infection.[146] With compromise of the barrier defense system, invasive candidiasis may occur because of deficient neutrophil number and function. Neutrophils provide the major role in antifungal defense, ingesting and killing *Candida* in a process requiring production of oxygen metabolites, antibodies, and cytokines and activation of the C3 complement component, all of which are decreased in preterm infants compared with term infants and adults. Also deficient are neutrophil granules and cytokines, which play a critical role in the lysis of *Candida* hyphae and pseudohyphae, which are too large to be engulfed by phagocytosis. Macrophages have impaired adherence, phagocytosis, and oxidative killing in preterm infants, thus affecting their ability to contain fungal colonization and infection. In addition to cell-mediated defenses, cytokines contribute to innate immunity against fungal infection through enhancement of cell-mediated fungicidal activity. Pulmonary host defense is primarily through alveolar macrophages, cilia and mucus, and surfactant, all of which are deficient in preterm infants.

Risk factors for *Candida* colonization and sepsis are similar.[264,265] Colonization of the skin, mucosal membranes, and/or vascular catheters commonly precedes infection. Biofilm formation on catheters inhibits the host's defense mechanisms and the penetration of antifungal agents. Using sterile precautions in preparation of parenteral nutrition, intravenous fluids, and medications and when performing line changes at the bedside is critical for infection control. Infusates may also become contaminated and directly seed the bloodstream. In vitro growth curves demonstrate that *Candida* spp. have a selective growth advantage compared with bacteria in parenteral nutrition fluid.[171] Invasive candidiasis is usually preceded by prior colonization, which may occur via vertical or horizontal transmission. Most fungal colonization occurs by 2 weeks of life.[21,130,261] Fungal colonization of the skin, respiratory tract, or gastrointestinal tract occurs in 10 percent of full-term infants compared with 26.7 to 62.5 percent of those of very low birth-weight (VLBW; <1500 g at birth) in the first weeks of life.[146] Fungal colonization and subsequent infection depends on exposure, size of inoculum, host susceptibility, and properties of the pathogen (Fig. 199-2).

Vertical transmission leading to colonization often occurs. *Candida* yeast cells adhere preferentially to intermediate layers of the vaginal tract that are increased during pregnancy, increasing maternal fungal colonization and exposure of vaginally delivered infants. The incidence of vaginal fungal colonization during pregnancy has been reported to be between 25 and 50 percent, with 85 to 90 percent due to *C. albicans* and the remainder predominantly *C. glabrata* and *C. tropicalis*.[146] Despite the frequent fungal colonization, chorioamnionitis caused by *Candida* is a rare event, with almost all vertical transmission occurring when the infant passes through the birth canal via mucocutaneous contact, swallowing, or aspiration of fungi.[195,338] Treatment of maternal *Candida* vaginosis and UTIs during pregnancy may decrease the inoculum the infant is exposed to and potentially prevent vertical transmission.[94,146]

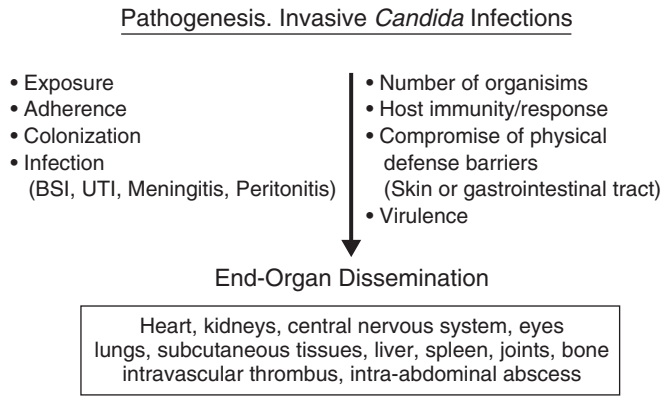

FIGURE 199-2 ■ Pathogenesis of invasive candidiasis.

Candida colonization also may be acquired horizontally, primarily from the hands of health care workers. In a multicenter trial examining fungal colonization in six NICUs, *Candida* spp. were isolated on the hands of 29 percent (859 of 2989) of health care workers.[264] Although *C. albicans* was the more common fungal isolate in all NICU patients (14% vs. 7%), *C. parapsilosis* was the most common species isolated from the hands of NICU staff. *C. parapsilosis* was isolated from 19 percent (similar to a single-center incidence of 20 percent), and *C. albicans* was isolated from 5 percent of the cultures from health care personnel (p <.001). *C. lusitaniae* (2%), *C. guilliermondii* (1%), *C. tropicalis* (<1%), and *C. glabrata* (<1%) were also recovered from hand cultures. Increased handling required by sicker preterm infants increases the risk for acquiring fungal colonization from health care workers' hands, contaminated infusates, and catheters or catheter-related devices because there are an average of 32 direct touches to an infant during a 12-hour shift.[63,264]

Candida spp. causing invasive infection rarely colonize environmental sources.[20,272] Despite the tendency of fungus to grow in moist environments, studies have not isolated fungi from isolettes, incubators, wash basins, water faucets, chest tubes, intravenous drug pumps, ventilators, pumped breast milk, inanimate objects, or radiant warmers.

The skin and gastrointestinal tract are the most common sites for *Candida* colonization.[20,144] Colonization is inversely proportional to gestational age, and fungal colonization occurs on the skin and gastrointestinal tract before the respiratory tract. Colonization at two or more sites occurs similarly with *C. albicans* (77%) and *C. parapsilosis* (85%), whereas colonization at three or more sites occurs more frequently with *C. albicans* (69%) compared with *C. parapsilosis* (23%) (p = .047).

Candida spp. produce proteases that may be lytic for the thin keratin layer produced by the immature stratum corneum and phospholipases against lipid membranes that both may facilitate epithelial invasion.[81,146] Increased transepidermal water loss from preterm skin creates a moist environment that facilitates fungal colonization and growth. Because of the increased permeability of the preterm skin, substrates such as glucose may diffuse to the epithelial surface, facilitating *Candida* growth. *Candida* may alter its surface structure in the presence of high glucose, thus increasing its adherence and proliferative properties. A thicker keratin layer is produced by 2 weeks of life in the extremely preterm infant, after which new fungal skin colonization occurs less frequently.

The microflora of the gastrointestinal tract plays an important role in fungal colonization and infection. Buccal candidal adherence is increased in preterm compared with term infants, facilitating colonization.[68] A normal bacterial flora inhibits *Candida* growth by competing for both adhesion sites and nutrients.[122,154] In a study of athymic mice given an oral challenge with *Candida*, fungal colonization was attenuated in mice with a normal gut flora compared with those with a germ-free microflora.[122] This highlights the fact that the intestinal microflora may be as important as an intact immune system in preventing fungal colonization.

Although skin and gastrointestinal colonization are more common and precede respiratory tract colonization, endotracheal colonization has a higher risk for infection.[152] Rowen and associates demonstrated that with endotracheal fungal colonization candidemia was 15.4 times more likely to occur than when infants were without any fungal colonization.[261] After controlling for fungal colonization at other sites was done, endotracheal colonization alone increased the risk for fungal sepsis 5.9-fold. (95% CI, 1.34 to 26).

When risk factors are examined by species, central vascular catheters, vaginal delivery, use of third-generation cephalosporins, and high acuity are the risk factors for *C. albicans* infection.[265] Histamine type 2 (H_2) antagonists, third-generation cephalosporins, central vascular catheters, parenteral nutrition and lipid emulsions, and high acuity are risk factors for *C. parapsilosis* infection. Gastrointestinal disease, exposure to fluconazole or antibiotics, prolonged hospitalization, and infection with other fungi increase the risk for sepsis due to *C glabrata*. Gastrointestinal mucosal injury, antibiotic suppression of bacterial flora, neutropenia, and parenteral nutrition increase the risk for sepsis due to *C. tropicalis*.

Medications such as broad-spectrum antibiotics and third- and fourth-generation cephalosporin and carbapenem antibiotics are associated with increased risk for candidemia.[29,67,265] It is hypothesized that these medications accumulate in high concentrations in an infant's bile and alter the balanced gastrointestinal microflora, eradicating competitive gram-negative and anaerobic bacteria and thus facilitating proliferation and dissemination of fungal organisms. Other classes of drugs found to be risk factors for neonatal fungal infection are H_2 antagonists and postnatal corticosteroids.[265,299] In infants weighing less than 1000 g, an association between lack of enteral feedings and infection has been reported.[32] For infants not feeding by the third day of life, 8.7 percent developed *Candida* BSI or meningitis, compared with 3.4 percent of those receiving enteral feedings.

Feja and associates examined risk factors in 2001 through 2002 and using multivariate analysis found that catheter use (OR, 1.06 per day of use; 95% CI, 1.02 to 1.10), previous bacterial BSIs (OR, 8.02; 95% CI, 2.76 to 23.30), and gastrointestinal pathologic processes as mentioned previously (OR, 4.57; 95% CI, 1.62 to 12.92) were significantly associated with candidemia.[87]

The extremely preterm infant has the unique combination of being immunocompromised (including an immature skin and gastrointestinal tract), requiring prolonged intensive care (parenteral nutrition, mechanical ventilation, central venous access), being exposed to medications that promote fungal growth (H_2 blocking agents, proton pump inhibiting agents, postnatal corticosteroids, and broad-spectrum antibiotics), and predisposed to gastrointestinal dysmotility and disease (NEC and focal bowel perforation).[33,87,265] Infants with complicated gastrointestinal diseases such as gastroschisis or NEC have combined risk factors of prolonged ileus, central venous access, parenteral nutrition, surgery, and exposure to prolonged and/or broad-spectrum antibiotics.[87]

Pathogenesis

Adherence and the slow-growing nature of *Candida* facilitate its ability to colonize and disseminate into the bloodstream and body tissues before clinical signs and symptoms of infection become apparent. Surface glycoproteins play a role in fungal adherence. One such surface adherence glycoprotein is INT1, which binds to β-integrins present on the endothelium and white blood cells. The absence of a functional INT1 gene diminishes adherence in yeast cells but not filamentous forms.

The preterm infant is immunocompromised and frequently exposed to broad-spectrum antibacterial medications. Investigators have studied the effect of corticosteroids and antibiotics in mice orally inoculated with *C. albicans* to mimic conditions in the preterm infant.[28] Antibiotic treatment alone led to increased *Candida* colonization but did not affect dissemination. When dexamethasone was added to the antibiotic regimen (presumably amplifying the inherent immunoincompetence), both colonization and dissemination increased in these animal models. Dexamethasone plus antibiotics led to an increase in the percentage of filamentous forms in the gastrointestinal tract compared with antibiotics alone. In addition, introduction of *C. albicans* strains with two functional copies of the INT1 gene increased the number of fungi colonizing the cecum and disseminating to extraintestinal sites.

C. albicans is dimorphic, having both yeast and filamentous forms (e.g., hyphae, pseudohyphae, germ tubes), and is assumed to have increased virulence in immunocompromised patients because of the filamentous forms. Filamentous forms may contribute to colonization and infection, although species that do not form filaments, such as *C. glabrata*, also can colonize and cause invasive disease in preterm infants.

To further examine the role of yeast and filamentous forms, researchers intravenously or orally infected antibiotic-treated and dexamethasone-treated mice using three strains of *C. albicans:* (1) a wild-type strain that had both yeast cell and filamentous forms, (2) a strain with only yeast cells, and (3) a strain that was constitutively filamentous.[27] The mortality rate was significantly greater in both the wild-type (92%) and yeast-cell (56%) strains compared with the filamentous strain alone (0%). The filamentous strain had no dissemination, and cecal colonization was significantly less than that of the other two strains. The wild-type strain had diffuse hyphal invasion with increased tissue necrosis compared with the yeast-cell strain. The researchers speculated that the yeast forms are critically important for adherence and tissue dissemination and that hyphal formation in the tissues contributes to parenchymal destruction.

Clinical Manifestations

Congenital Cutaneous Candidiasis

Congenital cutaneous candidiasis may manifest as pustules, vesicles, skin abscesses, and/or an erythematous maculopapular rash when the patient is born (or within a few days after birth) (Table 199-2, Fig. 199-3). The skin involvement covers one or more of the following areas: face/scalp, chest, abdomen, perineal area, one or more extremities, and/or the back. These lesions occasionally lead to desquamation. VLBW infants with congenital *Candida* infection are more likely to have severe infection, such as pneumonia and widespread dermatitis with focal areas of superficial erosion and desquamation. Dissemination can also occur in term infants (~10%), so treatment should be for 14 days. For affected term infants, it is reasonable to discuss the need for evaluation of the infant's immune system for a primary immunodeficiency. ELBW infants with congenital cutaneous candidiasis are at greater risk for developing dissemination to blood, urine, or CSF (66%) compared with those weighing 1000 to 2500 g (33%) or term infants (11%). For this reason, all should be treated with systemic antifungal therapy.

The skin involvement with congenital cutaneous candidiasis is invasive with a high burden of yeast and should be treated similarly as for other invasive fungal infections. Dissemination occurs if the infection is not treated for 14 days. In a study examining the pathogenesis and pathology of these infections, skin biopsies demonstrated invasion into the epidermis, with an inflammatory process ranging from dermal invasion and granuloma formation to focal necrosis and hemorrhage.[260] These data from biopsies give insight into the invasive nature of cutaneous involvement, demonstrating that these infections are invasive and need prompt intravenous antifungal therapy. Routine aerobic skin culture will isolate organisms causing rash (skin biopsies are not needed). By culturing for both fungal and bacterial organisms, the source of infection can be determined promptly and treatment started in a timely fashion. Differential diagnosis includes staphylococcal, other bacterial, and other fungal skin infections. Pathologic study with fungal staining of the umbilical cord and placenta can also aid in diagnosis.

Cutaneous Candidiasis

Cutaneous candidiasis, referred to in the past in the literature as mucocutaneous infection, occurs later than congenital cutaneous candidiasis and at the same time as late-onset sepsis, at between 2 and 3 weeks of age.[83] The incidence in one study reported an incidence of 7.8

TABLE 199-2 Neonatal Cutaneous Candidiasis Definition

Definition: Extensive *Candida* Rash Covering Two or More Affected Areas

Affected Areas

Skin
Chest
Abdomen
Back
Extremity
Groin or perineal area
Neck
Face or scalp
Umbilical cord and placenta
  White plaques on umbilical cord
  Placenta with yeast invasion

Timing

Congenital cutaneous candidiasis: presents in the first 48 hours of life and usually present at birth
Cutaneous candidiasis: presents after 2 to 7 days of life

Rash

Maculopapular
Scaly, erythematous
Dry flaking
Desquamating
Burnlike erythematous

Presentation

Extensive
Covering two or more affected areas
Covering one affected area *plus* umbilical plaques or placental pathology (silver or hematoxylin and eosin) would count as one affected area

Evaluation

Congenital cutaneous candidiasis: culture rash sites, send placental and/or cord for culture and pathology with silver stain for yeast; blood culture and cerebrospinal fluid culture (unless rash over lumbar area, if so treat empirically for meningitis)
Cutaneous candidiasis: Culture rash sites, blood, urine, and cerebrospinal fluid (unless rash over lumbar area, if so treat empirically for meningitis)

Diagnosis

Extensive Rash plus 1 or more of the following:
  Surface culture isolating *Candida* species
  Placental or cord identification (culture or silver stain) of yeast or *Candida* species for congenital cutaneous candidiasis
  Positive blood, urine, cerebrospinal fluid cultures for *Candida* species

Treatment

14-day course of intravenous antifungal therapy for end-organ dissemination

percent in VLBW infants in the era before antifungal prophylaxis.[83] Cutaneous candidiasis can also present as a diffuse burnlike erythematous rash that can be papulopustular and in some patients exfoliates. Risk factors include extreme prematurity, vaginal birth, postnatal corticosteroids, and hyperglycemia.[22,83,260] Cutaneous candidiasis is an invasive infection of the skin and needs prompt treatment for a minimum of 14 days in preterm infants. It also has a high rate of disseminating to the bloodstream and other fluids, and evaluation of blood, urine, and CSF is warranted.[22]

Neonatal Candidemia

Bloodstream infection with fungal species demonstrates clinical signs and symptoms similar to bacterial sepsis. The following signs and symptoms occur in the order of their prevalence with candidemia[86]; thrombocytopenia of less than 100,000/μL (84%), immature-to-total neutrophil ratio of greater than or equal to 0.2 (77%), increase in apnea and/or bradycardia (63%), oxygen requirement (56%), assisted ventilation (52%), lethargy and/or hypotonia (39%), gastrointestinal symptoms (e.g., gastric aspirates, distention, bloody stools) (30%), hypotension (15%), hyperglycemia (13%), elevated white blood cell count greater than 20,000/μL (12%), metabolic acidosis (11%), and absolute neutrophil count less than 1500/μL (3%). Most importantly, candidemia can be associated with disseminated disease. Evaluation for end-organ dissemination is warranted.

Neonatal Urinary Tract Infection

Evaluation of late-onset sepsis should include a urine culture obtained via sterile catheterization (or suprapubic bladder aspiration).[256] Signs and symptoms of UTIs can be similar to sepsis.[25] Additionally, an elevated creatinine value in absence of other pathologic processes should prompt evaluation for a UTI. If the urine culture is positive for fungus, renal ultrasonography should be performed to evaluate renal fungal abscesses. Candiduria develops in approximately 2.4 percent of VLBW infants and up to 6 percent of ELBW infants.[33,144] Recent studies have demonstrated similar mortality in infants with *Candida* UTI alone (26%) compared with *Candida* BSIs (28%) in ELBW infants.[33] Risk factors for candiduria in infants weighing less than 1000 g include vaginal birth, lower mean birth weight and gestational age, male sex, and receipt of prolonged initial empirical antibiotic therapy.[343] These findings emphasize the need for prompt treatment for a duration of 14 days.

Neonatal Meningitis

The reported frequency of fungal meningitis among VLBW infants is 1.6 percent.[207] The true incidence is likely higher because lumbar punctures are not obtained in many VLBW infants at the onset of sepsis.[297] Cell counts in preterm infants may not always be helpful because the results may not be abnormal in the presence of meningitis.[64,102] To reliably diagnose *Candida* meningitis, a lumbar puncture is needed before institution of antifungal therapy.

Neonatal Peritonitis

Invasive candidiasis can complicate the course of neonates with NEC or focal bowel perforation. Neonates with peritonitis secondary to bowel perforation due to NEC or focal bowel perforation often have abdominal

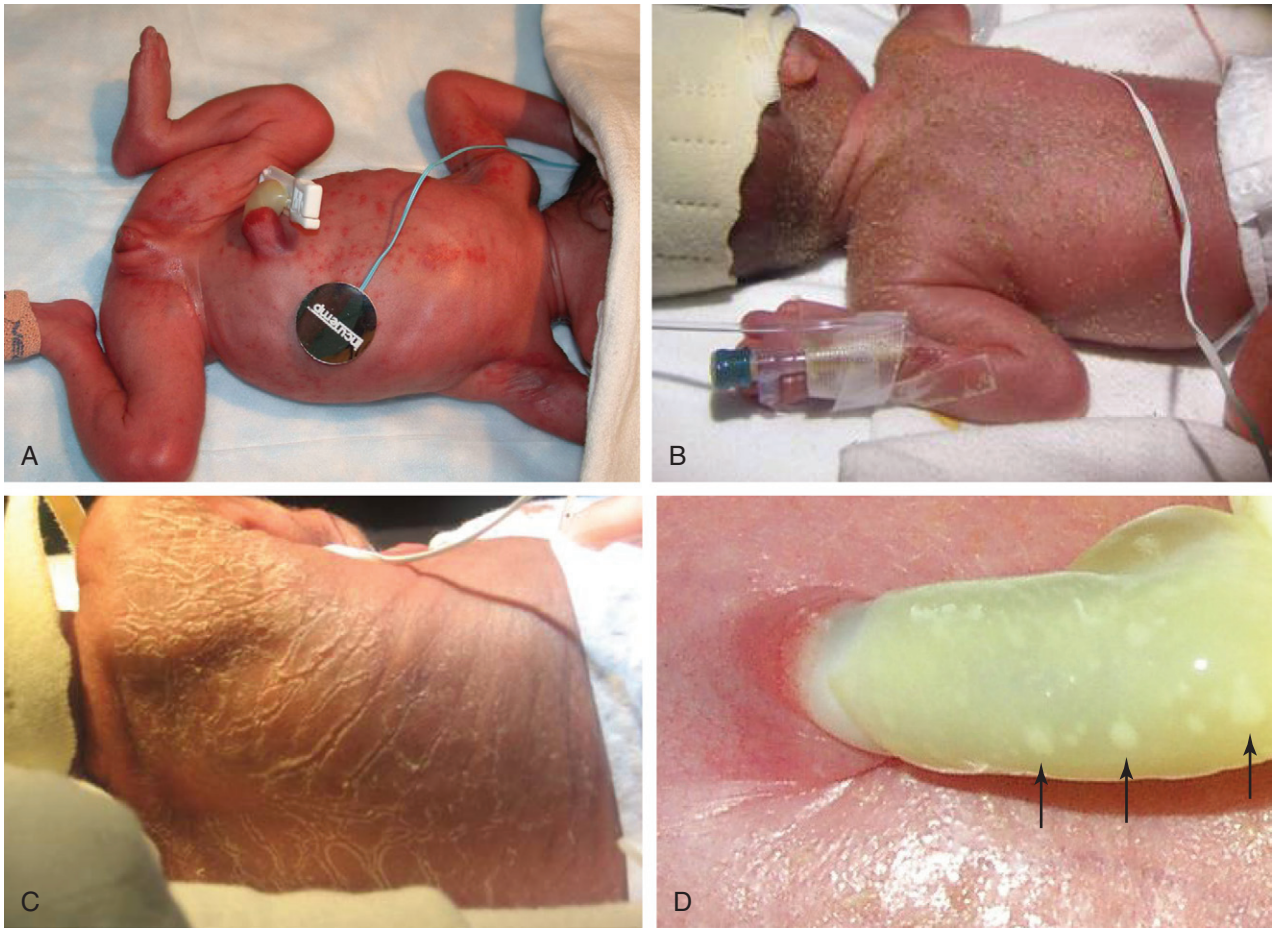

FIGURE 199-3 ■ Various presentations of congenital cutaneous candidiasis. **A,** Macular papular rash. **B,** Dry, flaking rash. **C,** Dry, cracking scaly rash. **D,** White plaques of the umbilical cord. (See companion Expert Consult website for color version.)

distention with or without erythema. In one study, *Candida* spp. were the predominant organism causing peritonitis in 15 percent of the perforated NEC cases and 44 percent of focal bowel perforation patients.[62] Radiographs help confirm perforation but at times may not be clear, and ultrasonography or an exploratory laparotomy may be needed if clinically indicated. When exploratory laparotomy is performed or drains are placed, cultures should be obtained to determine if *Candida* is associated with bowel perforation, peritonitis, and abscesses.

Neonatal Candida End-Organ Dissemination

At the time fungal infection is clinically apparent, the organisms have often disseminated from the blood, urine, or CSF to adhere and proliferate in body fluids, tissues, and organs. *Candida* spp. can cause endocarditis, endophthalmitis, dermatitis, peritonitis, osteomyelitis, and septic arthritis, and fungal abscesses may form in the CNS, kidneys, liver, spleen, skin, bowel, and peritoneum (Fig. 199-4). Fungal end-organ dissemination has been noted since the earliest reports of *Candida* sepsis in neonates. In an early review of 31 VLBW infants with invasive fungal infection (1972-1982), dissemination occurred to the bones or joints (26%), eye (23%), lung (13%), skin (10%), heart (6%), and peritoneum (3%).[134] More than one organ system was involved in 21 of these cases. The high prevalence of end-organ dissemination in older studies of neonatal fungal sepsis reflects prolonged periods of fungemia, because most of the diagnoses were made late or by autopsy. A meta-analysis of studies reporting fungal end-organ dissemination in neonates spanning both older and more recent practices from 1979 through 2002 found the median prevalence of cardiac vegetations or thrombi was 5 percent, with that of endophthalmitis 3 percent, of renal involvement 5 percent, and of CNS abscesses 4 percent.[30] In a more recent time period (1989-1999) of 86 cases of fungal sepsis in neonates, cardiac vegetations or thrombi occurred in 15.2 percent, endophthalmitis in 6 percent, renal involvement in 4.5 percent, and liver abscesses in 3 percent.[216] Dissemination may be higher in the lower birth-weight infant. In a study from 1988 through 1995 that examined 46 ELBW infants with fungal sepsis or meningitis, fungal abscesses of the CNS were found in 13 patients (28%).[97]

Noyola and colleagues[216] and Chapman and Faix[53] found that infants with candidemia for more than 5 and 7 days, respectively, were more likely to demonstrate ophthalmologic, renal, or cardiac abnormalities than those with a shorter duration of candidemia. When amphotericin B was administered and central vascular

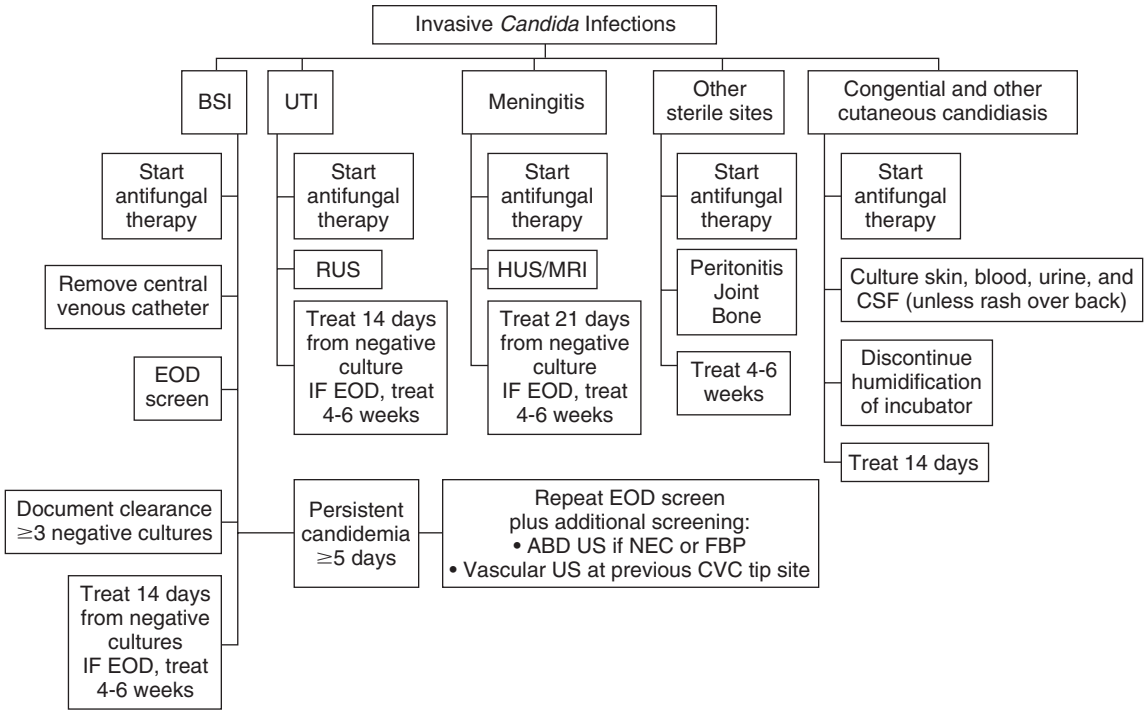

FIGURE 199-4 ■ Algorithm for evaluation and treatment of invasive *Candida* infections in neonates. Initial therapy pending susceptibilities: Amphotericin B deoxycholate (1 mg/kg/day). Alternatives: Amphotericin B lipid preparations (5 mg/kg/day) and fluconazole (12 g/kg/dose). With end-organ dissemination, meningitis, and persistent candidemia greater than 7 days, combination therapy with a second antifungal agent (fluconazole) is recommended. End-organ dissemination (EOD) includes performing an echocardiogram, renal ultrasonography (RUS), eye examination, and head ultrasonography (HUS). If bowel disease such as necrotizing enterocolitis (NEC) or focal bowel perforation (FBP), a complete abdominal ultrasound evaluation for abscess should be performed.

catheters were removed within 2 days of the first positive blood culture, outcomes such as end-organ dissemination and mortality were not increased.[216] With improved blood culture methods, the majority of blood cultures will grow *Candida* within 2 days and is associated with lower dissemination rates. One study of neonates demonstrated that isolation of fungus in blood cultures occurred at 37 ± 14 hours, and 97 percent of blood cultures were positive by 72 hours if the patient was not exposed to antifungal therapy.[273]

Neonatal Endocarditis and Infected Vascular Thrombi

Candida endocarditis has been reported in 5.5 to 15.2 percent of cases of fungal sepsis, with equal prevalence for *C. albicans* and *C. parapsilosis*. Fungal endocarditis may be associated with higher mortality than fungemia alone.[53,216] Use of central vascular catheters places neonates at increased risk for endocarditis and infected vascular thrombi. As discussed previously, they can cause local trauma to valvular, endocardial, or endothelial tissue, thus creating a nidus for thrombus and infection.

Neonatal Endophthalmitis and Retinopathy of Prematurity

Endophthalmitis begins as a chorioretinal lesion that gradually elevates and breaks free in the vitreous, appearing as a white fluffy ball. These solitary or multiple white lesions are most often seen in the posterior retina (zone I) and vitreous. In adults and children the clear cell-free vitreous becomes hazy, owing to an influx of inflammatory cells. This vitreous reaction is more difficult to recognize in preterm infants because of the vitreous haze that is present in the first weeks of life. The most immature infants appear to be at highest risk for fungal endophthalmitis. The incidence of retinal endophthalmitis with candidemia has decreased over the years, likely because of more rapid diagnosis and treatment. In a retrospective study from 1989 through 1999, 6 percent (4 of 67) of preterm infants with fungal sepsis who had indirect ophthalmoscopy had endophthalmitis.[216]

Even in absence of visible retinal abscesses or chorioretinitis there is some epidemiologic evidence that *Candida* sepsis may predispose preterm infants to severe retinopathy of prematurity. In a study of ELBW infants, threshold retinopathy of prematurity developed in 33 percent (19 of 58) of infants with a history of candidemia compared with 10 percent (39 of 391) without candidemia, but this was not statistically significant when controlled for gestational age.[139] Conversely, Noyola and colleagues,[215] in a case-control study of VLBW infants and those less than 28 weeks' gestation, found that 52 percent (24 of 46) of infants with candidemia developed threshold retinopathy of prematurity compared with 24 percent (11 of 46) of controls without candidemia (p = .008). Although the data regarding an association between fungal sepsis and retinopathy of prematurity are conflicting, early and frequent screening for retinal pathology is recommended in preterm infants with candidemia.

Neonatal Renal System and Fungal Infection

Candida UTIs may occur via the urinary tract as an ascending infection or dissemination via hematogenous spread. Renal factors that favor *Candida* growth include local immune factors and a delayed renal inflammatory response as well as hypertonicity and acidity of the tubular lumen. In preterm infants urine pH is increased in the first weeks of life owing to decreased bicarbonate reabsorption at the proximal tubule. Prematurity, antibiotics, and immunosuppressive therapy are also risk factors for renal fungal infections. In six studies of VLBW infants, the incidence of fungal UTI was 2.4 percent among all 1322 infants and 58.3 percent (28 of 48) in infants with fungal sepsis.[146] In one study of ELBW infants, the incidence of fungal UTI was 6 percent (3 of 50) and 1 of 8 infants with fungal sepsis had *Candida* UTI.[144]

Renal fungal abscess formation may complicate *Candida* UTIs in preterm infants. Fungal UTI may occur alone or in conjunction with fungal sepsis. Renal abscess formation in preterm infants may occur by dissemination of candidemia or as an ascending infection with candiduria. In one study, 41 of 8790 (0.5%) infants admitted to a NICU developed candiduria with median birth weight of 890 g and gestational age of 27 weeks.[45] Candiduria occurred in the more preterm infants, and renal abscesses developed in 36.6 percent (15 of 41) of the infants with candiduria. These 15 patients had a median birth weight of 770 g and gestational age of 26 weeks. Initial ultrasound examinations were normal in 6 patients, who developed renal abscesses 8 to 39 days later. This study suggests that in infants with candiduria, renal imaging studies should be performed at the time of infection and also at the end of antifungal treatment.

Neonatal Central Nervous System Candidiasis

Fungal infection of the CNS may result in meningitis, ventriculitis, or abscess formation in infants. Culture of the CSF is important in diagnosing fungal meningitis in infants because CSF cell counts and blood chemistry results may not be abnormal.[102] In one study of 25 infants with *Candida* sepsis or meningitis, CSF abnormalities occurred in only 25 percent of cases of culture-proven *Candida* meningitis.[174] In another study of VLBW infants with *Candida* meningitis detected by culture, the most common CSF abnormality was low glucose (<30 mg/dL), whereas the CSF protein level was elevated in only half of the patients (range, 30 to 480 mg/dL). CSF white blood cell counts ranged from 3 to 1030 cells/μL with varying percentages of neutrophils and mononuclear cells in these cases of culture-proven fungal meningitis.

Fungal abscesses of the CNS have been reported to be microscopic and not readily detectable by ultrasonography. In a study of 46 ELBW patients with fungal sepsis and/or meningoencephalitis, only 6 of 13 patients with fungal CNS abscesses had abnormal results on lumbar puncture.[97] Studies have found an association between invasive fungal infection and periventricular leukomalacia in preterm infants, possibly related to release of cytokines that may damage the periventricular white matter.[97] These findings and animal studies demonstrate the need for cranial imaging such as ultrasonography in all patients with fungal sepsis regardless of the results of CSF studies.[123]

Diagnosis

The majority (>99%) of fungal infections in neonates are due to *Candida* spp., the remainder are due to *Aspergillus* and *Zygomycetes*. Most common infections are of the blood, urine, skin, central nervous system, and/or other sterile body fluids. Dissemination can occur and screening for end-organ dissemination of abscess formation is warranted (see Fig. 199-4). Most commonly, with BSI, an initial screening including echocardiography, renal ultrasonography, cranial ultrasonography, and an ophthalmologic examination is needed. If there had been a significant bowel pathologic process such as NEC or focal bowel perforation, a complete abdominal ultrasound study should be performed to rule out peritoneal, liver, or spleen involvement. If signs and symptoms of septic arthritis (joint swelling) or osteomyelitis (swelling or immobility) are present, evaluation with joint aspiration, bone scan, or MRI is necessary. If candidemia persists, end-organ dissemination is even more likely and screening should be repeated as well as additional surveillance performed after 5 to 7 days, including (1) ultrasonography at the location of the tip of any central catheters for previous thrombus, (2) complete abdominal ultrasonography for abscesses (laparotomy is sometimes considered if there is high clinical suspicion), and (3) cranial ultrasonography/MRI to detect brain dissemination if meningitis. Treatment of invasive fungal infections in neonates requires intravenous administration of an antifungal agent and the removal of central venous catheters in infants with *Candida* BSIs.

Culture of blood, urine, CSF, and peritoneal fluid or other sterile body fluids remains the best method for diagnosing invasive *Candida* infections. Focus on obtaining sufficient blood culture volumes (≥1 mL) and performing urine and CSF cultures at the time of evaluation for sepsis remain critical to making a prompt diagnosis. Laboratory capabilities to culture fungi, identify *Candida* spp., and perform susceptibility testing are critical to diagnosis and management of invasive candidiasis.

Fungal PCR assay to detect the gene for 18S ribosomal RNA (rRNA) in VLBW infants has yielded promising results but requires additional study.[305] PCR results detect a broader number of infections because they not only detect patients with candidemia but are also positive in those with *Candida* peritonitis and candiduria.

In addition, investigators are examining the role of monitoring markers of fungal disease to diagnose and evaluate responses to antifungal therapy. These markers include β-glucan and mannan of the cell wall, anti-*Candida* antibodies, D-arabinitol (candidal metabolite), and fungal chitin synthase (assessed with PCR). These studies are challenging in neonates and need to studied in both non-infected colonized patients as well as infants with ICI.

Treatment and Prevention

Adequate dosing of antifungal therapy has been understudied in neonates and remains a critical issue. Most of

the literature lacks good data on dosing and outcomes, and we have likely been underdosing our patients, which may contribute to delayed clearance, treatment failures, and increased dissemination, morbidity, and mortality. In addition to dosing, prompt central line removal in patients with *Candida* BSIs is needed for clearance and to improve outcomes. Information on central line removal is often lacking, making it difficult to assess the efficacy of the antifungal agents being studied. In the area of pharmacokinetics, premature neonates, full-term neonates, pediatric patients, and adults often have different plasma clearance rates and drug half-lives, which affect dosing decisions as well as drug efficacy, safety, and toxicity.

Amphotericin B Preparations

Amphotericin B deoxycholate (AmBd) should be the first choice for treatment of invasive candidiasis owing to its excellent coverage for almost all *Candida* spp. causing invasive candidiasis in neonates, its low cost compared with the lipid preparations and other antifungal agents, its safety, and its better renal penetration compared with lipid preparations. This is the best choice for initial therapy, pending speciation. Almost all *Candida* spp. have favorable susceptibility to AmBd, whereas 97 percent of neonatal isolates are susceptible to fluconazole. When fluconazole is being used for prophylaxis in the NICU for any patient, a different antifungal agent should be used for treatment of invasive candidiasis in that NICU to decrease antifungal pressure that may lead to azole resistance.

AmBd should be started at 1 mg/kg and with lipid preparations (LFAB) at 5 mg/kg.[6,18,224] Serum electrolytes and urine output should be monitored as additional sodium and potassium supplementation is often required. Fluids and enteral feedings should be adjusted to maintain urine output of 2 ml/kg/hour. Several studies in the past decade have demonstrated no adverse renal or systemic effects of daily AmBd at 1 mg/kg. Linder and coworkers demonstrated that dosing of 1 mg/kg of AmBd (n = 34) did not affect renal function.[180] Creatinine levels in milligrams per deciliter remained unchanged from the start of treatment (0.8 ± 0.4), at 24 hours after beginning treatment (0.8 ± 0.4), and again at the end of therapy (0.6 ± 0.2). This is an important difference compared with older children and adults in whom infusion-related reactions and nephrotoxicity are frequent concerns with AmBd. Lower "test" dosages of AmBd are not needed in neonates, because adverse effects commonly seen in older patients do not occur in neonates. Moreover, lower dosages in neonates only delay the time to achieve optimal therapy and may contribute to morbidity and mortality by delaying clearance of *Candida* spp. If the initial treatment is ineffective, studies have demonstrated safety with dosing of AmBd increased to 1.5 mg/kg/day and LFAB to 7 mg/kg/day.[133]

A few retrospective studies have examined whether LFAB is better than AmBd, but none has shown superiority.[12,180] The major differences that still need to be evaluated in prospective studies include risk for phlebitis when given via peripheral intravenous line and whether AmBd has an advantage in treating UTIs and renal abscesses. Animal studies have demonstrated significantly higher renal concentrations of AmBd (when comparing 1 mg/kg dosing of each preparation), which has led most experts to recommend preference over lipid formulations for UTIs and renal candidiasis. None of the studies have compared optimal dosing of 1 to 1.5 mg/kg of amphotericin deoxycholate with 5 to 7 mg/kg of lipid preparations of amphotericin B. As mentioned previously, one major advantage of AmBd is cost, because it is significantly less expensive compared with LFAB.

Azole Antifungal Agents

Fluconazole has demonstrated similar efficacy to AmBd. Although AmBd is the treatment of choice in neonatal invasive candidiasis, fluconazole is used at some institutions. Fluconazole is readily bioavailable and has excellent tissue penetration. As mentioned earlier, fluconazole should be reserved and used primarily for prophylaxis and added when combination therapy is desired. Population pharmacokinetic study of fluconazole in 23- to 40-week-gestation infants younger than 120 days of age demonstrated that dosing in young infants requires adjustment for gestational age at birth and postnatal age to achieve therapeutic systemic drug exposures and that doses at the higher end of the recommended range (12 mg/kg) may yield better efficacy while the safety of this dosing awaits further clinical study.[329] Standard dosing of fluconazole for treatment of invasive candidiasis had previously been 6 mg/kg. Studies have also suggested that a loading dose may help achieve high levels rapidly.[239] With higher dosing, safety in relation to liver function, drug-drug interactions, and effect on fungal resistance should be monitored and studied.

Unlike fluconazole, voriconazole is mostly protein bound and also tends to accumulate in infants with renal insufficiency, and drug levels should be monitored. There is some concern for toxicity in neonates with a developing retina, and it has not been recommended or studied in neonates for the treatment of candidiasis. From a few case reports in the neonatal literature and the adult and pediatric data, suggested dosing is 6 mg/kg every 12 hours (for 24 hours), followed by 4 mg/kg every 12 hours with monitoring of serum hepatic enzymes weekly and drug levels obtained if possible.[93,210]

Echinocandin Agents

Neonatal data regarding echinocandins are just emerging. Early studies of caspofungin used doses of 1 to 5 mg/kg/day with variable efficacy at the lower doses and major toxicity at the high doses.[213,220,287] Pharmacokinetic data in neonates recommend dosing of 25 mg/m^2/day.[263] With any use currently, renal and liver function and electrolytes, including calcium and phosphorus, must be closely monitored and levels obtained if feasible. Micafungin pharmacokinetic and safety data point to a dose of 10 mg/kg/day, but only in a few patients with limited safety data.[31] Micafungin at high doses in animals and in one case report using 15 mg/kg in a preterm infant may have adverse effects.[156] All the echinocandins need to

address four major issues: optimal dose, resistance patterns with regard to *C. parapsilosis*, safety, and CNS penetration.[123,232,281]

Combination Antifungal Therapy

Linder and associates reported on the outcomes with combination therapy.[180] A second antifungal agent, most commonly fluconazole, was administered in cases of fungal sepsis, initially with the presence of any abscess, a positive urine culture, or after 10 days of persistent culture-positive infection. Patients were treated for 14 days after negative culture or more until radiographic resolution of abscess if present. Information on central line removal was not included. Sterilization occurred in 36 patients (67%) with monotherapy and increased to 52 patients (96%) with polytherapy. Currently, combination antifungal therapy is not recommended for neonatal candidiasis as the initial treatment but could be considered with persistent candidemia after 7 days of antifungal treatment or meningitis. Pediatric infectious disease consultation may help facilitate optimal management of these cases when combination therapy is considered.

Central Venous Catheter Removal with Bloodstream Infection

The question of whether the removal of central venous catheters with *Candida* BSI on presentation of infection improves outcomes has been the subject of multiple studies. Several studies have demonstrated prompt removal is associated with lower mortality rates, a shorter duration of infection, and reduced end-organ dissemination.[32,53,140,216] National Institute of Child Health and Human Development (NICHD) Neonatal Research Network data showed that mortality was reduced from 37 to 21 percent (p <.024) when catheters were removed promptly (<24 hours) compared with delayed removal.[32] The combined outcome of neurodevelopmental impairment and death was associated with delayed removal/replacement of catheters; although there was a trend, no difference in neurodevelopmental impairment alone was observed (45% prompt removal vs. 63% delayed; p = .08). The strongest effect of prompt catheter removal and mortality was with *C. parapsilosis*, in which case after prompt removal 10 percent of patients died compared with 31 percent when catheter removal was delayed.

Catheter removal, not simply catheter replacement, is recommended.[216] *Candida* can quickly form resilient biofilms on catheters and prevent the penetration of antifungal agents. The recommendation, therefore, is prompt catheter removal until clearance of *Candida* infection from the blood is documented with three or more cultures. Further study of catheter replacement at a different site is needed but may be an option when peripheral intravenous access cannot be maintained or central access is needed for medications such as vasopressors. It is better to immediately remove and replace the catheter rather than delay removal if intravenous access is a major concern.

Duration of Candidemia

Another controversial issue regarding neonatal candidiasis is how to determine that a patient's bloodstream is clear of infection. The NICHD Neonatal Research Network investigated complete clearance of BSI and found that intermittent negative cultures were obtained between positive cultures in 21 percent of cases of infants with *Candida* BSI.[32] Possible explanations may be the nature of clearing *Candida* infections, the theory that all infections have undetectable microabscesses that may be slowly dissolving, and/or low volume of blood (<1 mL) sent for culture. Based on this high incidence of false-negative results, daily blood cultures of 1 mL or more should be drawn until three negative cultures are documented, to confirm sterilization of the bloodstream; then a new central access device can be placed. Treatment is recommended for 14 days after the bloodstream is clear of candidemia.[180]

Adjunctive Therapies

Infection is affected by fungal load, virulence, and host factors, with neutrophil function being critical for *Candida* killing and clearance. Neutropenia should be corrected in the presence of active infection if persisting after 2 days of antifungal therapy. As an adjunctive therapy for invasive candidiasis in the absence of neutropenia, therapy with granulocyte colony-stimulating factor, other colony-stimulating factors, and immunoglobulins has not demonstrated efficacy.[44,146]

Empirical Therapy. The value of empirical therapy and its ability to improve outcomes in neonatal candidiasis continues to be investigated. Antibacterial agents are routinely prescribed when clinicians are concerned about late-onset sepsis in neonates, and perhaps in certain cases antifungal agents also should be added, pending culture results. Some studies have suggested that empirical antifungal therapy may lower mortality and improve outcomes in VLBW infants with fungal sepsis, but this has not been investigated in controlled trials.[29,185] One retrospective study reported the results of empirical antifungal therapy in neonates weighing less than 1500 g and considered "very sick" in the NICU.[243] Empirical antifungal therapy was started in those high-risk patients who had received vancomycin plus a third-generation cephalosporin for 7 days and had one or more of the following infection risk factors: total parenteral nutrition, mechanical ventilation, corticosteroid use, H_2 blocking agents, or signs of a *Candida* rash or thrush. The authors reported *Candida*-related mortality in 11 of 18 (61%) patients who did not receive empirical therapy compared with none of 6 (0%) patients receiving empirical therapy, suggesting that empirical therapy may have a role in improving mortality outcomes.

Retrospective studies have suggested that empirical antifungal therapy may lower mortality and improve outcomes in preterm infants with fungal sepsis. Although empirical or prompt standardized treatment (including prompt removal of central vascular catheters) may reduce *Candida*-related deaths, neurodevelopmental impairment

may still occur in almost 60 percent of the survivors, particularly those with birth weight less than 1000 g.[32,109] More prospective studies controlled for timing, dose, antifungal agent used, and central line removal need to be performed. In NICUs that are successful in infection prevention, infections are viewed as preventable and focus is placed on prevention instead of early detection and treatment.[79]

Preemptive Treatment

Preemptive antifungal treatment has been proposed in a few neonatal studies when early detection of colonization with *Candida* or mannan is isolated in endotracheal tubes.[240,325] As covered in the colonization section, the lung is a high-risk site for colonization. With this approach of early identification followed by 14 days of antifungal treatment, invasive candidiasis was reduced from 23 to 0 percent of these patients with *Candida* identified in their respiratory tracts. This may be an approach to be studied in patients with NEC in whom *Candida* is isolated in the gastrointestinal tract.

Prevention

As with other infectious diseases, prevention is key to achieving the best clinical outcomes. Mortality and morbidity is high, and for ELBW infants 73 percent either do not survive or survive with neurodevelopmental impairment.[33] To date, antifungal prophylaxis is the only intervention that has been subjected to evidence-based randomized, controlled trials for the prevention of invasive candidiasis. A summary of neonatal fluconazole and nystatin prophylaxis studies has been recently reviewed.[149]

Prophylactic antifungal therapy has been shown to reduce both colonization and invasive candidiasis in high-risk preterm infants. At birth, most infants are not colonized or have low colony counts of yeast, making them ideal candidates for antifungal prophylaxis. In the absence of antifungal prophylaxis, up to 60 percent of ELBW infants become colonized in the first 2 to 3 weeks after birth.[144] Prophylaxis prevents invasive candidiasis by decreasing *Candida* colonization of the skin, gastrointestinal and respiratory tracts, and central venous catheter as well as colonization at multiple (two or more) sites.[14,144,187,190] Additionally, subinhibitory concentrations of fluconazole improve white blood cell phagocytosis and killing of *C. albicans* from 50 to 90 percent.[119]

Fluconazole

Currently there are more than 20 studies examining fluconazole prophylaxis for the prevention of invasive candidiasis in more than 5000 neonates that are consistently demonstrating efficacy, with an overall reduction in invasive candidiasis of more than 80 percent and a 90 percent decrease in *Candida*-related mortality.* The overall incidence of invasive candidiasis in the placebo or control (untreated) groups was 9.1 percent compared with 1.3 percent in the fluconazole prophylaxis groups (p <.0001).[153]

Efficacy is highest in the smallest (<1000 g) and youngest infants (≤27 weeks' gestation) when prophylaxis is started shortly after birth and given to all high-risk patients. All of these studies have demonstrated the safety of fluconazole prophylaxis and lack of emergence of resistance.[144,145,149,150,188]

The first study of fluconazole prophylaxis against fungal colonization and infection in preterm infants was a prospective, randomized, double-blind clinical trial involving 100 high-risk preterm ELBW infants who required central venous access or endotracheal intubation.[144] Infants were randomly assigned during the first 5 days of life to receive either 3 mg/kg fluconazole intravenously or placebo for up to 6 weeks. Results demonstrated that fluconazole prophylaxis significantly decreased invasive fungal infection. None of the infants in the treatment group developed infection compared with 20 percent of infants in the placebo group (p = .008). A subanalysis of infants younger than 24 weeks of gestational age, approximately 10 percent of the cohort, revealed that fluconazole treatment was efficacious even in the most immature of infants.

From a system's viewpoint, the dosing schedule, although effective and safe in the first study, was complicated and changed every 2 weeks. Therefore, the efficacy of a twice-weekly dosing regimen was studied as a simplified schedule in a randomized controlled trial compared with the more frequent dosing schedule. The randomized controlled trial demonstrated that 3 mg/kg administered twice weekly was as effective in preventing invasive candidiasis as the more frequent dosing schedule used in the previous study.[145] This was recently confirmed in another study.[15] Twice-weekly dosing can occur on the same two days (e.g., Tuesday and Friday) and at a specific time, thus reducing pharmacy and nursing time, preparation and administration costs, and drug cost over the prophylaxis period. Twice-weekly administration of 3 mg/kg also is associated with a decreased risk for development of azole-resistance among fungi.[150]

Manzoni and colleagues in Italy performed a multicenter, randomized, placebo-controlled trial investigating two different fluconazole doses (3 mg/kg and 6 mg/kg) compared with a placebo group.[190] In 322 infants weighing less than 1500 g, investigators reported a significant difference between the fluconazole prophylaxis groups compared with the placebo patients. Fungal colonization was significantly decreased from 29.2 percent in the placebo patients to 7.7 percent and 9.8 percent in the 3- and 6-mg/kg groups, respectively. Invasive candidiasis was decreased from 13.2 percent in the placebo patients to 7.7 percent and 9.8 percent in the 3- and 6-mg/kg groups, respectively (p = .005 and p = .02, respectively, vs. the placebo group). Fluconazole prophylaxis had similar efficacy for both the 3-mg/kg and 6-mg/kg groups. The results also showed a significant effect in infants weighing less than 1000 g as well as in infants younger than 27 weeks' gestation.

Two studies by Healy and colleagues also demonstrated that, by targeting infants weighing less than 1000 g, they were able to eliminate all *Candida*-related

*References 3, 14, 15, 24, 35, 37, 120, 121, 144, 155, 186, 190, 192, 257, 316, 327, 336.

mortality in their NICU with fluconazole prophylaxis.[120,121] In their earlier study they assessed the impact of intravenous fluconazole prophylaxis over the course of 2 years and reported that the incidence of invasive candidiasis in infants weighing less than 1000 g decreased from 7 percent (15 of 206) to 2 percent (5 of 240) (p = .01); mortality related to infection also decreased from 12 percent (4 of 33) to 0 percent (0 of 40) (p = .04). They extended the study to a 4-year period (2002-2006), and compared their results using fluconazole prophylaxis in infants during that time to results obtained in their earlier study. For all patients in their NICU, both full-term and preterm infants, the incidence of invasive candidiasis significantly decreased from 0.6 percent (19 of 3012 infants) to 0.3 percent (22 of 6393 infants) ($p < .05$). The invasive candidiasis–related mortality rate for the entire NICU also decreased from 0.1 percent to 0 percent (p = .004). The decrease in incidence of infection occurred without the emergence of fluconazole-resistant *Candida* spp. during the time of study. This demonstrates that *Candida*-related mortality for the entire NICU can be eliminated by targeting high-risk patients.

Timing of Antifungal Prophylaxis. Most studies have targeted antifungal prophylaxis to the time period when high-risk patients have additional risk factors for *Candida* infections and when these infections most commonly occur.[32] These include (1) the need for intravenous access (central or peripheral), (2) the first 4 to 6 weeks after birth, and (3) during antibiotic administration. The initial studies targeted high-risk infants weighing less than 1000 g given prophylaxis during their high-risk period of needing intravenous access, which correlates with risk factors of receiving parenteral nutrition, antibiotics, gastrointestinal diseases, or having a central venous catheter.[144] This approach also individualizes prophylaxis for infants who achieve full enteral feedings and have their central venous catheter removed early versus those who need access for a longer time period. Studies using this criteria in infants weighing less than 1000 g have had infants on prophylaxis for an average of 4 to 5 weeks.[4,18] For example, an infant of 25 weeks' gestation on full enteral feedings by 3 weeks would only need a total of six doses of fluconazole prophylaxis. Conversely, those infants needing a central venous access for 2 months would be protected for a longer time period.

Starting prophylaxis later, during prolonged antibiotic courses for more than 3 or 10 days, has been examined in retrospective studies.[196,316] The goal of these studies is to prevent invasive candidiasis but further limit drug exposure, cost, and potentially fungal resistance. These studies have demonstrated efficacy but are not as effective as prophylaxis started in the first days after birth, which also makes the approach less cost effective. Delaying antifungal prophylaxis until after colonization has been detected is 30 percent less effective than starting within 72 hours after birth.[222] Finally, in these studies the investigators reported that they only had 75 percent compliance to their protocol, so eligible patients were being missed, making them less effective from a systems standpoint.[192]

Safety of Fluconazole Prophylaxis. No significant adverse effects have been reported in randomized trials in infants treated with fluconazole prophylaxis compared with control patients. Preventing invasive candidiasis has not led to an increased incidence in other infections. The incidences of bacterial infections and NEC are similar in all studies between treatment and control groups. Fluconazole-related cholestasis has been examined closely in several studies. No cholestasis was seen between groups in the randomized controlled trials, and retrospective studies have shown by multivariate analysis that cholestasis is related to other factors such as NEC and bacterial infections and not fluconazole prophylaxis.[121,144,190,192] In addition, dosing of 3 mg/kg twice a week may be associated with less cholestasis.[3,15]

Long-term safety of fluconazole prophylaxis has also been evaluated. Neurodevelopmental status and quality of life of survivors from the first randomized controlled trial were evaluated at 8 to 10 years of life. There were no differences in the Vineland Adaptive Behavior Scales-II scores for the fluconazole-treated group compared with the placebo group. Patients scored well in the four domains: communication (95 vs. 93), daily living skills (88 vs. 87), socialization (97 vs. 94), and motor skills (92 vs. 95). Additionally, there were no differences between the two groups regarding quality of life. Survivors were happy or satisfied with school (90% vs. 100%), friendships (90% vs. 88%), and life (95% vs. 100%). Self-esteem scores were 87 versus 90. There were also no differences between groups regarding emotional difficulties or behavior problems.[151]

Azole Resistance After Neonatal Antifungal Prophylaxis

Sarvikivi and colleagues studied the use of fluconazole prophylaxis over a 12-year period in an NICU and found no *C. albicans* BSIs or resistance, no *C. glabrata* or *C. krusei* BSIs, and only two cases of fluconazole-resistant *C. parapsilosis* BSIs.[270] Additional studies of MIC have demonstrated no significant resistance among fungal strains causing colonization or infection over 5-, 6-, and 11-year time periods.[144,145,150]

Further examination of the study by Sarvikivi and colleagues demonstrated that after 10 years of targeted prophylaxis in preterm infants, the protocol was expanded to the entire NICU and the daily dose was increased to 6 to 12 mg/kg. Two infants developed a fluconazole-resistant strain of *C. parapsilosis*, and it was concluded that this single strain with decreasing susceptibility was responsible for cross infections that caused BSIs in the NICU over the entire 12-year study period. This study and others have revealed a higher risk for emergence of resistance with higher (≥6 mg/kg) and more frequent (daily) dosing of fluconazole.[270,337] This study illustrated two important points. First, it is important to use a different antifungal agent for treatment than the one used for prophylaxis so that if an infection by a resistant organism occurs, treatment will be with an effective antifungal agent. Second, although a rare occurrence, high doses and total NICU exposure to fluconazole may have led to the resistant strains. By using fluconazole for both

prophylaxis and treatment, patients were exposed to higher doses for longer periods of time. Higher doses of daily fluconazole over longer time periods are associated with resistance in adult HIV-infected patients. These mechanisms of resistance include increases in efflux pumps, point mutations, and gene overexpression.[337]

Studies of the MIC have demonstrated no significant resistance among fungal strains causing colonization or infection over 5-, 6-, and 11-year time periods in various NICUs.[121,144,145,150] These studies have also shown a relationship with frequency of dosing and fungal resistance. Twice-weekly dosing of 3 mg/kg of fluconazole prophylaxis was associated with significantly less colonization with resistant fungal isolates.[150] A retrospective evaluation of all clinical and surveillance fungal isolates obtained from infants during a 10-year period (1997-2006) revealed no significant evidence of colonization or infection with *C. glabrata* or *C. krusei* strains.[188] A comparison was made between the 6-year prophylaxis period (2001-2006) and a 4-year period before the start of fluconazole prophylaxis. The rate of *C. glabrata* or *C. krusei* colonization remained approximately 4 percent and the rate of infection was 1 percent, and no significant differences were detected when comparing the two study periods. In these studies, fungal infections were treated with AmBd preparations, which decrease the overall exposure to fluconazole and may intermittently eliminate resistant organisms.

A 2007 Cochrane review of these trials demonstrated efficacy of this treatment, with a typical relative risk of 0.23 and a number needed to treat of 9. The number needed to treat could vary substantially depending on the incidence of invasive candidiasis in NICUs.

Another systematic review investigated the effect of fluconazole prophylaxis on invasive candidiasis and analyzed all-cause mortality, *Candida*-related mortality, combined outcomes, adverse drug reactions, and fluconazole sensitivities.[147] Meta-analyses were performed using Mantel-Haenszel methods dividing the patient population into infants weighing less than 1000 g and all infants.[147] This analysis demonstrated that fluconazole prophylaxis decreased the incidence of invasive candidiasis in infants less than 1000 g by 91 percent (OR, 0.09; 95% CI, 0.04 to 0.24; p = .0004) and in all infants weighing less than 1500 g by 85 percent (OR, 0.15; 95% CI, 0.08 to 0.26; $p < .0001$). The all-cause mortality was 11 percent for the fluconazole-treated group compared with 16.3 percent for controls (OR, 0.74; 95% CI, 0.58 to 0.95; p = .017). *Candida*-related mortality was virtually eliminated (OR, 0.04; 95% CI, 0.01 to 0.31, p = .006), with prophylaxis decreasing these deaths by 96 percent.

The combined outcome of ICI plus mortality was examined from the three published randomized, controlled trials. This analysis demonstrated that 10 percent (34/319) of the fluconazole-treated infants either developed an invasive infection or died, compared with 25 percent (51/206) of placebo patients (p <.001).[143]

Nystatin

Nystatin, an enteral or orally administered nonabsorbable antifungal agent, was the first drug studied for prophylaxis in preterm infants and has been the subject of randomized, quasi-randomized clinical trials, retrospective and epidemiologic studies. These studies have added to our understanding of antifungal prophylaxis by demonstrating the benefits of prophylaxis even in NICUs with low rates of infection, confirming both a greater efficacy of prophylaxis when it is started early (by 72 hours after birth) versus later when colonization is detected and a decrease in all-cause mortality.

Sims and colleagues performed the first randomized, controlled trial of nystatin prophylaxis targeted to 67 intubated infants with birth weights less than 1250 g and demonstrated the efficacy of antifungal prophylaxis against invasive candidiasis.[283] Although the study was underpowered to detect a difference in BSI, there was a significant difference in the rate of UTI between the nystatin-treated and placebo groups (p = .01).

One important observation of antifungal prophylaxis is that starting prophylaxis after colonization is detected is not as effective as starting it shortly after birth in preventing infections. Ozturk and colleagues importantly examined the efficacy of starting antifungal prophylaxis within 72 hours after birth compared with starting it later and targeting only colonized infants once colonization was detected by surveillance cultures.[222] In their multigroup study, they demonstrated that nystatin prophylaxis was more effective in reducing *Candida* BSI when started in the first 72 hours after birth compared with identifying colonization first before instituting nystatin. They studied three groups: control patients (n = 358), colonized patients who started prophylaxis at the time colonization was detected; (n = 115), and patients who received prophylactic therapy within the first 72 hours after birth (n = 475).[223] Fungal BSI decreased by 90 percent when nystatin prophylaxis was started in the first 72 hours (47 ± 12 hours, mean ± SD) compared with only 62 percent when started after colonization was detected (12.6 ± 2.4 days). The rates of candidemia, although high in the control group, decreased significantly with oral nystatin prophylaxis in both ELBW and VLBW infants. There was no benefit for infants weighing more than 1500 g at birth.

Two large retrospective studies of nystatin prophylaxis demonstrate the use of antifungal prophylaxis even in NICUs with low *Candida* BSI rates. In the largest multicenter epidemiologic study to date (n = 14,778) comparing preterm infants who received nystatin prophylaxis with those who did not receive antifungal prophylaxis (1993-2006; Australia and New Zealand), oral nystatin prophylaxis was associated with a decrease in BSI or meningitis in infants weighing less than 1500 g (1.23% to 0.54%, $p < .0001$) and also in the infants weighing less than 1000 g (2.67% to 1.23%, $p < 0.0001$).[129] Antifungal prophylaxis was used in NICUs with a low incidence of *Candida* BSI and/or meningitis, but *Candida* UTI data are missing. The second study was performed in the United Kingdom in preterm infants of less than 33 weeks' gestational age. In addition to demonstrating efficacy, this study also found that all-cause mortality was lower in the nystatin prophylaxis group (11.8%) compared with patients not receiving prophylaxis (17.8%) (p <.0001).[101]

Study data are still sparse with the use and efficacy of nystatin prophylaxis for infants at the lower extremes of

prematurity and with gastrointestinal diseases. Limitations of the nystatin studies are that few infants of less than 25 weeks' gestation or weighing less than 750 g have been studied and nystatin may not always be able to be administered when there is an ileus, gastrointestinal disease, feeding intolerance, or hemodynamic instability.

Antifungal prophylaxis with parenteral fluconazole is effective in preventing colonization of the skin, gastrointestinal and respiratory tracts, central venous catheter and dissemination to multisite colonization, as well as eradicating colonization with *C. albicans*, the most virulent yeast for preterm infants. Aydemir and associates[14] have demonstrated that nystatin (given 0.5 mL to the oral cavity and 0.5 mL to the feeding tube every 8 hours) decreases skin, gastrointestinal, and respiratory fungal colonization.

Many questions regarding fluconazole and nystatin prophylaxis remain unanswered, but there are two studies that have compared the two strategies. One randomized controlled trial of infants of less than 1500 g birth weight compared oral fluconazole with oral nystatin prophylaxis started in the first 7 days and continued until full enteral feedings were achieved.[327] Invasive candidiasis occurred in 2 of 38 (5.3%) fluconazole-treated infants compared with 6 of 42 (14.3%) nystatin-treated infants. There were no deaths in the fluconazole-treated group compared with 7.5 percent (all-cause mortality) in the nystatin-treated group ($p = .03$). Of the six deaths, three were due to NEC, one was due to focal bowel perforation, and two were sepsis related (one *Candida*-related death). A third of the eligible patients for this study (n = 44) were excluded owing to hemodynamic instability or gastrointestinal concerns. The oral suspension of nystatin has a very high osmolarity of 3002 mOsm/L, and there is a concern with the use of hyperosmolar medications and NEC.

Aydemir and associates[14] in a three-arm randomized controlled trial of antifungal prophylaxis compared intravenous fluconazole (n = 93) and oral/enteral nystatin (n = 94) with placebo (n = 91) in infants weighing less than 1500 g. Patients were randomized (1 : 1 : 1) to fluconazole, nystatin, or placebo. This study demonstrated that nystatin prophylaxis resulted in decreased *Candida* colonization of the gastrointestinal tract, skin, and respiratory tract. Invasive candidiasis was significantly lower in both the fluconazole (3.2%) and nystatin (4.3%) groups compared with the placebo (16.5%) group (p <.0001). Although a previous study had raised concerns regarding nystatin use and NEC and all-cause mortality,[327] this study demonstrated no difference between both groups regarding NEC (fluconazole [8.6%], nystatin [9.6%], placebo [9.9%]) or mortality (fluconazole [8.6%], nystatin [8.5%], placebo [12.1%]).

Although oral fluconazole for prophylaxis was used in one of the previously mentioned studies, almost all other fluconazole prophylaxis studies have used intravenous administration in the first weeks. Although enteral fluconazole is 90 percent absorbed, absorption characteristics in preterm infants are variable and not well studied. Additionally, as discussed earlier, oral prophylaxis may not always be able to be given if patients have an ileus, NEC, intestinal perforation, or hemodynamic instability. It is during these patient conditions that fungal colonization, proliferation, and dissemination are likely to occur. For these reasons and to prevent colonization of central venous catheters, the intravenous route is recommended for fluconazole prophylaxis in preterm infants when peripheral or central intravenous catheters are present. In some studies, fluconazole was changed to enteral administration once patients achieved full enteral feedings and intravenous access was discontinued to complete 4 to 6 weeks of prophylaxis, but this may only need to be done if postnatal corticosteroid therapy is necessary.

For antifungal prophylaxis, the evidence favors fluconazole for prophylaxis owing to superior evidence, greater efficacy compared with nystatin prophylaxis (80% to 90% compared with 50% to 60%), safety in infants weighing less than 1000 g, lower costs, twice-weekly administration, and ability to be administered to all high-risk infants weighing less than 1000 g, even in the presence of gastrointestinal disease or hemodynamic instability (Table 199-3). Several studies state that nystatin is less expensive,[14,129] which is based on comparing one dose of each medication, but for a complete course of prophylaxis, because nystatin is dosed three to four times a day compared with only twice a week for fluconazole, nystatin ends up being approximately twice as expensive in the United States.

Targeted Prophylaxis in High-Risk Patients

High-risk patients should be defined as NICU patients with a significant incidence, mortality, or neurodevelopmental morbidity from their infections. Mortality and neurodevelopmental impairment are substantial in infants weighing less than 1000 g. Preterm infants in each 100-g birth-weight group less than 1000 g and by each gestational week earlier than or equal to week 27 all have invasive candidiasis rates of 5 percent or more (see Fig. 199-1). High-risk infants weighing more than 1000 g in the NICU who also have rates of 5 percent or more include those with gastrointestinal diseases, including NEC and gastroschisis, and infants weighing 1000 to 1500 g with gram-negative infections being treated with a third- or fourth-generation cephalosporin.

Infection Control and Prevention Group Recommendations

According to the Centers for Disease Control and Prevention, there are an estimated 30,000 preterm infants weighing less than 1000 g or of less than or equal to 27 weeks' gestation born each year in the United States. Instituting antifungal prophylaxis would prevent 1350 to 2700 infections, 200 to 400 *Candida*-related deaths, and 280 to 570 infants with neurodevelopmental impairment yearly. A unified, dedicated approach similar to group B streptococcus prophylaxis in all NICUs could achieve the largest impact on the prevention of invasive candidiasis. For group B streptococcus, the number needed to treat to prevent one infection is 135. Using National Nosocomial Infections Surveillance System data, the overall number needed to treat would be 13 for each *Candida* BSI prevented. Including all invasive *Candida*, bloodstream,

TABLE 199-3 **Neonatal Antifungal Prophylaxis: Fluconazole and Nystatin**

	Fluconazole	Nystatin
Effect on Colonization	CVC, skin, GI, and respiratory colonization	Skin, GI, and respiratory colonization
Level of Evidence	Level A-1 RCT data	Level A-1 RCT data
Efficacy	>90% efficacy in <1000-g infants	Efficacy 50% to 60%
Efficacy in Most Extremely Preterm Infants	Efficacy in the most extreme preterm infants (<25 wk old, <750 g)	Fewer infants <750 g or <25 wk old have been studied
Can Be Given with GI Disease or Ileus	Can be given in infants with NEC, ileus, or GI disease	May not be effective or able to be given if GI disease or ileus
Resistance	No resistance	Lack of data on resistance
Dosing	Twice-a-week dosing	Dosing three to four times a day
Route of Administration	Intravenous	Oral or enteral
Start Prophylaxis	After birth	After birth
End Prophylaxis	No longer need intravenous access	Full enteral feedings
Cost	Cost effective	More expensive in United States

CVC, central venous catheter; GI, gastrointestinal; NEC, necrotizing enterocolitis; RCT, randomized controlled trial.

urine, cerebrospinal, and peritoneal infections, the number needed to treat is closer to 10 for infants weighing less than 1000 g. Owing to the severity of these infections, with 73 percent of infants less than 1000 g dying or having neurodevelopmental impairment, prevention is critical.

The *Red Book 2012* (The Report of the Committee on Infectious Diseases of the American Academy of Pediatrics) released guidelines regarding antifungal prophylaxis for preterm infants weighing less than 1000 g.[6a] This report states that: "On the basis of current data, fluconazole is the preferred agent for prophylaxis, because it has been shown to be effective and safe. Fluconazole prophylaxis is recommended for ELBW infants cared for in NICUs with ≥5% rates of invasive candidiasis. The recommended regimen for ELBW neonates is fluconazole administered intravenously during the first 48 to 72 hours after birth at a dose of 3 mg/kg, twice a week, for 4 to 6 weeks, or until intravenous access no longer is required for care. This dosage and duration of chemoprophylaxis has not been associated with emergence of fluconazole-resistant *Candida* species."

For NICUs with rates of less than 5 percent in preterm infants weighing less than 1000 g, invasive candidiasis rates should be analyzed by both gestational age and 100-g birth weight groups in those infants weighing less than 1000 g to find the break point where the rate is greater than or equal to 5 percent. Invasive candidiasis rates should be determined by including *Candida* cases caused by BSIs, UTIs, meningitis, and peritonitis over a 2- to 5-year period. It is advisable to consider gestational age as well because it has a more linear relationship compared with birth weight to both incidence and mortality. Some very extremely preterm infants may be excluded if birth weight is solely used to establish a different cutoff for prophylaxis. For example, an infant of 24 weeks' gestation can weigh between 468 and 940 g (the 3rd and 97th percentiles).[167]

NICUs transitioning to a policy of resuscitation of younger infants should use the literature to guide decision making regarding which infants should receive prophylaxis. Decisions to resuscitate and manage extremely preterm infants involve including antifungal prophylaxis in the care of these patients because prophylaxis would contribute to decreasing mortality and neurodevelopmental impairment.[315] Improving outcomes for these infants is multifactorial and needs to include all beneficial approaches as a "bundle" to have the largest effect, and antifungal prophylaxis should be included.

Targeted prophylaxis in ELBW infants to the time of intravenous access individualizes each infant to receive prophylaxis during the high-risk period based on individual risk factors. Intravenous access correlates to the time period patients have a central line, have an ileus, and are receiving antibiotics and/or parenteral nutrition. As mentioned previously, if a 26-week-gestation infant does well, achieves full enteral feedings at 3 weeks of life, and has intravenous (central or peripheral) access removed, the patient would require only 6 doses of fluconazole prophylaxis. Comparatively, if a 24-week-gestation infant takes 8 weeks to reach full enteral feedings, 16 doses of prophylaxis would be administered. If a patient needs intravenous access after a period of being on full enteral feedings, then antifungal prophylaxis would be restarted until intravenous access is no longer needed again. Targeting prophylaxis to individual patients' risk factors helps to limit drug exposure to the patient as well as fungi, which helps limit toxicity, costs, and the emergence of fungal resistance.

For NICUs with significant rates of ICI in their infants weighing 1000 to 1500 g, some retrospective studies have proposed either the presence of a central venous catheter or treatment with antibiotics for more than 3 days as guidelines for the use of antifungal prophylaxis.[35,257,316] A retrospective study by Bertini and colleagues reported that 10 percent of infants weighing 1000 to 1500 g had central venous catheters and were treated with fluconazole prophylaxis.[35] The other study targeted fluconazole prophylaxis (3 mg/kg) for 21 days to infants in whom a decision was made to give broad-spectrum antibiotics for more than 3 days. In this study,

40 percent of 1000- to 1500-g infants in their NICU were targeted for prophylaxis.[316] Data from nystatin trials and the recent multicenter fluconazole study indicate that approaches starting prophylaxis after the first week of life would be effective if fungal colonization has not already occurred.[190,222]

Fluconazole Prophylaxis Dosing Recommendations

Taking into consideration the issue of emerging antifungal resistance, a dosage of 3 mg/kg fluconazole administered parenterally twice weekly appears to be the safest and most effective schedule. This provides similar efficacy with less risk for resistance compared with higher and more frequent doses of treatment. Administering fluconazole prophylaxis (3 mg/kg) twice weekly on the same days, every Tuesday and Friday, at a specified time, also reduces pharmacy costs and may limit medication errors. This schedule continues until intravenous access is no longer required. Additionally, a different antifungal agent (e.g. amphotericin B) is selected for treatment or empirical therapy.

Infection Control Measures to Prevent Invasive Candidiasis (Table 199-4)

Prenatal Detection and Eradication of Maternal Vaginal Candidiasis. In pregnancies complicated by preterm labor or prolonged rupture of membranes, screening and treatment of vaginal candidiasis may be beneficial in preventing candidal colonization and subsequent infection in the newborn.[40,94,330]

Medication and Feeding Stewardship. Practices reducing medications that increase the risk for invasive candidiasis should be avoided if possible and instituted with stewardship, guidelines, tracking/auditing, and accountability.[153] Medications that increase risk for invasive candidiasis as discussed previously include broad-spectrum antibiotics (third- or fourth-generation cephalosporins or carbapenems), gastric acid inhibitors (H_2 blockers and proton pump inhibitors), and postnatal dexamethasone.[153] Data from a multicenter study of postnatal hydrocortisone in infants with a birth weight of less than 1000 g did not demonstrate an increase with hydrocortisone, with both treated and placebo groups having an incidence of *Candida* BSIs of 10 percent.[334] Development of starting feeding protocols (starting early and trophic and slow feed advances) and promoting breast milk feedings may aid in decreasing the risk for invasive candidiasis as well.

CLABSI Bundles. Standardized protocols for insertion and management of central venous catheters, attention to sterile practices, hub and dressing care, and closed medication delivery systems may reduce CLABSIs, including *Candida*-related BSIs.[5,57] Aly and associates reported significant reduction in all CLABSIs and elimination of *Candida* infection using a "bundled approach," which included antifungal prophylaxis in addition to line placement and maintenance interventions.[5]

TABLE 199-4 Strategies to Reduce Morbidity and Mortality of Neonatal Invasive *Candida* Infections

1. Use antifungal prophylaxis (IV fluconazole) while IV access is in use (central or peripheral) for infants with birth weight <1000 g and/or ≤27 weeks' gestational age (A-I).
   a. A-I evidence also exists for antifungal prophylaxis with nystatin but limited data are available in infants <750 g and <26 weeks' gestation. Because fluconazole prophylaxis has greater efficacy compared with nystatin, has efficacy in the most immature patients, and can be given to infants not feeding, current evidence would favor fluconazole prophylaxis in preterm infants <1000 g.
2. Start treatment of documented infections with appropriate antifungal dosing and prompt catheter removal for candidal BSI (A-II). Consider starting empirical antifungal therapy if ICI is suspected. Promptly treat congenital cutaneous candidiasis.
3. Decrease broad-spectrum antibiotic use (B-II). Restrict third- and fourth-generation cephalosporins and carbapenems to treatment of proven gram-negative infections. Add antifungal prophylaxis while patient is receiving these agents.
4. Decrease H2 blocker and proton-pump inhibitor use (B-II). Use only for proven gastritis, and restrict use to 3 days or until symptoms resolved.
5. Decrease postnatal dexamethasone use (B-II). Use only for severe lung disease.
6. Institute central line–associated bloodstream infection reduction bundles (B-II).
7. Monitor rates of ICI, including BSI, UTI, meningitis and peritonitis infections and feedback to staff (B-II).

Level of evidence from U.S. Public Health Service Grading System for ranking recommendations in clinical guidelines. Strength of recommendation and levels of evidence: A, good evidence; B, moderate evidence; C, poor evidence: I, at least one randomized clinical trial; II, at least one well-designed but nonrandomized trial; III, expert opinions based on experience or limited clinical reports.

BSI, bloodstream infection; IV, intravenous; ICI, invasive *Candida* infection; UTI, urinary tract infection.

Lactoferrin

In a randomized controlled trial of bovine lactoferrin (bLF) in infants with a birth weight of less than 1500 g the incidence of late-onset sepsis was significantly lower in the bLF and bLF-plus probiotics groups than in the placebo patients.[189] Subanalysis found that invasive fungal infections were also significantly less common with lactoferrin alone compared with placebo. One limitation of the study was that there were only 9 to 18 infants weighing less than or equal to 750 g in each group. Confirmation of the findings in extremely preterm infants is needed.

Outcomes

For preterm infants, invasive *Candida* infections are associated with high mortality and neurodevelopmental impairment.

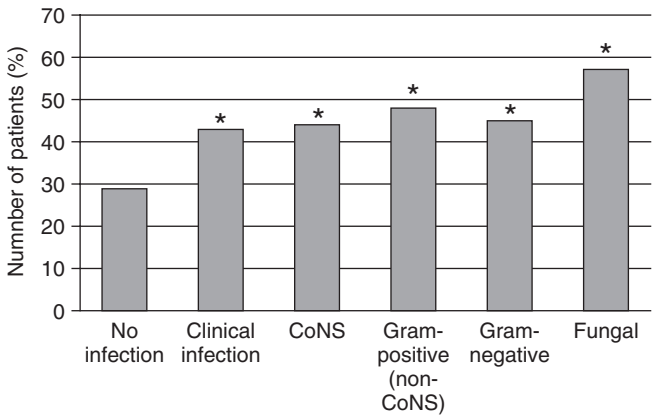

FIGURE 199-5 ■ Neurodevelopmental impairment and bloodstream infection in infants weighing less than 1000 grams. *$p \leq .001$ compared to no infection group. Neurodevelopmental impairment was determined by one or more of the following: Bayley Infants Score of Development physical or mental developmental index less than 70, cerebral palsy, or visual or hearing impairment. Clinical infection was defined as late-onset infection with negative cultures with antibiotic treatment for 5 or more days. CoNS, coagulase-negative staphylococci.

Neurodevelopmental Impairment

Invasive candidiasis may lead to significant neurodevelopmental impairment even in absence of documented fungal meningitis. Neurodevelopmental impairment in most studies is the presence of one or more of the following: low mental and motor development using Bayley Scales of Infant Development II, cerebral palsy, deafness, and/or blindness. With the use of these criteria, the incidence of neurodevelopmental impairment is 57 percent in infected infants versus 29 percent in noninfected ELBW infants (Fig. 199-5).[32,298] The incidence was similar for cases of *Candida* meningitis at 53 percent. These data also are similar to a study from an earlier time period.[98] Neurodevelopment was intact in 41 percent of *Candida*-infected patients and 65 percent of controls ($p = .02$). Periventricular leukomalacia was not significantly different between groups ($p = .06$). In this study, neurodevelopmental outcome was related to timing of initiation of antifungal therapy. Commencement of antifungal therapy was 5.1 ± 3.0 days after blood culture was drawn in those infants who had severe disabilities or died compared with 2.1 ± 1.3 days in those who had normal or mildly impaired outcomes ($p < .0001$).

Survival

Mortality is high with invasive *Candida* infections in ELBW infants. A recent multicenter study of infants weighing less than 1000 g at birth found that overall mortality was 34 percent for infants with invasive candidiasis compared with 14 percent in those without invasive candidiasis. [33] All-cause mortality rates were 28 percent for *Candida* BSI, 26 percent for *Candida* UTI, and 50 percent for other sterile sites (meningitis and peritonitis); and if two or more culture sites were involved (BSI +UTI or UTI + meningitis), the mortality rate was 57 percent.

There is a marked difference in overall and attributable mortality between infants weighing less than 1000 g and larger, more mature infants with invasive candidiasis. In infants weighing less than 1000 g with invasive candidiasis, a recent analysis reported an all-cause mortality rate of 26 percent compared with 13 percent in infants without candidiasis. For infants of more than 1000 g with invasive candidiasis versus those without, mortality was 2 percent compared with 0.4 percent.[347]

Candida*-Related Mortality*

Identifying *Candida*-related mortality is difficult and may include attributable mortality, death within a specified number of days from onset of infection (3, 7, 14, or 30 days), or review of each death using predetermined definitions. In infants weighing less than 1000 g with invasive candidiasis, these rates may range from an attributable mortality from epidemiology studies[33,347] of 13 to 15 percent to 23 to 66 percent when examining deaths of patients in the placebo or control groups in the prophylaxis studies.[149] Mortality increases at the lower ages of gestation of less than 26 weeks, and different mortality rates, similar to infection rates, will be influenced by resuscitation and admission rates of these extremely preterm infants.[135]

NEW REFERENCES SINCE THE SIXTH EDITION

2. Achkar JM, Fries BC. *Candida* infections of the genitourinary tract. *Clin Microbiol Rev* 2010;**23**:253–73.
6. American Academy of Pediatrics. Candidiasis. In: Pickering L, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Villiage, Ill.: American Academy of Pediatrics; 2009. p. 245–9.
8. Andes DR, Safdar N, Baddley JW, et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clin Infect Dis* 2012;**54**:1110–22.
11. Ascher SB, Smith PB, Clark RH, et al. Sepsis in young infants with congenital heart disease. *Early Hum Dev* 2012;**88**(Suppl. 2): S92–7.
12. Ascher SB, Smith PB, Watt K, et al. Antifungal therapy and outcomes in infants with invasive *Candida* infections. *Pediatr Infect Dis J* 2012;**31**:439–43.
13. Aun MV, Ribeiro MR, Costa Garcia CL, et al. Esophageal candidiasis—an adverse effect of inhaled corticosteroids therapy. *J Asthma* 2009;**46**:399–401.
14. Aydemir C, Oguz SS, Dizdar EA, et al. Randomised controlled trial of prophylactic fluconazole versus nystatin for the prevention of fungal colonisation and invasive fungal infection in very low birth weight infants. *Arch Dis Child Fetal Neonatal Ed* 2011; **96**(3):F164–8.
15. Aziz M, Patel AL, Losavio J, et al. Efficacy of fluconazole prophylaxis for prevention of invasive fungal infection in extremely low birth weight infants. *Pediatr Infect Dis J* 2010;**29**(4):352–6.
25. Bao Y, Du LZ, Shi LP, et al. [Clinical significance of oral fluconazole prophylaxis against invasive fungal infection in preterm neonates with peripherally inserted central catheters]. *Zhonghua Er Ke Za Zhi* 2010;**48**:510–13.
32. Benjamin DK Jr, Smith PB, Arrieta A, et al. Safety and pharmacokinetics of repeat-dose micafungin in young infants. *Clin Pharmacol Ther* 2010;**87**:93–9.
34. Benjamin DK Jr, Stoll BJ, Gantz MG, et al. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. *Pediatrics* 2010; **126**:e865–73.
37. Beyda ND, Lewis RE, Garey KW. Echinocandin resistance in *Candida* species: mechanisms of reduced susceptibility and therapeutic approaches. *Ann Pharmacother* 2012;**46**:1086–96.

38. Bhat V, Fojas M, Saslow JG, et al. Twice-weekly fluconazole prophylaxis in premature infants: association with cholestasis. *Pediatr Int* 2011;**53**(4):475–9.
39. Bizzarro MJ, Conrad SA, Kaufman DA, et al. Infections acquired during extracorporeal membrane oxygenation in neonates, children, and adults. *Pediatr Crit Care Med* 2011;**12**(3):277–81.
41. Bliss JM, Basavegowda KP, Watson WJ, et al. Vertical and horizontal transmission of *Candida* albicans in very low birth weight infants using DNA fingerprinting techniques. *Pediatr Infect Dis J* 2008;**27**:231–5.
43. Blyth CC, Chen SC, Slavin MA, et al. Not just little adults: candidemia epidemiology, molecular characterization, and antifungal susceptibility in neonatal and pediatric patients. *Pediatrics* 2009; **123**:1360–8.
45. Brocklehurst P, Farrell B, King A, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011; **365**:1201–11.
52. Chang TI, Kim HW, Park JT, et al. Early catheter removal improves patient survival in peritoneal dialysis patients with fungal peritonitis: results of ninety-four episodes of fungal peritonitis at a single center. *Perit Dial Int* 2011;**31**:60–6.
58. Chitnis AS, Magill SS, Edwards JR, et al. Trends in *Candida* central line–associated bloodstream infections among NICUs, 1999-2009. *Pediatrics* 2012;**130**(1):e46–52.
67. Conti HR, Gaffen SL. Host responses to *Candida* albicans: Th17 cells and mucosal candidiasis. *Microbes Infect* 2010;**12**:518–27.
73. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**:1813–21.
76. Dotis J, Prasad PA, Zaoutis T, et al. Epidemiology, risk factors and outcome of *Candida* parapsilosis bloodstream infection in children. *Pediatr Infect Dis J* 2012;**31**:557–60.
77. Dutta A, Palazzi DL. *Candida* non-*albicans* versus *Candida albicans* fungemia in the non-neonatal pediatric population. *Pediatr Infect Dis J* 2011;**30**:664–8.
78. Dvorak CC, Fisher BT, Sung L, et al. Antifungal prophylaxis in pediatric hematology/oncology: new choices & new data. *Pediatr Blood Cancer* 2012;**59**:21–6.
86. Falcone M, Barzaghi N, Carosi G, et al. *Candida* infective endocarditis: report of 15 cases from a prospective multicenter study. *Medicine* 2009;**88**:160–8.
89. Festekjian A, Neely M. Incidence and predictors of invasive candidiasis associated with candidaemia in children. *Mycoses* 2011;**54**: 146–53.
91. Fisher JF, Sobel JD, Kauffman CA, et al. *Candida* urinary tract infections—treatment. *Clin Infect Dis* 2011;**52**(Suppl. 6):S457–66.
92. Florescu DF, Islam KM, Grant W, et al. Incidence and outcome of fungal infections in pediatric small bowel transplant recipients. *Transpl Infect Dis* 2010;**12**:497–504.
96. Friberg LE, Ravva P, Karlsson MO, et al. Integrated population pharmacokinetic analysis of voriconazole in children, adolescents, and adults. *Antimicrob Agents Chemother* 2012;**56**:3032–42.
100. Gamaletsou MN, Kontoyiannis DP, Sipsas NV, et al. *Candida* osteomyelitis: analysis of 207 pediatric and adult cases (1970-2011). *Clin Infect Dis* 2012;**55**(10):1338–51.
101. Gammelsrud KW, Sandven P, Hoiby EA, et al. Colonization by *Candida* in children with cancer, children with cystic fibrosis, and healthy controls. *Clin Microbiol Infect* 2011;**17**:1875–81.
102. Ganesan K, Harigopal S, Neal T, et al. Prophylactic oral nystatin for preterm babies under 33 weeks gestation decreases fungal colonisation and invasive fungaemia. *Arch Dis Child Fetal Neonatal Ed* 2009;**94**(4):F275–8.
109. Gow NA, van de Veerdonk FL, Brown AJ, et al. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* 2012;**10**:112–22.
110. Greenberg RG, Benjamin DK Jr, Gantz MG, et al. Empiric antifungal therapy and outcomes in extremely low birth weight infants with invasive candidiasis. *J Pediatr* 2012;**161**:264–9.
111. Groll AH, Tragiannidis A. Update on antifungal agents for paediatric patients. *Clin Microbiol Infect* 2010;**16**:1343–53.
112. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev* 2011;**24**:247–80.
122. Healy CM, Campbell JR, Zaccaria E, et al. Fluconazole prophylaxis in extremely low birth weight neonates reduces invasive candidiasis mortality rates without emergence of fluconazole-resistant *Candida* species. *Pediatrics* 2008;**121**:703–10.
124. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous *Candida* meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008;**197**:163–71.
130. Howell A, Isaacs D, Halliday R. Oral nystatin prophylaxis and neonatal fungal infections. *Arch Dis Child Fetal Neonatal Ed* 2009;**94**(6):F429–33.
139. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, et al. Beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis* 2011;**52**:750–70.
143. Kauffman CA, Fisher JF, Sobel JD, et al. *Candida* urinary tract infections—diagnosis. *Clin Infect Dis* 2011;**52**(Suppl. 6):S452–6.
144. Kaufman D. Fluconazole prophylaxis decreases the combined outcome of invasive *Candida* infections or mortality in preterm infants. *Pediatrics* 2008;**122**:1158–9.
148. Kaufman DA. Fluconazole prophylaxis: can we eliminate invasive *Candida* infections in the neonatal ICU? *Curr Opin Pediatr* 2008;**20**:332–40.
149. Kaufman DA. Neonatal candidiasis: clinical manifestations, management, and prevention strategies. *J Pediatr* 2010;**156**:s53–67.
150. Kaufman DA. Aiming for zero: preventing invasive *Candida* infections in extremely preterm infants. *Neoreviews* 2011;**12**: e381–92.
151. Kaufman DA, Boyle R, Grossman LB, et al. Fluconazole prophylaxis dosing and the resistance patterns in preterm infants over a 11-year period. Presented before the annual meeting of the Pediatric Academic Society, 2010.
152. Kaufman DA, Cuff AL, Wamstad JB, et al. Fluconazole prophylaxis in extremely low birth weight infants and neurodevelopmental outcomes and quality of life at 8 to 10 years of age. *J Pediatr* 2011;**158**(5):759–65.e1.
154. Kaufman DA, Manzoni P. Strategies to prevent invasive candidal infection in extremely preterm infants. *Clin Perinatol* 2010;**37**: 611–28.
157. King KY, Edwards MS, Word BM. Hepatitis associated with micafungin use in a preterm infant. *J Perinatol* 2009;**29**:320–2.
159. Kisand K, Boe Wolff AS, Podkrajsek KT, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *J Exp Med* 2010;**207**:299–308.
164. Kollef M, Micek S, Hampton N, et al. Septic shock attributed to *Candida* infection: importance of empiric therapy and source control. *Clin Infect Dis* 2012;**54**:1739–46.
166. Kothavade RJ, Kura MM, Valand AG, et al. *Candida tropicalis:* its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol* 2010;**59**:873–80.
167. Kourkoumpetis TK, Fuchs BB, Coleman JJ, et al. Polymerase chain reaction–based assays for the diagnosis of invasive fungal infections. *Clin Infect Dis* 2012;**54**:1322–31.
172. Kuwahara T, Shimono K, Kaneda S, et al. Growth of microorganisms in total parenteral nutrition solutions containing lipid. *Int J Med Sci* 2010;**7**:101–9.
173. Lamoth F, Cruciani M, Mengoli C, et al. Beta-D-Glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin Infect Dis* 2012;**54**:633–43.
176. Legrand F, Lecuit M, Dupont B, et al. Adjuvant corticosteroid therapy for chronic disseminated candidiasis. *Clin Infect Dis* 2008; **46**:696–702.
179. Li PK, Szeto CC, Piraino B, et al. Peritoneal dialysis–related infections recommendations: 2010 update. *Perit Dial Int* 2010;**30**: 393–423.
180. Lim CS, Rosli R, Seow HF, et al. *Candida* and invasive candidiasis: back to basics. *Eur J Clin Microbiol Infect Dis* 2012;**31**:21–31.
182. Liu CY, Huang LJ, Wang WS, et al. Candidemia in cancer patients: impact of early removal of non-tunneled central venous catheters on outcome. *J Infect* 2009;**58**:154–60.

189. Manzoni P, Leonessa M, Galletto P, et al. Routine use of fluconazole prophylaxis in a neonatal intensive care unit does not select natively fluconazole-resistant *Candida* subspecies. *Pediatr Infect Dis J* 2008;**27**:731–7.
190. Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birthweight neonates: a randomized trial. *JAMA* 2009;**302**:1421–8.
192. Marom D, Levy I, Gutwein O, et al. Healthcare-associated versus community-associated infective endocarditis in children. *Pediatr Infect Dis J* 2011;**30**:585–8.
193. Martin A, Pappas A, Lulic-Botica M, et al. Impact of "targeted" fluconazole prophylaxis for preterm neonates: efficacy of a highly selective approach? *J Perinatol* 2012;**32**:21–6.
195. Matuszkiewicz-Rowinska J. Update on fungal peritonitis and its treatment. *Perit Dial Int* 2009;**29**(Suppl. 2):S161–5.
200. Melo NR, Taguchi H, Culhari VP, et al. Oral candidiasis of HIV-infected children undergoing sequential HIV therapies. *Med Mycol* 2009;**47**:149–56.
202. Mikulska M, Calandra T, Sanguinetti M, et al. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. *Crit Care* 2010;**14**:R222.
205. Mokaddas E, Burhamah MH, Khan ZU, et al. Levels of (1→3)-beta-D-glucan, *Candida* mannan and *Candida* DNA in serum samples of pediatric cancer patients colonized with *Candida* species. *BMC Infect Dis* 2010;**10**:292.
215. Nguyen MH, Wissel MC, Shields RK, et al. Performance of *Candida* real-time polymerase chain reaction, beta-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. *Clin Infect Dis* 2012;**54**:1240–8.
222. Olczak-Kowalczyk D, Pawlowska J, Garczewska B, et al. Oral candidiasis in immunosuppressed children and young adults after liver or kidney transplantation. *Pediatr Dent* 2010;**32**:189–94.
225. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:503–35.
228. Pasqualotto AC. *Candida* and the paediatric lung. *Paediatr Respir Rev* 2009;**10**:186–91.
230. Pedrosa AF, Rodrigues AG. Candidemia in burn patients: figures and facts. *J Trauma* 2011;**70**:498–506.
233. Petraitiene R, Petraitis V, Hope WW, et al. Cerebrospinal fluid and plasma (1→3)-beta-D-glucan as surrogate markers for detection and monitoring of therapeutic response in experimental hematogenous *Candida* meningoencephalitis. *Antimicrob Agents Chemother* 2008;**52**:4121–9.
235. Pfaller MA, Diekema DJ, Gibbs DL, et al. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. *J Clin Microbiol* 2008;**46**:515–21.
238. Pfeiffer CD, Samsa GP, Schell WA, et al. Quantitation of *Candida* CFU in initial positive blood cultures. *J Clin Microbiol* 2011;**49**:2879–83.
240. Piper L, Smith PB, Hornik CP, et al. Fluconazole loading dose pharmacokinetics and safety in infants. *Pediatr Infect Dis J* 2011;**30**(5):375–8.
241. Posteraro B, Sanguinetti M, Boccia S, et al. Early mannan detection in bronchoalveolar lavage fluid with preemptive treatment reduces the incidence of invasive *Candida* infections in preterm infants. *Pediatr Infect Dis J* 2010;**29**:844–8.
245. Puel A, Doffinger R, Natividad A, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med* 2010;**207**:291–7.
257. Robinson JL, Davies HD, Barton M, et al. Characteristics and outcome of infants with candiduria in neonatal intensive care—a Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study. *BMC Infect Dis* 2009;**9**:183.
258. Rolnitsky A, Levy I, Sirota L, et al. Targeted fluconazole prophylaxis for high-risk very low birth weight infants. *Eur J Pediatr* 2012;**171**:1481–7.
259. Rosenthal LB, Feja KN, Levasseur SM, et al. The changing epidemiology of pediatric endocarditis at a children's hospital over seven decades. *Pediatr Cardiol* 2010;**31**:813–20.
263. Sabino R, Sampaio P, Carneiro C, et al. Isolates from hospital environments are the most virulent of the *Candida* parapsilosis complex. *BMC Microbiol* 2011;**11**:180.
264. Saez-Llorens X, Macias M, Maiya P, et al. Pharmacokinetics and safety of caspofungin in neonates and infants less than 3 months of age. *Antimicrob Agents Chemother* 2009;**53**:869–75.
280. Shah CP, McKey J, Spirn MJ, et al. Ocular candidiasis: a review. *Br J Ophthalmol* 2008;**92**:466–8.
281. Sheppard DC, Locas MC, Restieri C, et al. Utility of the germ tube test for direct identification of *Candida* albicans from positive blood culture bottles. *J Clin Microbiol* 2008;**46**:3508–9.
282. Silva AP, Miranda IM, Lisboa C, et al. Prevalence, distribution, and antifungal susceptibility profiles of *Candida parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* in a tertiary care hospital. *J Clin Microbiol* 2009;**47**:2392–7.
283. Silva S, Negri M, Henriques M, et al. Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev* 2012;**36**:288–305.
285. Slenker AK, Keith SW, Horn DL. Two hundred and eleven cases of *Candida* osteomyelitis: 17 case reports and a review of the literature. *Diagn Microbiol Infect Dis* 2012;**73**:89–93.
292. Soysa NS, Samaranayake LP, Ellepola AN. Antimicrobials as a contributory factor in oral candidosis—a brief overview. *Oral Dis* 2008;**14**:138–43.
297. Steinbach WJ, Roilides E, Berman D, et al. Results from a prospective, international, epidemiologic study of invasive candidiasis in children and neonates. *Pediatr Infect Dis J* 2012;**31**(12):1252–7.
308. Tobudic S, Kratzer C, Lassnigg A, et al. Antifungal susceptibility of *Candida albicans* in biofilms. *Mycoses* 2012;**55**:199–204.
309. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective: preface. *Bone Marrow Transplant* 2009;**44**:453–5.
310. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009;**15**:1143–238.
312. Tragiannidis A, Dokos C, Lehrnbecher T, et al. Antifungal chemoprophylaxis in children and adolescents with haematological malignancies and following allogeneic haematopoietic stem cell transplantation: review of the literature and options for clinical practice. *Drugs* 2012;**72**:685–704.
313. Trofa D, Gacser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev* 2008;**21**:606–25.
314. Tronchin G, Pihet M, Lopes-Bezerra LM, et al. Adherence mechanisms in human pathogenic fungi. *Med Mycol* 2008;**46**:749–72.
316. Tyson JE, Parikh NA, Langer J, et al. Intensive care for extreme prematurity—moving beyond gestational age. *N Engl J Med* 2008;**358**:1672–81.
319. Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog* 2009;**5**:e1000639.
321. van de Veerdonk FL, Joosten LA, Shaw PJ, et al. The inflammasome drives protective Th1 and Th17 cellular responses in disseminated candidiasis. *Eur J Immunol* 2011;**41**:2260–8.
322. van de Veerdonk FL, Kullberg BJ, van der Meer JW, et al. Host-microbe interactions: innate pattern recognition of fungal pathogens. *Curr Opin Microbiol* 2008;**11**:305–12.
325. Velasco E, Portugal RD. Factors prompting early central venous catheter removal from cancer patients with candidaemia. *Scand J Infect Dis* 2011;**43**:27–31.
326. Vendettuoli V, Tana M, Tirone C, et al. The role of *Candida* surveillance cultures for identification of a preterm subpopulation at highest risk for invasive fungal infection. *Pediatr Infect Dis J* 2008;**27**:1114–16.
327. Vinh DC. Insights into human antifungal immunity from primary immunodeficiencies. *Lancet Infect Dis* 2011;**11**:780–92.
328. Violaris K, Carbone T, Bateman D, et al. Comparison of fluconazole and nystatin oral suspensions for prophylaxis of systemic fungal infection in very low birthweight infants. *Am J Perinatol* 2010;**27**(1):73–8.

330. Wade KC, Wu D, Kaufman DA, et al. Population pharmacokinetics of fluconazole in young infants. *Antimicrob Agents Chemother* 2008;**52**:4043–9.
337. Weitkamp JH, Ozdas A, Lafleur B, et al. Fluconazole prophylaxis for prevention of invasive fungal infections in targeted highest risk preterm infants limits drug exposure. *J Perinatol* 2008;**28**(6): 405–11.
340. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. *J Oral Microbiol* 2011:**3**.
344. Wynn JL, Tan S, Gantz MG, et al. Outcomes following candiduria in extremely low birth weight infants. *Clin Infect Dis* 2012;**54**: 331–9.
349. Zaoutis TE, Prasad PA, Localio AR, et al. Risk factors and predictors for candidemia in pediatric intensive care unit patients: implications for prevention. *Clin Infect Dis* 2010;**51**:e38–45.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 200

Coccidioidomycosis

Ziad M. Shehab • Kareem W. Shehab

Coccidioidomycosis is an infection caused by a dimorphic fungus of the genus *Coccidioides*. The primary pulmonary infection produced by this organism usually is self-limited, but disseminated and fatal disease may occur. In the United States, coccidioidomycosis is an endemic disease of the southwestern states and results in 150,000 infections per year.[68] In other parts of the United States, it also is seen in individuals who have traveled to or lived in the endemic areas of the Southwest or Mexico.[25,32,50] The disease is endemic in other areas of the Western Hemisphere, most notably in northern Mexico and in certain countries in South and Central America.[113] Pulmonary disease is underrecognized even in areas of high endemicity.[96,148] With population growth in the endemic areas of the Southwest and increased population mobility, clinicians in nonendemic areas are likely to encounter this disease, particularly in its more severe or disseminated forms. Reactivation, especially in the immunocompromised host, can result in disease long after primary infection, thus emphasizing the need for a thorough travel history.

Coccidioidal granuloma, a form of disseminated coccidioidomycosis, was described first by Posadas in 1892 in Argentina. The disease initially was thought to be a form of skin tumor. Rixford and Gilchrist associated it with what they thought was a protozoon resembling coccidia and named it *Coccidioides*. Ophüls and Moffitt, in 1900, were the first to attribute coccidioidal granuloma to the fungus *Coccidioides immitis*. Although earlier investigators had noted fungal growth on cultures from pathologic specimens, these were dismissed as contaminants. Between 1900 and 1936, numerous reports of coccidioidal granuloma appeared in the medical literature, but the association between coccidioidal granuloma and acute pulmonary infection remained unrecognized. In 1936, Gifford[73] and Dickson were the first to describe that *C. immitis* is the causative agent of what had previously been known in California as San Joaquin fever or valley fever.[66]

In the period that followed, the epidemiology of the disease[132,135] and ecology of the organism[57,104] were studied carefully by many investigators, led by the efforts of Charles Smith. Many different forms of therapy were tried for disseminated disease during this era, but none proved satisfactory.[61] In 1957, with the use of amphotericin B, effective antifungal therapy became available, and the prognosis associated with the disseminated form of the disease improved.

As with many pathogenic fungi, the life cycle of *C. immitis* demonstrates two distinct phases: a saprophytic, or vegetative, phase and a parasitic phase. In nature and on most laboratory media, the organism grows as mycelia with branching, septate hyphae. After 5 to 7 days, the aerial mycelia show development of rectangular spores (arthrospores, arthroconidia) separated by empty nonviable cells (Fig. 200-1). At this stage, the hyphae become fragile, and the arthroconidia easily become airborne. Their size, measuring 2 to 8 μm in diameter, allows them to reach the alveolar spaces of the lung when they are inhaled.

On gaining access to mammalian host tissues, the arthroconidia begin the parasitic phase of their life cycle. They enlarge and develop into spherules during the course of approximately 48 hours while undergoing internal segmentation to form endospores that are 2 to 5 μm in size. The spherules (Fig. 200-2) are round, double-walled structures measuring 20 to 100 μm in diameter. Endospores are released into the surrounding tissues by rupture of the spherule wall, where they mature

REFERENCES

1. Abelson JA, Moore T, Bruckner D, et al. Frequency of fungemia in hospitalized pediatric inpatients over 11 years at a tertiary care institution. *Pediatrics* 2005;**116**:61–7.
2. Achkar JM, Fries BC. *Candida* infections of the genitourinary tract. *Clin Microbiol Rev* 2010;**23**:253–73.
3. Aghai ZH, Mudduluru M, Nakhla TA, et al. Fluconazole prophylaxis in extremely low birth weight infants: association with cholestasis. *J Perinatol* 2006;**26**:550–5.
4. Al-Fattani MA, Douglas LJ. Penetration of *Candida* biofilms by antifungal agents. *Antimicrob Agents Chemother* 2004;**48**:3291–7.
5. Aly H, Herson V, Duncan A, et al. Is bloodstream infection preventable among premature infants? A tale of two cities. *Pediatrics* 2005;**115**:1513–18.
6. American Academy of Pediatrics. Candidiasis. In: Pickering L, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 245–9.

6a. American Academy of Pediatrics. *Red Book: 2012 Report of the Committee on Infectious Diseases*. In: Pickering LK, editor. 29th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2012. p. 265–9.

7. Anderson MR, Klink K, Cohrssen A. Evaluation of vaginal complaints. *JAMA* 2004;**291**:1368–79.
8. Andes DR, Safdar N, Baddley JW, et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clin Infect Dis* 2012;**54**:1110–22.
9. Arfania D, Everett ED, Nolph KD, et al. Uncommon causes of peritonitis in patients undergoing peritoneal dialysis. *Arch Intern Med* 1981;**141**:61–4.
10. Arisoy ES, Arisoy AE, Dunne WM Jr. Clinical significance of fungi isolated from cerebrospinal fluid in children. *Pediatr Infect Dis J* 1994;**13**:128–33.
11. Ascher SB, Smith PB, Clark RH, et al. Sepsis in young infants with congenital heart disease. *Early Hum Dev* 2012;**88**(Suppl. 2): S92–7.
12. Ascher SB, Smith PB, Watt K, et al. Antifungal therapy and outcomes in infants with invasive *Candida* infections. *Pediatr Infect Dis J* 2012;**31**:439–43.
13. Aun MV, Ribeiro MR, Costa Garcia CL, et al. Esophageal candidiasis—an adverse effect of inhaled corticosteroids therapy. *J Asthma* 2009;**46**:399–401.
14. Aydemir C, Oguz SS, Dizdar EA, et al. Randomised controlled trial of prophylactic fluconazole versus nystatin for the prevention of fungal colonisation and invasive fungal infection in very low birth weight infants. *Arch Dis Child Fetal Neonatal Ed* 2011; **96**(3):F164–8.
15. Aziz M, Patel AL, Losavio J, et al. Efficacy of fluconazole prophylaxis for prevention of invasive fungal infection in extremely low birth weight infants. *Pediatr Infect Dis J* 2010;**29**(4):352–6.
16. Badiee P, Kordbacheh P, Alborzi A, et al. Fungal infections in solid organ recipients. *Exp Clin Transplant* 2005;**3**:385–9.
17. Baillie GS, Douglas LJ. Effect of growth rate on resistance of *Candida albicans* biofilms to antifungal agents. *Antimicrob Agents Chemother* 1998;**42**:1900–5.
18. Baley JE. Neonatal candidiasis: the current challenge. *Clin Perinatol* 1991;**18**:263–80.
19. Baley JE, Ellis FJ. Neonatal candidiasis: ophthalmologic infection. *Semin Perinatol* 2003;**27**:401–5.
20. Baley JE, Kliegman RM, Boxerbaum B, et al. Fungal colonization in the very low birth weight infant. *Pediatrics* 1986;**78**:225–32.
21. Baley JE, Kliegman RM, Fanaroff AA. Disseminated fungal infections in very low-birth-weight infants: clinical manifestations and epidemiology. *Pediatrics* 1984;**73**:144–52.
22. Baley JE, Silverman RA. Systemic candidiasis: cutaneous manifestations in low birth weight infants. *Pediatrics* 1988;**82**:211–15.
23. Banerjee SN, Emori TG, Culver DH, et al. Secular trends in nosocomial primary bloodstream infections in the United States, 1980-1989. National Nosocomial Infections Surveillance System. *Am J Med* 1991;**91**:S86–9.
24. Bao Y, Du LZ, Shi LP, et al. [Clinical significance of oral fluconazole prophylaxis against invasive fungal infection in preterm neonates with peripherally inserted central catheters]. *Zhonghua Er Ke Za Zhi* 2010;**48**:510–13. In Chinese.
25. Bauer S, Eliakim A, Pomeranz A, et al. Urinary tract infection in very low birth weight preterm infants. *Pediatr Infect Dis J* 2003;**22**:426–30.
26. Beck-Sague C, Jarvis WR. Secular trends in the epidemiology of nosocomial fungal infections in the United States, 1980-1990. National Nosocomial Infections Surveillance System. *J Infect Dis* 1993;**167**:1247–51.
27. Bendel CM, Hess DJ, Garni RM, et al. Comparative virulence of *Candida albicans* yeast and filamentous forms in orally and intravenously inoculated mice. *Crit Care Med* 2003;**31**:501–7.
28. Bendel CM, Wiesner SM, Garni RM, et al. Cecal colonization and systemic spread of *Candida albicans* in mice treated with antibiotics and dexamethasone. *Pediatr Res* 2002;**51**:290–5.
29. Benjamin DK Jr, DeLong ER, Steinbach WJ, et al. Empirical therapy for neonatal candidemia in very low birth weight infants. *Pediatrics* 2003;**112**:543–7.
30. Benjamin DK Jr, Poole C, Steinbach WJ, et al. Neonatal candidemia and end-organ damage: a critical appraisal of the literature using meta-analytic techniques. *Pediatrics* 2003;**112**:634–40.
31. Benjamin DK Jr, Smith PB, Arrieta A, et al. Safety and pharmacokinetics of repeat-dose micafungin in young infants. *Clin Pharmacol Ther* 2010;**87**:93–9.
32. Benjamin DK Jr, Stoll BJ, Fanaroff AA, et al. Neonatal candidiasis among extremely low birth weight infants: risk factors, mortality rates, and neurodevelopmental outcomes at 18 to 22 months. *Pediatrics* 2006;**117**:84–92.
33. Benjamin DK Jr, Stoll BJ, Gantz MG, et al. Neonatal candidiasis: epidemiology, risk factors, and clinical judgment. *Pediatrics* 2010;**126**:e865–73.
34. Berenguer J, Buck M, Witebsky F, et al. Lysis-centrifugation blood cultures in the detection of tissue-proven invasive candidiasis: disseminated versus single-organ infection. *Diagn Microbiol Infect Dis* 1993;**17**:103–9.
35. Bertini G, Perugi S, Dani C, et al. Fluconazole prophylaxis prevents invasive fungal infection in high-risk, very low birth weight infants. *J Pediatr* 2005;**147**:162–5.
36. Beyda ND, Lewis RE, Garey KW. Echinocandin resistance in *Candida* species: mechanisms of reduced susceptibility and therapeutic approaches. *Ann Pharmacother* 2012;**46**:1086–96.
37. Bhat V, Fojas M, Saslow JG, et al. Twice-weekly fluconazole prophylaxis in premature infants: association with cholestasis. *Pediatr Int* 2011;**53**(4):475–9.
38. Bizzarro MJ, Conrad SA, Kaufman DA, et al. Infections acquired during extracorporeal membrane oxygenation in neonates, children, and adults. *Pediatr Crit Care Med* 2011;**12**(3):277–81.
39. Bjerke JW, Meyers JD, Bowden RA. Hepatosplenic candidiasis—a contraindication to marrow transplantation? *Blood* 1994;**84**: 2811–14.
40. Bliss JM, Basavegowda KP, Watson WJ, et al. Vertical and horizontal transmission of *Candida albicans* in very low birth weight infants using DNA fingerprinting techniques. *Pediatr Infect Dis J* 2008;**27**:231–5.
41. Blowey DL, Garg UC, Kearns GL, et al. Peritoneal penetration of amphotericin B lipid complex and fluconazole in a pediatric patient with fungal peritonitis. *Adv Perit Dial* 1998;**14**: 247–50.
42. Blyth CC, Chen SC, Slavin MA, et al. Not just little adults: candidemia epidemiology, molecular characterization, and antifungal susceptibility in neonatal and pediatric patients. *Pediatrics* 2009; **123**:1360–8.
43. Braun BR, Head WS, Wang MX, et al. Identification and characterization of TUP1-regulated genes in *Candida albicans*. *Genetics* 2000;**156**:31–44.
44. Brocklehurst P, Farrell B, King A, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011; **365**:1201–11.
45. Bryant K, Maxfield C, Rabalais G. Renal candidiasis in neonates with candiduria. *Pediatr Infect Dis J* 1999;**18**:959–63.
46. Buergelt CD, Cooley AJ, Hines SA, et al. Endocarditis in six horses. *Vet Pathol* 1985;**22**:333–7.
47. Buff SJ, McLelland R, Gallis HA, et al. *Candida albicans* pneumonia: radiographic appearance. *AJR Am J Roentgenol* 1982;**138**: 645–8.

48. Carstensen H, Nilsson KO, Nettelblad SC, et al. Common bile duct obstruction due to an intraluminal mass of candidiasis in a previously healthy child. *Pediatrics* 1986;**77**:858–61.
49. Castaldo P, Stratta RJ, Wood RP, et al. Clinical spectrum of fungal infections after orthotopic liver transplantation. *Arch Surg* 1991;**126**:149–56.
50. Chan TM, Chan CY, Cheng SW, et al. Treatment of fungal peritonitis complicating continuous ambulatory peritoneal dialysis with oral fluconazole: a series of 21 patients. *Nephrol Dial Transplant* 1994;**9**:539–42.
51. Chang TI, Kim HW, Park JT, et al. Early catheter removal improves patient survival in peritoneal dialysis patients with fungal peritonitis: results of ninety-four episodes of fungal peritonitis at a single center. *Perit Dial Int* 2011;**31**:60–6.
52. Chanock SJ, Pizzo PA. Infectious complications of patients undergoing therapy for acute leukemia: current status and future prospects. *Semin Oncol* 1997;**24**:132–40.
53. Chapman RL, Faix RG. Persistently positive cultures and outcome in invasive neonatal candidiasis. *Pediatr Infect Dis J* 2000;**19**: 822–7.
54. Chen J, Zhou S, Wang Q, et al. Crk1, a novel Cdc2-related protein kinase, is required for hyphal development and virulence in *Candida albicans*. *Mol Cell Biol* 2000;**20**:8696–708.
55. Chiou CC, Groll AH, Gonzalez CE, et al. Esophageal candidiasis in pediatric acquired immunodeficiency syndrome: clinical manifestations and risk factors. *Pediatr Infect Dis J* 2000;**19**:729–34.
56. Chiou CC, Groll AH, Mavrogiorgos N, et al. Esophageal candidiasis in human immunodeficiency virus–infected pediatric patients after the introduction of highly active antiretroviral therapy. *Pediatr Infect Dis J* 2002;**21**:388–92.
57. Chitnis AS, Magill SS, Edwards JR, et al. Trends in *Candida* central line–associated bloodstream infections among NICUs, 1999-2009. *Pediatrics* 2012;**130**(1):e46–52.
58. Chun CS, Turner RB. The outcome of candiduria in pediatric patients. *Diagn Microbiol Infect Dis* 1991;**14**:119–23.
59. Cimbaluk D, Scudiere J, Butsch J, et al. Invasive candidal enterocolitis followed shortly by fatal cerebral hemorrhage in immunocompromised patients. *J Clin Gastroenterol* 2005;**39**:795–7.
60. Clerihew L, Lamagni TL, Brocklehurst P, et al. Invasive fungal infection in very low birthweight infants: national prospective surveillance study. *Arch Dis Child Fetal Neonatal Ed* 2006;**91**:F188–92.
61. Clerihew L, McGuire W. Antifungal prophylaxis for very-low-birth-weight infants: UK national survey. *Arch Dis Child Fetal Neonatal Ed* 2008;**93**(3):F238–9.
62. Coates EW, Karlowicz MG, Croitoru DP, et al. Distinctive distribution of pathogens associated with peritonitis in neonates with focal intestinal perforation compared with necrotizing enterocolitis. *Pediatrics* 2005;**116**:e241–6.
63. Cohen B, Saiman L, Cimiotti J, et al. Factors associated with hand hygiene practices in two neonatal intensive care units. *Pediatr Infect Dis J* 2003;**22**:494–9.
64. Cohen-Wolkowiez M, Smith PB, Mangum B, et al. Neonatal *Candida* meningitis: significance of cerebrospinal fluid parameters and blood cultures. *J Perinatol* 2007;**27**:97–100.
65. Coker SB, Beltran RS. *Candida* meningitis: clinical and radiographic diagnosis. *Pediatr Neurol* 1988;**4**:317–19.
66. Conti HR, Gaffen SL. Host responses to *Candida* albicans: Th17 cells and mucosal candidiasis. *Microbes Infect* 2010;**12**:518–27.
67. Cotten CM, McDonald S, Stoll B, et al. The association of third-generation cephalosporin use and invasive candidiasis in extremely low birth-weight infants. *Pediatrics* 2006;**118**:717–22.
68. Cox F. *Candida albicans* adherence in newborn infants. *J Med Vet Mycol* 1986;**24**:121–5.
69. d'Ostiani CF, Del Sero G, Bacci A, et al. Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans:* implications for initiation of T helper cell immunity in vitro and in vivo. *J Exp Med* 2000;**191**:1661–74.
70. Dahl C, Fuursted K, Schroder H. A paediatric case of *Candida* pericarditis and eosophagus stricture during treatment for acute lymphatic leukaemia. *Acta Oncol* 2007;**46**:859–61.
71. Dato VM, Dajani AS. Candidemia in children with central venous catheters: role of catheter removal and amphotericin B therapy. *Pediatr Infect Dis J* 1990;**9**:309–14.
72. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008;**46**:1813–21.
73. Dendis M, Horvath R, Michalek J, et al. PCR-RFLP detection and species identification of fungal pathogens in patients with febrile neutropenia. *Clin Microbiol Infect* 2003;**9**:1191–202.
74. Denning DW. Echinocandin antifungal drugs. *Lancet* 2003;**362**: 1142–51.
75. Dotis J, Prasad PA, Zaoutis T, et al. Epidemiology, risk factors and outcome of *Candida parapsilosis* bloodstream infection in children. *Pediatr Infect Dis J* 2012;**31**:557–60.
76. Dutta A, Palazzi DL. *Candida* non-albicans versus *Candida* albicans fungemia in the non-neonatal pediatric population. *Pediatr Infect Dis J* 2011;**30**:664–8.
77. Dvorak CC, Fisher BT, Sung L, et al. Antifungal prophylaxis in pediatric hematology/oncology: new choices & new data. *Pediatr Blood Cancer* 2012;**59**:21–6.
78. Edmond MB, Wallace SE, McClish DK, et al. Nosocomial bloodstream infections in United States hospitals: a three-year analysis. *Clin Infect Dis* 1999;**29**:239–44.
79. Edwards WH. Preventing nosocomial bloodstream infection in very low birth weight infants. *Semin Neonatol* 2002;**7**:325–33.
80. Ellepola AN, Morrison CJ. Laboratory diagnosis of invasive candidiasis. *J Microbiol* 2005;**43**(Spec No):65–84.
81. Evans NJ, Rutter N. Development of the epidermis in the newborn. *Biology of the Neonate* 1986;**49**:74–80.
82. Fainstein V, Gilmore C, Hopfer RL, et al. Septic arthritis due to *Candida* species on patients with cancer: report of five cases and review of the literature. *Rev Infect Dis* 1982;**4**:78–85.
83. Faix RG, Kovarik SM, Shaw TR, et al. Mucocutaneous and invasive candidiasis among very low birth weight (less than 1,500 grams) infants in intensive care nurseries: a prospective study. *Pediatrics* 1989;**83**:101–7.
84. Falagas ME, Apostolou KE, Pappas VD. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. *Eur J Clin Microbiol Infect Dis* 2006;**25**:419–25.
85. Falcone M, Barzaghi N, Carosi G, et al. *Candida* infective endocarditis: report of 15 cases from a prospective multicenter study. *Medicine* 2009;**88**:160–8.
86. Fanaroff AA, Korones SB, Wright LL, et al. A controlled trial of intravenous immune globulin to reduce nosocomial infections in very-low-birth-weight infants. National Institute of Child Health and Human Development Neonatal Research Network. *N Engl J Med* 1994;**330**:1107–13.
87. Feja KN, Wu F, Roberts K, et al. Risk factors for candidemia in critically ill infants: a matched case-control study. *J Pediatr* 2005; **147**:156–61.
88. Festekjian A, Neely M. Incidence and predictors of invasive candidiasis associated with candidaemia in children. *Mycoses* 2011; **54**:146–53.
89. Filioti J, Spiroglou K, Panteliadis CP, et al. Invasive candidiasis in pediatric intensive care patients: epidemiology, risk factors, management, and outcome. *Intensive Care Med* 2007;**33**:1272–83.
90. Fisher JF, Sobel JD, Kauffman CA, et al. *Candida* urinary tract infections—treatment. *Clin Infect Dis* 2011;**52**(Suppl. 6):S457–66.
91. Florescu DF, Islam KM, Grant W, et al. Incidence and outcome of fungal infections in pediatric small bowel transplant recipients. *Transpl Infect Dis* 2010;**12**:497–504.
92. Foxman B. The epidemiology of vulvovaginal candidiasis: risk factors. *Am J Public Health* 1990;**80**:329–31.
93. Frankenbusch K, Eifinger F, Kribs A, et al. Severe primary cutaneous aspergillosis refractory to amphotericin B and the successful treatment with systemic voriconazole in two premature infants with extremely low birth weight. *J Perinatol* 2006;**26**:511–14.
94. Freydiere AM, Piens MA, Andre JM, et al. Successful treatment of *Candida* glabrata peritonitis with fluconazole plus flucytosine in a premature infant following in vitro fertilization. *Eur J Clin Microbiol Infect Dis* 2005;**24**:704–5.
95. Friberg LE, Ravva P, Karlsson MO, et al. Integrated population pharmacokinetic analysis of voriconazole in children, adolescents, and adults. *Antimicrob Agents Chemother* 2012;**56**:3032–42.

96. Fridkin SK, Kaufman D, Edwards JR, et al. Changing incidence of *Candida* bloodstream infections among NICU patients in the United States: 1995-2004. *Pediatrics* 2006;**117**:1680–7.
97. Friedman S, Richardson SE, Jacobs SE, et al. Systemic *Candida* infection in extremely low birth weight infants: short term morbidity and long term neurodevelopmental outcome. *Pediatr Infect Dis J* 2000;**19**:499–504.
98. Fukushima C, Matsuse H, Tomari S, et al. Oral candidiasis associated with inhaled corticosteroid use: comparison of fluticasone and beclomethasone. *Ann Allergy Asthma Immunol* 2003;**90**:646–51.
99. Gamaletsou MN, Kontoyiannis DP, Sipsas NV, et al. *Candida* osteomyelitis: analysis of 207 pediatric and adult cases (1970-2011). *Clin Infect Dis* 2012;**55**(10):1338–51.
100. Gammelsrud KW, Sandven P, Hoiby EA, et al. Colonization by *Candida* in children with cancer, children with cystic fibrosis, and healthy controls. *Clin Microbiol Infect* 2011;**17**:1875–81.
101. Ganesan K, Harigopal S, Neal T, et al. Prophylactic oral nystatin for preterm babies under 33 weeks gestation decreases fungal colonisation and invasive fungaemia. *Arch Dis Child Fetal Neonatal Ed* 2009;**94**(4):F275–8.
102. Garges HP, Moody MA, Cotten CM, et al. Neonatal meningitis: what is the correlation among cerebrospinal fluid cultures, blood cultures, and cerebrospinal fluid parameters? *Pediatrics* 2006;**117**: 1094–100.
103. Geers TA, Gordon SM. Clinical significance of *Candida* species isolated from cerebrospinal fluid following neurosurgery. *Clin Infect Dis* 1999;**28**:1139–47.
104. George BJ, Horvath LL, Hospenthal DR. Effect of inoculum size on detection of *Candida* growth by the BACTEC 9240 automated blood culture system using aerobic and anaerobic media. *J Clin Microbiol* 2005;**43**:433–5.
105. Gladdy RA, Richardson SE, Davies HD, et al. *Candida* infection in pediatric liver transplant recipients. *Liver Transplant Surg* 1999; **5**:16–24.
106. Glick SN. Barium studies in patients with *Candida* esophagitis: pseudoulcerations simulating viral esophagitis. *AJR Am J Roentgenol* 1994;**163**:349–52.
107. Goldberg PK, Kozinn PJ, Wise GJ, et al. Incidence and significance of candiduria. *JAMA* 1979;**241**:582–4.
108. Gow NA, van de Veerdonk FL, Brown AJ, et al. *Candida albicans* morphogenesis and host defence: discriminating invasion from colonization. *Nat Rev Microbiol* 2012;**10**:112–22.
109. Greenberg RG, Benjamin DK Jr, Gantz MG, et al. Empiric antifungal therapy and outcomes in extremely low birth weight infants with invasive candidiasis. *J Pediatr* 2012;**161**:264–9.
110. Groll AH, Tragiannidis A. Update on antifungal agents for paediatric patients. *Clin Microbiol Infect* 2010;**16**:1343–53.
111. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev* 2011;**24**:247–80.
112. Hachiya KA, Kobayashi RH, Antonson DL. *Candida* esophagitis following antibiotic usage. *Pediatr Infect Dis* 1982;**1**:168–70.
113. Hagensee ME, Bauwens JE, Kjos B, et al. Brain abscess following marrow transplantation: experience at the Fred Hutchinson Cancer Research Center, 1984-1992. *Clin Infect Dis* 1994;**19**: 402–8.
114. Haron E, Vartivarian S, Anaissie E, et al. Primary *Candida* pneumonia: experience at a large cancer center and review of the literature. *Medicine* 1993;**72**:137–42.
115. Harrington LE, Hatton RD, Mangan PR, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;**6**: 1123–32.
116. Harris MC, Pereira GR, Myers MD, et al. Candidal arthritis in infants previously treated for systemic candidiasis during the newborn period: report of three cases. *Pediatr Emerg Care* 2000; **16**:249–51.
117. Hawser SP, Douglas LJ. Resistance of *Candida albicans* biofilms to antifungal agents in vitro. *Antimicrob Agents Chemother* 1995;**39**: 2128–31.
118. Hazen KC. New and emerging yeast pathogens. *Clin Microbiol Rev* 1995;**8**:462–78.
119. Hazen KC, Mandell G, Coleman E, et al. Influence of fluconazole at subinhibitory concentrations on cell surface hydrophobicity and phagocytosis of *Candida* albicans. *FEMS Microbiol Lett* 2000; **183**:89–94.
120. Healy CM, Baker CJ, Zaccaria E, et al. Impact of fluconazole prophylaxis on incidence and outcome of invasive candidiasis in a neonatal intensive care unit. *J Pediatr* 2005;**147**:166–71.
121. Healy CM, Campbell JR, Zaccaria E, Baker CJ. Fluconazole prophylaxis in extremely low birth weight neonates reduces invasive candidiasis mortality rates without emergence of fluconazole-resistant *Candida* species. *Pediatrics* 2008;**121**:703–10.
122. Helstrom PB, Balish E. Effect of oral tetracycline, the microbial flora, and the athymic state on gastrointestinal colonization and infection of BALB/c mice with *Candida albicans*. *Infect Immun* 1979;**23**:764–74.
123. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous *Candida* meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008;**197**:163–71.
124. Hoppe JE. Treatment of oropharyngeal candidiasis and candidal diaper dermatitis in neonates and infants: review and reappraisal. *Pediatric Infect Dis J* 1997;**16**:885–94.
125. Hoppe JE, Frey P. Evaluation of six commercial tests and the germ-tube test for presumptive identification of *Candida albicans*. *Eur J Clin Microbiol Infect Dis* 1999;**18**:188–91.
126. Horn DL, Fishman JA, Steinbach WJ, et al. Presentation of the PATH Alliance registry for prospective data collection and analysis of the epidemiology, therapy, and outcomes of invasive fungal infections. *Diagnc Microbiol Infect Dis* 2007;**59**:407–14.
127. Horvath LL, George BJ, Murray CK, et al. Direct comparison of the BACTEC 9240 and BacT/ALERT 3D automated blood culture systems for candida growth detection. *J Clin Microbiol* 2004;**42**:115–18.
128. Horvath LL, Hospenthal DR, Murray CK, Dooley DP. Detection of simulated candidemia by the BACTEC 9240 system with plus aerobic/F and anaerobic/F blood culture bottles. *J Clin Microbiol* 2003;**41**:4714–17.
129. Howell A, Isaacs D, Halliday R. Oral nystatin prophylaxis and neonatal fungal infections. *Arch Dis Child Fetal Neonatal Ed* 2009;**94**(6):F429–33.
130. Huang YC, Li CC, Lin TY, et al. Association of fungal colonization and invasive disease in very low birth weight infants. *Pediatr Infect Dis J* 1998;**17**:819–22.
131. Huttova M, Kralinsky K, Horn J, et al. Prospective study of nosocomial fungal meningitis in children—report of 10 cases. *Scand J Infect Dis* 1998;**30**:485–7.
132. Jabra-Rizk MA, Torres SR, Rambob I, et al. Prevalence of oral *Candida* species ina North American pediatric population. *J Clin Pediatr Dent* 2007;**31**:260–3.
133. Jeon GW, Koo SH, Lee JH, et al. A comparison of AmBisome to amphotericin B for treatment of systemic candidiasis in very low birth weight infants. *Yonsei Med J* 2007;**48**:619–26.
134. Johnson DE, Thompson TR, Green TP, et al. Systemic candidiasis in very low-birth-weight infants (less than 1,500 grams). *Pediatrics* 1984;**73**:138–43.
135. Johnsson H, Ewald U. The rate of candidaemia in preterm infants born at a gestational age of 23-28 weeks is inversely correlated to gestational age. *Acta Paediatr* 2004;**93**:954–8.
136. Kalin M, Petrini B. Clinical and laboratory diagnosis of invasive *Candida* infection in neutropenic patients. *Med Oncol* 1996;**13**: 223–31.
137. Kanda N, Yasuba H, Takahashi T, et al. Prevalence of esophageal candidiasis among patients treated with inhaled fluticasone propionate. *Am J Gastroenterol* 2003;**98**:2146–8.
138. Karageorgopoulos DE, Vouloumanou EK, Ntziora F, et al. Beta-D-glucan assay for the diagnosis of invasive fungal infections: a meta-analysis. *Clin Infect Dis* 2011;**52**:750–70.
139. Karlowicz MG, Giannone PJ, Pestian J, et al. J: Does candidemia predict threshold retinopathy of prematurity in extremely low birth weight (</=1000 g) neonates? *Pediatrics* 2000;**105**:1036–40.
140. Karlowicz MG, Hashimoto LN, Kelly RE Jr, et al. Should central venous catheters be removed as soon as candidemia is detected in neonates? *Pediatrics* 2000;**106**:E63.
141. Kassner EG, Kauffman SL, Yoon JJ, et al. Pulmonary candidiasis in infants: clinical, radiologic, and pathologic features. *AJR Am J Roentgenol* 1981;**137**:707–16.
142. Kauffman CA, Fisher JF, Sobel JD, et al. *Candida* urinary tract infections—diagnosis. *Clin Infect Dis* 2011;**52**(Suppl. 6):S452–6.

143. Kaufman D. Fluconazole prophylaxis decreases the combined outcome of invasive *Candida* infections or mortality in preterm infants. *Pediatrics* 2008;**122**:1158–9.
144. Kaufman D, Boyle R, Hazen KC, et al. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. *N Engl J Med* 2001;**345**:1660–6.
145. Kaufman D, Boyle R, Hazen KC, et al. Twice weekly fluconazole prophylaxis for prevention of invasive *Candida* infection in high-risk Infants of <1000 grams birth weight. *J Pediatr* 2005;**147**: 172–9.
146. Kaufman D, Fairchild KD. Clinical microbiology of bacterial and fungal sepsis in very-low-birth-weight infants. *Clin Microbiol Rev* 2004;**17**:638–80.
147. Kaufman DA. Fluconazole prophylaxis: can we eliminate invasive *Candida* infections in the neonatal ICU? *Curr Opin Pediatr* 2008;**20**:332–40.
148. Kaufman DA. Neonatal candidiasis: clinical manifestations, management, and prevention strategies. *J Pediatr* 2010;**156**:s53–67.
149. Kaufman DA. Aiming for zero: preventing invasive *Candida* infections in extremely preterm infants. *Neoreviews* 2011;**12**:e381–92.
150. Kaufman DA, Boyle R, Grossman LB, et al. Fluconazole prophylaxis dosing and the resistance patterns in preterm infants over a 11-year period. Presented before the annual meeting of the Pediatric Academic Society, 2010.
151. Kaufman DA, Cuff AL, Wamstad JB, et al. Fluconazole prophylaxis in extremely low birth weight infants and neurodevelopmental outcomes and quality of life at 8 to 10 years of age. *J Pediatr* 2011;**158**(5):759–65.e1.
152. Kaufman DA, Gurka MJ, Hazen KC, et al. Patterns of fungal colonization in preterm infants weighing less than 1000 grams at birth. *Pediatr Infect Dis J* 2006;**25**:733–7.
153. Kaufman DA, Manzoni P. Strategies to prevent invasive candidal infection in extremely preterm infants. *Clin Perinatol* 2010;**37**: 611–28.
154. Kennedy MJ, Volz PA. Ecology of *Candida* albicans gut colonization: inhibition of *Candida* adhesion, colonization, and dissemination from the gastrointestinal tract by bacterial antagonism. *Infect Immun* 1985;**49**:654–63.
155. Kicklighter SD, Springer SC, Cox T, et al. Fluconazole for prophylaxis against candidal rectal colonization in the very low birth weight infant. *Pediatrics* 2001;**107**:293–8.
156. King KY, Edwards MS, Word BM. Hepatitis associated with micafungin use in a preterm infant. *J Perinatol* 2009;**29**:320–2.
157. Kirkpatrick CH. Chronic mucocutaneous candidiasis. *Pediatr Infect Dis J* 2001;**20**:197–206.
158. Kisand K, Boe Wolff AS, Podkrajsek KT, et al. Chronic mucocutaneous candidiasis in APECED or thymoma patients correlates with autoimmunity to Th17-associated cytokines. *J Exp Med* 2010; **207**:299–308.
159. Kleinpeter MA, Butt AA. Non–*Candida albicans* fungal peritonitis in continuous ambulatory peritoneal dialysis patients. *Adv Perit Dial* 2001;**17**:176–9.
160. Klingspor L, Stintzing G, Tollemar J. Deep *Candida* infection in children with leukaemia: clinical presentations, diagnosis and outcome. *Acta Paediatr* 1997;**86**:30–6.
161. Kobayashi RH, Rosenblatt HM, Carney JM, et al. *Candida* esophagitis and laryngitis in chronic mucocutaneous candidiasis. *Pediatrics* 1980;**66**:380–4.
162. Kojic EM, Darouiche RO. *Candida* infections of medical devices. *Clin Microbiol Rev* 2004;**17**:255–67.
163. Kollef M, Micek S, Hampton N, et al. Septic shock attributed to *Candida* infection: importance of empiric therapy and source control. *Clin Infect Dis* 2012;**54**:1739–46.
164. Kossoff EH, Buescher ES, Karlowicz MG. Candidemia in a neonatal intensive care unit: trends during fifteen years and clinical features of 111 cases. *Pediatr Infect Dis J* 1998;**17**:504–8.
165. Kothavade RJ, Kura MM, Valand AG, et al. *Candida tropicalis:* its prevalence, pathogenicity and increasing resistance to fluconazole. *J Med Microbiol* 2010;**59**:873–80.
166. Kourkoumpetis TK, Fuchs BB, Coleman JJ, et al. Polymerase chain reaction–based assays for the diagnosis of invasive fungal infections. *Clin Infect Dis* 2012;**54**:1322–31.
167. Kramer MS, Platt RW, Wen SW, et al. A new and improved population-based Canadian reference for birth weight for gestational age. *Pediatrics* 2001;**108**:E35.
168. Krause W, Matheis H, Wulf K. Fungaemia and funguria after oral administration of *Candida albicans*. *Lancet* 1969;**1**:598–9.
169. Krcmery V, Barnes AJ. Non-albicans *Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. *J Hosp Infect* 2002;**50**:243–60.
170. Kumamoto CA. *Candida* biofilms. *Curr Opin Microbiol* 2002;**5**: 608–11.
171. Kuwahara T, Shimono K, Kaneda S, et al. Growth of microorganisms in total parenteral nutrition solutions containing lipid. *Int J Med Sci* 2010;**7**:101–9.
172. Lamoth F, Cruciani M, Mengoli C, et al. Beta-D-glucan antigenemia assay for the diagnosis of invasive fungal infections in patients with hematological malignancies: a systematic review and meta-analysis of cohort studies from the Third European Conference on Infections in Leukemia (ECIL-3). *Clin Infect Dis* 2012;**54**: 633–43.
173. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005;**201**:233–40.
174. Lee BE, Cheung PY, Robinson JL, et al. Comparative study of mortality and morbidity in premature infants (birth weight, < 1,250 g) with candidemia or candidal meningitis. *Clin Infect Dis* 1998;**27**:559–65.
175. Legrand F, Lecuit M, Dupont B, et al. Adjuvant corticosteroid therapy for chronic disseminated candidiasis. *Clin Infect Dis* 2008;**46**:696–702.
176. Levine MS. Radiology of esophagitis: a pattern approach. *Radiology* 1991;**179**:1–7.
177. Levine MS, Woldenberg R, Herlinger H, et al. Opportunistic esophagitis in AIDS: radiographic diagnosis. *Radiology* 1987;**165**: 815–20.
178. Li PK, Szeto CC, Piraino B, et al. Peritoneal dialysis–related infections recommendations: 2010 update. *Perit Dial Int* 2010;**30**: 393–423.
179. Lim CS, Rosli R, Seow HF, et al. *Candida* and invasive candidiasis: back to basics. *Eur J Clin Microbiol Infect Dis* 2012;**31**:21–31.
180. Linder N, Klinger G, Shalit I, et al. Treatment of candidaemia in premature infants: comparison of three amphotericin B preparations. *J Antimicrob Chemother* 2003;**52**:663–7.
181. Liu CY, Huang LJ, Wang WS, et al. Candidemia in cancer patients: impact of early removal of non-tunneled central venous catheters on outcome. *J Infection* 2009;**58**:154–60.
182. Lo HJ, Kohler JR, DiDomenico B, et al. Nonfilamentous *C. albicans* mutants are avirulent. *Cell* 1997;**90**:939–49.
183. Ludviksson BR, Thorarensen O, Gudnason T, et al. *Candida albicans* meningitis in a child with myeloperoxidase deficiency. *Pediatr Infect Dis J* 1993;**12**:162–4.
184. MacDonald L, Baker C, Chenoweth C. Risk factors for candidemia in a children's hospital. *Clin Infect Dis* 1998;**26**:642–5.
185. Makhoul IR, Kassis I, Smolkin T, et al. Review of 49 neonates with acquired fungal sepsis: further characterization. *Pediatrics* 2001;**107**:61–6.
186. Manzoni P, Arisio R, Mostert M, et al. Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year, retrospective cohort study. *Pediatrics* 2006;**117**:e22–32.
187. Manzoni P, Farina D, Leonessa M, et al. Risk factors for progression to invasive fungal infection in preterm neonates with fungal colonization. *Pediatrics* 2006;**118**:2359–64.
188. Manzoni P, Leonessa M, Galletto P, et al. Routine use of fluconazole prophylaxis in a neonatal intensive care unit does not select natively fluconazole-resistant *Candida* subspecies. *Pediatr Infect Dis J* 2008;**27**:731–7.
189. Manzoni P, Rinaldi M, Cattani S, et al. Bovine lactoferrin supplementation for prevention of late-onset sepsis in very low-birthweight neonates: a randomized trial. *JAMA* 2009;**302**:1421–8.
190. Manzoni P, Stolfi I, Pugni L, et al. A multicenter, randomized trial of prophylactic fluconazole in preterm neonates. *N Engl J Med* 2007;**356**:2483–95.
191. Marom D, Levy I, Gutwein O, et al. Healthcare-associated versus community-associated infective endocarditis in children. *Pediatr Infect Dis J* 2011;**30**:585–8.
192. Martin A, Pappas A, Lulic-Botica M, et al. Impact of "targeted" fluconazole prophylaxis for preterm neonates: efficacy of a highly selective approach? *J Perinatol* 2012;**32**:21–6.

193. Masood A, Sallah S. Chronic disseminated candidiasis in patients with acute leukemia: emphasis on diagnostic definition and treatment. *Leuk Res* 2005;**29**:493–501.
194. Matuszkiewicz-Rowinska J. Update on fungal peritonitis and its treatment. *Perit dial Int* 2009;**29**(Suppl. 2):S161–5.
195. Maudsley RF, Brix GA, Hinton NA, et al. Placental inflammation and infection: a prospective bacteriologic and histologic study. *Am J Obstet Gynecol* 1966;**95**:648–59.
196. McCrossan BA, McHenry E, O'Neill F, et al. Selective fluconazole prophylaxis in high-risk babies to reduce invasive fungal infection. *Arch Dis Child Fetal Neonatal Ed* 2007;**92**:F454–8.
197. McCullers JA, Vargas SL, Flynn PM, et al. Candidal meningitis in children with cancer. *Clin Infect Dis* 2000;**31**:451–7.
198. McGinley KJ, Larson EL, Leyden JJ. Composition and density of microflora in the subungual space of the hand. *J Clin Microbiol* 1988;**26**:950–3.
199. Melo NR, Taguchi H, Culhari VP, et al. Oral candidiasis of HIV-infected children undergoing sequential HIV therapies. *Med Mycol* 2009;**47**:149–56.
200. Micames CG, Bentley R, Onken J. Clinical challenges and images in GI invasive candidal enterocolitis. *Gastroenterology* 2007;**133**:391, 731.
201. Mikulska M, Calandra T, Sanguinetti M, et al. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. *Crit Care* 2010;**14**:R222.
202. Millar BC, Jugo J, Moore JE. Fungal endocarditis in neonates and children. *Pediatr Cardiol* 2005;**26**:517–36.
203. Mok WY, Luizão RC, Barreto da Silva Mdo S. Isolation of fungi from bats of the Amazon basin. *Appl Environ Microbiol* 1982;**44**:570–5.
204. Mokaddas E, Burhamah MH, Khan ZU, et al. Levels of (1→3)-beta-D-glucan, *Candida* mannan and *Candida* DNA in serum samples of pediatric cancer patients colonized with *Candida* species. *BMC Infect Dis* 2010;**10**:292.
205. Montane BS, Mazza I, Abitbol C, et al. Fungal peritonitis in pediatric patients. *Adv Perit Dial* 1998;**14**:251–4.
206. Montenegro J, Aguirre R, Gonzalez O, et al. Fluconazole treatment of candida peritonitis with delayed removal of the peritoneal dialysis catheter. *Clin Nephrol* 1995;**44**:60–3.
207. Moylett EH. Neonatal *Candida* meningitis. *Semin Pediatr Infect Dis* 2003;**14**:115–22.
208. Mukherjee PK, Chandra J, Kuhn DM, et al. Mechanism of fluconazole resistance in *Candida* albicans biofilms: phase-specific role of efflux pumps and membrane sterols. *Infect Immun* 2003; **71**:4333–40.
209. Mukherjee PK, Zhou G, Munyon R, et al. *Candida* biofilm: a well-designed protected environment. *Med Mycol* 2005;**43**:191–208.
210. Muldrew KM, Maples HD, Stowe CD, et al. Intravenous voriconazole therapy in a preterm infant. *Pharmacotherapy* 2005;**25**: 893–8.
211. Myerowitz RL. Gastrointestinal and disseminated candidiasis: an experimental model in the immunosuppressed rat. *Arch Pathol Lab Med* 1981;**105**:138–43.
212. Naglik JR, Challacombe SJ, Hube B. *Candida* albicans secreted aspartyl proteinases in virulence and pathogenesis. *Microbiol Mol Biol Rev* 2003;**67**:400–28, table of contents.
213. Natarajan G, Lulic-Botica M, Rongkavilit C, et al. Experience with caspofungin in the treatment of persistent fungemia in neonates. *J Perinatol* 2005;**25**:770–7.
214. Nguyen MH, Wissel MC, Shields RK, et al. Performance of *Candida* real-time polymerase chain reaction, beta-D-glucan assay, and blood cultures in the diagnosis of invasive candidiasis. *Clin Infect Dis* 2012;**54**:1240–8.
215. Noyola DE, Bohra L, Paysse EA, et al. Association of candidemia and retinopathy of prematurity in very low birthweight infants. *Ophthalmology* 2002;**109**:80–4.
216. Noyola DE, Fernandez M, Moylett EH. Ophthalmologic, visceral, and cardiac involvement in neonates with candidemia. *Clin Infect Dis* 2001;**32**:1018–23.
217. Nucci M, Anaissie E. Revisiting the source of candidemia: skin or gut? *Clin Infect Dis* 2001;**33**:1959–67.
218. Nucci M, Anaissie E. Should vascular catheters be removed from all patients with candidemia? An evidence-based review. *Clin Infect Dis* 2002;**34**:591–9.
219. Nurbhai M, Grimshaw J, Watson M, et al. Oral versus intravaginal imidazole and triazole anti-fungal treatment of uncomplicated vulvovaginal candidiasis (thrush). *Cochrane Database Syst Rev* 2007;(4):CD002845.
220. Odio CM, Araya R, Pinto LE, et al. Caspofungin therapy of neonates with invasive candidiasis. *Pediatr Infect Dis J* 2004;**23**: 1093–7.
221. Olczak-Kowalczyk D, Pawlowska J, Garczewska B, et al. Oral candidiasis in immunosuppressed children and young adults after liver or kidney transplantation. *Pediatr Dent* 2010;**32**:189–94.
222. Ozturk MA, Gunes T, Koklu E, et al. Oral nystatin prophylaxis to prevent invasive candidiasis in Neonatal Intensive Care Unit. *Mycoses* 2006;**49**:484–92.
223. Pagano L, Mele L, Fianchi L, et al. Chronic disseminated candidiasis in patients with hematologic malignancies: clinical features and outcome of 29 episodes. *Haematologica* 2002;**87**:535–41.
224. Pappas PG, Kauffman CA, Andes D, et al. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**: 503–35.
225. Pappas PG, Rex JH, Lee J, et al. A prospective observational study of candidemia: epidemiology, therapy, and influences on mortality in hospitalized adult and pediatric patients. *Clin Infect Dis* 2003; **37**:634–43.
226. Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005;**6**:1133–41.
227. Pasqualotto AC. *Candida* and the paediatric lung. *Paediatr Respir Rev* 2009;**10**:186–91.
228. Pasqualotto AC, de Moraes AB, Zanini RR, et al. Analysis of independent risk factors for death among pediatric patients with candidemia and a central venous catheter in place. *Infect Control Hosp Epidemiol* 2007;**28**:799–804.
229. Pedrosa AF, Rodrigues AG. Candidemia in burn patients: figures and facts. *J Trauma* 2011;**70**:498–506.
230. Persat F, Topenot R, Piens MA, et al. Evaluation of different commercial ELISA methods for the serodiagnosis of systemic candidosis. *Mycoses* 2002;**45**:455–60.
231. Pestalozzi BC, Krestin GP, Schanz U, et al. Hepatic lesions of chronic disseminated candidiasis may become invisible during neutropenia. *Blood* 1997;**90**:3858–64.
232. Petraitiene R, Petraitis V, Hope WW, et al. Cerebrospinal fluid and plasma (1→3)-beta-D-glucan as surrogate markers for detection and monitoring of therapeutic response in experimental hematogenous *Candida* meningoencephalitis. *Antimicrob Agents Chemother* 2008;**52**:4121–9.
233. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007;**20**: 133–63.
234. Pfaller MA, Diekema DJ, Gibbs DL, et al. *Candida krusei*, a multidrug-resistant opportunistic fungal pathogen: geographic and temporal trends from the ARTEMIS DISK Antifungal Surveillance Program, 2001 to 2005. *J Clin Microbiol* 2008;**46**:515–21.
235. Pfaller MA, Diekema DJ, Jones RN, et al. Trends in antifungal susceptibility of *Candida* spp. isolated from pediatric and adult patients with bloodstream infections: SENTRY Antimicrobial Surveillance Program, 1997 to 2000. *J Clin Microbiol* 2002;**40**: 852–6.
236. Pfaller MA, Diekema DJ, Rinaldi MG, et al. Results from the ARTEMIS DISK Global Antifungal Surveillance Study: a 6.5-year analysis of susceptibilities of *Candida* and other yeast species to fluconazole and voriconazole by standardized disk diffusion testing. *J Clin Microbiol* 2005;**43**:5848–59.
237. Pfeiffer CD, Samsa GP, Schell WA, et al. Quantitation of *Candida* CFU in initial positive blood cultures. *J Clin Microbiol* 2011;**49**: 2879–83.
238. Pichova I, Pavlickova L, Dostal J, et al. Secreted aspartic proteases of *Candida* albicans, *Candida tropicalis*, *Candida parapsilosis* and *Candida lusitaniae:* inhibition with peptidomimetic inhibitors. *Eur J Biochem* 2001;**268**:2669–77.
239. Piper L, Smith PB, Hornik CP, et al. Fluconazole loading dose pharmacokinetics and safety in infants. *Pediatr Infect Dis J* 2011; **30**(5):375–8.

240. Posteraro B, Sanguinetti M, Boccia S, et al. Early mannan detection in bronchoalveolar lavage fluid with preemptive treatment reduces the incidence of invasive *Candida* infections in preterm infants. *Pediatr Infect Dis J* 2010;**29**:844–8.
241. Powell HL, Sand CA, Rennie RP. Evaluation of CHROMagar *Candida* for presumptive identification of clinically important *Candida* species. *Diagn Microbiol Infect Dis* 1998;**32**:201–4.
242. Prescott RJ, Harris M, Banerjee SS. Fungal infections of the small and large intestine. *J Clin Pathol* 1992;**45**:806–11.
243. Procianoy RS, Eneas MV, Silveira RC. Empiric guidelines for treatment of *Candida* infection in high-risk neonates. *Eur J Pediatr* 2006;**165**:422–3.
244. Puel A, Doffinger R, Natividad A, et al. Autoantibodies against IL-17A, IL-17F, and IL-22 in patients with chronic mucocutaneous candidiasis and autoimmune polyendocrine syndrome type I. *J Exp Med* 2010;**207**:291–7.
245. Raad I, Hanna H, Boktour M, et al. Management of central venous catheters in patients with cancer and candidemia. *Clin Infect Dis* 2004;**38**:1119–27.
246. Raaijmakers R, Schroder C, Monnens L, et al. Fungal peritonitis in children on peritoneal dialysis. *Pediatr Nephrol* 2007;**22**: 288–93.
247. Ragouilliaux CJ, Keeney SE, Hawkins HK, et al. Maternal factors in extremely low birth weight infants who develop spontaneous intestinal perforation. *Pediatrics* 2007;**120**:e1458–64.
248. Ramage G, Bachmann S, Patterson TF, et al. Investigation of multidrug efflux pumps in relation to fluconazole resistance in *Candida* albicans biofilms. *J Antimicrob Chemother* 2002;**49**:973–80.
249. Ramage G, Vande Walle K, Wickes BL, et al. Standardized method for in vitro antifungal susceptibility testing of *Candida* albicans biofilms. *Antimicrob Agents Chemother* 2001;**45**:2475–9.
250. Rebhandl W, Saadi S, Herneth AM, et al. Successful conservative treatment of severe renal candidosis with fungus balls. *Pediatr Nephrol* 1999;**13**:688–92.
251. Rebora A, Leyden JJ. Napkin (diaper) dermatitis and gastrointestinal carriage of *Candida albicans*. *Br J Dermatol* 1981;**105**: 551–5.
252. Reef SE, Levine WC, McNeil MM, et al. Treatment options for vulvovaginal candidiasis, 1993. *Clin Infect Dis* 1995;**20**(Suppl. 1):S80–90.
253. Rentz AM, Halpern MT, Bowden R. The impact of candidemia on length of hospital stay, outcome, and overall cost of illness. *Clin Infect Dis* 1998;**27**:781–8.
254. Rex JH, Bennett JE, Sugar AM, et al. Intravascular catheter exchange and duration of candidemia. NIAID Mycoses Study Group and the Candidemia Study Group. *Clin Infect Dis* 1995; **21**:994–6.
255. Robenshtok E, Gafter-Gvili A, Goldberg E, et al. Antifungal prophylaxis in cancer patients after chemotherapy or hematopoietic stem-cell transplantation: systematic review and meta-analysis. *J Clin Oncol* 2007;**25**:5471–89.
256. Robinson JL, Davies HD, Barton M, et al. Characteristics and outcome of infants with candiduria in neonatal intensive care—a Paediatric Investigators Collaborative Network on Infections in Canada (PICNIC) study. *BMC Infect Dis* 2009;**9**:183.
257. Rolnitsky A, Levy I, Sirota L, et al. Targeted fluconazole prophylaxis for high-risk very low birth weight infants. *Eur J Pediatr* 2012;**171**:1481–7.
258. Rosenthal LB, Feja KN, Levasseur SM, et al. The changing epidemiology of pediatric endocarditis at a children's hospital over seven decades. *Pediatr Cardiol* 2010;**31**:813–20.
259. Rotrosen D, Calderone RA, Edwards JE Jr. Adherence of *Candida* species to host tissues and plastic surfaces. *Rev Infect Dis* 1986;**8**: 73–85.
260. Rowen JL, Atkins JT, Levy ML, et al. Invasive fungal dermatitis in the < or = 1000-gram neonate. *Pediatrics* 1995;**95**:682–7.
261. Rowen JL, Rench MA, Kozinetz CA, et al. Endotracheal colonization with *Candida* enhances risk of systemic candidiasis in very low birth weight neonates. *J Pediatr* 1994;**124**:789–94.
262. Sabino R, Sampaio P, Carneiro C, et al. Isolates from hospital environments are the most virulent of the *Candida* parapsilosis complex. *BMC Microbiol* 2011;**11**:180.
263. Saez-Llorens X, Macias M, Maiya P, et al. Pharmacokinetics and safety of caspofungin in neonates and infants less than 3 months of age. *Antimicrob Agents Chemother* 2009;**53**:869–75.
264. Saiman L, Ludington E, Dawson JD, et al. Risk factors for *Candida* species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001;**20**:1119–24.
265. Saiman L, Ludington E, Pfaller M, et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. *Pediatr Infect Dis J* 2000;**19**:319–24.
266. Sallah S, Wan JY, Nguyen NP, et al. Analysis of factors related to the occurrence of chronic disseminated candidiasis in patients with acute leukemia in a non-bone marrow transplant setting: a follow-up study. *Cancer* 2001;**92**:1349–53.
267. Samonis G, Gikas A, Anaissie EJ, et al. Prospective evaluation of effects of broad-spectrum antibiotics on gastrointestinal yeast colonization of humans. *Antimicrob Agents Chemother* 1993;**37**: 51–3.
268. San Miguel LG, Cobo J, Otheo E, et al. Candidemia in pediatric patients with congenital heart disease. *Diagn Microbiol Infect Dis* 2006;**55**:203–7.
269. Sandovsky-Losica H, Barr-Nea L, Segal E. Fatal systemic candidiasis of gastrointestinal origin: an experimental model in mice compromised by anti-cancer treatment. *J Med Vet Mycol* 1992; **30**:219–31.
270. Sarvikivi E, Lyytikainen O, Soll DR, et al. Emergence of fluconazole resistance in a *Candida parapsilosis* strain that caused infections in a neonatal intensive care unit. *J Clin Microbiol* 2005;**43**: 2729–35.
271. Saville SP, Lazzell AL, Monteagudo C, et al. Engineered control of cell morphology in vivo reveals distinct roles for yeast and filamentous forms of *Candida albicans* during infection. *Eukaryot Cell* 2003;**2**:1053–60.
272. Saxen H, Virtanen M, Carlson P, et al. Neonatal *Candida parapsilosis* outbreak with a high case fatality rate. *Pediatr Infect Dis J* 1995;**14**:776–81.
273. Schelonka RL, Moser SA. Time to positive culture results in neonatal *Candida* septicemia. *J Pediatr* 2003;**142**:564–5.
274. Schrank JH Jr, Dooley DP. Purulent pericarditis caused by *Candida* species: case report and review. *Clin Infect Dis* 1995; **21**:182–7.
275. Schuman P, Sobel JD, Ohmit SE, et al. Mucosal candidal colonization and candidiasis in women with or at risk for human immunodeficiency virus infection. HIV Epidemiology Research Study (HERS) Group. *Clin Infect Dis* 1998;**27**:1161–7.
276. Schwartz RH, Knerr RJ. *Candida* esophagitis during tetracycline treatment for adolescent acne vulgaris. *Pediatr Infect Dis* 1982; **1**:374.
277. Semelka RC, Shoenut JP, Greenberg HM, Bow EJ. Detection of acute and treated lesions of hepatosplenic candidiasis: comparison of dynamic contrast-enhanced CT and MR imaging. *J Magn Reson Imaging* 1992;**2**:341–5.
278. Sendid B, Poirot JL, Tabouret M, et al. Combined detection of mannanaemia and antimannan antibodies as a strategy for the diagnosis of systemic infection caused by pathogenic *Candida* species. *J Med Microbiol* 2002;**51**:433–42.
279. Shah CP, McKey J, Spirn MJ, et al. Ocular candidiasis: a review. *Br J Ophthalmol* 2008;**92**:466–8.
280. Sheppard DC, Locas MC, Restieri C, et al. Utility of the germ tube test for direct identification of *Candida* albicans from positive blood culture bottles. *J Clin Microbiol* 2008;**46**:3508–9.
281. Silva AP, Miranda IM, Lisboa C, et al. Prevalence, distribution, and antifungal susceptibility profiles of *Candida parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* in a tertiary care hospital. *J Clin Microbiol* 2009;**47**:2392–7.
282. Silva S, Negri M, Henriques M, et al. *Candida glabrata*, *Candida parapsilosis* and *Candida tropicalis:* biology, epidemiology, pathogenicity and antifungal resistance. *FEMS Microbiol Rev* 2012;**36**: 288–305.
283. Sims ME, Yoo Y, You H, et al. Prophylactic oral nystatin and fungal infections in very-low-birthweight infants. *Am J Perinatol* 1988;**5**:33–6.
284. Slenker AK, Keith SW, Horn DL. Two hundred and eleven cases of *Candida* osteomyelitis: 17 case reports and a review of the literature. *Diagn Microbiol Infect Dis* 2012;**73**:89–93.
285. Smith PB, Benjamin DK Jr, Alexander BD, et al. Quantification of 1,3-beta-D-glucan levels in children: preliminary data for diagnostic use of the beta-glucan assay in a pediatric setting. *Clin Vaccine Immunol* 2007;**14**:924–5.

286. Smith PB, Morgan J, Benjamin JD, et al. Excess costs of hospital care associated with neonatal candidemia. *Pediatr Infect Dis J* 2007;**26**:197–200.
287. Smith PB, Steinbach WJ, Cotten CM, et al. Caspofungin for the treatment of azole resistant candidemia in a premature infant. *J Perinatol* 2007;**27**:127–9.
288. Sobel JD. Vaginitis. *N Engl J Med* 1997;**337**:1896–903.
289. Sobel JD. Vulvovaginal candidosis. *Lancet* 2007;**369**:1961–71.
290. Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstet Gynecol* 1998;**178**:203–11.
291. Soysa NS, Samaranayake LP, Ellepola AN. Antimicrobials as a contributory factor in oral candidosis—a brief overview. *Oral Dis* 2008;**14**:138–43.
292. Staab JF, Bradway SD, Fidel PL, et al. Adhesive and mammalian transglutaminase substrate properties of *Candida albicans* Hwp1. *Science* 1999;**283**:1535–8.
293. Staib F, Mishra SK, Tompak B, et al. Pathogenic yeast-like fungi in meat products. *Zentralbl Bakteriol* 1980;**248**:422–9.
294. Stamos JK, Rowley AH. Candidemia in a pediatric population. *Clin Infect Dis* 1995;**20**:571–5.
295. Steinbach WJ, Perfect JR, Cabell CH, et al. A meta-analysis of medical versus surgical therapy for *Candida* endocarditis. *J Infection* 2005;**51**:230–47.
296. Steinbach WJ, Roilides E, Berman D, et al. Results from a prospective, international, epidemiologic study of invasive candidiasis in children and neonates. *Pediatr Infect Dis J* 2012;**31**(12): 1252–7.
297. Stoll BJ, Hansen N, Fanaroff AA, et al. To tap or not to tap: high likelihood of meningitis without sepsis among very low birth weight infants. *Pediatrics* 2004;**113**:1181–6.
298. Stoll BJ, Hansen NI, Adams-Chapman I, et al. Neurodevelopmental and growth impairment among extremely low-birth-weight infants with neonatal infection. *JAMA* 2004;**292**:2357–65.
299. Stoll BJ, Temprosa M, Tyson JE, et al. Dexamethasone therapy increases infection in very low birth weight infants. *Pediatrics* 1999;**104**:e63.
300. Sudbery P, Gow N, Berman J. The distinct morphogenic states of *Candida albicans*. *Trends Microbiol* 2004;**12**:317–24.
301. Sullivan DJ, Westerneng TJ, Haynes KA, et al. *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology* 1995;**141**(Pt 7):1507–21.
302. Swanson H, Hughes PA, Messer SA, et al. *Candida albicans* arthritis one year after successful treatment of fungemia in a healthy infant. *J Pediatr* 1996;**129**:688–94.
303. Thaler M, Pastakia B, Shawker TH, et al. Hepatic candidiasis in cancer patients: the evolving picture of the syndrome. *Ann Intern Med* 1988;**108**:88–100.
304. Tie ML, Stephens DH. *Candida* jejunitis: a rare cause of intestinal pneumatosis in the immunocompromised patient. *Australas Radiol* 2000;**44**:206–7.
305. Tirodker UH, Nataro JP, Smith S, et al. Detection of fungemia by polymerase chain reaction in critically ill neonates and children. *J Perinatol* 2003;**23**:117–22.
306. Tissieres P, Jaeggi ET, Beghetti M, et al. Increase of fungal endocarditis in children. *Infection* 2005;**33**:267–72.
307. Tobudic S, Kratzer C, Lassnigg A, et al. Antifungal susceptibility of *Candida* albicans in biofilms. *Mycoses* 2012;**55**:199–204.
308. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplant recipients: a global perspective: preface. *Bone Marrow Transplant* 2009;**44**:453–5.
309. Tomblyn M, Chiller T, Einsele H, et al. Guidelines for preventing infectious complications among hematopoietic cell transplantation recipients: a global perspective. *Biol Blood Marrow Transplant* 2009;**15**:1143–238.
310. Torres HA, Bodey GP, Rolston KV, et al. Infections in patients with aplastic anemia: experience at a tertiary care cancer center. *Cancer* 2003;**98**:86–93.
311. Tragiannidis A, Dokos C, Lehrnbecher T, et al. Antifungal chemoprophylaxis in children and adolescents with haematological malignancies and following allogeneic haematopoietic stem cell transplantation: review of the literature and options for clinical practice. *Drugs* 2012;**72**:685–704.
312. Trofa D, Gacser A, Nosanchuk JD. *Candida parapsilosis*, an emerging fungal pathogen. *Clin Microbiol Rev* 2008;**21**:606–25.
313. Tronchin G, Pihet M, Lopes-Bezerra LM, et al. Adherence mechanisms in human pathogenic fungi. *Med Mycol* 2008;**46**: 749–72.
314. Turner RB, Donowitz LG, Hendley JO. Consequences of candidemia for pediatric patients. *Am J Dis Child* 1985;**139**:178–80.
315. Tyson JE, Parikh NA, Langer J, et al. Intensive care for extreme prematurity—moving beyond gestational age. *N Engl J Med* 2008; **358**:1672–81.
316. Uko S, Soghier LM, Vega M, et al. Targeted short-term fluconazole prophylaxis among very low birth weight and extremely low birth weight infants. *Pediatrics* 2006;**117**:1243–52.
317. Umenai T, Konno S, Ishida N. Systemic candidiasis from *Candida albicans* colonizing the gastrointestinal tract of mice. *Experientia* 1979;**35**:1331–2.
318. Urban CF, Ermert D, Schmid M, et al. Neutrophil extracellular traps contain calprotectin, a cytosolic protein complex involved in host defense against *Candida albicans*. *PLoS Pathog* 2009;**5**: e1000639.
319. Urban CF, Reichard U, Brinkmann V, et al. Neutrophil extracellular traps capture and kill *Candida* albicans yeast and hyphal forms. *Cell Microbiol* 2006;**8**:668–76.
320. van de Veerdonk FL, Joosten LA, Shaw PJ, et al. The inflammasome drives protective Th1 and Th17 cellular responses in disseminated candidiasis. *Eur J Immunol* 2011;**41**:2260–8.
321. van de Veerdonk FL, Kullberg BJ, van der Meer JW, et al. Host-microbe interactions: innate pattern recognition of fungal pathogens. *Curr Opin Microbiol* 2008;**11**:305–12.
322. van der Graaf CA, Netea MG, Verschueren I, et al. Differential cytokine production and Toll-like receptor signaling pathways by *Candida albicans* blastoconidia and hyphae. *Infect Immun* 2005; **73**:7458–64.
323. Vazquez JA, Sobel JD. Mucosal candidiasis. *Infect Dis Clin North Am* 2002;**16**:793–820.
324. Velasco E, Portugal RD. Factors prompting early central venous catheter removal from cancer patients with candidaemia. *Scand J Infect Dis* 2011;**43**:27–31.
325. Vendettuoli V, Tana M, Tirone C, et al. The role of *Candida* surveillance cultures for identification of a preterm subpopulation at highest risk for invasive fungal infection. *Pediatr Infect Dis J* 2008;**27**:1114–16.
326. Vinh DC. Insights into human antifungal immunity from primary immunodeficiencies. *Lancet Infect Dis* 2011;**11**:780–92.
327. Violaris K, Carbone T, Bateman D, et al. Comparison of fluconazole and nystatin oral suspensions for prophylaxis of systemic fungal infection in very low birthweight infants. *Am J Perinatol* 2010;**27**(1):73–8.
328. Vonk AG, Netea MG, van Krieken JH, et al. Endogenous interleukin (IL)-1 alpha and IL-1 beta are crucial for host defense against disseminated candidiasis. *J Infect Dis* 2006;**193**:1419–26.
329. Wade KC, Wu D, Kaufman DA, et al. Population pharmacokinetics of fluconazole in young infants. *Antimicrob Agents Chemother* 2008;**52**:4043–9.
330. Waggoner-Fountain LA, Walker MW, Hollis RJ, et al. Vertical and horizontal transmission of unique *Candida* species to premature newborns. *Clin Infect Dis* 1996;**22**:803–8.
331. Walsh TJ, Hutchins GM. Postoperative *Candida* infections of the heart in children: clinicopathologic study of a continuing problem of diagnosis and therapy. *J Pediatr Surgery* 1980;**15**: 325–31.
332. Walsh TJ, Whitcomb PO, Revankar SG, et al. Successful treatment of hepatosplenic candidiasis through repeated cycles of chemotherapy and neutropenia. *Cancer* 1995;**76**:2357–62.
333. Warady BA, Bashir M, Donaldson LA. Fungal peritonitis in children receiving peritoneal dialysis: a report of the NAPRTCS. *Kidney Int* 2000;**58**:384–9.
334. Watterberg KL, Gerdes JS, Cole CH, et al. Prophylaxis of early adrenal insufficiency to prevent bronchopulmonary dysplasia: a multicenter trial. *Pediatrics* 2004;**114**:1649–57.
335. Weems JJ Jr. *Candida* parapsilosis: epidemiology, pathogenicity, clinical manifestations, and antimicrobial susceptibility. *Clin Infect Dis* 1992;**14**:756–66.
336. Weitkamp JH, Ozdas A, Lafleur B, et al. Fluconazole prophylaxis for prevention of invasive fungal infections in targeted highest risk

preterm infants limits drug exposure. *J Perinatol* 2008;**28**(6): 405–11.
337. White TC, Marr KA, Bowden RA. Clinical, cellular, and molecular factors that contribute to antifungal drug resistance. *Clin Microbiol Rev* 1998;**11**:382–402.
338. Whyte RK, Hussain Z, deSa D. Antenatal infections with *Candida* species. *Arch Dis Child* 1982;**57**:528–35.
339. Williams D, Lewis M. Pathogenesis and treatment of oral candidosis. *J Oral Microbiol* 2011:**3**.
340. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004;**39**:309–17.
341. Wisplinghoff H, Seifert H, Tallent SM, et al. Nosocomial bloodstream infections in pediatric patients in United States hospitals: epidemiology, clinical features and susceptibilities. *Pediatr Infect Dis J* 2003;**22**:686–91.
342. Wolff K, Johnson RA, Suurmond D. *Candidiasis*. 5th ed. New York: McGraw-Hill; 2005. p. 716–29.
343. Wynn JL, Tan S, Gantz MG, et al. Outcomes following candiduria in extremely low birth weight infants. *Clin Infect Dis* 2012;**54**: 331–9.
344. Zaoutis TE, Argon J, Chu J, et al. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. *Clin Infect Dis* 2005; **41**:1232–9.
345. Zaoutis TE, Coffin SE, Chu JH, et al. Risk factors for mortality in children with candidemia. *Pediatr Infect Dis J* 2005;**24**: 736–9.
346. Zaoutis TE, Greves HM, Lautenbach E, et al. Risk factors for disseminated candidiasis in children with candidemia. *Pediatr Infect Dis J* 2004;**23**:635–41.
347. Zaoutis TE, Heydon K, Localio R, et al. Outcomes attributable to neonatal candidiasis. *Clin Infect Dis* 2007;**44**:1187–93.
348. Zaoutis TE, Prasad PA, Localio AR, et al. Risk factors and predictors for candidemia in pediatric intensive care unit patients: implications for prevention. *Clin Infect Dis* 2010;**51**: e38–45.
349. Zilberberg MD, Shorr AF, Kollef MH. Secular trends in candidemia-related hospitalization in the United States, 2000-2005. *Infect Control Hosp Epidemiol* 2008;**29**:978–80.

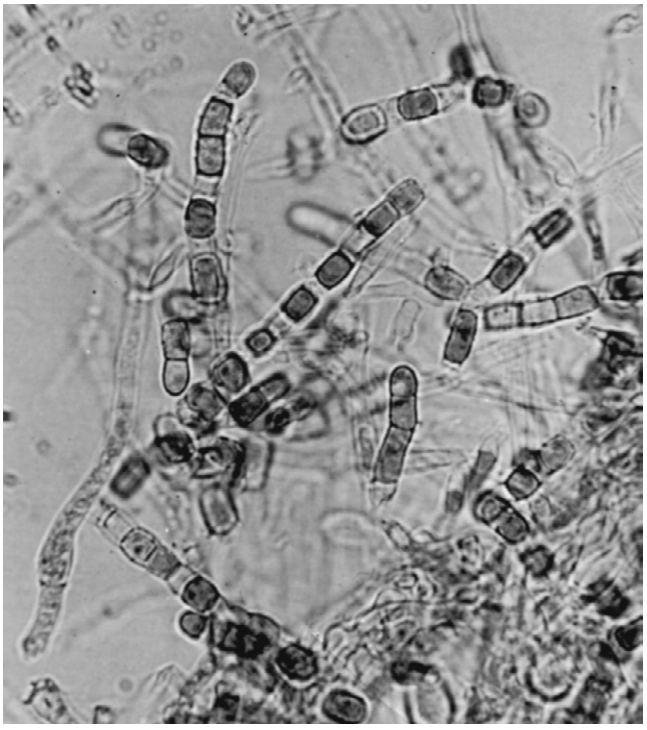

FIGURE 200-1 ■ Mycelial form of *Coccidioides immitis*. Arthroconidia are separated by vacuolated cells. (From Davis BD, Delbecco R, Eisen HW, et al. *Microbiology: including immunology and molecular genetics*. 3rd ed. Hagerstown, Md: Harper & Row; 1980.)

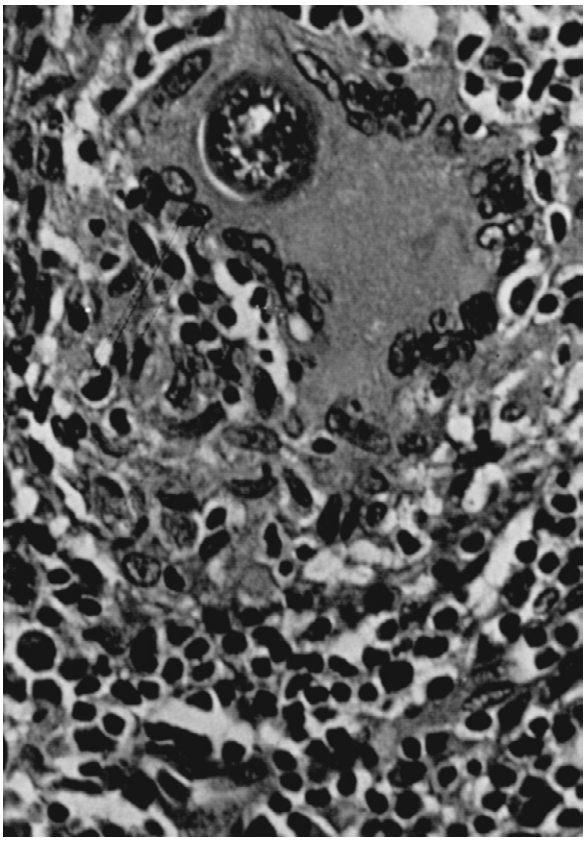

FIGURE 200-2 ■ Coccidioidal granuloma showing a multinucleated giant cell containing a spherule filled with endospores. The adjacent field contains lymphocytes and plasma cells.

into more spherules, repeating the tissue phase of the life cycle.[126]

Coccidioides spp. grow on laboratory media relatively easily, producing colonies that become visible in 3 to 4 days. They represent the mycelial phase of the fungus and appear as flat, smooth, and gray colonies from which spicules may project in some, whereas others have a velvety, membranous appearance. Colonies appear as tufts in the second week and then develop cobweb-like aerial hyphae. Pigmentation may be seen after 2 weeks and is observed best as a brownish undersurface. Variability in colony morphology can be significant. Of the two species of *Coccidioides*, *C. immitis* is prevalent in California; *C. posadasii* accounts for isolates from Arizona, Texas, Mexico, and parts of Central and South America.[62] No clinical differences have been recognized between the two species. Identification is accomplished by inoculation of the mycelia into laboratory animals and demonstration of the conversion of the fungus to the spherule phase. However, reliable techniques showing in vitro conversion to the spherule stage, demonstration of specific exoantigens, or identification of the organism by genetic probes have been developed and obviate the need for animal inoculation.[126]

EPIDEMIOLOGY

Coccidioidomycosis is endemic in the Western Hemisphere between 40 degrees of latitude north and south. In the United States, these areas lie in the southwestern states, especially in Arizona, California, western Texas, and southern New Mexico. The areas in which coccidioidomycosis is prevalent generally correspond to the lower Sonoran life zone.[113] This life zone is characterized by an arid to semiarid climate, with hot summers and relatively short winters with limited rainfall and few freezes. Most of the fungal growth occurs during the rainy season in alkaline soil and at low altitude. The fungus is found in the soil to a depth of 20 cm, especially in the walls of rodent burrows. Environmental conditions in endemic regions apparently inhibit growth of competitive organisms.[57,62] A variety of animals, including rodents, cattle, sheep, and dogs, have been shown to develop naturally acquired infection. Arthroconidia become airborne during wind storms or during disruption of soil by construction work and farming.[31,63] Prolonged droughts followed by heavy rains also have resulted in an increase in the number of cases, as occurred in California in 1992 and 1993.[30] Archeological excavations and digging by children in soil containing the organism have resulted in local outbreaks.[33,160] In addition, transmission has been reported to occur by contaminated fomites, such as dusty clothing and farm products.[1,124] The ease with which arthroconidia become airborne makes the organism dangerous to laboratory personnel. Epidemics have resulted from inadvertent opening of a single culture plate.[90] Guidelines for management of accidental laboratory exposures have been published.[140]

Primary coccidioidal infection occurs most frequently in summer and fall, after the rainy seasons. Arthroconidia are more likely to become dispersed during dry weather

after promotion of greater hyphal growth by a wet environment.[57,132,133] Variations in seasonal incidence are partially explained by the occurrence of dust storms[113,133] and earthquake activity.[31] Significant increases in the number of cases are being seen in the Southwest; high incidence periods in the winter are associated with environmental and climactic changes, such as construction activity and prolonged dry periods.[67,115]

Susceptibility to primary coccidioidal infection is unaffected by age, sex, or racial background.[61] Estimates of infection rates in endemic areas, which are based on the risk for development of infection in children, had declined as urbanization developed with dust abatement and paved roads. For example, infection rates measured by skin test reactivity to skin test antigens declined from approximately 10 percent in 1937-1939 to 2 percent in 1959 and to less than 1 percent thereafter in kindergarten and first-grade students who had lived all of their 5 to 7 years in Kern County, California.[100] Similarly, the annual risk has been estimated to be 2 and 4 percent in college students in Tucson, Arizona.[95] Incidence rates are higher in older male children, in rural areas, during wind storms, and for those with occupational exposure.[120,122,132] Significant increases in the number of reported cases have been observed in Arizona and California recently.[81] In contrast with susceptibility to primary infection, the frequency of dissemination and severe disease varies considerably, being higher in infants,[38,39,87,132] Filipinos, Hispanics, and blacks.* Immunosuppressed hosts also are at increased risk for development of disseminated infection. Persons receiving anti–tumor necrosis factor (TNF) alpha therapy are at increased risk for severe coccidioidomycosis.[13] In the era of potent antiretroviral therapy, the severity of the disease has decreased among human immunodeficiency virus (HIV)–infected individuals and is inversely associated with control of the infection.[107]

The tissue phase of the organism is the spherule, which is not infectious. Although mycelial growth can be found in cavities when they are carefully searched, no evidence exists of person-to-person spread of *C. immitis*,[78,159] except in special situations in which the fungus is allowed to revert to its airborne form, such as growth from wound drainage on a plaster cast.[56] Patients with coccidioidomycosis do not require isolation, even when draining wounds are present. In such circumstances, dressings should be changed frequently to prevent growth of the fungus and the formation of arthroconidia.

Once limited to the lower Sonoran life zone, the disease is seen throughout the country because of the ease and frequency of travel, increased populations in endemic areas, and reactivation of infection in immunocompromised hosts, including transplant recipients, patients with acquired immunodeficiency syndrome (AIDS), and recipients of TNF-α inhibitors. In these patients, delays in establishing the diagnosis can be fatal, and rapid recognition of the diagnosis and prompt institution of appropriate therapy mandate that a timely and accurate diagnosis be established.

*References 46, 63, 73, 81, 83, 123.

PATHOGENESIS AND PATHOLOGY

Acquisition of *Coccidioides* spp. infection usually is through the respiratory tract. It is surmised that most human infections result from exposure to only a single spore.[68] Growth of the organism stimulates an intense inflammatory response, and in most patients the infection remains localized to the lung and hilar nodes. In a minority of patients, clinically significant extrapulmonary dissemination occurs by way of lymphatics or the bloodstream. Rarely, direct cutaneous inoculation may occur by puncture of the skin with a contaminated object.[34,110]

The initial inflammatory response of acute pulmonary coccidioidomycosis predominantly is a polymorphonuclear leukocyte reaction, possibly related to a chemotactic effect of endospores or complement activation from other *Coccidioides* antigens. Tissue necrosis, spherules, and a few mononuclear cells are present at this stage of the disease, but epithelial giant cells are infrequently seen. This bronchopneumonic process can occur in any lobe of the lung. Although this response may slow the progress of the infection temporarily, it ultimately cannot arrest the disease process,[68] as neutrophils do not show any killing of coccidioidal forms. Killing has been demonstrated with natural killer cells and mononuclear leukocytes. Numerous studies have demonstrated the importance of T cell–mediated responses, particularly the Th1 response, in controlling the infection, as evidenced by delayed cutaneous hypersensitivity, peripheral lymphocyte transformation, and production of interleukin-12, interferon-γ, and TNF-α.[68] Dermal hypersensitivity correlates well with other measures of peripheral blood lymphocyte responsiveness, such as lymphocyte transformation and cytokine production.[2] In patients with progressive disease, cell-mediated immune defenses become defective, possibly because of defective antigen presentation, antigen overload, suppressor cells, immune complexes, or fungal immunomodulatory substances, resulting in an undesirable and ineffective Th2 response.[44]

Disseminated coccidioidomycosis resembles progressive tuberculosis of childhood, which usually occurs within weeks or months after the initial infection develops. However, endogenous reactivation may also occur, particularly among individuals with immunosuppressive conditions.[2,20,67] Extrapulmonary spread may occur anywhere in the body, but lesions are found most frequently in bone, soft tissue, lymph nodes, liver, spleen, and meninges. Bone lesions resemble chronic osteomyelitis (Fig. 200-3). Infection of the brain parenchyma is a rare event, but meningitis occurs commonly and frequently localizes to the basilar area.[21,83]

The pathologic findings of fatal coccidioidomycosis have been reviewed extensively.[84] The tissue reaction in disseminated disease predominantly is granulomatous but can be accompanied by elements of acute inflammation. Typically, the granulomatous lesions contain abundant giant cells and histiocytes. Caseous necrosis is a common occurrence, and spherules usually can be identified lying freely and within macrophages (Fig. 200-2). Fibrous tissue may surround areas of inflammation, but calcification is an infrequent occurrence.

FIGURE 200-3 ■ Osteomyelitis in a 14-year-old boy with disseminated coccidioidomycosis. An osteolytic lesion involves the distal radius.

Most cases of disseminated coccidioidal infection in the first months of life have been associated with heavy exposure to dust; these infants apparently acquire their infection by the respiratory route.[40,87] Nearly all the described infants in this age group have had severe disease,[38,39,87,145] but primary infection sometimes may go unrecognized.[39] Although women who develop coccidioidomycosis late in pregnancy may be at increased risk for development of disseminated disease,[154] with a few exceptions,[36,101] their infants are born free of infection.[40,41,131,149] In several patients with apparent perinatal transmission, the mothers had coccidioidal endometritis, and infected amniotic fluid was the most likely source of their infants' infections.[14,101,127,138]

Dissemination of coccidioidomycosis occurs more frequently in immunosuppressed patients who have resided in an endemic area,[67,105] but immunologic abnormalities have not been detected in other groups, such as Filipinos, who also are at high risk for development of disseminated disease. Once dissemination occurs, the patient's cell-mediated immunity is frequently impaired,[2,28,43,44,137] especially if extensive infection is present.[10,135] Dissemination can occur in patients who have a selective lack of response to coccidioidal antigens, as evidenced by negative skin test results to coccidioidin or spherulin, and who do not have evidence of generalized anergy. In vitro measurements of lymphocyte transformation to phytohemagglutinin or to specific cell wall antigens of *C. immitis* are also depressed.[10,43] In contrast, patients with disseminated disease with positive skin test results usually have normal in vitro lymphocyte responses that are similar to those found in healthy persons who have recovered from primary infection.[44,111,162] Furthermore, patients who have recovered from severe disseminated disease may show return of specific and nonspecific cell-mediated immunity.[10,28,61,137] Infected children demonstrate immunologic findings similar to those described in adults, but they have not been studied as extensively.

CLINICAL MANIFESTATIONS

Primary Infection

The clinical features of acute coccidioidomycosis in children are thought to be similar to the manifestations observed in adults.[134] Studies in Air Force personnel indicate that infection is subclinical or indistinguishable from a mild upper respiratory tract infection in 60 percent of infected persons.[135,137] Twenty-five percent experience an influenza-like illness lasting 1 to 2 days, whereas the remainder have more severe lower respiratory illnesses including lobar pneumonia, pleural effusions, and, occasionally, pericarditis.[61,68,116] With intensive exposure, such as occurs during military field exercises, the attack rate may be high and a large proportion of infections may be symptomatic.[46] The disease may mimic bacterial pneumonia and sepsis.[6,103] When serologic studies are used routinely in the evaluation of adults with community-acquired pneumonia in endemic areas, 17 to 28 percent of cases are the result of coccidioidal infection.[96,148] However, only 2 to 13 percent of adults with community-acquired pneumonia are tested for coccidioidomycosis, even in highly endemic areas.[35] The most common form of symptomatic infection is a subacute, self-limited pulmonary illness. Some patients, however, will experience more complicated pulmonary infections or even extrapulmonary disease. Outside of endemic areas, such patients are most likely to be encountered by physicians and may undergo extensive workups, and delays in establishing the diagnosis may occur if coccidioidomycosis is not considered.[24,25,50] Severe disease is more likely to develop in individuals with diabetes or those with a recent history of cigarette use.[123]

The usual incubation period is 10 to 16 days, but it may range from less than a week to almost a month.[61] In young adults, fatigue (77%), cough (64%), chest pain (53%), and dyspnea (17%) are the most common symptoms. Fever is present in 46 percent, with arthralgias, myalgias, and headaches each reported in 22 percent.[144] Chest pain sometimes is severe and is usually pleuritic.[144] It may be followed by vague chest pain that persists for several months.[68] In infants, stridor rarely may be present as a result of primary infection of subglottic tissue.[72,79]

Transient rashes probably occur more frequently in children than in adults and are observed in slightly more than half of symptomatic children[52,82,120]; the rash is manifested as erythema nodosum, erythema multiforme, toxic erythema, Sweet syndrome, and reactive interstitial granulomatous dermatitis. Erythema nodosum is the most characteristic lesion, usually appears 1 to 3 weeks after the onset of illness, and is associated with a lower risk

of disseminated disease.[9,52,82,61,132] Erythema multiforme–like lesions are common in children, as is toxic erythema, a macular, papular, urticarial, or morbilliform rash that is manifested within 48 hours of onset of symptoms. It may last for several weeks and is followed sometimes by palmar desquamation.[52] Rarely, primary cutaneous coccidioidomycosis is seen and results from cutaneous injury and direct inoculation of the skin. Lesions develop 1 to 3 weeks after an injury and are often associated with localized lymphadenitis. In adults, only mild constitutional symptoms are usually present, and the process spontaneously resolves within 2 to 3 months. However, in children, progressive and prolonged infection commonly occurs; antifungal therapy may be necessary.[110,158] Transmission by cat bite has been reported.[65]

Acute arthritis or arthralgia may accompany primary coccidioidal infection. These findings usually are transient, do not signify dissemination, and are thought to represent a hypersensitivity reaction.[54]

The radiographic appearance of primary coccidioidomycosis is nonspecific.[11,37,77,120] Bronchopneumonic infiltrates are the most frequent finding and often are associated with hilar lymphadenopathy. Segmental or lobar consolidation and nodular or patchy pulmonary infiltrates also can occur. Small pleural effusions or pleuropericardial reactions also occur in 15 percent of hospitalized patients (Fig. 200-4) and are usually sterile.[102,108] These radiographic findings resolve in 90 to 95 percent of symptomatic cases, albeit slowly in some, and usually do not necessitate specific therapy.

In neonates, the constellation of radiographic findings of focal consolidation with diffuse nodular densities associated with nonspecific symptoms and minimal clinical evidence of respiratory tract infection has been described but is not specific for coccidioidomycosis.[14,37] Chorioretinitis as a manifestation of systemic disease also has been reported.[74]

In a minority of patients, cavitation, nodule formation, bronchiectasis, or calcification may develop at the site of the pulmonic infiltrate.[16] The cavities usually are asymptomatic and rarely require surgical therapy. Nodules and thin-walled cavities develop in 5 percent of patients with coccidioidal pneumonia. These lesions are well circumscribed, typically single, and less than 6 cm in diameter. Many resolve spontaneously but result nonetheless in the need for prolonged care and convalescence. Rarely, the cavities lead to pneumothorax, empyema, or bronchopleural fistula, which is more likely to occur in immunosuppressed patients or in diabetics.[88] In adults, nodules may be confused with carcinoma of the lung, thus requiring excision or diagnostic fine-needle aspiration.[53] A miliary pattern, indicating hematogenous or lymphatic spread, sometimes can be appreciated in immunocompromised hosts and rarely in immunocompetent hosts.[8] On occasion, endotracheal or endobronchial lesions can be demonstrated by endoscopy.[117]

The manifestations of primary coccidioidal disease thus are varied, and no constellation of symptoms and signs is specific enough to establish a diagnosis, rendering the use of specific laboratory tests a requirement.

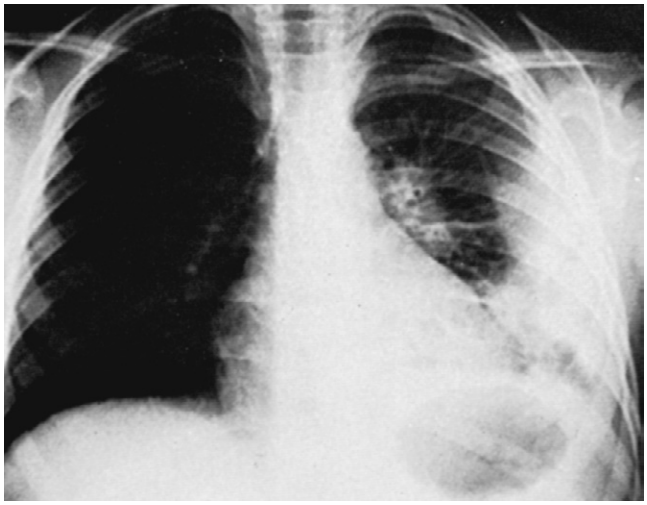

FIGURE 200-4 ■ Chest radiograph of a patient with acute pulmonary coccidioidomycosis showing pulmonary infiltrates, extensive pleural fluid, and enlargement of the left hilum.

Disseminated Coccidioidomycosis

Except in very young children, dissemination appears to occur less frequently in pediatric patients than in adults (0.5%), although this view has been questioned.[92,130] Spread of infection usually becomes apparent within a few weeks to a few months after the initial infection occurs and is heralded by persistent fever, toxicity, and insidious development of lesions outside the chest. An occasional patient develops disseminated disease after having an asymptomatic primary infection.[48,60,92] Disseminated disease rarely can be manifested with peritonitis, pericarditis, empyema, laryngeal lesions, or neck or gluteal abscesses.[22,47]

Skin Disease

The most common cutaneous manifestation of disseminated coccidioidomycosis is a verrucous granuloma that is characteristically located at the nasolabial fold.[61,82] These lesions may heal or may continue to progress. They can also take the form of papules, nodules, subcutaneous abscesses, pustules, or sinus tracks; they may be single or multiple and may ulcerate. Rarely they appear similar to mycosis fungoides. Cutaneous lesions result from blood-borne dissemination and contain the organism. These lesions mimic those caused by other fungi, tuberculosis, actinomycetes, and syphilis.[52]

Bone and Joint Disease

Involvement of bone results in chronic osteomyelitis,[17,49,89] which may drain into soft tissue (Fig. 200-5) and form fistulas to the overlying skin.[48] The bones most frequently infected are the vertebrae, tibias, metatarsals, skull, and metacarpals. The lesions are present in a single bone in 60 percent of cases; two bones are involved in 20 percent, three in another 10 percent, and four or more bones in the remainder. On radiographic examination, the lesions typically are lytic (Fig. 200-3). Involvement of the axial skeleton is common. Vertebral osteomyelitis is

FIGURE 200-5 ■ Swelling and chronic draining sinus over the proximal phalanx of the index finger. The infant has disseminated coccidioidomycosis with involvement of the underlying bone.

characterized by involvement of all parts of the vertebra with relative sparing of the disk, in contrast to tuberculosis. Skull involvement typically leads to erosion of the outer table. Spread of infection locally leading to meningitis is a serious concern with vertebral osteomyelitis. Arthritis has a waxing and waning course and usually involves the knee or ankle. It may also result from direct inoculation of the joint, such as with a cactus spine.

Meningitis

Meningitis may be the sole site of extrapulmonary disease, particularly in whites, but it also occurs as part of widespread dissemination.[21,91,128] It may be manifested acutely with the primary infection or appear up to 6 months later. The most common symptom is headache. Altered mental status, sluggishness, ataxia, and vomiting are other common symptoms. Children often lack signs of meningeal irritation and sometimes present with focal neurologic deficits. The pathologic process is that of granulomatous and suppurative basilar meningitis, with frequent presence of parenchymal involvement with granulomas and abscesses of the spinal cord and brain.[59,109] Vasculitic complications with infarction and stroke-like findings may be abrupt in onset.[59,157] Hydrocephalus is commonly present in children and is treated by placement of a ventriculoperitoneal shunt.[128] Before amphotericin B became available, coccidioidal meningitis was uniformly fatal, and the average length of survival in children was 5.5 months. Death with coccidioidal meningitis now is a relatively rare event. However, adults with neuroimaging abnormalities, especially hydrocephalus with or without cerebral infarction, have a high mortality rate.[7]

Examination of the cerebrospinal fluid (CSF) usually reveals moderate pleocytosis, usually between 100 and 500 white blood cells/mm^3 (with a range of a few to more than 10,000 white blood cells/mm^3) with mononuclear cell predominance, hypoglycorrhachia, and elevated protein level.[94,128] A polymorphonuclear cell predominance may be present early in the course of the illness. These findings are typical for CSF sampled from the lumbosacral space. However, considerable variation occurs in the cell count, chemistry, and antibody content of fluid obtained from the ventricles, cisterna magna, or lumbosacral space.[75,80,128] The lumbosacral space exhibits the most severe changes, that is, lower glucose concentration, higher protein level, higher cell count, and higher CSF coccidioidal complement fixation titer. Eosinophilic pleocytosis in the CSF is suggestive of coccidioidal meningitis and should prompt an appropriate diagnostic workup; it is of no prognostic value.[119] The CSF culture often is negative for the fungus, whereas CSF antibody is detectable in up to 95 percent of patients.[94] The diagnosis is confirmed by a positive CSF culture or serology and is supported by a positive serum serologic result or culture of *Coccidioides* spp. from a nonpulmonary site.

Coccidioidomycosis in Pregnancy

Like tuberculosis, coccidioidomycosis can involve the pelvic organs. Early studies had indicated an alarming risk of dissemination and death in women who acquired coccidioidomycosis, especially during the third trimester of pregnancy. In a population-based study, Wack and colleagues[154] showed that coccidioidomycosis occurs infrequently during pregnancy but remains associated with serious complications when the disease develops in the third trimester or soon after delivery. This is thought to be the result of immunosuppression and increased hormonal levels that favor the growth of spherules.[139] Pregnant women with erythema nodosum are much less likely to experience disseminated disease than are those without.[9] Wide variations are reported in the rates of dissemination and fatality among pregnant women, although all studies agree that the rate of dissemination in pregnant women is higher than that in the general population.[26,45] Maternal infection is more severe when it is acquired late in pregnancy and most severe in the immediate postpartum period.[12]

Coccidioidomycosis in the Immunocompromised Host

Control of coccidioidal infection depends primarily on cell-mediated immunity. Conditions that result in immune suppression, particularly T-lymphocyte dysfunction, such as those present in patients with lymphoma or bone marrow or solid-organ transplantation, predispose the individual to more fulminant forms of coccidioidomycosis.[20,121] Infections may be primary or the result of reactivation. Solid-organ transplant recipients are at the highest risk in the first year after undergoing transplantation, especially during primary infection. Dissemination also can occur late as a result of reactivation of a prior infection. The risk is increased by a prior history of coccidioidomycosis or a positive serologic reaction just before transplantation.[20] By use of targeted prophylaxis with fluconazole, however, the overall rate of coccidioidomycosis after liver transplantation (2.4%),

renal transplantation (3%), and hematopoietic stem cell transplantation (1.5%) can significantly be reduced.[23,153]

An increased risk of acquiring severe symptomatic disease was described in patients receiving TNF-α antagonists.[13] In patients infected with HIV, the severity of pulmonary disease is related inversely to virologic control, with greatly increased risk for development of severe pulmonary and extrapulmonary disease when the CD4 count is less than 250 cells/μL. The morbidity in HIV-1–infected patients receiving antiretroviral therapy and with CD4 counts of 250 cells/μL or higher is now similar to that in non–HIV-infected persons.[3,107] In endemic areas, the risk for coccidioidomycosis in solid-organ transplant recipients is 4 to 9 percent.[20,150,153] Most occurs in the first year after solid-organ transplantation. One third of such patients will have disseminated disease, with a mortality rate of 13 percent among those with posttransplantation coccidioidomycosis.[153] Genetic factors, such as deficiencies in interferon-γ receptor 1 or in interleukin-12 receptor β1, are being recognized in some patients with refractory disseminated coccidioidomycosis.[151,152]

DIAGNOSIS

In endemic areas where an awareness of the disease exists, the diagnosis of coccidioidomycosis usually is established readily by obtaining appropriate laboratory studies. However, even in these areas, coccidioidal pneumonia may be more prevalent than previously thought and may be underdiagnosed.[96,148] In nonendemic areas, the diagnosis is often not considered unless a travel history is obtained. Cases have occurred even after brief exposure in an endemic area. Primary pulmonary coccidioidomycosis resembles other lower respiratory illnesses, including those caused by viruses, bacteria, mycoplasma, *Mycobacterium tuberculosis*, and other fungi (e.g., *Histoplasma*).

The hematologic findings in primary coccidioidal infection consist of elevation of the erythrocyte sedimentation rate, leukocytosis, and, frequently, eosinophilia.[61] Specific diagnosis usually is based on the results of serologic reactions, cultures, sputum examination, and histologic examination of tissue.

Culture and Identification of the Fungus

The organism is detected readily by direct examination and culture from purulent material. The yield from other sources, such as pleural fluid, blood, and gastric aspirates, is somewhat lower. Only approximately one third of CSF samples are culture positive, and direct examination of the CSF almost always yields negative results.[82,144] Detection of the fungus from the blood is an uncommon occurrence and is associated with severe forms of disseminated disease.[4] Tissue biopsies of pleura, synovium, or meninges often yield the organism by culture or by histologic demonstration of coccidioidal spherules.

In severe pulmonary or disseminated disease, microscopic examination of bronchoalveolar lavage specimens, exudates, or biopsy specimens is diagnostic if typical spherules containing endospores are seen. Hematoxylin and eosin–stained sections can be used to demonstrate spherules, but they are used mainly to show the inflammatory process. The periodic acid–Schiff stain is useful for demonstration of spherule contents (Fig. 200-6), whereas methenamine silver stains highlight the wall of the spherule. Potassium hydroxide stains are useful but lack sensitivity and specificity; calcofluor white fluorescent stains are more sensitive but require experienced technologists.[126] Cytologic examination of sputa is more sensitive than are potassium hydroxide stains.[155] Cytologic examination of bronchial wash or bronchoalveolar fluid is diagnostic in only approximately one third of persons with or without HIV infection and is less sensitive than culture.[53] The Gram stain is not useful as it is not picked up by the spherules.

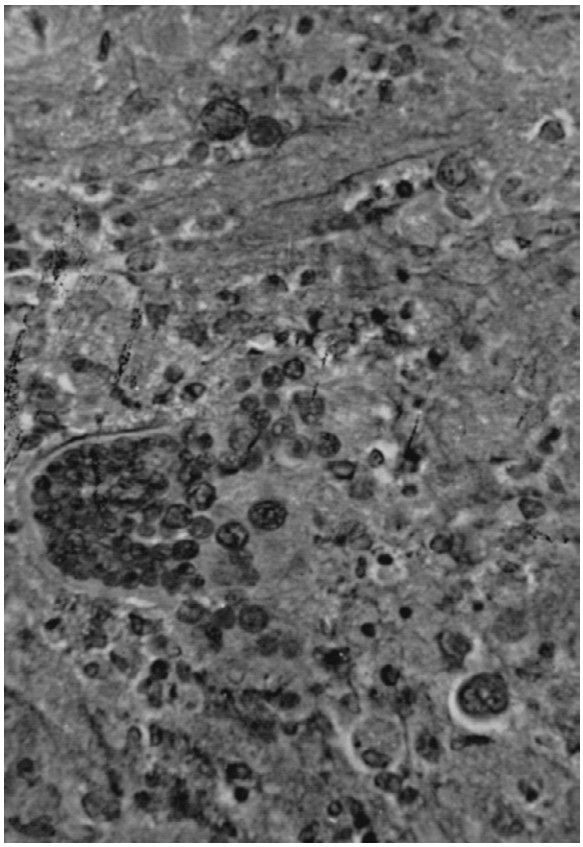

FIGURE 200-6 ■ A spherule that recently has ruptured and is in the process of releasing endospores.

With the rare exception of persons with primary cutaneous infection, the finding of spherules in tissues outside the thoracic cavity is evidence that the patient has disseminated disease. For this reason, biopsy may be useful to establish a diagnosis, particularly in patients with negative or low complement fixation antibody titers. Cultures of *Coccidioides* spp. from body fluids, sputum, or exudates can be performed on most laboratory media. If coccidioidomycosis is suspected, the use of mycologic media containing cycloheximide can be helpful.[90,142] The handling of the cultures is hazardous and requires special biosafety level 3 practices. *Coccidioides* spp. are no longer designated Select Agents by the Centers for Disease

Control and Prevention, *Coccidioides* spp. grow on routine bacterial, fungal, and *Legionella* media. Once a nonpigmented mold is grown, confirmation is achieved best by use of genus-specific genetic probes that allow rapid confirmation, usually within hours.[112] The probe does not differentiate between *C. immitis* and *C. posadasii*, and it is not clinically relevant to differentiate the two species. Real-time polymerase chain reaction assays are being developed for rapid detection of *Coccidioides* spp. in clinical specimens but are not commercially available.[15]

Skin Test

Intradermal skin tests using lysates of either the mycelial phase (coccidioidin) or the spherule phase (spherulin) have been used to elicit delayed hypersensitivity. They have primarily been used as epidemiologic tools and are no longer commercially available. Skin test reactions may be negative in immunocompromised individuals and in persons with disseminated disease.

Serologic Studies

The initial antibody response to coccidioidal infection predominantly is in the IgM fraction and is responsible for the positive precipitin test result that accompanies primary infection. Fifty percent of patients yield positive results in the first week, and 90 percent show precipitins within 2 to 3 weeks. Thereafter, antibody reversion occurs, and by 5 months, only 10 percent of patients with uncomplicated infection yield positive results.[136] These IgM responses can be measured by tube precipitins, latex agglutination, enzyme immunoassay, or immunodiffusion methods.[114] Precipitating IgM antibody detected by the tube precipitin assay or by immunodiffusion usually indicates acute infection.[136] Latex agglutination is sensitive, rapid, and easy to perform but frequently yields false-positive reactions[114]; positive results should be confirmed by a second method. Latex agglutination results in false-positives in spinal fluid and in diluted sera and should not be used for these specimens.[114] In contrast with that seen with complement fixation, the magnitude of the IgM titer does not correlate with an increased risk of dissemination.[135] IgM may persist in some patients with disseminated infection or may reappear with reactivation of infection. On occasion, IgM has been detected in cord blood of newborns whose mothers had detectable antibody. These infants had no evidence of infection on follow-up.[114] The enzyme immunoassay is a sensitive and rapid method for the detection of IgM. The interpretation of isolated positive IgM serologies is controversial. In a study of 706 enzyme immunoassays including 28 isolated IgM seropositives, Blair did not detect any false-positives.[19] In contrast, Kuberski found that 82 percent of patients with a positive IgM response and a negative IgG response had no evidence of coccidioidomycosis.[98]

Antibodies measured by complement fixation are slower to develop and primarily are in the IgG fraction.[114] Usually, they are not detected in serum samples obtained during the first week of illness, may appear as late as 3 months after onset of symptoms,[86,136] and typically last 6 to 8 months.[114] Early therapy with fluconazole may interfere with the development of IgG antibodies.[143] These antibodies are seen more commonly in symptomatic infections and are detected in 50 to 90 percent by 3 months after the onset of symptoms. At least 90 percent of persons show a positive IgM or IgG response after having symptomatic primary infections.[114,126] The main methods of detection of IgG antibodies are complement fixation, quantitative immunodiffusion, and enzyme immunoassay.[106,114,156,161] These assays correlate well, and immunodiffusion, particularly, is useful for detection of antibody in patients whose sera are anticomplementary.[114]

An IgM or IgG response is present in 90 percent of symptomatic coccidioidal infections.[115,127] The magnitude of the complement fixation antibody response correlates closely with the severity of infection and the likelihood of dissemination.[136] Although the antibody titer cannot be used as the sole indicator of dissemination, titers of 1:32 or greater in most laboratories are highly suggestive of extrapulmonary spread of infection[85,136]; 61 percent of these patients have a titer of at least 1:16, whereas 95 to 100 percent of those without dissemination have a titer lower than 1:16. The titer of complement fixation antibody parallels disease activity and is useful in following the progress of patients with disseminated disease. A few patients, especially those with extensive pulmonary involvement and pleural effusion, develop high titers without other clinical evidence of dissemination.[114] Conversely, some patients with dissemination, particularly those with single lesions in skin, bone, or meninges, have lower titers. These antibodies are measured by complement fixation or immunodiffusion.

More recently, enzyme immunoassays have become available. These assays compare well with more traditional assays and do not suffer from the subjectivity required to interpret the other assays.[64,106] The IgG enzyme immunoassay has a sensitivity of 92 percent, whereas the IgM assay has a sensitivity of 77 percent. When both assays are combined, the sensitivity is excellent,[93,106] but it is confounded by false-positive results in patients with blastomycosis or suspected noncoccidioidal pulmonary illness.[93]

Some patients with immunodeficiency disease, in particular HIV infection, may have extensive disease with low or undetectable antibody levels.[3,114] Bone marrow transplant recipients may yield no serologic or skin test response before or shortly after bone marrow transplantation, and aggressive attempts may be required to culture the fungus for diagnosis.[121]

The complement fixation antibody is detectable in the CSF of 70 percent of patients at the time of diagnosis of coccidioidal meningitis and eventually becomes detectable in almost all. The antibody measured is not a reflection of serum levels but rather is thought to indicate specific immunoglobulin biosynthesis by cells residing within or contiguous to the central nervous system. On occasion, patients with vertebral osteomyelitis or epidural abscesses yield low complement fixation titers in the CSF without other evidence of meningeal involvement.[114]

Antigen Detection

Antigen detection assays using *Coccidioides* galactomannan have been developed for detection of antigenuria and antigenemia. On the basis of preliminary data, they appear promising with detection rates of 50 to 71 percent for antigenuria and up to 73 percent for antigenemia when the specimen is treated with heat in the presence of EDTA. This assay still awaits validation.[55]

TREATMENT

Primary Infection

In more than 90 percent of children, primary coccidioidomycosis is a self-limited illness, and antifungal therapy is not needed. In some children with severe primary disease, therapy with antifungals may be justified either to abbreviate the period of morbidity or to lessen the chance of dissemination in those with an elevated complement fixation titer.

Which patients with primary uncomplicated pulmonary infections should receive therapy remains controversial. In general, evidence of severe infection or of concurrent risk factors such as immunosuppression should lead to the initiation of therapy. Severe infections usually are heralded in adults by weight loss of more than 10 percent, intense night sweats for a duration of longer than 3 weeks, infiltrates involving more than half of one lung or portions of both lungs, prominent or persistent hilar adenopathy, complement fixation titers of greater than 1:16, failure to develop normal skin test hypersensitivity to coccidioidal antigens, inability to work, or symptoms that persist for longer than 2 months.[69] Diagnosis of primary coccidioidomycosis during the third trimester of pregnancy or immediately post partum should prompt consideration of treatment. Amphotericin B is recommended for pregnant women in the first trimester because of the teratogenic potential of fluconazole, which is classified as a class D agent.[12] Thereafter, an azole antifungal or amphotericin B can be used. Fluconazole can be used in breast-feeding women, but other azoles should be avoided.[12] Because of their high risk for dissemination, persons of African or Filipino ancestry should be considered for antifungal therapy, which typically consists of oral azoles for 3 to 6 months. When reticulonodular or miliary disease is seen, therapy usually is initiated with amphotericin B or high-dose fluconazole. Amphotericin B seems to yield a faster response and is replaced by an azole after a few weeks.[69] The total course of therapy should last for at least 1 year and should be followed by oral azole therapy for secondary prophylaxis.[69] Immunocompromised patients, such as those with AIDS, recipients of solid-organ transplants, patients receiving high-dose steroids, or those receiving TNF-α inhibitors, warrant therapy.

Asymptomatic pulmonary cavities generally do not require therapy. Consideration should be given to excision of some cavities that persist for longer than 2 years, are progressively enlarging, or are adjacent to the pleural cavity. In patients with cavities that result in local discomfort, superinfection, or hemoptysis, therapy with oral azoles may alleviate the symptoms. Resection of localized cavities is an alternative to recurrent courses of antifungal therapy.[69] Pyopneumothorax results from rupture of a cavity into the pleural space. Its treatment is surgical with or without a course of antifungal therapy. Chronic fibrocavitary disease usually is treated with oral azoles for at least a year. In patients with inadequate responses, increasing the dose of azole, switching to a different azole, and administering amphotericin B are options. Surgical resection may be helpful in localized disease or when significant hemoptysis has occurred.[69]

Disseminated Disease

Nonmeningeal Dissemination

Patients with disseminated disease who have clinically apparent lesions outside the thoracic cavity almost always should receive antifungal therapy, usually with an azole (fluconazole or itraconazole). In fulminant infections, amphotericin B is the agent of choice as it is thought to result in more rapid improvement.[69]

The classic therapy consists of amphotericin B deoxycholate administered initially at a dose of 1 to 1.5 mg/kg/day and then tapered to 1 to 1.5 mg/kg/day three times a week. The usual duration of therapy is 3 months to 1 year. In children, a maximum duration of amphotericin therapy has not been defined because children are better able to tolerate the toxicities of amphotericin B. Local instillation or irrigation of abscesses and cavities with amphotericin B or other antifungal agents may be beneficial but has not been studied systematically.[17,89] Most patients respond to between 15 and 45 mg/kg, with total duration of therapy determined by the patient's response. Amphotericin lipid-complexed preparations offer the possibility of administering higher doses with less toxicity and have largely replaced intravenous amphotericin B deoxycholate.

The azoles have been tested extensively in this form of the disease. The main agents used are fluconazole and itraconazole. In a randomized, double-blind study of progressive nonmeningeal coccidioidomycosis, a trend toward better efficacy with itraconazole, especially in the subgroup of patients with skeletal infections, was demonstrated.[70] This approach must be weighed in children against the need for more frequent drug administration and dietary limitations related to the erratic absorption of itraconazole and its higher cost. Itraconazole has important drug interactions, most notably with tacrolimus, cyclosporine, and phenytoin.[76] Reports suggest that voriconazole and posaconazole can be effective therapies in patients refractory to fluconazole therapy.[5,97,141] A single case report demonstrated beneficial results with the concurrent use of interferon-γ.[99] In general, the azoles are used for a few months after resolution of symptoms or for at least a year of therapy. Fluconazole should not be used in the first trimester of pregnancy as cases of multiple congenital malformations somewhat reminiscent of the Antley-Bixler syndrome

have been described.[12] The role of echinocandins is unclear at present.

Surgical therapy seldom is required in patients with primary disease, but therapeutic thoracentesis may be indicated rarely when pneumonitis is complicated by large pleural effusions. Surgery also is necessary when pericardial involvement is complicated by tamponade. Patients with persistent coccidioidal cavities (>1 year) may require lobectomy, especially if the lesions are symptomatic.[69] Coccidioidal lymphadenopathy occasionally requires excision. In general, bone and joint disease requires a combined surgical and medical approach. Coccidioidal arthritis typically responds poorly to systemic therapy, and synovectomy may aid in the control of symptoms.[17,18] Osteomyelitis optimally is dealt with by curettage and drainage of the involved bone, with the addition of amphotericin B therapy, itraconazole, or fluconazole.[17,27,49,70]

Within endemic areas, routine serologic screening and primary prophylaxis are not indicated for HIV-infected children[29] and those receiving TNF-α inhibitors. Treatment is recommended for all HIV-infected individuals with active coccidioidomycosis and CD4 counts of less than 250 cells/μL. Once the infection is controlled, discontinuation of the therapy is reasonable if the CD4 count has risen to more than 250 cells/μL, except in cases of meningitis, in which the therapy should be lifelong.

Azoles have been used for both primary and secondary prophylaxis in endemic areas for patients with solid-organ transplants. The duration of prophylaxis usually is 6 to 12 months or more, but the optimal duration is unknown. Because of inhibition of the cytochrome P-450 system, levels of cyclosporine and tacrolimus should be monitored.[20]

Meningeal Disease

Amphotericin therapy for coccidioidal meningitis has been associated with a marked improvement in survival rates.[58,66] However, this requires prolonged intrathecal therapy through lumbar, ventricular, or cisternal administration. This therapy is associated with significant side effects that include headache, nausea, vomiting, chills and fever, arachnoiditis, and, occasionally, paralysis, seizures, and coma. The symptoms of arachnoiditis may be indistinguishable from those of microbiologic relapses.[80,91]

Studies using 400 mg of fluconazole or itraconazole in the treatment of meningitis without concurrent use of amphotericin B have shown good results, with response rates of approximately 80 percent.[71,146,147] Some experts prefer starting at a dose of 800 to 1200 mg of fluconazole or 600 mg of itraconazole.[91] Fluconazole currently is the preferred drug.[69] On the basis of limited data, the pediatric dose of fluconazole is approximately 12 mg/kg/day.[125] Patients generally respond clinically within 1 to 2 months, although abnormalities of the CSF may persist in some patients for a prolonged period. When therapy has been stopped, increases in CSF cell count or complement fixation antibody titer may be the only indications of reactivation of meningeal disease and signify a need to intensify therapy.[54] Some investigators use intra-CSF administration of amphotericin in conjunction with azole therapy in the hope of achieving a faster response.

Significant attention should be paid to CSF flow dynamics because almost all children with coccidioidal meningitis develop obstructive hydrocephalus, which requires ventriculoperitoneal shunting and rarely a second shunt to drain a "trapped" fourth ventricle.[80,128] Hence, the advantage of a drug such as fluconazole, which can be given systemically, obviates the problems of impaired CSF flow faced when intrathecal or intraventricular therapy is used. Monitoring of CSF pressure is important in assessing the timing of CSF shunting; persistent pressures above 200 mm of water herald the need for shunting.[91]

Studies have indicated that the relapse rate after discontinuation of azole therapy is high. Of 14 persons treated for coccidioidal meningitis with azoles (ketoconazole, itraconazole, or fluconazole) for periods ranging from 8 to 101 months, 11 (78%) had a relapse of disseminated coccidioidomycosis within 0.5 to 30 months after therapy was stopped. There were no clinical or laboratory predictors of patients at risk for having a relapse. The data thus indicate that moderately prolonged azole therapy for coccidioidal meningitis suppresses but does not eradicate the infection, suggesting the need for prolonged if not lifelong therapy.[51] Voriconazole and posaconazole have been used as rescue therapies in coccidioidal meningitis with some success; their role in central nervous system infections remains to be defined.[42,97,118,129,141]

PROGNOSIS

Primary pulmonary coccidioidal infection usually is self-limited, with complete recovery occurring in 1 to 3 weeks. In a small proportion of cases, localized complications of the primary infection, such as pleural effusion or pericarditis, prolong the clinical course. Dissemination is a rare event in whites and is less likely to occur in patients with erythema nodosum. Until the late 1950s, no effective therapy was available for the more severe forms of coccidioidomycosis, and the mortality rate of patients with disseminated disease was approximately 50 percent.[61] The mortality rate of patients with multiple sites of dissemination and those with coccidioidal meningitis approached 100 percent. Presently, therapy with amphotericin B or azoles may cure some of these patients and in many others prolongs useful life. Certain forms of disseminated infection, such as synovitis, are resistant to systemic therapy and may persist for many years without other signs of dissemination. For patients with HIV infection, therapy for disseminated coccidioidomycosis usually is not curative, and lifelong suppressive therapy with intermittent doses of amphotericin B or oral azoles may be required to prevent relapses in those with CD4 counts of less than 250 cells/μL. Those with counts above 250 cells/μL have a clinical course that is similar to that of immunocompetent individuals.[107] The prognosis for pregnant women has markedly improved with antifungal therapy.[12] The azoles are a major advance in the therapy

for coccidioidal meningitis, although they are fungistatic and thus require lifelong administration, sometimes failing to maintain remission of disease. Solid-organ transplant recipients remain at risk for disseminated disease and increased mortality when coccidioidomycosis develops after transplantation.

PREVENTION

Prevention of primary coccidioidal infection with antifungal agents or physical barriers such as masking is not practical. In endemic areas, control of dust, avoidance of wind storms, and avoidance of soil digging can decrease the risk for infection. Vaccine efforts are continuing but have not yielded a viable candidate vaccine to date.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Ampel NM. The complex immunology of human coccidioidomycosis. *Ann N Y Acad Sci* 2007;**1111**:245–58.
12. Bercovitch RS, Catanzaro A, Schwartz BS, et al. Coccidioidomycosis during pregnancy: a review and recommendations for management. *Clin Infect Dis* 2011;**53**:363–8.
18. Blair JE. State-of-the-art treatment of coccidioidal skeletal infections. *Ann N Y Acad Sci* 2007;**1111**:422–33.
19. Blair JE, Currier JT. Significance of isolated positive IgM serologic results by enzyme immunoassay for coccidioidomycosis. *Mycopathologia* 1988;**166**:77–82.
23. Braddy CM, Heilman RL, Blair JE. Coccidioidomycosis after renal transplantation in an endemic area. *Am J Transpl* 2006; **6**:340–5.
24. Bravo R, Pelayo-Katsanis LO, Shehab ZM, et al. Diagnostic and treatment challenges for the pediatric hematologist-oncologist in endemic areas for coccidioidomycosis. *J Pediatr Hematol Oncol* 2012;**34**:389–94.
29. Centers for Disease Control and Prevention. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children. *MMWR Recomm Rep* 2009;**58**:1–166.
34. Chang A, Tung RC, McGillis TS, et al. Primary cutaneous coccidioidomycosis. *J Am Acad Dermatol* 2003;**49**:944–9.
35. Chang DC, Anderson S, Wannemuehler K, et al. Testing for coccidioidomycosis among patients with community-acquired pneumonia. *Emerg Infect Dis* 2008;**14**:1053–9.
46. Crum NF, Lederman ER, Stafford CM, et al. Coccidioidomycosis: a descriptive survey of a reemerging disease. Clinical characteristics and current controversies. *Medicine (Baltimore)* 2004;**83**:149–75.
52. DiCaudo DJ. Coccidioidomycosis: a review and update. *J Am Acad Dermatol* 2006;**55**:929–42.
55. Durkin M, Estok L, Hospenthal D, et al. Detection of *Coccidioides* antigenemia following dissociation of immune complexes. *Clin Vaccine Immunol* 2009;**16**:1453–6.
58. Einstein HE, Holeman CW, Snadidge LL, et al. Coccidioidal meningitis: the use of amphotericin B in treatment. *Calif Med* 1961;**94**:339–43.
62. Fisher FS, Bultman MW, Johnson SM, et al. *Coccidioides* niches and habitat parameters in the southwestern United States—a matter of scale. *Ann N Y Acad Sci* 2007;**1111**:47–72.
65. Gaidici A, Saubolle MA. Transmission of coccidioidomycosis to a human via a cat bite. *J Clin Microbiol* 2009;**47**:505–6.
66. Galgiani JN. Coccidioidomycosis: changing perceptions and creating opportunities for its control. *Ann N Y Acad Sci* 2007; **1111**:1–18.
81. Hector RF, Rutherford GW, Tsang CA, et al. The public health impact of coccidioidomycosis in Arizona and California. *Int J Environ Res Public Health* 2011;**8**:1150–73.
94. Kelly PC. Coccidioidal meningitis. In: Stevens DA, editor. *Coccidioidomycosis: a text*. New York: Plenum; 1980. p. 163–93.
96. Kim MM, Blair JE, Carey EJ, et al. Coccidioidal pneumonia, Phoenix, Arizona, USA, 2000-2004. *Emerg Infect Dis* 2009;**15**: 397–401.
97. Kim MM, Vikram HR, Kusne S, et al. Treatment of refractory coccidioidomycosis with voriconazole or posaconazole. *Clin Infect Dis* 2011;**53**:1060–6.
98. Kuberski TT, Herig J, Pappagianis D. False-positive IgM serology for coccidioidomycosis. *J Clin Microbiol* 2010;**48**:2047–9.
107. Masannat FY, Ampel NM. Coccidioidomycosis in patients with HIV-1 infection in the era of potent antiretroviral therapy. *Clin Infect Dis* 2010;**50**:1–7.
108. Merchant M, Romero AO, Libke RD, et al. Pleural effusion in hospitalized patients with coccidioidomycosis. *Respir Med* 2008; **102**:537–40.
129. Schein R, Homans J, Larsen RA, et al. Posaconazole therapy for chronic refractory coccidioidal meningitis. *Clin Infect Dis* 2011;**53**:1252–4.
139. Spinello IM, Johnson RH, Baqi S. Coccidioidomycosis in pregnancy: a review. *Ann N Y Acad Sci* 2007;**1111**:358–64.
140. Stevens DA, Clemons KV, Levine HB, et al. Expert opinion: what to do when there is a *Coccidioides* exposure in a laboratory. *Clin Infect Dis* 2009;**49**:919–23.
141. Stevens DA, Rendon A, Gaona-Flores V, et al. Posaconazole therapy for chronic refractory coccidioidomycosis. *Chest* 2007; **132**:952–8.
142. Sutton DA. Diagnosis of coccidioidomycosis by culture. Safety considerations, traditional methods, and susceptibility testing. *Ann N Y Acad Sci* 2007;**1111**:315–25.
143. Thompson GR III, Lunetta JM, Johnson SM, et al. Early treatment with fluconazole may abrogate the development of IgG antibodies in coccidioidomycosis. *Clin Infect Dis* 2011;**53**: e20–4.
150. Vifram HR, Blair JE. Coccidioidomycosis in transplant recipients: a primer for clinicians in nonendemic areas. *Curr Opin Organ Transpl* 2009;**14**:606–12.
151. Vinh DC, Masannat F, Dzioba RB, et al. Refractory disseminated coccidioidomycosis and mycobacteriosis in interferon-γ receptor 1 deficiency. *Clin Infect Dis* 2009;**49**:e62–5.
152. Vinh DC, Schwartz B, Hsu AP, et al. Interleukin-12 receptor β1 deficiency predisposing to disseminated coccidioidomycosis. *Clin Infect Dis* 2011;**52**:e99–102.
153. Vucicevic D, Carey EJ, Blair JE. Coccidioidomycosis in liver transplant recipients in an endemic area. *Am J Transpl* 2011;**11**: 111–19.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Albert BL, Sellers TF Jr. Coccidioidomycosis from fomites. *Arch Intern Med* 1963;**112**:253–61.
2. Ampel NM. The complex immunology of human coccidioidomycosis. *Ann N Y Acad Sci* 2007;**1111**:245–58.
3. Ampel NM. Coccidioidomycosis in persons infected with HIV type 1. *Clin Infect Dis* 2005;**41**:1174–8.
4. Ampel NM, Ryan KJ, Carry PJ, et al. Fungemia due to *Coccidioides immitis*: an analysis of 16 episodes in 15 patients and a review of the literature. *Medicine (Baltimore)* 1986;**65**:312–21.
5. Anstead GM, Corcoran G, Lewis J, et al. Refractory coccidioidomycosis treated with posaconazole. *Clin Infect Dis* 2005;**40**: 1770–6.
6. Arsura AL, Bellinghausen PL, Kilgore WB, et al. Septic shock in coccidioidomycosis. *Crit Care Med* 1998;**26**:62–5.
7. Arsura EL, Johnson R, Penrose J, et al. Neuroimaging as a guide to predict outcome for patients with coccidioidal meningitis. *Clin Infect Dis* 2005;**40**:624–7.
8. Arsura EL, Kilgore WB. Miliary coccidioidomycosis in the immunocompetent. *Chest* 2000;**117**:404–9.
9. Arsura EL, Kilgore WB, Ratnayake SN. Erythema nodosum in pregnant patients with coccidioidomycosis. *Clin Infect Dis* 1998;**27**:1201–3.
10. Barbee RA, Hicks MJ. Clinical usefulness of lymphocyte transformation in patients with coccidioidomycosis. *Chest* 1988;**93**: 1003–7.
11. Batra P. Pulmonary coccidioidomycosis. *J Thorac Imaging* 1992;**7**:29–38.
12. Bercovitch RS, Catanzaro A, Schwartz BS, et al. Coccidioidomycosis during pregnancy: a review and recommendations for management. *Clin Infect Dis* 2011;**53**:363–8.
13. Bergstrom L, Yocum DE, Ampel NM, et al. Increased risk of coccidioidomycosis in patients treated with tumor necrosis factor α antagonists. *Arthritis Rheum* 2004;**50**:1959–66.
14. Bernstein DI, Tipton JR, Schott SF, et al. Coccidioidomycosis in a neonate: maternal-infant transmission. *J Pediatr* 1981;**99**:752–4.
15. Binnicker MJ, Buckwalter SP, Eisbrenner JJ, et al. Detection of *Coccidioides* species in clinical specimens by real-time PCR. *J Clin Microbiol* 2007;**45**:173–8.
16. Birsner JW. The roentgen aspects of five hundred cases of pulmonary coccidioidomycosis. *Am J Roentgenol Radium Ther Nucl Med* 1954;**72**:556–73.
17. Bisla RS, Taber TH Jr. Coccidioidomycosis of bone and joints. *Clin Orthop* 1976;**121**:196–204.
18. Blair JE. State-of-the-art treatment of coccidioidal skeletal infections. *Ann N Y Acad Sci* 2007;**1111**:422–33.
19. Blair JE, Currier JT. Significance of isolated positive IgM serologic results by enzyme immunoassay for coccidioidomycosis. *Mycopathologia* 1988;**166**:77–82.
20. Blair JE, Logan JL. Coccidioidomycosis in solid organ transplantation. *Clin Infect Dis* 2001;**33**:1536–44.
21. Bouza E, Dreyer JS, Hewitt WL, et al. Coccidioidal meningitis: an analysis of thirty-one cases and review of the literature. *Medicine (Baltimore)* 1981;**60**:139–72.
22. Boyle JO, Coulthard SW, Mandel RM. Laryngeal involvement in disseminated coccidioidomycosis. *Arch Otolaryngol Head Neck Surg* 1991;**117**:433–8.
23. Braddy CM, Heilman RL, Blair JE. Coccidioidomycosis after renal transplantation in an endemic area. *Am J Transpl* 2006;**6**: 340–5.
24. Bravo R, Pelayo-Katsanis LO, Shehab ZM, et al. Diagnostic and treatment challenges for the pediatric hematologist-oncologist in endemic areas for coccidioidomycosis. *J Pediatr Hematol Oncol* 2012;**34**:389–94.
25. Cairns L, Blythe D, Kao A, et al. Outbreak of coccidioidomycosis in Washington State residents returning from Mexico. *Clin Infect Dis* 2000;**30**:61–4.
26. Caldwell JW, Arsura EL, Kilgore WB, et al. Coccidioidomycosis during pregnancy during an epidemic in California. *Obstet Gynecol* 2000;**95**:236–9.
27. Catanzaro A, Galgiani JN, Levine BE, et al. Fluconazole in the treatment of chronic pulmonary and nonmeningeal disseminated coccidioidomycosis: NIAID Mycoses Study Group. *Am J Med* 1995;**98**:249–56.
28. Catanzaro A, Spitler LE, Moser KM. Cellular immune response in coccidioidomycosis. *Cell Immunol* 1975;**15**:360–71.
29. Centers for Disease Control and Prevention. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children. *MMWR Recomm Rep* 2009;**58**:1–166.
30. Centers for Disease Control and Prevention. Coccidioidomycosis—United States, 1991-1992. *MMWR Morb Mortal Wkly Rep* 1993; **42**:21–4.
31. Centers for Disease Control and Prevention. Coccidioidomycosis following the Northridge Earthquake—California, 1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**:164–5.
32. Centers for Disease Control and Prevention. Coccidioidomycosis in travelers returning from Mexico—Pennsylvania 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:1004–6.
33. Centers for Disease Control and Prevention. Coccidioidomycosis in workers at an archeologic site—Dinosaur National Monument, Utah, June-July 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**: 1005–8.
34. Chang A, Tung RC, McGillis TS, et al. Primary cutaneous coccidioidomycosis. *J Am Acad Dermatol* 2003;**49**:944–9.
35. Chang DC, Anderson S, Wannemuehler K, et al. Testing for coccidioidomycosis among patients with community-acquired pneumonia. *Emerg Infect Dis* 2008;**14**:1053–9.
36. Charlton V, Ramsdell K, Sehring S. Intrauterine transmission of coccidioidomycosis. *Pediatr Infect Dis J* 1999;**18**:561–3.
37. Child DC, Newell JD, Bjelland JC, et al. Radiographic findings of pulmonary coccidioidomycosis in neonates and infants. *A J R Am J Roentgenol* 1985;**145**:261–3.
38. Christian JR, Sarre SG, Peers JH, et al. Pulmonary coccidioidomycosis in a twenty-one-day-old infant. *Am J Dis Child* 1956;**92**: 66–73.
39. Cohen R. Coccidioidomycosis: case studies in children. *Arch Pediatr* 1949;**66**:241–65.
40. Cohen R. Placental coccidioides: proof that congenital coccidioides is nonexistent. *Arch Pediatr* 1951;**68**:59–66.
41. Cohen R, Burnip R. Coccidioidin skin testing during pregnancy and in infants and children. *Calif Med* 1950;**72**:31–3.
42. Cortez KJ, Walsh TJ, Bennett JE. Successful treatment of coccidioidal meningitis with voriconazole. *Clin Infect Dis* 2003;**36**: 1619–22.
43. Cox RA, Magee MM. Coccidioidomycosis: host response and vaccine development. *Clin Microbiol Rev* 2004;**17**:804–39.
44. Cox RA, Vivas JR, Gross A, et al. In vivo and in vitro cell-mediated responses in coccidioidomycosis: immunologic responses of persons with primary, asymptomatic infections. *Am Rev Respir Dis* 1976;**114**:937–43.
45. Crum NF, Ballon-Landa G. Coccidioidomycosis in pregnancy: case report and review of the literature. *Am J Med* 2006;**119**:993. e11–17.
46. Crum NF, Lederman ER, Stafford CM, et al. Coccidioidomycosis: a descriptive survey of a reemerging disease. Clinical characteristics and current controversies. *Medicine (Baltimore)* 2004;**83**:149–75.
47. Crum-Cianflone NF, Truett AA, Teneza-Mora N, et al. Unusual presentations of coccidioidomycosis. A case series and review of the literature. *Medicine (Baltimore)* 2006;**85**:263–77.
48. Dennis JL, Hansen AE. Coccidioidomycosis in children. *Pediatrics* 1954;**14**:481–94.
49. Deresinski SC. Coccidioidomycosis of bone and joints. In: Stevens DA, editor. *Coccidioidomycosis: a text*. New York: Plenum Medical; 1980. p. 195–224.
50. Desai SA, Minai OA, Gordon SM, et al. Coccidioidomycosis in non-endemic areas: a case series. *Respir Med* 2001;**95**:305–9.
51. Dewsnup DH, Galgiani JN, Graybill JR, et al. Is it ever safe to stop azole therapy for *Coccidioides immitis* meningitis? *Ann Intern Med* 1996;**124**:305–10.
52. DiCaudo DJ. Coccidioidomycosis: a review and update. *J Am Acad Dermatol* 2006;**55**:929–42.
53. DiTomasso JP, Ampel NM, Sobonya RE, et al. Bronchoscopic diagnosis of pulmonary coccidioidomycosis: comparison of cytology, culture, and transbronchial biopsy. *Diagn Microbiol Infect Dis* 1994;**18**:83–7.
54. Drutz DJ, Catanzaro A. Coccidioidomycosis. *Am Rev Respir Dis* 1978;**117**:559–85, 727–71.

55. Durkin M, Estok L, Hospenthal D, et al. Detection of *Coccidioides* antigenemia following dissociation of immune complexes. *Clin Vaccine Immunol* 2009;**16**:1453–6.
56. Eckmann BH, Schaefer GL, Huppert M. Bedside interhuman transmission of coccidioidomycosis via growth on fomites: an epidemic involving six persons. *Am Rev Respir Dis* 1964;**89**:175–85.
57. Egeberg RO, Ely AF. *Coccidioides immitis* in the soil of the southern San Joaquin Valley. *Am J Med Sci* 1956;**23**:151–4.
58. Einstein HE, Holeman CW, Snadidge LL, et al. Coccidioidal meningitis: the use of amphotericin B in treatment. *Calif Med* 1961;**94**:339–43.
59. Erly WK, Bellon RJ, Seeger JF, et al. MR imaging of acute coccidioidal meningitis. *Am J Neuroradiol* 1999;**20**:509–14.
60. Feigin RD, Shackelford PG, Lins RD, et al. Subcutaneous abscess due to *Coccidioides immitis*. *Am J Dis Child* 1972;**124**:734–5.
61. Fiese MJ. *Coccidioidomycosis*. Springfield, Ill: Charles C Thomas; 1958.
62. Fisher FS, Bultman MW, Johnson SM, et al. *Coccidioides* niches and habitat parameters in the southwestern United States—a matter of scale. *Ann N Y Acad Sci* 2007;**1111**:47–72.
63. Flynn NM, Hoeprich PD, Kawachi MM, et al. An unusual outbreak of windborne coccidioidomycosis. *N Engl J Med* 1979; **301**:358–61.
64. Gade W, Ledman DW, Wethington R, et al. Serological responses to various *Coccidioides* antigen preparations in a new enzyme immunoassay. *J Clin Microbiol* 1992;**30**:1907–12.
65. Gaidici A, Saubolle MA. Transmission of coccidioidomycosis to a human via a cat bite. *J Clin Microbiol* 2009;**47**:505–6.
66. Galgiani JN. Coccidioidomycosis: changing perceptions and creating opportunities for its control. *Ann N Y Acad Sci* 2007; **1111**:1–18.
67. Galgiani JN. Coccidioidomycosis: a regional disease of national importance. Rethinking approaches for control. *Ann Intern Med* 1999;**130**:293–300.
68. Galgiani JN. Coccidioidomycosis. *West J Med* 1993;**159**:153–71.
69. Galgiani JN, Ampel NM, Blair JE, et al. Coccidioidomycosis. *Clin Infect Dis* 2005;**41**:1217–23.
70. Galgiani JN, Catanzaro A, Cloud GA, et al. Comparison of oral fluconazole and itraconazole for progressive, nonmeningeal coccidioidomycosis. A randomized, double-blind trial. *Ann Intern Med* 2000;**133**:676–86.
71. Galgiani JN, Catanzaro A, Cloud GA, et al. Fluconazole therapy for coccidioidal meningitis: the NIAID-Mycoses Study Group. *Ann Intern Med* 1993;**119**:28–35.
72. Gardner S, Seilheimer D, Catlin F, et al. Subglottic coccidioidomycosis presenting with persistent stridor. *Pediatrics* 1980;**66**: 623–5.
73. Gifford MA, Buss WIC, Duds RJ. Data on *Coccidioides* fungus infection, Kern County, 1900-1936. Kern County Health Department Annual Report; 1936-1937. p. 39–54.
74. Golden SE, Morgan CM, Bartley DL, et al. Disseminated coccidioidomycosis with chorioretinitis in early infancy. *Pediatr Infect Dis* 1986;**5**:272–4.
75. Goldstein E, Winship MJ, Pappagianis D. Ventricular fluid and the management of coccidioidal meningitis. *Ann Intern Med* 1972;**77**:243–6.
76. Graybill JR. Treatment of coccidioidomycosis. *Curr Top Med Mycol* 1993;**5**:151–79.
77. Greendyke WH, Resnick DL, Harvey WC. The varied roentgen manifestations of primary coccidioidomycosis. *Am J Roentgenol Radium Ther Nucl Med* 1970;**109**:491–9.
78. Hagman HM, Madnick EG, D'Agostino AN, et al. Hyphal forms of the central nervous system of patients with coccidioidomycosis. *Clin Infect Dis* 2000;**30**:349–55.
79. Hajare S, Rakusan TA, Kalia A, et al. Laryngeal coccidioidomycosis causing airway obstruction. *Pediatr Infect Dis* 1989;**8**: 54–6.
80. Harrison HR, Galgiani JN, Sprunger L, et al. Amphotericin B and imidazole therapy for coccidioidal meningitis in children. *Pediatr Infect Dis* 1983;**2**:216–21.
81. Hector RF, Rutherford GW, Tsang CA, et al. The public health impact of coccidioidomycosis in Arizona and California. *Int J Environ Res Public Health* 2011;**8**:1150–73.
82. Hobbs ER. Coccidioidomycosis. *Dermatol Clin* 1989;**7**:227–39.
83. Huntington RW Jr. Morphology and racial distribution of fatal coccidioidomycosis: report of a 10-year autopsy series in an endemic area. *JAMA* 1959;**169**:115–18.
84. Huntington RW. Pathology of coccidioidomycosis. In: Stevens DA, editor. *Coccidioidomycosis: a text*. New York: Plenum Medical; 1980. p. 113–32.
85. Huppert M. Serology of coccidioidomycosis. *Mycopathol Mycol Appl* 1970;**41**:107–13.
86. Huppert M, Bailey JW. The use of immunodiffusion tests in coccidioidomycosis. I. The accuracy and reproducibility of the immunodiffusion test which correlates with complement fixation. *Am J Clin Pathol* 1965;**44**:364–73.
87. Hyatt HW Sr. Coccidioidomycosis in a 3-week-old infant. *Am J Dis Child* 1963;**105**:93–8.
88. Hyde L. Coccidioidal pulmonary cavitation. *Dis Chest* 1968; **54**(Suppl. 1):273–7.
89. Iger M. Coccidioidal osteomyelitis. In: Ajello L, editor. *Coccidioidomycosis*. Miami: Symposia Specialists; 1977. p. 177–90.
90. Johnson JE III, Perry JE, Fekety FR, et al. Laboratory-acquired coccidioidomycosis: a report of 210 cases. *Ann Intern Med* 1964;**60**:941–56.
91. Johnson RH, Einstein HE. Coccidioidal meningitis. *Clin Infect Dis* 2006;**42**:103–7.
92. Kafka JA, Catanzaro AT. Disseminated coccidioidomycosis in children. *J Pediatr* 1981;**98**:355–61.
93. Kaufman L, Sekhon AS, Moledina N, et al. Comparative evaluation of commercial Premier EIA and microimmunodiffusion and complement fixation tests for *Coccidioides immitis* antibodies. *J Clin Microbiol* 1995;**33**:618–19.
94. Kelly PC. Coccidioidal meningitis. In: Stevens DA, editor. *Coccidioidomycosis: a text*. New York: Plenum; 1980. p. 163–93.
95. Kerrick SS, Lundergan LL, Galgiani JN. Coccidioidomycosis at a university health service. *Am Rev Respir Dis* 1985;**131**:100–2.
96. Kim MM, Blair JE, Carey EJ, et al. Coccidioidal pneumonia, Phoenix, Arizona, USA, 2000-2004. *Emerg Infect Dis* 2009;**15**: 397–401.
97. Kim MM, Vikram HR, Kusne S, et al. Treatment of refractory coccidioidomycosis with voriconazole or posaconazole. *Clin Infect Dis* 2011;**53**:1060–6.
98. Kuberski TT, Herig J, Pappagianis D. False-positive IgM serology for coccidioidomycosis. *J Clin Microbiol* 2010;**48**:2047–9.
99. Kuberski TT, Servi RJ, Rubin PJ. Successful treatment of a critically ill patient with disseminated coccidioidomycosis, using adjunctive interferon-γ. *Clin Infect Dis* 2004;**38**:910–12.
100. Larwood TR. Coccidioidin skin testing in Kern County, California: decrease in infection rate over 58 years. *Clin Infect Dis* 2000;**30**:612–13.
101. Linsangan LC, Ross LA. *Coccidioides immitis* infection of the neonate: two routes of infection. *Pediatr Infect Dis J* 1999;**18**: 171–3.
102. Lonky SA, Catanzaro A, Moser KM, et al. Acute coccidioidal pleural effusion. *Am Rev Respir Dis* 1976;**114**:681–8.
103. Lopez AM, Williams PL, Ampel NM. Acute pulmonary coccidioidomycosis mimicking bacterial pneumonia and septic shock: a report of two cases. *Am J Med* 1993;**95**:236–9.
104. Lubarsky R, Plunkett OA. Some ecologic studies of Coccidioides immitis in soil. In: Sternberg TH, Newcomer VD, editors. *Therapy of fungus diseases, an international symposium*. Boston: Little, Brown; 1955. p. 308–10.
105. MacDonald N, Steinhoff MC, Powell KR. Review of coccidioidomycosis in immunocompromised children. *Am J Dis Child* 1981;**135**:553–6.
106. Martins TB, Jaskowski TD, Mouritsen CL, et al. Comparison of commercially available enzyme immunoassay with traditional serological tests for detection of antibodies to *Coccidioides immitis*. *J Clin Microbiol* 1995;**33**:940–3.
107. Masannat FY, Ampel NM. Coccidioidomycosis in patients with HIV-1 infection in the era of potent antiretroviral therapy. *Clin Infect Dis* 2010;**50**:1–7.
108. Merchant M, Romero AO, Libke RD, et al. Pleural effusion in hospitalized patients with coccidioidomycosis. *Respir Med* 2008; **102**:537–40.
109. Mischel PS, Vinters HV. Coccidioidomycosis of the central nervous system: neuropathological and vasculopathic manifestations and clinical correlates. *Clin Infect Dis* 1995;**20**:400–5.

110. O'Brien JJ, Gilsdorf JR. Primary cutaneous coccidioidomycosis in childhood. *Pediatr Infect Dis* 1986;**5**:485–6.
111. Opelz G, Scheer MI. Cutaneous sensitivity and in vitro responsiveness of lymphocytes in patients with disseminated coccidioidomycosis. *J Infect Dis* 1975;**132**:250–5.
112. Padhye AA, Smith G, Standard PG, et al. Comparative evaluation of chemiluminescent DNA probe assays and exoantigen tests for rapid identification of *Blastomyces dermatitidis* and *Coccidioides immitis*. *J Clin Microbiol* 1994;**32**:867–70.
113. Pappagianis D. Epidemiology of coccidioidomycosis. *Curr Top Med Mycol* 1988;**2**:199–238.
114. Pappagianis D, Zimmer DL. Serology of coccidioidomycosis. *Clin Microbiol Rev* 1990;**3**:247–68.
115. Park BJ, Sigel K, Vaz V, et al. An epidemic of coccidioidomycosis in Arizona associated with climactic changes, 1998-2001. *J Infect Dis* 2005;**191**:1981–7.
116. Pinckney L, Parker BR. Primary coccidioidomycosis in children presenting with massive pleural effusion. *A J R Am J Roentgenol* 1978;**130**:247–9.
117. Polesky A, Kirsch CM, Snyder LS, et al. Airway coccidioidomycosis—report of cases and review. *Clin Infect Dis* 1999;**28**:1273–80.
118. Proia LA, Tenorio AR. Successful use of voriconazole for treatment of *Coccidioides* meningitis. *Antimicrob Agents Chemother* 2004;**48**:2341.
119. Ragland AS, Arsura E, Ismail Y, et al. Eosinophilic pleocytosis in coccidioidal meningitis: frequency and significance. *Am J Med* 1993;**95**:254–7.
120. Richardson HB Jr, Anderson JA, McKay BM. Acute pulmonary coccidioidomycosis in children. *J Pediatr* 1967;**70**:376–82.
121. Riley DK, Galgiani JN, O'Donnell M, et al. Coccidioidomycosis in bone marrow transplant recipients. *Transplantation* 1993;**56**:1531–3.
122. Roberts PL, Lisciandro RC. A community epidemic of coccidioidomycosis. *Am Rev Respir Dis* 1967;**96**:766–72.
123. Rosenstein NE, Emery KW, Werner SB, et al. Risk factors for severe pulmonary and disseminated coccidioidomycosis: Kern County, California, 1995-1996. *Clin Infect Dis* 2001;**32**:708–15.
124. Rothman PE, Graw RG, Harris JC. Coccidioidomycosis—possible fomite transmission. A review and report of a case. *Am J Dis Child* 1969;**118**:792–801.
125. Saitoh A, Homans J, Kovacs A. Fluconazole treatment of coccidioidal meningitis in children: two case reports and a review of the literature. *Pediatr Infect Dis J* 2000;**19**:1204–8.
126. Saubolle MA, McKellar PP, Sussland D. Epidemiologic, clinical and diagnostic aspects of coccidioidomycosis. *J Clin Microbiol* 2007;**45**:26–30.
127. Shafai T. Neonatal coccidioidomycosis in premature twins. *Am J Dis Child* 1978;**132**:634.
128. Shehab ZM, Britton H, Dunn JH. Imidazole therapy of coccidioidal meningitis in children. *Pediatr Infect Dis J* 1988;**7**:40–4.
129. Schein R, Homans J, Larsen RA, et al. Posaconazole therapy for chronic refractory coccidioidal meningitis. *Clin Infect Dis* 2011;**53**:1252–4.
130. Sievers ML. Disseminated coccidioidomycosis among southwestern American Indians. *Am Rev Respir Dis* 1974;**109**:602–12.
131. Smale LE, Waechter KG. Dissemination of coccidioidomycosis in pregnancy. *Am J Obstet Gynecol* 1970;**107**:356–61.
132. Smith CE. Epidemiology of acute coccidioidomycosis with erythema nodosum ("San Joaquin" or "Valley Fever"). *Am J Public Health* 1940;**30**:600–11.
133. Smith CE, Beard RR, Rosenberger HG, et al. Effect of season and dust control on coccidioidomycosis. *JAMA* 1946;**132**:833–8.
134. Smith CE, Beard RR, Whiting EG, et al. Varieties of coccidioidal infection in relation to the epidemiology and control of the diseases. *Am J Public Health* 1946;**36**:1394–402.
135. Smith CE, Saito MT, Beard RR, et al. Serological tests in the diagnosis and prognosis of coccidioidomycosis. *Am J Hyg* 1950;**52**:1–21.
136. Smith CE, Saito MT, Simons SA. Pattern of 39,500 serologic tests in coccidioidomycosis. *JAMA* 1956;**160**:546–52.
137. Smith CE, Whiting EG, Baker EE, et al. The use of coccidioidin. *Am Rev Tuberc* 1948;**57**:330–60.
138. Spark RP. Does transplacental spread of coccidioidomycosis occur? *Arch Pathol Lab Med* 1981;**105**:347–50.
139. Spinello IM, Johnson RH, Baqi S. Coccidioidomycosis in pregnancy: a review. *Ann N Y Acad Sci* 2007;**1111**:358–64.
140. Stevens DA, Clemons KV, Levine HB, et al. Expert opinion: what to do when there is a *Coccidioides* exposure in a laboratory. *Clin Infect Dis* 2009;**49**:919–23.
141. Stevens DA, Rendon A, Gaona-Flores V, et al. Posaconazole therapy for chronic refractory coccidioidomycosis. *Chest* 2007;**132**:952–8.
142. Sutton DA. Diagnosis of coccidioidomycosis by culture. Safety considerations, traditional methods, and susceptibility testing. *Ann N Y Acad Sci* 2007;**1111**:315–25.
143. Thompson GR III, Lunetta JM, Johnson SM, et al. Early treatment with fluconazole may abrogate the development of IgG antibodies in coccidioidomycosis. *Clin Infect Dis* 2011;**53**:e20–4.
144. Tom PF, Long TJ, Fitzpatrick SB. Coccidioidomycosis in adolescents presenting as chest pain. *J Adolesc Health Care* 1987;**8**:365–71.
145. Townsend TE, McKey RW. Coccidioidomycosis in infants. *Am J Dis Child* 1953;**86**:51–3.
146. Tucker RM, Denning DW, Dupont B, et al. Itraconazole therapy for chronic coccidioidal meningitis. *Ann Intern Med* 1990;**112**:108–12.
147. Tucker RM, Galgiani JN, Denning DW, et al. Treatment of coccidioidal meningitis with fluconazole. *Rev Infect Dis* 1990;**12**:S380–9.
148. Valdivia L, Nix D, Wright M, et al. Coccidioidomycosis as a common cause of community-acquired pneumonia. *Emerg Infect Dis* 2006;**12**:958–62.
149. Vaughan JE, Ramirez H. Coccidioidomycosis as a complication of pregnancy. *Calif Med* 1951;**74**:121–5.
150. Vifram HR, Blair JE. Coccidioidomycosis in transplant recipients: a primer for clinicians in nonendemic areas. *Curr Opin Organ Transpl* 2009;**14**:606–12.
151. Vinh DC, Masannat F, Dzioba RB, et al. Refractory disseminated coccidioidomycosis and mycobacteriosis in interferon-γ receptor 1 deficiency. *Clin Infect Dis* 2009;**49**:e62–5.
152. Vinh DC, Schwartz B, Hsu AP, et al. Interleukin-12 receptor β1 deficiency predisposing to disseminated coccidioidomycosis. *Clin Infect Dis* 2011;**52**:e99–102.
153. Vucicevic D, Carey EJ, Blair JE. Coccidioidomycosis in liver transplant recipients in an endemic area. *Am J Transpl* 2011;**11**:111–19.
154. Wack EE, Ampel NM, Galgiani JN, et al. Coccidioidomycosis during pregnancy: an analysis of ten cases among 47,120 pregnancies. *Chest* 1988;**94**:376–9.
155. Warlick MA, Quan SF, Sobonya RE. Rapid diagnosis of pulmonary coccidioidomycosis: cytologic v. potassium hydroxide preparations. *Arch Intern Med* 1983;**143**:723–5.
156. Weiden MA, Galgiani JN, Pappagianis D. Comparison of immunodiffusion techniques with standard complement fixation assay for quantitation of coccidioidal antibodies. *J Clin Microbiol* 1983;**18**:529–34.
157. Williams PL, Johnson R, Pappagianis D, et al. Vasculitic and encephalitic complications associated with *Coccidioides immitis* infection of the central nervous system in humans: report of 10 cases and review. *Clin Infect Dis* 1992;**14**:673–82.
158. Winn RE. Primary cutaneous coccidioidomycosis. *Arch Dermatol* 1965;**9**:221–8.
159. Winn RE, Johnson R, Galgiani JN, et al. Cavitary coccidioidomycosis with fungus ball formation: diagnosis by fiberoptic bronchoscopy with coexistence of hyphae and spherules. *Chest* 1994;**105**:412–16.
160. Winn RE, Levine HB, Broderick JE, et al. A localized epidemic of coccidioidal infection: primary coccidioidomycosis occurring in a group of ten children infected in a backyard playground in the San Joaquin Valley of California. *N Engl J Med* 1963;**268**:867–70.
161. Zartarian M, Petersen EM, de la Maza LM. Detection of antibodies to *Coccidioides immitis* by enzyme immunoassay. *Am J Clin Pathol* 1997;**107**:148–53.
162. Zweiman B, Pappagianis D, Mailbach H, et al. Coccidioidin delayed hypersensitivity: skin test and in vitro lymphocyte reactivities. *J Immunol* 1969;**102**:1284–9.

CHAPTER 201

Paracoccidioidomycosis

Gil Benard • Maria José Soares Mendes-Giannini

Paracoccidioidomycosis, formerly known as South American blastomycosis, is usually a progressive chronic disease that preferentially affects the lungs, skin, mucous membranes, adrenals, and reticuloendothelial system. Benign, self-limited infections also have been occasionally documented. Two types of clinical presentations are described: the acute-subacute (juvenile) and the chronic (adult) forms of the disease. Children generally present with the acute form, which affects predominantly the reticuloendothelial system organs. Children account for about 5 to 10 percent of cases, and both sexes are infected; the systemic form of paracoccidioidomycosis occurs in about 70 percent of episodes.[16,83,168,169] The mycosis is limited geographically to various Latin American countries.*

The disease was described originally by Lutz in Brazil in 1908 and initially observed in children in 1911 by Montenegro.[110]

THE ORGANISM

The *Paracoccidioides* genus consists of dimorphic fungi, the etiologic agents of paracoccidioidomycosis. Currently, phylogenetic studies divide the *Paracoccidioides* genus into two species, *Paracoccidioides brasiliensis* (S1, PS2, and PS3 phylogenetic species) and *Paracoccidioides lutzii* (Pb01-like). Recombination events in *P. brasiliensis* species S1 and PS2 and *P. lutzii* were detected, whereas the *P. brasiliensis* species PS3 was considered to be clonal.[130,218,222]

The recognition of *Paracoccidioides* spp. as anamorphs in the phylum Ascomycota, order Onygenales, family Ajellomycetaceae has been accomplished by means of molecular tools.[27,230] *Paracoccidioides* spp. are phylogenetically closer to *Lacazia loboi* than to *Blastomyces dermatitidis*, *Emmonsia parva*, and *Histoplasma capsulatum*.[27,191] The fungus has four or five DNA chromosomes showing molecular sizes ranging from 2 to 10 Mb.[46,80,145] The genomes range in size from 29.1 to 32.9 Mb and encode 7610 to 8130 genes,[71] which is in agreement with the estimated gene number for ascomycete fungi genomes.

Paracoccidioides spp., thermally dimorphic fungi, appear as round to oval yeast cells of variable size (6 to 40 µm) in tissues and culture specimens at 37° C (98.6° F); in the environment or in vitro at temperatures lower than 24° C (75.2° F), morphologic features change to the mold form on culture under starvation conditions in minimal media. Conidia and mycelial fragments are considered the infectious particles. Once inhaled, they reach the alveoli, where, at the host tissue's temperature (37° C), they change from the saprophytic form to the parasitic multiple budding yeast form capable of producing disease.

Different groups have addressed the overall scenario of gene expression in *Paracoccidioides* spp. yeast versus mycelium or undergoing phase transition in vitro, making use of different molecular methods.* Many genes related to conidia[99] and in the parasitic phase in vitro[36,61,209] as well as in vivo models of infection† were also described. In the mycelial phase, growth is slow, requiring approximately 3 to 4 weeks; the colony is white to tan and compact, with short aerial mycelia. On microscopic examination, hyphae are fine (0.8 to 2.5 µm in diameter), septate, interlaced, and hyaline. Chlamydoconidia (20 to 50 µm in diameter), round to subspherical, terminal and intercalary, as well as arthroconidia may be observed.[110,184] Some isolates can produce aleurioconidia, uninucleate pear-shaped pedunculated or terminal structures, after long periods of incubation and under nutritional deprivation (media with reduced carbohydrate content).[37,110,184] The small size of these conidia (<5 µm) renders them compatible with alveolar deposition; furthermore, such conidia have been shown to be infectious when they are inhaled.[133]

The mycelial phase is not distinctive, and subcultures at 37° C are required for complete identification. At this temperature, *P. brasiliensis* produces soft, wrinkled, cream-colored colonies that develop in approximately 6 to 10 days. On microscopic examination, the most characteristic feature is the presence of multiple budding yeast cells; the parental cell produces various peripheral buds and acquires the appearance of a pilot's wheel. *P. brasiliensis* may possess alternative control mechanisms during cell growth to manage multiple budding and its multinucleate nature.[1] Single buds and short chains also are produced, but they are not diagnostic. Cells are variable in size (2 to 40 µm) and have a thick cell wall and internal vacuoles.[176] Elongated conidia were observed exclusively in *P. lutzii* isolates.[222] This phase of growth is identical to the one observed in tissue and pathologic materials.[37,88,110,177,184] The organism does not readily take most bacterial or hematologic stains. *P. brasiliensis* is aerobic and, in its mycelial phase, grows well in the regular mycologic media to which antibiotics and cycloheximide have been added to reduce growth of bacteria and saprophytic

*References 32, 37, 56, 88, 110, 113, 116, 119, 184.

*References 14, 81, 92, 100, 108, 121, 159, 165.
†References 9, 10, 49, 62, 76, 95, 117, 132, 140, 141, 158, 173, 210.

fungi.[37,110] Isolation should be attempted by the use of modified Sabouraud agar, yeast extract (15%) agar plus antibiotics and cycloheximide, yeast extract–phosphate agar, brain-heart infusion agar plus blood and antibiotics, or tryptic soy agar plus fungus water extract and horse serum, incubated at room temperature.[110,184]

TRANSMISSION

Paracoccidioidomycosis is not a contagious disease.[37,88,110,184,234] A single case of placental involvement that did not result in fetal infection has been reported.[31] Epidemic outbreaks have not been reported, and only a few cases in family members have been documented,[110,113,114,234] which could be attributed to the long periods of mycosis latency between the moment of infection and the appearance of overt clinical manifestations.[183] Consequently, the patient's memory of the activity contributing to the infection or of the accompanying individuals usually has been forgotten, which prompted Borelli[35] to coin the name "reservarea" for those places in the endemic areas where the fungus has its habitat and humans acquire the infection. This concept differs from that of an endemic area, which implicates the place where the disease is diagnosed.

P. brasiliensis infection in animals as proved by culture or histologic examination has been shown consistently in armadillos, mammals that probably acquire the infection when they disturb the terrestrial habitat of the fungus,* and more recently in a dog.[66,189,190] Unfortunately, the microniche of *P. brasiliensis* has not been identified properly; soil has been incriminated, but the number of isolates from this source is small.[211,234] The inhibitory effect of pesticides on *P. brasiliensis* may suggest that they can interfere with attempts to isolate *P. brasiliensis* from soil.[161,221] The role played by deforestation in increasing both exposure and incidence rates has been documented in Brazil in Amerindians and in children.[54,84,192] Some studies are related to presence of the fungus in environmental aerosol samples[3] and climate change.[12,13]

Species-specific inner primers derived from rDNA regions (internal transcribed spacer [ITS], 5.8S gene) have been described, and the nested polymerase chain reaction (PCR) sensitivity was higher for the detection of *P. brasiliensis* in soil than culture and animal inoculation. The fungus was detected in soil artificially seeded (positive soil control) and from environmental samples collected in an armadillo burrow.[221] This approach probably would improve the fungal microniche studies. The ecologic factors that prevail in the reservarea also have been characterized, with the following being significantly associated with the disease: altitude from 1000 to 1499 meters above sea level, annual rainfall from 2000 to 2999 mm, and presence of humid forests and of coffee and tobacco crops.[43] Environmental correlates (soil texture and precipitation) indicate that moisture availability plays an important role in paracoccidioidomycosis distribution.[212] The fertile regions of endemic areas have the highest incidence rates of paracoccidioidomycosis.[43,224]

*References 6, 7, 41, 152, 182, 192, 211, 219.

Paracoccidioidomycosis was thought, and still is by some, to be acquired by trauma. However, clinical and experimental data indicate that infection is acquired most commonly by inhalation.[37,96,112,178,185]

EPIDEMIOLOGY

Age

More than 70 percent of the patients are 30 years of age or older. The number of childhood cases was shown to vary from 2.1 to 3.4 percent in the first decade of life.* In 1976, Castro and del Negro[51] published one of the largest series of children with paracoccidioidomycosis diagnosed in a 30-year period. They found that among 1899 patients with this disease seen at the Hospital das Clinicas in São Paulo, Brazil, only 70 (3.6%) of the patients were 14 years of age or younger. However, in 1999 and in the same state, Blotta and colleagues[32] found that 5.6 percent of the paracoccidioidomycosis patients diagnosed between 1988 and 1996 were younger than 14 years, thus showing an increase from the 1976 figure. In 1998, Rios-Gonçalves and associates[192] analyzed the records of 36 children who had been diagnosed in the state of Rio de Janeiro during a period of 25 years. In a similar period, Fonseca's group[84] described 13 children, 12.7 percent of all cases, who had been diagnosed in the Amazonian states of Pará and Tocantins, forest areas that have been colonized since the 1970s and where paracoccidioidomycosis formerly had been regarded as rare. In certain places, the infection rates measured by a positive skin test reaction also were high. In 1976, Pedrosa[167] conducted a skin testing survey among rural children (6 to 11 years of age) in a particular county of Rio de Janeiro where paracoccidioidomycosis had been diagnosed previously in a 3-year-old boy. He found that 34 percent of the children tested positive for paracoccidioidin, a figure indicative of early exposure to *P. brasiliensis*.

In 1996, Londero and colleagues[114] compiled the records of 106 children with the mycosis, including those formerly analyzed by Castro and del Negro.[51] This series, some compilations,[24,156,168,169] several individual reports, and a thesis[11] have revealed that the disease occurs more frequently in individuals 12 to 15 years of age than in younger children (Table 201-1). More than 250 reports of paracoccidioidomycosis in children aged 3 to 14 years, including those analyzed by Londero and colleagues,[114] have been found in a literature search. Bellissimo-Rodrigues and colleagues[16] described, among 1219 patients studied, 196 patients (16%) younger than 21 years and 42 patients (3.4%) 3 to 10 years of age. Other studies appointed values of 15.4 to 20 percent in the midwest or southeast regions of Brazil.[32,164] The prevalence of infection with *P. brasiliensis* was evaluated in a cross-sectional study of 298 asymptomatic schoolchildren in the Brazilian Amazon region. The reactivity of children to two different *P. brasiliensis* antigen preparations, paracoccidioidin and a purified 43-kDa glycoprotein (gp43), was 4.6 percent. However, in the group of

*References 11, 16, 48, 51, 110, 114, 234.

TABLE 201-1 **Age and Gender Distribution of 354 Children with Paracoccidioidomycosis**

Gender*	Age		Total by Gender (%)
	3-9 yr	10-14 yr	
Male	95	125	220
Female	63	71	134
Total by age	158 (41.8%)	196 (58.2%)	354 (100%)

*Male-to-female ratio = 1.6:1.
Data from references 32, 53, 55, 84, 143, 157, 167, 168, 193, 194.

individuals receiving paracoccidioidin who had a positive histoplasmin skin test result, the prevalence of exposure to *P. brasiliensis* was 44 percent, suggesting that the use of the paracoccidioidin preparation may result in cross reactions with other environmental fungi.[105]

The relative paucity of children with this mycosis in comparison with the figures reported in adults* may be explained, in part, by the effective control exerted by most hosts on the progression of the primary infection, by the long incubation period characteristic of the mycosis, and by the establishment of latent foci that may become apparent clinically many years after initial exposure.† Benard and associates[23] considered that the association with different age groups is related to the epidemiology of paracoccidioidomycosis because in the endemic areas, as previously shown, children become exposed to the fungus at an early age. In healthy children, appropriate defenses usually curtail development of the mycosis; but in infants of low socioeconomic conditions, the transition from infection to overt disease may be accelerated and aggravated by the mycosis itself, by malnutrition, or by other associated conditions.‡ In addition, unknown susceptibility factors, the intensity of exposure to the infectious source, the virulence of certain *P. brasiliensis* isolates, and other factors may well contribute to the development of the disease in children.§

Gender

Adult men are afflicted with much greater frequency than are women.¶ In children, however, the disease affects boys and girls in approximately equal proportions.** In the review published by Londero and associates,[114] which included children up to 14 years of age, a slight predominance of male patients was noted in the older group (see Table 201-1). These findings suggest that sex hormones, hormone-dependent immunologic factors, or both play a role in determining the outcome of the host-parasite interaction.[4,5,19,37] Researchers have shown in vitro that estrogens inhibit *P. brasiliensis* mycelium to yeast transition, thereby hindering the progression of the infection.[4,37,202] Analysis of the mortality profile from paracoccidioidomycosis according to gender and age showed similar death totals for both genders in the groups younger than 20 years. The majority of deaths among male patients (97.14%) occurred in individuals older than 15 years, with only 2.37 percent among individuals younger than 15 years. Among female patients, most deaths from paracoccidioidomycosis (89.21%) also occurred in those older than 15 years, but 9.75 percent did occur in those younger than 15 years; that rate is nearly five times the percentage observed in males. These findings corroborate the literature, according to which the infection and disease occur equally between the genders during childhood, with a slight predominance of males with the disease, compared with an absolute predominance of males among adults with the chronic form of the mycosis.[59]

Occupation and Race

Almost half of the reported cases in adults occur in individuals whose occupations require extensive exposure to the soil. Adult residents of areas in which paracoccidioidomycosis is endemic generally develop disease less severe than that seen in immigrants or in people who migrate to highly endemic settings. These people often develop disseminated infection, much like the juvenile cases do. The proportion of acute-subacute cases observed in the study of Bellissimo-Rodrigues[16] (26.4%) might be explained by the higher proportion of children, women, and Afro-descendants. The influence of genetic traits on the mycosis has not been elucidated,[72] but in patients with the unifocal chronic form of the disease, a mild clinical presentation in which lesions are restricted or localized, the HLA allele most commonly seen was DRB1*11, suggesting that the participation of HLA antigens may influence the outcome of the host-parasite interaction in paracoccidioidomycosis.[196]

Geographic Distribution

Paracoccidioidomycosis is restricted to Latin America, from Mexico to Argentina; some countries within this area (Chile and some of the Caribbean islands), however, are free of the disease. In endemic countries, the disease distribution is uneven; most cases occur in individuals living in regions corresponding to the humid tropical and subtropical forests. The endemic area is centered in Brazil; 7000 of the about 10,000 patients described to date were natives of this country.* New endemic areas have been reported in the Amazon region, where the mycosis was considered to be rare. In Brazil, this fungal infection is considered to be an important cause of mortality among infectious diseases, with a rate of 1.45 cases per million inhabitants.[30,119,129] Paracoccidioidomycosis represented the most important cause of deaths among systemic mycoses[59,174] (approximately 51.2%).

*References 32, 51, 110, 112, 113, 124, 207, 228, 229.
†References 19, 37, 87, 90, 112, 183.
‡References 23, 24, 32, 51, 84, 90, 192.
§References 19, 55, 84, 85, 114, 125, 234.
¶References 32, 37, 84, 110, 112, 155, 234.
**References 32, 43, 51, 114, 157, 192, 193, 234.

*References 32, 88, 110, 112, 113, 119, 125, 155, 234.

Although paracoccidioidomycosis has been reported in patients not living in endemic areas, prior residence in Latin America has been documented in every case.* In some of these patients, the interval between residence in the endemic area and clinical manifestations of the disease has been 10 to 40 years.[39,110,184] Within endemic areas, 2.0 (children) to 82 percent react to the intradermal administration of paracoccidioidin, suggesting previous contact or subclinical infection with *P. brasiliensis*.†

P. brasiliensis is a complex species,[130] and phylogenetic studies have revealed that this species actually contains at least four cryptic phylogenetic species: two paraphyletic species, S1 (species 1) and PS2 (phylogenetic species 2); and two monophyletic species, PS3 (phylogenetic species 3) and the Pb01-like species for which the name *P. lutzii* sp. nov. has been proposed.[130,218] The cryptic species were present in different regions: S1, in Brazil, Argentina, Paraguay, Peru, and Venezuela; PS2, in Brazil and Venezuela; PS3, restricted to Colombia; and Pb01-like group, another species, *P. lutzii*, in the central region of Brazil.[218] Despite the apparently restricted ecologic niche, the habitat of the etiologic agent has not been determined precisely.[12,83,186]

PATHOGENESIS AND PATHOLOGY

The initial stages of the host-parasite interaction are unknown because of our inability to detect the precise moment when infection does occur.[8] In the past, traumatic implantation of the fungal propagules into skin and mucosa was thought to cause primary lesions, whereas other manifestations were regarded as secondary. However, through the study of many cases, including autopsies, what has become apparent is that pulmonary lesions are primary, with the infection taking place by inhalation of fungal propagules.[37,90] In experimental animals, the inhalatory route has been shown to give rise to disseminated disease.[133] In most cases, the initial pulmonary infection does not cause undue symptoms.[87,113] Once the conidia reach the terminal bronchi or the alveolar spaces, yeast transformation ensues.[133] In some cases, the fungus promptly disseminates by the lymphohematogenous route, producing distant subclinical and quiescent foci. Apparently, the local defenses are capable of controlling fungal spread, but some viable yeast cells may remain dormant in such foci in the pulmonary and mediastinal lymph nodes. Rarely, the initial pulmonary infection outweighs the host's immune defenses, causing an acute-subacute disease, the juvenile type, with predominant involvement of the reticuloendothelial system.[88] The disease in children falls into this category. Often, the quiescent foci remain so throughout life, as demonstrated by the high number of subclinical infections compared with the low disease incidence of this mycosis in the endemic regions.[86,234] Nevertheless, the most common clinical presentation of the disease is the chronic form, or adult-type disease, believed to result from fungal reactivation in these foci.[87] This reactivation frequently occurs when the patient has left the endemic area, as demonstrated by the cases reported outside Latin America, in countries where paracoccidioidomycosis is considered an imported disease.[52] Of note, these cases may be manifested decades after leaving the endemic area, leading to difficult and delayed diagnosis.[101]

Thus, in the acute or subacute form, the initial lung infection may pass unnoticed but is followed by prompt dissemination to other organs and tissues.[21] In the chronic form, lung disease frequently occurs. Disease is restricted to the lungs in some of these patients, but in most, other organs also are afflicted. This may be caused by lymphohematogenous dissemination from reactivated pulmonary foci or directly from foci at virtually any organ. Actually, paracoccidioidomycosis, in the acute, subacute, or chronic form, is more frequently a disseminated disease, even though clinical manifestations appear to be restricted to a sole organ.[88,135] However, determination of the degree of dissemination certainly is influenced by the availability of diagnostic procedures. After appropriate therapy, residual lesions, mostly fibrotic, become established.[87,135]

As already mentioned, the disease in children is acute or subacute and progressive (the juvenile form).[24,51,114] The time that elapses between infection and the onset of symptoms is not known precisely but has been estimated to be short in most cases, such as a few months.[87,88] In such patients, paracoccidioidomycosis is a severe, systemic disorder that involves preferentially the reticuloendothelial system to such an extent that it is the hallmark of the process.[11,24,114]

In adults, pulmonary lesions as seen on chest plain films may be defined as micronodular or miliary, nodular, infiltrative or interstitial, cavitary, fibrotic, and mixed types.[135,229] Emphysematous areas, pleural thickening, and enlarged hilar and mediastinal lymph nodes also can be observed. These alterations can be better visualized by computed tomography imaging.[89,213] Lung involvement probably is secondary to a chronic lymphangitic process provoked by the fungus itself and the host's response represented by formation of granulomas and fibrosis, the latter of which predominates at the perihilar region. This aspect correlates with the butterfly-like (perihilar) micronodular and interstitial infiltration observed on plain films.[228] Obstruction and reversal of lymphatic flow would lead to the spread of the inflammatory process throughout the lungs.[228]

In children and adolescents, the pulmonary component tends to pass unnoticed, and neither clinical examination nor radiologic studies reflect the real damage.[90] Actually, only a minor proportion of the cases reported have had lung symptoms[114] (Fig. 201-1). However, lack of notification may be more apparent than reality because the weight of the extrapulmonary lesions tends to minimize the less intense respiratory manifestations. Frequently, the mycosis is misdiagnosed, especially for tuberculosis and certain lymphomatous disorders.[114] Nonetheless, a careful search of pulmonary samples, including induced sputum, may reveal the characteristic multiple-budding *P. brasiliensis* yeast cells.[114,188] Moreover, gallium scanning and computed tomography have allowed

*References 39, 41, 101, 106, 116, 184, 212.
†References 37, 41, 54, 56, 105, 114.

FIGURE 201-1 ■ An 11-year-old boy with a 5-month history of fever and cervical and axillary lymph node enlargement, with fistulization.

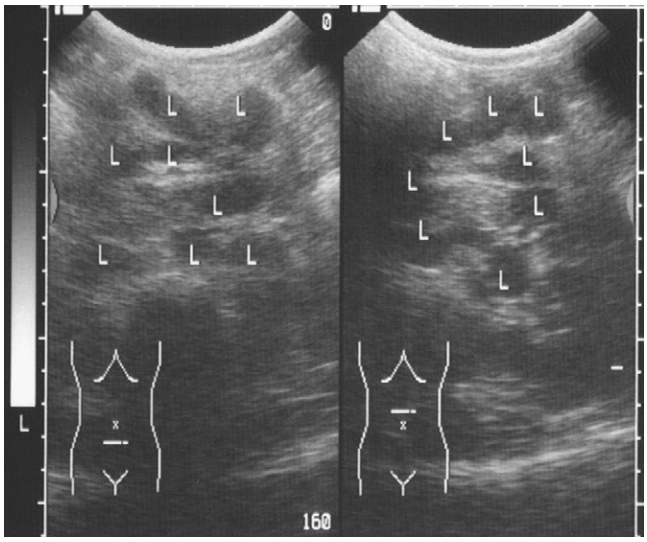

FIGURE 201-2 ■ Extensive mesenteric lymph node (L) enlargement in a 10-year-old boy as shown by ultrasonography imaging.

detection of incipient or discrete interstitial pulmonary lesions not revealed by plain radiography.[89,236]

Lesions in the oropharyngeal and laryngeal mucosa occur frequently in the chronic form. They may be infiltrative, ulcerated, nodular, or vegetative and usually have a granulomatous aspect and may mimic squamous cell carcinoma.[28,135,142,200] The base of the ulcerated lesions usually is covered by small abscesses (the mulberry-like lesions) that probably represent fungal dissemination through the lymphatic system because they usually are accompanied by regional lymph node involvement.[50]

In children, such mucosal lesions are rather exceptional, but skin involvement occurs more commonly and tends to be multiple, in contrast with the adult chronic form,[135] in which lesions are represented mostly by contiguous involvement of the periorificial mucosal lesions or draining lymph nodes. In children or young adults, and eventually in adults with the chronic severe disseminated disease, they represent hematogenous spread of the fungus. In this case, the lesions may appear as ulcerated or ulcerovegetative lesions, papules, or crust-covered ulcers and warts, usually at the same stage of development. Skin involvement may rarely be manifested as sarcoid-like lesions.[153] Reports of septic shock caused by septicemia by *P. brasiliensis* show that the fungus can be blood-borne.[135,227]

The reticuloendothelial system is the target organ in both children and young adults.[11] Almost every child with paracoccidioidomycosis exhibits involvement of the superficial or deep lymph node chains. Lymph nodes vary in size, number, consistency, and location; with time, they may liquefy, forming abscesses or fistulas (Fig. 201-2). The spleen and liver frequently are involved in these same groups. Abdominal lymph node involvement also is a common occurrence in children.[11,114] Hypertrophied lymph nodes, usually generalized but particularly periaortic, around the hepatic hilum and retroperitoneally, can be detected by ultrasonography, computed tomography, or magnetic resonance imaging.[126] Coalescent masses may become palpable and may result in disease caused by extrinsic compression of adjacent structures, such as jaundice by compression of the biliary duct,[33] pancreatitis,[33] or intestinal obstruction.[127]

Splenic lesions are nodular or miliary. Gross hepatic lesions may not be apparent, but histopathologic examination regularly reveals fungal invasion of this organ.[11,24,114] In a series of fatal cases of paracoccidioidomycosis, 56.7 percent revealed the presence of yeast cells in the liver, associated with a granulomatous tissue response. Thirty percent of the cases had only widening of the portal tracts by fibrosis (31.6%), whereas 11.6 percent had an essentially normal liver.[217]

The intestinal mucosa may be affected, but similar to the lungs, its involvement is secondary to blockade of the regional lymphatic flow, with retrograde progression of *P. brasiliensis* to the mucosa, a process resulting in mycotic enteritis.[33,85,127] In this case, the submucosal inflammatory process is granulomatous; fungal cells are visualized; and the intestinal changes may vary from dilated loops, edema, congestion, and nodule formation to multiple mucosal ulcers.[85,127]

Studies also have shown bone marrow infiltration mainly but not exclusively in the acute-subacute form of the disease; 25.7 percent of the patients examined during active disease and 36.4 percent of those who died of paracoccidioidomycosis revealed this type of involvement.[180] Bone marrow necrosis has also been reported in the juvenile form.[181] The histology pattern was variable, but appropriate staining always displayed fungal cells. Bone marrow invasion frequently is associated with marked eosinophilia.[180,205] In addition, bone (Fig. 201-3) and joint lesions are frequent occurrences in the acute-subacute form of the disease[2,24,75,114,227] and appear closely related to bone marrow infiltration.[180]

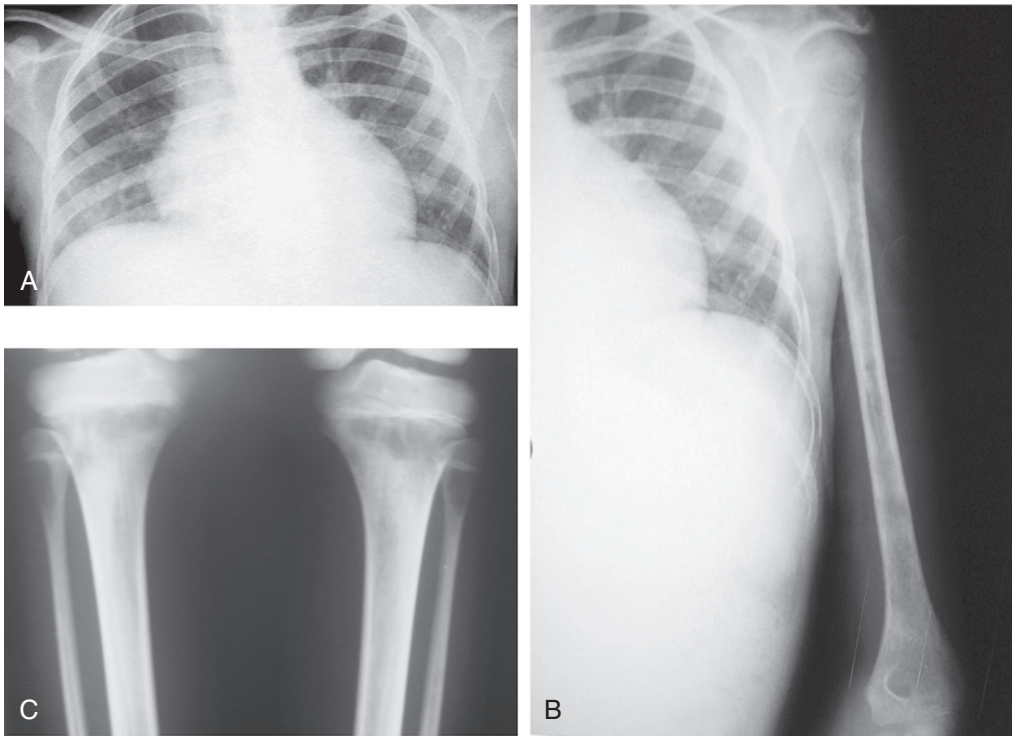

FIGURE 201-3 ■ Severe disseminated subacute paracoccidioidomycosis in a 9-year-old boy. The chest radiograph (**A**) shows mediastinal lymph node enlargement and mild bilateral infiltrates in the middle and lower fields. Osteolytic lesions of long bones: humerus (**B**) and tibia and fibula bilaterally (**C**).

The adrenals often are involved in patients with the chronic form of the mycoses, with a small proportion of the patients evolving to adrenal hypofunction or insufficiency (Addison disease). The glands contain multiple granulomatous foci, and diffuse necrosis may be seen in the most severe cases. Hyperplasia of the adrenal glands also occurs commonly.[135]

On histologic evaluation, formation of granulomas is the rule, except in patients with severe disseminated disease, such as those with the acute-subacute form.[88] The granulomatous inflammation is associated with a mixed pyogenic component, especially in the case of ulcerated skin lesions or ruptured lymph nodes. Caseation and central necrosis may be present. In compact granulomas, abundant epithelial cells, Langerhans or foreign body giant cells, plasmacytes, and lymphocytes are seen; often, phagocytosis of the yeast cells can be observed. $CD4^+$ lymphocytes dominate over $CD8^+$ lymphocytes and appear as peripheral mantles around aggregates of macrophages and histiocytes.[149] In the juvenile disseminated disease, the inflammatory reaction is diffuse, with abundance of both mononuclear and yeast cells but sparse formation of compact granulomas.[23,51,187] Loose granulomas appear unable to circumscribe fungal antigens, and at their periphery, *P. brasiliensis* antigens may permeate throughout the intercellular space.[199] Skin and mucous membrane lesions usually exhibit pseudoepitheliomatous hyperplasia and intraepithelial microabscesses.[163,166,199]

An interesting aspect drawn from the previously described histologic studies is the frequent description of areas of extremely active disease characterized by pyogenic reaction and loose granulomas, rich in budding fungal cells, intermingled with areas with compact granulomas, rare fungal cells, and variable degrees of fibrosis. This mixed aspect can be observed in lymph nodes, skin, or pulmonary lesions, suggesting that the disease evolves through localized new bouts of fungal multiplication and tissue invasion, whereas the adjacent older lesions are on their way to fibrotic resolution. Computed tomographic imaging of the lung confirmed this aspect by depicting areas with alveolar condensation along with fibrotic and emphysematous zones and the simultaneous presence of features of more recent and chronic lesions.[89,213]

Tissue reactions are nonspecific; thus, diagnosis depends on finding *P. brasiliensis*. If the parasite is abundant, it may be identified by hematoxylin and eosin stains. Special fungal stains (e.g., Grocott silver methenamine), however, always should be employed, especially when granulomas are examined. The typical multiple budding yeast cells must be found to establish a diagnosis. The finding of fungal cells of different sizes (2 to 40 μm) suggests the presence of *P. brasiliensis*. In some cases, short chains and cells with single buds also are observed, and in these patients, differentiation of *P. brasiliensis* from *Cryptococcus neoformans*, *Blastomyces dermatitidis*, and even *Histoplasma capsulatum* and *Pneumocystis jiroveci* must be made.[113,208] When the disease is chronic, most of the fungal cells are found inside the macrophages, but free yeast cells predominate in disseminated cases. Internalized yeast cells exhibit altered morphology.[183]

CLINICAL MANIFESTATIONS

Paracoccidioidomycosis is a polymorphic disorder that at a particular time may involve more than one organ system. Thus, making a topographic classification is unrealistic, and the classification of the mycosis currently accepted takes into consideration not only the organs involved but also the host's immune condition and the disease's natural history.[87] The infection is categorized as a *subclinical form*, and the overt process is subdivided into *acute-subacute* and *chronic disease*. The acute-subacute pattern predominates in children and young adults, although the disease is also called juvenile form, whereas the chronic disease affects adults and aged people and is thus called the adult form. According to the severity of the process, juvenile patients are assigned to two subgroups: severe and moderate. The adult form may be localized to pulmonary lesions, unifocal disease, or disseminated from its primary foci, the multifocal process. Disseminated disease is characterized by involvement of the skin, mucosa, reticuloendothelial system, adrenal glands, and, less frequently, the gastrointestinal or genitourinary tract, bones, and central nervous system.* The chronic form can be mild, moderate, or severe.[87]

Patients with acute or subacute disease develop signs and symptoms of a wasting process. Fever, malaise, listlessness, weight loss, and emaciation are recorded frequently. The severity of these symptoms is proportional to the degree of the organ involvement. Londero and associates[114] gathered the information published up to 1994 and found 269 cases in children younger than 14 years; however, sufficient clinical data for analysis of the prominent organ involvement were provided for only 77 children. The clinical characteristics exhibited by these children and by other patients described more recently, including the series of patients at two centers, are shown in Table 201-2. These findings support the notion that juvenile paracoccidioidomycosis is a disease of the reticuloendothelial system resulting in damage of the corresponding organs caused by severe macrophage dysfunction, as suggested previously.[88] Superficial lymph node enlargement was the predominant sign (81.6%) in these cases. Cervical and submandibular lymph node chains were involved most commonly, followed by those of the supraclavicular and axillary regions; however, any chain can be affected (Fig. 201-1). Lymph nodes may vary in size from slightly enlarged to large, painful, coalescent masses; they may be mobile and of elastic consistency or fixed to the adjacent tissues. Hypertrophied nodes may progress to fistulization and discharge purulent material rich in *P. brasiliensis* yeast cells.

The next most important finding was hepatomegaly or splenomegaly (60.7%), which usually was asymptomatic. Jaundice is an infrequent occurrence and more closely relates to extrinsic compression of the biliary tree.[33] Liver enzymes, especially alkaline phosphatase, were abnormal in some cases but not markedly increased.[33,156] Portal hypertension also was a rare occurrence. Findings and complaints relating to the abdomen and digestive tract, such as presence of abdominal masses, lymph node enlargement, diarrhea, vomiting, abdominal distention or pain, and ascites, also have been recorded in a sizable proportion of patients (see Table 201-2 and Fig. 201-2). Signs and symptoms of an acute abdomen, caused by masses formed by hypertrophied lymph nodes or perforation, also have been reported; this problem may lead to intestinal occlusion, blockage of lymphatic drainage, and, later, ascites.[11,24,114,157,169]

In a series comprising predominantly patients with the juvenile form of the disease, abdominal radiographic studies (double-contrast barium) revealed ileal and jejunal alterations in 42 to 51 percent.[85,127] The main findings were distortion and coarsening of the mucosal pattern and loop dilation. However, part of these alterations was probably nonspecific because jejunal biopsies performed in a small number of patients revealed neither granulomatous response nor *P. brasiliensis* yeast cells.[127] On the other hand, histopathologic examination of autopsies (probably the more severe cases) showed a specific granulomatous enteritis in 80 percent.[85] In addition, in a magnetic resonance imaging study of patients, mostly with the juvenile form, 48 percent had abdominal lymph node enlargement, even if no abdominal signs and symptoms had been recorded.[28] This lymph node involvement can cause mesenteric lymphatic stasis and enteric mucosal edema that may progress to fungal enteritis accompanied by abnormal intestinal function, such as reduced absorption of fat.[128,157,169] Computed tomography and magnetic resonance imaging have proven helpful in determining the extent and nature of abdominal involvement.[126]

TABLE 201-2 **Clinical Findings on Admission Diagnosis of Children with Paracoccidioidomycosis**

Clinical Findings	No. of Children (%)
Lymph node enlargement	
Superficial	81.6
Thoracic	25
Abdominal	36.6
Abdominal masses	19.9
Hepatomegaly or splenomegaly	60.7
Ascites	19.1
Jaundice	10.5
Diarrhea, vomiting, abdominal pain, or distention	19.1
Joint or bone lesions	37.8
Skin lesions	29.1
Respiratory symptoms	9.1
Pulmonary consolidations or infiltrates	10.2
Pleural effusion	5.1
Oral and upper respiratory tract mucosa lesions	9.2

Total number of children investigated for each clinical finding varied from 98 to 199.
Data from references 2, 16, 55, 75, 84, 116, 143, 156, 168, 184.

*References 11, 31, 90, 135, 143, 187.

Abdominal complications continue to be occasionally reported in more recent series.[157,169]

Bone damage and articular problems also are important components (37.8%) of disseminated disease, especially in younger children. In these patients, the long bones were frequently affected, with the lytic lesions located at the diaphyseal or metaphyseal-epiphyseal regions,[2] probably because of their higher vascularization, emphasizing the hematogenous dissemination that typically occurs in this form of the disease (Fig. 201-3). Ribs, skull, phalanges, and vertebral lytic lesions also have been documented (Fig. 201-3). Different from the painful, motion-restriction joint lesions, the bone lesions usually were silent.[2,24,77,144] A pathologic fracture occasionally may occur.[144]

Skin lesions were noted in 29.1 percent of the juvenile cases, with a tendency toward higher frequency with increasing age of the patient. Distribution of cutaneous lesions was variable, but face and trunk were involved more frequently.[135] Lung abnormalities were recorded in a smaller proportion of cases. However, even in the absence of clinical and radiologic involvement, colonization of the lung by *P. brasiliensis* can be demonstrated by direct examination and by culture.[188] When chest radiographs were abnormal, enlarged hilar lymph nodes and miliary infiltrates predominated.[90,114,188] In contrast with the adult form of the disease, in which adrenal involvement is a serious concern, it is uncommon in children. A survey of adrenal function before and after treatment in 23 children has shown normal adrenal function.[170]

Anemia, an increased erythrocyte sedimentation rate, severe hypoalbuminemia, and hypergammaglobulinemia with high IgG serum concentrations are found regularly.[11,24,88,156] Nonetheless, anti–*P. brasiliensis* antibodies may prove undetectable in some patients.[68,69,155] Eosinophilia and elevated IgE antibody titers have been detected in most patients.[156,205,237]

The association between human immunodeficiency virus (HIV) infection and paracoccidioidomycosis has been investigated.[19,147] However, the youngest patient described was a 15-year-old boy who had enlargement of superficial and mediastinal lymph nodes, skin nodules, and pulmonary infiltrates.[53] The mycosis was also diagnosed occasionally in other patients whose immune function was depressed by certain conditions or medications.[125] The incidence of this mycosis has not increased in HIV-infected patients as would have been expected.[19] No report of paracoccidioidomycosis and HIV coinfection in children has so far been published. In the chronic, progressive, adult form of paracoccidioidomycosis, signs and symptoms differ substantially from those found in children. Descriptions of the disease in adults are beyond the scope of this text but are referenced.[88,135,200]

DIAGNOSIS

Specific diagnosis depends solely on laboratory confirmation. The "gold standard" for paracoccidioidomycosis diagnosis is the direct observation of characteristic multiple-budding cells in biologic fluids and tissue sections or the isolation of the fungus from clinical specimens. The large cell, with multiple exosporulation, resembling a pilot's wheel, is pathognomonic of the disease. Detection of antibodies against certain fungal antigens or the presence of such antigens in the patient's body fluids constitutes indirect diagnostic criteria. The direct microscopic observation of the characteristic *Paracoccidioides* spp. should be done in a drop of physiologic saline or potassium hydroxide test (Fig. 201-4).[110] Usually, the sources of clinical specimens are sputum, secretion and scraps or debris from ulcerated skin and mucosal lesions, draining material from suppurating lymph nodes or abscesses, bronchoalveolar fluid, and biopsy tissue as well as exfoliative cytology specimens.[47,68] Bronchoalveolar, articular, cerebrospinal, or other fluids must be centrifuged before examination. Draining material from suppurating lymph nodes usually is rich in fungal cells with multiple buds. Sputum is one of the most important specimens used for diagnosis, and various procedures are used to improve the detection of the organism in this specimen. A cell block preparation method for sputum proved more sensitive (93%) than simple direct examination,[215] but few laboratories have employed it routinely. When the first examination of the sputum is negative, it can be homogenized with an equal volume of *N*-acetyl-L-cysteine and then centrifuged again. Sodium or potassium hydroxide can be used, but only in direct examination because this treatment reduces the fungus isolation. The sensitivity of the direct examination (wet mount, smears, and histopathologic examination) varies from 80 to 100 percent or less and depends on the laboratory.[37,110,148] Biopsy samples should be taken only when routine direct examinations have failed, and the specimen should be placed in two separate flasks: one with sterile saline and the other with formalin buffered for mycologic and histopathologic examinations. As the morphologic appearance of the parasitic forms of *P. brasiliensis* is pathognomonic, the diagnosis of the mycosis can be established by biopsy specimen and confirmed by either culture or serology. If the parasite is abundant, it may be identified by hematoxylin and eosin stain and confirmed by a special fungal stain, such as periodic acid–Schiff or Gomori methods.[88,110,199] Pereira and colleagues[169] established

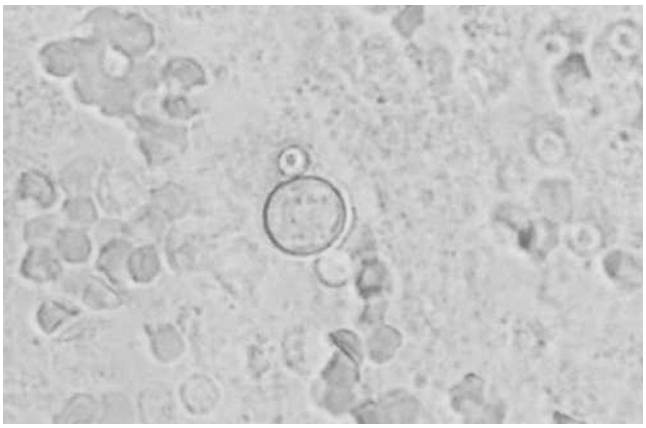

FIGURE 201-4 ■ Direct examination of a potassium hydroxide mount of a patient's sputum specimen showing a budding *Paracoccidioides brasiliensis* yeast form–like cell.

the diagnosis of 63 children with paracoccidioidomycosis by the identification of *P. brasiliensis* in lymph node biopsy (84%), bone biopsy (9%), or skin biopsy (7%) specimens.

When the disease is chronic, most of the fungal cells are found inside the macrophages, but free yeast cells predominate in disseminated cases. Pauciparasitic forms of the *Paracoccidioides* spp. granuloma that show a sarcoidic pattern with rare yeast forms may appear. In these cases, the diagnosis may rely on epidemiologic data plus serologic and molecular diagnostic tools such as PCR on paraffin-embedded tissue.[65,138,139] Visualization of the multiple budding yeast cells, in which the buds are connected to the parent cells by a narrow bridge with a well-defined refringent double wall, is mandatory because the *Paracoccidioides* spp. yeast cells may range from a few microns to 30 to 40 µm. Sometimes, in the lesions, the parasite is present as diminutive yeast cells, measuring about 3 to 5 µm.[110,183,184] *Paracoccidioides* spp. blastoconidia may be confused with *B. dermatitidis*, capsule-deficient *C. neoformans*, endospores and small *Coccidioides immitis* empty spherules, large *H. capsulatum* yeast cells,[37,110] or even *P. jiroveci*.[208] For this reason, the multiple budding structures always should be found for specific diagnosis. In these cases, the molecular tests such as PCR and immunocytochemistry with specific polyclonal or monoclonal anti–*P. brasiliensis* antibody could be used.[45,64,91,93,110]

Both disseminated and pulmonary paracoccidioidomycosis can be confused with tuberculosis, histoplasmosis, leukemia, malignant neoplasms, or Hodgkin disease. When the skin or mucous membranes are affected, paracoccidioidomycosis must be differentiated from histoplasmosis, leishmaniasis, leprosy, syphilis, lupus erythematosus, and a variety of malignant neoplasms. In children, tuberculosis, acute abdominal syndrome, intestinal obstruction, osteomyelitis, and rheumatic fever also are important considerations in the differential diagnosis of this disorder.

Culture samples should be obtained to support the diagnosis and to establish the viability of the fungus. However, they are not always positive because the presence of other more rapidly growing microorganisms in the samples renders isolation difficult. Culture methods, although essential to establish the diagnosis in some cases, have limitations for rapid diagnosis, namely, insensitivity, the need for invasive procedures, and delayed growth. At room temperature, *Paracoccidioides* spp. are slow-growing fungi that can be overgrown readily by bacteria, yeasts (especially *Candida*), and contaminant molds. Isolation should be attempted by the concomitant use of modified Sabouraud agar and yeast extract agar plus antibiotics and cycloheximide. In noncontaminated samples, the use of brain-heart infusion agar plus blood and antibiotics (without cycloheximide), a hemoglobin-containing agar incubated at 37°C, or both also is advisable. Cultures should be observed for 4 to 6 weeks, depending on temperature of incubation, with a definitive classification being accomplished only in the yeast phase.[37,110,155] The isolation rate of the fungus in culture varies from 80 to 100 percent but may be less in other nonspecialized centers.[37,110] As the mycelial form is not characteristic, subcultures in brain-heart infusion plus blood, Fava Netto agar, or peptone–yeast extract–glucose agar, for up to 14 days at 35°C to 37°C (95°F to 98.6°F), allow yeast conversion to occur and definitive diagnosis to be established.[110]

Paracoccidioides complex includes several different cryptic species, as observed by phylogenetic studies using multilocus sequence typing,[130] microsatellite analysis,[131] and PRP8 intein sequencing.[220] Phylogenetic analyses with several nuclear encoding regions showed a significant genetic distance between Pb01 and the remaining genetic groups.[130,131] More recently, the use of at least three single-nucleotide polymorphisms (*GP43*, *ARF*, and PRP8 intein genes), but not morphologic criteria, distinguished among the four cryptic species of the genus *Paracoccidioides*.[222] Morphologic evaluation indicated that elongated conidia were observed exclusively in *P. lutzii* isolates, whereas all other species (S1, PS2, and PS3) were indistinguishable.[222] *P. lutzii* isolates presented longer conidia (length ranged from 2.17 to 22.89 µm, with 11.07 µm being the median value) than S1 and PS2 conidia, whose lengths ranged from 2.25 to 7.99 µm (median value, 5.13 µm). Therefore, conidial morphology appears to be an important morphologic marker for differentiation between *P. lutzii* and the three cryptic species of the *P. brasiliensis* complex (S1, PS2, and PS3).[222]

Several sequences of *P. brasiliensis* DNA are of use in the identification of the fungus,* as is the exoantigen test with specific antiserum, mainly for atypical isolates.[107] The species-specific gp43 gene of *P. brasiliensis* was detected by loop-mediated isothermal amplification (LAMP) in DNA from *P. brasiliensis* strains in 3 hours, suggesting that this method may be applied to rapid identification of this fungus.[79,216] Recently, the identity of the *P. brasiliensis* typical and atypical strains could be confirmed by its dimorphism, by the expression of gp43, and by PCR using specific primers for gp43 as well as ribosomal internal transcribed spacer regions.[34,58,175] However, an important note is that one sequence (483 bp) of the *Lacazia loboi* gp43-like gene had 85 percent identity at the nucleotide level and 75 percent identity with the deduced amino acid sequences of the *P. brasiliensis* gp43 protein.[233]

Several *Paracoccidioides* spp. DNA sequences (Tables 201-3 and 201-4) of potential diagnostic use have been reported,† but few have been applied to clinical samples; further study is needed before they are introduced in diagnosis.‡ *Paracoccidioides* spp. DNA sequences of potential diagnostic use are species-specific sequences of the gene encoding gp43.[27,93,190,216] A PCR assay based on sequence of the gene coding for the gp43 antigen presented the highest sensitivity and specificity and gave a single band of 600 bp in sputum from a patient with chronic paracoccidioidomycosis.[93] Morais and colleagues[146] showed that some sequences of gp43 could undergo substitutions and need further testing to verify their universality and specificity for *P. brasiliensis*. The species-specific gp43 gene of *P. brasiliensis* was detected by LAMP in DNA extracted from a paraffin-embedded

*References 40, 74, 91, 102, 111, 151, 201, 221.
†References 27, 40, 64, 74, 91, 93, 102, 109, 111, 151, 190, 198, 201, 216, 221.
‡References 27, 40, 64, 93, 109, 190, 198, 216.

TABLE 201-3 **Molecular Applications to *Paracoccidioides* spp. Identification**

Primers/ Probes	Primer Sequences	Amplicon	Samples	Limit Detection	Test	Sensitivity (%)	Specificity (%)	Reference
LO/UP p27	5′-CACTCTTGG CTTTGGTTGA AG-3′ 5′-CTGTTGTTT CCGTCCTTG CGC-3′	536 bp	Cultures, soil	3 pg	PCR	40	100	74
ITS1s PbITS3a	5′-CCGCCGGGG ACACCGT TG-3′ 5′-AAGGGTGTC GATCGAG AG-3′	418 bp	Cultures	—	PCR	100	100	102
rRNA ITS1-OL5 OL3–UNI-R	5′-TCC GTA GGT GAA CCT GCG G-3′ 5′-TGT GAC GAA GCC CCA TAC G-3′ 5′-CTC AGC GGG CAC TT 3′ 5′-GGT CCG TGT TTC AAG ACG-3′	203 bp	Cultures	—	PCR	100	100	151
rRNA ITS3-B/Pb	5′GCATCGATGAA GAACGCAGC3′ 5′CACTCATGGAC CCCGG3′	—	Cultures	—	PCR-EIA	100	100	111
549 and 610 gp43 gene	5′-TTCCCAAAA CggCTTCgA-3′ 5′-TgTCACCCTT TTgCCAg TTg-3′	—	Cultures	10 copies	Real-time PCR	100	100	201
ITS4 ITS5 PbITSE PbITSR	5′GAGCTTTGACG TCTGAGACC′3 5′AAGGGTGTCGA TCGAGAGAG′3	387 bp	Soil from armadillo burrows	—	Nested PCR	75	100	220

EIA, enzyme immunoassay; PCR, polymerase chain reaction.

tissue sample of paracoccidioidomycosis, suggesting that this method may achieve clinical application.[79,216] Work employing sensitive nested PCR,[27] the LAMP method of greater sensitivity, has been used[79] and detected the gp43 gene with a combination of F3, B3, FIP, and BIP primers designed from the partial sequence of gp43.[216]

P. brasiliensis strain genotyping was carried out directly from the paraffin-embedded tissue samples by modification of the diagnostic nested PCR assay.[190] Other approaches have been developed, such as MG2(1)F and MG2(1) primers that produced positive identification bands,[198] mainly in sputum samples. Further experience will be necessary to include molecular tests in routine diagnosis. Real-time PCR was developed for the detection of *P. brasiliensis* DNA in both culture and patients' clinical samples, and this procedure proved to be reproducible and specific.[40] Semi-nested PCR, with the primers ITS1 and ITS4 and MJ03 and ITS1, respectively, first and in the second reaction, was used to investigate biopsy specimens of five patients with oral lesions that resembled paracoccidioidomycosis. The test result was positive for four samples and negative for a sample from a patient later diagnosed with leishmaniasis.[109]

Patients with paracoccidioidomycosis not only have a polyclonal B-cell activation but also produce large amounts of anti–*P. brasiliensis* antibodies, which are long-lasting and generally correlate with the severity of the disease.* Assessment of the serologic response has long been used in paracoccidioidomycosis. It has been used as a diagnostic tool mainly because in a significant proportion of the patients, it is difficult to obtain clinical specimens for visualization or cultivation of the fungus when only deep-seated lesions are present. On some occasions, a serologic result is the first indication of the mycotic nature of the patient's illness. In recent years, a great interest has been generated in the development of techniques to improve paracoccidioidomycosis diagnosis. These developments have come in the fields of either antibody or antigen detection. Thus, serologic tests are of value for the diagnosis and may be useful in

*References 8, 22, 29, 115, 150, 237.

TABLE 201-4 **Molecular Applications to Paracoccidioidomycosis Diagnosis**

Primers/ Probes	Primer Sequences	Amplicon	Samples	Limit Detection	Test	Sensitivity (%)	Specificity (%)	Reference
28S ribosomal	5′GCATCGATGAAGAACGCAGC3′ 5′CACTCATGGACCCCGG3′	—	Oral lesions Biopsy specimens	—	In situ hybridization	2-3	100	64
PC2-PC6	5′ ATA GAG GGA GAG CCA TAT GTA CAA GGT 3′ 5′ GGC TCC TCA AAG TCT GCC ATG AGG AAG 3′	600 bp	Sputum	10 cells/mL	PCR	100	100	93
gp43 para I para II para III para IV	5′-AAC TAG AAT ATC TCA CTC CA GTC C-3′ 5′-TGT AGA CGT TCT TGT ATG TCT TGG G-3′ 5′-GAT CGC CAT CCA TAC TCT CGC AAT C-3′ 5′-GGG CAG AGA AGC ATC CGA AAT TGC G-3′	196 bp	Lung homogenates	0.5 fg	Nested PCR	91	100	27
MG2(1)F MG2(1)R	5′GGGATTCCCTAGGCAAACACTTGTG TGA3′ 5′GTGCAGTTATCCACAAGCCATATATT C3′	286 bp	Sputa and CSF sample	—	PCR	75	100	198
F3 B3 FIP BIP gp43	5′TCA CGT CGC ATC TCA CAT TG′3 5′AAG CGC CTTGTC CAA ATA GTC GA′3 5′TGG CTC CAG CAA TAG CCAv CCC GTC AAG CAG GAT CAG CAA T′3 5′CAT GTC AGG ATC CCG ATC GGG CCT TGT ACA TAT GGC TCT CCC T′3	—	Sputum	100 fg	LAMP	58-85	100	216
gp43 para I para II para III para IV	5′-AAC TAG AAT ATC TCA CTC CA GTC C-3′ 5′-TGT AGA CGT TCT TGT ATG TCT TGG G-3′ 5′-GAT CGC CAT CCA TAC TCT CGC AAT C-3′ 5′-GGG CAG AGA AGC ATC CGA AAT TGC G-3′	355 bp	Human paraffin-embedded samples	—	Nested PCR/ genotype	6 of 10 samples	—	190
ITS1 OliPbMB1 OliPbMB2	5′ACCCTTGTCTATTCTACC3′ 5′TTACTGATTATGATAGGTCT3′	144 bp	Culture samples Human samples	10 and 1 fg/μl	Real-time PCR	Culture samples: 100 Human samples: 45	100	40
ITS1 MJ03	5′TCCGTAGGTGAACCTGCGG3′ 5′GTCTCAGACGTCAAAGCTCC3′	212 bp	Human biopsy specimens	0.25 pg/10 cells	Nested PCR	80	100	109

CSF, cerebrospinal fluid; LAMP, loop-mediated isothermal amplification; PCR, polymerase chain reaction.

monitoring the evolution of the disease and its response to treatment. Antibodies are encountered in patients with clinical symptoms, and generally the titers of antibodies can be related to the severity of the disease. Patients with paracoccidioidomycosis have a polyclonal activation of the humoral system with high serum concentrations of specific IgA, IgG, and IgE isotypes.* However, antibodies usually are impaired or absent in some patients with severe juvenile forms and in immunocompromised individuals.[69,232] On occasion, titer fluctuations may produce values below the level of detection by current methods, which may cause false-negative results. Experience over the years has resulted in various highly sensitive and widely employed serologic tests; the frequency of positive test results in patients with paracoccidioidomycosis varies from 70 to 95 percent and may even reach 100 percent in particular cases, depending on the type of test used and the stage of the disease. Of note is that discrepancies between serologic tests performed in different laboratories represent a problem because of the variation in reagents used, laboratory procedures adopted, and varying criteria for evaluation of the results obtained. Some of the most critical reasons for discrepancies are variations in the type of antigenic preparations, different strains, and, indeed, the mycelial or yeast cell phase chosen. Culture filtrates, cytoplasmic extracts, and antigens derived from the cell wall have been used as reagents in serologic tests for paracoccidioidomycosis, although culture filtrates are used in most laboratories.†

Remarkably, exoantigens from two *P. brasiliensis* strains isolated in two different geographic areas presented different results in immunodiffusion tests. When Ag 550B was used in an immunodiffusion test against sera of patients from Mato Grosso and São Paulo, positivity was 92.3 percent and 41.3 percent, respectively. On the other hand, when Ag B-339 was tested with the same sera, positivity was 26.2 percent and 100 percent, respectively. These results suggest that differences in the antigenic composition probably related to phylogenetic peculiarities in *P. brasiliensis* isolates from the central-western region of Brazil should be considered in the diagnosis of paracoccidioidomycosis.[15]

Various fractions that have been cloned, sequenced, and applied to the diagnosis include HSP60,[60] gp43,[175,225] gp70,[67] 27-kDa fractions,[57,162] P2,[42] combinations of the previously described 27-kDa recombinant antigen and the 87-kDa heat shock protein,[75] and rPb27 and anti-rPb40 antibodies, which showed a sensitivity of 96 percent with a specificity of 100 percent in relation to control normal human sera and to sera from patients with other systemic mycosis and 93.5 percent to sera from patients with diverse infections.[82] The main diagnostic antigen of paracoccidioidomycosis is the 43-kDa glycoprotein[175,225] because 100 percent of these patients' sera display antibodies against it, depending on the test.[45,138] This fraction has been extensively and successfully employed as an antigen in establishing the serologic diagnosis of paracoccidioidomycosis.

Many serologic tests have been developed and extensively studied. Agar gel immunodiffusion, complement fixation, and counterimmunoelectrophoresis tests have been employed extensively.[139] Complement fixation tends to be used less often because it is a difficult and time-consuming technique. Also available are indirect immunofluorescence, indirect hemagglutination, enzyme-linked immunosorbent assay (ELISA), and dot-blot immunobinding and immunoblotting.[139,214] Some of the newer tests have employed purified antigens, such as glycoprotein 43, which are well characterized and more specific.[175] Immunodiffusion and counterimmunoelectrophoresis, which employ crude antigens derived from the yeast phase, can detect 90 percent or more of active cases.[65] Most authorities recommend the use of two different tests because none of these tests guarantees more than 90 percent sensitivity when it is employed alone. Immunodiffusion, for instance, is simple and specific. Three precipitin bands, two of which are specific for *P. brasiliensis*, have been identified.[26,37]

A joint effort of several diagnostic centers assessing the value of this test with use of a reference crude antigen (Ag7) demonstrated it to be 84.3 percent sensitive and 98.9 percent specific[231]; but 15 percent represented false-negative results and constituted a failure of this diagnostic test, or they may be related to the production of low-avidity IgG2 antibodies directed against carbohydrate epitopes.[65] Patients with localized, benign disease may have negative results in these tests, but with more sensitive techniques (e.g., immunoblotting or gp43-ELISA), a diagnosis usually is reached. In our laboratory, three tests (immunodiffusion, counterimmunoelectrophoresis, and immunoblotting) are used in a routine serologic diagnosis. The sensitivities of these tests were 85.3 percent, 92.9 percent, and 100.0 percent, respectively, evaluated against 120 paracoccidioidomycosis sera (data not published). Other studies have also described Western blot analysis as a more sensitive test.[78,172] Limitations of these tests include cross reactivity to other mycotic disorders, especially histoplasmosis and *L. loboi* antigens, mainly in more sensitive tests,[175] and difficulties in standardization of the various tests and reagents.[44] The gp43 cross reactivities were predominantly attributed to periodate-sensitive carbohydrate epitopes containing galactosyl.[175]

The value of serologic tests in the follow-up of patients undergoing treatment also has been addressed by several centers.[136] The value of these tests in monitoring patients also is problematic, partly because of the diversity and complexity of the humoral response in patients with paracoccidioidomycosis. Serial serologic evaluations, usually done 3 months apart, together with the clinical, mycologic, and radiologic surveys can guide the treatment schedule, and two negative results in two of the serologic tests routinely employed is an additional criterion that helps in the difficult decision of therapy discontinuation.[139,207] The immunodiffusion, complement fixation, counterimmunoelectrophoresis, and ELISA tests were shown to be able to document the decline in antibody levels that parallels the clinical improvement; serum antibodies are cleared 1 to 2 years after cessation of therapy, depending on the clinical form.[38,70,139] Conversely, in

*References 8, 26, 29, 115, 138, 237.
†References 15, 44, 65, 70, 104, 139.

these circumstances, rising antibody levels indicate a relapse. Nonetheless, a considerable number of patients maintain, sometimes lifelong, the so-called healing titers (i.e., the persistence of low serologic titers in the tests). This is seen more frequently in patients with the acute form or the chronic multifocal form of the disease.[70] The debate on the clinical relevance of this phenomenon—whether it represents the persistence of residual active lesions, thus predicting possible future relapses—is inconclusive. During treatment of paracoccidioidomycosis, an important consideration is to be able to establish cure or remission criteria. A significant fall in reactivity of anti-gp43 antibodies has been shown to correlate with clinical improvement. During relapses, the levels of specific antibodies rose, and some patients considered cured exhibited a residual reactivity. Some patients have a negative immunodiffusion and counterimmunoelectrophoresis test result but a residual reactivity with gp43 and gp70 by immunoblotting.[37,70,75,138]

On the other hand, gp43 and gp70 can be detected in the biologic fluids. Furthermore, several studies have shown that the detection of antigens in blood and urine instead of antibodies is a potent means of establishing a diagnosis, monitoring treatment, and maintaining cure control.* They allow a more precise diagnosis to be established for patients with disseminated childhood and adult multifocal disease, in which antibodies are undetectable because of their coupling to excess antigen or the incapacity to raise antibodies. Antigenemia follow-up studies permit a more precise determination of improvement as antigen load decreases with treatment response. The detection of circulating antigen may represent a more practical approach to the early and rapid diagnosis of the disease.[63] It also will be helpful in establishing the diagnosis of immunocompromised patients, especially those with acquired immunodeficiency syndrome (AIDS), in which searching for an antibody response is not a completely reliable diagnostic tool.

The skin test with paracoccidioidin is not considered a diagnostic test because 30 to 50 percent of the patients prove nonreactive when they are tested initially.[26,29,56,86] Conversely, a positive skin test result indicates previous contact with the fungus but not necessarily active disease. When histoplasmin skin test material is used, cross reactions have been verified.[26,86] When the result of the skin test with paracoccidioidin is negative and the patient is treated, the skin test result may become positive, in which case the patient's prognosis is considered good.[37,110,155,184]

TREATMENT

The treatment of paracoccidioidomycosis is usually divided into two phases: acute and maintenance.[203] Two different drugs can be used for each phase, or the same drug can be used for the two phases but at different dosages. Among the reasons for this two-phase prolonged treatment are the chronic progressive nature of the disease and the knowledge that all currently available drugs, albeit with apparently different efficacies, are only fungistatic. Thus, an adequate humoral and cellular immune response is required to control the mycosis. Cellular immunity, however, may be impaired in a large number of patients because of malnutrition or the disease process itself.[11,24,150] Providing supportive therapy, therefore, is important. Rest, adequate nutrition, correction of anemia, and treatment of other concomitant infections are recommended.[136] Paracoccidioidomycosis is the only fungal disease that can be treated successfully with sulfa drugs. In children, sulfadiazine can be provided orally at daily doses of 60 to 100 mg/kg divided into four to six equal parts; in adults, a maximum daily dose of 6 g can be used. Water intake and urine alkalinization (usually by bicarbonated water intake) should be encouraged during therapy to prevent crystalluria and tubular deposits of sulfadiazine.[136,204] Duration of the acute treatment is dictated by the patient's response after clinical and serologic improvements have been attained; in general, in the chronic form, at least 6 months is required. A slow-acting sulfa drug (sulfamethoxypyridazine or sulfadimethoxine) can be provided as maintenance treatment, at a maximum daily dosage of 1 g. Resistance to sulfonamides has already been documented.[184]

In children, the precise total duration of therapy is variable according to the severity of the disease and as such should also be dictated by the clinical and serologic responses of the patient to treatment. The importance of continuous treatment must be emphasized because relapses occur if the drug is not taken regularly. Sulfonamides can be used by patients with mild or moderately severe disease. They also can be used for patients who have been treated initially with amphotericin B.[136] Brazilian physicians most often employ trimethoprim-sulfamethoxazole (80 mg of trimethoprim and 400 mg of sulfamethoxazole per tablet) given at a dose of two tablets and administered orally at 12-hour intervals with satisfactory results. This drug is also used in association with amphotericin B. Tolerability is good; myelotoxicity (leukopenia) is the main side effect, which can be monitored and controlled by folinic acid administration without modification of the therapeutic regimen. In general, children should be given half the dose given to adults.[11,136] A Brazilian consensus on paracoccidioidomycosis recommends 8 to 10 mg/kg of trimethoprim daily.[207] This combination has the advantage of permitting alternative parenteral administration whenever necessary. Duration of the acute treatment with this drug varies in each case, but it usually lasts for 6 months. Maintenance treatment in these cases can be achieved by using half the dose of the acute treatment or by using a slow-acting sulfa. Development of resistance to trimethoprim-sulfamethoxazole is occasionally clinically suspected but has rarely been documented in vitro. There is a single report of resistance of an isolate from an adult patient with the subacute form.[98]

Amphotericin B is effective but should be reserved for severely disseminated cases.[24,136] It also can be used by patients who relapse during the course of or after treatment with sulfonamide or any orally administered drug because gastrointestinal involvement may impair drug absorption in these cases. The effectiveness of therapy

*References 94, 118, 120, 137, 197, 231.

with azoles has curtailed the need for more aggressive regimens. More than one course of therapy may be required in some patients.

Some investigators have suggested the use of a combined amphotericin B–sulfonamide treatment. In adults, a total cumulative dose of amphotericin B of 1 to 2 g followed by sulfa drugs, given as indicated previously, usually is sufficient.[123] With this drug combination, clinical improvement can be achieved in about 75 percent of patients; about 10 percent do not respond as well, and the remainder die during treatment. Relapses may be expected in about 10 to 15 percent of optimally treated patients.[138] Data about effectiveness of the new amphotericin B lipid formulations in paracoccidioidomycosis are still required.[73]

Ketoconazole is rarely considered the therapy of choice because of its side effects and numerous drug interactions compared with other triazole derivatives for oral administration (itraconazole, fluconazole). Today, more experience has been gained with itraconazole.[136,154,157,169] This triazole is more potent and less toxic than ketoconazole and is equally effective as or even more effective than the parent compound. It is administered in 100-mg capsules that should be given with or shortly after a meal. One or two capsules, depending on the severity of the fungal process, taken daily for 6 months have been shown to be effective in reducing all active lesions. Most (98%) of the patients, including children and young adults, respond.[136,154] Post-therapy observations indicate variable proportions (ranging from 2.1% to 21%) of relapses, depending on the severity of the disease, duration of therapy, and time of follow-up.[122,223] Side effects have been few and include transient elevation of hepatic enzymes.[136,154,223] At present, several well-conducted (but noncomparative) trials have been reported favoring this triazole, which has become the first choice for the treatment of paracoccidioidomycosis in many reference centers. The experience in treating childhood paracoccidioidomycosis with itraconazole still is limited compared with that with amphotericin B and trimethoprim-sulfamethoxazole, but the few reports are encouraging.[157,169] A randomized trial with sulfadiazine, ketoconazole, and itraconazole for the treatment of adult patients with disease of moderate severity failed to show higher efficacy of a particular regimen over the others.[204] However, long-term follow-up was not evaluated in this study. Itraconazole maintenance therapy is recommended at a dose of 5 to 10 mg/kg daily, with a maximum dosage of 200 mg twice daily. A liquid formulation of itraconazole in cyclodextrin was initially suggested as a promising alternative for treatment of children, but it has not been marketed in countries where the disease is endemic, and thus far no clinical experience could be gathered to support its recommendation.

Fluconazole is not as effective in this disorder; higher doses, up to 600 mg/day, and longer treatment periods are required. Recrudescence and relapse of disease occur more frequently than when itraconazole is used.[24,136] Fluconazole may be useful in severely ill patients who must be treated intravenously.[136] No experience in children is available. The literature has only one report of the successful use of terbinafine in an adult patient.[160] Voriconazole is an extended-spectrum triazole antifungal agent that is available in both oral and intravenous formulations. In vitro susceptibility data show that *P. brasiliensis* is highly sensitive to voriconazole. A pilot open label study showed that it is well tolerated and as effective as itraconazole for treatment of chronic paracoccidioidomycosis.[179] However, this drug has not yet been approved for pediatric use.

Many questions remain to be answered about optimal treatment of paracoccidioidomycosis. The role of adjunct therapy with immunomodulators, such as peptides derived from the immunodominant glycoprotein of *P. brasiliensis* that act by augmenting the Th1-mediated immune responses, has been investigated mainly in experimental models of the disease and showed promising results.[226] Two studies demonstrated some reactivity of patients' T cells to these peptides.[103,195] Further studies are required to demonstrate their utility in promoting protective cell-mediated immune responses in the human host. α-Glucan obtained from *Saccharomyces cerevisiae* with immunoenhancing properties has been preliminarily investigated, but further studies are also needed.[134] In the opposite direction, some investigators have administrated short courses of corticosteroids as adjunct therapy in severe cases on the basis of the rationale that the failure to improve with antifungal therapy observed in these cases was due to uncontrolled or excessive inflammatory response.[17,97] Some fatalities were attributed to the exacerbated inflammatory response.[18,25] The clinical and laboratory criteria that allow the transition from the attack to the maintenance treatment phases have not been standardized, nor have the parameters that could be used to end treatment with the assurance that the patient is cured. Progressive decrease in the titers of the serologic tests currently available is one of the laboratory parameters used most frequently. Newer methods (e.g., antigenemia detection and molecular biology approaches) still need further standardization and clinical studies and have not yet become widely available.[94,118,121] Meanwhile, most specialists agree that treatment decisions need to be tailored according to the patient.

PROGNOSIS

Paracoccidioidomycosis has no prophylaxis. Vaccine strategies are being developed but are still restricted to experimental models of the disease.[226] The mycosis can be fatal if it is left untreated, but currently this has seldom been observed.[18,25,171] On the other hand, sequelae still remain a problem. Residual lesions have been observed in a few patients with no known history of active mycotic infection.[87,90,135] Prognosis depends on the status of the patient at the time of diagnosis. Children and young adults in whom fungemia has taken place and who have multiple organ involvement may respond less well to therapy. The fatality rate in young patients at one time was reported to be 31 percent,[51] but it now is much lower as a result of earlier diagnoses and better treatments. Nevertheless, it remains a life-threatening disease with 9.5 to 13 percent fatalities in the most recent series. This was attributed to delayed diagnosis or searching for

medical assistance, irregular treatment, or treatment not monitored. Abdominal complications, especially ascites and malnutrition, were associated with the lethal prognosis.[11,157,169] In the adult group, mortality rates are much lower. Once specific treatment is instituted, lesions regress promptly; skin lesions may heal completely in 2 to 4 weeks.[136] Complete remission is expected for most patients with the acute-subacute and chronic form of the disease. Prognosis has improved as a result of earlier diagnosis and new antifungal drugs that facilitate compliance; better knowledge of the disease has provided better clinical and laboratory follow-up and the notion that the disease needs prolonged surveillance, as shown by the decrease in positive mycologic test results during and after therapy.[136,223] Some investigators consider the term *cure* inappropriate because of the inability to confirm complete eradication of the organism; the term *apparent cure* should instead be used.[136]

Paracoccidioidomycosis persists as a disease with low mortality but high morbidity. Complications vary and, like prognosis, their occurrence depends on the extent of fungal invasion. In the juvenile form, early complications that may lead to surgical intervention are intestinal obstruction and jaundice, both of which result from enlarged mesenteric lymph nodes. Disabsorptive syndromes may be associated, aggravating the nutritional status of the patients.[20,206] Patients also may present with ascites or chylothorax. Lymph nodes may suppurate, and fistulas can develop. A late complication that has been described is abdominal malakoplakia.[194] In the adult form, acute complications of mucosal involvement are dysphagia and dysphonia,[235] edema of the glottis, respiratory insufficiency, and Addison disease, among others. Sequelae are not as common in children as in adults. In general, fibrosis is the cause of serious problems in patients who respond to therapy. Despite the newer, effective therapies, these sequelae preclude, in a proportion of the cases, the complete restoration of the patient's previous health status.[135,136,154,223] In the former group, scarring and fibrosis of the affected nodes and residual pulmonary fibrosis have been noted.[114] Malabsorption syndrome probably is the most serious sequela in the juvenile form because of the enteric loss of proteins and inflammatory cells that results in immunodeficiency and opportunistic infections.[20,206]

Acknowledgments

We would like to deeply acknowledge Prof. Angela Restrepo for her invaluable contribution to this version of the chapter and Drs. Maria Gorete Nogueira and Ricardo Pereira for their comments and sharing of unpublished material. In addition, we would like to thank Irani Coito for the bibliography revision.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Arantes TD, Theodoro RC, Da Graça Macoris SA, Bagagli E. Detection of *Paracoccidioides* spp. in environmental aerosol samples. *Med Mycol* 2013;**51**:83–92.
10. Bailão AM, Nogueira SV, Rondon Caixeta Bonfim SM, et al. Comparative transcriptome analysis of *Paracoccidioides brasiliensis* during in vitro adhesion to type I collagen and fibronectin: identification of potential adhesins. *Res Microbiol* 2012;**163**:182–91.
12. Barrozo LV, Mendes RP, Marques SA. Climate and acute/subacute paracoccidioidomycosis in a hyper-endemic area in Brazil. *Int J Epidemiol* 2009;**38**:1642–9.
13. Barrozo LV, Benard G, Silva ME, et al. First description of a cluster of acute/subacute paracoccidioidomycosis cases and its association with a climatic anomaly. *PLoS Negl Trop Dis* 2010; **4**:e643.
16. Bellissimo-Rodrigues F, Bollela VR, Da Fonseca BA, Martinez R. Endemic paracoccidioidomycosis: relationship between clinical presentation and patients' demographic features. *Med Mycol* 2013;**51**:313–18.
17. Benard G, Campos AF, Netto LC, et al. Treatment of severe forms of paracoccidioidomycosis: is there a role for corticosteroids? *Med Mycol* 2012;**50**:641–8.
18. Benard G, Costa AN, Ravanini J, et al. Fatal acute respiratory distress syndrome in a patient with paracoccidioidomycosis: first case report. *Med Mycol* 2010;**48**:542–5.
25. Benard G, Patzina RL, Schwab JB, et al. Fatal septic shock due to a disseminated chronic form of paracoccidioidomycosis in an aged woman. *Med Mycol* 2012;**50**:407–11.
36. Borges CL, Bailão AM, Báo SN, et al. Genes potentially relevant in the parasitic phase of the fungal pathogen *Paracoccidioides brasiliensis*. *Mycopathologia* 2011;**171**:1–9.
39. Buitrago MJ, Cuenca-Estrella M. Current epidemiology and laboratory diagnosis of endemic mycoses in Spain. *Enferm Infecc Microbiol Clin* 2012;**30**:407–13.
40. Buitrago MJ, Merino P, Puente S, et al. Utility of real-time PCR for the detection of *Paracoccidioides brasiliensis* DNA in the diagnosis of imported paracoccidioidomycosis. *Med Mycol* 2009;**47**: 879–82.
42. Caldini CP, Xander P, Kioshima ÉS, et al. Synthetic peptides mimic gp75 from *Paracoccidioides brasiliensis* in the diagnosis of paracoccidioidomycosis. *Mycopathologia* 2012;**174**:1–10.
48. Carneiro RC, Miranda BG, Camilo Neto C, et al. Juvenile paracoccidioidomycosis in urban area: report of two cases. *Braz J Infect Dis* 2010;**14**:77–80.
49. Caro E, Gonzalez A, Muñoz C, et al. Recognition of laminin by *Paracoccidioides brasiliensis* conidia: a possible mechanism of adherence to human type II alveolar cells. *Med Mycol* 2008;**46**:795–804.
56. Colombo AL, Tobón A, Restrepo A, et al. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol* 2011;**49**:785–98.
65. de Camargo ZP. Serology of paracoccidioidomycosis. *Mycopathologia* 2008;**165**:289–302.
68. de Souza Vianna LM, Pirani Carneiro F, Calvalca Tavares A, et al. Cytological diagnosis of paracoccidioidomycosis: a report of four cases. *Diagn Cytopathol* 2013;**41**:374–6.
71. Desjardins CA, Champion MD, Holder JW, et al. Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. *PLoS Genet* 2011;**7**:e1002345.
82. Fernandes VC, Coitinho JB, Veloso JM, et al. Combined use of *Paracoccidioides brasiliensis* recombinant rPb27 and rPb40 antigens in an enzyme-linked immunosorbent assay for immunodiagnosis of paracoccidioidomycosis. *J Immunol Methods* 2011;**367**: 78–84.
83. Ferreira MS. Paracoccidioidomycosis. *Paediatr Respir Rev* 2009; **10**:161–5.
95. González A, Caro E, Muñoz C, et al. *Paracoccidioides brasiliensis* conidia recognize fibronectin and fibrinogen which subsequently participate in adherence to human type II alveolar cells: involvement of a specific adhesin. *Microb Pathog* 2008;**44**:389–401.
96. Grosskaus DA, Tadano T, Breder SA, Hahn RC. Acute disseminated paracoccidioidomycosis in a 3 year-old child. *Braz J Infect Dis* 2009;**13**:242–4.
97. Gryschek RC, Pereira RM, Kono A, et al. Paradoxical reaction to treatment in 2 patients with severe acute paracoccidioidomycosis: a previously unreported complication and its management with corticosteroids. *Clin Infect Dis* 2010;**50**:e56–8.
99. Hernández O, Garcia AM, Almeida AJ, et al. Gene expression during activation of *Paracoccidioides brasiliensis* conidia. *Yeast* 2011; **28**:771–81.
100. Hernández O, Tamayo D, Torres I, et al. Kinetic analysis of gene expression during mycelium to yeast transition and yeast to mycelium germination in *Paracoccidioides brasiliensis*. *Biomedica* 2011;**31**:570–9.

109. Koishi AC, Vituri DF, Dionízio Filho PS, et al. A semi-nested PCR assay for molecular detection of *Paracoccidioides brasiliensis* in tissue samples. *Rev Soc Bras Med Trop* 2010;**43**:728–30.
117. Marcos CM, Silva JF, Oliveira HC, et al. Surface-expressed enolase contributes to the adhesion of *Paracoccidioides brasiliensis* to host cells. *FEMS Yeast Res* 2012;**12**:557–70.
119. Marques-da-Silva SH, Messias Rodrigues A, Sybren de Hoog G, et al. Occurrence of *Paracoccidioides lutzii* in the Amazon region: description of two cases. *Am J Trop Med Hyg* 2012;**87**:710–14.
129. Matos WB, Santos GM, Silva VE, et al. Paracoccidioidomycosis in the state of Maranhão, Brazil: geographical and clinical aspects. *Rev Soc Bras Med Trop* 2012;**45**:385–9.
132. Maza PK, Straus AH, Toledo MS, et al. Interaction of epithelial cell membrane rafts with *Paracoccidioides brasiliensis* leads to fungal adhesion and Src-family kinase activation. *Microbes Infect* 2008;**10**: 540–7.
140. Mendes-Giannini MJ, Monteiro da Silva JL, Silva JF, et al. Interactions of *Paracoccidioides brasiliensis* with host cells: recent advances. *Mycopathologia* 2008;**165**:237–48.
147. Morejón KM, Machado AA, Martinez R. Paracoccidioidomycosis in patients infected with and not infected with human immunodeficiency virus: a case-control study. *Am J Trop Med Hyg* 2009; **80**:359–66.
148. Moreto TC, Marques ME, de Oliveira ML, et al. Accuracy of routine diagnostic tests used in paracoccidioidomycosis patients at a university hospital. *Trans R Soc Trop Med Hyg* 2011;**105**:473–8.
153. Nascimento CR, Delanina WF, Soares CT. Paracoccidioidomycosis: sarcoid-like form in childhood. *An Bras Dermatol* 2012;**87**: 486–7.
158. Nogueira SV, Fonseca FL, Rodrigues ML, et al. *Paracoccidioides brasiliensis* enolase is a surface protein that binds plasminogen and mediates interaction of yeast forms with host cells. *Infect Immun* 2010;**78**:4040–50.
165. Parente JA, Borges CL, Bailão AM, et al. Comparison of transcription of multiple genes during mycelia transition to yeast cells of *Paracoccidioides brasiliensis* reveals insights to fungal differentiation and pathogenesis. *Mycopathologia* 2008;**165**:259–73.
168. Pereira GH, Santos AQ, Park M, et al. Bone marrow involvement in a patient with paracoccidioidomycosis: a rare presentation of juvenile form. *Mycopathologia* 2010;**170**:259–61.
172. Perenha-Viana MC, Gonzales IA, Brockelt SR, et al. Serological diagnosis of paracoccidioidomycosis through a Western blot technique. *Clin Vaccine Immunol* 2012;**19**:616–9.
173. Peres da Silva R, Matsumoto MT, Braz JD, et al. Differential gene expression analysis of *Paracoccidioides brasiliensis* during keratinocyte infection. *J Med Microbiol* 2011;**60**:269–80.
174. Prado M, Silva MB, Laurenti R, et al. Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: a review from 1996 to 2006. *Mem Inst Oswaldo Cruz* 2009;**104**:513–21.
175. Puccia R, McEwen JG, Cisalpino PS. Diversity in *Paracoccidioides brasiliensis*. The PbGP43 gene as a genetic marker. *Mycopathologia* 2008;**165**:275–87.
176. Puccia R, Vallejo MC, Matsuo AL, Longo LV. The paracoccidioides cell wall: past and present layers toward understanding interaction with the host. *Front Microbiol* 2011;**2**:257.
178. Queiroz-Telles F, Escuissato DL. Pulmonary paracoccidioidomycosis. *Semin Respir Crit Care Med* 2011;**32**:764–74.
181. Resende LS, Mendes RP, Bacchi MM, et al. Bone marrow necrosis related to paracoccidioidomycosis: the first eight cases identified at autopsy. *Histopathology* 2009;**54**:486–9.
185. Restrepo A, Benard G, Castro CC, et al. Pulmonary paracoccidioidomycosis. *Semin Respir Crit Care Med* 2008;**29**:182–97.
190. Ricci G, Zelck U, Mota F, et al. Genotyping of *Paracoccidioides brasiliensis* directly from paraffin embedded tissue. *Med Mycol* 2008;**46**:31–42.
191. Richini-Pereira VB, Bosco SM, Theodoro RC, et al. Molecular approaches for eco-epidemiological studies of *Paracoccidioides brasiliensis*. *Mem Inst Oswaldo Cruz* 2009;**104**:636–43.
202. Shankar J, Wu TD, Clemons KV, et al. Influence of 17β-estradiol on gene expression of *Paracoccidioides* during mycelia-to-yeast transition. *PLoS One* 2011;**6**:e28402.
209. Silva MG, Schrank A, Bailão EF, et al. The homeostasis of iron, copper, and zinc in *Paracoccidioides brasiliensis*, *Cryptococcus neoformans* var. *grubii*, and *Cryptococcus gattii*: a comparative analysis. *Front Microbiol* 2011;**2**:49.
216. Tatibana BT, Sano A, Uno J, et al. Detection of *Paracoccidioides brasiliensis* gp43 gene in sputa by loop-mediated isothermal amplification method. *J Clin Lab Anal* 2009;**23**:139–43.
219. Theodoro RC, Bagagli E, Oliveira C. Phylogenetic analysis of PRP8 intein in *Paracoccidioides brasiliensis* species complex. *Fungal Genet Biol* 2008;**45**:1284–91.
221. Theodoro RC, Teixeira Mde M, Felipe MS, et al. Genus *Paracoccidioides*: species recognition and biogeographic aspects. *PLoS One* 2012;**7**:e37694.
226. Travassos LR, Taborda CP. Paracoccidioidomycosis: advances in treatment incorporating modulators of the immune response. *J Invas Fungal Infect* 2011;**5**:1–6.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 202

Cryptococcosis

David Goldman • John Perfect

Cryptococcus neoformans is an encapsulated yeast that causes meningoencephalitis and pneumonia in both immunocompetent and immunocompromised children. Since its identification as a pathogen in 1894,[103] great strides have been made in our understanding of the biology and the pathogenesis of cryptococcosis. The incidence of cryptococcosis has greatly increased in association with the acquired immunodeficiency syndrome (AIDS) epidemic and improvements in health care, which have resulted in an increased number of individuals at risk

REFERENCES

1. Almeida AJ, Martins M, Carmona JA, et al. New insights into the cell cycle profile of *Paracoccidioides brasiliensis*. *Fungal Genet Biol* 2006;**43**:401–9.
2. Amstalden EM, Xavier R, Kattapuram SV, et al. Paracoccidioidomycosis of bone and joints. *Medicine (Baltimore)* 1996;**75**:212–25.
3. Arantes TD, Theodoro RC, Da Graça Macoris SA, Bagagli E. Detection of *Paracoccidioides* spp. in environmental aerosol samples. *Med Mycol* 2013;**51**:83–92.
4. Aristizábal BH, Clemons KV, Stevens DA, et al. Morphological transition of *Paracoccidioides brasiliensis* conidia to yeast cells: in vivo inhibition in females. *Infect Immun* 1998;**66**:5587–91.
5. Aristizábal BH, Clemons KV, Cock AM, et al. Experimental *Paracoccidioides brasiliensis* infection in mice: influence of the hormonal status of the host on tissue responses. *Med Mycol* 2002; **40**:169–78.
6. Bagagli E, Bosco SM, Theodoro RC, et al. Phylogenetic and evolutionary aspects of *Paracoccidioides brasiliensis* reveal a long coexistence with animal hosts that explain several biological features of the pathogen. *Infect Genet Evol* 2006;**6**:344–51.
7. Bagagli E, Theodoro RC, Bosco SM, McEwen JG. *Paracoccidioides brasiliensis*: phylogenetic and ecological aspects. *Mycopathologia* 2008;**165**:197–207.
8. Baida H, Biselli PJ, Juvenale M, et al. Differential antibody isotype expression to the major *Paracoccidioides brasiliensis* antigen in juvenile and adult form paracoccidioidomycosis. *Microb Infect* 1999;**1**: 273–8.
9. Bailão AM, Schrank A, Borges CL, et al. Differential gene expression by *Paracoccidioides brasiliensis* in host interaction conditions: representational difference analysis identifies candidate genes associated with fungal pathogenesis. *Microbes Infect* 2006;**8**:2686–97.
10. Bailão AM, Nogueira SV, Rondon Caixeta Bonfim SM, et al. Comparative transcriptome analysis of *Paracoccidioides brasiliensis* during in vitro adhesion to type I collagen and fibronectin: identification of potential adhesins. *Res Microbiol* 2012;**163**:182–91.
11. Barbosa GL. Paracoccidioidomicose na criança. *Rev Pat Trop (Brazil)* 1992;**21**:269–383.
12. Barrozo LV, Mendes RP, Marques SA. Climate and acute/subacute paracoccidioidomycosis in a hyper-endemic area in Brazil. *Int J Epidemiol* 2009;**38**:1642–9.
13. Barrozo LV, Benard G, Silva ME, et al. First description of a cluster of acute/subacute paracoccidioidomycosis cases and its association with a climatic anomaly. *PLoS Negl Trop Dis* 2010; **4**:e643.
14. Bastos KP, Bailão AM, Borges CL, et al. The transcriptome analysis of early morphogenesis in *Paracoccidioides brasiliensis* mycelium reveals novel and induced genes potentially associated to the dimorphic process. *BMC Microbiol* 2007;**10**:7:29.
15. Batista J Jr, de Camargo ZP, Fernandes GF, et al. Is the geographical origin of a *Paracoccidioides brasiliensis* isolate important for antigen production for regional diagnosis of paracoccidioidomycosis? *Mycoses* 2010;**53**:176–80.
16. Bellissimo-Rodrigues F, Bollela VR, Da Fonseca BA, Martinez R. Endemic paracoccidioidomycosis: relationship between clinical presentation and patients' demographic features. *Med Mycol* 2013;**51**:313–18.
17. Benard G, Campos AF, Netto LC, et al. Treatment of severe forms of paracoccidioidomycosis: is there a role for corticosteroids? *Med Mycol* 2012;**50**:641–8.
18. Benard G, Costa AN, Ravanini J, et al. Fatal acute respiratory distress syndrome in a patient with paracoccidioidomycosis: first case report. *Med Mycol* 2010;**48**:542–5.
19. Benard G, Duarte AJ. Paracoccidioidomycosis: a model for evaluation of the effects of human immunodeficiency virus infection on the natural history of endemic tropical diseases. *Clin Infect Dis* 2000;**31**:1032–9.
20. Benard G, Gryschek RC, Duarte AJ, et al. Cryptococcosis as an opportunistic infection in immunodeficiency secondary to paracoccidioidomycosis. *Mycopathologia* 1996;**133**:65–9.
21. Benard G, Kavakama J, Mendes-Giannini MJ, et al. Contribution to the natural history of paracoccidioidomycosis: identification of the primary pulmonary infection in the severe acute form of the disease—a case report. *Clin Infect Dis* 2005;**40**:e1–4.
22. Benard G, Mendes-Giannini MJ, Juvenale M, et al. Immunosuppression in paracoccidioidomycosis: T cell hyporesponsiveness to two *Paracoccidioides brasiliensis* glycoproteins that elicit strong humoral immune response. *J Infect Dis* 1997;**175**:1263–7.
23. Benard G, Neves CP, Gryschek RC, et al. Severe juvenile type paracoccidioidomycosis in an adult. *J Med Vet Mycol* 1995;**33**: 67–71.
24. Benard G, Orii NM, Marques HH, et al. Severe acute paracoccidioidomycosis in children. *Pediatr Infect Dis* 1994;**13**:510–15.
25. Benard G, Patzina RL, Schwab JB, et al. Fatal septic shock due to a disseminated chronic form of paracoccidioidomycosis in an aged woman. *Med Mycol* 2012;**50**:407–11.
26. Biagioni L, Souza MJ, Chamma LG, et al. Serology of paracoccidioidomycosis. II. Correlation between class-specific antibodies and clinical forms of the disease. *Trans R Soc Trop Med Hyg* 1984;**78**:617–21.
27. Bialek R, Ibricevic A, Fothergill A, Begerow D. Small subunit ribosomal DNA sequence shows *Paracoccidioides brasiliensis* closely related to *Blastomyces dermatitidis*. *J Clin Microbiol* 2000;**38**: 3190–3.
28. Bicalho RN, Santo MF, de Aguiar MC, et al. Oral paracoccidioidomycosis: a retrospective study of 62 Brazilian patients. *Oral Dis* 2001;**7**:56–60.
29. Biselli PJ, Juvenale M, Mendes-Giannini MJ, et al. IgE antibody response to the main antigenic component of *Paracoccidioides brasiliensis* in patients with paracoccidioidomycosis. *Med Mycol* 2001;**39**:475–8.
30. Bittencourt JI, de Oliveira RM, Coutinho ZF. Paracoccidioidomycosis mortality in the State of Paraná, Brazil, 1980/1998. *Cad Saude Publica* 2005;**21**:1856–64.
31. Blotta MH, Altermani AM, Amaral E, et al. Placental involvement in paracoccidioidomycosis. *J Med Vet Mycol* 1993;**31**:249–57.
32. Blotta MH, Mamoni RL, Oliveira SJ, et al. Endemic regions of paracoccidioidomycosis in Brazil: a clinical and epidemiologic study of 584 cases in the southeast region. *Am J Trop Med Hyg* 1999;**61**:390–4.
33. Boccalandro I, Albuquerque FJ. Icterícia e comprometimento hepático na blastomicose sulamericana. A propósito de 10 casos e revisão bibliográfica. *Rev Paul Med* 1960;**56**:350–66.
34. Borba CM, Vinhas EA, Lopes-Bezerra LM, Lucena-Silva N. Morphological, biochemical and molecular approaches for comparing typical and atypical *Paracoccidioides brasiliensis* strains. *Antonie Van Leeuwenhoek* 2005;**88**:257–66.
35. Borelli D. Some ecological aspects of paracoccidioidomycosis. *In* Paracoccidioidomycosis. Proceedings, Panamerican Symposium. Washington, DC: Pan American Health Organization; Scientific Publication 254; 1972. p. 59–64.
36. Borges CL, Bailão AM, Báo SN, et al. Genes potentially relevant in the parasitic phase of the fungal pathogen *Paracoccidioides brasiliensis*. *Mycopathologia* 2011;**171**:1–9.
37. Brummer E, Castañeda E, Restrepo A. Paracoccidioidomycosis: an update. *Clin Microbiol Rev* 1993;**6**:89–117.
38. Bueno JP, Assis CM, del Negro GM, et al. IgG, IgM and IgA antibody response for the diagnosis and follow up of paracoccidioidomycosis: comparison of counterimmunoelectrophoresis and complement fixation reactions. *J Med Vet Mycol* 1997;**35**: 213–17.
39. Buitrago MJ, Cuenca-Estrella M. Current epidemiology and laboratory diagnosis of endemic mycoses in Spain. *Enferm Infecc Microbiol Clin* 2012;**30**:407–13.
40. Buitrago MJ, Merino P, Puente S, et al. Utility of real-time PCR for the detection of *Paracoccidioides brasiliensis* DNA in the diagnosis of imported paracoccidioidomycosis. *Med Mycol* 2009;**47**: 879–82.
41. Cadavid D, Restrepo A. Factors associated with *Paracoccidioides brasiliensis* infection among permanent residents of 3 endemic areas in Colombia. *Epidemiol Infect* 1993;**111**:121–33.
42. Caldini CP, Xander P, Kioshima ÉS, et al. Synthetic peptides mimic gp75 from *Paracoccidioides brasiliensis* in the diagnosis of paracoccidioidomycosis. *Mycopathologia* 2012;**174**:1–10.
43. Calle D, Rosero DS, Orozco LC, et al. Paracoccidioidomycosis in Colombia: an ecological study. *Epidemiol Infect* 2001;**126**: 309–15.
44. Camargo ZP, Berzaghi R, Amaral CC, et al. Simplified method for producing *Paracoccidioides brasiliensis* exoantigens for use in immunodiffusion tests. *Med Mycol* 2003;**41**:539–42.

45. Camargo ZP, Unterkircher C, Travassos LR. Identification of antigenic polypeptides of *Paracoccidioides brasiliensis* by immunoblotting. *J Med Vet Mycol* 1989;**27**:407–12.
46. Cano MI, Cisalpino PS, Galindo I, et al. Electrophoretic karyotypes and genome sizing of the pathogenic fungus *Paracoccidioides brasiliensis*. *J Clin Microbiol* 1998;**36**:742–7.
47. Cardoso SV, Moreti MM, Costa IM, Loyola AM. Exfoliative cytology: a helpful tool for the diagnosis of paracoccidioidomycosis. *Oral Dis* 2001;**7**:217–20.
48. Carneiro RC, Miranda BG, Camilo Neto C, et al. Juvenile paracoccidioidomycosis in urban area: report of two cases. *Braz J Infect Dis* 2010;**14**:77–80.
49. Caro E, Gonzalez A, Muñoz C, et al. Recognition of laminin by *Paracoccidioides brasiliensis* conidia: a possible mechanism of adherence to human type II alveolar cells. *Med Mycol* 2008;**46**:795–804.
50. Castro CC, Benard G, Ygaki Y, et al. MRI of head and neck paracoccidioidomycosis. *Br J Radiol* 1999;**72**:717–22.
51. Castro RM, del Negro G. Particularidades clinicas da paracoccidioidomicose na crianca. *Rev Hosp Clin Fac Med São Paulo* 1976;**31**:194–8.
52. Chikamori T, Saka S, Nagano H, et al. Paracoccidioidomycosis in Japan. Report of a case. *Rev Inst Med Trop São Paulo* 1984;**26**: 267–71.
53. Cimerman S, Bacha HA, Ladeira MC, et al. Paracoccidioidomycosis in a boy infected with HIV. *Mycoses* 1997;**40**:434–44.
54. Coimbra CE Jr, Wanke B, Santos RV, et al. Paracoccidioidin and histoplasmin sensitivity in the Tupí-Mondé Amerindian populations from Brazilian Amazonia. *Ann Trop Med Parasitol* 1994;**88**: 197–207.
55. Colares SM, Marcantônio S, Zambonato S, et al. Paracoccidioidomicose aguda/subaguda disseminada. Primeiro caso no Rio Grande do Sul. *Rev Soc Bras Med Trop* l998;**31**:563–76.
56. Colombo AL, Tobón A, Restrepo A, et al. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol* 2011;**49**:785–98.
57. Correa MM, Bedoya AM, Guerrero MP, et al. Diagnosis of paracoccidioidomycosis by a dot blot assay using a recombinant *Paracoccidioides brasiliensis* p27 protein. *Mycoses* 2007;**50**:41–7.
58. Corredor GG, Peralta LA, Castano JH, et al. The naked-tailed armadillo *Cabassous centralis* (Miller 1899): a new host to *Paracoccidioides brasiliensis*. Molecular identification of the isolate. *Med Mycol* 2005;**43**:275–80.
59. Coutinho ZF, Silva D, Lazera M, et al. Paracoccidioidomycosis mortality in Brazil (1980-1995). *Cad Saude Publica* 2002;**18**:1441–54.
60. Cunha DA, Zancopé-Oliveira RM, Felipe MS, et al. Heterologous expression, purification, and immunological reactivity of a recombinant HSP60 from *Paracoccidioides brasiliensis*. *Clin Diagn Lab Immunol* 2002;**9**:374–7.
61. da Silva Castro N, Barbosa MS, Maia ZA, et al. Characterization of *Paracoccidioides brasiliensis* PbDfg5p, a cell-wall protein implicated in filamentous growth. *Yeast* 2008;**25**:141–54.
62. da Silva Neto BR, de Fátima da Silva J, Mendes-Giannini MJ, et al. The malate synthase of *Paracoccidioides brasiliensis* is a linked surface protein that behaves as an anchorless adhesin. *BMC Microbiol* 2009;**9**:272.
63. Da Silva SH, Colombo AL, Blotta MH, et al. Diagnosis of neuroparacoccidioidomycosis by detection of circulating antigen and antibody in cerebrospinal fluid. *J Clin Microbiol* 2005;**43**: 4680–3.
64. De Brito T, Sandhu GS, Kline BC, et al. In situ hybridization in paracoccidioidomycosis. *Med Mycol* 1999;**37**:207–11.
65. de Camargo ZP. Serology of paracoccidioidomycosis. *Mycopathologia* 2008;**165**:289–302.
66. de Farias MR, Condas LA, Ribeiro MG, et al. Paracoccidioidomycosis in a dog: case report of generalized lymphadenomegaly. *Mycopathologia* 2011;**172**:147–52.
67. De Mattos Grosso D, Almeida SR, Mariano M, et al. Characterization of gp70 and anti-gp70 monoclonal antibodies in *Paracoccidioides brasiliensis* pathogenesis. *Infect Immun* 2003;**71**:6534–42.
68. de Souza Vianna LM, Pirani Carneiro F, Calvalca Tavares A, et al. Cytological diagnosis of paracoccidioidomycosis: a report of four cases. *Diagn Cytopathol* 2013;**41**:374–6.
69. del Negro GM, Benard G, De Assis CM, et al. Lack of reactivity of paracoccidioidomycosis sera in the double immunodiffusion test with the gp43 antigen: report of two cases. *J Med Vet Mycol* 1995;**33**:113–16.
70. del Negro GM, Pereira CN, Andrade HF, et al. Evaluation of tests for antibody response in the follow-up of patients with acute and chronic forms of paracoccidioidomycosis. *J Med Microbiol* 2000;**49**:37–46.
71. Desjardins CA, Champion MD, Holder JW, et al. Comparative genomic analysis of human fungal pathogens causing paracoccidioidomycosis. *PLoS Genet* 2011;**7**:e1002345.
72. Dias MF, Pereira AC, Pereira A, et al. The role of HLA antigens in the development of paracoccidioidomycosis. *Eur Acad Dermatol Venereol* 2000;**14**:166–71.
73. Dietze R, Fowler VG, Steiner TS, et al. Failure of amphotericin B colloidal dispersion in the treatment of paracoccidioidomycosis. *Am J Trop Med Hyg* 1999;**60**:837–9.
74. Díez S, Garcia EA, Pino PA, et al. PCR with *Paracoccidioides brasiliensis* specific primers: potential use in ecological studies. *Rev Inst Med Trop São Paulo* 1999;**41**:351–8.
75. Díez S, Gomez BL, McEwen JG. Combined use of *Paracoccidioides brasiliensis* recombinant 27-kilodalton and purified 87-kilodalton antigens in an enzyme-linked immunosorbent assay for serodiagnosis of paracoccidioidomycosis. *J Clin Microbiol* 2003;**41**:1536–42.
76. Donofrio FC, Calil AC, Miranda ET, et al. Enolase from *Paracoccidioides brasiliensis*: isolation and identification as a fibronectin-binding protein. *J Med Microbiol* 2009;**58**:706–13.
77. Doria AS, Taylor GA. Bony involvement in paracoccidioidomycosis. *Pediatr Radiol* 1997;**27**:67–9.
78. Do Valle AC, Costa RL, Fialho Monteiro PC, et al. Interpretation and clinical correlation of serological tests in paracoccidioidomycosis. *Med Mycol* 2001;**39**:373–7.
79. Endo S, Komori T, Ricci G, et al. Detection of gp43 of *Paracoccidioides brasiliensis* by the loop-mediated isothermal amplification (LAMP) method. *FEMS Microbiol Lett* 2004;**234**(1):93–7.
80. Feitosa LS, Cisalpino PS, dos Santos MR, et al. Chromosomal polymorphism, syntenic relationships, and ploidy in the pathogenic fungus *Paracoccidioides brasiliensis*. *Fungal Genet Biol* 2003;**39**: 60–9.
81. Felipe MS, Andrade RV, Arraes FB, et al. Transcriptional profiles of the human pathogenic fungus *Paracoccidioides brasiliensis* in mycelium and yeast cells. *J Biol Chem* 2005;**280**:24706–14.
82. Fernandes VC, Coitinho JB, Veloso JM, et al. Combined use of *Paracoccidioides brasiliensis* recombinant rPb27 and rPb40 antigens in an enzyme-linked immunosorbent assay for immunodiagnosis of paracoccidioidomycosis. *J Immunol Methods* 2011;**367**:78–84.
83. Ferreira MS. Paracoccidioidomycosis. *Paediatr Respir Rev* 2009; **10**:161–5.
84. Fonseca ER, Pardal PP, Severo LC. Paracoccidioidomicose em crianças em Belém do Pará. *Rev Soc Bras Med Trop* 1999;**32**: 31–3.
85. Fonseca LC, Mignone C. Paracoccidioidomicose do intestino delgado, aspectos anatomo-clínicos e radiológicos de 125 casos. *Rev Hosp Clin Fac Med São Paulo* 1976;**31**:199–207.
86. Fornajeiro N, Maluf ML, Takahachi G, et al. Paracoccidioidomycosis epidemiological survey using gp43, in two cities of northwestern region of Paraná, Brazil. *Rev Soc Bras Med Trop* 2005;**38**: 191–3.
87. Franco M, Mendes RP, Dillon NL, Mota NG. Paracoccidioidomycosis, a recently proposed classification of its clinical forms. *Rev Soc Bras Med Trop* 1987;**20**:129–32.
88. Franco M, Mendes RP, Moscardi-Bacchi MM, et al. Paracoccidioidomycosis. *Baillieres Clin Trop Med Comm Dis* 1989;**4**:185–220.
89. Funari M, Kavakama J, Shikanai-Yasuda MA, et al. Chronic pulmonary paracoccidioidomycosis (South American blastomycosis): high-resolution CT findings in 41 patients. *Am J Roentgenol* 1999;**173**:59–64.
90. Giraldo R, Restrepo A, Gutierrez F, et al. Pathogenesis of paracoccidioidomycosis: a model based on the study of 46 patients. *Mycopathologia* 1976;**58**:63–70.
91. Goldani LZ, Sugar AM. Short report: use of the polymerase chain reaction to detect *Paracoccidioides brasiliensis* in murine paracoccidioidomycosis. *Am J Trop Med Hyg* 1998;**58**:152–3.
92. Goldman GH, dos Reis Marques E, Duarte Ribeiro DC, et al. Expressed sequence tag analysis of the human pathogen *Paracoccidioides brasiliensis* yeast phase: identification of putative

homologues of *Candida albicans* virulence and pathogenicity genes. *Eukaryot Cell* 2003;**2**:34–48.
93. Gomes GM, Cisalpino PS, Taborda CP, et al. PCR for diagnosis of paracoccidioidomycosis. *J Clin Microbiol* 2000;**38**:3478–80.
94. Gómez BL, Figueroa JI, Hamilton AJ, et al. Antigenemia in patients with paracoccidioidomycosis: detection of the 87 kDa determinant during and after antifungal therapy. *J Clin Microbiol* 1998;**36**:3309–16.
95. González A, Caro E, Muñoz C, et al. *Paracoccidioides brasiliensis* conidia recognize fibronectin and fibrinogen which subsequently participate in adherence to human type II alveolar cells: involvement of a specific adhesin. *Microb Pathog* 2008;**44**:389–401.
96. Grossklaus DA, Tadano T, Breder SA, Hahn RC. Acute disseminated paracoccidioidomycosis in a 3 year-old child. *Braz J Infect Dis* 2009;**13**:242–4.
97. Gryschek RC, Pereira RM, Kono A, et al. Paradoxical reaction to treatment in 2 patients with severe acute paracoccidioidomycosis: a previously unreported complication and its management with corticosteroids. *Clin Infect Dis* 2010;**50**:e56–8.
98. Hahn RC, Conceição YT, Santos NL, et al. Disseminated paracoccidioidomycosis: correlation between clinical and in vitro resistance to ketoconazole and trimethoprim sulphamethoxazole. *Mycoses* 2003;**46**:324–9.
99. Hernández O, Garcia AM, Almeida AJ, et al. Gene expression during activation of *Paracoccidioides brasiliensis* conidia. *Yeast* 2011; **28**:771–81.
100. Hernández O, Tamayo D, Torres I, et al. Kinetic analysis of gene expression during mycelium to yeast transition and yeast to mycelium germination in *Paracoccidioides brasiliensis*. *Biomedica* 2011;**31**: 570–9.
101. Horre R, Schumacher G, Alpers K, et al. A case of imported paracoccidioidomycosis in a German legionnaire. *Med Mycol* 2002;**40**:213–16.
102. Imai T, Sano A, Mikami Y, et al. A new PCR primer for the identification of *Paracoccidioides brasiliensis* based on rRNA sequences coding the internal transcribed spacers (ITS) and 5.8S regions. *Med Mycol* 2000;**38**:323–6.
103. Iwai LK, Yoshida M, Sadahiro A, da Silva WR. T-cell recognition of *Paracoccidioides brasiliensis* gp43-derived peptides in patients with paracoccidioidomycosis and healthy individuals. *Clin Vaccine Immunol* 2007;**14**:474–6.
104. Juvenale M, del Negro GM, Duarte AJ, Benar G. Antibody isotypes to a *Paracoccidioides brasiliensis* somatic antigen in sub-acute and chronic form paracoccidioidomycosis. *J Med Microbiol* 2001;**50**:127–34.
105. Kalmar EM, Alencar FE, Alves FP, et al. Paracoccidioidomycosis: an epidemiologic survey in a pediatric population from the Brazilian Amazon using skin tests. *Am J Trop Med Hyg* 2004;**71**:82–6.
106. Kamei K, Sano A, Kikuchi K, et al. The trend of imported mycoses in Japan. Review. *J Infect Chemother* 2003;**9**:16–20.
107. Kaufman L, Standard P, Padhye AA. Exoantigen tests for the immunoidentification of fungal cultures. *Mycopathologia* 1983;**82**: 3–12.
108. Klein BS, Tebbets B. Dimorphism and virulence in fungi. *Curr Opin Microbiol* 2007;**10**:314–19.
109. Koishi AC, Vituri DF, Dionízio Filho PS, et al. A semi-nested PCR assay for molecular detection of *Paracoccidioides brasiliensis* in tissue samples. *Rev Soc Bras Med Trop* 2010;**43**:728–30.
110. Lacaz CS, Porto E, Martins JE. Paracoccidioidomicose. In: Lacaz CS, Porto E, Martins JE, et al, editors. *Tratado de micologia médica Lacaz*. 9th ed. São Paulo, Brazil: Sarvier; 2002. p. 629–729.
111. Lindsley MD, Hurst SF, Iqbal NJ, Morrison CJ. Rapid identification of dimorphic and yeast-like fungal pathogens using specific DNA probes. *J Clin Microbiol* 2001;**39**:3505–11.
112. Londero AT. Paracoccidioidomicose: patogenia, formas clinicas, manifestacões pulmonares e diagnostico. *J Pneumol (Brazil)* 1986; **12**:41–57.
113. Londero AT, Melo IS. Paracoccidioidomicose. *J Brasil Med* 1988;**55**:96–111.
114. Londero AT, Rios-Gonçalves AJ, Terra GM, et al. Paracoccidioidomycosis in Brazilian children. A critical review (1911-1994). *Arq Bras Med* 1996;**70**:197–203.
115. Mamoni RL, Nouér SA, Oliveira SJ, et al. Enhanced production of specific IgG4, IgE, IgA, and TGF-beta in sera from patients with the juvenile form of paracoccidioidomycosis. *Med Mycol* 2002;**40**:153–9.
116. Manns BJ, Baylis BW, Urbanski SJ, et al. Paracoccidioidomycosis: case report and review. *Clin Infect Dis* 1996;**23**:1026–32.
117. Marcos CM, Silva JF, Oliveira HC, et al. Surface-expressed enolase contributes to the adhesion of *Paracoccidioides brasiliensis* to host cells. *FEMS Yeast Res* 2012;**12**:557–70.
118. Marques da Silva SH, Colombo A, Blotta MH, et al. Detection of circulating gp43 antigen in serum, cerebrospinal fluid, and bronchoalveolar lavage fluid of patients with paracoccidioidomycosis. *J Clin Microbiol* 2003;**41**:3675–80.
119. Marques-da-Silva SH, Messias Rodrigues A, Sybren de Hoog G, et al. Occurrence of *Paracoccidioides lutzii* in the Amazon region: description of two cases. *Am J Trop Med Hyg* 2012;**87**:710–14.
120. Marques da Silva SH, Queiroz-Telles F, Colombo AL, et al. Monitoring gp43 antigenemia in paracoccidioidomycosis patients during therapy. *J Clin Microbiol* 2004;**42**:2419–24.
121. Marques ER, Ferreira ME, Drummond RD, et al. Identification of genes preferentially expressed in the pathogenic yeast phase of *Paracoccidioides brasiliensis*, using suppression subtraction hybridization and differential macroarray analysis. *Mol Genet Genomics* 2004;**271**:667–77.
122. Marques SA. Treatment of paracoccidioidomycosis with itraconazole. *Annu Rev Biomed Sci* 2002;special issue:24–5.
123. Marques SA, Dillon NL, Franco MF, et al. Paracoccidioidomycosis: a comparative study of the evolutionary serologic, clinical and radiologic results for patients treated with ketoconazole or amphotericin B plus sulfonamides. *Mycopathologia* 1985;**89**:19–25.
124. Marques SA, Franco MF, Mendes RP, et al. Some epidemiological aspects of paracoccidioidomycosis in Botucatú endemic area, State of São Paulo, Brazil. *Rev Inst Med Trop São Paulo* 1983;**25**:87–92.
125. Marques SA, Shikanai-Yasuda MA. Paracoccidioidomycosis associated to immunosuppression, AIDS, and cancer. In: Franco M, Lacaz CS, Restrepo-Moreno A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 393–405.
126. Martinez R, Belluci AD, Fiorillo AM. A tomografia computadorizada na avaliação do comprometimento abdominal na paracoccidioidomicose. *Rev Soc Bras Med Trop* 1988;**21**:47.
127. Martinez R, Meneghelli UG, Dantas RO, et al. O comprometimento gastrintestinal na blastomicose sul-americana (paracoccidioidomicose). I. Estudo clínico, radiológico e histopatológico. *Rev Assoc Med Bras* 1979;**25**:31–4.
128. Martinez R, Meneghelli UG, Dantas RO, et al. O comprometimento gastrintestinal na blastomicose sul-americana (paracoccidioidomicose). II. Estudo funcional do intestino delgado. *Rev Assoc Med Bras* 1979;**25**:70–3.
129. Matos WB, Santos GM, Silva VE, et al. Paracoccidioidomycosis in the state of Maranhão, Brazil: geographical and clinical aspects. *Rev Soc Bras Med Trop* 2012;**45**:385–9.
130. Matute DR, McEwen JG, Puccia R, et al. Cryptic speciation and recombination in the fungus *Paracoccidioides brasiliensis* as revealed by gene genealogies. *Mol Biol Evol* 2006;**23**:65–73.
131. Matute DR, Sepulveda VE, Quesada LM, et al. Microsatellite analysis of three phylogenetic species of *Paracoccidioides brasiliensis*. *J Clin Microbiol* 2006;**44**:2153–7.
132. Maza PK, Straus AH, Toledo MS, et al. Interaction of epithelial cell membrane rafts with *Paracoccidioides brasiliensis* leads to fungal adhesion and Src-family kinase activation. *Microbes Infect* 2008;**10**: 540–7.
133. McEwen JG, Bedoya V, Patiño MM, et al. Experimental paracoccidioidomycosis induced by the inhalation of conidia. *J Med Vet Mycol* 1987;**25**:165–75.
134. Meira DA, Pereira PC, Marcondes-Machado J, et al. The use of glucan as immunostimulant in the treatment of paracoccidioidomycosis. *Am J Trop Med Hyg* 1996;**55**:496–503.
135. Mendes RP. The gamut of clinical manifestations. In: Franco M, Lacaz CS, Restrepo-Moreno A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 233–58.
136. Mendes RP, Negroni R, Arachevala A. Treatment and control of cure. In: Franco M, Lacaz CS, Restrepo-Moreno A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 373–92.
137. Mendes-Giannini MJ, Bueno JP, Shikanai-Yasuda MA, et al. Detection of the 43,000-molecular-weight glycoprotein in sera of patients with paracoccidioidomycosis. *J Clin Microbiol* 1989;**27**: 2842–5.
138. Mendes-Giannini MJ, Bueno JP, Shikanai-Yasuda MA, et al. Antibody response to the 43 kDa glycoprotein of *Paracoccidioides*

brasiliensis as a marker for the evaluation of patients under treatment. *Am J Trop Med Hyg* 1990;**43**:200–6.
139. Mendes-Giannini MJ, del Negro GMB, Siqueira AM. Serodiagnosis of paracoccidioidomycosis. In: Franco M, Lacaz CS, Restrepo-Moreno A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 343–63.
140. Mendes-Giannini MJ, Monteiro da Silva JL, Silva JF, et al. Interactions of *Paracoccidioides brasiliensis* with host cells: recent advances. *Mycopathologia* 2008;**165**:237–48.
141. Mendes-Giannini MJ, Taylor ML, Bouchara JB, et al. Pathogenesis II: fungal responses to host responses: interaction of host cells with fungi. *Med Mycol* 2000;**38**(Suppl 1):113–23.
142. Meneses-Garcia A, Mosqueda-Taylor A, Morales-de-la Luz R, et al. Paracoccidioidomycosis: report of 2 cases mimicking squamous cell carcinoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2002;**94**:609–13.
143. Migliari DA, Sugaya NN, Mimura MA, et al. Periodontal aspects of the juvenile form of paracoccidioidomycosis. *Rev Inst Med Trop São Paulo* 1998;**40**:15–18.
144. Miranda-Aires E, Costa Alves, CA, Ferreira AV, et al. Bone paracoccidioidomycosis in an HIV-positive patient. *Braz J Infect Dis* 1997;**1**:260–5.
145. Montoya AE, Moreno MN, Restrepo A, McEwen JG. Electrophoretic karyotype of clinical isolates of *Paracoccidioides brasiliensis*. *Fungal Genet Biol* 1997;**21**:223–7.
146. Morais FV, Barros TF, Fukada MK, et al. Polymorphism in the gene coding for the immunodominant antigen gp43 from the pathogenic fungus *Paracoccidioides brasiliensis*. *J Clin Microbiol* 2000;**38**:3960–6.
147. Morejón KM, Machado AA, Martinez R. Paracoccidioidomycosis in patients infected with and not infected with human immunodeficiency virus: a case-control study. *Am J Trop Med Hyg* 2009; **80**:359–66.
148. Moreto TC, Marques ME, de Oliveira ML, et al. Accuracy of routine diagnostic tests used in paracoccidioidomycosis patients at a university hospital. *Trans R Soc Trop Med Hyg* 2011;**105**: 473–8.
149. Moscardi-Bacchi M, Mendes RP, Marques SA, et al. In situ localization of T lymphocyte subsets in human paracoccidioidomycosis. *J Med Vet Mycol* 1989;**27**:149–58.
150. Mota NG, Rezkallah-Iwasso MT, Peraçoli MT, et al. Correlation between cell-mediated immunity and clinical forms of paracoccidioidomycosis. *Trans R Soc Trop Med Hyg* 1985;**79**:765–71.
151. Motoyama AB, Venancio EJ, Brandao GO, et al. Molecular identification of *Paracoccidioides brasiliensis* by PCR amplification of ribosomal DNA. *J Clin Microbiol* 2000;**38**:3106–9.
152. Naiff RD, Ferreira LC, Barret TV, et al. Enzootic paracoccidioidomycosis in armadillos (*Dasypus novemcinctus*) in the State of Para. *Rev Inst Med Trop São Paulo* 1986;**28**:19–27.
153. Nascimento CR, Delanina WF, Soares CT. Paracoccidioidomycosis: sarcoid-like form in childhood. *An Bras Dermatol* 2012;**87**: 486–7.
154. Naranjo MS, Trujillo M, Munera MI, et al. Treatment of paracoccidioidomycosis with itraconazole. *J Med Vet Mycol* 1990;**28**: 67–76.
155. Negroni R. Pathogenesis. In: Franco M, Lacaz CS, Restrepo A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 203–12.
156. Nogueira MG, Andrade GM, Tonelli E, et al. Laboratory evolutive aspects of children under paracoccidioidomycosis treatment. *Rev Soc Bras Med Trop* 2006;**39**:478–83.
157. Nogueira MG, Andrade GM, Tonelli E. Clinical evolution of paracoccidioidomycosis in 38 children and teenagers. *Mycopathologia* 2006;**161**:73–81.
158. Nogueira SV, Fonseca FL, Rodrigues ML, et al. *Paracoccidioides brasiliensis* enolase is a surface protein that binds plasminogen and mediates interaction of yeast forms with host cells. *Infect Immun* 2010;**78**:4040–50.
159. Nunes LR, Oliveira RO, Leite DB, et al. Transcription profiling of *Paracoccidioides brasiliensis* cells undergoing the mycelium-to-yeast transition. *Eukaryot Cell* 2005;**4**:2115–28.
160. Ollague JM, Zurita AM, Calero G. Paracoccidioidomycosis (South American blastomycosis) successfully treated with terbinafine: first case report. *Br J Dermatol* 2000;**143**:188–91.
161. Ono MA, Itano EN, Mizuno LT. Inhibition of *Paracoccidioides brasiliensis* by pesticides: is this a partial explanation for the difficulty in isolating this fungus from the soil? *Med Mycol* 2002; **40**:493–9.
162. Ortiz BL, Díez S, Urán ME, et al. Use of the 27-kilodalton recombinant protein from *Paracoccidioides brasiliensis* in serodiagnosis of paracoccidioidomycosis. *Clin Diagn Lab Immunol* 1998;**5**: 826–30.
163. Pagliari C, Sotto MN. Dendritic cells and pattern of cytokines in paracoccidioidomycosis skin lesions. *Am J Dermatopathol* 2003; **25**:107–12.
164. Paniago AMM, Aguiar JIA, Aguiar ES, et al. Paracoccidioidomycosis: a clinical and epidemiological study of 422 cases observed on the Mato Grosso do Sul State. *Rev Soc Bras Med Trop* 2003; **36**:455–9.
165. Parente JA, Borges CL, Bailão AM, et al. Comparison of transcription of multiple genes during mycelia transition to yeast cells of *Paracoccidioides brasiliensis* reveals insights to fungal differentiation and pathogenesis. *Mycopathologia* 2008;**165**:259–73.
166. Parise-Fortes MR, Marques SA, Soares AM, et al. Cytokines released from blood monocytes and expressed in mucocutaneous lesions of patients with paracoccidioidomycosis evaluated before and during trimethoprim-sulfamethoxazole treatment. *Br J Dermatol* 2006;**154**:643–50.
167. Pedrosa PN. *Paracoccidioidomicose. Inquérito intradérmico com paracoccidioidina em zona rural do Estado do Rio de Janeiro [MSc thesis]*. Brazil: Universidade Federal do Rio de Janeiro; 1976.
168. Pereira GH, Santos AQ, Park M, et al. Bone marrow involvement in a patient with paracoccidioidomycosis: a rare presentation of juvenile form. *Mycopathologia* 2010;**170**:259–61.
169. Pereira RM, Bucaretchi F, Barison EM, et al. Paracoccidioidomycosis in children: clinical presentation, follow-up and outcome. *Rev Inst Med Trop São Paulo* 2004;**46**:127–31.
170. Pereira RM, Guerra-Júnior G, Tresoldi AT. Adrenal function in 23 children with paracoccidioidomycosis. *Rev Inst Med Trop São Paulo* 2006;**48**:333–6.
171. Pereira RM, Tresoldi AT, Silva MT, et al. Fatal disseminated paracoccidioidomycosis in a two-year-old child. *Rev Inst Med Trop São Paulo* 2004;**46**:37–9.
172. Perenha-Viana MC, Gonzales IA, Brockelt SR, et al. Serological diagnosis of paracoccidioidomycosis through a Western blot technique. *Clin Vaccine Immunol* 2012;**19**:616–9.
173. Peres da Silva R, Matsumoto MT, Braz JD, et al. Differential gene expression analysis of *Paracoccidioides brasiliensis* during keratinocyte infection. *J Med Microbiol* 2011;**60**:269–80.
174. Prado M, Silva MB, Laurenti R, et al. Mortality due to systemic mycoses as a primary cause of death or in association with AIDS in Brazil: a review from 1996 to 2006. *Mem Inst Oswaldo Cruz* 2009;**104**:513–21.
175. Puccia R, McEwen JG, Cisalpino PS. Diversity in *Paracoccidioides brasiliensis*. The PbGP43 gene as a genetic marker. *Mycopathologia* 2008;**165**:275–87.
176. Puccia R, Vallejo MC, Matsuo AL, Longo LV. The paracoccidioides cell wall: past and present layers toward understanding interaction with the host. *Front Microbiol* 2011;**2**:257.
177. Queiroz-Telles F. *Paracoccidioides brasiliensis:* ultrastructural findings. In: Franco M, Lacaz CS, Restrepo-Moreno A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 27–48.
178. Queiroz-Telles F, Escuissato DL. Pulmonary paracoccidioidomycosis. *Semin Respir Crit Care Med* 2011;**32**:764–74.
179. Queiroz-Telles F, Goldani LZ, Schlamm HT, et al. An open-label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidioidomycosis. *Clin Infect Dis* 2007;**45**:1462–9.
180. Resende LS, Mendes RP, Bacchi MM, et al. Infiltrative myelopathy by paracoccidioidomycosis. A review and report of nine cases with emphasis on bone marrow morphology. *Histopathology* 2006; **48**:377–86.
181. Resende LS, Mendes RP, Bacchi MM, et al. Bone marrow necrosis related to paracoccidioidomycosis: the first eight cases identified at autopsy. *Histopathology* 2009;**54**:486–9.
182. Restrepo A. Ecology of *Paracoccidioides brasiliensis*. In: Franco M, Lacaz CS, Restrepo-Moreno A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 121–30.
183. Restrepo A. Morphological aspects of *Paracoccidioides brasiliensis* in lymph nodes: implications for the prolonged latency of paracoccidioidomycosis? *Med Mycol* 2000;**38**:317–22.

184. Restrepo A. *Paracoccidioides brasiliensis*. In: Mandell GL, Douglas R, Bennett JE, editors. *Principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2005. p. 3062–7.
185. Restrepo A, Benard G, Castro CC, et al. Pulmonary paracoccidioidomycosis. *Semin Respir Crit Care Med* 2008;**29**:182–97.
186. Restrepo A, McEwen JG, Castañeda E. The habitat of *Paracoccidioides brasiliensis*: how far from solving the riddle? *Med Mycol* 2001;**39**:233–41.
187. Restrepo A, Robledo M, Giraldo R, et al. The gamut of paracoccidioidomycosis. *Am J Med* 1976;**61**:33–42.
188. Restrepo A, Trujillo M, Gómez I. Inapparent lung involvement in patients with the subacute juvenile type of paracoccidioidomycosis. *Rev Inst Med Trop São Paulo* 1989;**31**:18–22.
189. Ricci G, Mota FT, Wakamatsu A, et al. Canine paracoccidioidomycosis. *Med Mycol* 2004;**42**:379–83.
190. Ricci G, Zelck U, Mota F, et al. Genotyping of *Paracoccidioides brasiliensis* directly from paraffin embedded tissue. *Med Mycol* 2008;**46**:31–42.
191. Richini-Pereira VB, Bosco SM, Theodoro RC, et al. Molecular approaches for eco-epidemiological studies of *Paracoccidioides brasiliensis*. *Mem Inst Oswaldo Cruz* 2009;**104**:636–43.
192. Rios-Gonçalves AJ, Londero AT, Terra GM, et al. Paracoccidioidomycosis in children in the state of Rio de Janeiro (Brazil). Geographic distribution and the study of a "reservarea." *Rev Inst Med Trop São Paulo* 1998;**40**:11–13.
193. Rios-Gonçalves AJ, Terra GM, Rosembaum R, et al. Paracoccidioidomicose disseminada "tipo juvenil." *Arq Brasil Med* 1992;**66**: 335–7.
194. Rocha N, Suguiama EH, Maia D, et al. Intestinal malakoplakia associated with paracoccidioidomycosis: a new association. *Histopathology* 1997;**30**:79–83.
195. Sadahiro A, Diogo CL, Oshiro TM, Shikanai-Yasuda MA. Kinetics of IFN-γ, TNF-α, IL-10 and IL-4 production by mononuclear cells stimulated with gp43 peptides, in patients cured of paracoccidioidomycosis. *Rev Soc Bras Med Trop* 2007;**40**:156–62.
196. Sadahiro A, Roque AC, Shikanai-Yasuda MA. Generic human leukocyte antigen class II (DRB1 and DQB1) alleles in patients with paracoccidioidomycosis. *Med Mycol* 2007;**45**:35–40.
197. Salina MA, Shikanai-Yasuda MA, Mendes RP, et al. Detection of circulating *Paracoccidioides brasiliensis* antigen in urine of paracoccidioidomycosis patients before and during treatment. *J Clin Microbiol* 1998;**36**:1723–8.
198. San-Blas G, Nino-Vega G, Barreto L. Primers for clinical detection of *Paracoccidioides brasiliensis*. *J Clin Microbiol* 2005;**43**: 4255–7.
199. Sandoval M, De Brito T, Sotto MN, et al. Antigen distribution in mucocutaneous biopsies of human paracoccidioidomycosis. *Int J Surg Pathol* 1996;**3**:181–8.
200. Sant'Anna GD, Mauri M, Arrarte H Jr, et al. Laryngeal manifestations of paracoccidioidomycosis (South American blastomycosis). *Arch Otolaryngol Head Neck Surg* 1999;**125**:1375–8.
201. Semighini CP, Camargo ZP, Puccia R, et al. Molecular identification of *Paracoccidioides brasiliensis* by 5′ nuclease assay. *Diagn Microbiol Infect Dis* 2002;**44**:383–6.
202. Shankar J, Wu TD, Clemons KV, et al. Influence of 17β-estradiol on gene expression of *Paracoccidioides* during mycelia-to-yeast transition. *PLoS One* 2011;**6**:e28402.
203. Shikanai-Yasuda MA. Pharmacological management of paracoccidioidomycosis. *Expert Opin Pharmacother* 2005;**6**:385–97.
204. Shikanai-Yasuda MA, Benard G, Higaki Y, et al. Randomized trial with itraconazole, ketoconazole and sulfadiazine in paracoccidioidomycosis. *Med Mycol* 2002;**40**:411–17.
205. Shikanai-Yasuda MA, Higaki Y, Uip DE, et al. Comprometimiento da medula ossea e eosinofilia na paracoccidioidomicose. *Rev Inst Med Trop São Paulo* 1992;**34**:85–90.
206. Shikanai-Yasuda MA, Segurado AA, Pinto WP, et al. Immunodeficiency secondary to juvenile paracoccidioidomycosis. *Mycopathologia* 1992;**120**:23–8.
207. Shikanai-Yasuda MA, Telles Filho FQ, Mendes RP, et al. Guidelines in paracoccidioidomycosis. *Rev Soc Bras Med Trop* 2006;**39**: 297–310.
208. Silletti RP, Glezerov V, Schwartz IS, et al. Pulmonary paracoccidioidomycosis misdiagnosed as *Pneumocystis* pneumonia in an immunocompromised host. *J Clin Microbiol* 1996;**34**:2328–30.
209. Silva MG, Schrank A, Bailão EF, et al. The homeostasis of iron, copper, and zinc in *Paracoccidioides brasiliensis*, *Cryptococcus neoformans* var. *grubii*, and *Cryptococcus gattii*: a comparative analysis. *Front Microbiol* 2011;**2**:49.
210. Silva SS, Tavares AH, Passos-Silva DG. Transcriptional response of murine macrophages upon infection with opsonized *Paracoccidioides brasiliensis* yeast cells. *Microbes Infect* 2008;**10**:12–20.
211. Silva-Vergara ML, Martinez R. Role of the armadillo *Dasypus novemcinctus* in the epidemiology of paracoccidioidomycosis. *Mycopathologia* 1999;**144**:131–3.
212. Simoes LB, Marques SA, Bagagli E. Distribution of paracoccidioidomycosis: determination of ecologic correlates through spatial analyses. *Med Mycol* 2004;**42**:517–23.
213. Souza AS Jr, Gasparetto EL, Davaus T, et al. High-resolution CT findings of 77 patients with untreated pulmonary paracoccidioidomycosis. *Am J Roentgenol* 2006;**187**:1248–52.
214. Taborda CP, Camargo ZP. Diagnosis of paracoccidioidomycosis by dot immunobinding assay for antibody detection using the purified and specific antigen gp43. *J Clin Microbiol* 1994;**32**: 554–6.
215. Tani EM, Franco M. Pulmonary cytology in paracoccidioidomycosis. *Acta Cytol* 1984;**28**:571–5.
216. Tatibana BT, Sano A, Uno J, et al. Detection of *Paracoccidioides brasiliensis* gp43 gene in sputa by loop-mediated isothermal amplification method. *J Clin Lab Anal* 2009;**23**:139–43.
217. Teixeira F, Gayotto LC, De Brito T. Morphological patterns of the liver in South American blastomycosis. *Histopathology* 1978;**2**: 231–7.
218. Teixeira MM, Theodoro RC, Carvalho MJ. Phylogenetic analysis reveals a high level of speciation in the *Paracoccidioides* genus. *Mol Phylogenet Evol* 2009;**52**:273–83.
219. Terçarioli GR, Bagagli E, Reis GM. Ecological study of *Paracoccidioides brasiliensis* in soil: growth ability, conidia production and molecular detection. *BMC Microbiol* 2007;**7**:92.
220. Theodoro RC, Bagagli E, Oliveira C. Phylogenetic analysis of PRP8 intein in *Paracoccidioides brasiliensis* species complex. *Fungal Genet Biol* 2008;**45**:1284–91.
221. Theodoro RC, Candeias JM, Araújo JP Jr, et al. Molecular detection of *Paracoccidioides brasiliensis* in soil. *Med Mycol* 2005;**43**: 725–9.
222. Theodoro RC, Teixeira Mde M, Felipe MS, et al. Genus *Paracoccidioides*: species recognition and biogeographic aspects. *PLoS One* 2012;**7**:e37694.
223. Tobón AM, Gómez I, Franco L, et al. Seguimiento post-terapia en pacientes con paracoccidioidomicosis tratados con itraconazol. *Rev Colombiana Neumol* 1995;**7**:74–8.
224. Torrado E, Castañeda E, de la Hoz F, et al. Paracoccidioidomicosis: definición de las áreas endémicas de Colombia. *Biomédica* 2000;**20**:327–34.
225. Travassos LR, Puccia R, Cisalpino P, et al. Biochemistry and molecular biology of the main diagnostic antigen of *Paracoccidioides brasiliensis*. Review. *Arch Med Res* 1995;**26**:297–304.
226. Travassos LR, Taborda CP. Paracoccidioidomycosis: advances in treatment incorporating modulators of the immune response. *J Invas Fungal Infect* 2011;**5**:1–6.
227. Tresoldi AT, Pereira RM, Castro LC, et al. Hypercalcemia and multiple osteolytic lesions in a child with disseminated paracoccidioidomycosis and pulmonary tuberculosis. *J Pediatr Rio Janeiro* 2005;**81**:349–52.
228. Tuder RM, El Ibrahim R, Godoy CE, et al. Pathology of the pulmonary paracoccidioidomycosis. *Mycopathologia* 1985;**92**:179–88.
229. Valle AC, Guimarães RR, Lopes DJ, et al. Aspectos radiológicos torácicos na paracoccidioidomicose. *Rev Inst Med Trop São Paulo* 1992;**34**:107–15.
230. Untereiner WA, Scott JA, Naveau FA. The Ajellomycetaceae, a new family of vertebrate-associate Onygenales. *Mycology* 2004;**96**: 812–21.
231. Verweij PE, Figueroa J, Van Burik J. Clinical applications of non–culture based methods for the diagnosis and management of opportunistic and endemic mycoses. *Med Mycol* 2000;**38**:161–71.
232. Vidal MS, Benard G, de Brito T, et al. Atypical serological response marked by a lack of detectable anti-gp43 antibodies in a patient with disseminated paracoccidioidomycosis. *J Clin Microbiol* 2005;**43**:3014–16.

233. Vilela R, Mendoza L, Rosa OS. Molecular model for studying the uncultivated fungal pathogen *Lacazia loboi*. *J Clin Microbiol* 2005;**43**:3657–61.
234. Wanke B, Londero AT. Epidemiology and paracoccidioidomycosis infection. In: Franco M, Lacaz CS, Restrepo-Moren A, del Negro G, editors. *Paracoccidioidomycosis*. Boca Raton, Fla: CRC Press; 1994. p. 109–20.
235. Weber SA, Brasolotto A, Rodrigues L, et al. Dysphonia and laryngeal sequelae in paracoccidioidomycosis patients: a morphological and phoniatric study. *Med Mycol* 2006;**44**:219–25.
236. Yamaga LY, Benard G, Hironaka FH, et al. The role of gallium-67 scan in defining the extent of disease in an endemic deep mycosis, paracoccidioidomycosis: a predominantly multifocal disease. *Eur J Nucl Med Mol Imaging* 2003;**30**:888–94.
237. Yarzábal L, Dessaint J P, Arango M, et al. Demonstration and qualification of IgE antibodies against *Paracoccidioides brasiliensis* in paracoccidioidomycosis. *Int Arch Allergy Immunol* 1980;**62**:346–51.

for cryptococcosis. We have also come to recognize *C. neoformans* comprises at least two separate species, *C. neoformans* and *C. gattii*, that evolved from each other more than 40 million years ago.[33] Disease caused by these two species is similar in many ways, but there are important differences. An outbreak of *C. gattii* disease in the Pacific Northwest was first identified in the late 1990s beginning on Vancouver Island. Despite the availability of antifungal therapy, the mortality associated with cryptococcosis remains high and new therapies and prevention strategies are needed.

THE ORGANISM

C. neoformans is an encapsulated basidiomycete that is evolutionarily distinct from yeasts like *Candida albicans* and more closely related to corn and grain plant fungal pathogens.[63] *C. neoformans* is a round yeast (5 to 10 μm diameter) that produces white (frequently mucoid) colonies on standard mycologic media. The capsule varies greatly in size depending on the local milieu. Recently, giant cells (80 to 100 μm diameter) called titan cells have been described in animal studies, although the clinical significance of these forms remains to be understood.[46] *C. neoformans* is capable of sexual reproduction in the laboratory, and mating types (alpha and A) have been identified. In the environment and during infection, the organism is typically haploid and replicates asexually. *C. neoformans* was originally classified into five serotypes (A, B, C, D, and AD) based on its reaction with anticapsular serum. Serotypes B and C, formally known as *C. neoformans* var. *gattii* are now collectively named *C. gattii*. Serotype A (classified as *C. neoformans* var. *grubii*) is responsible for the vast majority of *C. neoformans* disease. Serotype D (*C. neoformans* var. *neoformans*) is primarily found in Europe and occasionally hybrids such as serotype A/D and other diploid strains are found in nature. *C. neoformans* and *C. gattii* can be distinguished by a variety of biochemical, serologic, and molecular typing techniques, although these are not routinely available in clinical microbiology laboratories. For instance, *C. gattii* (but not *C. neoformans*) produces blue coloration on L-canavanine-glycine-bromothymol blue (CGB) medium. The advent of molecular typing techniques has further enhanced our understanding of cryptococcal pathogenesis. With the use of these techniques, both *C. neoformans* and *C. gattii* can each be classified into four molecular types: VN I to IV and VG I to IV, respectively, with further subtypes. Future work may actually divide these into separate species. Other species of *Cryptococcus* (e.g., *Cryptococcus laurentii*, *Cryptococcus albidus*) are less virulent and rarely have been reported to cause disease.[11,47]

Virulence

Several important virulence traits common to *C. neoformans* and *C. gattii* include encapsulation, melanization, the ability to grow at 37°C, and both urease and phospholipase production. These characteristics are not uniformly present in other cryptococcal species. The emergence of some of these traits has been hypothesized to have been selected for because of survival advantages they confer in their natural environment, where these yeasts are exposed to invertebrate predatory scavengers such as worms and amebae.

Capsule

The impressive capsules of *C. neoformans* and *C. gattii* are composed of long unbranched polymers of xylose and glucuronic acid. The capsule plays a central role in cryptococcal virulence, and acapsular/hypocapsular mutants exhibit greatly reduced virulence.[19] Although capsules can be produced by other cryptococcal species, studies suggest that there are important differences in the biophysical properties between the capsule of virulent and nonvirulent species.[7] Capsule size is actively regulated in response to local conditions such as pCO_2, serum, and iron. In nature, the capsule likely protects the yeast from desiccation and ingestion by soil amebae. The capsule gives the organism its distinctive appearance in India ink staining (Fig. 202-1). The substantial polysaccharide material forming the capsule is extensively shed into host tissue, cerebrospinal fluid (CSF), and serum during infection; and the detection of this polysaccharide forms the basis of cryptococcal antigen testing. Similar to prototypical pediatric pathogens (e.g., *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Neisseria meningitidis*), the cryptococcal capsule inhibits antibody- and complement-mediated phagocytosis and thereby interferes with the development of an effective immune response.[77] However, the capsule also exhibits a variety of other anti-inflammatory properties, including inhibition of leukocyte migration,[37] enhanced interleukin-10 secretion,[145] inhibition of T-cell proliferation,[153] and induction of macrophage apoptosis.[149] In addition, the cryptococcal capsule also has been hypothesized to contribute to cerebral edema[59] and to promote replication of the human immunodeficiency virus (HIV).[120] Given the central role of the capsule in cryptococcal pathogenesis, administration of capsular-specific antibody has been studied as potential adjunctive therapy in animal models.[104,126] However, this therapeutic approach has so far had limited success in humans.[81]

Melanin

Melanin is a pigment that is synthesized from catechol precursors (e.g., dopamine, epinephrine) by the laccase enzymes of *C. neoformans* and *C. gattii*. The laccase enzyme is similar to enzymes produced by wood-rotting fungi and is capable of digesting lignin, an important constituent of trees, and may thereby promote growth of the yeast in decaying trees. Melanin is produced during cryptococcal infection and is localized to the cell wall.[123] Melanin content can vary significantly among *C. neoformans* strains.[151] Yeast mutants lacking the ability to produce melanin are less virulent in animal models than their melanin-producing parental strains. Although melanin structure remains poorly understood, its chemistry endows it with the ability to stabilize free radicals and provides a stable framework even under extremely harsh environmental conditions. These characteristics

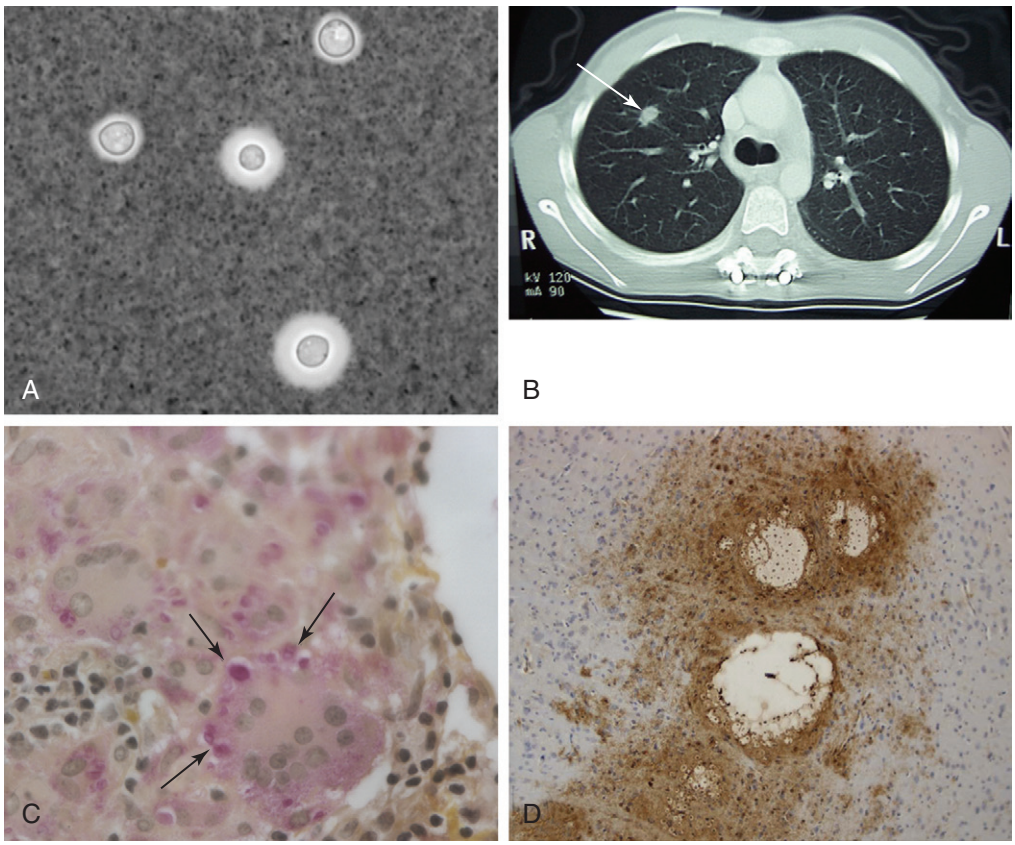

FIGURE 202-1 ■ **A,** India ink staining of CSF demonstrates *C. neoformans* and capsule, which excludes the stain and results in a halo around the organism. **B,** CT scan of child with rhabdomyosarcoma reveals nodule (white arrow) secondary to *C. neoformans*. **C,** Mucicarmine staining of a lung from a rat with experimentally-induced pulmonary cryptococcosis shows granulomas containing *C. neoformans* (black arrows). **D,** Immunostaining for cryptococccal polysaccharide reveals extensive shedding of polysaccharide within the brain parencyhma and around cryptococcomas of a rat with experimentally induced cryptococcal meningoencephalitis. (See Expert Consult website for color version.)

likely play an important role in the capacity of melanin to protect against damage caused by nitrogen- and oxygen-derived host oxidants in addition to exogenous factors such as ultraviolet light and antifungal agents. Melanin also appears to promote disease through its immunomodulatory properties[62] and its ability to bind to host antimicrobial peptides.[107] One explanation for cryptococcal neurotropism relates to the hypothesis that high concentrations of catechol precursors within the brain promote cryptococcal melanization, which in turn protects the organism from the host immune response within the brain.[121]

Extracellular Enzymes

C. neoformans and *C. gattii* also produce a variety of extracellular enzymes that have been linked to virulence, including proteinase, phospholipase, and urease. Urease catalyzes the hydrolysis of urea to ammonia and carbamate. The importance of urease in virulence and during infection appears to depend on the infection site and may be more important for pulmonary infection than brain infection.[31] Urease production by *C. neoformans* has been associated with the accumulation of immature dendritic cells and the production of a disease-enhancing T-helper cell type 2 (Th2) inflammation.[110] *C. neoformans* strains vary in their phospholipase production, and high phospholipase production has been associated with increase fungal burden in animal models.[147] Conversely, lack of phospholipase production has been linked to reduced virulence. Phospholipases have also been implicated in promoting entry into the brain[30] by promoting passage through brain endothelial cells.[96] Phospholipases may also promote intracellular growth by releasing arachidonic acid from host cells.[152]

Other virulence factors that have been identified, include mating type,[79] prostaglandin production,[42] and phenotypic switching.[48] The calcineurin signaling pathway has been implicated in induction of metabolic and oxidative stress genes in response to environmental stimuli. This pathway appears to play a role in promoting survival both within the environment and host.[109]

ECOLOGY AND EPIDEMIOLOGY

Cryptococcus neoformans

There are important differences in the ecology of *C. neoformans* and *C. gattii* (Table 202-1). *C. neoformans* (but not *C. gattii*) is well known for its association with bird droppings, including those from pigeons, canaries, and

TABLE 202-1 **Summary of Characteristics of *Cryptococcus gattii* and *Cryptococcus neoformans***

	Cryptococcus gattii	*Cryptococcus neoformans*
Microbiology	Serotypes B and C Molecular type VGI associated with Australia and VGIIa with outbreak in Pacific Northwest Bluish discoloration on CGB media	Serotypes A and D Molecular type VNI most common cause of *C. neoformans* diseases
Ecology	Eucalyptus and other trees	Bird droppings and trees
Epidemiology	Immunocompetent > Immunocompromised Endemic in certain tropical/subtropical regions Outbreak in Pacific Northwest	Immunocompromised (especially AIDS and T cell defects) > Immunocompetent High incidence in sub-Saharan Africa and Southeast Asia Decreased incidence in regions where HAART is available
Clinical Manifestations	Primary pulmonary infection and CNS cryptococcomas with associated complications	Meningoencephalitis and disseminated disease a common presentation, especially among AIDS patients

parrots. *C. neoformans* is capable of growth in the acidic environment of bird droppings. Nonetheless, birds are unlikely to be the primary environmental source for *C. neoformans* because their high internal temperatures inhibit *C. neoformans* growth and possibly possess innate avian host factors against this yeast. In addition, the *C. neoformans* density is much higher in aged pigeon droppings compared with fresh droppings, suggesting the possibility for secondary contamination as the mechanism for this ecologic niche.[91] Other noted environmental sources for *C. neoformans* include rotting trees and fruits.[26,92]

There are also important differences in the epidemiology of disease caused by these different species of cryptococcus. *C. neoformans* is generally considered an opportunistic pathogen that causes central nervous system (CNS) and respiratory disease in patients with depressed cellular immunity. However, cryptococcosis secondary to *C. neoformans* also occurs in individuals without any identified risk factors. A dramatic increase in the prevalence of cryptococcosis occurred in association with the AIDS epidemic. In patients with HIV infection, cryptococcal meningoencephalitis is an AIDS-defining diagnosis, typically occurring in both adults and children with profound immunodeficiency, as evidenced by a finding of $CD4^+$ T cells less than 100/μL. In countries where highly active antiretroviral therapy (HAART) has been successfully introduced, the prevalence of cryptococcosis has greatly decreased. Unfortunately, cryptococcosis has emerged as a significant cause of disease in AIDS patients in countries with limited resources. Worldwide, most cases of cryptococcosis occur in sub-Saharan Africa and Southeast Asia, affecting an estimated 1 million individuals annually[115] and up to 30 percent of AIDS patients.[12] In South Africa, *C. neoformans* is the leading cause of meningitis in adults, with an incidence more than twice that of tuberculous meningitis.[68] In developed countries, an increase in the incidence of *C. neoformans* disease among non–HIV-infected individuals has been recognized. This appears to be primarily associated with improvements in medical care and an increase in the number of individuals receiving immunosuppressive therapy, especially organ transplant recipients. Among solid-organ transplant recipients, cryptococcosis is the third most common invasive fungal infection. Among non–HIV-infected patients with cryptococcosis in the United States, 20 to 60 percent are transplant recipients.[148]

Overall, cryptococcosis secondary to *C. neoformans* is less common in children than adults but remains an important cause of pneumonia and meningoencephalitis, especially in certain parts of the world. Owing to the rarity of pediatric cryptococcosis, the precise prevalence is difficult to define and may vary by region. Studies of U.S. children with AIDS in the 1990s estimated an incidence of cryptococcosis to be about 1 percent.[50] A more recent U.S. series identified 63 cases of pediatric cryptococcosis from a consortium of 42 children's hospitals over a 5-year period, with an admission rate of 6.2 per million hospitalizations.[70] Most children (63.5%) in this cohort had underlying immunosuppression, whereas 21 percent were immunocompetent and 16 percent had AIDS. The 8-year point prevalence of cryptococcosis among Thai children with AIDS was 2.97 percent.[89] A study from South Africa reported 361 cases of pediatric cryptococcosis over a 3-year period, comprising 2 percent of all cases of cryptococcosis over this time.[99] The reported median age at time of diagnosis of pediatric cryptococcosis varies from 7 to 12 years. In most series, pediatric cryptococcosis rarely occurs in children younger than age 1 year, although in the South African cohort an increased incidence was noted within the first year of life. In addition to AIDS, cryptococcosis has been identified in children with a variety of primary and secondary immunodeficiency states, including organ transplantation, lymphoma, leukemia, lupus, glucocorticoid therapy, sarcoma, hyper-IgM syndrome, Bruton agammaglobulinemia, and severe combined immunodeficiency.[4,66,84,135,150] Neonatal cryptococcosis is rare but has been described (see later discussion).

Although the incidence of symptomatic cryptococcosis is disproportionately low in children, serologic studies suggest that children from certain regions of the country may be infected with *C. neoformans* at a very young age.[49]

The basis for this discrepancy between serologic findings and clinical disease is unknown. It is likely that most pediatric infections are asymptomatic or misdiagnosed as viral disease. Potential reasons for the low incidence of symptomatic pediatric cryptococcosis include the time needed to reactivate infection, the importance of repeated exposures, and differences in infecting strains.[101] Nonetheless, it remains a perplexing issue after serologic study as to why many children appear to be infected but rarely develop disease.

Cryptococcus gattii

In contrast to *C. neoformans*, *C. gattii* was initially recognized as a disease of tropical regions and endemic in Australasia, where it is associated with eucalyptus trees (both river red and forest red gum trees). However, recent studies have revealed that *C. gattii* causes disease in both tropical and temperate regions. This includes areas without eucalyptus trees, suggesting the possibility of other environmental reservoirs. In the Pacific Northwest, *C. gattii* has been isolated from non–eucalyptus tree species (Douglas fir, alder, and Garry oak), soil, air, fresh water, and sea water.

Cryptococcus gattii causes disease in both immunocompetent and immunocompromised individuals, although disease appears to be more common among immunocompetent individuals compared with *C. neoformans.* In an Australian study of *C. gattii* disease, 72 percent of patients had no recognizable immune deficit.[25] Some investigators, however, have hypothesized that affected individuals actually reported to be normal hosts have a subclinical immune deficit.[95] Because standard microbiologic techniques fail to distinguish between the two species, the precise prevalence of *C. gattii* disease is not known. Recently, *C. gattii* has been increasingly recognized to cause disease outside Australasia and tropical regions. Over the past 13 years, *C. gattii* has been linked to an outbreak of disease in the Pacific Northwest. This outbreak was initially recognized on Vancouver Island with subsequent extension into the United States (including the states of Washington and Oregon). In this outbreak, corticosteroid use and older age (>50 years) have been associated with increased severity.[56] Cryptococcosis secondary to *C. gattii* may occasionally occur in travelers returning from the Pacific Northwest. Disease typically presents within weeks to months after travel but may occur as long as a 1 year after travel.[55] There are also cases being reported outside the geographic boundaries typically associated with *C. gattii* disease.[97] This change in the environmental location of *C. gattii* may be the result of genetic changes in the yeast or climactic changes that allow the organism to successfully compete in new geographic regions.

The incidence of cryptococcosis secondary to *C. gattii* in children is not well characterized. Of 218 cases of cryptococcosis secondary to *C. gattii* in British Columbia between 1999 and 2007, 4 cases (1.8%) occurred in children younger than 18 years.[43] In contrast, among 43 cases of *C. gattii* meningoencephalitis in a series from Brazil, 19 percent were children younger than 12 years.[127]

PATHOGENESIS

C. neoformans and *C. gattii* infections are believed to be acquired through inhalation of infectious propagules within the environment. The precise infecting form remains to be understood and may involve either desiccated yeast cells or small basidiospores (1 to 2 μm), thus allowing alveolar deposition. Once within the lung, infection may cause disease through progression of pneumonia and dissemination to the CNS. Alternatively, infection may be contained in a dormant site within the lungs and reactivate in the setting of subsequent immunosuppression, such as HIV infection or corticosteroid use. Evidence for reactivation disease includes molecular typing[45] and pathologic[57] and serologic studies.[125] Furthermore, recent reports of very early cryptococcosis after organ transplantation highlight the tendency of *C. neoformans* to cause latent infections within transplanted organs.[137] Differences in the propensity for *C. neoformans* and *C. gattii* to cause primary progressive disease may exist. For example, molecular typing studies and cases of cryptococcosis among returning travelers to the Pacific Northwest highlight the potential for *C. gattii* to cause primary progressive disease.[15,18] These findings are consistent with findings from animal experimentation.[78] Within the lung, both innate and adaptive immune responses are essential to the effective control of infection. *C. neoformans* can be recognized by the cells of the innate immune system through a variety of receptors including, Toll-like, β-glucan, and mannose receptors. Macrophages play a central role in the host immune response.[129] Dendritic cells, natural killer cells, neutrophils, and activated macrophages can kill the yeast. *C. neoformans* is a facultative intracellular pathogen and is well adapted to survive and replicate within the acidic environment of the phagolysosome.[86] Unlike other intracellular pathogens, *C. neoformans* does not employ typical strategies to escape or modify the phagolysosomal compartment. However, intracellular survival within the harsh environment of the phagolysosome is supported by virulence traits, including capsule production, melanization, secretion of extracellular enzymes, and prostaglandin production. Interestingly, *C. neoformans* can escape the macrophage through induction of cell lysis or phagosomal extrusion.[5] The latter process allows the transfer of yeasts between macrophages without inducing macrophage death and associated inflammation. This novel mechanism likely promotes the intracellular persistence of *C. neoformans.*

Adaptive immunity and T-cell–mediated activation of macrophages is central to the effective host response. The importance of T-cell immunity in the protective host response to cryptococcosis is highlighted by the increased susceptibility of individuals with defective T-cell immunity to cryptococcosis. Animal experimentation highlights the protective nature of T-helper cell type 1 (Th1) immunity and nonprotective (disease-enhancing) nature of Th2 immunity.[60,73] In this regard, *C. neoformans* and *C. gattii* virulence has been linked to the ability to actively modulate the inflammatory response and to elicit nonprotective Th2 inflammation.[2,32]

PULMONARY CRYPTOCOCCOSIS

The lungs are the most commonly recognized body site of cryptococcal disease after the CNS. Pulmonary cryptococcosis can present as the primary manifestation of cryptococcosis (e.g., without extrapulmonary disease) or in association with disseminated disease/meningoencephalitis. Symptomatic pulmonary cryptococcosis typically occurs in individuals with underlying immunodeficiency or chronic lung disease. Nonetheless, pulmonary cryptococcosis in individuals without recognized risk factors is well described and appears to be more common for infection secondary to *C. gattii*. In one study of HIV-negative patients with cryptococcosis, 15 percent of patients with pulmonary cryptococcosis had no underlying disease.[114] Among AIDS patients with cryptococcal meningoencephalitis, pulmonary cryptococcosis has been reported to be present in a wide range (10% to 55%) of patients.[14] This variation may occur as a result of the nonspecific features of pulmonary disease along with differences in diagnostic evaluation.

Case reports and series describing adults with primary pulmonary cryptococcosis first appeared in the 1960s. Initial descriptions of this disorder highlighted the chronic, nonspecific nature of pulmonary cryptococcosis and a tendency for it to occur in older white men.[16] Although primary pulmonary cryptococcosis occurs in apparently normal hosts, several underlying conditions (in addition to HIV) have been identified as risk factors. These include corticosteroid use, chronic lung disease, Cushing syndrome, diabetes mellitus, malignancy, tuberculosis, rheumatologic disease, and chronic obstructive pulmonary disease.[5,114] Among patients with cancer, a lymphoid malignancy is present in the majority of patients with pulmonary cryptococcosis, as is corticosteroid usage and lymphopenia.[76] Reports highlight the risk for anti–tumor necrosis factor therapies and pulmonary cryptococcosis, similar to that for tuberculosis and other endemic mycosis.[54] In a multicenter study of organ transplant recipients, 39 percent of patients with cryptococcosis had primary pulmonary disease.[112] Primary pulmonary cryptococcosis has also been reported to be disproportionately more common in the British Columbia *C. gattii* outbreak, where it is the most common form of presentation.[44] Nonetheless, the presentation of *C. gattii* disease may still be primarily dependent on host immune status.[25]

Pulmonary Cryptococcosis in Children

Pulmonary disease as the primary manifestation of cryptococcosis in children is uncommon but has been described and affects both immunocompetent and immunocompromised children.[50,111] Infected children usually have chronic respiratory tract symptoms (i.e., cough, fever, chest pain, and weight loss) and are initially treated for bacterial pneumonia before the diagnosis is suspected. In one review of pulmonary cryptococcosis in children a predilection for lower lobe lung disease was apparent, with most children being asymptomatic, immunocompromised, and male.[140] Children may be asymptomatic and the diagnosis recognized as a result of imaging performed for other reasons. Several characteristic cases of pediatric pulmonary cryptococcosis in the context of soft tissue malignancy have been previously diagnosed as a result of imaging to evaluate for metastasis.[4,140] These children all had lung nodules that were initially thought to represent metastatic disease and were often lymphopenic.

Clinical Manifestations

The typical symptoms of symptomatic pulmonary cryptococcosis include cough, chest pain, dyspnea, fever, and weight loss.[16,74] Several studies indicate that a significant proportion of affected patients do not have respiratory symptoms. In a study of pulmonary cryptococcosis among solid-organ transplant recipients, 38 percent of the patients were asymptomatic.[131] Asymptomatic disease appears to be more common among immunocompetent individuals but does occur in immunosuppressed patients and may be initially confused with cancer. In patients with recognizable pulmonary disease, symptoms are typically mild and often chronic. In immunocompetent individuals, primary pulmonary cryptococcosis is often self-limited. In contrast, pulmonary cryptococcosis among immunocompromised individuals is more likely to occur in the context of disseminated disease or progress to involve the CNS. Constitutional signs, especially fever, weight loss, and anorexia, have been described, particularly in association with underlying immunosuppression. Rarely, pulmonary cryptococcosis can rapidly progress to acute respiratory distress syndrome. Coinfection with other pathogens, including *Mycobacterium tuberculosis* and *Aspergillus* spp. occasionally has been described.[41,144]

Radiology

The three most common radiologic appearances of pulmonary cryptococcosis are nodular (see Fig. 202-1), infiltrative, and interstitial, although pleural effusions and cavitary disease have also been described.[27,75] Nodules may be solitary or multiple and are nonspecific. Among AIDS patients, interstitial disease and large lobar infiltrates are the most common radiologic findings. Interstitial findings may be an indication of disseminated disease. Nodular densities or mass lesions are more likely to be asymptomatic or incidentally detected pulmonary cryptococcosis than pleural effusions and infiltrates.[131] Cavitation of cryptococcal nodules and infiltrates may occur especially in immunocompromised individuals and has been described in the context of immune reconstitution inflammatory syndrome (IRIS, see later discussion) in AIDS patients.[69]

Diagnosis

Sputum culture has an extremely limited sensitivity in the diagnosis of pulmonary cryptococcosis. In one study, only 19 of 101 cases of pulmonary cryptococcosis in adults were confirmed by sputum culture.[16] Bronchoscopy with fungal culture and antigen testing of lavage fluid may aid

in the diagnosis of cryptococcosis, although the sensitivity of this approach appears to be variable. Definitive diagnosis may require obtaining tissue through either transthoracic, transbronchial or open-lung biopsy. Serum cryptococcal antigen is typically nondetectable in isolated pulmonary cryptococcosis, and the presence of serum antigenemia supports consideration of disseminated disease.

CRYPTOCOCCAL MENINGOENCEPHALITIS

Cryptococcal meningoencephalitis typically involves the base of the brain and is usually associated with brain parenchymal involvement. Infection may extend through the Virchow-Robin space into the brain substance, most commonly affecting the midbrain and basal ganglia. Immunohistochemistry studies of brains from patients reveal extensive shedding of cryptococcal polysaccharide into the brain parenchyma (see Fig. 202-1).[82] Subarachnoid and parenchymal inflammation ranging from minimal to extensive granulomas may be present, depending on the host immune status and fungal burden.[83] Increased intracranial pressure (ICP) often complicates cryptococcal meningoencephalitis and contributes significantly to the morbidity and mortality of this disease. Cryptococcomas and CNS complications including hydrocephalus and cranial nerve palsies appear to be more common with *C. gattii* disease.[102,134] Differences between cryptococcal meningoencephalitis in HIV-infected and non–HIV-infected individuals have also been described. This includes a more acute presentation and the presence of a second site of infection in HIV-infected individuals.[87]

The mechanisms by which *C. neoformans* and *C. gattii* enter the CNS are not well understood. In general, organisms cross the blood-brain barrier by one of three mechanisms: transcellular, paracellular, or via infected phagocytes (Trojan horse). Evidence for both transcellular[20] and Trojan horse mechanisms exist for *Cryptococcus*. In this regard, *C. neoformans* adheres to and infects endothelial cells in vitro.[65] This process may be active and involve the secretion of enzymes (e.g., urease, phospholipase), which in turn induces cytoskeleton rearrangements, resulting in ingestion.[96] Evidence supporting the Trojan horse model comes from experiments in which CNS infection was successfully produced by infection of mice with *C. neoformans*–infected monocytes.[23]

Clinical Manifestations

Cryptococcal meningoencephalitis is a subacute to chronic process that generally presents symptomatically over weeks to even months. Occasionally, shorter and longer time intervals are described. Affected patients typically have a headache with or without a variety of other nonspecific symptoms, including fever, lethargy, nausea, and vomiting. Meningismus and photophobia, which are typical of bacterial meningitis, are often absent in adults. Symptoms of encephalitis may also be present and even predominate, including personality changes, memory loss, and altered mental status. Patients with cryptococcomas can have signs of increased ICP and localizing neurologic symptoms, consistent with an intracranial mass.

Pediatric Cryptococcal Meningoencephalitis

Because of the small number of cases, it is difficult to know if there are clinical differences in pediatric compared with adult meningoencephalitis. The most common symptoms reported in the largest pediatric series to date are headache and fever. Other commonly reported symptoms include nausea and vomiting. In one study of affected African children with AIDS, seizures were significantly more common in children when compared with adults (38% vs. 11%); however, this high incidence of seizures has not been reported in other pediatric series.[53] Additional reported symptoms include altered mental status, focal neurologic deficits, photophobia, abdominal pain, and respiratory symptoms. In some studies, meningismus is present in the majority of children. Like adults, it is common to see a lack of CSF inflammation in children with cryptococcal meningoencephalitis and CSF profiles may be relatively normal. Increased ICP in pediatric cryptococcal meningoencephalitis is well described, but the precise incidence is not well documented. In one study, more than 80 percent of children with cryptococcal meningoencephalitis had increased ICP.[89]

Diagnosis

Cerebrospinal Fluid Profile

The CSF profile of both adults and children with cryptococcal meningoencephalitis is characterized by a lack in both cellularity and alterations in biochemical profile, especially in HIV-infected individuals. In pediatric studies, mean CSF white blood cell counts vary from 4 to 16 cells/mm^3.[1,61,128] In another study, approximately 50 percent of infected children had normal CSF findings (with a normal white blood cell count of <7 cells/mm^3).[89] Likewise, CSF protein and glucose concentrations are often normal, although elevated protein concentrations may be present, especially with obstruction. In one study, decreased glucose or increased protein concentrations were present in 19 percent of infected children.[89] Given the normal appearance of CSF, a high suspicion and cryptococcal specific studies (see later discussion) are necessary for diagnosis.

Imaging

In cryptococcal meningoencephalitis, imaging often underrepresents the extent of disease, although magnetic resonance imaging results are more likely to be abnormal.[22] The presence of imaging abnormalities may be a marker of more extensive disease and high risk for death.[38] Meningeal enhancement is not uniformly present, which likely reflects the paucity of inflammation in this disease. In a study of solid-organ transplant recipients

with cryptococcal meningoencephalitis, meningeal enhancement (the most common finding) was present in only 15 percent of patients.[132] A similar frequency of enhancement has been reported in patients with AIDS. Other imaging features that may occasionally be noted include parenchymal masses (see later discussion), dilatation of the Virchow-Robin spaces, hydrocephalous, and cerebral edema.[35,122] Lesions of the Virchow-Robin spaces (called "soap bubble" lesions) are nonenhancing on magnetic resonance imaging and occur at the level of the thalamus, basal ganglia, periventricular white matter, and cerebellum. These lesions correlate with invasion through the perivascular Virchow-Robin spaces seen on autopsy and are characteristic of meningoencephalitis.[8]

India Ink Staining

India ink staining is based on the exclusion of the dye by the *C. neoformans/C. gattii* capsule, which creates a halo around the cell against a background. The sensitivity of India ink staining of CSF has been reported to vary from 50 percent to more than 80 percent. The sensitivity of this assay depends on the number of organisms in the CSF, which may in part be dependent on the immune state of the host.[29] False-positive results may be reported from leukocytes and tissue cells that can be removed by treatment with potassium hydroxide.

Culture and Histopathology

In adults with meningoencephalitis and HIV infection, CSF and blood cultures are positive in 75 to 90 percent and 70 percent of patients, respectively. Similar numbers have been reported in pediatric series.[1] For patients who undergo biopsy (e.g., brain biopsy for cryptococcoma) *C. neoformans/C. gattii* is best visualized with special stains, including silver stain and stains that label the capsule (e.g., mucicarmine and alcian blue). As with other fungi, the cell wall of this organism can be visualized with fluorescence using calcofluor, whereas melanin can be detected with a Fontana-Mason stain. Detection of the organism by immunohistochemistry using capsule-specific antibody and polymerase chain reaction has also been described on a research basis (see Fig. 202-1).[140,146] Although not part of routine clinical practice, quantitative CSF yeast counts have been used on a research basis to predict prognosis and treatment success.[13]

Antigen Detection

The polysaccharide capsule is shed during infection and can be measured in serum, CSF, and bronchoalveolar lavage fluid using two different antibody-based technologies (latex agglutination and enzyme-linked immunosorbent assay). Currently, latex agglutination is the most commonly used assay in clinical laboratories. This is a semi-quantitative assay, which is reported as a titer. The sensitivity and specificity of antigen detection (using CSF or serum) for patients with cryptococcal meningoencephalitis are both more than 95 percent. Antigen may also be detected in the serum in more than 90 percent of patients with disseminated disease.[10] As noted earlier, high CSF antigen titers have been associated with increased mortality, presumably reflecting a high fungal burden. In fact, CSF fungal burden can reach over a million yeasts per milliliter of CSF in advanced cases. In patients with extremely high antigen titers, a false-negative agglutination test may result from a prozone phenomenon. This can be addressed by dilution of samples. Care should therefore be taken in interpreting negative result in patients where there is a high clinical suspicion. Rare false-positive results have been reported in patients with infections secondary to other fungal pathogens such as *Trichosporon* species[98,142] and other microbes.[21] Although antigen detection assays are useful in diagnosis, they have limited utility in following response to therapy. Recently, a lateral flow assay, which shows good agreement with standard antigen testing, has been developed for the diagnosis of cryptococcosis, especially in resource-limited countries.[90] This assay offers the advantages of rapidity, ease of use, and decreased cost.

Complications

Mortality

Untreated cryptococcal meningoencephalitis is uniformly fatal. Despite the development of a variety of antifungal therapies, the mortality associated with cryptococcosis, particularly CNS and disseminated disease, remains high. Worldwide there are more than an estimated one-half million deaths annually, with the majority of these occurring in sub-Saharan Africa and Southeast Asia.[115] Overall, cryptococcal meningitis is the fourth leading infectious cause of death in sub-Saharan Africa, surpassing even tuberculosis.[115] In solid-organ transplant recipients, the mortality associated with cryptococcal meningoencephalitis can approach 40 percent.[64] This is comparable to the 10 to 30 percent mortality for HIV-associated cryptococcal disease in developed countries. In pediatric series, mortality rates have been reported to range between 23 and 38 percent.[1,53,128] Mortality is affected by several variables, including the underlying immune status, the presence of brain involvement, and the availability of appropriate antifungal therapies. Predictors of mortality reflect host factors and yeast burden and include high CSF cryptococcal antigen titer, increased ICP, altered mental status, positive India ink staining, and low CSF white blood cell counts at baseline.[6,36,124]

Increased Intracranial Pressure

Increased ICP is an important and common complication of cryptococcal meningoencephalitis, occurring in as many as 60 percent of affected adult AIDS patients. Increased ICP appears to be more common in *C. gattii* disease. The precise incidence of increased ICP in children with cryptococcal meningoencephalitis is not known but is likely to be similar to that in adults.[89] The entity is well described in children and includes cases of fulminant ICP.[105] The basis of increased ICP in cryptococcal meningoencephalitis remains unknown, but possible mechanisms include (1) inflammation, (2) osmotic effects of shed polysaccharide or mannitol, and (3) obstruction of

normal CSF drainage pathways (e.g., arachnoid villi) by cryptococcal polysaccharide or organisms. In support of this latter hypothesis, increased ICP has been associated in some studies with higher CSF fungal burden and CSF cryptococcal antigen levels but not with inflammation.[52] Furthermore, a difference in the tendencies of strains to elicit increased ICP has been demonstrated in animal models.[43]

Increased ICP is a recognized risk factor for early mortality in cryptococcal meningoencephalitis[52,143] and may correlate with increased CSF fungal burden, increased CSF cryptococcal antigen levels, and failure to sterilize CSF at 14 days. Patients with increased ICP may be symptomatic (e.g., hearing loss, altered mental status, cranial nerve palsy), but increased ICP may not be clinically apparent. Furthermore, computed tomography usually does not reveal ventricular dilatation, although occasional cases of obstructive hydrocephalous on presentation have been described. Appropriate treatment of cryptococcal meningoencephalitis therefore includes the documentation of CSF opening pressures at the initiation of therapy. Symptomatic increased opening pressure (≥250 mm^3 of H_2O) should be managed by serial lumbar punctures with daily withdrawal of CSF. Although there have been no controlled studies to determine the best approach to lowering ICP, the most recent Infectious Diseases Society of America (IDSA) guidelines suggest removal of enough CSF to decrease pressure by 50 percent if it is extremely high or to a normal pressure of less than 200 mm^3 H_2O.[119] Temporary percutaneous lumbar drains or ventriculostomy for persons who require repeated daily lumbar punctures should be considered. Permanent ventriculoperitoneal shunts may be indicated in patients who fail less-invasive methods. Acetazolamide, mannitol, and corticosteroids are generally not indicated in the treatment of increased ICP and may be detrimental. Nonetheless, successful treatment of increased ICP in association with IRIS with corticosteroids has been described in case reports and small series.[85]

Cryptococcoma

Intraparenchymal brain lesions (cryptococcomas) are a recognized complication of cryptococcal meningoencephalitis and have been associated with increased mortality.[22,132] Lesions may be singular or multiple and occur in 11 to 35 percent of patients[122] and can be associated with focal neurologic symptoms and hydrocephalus. Cryptococcomas appear to be more common in non–AIDS patients and with *C. gattii* disease. In a recent study of *C. gattii* meningoencephalitis in Australia, 37 percent of patients developed cryptococcomas.[25] The treatment of cryptococcomas has not been studied in a prospective manner, and current recommendations are based on small series and expert opinion. These recommendations highlight the importance of extended duration for induction and maintenance antifungal therapies. In patients with large lesions (≥3-cm lesion), consideration should be given to surgical debulkment.[119] Repeat imaging is helpful in monitoring the response of brain lesions to therapy, but lesions may persist for a long time and may develop surrounding edema in the context of effective antifungal therapy, which can interfere with the assessment of size. Intraparenchymal brain lesions also occur in the context of IRIS (see next discussion).

IMMUNE RECONSTITUTION INFLAMMATORY SYNDROME

IRIS is thought to occur as a result of improved immune function in the context of residual microbial antigens, which, in turn, produces symptoms secondary to enhanced inflammation. The current paradigm is that IRIS represents a dysregulated immune response with exaggerated Th1 response relative to Th2 inflammation. Support for this hypothesis comes from studies elucidating the CSF cytokine pattern in individuals with IRIS.[141] IRIS in association with cryptococcosis was initially described in AIDS patients treated with HAART, although it has subsequently been described in solid-organ transplant recipients,[138] pregnant patients,[40] and apparently normal hosts.[39] Cryptococcal IRIS may unmask a previously unrecognized infection or occur after treatment of cryptococcosis, resulting in a paradoxical worsening of symptoms. The lack of precise clinical criteria for a diagnosis of cryptococcal IRIS has hindered the study of this phenomenon. Several essential elements of this diagnosis have been proposed, including (1) appearance or worsening of clinical or radiologic manifestations consistent with an inflammatory process, (2) symptoms occurring during receipt of appropriate antifungal therapy that cannot be explained by a newly acquired infection, and (3) negative results of cultures or stable or reduced biomarkers for the initial fungal pathogen during the diagnostic workup for the inflammatory process.[133] Physicians considering a diagnosis of IRIS should exclude progression of infection (owing to inadequate antifungal therapy, antifungal resistance, or worsening immune function) and alternative diagnoses (e.g., other infection, malignancy). Cryptococcal IRIS may present in the form of lymphadenitis, pneumonia, meningitis, or space-occupying lesions in the CNS. Histopathology of affected lymph nodes typically reveals granulomatous inflammation with necrosis and few or absent fungal forms. Cultures typically reveal no growth. Patients with CNS IRIS may develop headache, fever, neck stiffness, and increased ICP, and the associated morbidity and mortality may be significant.[71] In patients with AIDS, cryptococcal IRIS has been reported to occur days to years after initiation of HAART, but typically it occurs weeks to months after HAART is begun. There is usually an associated decline in HIV viral load (at least 1 log_{10}), although changes in $CD4^+$ T cell counts are more variable. Risk factors for cryptococcal IRIS among HIV patients include lower baseline $CD4^+$ T cells, higher HIV viral load, and higher fungal burden at the initiation of therapy. There is considerable controversy on the role of early institution of HAART and the development of IRIS. Some studies highlight early initiation of HAART in the context of cryptococcal meningitis as an important risk factor for IRIS.[93,94,130,139] However, delayed introduction of HAART may be associated with increased mortality from progression of AIDS. Treatment of cryptococcal IRIS has not been studied rigorously, and IRIS

in pediatric cryptococcal disease has not been well documented. Minor IRIS manifestations may resolve spontaneously without specific therapy. Recent IDSA guidelines suggest that prednisone 0.5 to 1 mg/kg in conjunction with antifungal therapy may be helpful for the treatment of CNS cryptococcal IRIS.[119] Symptomatic increased ICP associated with IRIS may also be treated with daily lumbar punctures and CSF removal.

OTHER FORMS OF CRYPTOCOCCOSIS

Besides the CNS and pulmonary tract, *C. neoformans* disease may affect a wide range of organ systems. Primary cutaneous disease may occasionally occur as a result of direct inoculation.[106] More commonly, cutaneous disease represents secondary dissemination, especially in immunocompromised hosts.[118,136] The clinical presentation varies and may include acneiform lesions, purpura, vesicles, nodules, abscesses, ulcers, granulomas, pustules, draining sinuses, and cellulitis. In patients with AIDS, cutaneous cryptococcosis may appear similar to molluscum contagiosum. A high clinical suspicion along with tissue sampling is therefore needed to make a diagnosis. The presence of skin disease should prompt an evaluation for disseminated disease. The prostate has been hypothesized to serve as a sanctuary for *C. neoformans* during antifungal therapy and thereby promote recurrent infection. *C. neoformans* has been isolated from prostatic secretions of AIDS patients with disseminated infection, during and after treatment.[80] The clinical significance of these observations, however, remains unclear; and routine surveillance of prostatic secretions is not indicated.

Congenital Cryptococcosis

Congenital cryptococcosis is rare. Over a dozen cases of cryptococcosis in early infancy (e.g., within the first 3 months of life) have been reported, with a mortality greater than 50 percent.[117] Disease is typically multisystemic, usually with involvement of the central nervous and respiratory systems. Early (e.g., within hours to days of life)[17] and late presentations[72] of cryptococcosis (weeks to months) have been described in neonates. Based on the timing of disease, it has been hypothesized that infection can be transmitted both transplacentally and perinatally, possibly through the aspiration of infected material. The rarity of congenital cryptococcosis among women with active cryptococcosis during pregnancy indicates that the placenta is extremely effective in limiting maternal-fetal transmission. This notion is further supported by several descriptions of cryptococcal placentitis without neonatal disease.[34]

TREATMENT

The treatment of cryptococcosis depends on both the type of disease and the underlying immune status of the host. Although there are clinical differences in cryptococcosis secondary to *C. neoformans* and *C. gattii*, there are no differences in treatment recommendations at the present time. There are no clinical trials addressing the treatment of cryptococcosis in children. Therefore, current pediatric recommendations are based on extrapolations from adult studies using known pharmacokinetic profile of antifungal drugs in children. Routine testing for antifungal susceptibility is not indicated because resistance to amphotericin B and fluconazole is rare. Furthermore, minimal inhibitory concentration (MIC) cutoffs for fluconazole susceptibility have not been established and do not always correlate with clinical outcome. In one study of 487 isolates, only 3 (0.6%) demonstrated a high fluconazole MIC (i.e., ≥16 μg/mL) and all isolates were inhibited by very low concentrations of amphotericin B and exhibited very low MICs to voriconazole and posaconazole.[51] The IDSA guidelines suggest testing for antifungal susceptibilities only in the context of relapsing disease.[119]

Pulmonary Disease

In patients with pulmonary disease, it is essential to determine whether there is associated extrapulmonary involvement. To this end, testing serum for cryptococcal antigen and CSF analysis should be considered in all patients with CNS symptoms and in those with underlying immunosuppression. Isolated pulmonary disease, which is not severe, can be treated with fluconazole. The dosage of fluconazole for pulmonary disease in children is 6 to 12 mg/kg/day for 6 to 12 months based on resolution of symptoms (not to exceed the adult dosage of 400 mg daily). Itraconazole is an alternative therapy for individuals who cannot tolerate fluconazole, and the successful use of extended-spectrum azoles (e.g., voriconazole, posaconazole) has been described. Patients with evidence of extrapulmonary disease or severe pulmonary involvement should be treated with the same approach used to treat meningoencephalitis. Azoles are contraindicated in early pregnancy.

Central Nervous System Disease

The currently recommended treatment strategy for cryptococcal meningoencephalitis involves a staged approach using amphotericin B with flucytosine in combination for induction followed by consolidation treatment with fluconazole. The IDSA Guidelines for the Management of Cryptococcal Disease recommend amphotericin B at 0.7 to 1 mg/kg/day plus flucytosine 100 mg/kg/day for the treatment of cryptococcal meningitis.[119] In children, who tolerate amphotericin B better than adults, the higher dosage (e.g., 1 mg/kg /day) is preferred. The combination of amphotericin B and flucytosine has been demonstrated to be synergistic in vitro and result in improved outcomes in both animal models and clinical studies. Both amphotericin B and flucytosine have significant toxicities, and patients must be closely monitored for these. The duration of induction therapy is typically 2 weeks but should be individualized. A longer duration of induction should be considered under the following conditions: lack of clinical improvement, positive CSF cultures at 2 weeks, the inability to administer flucytosine, and non-HIV and nontransplant recipients. Most

meningoencephalitis studies have been done in patients with AIDS and transplant recipients, and the duration of induction therapy for other individuals has not been well studied. Repeated CSF examination at 14 days should be considered to document CSF sterilization. Patients with persistently positive cultures should continue induction therapy, and CSF analysis should be repeated every 2 weeks until CSF is sterile. If flucytosine cannot be administered, amphotericin B administered alone is an acceptable alternative. In patients whose renal function is impaired, lipid formulations of amphotericin B (liposomal amphotericin B, 3 to 6 mg/kg/day, or amphotericin B lipid complex, 5 mg/kg) may be substituted. Lipid formulations of amphotericin are also recommended as first-line agents for solid-organ transplant recipients with cryptococcosis. In resource-limited regions where amphotericin B is not readily available, high-dose fluconazole (1200 mg/day) with flucytosine has been studied as an alternative in adults.[108] After successful induction therapy, children should be treated with fluconazole 12 mg/kg/day (adult dosage, 400 mg) for 8 weeks.

Secondary Prophylaxis

After successful therapy for pneumonia and meningoencephalitis, children with AIDS should be maintained on suppressive fluconazole therapy (6 to 10 mg/kg/day) to prevent recurrence of disease. In adults, the current recommendations are to continue fluconazole until patients demonstrate a response to HAART, as manifested by a CD4 cell count greater than 100/μL and a sustained undetectable or very low HIV RNA level for 3 months (with a minimum of 12 months of antifungal therapy).[119] Although this approach is generally applied to children, there are little clinical data to support this practice and it should be undertaken with caution and with close observation for recurrence. A 6- to 12-month period of suppressive fluconazole therapy should also be considered in organ transplant recipients.[119]

Adjuvant Interferon Gamma

Consistent with its action as a Th1 cytokine, interferon (IFN)-γ plays a central role in the effective host response to cryptococcosis in animal models.[9,24] Furthermore, exogenously administered IFN-γ enhances resistance and potentiates the effects of amphotericin B in these models.[28,58] A phase II study demonstrated that adjuvant IFN-γ is well tolerated in patients with cryptococcal meningoencephalitis.[113] In one study it was suggested that the addition of IFN-γ to initial induction with amphotericin B/flucytosine results in quicker CSF sterilization, a marker that has previously been associated with clinical success.[67] This study, however, was not powered to detect differences in mortality. Current IDSA guidelines recommend that adjuvant IFN-γ should be considered in refractory cases.[119]

Primary Prophylaxis

In resource-limited regions where HAART is not widely available there has been increased interest in fluconazole prophylaxis to prevent cryptococcosis. The rationale for this approach lies in the high incidence and mortality of cryptococcosis in these regions. Two different strategies have been applied with promising results. With the first approach, at-risk HIV-infected individuals are regularly screened for serum cryptococcal antigenemia and treated with fluconazole if they are antigenemic but without overt cryptococcosis.[100] Cryptococcal antigenemia is a marker for the subsequent development of symptomatic cryptococcosis and for death from cryptococcosis and other causes.[88] In essence, this approach represents treatment of incipient disease and is similar to preemptive strategy used in CMV disease in solid-organ transplant recipients. This approach may become more attractive with the recent development of the lateral flow assay for antigen detection. With the second approach, all at-risk individuals (i.e., those with CD4 T cells <200/μL) are prophylactically treated with fluconazole. In a Ugandan prospective, double-blind randomized controlled trial, fluconazole prophylaxis for all patients with CD4 T cells less than 200/μL was effective in preventing cryptococcosis and cryptococcosis-associated death.[116]

NEW REFERENCES SINCE THE SIXTH EDITION

5. Alvarez M, Burn T, Luo Y, et al. The outcome of *Cryptococcus neoformans* intracellular pathogenesis in human monocytes. *BMC Microbiol* 2009;**9**:51.
6. Anekthananon T, Manosuthi W, Chetchotisakd P, et al. Predictors of poor clinical outcome of cryptococcal meningitis in HIV-infected patients. *Int J STD AIDS* 2011;**22**:665–70.
7. Araujo Gde S, Fonseca FL, Pontes B, et al. Capsules from pathogenic and non-pathogenic *Cryptococcus* spp. manifest significant differences in structure and ability to protect against phagocytic cells. *PLoS One* 2012;**7**:e29561.
12. Bicanic T, Harrison TS. Cryptococcal meningitis. *Br Med Bull* 2004;**72**:99–118.
13. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clin Infect Dis* 2009;**49**:702–9.
14. Brizendine KD, Baddley JW, Pappas PG. Pulmonary cryptococcosis. *Semin Respir Crit Care Med* 2011;**32**:727–34.
15. Byrnes EJ 3rd, Li W, Lewit Y, et al. First reported case of *Cryptococcus gattii* in the southeastern USA: implications for travel-associated acquisition of an emerging pathogen. *PLoS One* 2009;**4**:e5851.
18. Chambers C, MacDougall L, Li M, et al. Tourism and specific risk areas for *Cryptococcus gattii*, Vancouver Island, Canada. *Emerg Infect Dis* 2008;**14**:1781–3.
22. Charlier C, Dromer F, Leveque C, et al. Cryptococcal neuroradiological lesions correlate with severity during cryptococcal meningoencephalitis in HIV-positive patients in the HAART era. *PLoS One* 2008;**3**:e1950.
23. Charlier C, Nielsen K, Daou S, et al. Evidence of a role for monocytes in dissemination and brain invasion by *Cryptococcus neoformans*. *Infect Immun* 2009;**77**:120–7.
25. Chen SC, Slavin MA, Heath CH, et al. Clinical manifestations of *Cryptococcus gattii* infection: determinants of neurological sequelae and death. *Clin Infect Dis* 2012;**55**(6):789–98.
26. Chowdhary A, Rhandhawa HS, Prakash A, et al. Environmental prevalence of *Cryptococcus neoformans* and *Cryptococcus gattii* in India: an update. *Crit Rev Microbiol* 2012;**38**:1–16.
33. D'Souza CA, Kronstad JW, Taylor G, et al. Genome variation in *Cryptococcus gattii*, an emerging pathogen of immunocompetent hosts. *MBio* 2011;**2**:e00342–10.
34. Darko AD, Dim DC, Taylor G, et al. Placental *Cryptococcus neoformans* infection without neonatal disease: case report and review of the literature. *Pediatr Dev Pathol* 2009;**12**:249–52.

41. Enoki E, Maenishi O, Chikugo T, et al. Coinfection of *Aspergillus* and *Cryptococcus* in post-tuberculosis pulmonary cavity. *Pathol Int* 2012;**62**:574–6.
44. Galanis E, Macdougall L. Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999-2007. *Emerg Infect Dis* 2010;**16**: 251–7.
46. Garcia-Rodas R, Casadevall A, Rodriguez-Tudela JL, et al. *Cryptococcus neoformans* capsular enlargement and cellular gigantism during *Galleria mellonella* infection. *PLoS One* 2011;**6**:e24485.
51. Govender NP, Patel J, van Wyk M, et al. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates obtained through population-based surveillance in South Africa in 2002-2003 and 2007-2008. *Antimicrob Agents Chemother* 2011;**55**:2606–11.
55. Hagen F, van Assen S, Luijckx GJ, et al. Activated dormant *Cryptococcus gattii* infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach. *Med Mycol* 2010;**48**:528–31.
56. Harris JR, Lockhart SR, Debess E, et al. *Cryptococcus gattii* in the United States: clinical aspects of infection with an emerging pathogen. *Clin Infect Dis* 2011;**53**:1188–95.
61. Huang KY, Huang YC, Hung IJ, et al. Cryptococcosis in nonhuman immunodeficiency virus-infected children. *Pediatr Neurol* 2010;**42**:267–70.
67. Jarvis JN, Meintjes G, Rebe K, et al. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS* 2012;**26**:1105–13.
68. Jarvis JN, Meintjes G, Williams A, et al. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis* 2010;**10**:67.
70. Joshi NS, Fisher BT, Prasad PA, et al. Epidemiology of cryptococcal infection in hospitalized children. *Pediatr Infect Dis J* 2010; **29**:e91–5.
71. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clin Infect Dis* 2008;**46**:1694–701.
78. Krockenberger MB, Malik R, Ngamskulrungroj P, et al. Pathogenesis of pulmonary *Cryptococcus gattii* infection: a rat model. *Mycopathologia* 2010;**170**:315–30.
87. Liao CH, Chi CY, Wang YJ, et al. Different presentations and outcomes between HIV-infected and HIV-uninfected patients with cryptococcal meningitis. *J Microbiol Immunol Infect* 2012; **45**:296–304.
90. Lindsley MD, Mekha N, Baggett HC, et al. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin Infect Dis* 2011;**53**:321–5.
94. Makadzange AT, Ndhlovu CE, Takarinda K, et al. Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-Saharan Africa. *Clin Infect Dis* 2010;**50**:1532–8.
95. Marr KA, Datta K, Pirofski LA, et al. *Cryptococcus gattii* infection in healthy hosts: a sentinel for subclinical immunodeficiency? *Clin Infect Dis* 2012;**54**:153–4.
96. Maruvada R, Zhu L, Pearce D, et al. *Cryptococcus neoformans* phospholipase B1 activates host cell Rac1 for traversal across the blood-brain barrier. *Cell Microbiol* 2012;**14**(10):1544–53.
97. McCulloh RJ, Phillips R, Perfect JR, et al. *Cryptococcus gattii* genotype VGI infection in New England. *Pediatr Infect Dis J* 2011;**30**:1111–14.
99. Meiring ST, Quan VC, Cohen C, et al; Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA). A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. *AIDS* 2012;**26**(18):2307–14.
100. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count ≤100 cells/μL who start HIV therapy in resource-limited settings. *Clin Infect Dis* 2010;**51**:448–55.
101. Miglia KJ, Govender NP, Rossouw J, et al. Analyses of pediatric isolates of *Cryptococcus neoformans* from South Africa. *J Clin Microbiol* 2011;**49**:307–14.
105. Nagotkar L, Shanbag P, Mauskar A, et al. Fulminant intracranial hypertension due to cryptococcal meningitis in a child with nephrotic syndrome. *Indian J Crit Care Med* 2011;**15**:176–8.
106. Nasser N, Nasser Filho N, Vieira AG. Primary cutaneous cryptococcosis in an immunocompetent patient. *An Bras Dermatol* 2011;**86**:1178–80.
108. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin Infect Dis* 2010;**50**:338–44.
110. Osterholzer JJ, Surana R, Milam JE, et al. Cryptococcal urease promotes the accumulation of immature dendritic cells and a non-protective T2 immune response within the lung. *Am J Pathol* 2009;**174**:932–43.
112. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**:1101–11.
115. Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009;**23**:525–30.
116. Parkes-Ratanshi R, Wakeham K, Levin J, et al. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomised, placebo-controlled trial. *Lancet Infect Dis* 2011;**11**:933–41.
117. Patel M, Beckerman KP, Reznik S, et al. Transplacental transmission of *Cryptococcus neoformans* to an HIV-exposed premature neonate. *J Perinatol* 2012;**32**:235–7.
118. Patel NC, Ewanowski C, Kupiec-Banasikowska A, et al. Disseminated *Cryptococcus neoformans*: case report and review of the literature. *Cutis* 2009;**84**:93–6.
119. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
127. Santos WR, Meyer W, Wanke B, et al. Primary endemic *Cryptococcosis gattii* by molecular type VGII in the state of Para, Brazil. *Mem Inst Oswaldo Cruz* 2008;**103**:813–18.
128. Severo CB, Xavier MO, Gazzoni AF, et al. Cryptococcosis in children. *Paediatr Respir Rev* 2009;**10**:166–71.
131. Singh N, Alexander BD, Lortholary O, et al. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. *Clin Infect Dis* 2008;**46**:e12–18.
132. Singh N, Lortholary O, Dromer F, et al. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. *Transplantation* 2008; **86**:647–51.
136. Sun HY, Alexander BD, Lortholary O, et al. Cutaneous cryptococcosis in solid organ transplant recipients. *Med Mycol* 2010;**48**: 785–91.
137. Sun HY, Alexander BD, Lortholary O, et al. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. *Clin Infect Dis* 2010;**51**:1062–9.
138. Sun HY, Singh N. Opportunistic infection-associated immune reconstitution syndrome in transplant recipients. *Clin Infect Dis* 2011;**53**:168–76.
139. Sungkanuparph S, Filler SG, Chetchotisakd P, et al. Cryptococcal immune reconstitution inflammatory syndrome after antiretroviral therapy in AIDS patients with cryptococcal meningitis: a prospective multicenter study. *Clin Infect Dis* 2009;**49**:931–4.
141. Tan DB, Yong YK, Tan HY, et al. Immunological profiles of immune restoration disease presenting as mycobacterial lymphadenitis and cryptococcal meningitis. *HIV Med* 2008;**9**:307–16.
144. Van Tongeren L, Shaipanich T, Fleetham JA. Coinfection with *Cryptococcus gattii* and *Mycobacterium tuberculosis* in an otherwise healthy 18-year-old woman. *Can Respir J* 2011;**18**:e62–3.
146. Veron V, Simon S, Blanchet D, et al. Real-time polymerase chain reaction detection of *Cryptococcus neoformans* and *Cryptococcus gattii* in human samples. *Diagn Microbiol Infect Dis* 2009;**65**: 69–72.
149. Villena SN, Pinheiro RO, Pinheiro CS, et al. Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from *Cryptococcus neoformans* induce macrophage apoptosis mediated by Fas ligand. *Cell Microbiol* 2008;**10**:1274–85.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abadi J, Nachman S, Kressel AB, et al. Cryptococcosis in children with AIDS. *Clin Infect Dis* 1999;**28**:309–13.
2. Abe K, Kadota J, Ishimatsu Y, et al. Th1-Th2 cytokine kinetics in the bronchoalveolar lavage fluid of mice infected with *Cryptococcus neoformans* of different virulences. *Microbiol Immunol* 2000;**44**: 849–55.
3. Aberg JA, Mundy LM, Powderly WG. Pulmonary cryptococcosis in patients without HIV infection. *Chest* 1999;**115**:734–40.
4. Allende M, Pizzo PA, Horowitz M, et al. Pulmonary cryptococcosis presenting as metastases in children with sarcomas. *Pediatr Infect Dis J* 1993;**12**:240–3.
5. Alvarez M, Burn T, Luo Y, et al. The outcome of *Cryptococcus neoformans* intracellular pathogenesis in human monocytes. *BMC Microbiol* 2009;**9**:51.
6. Anekthananon T, Manosuthi W, Chetchotisakd P, et al. Predictors of poor clinical outcome of cryptococcal meningitis in HIV-infected patients. *Int J STD AIDS* 2011;**22**:665–70.
7. Araujo Gde S, Fonseca FL, Pontes B, et al. Capsules from pathogenic and non-pathogenic *Cryptococcus* spp. manifest significant differences in structure and ability to protect against phagocytic cells. *PLoS One* 2012;**7**:e29561.
8. Arnder L, Castillo M, Heinz ER, et al. Unusual pattern of enhancement in cryptococcal meningitis: in vivo findings with postmortem correlation. *J Comput Assist Tomogr* 1996;**20**:1023–6.
9. Arora S, Hernandez Y, Erb-Downward JR, et al. Role of IFN-gamma in regulating T2 immunity and the development of alternatively activated macrophages during allergic bronchopulmonary mycosis. *J Immunol* 2005;**174**:6346–56.
10. Asawavichienjinda T, Sitthi-Amorn C, Tanyanont V. Serum cyrptococcal antigen: diagnostic value in the diagnosis of AIDS-related cryptococcal meningitis. *J Med Assoc Thai* 1999;**82**:65–71.
11. Averbuch D, Boekhoutt T, Falk R, et al. Fungemia in a cancer patient caused by fluconazole-resistant *Cryptococcus laurentii*. *Med Mycol* 2002;**40**:479–84.
12. Bicanic T, Harrison TS. Cryptococcal meningitis. *Br Med Bull* 2004;**72**:99–118.
13. Bicanic T, Muzoora C, Brouwer AE, et al. Independent association between rate of clearance of infection and clinical outcome of HIV-associated cryptococcal meningitis: analysis of a combined cohort of 262 patients. *Clin Infect Dis* 2009;**49**:702–9.
14. Brizendine KD, Baddley JW, Pappas PG. Pulmonary cryptococcosis. *Semin Respir Crit Care Med* 2011;**32**:727–34.
15. Byrnes EJ 3rd, Li W, Lewit Y, et al. First reported case of *Cryptococcus gattii* in the southeastern USA: Implications for travel-associated acquisition of an emerging pathogen. *PLoS One* 2009; **4**:e5851.
16. Campbell GD. Primary pulmonary cryptococcosis. *Am Rev Respir Dis* 1966;**94**:236–43.
17. Campos JO. *Rev Clin Inst Matern Lisb* 1954;**6**:87–92. PMID: 14395719.
18. Chambers C, MacDougall L, Li M, et al. Tourism and specific risk areas for *Cryptococcus gattii*, Vancouver Island, Canada. *Emerg Infect Dis* 2008;**14**:1781–3.
19. Chang YC, Kwon-Chung KJ. Complementation of a capsule-deficient mutation of *Cryptococcus neoformans* restores its virulence. *Mol Cell Biol* 1994;**14**:4912–19.
20. Chang YC, Stins MF, McCaffery MJ, et al. Cryptococcal yeast cells invade the central nervous system via transcellular penetration of the blood-brain barrier. *Infect Immun* 2004;**72**:4985–95.
21. Chanock SJ, Toltzis P, Wilson C. Cross-reactivity between *Stomatococcus mucilaginosus* and latex agglutination for cryptococcal antigen. *Lancet* 1993;**342**:1119–20.
22. Charlier C, Dromer F, Leveque C, et al. Cryptococcal neuroradiological lesions correlate with severity during cryptococcal meningoencephalitis in HIV-positive patients in the HAART era. *PLoS One* 2008;**3**:e1950.
23. Charlier C, Nielsen K, Daou S, et al. Evidence of a role for monocytes in dissemination and brain invasion by *Cryptococcus neoformans*. *Infect Immun* 2009;**77**:120–7.
24. Chen GH, McDonald RA, Wells JC, et al. The gamma interferon receptor is required for the protective pulmonary inflammatory response to *Cryptococcus neoformans*. *Infect Immun* 2005;**73**:1788–96.
25. Chen SC, Slavin MA, Heath CH, et al. Clinical manifestations of *Cryptococcus gattii* infection: determinants of neurological sequelae and death. *Clin Infect Dis* 2012;**55**(6):789–98.
26. Chowdhary A, Rhandhawa HS, Prakash A, et al. Environmental prevalence of *Cryptococcus neoformans* and *Cryptococcus gattii* in India: an update. *Crit Rev Microbiol* 2012;**38**:1–16.
27. Clark RA, Greer DL, Valainis GT, et al. *Cryptococcus neoformans* pulmonary infection in HIV-1-infected patients. *J Acquir Immune Defic Syndr* 1990;**3**:480–4.
28. Clemons KV, Lutz JE, Stevens DA. Efficacy of recombinant gamma interferon for treatment of systemic cryptococcosis in SCID mice. *Antimicrob Agents Chemother* 2001;**45**:686–9.
29. Cohen J. Comparison of the sensitivity of three methods for the rapid identification of *Cryptococcus neoformans*. *J Clin Pathol* 1984; **37**:332–4.
30. Cox GM, McDade HC, Chen SC, et al. Extracellular phospholipase activity is a virulence factor for *Cryptococcus neoformans*. *Mol Microbiol* 2001;**39**:166–75.
31. Cox GM, Mukherjee J, Cole GT, et al. Urease as a virulence factor in experimental cryptococcosis. *Infect Immun* 2000;**68**:443–8.
32. Curtis JL, Huffnagle GB, Chen GH, et al. Experimental murine pulmonary cryptococcosis: differences in pulmonary inflammation and lymphocyte recruitment induced by two encapsulated strains of *Cryptococcus neoformans*. *Lab Invest* 1994;**71**:113–26.
33. D'Souza CA, Kronstad JW, Taylor G, et al. Genome variation in *Cryptococcus gattii*, an emerging pathogen of immunocompetent hosts. *MBio* 2011;**2**:e00342–10.
34. Darko AD, Dim DC, Taylor G, et al. Placental *Cryptococcus neoformans* infection without neonatal disease: case report and review of the literature. *Pediatr Dev Pathol* 2009;**12**:249–52.
35. Daunt N, Jayasinghe LS. Cerebral torulosis: clinical features and correlation with computed tomography. *Clin Radiol* 1985;**36**:485–90.
36. Diamond RD, Bennett JE. Prognostic factors in cryptococcal meningitis: a study in 111 cases. *Ann Intern Med* 1974;**80**:176–81.
37. Dong ZM, Murphy JW. Effects of the two varieties of *Cryptococcus neoformans* cells and culture filtrate antigens on neutrophil locomotion. *Infect Immun* 1995;**63**:2632–44.
38. Dromer F, Mathoulin-Pelissier S, Launay O, et al. Determinants of disease presentation and outcome during cryptococcosis: the CryptoA/D study. *PLoS Med* 2007;**4**:e21.
39. Ecevit IZ, Clancy CJ, Schmalfuss IM, et al. The poor prognosis of central nervous system cryptococcosis among nonimmunosuppressed patients: a call for better disease recognition and evaluation of adjuncts to antifungal therapy. *Clin Infect Dis* 2006;**42**: 1443–7.
40. Einsiedel L, Gordon DL, Dyer JR. Paradoxical inflammatory reaction during treatment of *Cryptococcus neoformans* var. *gattii* meningitis in an HIV-seronegative woman. *Clin Infect Dis* 2004; **39**:e78–82.
41. Enoki E, Maenishi O, Chikugo T, et al. Coinfection of *Aspergillus* and *Cryptococcus* in post-tuberculosis pulmonary cavity. *Pathol Int* 2012;**62**:574–6.
42. Erb-Downward JR, Huffnagle GB. *Cryptococcus neoformans* produces authentic prostaglandin E2 without a cyclooxygenase. *Eukaryot Cell* 2007;**6**:346–50.
43. Fries BC, Lee SC, Kennan R, et al. Phenotypic switching of *Cryptococcus neoformans* can produce variants that elicit increased intracranial pressure in a rat model of cryptococcal meningoencephalitis. *Infect Immun* 2005;**73**:1779–87.
44. Galanis E, Macdougall L. Epidemiology of *Cryptococcus gattii*, British Columbia, Canada, 1999-2007. *Emerg Infect Dis* 2010;**16**: 251–7.
45. Garcia-Hermoso D, Janbon G, Dromer F. Epidemiological evidence for dormant *Cryptococcus neoformans* infection. *J Clin Microbiol* 1999;**37**:3204–9.
46. Garcia-Rodas R, Casadevall A, Rodriguez-Tudela JL, et al. *Cryptococcus neoformans* capsular enlargement and cellular gigantism during *Galleria mellonella* infection. *PLoS One* 2011;**6**:e24485.
47. Gluck JL, Myers JP, Pass LM. Cryptococcemia due to *Cryptococcus albidus*. *South Med J* 1987;**80**:511–13.
48. Goldman DL, Fries BC, Franzot SP, et al. Phenotypic switching in the human pathogenic fungus *Cryptococcus neoformans* is associated with changes in virulence and pulmonary inflammatory

response in rodents. *Proc Natl Acad Sci U S A* 1998;**95**:14967–72.
49. Goldman DL, Khine H, Abadi J, et al. Serologic evidence for *Cryptococcus neoformans* infection in early childhood. *Pediatrics* 2001;**107**:E66.
50. Gonzalez CE, Shetty D, Lewis LL, et al. Cryptococcosis in human immunodeficiency virus-infected children. *Pediatr Infect Dis J* 1996;**15**:796–800.
51. Govender NP, Patel J, van Wyk M, et al. Trends in antifungal drug susceptibility of *Cryptococcus neoformans* isolates obtained through population-based surveillance in South Africa in 2002-2003 and 2007-2008. *Antimicrob Agents Chemother* 2011;**55**:2606–11.
52. Graybill JR, Sobel J, Saag M, et al. Diagnosis and management of increased intracranial pressure in patients with AIDS and cryptococcal meningitis. The NIAID Mycoses Study Group and AIDS Cooperative Treatment Groups. *Clin Infect Dis* 2000;**30**: 47–54.
53. Gumbo T, Kadzirange G, Mielke J, et al. *Cryptococcus neoformans* meningoencephalitis in African children with acquired immunodeficiency syndrome. *Pediatr Infect Dis J* 2002;**21**:54–6.
54. Hage CA, Wood KL, Winer-Muram HT, et al. Pulmonary cryptococcosis after initiation of anti-tumor necrosis factor-alpha therapy. *Chest* 2003;**124**:2395–7.
55. Hagen F, van Assen S, Luijckx GJ, et al. Activated dormant *Cryptococcus gattii* infection in a Dutch tourist who visited Vancouver Island (Canada): a molecular epidemiological approach. *Med Mycol* 2010;**48**:528–31.
56. Harris JR, Lockhart SR, Debess E, et al. *Cryptococcus gattii* in the United States: clinical aspects of infection with an emerging pathogen. *Clin Infect Dis* 2011;**53**:1188–95.
57. Haugen RK, Baker RD. The pulmonary lesions in cryptococcosis with special reference to subpleural nodules. *Am J Clin Pathol* 1954;**24**:1381–90.
58. Herrmann JL, Dubois N, Fourgeaud M, et al. Synergic inhibitory activity of amphotericin-B and gamma interferon against intracellular *Cryptococcus neoformans* in murine macrophages. *J Antimicrob Chemother* 1994;**34**:1051–8.
59. Hirano A, Zimmerman HM, Levine S. The fine structure of cerebral fluid accumulation, part III: extracellular spread of cryptococcal polysaccharides in the acute stage. *Am J Pathol* 1964; **45**:1–19.
60. Hoag KA, Lipscomb MF, Izzo AA, et al. IL-12 and IFN-gamma are required for initiating the protective Th1 response to pulmonary cryptococcosis in resistant C.B-17 mice. *Am J Respir Cell Mol Biol* 1997;**17**:733–9.
61. Huang KY, Huang YC, Hung IJ, et al. Cryptococcosis in nonhuman immunodeficiency virus-infected children. *Pediatr Neurol* 2010;**42**:267–70.
62. Huffnagle GB, Chen GH, Curtis JL, et al. Down-regulation of the afferent phase of T cell-mediated pulmonary inflammation and immunity by a high melanin-producing strain of *Cryptococcus neoformans*. *J Immunol* 1995;**155**:3507–16.
63. Hull CM, Heitman J. Genetics of *Cryptococcus neoformans*. *Ann Rev Genet* 2002;**36**:557–615.
64. Husain S, Wagener MM, Singh N. *Cryptococcus neoformans* infection in organ transplant recipients: variables influencing clinical characteristics and outcome. *Emerg Infect Dis* 2001;**7**:375–81.
65. Ibrahim AS, Filler SG, Alcouloumre MS, et al. Adherence to and damage of endothelial cells by *Cryptococcus neoformans* in vitro: role of the capsule. *Infect Immun* 1995;**63**:4368–74.
66. Iseki M, Anzo M, Yamashita N, et al. Hyper-IgM immunodeficiency with disseminated cryptococcosis. *Acta Paediatr* 1994;**83**: 780–2.
67. Jarvis JN, Meintjes G, Rebe K, et al. Adjunctive interferon-gamma immunotherapy for the treatment of HIV-associated cryptococcal meningitis: a randomized controlled trial. *AIDS* 2012;**26**:1105–13.
68. Jarvis JN, Meintjes G, Williams A, et al. Adult meningitis in a setting of high HIV and TB prevalence: findings from 4961 suspected cases. *BMC Infect Dis* 2010;**10**:67.
69. Jenny-Avital ER, Abadi M. Immune reconstitution cryptococcosis after initiation of successful highly active antiretroviral therapy. *Clin Infect Dis* 2002;**35**:e128–33.
70. Joshi NS, Fisher BT, Prasad PA, et al. Epidemiology of cryptococcal infection in hospitalized children. *Pediatr Infect Dis J* 2010;**29**: e91–5.
71. Kambugu A, Meya DB, Rhein J, et al. Outcomes of cryptococcal meningitis in Uganda before and after the availability of highly active antiretroviral therapy. *Clin Infect Dis* 2008;**46**:1694–701.
72. Kaur R, Mittal N, Rawat D, et al. Cryptococcal meningitis in a neonate. *Scand J Infect Dis* 2002;**34**:542–3.
73. Kawakami K, Tohyama M, Teruya K, et al. Contribution of interferon-gamma in protecting mice during pulmonary and disseminated infection with *Cryptococcus neoformans*. *FEMS Immunol Med Microbiol* 1996;**13**:123–30.
74. Kerkering TM, Duma RJ, Shadomy S. The evolution of pulmonary cryptococcosis: clinical implications from a study of 41 patients with and without compromising host factors. *Ann Intern Med* 1981;**94**:611–16.
75. Khoury MB, Godwin JD, Ravin CE, et al. Thoracic cryptococcosis: immunologic competence and radiologic appearance. *AJR Am J Roentgenol* 1984;**142**:893–6.
76. Kontoyiannis DP, Peitsch WK, Reddy BT, et al. Cryptococcosis in patients with cancer. *Clin Infect Dis* 2001;**32**:E145–50.
77. Kozel TR, Gotschlich EC. The capsule of *Cryptococcus neoformans* passively inhibits phagocytosis of the yeast by macrophages. *J Immunol* 1982;**129**:1675–80.
78. Krockenberger MB, Malik R, Ngamskulrungroj P, et al. Pathogenesis of pulmonary *Cryptococcus gattii* infection: a rat model. *Mycopathologia* 2010;**170**:315–30.
79. Kwon-Chung KJ, Edman JC, Wickes BL. Genetic association of mating types and virulence in *Cryptococcus neoformans*. *Infect Immun* 1992;**60**:602–5.
80. Larsen RA, Bozzette S, McCutchan JA, et al. Persistent *Cryptococcus neoformans* infection of the prostate after successful treatment of meningitis. California Collaborative Treatment Group. *Ann Intern Med* 1989;**111**:125–8.
81. Larsen RA, Pappas PG, Perfect J, et al. Phase I evaluation of the safety and pharmacokinetics of murine-derived anticryptococcal antibody 18B7 in subjects with treated cryptococcal meningitis. *Antimicrob Agents Chemother* 2005;**49**:952–8.
82. Lee SC, Casadevall A. Polysaccharide antigen in brain tissue of AIDS patients with cryptococcal meningitis. *Clin Infect Dis* 1996;**23**:194–5.
83. Lee SC, Dickson DW, Casadevall A. Pathology of cryptococcal meningoencephalitis: analysis of 27 patients with pathogenetic implications. *Hum Pathol* 1996;**27**:839–47.
84. Leggiadro RJ, Barrett FF, Hughes WT. Extrapulmonary cryptococcosis in immunocompromised infants and children. *Pediatr Infect Dis J* 1992;**11**:43–7.
85. Lesho E. Evidence base for using corticosteroids to treat HIV-associated immune reconstitution syndrome. *Expert Rev Anti Infect Ther* 2006;**4**:469–78.
86. Levitz SM, Nong SH, Seetoo KF, et al. *Cryptococcus neoformans* resides in an acidic phagolysosome of human macrophages. *Infect Immun* 1999;**67**:885–90.
87. Liao CH, Chi CY, Wang YJ, et al. Different presentations and outcomes between HIV-infected and HIV-uninfected patients with cryptococcal meningitis. *J Microbiol Immunol Infect* 2012; **45**:296–304.
88. Liechty CA, Solberg P, Were W, et al. Asymptomatic serum cryptococcal antigenemia and early mortality during antiretroviral therapy in rural Uganda. *Trop Med Int Health* 2007;**12**:929–35.
89. Likasitwattanakul S, Poneprasert B, Sirisanthana V. Cryptococcosis in HIV-infected children. *Southeast Asian J Trop Med Public Health* 2004;**35**:935–9.
90. Lindsley MD, Mekha N, Baggett HC, et al. Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. *Clin Infect Dis* 2011;**53**:321–5.
91. Littman ML, Schneierson SS. *Cryptococcus neoformans* in pigeon excreta in New York City. *Am J Hyg* 1959;**69**:49–59.
92. Lopez-Martinez R, Castanon-Olivares LR. Isolation of *Cryptococcus neoformans* var. *neoformans* from bird droppings, fruits and vegetables in Mexico City. *Mycopathologia* 1995;**129**:25–8.
93. Lortholary O, Fontanet A, Memain N, et al. Incidence and risk factors of immune reconstitution inflammatory syndrome complicating HIV-associated cryptococcosis in France. *AIDS* 2005;**19**: 1043–9.
94. Makadzange AT, Ndhlovu CE, Takarinda K, et al. Early versus delayed initiation of antiretroviral therapy for concurrent HIV infection and cryptococcal meningitis in sub-Saharan Africa. *Clin Infect Dis* 2010;**50**:1532–8.

95. Marr KA, Datta K, Pirofski LA, et al. *Cryptococcus gattii* infection in healthy hosts: a sentinel for subclinical immunodeficiency? *Clin Infect Dis* 2012;**54**:153–4.
96. Maruvada R, Zhu L, Pearce D, et al. *Cryptococcus neoformans* phospholipase B1 activates host cell Rac1 for traversal across the blood-brain barrier. *Cell Microbiol* 2012;**14**(10):1544–53.
97. McCulloh RJ, Phillips R, Perfect JR, et al. *Cryptococcus gattii* genotype VGI infection in New England. *Pediatr Infect Dis J* 2011; **30**:1111–14.
98. McManus EJ, Jones JM. Detection of a *Trichosporon beigelii* antigen cross-reactive with *Cryptococcus neoformans* capsular polysaccharide in serum from a patient with disseminated *Trichosporon* infection. *J Clin Microbiol* 1985;**21**:681–5.
99. Meiring ST, Quan VC, Cohen C, et al; Group for Enteric, Respiratory and Meningeal disease Surveillance in South Africa (GERMS-SA). A comparison of cases of paediatric-onset and adult-onset cryptococcosis detected through population-based surveillance, 2005-2007. *AIDS* 2012;**26**(18):2307–14.
100. Meya DB, Manabe YC, Castelnuovo B, et al. Cost-effectiveness of serum cryptococcal antigen screening to prevent deaths among HIV-infected persons with a CD4+ cell count ≤100 cells/μl who start HIV therapy in resource-limited settings. *Clin Infect Dis* 2010;**51**:448–55.
101. Miglia KJ, Govender NP, Rossouw J, et al. Analyses of pediatric isolates of *Cryptococcus neoformans* from South Africa. *J Clin Microbiol* 2011;**49**:307–14.
102. Mitchell DH, Sorrell TC, Allworth AM, et al. Cryptococcal disease of the CNS in immunocompetent hosts: influence of cryptococcal variety on clinical manifestations and outcome. *Clin Infect Dis* 1995;**20**:611–16.
103. Mitchell TG, Perfect JR. Cryptococcosis in the era of AIDS—100 years after the discovery of *Cryptococcus neoformans*. *Clin Microbiol Rev* 1995;**8**:515–48.
104. Mukherjee J, Pirofski LA, Scharff MD, et al. Antibody-mediated protection in mice with lethal intracerebral *Cryptococcus neoformans* infection. *Proc Natl Acad Sci U S A* 1993;**90**:3636–40.
105. Nagotkar L, Shanbag P, Mauskar A, et al. Fulminant intracranial hypertension due to cryptococcal meningitis in a child with nephrotic syndrome. *Indian J Crit Care Med* 2011;**15**:176–8.
106. Nasser N, Nasser Filho N, Vieira AG. Primary cutaneous cryptococcosis in an immunocompetent patient. *An Bras Dermatol* 2011;**86**:1178–80.
107. Nosanchuk JD, Casadevall A. The contribution of melanin to microbial pathogenesis. *Cell Microbiol* 2003;**5**:203–23.
108. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin Infect Dis* 2010;**50**:338–44.
109. Odom A, Muir S, Lim E, et al. Calcineurin is required for virulence of *Cryptococcus neoformans*. *EMBO J* 1997;**16**:2576–89.
110. Osterholzer JJ, Surana R, Milam JE, et al. Cryptococcal urease promotes the accumulation of immature dendritic cells and a non-protective T2 immune response within the lung. *Am J Pathol* 2009;**174**:932–43.
111. Pan AH, Janner D. Lung mass in a sixteen-year-old young man. *Pediatr Infect Dis J* 2003;**22**:293–4, 296.
112. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**:1101–11.
113. Pappas PG, Bustamante B, Ticona E, et al. Recombinant interferon-gamma 1b as adjunctive therapy for AIDS-related acute cryptococcal meningitis. *J Infect Dis* 2004;**189**:2185–91.
114. Pappas PG, Perfect JR, Cloud GA, et al. Cryptococcosis in human immunodeficiency virus-negative patients in the era of effective azole therapy. *Clin Infect Dis* 2001;**33**:690–9.
115. Park BJ, Wannemuehler KA, Marston BJ, et al. Estimation of the current global burden of cryptococcal meningitis among persons living with HIV/AIDS. *AIDS* 2009;**23**:525–30.
116. Parkes-Ratanshi R, Wakeham K, Levin J, et al. Primary prophylaxis of cryptococcal disease with fluconazole in HIV-positive Ugandan adults: a double-blind, randomised, placebo-controlled trial. *Lancet Infect Dis* 2011;**11**:933–41.
117. Patel M, Beckerman KP, Reznik S, et al. Transplacental transmission of *Cryptococcus neoformans* to an HIV-exposed premature neonate. *J Perinatol* 2012;**32**:235–7.
118. Patel NC, Ewanowski C, Kupiec-Banasikowska A, et al. Disseminated *Cryptococcus neoformans*: case report and review of the literature. *Cutis* 2009;**84**:93–6.
119. Perfect JR, Dismukes WE, Dromer F, et al. Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:291–322.
120. Pettoello-Mantovani M, Casadevall A, Kollmann TR, et al. Enhancement of HIV-1 infection by the capsular polysaccharide of *Cryptococcus neoformans*. *Lancet* 1992;**339**:21–3.
121. Polacheck I, Platt Y, Aronovitch J. Catecholamines and virulence of *Cryptococcus neoformans*. *Infect Immun* 1990;**58**:2919–22.
122. Popovich MJ, Arthur RH, Helmer E. CT of intracranial cryptococcosis. *AJR Am J Roentgenol* 1990;**154**:603–6.
123. Rosas AL, Nosanchuk JD, Feldmesser M, et al. Synthesis of polymerized melanin by *Cryptococcus neoformans* in infected rodents. *Infect Immun* 2000;**68**:2845–53.
124. Saag MS, Powderly WG, Cloud GA, et al. Comparison of amphotericin B with fluconazole in the treatment of acute AIDS-associated cryptococcal meningitis. The NIAID Mycoses Study Group and the AIDS Clinical Trials Group. *N Engl J Med* 1992; **326**:83–9.
125. Saha DC, Goldman DL, Shao X, et al. Serologic evidence for reactivation of cryptococcosis in solid-organ transplant recipients. *Clin Vaccine Immunol* 2007;**14**:1550–4.
126. Sanford JE, Lupan DM, Schlageter AM, et al. Passive immunization against *Cryptococcus neoformans* with an isotype-switch family of monoclonal antibodies reactive with cryptococcal polysaccharide. *Infect Immun* 1990;**58**:1919–23.
127. Santos WR, Meyer W, Wanke B, et al. Primary endemic *Cryptococcosis gattii* by molecular type VGII in the state of Para, Brazil. *Mem Inst Oswaldo Cruz* 2008;**103**:813–18.
128. Severo CB, Xavier MO, Gazzoni AF, et al. Cryptococcosis in children. *Paediatr Respir Rev* 2009;**10**:166–71.
129. Shao X, Mednick A, Alvarez M, et al. An innate immune system cell is a major determinant of species-related susceptibility differences to fungal pneumonia. *J Immunol* 2005;**175**:3244–51.
130. Shelburne SA, Visnegarwala F, Darcourt J, et al. Incidence and risk factors for immune reconstitution inflammatory syndrome during highly active antiretroviral therapy. *AIDS* 2005;**19**:399–406.
131. Singh N, Alexander BD, Lortholary O, et al. Pulmonary cryptococcosis in solid organ transplant recipients: clinical relevance of serum cryptococcal antigen. *Clin Infect Dis* 2008;**46**:e12–18.
132. Singh N, Lortholary O, Dromer F, et al. Central nervous system cryptococcosis in solid organ transplant recipients: clinical relevance of abnormal neuroimaging findings. *Transplantation* 2008; **86**:647–51.
133. Singh N, Perfect JR. Immune reconstitution syndrome associated with opportunistic mycoses. *Lancet Infect Dis* 2007;**7**:395–401.
134. Speed B, Dunt D. Clinical and host differences between infections with the two varieties of *Cryptococcus neoformans*. *Clin Infect Dis* 1995;**21**:28–34; discussion 35–6.
135. Stone BD, Wheeler JG. Disseminated cryptococcal infection in a patient with hyperimmunoglobulinemia E syndrome. *J Pediatr* 1990;**117**:92–5.
136. Sun HY, Alexander BD, Lortholary O, et al. Cutaneous cryptococcosis in solid organ transplant recipients. *Med Mycol* 2010;**48**: 785–91.
137. Sun HY, Alexander BD, Lortholary O, et al. Unrecognized pretransplant and donor-derived cryptococcal disease in organ transplant recipients. *Clin Infect Dis* 2010;**51**:1062–9.
138. Sun HY, Singh N. Opportunistic infection–associated immune reconstitution syndrome in transplant recipients. *Clin Infect Dis* 2011;**53**:168–76.
139. Sungkanuparph S, Filler SG, Chetchotisakd P, et al. Cryptococcal immune reconstitution inflammatory syndrome after antiretroviral therapy in AIDS patients with cryptococcal meningitis: a prospective multicenter study. *Clin Infect Dis* 2009;**49**:931–4.
140. Sweeney DA, Caserta MT, Korones DN, et al. A ten-year-old boy with a pulmonary nodule secondary to *Cryptococcus neoformans*: case report and review of the literature. *Pediatr Infect Dis J* 2003;**22**:1089–93.
141. Tan DB, Yong YK, Tan HY, et al. Immunological profiles of immune restoration disease presenting as mycobacterial lymphadenitis and cryptococcal meningitis. *HIV Med* 2008;**9**:307–16.

142. Thakur K, Singh G, Agarwal S, et al. Meningitis caused by *Rhodotorula rubra* in an human immunodeficiency virus infected patient. *Indian J Med Microbiol* 2007;**25**:166–8.
143. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Mycoses Study Group and AIDS Clinical Trials Group. *N Engl J Med* 1997;**337**:15–21.
144. Van Tongeren L, Shaipanich T, Fleetham JA. Coinfection with *Cryptococcus gattii* and *Mycobacterium tuberculosis* in an otherwise healthy 18-year-old woman. *Can Respir J* 2011;**18**:e62–3.
145. Vecchiarelli A, Retini C, Monari C, et al. Purified capsular polysaccharide of *Cryptococcus neoformans* induces interleukin-10 secretion by human monocytes. *Infect Immun* 1996;**64**:2846–9.
146. Veron V, Simon S, Blanchet D, et al. Real-time polymerase chain reaction detection of *Cryptococcus neoformans* and *Cryptococcus gattii* in human samples. *Diagn Microbiol Infect Dis* 2009;**65**:69–72.
147. Vidotto V, Sinicco A, Di Fraia D, et al. Phospholipase activity in *Cryptococcus neoformans*. *Mycopathologia* 1996;**136**:119–23.
148. Vilchez RA, Fung J, Kusne S. Cryptococcosis in organ transplant recipients: an overview. *Am J Transplant* 2002;**2**:575–80.
149. Villena SN, Pinheiro RO, Pinheiro CS, et al. Capsular polysaccharides galactoxylomannan and glucuronoxylomannan from *Cryptococcus neoformans* induce macrophage apoptosis mediated by Fas ligand. *Cell Microbiol* 2008;**10**:1274–85.
150. Wahab JA, Hanifah MJ, Choo KE. Bruton's agammaglobulinaemia in a child presenting with cryptococcal empyema thoracis and periauricular pyogenic abscess. *Singapore Med J* 1995;**36**:686–9.
151. Wang Y, Aisen P, Casadevall A. Melanin, melanin "ghosts," and melanin composition in *Cryptococcus neoformans*. *Infect Immun* 1996;**64**:2420–4.
152. Wright LC, Santangelo RM, Ganendren R, et al. Cryptococcal lipid metabolism: phospholipase B1 is implicated in transcellular metabolism of macrophage-derived lipids. *Eukaryot Cell* 2007;**6**:37–47.
153. Yauch LE, Lam JS, Levitz SM. Direct inhibition of T-cell responses by the *Cryptococcus* capsular polysaccharide glucuronoxylomannan. *PLoS Pathog* 2006;**2**:e120.

CHAPTER 203

Histoplasmosis

Martin B. Kleiman

Histoplasmosis is the most common pulmonary and systemic mycosis in humans and affects millions of people.[17,69,238,506] Although most infections occur in areas where the etiologic agent, *Histoplasma capsulatum*, is highly endemic, histoplasmosis is being recognized in areas not previously known to be endemic. A substantial proportion of infections with *H. capsulatum* occur in the United States, where approximately 500,000 individuals are infected annually.[487] Of these, an estimated 55,000 to 200,000 infected individuals become symptomatic and 1,500 to 4,000 require hospitalization.[77] The significance of *H. capsulatum* as a cause of opportunistic infection has escalated in proportion to the increasing numbers of individuals who are immunosuppressed.[304]

H. capsulatum was described first in 1906 by Darling[239] while he was examining autopsy specimens of a man from Martinique who died of a chronic, wasting illness. He observed organisms within histiocytes and thought them to be encapsulated plasmodia; the illness caused by this previously unrecognized pathogen was named reticuloendothelial cytomycosis.[239] In 1932, Dodd and Tompkins described intracellular *H. capsulatum* in the peripheral blood smear of a febrile child and DeMonbreum isolated the organism from this child and correctly classified it as a fungus. In 1944, Christie and Peterson[76] developed histoplasmin and, after cutaneous inoculation, showed a localized reaction in a child with histoplasmosis.

In 1945, Parsons and Zarafonetis characterized the disease as rare and usually fatal after studying 71 patients with disseminated histoplasmosis. Using histoplasmin skin testing, however, Christie and Peterson[76] showed histoplasmosis to be a common and usually a mild disease. They also determined that histoplasmosis was the cause of pulmonary calcifications in patients who were presumed to have had tuberculosis, but in whom tuberculin skin tests were negative. Demonstration of the organism in soil and air samples in the late 1940s and early 1950s confirmed the natural habitat and transmission of the pathogen.[13,382,488]

THE ORGANISM

Histoplasma capsulatum var. *capsulatum*, the anamorphic (asexual) form of the organism, is a thermally dimorphic, saprophytic fungus of the class Ascomycetes, family Gymnoascaceae, subdivision Ascomycotina. The mold form displays heterothallic sexual reproduction with plus (+) and minus (–) mating types. Ninety percent of infections are caused by the minus type. The ascomycetous teleomorph produced by mating opposite types is termed *Emmonsiella capsulata*; the current taxonomy of this perfect (sexual) form is *Ajellomyces capsulatus*. Because the fungus is isolated in the laboratory as the anamorph, the more common name *H. capsulatum* is used in this text.

At temperatures of 25° C to 30° C, the mold grows as a fluffy colony with an aerial mycelium that varies from white to buff brown. Mycelia, small oval microconidia (2 to 4 μm) that are attached laterally to hyphae, and tuberculate and nontuberculate macroconidia (8 to 16 μm) are seen microscopically (Fig. 203-1). Growth is slow, with 1 to 2 weeks required for laboratory strains and 8 to 12 weeks for growth from clinical specimens. When cultured at 37° C on enriched media containing cysteine, the mold transforms to the yeast form in 7 to 10 days. The small, heaped, yellow-white, pasty colonies appear microscopically as ovoid, budding yeasts (1 to 3 μm × 3 to 5 μm) with rare pseudohyphae.[332]

In its natural habitat, the soil, *H. capsulatum* exists as spore-bearing mycelia. It survives best in moist (95% to 100% humidity), nitrogen-rich soil at temperatures of 37° C or greater and can be found 1 mile below the soil surface.[467,487] The droppings of chickens, blackbirds, and starlings contain nutrients that promote growth, and, at least in the case of chickens, may contain substances that discourage the growth of competitive organisms. Many species of colonial New World bats become infected and excrete the fungus in their guano, which contains substances that encourage fungal growth.[134,277,442,443] The migratory behavior of bats has been shown to be associated with genetic variation and both the geographic distribution and the ecotype dispersion of *H. capsulatum*.[444] Birds excrete the fungus in droppings yet remain uninfected, probably because of their high body temperature. *H. capsulatum* can remain viable in droppings or other contaminated sites for many years.[516]

Early techniques used to differentiate strains of *H. capsulatum* included serotyping and chemotyping.[118] Examinations of restriction fragment length polymorphisms of DNA have delineated three broad classes that exhibit geographic and possibly clinical differences.[126,242,470] Class 2 strains are found most frequently in North America, and class 3 strains are found in Central and South America. Class 1 strains are isolated almost exclusively from immunocompromised patients. Individual strains within each class have been examined further with additional nucleic acid–based and genome-wide approaches to reveal considerable genetic diversity.[68,220,312,444] Such strain differences are useful for molecular epidemiologic investigations.[220,311,441,445,537] Studies have suggested that

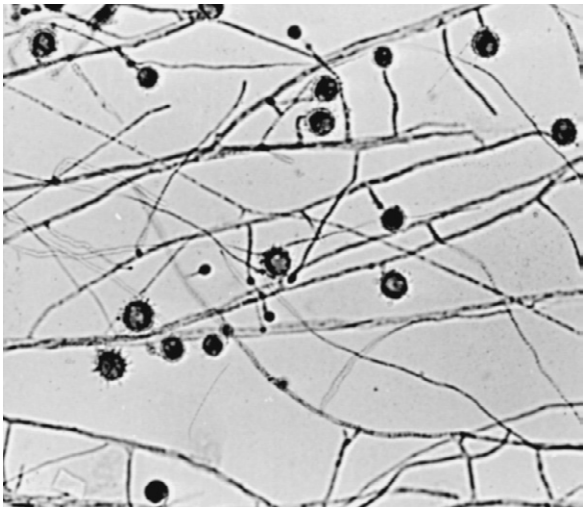

FIGURE 203-1 ■ Culture of *Histoplasma capsulatum* from sputum illustrating tuberculate macroaleuriospores and microaleuriospores (lactophenol cotton blue, ×52).

patients with acquired immunodeficiency syndrome (AIDS) may be infected with less virulent, temperature-sensitive variants of *H. capsulatum*.[427] Additional investigations using molecular methodology have suggested that genetic differences in the pathogen may underlie clinical differences.[197,235,313]

Histoplasma duboisii, the cause of African histoplasmosis, was reclassified as a variant of *H. capsulatum* when it was shown that mating of isolates from each type produced cleistothecia and ascospores identical to those of *E. capsulata* (now termed *A. capsulatus*).[382] *H. capsulatum* var. *duboisii* is indistinguishable from *H. capsulatum* var. *capsulatum* in its mycelial stage; in its tissue phase it appears as thick-walled oval yeast forms that are 10 to 15 μm in diameter. Infections caused by *H. capsulatum* var. *duboisii* have been described across central and western Africa, and cases occur along with histoplasmosis caused by *H. capsulatum*.[179,281,332] A more recent phylogeographic analysis[235] of both varieties that cause human disease and another, *H. capsulatum* var. *farciminosum* (an Old World horse pathogen), using DNA sequencing of four independent protein-coding genes questioned the validity of the classification.[65]

EPIDEMIOLOGY

Histoplasmosis is almost always acquired when sites contaminated with *H. capsulatum* are disturbed and spores are aerosolized and inhaled. The early understanding of its global epidemiology and clinical signs was skewed by analyses of localized outbreaks after exposure to large inocula. A more accurate picture emerged when skin test epidemiology, environmental cultures, vectors, and analyses of patient exposures were considered.[65]

Histoplasmosis has been reported worldwide[303] in tropical, subtropical, and, most frequently, temperate areas. Regions with high rates of infection are considered endemic. The majority of illnesses are sporadic and unassociated with exposure to a specific site or specific activity. They are presumed to result when environmental conditions are conducive to fungal growth and when dry, windy conditions or other events or activities facilitate the aerosolization of spores. Although early reports emphasized that the infection usually was associated with occupational exposure in primarily rural environments, infection is recognized commonly in urban areas,[221,283] where it may affect many people.[510] Asymptomatic infections are common in children and are usually unrecognized.[50,185,212] In a highly endemic area, skin test reactivity was 34 percent in children younger than 2 years, 55 percent between 2 and 4 years, and 77 percent in 10-year-olds.[538] Rates accelerated in summer.

The incidence of infection varies widely in endemic regions. Within short distances of areas in which residents show a moderate rate of skin test reactivity may be those in which the incidence is considerably higher. Still more localized and very heavily contaminated sites, termed *microfoci*, may be found in which localized, sometimes severe, outbreaks occur. Because the droppings of birds and bats[26,285] accelerate growth of the mycelial form in the environment, implicated sites have included blackbird and pigeon roosting areas, chicken houses, bat-infested caves,[214] attics, chimneys, old structures, and decaying woodpiles and trees. Activities that disturb these areas have been implicated in localized outbreaks.[212,363,487] Infections in children have been associated with exploring caves, playing in barns or hollow trees, cleaning abandoned buildings, cutting firewood or decayed tree stumps, renovation of older homes, digging in contaminated sites, excavation/demolition of buildings, cleaning seldom-used fireplaces, and exposure to fungal aerosols that gain access to air intakes.[70,283]

The geographic distribution of *H. capsulatum* skin test reactivity among 275,558 U.S. Navy recruits was determined[131,130] between 1958 and 1965. The highest incidence was found in residents of the Ohio-Mississippi-Missouri, the St. Lawrence, and the Rio Grande River Valleys. In four states (Arkansas, Kentucky, Missouri, and Tennessee), the percentage of positive adult reactors ranged from 57 to 68 percent. In the adjacent states (Illinois, Indiana, Ohio, and Oklahoma), reactivity ranged from 50 to 73 percent among adults in farm areas. Of the other seven states in the endemic area, lifetime prevalence rates exceeding 50 percent were shown in one or more counties. Epidemiologic studies have not been repeated, and population shifts and changes in land use may alter these patterns.[258] In Latin America,[66] areas of high prevalence include Venezuela,[293] Colombia,[23,314] Ecuador,[339] Brazil,[33] Paraguay, Uruguay, and Argentina.[86,293] Disseminated histoplasmosis is often recognized as AIDS defining.[315]

Analyses of point-source and sustained outbreaks offer some means of assessing the epidemiology and clinical spectrum of infections. An outbreak in Indianapolis, Indiana, affected an estimated 100,000 individuals within a 400-square-mile area and lasted nearly 1 year.[510] Illnesses were identified in 435 individuals (including 49 children younger than 15 years old), and disseminated disease developed in 46. Those aged 15 to 34 years were more likely to become infected than were other age groups. An equal sex distribution of infections

occurred before puberty; males predominated in a 3:1 ratio in older individuals. Localized outbreaks related to high-risk sites and activities[60,395,464] are well documented. Isolated cases may not be associated readily with such activities. Infections acquired during travel* and/or migration[17,54,215,334] to endemic regions[20,137,154,327,477] are being reported more frequently. Infections acquired in areas not recognized as endemic, such as occurred in college students whose only known common exposure was vacationing at a hotel in Mexico,[309] require consideration of the diagnosis in patients with compatible clinical findings.[18,57,63,394] Rarely, infections occur in nonendemic areas and may result from reactivation of quiescent infection in patients who become immunosuppressed.[17,53,218,457]

Although various wild[243] and domestic[51] animals may become infected, transmission of histoplasmosis to humans does not occur. Infection of an infant after exposure to a pillow that contained contaminated feathers has been reported.[64] Vertical transmission, in an area not endemic for histoplasmosis, of both disseminated histoplasmosis and HIV infection has been reported in the 5-week-old infant of a mother with both HIV infection and disseminated histoplasmosis.[14] Although a case of sexual transmission that resulted from exposure to mucosal lesions of a patient with disseminated histoplasmosis has been described,[415] human-to-human transmission is exceedingly rare. Transmission has been confirmed, with the use of molecular typing methodology, in the recipients of two cadaveric organs from a donor who had resided in an endemic area.[270] Infections caused by inoculation of contaminated material from either the environment or laboratory accidents are also rare.[55]

PATHOPHYSIOLOGY

H. capsulatum has unique interactions with human macrophages, dendritic cells, and neutrophils,[322,326,408] the outcomes of which contribute to its success as a pathogen. Key are its ability to infect and gain access to macrophages[526]; alter the intracellular environment thereby allowing it to survive, replicate, and disseminate[103,129,171]; remain viable during clinically unapparent infection; and become reactivated when host immune factors permit.[61] Inhalation of fungal spores induces a brisk innate immune response that is followed by an acquired immune response after about 2 weeks. During these events, the fungus induces an orderly modulation of the host inflammatory and cytokine and chemokine responses.[251] Production of cytokines begins within 24 hours of primary infection and continues for approximately 3 weeks, bridging both the innate and acquired immune responses. Successful clearance of the infection depends primarily on the functional integrity of cellular immunity.† The clinical manifestations and outcome are determined by this complex interplay of events. Variation of responses to infection in inbred murine models has shown that genetic factors both influence shifts of immune cell populations and alter disease outcomes.[294]

The length of the incubation period of histoplasmosis varies inversely with the size of the inoculum, the integrity of the host immune response, the presence of immunity from previous infection, and strain-related differences in fungal virulence.[128,168,248] Although the range of incubation periods is reported to be 1 to 3 weeks in nonimmune hosts, it has been based largely on data from point-source outbreaks in which the time of the event leading to exposure is readily determined. In these settings, the size of the infecting inocula is probably larger, however, than those resulting in sporadic infections.[65,98] Because most infections occur sporadically and either are asymptomatic or result in nonspecific and self-limited flulike illnesses that are not diagnosed, the upper range may be longer. In patients who retain specific cellular (protective) immunity from previous infection, reexposure results in milder symptoms and shorter incubation periods, usually 4 to 7 days.[65] A very small proportion of otherwise normal infants as well as individuals of any age with primary or acquired cellular immune dysfunction are more likely to experience symptomatic illness after exposure.[168]

In experimental animals, pneumonitis develops after inhalation of *H. capsulatum* microconidia or mycelial fragments.[34] Because of their smaller size, microconidia enter terminal alveoli and, within 2 to 3 days, germinate and convert to yeast forms. Dimorphism appears to be a major determinant of virulence[206]; chemical blockage of the transition has been shown to impair virulence[329] in a murine model.[306] Progress in understanding the molecular cell biology and genetics of the mold-to-yeast transition has been reviewed.[213] Molecular techniques have identified two genes *(URA5*, *CBPI)* that have a role in pathogenesis.[525,527]

Additional determinants of fungal virulence have been described and include roles for α-1,3-glucan.[378] In the first week, a neutrophil response occurs at the site of the infection[35]; it is followed in approximately 2 weeks by the accumulation of helper T lymphocytes and macrophages.[365] The yeast forms attach to integrins of the CD11/CD18 group of cell surface receptors and enter neutrophils[403,408] and macrophages.[56,439] This process may be an adaptive microbe entry mechanism that enables the organism to evade the host oxidative burst response associated with host-directed opsonophagocytosis.[527] Human neutrophils possess fungistatic activity that resides within azurophilic granules.[52,323] Neutrophils have been shown to play a role in the primary immune response in a murine model[542]; however, depletion of neutrophils in a systemic model of secondary infection did not alter the course. In an animal model, phagocyte-produced reactive oxygen species participate in the immune response while superoxide dismutase facilitates pathogenesis by detoxifying host-derived reactive oxygen, thereby enabling survival of *Histoplasma*.[536]

Traditional opsonophagocytosis facilitated by binding to complement or to immunoglobulin after the development of humoral immunity also occurs.[527] This process exposes the yeast to the full array of antimicrobial defense mechanisms.[526] Microbe-directed binding using a specific ligand (adhesin) on the yeast's surface has been identified

*References 21, 54, 64, 138, 229-231, 344.
†References 24, 168, 232, 333, 374, 540.

as *H. capsulatum* HSP60.[16,58,278] This heat shock protein also induces immunity to lethal and sublethal infection in a mouse model, a complex effect related to its ability to stimulate $CD4^+$ T lymphocytes.[106] HSP60 has also been shown to both orchestrate the adaptation of the fungus to temperature stress[181] and impact macrophage effector function.[180]

The yeast forms seem to have a competitive advantage within the membrane-bound vacuoles of macrophages,[320,321] and the asylum seems to protect it from pulmonary collectin-mediated killing.[295] Both fungal growth and virulence are exquisitely sensitive to the availability of iron.[204] Zinc deprivation is also thought to be a host defense mechanism utilized by macrophages in a mouse model.[522] Additional strategies used by the organism to survive and replicate in mouse models include increasing the alkalinity, tolerating nutrient starvation, and resisting the toxic effects of host reactive oxygen and nitrogen intermediates, degradative enzymes, and antimicrobial peptides.[205,527] Murine macrophages differ from human macrophages because the latter mount a respiratory burst without the need for opsonization, as is required for murine macrophages. Lysis of infected cells releases additional yeasts and results in infection of increased numbers of macrophages. During this period of fungal sequestration and replication, lymphohematogenous dissemination distributes yeast from the lungs to the reticuloendothelial system and to other organs.[320] Without the development of an effective cellular immune response, the infection is lethal.[321,365]

Although infection usually results in a brisk antibody response, humoral immunity does not contribute substantially to primary or secondary immunity.[288,329,396] A mouse model has shown, however, that antibodies to a surface protein of the fungus altered the intracellular fate of the fungus and mediated protection.[19] Human dendritic cells that reside in the lung serve to link the innate and adaptive immune responses. Because they are more efficient antigen-presenting cells than alveolar macrophages, dendritic cells use the fibronectin receptor, phagocytose and rapidly degrade the organism, present *H. capsulatum* antigen for lymphocyte proliferation, and facilitate the induction of cell-mediated immunity.[161,321,438] Dendritic cells also have been shown to cross present exogenous antigen through uptake of apoptotic macrophage-associated fungal antigens (cross presentation).[272] Some of the strategies used by the yeast that facilitate its survival and replication within macrophages, such as prevention of phagolysosomal fusion, do not occur in dendritic cells. Destruction of yeast within human dendritic cells is an important role within the innate immune response[321] and seems to result from exposure to lysosomal hydrolases.[160] Conidia transformation into yeastlike forms does not take place within dendritic cells.[326]

After they participate in the innate immune response, several endogenous cytokines[251] induced by the development of cellular immunity[321] activate macrophages, which then function as primary cellular effectors that control the infection.[103,320,408] Cytokines that play key roles in the resolution of primary or secondary infection (protective immunity) in animal models include tumor necrosis factor-α (TNF-α),* interferon-γ (IFN-γ),[10,11,83,255,469] interleukin (IL)-4,[101] IL-12,[11,62,543,544] IL-1,[110] and granulocyte-macrophage colony-stimulating factor.[9,12,104,109,422] The effect of IL-12 results from its induction of IFN-γ; it is active in primary infection but not in reinfection.[11] Deficiency of IL-10 has been shown to confer a salutary effect on histoplasmosis in a murine model.[107] Apoptosis, a crucial element of protective immunity in the mouse model, when inhibited, is accompanied by increased production of IL-4 and IL-10 as well as by exacerbation of the severity of infection.[8]

Cytokines that activate human macrophages and induce them to become fungistatic are granulocyte-macrophage colony-stimulating factor, macrophage-stimulating factor, and IL-3[101]; the mechanisms by which the colony-stimulating factors promote fungistatic activity are not fully elucidated but have been shown to not require phagosome acidification to kill and degrade yeast.[324] TNF-α[101,108,259,524] is a central and critical mediator of resistance in mice and humans, although its effects on cellular targets differ.[190] Human macrophages also express antifungal activity that is not cytokine dependent. The latter mechanism is stimulated by attachment of macrophages to type 1 collagen matrices and is mediated at least partly by overcoming the ability of yeasts to inhibit phagolysosomal fusion.[325] Leukotrienes are required for control of chronic infections, amplifying innate and adaptive immune responses in a mouse model.[407]

In addition to macrophages, specific T-cell subpopulations also are capable of killing *H. capsulatum*. $CD4^+$ cells have been shown to be essential for fungal clearance in mouse models.[102,164,271] This comports with observations showing that the risk for severe histoplasmosis developing in adults with human immunodeficiency virus (HIV) infection increases when the $CD4^+$ count is less than 200/μL.[501] Idiopathic deficiency of $CD4^+$ cells has also been associated with opportunistic infection caused by *H. capsulatum*.[125] $CD8^+$ cells also mediate immunity in mice,[271,272] but their contribution is not as significant as that of $CD4^+$ cells. $CD8^+$ cell clearance of *H. capsulatum* involves both perforin-dependent and perforin-independent mechanisms.[541] IFN-γ and TNF-α also work in concert with cytolytic mechanisms to provide protective immunity against *H. capsulatum*.[541] The role of natural killer cells has been studied in murine models.[349] Natural killer cells participate to a limited degree in the immune response in immunocompetent animals and play a more substantial role in animals depleted of T cells.[321]

In immunocompetent hosts, cellular immunity is suppressed[353] early in the course of histoplasmosis, especially in disseminated histoplasmosis of infancy.[79,333] Cellular immune function returns to normal after 4 to 6 weeks of treatment. In the mouse model, *H. capsulatum* infection is associated with the generation of suppressor T-cell activity; this activity depresses the production of T-cell–dependent, delayed-type hypersensitivity, mitogen-induced lymphocyte transformation, and cytotoxic activity.[25] T-cell immunity develops 10 to 21 days after

*References 10, 12, 13, 101, 104, 531, 542.

exposure, and splenic suppressor T-cell numbers decrease while helper T-cell numbers increase.

In a study of the cellular immune responses of patients infected with HIV-1 and with recent onset of histoplasmosis, all of the patients had nonreactive skin tests. Lymphoproliferative responses and IFN-γ production were depressed in patients with $CD4^+$ counts of 200 to 500 cells/μL; lymphoproliferative responses approached normal in patients with $CD4^+$ counts above 500 cells/μL.[463] In addition to having defective cellular immunity resulting from decreased numbers of $CD4^+$ cells, the macrophages of patients with HIV infection have impaired ability to control *H. capsulatum*. This results from a profound defect in their ability to recognize and bind to yeast and from permissiveness for intracellular fungal growth.[72] The HIV envelope glycoprotein gp120 seems to play a role in inhibiting phagocytosis of *H. capsulatum* by macrophages, but it is not responsible for the capacity for accelerated growth of yeast within macrophages.[73]

PATHOLOGY

With the exception of patients who have severe progressive disseminated infection, particularly those with preexisting primary or acquired cellular immune dysfunction, histopathologic findings in histoplasmosis are characterized by granulomatous inflammation, usually in association with caseating and noncaseating granulomas. The mature granuloma serves to both localize inflammation and inhibit fungal growth. The effectiveness of this pathologic response is reflected in the substantial prevalence of asymptomatic and self-limited infections in geographic regions in which skin test reactivity exceeds 90 percent. Another potential and clinically important outcome of this microenvironment is late reactivation of the infection in the event of acquired immune dysfunction.[200,466]

Granulomas appear after the development of an effective acquired immune response; the cellular elements consist primarily of mononuclear phagocytes and lymphocytes, largely T cells. Langerhans giant cells often are present; and typical yeast forms occasionally, but not consistently, are visible within macrophages. Inflammation ultimately progresses to fibrosis and often is accompanied by calcification.[168] The rate of calcification is age dependent, and it may occur within months in children and over several years in adults.[168,432] Exuberant granulomatous inflammation or fibrosis or both can result in obstruction or dysfunction of adjacent mediastinal or, less commonly, abdominal[379] structures. Although viable yeasts may remain in quiescent granulomas, they are few and rarely seen. In areas endemic for histoplasmosis, old granulomas in the lung, bone marrow, or other sites may be seen as incidental findings, the significance of which depends on clinical assessment. Yeasts almost never are observed in histologic sections of extensive fibrosis in late histoplasmosis.

In patients with acute progressive manifestations of histoplasmosis, especially those with preexisting cellular immune dysfunction,[140,307] or in otherwise normal infants, the inflammatory response is impaired and granuloma formation is poor.[307] In these instances, extensive parasitization of macrophages by yeasts occurs, and organisms may be seen readily in various reticuloendothelial structures, especially the bone marrow, and sometimes within leukocytes in the peripheral blood.[333] Many other organ systems are often involved. The histopathologic findings in central nervous system (CNS) infection consist of granulomatous basilar meningeal and vascular inflammation, perineural inflammatory changes in cranial nerves, and the presence of many organisms at the periphery of mass lesions.[493] In disseminated infections, the adrenals frequently are affected.[29,178,437] A spectrum of pathologic abnormalities of the gastrointestinal tract may also occur.[28,85,254,345]

CLINICAL MANIFESTATIONS

Approximately 95 percent of infections caused by *H. capsulatum* occur in normal individuals and either are asymptomatic or cause brief, self-limited illnesses with no sequelae. The only residual findings are the incidental radiographic demonstration of typical granulomas in the lung parenchyma and/or calcifications in the hilar mediastinal lymph nodes or spleen. In immunocompetent hosts, the size of the inoculum probably is the principal determinant of whether infection is accompanied by clinical symptoms. After exposure to small inocula, only 1 percent of individuals develop symptoms, whereas after heavy exposure,[487] 50 to 100 percent become ill. Additionally, the symptoms that develop are more severe in individuals who are exposed to large inocula. Severity may be reduced in individuals with preexisting immunity derived from previous infection. Protection is incomplete, however, and severe disease can occur after reexposure to a large inoculum.

A crucial determinant of susceptibility and disease severity also is host dependent. Individuals with primary or acquired disorders of cellular immunity and otherwise normal infants younger than 1 year old have a disproportionately high risk for developing symptoms after exposure. These groups are at far greater risk for progression of early dissemination. The precise factor or factors that predispose to a higher risk for progressive dissemination in infections acquired during infancy have not been determined.[372]

As with other endemic mycoses, histoplasmosis begins as an acute inflammatory pneumonitis, undergoes self-limited or progressive dissemination, and requires an effective host immune response for its control. Clinical manifestations (Table 203-1) vary because they result from any or all of the following: the nonspecific systemic effects caused by the infection, symptoms resulting from either the primary inflammatory focus or perturbation of function of anatomic structures adjacent to primary sites of inflammation, hypersensitivity phenomena resulting from the acquired immune response, symptoms caused by hematogenously infected sites, life-threatening multisystem symptoms resulting from progressive parasitization of the reticuloendothelial and other organ systems, symptoms caused by chronically progressive forms of

TABLE 203-1 Clinical Manifestations of Histoplasmosis

Asymptomatic infection
Pulmonary disease
Acute (mild, moderate, severe forms)
Mediastinal adenitis
Mediastinal granuloma*
Obstruction/dysfunction of contiguous mediastinal structures by granulomatous inflammation of lymph nodes
Pericarditis
Chronic disease with cavitation†
Broncholithiasis†
Lithoptysis†
Mediastinal fibrosis‡
Progressive disseminated infection
Acute
Infancy (PDH of infancy) with or without meningitis
Immunocompromised (non-HIV)
HIV
Subacute†
Chronic†
Presumed ocular histoplasmosis†
Primary cutaneous infection†
Isolated meningitis†

*Complications similar to those of mediastinal adenitis but presentation either unassociated with acute infection or after asymptomatic or unrecognized infection.
†Rare in children.
‡Infrequent manifestation during childhood.
HIV, human immunodeficiency virus; PDH, progressive disseminated histoplasmosis.

pneumonitis or dissemination, or exacerbation of quiescent disease. Recognition, confirmation of the diagnosis, and selection of prudent therapeutic options require understanding of these diverse clinical manifestations. Aside from patients with preexisting conditions or those receiving therapy that impairs immune function, all patients with serious disseminated disease, persistent antigenuria after completion of therapy, relapse, or recurrent infections should undergo comprehensive assessment of immune function.

Pulmonary Histoplasmosis

The most common symptoms of acute primary histoplasmosis encompass a spectrum[300,496] of respiratory and systemic complaints of varying severity. Eighty percent of infections that are accompanied by symptoms are mild, undifferentiated, flulike illnesses with cough, myalgia, headache, and variable low-grade fever. Symptoms usually are self-limited and resolve in 3 to 5 days.[487] In cases of more significant fungal exposure, fever is present, and symptoms can include headache, myalgia, chills, persistent cough, and nonpleuritic chest pain.[168] Illness may persist for 2 weeks and be associated with nausea, asthenia, weight loss, and/or fatigue. This manifestation also is self-limited, although fatigue and weight loss improve slowly after the fever resolves.

In primary infections that last longer than 2 weeks, fever persists and is often accompanied by greater weight loss, chills, night sweats, fatigue, and/or chest pain. The chest pain that occurs in histoplasmosis typically is nonpleuritic; may be substernal, posterior, or lateralized; usually is brief in duration; and recurs frequently. This pattern of chest pain may last from a week to several months.[168] Wheezing may be an early symptom.[59,476] Hepatosplenomegaly occasionally is present, although its occurrence should raise suspicion of the early onset of progressive dissemination. In these prolonged illnesses, resolution without treatment may occur, but with the safe and effective oral antifungal agents available, most experts recommend treatment with antifungal therapy.

Finally, in acute primary infections that occur after exposure to a large inoculum of spores, the resulting diffuse pneumonitis may be associated with severe symptoms, particularly dyspnea or adult respiratory distress syndrome early in the infection. In these instances, the ordinarily self-limited early dissemination has a high risk for becoming progressive, and treatment is required. After development of the respiratory complaints that accompany primary infection, rheumatologic syndromes may be seen within several months of infection. They usually consist of erythema multiforme,[305] erythema nodosum,[168,276,343,409] acute migratory polyarthritis, or any combination of these conditions.[81,388] Although histoplasmosis-induced erythema nodosum is seen during childhood and adolescence, arthritis is uncommon. The rheumatologic symptoms are immune mediated and usually self-limited or respond to mild anti-inflammatory therapy.

Various symptoms may result from complications arising from intrathoracic or, less commonly, intra-abdominal lymphadenitis caused by *H. capsulatum*. One of the most problematic, often termed *mediastinal lymphadenitis*, is acute primary infection accompanied by fever, weight loss, and a mediastinal mass visible on chest radiograph. The presence of mediastinal lymphadenopathy in the absence of any recognized clinical symptoms often require definitive diagnosis to exclude a neoplasm, especially lymphoma.[149,529] Pediatric series that have examined the definitive diagnosis of such manifestations have found rates of histoplasmosis to range from 1 to 57 percent,[44,149,475,529] a discrepancy best explained by considering whether the study population lived in an endemic region. In a highly endemic region, the cause of a mass within the middle mediastinum was histoplasmosis if the *Histoplasma* complement-fixation (CF) titer was greater than 1:16.[149]

Complications of acute primary histoplasmosis may be seen when granulomatous lymphadenitis results in inflammation, compression, or obstruction of contiguous structures within the thorax; this manifestation occasionally is termed *mediastinal granuloma*. Structures most commonly affected are, in decreasing order of frequency, the bronchi, trachea, pericardium, pulmonary vasculature and great vessels, lymphatics,[459] esophagus, nerves,[124,348,381,528] and endovascular infections of prosthetic endografts or valves.[196,222] Symptoms include bronchial compression or obstruction, or both,[429] which sometimes are associated with distal pneumonitis; pericarditis; pulmonary infarction; esophageal diverticula, fistula formation, or dysmotility, alone or in combination; tracheoesophageal fistula formation; phrenic or recurrent laryngeal nerve palsy; or, in endovascular infections,

embolic phenomena. Presumably because of their more pliable airways, children are more susceptible than adults to tracheobronchial compression as a result of encroachment by enlarged lymph nodes.[153] The risk for involvement of contiguous structures by such mediastinal granulomas may be related to the thickness of the capsule, rather than to the overall size of the node.[168]

In highly endemic regions, histoplasmosis is the cause of 25 percent of acute pericarditis cases; approximately one fourth of patients have symptoms of tamponade,[488] although it rarely is seen in children. In almost all instances, pericardial effusion results from inflammation caused by infected lymph nodes adjacent to the pericardium and not by frank fungal pericarditis.[535] The pericardial fluid is exudative, bloody, and almost always sterile.[510] Most patients with *Histoplasma* pericarditis quickly improve with administration of nonsteroidal anti-inflammatory drugs, and neither acute drainage nor pericardiectomy is needed. Rarely, contiguous adenitis or broncholiths erode through the pericardium and cause fungal contamination of the pericardial space.[168,535] Pleural effusions occur very rarely in children with histoplasmosis, and, similar to pericarditis, seem to result from pleural inflammatory reactions to adjacent granulomas; cultures of pleural fluid are negative.[371] Chylothorax caused by extrinsic compression of the thoracic duct by histoplasmosis-induced lymphadenitis has been reported to occur in a 3-year-old.[459] Broncholithiasis may result from calcifications that erode through bronchi[39] but rarely occurs in children. Lower cervical[296,488] or supraclavicular adenitis is an infrequent manifestation of histoplasmosis and usually is seen in association with mediastinal involvement. Lymphadenitis occasionally affects intra-abdominal lymph nodes and has been reported to cause biliary tract obstruction[5,350,379] in children.

Subacute or chronic manifestations of intrathoracic histoplasmosis may occur in children, although they most commonly affect older adults. Of these entities, diffuse mediastinal fibrosis may affect adolescents, albeit infrequently. In these rare instances, granulomatous inflammation progresses to dense fibrosis and may cause stenosis, obstruction, or malfunction of contiguous critical mediastinal structures. Although symptoms are similar to those caused by active granulomatous inflammation that impairs the function of these structures, the fibrosis is progressive and irreversible and responds to neither antifungal therapy nor anti-inflammatory agents.[282] Common complications include the superior vena cava syndrome and/or stenosis or obstruction of the trachea, bronchi, pulmonary artery, or esophagus.[190] Late constrictive pericarditis has been reported[168,530] to develop in 14 percent of adults with *Histoplasma* pericarditis, but data have been descriptive. Histoplasmosis has been documented to occur in a 14-year-old who presented with late constrictive peridarditis.[530] Long-term data in children are lacking; in my personal experience of 35 years of research in a highly endemic area I have failed to identify histoplasmosis-induced constrictive pericarditis during childhood.

Additional late complications of intrathoracic histoplasmosis are seen primarily in adults and include pulmonary histoplasmomas (lung nodules), which are granulomas in the peripheral lung fields that become encased in dense fibrous tissue, often with concentric layers of calcification. These may enlarge over several years and cause symptoms of a mass lesion[170] or manifest as an isolated pulmonary mass. Endobronchial lesions may result in stricture, obstruction, or hemoptysis and mimic bronchogenic neoplasms.[385,389] Unusual histologic features seen in adults may mimic those of lymphomatoid granulomatosis.[310]

Cavitary (also termed *chronic*) pulmonary histoplasmosis rarely has been reported in a child.[37,117] In an analysis of risk factors for cavitary disease in an outbreak estimated to have involved 150,000 persons, only 2 in 170 individuals with cavitary disease were younger than 19 years. Cavitary disease was seen primarily in adults with chronic obstructive pulmonary disease. Symptoms include low-grade fever, productive cough, weakness, weight loss, and fatigue.[173,238] Early pulmonary findings consist of apical interstitial pneumonitis that eventually progresses to fibrosis, cavitation, and gradual spread to uninvolved areas of the lung.[506] If chronic histoplasmosis is untreated, the disease is fatal.[238] Disseminated infection has been reported to develop in approximately 80 percent of patients with this disorder.[146,391]

Primary Cutaneous Histoplasmosis

Numerous reports[335,468] describe immunocompetent patients who present with mucosal,[155,387,415] corneal,[451] or cutaneous ulcerated lesions that show characteristic pathologic changes and morphologic and cultural documentation of *H. capsulatum* and resolve without treatment. These lesions have been considered to be primary, localized infections.[449] Aside from several cases that reasonably may be attributed to documented instances of direct inoculation,[252,415] many of these reports fail to provide sufficient evidence[6,136] to exclude low-grade, chronic dissemination with histoplasmosis.[506] Comprehensive clinical and laboratory evaluations as well as long-term follow-up[90,534] are needed to confidently attribute mucocutaneous lesions to localized infections with *H. capsulatum.*

Progressive Disseminated Histoplasmosis

Progressive disseminated histoplasmosis (PDH) occurs most frequently in immunodeficient patients and patients at the extremes of age.[169,506,513] The clinical entity is defined as an illness that is accompanied by active replication of *H. capsulatum* in multiple organ systems. Symptoms are prolonged and usually last longer than 3 weeks. Laboratory evidence of dissemination may include anemia, thrombocytopenia, and/or leukopenia; persistent antigenuria or antigenemia or both; demonstration of granulomas in extrapulmonary tissue; histopathologic demonstration of compatible yeast; or growth of the fungus in culture.[486] This definition excludes the transient fungal dissemination that uniformly occurs in early primary infection and is aborted by the host's acquired cellular immune response. It does encompass, however,

the rare instance in which intense exposure to a large fungal inoculum results in severe primary infection that overwhelms the host's ability to mount an effective cellular immune response, allowing the usually self-limited primary dissemination to progress.

Distinct clinical presentations of PDH in adults permit subclassification into acute, subacute, and chronic forms. In contrast, PDH in children occurs almost exclusively as an acute, progressive, life-threatening infection.[224] Most of these infections develop in immunocompetent infants younger than 1 year or in children with acquired or primary cellular immunodeficiency. Rarely, PDH occurs in an immunocompetent child, usually in association with exposure to a large fungal inoculum.[95] Transplacental infection has been reported to occur in infants of women with PDH complicating HIV infection.[515]

PDH may result from exogenous exposure of a susceptible or immune host or from reactivation of endogenous quiescent foci of infection. Precise differentiation of these mechanisms of pathogenesis is complicated by the risk for reexposure in endemic areas and the relative unavailability of a method that easily distinguishes strain differences in isolates. Although reactivation of infection may occur in an immunosuppressed host,[218,457,466] epidemiologic data in immunosuppressed individuals who reside in areas highly endemic for histoplasmosis favor exogenous exposure as the most common mechanism. Supporting evidence consists of observations showing that rates of PDH in immunocompromised patients increase only during periods in which infection rates increase in the general population and do not increase in interepidemic periods.[355,399,402,463] Using the results of histoplasmin skin testing obtained at the time of diagnosis of malignancy in a large cohort of children, Hughes[211] found that skin test reactivity was absent in all children in whom PDH subsequently developed and that in none of those known to be skin test positive at the time of diagnosis of neoplastic diseases did PDH develop later.

Progressive Disseminated Histoplasmosis of Infancy

PDH of infancy usually affects children younger than 1 year and has been reported to occur in a 6-week-old infant.[263,333] Rare instances of transplacental[515] infection have been reported. It usually is a subacute illness in which symptoms often are reported to have been present for 1 to 12 weeks before the patient undergoes an initial evaluation; as the disease progresses, patients eventually become profoundly ill and usually die unless treated. Early symptoms include variable fever, failure to thrive,[428] and hepatosplenomegaly in almost all instances. Pallor, cough, tachypnea, oropharyngeal ulcerations, lymphadenopathy, gastrointestinal bleeding, or hemorrhagic skin lesions may develop. Abnormalities that are seen frequently in laboratory studies, and inexorably worsen, include anemia, thrombocytopenia, leukopenia,[169,263,372] disseminated intravascular coagulopathy, and marked depression of immunoregulatory cells[79,333]; these abnormalities reflect the overwhelming fungal parasitization of the reticuloendothelial system and often mimic the presenting signs of a lymphoreticular malignancy.[2] The chest radiograph may show signs of focal pneumonitis, mediastinal adenopathy, or miliary disease, but more than 50 percent of chest radiographs are normal at the time the diagnosis is made. Anatomic sites commonly affected are the spleen, liver, bone marrow, lymph nodes, gastrointestinal tract, and adrenal glands.[68,178,267] Abnormalities in the cerebrospinal fluid (CSF) were found in 62 percent of a series of infections in Costa Rican infants.[333]

PDH of infancy was uniformly fatal before effective antifungal agents became available[263]; survival rates are now excellent. In a series of Costa Rican children that included some infants with malnutrition, 90 percent of patients treated with amphotericin B survived; of the four who died, two had received amphotericin B for either 33 or 35 days and thereafter were treated with orally administered ketoconazole. Both families declined further treatment and were eventually lost to follow-up*; each family returned, however, within 5 weeks of their children's discharge, and massive gastrointestinal hemorrhage and recurrent dissemination were seen in both patients. The other two children were gravely ill and died after having received only either two or three doses. In my experience, survival has been 100 percent in an Indiana series of PDH of infancy.

Progressive Disseminated Histoplasmosis in Immunocompromised Hosts

Immunocompromised individuals, including those in whom either a preexisting medical condition or therapy[279] has depressed cellular immunity, are at risk for progressive dissemination if they acquire histoplasmosis.[211] Common predisposing conditions include AIDS,[71] primary immunodeficiency disorders,[207] those receiving immunosuppressive therapy for reticuloendothelial malignancies,[211] organ transplantation,[176,297] rheumatologic disorders,[187] and chronic renal failure.[216] Severe PDH infections have been recognized in patients treated with TNF-α antagonists for inflammatory disorders.[524] This effect seems to be associated most closely with the use of infliximab,[120,132,259,269] a chimeric mouse monoclonal IgG1 antibody directed against soluble and cellular TNF-α that blocks it from binding with its endogenous cell surface TNF-α receptor.[101,148]

In children who are receiving chemotherapy for malignancies, disseminated histoplasmosis can occur during either remission or relapse.[2,211] The most common initial symptom is persistent fever, usually without localizing symptoms and unassociated with toxicity. As the illness evolves, fever persists and liver and spleen enlargement may occur. Laboratory abnormalities include progressively worsening anemia, leukopenia, and thrombocytopenia; disseminated intravascular coagulopathy may ensue. The chest radiograph may be normal.[211] *Histoplasma* antibody assays usually are negative, and the diagnosis can be confirmed most promptly and reliably by using the *Histoplasma* antigen assay of urine or serum.

Another common symptom complex consists of respiratory complaints and tachypnea. In these instances,

*C. Odio, 2007, personal communication.

persistent fever is followed in several days by dyspnea. Chest radiographs, although occasionally normal at the onset of symptoms, show diffuse interstitial infiltrates that progressively worsen.[2] Hypoxemia usually accompanies the symptoms and is progressive. This clinical complex is not specific for histoplasmosis, however, and may be indistinguishable from those caused by *Pneumocystis jiroveci*, cytomegalovirus, various viral respiratory pathogens, other opportunistic fungi, and bacterial septicemia.

The diagnosis of histoplasmosis may often be established promptly and noninvasively with the urine antigen assay. If this assay is negative, histologic examination and culture of a lung biopsy are the most sensitive and specific diagnostic methods and often provide diagnostic information about other potential causes. Examination of bronchoalveolar lavage (BAL) fluid[188] for *Histoplasma* antigen is sensitive and specific in adults but has not been studied adequately in children.

A subacute form of PDH is seen primarily in adults,[506] although in one report of 19 cases it was found in seven infants and one child.[169] The principal clinical features that distinguish the subacute from the acute form of PDH are a history of more prolonged symptoms at initial evaluation, a higher frequency of focal lesions, less pronounced fever, and fewer hematologic abnormalities in the subacute illness. Early complaints are nonspecific and may be present for 1 to 6 months; malaise and weight loss occur frequently. Hepatosplenomegaly is seen almost uniformly, and intestinal ulceration,[232] sometimes with perforation, occurs commonly.

Gastrointestinal histoplasmosis displays protean manifestations, including perforation,[227] that may occur in association with mediastinal histoplasmosis[228] or in the setting of PDH.[227,505] An autopsy study[182] of 93 adults with AIDS in an endemic region identified gastrointestinal histoplasmosis in 12 percent of cases. The terminal ileum often is involved, and findings may mimic those of Crohn disease.[67,430] Colonic lesions may result in bleeding or obstruction[202,410] and may mimic neoplasms.[260] Isolated colonic lesions without evidence of dissemination are rare.[217] Oropharyngeal ulcerations often occur and are large and deep and can mimic neoplasms.[22,136,169] Seventy-five percent of patients have adrenal involvement, which sometimes is unilateral[437]; adrenal insufficiency occurs less frequently but is reported in 15 to 50 percent of adults.[169,174,256,375,424] CNS infections are seen occasionally and may manifest as chronic meningitis, focal mass lesions, or focal areas of cerebritis. Meningitis has been reported to occur in 62 percent of infants with disseminated histoplasmosis. Endovascular infections occur and may affect normal or native valves. The aortic or mitral valves or prosthetic valves most commonly are affected.* Valvular disease generally is typical of fungal endocarditis, with large vegetations and a high frequency of embolic phenomena.[99]

Numerous isolated lesions[40,93,291,451,523] have been described; some occur in the setting of PDH, and others are found to be focal manifestations of infection.[228,385] Vaginal and penile ulcerations, soft tissue nodules, recurrent panniculitis, tenosynovitis, carpal tunnel syndrome, osteomyelitis and arthritis, enteropathies, immune hemolytic anemia, and epididymitis all have occurred.* Although the pace of subacute PDH is slower than that of acute PDH, it is nonetheless fatal in 2 to 24 months if untreated.[169]

Chronic disseminated histoplasmosis is seen almost exclusively in adults, usually in association with cavitary pulmonary lesions.[169] Oropharyngeal ulcers are accompanied by chronic, mild, intermittent constitutional symptoms. A chronic relapsing disseminated form of the disease has been described in two younger patients, 9 and 20 years old, with chronic mucocutaneous candidiasis[140,307,351] and in a child with IFN-γ receptor-1 deficiency.[540]

Progressive Disseminated Histoplasmosis in Human Immunodeficiency Virus–Infected Patients

Histoplasmosis is an AIDS-defining opportunistic infection and commonly occurs in HIV-infected patients residing in endemic areas. Prior to the development of effective antiretroviral therapy, disseminated infection developed in more than 90 percent of HIV-infected adults who acquired histoplasmosis.[501] In 1988, less than 0.5 percent of adult patients with AIDS had disseminated disease.[172] In endemic areas, it has been reported in approximately 5 percent of patients,[192,292,301] and in 21 to 53 percent of patients during epidemic periods.[43,478,501,512]

In a multivariate analysis of risk factors for the development of histoplasmosis in HIV-infected adults residing in endemic areas, receipt of antiretroviral therapy and triazole drugs was associated independently with decreased risk[193,302]; one study found the risk to be increased with positive baseline serology, $CD4^+$ cell counts less than 150/μL, and exposure to chicken coops.[301] Risk factors for death in two large cohorts of adults also have been determined.[91,99,494] Mortality rates were greater in HIV-infected individuals with PDH treated with antifungal therapy compared with HIV-negative patients with PDH; in HIV-positive patients receiving highly active antiretroviral (HAART) therapy in association with antifungal therapy, clinical outcomes were similar to those of patients with PDH unassociated with HIV infection.[453] Comparable studies have not been done in children. In Arkansas, a highly endemic area, histoplasmosis was the AIDS-defining illness in 8 percent of 40 children who acquired HIV in the perinatal period.[406] Histoplasmosis occasionally is seen in nonendemic regions, perhaps because of reactivation of quiescent foci of infection.[21,63,138,397,398]

In HIV-infected adults with disseminated histoplasmosis, early symptoms often are nonspecific; prolonged unexplained fever and weight loss are seen almost uniformly, and respiratory complaints occur in approximately half of patients.[186,224,398,501] Features are not distinctive, and in areas in which *Penicillium marneffei* also is endemic, clinical, laboratory, and radiographic findings often overlap.[261,308] Gastrointestinal symptoms[228]

*References 7, 40, 74, 133, 156, 196.

*References 226, 240, 245, 341, 366, 388, 415, 433, 476.

including perforation and those caused by partial obstruction[410] are reported commonly and usually are nonspecific; abdominal pain, weight loss, and diarrhea occur in 50 to 70 percent of patients.[28,191,194,254,434] Multiple intestinal pathogens may be present along with *H. capsulatum*.[361] Enlargement of the liver and spleen occurs in approximately 25 percent of patients. Ten percent of patients are initially gravely ill with signs of a septic shock–like syndrome.[501] Mucocutaneous lesions[22,339] occur in approximately 10 percent of adult patients and include nonspecific maculopapular rash, papules, nodules, pustules, ulcerative lesions, acneiform lesions, mucosal ulcers, and vegetative plaques.[145,189] Lesions may mimic those of secondary syphilis[347]; histopathologic examination of skin biopsy specimens often is diagnostic. The frequency of skin lesions in a cohort of Brazilian adult patients with disseminated infection was 66 percent, a difference attributed to geographic strain differences.[234] Histologic examination of the digestive tract in an autopsy study in this population identified histoplasmosis in 11.8 percent.[182] An adult series found that patients with AIDS complicated by disseminated histoplasmosis had a substantially higher mortality rate (32% vs. 14%) than those with other opportunistic infections.[94]

Isolated meningoencephalitis in an immunocompetent child is rarely seen.[452] CNS involvement, including meningitis, encephalitis, and focal brain lesions, occurs in approximately 18 percent of patients with PDH complicating HIV infection.* Symptoms include headache, encephalopathy, or complaints arising from focal neurologic abnormalities.[493] Published reports of histoplasmosis complicating HIV infection in children are few; in four HIV-infected children in Indiana, fever was present in all and cutaneous lesions were absent; hepatosplenomegaly was found in one child, and an abdominal mass caused by an infected abdominal lymph node was seen in one child. In adults, initial chest radiographs are normal in approximately 40 percent, show diffuse interstitial or reticulonodular infiltrates in approximately 50 percent, and show localized lesions in approximately 5 percent. Diffuse abnormalities may appear during treatment of individuals whose chest radiographs initially were normal.[398]

With the widespread use of HAART for treating advanced HIV infections, an immune reconstitution inflammatory syndrome has been described.[1,92,316,413] The immunopathogenesis is presumed to result from interaction between HAART-induced improvement of host immune function and residual microbial antigens.[274,384] *H. capsulatum* is among other granuloma-inducing pathogens reported to induce immune reconstitution inflammatory syndrome.[1,48,346,413]

Central Nervous System Infection

CNS manifestations occur in 5 to 10 percent of adults with PDH,[393,493,509] but they also may occur as chronic meningitis without other signs of apparent dissemination.† Clinical manifestations are varied and include chronic meningitis and arachnoiditis, hydrocephalus, focal parenchymal lesions, cranial nerve neuropathy, vasculitis, stroke, and/or diffuse encephalitis. Isolated meningitis in immunocompetent adults has been associated with presenting signs that include headache, signs of meningeal irritation, and mental status changes.[401] Meningitis is seen in association with disseminated infections in children, especially if acquired during infancy.[333] Isolated meningitis caused by histoplasmosis is extremely unusual in an immunocompetent child.[452] In adults, acute or chronic meningitis occurs in 60 percent of CNS infections; isolated or multiple focal lesions affecting the brain[30] or spinal cord,[208] stroke syndromes,[82] and encephalitis constitute the balance of manifestations. Symptoms of meningitis often are present within 6 months of diagnosis but have been reported to be present for 7 years.[157] Meningitis has been reported to occur years after apparent total resolution of PDH.[48,451] This may result from failure of antifungal agents to penetrate the CNS compartment sufficiently to eradicate the infection.[30,453,493] Commonly associated symptoms include headache, altered level of consciousness, confusion, and cranial nerve deficits in 28 to 56 percent of cases.[493]

Diagnosis is problematic in patients with histoplasmal meningitis and no associated manifestations of localized or disseminated infection.[393] It is suspected ante mortem in only 40 percent of cases. A case has been reported with the onset of symptoms 4 years before the diagnosis was confirmed.[38] Symptoms and findings often mimic those caused by other granulomatous infections, sarcoidosis,[493] cerebral vasculitis,[431] neoplasms,[223] or conditions leading to hydrocephalus.[375] CSF findings are nonspecific and include mild pleocytosis,[376] elevated protein, and depressed glucose concentration. The diagnosis is confirmed by isolating *H. capsulatum* from CSF, detecting *Histoplasma* antigen or antibody in CSF, or identifying the organism in non-CNS sites. Although the specificities of culture and antigen methodologies are high, sensitivity is variable. CSF antigen sensitivity is 38 percent in non–HIV-infected patients and 67 percent in HIV-infected patients. CSF antibody is present in 80 to 89 percent of cases; cross reactions with *Cryptococcus* occur in 28 percent of cases.[509]

CNS infection in children is unusual and, when seen, usually is reported in association with dissemination[405,411]; in one series,[333] meningitis was found in 62 percent of cases of PDH of infancy. A chronic, progressive infection of the CNS has been reported in a child.[383] Other reports describe isolated meningitis with focal neurologic abnormalities occurring after an acute respiratory illness,[36] cerebellar ataxia that was diagnosed only presumptively as histoplasmosis,[412] and cerebellar and medullary lesions.[471] A child has been described with a symptom complex of cervical lymphadenitis, CSF pleocytosis, arthritis, and interstitial nephritis.[476]

Presumed Ocular Histoplasmosis Syndrome

The entity termed *presumed ocular histoplasmosis syndrome* consists of a triad of findings: (1) discrete atrophic choroidal scars in the macula or midperiphery ("histo spots"), (2) peripapillary atrophy, and (3) choroidal

*References 15, 16, 43, 223, 287, 398, 493.
†References 38, 247, 391, 300, 424, 493, 509.

neovascularization[418] that can lead to loss of central vision.[78] In contrast to the acute endophthalmitis[166] or choroiditis[342] that occurs as a manifestation of disseminated infection, the abnormalities seen with presumed ocular histoplasmosis syndrome occur in the absence of inflammatory changes in the vitreous or anterior chambers. Smoking, level of educational attainment, and older age have been reported as risk factors for acquisition of presumed ocular histoplasmosis among a cohort of affected adults.[75]

Commonly diagnosed in endemic areas, the cause-and-effect relationship of the findings in presumed ocular histoplasmosis syndrome and histoplasmosis has been based largely on a weak epidemiologic association with histoplasmin skin test reactivity.[46] The clinical syndrome also occurs in individuals residing in nonendemic regions, however.[340,436,474] Little histopathologic evidence supports histoplasmosis as its cause.[244,338,368] A single report detected *H. capsulatum* DNA in cells of an enucleated eye from an affected patient; however, a nonstandardized polymerase chain reaction–based methodology was used (see section on molecular diagnosis). Another isolated report of the ocular syndrome used a nested PCR assay to detect *H. capsulatum* in serum.[201] Presumed ocular histoplasmosis does not seem to occur in children younger than 10 years. In a review of the operative management of the entity were 11 children aged 12 to 18 years and 1 child aged 7 years.[460] A good recovery of vision has been reported to follow surgical removal of the choroidal neovascularization that accompanies this entity.

Illness Caused by Infection with *Histoplasma capsulatum* var. *duboisii*

In patients infected with *H. capsulatum* var. *duboisii*, focal lesions are seen more commonly in bones (usually the femur, ribs, or skull) and skin (cutaneous or subcutaneous).[3] The lungs, gastrointestinal tract, liver, spleen, and lymph nodes rarely are involved. A progressive disseminated form of infection with *H. capsulatum* var. *duboisii* occurs and is associated with pyogranulomatous inflammation involving multiple organs.[84,152,284,517]

RADIOGRAPHIC FINDINGS

The radiographic findings seen most commonly in children with histoplasmosis are not pathognomonic[149,246] and may mimic the findings seen in tuberculosis or other granulomatous processes and, in some instances, neoplastic conditions,[359] especially lymphoma.[149] After small or moderate degrees of fungal exposure, the plain chest radiograph is normal in approximately 75 percent of skin test converters.[168,514] Computed tomography (CT) is more sensitive and likely to reveal parenchymal infiltrates that are not visualized in plain radiographs. The most common pulmonary parenchymal changes are "soft" single or multiple, poorly defined areas of airspace consolidation[184] often found in the basilar portions of the lungs (Fig. 203-2). CT may also reveal a "reversed halo" sign.[290] Findings either may fully resolve or consolidate into granulomas that persist, with or without calcification, in residents of, or travelers to, endemic areas.[336]

The appearance of enlarged hilar/mediastinal nodes, either in association with pulmonary infiltrates or as isolated findings,[440] also is a common radiographic finding of acute pulmonary histoplasmosis. Isolated hilar or mediastinal adenopathy occurs in 20 percent of cases, 80 percent of which are asymptomatic.[88] With CT, low signal intensity within nodes sometimes is present; however, frank suppuration is unusual. Infected nodes may enlarge and compress or obstruct adjacent structures. Nodes usually have reached their maximal size at the time of diagnosis and become smaller or return to normal in several months; calcification may, but does not consistently, occur. In infants and young children, clusters of small infiltrates occasionally coalesce into a larger bronchopneumonic lesion.[168] Although small pleural effusions are present in 10 percent of adults with acute histoplasmosis,[510] they occur infrequently in children.[2,369,459] Isolated calcifications may be seen in the spleen or liver months to years after infection. These calcifications result from the self-limited fungal dissemination that occurs during primary infection and often are appreciated as incidental findings in individuals who have lived in or visited endemic regions.

Three distinct chest radiographic patterns have been described in individuals who have had intense exposure to the fungus.[168] Individuals from nonendemic areas initially may have scattered infiltrates that later evolve into "buckshot" calcifications. The second and most common pattern is the presence of smaller, nodular lesions (Fig. 203-3). This form is often seen in individuals from endemic and nonendemic areas. Finally, a diffuse miliary or interstitial pattern (Fig. 203-4) may occur, often in a patient with protective immunity. In PDH, the chest radiograph in adults may show abnormalities in only 25 to 50 percent of patients.[87] Diffuse abnormalities commonly appear during treatment of individuals for whom chest radiographs initially were normal.[398] Diffuse interstitial or reticulonodular infiltrates are present in approximately 50 percent, and localized lesions are present in the remainder. In immunocompromised children, diffuse interstitial infiltrates are the most common radiographic findings; they worsen rapidly and in concert with progressive hypoxemia, especially in patients with HIV infection or those receiving immunosuppressive therapy.[87] The presence of interstitial infiltrates at admission has been reported in 60 percent of children with PDH of infancy[333] in a Costa Rican series, but in my experience chest radiographs were normal in a series of Indiana patients. Abdominal ultrasound evaluation or CT, or both, may show evidence of adrenal enlargement in chronic disseminated disease in adults.[29,521]

Fibrosing mediastinitis caused by histoplasmosis is seen radiographically as pronounced thickening of the mediastinum that often compresses or obstructs the superior vena cava, major bronchi, esophagus, and/or other critical structures. It can be found in a localized pattern that frequently contains calcification. It must be differentiated from idiopathic fibrosis or fibrosis not induced by infection, which may cause similar symptoms.[414] CT and magnetic resonance imaging help differentiate active

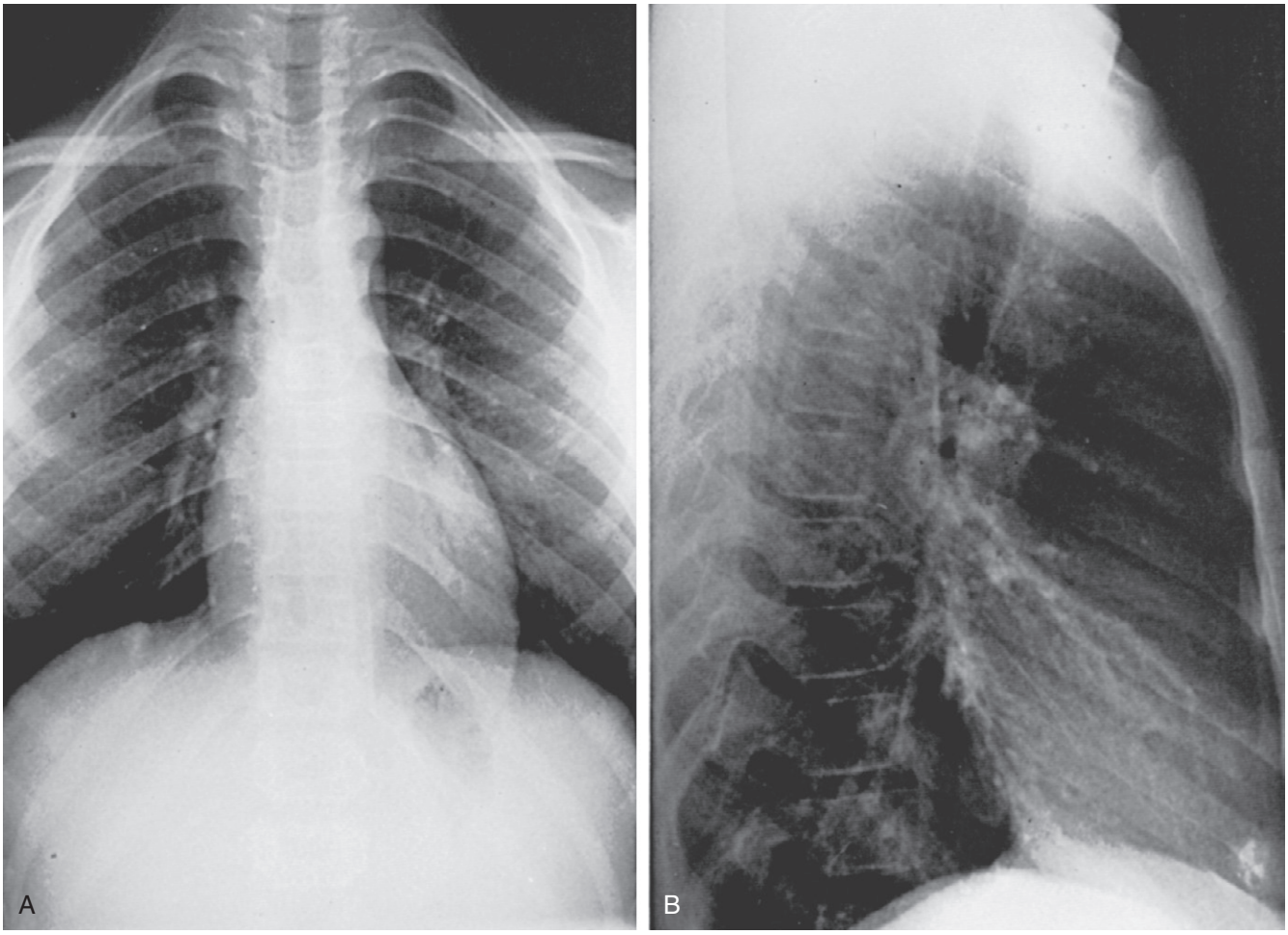

FIGURE 203-2 ■ Chest radiographs of an asymptomatic boy show a calcification in the right lower lobe overlying the diaphragm between ribs 10 and 11 in the posteroanterior view **(A)** and overlying the apex of the heart in the lateral view **(B)**. Punctate calcifications were found overlying the spleen and right axilla (not shown here).

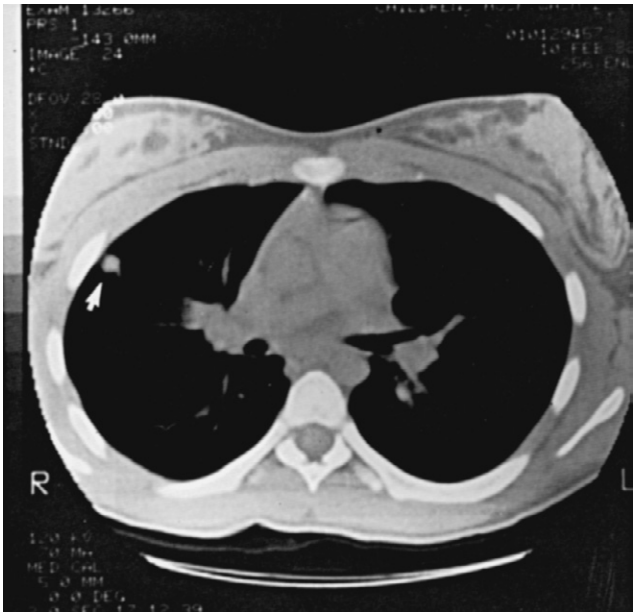

FIGURE 203-3 ■ CT scan of the chest of a teenage girl from Tennessee complaining of right arm pain when swimming. A nodular density is seen in the periphery of the right lung field *(arrow),* and the right hilum is enlarged slightly. Serologic tests and needle biopsy of the lesion were nondiagnostic, but excisional biopsy showed necrotizing granulomas with numerous histoplasmal yeast forms.

FIGURE 203-4 ■ CT scan of the chest of a 16-year-old with a history of recent demolition and renovation in the home. The patient developed fever, cough, and progressive respiratory failure requiring maximal ventilatory support using an oscillator. Note diffuse interstitial infiltrates and a pleural effusion. A urine *Histoplasma* antigen assay was strongly positive.

inflammation from fibrosis and aid in monitoring progression.[386,390] In my experience during a period of 36 years, diffuse mediastinal fibrosis caused by histoplasmosis has been confirmed in only one child in a highly endemic region.

DIAGNOSIS

Several laboratory tests play key roles in establishing the diagnosis of histoplasmosis. Although each has value, their optimal use necessitates an understanding of their differing sensitivities and specificities in various clinical settings.[490] General guidelines for their interpretation are provided in Table 203-2.

Organisms Shown on Histology

In clinical and epidemiologic settings compatible with histoplasmosis, observation of 2- to 4-μm typical yeast forms[183] in histopathologic specimens demonstrating granulomatous inflammation[310] is strong supportive evidence of histoplasmosis (Fig. 203-5). Rarely, typical yeast may be observed but result from past infection.[490] Care must be taken to differentiate *H. capsulatum* from the intracellular pathogens *Leishmania donovani* and *Toxoplasma gondii*, small variants of *Blastomyces dermatitidis*, endospores and young spherules of *Coccidioides immitis*, *P. marneffei*, and the yeast forms of *Cryptococcus neoformans*. Both Giemsa- and hematoxylin and eosin–stained specimens show intracellular yeasts in sputum, blood smears, bone marrow aspirates, and biopsy specimens. The

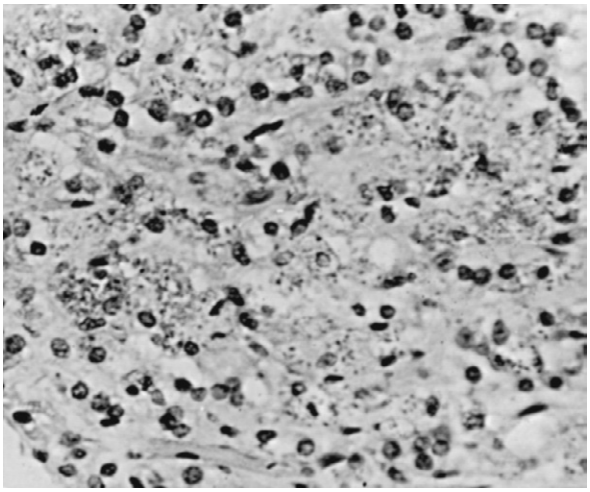

FIGURE 203-5 ■ Histoplasmosis of the adrenal gland illustrating the histiocytic response with numerous cells of *H. capsulatum* within the cytoplasm (hematoxylin and eosin, ×52).

TABLE 203-2 **Interpretive Guidelines for Laboratory Tests in Children with Acute Histoplasmosis**

		*Interpretation**	
Test	**Result**	NORMAL HOST	IMMUNOCOMPROMISED HOST
Complement fixation antibody assay†	≤1:8	–	(–)
	1:16	+	(–)
	≥1:32	++	++
Immunodiffusion antibody assay†	M bands and H bands negative	–	(–)
	M bands present only	+	+
	H bands present only	++	++
	M bands and H bands present	++	++
Urine antigen‡	Negative	(–)‖	(–)‖
	Positive (low) 0.6-4 ng/mL	+	+
	Positive >4 ng/mL	++§	++§¶
Histologic findings			
Node/mass	Negative	(–)	(–)
Infected organ	Granulomas, no yeast form	++	++
	Granulomas, yeast forms	+++	+++
Bone marrow/blood	No yeast forms	(–)	(–)
	Yeast forms seen	+++¶	+++¶
Culture any site	Negative	(–)	(–)
	Positive	+++	+++
Bone marrow/blood culture	Positive	+++¶	+++¶

*(–), does not exclude infection; –, recent infection unlikely (≤5% probability); +, recent infection possible; ++, strongly suggestive of acute or recent infection; +++, confirms current infection.
†Seroconversion confirms acute/recent infection.
‡Positive urine antigen tests should be confirmed, especially if additional laboratory examinations do not strongly support active infection.
§Compatible with early infection or disseminated infection.
‖Strongly suggestive of progressive disseminated infection or acute primary infection.
¶Progressive disseminated infection unlikely.
Modified from Long S, Pickering LK, Prober CG, editors. Principles and practice of pediatric infectious diseases. *New York: Churchill Livingstone; 2003, p. 1236.*

TABLE 203-3 **Sensitivity of Diagnostic Studies in Different Histoplasmosis Syndromes**

Test	Acute Pulmonary	Subacute Pulmonary	Progressive Disseminated
Antigen (%)	75-81	19-34	91-92
Antibody (%)	40-80	78-89	63-81
Histopathology (%)	47	9-38	12-43
Culture (%)	34	9-15	75-85

Modified from Wheat LJ. Improvements in diagnosis of histoplasmosis. Expert Opin Biol Ther *2006;**6**:1207-21.*

Gomori methenamine silver stain is the most sensitive reagent and has the added advantage that calcification artifacts dissolve during the staining procedure[332]; its disadvantage is that it does not provide adequate cellular detail.

Although observation of typical yeast forms in specimens provides important diagnostic information, it requires either a body fluid with a large fungal load or tissue that must be obtained by biopsy. Test sensitivity (Table 203-3) is better in disseminated forms of histoplasmosis, in which yeasts are readily observed in bone marrow, in affected tissue, and often in blood smears.[223] It is less sensitive in self-limited primary infections,[518] including acute pulmonary infection following heavy exposure, in which cytology and histopathology sensitivities are 25 percent.[490] Histopathologic demonstration of typical yeast forms ranged from 18 to 62 percent in three large Indianapolis outbreaks.[490] The technique usually is unrewarding in patients with calcified and/or fibrotic lesions.[286,292,518]

Culture

Normally, sterile specimens and minced or homogenized tissue can be inoculated onto suitable media, such as brain-heart infusion agar, inhibitory mold agar, Sabouraud glucose (dextrose) agar, and enriched broth such as brain-heart infusion broth. The optimal method for recovery of *H. capsulatum* from blood is the lysis-centrifugation technique,[337] which lyses white blood cells, inhibits complement, and prevents coagulation. After processing is completed, the resulting concentrate is transferred to culture media. Specimens from nonsterile sites should be cultured on media that inhibit bacteria and saprophytic fungi; non–cycloheximide-containing media also should be used to permit the growth of other opportunistic pathogens. Cultures are incubated aerobically at 25°C to 30°C and held for at least 12 weeks before being considered negative. Most isolates grow in 3 to 4 weeks.[209,480] Use of the lysis-centrifugation system for blood culture has shortened the time required for identification of a positive culture from approximately 16 days to 9 days.[42,352] The fungus is recovered in its mycelial form, and confirmation of identification necessitates conversion to the yeast form, being shown to produce the H or M exoantigen or reacting positively with a specific nucleic acid probe.[431] Laboratory-based rapid identification and differentiation of fungal pathogens using polymerase chain reaction methodology have been developed, but they are not yet available in clinical laboratories.[144,367,370]

Recovery of *H. capsulatum* from a clinical specimen obtained from a symptomatic patient with a compatible illness confirms the diagnosis of active histoplasmosis. The only exception is the infrequent event in which an incidental granuloma is found to be culture positive, but active histoplasmosis is not a likely cause of the clinical symptoms. The sensitivity of culture varies in direct proportion to the severity of the infection and the fungal burden (see Table 203-3).[519] In primary acute, self-limited histoplasmosis, 40 percent of patients have positive cultures when obtained from sputum (23%), BAL fluid (39%), lung, and extrapulmonary sites (37%).[490] In contrast, cultures are positive in 75 to 85 percent of adults with PDH. In the latter patients, sites from which *H. capsulatum* commonly is recovered include the lower respiratory tract, blood, bone marrow, CSF, liver, spleen, skin lesions, and synovium of affected joints.[253,333] The highest yield in PDH is from bone marrow, which is positive in 75 percent of instances.[399,488] Urine is positive in 40 to 70 percent, and sputum is positive in 60 percent.[424] In adults with PDH, rates of positive lysis-centrifugation cultures of peripheral blood are 90 to 100 percent in acute dissemination and 50 percent in subacute dissemination; positive cultures are very rarely seen in chronic dissemination.

In forms of pulmonary histoplasmosis other than low-inoculum infection, sputum may be a reliable site for recovery of the organism in culture but not the best choice among diagnostic methods. High-inoculum infection after exposure to heavily contaminated sites usually results in moderate-to-severe infection in which patients seek medical attention within 2 weeks of exposure.[70] In these instances, the fungal burden is high and specimens of respiratory secretions and/or lung tissue may show the organism, allowing the diagnosis to be made promptly; culture also may be positive, but the 2- to 4-week delay in achieving growth renders culture less desirable than other diagnostic methods, especially antigen detection. In adults with chronic cavitary pulmonary infection, sputum culture is positive in 50 to 85 percent of instances.[167,491] The sensitivity of sputum culture may be increased with multiple sampling.

Bronchoscopy with BAL may be helpful in patients with pulmonary disease. In a study of bronchoscopy in 71 adults, BAL was positive in only 4 percent of individuals with a single pulmonary nodule, but it increased to 55 percent in the remainder of the patient group. The highest yield of 88 percent (in 7 of 8 patients) occurred in the presence of infiltrates or cavitary disease.[369] Bronchoscopy did not appear to be helpful in evaluating patients with adenopathy, chronic pleural effusion, or bronchopleural fistulas. In children, culture plus staining of BAL fluid has been rewarding in diagnosing high fungal burden infection in patients with HIV, but it is less sensitive than lung biopsy in other immunocompromised patients, especially those receiving chemotherapy for reticuloendothelial malignancy. In adults with

mediastinal granuloma and fibrosis, cultures were positive in only 3.8 to 10 percent.[113,282]

Cultures usually are negative in rheumatologic manifestations of histoplasmosis, such as arthritis, erythema nodosum, erythema multiforme, and pericarditis. This is presumed to result from the fact that these reactive manifestations usually occur months after low-burden infection in patients whose acquired cellular immune response has controlled fungal replication. Isolated CNS infection is uncommon in children, but meningitis has been reported to occur in 25 of 40 cases of PDH of infancy; yeasts were observed in the CSF, and *H. capsulatum* was recovered in culture from four patients.[333] Recognizing CNS infection in adults is difficult unless concurrent dissemination, which occurs in 20 to 40 percent of cases, is also present.[491,493,509] Invasive procedures sometimes are needed to confirm the diagnosis.[377] In adults, culture has been reported to be positive in 27 to 65 percent of CSF specimens and in 50 percent of cultures obtained from other sites.[491] The number of organisms in CSF is small, so the yield is improved substantially by culturing large volumes (10 to 20 mL in adults) on at least two occasions.[509] In isolated CNS infections, culture is usually unrewarding.

Antibody and Antigen Detection

The serologic methods that are used most commonly for the diagnosis of histoplasmosis are immunodiffusion (ID) and CF. These tests are useful because, despite documentation of skin test reactivity in 50 to 70 percent of young healthy adults residing in an endemic area, seropositivity in this population has been found to be 0.5 percent by ID and 2 percent by CF using the mycelial antigen and 4 percent with the yeast antigen. Therefore, if these test results are elevated, they serve to substantially increase the probability of acute or recent histoplasmosis when performed in a patient with a compatible illness.

Both serologic methods have equal sensitivity (75% to 85%); however, ID is slightly more specific (>95% vs. 85% to 90%).[488,489] In immunocompetent patients, either or both tests are positive in 95 percent of patients with acute primary pulmonary infection. CF titers often become positive 2 to 4 weeks earlier, usually within 4 to 6 weeks after exposure.[488] In patients with CF antibody, 25 percent have a negative ID result; only 1 percent of patients reactive by ID are CF negative.[96] When the ID test is reactive, however, it remains so for a longer period of time. In addition to the 4- to 6-week lag in developing elevated titers, an important limitation of both serologic assays is their reduced sensitivity in immunosuppressed patients. Only 50 percent of immunosuppressed children and adults with disseminated histoplasmosis are seropositive.[96,211] Of immunocompetent infants and young children with severe PDH during infancy and early childhood, 93 percent had elevated antibody titers.[333]

Cross reactivity with other fungal antigens affects CF and ID assays.[480] CF cross reactivity occurs most commonly with *B. dermatitidis* (40%) and *C. immitis* (16%).[480] Cross reactions also occur, albeit rarely, with candidiasis, tuberculosis, aspergillosis, and cryptococcosis.[286,480] Cross reactions with blastomycosis are common in the histoplasmosis CF assay, in which titers of 1:8 to 1:16 frequently are observed; comparison of results of a single serum sample performed in the same assay often reveals CF antibody concentrations to be higher for the homologous infection. In these instances, ID tests sometimes are corroborative because the blastomycosis ID method is more specific and less likely to cross react in histoplasmosis.[273] ID has been noted to show false positivity in 5 percent of other fungal infections, however.[480] Cross reactivity also is seen occasionally in patients with chronic cavitary tuberculosis, although simultaneous infection with tuberculosis and fungal pathogens can occur.[147,480] Variability in the specificity of commercial reagents has been reported to cause false-negative results.[264]

Complement Fixation

CF uses sensitized sheep red blood cells, killed whole *H. capsulatum* yeast cells, and histoplasmin, a soluble mycelium-form filtrate antigen. In a common-source outbreak of acute primary pulmonary histoplasmosis, the titer becomes positive in 6 percent of individuals at 3 weeks, 73 percent at 4 weeks, and 77 percent at 6 weeks.[96,480] With resolution of infection, titers decrease to 1:8 to 1:16 within 4 to 6 months and, in most instances, become undetectable (<1:8) by 9 months. Reported sensitivities for a single titer vary from 70 to 95 percent and depend on the threshold for considering a result positive. Single titers of 1:32 performed by an experienced laboratory are strong supportive evidence of acute or recent infection, especially when the accompanying clinical symptoms are compatible. One report[448] showed that 12 of 28 patients with non-*Histoplasma* febrile pneumonia had false-positive CF titers of 1:32 or greater, so other laboratory and clinical data need to be considered.

The demonstration of a fourfold increase between acute and convalescent sera provides the best serologic evidence of recent infection. The individual yeast (CF-Y) and mycelial (CF-M) phases can be measured. The CF-Y phase is more sensitive than the CF-M phase when performed in recent or active infection. In a series of 11 children with acute pulmonary histoplasmosis, CF-Y was 1:32 or greater in 9 children, but the CF-M titer was 1:32 or greater in only 3 children.[529] In highly endemic areas, background low-titer CF serologic reactions may be present in 5 to 15 percent of adults[318] but they have been reported to occur in 30 percent.[158,168,481]

The serologic response seems to correlate with the severity of disease in immunocompetent individuals with acute infection.[257,506] In an extensive outbreak of histoplasmosis, seropositivity was 90 to 100 percent in severe acute histoplasmosis, 86 percent in moderate histoplasmosis, 75 percent in mild histoplasmosis, and 18 percent in asymptomatic histoplasmosis. Twenty-five percent of patients with acute histoplasmosis have CF titers that could be considered borderline positive, between 1:8 and 1:16.[96] Although they may result from previous infection, these titers should not be disregarded if clinical symptoms are compatible with acute infection. In adults with chronic pulmonary histoplasmosis, the CF titer is greater than 1:32 in almost all patients.[149,237] In children

who present with middle mediastinal masses caused by histoplasmosis, 67 percent have CF titers of 1:32 or greater.[149]

The serologic diagnosis of patients with isolated meningitis caused by *H. capsulatum* often is problematic[509] because no single test exhibits high sensitivity. CF and ID can be positive in CSF, but half of patients with other chronic fungal meningeal infections may show false-positive results.[364,479] CF-M antibody seems to be the most sensitive and specific test for the diagnosis of meningitis caused by histoplasmosis. In one study, no false-positive CSF CF-M titers occurred in patients with cryptococcal meningitis but CF-Y was false positive in 5 of 18 patients.[479] Individuals with fibrosing mediastinitis or mediastinal granuloma generally have negative or very low levels of CF antibody.[506]

Immunodiffusion

The micro-ID method detects precipitins (reported as bands) against the H and M glycoprotein antigens of *H. capsulatum*.[481] The M band is present in 25 percent of infections by the fourth week of infection and in 50 to 86 percent by the sixth week.[35,96,356] It can persist for 18 to 36 months after recovery, but eventually becomes nonreactive. In endemic areas, in which serologic surveys show that 24 percent of healthy adult blood donors have CF-Y titers of 1:8 or 1:16, ID serology is positive in less than 1 percent.[158] Because it is more specific, ID can be of value to confirm the diagnosis of histoplasmosis in patients with *Histoplasma* CF titers in the borderline 1:8 to 1:16 range.[473]

The H band is present infrequently in patients with histoplasmosis; when seen, it is transient, and its presence suggests active infection.[168] In patients with active pulmonary histoplasmosis, one half to three fourths have an M band alone. The H band is present in only 10 to 20 percent of acute infections,[173,481] and only 10 percent of individuals have M and H bands present. The presence of both is highly suggestive of active histoplasmosis. The H band is detected less consistently in children with histoplasmosis.[476] In adults with disseminated disease, both bands are present in 25 percent of individuals. The M band has been detected in 52 percent and 57 percent of patients with chronic pulmonary and disseminated disease.[173] The ID test is not approved for use in CSF specimens and has not been evaluated as a test for the diagnosis of meningitis.[490]

Antigen Detection

The development in 1986[507] and further refinements of the *Histoplasma* antigen assay have filled an important gap in laboratory diagnosis. When performed on serum, urine, or other selected body fluids, antigen detection provides rapid, accurate, noninvasive diagnostic information for the most serious manifestations of disease. It is especially useful for the evaluation of infections in immunocompromised hosts, in whom serologic methods often are negative.[507] Although evaluated almost exclusively in adults, antigen detection occupies the same diagnostic niche for managing infection during infancy and childhood.

Antigen detection is most sensitive in infections accompanied by high fungal burdens.[519] These include progressive disseminated infections in intensely exposed or immunocompromised hosts and primary pulmonary infection, in whom antigen detection reflects the early hematogenous dissemination that occurs before being aborted by an effective cellular immune response. In addition to its usefulness in diagnosis, antigen assay provides a quantitative parameter with which to assess the pace and adequacy of response to therapy and, thereafter, a sensitive monitor that promptly detects relapse in patients who are at high risk for recurrence.[497,500,502,503]

The sensitivity of urine antigen detection (see Table 203-3) in patients with acute primary pulmonary infection is 75 to 81 percent, with the highest rates seen in patients tested within a few weeks of exposure, in those with large inoculum exposure, and in patients with extensive pulmonary involvement.[491,518] Sensitivity is less in subacute pulmonary and chronic cavitary infection in adults. The sensitivity of antigen detection is very high in manifestations of progressive disseminating manifestations of histoplasmosis. In adults with disseminated infections, antigen is detected in 91 to 92 percent of immunosuppressed and nonimmunosuppressed patients and in 95 to 100 percent of patients with AIDS.[491,518] In 22 children with PDH, urinary antigen testing was positive in 100 percent.[141] From 1991 through 2011 at Riley Hospital for Children in Indianapolis, I found that urine antigen testing was positive in all instances of disseminated histoplasmosis.

Measurement of *Histoplasma* antigen utilizes a quantitative, third-generation enzyme-linked immunoassay.[127] Although *Histoplasma* antigen can be detected in serum, the sensitivity is substantially less than that of urine. Of immunosuppressed adults with disseminated histoplasmosis, antigen was detected in 50 percent of serum samples and 92 percent of urine specimens. Similarly, in patients with disseminated histoplasmosis complicating AIDS, antigenuria was seen in 95 percent versus 85 percent in serum.[507,518] Antigen often is found in BAL fluid of patients after high inoculum exposure to histoplasmal spores and in immunocompromised patients with hematogenous dissemination and lung involvement.[501,504] In adults with isolated meningitis, antigen detection in CSF may be the sole basis for confirming the diagnosis.[507-509] The range of antigen positivity in all cases of meningitis is 40 to 66 percent; the highest rates are found in severe infections with heavy fungal burdens, especially infections that occur in immunosuppressed patients.[509] In patients with clinical findings suggestive of meningitis but in whom antigen is not present in CSF; antigen detected in extrameningeal sites sometimes can confirm the diagnosis.

Antigen levels in blood or urine decrease during treatment and should clear completely in infections that are treated adequately.[328] Conversely, the persistence of moderate antigenuria has been associated with a risk for recrudescence. This risk has been shown best, but not exclusively,[199] in patients with AIDS and histoplasmosis,

in whom significant antigenuria often persists after cessation of therapy.[372,485,500,502] Monitoring urine antigen in such patients has shown that increasing concentrations foretell recurrence of active infection.[500] The sensitivity of the antigen assay is high, however, and the pattern of antigen clearance has not been established firmly in other clinical settings. Infants with PDH who have been treated adequately, have had a good clinical response, and in whom urine antigen levels have substantially decreased during therapy often have persistence of low levels of urine antigen after completion of amphotericin B therapy. In these infants, all of whom were immunocompetent, I have not observed a relapse after treatment with a total dose of 30 mg/kg of amphotericin B. Residual excretion of urine antigen after adequate treatment continues to decrease and eventually ceases; monitoring is recommended to confirm resolution of antigenuria.[141,372]

With the introduction in 2006 of a third-generation quantitative *Histoplasma* antigen method,[490] interassay variability was reduced by extrapolating antigen concentrations from a calibration curve that was created using the results of assays of known quantities of antigen. Because this calibration curve is linear at values less than a concentration of 40 ng/mL, measurements in excess of this are reported as greater than 39 ng/mL and changes cannot be determined until they fall below this value. Although the quantitative method has increased precision and eliminates the need to evaluate current specimens in tandem with earlier specimens,[490] as required in earlier enzyme-linked immunoassays, it also has necessitated changes in recommendations for using the antigen assay to monitor the effectiveness of therapy.[500] Antigen concentrations in urine measured with the quantitative assay often exceed the upper end of the calibration curve, especially in disseminated infections. If urine concentration exceeds 39 ng/mL, monitoring is more informative if antigen concentrations of serum are initially followed because concomitant serum concentrations are lower than those of concomitant urine samples and more likely to be within the range in which differences in concentration could be measured accurately. Thereafter, when antigenemia is resolved, urine concentrations can be followed with the quantitative assay. Testing should be performed routinely at 3- to 4-month intervals during treatment, at the end of therapy, if symptoms recur, and periodically for another year to monitor for relapse.

Antigen concentration decreases during effective therapy. If the baseline value is less than 20 ng/mL, antigen concentration should decline by 3 ng/mL or more, during the first month and during subsequent 3-month intervals. If the baseline antigen is greater than 20 ng/mL, the concentration should decrease by at least 15 percent during similar time periods. Failure of the antigen concentration to decline or documentation of progressive increase may indicate treatment failure. Persistent but decreasing concentrations of antigenuria may be present after completion of an appropriate and effective course of antifungal therapy. Patients who have completed appropriate courses of therapy for serious infections and have had resolution of their clinical symptoms yet demonstrated persistent but decreasing concentrations of antigenuria have done well after the completion of the planned course of antifungal therapy.[372]

Cross reactions in the *Histoplasma* antigen assay occur in paracoccidioidomycosis, African histoplasmosis caused by *H. capsulatum* var. *duboisii*, blastomycosis, sporotrichosis,[27] and infections caused by *P. marneffei*.[27] Because these infections have distinguishing epidemiologic and clinical features and because supportive diagnostic tests may aid further in differentiating them from histoplasmosis, such cross reactions usually create little difficulty in interpreting positive test results. Because treatment is similar for these endemic mycoses, appropriate therapy may be given while confirmatory diagnostic data are sought. Specimens of blood, urine, BAL fluid, and CSF yield reliable and reproducible results in the antigen assay. False-positive results may occur with pleural fluid and other high protein–containing body fluids, such as peritoneal, synovial, and pericardial fluids; these fluids and tissue specimens are unsuitable specimens. The *Histoplasma* antigen test is commercially available.

Skin Testing

A skin test for histoplasmosis has been a valuable epidemiologic and investigational tool but provided limited diagnostic usefulness[168] for distinguishing past from active infection; it no longer is commercially available. Skin testing was performed with a standardized histoplasmin antigen prepared from a mycelial-phase culture filtrate. After intradermal administration, an area of induration of 5 mm or larger at 48 hours is considered positive and indicative of cellular immunity to histoplasmal antigen. Skin test reactivity usually develops within 2 to 4 weeks after the onset of infection.[146] In chronic pulmonary disease the skin test is positive in three fourths of patients.[146] Individuals with progressive disseminated disease seldom have positive results; this is thought to result from the depression of cellular immune function that accompanies this disease manifestation. Eighty percent of children who are older than 12 years and reside in endemic areas may be reactive.[506] Cutaneous reactivity may diminish, wax, or wane with reexposure[168,506,538] but often persists indefinitely in most individuals. Serologic boosting resulting from intradermal skin testing also limited its diagnostic usefullness.[203,241] Cross reactions occur in patients with blastomycosis and coccidioidomycosis.[131,130]

Molecular Methods

Epidemiologic studies have used restriction fragment length polymorphisms of DNA. Nucleic acid–based and genome-wide approaches have been used to explore the genetic diversity of the pathogen.[242,312,470] Molecular methods also have been used to examine strains recovered from patients who have failed therapy[484] and to identify relatedness in isolates recovered after organ transplantation.[270]

Sensitive and specific oligonucleotide probes for yeast-like fungi have been developed and hold promise for application to clinical specimens.[273] Molecular methods

that reliably detect *H. capsulatum* in clinical samples have yet to show superiority, however, to standard diagnostic tests.[74] Polymerase chain reaction methodology has been evaluated for identifying *H. capsulatum*[31] in tissue and body fluids, but false-negative results were encountered in a third of specimens.[41] Polymerase chain reaction methodology used with clinical specimens (urine, serum, BAL, CSF) containing urine antigen showed specificities of 80 to 100 percent but sensitivities of only 0 to 22 percent.[492] More recent reports show promise for PCR methodology in laboratory identification of *H. capsulatum* in clinical samples.[31,100,144,416]

TREATMENT

The majority of patients with symptomatic histoplasmosis recover without receiving antifungal therapy.[168] Treatment is required for those with severe symptoms, prolonged illness, congenital or acquired immune dysfunction, or signs and/or symptoms suggestive of progressive dissemination. Evidence-based, consensus practice guidelines for treatment of histoplasmosis have been published[486] and revised in 2007[505] to include recommendations for treatment of children.

No controlled therapeutic trials have been conducted in children. Chronic pulmonary infection and subacute and chronic dissemination syndromes rarely occur in children. Recommendations for treating the clinical manifestations of histoplasmosis seen most frequently in children are summarized in Table 203-4. These recommendations have been derived from anecdotal published reports, clinical experience,[372] and extrapolation from experience in adults. Surgical management of complications of histoplasmosis rarely is indicated.[379,476]

Medical Management for Manifestations Requiring Antifungal Therapy

Because most patients with light or moderate exposure to fungal spores recover without treatment, the first decision is to determine whether to observe the patient carefully or to begin administering an antifungal agent. The criteria that are key to making this decision are clinical assessment of the character, severity, and duration of symptoms and assessment of the adequacy of the patient's cellular immunity. When treatment with antifungal agents has been elected, a regimen is selected that is appropriate for the initial findings.[421] Manifestations of histoplasmosis in children that most often require antifungal treatment are severe or protracted symptoms resulting from acute primary pulmonary

TABLE 203-4 **Summary of Treatment Recommendations for Children with Histoplasmosis**

	Treatment	
Manifestation	SEVERE ILLNESS	MODERATE OR MILD ILLNESS
Acute pulmonary	AmB,* 1-2 wk, then Itr for 12 wk†; use of concomitant corticosteroids is controversial	Symptoms <4 wk, none; persistent symptoms for >4 wk, Itr for 6-12 wk
Disseminated (non-HIV) ± meningitis	AmB,‡ or AmB for 2 wk, then Itr for 6 mo‡	Itr† for 6-8 mo or same as for severe§
Disseminated (with HIV)	AmB,‡‖ or AmB followed by Itr§	Same as for severe; Itr secondary prophylaxis if immune dysfunction persists (see text for criteria)
Meningitis, isolated	AmB‖ for 3 mo, then Itr for 12 mo	Some as for severe because of poor outcome
Fibrosing mediastinitis	Itr for 3 mo¶	Same as for severe
Pericarditis	Pericardial drainage for severe tamponade + NSAID for 2-12 wk**	NSAID for 2-12 wk
Rheumatologic	NSAID for 2-12 wk	Same as for severe
Compression of contiguous structures by mediastinal adenitis/granuloma	Prednisone 2 mg/kg/day, concurrent Itr (see text)	

*Amphotericin B deoxycholate is preferred, 1 mg/kg/day.
†In severe manifestations, itraconazole may be given at 150 percent of the recommended dose in three divided doses for the first 3 days of treatment only. Itraconazole levels are recommended for severe infections, and in patients in whom clinical response is suboptimal. Serum levels of 2 µg/mL are recommended when steady state is reached (approximately 2 weeks). Itraconazole dose is 5 to 10 mg/kg/day divided twice a day.
‡If amphotericin B is used for the entire course of treatment, 30 to 40 mg/kg should be given over 4 to 6 weeks.
§Therapy should continue until *Histoplasma* urine antigen concentrations are stable and <4 ng/mL; prolonged low-level excretions may persist (see text).
‖Liposomal amphotericin B (3 to 5 mg/kg/day) may be superior to amphotericin B deoxycholate in patients with HIV (see text).
¶Probably ineffective if fibrotic; when granulomatous mediastinitis could be present, it may be considered.
***Prompt administration of NSAID may obviate need for drainage in non–life-threatening manifestation of pericarditis.*
AmB, amphotericin B; HIV, human immunodeficiency virus; Itr, itraconazole; NSAID, nonsteroidal anti-inflammatory drug (indomethacin 1 to 3 mg/kg/day).
Modified from Wheat LJ, Freifeld A, Kleiman MB, et al. Practice guidelines for management of patients with histoplasmosis. Clin Infect Dis *2007;**45**:807-25.*

infection, mediastinal adenitis that is causing compression of adjacent structures, disseminated infection of infancy,[141,333,372] and infection in an immunocompromised host.[2,435] Treatment recommendations are summarized in Table 203-4.

The antifungal agents that play primary roles in medical management include amphotericin B deoxycholate,[97] liposomal amphotericin B,[225] amphotericin B lipid complex,[358] and itraconazole.[114,482] Amphotericin B preparations are fungicidal for *H. capsulatum*, whereas itraconazole is fungistatic. Combined use of the two agents does not result in synergy. Based largely on the results of its superiority in animal studies[89] and its faster clearance of fungemia in patients with AIDS,[225] amphotericin B is more effective for severe infection than is itraconazole.[372,482] Amphotericin B is used most commonly as "induction" therapy, especially in severe infections; after substantial clinical improvement has occurred, it is stopped and itraconazole is begun to complete treatment.[372] Monotherapy with itraconazole is effective for treating patients who have mild or only moderately severe symptoms. In addition to regimens that are used to treat acute infections, suppressive (secondary prophylaxis) regimens have been developed for treating patients with AIDS because the rate of relapse is high if antifungal treatment is stopped and if immune function has not been improved by HAART.[501] Primary prophylactic regimens also have been developed for use by patients with AIDS who live in highly endemic areas.[299]

A detailed discussion of the antifungal agents used to treat histoplasmosis is presented in Chapter 251. Amphotericin B deoxycholate usually is well tolerated and effective in children. Lipid formulations, although less nephrotoxic, are substantially more expensive.[446] Liposomal amphotericin B is the best evaluated of the lipid preparations. A comparative trial of amphotericin B deoxycholate and liposomal amphotericin B in adults with disseminated histoplasmosis and AIDS showed faster improvement and lower mortality rates with the liposomal preparation.[225] Liposomal, as well as standard, amphotericin B formulations clear fungemia faster than itraconazole does.[495]

If amphotericin B deoxycholate is used as monotherapy for severe or disseminated infection, a dosage of 1 mg/kg/day administered for 4 to 6 weeks is recommended. Failures of 30 and 35 mg/kg total dose have been reported in PDH of infancy,[333] and because relapse has been observed in immunosuppressed patients,[211] close follow-up with monitoring of urine antigen concentrations is required after completion of antifungal therapy. When amphotericin B is used as induction therapy in severely ill children, it should be continued for 2 to 4 weeks and until substantial improvement in clinical and laboratory findings is achieved. Short courses of therapy for PDH of infancy have been successful.[142,263,275] One report described cure in five infants (only two with microbiologic confirmation and another in which PDH was highly probable) with an initial dose of 0.25 mg/kg, 0.5 mg/kg given on the second day, and 1 mg/kg/day administered for 7 to 11 days thereafter.[142] Insufficient data are available to recommend short courses of amphotericin B for treatment as monotherapy; and because of the availability of itraconazole, such regimens seldom are needed.

Itraconazole generally is well tolerated by children and, in adults, is more effective[166] and less likely to induce resistance than the other azoles.* Although formal trials using itraconazole have not been conducted in children, clinical experience has confirmed its effectiveness as the oral azole of choice. The erratic bioavailability of the capsule form can be improved when it is taken with liquids with low pH and caloric content (concomitant food and a cola drink are recommended).[219] Serum levels should be monitored,[162] particularly if symptoms persist. The liquid solution of itraconazole is better absorbed but may have adverse gastrointestinal effects that affect compliance. The liquid solution is better absorbed when taken on an empty stomach. The 90 percent minimal inhibitory concentration (MIC_{90}) of itraconazole for *H. capsulatum* is less than 0.01 μg/mL; the optimal therapeutic level has not been determined, but serum concentrations greater than 2 μg/mL are effective, readily achieved, and well tolerated.[197] With its long half-life, serum concentrations vary little after steady state is achieved (approximately 2 weeks). When used as monotherapy or as step-down treatment after amphotericin B induction to treat mild to moderately severe infections in adults, a loading dose consisting of 150 percent of the total daily dose is recommended for the initial 3 days of therapy. Itraconazole is quite expensive. As with the other azoles, important drug-drug interactions need to be considered in patients receiving agents known to affect excretion of the azole or whose excretion is affected by the antifungal agent.

Fluconazole was less effective than itraconazole in treating adults with chronic pulmonary histoplasmosis[299] or disseminated histoplasmosis.[484] It also has been associated with relapse in disseminated infection,[485] is less effective than itraconazole for secondary prophylaxis in adults with disseminated infection,[302] and clears fungemia more slowly in adults with disseminated infection than does itraconazole.[497] Fluconazole is recommended for use in patients who either do not tolerate itraconazole or fail to absorb it. When fluconazole is used, careful follow-up with urine antigen monitoring is needed to promptly detect relapse. Because itraconazole does not enter the CSF easily, fluconazole may be considered in patients with CNS infection. The relationship between CSF concentrations and outcome has not been determined, however.[486] Antagonism is a concern when fluconazole is used in conjunction with amphotericin B to treat meningitis.[265] Fluconazole is reliably absorbed and metabolized, and drug monitoring seldom is needed.[485]

Ketoconazole is less well tolerated than either itraconazole or fluconazole.[47,112,199,268,458] It is reasonably effective in adults with chronic pulmonary infection[116,268,419,455] and in patients with disseminated infection, although relapse is common.[373] In histoplasmosis complicating AIDS, the response to ketoconazole was only 9 percent versus 74 to 88 percent with amphotericin B and 85 percent with itraconazole.[318,462,485] Ketoconazole is inexpensive and

*References 135, 151, 198, 318, 360, 425, 465.

may be an alternative consideration for treatment if high cost precludes the use of itraconazole.

Both voriconazole and posaconazole have in vitro activity against *H. capsulatum*. Posaconazole has shown greater activity[89,499] and seems to be more active in experimental models. Resistance that seems to have been induced by fluconazole when used in patients with AIDS also was accompanied by an increase in MIC values to voriconazole,[499] suggesting that resistance may emerge during treatment with voriconazole.[498] A recent review of isolates from 208 cases found no development of drug resistance during a 4-year period.[49] Voriconazole* and posaconazole have been effective in isolated reports describing differing manifestations of infection. These agents and fluconazole remain second-line alternatives to itraconazole.[80,362] If these agents are elected for use, therapeutic drug monitoring is recommended.

Primary Pulmonary Infection

Primary pulmonary histoplasmosis that occurs after exposure to a large inoculum of spores may result in life-threatening illness with high fever, diffuse pulmonary infiltrates, hypoxemia, and adult respiratory distress syndrome.[122,168,169,187,486] Infection of this severity should be treated promptly; induction with amphotericin B is indicated and it is continued until substantial clinical improvement occurs. Only anecdotal evidence indicates that concomitant use of corticosteroids may be beneficial in treating patients with severe inhalation and adult respiratory distress syndrome.[175,236,447,533] After improvement resulting from amphotericin B induction, itraconazole should be started and continued for at least 3 months. In patients who may not appear as ill, but in whom laboratory or clinical evidence is suggestive of progressive primary dissemination, empirical therapy should be initiated.

Patients without respiratory distress but in whom systemic complaints, such as fever, fatigue, and weight loss, persist for 2 to 4 weeks or longer also are candidates for antifungal therapy. In these instances, firm criteria to guide the timing of this decision are not established and treatment must be individualized. Still, as observed when amphotericin B was the only effective treatment, and its toxicity and inconvenience resulted in deferring its use for longer than currently practiced, a significant proportion of these symptoms could be expected to undergo spontaneous resolution. The convenience of oral administration of antifungal agents renders the decision less problematic because these protracted but mild to moderately severe symptoms may be treated with itraconazole. Itraconazole should be used for at least 6 weeks in these instances (see Table 203-4). No clinical studies have been conducted to confirm whether treatment shortens the duration of symptoms or prevents later complications.

Mediastinal Adenitis

One of the most common initial presentations of acute illness is the onset of symptoms caused by the mediastinal adenitis that commonly accompanies pulmonary infection caused by *H. capsulatum*. In this setting, affected nodes enlarge, may coalesce, and often compress or obstruct adjacent structures. Bronchi and the trachea are most often affected; less commonly, the superior vena cava and esophagus are affected. The phrenic or recurrent laryngeal nerves[159] rarely are affected. Symptoms usually associated with compression of an airway are cough, mild tachypnea, early exertion with activity, bronchospasm, and nonpleuritic chest pain. The superior vena cava syndrome may accompany obstruction of the superior vena cava. Fever is an uncommon manifestation, unless secondary bacterial pneumonitis caused by airway obstruction is present. The illness generally is self-limited, but symptoms may persist for several weeks. Although only anecdotal evidence supports the benefit of treatment, itraconazole may be helpful for patients with active inflammation. An elevated erythrocyte sedimentation rate and CF titer could be considered markers compatible with active inflammation. As also shown in a study involving dogs with mediastinal granuloma,[404] adjunctive treatment with corticosteroids sometimes is beneficial[175,447] and, if effective, results in prompt improvement. If a corticosteroidal agent is used, it should be given in association with an effective antifungal agent because of the risk for dissemination resulting from corticosteroid-induced suppression of cellular immunity. Itraconazole should be continued for 6 to 12 weeks. Although fibrosing mediastinitis is a late complication of histoplasmosis, no evidence has shown that focal mediastinal adenitis is its precursor and that antifungal therapy prevents its occurrence.

Mediastinal granuloma describes a group of mostly caseous lymph nodes that coalesce into a single lesion usually located in the paratracheal region (Fig. 203-6). It

FIGURE 203-6 ■ A 16-year-old boy was present when a barn was cleaned using a power blower. One week later he developed a low-grade fever followed by intermittent, nonpleuritic, right parasternal chest pain. CT scan of the chest shows a subcarinal mass with central low signal intensity. There is scattered calcification. Histoplasmosis was confirmed by laboratory studies and was compatible with reexposure to the fungus.

*References 4, 143, 208, 317, 380, 456.

affects adults and is very infrequently seen in children. Lesions often are asymptomatic and discovered as incidental findings in imaging studies. Symptoms usually result from impingement of bronchi, the esophagus, or the vena cava. Spontaneous drainage of caseous material may occur spontaneously and result in fistulous tracts to the esophagus,[429] bronchi, or skin. In this setting, treatment with itraconazole is appropriate, although its efficacy in this setting is unproven. Operative intervention or stenting of the affected portion of the superior vena cava sometimes is indicated in the event that compression of vital structures occurs and fails to respond to antifungal therapy. No evidence indicates that this entity is a precursor to mediastinal fibrosis (see section on surgical treatment). There are rare reports of histoplasmosis presenting as tracheal or endobronchial tumors in children.[385]

Disseminated Infection

Progressive disseminated infections in children occur in several settings, and all such occurrences require treatment with antifungal agents.[263,275] Among immunocompetent children, they occur primarily in infants younger than 1 year of age and, albeit infrequently, at any age after high inoculum exposure.[333] Among immunocompromised children, dissemination is seen most often in patients who are receiving immunosuppressive agents that impair the cellular immune system, in patients with AIDS, and less commonly in patients with primary disorders of cellular or combined immunity.[297,540] Treatment recommendations are summarized in Table 203-4.

Immunocompetent Patients and Immunosuppressed Patients Without Human Immunodeficiency Virus Infection

If not treated with antifungal agents,[169,263,275,498] patients with acute PDH have a mortality rate approaching 100 percent.[115,225,482] Treatment with amphotericin B results in survival of more than 90 percent of infants with PDH.[141,211,263,333] In contrast to adults, in whom monotherapy with itraconazole for patients with nonsevere dissemination has been successful,[114,482] only one report[454] has described the outcome of itraconazole monotherapy in children with disseminated histoplasmosis. Seven children 2 to 14 years old were treated. A 2-year-old died "shortly after" treatment was stopped at 1 month. "Marked improvement" was noted in patients treated for 3 months (four patients) and 6 months (one patient), and complete resolution was reported in one patient after 12 months of therapy. Monotherapy with itraconazole is not recommended for disseminated histoplasmosis.[505] Based largely on its demonstrated effectiveness in clinical trials in adults, however, and its ease of administration and tolerability in children, itraconazole is recommended for step-down therapy after induction and clinical improvement with amphotericin B. Serum levels of itraconazole should be monitored (see discussion on antifungal therapy) along with serum/urine antigen concentrations during therapy and in follow-up to confirm that the fungal burden is decreasing.[372] If amphotericin B is elected as monotherapy, a total dose of 30 to 40 mg/kg over 4 to 6 weeks is recommended.

Disseminated Infection in Patients with Acquired Immunodeficiency Syndrome

The severity of the initial signs of illness heavily influences the outcome and the treatment regimens selected for patients with disseminated histoplasmosis complicating HIV infection (see Table 203-4). Regimens are based on trials conducted in adults. Amphotericin B was effective in 74 to 88 percent of all cases,[478,501] whereas patients treated for infections that were sufficiently severe to require hospitalization had a 50 percent mortality rate.[478] In adult patients with moderate-to-severe disseminated histoplasmosis, liposomal amphotericin B provided a survival benefit compared with that of amphotericin B deoxycholate.[225] Adults with only mild or moderate symptoms were treated successfully with itraconazole monotherapy in 85 percent of instances.[482]

Recommendations for children are similar to the recommendations for adults; amphotericin B deoxycholate usually is well tolerated in children and is recommended as monotherapy at a dose of 1 mg/kg/day given for 4 to 6 weeks. Alternatively, it may be used for 2 weeks as induction therapy and, after a satisfactory clinical response, discontinued and followed by itraconazole 5 to 10 mg/kg/day for 12 months. Liposomal amphotericin B, a more costly preparation, may be substituted for the deoxycholate preparation with either regimen in the event of renal complications. Serum, followed by urine, antigen monitoring[328,500] (see discussion of antigen detection) is recommended during therapy and for 1 year after its completion to confirm clearance and, thereafter, to assess for relapse. After the completion of therapy, the significance of low levels of antigenuria in the absence of antigenemia and clinical symptoms is unclear and is not an indication for prolongation of therapy. Prolonged follow-up is recommended in these instances.[372]

Because of a high rate of relapse of histoplasmosis after cessation of antifungal therapy in patients with AIDS complicated by persistently impaired immune function, long-term maintenance therapy (secondary prophylaxis) is recommended.[224,501,511] In adults with mild to moderately severe disseminated infection, amphotericin B (50 mg weekly or biweekly) is effective in 81 to 97 percent of cases,[298,501] and itraconazole is effective in 90 percent.[197,483] Itraconazole is the azole of choice for mildly or moderately ill patients, in whom it may be used as both induction therapy and secondary prophylaxis. Fluconazole is not recommended for secondary prophylaxis (see discussion in the section on medical management) but may be considered if patients received amphotericin B as induction therapy or if itraconazole is poorly tolerated or its cost precludes its use.

A prospective observational study[163] of adults with AIDS and disseminated histoplasmosis found it safe to discontinue secondary prophylaxis after 12 months of primary antifungal therapy and the demonstration of sustained immunologic improvement resulting from HAART therapy. Specific criteria for withholding secondary prophylaxis include negative blood cultures, *Histoplasma* urine antigen concentration of less than

4 ng/mL, and $CD4^+$ T-cell counts greater than 150 cells/μL in patients continuing to receive HAART.[163] Primary prophylaxis with itraconazole is effective for patients with AIDS who live in hyperendemic areas[302] (areas in which the incidence of histoplasmosis is more than 5 cases per 100 patients per year).

Prophylaxis of Immunosuppressed Patients

Clusters of infection occasionally are reported in high-risk populations, sometimes in geographic regions with low background rates.[143] Based on the results of a large clinical trial, however, the need for prophylaxis for patients residing in a hyperendemic area and who are undergoing immunosuppression for management of neoplasms, inflammatory syndromes, or transplantation of allogeneic bone marrow or solid organs[33,417] seems to be low.[463] Included were patients with CF titers to *H. capsulatum* of 1:8 or 1:16, positive M bands by ID, or radiographic evidence suggestive of past infection (calcified splenic or lung nodules). In contrast, patients receiving TNF-α antagonists seem to be at greater risk for developing disseminated histoplasmosis,[420,472,524] perhaps attributable to reactivation of latent infection. Active histoplasmosis during the 2 years preceding the need for immunosuppression may be a basis for consideration of prophylaxis. Although firm evidence is lacking, criteria for such evidence may include chest radiographic findings compatible with histoplasmosis, *Histoplasma* antigenuria, or anti-*Histoplasma* antibody titers of 1:32 or greater. Duration has not been studied, but 12 to 24 weeks has been recommended.[505]

Central Nervous System Infection

With the exception of CNS infections that accompany PDH of infancy, isolated histoplasmosis of the CNS in children[208] rarely is seen.[333,357,452] Adults with CNS infections[30,401] can present with meningitis, focal infection of the brain or spinal cord, thrombotic events caused by focal vasculitis or emboli, or diffuse encephalitis. Active infection in an adult has been reported to be present for 22 years.[157] In children, some infections occur in association with disseminated infection, some as isolated illnesses, and others in the context of relapse involving a sequestered site that is penetrated inadequately by antifungal agents.[509] Mortality is almost certain if meningitis is untreated. Treatment is effective in only 20 to 40 percent of patients with meningitis, and 50 percent of responders experience relapse after cessation of treatment.[247]

Although amphotericin B deoxycholate and liposomal amphotericin B enter the CSF poorly,[123,177,262] liposomal amphotericin B concentrations are higher in brain parenchyma in animal models.[262] Comparative studies of these agents for the treatment of CNS infections caused by *H. capsulatum* have not been performed. In a more recent report,[401] five adults were treated initially with amphotericin B deoxycholate at doses of 40 mg/kg for 8 weeks followed by fluconazole 200 to 400 mg/day for 1 year, resulting in cure of four patients and minor residual sequelae in one. These reports, the ability of liposomal amphotericin B to achieve higher brain tissue levels than occur with amphotericin B deoxycholate, and the superiority of itraconazole over fluconazole in an animal model all contribute to the basis for the recommendation[254] that adults with CNS infections receive liposomal amphotericin B 5 mg/kg/day for 4 to 6 weeks followed by itraconazole for 1 year.

Meningitis was present in 25 of 40 children in a series[333] of PDH. All children received 40 mg/kg of amphotericin B deoxycholate followed by ketoconazole for 3 months. The overall mortality rate in the series was 10 percent; however, two deaths were in children who failed to complete therapy and 2 died within 4 days of presentation, suggesting that this regimen cured meningitis in most cases. In the rare setting in which meningitis is found in a child as an isolated infection, recommendations reasonably might include the use of liposomal amphotericin B (in place of amphotericin B deoxycholate) followed by itraconazole for 12 months.

The role of combination therapy for CNS infection has not been adequately examined in humans. A single case report of histoplasmal meningitis described successful treatment of basilar meningitis caused by histoplasmosis using liposomal amphotericin B and itraconazole.[377] Antagonism has been shown in an animal model[195] that used a regimen of fluconazole and amphotericin B. In another animal model of CNS infection, itraconazole in association with amphotericin B provided no benefit compared with amphotericin B alone.[265]

Despite isolated case reports that have documented success with ketoconazole, itraconazole, fluconazole, the combination of itraconazole and fluconazole, and voriconazole,[456,505] the efficacy of azole monotherapy for the treatment of CNS infections remains inadequately studied. Posaconazole[362,380] has been used successfully as salvage therapy in a patient after therapeutic failure with prior regimens. The evidence is insufficient to recommend azole monotherapy for CNS infection.

Medical Management of Manifestations That Do Not Require Antifungal Therapy

Several common and uncommon manifestations of histoplasmosis do not require antifungal treatment. Apart from mild primary pulmonary infection, one of the most common is pericarditis. Because it rarely is associated with frank fungal infection of the pericardium, it usually resolves spontaneously or with nonsteroidal anti-inflammatory drugs. Indomethacin usually is effective[535] in rapidly reducing inflammation and can reverse signs of impending tamponade. Corticosteroids have been reported to be effective, but, if used, should be accompanied by antifungal therapy. Rheumatologic symptoms of arthritis, with or without erythema multiforme or erythema nodosum, also respond to nonsteroidal anti-inflammatory drugs.[409] Fibrosing mediastinitis is a late complication of histoplasmosis, but it occurs occasionally in children. When granulomatous lesions evolve to become diffusely and densely fibrotic, both antifungal and anti-inflammatory therapies are ineffective. The clinical and laboratory criteria that are used to assess whether active inflammation is present are inexact, however, and

a trial of antifungal therapy often is warranted if clinical features or laboratory evidence of active inflammation, or both, is present. Treatment with antifungal agents almost always is unsuccessful, but improvement has been, albeit rarely, reported.[461] Presumed ocular histoplasmosis is seen almost exclusively in adults and does not improve with antifungal therapy; its management has been reviewed extensively.[78]

Surgical Treatment

Operative intervention rarely is needed to manage patients with histoplasmosis. In a review of 94 patients, 10 to 40 years old, the most common reason for evaluation was obstruction of thoracic structures by mediastinal masses.[153] Seventy-five patients underwent surgery or endoscopy to relieve obstruction of the pulmonary artery, superior vena cava, bronchus, or esophagus. Recurrent pneumonia, tracheoesophageal fistula, hemoptysis, carinal injury, and broncholithiasis were other indications for surgical procedures.[159,153,249,539] Because attempts to excise caseous nodes completely sometimes can damage contiguous structures, the preferable approach is to incise and evacuate debris from such lesions and leave the adherent portion of the capsule intact.[138,153,354,520]

Surgery has little place in the management of fibrosing mediastinitis caused by histoplasmosis. The dense fibrous consolidation of mediastinal structures coupled with the hypervascularity of mediastinal tissues renders the risk for development of hemorrhagic and other life-threatening operative complications high. Excisional biopsy carries a prohibitive risk.[98] Bronchoscopy coupled with coagulation of hyperemic vessels within bronchi in individuals presenting with hemoptysis has been useful.[289] When calcification has occurred in fibrosing mediastinitis, surgical repair is not feasible,[286] and the fibrosis continues after surgery. Some success has been achieved with relief of vascular obstruction by percutaneous placement of intravascular stents,[121,450] but complications occur. I have observed the successful use of an intravascular stent to relieve obstruction in a child with superior vena cava syndrome. Prophylactic excision of enlarged mediastinal nodes has not been shown to prevent fibrosis, and patients with large mediastinal lymph nodes who are otherwise asymptomatic rarely progress to develop mediastinal fibrosis.[506]

PROGNOSIS

Histoplasmosis is either unrecognized or self-limited in the majority of individuals who acquire this most common of the endemic mycoses. Histoplasmosis causes serious illness in infants,[333,371] immunocompromised patients, and older individuals, particularly those with emphysematous pulmonary disease.[45] The cure rate for children who receive therapy for serious acute manifestations is high. Little prospective information is available about the long-term outcome in acute pulmonary histoplasmosis. A study that examined pulmonary function in six children and their parents after intense exposure found restrictive (three patients) and obstructive (two patients) patterns and a reduction in carbon monoxide diffusing capacity in five of six tested. At 2 years, a diffusing capacity abnormality remained in three children and hypoxemia persisted in the most severely affected patient.[250] No data that can help predict the occurrence of long-term complications, such as mediastinal fibrosis, are available. When it does occur, mediastinal fibrosis is progressive and therapeutic options are few.[282] Since, by definition, mediastinal fibrosis impairs the function of both the airways and pulmonary vasculature,[423] its prognosis is influenced heavily by whether it affects one or both lungs.[98] The prognosis is poor in the latter instance.

PREVENTION

Complete prevention of histoplasmosis is currently impossible, but reasonable precautions can substantially decrease the risk to individuals with risk factors that predispose them to serious complications should they acquire the infection. Individuals with impaired cellular immunity who reside in, or plan travel to, endemic regions should be counseled about the potentially serious consequences of infection. This should include education about areas which are endemic for histoplasmosis, sites likely to be heavily contaminated with *H. capsulatum*, and the circumstances, activities, and events that may aerosolize spores and result in inhalation and infection. Primary prophylactic regimens using itraconazole may be considered for HIV-infected patients with exposure to soil mixed with bird or bat droppings (see earlier discussion of epidemiology).[193] If such activities are unavoidable, the use of National Institute for Occupational Safety and Health (NIOSH)–certified high-efficiency mask filtration devices[266] should be encouraged; however, effective devices appropriate for use by young children may be unavailable. Sterilization of sites by formaldehyde spray[266] have been attempted, but the toxicity of this agent renders its use undesirable and, in some settings, ineffective. When potentially contaminated sites, such as old or unused structures in which birds or bats have roosted, are disturbed, aerosols that may contain spores can be substantially reduced by thoroughly dampening the areas with water before they are manipulated.[32] Although educating and counseling high-risk patients affords some protection against acquiring infection from microfoci, some of which can result in intense exposure, this is not helpful in protecting against "sporadic" exposure to contaminated aerosols. For the general population residing in endemic areas, education also is beneficial, particularly for protecting individuals whose occupational and recreational activities may increase their risk for exposure to sites likely to be contaminated with *H. capsulatum*.

Although long an active area of research, vaccines directed against any of the endemic mycoses have not yet been developed. Among the challenges in this effort are both the need to target the general population in an extensive geographic region and to develop a vaccine product that provides lifelong protection, perhaps necessitating repeat immunization.[426] Investigations in animal models[210,330,392] are ongoing in an attempt to identify

vaccine targets[105,111,280,527,532] and to delineate the regulatory elements needed for developing immunity against *H. capsulatum*.[106,400] Investigations in such models have shown that monoclonal antibodies can alter the pathogenesis of histoplasmosis by targeting host costimulatory molecules or fungal cell surface proteins.[331] Another area of inquiry is that of identifying vaccine targets integral to dimorphism, a requisite for the pathogenicity of all the endemic fungi.[319] Additional efforts have explored the feasibility of developing vaccines against invasive fungal pathogens in the setting of severe immunodeficiency, including individuals with $CD4^+$ T-cell deficiency.[532] An animal model has shown that immunization with apoptotic phagocytes that contain heat-killed *H. capsulatum* antigen activated functional $CD8^+$ T cells as well as $CD4^+$ T cells and protected against infection.[210] Biodegradable microspheres that contain leukotriene B4 and cell-free antigens of *H. capsulatum* have been shown to activate murine bone marrow-derived macrophages.[119] A recombinant protein vaccine has been produced from cloned sequences of DNA coding for the cell wall glycoprotein HSP60 and found to be protective in a mouse model against a lethal intravenous inoculum of *H. capsulatum*.[165] This vaccine protected mice in a model of pulmonary infection. Recent experimental data have demonstrated that monoclonal antibodies modify the pathogenesis of histoplasmosis in animal models.[331]

NEW REFERENCES SINCE THE SIXTH EDITION

3. Ahogo KC, Sangare A, Gbery IP, et al. [Cutaneous histoplasmosis due to *Histoplasma capsulatum* variety *duboisii* in an immune competent child: about one case in Abidjan, Cote d'Ivoire]. *Bull Soc Pathol Exot* 2009;**102**(3):147–9.
5. Alave J, Bustamante B, Soto L, et al. Acalculous cholecystitis caused by *Histoplasma capsulatum* in a severely immunosuppressed HIV-infected patient. *J Infect Dev Ctries* 2011;**5**(3):235–8.
14. Alverson B, Alexander N, LeGolvan MP, et al. A human immunodeficiency virus–positive infant with probable congenital histoplasmosis in a nonendemic area. *Pediatr Infect Dis J* 2010; **29**(11):1055–7.
17. Anderson AM, Mehta AK, Wang YF, et al. HIV-associated histoplasmosis in a nonendemic area of the United States during the HAART era: role of migration from endemic areas and lack of antiretroviral therapy. *J Int Assoc Physicians AIDS Care (Chic)* 2010;**9**(5):296–300.
22. Antonello VS, Zaltron VF, Vial M, et al. Oropharyngeal histoplasmosis: report of eleven cases and review of the literature. *Rev Soc Bras Med Trop* 2011;**44**(1):26–9.
23. Arango M, Castaneda E, Agudelo CI, et al. Histoplasmosis: results of the Colombian National Survey, 1992-2008. *Biomedica* 2011; **31**(3):344–56.
27. Assi M, Lakkis IE, Wheat LJ. Cross-reactivity in the *Histoplasma* antigen enzyme immunoassay caused by sporotrichosis. *Clin Vaccine Immunol* 2011;**18**(10):1781–2.
29. Azhar JK, Jacqueline HS, Tony LK, et al. Bilateral adrenal histoplasmosis: endoscopic ultrasound-guided fine needle aspiration as a method of diagnosis and assessment. *Med J Malaysia* 2011; **66**(5):504–6.
33. Batista MV, Pierrotti LC, Abdala E, et al. Endemic and opportunistic infections in Brazilian solid organ transplant recipients. *Trop Med Int Health* 2011;**16**(9):1134–42.
38. Berger JR, Greenberg RN. Isolated central nervous system histoplasmosis in an immunocompetent patient: 53-month hiatus to diagnosis and treatment. *J Neurovirol* 2010;**16**(6):472–4.
49. Brilhante RS, Fechine MA, Mesquita JR, et al. Histoplasmosis in HIV-positive patients in Ceará, Brazil: clinical-laboratory aspects and in vitro antifungal susceptibility of *Histoplasma capsulatum* isolates. *Trans R Soc Trop Med Hyg* 2012;**106**(8):484–8.
54. Buitrago MJ, Bernal-Martinez L, Castelli MV, et al. Histoplasmosis and paracoccidioidomycosis in a non-endemic area: a review of cases and diagnosis. *J Travel Med* 2011;**18**(1):26–33.
55. Buitrago MJ, Gonzalo-Jimenez N, Navarro M, et al. A case of primary cutaneous histoplasmosis acquired in the laboratory. *Mycoses* 2011;**54**(6):e859–61.
69. Chakrabarti A, Slavin MA. Endemic fungal infections in the Asia-Pacific region. *Med Mycol* 2011;**49**(4):337–44.
71. Chandra H, Chandra S, Sharma A. Histoplasmosis on bone marrow aspirate cytological examination associated with hemophagocytosis and pancytopenia in an AIDS patient. *Korean J Hematol* 2012;**47**(1):77–9.
75. Chheda LV, Ferketich AK, Carroll CP, et al. Smoking as a risk factor for choroidal neovascularization secondary to presumed ocular histoplasmosis syndrome. *Ophthalmology* 2012;**119**(2):333–8.
82. Cleary JD, Pearson M, Oliver J, et al. Association between *Histoplasma* exposure and stroke. *J Stroke Cerebrovasc Dis* 2008;**17**(5): 312–19.
85. Colaiacovo R, de Castro AC, Shiang C, et al. Disseminated histoplasmosis: a rare cause of multiple ulcers in the gastrointestinal tract. *Endoscopy* 2011;**43**(Suppl 2 UCTN):E216.
86. Colombo AL, Tobon A, Restrepo A, et al. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol* 2011;**49**(8):785–98.
103. Deepe GS Jr, Buesing WR. Deciphering the pathways of death of *Histoplasma capsulatum*-infected macrophages: implications for the immunopathogenesis of early infection. *J Immunol* 2012; **188**(1):334–44.
119. Dos Santos DF, Bitencourt CS, Gelfuso GM, et al. Biodegradable microspheres containing leukotriene B(4) and cell-free antigens from *Histoplasma capsulatum* activate murine bone marrow-derived macrophages. *Eur J Pharm Sci* 2011;**44**(5):580–8.
120. Dotson JL, Crandall W, Mousa H, et al. Presentation and outcome of histoplasmosis in pediatric inflammatory bowel disease patients treated with antitumor necrosis factor alpha therapy: a case series. *Inflamm Bowel Dis* 2011;**17**(1):56–61.
128. Edwards JA, Rappleye CA. *Histoplasma* mechanisms of pathogenesis—one portfolio doesn't fit all. *FEMS Microbiol Lett* 2011;**324**(1):1–9.
129. Edwards JA, Zemska O, Rappleye CA. Discovery of a role for Hsp82 in *Histoplasma* virulence through a quantitative screen for macrophage lethality. *Infect Immun* 2011;**79**(8):3348–57.
144. Frias De Leon MG, Arenas Lopez G, Taylor ML, et al. Development of specific sequence-characterized amplified region markers for detecting *Histoplasma capsulatum* in clinical and environmental samples. *J Clin Microbiol* 2012;**50**(3):673–9.
145. Friedman A, Solomon G, Segal-Maurer S, et al. Sudden onset of verrucous plaques to the face and trunk: a case of cutaneous histoplasmosis in the setting of HIV. *Dermatol Online J* 2008; **14**(2):19.
152. Garcia-Guinon A, Torres-Rodriguez JM, Ndidongarte DT, et al. Disseminated histoplasmosis by *Histoplasma capsulatum* var. *duboisii* in a paediatric patient from the Chad Republic, Africa. *Eur J Clin Microbiol Infect Dis* 2009;**28**(6):697–9.
171. Gorocica P, Taylor ML, Alvarado-Vasquez N, et al. The interaction between *Histoplasma capsulatum* cell wall carbohydrates and host components: relevance in the immunomodulatory role of histoplasmosis. *Mem Inst Oswaldo Cruz* 2009;**104**(3): 492–6.
176. Grim SA, Proia L, Miller R, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. *Transpl Infect Dis* 2012;**14**(1):17–23.
180. Guimaraes AJ, Frases S, Pontes B, et al. Agglutination of *Histoplasma capsulatum* by IgG monoclonal antibodies against Hsp60 impacts macrophage effector functions. *Infect Immun* 2011;**79**(2):918–27.
181. Guimaraes AJ, Nakayasu ES, Sobreira TJ, et al. *Histoplasma capsulatum* heat-shock 60 orchestrates the adaptation of the fungus to temperature stress. *PLoS One* 2011;**6**(2):e14660.
182. Guimaraes LC, Silva AC, Micheletti AM, et al. Morphological changes in the digestive system of 93 human immunodeficiency virus positive patients: an autopsy study. *Rev Inst Med Trop Sao Paulo* 2012;**54**(2):89–93.
183. Gupta N, Arora SK, Rajwanshi A, et al. Histoplasmosis: cytodiagnosis and review of literature with special emphasis on differential diagnosis on cytomorphology. *Cytopathology* 2010;**21**(4):240–4.

187. Hage CA, Bowyer S, Tarvin SE, et al. Recognition, diagnosis, and treatment of histoplasmosis complicating tumor necrosis factor blocker therapy. *Clin Infect Dis* 2010;**50**(1):85–92.
196. Head SJ, Dewey TM, Mack MJ. Fungal endocarditis after transfemoral aortic valve implantation. *Catheter Cardiovasc Interv* 2011;**78**(7):1017–19.
201. Hernandez JM, Munoz-Cadavid CO, Hernandez DL, et al. Detection of *Histoplasma capsulatum* DNA in peripheral blood from a patient with ocular histoplasmosis syndrome. *Med Mycol* 2012;**50**(2):202–6.
204. Hilty J, George Smulian A, Newman SL. *Histoplasma capsulatum* utilizes siderophores for intracellular iron acquisition in macrophages. *Med Mycol* 2011;**49**(6):633–42.
205. Hilty J, Smulian AG, Newman SL. The *Histoplasma capsulatum* vacuolar ATPase is required for iron homeostasis, intracellular replication in macrophages and virulence in a murine model of histoplasmosis. *Mol Microbiol* 2008;**70**(1):127–39.
206. Holbrook ED, Rappleye CA. *Histoplasma capsulatum* pathogenesis: making a lifestyle switch. *Curr Opin Microbiol* 2008;**11**(4):318–24.
210. Hsieh SH, Lin JS, Huang JH, et al. Immunization with apoptotic phagocytes containing *Histoplasma capsulatum* activates functional CD8(+) T cells to protect against histoplasmosis. *Infect Immun* 2011;**79**(11):4493–502.
214. Igreja RP. Infectious diseases associated with caves. *Wilderness Environ Med* 2011;**22**(2):115–21.
215. Inojosa W, Rossi MC, Laurino L, et al. Progressive disseminated histoplasmosis among human immunodeficiency virus–infected patients from West-Africa: report of four imported cases in Italy. *Infez Med* 2011;**19**(1):49–55.
216. Jain M, Revankar SG. A case of peritoneal histoplasmosis in a patient receiving chronic ambulatory peritoneal dialysis. *Mycoses* 2012;**55**(1):99–100.
222. Jinno S, Gripshover BM, Lemonovich TL, et al. *Histoplasma capsulatum* prosthetic valve endocarditis with negative fungal blood cultures and negative *Histoplasma* antigen assay in an immunocompetent patient. *J Clin Microbiol* 2010;**48**(12):4664–6.
227. Kadaria D, Muthiah MP, Sinclair SE. Unusual presentations of disseminated histoplasmosis. *Tenn Med* 2012;**105**(3):39–40.
229. Kajfasz P, Basiak W. Outbreak of pulmonary histoplasmosis involving a group of four Polish travellers returning from Ecuador. *Int Marit Health* 2012;**63**(1):59–62.
238. Kauffman CA. Endemic mycoses: blastomycosis, histoplasmosis, and sporotrichosis. *Infect Dis Clin North Am* 2006;**20**(3):645–62, vii.
243. Keller DL, Steinberg H, Sladky KK. Disseminated histoplasmosis in a Bengal tiger *(Panthera tigris)*. *J Zoo Wildl Med* 2011;**42**(4):727–31.
251. Kroetz DN, Deepe GS. The role of cytokines and chemokines in *Histoplasma capsulatum* infection. *Cytokine* 2012;**58**(1):112–17.
256. Larbcharoensub N, Boonsakan P, Aroonroch R, et al. Adrenal histoplasmosis: a case series and review of the literature. *Southeast Asian J Trop Med Public Health* 2011;**42**(4):920–5.
261. Lee PP, Chan KW, Lee TL, et al. Penicilliosis in children without HIV infection—are they immunodeficient? *Clin Infect Dis* 2012;**54**(2):e8–e19.
269. Lim LT, Ruzmetova N, Ballinger SH, et al. Acute pulmonary histoplasmosis in a patient with uveitis after infliximab therapy. *Int Ophthalmol* 2011;**31**(4):349–51.
280. Lopes LC, Guimaraes AJ, de Cerqueira MD, et al. A *Histoplasma capsulatum*–specific IgG1 isotype monoclonal antibody, H1C, to a 70-kilodalton cell surface protein is not protective in murine histoplasmosis. *Clin Vaccine Immunol* 2010;**17**(7):1155–8.
290. Marchiori E, Zanetti G, Escuissato DL, et al. Reversed halo sign: high-resolution CT findings in 79 patients. *Chest* 2012;**141**(5):1260–6.
293. Mata-Essayag S, Colella MT, Rosello A, et al. Histoplasmosis: a study of 158 cases in Venezuela, 2000-2005. *Medicine (Baltimore)* 2008;**87**(4):193–202.
294. Mayfield JA, Fontana MF, Rine J. Genetic control of immune cell types in fungal disease. *Proc Natl Acad Sci U S A* 2010;**107**(51):22202–6.
300. McKinsey DS, McKinsey JP. Pulmonary histoplasmosis. *Semin Respir Crit Care Med* 2011;**32**(6):735–44.
303. McLeod DS, Mortimer RH, Perry-Keene DA, et al. Histoplasmosis in Australia: report of 16 cases and literature review. *Medicine (Baltimore)* 2011;**90**(1):61–8.
310. Mukhopadhyay S. Role of histology in the diagnosis of infectious causes of granulomatous lung disease. *Curr Opin Pulm Med* 2011;**17**(3):189–96.
312. Muniz Mde M, Morais ESTP, Meyer W, et al. Comparison of different DNA-based methods for molecular typing of *Histoplasma capsulatum*. *Appl Environ Microbiol* 2010;**76**(13):4438–47.
313. Munoz B, Martinez MA, Palma G, et al. Molecular characterization of *Histoplasma capsulatum* isolated from an outbreak in treasure hunters. *BMC Infect Dis* 2010;**10**:264.
314. Munoz C, Gomez BL, Tobon A, et al. Validation and clinical application of a molecular method for identification of *Histoplasma capsulatum* in human specimens in Colombia, South America. *Clin Vaccine Immunol* 2010;**17**(1):62–7.
315. Nacher M, Adenis A, Adriouch L, et al. What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. *Am J Trop Med Hyg* 2011;**84**(2):239–40.
326. Newman SL, Lemen W, Smulian AG. Dendritic cells restrict the transformation of *Histoplasma capsulatum* conidia into yeasts. *Med Mycol* 2011;**49**(4):356–64.
327. Norman FF, Martin-Davila P, Fortun J, et al. Imported histoplasmosis: two distinct profiles in travelers and immigrants. *J Travel Med* 2009;**16**(4):258–62.
331. Nosanchuk JD, Zancope-Oliveira RM, Hamilton AJ, et al. Antibody therapy for histoplasmosis. *Front Microbiol* 2012;**3**:21.
334. Ohji G, Kikuchi K, Inoue K, et al. Progressive disseminated histoplasmosis in an immunocompetent patient as an underrecognized imported mycosis in Japan. *J Infect Chemother* 2010;**16**(6):443–5.
339. Ollague Sierra JE, Ollague Torres JM. New clinical and histological patterns of acute disseminated histoplasmosis in human immunodeficiency virus-positive patients with acquired immunodeficiency syndrome. *Am J Dermatopathol* 2012. [Epub ahead of print].
345. Panchabhai TS, Bais RK, Pyle RC, et al. An apple-core lesion in the colon: an infectious etiology. *J Glob Infect Dis* 2011;**3**(2):195–8.
346. Passos L, Talhari C, Santos M, et al. Histoplasmosis-associated immune reconstitution inflammatory syndrome. *An Bras Dermatol* 2011;**86**(4 Suppl 1):S168–72.
347. Pastor TA, Holcomb MJ, Motaparthi K, et al. Disseminated histoplasmosis mimicking secondary syphilis. *Dermatol Online J* 2011;**17**(11):10.
357. Pereira GH, Padua SS, Park MV, et al. Chronic meningitis by histoplasmosis: report of a child with acute myeloid leukemia. *Braz J Infect Dis* 2008;**12**(6):555–7.
359. Perko R, Messinger Y, Moertel C. Pseudometastasis secondary to histoplasmosis infection: false-positive PET/CT findings. *Pediatr Blood Cancer* 2010;**54**(4):621–3.
372. Quilter LA, Kleiman MB, Kirsch E, et al. Disseminated histoplasmosis of infancy in one of twins. *Pediatr Infect Dis J* 2012;**31**(9):990–1.
375. Rana C, Kumari N, Krishnani N. Adrenal histoplasmosis: a diagnosis on fine needle aspiration cytology. *Diagn Cytopathol* 2011;**39**(6):438–42.
377. Rangel-Castilla L, Hwang SW, White AC, et al. Neuroendoscopic diagnosis of CNS histoplasmosis with basilar arachnoiditis. *World Neurosurg* 2012;**77**(2):399.E9–13.
385. Roby BB, Drehner D, Sidman JD. Pediatric tracheal and endobronchial tumors: an institutional experience. *Arch Otolaryngol Head Neck Surg* 2011;**137**(9):925–9.
393. Saccente M. Central nervous system histoplasmosis. *Curr Treat Options Neurol* 2008;**10**(3):161–7.
407. Secatto A, Rodrigues LC, Serezani CH, et al. 5-Lipoxygenase deficiency impairs innate and adaptive immune responses during fungal infection. *PLoS One* 2012;**7**(3):e31701.
408. Seider K, Heyken A, Luttich A, et al. Interaction of pathogenic yeasts with phagocytes: survival, persistence and escape. *Curr Opin Microbiol* 2010;**13**(4):392–400.
410. Shahani L. Disseminated *Histoplasma* and CMV infection presenting as subacute intestinal obstruction in an immunocompromised patient. *BMJ Case Rep* 2012;**2012**.
416. Simon S, Veron V, Boukhari R, et al. Detection of *Histoplasma capsulatum* DNA in human samples by real-time polymerase chain reaction. *Diagn Microbiol Infect Dis* 2010;**66**(3):268–73.
417. Singh N, Huprikar S, Burdette SD, et al. Donor-derived fungal infections in organ transplant recipients: guidelines of the

American Society of Transplantation, Infectious Diseases Community of Practice. *Am J Transplant* 2012;**12**(9):2414–28.
418. Sivaprasad S, Moore AT. Choroidal neovascularisation in children. *Br J Ophthalmol* 2008;**92**(4):451–4.
420. Smith JA, Kauffman CA. Endemic fungal infections in patients receiving tumour necrosis factor-alpha inhibitor therapy. *Drugs* 2009;**69**(11):1403–15.
422. Smith JG, Magee DM, Williams DM, et al. Tumor necrosis factor-alpha plays a role in host defense against *Histoplasma capsulatum*. *J Infect Dis* 1990;**162**(6):1349–53.
423. Smith JS, Kadiev S, Diaz P, et al. Pulmonary artery stenosis secondary to fibrosing mediastinitis: management with cutting balloon angioplasty and endovascular stenting. *Vasc Endovascular Surg* 2011;**45**(2):170–3.
426. Spellberg B. Vaccines for invasive fungal infections. *F1000 Med Rep* 2011;**3**:13.
429. Steiner SJ, Cox EG, Gupta SK, et al. Esophageal diverticulum: a complication of histoplasmosis in children. *J Pediatr* 2005;**146**(3):426–8.
438. Szymczak WA, Deepe GS Jr. Antigen-presenting dendritic cells rescue CD4-depleted CCR2–/– mice from lethal *Histoplasma capsulatum* infection. *Infect Immun* 2010;**78**(5):2125–37.
439. Tagliari L, Toledo MS, Lacerda TG, et al. Membrane microdomain components of *Histoplasma capsulatum* yeast forms, and their role in alveolar macrophage infectivity. *Biochim Biophys Acta* 2012;**1818**(3):458–66.
440. Takahashi K, Sasaki T, Nabaa B, et al. Pulmonary lymphatic drainage to the mediastinum based on computed tomographic observations of the primary complex of pulmonary histoplasmosis. *Acta Radiol* 2012;**53**(2):161–7.
444. Taylor ML, Hernandez-Garcia L, Estrada-Barcenas D, et al. Genetic diversity of *Histoplasma capsulatum* isolated from infected bats randomly captured in Mexico, Brazil, and Argentina, using the polymorphism of (GA)(n) microsatellite and its flanking regions. *Fungal Biol* 2012;**116**(2):308–17.
453. Threlkeld ZD, Broughton RA, Khan GQ, et al. Isolated *Histoplasma capsulatum* meningoencephalitis in an immunocompetent child. *J Child Neurol* 2012;**27**(4):532–5.
466. van Koeveringe MP, Brouwer RE. *Histoplasma capsulatum* reactivation with haemophagocytic syndrome in a patient with chronic lymphocytic leukaemia. *Neth J Med* 2010;**68**(12):418–21.
522. Winters MS, Chan Q, Caruso JA, et al. Metallomic analysis of macrophages infected with *Histoplasma capsulatum* reveals a fundamental role for zinc in host defenses. *J Infect Dis* 2010;**202**(7):1136–45.
536. Youseff BH, Holbrook ED, Smolnycki KA, et al. Extracellular superoxide dismutase protects *Histoplasma* yeast cells from host-derived oxidative stress. *PLoS Pathog* 2012;**8**(5):e1002713.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 204

Sporotrichosis

Bernhard L. Wiedermann

Sporotrichosis is an infection caused by the fungus *Sporothrix schenckii* that is manifested most commonly as an ulcerating nodule at a site of local trauma, with spread occurring along regional lymphatic channels. Infection of other tissues or widespread dissemination is rare. It is an uncommon problem in children, but young adults seem to be infected more frequently, presumably owing to more frequent exposure to soil, plants, and decaying vegetable matter that harbor the organism. The disease was described first by a medical student, Schenck, in 1898, and most of what is known about the clinical syndrome has been learned from studies of large outbreaks, particularly one occurring in gold miners near Johannesburg, South Africa, in the early 1940s.[94,108]

ORGANISM

S. schenckii is a dimorphic fungus found principally in decaying vegetable matter or plant debris, although it does not seem to be a plant pathogen. A molecular phylogenetic study of 60 isolates from South America and Europe showed three major clades that tended to group according to geographic origin.[72] The organism grows well on most culture media and is resistant to cycloheximide. Preferred culture media are Sabouraud glucose agar and blood agar, incubated at 25°C to 27°C. Most isolates grow readily from clinical material within 3 to 5 days, although occasional instances of slow growth have been reported, and cultures should be held at least 4 weeks before being considered negative. Incubation on blood agar at higher temperatures (37°C) allows growth of the yeast phase, which is necessary for specific identification of the organism in culture.[87] Another species, *Sporothrix mexicana*, also has caused disease in at least one individual.[25]

EPIDEMIOLOGY

Most cases of sporotrichosis are reported from Central and South America, especially from Mexico and Brazil,

REFERENCES

1. Ablanedo-Terrazas Y, Alvarado-de la Barrera C, Ormsby CE, et al. Head and neck manifestations of the immune reconstitution syndrome in HIV-infected patients: a cohort study. *Otolaryngol Head Neck Surg* 2012;**147**(1):52–6.
2. Adderson EE. Histoplasmosis in a pediatric oncology center. *J Pediatr* 2004;**144**(1):100–6.
3. Ahogo KC, Sangare A, Gbery IP, et al. [Cutaneous histoplasmosis due to *Histoplasma capsulatum* variety *duboisii* in an immune competent child: about one case in Abidjan, Cote d'Ivoire]. *Bull Soc Pathol Exot* 2009;**102**(3):147–9.
4. Al-Agha OM, Mooty M, Salarieh A. A 43-year-old woman with acquired immunodeficiency syndrome and fever of undetermined origin: disseminated histoplasmosis. *Arch Pathol Lab Med* 2006; **130**(1):120–3.
5. Alave J, Bustamante B, Soto L, et al. Acalculous cholecystitis caused by *Histoplasma capsulatum* in a severely immunosuppressed HIV-infected patient. *J Infect Dev Ctries* 2011;**5**(3):235–8.
6. Alcure ML, Di Hipolito Junior O, Almeida OP, et al. Oral histoplasmosis in an HIV-negative patient. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;**101**(2):e33–6.
7. Alexander WJ, Mowry RW, Cobbs CG, et al. Prosthetic valve endocarditis caused by *Histoplasma capsulatum*. *JAMA* 1979; **242**(13):1399–400.
8. Allen HL, Deepe GS Jr. Apoptosis modulates protective immunity to the pathogenic fungus *Histoplasma capsulatum*. *J Clin Invest* 2005;**115**(10):2875–85.
9. Allendoerfer R, Biovin GP, Deepe GS Jr. Modulation of immune responses in murine pulmonary histoplasmosis. *J Infect Dis* 1997; **175**(4):905–14.
10. Allendorfer R, Brunner GD, Deepe GS Jr. Complex requirements for nascent and memory immunity in pulmonary histoplasmosis. *J Immunol* 1999;**162**(12):7389–96.
11. Allendoerfer R, Deepe GS Jr. Intrapulmonary response to *Histoplasma capsulatum* in gamma interferon knockout mice. *Infect Immun* 1997;**65**(7):2564–9.
12. Allendoerfer R, Deepe GS Jr. Blockade of endogenous TNF-alpha exacerbates primary and secondary pulmonary histoplasmosis by differential mechanisms. *J Immunol* 1998;**160**(12):6072–82.
13. Allendoerfer R, Deepe GS Jr. Regulation of infection with *Histoplasma capsulatum* by TNFR1 and -2. *J Immunol* 2000;**165**(5): 2657–64.
14. Alverson B, Alexander N, LeGolvan MP, et al. A human immunodeficiency virus–positive infant with probable congenital histoplasmosis in a nonendemic area. *Pediatr Infect Dis J* 2010;**29**(11): 1055–7.
15. Anaissie E, Fainstein V, Samo T, et al. Central nervous system histoplasmosis: an unappreciated complication of the acquired immunodeficiency syndrome. *Am J Med* 1988;**84**(2):215–17.
16. Anders KH, Guerra WF, Tomiyasu U, et al. The neuropathology of AIDS. UCLA experience and review. *Am J Pathol* 1986; **124**(3):537–58.
17. Anderson AM, Mehta AK, Wang YF, et al. HIV-associated histoplasmosis in a nonendemic area of the United States during the HAART era: role of migration from endemic areas and lack of antiretroviral therapy. *J Int Assoc Physicians AIDS Care (Chic)* 2010;**9**(5):296–300.
18. Anderson H, Honish L, Taylor G, et al. Histoplasmosis cluster, golf course, Canada. *Emerg Infect Dis* 2006;**12**(1):163–5.
19. Andes D, Woods JP, Mejicano G. Fungal pathogenesis: bridging the gap between the bedside and the bench. *Mycopathologia* 2005;**159**(2):319–23.
20. Ansart S, Pajot O, Grivois JP, et al. Pneumonia among travelers returning from abroad. *J Travel Med* 2004;**11**(2):87–91.
21. Antinori S, Magni C, Nebuloni M, et al. Histoplasmosis among human immunodeficiency virus-infected people in Europe: report of 4 cases and review of the literature. *Medicine (Baltimore)* 2006;**85**(1):22–36.
22. Antonello VS, Zaltron VF, Vial M, et al. Oropharyngeal histoplasmosis: report of eleven cases and review of the literature. *Rev Soc Bras Med Trop* 2011;**44**(1):26–9.
23. Arango M, Castaneda E, Agudelo CI, et al. Histoplasmosis: results of the Colombian National Survey, 1992-2008. *Biomedica* 2011; **31**(3):344–56.
24. Artz RP, Bullock WE. Immunoregulatory responses in experimental disseminated histoplasmosis: depression of T-cell-dependent and T-effectory responses by activation of splenic suppressor cells. *Infect Immun* 1979;**23**(3):893–902.
25. Artz RP, Bullock WE. Immunoregulatory responses in experimental disseminated histoplasmosis: lymphoid organ histopathology and serological studies. *Infect Immun* 1979;**23**(3):884–92.
26. Ashford DA, Hajjeh RA, Kelley MF, et al. Outbreak of histoplasmosis among cavers attending the National Speleological Society Annual Convention, Texas, 1994. *Am J Trop Med Hyg* 1999;**60**(6): 899–903.
27. Assi M, Lakkis IE, Wheat LJ. Cross-reactivity in the *Histoplasma* antigen enzyme immunoassay caused by sporotrichosis. *Clin Vaccine Immunol* 2011;**18**(10):1781–2.
28. Assi M, McKinsey DS, Driks MR, et al. Gastrointestinal histoplasmosis in the acquired immunodeficiency syndrome: report of 18 cases and literature review. *Diagn Microbiol Infect Dis* 2006; **55**(3):195–201.
29. Azhar JK, Jacqueline HS, Tony LK, et al. Bilateral adrenal histoplasmosis: endoscopic ultrasound-guided fine needle aspiration as a method of diagnosis and assessment. *Med J Malaysia* 2011; **66**(5):504–6.
30. Azizirad O, Clifford DB, Groger RK, et al. Histoplasmoma: isolated central nervous system infection with *Histoplasma capsulatum* in a patient with AIDS: case report and brief review of the literature. *Clin Neurol Neurosurg* 2007;**109**(2):176–81.
31. Balada-Llasat JM, LaRue H, Kamboj K, et al. Detection of yeasts in blood cultures by the Luminex xTAG fungal assay. *J Clin Microbiol* 2012;**50**(2):492–4.
32. Bartlett PC, Weeks RJ, Ajello L. Decontamination of a *Histoplasma capsulatum*–infested bird roost in Illinois. *Arch Environ Health* 1982;**37**(4):221–3.
33. Batista MV, Pierrotti LC, Abdala E, et al. Endemic and opportunistic infections in Brazilian solid organ transplant recipients. *Trop Med Int Health* 2011;**16**(9):1134–42.
34. Baughman RP, Kim CK, Vinegar A, et al. The pathogenesis of experimental pulmonary histoplasmosis: correlative studies of histopathology, bronchoalveolar lavage, and respiratory function. *Am Rev Respir Dis* 1986;**134**(4):771–6.
35. Bauman DS, Smith CD. Comparison of immunodiffusion and complement fixation tests in the diagnosis of histoplasmosis. *J Clin Microbiol* 1976;**2**(2):77–80.
36. Bellin EL, Silva M, Lawyer T Jr. Central nervous system histoplasmosis in a Puerto Rican. *Neurology* 1962;**12**:148–52.
37. Bennish M, Radkowski MA, Ripon JW. Cavitation in acute histoplasmosis. *Chest* 1983;**84**(4):496–7.
38. Berger JR, Greenberg RN. Isolated central nervous system histoplasmosis in an immunocompetent patient: 53-month hiatus to diagnosis and treatment. *J Neurovirol* 2010;**16**(6):472–4.
39. Bhagavan BS, Rao DR, Weinberg T. Histoplasmosis producing broncholithiasis. *Arch Pathol* 1971;**91**(6):577–9.
40. Bhatti S, Vilenski L, Tight R, et al. *Histoplasma* endocarditis: clinical and mycologic features and outcomes. *J Infect* 2005; **51**(1):2–9.
41. Bialek R, Feucht A, Aepinus C, et al. Evaluation of two nested PCR assays for detection of *Histoplasma capsulatum* DNA in human tissue. *J Clin Microbiol* 2002;**40**(5):1644–7.
42. Bille J, Stockman L, Roberts GD, et al. Evaluation of a lysis-centrifugation system for recovery of yeasts and filamentous fungi from blood. *J Clin Microbiol* 1983;**18**(3):469–71.
43. Bonner JR, Alexander WJ, Dismukes WE, et al. Disseminated histoplasmosis in patients with the acquired immune deficiency syndrome. *Arch Intern Med* 1984;**144**(11):2178–81.
44. Bower RJ, Kiesewetter WB. Mediastinal masses in infants and children. *Arch Surg* 1977;**112**(8):1003–9.
45. Bradsher RW, Alford RH, Hawkins SS, et al. Conditions associated with relapse of amphotericin B–treated disseminated histoplasmosis. *Johns Hopkins Med J* 1982;**150**(4):127–31.
46. Brahmi Z, Wheat J, Rubin RH, Schaegel TF Jr. Humoral and cellular immune response in ocular histoplasmosis. *Ann Ophthalmol* 1985;**17**(7):440–4.
47. Brass C, Galgiani JN, Blaschke TF, et al. Disposition of ketoconazole, an oral antifungal, in humans. *Antimicrob Agents Chemother* 1982;**21**(1):151–8.
48. Breton G, Adle-Biassette H, Therby A, et al. Immune reconstitution inflammatory syndrome in HIV-infected patients with disseminated histoplasmosis. *AIDS* 2006;**20**(1):119–21.
49. Brilhante RS, Fechine MA, Mesquita JR, et al. Histoplasmosis in HIV-positive patients in Ceará, Brazil: clinical-laboratory aspects

and in vitro antifungal susceptibility of *Histoplasma capsulatum* isolates. *Trans R Soc Trop Med Hyg* 2012;**106**(8):484–8.
50. Brodsky AL, Gregg MB, Loewenstein MS, et al. Outbreak of histoplasmosis associated with the 1970 Earth Day activities. *Am J Med* 1973;**54**(3):333–42.
51. Bromel C, Sykes JE. Histoplasmosis in dogs and cats. *Clin Tech Small Anim Pract* 2005;**20**(4):227–32.
52. Brummer E, Kurita N, Yosihida S, et al. Fungistatic activity of human neutrophils against *Histoplasma capsulatum*: correlation with phagocytosis. *J Infect Dis* 1991;**164**(1):158–62.
53. Buck BE, Malinin TI, Davis JH. Transmission of histoplasmosis by organ transplantation. *N Engl J Med* 2001;**344**(4):310.
54. Buitrago MJ, Bernal-Martinez L, Castelli MV, et al. Histoplasmosis and paracoccidioidomycosis in a non-endemic area: a review of cases and diagnosis. *J Travel Med* 2011;**18**(1):26–33.
55. Buitrago MJ, Gonzalo-Jimenez N, Navarro M, et al. A case of primary cutaneous histoplasmosis acquired in the laboratory. *Mycoses* 2011;**54**(6):e859–61.
56. Bullock WE, Wright SD. Role of the adherence-promoting receptors, CR3, LFA-1, and p150,95, in binding of *Histoplasma capsulatum* by human macrophages. *J Exp Med* 1987;**165**(1): 195–210.
57. Bun Navy K, Prem Prey S, Lynen L, et al. The first reported cases of disseminated histoplasmosis in Cambodia, complicated by multiple opportunistic infections. *Southeast Asian J Trop Med Public Health* 2005;**36**(5):1272–4.
58. Burnie JP, Carter TL, Hodgetts SJ, et al. Fungal heat-shock proteins in human disease. *FEMS Microbiol Rev* 2006;**30**(1):53–88.
59. Butler JC, Heller R, Wright PF. Histoplasmosis during childhood. *South Med J* 1994;**87**(4):476–80.
60. Buxton JA, Dawar M, Wheat LJ, et al. Outbreak of histoplasmosis in a school party that visited a cave in Belize: role of antigen testing in diagnosis. *J Travel Med* 2002;**9**(1):48–50.
61. Cain JA, Deepe GS Jr. Evolution of the primary immune response to *Histoplasma capsulatum* in murine lung. *Infect Immun* 1998; **66**(4):1473–81.
62. Cain JA, Deepe GS Jr. Interleukin-12 neutralization alters lung inflammation and leukocyte expression of CD80, CD86, and major histocompatibility complex class II in mice infected with *Histoplasma capsulatum*. *Infect Immun* 2000;**68**(4):2069–76.
63. Calza L, Manfredi R, Donzelli C, et al. Disseminated histoplasmosis with atypical cutaneous lesions in an Italian HIV-infected patient: another autochtonous case. *HIV Med* 2003;**4**(2): 145–8.
64. Campbell CC, Hill GB, Falgout BT. *Histoplasma capsulatum* isolated from feather pillow associated with histoplasmosis in an infant. *Science* 1962;**136**:1050–1.
65. Cano MV, Hajjeh RA. The epidemiology of histoplasmosis: a review. *Semin Respir Infect* 2001;**16**(2):109–18.
66. Canteros CE, Zuiani MF, Ritacco V, et al. Electrophoresis karyotype and chromosome-length polymorphism of *Histoplasma capsulatum* clinical isolates from Latin America. *FEMS Immunol Med Microbiol* 2005;**45**(3):423–8.
67. Cappell MS, Mandell W, Grimes MM, et al. Gastrointestinal histoplasmosis. *Dig Dis Sci* 1988;**33**(3):353–60.
68. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 24-1984. Pancytopenia and fever in a renal-transplant recipient. *N Engl J Med* 1984;**310**(24): 1584–94.
69. Chakrabarti A, Slavin MA. Endemic fungal infections in the Asia-Pacific region. *Med Mycol* 2011;**49**(4):337–44.
70. Chamany S, Mirza SA, Fleming JW, et al. A large histoplasmosis outbreak among high school students in Indiana, 2001. *Pediatr Infect Dis J* 2004;**23**(10):909–14.
71. Chandra H, Chandra S, Sharma A. Histoplasmosis on bone marrow aspirate cytological examination associated with hemophagocytosis and pancytopenia in an AIDS patient. *Korean J Hematol* 2012;**47**(1):77–9.
72. Chaturvedi S, Frame P, Newman SL. Macrophages from human immunodeficiency virus-positive persons are defective in host defense against *Histoplasma capsulatum*. *J Infect Dis* 1995;**171**(2): 320–7.
73. Chaturvedi S, Newman SL. Modulation of the effector function of human macrophages for *Histoplasma capsulatum* by HIV-1. Role of the envelope glycoprotein gp120. *J Clin Invest* 1997;**100**(6): 1465–74.
74. Chemaly RF, Tomford JW, Hall GS, et al. Rapid diagnosis of *Histoplasma capsulatum* endocarditis using the AccuProbe on an excised valve. *J Clin Microbiol* 2001;**39**(7):2640–1.
75. Chheda LV, Ferketich AK, Carroll CP, et al. Smoking as a risk factor for choroidal neovascularization secondary to presumed ocular histoplasmosis syndrome. *Ophthalmology* 2012;**119**(2): 333–8.
76. Christie A, Peterson JC. Pulmonary calcification and sensitivity to histoplasmin, tuberculin and haplosporangin. *JAMA* 1946;**131**: 658–60.
77. Chu JH, Feudtner C, Heydon K, et al. Hospitalizations for endemic mycoses: a population-based national study. *Clin Infect Dis* 2006;**42**(6):822–5.
78. Ciulla TA, Piper HC, Xiao M, et al. Presumed ocular histoplasmosis syndrome: update on epidemiology, pathogenesis, and photodynamic, antiangiogenic, and surgical therapies. *Curr Opin Ophthalmol* 2001;**12**(6):442–9.
79. Clapp DW, Kleiman MB, Brahmi Z. Immunoregulatory lymphocyte populations in disseminated histoplasmosis of infancy. *J Infect Dis* 1987;**156**(4):687–8.
80. Clark B, Foster R, Tunbridge A, et al. A case of disseminated histoplasmosis successfully treated with the investigational drug posaconazole. *J Infect* 2005;**51**(3):e177–80.
81. Class RN, Cascio FS. Histoplasmosis presenting as acute polyarthritis. *N Engl J Med* 1972;**287**(22):1133–4.
82. Cleary JD, Pearson M, Oliver J, et al. Association between *Histoplasma* exposure and stroke. *J Stroke Cerebrovasc Dis* 2008; **17**(5):312–19.
83. Clemons KV, Darbonne WC, Curnutte JT, et al. Experimental histoplasmosis in mice treated with anti-murine interferon-gamma antibody and in interferon-gamma gene knockout mice. *Microbes Infect* 2000;**2**(9):997–1001.
84. Cockshott WP, Lucas AO. Histoplasmosis duboisii. *Q J Med* 1964;**33**:223–38.
85. Colaiacovo R, de Castro AC, Shiang C, et al. Disseminated histoplasmosis: a rare cause of multiple ulcers in the gastrointestinal tract. *Endoscopy* 2011;**43**(Suppl 2 UCTN):E216.
86. Colombo AL, Tobon A, Restrepo A, et al. Epidemiology of endemic systemic fungal infections in Latin America. *Med Mycol* 2011;**49**(8):785–98.
87. Conces DJ Jr, Stockberger SM, Tarver RD, et al. Disseminated histoplasmosis in AIDS: findings on chest radiographs. *AJR Am J Roentgenol* 1993;**160**(1):15–19.
88. Connell JV, Muhm JR. Radiographic manifestations of pulmonary histoplasmosis: a 10-year review. *Radiology* 1976;**121**(2):281–5.
89. Connolly P, Wheat LJ, Schnizlein-Bick C, et al. Comparison of a new triazole, posaconazole, with itraconazole and amphotericin B for treatment of histoplasmosis following pulmonary challenge in immunocompromised mice. *Antimicrob Agents Chemother* 2000;**44**(10):2604–8.
90. Cott GR, Smith TW, Hinthorn DR, et al. Primary cutaneous histoplasmosis in immunosuppressed patient. *JAMA* 1979;**242**(5): 456–7.
91. Couppie P, Sobesky M, Aznar C, et al. Histoplasmosis and acquired immunodeficiency syndrome: a study of prognostic factors. *Clin Infect Dis* 2004;**38**(1):134–8.
92. Crum-Cianflone NF. Immune reconstitution inflammatory syndromes: what's new? *AIDS Read* 2006;**16**(4):199–206, 13, 16–17; discussion 14–17.
93. Cucurull E, Sarwar H, Williams CS, et al. Localized tenosynovitis caused by *Histoplasma capsulatum*: case report and review of the literature. *Arthritis Rheum* 2005;**53**(1):129–32.
94. Daher EF, Silva GB Jr, Barros FA, et al. Clinical and laboratory features of disseminated histoplasmosis in HIV patients from Brazil. *Trop Med Int Health* 2007;**12**(9):1108–15.
95. Daubenton JD, Beatty DW. Disseminated histoplasmosis in an "immunocompetent" child. *S Afr Med J* 1998;**88**(3):270–1.
96. Davies SF. Serodiagnosis of histoplasmosis. *Semin Respir Infect* 1986;**1**(1):9–15.
97. Davies SF, Khan M, Sarosi GA. Disseminated histoplasmosis in immunologically suppressed patients: occurrence in a nonendemic area. *Am J Med* 1978;**64**(1):94–100.
98. Davis AM, Pierson RN, Loyd JE. Mediastinal fibrosis. *Semin Respir Infect* 2001;**16**(2):119–30.
99. de Francesco Daher E, de Sousa Barros FA, da Silva Junior GB, et al. Risk factors for death in acquired immunodeficiency

syndrome–associated disseminated histoplasmosis. *Am J Trop Med Hyg* 2006;**74**(4):600–3.
100. De Lima Sampaio I, Lima Freire AK, Morishi Ogusko M, et al. Selection and optimization of PCR-based methods for the detection of *Histoplasma capsulatum* var. *capsulatum*. *Rev Iberoam Micol* 2012;**29**(1):34–9.
101. Deepe GS Jr. Modulation of infection with *Histoplasma capsulatum* by inhibition of tumor necrosis factor-alpha activity. *Clin Infect Dis* 2005;**41**(Suppl 3):S204–7.
102. Deepe GS Jr. Protective immunity in murine histoplasmosis: functional comparison of adoptively transferred T-cell clones and splenic T cells. *Infect Immun* 1988;**56**(9):2350–5.
103. Deepe GS Jr, Buesing WR. Deciphering the pathways of death of *Histoplasma capsulatum*–infected macrophages: implications for the immunopathogenesis of early infection. *J Immunol* 2012; **188**(1):334–44.
104. Deepe GS Jr, Gibbons R. Recombinant murine granulocyte-macrophage colony-stimulating factor modulates the course of pulmonary histoplasmosis in immunocompetent and immunodeficient mice. *Antimicrob Agents Chemother* 2000;**44**(12):3328–36.
105. Deepe GS Jr, Gibbons R. Protective efficacy of H antigen from *Histoplasma capsulatum* in a murine model of pulmonary histoplasmosis. *Infect Immun* 2001;**69**(5):3128–34.
106. Deepe GS Jr, Gibbons RS. Cellular and molecular regulation of vaccination with heat shock protein 60 from *Histoplasma capsulatum*. *Infect Immun* 2002;**70**(7):3759–67.
107. Deepe GS Jr, Gibbons RS. Protective and memory immunity to *Histoplasma capsulatum* in the absence of IL-10. *J Immunol* 2003; **171**(10):5353–62.
108. Deepe GS Jr, Gibbons RS. T cells require tumor necrosis factor-alpha to provide protective immunity in mice infected with *Histoplasma capsulatum*. *J Infect Dis* 2006;**193**(2):322–30.
109. Deepe GS Jr, Gibbons R, Woodward E. Neutralization of endogenous granulocyte-macrophage colony-stimulating factor subverts the protective immune response to *Histoplasma capsulatum*. *J Immunol* 1999;**163**(9):4985–93.
110. Deepe GS Jr, McGuinness M. Interleukin-1 and host control of pulmonary histoplasmosis. *J Infect Dis* 2006;**194**(6):855–64.
111. Deepe GS Jr, Wuthrich M, Klein BS. Progress in vaccination for histoplasmosis and blastomycosis: coping with cellular immunity. *Med Mycol* 2005;**43**(5):381–9.
112. DeFelice R, Johnson DG, Galgiani JN. Gynecomastia with ketoconazole. *Antimicrob Agents Chemother* 1981;**19**(6):1073–4.
113. Dines DE, Payne WS, Bernatz PE, et al. Mediastinal granuloma and fibrosing mediastinitis. *Chest* 1979;**75**(3):320–4.
114. Dismukes WE, Bradsher RW Jr, Cloud GC, et al. Itraconazole therapy for blastomycosis and histoplasmosis. NIAID Mycoses Study Group. *Am J Med* 1992;**93**(5):489–97.
115. Dismukes WE, Royal SA, Tynes BS. Disseminated histoplasmosis in corticosteroid-treated patients: report of five cases. *JAMA* 1978;**240**(14):1495–8.
116. Dismukes WE, Stamm AM, Graybill JR, et al. Treatment of systemic mycoses with ketoconazole: emphasis on toxicity and clinical response in 52 patients. National Institute of Allergy and Infectious Diseases collaborative antifungal study. *Ann Intern Med* 1983;**98**(1):13–20.
117. Diveley W, McCracken R. Cavitary pulmonary histoplasmosis treated by pulmonary resection: 13-year experience with 29 cases. *Ann Surg* 1966;**163**(6):921–30.
118. Domer JE. Monosaccharide and chitin content of cell walls of *Histoplasma capsulatum* and *Blastomyces dermatitidis*. *J Bacteriol* 1971;**107**(3):870–7.
119. Dos Santos DF, Bitencourt CS, Gelfuso GM, et al. Biodegradable microspheres containing leukotriene B(4) and cell-free antigens from *Histoplasma capsulatum* activate murine bone marrow-derived macrophages. *Eur J Pharm Sci* 2011;**44**(5):580–8.
120. Dotson JL, Crandall W, Mousa H, et al. Presentation and outcome of histoplasmosis in pediatric inflammatory bowel disease patients treated with antitumor necrosis factor alpha therapy: a case series. *Inflamm Bowel Dis* 2011;**17**(1):56–61.
121. Doyle TP, Loyd JE, Robbins IM. Percutaneous pulmonary artery and vein stenting: a novel treatment for mediastinal fibrosis. *Am J Respir Crit Care Med* 2001;**164**(4):657–60.
122. Drutz DJ, Spickard A, Rogers DE, et al. Treatment of disseminated mycotic infections: a new approach to amphotericin B therapy. *Am J Med* 1968;**45**(3):405–18.
123. Dugoni BM, Guglielmo BJ, Hollander H. Amphotericin B concentration in cerebrospinal fluid of patients with AIDS and cryptococcal meningitis. *Clin Pharm* 1989;**8**(3):220–1.
124. Dukes RJ, Strimlan CV, Dines DE, et al. Esophageal involvement with mediastinal granuloma. *JAMA* 1976;**236**(20):2313–15.
125. Duncan RA, von Reyn CF, Alliegro GM, et al. Idiopathic CD4+ T-lymphocytopenia—four patients with opportunistic infections and no evidence of HIV infection. *N Engl J Med* 1993;**328**(6): 393–8.
126. Durkin MM, Connolly PA, Karimi K, et al. Pathogenic differences between North American and Latin American strains of *Histoplasma capsulatum* var. *capsulatum* in experimentally infected mice. *J Clin Microbiol* 2004;**42**(9):4370–3.
127. Durkin MM, Connolly PA, Wheat LJ. Comparison of radioimmunoassay and enzyme-linked immunoassay methods for detection of *Histoplasma capsulatum* var. *capsulatum* antigen. *J Clin Microbiol* 1997;**35**(9):2252–5.
128. Edwards JA, Rappleye CA. *Histoplasma* mechanisms of pathogenesis—one portfolio doesn't fit all. *FEMS Microbiol Lett* 2011;**324**(1):1–9.
129. Edwards JA, Zemska O, Rappleye CA. Discovery of a role for Hsp82 in *Histoplasma* virulence through a quantitative screen for macrophage lethality. *Infect Immun* 2011;**79**(8):3348–57.
130. Edwards LB, Acquaviva FA, Livesay VT, et al. An atlas of sensitivity to tuberculin, PPD-B, and histoplasmin in the United States. *Am Rev Respir Dis* 1969;**99**(4 Suppl):1–132.
131. Edwards LB, Acquaviva FA, Livesay VT. Further observations on histoplasmin sensitivity in the United States. *Am J Epidemiol* 1973;**98**(5):315–25.
132. Ehlers S. Tumor necrosis factor and its blockade in granulomatous infections: differential modes of action of infliximab and etanercept? *Clin Infect Dis* 2005;**41**(Suppl 3):S199–203.
133. Ellis ME, Al-Abdely H, Sandridge A, et al. Fungal endocarditis: evidence in the world literature, 1965-1995. *Clin Infect Dis* 2001;**32**(1):50–62.
134. Emmons CW, Klite PD, Baer GM, et al. Isolation of *Histoplasma capsulatum* from bats in the United States. *Am J Epidemiol* 1966;**84**(1):103–9.
135. Espinel-Ingroff A, Shadomy S, Gebhart RJ. In vitro studies with R 51,211 (itraconazole). *Antimicrob Agents Chemother* 1984;**26**(1): 5–9.
136. Ezzedine K, Accoceberry I, Malvy D. Oral histoplasmosis after radiation therapy for laryngeal squamous cell carcinoma. *J Am Acad Dermatol* 2007;**56**(5):871–3.
137. Faggian F, Lanzafame M, Lattuada E, et al. Histoplasmosis in two human immunodeficiency virus–positive immigrants to Italy: clinical features and management in the highly active antiretroviral therapy era. *South Med J* 2004;**97**(4):398–400.
138. Farina C, Rizzi M, Ricci L, et al. Imported and autochthonous histoplasmosis in Italy: new cases and old problems. *Rev Iberoam Micol* 2005;**22**(3):169–71.
139. Ferguson TB, Burford TH. Mediastinal granuloma: a 15-year experience. *Ann Thorac Surg* 1965;**24**:125–41.
140. Flynn PM, Barrett FF, Herrod HG. Disseminated histoplasmosis in two patients with chronic mucocutaneous candidiasis. *Pediatr Infect Dis J* 1987;**6**(7):691–3.
141. Fojtasek MF, Kleiman MB, Connolly-Stringfield P, et al. The *Histoplasma capsulatum* antigen assay in disseminated histoplasmosis in children. *Pediatr Infect Dis J* 1994;**13**(9):801–5.
142. Fosson AR, Wheeler WE. Short-term amphotericin B treatment of severe childhood histoplasmosis. *J Pediatr* 1975;**86**(1):32–6.
143. Freifeld AG, Iwen PC, Lesiak BL, et al. Histoplasmosis in solid organ transplant recipients at a large Midwestern university transplant center. *Transpl Infect Dis* 2005;**7**(3-4):109–15.
144. Frias De Leon MG, Arenas Lopez G, Taylor ML, et al. Development of specific sequence-characterized amplified region markers for detecting *Histoplasma capsulatum* in clinical and environmental samples. *J Clin Microbiol* 2012;**50**(3):673–9.
145. Friedman A, Solomon G, Segal-Maurer S, et al. Sudden onset of verrucous plaques to the face and trunk: a case of cutaneous histoplasmosis in the setting of HIV. *Dermatol Online J* 2008;**14**(2):19.
146. Furcolow ML. Tests of immunity in histoplasmosis. *N Engl J Med* 1963;**268**:357–61.
147. Furcolow ML, Schubert J, Tosh FE, et al. Serologic evidence of histoplasmosis in sanatoriums in the U.S. *JAMA* 1962;**180**: 109–14.

148. Furst DE, Wallis R, Broder M, et al. Tumor necrosis factor antagonists: different kinetics and/or mechanisms of action may explain differences in the risk for developing granulomatous infection. *Semin Arthritis Rheum* 2006;**36**(3):159–67.
149. Gaebler JW, Kleiman MB, Cohen M, et al. Differentiation of lymphoma from histoplasmosis in children with mediastinal masses. *J Pediatr* 1984;**104**(5):706–9.
150. Gaissert HA, Grillo HC, Mathisen DJ, et al. [Resection and reconstruction of the carina in children and adolescents]. *Langenbecks Arch Chir* 1995;**380**(3):166–70.
151. Ganer A, Arathoon E, Stevens DA. Initial experience in therapy for progressive mycoses with itraconazole, the first clinically studied triazole. *Rev Infect Dis* 1987;**9**(Suppl 1):S77–86.
152. Garcia-Guinon A, Torres-Rodriguez JM, Ndidongarte DT, et al. Disseminated histoplasmosis by *Histoplasma capsulatum* var. *duboisii* in a paediatric patient from the Chad Republic, Africa. *Eur J Clin Microbiol Infect Dis* 2009;**28**(6):697–9.
153. Garrett HE Jr, Roper CL. Surgical intervention in histoplasmosis. *Ann Thorac Surg* 1986;**42**(6):711–22.
154. Gascon J, Torres JM, Jimenez M, et al. Histoplasmosis infection in Spanish travelers to Latin America. *Eur J Clin Microbiol Infect Dis* 2005;**24**(12):839–41.
155. Gass M, Kobayashi GS. Histoplasmosis: an illustrative case with unusual vaginal and joint involvement. *Arch Dermatol* 1969; **100**(6):724–7.
156. Gaynes RP, Gardner P, Causey W. Prosthetic valve endocarditis caused by *Histoplasma capsulatum*. *Arch Intern Med* 1981;**141**(11): 1533–7.
157. Gelfand JA, Bennett JE. Active *Histoplasma* meningitis of 22 years' duration. *JAMA* 1975;**233**(12):1294–5.
158. George RB, Lambert RS. Significance of serum antibodies to *Histoplasma capsulatum* in endemic areas. *South Med J* 1984; **77**(2):161–3.
159. Gilbert EH, Murray KD, Lucas J, et al. Left recurrent laryngeal nerve paralysis: an unusual presentation of histoplasmosis. *Ann Thorac Surg* 1990;**50**(6):987–8.
160. Gildea LA, Ciraolo GM, Morris RE, et al. Human dendritic cell activity against *Histoplasma capsulatum* is mediated via phagolysosomal fusion. *Infect Immun* 2005;**73**(10):6803–11.
161. Gildea LA, Morris RE, Newman SL. *Histoplasma capsulatum* yeasts are phagocytosed via very late antigen-5, killed, and processed for antigen presentation by human dendritic cells. *J Immunol* 2001; **166**(2):1049–56.
162. Glasmacher A, Hahn C, Molitor E, et al. Itraconazole trough concentrations in antifungal prophylaxis with six different dosing regimens using hydroxypropyl-beta-cyclodextrin oral solution or coated-pellet capsules. *Mycoses* 1999;**42**(11-12):591–600.
163. Goldman M, Zackin R, Fichtenbaum CJ, et al. Safety of discontinuation of maintenance therapy for disseminated histoplasmosis after immunologic response to antiretroviral therapy. *Clin Infect Dis* 2004;**38**(10):1485–9.
164. Gomez AM, Bullock WE, Taylor CL, et al. Role of L3T4$^+$ T cells in host defense against *Histoplasma capsulatum*. *Infect Immun* 1988;**56**(7):1685–91.
165. Gomez FJ, Allendoerfer R, Deepe GS Jr. Vaccination with recombinant heat shock protein 60 from *Histoplasma capsulatum* protects mice against pulmonary histoplasmosis. *Infect Immun* 1995;**63**(7): 2587–95.
166. Gonzales CA, Scott IU, Chaudhry NA, et al. Endogenous endophthalmitis caused by *Histoplasma capsulatum* var. *capsulatum:* a case report and literature review. *Ophthalmology* 2000;**107**(4):725–9.
167. Goodwin RA Jr, Owens FT, Snell JD, et al. Chronic pulmonary histoplasmosis. *Medicine (Baltimore)* 1976;**55**(6):413–52.
168. Goodwin RA, Loyd JE, Des Prez RM. Histoplasmosis in normal hosts. *Medicine (Baltimore)* 1981;**60**(4):231–66.
169. Goodwin RA Jr, Shapiro JL, Thurman GH, et al. Disseminated histoplasmosis: clinical and pathologic correlations. *Medicine (Baltimore)* 1980;**59**(1):1–33.
170. Goodwin RA Jr, Snell JD Jr. The enlarging histoplasmoma: concept of a tumor-like phenomenon encompassing the tuberculoma and coccidioidoma. *Am Rev Respir Dis* 1969;**100**(1):1–12.
171. Gorocica P, Taylor ML, Alvarado-Vasquez N, et al. The interaction between *Histoplasma capsulatum* cell wall carbohydrates and host components: relevance in the immunomodulatory role of histoplasmosis. *Mem Inst Oswaldo Cruz* 2009;**104**(3):492–6.
172. Graybill JR. Histoplasmosis and AIDS. *J Infect Dis* 1988; **158**(3):623–6.
173. Graybill JR, Patino MM, Gomez AM, et al. Detection of histoplasmal antigens in mice undergoing experimental pulmonary histoplasmosis. *Am Rev Respir Dis* 1985;**132**(4):752–6.
174. Greene LW, Cole W, Greene JB, et al. Adrenal insufficiency as a complication of the acquired immunodeficiency syndrome. *Ann Intern Med* 1984;**101**(4):497–8.
175. Greenwood MF, Holland P. Tracheal obstruction secondary to *Histoplasma* mediastinal granuloma. *Chest* 1972;**62**(5):642–5.
176. Grim SA, Proia L, Miller R, et al. A multicenter study of histoplasmosis and blastomycosis after solid organ transplantation. *Transpl Infect Dis* 2012;**14**(1):17–23.
177. Groll AH, Mickiene D, Petraitis V, et al. Comparative drug disposition, urinary pharmacokinetics, and renal effects of multilamellar liposomal nystatin and amphotericin B deoxycholate in rabbits. *Antimicrob Agents Chemother* 2003;**47**(12):3917–25.
178. Grover SB, Midha N, Gupta M, et al. Imaging spectrum in disseminated histoplasmosis: case report and brief review. *Australas Radiol* 2005;**49**(2):175–8.
179. Gugnani HC. Histoplasmosis in Africa: a review. *Indian J Chest Dis Allied Sci* 2000;**42**(4):271–7.
180. Guimaraes AJ, Frases S, Pontes B, et al. Agglutination of *Histoplasma capsulatum* by IgG monoclonal antibodies against Hsp60 impacts macrophage effector functions. *Infect Immun* 2011;**79**(2):918–27.
181. Guimaraes AJ, Nakayasu ES, Sobreira TJ, et al. *Histoplasma capsulatum* heat-shock 60 orchestrates the adaptation of the fungus to temperature stress. *PLoS One* 2011;**6**(2):e14660.
182. Guimaraes LC, Silva AC, Micheletti AM, et al. Morphological changes in the digestive system of 93 human immunodeficiency virus positive patients: an autopsy study. *Rev Inst Med Trop Sao Paulo* 2012;**54**(2):89–93.
183. Gupta N, Arora SK, Rajwanshi A, et al. Histoplasmosis: cytodiagnosis and review of literature with special emphasis on differential diagnosis on cytomorphology. *Cytopathology* 2010;**21**(4): 240–4.
184. Gurney JW, Conces DJ. Pulmonary histoplasmosis. *Radiology* 1996;**199**(2):297–306.
185. Gustafson TL, Kaufman L, Weeks R, et al. Outbreak of acute pulmonary histoplasmosis in members of a wagon train. *Am J Med* 1981;**71**(5):759–65.
186. Gutierrez ME, Canton A, Sosa N, et al. Disseminated histoplasmosis in patients with AIDS in Panama: a review of 104 cases. *Clin Infect Dis* 2005;**40**(8):1199–202.
187. Hage CA, Bowyer S, Tarvin SE, et al. Recognition, diagnosis, and treatment of histoplasmosis complicating tumor necrosis factor blocker therapy. *Clin Infect Dis* 2010;**50**(1):85–92.
188. Hage CA, Davis TE, Egan L, et al. Diagnosis of pulmonary histoplasmosis and blastomycosis by detection of antigen in bronchoalveolar lavage fluid using an improved second-generation enzyme-linked immunoassay. *Respir Med* 2007;**101**(1):43–7.
189. Hage CA, Goldman M, Wheat LJ. Mucosal and invasive fungal infections in HIV/AIDS. *Eur J Med Res* 2002;**7**(5):236–41.
190. Hage CA, Wheat LJ, Twigg HL, et al. Infliximab does not affect dendritic cells' mediated lymphoproliferative response to *Histoplasma capsulatum*. *Clin Infect Dis* 2005;**41**(11):1685–7; author reply 7.
191. Haggerty CM, Britton MC, Dorman JM, et al. Gastrointestinal histoplasmosis in suspected acquired immunodeficiency syndrome. *West J Med* 1985;**143**(2):244–6.
192. Hajjeh RA. Disseminated histoplasmosis in persons infected with human immunodeficiency virus. *Clin Infect Dis* 1995;**21**(Suppl 1): S108–10.
193. Hajjeh RA, Pappas PG, Henderson H, et al. Multicenter case-control study of risk factors for histoplasmosis in human immunodeficiency virus-infected persons. *Clin Infect Dis* 2001;**32**(8): 1215–20.
194. Halline AG, Maldonado-Lutomirsky M, Ryoo JW, et al. Colonic histoplasmosis in AIDS: unusual endoscopic findings in two cases. *Gastrointest Endosc* 1997;**45**(2):199–204.
195. Haynes RR, Connolly PA, Durkin MM, et al. Antifungal therapy for central nervous system histoplasmosis, using a newly developed intracranial model of infection. *J Infect Dis* 2002;**185**(12): 1830–2.

196. Head SJ, Dewey TM, Mack MJ. Fungal endocarditis after transfemoral aortic valve implantation. *Catheter Cardiovasc Interv* 2011;**78**(7):1017–19.
197. Hecht FM, Wheat J, Korzun AH, et al. Itraconazole maintenance treatment for histoplasmosis in AIDS: a prospective, multicenter trial. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;**16**(2): 100–7.
198. Heeres J, Backx LJ, Van Cutsem J. Antimycotic azoles, part 7: synthesis and antifungal properties of a series of novel triazol-3-ones. *J Med Chem* 1984;**27**(7):894–900.
199. Heiberg JK, Svejgaard E. Toxic hepatis during ketoconazole treatment. *BMJ (Clin Res Ed)* 1981;**283**(6295):825–6.
200. Heninger E, Hogan LH, Karman J, et al. Characterization of the *Histoplasma capsulatum*–induced granuloma. *J Immunol* 2006; **177**(5):3303–13.
201. Hernandez JM, Munoz-Cadavid CO, Hernandez DL, et al. Detection of *Histoplasma capsulatum* DNA in peripheral blood from a patient with ocular histoplasmosis syndrome. *Med Mycol* 2012;**50**(2):202–6.
202. Hertan H, Nair S, Arguello P. Progressive gastrointestinal histoplasmosis leading to colonic obstruction two years after initial presentation. *Am J Gastroenterol* 2001;**96**(1):221–2.
203. Heusinkveld RS, Tosh FE, Newberry WM Jr, et al. Antibody response to the histoplasmin skin test. *Am Rev Respir Dis* 1967; **96**(5):1069–71.
204. Hilty J, George Smulian A, Newman SL. *Histoplasma capsulatum* utilizes siderophores for intracellular iron acquisition in macrophages. *Med Mycol* 2011;**49**(6):633–42.
205. Hilty J, Smulian AG, Newman SL. The *Histoplasma capsulatum* vacuolar ATPase is required for iron homeostasis, intracellular replication in macrophages and virulence in a murine model of histoplasmosis. *Mol Microbiol* 2008;**70**(1):127–39.
206. Holbrook ED, Rappleye CA. *Histoplasma capsulatum* pathogenesis: making a lifestyle switch. *Curr Opin Microbiol* 2008;**11**(4):318–24.
207. Hostoffer RW, Berger M, Clark HT, et al. Disseminated *Histoplasma capsulatum* in a patient with hyper IgM immunodeficiency. *Pediatrics* 1994;**94**(2 Pt 1):234–6.
208. Hott JS, Horn E, Sonntag VK, et al. Intramedullary histoplasmosis spinal cord abscess in a nonendemic region: case report and review of the literature. *J Spinal Disord Tech* 2003;**16**(2):212–15.
209. Hove MG, Woods GL. Duration of fungal culture incubation in an area endemic for *Histoplasma capsulatum*. *Diagn Microbiol Infect Dis* 1997;**28**(1):41–3.
210. Hsieh SH, Lin JS, Huang JH, et al. Immunization with apoptotic phagocytes containing *Histoplasma capsulatum* activates functional CD8(+) T cells to protect against histoplasmosis. *Infect Immun* 2011;**79**(11):4493–502.
211. Hughes WT. Hematogenous histoplasmosis in the immunocompromised child. *J Pediatr* 1984;**105**(4):569–75.
212. Huhn GD, Austin C, Carr M, et al. Two outbreaks of occupationally acquired histoplasmosis: more than workers at risk. *Environ Health Perspect* 2005;**113**(5):585–9.
213. Ignatov A, Keath EJ. Molecular cell biology and molecular genetics of *Histoplasma capsulatum*. *Int J Med Microbiol* 2002;**292**(5-6): 349–61.
214. Igreja RP. Infectious diseases associated with caves. *Wilderness Environ Med* 2011;**22**(2):115–21.
215. Inojosa W, Rossi MC, Laurino L, et al. Progressive disseminated histoplasmosis among human immunodeficiency virus–infected patients from West-Africa: report of four imported cases in Italy. *Infez Med* 2011;**19**(1):49–55.
216. Jain M, Revankar SG. A case of peritoneal histoplasmosis in a patient receiving chronic ambulatory peritoneal dialysis. *Mycoses* 2012;**55**(1):99–100.
217. Jain S, Koirala J, Castro-Pavia F. Isolated gastrointestinal histoplasmosis: case report and review of the literature. *South Med J* 2004;**97**(2):172–4.
218. Jain VV, Evans T, Peterson MW. Reactivation histoplasmosis after treatment with anti-tumor necrosis factor alpha in a patient from a nonendemic area. *Respir Med* 2006;**100**(7):1291–3.
219. Jaruratanasirikul S, Kleepkaew A. Influence of an acidic beverage (Coca-Cola) on the absorption of itraconazole. *Eur J Clin Pharmacol* 1997;**52**(3):235–7.
220. Jiang B, Bartlett MS, Allen SD, et al. Typing of *Histoplasma capsulatum* isolates based on nucleotide sequence variation in the internal transcribed spacer regions of rRNA genes. *J Clin Microbiol* 2000;**38**(1):241–5.
221. Jimenez RA, Uran ME, de Bedout C, et al. [Outbreak of acute histoplasmosis in a family group: identification of the infection source]. *Biomedica* 2002;**22**(2):155–9.
222. Jinno S, Gripshover BM, Lemonovich TL, et al. *Histoplasma capsulatum* prosthetic valve endocarditis with negative fungal blood cultures and negative *Histoplasma* antigen assay in an immunocompetent patient. *J Clin Microbiol* 2010;**48**(12):4664–6.
223. Johnson PC, Khardori N, Najjar AF, et al. Progressive disseminated histoplasmosis in patients with acquired immunodeficiency syndrome. *Am J Med* 1988;**85**(2):152–8.
224. Johnson PC, Sarosi GA, Septimus EJ, et al. Progressive disseminated histoplasmosis in patients with the acquired immune deficiency syndrome: a report of 12 cases and a literature review. *Semin Respir Infect* 1986;**1**(1):1–8.
225. Johnson PC, Wheat LJ, Cloud GA, et al. Safety and efficacy of liposomal amphotericin B compared with conventional amphotericin B for induction therapy of histoplasmosis in patients with AIDS. *Ann Intern Med* 2002;**137**(2):105–9.
226. Jones RC, Goodwin RA Jr. Histoplasmosis of bone. *Am J Med* 1981;**70**(4):864–6.
227. Kadaria D, Muthiah MP, Sinclair SE. Unusual presentations of disseminated histoplasmosis. *Tenn Med* 2012;**105**(3):39–40.
228. Kahi CJ, Wheat LJ, Allen SD, et al. Gastrointestinal histoplasmosis. *Am J Gastroenterol* 2005;**100**(1):220–31.
229. Kajfasz P, Basiak W. Outbreak of pulmonary histoplasmosis involving a group of four Polish travellers returning from Ecuador. *Int Marit Health* 2012;**63**(1):59–62.
230. Kamei K. [Imported mycoses in Japan: their present status and problems]. *Nihon Ishinkin Gakkai Zasshi* 2005;**46**(1):17–20.
231. Kamei K, Sano A, Kikuchi K, et al. The trend of imported mycoses in Japan. *J Infect Chemother* 2003;**9**(1):16–20.
232. Kane S, Brasitus T. Histoplasmosis capsulatum as a cause of lower gastrointestinal bleeding in common variable immunodeficiency. *Dig Dis Sci* 2000;**45**(11):2133–5.
233. Karalakulasingam R, Arora KK, Adams G, et al. Meningoencephalitis caused by *Histoplasma capsulatum:* occurrence in a renal transplant recipient and a review of the literature. *Arch Intern Med* 1976;**136**(2):217–20.
234. Karimi K, Wheat LJ, Connolly P, et al. Differences in histoplasmosis in patients with acquired immunodeficiency syndrome in the United States and Brazil. *J Infect Dis* 2002;**186**(11):1655–60.
235. Kasuga T, White TJ, Koenig G, et al. Phylogeography of the fungal pathogen *Histoplasma capsulatum*. *Mol Ecol* 2003;**12**(12):3383–401.
236. Kataria YP, Campbell PB, Burlingham BT. Acute pulmonary histoplasmosis presenting as adult respiratory distress syndrome: effect of therapy on clinical and laboratory features. *South Med J* 1981;**74**(5):534–7, 42.
237. Kauffman CA. Pulmonary histoplasmosis. *Curr Infect Dis Rep* 2001;**3**(3):279–85.
238. Kauffman CA. Endemic mycoses: blastomycosis, histoplasmosis, and sporotrichosis. *Infect Dis Clin North Am* 2006;**20**(3):645–62, vii.
239. Kauffman CA. Histoplasmosis. *Clin Chest Med* 2009;**30**(2):217–25, v.
240. Kauffman CA, Slama TG, Wheat LJ. *Histoplasma capsulatum* epididymitis. *J Urol* 1981;**125**(3):434–5.
241. Kaufman L, Terry RT, Schubert JH, et al. Effects of a single histoplasmin skin test on the serological diagnosis of histoplasmosis. *J Bacteriol* 1967;**94**(4):798–803.
242. Keath EJ, Kobayashi GS, Medoff G. Typing of *Histoplasma capsulatum* by restriction fragment length polymorphisms in a nuclear gene. *J Clin Microbiol* 1992;**30**(8):2104–7.
243. Keller DL, Steinberg H, Sladky KK. Disseminated histoplasmosis in a Bengal tiger *(Panthera tigris)*. *J Zoo Wildl Med* 2011; **42**(4):727–31.
244. Khalil MK. Histopathology of presumed ocular histoplasmosis. *Am J Ophthalmol* 1982;**94**(3):369–76.
245. King RW Jr, Kraikitpanitch S, Lindeman RD. Subcutaneous nodules caused by *Histoplasma capsulatum*. *Ann Intern Med* 1977;**86**(5):586–7.
246. Kirchner SG, Hernanz-Schulman M, Stein SM, et al. Imaging of pediatric mediastinal histoplasmosis. *RadioGraphics* 1991;**11**(3): 365–81.

247. Klein CJ, Dinapoli RP, Temesgen Z, et al. Central nervous system histoplasmosis mimicking a brain tumor: difficulties in diagnosis and treatment. *Mayo Clin Proc* 1999;**74**(8):803–7.
248. Klimpel KR, Goldman WE. Isolation and characterization of spontaneous avirulent variants of *Histoplasma capsulatum*. *Infect Immun* 1987;**55**(3):528–33.
249. Knight PJ, Mulne AF, Vassy LE. When is lymph node biopsy indicated in children with enlarged peripheral nodes? *Pediatrics* 1982;**69**(4):391–6.
250. Kritski AL, Lemle A, de Souza GR, et al. Pulmonary function changes in the acute stage of histoplasmosis, with follow-up: an analysis of eight cases. *Chest* 1990;**97**(5):1244–5.
251. Kroetz DN, Deepe GS. The role of cytokines and chemokines in *Histoplasma capsulatum* infection. *Cytokine* 2012;**58**(1):112–17.
252. Krunic AL, Carag H, Medenica MM, et al. A case of primary cutaneous histoplasmosis in a patient with diabetes and multi-infarct dementia. *J Dermatol* 2002;**29**(12):797–802.
253. Laboratory diagnosis of mycotic and specific fungal infections. Medical Section of the American Lung Association. *Am Rev Respir Dis* 1985;**132**(6):1373–9.
254. Lamps LW, Molina CP, West AB, et al. The pathologic spectrum of gastrointestinal and hepatic histoplasmosis. *Am J Clin Pathol* 2000;**113**(1):64–72.
255. Lane TE, Wu-Hsieh BA, Howard DH. Gamma interferon cooperates with lipopolysaccharide to activate mouse splenic macrophages to an antihistoplasma state. *Infect Immun* 1993; **61**(4):1468–73.
256. Larbcharoensub N, Boonsakan P, Aroonroch R, et al. Adrenal histoplasmosis: a case series and review of the literature. *Southeast Asian J Trop Med Public Health* 2011;**42**(4):920–5.
257. Larrabee WF, Ajello L, Kaufman L. An epidemic of histoplasmosis on the Isthmus of Panama. *Am J Trop Med Hyg* 1978;**27** (2 Pt 1):281–5.
258. Larwood TR. Coccidioidin skin testing in Kern County, California: decrease in infection rate over 58 years. *Clin Infect Dis* 2000;**30**(3):612–13.
259. Lee JH, Slifman NR, Gershon SK, et al. Life-threatening histoplasmosis complicating immunotherapy with tumor necrosis factor alpha antagonists infliximab and etanercept. *Arthritis Rheum* 2002;**46**(10):2565–70.
260. Lee JT, Dixon MR, Murrell Z, et al. Colonic histoplasmosis presenting as colon cancer in the nonimmunocompromised patient: report of a case and review of the literature. *Am Surg* 2004; **70**(11):959–63.
261. Lee PP, Chan KW, Lee TL, et al. Penicilliosis in children without HIV infection—are they immunodeficient? *Clin Infect Dis* 2012; **54**(2):e8–e19.
262. Leenders AC, Reiss P, Portegies P, et al. Liposomal amphotericin B (AmBisome) compared with amphotericin B both followed by oral fluconazole in the treatment of AIDS-associated cryptococcal meningitis. *AIDS* 1997;**11**(12):1463–71.
263. Leggiadro RJ, Barrett FF, Hughes WT. Disseminated histoplasmosis of infancy. *Pediatr Infect Dis J* 1988;**7**(11):799–805.
264. Leland DS, Zimmerman SE, Cunningham EB, et al. Variability in commercial *Histoplasma* complement fixation antigens. *J Clin Microbiol* 1991;**29**(8):1723–4.
265. LeMonte AM, Washum KE, Smedema ML, et al. Amphotericin B combined with itraconazole or fluconazole for treatment of histoplasmosis. *J Infect Dis* 2000;**182**(2):545–50.
266. Lenhart SW, Schafer MP, Singal M, et al. *Histoplasmosis—protecting workers at risk*. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2004. DHHS (NIOSH) publication No. 2005-109. Available at <http://www.cdc.gov/niosh/docs/2005-109/#a>; 2004.
267. Lerttumnongtum P, Muttarak M, Visrutaratna P, et al. Imaging features of unusual adrenal masses. *Australas Radiol* 2004;**48**(2): 107–13.
268. Lewis JH, Zimmerman HJ, Benson GD, et al. Hepatic injury associated with ketoconazole therapy: analysis of 33 cases. *Gastroenterology* 1984;**86**(3):503–13.
269. Lim LT, Ruzmetova N, Ballinger SH, et al. Acute pulmonary histoplasmosis in a patient with uveitis after infliximab therapy. *Int Ophthalmol* 2011;**31**(4):349–51.
270. Limaye AP, Connolly PA, Sagar M, et al. Transmission of *Histoplasma capsulatum* by organ transplantation. *N Engl J Med* 2000; **343**(16):1163–6.
271. Lin JS, Wu-Hsieh BA. Functional T cells in primary immune response to histoplasmosis. *Int Immunol* 2004;**16**(11):1663–73.
272. Lin JS, Yang CW, Wang DW, et al. Dendritic cells cross-present exogenous fungal antigens to stimulate a protective CD8 T cell response in infection by *Histoplasma capsulatum*. *J Immunol* 2005;**174**(10):6282–91.
273. Lindsley MD, Hurst SF, Iqbal NJ, et al. Rapid identification of dimorphic and yeast-like fungal pathogens using specific DNA probes. *J Clin Microbiol* 2001;**39**(10):3505–11.
274. Lipman M, Breen R. Immune reconstitution inflammatory syndrome in HIV. *Curr Opin Infect Dis* 2006;**19**(1):20–5.
275. Little J, Bruce J, Andrews H, et al. Treatment of disseminated infantile histoplasmosis with amphotericin B. *Pediatrics* 1959; **24**(1):1–6.
276. Little JA, Steigman AJ. Erythema nodosum in primary histoplasmosis. *JAMA* 1960;**173**:875–7.
277. Lockwood GF, Garrison RG. The possible role of uric acid in the ecology of *Histoplasma capsulatum*. *Mycopathol Mycol Appl* 1968;**35**(3):377–88.
278. Long KH, Gomez FJ, Morris RE, et al. Identification of heat shock protein 60 as the ligand on *Histoplasma capsulatum* that mediates binding to CD18 receptors on human macrophages. *J Immunol* 2003;**170**(1):487–94.
279. Lopes JO, Alves SH, Benevenga JP, et al. The second case of peritonitis due to *Histoplasma capsulatum* during continuous ambulatory peritoneal dialysis in Brazil. *Mycoses* 1994;**37**(5-6): 161–3.
280. Lopes LC, Guimaraes AJ, de Cerqueira MD, et al. A *Histoplasma capsulatum*–specific IgG1 isotype monoclonal antibody, H1C, to a 70-kilodalton cell surface protein is not protective in murine histoplasmosis. *Clin Vaccine Immunol* 2010;**17**(7):1155–8.
281. Loulergue P, Bastides F, Baudouin V, et al. Literature review and case histories of *Histoplasma capsulatum* var. *duboisii* infections in HIV-infected patients. *Emerg Infect Dis* 2007;**13**(11):1647–52.
282. Loyd JE, Tillman BF, Atkinson JB, et al. Mediastinal fibrosis complicating histoplasmosis. *Medicine (Baltimore)* 1988;**67**(5):295–310.
283. Luby JP, Southern PM Jr, Haley CE, et al. Recurrent exposure to *Histoplasma capsulatum* in modern air-conditioned buildings. *Clin Infect Dis* 2005;**41**(2):170–6.
284. Lucas AO. Cutaneous manifestations of African histoplasmosis. *Trans R Soc Trop Med Hyg* 1971;**65**(1):27.
285. Lyon GM, Bravo AV, Espino A, et al. Histoplasmosis associated with exploring a bat-inhabited cave in Costa Rica, 1998-1999. *Am J Trop Med Hyg* 2004;**70**(4):438–42.
286. Macher A. Histoplasmosis and blastomycosis. *Med Clin North Am* 1980;**64**(3):447–59.
287. Macher A, Rodrigues MM, Kaplan W, et al. Disseminated bilateral chorioretinitis due to *Histoplasma capsulatum* in a patient with the acquired immunodeficiency syndrome. *Ophthalmology* 1985; **92**(8):1159–64.
288. Magliani W, Conti S, Arseni S, et al. Antibody-mediated protective immunity in fungal infections. *New Microbiol* 2005;**28**(4):299–309.
289. Manali ED, Saad CP, Krizmanich G, et al. Endobronchial findings of fibrosing mediastinitis. *Respir Care* 2003;**48**(11):1038–42.
290. Marchiori E, Zanetti G, Escuissato DL, et al. Reversed halo sign: high-resolution CT findings in 79 patients. *Chest* 2012;**141**(5): 1260–6.
291. Marcic SM, Kammeyer PL, Aneziokoro C, et al. "Culture-negative" peritonitis due to *Histoplasma capsulatum*. *Nephrol Dial Transplant* 2006;**21**(10):3002.
292. Mashburn JD, Dawson DF, Young JM. Pulmonary calcifications and histoplasmosis. *Am Rev Respir Dis* 1961;**84**:208–16.
293. Mata-Essayag S, Colella MT, Rosello A, et al. Histoplasmosis: a study of 158 cases in Venezuela, 2000-2005. *Medicine (Baltimore)* 2008;**87**(4):193–202.
294. Mayfield JA, Fontana MF, Rine J. Genetic control of immune cell types in fungal disease. *Proc Natl Acad Sci U S A* 2010; **107**(51):22202–6.
295. McCormack FX, Gibbons R, Ward SR, et al. Macrophage-independent fungicidal action of the pulmonary collectins. *J Biol Chem* 2003;**278**(38):36250–6.
296. McGraw EP, Kane JM, Kleiman MB, et al. Cervical abscess and mediastinal adenopathy: an unusual presentation of childhood histoplasmosis. *Pediatr Radiol* 2002;**32**(12):862–4.

297. McGuinn ML, Lawrence ME, Proia L, et al. Progressive disseminated histoplasmosis presenting as cellulitis in a renal transplant recipient. *Transplant Proc* 2005;**37**(10):4313–14.
298. McKinsey DS, Gupta MR, Riddler SA, et al. Long-term amphotericin B therapy for disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1989;**111**(8):655–9.
299. McKinsey DS, Kauffman CA, Pappas PG, et al. Fluconazole therapy for histoplasmosis. The National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Clin Infect Dis* 1996;**23**(5):996–1001.
300. McKinsey DS, McKinsey JP. Pulmonary histoplasmosis. *Semin Respir Crit Care Med* 2011;**32**(6):735–44.
301. McKinsey DS, Spiegel RA, Hutwagner L, et al. Prospective study of histoplasmosis in patients infected with human immunodeficiency virus: incidence, risk factors, and pathophysiology. *Clin Infect Dis* 1997;**24**(6):1195–203.
302. McKinsey DS, Wheat LJ, Cloud GA, et al. Itraconazole prophylaxis for fungal infections in patients with advanced human immunodeficiency virus infection: randomized, placebo-controlled, double-blind study. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Clin Infect Dis* 1999;**28**(5): 1049–56.
303. McLeod DS, Mortimer RH, Perry-Keene DA, et al. Histoplasmosis in Australia: report of 16 cases and literature review. *Medicine (Baltimore)* 2011;**90**(1):61–8.
304. McNeil MM, Nash SL, Hajjeh RA, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980-1997. *Clin Infect Dis* 2001;**33**(5):641–7.
305. Medeiros AA, Marty SD, Tosh FE, et al. Erythema nodosum and erythema multiforme as clinical manifestations of histoplasmosis in a community outbreak. *N Engl J Med* 1966;**274**(8):415–20.
306. Medoff G, Kobayashi GS, Painter A, et al. Morphogenesis and pathogenicity of *Histoplasma capsulatum*. *Infect Immun* 1987;**55**(6): 1355–8.
307. Miller CR, Grossmann H. Disseminated histoplasmosis in chronic mucocutaneous candidiasis. *Pediatr Radiol* 1993;**23**(2):104–5.
308. Mootsikapun P, Srikulbutr S. Histoplasmosis and penicilliosis: comparison of clinical features, laboratory findings and outcome. *Int J Infect Dis* 2006;**10**(1):66–71.
309. Morgan J, Cano MV, Feikin DR, et al. A large outbreak of histoplasmosis among American travelers associated with a hotel in Acapulco, Mexico, spring 2001. *Am J Trop Med Hyg* 2003;**69**(6): 663–9.
310. Mukhopadhyay S. Role of histology in the diagnosis of infectious causes of granulomatous lung disease. *Curr Opin Pulm Med* 2011;**17**(3):189–96.
311. Muniz MM, Pizzini CV, Peralta JM, et al. Genetic diversity of *Histoplasma capsulatum* strains isolated from soil, animals, and clinical specimens in Rio de Janeiro State, Brazil, by a PCR-based random amplified polymorphic DNA assay. *J Clin Microbiol* 2001;**39**(12):4487–94.
312. Muniz Mde M, Morais ESTP, Meyer W, et al. Comparison of different DNA-based methods for molecular typing of *Histoplasma capsulatum*. *Appl Environ Microbiol* 2010;**76**(13):4438–47.
313. Munoz B, Martinez MA, Palma G, et al. Molecular characterization of *Histoplasma capsulatum* isolated from an outbreak in treasure hunters. *BMC Infect Dis* 2010;**10**:264.
314. Munoz C, Gomez BL, Tobon A, et al. Validation and clinical application of a molecular method for identification of *Histoplasma capsulatum* in human specimens in Colombia, South America. *Clin Vaccine Immunol* 2010;**17**(1):62–7.
315. Nacher M, Adenis A, Adriouch L, et al. What is AIDS in the Amazon and the Guianas? Establishing the burden of disseminated histoplasmosis. *Am J Trop Med Hyg* 2011;**84**(2): 239–40.
316. Nacher M, Sarazin F, El Guedj M, et al. Increased incidence of disseminated histoplasmosis following highly active antiretroviral therapy initiation. *J Acquir Immune Defic Syndr* 2006;**41**(4): 468–70.
317. Nath DS, Kandaswamy R, Gruessner R, et al. Fungal infections in transplant recipients receiving alemtuzumab. *Transplant Proc* 2005;**37**(2):934–6.
318. Negroni R, Palmieri O, Koren F, et al. Oral treatment of paracoccidioidomycosis and histoplasmosis with itraconazole in humans. *Rev Infect Dis* 1987;**9**(Suppl 1):S47–50.
319. Nemecek JC, Wuthrich M, Klein BS. Global control of dimorphism and virulence in fungi. *Science* 2006;**312**(5773):583–8.
320. Newman SL. Macrophages in host defense against *Histoplasma capsulatum*. *Trends Microbiol* 1999;**7**(2):67–71.
321. Newman SL. Cell-mediated immunity to *Histoplasma capsulatum*. *Semin Respir Infect* 2001;**16**(2):102–8.
322. Newman SL. Interaction of *Histoplasma capsulatum* with human macrophages, dendritic cells, and neutrophils. *Methods Mol Med* 2005;**118**:181–91.
323. Newman SL, Gootee L, Gabay JE. Human neutrophil–mediated fungistasis against *Histoplasma capsulatum:* localization of fungistatic activity to the azurophil granules. *J Clin Invest* 1993;**92**(2): 624–31.
324. Newman SL, Gootee L, Hilty J, et al. Human macrophages do not require phagosome acidification to mediate fungistatic/fungicidal activity against *Histoplasma capsulatum*. *J Immunol* 2006;**176**(3):1806–13.
325. Newman SL, Gootee L, Kidd C, et al. Activation of human macrophage fungistatic activity against *Histoplasma capsulatum* upon adherence to type 1 collagen matrices. *J Immunol* 1997;**158**(4): 1779–86.
326. Newman SL, Lemen W, Smulian AG. Dendritic cells restrict the transformation of *Histoplasma capsulatum* conidia into yeasts. *Med Mycol* 2011;**49**(4):356–64.
327. Norman FF, Martin-Davila P, Fortun J, et al. Imported histoplasmosis: two distinct profiles in travelers and immigrants. *J Travel Med* 2009;**16**(4):258–62.
328. Norris S, Wheat J, McKinsey D, et al. Prevention of relapse of histoplasmosis with fluconazole in patients with the acquired immunodeficiency syndrome. *Am J Med* 1994;**96**(6):504–8.
329. Nosanchuk JD. Protective antibodies and endemic dimorphic fungi. *Curr Mol Med* 2005;**5**(4):435–42.
330. Nosanchuk JD, Steenbergen JN, Shi L, et al. Antibodies to a cell surface histone-like protein protect against *Histoplasma capsulatum*. *J Clin Invest* 2003;**112**(8):1164–75.
331. Nosanchuk JD, Zancope-Oliveira RM, Hamilton AJ, et al. Antibody therapy for histoplasmosis. *Front Microbiol* 2012;**3**:21.
332. O'Hara M. Histopathologic diagnosis of fungal diseases. *Infect Control* 1986;**7**(2):78–84.
333. Odio CM, Navarrete M, Carrillo JM, et al. Disseminated histoplasmosis in infants. *Pediatr Infect Dis J* 1999;**18**(12):1065–8.
334. Ohji G, Kikuchi K, Inoue K, et al. Progressive disseminated histoplasmosis in an immunocompetent patient as an underrecognized imported mycosis in Japan. *J Infect Chemother* 2010;**16**(6): 443–5.
335. Ohnishi K, Nagai Y, Okada K, et al. Primary cutaneous histoplasmosis in papuloerythroderma (Ofuji). *J Dermatol* 1994;**21**(8): 586–9.
336. Okudaira M, Straub M, Schwarz J. The etiology of discrete splenic and hepatic calcifications in an endemic area of histoplasmosis. *Am J Pathol* 1961;**39**(5):599–611.
337. Oliveira Fde M, Fernandes SS, Severo CB, et al. *Histoplasma capsulatum* fungemia in patients with acquired immunodeficiency syndrome: detection by lysis-centrifugation blood-culturing technique. *Rev Inst Med Trop Sao Paulo* 2007;**49**(3):135–8.
338. Oliver A, Ciulla TA, Comer GM. New and classic insights into presumed ocular histoplasmosis syndrome and its treatment. *Curr Opin Ophthalmol* 2005;**16**(3):160–5.
339. Ollague Sierra JE, Ollague Torres JM. New clinical and histological patterns of acute disseminated histoplasmosis in human immunodeficiency virus-positive patients with acquired immunodeficiency syndrome. *Am J Dermatopathol* 2012. [Epub ahead of print].
340. Ongkosuwito JV, Kortbeek LM, Van der Lelij A, et al. Aetiological study of the presumed ocular histoplasmosis syndrome in the Netherlands. *Br J Ophthalmol* 1999;**83**(5):535–9.
341. Orchard JL, Luparello F, Brunskill D. Malabsorption syndrome occurring in the course of disseminated histoplasmosis: case report and review of gastrointestinal histoplasmosis. *Am J Med* 1979; **66**(2):331–6.
342. Ormerod LD, Qamar TU, Toller K, et al. Acute disseminated histoplasmosis with multifocal choroiditis in a child. *Pediatr Infect Dis J* 2000;**19**(5):479–81.
343. Ozols II, Wheat LJ. Erythema nodosum in an epidemic of histoplasmosis in Indianapolis. *Arch Dermatol* 1981;**117**(11): 709–12.

344. Panackal AA, Hajjeh RA, Cetron MS, et al. Fungal infections among returning travelers. *Clin Infect Dis* 2002;**35**(9):1088–95.
345. Panchabhai TS, Bais RK, Pyle RC, et al. An apple-core lesion in the colon: an infectious etiology. *J Glob Infect Dis* 2011;**3**(2): 195–8.
346. Passos L, Talhari C, Santos M, et al. Histoplasmosis-associated immune reconstitution inflammatory syndrome. *An Bras Dermatol* 2011;**86**(4 Suppl 1):S168–72.
347. Pastor TA, Holcomb MJ, Motaparthi K, et al. Disseminated histoplasmosis mimicking secondary syphilis. *Dermatol Online J* 2011;**17**(11):10.
348. Pate JW, Hammon J. Superior vena cava syndrome due to histoplasmosis in children. *Ann Surg* 1965;**161**:778–87.
349. Patino MM, Williams D, Ahrens J, et al. Experimental histoplasmosis in the beige mouse. *J Leukoc Biol* 1987;**41**(3):228–35.
350. Patrick CC, Flynn PM, Henwick S, Pui CH. Disseminated histoplasmosis presenting as a cystic duct obstruction. *Pediatr Infect Dis J* 1992;**11**(7):593–4.
351. Paya CV, Hermans PE, Van Scoy RE, et al. Repeatedly relapsing disseminated histoplasmosis: clinical observations during long-term follow-up. *J Infect Dis* 1987;**156**(2):308–12.
352. Paya CV, Roberts GD, Cockerill FR 3rd. Transient fungemia in acute pulmonary histoplasmosis: detection by new blood-culturing techniques. *J Infect Dis* 1987;**156**(2):313–15.
353. Payan DG, Wheat LJ, Brahmi Z, et al. Changes in immunoregulatory lymphocyte populations in patients with histoplasmosis. *J Clin Immunol* 1984;**4**(2):98–107.
354. Peabody JW Jr, Brown RB, Davis EW, et al. Surgical implications of mediastinal granulomas. *Am Surg* 1959;**25**(5):357–68.
355. Peddi VR, Hariharan S, First MR. Disseminated histoplasmosis in renal allograft recipients. *Clin Transplant [Case Reports]* 1996; **10**(2):160–5.
356. Penn RL, Lambert RS, George RB. Invasive fungal infections: the use of serologic tests in diagnosis and management. *Arch Intern Med* 1983;**143**(6):1215–20.
357. Pereira GH, Padua SS, Park MV, et al. Chronic meningitis by histoplasmosis: report of a child with acute myeloid leukemia. *Braz J Infect Dis* 2008;**12**(6):555–7.
358. Perfect JR. Treatment of non-*Aspergillus* moulds in immunocompromised patients, with amphotericin B lipid complex. *Clin Infect Dis* 2005;**40**(Suppl 6):S401–8.
359. Perko R, Messinger Y, Moertel C. Pseudometastasis secondary to histoplasmosis infection: false-positive PET/CT findings. *Pediatr Blood Cancer* 2010;**54**(4):621–3.
360. Phillips P, Fetchick R, Weisman I, et al. Tolerance to and efficacy of itraconazole in treatment of systemic mycoses: preliminary results. *Rev Infect Dis* 1987;**9**(Suppl 1):S87–93.
361. Piscoya Rivera A, Cedron Cheng H, Huerta-Mercado Tenorio J, et al. GI tuberculosis and histoplasmosis in an HIV (+) patient presenting with lower GI bleeding. *Am J Gastroenterol* 2005; **100**(8):1896–7.
362. Pitisuttithum P, Negroni R, Graybill JR, et al. Activity of posaconazole in the treatment of central nervous system fungal infections. *J Antimicrob Chemother* 2005;**56**(4):745–55.
363. Pladson TR, Stiles MA, Kuritsky JN. Pulmonary histoplasmosis: a possible risk in people who cut decayed wood. *Chest* 1984; **86**(3):435–8.
364. Plouffe JF, Fass RJ. *Histoplasma* meningitis: diagnostic value of cerebrospinal fluid serology. *Ann Intern Med* 1980;**92**(2 Pt 1): 189–91.
365. Porta A, Maresca B. Host response and *Histoplasma capsulatum*/ macrophage molecular interactions. *Med Mycol* 2000;**38**(6):399–406.
366. Pottage JC Jr, Trenholme GM, Aronson IK, et al. Panniculitis associated with histoplasmosis and alpha 1-antitrypsin deficiency. *Am J Med* 1983;**75**(1):150–3.
367. Pounder JI, Hansen D, Woods GL. Identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides* species by repetitive-sequence-based PCR. *J Clin Microbiol* 2006;**44**(8): 2977–82.
368. Prasad AG, Van Gelder RN. Presumed ocular histoplasmosis syndrome. *Curr Opin Ophthalmol* 2005;**16**(6):364–8.
369. Prechter GC, Prakash UB. Bronchoscopy in the diagnosis of pulmonary histoplasmosis. *Chest* 1989;**95**(5):1033–6.
370. Pryce TM, Palladino S, Price DM, et al. Rapid identification of fungal pathogens in BacT/ALERT, BACTEC, and BBL MGIT media using polymerase chain reaction and DNA sequencing of the internal transcribed spacer regions. *Diagn Microbiol Infect Dis* 2006;**54**(4):289–97.
371. Quasney MW, Leggiadro RJ. Pleural effusion associated with histoplasmosis. *Pediatr Infect Dis J* 1993;**12**(5):415–18.
372. Quilter LA, Kleiman MB, Kirsch E, Wheat LJ. Disseminated histoplasmosis of infancy in one of twins. *Pediatr Infect Dis J* 2012;**31**(9):990–1.
373. Quinones CA, Reuben AG, Hamill RJ, et al. Chronic cavitary histoplasmosis: failure of oral treatment with ketoconazole. *Chest* 1989;**95**(4):914–16.
374. Rachid A, Rezende LS, de Moura SF, et al. A case study of disseminated histoplasmosis linked to common variable immunodeficiency. *Braz J Infect Dis* 2003;**7**(4):268–72.
375. Rana C, Kumari N, Krishnani N. Adrenal histoplasmosis: a diagnosis on fine needle aspiration cytology. *Diagn Cytopathol* 2011; **39**(6):438–42.
376. Ranganath S, McGhie TA, Larosa S, et al. Clinical and laboratory diagnosis of central nervous system histoplasmosis. *Am J Med* 2012;**125**(10):e1–2.
377. Rangel-Castilla L, Hwang SW, White AC, et al. Neuroendoscopic diagnosis of CNS histoplasmosis with basilar arachnoiditis. *World Neurosurg* 2012;**77**(2):399.E9–13.
378. Rappleye CA, Engle JT, Goldman WE. RNA interference in *Histoplasma capsulatum* demonstrates a role for alpha-(1,3)-glucan in virulence. *Mol Microbiol* 2004;**53**(1):153–65.
379. Rescorla FJ, Kleiman MB, Grosfeld JL. Obstruction of the common bile duct in histoplasmosis. *Pediatr Infect Dis J* 1994; **13**(11):1017–19.
380. Restrepo A, Tobon A, Clark B, et al. Salvage treatment of histoplasmosis with posaconazole. *J Infect* 2007;**54**(4):319–27.
381. Riggs W Jr, Nelson P. The roentgenographic findings in infantile and childhood histoplasmosis. *Am J Roentgenol Radium Ther Nucl Med* 1966;**97**(1):181–5.
382. Riley HD Jr. The history of histoplasmosis. *J Okla State Med Assoc* 1983;**76**(2):31–40.
383. Rivera IV, Curless RG, Indacochea FJ, et al. Chronic progressive CNS histoplasmosis presenting in childhood: response to fluconazole therapy. *Pediatr Neurol* 1992;**8**(2):151–3.
384. Robertson J, Meier M, Wall J, et al. Immune reconstitution syndrome in HIV: validating a case definition and identifying clinical predictors in persons initiating antiretroviral therapy. *Clin Infect Dis* 2006;**42**(11):1639–46.
385. Roby BB, Drehner D, Sidman JD. Pediatric tracheal and endobronchial tumors: an institutional experience. *Arch Otolaryngol Head Neck Surg* 2011;**137**(9):925–9.
386. Rodriguez E, Soler R, Pombo F, et al. Fibrosing mediastinitis: CT and MR findings. *Clin Radiol* 1998;**53**(12):907–10.
387. Rodriguez N, Sandoval M, Alsina A, et al. [Genital histoplasmosis: presentation of a clinical case]. *Arch Esp Urol* 2003;**56**(5): 462–6.
388. Rosenthal J, Brandt KD, Wheat LJ, et al. Rheumatologic manifestations of histoplasmosis in the recent Indianapolis epidemic. *Arthritis Rheum* 1983;**26**(9):1065–70.
389. Ross P Jr, Magro CM, King MA. Endobronchial histoplasmosis: a masquerade of primary endobronchial neoplasia—a clinical study of four cases. *Ann Thorac Surg* 2004;**78**(1):277–81.
390. Rossi SE, McAdams HP, Rosado-de-Christenson ML, et al. Fibrosing mediastinitis. *RadioGraphics* 2001;**21**(3):737–57.
391. Rubin H, Furcolow ML, Yates JL, et al. The course and prognosis of histoplasmosis. *Am J Med* 1959;**27**:278–88.
392. Sa-Nunes A, Medeiros AI, Nicolete R, et al. Efficacy of cell-free antigens in evaluating cell immunity and inducing protection in a murine model of histoplasmosis. *Microbes Infect* 2005;**7**(4): 584–92.
393. Saccente M. Central nervous system histoplasmosis. *Curr Treat Options Neurol* 2008;**10**(3):161–7.
394. Saidinejad M, Burns MM, Harper MB. Disseminated histoplasmosis in a nonendemic area. *Pediatr Infect Dis J* 2004;**23**(8): 781–2.
395. Salomon J, Flament Saillour M, De Truchis P, et al. An outbreak of acute pulmonary histoplasmosis in members of a trekking trip in Martinique, French West Indies. *J Travel Med* 2003;**10**(2): 87–93.
396. Salvin SB. Acquired resistance in experimental histoplasmosis. *Trans N Y Acad Sci* 1956;**18**(5):462–8.

397. Salzman SH, Smith RL, Aranda CP. Histoplasmosis in patients at risk for the acquired immunodeficiency syndrome in a nonendemic setting. *Chest* 1988;**93**(5):916–21.
398. Sarosi GA, Johnson PC. Disseminated histoplasmosis in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1992;**14**(Suppl 1):S60–7.
399. Sathapatayavongs B, Batteiger BE, Wheat J, et al. Clinical and laboratory features of disseminated histoplasmosis during two large urban outbreaks. *Medicine (Baltimore)* 1983;**62**(5):263–70.
400. Scheckelhoff M, Deepe GS Jr. The protective immune response to heat shock protein 60 of *Histoplasma capsulatum* is mediated by a subset of V beta 8.1/8.2+ T cells. *J Immunol* 2002;**169**(10): 5818–26.
401. Schestatsky P, Chedid MF, Amaral OB, et al. Isolated central nervous system histoplasmosis in immunocompetent hosts: a series of 11 cases. *Scand J Infect Dis* 2006;**38**(1):43–8.
402. Schlech WF 3rd, Wheat LJ, Ho JL, et al. Recurrent urban histoplasmosis, Indianapolis, Indiana, 1980-1981. *Am J Epidemiol* 1983;**118**(3):301–12.
403. Schnur RA, Newman SL. The respiratory burst response to *Histoplasma capsulatum* by human neutrophils: evidence for intracellular trapping of superoxide anion. *J Immunol* 1990;**144**(12): 4765–72.
404. Schulman RL, McKiernan BC, Schaeffer DJ. Use of corticosteroids for treating dogs with airway obstruction secondary to hilar lymphadenopathy caused by chronic histoplasmosis: 16 cases (1979-1997). *J Am Vet Med Assoc* 1999;**214**(9):1345–8.
405. Schulz DM. Histoplasmosis of the central nervous system. *JAMA* 1953;**151**(7):549–51.
406. Schutze GE, Tucker NC, Jacobs RF. Histoplasmosis and perinatal human immunodeficiency virus. *Pediatr Infect Dis J* 1992;**11**(6): 501–2.
407. Secatto A, Rodrigues LC, Serezani CH, et al. 5-Lipoxygenase deficiency impairs innate and adaptive immune responses during fungal infection. *PLoS One* 2012;**7**(3):e31701.
408. Seider K, Heyken A, Luttich A, et al. Interaction of pathogenic yeasts with phagocytes: survival, persistence and escape. *Curr Opin Microbiol* 2010;**13**(4):392–400.
409. Sellers TF Jr, Price WN Jr, Newberry WM Jr. An epidemic of erythema multiforme and erythema nodosum caused by histoplasmosis. *Ann Intern Med* 1965;**62**:1244–62.
410. Shahani L. Disseminated *Histoplasma* and CMV infection presenting as subacute intestinal obstruction in an immunocompromised patient. *BMJ Case Rep* 2012;**2012**.
411. Shapiro JL, Lux JJ, Sprofkin BE. Histoplasmosis of the central nervous system. *Am J Pathol* 1955;**31**(2):319–35.
412. Shearer WT, Kobayashi G, Prensky AL. Presumptive histoplasmosis presenting as cerebellar ataxia with spontaneous recovery. *Pediatrics* 1976;**57**(1):150–2.
413. Shelburne SA 3rd, Visnegarwala F, Adams C, et al. Unusual manifestations of disseminated histoplasmosis in patients responding to antiretroviral therapy. *Am J Med* 2005;**118**(9):1038–41.
414. Sherrick AD, Brown LR, Harms GF, et al. The radiographic findings of fibrosing mediastinitis. *Chest* 1994;**106**(2):484–9.
415. Sills M, Schwartz A, Weg JG. Conjugal histoplasmosis: a consequence of progressive dissemination in the index case after steroid therapy. *Ann Intern Med* 1973;**79**(2):221–4.
416. Simon S, Veron V, Boukhari R, et al. Detection of *Histoplasma capsulatum* DNA in human samples by real-time polymerase chain reaction. *Diagn Microbiol Infect Dis* 2010;**66**(3):268–73.
417. Singh N, Huprikar S, Burdette SD, et al. Donor-derived fungal infections in organ transplant recipients: guidelines of the American Society of Transplantation, Infectious Diseases Community of Practice. *Am J Transplant* 2012;**12**(9):2414–28.
418. Sivaprasad S, Moore AT. Choroidal neovascularisation in children. *Br J Ophthalmol* 2008;**92**(4):451–4.
419. Slama TG. Treatment of disseminated and progressive cavitary histoplasmosis with ketoconazole. *Am J Med* 1983;**74**(1B):70–3.
420. Smith JA, Kauffman CA. Endemic fungal infections in patients receiving tumour necrosis factor-alpha inhibitor therapy. *Drugs* 2009;**69**(11):1403–15.
421. Smith JA, Kauffman CA. Pulmonary fungal infections. *Respirology* 2012;**17**(6):913–26.
422. Smith JG, Magee DM, Williams DM, et al. Tumor necrosis factor-alpha plays a role in host defense against *Histoplasma capsulatum*. *J Infect Dis* 1990;**162**(6):1349–53.
423. Smith JS, Kadiev S, Diaz P, et al. Pulmonary artery stenosis secondary to fibrosing mediastinitis: management with cutting balloon angioplasty and endovascular stenting. *Vasc Endovascular Surg* 2011;**45**(2):170–3.
424. Smith JW, Utz JP. Progressive disseminated histoplasmosis: a prospective study of 26 patients. *Ann Intern Med* 1972;**76**(4): 557–65.
425. Sobel JD, Muller G. Comparison of itraconazole and ketoconazole in the treatment of experimental candidal vaginitis. *Antimicrob Agents Chemother* 1984;**26**(2):266–7.
426. Spellberg B. Vaccines for invasive fungal infections. *F1000 Med Rep* 2011;**3**:13.
427. Spitzer ED, Keath EJ, Travis SJ, et al. Temperature-sensitive variants of *Histoplasma capsulatum* isolated from patients with acquired immunodeficiency syndrome. *J Infect Dis* 1990;**162**(1):258–61.
428. Steele CJ, Kleiman MB. Disseminated histoplasmosis, hypercalcemia and failure to thrive. *Pediatr Infect Dis J* 1994;**13**(5): 421–2.
429. Steiner SJ, Cox EG, Gupta SK, et al. Esophageal diverticulum: a complication of histoplasmosis in children. *J Pediatr* 2005; **146**(3):426–8.
430. Steiner SJ, Kleiman MB, Corkins MR, et al. Ileocecal histoplasmosis simulating Crohn disease in a patient with hyperimmunoglobulin E syndrome. *Pediatr Infect Dis J* 2009;**28**(8):744–6.
431. Stone JH, Pomper MG, Hellmann DB. Histoplasmosis mimicking vasculitis of the central nervous system. *J Rheumatol* 1998; **25**(8):1644–8.
432. Straub M, Schwarz J. The healed primary complex in histoplasmosis. *Am J Clin Pathol* 1955;**25**(7):727–41.
433. Strayer DS, Gutwein MB, Herbold D, et al. Histoplasmosis presenting as the carpal tunnel syndrome. *Am J Surg* 1981; **141**(2):286–8.
434. Suh KN, Anekthananon T, Mariuz PR. Gastrointestinal histoplasmosis in patients with AIDS: case report and review. *Clin Infect Dis* 2001;**32**(3):483–91.
435. Sutliff WD. Histoplasmosis cooperative study, part V: amphotericin B dosage for chronic pulmonary histoplasmosis. *Am Rev Respir Dis* 1972;**105**(1):60–7.
436. Suttorp-Schulten MS, Bollemeijer JG, Bos PJ, et al. Presumed ocular histoplasmosis in The Netherlands—an area without histoplasmosis. *Br J Ophthalmol* 1997;**81**(1):7–11.
437. Swartz MA, Scofield RH, Dickey WD, et al. Unilateral adrenal enlargement due to *Histoplasma capsulatum*. *Clin Infect Dis* 1996; **23**(4):813–15.
438. Szymczak WA, Deepe GS Jr. Antigen-presenting dendritic cells rescue CD4-depleted CCR2−/− mice from lethal *Histoplasma capsulatum* infection. *Infect Immun* 2010;**78**(5):2125–37.
439. Tagliari L, Toledo MS, Lacerda TG, et al. Membrane microdomain components of *Histoplasma capsulatum* yeast forms, and their role in alveolar macrophage infectivity. *Biochim Biophys Acta* 2012;**1818**(3):458–66.
440. Takahashi K, Sasaki T, Nabaa B, et al. Pulmonary lymphatic drainage to the mediastinum based on computed tomographic observations of the primary complex of pulmonary histoplasmosis. *Acta Radiol* 2012;**53**(2):161–7.
441. Taylor ML, Chavez-Tapia CB, Rojas-Martinez A, et al. Geographical distribution of genetic polymorphism of the pathogen *Histoplasma capsulatum* isolated from infected bats, captured in a central zone of Mexico. *FEMS Immunol Med Microbiol* 2005;**45**(3):451–8.
442. Taylor ML, Chavez-Tapia CB, Vargas-Yanez R, et al. Environmental conditions favoring bat infection with *Histoplasma capsulatum* in Mexican shelters. *Am J Trop Med Hyg* 1999;**61**(6): 914–19.
443. Taylor ML, Diaz S, Gonzalez PA, et al. Relationship between pathogenesis and immune regulation mechanisms in histoplasmosis: a hypothetical approach. *Rev Infect Dis* 1984;**6**(6):775–82.
444. Taylor ML, Hernandez-Garcia L, Estrada-Barcenas D, et al. Genetic diversity of *Histoplasma capsulatum* isolated from infected bats randomly captured in Mexico, Brazil, and Argentina, using the polymorphism of (GA)(n) microsatellite and its flanking regions. *Fungal Biol* 2012;**116**(2):308–17.
445. Taylor ML, Ruiz-Palacios GM, del Rocio Reyes-Montes M, et al. Identification of the infectious source of an unusual outbreak of histoplasmosis, in a hotel in Acapulco, state of Guerrero, Mexico. *FEMS Immunol Med Microbiol* 2005;**45**(3):435–41.

446. Taylor RL, Williams DM, Craven PC, et al. Amphotericin B in liposomes: a novel therapy for histoplasmosis. *Am Rev Respir Dis* 1982;**125**(5):610–11.
447. Tegeris AS, Smith DT. Acute disseminated pulmonary histoplasmosis treated with cortisone and MRD-112. *Ann Intern Med* 1958;**48**(6):1414–20.
448. Terry PB, Rosenow EC 3rd, Roberts GD. False-positive complement-fixation serology in histoplasmosis: a retrospective study. *JAMA* 1978;**239**(23):2453–6.
449. Tesh RB, Schneidau JD Jr. Primary cutaneous histoplasmosis. *N Engl J Med* 1966;**275**(11):597–9.
450. Thomas BP, Bream PR Jr, Milstone AP, et al. Treatment of SVC syndrome and hemoptysis in a patient with mediastinal fibrosis. *Emerg Radiol* 2006;**12**(5):240–3.
451. Thompson GR 3rd, LaValle CE 3rd, Everett ED. Unusual manifestations of histoplasmosis. *Diagn Microbiol Infect Dis* 2004; **50**(1):33–41.
452. Threlkeld ZD, Broughton RA, Khan GQ, et al. Isolated *Histoplasma capsulatum* meningoencephalitis in an immunocompetent child. *J Child Neurol* 2012;**27**(4):532–5.
453. Tobon AM, Agudelo CA, Rosero DS, et al. Disseminated histoplasmosis: a comparative study between patients with acquired immunodeficiency syndrome and non-human immunodeficiency virus-infected individuals. *Am J Trop Med Hyg* 2005;**73**(3):576–82.
454. Tobon AM, Franco L, Espinal D, et al. Disseminated histoplasmosis in children: the role of itraconazole therapy. *Pediatr Infect Dis J* 1996;**15**(11):1002–8.
455. Treatment of blastomycosis and histoplasmosis with ketoconazole: results of a prospective randomized clinical trial. National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Ann Intern Med* 1985;**103**(6 Pt 1):861–72.
456. Truong MT, Sabloff BS, Munden RF, et al. A patient with new-onset seizure and mediastinal adenopathy. *Chest* 2004;**126**(3): 982–5.
457. Tseng TC, Liaw SJ, Hsiao CH, et al. Molecular evidence of recurrent histoplasmosis with 9-year latency in a patient with Addison's disease. *J Clin Microbiol* 2005;**43**(9):4911–13.
458. Tucker WS Jr, Snell BB, Island DP, et al. Reversible adrenal insufficiency induced by ketoconazole. *JAMA* 1985;**253**(16):2413–14.
459. Tutor JD, Schoumacher RA, Chesney PJ. Chylothorax associated with histoplasmosis in a child. *Pediatr Infect Dis J* 2000;**19**(3): 262–3.
460. Uemura A, Thomas MA. Visual outcome after surgical removal of choroidal neovascularization in pediatric patients. *Arch Ophthalmol* 2000;**118**(10):1373–8.
461. Urschel HC Jr, Razzuk MA, Netto GJ, et al. Sclerosing mediastinitis: improved management with histoplasmosis titer and ketoconazole. *Ann Thorac Surg* 1990;**50**(2):215–21.
462. Utz JP, Shadomy HJ, Lurie HI. *Cryptococcus neoformans* and cryptococcosis. *Trans Am Clin Climatol Assoc* 1970;**81**:220–6.
463. Vail GM, Young RS, Wheat LJ, et al. Incidence of histoplasmosis following allogeneic bone marrow transplant or solid organ transplant in a hyperendemic area. *Transpl Infect Dis* 2002;**4**(3): 148–51.
464. Valdez H, Salata RA. Bat-associated histoplasmosis in returning travelers: case presentation and description of a cluster. *J Travel Med* 1999;**6**(4):258–60.
465. Van Cauteren H, Heykants J, De Coster R, et al. Itraconazole: pharmacologic studies in animals and humans. *Rev Infect Dis* 1987;**9**(Suppl 1):S43–6.
466. van Koeveringe MP, Brouwer RE. *Histoplasma capsulatum* reactivation with haemophagocytic syndrome in a patient with chronic lymphocytic leukaemia. *Neth J Med* 2010;**68**(12):418–21.
467. Vandiviere HM, Goodman NL, Melvin IG, et al. Histoplasmosis in Kentucky can it be prevented? *J Ky Med Assoc* 1981;**79**(11): 719–26.
468. Verma SB. Chronic disseminated cutaneous histoplasmosis in an immunocompetent individual—a case report. *Int J Dermatol* 2006; **45**(5):573–6.
469. Villarete L, de Fries R, Kolhekar S, et al. Impaired responsiveness to gamma interferon of macrophages infected with lymphocytic choriomeningitis virus clone 13: susceptibility to histoplasmosis. *Infect Immun* 1995;**63**(4):1468–72.
470. Vincent RD, Goewert R, Goldman WE, et al. Classification of *Histoplasma capsulatum* isolates by restriction fragment polymorphisms. *J Bacteriol* 1986;**165**(3):813–18.
471. Vos MJ, Debets-Ossenkopp YJ, Claessen FA, et al. Cerebellar and medullar histoplasmosis. *Neurology* 2000;**54**(7):1441.
472. Wallis RS, Broder M, Wong J, et al. Reactivation of latent granulomatous infections by infliximab. *Clin Infect Dis* 2005;**41**(Suppl 3):S194–8.
473. Ward JI, Weeks M, Allen D, et al. Acute histoplasmosis: clinical, epidemiologic and serologic findings of an outbreak associated with exposure to a fallen tree. *Am J Med* 1979;**66**(4):587–95.
474. Watzke RC, Klein ML, Wener MH. Histoplasmosis-like choroiditis in a nonendemic area: the northwest United States. *Retina* 1998;**18**(3):204–12.
475. Weber TR, Grosfeld JL, Kleiman MB, et al. Surgical implications of endemic histoplasmosis in children. *J Pediatr Surg* 1983;**18**(4): 486–91.
476. Weinberg GA, Kleiman MB, Grosfeld JL, et al. Unusual manifestations of histoplasmosis in childhood. *Pediatrics* 1983;**72**(1): 99–105.
477. Weinberg M, Weeks J, Lance-Parker S, et al. Severe histoplasmosis in travelers to Nicaragua. *Emerg Infect Dis* 2003;**9**(10):1322–5.
478. Wheat J. Histoplasmosis in the acquired immunodeficiency syndrome. *Curr Top Med Mycol* 1996;**7**(1):7–18.
479. Wheat J, French M, Batteiger B, et al. Cerebrospinal fluid *Histoplasma* antibodies in central nervous system histoplasmosis. *Arch Intern Med* 1985;**145**(7):1237–40.
480. Wheat J, French ML, Kamel S, et al. Evaluation of cross-reactions in *Histoplasma capsulatum* serologic tests. *J Clin Microbiol* 1986; **23**(3):493–9.
481. Wheat J, French ML, Kohler RB, et al. The diagnostic laboratory tests for histoplasmosis: analysis of experience in a large urban outbreak. *Ann Intern Med* 1982;**97**(5):680–5.
482. Wheat J, Hafner R, Korzun AH, et al. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. AIDS Clinical Trial Group. *Am J Med* 1995;**98**(4):336–42.
483. Wheat J, Hafner R, Wulfsohn M, et al. Prevention of relapse of histoplasmosis with itraconazole in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1993;**118**(8): 610–16.
484. Wheat J, Marichal P, Vanden Bossche H, et al. Hypothesis on the mechanism of resistance to fluconazole in *Histoplasma capsulatum*. *Antimicrob Agents Chemother* 1997;**41**(2):410–14.
485. Wheat J, MaWhinney S, Hafner R, et al. Treatment of histoplasmosis with fluconazole in patients with acquired immunodeficiency syndrome. National Institute of Allergy and Infectious Diseases Acquired Immunodeficiency Syndrome Clinical Trials Group and Mycoses Study Group. *Am J Med* 1997;**103**(3): 223–32.
486. Wheat J, Sarosi G, McKinsey D, et al. Practice guidelines for the management of patients with histoplasmosis. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**(4):688–95.
487. Wheat LJ. Systemic fungal infections: diagnosis and treatment, part I: histoplasmosis. *Infect Dis Clin North Am* 1988;**2**(4):841–59.
488. Wheat LJ. Diagnosis and management of histoplasmosis. *Eur J Clin Microbiol Infect Dis* 1989;**8**(5):480–90.
489. Wheat LJ. Histoplasmosis in Indianapolis. *Clin Infect Dis* 1992; **14**(Suppl 1):S91–9.
490. Wheat LJ. Improvements in diagnosis of histoplasmosis. *Expert Opin Biol Ther* 2006;**6**(11):1207–21.
491. Wheat LJ. Laboratory diagnosis of histoplasmosis: update 2000. *Semin Respir Infect* 2001;**16**(2):131–40.
492. Wheat LJ. Antigen detection, serology, and molecular diagnosis of invasive mycoses in the immunocompromised host. *Transpl Infect Dis* 2006;**8**(3):128–39.
493. Wheat LJ, Batteiger BE, Sathapatayavongs B. *Histoplasma capsulatum* infections of the central nervous system: a clinical review. *Medicine (Baltimore)* 1990;**69**(4):244–60.
494. Wheat LJ, Chetchotisakd P, Williams B, et al. Factors associated with severe manifestations of histoplasmosis in AIDS. *Clin Infect Dis* 2000;**30**(6):877–81.
495. Wheat LJ, Cloud G, Johnson PC, et al. Clearance of fungal burden during treatment of disseminated histoplasmosis with liposomal amphotericin B versus itraconazole. *Antimicrob Agents Chemother* 2001;**45**(8):2354–7.
496. Wheat LJ, Conces D, Allen SD, et al. Pulmonary histoplasmosis syndromes: recognition, diagnosis, and management. *Semin Respir Crit Care Med* 2004;**25**(2):129–44.

497. Wheat LJ, Connolly P, Haddad N, et al. Antigen clearance during treatment of disseminated histoplasmosis with itraconazole versus fluconazole in patients with AIDS. *Antimicrob Agents Chemother* 2002;**46**(1):248–50.
498. Wheat LJ, Connolly P, Smedema M, et al. Emergence of resistance to fluconazole as a cause of failure during treatment of histoplasmosis in patients with acquired immunodeficiency disease syndrome. *Clin Infect Dis* 2001;**33**(11):1910–13.
499. Wheat LJ, Connolly P, Smedema M, et al. Activity of newer triazoles against *Histoplasma capsulatum* from patients with AIDS who failed fluconazole. *J Antimicrob Chemother* 2006;**57**(6): 1235–9.
500. Wheat LJ, Connolly-Stringfield P, Blair R, et al. Histoplasmosis relapse in patients with AIDS: detection using *Histoplasma capsulatum* variety *capsulatum* antigen levels. *Ann Intern Med* 1991; **115**(12):936–41.
501. Wheat LJ, Connolly-Stringfield PA, Baker RL, et al. Disseminated histoplasmosis in the acquired immune deficiency syndrome: clinical findings, diagnosis and treatment, and review of the literature. *Medicine (Baltimore)* 1990;**69**(6):361–74.
502. Wheat LJ, Connolly-Stringfield P, Blair R, et al. Effect of successful treatment with amphotericin B on *Histoplasma capsulatum* variety *capsulatum* polysaccharide antigen levels in patients with AIDS and histoplasmosis. *Am J Med* 1992;**92**(2):153–60.
503. Wheat LJ, Connolly-Stringfield P, Kohler RB, et al. *Histoplasma capsulatum* polysaccharide antigen detection in diagnosis and management of disseminated histoplasmosis in patients with acquired immunodeficiency syndrome. *Am J Med* 1989;**87**(4): 396–400.
504. Wheat LJ, Connolly-Stringfield P, Williams B, et al. Diagnosis of histoplasmosis in patients with the acquired immunodeficiency syndrome by detection of *Histoplasma capsulatum* polysaccharide antigen in bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1992; **145**(6):1421–4.
505. Wheat LJ, Freifeld AG, Kleiman MB, et al. Clinical practice guidelines for the management of patients with histoplasmosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**(7):807–25.
506. Wheat LJ, Kauffman CA. Histoplasmosis. *Infect Dis Clin North Am* 2003;**17**(1):1–19, vii.
507. Wheat LJ, Kohler RB, Tewari RP. Diagnosis of disseminated histoplasmosis by detection of *Histoplasma capsulatum* antigen in serum and urine specimens. *N Engl J Med* 1986;**314**(2):83–8.
508. Wheat LJ, Kohler RB, Tewari RP, et al. Significance of *Histoplasma* antigen in the cerebrospinal fluid of patients with meningitis. *Arch Intern Med* 1989;**149**(2):302–4.
509. Wheat LJ, Musial CE, Jenny-Avital E. Diagnosis and management of central nervous system histoplasmosis. *Clin Infect Dis* 2005;**40**(6):844–52.
510. Wheat LJ, Slama TG, Eitzen HE, et al. A large urban outbreak of histoplasmosis: clinical features. *Ann Intern Med* 1981;**94**(3): 331–7.
511. Wheat LJ, Slama TG, Zeckel ML. Histoplasmosis in the acquired immune deficiency syndrome. *Am J Med* 1985;**78**(2):203–10.
512. Wheat LJ, Small CB. Disseminated histoplasmosis in the acquired immune deficiency syndrome. *Arch Intern Med* 1984;**144**(11): 2147–9.
513. Wheat LJ, Stein L, Corya BC, et al. Pericarditis as a manifestation of histoplasmosis during two large urban outbreaks. *Medicine (Baltimore)* 1983;**62**(2):110–19.
514. Whitehouse WM, Davey WN, Engelke OK, et al. Roentgen findings in histoplasmin-positive school children. *J Mich State Med Soc* 1959;**58**:1266–9.
515. Whitt SP, Koch GA, Fender B, et al. Histoplasmosis in pregnancy: case series and report of transplacental transmission. *Arch Intern Med* 2004;**164**(4):454–8.
516. Wilcox KR Jr, Waisbren BA, Martin J. The Walworth, Wisconsin, epidemic of histoplasmosis. *Ann Intern Med* 1958;**49**(2):388–418.
517. Williams AO, Lawson EA, Lucas AO. African histoplasmosis due to *Histoplasma duboisii*. *Arch Pathol* 1971;**92**(5):306–18.
518. Williams B, Fojtasek M, Connolly-Stringfield P, et al. Diagnosis of histoplasmosis by antigen detection during an outbreak in Indianapolis, Ind. *Arch Pathol Lab Med* 1994;**118**(12):1205–8.
519. Williams BJ, Connolly-Stringfield P, Bartlett M, et al. Correlation of *Histoplasma capsulatum* polysaccharide antigen with the severity of infection in murine histoplasmosis. *J Clin Lab Anal* 1991;**5**(2): 121–6.
520. Williams KR, Burford TH. Surgical treatment of granulomatous paratracheal lymphadenopathy. *J Thorac Cardiovasc Surg* 1964;**48**: 13–18.
521. Wilson DA, Nguyen DL, Tytle TL, et al. Sonography of the adrenal glands in chronic disseminated histoplasmosis. *J Ultrasound Med* 1986;**5**(2):69–73.
522. Winters MS, Chan Q, Caruso JA, et al. Metallomic analysis of macrophages infected with *Histoplasma capsulatum* reveals a fundamental role for zinc in host defenses. *J Infect Dis* 2010;**202**(7): 1136–45.
523. Wolf J, Blumberg HM, Leonard MK. Laryngeal histoplasmosis. *Am J Med Sci* 2004;**327**(3):160–2.
524. Wood KL, Hage CA, Knox KS, et al. Histoplasmosis after treatment with anti-tumor necrosis factor-alpha therapy. *Am J Respir Crit Care Med* 2003;**167**(9):1279–82.
525. Woods JP. *Histoplasma capsulatum* molecular genetics, pathogenesis, and responsiveness to its environment. *Fungal Genet Biol* 2002;**35**(2):81–97.
526. Woods JP. Knocking on the right door and making a comfortable home: *Histoplasma capsulatum* intracellular pathogenesis. *Curr Opin Microbiol* 2003;**6**(4):327–31.
527. Woods JP, Heinecke EL, Luecke JW, et al. Pathogenesis of *Histoplasma capsulatum*. *Semin Respir Infect* 2001;**16**(2):91–101.
528. Woods LP. Mediastinal *Histoplasma* granuloma causing tracheal compression in a 4-year-old child. *Surgery* 1965;**58**:448–52.
529. Woods WG, Singher LJ, Krivit W, et al. Histoplasmosis simulating lymphoma in children. *J Pediatr Surg* 1979;**14**(4):423–5.
530. Wooley CF, Hosier DM. Constrictive pericarditis due to *Histoplasma capsulatum*. *N Engl J Med* 1961;**264**:1230–2.
531. Wu-Hsieh BA, Lee GS, Franco M, et al. Early activation of splenic macrophages by tumor necrosis factor alpha is important in determining the outcome of experimental histoplasmosis in mice. *Infect Immun* 1992;**60**(10):4230–8.
532. Wuthrich M, Filutowicz HI, Warner T, et al. Vaccine immunity to pathogenic fungi overcomes the requirement for CD4 help in exogenous antigen presentation to $CD8^+$ T cells: implications for vaccine development in immune-deficient hosts. *J Exp Med* 2003;**197**(11):1405–16.
533. Wynne JW, Olsen GN. Acute histoplasmosis presenting as the adult respiratory distress syndrome. *Chest* 1974;**66**(2):158–61.
534. Yilmaz GG, Yilmaz E, Coskun M, et al. Cutaneous histoplasmosis in a child with hyper-IgM. *Pediatr Dermatol* 1995;**12**(3):235–8.
535. Young EJ, Vainrub B, Musher DM. Pericarditis due to histoplasmosis. *JAMA* 1978;**240**(16):1750–1.
536. Youseff BH, Holbrook ED, Smolnycki KA, et al. Extracellular superoxide dismutase protects *Histoplasma* yeast cells from host-derived oxidative stress. *PLoS Pathog* 2012;**8**(5):e1002713.
537. Zancope-Oliveira RM, Morais e Silva Tavares P, Muniz MM. Genetic diversity of *Histoplasma capsulatum* strains in Brazil. *FEMS Immunol Med Microbiol* 2005;**45**(3):443–9.
538. Zeidberg LD, Dillon A, Gass RS. Some factors in the epidemiology of histoplasmin sensitivity in Williamson County, Tennessee. *Am J Public Health Nations Health* 1951;**41**(1):80–9.
539. Zeiss J, Woldenberg LS, Morgan R, et al. Pulmonary histoplasmoma presenting as massive hemoptysis. *Pediatr Infect Dis J* 1987;**6**(7):689–91.
540. Zerbe CS, Holland SM. Disseminated histoplasmosis in persons with interferon-gamma receptor 1 deficiency. *Clin Infect Dis* 2005;**41**(4):e38–41.
541. Zhou P, Freidag BL, Caldwell CC, et al. Perforin is required for primary immunity to *Histoplasma capsulatum*. *J Immunol* 2001; **166**(3):1968–74.
542. Zhou P, Miller G, Seder RA. Factors involved in regulating primary and secondary immunity to infection with *Histoplasma capsulatum*: TNF-alpha plays a critical role in maintaining secondary immunity in the absence of IFN-gamma. *J Immunol* 1998; **160**(3):1359–68.
543. Zhou P, Sieve MC, Bennett J, et al. IL-12 prevents mortality in mice infected with *Histoplasma capsulatum* through induction of IFN-gamma. *J Immunol* 1995;**155**(2):785–95.
544. Zhou P, Sieve MC, Tewari RP, et al. Interleukin-12 modulates the protective immune response in SCID mice infected with *Histoplasma capsulatum*. *Infect Immun* 1997;**65**(3):936–42.

and cases in the United States appear to cluster in the Midwest, particularly along the Mississippi and Missouri river areas.[43,81] Disease in humans usually results from inoculation of minor wounds by debris containing *S. schenckii*, and gardeners and nursery workers, forestry workers, miners, and other individuals exposed to contaminated plant materials are at higher risk for acquiring the disease.[15,19] Disseminated disease has been the initial presentation of some human immunodeficiency virus (HIV)–infected individuals.[2] Laboratory personnel working with the organism have become infected after incurring needle-stick injuries.[106] The large South African epidemic in the gold mines of Transvaal probably resulted from the miners' brushing against rotting timbers in the mines, and a cluster of cases that occurred after a brick-throwing incident in Florida was traced to *S. schenckii* in the packing straw of the bricks.[90,108] Contaminated hay was the source of one outbreak linked to a Halloween haunted house.[26] Pulmonary disease may result from inhalation of spores.[81] Uncommonly, transmission of disease from animals, particularly domestic cats with cutaneous disease, or from family members occurs.[28,35,39,85] One adult acquired the disease after being bitten by a squirrel,[91] and a young girl developed cutaneous disease after a rodent bite.[50] In South America, armadillo hunters are at risk for development of sporotrichosis, but it is acquired from the decaying plant debris in armadillo nests rather than from the animals themselves.[71] Cats have been implicated in ongoing outbreaks in Brazil.[7,37,95,96] Molecular typing of isolates can be useful in investigations of outbreaks.[18,74,75]

PATHOGENESIS AND PATHOLOGY

As indicated, the most common mode of acquisition of sporotrichosis is by inoculation of the organism into skin structures, although disease also may develop from inhalation of spores of the organism. The incubation period varies considerably, commonly ranging from 7 to 30 days after cutaneous inoculation but possibly as long as 6 months.[87] The disease usually remains localized; of the 2825 cases of sporotrichosis in the Transvaal mine epidemic, none had systemic spread.[108]

S. schenckii, similar to other yeasts, seems to bind specifically to the glycosphingolipid lactosylceramide, which is present on the cell surface of animal cells.[54] This mechanism may be one by which the organism establishes a foothold in the host. Cell-mediated immune responses probably are important for containment of infection. One study documented intact responses in patients with cutaneous forms of the disease, whereas patients with systemic sporotrichosis had impaired cell-mediated immunity.[80] This study is supported by the observation that systemic disease tends to occur in individuals with underlying diseases or conditions that alter cell-mediated immunity. More recently, deficiencies in Toll-like receptor 4 have been suggested as contributing to severity of sporotrichosis.[6,92] Specific virulence factors also may contribute to likelihood of disseminated disease.[3,6,105,118]

Histopathologic examination of primary cutaneous lesions usually reveals changes in the epithelium, with hyperkeratosis, parakeratosis, and pseudoepitheliomatous hyperplasia. Intraepidermal microabscesses may be seen as well. In more established lesions, the pathologic process involves the dermis and below, with inflammatory infiltrate extending perivascularly.[68] The classic lesion on microscopic examination is a granuloma with an asteroid body at the center, although this picture is not pathognomonic for sporotrichosis. The asteroid body is an antigen-antibody complex deposited on the surface of the organism and may consist of several forms.[67,117] Visualization of fungi in tissue sections often is difficult, even with special staining techniques, because of the paucity of organisms in tissue. They may be seen on Gram stain as gram-positive but irregularly staining bodies, sometimes as cigar-shaped, 3- to 5-μm yeast forms. Periodic acid–Schiff and silver stains probably are better suited for detection of fungi in tissue sections.[68,87] Immunohistochemical staining techniques may prove superior to standard methods.[73,78]

CLINICAL MANIFESTATIONS

Sporotrichosis can occur in cutaneous and extracutaneous forms; the cutaneous varieties account for approximately 80 percent of all cases.[87] These categories can be broken down further into the organ system involved (Table 204-1).

Cutaneous Sporotrichosis

Cutaneous disease with *S. schenckii* can be either lymphocutaneous or fixed cutaneous, the latter of which occurs less frequently. In lymphocutaneous cases, the initial lesion appears as a firm, slightly tender subcutaneous nodule. It progresses along local lymphatic channels, with multiple nodules appearing. The lesions typically enlarge and may ulcerate and suppurate (Fig. 204-1). Untreated, they may heal slowly during months or persist; recurrences are frequent. Differential diagnosis includes cutaneous nocardiosis, atypical mycobacterial disease, leishmaniasis, rosacea, syphilis, pyoderma gangrenosum, disseminated *Acanthamoeba* disease, and cutaneous manifestations of other fungal diseases.[23,61,84,104,115]

The fixed cutaneous form of the disease is just as the name implies, with no evidence of lymphatic spread. The primary lesions are identical to the lesions seen in the lymphocutaneous form. Although some researchers have

TABLE 204-1 **Clinical Forms of Sporotrichosis**

Cutaneous	Extracutaneous
Lymphocutaneous	Osteoarticular
Fixed cutaneous	Pulmonary
	Muscular
	Ocular
	Genitourinary
	Central nervous system

Data from references 1, 46, 48, 63, 81, 88.

FIGURE 204-1 ■ Cutaneous lymphatic sporotrichosis of both arms. Note the characteristic involvement of lymphatics that drain the sites of the primary lesions. (Courtesy G. Medoff and G. S. Kobayashi.)

TABLE 204-2 **Summary of 42 Cases of Pediatric Sporotrichosis**

Duration of symptoms before diagnosis	3-10 weeks
Lymphatic involvement	28/47*
Culture positive	39/44*
Relapse after therapy	1/21*

*Numerator is number of patients positive; denominator is number of patients tested.
Data from references 13, 16, 21, 42, 69, 76.

suggested that sporotrichosis in children, compared with that in adults, is more likely to appear in the fixed cutaneous form, this theory has not been borne out in prior studies. Table 204-2 shows results from six reports of series of cases of sporotrichosis in children. The 60 percent rate for the lymphocutaneous form may not differ much from the 90 percent figure quoted for adults when one takes into account that many pediatric studies are reports of small numbers of cases and may have resulted from selection bias. A large series of patients from Peru documented a disproportionate number of children with facial lesions (86 of 143 children vs. 23 of 95 adults) but did not classify fixed versus lymphocutaneous forms by age.[79] Fixed cutaneous disease in children has been confused with impetigo.[13] A study from Peru also documented a predilection of children to develop lymphocutaneous lesions on the face and neck.[70] Risk factors for disease in children in this study included playing in fields, ownership of a cat, and residence in a house with dirt floors. Unusual dermatologic manifestations reported include molluscum-like lesions in a patient with alcoholic liver disease and Sweet syndrome in middle-aged women.[38,93]

Extracutaneous Sporotrichosis

Sporotrichosis occurring in extracutaneous sites either may be localized (related to an unusual area of trauma) or may represent disseminated disease. In the absence of trauma, the presence of extracutaneous sporotrichosis should raise suspicion of disseminated disease and consideration of immunodeficiency states. Overall, infection of bones and joints is the most common form of extracutaneous disease.[113] Sporotrichal arthritis usually is an indolent and slowly progressive disease that may occur with or without cutaneous or lymphatic disease, suggesting a hematogenous route of infection for most cases. Diagnosis generally requires synovial biopsy with culture to show the organism.[97,113] Two studies showed diagnostic delays averaging 17 and 25 months from the onset of symptoms.[8,20] Sporotrichal osteomyelitis usually occurs with concomitant arthritis, but isolated bone involvement has been recorded. Lytic lesions and periosteal changes are noted most frequently.[45,113]

Pulmonary sporotrichosis seldom occurs with cases of disseminated disease and probably develops after inhalation of spores, as with primary pulmonary histoplasmosis.[81] The presence of cavitary lesions in the upper lobes often leads to a diagnosis of tuberculosis, and fungal culture of sputum, bronchoscopic specimens, lung tissue, or gastric aspirate usually is needed to establish the diagnosis.[29,81,111] Pleural involvement is uncommon (3 of 47 cases in one review), and complications, such as massive hemoptysis, rarely occur.[31,49,81]

Sporotrichosis has been found to involve virtually every organ system in the body as part of disseminated disease.[113] Most commonly, underlying immunodeficiency states, such as diabetes, prolonged steroid therapy, alcoholism, immunosuppressive therapy, and acquired immunodeficiency syndrome (AIDS), are present.* In one Brazilian cohort of HIV-infected patients with sporotrichosis, 7 of 21 developed disseminated disease and 2 had meningitis.[36] Ocular and endocardial infection has been reported as well.[103] Disseminated disease, including meningitis, also has been reported as part of immune reconstitution syndrome in AIDS patients.[40,47] Fungemia in the absence of disseminated disease has been documented in one otherwise healthy adult with a lysis-centrifugation blood culture system, and laryngeal and cutaneous sporotrichosis have been reported in a healthy child.[60,62] Dissemination in patients with neoplasia seldom occurs.

DIAGNOSIS

A high index of suspicion is necessary to establish the diagnosis of sporotrichosis. In the largest outbreak in the United States, which occurred in 1988 among horticulturists and forestry workers, only 15 percent of cases were diagnosed at the time of initial presentation to a physician.[17] Culture is the "gold standard" for diagnosis of sporotrichosis. Although organisms occasionally are seen

*References 1, 32, 44, 51, 55, 63, 102, 107, 113, 116.

on pathologic specimens, the yield is low enough to render biopsy unnecessary. If the diagnosis is suspected, scrapings of cutaneous lesions for culture should be sufficient to establish the diagnosis.[87]

A skin test antigen has been available for many years, but as with the histoplasmin skin test for histoplasmosis, it is useful mainly as an epidemiologic tool. Polymerase chain reaction may prove useful for diagnosis in the future.[56] Serodiagnosis has been explored in a few studies. Immunoprecipitation or commercially available slide latex agglutination can be useful when material for culture is difficult to obtain or cultures are negative, such as with sporotrichal meningitis.[24,98] Similarly, an enzyme-linked immunosorbent assay has been used for diagnosis. Antibody titers in cerebrospinal fluid tend to decrease with successful therapy, and such tests might prove useful for monitoring of response to therapy.[9,98] Western blotting has been used to detect sporotrichal antibody.[99] Using a crude antigen preparation, Scott and Muchmore[99] determined that detection of antibody to three antigens, 32 kDa, 40 kDa, and 70 kDa, seems to be sensitive and specific for diagnosis of active sporotrichosis. Patients with extracutaneous disease seemed to form antibody to a greater number of *S. schenckii* organisms than did patients with cutaneous disease. Further studies of the immune response in sporotrichosis should enable development of better serodiagnostic tests than those that now are routinely available.

TREATMENT AND PROGNOSIS

Sporotrichosis may resolve spontaneously, but treatment is indicated in most circumstances.[5,82] The Infectious Diseases Society of America has published practice guidelines for management of patients with sporotrichosis.[59]

Heat applied to the site of cutaneous disease has been reported anecdotally to cause resolution of lesions in patients in whom medical therapy was contraindicated.[41,88,109] One prospective study showed good results with use of benzene pocket warmers for heat therapy of facial lesions in children.[52] Surgical removal of infected skin and soft tissue has been used, but skin grafting may be required.[12] This form of treatment usually is unnecessary with the availability of medical management except possibly for some cases of pulmonary infection (particularly if it is confined to one lobe) and other extracutaneous disease.[20,45,81] Hyperthermia remains an option for treatment of sporotrichosis in pregnancy, in situations in which other agents may be contraindicated.[57]

A saturated solution of potassium iodide (SSKI), a proteolytic agent for which the mechanism of action against sporotrichosis is unclear,[58,65] can be used for uncomplicated sporotrichosis. The in vitro growth of *S. schenckii* is not inhibited appreciably by iodide, but free iodine has a marked inhibitory effect on growth.[112] The small amount of free iodine in a saturated solution of potassium iodide may be sufficient to cause resolution of disease. The pediatric dosage of a saturated solution of potassium iodide is empirical; it usually is given three times daily in juice or milk, starting at a low dose (e.g., 1 to 2 drops per year of age) and increasing the dose during the course of several days to a maximum of 30 to 40 drops per dose.[16,69,76] For younger children, lower dosages may be acceptable.[69,83] Treatment is continued until a few weeks after all lesions have resolved. Adverse reactions, such as salivary gland swelling, excessive lacrimation or salivation, nausea, vomiting, and abdominal pain, may resolve with temporary cessation of therapy followed by reinstitution at a lower dosage. SSKI currently is only an alternative treatment for lymphocutaneous and cutaneous forms of the disease.[57]

In vitro susceptibility testing is not used widely, and results may not always agree with clinical response.[89,110] The Clinical and Laboratory Standards Institute has proposed a microdilution test, whereas the European Committee for Antimicrobial Susceptibility Testing uses a similar assay with a different inoculum and test medium.[6] Breakpoints have not been established.

Itraconazole, an oral azole derivative, is the drug of choice for treatment of cutaneous, lymphocutaneous, and osteoarticular forms of sporotrichosis.[11,53,54,57,101] Restrepo and colleagues[86] showed clinical cures in all 17 patients with cutaneous forms of sporotrichosis treated with itraconazole (100 mg/day), with no major side effects. Borelli[10] reported a treatment failure, however, in a patient treated with 100 mg/day, who subsequently responded when the dose was increased to 200 mg/day. Systemic disease also has been treated with itraconazole with favorable clinical responses, although all information is from cases in adults.[4,64,77,101,114]

Intravenous amphotericin B remains the drug of choice for treatment of patients with disseminated or severe sporotrichosis.[57,66,113] Duration of therapy in children has not been studied, but recommendations are to begin with liposomal amphotericin B at 3 to 5 mg/kg/day, with stepdown to itraconazole after clinical improvement to complete 12 months of total treatment.[59] On occasion, intra-articular amphotericin B is used to treat sporotrichal arthritis if response to other therapy is poor.[27] Amphotericin B therapy in patients with pulmonary sporotrichosis is less effective than with other forms of sporotrichosis, which has prompted many clinicians to use a combined medical-surgical approach.[59,81] Itraconazole is an alternative therapy and may be useful for long-term suppression.[59] Amphotericin B seems to be effective in treating sporotrichal meningitis when it is given early in the course of the disease, but adjunctive therapy with flucytosine or rifampin might be helpful.[97] Itraconazole and fluconazole also may have roles in treating meningitis.[53,59] Immunosuppressed individuals may require lifelong suppressive therapy with itraconazole to prevent recurrence, depending on the degree of immunosuppression and severity of disease.[59]

S. schenckii does not seem to be particularly susceptible to ketoconazole in vitro, and clinical experience with ketoconazole treatment of sporotrichosis has been mixed.[14,22,100] Possibly, relatively large doses of ketoconazole are needed to produce a good clinical response. Terbinafine, an allylamine, has looked promising in clinical studies.[33,34,53] Posaconazole has looked promising in a murine model of sporotrichosis.[30]

Prognosis for cutaneous forms of disease is excellent, but the extracutaneous forms are associated with significant morbidity and mortality, in part related to the underlying conditions predisposing these patients to disseminated disease. Many cases of osteoarticular disease result in permanent disability.[20]

PREVENTION

The key to prevention of sporotrichosis is the elimination of exposure to the organism, particularly with regard to skin surfaces and mucous membranes. Usually, elimination of exposure can be accomplished by the use of protective clothing during high-risk activities, such as working with sphagnum moss or other decaying, moist plant material.[15,17] Nursery workers should be educated about the hazards and early signs of sporotrichosis, and physicians and veterinarians should be aware of the uncommon circumstances of spread of the infection from family members and domestic cats. The epidemic in the Transvaal gold mines was stopped when timbers in the mine shafts were sprayed with a fungicide.[108] Although reporting of individual cases of sporotrichosis is not required in the United States, reporting of clusters of cases can aid epidemiologic investigations and stop the spread of the disease.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Arrillaga-Moncrieff I, Capilla J, Mayayo E, et al. Different virulence levels of the species of *Sporothrix* in a murine model. *Clin Microbiol Infect* 2009;**15**:651–5.
6. Barros MB, de Almeida Paes R, Schubach AO. *Sporothrix schenckii* and sporotrichosis. *Clin Microbiol Rev* 2011;**24**:633–54.
7. Barros MB, Schubach AO, Schubach TM, et al. An epidemic of sporotrichosis in Rio de Janeiro, Brazil: epidemiological aspects of a series of cases. *Epidemiol Infect* 2008;**136**:1192–6.
25. Dias NM, Oliveira MM, Santos C, et al. Sporotrichosis caused by *Sporothrix mexicana*, Portugal. *Emerg Infect Dis* 2011;**17**:1975–6.
30. Fernández-Silva F, Capilla J, Mayayo E, et al. Efficacy of posaconazole in murine experimental sporotrichosis. *Antimicrob Agents Chemother* 2012;**56**:2273–7.
33. Francesconi G, Francesconi do Valle AC, Passos SL, et al. Comparative study of 250 mg/day terbinafine and 100 mg/day itraconazole for the treatment of cutaneous sporotrichosis. *Mycopathologia* 2011;**171**:349–54.
34. Francesconi G, Valle AC, Passos S, et al. Terbinafine (250 mg/day): an effective and safe treatment of cutaneous sporotrichosis. *J Eur Acad Dermatol Venereol* 2009;**23**:1273–6.
36. Freitas DF, de Siqueira Hoagland B, do Valle AC, et al. Sporotrichosis in HIV-infected patients: report of 21 cases of endemic sporotrichosis in Rio de Janeiro, Brazil. *Med Mycol* 2012;**50**:170–8.
37. Freitas DF, do Valle AC, de Almeida Paes R, et al. Zoonotic sporotrichosis in Rio de Janeiro, Brazil: a protracted epidemic yet to be curbed. *Clin Infect Dis* 2010;**50**:453.
38. Freitas DF, Valle AC, Cuzzi T, et al. Sweet syndrome associated with sporotrichosis. *Br J Dermatol* 2012;**166**:212–13.
40. Galhardo MC, Silva MT, Lima MA, et al. *Sporothrix schenckii* meningitis in AIDS during immune reconstitution syndrome. *J Neurol Neurosurg Psychiatry* 2010;**81**:696–9.
47. Gutierrez-Galhardo MC, do Valle AC, Fraga BL, et al. Disseminated sporotrichosis as a manifestation of immune reconstitution inflammatory syndrome. *Mycoses* 2010;**53**:78–80.
50. Hayfron K, Wiedeman JA. A 7-year-old girl with ulcerative lesion after a rodent bite. *Pediatr Infect Dis J* 2010;**29**:185, 193.
55. Kamal A, Orenstein R. Disseminated sporotrichosis. *J Hosp Med* 2010;**5**:E29–30.
66. Limper AH, Knox KS, Sarosi GA, et al. An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011;**183**:96–128.
92. Sassá MF, Ferreira LS, Ribeiro LC, et al. Immune response against *Sporothrix schenckii* in TLR-4–deficient mice. *Mycopathologia* 2012;**174**:21–30.
93. Schechtman RC, Crignis GS, Pockstaller MP, et al. Molluscum-like lesions in a patient with sporotrichosis. *An Bras Dermatol* 2011;**86**:1217–19.
95. Schubach A, Barros MB, Wanke B. Epidemic sporotrichosis. *Curr Opin Infect Dis* 2008;**21**:129–33.
103. Silva-Vergara ML, de Camargo ZP, Silva PF, et al. Disseminated *Sporothrix brasiliensis* infection with endocardial and ocular involvement in an HIV-infected patient. *Am J Trop Med Hyg* 2012;**86**:477–80.
105. Teixeira PA, de Castro RA, Nascimento RC, et al. Cell surface expression of adhesins for fibronectin correlates with virulence in *Sporothrix schenckii*. *Microbiology* 2009;**155**(Pt 11):3730–8.
107. Tochigi M, Ochiai T, Mekata C, et al. Sporotrichosis of the face by autoinoculation in a patient undergoing tacrolimus treatment. *J Dermatol* 2012;**39**:796–8.
116. Yamaguchi T, Ito S, Takano Y, et al. A case of disseminated sporotrichosis treated with prednisolone, immunosuppressants, and tocilizumab under the diagnosis of rheumatoid arthritis. *Intern Med* 2012;**51**:2035–9.
117. Zhang YQ, Xu XG, Zhang M, et al. Sporotrichosis: clinical and histopathological manifestations. *Am J Dermatopathol* 2011;**33**:296–302.
118. Zhang Z, Liu X, Lv X, et al. Variation in genotype and higher virulence of a strain of *Sporothrix schenckii* causing disseminated cutaneous sporotrichosis. *Mycopathologia* 2011;**172**:439–46.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agger WA, Caplan RH, Maki DG. Ocular sporotrichosis mimicking mucormycosis in a diabetic. *Ann Ophthalmol* 1978;**10**: 767–71.
2. al-Tawfiq JA, Wools KK. Disseminated sporotrichosis and *Sporothrix schenckii* fungemia as the initial presentation of human immunodeficiency virus infection. *Clin Infect Dis* 1998;**26**:1403–6.
3. Arrillaga-Moncrieff I, Capilla J, Mayayo E, et al. Different virulence levels of the species of *Sporothrix* in a murine model. *Clin Microbiol Infect* 2009;**15**:651–5.
4. Baker JH, Goodpasture HC, Kuhns HR Jr, et al. Fungemia caused by an amphotericin B–resistant isolate of *Sporothrix schenckii*: successful treatment with itraconazole. *Arch Pathol Lab Med* 1989; **113**:1279–81.
5. Bargman HB. Sporotrichosis of the nose with spontaneous cure. *Can Med Assoc J* 1981;**124**:1027.
6. Barros MB, de Almeida Paes R, Schubach AO. *Sporothrix schenckii* and sporotrichosis. *Clin Microbiol Rev* 2011;**24**:633–54.
7. Barros MB, Schubach AO, Schubach TM, et al. An epidemic of sporotrichosis in Rio de Janeiro, Brazil: epidemiological aspects of a series of cases. *Epidemiol Infect* 2008;**136**:1192–6.
8. Bayer AS, Scott VJ, Guz CB. Fungal arthritis, III: sporotrichal arthritis. Semin. *Arthritis Rheum* 1979;**9**:66–74.
9. Bernardes-Engemann AR, Orofino Costa RC, Miguens BP, et al. Development of an enzyme-linked immunosorbent assay for the serodiagnosis of several clinical forms of sporotrichosis. *Med Mycol* 2005;**43**:487–93.
10. Borelli D. A clinical trial of itraconazole in the treatment of deep mycoses and leishmaniasis. *Rev Infect Dis* 1987;**9**(Suppl. 1): S57–63.
11. Breeling JL, Weinstein L. Pulmonary sporotrichosis treated with itraconazole. *Chest* 1993;**103**:313–14.
12. Bullpitt P, Weedon D. Sporotrichosis: a review of 39 cases. *Pathology* 1978;**10**:249–56.
13. Burch JM, Morelli JG, Weston WL. Unsuspected sporotrichosis in childhood. *Pediatr Infect Dis J* 2001;**20**:442–5.
14. Calhoun DL, Waskin H, White MP, et al. Treatment of systemic sporotrichosis with ketoconazole. *Rev Infect Dis* 1991;**13**:47–51.
15. Centers for Disease Control. Multistate outbreak of sporotrichosis in seedling handlers, 1988. *MMWR Morb Mortal Wkly Rep* 1988; **37**:652–3.
16. Chandler JW Jr, Kriel RL, Tosh FE. Childhood sporotrichosis. *Am J Dis Child* 1968;**115**:368–72.
17. Coles FB, Schuchat A, Hibbs JR, et al. A multistate outbreak of sporotrichosis associated with sphagnum moss. *Am J Epidemiol* 1992;**136**:475–87.
18. Cooper CR Jr, Breslin BJ, Dixon DM, et al. DNA typing of isolates associated with the 1988 sporotrichosis epidemic. *J Clin Microbiol* 1992;**30**:1631–5.
19. Cote TR, Kasten MJ, England AC III. Sporotrichosis in association with Arbor Day activities. *N Engl J Med* 1988;**319**:1290–1.
20. Crout JE, Brewer NS, Tompkins RB. Sporotrichosis arthritis: clinical features in seven patients. *Ann Intern Med* 1977;**86**: 294–7.
21. Dahl BA, Silberfarb PM, Sarosi GA, et al. Sporotrichosis in children: report of an epidemic. *JAMA* 1971;**215**:1980–2.
22. Dall L, Salzman G. Treatment of pulmonary sporotrichosis with ketoconazole. *Rev Infect Dis* 1987;**9**:795–8.
23. Day TW, Gibson GH, Guin JD. Rosacea-like sporotrichosis. *Cutis* 1984;**33**:549–52.
24. de Albornoz MB, Villanueva E, de Torres ED. Application of immunoprecipitation techniques to the diagnosis of cutaneous and extracutaneous forms of sporotrichosis. *Mycopathologia* 1984; **85**:177–83.
25. Dias NM, Oliveira MM, Santos C, et al. Sporotrichosis caused by *Sporothrix mexicana*, Portugal. *Emerg Infect Dis* 2011;**17**: 1975–6.
26. Dooley DP, Bostic PS, Beckius ML. Spook house sporotrichosis: a point-source outbreak of sporotrichosis associated with hay bale props in a Halloween haunted-house. *Arch Intern Med* 1997;**157**: 1885–7.
27. Downs NJ, Hinthorn DR, Mhatre VR, et al. Intra-articular amphotericin B treatment of *Sporothrix schenckii* arthritis. *Arch Intern Med* 1989;**149**:954–5.
28. Dunstan RW, Langham RF, Reimann KA, et al. Feline sporotrichosis: a report of five cases with transmission to humans. *J Am Acad Dermatol* 1986;**15**:37–45.
29. England DM, Hochholzer L. *Sporothrix* infection of the lung without cutaneous disease: primary pulmonary sporotrichosis. *Arch Pathol Lab Med* 1987;**111**:298–300.
30. Fernández-Silva F, Capilla J, Mayayo E, et al. Efficacy of posaconazole in murine experimental sporotrichosis. *Antimicrob Agents Chemother* 2012;**56**:2273–7.
31. Fields CL, Ossorio MA, Roy TM. Empyema associated with pulmonary sporotrichosis. *South Med J* 1989;**82**:910–13.
32. Fitzpatrick JE, Eubanks S. Acquired immunodeficiency syndrome presenting as disseminated cutaneous sporotrichosis. *Int J Dermatol* 1988;**27**:406–7.
33. Francesconi G, Francesconi do Valle AC, Passos SL, et al. Comparative study of 250 mg/day terbinafine and 100 mg/day itraconazole for the treatment of cutaneous sporotrichosis. *Mycopathologia* 2011;**171**:349–54.
34. Francesconi G, Valle AC, Passos S, et al. Terbinafine (250 mg/day): an effective and safe treatment of cutaneous sporotrichosis. *J Eur Acad Dermatol Venereol* 2009;**23**:1273–6.
35. Frean JA, Isaacson M, Miller GB, et al. Sporotrichosis following a rodent bite: a case report. *Mycopathologia* 1991;**116**:5–8.
36. Freitas DF, de Siqueira Hoagland B, do Valle AC, et al. Sporotrichosis in HIV-infected patients: report of 21 cases of endemic sporotrichosis in Rio de Janeiro, Brazil. *Med Mycol* 2012;**50**: 170–8.
37. Freitas DF, do Valle AC, de Almeida Paes R, et al. Zoonotic sporotrichosis in Rio de Janeiro, Brazil: a protracted epidemic yet to be curbed. *Clin Infect Dis* 2010;**50**:453.
38. Freitas DF, Valle AC, Cuzzi T, et al. Sweet syndrome associated with sporotrichosis. *Br J Dermatol* 2012;**166**:212–13.
39. Frumkin A, Tesserand ME. Sporotrichosis in a father and son. *J Am Acad Dermatol* 1989;**20**:964–7.
40. Galhardo MC, Silva MT, Lima MA, et al. *Sporothrix schenckii* meningitis in AIDS during immune reconstitution syndrome. *J Neurol Neurosurg Psychiatry* 2010;**81**:696–9.
41. Galiana J, Conti-Diaz IA. Healing effects of heat and a rubefacient on nine cases of sporotrichosis. *Sabouraudia* 1963;**3**:64–71.
42. Gluckman I. Sporotrichosis in children. *S Afr Med J* 1965; **39**:991–2.
43. Goncalves AP. Geopathology of sporotrichosis. *Int J Dermatol* 1973;**12**:115–18.
44. Gottlieb GS, Lesser CF, Holmes KK, et al. Disseminated sporotrichosis associated with treatment with immunosuppressants and tumor necrosis factor-alpha antagonists. *Clin Infect Dis* 2003;**37**: 838–40.
45. Govender S, Rasool MN, Ngcelwane M. Osseous sporotrichosis. *J Infect* 1989;**19**:273–6.
46. Gullberg RM, Quintanilla A, Levin ML, et al. Sporotrichosis: recurrent cutaneous, articular, and central nervous system infection in a renal transplant recipient. *Rev Infect Dis* 1987;**9**:369–75.
47. Gutierrez-Galhardo MC, do Valle AC, Fraga BL, et al. Disseminated sporotrichosis as a manifestation of immune reconstitution inflammatory syndrome. *Mycoses* 2010;**53**:78–80.
48. Halverson PB, Lahiri S, Wojno WC, et al. Sporotrichal arthritis presenting as granulomatous myositis. *Arthritis Rheum* 1985;**28**: 1425–9.
49. Haponik EF, Hill MK, Craighead CC. Case report: pulmonary sporotrichosis with massive hemoptysis. *Am J Med Sci* 1989;**297**: 251–3.
50. Hayfron K, Wiedeman JA. A 7-year-old girl with ulcerative lesion after a rodent bite. *Pediatr Infect Dis J* 2010;**29**:185, 193.
51. Heller HM, Fuhrer J. Disseminated sporotrichosis in patients with AIDS: case report and review of the literature. *AIDS* 1991;**5**: 1243–6.
52. Hiruma M, Kawada A, Noguchi H, et al. Hyperthermic treatment of sporotrichosis: experimental use of infrared and far infrared rays. *Mycoses* 1992;**35**:293–9.
53. Hull PR, Vismer HF. Treatment of cutaneous sporotrichosis with terbinafine. *Br J Dermatol* 1992;**126**(Suppl. 39):51–5.
54. Jimenez-Lucho V, Ginsburg V, Krivan HC. *Cryptococcus neoformans*, *Candida albicans*, and other fungi bind specifically to the glycosphingolipid lactosylceramide (GalB1-4GlcB1-1Cer), a possible adhesion receptor for yeasts. *Infect Immun* 1990;**58**:2085–90.

55. Kamal A, Orenstein R. Disseminated sporotrichosis. *J Hosp Med* 2010;**5**:E29–30.
56. Kanbe T, Natsume L, Goto I, et al. Rapid and specific identification of *Sporothrix schenckii* by PCR targeting the DNA topoisomerase II gene. *J Dermatol Sci* 2005;**38**:99–106.
57. Kauffman CA. Old and new therapies for sporotrichosis. *Clin Infect Dis* 1995;**21**:981–5.
58. Kauffman CA. Sporotrichosis. *Clin Infect Dis* 1999;**29**:231–7.
59. Kauffman CA, Bustamente B, Chapman SW, et al. Clinical practice guidelines for the management of sporotrichosis: 2007 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2007;**45**:1255–65.
60. Khabie N, Boyce TG, Roberts GD, et al. Laryngeal sporotrichosis causing stridor in a young child. *Int J Pediatr Otolaryngol* 2003; **67**:819–23.
61. Kibbi AG, Karam PG, Kurban AK. Sporotrichoid leishmaniasis in patients from Saudi Arabia: clinical and histologic features. *J Am Acad Dermatol* 1987;**17**:759–64.
62. Kosinski RM, Axelrod P, Rex JH, et al. *Sporothrix schenckii* fungemia without disseminated sporotrichosis. *J Clin Microbiol* 1992; **30**:501–3.
63. Kurosawa A, Pollock SC, Collins MP, et al. *Sporothrix schenckii* endophthalmitis in a patient with human immunodeficiency virus infection. *Arch Ophthalmol* 1988;**106**:376–80.
64. Lavalle P, Suchil P, De Ovando F, et al. Itraconazole for deep mycoses: preliminary experience in Mexico. *Rev Infect Dis* 1987; **9**(Suppl. 1):S64–70.
65. Lieberman J, Kurnick NB. Induction of proteolysis within purulent sputum by iodides. *Clin Res* 1963;**11**:81.
66. Limper AH, Knox KS, Sarosi GA, et al. An official American Thoracic Society statement: treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011;**183**:96–128.
67. Lurie HI. Histopathology of sporotrichosis: notes on the nature of the asteroid body. *Arch Pathol* 1963;**75**:421–37.
68. Lurie HI, Still WJS. The "capsule" of *Sporotrichum schenckii* and the evolution of the asteroid body: a light and electron microscopic study. *Sabouraudia* 1969;**7**:64–70.
69. Lynch PJ, Botero F. Sporotrichosis in children. *Am J Dis Child* 1971;**122**:325–7.
70. Lyon GM, Zurita S, Casquero I, et al. Population-based surveillance and a case-control study of risk factors for endemic lymphocutaneous sporotrichosis in Peru. *Clin Infect Dis* 2003; **36**:34–9.
71. Mackinnon JE, Conti-Diaz IA, Gezuele E, et al. Isolation of *Sporothrix schenckii* from nature and considerations on its pathogenicity and ecology. *Sabouraudia* 1969;**7**:38–45.
72. Marimon R, Gene J, Cano J, et al. Molecular phylogeny of *Sporothrix schenckii*. *J Clin Microbiol* 2006;**44**:3251–6.
73. Marques MEA, Coelho KIR, Sotto MN, et al. Comparison between histochemical and immunohistochemical methods for diagnosis of sporotrichosis. *J Clin Pathol* 1992;**45**:1089–93.
74. Neyra E, Fonteyne P-A, Swinne D, et al. Epidemiology of human sporotrichosis investigated by amplified fragment length polymorphism. *J Clin Microbiol* 2005;**43**:1348–52.
75. O'Reilly LC, Altman SA. Macrorestriction analysis of clinical and environmental isolates of *Sporothrix schenckii*. *J Clin Microbiol* 2006;**44**:2547–52.
76. Orr ER, Riley HD Jr. Sporotrichosis in childhood: report of ten cases. *J Pediatr* 1971;**78**:951–7.
77. Oscherwitz SL, Rinaldi MG. Disseminated sporotrichosis in a patient infected with human immunodeficiency virus. *Clin Infect Dis* 1992;**15**:568–9.
78. Padhye AA, Kaufman L, Durry E, et al. Fatal pulmonary sporotrichosis caused by *Sporothrix schenckii* var. *luriei* in India. *J Clin Microbiol* 1992;**30**:2492–4.
79. Pappas PG, Tellez I, Deep AE, et al. Sporotrichosis in Peru: description of an area of hyperendemicity. *Clin Infect Dis* 2000; **30**:65–70.
80. Plouffe JF Jr, Silva J Jr, Fekety R, et al. Cell-mediated immune responses in sporotrichosis. *J Infect Dis* 1979;**139**:152–7.
81. Pluss JL, Opal SM. Pulmonary sporotrichosis: review of treatment and outcome. *Medicine (Baltimore)* 1986;**65**:143–53.
82. Pueringer RJ, Iber C, Deike MA, et al. Spontaneous remission of extensive pulmonary sporotrichosis. *Ann Intern Med* 1986;**104**: 366–7.
83. Rafal ES, Rasmussen JE. An unusual presentation of fixed cutaneous sporotrichosis: a case report and review of the literature. *J Am Acad Dermatol* 1991;**25**(Pt. 2):928–32.
84. Readinger A, Blumberg M, Colome-Grimmer MI, et al. Disseminated *Acanthamoeba* infection with sporotrichoid nodules. *Int J Dermatol* 2006;**45**:942–3.
85. Reed KD, Moore FM, Geiger GE, et al. Zoonotic transmission of sporotrichosis: case report and review. *Clin Infect Dis* 1993; **16**:384–7.
86. Restrepo A, Robledo J, Gomez I, et al. Itraconazole therapy in lymphangitic and cutaneous sporotrichosis. *Arch Dermatol* 1986; **122**:413–17.
87. Rippon JW. *Medical mycology: the pathogenic fungi and the pathogenic actinomycetes*. 3rd ed. Philadelphia: WB Saunders; 1988.
88. Romig DA, Voth DW, Liu C. Facial sporotrichosis during pregnancy: a therapeutic dilemma. *Arch Intern Med* 1972;**130**: 910–12.
89. Sabatelli F, Patel R, Mann PA, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother* 2006;**50**:2009–15.
90. Sanders E. Cutaneous sporotrichosis: beer, bricks, and bumps. *Arch Intern Med* 1971;**127**:482–3.
91. Saravanakumar PS, Eslami P, Zar FA. Lymphocutaneous sporotrichosis associated with a squirrel bite: case report and review. *Clin Infect Dis* 1996;**23**:647–8.
92. Sassá MF, Ferreira LS, Ribeiro LC, et al. Immune response against *Sporothrix schenckii* in TLR-4–deficient mice. *Mycopathologia* 2012;**174**:21–30.
93. Schechtman RC, Crignis GS, Pockstaller MP, et al. Molluscum-like lesions in a patient with sporotrichosis. *An Bras Dermatol* 2011;**86**:1217–19.
94. Schenck RB. On refractory subcutaneous abscesses caused by a fungus possibly related to sporotricha. *Bull Johns Hopkins Hosp* 1898;**9**:286–90.
95. Schubach A, Barros MB, Wanke B. Epidemic sporotrichosis. *Curr Opin Infect Dis* 2008;**21**:129–33.
96. Schubach A, Schubach TMP, Barros MBL, et al. Cat-transmitted sporotrichosis, Rio de Janeiro, Brazil. *Emerg Infect Dis* 2005;**11**: 1952–4.
97. Schwartz DA. *Sporothrix* tenosynovitis: differential diagnosis of granulomatous inflammatory disease of the joints. *J Rheumatol* 1989;**16**:550–3.
98. Scott EN, Kaufman L, Brown AC, et al. Serologic studies in the diagnosis and management of meningitis due to *Sporothrix schenckii*. *N Engl J Med* 1987;**317**:935–40.
99. Scott EN, Muchmore HG. Immunoblot analysis of antibody responses to *Sporothrix schenckii*. *J Clin Microbiol* 1989;**27**: 300–4.
100. Shadomy S, White SC, Yu HP, et al. Treatment of systemic mycoses with ketoconazole: in vitro susceptibilities of clinical isolates of systemic and pathogenic fungi to ketoconazole. *J Infect Dis* 1985;**152**:1249–56.
101. Sharkey-Mathis PK, Kauffman CA, Graybill JR, et al. Treatment of sporotrichosis with itraconazole. *Am J Med* 1993;**95**:279–85.
102. Shaw JC, Levinson W, Montanaro A. Sporotrichosis in the acquired immunodeficiency syndrome. *J Am Acad Dermatol* 1989;**21**:1145–7.
103. Silva-Vergara ML, de Camargo ZP, Silva PF, et al. Disseminated *Sporothrix brasiliensis* infection with endocardial and ocular involvement in an HIV-infected patient. *Am J Trop Med Hyg* 2012;**86**:477–80.
104. Spiers EM, Hendrick SJ, Jorizzo JL, et al. Sporotrichosis masquerading as pyoderma gangrenosum. *Arch Dermatol* 1988;**122**: 691–4.
105. Teixeira PA, de Castro RA, Nascimento RC, et al. Cell surface expression of adhesins for fibronectin correlates with virulence in *Sporothrix schenckii*. *Microbiology* 2009;**155**(Pt 11):3730–8.
106. Thompson DW, Kaplan W. Laboratory-acquired sporotrichosis. *Sabouraudia* 1977;**15**:167–70.
107. Tochigi M, Ochiai T, Mekata C, et al. Sporotrichosis of the face by autoinoculation in a patient undergoing tacrolimus treatment. *J Dermatol* 2012;**39**:796–8.
108. Transvaal Mine Medical Officers' Association. *Sporotrichosis infection in mines of the Witwatersrand: a symposium*. Johannesburg: Transvaal Chamber of Mines; 1947.

109. Trejos A, Ramirez O. Local heat in the treatment of sporotrichosis. *Mycopathol Mycol Appl* 1966;**30**:47–53.
110. Trilles L, Fernandez-Torres B, Lazera MS, et al. In vitro antifungal susceptibilities of *Sporothrix schenckii* in two growth phases. *Antimicrob Agents Chemother* 2005;**49**:3952–4.
111. Velji AM, Hoeprich PD, Slovak R. Multifocal systemic sporotrichosis with lobar pulmonary involvement. *Scand J Infect Dis* 1988;**20**:565–9.
112. Wada R. Studies on mode of action of potassium iodide upon sporotrichosis. *Mycopathol Mycol Appl* 1968;**34**:97–107.
113. Wilson DE, Mann JJ, Bennett JE, et al. Clinical features of extracutaneous sporotrichosis. *Medicine (Baltimore)* 1967;**46**:265–79.
114. Winn RE, Anderson J, Piper J, et al. Systemic sporotrichosis treated with itraconazole. *Clin Infect Dis* 1993;**17**:210–17.
115. Wlodaver CG, Tolomeo T, Benear JB II. Primary cutaneous nocardiosis mimicking sporotrichosis. *Arch Dermatol* 1988;**124**:659–60.
116. Yamaguchi T, Ito S, Takano Y, et al. A case of disseminated sporotrichosis treated with prednisolone, immunosuppressants, and tocilizumab under the diagnosis of rheumatoid arthritis. *Intern Med* 2012;**51**:2035–9.
117. Zhang YQ, Xu XG, Zhang M, et al. Sporotrichosis: clinical and histopathological manifestations. *Am J Dermatopathol* 2011;**33**:296–302.
118. Zhang Z, Liu X, Lv X, et al. Variation in genotype and higher virulence of a strain of *Sporothrix schenckii* causing disseminated cutaneous sporotrichosis. *Mycopathologia* 2011;**172**:439–46.

CHAPTER 205

Mucormycosis and Entomopthoramycosis (Formerly Zygomycosis)

Theoklis E. Zaoutis • William J. Steinbach

The diseases originally known as mucormycosis or phycomycosis were previously renamed *zygomycosis*, in an effort to group uncommon but frequently fatal mycoses caused by fungi of the phylum Zygomycota. However, molecular phylogenetic analyses confirmed the phylum to be polyphyletic, and those taxa conventionally classified in Zygomycota are now distributed among the new phylum Glomeromycota and four subphyla.[65] Because the name *zygomycosis* was based on the phylum Zygomycota, this new classification alters the name of the clinical diseases. Instead, it is best to now use the names *mucormycosis* to refer to disease caused by members of the order Mucorales (not by organisms from the genus *Mucor* as the name might suggest) and *entomophthoramycosis* for disease caused by species from the order Entomophthorales. Therefore, the term *zygomycosis*, which has historically often been used incorrectly as a synonym of only mucormycosis, should not be used. As molecular phylogenetics continues to progress, these taxonomic definitions and clinical names may be subject to further change.

Organisms of the class Zygomycetes were first noted to cause disease in humans in publications from the 1800s. Plataut[102] is credited with the first description of mucormycosis in humans in his paper "Mycosis Mucorina." In this German language manuscript, he described a case of disseminated mycosis in a cancer patient. The first English-language description of the disease appeared in 1940 when Wade[149] described a case of cutaneous mucormycosis infection of the face.

The class Zygomycetes is divided into two orders, the Mucorales and the Entomophthorales.[112] The Mucorales are responsible for the majority of disease seen in humans, predominately affecting immunocompromised hosts and often characterized by a rapidly evolving clinical course with tissue destruction and invasion of blood vessels. The mycoses caused by Entomophthorales historically have been limited to immunocompetent patients residing in tropical and subtropical areas of the world. Infections caused by Entomophthorales are usually chronic, subcutaneous, and limited without blood vessel invasion.[112] This chapter will focus on the more relevant infections caused by Mucorales, with a brief description of infections caused by Entomophthorales.

THE ORGANISMS

The organisms of the class Zygomycetes are ubiquitous in nature and have a wide geographic distribution. The class consists of two orders—Mucorales and Entomophthorales—each containing several species reported to cause disease in humans (Fig. 205-1).[112] The most common causative Mucorales organisms in humans are the *Mucor*, *Rhizopus*, *Lichtheimia* (formerly *Absidia*), and *Rhizomucor* genera, but other genera in the order include *Saksenaea*, *Cunninghamella*, and *Apophysomyces*. These organisms grow readily on common laboratory media but are inhibited by cycloheximide. Growth of members of Mucorales on solid media demonstrates rapid growth of woolly colonies, often with spores seen as tiny dark dots to the naked eye. Hyphae characteristically are large (10 to 30 μm in diameter), nonseptate, and often twisted or ribbon-like. The lack of septation and the tendency of hyphae to branch at right angles usually serve to distinguish them from *Aspergillus* spp., which are septate, smaller, and branch at acute angles.[3] Genera usually are differentiated from one another by examination of mycelia. Speciating the members of Zygomycetes is often difficult. The Entomophthorales organisms that cause human infection to date are confined to two genera, *Conidiobolus* and *Basidiobolus*. Growth of members of Entomophthorales also occurs readily on solid media but appears as flat, gray, or pale yellow waxy colonies with velvety white mycelia on the surface.[112] The hyphae often are septate.

EPIDEMIOLOGY

All members of Zygomycetes are found in all parts of the world, regardless of climate or other factors. Most human disease caused by members of Mucorales is concentrated in North America, perhaps because of a concentration of immunocompromised hosts.[8] Infections caused by the Entomophthorales genera *Basidiobolus* and *Conidiobolus* occur predominantly in Africa, India, and the Far East, although a few cases have been reported in North and South America.[112]

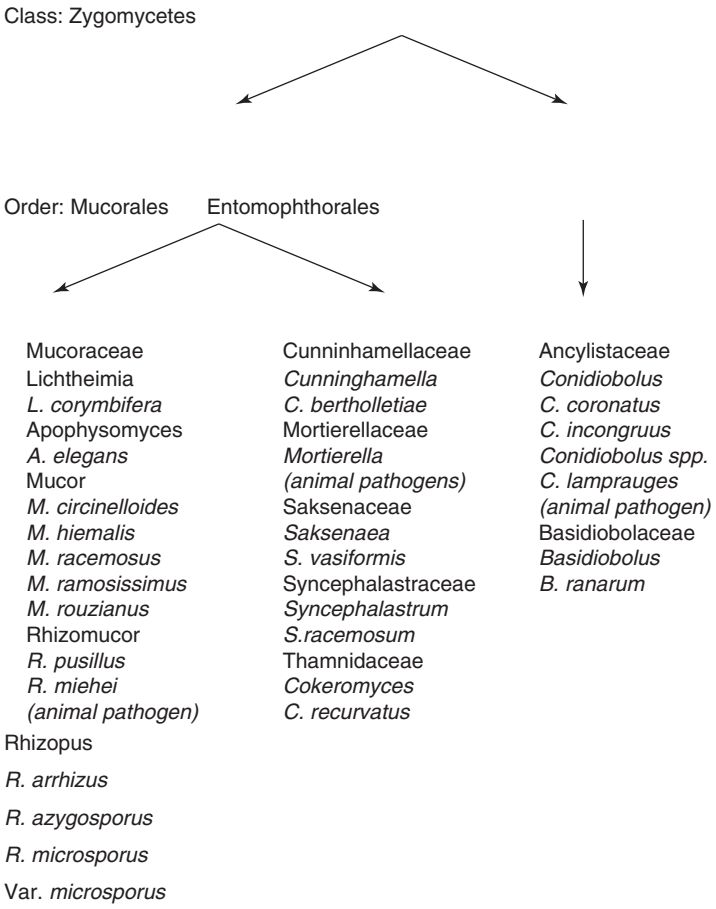

FIGURE 205-1 ■ **Classification of zygomycetes.** (From Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev* 2000;**13**:236-301.)

These fungi are commonly found in soil, in decaying organic material such as vegetation, bread, seeds and fruits and in manure. Many of them are animal pathogens. Members of Mucorales are also occasionally isolated from the hospital environment.[58,61,112,154,157] Studies have suggested a relationship between activities involved in hospital construction and development of mucormycosisin immunocompromised hosts.[61,118,157] *Rhizopus* spp. frequently are found in moldy bread and fruits.[112] Members of Entomophthorales commonly are found in feces of reptiles and other animals, decaying vegetable matter, and some are insect pathogens.[112]

The ubiquitous nature of these organisms, coupled with the paucity of human disease associated with them, is strong evidence for their relatively saprophytic characteristics. Mucormycosis and entomophthoramycosis infections are rare, occurring at an annual rate of 1.7 infections per million population in the United States.[109] In the past decade, however, the frequency and incidence of mucormycosis has increased, particularly among hematopoietic stem cell transplant (HSCT) recipients and patients with hematologic malignancies (Fig. 205-2A and B).[36,59,80] Despite this increase over the last decade, mucormycosis and entomophthoramycosis were seen at autopsy in 1.9 percent of patients dying of malignancy, contrasting with the high incidence of aspergillosis at autopsy (19.6%) at the same center.[60] The incidence of mucormycosis complicating HSCT was 2.5 percent in one study, and it may be less common than other molds because evaluation of indoor environments shows Zygomycetes are rarely isolated.[72] A review of 1500 patients who underwent HSCT over the span of 26 years at one center found that only 0.9 percent of patients had mucormycosis.[89] In the largest systematic review of reported cases, *Rhizopus* and *Mucor* spp. were the most commonly reported causative organisms, identified in 47 percent and 18 percent of cases, respectively.[116] A review of 157 published cases of pediatric mucormycosis revealed a similar distribution of species causing infection in both children and adults.[162] The overall cost of mucormycosis to the U.S. health care system is estimated at $50 million/yr.[44] The multicenter Transplant-Associated Infection Surveillance Network (TRANSNET) found that among 1208 invasive fungal infections identified among 1063 transplant recipients, of the mold infections, mucormycosis was responsible for only 2 percent of cases, in contrast to invasive aspergillosis (19%) and non-*Aspergillus* molds (8%).[99] The time to diagnose mucormycosis was a median of 312 days after transplantation.

A large European registry of 230 cases of mucormycosis from 2005 to 2007 found the most common underlying conditions to be hematologic malignancies (44%), with diabetes mellitus in 9 percent of cases. Of the cases, the majority (34%) were due to *Rhizopus* spp., with *Mucor* spp (19%) also identified frequently. On multivariate analysis, treatment with amphotericin B and surgery were associated with survival.[128] A large retrospective French study of 101 cases also between 2005 and 2007 found that

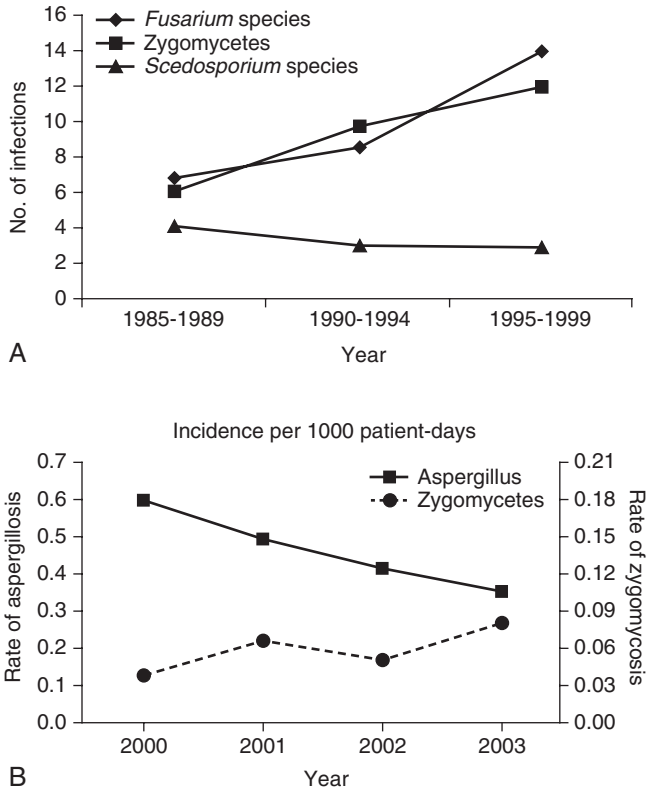

FIGURE 205-2 ■ **A,** Changing frequency of non-*Aspergillus* moulds in blood and marrow transplantation recipients at Fred Hutchinson Cancer Research Center (Seattle, Wash), 1985-1999. **B,** Changing spectrum of *Aspergillus* and Zygomycetes at M.D. Anderson Cancer Center (Houston, Tex). (**A,** from Marr KA, Carter RA, Crippa F, et al. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:909-17. **B,** from Kontoyiannis DP, Lionakis MS, Lewis RE, et al. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005;**191**:1350-60.)

again hematologic malignancies were the most common underlying condition (50%) and that the median time for mucormycosis was 8.8 months after disease occurrence. The main fungal species isolated were *Rhizopus oryzae* (32%) and *Lichtheimia* spp. (29%). Specifically, *R. oryzae* was found in 85 percent of rhinocerebral forms, in contrast to only 17 percent of nonrhinocerebral disease.[68] In a smaller registry of 41 patients with mucormycosis, the most common underlying conditions were malignancies (63%), diabetes mellitus (17%), and solid-organ transplantation (10%). The lung was the most commonly affected organ, and dissemination occurred in 15 percent of patients.[120] A retrospective analysis of children with mucormycosis (2003 to 2010) found 156 cases and noted that the prevalence did not significantly increase over time. Consistent with adult data, the most common underlying condition was malignancy (58%).[104]

Mucormycosis commonly presents as an opportunistic infection in patients with underlying risk factors. Predisposing risk factors include immunosuppression, metabolic disorders, breakdown of skin or soft tissue integrity such as in burn patients or surgical patients, and prematurity (Table 205-1).[58,60,116,162] The most commonly reported underlying conditions in both adults and children are diabetes mellitus and neutropenia.[60,116,162] Recent evidence has suggested that the increasing use of voriconazole as prophylaxis and as empirical, preemptive, and targeted therapy for invasive aspergillosis is associated with an increased risk for mucormycosis.[47,59,81,127] One hypothesis is that voriconazole, because of its lack of activity against Zygomycetes, has exerted selection pressure favoring the emergence of mucormycosis.[157]

TABLE 205-1 Underlying Diseases Commonly Associated with Increased Risk for Mucormycosis

Pediatric-Specific Risk Factors
Prematurity[162]
Methylmalonicaciduria
Malnourishment
Risk Factors Seen in Both Adults and Children[116,162]
Neutropenia or neutrophil dysfunction
Diabetes mellitus
Malignancy
Organ transplantation
Corticosteroid therapy
Human immunodeficiency virus infection
Deferoxamine therapy
Intravenous drug use
Trauma or surgery
Burns

MUCORMYCOSIS

Pathogenesis and Pathology

Mucormycosis usually is acquired by humans after inhalation of spores from environmental sources, and the organisms may colonize the sinuses and nasopharynx of some patients.[112] Occasionally, mucormycosis results from cutaneous inoculation of spores secondary to trauma, as has occurred with contaminated elastic adhesive tape[28,38,112] and ingestion of fermented milk or dried bread products.[69,93] After the inhalation of airborne spores, primary infection usually develops in the upper and lower respiratory tract and later by contiguous or hematogenous dissemination. Disseminated disease most commonly arises from the lungs and spreads hematogenously to the central nervous system (CNS), kidneys, gastrointestinal tract, and heart. Dissemination also can occur from cutaneous or subcutaneous sites of infection.[28,38,132] Person-to-person transmission has not been documented.

The immune status of the host is fundamental to production of disease. In normal hosts, Zygomycetes are killed by mononuclear cells and neutrophils via the generation of oxidative metabolites and cationic peptide defensins.[29,150,152] The neutrophil appears to be the primary component of the immune response against these organisms and may serve to prevent germination of inhaled spores.[29] Both neutropenia and qualitative deficiencies of neutrophil function are associated with an increased risk for mucormycosis.[19,132] In addition, corticosteroid treatment impairs the ability of macrophages to

prevent germination of the spores.[151] Mucormycosis reported in patients with acquired immunodeficiency syndrome (AIDS) tends to occur in intravenous drug users and may reflect direct inoculation as the risk factor, rather than the immunodeficiency itself.[90,130] Disease may follow a less fulminant course in patients with AIDS in contrast to other immunocompromised populations.[9]

Diabetes mellitus, in particular diabetic ketoacidosis, is the most common underlying disease in patients with mucormycosis.[57,58,116] Serum from patients with diabetic ketoacidosis does not inhibit the growth of *Rhizopus* spp., but serum from those same patients after treatment of the ketoacidosis does show inhibitory properties similar to those of normal human serum.[4,19,37,112] Furthermore, hyperglycemia and acidosis, commonly seen in diabetic patients, are known to impair the ability of phagocytes for chemotaxis and to kill the organisms by both oxidative and nonoxidative mechanisms.[19]

Iron levels in serum also may play a role in the pathogenesis and immune response to the organisms. Patients treated with the iron chelator deferoxamine have a markedly increased risk for invasive mucormycosis, as demonstrated by the tendency for deferoxamine therapy to result in progression of both natural and experimental mucormycosis.* This phenomenon seems to occur commonly, particularly in patients undergoing hemodialysis.[10,11,160] Studies in experimental animals suggest that deferoxamine may serve as a siderophore for *Rhizopus* spp., with resultant increased fungal growth and increased mortality in a guinea pig model.[12,27,145] A phase II clinical study found that patients with mucormycosis treated with deferoxamine had a higher mortality at 90 days, therefore not supporting a role for initial adjunctive deferoxamine therapy.[134] In diabetic patients, the presence of systemic acidosis can lead to elevated levels of serum iron, likely as a result of the release of iron from binding proteins.[4] In vitro data conflict, however, on the exact effects of iron or deferoxamine on promoting the growth of members of Mucorales.[95] Much remains to be understood about the pathogenesis of mucormycosis, and a unifying concept will have to incorporate the effects of steroids, acidosis, and iron on host neutrophils and macrophages in failing to suppress fungal replication.

The hallmark of histologic examination of mucormycosis is vascular invasion with resultant thrombosis and tissue necrosis and accompanying acute and chronic inflammation.[15,46,132] Most of the clinical findings of progressive disease can be related to these effects on blood vessels. Septic emboli may affect all parts of the body. Perineural invasion also occurs commonly.[35] In the rhinocerebral form, the disease may progress along nerve roots during intracranial spread. Although relatively sparse, hyphal forms may be seen in tissue on routine hematoxylin and eosin staining more commonly than with silver or other special fungal stains (Fig. 205-3).[114]

Clinical Manifestations

The clinical forms of mucormycosis are best considered by type of organ system involvement (Table 205-2). The primary site of infection at the time of initial diagnosis varies as a function of the host population (Table 205-3). Rhinocerebral, cutaneous, and pulmonary diseases are the most common clinical manifestations of mucormycosis.[116] Other forms of invasive mucormycosis include gastrointestinal, disseminated, and other rare presentations.[58,116]

*References 11, 13, 25, 27, 40, 111, 122, 145.

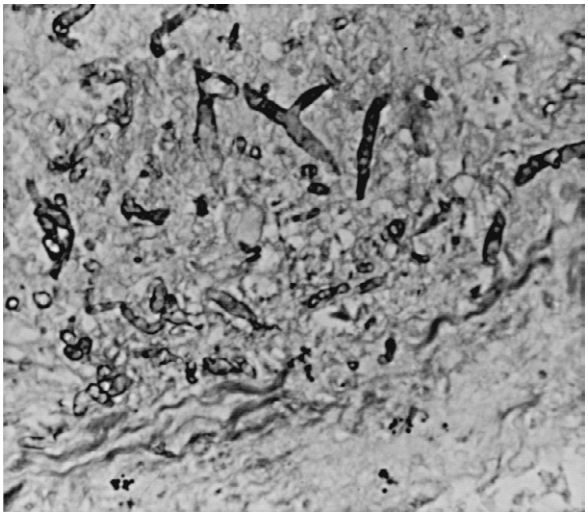

FIGURE 205-3 ■ Zygomycosis caused by *Rhizopus* spp. Observe the varied morphologic features of the coenocytic hyphae and large size. (Hematoxylin and eosin, original magnification ×64.)

TABLE 205-2 **Clinical Forms of Mucormycosis**

Rhinocerebral
Cutaneous
Pulmonary
Gastrointestinal
Disseminated
Miscellaneous

Acute Rhinocerebral Mucormycosis

The acute rhinocerebral form of mucormycosis, including sinus and sino-orbital infections, is the most common presentation of mucormycosis and occurs most often in the setting of diabetes mellitus.[60,116] Rhinocerebral mucormycosis spreads from the nares, palate, or sinuses to the paranasal sinuses, and then to the retro-orbital region and brain by hematogenous spread. Symptoms include facial pain and headache; physical examination reveals brownish, blood-tinged nasal discharge; black eschar on the palate; proptosis; or cranial nerve involvement.[33] Involved tissues become red, then violaceous, and finally black as vessels are thrombosed and tissues undergo necrosis. Extension into the periorbital region can be manifested as proptosis or orbital edema, and ocular or optic nerve involvement can be suggested by pain, blurring, or loss of vision.[131] Initial symptoms of invasive rhinocerebral disease include bloody nasal discharge,[112] seizures,[94] and cerebrovascular accident.[83] The mortality rate for rhinocerebral disease is greater than 50 percent.[116]

TABLE 205-3 **Patterns of Zygomycosis by Host Population**

Predisposing Condition	Predominant Sites of Infection (in Order of Frequency)
Diabetes mellitus	Rhinocerebral, pulmonary, sino-orbital cutaneous
Malignancy with neutropenia	Pulmonary, sinus, cutaneous, sino-orbital
Hematopoietic Stem Cell Transplantation	Pulmonary, disseminated, rhinocerebral
Solid-organ transplantation	Sinus, cutaneous, pulmonary, rhinocerebral, disseminated
Intravenous drug use or abuse	Cerebral, endocarditis, cutaneous, disseminated
Malnutrition	Gastrointestinal, disseminated
Deferoxamine-based therapy	Disseminated, pulmonary, rhinocerebral, cerebral, cutaneous, gastrointestinal
No underlying condition	Cutaneous, pulmonary, sino-orbital, rhinocerebral, gastrointestinal
No underlying condition	

Kontoyiannis DP, Lewis RE. Invasive zygomycosis: update on pathogenesis, clinical manifestations, and management. *Infect Dis Clin North Am* 2006;**20**:581-607.

FIGURE 205-4 ■ Cutaneous zygomycosis in a child with neutropenia.

Cutaneous Mucormycosis

Cutaneous mucormycosis represents approximately 20 percent of reported cases in adults and a slightly higher proportion of pediatric cases.[116,162] Primary cutaneous infection usually occurs at the site of trauma, burns, or adhesive dressings or invasive procedures in immunocompromised hosts.* Among neonates, the skin is the most common point of entry, with involvement of adhesive tape, monitor leads, or central venous access sites predominating. Premature infants represent most of the neonatal cases, and the overall mortality rate reported in the literature is 75 percent.[75,96,115] Secondary cutaneous disease results from hematogenous dissemination.[56] Primary disease may be very invasive locally and by direct extension affect adjacent bone and tissue. With vessel invasion, frankly disseminated disease may arise.

Primary cutaneous disease causes lesions that appear erythematous and indurated and progress to form black eschars (Fig. 205-4). In contrast, cutaneous disease arising from disseminated disease usually manifests in patients as nodular subcutaneous lesions that may ulcerate.[112] Cutaneous mucormycosis can be evidence of disseminated disease, but primary cutaneous mucormycosis associated with occlusive bandages, burns, or wounds rarely extends beyond local involvement.[49] Most cutaneous cases are caused by *Rhizopus* spp. and manifest with lesions that typically are black necrotic ulcers surrounded by erythematous and indurated painful cellulitis.[50] There can be rapid progression and skin necrosis caused by hyphae invading the vasculature and causing tissue infarction.[73] Cutaneous mucormycosis should be considered when skin necrosis continues despite administration of appropriate antibiotics.[98]

Pulmonary Mucormycosis

Pulmonary mucormycosis may develop as a result of inhalation or by hematogeneous or lymphatic spread. The clinical manifestations of pulmonary mucormycosis are similar to the manifestations of invasive pulmonary aspergillosis and often are indistinguishable.[18] Mucormycotic pneumonia typically develops in severely immunocompromised patients, such as patients with malignancy and neutropenia,[80,89,132] but it also has occurred in patients with juvenile diabetes mellitus and in normal hosts.[20,52,116] Patients with pulmonary mucormycosis often present with fever, cough, chest pain, and rapidly progressive dyspnea.[138] Angioinvasion results in necrosis of lung tissue, which may lead to cavitation and hemoptysis. Massive pulmonary hemorrhage may result from vascular erosion.[41,156] Chest radiographs and computed tomography (CT) scans may reveal a lobar pneumonia pattern, isolated masses, nodular disease, cavitation, or wedge-shaped areas of infarction.[55,79,84,138] Because of the lack of inflammatory cells that are capable of infiltrating the lung in neutropenic patients, however, radiography may not be dramatically abnormal.

A review of 58 solid-organ transplant recipients with mucormycosis found that pulmonary mucormycosis developed in 53 percent of the patients at a median of 5.5 months after transplantation. Those patients with disseminated disease most commonly had cutaneous soft tissue involvement, but the brain was not involved. The mortality at 90 days after treatment was over 45 percent.[135] Distinguishing pulmonary mucormycosis from invasive pulmonary aspergillosis is a clinical challenge. In patients with hematologic malignancies, clues for distinguishing pulmonary mucormycosis are the presence of concomitant pansinusitis, a history of antifungal prophylaxis with *Aspergillus*-active agents such as voriconazole and echinocandins, and possibly the repeated absence of detectable

*References 22, 38, 56, 142, 158, 159.

Aspergillus galatomannan antigen in serum. In addition, the presence of multiple nodules (>10) and, to a lesser degree, pleural effusions on CT scans favors the diagnosis of pulmonary mucormycosis over invasive pulmonary aspergillosis in patients with cancer.[18] Some atypical presentations of pulmonary mucormycosis include chronic infection with constitutional symptoms in relatively immunocompetent hosts.[84] Rarely, fungus balls have been noted within a preexisting lung cavity.[112] Hypersensitivity pneumonitis caused by *Rhizopus* spp. has been described in farm workers as well as Scandinavian sawmill workers (so-called wood trimmer disease).[31] The disease usually is fulminant, but may be milder in hosts with less severe immunocompromise.

Gastrointestinal Mucormycosis

Gastrointestinal involvement probably results from ingestion of fungal spores, from either the environment or colonized upper airways. Occasionally, hematogenous or direct extension routes result in intestinal tract involvement. Malnutrition, prematurity, uremia, and underlying gastrointestinal disease, such as typhoid fever or amebiasis, are predisposing factors.[74,85,87,93] Gastrointestinal mucormycosis is the most common clinical manifestation among neonates, representing two thirds of neonatal cases.[162] Most gastrointestinal disease in neonates is seen in premature neonates, some occurring in association with progressive necrotizing skin lesions and others manifesting with necrotizing entercolitis.[143,161] All sites along the intestinal tract can be involved, with the stomach and colon being the most common sites.[93] Nonspecific abdominal pain with hematemesis, hematochezia, or melena may occur. Dissemination to other sites may occur by hematogenous routes. Perforation after bowel wall necrosis is common. Diagnosis usually is made at autopsy.

Disseminated Mucormycosis

Disseminated mucormycosis occurs in the most severely immunocompromised patients and usually is diagnosed after death.[49,146] Clinical findings are nonspecific, and a high index of suspicion and readily accessible tissue for biopsy are needed to establish the diagnosis. The pulmonary system is affected most commonly, followed by the CNS, but virtually any organ can be involved. A subacute form of disseminated disease with a protracted but fatal course has been reported.[97] The combination of severe immunocompromise and subtle clinical findings renders this form of mucormycosis the most difficult to treat.

Miscellaneous Forms of Mucormycosis

A variety of case reports of mucormycosis involving isolated areas of the body are found in the literature and include reports of endocarditis,[86,119,163] brain abscess,[116] peritonitis,[16,91,103] osteomyelitis,[32] mediastinitis,[82] tracheitis,[124] corneal infection,[139] otitis externa,[139] superior vena cava syndrome,[14] and renal disease.[77] Common to these disease manifestations is some type of local accidental or surgical site that results in ready access for invasion of fungal organisms. Outcome varies highly and depends on the underlying condition of the host as well as the extent of disease.

Diagnosis

The presence of Zygomycetes in clinical specimens need not always represent clinically significant isolates. The spores from asexual reproduction are easily airborne and may be laboratory contaminants of culture medium or a result of oral ingestion in food or nasal inhalation of air before patient sampling. A positive culture linked to hyphal identification in cytologic specimens or tissue sections is considered diagnostic.[5,112] One study showed that the isolation of Zygomycetes from a patient with malignancy with no risk factors or clinically evident infection probably represents colonization; however, the pretest probability of a culture positive for Zygomycetes in a patient with leukemia was high.[60]

In most patients, the clinical presentation is assumed to be invasive aspergillosis, and in one series of 185 cases of disseminated mucormycosis, the correct diagnosis was made before death in only 17 cases.[49] In the largest single-center review of mucormycosis, chest radiographs were abnormal in all patients with pulmonary mucormycosis, showing patterns such as focal consolidation (30%), cavitation (24%), widespread infiltrates (24%), or nodules (18%).[60] Cavitation is a common finding on chest radiograph, but the "air crescent sign" is seen less frequently than in patients with invasive aspergillosis. In acidotic patients, pulmonary findings mostly manifest as lobar consolidation that may cavitate to form a lung abscess.[21] In one series, most manifestations in patients with pulmonary involvement were nodular or cavitary lesions.[89]

Noninvasive diagnostic procedures for mucormycosis are nonspecific, and a tissue diagnosis must be sought, even in situations in which other infectious agents have been isolated. Cultures of blood and bodily fluid rarely detect the disease. Immunofluorescent antibody staining of formaldehyde-fixed tissue is available through the mycology section of the Centers for Disease Control and Prevention, which might be helpful in confirming a suspected diagnosis.[33,49] Many specimens, including blood, sputum, gastric fluid, or nasal swabs, are either difficult to culture or of no diagnostic value, and invasive mucormycosis is correctly diagnosed with tissue biopsy.[33]

Antemortem respiratory tract specimens from autopsy-confirmed cases found 0/8 positive results in patients, including blood, urine, and cerebrospinal fluid cultures.[101] Cultures of lower respiratory tract specimens, including bronchoalveolar lavage and sputum specimens, were negative for mucormycosis in 75 percent of cases of histopathologically proved pulmonary mucormycosis, possibly owing to the high incidence of concomitant pulmonary infections.[60] Pulmonary mucormycosis has been diagnosed by fine-needle aspiration,[6] and an analysis of 87 cases of mucormycosis revealed that 40 percent of pulmonary disease was diagnosed by transbronchial biopsy and 20 percent required open lung biopsy or surgical resection for diagnosis.[71]

Serologic diagnostic tests for detecting mucormycosis are not clinically useful. Antibody tests lack specificity, whereas several antigen detection systems have been used in tissue specimens. Very few molecular techniques are in use for establishing the diagnosis of mucormycosis, and techniques that are available are still experimental. Polymerase chain reaction amplification of the 18S rRNA sequences may be useful in epidemiologic studies of outbreaks, but little is currently being offered for primary diagnosis.[112] Primers from ribosomal DNA for those Zygomycetes most implicated in clinical infection have been designed for use in a polymerase chain reaction assay for rapid and accurate identification.[113,148]

Treatment

Of overall great importance is the combination of early and aggressive surgical excision of the necrotic lesions, restoration of immune function, and intense antifungal therapy. Because tissue infarction is a prominent feature, removal of devitalized tissue is critical because antifungals alone, especially in the setting of continued immunosuppression, will not elicit cure.[72,101] All necrotic-appearing tissue should be removed, and often patients require repeated surgical procedures for removal of devitalized tissue. Surgical débridement alone may be sufficient to cure localized cutaneous disease.[106]

Currently, the recommended antifungal therapy for mucormycosis is amphotericin B, given at maximal dosages of 1 to 1.5 mg/kg/day, based on the individual's tolerance of side effects.[58] Amphotericin B lipid complex also has been used successfully,[100] including success with doses from 3 to 14 mg/kg/day, but there was no pattern of improved response in relation to dosage,[126] which correlates with the comparison of no advantage with 10 mg/kg versus 3 mg/kg of liposomal amphotericin B against invasive aspergillosis.[23] In a study of 41 cases, initial treatment with liposomal amphotericin B was associated with improved outcome ($p = 012$) and survival ($p = .004$) in contrast to initial treatment with amphotericin B, deoxycholate, or posaconazole.[120] Whether lipid formulations of amphotericin B definitively confer any advantage over conventional amphotericin B for mucormycosis is unclear,[60,100] but avoiding the nephrotoxicity associated with conventional amphotericin B may prove beneficial in delivering a longer duration of therapy. Delayed amphotericin B–based therapy (treatment ≥6 days after diagnosis) resulted in a two-fold increase in mortality (83% vs. 49%) and was an independent predictor of mortality in patients with hematologic malignancy and mucormycosis,[17] emphasizing the importance of aggressive management.

Most azoles, including fluconazole and voriconazole, have no meaningful activity against Zygomyecetes fungi. This point is critically important because voriconazole is an excellent antifungal against invasive aspergillosis, but has no clinical activity against mucormycosis. Multiple reports have suggested an increased risk for mucormycosis in patients receiving voriconazole prophylaxis.* One hypothesis posed to explain this association is that long-term exposure to voriconazole alters the nature of the fungal population in patients and provides a niche for new colonization by mucormycosis that are naturally resistant to voriconazole.[147] In addition, voriconazole may alter the virulence of mucormycosis. A study using murine and *Drosophila* models of mucormycosis found that exposure of Zygomycetes organisms to voriconazole by serial passage selectively enhanced their virulence.[66] Posaconazole, an approved orally available triazole, does possess potent activity against Zygomycetes.[140] However, in vitro time-kill studies have shown that although amphotericin B is rapidly fungicidal against Zygomycetes, with 95 percent killing in 6 hours and 99.9 percent killing at 24 hours, posaconazole has less than 70 percent killing at 6 hours and 99.9 percent killing only at 48 hours.[64] Therefore, in the critical early phase of therapy, the more rapidly fungicidal amphotericin B–based therapy is preferred. Another murine study found that posaconazole showed poor in vivo efficacy, which followed the very high mean fungicidal concentrations in contrast to the mean inhibitory concentrations.[121] Additional concerns regarding the effectiveness of posaconazole therapy for mucormycosis have been raised. It is unclear whether the posaconazole levels achieved in patients, particularly those with mucositis or diarrhea, are reliable and adequate to treat infections caused by mucormycosis.[62,63,70,141] Preclinical efficacy studies have shown poor activity of posaconazole despite the achievement of adequate serum levels (>5 μg/mL).[3] Furthermore, several investigators have found that posaconazole has virtually no activity against *R. oryzae*, one of the most common species causing infection.[26,45,117,136] Murine studies have failed to find evidence of an additive benefit of posaconazole when combined with polyene therapy.[45,117] In several more recent salvage therapy reports and studies, the overall success rate was 60 to 70 percent in patients with refractory disease.[107,144,125] Despite these encouraging results, its exact role in the treatment of mucormycosis remains unclear.

Echinocandins lack significant activity against Zygomycetes in vitro, and several cases of breakthrough mucormycosis have occurred in patients receiving echinocandin-based therapy.[59] However, *R. oryzae* expresses the gene encoding for the proteins of the 1,3-β-D-glucan synthase complex, and its enzyme activity is inhibited by caspofungin. Low dosage (0.5 mg/kg twice daily), but not higher doses of caspofungin, improved survival in a diabetic murine model of subacute disseminated zygomycosis.[43] When combined with amphotericin B lipid complex, caspofungin was more active than amphotericin B alone, or caspofungin alone, for the treatment of experimental, disseminated zygomycosis in diabetic mice.[133] Furthermore, Lamaris and colleagues[67] found that caspofungin uncovers β-glucan in the cell wall of *R. oryzae*. This effect of caspofungin is associated with enhanced activity of human neutrophils against the organism.[67] A clinical study of polyene-echinocandin combination therapy for mucormycoses found a benefit in patients who were predominantly non-neutropenic, and therefore this echinocandin-induced

*References 18, 47, 59, 81, 127, 147.

immunomodulatory activity of phagocytic cells may be particularly applicable to those with diabetes mellitus.[108] Whether the potentially beneficial effects of therapy with amphotericin B lipid complex plus an echinocandin are applicable to neutropenic patients or nonmyelosuppressed but immunosuppressed (e.g., corticosteroid-treated) hosts is not clear.

Hyperbaric oxygen therapy has been used as an adjunct to surgical and antifungal therapy for some patients in an attempt to limit the extent of gangrene and tissue necrosis.[24,34,53,105] The increased oxygen pressure achieved with hyperbaric therapy seems to improve the ability of neutrophils to kill organisms and inhibit fungal growth in vitro.[7] Although this regimen is apparently promising, the number of patients evaluated with this form of treatment is much too small to permit any firm conclusions on benefit. The role of other adjunctive therapies for mucormycosis has not been well studied. Granulocyte transfusions or cytokines that enhance phagocytic activity, such as interferon-γ and granulocyte-macrophage colony-stimulating factor, have been used occasionally to treat patients with mucormycosis.[1,39,129] Case reports have indicated favorable outcomes in patients treated with these adjunctive therapies,[1,129] but further studies are warranted before these therapies are recommended routinely.

Prognosis and Prevention

The disease site and host factors are key determinants of prognosis for mucormycosis. The reported mortality rate is highest for disseminated disease (100%), followed by gastrointestinal (85%), cerebral (79%), pulmonary (76%), and rhinocerebral (46%) disease.[116] Localized cutaneous disease is associated with a low mortality rate of 10 percent. Significant risk factors for mortality include disseminated disease and renal failure. Patients who undergo surgery as primary therapy and patients who receive antifungal therapy are significantly more likely to survive.[116] Among children, age younger than 1 year is associated with higher mortality.[162]

ENTOMOPHTHOROMYCOSIS

Pathogenesis and Pathology

Entomophthoromycosis presumably develops through inhalation of spores to cause sinus disease or cutaneous inoculation to cause cutaneous or subcutaneous infection. The exact mechanisms are unclear, however, and no specific animal model has been developed. The organisms are of extremely low virulence, as manifested by the rare occurrence of these infections despite the ubiquitous nature of the fungi. Although discrete clinical syndromes have been assigned to the two genera causing disease, this distinction is artificial, and the fact that either group could cause either form of entomophthoromycosis or even a syndrome more suggestive of mucormycosis should be recongnized.[112,123,153,155]

Histopathologic examination of affected tissues shows some similarities to that of mucormycosis. Areas of acute and chronic inflammation are found in association with broad hyphal elements that may or may not display septations. The hyphae are more visible with hematoxylin and eosin staining than with more specific fungal stains.[112] The tendency for vascular invasion typical of mucormycosis does not occur with entomophthoromycosis, however, and necrosis is uncommon. Instead, a Splendore-Hoeppli phenomenon, likely consisting of hyphae surrounded by eosinophilic material in a stellate pattern, occurs.[112] This feature is not pathognomonic of entomophthoromycosis, however, and has been seen in cases of mucormycosis.

Clinical Manifestations

Chronic Rhinofacial Entomophthoromycosis

Chronic rhinofacial entomophthoromycosis, also called *entomophthoromycosis conidiobolae* because most infections are caused by *Conidiobolus coronatus*, is an indolent subcutaneous infection involving the face.[42,88,92] Typically, bilateral intranasal swelling eventually progresses to invasion of the sinuses and soft tissue swelling of the face, unaccompanied by fever or pain. One infant developed orbital involvement from *C. coronatus* dacryocystitis.[76] Symptoms commonly persist for weeks or months, but deep tissue progression is unlikely to occur.

Chronic Subcutaneous Entomophthoromycosis

Chronic subcutaneous entomophthoromycosis, also known as *entomophthoromycosis basidiobolae*, is similar to chronic rhinofacial disease with the exception that the lesions usually are on the trunk or extremities. The etiologic agent is *Basidiobolus ranarum*. Painless subcutaneous nodules may progress to invade deeper soft tissues, and massive soft tissue swelling may develop. Facial infection similar to that of chronic rhinofacial entomophthoromycosis also may occur.[30] *Conidiobolus incongruus* has caused orbitofacial disease in a child residing in the Middle East.[2] With rare exceptions, most cases of subcutaneous entomophthoromycosis are slowly progressive.[51,112]

Gastrointestinal Basidiobolomycosis

Gastrointestinal basidiobolomycosis is an extremely rare condition resulting from *B. ranarum* infection of the gastrointestinal tract. A cluster of cases was identified in Arizona in the late 1990s, suggesting a possible emerging infection in the region. In a case-control study including seven adult patients in Arizona, infected patients tended to be more likely to use ranitidine and to have resided in Arizona for a longer time in contrast to controls.[78] Clinical features included abdominal pain, leukocytosis, and eosinophilia. Combined surgical resection and itraconazole appeared to be effective treatment.

Diagnosis

Entomophthoromycosis often can be diagnosed presumptively on the basis of clinical presentation and geographic origin of the patient, but usually biopsy is

required.[112] More recent work on serodiagnosis by immunodiffusion has had promising results.[48,54] Onchocerciasis, filariasis, and Burkitt lymphoma sometimes are in the differential diagnosis.[51,112]

Treatment

Evaluating therapy for this group of diseases is difficult because of occasional reports of spontaneous resolution. Many agents have been tried with variable success. Taylor and colleagues[137] treated a young man with rhinofacial disease with amphotericin B, corticosteroids, miconazole, and multiple surgical procedures before noting resolution some months after any therapy had been given. Susceptibility testing of the isolate on that patient showed minimal inhibitory effects of amphotericin B and no benefit of adding flucytosine or rifampin; miconazole, ketoconazole, and potassium iodide did not have impressive in vitro activity. Other investigators recommend potassium iodide therapy, with trimethoprim-sulfamethoxazole as an alternative.[110]

Other anecdotal reports of efficacy of trimethoprim-sulfamethoxazole, ketoconazole, and itraconazole raise hopes for effective therapy but must be tempered by the fact that only small numbers of patients have been treated.[42,78,137] Currently, no antifungal is the preferred treatment for this condition, although some type of medical therapy seems indicated for all cases. Data regarding the activity of posaconazole against this group of fungi are unavailable. The role of surgery is less for this group of disorders than for mucormycosis, but patients with well-circumscribed areas, such as nodular lesions, may benefit from surgical excision. Despite surgery, recurrence of disease after surgery commonly occurs in such patients.[137]

Prognosis and Prevention

Mortality caused by entomophthoromycoses is unlikely to occur, but morbidity and disfigurement are common. The efficacy of medical and surgical therapy is unclear, but all patients should likely receive attempts at treatment because the natural history of the disease is one of slow progression. Specific means for preventing disease are unavailable because so little is known about factors predisposing individuals to these chronic entomophthoromycotic syndromes.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Andes D, Marchillo K, Conklin R, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. *Antimicrob Agents Chemother* 2004;**48**:137–42.
17. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 15 2008;**47**:503–9.
23. Cornely OA, Maertens J, Bresnik M, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis* 2007;**44**:1289–97.
26. Dannaoui E, Meis JF, Loebenberg D, Verweij PE. Activity of posaconazole in treatment of experimental disseminated zygomycosis. *Antimicrob Agents Chemother* 2003;**47**:3647–50.
43. Ibrahim AS, Bowman JC, Avanessian V, et al. Caspofungin inhibits *Rhizopus oryzae* 1,3-beta-D-glucan synthase, lowers burden in brain measured by quantitative PCR, and improves survival at a low but not a high dose during murine disseminated zygomycosis. *Antimicrob Agents Chemother* 2005;**49**:721–7.
44. Ibrahim AS, Edwards JE Jr, Bryant R, et al. Economic burden of mucormycosis in the United States: can a vaccine be cost-effective? *Med Mycol* 2009;**47**:592–600.
45. Ibrahim AS, Gebremariam T, Schwartz JA, et al. Posaconazole mono- or combination therapy for treatment of murine zygomycosis. *Antimicrob Agents Chemother* 2009;**53**:772–5.
62. Krishna G, Martinho M, Chandrasekar P, et al. Pharmacokinetics of oral posaconazole in allogeneic hematopoietic stem cell transplant recipients with graft-versus-host disease. *Pharmacotherapy* 2007;**27**:1627–36.
63. Krishna G, Sansone-Parsons A, Martinho M, et al. Posaconazole plasma concentrations in juvenile patients with invasive fungal infection. *Antimicrob Agents Chemother* 2007;**51**:812–18.
64. Krishnan-Natesan S, Manavathu EK, Alangaden GJC, P.H. A comparison of the fungicidal activity of amphotericin B and posaconazole against Zygomycetes in vitro. *Diagn Microbiol Infect Dis* 2009;**63**:361–4.
65. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoramycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. *Clin Infect Dis* 2012;**54**(suppl 1):S8–S15.
66. Lamaris GA, Ben-Ami R, Lewis RE, et al. Increased virulence of Zygomycetes organisms following exposure to voriconazole: a study involving fly and murine models of zygomycosis. *J Infect Dis* 2009;**199**:1399–406.
67. Lamaris GA, Lewis RE, Chamilos G, et al. Caspofungin-mediated beta-glucan unmasking and enhancement of human polymorphonuclear neutrophil activity against *Aspergillus* and non-*Aspergillus* hyphae. *J Infect Dis* 2008;**198**:186–92.
68. Lanternier F, Dannaoui E, Morizot G, et al. A global analysis of mucormycosis in France: the RetroZygo Study (2005-2007). *Clin Infect Dis* 2012;**54**(suppl 1):S35–43.
70. Lebeaux D, Lanternier F, Elie C, et al. Therapeutic drug monitoring of posaconazole: a monocentric study with 54 adults. *Antimicrob Agents Chemother* 2009;**53**:5224–9.
99. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 15 2010;**50**:1101–11.
104. Prasad PA, Vaughan AM, Zaoutis TE. Trends in zygomycosis in children. *Mycoses* 2012;**55**:352–6.
108. Reed C, Bryant R, Ibrahim AS, et al. Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. *Clin Infect Dis* 2008;**47**:364–71.
120. Rüping MJ, Heinz WJ, Kindo AJ, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. *J Antimicrob Chemother* 2010;**65**:296–302.
121. Salas V, Pastor FJ, Calvo E, et al. In vitro and in vivo activities of posaconazole and amphotericin B in a murine invasive infection by *Mucor circinelloides:* poor efficacy of posaconazole. *Antimicrob Agents Chemother* 2012;**56**:2246–50.
126. Shoham S, Magill SS, Merz WG, et al. Primary treatment of zygomycosis with liposomal amphotericin B: analysis of 28 cases. *Med Mycol* 2010;**48**:511–17.
128. Skiada A, Pagano L, Groll A, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. *Clin Microbiol Infect* 2011; **17**:1859–67.
133. Spellberg B, Fu Y, Edwards JEJ, et al. Combination therapy with amphotericin B lipid complex and caspofungin acetate of disseminated zygomycosis in diabetic ketoacidotic mice. *Antimicrob Agents Chemother* 2005;**49**:830–2.
134. Spellberg B, Ibrahim AS, Chin-Hong PV, et al. The Deferasirox-AmBisome Therapy for Mucormycosis (DEFEAT Mucor) study: a randomized, double-blinded, placebo-controlled trial. *J Antimicrob Chemother* 2012;**67**:715–22.
135. Sun HY, Aguado JM, Bonatti H, et al. Pulmonary zygomycosis in solid organ transplant recipients in the current era. *Am J Transplant* 2009;**9**:2166–71.

136. Sun QN, Najvar LK, Bocanegra R, et al. In vivo activity of posaconazole against *Mucor* spp. in an immunosuppressed-mouse model. *Antimicrob Agents Chemother* 2002;**46**:2310–2.
141. Ullmann AJ, Cornely OA, Burchardt A, et al. Pharmacokinetics, safety, and efficacy of posaconazole in patients with persistent febrile neutropenia or refractory invasive fungal infection. *Antimicrob Agents Chemother* 2006;**50**:658–66.
147. Vigouroux S, Morin O, Moreau P, et al. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. *Clin Infect Dis* 2005;**40**: e35–7.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 206

Fusariosis and Scedosporiosis

Damian J. Krysan • Melanie Wellington • William J. Steinbach

Fusarium and *Scedosporium* are species of molds that cause hyalohyphomycosis. Hyalohyphomycoses, although not as common as invasive aspergillosis or mucormycosis, are invasive fungal infections caused by nondematiaceous molds that lack cell wall melanin and thus are nonpigmented, in contrast to phaeohyphomycoses, which are fungal infections caused by pigmented molds. In profoundly immunocompromised patients, fusariosis and scedosporiosis are increasing in prevalence and are associated with high mortality.

INFECTIONS CAUSED BY *FUSARIUM* SPECIES

Microbiology and Pathogenesis of Infection

Molds of the *Fusarium* genus are saprophytic environmental fungi that are well recognized as plant pathogens and, increasingly, as the cause of human infections.[17,47,50] *Fusarium* species are among the organisms that cause hyalohyphomycosis by virtue of their characteristic tissue form consisting of hyaline-like branching or nonbranching hyphal elements.[17] The most common clinical *Fusarium* isolates include *Fusarium solani* (50%), *Fusarium oxysporum* (20%), *Fusarium moniliforme* (10%), and *Fusarium verticillioidis* (10%).[47,50] In immunocompetent patients, *Fusarium* is the most common cause of fungal keratitis; *F. oxysporum* is the predominant organism.[17,47,50,64] Keratitis can progress to endophthalmitis, but this is more common in immunocompromised patients.[50] In addition, *Fusarium* is an important cause of onychomycosis.[48] Invasive fusarial disease can develop in immunocompetent patients who have had tissue breakdown due to trauma or burns[49] and in patients who have indwelling catheters, such as for vascular access, hemodialysis, or peritoneal dialysis.[20] Among immunocompromised patients, *Fusarium* is an emerging cause of infection[51]; according to data published before 2011, *Fusarium* was the second most common filamentous fungal pathogen in hematopoietic stem cell transplant recipients after *Aspergillus* species,[8,50] causing disseminated disease characterized by pneumonia, fungemia, and cutaneous manifestations. Data from the Transplant-Associated Infection Surveillance Network (TRANSNET) indicate that *Fusarium* species are now the third most common cause of mold infections in transplant recipients after *Aspergillus* and Mucorales.[54]

The portal of entry for most cases of disseminated *Fusarium* infections is the respiratory tract, but access to the patient may also be gained through vascular catheters.[1,8,19,47,51] *Fusarium* species have a propensity for vascular invasion, resulting in thrombosis and tissue necrosis, as well as for dissemination to extrapulmonary tissues.[49] One of the characteristics of *Fusarium* infections compared with other molds such as *Aspergillus* is that there is a high rate of fungemia. Many isolates of *Fusarium* species are capable of producing a yeast-like synanamorph when the microconidia enlarge; then, instead of germinating to form hyphae, these give rise directly to unicellular propagules, called adventitious sporulation (Fig. 206-1). This leads to a rapid dissemination and could explain the higher rate of positive blood cultures compared with other molds, such as *Aspergillus* species.[8] Another explanation for the high rate of positive blood cultures is the potential ability of *Fusarium* organisms to sporulate by forming phialides and phialoconidia within the human body.[58]

Risk Factors and Clinical Syndromes

One of the most common fusarial infections of immunocompetent patients is keratitis.[17,50,64] An outbreak of

REFERENCES

1. Abzug MJ, Walsh TJ. Interferon-gamma and colony-stimulating factors as adjuvant therapy for refractory fungal infections in children. *Pediatr Infect Dis J* 2004;**23**:769–73.
2. al-Hajjar S, Perfect J, Hashem F, et al. Orbitofascial conidiobolomycosis in a child. *Pediatr Infect Dis J* 1996;**15**:1130–2.
3. Andes D, Marchillo K, Conklin R, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. *Antimicrob Agents Chemother* 2004;**48**:137–42.
4. Artis WM, Fountain JA, Delcher HK, Jones HE. A mechanism of susceptibility to mucormycosis in diabetic ketoacidosis: transferrin and iron availability. *Diabetes* 1982;**31**:1109–14.
5. Ascioglu S, Rex JH, de Pauw B, et al. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus. *Clin Infect Dis* 2002;**34**:7–14.
6. Bakshi NA, Volk EE. Pulmonary mucormycosis diagnosed by fine needle aspiration cytology: a case report. *Acta Cytology* 2001;**45**: 411–14.
7. Barratt DM, Van Meter K, Asmar P, et al. Hyperbaric oxygen as an adjunct in zygomycosis: randomized controlled trial in a murine model. *Antimicrob Agents Chemother* 2001;**45**:3601–2.
8. Benbow EW, Stoddart RW. Systemic zygomycosis. *Postgrad Med J* 1986;**62**:985–96.
9. Blatt SP, Lucey DR, DeHoff D, et al. Rhinocerebral zygomycosis in a patient with AIDS. *J Infect Dis* 1991;**164**:215–16.
10. Boelaert JR, Fenves AZ, Coburn JW. Mucormycosis among patients on dialysis. *N Engl J Med* 1989;**321**:190–1.
11. Boelaert JR, Fenves AZ, Coburn JW. Deferoxamine therapy and mucormycosis in dialysis patients: report of an international registry. *Am J Kidney Dis* 1991;**18**:660–7.
12. Boelaert JR, de Locht M, Van Cutsem J, et al. Mucormycosis during deferoxamine therapy is a siderophore-mediated infection: in vitro and in vivo animal studies. *J Clin Invest* 1993;**91**:1979–86.
13. Boelaert JR, Van Cutsem J, de Locht M, et al. Deferoxamine augments growth and pathogenicity of *Rhizopus*, while hydroxypyridinone chelators have no effect. *Kidney Int* 1994;**45**:667–71.
14. Bosken CH, Szporn AH, Kleinerman J. Superior vena cava syndrome due to mucormycosis in a patient with lymphoma. *Mt Sinai J Med* 1987;**54**:508–11.
15. Bouchara JP, Oumeziane NA, Lissitzky JC, et al. Attachment of spores of the human pathogenic fungus *Rhizopus oryzae* to extracellular matrix components. *Eur J Cell Biol* 1996;**70**:76–83.
16. Branton MH, Johnson SC, Brooke JD, et al. Peritonitis due to *Rhizopus* in a patient undergoing continuous ambulatory peritoneal dialysis. *Rev Infect Dis* 1991;**13**:19–21.
17. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clin Infect Dis* 2008;**47**:503–9.
18. Chamilos G, Marom EM, Lewis RE, et al. Predictors of pulmonary zygomycosis versus invasive pulmonary aspergillosis in patients with cancer. *Clin Infect Dis* 2005;**41**:60–6.
19. Chinn RY, Diamond RD. Generation of chemotactic factors by *Rhizopus oryzae* in the presence and absence of serum: relationship to hyphal damage mediated by human neutrophils and effects of hyperglycemia and ketoacidosis. *Infect Immun* 1982;**38**:1123–9.
20. Cohen-Abbo A, Bozeman PM, Patrick CC. *Cunninghamella* infections: review and report of two cases of *Cunninghamella pneumonia* in immunocompromised children. *Clin Infect Dis* 1993;**17**: 173–7.
21. Connolly JE, McAdams HP, Erasmus JJ, et al. Opportunistic fungal pneumonia. *J Thorac Imaging* 1999;**14**:51–62.
22. Cooter RD, Lim IS, Ellis DH, et al. Burn wound zygomycosis caused by *Apophysomyces elegans*. *J Clin Microbiol* 1990;**28**:2151–3.
23. Cornely OA, Maertens J, Bresnik M, et al. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis* 2007;**44**:1289–97.
24. Couch L, Theilen F, Mader JT. Rhinocerebral mucormycosis with cerebral extension successfully treated with adjunctive hyperbaric oxygen therapy. *Arch Otolaryngol Head Neck Surg* 1988;**114**: 791–4.
25. Daly AL, Velazquez LA, Bradley SF, Kauffman CA. Mucormycosis: association with deferoxamine therapy. *Am J Med* 1989;**87**: 468–71.
26. Dannaoui E, Meis JF, Loebenberg D, Verweij PE. Activity of posaconazole in treatment of experimental disseminated zygomycosis. *Antimicrob Agents Chemother* 2003;**47**:3647–50.
27. de Locht M, Boelaert JR, Schneider YJ. Iron uptake from ferrioxamine and from ferrirhizoferrin by germinating spores of *Rhizopus microsporus*. *Biochem Pharmacol* 1994;**47**:1843–50.
28. Dennis JE, Rhodes KH, Cooney DR, et al. Nosocomical *Rhizopus* infection (zygomycosis) in children. *J Pediatr* 1980;**96**:824–8.
29. Diamond RD, Haudenschild CC, Erickson NF 3rd. Monocyte-mediated damage to Rhizopus oryzae hyphae in vitro. *Infect Immun* 1982;**38**:292–7.
30. Dworzack DL, Pollock AS, Hodges GR, et al. Zygomycosis of the maxillary sinus and palate caused by *Basidiobolus haptosporus*. *Arch Intern Med* 1978;**138**:1274–6.
31. Dykewicz MS, Laufer P, Patterson R, et al. Woodman's disease: hypersensitivity pneumonitis from cutting live trees. *J Allergy Clin Immunol* 1988;**81**:455–60.
32. Echols RM, Selinger DS, Hallowell C, et al. Rhizopus osteomyelitis: a case report and review. *Am J Med* 1979;**66**:141–5.
33. Espinel-Ingroff A, Oakley LA, Kerkering TM. Opportunistic zygomycotic infections. *Mycopathologica* 1987;**97**:33–41.
34. Ferguson BJ, Mitchell TG, Moon R, et al. Adjunctive hyperbaric oxygen for treatment of rhinocerebral mucormycosis. *Rev Infect Dis* 1988;**10**:551–9.
35. Frater JL, Hall GS, Procop GW. Histologic features of zygomycosis: emphasis on perineural invasion and fungal morphology. *Arch Pathol Lab Med* 2001;**125**:375–8.
36. Fridkin SK. The changing face of fungal infections in health care settings. *Clin Infect Dis* 2005;**41**:1455–60.
37. Gale GR, Welch AM. Inhibition of R. oryzae by human serum. *Am J Med Sci* 1971;**45**:604.
38. Gartenberg G, Bottone EJ, Keusch GT, et al. Hospital-acquired mucormycosis *(Rhizopus rhizopodiformis)* of skin and subcutaneous tissue: epidemiology, mycology and treatment. *N Engl J Med* 1978;**299**:1115–18.
39. Gil-Lamaignere C, Winn RM, Simitsopoulou M, et al. Inteferon gamma and granulocyte-macrophage colony-stimulating factor augment the antifungal activity of human polymorphonuclear leukocytes against *Scedosporium* spp.: comparison with *Aspergillus* spp. *Med Mycol* 2005;**43**:253–60.
40. Hamdy NA, Andrew SM, Shortland JR, et al. Fatal cardiac zygomycosis in a renal transplant patient treated with desferrioxamine. *Nephrol Dial Transplant* 1989;**4**:911–13.
41. Harada M, Manabe T, Yamashita K, et al. Pulmonary mucormycosis with fatal massive hemoptysis. *Acta Pathol Jpn* 1992;**42**: 49–55.
42. Herstoff JK, Bogaars H, McDonald CJ. Rhinophycomycosis entomophthorae. *Arch Dermatol* 1978;**114**:1674–8.
43. Ibrahim AS, Bowman JC, Avanessian V, et al. Caspofungin inhibits *Rhizopus oryzae* 1,3-beta-D-glucan synthase, lowers burden in brain measured by quantitative PCR, and improves survival at a low but not a high dose during murine disseminated zygomycosis. *Antimicrob Agents Chemother* 2005;**49**:721–7.
44. Ibrahim AS, Edwards JE Jr, Bryant R, et al. Economic burden of mucormycosis in the United States: can a vaccine be cost-effective? *Med Mycol* 2009;**47**:592–600.
45. Ibrahim AS, Gebremariam T, Schwartz JA, et al. Posaconazole mono- or combination therapy for treatment of murine zygomycosis. *Antimicrob Agents Chemother* 2009;**53**:772–5.
46. Ibrahim AS, Spellberg B, Avanessian V, et al. *Rhizopus oryzae* adheres to, is phagocytosed by, and damages endothelial cells in vitro. *Infect Immun* 2005;**73**:778–83.
47. Imhof A, Balajee SA, Fredricks DN, et al. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clin Infect Dis* 2004;**39**:743–6.
48. Imwidthaya P, Srimuang S. Immunodiffusion test for diagnosing basidiobolomycosis. *Mycopathologia* 1992;**118**:127–31.
49. Ingram CW, Sennesh J, Cooper JN, et al. Disseminated zygomycosis: report of four cases and review. *Rev Infect Dis* 1989;**11**: 741–54.
50. Jantunen E, Kolho E, Ruutu P, et al. Invasive cutaneous mucormycosis caused by *Absidia corymbifera* after allogeneic bone marrow transplantation. *Bone Marrow Transplantation* 1996;**18**:229–30.

51. Jelliffe DB, Burkitt D, O'Conor GT, et al. Subcutaneous phycomycosis in an East African child. *J Pediatr* 1961;**59**:124–7.
52. Johnson GM, Baldwin JJ. Pulmonary mucormycosis and juvenile diabetes. *Am J Dis Child* 1981;**135**:567–8.
53. Kajs-Wyllie M. Hyperbaric oxygen therapy for rhinocerebral fungal infection. *J Neurosci Nurs* 1995;**27**:174–81.
54. Kaufman L, Mendoza L, Standard PG. Immunodiffusion test for serodiagnosing subcutaneous zygomycosis. *J Clin Microbiol* 1990;**28**:1887–90.
55. Kawakami K, Watanabe Y, Kadowaki S. Early onset invasive pulmonary zygomycosis following allogeneic peripheral blood stem cell transplantation in a patient with therapy-related myelodysplastic syndrome. *Rinsho Ketsueki* 2004;**45**:319–21.
56. Khardori N, Hayat S, Rolston K, et al. Cutaneous *Rhizopus* and *Aspergillus* infections in five patients with cancer. *Arch Dermatol* 1989;**125**:952–6.
57. Kline MW. Mucormycosis in children: review of the literature and report of cases. *Pediatr Infect Dis* 1985;**4**:672–6.
58. Kontoyiannis DP, Lewis RE. Invasive zygomycosis: update on pathogenesis, clinical manifestations, and management. *Infect Dis Clin North Am* 2006;**20**:581–607.
59. Kontoyiannis DP, Lionakis MS, Lewis RE, et al. Zygomycosis in a tertiary-care cancer center in the era of *Aspergillus*-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis* 2005;**191**:1350–60.
60. Kontoyiannis DP, Wessel VC, Bodey GP, et al. Zygomycosis in the 1990s in a tertiary-care cancer center. *Clin Infect Dis* 2000; **30**:851–6.
61. Krasinski K, Holzman RS, Hanna B, et al. Nosocomial fungal infection during hospital renovation. *Infect Control* 1985;**6**:278–82.
62. Krishna G, Martinho M, Chandrasekar P, et al. Pharmacokinetics of oral posaconazole in allogeneic hematopoietic stem cell transplant recipients with graft-versus-host disease. *Pharmacotherapy* 2007;**27**:1627–36.
63. Krishna G, Sansone-Parsons A, Martinho M, et al. Posaconazole plasma concentrations in juvenile patients with invasive fungal infection. *Antimicrob Agents Chemother* 2007;**51**:812–18.
64. Krishnan-Natesan S, Manavathu EK, Alangaden GJC, P.H. A comparison of the fungicidal activity of amphotericin B and posaconazole against Zygomycetes in vitro. *Diagn Microbiol Infect Dis* 2009;**63**:361–4.
65. Kwon-Chung KJ. Taxonomy of fungi causing mucormycosis and entomophthoramycosis (zygomycosis) and nomenclature of the disease: molecular mycologic perspectives. *Clin Infect Dis* 2012; **54**(suppl 1):s8–15.
66. Lamaris GA, Ben-Ami R, Lewis RE, et al. Increased virulence of Zygomycetes organisms following exposure to voriconazole: a study involving fly and murine models of zygomycosis. *J Infect Dis* 2009;**199**:1399–406.
67. Lamaris GA, Lewis RE, Chamilos G, et al. Caspofungin-mediated beta-glucan unmasking and enhancement of human polymorphonuclear neutrophil activity against *Aspergillus* and non-*Aspergillus* hyphae. *J Infect Dis* 2008;**198**:186–92.
68. Lanternier F, Dannaoui E, Morizot G, et al. A global analysis of mucormycosis in France: the RetroZygo Study (2005-2007). *Clin Infect Dis* 2012;**54**(suppl 1):s35–43.
69. Lawson HH, Schmaman A. Gastric phycomycosis. *Br J Surg* 1974;**61**(9):743–6.
70. Lebeaux D, Lanternier F, Elie C, et al. Therapeutic drug monitoring of posaconazole: a monocentric study with 54 adults. *Antimicrob Agents Chemother* 2009;**5**:5224–9.
71. Lee FYW, Mossad SB, Adal KA. Pulmonary mucormycosis: the last 30 years. *Arch Intern Med* 1999;**159**:1301–9.
72. Leleu X, Sendid B, Fruit J, et al. Combined anti-fungal therapy and surgical resection as treatment of pulmonary zygomycosis in allogeneic bone marrow transplantation. *Bone Marrow Transplant* 1999;**24**:417–20.
73. Leong KW, Crowley B, White B, et al. Cutaneous mucormycosis due to *Absidia corymbifera* occurring after bone marrow transplantation. *Bone Marrow Transplant* 1997;**19**:513–15.
74. Levin SE, Isaacson C. Spontaneous perforation of the colon in the newborn infant. *Arch Dis Child* 1960;**35**:378–82.
75. Linder N, Keller N, Huri C, Kuint J, Goldshmidt-Reuven A, Barzilai A. Primary cutaneous mucormycosis in a premature infant: case report and review of the literature. *Am J Perinatol* 1998;**15**:35–8.
76. Lithander J, Louon A, Worthing E, et al. Orbital entomophthoramycosis in an infant: recovery following surgical debridement, combination antifungal therapy and use of hyperbaric oxygen. *Br J Ophthalmol* 2001;**85**:374–5.
77. Lussier N, Laverdiere M, Weiss K, et al. Primary renal mucormycosis. *Urology* 1998;**52**:900–3.
78. Lyon GM, Smilack JD, Komatsu KK, et al. Gastrointestinal basidiobolomycosis in Arizona: clinical and epidemiological characteristics and review of the literature. *Clin Infect Dis* 2001;**32**: 1448–55.
79. Marchevsky AM, Bottone EJ, Geller SA, et al. The changing spectrum of disease, etiology, and diagnosis of mucormycosis. *Hum Pathol* 1980;**11**:457–64.
80. Marr KA, Carter RA, Crippa F, et al. Epidemiology and outcome of mould infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:909–17.
81. Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. *N Engl J Med* 2004;**350**:950–2.
82. Marwaha RK, Banerjee AK, Thapa BR, et al. Mediastinal zygomycosis. *Postgrad Med J* 1985;**61**:733–5.
83. Mathur SC, Friedman HD, Kende AI, et al. Cryptic *Mucor* infection leading to massive cerebral infarction at initiation of antileukemic chemotherapy. *Ann Hematol* 1999;**78**:241–5.
84. McAdams HP, Rosado de Christenson M, Strollo DC, Patz EF Jr. Pulmonary mucormycosis: radiologic findings in 32 cases. *AJR Am J Roentgenol* 1997;**168**:1541–8.
85. Michalak DM, Cooney DR, Rhodes KH, et al. Gastrointestinal mucormycoses in infants and children: a cause of gangrenous intestinal cellulitis and perforation. *J Pediatr Surg* 1980;**15**:320–4.
86. Mishra B, Mandal A, Kumar N. Mycotic prosthetic-valve endocarditis. *J Hosp Infect* 1992;**20**:122–5.
87. Mooney JE, Wanger A. Mucormycosis of the gastrointestinal tract in children: report of a case and review of the literature. *Pediatr Infect Dis J* 1993;**12**:872–6.
88. Moretz ML, Grist WJ, Sewell CW. Zygomycosis presenting as nasal polyps in a healthy child. *Arch Otolaryngol Head Neck Surg* 1987;**113**:550–2.
89. Morrison VA, McGlave PB. Mucormycosis in the BMT populatioon. *Bone Marrow Transplant* 1993;**11**:383–8.
90. Mostaza JM, Barbado FJ, Fernandez-Martin J, et al. Cutaneoarticular mucormycosis due to *Cunninghamella bertholletiae* in a patient with AIDS. *Rev Infect Dis* 1989;**11**:316–18.
91. Nakamura M, Weil WB Jr, Kaufman DB. Fatal fungal peritonitis in an adolescent on continuous ambulatory peritoneal dialysis: association with deferoxamine. *Pediatr Nephrol* 1989;**3**:80–2.
92. Nathan MD, Keller AP Jr, Lerner CJ, et al. Entomophthorales infection of the maxillofacial region. *Laryngoscope* 1982;**92**(7 Pt 1):767–9.
93. Neame P, Rayner D. Mucormycosis: a report on twenty-two cases. *Arch Pathol* 1960;**70**:261–8.
94. Nenoff P, Kellerman S, Schober R, et al. Rhinocerebral zygomycosis following bone marrow transplantation in chronic myelogenous leukaemia: report of a case and review of the literature. *Mycoses* 1998;**41**:365–72.
95. Niimi O, Kokan A, Kashiwagi N. Effect of deferoxamine mesylate on the growth of mucorales. *Nephron* 1989;**53**:281–2.
96. Nissen MD, Jana AK, Cole MJ, et al. Neonatal gastrointestinal mucormycosis mimicking necrotizing enterocolitis. *Acta Paediatr* 1999;**88**:1290–3.
97. Nolan RL, Carter RR 3rd, Griffith JE, et al. Subacute disseminated mucormycosis in a diabetic male. *Am J Med Sci* 1989; **298**:252–5.
98. Nomura J, Ruskin J, Sahebi F, et al. Mucormycosis of the vulva following bone marrow transplantation. *Bone Marrow Transplant* 1997;**19**:859–60.
99. Pappas PG, Alexander BD, Andes DR, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clin Infect Dis* 2010;**50**:1101–11.
100. Perfect JR. Treatment of non-*Aspergillus* moulds in immunocompromised patients, with amphotericin B lipid complex. *Clin Infect Dis* 2005;**40**(suppl 6):S401–8.

101. Piliero PJ, Deresiewicz RL. Pulmonary zygomycosis after allogeneic bone marrow transplantation. *South Med J* 1995;**88**:1149–52.
102. Platauf A. Mycosis mucorina. *Virchows Arch* 1895;**102**:543–64.
103. Polo JR, Luno J, Menarguez C, et al. Peritoneal mucormycosis in a patient receiving continuous ambulatory peritoneal dialysis. *Am J Kidney Dis* 1989;**13**:237–9.
104. Prasad PA, Vaughan AM, Zaoutis TE. Trends in zygomycosis in children. *Mycoses* 2012;**55**:352–6.
105. Price JC, Stevens DL. Hyperbaric oxygen in the treatment of rhinocerebral mucormycosis. *Laryngoscope* 1980;**90**(5 Pt 1):737–47.
106. Pritchard RC, Muir DB, Archer KH, et al. Subcutaneous zygomycosis due to *Saksenaea vasiformis* in an infant. *Med J Aust* 1986;**145**:630–1.
107. Raad II, Graybill JR, Bustamante AB, et al. Safety of long-term oral posaconazole use in the treatment of refractory invasive fungal infections. *Clin Infect Dis* 2006;**42**:1726–34.
108. Reed C, Bryant R, Ibrahim AS, et al. Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. *Clin Infect Dis* 2008;**47**:364–71.
109. Rees JR, Pinner RW, Hajjeh RA, et al. The epidemiological features of invasive mycotic infections in the San Francisco Bay area, 1992-1993: results of population-based laboratory active surveillance. *Clin Infect Dis* 1998;**27**:1138–47.
110. Restrepo A. Treatment of tropical mycoses. *J Am Acad Dermatol* 1994;**31**(3 Pt 2):S91–102.
111. Rex JH, Ginsberg AM, Fries LF, et al. Cunninghamella bertholletiae infection associated with deferoxamine therapy. *Rev Infect Dis* 1988;**10**:1187–94.
112. Ribes JA, Vanover-Sams CL, Baker DJ. Zygomycetes in human disease. *Clin Microbiol Rev* 2000;**13**:236–301.
113. Rickerts V, Loeffler J, Bohme A, et al. Diagnosis of disseminated zygomycosis using a polymerase chain reaction assay. *Eur J Clin Microbiol Infect Dis* 2001;**20**:744–5.
114. Rippon J. *Medical Mycology: The Pathogenic Fungi and the Pathogenic Zygomycetes*. 3rd ed. Philadelphia: W.B. Saunders; 1988.
115. Robertson AF, Joshi VV, Ellison DA, et al. Zygomycosis in neonates. *Pediatr Infect Dis J* 1997;**16**:812–15.
116. Roden MM, Zaoutis TE, Buchanan WL, et al. Epidemiology and outcome of zygomycosis: a review of 929 reported cases. *Clin Infect Dis* 2005;**41**:634–53.
117. Rodríguez MM, Serena C, Mariné M, et al. Posaconazole combined with amphotericin B, an effective therapy for a murine disseminated infection caused by *Rhizopus oryzae*. *Antimicrob Agents Chemother* 2008;**52**:3786–8.
118. Rosen PP, Sternberg SS. Decreased frequency of aspergillosis and mucormycosis. *N Engl J Med* 1976;**295**:1319–20.
119. Roy TM, Anderson KC, Farrow JR. Cardiac mucormycosis complicating diabetes mellitus. *J Diabet Complications* 1990;**4**:132–5.
120. Rüping MJ, Heinz WJ, Kindo AJ, et al. Forty-one recent cases of invasive zygomycosis from a global clinical registry. *J Antimicrob Chemother* 2010;**65**:296–302.
121. Salas V, Pastor FJ, Calvo E, et al. In vitro and in vivo activities of posaconazole and amphotericin B in a murine invasive infection by *Mucor circinelloides:* poor efficacy of posaconazole. *Antimicrob Agents Chemother* 2012;**56**:2246–50.
122. Sane A, Manzi S, Perfect J, et al. Deferoxamine treatment as a risk factor for zygomycete infection. *J Infect Dis* 1989;**159**:151–2.
123. Schmidt JH, Howard RJ, Chen JL, et al. First culture-proven gastrointestinal entermophthoromycosis in the United States: a case report and review of the literature. *Mycopathologia* 1986;**95**:101–4.
124. Schwartz JR, Nagle MG, Elkins RC, et al. Mucormycosis of the trachea: an unusual cause of acute upper airway obstruction. *Chest* 1982;**81**:653–4.
125. Segal BH, Barnhart LA, Anderson VL, et al. Posaconazole as salvage therapy in patients with chronic granulomatous disease and invasive filamentous fungal infection. *Clin Infect Dis* 2005;**40**:1684–8.
126. Shoham S, Magill SS, Merz WG, et al. Primary treatment of zygomycosis with liposomal amphotericin B: analysis of 28 cases. *Med Mycol* 2010;**48**:511–17.
127. Siwek GT, Dodgson KJ, de Magalhaes-Silverman M, et al. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. *Clin Infect Dis* 2004;**39**:584–7.
128. Skiada A, Pagano L, Groll A, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. *Clin Microbiol Infect* 2011;**17**:1859–67.
129. Slavin MA, Kannan K, Buchanan MR, et al. Successful allogeneic stem cell transplant after invasive pulmonary zygomycosis. *Leuk Lymphoma* 2002;**43**:437–9.
130. Smith AG, Bustamante CI, Gilmor GD. Zygomycosis (absidiomycosis) in an AIDS patient: absidiomycosis in AIDS. *Mycopathologia* 1989;**105**:7–10.
131. Sohail MA, Al Khabori M, Hyder J, et al. Acute fulminant fungal sinusitis: clinical presentation, radiological findings, and treatment. *Acta Tropica* 2001;**80**:177–85.
132. Spellberg B, Edwards J Jr, Ibrahim A. Novel perspectives on mucormycosis: pathophysiology, presentation, and management. *Clin Microbiol Rev* 2005;**18**:556–69.
133. Spellberg B, Fu Y, Edwards JEJ, et al. Combination therapy with amphotericin B lipid complex and caspofungin acetate of disseminated zygomycosis in diabetic ketoacidotic mice. *Antimicrob Agents Chemother* 2005;**49**:830–2.
134. Spellberg B, Ibrahim AS, Chin-Hong PV, et al. The Deferasirox-AmBisome Therapy for Mucormycosis (DEFEAT Mucor) study: a randomized, double-blinded, placebo-controlled trial. *J Antimicrob Chemother* 2012;**67**:715–22.
135. Sun HY, Aguado JM, Bonatti H, et al. Pulmonary zygomycosis in solid organ transplant recipients in the current era. *Am J Transplant* 2009;**9**:2166–71.
136. Sun QN, Najvar LK, Bocanegra R, et al. In vivo activity of posaconazole against *Mucor* spp. in an immunosuppressed-mouse model. *Antimicrob Agents Chemother* 2002;**46**:2310–2.
137. Taylor GD, Sekhon AS, Tyrrell DL, et al. Rhinofacial zygomycosis caused by *Conidiobolus coronatus:* a case report including in vitro sensitivity to antimycotic agents. *Am J Trop Med Hyg* 1987;**36**:398–401.
138. Tedder M, Spratt JA, Anstadt MP, et al. Pulmonary mucormycosis: results of medical and surgical therapy. *Ann Thorac Surg* 1994;**57**:1044–50.
139. Tierney MR, Baker AS. Infections of the head and neck in diabetes mellitus. *Infect Dis Clin North Am* 1995;**9**:195–216.
140. Torres HA, Hachem RY, Chemaly RF, et al. Posaconazole: a broad-spectrum triazole antifungal. *Lancet Infect Dis* 2005;**5**:775–85.
141. Ullmann AJ, Cornely OA, Burchardt A, et al. Pharmacokinetics, safety, and efficacy of posaconazole in patients with persistent febrile neutropenia or refractory invasive fungal infection. *Antimicrob Agents Chemother* 2006;**50**:658–66.
142. Umbert IJ, Su WP. Cutaneous mucormycosis. *J Am Acad Dermatol* 1989;**21**:1232–4.
143. Vadeboncoeur C, Walton JM, Raisen J, Soucy P, Lau H, Rubin S. Gastrointestinal mucormycosis causing an acute abdomen in the immunocompromised pediatric patient: three cases. *J Pediatr Surg* 1994;**29**:1248–9.
144. van Burik JA, Hare RS, Solomon HF, et al. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. *Clin Infect Dis* 2006;**42**:e61–5.
145. Van Cutsem J, Boelaert JR. Effects of deferoxamine, feroxamine and iron on experimental mucormycosis (zygomycosis). *Kidney Int* 1989;**36**:1061–8.
146. Varricchio F, Reyes MG, Wilks A. Undiagnosed mucormycosis in infants. *Pediatr Infect Dis J* 1989;**8**:660.
147. Vigouroux S, Morin O, Moreau P, et al. Zygomycosis after prolonged use of voriconazole in immunocompromised patients with hematologic disease: attention required. *Clin Infect Dis* 2005;**40**:e35–7.
148. Voigt K, Cigelink E, O'Donnell K. Phylogeny and PCR identification of clinically important zygomycetes based on nuclear ribosomal-DNA sequence data. *J Clin Microbiol* 1999;**37**:3957–64.
149. Wade JMA. Cutaneous mucro infection of the face. *JAMA* 1940;**114**:410–11.
150. Waldorf AR. Pulmonary defense mechanisms against opportunistic fungal pathogens. *Immunol Ser* 1989;**47**:243–71.

151. Waldorf AR, Halde C, Vedros NA. Murine model of pulmonary mucormycosis in cortisone-treated mice. *Sabouraudia* 1982;**20**: 217–24.
152. Waldorf AR, Ruderman N, Diamond RD. Specific susceptibility to mucormycosis in murine diabetes and bronchoalveolar macrophage defense against Rhizopus. *J Clin Invest* 1984;**74**:150–60.
153. Walker SD, Clark RV, King CT, et al. Fatal disseminated *Conidiobolus coronatus* infection in a renal transplant patient. *Am J Clin Pathol* 1992;**98**:559–64.
154. Walsh TJ, Pizzo PA. Nosocomial fungal infections: a classification for hospital-acquired fungal infections and mycoses arising from endogenous flora or reactivation. *Annu Rev Microbiol* 1988;**42**: 517–45.
155. Walsh TJ, Renshaw G, Andrews J, et al. Invasive zygomycosis due to *Conidiobolus incongruus*. *Clin Infect Dis* 1994;**19**:423–30.
156. Watts WJ. Bronchopleural fistula followed by massive fatal hemoptysis in a patient with pulmonary mucormycosis: a case report. *Arch Intern Med* 1983;**143**:1029–30.
157. Weems JJ Jr, Davis BJ, Tablan OC, et al. Construction activity: an independent risk factor for invasive aspergillosis and zygomycosis in patients with hematologic malignancy. *Infect Control* 1987;**8**: 71–5.
158. White CB, Barcia PJ, Bass JW. Neonatal zygomycotic necrotizing cellulitis. *Pediatrics* 1986;**78**:100–2.
159. Wickline CL, Cornitius TG, Butler T. Cellulitis caused by *Rhizomucor pusillus* in a diabetic patient receiving continuous insulin infusion pump therapy. *South Med J* 1989;**82**:1432–4.
160. Windus DW, Stokes TJ, Julian BA, et al. Fatal *Rhizopus* infections in hemodialysis patients receiving deferoxamine. *Ann Intern Med* 1987;**107**:678–80.
161. Woodward A, McTigue C, Hogg G, et al. Mucormycosis of the neonatal gut: a "new" disease or a variant of necrotizing enterocolitis? *J Pediatr Surg* 1992;**27**:737–40.
162. Zaoutis TE, Roden M, Prasad P, et al. Zygomycosis in children: a systematic review of reported cases. Paper presented at the annual meeting of the Pediatric Academic Society, San Francisco, Calif, 2005.
163. Zhang R, Zhang JW, Szerlip HM. Endocarditis and hemorrhagic stroke caused by *Cunninghamella bertholletiae* infection after kidney transplantation. *Am J Kidney Dis* 2002;**40**:842–6.

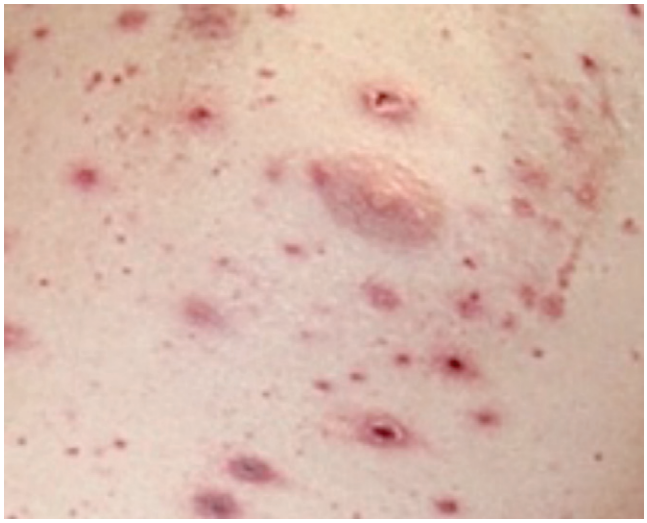

FIGURE 206-1 ■ Characteristic cutaneous lesions of disseminated fusariosis. (See companion Expert Consult website for color version.) (Courtesy Dr. John Perfect, Duke University.)

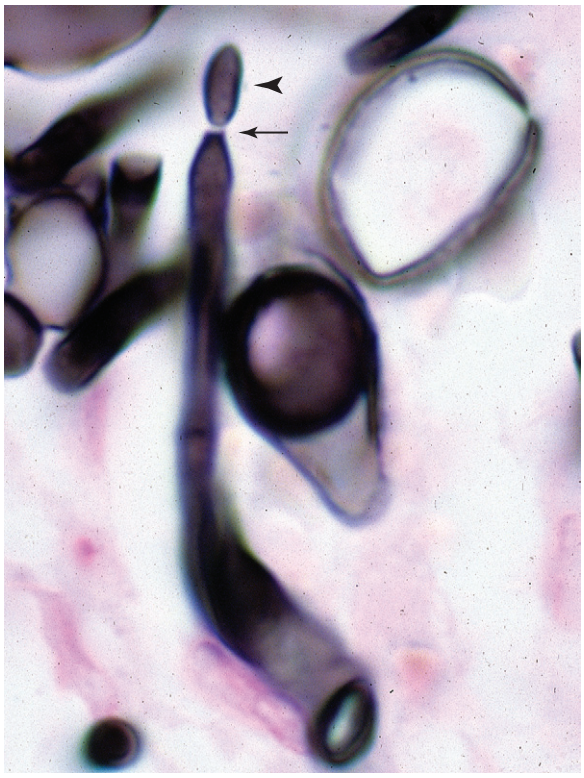

FIGURE 206-2 ■ Silver stain of a knee biopsy specimen from a patient with disseminated fusariosis showing adventitious sporulation. The arrow points to the phialide, and the arrowhead points to the released conidum. (See companion Expert Consult website for color version.) (Courtesy Wiley Schell, Duke University.)

fusarial keratitis occurred in 2006 in contact lens wearers[14,30] in association with a specific contact lens solution (ReNu with MoistureLoc) and resolved after the product was voluntarily removed from the market. Onychomycosis is a second common manifestation of fusarial infection in immunocompetent hosts and typically results from environmental contamination of the great toenail, presenting as proximal subungual onychomycosis.[61]

In immunocompromised patients, invasive fusariosis shares many characteristics with invasive aspergillosis, including the risk factors of neutropenia and corticosteroid exposure, which results in a similar bimodal distribution (before and after engraftment) in hematopoietic stem cell transplant recipients.[8,50,51] Among allogeneic hematopoietic stem cell transplant recipients with fusariosis, other risk factors identified in multivariate models include severe graft-versus-host disease and an underlying diagnosis of multiple myeloma.[42,50,51]

The incidence of fusariosis appears to be affected by both geography and season. A multicenter retrospective study found that fusariosis occurred more frequently in the summer and autumn months than in the winter and spring months. The infections were more likely to occur during the rainy season, which was thought to be due to the dispersal of the soil-borne fungi by the rain and wind.[47] More recently, reports have suggested that this organism may also be acquired through hospital water supplies.[1]

Invasive fusariosis in hematopoietic stem cell transplant recipients most frequently is manifested as invasive pulmonary disease, with dissemination to the extrapulmonary organs in as many as 70 percent of cases.[42] Widespread skin lesions occur in approximately 70 percent of cases (Fig. 206-2), in sharp contrast to invasive aspergillus, which has skin manifestations in less than 5 percent of cases.[49] Cutaneous lesions may take several forms, including painful, discrete, erythematous nodules; raised keratotic masses; and violaceous papules and nodules that break down to form black necrotic centers.[49] Although these skin lesions are typically larger, more erythematous, and more painful than those caused by *Aspergillus* species, the appearance is not specific for fusariosis. Other filamentous fungi, such as *Acremonium* species, can cause similar skin lesions, in which the characteristic yeast-like structures are noted on histopathologic examination. However, culture of skin lesions was the most important basis for the diagnosis of fusariosis in a series of immunocompromised patients[49]; therefore, in this population of patients, biopsy specimens of all skin lesions should be taken and evaluated by both culture and histology.

Localized cutaneous infections can also occur in immunocompromised patients; the most common manifestations are onychomycoses and cellulitis. Because localized infections may progress to involve cellulitis and may lead to disseminated infection, *Fusarium* isolated in nail cultures should not be dismissed as a contaminant in an immunocompromised patient.[5,8,47]

Diagnosis and Therapy

The clinical presentation of fusariosis has no features that reliably distinguish it from other invasive fungal infections. In addition, no specific diagnostic methods exist for fusariosis except for histopathology and the microbiologic examination of blood, sputum, and biopsy tissues. Major clues to *Fusarium* infections include blood cultures positive for mold and the presence of the characteristic skin lesions. In contrast to aspergillosis,

Fusarium species are isolated from the bloodstream in approximately 50 percent of patients.[49] Non–culture-based methods have been developed for the diagnosis of invasive fungal disease by the detection of galactomannan and (1→3)-β-D-glucan in the serum.[32,52] Like most other fungi, *Fusarium* species are generally positive in the (1→3)-β-D-glucan assay.[52] Some *Fusarium* species have been reported to contain galactomannan in vitro. For example, a report described a patient who had a positive galactomannan test result and was subsequently diagnosed with culture-confirmed fusariosis; additional in vitro testing confirmed that *Fusarium* isolates were positive for galactomannan.[44]

In general, *Fusarium* species are relatively resistant to most clinically used antifungal agents on the basis of in vitro studies. Among currently available agents, voriconazole and amphotericin B are the most active.[50] Keratitis is typically treated with topical natamycin, although studies of topical voriconazole indicate that it may be an alternative.[4] Echinocandins have very little activity in vitro against *Fusarium*.[63] Compared with other fungi, the minimum inhibitory concentrations (MICs) for amphotericin B–based agents are high (0.5 to 8 μg/mL); accordingly, animal studies indicate minimal therapeutic responses to lower doses of amphotericin B formulations.[22,36]

Reports have indicated that voriconazole and posaconazole have good in vitro and in vivo activity against experimental *Fusarium* infections.[22,36] Currently, both voriconazole and posaconazole are licensed by the Food and Drug Administration for salvage treatment of fusariosis.[45] A retrospective analysis of 73 cases of fusariosis treated with voriconazole suggested that voriconazole has efficacy that is comparable to the historical response rate reported for patients treated with amphotericin B.[35] Combination drug studies have also been reported, with in vitro synergy demonstrated with a combination of amphotericin B and either 5-flucytosine or rifampin.[3] Similarly, the combination of amphotericin B and caspofungin has been shown to yield synergistic or additive interactions with no antagonism observed in vitro.[34] In addition, there have been a number of case reports of the successful use of combinations of antifungals in the treatment of patients.[34]

As an approach to the treatment of fusariosis, high-dose intravenous amphotericin B lipid formulation has been generally regarded as a first-line option.[45] However, the case series of patients treated with voriconazole strongly support the notion that it is at least as effective as amphotericin B, and given its better tolerability, voriconazole may be a superior choice.[35]

Neutropenia is a prime risk factor for disseminated infection, and without the recovery of neutrophils, *Fusarium* infection is fatal.[46,51] A large clinical review, which included 43 cases of invasive fusariosis at one center and 54 cases from the literature, reported a response to therapy of 30 percent and 48 percent, respectively. Cure was observed only in those patients showing resolution of myelosuppression.[8] Attention to immune reconstitution is critical; granulocyte transfusions and the administration of colony-stimulating growth factors should be considered in all patients who are persistently neutropenic. Another review of fusariosis that was diagnosed among hematopoietic stem cell transplant recipients in nine centers in the United States and Brazil found that the survival after diagnosis was only 13 percent, with a median duration of 13 days.[51]

In summary, invasive fusariosis can be extremely difficult to diagnose and to treat. In the immunocompromised patient, the clinical presentation often mimics invasive aspergillosis, but it has some unique features. Because standard doses of amphotericin B do not appear to be consistently effective, voriconazole has emerged as an attractive option for the treatment of fusariosis. Similar to invasive aspergillosis, the attention to the recovery of neutrophils is paramount, and control of the underlying disease is essential to a favorable outcome. Surgery may decrease the fungal burden and may provide time for the recovery of immune function.[38] Because of the ability of *Fusarium* organisms to attach to and to degrade plastics, removal of infected catheters and prosthetic devices is, as always, strongly recommended.

INFECTIONS CAUSED BY *SCEDOSPORIUM* SPECIES

Microbiology and Pathogenesis

Scedosporium is a taxonomically complex genus, of which the two most medically significant species are *Scedosporium prolificans* and *Scedosporium apiospermum*.[15,31] This genus has morphologic characteristics of both hyaline and dematiaceous molds.[15] *S. apiospermum* is the anamorph, or asexual stage, of *Pseudallescheria boydii*, a species that has undergone several taxonomic name changes. *S. prolificans* is a relatively new species of mold that was first described in 1984 after being isolated from a deep, subcutaneous lesion in the right foot of a 6-year-old boy[15]; it was initially named *Scedosporium inflatum* and later renamed.[12,26,40] More recently, human infections involving a third species, *Scedosporium aurantiacum*, have been described in a large population-based survey of *Scedosporium* infections in Australia.[25]

Scedosporium species are ubiquitous fungi recovered worldwide from polluted water, soil, and poultry and cattle manure.* *S. apiospermum* appears to be more prevalent in temperate climates compared with tropical regions.[15] *S. prolificans* has mainly been isolated in the Iberian peninsula, Australia, southern United States, and California.[15] In the United States, for example, *S. prolificans*–associated bone and joint infections appear to be more common in those areas.[28] *S. prolificans* was found to be the most abundant pathogen in plant soils in hospitals.[59]

Like other pathogenic molds, *Scedosporium* species cause disease in both immunocompetent and immunocompromised people, with localized infections most common in immunocompetent patients and disseminated infections almost exclusively limited to immunocompromised patients.[15] The first disseminated case of *S. prolificans* was reported in 1991,[41] and since that time,

*References 15, 23, 25, 26, 31, 40, 60, 65.

FIGURE 206-3 ■ Pathology specimen from a patient with lumbar vertebral *Scedosporium apiospermum* infection. (See companion Expert Consult website for color version.) (Courtesy Wiley Schell, Duke University.)

Scedosporium species have emerged as the fourth most common cause of invasive mold infections in transplant recipients after *Aspergillus*, Mucorales, and *Fusarium*.[54] The mortality rates associated with disseminated *Scedosporium* infections in immunocompromised patients are extremely high, ranging between 58 percent and 100 percent in reported case series.*

The respiratory tract appears to be the portal of entry for disseminated infection in most cases, although the organism can also invade through ulcerative lesions in the gastrointestinal tract, through surgical wounds, or by inoculation from trauma.[15] In localized infection, the most common mechanism seems to be direct inoculation of wounds by organisms in the environment.[15,40] Germination into hyphae can result in hematogenous dissemination to other organs, particularly the kidneys, lungs, and brain. In the tissue, both the *Pseudallescheria* and *Scedosporium* species form slender, septate hyphae with parallel cell walls that cannot reliably be distinguished from other molds, such as *Aspergillus* or *Fusarium* species, unless conidia are present.[15,23,36]

Risk Factors and Clinical Syndromes

In immunocompetent patients, *P. boydii* is an important cause of mycetoma, a clinical syndrome characterized by infection of soft and connective tissue, joints, and bone by soil-borne fungi.[43] *P. boydii* has been estimated to cause approximately 10 percent of mycetoma worldwide.[15] Case series in the United States and Brazil found that *P. boydii* is the most common cause of fungal mycetoma in these regions.[21,57] Mycetoma displays a characteristic triad of symptoms[15]: tumefaction or edematous tissue; draining sinus track formation; and presence of "grains" (balls of fungal hyphae) in pus. Although localized nonmycetomatous infections involving a wide range of anatomic sites in immunocompetent patients have been reported (Fig. 206-3), they are uncommon.[15]

An important clinical manifestation of *P. boydii* disease in immunocompetent patients is its association with sinopulmonary and central nervous system infection of victims of near-drowning incidents in polluted waters.[10,15] The syndrome affects previously healthy individuals and generally is manifested within days to weeks of the incident, although presentation has been delayed up to nearly 5 months.[15] Dissemination from the lungs to a range of anatomic sites has been reported, but the syndrome is most commonly characterized by the formation of brain abscesses.[10,18] Although *P. boydii* is the most common mold associated with near-drowning–related infections, *Aspergillus* can also cause a similar syndrome[33]; in contrast, *S. prolificans* has not been associated with near-drowning.[15] Unfortunately, near-drowning–associated *P. boydii* infections carry a very poor prognosis with mortality greater than 70 percent.[15]

Consistent with the respiratory tract's being the most common portal of entry, *Scedosporium* species also appear to be transient colonizers of the sinopulmonary tract. A model for the host-pathogen interaction in pulmonary scedosporiosis was proposed by Cortez and associates,[15] in which transient colonization occurs that in the setting of a normal respiratory tract is clinically insignificant. If the pulmonary tract has been altered by the presence of a fixed cavity, damage due to tuberculosis, or cystic fibrosis, persistent colonization can result.[7] The first report of this occurrence was in 1955 and involved a patient with a previous pyogenic abscess that was subsequently colonized by *P. boydii*.[16] As with *Aspergillus*, persistent colonization of dysfunctional airways with *Scedosporium* species could trigger a syndrome similar to allergic bronchopulmonary aspergillosis; however, only three such cases have been reported in the literature.[15] *S. apiospermum* appears to be a relatively common component of respiratory cultures isolated from patients with cystic fibrosis. Although a case report has described the possible cerebral dissemination of *S. apiospermum* in a patient with cystic fibrosis,[56] the clinical significance of this colonization is not entirely clear.[57] However, the presence of molds such as *Scedosporium* species may be more relevant as more cystic fibrosis patients undergo lung transplantation, as illustrated by a case report describing the death of such a patient from disseminated *S. apiospermum* infection after double-lung transplantation.[62] This report emphasizes the notion that colonization of the airway can lead to dissemination when the immune status of the patient is altered.

The role of *Scedosporium* species in mold infections of immunocompromised patients has become more apparent as an increasing number of case series and large multicenter surveillance data have become available.* *Scedosporium* is among the four most commonly isolated molds associated with invasive disease in immunocompromised patients. The rate of disseminated disease in one study was 69 percent for hematopoietic stem cell transplant patients and 53 percent for solid-organ transplant patients.[27] Risk factors and clinical manifestations parallel those caused by other filamentous molds, such as *Aspergillus* and *Fusarium* species.† Prognosis is similarly

*References 6, 27, 37, 39, 53, 54, 55.

*References 6, 15, 25, 27, 28, 37, 53, 54, 65.
†References 6, 15, 25, 27, 28, 37, 39, 53, 54, 55, 65.

poor; for example, a large multicenter case series of infections involving transplant patients found that the overall mortality was 58 percent.[27]

In general, although *Scedosporium* infection is often clinically and radiographically indistinguishable from other filamentous fungi infections in neutropenic patients, several unique, salient features are present that reflect a large amount of fungal replication in vivo. These include a high incidence of positive blood cultures, characteristic although not pathognomonic erythematous cutaneous lesions, frequent hematogenous spread to the central nervous system, and a high incidence of visual complaints because of endophthalmitis.*

Diagnosis and Therapy

Histologic findings are insufficient to distinguish *Scedosporium* from other filamentous fungi, such as the *Aspergillus* species. However, scedosporiosis is frequently characterized by a high frequency of positive blood cultures. In fact, from 1990 to 1999, *S. prolificans* was the most common filamentous fungus isolated from blood culture at one hospital in Spain.[28] In another report, 12 of 16 patients with *Scedosporium* infections had growth in blood culture, 4 of 16 had growth in respiratory tract specimens, and 3 of 16 had growth in skin biopsy specimens.[6] No specific studies evaluating β-glucan or galactomannan assays in the setting of proven *Scedosporium* infections have been reported, so the utility of these methods in detecting this species of mold is unclear.

Scedosporium species have low in vitro susceptibility to currently available antifungal drugs, a fact that certainly contributes to the dismal outcomes associated with these infections. The excellent review by Cortez and associates presented a large set of susceptibility data from a variety of isolates.[15] In general, *S. apiospermum* appears more susceptible than *S. prolificans*. The agent most active[15] against *S. apiospermum* is voriconazole (median MIC_{50} of 0.25 μg/mL), whereas amphotericin B has poor activity with an MIC_{50} of 4 μg/mL.[15] As with other molds, a number of studies have examined the activity of combinations of antifungal drugs, but none yielded particularly encouraging results.[35]

The in vitro activity of a number of azoles currently under development (albaconazole [UR-9825], isavuconazole, and ravuconazole) against *Scedosporium* species has been reported.[12,24] Of these agents, albaconazole is the most active, with geometric mean MICs toward *S. prolificans* and *S. apiospermum* of 0.35 μg/mL and 1.0 μg/mL, respectively.[12] Encouragingly, albaconazole has been shown to have efficacy superior to that of amphotericin B in a rabbit model of disseminated *S. prolificans* infection.[11]

A case series describing the treatment of scedosporiosis with voriconazole collated from the global voriconazole clinical trials database suggested that voriconazole has promising activity against both *S. apiospermum* and *S. prolificans*.[62] In this uncontrolled, observational series, an overall response to therapy rate was 57 percent and overall mortality was 40 percent. Although it is difficult to make conclusions from this study, these rates are much better than those reported in previous case series.*

Because of the resistance of this fungus to antifungal drugs, immune function and surgical intervention play critical roles. The prognosis is particularly grim for patients with persistent neutropenia. For example, a case series of 16 patients with *S. prolificans* infections revealed that all patients who failed to recover neutrophils died.[6] Anecdotal reports of success have been noted with the adjunctive use of granulocyte colony-stimulating factor and interferon-γ.[9,29] These immunomodulation-based approaches are similar to those applied to other mold infections, but to date, none has progressed to the state at which it can be routinely recommended.[2] Surgical resection, combined with systemic antifungal therapy, should be pursued when feasible and was reported to be successful for a near-drowning victim.[13]

In summary, *Scedosporium* infections are difficult to treat and frequently occur in the immunocompromised host. The standard of care for localized infections in an immunocompetent host is surgical excision whenever possible, particularly because these organisms are highly resistant to antifungal therapy. In the immunocompromised host, in whom disseminated disease renders this approach useless, *Scedosporium* infections are generally rapidly fatal unless immune reconstitution occurs. Voriconazole appears to be the most active antifungal drug in this setting and should be considered the agent of choice.

REFERENCES

1. Anaissie EJ, Kuchar RT, Rex JH, et al. Fusariosis associated with pathogenic species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. *Clin Infect Dis* 2001;**33**:1871–8.
2. Antachopoulos C, Katragkou A, Rouilides E. Immunotherapy for invasive mold infections. *Imuunother* 2012;**4**:107–20.
3. Arikan S, Lozano-Chiu M, Paetznick V, et al. In vitro synergy studies with caspofungin and amphotericin B against *Aspergillus* and *Fusarium* spp. *Antimicrob Agents Chemother* 2002;**46**:245–7.
4. Arora R, Gupta D, Goyal J, Kaur R. Voriconazole versus natamycin as primary treatment in fungal corneal ulcers. *Clin Exp Ophthalmol* 2010;**128**:434–40.
5. Arrese JE, Pierard-Franchimont C, Pierard GE. Fatal hyalohyphomycosis following *Fusarium* onychomycosis in an immunocompromised patient. *Am J Dermatopathol* 1996;**18**:196–8.
6. Berenguer J, Rodriguez-Tudela JL, Richard C, et al. Deep infections caused by *Scedosporium prolificans*. A report on 16 cases in Spain and a review of the literature. *Scedosporium prolificans* Spanish Study Group. *Medicine (Baltimore)* 1997;**76**:256–65.
7. Borghi E, Manca A, Montagna MT, Morace G. Chronic airway colonization by *S. apiospermum* with a fatal outcome in a patient with cystic fibrosis. *Med Mycol* 2010;**48**:S108–13.
8. Boutati EI, Anaissie EJ. *Fusarium*, a significant emerging pathogen in patients with hematologic malignancy: ten years' experience at a cancer center and implications for management. *Blood* 1997; **90**:999–1008.
9. Bouza E, Munoz P, Vega L, et al. Clinical resolution of *Scedosporium prolificans* fungemia associated with reversal of neutropenia following administration of granulocyte colony-stimulating factor. *Clin Infect Dis* 1996;**23**:192–3.

*References 6, 15, 25, 27, 28, 37, 39, 53, 54, 55, 65.

*References 6, 15, 25, 27, 28, 37, 39, 53, 54, 55, 65.

10. Buzina W, Feierl G, Haas D, et al. Lethal brain abscess due to the fungus *Scedosporium apiospermum* (teleomorph *Pseudallescheria boydii*) after a near-drowning incident: case report and review of the literature. *Med Mycol* 2006;**44**:473–7.
11. Capilla J, Yustes C, Mayayo E, et al. Efficacy of albaconazole (UR-9825) in treatment of disseminated *Scedosporium prolificans* infection in rabbits. *Antimicrob Agents Chemother* 2003;**47**:1948–51.
12. Carrillo AJ, Guarro J. In vitro activities of four novel triazoles against *Scedosporium* spp. *Antimicrob Agents Chemother* 2001;**45**: 2151–3.
13. Chakraborty A, Workman MR, Bullock PR. *Scedosporium apiospermum* brain abscess treated with surgery and voriconazole. *J Neurosurg* 2005;**103**:83–7.
14. Chang DC, Grant GB, O'Donnell K, et al. Multistate outbreak of *Fusarium* keratitis associated with use of a contact lens solution. *JAMA* 2006;**296**:953–63.
15. Cortez KJ, Roilides E, Quiroz-Telles F, et al. Infections caused by *Scedosporium* spp. *Clin Microbiol Rev* 2008;**21**:157–97.
16. Creitz S, Harris HW. Isolation of *Allescheria boydii* from sputum. *Am Rev Tuberc* 1955;**71**:126–30.
17. Dignani MC, Anaissie E. Human fusariosis. *Clin Microbiol Infect* 2004;**10**(Suppl. 1):67–75.
18. Dworzack DL, Clark RB, Borkowski WJ, et al. *Pseudallescheria boydii* brain abscess: association with near drowning and efficacy of high-dose, prolonged miconazole therapy in patients with multiple abscesses. *Medicine (Baltimore)* 1989;**68**:218–24.
19. Ellis ME, Clink H, Younge D, et al. Successful combined surgical and medical treatment of *Fusarium* infection after bone marrow transplantation. *Scand J Infect Dis* 1994;**26**:225–8.
20. Flynn JT, Meislich B, Kaiser A, et al. *Fusarium* peritonitis in a child on peritoneal dialysis: case report and review of literature. *Perit Dial Int* 1996;**16**:52–7.
21. Green WO, Adams TE. Mycetoma in the United States: a review and report of seven additional cases. *Am J Clin Pathol* 1964;**42**: 75–91.
22. Guarro J, Pujol I, Mayayo E. In vitro and in vivo experimental activities of antifungal agents against *Fusarium solani*. *Antimicrob Agents Chemother* 1999;**43**:1256–7.
23. Gueho E, DeHoog GS. Taxonomy of the medical species of *Pseudallescheria* and *Scedosporium*. *J Mycol Med* 1991;**1**:3–9.
24. Guinea J, Pelaez T, Recio S, et al. In vitro antifungal activities of isavuconazole (BAL4815), voriconazole, and fluconazole against 1,007 isolates of zygomycete, *Candida*, *Aspergillus*, *Fusarium* and *Scedosporium* species. *Antimicrob Agents Chemother* 2008;**52**:1396–400.
25. Heath CH, Slavin MA, Sorrell TC, et al. Population-based surveillance for scedosporiosis in Australia: epidemiology, disease manifestations and emergence of *Scedosporium aurantiacum* infection. *Clin Microbiol Infect* 2009;**15**:689–93.
26. Hennebert GL, Desai BG. *Lomentospora prolificans*, a new hyphomycetes from greenhouse soil. *Mycotaxon* 1974;**1**:45–50.
27. Husain S, Munoz P, Forrest G, et al. Infections due to *Scedosporium apiospermum* and *Scedosporium prolificans* in transplant recipients: clinical characteristics and impact of antifungal agent on therapy outcome. *Clin Infect Dis* 2005;**40**:89–99.
28. Idigoras P, Perez-Trallero E, Pineiro L, et al. Disseminated infection and colonization by *Scedosporium prolificans*: a review of 18 cases, 1990-1999. *Clin Infect Dis* 2000;**32**:E158–65.
29. Jabado N, Casanova JL, Haddad E, et al. Invasive pulmonary infection due to *Scedosporium apiospermum* in two children with chronic granulomatous disease. *Clin Infect Dis* 1998;**27**:1437–41.
30. Khor W-B, Aung T, Saw S-M, et al. An outbreak of *Fusarium* keratitis associated with contact lens wear in Singapore. *JAMA* 2006;**295**:2867–73.
31. Kwon-Chung KJ, Bennett JE. Pseudallescheriasis and *Scedosporium* infection. In: Kwon-Chung KJ, Bennett JE, editors. *Medical mycology*. Philadelphia: Lea & Febiger; 1992. p. 678.
32. Leeflang MM, Debets-Ossenkopp YJ, Scholten RJ, et al. Galactomannan detection for invasive aspergillosis in immunocompromised patients. *Cochrane Database Syst Rev* 2008;(4):CD007394.
33. Leroy P, Smismans A, Seute T. Invasive pulmonary and central nervous system aspergillosis after near-drowning of a child: case-report and review of the literature. *Pediatrics* 2006;**118**:e509–13.
34. Liu J-Y, Chen W-T, Bor-Sheng K, et al. Combination antifungal therapy for disseminated fusariosis in immunocompromised patients: a case report and literature review. *Med Mycol* 2011;**49**: 872–8.
35. Lortholary O, Obenga G, Biswas P, et al. International retrospective analysis of 73 cases of invasive fusariosis treated with voriconazole. *Antimicrob Agents Chemother* 2010;**54**:4446–50.
36. Lozano-Chiu M, Arikan S, Paetznick VL, et al. Treatment of murine fusariosis with SCH 56592. *Antimicrob Agents Chemother* 1999;**43**:589–91.
37. Lumaris GA, Chamilos G, Lewis RE, et al. *Scedosporium* infection in a tertiary care cancer center: a review of 25 cases from 1989-2006. *Clin Infect Dis* 2006;**43**:1580–4.
38. Lupinetti FM, Giller RH, Trigg ME. Operative treatment of *Fusarium* fungal infection of the lung. *Ann Thorac Surg* 1990;**49**: 991–2.
39. Maertens J, Lagrou K, Deweerdt H, et al. Disseminated infection by *Scedosporium prolificans*: an emerging fatality among hematology patients. Case report and review. *Ann Hematol* 2000;**79**:304–44.
40. Malloch D, Salkin IF. A new species of *Scedosporium* associated with osteomyelitis in humans. *Mycotaxon* 1984;**21**:247–55.
41. Marin J, Sanz MA, Guarro J, et al. Disseminated *Scedosporium inflatum* infection in a patient with acute myeloblastic leukemia. *Eur J Clin Microbiol Infect Dis* 1991;**10**:759–61.
42. Marr KA, Carter RA, Crippa F, et al. Epidemiology and outcome of mold infections in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2002;**34**:909–17.
43. McGinnis MR, Fader RC. Mycetoma: a contemporary concept. *Infect Dis Clin North Am* 1988;**2**:939–54.
44. Mikulska M, Furfaro E, Del Bono V, et al. Galactomannan testing might be useful for early diagnosis of fusariosis. *Diagn Microbiol Infect Dis* 2012;**72**:367–9.
45. Muhammed M, Coleman JJ, Carneiro HA, Mylonakis E. The challenge of fusariosis. *Virulence* 2011;**2**:91–6.
46. Musa MO, Al Eisa A, Halim M, et al. The spectrum of *Fusarium* infection in immunocompromised patients with haematological malignancies and in non-immunocompromised patients: a single institution experience over 10 years. *Br J Haematol* 2000;**108**: 544–8.
47. Nelson PE, Dignani MC, Anaissie EJ. Taxonomy, biology, and clinical aspects of *Fusarium* species. *Clin Microbiol Rev* 1994;**7**: 479–504.
48. Ninet B, Jan I, Bontems O, et al. Molecular identification of *Fusarium* species in onychomycoses. *Dermatology* 2005;**210**:21–5.
49. Nucci M, Anaissie. Cutaneous infection by *Fusarium* species in healthy and immunocompromised hosts: implications for diagnosis and management. *Clin Infect Dis* 2002;**35**:909–20.
50. Nucci M, Anaissie E. *Fusarium* infections in immunocompromised patients. *Clin Microbiol Rev* 2007;**20**:695–704.
51. Nucci M, Marr KA, Queiroz-Telles F, et al. *Fusarium* infection in hematopoietic stem cell transplant recipients. *Clin Infect Dis* 2004;**38**:1237–42.
52. Ostrosky-Zeichner L, Alexander BD, Kett DJ, et al. Multicenter clinical evaluation of the (1→3)β-D-glucan assay as an aid to the diagnosis of fungal infections in humans. *Clin Infect Dis* 2005; **37**:645–59.
53. Panackal AA, Marr KA. *Scedosporium /Pseudallescheria* infections. *Semin Respir Crit Care Med* 2004;**25**:171–81.
54. Park BJ, Pappas PG, Wanemuehler KA, et al. Invasive non-aspergillus mold infections in transplant recipients, United States, 2001-2006. *Emerg Infect Dis* 2011;**10**:1856–64.
55. Patterson TF, Andriole VT, Zervos MJ, et al. The epidemiology of pseudallescheriasis complicating transplantation: nosocomial and community acquired infection. *Mycoses* 1990;**30**:297–302.
56. Pihet M, Carrere J, Chabasse D. Occurrence and relevance of filamentous fungi in respiratory secretions of patients with cystic fibrosis—a review. *Med Mycol* 2009;**47**:387–97.
57. Queiroz-Telles F, McGinnis MR, Salkin I, Graybill JR. Subcutaneous mycoses. *Infect Dis Clin North Am* 2003;**17**:59–85.
58. Schell WA. New aspects of emerging fungal pathogens: a multifaceted challenge. *Clin Lab Med* 1995;**15**:365–87.
59. Summerbell RC, Krajden S, Kane J. Potted plants in hospitals as reservoirs of pathogenic fungi. *Mycopathologica* 1989;**106**: 13–22.
60. Tadros TS, Workowski KA, Siegel RJ, et al. Pathology of hyalohyphomycosis caused by *Scedosporium apiospermum* (*Pseudallescheria boydii*): an emerging mycosis. *Hum Pathol* 1998;**29**:1266–72.

61. Tosti A, Piraccini BM, Lorenzi S. Onychomycosis caused by nondermatophytic molds: clinical features and response to treatment of 59 cases. *J Am Acad Dermatol* 2000;**42**:217–24.
62. Troke P, Aguirrebengoa K, Arteaga C, et al. Treatment of scedosporiosis with voriconazole: clinical experience with 107 patients. *Antimicrob Agents Chemother* 2008;**52**:1743–50.
63. Wang H, Xiao M, Kong F, et al. Accurate and practical identification of 20 *Fusarium* species by seven-locus sequence analysis and reverse line blot hybridization, and an in vitro antifungal susceptibility study. *Antimicrob Agents Chemother* 2011;**49**:1890–5.
64. Wong Ty, Fong KS, Tan DT. Clinical and microbial spectrum of fungal keratitis in Singapore: a 5-year retrospective study. *Int Ophthalmol* 1997;**21**:127–30.
65. Wood GM, McCormack JG, Muir DB, et al. Clinical features of human infection with *Scedosporium* inflatum. *Clin Infect Dis* 1992;**14**:1027–33.

CHAPTER 207

Miscellaneous Mycoses

William J. Steinbach

The landscape of medical mycology is changing. Fungi that were once innocent or only contaminants are now emerging to lead to lethal disseminated disease in immunocompromised patients. Although we are only beginning to learn about the full implications of some of these fungi, there is also the realization that nearly all of the therapeutic evaluations in these emerging pathogens are in vitro analyses with limited case report clinical experience. Every miscellaneous mycosis and fungal opportunist cannot be listed, and the field of taxonomy is dynamic, coupled with continued reports of previously nonpathogenic fungi now infecting severely immunocompromised patients. This chapter will highlight the most relevant miscellaneous fungi causing disease. Often these diseases can be classified as infection through trauma or inoculation, such as keratitis, myectoma, or soft tissue infection or as more invasive disease such as sinusitis, peritonitis, or disseminated infection.

YEASTS

Malassezia Species

Fourteen *Malassezia* spp. have been isolated from normal and diseased human and animal skin, with *Malassezia furfur* the most common species. Most microbiologists identify *M. furfur* as the "spaghetti and meatball"–appearing yeast that causes the prototypical skin disease tinea versicolor (also known as pityriasis versicolor), characterized by hypopigmented or hyperpigmented plaques covered in scales and generally distributed over the back, chest, and neck. Evidence also suggests that seborrheic dermatitis results from a nonspecific immune response to *Malassezia* yeasts, and dandruff is linked to this yeast as well, while research continues as to the role of this yeast in atopic eczema and psoriasis. However, this yeast is also responsible for intravascular catheter-related sepsis in immunocompromised patients, particularly neonates, receiving parenteral fat emulsions for nutrition.

M. furfur is a lipophilic yeast that lacks the ability to synthesize long-chain fatty acids and requires an exogenous supply of lipids for growth, growing in culture only if C12 through C24 fatty acids are added to the medium. The great majority of the lipids in parenteral intralipid formulations are C16 and C18 fatty acids, chiefly linoleic acid and oleic acid.[41] This absolute requirement for long fatty acid chains leads to specific procedures required for isolation, conservation, and identification. Whereas *M. furfur* is obligatory lipophilic, another major species, *Malassezia pachydermatis*, does not require lipids for growth.

The first invasive disease reported with *Malassezia* spp. was a patient with chronic renal failure treated with peritoneal dialysis who had repeated episodes of "sterile" peritonitis, with high levels of cholesterol and triglyceride found in the peritoneal fluid after meals, thought to have originated from the small intestinal lymphatics. The yeast did not grow in culture until the agar was overlaid with olive oil.[70] Neonatal systemic *Malassezia* disease was first reported in 1981 in a 740-g premature infant on long-term intralipid therapy.[49]

Lipophilic *Malassezia* spp. infections (e.g., with *M. furfur*) can be divided in two major groups: the first comprising children and adults with various forms of immunosuppression, and the second involving infants on parenteral nutrition. The majority of published case reports and mini-epidemics have involved infants, children, and adults with profound immunosuppression, serious concurrent health problems, and the infusion of

total parenteral nutrition with lipid supplementation through central vascular catheters.[20] There have been numerous outbreaks in neonates involving *M. furfur*; and fewer outbreaks involving *M. pachydermatis;* it is more commonly involved in otitis externa of dogs, and one outbreak was associated with the colonized dogs of health care workers.[5] A review of 55 neonates infected with *Malassezia* spp. found that prematurity was the most common underlying condition, followed by short-gut syndrome, but that all infected infants were receiving intralipid supplementation.[34]

Adults are nearly universally colonized with *Malassezia* spp. A study of the prevalence of *M. furfur* skin colonization of infants hospitalized in the intensive care unit found that of the 361 infants studied over 1 year, 133 (36.8%) had at least one positive culture for *M. furfur.* Colonized infants in contrast to noncolonized infants, had younger mean gestational age, lower mean birth weight, a longer stay in the hospital, and more mean days use of an incubator, lamb wool, paper tape, and Op-Site tape, suggesting that hospitalization in an intensive care unit often leads to *M. furfur* colonization.[47]

The use of intravenous fat emulsions appears to alter the microenvironment of the catheter and allowed colonization and subsequent infection. Dissemination of the organism appears to be limited to the lungs, which at autopsy show lipid deposits in the arterial walls. Because of the serious underlying disease(s) of patients with *M. furfur* catheter sepsis, it is difficult to determine the exact role of the organism in the overall status of the patients.[33] In an effort to determine if intralipid contamination was the source of these outbreaks, an experimental study found that six blood culture isolates of *M. furfur* from infected patients grow well in both 10 and 20 percent parenteral fat emulsions at 35° C (95° F) for 6 days. Room temperature growth was much slower, but there was no loss of viability. The authors concluded that intralipid solution provided more optimal supplementation than vegetable oils.[46]

A review of 16 infants infected with *M. furfur* revealed most presented with classic signs of sepsis, including apnea and bradycardia, and 9 had radiographic film evidence of interstitial pneumonitis to accompany worsening pulmonary status. Abnormal laboratory tests included leukocytosis, elevated neutrophil band counts, and thrombocytopenia. Blood cultures were most commonly positive from the central catheter, with few peripheral (noncatheter) blood cultures positive and few organisms found on those positive cultures.[33]

Clinicians must maintain a high index of suspicion of *M. furfur* catheter sepsis in the appropriate clinical setting, and laboratory investigators must be prepared to provide appropriate diagnostic methods.[33] Culture of biologic fluids and biopsy specimens in selective media, as well as an increased incubation time (10 to 15 days), would increase the rate of isolation of lipophilic *Malassezia* spp. The blood of infants receiving lipid emulsions probably contains sufficient lipid to support initial growth of the organism, but would not be sufficient for routine identification. On subculturing, a few drops of olive oil placed on the agar surface will help in isolation.[33]

The diagnosis should not be overlooked when evaluating symptomatic, low-birth-weight, premature infants receiving total parenteral nutrition with lipid supplementation, because simple removal of the catheter after the identification of the causative agent can lead to clinical improvement. In fungemia, removal of the catheter is the key to therapy and appears to be the only therapy necessary for *M. furfur* catheter sepsis. Most treatments in the literature focus on topical or systemic agents for the minor skin infection and not on disseminated disease. In vitro antifungal susceptibility testing against 95 *Malassezia* spp. isolates (including 74 *M. furfur* strains) revealed excellent antifungal activity with itraconazole, voriconazole, and ketoconazole, and limited activity with fluconazole.[37] The poor response to amphotericin B is supported by the in vitro high minimum inhibitory concentration (MIC) values of this agent.[33] Catheter removal or discontinuance of the fat emulsion therapy is required, and antifungal therapy without either of these two steps has not been shown to be efficacious.

Trichosporon *Species*

Fungemia caused by *Trichosporon* spp. is increasing in patients with hematologic malignancies and neutropenia and has been reported as the second most common yeast infection in cancer patients after *Candida* spp.[29] One study showed 10 percent of all fungemias at one institution were caused by *Trichosporon* spp.[29] *Trichosporon* spp. are able to colonize and proliferate in various parts of the human body, including the gastrointestinal system, respiratory tract, skin, and vagina. More than 10 percent of people have been shown to be colonized by *Trichosporon* spp. on their normal perigenital skin (i.e., scrotal, perianal, and inguinal sites of the body).[7] The opportunistic *Trichosporon* spp. are therefore part of the cutaneous fungal microbiota in humans, and this cutaneous position may be one of the routes through which deep-seated trichosporonosis is acquired. *Trichosporon* spp. may cause deep-seated, mucosa-associated, or superficial, infections, with invasive trichosporonosis documented in patients with hematologic malignancies and other immunosuppressive states, whereas superficial infections and allergic pneumonia are found predominantly in immunocompetent hosts.[7]

The taxonomy of *Trichosporon* has been revised several times. The most frequently reported species are *Trichosporon asahii* (formerly *Trichosporon beigelii* and *Trichosporon cutaneum*), *Trichosporon pullulans*, and *Trichosporon capitatum* (formerly *Blastoschizomyces capitatus*).[38] Culture identification of *Trichosporon* spp. may be difficult; it is often morphologically confused with *Candida* spp., which is worrisome because *Trichosporon* organisms have decreased susceptibility to amphotericin B.

The clinical presentation of superficial *Trichosporon* infection in humans is often benign superficial lesions of hair, called white piedra, characterized by the presence of irregular nodules on the affected hair. These nodules are loosely attached to the hair shaft, have a soft texture, and may be white or light brown. White piedra is a disease affecting children and adults from areas with tropical and temperate climates, and most cases have been reported

in children and young adults, particularly females who frequently use headbands.[7]

The first case of invasive trichosporonosis was described in 1970.[72] Clinically, the genus often causes catheter-related fungemia, but there are reports of endocarditis,[6] peritonitis,[31] meningitis,[2] and pneumonitis.[58] Infections with invasive *Trichosporon* spp. usually are associated with central venous catheters, bladder catheters, and peritoneal catheter-related devices. The ability to form biofilms on implanted devices aids in the development of invasive trichosporonosis, because it can promote escape from antifungals and host immune responses. Similar to biofilms of *Candida* spp., *T. asahii* cells are able to rapidly adhere to polystyrene after a 30-minute incubation.[10]

A multicenter retrospective Italian study was conducted to characterize cases of proved or probable invasive trichosporonosis diagnosed over the past 20 years in patients with hematologic diseases. Of 17 cases classified as *Trichosporon* spp. infections, acute myeloid leukemia accounted for 65.4 percent of the cases, leading to an incidence of 0.4 percent of *Trichosporon* spp. in patients with acute leukemia, including 76.9 percent of cases with a positive blood culture. Pulmonary involvement was documented in 26.9 percent of cases, and death was reported for 64.7 percent of *Trichosporon* spp. infections. A literature review on 287 trichosporonosis published cases with any underlying disease revealed *Trichosporon* spp. infections are seen with similar frequencies on all continents. The majority of published *Trichosporon* spp. infections occurred in patients with hematologic diseases (62.8%), with more than half having acute leukemia (68% of all patients with *Trichosporon* spp.). Crude mortality rates were 77 percent for *Trichosporon* spp.[23] One study of 39 *Trichosporon* clinical isolates showed high MICs for amphotericin B, with fluconazole and itraconazole relatively lower. Posaconazole and voriconazole appeared to be more active than amphotericin B or fluconazole and have activity similar to that of itraconazole. Fungicidal effects were also possible with the triazoles.[42] An in vitro study with 8 *Trichosporon* spp. isolates showed voriconazole was most effective and slightly more active than itraconazole, and amphotericin B, and fluconazole.[65] Another study demonstrated that voriconazole showed excellent activity, superior to that of amphotericin B, itraconazole, and fluconazole.[15] In vitro activity against several *Trichosporon* spp. showed voriconazole to be far superior to amphotericin B, fluconazole, and itraconazole. Notably, *T. pullulans* was generally more resistant to antifungals, including total resistance to amphotericin B and fluconazole.[36] In vitro studies against *T. beigelii* showed the echinocandins caspofungin and anidulafungin to have no activity.[14] This inactivity was validated in further studies with micafungin[63] and anidulafungin.[66] Invasive *T. beigelii* infection has also developed in a bone marrow transplant patient who was receiving caspofungin prophylaxis against *Aspergillus* infection.[24]

Trichosporon spp. have limited susceptibility to amphotericin B, and earlier clinical studies and animal models showed azoles were effective therapy[3] and azoles plus polyenes were potentially additive.[4] In one study, 5/12 reported cases occurred while the patient was on itraconazole prophylaxis and 11/12 patients died despite amphotericin B therapy.[29] The mortality of hematogenous trichosporonisis was higher than that of candidiasis in that study, confirmed by a review of the published literature in the last 20 years showing *Trichosporon* spp. mortality of 64 to 80 percent[29] and central venous catheters the most frequent risk factor for fungemia.

Girmenia and colleagues[23] reported the clinical outcomes for 55 patients with hematologic diseases and disseminated trichosporonosis who were treated with amphotericin B. A clinical response to amphotericin B was documented in only 13/55 (24%) of the patients evaluated. Echinocandins alone have little to no activity against *Trichosporon* spp. and are not recommended for trichosporonosis treatment. Indeed, breakthrough trichosporonosis in immunocompromised patients after administration of amphotericin B and echinocandins, and more rarely after the use of triazoles, has been extensively reported.[7]

Rhodotorula mucilaginosa

Rhodotorula mucilaginosa (formerly *Rhodotorula rubra*) is considered ubiquitous because of its worldwide distribution in terrestrial, freshwater, and marine habitats, and to its ability to colonize a large variety of substrates. *Rhodotorula* is naturally found in soil, water, and plants and is a constituent of the normal human respiratory, gastrointestinal, and genitourinary flora.[56] Previously this organism was considered only a harmless colonizer when isolated from healthy individuals, but it is now reported in immunocompromised patients, causing fungemia, endocarditis, meningitis, and peritonitis.[28] This is also the fungus that is chiefly responsible for the pink ring left in toilet bowls.

In vitro testing of 35 clinical samples of *Rhodotorula* spp. showed low MICs against all agents tested, including amphotericin B, ketoconazole, fluorocytosine, and itraconazole. The azoles were generally less effective than amphotericin B, and fluconazole showed an MIC greater than 32 μg/mL against all isolates.[21] In vitro amphotericin B is slightly superior to voriconazole and itraconazole, with general fluconazole resistance.[15]

In review of all 43 reported cases from 1960 to 2000, the most common risk factor was central venous catheter insertion. It appears that the yeast is already an inhabitant of the skin and mucosa and is introduced into the bloodstream after a disruption of normal anatomic barriers. In contrast to *Trichosporon*, mortality with *Rhodotorula* is uncommon. In a review, 10/36 survivors of infection were not treated with antifungals and appeared to respond to removal of the infected catheter.[56] This yeast has a strong affinity for plastic,[1] and almost all published cases involve long-term catheter placement. One single series reported 23 cases of fungemia; only 2 of the patients were neutropenic, and outcome was favorable in all using various treatment strategies of line removal alone, removal with amphotericin B, or antifungal therapy alone.[28]

A review of 29 cases of *Rhodotorula* fungemia in Spain found that the most common underlying hematologic disorder was acute leukemia (65.5%). *R. mucilaginosa* was the species found more frequently (79.3%), and the

most common predisposing factors were the presence of central venous catheter (100%) and neutropenia (62.1%). Most patients (81.5%) were treated with amphotericin B, and the overall mortality was higher (13.8%) than that described in patients with nonhematologic disease (5.8% in solid-organ neoplasms and 9% in acquired immunodeficiency syndrome [AIDS] or other chronic diseases). Patients with acute leukemia had a higher mortality rate (15.7%) than patients with non-Hodgkin lymphoma (0%).[22]

A review of 25 Brazilian cases of *Rhodotorula* spp. isolated from blood cultures found that the majority of patients (88%) had a central venous catheter and 40 percent were bone marrow transplant recipients; 80 percent of cases were classified as a bloodstream infection. Amphotericin B was the most common antifungal used; the catheter was removed in 89.5 percent of the patients, and death occurred in 4 patients (17.4%). All strains were identified as *R. mucilaginosa* and amphotericin B demonstrated good in vitro activity, and fluconazole had no activity.[8] Because of *Rhodotorula* spp. resistance to fluconazole and echinocandin antifungal agents, patients receiving fluconazole and caspofungin might be susceptible to the development of breakthrough *Rhodotorula* fungemia.[32]

Penicillium marneffei

Penicillium marneffei, the only dimorphic and human pathogenic *Penicillium* spp., is endemic in tropical Asia, especially Thailand, China, and Vietnam. It has emerged as the third most common AIDS-defining illness in parts of Southeast Asia.[26] Incidence of this disease has increased markedly after the first reported natural infection in 1973.[11] From approximately 30 cases from 1973 to 1990, this infection has risen to more than 160 reported cases by 1995.[13] In approximately 85 percent of cases, disseminated disease can begin as numerous papules with central necrosis and all on the face and neck (Fig. 207-1).[68] Lesions also can appear on the upper extremities, trunk, and lower extremities. The disease can present with nonproductive cough, generalized lymphadenopathy, fever, anemia, and weight loss.[13] Approximately half of the cases involve fungemia.[13] *P. marneffei* can mimic and often be misdiagnosed as other infections such as tuberculosis, *Pneumocystis jiroveci* pneumonia, cryptococcosis, and histoplasmosis. Most patients with human immunodeficiency virus (HIV) have a low $CD4^+$ count. *P. marneffei* has temperature-dependent dimorphic growth; below 37° C (98.6° F) it grows as mycelia (mold), with the formation of septate hyphae and bearing conidia, but at 37° C it grows in a yeastlike form. *P. marneffei* is the only dimorphic *Penicillium* spp., and the conidia in the environment likely enter through the respiratory tract and convert to the yeast form; pulmonary alveolar macrophages appear to be the primary pulmonary host defense.

An in vitro study of 30 isolates revealed susceptibility to itraconazole and ketoconazole, and intermediate activity to amphotericin B and fluconazole.[26] Two comparative in vitro studies showed that terbinafine and itraconazole had similar activity[36] and itraconazole and voriconazole possessed identical activity.[48] In another study, posaconazole showed better in vitro activity than itraconazole and amphotericin B against four isolates.[64] Micafungin showed the best in vitro activity in contrast to fluconazole, itraconazole, miconazole, and amphotericin B.[67]

Amphotericin B has been used as successful initial management of disseminated penicilliosis in immunocompromised patients, but it requires prolonged intravenous therapy and relapse is common. Amphotericin B followed by itraconazole is also effective (97.3%),[59] but itraconazole takes an average of 57 days to clear fungemia.[60] A review of 74 clinical isolates showed itraconazole generally possessed the best in vitro activity over amphotericin B, fluconazole, and ketoconazole and is generally recommended for treatment.[26]

The increasing incidence of *P. marneffei* appears to be linked to the HIV pandemic. Unlike some other emerging fungal pathogens, the infection remains sensitive to many antifungals. For severe cases, initial induction treatment with amphotericin B is necessary for 2 weeks, followed by an oral azole, such as itraconazole, for the next 10 weeks.[69] Although itraconazole is presently the azole of choice, voriconazole can be effective for chronic suppressive therapy.

FIGURE 207-1 ■ Skin lesions from *Penicillium marneffei*. (Courtesy S. Chiewchanvit, provided by http://www.doctorfungus.org.)

MOLDS

Phaeohyphomycosis: *Cladophialophora bantiana, Bipolaris* species, and Others

Phaeohyphomycosis is a heterogeneous group of fungal diseases characterized by dematiaceous (darkly pigmented or black) hyphal forms in tissue involving the skin, subcutaneous and deep tissue, and central nervous system (CNS). The characteristic color of the hyphae is related to the presence of melanin in the fungal cell wall, which likely plays a role in fungal pathogenesis.[40] *Phaeo* comes from Greek, meaning "dark," and has been commonly used, particularly when describing infections caused by these fungi as phaeohyphomycosis. It has been suggested that the term *dematiaceous* is not appropriate given its etymologic derivation from the Greek word *deme*, meaning bundle, although it has become fairly entrenched

in medical mycology literature. The term *melanized* has become more recently used, given its specific meaning.[50] Phaeohyphomycosis has been attributed to more than 150 species of fungi, spanning several orders in the kingdom Fungi. Among the most prevalent causes of human infection are *Bipolaris* spp., *Cladophialophora bantania* (previously *Xylohypha bantania*), *Ochroconis gallopava* (previously *Dactylaria gallopava*), *Alternaria* spp., *Wangiella dermatitidis* (previously *Exophiala dermatitidis*), *Phialophora* spp., and *Curvularia* spp.[40]

C. bantiana is the etiology for the majority of CNS phaeohyphomycosis, including patients who may have no apparent immunosuppression.[12] CNS disease commonly presents with a headache and a focal neurologic deficit, frequently a hemiparesis. *Bipolaris* spp. are the most common cause of phaeomycotic sinusitis, but also may cause pneumonia, fungemia, and disseminated infections.[71] Diagnosis of phaeohyphomycosis is primarily based on histopathologic findings in affected tissue and corresponding culture.

In vitro studies with *C. bantiana* show similar low MICs for voriconazole, itraconazole, and amphotericin B.[17] One study showed posaconazole had activity slightly worse than that of itraconazole but much better than with either anidulafungin or caspofungin.[15] Another study showed itraconazole MICs better than those of voriconazole against seven isolates[48]; in another set of experiments, voriconazole had in vitro activity superior to that of itraconazole and amphotericin B.[27] The in vitro activity of drugs against *Bipolaris* spp. can give mixed results. Amphotericin B possessed better activity than voriconazole and itraconazole against *Bipolaris hawaiiensis* and *Bipolaris spicifera*.[17] However, in another study, voriconazole showed the best in vitro activity in contrast to amphotericin B, fluconazole, and itraconazole.[36] In contrast, a study with six *Bipolaris* spp. isolates showed itraconazole with the best activity, followed closely by voriconazole and amphotericin B[15] and another study with 43 isolates showed itraconazole had great in vitro activity.[35] In the evaluation of the echinocandins, itraconazole retained the best activity, with both voriconazole and posaconazole displaying good activity, and all were far better than caspofungin or anidulafungin.[14] Comparing all available triazoles, posaconazole had the most activity, followed by itraconazole, and lastly voriconazole.[45]

Including only culture-positive cases of CNS phaeohyphomycosis, the survival rate for this infection is only 35 percent.[12] CNS lesions may be best managed surgically, and a series of 30 cases revealed those patients with single, encapsulated lesions did much better than those with multifocal disease. However, no patient who did not undergo surgery survived. Whether patients underwent neurosurgery alone or with antifungal therapy, the most important factor for cure was resectability of the lesion; antifungal therapy itself was not associated with improved survival.

CNS phaeohyphomycosis continues to possess a high mortality rate without surgical resection, and this may be the case for all localized phaeohyphomycosis. The extended-spectrum triazoles such as voriconazole and posaconazole show good in vitro activity against *C. bantiana*, *Bipolaris* spp., and several other dematiaceous fungi with some positive correlating clinical experience. Although the triazoles are probably more effective, the echinocandins do have some limited activity, and these agents will likely be tried in refractory cases as combination therapy. A review of 101 cases of culture-proved CNS phaeohyphomycosis found that the most frequently isolated species was *C. bantiana*, followed by *Ramichloridium mackenziei*, which is seen exclusively in patients from the Middle East (Fig. 207-2). More than half of the cases occurred in patients with no known underlying immunodeficiency. Mortality rates were high, and therapy, although not standardized, was best with the combination of amphotericin B, flucytosine, and itraconazole. Complete excision of brain lesions may provide better results than simple aspiration, underscoring that a very aggressive medical and surgical approach is needed.[51]

FIGURE 207-2 ■ Brain computed tomography showing six enhancing lesions at initial diagnosis.

Paecilomyces *Species*

In contrast to phaeohyphomycosis, hyalohyphomycosis refers to infections caused by colorless (nonpigmented, nonmelanized) septate fungal hyphae in affected tissue. The major hyalohyphomycotic pathogens are *Fusarium* spp. and *Scedosporium* spp. (both covered in Chapter 206), *Paecilomyces* spp., and *Trichoderma* spp. These four species are often misidentified as *Aspergillus* spp., but they can be differentiated by their conidia and phialide morphologies.[40]

Paecilomyces spp. are common soil saprophytes and can be recovered from the air because they are resistant to common methods of sterilization.[40] The two most

common species associated with clinical infection are *Paecilomyces lilacinus* and *Paecilomyces variotii*. They are often confused with the *Penicillium* spp. and clinically difficult to identify because their presentation can mimic better-known fungi such as dematiaceous fungi and certain hyalohyphomycoses.[55] They can cause keratitis, endophthalmitis, and subcutaneous infections in the healthy host. In immunocompromised patients they can manifest as cutaneous disease, catheter-related fungemia, sinusitis, and disseminated disease. Many immunocompromised patients obtain infection by integument breaks with intravascular catheters or chemotherapy.[44] In fact, contaminated skin lotion has caused a nosocomial outbreak of infection in a bone marrow transplant unit.[61] Of the approximately 160 cases of invasive *Paecilomyces* spp. infection reported,[43] approximately 50 percent are keratitis or endophthalmitis.

Fungemia can be seen, similar to *Fusarium* spp. and *Acremonium* spp. When *Fusarium* spp., *Paecilomyces* spp., and *Acremonium* spp. invade tissue they produce hyphae and adventitious structures similar to microconidia that hematogenously disseminate and are discovered on blood culture.[30] *Aspergillus* does not do this, except *Aspergillus terreus*, which can elaborate lateral conidia in vitro and in vivo.[44]

Amphotericin B is very potent against *P. variotii* but has poor in vitro activity against the most common species, *P. lilacinus*. Voriconazole MICs are much lower than itraconazole against *P. lilacinus*, including superior fungicidal activity[17] which was confirmed in another study, but the reverse was true for *P. variotii*, with itraconazole activity better than voriconazole.[48] In a study with posaconazole, it was found to have activity similar to that of itraconazole and better than that of amphotericin B against *P. variotii*. Posaconazole displayed significantly better activity against *P. lilacinus* than itraconazole, and amphotericin B showed no activity against that species.[64] Comparing all triazoles, posaconazole had the most activity, followed by itraconazole, ravuconazole, and finally voriconazole.[45] Caspofungin was inactive in vitro against *P. lilacinus*, but showed excellent activity against *P. variotii*.[9] On the other hand, micafungin showed good in vitro activity against *P. lilacinus* and *P. variotii*, and better than fluconazole, itraconazole, miconazole, and amphotericin B.[67]

In an immunosuppressed murine model of disseminated infection with *P. lilacinus*, voriconazole significantly prolonged survival with respect to the group treated with amphotericin B, reducing the fungal load in the spleen, kidneys, and liver of infected mice. Survival of mice treated with amphotericin B did not differ from that of the control group, and amphotericin B was not able to reduce the tissue burden in any organ with respect to the control group.[54] In another murine model of paecilomycosis, posaconazole was superior to amphotericin B or liposomal amphotericin B and was the only treatment able to significantly reduce fungal loads in the spleen, kidneys, and liver of the mice infected by each of the two strains.[53]

The cornerstone of therapy has been surgical resection and removal of any foreign object. In vitro data suggest the triazoles might be effective treatment for *Paecilomyces* infection, but large datasets of clinical experience are lacking to support the recommendation and it remains even less clear whether the echinocandins have any place in the management of these infections.

Trichoderma longibachiatum

Trichoderma spp. are a ubiquitous component of soil microflora with five species of the genus *Trichoderma* identified as etiologic agents of infection in immunocompromised hosts: *T. longibrachiatum*, *T. harzianum*, *T. koningii*, *T. pseudokoningii*, and *T. viride*.[52] *T. longibrachiatum* causes virtually all human infections, similar to *P. marneffei* as virtually the only pathogenic *Penicillium* spp. and has been reported to cause pulmonary, cerebral, soft tissue, and disseminated disease in immunocompromised patients. It has been suggested that *T. longibrachiatum* can be acquired through the gastrointestinal tract, and prolonged therapy with fluconazole and antibacterial agents may selectively favor this filamentous fungus.[52] Another likely source may be aerosols or contaminated water.[40]

Itraconazole displays the best in vitro activity amongst older antifungals.[19,39,57,62] Other studies showed itraconazole with no activity, and amphotericin B had better activity than voriconazole.[16] However, amphotericin B resistance is uniform in reported clinical cases. The first case of *Trichoderma* infection in an immunocompromised patient was reported in 1976, and a review lists only 10 cases reported in immunocompromised hosts.[52]

Trichoderma infections appear to be slowly increasing in immunocompromised patients. Extended-spectrum triazoles should be considered over the polyenes for treatment, but recovery from immunosuppression appears crucial to success. Stool surveillance cultures for early identification of this fungus remain unproved for clinical management, but may play a role with future studies.

Acremonium *Species*

Acremonium spp. are hyalohyphomycoses, mycoses caused by filamentous fungi that have colorless septate hyphae in tissue. They are common in soil, plant debris, and rotting mushrooms. *Acremonium kiliense* is the most important among the 10 species reported as infectious etiologies in vertebrates. The immunocompetent host can form a mycetoma or develop an ocular infection after sustaining a penetrating injury.[25] *Acremonium* spp. can lead to a wide range of infections from keratitis to disseminated disease, with the lung and gastrointestinal tract as apparent portals of entry.[71]

The first case of *Acremonium* spp. infection other than mycetoma or ocular infection was described in 1932, but most have arisen since 1985.[25] The largest review of 36 localized or disseminated cases showed the majority of disseminated cases occurred in immunocompromised patients and only approximately half could be cured, with complete resolution of disease dependent on neutrophil recovery.[25] Recognition of disseminated *Acremonium* infection is difficult because specific signs and symptoms do not exist. Patients may present with a cutaneous nodule, papular skin rash, or fungemia.

In one study, itraconazole had no activity and voriconazole had a marginally effective MIC for *A. kiliense*.[48] Another study showed voriconazole with better in vitro activity than amphotericin B, fluconazole, and itraconazole for *Acremonium alabamensis* and *Acremonium strictum*.[36] Anidulafungin has no activity against *Acremonium* spp., but posaconazole and caspofungin both possess some fungistatic activity against *A. strictum*.[14] A more recent study showed voriconazole and ravuconazole with similar activity and superior to posaconazole, and the strains tested were resistant to amphotericin B and itraconazole.[45]

The rarity of *Acremonium* infections makes it difficult to adequately assess the best treatment regimen. Amphotericin B was previously recommended as initial therapy[18] and despite the in vitro resistance to azole treatment, the combination of surgery and azoles has often been successful.[25] Voriconazole appears to have the greatest in vitro activity against *Acremonium*, but clinical reports of its effectiveness are lacking. Similar to other fungal pathogens, the status of the host defense system is crucial. Acremonium infections are still rare but deadly, and the limited amount of clinical experience makes firm recommendations difficult. The echinocandins appear to have limited activity, whereas the newer triazoles may be beneficial adjunctive therapy in concert with boosting the host immune response.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Alliot C, Desablens B, Garidi R, et al. Opportunistic infection with *Rodotorula* in cancer patients treated with chemotherapy: two case reports. *Clin Oncol* 2000;**12**:115–17.
6. Chaumentin G, Boibieux A, Piens MA, et al. Trichosporon inkin endocarditis: short-term evolution and clinical report. *Clin Infect Dis* 1996;**23**:396–7.
7. Colombo AL, Padovan AC, Chaves GM. Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clin Microbiol Rev* 2011; **24**:682–700.
8. De Almeida GM, Costa SF, Melhem M, et al. *Rhodotorula* spp. isolated from blood cultures: clinical and microbiological aspects. *Med Mycol* 2008;**46**:547–56.
9. Del Poeta M, Schell WA, Perfect JR. In vitro activity of pneumocandin L-743-872 against a variety of clinically important molds. *Antimicrob Agents Chemother* 1997;**41**:1835–6.
10. Di Bonaventura G, Pompilio A, Picciani C, et al. Biofilm formation by the emerging fungal pathogen *Trichosporon asahii:* development, architecture, and antifungal resistance. *Antimicrob Agents Chemother* 2006;**50**:3269–76.
11. DiSalvo AF, Fickling AM, Ajello L. Infection caused by *Penicillium marneffei:* description of first natural infection in man. *Am J Clin Pathol* 1973;**60**:259–63.
12. Dixon DM, Walsh TJ, Merz WG, et al. Infections due to *Xylohypha bantiana* (*Cladosporium trichoides*). *Rev Infect Dis* 1989;**11**: 515–25.
14. Espinel-Ingroff A. Comparison of in vitro activities of the new triazole SCH56592 and the echinocandins MK-0991 (L-743,872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. *J Clin Microbiol* 1998;**36**:2950–6.
16. Espinel-Ingroff A. Comparison of the E-test with NCCLS M38-P method for antifungal susceptibility testing of common and emerging pathogenic filamentous fungi. *J Clin Microbiol* 2001;**39**: 1360–7.
17. Espinel-Ingroff A. In vitro fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dermatiaceous fungi. *J Clin Microbiol* 2001;**39**:954–8.
19. Furukawa H, Kusne S, Sutton DA, et al. Acute invasive sinusitis due to *Trichoderma longibrachiatum* in a liver and small bowel transplant recipient. *Clin Infect Dis* 1998;**26**:487–9.
20. Gaitanis G, Magiatis P, Hantschke M, et al. The *Malassezia* genus in skin and systemic diseases. *Clin Microbiol Rev* 2012;**25**: 106–41.
21. Galan-Sanchez F, Garcia-Martos P, Rodriguez-Ramos C, et al. Microbiological characteristics and susceptibility patterns of strains of *Rhodotorula* isolated from clinical samples. *Mycopathologia* 1999; **145**:109–12.
22. García-Suárez J, Gómez-Herruz P, Cuadros JA, et al. Epidemiology and outcome of *Rhodotorula* infection in haematological patients. *Mycoses* 2011;**54**:318–24.
26. Imwidthaya P, Thipsuvan K, Chaiprasert A, et al. *Penicillium marneffei:* types and drug susceptibility. *Mycopathologia* 2000;**149**: 109–15.
27. Johnson EM, Szekely A, Warnock DW. In-vitro activity of voriconazole, itraconazole and amphotericin B against filamentous fungi. *J Antimicrob Chemother* 1998;**42**:741–5.
29. Kremery VJ, Mateicka F, Kunova A, et al. Hematogenous trichosporonosis in cancer patients: report of 12 cases including 5 during prophylaxis with itraconazole. *Support Care Cancer* 1999;**7**:39–43.
31. Lopes JO, Alves SH, Klock C, et al. *Trichosporon inkin* peritonitis during continuous ambulatory peritoneal dialysis with bibliography review. *Mycopathologia* 1997;**139**:15–18.
32. Lunardi LW, Aquino VR, Zimerman RA, et al. Epidemiology and outcome of *Rhodotorula fungemia* in a tertiary care hospital. *Clin Infect Dis* 2006;**43**:e60–3.
35. McGinnis MR, Nordoff NG, Ryder NS, et al. In vitro comparison of terbinafine and itraconazole against *Penicillium marneffei*. *Antimicrob Agents Chemother* 2000;**44**:1407–8.
37. Miranda KC, de Araujo CR, Costa CR, et al. Antifungal activities of azole agents against the *Malassezia* species. *Int J Antimicrob Agents* 2007;**29**:281–4.
38. Moretti-Branchini ML, Fukushima K, Schreiber AZ, et al. *Trichosporon* species infection in bone marrow transplanted recipients. *Diagn Microbiol Infect Dis* 2001;**39**:161–4.
39. Munoz FM, Demmler GJ, Travis WR, et al. *Trichoderma longibrachiatum* infection in a pediatric patient with aplastic anemia. *J Clin Microbiol* 1997;**35**:499–503.
40. Naggie S, Perfect JR. Molds: Hyalohyphomycosis, phaeohyphomycosis, and zygomycosis. *Clin Chest Med* 2009;**30**:337–53.
41. Nazzaro Porro M, Passi S, Caprilli F, et al. Growth requirements and lipid metabolism of *Pityrosporum orbiculare*. *J Invest Dermatol* 1976;**66**:178–82.
42. Paphitou NI, Ostrosky-Zeichner L, Paetznick V, et al. In vitro antifungal susceptibilities of *Trichosporon* species. *Antimicrob Agents Chemother* 2002;**46**:1144–6.
45. Pfaller MA, Messer SA, Hollis RJ, et al. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY antimicrobial surveillance program, 2000. *Antimicrob Agents Chemother* 2002;**46**:1032–7.
46. Powell DA, Durrell DE, Marcon MJ. Growth of *Malassezia furfur* in parenteral fat emulsions. *J Infect Dis* 1986;**153**:640–1.
48. Radford SA, Johnson EM, Warnock DW. In vitro studies of activity of voriconazole (UK-109,496), a new triazole antifungal agent, against emerging and less-common mold pathogens. *Antimicrob Agents Chemother* 1997;**41**:841–3.
50. Revankar SG, Sutton DA. Melanized fungi in human disease. *Clin Microbiol Rev* 2010;**23**:884–928.
52. Richter S, Cormican MG, Pfaller MA, et al. Fatal disseminated *Trichoderma longibrachiatum* infection in an adult bone marrow transplant patient: species identification and review of the literature. *J Clin Microbiol* 1999;**37**:1154–60.
53. Rodríguez MM, Pastor FJ, Serena C, et al. Posaconazole efficacy in a murine disseminated infection caused by *Paecilomyces lilacinus*. *Int J Antimicrob Agents* 2009;**63**:361–4.
54. Rodríguez MM, Pastor FJ, Serena C, et al. Efficacy of voriconazole in a murine model of invasive paecilomycosis. *Int J Antimicrob Agents* 2010;**35**:362–5.
56. Samonis G, Anatoliotaki M, Apostolakou H, et al. Transient fungemia due to *Rhodotorula rubra* in a cancer patient: case report and review of the literature. *Infection* 2001;**29**:173–6.
57. Sequin P, Degeilh B, Grulois I, et al. Successful treatment of a brain abscess due to *Trichoderma longibrachiatum* after surgical resection. *Eur J Clin Microbiol Infect Dis* 1995;**14**:445–8.

58. Shimazu K, Ando M, Sakata T, et al. Hypersensitivity pneumonitis induced by *Trichosporon cutaneum*. *Am Rev Resp Dis* 1984;**130**: 407–11.
62. Tanis BC, van der Pijl H, van Ogtrop ML, et al. Fatal fungal peritonitis by *Trichoderma longibrachiatum* complicating peritoneal dialysis. *Nephrol Dial Transplant* 1995;**10**:114–16.
63. Tawara S, Ikeda F, Maki K, et al. In vitro activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi. *Antimicrob Agents Chemother* 2000;**44**:57–62.
64. Uchida K, Yokota N, Yamaguchi H. In vitro antifungal activity of posaconazole against various pathogenic fungi. *Int J Antimicrob Agents* 2001;**18**:167–72.
65. Uzun O, Arikan S, Kocagoz S, et al. Susceptibility testing of voriconazole, fluconazole, itraconazole and amphotericin B against yeast isolates in a Turkish University hospital and effect of time of reading. *Diagn Microbiol Infect Dis* 2000;**38**:101–7.
66. Uzun O, Kocagoz S, Cetinkaya Y, et al. In vitro activity of a new echinocandin, LY303366, compared with those of amphotericin B and fluconazole against clinical yeast isolates. *Antimicrob Agents Chemother* 1997;**41**:1156–7.
67. Uchida K, Nishiyama Y, Yokota N, et al. In vitro antifungal activity of a novel lipopeptide antifungal agent, FK463, against various fungal pathogens. *J Antibiot (Tokyo)* 2000;**53**:1175–81.
68. Vanittanakom N, Sirisanthana T. Penicillium marneffei infection in patients infected with human immunodeficiency virus. *Curr Top Med Mycol* 1997;**8**:35–42.
70. Wallace M, Bagnall H, Glen D, et al. Isolation of lipophilic yeast in "sterile" peritonitis. *Lancet* 1979;**2**:956.
71. Walsh TJ, Groll AH. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transplant Infect Dis* 1999;**1**:247–61.
72. Watson KC, Kallichurum S. Brain abscess due to *Trichosporon cutaneum*. *J Med Microbiol* 1970;**3**:191–3.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alliot C, Desablens B, Garidi R, et al. Opportunistic infection with *Rodotorula* in cancer patients treated with chemotherapy: two case reports. *Clin Oncol* 2000;**12**:115–17.
2. Anaissie E, Bodey G. Disseminated trichosporonosis: meeting the challenge. *Eur J Clin Microbiol Infect Dis* 1991;**10**:711–13.
3. Anaissie E, Gokaslan A, Hachem R, et al. Azole therapy for trichosporonosis: clinical evaluation of eight patients, experimental therapy for murine infection, and review. *Clin Infect Dis* 1992;**15**: 781–7.
4. Anaissie EJ, Hachem R, Karyotakis NC, et al. Comparative efficacies of amphotericin B, triazoles, and combination of both as experimental therapy for murine trichosporonosis. *Antimicrob Agents Chemother* 1994;**38**:2541–4.
5. Chang HJ, Miller HL, Watkins N, et al. An epidemic of *Malassezia pachydermatis* in an intensive care nursery associated with colonization of health care workers' pet dogs. *N Engl J Med* 1998;**338**: 706–11.
6. Chaumentin G, Boibieux A, Piens MA, et al. *Trichosporon inkin* endocarditis: short-term evolution and clinical report. *Clin Infect Dis* 1996;**23**:396–7.
7. Colombo AL, Padovan AC, Chaves GM. Current knowledge of *Trichosporon* spp. and trichosporonosis. *Clin Microbiol Rev* 2011; **24**:682–700.
8. De Almeida GM, Costa SF, Melhem M, et al. *Rhodotorula* spp. isolated from blood cultures: clinical and microbiological aspects. *Med Mycol* 2008;**46**:547–56.
9. Del Poeta M, Schell WA, Perfect JR. In vitro activity of pneumocandin L-743-872 against a variety of clinically important molds. *Antimicrob Agents Chemother* 1997;**41**:1835–6.
10. Di Bonaventura G, Pompilio A, Picciani C, et al. Biofilm formation by the emerging fungal pathogen *Trichosporon asahii:* development, architecture, and antifungal resistance. *Antimicrob Agents Chemother* 2006;**50**:3269–76.
11. DiSalvo AF, Fickling AM, Ajello L. Infection caused by *Penicillium marneffei:* description of first natural infection in man. *Am J Clin Pathol* 1973;**60**:259–63.
12. Dixon DM, Walsh TJ, Merz WG, et al. Infections due to *Xylohypha bantiana* (*Cladosporium trichoides*). *Rev Infect Dis* 1989;**11**:515–25.
13. Duong TA. Infection due to Penicillium marneffei, an emerging pathogen: review of 155 reported cases. *Clin Infect Dis* 1996;**23**: 125–30.
14. Espinel-Ingroff A. Comparison of in vitro activities of the new triazole SCH56592 and the echinocandins MK-0991 (L-743,872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. *J Clin Microbiol* 1998;**36**:2950–6.
15. Espinel-Ingroff A. In vitro activity of the new triazole voriconazole (UK-109,496) against opportunistic filamentous and dimorphic fungi and common and emerging yeast pathogens. *J Clin Microbiol* 1998;**36**:198–202.
16. Espinel-Ingroff A. Comparison of the E-test with NCCLS M38-P method for antifungal susceptibility testing of commonn and emerging pathogenic filamentous fungi. *J Clin Microbiol* 2001;**39**: 1360–7.
17. Espinel-Ingroff A. In vitro fungicidal activities of voriconazole, itraconazole, and amphotericin B against opportunistic moniliaceous and dermatiaceous fungi. *J Clin Microbiol* 2001;**39**:954–8.
18. Fincher R-ME, Fisher JF, Lovell RD, et al. Infection die to the fungus *Acremonium* (*Cephalosporium*). *Medicine (Baltimore)* 1991;**70**: 398–409.
19. Furukawa H, Kusne S, Sutton DA, et al. Acute invasive sinusitis due to *Trichoderma longibrachiatum* in a liver and small bowel transplant recipient. *Clin Infect Dis* 1998;**26**:487–9.
20. Gaitanis G, Magiatis P, Hantschke M, et al. The *Malassezia* genus in skin and systemic diseases. *Clin Microbiol Rev* 2012;**25**:106–41.
21. Galan-Sanchez F, Garcia-Martos P, Rodriguez-Ramos C, et al. Microbiological characteristics and susceptibility patterns of strains of *Rhodotorula* isolated from clinical samples. *Mycopathologia* 1999; **145**:109–12.
22. García-Suárez J, Gómez-Herruz P, Cuadros JA, et al. Epidemiology and outcome of *Rhodotorula* infection in haematological patients. *Mycoses* 2011;**54**:318–24.
23. Girmenia C, Pagano L, Martino B, et al. Invasive infections caused by *Trichosporon* species and *Geotrichum capitatum* in patients with hematological malignancies: a retrospective multicenter study from Italy and review of the literature. *J Clin Microbiol* 2005;**43**: 1818–28.
24. Goodman D, Pamer E, Jakubowski A, et al. Breakthrough trichosporonosis in a bone marrow transplant patient receiving caspofungin acetate. *Clin Infect Dis* 2002;**35**:e35–6.
25. Guarro J, Gams W, Pujol I, et al. *Acremonium* species: new emerging fungal opportunists: in vitro antifungal susceptibilities and review. *Clin Infect Dis* 1997;**25**:1222–9.
26. Imwidthaya P, Thipsuvan K, Chaiprasert A, et al. *Penicillium marneffei:* types and drug susceptibility. *Mycopathologia* 2000;**149**: 109–15.
27. Johnson EM, Szekely A, Warnock DW. In-vitro activity of voriconazole, itraconazole and amphotericin B against filamentous fungi. *J Antimicrob Chemother* 1998;**42**:741–5.
28. Kiehn TE, Gorey E, Brown AE, et al. Sepsis due to *Rhodotorula* related use of indwelling central venous catheters. *Clin Infect Dis* 1992;**14**:841–6.
29. Kremery VJ, Mateicka F, Kunova A, et al. Hematogenous trichosporonosis in cancer patients: report of 12 cases including 5 during prophylaxis with itraconazole. *Support Care Cancer* 1999;**7**:39–43.
30. Liu K, Howell DN, Perfect JR, et al. Morphologic criteria for the preliminary identification of *Fusarium*, *Paecilomyces* and *Acremonium* species by histopathology. *Am J Clin Pathol* 1998;**109**:45–54.
31. Lopes JO, Alves SH, Klock C, et al. *Trichosporon inkin* peritonitis during continuous ambulatory peritoneal dialysis with bibliography review. *Mycopathologia* 1997;**139**:15–18.
32. Lunardi LW, Aquino VR, Zimerman RA, et al. Epidemiology and outcome of *Rhodotorula fungemia* in a tertiary care hospital. *Clin Infect Dis* 2006;**43**:e60–3.
33. Marcon MJ, Powell DA. Epidemiology, diagnosis, and management of *Malassezia furfur* systemic infection. *Diagn Microbiol Infect Dis* 1987;**7**:161–75.
34. Marcon MJ, Powell DA. Human infections due to *Malassezia*. *Clin Microbiol Rev* 1992;**5**:101–19.
35. McGinnis MR, Nordoff NG, Ryder NS, et al. In vitro comparison of terbinafine and itraconazole against *Penicillium marneffei*. *Antimicrob Agents Chemother* 2000;**44**:1407–8.
36. McGinnis MR, Pasarell L, Sutton DA, et al. In vitro activity of voriconazole against selected fungi. *Med Mycol* 1998;**36**:239–42.
37. Miranda KC, de Araujo CR, Costa CR, et al. Antifungal activities of azole agents against the *Malassezia* species. *Int J Antimicrob Agents* 2007;**29**:281–4.
38. Moretti-Branchini ML, Fukushima K, Schreiber AZ, et al. *Trichosporon* species infection in bone marrow transplanted recipients. *Diagn Microbiol Infect Dis* 2001;**39**:161–4.
39. Munoz FM, Demmler GJ, Travis WR, et al. *Trichoderma longibrachiatum* infection in a pediatric patient with aplastic anemia. *J Clin Microbiol* 1997;**35**:499–503.
40. Naggie S, Perfect JR. Molds: Hyalohyphomycosis, phaeohyphomycosis, and zygomycosis. *Clin Chest Med* 2009;**30**:337–53.
41. Nazzaro Porro M, Passi S, Caprilli F, et al. Growth requirements and lipid metabolism of *Pityrosporum orbiculare*. *J Invest Dermatol* 1976;**66**:178–82.
42. Paphitou NI, Ostrosky-Zeichner L, Paetznick V, et al. In vitro antifungal susceptibilities of *Trichosporon* species. *Antimicrob Agents Chemother* 2002;**46**:1144–6.
43. Pastor FJ, Guarro J. Clinical manifestations, treatment, and outcome of *Paecilomyces lilacinus* infections. *Clin Microbiol Rev* 2006;**12**:948–60.
44. Perfect JR, Schell WA. The new fungal opportunists are coming. *Clin Infect Dis* 1996;**22**:S112–18.
45. Pfaller MA, Messer SA, Hollis RJ, et al. Antifungal activities of posaconazole, ravuconazole, and voriconazole compared to those of itraconazole and amphotericin B against 239 clinical isolates of *Aspergillus* spp. and other filamentous fungi: report from SENTRY antimicrobial surveillance program, 2000. *Antimicrob Agents Chemother* 2002;**46**:1032–7.
46. Powell DA, Durrell DE, Marcon MJ. Growth of *Malassezia furfur* in parenteral fat emulsions. *J Infect Dis* 1986;**153**:640–1.
47. Powell DA, Hayes J, Durrell DE, et al. *Malassezia furfur* skin colonization of infants hospitalized in intensive care units. *J Pediatr* 1987;**111**:217–20.
48. Radford SA, Johnson EM, Warnock DW. In vitro studies of activity of voriconazole (UK-109,496), a new triazole antifungal agent, against emerging and less-common mold pathogens. *Antimicrob Agents Chemother* 1997;**41**:841–3.

49. Redline RW, Dahms BB. Malassezia pulmonary vasculitis in an infant on long-term intralipid therapy. *N Engl J Med* 1981;**305**: 1395–8.
50. Revankar SG, Sutton DA. Melanized fungi in human disease. *Clin Microbiol Rev* 2010;**23**:884–928.
51. Revankar SG, Sutton DA, Rinaldi MG. Primary central nervous system phaeohyphomycosis: a review of 101 cases. *Clin Infect Dis* 2004;**38**:206–16.
52. Richter S, Cormican MG, Pfaller MA, et al. Fatal disseminated *Trichoderma longibrachiatum* infection in an adult bone marrow transplant patient: species identification and review of the literature. *J Clin Microbiol* 1999;**37**:1154–60.
53. Rodríguez MM, Pastor FJ, Serena C, et al. Posaconazole efficacy in a murine disseminated infection caused by *Paecilomyces lilacinus*. *Int J Antimicrob Agents* 2009;**63**:361–4.
54. Rodríguez MM, Pastor FJ, Serena C, et al. Efficacy of voriconazole in a murine model of invasive paecilomycosis. *Int J Antimicrob Agents* 2010;**35**:362–5.
55. Saberhagen C, Klotz SA, Bartholomew W, et al. Infection due to *Paecilomyces lilacinus:* a challenging clinical identification. *Clin Infect Dis* 1997;**25**:1411–13.
56. Samonis G, Anatoliotaki M, Apostolakou H, et al. Transient fungemia due to *Rhodotorula rubra* in a cancer patient: case report and review of the literature. *Infection* 2001;**29**:173–6.
57. Sequin P, Degeilh B, Grulois I, et al. Successful treatment of a brain abscess due to *Trichoderma longibrachiatum* after surgical resection. *Eur J Clin Microbiol Infect Dis* 1995;**14**:445–8.
58. Shimazu K, Ando M, Sakata T, et al. Hypersensitivity pneumonitis induced by *Trichosporon cutaneum*. *Am Rev Resp Dis* 1984;**130**: 407–11.
59. Sirisanthana T, Supparatpinyo K, Perriens J, et al. Amphotericin B and itraconazole for treatment of disseminated *Penicillium marneffi* infection in human immunodeficiency virus-infected patients. *Clin Infect Dis* 1998;**26**:1107–10.
60. Supparatpinyo K, Chiewchanvit S, Hirunsri P, et al. An efficacy study of itraconazole in the treatment of *Penicillium marneffei* infection. *J Med Assoc Thailand* 1992;**75**:688–91.
61. Tan TQ, Ogden AK, Tillman J, et al. *Paecilomyces lilacinus* catheter-related fungemia in an immunocompromised pediatric patient. *J Clin Microbiol* 1992;**30**:2479–83.
62. Tanis BC, van der Pijl H, van Ogtrop ML, et al. Fatal fungal peritonitis by *Trichoderma longibrachiatum* complicating peritoneal dialysis. *Nephrol Dial Transplant* 1995;**10**:114–16.
63. Tawara S, Ikeda F, Maki K, et al. In vitro activities of a new lipopeptide antifungal agent, FK463, against a variety of clinically important fungi. *Antimicrob Agents Chemother* 2000;**44**:57–62.
64. Uchida K, Yokota N, Yamaguchi H. In vitro antifungal activity of posaconazole against various pathogenic fungi. *Int J Antimicrob Agents* 2001;**18**:167–72.
65. Uzun O, Arikan S, Kocagoz S, et al. Susceptibility testing of voriconazole, fluconazole, itraconazole and amphotericin B against yeast isolates in a Turkish University hospital and effect of time of reading. *Diagn Microbiol Infect Dis* 2000;**38**:101–7.
66. Uzun O, Kocagoz S, Cetinkaya Y, et al. In vitro activity of a new echinocandin, LY303366, compared with those of amphotericin B and fluconazole against clinical yeast isolates. *Antimicrob Agents Chemotherapy* 1997;**41**:1156–7.
67. Uchida K, Nishiyama Y, Yokota N, et al. In vitro antifungal activity of a novel lipopeptide antifungal agent, FK463, against various fungal pathogens. *J Antibiot* 2000;**53**:1175–81.
68. Vanittanakom N, Sirisanthana T. *Penicillium marneffei* infection in patients infected with human immunodeficiency virus. *Curr Top Med Mycol* 1997;**8**:35–42.
69. Vanittanakom N, Cooper CR Jr, Fisher MC, et al. *Penicillium marneffei* infection and recent advances in the epidemiology and molecular biology aspects. *Clin Microbiol Rev* 2006;**19**:95–110.
70. Wallace M, Bagnall H, Glen D, et al. Isolation of lipophilic yeast in "sterile" peritonitis. *Lancet* 1979;**2**:956.
71. Walsh TJ, Groll AH. Emerging fungal pathogens: evolving challenges to immunocompromised patients for the twenty-first century. *Transpl Infect Dis* 1999;**1**:247–61.
72. Watson KC, Kallichurum S. Brain abscess due to *Trichosporon* cutaneum. *J Med Microbiol* 1970;**3**:191–3.

SECTION **XXII**

PARASITIC DISEASES

CHAPTER 208

Classification and Nomenclature of Human Parasites

Lynne S. Garcia

Although common names frequently are used to describe parasitic organisms, these names may represent different parasites in different parts of the world. To eliminate these problems, a binomial system of nomenclature in which the scientific name consists of the genus and species is used.[1-3,8,12,14,17] These names generally are of Greek or Latin origin. In certain publications, the scientific name often is followed by the name of the individual who originally named the parasite. The date of naming also may be provided. If the name of the individual is in parentheses, it means that the person used a generic name no longer considered to be correct.

On the basis of life histories and morphologic characteristics, systems of classification have been developed to indicate the relationship among the various parasite species. Closely related species are placed in the same genus, related genera in the same family, related families in the same order, related orders in the same class, and related classes in the same phylum, one of the major categories in the animal kingdom. As one moves up the classification schema, each category becomes more broad; however, each category still has characteristics in common.

Parasites of humans are classified in a number of major divisions. They include the Protozoa (amebae, flagellates, ciliates, sporozoans, and coccidia), the Fungi (microsporidia), the Platyhelminthes or flatworms (cestodes, trematodes), the Acanthocephala or thorny-headed worms, the Nematoda or roundworms, and the Arthropoda (insects, spiders, mites, ticks, and so on). Although these categories appear to be well defined, often considerable confusion occurs in attempting to classify parasitic organisms. One of the primary reasons is the lack of known specimens. Some organisms recovered from humans are very rare; thus, difficulty arises in determining morphologic and physiologic variation among such groups. Type specimens must be deposited for study before a legitimate species name can be given. Even when certain parasites are numerous, they may represent strains or races of the same species with slightly different characteristics.

In general, reproductive mechanisms are a valid concept in determining definitions of species, but so many exceptions exist within parasite groups that taking into consideration properties such as sexual reproduction, parthenogenesis, and asexual reproduction is difficult. Another difficulty in recognition of species is the ability and tendency of the organisms to alter their morphologic forms according to age, host, or nutrition, which often results in several names being given to the same organism. An additional problem involves alternation of parasitic and free-living phases in the life cycle. These organisms may be very different and difficult to recognize as belonging to the same species. Despite these difficulties, newer, more sophisticated molecular methods of grouping organisms often have confirmed taxonomic conclusions reached hundreds of years earlier by experienced taxonomists.

As investigations continue in parasitic genetics, immunology, and biochemistry, the species designation will be defined more clearly. Originally, these species designations were determined primarily by morphologic differences, resulting in a phenotypic approach. With the use of highly sophisticated molecular techniques, the approach will continue to be more genotypic. Benefits of these studies also include the development of highly specific and sensitive diagnostic tests and the ability to diagnose parasitic infections on the basis of molecular parameters rather than merely phenotypic characteristics.

Although gaps in our knowledge concerning classification of all human parasites remain, the binomial system has allowed the classification of 1.5 million species of organisms in the animal kingdom such that all published information can be retrieved, regardless of the language spoken. The difficulty for the clinician arises when one considers the rapid increase in information concerning microbiology during the past few years and changing considerations, such as the role of immunosuppression in the host-parasite interaction and the modified definitions of "normal flora" and "nonpathogenic" in this population of patients.

The classification of parasites is presented in tabular form. Although certain designations of species may be somewhat controversial, this classification scheme is designed to provide some order and meaning to a widely divergent group of organisms. No attempt has been made to include every possible organism, and only those considered clinically relevant in the context of human parasitology are listed. The main groups that are presented include protozoa, fungi, nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). Some relevant information on arthropods is presented in Tables 208-1 and 208-2. The hope is that this information will provide some insight into the parasite groupings, thus leading to a better understanding of parasitic infections and the appropriate diagnostic and clinical approach.

TABLE 208-1 **Human Vector-Borne Infections**

Infection (Disease)	Causative Agent	Vector (Common Name)
Protozoal		
Malaria	*Plasmodium* spp.	Mosquitoes
Leishmaniasis	*Leishmania* spp.	Sandflies
Chagas disease	*Trypanosoma cruzi*	Triatomid bugs
East African trypanosomiasis	*Trypanosoma brucei rhodesiense*	Tsetse flies
West African trypanosomiasis	*Trypanosoma brucei gambiense*	Tsetse flies
Babesiosis	*Babesia* spp.	Ticks
Helminthic		
Filariasis	*Wuchereria bancrofti*	Mosquitoes
Filariasis	*Brugia malayi*	Mosquitoes
Filariasis	*Dirofilaria* spp.	Mosquitoes
Filariasis	*Mansonella perstans*	Biting midges
Filariasis	*Mansonella streptocerca*	Biting midges
Filariasis	*Mansonella ozzardi*	Biting midges
Onchocerciasis	*Onchocerca volvulus*	Black flies
Loiasis	*Loa loa*	Deer flies
Dog tapeworm infection	*Dipylidium caninum*	Dog lice and fleas, human fleas
Rat tapeworm infection	*Hymenolepis diminuta*	Rat fleas, beetles, grain beetles
Dwarf tapeworm	*Hymenolepis nana*	Grain beetles (rare)

PROTOZOA

Amebae—Intestinal

These organisms are characterized by having pseudopods (motility) and trophozoite and cyst stages in the life cycle and include some exceptions in which a cyst form has not been identified. Amebae usually are acquired by humans through fecal-oral transmission or mouth-to-mouth contact (*Entamoeba gingivalis*).

Current Name

*Entamoeba histolytica**
*Entamoeba dispar**
Entamoeba hartmanni†
Entamoeba coli
Entamoeba polecki
Entamoeba moshkovskii
Entamoeba gingivalis
Endolimax nana
Iodamoeba bütschlii
*Blastocystis hominis**

Flagellates—Intestinal

These organisms move by means of flagella and are acquired by fecal-oral transmission. With the exception of *Dientamoeba fragilis* (internal flagella) and those in the genera *Trichomonas* and *Pentatrichomonas*, they have the trophozoite and cyst stages in the life cycle. *D. fragilis*, *Trichomonas*, and *Pentatrichomonas* species do not have a cyst stage.

Current Name

Giardia lamblia†
Chilomastix mesnili
Dientamoeba fragilis
Pentatrichomonas hominis
Trichomonas tenax

**Entamoeba histolytica* is being used to designate the true pathogen, whereas *Entamoeba dispar* now is being used to designate a nonpathogen. Unless trophozoites containing ingested red blood cells (*E. histolytica*) are seen during microscopic examination, the two organisms cannot be differentiated on the basis of morphologic features seen in permanent stained smears of fecal specimens and will be reported as *E. histolytica/E. dispar*. Fecal immunoassays for antigen detection are available commercially for differentiation of *E. histolytica* from *E. dispar* and for detection of the *E. histolytica/E. dispar* group.[7] Because the differences in pathogenicity are genetic and not just phenotypic, the decision to treat is one that must be determined by the physician. The finding of organisms in the *E. histolytica/E. dispar* group in patient specimens must continue to be reported to state and county Departments of Public Health (follow your particular state reporting regulations).

†*Entamoeba hartmanni* is nonpathogenic and is totally different from *E. histolytica*. "Small race *E. histolytica*" is incorrect and should not be used at any time to designate *E. hartmanni*.

*The taxonomic position of *Blastocystis* has always been somewhat confusing, but it is now clear that this organism exists as a number of serotypes or species. It has been recommended that all isolates from birds and mammals, including humans, be designated in the future as *Blastocystis* spp., serotypes ST1 to ST10 plus ST Unknown. This organism has now been placed in the Stramenopila.[21]

†Although some individuals have changed the species designation for the genus *Giardia* to *G. duodenalis* or *G. intestinalis*, no consensus exists. Therefore, for this listing, the name *Giardia lamblia* is retained. However, molecular and epidemiologic evidence suggests that at least two assemblages of *Giardia* infect humans, one of which is *Giardia duodenalis* and the other, possibly a new species, *Giardia enterica*.[16]

TABLE 208-2 **Medically Important Arthropods**

Local or Systemic Problems	Vector (Common Name)
Skin reaction to bites	Sucking lice
	Bedbugs
	Kissing bugs
	Biting midges
	Sandflies
	Black flies
	Mosquitoes
	Deer flies
	Tsetse flies
	Soft ticks
	Hard ticks
Painful bite	Horseflies
	Fire ants
	Centipedes
Intense itching	Human itch mites
	Chiggers
Painful sting, potential anaphylaxis	Honeybees
	Bumblebees
	Wasps, hornets, yellow jackets
	Fire ants
	Scorpions
Dermatitis, ulcerations	Fleas
Nodular ulceration with subsequent secondary infection	Chigoe flea
Blistering of skin after contact with adult beetles	Blister beetles
Bite, usually painless, delayed systemic reaction	Black widow spiders
Initial blister followed by extensive necrosis and slow healing	Brown recluse spiders
	South American brown spider

Enteromonas hominis
Retortamonas intestinalis

Ciliates—Intestinal

These organisms, which move by means of cilia, are acquired by humans through fecal-oral transmission. They have both the trophozoite and cyst forms in the life cycle.

Current Name

Balantidium coli

Apicomplexa, Sporozoa (Coccidia)—Intestinal

These organisms are acquired by humans by ingestion of various meats or through fecal-oral transmission through contaminated food or water.

Current Name

Coccidia
Cryptosporidium hominis
Cryptosporidium parvum
Cryptosporidium spp.
Cyclospora cayetanensis[18,19]
Isospora (Cystoisospora) belli
Sarcocystis hominis
Sarcocystis suihominis
Sarcocystis bovihominis

Fungi (Microsporidia*)—Intestinal

Enterocytozoon bieneusi
Encephalitozoon (Septata) intestinalis†

Amebae, Flagellates—Other Body Sites

The amebae are pathogenic, free-living organisms that may be associated with warm, freshwater areas. They have been found in the central nervous system, the eye, and other sites. *Trichomonas vaginalis* usually is acquired by sexual transmission. This particular flagellate is found in the genitourinary system.

Current Name

Amebae
Naegleria fowleri
Acanthamoeba spp.
Hartmannella spp.
Balamuthia mandrillaris
Sappinia diploidea
Flagellates
Trichomonas vaginalis

Apicomplexa, Sporozoa (Coccidia)—Other Body Sites

The coccidia are particularly important in the compromised patient. They also may infect many individuals who have no apparent symptoms.[6,7] On the basis of molecular studies, the microsporidia are linked more closely to the fungi and have been reclassified with those organisms. However, during this transition phase, the listing will remain with the parasites.

Current Name

Coccidia
Toxoplasma gondii

*The microsporidia are now thought to be more closely related to fungi than to protozoa; however, parasitologists have been reluctant to part with this group, whereas mycologists have been equally reluctant to accept it. Consequently, the microsporidia will be retained within this list for parasites, although they are classified within the Fungi.[11,22,23]
†Formerly called *Septata intestinalis*.[10,15]

Fungi (Pneumocystis,* Microsporidia)—Other Body Sites

Microsporidia
- *Nosema ocularum*
- *Anncaliia (Brachiola) connori*
- *Anncaliia (Brachiola) algerae*
- *Anncaliia (Brachiola) vesicularum*
- *Vittaforma corneae*
- *Pleistophora ronneafiei*
- *Trachipleistophora hominis*
- *Trachipleistophora anthropophthera*
- *Encephalitozoon hellem*
- *Encephalitozoon cuniculi*
- *Encephalitozoon (Septata) intestinalis*†
- *Enterocytozoon bieneusi*‡
- *Microsporidium africanum*§
- *Microsporidium ceylonensis*
- *Tubulinosema acridophagus*¶

Sporozoa, Flagellates—Blood and Tissues

All of these organisms are arthropod borne. Diagnosis may be somewhat more difficult to make than is that of the intestinal protozoa, particularly if automated blood differential systems are used. The *Leishmania* have undergone extensive revisions in classification. However, from a clinical perspective, recovery and identification of the organisms still are related to body site. Recovery of the organisms is limited to the site of the lesion in infections other than those caused by the *Leishmania donovani* complex (visceral leishmaniasis).

Current Name

Apicomplexa, Sporozoa (malaria, babesiosis)
- Malaria
  - *Plasmodium vivax*
  - *Plasmodium ovale*
  - *Plasmodium malariae*
  - *Plasmodium falciparum*
  - *Plasmodium knowlesi***
- Babesiosis*
  - *Babesia microti*
  - *Babesia divergens*
  - *Babesia duncani*
  - *Babesia* spp.

Flagellates (leishmaniasis, trypanosomiasis)
- Leishmaniasis
  - *Leishmania tropica* complex (cutaneous leishmaniasis)
  - *Leishmania infantum* complex (cutaneous leishmaniasis)
  - *Leishmania major* complex (cutaneous leishmaniasis)
  - *Leishmania mexicana* complex (cutaneous leishmaniasis)
  - *Leishmania braziliensis* complex (mucocutaneous leishmaniasis)
  - *Leishmania donovani* complex (visceral leishmaniasis)
  - *Leishmania infantum/Leishmania chagasi* group (visceral leishmaniasis)
- Trypanosomiasis
  - *Trypanosoma brucei gambiense* (West African trypanosomiasis)
  - *Trypanosoma brucei rhodesiense* (East African trypanosomiasis)
  - *Trypanosoma cruzi* (American trypanosomiasis)
  - *Trypanosoma rangeli*

NEMATODES

Nematodes—Intestinal

These organisms normally are acquired by ingestion of eggs or penetration of the skin by larval forms from the soil.

Current Name

- *Ascaris lumbricoides*
- *Enterobius vermicularis* (pinworm)
- *Ancylostoma duodenale*
- *Necator americanus*
- *Strongyloides stercoralis*
- *Strongyloides fuelleborni*
- *Trichostrongylus* spp.
- *Trichuris trichiura* (whipworm)
- *Capillaria philippinensis*
- *Oesophagostomum* spp. (*O. bifurcum* most common in humans—West Africa)
- *Ternidens diminutus* (as high as 80% in Zimbabwe)

Nematodes—Tissue

For the most part, these organisms rarely are seen within the United States; however, the first three are more important.

**Pneumocystis carinii* has been reclassified with the fungi and renamed *Pneumocystis jiroveci*.[5,20] Consequently, it will be removed from future parasite listings.

†Formerly called *Septata intestinalis.*

‡*Enterocytozoon bieneusi* has been recovered from sites other than the intestinal tract.

§This designation is now written as a true genus but remains a "catch-all" for those organisms that have not been (or may never be) identified to the true genus or species levels.

¶New human pathogen, disseminated infection in a patient with multiple myeloma (severely immunosuppressed, allogeneic stem cell transplant recipient).[13]

***Plasmodium knowlesi*, a malaria parasite of macaque monkeys in Southeast Asia that is now established as a naturally transmitted parasite of humans in Malaysia and other parts of Southeast Asia, has been responsible for a number of deaths.[4] Thus, there are now five species of malaria that infect humans.

*Molecular studies confirm that humans can also harbor a number of *Babesia* parasites that have not yet been identified.[9]

Current Name

Trichinella spiralis
Trichinella spp.
Toxocara canis or *Toxocara cati* (visceral or ocular larva migrans)
Ancylostoma braziliense (cutaneous larva migrans)
Ancylostoma caninum (eosinophilic enteritis)
Baylisascaris procyonis (severe systemic visceral larva migrans, neural larva migrans)
Dracunculus medinensis
Angiostrongylus cantonensis
Angiostrongylus costaricensis
Gnathostoma spinigerum
Gnathostoma spp.
Anisakiasis (larvae from saltwater fish)
Anisakis spp.
Phocanema spp.
Contracaecum spp.
Pseudoterranova spp.
Hysterothylacium spp.
Porrocaecum spp.
Capillaria hepatica
Thelazia spp.

Nematodes (Filarial Worms)—Blood, Other Body Fluids, Skin

These organisms also are arthropod borne. The adult worms tend to live in the tissues of lymphatics. Diagnosis is made on the basis of the recovery and identification of the larval worms (microfilariae) in the blood, other body fluids, or skin. Elephantiasis may be associated with some of the organisms listed.

Current Name

Wuchereria bancrofti
Brugia malayi
Brugia timori
Loa loa
Onchocerca volvulus
Mansonella ozzardi
Mansonella streptocerca
Mansonella perstans
Dirofilaria immitis ("coin" lesion in the lung) (dog heartworm)
Dirofilaria spp. (may be found in subcutaneous nodules)

CESTODES

Cestodes—Intestinal

The adult form of these organisms is acquired by humans through ingestion of the larval forms contained in poorly cooked or raw meats or freshwater fish. In the case of *Dipylidium caninum*, infection is acquired by the accidental ingestion of dog fleas. Both *Hymenolepis nana* and *Hymenolepis diminuta* are transmitted by ingestion of certain arthropods (fleas, beetles). Also, *H. nana* can be transmitted through egg ingestion (life cycle in the human can bypass the intermediate beetle host). Humans can serve as both the intermediate and definitive hosts in *H. nana* and *Taenia solium* infections.

Current Name

Diphyllobothrium latum (broad, fish tapeworm)
Dipylidium caninum (dog tapeworm)
Hymenolepis (Rodentolepis) nana (dwarf tapeworm)
Hymenolepis diminuta (rat tapeworm)
Taenia solium (pork tapeworm)
Taenia saginata (beef tapeworm)
Taenia asiatica (Taiwanese variant of *T. saginata*)

Cestodes (Larval Forms)—Tissue

The ingestion of certain tapeworm eggs or accidental contact with certain larval forms can lead to the diseases shown in parentheses.

Current Name

Taenia solium (cysticercosis)
Echinococcus granulosus (hydatid disease)
Echinococcus multilocularis (alveolar hydatid disease)
Echinococcus oligarthrus (polycystic hydatid disease)
Multiceps multiceps (coenurosis)
Diphyllobothrium spp. (sparganosis)
Spirometra mansonoides (sparganosis)

TREMATODES

Trematodes—Intestinal

These organisms are uncommon within the United States, except for four species of *Alaria*, which are endemic within North America.

Current Name

Fasciolopsis buski (giant intestinal fluke)
Echinostoma ilocanum
Eurytrema pancreaticum
Heterophyes heterophyes
Metagonimus yokogawai
Alaria spp.

Trematodes—Liver, Lung

These organisms are not seen commonly within the United States; however, some Southeast Asian refugees do harbor some of these parasites.

Current Name

Clonorchis (Opisthorchis) sinensis (Chinese liver fluke)
Opisthorchis viverrini
Opisthorchis felineus
Fasciola hepatica (sheep liver fluke)
Paragonimus westermani (lung fluke)

Paragonimus kellicotti (lung fluke endemic in the United States)
Paragonimus spp.
Metorchis conjunctus (North American liver fluke)

Trematodes—Blood

The schistosomes are acquired by penetration of the skin by the cercarial forms that are released from freshwater snails. Although they are not endemic within the United States, occasionally patients are seen who may have these infections.

Current Name

Schistosoma mansoni
Schistosoma haematobium
Schistosoma japonicum
Schistosoma intercalatum
Schistosoma mekongi

ARTHROPODS

See Tables 208-1 and 208-2.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Cox FEG. Taxonomy and classification of human parasitic protozoa and helminths. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology. Section VIII. Parasitology*. 10th ed. Washington, DC: ASM Press; 2011. p. 2041–6.
4. Cox-Singh J, Singh B. Knowlesi malaria. newly emergent and of public health importance? *Trends Parasitol* 2008;**24**:406–10.
9. Gray JS, Weiss LM. Babesia microti. In: Khan NA, editor. *Emerging protozoan pathogens*. New York: Taylor and Francis; 2008. p. 303–49.
11. Hirt RP, Logsdon JM, Healey B, et al. Microsporidia are related to fungi. evidence from the largest subunit of RNA polymerase II and other proteins. *Proc Natl Acad Sci U S A* 1999;**96**:580–5.
13. Meissner EG, Bennett JE, Qvarnstron Y, et al. Disseminated microsporidiosis in an immunosuppressed patient. *Emerg Infect Dis* 2012;**18**:1155–8.
16. Monis PT, Caccio SM, Thompson RCA. Variation in *Giardia*. towards a taxonomic revision of the genus. *Trends Parasitol* 2009; **25**:93–100.
21. Tan KS. New insights on classification, identification and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* 2008;**21**:639–65.
22. Webster J, Weber RWS. *Introduction to fungi*. 3rd ed. Cambridge, UK: Cambridge University Press; 2007.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 1 *PROTOZOA*

CHAPTER 209

Amebiasis

Shinjiro Hamano • William A. Petri, Jr.

Diarrheal diseases continue to be major causes of morbidity and mortality in children in developing countries. In Bangladesh, 10 percent of children in the first year of life have amebic diarrhea and 1 in 30 children dies of diarrhea or dysentery by age 5 years.[78] Amebiasis is an infection caused by the protozoan parasite *Entamoeba histolytica*. Infection occurs via ingestion of the parasite's cyst from fecally contaminated food, water, or hands. Approximately 50 million illnesses and 100,000 deaths occur annually from amebiasis, rendering it the third leading cause of death by parasitic disease in humans.[78] Although amebiasis is present worldwide, it occurs most commonly in underdeveloped areas, especially Asia, sub-Saharan Africa, and Central and South America. In the United States and other developed countries, cases of amebiasis are most likely to occur in immigrants from and travelers to endemic regions, but it can affect populations of the developed world, as shown by the epidemic that occurred

REFERENCES

1. Beaver CB, Jung RC, Cupp EW. *Clinical parasitology*. Philadelphia: Lea & Febiger; 1984.
2. Cox FEG. Classification of the parasitic protozoa. In: Cox FEG, Despommier DD, Gillespie S, Wakelin D, editors. *Topley and Wilson's microbiology and microbial infections. Part 6. Parasitology*. 10th ed. London: Arnold; 2005. p. 186–99.
3. Cox FEG. Taxonomy and classification of human parasitic protozoa and helminths. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology. Section VIII. Parasitology*. 10th ed. Washington, DC: ASM Press; 2011. p. 2041–6.
4. Cox-Singh J, Singh B. Knowlesi malaria. newly emergent and of public health importance? *Trends Parasitol* 2008;**24**:406–10.
5. Edman JC, Kovacs JA, Masur H, et al. Ribosomal RNA sequence shows *Pneumocystis carinii* to be a member of the fungi. *Nature* 1988;**334**:519–22.
6. Fayer R. *Cryptosporidium and cryptosporidiosis*. Boca Raton, Fla: CRC Press; 1997.
7. Garcia LS. *Diagnostic medical parasitology*. 5th ed. Washington, DC: ASM Press; 2007.
8. Gibson DI. Nature and classification of parasitic helminths. In: Cox FEG, Despommier DD, Gillespie S, Wakelin D, editors. *Topley and Wilson's microbiology and microbial infections. Part 6. Parasitology*. 10th ed. London: Arnold; 2005. p. 573–99.
9. Gray JS, Weiss LM. Babesia microti. In: Khan NA, editor. *Emerging protozoan pathogens*. New York: Taylor and Francis; 2008. p. 303–49.
10. Hartskeerl RA, Van Gool T, Schuitema ARJ, et al. Genetic and immunological characterization of the microsporidian *Septata intestinalis* Cali, Kotler, and Orenstein, 1993. Reclassification to *Encephalitozoon intestinalis*. *Parasitology* 1995;**110**:277–85.
11. Hirt RP, Logsdon JM, Healey B, et al. Microsporidia are related to fungi. evidence from the largest subunit of RNA polymerase II and other proteins. *Proc Natl Acad Sci U S A* 1999;**96**:580–5.
12. Levine ND. *Veterinary parasitology*. Minneapolis, Minn: Burgess Publishing; 1978.
13. Meissner EG, Bennett JE, Qvarnstron Y, et al. Disseminated microsporidiosis in an immunosuppressed patient. *Emerg Infect Dis* 2012;**18**:1155–8.
14. Meyers WM, Neafie RC, Marty AM, et al, editors. *Pathology of infectious diseases helminthiases*, vol. 1. Washington, DC: Armed Forces Institute of Pathology; 2000.
15. Molina JM, Oksenhendler E, Beauvais B, et al. Disseminated microsporidiosis due to *Septata intestinalis* in patients with AIDS. clinical features and response to albendazole therapy. *J Infect Dis* 1995;**171**:245–9.
16. Monis PT, Caccio SM, Thompson RCA. Variation in *Giardia*. towards a taxonomic revision of the genus. *Trends Parasitol* 2009; **25**:93–100.
17. Murray PR, Baron EJ, Pfaller MA, et al, editors. *Manual of clinical microbiology*. 8th ed. Washington, DC: ASM Press; 2003.
18. Ortega Y, Sterling CR, Gilman RH, et al. *Cyclospora* species. a new protozoan pathogen of humans. *N Engl J Med* 1993;**328**:1308–12.
19. Pape JW, Verdier RI, Boncy M, et al. *Cyclospora* infection in adults infected with HIV. clinical manifestations, treatment, and prophylaxis. *Ann Intern Med* 1994;**121**:654–7.
20. Stringer SL, Hudson K, Blase MA, et al. Sequence from ribosomal RNA of *Pneumocystis carinii* compared to those of four fungi suggests an ascomycetous affinity. *J Protozool* 1989;**36**:14S–16S.
21. Tan KS. New insights on classification, identification and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* 2008;**21**:639–65.
22. Webster J, Weber RWS. *Introduction to fungi*. 3rd ed. Cambridge, UK: Cambridge University Press; 2007.
23. Wittner M, Weiss LM. *The microsporidia and microsporidiosis*. Washington, DC: ASM Press; 1999.

in Tbilisi, Republic of Georgia, caused by contaminated municipal water.[13] Currently, there is no vaccine to prevent the childhood morbidity and mortality resulting from infection with *E. histolytica*.

ETIOLOGY

E. histolytica is the cause of amebiasis and was named for the pathologic evidence of "lysis" of tissues. Additional *Entamoeba* spp. that infect humans and that are identical in appearance microscopically to *E. histolytica* include *E. dispar*, which is nonpathogenic, *E. moshkovskii*, which may cause diarrhea,[95] and the recently identified enteric parasite *E. bangladeshi*.[91] The first demonstration of the organism in human tissues was made by Lambl in 1859 in the postmortem examination of the colon of a child who died as a result of having excessive diarrhea.[16,74] No connection of the organism with the disease was made until 1875, when Losch, in St. Petersburg, Russia, found the organism at autopsy in the colon of a woodcutter. Losch induced diarrhea and ulcerations in a dog given feces from the patient.[61] He did not think, however, that a connection existed between the organism and the disease. The first patient described in the United States was a physician treated by Osler for an amebic liver abscess in 1890.[74] Councilman and Lafleur described the organism and the disease in 1891.[20,45] Further investigation of the disease was delayed until a better understanding of the life cycle of *E. histolytica* could be obtained.[27] In recent years, the application of modern molecular biology techniques to the study of *E. histolytica* and *E. dispar* has resulted in an explosion of information about the mechanisms of virulence, pathogenicity, and immune responses to these organisms.[88,90]

E. histolytica is the pathogenic species, having the capacity to invade tissue and cause symptomatic disease, whereas *E. dispar* (and *E. histolytica*) is associated with the asymptomatic carrier state.[6,88] More recently, a study revealed that all genotypes of *E. histolytica* are not equally capable of causing disease.[6] Morphologically distinct members of the genus *Entamoeba*, such as *Entamoeba coli* and *Entamoeba hartmanni*, also are nonpathogenic. *Entamoeba moshkovskii*, *Dientamoeba fragilis*, and *Entamoeba polecki* have been associated with diarrhea, and *Entamoeba gingivalis* has been associated with periodontal disease.

Members of the genus *Entamoeba*, which are protozoan organisms belonging to the subphylum Sarcodina and close to *Dictyostelium discoideum* on one of the lowest branches of the eukaryotic tree, have trophozoite and cyst forms.[39] The cysts of *E. histolytica*, *E. dispar*, *E. moshkovskii*, and *E. bangladeshi* are almost spherical, being surrounded by a cell wall composed of chitin. The cysts may have one to four nuclei, although quadrinucleate cysts are most typical. This feature allows differentiation from *Escherichia coli*, which usually has 6 to 8 nuclei in the cysts and may have 32 nuclei.[75] Cysts of *E. histolytica* are 5 to 20 μm in diameter (average, 12 μm) and have a greenish tint in the unstained condition.[62] Young cysts contain chromatoid bodies, which are composed of ribosome particles in crystalline arrays.[12] The cysts of *E. hartmanni* appear identical to those of *E. histolytica* except for being a smaller size (4 to 10 μm). *E. histolytica* cysts can survive for days in the dried state at 30° C or for months at 0° C to 4° C. They can be killed by temperatures greater than 50° C retained for 5 minutes.[45] They are completely resistant to the concentrations of chlorine used in water supplies but may be killed with hyperchlorination or with iodine solutions.[62,75] They are filtered from water supplies that pass through a sand filtration phase. They resist acids well.

When these quadrinucleate cysts are ingested, they resist the acid pH of the stomach and ultimately excyst in the alkaline environment of the bowel. The process of excystation results in the release of four trophozoites that divide by binary fission to produce eight trophozoites. The usual trophozoites have a diameter of 25 μm (range, 10 to 60 μm).[27,88] They have a single nucleus that is 3 to 5 μm in diameter and contains fine peripheral chromatin with a slightly eccentric karyosome. They have a granular endoplasm that typically contains vacuoles in which bacteria and debris can be seen. Some glycogen is present and can be stained with periodic acid–Schiff stain.

Although amebae were thought to lack organelles, such as mitochondria, endoplasmic reticulum, and Golgi apparatus, evidence to the contrary is coming to light. The existence of nuclear-encoded mitochondrial genes and a remnant mitochondrial organelle was reported more recently.[63,102] The presence of ingested erythrocytes is a characteristic feature of *E. histolytica* but not *E. dispar*.[88] Movement is accomplished by extension of clear pseudopodia. Replication is by binary fission. These protozoa live in the colon of humans and other mammals. Trophozoites die quickly outside the body and are quite sensitive to acid—they generally are not considered to be infective.[32] When cooled (as when feces are expelled and gradually cooled from body temperature) or stimulated by as-yet-undefined luminal conditions, the trophozoites form cysts that can remain viable for weeks to months on excretion.[88]

Trophozoites of *E. coli* are 15 to 50 μm in diameter; have much more sluggish motility than the trophozoites of *E. histolytica;* and have blunt pseudopodia, rather than the sharp, finger-like pseudopodia of *E. histolytica*. Trophozoites of *E. hartmanni* are 4 to 14 μm in diameter and have much less glycogen than the trophozoites of *E. histolytica*.[27]

EPIDEMIOLOGY

Amebiasis is distributed throughout the world. The number of people infected with either *E. histolytica* or *E. dispar* per year is estimated to be 500 million. Although most individuals remain asymptomatic, perpetuating the natural cycle of the organism through fecal excretion of infective cysts, approximately 50 million people experience the severe morbidity associated with invasive disease, with an estimated 100,000 dying annually.[78,99] In the United States, 50 percent of amebiasis is observed in Hispanics, Asians, and Pacific Islanders. Travelers from developing countries, malnourished individuals,[72] men, and residents of institutions for the mentally retarded are considered to be at higher risk for amebiasis (Table 209-1).

TABLE 209-1 Risk Factors for Amebiasis in the United States

Hispanic/Asian/Pacific Islanders—50% of U.S. cases reported to CDC
Travelers to endemic regions of the world—0.3% incidence in one study
Institutions for mentally retarded
Men who have sex with men
Men—90% amebic liver abscesses in men, but rare in children

CDC, Centers for Disease Control and Prevention.

During the 1990s, enough evidence had accumulated to support the formal separation of two morphologically identical species of ameba: the nonpathogenic *E. dispar* from the potentially pathogenic *E. histolytica*.* Morbidity and mortality data in absolute numbers that existed before this time pertaining to cases of invasive disease were not greatly affected by this reclassification because all invasive disease was known to be caused by *E. histolytica*.[99] Because most prevalence and incidence data previously collected pertained to asymptomatic individuals, however, and it was clear that most asymptomatic individuals with cysts detected in their stool were infected with nonpathogenic *E. dispar*, the true prevalence and incidence of *E. histolytica* became a matter of speculation.[99]

Estimates of *E. histolytica* infections have been based primarily on examinations of stool for cysts and parasites, but these tests are insensitive and cannot differentiate *E. histolytica* from morphologically identical species that are nonpathogenic, such as *E. dispar* and *Entamoeba moshkovskii*.[5,22] Specific and sensitive means to detect *E. histolytica* in stool are now available and include antigen detection and polymerase chain reaction (PCR) analysis.[36,44,58]

A prospective study of preschool children in a slum of Dhaka, Bangladesh, showed *E. histolytica*–associated diarrhea in 9 percent and *E. histolytica*–associated dysentery in 3 percent of the children annually.[42] Not all individuals are equally susceptible to amebiasis, with a leptin receptor polymorphism and certain HLA-DR and HLA-DQ alleles associated with resistance to infection and disease.[23,24] The annual incidence of amebic liver abscess was reported to be 21 cases per 100,000 inhabitants in Hue City, Vietnam.[14] Carefully conducted serologic studies in Mexico, where amebiasis is endemic, showed antibody to *E. histolytica* in 8.4 percent of the population.[17] In the urban slum of Fortaleza, Brazil, 25 percent of all individuals tested carried antibody to *E. histolytica;* the prevalence of anti-amebic antibodies in children 6 to 14 years old was 40 percent.[15]

PATHOGENESIS AND PATHOLOGY

The cysts are transported through the digestive tract to the intestine, where they release their mobile, disease-producing form, the trophozoite. *E. histolytica* trophozoites can live in the large intestine and form new cysts without causing disease. They also can invade the lining of the colon, killing host cells and causing diarrhea, amebic colitis, acute dysentery, or chronic diarrhea. The trophozoites also can be carried through the blood to other organs, most commonly the liver and occasionally the brain, where they form potentially life-threatening abscesses (Fig. 209-1). Important virulence factors include the trophozoite cell surface galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin that mediates adherence to colonic mucins and host cells,[77,89] cysteine proteinases that likely promote invasion by degrading extracellular matrix and serum components, and amebapore pore-forming proteins involved in killing of bacteria and host cells.[59,92]

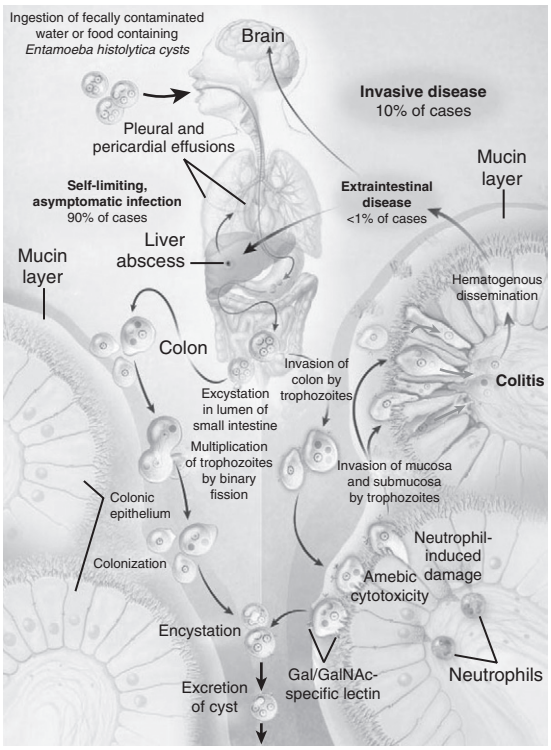

FIGURE 209-1 ■ Life cycle of *Entamoeba histolytica.* Infection normally is initiated by the ingestion of fecally contaminated water or food containing *E. histolytica* cysts. The infective cyst form of the parasite survives passage through the stomach and small intestine. Excystation occurs in the bowel lumen, where motile and potentially invasive trophozoites are formed. In most infections, the trophozoites aggregate in the intestinal mucin layer and form new cysts, resulting in a self-limited and asymptomatic infection. In some cases, adherence to and lysis of the colonic epithelium, mediated by the galactose and *N*-acetyl-D-galactosamine (Gal/GalNAc)–specific lectin, initiates invasion of the colon by trophozoites. Neutrophils responding to the invasion contribute to cellular damage at the site of invasion. When the intestinal epithelium is invaded, extraintestinal spread to the peritoneum, liver, and other sites may follow. Factors controlling invasion, as opposed to encystation, most likely include parasite "quorum sensing" signaled by the Gal/GalNAc-specific lectin, interactions of amebae with the bacterial flora of the intestine, and innate and acquired immune responses of the host. (From Haque R, Huston CD, Hughes E, et al. Amebiasis. *N Engl J Med* 2003;**348**:1565-73.)

*References 1, 14, 30, 31, 38, 99.

The interface of the Gal/GalNAc lectin with the host mucins lining the intestine is the defining moment of the infection.[19] If the parasite lectin attaches to the host mucin glycoproteins that line the intestinal lumen, a noninvasive gut infection ensues. The life cycle continues as the trophozoites reproduce by clonal expansion in the mucin layer. Subsequently, the Gal/GalNAc lectin, along with mucin glycoproteins or other gut bacteria, initiates the developmental pathway leading to encystment.[26,99]

Colitis is caused when the trophozoite penetrates the intestinal mucous layer, which otherwise acts as a barrier to invasion by inhibiting amebic adherence to the underlying epithelium and by slowing trophozoite motility.[19] Invasion is mediated by the killing of epithelial cells, neutrophils, and lymphocytes by trophozoites, which occurs only after the parasite lectin engages host GalNAc on O-linked cell surface oligosaccharides.[78] The interaction of the lectin with glycoconjugates is stereospecific and multivalent.[107] The identity of the high-affinity intestinal epithelial cell receptor is unknown. Secretion of amebapore, a 5-kDa pore-forming protein, by the ameba may contribute to killing.[57] Activation of human caspase 3, a distal effector molecule in the apoptotic pathway, occurs rapidly after amebic contact, and caspases are required for cell killing in vitro and for the formation of amebic liver abscesses in vivo.[47,106]

Interaction of the parasite with the intestinal epithelium causes an inflammatory response marked by the activation of nuclear factor κB and the secretion of cytokines.[25,93] The development of this epithelial response may depend on trophozoite virulence factors, such as cysteine proteinase, and leads to intestinal abnormalities through neutrophil-mediated damage. Neutrophils also can be protective, and activation of neutrophils or macrophages by tumor necrosis factor-α or interferon-γ kills amebae in vitro and limits the size of amebic liver abscesses.[7,21] In contrast to the intense inflammatory response typical of early invasive amebiasis, inflammation surrounding well-established colonic ulcers and liver abscesses is minimal, given the degree of tissue damage.[16]

The initial lesions of clinical amebiasis often are small interglandular ulcers with a diameter of approximately 1 mm. They extend only to the muscularis mucosa.[16,66] The margins may be hyperemic, and slight edema of the surrounding mucosa is present. *E. histolytica* organisms seen in these ulcers stain well with periodic acid–Schiff stain.[79] Bleeding and friability are not prominent at this stage, although proctoscopic examination may find mucus coming from these ulcers, with an abundant number of amebae present.

The next stage of intestinal disease is the production of deeper ulcers. These "buttonhole" ulcers may be 1 cm in diameter and may extend into the submucosa.[16,79] The ulcer often extends laterally under normal-appearing mucosa, forming a characteristic flask shape. Occasional perforation through the serosa leads to peritonitis or pneumoperitoneum.[96] Extensive necrosis may be present, but usually only very little inflammation occurs. The edema is more intense, but the mucosa between ulcers is normal, in contrast to the marked inflammatory response seen in bacterial enteritis. When ulceration is more extensive, the edema surrounding the ulcers becomes confluent and the mucosa appears gelatinous. In young children, this condition can progress to a fulminant necrotizing colitis associated with transmural necrosis. The pathologic events associated with this phenomenon are not understood. Rarely, an inflammatory response is present, resulting in granulation of the tissue with a fibrous outer wall.[75] It is given the name *ameboma*. Occasionally, an ameboma fills a significant portion of the lumen, which causes stricture or obstruction. Other complications of intestinal amebiasis result from direct extension of the ulcers. This extension may result in cutaneous involvement of the perianal area or lesions of the penis, vulva, vagina, or cervix.[2,75] Cutaneous and ophthalmologic amebiasis also is caused by fecal contamination of the face.[69]

Amebae disseminate to the liver in 50 percent of patients with fulminant amebiasis.[2,3] Dissemination to other organs directly from the intestine probably does not occur, but dissemination from the liver to lung, heart, brain, spleen, scapula, larynx, stomach, and aorta has been described.[16] Amebic abscess of the liver occurs more often in men than in women by a ratio of 16:1 but occurs equally often in prepubertal children of both sexes.[3,16] Abscesses occur more commonly in adults but occur in children as young as 4 months of age.[73] These abscesses vary from microscopic lesions to massive necrosis of 90 percent of the liver. Fever, right upper quadrant pain, and the presence of serum antibodies to amebae point to hepatic amebic abscess.[87] Examination of the fluid from such an abscess frequently reveals a reddish, "anchovy paste" fluid that rarely may appear white or green. The fluid is acidic, with a pH ranging from 5.2 to 6.7.[85] Amebae are found in the walls of the abscess and only rarely in the fluid of the abscess. Many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid.[86] The walls are composed of a thin connective tissue capsule. The right lobe of the liver is involved with amebic liver abscess about six times as often as the left lobe. Abscesses in the right lobe can perforate and cause disease below the diaphragm or in the thoracic cavity. Abscesses in the left lobe can lead to pericardial effusions, which are less common than pleural effusions.[34,49]

Pleural effusions can remain loculated or lead to cutaneous fistulas or to bronchopleural fistulas. Drainage from these fistulas is acidic, in contrast to the neutral secretions in the normal lung. Seeding of the cardiac valves and of the brain has been described.[16] Cerebral abscesses have the same microscopic findings as do liver abscesses, with a thin capsule of connective tissue surrounding a fluid with little or no associated inflammatory response.

IMMUNITY

Protection from amebiasis, including acquired immunity to infection and invasion by *E. histolytica*, is associated with a mucosal IgA antibody response against the carbohydrate recognition domain of the parasite Gal/GalNAc lectin.[33,37,39,55] Cell-mediated immunity in protection

from invasive amebiasis, but not infection per se, also has been shown. There is substantial evidence from an in vitro animal model and most recently from human studies of an important role for interferon-γ and IL-17 in protection from amebic colitis, acting in part by activating of macrophages to kill the parasite.[33,43,46] Invasive amebiasis rarely occurs in individuals with human immunodeficiency virus infection/acquired immunodeficiency syndrome, even in areas where amebiasis is common, suggesting an important role also exists for natural immunity or innate immune responses, or both, in protection from infection.[7,36]

CLINICAL MANIFESTATIONS

Intestinal Amebiasis

Asymptomatic Intraluminal Amebiasis

The most common type of amebic infestation is an asymptomatic cyst-passing carrier state. All *E. dispar* infections and 90 percent of *E. histolytica* infections are asymptomatic, manifesting as only *Entamoeba* cysts in the feces.[30,81]

Entamoeba histolytica–*Associated Diarrhea*

Diarrhea is the most common manifestation of amebic disease, present in 9 percent of children in the Mirpur cohort each year, compared with only 3 percent of children having amebic colitis each year.[42] *E. histolytica*–associated diarrhea is defined as three or more unformed stools in a 24-hour period accompanied by a new episode of *E. histolytica* infection. This definition was validated previously in the cohort by (1) showing that diarrhea was approximately five times more common in the setting of a new infection (age-adjusted odds ratio for the association of new *E. histolytica* infection with diarrhea of 4.7; 95% confidence interval 2.9 to 7.6), and (2) showing by a complete bacteriologic, virologic, and parasitic workup that only 32 percent of patients with *E. histolytica*–associated diarrhea were coinfected with another pathogen compared with identification of an enteropathogen in 59 percent of all cases of diarrhea.[39]

Acute Amebic Colitis

Amebic dysentery was defined as a diarrheal stool sample containing occult or gross blood that was positive for *E. histolytica* antigen. Seventy percent of patients have a gradual onset of symptoms over 3 or 4 weeks after infestation, with increasingly severe diarrhea as the primary complaint, accompanied by general abdominal tenderness. Occasionally, the onset may be acute or may be delayed for several months after infestation. This onset differs from bacterial causes of dysentery, in which patients usually have only symptoms of 1 to 2 days' duration. The diarrhea is usually associated with pain in children. Pain may be of such severity that an acute abdomen is suspected.[50,79] The stools contain blood and mucus in virtually all cases.[2,79,80] Fever is present in only a few patients with amebic colitis. Abdominal distention and dehydration occur in less than 10 percent of patients. In young children, intussusception, perforation, peritonitis, or necrotizing colitis may develop rapidly.[10,50,96] Amebic colitis has been shown to be associated with cognitive disability as a long-term sequelae.[101]

Ameboma

Unusual manifestations of amebic colitis include toxic megacolon (0.5% of cases, usually requires surgical intervention), ameboma (granulation tissue in colonic lumen mimicking colonic cancer in appearance), and a chronic nondysenteric form of infection that can manifest as years of waxing and waning diarrhea, abdominal pain, and weight loss (easily misdiagnosed as inflammatory bowel disease).

Extraintestinal Amebiasis

Amebic Liver Abscess

The typical patient with an amebic liver abscess in the United States is an immigrant, usually a Hispanic/Asian/Pacific Islander; is male; is 20 to 40 years old; and has fever, right upper quadrant pain, leukocytosis, abnormal serum aminotransferase and alkaline phosphatase levels, and a defect on hepatic imaging study. Roughly 90 percent of patients with liver abscesses are men. The abscess usually is single and is in the right lobe of the liver 80 percent of the time.[51] Most frequently, patients have liver abscess without concurrent colitis. Amebae are seen infrequently in the stool at the time of diagnosis of liver abscess.[3] Liver abscess can manifest acutely as fever and right upper abdominal tenderness and pain or subacutely as prominent weight loss and less frequently fever and abdominal pain. The peripheral white blood cell count is elevated, as is the alkaline phosphatase level, in many patients.

Early evaluation of the hepatobiliary system by ultrasonography or computed tomography (CT) is essential to show the abscess in the liver. The differential diagnosis of the lesion in the liver includes pyogenic abscess, hepatoma, and echinococcal cyst. Aspiration of the abscess occasionally is required to diagnose amebiasis (although amebae are visualized in the pus in only a few cases; if the abscess is pyogenic, the responsible bacteria are seen or cultured). Antibodies to *E. histolytica* are present in the serum of 70 to 90 percent of patients on acute presentation with amebic liver abscess and are useful diagnostically, especially in combination with antigen detection or PCR tests.[41] Unusual extraintestinal manifestations of amebiasis include direct extension of the liver abscess to pleura or pericardium and brain abscess. In a patient who has right upper quadrant pain, ultrasonography, CT, or magnetic resonance imaging (MRI) should be performed to examine the liver and gallbladder.

If a space-filling defect in the liver is observed, the differential diagnosis includes (1) amebiasis (most common in men with a history of travel or residence in a developing country); (2) pyogenic or bacterial abscess (suspected in women, patients with cholecystitis, elderly individuals, individuals with diabetes, and patients with jaundice); (3) echinococcal abscess (an incidental finding

because echinococcal abscess should not cause pain or fever); and (4) cancer. Most patients with amebic liver abscess have detectable circulating antigen in serum and serum anti-amebic antibodies.[36]

In children, abdominal pain is reported infrequently with amebic liver abscess.[35,70] More commonly, high fever, abdominal distention, irritability, and tachypnea are noted. Some children are admitted to the hospital with a fever of unknown origin. Hepatomegaly occurs frequently, but elicitation of hepatic tenderness is not well documented. In one report, four of five children younger than age 5 years died with amebic liver abscesses because the diagnosis was not suspected.[56] Death usually results from rupture of the liver abscess into the peritoneum, thorax, or pericardium but may follow extensive hepatic damage and liver failure.[3,84]

Metastatic Amebiasis

Extra-abdominal amebiasis presumably follows direct extension from liver abscesses, rather than direct dissemination from the intestine.[3,16] Thoracic amebiasis is the most common type of extra-abdominal amebiasis and occurs in less than 10 percent of patients with amebic liver abscess.[16,49] Symptoms depend on the type of involvement. Empyema, bronchohepatic fistulas, or extension of a pleuropulmonary abscess into the pericardium may occur.

Pericardial amebiasis is the next most common form of extraintestinal involvement and may result from rupture of a liver abscess in the left lobe of the liver into the pericardium or through extension of the right-sided pleural amebiasis.[16,28,29,34] It is estimated to occur in 3 percent of patients with hepatic abscesses.[29] It manifests as acute pericarditis with tamponade and, occasionally, as pneumopericardium.[28] Amebic liver abscess in the left lobe also may rupture directly into the left side of the chest.[65]

Cerebral amebic abscesses are seen only in individuals with amebic liver abscess and were found in 8 percent of patients with liver abscess discovered at autopsy in one study.[60] In other studies, lower rates of 0.66 to 4.7 percent of patients with amebic liver abscess having brain abscesses were reported.[48,98] Patients with cerebral amebiasis frequently are so ill from the intestinal, liver, and possibly lung involvement that neurologic signs are not always assessed easily. In 18 patients with proven cerebral amebiasis, initial neurologic examination was normal in 13 and only 1 patient later developed seizures.

Other foci of infection are rare findings, but amebic rectovesical fistula formation and involvement of pharynx, heart, aorta, and scapula have been reported. Cutaneous extension after the adherence of perforated, inflamed bowel to the skin is an extremely painful and rare complication.[16,75] This situation also may arise after invasion of the skin occurs from trophozoites emerging out from the rectum.[69]

DIAGNOSIS

The prevalence of diarrhea due to *E. histolytica* in the first year of life in impoverished children in the developing world may be as high as 10 percent.[71] A heightened suspicion of amebiasis should be present if the patient has been in a developing country as a resident or traveler. The diagnosis of amebiasis should be considered in any child with risk factors who has diarrhea, bloody stools, or stools with mucus; with a hepatic abscess; and/or with right upper quadrant pain, abdominal distention, or tachypnea.[56,70] In a patient with diarrhea, if blood is present in the stool (grossly bloody or occult blood positive), infectious (Shiga toxin–producing *E. coli*, *Salmonella*, *Shigella*, *Campylobacter*, and *E. histolytica*) and noninfectious (inflammatory bowel disease, diverticulosis, arteriovenous malformations, cancer) causes should be considered.

Serologic Tests

Serologic tests for anti-amebic antibodies also are a very useful tool in diagnosis, with sensitivity of 70 to 80 percent early in disease and approaching 100 percent sensitivity on convalescence.[97,100] The combined use of serology and stool antigen detection or PCR analysis offers the best diagnostic approach.

Microscopic Examination of Stool

Before the development of new antigen detection tests and PCR analysis, amebiasis was diagnosed by examining a stool sample through a microscope to determine whether *E. histolytica* cysts were present. This method often requires more than one specimen, however, because the number of cysts in the stool varies greatly. In addition, stool microscopy has limited sensitivity and specificity. The body's own immune system produces macrophage cells that can look like the amebae. Four different amebae—*E. histolytica*, which causes amebiasis, *E. dispar*, which does not cause disease, and *E. moshkovskii* and *E. bangladeshi*, which may cause diarrhea—look identical under a microscope.[22,91,95]

Noninvasive Diagnosis of Extraintestinal Amebiasis

Amebiasis outside the intestine has been even more difficult to diagnose. Clinical manifestations of extraintestinal disease vary widely, and less than 10 percent of individuals with amebic liver abscesses have identifiable *E. histolytica* in their stools. The TechLab *E. histolytica* II test, which differentiates the true pathogen *E. histolytica* from *E. dispar*, was reported to detect Gal/GalNAc lectin in the sera of 22 of 23 (96%) patients with amebic liver abscess tested before treatment with the anti-amebic drug metronidazole and 0 of 70 (0%) controls. After 1 week of treatment with metronidazole, more than 80 percent of patients became serum lectin antigen negative. Detection of *E. histolytica* Gal/GalNAc lectin in the sera using the TechLab *E. histolytica* II kit is sensitive to diagnose hepatic and intestinal amebiasis before the institution of metronidazole treatment.[41] A real-time PCR assay detected *E. histolytica* DNA in 49, 77, and 69 percent of blood, urine, and saliva specimens, respectively, from the amebic liver abscess patients.[40]

Noninvasive diagnostic procedures such as ultrasound, CT, and MRI can detect extracolonic amebiasis in the liver, paracecal masses, brain, and other sites, but they cannot distinguish between abscesses caused by amebae and those caused by bacteria, hampering proper treatment of the condition. Most patients with amebic liver abscess have a single abscess in the right lobe of the liver, although multiple lesions also can occur.[4] Chest radiographs show elevation of the right diaphragm in 56 percent of patients with hepatic abscess.[3] The diagnosis of cerebral amebiasis requires careful neurologic evaluation and radiographic evaluation with either CT or MRI.[16,48,60] In one case, *E. histolytica* DNA was detected by PCR in CNS to make the diagnosis.[98] Because of the risk for perforation, barium studies are relatively contraindicated in patients with amebic colitis.

Biopsy Studies

The colonic and rectal mucosa in amebic colitis usually reveals ulcerations with a diameter of 1 to 10 mm. Amebic trophozoites often are at the periphery of these necrotic areas, which can be sampled through a biopsy specimen taken during sigmoidoscopy or colonoscopy.[45,51] Because of the potential for perforation, colonoscopy should be undertaken with caution.

In patients with amebic liver abscesses, amebic trophozoites are found near the capsule of the abscess. Until more recently, the most accurate diagnostic test involved the examination by microscopy of a sample collected from the abscess tissue by needle aspiration, a procedure that is relatively insensitive, identifying amebic trophozoites only 20 percent of the time. PCR in contrast is a sensitive and specific means to identify *E. histolytica* in liver abscess material.[40]

DIFFERENTIAL DIAGNOSIS

Invasive amebic colitis may resemble ulcerative colitis, Crohn disease of the colon (inflammatory bowel disease), bacillary dysentery, or tuberculous colitis.[11,18,45,93] Stool *E. histolytica* antigen or PCR analysis, colonoscopic examination with biopsies, and serologic examination should be able to differentiate amebic colitis from these diseases. Histologic examination of involved colonic mucosa should differentiate amebic colitis, with its lack of inflammation and rare granulation tissue, from the inflammatory responses seen in ulcerative colitis, bacillary dysentery, and Crohn disease of the colon. Tuberculous colitis and Crohn disease are more likely to show granuloma formation than amebiasis. Ileocecal or small bowel involvement as seen on barium studies would suggest Crohn disease or tuberculosis of the gastrointestinal tract, rather than amebiasis. Tuberculous colitis usually is associated with pulmonary tuberculosis and with a strong reaction to tuberculin skin testing. In some cases, differentiating between invasive amebic colitis and inflammatory bowel disease may be impossible. If a patient with this differential diagnosis is placed on corticosteroids and his or her condition deteriorates, the corticosteroids should be stopped and repeat investigation for amebiasis should be performed.[18,70,75]

Amebic liver abscess must be differentiated from pyogenic abscesses and neoplastic lesions. Detection of *E. histolytica* Gal/GalNAc lectin in the sera using the TechLab *E. histolytica* II kit was helpful in one study to diagnose hepatic and intestinal amebiasis before the institution of metronidazole treatment.[41] Total leukocyte counts and cultures of blood may help to differentiate pyogenic and amebic abscesses. Many children with pyogenic liver abscesses have negative blood cultures, however. Often, amebic and pyogenic liver abscesses show similar features on CT and MRI. Occasionally, nuclear imaging with gallium is helpful because, in contrast to a pyogenic abscess, very few neutrophils are contained within an amebic liver abscess.[88,90] Gallium scanning of an amebic liver abscess may reveal a cold spot, possibly with a bright rim. For an individual with risk factors for amebiasis, several investigators recommend instituting treatment for amebic abscess for 3 or 4 days while serologic, antigen detection, or PCR results are awaited.[40,70] Patients with amebic liver abscess should respond to treatment in this length of time by becoming afebrile. No change in size of the liver or size of the abscess should be noted at this time because resolution of the abscess usually takes 2 months to several years.[4,82,83,94,105]

COMPLICATIONS

Complications of amebiasis may be prevented by early establishment of diagnosis and initiation of treatment with appropriate agents.[48,70] When complications occur, the prognosis generally is worse.

Invasive intestinal amebiasis has been associated most commonly with perforation and peritonitis,* which apparently are an end result of "necrotizing" or "toxic" amebic colitis. In children, perforation may be heralded by the appearance of an acute abdomen or pneumoperitoneum, with rapid progression to death, presumably from sepsis.[8,70,104] Surgical resection and therapy for endotoxic shock improve the prognosis.[104] This complication is not rare and accounts for more than 30 percent of deaths from amebiasis in children.[11,52] Massive intestinal hemorrhage causes approximately 3 percent of deaths from amebiasis. Multiple colonic strictures also can occur and cause obstructive symptoms. Fistulas to other organs or to the skin may develop.

Liver abscesses are an unusual manifestation of amebiasis in children, but their resultant complications account for approximately 40 percent of all deaths from amebiasis.[52] Liver abscess also was found in 13 percent of patients with amebiasis at postmortem examinations. Liver abscess with rupture into the abdomen was present in 8 percent of patients who died with amebiasis, and rupture of a liver abscess into the right pleural space was found in 12 percent.[52] Many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid.[86]

*References 8, 10, 50, 70, 96, 104.

In cases free of bacterial contamination, the fluid has few inflammatory cells and an acidic pH. Amebic pericarditis or pneumopericardium occurs rarely and is found in only 1 percent of patients whose deaths were caused by amebiasis.[28,29,34,52] The fluid is similar to that found in the pleural space. A cerebral abscess was found in 4 percent of patients with amebiasis who died.[52] It has been reported in fewer than 10 children, only 1 of whom survived.[9,16,48,60] Other complications include infections of the retroperitoneal space, stomach, spleen, esophagus, and duodenum.[60]

TREATMENT

Intestinal Amebiasis

Asymptomatic Intraluminal Amebiasis

Therapy for asymptomatic colonization differs from therapy for invasive infection. Asymptomatic infections may be treated with intraluminal agents, such as paromomycin or diloxanide furoate. Each agent has a high rate of success for eradication of cyst passage.[67,68] Paromomycin is a nonabsorbable aminoglycoside that is active against the cyst and trophozoite stages. High cure rates have been reported with a 7-day oral dose of paromomycin at 25 to 35 mg/kg/day in three divided doses (Table 209-2). Diloxanide furoate (Furamide) is a poorly absorbed agent that is quite active against only intraluminal amebiasis.[64] Cure rates have been greater than 90 percent with a 10-day oral course of diloxanide furoate at 20 mg/kg/day in three divided doses (maximum, 1500 mg/day).[67,68,76]

Acute Amebic Colitis

Nitroimidazoles, particularly metronidazole, are the mainstay of therapy for invasive amebiasis.[39] The oral dosage of metronidazole is 35 to 50 mg/kg/day (maximum, 2250 mg/day) in three divided doses for 7 to 10 days for severe intestinal or extraintestinal amebiasis. Metronidazole is concentrated in the ameba, probably via reduction of its nitro group by ferredoxin or flavodoxin-like electron transport proteins, which maintain a gradient for the entry of the unchanged drug. Metabolic intermediates of metronidazole damage DNA and possibly other macromolecules, and they deprive the organism of reducing equivalents by acting as an electron sink. Nitroimidazoles with longer half-lives (tinidazole, secnidazole, and ornidazole) are better tolerated and allow shorter periods of treatment.[41] For children 3 years of age or older, the oral dosage of tinidazole is 50 mg/kg/day (to a maximum of 2000 mg/day) for 5 days for severe intestinal or extraintestinal amebiasis (see Table 209-2).

Approximately 90 percent of patients who present with mild-to-moderate amebic dysentery have a response to nitroimidazole therapy. In the rare case of fulminant amebic colitis, adding broad-spectrum antibiotics to treat intestinal bacteria that may spill into the peritoneum is prudent; surgical intervention occasionally is required for acute abdomen, gastrointestinal bleeding, or toxic megacolon.[39] Agents such as metronidazole that are active against invasive and extraintestinal amebiasis are well absorbed and do not stay in the lumen long enough to have an effect on intestinal amebiasis. Parasites persist in the intestine in 40 to 60 percent of patients who receive nitroimidazole. Nitroimidazole treatment should be followed with paromomycin or the second-line agent diloxanide furoate to cure luminal infection.[41] Metronidazole and paromomycin should not be given at the same time because the diarrhea that is a common side effect of paromomycin may render assessing the patient's response to therapy difficult.[52-54]

Extraintestinal Amebiasis

Amebic Liver Abscess and Metastatic Amebiasis

Extraintestinal and severe intestinal amebiasis must be treated with the tissue-active agents. Metronidazole 35 to 50 mg/kg/day in three divided doses for 7 to 10 days is preferred because it is effective and relatively free of serious side effects (see Table 209-2).[2,3,64,88,90] It is effective for extraintestinal amebiasis in any location, although amebic brain abscesses usually are not treated successfully with any medications. Most patients with amebic liver abscess respond to metronidazole within 72 hours. For amebic colitis, follow-up therapy with a luminal agent is very important because of the high rates of asymptomatic intestinal colonization in patients with amebic liver abscess.

Therapeutic aspiration of an amebic liver abscess occasionally is required as an adjunct to antiparasitic therapy. Drainage of the abscess should be considered in patients who have no clinical response to drug therapy within 5 to 7 days or patients with a high risk of experiencing rupture of the abscess, as defined by a cavity with

TABLE 209-2 **Pediatric Dosage of Drugs for Amebiasis**

Type of Disease	Drug	Dosage
Asymptomatic colonization	Paromomycin	25-35 mg/kg/day in 3 doses × 7 days
Mild-to-moderate intestinal disease	Metronidazole	35-50 mg/kg/day in 3 doses × 7-10 days
	Tinidazole	50 mg/kg/day (maximum, 2000 mg) × 3 days
Severe intestinal and extraintestinal disease	Metronidazole	35-50 mg/kg/day in 3 doses × 7-10 days
	Tinidazole	50 mg/kg/day (maximum, 2000 mg) × 3 days

a diameter of more than 5 cm or by the presence of lesions in the left lobe.[103] Because many patients with amebic liver abscess also have anaerobic bacteria in the abscess fluid,[86] addition of antibiotics, drainage, or both to the treatment regimen in the absence of a prompt response to nitroimidazole therapy is reasonable. Image-guided percutaneous treatment (needle aspiration or catheter drainage) has replaced surgical intervention as the procedure of choice for reducing the size of an abscess.[103]

PROGNOSIS

Invasive disease develops in 50 million people each year, and 50,000 to 100,000 deaths result.[81,87,88] The case-fatality ratio is between 1 in 500 and 1 in 1000 diagnosed cases. Among patients with illness severe enough to require hospitalization, the case-fatality ratio is higher. One small study in children reported a 9 percent mortality rate and a 27 percent morbidity rate.[70]

Bowel necrosis or perforation is the cause of death from purely intestinal amebiasis, and early surgical intervention can reduce the mortality rate of these complications from 100 to 28 percent.[104] Amebic liver abscess has a case-fatality rate of 10 to 15 percent in combined figures of adults and children.[56,73,84] The mortality rate when pleural involvement is noted is 14 percent.[49,56] Amebic pericarditis has a case-fatality rate of 40 percent.[34] Cerebral amebiasis is fatal if untreated.[98]

FUTURE CONSIDERATIONS

Amebiasis would be prevented by eradicating fecal contamination of food and water. Providing sanitation and safe food and water for all children in developing countries is an important and achievable goal but will require massive societal changes and monetary investments. An effective amebiasis vaccine is a desirable and feasible goal to help the approximately 1 billion people living in unsanitary conditions. The high incidence of amebiasis in community-based developing country studies of infants, who bear the brunt of morbidity and mortality from diarrhea, suggests that an effective vaccine would improve child health.

The fact that humans naturally acquire partial immunity against intestinal infection indicates that barriers to stimulating an effective acquired immune response should not be insurmountable. Aiding vaccine design is the demonstration that several recombinant antigens, including the Gal/GalNAc-specific lectin, provide protection in animal models of amebiasis and that human immunity is linked to intestinal IgA against the lectin.[33,37,39] The high degree of sequence conservation of the Gal/GalNAc-specific lectin suggests that a vaccine could be broadly protective. Finally, the absence of epidemiologically significant animal reservoirs suggests that herd immunity could interrupt fecal-oral transmission in humans. The challenges will be to design vaccines capable of eliciting durable mucosal immunity, to understand the correlates of acquired immunity, and, most important, to enlist the continued support of industrialized nations to combat diarrheal diseases of children in developing countries.

NEW REFERENCES SINCE THE SIXTH EDITION

23. Duggal P, Guo X, Haque R, et al. A mutation in the leptin receptor is associated with *Entamoeba histolytica* infection in children. *J Clin Invest* 2011;**121**:1191–8.
33. Guo X, Barroso L, Lyerly D, et al. $CD4^+$ and $CD8^+$ T cell- and IL-17–mediated protection against *Entamoeba histolytica* induced by a recombinant vaccine. *Vaccine* 2011;**29**:772–7.
40. Haque R, Kabir M, Noor Z, et al. Diagnosis of amebic liver abscess and colitis by detection of *Entamoeba histolytica* DNA in blood, urine and saliva by real-time PCR assay. *J Clin Microbiol* 2010;**48**:2798–801.
71. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012;**54**:185–92.
91. Royer TL, Gilchrist C, Kabir M, et al. *Entamoeba bangladeshi* n. sp.: a novel *Entamoeba* species identified in children in Bangladesh through small subunit rRNA gene sequencing. *Emerg Infect Dis* 2012;**18**:1543–5.
95. Shimokawa C, Kabir M, Taniuchi M, et al. *Entamoeba moshkovskii* is associated with diarrhea in infants and causes diarrhea and colitis in mice. *J Infect Dis* 2012;**206**:744–51.
97. Solaymani-Mohammadi S, Factor SM, Coyle CM, et al. Amebic colitis in an antigenically and serologically negative patient: usefulness of a small-subunit ribosomal RNA (SSU-rRNA) gene-based PCR in diagnosis. *Diagn Microbiol Infect Dis* 2008;**62**:333–5.
98. Solaymani-Mohammadi S, Lam M, Zunt JR, et al. *Entamoeba histolytica* encephalitis diagnosed by polymerase chain reaction of cerebrospinal fluid. *Trans R Soc Trop Med Hyg* 2007;**101**:311–13.
100. Tanyuksel M, Petri WA Jr. Laboratory diagnosis of amebiasis. *Clin Microbiol Rev* 2003;**16**:713–29.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abd-Alla M, Wahib A, Ravdin JI. Diagnosis of amoebic colitis by antigen capture ELISA in patients presenting with acute diarrhoea in Cairo, Egypt. *Trop Med Int Health* 2002;**7**:1–6.
2. Adams EB, MacLeod IN. Invasive amebiasis, part I: Amebic dysentery and its complications. *Medicine* 1977;**56**:315–23.
3. Adams EB, MacLeod IN. Invasive amebiasis, part II: Amebic liver abscess and its complications. *Medicine* 1977;**56**:325–34.
4. Ahmed L, Salama ZA, El Rooby A, et al. Ultrasonographic resolution time for amebic liver abscess. *Am J Trop Med Hyg* 1989; **41**:406–10.
5. Ali IKM, Hossain MB, Roy S, et al. *Entamoeba moshkovskii* infections in children in *Bangladesh Emerg Infect Dis* 2003;**9**:580–4.
6. Ali IKM, Mondal D, Roy S, et al. Evidence for a link between parasite genotype and outcome of infection with *Entamoeba histolytica*. *J Clin Microbiol* 2007;**45**:285–9.
7. Asgharpour A, Gilchrist C, Baba D, et al. Resistance to intestinal *Entamoeba histolytica* infection is conferred by innate immunity and Gr-1$^+$ cells. *Infect Immun* 2005;**73**:4522–9.
8. Azar H, Nazarian I, Sadrieh M. A study of cause of death in patients with fulminant amebic colitis. *Am J Proctol* 1977;**28**: 80–4.
9. Bachy A. Cerebral abscesses in amebiasis. *J Pediatr* 1976;**88**: 364–5.
10. Balikian JP, Bitar JG, Rishani KK, et al. Fulminant necrotizing amebic colitis in children. *Am J Proctol* 1977;**28**:69–73.
11. Balikian JP, Uthman SM, Kabakian HA. Tuberculous colitis. *Am J Proctol* 1977;**28**:75–9.
12. Barker DC. Differentiation of *Entamoeba*: Patterns of nucleic acids and ribosomes during encystation and excystation. In: Van den Bossche H, editor. *Biochemistry of parasites and host-parasite relationships*. Amsterdam: Elsevier Biomedical; 1976. p. 253.
13. Barwick R, Uzicanin A, Lareau S, et al. Outbreak of amebiasis in Tbilisi, Republic of Georgia, 1998. *Am J Trop Med Hyg* 2002; **67**:623–31.
14. Blessmann J, Van Linh P, Nu PA, et al. Epidemiology of amebiasis in a region of high incidence of amebic liver abscess in central Vietnam. *Am J Trop Med Hyg* 2002;**66**:578–83.
15. Braga LL, Lima AA, Sears CL, et al. Seroepidemiology of *Entamoeba histolytica* in a slum in northeastern *Brazil Am J Trop Med Hyg* 1996;**55**:693–7.
16. Brandt H, Tamayo RP. Pathology of human amebiasis. *Hum Pathol* 1970;**1**:351–85.
17. Caballero-Salcedo A, Viveros-Rogel M, Salvatierra B, et al. Seroepidemiology of amebiasis in *Mexico Am J Trop Med Hyg* 1994; **50**:412–19.
18. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises: Case 32-1977. *N Engl J Med* 1977; **297**:322–30.
19. Chadee K, Petri WA Jr, Innes DJ, et al. Rat and human colonic mucins bind to and inhibit the adherence lectin of *Entamoeba histolytica*. *J Clin Invest* 1987;**80**:1245–54.
20. Councilman WT, Lafleur HA. Amoebic dysentery. *Johns Hopkins Hosp Rep* 1891;**2**:395–548.
21. Denis M, Chadee K. Human neutrophils activated by interferon-gamma and tumour necrosis factor-alpha kill *Entamoeba histolytica* trophozoites in vitro. *J Leukoc Biol* 1989;**46**:270–4.
22. Diamond LS, Clark CG. A redescription of *Entamoeba histolytica* (Schaudinn, 1903) (Emended Walker, 1911) separating it from *Entamoeba dispar* (Brumpt, 1925). *J Eukaryot Microbiol* 1993;**40**: 340–4.
23. Duggal P, Guo X, Haque R, et al. A mutation in the leptin receptor is associated with *Entamoeba histolytica* infection in children. *J Clin Invest* 2011;**121**:1191–8.
24. Duggal P, Haque R, Roy S, et al. Influence of human leukocyte antigen class II alleles on susceptibility to *Entamoeba histolytica* infection in Bangladeshi children. *J Infect Dis* 2004;**189**:520–6.
25. Eckmann L, Reed SL, Smith JR, et al. *Entamoeba histolytica* trophozoites induce an inflammatory cytokine response by cultured human cells through the paracrine action of cytolytically released interleukin-1 alpha. *J Clin Invest* 1995;**96**:1269–79.
26. Eichinger D. A role for a galactose lectin and its ligands during encystment of *Entamoeba*. *J Eukarot Microbiol* 2001;**48**:17–21.
27. Elsdon-Dew R. The epidemiology of amoebiasis. *Adv Parasitol* 1968;**6**:1–62.
28. Freeman AL, Bhoola KD. Pneumopericardium complicating amoebic liver abscess: a case report. *S Afr Med J* 1976;**50**: 551–3.
29. Ganesan TK, Kandaswamy S. Amebic pericarditis. *Chest* 1975; **67**:112–13.
30. Gathiram V, Jackson TF. Frequency distribution of *Entamoeba histolytica* zymodemes in a rural South African population. *Lancet* 1985;**1**:719–21.
31. Gathiram V, Jackson TF. A longitudinal study of asymptomatic carriers of pathogenic zymodemes of *Entamoeba histolytica*. *S Afr Med J* 1987;**72**:669–72.
32. Griffin JL. Human amebic dysentery: electron microscopy of *Entamoeba histolytica* contacting, ingesting, and digesting inflammatory cells. *Am J Trop Med Hyg* 1972;**21**:895–906.
33. Guo X, Barroso L, Lyerly DM, et al. CD4$^+$ and CD8$^+$ T cell- and IL-17-mediated protection against *Entamoeba histolytica* induced by a recombinant vaccine. *Vaccine* 2011;**29**:772–7.
34. Guimaraes AC, Vinhaes LA, Filho AS, et al. Acute suppurative amebic pericarditis. *Am J Cardiol* 1974;**34**:103–6.
35. Haffar A, Boland J, Edwards MS. Amebic liver abscess in children. *Pediatr Infect Dis* 1982;**1**:322–7.
36. Hamano S, Asgharpour A, Stroup SE, et al. Resistance of C57BL/6 mice to amoebiasis is mediated by nonhemopoietic cells but requires hemopoietic IL-10 production. *J Immunol* 2006;**177**:1208–13.
37. Haque R, Ali IKM, Sack RB, et al. Amebiasis and mucosal IgA antibody against the *Entamoeba histolytica* adherence lectin in Bangladeshi children. *J Infect Dis* 2001;**183**:1787–93.
38. Haque R, Duggal P, Ali IKM, et al. Innate and acquired resistance to amebiasis in Bangladeshi children. *J Infect Dis* 2002;**86**: 547–52.
39. Haque R, Huston CD, Hughes M, et al. Amebiasis. *N Engl J Med* 2003;**348**:1565–73.
40. Haque R, Kabir M, Noor Z, et al. Diagnosis of amebic liver abscess and colitis by detection of *Entamoeba histolytica* DNA in blood, urine and saliva by real-time PCR assay. *J Clin Microbiol* 2010;**48**:2798–801.
41. Haque R, Mollah NU, Ali IKM, et al. Diagnosis of amebic liver abscess and intestinal infection with the TechLab *Entamoeba histolytica* II antigen detection and antibody tests. *J Clin Microbiol* 2000;**38**:3235–9.
42. Haque R, Mondal D, Duggal P, et al. *Entamoeba histolytica* infection in children and protection from subsequent amebiasis. *Infect Immun* 2006;**74**:904–9.
43. Haque R, Mondal D, Shu J, et al. Correlation of IFN-γ production by peripheral blood mononuclear cells with childhood malnutrition and susceptibility to amebiasis. *Am J Trop Med Hyg* 2007;**76**:340–4.
44. Haque R, Roy S, Siddique A, et al. Multiplex real-time PCR assay for detection of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* spp. *Am J Trop Med Hyg* 2007;**76**:713–17.
45. Harries J. Amoebiasis: a review. *J R Soc Med* 1982;**75**:190–7.
46. Houpt ER, Glembocki DJ, Obrig TG, et al. The mouse model of amebic colitis reveals mouse strain susceptibility to infection and exacerbation of disease by CD4$^+$ T cells. *J Immunol* 2002; **169**:4496–503.
47. Huston CD, Houpt ER, Mann BJ, et al. Caspase 3–dependent killing of host cells by the parasite *Entamoeba histolytica*. *Cell Microbiol* 2000;**2**:617–25.
48. Huston CD, Petri WA Jr. Host-pathogen interaction in amebiasis and progress in vaccine development. *Eur J Clin Microbiol Infect Dis* 1998;**17**:601–14.
49. Ibarra-Perez C, Selman-Lama M. Diagnosis and treatment of amebic "empyema": report of eighty-eight cases. *Am J Surg* 1977;**134**:283–7.
50. Kala PC, Sharma GC, Haldia KN. Fulminating amoebic colitis with multiple perforations. *Am J Proctol* 1977;**28**:31–4.
51. Katzenstein D, Rickerson V, Braude A, et al. New concepts of amebic liver abscess derived from hepatic imaging, serodiagnosis, and hepatic enzymes in 67 consecutive cases in San Diego. *Medicine (Baltimore)* 1982;**61**:237–46.
52. Kean BH, Gilmore HR Jr, Van Stone WW. Fatal amebiasis: report of 148 fatal cases from the Armed Forces Institutes of Pathology. *Ann Intern Med* 1956;**44**:831–43.
53. Keene WE, Petitt MG, Allen S, et al. The major neutral proteinase of *Entamoeba histolytica*. *J Exp Med* 1986;**163**:536–49.

54. Kelsall BL, Ravdin JI. Proteolytic degradation of human IgA by *Entamoeba histolytica*. *J Infect Dis* 1993;**168**:1319–22.
55. Kelsall BL, Ravdin JI. Immunization of rats with the 260-kilodalton *Entamoeba histolytica* galactose-inhibitable lectin elicits an intestinal secretory immunoglobulin A response that has in vitro adherence-inhibitory activity. *Infect Immun* 1995;**63**:686–9.
56. Lamont AC, Wicks ACB. Amoebic liver abscess in Rhodesian Africans. *Trans R Soc Trop Med Hyg* 1976;**70**:302–5.
57. Leippe M, Ebel S, Schoenberger OL, et al. Poreforming peptide of pathogenic *Entamoeba histolytica*. *Proc Natl Acad Sci U S A* 1991;**88**:7659–63.
58. Leo M, Haque R, Kabir M, et al. Evaluation of *Entamoeba histolytica* antigen and antibody point-of-care tests for the rapid diagnosis of amebiasis. *J Clin Microbiol* 2006;**44**:4569–71.
59. Lidell ME, Moncada DM, Chadee K, et al. *Entamoeba histolytica* cysteine proteases cleave the MUC2 mucin in its C-terminal domain and dissolve the protective colonic mucus gel. *Proc Natl Acad Sci U S A* 2006;**103**:9298–303.
60. Lombardo L, Alonso P, Arroyo LS, et al. Cerebral amebiasis: report of 17 cases. *J Neurosurg* 1964;**21**:704–9.
61. Losch F. Massenhafte entwicklung von amoben in dickdarm. *Virchows Arch [A]* 1975;**65**:196–211.
62. Mahmoud AAF, Warren KS. Algorithms in the diagnosis and management of exotic diseases, part XVII: amebiasis. *J Infect Dis* 1976;**134**:639–43.
63. Mai Z, Ghosh S, Frisardi M, et al. Hsp60 is targeted to a cryptic mitochondrion-derived organelle ("crypton") in the microaerophilic protozoan parasite *Entamoeba histolytica*. *Mol Cell Biol* 1999;**19**:2198–205.
64. Mandell WF, Neu HC. Parasitic infections: therapeutic considerations. *Med Clin North Am* 1988;**72**:669–90.
65. Markwalder K. Left-side pleuropulmonary amoebiasis: a case report from Chad. *Trans R Soc Trop Med Hyg* 1981;**75**:308–9.
66. Martinez-Palomo A, Tsutsumi V, Anaya-Velazquez F, et al. Ultrastructure of experimental intestinal invasive amebiasis. *Am J Trop Med Hyg* 1989;**41**:273–9.
67. McAuley JB, Herwaldt BL, Stokes SL, et al. Diloxanide furoate for treating asymptomatic *Entamoeba histolytica* cyst passers: 14 years' experience in the United States. *Clin Infect Dis* 1992;**15**:464–8.
68. McAuley JB, Juranek DD. Luminal agents in the treatment of amebiasis. *Clin Infect Dis* 1992;**14**:1161–2.
69. Mendoza JB, Barba EJR. Cutaneous amebiasis of the face: a case report. *Am J Trop Med Hyg* 1986;**35**:69–71.
70. Merritt RJ, Coughlin E, Thomas DW, et al. Spectrum of amebiasis in children. *Am J Dis Child* 1982;**136**:785–9.
71. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012;**54**:185–92.
72. Mondal D, Petri WA Jr, Sack RB, et al. *Entamoeba histolytica*-associated diarrheal illness is negatively associated with the growth of preschool children: evidence from a prospective study. *Trans R Soc Trop Med Hyg* 2006;**100**:1032–8.
73. Moorthy B, Mehta S, Mitra SK, et al. Amoebic liver abscess in a four-month-old infant. *Aust Paediatr J* 1977;**13**:53–5.
74. Osler W. On the *Amoeba coli* in dysentery and in dysenteric liver abscess. *Johns Hopkins Hosp Bull* 1890;**1**:53–4.
75. Patterson M, Schoppe LE. The presentation of amoebiasis. *Med Clin North Am* 1982;**66**:689–705.
76. Pehrson P, Bengtsson E. Treatment of non-invasive amoebiasis: A comparison between tinidazole alone and in combination with diloxanide furoate. *Trans R Soc Trop Med Hyg* 1983;**77**:845–6.
77. Petri WA Jr, Haque R, Mann BJ. The bittersweet interface of parasite and host: Lectin-carbohydrate interactions during human invasion by the parasite *Entamoeba histolytica*. *Annu Rev Microbiol* 2002;**56**:39–64.
78. Petri WA Jr, Smith RD, Schlesinger PH, et al. Isolation of the galactose binding lectin which mediates the in vitro adherence of *Entamoeba histolytica*. *J Clin Invest* 1987;**80**:1238–44.
79. Pittman FE, El-Hashimi WK, Pittman JC. Studies of human amebiasis, part I: clinical and laboratory findings in eight cases of acute amebic colitis. *Gastroenterology* 1973;**65**:581–7.
80. Pittman FE, El-Hashimi WK, Pittman JC. Studies of human amebiasis, part II: light and electron microscopic observations of colonic mucosa and exudate in acute amebic colitis. *Gastroenterology* 1973;**65**:588–603.
81. Prevention and control of intestinal parasitic infections. *WHO Tech Rep* 1987;**749**:1–80.
82. Radin DR, Ralls PW, Colletti PM, et al. CT of amebic liver abscess. *AJR Am J Roentgenol* 1988;**150**:1297–301.
83. Ralls PW, Henley DS, Colletti PM, et al. Amebic liver abscess: MR imaging. *Radiology* 1987;**165**:801–4.
84. Ramachandran S, Goonatillake HD, Induruwa PAC. Syndromes in amoebic liver abscess. *Br J Surg* 1976;**63**:220–5.
85. Ramachandran S, Induruwa PAC, Perera MVF. pH of amoebic liver pus. *Trans R Soc Trop Med Hyg* 1976;**70**:159–60.
86. Rani R, Murthy RS, Bhattacharya S, et al. Changes in bacterial profile during amebiasis: demonstration of anaerobic bacteria in ALA pus samples. *Am J Trop Med Hyg* 2006;**75**:880–5.
87. Ravdin JI. *Entamoeba histolytica*: from adherence to enteropathy. *J Infect Dis* 1989;**159**:420–9.
88. Ravdin JI. *Amebiasis Clin Infect Dis* 1995;**20**:1453–66.
89. Ravdin JI, Guerrant RL. The role of adherence in cytopathogenic mechanisms of *Entamoeba histolytica*: study with mammalian tissue culture cells and human erythrocytes. *J Clin Invest* 1981;**68**:1305–13.
90. Reed SL, Ravdin JI. Amebiasis. In: Blaser MJ, Smith PD, Ravdin JI, et al, editors. *Infections of the gastrointestinal tract*. New York: Raven Press; 1995. p. 1065–80.
91. Royer TL, Gilchrist C, Kabir M, et al. *Entamoeba bangladeshi* n. sp.: a novel *Entamoeba* species identified in children in Bangladesh through small subunit rRNA gene sequencing. *Emerg Infect Dis* 2012;**18**:1543–5.
92. Saito-Nakano Y, Yasuda T, Nakada-Tsukui K, et al. Rab5-associated vacuoles play a unique role in phagocytosis of the enteric protozoan parasite *Entamoeba histolytica*. *J Biol Chem* 2004;**279**:49497–507.
93. Seydel KB, Li E, Zhang Z, et al. Epithelial cell-initiated inflammation plays a crucial role in early tissue damage in amebic infection of human intestine. *Gastroenterology* 1998;**115**:1446–53.
94. Sheen IS, Chien CSC, Lin DY, et al. Resolution of liver abscesses: comparison of pyogenic and amebic liver abscesses. *Am J Trop Med Hyg* 1989;**40**:384–9.
95. Shimokawa C, Kabir M, Taniuchi M, et al. *Entamoeba moshkovskii* is associated with diarrhea in infants and causes diarrhea and colitis in mice. *J Infect Dis* 2012;**206**:744–51.
96. Sotela-Avila C, Kline M, Silberstein MJ, et al. Bloody diarrhea and pneumoperitoneum in a 10-month-old girl. *J Pediatr* 1988;**113**:1098–104.
97. Solaymani-Mohammadi S, Factor SM, Coyle CM, et al. Amebic colitis in an antigenically and serologically negative patient: usefulness of a small-subunit ribosomal RNA (SSU-rRNA) gene-based PCR in diagnosis. *Diagn Microbiol Infect Dis* 2008;**62**:333–5.
98. Solaymani-Mohammadi S, Lam M, Zunt JR, et al. *Entamoeba histolytica* encephalitis diagnosed by polymerase chain reaction of cerebrospinal fluid. *Trans R Soc Trop Med Hyg* 2007;**101**:311–13.
99. Stauffer W, Ravdin JI. *Entamoeba histolytica*: an update. *Curr Opin Infect Dis* 2003;**16**:479–85.
100. Tanyuksel M, Petri WA Jr. Laboratory diagnosis of amebiasis. *Clin Microbiol Rev* 2003;**16**:713–29.
101. Tarleton JL, Haque R, Mondal D, et al. Cognitive effects of diarrhea, malnutrition, and *Entamoeba histolytica* infection on school age children in Dhaka, Bangladesh. *Am J Trop Med Hyg* 2006;**74**:475–81.
102. Tovar J, Fischer A, Clark CG, et al. The mitosome, a novel organelle related to mitochondria in the amitochondrial parasite *Entamoeba histolytica*. *Mol Microbiol* 1999;**32**:1013–21.
103. vanSonnenberg E, Mueller PR, Schiffman HR, et al. Intrahepatic amebic abscesses: indications for and results of percutaneous catheter drainage. *Radiology* 1985;**156**:631–5.
104. Vargas M, Pena A. Toxic amoebic colitis and amoebic colon perforation in children: an improved prognosis. *J Pediatr Surg* 1976;**11**:223–5.
105. Watt G, Padre LP, Adapon B, et al. Nonresolution of an amebic liver abscess after parasitologic cure. *Am J Trop Med Hyg* 1986;**35**:501–4.
106. Yan L, Stanley SL Jr. Blockade of caspases inhibits amebic liver abscess formation in a mouse model of disease. *Infect Immun* 2001;**69**:7911–14.
107. Yi D, Lee RT, Longo P, et al. Substructural specificity and polyvalent carbohydrate recognition by the *Entamoeba histolytica* and rat hepatic *N*-acetylgalactosamine/galactose lectins. *Glycobiology* 1998;**8**:1037–43.

CHAPTER 210

Blastocystis hominis and *Blastocystis* spp. Infection

Peter J. Hotez

Blastocystis hominis and related *Blastocystis* species are among the most common gastrointestinal protozoa of humans, with a worldwide prevalence that may be greater than 50 percent.[27,29] The infection is believed to be more common in low- and middle-income countries. Other *Blastocystis* spp. have been described from numerous other vertebrates and insects.[28] Although new information about the molecular and cell biology of this organism has been acquired in recent years, considerable controversy about its true taxonomy and life cycle remains.[4,27,29,31,32] The pathogenicity of *B. hominis* and its ability to cause gastrointestinal illness in humans are equally controversial.[10,11,13,16,24]

ETIOLOGY AND PATHOGENESIS

Since its discovery in the early part of the 20th century by Alexeieff[1] and then Brumpt,[6] *B. hominis* has been assigned to many different phyla in animal and plant kingdoms. Early on, it was identified by various workers as vegetable material, a yeast, a fungus, or a protozoan. Although the organism was identified as a polymorphic protozoan parasite in 1967, its subphylum status bounced between the Sporozoa and the Sarcodina.[4] Nucleic acid sequencing data now suggest that *B. hominis* does not belong to either category[13,14] but instead probably constitutes its own group (class Blastocystea) more closely related to the stramenopiles, a heterogeneous group of unicellular and multicellular protists that includes slime nets, water molds, and brown algae.[29] Additional molecular taxonomic data suggest that the intraspecific variation among stocks of *B. hominis* is sufficiently different to warrant multiple separate species assignments for the organism.[5,29] There are at least 10 recognized subtypes within the *Blastocystis* genus, with ST1 and ST3 being the most common ones isolated from humans.[26] The difference between the subtypes ultimately may have a bearing on the current controversies surrounding the pathogenicity of the organism and different modes of transmission (i.e., zoonotic and human to human).[29] ST3 is believed to be of human origin, whereas the others may be zoonotically transmitted to humans from a variety of animal sources.[28]

Ultrastructural data gathered from light and electron microscopy on organisms obtained from in vitro culture and from fresh fecal material indicate the existence of at least six different parasite forms. These include cyst forms; ameboid forms; and the so-called granular, avacuolar, vacuolar, and multivacuolar forms.[4,26] The *B. hominis* vacuolar cell is the most distinctive and appears as a thin peripheral band of cytoplasm surrounding a large membrane-enclosed central vacuole.[4] The central vacuole may have a storage function. Although *B. hominis* is thought to have predominantly anaerobic metabolism, structures that look like mitochondria have been identified on transmission electron microscopy. *Blastocystis* mitochondria may function in only lipid biosynthesis and not oxidative phosphorylation.[4,31] Alternatively, what appears to be a *Blastocystis* mitochondrion on electron microscopy actually may be a hydrogenosome.[4]

Several highly speculative life cycles of *B. hominis* have been proposed.[4,14] The infective stage probably is a dormant cyst form that undergoes excystation in response to host gastric acid and intestinal enzymes. Excystation may result in the release of avacuolar forms that can undergo facultative transformation to either an ameboid or a multivacuolar form. The multivacuolar form either may encyst to an infective stage or may coalesce to the vacuolar form.[4] These stages are thought to predominate in the large intestine, although organisms also have been recovered from duodenal aspirates. Which if any of these life cycle stages invades tissue or causes disease is unknown. Acquisition of knowledge in this area has been hampered by lack of a suitable animal model, although infection of gnotobiotic guinea pigs with this organism was reported to result in mild intestinal hyperemia and superficial invasion of *B. hominis* into the mucosa of the cecum. Nonspecific inflammation (infiltration of lymphocytes and plasmocytes) and edema of the colonic mucosa have been seen during sigmoidoscopy with biopsy in some patients.[4] So far, except in a subset of patients with irritable bowel syndrome who reportedly show elevated IgG2 antibody,[12] no serologic antibody response to the organism has been shown.[7] The possibility remains that *B. hominis* is entirely commensal in humans.

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS

B. hominis has a worldwide distribution in tropical and temperate regions, with infection rates of 54 percent occurring among some populations.* Developing countries,

*References 2-4, 8, 9, 20, 21, 24, 30.

including Brazil, Egypt, and Indonesia, appear to have a higher prevalence than developed countries.[3,28] High rates of infection also have been reported in individuals with a recent history of travel,[23] with exposure to pets or farm animals,[8] and living in institutionalized settings.[4] In many of these individuals, however, *B. hominis* probably is a commensal parasite. Although *B. hominis* is found commonly in preschool-age and school-age children,[20-22] children overall do not seem to be at increased risk for acquisition of infection.[4] Contaminated water may represent the most common source of *B. hominis* transmission.[28]

Most studies investigating the association between *B. hominis* infection and disease are based primarily on clinical laboratory isolates of the organism in patients exhibiting gastrointestinal symptoms. Common clinical complaints include abdominal discomfort, bloating, cramping, diarrhea, and vomiting.[2] As noted earlier, IgG2 seroconversion to *B. hominis* has been linked to irritable bowel syndrome.[12] Weight loss associated with a protein-losing enteropathy also has been described. Infective arthritis also has been reported.[16] Because *B. hominis* commonly is found in symptomatic and asymptomatic individuals, some investigators have proposed that only very heavy infections result in disease.[17,25] Many of these studies were not controlled, however. Overall, the most common symptoms ascribed to *B. hominis* infection are abdominal pain and diarrhea.[28]

Shlim and colleagues[25] conducted a large prospective controlled study among a population of expatriates and tourists in Katmandu, Nepal, who were at high risk for development of traveler's diarrhea. They concluded that *B. hominis* in high concentrations was not associated with diarrhea and that the presence of higher concentrations of the organism in stool was not associated with more severe symptoms.[25] In a subsequent editorial, several design features were cited that may "weaken the authors' conclusions."[15] At least four other prospective trials have been conducted; one supported *B. hominis* as a cause of diarrhea, one argued against it, and two others were inconclusive.[23] The confusion may be resolved partly by the increasing recognition of separate human subtypes of *Blastocystis*,[5,14] which might lead to improved molecular diagnostic techniques to distinguish pathogenic from nonpathogenic species. This situation is analogous to the morphologically identical species of *Entamoeba* (*Entamoeba histolytica* and *Entamoeba dispar*, only the former of which causes colitis). Some investigators believe that by distinguishing between the different subtypes, at least one or more may be identified as pathogenic, especially among immunocompromised patients.[28]

DIAGNOSIS

Light microscopy of wet preparations of fresh or concentrated stool usually identify *B. hominis*. Staining of preparations with iodine or trichrome also is beneficial. Many laboratories attempt to identify the characteristic vacuolar forms, which may be underrepresented in clinical material.[4] Under these circumstances, the services of an experienced technologist are required to identify the less distinctive fecal cyst form.[29] Organisms also can be recovered from biopsy material obtained during sigmoidoscopy and colonoscopy.

TREATMENT

Given the controversy surrounding the pathogenicity of *B. hominis*, a prudent approach is to refrain from treating asymptomatic immunocompetent individuals.[18] In individuals who have gastrointestinal illness and in whom other pathogens have been excluded, administering a course of antiprotozoal chemotherapy may be reasonable. The results of several drug studies, including three that were placebo controlled, indicate that therapeutic improvements with parasite clearance in symptomatic patients were noted with the use of metronidazole (15 to 30 mg/kg/day for 7 to 10 days), nitazoxanide (200 mg twice daily for children 4 to 11 years old and 100 mg twice daily for children 1 to 3 years old), and trimethoprim-sulfamethoxazole (6 mg/kg trimethoprim and 30 mg/kg sulfamethoxazole daily in two equal doses for 7 days in children; 320 mg trimethoprim and 1600 mg sulfamethoxazole daily in two equal doses for 7 days in adults).[19,28] However, because these drugs are broad spectrum, it is possible that clinical improvement may have been a consequence of treating an unidentified enteric pathogen.[28] To date, the scientific community has been unable to fulfill Koch's postulates for members of the genus *Blastocystis*.[28] Some investigators have reported symptomatic improvement in patients receiving either metronidazole or tinidazole.[4,9] Using an in vitro assay that employed metabolic labeling, researchers found that the drugs emetine, satranidazole, furazolidone, and quinacrine were superior in activity to either metronidazole or tinidazole.[4] The authors caution, however, that the in vitro assay does not take into account the pharmacokinetic properties of the drugs. Furazolidone is available in suspension and may be suitable for pediatric use.[11] Tan[28] recommends metronidazole as a first-line treatment despite some evidence of treatment failures, followed by cotrimoxazole or nitazoxanide as second-line drugs. He recommends treatment if diarrhea is persistent and other pathogens are rigorously excluded.[28] Among the other drugs that have been tried with variable success in patients with *B. hominis* infection are iodoquinol, tinidazole, nitazoxanide, paromomycin, ketoconazole, and ornidazole.[26] Newer studies that examine drug sensitivities of different *Blastocystis* subtypes might help clarify this situation, although currently the status of treating human *Blastocystis* infection remains controversial.[26]

NEW REFERENCES SINCE THE SIXTH EDITION

26. Stensvold CR, Smith HV, Nagel R, et al. Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? *J Clin Gastroenterol* 2010;**44**:85–90.
28. Tan KSW. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* 2008;**21**:639–65.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alexeieff A. Sur la nature des formations dites "kystes de *Trichomonas intestinalis*." *C R Soc Biol* 1911;**71**:296–8.
2. Al-Tawil YS, Gilger MA, Gopalakrishna GS, et al. Invasive *Blastocystis hominis* infection in a child. *Arch Pediatr Adolesc Med* 1994; **148**:882–5.
3. Ashford RW, Atkinson EA. Epidemiology of *Blastocystis hominis* infection in Papua New Guinea: age-prevalence and associations with other parasites. *Ann Trop Med Parasitol* 1992;**86**:129–36.
4. Boreham PF, Stenzel DJ. *Blastocystis* in humans and animals: morphology, biology, and epizootiology. *Adv Parasitol* 1993;**32**:1–70.
5. Boreham PF, Upcroft JA, Dunn LA. Protein and DNA evidence for two demes of *Blastocystis hominis* from humans. *Int J Parasitol* 1992;**22**:49–53.
6. Brumpt E. *Blastocystis hominis* n. sp. et formes voisines. *Bull Soc Pathol Exot Filiales* 1912;**5**:725–30.
7. Chen J, Vaudry WL, Kowalewska K, Wenman W. Lack of serum immune response to *Blastocystis hominis*. *Lancet* 1987;**1**:1021.
8. Doyle PW, Helgason MM, Mathias RG, Proctor EM. Epidemiology and pathogenicity of *Blastocystis hominis*. *J Clin Microbiol* 1990;**28**:116–21.
9. El Masry NA, Bassily S, Farid Z. *Blastocystis hominis*: clinical and therapeutic aspects. *Trans R Soc Trop Med Hyg* 1988;**82**:173.
10. Garcia LS, Bruckner DA, Clancy MN. Clinical relevance of *Blastocystis hominis*. *Lancet* 1984;**1**:1233–4.
11. Hotez P. The other intestinal protozoa: enteric infections caused by *Blastocystis hominis*, *Entamoeba coli*, and *Dientamoeba fragilis*. *Semin Pediatr Infect Dis* 2000;**11**:178–81.
12. Hussain R, Jaferi W, Zuberi S, et al. Significantly increased IgG2 subclass antibody levels to *Blastocystis hominis* in patients with irritable bowel syndrome. *Am J Trop Med Hyg* 1997;**56**:301–6.
13. Jiang JH, He JG. Taxonomic status of *Blastocystis hominis*. *Parasitol Today* 1993;**9**:2–3.
14. Johnson AM, Thanou A, Boreham PF, Baverstock PR. *Blastocystis hominis*: phylogenetic affinities determined by rRNA sequence comparison. *Exp Parasitol* 1989;**68**:283–8.
15. Keystone JS. *Blastocystis hominis* and traveler's diarrhea [editorial]. *Clin Infect Dis* 1995;**21**:102–3.
16. Lee MG, Rawlins SC, Didier M, et al. Infective arthritis due to *Blastocystis hominis*. *Ann Rheum Dis* 1990;**49**:192–3.
17. Logar J, Andlovic A, Poljsak-Prijatel M. Incidence of *Blastocystis hominis* in patients with diarrhoea. *J Infect* 1994;**28**:151–4.
18. Markell EK. Is there any reason to continue treating *Blastocystis* infections [editorial]? *Clin Infect Dis* 1995;**21**:104–5.
19. Moghaddam DD, Ghadirian E, Azami M. *Blastocystis hominis* and the evaluation of efficacy of metronidazole and trimethoprim/sulfamethoxazole. *Parasitol Res* 2005;**96**:273–5.
20. Nimri LF. Evidence of an epidemic of *Blastocystis hominis* infections in preschool children in northern Jordan. *J Clin Microbiol* 1993; **31**:2706–8.
21. Nimri LF, Batchoun R. Intestinal colonization of symptomatic and asymptomatic schoolchildren with *Blastocystis hominis*. *J Clin Microbiol* 1994;**32**:2865–6.
22. O'Gorman MA, Orenstein SR, Proujansky R, et al. Prevalence and characteristics of *Blastocystis hominis* infection in children. *Clin Pediatr* 1993;**32**:91–6.
23. Rossignol JF, Kabil SM, Said M, et al. Effect of nitazoxanide in persistent diarrhea and enteritis associated with *Blastocystis hominis*. *Clin Gastroenterol Hepatol* 2005;**3**:987–91.
24. Senay H, MacPherson D. *Blastocystis hominis*: epidemiology and natural history. *J Infect Dis* 1990;**162**:987–90.
25. Shlim DR, Hoge CW, Rajah R, et al. Is *Blastocystis hominis* a cause of diarrhea in travelers? A prospective controlled study in Nepal. *Clin Infect Dis* 1995;**21**:97–101.
26. Stensvold CR, Smith HV, Nagel R, et al. Eradication of *Blastocystis* carriage with antimicrobials: reality or delusion? *J Clin Gastroenterol* 2010;**44**:85–90.
27. Stenzel DJ, Boreham PF. *Blastocystis hominis* revised. *Clin Microbiol Rev* 1996;**9**:563–84.
28. Tan KSW. New insights on classification, identification, and clinical relevance of *Blastocystis* spp. *Clin Microbiol Rev* 2008;**21**: 639–65.
29. Tan KSW, Singh M, Yap EH. Recent advances in *Blastocystis hominis* research: hot spots in terra incognita. *Int J Parasitol* 2002;**32**: 789–804.
30. Udkow MP, Markell EK. *Blastocystis hominis*: prevalence in asymptomatic versus symptomatic hosts. *J Infect Dis* 1993;**168**:242–4.
31. Zierdt CH. *Blastocystis hominis*, a protozoan parasite and intestinal pathogen of human beings. *Clin Microbiol Newsl* 1983;**5**:57–9.
32. Zierdt CH. *Blastocystis hominis*: past and future. *Clin Microbiol Rev* 1992;**4**:61–79.

CHAPTER 211

Entamoeba coli Infection

Peter J. Hotez

Until recently, *Entamoeba coli* was considered to be entirely nonpathogenic and was of interest to the clinician only because of its morphologic similarities to *Entamoeba histolytica* that might result in misdiagnosis. In 1991, several case reports from northern Europe appeared, however, that implicated *Entamoeba coli* as a possible cause of infectious diarrhea.[2,5,9] Two cases of diarrhea associated with *Entamoeba coli* have been described in children.[2]

ETIOLOGY AND PATHOGENESIS

Entamoeba coli, similar to other members of the genus *Entamoeba*, has trophozoite and cyst forms. The trophozoite is similar in size to that of *E. histolytica* (15 to 50 μm) but has a more sluggish motility with short pseudopodia.[4] The cytoplasm is described as granular, coarse, or frothy and contains numerous bacteria, yeasts, and other food materials.[4,8] Occasionally, red blood cells are seen in the cytoplasm, but their occurrence is not nearly as common as in pathogenic strains of *E. histolytica*. During passage through the colon, the trophozoite rounds up and synthesizes a chitin-containing cyst wall. *Entamoeba coli* cysts measure 10 to 35 μm and usually contain 8 to 16 nuclei, although occasionally 32 nuclei are seen.

Transmission of *Entamoeba coli* infection occurs through the fecal-oral route in the same manner as *E. histolytica* infection. On cyst ingestion, the total number of excysting trophozoites usually is fewer than eight.[4] The *Entamoeba coli* trophozoites colonize the lumen of the large intestine. Very little is known about the events by which *Entamoeba coli* trophozoites occasionally cause human gastrointestinal illness. Even with a pathogenic *Entamoeba coli* strain, the invasive potential of this organism is presumed not to be nearly as great as that of *E. histolytica* because diarrheal disease in patients infected with *Entamoeba coli* is not associated with dysentery or accompanied by a leukocytosis or elevated serum IgA level.[9]

EPIDEMIOLOGY AND CLINICAL MANIFESTATIONS

Entamoeba coli is worldwide in distribution, although it occurs more commonly in warmer climates, especially in tropical urban slums, and in some populations of homosexual men.[4,6] In 1991, Wahlgren[9] described eight patients from Sweden with mild or persistent diarrhea who harbored *Entamoeba coli*. Before receiving specific antiamebic chemotherapy, all eight patients had their stools examined repeatedly by (1) light microscopy, to exclude other protozoa and helminths; (2) electron microscopy, to exclude the presence of some pathogenic viruses; and (3) aerobic and anaerobic culture, to exclude pathogenic bacteria. These patients typically complained of a long history of loose but not watery stools (without blood or mucus), flatulence, and colicky pain. One patient was a parasitology laboratory technician who had symptoms for more than 15 years. Every patient responded to specific antiamebic chemotherapy.[9] Two children with similar symptoms who also responded to antiamebic chemotherapy subsequently were described in Ireland.[2]

DIAGNOSIS AND TREATMENT

Entamoeba coli sometimes is difficult to distinguish from *E. histolytica*, particularly because the nuclear structures of their trophozoite stages are similar.[3] Some differences exist, however, including the karyosome, which is eccentric in *Entamoeba coli* but central in *E. histolytica*, and the cytoplasm, which is coarse and seldom contains red blood cells in *Entamoeba coli*, in contrast to *E. histolytica*.[3] The differences between the cyst stages of *Entamoeba coli* and *E. histolytica* (and *Entamoeba dispar*) are more apparent. The *Entamoeba coli* cyst typically has two to four times more nuclei than cysts in *E. histolytica*. The *Entamoeba coli* cyst has been reported to become more refractive during fixation so that it often is visualized better in a wet preparation.[4] With use of the polymerase chain reaction assay it is possible to distinguish *Entamoeba coli* from other pathogenic amebas.[7]

Generally, *Entamoeba coli* still is regarded by most investigators as a commensal organism. In patients with persistent diarrhea whose diagnostic fecal evaluation reveals only the presence of *Entamoeba coli*, administering a course of specific antiamebic therapy is reasonable.[1] All of the Swedish patients were reported to respond to a 10-day course of diloxanide furoate in a dose of 500 mg three times daily.[7] Children also may respond to an equivalent pediatric dose of 20 mg/kg/day in three divided doses for 10 days. As of 1994, diloxanide furoate was available in the United States from the Centers for Disease Control and Prevention Drug Service. Alternatively, two children from Ireland (where diloxanide

furoate was unavailable) were treated successfully for *Entamoeba coli*–associated diarrhea with metronidazole.[2]

NEW REFERENCES SINCE THE SIXTH EDITION

6. Korkes F, Kumagai FU, Belfort RN, et al. Relationship between intestinal parasitic infection in children and soil contamination in an urban slum. *J Trop Pediatr* 2009;**55**:42–5.

7. Santos HL, Bandea R, Martins LA, et al. Differential identification of *Entamoeba* spp. based on the analysis of 18S rRNA. *Parasitol Res* 2010;**106**:883–8.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 212

Giardiasis

Tina Q. Tan

Giardiasis is caused by an infection with the flagellated, binucleated protozoan parasite *Giardia lamblia* (also known as *Giardia duodenalis* or *Giardia intestinalis*). Worldwide, it is one of the most prevalent and important infectious causes of diarrheal illness in humans. Anton van Leeuwenhoek first described the parasite when examining his own stool under a microscope in 1681. Vilem Lambl described the trophozoite in 1859, and Grassi described the cyst in 1879.[70] This flagellated parasite, which belongs to the genus *Giardia*, family *Hexamitidae*, and supergroup *Excavata*,[98] is one of the most ancient eukaryotes and lacks many organelles, including Golgi apparatus, peroxisomes, and mitochondria.[36] In 2003, however, mitochondrial genes were discovered in the organism; and the organism has been shown to possess specialized membrane structures, known as mitosomes, that perform the respiratory chain activities of the inner mitochondrial membrane. This lends support to the theory that *Giardia* organisms have become highly evolved and have lost many ancestral characteristics.* For centuries, *Giardia* organisms were thought to be nonpathogenic, but, over the past 30 years, data have shown that *G. lamblia* can be a pathogen that causes both sporadic and epidemic disease.[28,46]

THE ORGANISM AND ETIOLOGY

This organism has two morphologic forms: the infectious cyst, which is relatively resistant to chemicals and environmental stresses, and the trophozoite, which is responsible for the disease. The trophozoite is pear shaped, with a dorsal convexity and a spiral organelle on its ventral surface. The spiral organelle or sucking disk is the way in which the organism attaches to the epithelial microvillus of the upper small intestine.[80] The trophozoite usually measures 10 to 21 μm in length and 5 to 15 μm in width. It has a complex cytoskeleton that maintains the shape of the parasite and anchors the four pairs of flagella, which emerge ventrally, anterolaterally, posterolaterally, and caudally from the cell body. It also has two diploid oval symmetric nuclei surrounded by nuclear envelopes. These nuclei are each about 1 μm in diameter and are located in the anterior half of the cell, at the right and left sides of the longitudinal axis. They live in the small intestine and divide by binary fission. The cyst form is most commonly seen in stool samples and is oval, measuring 8 to 12 μm in length and 5 to 10 μm in width. It contains two to four nuclei and remnants of organelles. Cysts can remain viable for long periods of time (≥3 months) in cold water and are resistant to killing by iodine and chlorine, but they can be destroyed by heating to 50° C and by desiccation. This form of the parasite is responsible for transmission among susceptible hosts.[2,14,15] There are six species of *Giardia* that have been identified based on the morphologic characteristics of the trophozoite: *Giardia duodenalis*, *Giardia agilis*, *Giardia muris*, *Giardia ardeae*, *Giardia psittaci*, and *Giardia microti*. *G. lamblia*, also called *G. duodenalis*, is the primary pathogenic species in humans and other mammals. All the other species primarily infect animal species and cause no significant disease in humans. The species that infects humans is morphologically indistinguishable from that of other mammals.[2,46] Isoenzyme and genetic analyses indicate that isolates from infected individuals exhibit high levels of genetic diversity and comprise a large number of genotypes, which can be placed in four groups.[16] The

*References 1, 4, 65, 66, 83, 103, 104.

REFERENCES

1. Cooperstock M, DuPont HL, Corrado ML, et al. Evaluation of new anti-infective drugs for the treatment of diarrhea caused by *Entamoeba histolytica*. *Clin Infect Dis* 1992;**15**(Suppl. 1): S254–8.
2. Corcoran GD, O'Connell B, Gilleece A, Mulvihill TE. *Entamoeba coli* as possible cause of diarrhea. *Lancet* 1991;**338**:254.
3. Despommier DD, Gwadz RW, Hotez PJ. *Parasitic diseases*. 3rd ed. New York: Springer-Verlag; 1995. p. 232–3.
4. Garcia LS, Bruckner DA. *Diagnostic medical parasitology*. 2nd ed. Washington, D.C.: American Society for Microbiology; 1992. p. 18–20.
5. Hotez P. The other intestinal protozoa: enteric infections caused by *Blastocystis hominis*, *Entamoeba coli*, and *Dientamoeba fragilis*. *Semin Pediatr Infect Dis* 2000;**11**:178–81.
6. Korkes F, Kumagai FU, Belfort RN, et al. Relationship between intestinal parasitic infection in children and soil contamination in an urban slum. *J Trop Pediatr* 2009;**55**:42–5.
7. Santos HL, Bandea R, Martins LA, et al. Differential identification of *Entamoeba* spp. based on the analysis of 18S rRNA. *Parasitol Res* 2010;**106**:883–8.
8. Proctor EM. Laboratory diagnosis of amebiasis. *Clin Lab Med* 1991;**11**:829–59.
9. Wahlgren M. *Entamoeba coli* as cause of diarrhoea? *Lancet* 1991;**337**:675.

different isolates within groups demonstrate some specificity for infection in various mammals versus humans, but there are no consistent genetic differences between isolates that cause symptomatic versus asymptomatic disease. The genotypes are grouped into two major genetic assemblages—assemblage A and assemblage B—and several minor assemblages—assemblages C, D, E, F, and G—that have very strong host specificities and narrow host ranges. It is believed that humans are infected only with assemblages A and B.[28] Studies have demonstrated that in symptomatic infection there is a strong correlation between isolate assemblage type and severity of infection; however, the infectivity and pathogenicity of *Giardia* genotypes seem to vary depending on the age, clinical and nutritional status, and immune responses of the host.[2,50,75,86]

EPIDEMIOLOGY

Giardia infections are ubiquitous, and outbreaks occur in developed and underdeveloped countries throughout the world. Cross-transmission and infection occur between domestic and wild animals and humans and between humans.[24,94] Transmission involves the fecal-oral, waterborne, and foodborne transmission of cysts; thus, the level of sanitation and a high intradomiciliary/peridomiciliary concentration of domestic animals are directly related to the prevalence of infection.* *Giardia* is one of the most common parasites in the United States, affecting persons of all ages, and is found in 4.2 to 7 percent of specimens submitted to the laboratory for examination.[56] The populations that are affected most frequently include children from birth to 5 years of age, adults 31 to 40 years of age, and backpackers, campers, hunters, and travelers to areas where the disease is endemic.[51,73] Data from the National Giardiasis Surveillance System of the Centers for Disease Control and Prevention estimate that 2.5 million cases occur each year in the United States.[118] Cases per 100,000 population range from 1.4 to 30, with seven states reporting more than 15 cases per 100,000 population. In 2005, Vermont had the highest incidence, with 30 cases per 100,000 population. Cases are more prevalent in males, and rates are highest in children 1 to 4 years of age (children in daycare and their close contacts), followed closely by children 5 to 9 years of age and adults 31 to 39 years of age. Most cases are reported in the early summer through early fall.[118]

Giardia is a frequent cause of diarrhea in daycare centers around the world, with infection rates of 1 to 55 percent.† Most children are symptomatic, and chronic passage of cysts in some preschool-aged children in daycare centers may last for as long as 5 to 6 months after the initial diagnosis. Transmission from these infants and children to adult family members is not an uncommon occurrence; up to 25 percent of the children have *Giardia*-positive stools. The prevalence rates are highest in centers with many non–toilet-trained children and staff who change diapers and prepare food without adequate hand washing. Prevalence rates decline after children are toilet trained.[9] Sexual transmission may occur with both heterosexual and homosexual contact, but male homosexual behavior is an established risk factor for acquiring *G. lamblia* infection and cyst passage rates may be as high as 20 percent.[49,58,63] Other persons at risk include campers, backpackers, hunters, and other travelers to national parks, wilderness areas, and disease-endemic areas because of vertical transmission from animals and ingestion of untreated drinking water.[114] Patients who were infected during travel had longer exposures in countries where the prevalence was high.[54] In addition, many outbreaks have been reported in municipal water supplies that have not been treated with flocculation or filtration. From 1985 to 1994, *Giardia* was responsible for 44 percent of the outbreaks of waterborne diarrheal illness in which a cause could be determined. Sixty-four percent of these outbreaks were associated with unfiltered water. According to a sample survey of 66 reservoir sites in Canada and the United States, 81 percent contained *Giardia* cysts at a concentration of 3 cysts per liter before treatment and 1.7 cysts per liter after treatment.[60,61] Treatment of water with iodine preparations kills most cysts, but the cysts are relatively resistant to chlorination. Swimming pools may be another source of infection when used by diapered infants and developmentally delayed individuals.[84] A less common mode of transmission is through ingestion of contaminated food, in which as few as 10 cysts may be required to establish infection.[72,88]

PATHOGENESIS

Humans are infected by the oral ingestion of as few as 10 to 25 cysts.[88] Excystation occurs in the stomach and small intestine, and the trophozoites are found in large numbers in the upper part of the small intestine, where they closely apply their sucking disks to the mucosa (Fig. 212-1). They may penetrate into the secretory tubules of the mucosa and, at times, are found in the gallbladder and in the biliary drainage. Scanning electron micrographs of the intestinal mucosa (Fig. 212-2) demonstrate the mechanical damage caused by the presence of the organisms on the mucosal surface. The histologic changes seen in the tissues do not always correlate with the presence or absence of symptoms. Biopsy specimens of the small intestine in children and adults show normal histology despite the presence of diarrhea and other symptoms.[22] On the other hand, flattening of the brush border, damage to mucosal epithelial cells, and slight flattening of the villi with increased mitotic index, increased goblet cells, alteration of bile content or duodenal flora, and infiltration with inflammatory cells also may be present.[23,77,89,117] Studies have demonstrated that *Giardia* disrupts tight junctional zona occludens, increases permeability, and induces apoptosis in small intestinal epithelial cells. Disruption of the intestinal epithelial cell brush border and inhibition of brush-border enzymes and trypsin may explain the lactose intolerance that

*References 30, 52, 57, 67, 93, 97.
†References 3, 9, 59, 76, 79, 82.

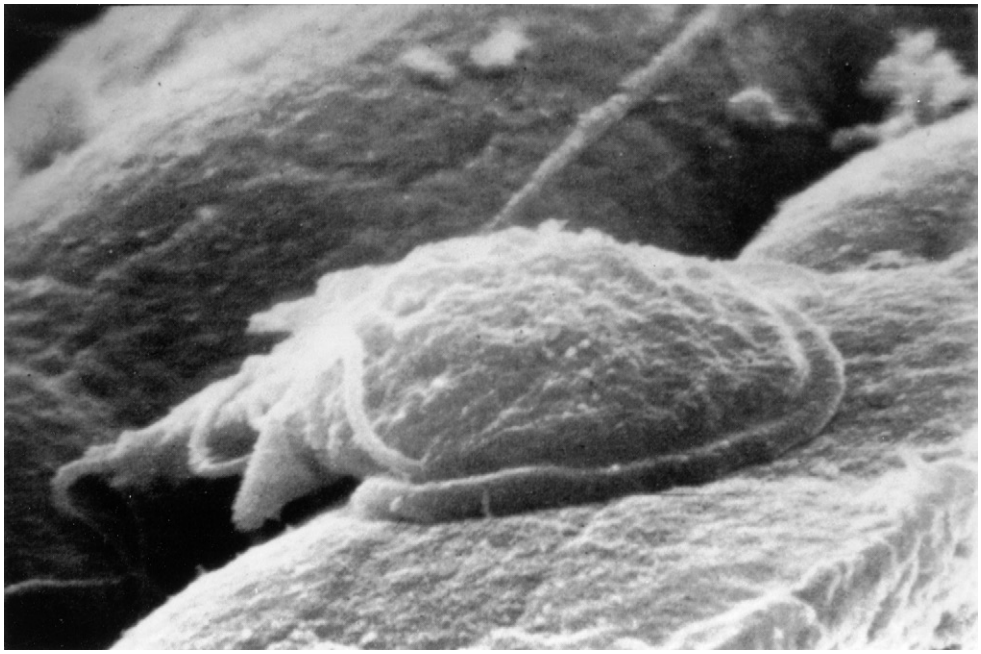

FIGURE 212-1 ■ Trophozoite of *Giardia lamblia* adhering to the intestinal surface.

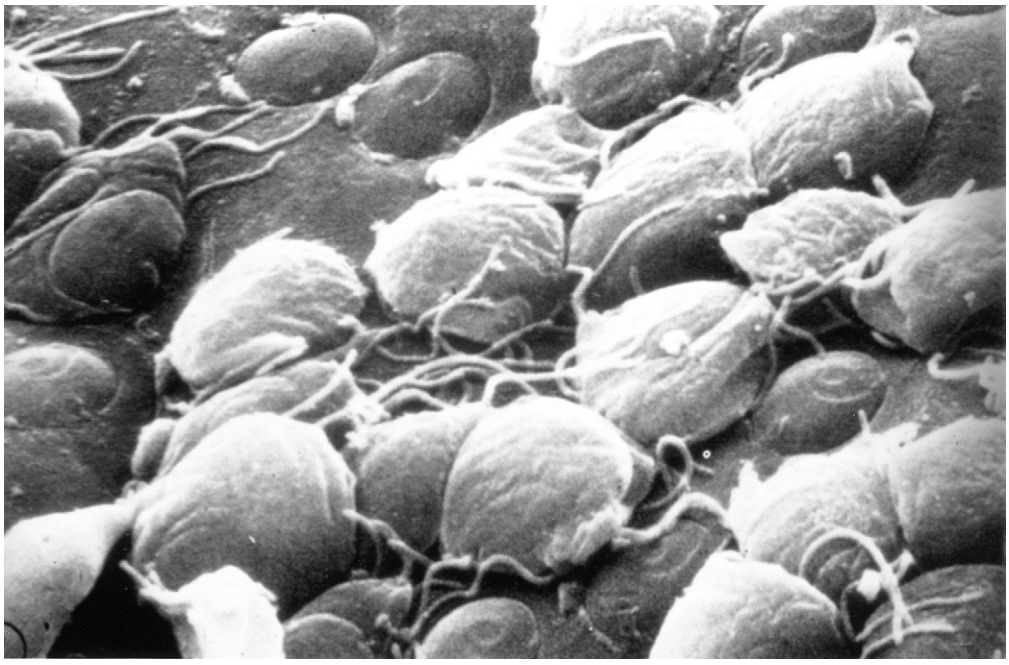

FIGURE 212-2 ■ Scanning electron micrograph of trophozoites of *Giardia lamblia* from an intestinal biopsy. Note the indentations left on the mucosal surface by the parasite's sucking disk.

commonly develops.[12,17] In vivo and in vitro studies have established that *Giardia* causes malabsorption of glucose, sodium, and water and hypersecretion of chloride and reduces disaccharidase activity, owing to shortening of the microvilli in a $CD8^+$ lymphocyte–dependent manner, resulting in loss of epithelial absorptive surface area.[6,11,18,95,96] Disruption of digestion by bile and proteases may produce malabsorption, diarrhea, and resultant malnutrition.

Giardia-infected individuals may have a spectrum of manifestations ranging from asymptomatic carriage to acute or chronic diarrheal disease, the causes of which are not well understood. Hypotheses include (1) the parasite as a physical barrier to absorption, (2) disruption of the brush border with loss of enzymatic activity, (3) elaboration of toxins by the parasite, (4) changes in fat absorption in the small intestine, and (5) activation of cytokines with mucosal inflammation after damage to enterocytes.

Host immunity, both the humoral and cellular components, is important in the variable manifestations of giardiasis and in clearance and protection against reinfection with *Giardia*. Persistence of infection in nude mice suggests a role for T-cell activity.[25,90,102] Humans produce serum immunoglobulin (Ig) G, IgM, IgA, IgE, mast cells, T cells, dendritic cells, and nitric oxide in response to infection.* Studies have shown that IgM and IgG antibody with complement is lethal to *Giardia* trophozoites. In the intestinal lumen, the secretory IgA response appears to play a role in the modulation of infection, as does the migration of infiltrating lymphocytes in the intestinal mucosa. The absence of secretory IgA is associated with the inability to clear *Giardia* infection and is associated with the development of chronic giardiasis in humans.[16,85] Eighty-four percent of human volunteers experienced self-cure in 18.4 days, whereas the remainder became chronically infected.[88] Human milk has been shown to kill the trophozoites of *G. lamblia* by generating toxic lipolytic products.[36,37,87]

Infection may be severe and difficult to eradicate in immunodeficient individuals with antibody deficiencies (e.g., common variable immunodeficiency, X-linked agammaglobulinemia) or in patients with reduced gastric acidity. Giardiasis long has been associated with hypogammaglobulinemia, nodular lymphoid hyperplasia of the small intestine, and chronic diarrhea.[47] Patients with hypogammaglobulinemia, absence of plasma cells in the intestinal lamina propria, cystic fibrosis, protein-calorie malnutrition, and human immunodeficiency virus (or acquired immunodeficiency syndrome) and those who are on immunosuppressive therapy are more likely to develop chronic giardiasis than individuals with X-linked hypogammaglobulinemia, selective IgA deficiency, and Wiskott-Aldrich or Nezelof syndrome.[53,78,99]

CLINICAL MANIFESTATIONS

Infection with *Giardia* can result in asymptomatic infection (5% to 15%), an acute self-limited diarrheal illness (25% to 50%), or chronic diarrhea and malabsorption depending on host susceptibility and pathogen genotype virulence.[6,44,51] The incubation period for giardiasis is 7 to 14 days. Symptoms may vary from mild abdominal discomfort and diarrhea to severe cramping, bloating, and severe, explosive, watery, greasy, foul-smelling diarrhea (Table 212-1).[49] Patients and caretakers describe a constellation of signs and symptoms, including abdominal bloating, flatulence, and frequent foul-smelling diarrhea. Young infants may exhibit anorexia, weight loss, or a malabsorption syndrome that resembles sprue. Symptoms usually last for more than 7 to 10 days and in the majority of cases clear spontaneously. Stooling patterns vary from normal to mushy, foul-smelling diarrhea (Table 212-2). Gross blood, pus, and mucus are usually not present in the stool and signs of inflammatory diarrhea, such as tenesmus and bloody diarrhea, do not occur. *Giardia* infection also may lead to disorders at extraintestinal sites. Extraintestinal symptoms may involve the eyes, joints, and muscles, skin, circulation, and micronutrient deficiencies.[18] A minority of patients will develop persistent, chronic infection that is associated with severe malaise, headache, weight loss, and diffuse abdominal and epigastric discomfort that is exacerbated by eating. Episodes of diarrhea typically alternate with periods of normal bowel movements and may persist over a period of months. Lactose intolerance may develop in 20% to 40% of cases and persist for weeks after the infection has been eradicated.[99,115] Children with symptomatic giardiasis may develop steatorrhea and malabsorption of vitamin A, vitamin B_{12}, protein, D-xylose, and iron.[39,101] Anecdotal reports have attributed a variety of other symptoms, including rash, urticaria, arthralgia, reactive arthritis, constipation, biliary tract disease, and gastric infection to giardiasis, but no firm evidence supports *G. lamblia* as the cause of these symptoms.

Cyst passers may be asymptomatic and may serve as a reservoir of infection for others. This fact is particularly important for food handler and daycare center outbreaks. Severe prolonged illness that requires hospitalization is found in 2 per 100,000 cases, which is similar to that for shigellosis.[62] Hospital admission for young children and pregnant women usually is required because of volume depletion and failure to thrive.[101]

Parasitic infection, including giardiasis, should be considered in the evaluation of the child who fails to thrive and in immunocompromised infants and children who

TABLE 212-1 **Symptoms in Patients with Giardiasis**

Symptom	Frequency (%)
Flatulence	56% to 74%
Anorexia	40% to 64%
Abdominal cramps	55% to 80%
Foul-smelling stool	57% to 72%
Abdominal distention	31%
Bloating	55% to 69%
Nausea	58% to 68%
Malaise	84%
Diarrhea	89%
Belching	30%
Weight loss	48% to 64%
Fever	17% to 28%

TABLE 212-2 **Stool Characteristics in Patients with Giardiasis**

Stool Characteristic	Frequency (%)
Mushy	52
Formed	33
Watery	12
Mucous	3
Blood	0

*References 32, 35, 37, 40, 75, 81, 88, 92, 97, 100, 112.

have diarrhea or gastrointestinal complaints. It also should be considered in the differential diagnosis of any child who is in daycare or who has traveled outside the United States and has gastrointestinal symptoms.[45,76]

DIAGNOSIS

Obtaining a thorough travel and potential exposure history is important in making the diagnosis of giardiasis. Recent travel to wilderness areas or a national park and travel to developing areas or other endemic areas of the world where fecal-oral hygiene is a problem are important parts of the history in any patient with persistent diarrhea. Demonstration of trophozoites or cysts in the stool on ova and parasite (O&P) examination is the traditional standard of diagnosis.[71] Examination of preserved fecal specimens reveals cysts or trophozoites in most infections. Organisms are excreted in a highly variable pattern; therefore, multiple samples taken on different days are required for detection.[21] A single stool sample misses 20 to 40 percent of the infections, whereas three stool samples miss less than 10 percent of infections.[48]

Commercially available tests to detect antigen by an enzyme-linked immunosorbent assay (ELISA) have been shown to be rapid and highly sensitive and specific but are qualitative and are unable to distinguish between genotypes or detect low levels of infection.[55,113] The ELISA may be particularly useful in screening mass outbreaks and assessing cure. Monoclonal antibodies usually are employed to detect antigen either by an ELISA or immunofluorescence assay. Some of these assays have a sensitivity of 91 to 95 percent and specificities of over 98 percent.[27,68,113] Antigen may be best detected when the stool is preserved in formalin.[27] These tests do not replace microscopic examination of the stool, which is considered to be the standard, because of the possibility of infection with multiple organisms in travelers.

Molecular techniques such as polymerase chain reaction (PCR) are alternative methods that may be used for the specific detection of *Giardia* in the stool. These techniques in combination with restriction fragment length polymorphism or nested PCR may be used to genotype the organisms. The sensitivity of detection by PCR is greater than that of microscopy and is much better for detection of low number of parasites in stool samples.[8] A recent advancement in PCR-based technology for the detection of *G. lamblia* in stool is the use of real-time PCR. This method, using dual-labeled fluorescent probes targeting the β-giardin gene, is sensitive, is rapid, and may be used to detect *G. lamblia* in stool and to differentiate the major genotypes of the organism. It can be adapted to high-throughput detection for the screening of large numbers of samples, especially in outbreak situations.[13,42]

Even with the available diagnostic tests, infections may be missed in some patients; and in cases with a high index of suspicion, the organism may be recovered by duodenal biopsy or duodenal aspiration. Invasive techniques may be an important diagnostic adjunct in immunosuppressed individuals and in those with sprue, for whom histology of the bowel also may be important in planning therapy.

TREATMENT

All patients with either cysts or trophozoites in the stool should be treated. Multiple classes of drugs are available for the treatment of giardiasis, each with different clinical properties and efficacies, as shown in Table 212-3.[5,33,116] The nitroimidazole class of agents includes metronidazole, tinidazole, ornidazole, and secnidazole and is the mainstay of therapy for the treatment of giardiasis. These drugs are administered orally in their inactive probiotic form.[33] Of the nitroimidazoles, metronidazole has been the most extensively studied and widely used worldwide. The mechanism of killing of *Giardia* by the nitroimidazoles involves the utilization of the anaerobic metabolic pathways of the organism. The drug diffuses into the trophozoite, where it is activated and inhibits trophozoite respiration and causes cellular damage by the production of toxic radicals and loss of DNA helical structure, resulting in trophozoite death.[38,107] Of the nitroimidazoles, tinidazole and metronidazole have demonstrated the greatest in vitro activity.[20,41] Metronidazole is considered the drug of choice for treatment, with tinidazole being an alternative.[31,33] Although albendazole has been shown to be curative either in single doses or a 5-day course, it is not approved for use in giardiasis in the United States.[43,120] Quinacrine, which is inexpensive and has excellent efficacy, also is no longer available in the United States.[33] It may cause hemolysis in children with glucose-6-phosphate dehydrogenase deficiency. Furazolidone is an effective alternative and is available in a liquid form. However, it is administered four times a day for 7 to 10 days and is associated with side effects that include rash, nausea, and vomiting. Tinidazole has been shown to eliminate the parasite after single-dose therapy.[31] Another member of the 5-nitromidazoles, secnidazole has been shown to be well tolerated at 30-mg/kg/day (maximum, 2 g) and is an option for therapy.[38] Paromomycin, an aminoglycoside that is not well absorbed, is the only drug that can be used in a pregnant patient. It is not as effective as other medications in eradicating the parasite but may induce clinical improvement. A 3-day course of nitazoxanide has been shown to treat successfully 85 percent of children with diarrhea caused by *Giardia*.[29,91] Although outbreaks in daycare centers are common, treating all children in the daycare center is not efficacious because recurrence rates are high and a chance of a drug reaction always exists. Treatment is reserved for symptomatic children.

Treatment failures and the development of drug resistance have been reported with all of the common agents used to treat *Giardia*, including albendazole, furazolidone, metronidazole, and quinacrine.[5] The prevalence of clinical metronidazole-resistant cases is reported to be up to 20 percent[10,26,106,109,111] with recurrence rates up to 90 percent.[119] Metronidazole-resistant *Giardia* strains have also manifested cross-resistance to tinidazole.[106,109,111] Furazolidone-resistant *Giardia* species can be induced both in vitro and in vivo, and this may induce the organism to become resistant more easily to quinacrine.[69,108,110] *Giardia* resistant to albendazole has been reported to develop rapidly in vitro. Albendazole resistance also

TABLE 212-3 **Drugs for the Treatment of Giardiasis**

Drug	Dose	Efficacy (%)	Side Effects
Metronidazole	15 mg/kg/day orally divided three times a day for 5-10 days (max, 750 mg/day)	80-100	Metallic taste, headache, nausea, vomiting, rash, peripheral neuropathy, neutropenia, disulfiram-like effects
Tinidazole	50 mg/kg, single dose (max, 2 g)	80-96	Metallic taste, nausea, vomiting, headache, rash, peripheral neuropathy, neutropenia, disulfiram-like effects
Ornidazole*	40-50 mg/kg, single dose (max, 2 g)	96-100	Metallic taste, nausea, vomiting, headache, rash, peripheral neuropathy, neutropenia, disulfiram-like effects
Secnidazole	2 g, single dose	90	Nausea, anorexia, abdominal pain
Albendazole†	15 mg/kg/day for 5-7 days (max, 400 mg/day)	94-100	Anorexia, constipation, neutropenia, elevated liver function test results
Furazolidone	6 mg/kg/day orally divided four times a day for 10 days	81-96	Allergic reactions, headache, nausea, vomiting, diarrhea, brown discoloration of urine, hemolysis, disulfiram-like effects
Paromomycin	30 mg/kg/day divided three times a day for 5-10 days (max 1.5 g/day)	55-88	Nausea, vomiting, ototoxicity, nephrotoxicity
Quinacrine*	6 mg/kg/day divided three times a day for 7 days	92-95	Nausea, vomiting, dizziness, headache, yellow/orange skin and mucous membrane discoloration, hemolysis, toxic psychosis
Nitazoxanide	1 to 3 years: 200 mg/day divided twice daily for 3 days 4 to 11 years: 400 mg/day divided twice daily for 3 days ≥12 years: 1000 mg/day divided twice daily for 3 days	85-90	Nausea, abdominal pain, diarrhea, anorexia, flatulence, headache, yellow eyes, discolored urine, increased creatinine and serum glutamic-pyruvic transaminase levels

*No longer produced in United States. Can be obtained from Panorama Pharmacy, Panorama City, Calif.
†Not FDA approved for this indication in the United States.

develops more easily in *Giardia* strains that are furazolidone resistant, giving rise to multidrug-resistant phenotypes.[64,105]

Patients with clinically resistant strains have been treated with longer repeat courses or higher doses of the initial agent[34,74]; however, the most effective means of eradicating these infections seems to be the use of an anti-*Giardia* agent from a different class to avoid potential cross-resistance.[9,19,35] For *Giardia* strains that are phenotypically considered to be multidrug resistant, the administration of anti-*Giardia* drugs in combination is a method that may be used for the successful treatment of the infection.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Ankarklev J, Jerlstrom-Hultqvist J, Ringqvist E, et al. Behind the smile: cell biology and disease mechanisms of *Giardia* species. *Nature Rev Microbiol* 2010;**8**:413–22.
14. Carranza PG, Lujan HD. New insights regarding the biology of *Giardia lamblia*. *Microbes Infect* 2010;**12**:70–80.
18. Cotton JA, Beatty JK, Buret AG. Host-parasite interactions and pathophysiology of *Giardia* infections. *Int J Parasitol* 2011;**41**: 925–33.
28. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin Microbiol Rev* 2011;**24**(1): 110–40.
83. Plutzer J, Ongerth J, Karanis P. *Giardia* taxonomy, phylogeny and epidemiology: facts and open questions. *Int J Hyg Environ Health* 2010;**213**:321–33.
85. Prucca CG, Lujan HD. Antigenic variation in *Giardia lamblia*. *Cell Microbiol* 2009;**11**:1706–15.
103. Touz MC, Rivero MR, Miras SL, Bonifacino JS. Lysosomal protein trafficking in *Giardia lamblia*: common and distinct features. *Front Biosci (Elite Ed)* 2012;**4**:1898–901.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adam RD. The *Giardia lamblia* genome. *Int J Parasitol* 2000; **30**:475–84.
2. Adam RD. Biology of *Giardia lamblia*. *Clin Microbiol Rev* 2001; **14**:447–75.
3. Addis DG, Stewart JM, Finton RJ, et al. *Giardia lamblia* and *Cryptosporidium* infections in child day-care centers in Fulton County, Georgia. *Pediatr Infect Dis* 1991;**10**:907–11.
4. Ali SA, Hill DR. *Giardia* intestinalis. *Curr Opin Infect Dis* 2003; **16**:453–60.
5. Ali V, Nozaki T. Current therapeutics, their problems, and sulfur-containing amino-acid metabolism as a novel target against infections by "amitochondriate" protozoan parasites. *Clin Microbiol Rev* 2007;**20**(1):164–7.
6. Ankarklev J, Jerlstrom-Hultqvist J, Ringqvist E, et al. Behind the smile: cell biology and disease mechanisms of *Giardia* species. *Nature Rev Microbiol* 2010;**8**:413–22.
7. Centers for Disease Control and Prevention. Waterborne giardiasis—California, Colorado, Oregon, Pennsylvania. *MMWR Morb Mortal Wkly Rep* 1980;**29**:121.
8. Bialek R, Binder N, Dietz A, et al. Comparison of fluorescence, antigen and PCR assays to detect *Cryptosporidium parvum* in fecal specimens. *Diagn Microbiol Infect Dis* 2002;**43**:283–8.
9. Black RE, Sinclair SP, Wells JG. Giardiasis in day care centers: evidence of person-to-person transmission. *Pediatrics* 1977;**60**: 486–91.
10. Boreham PF, Phillips RE, Shepherd RS. Altered uptake of metronidazole *in vitro* by stocks of *Giardia intestinalis* with different drug sensitivities. *Trans R Soc Trop Med Hyg* 1988;**82**: 104–6.
11. Buret AG. Mechanisms of epithelial dysfunction in giardiasis. *Gut* 2007;**56**:316–17.
12. Buret AG, Mitchell K, Muench DG, et al. *Giardia lamblia* disrupts tight junctional ZO-1 and increases permeability in non-transformed human small intestinal epithelial monolayers: effects of epidermal growth factor. *Parasitology* 2002;**125**:11–19.
13. Caccio SM, DeGiacomo M, Pozio E. Sequence analysis of the β-giardin gene and development of a polymerase chain reaction-restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. *Int J Parasitol* 2002;**32**:1023–30.
14. Carranza PG, Lujan HD. New insights regarding the biology of *Giardia lamblia*. *Microbes Infect* 2010;**12**:70–80.
15. Chadhuri P, De A, Bhattacharya A, Pal S. Identification of heterogeneity in human isolates of *Giardia lamblia* by isoenzyme studies. *Zentralbl Bakteriol* 1991;**274**:490–5.
16. Char S, Cevallos AM, Yamson P, et al. Impaired IgA response to *Giardia* heat shock antigen in children with persistent diarrhea and giardiasis. *Gut* 1993;**34**:38–40.
17. Chin AC, Teoh DA, Scott KG, et al. Strain-dependent induction of enterocyte apoptosis by *Giardia lamblia* disrupts epithelial barrier function in a caspase-3-dependent manner. *Infect Immun* 2002;**70**:3673–80.
18. Cotton JA, Beatty JK, Buret AG. Host-parasite interactions and pathophysiology of *Giardia* infections. *Int J Parasitol* 2011;**41**: 925–33.
19. Craft JC, Murphy T, Nelson JD. Furazolidone and quinacrine: comparative study of therapy for giardiasis in children. *Am J Dis Child* 1981;**135**:164–6.
20. Crouch AA, Seow WK, Thong YH. Effect of twenty-three chemotherapeutic agents on the adherence and growth of *Giardia lamblia* in vitro. *Trans R Soc Trop Med Hyg* 1986;**80**:893–6.
21. Danciger M, Lopez M. Numbers of *Giardia* in the feces of infected children. *Am J Trop Med* 1975;**24**:237–41.
22. Da Silva JR, Coutinho SG, Dias L, et al. Histopathologic changes in giardiasis: a biopsy study. *Am J Dig Dis* 1964;**9**:355–65.
23. Duncombe VM, Bolin TD, Davis AE, et al. Histopathology in giardiasis: a correlation with diarrhea. *Aust N Z J Med* 1978;**8**: 392–6.
24. Dykes AC, Juranekl DD, Lorenz RA, et al. Municipal waterborne giardiasis: an epidemiological investigation: beavers implicated as a possible reservoir. *Ann Intern Med* 1980;**92**:165–70.
25. Eckmann L. Mucosal defenses against *Giardia*. *Parasite Immunol* 2003;**25**:259–70.
26. Farthing MJ. Giardiasis. *Gastroenterol Clin N Am* 1996;**25**:493–515.
27. Fedorko DP, Williams EC, Nelson NA, et al. Performance of three enzyme immunoassays and two different fluorescence assays for detection of *Giardia lamblia* in stool specimens preserved in ECOFIX. *J Clin Microbiol* 2000;**38**:2781–3.
28. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and Giardiasis. *Clin Microbiol Rev* 2011;**24**(1): 110–40.
29. Fox LM, Saravolatz LD. Nitazoxanide: a new thiazolide antiparasitic agent. *Clin Infect Dis* 2005;**40**:1173–80.
30. Fraser CT, Cooke K. Epidemic giardiasis and municipal water supply. *Am J Public Health* 1991;**81**:760–2.
31. Fung HB, Doan TL. Tinidazole: a nitroimidazole antiprotozoal agent. *Clin Ther* 2005;**27**(12):1859–84.
32. Furness BW, Beach MJ, Roberts JM. Giardiasis surveillance—United States 1992–1997. *MMWR Morb Mortal Wkly Rep* 2000; **49**:1–13.
33. Gardner TB, Hill DR. Treatment of giardiasis. *Clin Microbiol Rev* 2001;**14**:114–28.
34. Garg BK. Furzolidone and metronidazole in the treatment of giardiasis. *Indian J Pediatr* 1972;**39**:264–6.
35. Gascon J, Abos R, Valls ME, et al. Mebendazole and metronidazole in giardial infections. *Trans R Soc Trop Med Hyg* 1990; **84**:694.
36. Gillin FD, Reiner DS, McCaffery JM. Cell biology of the primitive eucaryote *Giardia lamblia*. *Annu Rev Microbiol* 1996; **50**:679.
37. Gillin FD, Reiner DS, Wang CS. Killing of *Giardia lamblia* trophozoites by normal human milk. *J Cell Biochem* 1983;**23**:47–56.
38. Gillis JC, Wiseman LR. Secnidazole: a review of its antimicrobial activity, pharmacokinetic properties and therapeutic use in the management of protozoal infections and bacterial vaginosis. *Drugs* 1996;**51**:621–8.
39. Gillon J. Clinical studies in adults presenting with giardiasis to a gastrointestinal unit. *Scott Med J* 1985;**30**:89–95.
40. Goka JAK, Mathan VI, Rolston DDK, et al. Diagnosis of giardiasis by specific IgM antibody enzyme-linked immunosorbent assay. *Lancet* 1989;**2**:184–6.
41. Gordts B, Hemelhof W, Asselman C, et al. In vitro susceptibilities of 25 *Giardia lamblia* isolates of human origin to six commonly used antiprotozoal agents. *Antimicrob Agents Chemother* 1985;**28**: 378–80.
42. Guy RA, Xiao C, Horgen PA. Real-time PCR assay detection and genotype differentiation of *Giardia lamblia* in stool specimens. *J Clin Microbiol* 2004;**42**(7):3317–20.
43. Hall A, Nahar Q. Albendazole as a treatment for infections with *Giardia duodendalis* in children in Bangladesh. *Trans R Soc Trop Med Hyg* 1993;**87**:84–6.
44. Haque R, Roy S, Kabir M, et al. *Giardia* assemblage A infection and diarrhea in Bangladesh. *J Infect Dis* 2005;**192**:2171–3.
45. Hardie RM, Wall PG, Gott P, et al. Infectious diarrhea in tourists staying in a resort hotel. *Emerg Infect Dis* 1999;**5**:168–71.
46. Heresi G, Cleary TG. *Giardia*. *Pediatr Rev* 1997;**18**:243–7.
47. Hermans PE, Huizemnga KA, Hoffman HN, et al. Dysgammaglobinemia associated with nodular hyperplasia of the small intestine. *Am J Med* 1965;**40**:78–89.
48. Hiatt RA, Markell EK, Ng E. How many stool examinations are necessary to detect pathogenic intestinal protozoa? *Am J Trop Med Hyg* 1995;**53**:36–9.
49. Hill DR. Giardiasis: issues in diagnosis and management. *Infect Dis Clin North Am* 1993;**7**:503–25.
50. Homan WL, Mank TG. Human giardiasis: genotype linked differences in clinical symptomatology. *Int J Parsitol* 2001;**31**: 822–6.
51. Huang DB, White AC. An updated review on *Cryptosporidium* and *Giardia*. *Gastroenterol Clin Nortth Am* 2006;**35**:291–314.
52. Issac-Renton JL, Cordiero C, Sarafis K, et al. Characteristics of *Giardia duodenalis* isolates from a waterborne outbreak. *J Infect Dis* 1993;**167**:431–40.
53. Janoff EN, Smith PD, Blaser MJ. Acute antibody responses to *Giardia lamblia* are depressed in patients with AIDS. *J Infect Dis* 1988;**157**:798–804.
54. Jellinek T, Loscher T. Epidemiology of giardiasis in German travelers. *J Trav Med* 2000;**7**:70–3.

55. Johnston JP, Ballard MM, Beach MJ, et al. Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. *J Clin Microbiol* 2003;**41**: 623–6.
56. Kappus KD, Lundgren RGJ, Juranek DD, et al. Intestinal parasitism in the United States: update on a continuing problem. *Am J Trop Med Hyg* 1994;**50**:705–13.
57. Kent GP, Greenspan JR, Hendon JL, et al. Epidemic giardiasis caused by a contaminated public water supply. *Am J Public Health* 1988;**78**:139–43.
58. Keystone JS, Keystone DL, Proctor EM. Intestinal parasitic infections in homosexual men: prevalence, symptoms and factors in transmission. *Can Med Assoc J* 1980;**123**:512–14.
59. Keystone JS, Krajden S, Warren MR. Person-to-person transmission of *Giardia lamblia* in day-care nurseries. *Can Med Assoc J* 1978;**12**:241–2.
60. LeChevallier MW, Norton WE, Lee RG. *Giardia* and *Cryptosporidium* in filtered drinking water supplies. *Appl Environ Microbiol* 1991;**57**:2617–21.
61. LeChevallier MW, Norton WE, Lee RG. Occurrence of *Giardia* and *Cryptosporidium* spp. in surface water supplies. *Appl Environ Microbiol* 1991;**57**:2610–16.
62. Lengerich EJ, Addiss DG, Juranek DD. Severe giardiasis in the United States. *Clin Infect Dis* 1994;**18**:760–3.
63. Levine GI. Sexually transmitted parasitic diseases. *Prim Care Clin Office Practice* 1991;**18**:101–28.
64. Lindquist HD. Induction of albendazole resistance in *Giardia lamblia*. *Microb Drug Resist* 1996;**2**:433–4.
65. Lloyd D, Harris JC. *Giardia*: highly evolved parasite or early branching eukaryote? *Trends Microbiol* 2002;**10**:1220127.
66. Lloyd D, Harris JC, Maroulis S, et al. The "primitive" microaerophile *Giardia intestinalis* (syn. *Lamblia duodenalis*) has specialized membranes with electron transport and membrane-potential-generating functions. *Microbiology* 2002;**148**:1349–54.
67. Lopez CE, Dykes AC, Juranek DD, et al. Waterborne giardiasis: a communitywide outbreak of disease and a high rate of asymptomatic infection. *Am J Epidemiol* 1980;**112**:495–507.
68. Maraha B, Buiting AG. Evaluation of four enzyme immunnoassays for detection of *Giardia lamblia* antigen in stool specimens. *Eur J Clin Microbiol Infect Dis* 2000;**19**:485–7.
69. McIntyre P, Boreham PFL, Phillips RE, et al. Chemotherapy in giardiasis: clinical responses and *in vitro* drug sensitivity of human isolates in axenic culture. *J Pediatr* 1986;**108**:1005–10.
70. Meyer EA, editor. Giardiasis. Amsterdam: Elsevier Science; 1990. p. 51.
71. Meyers EA, Radulescu S. Giardia and giardiasis. *Adv Parasitol* 1979;**17**:1–47.
72. Mintz ED, Hudson Wragg M, Mshar P, et al. Foodborne giardiasis in a corporate office setting. *J Infect Dis* 1993;**167**:250–3.
73. Moore GT, Cross WM, McGuire D, et al. Epidemic giardiasis at a ski resort. *N Engl J Med* 1969;**282**:402–7.
74. Murphy TV, Nelson JD. Five vs. ten days therapy with furazolidone for giardiasis. *Am J Dis Child* 1983;**137**:267–70.
75. Nash TE, Herrington DA, Losonsky GA, Levine MM. Experimental human infections with *Giardia lamblia*. *J Infect Dis* 1987; **156**:974–84.
76. Nunez FA, Hernandez M, Finlay CM. Longitudinal study of giardiasis in three day care centers of Havana City. *Acta Trop* 1999; **73**:237–42.
77. Oberhuber G, Kastner N, Stolte M. Giardiasis: a histologic analysis of 567 cases. *Scand J Infect Dis* 1997;**32**:48–58.
78. Ochs HD, Ament ME, Davis SD. Giardiasis with malabsorption in X-linked agammaglobulinemia. *N Engl J Med* 1972;**287**: 341–2.
79. Overturf GD. Endemic giardiasis in the United States: role of the day care center [editorial]. *Clin Infect Dis* 1994;**18**:764.
80. Palm D, Weiland M, McArthur AG, et al. Developmental changes in the adhesive disk during *Giardia* differentiation. *Mol Biochem Parasitol* 2005;**141**:199–207.
81. Perez O, Lastre M, Bandera F, et al. Evaluation of the immune response to symptomatic and asymptomatic human giardiasis. *Arch Med Res* 1994;**25**:171–7.
82. Pickering LK, Woodward WE, DuPont HL, et al. Occurrence of *Giardia lamblia* in children in day care centers. *J Pediatr* 1984;**104**:522–6.
83. Plutzer J, Ongerth J, Karanis P. *Giardia* taxonomy, phylogeny and epidemiology: facts and open questions. *Int J Hyg Environ Health* 2010;**213**:321–33.
84. Porter JD, Ragassoni HP, Buchanin JD, et al. *Giardia* transmission in a swimming pool. *Am J Public Health* 1988;**78**:659–62.
85. Prucca CG, Lujan HD. Antigenic variation in *Giardia lamblia*. *Cell Microbiol* 2009;**11**:1706–151.
86. Read C, Walters J, Robertson ID, Thompson RC. Correlation between genotype of *Giardia duodenalis* and diarrhea [letter]. *Int J Parasitol* 2002;**32**:229–31.
87. Reiner DS, Wang CS, Gillin FD. Human mold kills *Giardia lamblia* by generating toxic lipolytic products. *J Infect Dis* 1986; **154**:825–32.
88. Rendtorff RC. The experimental transmission of human intestional protozoan parasites, part II: *Giardia lamblia* cysts given in capsules. *Am J Hyg* 1954;**59**:209–12.
89. Ridley MJ, Ridley DS. Serum antibiodies and jejunal histology in giardiasis. *J Clin Pathol* 1976;**29**:30–4.
90. Roberts-Thomson IC, Mitchel GF. Giardiasis in mice, part I: prolonged infection in certain mouse strains and hypothymic (nude) mice. *Gastroenterology* 1978;**75**:42–6.
91. Rossignol JF, Ayoub A, Ayers MSD. Treatment of diarrhea caused by *Giardia intestinalis* and *Entamoeba histolytica* or *E. dispar*: a randomized double-blind, placebo-controlled study of nitazoxanide. *J Infect Dis* 2001;**184**:381–4.
92. Roxstrom-Lindquist K, Palm D, Reiner D, et al. *Giardia* immunity—an update. *Trends Parasitol* 2006;**22**:26–31.
93. Sackey ME, Weigel MM, Armijos RX. Predictors and nutritional consequences of intestinal parasitic infections in rural Ecuadorian children. *J Trop Pediatr* 2003;**49**:17–23.
94. Savioli L, Smith H, Thompson A. *Giardia* and *Crptosporidium* join the "Neglected Diseases Initiative." *Trends Parasitol* 2006; **22**:203–8.
95. Scott KG, Meddings JB, Kirk DR, et al. Intestinal infection with *Giardia* spp. reduces epithelial barrier function in a myosin light chain kinase-dependent fashion. *Gastroenterology* 2002;**123**: 1179–90.
96. Scott KG, Yu LC, Buret AG. Role of $CD8^+$ and $CD4^+$ T lymphocytes in jejunal mucosal injury during murine giardiasis. *Infect Immun* 2004;**72**:3536–42.
97. Shaw PK, Brodsky RE, Lyman DO, et al. A community-wide outbreak of giardiasis with evidence of transmission by a municipal water supply. *Ann Intern Med* 1977;**87**:426.
98. Simpson AG. Cytoskeletal organization, phylogenetic affinities and systematics in the contentious taxon Excavata (Eukaryota). *Int J Syst Evol Microbiol* 2003;**53**:1759–77.
99. Singh KD, Bhasin DK, Rana SV, et al. Effect of *Giardia lamblia* on duodenal disaccharidase levels in humans. *Trop Gastroenterol* 2000;**21**:174–6.
100. Smith PD, Gillin FD, Brown WR, et al. IgG antibody to *Giardia lamblia* detected by enzyme-linked immunosorbent assay. *Gastroenterology* 1982;**80**:1476–80.
101. Solomons NW. Giardiaisis nutritional implications. *Rev Infect Dis* 1982;**4**:859–69.
102. Stevens DP, Frank DM, Mahmoud AAF. Thymus dependency of host resistance to *Giardia muris* infection: studies in nude mice. *J Immunol* 1978;**120**:680–2.
103. Touz MC, Rivero MR, Miras SL, Bonifacino JS. Lysosomal protein trafficking in *Giardia lamblia*: common and distinct features. *Front Biosci (Elite Ed)* 2012;**4**:1898–901.
104. Tovar J, León-Avila G, Sánchez LB, et al. Mitochondrial remnant organelles of *Giardia* function in iron-sulphur protein maturation. *Nature* 2003;**426**:172–6.
105. Upcroft J, Mitchell R, Chen N, et al. Albendazole resistance in *Giardia* is correlated with cytoskeletal changes but not with a mutation at amino acid 200 in beta-tubulin. *Microb Drug Resist* 1996;**2**:303–8.
106. Upcroft J, Upcroft P. Drug-resistance and *Giardia*. *Parasitol Today* 1993;**9**:187–90.
107. Upcroft J, Upcroft P. My favorite cell: *Giardia*. *Bioessays* 1998; **20**:256–63.
108. Upcroft JA, Upcroft P, Boreham PRL. Drug resistance in *Giardia intestinalis*. *Int J Parasitol* 1990;**20**:489–96.
109. Upcroft JA, Campbell RW, Benakli K, et al. Efficacy of new 5-nitroimidazoles against metronidazole-susceptible and -resis-

tant *Giardia*, *Trichomonas*, and *Entamaeba* spp. *Antimicrob Agents Chemother* 1999;**43**:73–6.
110. Upcroft JA, Campbell RW, Upcroft P. Quinacrine-resistant *Giardia duodenalis*. *Parasitology* 1996;**112**:309–13.
111. Upcroft P, Upcroft JA. Drug targets and mechanisms of resistance in the anaerobic protozoa. *Clin Microbiol Rev* 2001;**14**:150–64.
112. Visvesvera GS, Smith PD, Healy GR, et al. An immunofluorescence test to detect serum antibody to *Giardia lamblia*. *Ann Intern Med* 1980;**93**:802–5.
113. Weitzel T, Dittrich S, Mohl I, et al. Evaluation of seven commercial antigen detection test for *Giardia* and *Cryptosporidium* in stool samples. *Clin Microbiol Infect* 2006;**12**:656–9.
114. Welch TP. Risk of giardiasis from consumption of wilderness water in North America: a systematic review of the epidemiologic data. *Int J Infect Dis* 2000;**4**:100–3.
115. Welsh JD, Poley JR, Hensley J, et al. Intestinal disaccharidase and alkaline phosphatase activity in giardiasis. *J Pediatr Gastroenterol Nutr* 1984;**3**:37–40.
116. Wright JM, Dunn LA, Upcroft P, Upcroft JA. Efficacy of antigiardial drugs. *Expert Opin Drug Saf* 2003;**2**(6):529–41.
117. Yardley JH, Takano J, Hendrix TR. Epithelial and other mucosal lesions of the jejunum in giardiasis: jejunal biopsy studies. *Bull Johns Hopkins Hosp* 1964;**115**:389–406.
118. Yoder JS, Beach MJ. Giardiasis surveillance—United States, 2003-2005. *MMWR Morb Mortal Wkly Rep* 2007;**56**(SS07): 11–18.
119. Zaat JO, Mank TG, Assendelft WJ. A systematic review on the treatment of giardiasis. *Trop Med Int Health* 1997;**2**:63–82.
120. Zaat JO, Mank TH, Assendelft WJ. Drugs for treating giardiasis. *Cochrane Database Syst Rev* 2000;71:CD000217.

CHAPTER 213

Dientamoeba fragilis Infections

Damien Stark

Dientamoeba fragilis is a protozoan parasite that may inhabit the human gastrointestinal tract and is associated with gastrointestinal disease.[6,7] Although discovered in 1908, it was not described in the scientific literature until 1918, by Jepps and Dobell.[38] At that time it was considered to be a rare intestinal commensal.[38] In contrast to most other intestinal protozoa, *Dientamoeba* has no known cyst form. In unpreserved feces, the morphologic characteristics of *D. fragilis* do not persist,[39] which most likely accounts for its perceived rarity in some surveys. Studies using appropriate diagnostic methods have found an association between *D. fragilis* infection and acute and chronic gastrointestinal symptoms,[70] which led to its recognition as a pathogenic protozoan.

THE ORGANISM

The trophozoite was classified initially in the genus *Entamoeba*. The similarity of *D. fragilis* to flagellates, however, specifically to *Histomonas meleagridis*, the cause of "blackhead" enterohepatitis in fowl, was noted on careful examination under the light microscope.[24] Later studies using various techniques, including microscopy, immunologic,[26] antigenic,[25] electron microscopy,[4,8,14] and molecular studies,[21,31,63] resulted in the reclassification of *D. fragilis* as a nonflagellate trichomonad.[14,45] Genetic studies confirmed recent evolution with trichomonads,[21,31,63] with two genotypes detected in DNA encoding ribosomal RNA.[40] Genotype 1 seems to predominate in epidemiologic studies.[19,55,80]

D. fragilis infects the mucosal crypts of the large intestine in close proximity to mucosal epithelium from the cecum to the rectum. The organism ranges in size from 3 to 18 μm in diameter, but usually is 7 to 12 μm (Fig. 213-1). The pseudopodia of *D. fragilis* have a delicate, leaflike appearance and serrated margins.[37] This protozoan is seen to move actively in fresh feces, but quickly becomes rounded after standing. *D. fragilis* is easy to isolate and grow in vitro in media containing solid rice starch.[24,52] Cultures should be maintained at 37° to 38° C (98.6° to 100.4° F) because the organism rounds up and stops moving and feeding at lower temperatures. *D. fragilis* thrives in temperatures up to 41° C (105.8° F).[5] This organism feeds on bacteria and starch grains and ingests human red blood cells.

D. fragilis reproduces by binary fission.[4,24] Although the organisms are found most commonly in the binucleate form, approximately 20 percent are in the uninucleate form and a few are multinucleate.[38,43,78,85] Each nucleus contains a large, fragmented (four to eight granules) karyosome surrounded by a clear zone with no peripheral chromatin and a fine nuclear membrane.[24,38,84] Humans seem to be the natural host of *D. fragilis*. *D. fragilis* infection has been reported in a limited number of animals, including nonhuman primates[24,73] and pigs.[13] Multiple attempts to infect other animals have been unsuccessful so far.[6] The atypical morphologic and biologic characteristics of the organism and the absence of an animal model have impeded classification and defining the epidemiology and pathogenicity of *D. fragilis*.[37]

EPIDEMIOLOGY AND TRANSMISSION

Despite the discovery of *D. fragilis* over a century ago, both its life cycle and mode of transmission are poorly defined. The mode of transmission of *D. fragilis* is unknown; however, two mechanisms have been postulated. One hypothesis is that *D. fragilis* is transmitted in the eggs of *Enterobius vermicularis* (pinworm) in a manner similar to the transmission of *H. meleagridis* in the eggs of the avian nematode *Heterakis gallinae*.[11,24,32,77,83] In support of this theory, many investigators have noted a high frequency of concomitant infection with *D. fragilis* and *E. vermicularis*.[11,32,34] Ockert[54] provided the most convincing support for this theory when, by ingesting eggs of *E. vermicularis*, which he had washed with water and exposed to pepsin and hydrochloric acid, he became infected with *E. vermicularis* and *D. fragilis*. Other investigators, noting a high rate of concomitant infection of *D. fragilis* with organisms causing intestinal infections[47,48,51,83] and negative tests for *E. vermicularis*,[20,70,74] suggest fecal-oral transmission.

D. fragilis was thought to be uncommon until improved diagnostic techniques were used for detection, including prompt fixation for preserving the organism[30,33,61] and molecular methods.[68,71,82] The trophozoites of *D. fragilis* have been noted to be quite sensitive to an aerobic environment.[18] They die and disintegrate within 1 hour in an isotonic salt solution at room temperature; when smeared on slides, they round up and become granular within 15 minutes during microscopic examination at room temperature and low humidity.[18]

D. fragilis has been reported worldwide with a prevalence in selected populations of 1.4 to 38 percent.[1] Higher prevalence rates of 19 to 69 percent have been reported in crowded living situations, such as institutions and communal groups,[10,48,50,51,76] extended families in close contact,[74] and in individuals traveling outside the United

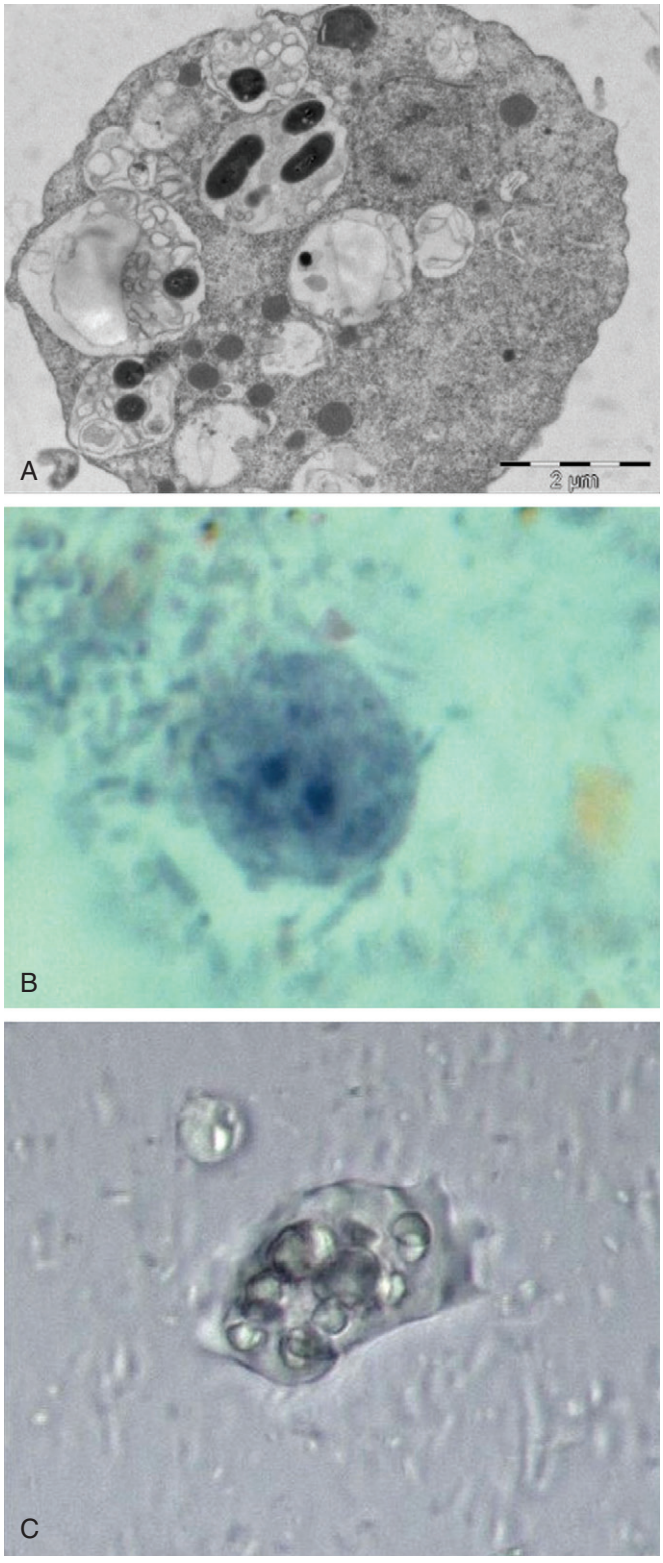

FIGURE 213-1 ■ **A,** *Dientamoeba fragilis* trophozoites on electron micrography. **B,** Light microscopy of trophozoite on modified iron-haematoxylin stain. **C,** Phase contrast microscopy of trophozoite in culture (with ingested rice starch) (See companion Expert Consult website for color version.)

States.[46,58,66,72] A serologic survey in Canada using indirect immunofluorescence techniques detected antibodies to *D. fragilis* in 87 to 100 percent of healthy children 1 to 19 years old,[15] suggesting that infection occurs in most individuals during childhood. Although the antibodies detected in this survey did not absorb when incubated with *Klebsiella pneumoniae* or *Bacteroides vulgatus*, known to contaminate the antigen source of *D. fragilis*, additional studies are needed to confirm the specificity of the assay and to define the seroprevalence in other populations.

CLINICAL MANIFESTATIONS

The clinical presentation of dientamoebiasis ranges from asymptomatic carriage to a variety of symptoms. Gastrointestinal and, less frequently, systemic symptoms have been reported to occur in association with *D. fragilis* in the fecal specimens of children and adults.[2,3] Symptomatic infections occur in 15 to 85 percent of infected individuals.[23,88] Acute watery diarrhea and a chronic recurrent abdominal pain syndrome have been associated with *D. fragilis* in children and adults.[9,12]

Individuals with acute diarrhea also have reported abdominal pain, anorexia, nausea, and vomiting, and, less frequently, fever, weight loss, headache, malaise, fatigue, irritability, and weakness.* The stools have been described as greenish brown, mushy or sticky, with a foul odor and sometimes bloody and with mucus.[41,42] Abdominal tenderness has been found commonly on physical examination[20,41,65] and may mimic appendicitis.[62,77]

When symptomatic, *D. fragilis* infection is associated most frequently with chronic abdominal pain,[20,49,64,65] which may persist for months to years.[35,85] The pain is described commonly as dull, achy, crampy, or colicky and usually is located in the lower abdominal quadrants.[65,85] Complaints of flatulence, fatigue, and alternating diarrhea and constipation are common in individuals with *D. fragilis* infections.[38,65] Laboratory and radiologic studies usually are normal. Eosinophilia, not usually seen with protozoal infections, has been reported in infected children and adults, most commonly in association with chronic symptoms.[20,64,65,75] Eosinophilia has not been observed by others, however.[41] The high prevalence of *D. fragilis* in fecal surveys generally without symptoms and the nonprogressive nature of symptomatic infections should steer clinicians to look for alternative diagnoses in seriously ill children.

DIAGNOSIS

Infection with *D. fragilis* should be considered when abdominal pain, diarrhea, or both persist beyond 1 week. Diagnosis of dientamoebiasis may be problematic if inadequate laboratory methods are used. Investigation for *D. fragilis* infection should include the collection of at least three stool specimens that are immediately placed in a stool preservative such as polyvinyl alcohol stool preservative to retain the morphologic characteristics of the delicate trophozoite.[60,61,79] Diagnosis also can be made by permanent stained smear of a fresh or purged fecal specimen.[11,33,78] Detection of the organism seems not to be compromised by use of "environmentally friendly"

*References 3, 20, 22, 23, 35, 41, 42, 57, 62, 64, 65, 72.

mercury-free stool preservative and stain (EcoFix and EcoStain).[29]

Three fecal specimens properly collected and stained leads to the identification of this intestinal protozoan in 70 to 93 percent of infected individuals.[36,60] Stool specimens should be collected on alternate days because excretion of *D. fragilis* seems to manifest a cyclic pattern.[22] Stool samples should be collected before radiologic studies with barium are done because barium interferes with detection of the protozoa.[28] Other medications interfering with parasite identification include antibiotics, mineral oil, antimalarials, antiprotozoan agents, nonabsorbable diarrheal preparations, and bismuth.[28] These substances may interfere with the detection of parasites for 3 weeks.[28]

After arrival in the laboratory, stool specimens are processed with the use of a formalin-ether sedimentation concentration technique and stained with either iron hematoxylin or trichrome and examined by qualified and experienced individuals for the proper identification of *D. fragilis*.[22,26] Diagnostic characteristics of *D. fragilis* on a permanently stained smear include a high percentage of binucleate trophozoites and nuclei without peripheral chromatin, but with four to eight chromatin granules in a central mass.[21]

Other methods used for the detection of *D. fragilis* include indirect immunofluorescence,[16] xenic culture,[52,59,86] and molecular methods.[55,69,82] Both conventional and real-time polymerase chain reaction assays have been developed and show excellent sensitivity and specificity in contrast to microscopy.[67,68,71] One serologic test has been developed; however, it is not commercially available and the high seroprevalence of *D. fragilis* precludes its use as a diagnostic tool.[10]

TREATMENT

Several agents have been used in the treatment of *D. fragilis* infection. However, it should be noted that most treatment data are based only on a small number of case reports. No large-scale double-blind, randomized placebo-controlled trials testing the efficacy of antimicrobial agents against *D. fragilis* have been undertaken. Presently, one of four drugs is recommended for the treatment of *D. fragilis* infection (Table 213-1): iodoquinol, tetracycline, paromomycin, or metronidazole.[17,44,56,81] However, owing to a paucity of clinical studies, tetracycline and paromomycin are considered investigational by the U.S. Food and Drug Administration.[1,2] Among newer agents, members of the 5-nitroimidazole derivatives secnidazole[28] and ornidazole seem effective in limited studies, and have the advantage of longer half-lives, single dosages, and fewer side effects. Laboratory studies have found that ronidazole,[53] tinidazole,[27,53] and nitazoxanide[53] are active in vitro.

Iodoquinol 650 mg three times daily for 20 days is recommended for adults and 30 to 40 mg/kg/day divided three times daily for children. The tablets should be taken with meals. Side effects include abdominal discomfort, diarrhea, anal irritation and pruritus, headache, and dysesthesias of the hands and feet. Paromomycin 500 mg three times daily for adults and 25 to 35 mg/kg/day in three divided doses in children for 7 days may be more effective than iodoquinol.[87] Adverse reactions to paromomycin include nausea, abdominal cramps, and diarrhea. It is absorbed poorly after oral administration and is no longer available in syrup form in the United States.

Recrudescence or relapse has been reported after one or more 7-day courses of paromomycin in a few treated children.[81] Alternative therapy is tetracycline hydrochloride or metronidazole. Tetracycline is recommended at a dosage of 500 mg four times daily for adults and 40 mg/kg/day in four divided doses for children for 10 days. Tetracycline may cause gastrointestinal and central nervous system symptoms (CNS). It should not be given to children younger than 9 years of age because it may cause discoloration of the teeth. Metronidazole treatment is recommended at a dosage of 500 to 750 mg three times daily for 10 days for adults and 20 to 40 mg/kg/day divided into three doses for children.[3] Treatment with metronidazole may cause gastrointestinal and CNS symptoms. Because of the adverse reactions associated with these drugs, the clinician should evaluate carefully the need for therapy in each case and discuss treatment options with the child's family.

TABLE 213-1 **List of Treatment Options for Dientamoebiasis**

Drug of Choice	Alternative Drugs
Iodoquinol Adults: 650 mg PO three times daily × 20 days Pediatric: 30-40 mg/kg/day (maximum 2 g) PO in three doses × 20 days	**Paromomycin** Adults: 500 mg PO three times daily × 7 days Pediatric: 25-35 mg/kg/day PO in three doses × 7 days **Tetracycline** Adults: 500 mg PO four times daily × 10 days Pediatric: 40 mg/kg/day (maximum 2 g) PO in four doses × 10 days **Metronidazole** Adults: 500-750 mg PO three times daily × 10 days Pediatric: 35-50 mg/kg/day PO in three doses × 10 days

PO, Orally.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Banik GR, Barratt JL, Marriott D, et al. A case-controlled study of *Dientamoeba fragilis* infections in children. *Parasitology* 2011; 1–5.
4. Banik GR, Birch D, Stark D, et al. A microscopic description and ultrastructural characterisation of *Dientamoeba fragilis*: an emerging cause of human enteric disease. *Int J Parasitol* 2012;**42**:139–53.
5. Barratt JL, Banik GR, Harkness J, et al. Newly defined conditions for the in vitro cultivation and cryopreservation of *Dientamoeba fragilis*: new techniques set to fast track molecular studies on this organism. *Parasitology* 2010;**137**:1867–78.
6. Barratt JL, Harkness J, Marriott D, et al. A review of *Dientamoeba fragilis* carriage in humans: several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. *Gut Microbes* 2011;**2**:3–12.

7. Barratt JL, Harkness J, Marriott D, et al. The ambiguous life of *Dientamoeba fragilis:* the need to investigate current hypotheses on transmission. *Parasitology* 2011;**138**:557–72.
8. Bird RG, Sargeaunt P, Upton CP. Uni- and binucleate trophozoites of *Dientamoeba fragilis*. *Trans R Soc Trop Med Hyg* 1970;**64**:18.
13. Caccio SM, Sannella AR, Manuali E, et al. Pigs as natural hosts of *Dientamoeba fragilis* genotypes found in humans. *Emerg Infect Dis* 2012;**18**:838–41.
15. Chan F, Stewart N, Guan M, et al. Prevalence of *Dientamoeba fragilis* antibodies in children and recognition of a 39 kDa immunodominant protein antigen of the organism. *Eur J Clin Microbiol Infect Dis* 1996;**15**:950–4.
19. Crotti D, D'Annibale ML, Fonzo G, et al. *Dientamoeba fragilis* is more prevalent than *Giardia duodenalis* in children and adults attending a day care centre in Central Italy. *Parasite* 2005;**12**: 165–70.
45. Levine ND, Corliss JO, Cox FE, et al. A newly revised classification of the protozoa. *J Protozool* 1980;**27**:37–58.
52. Munasinghe VS, Stark D, Ellis JT. New advances in the in-vitro culture of *Dientamoeba fragilis*. *Parasitology* 2012;**139**:864–9.
53. Nagata N, Marriott D, Harkness J, et al. In vitro susceptibility testing of *Dientamoeba fragilis*. *Antimicrob Agents Chemother* 2012; **56**:487–94.
56. Preiss U, Ockert G, Broemme S, et al. On the clinical importance of *Dientamoeba fragilis* infections in childhood. *J Hyg Epidemiol Microbiol Immunol* 1991;**35**:27–34.
67. Stark D, Al-Qassab SE, Barratt JL, et al. Evaluation of multiplex tandem real-time PCR for detection of *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia intestinalis* in clinical stool samples. *J Clin Microbiol* 2010;**49**:257–62.
68. Stark D, Barratt J, Roberts T, et al. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of *Dientamoeba fragilis* in clinical stool samples. *Eur J Clin Microbiol Infect Dis* 2010;**29**:411–16.
69. Stark D, Beebe N, Marriott D, et al. Detection of *Dientamoeba fragilis* in fresh stool specimens using PCR. *Int J Parasitol* 2005; **35**:57–62.
73. Stark D, Phillips O, Peckett D, et al. Gorillas are a host for *Dientamoeba fragilis*: an update on the life cycle and host distribution. *Vet Parasitol* 2008;**151**:21–6.
74. Stark D, Roberts T, Marriott D, et al. Detection and transmission of *Dientamoeba fragilis* from environmental and household samples. *Am J Trop Med Hyg* 2012;**86**:233–6.
79. van Gool T, Weijts R, Lommerse E, et al. Triple Faeces Test: an effective tool for detection of intestinal parasites in routine clinical practice. *Eur J Clin Microbiol Infect Dis* 2003;**22**:284–90.
82. Verweij JJ, Mulder B, Poell B, et al. Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. *Mol Cell Probes* 2007;**21**:400–4.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 214

Trichomonas Infections

Joan S. Purcell • Mariam R. Chacko

Trichomonas spp. are found in animals and humans. The most widely studied member of this species is *Trichomonas vaginalis* because it is the trichomonad most relevant to human disease. Donne first described *T. vaginalis* in 1836 as motile microorganisms in the purulent frothy leukorrhea of women with vaginal discharge and genital irritation.[24] Epidemiologic information concerning *Trichomonas* infection is most detailed in the adult population; nonetheless, studies that document the epidemiology of *T. vaginalis* in children and adolescents are available.

MICROBIOLOGY

Trichomonads are acellular flagellated protozoans. The Trichomonadidae family is characterized as mononucleate with an axial organelle that has an undulating membrane. The *Trichomonas* genus includes protozoans that have organelles with three to four anterior flagella and an undulating membrane comprising the posterior flagellum. Five *Trichomonas* spp. infect humans, and three *Trichomonas* spp. infect animals. In humans, the five species of *Trichomonas* include *T. tenax*, *T. ardin delteili*, *T. faecalis*, *T. hominis*, and *T. vaginalis*.[79]

T. vaginalis is the most clinically relevant human trichomonad; the other four species are nonpathogenic. *T. vaginalis* consists of four anterior flagella and a posterior flagellum incorporated into the undulating membrane. *T. vaginalis* can survive but cannot multiply at room temperature. In contrast, *T. hominis* has five anterior flagella and a trailing posterior flagellum, and can survive and multiply at room temperature. Only *T. hominis* can survive in media without serum and in feces for 24 hours.[79]

EPIDEMIOLOGY

Prevalence

Trichomonas infection is found in all age groups, from neonates to adults. Prevalence data concerning *Trichomonas* infection vary widely because it is not as well

REFERENCES

1. Abromowicz M, editor. *Handbook of Antimicrobial Therapy*. 17th ed. New Rochelle, NY, 2005.
2. American Academy of Pediatrics. *2006 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2007.
3. Banik GR, Barratt JL, Marriott D, et al. A case-controlled study of *Dientamoeba fragilis* infections in children. *Parasitology* 2011;1–5.
4. Banik GR, Birch D, Stark D, et al. A microscopic description and ultrastructural characterisation of *Dientamoeba fragilis*: an emerging cause of human enteric disease. *Int J Parasitol* 2012;**42**:139–53.
5. Barratt JL, Banik GR, Harkness J, et al. Newly defined conditions for the in vitro cultivation and cryopreservation of *Dientamoeba fragilis*: new techniques set to fast track molecular studies on this organism. *Parasitology* 2010;**137**:1867–78.
6. Barratt JL, Harkness J, Marriott D, et al. A review of *Dientamoeba fragilis* carriage in humans: several reasons why this organism should be considered in the diagnosis of gastrointestinal illness. *Gut Microbes* 2011;**2**:3–12.
7. Barratt JL, Harkness J, Marriott D, et al. The ambiguous life of *Dientamoeba fragilis:* the need to investigate current hypotheses on transmission. *Parasitology* 2011;**138**:557–72.
8. Bird RG, Sargeaunt P, Upton CP. Uni- and binucleate trophozoites of *Dientamoeba fragilis*. *Trans R Soc Trop Med Hyg* 1970;**64**:18.
9. Boe J. The occurrence of human intestinal protozoa in Norway. *Acta Med Scand* 1943;**113**:321–8.
10. Brug SL. Observation on *Dientamoeba fragilis*. *Ann Trop Med Parasitol* 1936;**30**:441–52.
11. Burrows RB, Swerdlow HA. *Enterobius vermicularis:* A probable vector of *Dientamoeba fragilis*. *Am J Trop Med Hyg* 1956;**5**: 256–64.
12. Butler WP. *Dientamoeba fragilis*: an unusual intestinal pathogen. *Dig Dis Sci* 1996;**41**:1811–13.
13. Caccio SM, Sannella AR, Manuali E, et al. Pigs as natural hosts of *Dientamoeba fragilis* genotypes found in humans. *Emerg Infect Dis* 2012;**18**:838–41.
14. Camp RR, Mattern CF, Honigberg BM. Study of *Dientamoeba fragilis* Jepps & Dobell. I. Electronmicroscopic observations of the binucleate stages. II. Taxonomic position and revision of the genus. *J Protozool* 1974;**21**:69–82.
15. Chan F, Stewart N, Guan M, et al. Prevalence of *Dientamoeba fragilis* antibodies in children and recognition of a 39 kDa immunodominant protein antigen of the organism. *Eur J Clin Microbiol Infect Dis* 1996;**15**:950–4.
16. Chan FT, Guan MX, Mackenzie AM. Application of indirect immunofluorescence to detection of *Dientamoeba fragilis* trophozoites in fecal specimens. *J Clin Microbiol* 1993;**31**:1710–14.
17. Chan FT, Guan MX, Mackenzie AM, et al. Susceptibility testing of *Dientamoeba fragilis* ATCC 30948 with iodoquinol, paromomycin, tetracycline, and metronidazole. *Antimicrob Agents Chemother* 1994;**38**:1157–60.
18. Chang SL. Parasitization of the parasite. *JAMA* 1973;**223**:1510.
19. Crotti D, D'Annibale ML, Fonzo G, et al. *Dientamoeba fragilis* is more prevalent than *Giardia duodenalis* in children and adults attending a day care centre in Central Italy. *Parasite* 2005;**12**: 165–70.
20. Cuffari C, Oligny L, Seidman EG. *Dientamoeba fragilis* masquerading as allergic colitis. *J Pediatr Gastroenterol Nutr* 1998;**26**:16–20.
21. Delgado-Viscogliosi P, Viscogliosi E, Gerbod D, et al. Molecular phylogeny of parabasalids based on small subunit rRNA sequences, with emphasis on the Trichomonadinae subfamily. *J Eukaryot Microbiol* 2000;**47**:70–5.
22. Desser SS, Yang YJ. Letter: *Dientamoeba fragilis* in idiopathic gastrointestinal disorders. *Can Med Assoc J* 1976;**114**:290, 293.
23. Dickinson EC, Cohen MA, Schlenker MK. *Dientamoeba fragilis*: a significant pathogen. *Am J Emerg Med* 2002;**20**:62–3.
24. Dobell C. Researches on the intestinal protozoa of monkeys and man. X. The life-history of *Dientamoeba fragilis*: observations, experiments, and speculations. *Parasitol Res* 1940;**32**:417–61.
25. Dwyer DM. Analysis of the antigenic relationships among *Trichomonas*, *Histomonas*, *Dientamoeba*, and *Entamoeba*. I. Quantitative fluorescent antibody methods. *J Protozool* 1972;**19**:316–25.
26. Dwyer DM. Analysis of the antigenic relationships among *Trichomonas*, *Histomonas*, *Dientamoeba* and *Entamoeba*. III. Immunoelectrophoresis technics. *J Protozool* 1974;**21**:139–45.
27. Fung HB, Doan TL. Tinidazole: a nitroimidazole antiprotozoal agent. *Clin Ther* 2005;**27**:1859–84.
28. Garcia LS, Ash LR. *Diagnostic Parasitology*. St. Louis, C. V.: Mosby; 1975.
29. Garcia LS, Shimizu RY. Evaluation of intestinal protozoan morphology in human fecal specimens preserved in EcoFix: comparison of Wheatley's trichrome stain and EcoStain. *J Clin Microbiol* 1998;**36**:1974–6.
30. Garcia LS, Brewer TC, Bruckner DA. A comparison of the formalin-ether concentration and trichrome-stained smear methods for the recovery and identification of intestinal protozoa. *Am J Med Technol* 1979;**45**:932–5.
31. Gerbod D, Edgcomb VP, Noel C, et al. Phylogenetic position of the trichomonad parasite of turkeys, *Histomonas meleagridis* (Smith) Tyzzer, inferred from small subunit rRNA sequence. *J Eukaryot Microbiol* 2001;**48**:498–504.
32. Girginkardesler N, Kurt O, Kilimcioglu AA, et al. Transmission of *Dientamoeba fragilis*: evaluation of the role of Enterobius vermicularis. *Parasitol Int* 2008;**57**:72–5.
33. Goldman M, Brooke MM. Protozoans in stools unpreserved and preserved in PVA-fixative. *Public Health Rep* 1953;**68**:703–6.
34. Grendon JH, DiGiacomo RF, Frost FJ. Descriptive features of *Dientamoeba fragilis* infections. *J Trop Med Hyg* 1995;**98**: 309–15.
35. Hakansson EG. *Dientamoeba fragilis*, some further observations. *Am J Trop Med Hyg* 1936;**16**:175–83.
36. Hiatt RA, Markell EK, Ng E. How many stool examinations are necessary to detect pathogenic intestinal protozoa? *Am J Trop Med Hyg* 1995;**53**:36–9.
37. Hood M. Diarrhea caused by *Dientamoeba fragilis*. *J Laparoendosc Adv Surg Tech A* 1940;**25**:914–18.
38. Jepps MW, Dobell C. *Dientamoeba fragilis*, a new intestinal amoeba from man. *Parasitol Res* 1918;**10**:352–67.
39. Johnson EH, Windsor JJ, Clark CG. Emerging from obscurity: biological, clinical, and diagnostic aspects of *Dientamoeba fragilis*. *Clin Microbiol Rev* 2004;**17**:553–70, table of contents.
40. Johnson JA, Clark CG. Cryptic genetic diversity in *Dientamoeba fragilis*. *J Clin Microbiol* 2000;**38**:4653–4.
41. Kean BH, Moloch CL. The neglected ameba: dientamoeba fragilis: a report of 100 "pure" infections. *Am J Dig Dis* 1966;**11**: 735–46.
42. Knoll EW, Howell KM. Studies on *Dientamoeba fragilis*: its incidence and possible pathogenicity. *Am J Clin Pathol* 1945;**15**: 178–83.
43. Kudo R. Observation on *Dientamoeba fragilis*. *Am J Trop Med Hyg* 1926;**6**:299–305.
44. Kurt O, Girginkardesler N, Balcioglu IC, et al. A comparison of metronidazole and single-dose ornidazole for the treatment of dientamoebiasis. *Clin Microbiol Infect* 2008;**14**:601–4.
45. Levine ND, Corliss JO, Cox FE, et al. A newly revised classification of the protozoa. *J Protozool* 1980;**27**:37–58.
46. Mackie TT, Larsh JE Jr, Mackie JW. A survey of intestinal parasitic infections in the Dominican Republic. *Am J Trop Med Hyg* 1951; **31**:825–32.
47. McQuay RM. Parasitologic studies in a group of furloughed missionaries. I. Intestinal protozoa. *Am J Trop Med Hyg* 1967;**16**: 154–60.
48. Melvin DM, Brooke MM. Parasitologic surveys on Indian reservations in Montana, South Dakota, New Mexico, Arizona, and Wisconsin. *Am J Trop Med Hyg* 1962;**11**:765–72.
49. Miller MJ. The intestinal protozoa of man in midwestern Canada. *J Parenter Sci Technol* 1939;**25**:355–7.
50. Millet V, Spencer MJ, Chapin M, et al. *Dientamoeba fragilis*, a protozoan parasite in adult members of a semicommunal group. *Dig Dis Sci* 1983;**28**:335–9.
51. Millet VE, Spencer MJ, Chapin MR, et al. Intestinal protozoan infection in a semicommunal group. *Am J Trop Med Hyg* 1983; **32**:54–60.
52. Munasinghe VS, Stark D, Ellis JT. New advances in the in-vitro culture of *Dientamoeba fragilis*. *Parasitology* 2012;**139**:864–9.
53. Nagata N, Marriott D, Harkness J, et al. In vitro susceptibility testing of *Dientamoeba fragilis*. *Antimicrob Agents Chemother* 2012; **56**:487–94.
54. Ockert G. Zur epidemiologie von *Dientamoeba fragilis*. II. Mitteilung: Versuche über die Übertragung der Art mit Enterobius-Eiern. *J Hyg Epidemiol Immunol* 1972;**16**:222–5.

55. Peek R, Reedeker FR, van Gool T. Direct amplification and genotyping of *Dientamoeba fragilis* from human stool specimens. *J Clin Microbiol* 2004;**42**:631–5.
56. Preiss U, Ockert G, Broemme S, et al. On the clinical importance of *Dientamoeba fragilis* infections in childhood. *J Hyg Epidemiol Microbiol Immunol* 1991;**35**:27–34.
57. Robertson A. Note on a case infected with *Dientamoeba fragilis*. Jepps and Dobell. *Am J Trop Med Hyg* 1917;**1923**:26–243.
58. Sapero JJ. Clinical studies in nondysenteric intestinal amebiasis. *Am J Trop Med Hyg* 1939;**19**:497–514.
59. Sawangjaroen N, Luke R, Prociv P. Diagnosis by faecal culture of *Dientamoeba fragilis* infections in Australian patients with diarrhoea. *Trans R Soc Trop Med Hyg* 1993;**87**:163–5.
60. Sawitz WG, Faust EC. The probability of detecting intestinal protozoa by successive stool examinations. *Am J Trop Hyg* 1942; **22**:131–6.
61. Scholten TH, Yang J. Evaluation of unpreserved and preserved stools for the detection and identification of intestinal parasites. *Am J Clin Pathol* 1974;**62**:563–7.
62. Schwartz MD, Nelson ME. *Dientamoeba fragilis* infection presenting to the emergency department as acute appendicitis. *J Emerg Med* 2003;**25**:17–21.
63. Silberman JD, Clark CG, Sogin ML. *Dientamoeba fragilis* shares a recent common evolutionary history with the trichomonads. *Mol Biochem Parasitol* 1996;**76**:311–14.
64. Spencer MJ, Chapin MR, Garcia LS. *Dientamoeba fragilis:* a gastrointestinal protozoan infection in adults. *Am J Gastroenterol* 1982;**77**: 565–9.
65. Spencer MJ, Garcia LS, Chapin MR. *Dientamoeba fragilis:* an intestinal pathogen in children? *Am J Dis Child* 1979;**133**:390–3.
66. Spencer MJ, Millet VE, Garcia LS, et al. Parasitic infections in a pediatric population. *Pediatr Infect Dis* 1983;**2**:110–13.
67. Stark D, Al-Qassab SE, Barratt JL, et al. Evaluation of multiplex tandem real-time PCR for detection of *Cryptosporidium* spp., *Dientamoeba fragilis*, *Entamoeba histolytica*, and *Giardia intestinalis* in clinical stool samples. *J Clin Microbiol* 2010;**49**:257–62.
68. Stark D, Barratt J, Roberts T, et al. Comparison of microscopy, two xenic culture techniques, conventional and real-time PCR for the detection of *Dientamoeba fragilis* in clinical stool samples. *Eur J Clin Microbiol Infect Dis* 2010;**29**:411–16.
69. Stark D, Beebe N, Marriott D, et al. Detection of *Dientamoeba fragilis* in fresh stool specimens using PCR. *Int J Parasitol* 2005;**35**: 57–62.
70. Stark D, Beebe N, Marriott D, et al. Dientamoeba fragilis as a cause of travelers' diarrhea: report of seven cases. *J Travel Med* 2007;**14**:72–3.
71. Stark D, Beebe N, Marriott D, et al. Evaluation of three diagnostic methods, including real-time PCR, for detection of *Dientamoeba fragilis* in stool specimens. *J Clin Microbiol* 2006;**44**:232–5.
72. Stark D, Beebe N, Marriott D, et al. Prospective study of the prevalence, genotyping, and clinical relevance of *Dientamoeba fragilis* infections in an Australian population. *J Clin Microbiol* 2005;**43**:2718–23.
73. Stark D, Phillips O, Peckett D, et al. Gorillas are a host for *Dientamoeba fragilis*: an update on the life cycle and host distribution. *Vet Parasitol* 2008;**151**:21–6.
74. Stark D, Roberts T, Marriott D, et al. Detection and transmission of *Dientamoeba fragilis* from environmental and household samples. *Am J Trop Med Hyg* 2012;**86**:233–6.
75. Stark D, van Hal S, Marriott D, et al. Irritable bowel syndrome: a review on the role of intestinal protozoa and the importance of their detection and diagnosis. *Int J Parasitol* 2007;**37**:11–20.
76. Svennson RM. A survey of human intestinal protozoa in Sweden and Finland. *Parasitol Res* 1928;**20**:237–49.
77. Swerdlow MA, Burrows RB. *Dientamoeba fragilis*, an intestinal pathogen. *J Am Med Assoc* 1955;**158**:176–8.
78. Thompson JG. Dientamoeba fragilis, Jepps and Dobell, 1917: a case of human infection in England. *J Trop Pediatr* 1923;**26**: 135–6.
79. van Gool T, Weijts R, Lommerse E, et al. Triple Faeces Test: an effective tool for detection of intestinal parasites in routine clinical practice. *Eur J Clin Microbiol Infect Dis* 2003;**22**:284–90.
80. Vandenberg O, Peek R, Souayah H, et al. Clinical and microbiological features of dientamoebiasis in patients suspected of suffering from a parasitic gastrointestinal illness: a comparison of *Dientamoeba fragilis* and *Giardia lamblia* infections. *Int J Infect Dis* 2006;**10**:255–61.
81. Vandenberg O, Souayah H, Mouchet F, et al. Treatment of *Dientamoeba fragilis infection* with paromomycin. *Pediatr Infect Dis J* 2007;**26**:88–90.
82. Verweij JJ, Mulder B, Poell B, et al. Real-time PCR for the detection of *Dientamoeba fragilis* in fecal samples. *Mol Cell Probes* 2007;**21**:400–4.
83. Weiner D, Brooke MM, Witkow A. Investigation of parasitic infections in the central area of Philadelphia. *Am J Trop Med Hyg* 1959;**8**:625–9.
84. Wenrich DH. Studies on *Dientamoeba fragilis* (protozoa). I. Observations with special reference to nuclear structure. *J Parasitol* 1936;**22**:76–83.
85. Wenrich DH. Studies on *Dientamoeba fragilis* (protozoa). II. Report on unusual morphology in one case with suggestions as to pathogenicity. *J Parenter Sci Technol* 1937;**23**:183–96.
86. Windsor JJ, Macfarlane L, Hughes-Thapa G, et al. Detection of *Dientamoeba fragilis* by culture. *Br J Biomed Sci* 2003;**60**:79–83.
87. Wolfe MS. *Hunter's Tropical Medicine*. Philadelphia, W. B.: Saunders; 1991.
88. Yoeli M. A report on intestinal disorders accompanied by large numbers of *Dientamoeba fragilis*. *J Trop Med Hyg* 1955;**58**:38–41.

documented as other sexually transmitted infections (STIs) because of the absence of screening programs and limitations and variations with diagnostic tests.[33] A vast amount of international prevalence data on *T. vaginalis* STIs stems from research done in clinics and populations in Africa.[5,19] Studies reporting the epidemiology of *T. vaginalis* in children and adolescents are available. Many of the prevalence studies of urogenital trichomoniasis in adolescents and adults are influenced by lack of random sampling, variance in the sensitivity and specificity of the diagnostic tests used, and sample selection, often from sexually transmitted infection (STI) clinics.[33] However the emergence of point-of-care and nucleic acid amplification tests (NAATs) has enhanced epidemiologic information in this field of study. For details on newborns, infants, and premenarcheal children see Chapter 42.

The incidence of trichomonal infections is greater in females than males. Approximately 180 million women worldwide may be infected with *T. vaginalis;* prevalence data depend on the population studied. *T. vaginalis* is seen most commonly in postmenarchal sexually active adolescent girls and women. Up to 700,000 cases of trichomonas infections per year have been reported to the Centers for Disease Control and Prevention (CDC) among women in the United States at their initial physician office visits, and this number has been relatively steady since 1988.[15] The prevalence of *T. vaginalis* urogenital infection varies from 5 to 74 percent in women, with the highest rates reported from STI clinics and other high-risk populations.[33] The 2003 to 2004 National Health and Nutrition Examination Survey, which randomly samples the noninstitutionalized civilian population in the United States, found a 3.6 percent weighted prevalence rate for *T. vaginalis* in sexually active females 14 to 19 years of age by polymerase chain reaction (PCR) testing.[23] In an inner city primary care clinic the prevalence of *T. vaginalis* was 14.4 percent by wet mount and culture in 13- to 19-year-old females.[43] In an urban pediatric emergency department a prevalence rate of *T. vaginalis* by OSOM Trichomonas Rapid enzyme test (Genzyme) was 9.9 percent in 14- to 19-year-old females; 25 percent of the positive tests were among 14- and 15-year-olds.[27] In contrast to females, the prevalence in males is not well studied. In men attending STI clinics, the prevalence of *T. vaginalis* was 22 to 29 percent in sexual contacts of women with trichomoniasis and 6 percent in homosexual men attending the same clinic.[33,44] *T. vaginalis* was found in 58 percent of 85 young black men 16 to 22 years of age who were sexually active, of whom 69 percent previously had an STI.[63]

Risk Factors

Urogenital trichomoniasis is seen more commonly in inner-city patients and patients in the 20- to 30-year-old age group.[53] A large epidemiologic neighborhood study isolated *T. vaginalis* in young adults living in neighborhoods with a higher concentration of blacks, which was mediated by neighborhood poverty. Factors such as immigrant concentration and residential instability were not associated with *T. vaginalis.*[22] Trichomoniasis apparently does not have a seasonal pattern of infection.[53] The rate of *T. vaginalis* infection is increased in black women; women with other STIs, including gonococcal cervicitis; a vaginal pH shift greater than 4.5; pregnancy; menarche; and after menopause.[57,79]

Infection with *T. vaginalis* increases in direct relation to number of sexual partners. An increased number of sexual partners also increases the risk for coinfection with other sexually transmitted organisms, including, but not limited to, *Chlamydia trachomatis*, *Neisseria gonorrhea*, *Mycoplasma hominis*, and human immunodeficiency virus (HIV).[3] Foster care status may be a correlate for higher rates of *T. vaginalis* infection in adolescents. In Wave III of the National Longitudinal Study of Adolescent Health, using a urine PCR enzyme-linked immunosorbent assay (ELISA) test, female participants exposed to foster care were significantly more likely to test positive for *T. vaginalis* infection (2.8%) than their male counterparts in foster care (1.8%).[1] Oral contraceptives diminish the rate of infection with *T. vaginalis* in contrast to an intrauterine device or tubal ligation.[53] The use of nonoxynol-9 spermicidal cream was not related significantly to a decrease in *Trichomonas* infection.[7] In another study of 226 women attending an STI clinic, no association was found among patient age, frequency of coitus, date of most recent coitus, day of menstrual cycle, antibiotic use, contraceptive methods, or symptoms of discharge or pruritus.[57] The risk for *T. vaginalis* is decreased in the wives of circumcised males.[30]

Other risk factors for women may involve a change in the normal vaginal flora, such as overgrowth of *Gardnerella vaginalis*, *Bacteroides*, or *Peptostreptococcus*. These bacteria may serve as sources of nutrients for trichomonads and allow them to thrive. In men, sexual contact with a woman infected with *T. vaginalis*, nongonococcal urethritis, or nongonococcal nonchlamydial urethritis was associated with an increased risk for acquiring infection with *T. vaginalis.*[47] In trichomoniasis, lactobacilli are absent from the vagina. This absence promotes alkalinity of the vaginal pH, enhancing the overgrowth of anaerobes and trichomonads.[72] Another study performed in Africa failed to show a significant role for *Lactobacillus* in patients with *T. vaginalis* infection.[20,55]

TRANSMISSION

Trichomoniasis in newborns through perinatal acquisition with recovery of *T. vaginalis* from urine, vaginal, and respiratory tract specimens has been described. The mode of transmission in children older than 1 year of age is controversial as a history or no history of sexual abuse has been reported. In one study *T. vaginalis* was not identified in any of the asymptomatic healthy control group of prepubertal children.[29] Nonsexual transmission of *T. vaginalis* has been reported in children. In rural India, a point prevalence survey of random samples from preadolescent and adolescent girls complaining of leukorrhea revealed that 76 percent were infected with *T. vaginalis.* Of the girls who were infected, 38 percent were younger than 12 years of age. In this study, poor genital hygiene and underwear use were correlated with a higher incidence of infection. In addition, a significantly higher risk

occurred in girls who washed or bathed in tanks or rivers versus girls who used pipe or well water. In the tropics, nonsexual transmission may account for infection with *T. vaginalis* in preadolescent and adolescent girls.[16] *T. vaginalis* can survive on toilet seats for 1 hour, on wet clothes for 3 hours, in fresh water for 30 minutes, in warm mineral water for 2 or 3 days, and in urine for hours. According to the literature, transmission of *Trichomonas* may occur via fomites (e.g., wash cloths and towels), particularly when people are living together in crowded, confined spaces.[60] For details see Chapter 42.

In summary, *T. vaginalis* conceivably is transmitted to children nonsexually. The likelihood of perinatal transmission is high in infants younger than 1 year of age. A child or an infant younger than 1 year with *T. vaginalis* also may have been sexually abused. In a child older than 1 year of age, suspicion must be high for the probability of sexual abuse. Perinatal transmission and transmission of infection through fomites should not be assumed without an investigation for sexual abuse.

T. vaginalis usually is transmitted sexually in adolescents and adults. In a clinic for patients with sexually transmitted diseases, 60 percent of husbands of women who had chronic, repeated *T. vaginalis* infection were culture-positive for *T. vaginalis*, in contrast to 8 percent in a control group of men attending the same clinic. Large numbers of trichomonads are found in the prostatic secretions of husbands of women with recurrent *T. vaginalis* infection.[77] In a study from the United Kingdom of child and adolescent sexual assault victims, *T. vaginalis* was found in 1 of the 16 girls who were 12 years of age or younger.[38] These data support the concept of sexual transmission of *T. vaginalis*. *T. vaginalis* has been isolated in two males reporting receptive anal intercourse[17] and in a lesbian couple who denied penetrative sex toys or male partners, suggesting transmission may occur through mutual masturbation.[33]

PATHOLOGY AND PATHOGENESIS

As noted previously, five species of *Trichomonas* have been identified in humans; the most clinically important is *T. vaginalis*. *T. vaginalis* is not part of the normal flora, but is found in the human vagina, urethra, and prostate. Inoculation experiments with *T. vaginalis* in the mouth and intestines failed to establish infection in these sites.[79] *T. tenax* is found in the mouth, and *T. hominis*, *T. ardin delteili*, and *T. faecalis* are found in the bowel. *T. tenax* is detected in approximately 5 percent of patients with *T. vaginalis*. *T. tenax*, *T. ardin delteili*, and *T. faecalis* are part of the normal flora and are nonpathogenic. *T. hominis* can infect humans and is associated with gastrointestinal dysentery. The incidence of *T. hominis* in humans is 0.4 to 3.5 percent. *T. hominis* is found only rarely in the stools of patients who concomitantly have *T. vaginalis*.[79]

Virulence factors associated with *T. vaginalis* infection have been defined and include adherence, contact-independent factors, hemolysis, and host macromolecule acquisition. Epithelial cell adherence depends on an intact cytoskeleton and *Trichomonas* protein ligands and proteases, which are necessary to activate adherence molecules.[18,26] Cell contact-independent factors include pH variability and cell-detaching factor, which in vitro inhibit reorganization of cells infected with *T. vaginalis*. *T. vaginalis* produces lactic and acetic acid as by-products of glucose metabolism.[18] These acids lower the pH, which is cytotoxic to epithelial cells. Another metabolic by-product of *T. vaginalis* is cell-detaching factor, which has a cytopathic effect on epithelial cells and increases subepithelial vascularity, producing the clinical sign of "strawberry cervix."[18] The activity of cell-detaching factor is optimal at pH 5.0 or greater. Hemolysis is seen only in the presence of live trichomonads. Cysteine proteases seem to be important for hemolysis because introduction of their inhibitors in vitro eliminates hemolysis by *T. vaginalis*.[12,18]

The addition of metronidazole reduces levels of hemolysis by 50 percent.[18] The hemolytic activity of *T. vaginalis* is temperature-dependent, with maximal hemolysis at 37° C (98.6° F). Hemolysis is inhibited via separation of trichomonads from erythrocytes by a 3-mm filter, suggesting a contact-dependent mechanism.[18] As a parasite, *T. vaginalis* also depends on host macromolecules for nutrition, including plasma proteins and lactoferrin.

The host responds to *T. vaginalis* infection at the cellular level with polymorphonuclear cells and lymphocyte activity. *T. vaginalis* secretes proteases that are chemotactic to polymorphonuclear leukocytes, with resultant phagocytosis and killing of the trichomonad by oxidative mechanisms.[18] *T. vaginalis* secretions are mitogenic to lymphocytes; they enhance phagocytosis by polymorphonuclear cells and may suppress the host immune response if numerous suppressor lymphocytes are activated.

Clinically, women are largely symptomatic, whereas only a few men have symptoms with *T. vaginalis* infection. Estrogen levels in females directly correlate with infection at peak estradiol levels, and symptoms may be influenced by vaginal concentration of estrogen.[26] In an early study of premenarcheal vaginitis in children 3 months to 9 years of age, *T. vaginalis* infection accounted for only 2.8 to 4.4 percent of cases of vaginitis in unestrogenized vaginas in contrast to 50 percent of infections in fully estrogenized vaginas of patients nearing puberty.[28] In asymptomatic males infected with *T. vaginalis*, the prostate gland serves as a reservoir. Men may remain asymptomatic because of the concentration of zinc salts in prostatic fluid, which is cytocidal for trichomonads.[44] In symptomatic males, *T. vaginalis* may be isolated in specimens from urine, urethral discharge, and semen.[39,44]

In the host, trichomonads may serve as a vector for bacteria and viruses, as shown by the high coinfection rate with *T. vaginalis* and human papillomavirus. Although bacteria contaminate trichomonads externally, trichomonads are thought to ingest virus-infected cells and destroy them, with the active virus left intact.[32] *T. vaginalis* has been implicated in pelvic inflammatory disease (PID) via ascension from the vagina to the fallopian tubes. In vitro studies have shown *Escherichia coli* strains, a part of the normal vaginal flora, intimately attached to trichomonads by glycoprotein strands.[40] Trichomonads contaminated with bacteria serve as a vector for the bacteria to produce

pelvic infection on reaching the uterus, fallopian tubes, or peritoneum.[40] Likewise, a more recent study involving Dutch women attending an STI treatment clinic found that 71 percent of the women infected with *T. vaginalis* were coinfected with *M. hominis*. Because of the small sample size, however, whether infection with *T. vaginalis* predisposes females to infection with *M. hominis*, or whether this coinfection reflects the sexual behavior of the individual, remains unclear.[8]

In contrast, vaginal colonization with *Lactobacillus* spp. was thought to inhibit trichomonal invasion by lowering vaginal pH. In a study of 336 African women 15 to 49 years of age, 199 of whom were pregnant, 31 percent were culture-positive for *T. vaginalis* and only 40 percent of the patients tested positive for *Lactobacillus*. Although the rate of *Lactobacillus* colonization in these African women was low, the high rate of *T. vaginalis* infection was not related to the absence of *Lactobacillus*. Trichomonads may not alter the vaginal flora substantially.[55] In women infected with *T. vaginalis* in the lower genital tract, however, the aggressive inflammatory response with resultant punctate hemorrhages allows transmission and infection with HIV-1.[70]

A spectrum of severity of disease exists in patients infected with *T. vaginalis*. Some patients are asymptomatic, whereas others experience severe symptomatic inflammation and discomfort. The virulence of *T. vaginalis* isolates varies; whether this variance is caused by the host response or inherent properties of the parasite is unknown. The Golgi apparatus in *T. vaginalis* is a prominent structure and seems to be a key station in the production of adhesins.[9] Evidence indicates, however, that the dramatic heterogeneity that exists on the surface of the parasite leads to antigenic diversity among different isolates of *T. vaginalis*. In one study, prominent immunogens absent on the surface of *T. vaginalis* isolates led to an enhanced ability of the parasite to cause cytoadherence-dependent killing of HeLa cells in monolayer culture. In addition, only the adherent parasites possessed adhesins, which directly affected cytoadherence and cytotoxicity of the parasites.[2] The cysteine proteases of *T. vaginalis* may be responsible for the cytoadherence, nutrient acquisition, and cytotoxicity of *T. vaginalis*. These proteinases are shed during the life cycle and growth of *T. vaginalis*. One hundred percent of serum samples from women infected with *T. vaginalis*, but none from normal uninfected women, possessed immunoglobulin (Ig)G to numerous trichomonad cysteine proteinases. This serum antiproteinase antibody disappeared after the women received effective therapy for the infection.[3]

Some recent studies provide insight into the relationship between *T. vaginalis* and HIV infection. Potentially biologic synergy is present between *T. vaginalis* and HIV infection, with a 1.5 to 1.7-fold increased risk for HIV acquisition when *T. vaginalis* is present. *T. vaginalis* probably alters the cervicovaginal epithelium by several interacting mechanisms mediated by Toll-like receptors, cytokines, chemokines, and innate immune factors, thereby increasing risk for HIV infection.[76] Women with HIV infection with *T. vaginalis* are found to have a higher prevalence of HIV RNA in vaginal secretions than those without *T. vaginalis* infection. A decrease in mean HIV RNA genital shedding among HIV-infected females also has been noted after *T. vaginalis* treatment and over a 1- to 3-month period.[6] HIV-infected females with *T. vaginalis* alone or with gonorrhea and *Chlamydia* infection also have a 1.9 times higher risk for PID.[6]

IMMUNOLOGY

The interaction between *T. vaginalis* and host immunoglobulins is unclear. In women infected with *T. vaginalis*, specific local antibodies—IgG and IgA—are seen in vaginal secretions. IgA may serve to increase opsonization of the parasite by IgG and result in enhanced phagocytosis. IgG specific for *Trichomonas* cysteine proteases and surface proteins is seen, but it does not help rid the host of infection.[3] *T. vaginalis* synthesizes high-molecular-weight proteins with variable surface expression.[4] Of samples obtained from women infected with *T. vaginalis*, 70 percent of vaginal washes and 80 percent of vaginal mucus samples had IgG to a specific *T. vaginalis* surface protein immunogen with a molecular mass of 230,000 Da (P230). In contrast, no antibody to P230 was detected in uninfected women or in detergent extract depleted of P230, suggesting a highly specific antibody.[4] Clinically, this finding may account for the lack of resistance to repeated *Trichomonas* infections and variable host antibody titers in infected individuals.

CLINICAL MANIFESTATIONS

Trichomonas has been isolated from the tracheal aspirates in preterm neonates with respiratory failure and from nasal secretions in a full-term newborn with suppurative nasal discharge and respiratory distress.[62,74,75] The vagina of an infant may serve as a reservoir of infection that goes unnoticed until the infant is evaluated for fever 5 to 6 weeks after birth. Motile trichomonads and pyuria are seen on examination of the urine. Symptoms resolve after treatment with metronidazole.[64] Premenarcheal girls have diffuse bubbly leukorrhea and pruritus.[51] Vaginitis with a purulent foul-smelling discharge is the most common manifestation of infection with *T. vaginalis* in girls; in some patients, trichomonads may be found in the urine initially, with the development of frank signs of vaginitis 7 to 28 days later.[37]

T. vaginalis causes an infection of the vagina, the urethra, Bartholin's and Skene's glands and the cervix in females. In males it causes an infection of the urethra, urine, semen, Skene's glands, epididymis, and prostate. However, 50 percent of adolescent girls and women and 90 percent of adolescent boys and men infected with *T. vaginalis* are asymptomatic.[73] With control for coinfection with other organisms, *T. vaginalis* infection is associated significantly with purulent discharge, vulvar itching, colpitis macularis (strawberry cervix), and vaginal and vulvar erythema. The sensitivity of the other signs and symptoms of *Trichomonas* vaginitis, including vaginal burning, dysuria, urinary frequency, dyspareunia, frothy

discharge, and cervical friability, is low.[78] Although frothy leukorrhea was associated most frequently with *Trichomonas* infection, 29 percent of the patients with frothy discharge in one study did not have *Trichomonas* infection.[78] Strawberry cervix was pathognomonic of *T. vaginalis* infection, but it was noted in only 2 to 3 percent of patients. Thus, this finding cannot be depended on to establish a clinical diagnosis of *T. vaginalis* vaginitis in most patients.[78] Using an NAAT, *T. vaginalis* has been isolated from the anorectal canal in 8.8 percent of women and 0.9 percent of males who reported anorectal intercourse. The significance of this finding is not completely understood, but it is possible that in women perianal contamination from the vagina occurred.[17]

Trichomonads may ascend the fallopian tubes and, if contaminated with bacteria, can produce PID.[12,40] In HIV-infected girls and women, the risk for developing PID increases significantly with *T. vaginalis* vaginitis.[59] In boys and men, *T. vaginalis* may be manifested as symptomatic urethritis with dysuria secondary to urethral inflammation and discharge. On examination, the discharge often is not visualized. When a discharge is present, it is clear to cloudy, but not grossly purulent. On microscopy, numerous inflammatory cells are seen.[45,49] *T. vaginalis* also may be a cause of chronic nonbacterial prostatitis and may be manifested as chronic prostatitis resistant to standard therapy.[36,49] In addition, *T. vaginalis* has been reported to be an etiologic agent of epididymitis in men, with purulent urethral discharge, scrotal swelling, and enlargement of the epididymis.[21] Although a rare finding, *T. vaginalis* has been reported to infect the median raphe of the penis.[71]

CLINICAL DIAGNOSIS

In girls and women, report of vaginal discharge, the presence of a frothy purulent discharge, vulvar or vaginal erythema, and a strawberry cervix should be suspicious for *Trichomonas* infection. Boys and men tend to be asymptomatic or, if symptomatic, have a purulent urethral discharge and urethral inflammation. If the physician relies on clinical examination alone, 80 percent of infections may be missed.[24,72]

Screening and Diagnosis

No studies have adequately tested screening for asymptomatic females and males. Because of the high prevalence of *Trichomonas* infections in females, testing should be performed in females seeking care for a vaginal discharge. The CDC recommends that screening can be considered in those at high risk for infection (i.e., new or multiple partners, history of STIs, exchange sex for payment, and intravenous drug use).[14] Female foster care attendees also should be considered for routine screening for *T. vaginalis* infections.[31]

Laboratory Tests

Several tests are currently available, including culture, point-of-care tests, and NAAT.

Culture

Culture using Diamond medium is the gold standard for detecting *T. vaginalis* infections.[61] The CDC recommends both culture and vaginal wet mount as acceptable diagnostic testing for *T. vaginalis*. However, cost of and time taken to process this test in the laboratory, storage requirements, and transport requirements of using Diamond media may be prohibitive for routine office use; such settings mainly rely on wet mount testing.[67]

A plastic envelope or pouch method, InPouch (Biomed Diagnostics), is now a standard commercially available method for culture of *T. vaginalis*. This method does not promote the growth of *T. hominis*, a common commensal organism. Vaginal secretions are placed in a liquid with a growth medium that allows the organism to grow and are visualized on direct microscopy. The sensitivity and specificity of the pouch method are 94 percent and 96 percent, respectively; the sensitivity and specificity of wet mount are 58 percent and 100 percent, respectively.[52,61] In contrast to wet-mount examination, the InPouch method also has been found to be significantly more sensitive and specific in one study conducted in young adolescent females (median age, 13.6 years). This underscores the need for accurate testing when using culture-based testing, using InPouch or Diamond medium in cases of sexual abuse.[25] In men, the combination of prostatic massage before collection of the specimen and culture of urethral samples and urinary sediment have a sensitivity of 94 to 98 percent.[47,63]

Point-of-Care Tests

Vaginal microscopy of vaginal secretions continues to be a popular and efficient point-of-care test in the clinical setting. Wet-mount preparations are obtained by swabbing the lateral and anterior vaginal fornices to obtain discharge material with vaginal epithelial cells; the secretions are placed in a tube with normal saline and mounted on a slide as soon as possible to detect motile trichomonads with flagella. These preparations are easy to perform, and the method is cost-effective, but in women its sensitivity is only approximately 60 to 70 percent.[14] The sensitivity of wet-mount preparations decreased from 57 to 22 percent after douching.[24] This method also may be used for urethral specimens in men, but it is insensitive. In addition, the validity of the result depends on the technical skill of the examiner and the rapidity with which the specimen is examined; cooling greatly affects the motility of trichomonads, which may hinder identification.[48] Wet-mount examinations are tests now regulated by the Clinical Laboratory Improvement Amendments, which may be a barrier in performing them in office-based laboratories.

Other point-of-care diagnostic testing for *T. vaginalis* in vaginal secretions in sexually active adolescent and young adult women include an immunochromatographic capillary flow dipstick technology, the OSOM Trichomonas Rapid Test (Genzyme), and a direct nucleic acid probe hybridization test for *T. vaginalis*, *G. vaginalis*, and *Candida* spp., Affirm VP III Microbial Identification System (Becton Dickinson).[11] The results with these tests

can be obtained in 10 minutes and 45 minutes, respectively. Although the sensitivity of both tests on vaginal secretions is greater than 83 percent and the specificity is greater than 97 percent, false-positive test results might occur in low-prevalence populations.[8,14] The direct nucleic acid probe hybridization test has not been validated or approved for use in genital specimens from prepubertal girls or male urethral specimens.[14,31]

Positive attitude toward home testing by adolescent females 14 to 22 years of age predicted high self-test acceptability, and tampon use was associated with increased self-test comfort.[34] Adolescents are capable of performing a point-of-care test with self-collected vaginal swabs with results in 100 percent agreement with swabs and tests conducted by a physician.[35] This is helpful for purposes of clinical research and home-based clinical services.

Nucleic Acid Amplification Tests

NAATs, including PCR assay and APTIMA *T. vaginalis* test (ASR, Gen-Probe), are available through commercial laboratories and frequently combined with tests for bacterial vaginosis, yeast, gonorrhea, and chlamydia. A first-void urine test for *T. vaginalis* by APTIMA is available commercially for females and males. The sensitivity of the PCR assay in detecting *T. vaginalis* in vaginal or endocervical swabs and urine in females and males is 88 percent to 97 percent, and the specificity is 98 percent to 99 percent.[14] The sensitivity of the APTIMA *T. vaginalis* test is 74 percent to 98 percent, and the specificity is 87 percent to 98 percent.[14] However, no data are available in children.[31]

Papanicolaou Smear

Conventional Papanicolaou (Pap) smears can detect trichomonads and have the advantage of being fixed and read at a later time. This method is considered unreliable, however, for the diagnosis of trichomoniasis. *T. vaginalis* does not always appear in its typical pear-shaped morphology after fixation on the slide. Rather, it may appear to be more rounded, similar to a polymorphonuclear leukocyte. Cytopathologic diagnostic criteria for *Trichomonas* infection include the presence of perinuclear halos and a dirty granular background. These features are nonspecific, however, and a similar background and halos can be found with certain other conditions.

The sensitivity of Pap smears for diagnosing *Trichomonas* in culture-proved infection is 56 percent.[46] In a study of 1199 women, *T. vaginalis* infection would have been diagnosed falsely in 37 percent based on Pap smear results. Data show the liquid-based Pap smears to be highly sensitive for *T. vaginalis.* However false-positive test results can occur, and confirmatory testing might be needed in certain circumstances.[14]

Other Diagnostic Tests

Since the availability of NAAT, direct immunofluorescence and enzyme immunoassays are not readily available in the United States for clinical or research purposes. Acridine orange stain is also rarely used in the United States; however, this technique continues to be a useful technique in clinical settings around the world with very basic laboratory resources. The dye stains DNA (yellow-green) and RNA (bright red). *T. vaginalis* stains brick-red with an oval, yellow-green nucleus. The flagella do not stain. Unfixed smears are kept at room temperature for 24 hours; fixed slides may be kept 5 days. Acridine orange stains may permit a rapid, accurate diagnosis of *T. vaginalis* infection. The diagnosis can be confirmed by other diagnostic methods in 93 percent of cases.[56] Acridine orange staining seems to be at least as sensitive as wet-mount examination.[10,65]

TREATMENT

Metronidazole or tinidazole are the treatments of choice for *T. vaginalis* infection and are available for treatment of trichomoniasis in the United States. Worldwide, other drugs used for trichomoniasis include nifuratel, nimorazole, secnidazole, and carnidazole. Despite the availability of these other drugs, metronidazole remains the standard therapy for trichomoniasis. Metronidazole enters the trichomonad via passive diffusion, and its nitro group is reduced to a cytotoxic intermediate that reacts with DNA and causes cell death. Metronidazole is 93 to 95 percent bioavailable, and after oral administration, peak serum levels are attained in 1 to 3 hours and a steady state is attained in 2 to 3 days. Metronidazole is metabolized by the liver, with only 20 percent being protein-bound; the drug is distributed well in the body.[54]

Side effects reported with the use of metronidazole include nausea, vomiting, anorexia, a metallic taste, headache, dizziness, diarrhea, and darkening of the urine. Urticaria, reversible peripheral neuropathy, seizures, and ataxia have been reported with intravenous use. The side effects tend to be dose-related and self-limited.[54] In a study of 1199 women with *T. vaginalis* infection treated with metronidazole, only 4 to 5 percent experienced symptoms of nausea, coated tongue, dryness of the mouth, anorexia, or diarrhea. All symptoms disappeared within a few days of completion of treatment, and in only one case was treatment discontinued because of side effects. In addition, relative and absolute leukopenia was not observed in these subjects. Metronidazole enhances or reactivates the growth of *Candida albicans* in the vagina. Metronidazole may potentiate the actions of anticonvulsants and warfarin. Because a significant disulfiram-like effect is produced when the drug is combined with moderate intake of alcohol, alcohol should be avoided during and for 48 hours after completion of a course of therapy with metronidazole.[54]

In the United States, two treatment regimens are recommended for treatment of *T. vaginalis.* The first is metronidazole 2 g orally in a single dose in adolescents. This regimen is 90 to 95 percent effective.[14] Metronidazole vaginal suppositories are not recommended for the treatment of trichomoniasis in adolescents. The second regimen is tinidazole 2 g orally in a single dose. This regimen is 86 to 100 percent effective. An alternative regimen is metronidazole 500 mg twice daily for 7 days.

Some strains of *T. vaginalis* have reduced susceptibility to metronidazole, with low-level metronidazole-resistant strains reported in 2 to 5 percent of infected cases. Although rare, higher level resistance also has been reported.[58,66,69] Treatment options for metronidazole-resistant cases are limited. If the infection fails to respond to metronidazole, it should be re-treated with tinidazole or metronidazole 500 mg twice daily for 7 days.[14] If treatment failure continues, the patient can be re-treated with 2 g of metronidazole or tinidazole once daily for 5 days.[14]

Treatment of *T. vaginalis* in an HIV-positive adolescent is the same as the standard treatment.[14] However, a randomized clinical trial has demonstrated that the 7-day regimen of metronidazole 500 mg twice daily for 7 days is better than the single-dose regimen in HIV-positive individuals.[41] Metronidazole is a Pregnancy Category B drug. To date, studies have been unable to show consistently an association between metronidazole use during pregnancy and teratogenic effects in infants. In addition, although some data suggest increased risk for having a premature or low-birth-weight infant after undergoing metronidazole treatment, limitations of the studies prevent definitive conclusions regarding risks involved in treatment.[14,42] Treatment during pregnancy may relieve symptoms and prevent pregnancy and perinatal complications. The CDC recommends counseling all pregnant patients on the potential risks and benefits of treatment. Treatment of all symptomatic women is recommended. In asymptomatic women, deferring treatment after 37 weeks of gestation is an option.[14]

Tinidazole is a Pregnancy Category C drug, and its safety in pregnancy has not been evaluated. It is not offered during pregnancy.[14] A breast-fed infant consumes approximately 1 percent of a single 2-g oral dose of metronidazole; infants of mothers who are breast-feeding and who are treated with a single dose of metronidazole for trichomoniasis should be removed from the breast for at least 24 hours after treatment.[14,54] In the case of treatment with tinidazole, breast-feeding can be resumed 3 days after the last dose.

In children with *T. vaginalis* infection of the vulvovaginal area, local therapy with metronidazole is preferred because it has fewer systemic side effects. Cotton-tipped swabs saturated with metronidazole are introduced into the hymen and applied locally. In multifocal infections, genital infections that fail to respond to local therapy, and infections in newborn or infant boys, oral therapy with metronidazole is given (Table 214-1).[50] Before administering oral or intravenous metronidazole to children, the clinician should order baseline hematologic, renal, and liver function tests to monitor changes as therapy continues. Resolution of the signs and symptoms of infection indicates a response to treatment. Eradication of *T. vaginalis* should be confirmed by wet-mount examination or culture (if available) 3 to 5 days after completion of treatment in children.[50]

To date the CDC has not recommended partner treatment for *Trichomonas* infection because of inadequate data to support this approach.[13,14] Despite this, it is not unusual for practitioners to prescribe expedited partner therapy for *T. vaginalis*. As more data emerge to demonstrate a decrease in repeat infection with partner treatment, a national policy on expedited partner therapy for *T. vaginalis* may be reconsidered.[68]

TABLE 214-1 Dosing of Metronidazole in Children

		Metronidazole Administered (mg)	
Age (yr)	Weight (kg)	Orally*	Locally†
0-1	10	150	10
1-6	20.5	250	50
7-12	40	500	150
>12	>40	500-1000	250-500

Modified from Kurnatowska A, Komorowska A. Urogenital trichomoniasis in children. In: Honigberg BM, editor. *Trichomonads parasitic in humans*. New York: Springer-Verlag; 1989, p. 268.
*Oral dose = one third of the total dose administered every 8 hours for 5 to 10 days.
†Local dose = total dose administered intravaginally once daily or half the total dose administered twice daily for 5 to 10 days.

PROGNOSIS

If untreated, *T. vaginalis* can lead to chronic inflammation of the Bartholin and Skene glands in adolescent females and to prostatitis and urethritis with urethral stricture formation in adolescent males.[19] *T. vaginalis* has been associated with adverse pregnancy outcomes, primarily premature rupture of membranes, preterm labor, and low-birth-weight infants.[42] Complete resolution of symptoms plus eradication of *T. vaginalis* usually is noted when treatment is provided promptly in all situations.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Ahrens KR, Richardson LP, Courtney ME, et al. Laboratory-diagnosed sexually transmitted infections in former foster youth compared with peers. *Pediatrics* 2010;**126**:e97–103.
6. Bachmann LH, Hobbs MM, Sena AC, et al. *Trichomonas vaginalis* genital infections: progress and challenges. *Clin Infect Dis* 2011; **53**(Suppl. 3):S160–70.
11. Briseldon AM, Hillier SL. Evaluation of affirm VP microbial identification system test for *Gardnerella vaginalis* and *Trichomonas vaginalis*. *J Clin Microbiol* 1994;**32**:148–52.
13. Centers for Disease Control and Prevention. *Expedited partner therapy in the management of sexually transmitted diseases*. Atlanta: U.S. Department of Health and Human Services; 2006.
14. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines. 2010. *MMWR Recomm Rep* 2010; **59**(RR-12).
15. Centers for Disease Control and Prevention. *Sexually transmitted disease surveillance 2010*. Atlanta: U.S. Department of Health and Human Services; 2011.
17. Cosentino LA, Campbell T, Jett A, et al. Use of nucleic acid amplification testing for diagnosis of ano rectal sexually transmitted infections. *J Clin Microbiol* 2012;**50**:2005–8.
22. Ford JL, Browning CR. Neighbourhood social disorganization and acquisition of trichomoniasis among young adults in the United States. *Am J Public Health* 2011;**101**:1696–703.
23. Forhan SE, Gottlieb SL, Sternberg MR, et al. Prevalence of sexually transmitted infections among female adolescents aged 14 to 19 in the United States. *Pediatrics* 2009;**124**:1505–12.

25. Gallion HR, Dupree LJ, Scott TA, et al. Diagnosis of *Trichomonas vaginalis* in female children and adolescent evaluated for possible sexual abuse: a comparison of the InPouch TV culture method and wet mount microscopy. *J Pediatr Adolesc Gynecol* 2009;**22**:300–5.
27. Goyal M, Hayes K, McGowen KL, et al. Prevalence of *Trichomonas vaginalis* infection in symptomatic adolescent females presenting to an emergency department. *Acad Emerg Med* 2011;**18**:763–6.
30. Gray RH, Kigozi G, Serwadda D, et al. The effects of male circumcision on female partner's genital tract symptoms and vaginal infections in a randomized trial in Rakai, Uganda. *Am J Obstet Gynecol*;2009;**200**:42.e1–7.
32. Hammerschlag MR. Sexual assault and abuse of children. *Clin Infect Dis* 2011;**53**(Suppl. 3):S103–9.
33. Hobbs MM, Sena AC, Swygard,H, et al. *Trichomonas vaginalis* and trichomoniasis. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually transmitted diseases*. New York: McGraw-Hill Medical; 2008. p. 771–93.
34. Huppert JS, Hesse EA, Bernard MA, et al. Acceptability of self-testing for trichomoniasis increases with experience. *Sex Transm Infect* 2011;**87**:494–500.
35. Huppert JS, Hesse E, Kim G, et al. Adolescent women can perform a point-of-care test for trichomoniasis as accurately as clinicians. *Sex Transm Infect* 2010;**86**:514–19.
41. Kissinger P, Mena L, Levison J, et al. A randomized treatment trial: single versus 7 day dose of metronidazole for the treatment of *Trichomonas vaginalis* among HIV-infected women. *J Acquir Immune Defic Syndr* 2010;**55**:565–71.
43. Krashin JW, Koumans EH, Bradshaw-Sydnor AC. Trichomonas vaginalis prevalence, incidence, risk factors and antibiotic resistance in an adolescent population. *Sex Transm Dis* 2010;**37**:440–4.
68. Schwebke JR, Desmond RA. A randomized clinical trial of partner notification methods for prevention of trichomoniasis in women. *Sex Transm Dis* 2010;**37**:392–6.
76. Thurman AR, Doncel GF. Innate immunity and inflammatory response to *Trichomonas vaginalis* and bacterial vaginosis: relationship to HIV acquisition. *Am J Reprod Immunol* 2011;**65**:89–98.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 215

Balantidium coli Infection

Peter J. Hotez

Balantidium coli is the largest protozoan parasite and the only ciliate to infect humans.[7,11,18] The organism has a worldwide distribution, but usually it is found in less developed nations of the tropics. Because pigs are the principal animal reservoir, most human infections have been reported in tropical regions where swine have close contact with humans, such as the islands of East Asia and the South Pacific (including the Philippines, Papua New Guinea, and West Irian) and Central and South America (including the Altiplano of Bolivia).[12,13] High prevalence rates of this infection can be found among pig farmers in this region. Balantidiasis occurs in areas where other swine-associated parasitic zoonoses (e.g., taeniasis, trichinellosis, ascariasis) also are prevalent, although infection with *B. coli* occurs rarely. In Iran, where pig raising no longer is permitted, wild boars serve as an animal reservoir.[14] The organism first was described in 1857 by Malmsten, who observed the ciliates from two patients in Sweden.[3]

ETIOLOGY AND PATHOGENESIS

The organism has trophozoite and cyst stages. The trophozoite is a large, pear-shaped organism covered with cilia. Estimates of size range from 50 to 100 μm in length and 40 to 70 μm in width.[7] This enormous protozoan organism typically can be seen by light microscopy using only low-power magnification.[7] Subcellular organelles, such as a cytostome and many large vacuoles containing bacteria and debris, are visualized under higher magnification. Similar to many other ciliates, *B. coli* trophozoites have a macronucleus and a micronucleus. The trophozoites colonize the large intestine, where ultimately they round up and secrete a cyst wall as they pass down the lumen. The cysts measure 50 to 70 μm and also contain a macronucleus and a micronucleus. The cyst stages can survive in the outside environment and are infectious to a wide range of animals, including humans (see later discussion).

REFERENCES

1. Ahrens KR, Richardson LP, Courtney ME, et al. Laboratory-diagnosed sexually transmitted infections in former foster youth compared with peers. *Pediatrics* 2010;**126**:e97–103.
2. Alderete JF. Alternating phenotypic expression of two classes of *Trichomonas vaginalis* surface markers. *Rev Infect Dis* 1988; **10**(Suppl.):408–12.
3. Alderete JF, Newton E, Dennis C, et al. Antibody in sera of patients infected with *Trichomonas vaginalis* is to trichomonad proteinases. *Genitourin Med* 1991;**67**:331–4.
4. Alderete JF, Newton E, Dennis C, et al. Vaginal antibody of patients with trichomoniasis is to a prominent surface immunogen of *Trichomonas vaginalis*. *Genitourin Med* 1991;**67**:220–5.
5. Anrolu RI, Fagbenro Beyioku AF, et al. Prevalence of *Trichomonas vaginalis* in patients with vaginal discharge in Lagos, Nigeria. *Niger Postgrad Med J* 2001;**8**:183–6.
6. Bachmann LH, Hobbs MM, Sena AC, et al. *Trichomonas vaginalis* genital infections: progress and challenges. *Clin Infect Dis* 2011; **53**(Suppl. 3):S160–70.
7. Barbone F, Austin H, Louv WC, et al. A follow-up study of methods of contraception, sexual activity, and rates of trichomoniasis, candidiasis, and bacterial vaginosis. *Am J Obstet Gynecol* 1990;**163**: 510–14.
8. Belkum van A, van der Schee C, van der Meijden WI. A clinical study on the association of *Trichomonas vaginalis* and *Mycoplasma hominis* infections in women attending a sexually transmitted disease (STD) outpatient clinic. *FEMS Immunol Med Microbiol* 2001;**32**:27–32.
9. Benchimol M, Ribeiro KC, Mariante RM, et al. Structure and division of the Golgi complex in *Trichomonas vaginalis* and *Trichomonas fetus*. *Eur J Cell Biol* 2001;**80**:593–607.
10. Bickley LS, Krisher KK, Punsalang A Jr, et al. Comparison of direct fluorescent antibody, acridine orange, wet mount, and culture for detection of *Trichomonas vaginalis* in women attending a public sexually transmitted diseases clinic. *Sex Transm Dis* 1989;**16**: 127–31.
11. Briseldon AM, Hillier SL. Evaluation of affirm VP microbial indentification system test for *Gardnerella vaginalis* and *Trichomonas vaginalis*. *J Clin Microbiol* 1994;**32**:148–52.
12. Cates W Jr, Rauh JL. Adolescents and sexually transmitted diseases: an expanding problem. *J Adolesc Health Care* 1985;**6**:257–61.
13. Centers for Disease Control and Prevention. *Expedited partner therapy in the management of sexually transmitted diseases*. Atlanta: United States Department of Health and Human Services; 2006.
14. Centers for Disease Control and Prevention. Sexually transmitted disease treatment guidelines 2010. *MMWR Recomm Rep* 2010; **59**(RR-12).
15. Centers for Disease Control and Prevention. *Sexually transmitted disease surveillance 2010*. Atlanta: U.S. Department of Health and Human Services; 2011.
16. Charles SX. Epidemiology of *Trichomonas vaginalis* in rural adolescent and juvenile children. *J Trop Pediatr* 1991;**37**:90.
17. Cosentino LA, Campbell T, Jett A, et al. Use of nucleic acid amplification testing for diagnosis of anorectal sexually transmitted infections. *J Clin Microbiol* 2012;**50**:2005–8.
18. Dailey DC, Chang T, Alderete JF. Characterization of *Trichomonas vaginalis* hemolysis. *Parasitology* 1990;**101**:171–5.
19. Daly CC, Maggwa N, Mati JK, et al. Risk factors for gonorrhoea, syphilis, and trichomonas infections among women attending family planning clinics in Nairobi, Kenya. *Genitourin Med* 1994; **70**:155–61.
20. Ekwempu CC, Lawande RV, Egler LJ. Microbial flora of the lower genital tract of women in labour in Zaria, Nigeria. *J Clin Pathol* 1981;**34**:82–3.
21. Fisher I, Morton RS. Epididymitis due to *Trichomonas vaginalis*. *Br J Vener Dis* 1969;**45**:252–3.
22. Ford JL, Browning CR. Neighbourhood social disorganization and acquisition of trichomoniasis among young adults in the United States. *Am J Public Health* 2011;**101**:1696–703.
23. Forhan SE, Gottlieb SL, Sternberg MR, et al. Prevalence of sexually transmitted infections among female adolescents aged 14 to 19 in the Unites States. *Pediatr* 2009;**124**:1505–12.
24. Fouts AC, Kraus SJ. *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. *J Infect Dis* 1980;**141**: 137–43.
25. Gallion HR, Dupree LJ, Scott TA, et al. Diagnosis of *Trichomonas vaginalis* in female children and adolescent evaluated for possible sexual abuse: a comparison of the In Pouch TV culture method and wet mount microscopy. *J Pediatr Adolesc Gynecol* 2009;**22**:300–5.
26. Garber GE, Lemchuk-Favel LT, Rousseau G. Effect of estradiol on cell detaching factor of *Trichomonas vaginalis*. *Clin Microbiol* 1991; **29**:1847–9.
27. Goyal. M, Hayes. K, McGowen KL, et al. Prevalence of *Trichomonas vaginalis* infection in symptomatic adolescent females presenting to an emergency department. *Acad Emerg Med* 2011;**18**: 763–6.
28. Graves A, Gardner WA Jr. Pathogenicity of *Trichomonas vaginalis*. *Clin Obstet Gynecol* 1993;**36**:145–52.
29. Gray LA, Kotcher E. Vulvovaginitis in childhood. *Clin Obstet Gynecol* 1960;**3**:165–74.
30. Gray RH, Kigozi G, Serwadda D, et al. The effects of male circumcision on female partner's genital tract symptoms and vaginal infections in a randomized trial in Rakai, Uganda. *Am J Obstet Gynecol* 2009;**200**:42.e1–7.
31. Hammerschlag MR. Sexual assault and abuse of children. *Clin Infect Dis* 2011;**53**(Suppl. 3):S103–9.
32. Hammerschlag MR, Alpert S, Rosner I, et al. Microbiology of the vagina in children: normal and potentially pathogenic organisms. *Pediatrics* 1978;**62**:57–62.
33. Hobbs MM, Sena AC,Swygard,H, et al. *Trichomonas vaginalis* and trichomoniasis. In: Holmes KK, Sparling PF, Stamm WE, et al, editors. *Sexually transmitted diseases*. New York: McGraw-Hill Medical; 2008. p. 771–93.
34. Huppert JS, Hesse EA, Bernard MA, et al. Acceptability of self-testing for trichomoniasis increases with experience. *Sex Transm Infect* 2011;**87**:494–500.
35. Huppert JS, Hesse E, Kim G, et al. Adolescent women can perform a point-of-care test for trichomoniasis as accurately as clinicians. *Sex Transm Infect* 2010;**86**:514–19.
36. Ireton RC, Berger RE. Prostatitis and epididymitis. *Urol Clin North Am* 1984;**13**:87–8.
37. Jones JG, Yamauchi T, Lambert B. *Trichomonas vaginalis* infestation in sexually abused girls. *Am J Dis Child* 1985;**139**:846–7.
38. Kawsar M, Anfield A, Walters E, et al. Prevalence of sexually transmitted infections and mental health needs of female child and adolescent survivors of rape and sexual assault attending a specialist clinic. *Sex Transm Infect* 2004;**80**:138–41.
39. Kaydos-Daniels SC, Miller WC, Hoffman I. The use of specimens from various genitourinary sites in men, to detect *Trichomonas vaginalis* infection. *J Infect Dis* 2004;**189**:1926–31.
40. Keith LG, Berger GS, Edelman DA, et al. On the causation of pelvic inflammatory disease. *Am J Obstet Gynecol* 1983;**149**: 215–24.
41. Kissinger P, Mena L, Levison J, et al. A randomized treatment trial: single versus 7 day dose of metronidazole for the treatment of *Trichomonas vaginalis* among HIV-infected women. *J Acquir Immune Defic Syndr* 2010;**55**:565–71.
42. Klebanoff MA, Carey JC, Hauth JC, et al. Failure of metronidazole to prevent preterm delivery among pregnant women with asymptomatic *Trichomonas vaginalis* infection. *N Engl J Med* 2001;**16**: 487–93.
43. Krashin JW, Koumans EH, Bradshaw-Sydnor AC. *Trichomonas vaginalis* prevalence, incidence, risk factors and antibiotic resistance in an adolescent population. *Sex Transm Dis* 2010;**37**:440–4.
44. Krieger JN, Rein MF. Zinc sensitivity of *Trichomonas vaginalis*: in vitro studies and clinical implications. *J Infect Dis* 1982;**146**: 341–5.
45. Krieger JN, Jenny C, Verdon M, et al. Clinical manifestations of trichomoniasis in men. *Ann Intern Med* 1993;**118**:844–9.
46. Krieger JN, Tam MR, Stevens CE, et al. Diagnosis of trichomoniasis: comparison of conventional wet-mount examination with cytologic studies, cultures, and monoclonal antibody staining of direct specimens. *JAMA* 1988;**259**:1223–7.
47. Krieger JN, Verdon M, Siegel N, et al. Risk assessment and laboratory diagnosis of *Trichomoniasis* in men. *J Infect Dis* 1992;**166**: 1362–6.
48. Krowchuk DP, Anglin TM, Kumar ML. Rapid diagnosis of common sexually transmitted diseases in adolescents: a review. *Pediatr Dermatol* 1989;**6**:278–9.
49. Kuberski T. *Trichomonas vaginalis* associated with nongonococcal urethritis and prostatitis. *Sex Transm Dis* 1980;**7**:135–6.

50. Kurnatowska A, Komorowska A. Urogenital trichomoniasis in children. In: Honigberg BM, editor. *Trichomonads parasitic in humans*. New York: Springer-Verlag; 1989. p. 246–73.
51. Lang WR. Premenarchal vaginitis. *Obstet Gynecol* 1959;**13**:723–9.
52. Lawing LF, Hedges SR, Schwebke JR. Detection of trichomoniasis in vaginal and urine specimens from women by culture and PCR. *J Clin Microbiol* 2000;**38**:3585–8.
53. Lossick J. Epidemiology of urogenital trichomoniasis. In: Honigberg BM, editor. *Trichomonads parasitic in humans*. New York: Springer-Verlag; 1989. p. 311–23.
54. Lossick J. Therapy of urogenital trichomoniasis. In: Honigberg BM, editor: *Trichomonads parasitic in humans*. New York: Springer-Verlag; 1989. p. 324–41.
55. Mason PR, MacCallum MJ, Poynter B. Association of *Trichomonas vaginalis* with other microorganisms. *Lancet* 1982;**1**:1067.
56. Mason PR, Super H, Fripp PJ. Comparison of four techniques for the routine diagnosis of *Trichomonas vaginalis* infection. *J Clin Pathol* 1976;**29**:154–7.
57. McLellan R, Spence MR, Brockman M, et al. The clinical diagnosis of trichomoniasis. *Obstet Gynecol* 1982;**60**:30–4.
58. Moldwin RM. Sexually transmitted protozoal infections. *Urol Clin North Am* 1992;**19**:93–6.
59. Moodley P, Wilkinson D, Connolly C. *Trichomonas vaginalis* is associated with pelvic inflammatory disease in women infected with human immunodeficiency virus. *Clin Infect Dis* 2002;**34**: 519–22.
60. Neinstein LS, Goldenring J, Carpenter S. Nonsexual transmission of sexually transmitted diseases: an infrequent occurrence. *Pediatrics* 1984;**74**:67–76.
61. Ohlemeyer CL, Hornberger LL, Lynch DA, et al. Diagnosis of *Trichomonas vaginalis* in adolescent females: In Pouch TV culture versus wet-mount microscopy. *J Adolesc Health* 1998;**22**:205–8.
62. Redman R, Johnson D. Newborn with suppurative nasal discharge. *Pediatr Infect Dis J* 2003;**22**:933, 937–8.
63. Saxena SB, Jenkins RR. Prevalence of *Trichomonas vaginalis* in men at high risk for sexually transmitted diseases. *Sex Transm Dis* 1991;**18**:138–42.
64. Schares T, Machtinger S, D'Harlingue AE, et al. *Trichomonas vaginalis* urinary tract infection in an infant. *Pediatr Infect Dis* 1982;**1**:340–1.
65. Schee van der C, Belkum van A, Zwijgers L, et al. Improved diagnosis of *Trichomonas vaginalis* infection by PCR using vaginal swabs and urine specimens compared to diagnosis by wet-mount microscopy, culture, and fluorescent staining. *J Clin Microbiol* 1999;**37**: 4127–30.
66. Schmid G, Narcisi E, Mosure D, et al. Prevalence of metronidazole-resistant *Trichomonas vaginalis* in a gynecology clinic. *J Reprod Med* 2001;**46**:545–9.
67. Schmid GP, Matheny LC, Zaidi AA, et al. Evaluation of six media for the growth of *Trichomonas vaginalis* from vaginal secretions. *J Clin Microbiol* 1989;**6**:1230–3.
68. Schwebke JR, Desmond RA. A randomized clinical trial of partner notification methods for prevention of trichomoniasis in women. *Sex Transm Dis* 2010;**37**:392–6.
69. Sobel JD, Nagappan V, Nyirjesy P. Metronidazole-resistant vaginal trichomoniasis: an emerging problem. *N Engl J Med* 1999;**341**: 292–3.
70. Sorvillo F, Kerndt P. *Trichomonas vaginalis* and amplification of HIV-1 transmission. *Lancet* 1998;**351**:213–14.
71. Sowmini CN, Vijayalakshmi K, Chellamuthiah C, et al. Infections of the median raphe of the penis: report of three cases. *Br J Vener Dis* 1972;**49**:469–74.
72. Spence MR, Hollander DH, Smith J, et al. The clinical and laboratory diagnosis of *Trichomonas vaginalis* infection. *Sex Transm Dis* 1980;**7**:168–71.
73. Spiegel C. Microflora associated with *Trichomonas vaginalis* and vaccination against vaginal trichomoniasis. In: Honigberg BM, editor. *Trichomonads parasitic in humans*. New York: Springer-Verlag; 1989. p. 213–24.
74. Szarka K, Temesvari P, Kerekes A, et al. Neonatal pneumonia caused by *Trichomonas vaginalis*. *Acta Microbiol Immunol Hung* 2004;**49**:15–19.
75. Temesvari P, Kerekes A, Tege A, et al. Demonstration of *Trichomonas vaginalis* in tracheal aspirates in infants with early respiratory failure. *J Matern Fetal Neonat Med* 2001-2002;**11**:347–9.
76. Thurman AR, Doncel GF. Innate immunity and inflammatory response to *Trichomonas vaginalis* and bacterial vaginosis: relationship to HIV acquisition. *Am J Reprod Immunol* 2011;**65**:89–98.
77. Watt L, Jennison RF. Incidence of *Trichomonas vaginalis* in marital partners. *Br J Vener Dis* 1960;**36**:163–6.
78. Wolner-Hanssen PW, Krieger JN, Stevens CE, et al. Clinical manifestations of vaginal trichomoniasis. *J A M A* 1989;**261**:571–6.
79. Wilcox RR. Epidemiological aspects of human trichomoniasis. *Br J Vener Dis* 1960;**36**:167–74.

Frequently, *B. coli* does not invade human tissues and does not cause clinical disease. Under conditions that are not well understood, however, *B. coli* also has the potential for highly aggressive invasion and destruction of tissue. Whether the invasive potential of *B. coli* results from parasite virulence, compromised host defenses, or some combination of these two factors is unclear. The observation that invasive disease occurs more commonly in debilitated patients and in patients with polyparasitism suggests that host defenses have an important role in limiting tissue destruction by *B. coli*.[1,11]

When parasite invasion occurs, it begins in the colonic mucosa, where ulcerations and secondary microabscesses can result. Extensive tissue damage in the cecum and appendix results in clinical presentations of typhlitis and appendicitis.[4,5,11] Histopathologic examination of these tissues reveals flask-shaped ulcerations and necrosis with an extensive inflammatory infiltrate composed predominantly of polymorphonuclear leukocytes.[4,11] *B. coli* probably creates mucosal ulcerations through the release of histolytic enzymes similar to those described from *Entamoeba histolytica*.[15] Ulcerations can lead to hemorrhage or even colonic perforation. A second type of histopathology has been reported in which patients harboring *B. coli* develop inflammatory polyposis of the rectum and sigmoid colon.[10]

When tissue invasion is extensive, the organism can metastasize to extraintestinal sites and cause hepatic and pulmonary involvement. Polymorphonuclear inflammatory cell infiltration results in abscesses at these sites.[3,5,10] Most patients with metastatic balantidiasis have recognizable defects in host defenses.[10]

EPIDEMIOLOGY

As noted earlier, human balantidiasis has a worldwide distribution, but epidemic foci have been reported in the swine-producing areas of Papua New Guinea, West Irian, Micronesia, the Seychelles Islands, the Philippines, and Central and South America.[3,12,16] Incidence rates can be high among swine farmers and slaughterhouse workers. The potential for development of human *B. coli* infections is thought to be high in areas of poor hygiene where extensive contact occurs between humans and pigs. The major risk factors include close contact between pigs and humans, inadequate sanitation, and subtropical or tropical climates that favor survival of the infective cyst stages.[13] A notorious outbreak of human balantidiasis occurred after a devastating typhoon on the Pacific island of Truk caused widespread contamination of ground and surface water supplies with pig feces.[16] Many other animals, including nonhuman primates, guinea pigs, horses, cattle, and rats, also potentially can serve as reservoir hosts. *B. coli* also colonizes many great apes, including baboons, orangutans, chimpanzees, and gorillas, and clinical balantidiasis has been reported in these primates when they are maintained in captivity.[11] Human epidemics also have been described in institutional settings, especially where crowding mixes with low levels of personal hygiene.[16] In these instances, human-to-human spread has been postulated. Although *B. coli* is not known as an opportunistic pathogen in patients infected with human immunodeficiency virus, at least one case of *B. coli* in this setting has been described.[3]

CLINICAL MANIFESTATIONS

Asymptomatic Infection

Most infections are asymptomatic or cause occasional loose stools. This situation probably accounts for 85 percent of patients harboring *B. coli*.[11] Asymptomatic infection may occur more commonly in children than in adults.[17]

Diarrhea

The next most frequent presentation of *B. coli* infections is in patients who have intermittent diarrhea, abdominal pain, and weight loss.[17] Sometimes, discrete ulcerations can be observed during sigmoidoscopy.[11] Chronic diarrhea has been described in a patient with acquired immunodeficiency syndrome.[2] A subset of patients with balantidiasis subsequently develop invasive disease.

Invasive Colonic Balantidiasis

The hallmark of *Balantidium* colitis is dysentery with bloody and mucous stools, colonic tenderness, leukocytosis, and fever. Sigmoidoscopy and colonoscopy of these patients reveal ulcerations and formation of mucosal granulomas.[1,11,16] Involvement of the large intestine can be diffuse, although in some cases, right-sided colonic lesions predominate. Right-sided colonic lesions can progress to typhlitis or appendicitis.[4,5,11] Transmural involvement of the colon frequently results in intestinal obstruction, hemorrhage, and balantidial peritonitis. Colonic perforation is an ominous complication that is associated with extremely high mortality.[11]

Metastatic and Extraintestinal Balantidiasis

Highly invasive balantidiasis leading to metastatic disease of the mesenteric lymph nodes, liver, and lung is a rare complication that can occur in malnourished, debilitated, and immunocompromised patients. Pulmonary infection with *B. coli* has been described.[13] A case of extraintestinal disease associated with pulmonary hemorrhage and iron deficiency was described recently, possibly as a result of inhalation of pig manure.[9]

DIAGNOSIS

Stools from patients harboring *B. coli* have been described as having a pigpen odor.[17] The examination of wet preparations of fresh or concentrated stools usually reveals cyst and trophozoite forms of *B. coli*. Cilia motility and rapid rotary motion of the trophozoites occasionally can be appreciated under low-power magnification. Because the organism takes up heavy concentrations of dye, stained

preparations typically do not reveal internal structures or even cilia.[7] These large organisms can be confused with helminth ova, especially on stained preparations.[7] As an adjunct to direct fecal examinations, sigmoidoscopy can show ulcerations from which abundant trophozoites may be obtained for diagnosis.[17] Bronchoalveolar wash fluid containing *B. coli* has been reported; it has been suggested that diagnosing such lung infections can be problematic because of the potential to confuse ciliated *B. coli* with ciliated pulmonary epithelial cells.[13]

TREATMENT

For the treatment of intestinal balantidiasis, numerous chemotherapeutic regimens have been tried, usually with some improvement. In many cases, the parasite is not eradicated, however.[11] For children older than 8 years, tetracycline 40 mg/kg/day in four doses for 10 days (maximum, 2 g/day) is the treatment of choice. Tetracycline is considered to be investigational for this condition by the U.S. Food and Drug Administration. Also considered investigational for balantidiasis are the drugs iodoquinol 30-40 mg/kg/day (maximum, 2 g) in three doses for 20 days and metronidazole 35 to 50 mg/kg/day in three doses for 5 days (maximum dosage, 2 g).[6,8] Nitazoxanide also may be effective.[13] Alternative chemotherapeutic agents that have been tried with variable success include paromomycin and chloroquine.[11] Surgical intervention often is required for gastrointestinal invasive complications of *B. coli*, such as typhlitis, appendicitis, and peritonitis.

NEW REFERENCES SINCE SIXTH EDITION

6. *Drugs for parasitic infections from the Medical Letter*. New Rochelle NY: The Medical Letter; 2010.
9. Koopowitz A, Smith P, van Rensburg N, Rudman A. *Balantidium coli*-induced pulmonary haemorrhage with iron deficiency. *S Afr Med J* 2010;**100**:534–8.
13. Schuster FL, Ramirez-Avila L. Current world status of *Balantidium coli*. *Clin Microbiol Rev* 2008;**21**:626–38.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 216

Cryptosporidiosis

Theresa J. Ochoa • A. Clinton White, Jr.

Cryptosporidium is an important cause of acute diarrhea in normal hosts worldwide, of persistent diarrhea in children in developing countries, and of chronic diarrhea in immunocompromised hosts, including patients with acquired immunodeficiency syndrome (AIDS).[81,119,187,210] These protozoan parasites were identified first in the stomach of mice in 1907[200] and for decades were considered to be veterinary pathogens. The first human cases of cryptosporidiosis were reported in 1976.[134,155] In the early 1980s, large numbers of cases were noted with the emerging epidemic of AIDS.[49] Thereafter, *Cryptosporidium* was identified in animal handlers and children[49,214] and was associated with large waterborne outbreaks of diarrhea.[50,124] Studies have shown that *Cryptosporidium* is a common cause of diarrheal disease in immunocompetent and immunocompromised hosts. Recent studies using more sensitive techniques suggest that it is an underrecognized cause of moderate-to-severe diarrhea in Africa and Asia. *Cryptosporidium* is thought to be second to rotavirus as a cause of diarrhea morbidity worldwide.[149,187]

MICROBIOLOGY

Cryptosporidium spp. are ubiquitous, small (2 to 6 μm), obligate intracellular protozoan parasites that infect the epithelium of the gastrointestinal tract of vertebrates. *Cryptosporidium* was previously classified within the subclass Coccidia, along with *Isospora*, *Cyclospora*, and *Toxoplasma*, but this may soon change.[210] Species names were given according to the animal that they infected.[59] Initial cross-transmission studies revealed that little host specificity exists. Subsequent genetic studies have identified at least 26 accepted species of *Cryptosporidium* and more than 45 *Cryptosporidium* genotypes.[33,116] At least 15 species have been documented to cause human infection.[18,33] Most human infections are caused by separate

REFERENCES

1. Arean VM, Koppisch E. Balantidiasis: a review and report of cases. *Am J Pathol* 1956;**32**:1089–116.
2. Cermeno JR, Hernandez De Cuesta I, Uzcategui O, et al. *Balantidium coli* in an HIV-infected patient with chronic diarrhoea. *AIDS* 2003;**17**:941–2.
3. Clyti E, Aznar C, Couppie P, et al. A case of coinfection by *Balantidium coli* and HIV in French Guiana. *Bull Soc Pathol Exot* 1998;**91**:309–11.
4. Dodd LG. *Balantidium coli* infestation as a cause of acute appendicitis. *J Infect Dis* 1991;**163**:1392.
5. Dorfman S, Rangel O, Bravo LG. Balantidiasis: report of a fatal case with appendicular and pulmonary involvement. *Trans R Soc Trop Med Hyg* 1984;**78**:833–4.
6. *Drugs for parasitic infections from the Medical Letter*. New Rochelle NY: The Medical Letter; 2010.
7. Garcia LS, Bruckner DA. *Diagnostic medical parasitology*. 2nd ed. Washington DC: American Society for Microbiology; 1993.
8. Garcia-Laverde A, DeBonilla L. Clinical trials with metronidazole in human balantidiasis. *Am J Trop Med Hyg* 1975;**24**: 781–3.
9. Koopowitz A, Smith P, van Rensburg N, Rudman A. *Balantidium coli*-induced pulmonary haemorrhage with iron deficiency. *S Afr Med J* 2010;**100**:534–8.
10. Ladas SD, Savva S, Frydas A, et al. Invasive balantidiasis presented as chronic colitis and lung involvement. *Dig Dis Sci* 1989;**34**: 1621–3.
11. Lee RV, Prowten AW, Anthone S, et al. Typhlitis due to *Balantidium coli* in captive lowland gorillas. *Rev Infect Dis* 1990;**12**:1052–9.
12. Radford AJ. Balantidiasis in Papua New Guinea. *Med J Aust* 1971;**1**:238–41.
13. Schuster FL, Ramirez-Avila L. Current world status of *Balantidium coli*. *Clin Microbiol Rev* 2008;**21**:626–38.
14. Solaymani-Mohammadi S, Rezaian M, Ali Anwar M. Human balantidiasis in Iran: an unresolved enigma? *Trends Parasitol* 2005;**21**: 160–1.
15. Tempelis CH, Lysenko MG. The production of a hyaluronidase by *B. coli*. *Exp Parasitol* 1957;**6**:31–6.
16. Walzer PD, Judson FN, Murphy KB, et al. Balantidiasis outbreak in *Truk Am J Trop Med Hyg* 1973;**22**:33–41.
17. Woody NC, Woody HB. Balantidiasis in infancy: review of literature and report of a case. *J Pediatr* 1960;**56**:485–9.
18. Young MD. Balantidiasis. *JAMA* 1939;**113**:580–4.

species, *C. hominis* and *C. parvum*. *C. hominis* is found only in humans (but can infect gnotobiotic pigs), and *C. parvum* is found in a wide range of animals (particularly cattle and sheep) and in humans. The relative frequency of *C. hominis* and *C. parvum* in humans differs in geographic regions, probably as the result of differences in transmission routes. In European countries, *C. parvum* is found more commonly in human cases than *C. hominis*, although a more recent study in the United Kingdom has shown a comparable rate for both pathogens.[114] In the rest of the world, *C. hominis* is the predominant species in humans.[22,24,216] *C. meleagridis* has been identified in most case series. Humans are less frequently infected with *C. cuniculus*, *C. canis*, *C. felis*, *C. muris*, *C. ubiquitum*, and *C. viatorum*.[23,37,56,57,68-70] Rare infections have been noted with six other species and four other genotypes.[33]

LIFE CYCLE

Cryptosporidium spp. can complete their entire life cycles, including asexual (merogony) and sexual (sporogony) reproductive cycles, within a single host (homoxenous). The life cycle is characterized by six major developmental stages: (1) excystation, or release of infective sporozoites; (2) merogony, or asexual replication in the host; (3) gametogony, or the formation of microgametocytes and macrogametocytes; (4) fertilization, or the union of microgametocytes and macrogametocytes; (5) oocyst formation; and (6) sporogony, or the formation of infectious sporozoites within the oocyst wall (Fig. 216-1).[18,59,156]

The life cycle begins with ingestion of the infectious oocyst. The oocysts are activated in the stomach (temperature and acid) and upper intestines (bicarbonate, bile, and pancreatic enzymes). This allows the organisms to excyst, releasing four infective sporozoites.[156] The motile sporozoites glide over the surface of the epithelium and bind to receptors on the surface of the intestinal epithelium, inducing actin polymerization and protrusion of the intestinal epithelial cell membrane.[156] The membrane surrounds the sporozoite and fuses to form the parasitophorous vacuole, which remains in the microvillus layer on the surface of the epithelium. Inside the parasitophorous vacuole, the parasites undergo asexual reproduction (merogony). They enlarge into trophozoite forms and divide to form type I meronts, which mature and rupture to release the motile merozoites. The merozoites bind to receptors on the epithelial cells and are engulfed by the cells. They then either repeat the process of merogony or undergo sexual differentiation (sporogony).

In the case of sexual differentiation, the merozoites differentiate into the microgametocytes and macrogametocytes. The microgametocyte releases the microgametes, which penetrate the cells infected with macrogametocytes. The macrogametocyte and microgametes fuse to form the zygote form, which then undergoes meiosis to form the oocyst, containing four sporozoites. Two morphologic forms of the oocyst have been described: thin-walled oocysts, which excyst within the same host in a process of self-infection, and thick-walled oocysts, which are shed into the environment.

EPIDEMIOLOGY

Cryptosporidium is distributed worldwide. Infection generally is more common in warm, humid months. Prevalence rates generally are higher in resource-poor countries than in industrialized countries.[119] Older surveys not involving patients with human immunodeficiency virus (HIV) documented oocysts in 1 to 3 percent of specimens from industrialized countries of Europe and North America and in about 10 to 15 percent of specimens from developing countries.[119] Most studies on the prevalence of infection have relied on detection of oocysts in fecal samples using acid-fast stains, which are insensitive. With the development of improved diagnostic techniques, more cases are being identified. Using direct immunofluorescence, *Cryptosporidium* was found in 19 percent of children with diarrhea in Brazil.[164] By antigen assay, 19 percent of children with diarrhea in Tanzania has cryptosporidiosis.[148] With the use of polymerase chain reaction (PCR), *Cryptosporidium* was found in 18 percent of hospitalized patients and schoolchildren in South Africa,[182] in 25 percent of children with diarrhea in Uganda,[199] in 36 percent of children prospectively followed in an urban slum in Brazil,[23] and in 15 percent of children with diarrhea in India[5] Thus, the prevalence may be higher than suggested by earlier stool studies.[187]

In wealthy countries, cryptosporidiosis is an important, but underdiagnosed problem. The number of cases reported in the United States tripled in the early 2000s, in part because of increased use of diagnostic tests. Currently, approximately 8000 cases are reported per year in the United States (incidence of ~3/100,000).[217,218] However, the actual number of cases is estimated to be 100-fold higher because of underdiagnosis and underreporting.[184] In England and Wales, over 4000 cases are reported per year (incidence 8/100,000/yr).[38] In both countries, more cases are detected when laboratories employ routing testing for *Cryptosporidium* in all stools from children and when more sensitive immunologic techniques are used (instead of stool microscopy).[35,129,167]

Cryptosporidium is more frequent in children than in adults. Studies from Peru, Brazil, India, Bangladesh, Guinea-Bissau, and Zimbabwe have documented frequent infections in children during their first 2 years of life.* In a Peruvian birth cohort study of diarrheal disease, the incidence rate of cryptosporidiosis determined by a serologic assay was higher than the rate determined by stool microscopy (0.77 vs. 0.41 infection/child-year of surveillance).[170] Similarly high seroprevalence rates were noted along the U.S.–Mexico border.[112] Young children in daycare centers are at high risk for acquiring infection. Outbreaks of cryptosporidiosis in daycare centers have been reported from numerous countries.[10,45,75,136,205]

Cryptosporidium is a common cause of diarrhea in patients infected with HIV. Cryptosporidiosis occurs in an estimated 10 to 15 percent of patients with AIDS in developed countries and in 30 to 50 percent of patients with AIDS in developing countries.[119] Among patients

*References 4, 5, 41, 100, 139, 145, 153, 170, 204.

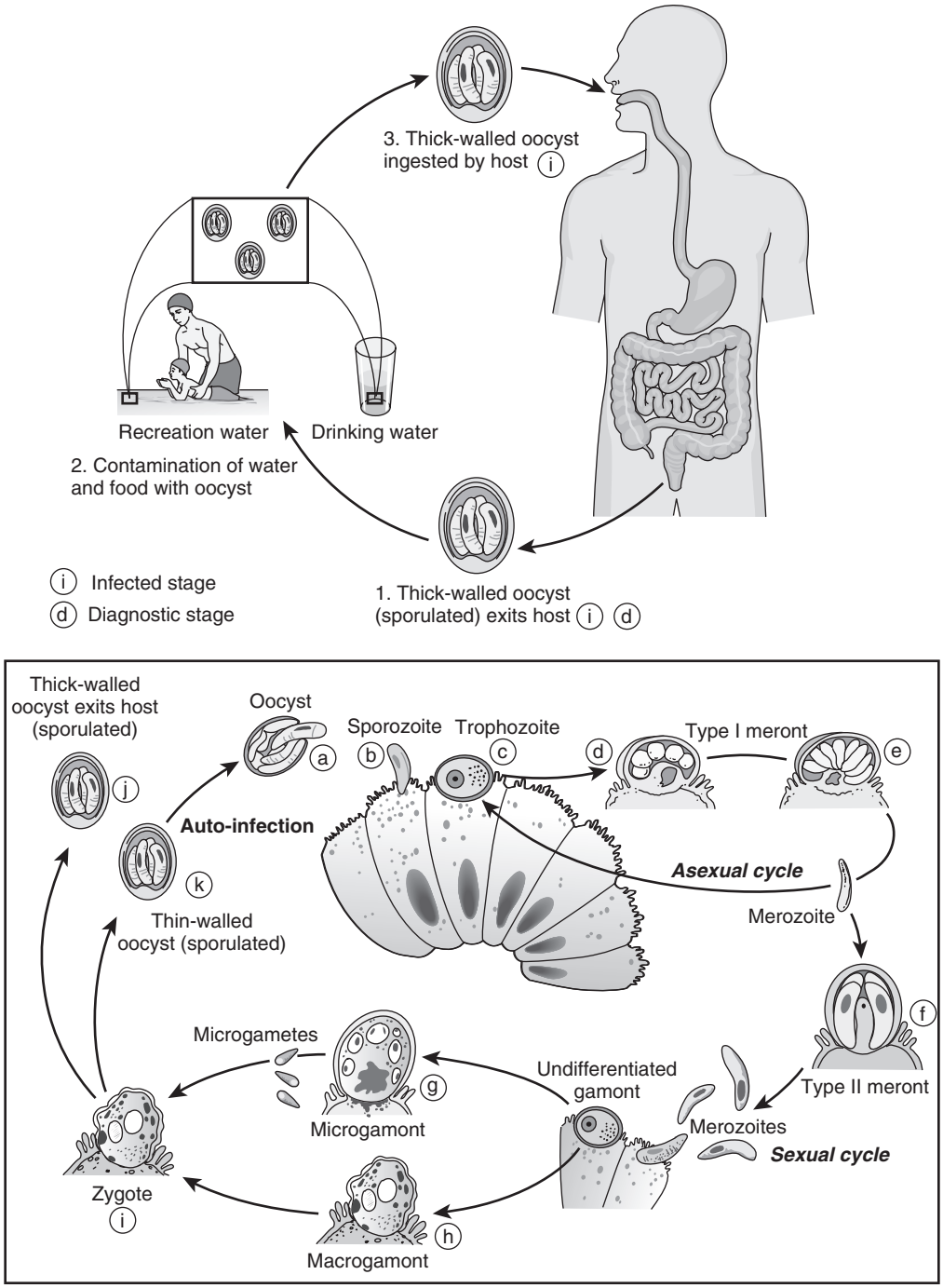

FIGURE 216-1 ■ Life cycle of oocyst. After ingestion, the oocysts (a) release the sporozoite (b), which attach to and are engulfed by the epithelial cell. Inside the parasitophorous vacuole, the organisms enlarge to form trophozoites (c) and divide to form meronts (d, e, and f). After reinvasion, they may differentiate into the sexual forms (microgamont and macrogamont, g and h), which fuse to form the zygote (i). The zygote releases the thin-walled oocysts (k), which cause autoinfection, or thick-walled oocysts (j), which are the infectious form.

with HIV, the infection rate is proportional to the $CD4^+$ cell count. In a longitudinal study of patients with HIV, the infection rate varied from 23 percent of patients with $CD4^+$ cell counts greater than 1000/μL to 46 percent with cell counts less than 100/μL.[168] In recent years, the widespread use of highly active antiretroviral therapy has resulted in the restoration of immune function and a reduction in the incidence of cryptosporidiosis and other opportunistic infections.[111]

Cryptosporidium is a common cause of persistent diarrhea in immunocompetent and immunocompromised children, especially in developing countries.* A study

*References 70, 146, 153, 157, 190, 198, 199.

in West African children investigated episode-specific determinants for the progression of an acute episode of diarrhea to chronic diarrhea; current infection with *C. parvum* was the most significant risk factor.[190] Among 243 children with persistent diarrhea in Uganda, 76 (31%) had *Cryptosporidium*.[198] Of the 243 children, 91 had HIV, of whom 67 (74%) had *Cryptosporidium*. Children with $CD4^+$ cell counts less than 25 percent were more likely to have *Cryptosporidium*.[198]

Cryptosporidiosis also has been noted in other immunodeficient hosts, including patients with primary immunodeficiencies, organ transplantation, cancer, and diabetes.[93] A particularly high prevalence of cryptosporidiosis occurs in patients with X-linked hyper–immunoglobulin (Ig)M syndrome. In two case series of hyper-IgM syndrome, the prevalence of cryptosporidiosis was 24 percent.[85,115] Cryptosporidiosis is also increasingly recognized after solid-organ or stem cell transplantation.[17,108,113]

TRANSMISSION

Humans can acquire *Cryptosporidium* infections through several transmission routes, such as direct contact with infected individuals (person-to-person transmission) or animals (zoonotic transmission) and ingestion of contaminated water (waterborne transmission) and food (foodborne transmission). The relative importance of these transmission routes varies by species and from country to country.[18,38,217] For example, foodborne disease has been noted more frequently in Scandinavia than in other regions.[73] Transmission dynamics are modulated by the low infectious dose, the fact that oocysts are immediately infectious when shed, the resistance of oocysts to chlorination, the stability of oocysts in the environment (especially in water), and the different dynamics of anthroponotic and zoonotic species.

The infectious dose of *Cryptosporidium* is low. In human challenge studies (including *C. hominis*, *C. parvum*, *C. ubiquitum*, and *C. meleagridis*), all demonstrated a low infections dose, but there was considerable variability among isolates, ranging from approximately 1000 oocysts (UCP strain) to 10 oocysts (Texas isolate, *C. hominis*).[39,55,158,159] Because the infectious dose is low, *Cryptosporidium* is transmitted easily from person to person. This route of transmission was recognized initially in outbreaks associated with contact with daycare centers[45,214] and with nosocomial transmission.[61,105,152,183] Secondary transmission within households also is common.[125] During the acute diarrheal illness, large quantities of oocytes are excreted in stool and are highly infectious. Asymptomatic shedding of oocytes may continue for 5 weeks after an acute episode of diarrhea.[192] Contact with an ill individual is a major risk factor for cryptosporidiosis.[18,217] Sexual transmission has been postulated to occur in association with anogenital sex.[86]

Cryptosporidium parasites are well adapted to waterborne transmission.[11,33,91] *Cryptosporidium* oocysts are resistant to environmental conditions; oocysts can remain infectious for at least 6 months if kept moist, but viability decreases rapidly with desiccation.[11,59,156] Oocysts are killed by heat, pasteurization, hydrogen peroxide, ozone, and ultraviolet radiation.[59,102,156] Oocysts are highly resistant to chlorination, however.[106] Surveys have shown that most surface sources of drinking water are contaminated with oocysts before treatment, and even a low-grade contamination has been documented in samples of treated water.

Waterborne outbreaks of cryptosporidiosis have been linked to contaminated drinking water, such as water from artesian wells, surface water, and filtered public drinking water[52,60] and to contaminated recreational water, such as swimming pools, water parks, lakes, rivers, beaches, and fountains.[33,91] The largest documented waterborne outbreak of diarrhea occurred in Milwaukee in 1993, affecting an estimated 400,000 individuals.[124] Many of the waterborne outbreaks, including the outbreak in Milwaukee, have been caused by *C. hominis*. However, both *C. hominis* and *C. parvum* have been implicated in spread by drinking water.[33,38,109] With improvements in filtration, drinking water is less often implicated in cases in the United States and Europe, but *Cryptosporidium* remains the main cause of illness associated with recreational water (e.g., swimming pools, rivers, lakes, fountains).[33,91]

The epidemiology varies considerably by parasite species. Studies in the United Kingdom demonstrated that *C. hominis* is associated with the population of young children, international travel, and recreational water.[38] Incidence peaks in the fall. By contrast, *C. parvum* infection peaked in the spring is associated with animal contact, surface water, and private water supplies. Numerous outbreaks have been attributed to animal contact.[18,38,109] Other species are more frequent in older patients, immunosuppressed hosts (such as AIDS patients), and those with animal contact.[24,56,57,68,171]

Foodborne infections occur less commonly.[217] Outbreaks have been associated with contaminated apple cider, unpasteurized milk, chicken salad, and raw produce. Oocysts are found commonly on vegetables in developing countries.[161] *Cryptosporidium* is associated with international travel and causes approximately 6 percent of traveler's diarrhea.[151]

PATHOLOGY AND PATHOGENESIS

In immunocompetent hosts, the organisms are localized primarily to the distal small intestines and proximal colon. In immunodeficient hosts, the parasites have been identified throughout the gut, in the biliary tract, and in the respiratory tract.[44,77,99] Recent studies employing PCR suggest that respiratory involvement may be underappreciated in children with cryptosporidiosis.[147] Children with persistent cryptosporidiosis may have villous atrophy and a mild increase in lamina propria lymphocytes.[166] Heavier infection is associated with villous atrophy; crypt hyperplasia; marked infiltration with lymphocytes, plasma cells, and neutrophils[44,72,76,77,123]; and with extraintestinal involvement.

Cryptosporidium causes watery diarrhea, malabsorption, and weight loss. These symptoms are thought to result from sodium malabsorption, electrogenic chloride secretion, and increased intestinal permeability.[119] In

vitro studies demonstrated that *Cryptosporidium* infection directly induces defects in intestinal epithelial cell barrier function in vitro.[2,78,175] Cryptosporidiosis also is characterized by defects in intestinal permeability.[76,118,186,219] Increased permeability may result in decreased absorption of fluids and electrolytes and solute fluxes into the gut. Studies in children with cryptosporidiosis have shown a correlation between the severity of disease and altered intestinal permeability.[219] Increased permeability is associated with circulating endotoxin and childhood malnutrition.[145]

Infection of intestinal epithelial cells leads to activation of nuclear factor κB.[135,173,180] Toll-like receptors and MyD88 are key mediators of this pathway.[42,177] The main effect of this activation is to inhibit apoptosis of the infected cells. This signaling pathway leads to modulation of microRNAs and secretion of defensins.[42,156] Increased expression of proinflammatory cytokines and markers of inflammation also occurs, including tumor necrosis factor (TNF)-α and increased expression of proinflammatory CXCL8, CXCL10, and lactoferrin.[103,119,156,163] The chemokines and cytokines recruit inflammatory and immune cells into the intestines. Infection also leads to increased expression of cyclooxygenase-2, production of prostaglandins by the epithelial cells, and production of neuropeptides by the inflammatory cells.[87,110,173] Prostaglandins are thought to mediate decreased sodium absorption and to stimulate chloride secretion. However, prostaglandin inhibitors have not proved to be effective in therapy of human cryptosporidiosis. The neuropeptide substance P correlates with severity of diarrhea in patients with cryptosporidiosis and AIDS and causes many of the defects associated with cryptosporidiosis in a monkey model.[87,173] Octreotide, a substance P antagonist, partially suppresses diarrhea in patients with chronic cryptosporidiosis.[64]

Cryptosporidiosis leads to villous atrophy and crypt hyperplasia.[76,123,166] Increased epithelial cell apoptosis has been shown in cells from tissue cultures and in biopsy specimens from infected intestines.[123] Loss of villous surface area is associated with malabsorption.[44,76,186]

IMMUNOLOGY

Both innate and acquired immunity are important in the defense and clearance of *Cryptosporidium*.[163] Innate responses play a key role in clearance of initial infections.[130] Some host factors have been implicated in innate responses, including mannose-binding lectins, complement, defensins, natural killer cells, and nonspecific inflammatory responses.[27,29,42,51,165]

$CD4^+$ T cells play a key role in the control of cryptosporidiosis, mainly associated with acquired immunity. Among patients with HIV infection, cryptosporidiosis is self-limited in patients with $CD4^+$ cell counts greater than 180/μL, chronic in patients with cell counts less than 100/μL, and fulminant in some patients with cell counts less than 50/μL.[16,62,83,127] Resolution of cryptosporidiosis in patients with AIDS in response to highly active antiretroviral therapy is associated with an influx of $CD4^+$ cells into the intestines.[185] Similarly, Kirkpatrick and colleagues[104] noted an association between MHC class II types (which present antigen to CD4 cells) and susceptibility to cryptosporidiosis.

IFN-γ is an important mediator of the immune clearance of *Cryptosporidium* in murine models.[11,13,14,18] Similarly, lymphocytes from individuals who have recovered from cryptosporidiosis produced IFN-γ after antigen stimulation in vitro.[2] While seropositive volunteers challenged with *C. parvum* express IFNγ in the intestinal mucosa, biopsies from seronegative volunteers in human challenge studies, lymphocytes obtained during active cryptosporidiosis, and stool from children with active cryptosporidiosis lacked IFN-γ expression, despite the fact that all had self-limited disease.[2,5,15] Thus, the role of IFN-γ in human infections is predominantly in acquired immunity and memory responses. CD8 cells may be important in human infection because they produce IFN-γ in response to parasite antigen, MHC class I polymorphisms are associated with risk of infections, and in vitro studies suggest CD8 T cells clear infection in vitro.[6,10-12]

Some IFN-γ–seronegative, normal volunteers were experimentally infected, and in patients with AIDS recovering from cryptosporidiosis, IL-15 was expressed in the intestines in association with IFN-γ-independent control of infection.[160,172] This effect likely is mediated by activation of natural killer cells.[51,130]

The role of antibody in the immune response to cryptosporidiosis is controversial.[163] High levels of serum and fecal antibodies to *C. parvum* have been found in patients with AIDS with chronic cryptosporidiosis and in volunteers challenged with *C. parvum*.[13,46] The presence and timing of antibody correlated with shedding of oocysts, however, rather than with clearance or resistance to infection, and cryptosporidiosis has not been associated with immunoglobulin defects.[93] However, recent studies suggest that maternal antibodies in breast milk may be protective.[107]

Hyper-IgM syndrome, owing to defects in CD40 ligand, is associated with increased frequency and severity of *Cryptosporidium* infection.[3,7] This susceptibility is thought to result from profound defects of antigen-presenting cells, production of IL-12 and TNF-α, and defective stimulation of interferon-γ production.[4] Other primary immunodeficiencies associated with cryptosporidiosis include CD4 cell deficiency, deficient major histocompatibility class (MHC) II, and combined immunodeficiencies.[93,131,215]

CLINICAL MANIFESTATIONS

The clinical spectrum of disease caused by *Cryptosporidium* is broad and depends on the immunologic status of the host, prior exposure, and parasite species (Table 216-1). Symptoms of cryptosporidiosis develop after a prepatent period, during which the parasites invade the intestinal epithelium and proliferate. The prepatent period, determined from point-source exposures and human challenge studies, is approximately 1 week (range 1 to 30 days).[34,124] However, this varies considerably with parasite strain and intensity of exposure. Regardless of the immunologic status of the patient, diarrhea is the most common manifestation of cryptosporidiosis.

TABLE 216-1 **Clinical Manifestation of Cryptosporidiosis**

Host	Clinical Manifestations	Comments
Normal host	Acute watery diarrhea	Relapses are common Persistent diarrhea is common
Children in developing countries	Acute watery diarrhea	Diarrhea more severe in children with malnutrition
	Persistent diarrhea	Persistent diarrhea affects nutritional status, growth, and intellectual function
Immunocompromised host	Acute watery diarrhea	Transient, self-limited, similar to normal host
	Relapsing diarrhea	Very common
	Persistent or chronic diarrhea	Usually found in patients with low CD4 or malnutrition
	Cholera-like illness	Voluminous watery diarrhea, only with very low CD4
	Extraintestinal involvement	Respiratory tract, biliary tract, and pancreas

Children present with an acute diarrheal syndrome characterized by watery diarrhea, cramps, and abdominal pain.[36,119,210] Other, less common clinical findings include fever, flatulence, nausea, vomiting, shortness of breath, and cough. The stools are occasionally described as foul-smelling, bulky stools, or with mucus, but rarely contain blood and fecal leukocytes. Respiratory symptoms are not uncommon, and lung involvement has been noted frequently.[147] In immunocompetent patients, the onset of diarrhea is often abrupt, and the illness usually is self-limited. Recurrent symptoms develop in 40 percent of cases after initial resolution. Relapses may occur after a diarrhea-free period of several days to weeks.[124,125,153] Forty-five percent of cases develop diarrhea lasting more than 14 days.[154,190]

Cryptosporidium is one of the most common causes of prolonged and persistent diarrhea in developing countries, causing about one third of cases.* Children with persistent diarrhea, especially if caused by *Cryptosporidium*, are at high risk for developing additional gastrointestinal infections after the initial illness, weight loss, and premature death.[3,7,117,143,144] Persistent diarrhea in children seriously affects nutritional status, growth, and intellectual function.[14,80] A long-term follow-up study of children with onset of cryptosporidiosis before 1 year of age suggests an association with poorer physical fitness and poorer cognitive development that persists for years.[80] In a second study, long-term effects of early childhood malnutrition were noted, but no significant association with *Cryptosporidium* in contrast to other pathogens was found.[14]

Cryptosporidiosis is more severe in children with malnutrition, and malnourished children tend to have a protracted course and shed oocysts longer.[142,144,145,181] They often require hospitalization, and their diseases may have a fatal outcome.[8,47,183] Prospective cohort studies of children followed from birth have shown significant differences in nutritional status before acquisition of *Cryptosporidium* infection.[3,40,142] In addition, the onset of cryptosporidiosis is associated with growth faltering. Older children eventually recovered and experienced catch-up growth, but children infected before 1 year of age often never recovered.[3,40,142] Asymptomatic infection can lead to malnutrition even without diarrhea.[14,41] *Cryptosporidium* infection causes acute malnutrition, and the long-term consequences of this interaction are likely to be worse in children infected in infancy or with prior malnutrition.

The clinical manifestations also vary somewhat by parasite species. In general, *C. hominis* causes more severe disease than other species. Cama and colleagues[23] noted more nausea, vomiting, and malaise with *C. hominis* than with zoonotic species. Hunter and colleagues[92a,93] noted that *C. hominis* was more strongly associated with extraintestinal sequelae (e.g., joint pain, eye pain, fatigue).[34,92a]

The clinical manifestations of cryptosporidiosis in patients with HIV vary. Four distinct gastrointestinal syndromes have been described in HIV-infected patients: (1) transient, self-limited diarrhea, similar to diarrhea in normal hosts, usually in patients with $CD4^+$ cell counts greater than 150/μL counts; (2) voluminous watery diarrhea or cholera-like illness that requires rehydration, seen in a few cases, usually in patients with CD4+ cell counts less than 50/μL; (3) relapsing diarrhea; and (4) a chronic diarrheal illness resulting in wasting, usually in patients with CD4+ cell counts less than 150/μL.[16,83,123,127,198] The severity and duration of illness in both AIDS patients and other patients with immunodeficiencies generally depend on the degree of immunodeficiency.

In immunocompromised patients, extraintestinal involvement may occur in the respiratory tract, biliary tract, and pancreas.[43,83,122,137,203] Respiratory tract involvement often is asymptomatic, but may cause cough, shortness of breath, wheezing, croup, and hoarseness. *Cryptosporidium* has been reported as a cause of laryngobronchitis in children.[82] Biliary tract involvement in cryptosporidiosis has been limited to patients with profound immunodeficiency.[83,203] Patients may have acalculous cholecystitis or cholangitis and, less frequently, sclerosing cholangitis or hepatitis.[83,194,203] Pancreatitis is an uncommon finding, but it has been reported in adults and children infected with HIV.[84,122,140] Concomitant infection with cytomegalovirus can occur and frequently is associated with irregularities of the intrahepatic duct.[12,90,97]

DIAGNOSIS

Many laboratories do not test for *Cryptosporidium* unless the tests are specifically requested (Table 216-2). *Cryptosporidium* infection usually is diagnosed by microscopic examination of stools. Fresh, preserved, and frozen stools can be used for testing, but preservatives may interfere with some assays.[32,36,189] Numerous concentration

*References 100, 117, 146, 153, 157, 190.

TABLE 216-2 **Diagnosis of *Cryptosporidium* Infection**

Test Type	Method	Comments
Microscopic examination of stools	Modified acid-fast stain of stools	Inexpensive and widely available diagnostic test
	Fluorescent stains (auramine O, auramine-rhodamine)	Faster than other acid-fast stains and may improve sensitivity
	Immunofluorescent assays (IFA)	More sensitive than acid-fast staining but also more expensive
Antigen-detection assays	Enzyme immunoassay (EIA), and immunochromatographic tests: direct and indirect immunofluorescence assay (DFA and IIF)	Good sensitivity (66%-100%) and excellent specificity (93%-100%), but occasional lots associated with false positive tests
Molecular methods	Polymerase chain reaction (PCR)	Increased sensitivity in contrast to microscopic or antigen-detection studies

methods have been attempted to improve the yield, but formalin-ethyl acetate concentration is the most widely used.[32,189] The oocysts are small, 4 to 6 μm, and similar in size and shape to yeast forms, and stains that are used routinely to detect other intestinal parasites (i.e., iodine, trichrome, and iron hematoxylin) do not detect *Cryptosporidium*. The method most commonly used by clinical laboratories to detect *Cryptosporidium* oocysts in stools has been the modified acid-fast stain.[35,167] Oocysts stain pink or red, whereas yeast cells and fecal debris stain green or blue (Fig. 216-2).[32,36,289] The sensitivity of stool examination with acid-fast staining is poor, requiring an oocyst concentration of greater than 500,000/mL in formed stools,[207] with fewer cases detected than with other methods.[36] Fluorescent stains (e.g., auramine O, auramine-rhodamine) can be read more quickly than other acid-fast stains and may have improved sensitivity, but positive results require confirmation by a second method.[36,101,189] Immunofluorescent assays are more sensitive than other microscopic methods.[36,65,101]

Antigen-detection assays are being used increasingly for diagnosis. Commercial kits for *Cryptosporidium* are available in enzyme-linked immunosorbent assay (ELISA) and immunochromatographic formats. The ELISA kits for *Cryptosporidium* have good sensitivity (66% to 100%) and excellent specificity (93% to 100%).[36,65,66,189] The sensitivity has been poor in some studies, however, and pseudo-outbreaks from false-positive results have been reported.[30,54] Commercial ELISA kits also may test for *Giardia* and *Entamoeba* antigens. Immunochromatographic tests are rapid tests for *Cryptosporidium* and *Giardia* antigen.[66,95] The sensitivity is less than with other assays, and results are available in minutes.[66,95] The specificity was initially reported to be excellent, but a recent study from Minnesota suggested a poor positive predictive value.[66,95,174] Antigen assays as a group have the advantage of not requiring skills in microscopic identification of organisms.

Molecular methods such as PCR for *C. parvum* DNA also have been employed to detect organisms. They have increased sensitivity in contrast to microscopic or antigen-detection studies of stool.[5,131,151,189,195] In one study of children with primary immunodeficiencies, PCR was able to detect significantly more cases of biliary cryptosporidiosis than were detected by stool studies.[131] In general, the number of cases detected increases significantly with molecular methods. Multiplex assays, including for a wide range of enteric pathogens, are in development.[120] A multiplex assay for enteric pathogens using polymerase amplification and detection with probes attached to beads was recently approved in the United States.[193]

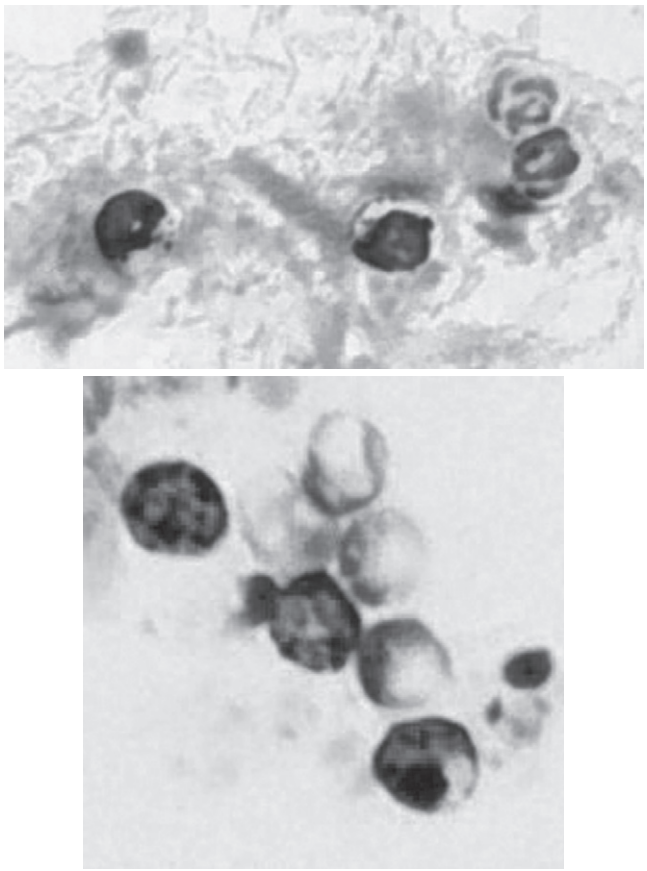

FIGURE 216-2 ■ Staining of oocysts.

Biopsy of the intestine typically is unnecessary for the diagnosis of intestinal infection, although the unique apical location within the intestinal epithelium is distinctive. The parasite can be visualized by light microscopy after staining with hematoxylin and eosin or by electron microscopy.[48]

MANAGEMENT

Replacement of fluids and electrolytes is a crucial first step in management, as with all cases of diarrhea. Oral rehydration is preferred, but severely ill patients may require parenteral fluids. Glutamine supplementation may improve fluid absorption.[25] Dietary management is crucial in cryptosporidiosis, especially if it is associated with persistent diarrhea. Supportive care should include initially a lactose-free diet because lactase activity is decreased on the apical border of epithelial cells.[166]

Cryptosporidiosis is associated with increased intestinal transit, which could interfere with absorption of fluids, electrolytes, and drugs.[19,186] Antimotility and antisecretory agents (i.e., tincture of opium, loperamide, octreotide, and acetorphan) have roles in therapy, but their use generally is limited to refractory cases in adults with AIDS.[64,188] These agents may control the diarrhea, but they do not eradicate the parasite.

In immunocompromised hosts, management of intestinal cryptosporidiosis is problematic. For patients with AIDS with chronic cryptosporidiosis, effective antiretroviral therapy can result in dramatic improvement in diarrhea.* The HIV protease inhibitors have anticryptosporidial activity in vitro and reduced infection by 90 percent in an animal model,[92,135] and most of the studies reporting responses have used protease inhibitors. By contrast, cryptosporidiosis is associated with malabsorption of nucleosides.[21] Similarly, reversal of immunosuppression may be a key component of management of severe cryptosporidiosis in transplant patients.[108,113] Patients with X-linked immunodeficiency with hyper-IgM may respond to a CD40 agonist antibody.[58]

The role of antiparasitic therapy in cryptosporidiosis is controversial. Because cryptosporidiosis usually is self-limited in immunocompetent hosts and can be variable in compromised hosts, controlled trials are crucial. No agent has proved reliably curative in patients with advanced AIDS. A meta-analysis of seven randomized controlled clinical trials found no clear evidence for efficacy of antiparasitic agents in the management of cryptosporidiosis in compromised hosts.[1]

Nitazoxanide is a nitrothiazolyl-salicylamide derivative with a broad spectrum of antiprotozoal and antihelminthic activity.[157a] Nitazoxanide is active against *Cryptosporidium* in vitro and in animal models.[67,196] A placebo-controlled study of nitazoxanide in patients with cryptosporidiosis showed significant clinical improvement in apparently immunocompetent children and adults with persistent diarrhea.[178] By day 7, diarrhea had resolved in 39/49 (80%) patients treated with nitazoxanide in contrast to 20/49 (41%) treated with placebo. Nitazoxanide also reduced the duration of shedding of oocysts. In parallel randomized studies in severely malnourished HIV-infected and HIV-negative children in Zambia with chronic cryptosporidiosis, a 3-day course of therapy not only led to clinical and parasitologic improvement, but also improved survival in HIV-negative children.[8] All children were treated with nitazoxanide suspension (100 mg twice daily for 3 days) or matching placebo. Among non–HIV-infected patients, resolution by day 7 was noted in 14/25 (56%) of patients treated with nitazoxanide in contrast to 5/22 (23%) for the placebo group. Similar findings were reported in immunocompetent patients 12 years of age and older. A good clinical response was seen in 27/28 (96%) patients receiving nitazoxanide in contrast to 11/27 (41%) receiving placebo.[179] Among the HIV-infected children, most with severe malnutrition, no significant response occurred.[8] A second study using higher doses and prolonged therapy in children with HIV and cryptosporidiosis also did not demonstrate efficacy.[9]

Nitazoxanide suspension was approved in the United States in 2002 for treatment of cryptosporidiosis and giardiasis in children. For most indications, it is dosed every 12 hours for 3 days. The recommended dose is 100 mg (5 mL) for children 12 to 47 months of age, 200 mg for children 4 to 11 years, and 500 mg for older children and adults (~15 mg/kg/day).[208] Nitazoxanide generally is well tolerated; adverse events occur at a frequency similar to that for placebo and include abdominal pain, diarrhea, vomiting, and headache.[208] Although unproved, nitazoxanide and other antiparasitic drugs should be considered as an adjunct to antiretrovirals or reversal of immunosuppression in children with severe cryptosporidiosis.[141] Combinations of nitazoxanide with other agents (e.g., paromomycin and azithromycin) have been used in some severely compromised patients.

Paromomycin is an orally administered nonabsorbable aminoglycoside. Initial in vitro studies noted poor activity against *C. parvum*. When patients with AIDS and cryptosporidiosis were treated with available antiparasitic drugs, however, some improved when treated with paromomycin.[71] Three randomized, controlled trials examined the effects of paromomycin in patients with AIDS and cryptosporidiosis.[88,98,211] Paromomycin was associated with a significant reduction in shedding of oocysts and decreased stool frequency, but not cure.[211] In another study, no significant difference was found between groups.[88]

Macrolide antibiotics, including spiramycin, azithromycin, roxithromycin, and clarithromycin, have some activity against *Cryptosporidium*.[15] In one study, children treated with spiramycin had shorter duration of symptoms and shedding of oocysts,[180] but a second trial showed no effect.[213] Case series noted improvement in cryptosporidiosis in patients with HIV and cancer treated with azithromycin,[53,89,96,150] but subsequent trials in patients with AIDS did not show changes in frequency of stools or shedding of oocysts.[15] A study in Egyptian schoolchildren suggested more rapid resolution of symptoms was achieved with azithromycin.[6] Roxithromycin treatment was associated with improvement in AIDS-associated cryptosporidiosis in two uncontrolled studies.[191,201]

PREVENTION

In hospitals and daycare centers, hand washing is the most important measure to prevent the spread of any enteric pathogen. Alcohol rubs may not be adequate. Enteric precautions (i.e., washing hands, wearing gloves, and wearing gowns if soiling is likely) are important measures to prevent nosocomial spread.

*References 28, 63, 79, 126, 138, 160.

Swimming pools are an important source of infection. Contamination of treated recreational water, such as a fecal accident in a swimming pool, should prompt aggressive measures, including closing the pool temporarily.[31,33] Recreational waters, such as lakes, may pose a danger for compromised hosts, who should avoid contact with untreated water.[141]

Control of waterborne transmission of cryptosporidiosis is a major public health concern. This issue is complicated by the ability of *Cryptosporidium* to escape the filtration system used in most public water facilities and its resistance to chlorination. The Centers for Disease Control and Prevention (CDC) recommends that during outbreak situations, compromised patients boil tap water for 1 minute or filter tap water using a filtration system to remove particles 1 μ or larger, or use bottled water prepared by distillation or reverse osmotic filtration.[32] In situations not associated with an outbreak, no special measures are recommended. However, routinely using the outbreak measures outlined earlier for severe immunosuppressed patients may be prudent.[141] The CDC website contains an excellent patient fact sheet about *Cryptosporidium* and its prevention (http://www.cdc.gov/parasites/crypto). Currently, no vaccine is available to protect against *Cryptosporidium*. Vaccines for cryptosporidiosis are in development stages, but, as of this writing, none have progressed to human trials.*

NEW REFERENCES SINCE THE SIXTH EDITION

5. Ajjampur SS, Rajendran P, Ramani S, et al. Closing the diarrhoea diagnostic gap in Indian children by the application of molecular techniques. *J Med Microbiol* 2008;**57**:1364–8.
9. Amadi B, Mwiya M, Sianongo S, et al. High dose prolonged treatment with nitazoxanide is not effective for cryptosporidiosis in HIV positive Zambian children: a randomised controlled trial. *BMC Infect Dis* 2009;**9**:195.
10. Artieda J, Basterrechea M, Arriola L, et al. Outbreak of cryptosporidiosis in a child day-care centre in Gipuzkoa, Spain, October to December 2011. *Eur Surveill* 2012;17.
11. Beach MJ. Waterborne: recreational water. In: Fayer R, Xiao L, editors. *Cryptosporidium and cryptosporidiosis*. 2nd ed. Boca Raton, Fla: CRC Press; 2008. p. 335–69.
17. Bonatti H, Barroso LF 2nd, Sawyer RG, et al. *Cryptosporidium* enteritis in solid organ transplant recipients: multicenter retrospective evaluation of 10 cases reveals an association with elevated tacrolimus concentrations. *Transpl Infect Dis* 2012;**14**:635–48.
18. Bouzid M, Hunter PR, Chalmers RM, et al. *Cryptosporidium* pathogenicity and virulence. *Clin Microbiol Rev* 2013;**26**:115–34.
20. Burton AJ, Nydam DV, Jones G, et al. Antibody responses following administration of a *Cryptosporidium parvum* rCP15/60 vaccine to pregnant cattle. *Vet Parasitol* 2011;**175**:178–81.
29. Carryn S, Schaefer DA, Imboden M, et al. Phospholipases and cationic peptides inhibit *Cryptosporidium parvum* sporozoite infectivity by parasiticidal and non-parasiticidal mechanisms. *J Parasitol* 2012;**98**:199–204.
31. Centers for Disease Control and Prevention. Promotion of healthy swimming after a statewide outbreak of cryptosporidiosis associated with recreational water venues: Utah, 2008-2009. *MMWR Morb Mortal Wkly Rep* 2012;**61**:348–52.
32. Centers for Disease Control and Prevention. *Cryptosporidium* infection, http://www.cdc.gov/parasites/crypto/. 2013. Accessed April 1, 2013, 2013.
33. Chalmers RM. Waterborne outbreaks of cryptosporidiosis. *Ann Ist Super Sanita* 2012;**48**:429–46.
34. Chalmers RM, Davies AP. Minireview: clinical cryptosporidiosis. *Exp Parasitol* 2010;**124**:138–46.
35. Chalmers RM, Campbell B, Crouch N, et al. Clinical laboratory practices for detection and reporting of *Cryptosporidium* in community cases of diarrhoea in the United Kingdom, 2008. *Euro Surveill* 2010;15.
36. Chalmers RM, Campbell BM, Crouch N, et al. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. *J Med Microbiol* 2011;**60**:1598–604.
37. Chalmers RM, Elwin K, Hadfield SJ, et al. Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus*, United Kingdom, 2007-2008. *Emerg Infect Dis* 2011;**17**:536–8.
38. Chalmers RM, Smith R, Elwin K, et al. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004-2006. *Epidemiol Infect* 2011;**139**:700–12.
47. Creek TL, Kim A, Lu L, et al. Hospitalization and mortality among primarily nonbreastfed children during a large outbreak of diarrhea and malnutrition in Botswana, 2006. *J Acquir Immune Defic Syndr* 2010;**53**:14–19.
56. Elwin K, Hadfield SJ, Robinson G, et al. *Cryptosporidium viatorum* n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007-2011. *Int J Parasitol* 2012;**42**:675–82.
57. Elwin K, Hadfield SJ, Robinson G, et al. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. *Epidemiol Infect* 2012;**140**:673–83.
58. Fan X, Upadhyaya B, Wu L, et al. CD40 agonist antibody mediated improvement of chronic *Cryptosporidium* infection in patients with X-linked hyper IgM syndrome. *Clin Immunol* 2012;**143**:152–61.
59. Fayer R. General biology. In: Fayer R, Xiao L, editors. *Cryptosporidium and cryptosporidiosis*. 2nd ed. Boca Raton, Fla: CRC Press; 2008. p. 1–42.
61. Feng Y, Wang L, Duan L, et al. Extended outbreak of cryptosporidiosis in a pediatric hospital, China. *Emerg Infect Dis* 2012;**18**:312–14.
73. Gherasim A, Lebbad M, Insulander M, et al. Two geographically separated food-borne outbreaks in Sweden linked by an unusual *Cryptosporidium parvum* subtype, October 2010. *Euro Surveill* 2012;17.
81. Guerrant RL, Deboer MD, Moore SR, et al. The impoverished gut: a triple burden of diarrhoea, stunting and chronic disease. *Nat Rev Gastroenterol Hepatol* 2013;**10**:220–9.
91. Hlavsa MC, Roberts VA, Anderson AR, et al. Surveillance for waterborne disease outbreaks and other health events associated with recreational water: United States, 2007-2008. *MMWR Surveill Summ* 2011;**60**:1–32.
101. Khurana S, Sharma P, Sharma A, et al. Evaluation of Ziehl-Neelsen staining, auramine phenol staining, antigen detection enzyme linked immunosorbent assay and polymerase chain reaction, for the diagnosis of intestinal cryptosporidiosis. *Trop Parasitol* 2012;**2**:20–3.
104. Kirkpatrick BD, Haque R, Duggal P, et al. Association between *Cryptosporidium* infection and human leukocyte antigen class I and class II alleles. *J Infect Dis* 2008;**197**:474–8.
107. Korpe PS, Liu Y, Siddique A, et al. Breast milk parasite-specific antibodies and protection from amebiasis and cryptosporidiosis in Bangladeshi infants: a prospective cohort study. *Clin Infect Dis* 2013;**56**:988–92.
108. Krause I, Amir J, Cleper R, et al. Cryptosporidiosis in children following solid organ transplantation. *Pediatr Infect Dis J* 2012;**31**:1135–8.
119. Lima AAM, Samie A, Guerrant RL. Cryptosporidiosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. 3rd ed. Edinburgh: Saunders; 2011. p. 633–40.
120. Liu J, Gratz J, Amour C, et al. A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. *J Clin Microbiol* 2013;**51**:472–80.
121. Liu K, Zai D, Zhang D, et al. Divalent Cp15-23 vaccine enhances immune responses and protection against *Cryptosporidium parvum* infection. *Parasite Immunol* 2010;**32**:335–44.
128. Manque PA, Tenjo F, Woehlbier U, et al. Identification and immunological characterization of three potential vaccinogens against *Cryptosporidium* species. *Clin Vaccine Immunol* 2011;**18**:1796–802.
129. Martin-Ampudia M, Mariscal A, Lopez-Gigosos RM, et al. Under-notification of cryptosporidiosis by routine clinical and laboratory practices among non-hospitalised children with acute diarrhoea in Southern Spain. *Infection* 2012;**40**:113–19.

*References 20, 121, 128, 132, 176, 206.

132. Mead JR. Challenges and prospects for a *Cryptosporidium* vaccine. *Future Microbiol* 2010;**5**:335–7.
141. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1–166.
144. Mondal D, Haque R, Sack RB, et al. Attribution of malnutrition to cause-specific diarrheal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. *Am J Trop Med Hyg* 2009;**80**:824–6.
145. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012;**54**:185–92.
146. Moore SR, Lima NL, Soares AM, et al. Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children. *Gastroenterology* 2010;**139**:1156–64.
147. Mor SM, Tumwine JK, Ndeezi G, et al. Respiratory cryptosporidiosis in HIV-seronegative children in Uganda: potential for respiratory transmission. *Clin Infect Dis* 2010;**50**:1366–72.
148. Moyo SJ, Gro N, Matee MI, et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatr* 2011;**11**:19.
149. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013;doi:pii: S0140-6736(13)60844-2. [Epub ahead of print] PMID: 23680352. Can be found at http://www.ncbi.nlm.nih.gov/pubmed/23680352.
151. Nair P, Mohamed JA, DuPont HL, et al. Epidemiology of cryptosporidiosis in North American travelers to Mexico. *Am J Trop Med Hyg* 2008;**79**:210–14.
156. O'Hara SP, Chen XM. The cell biology of cryptosporidium infection. *Microbes Infect* 2011;**13**:721–30.
162. Pantenburg B, Castellanos-Gonzalez A, Dann SM, et al. Human CD8(+) T cells clear *Cryptosporidium parvum* from infected intestinal epithelial cells. *Am J Trop Med Hyg* 2010;**82**:600–7.
163. Pantenburg B, Dann S, Wang H, et al. Intestinal immune response to human *Cryptosporidium* sp. infection. *Infect Immun* 2008;**76**:23–9.
165. Petry F, Jakobi V, Wagner S, et al. Binding and activation of human and mouse complement by *Cryptosporidium parvum* (Apicomplexa) and susceptibility of C1q- and MBL-deficient mice to infection. *Mol Immunol* 2008;**45**:3392–400.
167. Polage CR, Stoddard GJ, Rolfs RT, et al. Physician use of parasite tests in the United States from 1997 to 2006 and in a Utah *Cryptosporidium* outbreak in 2007. *J Clin Microbiol* 2011;**49**:591–6.
174. Robinson TJ, Cebelinski EA, Taylor C, et al. Evaluation of the positive predictive value of rapid assays used by clinical laboratories in Minnesota for the diagnosis of cryptosporidiosis. *Clin Infect Dis* 2010;**50**:e53–55.
176. Roche JK, Rojo AL, Costa LB, et al. Intranasal vaccination in mice with an attenuated *Salmonella enterica* Serovar 908htr A expressing Cp15 of *Cryptosporidium*: impact of malnutrition with preservation of cytokine secretion. *Vaccine* 2013;**31**:912–18.
184. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States: major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.
187. Shirley DA, Moonah SN, Kotloff KL. Burden of disease from cryptosporidiosis. *Curr Opin Infect Dis* 2012;**25**:555–63.
193. Taniuchi M, Verweij JJ, Noor Z, et al. High throughput multiplex PCR and probe-based detection with Luminex beads for seven intestinal parasites. *Am J Trop Med Hyg* 2011;**84**:332–7.
206. Wang C, Luo J, Amer S, et al. Multivalent DNA vaccine induces protective immune responses and enhanced resistance against *Cryptosporidium parvum* infection. *Vaccine* 2010;**29**:323–8.
210. White A Jr. Cryptosporidium species. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 3547–60.
217. Yoder JS, Beach MJ. *Cryptosporidium* surveillance and risk factors in the United States. *Exp Parasitol* 2010;**124**:31–9.
218. Yoder JS, Wallace RM, Collier SA, et al. Cryptosporidiosis surveillance: United States, 2009-2010. *MMWR Surveill Summ* 2012;**61**:1–12.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 217

Cyclosporiasis, Cystoisosporiasis, and Microsporidiosis

A. Clinton White, Jr. • Carlos Seas • Martin Montes

CYCLOSPORA CAYETANENSIS

Cyclospora cayetanensis is an intestinal coccidian protozoan parasite, related to *Toxoplasma*, *Cryptosporidium*, *Sarcocystis*, and *Cystoisospora*. This parasite infects the gastrointestinal tract of returning travelers, and immunocompromised patients causing acute diarrheal disease.[2,54,55,117,169] In 1979, *C. cayetanensis* was first identified by Ashford[10] in the stool of a woman and two children from Papua New Guinea. *C. cayetanensis* was identified in four patients with diarrhea after travel to Mexico and Haiti.[144] The organisms were identified in stools from travelers and expatriates in Nepal. Subsequent studies noted an association with diarrhea in patients with acquired immunodeficiency syndrome (AIDS) patients.[2,117,140,168,169]

REFERENCES

1. Abubakar I, Aliyu S, Arumugam C, et al. Prevention and treatment of cryptosporidiosis in immunocompromised patients. *Cochrane Database Syst Rev* 2007;(1):CD004932.
2. Adams RB, Guerrant RL, Zu S, et al. *Cryptosporidium parvum* infection of intestinal epithelium: morphologic and functional studies in an in vitro model. *J Infect Dis* 1994;**169**:170–7.
3. Agnew DG, Lima AA, Newman RD, et al. Cryptosporidiosis in northeastern Brazilian children: association with increased diarrhea morbidity. *J Infect Dis* 1998;**177**:754–60.
4. Ajjampur S, Gladstone B, Selvapandian D, et al. Molecular and spatial epidemiology of cryptosporidiosis in children in a semiurban community in South India. *J Clin Microbiol* 2007;**45**: 915–20.
5. Ajjampur SS, Rajendran P, Ramani S, et al. Closing the diarrhoea diagnostic gap in Indian children by the application of molecular techniques. *J Med Microbiol* 2008;**57**:1364–8.
6. Allam AF, Shehab AY. Efficacy of azithromycin, praziquantel and mirazid in treatment of cryptosporidiosis in school children. *J Egypt Soc Parasitol* 2002;**32**:969–78.
7. Amadi B, Kelly P, Mwiya M, et al. Intestinal and systemic infection, HIV, and mortality in Zambian children with persistent diarrhea and malnutrition. *J Pediatr Gastroenterol Nutr* 2001;**32**: 550–4.
8. Amadi B, Mwiya M, Musuku J, et al. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. *Lancet* 2002;**360**: 1375–80.
9. Amadi B, Mwiya M, Sianongo S, et al. High dose prolonged treatment with nitazoxanide is not effective for cryptosporidiosis in HIV positive Zambian children: a randomised controlled trial. *BMC Infect Dis* 2009;**9**:195.
10. Artieda J, Basterrechea M, Arriola L, et al. Outbreak of cryptosporidiosis in a child day-care centre in Gipuzkoa, Spain, October to December 2011. *Eur Surveill* 2012;17.
11. Beach MJ. Waterborne: Recreational water. In: Fayer R, Xiao L, editors. *Cryptosporidium and cryptosporidiosis*. 2nd ed. Boca Raton, Fla: CRC Press; 2008. p. 335–69.
12. Benhamou Y, Caumes E, Gerosa Y, et al. AIDS-related cholangiopathy: critical analysis of a prospective series of 26 patients. *Digest Dis Sci* 1993;**38**:1113–18.
13. Benhamou Y, Kapel N, Hoang C, et al. Inefficacy of intestinal secretory immune response to *Cryptosporidium* in acquired immunodeficiency syndrome. *Gastroenterology* 1995;**108**:627–35.
14. Berkman DS, Lescano AG, Gilman RH, et al. Effects of stunting, diarrhoeal disease, and parasitic infection during infancy on cognition in late childhood: a follow-up study. *Lancet* 2002;**359**: 564–71.
15. Blagburn BL, Soave R. Prophylaxis and chemotherapy: human and animal. In: Fayer R, editor. *Cryptosporidium and cryptosporidiosis*. Boca Raton, Fla: CRC Press; 1997. p. 111–28.
16. Blanshard C, Jackson AM, Shanson DC, et al. Cryptosporidiosis in HIV-seropositive patients. *Q J Med* 1992;**85**:813–23.
17. Bonatti H, Barroso LF 2nd, Sawyer RG, et al. Cryptosporidium enteritis in solid organ transplant recipients: multicenter retrospective evaluation of 10 cases reveals an association with elevated tacrolimus concentrations. *Transpl Infect Dis* 2012;**14**:635–48.
18. Bouzid M, Hunter PR, Chalmers RM, et al. *Cryptosporidium* pathogenicity and virulence. *Clin Microbiol Rev* 2013;**26**:115–34.
19. Brantley RK, Williams KR, Silva TM, et al. AIDS-associated diarrhea and wasting in Northeast Brazil is associated with subtherapeutic plasma levels of antiretroviral medications and with both bovine and human subtypes of *Cryptosporidium parvum*. *Brazil J Infect Dis* 2003;**7**:16–22.
20. Burton AJ, Nydam DV, Jones G, et al. Antibody responses following administration of a *Cryptosporidium parvum* rCP15/60 vaccine to pregnant cattle. *Vet Parasitol* 2011;**175**:178–81.
21. Bushen OY, Davenport JA, Lima AB, et al. Diarrhea and reduced levels of antiretroviral drugs: improvement with glutamine or alanyl-glutamine in a randomized controlled trial in northeast Brazil. *Clin Infect Dis* 2004;**38**:1764–70.
22. Bushen OY, Kohli A, Pinkerton RC, et al. Heavy cryptosporidial infections in children in northeast Brazil: comparison of *Cryptosporidium hominis* and *Cryptosporidium parvum*. *Trans R Soc Trop Med Hyg* 2007;**101**:378–84.
23. Cama VA, Bern C, Roberts J, et al. *Cryptosporidium* species and subtypes and clinical manifestations in children, Peru. *Emerg Infect Dis* 2008;**14**:1567–74.
24. Cama VA, Bern C, Sulaiman IM, et al. *Cryptosporidium* species and genotypes in HIV-positive patients in Lima, Peru. *J Eukaryot Microbiol* 2003;**50**(suppl):531–3.
25. Cameiro-Filho BA, Bushen OY, Brito GA, et al. Glutamine analogues as adjunctive therapy for infectious diarrhea. *Curr Infect Dis Rep* 2003;**5**:114–19.
26. Reference deleted in proofs.
27. Carmolli M, Duggal P, Haque R, et al. Deficient serum mannose-binding lectin levels and MBL2 polymorphisms increase the risk of single and recurrent *Cryptosporidium* infections in young children. *J Infect Dis* 2009;**200**:1540–7.
28. Carr A, Marriott D, Field A, et al. Treatment of HIV-1-associated microsporidiosis and cryptosporidiosis with combination antiretroviral therapy. *Lancet* 1998;**351**:256–61.
29. Carryn S, Schaefer DA, Imboden M, et al. Phospholipases and cationic peptides inhibit *Cryptosporidium parvum* sporozoite infectivity by parasiticidal and non-parasiticidal mechanisms. *J Parasitol* 2012;**98**:199–204.
30. Centers for Disease Control and Prevention. Manufacturer's recall of rapid assay kits based on false positive *Cryptosporidium* antigen tests: Wisconsin, 2001-2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:189.
31. Centers for Disease Control and Prevention. Promotion of healthy swimming after a statewide outbreak of cryptosporidiosis associated with recreational water venues: Utah, 2008-2009. *MMWR Morb Mortal Wkly Rep* 2012;**61**:348–52.
32. Centers for Disease Control and Prevention. *Cryptosporidium* infection, http://www.cdc.gov/parasites/crypto/. 2013. Accessed: April 1, 2013, 2013.
33. Chalmers RM. Waterborne outbreaks of cryptosporidiosis. *Ann Ist Super Sanita* 2012;**48**:429–46.
34. Chalmers RM, Davies AP. Minireview: clinical cryptosporidiosis. *Exp Parasitol* 2010;**124**:138–146.
35. Chalmers RM, Campbell B, Crouch N, et al. Clinical laboratory practices for detection and reporting of *Cryptosporidium* in community cases of diarrhoea in the United Kingdom, 2008. *Eur surveill* 2010;15.
36. Chalmers RM, Campbell BM, Crouch N, et al. Comparison of diagnostic sensitivity and specificity of seven *Cryptosporidium* assays used in the UK. *J Med Microbiol* 2011;**60**:1598–604.
37. Chalmers RM, Elwin K, Hadfield SJ, et al. Sporadic human cryptosporidiosis caused by *Cryptosporidium cuniculus*, United Kingdom, 2007-2008. *Emerg Infect Dis* 2011;**17**:536–8.
38. Chalmers RM, Smith R, Elwin K, et al. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004-2006. *Epidemiol Infect* 2011;**139**:700–12.
39. Chappell CL, Okhuysen PC, Langer-Curry R, et al. *Cryptosporidium hominis:* experimental challenge of healthy adults. *Am J Trop Med Hyg* 2006;**75**:851–7.
40. Checkley W, Epstein LD, Gilman RH, et al. Effects of *Cryptosporidium parvum* infection in Peruvian children: growth faltering and subsequent catch-up growth. *Am J Epidemiol* 1998;**148**:497–506.
41. Checkley W, Gilman RH, Epstein LD, et al. Asymptomatic and symptomatic cryptosporidiosis: their acute effect on weight gain in Peruvian children. *Am J Epidemiol* 1997;**145**:156–63.
42. Chen XM, O'Hara SP, Nelson JB, et al. Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to *Cryptosporidium parvum* via activation of NF-kappaB. *J Immunol* 2005;**175**:7447–56.
43. Clavel A, Arnal AC, Sanchez EC, et al. Respiratory cryptosporidiosis: case series and review of the literature. *Infection* 1996;**24**: 341–6.
44. Clayton F, Heller T, Kotler DP. Variation in the enteric distribution of cryptosporidia in acquired immunodeficiency syndrome. *Am J Clin Pathol* 1994;**102**:420–5.
45. Cordell RL, Addiss DG. Cryptosporidiosis in child care settings: a review of the literature and recommendations for prevention and control. *Pediatr Infect Dis J* 1994;**13**:310–17.
46. Cozon G, Biron F, Jeannin M, et al. Secretory IgA antibodies to *Cryptosporidium* pairvum in AIDS patients with chronic cryptosporidiosis. *J Infect Dis* 1994;**169**:696–9.
47. Creek TL, Kim A, Lu L, et al. Hospitalization and mortality among primarily nonbreastfed children during a large outbreak of

diarrhea and malnutrition in Botswana, 2006. *J Acquir Immune Defic Syndr* 2010;**53**:14–19.
48. Current WL, Garcia LS. Cryptosporidiosis. *Clin Microbiol Rev* 1991;**4**:325–58.
49. Current WL, Reese NC, Ernst JV, et al. Human cryptosporidiosis in immunocompetent and immunodeficient persons: studies of an outbreak and experimental transmission. *New Engl J Med* 1983; **308**:1252–7.
50. D'Antonio RG, Winn RE, Taylor JP, et al. A waterborne outbreak of cryptosporidiosis in normal hosts. *Ann Intern Med* 1985; **103**:886–8.
51. Dann SM, Wang HC, Gambarin KJ, et al. IL-15 activates human NK cells to clear the intestinal protozoan *Cryptosporidium*. *J Infect Dis* 2005;**192**:1294–302.
52. Dillingham RA, Lima AA, Guerrant RL. Cryptosporidiosis: epidemiology and impact. *Microbes Infect* 2002;**4**:1059–66.
53. Dionisio D, Orsi A, Sterrantino G, et al. Chronic cryptosporidiosis in patients with AIDS: stable remission and possible eradication after long-term, low dose azithromycin. *J Clin Pathol* 1998; **51**:138–42.
54. Doing KM, Hamm JL, Jellison JA, et al. False-positive results obtained with the Alexon ProSpecT *Cryptosporidium* enzyme immunoassay. *J Clin Microbiol* 1999;**37**:1582–3.
55. DuPont HL, Chappell CL, Sterling CR, et al. The infectivity of *Cryptosporidium* parvum in healthy volunteers. *N Engl J Med* 1995;**332**:855–9.
56. Elwin K, Hadfield SJ, Robinson G, et al. *Cryptosporidium viatorum* n. sp. (Apicomplexa: Cryptosporidiidae) among travellers returning to Great Britain from the Indian subcontinent, 2007-2011. *Int J Parasitol* 2012;**42**:675–82.
57. Elwin K, Hadfield SJ, Robinson G, et al. The epidemiology of sporadic human infections with unusual cryptosporidia detected during routine typing in England and Wales, 2000-2008. *Epidemiol Infect* 2012;**140**:673–83.
58. Fan X, Upadhyaya B, Wu L, et al. CD40 agonist antibody mediated improvement of chronic *Cryptosporidium* infection in patients with X-linked hyper IgM syndrome. *Clin Immunol* 2012;**143**: 152–61.
59. Fayer R. General Biology. In: Fayer R, Xiao L, editors. *Cryptosporidium and cryptosporidiosis*. 2nd ed. Boca Raton, FL: CRC Press; 2008. p. 1–42.
60. Fayer R, Morgan U, Upton SJ. Epidemiology of *Cryptosporidium:* transmission, detection and identification. *Int J Parasitol* 2000;**30**: 1305–22.
61. Feng Y, Wang L, Duan L, et al. Extended outbreak of cryptosporidiosis in a pediatric hospital, China. *Emerg Infect Dis* 2012;**18**:312–14.
62. Flanigan T, Whalen C, Turner J, et al. *Cryptosporidium* infection and CD4 counts. *Ann Intern Med* 1992;**116**:840–2.
63. Foudraine NA, Weverling GJ, van Gool T, et al. Improvement of chronic diarrhoea in patients with advanced HIV-1 infection during potent antiretroviral therapy. *AIDS* 1998;**12**:35–41.
64. Garcia Compean D, Ramos Jimenez J, Guzman de la Garza F, et al. Octreotide therapy of large-volume refractory AIDS-associated diarrhea: a randomized controlled trial. *AIDS* 1994;**8**: 1563–7.
65. Garcia LS, Shimizu RY. Evaluation of nine immunoassay kits (enzyme immunoassay and direct fluorescence) for detection of *Giardia lamblia* and *Cryptosporidium parvum* in human fecal specimens. *J Clin Microbiol* 1997;**35**:1526–9.
66. Garcia LS, Shimizu RY, Novak S, et al. Commercial assay for detection of *Giardia lamblia* and *Cryptosporidium parvum* antigens in human fecal specimens by rapid solid-phase qualitative immunochromatography. *J Clin Microbiol* 2003;**41**:209–12.
67. Gargala G, Delaunay A, Li X, et al. Efficacy of nitazoxanide, tizoxanide and tizoxanide glucuronide against *Cryptosporidium parvum* development in sporozoite-infected HCT-8 enterocytic cells. *J Antimicrob Chemother* 2000;**46**:57–60.
68. Gatei W, Greensill J, Ashford RW, et al. Molecular analysis of the 18S rRNA gene of *Cryptosporidium* parasites from patients with or without human immunodeficiency virus infections living in Kenya, Malawi, Brazil, the United Kingdom, and Vietnam. *J Clin Microbiol* 2003;**41**:1458–62.
69. Gatei W, Suputtamongkol Y, Waywa D, et al. Zoonotic species of *Cryptosporidium* are as prevalent as the anthroponotic in HIV-infected patients in Thailand. *Ann Trop Med Parasitol* 2002; **96**:797–802.
70. Gatei W, Wamae CN, Mbae C, et al. Cryptosporidiosis: prevalence, genotype analysis, and symptoms associated with infections in children in Kenya. *Am J Trop Med Hyg* 2006;**75**:78–82.
71. Gathe J, Piot D, Hawkins K, et al. Treatment of gastrointestinal cryptosporidiosis with paromomycin. Presented at the VIth Annual Conference on AIDS. San Francisco, 1990.
72. Genta RM, Chappell CL, White AC Jr, et al. Duodenal morphology and intensity of infection in AIDS-related intestinal cryptosporidiosis. *Gastroenterology* 1993;**105**:1769–75.
73. Gherasim A, Lebbad M, Insulander M, et al. Two geographically separated food-borne outbreaks in Sweden linked by an unusual *Cryptosporidium parvum* subtype, October 2010. *Eur Surveill* 2012;17.
74. Gomez Morales MA, La Rosa G, Ludovisi A, et al. Cytokine profile induced by *Cryptosporidium* antigen in peripheral blood mononuclear cells from immunocompetent and immunosuppressed persons with cryptosporidiosis. *J Infect Dis* 1999;**179**: 967–73.
75. Goncalves EM, da Silva AJ, Eduardo MB, et al. Multilocus genotyping of *Cryptosporidium hominis* associated with diarrhea outbreak in a day care unit in São Paulo. *Clinics (São Paulo)* 2006; **61**:119–26.
76. Goodgame RW, Kimball K, Ou CN, et al. Intestinal function and injury in acquired immunodeficiency syndrome-related cryptosporidiosis. *Gastroenterology* 1995;**108**:1075–82.
77. Greenberg PD, Koch J, Cello JP. Diagnosis of *Cryptosporidium parvum* in patients with severe diarrhea and AIDS. *Digest Dis Sci* 1996;**41**:2286–90.
78. Griffiths JK, Moore R, Dooley S, et al. *Cryptosporidium parvum* infection of Caco-2 cell monolayers induces an apical monolayer defect, selectively increases transmonolayer permeability, and causes epithelial cell death. *Infect Immun* 1994;**62**:4506–14.
79. Grube H, Ramratnam B, Ley C, et al. Resolution of AIDS associated cryptosporidiosis after treatment with indinavir. *Am J Gastroenterol* 1997;**92**:726.
80. Guerrant DI, Moore SR, Lima AA, et al. Association of early childhood diarrhea and cryptosporidiosis with impaired physical fitness and cognitive function four-seven years later in a poor urban community in northeast Brazil. *Am J Trop Med Hyg* 1999;**61**:707–13.
81. Guerrant RL, Deboer MD, Moore SR, et al. The impoverished gut-a triple burden of diarrhoea, stunting and chronic disease. *Nat Rev Gastroenterol Hepatol* 2012.
82. Harari MD, West B, Dwyer B. *Cryptosporidium* as cause of laryngotracheitis in an infant. *Lancet* 1986;**1**:1207.
83. Hashmey R, Smith NH, Cron S, et al. Cryptosporidiosis in Houston, Texas: a report of 95 cases. *Medicine* 1997;**76**: 118–39.
84. Hawkins SP, Thomas RP, Teasdale C. Acute pancreatitis: a new finding in cryptosporidium enteritis. *Brit Med J* 1987;**294**:483–4.
85. Hayward AR, Levy J, Facchetti F, et al. Cholangiopathy and tumors of the pancreas, liver, and biliary tree in boys with X-linked immunodeficiency with hyper-IgM. *J Immunol* 1997;**158**:977–83.
86. Hellard M, Hocking J, Willis J, et al. Risk factors leading to *Cryptosporidium* infection in men who have sex with men. *Sex Trans Infect* 2003;**79**:412–14.
87. Hernandez J, Lackner A, Aye P, et al. Substance P is responsible for physiological alterations such as increased chloride ion secretion and glucose malabsorption in cryptosporidiosis. *Infect Immun* 2007;**75**:1137–43.
88. Hewitt RG, Yiannoutsos CT, Higgs ES, et al. Paromomycin: no more effective than placebo for treatment of cryptosporidiosis in patients with advanced human immunodeficiency virus infection. AIDS Clinical Trial Group. *Clin Infect Dis* 2000;**31**:1084–92.
89. Hicks P, Zwiener RJ, Squires J, et al. Azithromycin therapy for *Cryptosporidium parvum* infection in four children infected with human immunodeficiency virus. *J Pediatr* 1996;**129**:297–300.
90. Hinnant K, Schwartz A, Rotterdam H, et al. Cytomegaloviral and cryptosporidial cholecystitis in two patients with AIDS. *Am J Surg Pathol* 1989;**13**:57–60.
91. Hlavsa MC, Roberts VA, Anderson AR, et al. Surveillance for waterborne disease outbreaks and other health events associated with recreational water: United States, 2007–2008. *MMWR Surveill Summ* 2011;**60**:1–32.
92. Hommer V, Eichholz J, Petry F. Effect of antiretroviral protease inhibitors alone, and in combination with paromomycin, on the

excystation, invasion and in vitro development of *Cryptosporidium parvum*. *J Antimicrob Chemother* 2003;**52**:359–64.
92a. Hunter PR, Hughes S, Woodhouse S, et al. Health sequelae of human cryptosporidiosis in immunocompetent patients. *Clin Infect Dis* 2004;**39**:504–10.
93. Hunter PR, Nichols G. Epidemiology and clinical features of *Cryptosporidium* infection in immunocompromised patients. *Clin Microbiol Rev* 2002;**15**:145–54.
94. Jain A, Atkinson TP, Lipsky PE, et al. Defects of T-cell effector function and post-thymic maturation in X-linked hyper-IgM syndrome. *J Clin Invest* 1999;**103**:1151–8.
95. Johnston SP, Ballard MM, Beach MJ, et al. Evaluation of three commercial assays for detection of *Giardia* and *Cryptosporidium* organisms in fecal specimens. *J Clin Microbiol* 2003;**41**:623–6.
96. Kadappu KK, Nagaraja MV, Rao PV, et al. Azithromycin as treatment for cryptosporidiosis in human immunodeficiency virus disease. *J Postgrad Med* 2002;**48**:179–81.
97. Kahn DG, Garfinkle JM, Klonoff DC, et al. Cryptosporidial and cytomegaloviral hepatitis and cholecystitis. *Arch Pathol Lab Med* 1987;**111**:879–81.
98. Kanyok TP, Novak RM, Danziger LH. Preliminary results of a randomized, blinded control study of paromomycin versus placebo for treatment of *Cryptosporidium* diarrhea in AIDS patients. IX International Conference on AIDS. Berlin, June 6-11, 1993.
99. Kelly P, Makumbi FA, Carnaby S, et al. Variable distribution of *Cryptosporidium parvum* in the intestine of AIDS patients revealed by polymerase chain reaction. *Eur J Gastroenterol Hepatol* 1998; **10**:855–8.
100. Khan WA, Rogers KA, Karim MM, et al. Cryptosporidiosis among Bangladeshi children with diarrhea: a prospective, matched, case-control study of clinical features, epidemiology and systemic antibody responses. *Am J Trop Med Hyg* 2004;**71**:412–19.
101. Khurana S, Sharma P, Sharma A, et al. Evaluation of Ziehl-Neelsen staining, auramine phenol staining, antigen detection enzyme linked immunosorbent assay and polymerase chain reaction, for the diagnosis of intestinal cryptosporidiosis. *Trop Parasitol* 2012;**2**:20–3.
102. King BJ, Monis PT. Critical processes affecting *Cryptosporidium* oocyst survival in the environment. *Parasitology* 2007;**134**: 309–23.
103. Kirkpatrick BD, Daniels MM, Jean SS, et al. Cryptosporidiosis stimulates an inflammatory intestinal response in malnourished Haitian children. *J Infect Dis* 2002;**186**:94–101.
104. Kirkpatrick BD, Haque R, Duggal P, et al. Association between *Cryptosporidium* infection and human leukocyte antigen class I and class II alleles. *J Infect Dis* 2008;**197**:474–8.
105. Koch KL, Phillips DJ, Aber RC, et al. Cryptosporidiosis in hospital personnel: evidence for person-to-person transmission. *Ann Intern Med* 1985;**102**:593–6.
106. Korich DG, Mead JR, Madore MS, et al. Effects of ozone, chlorine dioxide, chlorine, and monochloramine on *Cryptosporidium parvum* oocyst viability. *Appl Environ Microbiol* 1990;**56**: 1423–8.
107. Korpe PS, Liu Y, Siddique A, et al. Breast milk parasite-specific antibodies and protection from amebiasis and cryptosporidiosis in Bangladeshi infants: a prospective cohort study. *Clin Infect Dis* 2013;**56**:988–92.
108. Krause I, Amir J, Cleper R, et al. Cryptosporidiosis in children following solid organ transplantation. *Pediatr Infect Dis J* 2012;**31**: 1135–8.
109. Lake IR, Harrison FC, Chalmers RM, et al. Case-control study of environmental and social factors influencing cryptosporidiosis. *Eur J Epidemiol* 2007;**22**:805–11.
110. Laurent F, Kagnoff MF, Savidge TC, et al. Human intestinal epithelial cells respond to *Cryptosporidium parvum* infection with increased prostaglandin H synthase 2 expression and prostaglandin E2 and F2alpha production. *Infect Immun* 1998;**66**:1787–90.
111. Le Moing V, Bissuel F, Costagliola D, et al. Decreased prevalence of intestinal cryptosporidiosis in HIV-infected patients concomitant to the widespread use of protease inhibitors. *AIDS* 1998; **12**:1395–7.
112. Leach CT, Koo FC, Kuhls TL, et al. Prevalence of *Cryptosporidium parvum* infection in children along the Texas-Mexico border and associated risk factors. *Am J Trop Med Hyg* 2000;**62**:656–61.
113. Legrand F, Grenouillet F, Larosa F, et al. Diagnosis and treatment of digestive cryptosporidiosis in allogeneic haematopoietic stem cell transplant recipients: a prospective single centre study. *Bone marrow transplantation* 2011;**46**:858–62.
114. Leoni F, Amar C, Nichols G, et al. Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. *J Med Microbiol* 2006;**55**:703–7.
115. Levy J, Espanol-Boren T, Thomas C, et al. Clinical spectrum of X-linked hyper-IgM syndrome. *J Pediatr* 1997;**131**:47–54.
116. Li N, Xiao L, Wang L, et al. Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneusi* by genotyping and subtyping parasites in wastewater. *PLoS Negl Trop Dis* 2012;**6**:e1809.
117. Lima AA, Moore SR, Barboza MS Jr, et al. Persistent diarrhea signals a critical period of increased diarrhea burdens and nutritional shortfalls: a prospective cohort study among children in northeastern Brazil. *J Infect Dis* 2000;**181**:1643–51.
118. Lima AA, Silva TM, Gifoni AM, et al. Mucosal injury and disruption of intestinal barrier function in HIV-infected individuals with and without diarrhea and cryptosporidiosis in northeast Brazil. *Am J Gastroenterol* 1997;**92**:1861–6.
119. Lima AAM, Samie A, Guerrant RL. Cryptosporidiosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. 3rd ed. Edinburgh: Saunders; 2011. p. 633–40.
120. Liu J, Gratz J, Amour C, et al. A laboratory-developed TaqMan Array Card for simultaneous detection of 19 enteropathogens. *J Clin Microbiol* 2013;**51**:472–80.
121. Liu K, Zai D, Zhang D, et al. Divalent Cp15-23 vaccine enhances immune responses and protection against *Cryptosporidium parvum* infection. *Parasite Immunol* 2010;**32**:335–44.
122. Lopez-Velez R, Tarazona R, Garcia Camacho A, et al. Intestinal and extraintestinal cryptosporidiosis in AIDS patients. *Eur J Clin Microbiol Infect Dis* 1995;**14**:677–81.
123. Lumadue JA, Manabe YC, Moore RD, et al. A clinicopathologic analysis of AIDS-related cryptosporidiosis. *AIDS* 1998;**12**:2459–66.
124. MacKenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994;**331**: 161–7.
125. MacKenzie WR, Schell WL, Blair KA, et al. Massive outbreak of waterborne cryptosporidium infection in Milwaukee, Wisconsin: recurrence of illness and risk of secondary transmission. *Clin Infect Dis* 1995;**21**:57–62.
126. Maggi P, Larocca AM, Quarto M, et al. Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1. *Eur J Clin Microbiol* 2000;**19**:213–17.
127. Manabe YC, Clark DP, Moore RD, et al. Cryptosporidiosis in patients with AIDS: correlates of disease and survival. *Clin Infect Dis* 1998;**27**:536–42.
128. Manque PA, Tenjo F, Woehlbier U, et al. Identification and immunological characterization of three potential vaccinogens against *Cryptosporidium* species. *Clin Vaccine Immunol* 2011;**18**:1796–802.
129. Martin-Ampudia M, Mariscal A, Lopez-Gigosos RM, et al. Under-notification of cryptosporidiosis by routine clinical and laboratory practices among non-hospitalised children with acute diarrhoea in Southern Spain. *Infection* 2012;**40**:113–19.
130. McDonald V, Korbel DS, Barakat FM, et al. Innate immune responses against *Cryptosporidium parvum* infection. *Parasite Immunol* 2013;**35**:55–64.
131. McLauchlin J, Amar CF, Pedraza-Diaz S, et al. Polymerase chain reaction-based diagnosis of infection with *Cryptosporidium* in children with primary immunodeficiencies. *Pediatr Infect Dis J* 2003; **22**:329–35.
132. Mead JR. Challenges and prospects for a *Cryptosporidium* vaccine. *Future Microbiol* 2010;**5**:335–7.
133. Mead JR, You X. Susceptibility differences to *Cryptosporidium parvum* infection in two strains of gamma interferon knockout mice. *J Parasitol* 1998;**84**:1045–8.
134. Meisel JL, Perera DR, Meligro C, et al. Overwhelming watery diarrhea associated with a *Cryptosporidium* in an immunosuppressed patient. *Gastroenterology* 1976;**70**:1156–60.
135. Mele R, Gomez Morales MA, Tosini F, et al. Indinavir reduces *Cryptosporidium parvum* infection in both in vitro and in vivo models. *Int J Parasit* 2003;**33**:757–64.

136. Melo Cristino JA, Carvalho MI, Salgado MJ. An outbreak of cryptosporidiosis in a hospital day-care centre. *Epidemiol Infect* 1988;**101**:355–9.
137. Meynard JL, Meyohas MC, Binet D, et al. Pulmonary cryptosporidiosis in the acquired immunodeficiency syndrome. *Infection* 1996;**24**:328–31.
138. Miao YM, Awad-El-Kariem FM, Franzen C, et al. Eradication of cryptosporidia and microsporidia following successful antiretroviral therapy. *J Acquir Immune Defic Syndr* 2000;**25**:124–9.
139. Miller SA, Rosario CL, Rojas E, et al. Intestinal parasitic infection and associated symptoms in children attending day care centres in Trujillo, Venezuela. *Trop Med Int Health* 2003;**8**:342–7.
140. Miller TL, Winter HS, Luginbuhl LM, et al. Pancreatitis in pediatric human immunodeficiency virus infection. *J Pediatr* 1992;**120**:223–7.
141. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1–166.
142. Molbak K, Andersen M, Aaby P, et al. *Cryptosporidium* infection in infancy as a cause of malnutrition: a community study from Guinea-Bissau, West Africa. *Am J Clin Nutr* 1997;**65**:149–52.
143. Molbak K, Hojlyng N, Gottschau A, et al. Cryptosporidiosis in infancy and childhood mortality in Guinea Bissau, west Africa. *BMJ* 1993;**307**:417–20.
144. Mondal D, Haque R, Sack RB, et al. Attribution of malnutrition to cause-specific diarrheal illness: evidence from a prospective study of preschool children in Mirpur, Dhaka, Bangladesh. *Am J Trop Medicine and Hygiene* 2009;**80**:824–6.
145. Mondal D, Minak J, Alam M, et al. Contribution of enteric infection, altered intestinal barrier function, and maternal malnutrition to infant malnutrition in Bangladesh. *Clin Infect Dis* 2012;**54**: 185–92.
146. Moore SR, Lima NL, Soares AM, et al. Prolonged episodes of acute diarrhea reduce growth and increase risk of persistent diarrhea in children. *Gastroenterology* 2010;**139**:1156–64.
147. Mor SM, Tumwine JK, Ndeezi G, et al. Respiratory cryptosporidiosis in HIV-seronegative children in Uganda: potential for respiratory transmission. *Clin Infect Dis* 2010;**50**:1366–72.
148. Moyo SJ, Gro N, Matee MI, et al. Age specific aetiological agents of diarrhoea in hospitalized children aged less than five years in Dar es Salaam, Tanzania. *BMC Pediatr* 2011;**11**:19.
149. Kotloff KL, Nataro JP, Blackwelder WC, et al. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 2013;doi:pii: S0140-6736(13)60844-2. [Epub ahead of print] PMID: 23680352. Can be found at http://www.ncbi.nlm.nih.gov/pubmed/23680352.
150. Nachbaur D, Kropshofer G, Feichtinger H, et al. Cryptosporidiosis after CD34-selected autologous peripheral blood stem cell transplantation (PBSCT): treatment with paromomycin, azithromycin and recombinant human interleukin-2. *Bone Marrow Transplant* 1997;**19**:1261–3.
151. Nair P, Mohamed JA, DuPont HL, et al. Epidemiology of cryptosporidiosis in North American travelers to Mexico. *Am J Trop Med Hyg* 2008;**79**:210–14.
152. Navarrete S, Stetler HC, Avila C, et al. An outbreak of *Cryptosporidium* diarrhea in a pediatric hospital. *Pediatr Infect Dis J* 1991;**10**:248–50.
153. Newman RD, Sears CL, Moore SR, et al. Longitudinal study of *Cryptosporidium* infection in children in northeastern Brazil. *J Infect Dis* 1999;**180**:167–75.
154. Newman RD, Zu SX, Wuhib T, et al. Household epidemiology of *Cryptosporidium parvum* infection in an urban community in northeast Brazil. *Ann Intern Med* 1994;**120**:500–5.
155. Nime FA, Burek JD, Page DL, et al. Acute enterocolitis in a human being infected with the protozoan *Cryptosporidium*. *Gastroenterology* 1976;**70**:592–8.
156. O'Hara SP, Chen XM. The cell biology of cryptosporidium infection. *Microbes Infect* 2011;**13**:721–30.
157. Ochoa TJ, Salazar-Lindo E, Cleary TG. Management of children with infection-associated persistent diarrhea. *Semin Pediatr Infect Dis* 2004;**15**:229–36.
157a. Ochoa TJ, White Jr AC. Nitazoxanide for treatment of intestinal parasites in children. *Ped Infect Dis J* 2005;**24**:641–2.
158. Okhuysen PC, Chappell CL, Crabb JH, et al. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *J Infect Dis* 1999;**180**:1275–81.
159. Okhuysen PC, Rich SM, Chappell CL, et al. Infectivity of a *Cryptosporidium parvum* isolate of cervine origin for healthy adults and interferon-gamma knockout mice. *J Infect Dis* 2002;**185**: 1320–5.
160. Okhuysen PC, Robinson P, Nguyen MT, et al. Jejunal cytokine response in AIDS patients with chronic cryptosporidiosis and during immune reconstitution. *AIDS* 2001;**15**:802–4.
161. Ortega YR, Roxas CR, Gilman RH, et al. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in markets of an endemic region in Peru. *Am J Trop Med Hyg* 1997;**57**:683–6.
162. Pantenburg B, Castellanos-Gonzalez A, Dann SM, et al. Human CD8(+) T cells clear *Cryptosporidium parvum* from infected intestinal epithelial cells. *Am J Trop Med Hyg* 2010;**82**:600–7.
163. Pantenburg B, Dann S, Wang H, et al. Intestinal immune response to human *Cryptosporidium* sp. infection. *Infect Immun* 2008;**76**: 23–9.
164. Pereira SJ, Ramirez NE, Xiao L, et al. Pathogenesis of human and bovine *Cryptosporidium parvum* in gnotobiotic pigs. *J Infect Dis* 2002;**186**:715–18.
165. Petry F, Jakobi V, Wagner S, et al. Binding and activation of human and mouse complement by *Cryptosporidium parvum* (Apicomplexa) and susceptibility of C1q- and MBL-deficient mice to infection. *Mol Immunol* 2008;**45**:3392–400.
166. Phillips AD, Thomas AG, Walker-Smith JA. *Cryptosporidium*, chronic diarrhoea and the proximal small intestinal mucosa. *Gut* 1992;**33**:1057–61.
167. Polage CR, Stoddard GJ, Rolfs RT, et al. Physician use of parasite tests in the United States from 1997 to 2006 and in a Utah *Cryptosporidium* outbreak in 2007. *J Clin Microbiol* 2011;**49**:591–6.
168. Pozio E, Rezza G, Boschini A, et al. Clinical cryptosporidiosis and human immunodeficiency virus (HIV)-induced immunosuppression: findings from a longitudinal study of HIV-positive and HIV-negative former injection drug users. *J Infect Dis* 1997;**176**: 969–75.
169. Preidis GA, Wang HC, Lewis DE, et al. Seropositive human subjects produce interferon gamma after stimulation with recombinant *Cryptosporidium hominis* gp15. *Am J Trop Med Hyg* 2007; **77**:583–5.
170. Priest JW, Bern C, Roberts JM, et al. Changes in serum immunoglobulin G levels as a marker for *Cryptosporidium* sp. infection in Peruvian children. *J Clin Microbiol* 2005;**43**:5298–300.
171. Rao Ajjampur SS, Asirvatham JR, Muthusamy D, et al. Clinical features & risk factors associated with cryptosporidiosis in HIV infected adults in India. *Indian J Med Res* 2007;**126**: 553–7.
172. Robinson P, Okhuysen PC, Chappell CL, et al. Expression of tumor necrosis factor alpha and interleukin 1 beta in jejuna of volunteers after experimental challenge with *Cryptosporidium parvum* correlates with exposure but not with symptoms. *Infect Immun* 2001;**69**:1172–4.
173. Robinson P, Okhuysen PC, Chappell CL, et al. Substance P expression correlates with severity of diarrhea in cryptosporidiosis. *J Infect Dis* 2003;**188**:290–6.
174. Robinson TJ, Cebelinski EA, Taylor C, et al. Evaluation of the positive predictive value of rapid assays used by clinical laboratories in Minnesota for the diagnosis of cryptosporidiosis. *Clin Infect Dis* 2010;**50**:e53–55.
175. Roche JK, Martins CA, Cosme R, et al. Transforming growth factor beta1 ameliorates intestinal epithelial barrier disruption by *Cryptosporidium parvum* in vitro in the absence of mucosal T lymphocytes. *Infect Immun* 2000;**68**:5635–44.
176. Roche JK, Rojo AL, Costa LB, et al. Intranasal vaccination in mice with an attenuated *Salmonella enterica* Serovar 908htr A expressing Cp15 of *Cryptosporidium*: impact of malnutrition with preservation of cytokine secretion. *Vaccine* 2013;**31**:912–18.
177. Rogers KA, Rogers AB, Leav BA, et al. MyD88-dependent pathways mediate resistance to *Cryptosporidium parvum* infection in mice. *Infection and immunity* 2006;**74**:549–56.
178. Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by *Cryptosporidium parvum:* a prospective randomized,

double-blind, placebo-controlled study of nitazoxanide. *J Infect Dis* 2001; **184**:103–6.
179. Rossignol JF, Kabil SM, el-Gohary Y, et al. Effect of nitazoxanide in diarrhea and enteritis caused by *Cryptosporidium* species. *Clin Gastroenterol Hepatol* 2006;**4**:320–4.
180. Saez-Llorens X, Odio CM, Umana MA, et al. Spiramycin vs. placebo for treatment of acute diarrhea caused by *Cryptosporidium*. *Pediatr Infectious Dis J* 1989;**8**:136–40.
181. Sallon S, Deckelbaum RJ, Schmid II, et al. Cryptosporidium, malnutrition, and chronic diarrhea in children. *Am J Dis Child* 1988;**142**:312–15.
182. Samie A, Bessong PO, Obi CL, et al. *Cryptosporidium* species: preliminary descriptions of the prevalence and genotype distribution among school children and hospital patients in the Venda region, Limpopo Province, South Africa. *Exp Parasitol* 2006;**114**: 314–22.
183. Sarabia-Arce S, Salazar-Lindo E, Gilman RH, et al. Case-control study of *Cryptosporidium parvum* infection in Peruvian children hospitalized for diarrhea: possible association with malnutrition and nosocomial infection. *Pediatr Infect Dis J* 1990;**9**:627–31.
184. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States: major pathogens. *Emerg Infect Dis* 2011;**17**:7–15.
185. Schmidt W, Wahnschaffe U, Schafer M, et al. Rapid increase of mucosal CD4 T cells followed by clearance of intestinal cryptosporidiosis in an AIDS patient receiving highly active antiretroviral therapy. *Gastroenterology* 2001;**120**:984–7.
186. Sharpstone D, Neild P, Crane R, et al. Small intestinal transit, absorption, and permeability in patients with AIDS with and without diarrhoea. *Gut* 1999;**45**:70–6.
187. Shirley DA, Moonah SN, Kotloff KL. Burden of disease from cryptosporidiosis. *Curr Opin Infect Dis* 2012;**25**:555–63.
188. Simon DM, Cello JP, Valenzuela J, et al. Multicenter trial of octreotide in patients with refractory acquired immunodeficiency syndrome-associated diarrhea. *Gastroenterology* 1995;**108**: 1753–60.
189. Smith H. Diagnostics. In: Fayer R, Xiao L, editor. *Cryptosporidium and cryptosporidiosis*. Boca Raton, Fla: CRC Press; 2007. p. 173–207.
190. Sodemann M, Jakobsen MS, Molbak K, et al. Episode-specific risk factors for progression of acute diarrhoea to persistent diarrhoea in west African children. *Trans R Soc Trop Med Hyg* 1999;**93**: 65–8.
191. Sprinz E, Mallman R, Barcellos S, et al. AIDS-related cryptosporidial diarrhoea: an open study with roxithromycin. *J Antimicrob Chemother* 1998;**41**(suppl B):85–91.
192. Stehr-Green JK, McCaig L, Remsen HM, et al. Shedding of oocysts in immunocompetent individuals infected with *Cryptosporidium*. *Am J Trop Med Hyg* 1987;**36**:338–42.
193. Taniuchi M, Verweij JJ, Noor Z, et al. High throughput multiplex PCR and probe-based detection with Luminex beads for seven intestinal parasites. *Am J Trop Med Hyg* 2011;**84**:332–7.
194. Teare JP, Daly CA, Rodgers C, et al. Pancreatic abnormalities and AIDS related sclerosing cholangitis. *Genitourin Med* 1997; **73**:271–3.
195. ten Hove R, Schuurman T, Kooistra M, et al. Detection of diarrhoea-causing protozoa in general practice patients in the Netherlands by multiplex real-time PCR. *Clin Microbiol Infect* 2007;**13**:1001–7.
196. Theodos CM, Griffiths JK, D'Onfro J, et al. Efficacy of nitazoxanide against *Cryptosporidium parvum* in cell culture and in animal models. *Antimicrob Agents Chemother* 1998;**42**:1959–65.
197. Theodos CM, Sullivan KL, Griffiths JK, et al. Profiles of healing and nonhealing *Cryptosporidium parvum* infection in C57BL/6 mice with functional B and T lymphocytes: the extent of gamma interferon modulation determines the outcome of infection. *Infect Immun* 1997;**65**:4761–9.
198. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, et al. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. *Am J Trop Med Hyg* 2005;**73**:921–5.
199. Tumwine JK, Kekitiinwa A, Nabukeera N, et al. *Cryptosporidium parvum* in children with diarrhea in Mulago Hospital, Kampala, Uganda. *Am J Trop Med Hyg* 2003;**68**:710–15.
200. Tyzzer EE. A sporozoan found in the peptic glands of the common mouse. *Proc Soc Exp Biol Med* 1907;**5**:12–13.
201. Uip DE, Lima AL, Amato VS, et al. Roxithromycin treatment for diarrhoea caused by *Cryptosporidium* spp. in patients with AIDS. *J Antimicrob Chemother* 1998;**41**(suppl B):93–7.
202. Urban JF Jr, Fayer R, Sullivan C, et al. Local TH1 and TH2 responses to parasitic infection in the intestine: regulation by IFN-gamma and IL-4. *Vet Immunol Immunopathol* 1996;**54**:337–44.
203. Vakil NB, Schwartz SM, Buggy BP, et al. Biliary cryptosporidiosis in HIV-infected people after the waterborne outbreak of cryptosporidiosis in Milwaukee. *N Engl J Med* 1996;**334**:19–23.
204. Valentiner-Branth P, Steinsland H, Fischer TK, et al. Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life. *J Clin Microbiol* 2003;**41**:4238–45.
205. Walters IN, Miller NM, van den Ende J, et al. Outbreak of cryptosporidiosis among young children attending a day-care centre in Durban. *South Afr Med J* 1988;**74**:496–9.
206. Wang C, Luo J, Amer S, et al. Multivalent DNA vaccine induces protective immune responses and enhanced resistance against *Cryptosporidium parvum* infection. *Vaccine* 2010;**29**:323–8.
207. Weber R, Bryan RT, Bishop HS, et al. Threshold of detection of *Cryptosporidium* oocysts in human stool specimens: evidence for low sensitivity of current diagnostic methods. *J Clin Microbiol* 1991;**29**:1323–7.
208. White AC Jr. Nitazoxanide: an important advance in anti-parasitic therapy. *Am J Trop Med Hyg* 2003;**68**:382–3.
209. Deleted in proofs.
210. White A Jr. *Cryptosporidium* species. In: Mandell GL, Bennett JE, Dolin R, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 7th ed. Philadelphia: Churchill Livingstone; 2010. p. 3547–60.
211. White AC Jr, Chappell CL, Hayat CS, et al. Paromomycin for cryptosporidiosis in AIDS: a prospective, double-blind trial. *J Infect Dis* 1994;**170**:419–24.
212. White AC, Robinson P, Okhuysen PC, et al. Interferon-gamma expression in jejunal biopsies in experimental human cryptosporidiosis correlates with prior sensitization and control of oocyst excretion. *J Infect Dis* 2000;**181**:701–9.
213. Wittenberg DF, Miller NM, van den Ende J. Spiramycin is not effective in treating *Cryptosporidium* diarrhea in infants: results of a double-blind randomized trial. *J Infect Dis* 1989;**159**:131–2.
214. Wolfson JS, Richter JM, Waldron MA, et al. Cryptosporidiosis in immunocompetent patients. *N Engl J Med* 1985;**312**:1278–82.
215. Wolska-Kusnierz B, Bajer A, Caccio S, et al. *Cryptosporidium* infection in patients with primary immunodeficiencies. *J Pediatr Gastroenterol Nutr* 2007;**45**:458–64.
216. Xiao L, Morgan UM, Fayer R, et al. Cryptosporidium systematics and implications for public health. *Parasitol Today* 2000;**16**: 287–92.
217. Yoder JS, Beach MJ. *Cryptosporidium* surveillance and risk factors in the United States. *Exp Parasitol* 2010;**124**:31–9.
218. Yoder JS, Wallace RM, Collier SA, et al. *Cryptosporidiosis* surveillance: United States, 2009-2010. *MMWR Surveill Summ* 2012;**61**: 1–12.
219. Zhang Y, Lee B, Thompson M, et al. Lactulose-mannitol intestinal permeability test in children with diarrhea caused by rotavirus and cryptosporidium. Diarrhea Working Group, Peru. *J Pediatr Gastroenterol Nutr* 2000;**31**:16–21.

Microbiology and Life Cycle

The oocysts of *C. cayetanenesis* found in stool are round, measuring 8 to 10 m.[109] In reports from the late 1980s and early 1990s, the taxonomy was in doubt and the organisms were referred to as *Cyanobacterium*-like body, a blue-green algae, a coccidian-like body, *Cryptosporidium*-like, or big *Cryptosporidium*.* Ortega and colleagues[109,113] demonstrated sporulation and excystation of the organisms, characterizing them as a coccidian species and suggesting the name *C. cayetanensis*.[168] Molecular studies have confirmed that the organisms are coccidian parasites closely related to the *Eimeria*.[120] Humans are the only known host of *C. cayetanensis*.

Infected patients pass unsporulated oocysts into the environment. A period of 1 to 2 weeks in a warm, moist environment is required for maturation into an infectious, sporulated oocyst.[142,146] In vivo, excystation of mature sporulated oocysts most likely occurs in the small bowel. In vitro, a combination of bile salts, sodium taurocholate, and mechanical pressure is required for excystation.[146] After excystation, the sporozoites invade the intestinal epithelical cells, where they undergo first asexual and later sexual reproduction. Sexual and asexual stages of *Cyclospora* are found in jejunal biopsy specimens from infected individuals, showing that this coccidian parasite can complete its entire life cycle in a single host.[111]

Epidemiology and Transmission

Cyclospora is found worldwide. Initial reports of cyclosporiasis involved residents of or travelers returning from resource-limited countries.[59,109,113,122,139] The organism has been acquired in numerous geographic areas, including North, Central, and South America; the Caribbean islands; Europe; Turkey; northern, central, and southern Africa; southwest, southern, and eastern Asia; and Papua New Guinea and Australia.† In one study of diarrheic stool from subjects returning from tropical areas, *C. cayetanensis* was identified in 5 percent of samples, rendering it the second most common pathogen after *Giardia*.[156] Seasonal variation has been described.[59,109,172] In Nepal, most cases occur during the rainy season, between May and October.[59] In Peru, the peak is during the fall, between April and June.[109] The prevalence in endemic regions varies with the age and immunologic status of the host. Prevalence rates in children from Peru and Nepal are reported to range from 2 to 18 percent.[59,109] In a Venezuelan review, Chacin-Bonilla and associates[26] report a prevalence that ranges from 0 to 41.6 percent. In one series from Haiti, *Cyclospora* was reported in 11 percent of adults infected with human immunodeficiency virus (HIV), but was not detected in non–HIV-infected adults or infants younger than 6 months.[117]

Hall and associates[54] published an active surveillance of *C. cayetanensis* cases in the United States. They noted 370 cases and significant association to travels and outbreaks.[54]

Direct person-to-person transmission is unlikely to occur because a period outside the host is required for maturation into a sporulated oocyst.[146] Contamination of the environment with oocysts plays a major role in the transmission of *Cyclospora*. Foodborne and waterborne transmission of *Cyclospora* have been described.[52,65,98,146] Waterborne outbreaks have been reported in Chicago and Nepal.[24,62,168] In one area of Egypt, the average prevalence of *Cyclospora* spores in stool samples was 5.6 percent in patients with diarrhea, in contrast to 2.3 percent in asymptomatic subjects, with higher prevalence in places with higher water contamination.[45] High levels of aquatic contamination also were documented in Hanoi (Vietnam), reaching 63.6 percent in certain areas, again emphasizing the important role of contaminated water in the spread of the pathogen.[97]

Foodborne outbreaks have been reported frequently.[52,55-57,65,98] Eleven foodborne outbreaks occurred in North America between 1990 and 2000, affecting 3600 people.[92] For example, widespread outbreaks of cyclosporiasis occurred in the United States and Canada in 1996 and 1997 associated with the consumption of raspberries imported from Guatemala.[55-57] Researchers postulated that the raspberries were contaminated with insecticide or fungicides that were mixed with contaminated water or that the irrigation water was contaminated with *Cyclospora* oocysts.[114,142] During 1997, clusters of cases were identified in which fresh basil and mesclun lettuce were implicated.[124] *Cyclospora* oocysts contaminating vegetables are not removed easily by routine washing.[111]

Clinical Manifestations

Cyclospora affects both immunocompetent and immunocompromised patients.* The incubation period typically is 2 to 11 days.[24] In immunocompetent host, diarrhea may last up to 7 weeks.[168] Relapsing diarrhea alternating with constipation is the rule.[142] Resolution of symptoms usually correlates with disappearance of the organism from stool.[59,116] Asymptomatic oocysts also occurs.[17,122] In one prevalence study in a Venezuelan community, *C. cayetanensis* oocysts were identified in 6.1 percent of subjects, of whom 84.6 percent were asymptomatic.[27]

In immunocompromised hosts, especially, onset of watery diarrhea may be abrupt or gradual. Malaise, myalgia, and anorexia, are common.[30,168] Low-grade fever is reported in approximately 25 percent of patients.[24,59,139,168] Vomiting may occur but is less common than diarrhea. Other associated symptoms include abdominal cramping, heartburn, and indigestion.[142] Weight loss and malabsorption occur with prolonged illness.[59,60,144,169]

The median duration of disease in various U.S. outbreaks has been 10 to 20 days (range, 1 to 60 days).[55] In HIV-infected patients, *Cyclospora* causes symptoms that are indistinguishable from symptoms of *Cryptosporidium* and *Cystoisospora* infection.[168] Biliary disease was reported in two patients with AIDS infected with *Cyclospora*. These patients had clinical and radiographic confirmation of biliary disease and did not have evidence of infection with

*References 1, 17, 61, 109, 112, 113, 122, 139, 143.
†References 5, 6, 20, 45, 66, 93, 104, 136, 142, 146, 170, 171.

*References 66, 93, 104, 109, 117, 122, 139, 156, 168.

other pathogens. Both patients had acalculous cholecystitis that was responsive to therapy with trimethoprim-sulfamethoxazole (TMP-SMX).[144] *Cyclospora* also has been identified in respiratory secretions of immunocompromised patients.[64]

Diagnosis

Cyclospora can be visualized by light microscopy on wet mounts or after formol-ether concentration of the stool. In fresh stool, the oocysts are unsporulated. They appear as refractile spherical bodies measuring 8 to 10 μm and have a central greenish morula that contains six to nine refractile globules.[113] Safranin staining enhances the outline of the membrane, but does not stain internal structures. Galvan-Diaz and colleagues[49] compared safranin and Ziehl-Neelsen stains using as gold standard the direct visualization of the parasite. They found a sensitivity of 95 percent and specificity of 98 percent with safranin staining and a sensitivity 90 percent and specificity of 100 percent for Ziehl-Neelsen staining.[49]

The sensitivity of wet mounts is 75 percent.[117] Oocysts exhibit bright blue autofluorescence when exposed to ultraviolet light. Auramine-rhodamine staining enhances the visualization of internal structures. Staining is variable with modified Ziehl-Neelsen stain (Fig. 217-1).[109] *Cyclospora* is not visualized by Gram, Giemsa, Grocott-Gomori methenamine–silver nitrate, Lugol iodine, periodic acid–Schiff, or hematoxylin and eosin staining.[118,168]

A direct comparison of formalin-ether sedimentation, direct smear examination, and sucrose centrifugal flotation yielded the highest recovery of *Cyclospora* oocysts in stool with the last method.[76] More recently, polymerase chain reaction (PCR)[63,79,81,154,156] and flow cytometry[44] have been studied as more sensitive detection methods.

Treatment

When treatment is indicated because of the severity or persistence of symptoms, TMP-SMX is the drug of choice.[60,88,89,155] No clear alternatives are available in patients who are intolerant of TMP-SMX. Ciprofloxacin was inferior to TMP-SMX in patients with AIDS.[155] Nitazoxanide has been used in a limited number of patients.[173] Prophylaxis with TMP-SMX 3 days per week seems to prevent recurrent episodes in HIV-infected patients.[117]

CYSTOISOSPORIASIS

Cystoisospora belli (previously known as *Isospora belli*) is an intestinal coccidian parasite (Fig. 217-2). Human infection was first recorded in U.S. military personnel during World War I. The first U.S. cases were described in children with chronic diarrhea and malnutrition.[21] *C. belli* causes acute and chronic diarrhea in immunocompetent children but can cause prolonged, life-threatening diarrhea and volume depletion in patients with compromised cellular immunity, particularly among patients infected with HIV.[58,129]

Microbiology and Life Cycle

C. belli is a coccidian parasite.[84] The taxonomy was recently revised separating the genus *Isospora* with organisms that infect birds reclassified as *Atoxoplasma* and those infecting mammals as *Cystoisospora*.[15] Some species of *Cystoisospora* infect other mammals, but only *C. belli* infects humans.[84] Mature oocysts of *C. belli* are oval, measure 10 to 20 mm × 20 to 33 μm, with a translucent thin wall that contains two round sporoblasts, each with four crescent-shaped sporozoites (Fig. 217-3).[31] Humans acquire the infection by ingesting food or water contaminated with the mature sporulated oocyst. Excystation occurs in the proximal part of the small intestine, leading to the release of sporozoites that invade enterocytes of the distal duodenum and proximal jejunum and develop into trophozoites. The trophozoites reproduce asexually to form merozoites. The merozoites undergo asexual replication (schizogony or merogony) or sexual replication (gametogony) that results in the production of an immature unsporulated oocyst. This latter form is excreted in stool

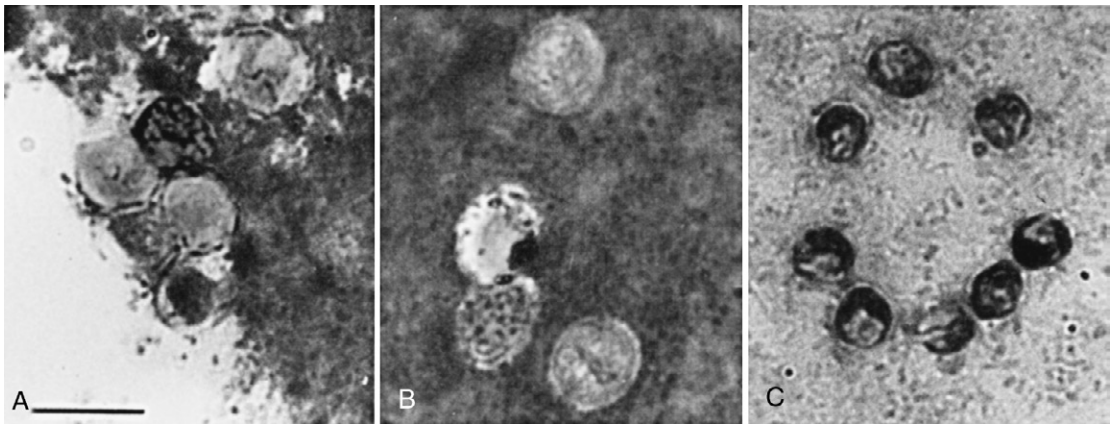

FIGURE 217-1 ■ Modified acid-fast stain of *Cyclospora* sp. **(A),** *Cryptosporidium muris* **(B),** *Cryptosporidium parvum* **(C).** Bar = 10 μm. (From Ortega YR, Sterling CR, Gilman RH, et al. *Cyclospora* species: a new protozoan pathogen of humans. *N Engl J Med* 1993;**328**:1308–12.)

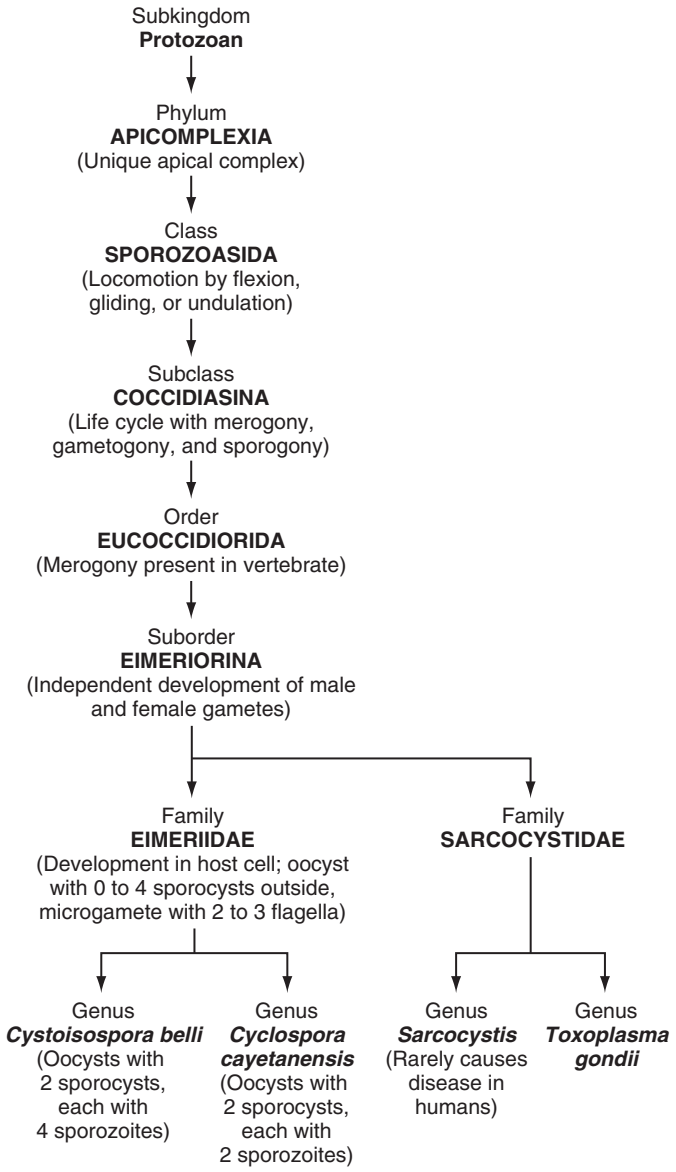

FIGURE 217-2 ■ Taxonomic classification of *Cryptosporidium, Isospora, Cyclospora,* and *Sarcocystis*.

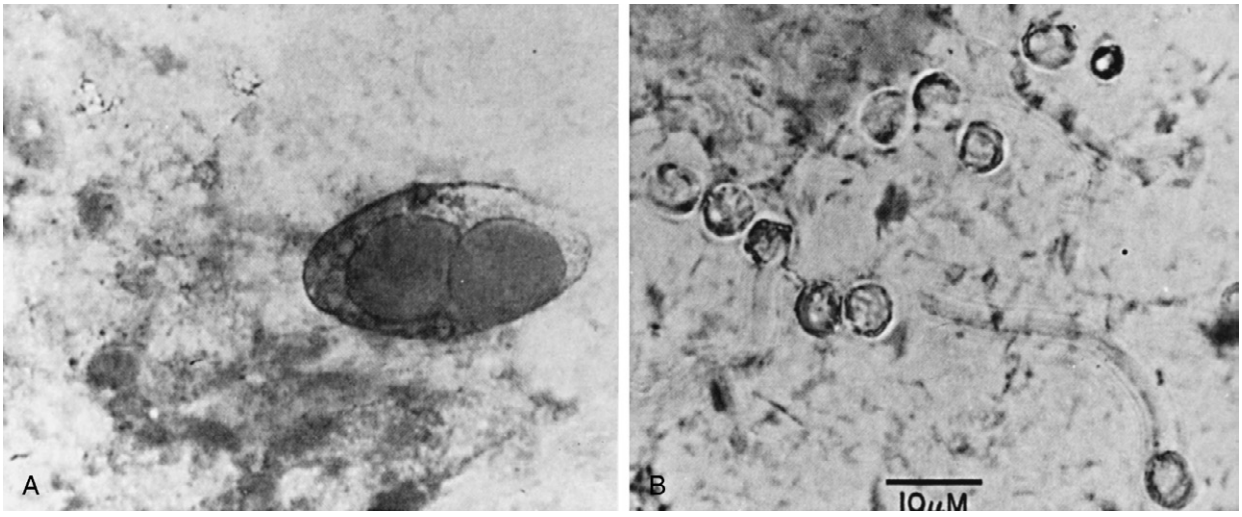

FIGURE 217-3 ■ Unconcentrated fresh stool sample from two patients with AIDS stained with modified Kinyoun stain. **A,** *Isospora belli*. **B,** *Cryptosporidium*. Note the difference in size and shape of the two coccidian parasites; *Isospora* averages 25 × 15 μm and contains two sporoblasts, whereas *Cryptosporidium* averages about 5 μm in diameter. (From DeHovitz JA, Pape JW, Boncy M, et al. Clinical manifestations and therapy of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1986;**315**:87–90.)

and requires 12 to 48 hours to mature into the infectious form, a sporulated oocyst.[84]

Epidemiology and Transmission

C. belli is distributed worldwide but most cases are reported from tropical and subtropical regions. Travel to or immigration from resource-poor areas is the main risk factor for infection among cases in Europe and North America, including in patients with HIV and AIDS.[4,53,78,137,145] The incidence of *C. belli* among HIV-infected patients with chronic diarrhea in the developing world varies markedly, with very high rates of 13 to 26 percent of patients with diarrhea in Venezuela, Haiti, and India.[1,25,37,158] Infection is also common in Egypt, sub-Saharan Africa, Brazil, and other parts of Asia, but recorded rates have been lower.[141,161,167] On the other hand, *C. belli* is less common among HIV-infected patients taking cotrimoxazole prophylaxis.[23,53]

Transmission is by the fecal-oral route. However, direct person-to-person transmission is considered very uncommon, because the immature oocysts shed by humans require at least 1 to 2 days in the environment to become infectious.[37,84] The incubation period is thought to be 3 to 14 days.[84] The infectious dose is largely unknown. The oocysts of *C. belli* are resistant to commonly used disinfectants and may remain viable for months.

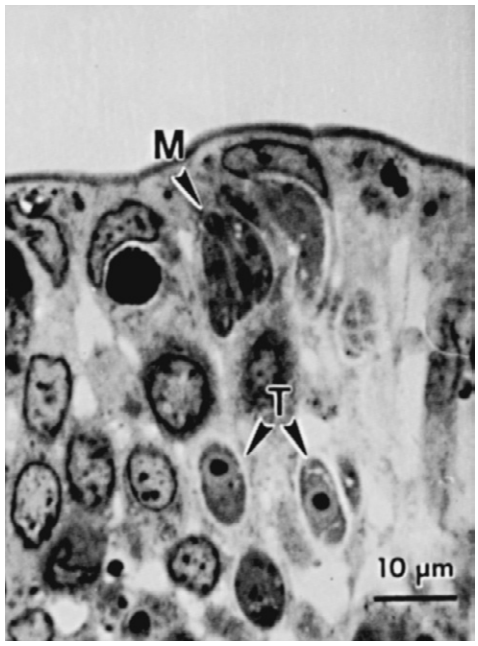

FIGURE 217-4 ■ *Isospora* infecting the jejunum of a patient with severe diarrhea. Trophozoites *(T)* divide within enterocytes by schizogony to form merozoites *(M).* (From Garcia LS, Owen RL. Current WL. Isosporiasis. In: Balows A, Hausler WJ Jr, Ohashi M, Turano A, editors. *The laboratory diagnosis of infectious diseases: principles and practice.* vol. 1. New York: Springer-Verlag, 1988, p. 899–903.)

Pathophysiology

The pathologic findings are nonspecific. Typical histopathologic findings include shortening of the villi; hypertrophy of the crypts; and infiltration of the lamina propria with plasma cells, lymphocytes, polymorphonuclear leukocytes, and eosinophils.[46,103] All stages of the life cycle have been identified within the villous epithelium (Fig. 217-4). *C. belli* also can cause biliary tract infection.[159] The pathophysiology of cystoisosporiasis has not been defined, but the organisms are strongly associated with diarrhea and malabsorption syndromes.[9,16,153]

Clinical Manifestations

The main clinical manifestation of cystoisosporiasis is nonbloody, watery diarrhea. However, the spectrum of disease ranges from asymptomatic infection to life-threatening diarrhea. Immunocompetent patients usually have a self-limited illness, although diarrhea may not resolve for several weeks.[74,99,153] Some children have chronic malabsorption.[16] Headache, malaise, vomiting, fever, and dehydration may be noted. The disease is more severe in infants and children than in adults. In contrast to other protozoan parasites, *C. belli* is strongly associated with peripheral eosinophilia, which can be marked.[25,72]

The clinical course of cystoisosporiasis in immunosuppressed children depends on their cellular immunity. HIV-infected patients with CD4 cell counts below 200 cells/mm^3 are particularly at risk for becoming infected and to present symptoms, usually for prolonged periods with intermittent bouts of reactivation.[9,13,78] Patients who have a reconstituted immune system as a result of effective antiretroviral therapy are seldom symptomatic.[14] Seasonality has been observed in some but not all studies from developing countries.[25,35] Other conditions associated with depressed cellular immunity, including lymphomas and organ transplantation, predispose to a more severe illness.[127,128] The onset of illness is insidious and is associated with nonspecific symptoms, such as low-grade fever, headache, malaise, myalgia, and anorexia. Nausea, vomiting, and diffuse abdominal pain may be present as well. The diarrhea is typically watery. Severe dehydration, hypokalemia, metabolic acidosis resulting from bicarbonate losses, and azotemia may occur.[129,165] Marked weight loss, reaching 30 percent of body weight, has been reported in a patient with a 9-month clinical course.[129] Elevated stool osmolality and steatorrhea, are also common findings.

Extraintestinal manifestations of *C. belli* are rare; gallbladder and biliary tract infections have been reported in both HIV-infected and immunocompetent patients.[18,48,159] Cholangitis defined as elevation of alkaline phosphatase and dilation of the common bile duct detected by ultrasound was found in 4/28 patients chronically infected in France.[78] The organism has been identified in tracheobronchial, mediastinal, and mesenteric lymph nodes; the spleen; and the liver.[96]

Diagnosis

C. belli infection is usually diagnosed by observing oocysts in a stool wet-mount preparation or using modified acid-fast or auramine-rhodamine stain (http://dpd.cdc.gov/

dpdx/Html/Cystoisosporiasis.htm).[110] Concentration techniques such as zinc sulfate, hypertonic sodium chloride, formalin-ether sedimentation, and Sheather sucrose solution are recommended to increase the sensitivity. Multiple stool samples may be required to make the proper diagnosis. The oocysts are oval, contain one or two sporoblasts, and are 10 times larger than *Cryptosporidium* oocysts. Like *Cyclospora*, the oocysts of *C. belli* exhibit autofluorescence with an ultraviolet epifluorescent illuminator (450- to 490-nm excitation filter). Histologic examination of the small bowel shows nonspecific findings, but asexual and sexual stages of the parasite can be observed within the enterocytes[22] (Fig. 217-5). *C. belli* can be found infecting the small and large intestine in patients with HIV and AIDS. Peripheral blood eosinophilia and Charcot-Leyden crystals in stool specimens are present in most cases.[72] PCR can increase detection of *C. belli* and multiplex PCR methods allow for detection of other coccidian parasites at the same time.[103,149,150] However, these methods are expensive and not widely available.

Treatment

Treatment includes careful attention to fluid replacement, nutritional support, antimicrobial therapy, and, in patients with HIV, antiretroviral therapy. Immunosuppressed patients tend to experience higher degrees of

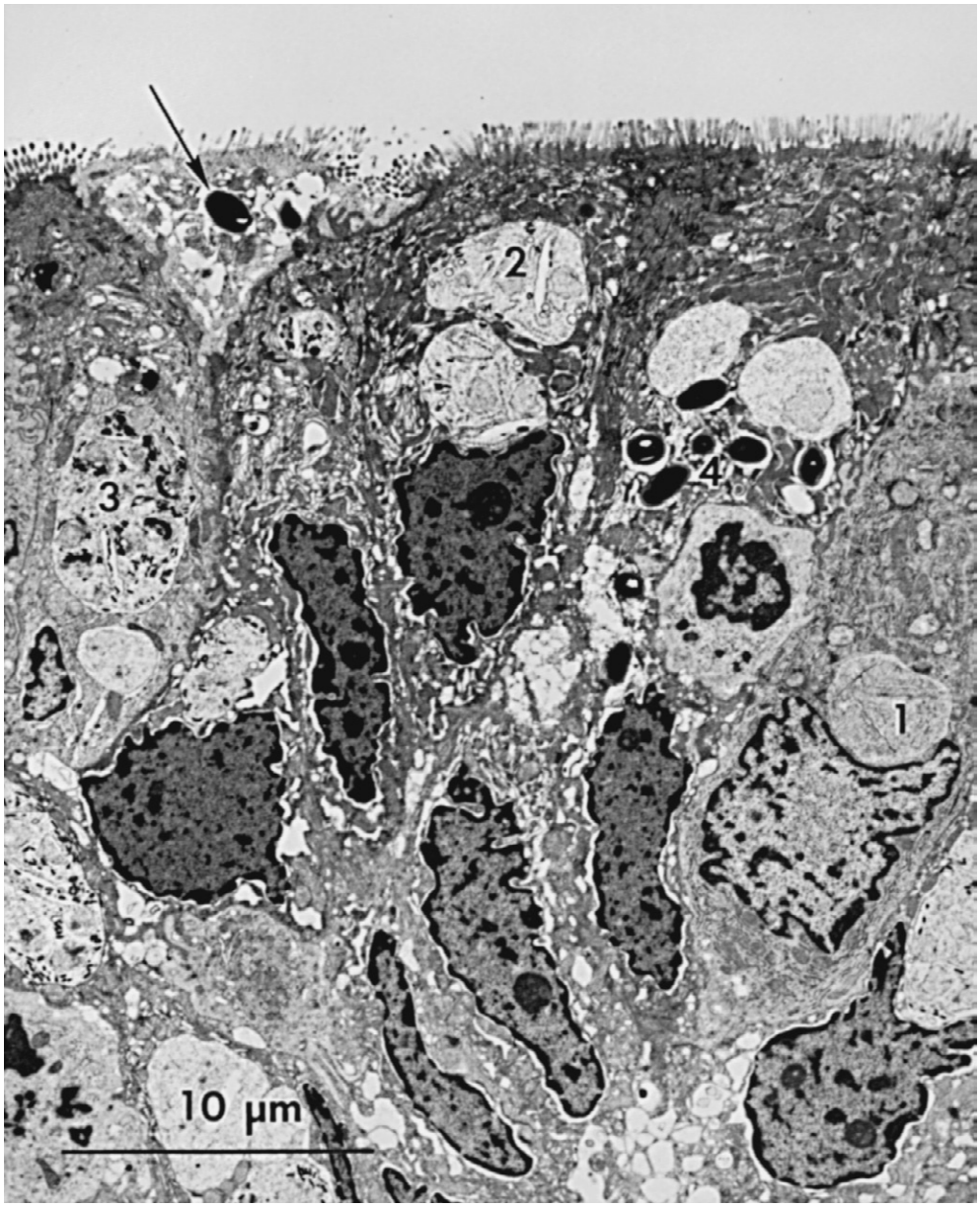

FIGURE 217-5 ■ Jejunal biopsy from a patient with AIDS and severe diarrhea. Various developmental stages of the intestinal microsporidian *Enterocytozoon* infect almost every enterocyte. Stages include proliferative plasmodia (1), early sporogonial plasmodia (2), late sporogonial plasmodia (3), and mature spores (4). A spore *(arrow)* can be seen within a necrotic enterocyte, which appears ready to slough into the lumen. (From Cali A, Owen RL. Intracellular development of *Enterocytozoon:* a unique microsporidian found in the intestine of AIDS patients. *J Protozool* 1990;**37**:145.)

dehydration than immunocompetent patients. In addition, the presence of *C. belli* and other coccidian parasites among HIV and AIDS patients has been associated with higher mortality rates.[43] Patients with severe dehydration require intravenous fluids and electrolyte support, with particular consideration to correction of hypokalemia, metabolic acidosis, and children coinfected with *C. belli* and other coccidian parasites, hypoalbuminemia, and malabsorption of vitamins and fats are commonly described. Hence, careful nutritional support is highly advised in these patients. Patients with mild-to-moderate dehydration who can tolerate the oral route should be offered oral rehydration solutions.

TMP-SMX (TMP 10 mg/kg/day and SMX 50 mg/kg/day or TMP 160-mg and SMX 800-mg tablet for adolescents) is the drug of choice. For immunocompetent hosts, one dose two times per day for 7 to 10 days is curative in most cases.[37,155] Patients with HIV or AIDS may require higher doses. The current recommendations for these patients are to start with TMP 5 mg/kg and SMX 25 mg/kg two times per day and observe the response, which usually occurs within 2 to 3 days; if no response is observed the recommendation is to increase the dose to one tablet four times per day for 7 to 10 days. Children who do not respond may have malabsorption of the drug, and, in these patients, parenteral therapy may be considered. Patients with HIV or AIDS present more frequent drug reactions to the sulfa component (SMX) than normal hosts.[71] Clinical data are very limited on treatments alternative to TMP-SMX. Ciprofloxacin may have some activity, but it is inferior to TMP-SMX.[155] Pyrimethamine with or without a long-acting sulfonamide is an alternative agent, and has worked in some cases refractory to TMP-SMX.[67,162] Nitazoxanide has in vitro activity, but clinical data are limited to case reports with both successes and failure.[19,108] Recurrences are very common among patients with HIV or AIDS; therefore, secondary prophylaxis is indicated. Current recommendation includes either TMP 5 mg/kg and SMX 25 mg/kg three times per week or pyrimethamine (25 mg) plus sulfadoxine (500 mg) once a week. Prophylaxis should be continued until the CD4 cell count is out of the AIDS range. However, failure to eradicate *C. belli* even in patients with immune reconstitution has been reported.[22]

MICROSPORIDIOSIS

Microsporidiosis comprises a diverse group of infectious diseases caused by the single-cell eukaryotic pathogens from the phylum Microsporidia, formerly termed Microspora.[42,162,166] *Microsporidia* were first identified in 1857 as pathogens of silkworms. The first human infection was described only in 1959, when the organisms were found in the spinal fluid of a Japanese child with seizures. Widespread human infection was initially recognized during the 1980s in AIDS patients. For over a century, the organisms were misclassified as protozoan parasites. However, molecular studies have clarified that the organisms were recognized as a unique phylum, closely related to the fungi. *Microsporidia* have been associated with a wide range of human infections, but most cases present with keratoconjunctivitis or diarrheal disease.

Microbiology and Lifecycle

The phylum Microsporidia includes over 170 genera with more than 1200 species.[163] Eight genera and 14 species have been identified in human infections (Table 217-1). Recent classification schemes reflecting emerging genomic sequences include them with fungi in the Opisthokonta supergroup. All are obligate intracellular pathogens. Their hosts include every animal phyla and some protozoa. Their genomes are highly compact, ranging from 2.3 to 19.5 Mb, with some of the smallest eukaryotic genomes, with few introns, high gene density, and shorter protein sequences than noted for corresponding genes in *Saccharomyces cerevisiae*.[33,75,119,123] Although the organisms were initially thought to be primitive eukaryotic

TABLE 217-1 **Microsporidia Implicated in Human Disease**

Genus	Species	Main Clinical Manifestation
Enterocytozoon	*E. bieneusi*	Chronic diarrhea
Encephalitozoon	*E. intestinalis* (syn *Septata*)	Chronic diarrhea
	E. hellem	Keratoconjunctivitis, disseminated infection
	E. curriculi	Disseminated infection, seizure
Trachipleistophora	*T. anthropophthera*	Disseminated infection
	T. hominis	Myositis
Pleistophora	*Pleistophora* spp.	Myositis
Annacaliia (syn. *Brachiola*)	*A. vesicularum*	Myositis
	A. connori	Disseminated infection
	A. algerae	Keratoconjunctivitis
Nosema	*N. ocularum*	Keratoconjunctivitis
Vittaforma	*V. corneae*	Keratoconjunctivitis
Microsporidium	*M. africanus*	Corneal ulcer
	M. ceylonensis	Corneal ulcer

organisms, recent studies have demonstrated that they are a highly evolved and divergent group of fungal organisms that are highly adapted and specialized for intracellular parasitism.

Microsporidia form characteristic unicellular spores (ranging in size from 1.0 × 3.0 μ to 1.5 × 4.0 μ) with a proteinaceous exospore, an endospore made of chitin and protein, and a plasma membrane. All spores contain a nucleus, a polaroplast, and a coiled microtubular extrusion apparatus anchored to one end of the spore. The host is infected by ingestion or inhalation of spores. Within the host, the spores undergo extrusion of the polar tube and injection of the sporoplasma into the host cell cytoplasma. Within the host cells, the pathogen undergoes cell division by either binary or multiple fission (merogony). This leads to formation of spores (sporogony), during which meront cell membranes thicken and divide to form spores. The spores are released usually by lysis of the host cell.

Epidemiology

Microsporidiosis has been identified on all continents except Antarctica.[162] Microsporidia infection appears to be common, with mainly asymptomatic or mild self-limited illnesses in normal hosts.* Prevalence is variable and can be as high as 80 percent in some populations and not detected in others. Clinical disease was initially recognized in compromised hosts, especially patients with AIDS. Microsporidia, especially *Enterocytozoon bieneusi*, are commonly detected in stools from HIV-infected children with persistent diarrhea not on effective antiretroviral therapy (up to 77% of cases, but typically about 15%).[152] Microsporidiosis is increasingly recognized in children on immunosuppression for organ transplantation.[3,51,80] However, recent studies have emphasized underdiagnosis in normal hosts, including travelers and children, especially those living in resource-limited countries.† Even minor increase in economic status can protect against disease.[107] However, a foodborne outbreak was associated with diarrhea in normal hosts in Sweden.[36,39]

Most human infections appear to be acquired by ingestion or inoculation in the eye.[39,42] Sources of ingested organisms include other people, food, and water. Person-to-person transmission was documented in an orphanage.[82] Pathogenic microsporidial spores are commonly found in surface and drinking water.[29,50] A foodborne outbreak has been described.[36] Encephalitozoon species are common in mammals and birds.[38,40,94,147] *Enterocytozoon bienuesi* has also been reported from pigs and a number of other mammalian and avian hosts.[38,39,94,134,147]

Clinical Manifestations

Microsporidiosis has been associated with a wide range of clinical manifestations. Most cases present with either diarrhea or keratoconjunctivitis. Less common manifestations include disseminated infection (including neurologic disease) and myositis. Infection of a wide range of other organs has been rarely described, including kidney, liver, central nervous system, and sinuses.

Intestinal and Biliary Tract Microsporidiosis

Human microsporidiosis was first recognized as a cause of chronic diarrhea in patients with AIDS. Most cases of intestinal microsporidiosis are caused by *E. bieneusi* and, less frequently, *Enterocytozoon intestinalis*. Other species have rarely been identified as causes of diarrhea. Cases typically present with severe immunodeficiency ($CD4^+$ cell count $<50/mm^3$).* AIDS patients typically have chronic diarrhea, often with 3 to 10 loose or watery bowel movements per day. Stools are not typically bloody or mucoid. Affected children also may have anorexia, wasting or failure to thrive, and abdominal pain. Fever is unusual. Intestinal biopsies typically reveal villus atrophy and crypt hyperplasia, and malabsorption of carbohydrates (e.g., d-xylose), zinc, and fats frequently occurs.[12,83] By contrast, only a limited degree of inflammation is noted.[133] Infection may be fatal without aggressive treatment, but may also be self-limited or asymptomatic, especially in children with higher CD4 cell counts or on combination antiretroviral therapy.[18,115] Children with malignancies or who have undergone organ transplantation also may have chronic diarrhea.[1,3,8,85] In immunocompetent children, most cases are either asymptomatic or self-limited.[106,130] However, intestinal microsporidiosis has been associated with stunting in some series.[48,102,121]

Disease may spread to the biliary tract. Biliary tract disease with acalculous cholecystitis and sclerosing cholangitis have been associated with very low CD4 counts.[48,121] Children have abdominal pain, fever, and right upper quadrant tenderness. Imaging studies may reveal dilation of the intrahepatic and common bile ducts or irregularities of the bile duct and gallbladder wall. AIDS cholangiopathy is similar to cholangiopathy associated with cytomegalovirus and cryptosporidial infection.[142]

Ocular Infection

Ocular involvement is also a common manifestation of microsporidiosis. The main manifestations are keratoconjunctivitis and stromal keratitis. Keratoconjunctivitis was initially recognized in patients with AIDS and subsequently in other compromised hosts. However, recent studies from Asia have demonstrated that it is a common and usually self-limited illness.† Most patients have a history of local trauma or exposure to contaminated water or dirt. Many cases were previously treated with topical steroids. Immunocompetent individuals usually present with a gradual onset in the week before presentation. Most complain of tearing and redness. Other frequent symptoms include the sensation of a foreign body in the eye, ocular pain, and decreased visual acuity, Most cases are unilateral. On examination, all patients have nonpurulent conjunctivitis. Most have a coarse punctate keratitis often accompanied by a superficial scar. Most cases have a

*References 39, 42, 91, 105, 130-132, 162.
†References 7, 38, 85, 87, 115, 133, 164.

*References 12, 32, 77, 125, 152, 160, 162.
†References 34, 69, 86, 126, 135, 138, 151.

full recovery without loss of visual acuity. The clinical presentation of keratoconjunctivitis in AIDS patients and other compromised hosts is more often chronic and bilateral. However, the clinical presentation is still typically with a superficial keratoconjunctivitis.[138,148,162] Most cases in AIDS patients are thought to be caused by *Encephalitozoon* spp. However, *Anncaliia*, *Nosema*, *Vittaforma*, and *Trachipleistophora* also have been identified in immunocompetent patients with keratoconjunctivitis.

Stromal keratitis often with corneal ulceration is a more severe, but less frequent form of ocular microsporidiosis.[138] Stromal keratitis has been described only in hosts thought to be immunocompetent. Cases have been attributed to *Microsporidium* spp., *Vittaforma corneae*, and *Trachipleistophora hominis*. Patients have redness, tears, eye pain, and decreased visual acuity. Cases are often confused with herpetic keratitis. Cure usually requires surgery.

Other Clinical Presentations

Disseminated infection has been described commonly with *Encephalitozoon* spp. Many people are seropositive for *Encephalitozoon* spp., and infection has been associated with seizures, hepatitis, sinusitis, and respiratory infection.[162] Spores are frequently found in urine, reflecting common involvement of the urinary tract, even in patients with diarrhea. *Pleistophora* species, *T. hominis*, and *Anncaliia* spp. have been associated with myositis. Patients present with myalgia, weakness, and elevated muscle enzymes.

Diagnosis

The mainstay of diagnosis is the demonstration of spores or intracellular organisms in specimens from appropriate tissues. Stool microscopy remains the most commonly used technique for diagnosis in patients with diarrhea. The spores are similar in size to bacteria and do not readily stain with routine parasologic stains, so demonstration of the organisms in stool requires special stains. Chromotrope 2R (modified trichrome), Uvitex 2B, calcofluor white, and the quick hot Gram-chromotrope stains have been used to visualize spores in the stool.[40,95,162] The fluorescent stains (Uvitex 2B and calcofluor white) are more sensitive but less specific than the chromotrope stains. Fluorescent antibody kits are commercially available for identification of *Encephalitozoon* and *E. bieneusi* in water and stool. Urine specimens are frequently positive in cases with *Enterocytozoon* infection.

Organisms can be identified in tissues. Spores can be identified in conjunctival scrapings by Giemsa, Gram-chromotrope, or calcofluor white staining.[34,70] Tissue Gram stains or chromotrope 2R can be used to stain the organisms in biopsy or autopsy tissues. Occasionally, organisms are identified in intestinal biopsies when missed on stool studies. Electron microscopy can provide more precise anatomic detail but is rarely required.

Molecular assays such as PCR are increasingly being used to diagnose microsporidiosis.[47,68,126,157] The assays are more sensitive than microscopic methods. Primers are available to identify organisms to the species level.

Treatment

Microsporidiosial diarrhea is usually self-limited in immunocompetent hosts. Thus, when possible, reconstitution of the immune response plays a critical role in management. Children with AIDS should be treated with effective combination antiretroviral therapy, which often will lead to resolution of illness.[73,90]

Albendazole 400 mg twice daily is effective therapy for most microsporidial species, including a small randomized trial in intestinal infection with *E. intestinalis*.[100] It has been used in intestinal and systemic infections.[163] However, albendazole does not work for *E. bieneusi*, the most common cause of intestinal microsporidiosis.[162]

Fumagillin is active in vitro against most microsporidial species. A small randomized trial demonstrated efficacy of fumagillin 20 mg three times daily in diarrhea caused by *E. bieneusi*, mainly in AIDS patients.[101] Similar response rates were noted in transplant patients.[28] However, a substantial rate of thrombocytopenia occurs during therapy. Fumagillin is licensed in France, but not in the United States.

Keratoconjunctivitis may be self-limited or respond to minor débridement. Topical fumagillin is available for ocular use in cases of severe keratoconjunctivitis.[34] Topical quinolone antibiotics also may be effective. Systemic albendazole may improve some cases and is recommended with *Encephalitozoon* infections, which may be associated with systemic infection. Most cases with stromal keratis require keratoplasty and may require corneal transplantation.[148]

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdel-Hafeez EH, Ahmad AK, Ali BA, et al. Opportunistic parasites among immunosuppressed children in Minia District, Egypt. *Korean J Parasitol* 2012;**50**:57–62.
2. Agholi M, Hatam GR, Motazedian MH. HIV/AIDS-associated opportunistic protozoal diarrhea. *AIDS Res Hum Retroviruses* 2013;**29**:35–41.
3. Agholi M, Hatam GR, Motazedian MH. Microsporidia and coccidia as causes of persistence diarrhea among liver transplant children: incidence rate and species/genotypes. *Pediatr Infect Dis J* 2013;**32**:185–7.
4. Agnamey P, Djeddi D, Oukachbi Z, et al. *Cryptosporidium hominis* and *Isospora belli* diarrhea in travelers returning from West Africa. *J Travel Med* 2010;**17**:141–2.
7. Anane S, Attouchi H. Microsporidiosis: epidemiology, clinical data and therapy. *Gastroenterol Clin Biol* 2010;**34**:450–64.
8. Antonios SN, Tolba OA, Othman AA, et al. A preliminary study on the prevalence of parasitic infections in immunocompromised children. *J Egypt Soc Parasitol* 2010;**40**:617–30.
13. Assefa S, Erko B, Medhin G, Assefa Z, et al. Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count. *BMC Infect Dis* 2009;**9**:155.
14. Bachur TP, Vale JM, Coelho IC, et al. Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. *Braz J Infect Dis* 2008;**12**:115–22.
16. Behera B, Mirdha BR, Makharia GK, et al. Parasites in patients with malabsorption syndrome: a clinical study in children and adults. *Dig Dis Sci* 2008;**53**:672–9.
21. Boyles TH, Black J, Meintjes G, et al. Failure to eradicate *Isospora belli* diarrhoea despite immune reconstitution in adults with HIV: a case series. *PLoS One* 2012;**7**:e42844.
26. Chacin-Bonilla L, Barrios F. [*Cyclospora cayetanensis:* biology, environmental distribution and transfer]. *Biomedica* 2011;**31**:132–44.
28. Champion L, Durrbach A, Lang P, et al. Fumagillin for treatment of intestinal microsporidiosis in renal transplant recipients. *Am J Transplant* 2010;**10**:1925–30.

29. Cheng HW, Lucy FE, Graczyk TK, et al. Municipal wastewater treatment plants as removal systems and environmental sources of human-virulent microsporidian spores. *Parasitol Res* 2011;**109**: 595–603.
33. Cuomo CA, Desjardins CA, Bakowski MA, et al. Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res* 2012;**22**:2478–88.
34. Das S, Sharma S, Sahu SK, et al. Diagnosis, clinical features and treatment outcome of microsporidial keratoconjunctivitis. *Br J Ophthalmol* 2012;**96**:793–5.
36. Decraene V, Lebbad M, Botero-Kleiven S, et al. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. *Epidemiol Infect* 2012;**140**:519–27.
38. Didier ES. Microsporidiosis: an emerging and opportunistic infection in humans and animals. *Acta Trop* 2005;**94**:61–76.
39. Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr Opin Infect Dis* 2011;**24**:490–5.
43. Dillingham RA, Pinkerton R, Leger P, et al. High early mortality in patients with chronic acquired immunodeficiency syndrome diarrhea initiating antiretroviral therapy in Haiti: a case-control study. *Am J Trop Med Hyg* 2009;**80**:1060–4.
49. Galvan-Diaz AL, Herrera-Jaramillo V, Santos-Rodriguez ZM, et al. [Modified Ziehl-Neelsen and modified safranin staining for diagnosing *Cyclospora cayetanensis*]. *Rev Salud Publica (Bogota)* 2008;**10**:488–93.
50. Galván A, Magnet A, Izquierdo F, et al. Molecular characterization of human pathogenic microsporidia and *Cyclospora cayetanensis* in different water sources from Spain: a year-long longitudinal study. *Appl Environ Microbiol* 2013;**79**:449–59.
51. Galván AL, Sánchez AM, Valentín MA, et al. First cases of microsporidiosis in transplant recipients in Spain and review of the literature. *J Clin Microbiol* 2011;**49**:1301–6.
52. Gibbs RA, Nanyonjo R, Pingault NM, et al. An outbreak of *Cyclospora* infection on a cruise ship. *Epidemiol Infect* 2012; **12**:1–9.
54. Hall RL, Jones JL, Hurd S, et al. Population-based active surveillance for *Cyclospora* infection; United States, Foodborne Diseases Active Surveillance Network (FoodNet), 1997-2009. *Clin Infect Dis* 2012;**54**(suppl 5):S411–417.
65. Insulander M, Svenungsson B, Lebbad M, et al. A foodborne outbreak of *Cyclospora* infection in Stockholm, Sweden. *Foodborne Pathog Dis* 2010;**7**:1585–7.
66. Iqbal J, Hira PR, Al-Ali F, et al. *Cyclospora cayetanensis:* first report of imported and autochthonous infections in Kuwait. *J Infect Dev Ctries* 2011;**5**:383–90.
73. Kaplan JE, Benson C, Holmes KH, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009;**58**:1–207; quiz CE201–4.
75. Keeling PJ, Corradi N. Shrink it or lose it: balancing loss of function with shrinking genomes in the microsporidia. *Virulence* 2011;**2**:67–70.
78. Lagrange-Xélot M, Porcher R, Sarfati C, et al. Isosporiasis in patients with HIV infection in the highly active antiretroviral therapy era in France. *HIV Med* 2008;**9**:126–30.
79. Lalonde LF, Gajadhar AA. Highly sensitive and specific PCR assay for reliable detection of *Cyclospora cayetanensis* oocysts. *Appl Environ Microbiol* 2008;**74**:4354–8.
80. Lanternier F, Boutboul D, Menotti J, et al. Microsporidiosis in solid organ transplant recipients: two *Enterocytozoon bieneusi* cases and review. *Transpl Infect Dis* 2009;**11**:83–8.
81. Lee SH, Joung M, Yoon S, et al. Multiplex PCR detection of waterborne intestinal protozoa: microsporidia, *Cyclospora*, and *Cryptosporidium*. *Korean J Parasitol* 2010;**48**:297–301.
85. Lobo ML, Xiao L, Antunes F, et al. Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and -negative patients. *Int J Parasitol* 2012;**42**: 197–205.
86. Loh RS, Chan CM, Ti SE, et al. Emerging prevalence of microsporidial keratitis in Singapore: epidemiology, clinical features, and management. *Ophthalmology* 2009;**116**:2348–53.
91. Mahanty S, Maclean JD, Cross JH. Liver, lung, and intestinal fluke infections. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases*. 3rd ed. Edinburgh: Saunders; 2011. p. 854–67.
93. Masucci L, Graffeo R, Siciliano M, et al. First Italian case of cyclosporiasis in an immunocompetent woman: local acquired infection. *New Microbiol* 2008;**31**:281–4.
98. Milord F, Lampron-Goulet E, St-Amour M, et al. *Cyclospora cayetanensis:* a description of clinical aspects of an outbreak in Quebec, Canada. *Epidemiol Infect* 2012;**140**:626–32.
102. Mor SM, Tumwine JK, Naumova EN, et al. Microsporidiosis and malnutrition in children with persistent diarrhea, Uganda. *Emerg Infect Dis* 2009;**15**:49–52.
103. Murphy SC, Hoogestraat DR, Sengupta DJ, et al. Molecular diagnosis of cystoisosporiasis using extended-range PCR screening. *J Mol Diagn* 2011;**13**:359–62.
104. Naito T, Mizue S, Misawa S, Nakamura A, et al. *Cyclospora* infection in an immunocompetent patient in Japan. *Jpn J Infect Dis* 2009;**62**:57–8.
106. Norhayati M, Azlin M, Al-Mekhlafi MH, et al. A preliminary study on the prevalence of intestinal microsporidiosis in patients with and without gastrointestinal symptoms in Malaysia. *Trans R Soc Trop Med Hyg* 2008;**102**:1274–8.
107. Nundy S, Gilman RH, Xiao L, et al. Wealth and its associations with enteric parasitic infections in a low-income community in Peru: use of principal component analysis. *Am J Trop Med Hyg* 2011;**84**:38–42.
110. Ortega YR, Pape JW. *Cyclospora*, *Isospora*, and *Sarcocystis* infections. In: Guerrant RL, Walker DH, Weller PT, editor. *Tropical infectious diseases: principles, pathogens, and practice*. 3rd ed. Edinburgh: Saunders; 2011. p. 641–5.
112. Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a foodborne and waterborne parasite. *Clin Microbiol Rev* 2010;**23**: 218–34.
115. Pagornrat W, Leelayoova S, Rangsin R, et al. Carriage rate of *Enterocytozoon bieneusi* in an orphanage in Bangkok, Thailand. *J Clin Microbiol* 2009;**47**:3739–41.
119. Peyretaillade E, El Alaoui H, Diogon M, et al. Extreme reduction and compaction of microsporidian genomes. *Res Microbiol* 2011;**162**:598–606.
123. Pombert JF, Selman M, Burki F, et al. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. *Proc Natl Acad Sci U S A* 2012;**109**:12638–43.
126. Reddy AK, Balne PK, Gaje K, et al. PCR for the diagnosis and species identification of microsporidia in patients with keratitis. *Clin Microbiol Infect* 2011;**17**:476–8.
129. Ryan ET, Cronin CG, Branda JA. Case records of the Massachusetts General Hospital: case 38-2011—a 34-year-old man with diarrhea and weakness. *N Engl J Med* 2011;**365**: 2306–16.
130. Sak B, Brady D, Pelikánová M, et al. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *J Clin Microbiol* 2011;**49**:1064–70.
131. Sak B, Kucerova Z, Kvac M, et al. Seropositivity for *Enterocytozoon bieneusi*, Czech Republic. *Emerg Infect Dis* 2010;**16**: 335–7.
132. Sak B, Kváč M, Kučerová Z, et al. Latent microsporidial infection in immunocompetent individuals: a longitudinal study. *PLoS Negl Trop Dis* 2011;**5**:e1162.
134. Santín M, Fayer R. Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Res Vet Sci* 2011;**90**:363–71.
135. Sengupta J, Saha S, Khetan A, Pal D, et al. Characteristics of microsporidial keratoconjunctivitis in an eastern Indian cohort: a case series. *Indian J Pathol Microbiol* 2011;**54**:565–8.
138. Sharma S, Das S, Joseph J, et al. Microsporidial keratitis: need for increased awareness. *Surv Ophthalmol* 2011;**56**:1–22.
141. Silva CV, Ferreira MS, Borges AS, et al. Intestinal parasitic infections in HIV/AIDS patients: experience at a teaching hospital in central Brazil. *Scand J Infect Dis* 2005;**37**:211–15.
148. Taju S, Tilahun Y, Ayalew M, et al. Diagnosis and treatment of microsporidial keratoconjunctivitis: literature review and case series. *J Ophthalmic Inflamm Infect* 2011;**1**:105–10.
149. Taniuchi M, Verweij JJ, Sethabutr O, et al. Multiplex polymerase chain reaction method to detect *Cyclospora*, *Cystoisospora*, and *Microsporidia* in stool samples. *Diagn Microbiol Infect Dis* 2011; **71**:386–90.

150. ten Hove RJ, Van Lieshout L, Beadsworth MB, et al. Characterization of genotypes of *Enterocytozoon bieneusi* in immunosuppressed and immunocompetent patient groups. *J Eukaryot Microbiol* 2009;**56**:388–93.
151. Tham AC, Sanjay S. Clinical spectrum of microsporidial keratoconjunctivitis. *Clin Experiment Ophthalmol* 2012;**40**:512–18.
159. Walther Z, Topazian MD. *Isospora* cholangiopathy: case study with histologic characterization and molecular confirmation. *Hum Pathol* 2009;**40**:1342–6.
162. Weiss LM, Schwartz DA. Microsporidiosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases*. 3rd ed. New York: Saunders; 2011. p. 714–21.
165. Williams DT, Smith RS, Mallon WK. Severe hypokalemia, paralysis, and AIDS-associated *Isospora belli* diarrhea. *J Emerg Med* 2011;**41**:e129–132.
167. Wumba R, Longo-Mbenza B, Mandina M, et al. Intestinal parasites infections in hospitalized AIDS patients in Kinshasa, Democratic Republic of Congo. *Parasite* 2010;**17**:321–8.
171. Yazar S, Mistik S, Yaman O, et al. [Three diarrheal cases caused by *Cyclospora cayetanensis* in Kayseri]. *Turkiye Parazitol Derg* 2009;**33**:85–8.
172. Zhou Y, LV B, Wang Q, et al. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. *Emerg Infect Dis* 2011;**17**:1887–90.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 218

BABESIOSIS

Peter J. Krause

The first reference to babesiosis may have been in the Bible, where a widespread murrain or plague in cattle and other domestic animals is described in Exodus, Chapter 9, Verse 3: "Behold, the hand of the Lord is upon thy cattle which is in the field, upon the horses, upon the asses, upon the camels, upon the oxen, and upon the sheep: there shall be a very grievous murrain." The word "murrain" still is used to describe red-water fever, a form of babesiosis found in cattle in parts of Ireland.[30] Babesiosis long has been recognized as an important disease in livestock, having significant economic impact in many parts of the world; the health burden of babesiosis in humans has been recognized only more recently.[30,40,70,152]

Babesiosis is a disease caused by intraerythrocytic protozoa that are transmitted by ticks from animal reservoirs. It has many clinical features similar to those of malaria. The parasite first was described in cattle in 1888 by Babes.[5] In 1893, it became the first microorganism shown to be transmitted by arthropods when Smith and Kilbourne[135] identified a tick as the vector for a species of babesiosis *(Babesia bigemina)* in Texas cattle. The first human case was described in 1957.[132] During the past 40 years, the epidemiology of the disease has changed from a few isolated cases to the establishment of endemic areas in New England, New York, and the north-central Midwest, with reports of cases from a wide geographic range in North America, Europe, Asia, Africa, Australia, and South America. Evidence indicates that the disease is more common in children and adults than is currently reported.[74,77,152]

EPIDEMIOLOGY

Worldwide, more than 100 species in the genus *Babesia* infect a wide variety of wild and domestic animals. *B. bigemina*, *Babesia bovis*, *Babesia divergens*, and *Babesia major* are found in cattle; *Babesia equi* is found in horses; *Babesia canis* is found in dogs; *Babesia felis* is found in cats; and *Babesia microti* is found in rodents.[86] Each species previously was thought to be host specific, but the host range of some species now is recognized to be quite broad.[53,85,86,139,146] Some confusion has occurred in taxonomy because the identification of different *Babesia* spp. has been based largely on morphology and the vertebrate host.[51] Most *Babesia* spp. are small (1 to 5 μm in length) and pear-shaped, round, or oval.[51,85] Six *Babesia* spp. have been found to cause disease in humans: *B. microti*, *B. divergens*, and *B. divergens*–like, *B. duncani*, *B. venatorum*, and KO1.* The complete genome of *B. microti* recently has been sequenced. It has the smallest nuclear genome of all parasites in the phylum Apicomplexa, including *Plasmodium*, *Toxoplamsma*, and *Cryptosporidia*, and has the minimal metabolic requirement for intraerythrocytic protozoan parasitism.[28] These and other data suggest that *B. microti* should be renamed, but additional phenotypic and genotypic characterization of this organism and *B. microti*–like strains and isolates are needed.

Human babesiosis is a zoonotic disease transmitted by a tick vector from an infected animal reservoir

*References 29, 46, 57, 81, 110, 131, 153.

REFERENCES

1. Abdel-Hafeez EH, Ahmad AK, Ali BA, et al. Opportunistic parasites among immunosuppressed children in Minia District, Egypt. *Korean J Parasitol* 2012;**50**:57–62.
2. Agholi M, Hatam GR, Motazedian MH. HIV/AIDS-associated opportunistic protozoal diarrhea. *AIDS Res Hum Retroviruses* 2013;**29**:35–41.
3. Agholi M, Hatam GR, Motazedian MH. Microsporidia and coccidia as causes of persistence diarrhea among liver transplant children: incidence rate and species/genotypes. *Pediatr Infect Dis J* 2013;**32**:185–7.
4. Agnamey P, Djeddi D, Oukachbi Z, et al. *Cryptosporidium hominis* and *Isospora belli* diarrhea in travelers returning from West Africa. *J Travel Med* 2010;**17**:141–2.
5. Alakpa G, Fagbenro-Beyioku AF, Clarke SC. *Cyclospora cayetanensis* in stools submitted to hospitals in Lagos, Nigeria. *Int J Infect Dis* 2002;**6**:314–18.
6. Alakpa GE, Clarke SC, Fagbenro-Beyioku AF. *Cyclospora cayetanensis* infection in Lagos, Nigeria. *Clin Microbiol Infect* 2003;**9**: 731–3.
7. Anane S, Attouchi H. Microsporidiosis: epidemiology, clinical data and therapy. *Gastroenterol Clin Biol* 2010;**34**:450–64.
8. Antonios SN, Tolba OA, Othman AA, Saad MA. A preliminary study on the prevalence of parasitic infections in immunocompromised children. *J Egypt Soc Parasitol* 2010;**40**:617–30.
9. Arenas-Pinto A, Certad G, Ferrara G, et al. Association between parasitic intestinal infections and acute or chronic diarrhoea in HIV-infected patients in Caracas, Venezuela. *Int J STD AIDS* 2003;**14**:487–92.
10. Ashford RW. Occurrence of an undescribed coccidian in man in Papua New Guinea. *Ann Trop Med Parasitol* 1979;**73**:497–500.
11. Reference deleted in proofs.
12. Asmuth DM, DeGirolami PC, Federman M, et al. Clinical features of microsporidiosis in patients with AIDS. *Clin Infect Dis* 1994;**18**:819–25.
13. Assefa S, Erko B, Medhin G, et al. Intestinal parasitic infections in relation to HIV/AIDS status, diarrhea and CD4 T-cell count. *BMC Infect Dis* 2009;**9**:155.
14. Bachur TP, Vale JM, Coelho IC, et al. Enteric parasitic infections in HIV/AIDS patients before and after the highly active antiretroviral therapy. *Braz J Infect Dis* 2008;**12**:115–22.
15. Barta JR, Schrenzel MD, Carreno R, et al. The genus *Atoxoplasma* (Garnham 1950) as a junior objective synonym of the genus *Isospora* (Schneider 1881) species infecting birds and resurrection of *Cystoisospora* (Frenkel 1977) as the correct genus for *Isospora* species infecting mammals. *J Parasitol* 2005;**91**:726–7.
16. Behera B, Mirdha BR, Makharia GK, et al. Parasites in patients with malabsorption syndrome: a clinical study in children and adults. *Dig Dis Sci* 2008;**53**:672–9.
17. Bendall RP, Lucas S, Moody A, et al. Diarrhoea associated with cyanobacterium-like bodies: a new coccidian enteritis of man. *Lancet* 1993;**341**:590–2.
18. Bern C, Kawai V, Vargas D, et al. The epidemiology of intestinal microsporidiosis in patients with HIV/AIDS in Lima, Peru. *J Infect Dis* 2005;**191**:1658–64.
19. Bialek R, Overkamp D, Rettig I, et al. Case report: nitazoxanide treatment failure in chronic isosporiasis. *Am J Trop Med Hyg* 2001;**65**:94–5.
20. Blans MC, Ridwan BU, Verweij JJ, et al. Cyclosporiasis outbreak, Indonesia. *Emerg Infect Dis* 2005;**11**:1453–5.
21. Boyles TH, Black J, Meintjes G, et al. Failure to eradicate *Isospora belli* diarrhoea despite immune reconstitution in adults with HIV: a case series. *PLoS One* 2012;**7**:e42844.
22. Brandborg LL, Goldberg SB, Breidenbach WC. Human coccidiosis: a possible cause of malabsorption. *N Engl J Med* 1970;**283**: 1306–13.
23. Carcamo C, Hooton T, Wener MH, et al. Etiologies and manifestations of persistent diarrhea in adults with HIV-1 infection: a case-control study in Lima, Peru. *J Infect Dis* 2005;**191**:11–19.
24. Centers for Disease Control and Prevention. Outbreak of diarrheal illness associated with cyanobacteria (blue-green algae)-like bodies: Chicago and Nepal, 1989 and 1990. *MMWR Morb Mortal Wkly Rep* 1991;**40**:325–7.
25. Certad G, Arenas-Pinto A, Pocaterra L, et al. Isosporiasis in Venezuelan adults infected with human immunodeficiency virus: clinical characterization. *Am J Trop Med Hyg* 2003;**69**:217–22.
26. Chacin-Bonilla L, Barrios F. [*Cyclospora cayetanensis:* biology, environmental distribution and transfer]. *Biomedica* 2011;**31**:132–44.
27. Chacin-Bonilla L, Mejia de Young M, Estevez J. Prevalence and pathogenic role of *Cyclospora cayetanensis* in a Venezuelan community. *Am J Trop Med Hyg* 2003;**68**:304–6.
28. Champion L, Durrbach A, Lang P, et al. Fumagillin for treatment of intestinal microsporidiosis in renal transplant recipients. *Am J Transplant* 2010;**10**:1925–30.
29. Cheng HW, Lucy FE, Graczyk TK, et al. Municipal wastewater treatment plants as removal systems and environmental sources of human-virulent microsporidian spores. *Parasitol Res* 2011;**109**: 595–603.
30. Chiodini PL. A 'new' parasite: human infection with *Cyclospora cayetanensis*. *Trans R Soc Trop Med Hyg* 1994;**88**:369–71.
31. Comin CE, Santucci M. Submicroscopic profile of *Isospora belli* enteritis in a patient with acquired immune deficiency syndrome. *Ultrastruct Pathol* 1994;**18**:473–82.
32. Coyle CM, Wittner M, Kotler DP, Noyer C, et al. Prevalence of microsporidiosis due to *Enterocytozoon bieneusi* and *Encephalitozoon (Septata) intestinalis* among patients with AIDS-related diarrhea: determination by polymerase chain reaction to the microsporidian small-subunit rRNA gene. *Clin Infect Dis* 1996; **23**:1002–6.
33. Cuomo CA, Desjardins CA, Bakowski MA, et al. Microsporidian genome analysis reveals evolutionary strategies for obligate intracellular growth. *Genome Res* 2012:222478–88.
34. Das S, Sharma S, Sahu SK, Nayak SS, et al. Diagnosis, clinical features and treatment outcome of microsporidial keratoconjunctivitis. *Br J Ophthalmol* 2012;**96**:793–5.
35. de Oliveira-Silva MB, de Oliveira LR, Resende JC, et al. Seasonal profile and level of $CD4^+$ lymphocytes in the occurrence of cryptosporidiosis and cystoisosporidiosis in HIV/AIDS patients in the Triangulo Mineiro region, Brazil. *Rev Soc Bras Med Trop* 2007;**40**:512–15.
36. Decraene V, Lebbad M, Botero-Kleiven S, et al. First reported foodborne outbreak associated with microsporidia, Sweden, October 2009. *Epidemiol Infect* 2012;**140**:519–27.
37. DeHovitz JA, Pape JW, Boncy M, et al. Clinical manifestations and therapy of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1986;**315**:87–90.
38. Didier ES. Microsporidiosis: an emerging and opportunistic infection in humans and animals. *Acta Trop* 2005;**94**:61–76.
39. Didier ES, Weiss LM. Microsporidiosis: not just in AIDS patients. *Curr Opin Infect Dis* 2011;**24**:490–5.
40. Didier ES, Didier PJ, Snowden KF, et al. Microsporidiosis in mammals. *Microbes Infect* 2000;**2**:709–20.
41. Reference deleted in proofs.
42. Didier ES, Stovall ME, Green LC, et al. Epidemiology of microsporidiosis: sources and modes of transmission. *Vet Parasitol* 2004; **126**:145–66.
43. Dillingham RA, Pinkerton R, Leger P, et al. High early mortality in patients with chronic acquired immunodeficiency syndrome diarrhea initiating antiretroviral therapy in Haiti: a case-control study. *Am J Trop Med Hyg* 2009;**80**:1060–4.
44. Dixon BR, Bussey JM, Parrington LJ, et al. Detection of *Cyclospora cayetanensis* oocysts in human fecal specimens by flow cytometry. *J Clin Microbiol* 2005;**43**:2375–9.
45. el-Karamany EM, Zaher TI, el-Bahnasawy MM. Role of water in the transmission of cyclosporiarsis in Sharkia Governorate, Egypt. *J Egypt Soc Parasitol* 2005;**35**:953–62.
46. Field AS. Light microscopic and electron microscopic diagnosis of gastrointestinal opportunistic infections in HIV-positive patients. *Pathology* 2002;**34**:21–35.
47. Franzen C, Müller A. Molecular techniques for detection, species differentiation, and phylogenetic analysis of microsporidia. *Clin Microbiol Rev* 1999;**12**:243–85.
48. French AL, Beaudet LM, Benator DA, et al. Cholecystectomy in patients with AIDS: clinicopathologic correlations in 107 cases. *Clin Infect Dis* 1995;**21**:852–8.
49. Galván-Diaz AL, Herrera-Jaramillo V, Santos-Rodriguez ZM, et al. [Modified Ziehl-Neelsen and modified safranin staining for diagnosing *Cyclospora cayetanensis*]. *Rev Salud Publica (Bogota)* 2008; **10**:488–93.

50. Galván A, Magnet A, Izquierdo F, et al. Molecular characterization of human pathogenic microsporidia and *Cyclospora cayetanensis* in different water sources from Spain: a year-long longitudinal study. *Appl Environ Microbiol* 2013;**79**:449–59.
51. Galván AL, Sánchez AM, Valentín MA, et al. First cases of microsporidiosis in transplant recipients in Spain and review of the literature. *J Clin Microbiol* 2011;**49**:1301–6.
52. Gibbs RA, Nanyonjo R, Pingault NM, et al. An outbreak of Cyclospora infection on a cruise ship. *Epidemiol Infect* 2012; **12**:1–9.
53. Guiguet M, Furco A, Tattevin P, et al; French Hospital Database on HIV Clinical Eidemiology Group. HIV-associated *Isospora belli* infection: incidence and risk factors in the French Hospital Database on HIV. *HIV Med* 2007;**8**:124–30.
54. Hall RL, Jones JL, Hurd S, et al. Population-based active surveillance for *Cyclospora* infection: united States, Foodborne Diseases Active Surveillance Network (FoodNet), 1997-2009. *Clin Infect Dis* 2012;**54**(suppl 5):S411–17.
55. Herwaldt BL. *Cyclospora cayetanensis:* a review, focusing on the outbreaks of cyclosporiasis in the 1990s. *Clin Infect Dis* 2000; **31**:1040–57.
56. Herwaldt BL, Ackers ML. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. The Cyclospora Working Group. *N Engl J Med* 1997;**336**:1548–56.
57. Herwaldt BL, Beach MJ. The return of *Cyclospora* in 1997: another outbreak of cyclosporiasis in North America associated with imported raspberries. Cyclospora Working Group. *Ann Intern Med* 1999;**130**:210–20.
58. Heyworth MF. Parasitic diseases in immunocompromised hosts: cryptosporidiosis, isosporiasis, and strongyloidiasis. *Gastroenterol Clin North Am* 1996;**25**:691–707.
59. Hoge CW, Echeverria P, Rajah R, et al. Prevalence of *Cyclospora* species and other enteric pathogens among children less than 5 years of age in Nepal. *J Clin Microbiol* 1995;**33**:3058–60.
60. Hoge CW, Shlim DR, Ghimire M, et al. Placebo-controlled trial of co-trimoxazole for *Cyclospora* infections among travellers and foreign residents in Nepal. *Lancet* 1995;**345**:691–3.
61. Hoge CW, Shlim DR, Rajah R, et al. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. *Lancet* 1993;**341**:1175–9.
62. Huang P, Weber JT, Sosin DM, et al. The first reported outbreak of diarrheal illness associated with *Cyclospora* in the United States. *Ann Intern Med* 1995;**123**:409–14.
63. Hussein EM. Molecular identification of *Cycospora* spp. using multiplex PCR from diarrheic children compared to others conventional methods. *J Egypt Soc Parasitol* 2007;**37**:585–98.
64. Hussein EM, Abdul-Manaem AH, el-Attary SL. *Cyclospora cayetanensis* oocysts in sputum of a patient with active pulmonary tuberculosis: case report in Ismailia, Egypt. *J Egypt Soc Parasitol* 2005; **35**:787–93.
65. Insulander M, Svenungsson B, Lebbad M, et al. A foodborne outbreak of *Cyclospora* infection in Stockholm, Sweden. *Foodborne Pathog Dis* 2010;**7**:1585–7.
66. Iqbal J, Hira PR, Al-Ali F, Khalid N. *Cyclospora cayetanensis:* first report of imported and autochthonous infections in Kuwait. *J Infect Dev Ctries* 2011;**5**:383–90.
67. Jongwutiwes S, Sampatanukul P, Putaporntip C. Recurrent isosporiasis over a decade in an immunocompetent host successfully treated with pyrimethamine. *Scand J Infect Dis* 2002;**34**: 859–62.
68. Joseph J, Sharma S, Murthy SI, et al. Microsporidial keratitis in India: 16S rRNA gene-based PCR assay for diagnosis and species identification of microsporidia in clinical samples. *Invest Ophthalmol Vis Sci* 2006;**47**:4468–73.
69. Joseph J, Sridhar MS, Murthy S, et al. Clinical and microbiological profile of microsporidial keratoconjunctivitis in southern India. *Ophthalmology* 2006;**113**:531–7.
70. Joseph J, Vemuganti GK, Garg P, et al. Histopathological evaluation of ocular microsporidiosis by different stains. *BMC Clin Pathol* 2006;**6**:6.
71. Jung AC, Paauw DS. Management of adverse reactions to trimethoprim-sulfamethoxazole in human immunodeficiency virus-infected patients. *Arch Intern Med* 1994;**154**:2402–6.
72. Junod C. [*Isospora belli* coccidiosis in immunocompetent subjects (a study of 40 cases seen in Paris)]. *Bull Soc Pathol Exot Filiales* 1988;**81**:317–25.
73. Kaplan JE, Benson C, Holmes KH, et al. Guidelines for prevention and treatment of opportunistic infections in HIV-infected adults and adolescents: recommendations from CDC, the National Institutes of Health, and the HIV Medicine Association of the Infectious Diseases Society of America. *MMWR Recomm Rep* 2009;**58**:1–207; quiz CE201–4.
74. Kassi RR, Kouassi RA, Yavo W, et al. [Cryptosporidiosis and isosporiasis in children suffering from diarrhoea in Abidjan]. *Bull Soc Pathol Exot* 2004;**97**:280–2.
75. Keeling PJ, Corradi N. Shrink it or lose it: balancing loss of function with shrinking genomes in the microsporidia. *Virulence* 2011;**2**:67–70.
76. Kimura K, Kumar Rai S, Takemasa K, et al. Comparison of three microscopic techniques for diagnosis of *Cyclospora cayetanensis*. *FEMS Microbiol Lett* 2004;**238**:263–6.
77. Kotler DP, Orenstein JM. Clinical syndromes associated with microsporidiosis. *Adv Parasitol* 1998;**40**:321–49.
78. Lagrange-Xélot M, Porcher R, Sarfati C, et al. Isosporiasis in patients with HIV infection in the highly active antiretroviral therapy era in France. *HIV Med* 2008;**9**:126–30.
79. Lalonde LF, Gajadhar AA. Highly sensitive and specific PCR assay for reliable detection of *Cyclospora cayetanensis* oocysts. *Appl Environ Microbiol* 2008;**74**:4354–8.
80. Lanternier F, Boutboul D, Menotti J, et al. Microsporidiosis in solid organ transplant recipients: two *Enterocytozoon bieneusi* cases and review. *Transpl Infect Dis* 2009;**11**:83–8.
81. Lee SH, Joung M, Yoon S, et al. Multiplex PCR detection of waterborne intestinal protozoa: microsporidia, *Cyclospora*, and *Cryptosporidium*. *Korean J Parasitol* 2010;**48**:297–301.
82. Leelayoova S, Subrungruang I, Rangsin R, et al. Transmission of *Enterocytozoon bieneusi* genotype a in a Thai orphanage. *Am J Trop Med Hyg* 2005;**73**:104–7.
83. Lima AA, Silva TM, Gifoni AM, et al. Mucosal injury and disruption of intestinal barrier function in HIV-infected individuals with and without diarrhea and cryptosporidiosis in northeast Brazil. *Am J Gastroenterol* 1997;**92**:1861–6.
84. Lindsay DS, Dubey JP, Blagburn BL. Biology of *Isospora* spp. from humans, nonhuman primates, and domestic animals. *Clin Microbiol Rev* 1997;**10**:19–34.
85. Lobo ML, Xiao L, Antunes F, Matos O. Microsporidia as emerging pathogens and the implication for public health: a 10-year study on HIV-positive and -negative patients. *Int J Parasitol* 2012;**42**:197–205.
86. Loh RS, Chan CM, Ti SE, et al. Emerging prevalence of microsporidial keratitis in Singapore: epidemiology, clinical features, and management. *Ophthalmology* 2009;**116**:2348–53.
87. López-Vélez R, Turrientes MC, Garrón C, et al. Microsporidiosis in travelers with diarrhea from the tropics. *J Travel Med* 1999;**6**:223–7.
88. Madico G, Gilman RH, Miranda E, et al. Treatment of *Cyclospora* infections with co-trimoxazole. *Lancet* 1993;**342**:122–3.
89. Madico G, McDonald J, Gilman RH, et al. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. *Clin Infect Dis* 1997;**24**:977–81.
90. Maggi P, Larocca AM, Quarto M, et al. Effect of antiretroviral therapy on cryptosporidiosis and microsporidiosis in patients infected with human immunodeficiency virus type 1. *Eur J Clin Microbiol Infect Dis* 2000;**19**:213–17.
91. Mahanty S, Maclean JD, Cross JH. Liver, lung, and intestinal fluke infections. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases*. 3rd ed. Edinburgh: Saunders; 2011. p. 854–67.
92. Mansfield LS, Gajadhar AA. *Cyclospora cayetanensis:* a food- and waterborne coccidian parasite. *Vet Parasitol* 2004;**126**:73–90.
93. Masucci L, Graffeo R, Siciliano M, et al. First Italian case of cyclosporiasis in an immunocompetent woman: local acquired infection. *New Microbiol* 2008;**31**:281–4.
94. Mathis A, Weber R, Deplazes P. Zoonotic potential of the microsporidia. *Clin Microbiol Rev* 2005;**18**:423–45.
95. Matos O, Lobo ML, Gonçalves L, et al. Diagnostic use of 3 techniques for identification of microsporidian spores among AIDS patients in Portugal. *Scand J Infect Dis* 2002;**34**:591–3.
96. Michiels JF, Hofman P, Bernard E, et al. Intestinal and extraintestinal Isospora belli infection in an AIDS patient: a second case report. *Pathol Res Pract* 1994;**190**:1089–1093; discussion 1094.

97. Miegeville M, Koubi V, Dan LC, et al. [*Cyclospora cayetanensis* presence in aquatic surroundings in Hanoi (Vietnam): environmental study (well water, lakes and rivers)]. *Bull Soc Pathol Exot* 2003;**96**:149–52.
98. Milord F, Lampron-Goulet E, St-Amour M, et al. *Cyclospora cayetanensis:* a description of clinical aspects of an outbreak in Quebec, Canada. *Epidemiol Infect* 2012;**140**:626–32.
99. Mirdha BR, Kabra SK, Samantray JC. Isosporiasis in children. *Indian Pediatr* 2002;**39**:941–4.
100. Molina JM, Chastang C, Goguel J, et al. Albendazole for treatment and prophylaxis of microsporidiosis due to *Encephalitozoon intestinalis* in patients with AIDS: a randomized double-blind controlled trial. *J Infect Dis* 1998;**177**:1373–7.
101. Molina JM, Tourneur M, Sarfati C, et al; and Agence Nationale de Recherches sur le SIDA 090 Study Group. Fumagillin treatment of intestinal microsporidiosis. *N Engl J Med* 2002;**346**:1963–9.
102. Mor SM, Tumwine JK, Naumova EN, et al. Microsporidiosis and malnutrition in children with persistent diarrhea, Uganda. *Emerg Infect Dis* 2009;**15**:49–52.
103. Murphy SC, Hoogestraat DR, Sengupta DJ, et al. Molecular diagnosis of cystoisosporiasis using extended-range PCR screening. *J Mol Diagn* 2011;**13**:359–62.
104. Naito T, Mizue S, Misawa S, et al. *Cyclospora* infection in an immunocompetent patient in Japan. *Jpn J Infect Dis* 2009;**62**: 57–8.
105. Nkinin SW, Asonganyi T, Didier ES, et al. Microsporidian infection is prevalent in healthy people in Cameroon. *J Clin Microbiol* 2007;**45**:2841–6.
106. Norhayati M, Azlin M, Al-Mekhlafi MH, et al. A preliminary study on the prevalence of intestinal microsporidiosis in patients with and without gastrointestinal symptoms in Malaysia. *Trans R Soc Trop Med Hyg* 2008;**102**:1274–8.
107. Nundy S, Gilman RH, Xiao L, et al. Wealth and its associations with enteric parasitic infections in a low-income community in Peru: use of principal component analysis. *Am J Trop Med Hyg* 2011;**84**:38–42.
108. Ochoa TJ, White AC. Nitazoxanide for treatment of intestinal parasites in children. *Pediatr Infect Dis J* 2005;**24**:641–2.
109. Ortega YR, Gilman RH, Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. *J Parasitol* 1993;**80**: 625–9.
110. Ortega YR, Pape JW. *Cyclospora*, *Isospora*, and *Sarcocystis* infections. In: Guerrant RL, Walker DH, Weller PT, et al, editors. *Tropical infectious diseases: principles*, *pathogens*, *and practice*. 3rd ed. Edinburgh: Saunders; 2011. p. 641–5.
111. Ortega YR, Roxas CR, Gilman RH, et al. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected from markets of an endemic region in Peru. *Am J Trop Med Hyg* 1997;**57**:633–6.
112. Ortega YR, Sanchez R. Update on *Cyclospora cayetanensis*, a foodborne and waterborne parasite. *Clin Microbiol Rev* 2010;**23**: 218–34.
113. Ortega YR, Sterling CR, Gilman RH, et al. *Cyclospora* species: a new protozoan pathogen of humans. *N Engl J Med* 1993;**328**: 1308–12.
114. Osterholm MT. Lessons learned again: cyclosporiasis and raspberries. *Ann Intern Med* 1999;**130**:233–4.
115. Pagornrat W, Leelayoova S, Rangsin R, et al. Carriage rate of *Enterocytozoon bieneusi* in an orphanage in Bangkok, Thailand. *J Clin Microbiol* 2009;**47**:3739–41.
116. Pape JW, Verdier RI, Johnson WD Jr. Treatment and prophylaxis of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome. *N Engl J Med* 1989;**320**:1044–7.
117. Pape JW, Verdier RI, Boncy M, et al. *Cyclospora* infection in adults infected with HIV: clinical manifestations, treatment, and prophylaxis. *Ann Intern Med* 1994;**121**:654–7.
118. Parija SC, Shivaprakash MR, Jayakeerthi SR. Evaluation of lactophenol cotton blue (LPCB) for detection of *Cryptosporidium*, *Cyclospora* and *Isospora* in the wet mount preparation of stool. *Acta Trop* 2003;**85**:349–54.
119. Peyretaillade E, El Alaoui H, Diogon M, et al. Extreme reduction and compaction of microsporidian genomes. *Res Microbiol* 2011; **162**:598–606.
120. Pieniazek NJ, Herwaldt BL. Reevaluating the molecular taxonomy: is human-associated *Cyclospora* a mammalian *Eimeria* species? *Emerg Infect Dis* 1997;**3**:381–3.
121. Pol S, Romana CA, Richard S, et al. Microsporidia infection in patients with the human immunodeficiency virus and unexplained cholangitis. *N Engl J Med* 1993;**328**:95–9.
122. Pollok RC, Bendall RP, Moody A, et al. Traveller's diarrhoea associated with cyanobacterium-like bodies. *Lancet* 1992;**340**: 556–7.
123. Pombert JF, Selman M, Burki F, et al. Gain and loss of multiple functionally related, horizontally transferred genes in the reduced genomes of two microsporidian parasites. *Proc Natl Acad Sci U S A* 2012;**109**:12638–43.
124. Pritchett R, Gossman C, Radke V, et al. Outbreak of cyclosporiasis: Northern Virginia–Washington, D.C., Baltimore, Maryland, Metropolitan Area, 1997. *MMWR Morb Mortal Wkly Rep* 1997; **46**:689–91.
125. Rabeneck L, Genta RM, Gyorkey F, et al. Observations on the pathological spectrum and clinical course of microsporidiosis in men infected with the human immunodeficiency virus: follow-up study. *Clin Infect Dis* 1995;**20**:1229–35.
126. Reddy AK, Balne PK, Gaje K, et al. PCR for the diagnosis and species identification of microsporidia in patients with keratitis. *Clin Microbiol Infect* 2011;**17**:476–8.
127. Resiere D, Vantelon JM, Bouree P, et al. *Isospora belli* infection in a patient with non-Hodgkin's lymphoma. *Clin Microbiol Infect* 2003;**9**:1065–7.
128. Rotterdam H, Tsang P. Gastrointestinal disease in the immunocompromised patient. *Hum Pathol* 1994;**25**:1123–40.
129. Ryan ET, Cronin CG, Branda JA. Case records of the Massachusetts General Hospital: case 38-2011—a 34-year-old man with diarrhea and weakness. *N Engl J Med* 2011;**365**:2306–16.
130. Sak B, Brady D, Pelikánová M, Květoňová D, et al. Unapparent microsporidial infection among immunocompetent humans in the Czech Republic. *J Clin Microbiol* 2011;**49**:1064–70.
131. Sak B, Kucerova Z, Kvac M, et al. Seropositivity for *Enterocytozoon bieneusi*, Czech Republic. *Emerg Infect Dis* 2010;**16**:335–7.
132. Sak B, Kváč M, Kučerová Z, et al. Latent microsporidial infection in immunocompetent individuals: a longitudinal study. *PLoS Negl Trop Dis* 2011;**5**:e1162.
133. Samie A, Obi CL, Tzipori S, et al. Microsporidiosis in South Africa: PCR detection in stool samples of HIV-positive and HIV-negative individuals and school children in Vhembe district, Limpopo Province. *Trans R Soc Trop Med Hyg* 2007;**101**:547–54.
134. Santín M, Fayer R. Microsporidiosis: *Enterocytozoon bieneusi* in domesticated and wild animals. *Res Vet Sci* 2011;**90**:363–71.
135. Sengupta J, Saha S, Khetan A, et al. Characteristics of microsporidial keratoconjunctivitis in an eastern Indian cohort: a case series. *Indian J Pathol Microbiol* 2011;**54**:565–8.
136. Shadduck JA, Meccoli RA, Davis R, et al. Isolation of a microsporidian from a human patient. *J Infect Dis* 1990;**162**:773–6.
137. Shaffer N, Moore L. Chronic travelers' diarrhea in a normal host due to *Isospora belli*. *J Infect Dis* 1989;**159**:596–7.
138. Sharma S, Das S, Joseph J, et al. Microsporidial keratitis: need for increased awareness. *Surv Ophthalmol* 2011;**56**:1–22.
139. Shlim DR, Cohen MT, Eaton M, et al. An alga-like organism associated with an outbreak of prolonged diarrhea among foreigners in Nepal. *Am J Trop Med Hyg* 1991;**45**:383–9.
140. Sifuentes-Osornio J, Porras-Cortés G, Bendall RP, et al. *Cyclospora cayetanensis* infection in patients with and without AIDS: biliary disease as another clinical manifestation. *Clin Infect Dis* 1995;**21**: 1092–7.
141. Silva CV, Ferreira MS, Borges AS, et al. Intestinal parasitic infections in HIV/AIDS patients: experience at a teaching hospital in central Brazil. *Scand J Infect Dis* 2005;**37**:211–15.
142. Soave R. *Cyclospora:* an overview. *Clin Infect Dis* 1996;**23**:429–35; quiz 436–427.
143. Soave R, Johnson WD Jr. *Cyclospora:* conquest of an emerging pathogen. *Lancet* 1995;**345**:667–8.
144. Soave R, Dubey JP, Ramos LL, et al. A new intestinal pathogen? (Abstract.) *Clin Res* 1986;**34**:533.
145. Sorvillo FJ, Lieb LE, Seidel J, et al. Epidemiology of isosporiasis among persons with acquired immunodeficiency syndrome in Los Angeles County. *Am J Trop Med Hyg* 1995;**53**:656–9.
146. Sterling CR, Ortega YR. *Cyclospora:* an enigma worth unraveling. *Emerg Infect Dis* 1999;**5**:48–53.
147. Sulaiman IM, Bern C, Gilman R, et al. A molecular biologic study of *Enterocytozoon bieneusi* in HIV-infected patients in Lima, Peru. *J Eukaryot Microbiol* 2003;**50**(Suppl):591–6.

148. Taju S, Tilahun Y, Ayalew M, et al. Diagnosis and treatment of microsporidial keratoconjunctivitis: literature review and case series. *J Ophthalmic Inflamm Infect* 2011;**1**:105–10.
149. Taniuchi M, Verweij JJ, Sethabutr O, et al. Multiplex polymerase chain reaction method to detect *Cyclospora*, *Cystoisospora*, and microsporidia in stool samples. *Diagn Microbiol Infect Dis* 2011; **71**:386–90.
150. ten Hove RJ, Van Lieshout L, Beadsworth MB, et al. Characterization of genotypes of *Enterocytozoon bieneusi* in immunosuppressed and immunocompetent patient groups. *J Eukaryot Microbiol* 2009;**56**:388–93.
151. Tham AC, Sanjay S. Clinical spectrum of microsporidial keratoconjunctivitis. *Clin Experiment Ophthalmol* 2012;**40**:512–18.
152. Tumwine JK, Kekitiinwa A, Bakeera-Kitaka S, et al. Cryptosporidiosis and microsporidiosis in Ugandan children with persistent diarrhea with and without concurrent infection with the human immunodeficiency virus. *Am J Trop Med Hyg* 2005;**73**: 921–5.
153. Valentiner-Branth P, Steinsland H, Fischer TK, et al. Cohort study of Guinean children: incidence, pathogenicity, conferred protection, and attributable risk for enteropathogens during the first 2 years of life. *J Clin Microbiol* 2003;**41**:4238–45.
154. Varma M, Hester JD, Schaefer FW 3rd, et al. Detection of *Cyclospora cayetanensis* using a quantitative real-time PCR assay. *J Microbiol Methods* 2003;**53**:27–36.
155. Verdier RI, Fitzgerald DW, Johnson WD, et al. Trimethoprim-sulfamethoxazole compared with ciprofloxacin for treatment and prophylaxis of *Isospora belli* and *Cyclospora cayetanensis* infection in HIV-infected patients: a randomized, controlled trial. *Ann Intern Med* 2000;**132**:885–8.
156. Verweij JJ, Laeijendecker D, Brienen EA, et al. Detection of *Cyclospora cayetanensis* in travellers returning from the tropics and subtropics using microscopy and real-time PCR. *Int J Med Microbiol* 2003;**293**:199–202.
157. Verweij JJ, ten Hove R, Brienen EA, et al. Multiplex detection of *Enterocytozoon bieneusi* and *Encephalitozoon* spp. in fecal samples using real-time PCR. *Diagn Microbiol Infect Dis* 2007;**57**: 163–7.
158. Vignesh R, Balakrishnan P, Shankar EM, et al. High proportion of isosporiasis among HIV-infected patients with diarrhea in southern India. *Am J Trop Med Hyg* 2007;**77**:823–4.
159. Walther Z, Topazian MD. Isospora cholangiopathy: case study with histologic characterization and molecular confirmation. *Hum Pathol* 2009;**40**:1342–6.
160. Wanachiwanawin D, Chokephaibulkit K, Lertlaituan P, et al. Intestinal microsporidiosis in HIV-infected children with diarrhea. *Southeast Asian J Trop Med Public Health* 2002;**33**:241–5.
161. Waywa D, Kongkriengdaj S, Chaidatch S, et al. Protozoan enteric infection in AIDS related diarrhea in Thailand. *Southeast Asian J Trop Med Public Health* 2001;**32**(suppl 2):151–5.
162. Weiss LM, Schwartz DA. Microsporidiosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases*. 3rd ed. New York: Saunders; 2011. p. 714–21.
163. Weiss LM, Perlman DC, Sherman J, et al. *Isospora belli* infection: treatment with pyrimethamine. *Ann Intern Med* 1988;**109**:474–5.
164. Wichro E, Hoelzl D, Krause R, et al. Microsporidiosis in travel-associated chronic diarrhea in immune-competent patients. *Am J Trop Med Hyg* 2005;**73**:285–7.
165. Williams DT, Smith RS, Mallon WK. Severe hypokalemia, paralysis, and AIDS-associated *Isospora belli* diarrhea. *J Emerg Med* 2011;**41**:e129–32.
166. Wittner M, Weiss LM. *The microsporidia and microsporidiosis*. Washington, D.C.: ASM Press; 1999.
167. Wumba R, Longo-Mbenza B, Mandina M, et al. Intestinal parasites infections in hospitalized AIDS patients in Kinshasa, Democratic Republic of Congo. *Parasite* 2010;**17**:321–8.
168. Wurtz R. *Cyclospora:* a newly identified intestinal pathogen of humans. *Clin Infect Dis* 1994;**18**:620–3.
169. Wurtz RM, Kocka FE, Peters CS, et al. Clinical characteristics of seven cases of diarrhea associated with a novel acid-fast organism in the stool. *Clin Infect Dis* 1993;**16**:136–8.
170. Yazar S, Yalcln S, Sahin I. Human cyclosporiosis in Turkey. *World J Gastroenterol* 2004;**10**:1844–7.
171. Yazar S, Mistik S, Yaman O, et al. [Three diarrheal cases caused by *Cyclospora cayetanensis* in Kayseri]. *Turkiye Parazitol Derg* 2009; **33**:85–8.
172. Zhou Y, LV B, Wang Q, et al. Prevalence and molecular characterization of *Cyclospora cayetanensis*, Henan, China. *Emerg Infect Dis* 2011;**17**:1887–90.
173. Zimmer SM, Schuetz AN, Franco-Paredes C. Efficacy of nitazoxanide for cyclosporiasis in patients with sulfa allergy. *Clin Infect Dis* 2007;**44**:466–7.

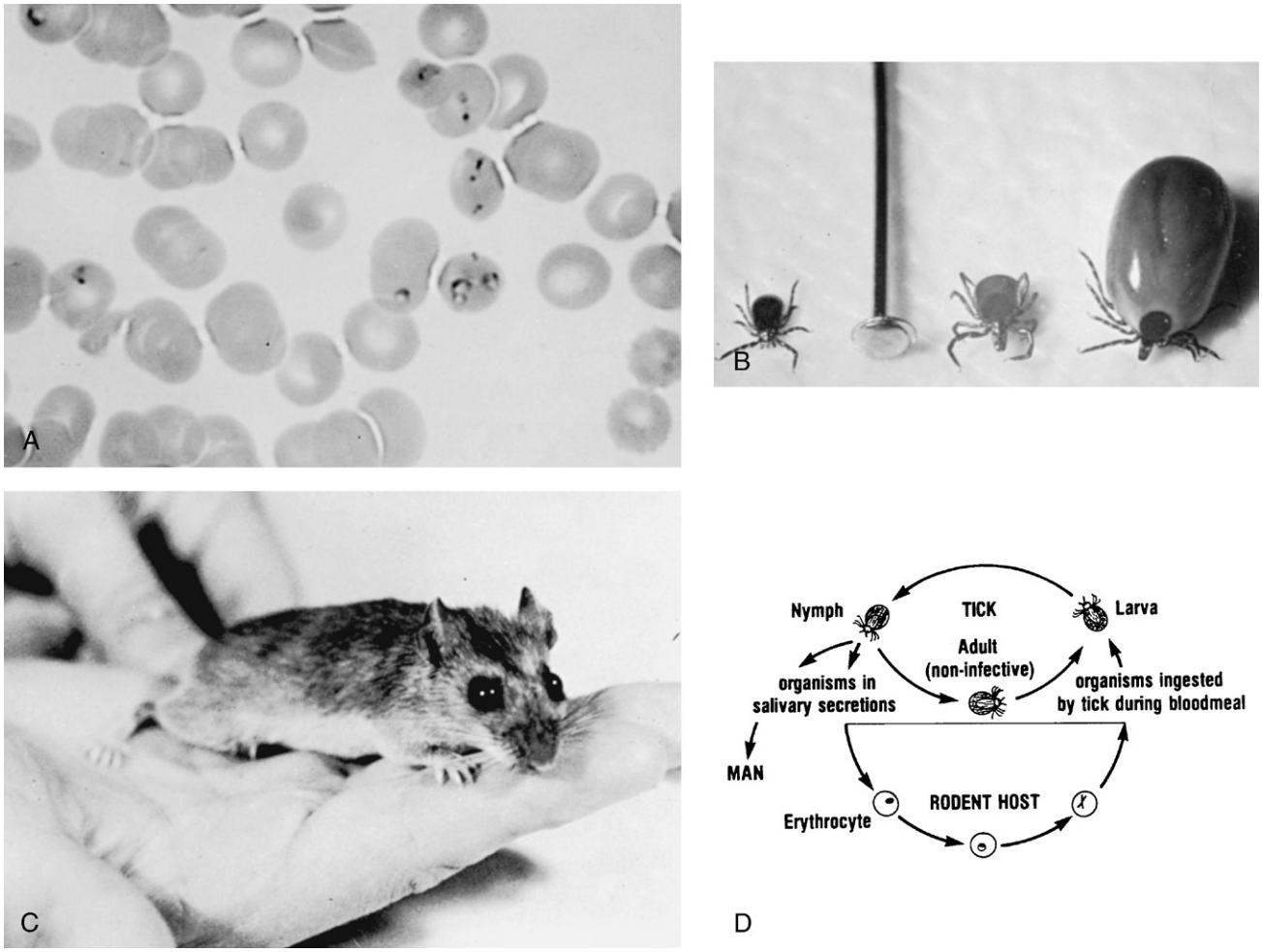

FIGURE 218-1 ■ Life cycle of *Babesia microti*. **A,** Ring forms of *B. microti* in human blood film (original magnification ×1000). **B,** *Ixodes dammini* ticks and a common pin. The ticks are an adult male, an adult female, and an engorged female. **C,** White-footed mouse *(Peromyscus leucopus).* **D,** Life cycle of *B. microti.* (**B,** Courtesy Mike Frigione, Pfizer, Inc., New York. **D,** Modified from Ruebush TK II. Babesiosis. In: Strickland GT, editor. *Hunter's tropical medicine.* Philadelphia: Saunders; 1984.)

(Fig. 218-1). Humans are an uncommon and terminal host for *Babesia* spp., which depend on other species for survival. The primary reservoir for *B. microti* in eastern North America is the white-footed mouse *(Peromyscus leucopus)*, but the parasite also has been found in shrews, chipmunks, voles, and rats.[52,139,147] Two thirds of *P. leucopus* have been found to be parasitemic in endemic areas.[140] *Babesia* spp. are transmitted by hard-bodied (ixodid) ticks. The primary vector in the northeastern United States is *Ixodes scapularis* (also known as *Ixodes dammini*), which is the same tick that transmits *Borrelia burgdorferi*, the etiologic agent of Lyme disease, and the agent of human granulocytic anaplasmosis.[64,66,136-141,145,154] *I. scapularis* ticks may be infected simultaneously with *B. burgdorferi*, *B. microti*, and the agent of human granulocytic anaplasmosis.[91,106,154] Clinical and serologic evidence exists for simultaneous human infection with two or more of these pathogens.*

Each of the three active stages in the life cycle (i.e., larva, nymph, and adult) of *I. scapularis* takes a blood meal from a vertebrate host to mature to the next stage.[140] Ingested babesial organisms infect intestinal tissue of the tick and subsequently travel to the salivary glands, from which they may be introduced into a new vertebrate host.[105] *Babesia* spp. are transmitted to the subsequent tick stage (trans-stadial passage). In some species of *Babesia*, such as *B. bovis*, the organisms may invade the ovaries and pass transovarially to the larvae. The tick transmission cycle begins in late summer, when newly hatched larvae ingest the parasite with a blood meal from an infected rodent and maintain the parasite to the nymphal stage. Nymphs transmit the *Babesia* spp. to rodents in late spring and summer of the following year.[137,140] Larvae, nymphs, and adults can feed on humans, but the nymph is the primary vector.[105] All active tick stages also feed on the white-tailed deer *(Odocoileus virginianus)*, which is an important host for the tick, but is not a reservoir for *B. microti*.[107,140] An increase in the deer population during the past few decades is thought to be a major factor in the spread of *I. scapularis* and the resulting increase in human cases.[137,140] Domestic animals such as the dog may carry the adult *I. scapularis*, but do not seem to be important hosts for the tick and are not infected with *B. microti*.[124,138]

*References 8, 10, 77, 81, 88, 97, 148.

Since the 1980s, human babesiosis has been described with increasing frequency at mainland sites, and more recent studies suggest that the endemic range continues to expand.[34,63,78,152] In certain sites, in certain years of high transmission, babesiosis may constitute a significant public health burden.[74] *B. microti* has been identified in rodent populations in several regions of the United States,[2,3,41,119,139] and human cases have been reported along the East Coast from Maryland to Maine and in Minnesota and Wisconsin.* Moderately severe illness caused by *Babesia duncani* and *B. duncani*–type organisms have been reported on the Pacific Coast from northern California to Washington State.[27,68,103,109] Cases of human babesiosis caused by *B. divergens*–like organisms have been reported in Missouri, Kentucky, and Washington State.[6,54-56] The tick vectors and reservoir hosts for *B. duncani* and *B. duncani*–type and *B. divergens*–like organisms have not been definitively identified.[59,152] In Europe, *B. divergens* and *Babesia venatorum* infections are primarily transmitted by the cattle tick *Ixodes ricinus*.[13,38,39,45,60] Human cases also have been reported in Asia, Africa, Australia, and South America.† An absence of clinical cases of babesiosis in the tropics may be due to cross immunity from other endemic protozoal diseases.[65] Most human cases of babesiosis occur in the summer and in areas where the vector tick, rodents, and deer are in close proximity to humans.[137,139,142]

Babesiosis also may be acquired through blood transfusion, and more than 150 cases have been identified.‡ *B. microti* is the most common transfusion-transmitted pathogen reported in the United States.[83,151,165] Whole blood, frozen erythrocytes, and platelets have been implicated. The incubation period in these cases is usually 6 to 9 weeks but may be as long as 6 months. Transfusion-transmitted babesiosis has been reported throughout the year and in nonendemic areas. About a fifth of the cases have been fatal.[55,150,165] Although most cases of babesiosis in neonates have been acquired through blood transfusion, a few cases of transplacental transmission of babesiosis have been described.[33,36,62,152] The outcome in these cases has been good after treatment with appropriate antibiotic therapy.

PATHOGENESIS AND PATHOLOGY

Understanding of the pathogenesis and pathology of babesiosis in humans is incomplete and is based largely on information gathered from human case studies and studies of babesiosis in other animals. Rudzinska and colleagues[116,117] and Karakashian and associates[66] studied the life cycle of *B. microti* in erythrocytes using electron microscopy. After adhesion and entry into the erythrocyte occurs, the organism multiplies by asexual budding into two to four daughter cells, or merozoites. In contrast to *Plasmodium* spp. merozoites, which are released from the erythrocytes all at once (synchrony), *Babesia* spp. merozoites are released at varying intervals. New erythrocytes are infected, and the cycle is repeated.

The pathogenesis of babesia infection is closely linked to the host response to infection and modification of the erythrocyte membrane.[71] Investigation of *Babesia rodhani* suggests that activation of the alternative complement pathway allows entry of parasite into red blood cells through the C3b receptor.[60] It is unknown whether the initial merozoite release leads to destruction of the host erythrocyte, but alteration of the erythrocyte membrane and eventual lysis occur.[116,143] Erythrocyte lysis is associated with many of the clinical manifestations and complications of the disease, including fever, hemolytic anemia, jaundice, hemoglobinemia, hemoglobinuria, and renal insufficiency. The absence of synchrony decreases the possibility of massive hemolysis and may explain why patients heavily parasitized with *Babesia* spp. may be less ill than patients with *Plasmodium* spp.[143]

Babesia spp. are intraerythrocytic protozoa, and extracellular forms are seen only in heavily parasitized cases.[1,143] *Babesia* organisms do not invade fixed tissue, and evidence in animals suggests that disease results from the excessive production of proinflammatory cytokines (e.g., tumor necrosis factor), as is thought to occur with malaria.[22,25,71] The release of proinflammatory cytokines in patients with babesiosis may be initiated by contact with an important surface protein, glycosylphosphatidylinositol (GPI).[23,28] These cytokines subsequently stimulate release of downstream mediators, such as nitric oxide, that kill parasites but also may cause direct cellular damage if produced in excess.[22,24]

In addition to proinflammatory cytokine release, obstruction of blood vessels as a result of adherence of parasitized erythrocytes to vascular endothelium with accompanying ischemia and necrosis may result in cerebral abnormalities, hepatomegaly and hepatic dysfunction, and splenomegaly.[30,113,121] Although such cytoadherence appears to occur in cattle infected with *B. bovis*, it has yet to be demonstrated in human babesial infection.[25,71] A report of fatal human babesiosis with cerebral involvement was not associated with erythrocyte adherence and vascular occlusion.[25] Clinical manifestations such as hypotension, vascular congestion, and anoxia also may result from the activation of fibronectin, kallikreins, and complement.[32,162,163]

Innate and adaptive immune mechanisms limit the severity of babesial infections. The spleen plays a critical role in protection against *Babesia* spp. by (1) removing parasites from infected erythrocytes through a process known as "pitting," (2) ingesting parasites by resident reticuloendothelial cells and mononuclear phagocytes, and (3) producing antibabesial antibody.* It long has been known that splenectomized animals have more severe babesiosis than animals with intact spleens. Animals that have recovered from babesiosis and have had negative blood smears have developed parasitemia again after undergoing splenectomy.[87,125] Most fatal cases of babesiosis in humans have occurred in splenectomized individuals, although asplenia does not always result in death or even severe illness.[16,115] Other host defense mechanisms

*References 19, 31, 34, 63, 78, 95, 119, 142, 152
†References 14, 114, 128, 129, 152, 155.
‡References 4, 12, 44, 48, 47, 55, 83, 85, 89, 102, 134, 150, 151, 165.

*References 8, 17, 29, 32, 115, 152, 163.

that may help limit babesial infection include macrophages and macrophage products, such as tumor necrosis factor,[22,32,152,163] B lymphocytes,[94] T lymphocytes,[17,94,118,159] polymorphonuclear leukocytes,[131] antibody,[17] and complement.[8,163]

Age is an important factor in host defense against babesial disease in animals and humans. Most clinically apparent cases are reported in adults, but serologic surveys indicate that children are equally susceptible to infection and presumably are exposed to ticks to the same extent.[77,119,124] Almost all of the pediatric cases reported have been in neonates.[36,92,158] More recent data from a murine model of babesiosis have suggested that resistance to *B. microti* infection conferred by the adaptive immune system is genetically determined and associated with age.[153] Immunity is incomplete because parasitemia may exist for months to years in animals and up to 27 months in humans after recovery from the initial illness.[77] Reinfection may occur, although it is uncommon.

CLINICAL MANIFESTATIONS

Clinical manifestations of babesiosis range from subclinical illness to fulminating disease resulting in death. Overt signs and symptoms begin after an incubation period of 1 to 6 weeks from the beginning of tick feeding or 6 weeks to 6 months after transfusion.[55,124] The unengorged *I. scapularis* nymph is about 2 mm in length, and the patient often has no recollection of having a tick bite. In most cases, the patient has a gradual onset of malaise, anorexia, and fatigue followed by intermittent fever as high as 40° C (104° F) and one or more of the following: chills, sweats, myalgia, arthralgia, nausea, and vomiting.* Less commonly noted are emotional lability and depression, hyperesthesia, headache, sore throat, abdominal pain, conjunctival injection, photophobia, weight loss, and nonproductive cough.[69,121,142,143] In contrast to other tick-borne illnesses, such as Lyme disease, Rocky Mountain spotted fever, or tularemia, rash seldom is noted.[31] Ecchymoses and petechiae have been described.[67,143]

The findings on physical examination generally are minimal, often consisting only of fever.[1,56,81,123,142] Mild splenomegaly, hepatomegaly, or both are noted occasionally.[121,158] Slight pharyngeal erythema, jaundice, and retinopathy with splinter hemorrhages and retinal infarcts also have been reported.[69,92,100] Several abnormal laboratory findings in patients with babesiosis reflect the invasion and subsequent lysis of erythrocytes by the parasite.[69,92,119,121] Mild to moderately severe hemolytic anemia occurs, with an elevated reticulocyte count. Elevated liver enzyme levels may be detected in serum.[119] The leukocyte count is normal to slightly decreased, with a "left shift." Thrombocytopenia is common.[121] The erythrocyte sedimentation rate is elevated. Proteinuria and an elevated blood urea nitrogen and creatinine also may be noted.[67,69,89,121] The illness usually lasts a few weeks to several months, with prolonged recovery taking up to 18 months.[1,7,76,121,123,142] Parasitemia may continue even after the patient feels well. Persistent parasitemia and relapse of illness, as noted with malaria, has been described 27 months after the initial episode.[76] Highly immunocompromised patients may experience a prolonged relapsing course of illness despite standard antibiotic therapy.[72]

Some patients, especially patients who are immunocompromised or patients with *B. divergens* or *B. duncani* infection, have a more severe form of the disease consisting of fulminant illness lasting approximately 1 week and ending in death or a prolonged convalescence.* Signs and symptoms include high fever, hemolytic anemia, hemoglobinemia and hemoglobinuria, jaundice, ecchymoses, petechiae, congestive heart failure, pulmonary edema, renal failure, acute respiratory distress syndrome, and coma.† Patients coinfected with babesiosis and Lyme disease experience more severe acute illness than patients with Lyme disease alone but not babesiosis alone.[46,81,88,148] Coinfected patients usually experience moderate to severe acute illness often followed by persistent fatigue. Of patients with antibody to *B. burgdorferi*, 10 to 66 percent also have been found to have antibody to *B. microti*.[10,77,81]

Inapparent infection occurs in approximately a quarter of adults and half of children.[74] Serosurveys provide evidence of asymptomatic infection because of the disparity between seroprevalence rates and the number of indigenous reported cases of babesiosis. In a survey on Nantucket Island in Massachusetts, 2 percent of 577 random blood samples and 7.5 percent of 133 blood samples from patients with a history of tick bite or fever had *B. microti* indirect immunofluorescent antibody (IFA) titers of 1:64 or greater.[123] A survey of adults living on Shelter Island, New York showed that 6/136 (4.4%) and 7/102 (6.9%) had *B. microti* IFA titers of 1:64 or greater.[35] In a survey of Massachusetts blood donors, 29/779 (37%) from Cape Cod had *B. microti* IFA titers of 1:16 in contrast to 7/148 (4.7%) from metropolitan Boston.[108] In a serosurvey in Connecticut, 72/735 (9.5%) residents who were seropositive for *B. burgdorferi* had positive *B. microti* IFA titers of 1:64 in contrast to 8/299 (2.7%) seronegative for *B. burgdorferi*.[78] Serosurveys in Mexico, Nigeria, and Taiwan also showed high *B. microti* seroprevalence rates in contrast to the number of indigenous reported cases of babesiosis.[57,82,101]

DIAGNOSIS

Specific diagnosis of babesiosis is made by microscopic identification of the organism by Giemsa or Wright stains of thick or thin blood smears and by detection of babesial antibodies by one of several serologic tests. *Babesia* spp. are round, oval, or pear-shaped and have a blue cytoplasm with a red chromatin. The ring form is most common and is similar to the rings of *Plasmodium falciparum*.[51] *Babesia* spp. can be distinguished from *Plasmodium* spp. by (1) the absence of hemozoin pigment, which is present in older trophozoites of *Plasmodium* spp.;

*References 1, 38, 73, 81, 92, 119, 123, 142.

*Restaurant 39, 49, 59, 67, 68, 88, 128, 143.
†Restaurant 42, 43, 49, 67, 70, 142, 143, 156.

(2) the absence of identifiable gametocytes; (3) the absence of synchronous stages within the erythrocytes; and (4) the presence of the infrequently noted tetrad or Maltese cross forms, in which four compact masses, each containing nuclear material, are joined by strands of cytoplasm.[51]

Multiple thick and thin blood smears should be examined because only a few erythrocytes are infected in the early stage of the illness, when most people seek medical attention.[51] Rapid automated differential blood analyzers may fail to distinguish erythrocytic inclusions.[14] In thick smears, the *Babesia* organism appears as a tiny red-to-purple nucleus with a thin tail of light blue cytoplasm. Maximum erythrocyte infection is approximately 10 percent in normal hosts, but up to 85 percent in asplenic individuals.[142] Usually less than 1 percent of erythrocytes are parasitized early in the course of the illness, and the laboratory investigation of possible babesiosis should include more than an examination of blood smears.

Several serologic tests have been developed to detect babesial antibodies, but the IFA assay is the most reliable.[21,75,79,121] During the acute phase of the illness, titers usually exceed 1:1024, but decline to 1:64 or less within 8 to 12 months. A babesial IFA titer of 1:1024 or greater usually signifies active or recent infection.[21,122] Although cross reactions occur to different *Babesia* spp. and *Plasmodium* spp. with the IFA test, these titers almost always are low (≤1:16).[21,20] The problem of cross reactivity with *Plasmodium* spp. is minimized in areas that have no indigenous malaria. A reliable immunoblot assay for detection of *B. microti* antibody has been developed.[127] Enzyme-linked immunosorbent assays for detection of *B. divergens* and *B. major* have been found to be superior to complement fixation and IFA procedures.[11,112,146,149]

In cases in which the presence of *Babesia* spp. is suspected but not shown by blood smears or antibody studies, babesial DNA can be amplified and detected using the polymerase chain reaction (PCR).[80,104,144] Blood from the patient also can be injected by the intravenous or intraperitoneal route into small laboratory animals such as hamsters or gerbils. If present in the patient, *B. microti* usually appears in the blood of the inoculated animal within 2 to 4 weeks.[9] This diagnostic technique is less sensitive and more time-consuming and costly than the PCR.[80]

PREVENTION AND TREATMENT

The combination of either atovaquone plus azithromycin or clindamycin plus quinine for 7 to 10 days is the recommended therapy for patients with babesiosis. Clindamycin and quinine should be given to patients with severe babesiosis. In such patients, clindamycin should be administered intravenously rather than orally, and exchange transfusion should be considered. The dosage recommendations are as follows[18,36,42,110,160]:

- Atovaquone: Children, 20 mg/kg every 12 hours (maximum 750 mg/dose); adults, 750 mg orally every 12 hours
- Azithromycin: Children, 10 mg/kg once per day on day 1 (maximum 500 mg/dose) and 5 mg/kg once per day (maximum 250 mg/dose) and orally thereafter; adults, 500 to 1000 mg on day 1 and 250 mg orally once per day thereafter; immunocompromised adults, 600 to 1000 mg/day
- Clindamycin: Children, 7 to 10 mg/kg given intravenously or orally every 6 to 8 hours (maximum 600 mg/dose); adults, 300 to 600 mg every 6 hours intravenously or 600 mg every 8 hours orally
- Quinine: Children, 8 mg/kg given orally every 8 hours (maximum 650 mg/dose); adults, 650 mg every 6 to 8 hours orally

The clindamycin and quinine combination first was used for an 8-week-old infant girl who contracted babesiosis from a blood transfusion.[158] Initially, she was thought to have malaria. Clindamycin and quinine were given after failure with chloroquine. Her favorable outcome suggested the prospective use of this combination in adults. Numerous children and adults subsequently have been treated with clindamycin and quinine, with prompt clearing of parasitemia and resolution of clinical signs and symptoms.[18,19,81,92,142]

The successful use of atovaquone and azithromycin for the treatment of malaria and for babesiosis in hamsters prompted a clinical trial to determine whether the combination would be effective in human babesiosis.[58,73,157] In the first prospective trial of antibabesial therapy in humans, atovaquone and azithromycin were compared with clindamycin and quinine in adults.[73] Adverse effects were reported in 15 percent of subjects who received atovaquone and azithromycin in contrast to 72 percent of subjects who received clindamycin and quinine. In approximately one third of subjects taking clindamycin and quinine, the apparent drug reactions were severe enough that the drugs were discontinued or the dosages decreased in contrast to only 2 percent of subjects taking atovaquone and azithromycin. The drug combinations were equally effective in clearing symptoms and parasitemia. Although rare cases of resistance to atovaquone and azithromycin have been reported, subsequent use of this combination in children and adults has shown that this combination is effective and should be used in those who have mild to moderate disease.[36,110,160,161]

Other antimicrobial agents that have been used to treat babesiosis generally are ineffective. Although chloroquine may give some symptomatic relief of fever and myalgia by its anti-inflammatory action, it often fails to clear parasitemia in guinea pigs and humans and is not recommended.[18,96] Other antimalarial drugs, such as quinacrine, primaquine, pyrimethamine, pyrimethamine and sulfadiazine, sulfadiazine, and tetracycline, have no effect on parasitemia in animals. Pentamidine isothionate has been found to decrease fever and parasitemia, but the organisms are not eradicated and the drug has proved to be ineffective in animals and humans.[37] Diminazene aceturate was effective in clearing parasitemia and clinical symptoms in one patient, but he developed Guillain-Barré syndrome during recovery, possibly as a result of receiving the drug.[126] Pentamidine and trimethoprim-sulfamethoxazole were used successfully to treat a case of *B. divergens* infection in France.[111]

Additional therapeutic measures may be necessary for people with severe babesiosis, especially those who are

immunocompromised. Partial or complete red blood cell exchange transfusion is an important adjunct therapy that can decrease the degree of parasitemia rapidly and remove toxic by-products of babesial infections.[16,61,143,152] Exchange transfusion is indicated for patients with severe babesiosis, as indicated by high-grade parasitemia (≥10%); significant hemolysis; or renal, hepatic, or pulmonary compromise.[160] No data are available to determine whether partial exchange transfusion is preferable to whole-blood exchange transfusion. For highly immunocompromised patients who experience therapeutic failure with an initial course of standard antibiotic therapy, resolution of infection may require at least 6 weeks of antimicrobial therapy, including 2 weeks after *Babesia* organisms are no longer detected on blood smears.[72]

Babesiosis can be prevented by avoiding areas in May through September where ticks, deer, and mice are known to thrive. It is especially important for asplenic individuals or other immunocompromised patients who are at increased risk for severe babesiosis, and who live or travel in endemic areas, to avoid tall grass and brush where ticks may abound. Use of clothing that covers the lower part of the body and that is sprayed or impregnated with diethyltoluamide, dimethyl phthalate, or permethrin (Permanone) is recommended for individuals who travel into the foliage of endemic areas.[30,133] A search for ticks on people and pets should be carried out and the ticks removed as soon as possible.[30] Tick removal is accomplished best with tweezers by grasping the mouth parts without squeezing the body of the tick.[31,99] Landscape-management approaches such as keeping grass mowed, clearing leaf litter, and spraying property where there is high tick density may help reduce the risk for infection.[50,90] Attempts to reduce the tick, mouse, or deer populations in endemic areas are less effective.[91,133,137] It is recommended that prospective blood donors who reside in endemic areas and who have a history of fever within the preceding 1 to 2 months be excluded from giving blood to prevent transfusion-related cases.[120] Blood donors who have had a history of babesiosis are permanently deferred from donating.[93,165] Effective *B. bovis* and *B. bigemina* vaccines have been developed for use in cattle, but no *B. microti* vaccine has been developed.[98,164]

Babesiosis is a worldwide tick-borne zoonosis that is endemic in the northeastern and northern Midwestern United States. The incidence is increasing and is greater than currently recognized. Babesiosis presents as a viral-like illness with generalized nonspecific symptoms, and diagnosis may be delayed. The diagnosis is confirmed by visualizing the organism on blood smears or indirectly by detection of *B. microti* antibody or detection of *B. microti* DNA with PCR. The disease may be severe or fatal, primarily in immunocompromised hosts, but complete recovery with antibabesial chemotherapy is the rule.

NEW REFERENCES SINCE THE SIXTH EDITION

4. Asad S, Sweeney J, Mermel LA. Transfusion-transmitted babesiosis in Rhode Island. *Transfusion* 2009;**49**:2564–73.
6. Beattie JF, Michelson ML, Holman PJ. Acute babesiosis caused by *Babesia divergens* in a resident of Kentucky. *N Engl J Med* 2002;**347**:697–8.
12. Bloch EM, Herwaldt BL, Leiby DA, et al. The third described case of transfusion transmitted *Babesia duncani*. *Transfusion* 2012;**52**:1517–22.
13. Bonnet S, Jouglin M, L'Hostis M, et al. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. *Emerg Infect Dis* 2007;**13**:1208–10.
23. Clark IA, Budd AC, Alleva LM, et al. Human malarial disease: a consequence of inflammatory cytokine release. *Malaria J* 2006; **5**:85.
27. Conrad PA, Kjemtrup AM, Carreno RA, et al. Description of *Babesia duncani* n. sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. *Int J Parasitol* 2006;**36**:779–89.
28. Cornillot E, Hadj-Kaddour K, Dassouli A. et al. Sequencing of the smallest Apicomplexan genome from the human pathogen *Babesia microti*. *Nucl Acids Res* 2012;**40**:9102–14.
45. Gray J, Zintl A, Hildebrandt A, et al. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. *Ticks Tick Borne Dis* 2010;**1**:3–10.
47. Gubernot DM, Lucey CT, Lee KC, et al. *Babesia* infection through blood transfusions: reports received by the US Food and Drug Administration, 1997–2007. *Clin Infect Dis* 2009;**48**:25–30.
48. Gubernot DM, Nakhasi HL, Mied PA, et al. Transfusion-transmitted babesiosis in the United States: summary of a workshop. *Transfusion* 2009;**49**:2759–71.
54. Herwaldt BL, de Bruyn G, Pieniazek NJ, et al. *Babesia divergens*-like infection, Washington State. *Emerg Infect Dis* 2004;**10**: 622–9.
55. Herwaldt BL, Linden JV, Bosserman E, et al. Transfusion-associated babesiosis in the United States: a description of cases. *Ann Intern Med* 2011;**155**:509–19.
59. Hunfeld KP, Hildebrandt A, Gray JS Babesiosis: recent insights into an ancient disease. *Int J Parasitol* 2008;**38**:1219–37.
62. Joseph JT, Purtill K, Wong SJ, et al. Vertical transmission of *Babesia microti*, United States. *Emerg Infect Dis* 2012;**18**:1318–21.
63. Joseph JT, Roy SS, Shams N, et al. Babesiosis in Lower Hudson Valley, New York, USA. *Emerg Infect Dis* 2011;**17**:843–7.
68. Kjemtrup AM, Conrad PA. Human babesiosis: an emerging tick-borne disease. *Int J Parasitol* 2000;**30**:1323–37.
71. Krause PJ, Daily J, Telford SR, et al. Shared features in the pathobiology of babesiosis and malaria. *Trends Parasitol* 2007;**23**: 605–10.
72. Krause PJ, Gewurz BE, Hill D, et al. Persistent and relapsing babesiosis in immunocompromised patients. *Clin Infect Dis* 2008; **46**:370–6.
83. Leiby DA. Transfusion-associated babesiosis: shouldn't we be ticked off? *Ann Intern Med* 2011;**155**:556–7.
84. Leiby DA. Transfusion-transmitted *Babesia* spp.: bull's-eye on *Babesia microti*. *Clin Microbiol Rev* 2011;**24**:14–28.
102. Perkins HA, Busch MP. Transfusion associated infections: 50 years of relentless challenges and remarkable progress. *Transfusion* 2010;**50**:2080–99.
129. Senanayake SN, Paparini A, Latimer M, et al. First report of human babesiosis in Australia. *Med J Aust* 2012;**196**:350–2.
144. Teal AE, Habura A, Ennis J, et al. A new real-time PCR assay for improved detection of the parasite *Babesia microti*. *J Clin Microbiol* 2012;**50**:903–8.
149. Tonnetti L, Eder AF, Dy B, et al. Transfusion-transmitted *Babesia microti* identified through hemovigilance. *Transfusion* 2009;**49**: 2557–63.
151. U.S. Food and Drug Administration. *Biological product and hematopoietic cell therapy/product (HCT/P) deviation report: annual summary for fiscal year 2008–9*. Silver Spring, Md: USFDA; 2009. (http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/BiologicalProductDeviations/ucm169990.htm).
153. Vannier E, Krause PJ. Human babesiosis. *New Engl J Med* 2012;**366**:2397–407.
160. Wormser GP, Prasad A, Neuhaus E, et al. Emergence of resistance to azithromycin-atovaquone in immunocompromised patients with *Babesia microti* infection. *Clin Inf Dis* 2010;**50**:381–6.
165. Young C, Krause PJ. The problem of transfusion-transmitted babesiosis. *Transfusion* 2009;**49**:2548–50.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Anderson AE, Cassaday PB, Healy GR. Babesiosis in man: sixth documented case. *Am J Clin Pathol* 1974;**62**:612–18.
2. Anderson JF, Magnarelli LA. Spirochetes in *Ixodes dammini* and *Babesia microti* on Prudence Island, Rhode Island. *J Infect Dis* 1983;**148**:1124.
3. Anderson JF, Magnarelli LA, Kurz J. Intraerythrocytic parasites in rodent populations of Connecticut: *Babesia* and *Grahamella* species. *J Parasitol* 1979;**65**:599–604.
4. Asad S, Sweeney J, Mermel LA. Transfusion-transmitted babesiosis in Rhode Island. *Transfusion* 2009;**49**:2564–73.
5. Babes V. Sur l'hemoglubinurie bacterienne boeuf. *Compt Rend Acad Sci* 1888;**107**:692–4.
6. Beattie JF, Michelson ML, Holman PJ. Acute babesiosis caused by *Babesia divergens* in a resident of Kentucky. *N Engl J Med* 2002;**347**:697–8.
7. Benach JL, Habicht GS. Clinical characteristics of human babesiosis. *J Infect Dis* 1981;**144**:481.
8. Benach JL, Habicht GS, Manburger MI. Immunoresponsiveness in acute babesiosis in humans. *J Infect Dis* 1982;**146**:369–80.
9. Benach JL, White DJ, McGovern JP. Babesiosis in Long Island: host-parasite relationship of rodent and human-derived *Babesia microti* isolates in hamsters. *Am J Trop Med Hyg* 1978;**27**: 1073–8.
10. Benach JL, Coleman JL, Habicht GS, et al. Serological evidence for simultaneous occurrences of Lyme disease and babesiosis. *J Infect Dis* 1985;**152**:473–7.
11. Bidwell DE, Turp P, Joyner LP, et al. Comparisons of serologic tests for babesia in British cattle. *Vet Rec* 1978;**103**:446–9.
12. Bloch EM, Herwaldt BL, Leiby DA, et al. The third described case of transfusion transmitted *Babesia duncani*. *Transfusion* 2012;**52**:1517–22.
13. Bonnet S, Jouglin M, L'Hostis M, Chauvin A. *Babesia* sp. EU1 from roe deer and transmission within *Ixodes ricinus*. *Emerg Infect Dis* 2007;**13**:1208–10.
14. Bruckner DA, Garcia LS, Shimizu RY, et al. Babesiosis: problems in diagnosis using autoanalyzers. *Am J Clin Pathol* 1985;**83**: 320–1.
15. Bush JB, Isaacson M, Mohamed AS. Human babesiosis: a preliminary report of two suspect cases in southern Africa. *S Afr J Med* 1990;**78**:699.
16. Cahill KM, Benach JL, Reich LM, et al. Red cell exchange: treatment of babesiosis in a splenectomized patient. *Transfusion* 1981; **21**:193–8.
17. Carson CA, Phillips RS. Immunologic response of the vertebrate host to babesia. In: Ristic M, Kreier JP, editors. *Babesiosis*. New York: Academic Press; 1981. p. 411–43.
18. Centers for Disease Control and Prevention. Clindamycin and quinine treatment for *Babesia microti* infections. *MMWR Morb Mortal Wkly Rep* 1983;**32**:65–72.
19. Centers for Disease Control and Prevention. Babesiosis in Connecticut. *MMWR Morb Mortal Wkly Rep* 1989;**38**:649–50.
20. Chisholm ES, Sulzer AJ, Ruebush T. Indirect immunofluorescence test for human *Babesia microti* infection: antigenic specificity. *Am J Trop Med Hyg* 1986;**35**:921–5.
21. Chisholm ES, Ruebush TK, Sulzer AJ, et al. *Babesia microti* infection in man: evaluation of an indirect immunofluorescent antibody test. *Am J Trop Med Hyg* 1978;**27**:14–19.
22. Clark IA, Jacobson LS. Do babesiosis and malaria share a common disease process? *Ann Trop Med Parasitol* 1998;**92**:483–8.
23. Clark IA, Budd AC, Alleva LM, et al. Human malarial disease: a consequence of inflammatory cytokine release. *Malaria J* 2006; **5**:85.
24. Clark IA, Alleva LM, Mills AC, et al. Pathogenesis in malaria and clinically similar conditions. *Clin Microbiol Rev* 2004;**17**: 509–39.
25. Clark IA, Budd AC, Hsue G, et al. Absence of erythrocyte sequestration in a case of babesiosis in a splenectomized human patient. *Malar J* 2006;**5**:69.
26. Clark IA, Wills EJ, Richmond JE, et al. Immunity to intraerythrocytic protozoa. *Lancet* 1973;**2**:1128–9.
27. Conrad PA, Kjemtrup AM, Carreno RA, et al. Description of *Babesia duncani* n. sp. (Apicomplexa: Babesiidae) from humans and its differentiation from other piroplasms. *Int J Parasitol* 2006;**36**:779–89.
28. Cornillot E, Hadj-Kaddour K.; Dassouli A. et al. Sequencing of the smallest Apicomplexan genome from the human pathogen *Babesia microti*. *Nucl Acids Res* 2012;**40**:9102–14.
29. Cullen JM, Levine JF. Pathology of experimental *Babesia microti* infection in the Syrian hamster. *Lab Anim Sci* 1987;**37**:640–3.
30. Dammin GJ. Babesiosis. In: Weinstein L, Fields BN, editors. *Seminars in Infectious Disease*. New York: Stratton; 1978. p. 169–99.
31. Dammin GJ, Spielman A, Benach JL, et al. The rising incidence of clinical *Babesia microti* infection. *Hum Pathol* 1981;**12**:398–400.
32. DeVos AJ, Dalgliesh RJ, Callow LL. Babesia. In: Soulsby EJL, editor. *Immune Responses in Parasitic Infections: Immunology, Immunopathology, and Immunoprophylaxis*, Vol. 3. Boca Raton, Fla: CRC Press; 1987. p. 183–222.
33. Esernio-Jenssen D, Scimeca PG, Benach JL, et al. Transplacental/perinatal babesiosis. *J Pediatr* 1987;**110**:570–2.
34. Eskow ES, Krause PJ, Spielman A, et al. Southern extension of the range of human babesiosis in the eastern United States. *J Clin Microbiol* 1999;**37**:2051–2.
35. Filstein MR, Benach JL, White D, et al. Serosurvey for human babesiosis in New York. *J Infect Dis* 1980;**141**:518–21.
36. Fox L, Wingerter S, Ahmed A, et al. Neonatal babesiosis: case report and review of the literature. *Pediatr Infect Dis J* 2006;**25**: 169–73.
37. Francioli PB, Keithly JS, Jones TC, et al. Response of babesiosis to pentamidine therapy. *Ann Intern Med* 1981;**94**:326–30.
38. Garnham PCC. Human babesiosis: European aspects. *Trans R Soc Trop Med Hyg* 1980;**74**:153–5.
39. Garnham PCC, Donelly J, Hoogstraal H, et al. Human babesiosis in Ireland: further observations and the medical significance of this infection. *Br Med J* 1969;**4**:768–70.
40. Gibbons WJ. *Diseases of cattle*. 2nd ed. Wheaton, Ill: Veterinary Publications; 1963.
41. Goethert HK, Lubelcyzk C, LaCombe E, et al. Enzootic *Babesia microti* in Maine. *J Parasitol* 2003;**89**:1069–71.
42. Golightly LM, Hirschhorn LR, Weller PF. Fever and headache in a splenectomized woman. *Rev Infect Dis* 1989;**11**:629–37.
43. Gordon S, Cordon RA, Mazdzer EJ, et al. Adult respiratory distress syndrome in babesiosis. *Chest* 1984;**86**:633–4.
44. Grabowski EF, Giardina PJV, Goldberg D, et al. Babesiosis transmitted by a transfusion of frozen-thawed blood. *Ann Intern Med* 1982;**96**:466–7.
45. Gray J, Zintl A, Hildebrandt A, et al. Zoonotic babesiosis: overview of the disease and novel aspects of pathogen identity. *Ticks Tick Borne Dis* 2010;**1**:3–10.
46. Grunwaldt E, Barbour AG, Benach JL. Simultaneous occurrence of babesiosis and Lyme disease. *N Engl J Med* 1983;**308**:1166.
47. Gubernot DM, Lucey CT, Lee KC, et al. *Babesia* infection through blood transfusions: reports received by the US Food and Drug Administration, 1997-2007. *Clin Infect Dis* 2009;**48**:25–30.
48. Gubernot DM, Nakhasi HL, Mied PA, et al. Transfusion-transmitted babesiosis in the United States: summary of a workshop. *Transfusion* 2009;**49**:2759–71.
49. Hatcher JC, Greenberg PD, Antique J, et al. Severe babesiosis in Long Island: review of 34 cases and their complications. *Clin Infect Dis* 2001;**32**:1117–25.
50. Hayes EB, Piesman J. How can we prevent Lyme disease? *N Engl J Med* 2003;**348**:2424–30.
51. Healy GR, Ruebush TK. Morphology of *Babesia microti* in human blood smears. *Am J Clin Pathol* 1980;**73**:107–9.
52. Healy GR, Spielman A, Gleason N. Human babesiosis: reservoir of infection on Nantucket Island. *Science* 1976;**192**:479–80.
53. Healy GR, Walzer PD, Sulzer AJ. A case of asymptomatic babesiosis in Georgia. *Am J Trop Med Hyg* 1976;**25**:376–8.
54. Herwaldt BL, de Bruyn G, Pieniazek NJ, et al. *Babesia divergens*-like infection, Washington State. *Emerg Infect Dis* 2004;**10**: 622–9.
55. Herwaldt BL, Linden JV, Bosserman E, et al. Transfusion-associated babesiosis in the United States: a description of cases. *Ann Intern Med* 2011;**155**:509–19.
56. Herwaldt BL, Persing DH, Precigont EA, et al. A fatal case of babesiosis in Missouri: identification of another piroplasm that infects humans. *Ann Intern Med* 1996;**124**:643–50.
57. Hsu NH, Cross JH. Serologic survey for human babesiosis on Taiwan. *J Formos Med Assoc* 1977;**76**:950–4.

58. Hughes WT, Oz HS. Successful prevention and treatment of babesiosis with atovaquone. *J Infect Dis* 1995;**172**:1042–6.
59. Hunfeld KP, Hildebrandt A, Gray JS. Babesiosis: recent insights into an ancient disease. *Int J Parasitol* 2008;**38**:1219–37.
60. Jack RM, Ward PA. *Babesia rodhaini* interactions with complement: relationship to parasitic entry into red cells. *J Immunol* 1980;**124**:1566–73.
61. Jacoby GA, Hunt JV, Kosinski KS, et al. Treatment of transfusion transmitted babesiosis by exchange transfusion. *N Engl J Med* 1980;**303**:1098–100.
62. Joseph JT, Purtill K, Wong SJ et al. Vertical Transmission of *Babesia microti*, United States. *Emerg Infect Dis* 2012;**18**:1318–21.
63. Joseph JT, Roy SS, Shams N, et al. Babesiosis in Lower Hudson Valley, New York, USA. *Emerg Infect Dis* 2011;**17**:843–7.
64. Johnson RC, Schmid GP, Hyde FW, et al. *Borrelia burgdorferi:* etiologic agent of Lyme disease. *Int J Syst Bacteriol* 1984;**34**: 496–7.
65. Kakoma I, Ristic M. Pathogenesis of babesiosis. In: Ristic M, Ambroise-Thomas P, Kreier J, editors. *Malaria and babesiosis: research findings and control measures*. Dordrecht: Martinus Nijhoff; 1984. p. 85–93.
66. Karakashian SJ, Rudzinska MA, Spielman A, et al. Primary and secondary ookinetes of *Babesia microti* in the larval and nymphal stages of the tick *Ixodes dammini*. *Can J Zool* 1986;**64**:328–39.
67. Kennedy CC. Human babesiosis: summary of a case in Ireland. *Trans R Soc Trop Med Hyg* 1980;**74**:156.
68. Kjemtrup AM, Conrad PA. Human babesiosis: an emerging tick-borne disease. *Int J Parasitol* 2000;**30**:1323–37.
69. Kogut SJ, Thill CD, Prusinski MA, et al. *Babesia microti*, upstate New York. *Emerg Infect Dis* 2005;**11**:476–8.
70. Krause PJ. Babesiosis. *Med Clin North Am* 2002;**86**:361–73.
71. Krause PJ, Daily J, Telford SR, et al. Shared features in the pathobiology of babesiosis and malaria. *Trends Parasitol* 2007;**23**: 605–10.
72. Krause PJ, Gewurz BE, Hill D, et al. Persistent and relapsing babesiosis in immunocompromised patients. *Clin Infect Dis* 2008;**46**:370–6.
73. Krause PJ, Lepore T, Sikand VJ, et al. Atovaquone and azithromycin for the treatment of human babesiosis. *N Engl J Med* 2000;**343**:1454–8.
74. Krause PJ, McKay K, Gadbaw J, et al. Increasing health burden of human babesiosis in endemic sites. *Am J Trop Med Hyg* 2003;**68**:431–6.
75. Krause PJ, Ryan R, Telford SR, et al. Efficacy of immunoglobulin M serodiagnostic test for rapid diagnosis of acute babesiosis. *J Clin Microbiol* 1996;**34**:2014–16.
76. Krause PJ, Spielman A, Telford S, et al. Persistent parasitemia after acute babesiosis. *N Engl J Med* 1998;**339**:160–5.
77. Krause PJ, Telford SR, Pollack RJ, et al. Babesiosis: an underdiagnosed disease of children. *Pediatrics* 1992;**89**:1045–8.
78. Krause PJ, Telford SR, Ryan R, et al. Geographical and temporal distribution of babesial infection in Connecticut. *J Clin Microbiol* 1991;**29**:1–4.
79. Krause PJ, Telford S, Ryan R, et al. Diagnosis of babesiosis: evaluation of a serologic test for the detection of *Babesia microti* antibody. *J Infect Dis* 1994;**169**:923–6.
80. Krause PJ, Telford SR, Spielman A, et al. Comparison of PCR with blood smear and inoculation of small animals for diagnosis of *Babesia microti* parasitemia. *J Clin Microbiol* 1996;**34**: 2791–4.
81. Krause PJ, Telford SR, Spielman A, et al. Concurrent Lyme disease and babesiosis: evidence for increased severity and duration of illness. *JAMA* 1996;**275**:1657–60.
82. Leeflang P, Oomen JMV, Zwart D, et al. The prevalence of *Babesia* antibody in Nigerians. *Int J Parasitol* 1976;**6**:159–61.
83. Leiby DA. Transfusion-associated babesiosis: shouldn't we be ticked off? *Ann Intern Med* 2011;**155**:556–7.
84. Leiby DA. Transfusion-transmitted *Babesia* spp.: bull's-eye on *Babesia microti*. *Clin Microbiol Rev* 2011;**24**:14–28.
85. Levine ND. *Protozoan parasites of domestic animals and of man*. Minneapolis: Burgess; 1966.
86. Levine ND. Taxonomy of the piroplasms. *Trans Am Microsc Soc* 1971;**90**:2–33.
87. Lykins JD, Ristic M, Weisiger RM. *Babesia microti*: pathogenesis of parasite of human origin in the hamster. *Exp Parasitol* 1975; **37**:388–97.
88. Marcus LC, Steere AC, Duray PH, et al. Fatal pancarditis in a patient with coexistent Lyme disease and babesiosis: demonstration of spirochetes in the myocardium. *Ann Intern Med* 1985;**103**:374–6.
89. Marcus LC, Valigorsky JM, Fanning WL, et al. A case report of transfusion-induced babesiosis. *JAMA* 1982;**248**:465–7.
90. Mather TN, Ribeiro JMC, Spielman A. Lyme disease and babesiosis: acaricide focused on potentially infected ticks. *Am J Trop Med Hyg* 1987;**36**:609–14.
91. Mather TN, Telford SR, Moore SI, et al. *Borrelia burgdorferi* and *Babesia microti:* efficiency of transmission from reservoirs to vector ticks (*Ixodes dammini*). *Exp Parasitol* 1990;**70**:55–61.
92. Mathewson HO, Anderson AE, Hazard GW. Self-limited babesiosis in a splenectomized child. *Pediatr Infect Dis* 1984;**3**: 148–9.
93. McQuiston JH, Childs JE, Chamberland ME, et al. Transmission of tickborne agents by blood transfusions: a review of known and potential risks in the United States. *Transfusion* 2000;**40**: 274–84.
94. Meeusen E, Lloyd S, Soulsby EJL. *Babesia microti* in mice: adoptive transfer of immunity with serum and cells. *Aust J Exp Biol Med Sci* 1984;**62**:551–66.
95. Meldrum SC, Birkhead GS, White DJ, et al. Human babesiosis in New York state: an epidemiological description of 136 cases. *Clin Infect Dis* 1992;**15**:1019–23.
96. Miller LH, Neva FA, Gill F. Failure of chloroquine in human babesiosis (*Babesia microti*): case report and chemotherapeutic trials in hamsters. *Ann Intern Med* 1978;**88**:200–2.
97. Mitchell PD, Reed KD, Hofkes JM. Immunoserologic evidence of coinfection with *Borrelia burgdorferi*, *Babesia microti*, and human granulocytic *Ehrlichia* species in residents of Wisconsin and Minnesota. *J Clin Microbiol* 1996;**34**:724–7.
98. Montenegro-James S. Immunoprophylactic control of bovine babesiosis: role of exoantigens of *Babesia*. *Trans R Soc Trop Med Hyg* 1989;**83**(suppl.):85–94.
99. Needham GR. Evaluation of five popular methods for tick removal. *Pediatrics* 1985;**75**:997–1002.
100. Ortiz JM, Eagle RC Jr. Ocular findings in human babesiosis (Nantucket fever). *Am J Ophthalmol* 1982;**93**:307–11.
101. Osorno BM, Vega C, Ristic M, et al. Isolation of *Babesia* spp. from asymptomatic human beings. *Vet Parasitol* 1976;**2**:111–20.
102. Perkins HA, Busch MP. Transfusion associated infections: 50 years of relentless challenges and remarkable progress. *Transfusion* 2010;**50**:2080–99.
103. Persing DH, Herwaldt BL, Glaser C, et al. Infection with a *Babesia*-like organism in northern California. *N Engl J Med* 1995;**332**:298–303.
104. Persing DH, Mathiesen D, Marshall WF, et al. Detection of *Babesia microti* by polymerase chain reaction. *J Clin Microbiol* 1992;**30**:2097–103.
105. Piesman J, Spielman A. Human babesiosis on Nantucket Island: prevalence of *Babesia microti* in ticks. *Am J Trop Med Hyg* 1980; **29**:742–6.
106. Piesman J, Hicks TC, Sinsky RJ, et al. Simultaneous transmission of *Borrelia burgdorferi* and *Babesia microti* by individual nymphal *Ixodes dammini* ticks. *J Clin Microbiol* 1987;**25**:2012–13.
107. Piesman J, Spielman A, Etkind P, et al. Role of deer in the epizootiology of *Babesia microti* in Massachusetts, USA. *J Med Entomol* 1979;**15**:537–40.
108. Popovsky MA, Lindbert LE, Syrek AL, et al. Prevalence of *Babesia* antibody in a selected blood donor population. *Transfusion* 1987; **28**:59–61.
109. Quick RE, Herwaldt BL, Thomford JW, et al. Babesiosis in Washington State: a new species of *Babesia*? *Ann Intern Med* 1993;**119**:284–90.
110. Raju M, Salazar JC, Leopold H, et al. Atovaquone and azithromycin treatment for babesiosis in an infant. *Pediatr Infect Dis J* in press.
111. Raoult D, Soulayrol L, Toga B, et al. Babesiosis, pentamidine, and cotrimoxazole. *Ann Intern Med* 1987;**107**:944.
112. Reiter I, Weiland G. Recently developed methods for the detection of babesial infections. *Trans R Soc Trop Med Hyg* 1989; **83**(suppl.):21–3.
113. Riek RF. Babesiosis. In: Weinman D, Ristic M, editors. *Infectious blood diseases of man and animals*, Vol. 2. New York: Academic Press; 1968. p. 219–68.

114. Rios L, Alvarez G, Blair S. Serological and parasitological study and report of the first case of human babesiosis in Colombia. *Rev Soc Bras Med Trop* 2003;**36**:493–8.
115. Rosner F, Zarrabi MH, Benach JL, et al. Babesiosis in splenectomized adults: review of 22 reported cases. *Am J Med* 1984;**76**: 696–701.
116. Rudzinska MA. Morphological aspects of host-cell-parasite relationships in babesiosis. In: Ristic M, Kreier JP, editors. *Babesiosis*. New York: Academic Press; 1981. p. 87–141.
117. Rudzinska MA, Spielman A, Lewengrub S, et al. Sexuality in piroplasms as revealed by electron microscopy in *Babesia microti*. *Proc Natl Acad Sci U S A* 1983;**80**:2966–70.
118. Ruebush MJ, Hanson WL. Thymus dependence of resistance to infection with *Babesia microti* of human origin in mice. *Am J Trop Med Hyg* 1980;**29**:507–15.
119. Ruebush TK. Human babesiosis in North America. *Trans R Soc Trop Med Hyg* 1980;**74**:149–52.
120. Ruebush TK. Babesiosis. In: Strickland GT, editor. *Hunter's tropical medicine*. Philadelphia: Saunders; 1984. p. 608–11.
121. Ruebush TK, Cassaday PB, Marsh HJ, et al. Human babesiosis on Nantucket Island: clinical features. *Ann Intern Med* 1977; **86**:6–9.
122. Ruebush TK, Chisholm ES, Sulzer AJ, et al. Development and persistence of antibody in persons infected with *Babesia microti*. *Am J Trop Med Hyg* 1981;**30**:291–2.
123. Ruebush TK, Juranek DD, Chisholm ES, et al. Human babesiosis on Nantucket Island: evidence for self-limited and subclinical infections. *N Engl J Med* 1977;**297**:825–7.
124. Ruebush TK, Juranek DD, Spielman A, et al. Epidemiology of human babesiosis on Nantucket Island. *Am J Trop Med Hyg* 1981;**30**:937–41.
125. Ruebush TK, Piesman J, Collins WE, et al. Tick transmission of *Babesia microti* to rhesus monkeys (*Macaca mulatta*). *Am J Trop Med Hyg* 1981;**30**:555–9.
126. Ruebush TK, Rubin RH, Wolpow ER, et al. Neurologic complications following the treatment of human *Babesia microti* infection with diminazene aceturate. *Am J Trop Med Hyg* 1979; **28**:184–9.
127. Ryan R, Krause PJ, Radolf J, et al. Immunoblot assay for detection of *Babesia microti* antibody. *Clin Diag Lab Immunol* 2001;**8**: 1177–80.
128. Scholtens RG, Braff EH, Healy GR, et al. A case of babesiosis in man in the United States. *Am J Trop Med Hyg* 1968;**17**:810–13.
129. Senanayake SN, Paparini A, Latimer M, et al. First report of human babesiosis in Australia. *Med J Aust* 2012;**196**:350–2.
130. Shih C, Liu L, Chung W, et al. Human babesiosis in Taiwan: asymptomatic infection with a *Babesia microti*-like organism in a Taiwanese woman. *J Clin Microbiol* 1997;**35**:450–4.
131. Simpson CF. Phagocytosis of *Babesia canis* by neutrophils in the peripheral circulation. *Am J Vet Res* 1974;**35**:701–4.
132. Skrabalo A, Deanovic A. Piroplasmosis in man: report on a case. *Doc Med Geogr Trop* 1957;**9**:11–16.
133. Smith RD, Kakoma I. A reappraisal of vector control strategies for babesiosis. *Trans R Soc Trop Med Hyg* 1989;**83**(suppl.):43–52.
134. Smith RP, Evans AT, Popovsky M, et al. Transfusion-acquired babesiosis and failure of antibiotic treatment. *JAMA* 1986;**256**: 2726–7.
135. Smith T, Kilbourne FL. Investigation into the nature, causation, and prevention of southern cattle fever. *U S Dept Agr Bur Anim Indust Bull* 1893;**1**:1–301.
136. Spielman A. Human babesiosis on Nantucket Island: transmission by nymphal *Ixodes* ticks. *Am J Trop Med Hyg* 1976;**25**:784–7.
137. Spielman A. Lyme disease and human babesiosis: evidence incriminating vector and reservoir hosts. In: Englund PT, Sher A, editors. *The biology of parasitism*. New York: Alan R. Liss; 1988. p. 147–65.
138. Spielman A, Clifford CM, Piesman J, et al. Human babesiosis on Nantucket Island, U.S.A.: description of the vector, *Ixodes* (Ixodes) *dammini*, n. sp. (Acarina: Ixodidae). *J Med Entomol* 1979; **15**:218–34.
139. Spielman A, Etkind P, Piesman J, et al. Reservoir hosts of human babesiosis on Nantucket Island. *Am J Trop Med Hyg* 1981;**30**: 560–5.
140. Spielman A, Wilson ML, Levine JF, et al. Ecology of *Ixodes dammini* borne human babesiosis and Lyme disease. *Ann Rev Entomol* 1985;**30**:439–60.
141. Steere AC, Malawista SE. Cases of Lyme disease in the United States: locations correlated with distribution of *Ixodes dammini*. *Ann Intern Med* 1979;**91**:730–3.
142. Steketee RW, Eckman MR, Burgess EC, et al. Babesiosis in Wisconsin: a new focus of disease transmission. *JAMA* 1985; **253**:2675–8.
143. Sun T, Tenenbaum MJ, Greenspan J, et al. Morphologic and clinical observations in human infection with *Babesia microti*. *J Infect Dis* 1983;**148**:239–48.
144. Teal AE, Habura A, Ennis J, et al. A new real-time PCR assay for improved detection of the parasite *Babesia microti*. *J Clin Microbiol* 2012;**50**:903–8.
145. Telford SR, Dawson JE, Katavolos P, et al. Perpetuation of the agent of human granulocytic ehrlichiosis in a deer tick-rodent cycle. *Proc Natl Acad Sci U S A* 1996;**93**:6209–14.
146. Telford SR, Gorenflot A, Brasseur P, et al. Babesial infections in man and wildlife. In: Kreirer JP, Baker, JR, editors. *Parasitic protozoa*. 2nd ed. San Diego: Academic Press; 1991.
147. Telford SR, Mather TN, Adler GH, et al. Short-tailed shrews as reservoirs of the agent of Lyme disease and human babesiosis. *J Parasitol* 1990;**76**:681–3.
148. Thompson C, Spielman A, Krause PJ. Coinfecting deer associated zoonoses: Lyme disease, babesiosis, and ehrlichiosis. *Clin Infect Dis* 2001;**33**:676–85.
149. Todorovic RA, Carson CA. Methods for measuring the immunological response to Babesia. In: Ristic M, Kreier JP, editors. *Babesiosis*. New York: Academic Press; 1981. p. 381–410.
150. Tonnetti L, Eder AF, Dy B, et al. Transfusion-transmitted *Babesia microti* identified through hemovigilance. *Transfusion* 2009;**49**: 2557–63.
151. U.S. Food and Drug Administration. *Biological product and hematopoietic cell therapy/product (HCT/P) deviation report: annual summary for Fiscal Year 2008-9*. Silver Spring, Md: USFDA; 2009. (http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/ReportaProblem/BiologicalProductDeviations/ucm169990.htm).
152. Vannier E, Krause PJ. Human babesiosis. *New Engl J Med* 2012;**366**:2397–407.
153. Vannier E, Borggraefe I, Telford SR, et al. Age-associated decline in resistance to *Babesia microti* is genetically determined. *J Infect Dis* 2004;**189**:1721–8.
154. Varde S, Beckley J, Schwartz I. Prevalence of tick-borne pathogens in *Ixodes scapularis* in a rural New Jersey County. *Emerg Infect Dis* 1998;**4**:97–9.
155. Wei Q, Tsuji M, Zamoto A. Human babesiosis in Japan: isolation of *Babesia microti*-like parasites from an asymptomatic transfusion donor and from a rodent from an area where babesiosis is endemic. *J Clin Microbiol* 2001;**39**:2178–83.
156. William H. Human babesiosis. *Trans R Soc Trop Med Hyg* 1980; **74**:157.
157. Wittner M, Lederman J, Tanowitz HB. Atovaquone in the treatment of *Babesia microti* infections in hamsters. *Am J Trop Med Hyg* 1996;**55**:219–22.
158. Wittner M, Rowin KS, Tanowitz HB, et al. Successful chemotherapy of transfusion babesiosis. *Ann Intern Med* 1982;**96**:601–4.
159. Wolf RE. Effects of antilymphocyte serum and splenectomy on resistance to *Babesia microti* infection in hamsters. *Clin Immunol Immunopathol* 1974;**2**:381–94.
160. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006; **43**:1089–94.
161. Wormser GP, Prasad A, Neuhaus E, et al. Emergence of resistance to azithromycin-atovaquone in immunocompromised patients with *Babesia microti* infection. *Clin Inf Dis* 2010;**50**:381–6.
162. Wright IG, Mahoney DF. The activation of kallikrein in acute *Babesia argentina* infections of splenectomized calves. *Z Parasitenkd* 1974;**43**:271–8.
163. Wright IG, Goodger BV, Buffington GD, et al. Immunopathophysiology of babesial infections. *Trans R Soc Trop Med Hyg* 1989;**83**(suppl.):11–13.
164. Wright IG, Mirre GB, Rode-Bramanis K, et al. Protective vaccination against virulent *Babesia bovis* with a low-molecular-weight antigen. *Infect Immun* 1985;**48**:109–13.
165. Young C, Krause PJ. The problem of transfusion-transmitted babesiosis. *Transfusion* 2009;**49**:2548–50.

CHAPTER 219

Malaria

Rosemary M. Olivero • Elizabeth D. Barnett

Malaria is a disease of global importance. An estimated 216 million episodes of malaria resulting in 655,000 deaths occurred in 2010.[167] Worldwide, most deaths from malaria occur in children.[167] Regular transmission of malaria occurs in parts of Africa, Asia, the Middle East, Central and South America, Hispaniola, and Oceania. The infection has been imported by travel to nearly every part of the world. Because of the ubiquity of the infection and the increasing volume of international travel, being able to recognize the signs and symptoms of malaria and knowing about methods of prevention and treatment are important for health care professionals wherever they practice.[42,51]

Malaria usually is transmitted by bites of infected female anopheline mosquitoes. Disease typically results from infection with one or more of the four species of *Plasmodium* (*Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae*) that commonly infect humans. A fifth species, *Plasmodium knowlesi*, which is a malaria parasite of monkeys, has also been identified to cause human malaria.[27] These protozoa have complex life cycles involving arthropod and vertebrate hosts. If untreated, *falciparum* malaria can progress to coma, renal failure, pulmonary edema, and death. Asymptomatic carriage may last decades in the case of infection with *P. malariae*. Relapses are common after infection with *P. vivax* and *P. ovale*. Resistance of the parasites to antimalarial agents and incomplete success in developing and maintaining programs to eradicate the mosquito vectors have contributed to making malaria a persistent worldwide challenge.

HISTORY

Malaria has been known since antiquity and probably affected prehistoric humans. Fossilized mosquitoes have been found in geologic strata 30 million years old. Descriptions of the signs and symptoms of malaria have been found in early Hindu and Chinese writing, and Hippocrates described seasonal and geographic aspects of the disease.[150] Numerous references to malaria occur in literature, ranging from Shakespeare's *The Tempest* to Laura Ingalls Wilder's *Little House on the Prairie*. The name is derived from the Italian *mal aria* ("bad air") based on recognition of the connection between malaria and swamps. One of the first methods of malaria control, draining swamps, was based on this association.

The first malaria treatment known to Europeans was bark of the cinchona tree, which was identified in the early 17th century almost 200 years before the active ingredient (quinine) was isolated. Not until the late 1800s was the vector-borne nature of malaria understood and described; for their roles in this discovery, Laveran, a French military surgeon working in Algeria, and Ross, a British military physician working in India, were awarded Nobel prizes. Further work by many investigators rounded out the current understanding of the life cycle of the malaria parasites and the complex interrelationship with vector mosquitoes.

During the 20th century, parallel efforts directed toward vector control and discovery and development of drugs to treat malaria were undertaken. The development of larvicides and insecticides permitted control of the mosquito vector. The spectacular success of house spraying with dichlorodiphenyltrichloroethane (DDT) was instrumental in eradicating malaria from most of North America and Europe and in decreasing its prevalence significantly in the Mediterranean area, the Middle East, the Far East, and parts of southern Africa.[130] The development of antimalarial drugs, stimulated largely by the need to protect soldiers during World Wars I and II during shortages of the standard antimalarial agent quinine, allowed for successful treatment of malaria cases.

The World Health Organization (WHO) launched a global campaign for eradication of malaria in 1957. Early successes, attributable to the efficacy of DDT and development of new antimalarial agents, subsequently were hindered by resistance of mosquito vectors to DDT and resistance of the parasites to antimalarial drugs. In 1969, the WHO philosophy on malaria was altered to emphasize the development of health services and research. The goals of malaria control since then have been refined and broadened to include implementation of selective and sustainable preventive measures, early diagnosis and prompt treatment, early detection or prevention of epidemics, and strengthening of local infrastructures to allow better understanding of the determinants of local transmission and malaria control.[130,150]

During 2000 through 2010, reductions of more than 50 percent in malaria cases have occurred in almost half (43/99) of the countries with ongoing transmission; eight additional countries showed downward trends. These successes are likely due to renewed global commitment to financing of malaria programs, allowing increased access to insecticide-treated mosquito nets (from 3% in 2000 to 50% in 2011), and indoor residual spraying (from 5% in 2005 to 11% in 2010), as well as increased availability of rapid diagnostic tests and artemisinin-based combination therapies.[167] Reductions in malaria control

have proven most challenging in countries with the most intense transmission, such as Comoros, Malawi, Uganda, and United Republic of Tanzania.[131,167] Ongoing challenges to global malaria elimination include maintaining local control measures; resistance to antimalarial drugs, especially to artemisinin derivatives; changes in climate; and population movement as a result of urbanization, mass displacement of populations, and international travel.[12,80,88,93,167]

Mortality from malaria, particularly in adults, was recently found to be larger than previously estimated, with increases in malaria deaths from 995,000 in 1980 to a peak of 1,817,000 in 2004. Corresponding to the decreases in malaria cases attributed to financing of global malaria programs, mortality rates decreased to 1,238,000 in 2010. The majority of malaria deaths are reported from Africa.[104]

Despite reductions in many areas of the world, in the United States in 2010 there were 1,691 reported cases of malaria, an increase of 14 percent compared with the number reported in 2009; this represents the highest number of cases reported in the United States since 1980. This trend is thought to reflect the steady growth of international travel with inadequate use of appropriate prevention measures by travelers but may also represent increased reporting.[90]

THE ORGANISM

The life cycle of malaria parasites is complex and requires a suitable population of anopheline mosquitoes and infected humans for completion (Fig. 219-1). To begin the cycle, the female anopheline mosquito injects sporozoites along with saliva in preparation for taking a blood meal from a vertebrate host. Sporozoites, the infective stage of *Plasmodium*, remain in the circulation for less than 1 hour and then migrate to the liver, where they invade hepatocytes and multiply asexually. Proliferation within hepatocytes takes approximately 1 week for

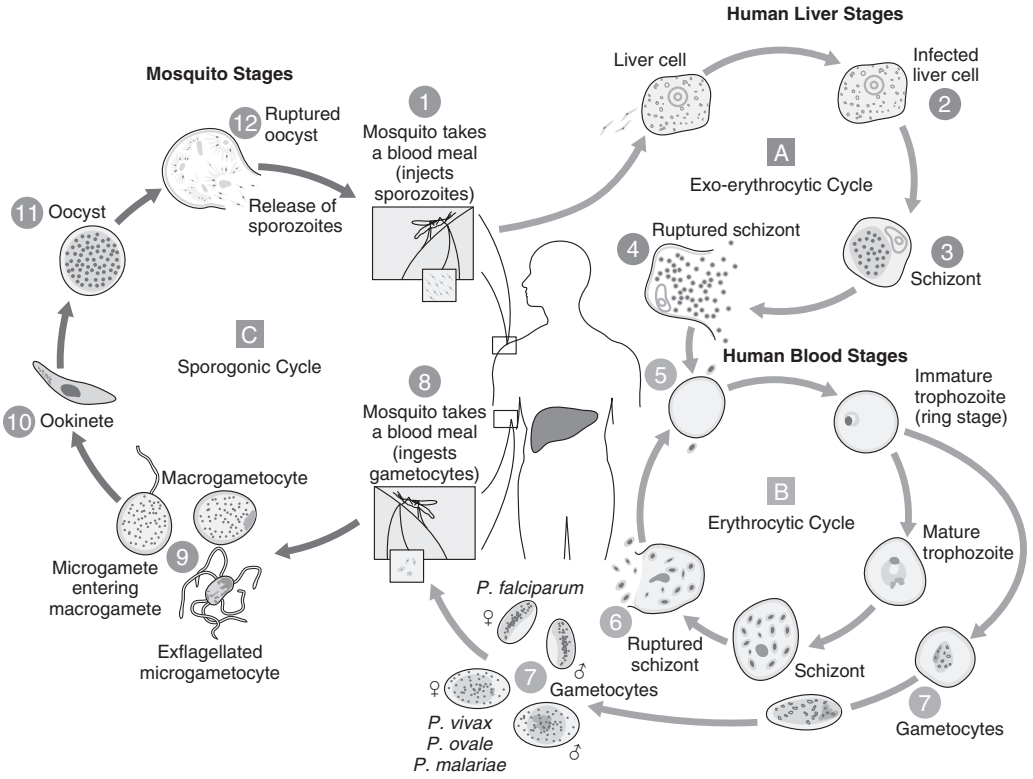

FIGURE 219-1 ■ Life cycle of the human malaria parasites. The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host (1). Sporozoites infect liver cells (2) and mature into schizonts (3), which rupture and release merozoites (4). (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years, later.) After this initial replication in the liver (exoerythrocytic schizogony [**A**]), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony [**B**]). Merozoites infect red blood cells (5). The ring stage trophozoites mature into schizonts, which rupture, releasing merozoites (6). Some parasites differentiate into sexual erythrocytic stages (gametocytes) (7). Blood stage parasites are responsible for the clinical manifestations of the disease. The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal (8). The parasites' multiplication in the mosquito is known as the sporogonic cycle (**C**). While in the mosquito's stomach, the microgametes penetrate the macrogametes, generating zygotes (9). The zygotes, in turn, become motile and elongated (ookinetes) (10) and invade the midgut wall of the mosquito, where they develop into oocysts (11). The oocysts grow, rupture, and release sporozoites (12), which make their way to the mosquito's salivary glands. Inoculation of the sporozoites (1) into a new human host perpetuates the malaria life cycle. (From Centers for Disease Control and Prevention. *Malaria: biology,* <http://www.cdc.gov/malaria/about/biology/index.html> [accessed 11.06.13].)

P. falciparum and *P. vivax* and approximately 2 weeks for *P. malariae*. At the end of this period, mature tissue schizonts rupture and release thousands of merozoites, which then invade red blood cells (RBCs). *P. vivax* and *P. ovale* have a second type of exoerythrocytic form, the hypnozoite, which can remain dormant for weeks to years. Dormant hypnozoites may develop weeks, months, or years later into merozoites, which then can enter RBCs and cause relapse of malaria. The factors that influence which exoerythrocytic form develops are not understood.

Merozoites released from tissue schizonts invade RBCs, where the erythrocytic phase of the life cycle occurs. Two pathways exist in the erythrocytic, or blood, phase: asexual and sexual. In the asexual phase, development of the parasite begins with the youngest stage, the trophozoite or ring form. The parasite undergoes nuclear division to form schizonts and then merozoites in the asexual multiplication process, called *erythrocytic schizogony/merogony*. Lysis of RBCs releases the merozoites, which invade other RBCs, perpetuating the asexual erythrocytic cycle. The cycle continues until interrupted by treatment or by the host's immune response.

In the sexual phase, subpopulations of merozoites in the erythrocytic phase differentiate into gametocytes, or sexual forms, which then are available for ingestion by mosquitoes to complete the life cycle within the mosquito. Female macrogametocytes and male microgametocytes appear in the circulation within 3 to 15 days of the onset of symptoms. Gametocytes of *P. vivax* may appear in 4 days, whereas gametocytes of *P. falciparum* may require 10 days for development.

In the stomach (midgut) of the mosquito, male gametocyte nuclei divide into four to eight nuclei and form motile gametocytes that fertilize the female gametocytes. The zygotes become motile ookinetes that migrate through the wall of the midgut, attach to its outer surface, and form oocysts. The oocysts rupture 9 to 14 days later and release sporozoites that invade the mosquito salivary glands, where they are ready for inoculation into the next vertebrate host.

EPIDEMIOLOGY

Transmission of malaria occurs in large parts of Africa, the Indian subcontinent, Southeast Asia, the Middle East, Oceania, and Central and South America (Fig. 219-2). Although indigenous transmission of malaria has been eradicated almost completely from the United States, Canada, northern Europe, most of the Caribbean, parts of South America, Israel, Turkmenistan, Morocco, the United Arab Emirates, Armenia, Lebanon, Reunion, Singapore, Hong Kong, Japan, Korea, Taiwan, Brunei, and Australia, many cases of imported malaria occur in these countries each year.[167]

P. falciparum is the major malaria species in sub-Saharan Africa and the island of Hispaniola, with *P. malariae* assuming a more minor role. *P. vivax* occurs alongside *P. falciparum* in the Indian subcontinent, Central and South America, Mexico, Southeast Asia, and Oceania. *P. ovale* occurs mainly in Africa. *P. vivax* occurs rarely in sub-Saharan Africa because most Africans lack the Duffy blood group antigen necessary for parasite invasion.[73] *P. knowlesi* has been isolated only in Southeast Asia.[167]

TRANSMISSION

Epidemiologic Terminology

Patterns of transmission of malaria include stable endemic malaria (natural transmission occurring over many years, with a predictable incidence of illness and prevalence of infection) and unstable malaria (transmission rates vary from year to year, and immunity is low, with a greater likelihood of epidemics occurring). The degree of endemic malaria is based on the parasite rate in children 2 to 9 years old. Types of endemic malaria include hypoendemic (parasite rate of 0% to 10%), mesoendemic (parasite rate of 11 to 50%), hyperendemic (parasite rate consistently >50%, with a high proportion of adults having enlarged spleens), and holoendemic (parasite rate consistently >75%, with a low proportion of adults having enlarged spleens).[150]

Autochthonous malaria is acquired locally and may be indigenous or introduced. Introduced malaria may occur when migrant populations with asymptomatic infection provide blood meals for feeding anopheline mosquitoes under conditions that allow for the life cycle to be completed in the mosquito and enable the mosquito to infect others. Imported malaria cases may occur in nonendemic areas but result from infection in an endemic area. Induced malaria is acquired by exposure to infected blood, such as from blood transfusion, needlestick injury, laboratory accident, or, historically, medical treatments. Cryptic malaria cases are cases for which no explanation can be found and no epidemiologic link to other cases can be identified.

Mosquito-borne Transmission

The most typical means of transmission of malaria is through the bite of an infected female anopheline mosquito. Although more than 450 species of anopheline mosquito exist, only approximately 60 have been shown to be effective vectors of malaria.[24] A population of infected humans is necessary to sustain transmission because of the short life span of mosquitoes (5 to 20 days) and the long incubation period required in the mosquito (8 to ≥10 days).

Bloodborne Transmission

Transmission of malaria via blood transfusion is well documented.[103,116] Transmission also may occur through organ donation or needlestick injury.[53,163] Relapses of malaria cannot occur with bloodborne transmission, even if the infecting species is *P. vivax* or *P. ovale*, because the infection is produced by the transmission of infected RBCs rather than forms that invade the liver.

FIGURE 219-2 ■ Malaria-endemic countries in the Western **(A)** and Eastern **(B)** Hemispheres, 2007. (See companion Expert Consult website for color version of part **B**). (From Centers for Disease Control and Prevention. *Infectious diseases related to travel,* <http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/malaria> [accessed 10.05.12].)

Congenital Malaria

Infants can acquire malaria from their mothers during pregnancy. Transplacental transmission of parasites has been proposed as the most likely route of transmission, although breakdown of placental barriers allowing transmission of maternal blood cells to the infant during labor or delivery also has been suggested as a mechanism of transmission.[61,89,99] *P. vivax* most often is associated with this phenomenon, but it may occur with all species. Congenital malaria is less likely to occur in infants of semi-immune mothers because of passage of maternal antibody at the time of birth. As with transfusion-associated malaria, relapses do not occur.

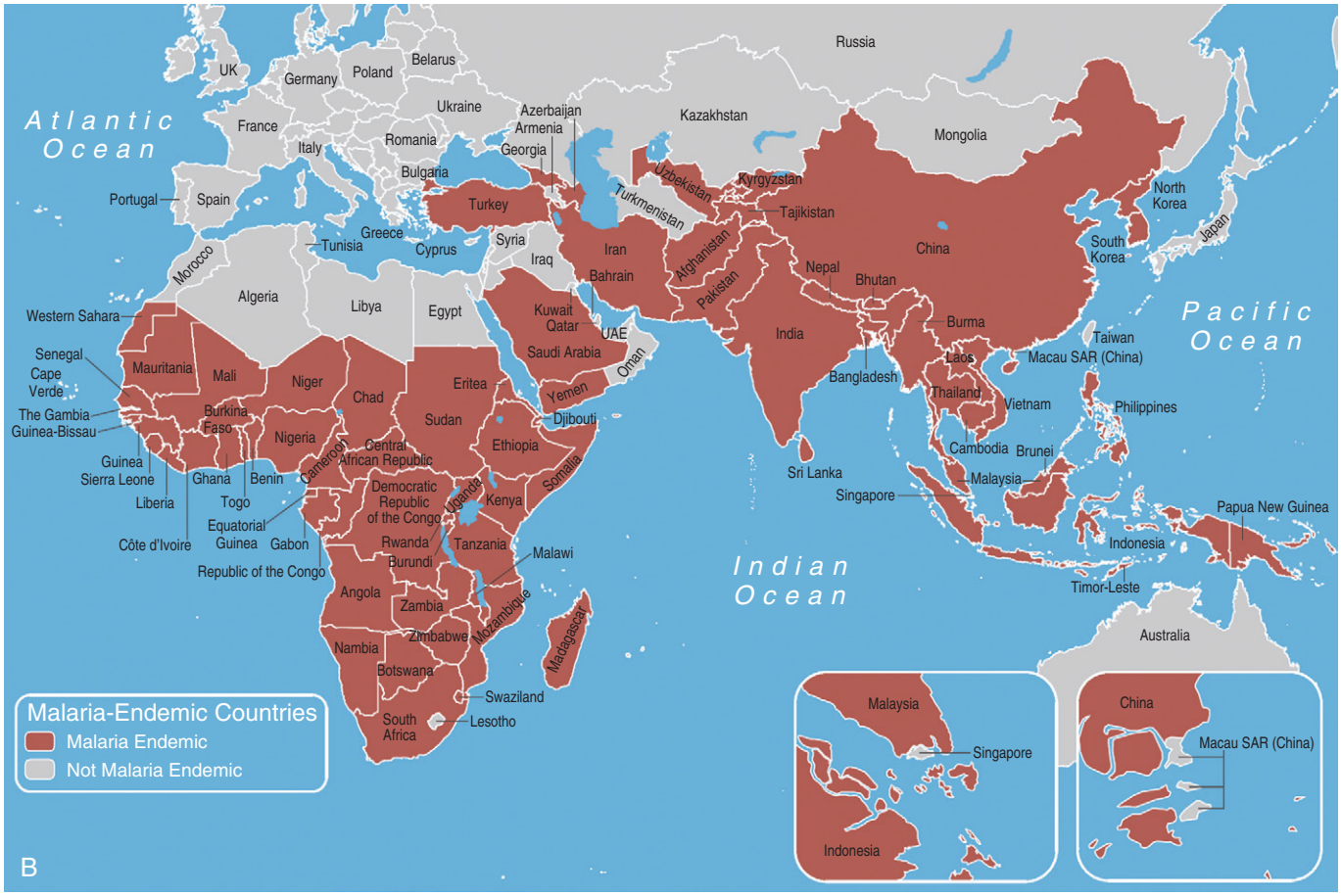

FIGURE 219-2, Cont'd

Cryptic Malaria

The category of cryptic malaria includes cases for which no source of infection can be identified. Typical cases include confirmed malaria in U.S. residents who have never traveled to or resided in malarial areas, who have not received blood transfusions, and who are not linked epidemiologically with other cases.[15,78,163] Airport malaria, one kind of cryptic malaria, occurs in proximity to international airports and is thought to occur when mosquitoes arriving with airplanes from endemic areas infect individuals working in or living near airports.[72]

HOST-PARASITE INTERACTION

The intensity of transmission of malaria depends on factors that affect the density of vectors and the extent of vector-human contact. Transmission of malaria may be continuous or seasonal, or it may depend on local site-specific factors, such as the presence of irrigation projects or intermittent flooding. Mosquito vectors differ in their efficiency in transmitting malaria; the principal vector in sub-Saharan Africa, *Anopheles gambiense*, is known for being a highly effective vector. Variations in climate may affect the viability of mosquitoes.

The incidence and severity of malaria are affected by the intensity of exposure, the presence of immunity, and genetic factors. Distinction must be made between malaria infection (presence of parasitemia) and malaria illness. A major puzzle in malaria is why individuals with similar degrees of parasitemia may exhibit radically different clinical manifestations.

Individuals who reside in endemic areas and are exposed continually to infected mosquitoes acquire some degree of immunity to malaria illness. Adults in these areas continue to become infected, but they have lower levels of parasitemia. Infants born to mothers with acquired immunity may be protected transiently by placental passage of maternal antibody. The highest incidence of infection with malaria occurs in infants and young children who are no longer protected by maternal antibody but who are too young for significant acquired immunity to have developed. Children also are more susceptible to certain manifestations of severe illness, such as cerebral malaria. The lack of acquired immunity in infants and young children accounts partly for their increased risk for acquiring disease and having severe manifestations. Acquired immunity diminishes during pregnancy, and pregnant women are at high risk for development of severe complications of malaria. Individuals in the population who remain asymptomatic but harbor gametocytes in their blood are reservoirs of infection when bitten by mosquitoes. With the exception of *P. knowlesi*, which infects monkeys, there are no significant animal reservoirs of malaria.

Clinical manifestations of malaria may be severe in nonimmune patients. Malaria is the most common life-threatening infection acquired by travelers to malaria-endemic regions. Individuals with acquired immunity who then leave endemic areas for long periods may lose their immunity and be at risk for severe disease if reexposed.

Genetic factors determine the risk for acquiring malaria and having severe infection. Individuals who have a Duffy-negative blood type lack the most important specific receptors for invasion of the merozoites of *P. vivax*, and these individuals have markedly reduced susceptibility to infection with this species.[73,100] This resistance is the basis for the low incidence of vivax malaria in Africa. Specific human leukocyte antigens present in individuals from West Africa may protect against the development of severe complications of malaria, including cerebral malaria and severe anemia. The best-known example of the relationship between malaria and genetics is the association between sickle hemoglobinopathies and protection against severe falciparum malaria. This balanced polymorphism is thought to have helped ensure survival of the gene for hemoglobin S in the population because of the selective advantage provided on a population basis to those who are heterozygous for sickle-cell disease. Individuals with sickle hemoglobinopathies still may be infected and manifest signs and symptoms of malaria, although the risk for acquiring severe malaria or dying of malaria may be 60-fold to 70-fold less in children with hemoglobin AS than in children with hemoglobin AA.[75]

PATHOPHYSIOLOGY

Pathophysiologic changes in malaria are caused by destruction of RBCs, production of cytokines, stimulation of intravascular synthesis of nitric oxide, and sequestration of infected erythrocytes.

Lysis of RBCs leads to anemia, which may be severe, and its attendant hemodynamic consequences. Anemia may develop as a result of hemolysis, impaired erythropoiesis, or bone marrow depression secondary to folic acid deficiency.[119] Intravascular hemolysis may be so severe that it results in pronounced hemoglobinuria (blackwater fever), which may be a precipitating event in the development of renal failure. This complication has been noted in association with treatment using quinine. Hematopoiesis is suppressed during acute infection, and such suppression may not be reversed as readily in iron-deficient individuals, contributing to chronic anemia.

Cytokines such as tumor necrosis factor (TNF) and interleukin-1 have important roles in the pathogenesis of malaria.[10,76] Severe disease has been associated with higher concentrations of TNF, and TNF polymorphisms may play a role in the development of specific complications.[95,96] Parasite factors responsible for release of cytokines have not been identified. A possible mechanism for the role of TNF in malaria pathogenesis is that the binding of TNF to its receptors is known to cause signaling to amplify cytokines of inflammatory cascades.[125] Another possible role of TNF in malaria is to stimulate nitric oxide, a short-lived neurotransmitter. Nitric oxide may have a role in cerebral malaria, and its transient nature may provide a partial explanation for the complete recovery noted in some patients with severe cerebral malaria. TNF and nitric oxide have harmful and beneficial roles in the pathogenesis of malaria; both have been shown to be correlated with clearance of parasites and eventual recovery and the severity of illness.

Sequestration of infected RBCs has long been thought to contribute to the clinical manifestations of malaria, particularly that caused by *P. falciparum*. Late-stage parasites induce host cells to develop knobs on the surface of erythrocytes that facilitate adherence of these cells to vascular endothelium. The effects of these sequestered RBCs on the perfusion, nutrition, and oxygenation of surrounding tissues may be responsible for the complications of *P. falciparum* infection, including cerebral malaria, renal failure, and watery diarrhea.[106,122] Consumption of glucose by metabolically active late-stage parasites contributes to hypoglycemia and lactic acidosis. Despite these changes, the majority of histopathologic appearances of tissue is remarkably benign, consistent with the reversible nature of the changes and supportive of the role of cytokines and secondary messengers in the pathogenesis of complications of cerebral malaria.[75,150] Recent postmortem microscopy of brain sections from adults who died of malaria confirmed that sequestration of cerebral microvasculature was significantly higher in patients with cerebral malaria and was also correlated with deeper levels of premortem coma and shorter time to death.[123]

CLINICAL MANIFESTATIONS AND LABORATORY FINDINGS

The clinical manifestations of malaria depend on the species of malaria parasite causing the infection, the immune status of the individual, the mode of transmission of infection, whether the individual was taking prophylaxis, and host immune factors (Table 219-1). Acute malaria generally is understood to refer to the signs and symptoms associated with disease caused by infection with malaria parasites.

Recurrent infections are of three types: relapse, recrudescence, and reinfection. Relapses occur as a result of delayed maturation of the dormant liver stages (hypnozoites) of *P. vivax* or *P. ovale*. Recrudescence occurs when parasitemia caused by the same parasite responsible for the initial infection recurs after clearance or a significant reduction in the initial parasitemia. It occurs most commonly with *P. falciparum* because of drug resistance. Reinfection with different parasites and infection with more than one type of *Plasmodium* occur especially in areas with a high intensity of transmission. Persistent infection is noted with *P. malariae*. Hypnozoites have not been identified with *P. malariae*, so the organism is thought to persist as a low-level parasitemia that can exist for years without causing symptoms.

A classic description of malaria includes features of the malaria paroxysm resulting from the lysis of parasitized RBCs and release of merozoites into the circulation at

TABLE 219-1 **Characteristics of the *Plasmodium* Species Responsible for Human Malaria**

Characteristic	Species				
	P. FALCIPARUM	*P. VIVAX*	*P. OVALE*	*P. MALARIAE*	*P. KNOWLESI*
Incubation period in days (range)	12 (8-25)	14 (8-27)	17 (15-≥18)	28 (15-≥40)	11 (9->12)
Periodicity of febrile attacks (hr)	None	48	48	72	24
Earliest appearance of gametocytes (days)	10	3	?	?	?
Relapse	No	Yes	Yes	No	No
Duration of untreated infection (yr)	1-2	1.5-4	1.5-4	3-50	?
RBC preference	Younger cells (but can invade cells of all ages)	Reticulocytes	Reticulocytes	Older cells	?
Characteristic morphology	Ring forms Multiply infected cells Banana-shaped gametocytes	Schüffner dots Enlarged RBCs	Schüffner dots Enlarged RBCs	Normal-sized cells Band or rectangular forms of trophozoites	Ring forms Occasional multiply infected cells Band forms

RBC, Red blood cell.

the completion of asexual reproduction. The paroxysm is characterized by fever and chills accompanied by constitutional symptoms of headache, body ache, fatigue, dizziness, and malaise. Gastrointestinal symptoms include nausea and vomiting, abdominal pain, and diarrhea. Cough and dyspnea may accompany an attack. Although periodicity of the paroxysms in primary attacks is thought to be pathognomonic for malaria species, this periodicity may take several days to become established, may not occur at all in asynchronous infections, or may be modified by previous immunity or treatment.

In children, fever and headache may be the sole symptoms or gastrointestinal symptoms may predominate. Physical signs of malaria include pallor, jaundice, and hepatosplenomegaly. Rash and lymphadenopathy typically are not associated with malaria, although malaria may precipitate recrudescence of latent herpes infections.

Anemia is the most common abnormality in malaria. Thrombocytopenia is common and may be the first manifestation in patients with uncomplicated malaria.[75] Leukopenia also may occur, but high white blood cell counts are less usual and should provoke investigation for other conditions. Liver function test abnormalities may be present and can be mistaken for evidence of hepatitis, especially in patients with jaundice and tender hepatosplenomegaly. Hypoglycemia occurs frequently with falciparum malaria and may develop before or as a consequence of treatment with quinine. When present in children before treatment, hypoglycemia is associated with a poor prognosis.[149] Hyponatremia may occur as part of the syndrome of inappropriate secretion of antidiuretic hormone in some patients. Serum creatinine and blood urea nitrogen values may be elevated transiently or may increase significantly with acute renal failure.

Malaria stimulates a polyclonal increase in immunoglobulins associated with rapid production of malaria-specific antibodies and reduced complement levels. False-positive tests for syphilis, rheumatoid factor, heterophil agglutinins, and cold agglutinins may occur.[150]

Severe and Complicated Malaria

Nonimmune individuals are most susceptible to the severe complications of falciparum malaria, which include cerebral malaria, pulmonary failure or acute respiratory distress syndrome, renal failure, and severe anemia.[85] Hypoglycemia and metabolic acidosis may occur. Falciparum malaria in nonimmune patients should be considered a medical emergency, and treatment of *P. falciparum* should be initiated in all ill patients with malaria until the species can be confirmed. The highest mortality is seen in subjects who have a combination of severe respiratory distress, impaired consciousness, and severe anemia. The Centers for Disease Control and Prevention (CDC) and WHO criteria for the diagnosis of severe malaria are outlined in Table 219-2.

Severe Anemia

Children younger than 1 year old, especially in sub-Saharan Africa, are those most likely to experience severe malarial anemia. This complication is thought to occur most often in areas with year-round transmission. Clinical consequences of anemia are determined by rate of development of anemia and by severity of anemia, with high risk for complications as hemoglobin decreases to less than 5 g/dL.[160]

TABLE 219-2 **Manifestations of Severe Malaria**

Centers for Disease Control Definition*	World Health Organization Definition[165]
Cerebral malaria	Prostration, impaired consciousness, coma (cerebral malaria) or multiple convulsions
Severe anemia	Severe anemia
Acute renal failure	Renal impairment
Hemoglobinuria	Hemoglobinuria
Hypoglycemia	Hypoglycemia (<40 mg/dL)
Metabolic acidosis	Acidosis or hyperlactatemia
Hypotension caused by cardiovascular collapse	Circulatory collapse or shock
Acute respiratory distress syndrome	Respiratory distress or pulmonary edema
Coagulopathy	Abnormal bleeding
Hyperparasitemia (>5% infected RBCs)	Hyperparasitemia (>2% infected RBCs in low-transmission areas or >5% in high-transmission areas
	Jaundice
	Failure to feed

*From www.cdc.gov/malaria/about/disease/html#severe.
RBCs, Red blood cells.

Hypoglycemia

Hypoglycemia (blood glucose <40 mg/dL) may be present on initial evaluation in 20 percent of children with severe malaria and is associated with a poor prognosis. The etiology of pretreatment hypoglycemia is thought to be a combination of parasite consumption of glucose and inadequate gluconeogenesis in the liver. Hypoglycemia also can occur as a result of treatment, most typically with quinine. Intravenous or oral administration of quinine may lead to hypoglycemia by stimulating insulin secretion. Pregnant women seem to be especially susceptible to this complication.

Acid-Base Changes

Metabolic acidosis is a marker of severity and clinically may be manifested as hyperpnea. Acidosis often is associated with hypoglycemia. Fluid resuscitation and treatment with antimalarial drugs often result in rapid resolution of acidosis, although persistence of acidosis may occur in patients who eventually die of malaria.

Renal Complications

Acute renal failure is a potentially life-threatening consequence of acute malaria that occurs more commonly in adults than in children. It typically is oliguric and often is reversible if the patient can be supported by dialysis through the oliguric phase. Acute renal failure is a rare development in residents of endemic areas and long has been thought to occur more frequently in patients treated with quinine or quinidine (blackwater fever). The histologic changes resemble those of acute tubular necrosis.

Nephrotic syndrome and chronic renal failure occur more frequently in areas where malaria is endemic and usually are associated with infection with *P. malariae.* Symptoms occur in individuals younger than 15 years old in approximately half the cases, with gradual progression to renal failure over 3 to 5 years. Most patients have asymptomatic proteinuria and the gradual development of hypertension and deterioration in renal function. Adults more commonly have hematuria and azotemia; adults and children may have hematuria. The disease does not respond to antimalarial agents. Treatment with corticosteroids, cyclophosphamide, and azathioprine has had variable results, with remission occurring only in patients with mild changes on renal biopsy.[150]

Respiratory Complications

Pulmonary edema typically develops late in the course of severe malaria when other complications are already present, and it may occur when parasitemia has resolved and the patient appears to be clinically improving. Pulmonary edema occurs more commonly in adults than in children. The pathogenesis is consistent with capillary leak syndrome. Supplemental oxygen or mechanical ventilation with positive end-expiratory pressure may be necessary to manage respiratory complications. Because of the presence of alveolar macrophages, there is a well-characterized pulmonary inflammatory response that leads to respiratory distress in severe malaria.[113] Respiratory distress is the most dangerous presentation of severe malaria, especially in combination with severe anemia and cerebral malaria.[49]

Cerebral Malaria

Cerebral malaria is the most common complication of falciparum malaria in children and occurs most often in children 3 to 6 years old.[63,120] In general, it presents as alteration of consciousness and diffuse encephalopathy with prolonged multiple seizures in a patient with no other explanation for the condition. Patients may be comatose without response to stimuli and may assume an opisthotonic posture. Generalized convulsions may occur, but focal findings are uncommon. Serial electroencephalographic monitoring of 65 Kenyan children uncovered a large range of clinical and electrographic seizures with diffuse background slow wave activity, which are nonspecific features; electrical seizure activity consistently arose from the posterior temporoparietal region.[28] Intracranial pressure often is increased in children with cerebral malaria, but a relationship between the presence of increased intracranial pressure and morbidity and mortality has not been established. Factors associated with neurologic sequelae include prolonged coma, severe anemia, and multiple seizures. Mortality rates range from 15 to 30 percent of affected children; most survivors recover completely, but approximately 10 percent may have neurologic sequelae. The most common neurologic sequelae in children noted in a study in the Gambia were hemiplegia, cortical blindness, aphasia, and ataxia.[14]

Many factors, including hypoglycemia, anemia, microvascular obstruction, acidosis, and elaboration of inflammatory mediators, contribute to the syndrome of cerebral malaria. Histopathologic features are minor, with occasional hemorrhages and perivascular infiltrates.

Hyperreactive Malarial Syndrome (Tropical Splenomegaly Syndrome, Hyperreactive Malarial Splenomegaly)

Hyperreactive malarial syndrome is characterized by massive splenomegaly, high concentrations of total serum IgM and malarial antibodies of multiple immunoglobulin classes, and clinical and immunologic response to antimalarial agents.[170] Hyperreactive malarial syndrome is correlated with malaria endemicity, with an incidence ranging from 0.5 to 80 percent of the adult population. The pathogenesis is unknown but seems to involve chronic exposure to malaria, resulting in chronic stimulation of the immune system and genetic factors. Findings on physical examination include a huge spleen (>10 cm below the costal margin) and an enlarged liver. Laboratory findings include anemia and an increased reticulocyte count, elevated IgM (>2 standard deviations above the mean), and high antibody levels of antimalarial antibodies.[143] Some patients may have thrombocytopenia or neutropenia. Patients may have an increased risk for acquiring bacterial infections, and some researchers have suggested that hyperreactive malarial syndrome is a premalignant condition.[7] Lifelong treatment with antimalarial agents is the treatment of choice for patients who reside in endemic areas.[150] Treatment of patients who have left endemic areas has not been standardized; a publication including several case reports shows success with a single treatment course of antimalarial agents followed by close monitoring of the size of the spleen.[143]

MALARIA IN SPECIAL POPULATIONS

Malaria in Children

The signs and symptoms of malaria in children range from asymptomatic infection to life-threatening illness. Severe anemia is most likely to develop in children younger than 2 years old, whereas cerebral malaria is most likely to occur in older children (mean age 3.5 years). The greatest burden of disease is borne by infants and young children, although the impact of disease on adolescents has not been studied extensively. Malaria in pregnant adolescents may be of particular concern.[77]

In endemic areas, lack of diagnostic facilities, limited resources, and encroaching drug resistance complicate the ability to make a rapid, accurate diagnosis and provide expedient treatment to children with malaria. Malaria often is difficult to distinguish from other common illnesses such as pneumonia. Clinical algorithms that can distinguish children with malaria from children with pneumonia or other illnesses have been developed and studied but do not eliminate completely the overlap with conditions having similar clinical manifestations.[126,127,133] Risk factors for a fatal outcome in children with malaria were studied in Kenya; the presence of coma or respiratory distress, or both, at initial evaluation identified children at high risk for dying.[92,49]

Imported malaria occurs in children in many nonendemic countries. The diagnosis often is delayed because of lack of consideration of malaria as a cause of illness and unfamiliarity with the disease. In children with acquired immunity, the signs and symptoms of disease may be subtle and nonspecific but fever or a history of fever is universal.[36,84] Other symptoms include anorexia, vomiting, diarrhea, headache, lethargy, and abdominal pain. Laboratory findings include anemia, thrombocytopenia, and leukopenia. The diagnosis of malaria should be considered in every child with fever or a history of recent fever who has visited an area where malaria occurs.

Congenital Malaria

All four types of human malaria can be transmitted congenitally, but the disease most often is associated with *P. vivax*. Congenital malaria has not been reported with *P. knowlesi*. That congenital malaria is not seen more frequently is due partly to the effective barrier function of the placenta. Congenital malaria develops in infants of approximately 0.1 percent of immune mothers and 10 percent of nonimmune mothers in endemic areas, although placental infection occurs in one third of pregnant women.[67] In endemic areas, distinguishing malaria acquired congenitally from malaria acquired by transmission from mosquitoes is difficult. In non–malaria-endemic countries, congenital malaria is very rare; five cases have been reported in the United States since 2000.[18]

The onset of symptoms is insidious and usually occurs at 2 to 8 weeks of age. The typical malaria paroxysm is absent, with the infant instead having poor feeding, fever, vomiting, diarrhea, irritability, and hepatosplenomegaly on physical examination.[61] The most common laboratory finding is anemia, but thrombocytopenia and hyperbilirubinemia also occur. Therapy for the infecting species of malaria is curative, and the infant does not need treatment of the exoerythrocytic stages of the parasite (although the mother does).

Malaria in Pregnancy

The effects of malaria on the mother depend on the degree of immunity that she has attained and her parity.[99] Relapses and recrudescence of malaria are common during pregnancy, probably because of the immunosuppression associated with the pregnant state. Malaria can exacerbate the anemia occurring during pregnancy, and hypoglycemia and renal insufficiency may complicate falciparum malaria during pregnancy.[48]

Placental infection during pregnancy may be associated with low birth-weight, particularly in primigravidas. Vivax malaria has been linked to maternal anemia and low birth-weight in multigravidas and primigravidas.[109] Severe falciparum malaria is harmful to the fetus because of fetal tachycardia and distress secondary to maternal fever; disruption of maternal-fetal blood flow and exchange of metabolic substrates by malaria parasites trapped in the placenta; and the potential for reduction in fetal glucose supply, which may be exacerbated by treatment with quinine, a drug that is able to stimulate the release of insulin.[82] Heavy infection of the placenta interferes with transfer of tetanus antibodies from the mother to the fetus, but the effect on other antibodies is unknown.[13]

Prompt treatment of pregnant women with severe malaria is critical to survival of the mother and the fetus.

DIAGNOSIS

The most important first step in establishing the diagnosis of malaria is to consider the diagnosis in all individuals with febrile illness, especially febrile individuals with a history of travel to endemic areas. Having a high index of suspicion for the diagnosis of malaria cannot be overemphasized. Failure to diagnose and treat malaria promptly contributed to fatal outcomes in U.S. civilians who died of malaria between 1963 and 2001.[107] In one series of fatal falciparum malaria cases in the United States, 40 percent of patients were not recognized as having malaria during their initial contact with a physician.[46] Manifestations of the disease are most classic in nonimmune individuals or in individuals in areas where malaria transmission is seasonal. Signs and symptoms of disease may be nonspecific in semi-immune individuals, those who have received malaria prophylaxis, or individuals who have been partially treated.

In nonendemic areas, a history of travel to an endemic area should suggest the diagnosis in all individuals with a febrile illness, regardless of the accompanying signs and symptoms.[164] Common diagnoses mistakenly assigned to patients ultimately determined to have malaria include gastroenteritis and viral syndrome. The course of disease may be modified by exposure to antimalarial drugs, such as agents that may have been used for prophylaxis, and the incubation period may be prolonged after the administration of antimalarial chemoprophylaxis. Because malaria may be transmitted by blood transfusion or an organ transplant, may be congenitally acquired, and, rarely, occurs cryptically, the diagnosis also should be considered in patients with compatible signs and symptoms of malaria, anemia, or thrombocytopenia and no other explanation for their illness.

Microscopy is the technique used most commonly for establishing the diagnosis of malaria. Rapid diagnostic tests to detect parasite antigens are important tools in centers lacking expertise in malaria diagnostic microscopy. Other alternatives to microscopic diagnostic techniques for malaria include tests using fluorescent microscopy, DNA probes, polymerase chain reaction (PCR), antibody detection, and flow cytometry. Only a few of these methods meet the requirements of low cost, high reliability and reproducibility, and rapid turnaround time. Some of them are suitable for use in field conditions, however, and have been used in endemic areas and for self-diagnosis.

Diagnosis of cerebral malaria is largely clinical. Patients with cerebral malaria typically do not have marked cerebrospinal fluid (CSF) pleocytosis, differentiating this entity from bacterial meningitis. In comparing CSF parameters in children with cerebral malaria to those with viral encephalitis, those with cerebral malaria have a lower CSF glucose level and higher protein and lactate dehydrogenase (LDH) levels, CSF-to-blood LDH ratio, and CSF-to-serum adenosine deaminase ratio.[65]

Microscopy

Microscopy is the gold standard for establishing the diagnosis of malaria. Identification of typical parasite forms by an experienced microscopist is the mainstay of diagnosis worldwide (Figs. 219-3 through 219-7). There are many advantages of using light microscopy: it can be performed at low cost, it can be done rapidly, it allows identification of the infecting species and estimation of parasite load, and it can be performed with a small sample of blood. The major disadvantage of microscopy is the need for an experienced microscopist. In settings where malaria is not endemic, the need for specialists to review microscope slides may lead to a delay in establishing the diagnosis.

Thin smears are the most useful in diagnosing malaria. They are easy to prepare, with only a single drop of blood required. The drop of blood is placed at one end of the slide, and the edge of a second slide is placed at the edge of the blood smear and drawn across the slide.[114] RBC morphology is preserved by methanol fixation, so invasion of large RBCs by the parasites can be identified and speciation of the organism is possible (see Table 219-1). Oil-immersion magnification (1000×) should be used for viewing the slide because many young asexual intraerythrocytic parasites are only 2 to 3 μm in diameter and may be missed when using high-dry (440×) magnification. The microscopist begins by looking at the thin edge of the blood film farthest from where the drop of blood was placed. Giemsa stain is preferred to Wright stain when available because it preserves details such as Schüffner dots in *P. vivax* and *P. ovale* infections. When individual RBCs can be seen at low magnification (100×), switching to oil immersion allows examination for parasites within the cells. The major disadvantage of thin films is low sensitivity. With low parasite loads (<100 to 300/μL), the amount of blood on the smear may be too small to detect the parasites.

Thick smears have greater sensitivity than thin smears as a result of the larger quantity of blood used. Because the RBCs are lysed during preparation of the slide, the types of cells containing parasites cannot be identified. To make a thick smear, a drop of blood is placed on the slide and spread in a circle. The slide is stained without using methanol fixation so that RBCs lyse.

Estimating parasite density is useful for assessing parasite density and for evaluating response to therapy. The density of parasites can be determined on thick or thin smears.[75] When thin smears are used, the proportion of RBCs infected is counted while the smear is viewed under an oil-immersion lens.

Fluorescent Microscopy

The quantitative Buffy coat test relies on identification of parasitized RBCs stained with acridine orange in the RBC layer of centrifuged blood.[147] Experienced personnel can perform the test rapidly, but the reagents are costly when compared with those needed for microscopy and the species of parasite cannot be identified. If a fluorescent microscope is available, identification of

Text continued on p. 2935

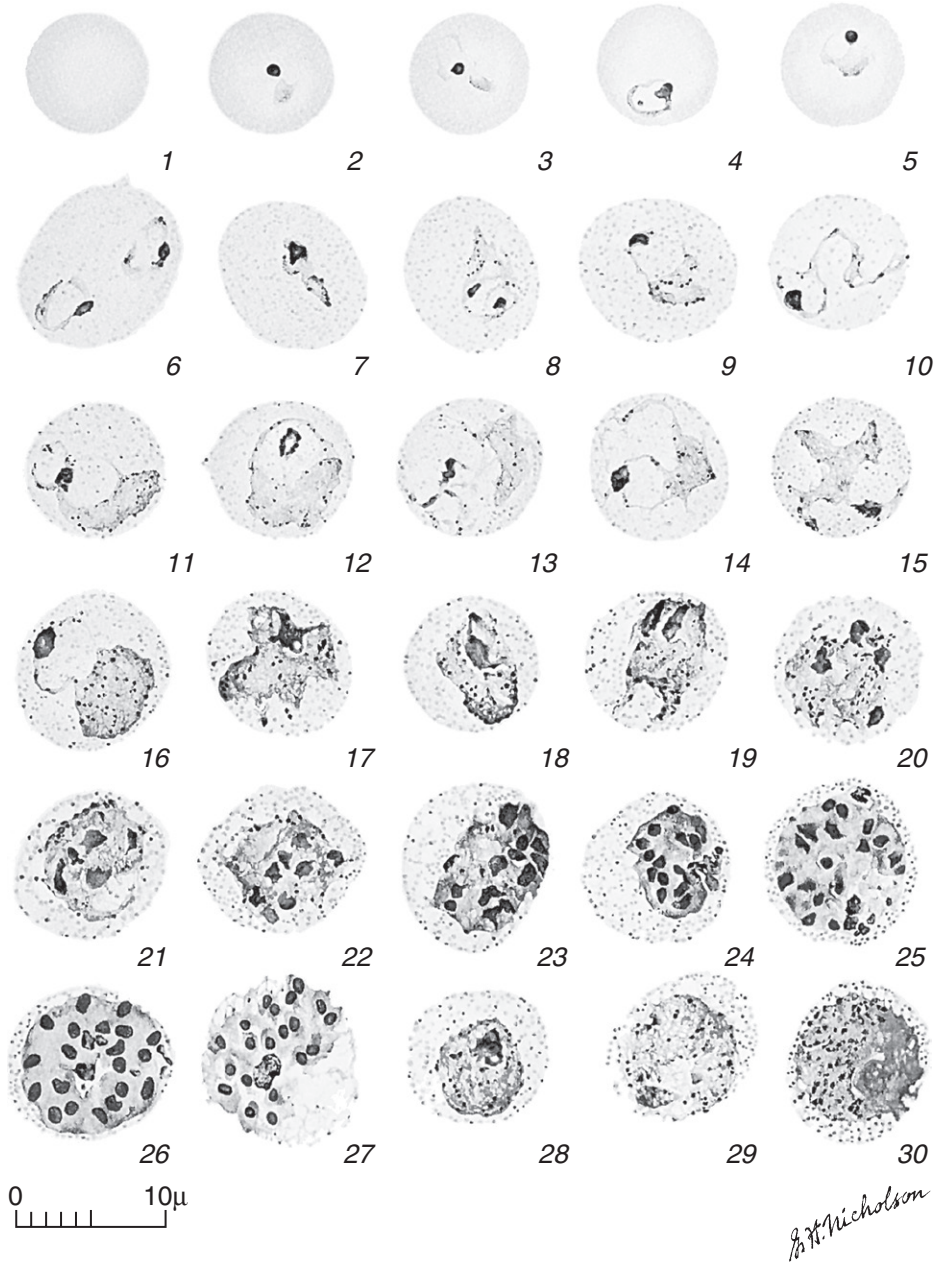

FIGURE 219-3 ■ *Plasmodium vivax.* 1, Normal erythrocyte; 2-5, young trophozoites; 6-16, growing trophozoites; 17, 18, mature trophozoites; 19-21, early schizonts; 22, 23, developing schizonts; 24-27, nearly mature and mature schizonts; 28, 29, nearly mature and mature macrogametocytes; 30, mature microgametocyte. (From Coatney GR, Collins WE, Warren M, et al. *The primate malarias.* Bethesda [MD]: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases; 1971.)

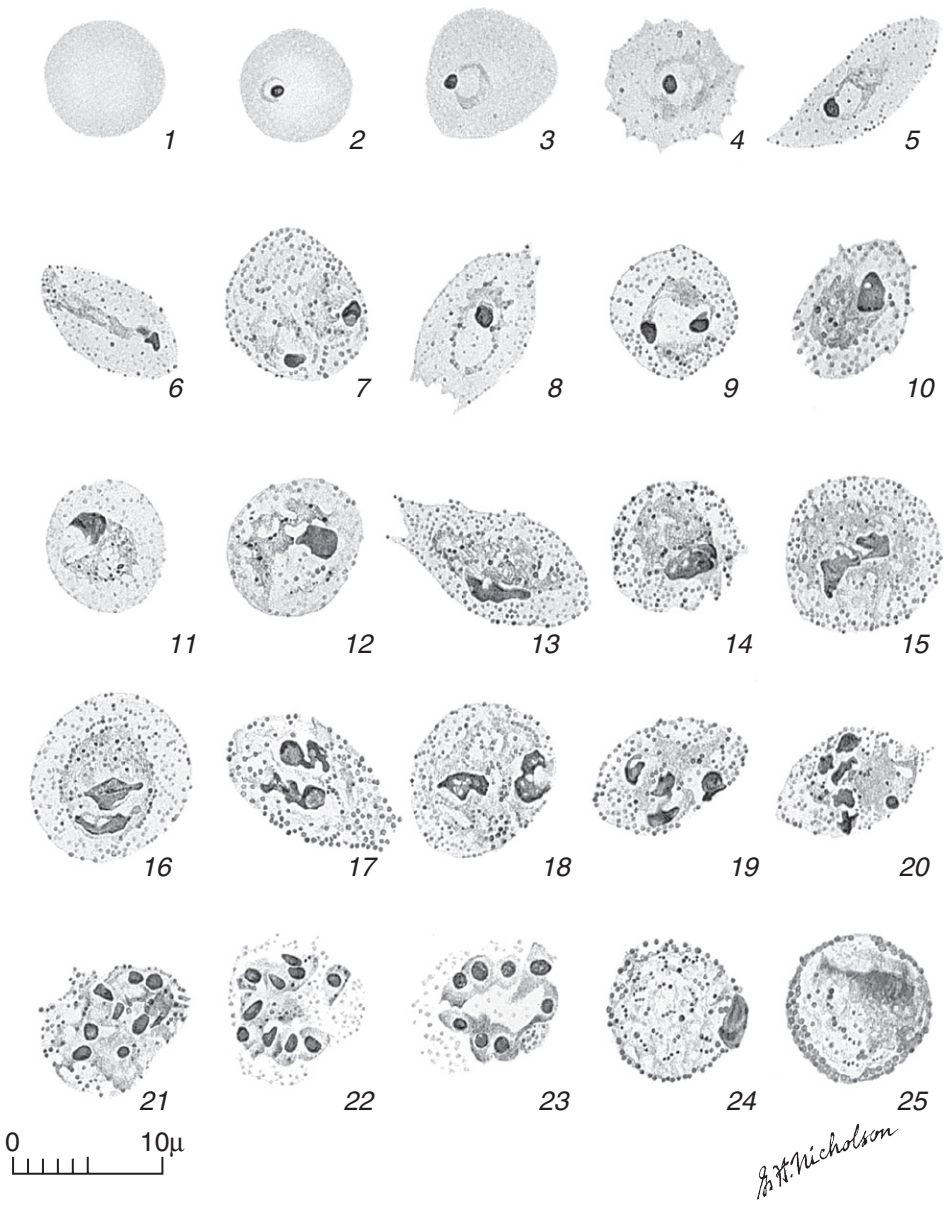

FIGURE 219-4 ■ *Plasmodium ovale*. 1, Normal erythrocyte; 2-5, young trophozoites; 6-12, growing trophozoites; 13-15, mature trophozoites; 16-22, developing schizonts; 23, mature schizont; 24, adult macrogametocyte; 25, adult microgametocyte. (From Coatney GR, Collins WE, Warren M, et al. *The primate malarias.* Bethesda [MD]: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases; 1971.)

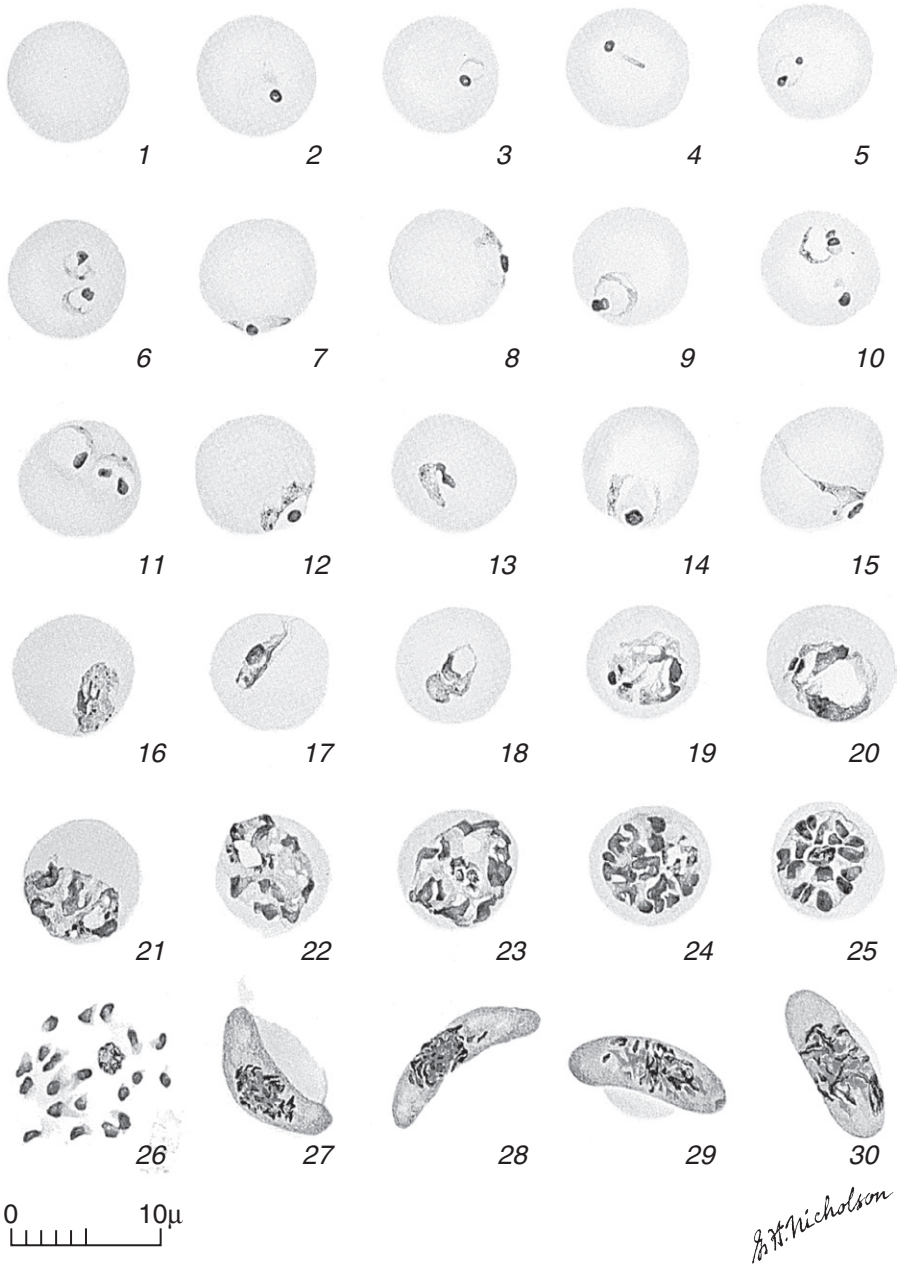

FIGURE 219-5 ■ *Plasmodium falciparum*. 1, Normal erythrocyte; 2-11, young trophozoites; 12-15, growing trophozoites; 16-18, mature trophozoites; 19-22, developing schizonts; 23-26, nearly mature and mature schizonts; 27, 28, mature macrogametocytes; 29, 30, mature microgametocytes. (From Coatney GR, Collins WE, Warren M, et al. *The primate malarias*. Bethesda [MD]: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases; 1971.)

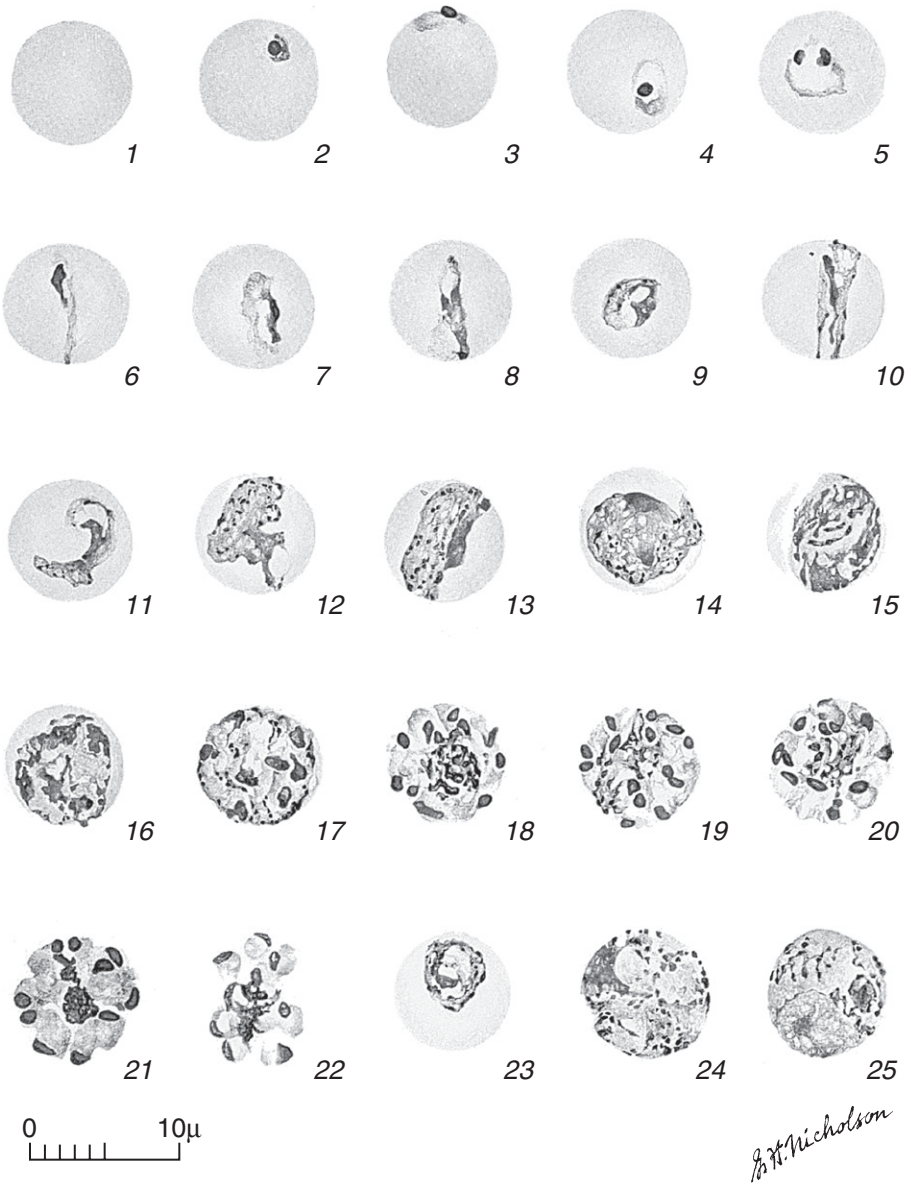

FIGURE 219-6 ■ *Plasmodium malariae*. 1, Normal erythrocyte; 2-5, young trophozoites; 6-11, growing trophozoites; 12, 13, nearly mature and mature trophozoites; 14-20, developing schizonts; 21, 22, mature schizonts; 23, developing gametocyte; 24, mature macrogametocyte; 25, mature microgametocyte. (From Coatney GR, Collins WE, Warren M, et al. *The primate malarias.* Bethesda [MD]: U.S. Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases; 1971.)

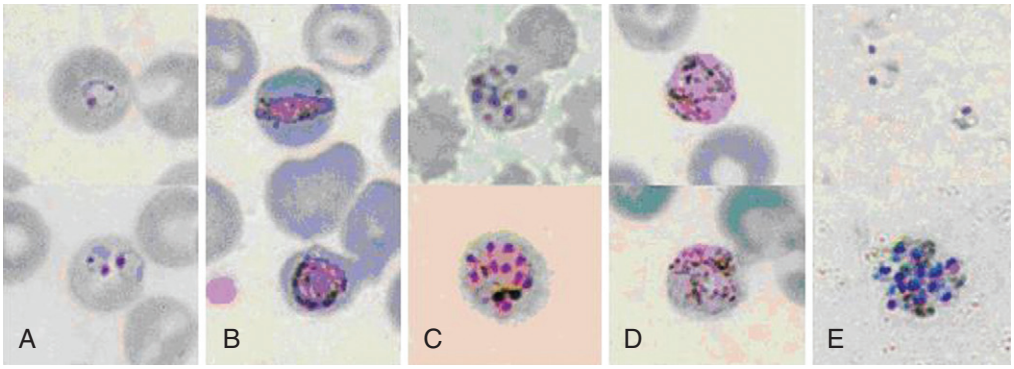

FIGURE 219-7 ■ *Plasmodium knowlesi.* **A,** Early trophozoite or ring form with double chromatin dot *(top panel)* and erythrocyte infected with two trophozoites *(bottom panel).* **B,** Band for late trophozoite *(top panel)* and spherical late trophozoite *(bottom panel).* **C,** Schizonts. **D,** Gametocytes. **E,** Early trophozoites *(top panel)* and schizont *(bottom panel).* (See companion Expert Consult website for color version.) (From Singh B, Kim Sung L, Matusop A, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. *Lancet* 2004;**363**:1017.)

parasitized RBCs can be accomplished by staining a thick smear with acridine orange and examining it under fluorescent light.

Detection of Parasite Antigen

Antigen capture dipstick assays based on detection of histidine-rich protein 2 (HRP-2) (ParaSight F), HRP-2 and aldolase (ICT Malaria Pf/Pv), or parasite LDH (OptiMAL-IT) have been developed.[33,86,102] These assays use a finger-prick sample of blood and give a result in 10 to 15 minutes. Assays detecting HRP-2 are specific for *P. falciparum*, whereas assays based on parasite LDH or HRP-2 and aldolase can identify four species of *Plasmodium*.[30,102] The Binax Now malaria test, based on detection of a pan-malaria antigen and a circulating antigen specific to *P. falciparum*, was licensed in the United States in 2007.[40] The ParaSight F dipstick test, when studied in field trials in Kenya, showed a sensitivity of 96.5 to 100 percent in patients whose parasitemia was greater than 60 parasites/μL, with lower sensitivity noted at lower levels of parasitemia. Antigen persisted to day 6 in 11.9 percent of children whose blood smears were clear at that time.[8] When studied in travelers, the sensitivity of the ParaSight F test ranged from 40 percent in subjects with less than 50 parasites/μL to 93 percent or more in subjects with more than 100 parasites/μL, and positive dipstick tests occurred in 68 percent of blood smear–negative patients on day 7 and in 20 percent on day 28.[62]

Compared with the PCR assay, the HRP-2 assays performed well, with sensitivity of 88 to 95 percent and specificity of 95 to 97 percent; when the HRP-2 assays were compared with microscopy, sensitivity ranged from 80 to 95 percent and specificity ranged from 85 to 100 percent.[114] The tests also performed well when used in nonimmune travelers; a meta-analysis of 21 studies identified sensitivity of 88 to 98 percent and specificity of 95 to 100 percent for detection of *P. falciparum*.[94] The HRP-2/aldolase-based assay that can detect four species of malaria has a lower sensitivity for diagnosis of vivax malaria, although this assay has a higher sensitivity for detection of malaria than the HRP-2 assay.[25,64,102] A limitation of HRP-2–based assays is that they remain positive after therapy because HRP persists in the blood after acute infection.

LDH-based assays are able to distinguish between *P. falciparum* and other malaria species, although the test is unreliable in detecting *P. vivax* in the presence of *P. falciparum*.[124] Sensitivity of OptiMAL-IT was 85 to 95 percent, with specificity of nearly 100 percent for *P. falciparum* and 75 to 85 percent for *P. vivax*. The Clearview malaria test has an overall sensitivity of 93 percent and specificity of 100 percent, with sensitivities of 99, 90.0, 86, and 60 percent, respectively, for *P. falciparum*, *P. vivax*, *P. malariae*, and *P. ovale*.[60] For both OptiMAL-IT and the Clearview malaria tests, sensitivity decreased with decreasing parasite density. An advantage of LDH-based assays is the ability to distinguish between viable and nonviable parasites, rendering the test useful for monitoring response to therapy.[23,26,64,102,117]

Dipstick tests may have a role in cost-effective diagnosis of malaria in situations in which laboratory services are inadequate, in mobile clinics, in locations where levels of malaria transmission are low and drug resistance is high, when the cost of treatment exceeds the cost of the dipstick test, and when blood smears are negative and determining the diagnosis is critical. In developed countries, dipstick assays would benefit laboratories with less experienced microscopists by helping them establish a rapid diagnosis, confirm a diagnosis made by microscopy, or determine the infecting species. Current limitations of the tests are decreased sensitivity compared with expert microscopy, especially at low levels of parasite density; cost; and lack of approval by the U.S. Food and Drug Administration (FDA) of some of the tests.

Dipstick malaria tests have been proposed for use by travelers for self-diagnosis of malaria and have been tested for this purpose. The accuracy of both test performance and interpretation of results by travelers has not been demonstrated as high enough to recommend this strategy routinely for travelers. Most experts suggest using the test to guide initial treatment while the traveler is heading to a source of health care for more definitive diagnosis and treatment.[66,108]

DNA Probe

Specific DNA probes for identification of *P. falciparum* have been developed and tested in field conditions in Thailand.[5] The technique compared favorably with microscopy and could be used to test large numbers of samples at a time, but it had the disadvantage of needing a radiolabeled probe. Nonisotope probes have been used in other field trials in Madagascar, where an enzyme-linked probe was compared with microscopy for diagnosis of falciparum malaria. Results were comparable to examination of thick smears by microscopy for *P. falciparum* infection, but the assay could not identify infection with *P. vivax*, *P. ovale*, or *P. malariae* alone.[97]

Polymerase Chain Reaction Assay

PCR-based techniques used for establishing the diagnosis of malaria are highly sensitive and now are able to detect mixed infections. Compared with thick and thin smears, sensitivity and specificity are nearly 100 percent. PCR techniques can be useful for determining the species of an infection when microscopy is equivocal. Early detection of resistance to antimalarial drugs is another benefit of PCR-based techniques, which have been used to detect a mutation of *P. falciparum* associated with resistance to chloroquine.[39,69] Currently, PCR is the only definite and validated means of diagnosing *P. knowlesi*, because this species may be confused with *P. malariae* on microscopy.[167]

Major disadvantages of the PCR assay include the need for expensive equipment, reagents, and significant technical expertise and the length of time required to perform the testing. At this time, the PCR assay may be better suited for use in large-scale epidemiologic surveys, rather than for establishing the diagnosis of acute malaria in the clinical setting.

Flow Cytometry

Automated blood cell analyzers based on flow cytometry have been shown to display cells containing malaria pigment as a population of cells distinct from other blood components. To date, this discovery has been serendipitous in patients not suspected to have malaria and the diagnosis must be confirmed by conventional methods.[51,70] A future application of this technology could be to modify automated blood cell analyzers to flag specimens that need to be examined further by thin or thick smears (or both).[70]

Antibody Detection

Malaria antibodies develop rapidly after infection and remain present for years; they are of limited value in diagnosing malaria in individual patients. Occasionally, antibody tests might prove useful in the diagnosis of nonimmune individuals with cryptic febrile illnesses or on a population basis to assess the degree of community-wide immunity. Measurement of antibody may have a role in the diagnosis of hyperreactive malarial syndrome.[170]

TREATMENT

Treatment of malaria depends on identification of the species of *Plasmodium* causing infection, knowledge of the presence of resistance to chloroquine and other drugs in the area in which malaria was contracted, and therapeutic goals (treatment of acute illness, eradication of the exoerythrocytic phase of malaria, or prevention of infection). Drugs and classes of drugs currently in use for treating malaria include 4-aminoquinolines and related compounds (chloroquine, mefloquine [Lariam], amodiaquine); primaquine; sulfadoxine-pyrimethamine (Fansidar); halofantrine; atovaquone-proguanil (Malarone); artemisinin (qinghaosu) and other artemisinins (artemether, artesunate) and artemisinin drugs in combination with other agents; cinchona alkaloids (quinine, quinidine); and the antimicrobial agents doxycycline, tetracycline, and clindamycin. Investigational compounds and combination drugs are in various stages of development. Drugs that may be used for the treatment of malaria in the United States are listed in Table 219-3. Drugs used for the prevention of malaria are discussed in a subsequent section.

The choice of agent to treat symptomatic malaria depends on the presence of resistance to chloroquine and other antimalarial drugs, the availability of drugs in the local area, and the age of the patient. Local and national guidelines for the treatment of malaria differ, and practitioners treating patients with malaria should consult local resources and experts. Country-specific information on the local areas with malaria, the estimated risk for malaria, whether there is chloroquine-resistant malaria, and which malaria species are present is available in the United States from the CDC* and outside the United States from local and national health organizations and the WHO.*

In malaria-endemic areas, strategies such as intermittent treatment of infants and pregnant women are being employed in combination with use of insecticides and treated bed nets to prevent and treat malaria.[136] A full discussion of these programs is beyond the scope of this chapter.

Antimalarial Agents Available for Use in the United States

Chloroquine

Chloroquine is the drug of choice for chloroquine-susceptible malaria caused by *P. falciparum* and *P. vivax* and for all infections with *P. ovale* and *P. malariae*. Chloroquine is a 4-aminoquinoline with rapid schizonticidal activity. It is not effective against exoerythrocytic forms of the parasite, and patients treated with chloroquine after acquiring *P. vivax* or *P. ovale* infection need to have additional treatment to eliminate these forms. Chloroquine is most active against late ring stages and mature trophozoites. Response to treatment is rapid, with disappearance of parasitemia within 48 to 72 hours after initiation of treatment when the parasite is susceptible.

Chloroquine is available as an oral preparation and, in many areas, as intramuscular and intravascular formulations. Oral absorption is excellent, and the drug is safe and effective when given by nasogastric or orogastric tube in comatose children.[161] Intramuscular and intravenous administration may be associated with an increased risk for cardiac arrhythmias.

Adverse reactions to chloroquine rarely occur at the doses used for suppression or treatment of malaria. Side effects include gastrointestinal symptoms such as nausea, vomiting, and diarrhea; dizziness; headache; and fatigue. Pruritus may be a troubling adverse event, especially in black patients. Chloroquine has no known teratogenic effects, and it may be used in pregnant and lactating women. The drug is not contraindicated in glucose-6-phosphate dehydrogenase (G6PD)–deficient individuals. Serious adverse events, including hypotension, cardiac arrest, and circulatory failure, may occur after intravenous or intramuscular use of the drug, especially in children. Psychotic symptoms may occur rarely with chloroquine.[44] Chloroquine always should be given slowly when administered intravenously and in small doses when given by intramuscular injection; some experts discourage the parenteral administration of chloroquine. Oral doses are absorbed rapidly. The risk for aspiration occurring while giving a dose by tube to a comatose patient must be weighed against the risk for delaying therapy if parenteral treatment is unavailable or of complications developing when giving chloroquine by the intravenous or intramuscular route. The lethal dose of chloroquine is 1 g in children and 4 g in adults. Cumulative doses exceeding 100 g are associated with an increased

*http://www.cdc.gov/malaria/travelers/country_table/a.html

*http://www.who.int/malaria/diagnosisandtreatment.html

Text continued on p. 2941

TABLE 219-3 **Guidelines for Treatment of Malaria in the United States***

Clinical Diagnosis and *Plasmodium* spp.	Region Infection Acquired	Recommended Drug and Adult Dose[1,8]	Recommended Drug and Pediatric Dose[1,8] *Pediatric dose should NEVER exceed adult dose*
Uncomplicated malaria: *P. falciparum* or species not identified If "species not identified" is subsequently diagnosed as *P. vivax* or *P. ovale*: see *P. vivax* and *P. ovale* (below) for treatment with primaquine.	**Chloroquine-resistant or unknown resistance[2]** (All malarious regions except those specified as chloroquine-sensitive listed below.)	**A. Atovaquone-proguanil (Malarone)[3]** Adult tab = 250 mg atovaquone/100 mg proguanil: 4 adult tabs PO daily × 3 days	**A. Atovaquone-proguanil (Malarone)[3]** Adult tab = 250 mg atovaquone/100 mg proguanil Pediatric tab = 62.5 mg atovaquone/25 mg proguanil 5-8 kg: 2 pediatric tabs PO daily × 3 days 9-10 kg: 3 pediatric tabs PO daily × 3 days 11-20 kg: 1 adult tab PO daily × 3 days 21-30 kg: 2 adult tabs PO daily × 3 days 31-40 kg: 3 adult tabs PO daily × 3 days >40 kg: 4 adult tabs PO daily × 3 days
		B. Artemether-lumefantrine (Coartem)[3] 1 tablet = 20 mg artemether and 120 mg lumefantrine A 3-day treatment schedule with a total of six oral doses is recommended for both adult and pediatric patients based on weight. The patient should receive the initial dose, followed by the second dose 8 hr later, then one dose PO twice daily for the following 2 days. 5-15 kg: 1 tablet per dose 15-25 kg: 2 tablets per dose 25-35 kg: 3 tablets per dose ≥35 kg: 4 tablets per dose	
		C. Quinine sulfate plus one of the following: doxycycline tetracycline, or clindamycin **Quinine sulfate:** 542 mg base (= 650 mg salt)[4] PO three times a day × 3 or 7 days[5] **Doxycycline:** 100 mg PO twice daily × 7 days **Tetracycline:** 250 mg PO four times a day × 7 days **Clindamycin:** 20 mg base/kg/day PO divided three times a day × 7 days	**C. Quinine sulfate[4] plus one of the following: doxycycline[6], tetracycline,[6] or clindamycin** **Quinine sulfate:** 8.3 mg base/kg (= 10 mg salt/kg) PO × 3 or 7 days[5] **Doxycycline:** 2.2 mg/kg PO every 12 hr × 7 days **Tetracycline:** 25 mg/kg/day PO divided four times a day × 7 days **Clindamycin:** 20 mg base/kg/day PO divided three times a day × 7 days
		D. Mefloquine (Lariam and generics)[7] 684 mg base (= 750 mg salt) PO as initial dose, followed by 456 mg base (= 500 mg salt) PO given 6-12 hr after initial dose Total dose = 1250 mg salt	**D. Mefloquine (Lariam and generics)[7]** 13.7 mg base/kg (= 15 mg salt/kg) PO as initial dose, followed by 9.1 mg base/kg (= 10 mg salt/kg) PO given 6-12 hr after initial dose. Total dose = 25 mg salt/kg
Uncomplicated malaria: *P. falciparum* or species not identified	**Chloroquine-sensitive (Central America west of Panama Canal; Haiti; the Dominican Republic; and most of the Middle East)**	**Chloroquine phosphate (Aralen and generics)[8]** 600 mg base (= 1000 mg salt) PO immediately, followed by 300 mg base (= 500 mg salt) PO at 6, 24, and 48 hr Total dose: 1500 mg base (= 2500 mg salt) *or* **Hydroxychloroquine (Plaquenil and generics)** 620 mg base (= 800 mg salt) PO immediately, followed by 310 mg base (= 400 mg salt) PO at 6, 24, and 48 hr Total dose: 1550 mg base (= 2000 mg salt)	**Chloroquine phosphate (Aralen and generics)[8]** 10 mg base/kg PO immediately, followed by 5 mg base/kg PO at 6, 24, and 48 hr Total dose: 25 mg base/kg *or* **Hydroxychloroquine (Plaquenil and generics)** 10 mg base/kg PO immediately, followed by 5 mg base/kg PO at 6, 24, and 48 hr Total dose: 25 mg base/kg

Continued on following page

TABLE 219-3 **Guidelines for Treatment of Malaria in the United States** (Continued)

Clinical Diagnosis and *Plasmodium* spp.	Region Infection Acquired	Recommended Drug and Adult Dose[1,8]	Recommended Drug and Pediatric Dose[1,8] *Pediatric dose should NEVER exceed adult dose*
Uncomplicated malaria: *P. malariae* or *P. knowlesi*	**All regions**	**Chloroquine phosphate[8]:** treatment as above *or* **Hydroxychloroquine:** treatment as above	**Chloroquine phosphate[8]:** treatment as above *or* **Hydroxychloroquine:** treatment as above
Uncomplicated malaria: *P. vivax* or *P. ovale*	**All regions** *Note:* for suspected chloroquine-resistant *P. vivax*, see below.	**Chloroquine phosphate[8] plus primaquine phosphate[9]** **Chloroquine phosphate:** treatment as above **Primaquine phosphate:** 30 mg base PO daily × 14 days *or* **Hydroxychloroquine plus primaquine phosphate[9]** **Hydroxychloroquine:** treatment as above **Primaquine phosphate:** 30 mg base PO daily × 14 days	**Chloroquine phosphate[8] plus primaquine phosphate[9]** **Chloroquine phosphate:** treatment as above **Primaquine:** 0.5 mg base/kg PO daily × 14 days *or* **Hydroxychloroquine plus primaquine phosphate[9]** **Hydroxychloroquine:** treatment as above **Primaquine phosphate:** 0.5 mg base/kg PO daily × 14 days
Uncomplicated malaria: *P. vivax*	**Chloroquine-resistant[10]** (Papua New Guinea and Indonesia)	**A. Quinine sulfate plus either doxycycline or tetracycline plus primaquine phosphate[9]** **Quinine sulfate:** treatment as above **Doxycycline or tetracycline:** treatment as above **Primaquine phosphate:** treatment as above B. Atovaquone-proguanil plus primaquine phosphate[9] Atovaquone-proguanil: treatment as above Primaquine phosphate: treatment as above C. Mefloquine plus primaquine phosphate[9] Mefloquine: treatment as above Primaquine phosphate: treatment as above	**A. Quinine sulfate plus either doxycycline[6] or tetracycline[6] plus primaquine phosphate[9]** **Quinine sulfate:** treatment as above **Doxycycline or tetracycline:** treatment as above **Primaquine phosphate:** treatment as above B. Atovaquone-proguanil plus primaquine phosphate[9] Atovaquone-proguanil: treatment as above Primaquine phosphate: treatment as above C. Mefloquine plus Primaquine phosphate[9] Mefloquine: treatment as above Primaquine phosphate: treatment as above
Uncomplicated malaria: alternatives for pregnant women[11,12,13]	**Chloroquine-sensitive** (see uncomplicated malaria sections above for chloroquine-sensitive species by region)	**Chloroquine phosphate:** treatment as above *or* **Hydroxychloroquine:** treatment as above	Not applicable
	Chloroquine-resistant (see sections above for regions with chloroquine-resistant *P. falciparum* and *P. vivax*)	**Quinine sulfate plus clindamycin** **Quinine sulfate:** treatment as above **Clindamycin:** treatment as above *or* **Mefloquine:** treatment as above	Not applicable

Severe malaria[14,15,16]	**All regions**	**Quinidine gluconate**[14] **plus one of the following: doxycycline**[4]**, tetracycline**[4]**, or clindamycin** **Quinidine gluconate:** 6.25 mg base/kg (= 10 mg salt/kg) loading dose IV over 1-2 hr, then 0.0125 mg base/kg/min (= 0.02 mg salt/kg/min) continuous infusion for at least 24 hr. An *alternative regimen* is 15 mg base/kg (= 24 mg salt/kg) loading dose IV infused over 4 hr, followed by 7.5 mg base/kg (= 12 mg salt/kg) infused over 4 hr every 8 hr, starting 8 hr after the loading dose (see package insert). Once parasite density <1% and patient can take oral medication, complete treatment with oral quinine, dose as above. Quinidine/quinine course = 7 days in Southeast Asia; = 3 days in Africa or South America. **Doxycycline:** treatment as above. If patient not able to take oral medication, give 100 mg IV every 12 hr and then switch to oral doxycycline (as above) as soon as patient can take oral medication. For IV use, avoid rapid administration. Treatment course = 7 days. **Tetracycline:** treatment as above **Clindamycin:** treatment as above. If patient not able to take oral medication, give 10 mg base/kg loading dose IV followed by 5 mg base/kg IV every 8 hr. Switch to oral clindamycin (oral dose as above) as soon as patient can take oral medication. For IV use, avoid rapid administration. Treatment course = 7 days. ***Investigational new drug (contact CDC for information):*** **artesunate followed by one of the following: atovaquone-proguanil (Malarone), doxycycline (clindamycin in pregnant women), or mefloquine**	**Quinidine gluconate**[14] **plus one of the following: doxycycline**[4]**, tetracycline**[4]**, or clindamycin** **Quinidine gluconate:** same mg/kg dosing and recommendations as for adults. **Doxycycline:** treatment as above. If patient not able to take oral medication, may give IV. For children <45 kg, give 2.2 mg/kg IV every 12 hr and then switch to oral doxycycline (dose as above) as soon as patient can take oral medication. For children >45 kg, use same dosing as for adults. For IV use, avoid rapid administration. Treatment course = 7 days. **Tetracycline:** treatment as above **Clindamycin:** treatment as above. If patient not able to take oral medication, give 10 mg base/kg loading dose IV followed by 5 mg base/kg IV every 8 hr. Switch to oral clindamycin (oral dose as above) as soon as patient can take oral medication. For IV use, avoid rapid administration. Treatment course = 7 days. ***Investigational new drug (contact CDC for information):*** **artesunate followed by one of the following: atovaquone-proguanil (Malarone), clindamycin, or mefloquine**

*Based on drugs currently available for use in the United States. If outside the United States, contact national authorities about current recommendations.

[†]*Note:* There are three options (A, B, or C) available for treatment of uncomplicated malaria caused by chloroquine-resistant *P. falciparum*. Options A and B are equally recommended. Because of a higher rate of severe neuropsychiatric reactions seen at treatment doses, we do not recommend option C (mefloquine) unless options A and B cannot be used. For option A, because there are more data on the efficacy of quinine in combination with doxycycline or tetracycline, these treatment combinations are generally preferred to quinine in combination with clindamycin.

[2]For infections acquired in Southeast Asia, quinine treatment should continue for 7 days. For infections acquired in Africa and South America, quinine treatment should continue for 3 days.

[3]U.S.-manufactured quinine sulfate capsule is in a 324-mg dosage; therefore, 2 capsules should be sufficient for adult dosing. Pediatric dosing may be difficult due to unavailability of noncapsule forms of quinine. If unable to provide pediatric doses of quinine, consider Malarone (recommendation B) or mefloquine (recommendation C).

[4]Doxycycline and tetracycline are not indicated for use in children younger than 8 years of age. For children younger than 8 years of age with chloroquine-resistant *P. falciparum* malaria, quinine (given alone for 7 days or given in combination with clindamycin) and atovaquone-proguanil are recommended treatment options; mefloquine can be considered if no other options are available. For children younger than 8 years of age with chloroquine-resistant *P. vivax* malaria, quinine (given alone for 7 days) or mefloquine is the recommended treatment option. If none of these treatment options is available or is not being tolerated and if the treatment benefits outweigh the risks, doxycycline or tetracycline may be given to children younger than 8 years of age.

[5]Give atovaquone-proguanil with food. If patient vomits within 30 minutes of taking a dose, then the dose should be repeated.

[6]Treatment with mefloquine is not recommended in persons who have acquired infections from the Southeast Asian region including Myanmar, Thailand, and Cambodia due to resistant strains.

[7]Primaquine is used to eradicate any hypnozoite forms that may remain dormant in the liver, and thus prevent relapses, in *P. vivax* and *P. ovale* infections. Because primaquine can cause hemolytic anemia in persons with G6PD deficiency, patients must be screened for G6PD deficiency before starting treatment with primaquine. For persons with borderline G6PD deficiency, or as an alternate to the above regimen, primaquine may be given 45 mg orally one time per week for 8 weeks; consultation with an expert in infectious disease and/or tropical medicine is advised if this alternative regimen is considered in G6PD-deficient persons. Primaquine must not be used during pregnancy.

[8]*Note:* There are two options (A or B) available for treatment of uncomplicated malaria caused by chloroquine-resistant *P. vivax*. High treatment failure rates due to chloroquine-resistant *P. vivax* have been well documented in Papua New Guinea and Indonesia. Rare case reports of chloroquine-resistant *P. vivax* malaria have also been documented in Burma (Myanmar), India, and Central and South America. Persons acquiring *P. vivax* infections outside Papua New Guinea or Indonesia should be started on chloroquine. If the patient does not respond, the treatment should be changed to a chloroquine-resistant *P. vivax* regimen and the CDC should be notified (Malaria Hotline number: 770-488-7788 or 770-488-7100). For treatment of chloroquine-resistant *P. vivax* infections, options A and B are equally recommended.

[9]For pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant *P. falciparum* or chloroquine-resistant *P. vivax*, treatment with doxycycline or tetracycline is generally not indicated. However, doxycycline or tetracycline may be used in combination with quinine (as recommended for nonpregnant adults) if other treatment options are not available or are not being tolerated and if the benefit is judged to outweigh the risks.

[10]Because there are no adequate, well-controlled studies of atovaquone and/or proguanil hydrochloride in pregnant women, atovaquone-proguanil is generally not recommended for use in pregnant women. For pregnant women diagnosed with uncomplicated malaria caused by chloroquine-resistant *P. falciparum,* atovaquone-proguanil may be used if other treatment options are not available or are not being tolerated and if the potential benefit is judged to outweigh the potential risks. There are no data on the efficacy of atovaquone-proguanil in the treatment of chloroquine-resistant *P. vivax* infections.

[11]Because of a possible association with mefloquine treatment during pregnancy and an increase in stillbirths, mefloquine is generally not recommended for treatment in pregnant women. However, mefloquine may be used if it is the only treatment option available and if the potential benefit is judged to outweigh the potential risks.

[12]For *P. vivax* and *P. ovale* infections, primaquine phosphate for radical treatment of hypnozoites should not be given during pregnancy. Pregnant patients with *P. vivax* and *P. ovale* infections should be maintained on chloroquine prophylaxis for the duration of their pregnancy. The chemoprophylactic dose of chloroquine phosphate is 300 mg base (= 500 mg salt) orally once per week. After delivery, pregnant patients who do not have G6PD deficiency should be treated with primaquine.

[13]Persons with a positive blood smear *or* history of recent possible exposure and no other recognized pathology who have one or more of the following clinical criteria (impaired consciousness/coma, severe normocytic anemia, renal failure, pulmonary edema, acute respiratory distress syndrome, circulatory shock, disseminated intravascular coagulation, spontaneous bleeding, acidosis, hemoglobinuria, jaundice, repeated generalized convulsions, and/or parasitemia of >5%) are considered to have manifestations of more severe disease. Severe malaria is practically always due to *P. falciparum.*

[14]Patients diagnosed with severe malaria should be treated aggressively with parenteral antimalarial therapy. Treatment with IV quinidine should be initiated as soon as possible after the diagnosis has been made. Patients with severe malaria should be given an intravenous loading dose of quinidine unless they have received more than 40 mg/kg of quinine in the preceding 48 hours or if they have received mefloquine within the preceding 12 hours. Consultation with a cardiologist and a physician with experience in treating malaria is advised when treating malaria patients with quinidine. During administration of quinidine, blood pressure monitoring (for hypotension) and cardiac monitoring (for widening of the QRS complex and/or lengthening of the QTc interval) should be done continuously and blood glucose (for hypoglycemia) should be monitored periodically. Cardiac complications, if severe, may warrant temporary discontinuation of the drug or slowing of the intravenous infusion.

[15]Consider exchange transfusion if the parasite density (i.e., parasitemia) is >10% *or* if the patient has altered mental status, non–volume overload pulmonary edema, or renal complications. The parasite density can be estimated by examining a monolayer of red blood cells (RBCs) on the thin smear under oil immersion magnification. The slide should be examined where the RBCs are more or less touching (approximately 400 RBCs per field). The parasite density can then be estimated from the percentage of infected RBCs and should be monitored every 12 hours. Exchange transfusion should be continued until the parasite density is <1% (usually requires 8-10 units). IV quinidine administration should not be delayed for an exchange transfusion and can be given concurrently throughout the exchange transfusion.

[16]Pregnant women diagnosed with severe malaria should be treated aggressively with parenteral antimalarial therapy.

Modified from http://www.cdc.gov/malaria/pdf/treatmenttable.pdf.

risk for development of retinopathy. Dose reduction may be required for patients with renal and hepatic disease.

Artemether-Lumefantrine

Artemesinin (qinghaosu) is a sesquiterpene lactone that, along with its derivatives (artemether, artesunate, arteether, and others), has antimalarial activity thought to be due to the ability to cause free radical damage in parasite membrane systems.[56] These compounds are unique in their ability to cure malaria more rapidly than other agents, with no apparent adverse events. There is no consensus on the mechanisms through which artemisinin derivatives kill malaria parasites. Lumefantrine, a dichlorobenzylidine derivative, also has an unclear mechanism of action. The fixed dose combination of artemether (20 mg) and lumefantrine (120 mg) [Coartem] is available in the United States and is FDA approved, and it is considered a first-line agent for treatment of uncomplicated falciparum malaria in countries where it is available. It is well tolerated and effective against multidrug-resistant falciparum malaria.[128] A six-dose regimen compared favorably with other agents in African children and adults with uncomplicated falciparum malaria.[105,121] Its side effect profile along with rapid parasite clearance and high cure rate for uncomplicated falciparum malaria in African infants and children make this regimen favorable.[38] Bioavailability of lumefantrine is enhanced when taken with food.[37]

Artemether-lumefantrine is not approved for use in treatment of infection with *P. vivax*, *P. ovale*, or *P. malariae* because there are few data on its efficacy for infection with these species. There may be a role for the use of artemether-lumefantrine in treating chloroquine-resistant vivax malaria, because this drug combination produces rapid clearance of the blood stage. The short half-life of lumefantrine may, however, allow for a shorter time to relapse by dormant hypnozoites.[6,169]

Atovaquone-Proguanil

A fixed-dose combination (available in adult and pediatric formulations) of atovaquone and proguanil hydrochloride is available for treatment of malaria caused by *P. falciparum* for individuals who were not taking this agent for prophylaxis. The combination is significantly more effective than either drug alone, and is effective for treatment of malaria strains resistant to other antimalarial drugs. It has fewer reported adverse events than quinine-based regimens. The combination also is likely to be active against the blood stages of *P. vivax*, *P. ovale*, and *P. malariae*, but the regimen does not have activity against the hypnozoites of *P. vivax*. Atovaquone-proguanil is significantly more effective than mefloquine, chloroquine, amodiaquine, or pyrimethamine-sulfadoxine in locations where parasites are resistant to these drugs.[81]

Mefloquine

The development of mefloquine was a major advance in the treatment of chloroquine-resistant malaria, although resistance has developed rapidly in some parts of the world where it is used widely. Mefloquine is a 4-quinolone-carbinolamine whose exact mechanism of action is unknown. Its main target is early trophozoites. Similar to chloroquine, it is not active against exoerythrocytic phases. Mefloquine has been associated with neurologic and psychiatric side effects when used for treatment or prophylaxis.[150] Vomiting occurs commonly when mefloquine is given to children younger than 5 years old and may decrease its efficacy.[145] Mefloquine should not be administered concurrently with quinine, quinidine, or halofantrine because of concern about arrhythmias. Pregnancy is not a contraindication to treatment in any trimester, although with the development of other well-tolerated medications, mefloquine is no longer the first choice for treatment of malaria in pregnancy.

Quinine and Quinidine

Quinine has been used for the treatment of malaria for centuries.[150] It is active against the mature asexual erythrocytic forms of all four species of human malaria and against the gametes of all species except *P. falciparum*, where it has activity against only immature gametes. It is not effective against exoerythrocytic forms. It may be given orally, but intravenous and intramuscular forms are available. Quinine crosses the placenta easily and may be used in the treatment of pregnant women, although close monitoring for hypoglycemia is warranted.[82]

Adverse reactions to quinine include tinnitus, headache, nausea, visual and hearing disturbances, and tremors, a constellation of symptoms called *cinchonism*. Symptoms may appear during the first 1 to 3 days of therapy and stop when treatment is terminated. Hypoglycemia may occur, particularly when the drug is used to treat pregnant women. Severe adverse events are rare but may occur idiosyncratically. Blackwater fever (hemoglobinuria) seems to occur after treatment with quinine, but the causal mechanism is unknown.

Recrudescence rates may be high when quinine is used alone because of the presence of quinine-resistant strains of *P. falciparum*. A second drug is added to treat the remaining forms. Drugs that may be used with quinine include doxycycline, tetracycline, and clindamycin.

Quinidine is a related drug that may be given intravenously; in some developed countries where intravenous quinine is unavailable, it is a treatment option for severe malaria. The mechanisms of action and adverse events are similar, although cardiac arrhythmias occur more commonly with quinidine and cardiac monitoring is advised.[159]

Primaquine

Primaquine, an 8-aminoquinoline derivative that is effective against exoerythrocytic hypnozoites, is used primarily to prevent relapses of infections with *P. vivax* and *P. ovale*, although it may not be completely effective against some strains of *P. vivax*. Despite adequate treatment with primaquine, relapses of infections with *P. vivax* and *P. ovale* do occur[11]; in some instances this may be due to inadequate dosing. It is not an effective blood schizonticide but is an active gametocidal and sporontocidal drug

for the four main species of *Plasmodium* that cause human malaria. Primaquine can cause intravascular hemolysis in individuals with G6PD deficiency. Patients should be tested for G6PD deficiency and confirmed to have normal G6PD status before being treated with primaquine. Other adverse events include gastrointestinal symptoms such as nausea, epigastric pain, anorexia, and abdominal cramps. Other rare side effects include methemoglobinemia, hemoglobinuria, and bone marrow suppression. Safety in pregnancy has not been established, and the drug should not be used by pregnant women. After delivery, women with *P. vivax* or *P. ovale* infections and without G6PD deficiency may be treated with primaquine.

Tetracycline and Doxycycline

Tetracycline, doxycycline, and the related drug minocycline have very slow activity against malarial schizonts.[150] Doxycycline and minocycline may be given once daily. They are active against chloroquine-resistant and pyrimethamine-sulfadoxine–resistant *P. falciparum*. Resistance to tetracycline has not been shown. Because of their slow activity, these agents should be given in combination with a rapidly acting drug such as quinine. These drugs should not be used in children younger than 8 years old or by pregnant or nursing women.

Antimalarial Agents Not Currently Available or Recommended for Treatment of Malaria in the United States

Other Artemisinin Derivatives

Aside from artemether-lumefantrine, the only artemisinin derivative available in the United States is parenteral artesunate, which is available on an emergency basis only from the CDC. Other artemisinin derivatives are available in other parts of the world. When used alone, recrudescence rates are high, and the drug class seems to have limited potential for prophylaxis. Oral preparations, suppositories, and oil-based preparations for intramuscular injection are available, although standardization of preparations is lacking.[128] Combinations including artemisinins are the preferred antimalarial agents worldwide[166] and have shown major utility in areas of multidrug-resistant *P. falciparum*.[1,55] In clinical trials in the Gambia, artemether was as effective as quinine and chloroquine for treatment of cerebral malaria in children.[156,162] When used in sequence with mefloquine, the combination was effective and tolerated well by patients with acute, uncomplicated falciparum malaria in Thailand, an area with multidrug resistance.[83] Later studies showed that the use of this combination may have helped halt the progressions of mefloquine resistance in the region.[112] The use of artemisinin-based combination therapies has contributed to significant reductions in malaria burden in many parts of the world. There is, however, reduced susceptibility of *P. falciparum* to artemisinin derivatives in the Cambodia-Thailand border region, likely owing to use of the artemisinins as single-drug therapy as opposed to combination therapy, leading to a WHO-led program to contain artemisinin resistance in this region.[35]

Pyrimethamine-Sulfadoxine

The combination pyrimethamine-sulfadoxine, a sulfonamide plus a dihydrofolate reductase inhibitor, exhibits synergy in its action against malaria parasites; the combination is active even against strains resistant to the individual drug components. Although pyrimethamine-sulfadoxine has been used for presumptive treatment of malaria in travelers who are taking other drugs for prophylaxis in areas where chloroquine resistance occurs, widespread resistance to the drug combination and its slow action should limit its use in nonimmune individuals with falciparum malaria. The CDC no longer recommends pyrimethamine-sulfadoxine for treatment of malaria. Although safety has not been established in pregnancy, the drug has been used widely and effectively for intermittent presumptive treatment of pregnant women in endemic areas to decrease the risk for severe anemia and placental malaria.[87,142,144]

Amodiaquine

Amodiaquine, similar to chloroquine, is a 4-aminoquinolone. It has similar efficacy but may have increased activity compared with chloroquine for the treatment of strains of *P. falciparum* resistant to chloroquine.[115] It is available in an oral preparation and is more palatable. Significant adverse events, including agranulocytosis, hepatotoxicity, and death, when used as a prophylactic agent have limited its use as a first-line agent. It is not licensed or marketed in the United States.

Halofantrine

Halofantrine is a 9-phenanthrene-methanol that has been used to treat malaria caused by chloroquine-resistant *P. falciparum*. Its major side effect is dose-related prolongation of the QT interval, and it has been associated with cardiac arrhythmias and sudden death, especially in individuals taking mefloquine prophylaxis.[16,110] Its major use was in uncomplicated malaria in areas of multidrug resistance, but cardiac toxicity at the doses required to be effective may limit its widespread use.[151] It is contraindicated in pregnant and lactating women because of embryotoxic effects. Resistance to halofantrine has limited its use in some areas. Travelers who are taking mefloquine for prophylaxis should be cautioned against accepting treatment with halofantrine if they are diagnosed with malaria during their travels. Halofantrine is unavailable in the United States.

Supportive Therapy

Malaria is a potentially fatal illness even in patients who may have developed partial immunity. Patients, especially children and those without prior immunity, with falciparum malaria should have immediate treatment and generally should be admitted to the hospital, especially in nonendemic countries such as the United States.

Patients with severe malaria should be managed in intensive care units, and experts in treatment of malaria and its complications should be consulted. Blood smears should be examined at least every 12 hours (every 4 to 6 hours for severe malaria) until parasitemia is less than 1 percent. Most patients can be treated with oral medication, although patients should be switched promptly to parenteral medication if oral medication is not tolerated. CDC* and WHO† guidelines for treatment of malaria, including management of severe malaria, are available.

Goals of supportive therapy are to maintain oxygenation and treat acidosis and hypoglycemia. Treatment with glucose infusion, oxygen, and blood transfusion may be necessary. Mechanical ventilation with positive-pressure ventilation may be needed to manage acidosis and acute respiratory distress syndrome. In some cases, hemodialysis may be needed. A high index of suspicion should be maintained for other concomitant or nosocomial infections, such as meningitis or septicemia.

Investigational Drugs and Adjunctive Therapy

Targets for new antimalarial drugs include malaria proteases and protein kinases, which play a crucial role in malaria pathogenesis.[34,132] These drugs are largely in the development phase.

Adjunctive therapies that have been shown to be beneficial include antipyretic and anticonvulsant drugs. Exchange transfusion remains a controversial option[75,159] largely owing to the lack of sufficient randomized controlled trials. Exchange transfusion may decrease parasite clearance times compared with quinine alone,[154] but there is a lack of clear survival benefit with this adjunctive therapy.[129] Adjunctive therapies that would modify or prevent the deleterious effects of inflammatory mediators, oxidative stress, iron overload, acidosis, and hypercoagulability in severe and cerebral malaria have been proposed, but limited clinical studies have not shown significant benefit.[68] A randomized study of inhaled nitric oxide in severe malaria is ongoing.[52] Some therapies, such as high-dose corticosteroids for cerebral malaria or heparin, have been shown to be harmful.[159,168] The search for effective adjunctive therapy for severe and complicated malaria continues.

PREVENTION

Prevention of malaria can be accomplished by reducing the mosquito population, using personal protection methods to prevent mosquito bites, and initiating chemoprophylaxis. Reduction of mosquito vectors is most successful when locally controlled programs are developed and maintained.[74] Strategies that combine personal protective measures with community-based control measures, such as distribution of insecticide-treated mosquito nets and indoor residual spraying, are most likely to be successful.[167] Most programs in endemic areas require a combination of preventive strategies and effective treatment modalities. Many nonendemic countries have developed recommendations for prevention of malaria in travelers.

Personal Protective Measures

A significant reduction in the incidence of malaria in children in communities where bed nets, especially when impregnated with insecticide, are used consistently has been reported from many countries.[31,45,146] In northern Ghana, community-wide use of bed nets was associated with a reduction in all-cause child mortality in young children.[10] Use of bed nets, especially those impregnated with insecticide, is recommended for travelers to malarial areas. Other personal protective measures include using mosquito repellents and insecticide-impregnated clothing or gear; wearing clothing that covers areas likely to be bitten; and remaining, if possible, in air-conditioned or well-screened areas during times when mosquitoes are biting. DEET-containing repellents are recommended most commonly for prevention of mosquito bites and are highly effective.[41] Other agents, such as picaridin, oil of lemon eucalyptus, and IR3535 are available. Picaridin is available in a 20 percent formulation, which may be used as an alternative to DEET.[98] Other agents have not been studied extensively against *Anopheles* mosquitoes.

Chemoprophylaxis

Country-specific information regarding recommendations for the use of antimalarial drugs may differ, and readers are urged to seek recommendations from national or local authorities. In the United States, these recommendations are available widely in publications from the CDC[19*] or national health information sources. Many excellent sources of health information for travelers include discussions of malaria prevention.† The Infectious Diseases Society of America has published detailed pre-travel guidelines that include recommendations for malaria prevention.[57] A list of drugs available for malaria prevention is presented in Table 219-4. Prevention of malaria for travelers is best discussed in the context of providing complete pre-travel services, advice, and information.

Antimalarial Agents Available in the United States for Prevention of Malaria

Chloroquine

A major determinant of appropriate chemoprophylaxis is whether a child is to travel to an area of chloroquine resistance. In areas of the world that have no resistance to chloroquine, the drug of choice for prevention of malaria is chloroquine. This drug is tolerated well and may be given to children of all ages, including infants.

*http://www.cdc.gov/malaria/pdf/treatmenttable.pdf
†http://www.who.int/malaria/diagnosisandtreatment.html

*http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/malaria.htm
†References 20-22, 91, 135, 138, 141, 148.

TABLE 219-4 **Drug Regimens Used for Prevention of Malaria**

Drug	Adult Dosage	Pediatric Dosage	Comments
Chloroquine-Sensitive Areas			
Chloroquine phosphate (drug of choice)	500 mg salt (300 mg base) orally once/wk	8.3 mg/kg salt (5 mg/kg base) once/wk, up to adult dose of 300 mg base	Begin 1-2 wk before exposure, continue during exposure, and continue for 4 wk after exposure May be used in pregnant women
Hydroxychloroquine sulfate	400 mg salt (310 mg base) orally once/wk	6.5 mg/kg salt (5 mg/kg base) orally once/wk	Begin 1-2 wk before exposure, continue during exposure, and continue for 4 wk after exposure An alternative to chloroquine for use only in areas with chloroquine-sensitive malaria
Chloroquine-Resistant Areas			
Mefloquine	250 mg salt (228 mg base) orally once/wk	≤9 kg: 5 mg/kg salt (4.6 mg/kg base) orally once/wk 10-19 kg: ¼ tablet orally once/wk 20-30 kg: ½ tablet orally once/wk 31-45 kg: ¾ tablet orally once/wk >45 kg: 1 tablet orally once/wk	Begin 1-2 wk before exposure, continue during exposure, and continue for 4 wk after exposure See text for contraindications
or			
Doxycycline	100 mg daily	2.2 mg/kg/day up to 100 mg/day	Begin 1-2 days before exposure, continue during exposure, and for 4 wk after exposure; not to be used in children >8 yr old or pregnant women
or			
Atovaquone-proguanil	250 mg/100 mg (1 tablet) daily	5-8 kg: ½ pediatric tablet daily 9-10 kg: ¾ pediatric tablet daily 11-20 kg: 1 pediatric tablet daily 21-30 kg: 2 pediatric tablets daily 31-40 kg: 3 pediatric tablets daily >40 kg: 1 adult tablet daily	Begin 1-2 days before exposure, continue during exposure, and continue for 7 days after exposure Pediatric tablets contain 62.5 mg atovaquone and 25 mg proguanil hydrochloride See text for contraindications
Alternative			
Primaquine	52.6 mg salt (30 mg base) orally daily	0.8 mg/kg salt (0.5 mg/kg base) up to adult dose orally daily	Begin 1-2 days before exposure, continue during exposure, and continue for 7 days after exposure Contraindicated in people with G6PD deficiency, in pregnancy, and during lactation Prophylaxis to areas principally with *P. vivax*

Dosing of chloroquine in children is based on the child's weight and can be found in Table 219-3. In the United States, chloroquine is available only in tablets that have a very bitter taste, although some compounding pharmacies may be willing to make liquid preparations. Pharmacies can also prepare appropriate doses in capsules, and the contents of the capsule can be mixed with a small amount of liquid or semisolid food for administration. Although liquid preparations are widely available overseas and are stable for long periods,[101] many U.S. pharmacies are reluctant to prepare them. If patients are considering purchasing liquid preparations overseas, the family should be given information with the child's medication dose calculated as the base and the salt because concentrations of liquid preparations may vary. Families also should be cautioned about the greater incidence of ineffective or counterfeit medications available in some areas.

Adverse events caused by chloroquine are uncommon when the medication is used at doses needed for malaria prophylaxis. Minor side effects include gastrointestinal upset, headache, dizziness, blurred vision, and pruritus. Adverse events rarely require discontinuation of medication. Chloroquine may be taken during pregnancy and is not contraindicated in children with G6PD deficiency.

The choice of antimalarial agents in locations where drug-resistant parasites have been identified is more challenging. Chloroquine-containing regimens are not recommended for travel to areas with known chloroquine-resistant *P. falciparum*, and deaths from malaria have occurred when these regimens have been used.[17] The choice of regimen depends on the type of drug resistance, length of stay, age of the traveler, and individual medical history. Drugs currently available in the United States include mefloquine, doxycycline, atovaquone-proguanil, and primaquine.

Atovaquone-Proguanil

Atovaquone-proguanil is becoming one of the most popular choices for malaria chemoprophylaxis because of the favorable profile of side effects and the need to take the medication once daily for only 1 to 2 days before and 7 days after leaving the area of risk for malaria. Once-weekly dosing of atovaquone-proguanil for prophylaxis may be possible, but larger field trials are needed before this can be recommended.[32] The drug is effective against most *P. falciparum*, including resistant strains. It is available in adult and pediatric dosing forms and is administered daily. Adverse events most commonly reported with atovaquone-proguanil are abdominal pain, nausea, vomiting, and headache. It is not recommended at this time for prophylaxis of infants weighing less than 5 kg, for mothers nursing such infants, or for pregnant women.

Mefloquine

Mefloquine generally is well tolerated in children and can be given to infants. Dosing is based on weight, and no liquid preparation is available. Doses for older children can be supplied by breaking tablets; when small fractions of tablets are needed, pharmacists can provide individual doses in capsules. The medication has a pasty consistency but does not taste bitter. Infants tend to swallow doses better when given liquids or semisolid foods after the medication. Adverse events with mefloquine are self-limited in most cases and include gastrointestinal disturbance, insomnia, and dizziness.[79] Withdrawal rates in adults, presumably caused by the incidence of these adverse events, may impair the efficacy of mefloquine.[29] Rarely, serious adverse events, such as psychosis or seizures, have occurred after prophylactic doses of mefloquine. Consequently, mefloquine should not be used in individuals who have seizures or neuropsychiatric disorders or in individuals who have had previous adverse events with mefloquine. Mefloquine also is contraindicated in patients with cardiac conduction abnormalities, although it may be used in individuals concurrently taking β blockers for indications other than arrhythmias.

Mefloquine must be taken weekly, beginning 1 to 2 weeks before travel (with some experts recommending initiation up to 4 weeks before travel) and continued for 4 weeks after leaving the malarial area. Families may be counseled to choose a day of the week to take the medicine and to take their first dose or doses on that day of the week 1 to 2 weeks before departure. Mefloquine may be used by pregnant women in the second and third trimester and also in the first trimester if traveling to a chloroquine-resistant area.[111,155]

Doxycycline

Doxycycline is an effective antimalarial drug in parts of the world where chloroquine resistance is present. It also has been shown to be effective in areas where mefloquine-resistant *P. falciparum* exists, such as portions of Cambodia, Myanmar (Burma), Vietnam, and Thailand. Limitations in the use of this drug include that it is contraindicated in children younger than 8 years, need for daily dosing, and photosensitivity. In women, daily use of doxycycline may be associated with an increased risk for *Candida* vulvovaginitis. Gastrointestinal side effects, including nausea and vomiting, can be limited by taking the medication with meals and by avoiding bedtime dosing. Doxycycline must not be used by pregnant or lactating women. Prophylaxis with doxycycline needs to begin 1 to 2 days before travel and should be continued daily for 4 weeks after leaving the malarial area.

Primaquine

Primaquine is used most often to eradicate exoerythrocytic stages of *P. vivax* and *P. ovale*. The CDC recommends primaquine for use primarily in regions with predominantly vivax malaria. Individuals must be tested and shown not to be deficient in G6PD before they take this drug. Efficacy of primaquine compared favorably with that of mefloquine, doxycycline, and chloroquine plus proguanil in children in the holoendemic area of western Kenya.[43,139,158] It cannot be used in pregnant women.

Antimalarial Agents Not Available or Recommended in the United States for Prevention of Malaria

Tafenoquine

Tafenoquine is an investigational long-acting primaquine analog that kills parasites in the liver and in blood. It is thought to be more effective and less toxic than primaquine, but it cannot be used by individuals with G6PD deficiency or by pregnant women. No dosing regimen has been established, and it is not licensed for use,[50,140,157] but it may be available in the future.[59,71,140]

Proguanil

Proguanil has been used in combination with chloroquine to prevent chloroquine-resistant *P. falciparum*, but is less effective than mefloquine, and is not recommended or available as a single agent in the United States.

Pyrimethamine-Sulfadoxine

Pyrimethamine-sulfadoxine has been used for prevention of malaria in areas of chloroquine-resistant *P. falciparum*. An unacceptably high incidence of Stevens-Johnson reactions (fatalities in 1 in 11,000 to 25,000 users) resulted in withdrawal of the indication for using this drug for malaria prophylaxis.

Stand-By Emergency Self-Treatment

Stand-by emergency treatment (SBET) is the self-administration of antimalarial drugs when malaria is expected and prompt medical attention is unavailable within 24 hours of the onset of symptoms. This strategy is intended for emergency use only and is considered when the risks of toxicity from chemoprophylactic drugs

outweigh the benefit of avoided infection. SBET should not be recommended for travelers to high-risk *P. falciparum*-endemic areas, where chemoprophylaxis is the preferred strategy. SBET is an option for clearly defined situations, and its use must be weighed carefully with the traveler's ability to use SBET as directed.[4,137]

Vaccine

Vaccination against malaria has been a subject of active research for several decades, but a licensed vaccine is not yet available. Development of a vaccine is challenging because of the complexity of the parasite life cycle, heterogeneity of the host immune response, lack of animal models, and absence of surrogate markers of protection. Testing of vaccines is difficult because vaccine efficacy depends on conditions of transmission and the degree of immunity present in the population being studied.[152] Targets for development of a vaccine include the sporozoite (prevention of infection), the merozoite (prevention of disease manifestations by preventing invasion of RBCs), or the gametocyte (prevention of transmission by interfering with development of the parasite within the mosquito). Hundreds of candidate vaccine antigens have been identified by the *P. falciparum* genome project; choosing the most promising ones is challenging.[47]

The first malaria vaccines were directed against circumsporozoite antigen, an antigen present over most of the surface of the sporozoite. Development of these vaccines was based on the concept that sporozoites should be susceptible to antibody-mediated destruction in the extracellular space. Results of early studies with these vaccines were disappointing, with inadequate protection provided against sporozoite challenge, inconsistent relationships between antibody response and clinical efficacy, and numerous local side effects.[54,58] More recently, the candidate vaccines RTS,S/AS02A and RTS,S/ASOIE have shown promise in trials in Mozambique[2] and Kenya.[9] The candidate vaccine RTS,S/AS01, currently being studied in a phase 3 trial in seven African countries, has shown vaccine efficacy of 50.4 percent against malaria, with efficacy against severe malaria of 45.1 percent.[134] Merozoite surface proteins are another group of antigens that have been studied in connection with the development of a blood-stage, or asexual, malaria vaccine.[118] The SPf66 vaccine, derived from three merozoite proteins linked to a 4-amino acid from a *P. falciparum* circumsporozoite protein, has been tested in clinical trials in Colombia, Tanzania, and the Gambia.[3,30,153] Although initial studies in countries in South America with low malaria endemicity showed promise, investigations in African countries with high endemicity have been disappointing, with protective efficacy ranging from 31 percent (95% confidence interval [CI], 0% to 52%) in Tanzania to 8 percent (95% CI, 18% to 29%) for first or only clinical episodes and 3 percent (95% CI, –24% to 24%) in overall incidence of clinical episodes in the Gambia.

Vaccines that block transmission from humans to mosquitoes would reduce the reservoir of infected mosquitoes and reduce transmission on a population level. The poor immunogenicity and diversity of gametocyte antigens have proved challenging to this approach to the development of a vaccine, and no vaccines of this type have been used yet in clinical trials. Future vaccines are likely to use a combination approach that incorporates target antigens from all three stages of the parasite. To have a significant impact on decreasing worldwide burden of malaria, vaccines ultimately may need to be combined with other prevention strategies, including protection against mosquito bites with insecticides and treated bed nets and intermittent preventive treatment with antimalarial drugs.[47]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Arguin PM, Mali S. Malaria. In: Brunette G, Kozarsky PE, Magill AJ, et al, editors. *CDC health information for international travel 2012*. Oxford: Oxford University Press; 2012. p. 223–43.
6. Bassat Q. The use of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium vivax* malaria. *PLoS Negl Trop Dis* 2011;**5**(12):1–10.
11. Bottieau E, Clerinx J, Van den Enden E, et al. Imported non-*Plasmodium falciparum* malaria: a five-year prospective study in a European referral center. *Am J Trop Med Hyg* 2006;**75**(1):133–8.
24. Cohuet A, Harris C, Robert V, et al. Evolutionary forces on *Anopheles*: what makes a malaria vector? *Trends Parasitol* 2010;**26**(3): 130–6.
28. Crawley J, Smith S, Muthinji P, et al. Electroencephalographic and clinical features of cerebral malaria. *Arch Dis Child* 2001;**84**: 247–53.
32. Deye GA, Miller SA, Miller L, et al. Prolonged protection provided by a single dose of atovaquone-proguanil for the chemoprophylaxis of *Plasmodium falciparum* malaria in a human challenge model. *Clin Infect Dis* 2012;**54**(2):232–9.
34. Doerig C, Billker O, Haystead T, et al. Protein kinases of malaria parasites: an update. *Trends Parasitol* 2008;**24**(12):570–7.
35. Dondorp AM, Fairhurst RM, Slutsker L, et al. The threat of artemisinin-resistant malaria. *N Engl J Med* 2011;**365**(12): 1073–5.
37. Ezzet F, Van Vugt M, Nosten F, et al. Pharmacokinetics and pharmacodynamics of lumefantrine (Benflutemol) in acute falciparum malaria. *Antimicrob Agents Chemother* 2000;**44**(3):697–704.
38. Falade C, Makanga M, Premji Z, et al. Efficacy and safety of artemether-lumefantrine (Coartem®) tablets (six-dose regimen) in African infants and children with acute, uncomplicated falciparum malaria. *Trans R Soc Trop Med Hyg* 2005;**99**(6):459–67.
46. Greenberg A, Lobel HO. Mortality from *Plasmodium falciparum* malaria in travelers from the United States. *Ann Intern Med* 1990;**113**:326–7.
52. Hawkes M, Opoka RO, Namasopo S, et al. Inhaled nitric oxide for the adjunctive therapy of severe malaria: protocol for a randomized controlled trial. *Trials* 2011;**12**:176–88.
60. Houze S, Hubert V, Cohen DP, et al. Evaluation of the Clearview® malaria pLDH malaria rapid diagnostic test in a non-endemic setting. *Malaria J* 2011;**10**:284–8.
65. Jakka SR, Veena S, Atmakuri RM, et al. Characteristic abnormalities in cerebrospinal fluid biochemistry in children with cerebral malaria compared to viral encephalitis. *Cerebrospinal Fluid Res* 2006;**3**:1–7.
66. Jelinek J, Amsler L, Grobusch MP, et al. Self-use of rapid tests for malaria diagnosis by tourists. *Lancet* 1999;**354**:1609.
68. John CC, Kutamba E, Mugarura K, et al. Adjunctive therapy for cerebral malaria and other severe forms of *Plasmodium falciparum* malaria. *Expert Rev Anti Infect Ther* 2010;**8**(9):997–1008.
73. King CL, Adams JH, Xianli J, et al. Fya/Fyb antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to *Plasmodium vivax* malaria. *Proc Natl Acad Sci U S A* 2001; **108**(50):20113–18.
87. Maiga OM, Kayentao K, Traore BT, et al. Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in Mali: a randomized controlled trial. *Clin Infect Dis* 2011;**53**(3): 215–23.
89. Malhotra I, Mungai P, Muchiri E, et al. Umbilical cord-blood infections with *Plasmodium falciparum* malaria are acquired antenatally in Kenya. *J Infect Dis* 2006;**194**:176–83.

90. Mali S, Kachur SP, Arguin PM. Malaria surveillance—United States, 2010. *Morb Mortal Wkly Rep Surveill Summ* 2012;**61**(2): 1–17.
104. Murray CJ, Rosenfeld LC, Lim SS, et al. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* 2012;**379**: 413–31.
108. Nothdurft HD, Jelinek T, Chiodini PL. Use of malaria rapid diagnostic tests for and by travelers. In: Schlagenhauf-Lawlor P, editor. *Travelers' malaria*. 2nd ed. Hamilton, Ontario: BC Decker; 2008. p. 300–5.
113. Oakley MS, Gerald N, McCutchan TF, et al. Clinical and molecular aspects of malaria fever. *Trends Parasitol* 2011;**27**(10):442–9.
123. Ponsford MJ, Medana IM, Prapansilp P, et al. Sequestration and microvascular congestion are associated with coma in human cerebral malaria. *J Infect Dis* 2011;**204**(13):1–9.
125. Randall LM, Engwerda CR. TNF family members and malaria: old observations, new insights and future directions. *J Parasitol* 2010;**126**:326–31.
131. Roca-Feltrer A, Kwizome CJ, Sanjoaquin MA, et al. Lack of decline in childhood malaria, Malawi, 2001-2010. *Emerg Infect Dis* 2012;**18**(2):272–8.
134. The RTS, S Clinical Trials Partnership. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 2011;**365**(20):1863–75.
137. Schlagenhauf-Lawlor P. Stand-by emergency self-treatment of malaria by travelers. In: Schlagenhauf-Lawlor P, editor. *Travelers' malaria*. 2nd ed. Hamilton, Ontario: BC Decker; 2008. p. 316–22.
143. Singh RK. Hyperreactive malarial splenomegaly in expatriates. *Travel Med Infect Dis* 2007;**5**(1):24–9.
154. van Genderen PJJ, Hesselink DA, Bezemer JM, et al. Efficacy and safety of exchange transfusion as an adjunct therapy for severe *Plasmodium falciparum* malaria in nonimmune travelers: a 10-year single-center experience with a standardized treatment protocol. *Transfusion* 2010;**50**(4):787–94.
164. Wilson ME, Weld LH, Boggild A, et al. Fever in returned travelers: results from the GeoSentinel Surveillance Network. *Clin Infect Dis* 2006;**44**(12):1560–8.
165. World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2000;**94**(Suppl. 2):S1–90.
166. World Health Organization. *Guidelines for the treatment of malaria*. 2nd ed. Geneva: World Health Organization; 2010. http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf. Accessed June 10, 2013.
167. World Health Organization. *World malaria report 2011*. Geneva: World Health Organization; 2011. http://www.who.int/malaria/world_malaria_report_2011/9789241564403_eng.pdf. Accessed June 10, 2013.
169. Yohannes AM, Teklehaimanot A, Bergqvist Y, et al. Confirmed vivax resistance to chloroquine and effectiveness of artemether-lumefantrine for the treatment of vivax malaria in Ethiopia. *Am J Trop Med Hyg* 2011;**84**(1):137–40.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 220

Leishmaniasis

Lynne S. Garcia

Leishmaniasis is a parasitic disease affecting more than 12 million people in 88 countries, with 350 million more at risk and more than 400,000 new cases reported each year.[4] The infection consists of a group of diseases that may affect the skin, mucous membranes, or viscera with a wide range of clinical presentations caused by obligate intracellular hemoflagellates of the genus *Leishmania*. Infection is transmitted by several genera and species of phlebotomine sandflies. Three major clinical syndromes usually are recognized: cutaneous (CL), mucocutaneous (MCL), and visceral (VL) leishmaniasis. Macrophages of the skin, mucous membranes, or spleen, liver, and bone marrow (reticuloendothelial system) are parasitized. New species continue to be identified, particularly in the New World. However, the taxonomy of leishmaniasis is controversial, and species differentiation is currently based on molecular techniques rather than morphology and clinical presentation.[9]

Studies on the components of sandfly saliva suggest that substances may partially influence the tropism of the parasite and therefore the resulting disease. The amount of a vasodilatory peptide, maxadilan, in sandfly saliva appears to correlate with the type of human infection.[47] Sandflies in Brazil that transmit *Leishmania chagasi* VL have relatively large amounts of maxadilan in their saliva that causes marked vasodilation and may encourage visceralization of the parasites. The same complex of sandflies in Costa Rica, which have relatively small amounts of the vasodilatory peptide in their saliva, transmit organisms that produce a nonulcerating and nonvisceralizing *L. chagasi* infection that is limited to the dermis.

The clinical manifestations of leishmaniasis appear to depend on a complex set of factors, including tropism and virulence of the parasite strain, as well as the susceptibility of the host, which may be determined genetically.[32] Cell-mediated immune mechanisms appear to be the

REFERENCES

1. Adjuik M, Babiker A, Garner P, et al. Artesunate combinations for treatment of malaria: meta-analysis. *Lancet* 2004;**363**:9–17.
2. Alonso PL, Sacarial J, Aponte JJ, et al. Efficacy of the RTS,S/AS02A vaccine against *Plasmodium falciparum* infection and disease in young African children: randomized controlled trial. *Lancet* 2004;**364**:1411–20.
3. Alonso PL, Smith T, Armstrong Schellenberg JRM, et al. Randomised trial of efficacy of SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. *Lancet* 1994; **344**:1175–81.
4. Arguin PM, Mali S. Malaria. In: Brunette G, Kozarsky PE, Magill AJ, et al, editors. *CDC health information for international travel 2012*. Oxford: Oxford University Press; 2012. p. 223–43.
5. Barker RH, Suebsaeng L, Rooney W, et al. Detection of *Plasmodium falciparum* infection in human patients: a comparison of the DNA probe method to microscopic diagnosis. *Am J Trop Med Hyg* 1989;**41**:266–72.
6. Bassat Q. The use of artemether-lumefantrine for the treatment of uncomplicated *Plasmodium vivax* malaria. *PLoS Negl Trop Dis* 2011;**5**(12):1–10.
7. Bates I, Bedu-Addo G, Bevan DH, et al. Use of immunoglobulin gene arrangements to show clonal lymphoproliferation in hyperreactive malarial splenomegaly. *Lancet* 1991;**337**:505.
8. Beadle C, Long GW, Weiss WR. Diagnosis of malaria by detection of *Plasmodium falciparum* HRP-2 antigen with a rapid dipstick antigen-capture assay. *Lancet* 1994;**343**:564–8.
9. Bejon P, Lusingu J, Olotu A, et al. Efficacy of RTS,S/ASOIE vaccine against malaria in children 5 to 17 months of age. *N Engl J Med* 2008;**359**:2521–32.
10. Binka FN, Kubaje A, Adjuik M, et al. Impact of permethrin impregnated bednets on child mortality in Kassena-Nankana district, Ghana: a randomized controlled trial. *Trop Med Int Health* 1996;**1**:147–54.
11. Bottieau E, Clerinx J, Van den Enden E, et al. Imported non-*Plasmodium falciparum* malaria: a five-year prospective study in a European referral center. *Am J Trop Med Hyg* 2006;**75**(1):133–8.
12. Bouma MJ, Dye C. Cycles of malaria associated with El Niño in Venezuela. *JAMA* 1997;**278**:1772–4.
13. Brair ME, Brabin BJ, Milligan P, et al. Reduced transfer of tetanus antibodies with placental malaria. *Lancet* 1994;**343**:208–9.
14. Brewster DR, Kwiatkowski D, White NJ. Neurological sequelae of cerebral malaria in children. *Lancet* 1990;**336**:1039–43.
15. Centers for Disease Control and Prevention. Probable locally acquired mosquito-transmitted *Plasmodium vivax* infection—Suffolk County, New York, 1999. *Morb Mortal Wkly Rep* 2000;**49**: 495–8.
16. Centers for Disease Control and Prevention. Sudden death in a traveler following halofantrine administration—Togo, 2000. *Morb Mortal Wkly Rep* 2001;**50**:169–79.
17. Centers for Disease Control and Prevention. Malaria deaths following inappropriate malaria chemoprophylaxis—United States, 2001. *Morb Mortal Wkly Rep* 2001;**50**:597–9.
18. Centers for Disease Control and Prevention. Congenital malaria—Nassau County, New York, 2004. *Morb Mortal Wkly Rep* 2005; **54**:383.
19. Centers for Disease Control and Prevention. *Health Information for International Travel, 2005-2006*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2005.
20. Chen LH, Keystone JS. New strategies for the prevention of malaria in travelers. *Infect Dis Clin North Am* 2005;**19**:185–210.
21. Chen LH, Wilson ME, Schlagenhauf P. Prevention of malaria in long-term travelers. *JAMA* 2006;**296**:2234–44.
22. Chiodini J. Malaria in UK travellers: assessment, prevention and treatment. *Nursing Standard* 2006;**20**:49–57.
23. Chiodini PL. Non-microscopic methods for diagnosis of malaria. *Lancet* 1998;**351**:80–1.
24. Cohuet A, Harris C, Robert V, et al. Evolutionary forces on *Anopheles*: what makes a malaria vector? *Trends Parasitol* 2010; **26**(3):130–6.
25. Coleman RE, Maneechai N, Rachapaew N, et al. Field evaluation of the ICT Malaria Pf/Pv immunochromatographic test for the detection of asymptomatic malaria in a *Plasmodium falciparum/vivax* endemic area in Thailand. *Am J Trop Med Hyg* 2002; **66**:379–83.
26. Cooke AH, Chiodini PL, Doherty T, et al. Comparison of a parasite lactate dehydrogenase-based immunochromatographic antigen detection assay (OptiMAL) with microscopy for the detection of malaria parasites in human blood samples. *Am J Trop Med Hyg* 1999;**60**:173–6.
27. Cox-Singh J, Davis TME, Lee K-S, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. *Clin Infect Dis* 2008;**46**:165–71.
28. Crawley J, Smith S, Muthinji P, et al. Electroencephalographic and clinical features of cerebral malaria. *Arch Dis Child* 2001;**84**: 247–53.
29. Croft A, Garner P. Mefloquine to prevent malaria: a systematic review of trials. *BMJ* 1998;**315**:1412–16.
30. D'Alessandro U, Leach A, Drakeley CJ, et al. Efficacy trial of malaria vaccine SPf66 in Gambian infants. *Lancet* 1995;**346**: 462–7.
31. D'Alessandro U, Olaleye BO, McGuire W, et al. Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet* 1995;**345**:479–83.
32. Deye GA, Miller SA, Miller L, et al. Prolonged protection provided by a single dose of atovaquone-proguanil for the chemoprophylaxis of *Plasmodium falciparum* malaria in a human challenge model. *Clin Infect Dis* 2012;**54**(2):232–9.
33. Dietze R, Perkins M, Boulos M, et al. The diagnosis of *Plasmodium falciparum* infection using a new antigen detection system. *Am J Trop Med Hyg* 1995;**52**:45–9.
34. Doerig C, Billker O, Haystead T, et al. Protein kinases of malaria parasites: an update. *Trends Parasitol* 2008;**24**(12):570–7.
35. Dondorp AM, Fairhurst RM, Slutsker L, et al. The threat of artemisinin-resistant malaria. *N Engl J Med* 2011;**365**(12): 1073–5.
36. Emanuel B, Aronson N, Shulman S. Malaria in children in Chicago. *Pediatrics* 1993;**92**:83–5.
37. Ezzet F, Van Vugt M, Nosten F, et al. Pharmacokinetics and pharmacodynamics of lumefantrine (Benflutemol) in acute falciparum malaria. *Antimicrob Agents Chemother* 2000;**44**(3):697–704.
38. Falade C, Makanga M, Premji Z, et al. Efficacy and safety of artemether-lumefantrine (Coartem®) tablets (six-dose regimen) in African infants and children with acute, uncomplicated falciparum malaria. *Trans R Soc Trop Med Hyg* 2005;**99**(6):459–67.
39. Farcas GA, Soeller R, Zhong K, et al. Real-time polymerase chain reaction assay for the rapid detection and characterization of chloroquine-resistant *Plasmodium falciparum* malaria in returned travelers. *Clin Infect Dis* 2006;**42**:622–7.
40. Farcas GA, Zhong KJY, Lovegrove F, et al. Evaluation of the Binax Now ICT test versus polymerase chain reaction and microscopy for the detection of malaria in returned travelers. *Am J Trop Med Hyg* 2003;**69**:589–92.
41. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med* 2002;**347**:13–18.
42. Franco-Paredes C, Santos-Preciado JI. Problem pathogens: prevention of malaria in travellers. *Lancet Infect Dis* 2006;**6**:139–49.
43. Fryauff DJ, Baird JK, Basri H, et al. Randomised placebo-controlled trial of primaquine for prophylaxis of falciparum and vivax malaria. *Lancet* 1995;**346**:1190–3.
44. Garg P, Mody P, Lall KB. Toxic psychosis due to chloroquine—not uncommon in children. *Clin Pediatr (Phila)* 1990;**29**:448–50.
45. Graves PM, Brabin BJ, Charlwood JD, et al. Reduction in incidence and prevalence of *Plasmodium falciparum* in under-5-year-old children by permethrin impregnation of mosquito nets. *Bull World Health Organ* 1987;**65**:869–77.
46. Greenberg A, Lobel HO. Mortality from *Plasmodium falciparum* malaria in travelers from the United States. *Ann Intern Med* 1990;**113**:326–7.
47. Greenwood B. Malaria vaccines evaluation and implementation. *Acta Trop* 2005;**95**:298–304.
48. Greenwood B, Alonso P, ter Kuile FO, et al. Malaria in pregnancy: priorities for research. *Lancet Infect Dis* 2007;**7**:169–74.
49. Greenwood BM, Bojang K, Whitty CJ, Targett GA. Malaria. *Lancet* 2005;**365**:1487–98.
50. Hale BR, Owusu-Agyei S, Fryauff DJ, et al. A randomized, double-blind, placebo-controlled, dose-ranging trial of tafenoquine for weekly prophylaxis against *Plasmodium falciparum*. *Clin Infect Dis* 2003;**36**:541–9.
51. Hänscheid T, Pinto BG, Pereira I, et al. Avoiding misdiagnosis of malaria: a novel automated method allows specific diagnosis, even

in the absence of clinical suspicion. *Emerg Infect Dis* 1999;**5**: 836–7.
52. Hawkes M, Opoka RO, Namasopo S, et al. Inhaled nitric oxide for the adjunctive therapy of severe malaria: protocol for a randomized controlled trial. *Trials* 2011;**12**:176–88.
53. Haworth FLM, Cook GC. Needlestick malaria. *Lancet* 1995; **346**:1361.
54. Heppner DG, Gordon DM, Gross M, et al. Safety, immunogenicity, and efficacy of *Plasmodium falciparum* repeatless circumsporozoite protein vaccine encapsulated in liposomes. *J Infect Dis* 1996;**174**:361–6.
55. Hien TT, Day NPJ, Phu NH, et al. A controlled trial of artemether or quinine in Vietnamese adults with severe falciparum malaria. *N Engl J Med* 1996;**335**:76–83.
56. Hien TT, White NJ. Qinghaosu. *Lancet* 1993;**341**:603–8.
57. Hill DR, Ericsson CD, Pearson RD, et al. The practice of travel medicine: guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006;**43**:1499–539.
58. Hoffman SL, Edelman R, Bryan JP, et al. Safety, immunogenicity, and efficacy of a malaria sporozoite vaccine administered with monophosphoryl lipid A, cell wall skeleton of mycobacteria, and squalane as adjuvant. *Am J Trop Med Hyg* 1994; **51**:603–12.
59. Hogh B, Clarke PD, Camus D, et al. Atovaquone-proguanil versus chloroquine-proguanil for malaria prophylaxis in non-immune travellers: a randomised, double-blind study. *Lancet* 2000; **356**:1888–94.
60. Houze S, Hubert V, Cohen DP, et al. Evaluation of the Clearview® malaria pLDH malaria rapid diagnostic test in a non-endemic setting. *Malaria J* 2011;**10**:284–8.
61. Hulbert TV. Congenital malaria in the United States: report of a case and review. *Clin Infect Dis* 1992;**14**:922–6.
62. Humar A, Ohrt C, Harrington MA, et al. Parasight F test compared with the polymerase chain reaction and microscopy for the diagnosis of *Plasmodium falciparum* malaria in travelers. *Am J Trop Med Hyg* 1997;**56**:44–8.
63. Idro R, Jenkins NE, Newton CR. Pathogenesis, clinical features, and neurological outcome of cerebral malaria. *Lancet Neurol* 2005;**4**:827–40.
64. Iqbal J, Khalid N, Hira PR. Comparison of two commercial assays with expert microscopy for confirmation of symptomatically diagnosed malaria. *J Clin Microbiol* 2002;**40**:4675–8.
65. Jakka SR, Veena S, Atmakuri RM, et al. Characteristic abnormalities in cerebrospinal fluid biochemistry in children with cerebral malaria compared to viral encephalitis. *Cerebrospinal Fluid Res* 2006;**3**:1–7.
66. Jelinek J, Amsler L, Grobusch MP, et al. Self-use of rapid tests for malaria diagnosis by tourists. *Lancet* 1999;**354**:1609.
67. Joffe A, Jadavji T. Congenital malaria: a case report of a preventable disease. *Pediatr Infect Dis J* 1990;**9**:522–3.
68. John CC, Kutamba E, Mugarura K, et al. Adjunctive therapy for cerebral malaria and other severe forms of *Plasmodium falciparum* malaria. *Expert Rev Anti Infect Ther* 2010;**8**(9):997–1008.
69. Johnston SP, Pieniazek NJ, Xayavong MV, et al. PCR as a confirmatory technique for laboratory diagnosis of malaria. *J Clin Microbiol* 2006;**44**:1087–9.
70. Jones KN, Mascia B, Waggoner-Fountain L, Pearson RD. Diagnosis by automated blood analyzer (photo quiz). *Clin Infect Dis* 2001;**33**:1886, 1944–5.
71. Kain KC, Shanks GD, Keystone JS. Malaria chemoprophylaxis in the age of drug resistance, part I: currently recommended drug regimens. *Clin Infect Dis* 2001;**33**:226–34.
72. Karch S, Dellile M-F, Guillet P, Mouchet J. African malaria vectors in European aircraft. *Lancet* 2001;**357**:235.
73. King CL, Adams JH, Xianli J, et al. Fya/Fyb antigen polymorphism in human erythrocyte Duffy antigen affects susceptibility to *Plasmodium vivax* malaria. *Proc Natl Acad Sci U S A* 2001; **108**(50):20113–18.
74. Kitron U, Spielman A. Suppression of transmission of malaria through source reduction: antianopheline measures applied in Israel, the United States, and Italy. *Rev Infect Dis* 1989;**11**:391–406.
75. Krogstad DJ. Malaria. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens and practice*. 2nd ed. New York: Churchill Livingstone; 2006.
76. Kwiatkowski D, Hill AVS, Sambou I, et al. TNF concentration in fatal cerebral, non-fatal cerebral, and uncomplicated *Plasmodium falciparum* malaria. *Lancet* 1990;**336**:1201–4.
77. Lalloo DG, Olukoya P, Olliaro P. Malaria in adolescence: burden of disease, consequences, and opportunities for intervention. *Lancet Infect Dis* 2006;**6**:780–93.
78. Layton M, Parise ME, Campbell CC, et al. Mosquito-transmitted malaria in New York City, 1993. *Lancet* 1995;**346**:729–31.
79. Lobel HO, Miani M, Eng T, et al. Long-term malaria prophylaxis with weekly mefloquine. *Lancet* 1993;**341**:848–51.
80. Loevinsohn ME. Climatic warming and increased malaria incidence in Rwanda. *Lancet* 1994;**343**:714–18.
81. Looareesuwan S, Chulay JD, Canfield CJ, Hutchinson D. Malarone (atovaquone and proguanil hydrochloride): a review of its clinical development for treatment of malaria. *Am J Trop Med Hyg* 1999;**60**:533–41.
82. Looareesuwan S, Phillips RE, White NJ, et al. Quinine and severe falciparum malaria in late pregnancy. *Lancet* 1985;**2**:4–7.
83. Looareesuwan S, Viravan C, Vanijanonta S, et al. Randomised trial of artesunate and mefloquine alone and in sequence for acute uncomplicated falciparum malaria. *Lancet* 1992;**339**:821–4.
84. Lynk A, Gold R. Review of 40 children with imported malaria. *Pediatr Infect Dis J* 1989;**8**:745–50.
85. Mackintosh CL, Beeson JG, Marsh K. Clinical features and pathogenesis of severe malaria. *Trends Parasitol* 2004;**20**:597–603.
86. Makler MT, Hinrichs DJ. Measurement of the lactate dehydrogenase activity of *Plasmodium falciparum* as an assessment of parasitemia. *Am J Trop Med Hyg* 1993;**48**:205–10.
87. Maiga OM, Kayentao K, Traore BT, et al. Superiority of 3 over 2 doses of intermittent preventive treatment with sulfadoxine-pyrimethamine for the prevention of malaria during pregnancy in Mali: a randomized controlled trial. *Clin Infect Dis* 2011;**53**(3): 215–23.
88. Malakooti MA, Biomndo K, Shanks GD. Reemergence of epidemic malaria in the highlands of western Kenya. *Emerg Infect Dis* 1998;**4**:671–6.
89. Malhotra I, Mungai P, Muchiri E, et al. Umbilical cord-blood infections with *Plasmodium falciparum* malaria are acquired antenatally in Kenya. *J Infect Dis* 2006;**194**:176–83.
90. Mali S, Kachur SP, Arguin PM. Malaria surveillance—United States, 2010. *Morb Mortal Wkly Rep Surveill Summ* 2012;**61**(2): 1–17.
91. Maloney SA, Weinberg M. Prevention of infectious diseases among international pediatric travelers: considerations for clinicians. *Semin Pediatr Infect Dis* 2004;**15**:137–49.
92. Marsh K, Forster D, Waruiru C, et al. Indicators of life-threatening malaria in African children. *N Engl J Med* 1995; **332**:1399–404.
93. Martens P, Hall L. Malaria on the move: human population movement and malaria transmission. *Emerg Infect Dis* 2000;**6**: 103–9.
94. Marx A, Pewsner D, Egger M, et al. Meta-analysis: accuracy of rapid tests for malaria in travelers returning from endemic areas. *Ann Intern Med* 2005;**142**:836–46.
95. McGuire W, Hill AV, Allsopp CE, et al. Variation in the TNF-alpha promoter region associated with susceptibility to cerebral malaria. *Nature* 1994;**371**:508–10.
96. McGuire W, Knight JC, Hill AV, et al. Severe malarial anemia and cerebral malaria are associated with different tumor necrosis factor promoter alleles. *J Infect Dis* 1999;**179**:287–90.
97. McLaughlin GL, Subramanian S, Lepers JP, et al. Evaluation of a nonisotopic DNA assay kit for diagnosing *Plasmodium falciparum* malaria in Madagascar. *Am J Trop Med Hyg* 1993;**48**: 211–15.
98. Medical Letter: Picaridin: a new insect repellent. *Med Lett Drugs Ther* 2005;**47**:46–7.
99. Menendez C. Malaria during pregnancy: a priority area of malaria research and control. *Parasitol Today* 1995;**11**:178–83.
100. Miller LH, Mason SJ, Clyde DF, McGinniss MH. The resistance factor to *Plasmodium vivax* in blacks: the Duffy-blood-group genotype, FyFy. *N Engl J Med* 1976;**295**:302–4.
101. Mirochnick M, Barnett ED, Clarke DF, et al. Stability of chloroquine in an extemporaneously prepared suspension stored at three temperatures. *Pediatr Infect Dis J* 1994;**13**:827–8.
102. Moody A. Rapid diagnostic tests for malaria parasites. *Clin Microbiol Rev* 2002;**15**:66.

103. Mungai M, Tegtmeier G, Chamberland M, et al. Transfusion-transmitted malaria in the United States from 1963 through 1999. *N Engl J Med* 2001;**344**:1973–8.
104. Murray CJ, Rosenfeld LC, Lim SS, et al. Global malaria mortality between 1980 and 2010: a systematic analysis. *Lancet* 2012;**379**: 413–31.
105. Mutabingwa TK, Anthony D, Heller A, et al. Amodiaquine alone, amodiaquine + sulfadoxine-pyrimethamine, amodiaquine + artesunate, and artemether-lumefantrine for outpatient treatment of malaria in Tanzanian children: a four-arm randomized effectiveness trial. *Lancet* 2005;**365**:1474–80.
106. Nagatake T, Van Thuc H, Tegoshi T, et al. Pathology of falciparum malaria in Vietnam. *Am J Trop Med Hyg* 1992;**47**: 259–64.
107. Newman RD, Parise ME, Barber AM, et al. Malaria-related deaths among US travelers, 1963-2001. *Ann Intern Med* 2004; **141**:547–55.
108. Nothdurft HD, Jelinek T, Chiodini PL. Use of malaria rapid diagnostic tests for and by travelers. In: Schlagenhauf-Lawlor P, editor. *Travelers' malaria*. 2nd ed. Hamilton, Ontario: BC Decker; 2008. p. 300–5.
109. Nosten F, McGready R, Simpson JA, et al. Effects of *Plasmodium vivax* malaria in pregnancy. *Lancet* 1999;**354**:546–9.
110. Nosten F, ter Kuile FO, Luxemburger C, et al. Cardiac effects of antimalarial treatment with halofantrine. *Lancet* 1993;**341**: 1054–6.
111. Nosten F, ter Kuile F, Maelankiri L, et al. Mefloquine prophylaxis prevents malaria during pregnancy: a double-blind, placebo-controlled study. *J Infect Dis* 1994;**169**:595–603.
112. Nosten F, van Vugt M, Price R, et al. Effects of artesunate-mefloquine combination on incidence of *Plasmodium falciparum* malaria and mefloquine resistance in western Thailand: a prospective study. *Lancet* 2000;**356**:297–302.
113. Oakley MS, Gerald N, McCutchan TF, et al. Clinical and molecular aspects of malaria fever. *Trends Parasitol* 2011;**27**(10): 442–9.
114. Ochola L, Vounatsou P, Smith T, et al. The reliability of diagnostic techniques in the diagnosis and management of malaria in the absence of a gold standard. *Lancet Infect Dis* 2006;**6**:582–8.
115. Olliaro P, Nevill C, LeBras J, et al. Systematic review of amodiaquine treatment in uncomplicated malaria. *Lancet* 1996;**348**: 1196–201.
116. Owusu-Ofori AK, Parry C, Bates I. Transfusion-transmitted malaria in countries where malaria is endemic: a review of the literature from sub-Saharan Africa. *Clin Infect Dis* 2010;**51**(10): 1192–8.
117. Palmer CJ, Lindo JF, Klaskala WI, et al. Evaluation of the OptiMAL test for the rapid diagnosis of *Plasmodium vivax* and *Plasmodium falciparum* malaria. *J Clin Microbiol* 1998;**36**:203–6.
118. Pasloske BL, Howard RJ. The promise of asexual malaria vaccine development. *Am J Trop Med Hyg* 1994;**50**(Suppl.):3–10.
119. Perrin LH, Mackey LJ, Miescher PA. The hematology of malaria in man. *Semin Hematol* 1982;**19**:70–82.
120. Phillips RE, Solomon T. Cerebral malaria in children. *Lancet* 1990;**336**:1355–60.
121. Piola P, Fogg C, Bajunirwe F, et al. Supervised versus unsupervised intake of six-dose artemether-lumefantrine for treatment of acute, uncomplicated *Plasmodium falciparum* malaria in Mbarara, Uganda: a randomized trial. *Lancet* 2005;**365**:1467–73.
122. Pongponratn E, Riganti M, Punpoowong B, Aikawa M. Microvascular sequestration of parasitized erythrocytes in human falciparum malaria: a pathological study. *Am J Trop Med Hyg* 1991; **44**:168–75.
123. Ponsford MJ, Medana IM, Prapansilp P, et al. Sequestration and microvascular congestion are associated with coma in human cerebral malaria. *J Infect Dis* 2011;**204**(13):1–9.
124. Quintana M, Piper R, Bloing HL, et al. Malaria diagnosis by dipstick assay in a Honduran population with coendemic *Plasmodium falciparum* and *Plasmodium vivax*. *Am J Trop Med Hyg* 1998;**59**:868–71.
125. Randall LM, Engwerda CR. TNF family members and malaria: old observations, new insights and future directions. *J Parasitol* 2010;**126**:326–31.
126. Redd SC, Bloland PB, Kazembe PN, et al. Usefulness of clinical case-definitions in guiding therapy for African children with malaria or pneumonia. *Lancet* 1992;**340**:1140–3.
127. Redd SC, Kazembe PN, Luby SP, et al. Clinical algorithm for treatment of *Plasmodium falciparum* malaria in children. *Lancet* 1996;**347**:223–7.
128. Rediscovering wormwood: qinghaosu for malaria. *Lancet* 1992; **339**:649–51.
129. Riddle MS, Jackson JL, Sanders JW, et al. Exchange transfusion as an adjunct therapy in severe *Plasmodium falciparum* malaria: a meta-analysis. *Clin Infect Dis* 2002;**34**(9):1192–8.
130. Roberts DR, Manguin S, Mouchet J. DDT house spraying and re-emerging malaria. *Lancet* 2000;**356**:330–2.
131. Roca-Feltrer A, Kwizome CJ, Sanjoaquin MA, et al. Lack of decline in childhood malaria, Malawi, 2001-2010. *Emerg Infect Dis* 2012;**18**(2):272–8.
132. Rosenthal PJ. Proteases of malaria parasites: new targets for chemotherapy. *Emerg Infect Dis* 1998;**4**:49–57.
133. Rougemont A, Breslow N, Brenner E, et al. Epidemiological basis for clinical diagnosis of childhood malaria in endemic zone in West Africa. *Lancet* 1991;**338**:1292–5.
134. The RTS,S Clinical Trials Partnership. First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *N Engl J Med* 2011;**365**(20):1863–75.
135. Ryan ET, Kain KC. Health advice and immunizations for travelers. *N Engl J Med* 2000;**342**:1716–25.
136. Schellenberg D, Cisse B, Menendez C. The IPTi Consortium: research for policy and action. *Trends Parasitol* 2006;**22**:296–300.
137. Schlagenhauf-Lawlor P. Stand-by emergency self-treatment of malaria by travelers. In: Schlagenhauf-Lawlor P, editor. *Travelers' malaria*. 2nd ed. Hamilton, Ontario: BC Decker; 2008. p. 316–22.
138. Schuman AJ. Preparing children and families to travel overseas. *Contemp Pediatr* 2001;**18**:45–59.
139. Schwartz E, Regev-Yochay G. Primaquine as prophylaxis for malaria for nonimmune travelers: a comparison with mefloquine and doxycycline. *Clin Infect Dis* 1999;**29**:1502–6.
140. Shanks GD, Kain KC, Keystone JS. Malaria chemoprophylaxis in the age of drug resistance, part II: drugs that may be available in the future. *Clin Infect Dis* 2001;**33**:381–5.
141. Shetty AK, Woods CR. Prevention of malaria in children. *Pediatr Infect Dis J* 2006;**25**:1173–6.
142. Shulman CE, Dorman EK, Cutts F, et al. Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: a randomised placebo-controlled trial. *Lancet* 1999;**353**:632–6.
143. Singh RK. Hyperreactive malarial splenomegaly in expatriates. *Travel Med Infect Dis* 2007;**5**(1):24–9.
144. Sirima SB, Cotte AH, Konate A, et al. Malaria prevention during pregnancy: assessing the disease burden one year after implementing a program of intermittent preventive treatment in Koupela District, Burkina Faso. *Am J Trop Med Hyg* 2006;**75**:205–11.
145. Slutsker LM, Khoromana CO, Payne D, et al. Mefloquine therapy for *Plasmodium falciparum* malaria in children under 5 years of age in Malawi: in vivo/in vitro efficacy and correlation of drug concentration with parasitological outcome. *Bull World Health Organ* 1990;**68**:53–9.
146. Snow RW, Lindsay SW, Hayes RJ, Greenwood BM. Permethrin-treated bed nets (mosquito nets) prevent malaria in Gambian children. *Trans R Soc Trop Med Hyg* 1988;**82**:838–42.
147. Spielman A, Perrone JB, Teklehaimanot A, et al. Malaria diagnosis by direct observation of centrifuged samples of blood. *Am J Trop Med Hyg* 1988;**39**:337–42.
148. Spira AM. Preparing the traveller. *Lancet* 2003;**361**:1368–81.
149. Taylor TE, Molyneux ME, Wirima JJ, et al. Blood glucose levels in Malawian children before and during the administration of intravenous quinine for severe falciparum malaria. *N Engl J Med* 1988;**319**:1040–7.
150. Taylor TE, Strickland GT. Malaria. In: Strickland GT, editor. *Hunter's tropical medicine and emerging infectious diseases*. 8th ed. Philadelphia: WB Saunders; 2000. p. 614–43.
151. ter Kuile FO, Dolan G, Nosten F, et al. Halofantrine versus mefloquine in treatment of multidrug-resistant falciparum malaria. *Lancet* 1993;**341**:1044–9.
152. Towards a malarial vaccine. *Lancet* 1992;**339**:586–7.
153. Valero MV, Amador LR, Galindo C, et al. Vaccination with SPf66, a chemically synthesised vaccine, against *Plasmodium falciparum* malaria in Colombia. *Lancet* 1993;**341**:705–10.

154. van Genderen PJJ, Hesselink DA, Bezemer JM, et al. Efficacy and safety of exchange transfusion as an adjunct therapy for severe *Plasmodium falciparum* malaria in nonimmune travelers: a 10-year single-center experience with a standardized treatment protocol. *Transfusion* 2010;**50**(4):787–94.
155. Vanhauwere B, Maradit H, Kerr L. Post-marketing surveillance of prophylactic mefloquine (Lariam) use in pregnancy. *Am J Trop Med Hyg* 1998;**58**:17–21.
156. van Hensbroek MB, Onyiorah E, Jaffar S, et al. A trial of artemether or quinine in children with cerebral malaria. *N Engl J Med* 1996;**335**:69–75.
157. Walsh DS, Eamsila C, Sasiprapha T, et al. Efficacy of monthly tafenoquine for prophylaxis of *Plasmodium vivax* and multidrug-resistant *P. falciparum* malaria. *J Infect Dis* 2004;**190**: 1456–63.
158. Weiss WR, Oloo AJ, Johnson A, et al. Daily primaquine is effective for prophylaxis against falciparum malaria in Kenya: comparison with mefloquine, doxycycline, and chloroquine plus proguanil. *J Infect Dis* 1995;**171**:1569–75.
159. White NJ. The treatment of malaria. *N Engl J Med* 1996; **335**:800–6.
160. White NJ. Malaria. In: Cook GC, Zumla A, editors. *Manson's tropical diseases*. 21st ed. London: WB Saunders; 2003. p. 1205–95.
161. White NJ, Miller KD, Churchill FC, et al. Chloroquine treatment of severe malaria in children. *N Engl J Med* 1988;**319**:1493–500.
162. White NJ, Waller D, Crawley J, et al. Comparison of artemether and chloroquine for severe malaria in Gambian children. *Lancet* 1992;**339**:317–21.
163. Williams HA, Roberts J, Kachur SP, et al. Malaria surveillance—United States, 1995. *Morb Mortal Wkly Rep C D C Surveill Summ* 1998;**48**(SS-1):1–23.
164. Wilson ME, Weld LH, Boggild A, et al. Fever in returned travelers: results from the GeoSentinel Surveillance Network. *Clin Infect Dis* 2006;**44**(12):1560–8.
165. World Health Organization. Severe falciparum malaria. *Trans R Soc Trop Med Hyg* 2000;**94**(Suppl. 2):S1–90.
166. World Health Organization. *Guidelines for the treatment of malaria*. 2nd ed. Geneva: World Health Organization; 2010. http://whqlibdoc.who.int/publications/2010/9789241547925_eng.pdf. Accessed June 10, 2013.
167. World Health Organization. *World malaria report 2011*. Geneva: World Health Organization; 2011. http://www.who.int/malaria/world_malaria_report_2011/9789241564403_eng.pdf. Accessed June 10, 2013.
168. Wyler DJ. Bark, weeds, and iron chelators—drugs for malaria. *N Engl J Med* 1992;**327**:1519–21.
169. Yohannes AM, Teklehaimanot A, Bergqvist Y, et al. Confirmed vivax resistance to chloroquine and effectiveness of artemether-lumefantrine for the treatment of vivax malaria in Ethiopia. *Am J Trop Med Hyg* 2011;**84**(1):137–40.
170. Zingman BS, Viner BL. Splenic complications in malaria: case report and review. *Clin Infect Dis* 1993;**16**:223–32.

major factors in modulating these diseases (Table 220-1). Disease syndromes range from self-healing cutaneous lesions to debilitating mucocutaneous infections, subclinical viscerotropic dissemination, and fatal visceral involvement.

In CL, *L. tropica* (with some notable exceptions), *L. major*, *L. aethiopica*, and *L. mexicana* tend to be restricted to reticuloendothelial cells of the skin, and infections generally are self-limited, with spontaneous healing. Similarly, in MCL *L. braziliensis* can invade the

TABLE 220-1 **Clinical Syndromes Caused by *Leishmania* Species and Their Geographic Distribution***

Clinical Syndromes	Leishmania Species†	Location
Visceral Leishmaniasis		
Kala-azar: Generalized involvement of the reticuloendothelial system (i.e., spleen, bone marrow, liver)	*L. (L.) donovani*	Indian subcontinent, northern and eastern China, Pakistan, Nepal
	L. (L.) infantum	Middle East, Mediterranean littoral, Balkans, Central and southwestern Asia, northern and northwestern China, northern and sub-Saharan Africa
	Canine visceral leishmaniasis	Foxhounds in 18 states (United States)
	L. (L.) donovani (archibaldi)	Sudan, Kenya, Ethiopia
	L. (L.) spp.*	Kenya, Ethiopia, Somalia
	L. (L.) chagasi	Latin America
	L. (L.) amazonensis	Brazil (Bahia State)
	L. (L.) tropica	Israel, India, and viscerotropic disease in Saudi Arabia (U. S. troops)
Post–kala-azar dermal leishmaniasis	*L. (L.) donovani*	Indian subcontinent, East Africa
Old World Cutaneous Leishmaniasis		
Single or limited number of skin lesions	*L. (L.) major*	Middle East, northwestern China, northwestern India, Pakistan, Africa
	L. (L.) tropica	Mediterranean littoral, Middle East, western Asiatic area, Indian subcontinent
	L. (L.) aethiopica	Ethiopian highlands, Kenya, Yemen
	L. (L.) infantum	Mediterranean basin
	L. (L.) donovani (archibaldi)	Sudan and East Africa
	L. (L.) spp.	Kenya, Ethiopia, Somalia
Diffuse cutaneous leishmaniasis	*L. (L.) aethiopica*	Ethiopian highlands, Kenya, Yemen
New World Cutaneous Leishmaniasis		
Single or limited number of skin lesions	*L. (L.) mexicana* (chiclero ulcer)	Central America, Mexico, Texas, Oklahoma, Arizona
	L. (L.) amazonensis	Amazon basin and neighboring areas, Bahia and other states in Brazil
	L. (V.) braziliensis	Multiple areas of Central and South America
	L. (V.) guyanensis (forest yaws)	Guyana, Suriname, northern Amazon basin
	L. (V.) peruviana (uta)	Peru (western Andes) and Argentinean highlands
	L. (V.) panamensis	Panama, Costa Rica, Colombia
	L. (V.) pifanoi	Venezuela
	L. (V.) venezuelensis	Venezuela
	L. (V.) colombiensis	Colombia and Panama
	L. (L.) chagasi	Central and South America
Diffuse cutaneous leshmaniasis	*L. (L.) amazonensis*	Amazon basin and neighboring areas, Bahia and other states in Brazil
	L. (V.) pifanoi	Venezuela
	L. (L.) mexicana	Mexico and Central America
	L. (L.) spp.	Dominican Republic
Mucosal leishmaniasis	*L. (V.) braziliensis* (espundia)	Multiple areas in Latin America

Data from Laison R, Shaw JJ. Evolution, classification and geographic distribution. In: Peters W, Killick-Kendrick R, editors. *The leishmaniases in biology and medicine*. London: Academic Press; 1987, p. 1–120; modified from Pearson RD, Sousa AQ. Clinical spectrum of leishmaniasis. *Clin Infect Dis* 1996;**22**:1–13; from Pearson RD, Jeronimo SMB, de Queiroz A. Leishmaniasis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens and practice*. Philadelphia: Churchill Livingstone; 1999; and Alva J, Vélez ID, Ber C, et al. Leishmaniasis worldwide and global estimates of its incidence, *PLoS One* 2012;**7**:e35671.

*This table contains the most common species; not every organism is listed above.

†>*(L.)* subgenus *Leishmania; (V.)* subgenus *Viannia.*

reticuloendothelial cells of the skin and the mucous membranes of the nose, mouth, and pharynx, resulting in serious disfigurement, if not death. Some strains of *L. tropica* may cause viscerotropic disease. In cases of VL (kala-azar), *L. donovani*, *L. chagasi*, and *L. infantum* invade cells of the reticuloendothelium of viscera, usually causing splenomegaly and occasionally hepatomegaly and pancytopenia; untreated, the disease is progressive and generally fatal.

Individuals with acquired immunodeficiency syndrome (AIDS) may have atypical manifestations. Dermotropic strains of *L. infantum* are known to cause visceral infection in immunocompromised patients with AIDS.[45] VL is quite prevalent among AIDS patients in certain geographic areas (southern Spain), with a high proportion of cases being subclinical. Like other opportunistic infections, subclinical VL can be found at any stage of human immunodeficiency virus (HIV)-1 infection, but symptomatic cases appear mainly when severe immunosuppression is present.[5] HIV-*Leishmania* coinfection is being seen increasingly frequently in the Mediterranean basin, especially in Spain, France, and Italy.

THE ORGANISM

In vertebrate hosts, *Leishmania* spp. are obligate intracellular parasites that exist only in the amastigote stage. The species that infect humans are usually morphologically indistinguishable from one another at both the light microscopic and ultrastructural levels. The organisms are round to oval bodies approximately 2 to 4 µm in diameter, with a single nucleus, a specialized mitochondrial structure that has extranuclear DNA termed a *kinetoplast*, and no free flagellum. Amastigotes are engulfed by macrophages and reside within the parasitophorous vacuole of the macrophage host. They multiply by binary fission and eventually destroy the host cell. Subsequently, they are phagocytized, and the process occurs repeatedly.[22]

When the vector, a female sandfly, feeds on an infected person, it may ingest an infected cell from blood or tissue. Amastigotes are liberated in the fly's midgut, and within a few hours, transformation to the promastigote stage occurs.[26] Promastigotes are elongated flagellates 15 to 25 µm long × 1.5 to 3.5 µm wide, each with an anterior, free flagellum that measures approximately 15 to 28 µm in length and may vary morphologically from a short and stumpy to an elongated form. Binary fission then begins, and large numbers of promastigotes are produced and gradually move forward to the pharynx, buccal cavity, and mouth parts. Depending on temperature and the species of sandfly, at 8 to 20 days, the mouth parts of the fly may be blocked partially or completely by huge numbers of promastigotes. These organisms may be dislodged into the bite wound when the female sandfly (*Phlebotomus*, *Lutzomyia*) next takes a blood meal. Promastigotes have surface molecules that bind to several macrophage receptors. The phagocytized promastigote forms transform into amastigotes within the parasitophorous vacuole, and multiplication takes place.

Amastigotes can be seen in Giemsa- or Wright-stained tissues or smears by light microscopy, with the nucleus and kinetoplast staining bright red and the cytoplasm pale blue. In Novy-McNeal-Nicolle (NNN) culture medium at 24°C (75.2°F), the organisms grow readily and assume the promastigote or insect form.

In the past, specific and subspecific taxonomy designations have been determined by the clinical syndrome caused by an isolate in a particular geographic area. Also, various molecular techniques have been used to characterize the strains and species of clinical isolates: endonuclease restriction studies of kinetoplast DNA (K-DNA), buoyant density of K-DNA and mitochondrial DNA (M-DNA) on cesium chloride, leishmanial isozyme patterns, monoclonal antibody specificity, and exoantigen secretory factor 4 serotyping.[3]

EPIDEMIOLOGY

Leishmaniasis is mainly a zoonosis, although in certain areas of the world primarily human-vector-human transmission occurs. The World Health Organization (WHO) estimates that 1.5 million cases of CL and 500,000 cases of VL occur every year.[19] With recent outbreaks in many areas of the world, including Brazil, India, Italy, Spain, Sudan, and Kenya, leishmaniasis has become more widely recognized as an important emerging infectious disease in many developed as well as underdeveloped countries.[12,27] Although the parasite usually is transmitted via the bite of the sandfly vector, it may be transmitted also as a result of a laboratory accident, direct person-to-person transmission, and blood transfusion.[34] In addition, evidence indicates that it may be transmitted either in utero or during the peripartum period.

The phlebotomine sandfly, the vector for leishmaniasis, was first identified by Bonanni in 1691.[18] The name of the genus, *Phlebotomus*, however, was coined in 1840 by Rondani and Berte. In 1907, the first description of an American phlebotomine sandfly was made by Coquillet. In 1909, Gaspar Vianna in Brazil identified the parasites responsible for cutaneous leishmaniasis later identified as *L. braziliensis* in 1911.[17] Concurrently, Lutz and Neiva identified the first three species of sandflies in Brazil. *Lutzomyia* sandflies were later named after Adolfo Lutz. To date, 229 species have been identified. It was not until 1921, however, that the Sergent brothers implicated phlebotomine sandflies in the transmission of cutaneous leishmaniasis in Algeria. The evidence was substantiated by the work of Aragao in 1922 and Pessoa and Pestana in Brazil in 1940. Later, in 1950, it was discovered that the different forms of leishmaniasis infesting the American continent were caused by distinct species of the parasite. The research on leishmaniasis and sandflies has shed light on the transmission of other zoonotic illnesses, including Trypanosomatids (Crithidia, Endotrypanum, and Trypanosoma), and several viruses including Rhabdoviridae, Bunyaviridae, and Reoviridae, all isolated from phlebotomine sandflies. Sixty nine serotypes of different arboviruses isolated from sandflies have been identified in the Amazon region.[18]

OLD WORLD CUTANEOUS LEISHMANIASIS

Definition and Epidemiology

Old World CL is caused by *L. major* (rural), *L. tropica* (urban), and *L. aethiopica*. It is found throughout the Middle East, along the Mediterranean basin and islands, and in East and West Africa, India, and southwestern Asia.[50] In humans, infection by *L. tropica* usually produces skin ulcers in which intracellular (amastigote) parasites can be found within macrophages in and around the lesions. These organisms are rarely found in spleen, liver, or bone marrow; however, several recent reports have described *L. tropica* isolates from patients with VL. In many areas, dogs or rodents are found to be naturally infected and are thought to be the natural reservoirs of infection. Various *Phlebotomus* spp. transmit the infection, although person-to-person transmission is possible and is the basis of the long-time practice in middle and central Asia of immunizing inoculation, that is, "leishmanization" to prevent possible disfigurement by a natural infection (Fig. 220-1).

Old World leishmaniasis, or Oriental sore (Delhi boil or Aleppo button), can be classified into "wet" and "dry" types. The wet, or rural, form is caused by *L. major* and is found chiefly in various rodents on the edge of deserts. The dry, or urban, type is caused by *L. tropica*, and is transmitted by phlebotomine species that frequently feed on humans and dogs. The dry, or urban, form of Oriental sore has a long incubation period, long duration of active infection, and large numbers of parasites in the dermis. The moist, or rural, type has a short incubation period, with rapid healing and few parasites in the skin. *L. aethiopica* is restricted to the mountain valleys of the Rift Valley of Ethiopia and Kenya, where rock and tree hyraxes are infected regularly. Humans become infected when they intrude in these areas. This form of CL is usually self-limited,[62] although in a small number of individuals (1/100,000), nonhealing diffuse cutaneous (DCL) disease has been reported.

FIGURE 220-1 ■ *Leishmania tropica.* Immunization by an induced lesion. (From a nonprofit cooperative endeavor by numerous colleagues under the editorship of Dr. Herman Zaiman, New York.)

Pathology

At the bite site, promastigotes are engulfed by histiocytes, in which they multiply. The histiocytes are destroyed, and amastigotes are released into tissues, where the process is repeated. Lymphocytic and plasma cell infiltration along with histiocytic hyperplasia occurs, and in some lesions, epithelioid and giant cells may be seen. Hypertrophy of the stratum corneum and hyperplasia of dermal papillae occur early, usually followed by necrosis of the area caused by capillary obstruction and endothelial proliferation; eventually the epithelium overlying the center of the lesion becomes necrotic and is sloughed, with the formation of a characteristic ulcer. Secondary neutrophil infiltration also occurs. At this point, a depressed ulcer with a raised purpuric indurated border and a base of friable granulation tissue is present. Amastigotes are located within the cells, although during the period of necrosis, organisms may be seen outside cells but division does not occur.

With *L. tropica*, development of the cutaneous lesion may take weeks to months, with large numbers of parasites present in nests of macrophages. In *L. major* infection, the onset is rapid, with an outpouring of lymphocytes and plasma cells; parasites are sometimes difficult to find. In some cases, satellite lesions form close to the primary lesion so that local spread is seen. The pathologic reaction may be florid, with marked pseudoepitheliomatous hyperplasia that can be mistaken for carcinoma. Secondary bacterial infection may complicate the lesion and delay healing. Once the ulcer heals, however, usually by fibrosis, the patient has long-lasting immunity (Fig. 220-2).

Several manifestations of CL have been described and seem to be associated with the ability of the patient to respond to the infection by cell-mediated immune mechanisms. Whether these mechanisms are directly responsible for protection in humans is still not certain. However, in a small number of patients, the inability to mount a suitable cell-mediated immune reaction is associated with specific anergy to leishmanin and an indolent nonhealing lesion.[14] This condition is known as diffuse cutaneous leishmaniasis (DCL). Characteristically, lesions in DCL are filled with large, parasite-containing histiocytes, and lymphocytes are absent. Recent studies of DCL from the Dominican Republic suggest that immune suppression plays an important role in this form of the disease.

At the other extreme is a small group of patients whose cell-mediated immune response to infection with leishmanial organisms is exaggerated and the lesions heal by scarring. At the edge of the scar, however, new lesions appear, so the disease seems to extend from the margins. Eventually, tissue damage may be rather extensive. On histologic examination, many lymphocytes, plasma cells, epithelioid cells, and large multinucleated giant cells are seen. Organisms are difficult to locate but sometimes can be cultured from these lesions. This form of CL is called

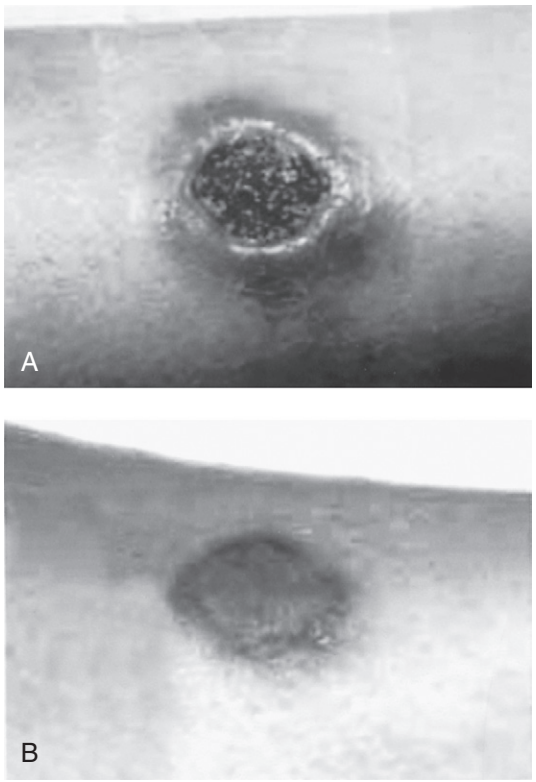

FIGURE 220-2 ■ **A,** Typical cutaneous leishmaniasis ulcer. **B,** Healed cutaneous leishmaniasis ulcer.

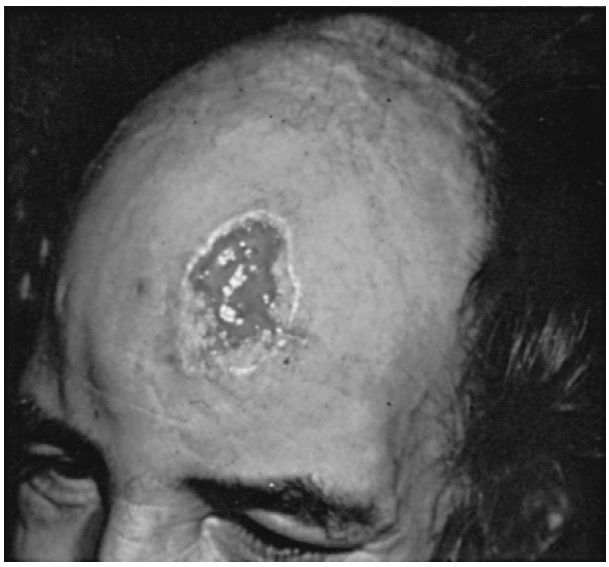

FIGURE 220-3 ■ Healing cutaneous leishmaniasis in a patient from Venezuela.

leishmaniasis recidivans. Patients exhibit marked delayed hypersensitivity to leishmanin. Studies by Turk and Bryceson[58] have provided the concept that CL may be a spectrum of diseases analogous to leprosy. They consider DCL to be at one end of the spectrum and representative of anergy and leishmaniasis recidivans to be at the other end and representative of marked delayed hypersensitivity, with an ordinary Oriental sore as the center in which balance exists.

Clinical Manifestations

The disease usually begins with the appearance of a pruritic, red, vesicular papule that appears weeks to months after the bite of a sandfly. The papule gradually enlarges, often measuring 1 to 2 cm in diameter. When the surface of the papule dries, it encrusts and drops off to reveal a shallow ulcer. The ulcer may or may not enlarge progressively and characteristically has raised, sharp, indurated, deep purpuric margins. Healing usually occurs in 3 to 18 months, with an obvious hypopigmented or hyperpigmented depressed scar frequently remaining (Fig. 220-3). However, single or multiple papules often heal directly without extensive ulceration. If the lesions do not become infected secondarily, usually no complications occur.

Diagnosis

Definitive diagnosis depends on demonstrating the amastigotes in tissue specimens or the promastigotes in culture. Microscopic examination of Giemsa-, Wright-, or other rapid blood-stained smears of tissue obtained from non-necrotic areas of the ulcer or from the base should be performed. Aspiration and culture of tissue fluid taken from the ulcer margin can be positive; however, biopsy material taken from the edge of the ulcer is preferred and should be examined histologically, as should small fragments macerated in saline and inoculated into NNN medium or Schneider insect medium supplemented with antibiotics. It is very important to make sure the lesion is cleaned thoroughly to remove all secondary infectious organisms (i.e., contaminating bacteria or fungi) before taking the punch biopsy specimen for culture. The specimen of choice would be a collection of several punch biopsy specimens taken from the most active lesion areas. Clinically, the lesions are often characteristic, so the diagnosis should be suspected in a patient who has visited an endemic area.

Although the leishmanin test is usually positive in patients with ulcerated lesions, the material is not readily available in the United States. However, a positive leishmanin skin test may help distinguish a variety of skin lesions such as syphilis, tropical phagedenic ulcer, yaws, tuberculosis, and various fungal diseases. The indirect fluorescent antibody test or direct agglutination test may be positive in this infection, although often at low titer and, therefore, of little value.

Treatment

Uncomplicated *L. tropica* lesions generally respond well to chemotherapy or conservative management. Because Old World CL usually remains a local lesion, if the ulcer is not disfiguring and appears to be healing, allowing the lesion to heal spontaneously is appropriate.[62] In most cases, systemic therapy with[28] stibogluconate sodium is effective.

Liposomal amphotericin B (AmBisome) has been used for treatment of visceral leishmaniasis, and more recently

for treatment of cutaneous leishmaniasis.[24] However, a healthy 38-year-old man treated with liposomal amphotericin B for cutaneous leishmaniasis acquired during military duties in Iraq reported memory difficulties and confusion after completion of his therapy. Extensive evaluation revealed no other source, and his cognitive issues were attributed to liposomal amphotericin B toxicity (mentioned in the package insert). The patient's symptoms resolved over a few weeks, which is consistent with published data about the drug's tissue penetration and metabolism. This is a potential side effect of liposomal amphotericin B that can be observed in otherwise healthy patients.[24]

Various imidazoles (e.g., ketoconazole, itraconazole) have been used and appear to have limited antileishmanial activity. An ointment containing paromomycin (aminosidine) and methylbenzethonium (Leshcutan) has been reported to show some promise in treating cutaneous lesions, especially in Israel.[9,53]

A 31-year-old man with DCL from 3 years of age experienced profound physical and psychosocial repercussions, and with no effective treatment available at present, was treated with miltefosine.[65] The patient was treated for 120 days, 100 mg/day for 1 week, then 150 mg/day subsequently. Lesions were free of parasites at 43 days, and no signs of infiltration were present at day 76. No adverse side effects were observed. The dramatic clinical effect of miltefosine in this patient appears to fully justify further evaluation of this experimental therapy in DCL.

Prevention

Residual spraying for sandflies, eradication of reservoir hosts, and vaccination procedures have reduced and limited this disease in many areas of the Middle East and central Asia. Because of the indolent nature of the healing with vaccination and the possibility of visceralization, this practice has been discontinued in Israel. The customary method for controlling leishmaniasis and sand fly bites in Israel involves the spraying of large quantities of residual insecticides on walls of houses and neighboring surfaces. However, the high summer temperatures, strong radiation, and dust limit the efficacy of the method.[41]

AMERICAN CUTANEOUS LEISHMANIASIS

The epidemiology and etiology of American CL are quite complex. In South and Central America, many varieties of leishmaniasis exist, including the mucocutaneous form, or espundia. In contrast to Old World CL, American cutaneous disease is tied closely to the forests of South and Central America, and each variety has its own distinct epidemiologic, pathologic, and clinical picture.[25] Whether designating each of the clinical types of American CL by a separate species of *Leishmania* is justified remains unclear. With regard to the American cutaneous forms, two main groups of organisms are distinguished: the *L. mexicana* and *L. braziliensis* complexes (Figs. 220-4 and 220-5). The former are often characterized by rapid growth in culture medium and in hamsters, and the latter are organisms that grow slowly in culture and hamsters.

FIGURE 220-4 ■ Chiclero ulcer *Leishmania (mexicana) mexicana.* A typical chronic lesion of the external ear is evident. Such lesions never metastasize.

FIGURE 220-5 ■ Mucocutaneous leishmaniasis: *Leishmania (braziliensis) braziliensis.* The entire nasal septum has been eroded. Few organisms could be found by smear or biopsy; however, promastigote forms were cultured from the lesion.

Leishmania Mexicana Complex

L. (mexicana) mexicana is transmitted by species of sandflies of the genus *Lutzomyia* (Fig. 220-6). Many rodent reservoir hosts exist. This species is found in Mexico, Guatemala, and Belize. It causes mild infection, often a single cutaneous lesion that is self-limited, or persistent chronic ear lesions, as well as chiclero ulcer.[2] One case of disseminated disease has been reported. This species is probably responsible for the occasional cases of CL found in the southern portion of the United States. The range of endemic infection has now been expanded within the United States to include areas within Arizona that are farther north than the southern Texas site previously

FIGURE 220-6 ■ Sandfly.

identified.[51] Apparently, wood rats serve as the primary wild reservoir hosts in these two states.

CL is endemic in south-central Texas and appears to be spreading northward into the Dallas–Ft. Worth metropolitan area, affecting humans, cats, and dogs. Multiple vectors and rodent reservoir hosts exist within Texas; vector-borne sand fly–based transmission is probably the primary means of disease spread in this area. Because of poor surveillance and infrequent diagnosis in the United States, whether leishmaniasis may have been present within at-risk canine and feline populations before the more recently recognized "outbreaks" in foxhounds and humans is not known. Evidence suggests that this disease may continue to emerge as a result of changes in the environment and closer contact between pets and sylvatic ecosystems.[43,63]

L. (mexicana) amazonensis is found along the Amazon basin and in Trinidad. It rarely infects humans but is transmitted by various species of *Lutzomyia* in rodents. In humans, it causes a mild, self-limited skin lesion. Occasionally, disseminated disease reportedly develops.

Leishmania Braziliensis Complex

L. (braziliensis) braziliensis or *L. (Viannia) braziliensis* is transmitted by various species of *Lutzomyia* in Brazil and the forest areas east of the Andes. It was originally described by Gaspar Vianna in Brazil as the cause of Bauru's ulcer.[17] This organism is the "prototype" of American CL or MCL, or espundia. It may cause destructive ulcerative lesions of the naso-oropharynx as a result of early or late metastases from a more superficial site.

L. (braziliensis) guyanensis is transmitted by species of *Lutzomyia* in Guyana, Suriname, Brazil, and Venezuela. It causes single or multiple spreading cutaneous ulcers over many parts of the body and is believed to metastasize along the lymphatics but does not visceralize. The organism sometimes spreads to the naso-oropharynx and causes mucosal disease. It sometimes is referred to as pian bois and forest yaws.

L. (braziliensis) panamensis is transmitted by species of *Lutzomyia* in Panama and possibly farther north and south. It may cause single to several superficial ulcers and may metastasize along the lymphatics to the naso-oropharynx. A cluster of five cases was reported of CL with *L. (Viannia) panamensis* among men attempting emigration from East Africa, who acquired the infection while traveling from Central America to the United States. Identification of the infecting species revealed important information related to a well-defined human smuggling route with potential public health consequences from this emerging infectious disease risk.[10]

L. (braziliensis) peruviana is seen in Peru on the western slopes of the Andes to an altitude of 3000 m. It causes a single or a few self-healing ulcers. No oronasopharyngeal spread occurs. Often, it is referred to as *uta*. Dogs are regarded as the reservoir hosts.

In MCL as represented by espundia, researchers estimate that nasal involvement may occur in as many as 80 percent of infections, up to 30 percent of which eventually mutilate the mucous membranes of the mouth, nose, palate, larynx, and trachea. These cases are often fatal because of the intervening sepsis. Lesions of the mucous membranes often occur years to decades after a cutaneous ulcer has healed. Once mucous membrane involvement occurs, the infection may be difficult to cure using chemotherapy.

Diagnosis

The discussion of Old World CL includes the various methods for diagnosis. In mucocutaneous disease, the fluorescent antibody test using amastigote antigen is most useful in that it is positive in 75 to 85 percent of cases, with declining titers after therapeutic cure.[61] A direct agglutination test using promastigotes also is used frequently, as is enzyme-linked immunosorbent assay (ELISA).[61] A DNA–DNA hybridization or dot-blot test that is highly sensitive and species-specific in tissue or biopsy specimens has been used.[37] Isozyme analysis of isolated organisms currently is being used to help identify the species causing the infection.

Treatment

As in Old World CL, treatment of most lesions is effective using pentavalent antimonials. Because prompt and adequate therapy for primary cutaneous lesions may reduce the risk for subsequent metastatic disease in potentially mucocutaneous infections, pentavalent antimony should be used. If lesions should prove unresponsive to antimony therapy, amphotericin B should be tried. Liposomal amphotericin treatment has been useful in selected studies.[15] Relapses with this form of leishmaniasis are common and must be retreated. Pentavalent antimonials are moderately effective in treating mild mucosal disease but are often unsatisfactory with severe mucosal involvement. Diffuse cutaneous leishmaniasis, a disseminated form of the disease, should be treated with stibogluconate sodium[28]; when relapses occur, amphotericin B should be used next because this disease is usually refractory to further antimony therapy.[15,48] Pentamidine also has been used with limited success when lesions have proved resistant to antimony compounds. Pentavalent

antimony–resistant cutaneous disease, such as leishmania recidivans, has been treated with limited success with ketoconazole 400 to 600 mg daily for 4 weeks. Miltefosine, an oral antileishmanial agent effective against VL, has shown promise for the treatment of American CL (*L. panamensis* and *amazonensis*).[15,55]

Prevention

This disease is extremely difficult to prevent because it is a forest disease. It can be avoided only by sleeping in tents under fine-mesh netting, wearing long-sleeved clothing, and using insect repellents. The presence of *Leishmania* parasites in the unaffected skin and peripheral-blood monocytes of a high proportion of patients even after treatment and the acquisition of infection by sand flies support the plausibility of anthroponotic transmission of American CL.[60] As travel to Latin America has become increasingly common, cutaneous leishmaniasis is increasingly seen among returning travelers; for instance, the number of observed cases has doubled in the Netherlands and tripled in the United Kingdom in the past decade.[52] A high proportion of cases are acquired in rural or jungle areas of the Amazon basin. In children acquiring *L. (Viannia) braziliensis* infections, the major risk factor for acquisition of disease was the presence of an adult in the household with CL within the last year.[1] Humans may serve as both reservoir and source of infection for young children. Vaccine development against American CL is underway.[30,44,49]

VISCERAL LEISHMANIASIS

VL is caused by various organisms in the *L. donovani* spp. complex in Asia and Africa, as well as by *L. infantum* in southern Europe and North Africa and the related *L. chagasi* in Brazil and elsewhere in the New World. Strains of *L. tropica* from the Middle East and *L. amazonensis* from Latin America also have been found to cause this syndrome.

VL, or kala-azar, is found in a broad belt that extends from the Strait of Gibraltar across the Mediterranean through Asia to the east coast of China, at latitude between 30 and 48 degrees north. It is transmitted by various species of sandfly, although congenital and blood-borne infections also can occur.[64] VL has been reported from 47 countries, but the Sudan and India account for more than half the cases. In the Western Hemisphere, it is found in Brazil, northern Argentina, Paraguay, Venezuela, Colombia, Guatemala, and Mexico. Kala-azar appears to exist in at least three epidemiologic forms.

The Mediterranean type of VL, with a canine reservoir, infects young children (1 to 4 years of age); dogs, foxes, and feral animals are the reservoirs *(L. infantum)*. This type extends from the Mediterranean littoral through central Asia into China; it is also present in parts of South America *(L. chagasi)*, where foxes and dogs are reservoir hosts. In Brazil, young males most often are infected. Infected dogs are the primary reservoir for zoonotic visceral leishmaniasis in endemic regions and the most significant risk factor predisposing humans to infection.[43] Dogs have a wide range of clinical presentation with infection with *L. infantum*, ranging from asymptomatic to fatal visceral disease.

An Indian type *(L. donovani)* of VL with a human reservoir predominates in Indian children between 5 and 15 years of age; humans are the only known reservoir. Although sought, evidence of natural infection in dogs has not been found. No evidence of rodent reservoirs exists.

The African type of VL uses rodents as the reservoir hosts. The Nile rat in the Sudan and probably the gerbil in Kenya are the reservoirs. In Kenya, researchers have noted that kala-azar often is related to old or eroded termite mounds where young males often congregate (see Fig. 220-5).

Pathology

The main pathologic lesions are the result of reticuloendothelial cell hyperplasia, especially in the spleen and liver (Fig. 220-7). Later, the bone marrow and lymph nodes are filled with infected macrophages, and a concomitant leukopenia and anemia develop (i.e., pancytopenia). Also, the kidneys may be filled with infected macrophages, and invasion of the submucosa and mucosa of the duodenum and jejunum results in hypertrophic congested and edematous villi. Small ulcerations and

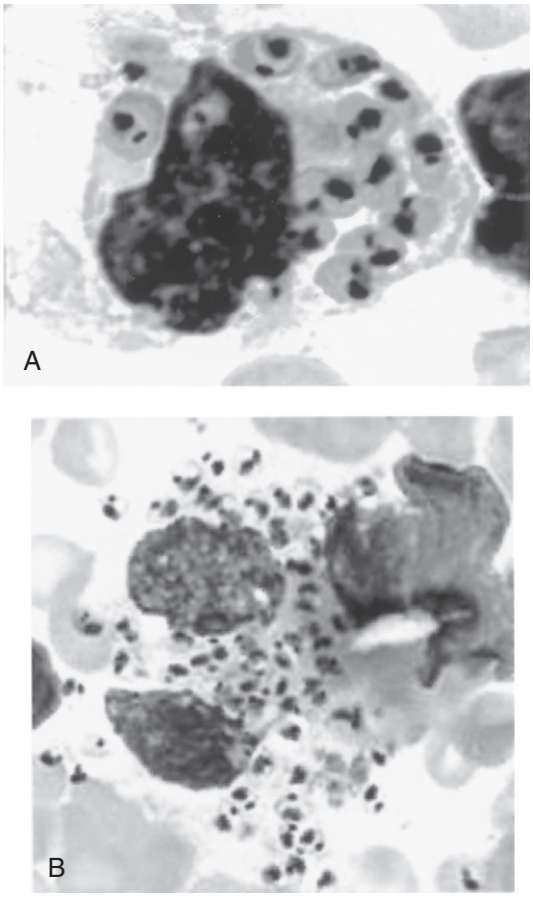

FIGURE 220-7 ■ **A,** *Leishmania tropica* in a macrophage. **B,** *Leishmania donovani* in a liver touch preparation.

hemorrhages may occur. The spleen gradually enlarges, sometimes assuming enormous proportions, and eventually extends into the pelvis. Splenic infarcts are a frequent development. The capsule is thickened, and, more deeply, the sinuses are dilated. Erythrophagocytosis by histiocytes is seen commonly, and the anemia so typical of kala-azar may in part be the result of such sequestration of red cells. Kupffer cells of the liver, filled with amastigotes, are swollen and hyperplastic, and centrilobular necrosis or fatty infiltration of the hepatic parenchyma often is observed. In late-stage or chronic disease, increased hepatic fibrosis may give a nodular cirrhotic appearance. Lymphadenopathy, especially of the mesenteric glands, is an early finding, and large numbers of parasite-filled macrophages are present. The bone marrow often is filled with parasitized histiocytes that replace the normal marrow elements; such replacement results in myelophthisic anemia.

The immunologic response to kala-azar infection is imperfectly understood. At the bite site, a small, pea-sized, dermal lesion may form (i.e., a leishmanioma); the parasites, initially localized in dermal macrophages, disseminate within the macrophages to the spleen, liver, bone marrow, and lymph nodes.

The infection outcome depends on the host's ability to raise a suitable cell-mediated immune response and the virulence of the invading organism. Experimentally, resistance in mice appears to be determined by a single autosomal gene. Researchers have shown in experimental infections that the disease is controlled by the T_H1 subset of $CD4^+$ and $CD8^+$ T cells; these cells are related to the production of cytokines such as interferon (IFN)-γ, interleukin-2, and tumor necrosis factor–α.[36] Infection with *L. donovani* amastigotes induces a Th1 cytokine milieu in both dendritic cells and T cells.[23] Cytokines activate macrophages to kill intracellular amastigotes by oxidative and nonoxidative mechanisms. Studies in mice indicate that nitric oxide is an important factor in killing amastigotes. If the infection is not eliminated or controlled by the host's cellular immune response, it then becomes clinically evident. Lymphocytogenesis and histiocytogenesis occur in affected organs, with resultant hepatosplenomegaly and lymphadenopathy. Polyclonal B-cell activation ensues and causes hyperglobulinemia. This outpouring of humoral antibodies, chiefly IgG and largely nonspecific, is not protective and may represent more than half the total serum proteins of the patient. The specific antibodies produced during active disease have diagnostic significance. Fluorescent antibody, ELISA, indirect hemagglutination, and complement-fixation tests are reasonably reliable diagnostic procedures.

Resistance to kala-azar is essentially absent once the infection has become evident clinically. However, after chemotherapeutic cure, acquired immunity emerges; delayed hypersensitivity, as demonstrated by the Montenegro (leishmanin) skin test (see later), also becomes apparent. Moreover, the hypergammaglobulinemia abates concomitant with chemical cure and the appearance of delayed hypersensitivity. Usually, immunity to VL is complete and long-lasting after chemotherapeutic cure. However, relapse, as seen in post–kala-azar dermal leishmaniasis, is characterized by delayed hypersensitivity, dermal localization of parasites, and moderate hypergammaglobulinemia. Although macrophage activation results in enhanced phagocytosis of parasites, macrophages remain unable to eliminate parasites. The appearance of dermal delayed hypersensitivity at the time that acquired immunity appears suggests that cell-mediated immunity plays an important role in protection. Further work on this aspect of VL is needed. The genetics of resistance in humans remains unclear.

Clinical Manifestations

The incubation period varies widely from 6 weeks to 6 months but has been reported to be as short as 10 to 14 days and as long as 10 years. A primary skin nodule is rarely seen, although in African leishmaniasis it is a more regular feature. Infantile VL may begin either suddenly with high fever and vomiting or insidiously with irregular daily fever, anorexia, weight loss, lassitude, and pallor. When fever is present, double daily spikes are a characteristic sign, with temperatures reaching 40° to 40.6° C (104° F to 105° F). The spleen gradually enlarges, so that by the end of the first month it can be palpated. If the symptoms continue unabated, the spleen may extend to the umbilicus or even into the pelvis. Diarrhea or frank dysentery is not unusual, and blood sometimes is observed. A general bleeding diathesis often becomes evident shortly before death. After several months, if the disease is untreated, patients usually die. Acute fulminant disease is seen more often in infants and young children.

In other cases, the clinical course is more protracted and generally ends fatally after a year or two. In older age groups, the disease tends to assume a more chronic course, with marked emaciation, brittle hair, massive splenomegaly, lymphadenopathy, and a dusky slate-gray complexion. Hyperglobulinemia, leukopenia, and anemia typically are found. As a result of general debility, death often results from concurrent infections such as pneumonia, amebic or bacillary dysentery, malaria, or cancrum oris in more than 90 percent of cases. Infantile VL has been associated with alterations in lipoprotein metabolism.[6] A handful of cases of presumed congenital VL have been reported.[21,64] These infants were born of infected mothers, and in some, evidence of parasitism of the placenta was found.[21] However, whether these cases represent congenital infection or peripartum infection is unclear because sophisticated serologic techniques were not available.

In a study of hormonal disturbances in visceral leishmaniasis, primary adrenal insufficiency was observed in 45.8 percent of patients; low aldosterone/renin plasma ratio in 69.4 person; low daily urinary aldosterone excretion in 61.1 percent; and low transtubular potassium gradient in 68.0 percent. All patients had normal plasma antidiuretic hormone (ADH) concentrations, hyponatremia, and high urinary osmolality. Plasma parathyroid hormone was low in 63 percent; hypomagnesemia was present in 46.4 percent, and increased magnesium excretion factor was present in 100 percent. Primary thyroid insufficiency was observed in 24.6 percent and secondary

thyroid insufficiency in 14.1 percent. Normal follicle-stimulating hormone plasma levels were present in 81.4 percent; high luteinizing hormone and low testosterone plasma levels were present in 58.2 percent of men. Hypothalamus-pituitary-adrenal axis abnormalities, inappropriate aldosterone and ADH secretions, and presence of hypoparathyroidism, magnesium depletion, and thyroid and testicular insufficiencies also were seen.[59]

Cutaneous manifestations of kala-azar are encountered frequently. In India, the dark gray appearance of the skin is known as kala-azar (black sickness). In some cases of inadequately treated VL, a skin condition termed *post–kala-azar dermal leishmaniasis* may ensue. In Indian VL, this complication is encountered in 15 to 20 percent of cases and appears several years after therapy. Individuals with VL, or kala-azar, and individuals with post–kala-azar dermal leishmaniasis (PKDL) are considered to be reservoirs of transmission of *L. donovani* in India.[54] In African disease, PKDL occurs much less commonly, often during therapy in approximately 2 to 3 percent of cases, and it heals spontaneously in a few months. The lesions are characterized by the appearance of hypopigmented, erythematous, or nodular lesions on the skin of the face, chest, neck, and buttocks. At times, the nodular lesions of the face may resemble lepromatous leprosy.[58] The lesions are thought to represent a modified form of *L. (L.) donovani* infection in which the parasites no longer invade the viscera and are localized to the skin. These lesions seem to be related to the host's immune response. This change to dermal tropism is said to coincide with recovery from visceral disease and to disappear with relapse. Infection with *L. (L.) chagasi* causes VL in young children. Atypical CL has been reported in older children with this infection.[7]

Pancytopenia is not unusual. Characteristically, anemia is always evident, with hemoglobin levels below 8 g/dL. Survival of red cells is shortened as a result of several possible factors, including Coombs-positive hemolytic anemia and hypersplenism. Leukopenia of 2000 to 3000 cells/mm^3 typically is found with neutropenia, relative lymphocytosis, an almost total absence of eosinophils, and thrombocytopenia. Serum albumin is usually less than 3 g/dL, and globulin levels (mostly immunoglobulin [IgG]) are often greater than 5 g/dL (5 to 10 g/dL).

Kala-azar has been reported as an important opportunistic infection in patients infected with HIV-1 and not known to have contracted *Leishmania* infection previously. These patients appear to have a more severe and fulminant form of kala-azar. In this regard, inapparent *Leishmania* infection may become evident after immunosuppression, such as chemotherapy for malignant disease. The diagnosis may be particularly difficult to make inasmuch as the findings are often atypical and consist of low-grade fever, fatigue, cough, and gastrointestinal complaints. Certainly in AIDS patients, VL is a recurrent disease that is highly prevalent and whose clinical course is modified by HIV. VL is very prevalent among HIV-1–infected patients in southern Spain, with a high proportion of cases being subclinical.[38] Symptomatic cases appear mainly when severe immunosuppression is present. There is also an association between VL, the male sex, and intravenous drug use. Similarly, atypical visceral disease caused by *L. tropica* was seen in individuals who participated in Operation Desert Storm in the Persian Gulf.[33]

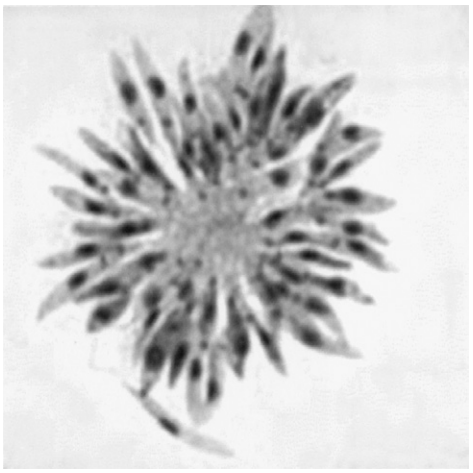

FIGURE 220-8 ■ *Leishmania* sp. promastigotes from culture system.

Diagnosis

VL is diagnosed by finding the organism in stained smears of spleen aspirate,[13] peripheral blood, or bone marrow (see Fig. 220-7). In Indian kala-azar, the parasites may be found regularly in peripheral blood monocytes (i.e., buffy coat), but in the African and Mediterranean forms, they may be difficult to find by this technique. Blood and marrow cultures grown on NNN medium or in Schneider insect medium with 15 to 20 percent fetal calf serum are most useful (Fig. 220-8). Some investigators regard splenic rather than bone marrow aspiration as the most sensitive procedure, although it can be especially hazardous in individuals with a bleeding diathesis. Contraindications include a soft or diffluent, acutely enlarging spleen. Patients with low platelet counts, a prolonged prothrombin time, or both should not undergo the needle biopsy procedure. In children younger than 5 years, splenic aspiration should be performed only by a physician fully experienced in the procedure.

Spleen and bone marrow aspirates should be placed in culture medium and smeared on slides, and saline-diluted aspirates should be inoculated into the peritoneal cavity of hamsters (see Fig. 220-6).

In a study conducted in pediatric patients in the Mediterranean region, antibody detection techniques, the antigen detection in urine (KAtex kit), and *Leishmania* nested polymerase chain reaction (LnPCR) analysis of the blood were useful for diagnosis of the first clinical episode.[16] After treatment, clinical improvement was associated with negative NNN cultures and microscopy of bone marrow aspirate, KAtex test, and LnPCR blood analysis results. New noninvasive techniques tested showed high diagnostic sensitivity. However, LnPCR analysis of the bone marrow was the most sensitive and was able to detect the persistence of parasites and predict potential relapses.[16]

Diagnostic approaches for visceral leishmaniasis have been improved in the last decade with the development of serologic and molecular tests. However, in many parts of the world, a definitive diagnosis still relies on the older parasitologic methods. Recombinant antigens have led to improved performance of serodiagnostic methods. Serology-based tests, rK39 antigen dipstick, and direct agglutination test are highly sensitive methods; however, they fail to distinguish present from past infections. Molecular approaches have become increasingly relevant because of the remarkable sensitivity, specificity, and flexibility in sample selection. Quantitative PCR is a highly sensitive and specific tool used in referral laboratories for detection and assessment of parasite load in VL patients and in monitoring treatment response. This method has the potential to distinguish asymptomatic patients in endemic areas. Techniques such as loop-mediated isothermal amplification offer a reliable molecular diagnostic method for field application.[46,56]

Nonspecific tests detecting the markedly elevated serum globulins such as the formol gel test is helpful in acute disease and is performed readily in the field. Antileishmanial antibodies are usually present and can be used to aid in the diagnosis. The fluorescent antibody test is highly specific, as are the indirect hemagglutination and gel diffusion tests. The complement-fixation test, however, is positive in only 65 to 70 percent of cases. Sera from patients with VL are known to give false-positive results when antibodies to *Trypanosoma cruzi* are present; consequently, in the Western Hemisphere, absorbing out these antibodies may be necessary. Of importance is to recognize that fluorescent antibody titers usually fall after complete cure, so a negative titer often is regarded as a sign of successful therapy. DNA–DNA hybridization tests are being evaluated and promise exquisite specificity and sensitivity for the diagnosis of leishmaniasis.[37] Serologic tests may be positive as a result of past or subclinical inapparent infection. An ELISA that detects antibodies to a cloned recombinant antigen, K39, of *L. chagasi* has been shown to be specific in active VL and could detect antibodies in AIDS patients. A recent study has demonstrated that this test could be applied to field situations.

In HIV-1–infected patients with active VL, the sensitivity of a peripheral blood smear is about 5 percent, because of a high parasitemia in these patients. However, in patients with HIV-1 and subclinical VL, the sensitivity of a routine blood smear is less than 10 percent.[5] Unfortunately, in these patients serology and *Leishmania* skin tests have low sensitivities and are not very helpful. Cultures have proved to be effective in VL patients who are coinfected with HIV-1. The usual diagnostic tests also may be negative in patients with *L. tropica* infection or in otherwise profoundly immunocompromised patients. However, two genomic fragments encoding portions of a single 210-kDa *L. tropica* protein have proved useful for the diagnosis of viscerotropic *L. tropica* infection in Desert Storm patients.[20]

The leishmanin or Montenegro skin test, like the lepromin and tuberculin skin tests, is a measure of delayed hypersensitivity to leishmanial antigen. It consists of 10^6 phenol-killed, culture-grown promastigotes in 1 mL of 0.5 percent phenol in saline. The test is performed like the tuberculin test, that is, 0.1 mL is injected intradermally. A positive result is a palpable area of induration at least 5 mm in diameter in 48 to 72 hours. Because the leishmanin test can be positive in CL or VL, the results must be evaluated carefully. In VL, the test remains negative throughout the period of active disease. Once chemotherapeutic control starts to take effect and immunocompetent lymphocytes are able to respond, the test begins to turn positive.[11] Thus, recovery from kala-azar is characterized by the development of cell-mediated immunity. The change from a negative to a positive leishmanin test in VL is regarded as an important prognostic sign that protective immunity is developing or has developed. Because numerous reports noting positive leishmanin tests in individuals who have had no history of VL have appeared, researchers have postulated that many individuals in an endemic area may become immune by previous inapparent infection.

Prognosis

Untreated VL is fatal in 75 to 85 percent of infantile and 90 percent of adult cases. Properly treated at an early stage, it can be cured in 85 to 95 percent of cases. The prognosis for patients in whom pancytopenia or bleeding diatheses develop or in whom a delayed hypersensitivity skin reaction fails to develop usually is poor.

Treatment

VL generally responds to treatment with pentavalent antimonials such as stibogluconate sodium (Pentostam, Triostam), which is the usual drug of choice in the United States and is available through the Drug Service of the Centers for Disease Control and Prevention.[28] Meglumine antimoniate (Glucantime) is available in French-speaking countries and Latin America.[8] The pediatric and adult dose is 20 mg/kg daily administered intramuscularly or intravenously for 28 days. Treatment can be repeated. In areas where leishmanial parasites may have acquired relative resistance to pentavalent antimonials, such as India, Nepal, and East Africa, extending therapy for more than 4 weeks may be necessary.[31] Side effects occur commonly and include nausea, vomiting, headache, anorexia, and abdominal pain. Elevated levels of serum amylase and lipase, indicative of pancreatitis, are encountered occasionally and can be severe. Electrocardiographic changes, including decreased T wave amplitude and T wave inversion, prolongation of the QTc, and nonspecific ST-T wave changes, are seen. They resolve shortly after therapy is concluded. (Treatment should be discontinued if the QTc interval exceeds 0.5 second.) Deaths, presumably caused by arrhythmias, have been reported in patients who were receiving more than 20 mg/kg/day. Primary antimony resistance, as well as relapses after receiving pentavalent antimony therapy, occurs with all types of VL but is most often encountered with the Indian form. The mechanism of antimonial resistance is unclear.[26] In HIV-infected patients, almost 25 percent fail to respond to antimony therapy and almost 40 percent of responders subsequently relapse.[40,42]

Liposomal amphotericin B (AmBisome) has been approved by the U.S. Food and Drug Administration for the treatment of VL.[39] This compound is taken up by cells of the reticuloendothelial system, where amastigotes reside, and is less nephrotoxic, thereby allowing for higher daily doses with shorter courses of therapy. The approved, recommended regimen for immunocompetent VL patients is 3 mg/kg/day on days 1 through 5 and days 14 and 21. In immunosuppressed and HIV patients, treatment of VL with AmBisome has resulted in an almost 100 percent response rate. However, most of these patients relapse.[40,42] The recommended treatment is 4 mg/kg/day on days 1 through 5, 10, 17, 24, 31, and 38. Without maintenance therapy, almost all of these patients relapse. Because resistance to pentavalent antimonials has developed, amphotericin B is used and is highly efficacious. Several recent studies have used liposomal amphotericin B successfully for the treatment of VL presumably caused by *L. infantum* in immunocompetent children.[35]

Parenteral pentamidine isethionate (Pentam 300) is administered intramuscularly (4 mg/kg is given three times weekly for up to 15 doses, depending on side effects such as hypotension, vomiting, and blood dyscrasias). Pentamidine occasionally may exacerbate diabetes mellitus or precipitate latent diabetes. Shock and liver and renal damage have been reported.

In several clinical trials, miltefosine, an oral alkyl phospholipid agent initially developed as an oral antineoplastic drug, has been used as an effective agent for the treatment of antimony-resistant Indian VL.[29,57] The treatment dose was 2.5 mg/kg/day for 4 weeks.

Patients may require hospitalization for therapy; supportive and corrective measures should be instituted in the event that other infections are present. An occasional patient may be encountered who may require splenectomy to relieve the profound hypersplenism and the resulting anemia. Response to therapy often can be assessed by return of the patient's temperature to normal, a brisk reticulocytosis, a gradual reduction in spleen size, and the reappearance of eosinophils on the peripheral blood smear.

Allopurinol has been used with pentavalent antimonials to treat cases of VL that did not respond to pentavalent antimonials alone. Several reports suggest that recombinant IFN-γ is helpful, along with pentavalent antimonial drugs, in successfully treating this disease. Because assessing whether a cure has been achieved is difficult, patients must be monitored at 6-month intervals for as long as 2 years. Fluorescent antibody titers should be absent by the end of 1 year and complement-fixation titers by 6 to 8 months. If post–kala-azar dermal leishmaniasis occurs, treatment should be reinstituted.

Prevention

Control of VL has many aspects. Sandflies (*Phlebotomus* and *Lutzomyia*) can be eliminated readily by residual spraying. Because sandflies ordinarily do not fly very high, sleeping quarters should be above ground level. Permethrin-impregnated bed nets can be highly effective in preventing sandfly bites. Animal reservoirs, such as infected dogs and rodents, should be destroyed. Early therapy will prevent family and neighborhood transmission. A vaccine against different forms of leishmaniasis should be feasible, considering the wealth of information on genetics and biology of the parasite, clinical and experimental immunology of leishmaniasis, and the availability of vaccines that can protect experimental animals against challenge with different *Leishmania* spp. At the present time, however, no vaccine against any form of leishmaniasis is available for human use. One major obstacle is the lack of a conceived market for human leishmaniasis vaccines. Ninety percent of visceral leishmaniasis occurs in five countries (i.e., Bangladesh, Brazil, India, Nepal, and Sudan). Nonetheless, local studies for vaccine development are ongoing in India and Brazil.

NEW REFERENCES SINCE THE SIXTH EDITION

9. Bruckner DA, LaBarca JA. *Leishmania* and *Trypanosoma*. In: Versalovic J, Carroll KC, Funke, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press; 2011.
10. Cannella AP, Nguyen BM, Piggott CD, et al. Short report: a cluster of cutaneous leishmaniasis associated with human smuggling. *Am J Trop Med Hyg* 2011;**84**:847–50.
15. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. *Clin Microbiol Rev* 2006;**19**:111–26.
22. Garcia LS. *Diagnostic medical parasitology*. 5th ed. Washington, D.C.: ASM Press; 2007.
24. Glasser JS, Murray CK. Case report: Central nervous system toxicity associated with liposomal amphotericin B therapy for cutaneous leishmaniasis. *Am J Trop Med Hyg* 2011;**84**:566–8.
43. Petersen CA. Leishmaniasis, an emerging disease found in companion animals in the United States. *Top Companion Anim Med* 2009;**24**:182–8.
44. Pinheiro RO, Pinto EF, de Matos Guedes HL, et al. Protection against cutaneous leishmaniasis by intranasal vaccination with lipophosphoglycan. *Vaccine* 2007;**25**:2716–22.
46. Reithinger R, Dujardin J-C. Molecular diagnosis of leishmaniasis: current status and future applications. *J Clin Microbiol* 2007;**45**: 21–5.
56. Srividya G, Kulshrestha A, Singh R, et al. Diagnosis of visceral leishmaniasis: developments over the last decade. *Parasitol Res* 2012;**110**:1065–78.
59. Verde FA, Verde FA, Neto AS, et al. Hormonal disturbances in visceral leishmaniasis (kala-azar). *Am J Trop Med Hyg* 2011;**84**: 668–73.
63. Wright AM, Fedorko DP, Li L, et al. Cutaneous leishmaniasis in Texas: a northern spread of endemic areas. *J Am Acad Dermatol* 2008;**58**:650–2.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ampuero J, Urdaneta M, Macedo Vde O. Risk factors for cutaneous leishmaniasis transmission in children aged 0 to 5 years in an endemic area of *Leishmania (Viannia) braziliensis*. *Cad Saude Publica* 2005;**21**:161–70.
2. Andrade-Narvaez FJ, Medina-Peralta S, Vargas-Gonzalez A, et al. The histopathology of cutaneous leishmaniasis due to *Leishmania (Leishmania) mexicana* in the Yucatan peninsula, Mexico. *Rev Inst Med Trop Sao Paulo* 2005;**47**:191–4.
3. Arnot DE, Barker DC. Biochemical identification of cutaneous leishmaniasis by analysis of kinetoplast DNA. II. Sequence homologies in Leishmania kDNA. *Mol Biochem Parasitol* 1981;**3**:47–56.
4. Ashford RW, Desjeux P, deRaadt P. Estimation of population at risk of infection and number of cases of leishmaniasis. *Parasitol Today* 1992;**8**:104.
5. Badaro R, Jones TC, Carvalho EM. New perspectives on a subclinical form of VL. *J Infect Dis* 1986;**154**:1003–11.
6. Bekaert ED, Dole E, Dubois DY, et al. Alterations in lipoprotein density classes in infantile leishmaniasis: presence of apolipoprotein SAA. *Eur J Clin Invest* 1992;**22**:190–9.
7. Belli A, Garcia D, Palacios X, et al. Widespread atypical cutaneous leishmaniasis caused by *Leishmania (L.) chagasi* in Nicaragua. *Am J Trop Med Hyg* 1999;**61**:380–5.
8. Berman JD. Chemotherapy for leishmaniasis: biochemical mechanisms, clinical efficacy, and future strategies. *Rev Infect Dis* 1988; **10**:560–86.
9. Bruckner DA, LaBarca JA. *Leishmania* and *Trypanosoma*. In: Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press; 2011.
10. Cannella AP, Nguyen BM, Piggott CD, et al. Short report: a cluster of cutaneous leishmaniasis associated with human smuggling. *Am J Trop Med Hyg* 2011;**84**:847–50.
11. Carvalho EM, Teixeira RS, Johnson WD Jr. Cell- mediated immunity in American visceral leishmaniasis: reversible immunosuppression during acute infection. *Infect Immun* 1981;**33**:498–500.
12. Centers for Disease Control. 2004. Update: cutaneous leishmaniasis in U. S. military personnel—Southwest/Central Asia, 2002-2004. *MMWR Morbid Mortal Wkly Rep* 2004;**53**:264–5.
13. Chulay JD, Bryceson ADM. Quantitation of amastigotes of *Leishmania donovani* in smears of splenic aspirates from patients with VL. *Am J Trop Med Hyg* 1983;**32**:475–9.
14. Convit J, Pinardi ME, Rondon AJ. Diffuse cutaneous leishmaniasis: a disease due to an immunologic defect of the host. *Trans R Soc Trop Med Hyg* 1972;**66**:603–10.
15. Croft SL, Sundar S, Fairlamb AH. Drug resistance in leishmaniasis. *Clin Microbiol Rev* 2006;**19**:111–26.
16. Cruz I, Chicharro C, Nieto J, et al. Comparison of new diagnostic tools for management of pediatric Mediterranean visceral leishmaniasis. *J Clin Microbiol* 2006;**44**:2343–7.
17. Da Silva LJ. Vianna and the discovery of *Leishmania braziliensis:* the role of Brazilian parasitologists in the identification of Bauru's ulcer as American leishmaniasis. *Parassitologia* 2005;**47**:335–41.
18. Dedet JP. Stages in the identification of phlebotomine sandflies as vectors of leishmaniases and other tropical diseases. *Parassitologia* 2005;**47**:291–5.
19. Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp Immunol Microbiol Infect Dis* 2004;**27**:305–18.
20. Dillon DC, Day CH, Whittle JA, et al. Characterization of a *Leishmania tropica* antigen that detects immune responses in Desert Storm viscerotropic leishmaniasis patients. *Proc Natl Acad Sci U S A* 1995;**92**:7981–5.
21. Eltoum IA, Zijlstra EE, Ali MS, et al. Congenital kala-azar and leishmaniasis in the placenta. *Am J Trop Med Hyg* 1992;**46**:57–62.
22. Garcia LS. *Diagnostic medical parasitology*. 5th ed. Washington, D.C.: ASM Press; 2007.
23. Ghosh M, Mandal L, Maitra S, et al. *Leishmania donovani* infection of human myeloid dendritic cells leads to a Th1 response in $CD4^+$ T cells from healthy donors and patients with kala-azar. *J Infect Dis* 2006;**194**:294–301.
24. Glasser JS, Murray CK. Case report: central nervous system toxicity associated with liposomal amphotericin B therapy for cutaneous leishmaniasis. *Am J Trop Med Hyg* 2011;**84**:566–8.
25. Grimaldi G Jr, Tesh RB. Leishmaniasis of the New World: current concepts and implications for future research. *Clin Microbiol Rev* 1993;**6**:230–50.
26. Handman E. Cell biology of *Leishmania*. *Adv Parasitol* 1999; **44**:1–39.
27. Herwaldt BL. Leishmaniasis. *Lancet* 1999;**354**:1191–9.
28. Herwaldt BL, Berman JD. Recommendations for treating leishmaniasis with sodium stibogluconate (Pentostam) and review of pertinent clinical studies. *Am J Trop Med Hyg* 1992;**46**:296–306.
29. Jha MD, Sundar S, Thakur CP, et al. Miltefosine, an oral agent for the treatment of Indian VL. *N Engl J Med* 1999;**341**:1795–800.
30. Khamesipour A, Rafati S, Davoudi N, et al. Leishmaniasis vaccine candidates for development: a global overview. *Indian J Med Res* 2006;**123**:423–38.
31. Laurent T, Rijal S, Yardley V, et al. Epidemiological dynamics of antimonial resistance in *Leishmania donovani:* genotyping reveals a polyclonal population structure among naturally-resistant clinical isolates from Nepal. *Infect Genet Evol* 2006. Epub ahead of print.
32. Locksley RM, Pingel S, Lacy D, et al. Susceptibility to infectious diseases: leishmania as a paradigm. *J Infect Dis* 1999;**179**(suppl. 2):305–8.
33. Magill AJ, Grogl M, Gasser RA, et al. Visceral infection caused by *Leishmania tropica* in veterans of Operation Desert Storm. *N Engl J Med* 1993;**328**:1383–7.
34. Martín-Sánchez J, Pineda JA, Morillas-Márquez F, et al. Detection of *Leishmania infantum* kinetoplast DNA in peripheral blood from asymptomatic individuals at risk for parenterally transmitted infections: relationship between polymerase chain reaction results and other *Leishmania* infection markers. *Am J Trop Med Hyg* 2004;**70**: 545–8.
35. Martino L, Davidson RN, Giacchino R et al. Treatment of VL in children with liposomal amphotericin B. *J Pediatr (on-line)* 1997; **131**:1–8.
36. Mary C, Auriault V, Faugere B, et al. Control of Leishmania infantum infection is associated with $CD8^+$ and gamma interferon– and interleukin-5—producing $CD4^+$ antigen-specific T cells. *Infect Immun* 1999;**67**:5559–66.
37. Maurya R, Singh RK, Kumar B, et al. Evaluation of PCR for diagnosis of Indian kala-azar and assessment of cure. *J Clin Microbiol* 2005;**43**:3038–41.
38. Medrano FJ, Hernandez-Quero J, Jimenez E, et al. VL in HIV-1–infected individuals: a common opportunistic infection in Spain? *AIDS* 1992;**6**:1499–503.
39. Meyerhof A. U.S. Food and Drug Administration approval of AmBisome (liposomal amphotericin B) for treatment of VL. *Clin Infect Dis* 1999;**28**:42.
40. Mira JA, Corzo JE, Rivero A, et al. Frequency of visceral leishmaniasis relapses in human immunodeficiency virus-infected patients receiving highly active antiretroviral therapy. *Am J Trop Med Hyg* 2004;**70**:298–301.
41. Orshan L, Szekely D, Schnur H, et al. Attempts to control sand flies by insecticide-sprayed strips along the periphery of a village. *J Vector Ecol* 2006;**31**:113–17.
42. Pasquau F, Ena J, Sanchez R, et al. Leishmaniasis as an opportunistic infection in HIV-infected patients: determinants of relapse and mortality in a collaborative study of 228 episodes in a Mediterranean region. *Eur J Clin Microbiol Infect Dis* 2005;**24**:411–18.
43. Petersen CA. Leishmaniasis, an emerging disease found in companion animals in the United States. *Top Companion Anim Med* 2009;**24**:182–8.
44. Pinheiro RO, Pinto EF, de Matos Guedes HL, et al. Protection against cutaneous leishmaniasis by intranasal vaccination with lipophosphoglycan. *Vaccine* 2007;**25**:2716–22.
45. Pratlong F, Rioux JA, Marty P, et al. Isoenzymatic analysis of 712 strains of *Leishmania infantum* in the south of France and relationship of enzymatic polymorphism to clinical and epidemiological features. *J Clin Microbiol* 2004;**42**:4077–82.
46. Reithinger R, Dujardin J-C. Molecular diagnosis of leishmaniasis: current status and future applications. *J Clin Microbiol* 2007;**45**: 21–5.
47. Sacks D, Kamhawi S. Molecular aspects of parasite-vector and vector-host interactions in leishmaniasis. *Annu Rev Microbiol* 2001; **55**:453–83.
48. Sampiao SA, Castro MR, Dillon NL, et al. Treatment of mucocutaneous (American) leishmaniasis with amphotericin B: report of 70 cases. *Int J Dermatol* 1971;**10**:179–81.
49. Selvapandiyan A, Duncan R, Debrabant A, et al. Genetically modified live attenuated parasites as vaccines for leishmaniasis. *Indian J Med Res* 2006;**123**:455–66.

50. Shaw JJ, Lainson R. Ecology and epidemiology: New World. In: Peters W, Killick-Kendrick R, editors. *The leishmaniases in biology and medicine*, vol. I. London: Academic Press; 1987. p. 78–87.
51. Shaw PK, Quigg LT, Allain DS, et al. Autochthonous dermal leishmaniasis in Texas. *Am J Trop Med Hyg* 1976;**25**:788–96.
52. Schwartz E, Hatz C, Blum J. New world cutaneous leishmaniasis in travellers. *Lancet Infect Dis* 2006;**6**:342–9.
53. Shazad B, Abbaszadeh B, Khamesipour A. Comparison of topical paromomycin sulfate (twice/day) with intralesional meglumine antimoniate for the treatment of cutaneous leishmaniasis caused by L. major. *Eur J Dermatol* 2005;**15**:85–7.
54. Singh R, Kumar D, Ramesh V et al. Visceral leishmaniasis, or kala Aazar (KA): high incidence of refractoriness to antimony is contributed by anthroponotic transmission via post-KA dermal leishmaniasis. *J Infect Dis* 2006;**194**:302–6.
55. Soto J, Toledo J, Gutierrez P, et al. Treatment of American cutaneous leishmaniasis with miltefosine, an oral agent. *Clin Infect Dis* 2001;**33**:e57–61.
56. Srividya G, Kulshrestha A, Singh R, et al. Diagnosis of visceral leishmaniasis: developments over the last decade. *Parasitol Res* 2012;**110**:1065–78.
57. Sundar S, Rosenkaimer F, Makharia MK, et al. Trial of oral miltefosine for VL. *Lancet* 1998;**352**:1821–3.
58. Turk JL, Bryceson AD. Immunological phenomenon in leprosy and related diseases. *Adv Immunol* 1971;**13**:209–66.
59. Verde FA, Verde FA, Neto AS, et al. Hormonal disturbances in visceral leishmaniasis (kala-azar). *Am J Trop Med Hyg* 2011;**84**: 668–73.
60. Vergel C, Palacios R, Cadena H, et al. Evidence for leishmania (viannia) parasites in the skin and blood of patients before and after treatment. *J Infect Dis* 2006;**194**(4):503–11.
61. Weigle KA, de Davalaos M, Heredia P, et al. Diagnosis of cutaneous and mucocutaneous leishmaniasis in Colombia: a comparison of seven methods. *Am J Trop Med Hyg* 1987;**36**:489–96.
62. Willard RJ, Jeffcoat AM, Benson PM et al. Cutaneous leishmaniasis in soldiers from Fort Campbell, Kentucky returning from Operation Iraqi Freedom highlights diagnostic and therapeutic options. *J Am Acad Dermatol* 2005;**52**:977–87.
63. Wright AM, Fedorko DP, Li L, et al. Cutaneous leishmaniasis in Texas: a northern spread of endemic areas. *J Am Acad Dermatol* 2008;**58**:650–2.
64. Yadav TP, Gupta H, Satteya U, et al. Congenital kala-azar. *Ann Trop Med Parasitol* 1989;**83**:535–7.
65. Zerpa O, Blanco B, Kannee C, et al. Treatment of diffuse cutaneous leishmaniasis with miltefosine: a case report. *Int J Dermatol* 2006; **45**:751–3.

CHAPTER 221

Trypanosomiasis

Lawrence A. Ross • Lynne S. Garcia • Murray Wittner • Herbert B. Tanowitz

AMERICAN TRYPANOSOMIASIS

American trypanosomiasis, or Chagas disease, is caused by the protozoan hemoflagellate *Trypanosoma cruzi*, which was discovered first by Chagas in 1909 in the blood of a seriously ill, wasted Brazilian child with fever, lymphadenopathy, and anemia.[89] The disease, transmitted by reduviid bugs, is limited to the Western Hemisphere and is a major health problem in Latin American countries. Chagas disease is currently identified within 21 countries in the Western Hemisphere; geopolitical, economic, ecologic, and vector-related factors have affected epidemiologic trends of this disease.[93,101] During the preceding decade, cessation of transmission of *T. cruzi* by the main vector, *Triatoma infestans*, has been recognized in several countries, including Uruguay, Chile, Brazil, and Guatemala.[63] In endemic areas, it occurs most commonly in the rural poor who live in an environment with many feral reservoir hosts. Maternal to child transmission and laboratory-acquisition are recognized routes of infection.[28,82] It is established that *T. cruzi* may be transmitted by blood transfusion, organ donation, and congenital infection.[52] In addition, outbreaks attributed to contaminated food have been identified.[72] Indigenous infections have been reported from the southwestern United States, Belize, Guyana, and Trinidad and Tobago[6,57] Chagas disease in some endemic areas is the most important cause of heart disease and may result in serious chronic digestive tract pathology.

Collaborative efforts, including control initiatives in the Southern Cone, Andean, Amazon, and Central America regions have resulted in substantial advances in vector control. An initiative for control of Chagas disease in nonendemic regions was undertaken by the World Health Organization (WHO) in 2007. An estimated 8 to 10 million people are infected, a dramatic decrease from previous projections of 16 to 18 million persons. Further, it is estimated that new cases have diminished from 200,000/year to 40,000/year and deaths from 45,000/year to 12,500/year.[68,97,102] It is estimated that 300,000 infected individuals reside in the United States, largely reflecting immigration patterns from Latin America.[8,58] In certain areas of endemic infection, approximately 10 percent of all adult deaths are caused by Chagas disease. The southern United States, particularly Texas, is identified as having many cases. The geographic distribution of *T. cruzi* infection overlaps that of *Trypanosoma rangeli* (a nonpathogen), the trypomastigotes may be misidentified, and the parasitemia is mild.

The Organism

Although six evolutionary lineages have been identified in *T. cruzi*, *T. cruzi* lineages I and II have been the most widely studied. Before 2005, epidemiologic and immunologic data suggested that chronic infections occurring in Brazil and Argentina were caused primarily by *T. cruzi* II strains. Using polymerase chain reaction (PCR) techniques, this hypothesis has been confirmed, and future studies can establish which strains are responsible for Chagas disease in other geographic locations.[49] A third lineage, termed *T. cruzi* III also exists and is equivalent to the sublineage called TCIIc,[16] thus supporting the distinction of six *T. cruzi* lineages, categorized as TcI to TcVI.

T. cruzi is a pleomorphic, spindle-shaped organism with a quite variable size, depending on the strain. The length of the blood forms (trypomastigote) in humans may vary widely, from 11 to 30 μm, including a free anterior flagellum. Most trypomastigotes assume a "C" or "S" shape, with the nucleus located just anterior to the middle of the cell. Trypomastigotes occur in the blood in two forms, a long slender form and a short stubby one. The nucleus is situated in the center of the body, with a large oval kinetoplast located at the posterior extremity. The kinetoplast consists of a small blepharoplast and a large, oval parabasal body; the kinetoplast often appears to protrude from the cell surface (Fig. 221-1). The kinetoplast contains thousands of mini-circle and maxi-circle DNAs that serve in the synthesis of mitochondrial proteins. A flagellum arises from the blepharoplast and extends along the outer edge of an undulating membrane until it reaches the anterior end of the body, where it projects as a free flagellum. When the trypomastigotes are stained with Giemsa stain, the cytoplasm stains blue and the nucleus, kinetoplast, and flagellum stain red or violet.

Trypomastigotes are ingested by the reduviid bug as it obtains a blood meal. Within a few hours after taking an infective blood meal, short, spindle-shaped forms lacking a free flagellum can be found in the insect's foregut. These forms develop into small epimastigotes that continue to divide and give rise to large (35 to 40 μm) epimastigotes; by the third or fourth day after the blood meal, these organisms can be found attached to the rectal epithelium. By about the fifth day, the epimastigotes have begun to round up and gradually develop into short, stout trypomastigotes, which then proceed to elongate, and by the seventh and eighth days, they become the long,

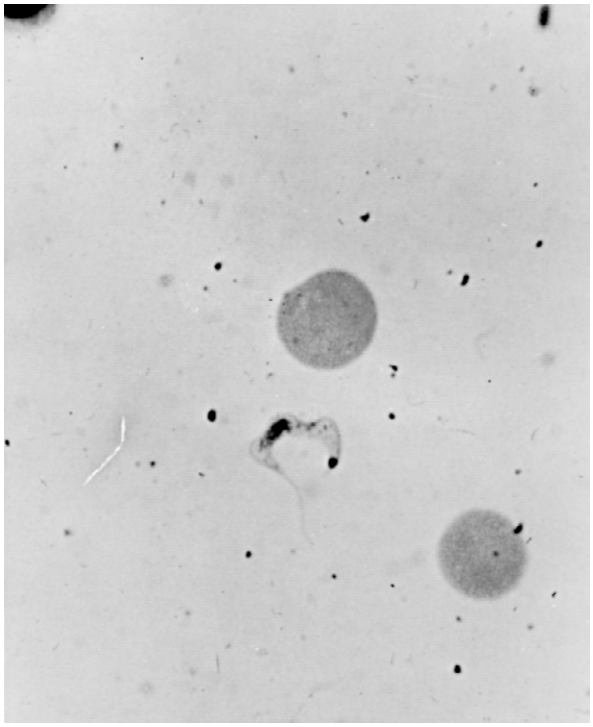

FIGURE 221-1 ■ *Trypanosoma cruzi* trypomastigote forms in a blood smear. Note the large posterior kinetoplast (original magnification ×288).

slender (17 to 22 μm), infective, metacyclic trypomastigotes. After 8 to 10 days, metacyclic trypomastigotes develop from the epimastigotes and are passed in the feces to infect humans when rubbed into the insect's puncture wound or rubbed onto exposed mucous membranes. These forms do not divide again, but are passed out with the feces sometime after the blood meal. There may be 3000 to 4000 organisms per microliter of excreta. Transmission may occur by means of infected insect excreta, and metacyclic forms readily negotiate mucosa, conjunctiva, and abraded or otherwise broken skin (puncture site). Extensive experimental evidence has shown that infection is by contamination rather than insect inoculation.

On invasion of a cell, the trypomastigote loses its flagellum and undulating membrane and divides by binary fission to form an amastigote. The amastigote continues to divide and eventually fills and destroys the infected cell. Amastigote and trypomastigote forms are released from the cell. The amastigote is indistinguishable from those found in leishmanial infections. It is 2 to 6 μm in diameter and contains a large nucleus and rod-shaped kinetoplast that stains red or violet with Giemsa stain. The cytoplasm stains blue. Only the trypomastigotes are found free in the peripheral blood.

When the infective metacyclic trypomastigotes traverse the skin, they invade and multiply locally in many cell types after having transformed into intracellular amastigote forms. Host-cell entry by the parasite is a complex process involving many host and parasite factors. Trypomastigotes released from infected host cells may infect adjacent uninfected cells or enter the bloodstream and lymphatics to infect distant tissues. No tissue is spared from infection, but *T. cruzi* strains may vary in tissue tropism. The cells of the reticuloendothelial system and nervous and muscular (striated and cardiac) systems seem to be particularly vulnerable. In the ensuing weeks, during which time repeated cycles of multiplication, cell destruction, and cell reinvasion have occurred, flagellates enter the bloodstream as trypomastigotes, and the infection becomes disseminated. Trypomastigotes of *T. cruzi* do not divide in peripheral blood; however, the trypomastigotes of *T. rangeli* and the African trypanomastigotes do continue to divide.

Epidemiology

Chagas disease is a zoonosis occurring throughout American continents and involves reduviid bugs living in close association with human reservoirs (e.g., dogs, cats, armadillos, opossums, raccoons, and rodents). The most ubiquitous sylvatic reservoir host is the opossum, *Didelphis*, which is found throughout much of the range of *T. cruzi* in the Americas. Multiple nesting or resting sites of the opossum encompass many types of triatomine habitat. High *T. cruzi* prevalence rates are due partly to the fact that opossums eat triatomid bugs and may transmit infection via anal gland secretions. Sylvatic cycles of *T. cruzi* transmission extend from southern Argentina and Chile to northern California.

The vectors are distributed in rural wooded areas closely associated with feral animal species; humans intervene only occasionally. This infection usually is a zoonosis. Many species have become adapted to houses, however, where they may live in cracks; on roofs, walls, and floors; and beneath seldom-moved objects. Other triatomine species have developed regular visiting habits to human dwellings, but have not taken up residence as yet. Although Chagas disease always has been associated with rural areas and low socioeconomic groups, some vectors have adapted to areas undergoing urbanization. Various suitable hosts that often live close to humans, such as the opossum, may provide the transition between wild and domestic infection. Investigators frequently have observed that domestic animals such as pigs, cattle, goats, and sheep are kept in close contact with the domicile, and that triatomid bugs closely associated with these animals can be carried into the home.

Although 12 species of reduviids occur within the United States, they have not adapted themselves to household habitation. Humans should avoid sleeping in thatch, mud, or adobe houses; bed nets should be used by individuals sleeping in these types of houses. Travelers planning to stay in hotels, resorts, or other well-constructed housing facilities are not at high risk for contracting Chagas disease. Insecticides can be used to kill the vectors and reduce the risk for transmission. The reduviid bug life stages include egg, five instars, and adult. A full blood meal is required for molting to the next stage; development from egg to adult may take a year or longer. Aside from natural transmission via the vector, other modes of transmission include congenital, blood transfusion, breast milk, organ transplantation, laboratory accident, and contaminated food.[10,48] Reported

rates of congenital infection vary substantially, ranging from 1 to 12 percent of infants born to infected mothers.*

Blood transfusion is an important mode of transmission. In many large urban areas of South America, 3 to 15 percent of blood donors were reported to have positive serologic findings. The problem is compounded because blood forms can live for weeks in stored refrigerated blood without losing their infectivity. Concerted efforts to evaluate blood donors for Chagas disease have achieved screening levels of 99 percent in most areas of Latin America.[84] In recent years, immigration from endemic regions to North America, Europe, Australia, and Japan has resulted in increasing recognition of Chagas disease in these nonendemic regions, with concomitant risk for transfusion-associated transmission outside the classic endemic regions.[70,51,83] The prevalence of *T. cruzi* in blood donors in the United States is approximately 1/30,000; transmission from blood transfusion and organ donation have been confirmed.[59] In areas where seroprevalence is high, rather than discarding all positive blood units, laboratories add gentian violet to the units, which are stored at 4°C (39.2°F) for 24 hours before use, to kill the organism.

Ingestion of infected mice by cats or cannibalism among rodents has been shown to transmit the infection successfully. Several reports have indicated the acquisition of infection by humans from infected meat. However, the importance of these routes of infection in maintaining the disease in nature or in humans is unclear. In endemic areas, large numbers of feral and domestic reservoirs of *T. cruzi* exist. Dogs and cats, especially in Brazil and Chile, have been shown to have a high index of infection and are thought to represent important domestic reservoirs. In some areas, armadillos have been implicated. In the United States, naturally occurring Chagas disease has been reported from Louisiana, Oklahoma, and Texas. In addition, the wood rat, raccoon, armadillo, and opossum are important feral hosts.

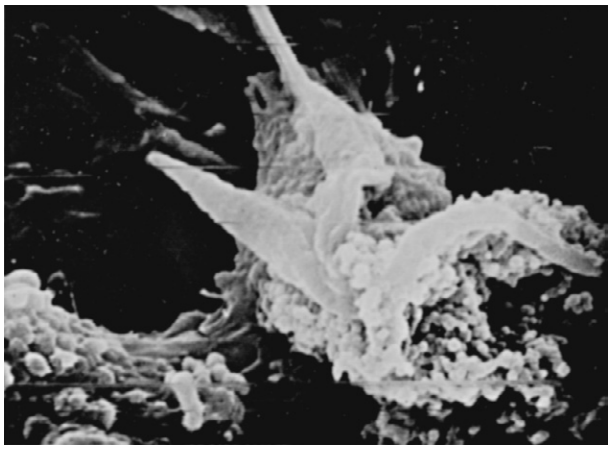

FIGURE 221-2 ■ *Trypanosoma cruzi* phagocytized by a mouse peritoneal macrophage (scanning electron micrograph ×6000).

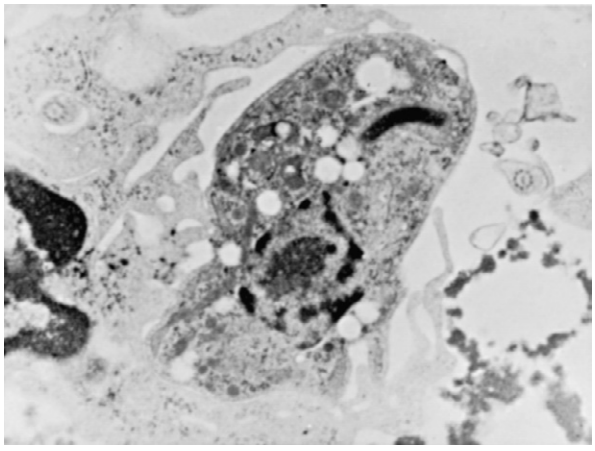

FIGURE 221-3 ■ *Trypanosoma cruzi* phagocytized by a mouse peritoneal macrophage (transmission electron micrograph ×7000).

Pathogenesis and Pathology

When metacyclic trypomastigotes penetrate the skin or mucous membrane, they may be phagocytized by tissue macrophages or actively penetrate other cells (Figs. 221-2 and 221-3).[76] Within the cell, trypomastigotes rapidly transform into rounded amastigotes and division begins (Fig. 221-4). In vitro studies have shown that parasites within macrophage vacuoles may be killed by the cytocidal mechanisms of the host cell, which include nitric oxide. The parasite may escape the parasitophorous vacuole by elaborating a hemolysin and reach the cytoplasm, where it reproduces. Within these cells, large numbers of amastigotes are formed, and within a few days, the greatly distended cells rupture and free trypomastigotes and amastigotes into tissues. They actively invade previously uninfected cells, and the process is repeated.

A nodular swelling, or chagoma, develops at the site of entry (Fig. 221-5). This area soon is infiltrated with macrophages surrounded by lymphocytes, eosinophils, and neutrophils. The process spreads to the regional lymph nodes, where focal lymphadenitis may be seen. Shortly thereafter, blood forms appear and disseminate throughout the body. An acute pathologic condition is caused by the invasion and subsequent destruction of cells by the replicating intracellular parasites. A marked host inflammatory reaction occurs characterized by local accumulation of neutrophils, lymphocytes, and plasma cells. Although any tissue can be involved, cardiac and skeletal muscle and the cells of the reticuloendothelial and nervous systems are infected more frequently.[71]

The myocardium reveals focal myonecrosis, contraction band necrosis, interstitial fibrosis, and lymphocytic infiltration. Interspersed among the degenerating fibers is a marked mixed inflammatory cell exudate, which with time becomes predominantly mononuclear. The course of the acute infection can be quite variable, with severe tissue destruction, or the infection can be silent, with few or no obvious pathologic findings. Nonetheless, the parasites have entered various cells of the body successfully

*References 5, 14, 19, 24, 28, 82.

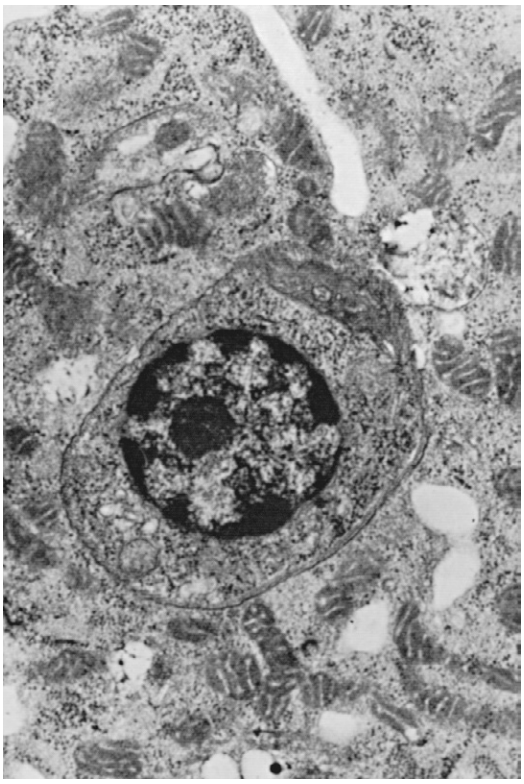

FIGURE 221-4 ■ *Trypanosoma cruzi* amastigote forms in the cytoplasm of a macrophage (transmission electron micrograph ×7000).

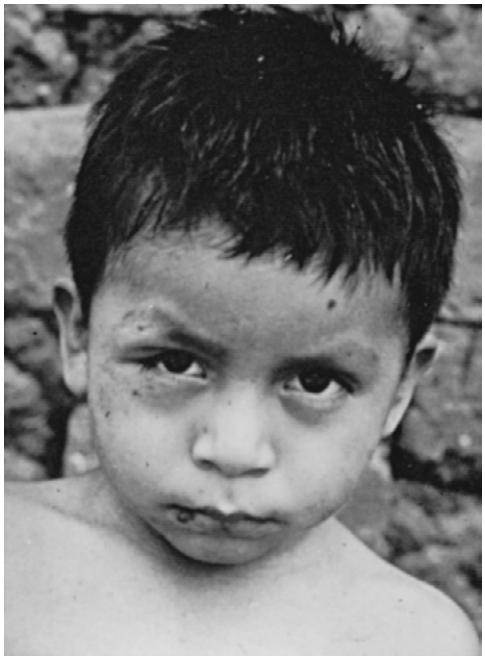

FIGURE 221-5 ■ Chagas disease. A chagoma on the mucocutaneous junction of the lip and moderate unilateral periorbital edema are present. (From a nonprofit cooperative endeavor by numerous colleagues under the editorship of Dr. Herman Zaiman, New York.)

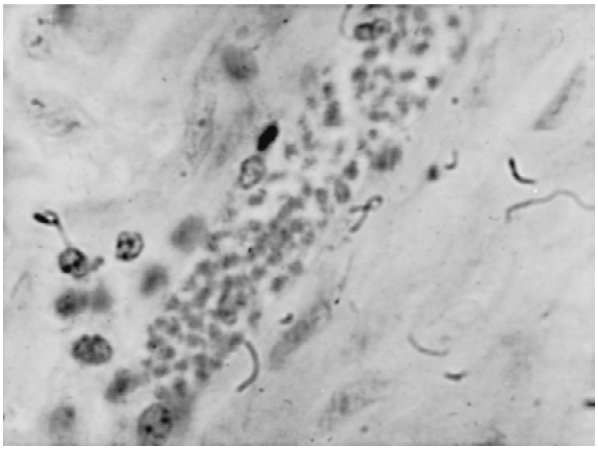

FIGURE 221-6 ■ *Trypanosoma cruzi* amastigote forms in cardiac muscle (original magnification ×288).

and have formed pseudocysts, each containing hundreds to thousands of amastigotes (Fig. 221-6). Individuals who recover from the acute episode, including those who have remained asymptomatic, probably harbor these intracellular parasites for the rest of their lives.

The immunology of Chagas disease in experimental animals and humans is very complex. At present, the disease is conceptualized as representing a persistent but variable interaction between *T. cruzi* and the host.[42] Immunologic investigation suggests that both innate immune response (NK cell activity) and adaptive immune responses (cell-mediated response and antibody formation) are apparent in the host response and contribute to tissue injury.[35,76] Cytokines such as interferon-γ and nitric oxide contribute to the intracellular killing of this parasite and to myocardial and gastrointestinal tract dysfunction.

Chagasic heart disease represents the interplay of inflammation and ischemia. During acute infection, foci of myonecrosis, myocytolysis, and vasculitis are observed, along with an inflammatory exudate consisting primarily of leukocytes. Pseudocysts containing amastigotes can be found interspersed among the degenerating fibers. *T. cruzi* gains access to cardiac myocytes by first invading endothelial cells, the interstitial areas of the vascular wall, and the myocardium. Parasites can be seen in and around the endothelium of infected mice. The trypomastigotes pass two basal laminae and two layers of extracellular matrix. Parasite enzymes such as proteases, gelatinases, and collagenases degrade native type I collagen, heat-denatured type I collagen, and native type IV collagen. Proteolytic activities against laminin and fibronectin also have been detected. These enzymes may play an important role in the degradation of extracellular matrix and subsequent parasite invasion.

Degradation of the collagen matrix, evident in acute murine Chagas disease, has been proposed to result in chronic pathologic processes such as apical thinning of the ventricle. When damage such as that caused by ischemia and necrosis occurs, extracellular matrix degradation ensues and leads to slippage of the ventricular layers,

with mural thinning and formation of an aneurysm. Damage to this area of the heart and remodeling of the wall frequently are encountered in chagasic heart disease. Remodeling refers to the structural changes associated with inflammation, necrosis, hypertrophy, and ventricular dilation. In the course of chronic chagasic heart disease, myonecrosis, myocytolysis, and contraction band necrosis are evident. The necrosis results from transient hypoperfusion followed by reperfusion, such as occurs after local spasm of the coronary microvasculature. Focal and diffuse areas of myocellular hypertrophy may be observed with or without inflammatory infiltrates. In other areas, focal fibrosis replacing previously damaged myocardial tissue is evident.

An important feature of chagasic heart disease is a dense accumulation of extracellular collagen that encloses fibers or groups of fibers. All areas of the heart, including the conduction pathways, may be involved. Microvascular involvement, manifested by basement membrane thickening, has been shown. These irreversible changes lead to functional disturbances. The remodeling process results in replacement of cardiac myocytes and cells of the vasculature by fibrous tissue, which leads to thinning of the myocardium and hypertrophy of the remaining cardiac myocytes. Chronic chagasic cardiomyopathy is characterized by focal lymphocytic infiltrates (predominately $CD8^+$), myocyte necrosis, and marked reactive and reparative fibrosis. The role of apoptosis in the pathogenesis of chronic chagasic cardiomyopathy is unclear.

In the chronic stage of Chagas disease, the heart usually is enlarged. The chambers may be dilated and associated with an apical left or right ventricular aneurysm, which rarely ruptures. Mural thrombi, especially of the right atrium and apex of the left ventricle, commonly are seen. They may cause widespread embolization, especially to the brain, lungs, spleen, and kidneys. The myocardium often reveals diffuse fibrosis with small numbers of mononuclear cells scattered throughout, and in many patients, finding parasites in the myocardium may be quite difficult.

The apparent absence of parasites in the myocardium during routine histologic examination in chronic Chagas cardiomyopathy, even in the presence of marked morphologic and functional changes, has engendered much speculation regarding possible pathogenic etiologies. Parasite persistence, autoimmunity, disturbances in the autonomic nervous system, and vascular compromise have emerged as possible important factors. Experimental studies indicate that endothelin-1 and thromboxane A_2, potent vasoconstrictors, and nitric oxide may contribute to the pathogenesis of Chagas cardiomyopathy.[73] In addition, genetic epidemiologic studies suggest that susceptibility to infection and progression to chronic disease may be partly genetically determined. Researchers have estimated that destruction of more than 95 percent of the ganglion cells of the myenteric plexus in the esophagus and colon is required for evidence of megaesophagus and megacolon. In experimental studies, destruction of ganglion cells mediated by inflammatory mediators is the result of parasitic destruction during the acute phase of the disease.

Clinical Manifestations

Clinically, Chagas disease can be viewed as having three phases: acute, indeterminate, and chronic. Seroepidemiologic studies indicate that overt or clinically recognizable disease fails to develop in most individuals infected with *T. cruzi*, and researchers estimate that 99 percent of individuals infected have inapparent or subclinical infection. Most individuals in whom clinical disease develops as a result of natural infection are infants unable to ward off the bite of triatomes, or "kissing bugs," and other young children up to 10 years old. After an incubation period of approximately 7 to 14 days, acute clinical symptoms may begin with anorexia, lassitude, headache, and intermittent or remitting fever with temperatures of 38° to 40.5° C (100° to 104.9° F). In young infants, swelling and edema of Bichat's fat pad may make feeding painful.

Early Disease

Locally, or near the site of initial infection, a characteristic unilateral, painless palpebral edema, often with conjunctivitis, may be recognized. This so-called Romaña's sign, when present in an endemic area (25% to 30% of patients), is highly suggestive of early acute Chagas disease. The edema may be generalized and mistaken for nephrotic syndrome, however, or swelling of the preauricular region may be so severe that it suggests mumps. The edema does not pit on pressure, and the skin is dry. Generalized lymphadenopathy, hepatosplenomegaly, vomiting, diarrhea, and signs of meningeal irritation commonly are encountered. Skin lesions can vary from a generalized maculopapular or morbilliform eruption to urticaria. Neurologic symptoms, found more often in younger infants than in older patients, include focal to generalized seizures.

Acute Stage

Acute-stage symptoms occur in only approximately 1 percent of patients, usually are seen in younger children and are less obvious in older individuals because of the nonspecific nature of the symptoms and the lack of availability of health care. Symptoms generally last 4 to 8 weeks and then may subside, even without therapy. Acute systemic signs occur around week 2 to 3 of infection and are characterized by high fevers, which may be intermittent, remitting, or continuous; hepatosplenomegaly; myalgia; erythematous rash; acute myocarditis; lymphadenopathy; keratitis; and subcutaneous edema of the face, legs, and feet. There may be signs of central nervous system (CNS) involvement, including meningoencephalitis, which has a very poor prognosis. Myocarditis is manifested by electrocardiographic changes, tachycardia, chest pain, and weakness. Amastigotes proliferate within the cardiac muscle cells and destroy the cells, leading to conduction defects and a loss of heart contractility. Death may occur secondary to myocardial insufficiency or cardiac arrest. In infants and very young children with acute Chagas disease, swelling of the brain can develop, causing death.[67] Also, in some areas of

endemic infection, a high frequency of early electrocardiographic abnormalities in children who are seropositive for *T. cruzi* suggests a rapid evolution from infection to disease. Under such conditions, a public health chemotherapy program targeted to this population would be recommended.

During acute infection, echocardiography may reveal abnormal segmental left ventricular wall motion, most commonly in the anterior or apical regions (or both). The overall size of the left ventricle and ejection fraction are typically normal. Fatal acute myocarditis may occur in a few patients. Examination of blood during the acute infection often reveals trypomastigote forms, leukocytosis with a relative lymphocytosis, and an elevated erythrocyte sedimentation rate. Trypomastigotes may be present in cerebrospinal fluid (CSF), even in the absence of neurologic abnormalities. Although most patients recover, apparently without sequelae, 2 to 10 percent may die of severe myocarditis, meningoencephalitis, or an underlying bacterial or viral infection. As the acute stage subsides in 8 to 12 weeks, increasingly fewer parasites can be found in the peripheral blood as antibody levels increase. The disease enters the indeterminate period, and evidence of infection may be detected by special techniques described later. During acute infection, most of the antibody is immunoglobulin (Ig)M. Later, in the indeterminate and chronic stages, antibodies predominantly are IgG. From a global perspective, *T. cruzi* infection is recognized as the most common cause of myocarditis.

Indeterminate Phase

The indeterminate phase of Chagas disease represents the period between acute infection and the onset of chronic signs and symptoms. The duration of this period can span 10 to 40 years, and in most cases, overt clinical disease never develops despite the fact that in asymptomatic individuals, organisms often can be identified by culture or molecular methods and by xenodiagnosis. During the indeterminate phase, electrocardiographic or radiologic abnormalities may not be detectable. However, a minority of patients will have demonstrable abnormalities identified using echocardiography or magnetic resonance imaging modalities.[78,99] Many of these individuals (10% to 30%) eventually experience the serious and potentially fatal chronic phase of the disease. A report from a hospital in Brazil identified Chagas heart disease in 11 percent of patients who died of a cardiovascular cause.[45,99]

The patient may have a subpatent parasitemia and develop antibodies to various *T. cruzi* antigens. Chronic Chagas disease is diagnosed more commonly than the acute disease. The chronic stage may be initially asymptomatic (indeterminate stage), and although trypomastigotes seldom are seen in peripheral blood, transmission by blood transfusion is a serious problem in areas where the disease is endemic. There are regional variations in disease severity, which may account for the geographic differences in morbidity. Approximately 8 to 10 weeks after infection, the indeterminate stage begins, during which the patients do not have any symptoms.

Chronic Stage

Chronic Chagas disease shows close interaction between the parasite and the host, causing many different clinical syndromes. Approximately 30 percent of patients may develop chronic Chagas disease, including cardiomyopathy, megacolon, and megaesophagus. Symptoms of the chronic stage are related to the damage sustained during the acute stage of the disease, the state of the host's immune system, and the inflammatory response. Other contributing factors include the host genetic background, environmental and social factors, the genetic composition of the parasite, and mixed infections and reinfections. The inflammatory response undergoes periods of exacerbation and is probably responsible for neuronal damage, microcirculatory changes, heart matrix deformations, and possible cardiac failure. Selenium deficiency also has been linked with some cardiomyopathies and apparently is a biologic marker for Chagas disease and related to progressive pathology.

Chronic chagasic heart disease occurs insidiously or abruptly with an arrhythmia, dilated congestive cardiomyopathy, thromboembolic phenomena, and sudden death. An apical aneurysm, with or without thrombus formation, is a hallmark of this disease and is commonly found, although not exclusively, in the left ventricle. Although significant major coronary artery lesions are usually absent, several instances of myocardial infarction in individuals with Chagas disease have been reported. Destruction of conduction tissue results in conduction abnormalities.[64] Chronic Chagas disease is now established as predisposing to cerebrovascular ischemic events and may be the initial indication of cardiomyopathy.[25] Microscopically, there is interstitial fibrosis of the myocardium and rare amastigotes; focal infiltrates of lymphocytes, monocytes, and plasma cells are present. Focal neutrophils and occasionally eosinophils may also be seen.

In endemic areas, electrocardiographic abnormalities may suggest chagasic cardiomyopathy. A right bundle branch block alone and a right bundle branch block together with an anterior fascicular block are highly suggestive of chagasic heart disease. These abnormalities have been reported in teenagers and young adults. Premature ventricular contractions and right bundle branch block occur in 50 percent of cases, atrioventricular block of varying degrees develops in about 30 percent of cases, complete atrioventricular block is seen much less commonly, and left bundle branch block is not characteristic. Chest radiographs may reveal cardiomegaly. Heart sounds may be distant, and murmurs of functional mitral and tricuspid insufficiency sometimes are heard. More recently, echocardiography and cardiac magnetic resonance imaging have been used to diagnose and suggest prognosis of chronic chagasic cardiomyopathy.[99] When congestive heart failure ensues, it is usually intractable and difficult to treat. Models have been formulated to identify risk scores predictive of survival

probabilities from Chagas heart disease. Such models may facilitate development of clinical trials for therapeutic intervention.[75]

Megaesophagus and megacolon are the result of destruction of the ganglion cells of the autonomic plexus. Although these complications are not usually fatal except in congenital disease, the marked dilation of the colon and esophagus can be debilitating.[37] Any hollow viscus, including the gallbladder and ureters, can be involved, but esophageal and colonic disease are encountered most frequently. Dysphagia, gastroesophageal reflux, and paroxysmal night coughs, presumably caused by aspiration while sleeping, are associated with megaesophagus; chronic constipation, long-term fecal retention, impaction, and volvulus are observed with megacolon. As megadisease progresses, the thin walls of the esophagus and colon begin to thicken, and the smooth muscle fibers become hyperplastic. There is also a dramatic decrease in the number of ganglion cells of the myenteric plexus. At this point in the disease process, it is very difficult to find amastigotes in either the esophagus or colon.

In some regions, patients with digestive disease have symptomatic chagasic heart disease as well. Wide geographic variation in the prevalence of cardiac and gastrointestinal disease has been noted. In Panama, Venezuela, and Colombia, megaesophagus and megacolon are rarely encountered, whereas in parts of Brazil, both forms of Chagas disease may be seen in the same individual.

Congenital Disease

Congenital Chagas disease is caused by vertical transmission of the infection.[13,18,19,79,92] Pregnant women in endemic areas may experience abortion or placentitis and may give birth to infants with congenital infection associated with prematurity. The risk for maternal-fetal transmission of *T. cruzi* appears to be increased by multiple pregnancies, maternal reinfection, and degree of parasitemia and diminished by adequate maternal innate response.[79,92] Similar rates of maternal-fetal transmission of about 5 percent have been observed in endemic and nonendemic regions.[18] In its acute stage, congenital Chagas disease often resembles the acquired disease. The onset may be at birth or a few months later, with the infant manifesting hepatosplenomegaly, anemia, jaundice, edema, thrombocytopenia, petechiae, tremors, and seizure disorders. The clinical and laboratory findings may resemble those of congenital toxoplasmosis, cytomegalovirus and herpes simplex virus, or erythroblastosis fetalis. Overt clinical disease is observed in 10 to 30 percent of infected newborns and early mortality rates in untreated infants have been reported as 10 percent.[18] Although meningoencephalitis has been observed, it is more likely to be silent. The prognosis of meningoencephalitis in congenital disease is better than in the acquired form of disease. Examination of CSF often reveals trypomastigotes. Necrotic and hemorrhagic lesions of the oral mucosa and skin are thought to represent hematogenously disseminated chagomas. Megacolon and megaesophagus can be present and cause constipation and aspiration pneumonitis.[12,36]

Immunocompromised Patients

Chagas disease has been reported in patients with human immunodeficiency virus (HIV), and neurologic sequelae caused by *T. cruzi* have been major findings.[32,33,60] Transmission of *T. cruzi* has been associated with injecting drug use in HIV-infected persons. Individuals who were previously infected with *T. cruzi* and later became positive for HIV are at risk for reactivation of Chagas disease.[80] These patients develop a severe multifocal or diffuse meningoencephalitis with numerous tissue parasites.[81] Meningoencephalitis is rare in patients without acquired immunodeficiency syndrome (AIDS) and is seen most frequently in immunocompetent children younger than 4 years. Because of the immunodeficiency associated with HIV infections, concomitant infection with *T. cruzi* may be difficult to recognize, particularly in individuals who have moved to areas where the disease is not endemic. *T. cruzi* infection also may have a protracted asymptomatic course in immunosuppressed HIV patients. Although a positive blood smear has been considered the key indicator of Chagas disease reactivation in immunocompromised patients with chronic disease, this finding may occur late, rather than early, in the reactivation process. It has been suggested that *T. cruzi* type I genotype may be associated with cerebral tropism in HIV-infected persons.[20]

Recrudescence of *T. cruzi* infections in immunosuppressed patients, particularly transplantation patients, is concerning. Transplant recipients also can become infected through receipt of infected organs.[27] In endemic regions, transplantation of bone marrow, liver, and kidneys from infected donors is controversial; hearts should not be transplanted. Treatment guidelines for HIV-infected adults and adolescents with Chagas disease have been formulated.[7] In patients with end-stage Chagas cardiomyopathy, heart transplantation is an option that has had variable success. Reactivation of the disease with the development of cutaneous lesions has been seen.[22] Bone marrow transplant recipients also are at risk for Chagas disease because of reactivation or transfusion. Prophylactic treatment of these patients has led to favorable outcomes.

There seems to be a link between *T. cruzi* seropositivity and human T-cell lymphotrophic virus 2 (HTLV-2) infection in studies of the Indians in western Paraguay. In this group, an individual infected with HTLV-2 is 2.28 times more likely to be *T. cruzi*–positive than an HTLV-2–negative individual. The public health significance of these findings is emphasized by the fact that approximately 8 to 10 million people are infected with *T. cruzi* in the Americas, and the frequency of HTLV-2 continues to increase.[46]

Diagnosis

Routine Methods

The diagnosis of Chagas disease should be considered if there is a history of consistent exposure to reduviid bug bites, residence in or travel to areas where the disease is endemic, a laboratory accident or recent blood

transfusion in an area where the disease is endemic, or if cardiac or digestive tract lesions are compatible with Chagas disease. A definitive diagnosis depends, however, on demonstration of the trypomastigotes in the blood, amastigote stages in tissues, or positive serologic reactions. Methods used for examination of blood are similar to the methods used for the diagnosis of African trypanosomiasis. Trypomastigote stages may be detected easily in the blood in young children; however, in chronic disease, this stage is rare or absent except during febrile exacerbations. Trypomastigotes may be detected in blood by using thin and thick blood films or by buffy coat concentration techniques. The most sensitive methods to detect trypomastigotes are concentration techniques, such as the Strout method of buffy coat preparations.[50] With the Strout method, blood is collected without anticoagulant and allowed to clot. The serum is centrifuged at low speed to remove the remaining blood cells and then at a higher speed ($600 \times g$) to concentrate the parasites in the sediment. The stain of choice is Giemsa for trypomastigote and amastigote stages; other blood stains also are acceptable.

Examination of peripheral blood is valuable only during the initial acute disease (6 to 12 weeks) or during chronic exacerbation. Although inoculation of animals with the patient's blood often aids in the diagnosis, this is not a practical approach in most diagnostic laboratories. In chronic stages, the investigator frequently must take 30 to 50 mL of the patient's blood and inoculate numerous cultures to obtain a positive diagnosis. Aspirates, blood, and tissues also can be cultured, which is valuable in detecting low-grade parasitemias. The medium of choice is Novy, McNeal, and Nicolle medium. Cultures should be incubated at 25°C (77°F) and observed for epimastigote stages for 30 days before they are considered negative.

Aspirates from chagomas and enlarged lymph nodes can be examined for amastigotes and trypomastigotes. Histologic examination of biopsy specimens also may be performed. Liver biopsy, bone marrow aspiration, or splenic puncture also can be tried to confirm the diagnosis. Examination of autopsy specimens from immunocompromised patients and patients who die of acute Chagas' disease usually reveals large numbers of amastigotes. Sometimes, xenodiagnosis may be the only means by which organisms can be found. In this technique, laboratory-reared reduviid bugs are permitted to feed on the patient or the patient's fresh blood; subsequently, the bugs are allowed to feed on uninfected guinea pigs. The guinea pigs are examined for trypomastigotes after approximately 45 days. Although 100 percent specific, this test is less than 50 to 80 percent sensitive. If organisms are present in the blood meal, the parasites multiply and are detected in the bug's intestinal contents, which should be examined monthly for flagellated forms over 3 months.

Serologic Tests

Serologic tests can be useful during various stages of the disease. The precipitin test may be positive during the acute episode, whereas complement fixation (Machado-Guerreiro test) is useful for the diagnosis of chronic disease. The most commonly used techniques for serologic diagnosis of chronic-phase Chagas disease include indirect hemagglutination, indirect immunofluorescence and enzyme-linked immunosorbent assay (ELISA). ELISA appears to provide favorable sensitivity and reproducibility.[91] Patients with leishmaniasis, malaria, collagen vascular disease, and syphilis may give false-positive reactions, however. The WHO recommends that at least two techniques be concomitantly positive to establish a diagnosis of Chagas disease.[102] Pregnant women at risk must be screened for Chagas disease, and a venous or umbilical cord blood specimen from the neonate should be evaluated for the parasite. Direct methods (i.e., microhaematocrit) and PCR are quite sensitive during the neonatal and early infancy time frame. Serologic assays based on two different techniques should be performed at least 8 months after birth.[31,39,61]

Polymerase Chain Reaction

Tests using recombinant methods that have the potential for high specificity and high sensitivity have been developed. During chronic infection, the number of circulating parasites is very low, but PCR-based assays can detect low numbers of organisms because the parasite has highly repetitive nuclear and kinetoplast DNA sequences that can be amplified by PCR.[69] During the chronic stage, serologic methods may be the only means to arrive at a diagnosis. The differential diagnosis of chronic chagasic cardiomyopathy includes rheumatic and arteriosclerotic heart disease and cardiomyopathies of other etiologies.

Immunoassays

Immunoassays have been used to detect antigens in urine and sera in patients with congenital infections and patients with chronic Chagas disease.[11] Determination of antigenuria can be valuable for early diagnosis of Chagas disease and for diagnosis of chronic cases in patients with conflicting serologic test results. A highly sensitive and specific chemiluminescent ELISA has been developed for blood bank screening and to monitor patients who are undergoing chemotherapy.[29] Information from screening U.S. blood donors for *T. cruzi* infection has identified autochthonous, chronic infections. This report adds 16 cases to the previously reported 7 cases; currently the estimated prevalence of autochthonous infections is 1/354,000 donors. It is hoped that ongoing studies will be better able to better assess the risk for infection.[23]

Treatment

Although numerous drugs have been tried, including agents used to treat African trypanosomiasis and leishmaniasis, only two medications–benznidazole and nifurtimox–have proved to be effective for therapy of Chagas disease.[56,94] In acute and congenital Chagas disease and infections caused by laboratory accidents, treatment should be administered as soon as possible, even though in some cases symptoms are self-limited. At

present, therapy for Chagas disease is recommended for patients with acute disease, children with congenital infection, and patients in the early phase of chronic disease (in particular, children younger than 15 years of age).[3,87,88] In addition, therapy should be prescribed for patients coinfected with HIV who experience Chagas reactivation. Whether therapy can modify the progression of chronic Chagas disease has not been conclusively shown. However, data published more recently suggest a beneficial effect of therapy in diminishing the risk for progression to severe chronic cardiomyopathy,[98] leading some experts to recommend therapy for adults aged 19 to 50 years with absence of severe heart disease.[9,10] A randomized trial in progress is expected to define more clearly the role of therapy on cardiac outcomes in chronic Chagas disease.[40,65]

Nifurtimox (Lampit), a nitrofurfurylidine derivative, is tolerated better in younger than older patients and should not be used during pregnancy. It reduces the duration and severity of illness and decreases mortality resulting from acute and congenital Chagas disease. Reversible gastrointestinal, cutaneous, and neurologic adverse effects are common. Treatment success varies from one country to another, possibly indicating differences in the susceptibility of strains of *T. cruzi*. There is no indication that treatment of patients with chronic Chagas disease is beneficial. Nifurtimox must be taken orally for prolonged periods, and severe side effects can occur, including abdominal pain, nausea, vomiting, anorexia, and neurologic symptoms. Nifurtimox is available in the United States from the Parasitic Drug Service of the Centers for Disease Control and Prevention. It is supplied in 30-mg and 120-mg tablets. The usual dose for adults is 8 to 10 mg/kg body weight per day. For adolescents (11 to 16 years of age), it is 12.5 to 15 mg/kg/day, and for children (1 to 10 years of age), the dose is 15 to 20 mg/kg/day. It is given in four divided doses daily for 90 to 120 days. This drug has many untoward reactions, including abdominal pain, nausea, vomiting, anorexia, restlessness, disorientation, insomnia, twitching, paresthesia, polyneuritis, and seizures. Skin reactions also may be observed.

Benznidazole (Rochagan) is similar to nifurtimox and seems to have similar efficacy. Some researchers consider it to be tolerated well, with fewer adverse reactions. It is used extensively in Brazil at 5 mg/kg/day for 30 to 120 days in two divided doses (adult dose). For children up to 12 years of age, the dose is 10 mg/kg/day in two doses for 30 to 90 days. Bone marrow depression and peripheral neuritis have limited its use, however. Additional chemotherapeutic agents are urgently needed and are under investigation.[93,77]

Interferon-γ has been used in conjunction with drug therapy because experimental data suggest that it is a useful adjunct in treatment by activating macrophage killing. Allopurinol and antifungal drugs have been suggested as alternatives in some patients. Patients with chronic chagasic cardiomyopathy may be helped for a varying period by implantation of a cardiac pacemaker, but the congestive heart failure is often refractory to cardiac glycosides and vasoactive drugs. More recently, inhibitors of angiotensin-converting enzymes have been advocated to reduce cardiac remodeling in these individuals. Heart transplantation also has been performed for the management of chronic cardiomyopathy. The use of high-fiber diets, occasional laxatives, and enemas may assist in the management of megacolon. When these measures fail, surgical resection may be required. Megaesophagus is usually amenable to therapy consisting of a combination of diet and dilation of the esophagogastric region. In more severe disease, various surgical procedures have been used with variable success to relieve the symptoms of achalasia.

Prevention

A strategy for elimination of Chagas disease, with four core components, was formulated by the WHO in 2007. The components include (1) vector control, (2) screening of blood donors in endemic countries, (3) detection and treatment of congenitally infected patients, and (4) treatment of all infected children and all patients with acute disease.[103] Regular insecticide spraying programs with benzene hexachloride could reduce transmission of the infection, at least around domiciles. Proper screening and improved housing for individuals in endemic areas also help reduce transmission. In endemic areas, thorough screening of blood donors attenuates the risk for transmission via transfusion. In 2010, the U.S. Department of Health and Human Services published guidelines for use of serologic tests to reduce the transmission of *T. cruzi* via blood donations.[96]

The development of a chemoprophylactic agent or vaccine holds the best hope for prevention and subsequent eradication of Chagas disease. Vaccine development is challenged by the recognition that host response to *T. cruzi* infection is characterized as inadequate.[26]

As previously noted, transfusion-acquired disease is a major public health problem in endemic areas. Treatment of blood with gentian violet inactivates the parasite.

Travelers to the Americas should be counseled regarding the transmission risks for Chagas disease, as well as the protective benefit provided by use of pyrethroid-impregnated insect nets when sleeping in unmortared cabins.[40,41]

AFRICAN TRYPANOSOMIASIS

African sleeping sickness is caused by two morphologically identical subspecies of hemoflagellate protozoans: *Trypanosoma brucei gambiense*, the cause of West African or Gambian sleeping sickness, and *Trypanosoma brucei rhodesiense*, the cause of East African or Rhodesian disease. Although these parasites produce similar disease, the Gambian form is usually chronic and evolves slowly, often over many years, and ends fatally if untreated, whereas the Rhodesian form is characterized by being acute, usually killing the host in a matter of weeks or months.[1] Recent findings have indicated the presence of human trypanotolerance in *T. b. gambiense* infections, now considered a major issue for future research.[55] These diseases exist wherever the various species of *Glossina*, the tsetse fly, are found. The completion of the *T. brucei* genome should facilitate greatly the discovery of new

drug targets and genetic markers.[17] The WHO has identified human African trypanosomiasis (HAT) as well as Chagas disease, leishmaniasis, and Buruli ulcer as neglected tropical diseases requiring innovative and intensified disease management.[104]

The Organism

T. b. gambiense and *T. b. rhodesiense* are pleomorphic flagellates 15 to 30 μm in length × 1.5 to 3.5 μm in breadth. In Giemsa-stained blood smears, they may appear long and slender with an undulating membrane and free anterior flagellum or appear short and broad without a free anterior flagellum. No intracellular forms exist. At various stages of disease, trypomastigote forms may be found in the peripheral blood, lymphatics, lymph nodes, CSF, and neural tissue. Although the two species are morphologically indistinguishable and share major biochemical features, isozyme and molecular analysis has shown substantial variation among species, particularly regarding clinical disease. In addition, the two species maintain separate biologic characteristics, especially with regard to virulence in cross-inoculation experiments. Humans are the only important reservoir host for *T. b. gambiense*, whereas *T. b. rhodesiense* naturally infects wild game animals.

The haploid genome size of *T. brucei* spp. is approximately 40 Mb, with 14 percent variation within the same subspecies and 29 percent variation among different subspecies. Homologous chromosomes, when probed by Southern blot, can differ in size by 20 percent. In addition to the large chromosome pairs, *T. brucei* has approximately 100 linear mini-chromosomes ranging in size from 50 to 150 kb. These mini-chromosomes contain transcriptionally silent copies of variant surface glycoprotein (VSG) genes. *T. brucei* has approximately 1000 genes capable of coding for *VSG* genes; these genes are switched at a rate of 10^{-2} to 10^{-6} switches per generation, which serves as the main mechanism of immune evasion for *T. brucei*.[43] Only one VSG expression site is active at any given time. Studies comparing homologous nuclear genes between *T. cruzi* and *T. brucei* have shown large evolutionary divergence in codon usage. Comparison of the nuclear small-subunit and large-subunit rRNA gene sequences yields genetic distances comparable to that between plants and animals.

Within the male and female insect vector, the tsetse fly, the blood meal settles in the posterior portion of the midgut and organisms multiply by binary fission for approximately 7 to 10 days. The slender trypomastigotes migrate anteriorly to the foregut, where they remain for the next 2 to 3 weeks. They next move further forward and finally enter the salivary glands, in which they transform into epimastigote forms—forms in which the kinetoplast has migrated just anterior to the nucleus—and continue to replicate. After multiplication cycles, they transform into infective metacyclic trypomastigote forms, which are the small, broad or "stumpy" forms that lack a free anterior flagellum. When next feeding, the infective tsetse fly may inoculate thousands of these infective trypomastigotes into the bite wound. The entire life cycle of the tsetse fly spans 15 to 35 days. A single tsetse fly can be infected with multiple species of trypanosomes and remains infected for life. Within the human host, trypomastigotes multiply by binary fission in blood, lymph, and extracellular spaces. The CNS eventually is invaded, at which time multiplication continues unabated. Transmission by blood transfusion, hypodermic needle, other insects, and congenital transmission has been reported.

Epidemiology

African sleeping sickness (*T. b. gambiense* and *T. b. rhodesiense*) and veterinary trypanosomiasis caused by *T. brucei* subgroup parasites are responsible for much human suffering and economic loss. Factors that have historically contributed to disease resurgence include socioeconomic unrest resulting in population shifts of animal reservoirs, insufficient financial allocations, emergence of drug-resistant parasitic strains, climatologic alterations, and altered susceptibility of populations at risk.[55] Substantial efforts by the WHO in collaboration with national programs and public-private partnerships, as well as with contributions from nongovernmental organizations in the 21st century have resulted in diminishing the number of new cases.[105] Because the larval stages of the tsetse fly are vulnerable to desiccation, *Trypanosoma* spp. are restricted to an area spanning 10 million km^2 south of the Sahara, encompassing 200 separate foci from 36 countries, where the annual rainfall exceeds 500 mm (20 inches). The most recent reports indicate that, in 2009 and 2010, fewer than 10,000 new reported cases were identified; currently, the estimated number of new cases is approximately just under 10,000/year.[106] *T.b. gambiense* is endemic in 24 nations of west and central Africa and is the reported cause of HAT in more than 90 percent of cases; *T. b. rhodesiense* is endemic in 13 nations in eastern and southern Africa. The recent observation that Uganda harbors both forms of HAT is of great concern because control of HAT requires different strategies for the two parasites; emergence of geographic overlap would add greatly to costs.[47,74,106] The Gambian form occurs mainly in the western portion of tropical Africa, with focal incursions eastward north of Lake Victoria into the Sudan. *Glossina palpalis* is the main tsetse fly vector, although other related species, such as *Glossina tachinoides* and *Glossina fuscipes*, are implicated as well.

Although *T. b. gambiense* can infect various mammals, humans seem to be more susceptible and maintain a high enough parasitemia to sustain the fly-human-fly cycle. The prolonged chronicity of Gambian disease with infectious individuals continually exposed to tsetse flies undoubtedly helps sustain the disease. Asymptomatic carriers of the infection also may be an important factor in maintaining the disease in a community. Among the important factors that may limit Gambian infection to humans is the transient and low parasitemia that results from an infective tsetse fly bite in mammals, usually ungulates. Researchers have observed that Gambian disease typically is sustained only when a close and

repeated relationship exists between humans and tsetse flies. The practical result of all this is that West African sleeping sickness is maintained by members of the *G. palpalis* group of tsetse flies that use or prefer human blood almost exclusively.

In contrast to Gambian disease, *T. b. rhodesiense* infection generally is maintained in wild mammals. The fly-human-fly cycle tends to be unimportant inasmuch as the severe nature of the disease usually quickly removes acutely ill humans as an infective source. The intervention of wild game animals, in which the disease tends to be less acute and whose blood seems to be more attractive, appears to have relegated humans to an occasional or facultative host for *T. b. rhodesiense*. The *Glossina morsitans* group of tsetse flies that inhabit the dry East African savannah readily feed on wild ungulates, especially the bushbuck, *Tragelaphus scriptus*, and the hartebeest, *Alcelaphus buselaphus*. Occasionally, Rhodesian disease may reach epidemic proportions, and during these epidemics, direct human-fly-human cycles may occur.

In endemic countries there tend to be regions where both infected and parasite-free tsetse flies are found, A disease focus can be as limited as a single village or can encompass an entire region, and epidemics tend to be followed by extended periods of endemicity. It is estimated that only 10 percent of new cases are identified or treated. In some endemic areas, HAT is the leading cause of death, even when considering the mortality rate caused by HIV and AIDS.

All age groups are susceptible to infection. The factors that influence human prevalence rest more on occupational exposure to suitable tsetse flies and the flies' breeding and feeding habits. Young men are found to be infected most frequently. During epidemics, all groups are infected, however, and mechanical transmission probably occurs. Tourists on safari are possibly at risk. In that regard, reports of African trypanosomiasis in Europe, the United States, and Australia have been increasing, although fewer than 50 cases/year of human African trypanosomiasis are diagnosed outside of Africa.[95]

Pathogenesis and Pathology

The metacyclic infective trypomastigote forms are inoculated by the tsetse fly into the skin, where they multiply at the inoculation site. A characteristic, hard, sometimes painful chancre develops. By approximately the 10th day, long slender forms are found in the bloodstream and lymphatics, and for the next several days, their numbers increase logarithmically. Soon thereafter, the organisms nearly disappear from the bloodstream as a result of immune lysis, only to reappear again.

The interval between waves of parasitemia may vary from 1 to 8 days, with clinical symptoms accompanying each bout of parasitemia. These parasitemic waves can be accounted for by the highly developed antigenic variation strategy of the parasite. The trypomastigote is covered with viral coat protein VSG, and with each peak of parasitemia, a predominant variable antigen type is displayed by the organism. The specific antibody response to VSG leads to the destruction of parasites that display the predominant variable antigen type or homotype. Numerous heterotypes are found within each population of parasites, one of which, not recognized by the host's immune system, becomes the next homotype. The parasite in each successive wave of parasitemia bears a different variable antigen type. A single trypomastigote may contain 1000 genes, each encoding for a specific VSG. Each successive parasitemic wave represents a new antigenic variant that has emerged to elude the host's antibody response to the previous antigen.

As a result of successive waves of immune lysis and parasitemia, a marked early humoral antibody response, predominantly involving IgM, is seen regularly. *In an immunocompetent host, the absence of elevated IgM levels in serum rules out trypanosomiasis.* These macroglobulins contain not only antitrypanosomal antibodies, which are directed against the surface antigens, but also a variety of other antibodies such as heterophile and rheumatoid factor. Researchers have shown experimentally that because of polyclonal B-cell activation, many antibodies are produced to a wide variety of antigens, including brain-specific autoantibodies directed against myelin basic protein, gangliosides, and cerebrosides. Circulating immune complexes have been reported regularly and may be responsible for the glomerulonephritis, hypocomplementemia, and hemolytic anemia that often accompany acute and chronic disease. Cell-mediated immunity also is important in African trypanosomiasis, and increased production of nitric oxide may be important in depression of T-cell responsiveness and generalized immunodepression.

The main pathologic lesions involve the posterior cervical, submaxillary, supraclavicular, and mesenteric lymph nodes and the CNS. The lymphatic tissue usually reveals generalized hyperplasia with diffuse proliferation of lymphocytes. Later, the nodes may become small and fibrotic; however, they initially are markedly hemorrhagic and contain large numbers of trypomastigotes. The CNS remains normal until invaded by organisms, but then a progressive chronic leptomeningitis develops. The brain becomes edematous, and prominent perivascular cuffing by glial cells, lymphocytes, and plasma cells is present. When the latter become vacuolated with pyknotic nuclei, they often are referred to as the morula cells of Mott and Marshalko. Organisms often can be found in brain tissue in proximity to vessels and may be detected in CSF. Glomerulonephritis, myocarditis, pericardial effusion, pulmonary edema, and hypoplastic bone marrow with associated anemia may be seen.

The pathogenesis of the neuropsychiatric manifestations is not understood. More recent experimental studies suggest that changes in levels of brain neurotransmitters; deposition of immune complexes; and alteration in production of prostaglandin, cytokine, and nitric oxide may account partly for these behavioral changes.[2,85]

Clinical Manifestations

The clinical manifestations of *T. b. gambiense* and *T. b. rhodesiense* disease are similar except that Rhodesian infection is a more fulminant, acute disease that may run

its course in several weeks to 6 to 9 months, whereas Gambian infection may last for years. Typically, the incubation period in Rhodesian infection is brief (3 to 21 days), whereas the onset of symptoms with Gambian infection may be delayed for several weeks or years. Approximately 1 week after infection and at the site of the tsetse fly bite, a hard and painful chancre sometimes appears and lasts several weeks. During the early stages, when recurrent bouts of fever may be the only symptom, the blood and lymphatics primarily are involved, and infection often is mistaken for malaria, especially if a chancre is not obvious or a history of exposure to tsetse flies is not obtained. The period of intermittent fever may last months to years with Gambian infection. During this time, persistent headache and tachycardia often are encountered, and a circinate erythematous rash or erythema multiforme sometimes is noted. The fever abates gradually.

In many patients, characteristic posterior cervical lymphadenopathy becomes evident (Winterbottom's sign). The nodes are nontender and attain a diameter of approximately 1 cm. They tend to become small and fibrotic in approximately 6 months.

Signs of CNS invasion often develop in untreated patients with Gambian disease. The initial signs and symptoms of neurologic involvement can be difficult to assess. They may consist of alterations in behavior or personality (or both) that eventually may be manifested as a severe psychosis. Severe headache, loss of nocturnal sleep, and a feeling of impending doom typically are described. Next, progressive mental deterioration may occur, and with unrelenting deterioration, patients become incapable of caring for themselves. Tremors, especially of the tongue, hands, or feet, and generalized or focal convulsive episodes may occur. Almost any neurologic or psychiatric manifestation can be seen, and with progressive mental deterioration, patients finally lapse into a coma and die.

During the final period of the disease, patients often die of concurrent infections such as bacterial pneumonia, amebiasis, and malaria. Wasting and malnutrition are a large component of the progressive deterioration in these patients. Sleeping sickness can be a difficult disease to diagnose early in young children. It usually is found only after a child is evaluated for obtundation, seizures, or psychomotor retardation.[21,67]

T. b. rhodesiense produces a more rapid, fulminating disease than *T. b. gambiense*. Fever, severe headaches, irritability, extreme fatigue, swollen lymph nodes, and aching muscles and joints are common symptoms. Progressive confusion, personality changes, slurred speech, seizures, and difficulty walking and talking occur as the organisms invade the CNS. The early stages of the pathologic process parallel those of *T. b. gambiense* infections; however, the disease progresses more rapidly, such that death may occur before there is extensive CNS involvement, even though CNS invasion occurs early. The incubation period is short, often within 1 to 4 weeks, with trypomastigotes being more numerous and appearing earlier in the blood. Lymph node involvement is less pronounced, and Winterbottom's sign may be absent. Febrile paroxysms are more frequent, and the patients are more anemic and more likely to develop myocarditis or jaundice. Some patients may develop persistent tachycardia, and death may result from arrhythmia and congestive heart failure owing to pancarditis.

Second Stage

The clinical symptoms and signs of patients with second-stage human African trypanosomiasis are described for a large cohort ($N = 2541$) of patients treated in a prospective multicenter, multinational study.[15] Special emphasis was given to the influence of disease stage (i.e., duration and number of white blood cells in CSF) and patient age on the clinical picture. Although the frequencies of symptoms and signs varied greatly among centers, the clinical picture of the disease was similar for all countries. Headache (78.7%), sleeping disorder (74.4%), and lymphadenopathy (56.1%) were the most frequent symptoms and signs, and they were similar for all stages of the disease. Lymphadenopathy tended to be highest in the advanced second stage (59%). Neurologic and psychiatric symptoms increased significantly, with the number of white blood cells in the CSF indicating the stage of progression of the disease. Pruritus was observed in all stages and increased with the number of white blood cells in CSF from 30 to 55 percent. In children younger than 7 years of age, lymphadenopathy was reported less frequently (11.8% to 37.3%) than in older children or adults (56.4% to 61.2%). Fever was reported most frequently in children 2 to 14 years of age (26.1% to 28.7%), and malnutrition was observed significantly more frequently in children of all ages (43% to 56%) than in adults (23.5%).

Diagnosis

Diagnostic options for human African trypanosomiasis are limited in field settings.[30] Even in field settings, however, positive results from screening assays, such as the card agglutination test, must be confirmed by demonstration of the parasite. Absence of confirmatory testing may lead to unnecessary administration of toxic drugs.[53] It is clear that substantial progress is needed to greatly improve diagnostics for point-of-care use.[66]

Routine Methods

As indicated earlier, a definitive diagnosis can be made only by finding trypanosomes in blood and bone marrow smears, in lymph node aspirates in early or acute disease, and in CSF in late or chronic disease.[100] For examination of thick and thin smears and the buffy coat, 10 to 20 mL of citrated whole blood or the sediment from 5 mL of centrifuged CSF is essential. Numerous microscopic techniques, including capillary tube centrifugation, quantitative buffy coat examination, miniature anion-exchange centrifugation, density gradient centrifugation, and acridine orange staining, have been used to enhance parasite detection. Culture or animal inoculation, or both, is sometimes the only successful means of making a diagnosis. In advanced CNS disease, lymphocytosis and elevated IgM are detected in the CSF.

Serologic Tests

A serologic diagnosis of African trypanosomiasis usually depends on detection of high levels of nonspecific serum IgM. Detection of specific antibody by indirect fluorescent antibody is helpful, especially when the serum is tested with trypanosomes of the homologous human species. Tests to determine antibody levels include the card agglutination trypanosomiasis test, rapid latex agglutination test, indirect immunofluorescence test, and ELISA. Direct agglutination and a microscale version of ELISA are highly reliable methods for making a specific diagnosis of trypanosomiasis.[4]

Polymerase Chain Reaction

Antigen-detection and PCR tests have been developed to detect circulating parasites.[34] The development of a peptide nucleic acid fluorescence in situ hybridization probe seems to offer an excellent diagnostic tool. By combining this test with a cytospin step, the limit of detection from blood can be improved to 5 parasites/mL, a level of sensitivity that equals an optimal PCR detection limit on blood specimens. A newer PCR technique targets the gene encoding the small ribosomal subunit to identify and differentiate all clinically important African trypanosome species and some subspecies. This method seems to be more economical, simple, and sensitive than some other screening methods and yields more detailed information.[34]

Immunoglobulin Levels

In advanced, untreated sleeping sickness, the IgM level in CSF often is elevated, but it has no relationship to the presence of trypanosomes in CSF. After successful treatment, the IgM level declines gradually and disappears after approximately 1 year. However, if 1 year after therapy a constant high level is present, or an abrupt increase in IgM occurs, relapse should be suspected. Nonetheless, the IgM level should not be used as the sole method to arrive at a diagnosis or prognosis.

The differential diagnosis of African trypanosomiasis includes many diseases, but chronic relapsing fever associated with enlarged cervical lymph nodes in an individual who has been to Africa is suggestive. As indicated earlier, considering diseases such as malaria, syphilis, lymphoma, visceral leishmaniasis, and leprosy is not unusual. Later, encephalitis of other etiologies must be excluded. An important diagnostic feature is the frequent finding of a strikingly elevated erythrocyte sedimentation rate, which is a reflection of the markedly increased serum macroglobulins.

Molecular Dipstick

The development and the first-phase evaluation of a simple and rapid test (human African trypanosomiasis–PCR–oligochromatography) for detection of amplified *T. brucei* DNA has been reported more recently.[38] Through hybridization with a gold-conjugated probe (oligochromatography), PCR products are visualized on a dipstick. Visualization is simple and takes only 5 minutes. Controls for the PCR and for DNA migration are incorporated into the assay. The lower detection limit of the test is 5 fg of pure *T. brucei* DNA. One parasite in 180 μL of blood is still detectable. Sensitivity and specificity for *T. brucei* were calculated at 100 percent when tested on blood samples from 26 patients with confirmed sleeping sickness, 18 negative controls (nonendemic region), and 50 negative control blood samples from an endemic region. Human African trypanosomiasis–PCR–oligochromatography is a promising new tool for diagnosis of sleeping sickness in laboratory settings.

Animal Inoculation and Culture

T. b. gambiense isolation in small laboratory animals is usually unsuccessful; in contrast, *T. b. rhodesiense* readily infects animals. Cultivation is not practical for most diagnostic laboratories, but is more successful than animal inoculation.[50]

Prognosis

If therapy is initiated before significant CSF involvement, the outcome is usually favorable. Untreated infection often ends fatally.[54] Patients with severe brain inflammation at time of diagnosis of second-stage disease are at substantial risk for treatment failure. Assessment of pretreatment as well as posttreatment CSF white blood cell counts, IgM titers, and protein concentrations may allow accurate prediction of treatment failure.[62]

Treatment

All drugs currently used in the treatment of African trypanosomiasis are toxic and require prolonged administration. Treatment should be started as soon as possible and is based on the patient's symptoms and laboratory findings. The choice of antiparasitic drug depends on whether the CNS is infected.[56] Pentamidine isethionate or suramin can be used when the CNS is not infected. The main chemotherapeutic agents for human trypanosomiasis are pentamidine and suramin for early-stage disease. Treatment options have expanded for second-stage HAT. Melarsoprol has been used since 1949. Eflornithine, which is effective only against *T. b. gambiense*, was accepted for second-stage therapy in 1990. The combination of nifurtimox and eflornithine was introduced in 2009 after clinical documentation of noninferiority to eflornithine monotherapy with identified safety and ease of administration advantages. This therapeutic combination is now accepted for treatment of *T. b. gambiense* and is included in the WHO List of Essential Medications.[107]

Pentamidine Isethionate

Pentamidine is a water-soluble aromatic diamidine that has been in use for nearly 60 years. It is administered as either the isethionate form (Pentam 300) or methanesulfonate (Lomidine). The dose is 4 mg/kg/day for 10 days

for adults and children. Pentamidine is effective against early-stage *T. b. gambiense* infection, but less effective against *T. b. rhodesiense* infection and ineffective against late-stage disease. African trypanosomes have a nucleoside (adenine/adenosine: P_2) transporter that takes up pentamidine, which results in concentrations of the agent many times that in plasma. Although laboratory-induced resistant strains seem to have reduced transport properties, naturally derived clinical isolates seem to have retained transport, indicating that most resistance may be caused by alteration of a metabolic target, rather than drug uptake. Pentamidine isethionate does not cross the blood-brain barrier and is administered intramuscularly; side effects include an immediate hypotensive reaction, nausea, vomiting, and Herxheimer-type (inflammatory) reactions.

Suramin

Suramin is a sulfonated naphthylamine that has been used successfully against early-stage sleeping sickness caused chiefly by *T. b. rhodesiense*. It was first used in 1922 and was developed from the closely related azo dyes trypan red and trypan blue. Suramin is administered intravenously. The adult dose is 100 to 200 mg (test dose), then 1 g on days 1, 3, 7, 14, and 21. The pediatric dose is 20 mg/kg on days 1, 3, 7, 14, and 21. Suramin has an extremely long half-life in humans (44 to 54 days), which is the result of avid binding to serum proteins. Suramin binds to many plasma proteins, including low-density lipoprotein, which trypanosomes avidly bind and endocytose as a result of specific membrane receptors. Low-density lipoprotein is a prime source of sterols for bloodstream trypanosomes. Suramin is taken up as a protein complex and has been shown to inhibit all the glycolytic enzymes in *T. b. brucei* in low concentrations, which in most cases are severalfold lower than for the corresponding mammalian enzyme. Suramin also has been found to affect thymidine kinase and dihydrofolate reductase.

Suramin (Bayer 205), a diamidine, also is effective in treating the hemolymphatic stage and CNS disease of *T. b. gambiense*. When suramin is used, a test dose should be given to the patient to ensure the drug can be tolerated. It should not be given to patients with renal disease, and if protein, red blood cells, or casts are detected in the urine, treatment should be stopped. Suramin is given intravenously; its side effects include nausea, vomiting, loss of consciousness, seizures, pruritus, edema, and hepatitis. Although rare, fatalities have been reported during suramin therapy. Suramin can be obtained under an investigational new drug protocol from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, Georgia, 30333; 404-639-3670 (evenings, weekends, or holidays: 404-639-2888).

Melarsoprol

Melarsoprol (mel B; Arsobal) is an arsenical created by the efforts of Freidheim in the late 1940s. His initial compound, melarsen oxide, *p*-(4,6-diamino-*s*-triazinyl-2-yl) aminophenyl arsenoxide, was complexed with dimercaptopropanol (British antilewisite) to form a less toxic complex, melarsoprol. Until 1990, it was the only agent available for curing late-stage (CNS) disease of East African and West African origin. Usually, each series is followed by a week interval before the next series. Melarsoprol is insoluble in water and must be dissolved in propylene glycol. It must be given intravenously; otherwise, it induces a severe localized inflammatory reaction. This drug is administered intravenously. The adult dosing schedule is 3.6 mg/kg at 3 days. This is followed in 1 week with 3.6 mg/kg/day for 3 days. This schedule is repeated after 10 to 21 days. The pediatric dose is 18 to 25 mg/kg total over 1 month; the initial dose is 0.36 mg/kg at intervals of 1 to 5 days for a total of 9 to 10 doses.

Side effects include nausea, vomiting, encephalopathy, and exfoliative dermatitis. Melarsoprol has been used for CNS involvement for years; however, relapse occurs in 6 percent of patients. Arsenic-related encephalopathy occurs in 10 percent of patients, and prednisolone treatment may help reduce the incidence. The mortality resulting from this side effect depends on whether supportive treatment is given, but it is often greater than 50 percent. Resistance to melarsoprol by *T. b. gambiense* and *T. b. rhodesiense* has been noted.

Reactive arsenical-induced encephalopathy is an important toxicity of melarsoprol and is followed by pulmonary edema and death within 48 hours in more than half the cases. The incidence of reactive arsenical-induced encephalopathy has been estimated at 2 to 10 percent. In one clinical study, the coadministration of steroids significantly reduced the incidence of reactive arsenical-induced encephalopathy–related deaths during therapy with melarsoprol. Although the mechanism of action of melarsoprol has been studied extensively, it still remains unclear.[1] Clinical signs and symptoms of African trypanosomiasis must be differentiated from those of reactive arsenical encephalopathy, a complication of treatment with melarsoprol.

Eflornithine

Eflornithine (DL-a-difluoromethylornithine [DFMO]) is an enzyme-activated inhibitor of ornithine decarboxylase, the lead enzyme of polyamine biosynthesis. DFMO was developed as an antitumor agent. After initial testing in model infections, DFMO was studied extensively in human trials in Africa, with some trials in Europe and the United States. The standard treatment regimens resulting from trials indicate that DFMO is more than 95 percent active when given intravenously at 400 mg/kg/day in four doses every 6 hours for 14 days. In these studies, DFMO cured children and adults, patients with melarsoprol-refractory strains, and patients with late-stage disease.[44] The short plasma half-life of DFMO necessitates constant dosing when given as an intravenous drip. The most frequent toxic reaction was reversible bone marrow suppression, which was reversed by reducing the dosage.

The major drawbacks of DFMO are its cost and the duration of treatment and availability. DFMO is not trypanocidal and depends on a functional immune system to rid the host of nondividing forms. It is curative for laboratory infections of *T. b. brucei* and *T. b. gambiense*, but not all strains of *T. b. rhodesiense*. The reason for this selectivity is not evident, although it is not caused by uptake of DFMO because the drug enters by passive diffusion, not transport. DFMO treatment leads to intracellular concentrations of approximately 5 mmol/L, an increase of approximately 50-fold over untreated parasites. The basis for DFMO selectivity lies in the rapid turnover of the mammalian enzyme as opposed to trypanosome ornithine decarboxylase. Because mammalian ornithine decarboxylase is synthesized constantly at a high rate, DFMO must be continuously available, and this availability is made more difficult by rapid excretion.

DFMO has been used for melarsoprol-resistant *T. b. gambiense* infection with or without CNS involvement; treatment is started intravenously and is followed by oral therapy. In one study, 47 patients with a relapse after a first treatment were treated with a 7-day course of intravenous eflornithine 100 mg/kg every 6 hours and monitored for 2 years; the failure rate was 6.5 percent. This approach seems to provide adequate treatment in cases of Gambian trypanosomiasis relapsing after treatment with another drug. With adequate treatment, non-CNS disease has a cure rate greater than 80 percent. As previously noted, combination therapy with eflornithine and nifurtimox is a significant advance for therapy of *T. b. gambiense*.

Newer Therapeutic Agents

Available treatment regimens are limited, are associated with serious adverse effects, and are potentially compromised by the emergence of drug-resistant parasites. These considerations underscore the urgent need to develop inexpensive, safe, and efficacious new drugs.[90] It is hoped that efforts led by the Tropical Disease Research/World Health Organization Committee on Genomics and Discovery Research and the Drugs for Neglected Diseases Initiative will encourage further pharmaceutical efforts.[17]

Prophylaxis and Prevention

Chemoprophylaxis with suramin 0.3 to 0.7 g intravenously every 2 to 3 months is reported to be highly effective. With few exceptions, the vector has proved to be difficult to control. Preventing tsetse fly bites and chemoprophylaxis may be the best means of eliminating the disease from an area. Development of a vaccine has been hindered by antigenic variation.

At present, there are no endemic areas where *T. b. gambiense* and *T. b. rhodesiense* coexist. Although foci of both exist in Uganda, they apparently are geographically separate. Based on evidence of the spread of cattle carrying *T. b. rhodesiense* further north, a merger of the two foci could create tremendous problems because the diagnosis and treatment of the two diseases differ significantly.

Vector control measures have met with limited success. Methods have included clearing streams of underbrush, eliminating breeding grounds, using insecticides, using fly traps, and releasing sterile male flies. Because tsetse flies have a slow reproductive rate and have not developed insecticide resistance, *Glossina* spp. are tempting control targets. The most effective control measures include an integrated approach to reduce the human reservoir of infection and to use insecticides and fly traps.

In regions where the disease is endemic, natives seem to be more resistant to infection than new arrivals to the area, although there is no evidence of acquired immunity. West African sleeping sickness affects primarily rural populations, and tourists are rarely infected. Chemoprophylaxis is not recommended because of drug toxicity, and vaccines are unavailable. Despite antigenic variation, it may be possible to develop a vaccine because immunity to reinfection occurs with *T. b. gambiense*.

Tourists are usually not at great risk unless they are spending long periods in rural areas of western and central Africa.[86] Individuals visiting areas where the disease is endemic should avoid tsetse fly bites by wearing protective clothing (i.e., long-sleeved shirts and long pants); khaki or olive-colored clothing is optimal because the tsetse fly is attracted to bright colors and very dark colors. Because heavy clothing is not always practical owing to heat and humidity, other measures, including the use of insect repellents, bed netting, and screens, are recommended. It also has been recommended not to ride in the back of jeeps, pickup trucks, or other open vehicles; the tsetse fly is attracted to the dust created by moving vehicles and wild animals. Bushes should be avoided because the tsetse fly is less active during the hot period of the day and rests in bushes, but bites if disturbed.

NEW REFERENCES SINCE THE SIXTH EDITION

6. Beard CB, Pye G, Steurer FJ, et al. Chagas disease in a domestic transmission cycle in southern Texas, U.S.A. *Emerg Infect Dis* 2003;**9**:103–5.
8. Bern C. Antitrypanosomal therapy for chronic Chagas disease. *N Engl J Med* 2011;**364**:2527–33.
9. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis* 2009;**49**:e52–4.
10. Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of Chagas disease in the U.S.: a systematic review. *JAMA* 2007;**298**:2171–81.
16. Brisse S, Verhoef J, Tibayrenc M. Characterization of large and small subunit rRNA and mini-exon genes further supports the distinction of six Trypanosoma cruzi lineages. *Int J Parasitol* 2001;**31**:1218–26.
18. Brutus L, Schneider D, Portigo J, et al. Congenital Chagas disease: diagnostic and clinical aspects in an area without vectorial transmission, Bernejo, Bolivia. *Acta Trop* 2008;**106**:195–9.
19. Buekens P, Almandares O, Carlier Y, et al. Mother-to-child transmission of Chagas disease in North America: why don't we do more? *Maternal Child Health J* 2008;**12**:283–6.
20. Burgos JM, Begher S, Silva HM, et al. Molecular identification of *T. cruzi* 1 tropism for central nervous system in Chagas reactivation due to AIDS. *Am J Trop Med Hyg* 2008;**78**:294–7.
22. Campos SV, Strabilli TM, Amato Neto V, et al. Risk factors for Chagas disease reactivation after heart transplantation. *J Heart Lung Transplant* 2008;**27**:597–602.

23. Cantey P, Stramer SL, Townsend RL et al. The United States Trypanosoma cruzi infection study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. *Transfusion* 2012;**52**:1922–30.
24. Carlier Y, Torrico F. Congenital infection with *T. cruzi:* from mechanism of transmission to strategies for diagnosis and control. *Rev Soc Brasil Med* 2003;**36**:767–71.
25. Carod-Artal FJ. Stroke: a neglected complication of American trypanosomiasis. *Trans R Soc Trop Med Hy* 2007;**101**:1070–80.
26. Cazorla SI, Frank FM, Malchiodi EL. vaccination approaches against *T. cruzi* infection. *Expert Rev Vaccine* 2009;**8**:921–35.
28. Centers for Disease control and Prevention. Congenital transmission of Chagas disease-Virginia, 2010. *MMWR Morbid Mortal Wkly Rep* 2012;**61**:477–9.
31. Chippaux JP, Chavizo AN, Santilla JA, et al. Antibody drop in newborns congenitally infected by T. cruzi treated with benznidazole. *Trop Med Int Health* 2010;**15**:87–93.
32. Cordova E, Boschi A, Ambrosoni J, et al. Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992-2007. *Int J Infect Dis* 2008;**12**:587–92.
35. Cruz-Robles D, Chavez-Gonzalez JP, Cavazos-Guero MM, et al. Association between IL-1B and IL-1 RN gene polymorphisms and Chagas disease development susceptibility. *Immunol Invest* 2009;**38**:231–9.
41. Diaz JH. Recognizing and reducing the risks of Chagas disease in travelers. *J Travel Med* 2008;**15**:184–95.
42. Dietra WO, Gollob KJ. Current concepts in immunoregulation and pathology of Chagas disease. *Curr Opin Infect Dis* 2008;**21**: 287–92.
54. Janonneau V, Ilboudo H, Kabore J et al. Untreated human infections by Trypanosoma brucei gambiense are not 100% fatal. *PLos Negl Trop Dis* 2012;**6**:e1691.
59. Kun H, Moore A, Mascola L, et al. Transmission of *T. cruzi* by heart transplantation. *Clin Infect Dis* 2009;**48**:1534–40.
62. Lejon V, Roger I, Ngoyi D, et al. Novel markers for treatment outcome in late-stage *T. brucei gambiense* trypanosomiasis. *Clin Infect Dis* 2008;**47**:15–22.
63. Lescure F-X, Leloup G, Freilij H, et al. Chagas disease: changes in knowledge and management. *Lancet Infect Dis* 2010;**10**: 556–70.
65. Marin-Neto JA, Rossi A, Morillo CA, et al. Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the Benznidazole Evaluation for Interrupting Trypanosomiasis (BENEFIT). *Am Heart J* 2008;**156**:37–43.
66. Matovu E, Kazibwe AJ, Mugasa CM, et al. Towards point-of-care diagnostic and staging tools for human African trypanaosomiasis. *J Trop Med* 2012;Article ID 340538.
68. Moncayo A, Silveira AC,. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. *Mem Inst Oswaldo Cruz* 2009;**104**(suppl 1):17–30.
72. Pereira KS, Schmidt FL, Guaraldo AM, et al. Chagas disease as a foodborne illness. *J Food Prot* 2009;**72**:441–6.
77. Ribeiro I Sevcsik A-M, Alver F, et al. New, improved treatments for Chagas disease: from the Rand D pipeline to the patients. *PLoS Negl Trop Dis* 2009;**3**:e484.
78. Rochitte CE, Oliveira PF, Andrade JM, et al. Myocardial delayed enhancement by magnetic resonance imaging in patients with Chagas disease. *J Am Coll Cardiol* 2005;**46**:1553–8.
79. Salas NA, Cot M, Schneider D, et al. Risk factors and consequences of congenital Chagas disease in Yacuiba, south Bolivia. *Trop Med Int Health* 2007;**12**:1498–505.
84. Schmunis GA, Cruz JR. Safety of the blood supply in Latin America. *Clin Microbiol Rev* 2005;**18**:22–9.
83. Schmunis GA. Epidemiology of Chagas disease in non-endemic countries: the role of international migration. *Mem Inst Oswaldo Cruz* 2007;**102**(suppl 1):75–85.
87. Sosa-Estani S, Seguera EL. Etiologic treatment in patients infected by *T. cruzi:* experiences in Argentina. *Curr Opin Infect Dis* 2006;**19**:583–7.
91. Tobler LH, Contestable P, Pitina L, et al. Evaluation of a new ELISA for detection of Chagas antibody in U.S. blood donors. *Transfusion* 2007;**47**:90–6.
92. Torrico F, Vega CA, Suarez E, et al. Are maternal reinfections with *T. cruzi* associated with higher morbidity and mortality of congenital Chagas disease? *Trop Med Int health* 2006;**11**: 628–35.
95. Urech K, Neunayn A, Blum J. Sleeping sickness in travelers: do they really sleep? *PLoS Negl Trop Dis* 2011;**5**:e1358.
96. U.S. Food and Drug Administration. *Guidelines for industry use of serological tests to reduce the risk of transmission of* T. cruzi *infection in whole blood and blood components intended for transfusion*. December, 2010. http://www.fda.gov/Biologics/BloodVaccines/GuidanceRegulatingInfromation/Guidances/default.htm (accessed June 5, 2012).
102. World Health Organization. *Chagas disease report*, 2007. http://www.who.int/mediacentre/news/releases/2007/pr36/en/index.html (accessed June 5, 2012).
104. World Health Organization. *WHO consultation on international biological reference preparation for Chagas diagnostic tests*. 2007. http://www.who.int/bloodproducts/ref_materials/WHO_Report_1st_Chagas_BPP_consultation_7-2007_final.pdf (accessed June 8, 2012).
105. World Health Organization. *Neglected tropical diseases: innovative and intensified disease management (IDM)*. 2010. http://www.who.int/neglected_diseases/disease_management/en. (accessed June 13, 2012).
106. World Health Organization. *Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases*. 2010. http://www.who.int/neglected_diseases/2010report/en/ (accessed June 13, 2012).
107. World Health Organization. *Human African trypanosomiasis*. 2012. http://www.who.int/mediacentre/factsheets/fs259/en/ (accessed June 13, 2012).
108. Yun O, Priotto G, Tong J, et al. NECT is next implementing the new drug combination therapy for *T. gambiense* sleeping sickness. *PLoS Negl Trop Dis* 2010;**4**:e720.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adams JH, Haller L, Boa FY, et al. Human African trypanosomiasis (*T. b. gambiense*): a study of 16 fatal cases of sleeping sickness with some observations on acute reactive encephalopathy. *Neuropathol Appl Neurobiol* 1986;**12**:81–94.
2. Amole B, Sharpless N, Wittner M, et al. Neurochemical measurements in the brains of mice infected with *Trypanosoma brucei brucei* (TREU 667). *Ann Trop Med Parasitol* 1989;**83**:225–32.
3. Andrade AL Martelli CMT, Oliveira RM, et al. Short report: benznidazole efficacy among *Trypanosoma cruzi*-infected adolescents after a 6-year follow-up. *Am J Trop Med Hyg* 2004;**71**: 594–7.
4. Asonganyi T, Doua F, Kibona SN, et al. A multicenter evaluation of the indirect agglutination test for trypanosomiasis (TrypTect CIATT). *Ann Trop Med Parasitol* 1998;**92**:837–44.
5. Azogue E, LaFuente C, Darras C. Congenital Chagas' disease in Bolivia: epidemiological aspects and pathological findings. *Trans R Soc Trop Med Hyg* 1985;**79**:176–80.
6. Beard CB, Pye G, Steurer FJ, et al. Chagas disease in a domestic transmission cycle in southern Texas, U.S.A. *Emerg Infect Dis* 2003;**9**:103–5.
7. Benson CA, Kaplan JE, Masur H, et al. Treating opportunistic infection among HIV-infected adults and adolescents: recommendations from CDC, NIH, HIV Medicine Association/IDSA. *Clin Infect Dis* 2005;**40**:S131–235.
8. Bern C. Antitrypanosomal therapy for chronic Chagas disease. *N Engl J Med* 2011;**364**:2527–33.
9. Bern C, Montgomery SP. An estimate of the burden of Chagas disease in the United States. *Clin Infect Dis* 2009;**49**:e52–4.
10. Bern C, Montgomery SP, Herwaldt BL, et al. Evaluation and treatment of Chagas disease in the U.S.: a systematic review. *JAMA* 2007;**298**:2171–81.
11. Bertot GM, Corral RS, Fresno M, et al. *Trypanosoma cruzi* tubulin eliminated in the urine of the infected host. *J Parasitol* 1998;**84**: 608–14.
12. Bittencourt AL. Congenital Chagas' disease. *Am J Dis Child* 1976;**130**:97–103.
13. Bittencourt AL, Vieira GO, Tavares HC, et al. Esophageal involvement in congenital Chagas' disease: report of a case with megaesophagus. *Am J Trop Med Hyg* 1984;**33**:30–3.
14. Blanco SB, Seguro EL, Cura EN, et al. Congenital transmission of *Trypanosoma cruzi*: an operational outline for detecting and treating infected infants in northwestern Argentina. *Trop Med Int Health* 2000;**5**:293–301.
15. Blum J, Schmid C, Burri C. Clinical aspects of 2541 patients with second stage human African trypanosomiasis. *Acta Trop* 2006;**97**: 55–64.
16. Brisse S, Verhoef J, Tibayrenc M. Characterization of large and small subunit rRNA and mini-exon genes further supports the distinction of six *Trypanosoma cruzi* lineages. *Int J Parasitol* 2001; **31**:1218–26.
17. Brun R, Balmer O. New developments in human African trypanosomiasis. *Curr Opin Infect Dis* 2006;**19**:415–20.
18. Brutus L, Schneider D, Portigo J, et al. Congenital Chagas disease: diagnostic and clinical aspects in an area without vectorial transmission, Bernejo, Bolivia. *Acta Trop* 2008;**106**:195–9.
19. Buekens P, Almandares O, Carlier Y, et al. Mother-to-child transmission of Chagas disease in North America: why don't we do more? *Maternal Child Health J* 2008;**12**:283–6.
20. Burgos JM, Begher S, Silva HM, et al. Molecular identification of *T. cruzi* 1 tropsim for central nervous system in Chagas reactivation due to AIDS. *Am J Trop Med Hyg* 2008;**78**:294–7.
21. Buyst H. Sleeping sickness in children. *Ann Soc Belg Med Trop* 1977;**57**:201–12.
22. Campos SV, Strabilli TM, Amato Neto V, et al. Risk factors for Chagas disease reactivation after heart transplantation. *J Heart Lung Transplant* 2008;**27**:597–602.
23. Cantey P, Stramer SL, Townsend RL et al. The United States *Trypanosoma cruzi* infection study: evidence for vector-borne transmission of the parasite that causes Chagas disease among United States blood donors. *Transfusion* 2012;**52**:1922–30.
24. Carlier Y, Torrico F. Congenital infection with *T. cruzi:* from mechanism of transmission to strategies for diagnosis and control. *Rev Soc Brasil Med* 2003;**36**:767–71.
25. Carod-Artal FJ. Stroke: a neglected complication of American trypanosomiasis. *Trans R Soc Trop Med Hy* 2007;**101**:1070–80.
26. Cazorla SI, Frank FM, Malchiodi EL. Vaccination approaches against *T. Cruzi* infection. *Expert Rev Vaccine* 2009;**8**:921–35.
27. Centers for Disease Control and Prevention. Chagas disease after organ transplantation: Los Angeles, California, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:798–800.
28. Centers for Disease control and Prevention. Congenital transmission of Chagas disease-Virginia, 2010. *MMWR Morbid Mortal Wkly Rep* 2012;**61**:477–9.
29. Chang CD, Cheng KY, Jiang LX, et al. Evaluation of a prototype *Trypanosoma cruzi* antibody assay with recombinant antigens on a fully automated chemiluminescence analyzer for blood donor screening. *Transfusion* 2006;**46**:1737–44.
30. Chappuis F, Loutan L, Simarro P, et al. Options for field diagnosis of human African trypanosomiasis. *Clin Microbiol Rev* 2005;**18**: 133–46.
31. Chippaux JP, Chavizo AN, Santilla JA, et al. Antibody drop in newborns congenitally infected by *T. cruzi* treated with benznidazole. *Trop Med Int Health* 2010;**15**:87–93.
32. Cordova E, Boschi A, Ambrosoni J, et al. Reactivation of Chagas disease with central nervous system involvement in HIV-infected patients in Argentina, 1992-2007. *Int J Infect Dis* 2008;**12**:587–92.
33. Corti M. AIDS and Chagas' disease. *AIDS Patient Care STDS* 2000;**14**:581–8.
34 Cox A, Tilley A, McOdimba F, et al. A PCR based assay for detection and differentiation of African trypanosome species in blood. *Exp Parasitol* 2005;**111**:24–9.
35. Cruz-Robles D, Chavez-Gonzalez JP, Cavazos-Guero MM, et al. Association between IL-1B and IL-1 RN gene polymorphisms and Chagas disease development susceptibility. *Immunol Invest* 2009;**38**:231–9.
36. Da-Costa-Pinto EAL, Almeida EA, Figueiredo D, et al. Chagasic megaesophagus and megacolon diagnosed in childhood and probably caused by vertical transmission. *Rev Int Med Trop Sao Paulo* 2001;**43**:227–30.
37. de Oliviera RB, Troncon LE, Dantas PR, et al. Gastrointestinal manifestations of Chagas' disease. *Am J Gastroenterol* 1998;**93**: 884–9.
38. Deborggraeve S, Claes F, Laurent T, et al. Molecular dipstick test for diagnosis of sleeping sickness. *J Clin Microbiol* 2006;**44**: 2884–9.
39. Di Pentima MC, Edwards MS. Enzyme-linked immunosorbent assay for IgA antibodies to *Trypanosoma cruzi* in congenital infection. *Am J Trop Hyg* 1999;**60**:211–14.
40. Dias JCP. The treatment of Chagas' disease (South American trypanosomiasis). *Ann Intern Med* 2006;**144**:772–4.
41. Diaz JH. Recognizing and reducing the risks of Chagas disease in travelers. *J Travel Med* 2008;**15**:184–95.
42. Dietra WO, Gollob KJ. Current concepts in immunoregulation and pathology of Chagas disease. *Curr Opin Infect Dis*: 2008;**21**: 287–92.
43. Donelson IE, Hill KL, El Sayed NMA. Multiple mechanisms of immune evasion of African trypanosomes. *Mol Biochem Parasitol* 1998;**91**:51–66.
44. Doua F, Boa FY, Schechter PF, et al. Treatment of human late stage gambiense trypanosomiasis with alpha-difluoromethylornithine (eflornithine): efficacy and tolerance in 14 cases in Cote d'Ivoire. *Am J Trop Med Hyg* 1987;**37**:525–33.
45. Feldman AM, McNamara D. Myocarditis. *N Engl J Med* 2000; **343**:1388–98.
46. Ferrer JF, Esteban E, Nurua A, et al. Association and epidemiologic features of *Trypanosoma cruzi* and human T cell lymphotropic A virus type II in inhabitants of the Paraguayan Gran Chaco. *Am J Trop Med Hyg* 2003;**68**:235–41.
47. Fevre EM, Picozzi K, Fyfe J, et al. A burgeoning epidemic of sleeping sickness in Uganda. *Lancet* 2005;**366**:745–7.
48. Freilij H, Altcheh J. Congenital Chagas' disease: diagnosis and clinical aspects. *Clin Infect Dis* 1995;**21**:551–5.
49. Freitas JM, Lages-Silva E, Crema E, et al. Real time PCR strategy for the identification of major lineages of *Trypanosoma cruzi* directly in chronically infected human tissues. *Int J Parasitol* 2005;**35**:411–17.
50. Garcia LS. *Diagnostic medical parasitology*. 5th ed. Washington, D.C.: ASM Press; 2007.

51. Grant IH, Gold JWM, Wittner M, et al. Transfusion associated acute Chagas' disease acquired in the United States. *Ann Intern Med* 1989;**111**:849–51.
52. Greenwood BM, Whittle HC. The pathogenesis of sleeping sickness. *Trans R Soc Trop Med Hyg* 1980;**74**:716–25.
53. Inojosa WO, Augusto I, Bisoffi Z, et al. Diagnosing human African trypanosomiasis in Angola using a card agglutination test: observational study of active and passive case-finding strategies. *BMJ* 2006;**232**:1479–83.
54. Janonneau V, Ilboudo H, Kabore J et al. Untreated human infections by *Trypanosoma brucei gambiense* are not 100% fatal. *PLos Negl Trop Dis* 2012;**6**:e1691.
55. Kennedy GEP. Human African trypanosomiasis of the CNS: current issues and challenges. *J Clin Invest* 2004;**113**:498–504.
56. Kirchhoff LV, Bacchi CJ, Wittner M, et al. African trypanosomiasis (sleeping sickness). *Curr Treat Options Infect Dis* 2000;**2**:66–9.
57. Kirchhoff LV, Bacchi CJ, Wittner M, et al. American trypanosomiasis (Chagas' disease). *Curr Treat Options Infect Dis* 2000;**2**: 59–65.
58. Kirchhoff LV. American trypanosomiasis (Chagas' disease): a tropical disease now in the United States. *N Engl J Med* 1993; **329**:639–44.
59. Kun H, Moore A, Mascola L, et al. Transmission of *T. cruzi* by heart transplantation. *Clin Infect Dis* 2009;**48**:1534–40.
60. Lambert N, Mehta B, Walters R, et al. Chagasic encephalitis as the initial manifestation of AIDS. *Ann Intern Med* 2006;**144**: 941–3.
61. Leiby DA, Wendel S, Takaoka DT, et al. Serologic testing for *Trypanosoma cruzi*: comparison of radioimmune precipitation assay with commercially available immunofluorescence assay, indirect hemagglutination assay, and enzyme-linked immunosorbent assay kits. *J Clin Microbiol* 2000;**38**:639–42.
62. Lejon V, Roger I, Ngoyi DM, et al. Novel markers for treatment outcome in late-stage *T. brucei gambiense* trypanosomiasis. *Clin Infect Dis* 2008;**47**:15–22.
63. Lescure F-X, Leloup G, Freilij H, et al. Chagas disease: changes in knowledge and management. *Lancet Infect Dis* 2010;**10**:556–70.
64. Maguire IH, Hoff R, Sherlock I, et al. Cardiac morbidity and mortality due to Chagas' disease: prospective electrocardiographic study of a Brazilian community. *Circulation* 1987;**75**:1140–5.
65. Marin-Neto JA, Rossi A, Morillo CA, et al. Rationale and design of a randomized placebo-controlled trial assessing the effects of etiologic treatment in Chagas' cardiomyopathy: the Benznidazole Evaluation for Interrupting Trypanosomiasis (BENEFIT). *Am Heart J* 2008;**156**:37–43.
66. Matovu E, Kazibwe AJ, Mugasa CM, et al. Towards point-of-care diagnostic and staging tools for human African trypanaosomiasis. *J Trop Med* 2012;Article ID 340538, 9 pages.
67. Mbala L, Matendo R, Kinkela T, et al. Congenital African trypanosomiasis in a newborn child with current neurologic symptomatology. *Trop Doctor* 1996;**26**:186–7.
68. Moncayo A, Silveira AC. Current epidemiological trends for Chagas disease in Latin America and future challenges in epidemiology, surveillance and health policy. *Mem Inst Oswaldo Cruz* 2009;**104**(suppl 1):17–30.
69. Mora MC, Sanchez Negrette O, Marco D, et al. Early diagnosis of congenital *Trypanosoma cruzi* infection using PCR, hemoculture, and capillary concentration, as compared with delayed serology. *J Parasitol* 2005;**91**:1468–73.
70. Nowicki MJ, Chinchilla C, Corado L, et al. Prevalence of antibodies to *Trypanosoma cruzi* among solid organ donors in Southern California: a population at risk. *Transplantation* 2006;**81**:477–9.
71. Parada H, Carrasco H, Anez N, et al. Cardiac involvement is a constant finding in acute Chagas' disease: a clinical, parasitological and histopathological study. *Int J Cardiol* 1997;**60**:49–54.
72. Pereira KS, Schmidt FL, Guaraldo AM, et al. Chagas disease as a foodborne illness. *J Food Prot* 2009;**72**:441–6.
73. Petkova SB, Huang H, Factor SM, et al. Role of endothelin in the pathogenesis of Chagas' disease. *Int J Parasitol* 2000;**31**: 499–511.
74. Picozzi K, Fevre EM, Odiit M, et al. Sleeping sickness in Uganda: a thin line between two fatal diseases. *B M J* 2005;**331**:1238–42.
75. Rassi A Jr, Rassi A, Little WC, et al. Development and validation of a risk score for predicting death in Chagas' heart disease. *N Engl J Med* 2006;**355**:799–808.
76. Reed SG. Immunology of *Trypanosoma cruzi*. *Chem Immunol* 1998;**70**:124–43.
77. Ribeiro I, Sevcsik A-M, Alver F, et al. New, improved treatments for Chagas disease: from the Rand D pipeline to the patients. *PLoS Negl Trop Dis* 2009;**3**:e484.
78. Rochitte CE, Oliveira PF, Andrade JM, et al. Myocardial delayed enhancement by magnetic resonance imaging in patients with Chagas disease. *J Am Coll Cardiol* 2005;**46**:1553–8.
79. Salas NA, Cot M, Schneider D, et al. Risk factors and consequences of congenital Chagas disease in Yacuiba, south Bolivia. *Trop Med Int Health* 2007;**12**:1498–505.
80. Sartori AMC, Caiaffa-Filho HH, Bezerra RC, et al. Exacerbation of HIV viral load simultaneous with asymptomatic reactivation of chronic Chagas' disease. *Am J Trop Med Hyg* 2002;**67**:521–3.
81. Sartori AM, Shikanai-Yasuda MA, Amato Neto V, et al. Follow-up of 18 patients with human immunodeficiency virus infection and chronic Chagas' disease, with reactivation of Chagas' disease causing cardiac disease in three patients. *Clin Infect Dis* 1998;**26**: 177–9.
82. Schijman AG, Altcheh J, Burgos JM, et al. Aetiological treatment of congenital Chagas' disease diagnosed and monitored by the polymerase chain reaction. *J Antimicrob Chemother* 2003;**52**: 441–9.
83. Schmunis GA. Epidemiology of Chagas disease in non-endemic countries: the role of international migration. *Mem Inst Oswaldo Cruz* 2007;**102** (suppl 1):75–85.
84. Schmunis GA, Cruz JR. Safety of the blood supply in Latin America. *Clin Microbiol Rev* 2005;**18**:22–9.
85. Sharafeldin A, Eltayeb R, Pashenkov M, et al. Chemokines are produced in the brain early during the course of experimental African trypanosomiasis. *J Neuroimmunol* 2000;**103**:165–70.
86. Sinha A, Grace C, Alston WK, et al. African trypanosomiasis in two travelers from the United States. *Clin Infect Dis* 1999;**29**: 840–4.
87. Sosa-Estani S, Seguera EL. Etiologic treatment in patients infected by *T. cruzi*: experiences in Argentina. *Curr Opin Infect Dis* 2006;**19**:583–7.
88. Streiger ML, del Marco ML, Fabbio DL, et al. Longitudinal study and specific chemotherapy in children with chronic Chagas' disease residing in a low endemicity area of Argentina. *Rev Soc Bras Med Trop* 2004;**37**:365–75.
89. Tanowitz HB, Kirchhoff LV, Simon D, et al. Chagas' disease. *Clin Microbiol Rev* 1992;**5**:400–19.
90. Tasdemir D, Kaiser M, Brun R, et al. Antitrypanosomal and antileishmanial activities of flavonoids and their analogues: in vitro, in vivo, and quantitative structure-activity relationship studies. *Antimicrob Agents Chemother* 2006;**50**:1352–64.
91. Tobler LH, Contestable P, Pitina L, et al. Evaluation of a new ELISA for detection of Chagas antibody in U.S. blood donors. *Transfusion* 2007;**47**:90–6.
92. Torrico F, Vega CA, Suarez E, et al. Are maternal reinfections with *T. cruzi* associated with higher morbidity and mortality of congenital Chagas disease? *Trop Med Int health* 2006;**11**:628–35.
93. Urbina JA. Chemotherapy of Chagas' disease; the how and the why. *J Mol Med* 1999;**77**:332–8.
94. Urbina JA, Docampu R. Specific chemotherapy of Chagas' disease: controversies and advances. *Trends Parasitol* 2003;**19**:495–501.
95. Urech K, Neunayn A, Blum J. Sleeping sickness in travelers-do they really sleep? *PLoS Negl Trop Dis* 2011;**5**:e1358.
96. U.S. Food and Drug Administration. *Guidelines for industry use of serological tests to reduce the risk of transmission of* T. cruzi *infection in whole blood and blood components intended for transfusion*. Silver Spring, Md: USFDA; 2010. http://www.fda.gov/Biologics/BloodVaccines/GuidanceRegulatingInfromation/Guidances/default.htm (accessed June 5, 2012).
97. Vinhaer MC, Schofield CJ. Trypanosomiasis control: surmounting diminishing returns. *Trends Parasitol* 2003;**19**:112–13.
98. Viotti R, Vigliano C, Lococo B, et al. Long-term outcomes of treating chronic Chagas' disease with benznidazole versus no treatment. *Ann Intern Med* 2006;**144**:724–34.
99. Viotti RJ, Vigliano C, Laucella S, et al. Value of echocardiography for diagnosis and prognosis of chronic Chagas' disease cardiomyopathy without heart failure. *Heart* 2004;**90**:655–60.
100. Wery M, Mulumba PM, Lambert PH, et al. Hematologic manifestations, diagnosis and immunopathology of African trypanosomiasis. *Semin Hematol* 1982;**19**:83–92.

101. World Health Organization. Control of Chagas' disease: second report of the WHO expert committee. *WHO Tech Rep Ser* 2002; **905**:1–109.
102. World Health Organization. *Chagas disease report 2007*. Geneva: WHO; 2007. http://www.who.int/mediacentre/news/releases/2007/pr36/en/index.html (accessed June 5, 2012).
103. World Health Organization. *WHO consultation on international biological reference preparation for Chagas diagnostic tests*. Geneva: WHO; 2007. http://www.who.int/bloodproducts/ref_materials/WHO_Report_1st_Chagas_BPP_consultation_7-2007_final.pdf. (accessed June 8, 2012).
104. World Health Organization. *Neglected tropical diseases: innovative and intensified disease management (IDM)*. Geneva: WHO; 2010. http://www.who.int/neglected_diseases/disease_management/en. (accessed June 13, 2012).
105. World Health Organization. *Working to overcome the global impact of neglected tropical diseases: first WHO report on neglected tropical diseases*. Geneva: WHO; 2010. http://www.who.int/neglected_diseases/2010report/en/ (accessed June 13, 2012).
106. World Health Organization. *Human African trypanosomiasis*. Geneva: WHO; 2012. http://www.who.int/mediacentre/factsheets/fs259/en/ (accessed June 13, 2012).
107. Yun O, Priotto G, Tong J, et al. NECT is next implementing the new drug combination therapy for T. gambiense sleeping sickness. *PLoS Negl Trop Dis* 2010;**4**:e720.

CHAPTER 222

Naegleria, *Acanthamoeba*, and *Balamuthia* Infections

Patrick J. Gavin • Tina Q. Tan

Interest in free-living amebae has increased since demonstration of their pathogenicity and, more recently, their role as reservoirs and vectors for potentially pathogenic bacteria, such as *Legionella pneumophila* and *Mycobacterium avium*. Small free-living amebae of the genera *Naegleria*, *Acanthamoeba*, and *Balamuthia* cause severe central nervous system (CNS) infections in humans and animals that are characterized by few distinguishing symptoms and an almost uniformly poor prognosis. Although these severe infections are uncommon, for optimal treatment it is important to recognize and diagnose them early.

Although small free-living amebae share structural and pathogenic similarities, the infections they cause have distinctive epidemiology, clinical presentation and course, neuroimaging, immunology, and pathology. Primary amebic meningoencephalitis caused by *Naegleria fowleri* is an acute fulminant illness that affects healthy children and young adults and usually results in death within 1 week of presentation. Almost invariably, the patient has a recent history of swimming in bodies of warm fresh water, although there are recent reports of acquiring the infection through nasal irrigation using a neti pot. In contrast, granulomatous amebic encephalitis caused by *Acanthamoeba* spp. or *Balamuthia mandrillaris* (formerly leptomyxid amebae) is an insidious, subacute, and protracted illness that affects primarily, but not exclusively, immunocompromised or debilitated patients and leads to death in weeks to months. Typically, the patient has no history of recent exposure to fresh water. One other species of ameba belonging to the genus *Sappinia* has also been identified as a cause of encephalitis.[128]

Despite these differences, the prognosis of CNS infection caused by *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* is similarly dismal, with few reports of survival. In the handful of cases that survived from the hundreds reported, recognition, early diagnosis, and timely administration of appropriate antimicrobial treatment were crucial to achieving a successful outcome. *Acanthamoeba* spp. also are associated with disseminated cutaneous, pulmonary, or sinus infection in immunocompromised hosts and with chronic painful and potentially sight-threatening keratitis in association with use of contact lenses or corneal trauma in otherwise healthy patients.

The pathogenic potential of small free-living amebae was recognized first during polio vaccine trials in the 1950s, when they were found to cause cytotoxicity in contaminated cell cultures.[23,54] Subsequently, the fulminant fatal encephalitis that followed intracerebral injection or intranasal instillation of tissue culture fluid in experimental mice and monkeys showed pathogenicity and a potential route for human infection.[19,22,23] The first cases of human infection caused by small free-living amebae were recognized in four children from southern Australia by Fowler and Carter[36] in 1965 and soon afterward by Butt[12] in Florida.

More recently, Visvesvara and colleagues[130,131] isolated and named *B. mandrillaris*, a new agent of amebic meningoencephalitis, from the brain of a mandrill baboon. The first reported human case of *B. mandrillaris* encephalitis was in a patient with acquired immunodeficiency syndrome (AIDS).[3] Reports of infection in immunocompetent hosts have followed.[6,25,38,60,95] Subsequently, infections caused by free-living amebae have been reported worldwide.* Finally, the first case report of nonfatal CNS disease, in an otherwise healthy adult, caused by *Sappinia diploidea*, a free-living ameba normally found in soil contaminated with elk and buffalo dung, adds to the list of pathogenic small free-living amebae and suggests that others may be identified in the future.[41]

EPIDEMIOLOGY

Free-living amebae are found worldwide and are cosmopolitan in their distribution, living primarily in soil, but also in water, inside vertebrates, on plants, and in the air.[94] They are spread readily by wind and water currents. In humans, infection with free-living amebae causes primary amebic meningoencephalitis, granulomatous amebic meningoencephalitis, disseminated granulomatous disease (cutaneous, pulmonary, or sinus), and keratitis.[70,73,107]

Although they are rare, cases of infection caused by *N. fowleri* have been reported throughout the world.[35] As of 2004, more than 200 cases of primary amebic encephalitis were documented worldwide, with approximately half occurring in the United States.[20,107] In the United States between 1989 and 2000, 24 fatal cases of primary amebic meningoencephalitis were reported to the Centers for Disease Control and Prevention (CDC).[83] Most of them occurred in the summer and in young children. Increased water temperatures, whether from thermal pollution or sunlight, provide the ideal environment for proliferation of *N. fowleri*.[123,132] Cases typically are associated with a

*References 4, 61, 70, 73, 80, 97.

history of swimming, diving, or playing in bodies of warm fresh water, brackish habitats with a soil and water interface, artificial lakes, hot springs, and thermally polluted rivers and streams.* Researchers have postulated that thermal pollution, particularly from overflow of power plants, or inadequate chlorination of water kills off normal thermosensitive and chlorine-sensitive fauna, shifting the balance in favor of thermotolerant, less chlorine-sensitive, pathogenic *N. fowleri.*[107]

In North America, cases of primary amebic meningoencephalitis tend to be clustered in the warmer southern states and Mexico.[107] In one study, almost 50 percent of freshwater lakes in Florida contained *N. fowleri* when the temperature was 30°C or higher.[132] *N. fowleri* may be found in colder areas, however. In temperate regions, distribution of *N. fowleri* is limited by seasons, being greatest during summer and early fall.[123] *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* were present year-round in lakes in the Tulsa region of Oklahoma.[56] Although it is most prevalent in areas where water temperatures are higher, *N. fowleri* has the ability to overwinter, in cyst form, in sediment at the bottom of lakes.[132]

Small outbreaks of primary amebic meningoencephalitis have been described. Over a 3-year period, from 1962 to 1965, 16 swimmers died after being exposed to warm contaminated and inadequately chlorinated water in an indoor pool in Czechoslovakia.[16,107] Similarly, eight cases of fulminant meningoencephalitis associated with exposure to the water of an artificial lake in Richmond, Virginia, were recognized retrospectively as being caused by *N. fowleri.*[14] Finally, five cases of primary amebic meningoencephalitis caused by *N. fowleri* occurred in 1990 in patients swimming in a drainage canal in Mexicali, Mexico.[66]

Disease also has been documented in patients with no history of exposure to fresh water. Atypical exposures have included bathing in domestic bath water, playing in a warm muddy puddle, and being baptized by full-body immersion.[4,8,75] *N. fowleri* also has been shown in domestic tap water.[29,75] Although cases of infection have not followed ingestion of drinking water contaminated by amebae, fatal infection was associated with washing in tap water heated to high temperatures in pipes by the summer sun in South Australia and with exposure to contaminated domestic water in Arizona.[2,29,75]

Infections occur far more frequently in previously healthy children and young adults than in other age groups, presumably because youngsters are most likely to remain in water for prolonged periods and to disturb sediment containing amebae by diving and swimming underwater.[20,83] In one series of primary amebic meningoencephalitis cases, boys outnumbered girls 12 to 1, presumably because boys are more likely than girls to engage in aquatic horseplay.[129] The incubation period in natural human infection is 2 to 15 days after exposure.[8,20,83] Death usually ensues within 1 week of presentation (generally within 72 hours).

Acanthamoeba spp. are among the most prevalent protozoa found in the environment, being found in soils from tropical to arctic regions. They are important components of the food chain and promote plant growth by predation on soil bacteria. *Acanthamoeba* spp. are prevalent in natural and artificial habitats that include fresh, brackish, potable, or stagnant water; in sewage; in improperly treated swimming pools; and in taps, sinks, hot tubs, air-conditioning units, aquaria, flowerpots, showers, ventilators, and ventilation units in homes and hospitals.[17,33,73,105,107] Although warm waters might enhance their numbers, pathogenic amebae may be isolated year-round from water samples in the United States.[132] Their distribution and viability are governed primarily by availability of a bacterial food source; soil texture; water temperature, pH, and salinity and by ultraviolet light and desiccation.[94,107] Growth of *Acanthamoeba* spp. is inhibited by temperatures of 35°C to 39°C. Although *Acanthamoeba* spp. seemingly are ubiquitous in water, *Acanthamoeba* infections generally are not associated with a history of recent swimming or water activities.[107] Acquisition occurs by inhalation or direct contact with contaminated soil or water.

In contrast to *N. fowleri*, *Acanthamoeba* spp. are opportunistic pathogens. Granulomatous amebic encephalitis and disseminated disease caused by *Acanthamoeba* spp. generally are seen in immunocompromised hosts or in patients with underlying disease or debilitation, such as that due to malignancy, cancer chemotherapy, corticosteroid treatment, organ transplantation, human immunodeficiency virus, diabetes, or malnutrition.[70,73] *Acanthamoeba* spp. cause keratitis in otherwise healthy individuals by directly attacking the corneal surface, usually in association with poor contact lens care or corneal trauma. Although cases of CNS or disseminated *Acanthamoeba* infection are extremely rare, in 2004, estimates of cases of amebic keratitis worldwide exceeded 3000.[107]

Comparatively less is known about *B. mandrillaris.* It is closely related to *Acanthamoeba* and causes a similar spectrum of infections that includes granulomatous amebic encephalitis and cutaneous and sinus disease.[107] Although initial cases of *B. mandrillaris* infection were confined to immunocompromised and debilitated hosts, its ability to cause disease in apparently healthy children is well documented.[6,25,39,60,95] By 2004, more than 100 cases of human infection had been reported.[107] *Balamuthia* is assumed to inhabit soil and water; it has been isolated more recently in soil from a flowerpot associated with a case of amebic encephalitis in a child from northern California.[101]

As with *Acanthamoeba* spp., granulomatous encephalitis caused by *B. mandrillaris* seems to occur after inhalation of airborne cysts or soil contamination of breaks in the skin. The patient generally has no history of recent exposure to fresh water. To date, 50 percent of cases of *Balamuthia* infection reported in the United States have occurred in patients of Hispanic ethnicity.[6,103] Although this statistic most likely is a result of environmental exposure, a genetic predisposition may exist. Among California cases of balamuthiasis, most cluster in the southern part of the state. Researchers have postulated that the area's dry warm soil is conducive for the amebae and that large-scale agriculture in these areas increases the likelihood of workers being exposed to soil or wind-blown

*References 20, 26, 35, 71, 83, 123.

particles. Cases of *B. mandrillaris* infection linked directly to incidental exposure to soil or to blowing soil while traveling in an open car or by motorcycle are reported.[25,60,101,105] Although *Balamuthia* encephalitis is a zoonosis, animals do not represent a source of infection for humans.

Despite their seeming ubiquity in the environment and ample opportunity for contact with humans compared with other parasitic protozoa, such as *Entamoeba*, malaria and trypanosome infections caused by free-living amebae are extremely rare. Given the enormous number of exposures to water containing *Naegleria*, why so few individuals become infected remains unknown. Only seven cases of primary amebic meningoencephalitis were reported in Florida during a 14-year period, despite estimates of billions of potential exposures in contaminated freshwater lakes during that time period.[132] Epidemiologic investigations of cases typically find that many other individuals swam in the same water at the same time but did not become ill. Factors other than the presence of amebae, such as host susceptibility, anatomy, inoculum size, and activity at the time of exposure, have been implicated.[132] Nonetheless, in recent years, an increase in the number of diagnoses of primary amebic meningoencephalitis cases has been noted.[68,137] The increase probably reflects a combination of greater recognition of the pathogenic potential of free-living amebae, improved diagnostic techniques, and a growth in the numbers of immunocompromised hosts.

Acanthamoeba organisms can infect the cornea directly, after trauma, or, most commonly, in association with the combination of extended-wear soft contact lens use and subsequent exposure to contaminated water.[109,118] Major risk factors for development of amebic keratitis include poor compliance with care of daily wear soft contact lenses, particularly use of homemade saline solutions using nonsterile tap water that may be contaminated with amebae; use of chlorine release lens disinfection systems; and inadequate cleansing of lens cases, which encourages growth of a bacterial biofilm that acts as a nidus and a food source for amebae.[91,117] Wearing contact lenses while swimming or in hot tubs, which potentially may be contaminated with amebae, is similarly risky.[96]

Since the first cases of amebic keratitis were described in the early 1970s, the incidence has increased dramatically. Between 1973 and 1984, slightly more than 200 cases of amebic keratitis were reported to the CDC. By 2002, in the United States, more than 3000 cases had been reported and 1 in 250,000 people were estimated to be affected.[58,65,85,107] The increased incidence most likely is due to increased use of contact lenses, particularly of the soft, extended-wear variety. Although many of these lenses were marketed as products requiring "low care," many patients interpreted this designation as meaning "no care."[91]

ORGANISMS

In contrast to the related true parasite protozoa *Entamoeba* spp., *Naegleria*, *Acanthamoeba*, and *Balamuthia* spp. are amphizoic protozoa, capable of existing as free-living amebae and as parasitic pathogens.[107] *Naegleria* spp. are ameboflagellates found primarily in moist soil and warm fresh water.[71] Although more than 27 separate *Naegleria* spp. have been identified, to date only *N. fowleri* is associated with human infections.[26,107] *Naegleria* spp. can exist in three forms: an active feeding trophozoite, a rarely seen dormant cyst (Fig. 222-1), and a flagellate form (Fig. 222-2).[71] Of these forms, only the trophozoite is found in tissue or cerebrospinal fluid (CSF). The trophozoite of *N. fowleri* is smaller than that of *Acanthamoeba* spp., measuring 8 to 15 μm in diameter, with a conspicuous nucleus containing a large, dense central karyosome without peripheral chromatin lining the membrane.[79] *Naegleria* trophozoites move slowly by extension of broad, rounded anterior pseudopodia (lobopodia). This feature may be noted on a warm saline preparation or in CSF specimens. *Naegleria* cysts are spherical, measure 7 to 12 μm in diameter, and have a single-layered outer membrane.

Approximately 20 *Acanthamoeba* spp. are identified by morphologic criteria.[107] Because morphologic distinctions do not always correlate with results of molecular typing, however, classification is complex and is currently

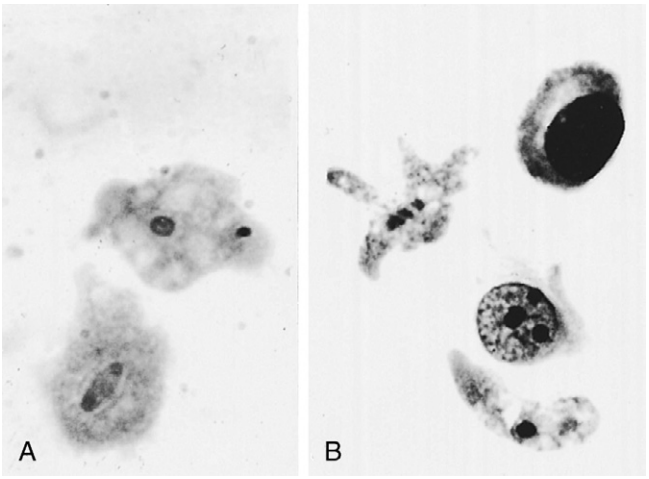

FIGURE 222-1 ■ **A** and **B,** Trophozoites of *Naegleria.*

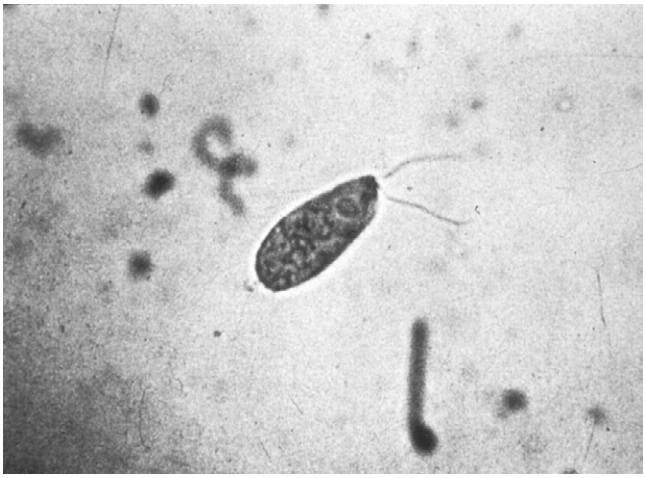

FIGURE 222-2 ■ Flagellate form of *Naegleria fowleri.*

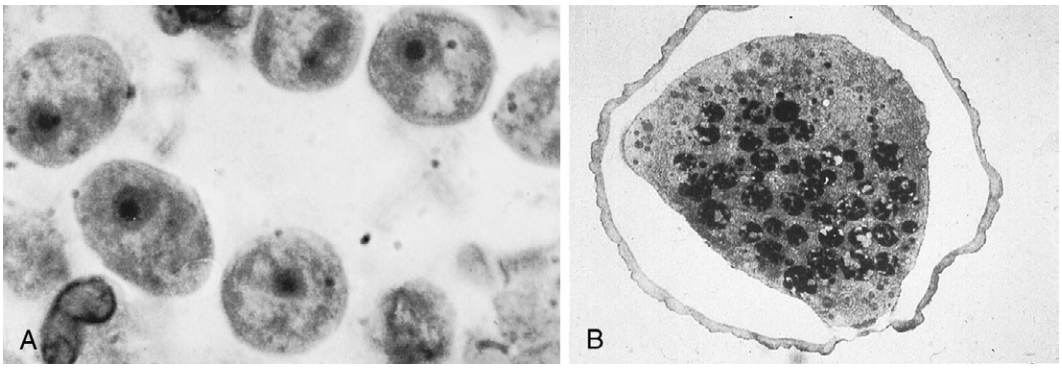

FIGURE 222-3 ■ **A,** Trophozoite of *Acanthamoeba*. **B,** Cyst of *Acanthamoeba* (scanning electron micrograph). (**A,** From Jager BV, Stamm WP. Brain abscesses caused by free-living amoeba probably of the genus *Hartmannella* living in a patient with Hodgkin disease. *Lancet* 1972;**2**:1343–5. Copyright © by the Lancet Ltd.)

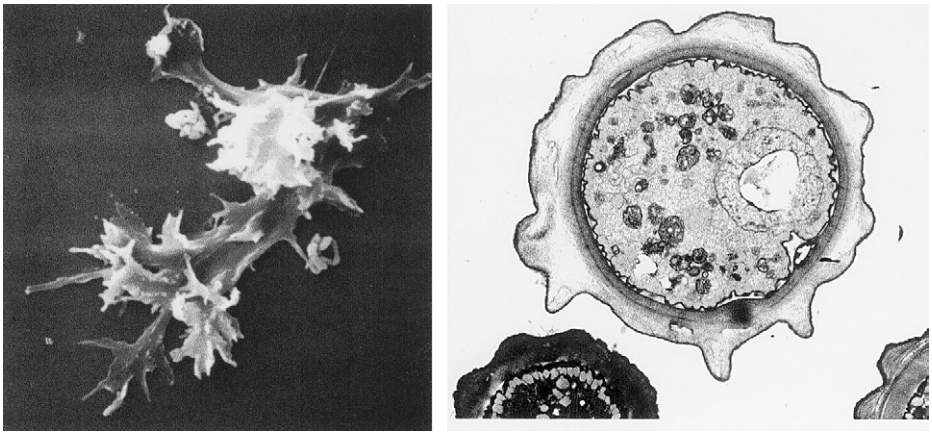

FIGURE 222-4 ■ Trophozoite and cyst of *Balamuthia mandrillaris*. (Courtesy Dr. G. S. Visvesvara, Centers for Disease Control and Prevention.)

under review.[73] Molecular classification is ongoing. The life cycle of *Acanthamoeba* spp. consists of two stages: an actively feeding and dividing trophozoite and a dormant cyst stage (Fig. 222-3).[87] The trophozoite is 15 to 40 µm. The nucleus is vesicular; has a large, dense central "targetoid" nucleolus; and is surrounded by vacuolated cytoplasm. Locomotion typically is sluggish, by extrusion of multiple fine spiny pseudopodia (acanthopodia).[79] *Acanthamoeba* trophozoites feed on bacteria, yeasts, and algae. *Acanthamoeba* cysts are smaller, 15 to 20 µm, with a distinct, wrinkled, thick double wall. Encystation occurs in response to adverse environmental conditions, such as desiccation, scarcity of food, and changes in pH or temperature. *Acanthamoeba* cysts are particularly hardy; they are intrinsically resistant to chlorination and sterilization of potable water, to biocides used for disinfecting bronchoscopes and contact lenses, and to antibiotics; they may remain viable for decades after freezing.[27,63,81] *Acanthamoeba* is the only pathogenic free-living species isolated from marine water. *Naegleria* spp. do not tolerate sea water.

Few *Acanthamoeba* spp. have been associated with human infections. Among the potentially pathogenic species, *Acanthamoeba culbertsoni*, *Acanthamoeba castellanii*, *Acanthamoeba astronyxis*, and *Acanthamoeba polyphaga* are prominent.[80] On the basis of 18S rRNA gene sequences *Acanthamoeba* spp. are divided into 15 genotypes (T1 through T15). At present, no clear consensus has been reached regarding which genotypes are pathogenic. Genotypes T1, T4, T7, T10, and T12 are found among cases of granulomatous encephalitis, whereas isolates associated with keratitis fall into genotypes T3, T4, and T11.[10,34,73,120] Genotype T4 makes up 94 percent and 80 percent of keratitis and nonkeratitis *Acanthamoeba* isolates, respectively,[10] presumably reflecting the preponderance of this genotype in the environment. Rare genotypes, such as T1, T10, and T12, are found only in CNS disease and are not found in the environment.

B. mandrillaris is the only pathogenic *Balamuthia* spp. identified to date.[107] It exists as either a vegetative trophozoite or a dormant cyst (Fig. 222-4). Trophozoites are similar in appearance to *Acanthamoeba* spp. but larger (50 to 60 µm in diameter), and the spherical cyst stage (10 to 30 µm in diameter) has a triple-layered wall on electron microscopy. *Balamuthia* trophozoites typically have a single vesicular nucleus, often with multiple large, dense nucleoli. Movement occurs by extension of broad pseudopodia. *Balamuthia* has been isolated more recently from environmental soil samples.[31] Because of its slow growth, it may have been obscured in environmental

cultures by overgrowth of more rapidly growing bacteria, fungi, or other amebae. In contrast to *Naegleria* and *Acanthamoeba* spp., *Balamuthia* feeds on smaller amebae and protozoa and does not prey on bacteria.

S. diploidea is a species of free-living amebae that is found worldwide. The life cycle of this organism involves animal feces. The trophozoites are 40 to 70 μm in diameter and can be distinguished by the presence of a large cytoplasmic vacuole, mitochondria with characteristic tubular crystal patterns, a juxtanuclear Golgi-like network, and a double nucleus.[41]

CLINICAL MANIFESTATIONS

Naegleria fowleri

Primary amebic meningoencephalitis caused by *N. fowleri* is an acute, rapidly progressive illness that almost uniformly is fatal. Patients have an abrupt onset of severe headache, fever, nausea and vomiting, malaise, rhinitis, meningitis, and changes in mental status 1 to 15 days after exposure (typically 5 to 8 days), most frequently after swimming or bathing in warm contaminated fresh water.[35,70,83,111] A history of disturbances of taste and smell has been noted in many cases, although for the most part the early clinical features of primary amebic meningoencephalitis are not distinctive.[49] Progression from fever to signs of meningitis and encephalitis is unrelenting and rapid. Seizures are common. Coma is present at or develops soon after hospital admission. In the absence of early diagnosis and appropriate treatment, the illness characteristically progresses rapidly to death within 1 week of onset of symptoms (median, 72 hours).[8]

Other than the clue of a recent history of exposure to warm water, there is little to distinguish primary amebic meningoencephalitis from fulminant bacterial meningitis. A high peripheral polymorphonuclear leukocytosis may be present. CSF findings are similarly nonspecific, with increased pressure, elevated protein level, a normal to low glucose value, and modest polymorphonuclear pleocytosis, but no bacteria, mycobacteria, or fungi are identified. Patients with a suggestive history and CSF indices suggestive of purulent meningitis, but without bacteria on Gram stain, should have a wet mount examined for the presence of motile trophozoites (Fig. 222-5).

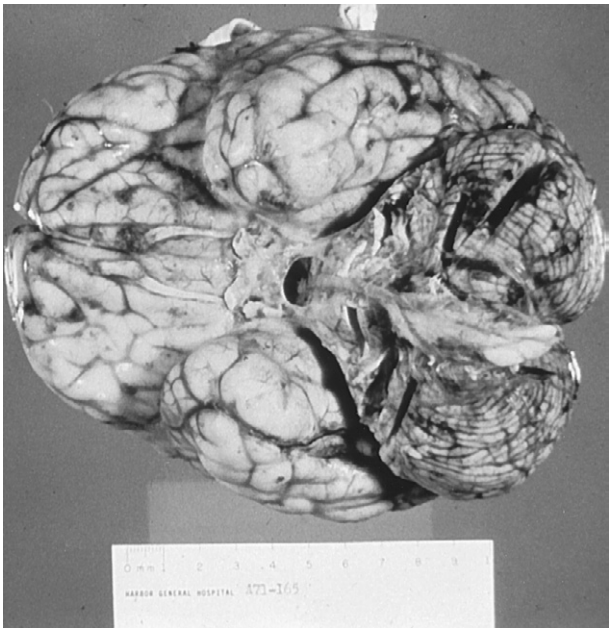

FIGURE 222-5 ■ Brain from a fatal case of *Naegleria* meningoencephalitis. Note the areas of necrosis on the basilar surface of the brain.

Acanthamoeba Species

Acanthamoeba spp. and *B. mandrillaris* cause granulomatous amebic encephalitis. The course typically is prolonged, with progression from focal neurologic signs to diffuse meningoencephalitis over weeks to months after exposure.[73,107] Patients most susceptible to contracting this disease include very young, old, debilitated, immunosuppressed, and chronically ill patients. The clinical picture resembles that of a bacterial brain abscess or brain tumor, with insidious headache, intermittent low-grade fever, nausea, vomiting, and symptoms of increased intracranial pressure. Altered mental status is particularly prominent. As the infection progresses, depending on the area of the brain affected, focal neurologic signs, hemiparesis, drowsiness, aphasia, behavioral and personality changes, and seizures develop. The incubation period after exposure to onset of CNS disease is unknown but is of the order of several weeks to months.

Acanthamoeba skin lesions often have been present for months before the onset of CNS disease. Routine CSF findings frequently are abnormal but nonspecific, with elevated protein level, low glucose value, and, typically, a mononuclear pleocytosis. *Acanthamoeba* spp. also may cause cutaneous, pulmonary, bone, and nasopharyngeal or sinus disease.[42,69,77] Cutaneous *Acanthamoeba* infection is characterized by pustules and hard erythematous nodules, which develop into nonhealing indurated draining ulcers or subcutaneous abscesses.[37,73] Disseminated disease without CNS involvement has a slightly better prognosis. In patients with AIDS, mortality rates of 70 percent and 100 percent are reported for *Acanthamoeba* skin disease alone and in association with CNS disease, respectively.[59]

Acanthamoeba Keratitis

In contrast to its role as an opportunistic pathogen in CNS and skin disease, most cases of *Acanthamoeba* keratitis occur in otherwise healthy individuals who wear contact lenses or who have a history of corneal trauma. Contact lenses of any type, including daily wear and disposable soft and extended-wear lenses, have been implicated in infection. All soft contact lenses contain 50 to 75 percent water. These lenses can absorb pathogens from contaminated cleaning solutions, carrying cases, and hands. When the lens comes into contact with the contaminated fluid, the amebae quickly adhere to the lens surface. If corneal trauma is present, the organisms invade the corneal tissue and produce infection.[57] The nidus for

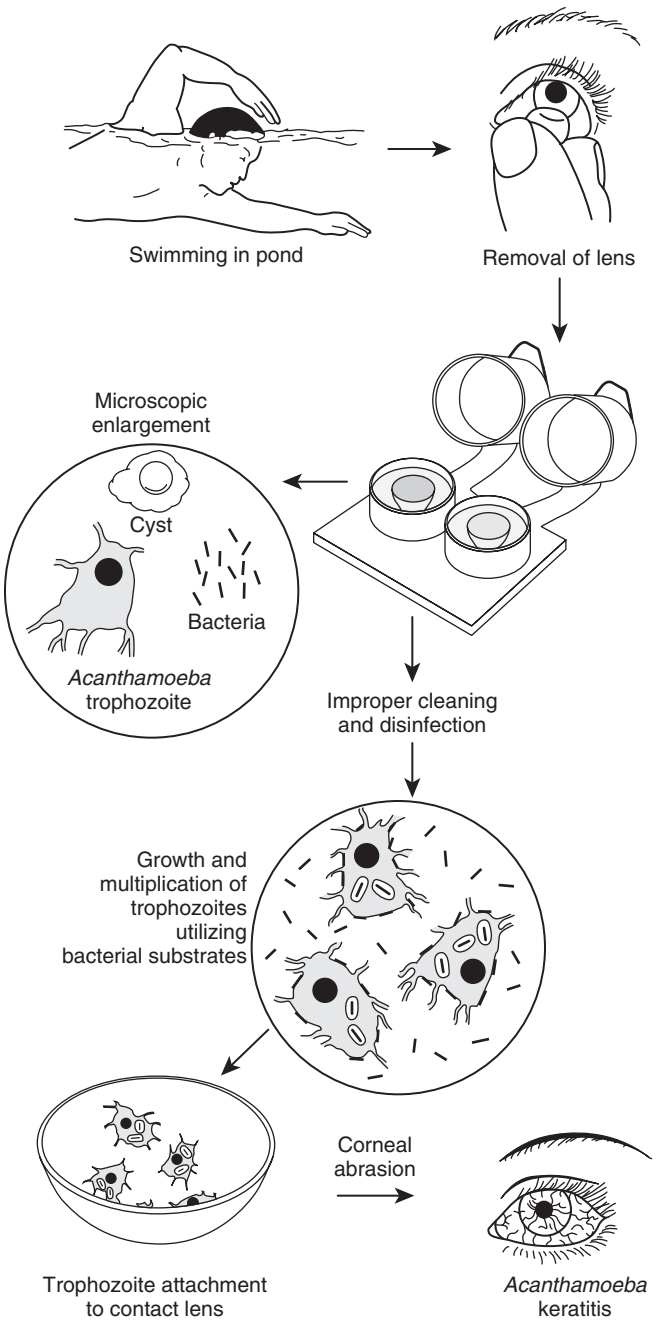

FIGURE 222-6 ■ Proposed mechanism for *Acanthamoeba* keratitis. Bacterial contaminants in the water on the lens support the growth of amoebae, which leads to an increased number of invading organisms. The organisms adhere to the contact lens and enter tissue through the damaged cornea, with the subsequent development of keratitis.

infection most likely is trauma to the cornea but also may be related to preexisting herpesviral or bacterial conjunctivitis (Fig. 222-6).

Amebic keratitis characteristically is painful, progressive, and sight threatening and fails to respond to conventional antibacterial or antiviral treatment.[5,73] Typically, only one eye is involved. Early symptoms include severe eye pain, lacrimation, foreign body sensation, and photophobia. *Acanthamoeba* keratitis frequently is misdiagnosed, initially, as herpetic, bacterial, or fungal infection. The course usually is more indolent, however. Periods of temporary remission delay establishing the diagnosis further. Recurrent corneal epithelial breakdown with dendritic infiltrates progresses to nonsuppurative keratitis, which waxes and wanes over several months and ends in a characteristic ring-shaped stromal abscess with secondary uveitis leading to loss of the cornea.[5,125] Infection that follows severe trauma deteriorates more rapidly than that which follows contact lens wear. In addition, complications of infection include iritis, cataracts, hypopyon, glaucoma, scleritis, and penetrating keratitis.[5]

Balamuthia mandrillaris

B. mandrillaris causes granulomatous encephalitis and cutaneous, sinus, and nasopharyngeal disease.[107] The incubation period of CNS disease is unknown but is thought to be weeks to months (not <10 days).[80] In animal models, extensive CNS necrosis develops 1 to 3 weeks after inoculation.[78] Onset of symptoms of granulomatous encephalitis is insidious, with low-grade fever, severe frontal headache, nausea and vomiting, meningismus, personality change, and prominent focal neurologic signs that have included cranial nerve palsies, hemiparesis, dysarthria/aphasia, focal seizures, and ataxia.[6,47] A history of recent otitis was noted by many authors.[6,47] A slow relentless deterioration to coma and death ensues over several weeks to months (as long as 2 years afterward). Death usually results from cardiorespiratory failure secondary to severe cerebral edema. In many reports, manifestation of cutaneous lesions (facial, nasal, and limb) has preceded development of CNS disease.[25,53,89,92,133] Although normal CSF parameters have been described in *B. mandrillaris* CNS infection, moderately increased protein levels (may be >1 g/dL), normal or mildly decreased glucose values, and a pleocytosis (about 500 cells/mm^3), which commonly but not invariably has a mononuclear predominance, are the typical findings.[6,39,47,78,107]

NEUROIMAGING

Neuroimaging findings in infections caused by free-living amebae are nonspecific and alone do not provide clues to an earlier diagnosis. In addition, in the early stages of infection, results of computed tomography (CT) and magnetic resonance imaging (MRI) may be normal. In primary amebic meningoencephalitis, cranial CT and MRI show obliteration of basal cisterns and signs of diffuse brain edema, focal and basilar meningeal enhancement, and infarction.[64,98,112] In granulomatous encephalitis, neuroimaging reveals variable but nondiagnostic abnormalities that include, alone or in combination, discrete corticomedullary lesions; large, solitary, masslike lesions (which may be confused with brain abscess or tumor); large arterial occlusions; and spinal cord infarctions.[64,98,112] Similarly, in *Balamuthia* infection, neuroimaging is helpful but nondiagnostic; CT scans show a focal enhancing mass or ring-enhancing cystic lesions or both, vascular occlusion, and diffuse edema that frequently progresses to communicating hydrocephalus.[6,25,47]

PATHOGENESIS

Naegleria trophozoites or cysts enter the nasal cavity by aspiration of water or inhalation of soil. After active phagocytosis by sustentacular neuroepithelial cells of the olfactory nerve occurs, trophozoites migrate along the nerve, pass through the cribriform plate, and gain entry to the CNS.[55,80] From the olfactory bulb, which is bathed in CSF and lies in the highly vascularized subarachnoid space, *N. fowleri* disseminate throughout the CSF and CNS. Only *Naegleria* trophozoites are found in the CNS lesions. In contrast, most *Acanthamoeba* spp. infections seem to follow hematogenous dissemination from a primary lower respiratory or cutaneous focus.[80] Researchers have postulated that *Acanthamoeba* spp. are inhaled or inoculated through skin in the cyst phase. Less commonly, *Acanthamoeba* is capable of causing CNS infection directly via the olfactory neuroepithelium.

Virulence factors described for free-living amebae include the ability to adhere to brain microvascular epithelium, production of extracellular protease and elastases, and phagocytosis by food-cups or amebostomes.* Among *Naegleria* spp., pathogenicity is closely related to thermotolerance.[26] *N. fowleri*, the only species pathogenic for humans, is unusually thermophilic and can tolerate temperatures of 45°C.[107] In addition, *N. fowleri* has less demanding growth requirements than nonpathogenic *Naegleria* spp. Trophozoites of pathogenic *Acanthamoeba* spp. also are thermophilic, being able to tolerate temperatures of 37°C, relative to their nonpathogenic counterparts.[99,107] Thermotolerance seems to be less important for *Acanthamoeba* strains that cause keratitis, presumably because corneal temperatures are lower (32°C to 35°C).[107]

PATHOLOGY

Pathologic changes of infection with small free-living amebae are most marked in the CNS. The pathology of primary and granulomatous amebic encephalitis differs fundamentally.[80] Macroscopically, *N. fowleri* produces diffuse meningoencephalitis, most severely affecting the cortical gray matter. It is characterized by marked cerebral edema and purulent leptomeninges, particularly adjacent to the olfactory bulbs, at the base of the frontal and temporal lobes, and at the hypothalamus.[79] Frequently, the olfactory bulbs are necrotic and hemorrhagic. Microscopically, an acute mononuclear and polymorphonuclear inflammatory infiltrate is seen throughout the cerebrum, brain stem, and cerebellum and the upper spinal cord. Trophozoites tend to concentrate in the perivascular spaces and adventitia of arteries, and they may contain ingested erythrocytes and brain tissue. Characteristically, only trophozoites are seen in the CSF or brain tissue.[80] Presumably, *N. fowleri* cysts are not found in clinical specimens because progression of *Naegleria* infection is so rapid and fatal that the patient dies before transformation from trophozoite into the cyst can occur.[70]

CNS infections caused by *Acanthamoeba* spp. and *B. mandrillaris* are characterized macroscopically by diffuse cerebral edema and hemorrhage, with areas of softening and abscess formation. The meninges are spared except for areas overlying involved cerebrum. Additional areas of hemorrhagic necrosis are concentrated in the posterior fossa, brain stem, cerebellum, midbrain, cerebral cortex, and basal ganglia.[6,25,28,70] Narrowing of the sulci and flattening of the gyri are noted in areas of active infection. Microscopic changes consist of multinucleated giant cells, with trophozoites and cysts seen within the lesions.[79] The olfactory bulbs and spinal cord are relatively spared.

Microscopically, necrotizing hemorrhagic encephalitis and subacute or chronic necrotizing granulomatous infiltrates and multinucleate giant cells are scattered throughout the CNS, particularly in the cerebral hemispheres, basal ganglia, midbrain, and brain stem. Typically, trophozoites and cysts of *Acanthamoeba* spp. or *B. mandrillaris* are present within necrotic cerebral tissue, particularly in perivascular spaces. Invasion of blood vessels causes severe necrotizing arteritis and hemorrhagic necrosis. The angiotropic distribution of *Acanthamoeba* or *Balamuthia* infection supports a hematogenous mechanism of spread. In severely immunocompromised patients, the inflammatory reaction may be negligible and lacks a granulomatous component.[9,78] In the absence of a vigorous granulomatous response, hemorrhagic necrosis that resembles primary amebic meningoencephalitis may occur.[134]

Skin lesions of acanthamebiasis and balamuthiasis are characterized by nodules, ulcers, and abscesses and by the presence of amebic trophozoites, cysts, and granulomas in tissue.[77,80,133] Amebic keratitis is characterized by moderate chronic inflammation of corneal stroma, a mixed polymorphonuclear and lymphocytic infiltrate, and the presence of amebic trophozoites and cysts within the corneal ulcerations.[129]

DIAGNOSIS

Previously, most cases of amebic meningoencephalitis were diagnosed with certainty only at autopsy or by brain biopsy.[9] Routine laboratory tests did not distinguish amebic meningoencephalitis from other causes of meningoencephalitis. The first and most important diagnostic step is to recognize the possibility that small free-living amebae may cause infection in the appropriate clinical setting. Among the few reported therapeutic successes, all are notable for early diagnosis and timely institution of appropriate antimicrobial therapy.[25,88]

Primary amebic meningoencephalitis should be included in the differential diagnosis of any child or young adult with meningoencephalitis. A history of recent exposure to fresh water should be sought, and a wet mount should be performed on centrifuged CSF. *Naegleria* is isolated most readily from CSF but also may be isolated from brain tissue, particularly olfactory lobes. In contrast, *Acanthamoeba* trophozoites characteristically are absent from CSF and *B. mandrillaris* has yet to be seen

*References 1, 33, 48, 62, 71, 73.

in CSF. *Acanthamoeba* spp. are more likely to be isolated from brain tissue or skin biopsy or from corneal scrapings in cases of keratitis.[99] Diagnostic techniques include direct microscopy of wet-mount and stained CSF specimens, histologic stains of tissue, fluorescent and immunofluorescent antibody stains of CSF and tissue, direct immunohistochemical stains of tissue, serology, molecular diagnostics, and culture.[127]

Direct Microscopic Identification

If amebic infection of the CNS is suspected, a wet-mount preparation of fresh CSF should be examined for the presence of motile amebae.[70,79,80,127] This method remains the most rapid means of diagnosis. Ideally, the CSF specimen should be kept at room temperature and examined soon after it has been collected. *Naegleria* is particularly fragile. Although the specimen may be kept at 4°C for short periods, it never should be frozen.[127] If present, amebae tend to attach to the test tube wall or surface of the container, so gentle shaking and low centrifugation at 150 *g* for 5 minutes is recommended to dislodge and concentrate the amebae.[79,127] The supernatant should be placed gently on a slide. The wet mount is examined best by light microscopy under low power (10× and 40× objectives), phase-contrast microscopy, or darkfield illumination and may be warmed to promote amebic motility. *Naegleria* trophozoites move in a characteristic "sluglike" fashion by extrusion of a few broad pseudopodia (lobopodia) anteriorly.[107] Leftover specimens can be frozen at –20°C for later antigen or antibody testing. Although *N. fowleri* may be shown by histologic stains of fixed CSF smears, Gram stain is not useful because it does not stain amebae and the heat fixation causes lysis.

Naegleria, *Acanthamoeba* spp., and *Balamuthia* may be shown in fixed sections of brain tissue biopsy or autopsy specimens by hematoxylin and eosin, Masson trichrome, periodic acid–Schiff, Wright-Giemsa stain, and Gomori–methenamine silver stains (Fig. 222-7).[70,73,79,80] Reliably differentiating *Acanthamoeba* and *Balamuthia* by histology alone is difficult. In addition, *Acanthamoeba* trophozoites have been variously mistaken for macrophages or "atypical mononuclear" or epithelial cells on routine histology.[79,134]

Immunofluorescent stains using monoclonal and polyclonal anti-*Acanthamoeba* and anti–*B. mandrillaris* antibodies (available at the CDC), immunoperoxidase staining, and electron microscopy of tissue sections have been used to confirm the diagnosis (Fig. 222-8).[25,59,72,127,128] Amebic keratitis may be diagnosed by direct detection of trophozoites or cysts in smears of deep corneal scraping or biopsy using Wright-Giemsa, trichrome, periodic acid–Schiff, calcofluor white, or indirect fluorescent antibody stains.[70,79,135]

Culture

Culture has a limited role in diagnosis but is useful for confirmation, speciation, and antimicrobial susceptibility testing. *N. fowleri* and *Acanthamoeba* spp. can be cultivated using bacteria as a food source (xenic cultures) or without bacteria, in an enriched cell-free nutrient (axenic) medium that contains antimicrobial agents to inhibit growth of contaminating bacteria.[21,99,110,127] *N. fowleri* and *Acanthamoeba* spp. are readily cultured from CSF, brain, and lung tissue on various non-nutrient agars or with low concentrations of nutrients, in the presence of a lawn of living or killed bacteria (e.g., most commonly nonmucoid strains of *Escherichia coli* or *Enterobacter* spp.).[79,99,127] Amebae feed on the lawn of bacteria and grow to cover the plate after 1 to 2 days' incubation at 37°C. Culture plates are incubated at 37°C and examined under low power daily for 10 days.[79,127]

Amebae can be visualized directly through the back of the inverted agar plate with a conventional microscope under low power.[99] The characteristic slow, sluglike movement of *Naegleria* trophozoites that is readily seen in a wet mount of CSF is difficult to distinguish on an agar plate.[99] The ability of *Naegleria* to transform into the

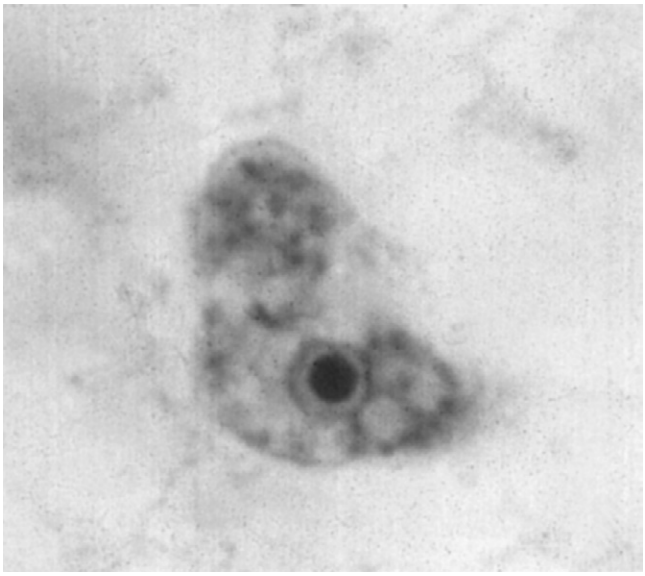

FIGURE 222-7 ■ Trichrome stain of a trophozoite of *Naegleria fowleri.*

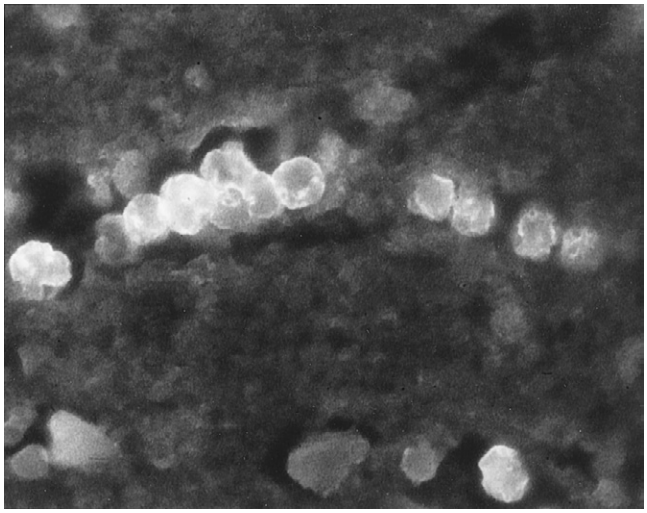

FIGURE 222-8 ■ Indirect fluorescent antibody examination of *Naegleria fowleri.* (Courtesy Dr. G. S. Visvesvara, Centers for Disease Control and Prevention.)

flagellated stage after suspension in distilled water is useful diagnostically.[99,127] Trophozoites of *Acanthamoeba* spp. move slowly by extrusion of multiple, finger-like pseudopodia (acanthopodia).

Culture and isolation of *Balamuthia* is more challenging. Generally, *B. mandrillaris* is more fastidious, does not use bacteria as a food source, grows slowly, and requires a heavily enriched basic medium. *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* all grow vigorously, however, and produce detectable cytopathic effects in mammalian cell cultures (monkey kidney, HeLa, and lung fibroblast tissue), which can be examined for cytopathic effect.[79,130] Typically, *Balamuthia* grows slowly and may require several weeks to proliferate.[130] Use of a positive control culture is advised to ensure correct methodology and to aid recognition.[99]

Although no outbreaks of infection caused by free-living amebae in laboratory workers have been reported, care must be taken when handling specimens to avoid getting culture materials on skin and in cuts or abrasions. Specimens should be handled in a biologic safety cabinet by technologists wearing masks and gloves.[79,127]

Serology

Although specific *N. fowleri* antibody has been shown in survivors of primary encephalitis, serologic tests are of no use diagnostically.[111] Onset of primary amebic meningoencephalitis is rapid, and almost all patients die before significant antibody production can occur. In contrast, because the clinical courses of *Acanthamoeba* spp. and *B. mandrillaris* infections usually are chronic, robust antibody responses have time to develop. Detection of serum or CSF antibody against *Acanthamoeba* spp. or *Balamuthia* by indirect immunofluorescence staining has helped establish the diagnosis in many cases.[25,60,107,124] By this method, amebae fixed on a slide bind antibody present in the patient's serum or CSF, which then is detected by fluorescein-conjugated, antihuman antibody.

Serologic testing has been most useful as an epidemiologic tool. Although the incidence of infection with free-living amebae seems low, results of seroepidemiology studies suggest that human exposure to amebae occurs frequently. Sera from healthy adults and children contain antibody against *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris.*[24,51,74,102,105] Detection of antibodies to *N. fowleri* and *Acanthamoeba* spp. in asymptomatic adult and pediatric populations from North Carolina, Virginia, and Pennsylvania in the United States and from Czechoslovakia and New Zealand presumably reflects unavoidable environmental contact with these ubiquitous organisms.[15,74] All specimens from a group of 93 asymptomatic patients from New Zealand contained anti-*Naegleria* and anti-*Acanthamoeba* IgG and IgM antibodies.[24] Antibodies were neutralizing against *Acanthamoeba* spp. but not against *N. fowleri.* In virtually all cases of infection caused by *Acanthamoeba* spp., disease occurs in the setting of immunocompromise or in an immunologically privileged site, such as the cornea. Whether neutralizing antibodies play a role in the very low incidence of infections in healthy individuals, despite seemingly frequent environmental exposure, is unknown.

Immunofluorescent antibody staining of serum for antibodies against *Acanthamoeba* spp. and *B. mandrillaris* has proved to be a rapid noninvasive diagnostic test in many clinical cases.[29,60,135] Potentially, this test may be useful clinically for establishing an earlier definitive diagnosis and initiation of therapy. Because the presence of *Balamuthia* antibodies may represent asymptomatic environmental exposure, confirmation of results of immunofluorescent antibody testing by histology, indirect immunofluorescent staining of tissue, or polymerase chain reaction (PCR) of CSF is advised. More recently, immunofluorescent antibody staining of serum from patients with encephalitis, predominantly from California, revealed seven previously unrecognized or misdiagnosed cases of *Balamuthia* and one case of *Acanthamoeba* encephalitis.[105] Most serum specimens showed some evidence of antibody (<1 : 64), which was attributed to cross-reactivity or subclinical exposure to contaminated soil. A cutoff value of greater than 1 : 64 for *Balamuthia* serology was considered positive pending results of confirmatory tests (e.g., histology, immunohistochemistry, or PCR).[105] Low levels of antibody may reflect preceding subacute disease or contact with environmental amebae.[105]

Molecular Diagnostics

Although microscopic assessment of a wet-mount preparation or histology may be useful in experienced hands, definitive identification in most centers awaits results of specimens sent out for immunofluorescence tests. Advances in molecular diagnostics offer the real possibility, however, of sensitive, specific, and rapid diagnosis being made by any laboratory with the capability to perform PCR. PCR of DNA extracted from frozen brain tissue has been used to confirm *N. fowleri* infection diagnosed by histopathology and immunofluorescent staining.[20] Similarly, nested and real-time PCR provide rapid, sensitive, and specific screening of environmental and recreational water sources for the presence of *N. fowleri.*[75,93] PCR, using mitochondrial 16S rRNA gene DNA as the target, has been used to detect *B. mandrillaris* in clinical samples of CSF and formalin-fixed brain and lung tissue of patients with encephalitis.[119,136] These assays have the ability to detect a single ameba per reaction mixture.[136]

Finally, the development of a multiplex real-time PCR assay for simultaneous detection of *N. fowleri*, *Acanthamoeba* spp., and *B. mandrillaris* in clinical specimens of CSF and brain tissue offers the possibility of highly sensitive and specific rapid confirmatory diagnosis of CNS infection by free-living ameba and the chance of initiating earlier, more effective treatment.[90] The assay is sensitive down to detection of a single ameba in a specimen and is species specific for *N. fowleri* and *B. mandrillaris* and genus specific for *Acanthamoeba.*[90]

TREATMENT

Mortality among patients with primary and granulomatous amebic encephalitis is greater than 95 percent.[8,108] Indeed, there are only a handful of known survivors of

primary amebic meningoencephalitis.[49] The high fatality rate undoubtedly is due partly to late diagnosis or misdiagnosis and late initiation of potentially effective treatment. Inclusion of amebic infection in the differential diagnosis, in the appropriate clinical setting, and timely administration of effective antiamebic treatment are emphasized in successful cases.[11,88] In the future, availability of real-time PCR may facilitate accurately establishing an early diagnosis. Although no randomized trials of optimal treatment for infection caused by free-living amebae have been performed, potentially effective treatment is available if the condition is recognized early.

If infection is suspected, treatment should not be withheld pending the results of confirmatory testing. Treatment regimens are based on results of in vitro susceptibility, in vivo animal experiments, and empirical treatment of individual cases. If amebic infection is suspected, accurate determination of the species is important to ensure optimal treatment. No one drug is effective against all free-living amebae (e.g., amphotericin is the drug of choice for *N. fowleri* infection but is neither amebistatic nor amebicidal for *Acanthamoeba* spp. and varies in its efficacy against *Balamuthia*).[30,106] Previously, authors have suggested initiating treatment with a combination of agents such as amphotericin B and flucytosine, which have activity against *Naegleria* and *Acanthamoeba* or *Balamuthia*, until amebae can be identified definitively.[134] Among newer antibiotics, miltefosine and voriconazole are potentially attractive as treatments for infection caused by free-living amebae. Both drugs penetrate into brain tissue and have a low toxicity profile. Although differences in susceptibility exist among strains, voriconazole has activity in vitro against *Acanthamoeba* spp. and *N. fowleri*, whereas miltefosine has in vitro activity against *Acanthamoeba* spp. and *Balamuthia*.[104]

Amebic Meningoencephalitis

N. fowleri is exquisitely susceptible in vitro to amphotericin B, which remains the treatment of choice for primary amebic meningoencephalitis.[108] All successfully treated cases have included amphotericin B in the treatment regimen. *Naegleria* also is susceptible in vitro to azithromycin and miconazole, with synergism in vitro and in the animal model with the combination of amphotericin and miconazole or tetracycline.[30,43,111,115,126] Treatment regimens in cases with successful outcomes have included intravenous amphotericin, high-dose intravenous and intrathecal amphotericin and miconazole, oral rifampin, intravenous sulfisoxazole, and intravenous and intrathecal amphotericin in combination with oral rifampin.[4,11,111] In at least one reported case, intravenous and intrathecal amphotericin B was combined with intravenous and intrathecal miconazole and with oral rifampin.[49] Rifampin has yielded conflicting results in in vitro testing.[44] Liposomal amphotericin B seems less effective in vitro and in the animal model than conventional amphotericin (10-fold higher minimum inhibitory concentration) and is not recommended.[44] Promising results in mice have been reported with chlorpromazine and to a lesser extent miltefosine and voriconazole.[128] In contrast to *Acanthamoeba* spp. and *B. mandrillaris*, *N. fowleri* remains a trophozoite and does not encyst in tissues, so after it is destroyed by antimicrobial therapy, infection with *N. fowleri* should not recur.

Optimal treatment for CNS disease caused by *Acanthamoeba* spp. has not been established, and the prognosis remains grim, with few reports of survivors.[108] Most cases are diagnosed at autopsy or a few days before death, leaving little time to evaluate treatment. Treatment successes have been attributed to initiation of treatment before spread of infection into the CNS. Combination treatment is favored because no single drug is active against trophozoites and cysts, and many drugs are amebistatic rather than amebicidal.[73] Among the few reports of successful treatment, orally administered trimethoprim-sulfamethoxazole, ketoconazole, and rifampin were associated with a favorable outcome in two apparently immunocompetent children with CNS disease.[113] Fluconazole, rifampin, metronidazole, and sulfadiazine were used successfully in a 64-year-old immunocompetent host with early disease.[88] Still other regimens have included pentamidine isethionate, 5-fluorocytosine, and itraconazole.[128]

Similarly, Slater and colleagues[114] reported successful treatment of disseminated cutaneous acanthamebiasis in a renal transplant recipient using 4 weeks of intravenous pentamidine, topical chlorhexidine, and 2 percent ketoconazole followed by maintenance oral itraconazole. A combination of pentamidine, 5-fluorocytosine, itraconazole, and topical chlorhexidine gluconate/ketoconazole cream was used to treat disseminated acanthamebiasis successfully in a lung transplant recipient.[86] Pentamidine is considered the most suitable treatment before CNS invasion has occurred because of its low penetration into the CNS. Because of its low penetration across the blood-brain barrier and nephrotoxicity, 5-fluorocytosine was preferred to pentamidine for CNS infection and in renal transplant recipients.[30,73] The anti-*Leishmania* drug miltefosine has been found to be useful both for systemic infections and amebic keratitis.[128]

Optimal treatment for *B. mandrillaris* infection also remains to be determined. Reports of successful treatment for granulomatous encephalitis caused by *Balamuthia* based on empirical regimens are few. If it is recognized early in its course, however, a more favorable outcome apparently is possible. According to Visvesvara, in the 100 cases reported only seven patients have survived, including four patients treated with a combination of flucytosine, pentamidine isethionate, fluconazole, sulfadiazine, and a macrolide such as azithromycin switched to clarithromycin (better CSF penetration), whereas some patients also received a phenothiazine (thioridazine or trifluoperazine).[25,128] Pentamidine treatment frequently was discontinued or interrupted because of side effects. Treatment failures have been reported in patients treated with similar regimens, however, possibly because of late initiation of treatment.[39] Pentamidine and azithromycin were more effective in vitro than fluconazole, flucytosine, or sulfasalazine.[108] Some researchers have postulated, however, that some of these drugs may be synergistic in vivo.[108] Because of concern about reactivation from dormant cysts in the brain, both patients remained on long-term fluconazole and on sulfadiazine

or clarithromycin prophylaxis. The drug miltefosine has also been used. One patient underwent excision of a brain abscess while undergoing treatment.

Use of corticosteroids to treat cerebral edema and inflammation seems to exacerbate *Acanthamoeba* infection. A precipitous decline in the clinical condition of one patient has been reported after administration of corticosteroid therapy.[39] At present, the risk of corticosteroid treatment probably outweighs any potential benefit and is best avoided.[39,73,95]

Amebic Keratitis

If untreated, amebic keratitis invariably progresses to visual loss and enucleation. Although amebic keratitis is more amenable to antimicrobial treatment than systemic infection, treating it is still difficult. The presence of *Acanthamoeba* cysts in deeper corneal layers renders exposure to adequate drug levels problematic. In addition, *Acanthamoeba* cysts are resistant to most antibacterial agents at concentrations that are achievable but nontoxic to the cornea. As with systemic infection, establishing the diagnosis early is required for treatment to be most effective.

In the early stages of infection, prolonged frequent application of drug is recommended. Combination treatment is preferred during prolonged treatment because of concerns for development of resistance. Nonetheless, cures have been reported with a variety of topical agents; the cationic antiseptic chlorhexidine gluconate and 0.02 percent polyhexamethylene biguanide (PHMB), a swimming pool disinfectant, alone or in combination with propamidine are among the drugs of choice for amebic keratitis.[32,67,108,109] Both drugs are effective against trophozoites and are well tolerated in the eye.[67] Alternatively, two diamidines, propamidine isethionate and hexamidine, can be used or chlorhexidine can be used along with diamidine.[128] Visvesvara reports that they should be used hourly as drops day and night for 48 hours initially, followed by hourly drops by day only for another 72 hours and, then, because of epithelial toxicity, tapered to 2 hours by day 5 for 3 to 4 weeks. Neomycin and oral azoles (e.g., miconazole or itraconazole) also have proved useful. Newer agents, such as myristamidopropyl dimethylamine and miltefosine, are effective *Acanthamoeba* cysticidal agents, possess excellent antifungal and antibacterial activity, and show promise in treating recalcitrant keratitis.[52,100,108]

Many cases of keratitis, particularly when chronic or where medical treatment alone fails, require keratoplasty and corneal grafting. In these cases, prolonged medical treatment aims to cure or control disease to allow for successful transplantation. Timing of surgery is controversial. Antiamebic drugs usually are continued for months after surgery to prevent late excystation of residual dormant cysts.[5] Use of topical or systemic corticosteroid to treat severe pain or inflammation is controversial. Severe complications have been described in clinical cases, and dexamethasone induces encystment and increases cytopathogenicity of emerging trophozoites in animal models.[82,120]

Role of *Acanthamoeba* Species as Reservoirs of Intracellular Pathogens

Although most bacteria are prey for free-living amebae, some bacteria have evolved to survive uptake by amebae. Many *Acanthamoeba* spp. act as environmental reservoirs of recognized intracellular pathogens for humans, including *Coxiella burnetii*, *Legionella pneumophila*, *Mycobacterium* spp., *Pseudomonas aeruginosa*, and *Cryptococcus neoformans*, and for potential emerging pathogens, such as *Rickettsia*-like organisms and *Parachlamydia* spp.[45,46,50,76,122] Some ameba-resistant microorganisms coexist with amebae as endobacterial symbionts, whereas others exploit the amebae as hosts for survival, for multiplication, or as vectors of disease.[7] Free-living amebae act as a "Trojan horse" in the transmission of bacterial passengers, such as for *L. pneumophila* or *M. avium*, or as an "evolutionary crib" for the selection of virulence traits and for adaptation of microorganisms to life within human macrophages, such as for *C. neoformans*.[7,46]

For *L. pneumophila*, prior multiplication within free-living amebae, principally *Acanthamoeba* spp., seems to be a prerequisite for acquisition of infection in humans.[121] In addition, many bacteria, such as *M. avium*, survive protected from chlorination, biocides, antibiotics, and desiccation within ameba cysts.[72,119] Similarly, growth of *M. avium* in amebae results in enhanced survival in macrophages. Researchers also have postulated that free-living amebae may have adapted some microorganisms for successful intracellular survival within macrophages.[40,116] Free-living amebae also apparently promote virulence traits and antimicrobial resistance in *L. pneumophila*, *M. avium*, and *C. neoformans*.[18,19,84] Researchers have postulated that capsule formation evolved in *C. neoformans* in response to selective pressure to resist environmental amebae. Production and maintenance of a capsule allows *Cryptococcus* to survive in human macrophages.[116] Some microorganisms resistant to amebae seem to behave as endosymbionts and to influence development and maintenance of virulence traits that enhance pathogenicity of free-living amebae.[38,46] Clinical and environmental *Naegleria* spp. do not support bacterial endosymbionts.

PREVENTION

Infections caused by free-living amebae are reported to be characterized by an absence of distinguishing symptoms, delayed diagnosis or misdiagnosis, and an almost uniformly poor prognosis without early appropriate treatment. Prevention of infection is of paramount importance. Strategies to prevent *Naegleria* infection exist; *N. fowleri* is susceptible to 1 μg/mL or less of chlorine, and adequate chlorination of water in swimming pools or hot tubs is a simple and sensible preventive measure. If water temperatures are higher, use of increased concentrations of chlorine (>2 to 3 mg/L) is recommended.[80] In certain parts of the world, environmental levels of *N. fowleri* are monitored to ensure it is safe to swim and that the probability of acquiring an infection is

low.[13] A prudent measure is to educate the public on the potential dangers associated with exposure to warm bodies of fresh or thermally polluted water, especially when temperatures are high and water levels are low, and to avoid immersion, diving, jumping, or horseplay that might force water up the nasal passages or disturb sediment.[137] If a neti pot is used for nasal irrigation, only bottled or filtered water should be used.

Prevention of infection caused by *Acanthamoeba* spp. or *B. mandrillaris* is problematic and involves regular inspections of hot-water tanks, plumbing, and eye-wash stations. *Acanthamoeba* keratitis associated with use of contact lenses is preventable, however. Contact lens wearers should be aware of the importance of proper care of the lenses. Lenses should be cleansed in sterile benzalkonium-preserved saline or disinfected by heat. Use of homemade cleansing solutions and wearing lenses while swimming are contraindicated.

Although the incidence of infections caused by free-living amebae is small at present, the number of these infections is likely to grow with increasing numbers of transplant patients and patients with AIDS. Similarly, some researchers have predicted that climate change associated with global warming may increase further the numbers of free-living amebae in the environment.[20]

NEW REFERENCES SINCE THE SIXTH EDITION

49. Heggie TW. Swimming with death: *Naegleria fowleri* infections in recreational waters. *Travel Med Infect Dis* 2010;**8**:201–6.
128. Visvesvara GS. Amebic meningoencephalitides and keratitis: challenges in diagnosis and treatment. *Curr Opin Infect Dis* 2010;**23**: 590–4.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 223

Toxoplasmosis

James B. McAuley • Kenneth M. Boyer • Jack S. Remington • Rima L. McLeod

Toxoplasma gondii is an obligate intracellular protozoan parasite (phylum Apicomplexa, class Sporozoasida, order Eucoccidiida).[214] Infection may be clinically inapparent or result in disease, called toxoplasmosis. This parasite was observed first in 1908 by Nicolle and Manceaux[159,160,196] in mononuclear cells in the spleen and liver of a North African rodent, the gundi *(Ctenodactylus gundi)*. The organism soon was identified as a cause of disease in other animals,[209] and in 1923, Janku[96] first recognized a case in a human. He described a parasite found in the retina of an infant; it was recognized later by Levaditi[121] as *Toxoplasma*.

In 1937, Wolf and Cowen[222] reported a case of congenital granulomatous encephalitis that they considered to be caused by an "encephalitozoon." Sabin,[185] who previously had encountered *T. gondii* in guinea pigs, made the correct diagnosis. The discovery of *Toxoplasma* as a cause of disease acquired later in life has been credited to Pinkerton and Weinman,[166] who in 1940 described a generalized fatal illness caused by this organism in a young man. In retrospect, a case of acquired toxoplasmosis had been reported in 1908 by Darling.[47] In 1948, Sabin and Feldman[186] described a serologic test, the dye test that allowed numerous investigators to study the epidemiologic and clinical aspects of toxoplasmosis and to define the spectrum of disease in humans. In 1969, some 60 years after the parasite was discovered, Frenkel and colleagues[60,69,70] established that *Toxoplasma* is a coccidian protozoan and that its definitive host is the cat.

ORGANISM AND TRANSMISSION

T. gondii exists in three forms or stages: the proliferative stage, or tachyzoite; a tissue cyst that contains bradyzoites; and an oocyst, within which sporozoites develop. The oocyst is formed during the intestinal epithelial stage of infection, exclusively in members of the cat family, the definitive host. The tachyzoite and tissue cyst are found in the extraintestinal tissues of cats and in other mammalian and avian hosts. Each stage of the organism has antigens in common with the other stages and unique antigens. Many of these antigens have been cloned, sequenced, and localized to microanatomic structures.[144] Humans may become infected from any of the three forms as detailed later.

The tachyzoite form (Fig. 223-1A-C) is crescent shaped or oval, is approximately 3 by 7 μm, and is seen

REFERENCES

1. Alsam S, Kim KS, Stins MA, et al. *Acanthamoeba* interactions with human brain microvascular endothelial cells. *Microb Pathogen* 2003;**35**:235–41.
2. Anderson K, Jamieson A. Primary amoebic meningoencephalitis. *Lancet* 1972;**7756**:902–3.
3. Anzil AP, Rao C, Wzolek MA, et al. Amebic meningoencephalitis in a patient with AIDS caused by a newly recognized opportunistic pathogen, *Leptomyxid amoeba*. *Arch Pathol Lab Med* 1991;**115**: 21–5.
4. Apley J, Clarke SK, Roome AP, et al. Primary amoebic meningoencephalitis in Britain. *BMJ* 1970;**1**:596–9.
5. Auran JD, Starr MB, Jakobiec FA. *Acanthamoeba* keratitis. *Cornea* 1987;**6**:2–26.
6. Bakardjiev A, Azimi PH, Ashouri N, et al. Amebic encephalitis caused by *Balamuthia mandrillaris*: report of four cases. *Pediatr Infect Dis J* 2003;**22**:447–52.
7. Barker J, Brown MRW. Trojan horses of the microbial world: protozoa and the survival of bacterial pathogens in the environment. *Microbiology* 1994;**140**:1253–9.
8. Barnett NDP, Kaplan AM, Hopkin RJ, et al. Primary amoebic meningoencephalitis with *Naegleria fowleri*: clinical review. *Pediatr Neurol* 1996;**15**:230–4.
9. Bloch KC, Schuster FL. Inability to make a premortem diagnosis of *Acanthamoeba* species infection in a patient with fatal granulomatous amebic encephalitis. *J Clin Microbiol* 2005;**43**:3003–6.
10. Booton GC, Visvesvara GS, Byers TJ, et al. Identification and distribution of *Acanthamoeba* species genotypes associated with nonkeratitis infections. *J Clin Microbiol* 2005;**43**:1689–93.
11. Brown RL. Successful treatment of primary amebic meningoencephalitis. *Arch Intern Med* 1991;**151**:1201–2.
12. Butt CG. Primary meningoencephalitis. *N Engl J Med* 1966; **274**:1473–6.
13. Cabanes P-A, Wallet F, Pringuez E, Perrin P. Assessing the risk of primary amoebic meningoencephalitis from swimming in the presence of environmental *Naegleria fowleri*. *Appl Environ Microbiol* 2001;**67**:2927–31.
14. Callicott JH. Amebic meningoencephalitis due to free-living amoebas of the *Hartmanella* (*Acanthamoeba-Naegleria* group). *Am J Clin Pathol* 1968;**49**:84.
15. Cerva L. *Acanthamoeba culbertsoni* and *Naegleria fowleri*: occurrence of antibodies in man. *J Hyg Epidemiol Microbiol* 1989;**33**:99–103.
16. Cerva L, Novak K. Amoebic meningoencephalitis: sixteen fatalities. *Science* 1989;**160**:92.
17. Cerva L, Novak K, Culbertson GC. An outbreak of acute fatal amoebic meningoencephalitis. *Am J Epidemiol* 1968;**88**:336–44.
18. Cirillo JD, Cirillo SL, Falkow S, Tomkins LS. Growth of *Legionella pneumophila* in *Acanthamoeba castellani* enhances invasion. *Infect Immun* 1994;**62**:3254–61.
19. Cirillo JD, Falkow S, Tomkins LS, Bermudez LE. Interaction of *Mycobacterium avium* with environmental amoebae enhances virulence. *Infect Immun* 1997;**65**:3759–67.
20. Cogo PE, Scaglia M, Gatti S, et al. Fatal *Naegleria fowleri* meningoencephalitis, Italy. *Emerg Infect Dis* 2004;**10**:1835–7.
21. Culbertson CG, Ensminger PW, Overton WM. The isolation of additional strains of pathogenic *Hartmennella* sp.: proposed culture method for application of biological material. *Am J Pathol* 1965; **35**:383–7.
22. Culbertson CG, Smith JW, Cohen HK, Minner J. Experimental infection of mice and monkeys by *Acanthamoeba*. *Am J Pathol* 1959;**35**:185–97.
23. Culbertson CG, Smith JW, Minner JD. *Acanthamoeba*: observations on animal pathogenicity. *Science* 1958;**127**:1506.
24. Cursons RT, Brown TJ, Keys EA, et al. Immunity to pathogenic free-living amoebae: role of humoral antibody. *Infect Immun* 1980;**29**:401–7.
25. Deetz TR, Sawyer MH, Billman G, et al. Successful treatment of *Balamuthia* amoebic encephalitis: presentation of two cases. *Clin Infect Dis* 2003;**37**:1304–12.
26. De Jonckheere JF. A century of research on the amoeboflagellate genus *Naegleria*. *Acta Protozool* 2002;**41**:309–42.
27. De Jonckheere JF, van de Voorde H. Differences in destruction of cysts of pathogenic and nonpathogenic *Naegleria* and *Acanthamoeba* by chlorine. *Appl Environ Microbiol* 1976;**31**:294–7.
28. Denney CF, Iragui VJ, Uber-Zak LD, et al. Amebic meningoencephalitis caused by *Balamuthia mandrillaris*: case report and review. *Clin Infect Dis* 1997;**25**:1354–8.
29. Dorsch MM, Cameron AS, Robinson BS. The epidemiology and control of primary amoebic meningoencephalitis with particular reference to South Australia. *Trans R Soc Trop Med Hyg* 1983; **77**:372–7.
30. Duma RJ, Finley R. In vitro susceptibility of pathogenic *Naegleria* and *Acanthamoeba* species to a variety of therapeutic agents. *Antimicrob Agents Chemother* 1976;**10**:370–6.
31. Dunnebacke TH, Schuster FL, Yagi S, Bootone GC. Isolation of *Balamuthia* amoebas from the environment. *J Eukarot Microbiol* 2003;**50**:510–11.
32. Elder MJ, Dart K. Chemotherapy for *Acanthamoeba* keratitis. *Lancet* 1995;**345**:791–2.
33. Ferrante A, Bates EJ. Elastase in the pathogenic free-living amoebae *Naegleria* and *Acanthamoebae* spp. *Infect Immunol* 1988; **56**:3320–1.
34. Feurst PA, Booton GC, Visvesvara GS, Byers TJ. Genotypic identification of non-keratitis infections caused by the opportunistically pathogenic amoeba genus *Acanthamoeba*. *J Eukaryot Microbiol* 2003;**50**(Suppl.):512–13.
35. Fiordalisi I, Christie J, Moffitt C. Primary amebic meningoencephalitis—North Carolina, 1991. *MMWR Morb Mortal Wkly Rep* 1992;**41**:437–40.
36. Fowler M, Carter RF. Acute pyogenic meningitis probably due to *Acanthamoeba* sp.: a preliminary report. *BMJ* 1965;**2**:740–2.
37. Friedland LR, Raphael SA, Deutsch ES, et al. Disseminated acanthamoebiasis infection in a child with symptomatic human immunodeficiency virus infection. *Pediatr Infect Dis J* 1992;**11**:404–7.
38. Fritsche TR, Sobek D, Gautom RK. Enhancemant of in vitro cytopathogenicity by *Acanthamoeba* spp. following acquisition of bacterial endosymbionts. *FEMS Microbiol Lett* 1998;**166**:231–6.
39. Galarza M, Cuccia V, Sosa FP, Monges JA. Pediatric granulomatous cerebral amebiasis: a delayed diagnosis. *Pediatr Neurol* 2002; **26**:153–6.
40. Gao LY, Harb OS, Kwaik YA. Utilization of similar mechanisms by *Legionella pneumophila* to parasitize two evolutionarily distinct host cells, mammalian macrophages and protozoa. *Infect Immunol* 1997;**65**:4738–46.
41. Gelman BB, Rauf SJ, Nader R, et al. Amoebic encephalitis due to *Sappinia diploidea*. *JAMA* 2001;**285**:2450–1.
42. Gonzalez MD, Gould E, Dickinson G, et al. Acquired immunodeficiency syndrome associated with *Acanthamoeba* and other opportunistic organisms. *Arch Pathol Lab Med* 1986;**110**:749–51.
43. Goswick SM, Brenner GM. Activities of azithromycin and amphotericin B against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. *Antimicrob Agents Chemother* 2003;**47**:524–8.
44. Goswick SM, Brenner GM. Activities of therapeutic agents against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. *J Parasitol* 2003;**89**:837–42.
45. Greub G, Raoult D. *Parachlamydiaceae*: potential emerging pathogens. *Emerg Infect Dis* 2002;**8**:625–30.
46. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. *Clin Microbiol Rev* 2004;**17**:413–33.
47. Griesemer DA, Barton LL, Reese CM, et al. Amebic meningoencephalitis caused by *Balamuthia mandrillaris*. *Pediatr Neurol* 1994; **10**:249–54.
48. Hadas E, Mazur T. Proteolytic enzymes of pathogenic and nonpathogenic strains of *Acanthamoebae* spp. *Trop Med Parasitol* 1993; **44**:197–200.
49. Heggie TW. Swimming with death: *Naegleria fowleri* infections in recreational waters. *Travel Med Infect Dis* 2010;**8**: 201–6.
50. Horn M, Wagner M. Bacterial endosymbionts of free-living amoebae. *J Eukaryot Microbiol* 2004;**51**:509–14.
51. Huang ZH, Ferrante A, Carter RF. Serum antibodies to *Balamuthia mandrillaris*, a free-living amoeba recently demonstrated to cause granulomatous amoebic encephalitis. *J Infect Dis* 1999;**179**: 1305–8.
52. Hughes R, Dart J, Kilvington S. Activity of the amidoamine myristamidopropyl diethylamine against keratitis pathogens. *J Antimicrob Chemother* 2003;**51**:1415–18.
53. Intalapaporn P, Suankratay C, Shuangshoti S, et al. *Balamuthia mandrillaris* meningoencephalitis: the first case in Southeast Asia. *Am J Trop Med Hyg* 2004;**70**:666–9.

54. Jahnes WG, Fullmer HM, Li CP. Free-living amoebae as contaminants in monkey tissue culture. *Proc Soc Exp Biol Med* 1957; **96**:484–8.
55. Jarolim KL, McCosh JK, Howard MJ, John DT. A light microscopy study of the migration of *Naegleria fowleri* from the nasal submucosa to the central nervous system during the early stage of primary amebic meningoencephalitis in mice. *J Parasitol* 2000;**86**: 50–5.
56. John DT, Howard MJ. Seasonal distribution of pathogenic free-living amoebae in Oklahoma waters. *Parasitol Res* 1995;**81**: 193–201.
57. John T, Desai D, Sahm D. Adherence of *Acanthamoeba castellani* cysts and trophozoites to unworn soft contact lenses. *Am J Ophthalmol* 1989;**108**:658–64.
58. Jones BR, Visvesvara GS, Robinson NM. *Acanthamoeba polyphaga* and *Acanthamoeba* uveitis associated with fatal meningoencephalitis. *Trans Ophthalmol Soc* 1975;**95**:221–32.
59. Jorno MS Jr, Babapour R, Gurevitch A, Witt MD. Cutaneous acanthamoebiasis in AIDS. *J Am Acad Dermatol* 2000;**42**:351–4.
60. Jung S, Schelper RL, Visvesvara GS, Chang HT. *Balamuthia mandrillaris* meningoencephalitis in an immunocompetent patient: an unusual clinical course and a favorable outcome. *Arch Pathol Lab Med* 2004;**18**:466–8.
61. Katz JD, Ropper AH, Adelman L, et al. A case of *Balamuthia mandrillaris* meningoencephalitis. *Arch Neurol* 2000;**57**:1210–12.
62. Khan NA, Jarroll EL, Panjwani N, et al. Proteases as markers of differentiation of pathogenic and non-pathogenic *Acanthamoebae*. *J Clin Microbiol* 2000;**38**:2858–61.
63. Khunkitti WD, Lloyd D, Furr JR, Russell AD. *Acanthamoeba castellani*: growth, encystment, excystment and biocide susceptibility. *J Infect* 1998;**36**:43–8.
64. Kidney DD, Kim SH. CNS infections with free-living amoebas: neuroimaging findings. *AJR Am J Roentgenol* 1998;**171**:809–12.
65. Kumar R, Lloyd D. Recent advances in the treatment of amebic keratitis. *Clin Infect Dis* 2002;**35**:434–41.
66. Lares-Villa F, De Jonckheere JF, Moura HD, et al. Five cases of primary amebic meningoencephalitis in Mexicali, Mexico: study of isolates. *J Clin Microbiol* 1993;**31**:685–8.
67. Larkin DF, Kilvington S, Dart JK. Treatment of *Acanthamoeba* keratitis with polyhexamethylene biguanide. *Ophthalmology* 1992; **99**:185–91.
68. Lee SH, Levy DA, Craun GF, et al. Surveillance for waterborne-disease outbreaks—United States, 1999-2000. *MMWR Surveill Summ* 2002;**51**:1–47.
69. Lengy J, Jakovljevich R, Talis B. Recovery of hartmannelloid amoeba from purulent ear discharge. *Trop Dis Bull* 1971;**68**: 818–19.
70. Ma P, Visvesvara GS, Martinez AJ, et al. *Naegleria* and *Acanthamoeba* infection. *Rev Infect Dis* 1990;**12**:490–513.
71. Marciano-Cabral F. Biology of *Naegleria* spp. *Microbiol Rev* 1988;**52**:114–33.
72. Marciano-Cabral F. Introductory remarks: bacterial endosymbionts or pathogens of free-living amoebae. *J Eukaryot Microbiol* 2004;**51**:497–501.
73. Marciano-Cabral F, Cabral G. *Acanthamoeba* spp. as agents of disease in humans. *Clin Microbiol Rev* 2003;**16**:273–307.
74. Marciano-Cabral F, Cline ML, Bradley SG. Specificity of antibodies from human sera for *Naegleria* species. *J Clin Microbiol* 1987; **25**:692–7.
75. Marciano-Cabral F, Maclean R, Mansah A, LaPat-Polasko L. Identification of *Naegleria fowleri* in domestic water sources by nested PCR. *Appl Environ Microbiol* 2003;**69**:5864–9.
76. Marrie TJ, Raoult D, Scola BL, et al. Canadian Community-Acquired Pneumonia Study Group: *Legionella*-like and other amoebal pathogens as agents of community-acquired pneumonia. *Emerg Infect Dis* 2001;**7**:1026–9.
77. Martinez AJ. Free living amoebas: Infection of the central nervous system. *Mt Sinai J Med* 1993;**60**:271–8.
78. Martinez AJ, Schuster FL, Visvesvara GS. *Balamuthia mandrillaris*: its pathogenic potential. *J Eukaryot Microbiol* 2001;**50**(Suppl.): 6–9.
79. Martinez AJ, Visvesvara GS. Laboratory diagnosis of pathogenic free-living amoebas: *Naegleria*, *Acanthamoeba*, and *Leptomyxid*. *Clin Lab Med* 1991;**11**:861–72.
80. Martinez AJ, Visvesvara GS. Free-living, amphizoic and opportunistic amoebas. *Brain Pathol* 1997;**7**:583–98.
81. Mazur TE, Hadas E, Iwanicka I. The duration of the cyst stage and the viability and virulence of *Acanthamoeba* isolates. *Trop Med Parasitol* 1995;**46**:106–8.
82. McClellan K, Howard K, Niederkorn JY, Alizadeh H. Effect of steroids on *Acanthamoeba* cysts and trophozoites. *Invest Ophthalmol Vis Sci* 2001;**42**:2885–93.
83. McKee T, Davis L, Blake P, et al. Primary amoebic meningoencephalitis—Georgia, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:962–4.
84. Miltner EC, Bermudez LE. *Mycobacterium avium* grown in *Acanthamoeba castellani* is protected from the effects of antimicrobials. *Antimicrob Agents Chemother* 2000;**44**:1990–4.
85. Nagington J, Watson PG, Playfair TJ, et al. Amoebic infection of the eye. *Lancet* 1974;**2**:1537–40.
86. Oliva S, Jantz M, Tiernan R, et al. Successful treatment of widely disseminated acanthamoebiasis. *South Med J* 1999;**92**:55–7.
87. Page FC. *A new key to freshwater and soil Gymnamoebae*. Ambleside, Cumbria, U.K.: Freshwater Biological Association; 1988.
88. Petry F, Torzewski M, Bohl J, et al. Early diagnosis of *Acanthamoeba* infection during routine cytological examination of cerebrospinal fluid. *J Clin Microbiol* 2006;**44**:1903–4.
89. Pritzker AS, Kim BK, Agrawal D, et al. Fatal granulomatous amebic encephalitis caused by *Balamuthia mandrillaris* presenting as a skin lesion. *J Am Acad Dermatol* 2004;**50**(Suppl.):38–41.
90. Qvarnstrom Y, Visvesvara GS, Sriram R, de Silva AJ. Mutliplex real-time PCR assay for simultaneous detection of *Acanthamoeba* spp., *Balamuthia mandrillaris*, and *Naegleria fowleri*. *J Clin Microbiol* 2006;**44**:3589–95.
91. Radford CF, Bacon AS, Dart JKG, Minassian DC. Risk factors for *Acanthamoeba* keratitis in contact lens users: a case-control study. *BMJ* 1995;**310**:1567–70.
92. Reed RP, Cooke-Yarborough CM, Jaquiery AL, et al. Fatal granulomatous amoebic encephalitis caused by *Balamuthia mandrillaris*. *Med J Aust* 1997;**115**:21–5.
93. Robinson BS, Monis PT, Dobson PJ. Rapid, sensitive, and discriminating identification of *Naegleria* spp. by real-time PCR and melting-curve analysis. *Appl Environ Microbiol* 2006;**72**:5858–63.
94. Rodriguez-Zaragoza S. Ecology of free-living amoebae. *Crit Rev Microbiol* 1994;**20**:225–41.
95. Rowen JL, Doerr CA, Vogel H, Baker CJ. *Balamuthia mandrillaris*: a newly recognized agent for amoebic meningoencephalitis. *Pediatr Infect Dis J* 1995;**14**:705–10.
96. Samples JR, Binder PS, Luibel FJ, et al. *Acanthamoeba* keratitis possibly acquired from a hot tub. *Arch Ophthalmol* 1984;**102**: 707–10.
97. Schoeman CJ, van der Vyver AE, Visvesvara GC. Primary amoebic meningoencephalitis in South Africa. *J Infect* 1993;**26**:211–14.
98. Schumacher DJ, Tien RD, Lane K. Neuroimaging findings in rare amebic infections of the central nervous system. *AJNR Am J Neuroradiol* 1995;**16**:930–5.
99. Schuster FL. Cultivation of pathogenic and opportunistic free-living amoebas. *Clin Microbiol Rev* 2002;**15**:342–54.
100. Schuster FL, Buck S, Rosenthal RA, Schlech BA. Efficacy of myristamidopropyl dimethylamine. *J Eukaryot Microbiol* 2003; **50**(Suppl.):520–1.
101. Schuster FL, Dunnebacke TH, Booton GC, et al. Environmental isolation of *Balamuthia mandrillaris* associated with a case of amoebic encephalitis. *J Clin Microbiol* 2003;**41**:3175–80.
102. Schuster FL, Glaser C, Gilliam S, Visvesvara GS. Survey of sera from encephalitis patients for *Balamuthia mandrillaris* antibody. *J Eukaryot Microbiol* 2001;**48**:10S–12S.
103. Schuster FL, Glaser C, Honarmand S, et al. *Balamuthia* amebic encephalitis risk among Hispanic Americans. *Emerg Infect Dis* 2004;**10**:1510–12.
104. Schuster FL, Guglielmo BJ, Visvesvara GS. In-vitro activity of miltefosine and voriconazole on clinical isolates of free-living amoebas: *Balamuthia mandrillaris*, *Acanthamoeba* spp., and *Naegleria fowleri*. *J Eukaryot Microbiol* 2006;**53**:121–6.
105. Schuster FL, Honarmand S, Visvesvara GS, Glaser CA. Detection of antibodies against free-living amoebae *Balamuthia mandrillaris* and *Acanthamoeba* species in a population of patients with encephalitis. *Clin Infect Dis* 2006;**42**:1260–5.
106. Schuster FL, Visvesvara GS. Axenic growth and drug sensitivity studies of *Balamuthia mandrillaris*, an agent of amebic meningoencephalitis in humans and other animals. *J Clin Microbiol* 1996; **34**:385–8.

107. Schuster FL, Visvesvara GS. Free-living amoeba as opportunistic and non-opportunistic pathogens of humans and animals. *Int J Parasitol* 2004;**34**:1001–27.
108. Schuster FL, Visvesvara GS. Opportunistic amoebae: challenges in prophylaxis and treatment. *Drug Resist Updates* 2004;**7**:41–51.
109. Seal DV. *Acanthamoeba* keratitis. *BMJ* 1994;**308**:1116–17.
110. Seidel JS. Primary amoebic meningoencephalitis. *Pediatr Clin North Am* 1985;**32**:881–92.
111. Seidel JS, Harmatz P, Visvesvara GS, et al. Successful treatment of primary amebic meningoencephalitis. *N Engl J Med* 1982;**306**:346–8.
112. Singh P, Kochhar R, Vashista RK, et al. Amebic meningoencephalitis: spectrum of imaging findings. *AJNR Am J Neuroradiol* 2006;**27**:1217–21.
113. Singhal T, Bajpai A, Kalra V, et al. Successful treatment of *Acanthamoeba* meningitis with combination oral antimicrobials. *Pediatr Infect Dis J* 2001;**20**:623–7.
114. Slater CA, Sickel JZ, Visvesvara GS, et al. Brief report: successful treatment of disseminated *Acanthamoeba* infection in an immunocompromised patient. *N Engl J Med* 1994;**331**:85–7.
115. Soltow SM, Brenner GM. Synergistic activity of azithromycin and amphotericin B against *Naegleria fowleri* in vitro and in a mouse model of primary amebic meningoencephalitis. *Antimicrob Agents Chemother* 2007;**51**:23–7.
116. Steenbergen JN, Shuman HA, Casadevall A. *Cryptococcus neoformans* interactions with amoebae suggest an explanation for its virulence and intracellular pathogenic strategy in macrophages. *Proc Natl Acad Sci U S A* 2001;**98**:15245–50.
117. Stehr-Green JK, Bailey TM, Visvesvara GS. *Acanthamoeba* keratitis in soft contact lens wearers: a case control study. *JAMA* 1987;**258**:57–60.
118. Stehr-Green JK, Bailey TM, Visvesvara GS. The epidemiology of *Acanthamoeba* keratitis in the United States. *Am J Ophthalmol* 1989;**107**:331–6.
119. Steinert M, Birkness K, White E, et al. *Mycobacterium avium* bacilli grow saphrophytically in coculture with *Acanthamoeba polyphaga* and survive within cyst walls. *Appl Environ Microbiol* 1998;**64**:567–613.
120. Stern GA, Butross M. Use of corticosteroids in combination with antimicrobial drugs in the treatment of infectious corneal disease. *Ophthalmology* 1991;**98**:847–53.
121. Stothard DR, Schroeder-Diedrich JM, Awwad MH, et al. The evolutionary history of the genus *Acanthamoeba* and the identification of eight new 18S rRNA gene sequence types. *J Eukaryot Microbiol* 1998;**45**:45–54.
122. Swanson MS, Hammer BK. *Legionella pneumophila* pathogenesis: a fateful journey from amoebae to macrophages. *Annu Rev Microbiol* 2000;**54**:567–613.
123. Sykora JL, Keleti G, Martinez AJ. Occurrence and pathogenicity of *Naegleria fowleri* in artificially heated waters. *Appl Environ Microbiol* 1983;**45**:974–9.
124. Tavares M, da Costa JMC, Carpenter SS, et al. Diagnosis of first case of *Balamuthia* amoebic encephalitis in Portugal by immunofluorescence and PCR. *J Clin Microbiol* 2006;**44**:2660–3.
125. Theodore FH, Jakobiec FA, Juechter KB, et al. The diagnostic value of a ring infiltrate in *Acanthamoeba* keratitis. *Ophthalmology* 1985;**92**:1471–9.
126. Thong YH, Rowan-Kelly B, Shephard C, Ferrante A. Growth inhibition of *Naegleria fowleri* by tetracycline, rifamycin, and miconazole. *Lancet* 1977;**2**:876.
127. Visvesvara GS, editor. Pathogenic and opportunistic free-living amoebae. In: *Manual of clinical microbiology*, vol. 2. 8th ed. Washington, D.C.: ASM Press; 2003.
128. Visvesvara GS. Amebic meningoencephalitides and keratitis: challenges in diagnosis and treatment. *Curr Opin Infect Dis* 2010;**23**:590–4.
129. Visvesvara GS, Jones DB, Robinson NM. Isolation, identification and biologic characterization of *Acanthamoeba polyphaga* from the human eye. *Am J Trop Med Hyg* 1975;**24**:784–90.
130. Visvesvara GS, Martinez AJ, Schuster FL, et al. *Leptomyxid* amoeba, a new agent of amebic meningoencephalitis in humans and animals. *J Clin Microbiol* 1990;**28**:2750–6.
131. Visvesvara GS, Schuster FL, Martinez AJ. *Balamuthia mandrillaris* N.G., N. Sp., agent of amoebic meningoencephalitis in humans and other animals. *J Eukaryot Microbiol* 1993;**40**:504–14.
132. Wellings FM, Amuso PT, Chang SL, Lewis AL. Isolation and identification of pathogenic *Naegleria* from Florida lakes. *Appl Environ Microbiol* 1977;**34**:661–7.
133. White JML, Barker RD, Salisbury JR, et al. Granulomatous amoebic encephalitis. *Lancet* 2004;**364**:220.
134. Wiley CA, Safrin RE, Davis CE, et al. *Acanthamoeba* meningoencephalitis in a patient with AIDS. *J Infect Dis* 1987;**155**:130–3.
135. Wilhelmus KR, Osato MS, Font RL, et al. Rapid diagnosis of *Acanthamoeba* keratitis using calcofluor white. *Arch Ophthalmol* 1986;**104**:1309–12.
136. Yagi S, Booton GC, Visvesvara GS, Schuster FL. Detection of *Balamuthia* mitochondrial 16S rRNA gene DNA in clinical specimens by PCR. *J Clin Microbiol* 2005;**43**:3192–7.
137. Yoder JS, Blackburn BG, Craun GF, et al. Surveillance for waterborne-disease outbreaks associated with recreational water—United States, 2001-2002. *MMWR Surveill Summ* 2004;**53**:1–46.

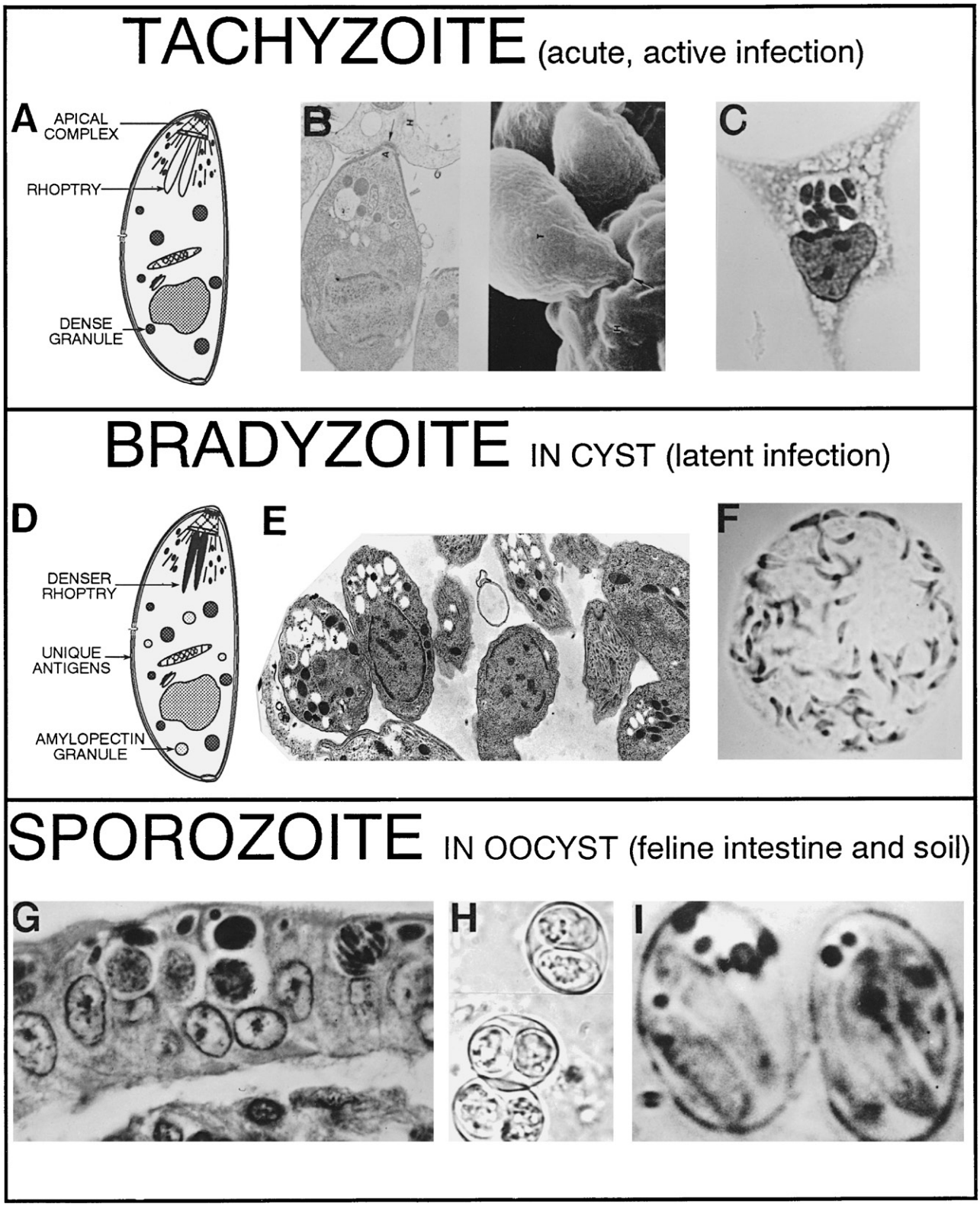

FIGURE 223-1 ■ Stages of *Toxoplasma gondii*. **A,** Schematic diagram of a tachyzoite. **B,** Transmission and scanning electron micrographs of a tachyzoite invading a host cell. **C,** Light micrograph of tachyzoites replicating within a parasitiferous vacuole in the host cell cytoplasm. **D,** Schematic diagram of a bradyzoite. **E,** Transmission electron micrograph of a cyst containing bradyzoites (*arrow* indicates amylopectin granules). **F,** Light micrograph of a cyst containing bradyzoites. **G,** Development of oocysts in cat intestine. **H,** Oocysts in the lumen of cat intestine. **I,** Sporulating oocysts that contain sporozoites. (From Boyer KM, McLeod RL. *Toxoplasma gondii* [toxoplasmosis]. In: Long SS, Pickering LK, Prober CG, editors. *Principles and practice of pediatric infectious diseases*. New York: Churchill Livingstone; 1997, p. 1423.)

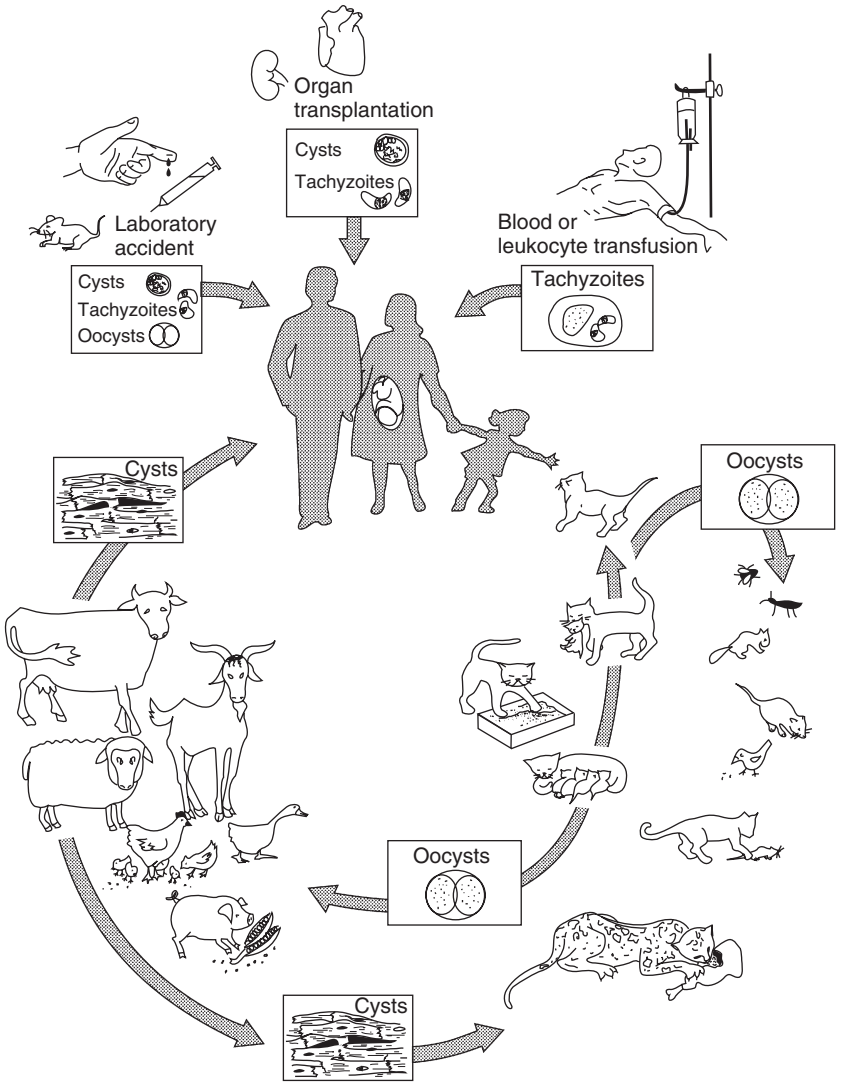

FIGURE 223-2 ■ Life cycle of *Toxoplasma gondii*. Cats are definitive hosts, with humans and other mammals being intermediate hosts. (From Remington JS, McLeod R. Toxoplasmosis. In: Braude AI, editor. *International textbook of medicine. Medical microbiology and infectious disease.* vol. II. Philadelphia: Saunders; 1981, p. 1818.)

during the acute stage of infection. It stains well with Wright or Giemsa stain. Ultrastructural features include the apical complex of microtubules and rings, secretory organelles called rhoptries, and a chloroplast-like structure with its own unique DNA.[31,61,214] Tachyzoites can invade all mammalian cells except perhaps non-nucleated red blood cells. They cannot withstand freezing and thawing, desiccation, or brief exposure to gastric or duodenal digestive juices. After penetration occurs, the tachyzoite multiplies by endodyogeny, ultimately causing disruption of the cell and cell death.

The bradyzoite (Fig. 223-1D-F) is able to persist in encysted form in all tissues and cause a chronic (latent) infection for the entire lifespan of the infected host. Cysts are demonstrable in tissues the first week of infection and range in size from approximately 10 to 100 μm. They have an argyrophilic wall but stand out most clearly from surrounding tissue when stained with periodic acid–Schiff stain. Usually, no inflammatory reaction occurs around cysts. Because this form may persist for many years in the tissues of clinically normal children and adults, its demonstration in histologic sections does not signify recent infection. Peptic or tryptic digestive fluids immediately disrupt the cyst wall, but the liberated bradyzoites (which resemble the tachyzoite form under light microscopy) can survive in these fluids for several hours, which allows time for invasion of local cells. The cyst is destroyed by heating to 66°C (150°F), by freezing (below –20°C [–4°F]) and thawing, and by desiccation. It can survive for some months at refrigeration temperatures (4°C [39°F]) if it is in tissue. Infection in humans may be acquired by eating inadequately cooked meat that contains cysts (Fig. 223-2). In carnivorous animals, infection may be acquired by eating raw meat or prey species that contain encysted organisms or by ingesting sporulated oocysts as discussed subsequently.

The oocyst form (Fig. 223-1G-I) is found only in feces of members of the cat family, the definitive host for *Toxoplasma*, and is the result of gametogony and schizogony, which occur in the intestinal epithelium.[71,94] The oocyst is ovoid and approximately 10 to 12 μm. Infected cats may shed 10 million oocysts each day, which may be excreted for 3 weeks after primary (acute) infection develops but rarely thereafter. Excreted oocysts become

infectious only after they undergo sporulation (eight sporozoites form in each oocyst); sporulation occurs 1 to 21 days (most commonly 2 to 8 days) after excretion, depending on temperature and the availability of oxygen. The oocyst is far more resilient than the other life cycle forms and can survive for months in water and for 1 year or more in moist soil. Ingestion of sporulated oocysts transmits the infection. This fact suggests that the oocyst plays a major role in transmission by the fecal-oral route in animal reservoirs and by inadvertent ingestion in humans.

The genome of *T. gondii* consists of 8×10^7 base pairs distributed among 12 chromosomes. Much of the genome has been sequenced, a genetic linkage map has been constructed, and three clonal genotypes (I, II, and III) have been identified.[46,111,193,214] Some of the factors that regulate stage conversion from tachyzoite to bradyzoite have been defined.[144,214] The shikimate pathway, an enzyme system that is absent in animals, is important for the production of many essential aromatic compounds in plants, bacteria, and fungi. This biochemical pathway has been identified in *Toxoplasma* and in other apicomplexan parasites, such as *Plasmodium falciparum* and *Cryptosporidium parvum*.[178] Biochemical inhibition of this pathway suppresses the growth of these parasites.[175] Understanding of the regulation of life cycle stages and the unique pathways of intermediary metabolism in *T. gondii* may provide new approaches to therapy.

EPIDEMIOLOGY

Acquired Infection

The cat is central in the parasite's life cycle, and humans and other mammals are intermediate hosts. If infected tissue (e.g., a mouse) is consumed by a susceptible cat, the sexual cycle is induced in the cat intestine; oocysts are excreted and are infectious for mammals and birds, in which the life cycle (tachyzoites and cysts) is perpetuated. Cats shed oocysts for only brief durations (days to weeks) but in extremely large numbers (1×10^7/day). A cat is more likely to become infected if it is an outdoor cat or a predator or is fed fresh, uncooked table scraps. Humans come in contact with cat excrement either directly (e.g., emptying the litter pan) or by more insidious means (e.g., cleaning a horse stall, weeding the garden, playing in a sandbox). Meat for human consumption containing tissue cysts may serve as a source of infection if it is eaten raw or undercooked. Accidental ingestion may occur under circumstances that are unsuspected. In a recent report, only 49 percent of women with acute primary toxoplasmosis during pregnancy who gave birth to infected children could identify significant risk factors for sporozoite acquisition.[18] Examples of inadvertent exposures include an Amish farm wife preparing sausage, a couple consuming steak tartare in an expensive restaurant, and a rancher butchering a deer.[19] Mode of infection probably varies geographically according to cultural dietary and soil exposure practices. In Chile, 43 percent of women with recent infection had antibody to sporozoite-specific protein (oocysts), suggesting ingestion of undercooked infected meat as the main mode of acquisition.[155]

Infection with *Toxoplasma* occurs commonly in humans. In the United States, the prevalence of seropositivity has been determined from studies of military recruits, from surveys in major cities, and most comprehensively from the National Health and Nutrition Examination Surveys (NHANES). The NHANES are periodic cluster sample surveys representing the entire population of the United States. In the early 1960s, the overall prevalence in military recruits was 14 percent, with the lowest rates in the Mountain (3%) and Pacific (8%) states and the highest rates in the Northeast (20%) and East South Central (19%) states.[194] A more recent study from the late 1980s showed a similar geographic distribution, but prevalence rates were approximately a third lower throughout the country.[194] This decline has continued as documented in the NHANES.

NHANES III (1988-1994) showed an overall seroprevalence in individuals of child-bearing age (12 to 49 years old) of 14.1 percent compared with a rate of 9 percent for the same age cohort in the 1999-2004 NHANES.[101] The consistent gradual increase in seropositivity with increasing age in the surveys suggests dietary exposure as a major mode of acquisition in the United States.[102] Previous estimates were that approximately 8 percent of commercial beef, 20 percent of commercial pork, and 25 percent of commercial lamb contain encysted *Toxoplasma* bradyzoites.[172] More recent data suggest much lower levels of viable tissue cysts in commercial meat.[59] The NHANES III failed to show an association, however, with specific dietary habits such as meat consumption. *Toxoplasma* infection was associated with foreign birth, low educational level, living in crowded conditions, and having a soil-related occupation.[102]

During these decades of declining prevalence of *Toxoplasma* infection, the rate of cat ownership has increased in the United States.[101,102] The decline may be explained partly by improved meat safety, with decreasing prevalence of viable *Toxoplasma* in commercial meat.[59] A common practice is to freeze commercial meat before releasing it for sale, which, combined with the use of home freezers, also may be having a beneficial effect.[193] However, recent trends toward free-range organically raised meat could increase the risk of *T. gondii* contamination of meat.[99] Additional modes of transmission, such as waterborne,[11,16,58] have been described. Their exact contribution to the overall prevalence of toxoplasmosis in the United States is unclear, however. During the decades of the NHANES studies, an increasing proportion of U.S. water supplies have added filtration to decrease *Giardia* and *Cryptosporidium* transmission. This filtration also is likely to eliminate *Toxoplasma* oocysts.[102]

Urban studies in U.S. women of child-bearing age have yielded variable prevalence rates: Denver, 3 percent; Palo Alto, 10 percent; Chicago, 12 percent; Boston, 14 percent; and Birmingham, 30 percent.[172] Internationally, rates also vary: Japan, 6 percent; Australia, 23 percent; United Kingdom, 35 percent; Poland, 36 percent; Belgium, 53 percent; Tahiti, 77 percent; and France, 87 percent.[172] The prevalence of infection is determined partly by climate; colder regions and regions that are hot and dry or at high altitude have lower rates of human infection than warmer and moister regions do.

Prevalences are declining in Europe, as in the United States, owing to educational efforts and improved meat safety.

Despite declining prevalence in the United States, it is estimated that more than 1 million new infections occur each year with resultant ocular lesions in more than 21,500 persons, of which approximately 4800 are symptomatic.[100] The rate of hospitalization for human immunodeficiency virus (HIV)–associated toxoplasmosis has declined since the advent of effective antiretroviral therapy; however, hospitalization for non–HIV-associated toxoplasmosis has remained steady from 1993 to 2008.[103]

Common-source outbreaks of acute acquired *Toxoplasma* infection have been authenticated. Unique and clear-cut sources that have been documented to be highly likely[16] include exposure to aerosolized cat excrement in a riding stable[204] and consumption of unpasteurized goat milk.[187] In other circumstances, extensive investigations have failed to yield convincing answers.[130,191] Because of the possibility of common-source exposure, however, families of patients with acute acquired infection should be evaluated for subclinical infection.[127] In 2012, the Centers for Disease Control and Prevention included an exercise in foodborne toxoplasmosis on a college campus in its tabletop training for emerging infectious disease threats.[154]

Accidental self-inoculation with a needle contaminated with *Toxoplasma* has resulted in many acquired infections in laboratory workers. Infections also have been ascribed to blood transfusions and organ transplantation.[68]

Congenital Infection

Infection of the fetus occurs during maternal parasitemia, with subsequent infection of the placenta caused by tachyzoites.[70,172] Placental infection represents an important intermediary step between maternal and fetal infection. A delay of 16 weeks between placental infection and subsequent infection of the fetus has been noted and is termed the prenatal incubation period.[172] Congenital infection is thought to occur, with rare exception, only after primary maternal infection with *Toxoplasma*.[52,172] The only two well-documented exceptions to this rule occurred in immunodeficient pregnant women.[148,172] Maternal reinfection leading to congenital toxoplasmosis also has been reported very rarely.[75,172] In a large series that carefully studied more than 800 women who had given birth to congenitally infected children, not a single congenital infection occurred in subsequent pregnancies.[172] A general consensus is that only acute infection beginning *during* pregnancy can lead to congenital infection. Despite the existence of a few well-documented cases of biopsy-proven lymphadenopathic toxoplasmosis occurring 2 months *before* conception that resulted in congenitally infected infants,[209] this pattern of occurrence also seems to be extremely rare.[75] Congenital toxoplasmosis has occurred in twins and triplets, with occasional discordance.[37,172]

The incidence of congenital toxoplasmosis in a population is determined by the risk of a woman's experiencing primary infection while she is pregnant. This incidence depends on three factors: (1) the age-specific incidence of primary infection during the child-bearing years, (2) the age distribution of pregnant women in the population, and (3) the fetal transmission rate in primary infection. A theoretical analysis by Frenkel[69] based on these three factors, assuming a child-bearing age group of 20 to 29 years and an overall fetal transmission rate of 40 percent, showed that maximal risk occurs when the age-specific incidence rate in a population is 3 to 5 percent per year, which corresponds to a seropositivity rate of 50 to 80 percent in women of child-bearing age. In such a population, the predicted prevalence of congenital toxoplasmosis would be 4.4 to 4.6 per 1000 pregnancies. At higher age-specific incidences, rates of congenital toxoplasmosis would be lower because nearly all pregnant women already would be infected chronically. At lower incidences, rates also would be lower but because of less frequent infection.

Studies in the early 1970s revealed an incidence of congenital toxoplasmosis in the United States of approximately 2 per 1000 births.[1,12,112,172] More recently the incidence of congenital infection in the United States has been documented to be 0.08 per 1000 births (1 in 12,000 births) by IgM screening of blood specimens collected on filter paper from newborns in Massachusetts and New Hampshire.[87] This is a conservative estimate as the methodology detects approximately 75 percent of infections. The figure compares with a rate of 3 to 10 per 1000 live births in Paris and Vienna. In Massachusetts, a case-control study involving 14 years of newborn screening for congenital toxoplasmosis found that birth of the mother outside the United States, particularly in Cambodia and Laos, and the educational level and higher gravidity of the mother were strongly predictive of congenital infection.[97]

PATHOLOGY

After intracellular multiplication at the site of entry occurs, tachyzoites are disseminated in blood and may invade all organs and tissues. The severity of infection probably is a function of strain virulence, host susceptibility, tissue tropism, and the immune privilege of the eyes and central nervous system (CNS).[84,85,129,172] Proliferation of tachyzoites results in death of the invaded cells and, eventually, small necrotic foci surrounded by intense cellular reaction. With recovery, cysts without an inflammatory response around them may persist in the brain, retinas, bone marrow, lymph nodes, liver, spleen, and lungs and in skeletal, heart, and smooth muscle.[68-70]

In active infection of the CNS, *Toxoplasma*-filled cells are scattered throughout the gray matter, where they produce diffuse meningoencephalitis with miliary microglial nodules and foci of perivascular inflammation.[35] Large lesions may mimic cerebral tumors. Areas of basal ganglial and periventricular inflammation may calcify in the fetus.[56,163] Obstruction of the aqueduct of Sylvius or the foramen of Monro may result in hydrocephalus in congenital infection.

The enlarged lymph nodes in acute acquired toxoplasmosis show characteristic pathologic changes that may

warrant making a presumptive diagnosis.[57,209] Such changes include reactive follicular hyperplasia, epithelioid histiocytes encroaching on and blurring the margins of germinal centers, and distention of subcapsular and trabecular sinuses by monocytoid cells. Tachyzoites and cysts rarely are seen.[57]

In the eye, active chorioretinitis begins in the retina with severe inflammation and necrosis and exudation into the vitreous. Single or multiple foci occur, and secondary involvement of the choroid is always present. Tachyzoites and cysts have been found in these lesions.[20,172,176,177]

In immunocompromised hosts, widespread necrotizing lesions may be seen in the heart, muscle, brain, and other organs. These lesions, particularly those involving the CNS, have been found frequently with reactivation of toxoplasmosis in patients with acquired immunodeficiency syndrome (AIDS).[125]

IMMUNOLOGY

Cell-mediated immune responses are the major immunologic mechanisms that prevent reactivation of *T. gondii* in a chronically infected normal host.[32,45] Numerous effector mechanisms contribute to protection in murine models and human infection: $CD8^+$ cytotoxic T lymphocytes, $CD4^+$ T lymphocytes, monocyte oxidative mechanisms, production of interferon-γ by natural killer cells, and activation of macrophages by interferon-γ and tumor necrosis factor-α.* Killing of the parasite within activated macrophages is associated with the intracellular production of nitric oxide. Interleukin-2 and interleukin-12 enhance host resistance to *T. gondii;* interleukin-10 impairs the ability of macrophages to kill the parasite.[77]

In murine models, evidence exists for genetic determination of host resistance to infection.[21,22,129,139] Presence of the DQ3 allele seems to be associated with toxoplasmic encephalitis in patients with AIDS and hydrocephalus in infants with congenital toxoplasmosis.[22] Successful immunization of mice[23] and sheep[24,25] with *T. gondii* components and live tachyzoites of an "incomplete" strain[26] raises the possibility of developing a human vaccine.[98,140,214]

CLINICAL SYNDROMES

The disease in children may be considered in three categories: postnatally acquired, congenital, and ocular (which may be congenital or acquired).[171] Clinically apparent infection in older children may have been acquired recently or caused by reactivation of latent congenital or postnatally acquired infection. Congenital and acquired infections usually are subclinical, but congenital infection ultimately leads to serious sequelae in most cases.

Acute Acquired Toxoplasmosis

Acquired *Toxoplasma* infection is asymptomatic in most cases and frequently goes unrecognized because only 10 to 15 percent of infected individuals have clinical symptoms and signs. In certain outbreaks related to infection by oocysts, however, more than half of infected patients have been symptomatic.[204] The most common findings are lymphadenopathy and fatigue without fever.[132] The nodes are discrete and may or may not be tender. They do not suppurate. The groups of nodes most commonly involved are the cervical, suboccipital, supraclavicular, axillary, and inguinal. The adenopathy may be localized or involve multiple areas, including the retroperitoneal and mesenteric nodes. Uncommonly, the lymphadenopathy is accompanied by fever, malaise, fatigue, sore throat, and myalgia, a picture that closely simulates that of infectious mononucleosis but without serologic evidence of acute Epstein-Barr virus infection. The differential diagnosis of the lymphadenopathy often includes lymphoma. Chorioretinitis may develop during acute acquired infection, but it does not occur commonly.[130,153] The liver may be involved, and liver function test results may reflect hepatocellular damage. In individuals with normal immunologic function and no severe underlying disease, the infection usually is self-limited and rarely requires treatment.

In contrast, more severe and frequently fulminant infections are seen in patients receiving immunosuppressive therapy, in patients who have disease of the bone marrow or reticuloendothelial system, in patients with agammaglobulinemia, in recipients of bone marrow or stem cell transplants, and in patients with AIDS.* Encephalitis and rarely pneumonitis and myocarditis are the most important localized forms that may be encountered in immunocompromised patients. In toxoplasmic encephalitis, the predominant neurologic symptoms are headache, disorientation, and drowsiness. These symptoms may simulate aseptic meningitis or a mass lesion. In view of the various clinical manifestations of CNS involvement, it is important to consider toxoplasmosis whenever evidence of acute CNS disease is present.

Epidemiologic studies have suggested a correlation between *Toxoplasma* infection and a variety of neurologic and psychiatric illnesses in adults, including schizophrenia, suicide rates in women, and Alzheimer disease.[8,115,124] These intriguing findings require additional research before any conclusions can be reached.

Congenital Toxoplasmosis

Congenital infection usually is the result of an asymptomatic acute infection in the mother.[19,65] In a small proportion of cases, spontaneous abortion, prematurity, or stillbirth may result. Congenital toxoplasmosis has a wide spectrum of clinical manifestations but is subclinical in approximately 75 percent of infected newborns. When it is clinically apparent, it may mimic other diseases of the newborn.

Involvement of the CNS is a hallmark of congenital *Toxoplasma* infection. The presence of chorioretinitis, intracranial calcifications, and hydrocephalus is referred to as the classic triad of congenital toxoplasmosis. Fever, hydrocephalus or microcephaly, hepatosplenomegaly,

*References 78, 88, 105, 107, 109, 192, 200.

*References 49, 76, 95, 122, 123, 125, 131, 184, 208.

TABLE 223-1 **Vertical Transmission and Severity of Disease for *Toxoplasma gondii* by the Timing of Maternal Infection during Pregnancy**

	Trimester of Maternal Infection		
	First	Second	Third
Mild disease (%)	6	18	10
Overall transmission rate (%)	15	30	60
Proportion of infected children with specific disease severity			
Severe disease (%)	41	8	0
Stillborn or perinatal death (%)	35	7	0
Subclinical (asymptomatic) (%)	18	67	90

jaundice, convulsions, chorioretinitis (usually bilateral), cerebral calcifications, and cerebrospinal fluid (CSF) abnormalities (markedly increased protein concentration and mononuclear pleocytosis) are considered the classic features of severe congenital toxoplasmosis.[56,146,172] These manifestations occurred commonly in an early series of patients reported by Eichenwald[64] (Table 223-1). The case-fatality rate was 12 percent. In survivors in this series, sequelae included mental retardation in 86 percent; convulsions, spasticity, and palsies in almost 75 percent; and severely impaired vision in 60 percent.[64,74,180] Other occasional findings included rash (maculopapular, petechial, or both), myocarditis, pneumonitis and respiratory distress, hearing defects, an erythroblastosis-like picture, thrombocytopenia, lymphocytosis, monocytosis, and nephrotic syndrome. *Toxoplasma* has been detected in the inner ear and mastoid, with the associated inflammation resulting in deafness. An ascending flaccid paralysis with myelitis also has been reported.[172] These signs now are known to be most typical of the severe form of the infection in the absence of treatment.

The often subclinical nature of congenital toxoplasmosis in the newborn was shown in a French prospective study of 154 mothers who had acquired *Toxoplasma* infection during pregnancy and did not receive treatment.[52] Nine pregnancies (6%) ended in stillbirth, and 85 (55%) resulted in the birth of infected live-born infants. Of the live-born infants who were infected, 64 (75%) had subclinical infection, 14 (16%) had mild disease, and only 7 (8%) had clinically obvious severe disease at birth.[52,172]

The risk for transmission to the fetus varies significantly with the trimester of gestation in which the mother becomes infected. For untreated women, the rate is approximately 25 percent in the first trimester, 54 percent in the second trimester, and 65 percent in the third trimester; these figures are minimal estimates derived from placental isolation studies.[52] In contrast, the severity of clinical disease in congenitally infected infants is related inversely to the gestational age at the time of primary maternal infection (Table 223-2). In two studies from France,[40,52] severe disease or fetal or neonatal death occurred in approximately 40 to 79 percent of infants born to mothers with first-trimester infection, in 15 to

TABLE 223-2 **Signs and Symptoms Occurring before Diagnosis or during the Course of Untreated Acute Congenital Toxoplasmosis***

Signs and Symptoms	Frequency of Occurrence† in Patients with Neurologic Disease‡	Frequency of Occurrence† in Patients with Generalized Disease§
Infants	***N* = 108**	***N* = 44**
Chorioretinitis	102 (94)	29 (66)
Abnormal spinal fluid	59 (55)	37 (84)
Anemia	55 (51)	34 (77)
Jaundice	31 (29)	35 (80)
Splenomegaly	23 (21)	40 (90)
Convulsions	54 (50)	8 (18)
Fever	27 (25)	34 (77)
Intracranial calcification	54 (50)	2 (4)
Hepatomegaly	18 (17)	34 (77)
Lymphadenopathy	18 (17)	30 (68)
Vomiting	17 (16)	21 (48)
Hydrocephalus	30 (28)	0 (0)
Diarrhea	7 (6)	11 (25)
Pneumonitis	0 (0)	18 (41)
Microcephalus	14 (13)	0 (0)
Eosinophilia	6 (4)	8 (18)
Rash	1 (1)	11 (25)
Abnormal bleeding	3 (3)	8 (18)
Hypothermia	2 (2)	9 (20)
Cataracts	5 (5)	0 (0)
Glaucoma	2 (2)	0 (0)
Optic atrophy	2 (2)	0 (0)
Microphthalmos	2 (2)	0 (0)
Children ≥4 Years Old	***N* = 70**	***N* = 31**
Mental retardation	62 (89)	25 (81)
Convulsions	58 (83)	24 (77)
Spasticity and palsies	53 (76)	18 (58)
Severely impaired vision	48 (69)	13 (42)
Hydrocephalus or microcephalus	31 (44)	2 (6)
Deafness	12 (17)	3 (10)
Normal	6 (9)	5 (16)

*In 152 infants and 101 of these same patients after ≥4 years of follow-up.
†Data indicate numbers of patients, with percentages in parentheses.
‡Patients with central nervous system diseases in the first year of life.
§Patients with non-neurologic diseases during the first 2 months of life.
Modified from Eichenwald HG. A study of congenital toxoplasmosis, with particular emphasis on clinical manifestations, sequelae, and therapy. In: Siim JC, editor. Human toxoplasmosis. Copenhagen: Munksgaard; 1960, p. 44.

18 percent with second-trimester infection, and in 0 to 3 percent with third-trimester infection.

Another French prospective study of 210 congenitally infected infants born to mothers who were identified to have primary infection acquired during pregnancy revealed significant morbidity in 94 newborns.[40] Overall, 2 (0.9%) cases were fatal, 21 (10%) were severe, and 71 (33.8%) were mild; 116 cases were asymptomatic. Approximately 40 to 45 percent of the mothers in this study had been treated with spiramycin during pregnancy. These observations confirm that most congenital infections are subclinical at birth. Obvious manifestations occur infrequently. In the same study by Couvreur and associates,[40] 116 infants initially were thought not to be infected on the basis of a routine newborn physical examination. On more intensive examination, however, 39 (34%) of them were found to have one or more abnormalities. Twenty-two (19%) had abnormal CSF findings on lumbar puncture, 17 (15%) had chorioretinitis on indirect ophthalmoscopic examination, and 10 (9%) had intracranial calcifications on head radiographs or computed tomography (CT) scans. Guerina and colleagues[87] made remarkably similar observations in the congenitally infected newborns that they identified in New England by heel-stick blood sampling.

Some infected children without overt disease as neonates may escape serious sequelae of the infection; however, a significant number (24% to 85%) develop chorioretinitis, strabismus, blindness, hydrocephalus or microcephaly, cerebral calcifications, developmental delay, epilepsy, or deafness months or years later. Three studies provide data that define the incidence of these late sequelae.[112,113,116]

In a study from Paris,[116] 26,402 apparently healthy infants were tested routinely for serologic evidence of *Toxoplasma* infection at 10 months of age. Of these infants, 51 had positive serologic results for *Toxoplasma*, indicative of congenital infection. None had been treated for *Toxoplasma* infection in infancy. Of the 51, 5 were found to have chorioretinal scars by ophthalmologic examination, and chorioretinal lesions had developed in another 4 children by the time they reached 4 years of age, the longest period of follow-up. Some eventually lost functional vision in one eye. Three had intracranial calcifications.

Similarly, in a study from Holland in which a cohort of 1821 pregnant women were screened serologically, 12 congenitally infected infants were detected, and 11 of them were monitored for 20 years.[112,113] Of the 11, 5 were treated as neonates for 1 month only and 6 were not. Of the 5 treated infants, 4 had eye disease as neonates and 1 had parasites in CSF, which prompted therapy. Of these 11, 9 (82%) had chorioretinal scars by the time they reached 20 years of age, and 4 of them, including 2 who were initially normal, had severe visual impairment or blindness in one eye. The onset of disease leading to blindness occurred as late as 18 years of age. No neurologic or cognitive sequelae were observed.

The results of this prospective study are similar to those previously reported by Wilson and associates[220] from Alabama in a retrospective analysis of patients from the United States. During a mean follow-up period of 8.3 years, sequelae developed in 11 of 13 congenitally infected children (85%) who had no signs of disease on detailed examination in the newborn period. Sequelae included chorioretinal lesions in 11 children (85%), severe neurologic disability in 1 child (8%), and mental retardation in 2 children (15%). Sequelae first were noted at ages ranging from 1 month to 9 years. These 13 children were detected either as a result of routine screening of cord serum for IgM antibodies to *Toxoplasma*,[10] performed because acute *Toxoplasma* infection was diagnosed in the mother,[2,221] or as a result of nonspecific findings in the neonatal period (2 were small for gestational age and 1 had transient borderline thrombocytopenia).

That treatment may decrease the frequency or severity of sequelae is suggested by the Alabama and Paris studies, in which chorioretinitis developed only in untreated infants between 10 months and 4 years old. Taken together, these data indicate that most congenitally infected children who receive no or brief treatment, including children with inapparent infection as neonates, experience untoward sequelae during childhood. Current treatment regimens—prolonged for at least 1 year and often initiated before birth—seem to be associated with substantially less frequent and severe sequelae (see section on treatment).

Congenital toxoplasmosis may mimic or coexist with infection by other organisms. It must be differentiated from other perinatal infections caused by cytomegalovirus, herpes simplex virus, rubella virus, *Treponema pallidum* (syphilis), HIV-1, lymphocytic choriomeningitis virus, and certain bacteria (e.g., *Listeria*). Herpesvirus and cytomegalovirus infections, syphilis, and rubella may cause chorioretinitis; cytomegalovirus and HIV-1 infections may cause encephalopathies associated with cerebral calcifications. Degenerative encephalopathies and storage diseases in older children also may resemble congenital toxoplasmosis.

Many infants or preschool children with coexisting HIV infection and toxoplasmosis have been described.[147,148] In at least six of these patients, HIV and *Toxoplasma* infections seem to have been acquired in utero. Of these six patients, all but one had clinical evidence of CNS disease, and in most of these children, the CNS disease was associated with other findings common in congenital infection. These findings included hepatosplenomegaly, fever, and chorioretinitis and were evident at birth (one infant) or developed by the time the infant reached 4 months of age. Two of the other infants remained asymptomatic; one was treated for *Toxoplasma* infection and the other was not treated. One additional infant who acquired HIV infection at 18 months of age from a blood transfusion died at 5 years of age of toxoplasmic encephalitis. The findings in this patient resembled those in adults with AIDS and toxoplasmic encephalitis.[125] The advent of highly active antiretroviral therapy (HAART) along with the widespread use of trimethoprim-sulfamethoxazole for *Pneumocystis jiroveci* pneumonia prophylaxis has decreased greatly the frequency of clinical toxoplasmic encephalitis in children and adults with HIV infection.

Ocular Toxoplasmosis

In active congenital toxoplasmosis, the retinal lesions usually are bilateral.[88,146,161] In older children, chorioretinitis may involve only one eye and may be the sole manifestation of congenital toxoplasmosis. Toxoplasmic chorioretinitis, even in older children and adults, usually is considered to be the result of congenital infection.[153] A report of 38 children diagnosed with symptomatic chorioretinitis between 2002 and 2004 in the United Kingdom confirmed that 58 percent of cases were the result of congenital infection.[80] In some studies, *Toxoplasma* infection has accounted for 5 percent of severe visual impairments in children.[106] Active lesions on the fundus appear as white or yellowish foci with elevated, edematous margins surrounded by a zone of hyperemia (Fig. 223-3). Cells and fibrinous exudate in the vitreous may obscure the fundus. Older lesions appear as glial scars, and in areas in which the retina has been destroyed, the choroid and sclera are visible. Around the depigmented areas, deposition of pigment from the destroyed retina is present. The position of the lesion may be macular, juxtapapillary, or peripheral.

Patients may experience loss of central vision (caused by a perimacular lesion), hazy vision (caused by accumulated exudate), or "floaters" (caused by reactivation of peripheral foci). Neonates or infants with toxoplasmic eye disease may have microphthalmos, small corneas, posterior cortical cataract, anisometropia, strabismus, and nystagmus.[146,212] Strabismus and nystagmus in a child of any age should raise the possibility of congenital toxoplasmosis. The appearance of lesions in the fundus is not specific for toxoplasmosis. Similar lesions may occur with other, less common granulomatous diseases in the eye, such as toxocariasis, lymphocytic choriomeningitis, cat-scratch disease, and tuberculosis. Chorioretinitis may be recurrent, most commonly with reactivation at the margins of preexisting lesions.

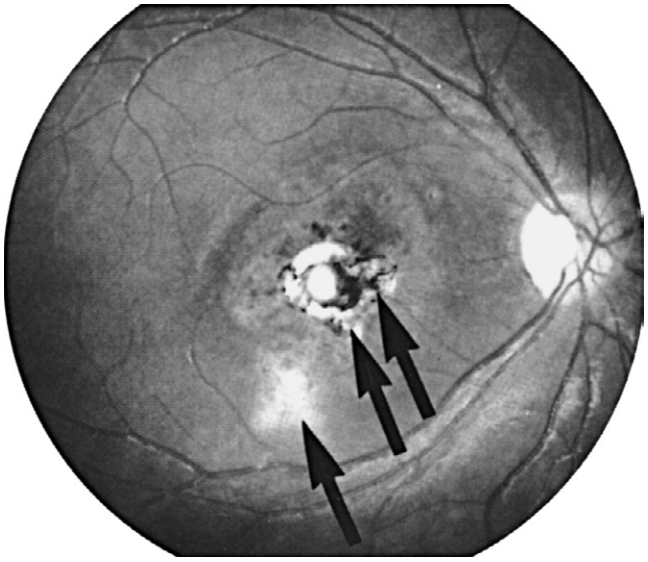

FIGURE 223-3 ■ Example of active and quiescent chorioretinitis caused by congenital toxoplasmosis in a 12-year-old patient. The active lesion *(single arrow)* is a satellite of an old chorioretinal scar *(two arrows)*. (From Mets M, Holfels E, Boyer KM, et al. Eye manifestations of congenital toxoplasmosis. *Am J Ophthalmol* 1996;**122**:309-24. Published with permission from the American Journal of Ophthalmology. Copyright © by The Ophthalmic Publishing Company.)

LABORATORY DIAGNOSIS

Acute infection can be diagnosed by isolation of *T. gondii* from blood or body fluids; demonstration of tachyzoites in histologic sections of tissue or cytologic preparations of body fluids; characteristic lymph node histology; demonstration of *Toxoplasma* cysts in the placenta, fetus, or neonate; detection of the *Toxoplasma* genome by polymerase chain reaction (PCR) in body fluids; and characteristic serologic test results.[17,150,151,173,221] Each of these methods is discussed, but serologic tests are emphasized because they are the most common and readily available method to establish the diagnosis (Table 223-3).[221]

Serologic Methods

Measurements of IgG Antibody

The most useful tests for detection of IgG antibodies to *Toxoplasma* include the Sabin-Feldman dye test,[186] indirect immunofluorescent antibody (IFA) test, agglutination tests, and enzyme-linked immunosorbent assay (ELISA). Titers in ELISA are expressed in different terms for different commercial kits, precluding a discussion of IgG ELISA titers in relation to the diagnosis of acute infection.[55,213,221,226] In these tests, IgG antibodies appear within the first week of primary infection and reach peak titers (usually ≥1 : 500) within 1 to 2 months; detectable titers usually persist for life. Although the dye test is the most reliable method, it is available in only a few reference laboratories. Immunoblot compares well to the dye test and may detect antibody slightly earlier than other methods, but it is also not widely available.[104,120]

IFA test and ELISA are the most widely available and, when properly performed, yield results similar to those obtained with the dye test; however, many laboratories use commercially available kits that are not consistently reliable. Because the performance of kits varies greatly, clinicians must be familiar with the variations if they are comparing results from different laboratories.[120] Some sera that contain antinuclear antibodies yield false-positive IFA test results. The direct agglutination tests that currently are available use formalin-fixed tachyzoites or antigen-coated latex particles, are simple to perform, and are accurate.[194,221]

Initially after primary infection occurs, the avidity of IgG antibody for *T. gondii* antigen is low. Urea dissociates low-avidity antibodies, and a test has been developed that determines the percentage of antibodies that resist elution by 6 mol/L urea. This test is useful in the first 12 weeks of gestation in that the presence of high-avidity antibodies excludes the acquisition of infection in the previous 3 months.[26,221,225]

TABLE 223-3 **Guidelines for Interpretation of Serologic Tests for Toxoplasmosis**

IgG	IgM	IgG Avidity	Interpretation
Positive	Negative	—	Remote infection, immune. IgG avidity testing is best used when both IgG and IgM are positive and timing of infection is crucial, as in pregnancy (see below).
			For evaluation of infection in the newborn, false-negative IgM results occur in approximately 25% of cases. If infection is suspected in this setting, further testing (dye test, IgM EIA, IgA EIA, IgE EIA/ISAGA, PCRs, ideally with paired maternal serologies—see text) is necessary in a reference laboratory (*Toxoplasma* Serology Laboratory, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301, 415-326-8120).
Positive	Positive or equivocal	High	Infection within the past 18 mo but likely >12 wk ago. If pregnant and beyond first trimester, consider sending specimen to reference laboratory for dye test, repeat IgG avidity and IgM EIA, IgA EIA, IgE EIA/ISAGA, and AC/HS testing (see above).
Positive	Positive or equivocal	Low	Infection within the past 12 wk. Consider sending specimen to a reference laboratory (see above) to time infection more accurately (dye test, repeat IgG avidity and IgM EIA, IgA EIA, IgE EIA/ISAGA, and AC/HS) in the setting of pregnancy.
Equivocal	Negative	—	Indeterminate. Test a new specimen or consider a different assay (IFA or ELISA).
Equivocal	Equivocal	—	Indeterminate. Test a new specimen or consider a different assay (IFA or ELISA).
Equivocal	Positive	—	Acute infection or false-positive IgM result. Test a second specimen; if IgG becomes positive or remains equivocal, consider sending specimen to a reference laboratory to time infection more accurately (dye test, IgG avidity, IgM EIA, IgA EIA, IgE EIA/ISAGA, AC/HS—see text) in the setting of pregnancy.
Negative	Negative	—	No evidence of *Toxoplasma* infection. Not immune.
Negative	Equivocal	—	Either false-positive IgM result or possible recent infection. Obtain a new specimen and retest. If infection is recent, IgM and IgG should become positive, with low IgG avidity. If repeated testing is still IgG negative and IgM equivocal, patient is likely uninfected. Consider IgM ISAGA.
Negative	Positive	—	Acute infection or false-positive IgM result. Repeat testing on new specimen. If the result is the same, it is likely a false-positive IgM result. Consider IgM ISAGA.

AC/HS, Differential agglutination test; *EIA,* enzyme immunoassay; *ELISA,* enzyme-linked immunosorbent assay; *IFA,* immunofluorescence assay; *ISAGA,* immunosorbent agglutination assay; *PCR,* polymerase chain reaction.

Other tests vary in their reliability. Indirect hemagglutination is widely available, but the results frequently are negative in newborns with congenital infection.[221] This test should *not* be used for screening of pregnant women because detectable increases in titer are delayed compared with the increases detected by ELISA and by the dye, IFA, and agglutination tests. Meaningful interpretation of changes in titer on sequential sera requires that assays on each sample be performed in the same run by a reliable laboratory.[221]

Measurements of IgM Antibody

IgM antibodies are detected most commonly by the IgM IFA test, IgM immunosorbent agglutination assay (ISAGA), or IgM ELISA. IgM antibodies appear in the first week of primary infection and peak within 1 month.[158] Depending on the sensitivity of the method used, IgM antibodies may be demonstrable for 2 to 3 months or 1 year or longer. This prolonged presence of IgM leads to the most common diagnostic error in pregnant women—the assumption that a positive IgM result indicates fetal exposure, when the maternal infection may have occurred well before pregnancy began. IgM ELISA and IgM ISAGA are much more sensitive than the IgM IFA test. Absence of IgM ELISA or IgM ISAGA antibodies in an immunologically normal older child (>1 year old) or adult essentially rules out a recently acquired infection.[220] A negative IgM IFA test result is not as sensitive in ruling out recently acquired infection. Of sera that were negative with the IgM IFA test and obtained from adults who had acquired toxoplasmosis recently, 93 percent were strongly positive in IgM ELISA.[157] IgM IFA testing detects specific IgM antibody in only 25 percent of infants with proven congenital infection, whereas IgM ELISA detects antibody in approximately 75 percent of such cases.[157] The presence of rheumatoid factor or antinuclear antibodies may cause false-positive results in IgM IFA testing.[2]

The "double-sandwich" IgM ELISA avoids the false-positive results caused by the presence of rheumatoid factor, which the infant can produce in utero, and the false-negative results caused by competition from the high levels of maternal IgG antibody that occur in the IgM IFA test.[156,221] In addition, the false-positive results in the IgM IFA test caused by antinuclear antibodies are not found in IgM ELISA.[2,156]

ISAGAs are used widely in Europe. Similar to IgM ELISA, they capture IgM on a solid surface, detect specific IgM, and involve the addition of whole formalin-fixed organisms or *Toxoplasma* antigen–coated latex particles.[54,170] These assays are available commercially

and give results comparable to those of IgM ELISA, are simpler to perform, and do not require expensive equipment.

Measurements of IgA and IgE Antibodies

Demonstration of IgA and IgE antibodies in the fetus or newborn by ELISA and ISAGA seems to be at least comparable to the demonstration of IgM antibody in sensitivity for the diagnosis of congenital *Toxoplasma* infection.[48,167,197,223] The IgA test also seems to be more sensitive than the IgM test for detection of acquired infection. Specificity remains an issue, however, so neither IgA/IgE ELISA nor IgA/IgE ISAGA has superseded the IgM test for diagnosis of acquired infection. IgA and IgE antibodies persist longer than IgM antibodies and may be useful in cases of subacute illness or when IgM titers are low.

Differential Agglutination

Acetone-fixed and formalin-fixed *T. gondii* tachyzoites may yield differing agglutination titers, depending on the acuity of the infection. The test based on this phenomenon is called the AC/HS differential agglutination test.[44] In general, a disproportionately high agglutination titer with acetone-fixed organisms suggests acute infection; a disproportionately high titer with formalin-fixed organisms suggests chronic infection. Interpretative norms for this test have been established.[44] This test, when it is combined with the dye test, IgM ELISA, IgA ELISA, IgE ELISA/ISAGA, and avidity studies, yields a "toxoplasmic serologic profile" that permits the most accurate evaluation of infection acuity in pregnant women,[152,221,224] and often it can resolve any inconclusive or discrepant results obtained in a hospital or commercial laboratory.

Nonserologic Methods

Nonserologic methods are used less commonly to establish the diagnosis of *Toxoplasma* infection because they are not widely available and require tissue specimens.[188]

Isolation of the Organism

Isolation of *Toxoplasma* from blood or body fluids (e.g., CSF) establishes that the infection is acute. In the case of a neonate, isolation from the placenta or the infant's tissues is sufficient to diagnose congenital *Toxoplasma* infection. In 90 percent of placentas from which *T. gondii* is isolated, congenital infection occurs.[172] Sensitivity of placental detection of *Toxoplasma* by isolation for the diagnosis of congenital infection appears to vary, with reports from 25 to 67 percent, and depends on the trimester of acquisition of infection.[66,174] Isolation of *Toxoplasma* from the tissues of older children or adults may reflect, however, only the presence of latent infection (cyst form). The organism may be isolated by inoculation of body fluids, leukocytes, or tissue specimens into the peritoneal cavities of mice or into tissue cultures. Specimens should be processed and inoculated immediately; however, tissue and blood may be stored at 4° C (39° F) overnight. Freezing and thawing or formalin treatment kills the organism. Establishment of definitive diagnosis by isolation of *Toxoplasma* from tissues usually takes 4 to 6 weeks by mouse inoculation; tissue culture is less sensitive for recovery of *Toxoplasma*, but the results are available sooner.

Histology

Demonstration of tachyzoites, but not cysts, in tissue sections or smears of body fluids (e.g., CSF) establishes a diagnosis of acute infection. The organism may be difficult to see with routine stains. The peroxidase-antiperoxidase technique is exquisitely sensitive and has been used with a high degree of sensitivity and specificity to show the organism in the CNS of patients with AIDS.[55] In older children and adults, the histopathologic changes in toxoplasmic lymphadenitis are sufficiently distinctive to enable pathologists to make a presumptive diagnosis of acute acquired toxoplasmosis (see section on pathology).[57,209] Histologic demonstration of cysts establishes that a patient has *Toxoplasma* infection, but they are diagnostic of toxoplasmosis only in the placenta, fetus, or newborn.

Antigen-Specific Lymphocyte Transformation

Lymphocyte transformation in response to *Toxoplasma* antigens is a specific and sensitive indicator of previous *Toxoplasma* infection in adults and has been used successfully to diagnose congenital *Toxoplasma* infection in infants 2 months or older.[136,199,218] Lymphocyte transformation often is absent in the newborn period, however, particularly in more severely affected infants, because of specific immune tolerance.[136,143]

Polymerase Chain Reaction

Amplification of the B1 genome of *T. gondii* DNA by PCR permits detection of the parasite in body fluids or tissues such as CSF, amniotic fluid, and lymph nodes.* Experience with the test in France, where PCR analysis of amniotic fluid was compared with the results of percutaneous umbilical blood sampling in 339 pregnant women, has shown that agreement of the two methods is almost 100 percent for the diagnosis of intrauterine infection.[89] In view of the considerably lower risk accompanying amniocentesis than with percutaneous umbilical blood sampling and the fact that amniocentesis potentially can be performed earlier in gestation, this diagnostic procedure seems to be a major advance in prenatal diagnosis. An early prospective study in France has shown, however, that only 48 (64%) of 75 amniotic fluid specimens from congenitally infected infants were positive by PCR analysis of a single sample obtained soon after maternal seroconversion. The test had a 100 percent positive predictive value, but a single negative assay did not rule out fetal infection.[183] A more recent report from the same institution on real-time PCR analysis of

*References 33, 86, 89, 182, 183, 209.

amniotic fluid of 261 acutely infected pregnant women indicated an overall sensitivity of 92 percent and a negative predictive value of 98 percent, with specificity and positive predictive values of 100 percent.[211]

A more recently developed nucleic acid amplification test, the loop-mediated isothermal amplification method, uses the same general principle as conventional PCR but may offer a simpler and less expensive alternative if initial encouraging reports are confirmed prospectively.[118]

Interferon-γ Release Assay

In recent years, interferon-γ release assays on whole blood have been approved for the diagnosis of latent tuberculosis infection. A report from France suggests that this same technique, using *T. gondii* antigen instead of *Mycobacterium tuberculosis* antigens to stimulate white blood cells and then measuring the release of interferon-γ, has a sensitivity of 94 percent and a specificity of 98 percent for diagnosis of congenital toxoplasmosis.[34]

Diagnosis in Specific Clinical Situations

Acute Acquired Toxoplasmosis

If IgM and IgG antibodies are not detectable, the diagnosis of acute *Toxoplasma* infection in an immunocompetent child virtually is excluded.[216,221] The diagnosis of recently acquired infection is confirmed if seroconversion from a negative to a positive titer is noted or if a serial fourfold increase in titer to high levels is observed when sera drawn at 3-week intervals are run in parallel. A single high titer in any test is not diagnostic. A dye test or IFA titer of 1:500 or greater in the presence of a high IgM antibody titer probably is diagnostic of recent acute infection. Because IgM can remain positive for 18 months after acute infection, a serum IgG avidity test can be performed to time the infection more accurately. Low IgG avidity suggests that infection has occurred within the past 12 weeks, whereas high avidity suggests more remote infection. The absence of IgM antibodies in IgM ELISA or IgM ISAGA essentially excludes the diagnosis of acute infection. In contrast, the absence of IgM antibodies in the IgM IFA test does not mean that the infection is not acute; in one series, 25 percent of results in adults with acute infection were negative by the IgM IFA test.[157]

Toxoplasma *Infection in Immunodeficient Children*

Serologic tests should be done to identify individuals at risk for acquiring toxoplasmosis, such as recipients of organ, stem cell, and bone marrow transplants. The available serologic tests may be inadequate to detect acute active infection in some immunodeficient patients because their antibody response may be abnormal, which is especially the case in stem cell and bone marrow transplant recipients.[50,131] Experience in the Palo Alto, California, reference laboratory has revealed that acute infection may be present in patients with AIDS and in bone marrow transplant recipients without any demonstrable IgM antibody and in some immunocompromised patients who have little or no IgG antibody.[126] In patients with AIDS and active *Toxoplasma* infection, antibody titers in the modified direct agglutination test[55] may be elevated in the presence of low or undetectable titers in the dye or IFA test.[133] These and other immunodeficient patients can have progressive, lethal toxoplasmosis. In almost all cases, encephalitis, brain abscesses, or both are the predominant findings; hepatic involvement, pneumonitis, and myocarditis may be present. A high index of suspicion is necessary in these patients, and immunoperoxidase staining of appropriate biopsy specimens often is required to establish the diagnosis. Real-time PCR may be a valuable aid in these situations, although tissue immunoperoxidase staining remains the most reliable test.[33]

Toxoplasma *Infection in Pregnant Women*

Toxoplasma infection acquired during pregnancy is associated with clinical signs (e.g., lymphadenopathy) in only 10 to 15 percent of patients. The fetus is at risk, however, for contracting the infection regardless of whether the mother is symptomatic. To detect acute infection in a pregnant woman in the absence of a routine screening program in which serologic tests are performed periodically throughout pregnancy, a suitable test for IgM antibody (IgM ELISA or IgM ISAGA) should be performed if other serologic test results are positive at any titer. If a suitable IgM antibody test is unavailable and the original serum contains IgG antibodies, the IgG antibody test should be repeated in 3 weeks, in parallel with the original serum, to determine whether the titer is stable or increasing.[173,221] More recent experience with IgG avidity testing on a single maternal specimen suggests that this additional tool may be helpful in attempting to define maternal infection as acute (within the previous 12 weeks) or remote.[26,173]

If the IgM ELISA or IgM ISAGA result is negative and the IgG antibody titer is stable and less than 1:500, no further evaluation is necessary. Because IgG titers usually stabilize at high levels (e.g., the dye test or IFA titer ≥1:500) at 6 to 8 weeks or longer after acquisition of the infection, if the dye test or IFA titer is 1:500 or less and stable (regardless of IgM antibody titer), infection was acquired at least 4 weeks and probably more than 8 weeks before the serum was obtained. In the United States, an asymptomatic woman commonly is evaluated for the first time more than 8 weeks after conception, however. If her dye test or IFA titer is 1:500 or greater, her IgM ELISA or IgM ISAGA result is negative, and no significant increase in titer in any test can be shown, her infection almost certainly was acquired before conception. In women with elevated IgM titers or increasing IgG titers, infection possibly was acquired during pregnancy. A complete toxoplasmic serologic profile[152] in a reference serologic laboratory is recommended to settle the question.

Fetal Diagnosis

As noted earlier, severe disease almost always is associated with primary maternal infection in the first or second

trimester of pregnancy, but only 25 percent and 54 percent of such maternal infections result in fetal infection. These rates in exposed fetuses may be reduced by half by maternal treatment with spiramycin. Identification of cases in which the fetus already is infected permits a parental decision to terminate the pregnancy or to treat the fetal infection more aggressively with pyrimethamine-sulfadiazine.

Studies by workers in Paris have established an approach that allows definitive diagnosis and treatment of fetal infection in utero.[42,53,89,168] These researchers initially sought to establish the diagnosis of fetal infection at 20 to 29 weeks of gestation by isolation of *Toxoplasma* from amniotic fluid or from fetal blood obtained by percutaneous umbilical blood sampling and use of the sensitive mouse inoculation method. Prenatal diagnosis was attempted in 746 pregnancies in which seroconversion occurred near the time of conception or before the 26th week of gestation. In 39 of these pregnancies, fetal infection was diagnosed in utero. *Toxoplasma* was isolated from fetal blood alone in 12 cases, from amniotic fluid alone in 7 cases, and from both in 15 cases, for a total of 34. *Toxoplasma*-specific IgM antibodies were detected in fetal blood in only 9 cases with the highly sensitive ISAGA, and none was positive before 24 weeks of gestation. By follow-up examination until 3 months post partum or by examination of aborted fetal tissue, a total of 42 cases were proved to have been infected. These researchers were able to detect 39 (93%) of 42 cases of fetal infection occurring before the 26th week of gestation; no false-positive diagnoses occurred.

These investigators subsequently extended their series and reported that a definitive diagnosis of infection was established in utero in 80 (90%) of 89 cases in which the fetus was infected.[90] These results may not be reproduced easily by other investigators for various reasons: monthly monitoring for new maternal infection to detect maternal infection soon after it occurs, sampling of fetal blood and amniotic fluid on more than one occasion, the use of optimal laboratory methods and conditions, and experience in these procedures.

With the use of PCR to amplify the B1 gene of *Toxoplasma* in amniotic fluid obtained after 18 weeks of gestation, these same investigators were able to achieve a sensitivity and specificity for the diagnosis of fetal infection that approached 100 percent.[89] In the most recent multicenter collaborative French study, in which a single specimen was prospectively studied in 261 pregnant women with acute toxoplasmosis, the specificity and positive predictive value remained 100 percent, with a sensitivity of 92 percent and a negative predictive value of 98 percent.[211] The sensitivity and specificity of this test as specimens currently are processed and handled in the United States remain to be determined.

Most of the mothers in these French studies had received treatment with spiramycin soon after primary maternal infection was diagnosed, with the intention of continuing therapy until delivery. In contrast to its beneficial effect on maternal-to-fetal transmission, spiramycin does not seem to decrease the severity of fetal infection after it has been established. The impetus for proving fetal infection in early to middle gestation is to allow intervention either to terminate the pregnancy or to treat the fetus more aggressively. In an extended series of 89 infected fetuses reported by Hohlfeld and colleagues,[90] 34 pregnancies were terminated at the request of the parents. In each of these cases, which were selected either for detectable CNS disease on ultrasound examination or for onset of maternal infection before 12 weeks of gestation, brain necrosis was found on examination of tissue.

These data indicate the value of specifically identifying fetuses that truly are infected and are at high risk for acquiring severe disease because selective termination of these pregnancies allowed successful completion of most pregnancies in which fetal infection did not occur. In addition to the 34 terminated pregnancies with proven fetal infection, 52 pregnancies with 55 offspring were carried to term. In these 52 pregnancies, 43 had fetal infection proven prenatally, and 9 additional offspring were found to be infected by postnatal examination. In the 43 pregnancies in which prenatal diagnosis of fetal infection was established, more aggressive therapy with pyrimethamine and a sulfonamide compound alternating with spiramycin was given in the latter part of pregnancy. This treatment seemed to decrease the number of parasites, as indicated by an approximate 50 percent reduction in the fraction of placentas from which *Toxoplasma* was isolated at delivery compared with cases in which no treatment or spiramycin alone was given.

Regarding clinical outcome, the authors interpreted their data to indicate that such treatment decreased the incidence of severe disease.[90] This conclusion was based, however, on a retrospective comparison with a group of historical controls in which data regarding fetal deaths and termination of pregnancy were unavailable. Because pregnancies with proven or probable severe fetal infection were terminated selectively at the parents' request, one cannot conclude with certainty that the outcome of the infants born to mothers with first-trimester infection was improved by the administration of more aggressive therapy; the outcome of infants born to mothers with second-trimester infection seems to have been improved.[217] A meta-analysis of selected published cohorts on prenatal treatment of congenital toxoplasmosis showed only a weak association between early treatment and a reduced risk of acquiring congenital toxoplasmosis.[202] However, a more recent report of an observational cohort from the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT) showed a significant reduction in serious neurologic sequelae for children with congenital toxoplasmosis who were treated prenatally, with the most significant impact for those women who seroconverted in the first 10 weeks of gestation.[36] Variations in outcomes for such studies need to be interpreted in light of the observation that the disease impact for toxoplasmosis varies across geographic regions, possibly related to virulence characteristics of different strains of *T. gondii* as well as other factors.[207]

It would be desirable to have data from studies in which the effects of such therapy are compared directly in a randomized, concurrent study[205,206]; such studies are unlikely to be performed, however. Nonetheless, these published studies have established the utility of an

aggressive approach to prenatal diagnosis in allowing physicians to make an accurate, early diagnosis of fetal infection. If the fetus is infected and affected, as determined by ultrasound examination, a decision for selective termination of pregnancy can be made rationally. Aggressive therapy with pyrimethamine-sulfadiazine may be offered in cases in which termination is not considered desirable, and it may improve the outcome.

Diagnosis of Congenital Toxoplasmosis after Birth

A thorough clinical and laboratory examination is necessary to evaluate fully the existence and extent of congenital toxoplasmosis in a newborn (Table 223-4). Demonstration of IgM, IgA, or IgE antibody in an infant's blood or CSF at any time is diagnostic of congenital infection if contamination by maternal blood can be reasonably excluded.[221] Specimens obtained after the infant reaches 10 days of age are more reliable in this regard. If the much less sensitive IgM IFA test is used, the presence of antinuclear antibody and rheumatoid factor also must be excluded. As mentioned earlier, the detection rate of congenitally infected infants is 25 percent for the IgM IFA test and 75 percent for IgM ELISA and IgM ISAGA. Data are insufficient for predicting how often IgA and IgE antibodies are detected. IgM antibodies may be demonstrable in the first few days of life or may appear at variable times after birth. Investigators from France recently reported on their use of likelihood ratios of the various tests used to confirm congenital infection in 767 children born after acute maternal toxoplasmosis during pregnancy. The probability of a child's being infected was essentially 100 percent if the amniotic fluid was PCR positive and the IgM was positive in the newborn baby.[169] If both test results were negative, there was still a 10 percent probability of infection in their cohort.

If *Toxoplasma* is not isolated and IgM, IgA, or IgE antibodies are not detected, follow-up serologic testing is the only means of establishing the diagnosis. Maternally transmitted IgG antibodies may persist for 6 to 12 months or longer, depending on the original titer. The higher the original titer, the longer maternal antibody is detectable in the infant. The presence of IgG antibody at 8 to 12 months of age does not prove that the infant is infected. Synthesis of IgG *Toxoplasma* antibody usually can be shown by the third month of life if the infant is not treated; it may be delayed until the sixth or ninth month if the infant is treated. At the time that the infant begins to synthesize IgG antibody, infection may be documented by computing the specific "antibody load"—the ratio of specific serum antibody titer to the level of serum IgG in the infant.[172] In the absence of infection, the antibody load decreases in the second or third month as the infant begins to produce IgG that does not contain specific *Toxoplasma* antibodies. In the presence of *Toxoplasma* infection, the infant produces specific antibodies, and the antibody load remains the same or increases. Most infected infants who are treated during the first year of life have a substantial increase in antibody after termination of therapy (serologic rebound).[210] This phenomenon permits the diagnosis to be confirmed in some uncertain cases.

TABLE 223-4 Evaluation of a Neonate When Serology of the Mother or Illness of the Neonate Indicates That a Diagnosis of Congenital Toxoplasmosis Is Suspected or Probable

In addition to a careful general examination, the infant is examined by the following:

Clinical Evaluation and Nonspecific Tests

Pediatric ophthalmologist
Pediatric neurologist
Brain CT (data suggest excellent agreement between CT and ultrasonography,[117] which can be obtained more quickly and without the need for sedation)
Blood tests
Complete blood cell count with differential and platelet counts
Serum total IgM, IgG, IgA, and albumin
Serum alanine aminotransferase, total and direct bilirubin
CSF cell count, glucose, protein, and total IgG

***Toxoplasma gondii*–Specific Tests**

Newborn serum analyzed for antibody detected by Sabin-Feldman dye test, IgM ISAGA, IgA EIA, IgE EIA/ISAGA (0.5 mL serum to *Toxoplasma* Serology Laboratory, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, CA 94301, 415-326-8120)
Newborn blood for inoculation into mice (1-2 mL clotted whole blood in red-topped tube to *Toxoplasma* Serology Laboratory)
Lumbar puncture: CSF dye test and IgM EIA (0.5 mL CSF to *Toxoplasma* Serology Laboratory); consider PCR (1 mL frozen CSF to *Toxoplasma* Serology Laboratory)
Sterile placental tissue (100 g in saline, from fetal side near insertion of cord, no formalin, to *Toxoplasma* Serology Laboratory for subinoculation)
Maternal serum analyzed for antibody detected by dye test, IgM EIA, IgA EIA, IgE EIA/ISAGA, and AC/HS

AC/HS, Differential agglutination test; *CSF,* cerebrospinal fluid; *CT,* computed tomography; *EIA,* enzyme immunoassay; *ISAGA,* immunosorbent agglutination assay; *PCR,* polymerase chain reaction.
Modified from McLeod R, Wisner J, Boyer K. Toxoplasmosis. In: Krugman S, Katz SL, Gershon AA, editors. Infectious diseases of children. *St. Louis: Mosby; 1992, p. 539.*

Ocular Toxoplasmosis

Toxoplasma has been estimated to cause 35 percent of cases of chorioretinitis in the United States and central and western Europe.[92,188,203] Acquired toxoplasmosis usually is not accompanied by chorioretinitis. Most cases are thought to result from congenital infection that does not become clinically apparent until after reactivation. This event occurs most commonly in adolescence. Although the presence of chorioretinitis should prompt a search for *Toxoplasma* infection, proof that *Toxoplasma* caused the eye disease often is lacking. The titer of antibody in serum does not correlate with the presence of active lesions in the fundus. Low titers of IgG antibody are the usual finding in patients with reactivation

Toxoplasma chorioretinitis. IgM antibodies generally are absent.

Toxoplasma probably is excluded as a cause of chorioretinitis if the results of serologic tests are negative in undiluted serum. If the retinal lesions are characteristic and serologic test results are positive, the diagnosis is probable. If the retinal lesions are atypical and the serologic test results are positive, the diagnosis of *Toxoplasma* chorioretinitis is less certain because of the increasing prevalence of *Toxoplasma* antibodies with age in the normal population. Finding *Toxoplasma* antibodies in the child's mother supports the possibility of congenital infection, as does detection of intracranial calcification on CT scan of the patient. Demonstration of local antibody production in aqueous humor obtained by paracentesis of the anterior chamber can be used to establish the diagnosis of *Toxoplasma* chorioretinitis in equivocal cases.[172,221] The risk of this procedure in a situation of threatened vision, weighed against the low risk of a short course of treatment, is such that it seldom is performed.

TREATMENT

The need for therapy and the duration of therapy are determined by the nature and severity of the clinical illness and by the immune status of the infected patient. Antibody titers are not useful indicators of therapeutic response, and an increasing antibody titer soon after discontinuation of therapy (serologic rebound) is not an indication of therapeutic failure. Specific therapy acts primarily against the tachyzoite form; the drugs currently available do not eradicate the encysted form containing bradyzoites. Close, longitudinal follow-up and supportive interventions are crucial contributors to therapeutic success.

Therapeutic Agents

The therapeutic agents used, their dosages, and indications for their use in the management of toxoplasmosis are included in Table 223-5 and discussed next.

Spiramycin

Spiramycin is a macrolide that has been used extensively in Europe to reduce transmission of infection from an acutely infected mother to the fetus in utero.[37] It is concentrated in the placenta and is reported to reduce transmission by 50 to 60 percent. It reduces the ability to isolate the organism from the placentas of definitively infected newborns from 95 to 80 percent.[39] Spiramycin is less effective than pyrimethamine-sulfadiazine in the treatment of congenital infection and toxoplasmic encephalitis.[38,39,51,189,190] Toxoplasmic encephalitis has developed in patients receiving spiramycin and has been treated effectively with pyrimethamine-sulfadiazine. Toxicities include allergic manifestations, gastrointestinal intolerance, and paresthesias.

Spiramycin apparently does not treat manifestations of *T. gondii* infection in the fetus in utero.[39,51,90,189] It formerly was used in alternate-month regimens with pyrimethamine and sulfadiazine for treatment of congenital toxoplasmosis in France.[172] Spiramycin is not approved by the U.S. Food and Drug Administration but may be obtained with compassionate clearance by calling 301-827-2127. The manufacturer (Aventis-Pasteur) provides the drug after the diagnosis has been documented and Food and Drug Administration forms have been completed.

Pyrimethamine

Pyrimethamine has been shown to be effective against *T. gondii* in vitro,[142,145] in animal models,[72] and in human infections.* When it is used in conjunction with sulfadiazine, synergy can be shown. The pharmacokinetics of pyrimethamine has been studied in infants and adults.[145,215] Pyrimethamine is metabolized in the liver. Its pharmacokinetics is not altered by renal insufficiency but is affected by concomitantly administered drugs (e.g., phenobarbital). Pyrimethamine toxicities include reversible marrow suppression (most commonly) and allergy. Aplastic anemia, hepatotoxicity, and various allergic manifestations (including Stevens-Johnson syndrome) also have been listed as toxicities of this medication. Pyrimethamine always should be administered in conjunction with leucovorin (i.e., folinic acid) because human cells can use folinic acid for synthesis of nucleic acids but *T. gondii* cannot.[145]

Leucovorin

Leucovorin (folinic acid) always is administered during treatment with pyrimethamine. Folinic acid has a protective effect for human cells that folic acid does not. Increased doses of leucovorin are used in the event of marrow suppression. Because of the long half-life of pyrimethamine, continuation of leucovorin therapy for 1 week after discontinuation of pyrimethamine is recommended.

Sulfadiazine, Sulfamerazine, and Sulfamethazine

The three sulfonamides sulfadiazine, sulfamerazine, and sulfamethazine (known as triple sulfa when used in combination) are the most active of the sulfonamides against *T. gondii* and are synergistic with pyrimethamine in their activity against *T. gondii*. Of the three, only sulfadiazine is available in the United States. All other sulfonamides are less active in vitro.[142] The sulfonamides are excreted by the kidney, and the dosage must be adjusted for patients with renal insufficiency. Nephrolithiasis can occur in older children and adults, in whom urinary acidification is more effective, and fluid requirements are lower on a weight basis than in infancy. The risk for development of stones in these groups can be reduced by aggressive fluid supplementation (1 to 2 L above maintenance) and urine alkalinization.[29] In older children who weigh more than 20 kg, a dose of 75 mg/kg/day (rather

*References 41, 43, 72, 128, 145, 224.

TABLE 223-5 **Treatment of Toxoplasmosis**

Disease	Medication	Dosage	Length of Therapy
Acute acquired—generally not treated unless severe or persistent symptoms, vital organ damage, or host is immunosuppressed	Pyrimethamine *plus*	2 mg/kg/day for 2 days, then 1 mg/kg/day	4-6 wk or 2 wk after symptoms resolve for normal host; 4-6 wk beyond resolution for immunosuppressed hosts. In AIDS, treat until $CD4^+$ count >200.
	Sulfadiazine *plus*	75-100 mg/kg/day divided twice daily (maximum 4 g/day); consider the lower dose in children >20 kg (see text)	
	Folinic acid	5-20 mg 3 times weekly; use higher doses if marrow suppression	
Ocular, older child	Pyrimethamine *plus*	2 mg/kg/day for 2 days, then 1 mg/kg/day (maximum 50 mg/day)	4-6 wk or 2 wk after symptoms resolve
	Sulfadiazine *plus*	75-100 mg/kg/day divided twice daily (maximum 4 g/day); consider the lower dose in children >20 kg (see text)	Prednisone should be continued until resolution of sight-threatening active chorioretinitis
	Folinic acid *plus*	5-20 mg 3 times weekly	
	Prednisone	1 mg/kg/day divided twice daily	
Congenital	Pyrimethamine *plus*	2 mg/kg/day for 2 days, then 1 mg/kg/day for 6 mo, then 3 times weekly (M-W-F) for 6 mo	1 yr
	Sulfadiazine *plus*	100 mg/kg/day divided twice daily	
	Folinic acid *plus*	5-10 mg 3 times weekly	
	Prednisone	1 mg/kg/day divided twice daily	Until resolution of elevated CSF protein level or sight-threatening active chorioretinitis
Pregnant women—acute infection first 21 wk of gestation	Spiramycin	3 g/day divided twice daily without food	Until fetal infection documented or excluded at 21 wk of gestation; if fetus infected, change to pyrimethamine plus sulfadiazine plus folinic acid until delivery
Pregnant women—fetal infection confirmed (amniotic fluid PCR positive)	Pyrimethamine *plus*	100 mg/day divided twice daily for 2 days, then 50 mg/day	Until delivery
	Sulfadiazine *plus*	3 g/day divided twice daily	
	Folinic acid	5-20 mg/day	

AIDS, Acquired immunodeficiency syndrome; *CSF,* cerebrospinal fluid; *PCR,* polymerase chain reaction.

than the conventional 100 mg/kg/day) should be considered because this dose more closely approximates the calculated adult dose based on body surface area. Other sulfonamide toxicities include allergy (including Stevens-Johnson and DRESS syndromes), marrow suppression, and hepatotoxicity.[141] Sulfonamide pharmacokinetics has been studied in infants.[172]

Clindamycin

Although the effect of clindamycin is delayed, it does have an effect in vitro against *T. gondii* with prolonged time in culture.[165] It also has been shown to be effective in murine models. Clindamycin has been found to be comparable in efficacy to sulfadiazine for the treatment of toxoplasmic encephalitis in adult patients with AIDS when it is used in a combined high-dose regimen with pyrimethamine.[43,128] High-dose pyrimethamine also is effective alone. It was not compared directly with the other two regimens in the latter study.

Other Antimicrobial Agents

Numerous other antimicrobial agents have been shown to be effective in vitro or in animal models against either tachyzoites or encysted bradyzoites,[3-6,91,93] but their role, if any, in the treatment of human disease remains to be defined. Atovaquone (5-hydroxynaphthoquinone) was effective against bradyzoites within cysts in vitro.[93] Forty percent of patients with AIDS had a relapse of toxoplasmic encephalitis, however, while being treated with this antimicrobial agent. Other antimicrobial agents with an effect on *T. gondii* in vitro or in vivo include cycloguanil[91]; artemisinin[91]; pyrimethamine-sulfadoxine (Fansidar)[142]; rifabutin[7]; trovafloxacin (no longer available owing to liver toxicity)[108]; and the newer macrolides

clarithromycin, azithromycin, and roxithromycin.[3,5,6] Isolated reports of clinical use of some of these agents, most typically when standard agents are not available or not tolerated, have appeared in the literature with mixed results.

Because the activity of sulfamethoxazole is less than that of sulfadiazine, trimethoprim-sulfamethoxazole has been considered less effective for treatment of toxoplasmosis. Many investigators have used this combination successfully, however, to treat toxoplasmic encephalitis in adults with AIDS. Doses of trimethoprim-sulfamethoxazole, as used to prevent *P. jiroveci* pneumonia in the context of HIV infection, also seem to prevent episodes of reactivated toxoplasmosis.[27] A randomized controlled trial has shown that trimethoprim-sulfamethoxazole is as effective as pyrimethamine-sulfadiazine in the treatment of isolated ocular toxoplasmosis in young adults.[195] Pyrimethamine combined with sulfadoxine, despite having lower in vitro activity than that of pyrimethamine-sulfadiazine, also has been used in Europe to treat reactivated and congenital infection.[13]

Therapy in Specific Clinical Settings

Acquired Toxoplasmosis

Most immunologically normal patients with the lymphadenopathic form of toxoplasmosis do not require specific treatment. Indications for treatment in these cases are the presence of severe and persistent symptoms and damage to vital organs. Because of the high incidence of severe morbidity and mortality in immunocompromised patients, toxoplasmosis should be treated in this population. Most immunocompromised patients in whom the diagnosis is established ante mortem improve when specific therapy is administered. The major problem lies in establishing the diagnosis early enough to institute treatment.

The optimal duration of specific therapy for toxoplasmosis is unknown. Patients who seem to be immunologically normal but who have severe and persistent symptoms or damage to vital organs should receive specific therapy for 2 to 6 weeks, provided symptoms resolve. In immunocompromised patients, therapy should continue at least 4 to 6 weeks beyond complete resolution of all signs and symptoms of active disease. Careful follow-up of these patients is imperative because relapses may occur and require prompt reinstitution of therapy. In patients with AIDS in whom toxoplasmosis develops, suppressive therapy with pyrimethamine-sulfadiazine, pyrimethamine-clindamycin, or trimethoprim-sulfamethoxazole should be continued for life or until immune reconstitution has been sustained for at least 6 months.

Pregnant Women

Treatment of an acutely infected woman during pregnancy may prevent transmission of the infection to her fetus. The rationale for such treatment is derived from the observation that the incubation period between the onset of maternal infection and acquisition of infection in the fetus may be significant. Data from France,[51,52] where women were treated with spiramycin, and from Austria[9,10] and Germany,[114] where women were treated with pyrimethamine and sulfonamides, indicate that the incidence of congenital infection in the offspring of mothers treated during gestation is at least 50 percent less than that in the offspring of untreated mothers. None of these studies was controlled rigidly. A meta-analysis including 20 cohorts with 1721 infected mothers and 506 infected children suggested that a reduction in the incidence of transmission (odds ratio, 0.48; 95% confidence interval, 0.28 to 0.80) was achieved if therapy was started within 3 weeks of maternal seroconversion.[205] These results combined with the numerous women studied by the group from France (154 untreated and 388 treated patients) strongly suggest that intrauterine treatment does reduce the incidence of transmission of maternal infection to the fetus.[57,197] The meta-analysis failed to show that intrauterine treatment ameliorated the manifestations of congenital infection in children who were born infected, but the large single-site studies from France suggest a benefit. The more recent report of an observational cohort included in the analysis from the European Multicentre Study on Congenital Toxoplasmosis (EMSCOT) showed a significant reduction in serious neurologic sequelae for children with congenital toxoplasmosis who were treated prenatally, with the most significant impact for those women who seroconverted in the first 10 weeks of gestation.[36] Long-term follow-up of young adults who were treated antenatally and postnatally for congenital toxoplasmosis at a single center in France showed remarkably normal quality of life and visual function of the affected individuals.[164] An additional report from a different center in France likewise found minimal disability (74% asymptomatic, 26% with chorioretinitis, and 1 child with serious neurologic involvement) in 107 children treated both prenatally and postnatally.[14]

Spiramycin treatment of pregnant women with recently acquired primary infection should be instituted empirically in the hope of preventing spread of infection to the fetus. When fetal infection has occurred, however, maternal treatment with spiramycin does not seem to alter the evolution and severity of disease in the fetus, which is why evaluation of a potentially exposed fetus by PCR amplification of amniotic fluid permits informed decisions to be made about termination of pregnancy or treatment of the fetus in utero with pyrimethamine-sulfadiazine.

Congenital Infection

Postnatal Treatment. Data regarding the efficacy of postnatal treatment of infants with congenital *Toxoplasma* infection are becoming available.[81,138] The improved outcome reported from several centers in France often includes prenatal treatment as well as postnatal therapy. However, uncontrolled studies in humans and controlled studies in experimental animals[172] have been interpreted as indicating beneficial effects of postnatal treatment on the development of sequelae in symptomatic and asymptomatic infants with congenital *Toxoplasma* infection. The controlled National Collaborative Treatment Trial

is now in progress in Chicago. This study seeks to define optimal therapeutic regimens. Physicians treating patients with congenital *Toxoplasma* infection who are younger than 2.5 months may wish to contact this multidisciplinary group about potential enrollment of their patients in that study (773-834-4152).

Outcomes to date from the National Collaborative Treatment Trial are substantially better for most but not all infants treated from the neonatal period for 12 months with pyrimethamine-sulfadiazine and leucovorin than for historical controls receiving no or short-course therapy.[137] Signs of active infection resolve within weeks of initiation of treatment. In many children, the appearance of brain CT scans has improved remarkably. Cerebral calcifications have diminished in size or resolved in most such treated children.[163] In conjunction with this improvement in brain CT scans, cognitive function has been in the normal range for 69 percent of treated children.[181,201] Overall, 18 (27%) of 66 children in the National Collaborative Treatment Trial have IQ results below 70 compared with 86 (85%) of 101 in the reported literature (see Table 223-1).[54,137]

No significant diminution in cognitive function occurs over time, and most treated children function well in regular school classrooms. Although the number of children compared is limited, for a small subset of these children, measures of cognitive function seem to be less than those for their siblings. No sensorineural hearing loss has been ascribable to congenital toxoplasmosis in treated children.[135,137] Despite the much improved neurologic outlook for most of these children, a subset of children with significant irreversible neurologic damage already present in the perinatal period have manifested profound developmental delay, motor impairment, and seizures. For the most part, these were children with hydrocephalus, high CSF protein concentration, minimal improvement in brain CT scans after shunting, and often substantial delays in shunt placement or needed revision for shunt failure or other intercurrent medical problems.[201] This experience emphasizes the importance of recognizing hydrocephalus and managing it aggressively.

Although treatment during the first year of life arrests all signs of active disease, results in normal cognitive and motor outcomes for most children, and may result in resolution of seizures without recurrence for some treated children, the drugs currently available do not eradicate all cysts containing bradyzoites. In most children, serologic titers of *T. gondii*–specific antibodies rebound in the 3 to 4 months after treatment is discontinued.[137,210]

To date, new retinal lesions have occurred in 17 of 58 children in the National Collaborative Treatment Trial during 3 to 10 years' follow-up after the 1-year course of treatment.[137] These active lesions have responded to brief courses of treatment with pyrimethamine, sulfadiazine, and leucovorin without subsequent loss of visual acuity. Although follow-up durations are shorter, this result contrasts with the almost uniform eventual development of retinal lesions in studies of untreated or briefly treated children.[37,64,112,113,220] We recommend that infected children undergo retinal examination each month for 3 months after discontinuing treatment around their first birthday, then every 3 months until they are old enough to describe visual symptoms accurately, and then every 6 months. In addition, an ophthalmologic evaluation should be performed promptly for any acute visual signs or symptoms that may be related to recrudescence of congenital ocular toxoplasmosis.[146]

Sequential Fetal and Postnatal Treatment. Several studies[42,73,83,90] have described outcomes in patients treated in utero with continuing treatment during the first year of life. As noted earlier, however, pregnancies in which fetuses had obvious manifestations on ultrasound examination and most pregnancies with definite first-trimester infection were terminated.

When the French method of initiating aggressive treatment of fetuses in utero was applied, retinal disease was reported in only 3 of 50 such infants monitored to 2 years of age. This finding contrasts with the presence of retinal or neurologic involvement in 50 percent of asymptomatic newborns detected by serologic screening in Massachusetts[87] and in 75 percent of children whose pediatricians referred them to our National Collaborative Treatment Trial for treatment in the perinatal period.[146] A prospective, carefully controlled study (as part of the National Collaborative Study) is under way to compare directly outcomes in infants identified and treated in utero, as detected by systematic neonatal screening and by pediatricians in the neonatal period and then referred. The outcome of pregnancies with infection acquired in the first trimester after in utero treatment also has been reported to be favorable in another study,[15] in which only pregnancies in which the fetus had hydrocephalus were terminated.

Toxoplasmosis with coexistent HIV infection in children is increasingly rare since the advent of HAART.[62,63,149,162] Most children described with toxoplasmosis and HIV infection have had congenital toxoplasmosis and have been symptomatic. For such children, therapy with pyrimethamine and sulfadiazine plus folinic acid is recommended in the doses described in Table 223-5. Adult and adolescent patients receiving secondary prophylaxis (i.e., long-term maintenance therapy) for acquired toxoplasmic encephalitis are at low risk for recurrence when they have successfully completed initial therapy, remain asymptomatic with regard to signs and symptoms of toxoplasmic encephalitis, and have a sustained increase in $CD4^+$ T lymphocyte counts of more than 200 cells/μL after receiving HAART (e.g., >6 months).

Although the numbers of patients who have been evaluated remain limited and occasional recurrences have been reported, on the basis of these observations and inference from more extensive cumulative data indicating the safety of discontinuing secondary prophylaxis for other opportunistic infections during advanced HIV disease, discontinuation of long-term maintenance therapy among such patients is a reasonable consideration (see http://aidsinfo.nih.gov/guidelines/ for up-to-date Department of Health and Human Services recommendations). The highest risk for relapse seems to occur within the first 6 months of discontinuing secondary prophylaxis. Certain specialists would obtain a magnetic resonance image of the brain as part of their

evaluation to determine whether it is appropriate to discontinue therapy. Zidovudine antagonizes the toxoplasmacidal effect of pyrimethamine and its in vitro synergy with sulfonamide. Whether this effect occurs in vivo is unknown.[119]

Prompt initiation of specific treatment in active ocular toxoplasmosis is mandatory to preserve vision. Inflammatory reactions in the vitreous frequently are a major pathogenetic phenomenon in patients with active disease, and in such cases, administration of corticosteroids in addition to specific anti-*Toxoplasma* therapy is recommended strongly.[190] Their use also is recommended for cases of retinochoroiditis involving the macula, maculopapillary bundle, or optic nerve. The initial daily dosage of prednisone is 1 mg/kg orally to a maximum of 75 mg in 24 hours. The equivalent dosage of another corticosteroid may be given. The dosage of corticosteroid may be reduced gradually when the lesion appears to be well demarcated and pigmentation has begun. Some physicians have administered systemic or intraocular clindamycin to treat patients in whom the use of corticosteroids and pyrimethamine plus sulfadiazine has failed[202]; its efficacy has not been proved in humans.

PREVENTION

Congenital infection may be avoided by preventing primary *Toxoplasma* infection during pregnancy.[67,134,219,224] The responsibility of all physicians caring for pregnant women at risk is to inform them of specific hygienic measures (Table 223-6) for avoiding *Toxoplasma* infection. Similar measures are useful for prevention of acquired infection in other settings as well. The effectiveness of a 10-minute education program, offered as part of prenatal care, to reduce the risk of acquiring *Toxoplasma* infection by modifying the behavior of pregnant women with regard to cats, food, and personal hygiene has been shown.[28] Pamphlets that describe methods to prevent toxoplasmosis in pregnant women are available from the March of Dimes (312-435-4007), from Abbott Diagnostics (800-323-9100), and on the Internet (www.cdc.gov/parasites/toxoplasmosis/resources/ToxoWomen_2.2003.pdf).

When primary maternal infection has occurred, several problems are inherent in the secondary prevention of congenital toxoplasmosis by therapeutic abortion or by treatment of the pregnant woman.[30] Because 80 to 90 percent of women with primary *Toxoplasma* infection are asymptomatic, most primary infections are overlooked, unless sequential serologic testing is performed routinely in pregnant women. The cost-effectiveness of routine screening in the prevention of congenital toxoplasmosis is clear in some European countries where screening is mandated by law.[51] In countries with a lower incidence, cost-efficiency has not been proved.[79,82,178] A recent analysis of the cost of a universal screening program for pregnant women in the United States modeled after the French approach suggests cost savings for the U.S. population even with current levels of congenital infection.[110,179,198] In the absence of national guidelines, physicians may choose to screen patients on an individual basis.[218] If screening is undertaken, a reliable serologic test for IgG antibodies (see section on laboratory diagnosis) should be performed before conception occurs or as soon as possible thereafter and then repeated every 2 to 3 months until the time of delivery. Serologic test results that suggest the acquisition of primary infection during pregnancy should be confirmed by a reference laboratory. Decisions about treatment or therapeutic abortion should be based on a consideration of whether the fetus is infected or affected, as determined by amniocentesis and ultrasonography.

TABLE 223-6 **Prevention of Toxoplasma Infection**

Prevention of Acquired Infection (Primary Prevention)
Cook meat to medium (66°C [150°F]), smoke it, or cure it in brine.
Wash fruits and vegetables before consumption.
Avoid touching mucous membranes of the mouth and eyes while handling uncooked meat or unwashed fruits or vegetables.
Wash hands and kitchen surfaces thoroughly after contact with raw meat or unwashed fruits or vegetables.
Prevent access of flies, cockroaches, and other coprophagous insects to fruits and vegetables.
Avoid contact with materials that potentially are contaminated with cat feces, such as cat litter boxes, or wear gloves when handling such materials and when gardening.
Disinfect cat litter boxes for 5 minutes with nearly boiling water.
Prevention of Congenital Infection (Secondary Prevention)
Identify women at risk by serologic testing.
Treatment during pregnancy results in an approximately 50% reduction in the incidence of infection in infants.
Therapeutic abortion prevents birth of an infected infant—consider only for women who acquire infection in the first or second trimester.

Modified from Remington JS, Wilson CB. Toxoplasmosis. In: Kass EH, Platt R, editors. Current therapy in infectious disease: 1983-1984. *Philadelphia: BC Decker; 1983, p. 149-53.*

The authors of this chapter would like to acknowledge Dr. Jack Remington as a contributor to the previous chapter from which this chapter was constructed.

NEW REFERENCES SINCE THE SIXTH EDITION

14. Berrebi A, Assouline C, Bessieres MH, et al. Long-term outcome of children with congenital toxoplasmosis. *Am J Obstet Gynecol* 2010;**203**:552.e1–6.
18. Boyer KM, Hill D, Mui E, et al. Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. *Clin Infect Dis* 2011;**53**:1081–9.
30. Centers for Disease Control and Prevention. Preventing congenital toxoplasmosis. *MMWR Morb Mortal Wkly Rep* 2000;**49**(RR-2):57–75.
34. Chapey E, Wallon M, Debize G, et al. Diagnosis of congenital toxoplasmosis by using a whole-blood gamma interferon release assay. *J Clin Microbiol* 2010;**48**:41–5.
36. Cortina-Borja M, Tan HK, Wallon M, et al. Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. *PLoS Med* 2010;**7**:e1000351.

62. Dunn D, Newell ML, Gilbert R. Low risk of congenital toxoplasmosis in children born to women infected with human immunodeficiency virus. *Pediatr Infect Dis J* 1997;**16**:84.
63. Dunn D, Wallon M, Peyron F, et al. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counseling. *Lancet* 1999;**353**:1829.
64. Eichenwald HG. A study of congenital toxoplasmosis. In: Siim JC, editor. *Human toxoplasmosis*. Copenhagen: Munksgaard; 1960. p. 41–9.
66. Filisetti D, Cocquerelle V, Pfaff A, et al. Placental testing for *Toxoplasma gondii* is not useful to diagnose congenital toxoplasmosis. *Pediatr Infect Dis J* 2010;**29**:665–7.
74. Galli-Tsinopoulou A, Kyrgios I, Giannopoulou EZ, et al. Acquired toxoplasmosis accompanied by facial nerve palsy in an immunocompetent 5-year-old child. *J Child Neurol* 2010;**25**:1525–8.
76. Garcia de la Fuente I, Ansari M, Rougemont AL, et al. Acute disseminated fatal toxoplasmosis after haploidentical stem cell transplantation despite atovaquone prophylaxis in a young man. *Pediatr Infect Dis J* 2010;**29**:1059–60.
97. Jara M, Hsu HW, Eaton RB, Demaria A Jr. Epidemiology of congenital toxoplasmosis identified by population-based newborn screening in Massachusetts. *Pediatr Infect Dis J* 2001;**20**:1132.
99. Jones JL, Dubey JP. Foodborne toxoplasmosis. *Clin Infect Dis* 2012;**55**:845–51.
100. Jones JL, Holland GN. Short report: annual burden of ocular toxoplasmosis in the United States. *Am J Trop Med Hyg* 2010; **82**:464–5.
103. Jones JL, Roberts JM. Toxoplasmosis hospitalizations in the United States, 2008, and trends, 1993–2008. *Clin InfectDis* 2012; **54**:e58–61.
104. Jost C, Touafek F, Fekkar A, et al. Utility of immunoblotting for early diagnosis of toxoplasmosis seroconversion in pregnant women. *Clin Vaccine Immunol* 2011;**18**:1908–12.
115. Kusbeci OY, Miman O, Yaman M, et al. Could *Toxoplasma gondii* have any role in Alzheimer disease? *Alzheimer Dis Assoc Disord* 2011;**25**:1–3.
118. Lau YL, Meganathan P, Sonaimuthu P, et al. Specific, sensitive, and rapid diagnosis of active toxoplasmosis by loop-mediated isothermal amplification method using blood samples from patients. *J Clin Microbiol* 2010;**48**:3698–702.
124. Ling VJ, Lester D, Mortensen PB, et al. *Toxoplasma gondii* seropositivity and suicide rates in women. *J Nerv Ment Dis* 2011;**199**: 440–4.
154. Morris JG, Greenspan A, Howell K, et al. Southeastern Center for Emerging Biologic Threats tabletop exercise: foodborne toxoplasmosis outbreak on college campuses. *Biosecur Bioterror* 2012;**10**:89–102.
155. Munoz-Zanzi CA, Fry P, Lesina B, Hill D. *Toxoplasma gondii* oocyst-specific antibodies and source of infection. *Emerg Infect Dis* 2010;**16**:1591–7.
162. O'Donohoe JM, Brueton MJ, Holliman RE. Concurrent congenital human immunodeficiency virus infection and toxoplasmosis. *Pediatr Infect Dis J* 1991;**10**:627.
164. Peyron F, Garweg JG, Wallon M, et al. Long-term impact of treated congenital toxoplasmosis on quality of life and visual performance. *Pediatr Infect Dis J* 2011;**30**:597–600.
167. Pinon JM, Chemla C, Villena I, et al. Early neonatal diagnosis of congenital toxoplasmosis: value of comparative enzyme linked immunofiltration assay immunological profiles and anti–*Toxoplasma gondii* immunoglobulin M (IgM) or IgA immunocapture and implications for postnatal therapeutic strategies. *J Clin Microbiol* 1996;**34**:579–83.
168. Pinon JM, Dumon H, Chemla C, et al. Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies. *J Clin Microbiol* 2001;**39**:2267.
169. Rabilloud M, Wallon M, Peyron F. In utero and at birth diagnosis of congenital toxoplasmosis: use of likelihood ratios for clinical management. *Pediatr Infect Dis J* 2010;**29**:421–5.
173. Robert-Gangneux F, Darde ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev* 2012;**25**:264–96.
174. Robert-Gangneux F, Dupretz P, Yvenou C, et al. Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. *Pediatr Infect Dis J* 2010;**29**:33–8.
198. Stillwaggon E, Carrier CS, Sautter M, McLeod R. Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. *PLoS Negl Trop Dis* 2011;**5**:e1333–9.
207. Vasconcelos-Santos DV, Queiroz-Andrade GM. Geographic differences in outcomes of congenital toxoplasmosis. *Pediatr Infect Dis J* 2011;**30**:816–17.
210. Wallon M, Cogan G, Ecochard R, et al: Serological rebound in congenital toxoplasmosis: long-term follow-up of 133 children. *Eur J Pediatr* 2001;**160**:534–40.
225. Yamada H, Nishikawa A, Yamamoto T, et al. Prospective study of congenital toxoplasmosis screening with use of IgG avidity and multiplex nested PCR methods. *J Clin Microbiol* 2011;**49**: 2552–6.
226. Zufferey J, Hohlfeld P, Bille J, et al. Value of the comparative enzyme-linked immunofiltration assay for early neonatal diagnosis of congenital *Toxoplasma* infection. *Pediatr Infect Dis J* 1999;**18**:971.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alford CA Jr, Stagno S, Reynolds DW. Congenital toxoplasmosis: clinical, laboratory and therapeutic considerations, with special reference to subclinical disease. *Bull N Y Acad Med* 1974;**50**: 160–81.
2. Araujo FG, Barnett EV, Gentry LO, et al. False positive anti-*Toxoplasma* fluorescent antibody tests in patients with antinuclear antibodies. *Appl Microbiol* 1971;**22**:270–5.
3. Araujo FG, Guptill DR, Remington JS. Azithromycin, a macrolide antibiotic with potent activity against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 1988;**32**:755–7.
4. Araujo FG, Lin T, Remington JS. The activity of atovaquone (566C80) in murine toxoplasmosis is markedly augmented when used in combination with pyrimethamine or sulfadiazine. *J Infect Dis* 1993;**167**:494–7.
5. Araujo F, Prokocimer P, Remington J. Clarithromycin-minocycline is synergistic in a murine model of toxoplasmosis. *J Infect Dis* 1992;**165**:788.
6. Araujo F, Remington J. Recent advances in the search for new drugs for treatment of toxoplasmosis. *Int J Antimicrob Agents* 1992;**1**:153–64.
7. Araujo FG, Slifer T, Remington JS. Rifabutin is active in murine models of toxoplasmosis. *Antimicrob Agents Chemother* 1994;**38**: 570–5.
8. Arias I, Sorlozano A, Villegas E, et al. Infectious agents associated with schizophrenia: a meta-analysis. *Schizophr Res* 2012;**136**: 128–39.
9. Aspock H. Prevention of congenital toxoplasmosis by serological surveillance during pregnancy: current strategies and future perspectives. In: Marget W, Lang W, Gabler-Sandberger E, editors. *Parasitic infections, immunology, mycotic infections, general topics*, vol. 3. Munich: Medizin Verlag; 1986. p. 69–72.
10. Aspock H, Flamm H, Pilcher O. Die Toxoplasmose-Überwachung während der Schwangerschaft 10-jahre Erfahrungen in Österreich. *Mitt Oesterr Ges Trophenmed Parasitol* 1986;**8**: 105–13.
11. Bahia-Oliveira LM, Jones JL, Azevedo-Silva J, et al. Highly endemic, waterborne toxoplasmosis in North Rio de Janeiro State, *Brazil Emerg Infect Dis* 2003;**9**:55–62.
12. Beach PG. Prevalence of antibodies to *Toxoplasma gondii* in pregnant women in Oregon. *J Infect Dis* 1979;**140**:780–3.
13. Berger R, Merkel S, Rudin C. Toxoplasmosis and pregnancy: findings from umbilical cord blood screening in 30,000 newborn infants. *Schweiz Med Wochenschr* 1995;**125**:1168–73.
14. Berrebi A, Assouline C, Bessieres MH, et al. Long-term outcome of children with congenital toxoplasmosis. *Am J Obstet Gynecol* 2010;**203**:552.e1–6.
15. Berrebi A, Kobuch WE, Bessieres MH, et al. Termination of pregnancy for maternal toxoplasmosis. *Lancet* 1994;**344**:36–8.
16. Bowie WR, King AS, Werker DH, et al. Outbreak of toxoplasmosis associated with municipal drinking water. *Lancet* 1997;**350**: 173–7.
17. Boyer KM. Diagnostic testing for congenital toxoplasmosis. *Pediatr Infect Dis J* 2001;**20**:59–60.
18. Boyer KM, Hill D, Mui E, et al. Unrecognized ingestion of *Toxoplasma gondii* oocysts leads to congenital toxoplasmosis and causes epidemics in North America. *Clin Infect Dis* 2011;**53**:1081–9.
19. Boyer KM, Holfels E, Roizen N, et al. Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: implications for prenatal management and screening. *Am J Obstet Gynecol* 2005;**192**:564–71.
20. Brezin AP, Kasner L, Thulliez P, et al. Ocular toxoplasmosis in the fetus: immunohistochemistry analysis and DNA amplification. *Retina* 1994;**14**:19–26.
21. Brown CR, Estes RG, Bechmann E, et al. Definitive identification of a toxoplasmosis resistance gene. *Res Immunol Ann Inst Pasteur* 1993;**144**:61–6.
22. Brown C, McLeod R. Class I MHC genes and $CD8^+$ T cells determine cyst number in *Toxoplasma gondii* infection. *J Immunol* 1990;**145**:3438–41.
23. Bulow R, Boothroyd JC. Protection of mice from fatal *Toxoplasma gondii* infection by immunization with p30 antigen in liposomes. *J Immunol* 1991;**147**:3496–500.
24. Buxton D. Toxoplasmosis: the first commercial vaccine. *Parasitol Today* 1993;**9**:335–7.
25. Buxton D, Innes EA. A commercial vaccine for ovine toxoplasmosis. *Parasitology* 1995;**110**(Suppl.):11–16.
26. Candolfi E, Pastor R, Huber R, et al. IgG avidity assay firms up the diagnosis of acute toxoplasmosis on the first serum sample in immunocompetent pregnant women. *Diagn Microbiol Infect Dis* 2007;**58**:83–8.
27. Carr A, Tindall B, Brew BJ, et al. Low-dose trimethoprim-sulfamethoxazole prophylaxis for toxoplasmic encephalitis in patients with AIDS. *Ann Intern Med* 1992;**117**:106–11.
28. Carter AO, Gelmon SB, Wells GA, et al. The effectiveness of a prenatal education programme for the prevention of congenital toxoplasmosis. *Epidemiol Infect* 1989;**103**:539–45.
29. Catalano-Pons C, Bargy S, Schlecht D, et al. Sulfadiazine-induced nephrolithiasis in children. *Pediatr Nephrol* 2004;**19**:928–31.
30. Centers for Disease Control and Prevention. Preventing congenital toxoplasmosis. *MMWR Morb Mortal Wkly Rep* 2000;**49**(RR-2): 57–75.
31. Cesbron-Delau MF. Dense-granule organelles of *Toxoplasma gondii*: their role in the host-parasite relationship. *Parasitol Today* 1994;**10**:293–6.
32. Cesbrons MF, Dubremetz JF, Sher A. The immunobiology of toxoplasmosis. *Res Immunol* 1993;**144**:7–8.
33. Chandraekar PH. Real-time polymerase chain reaction for early diagnosis of toxoplasmosis in stem cell transplant recipients: ready for prime time? *Clin Infect Dis* 2007;**40**:79–81.
34. Chapey E, Wallon M, Debize G, et al. Diagnosis of congenital toxoplasmosis by using a whole-blood gamma interferon release assay. *J Clin Microbiol* 2010;**48**:41–5.
35. Conley FK, Jenkins HT, Remington JS. *Toxoplasma gondii* infection of the central nervous system: use of the peroxidase-antiperoxidase method to demonstrate toxoplasma in formalin fixed paraffin embedded tissue sections. *Hum Pathol* 1981;**12**: 690–8.
36. Cortina-Borja M, Tan HK, Wallon M, et al. Prenatal treatment for serious neurological sequelae of congenital toxoplasmosis: an observational prospective cohort study. *PLoS Med* 2010;**7**: e1000351.
37. Couvreur J, Desmonts G. Congenital and maternal toxoplasmosis: a review of 300 congenital cases. *Dev Med Child Neurol* 1962;**4**: 519–30.
38. Couvreur J, Desmonts G, Aron-Rosa D. Le pronostic oculaire de la toxoplasmosis congenital: role du traitement. *Ann Pediatr (Paris)* 1994;**31**:855–8.
39. Couvreur J, Desmonts G, Thulliez P. Prophylaxis of congenital toxoplasmosis: effect of spiramycin on placental infection. *J Antimicrob Chemother* 1988;**22**:193–200.
40. Couvreur J, Desmonts G, Tournier G, et al. Etude d'une serie homogene de 210 cas de toxoplasmose congenitale chez des nourrissons ages de 0 a 11 mois et depistes de facon prospective. *Ann Pediatr (Paris)* 1984;**31**:815.
41. Couvreur J, Thulliez P, Daffos F, et al. Foetopathie toxoplasmique: traitment in utero par l'association pyrimethamine-sulfamides. *Arch Fr Pediatr* 1991;**48**:397–403.
42. Daffos F, Forestier F, Capella-Pavlovsky M, et al. Prenatal management of 746 pregnancies at risk for congenital toxoplasmosis. *N Engl J Med* 1988;**318**:271–5.
43. Dannemann B, McCutchan JA, Israelski D, et al. Treatment of toxoplasmic encephalitis in patients with AIDS: a randomized trial comparing pyrimethamine plus clindamycin to pyrimethamine plus sulfonamides. *Ann Intern Med* 1992;**116**:33–43.
44. Dannemann BR, Vaughan WC, Thulliez P, et al. The differential agglutination test for diagnosis of recently acquired infection with *Toxoplasma gondii*. *J Clin Microbiol* 1990;**28**:1928–33.
45. Darcy F, Santoro F. Toxoplasmosis. In: Kierszenbaum F, editor. *Parasitic infections and the immune system*. San Diego, Calif: Academic Press; 1994. p. 163–90.
46. Darde ML. Genetic analysis of the diversity in *Toxoplasma gondii*. *Ann Ist Super Sanita* 2004;**40**:57–63.
47. Darling ST. Sarcosporidiosis: with report of a case in man. *Proc Canal Zone Med Assoc* 1908;**1**:141.
48. Decoster A, Darcy F, Caron A, et al. IgA antibodies against P30 as markers of congenital and acute toxoplasmosis. *Lancet* 1988;**2**:1104–7.
49. Derouin F, Devergie A, Auber P. Toxoplasmosis in bone marrow–transplant recipients: report of seven cases and review. *Clin Infect Dis* 1992;**15**:267–70.

50. Derouin F, Gluckman E, Beauvais B, et al. *Toxoplasma* infection after human allogeneic bone marrow transplantation: clinical and serological study of 80 patients. *Bone Marrow Transplant* 1986;**1**:67.
51. Desmonts G, Couvreur J. Congenital toxoplasmosis: a prospective study of 378 pregnancies. *N Engl J Med* 1974;**290**:1110–16.
52. Desmonts G, Couvreur J. Congenital toxoplasmosis: a prospective study of the offspring of 54 women who acquired toxoplasmosis during pregnancy: pathophysiology of congenital disease. In: Thalhammer O, Baumgarten K, Pollak A, editors. *Perinatal medicine*. Sixth European Congress, Vienna, 1978. Stuttgart: Georg Thieme; 1979.
53. Desmonts G, Daffos F, Forestier F, et al. Prenatal diagnosis of congenital toxoplasmosis. *Lancet* 1985;**1**:500–4.
54. Desmonts G, Naot Y, Remington JS. Immunoglobulin M–immunosorbent agglutination assay for diagnosis of infectious disease: diagnosis of acute congenital and acquired *Toxoplasma* infections. *J Clin Microbiol* 1981;**14**:486–91.
55. Desmonts G, Remington JS. Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *J Clin Microbiol* 1980;**11**:562–8.
56. Diebler C, Dussler A, Dulac O. Congenital toxoplasmosis: clinical and neuroradiological evaluation of the cerebral lesions. *Neuroradiology* 1985;**27**:125–30.
57. Dorfman RF, Remington JS. Value of lymph node biopsy in the diagnosis of acute acquired toxoplasmosis. *N Engl J Med* 1973;**289**:878.
58. Dubey JP. Toxoplasmosis—a waterborne zoonosis. *Vet Parasitol* 2004;**126**:57–72.
59. Dubey JP, Hill DE, Jones JL, et al. Prevalence of viable *Toxoplasma gondii* in beef, chicken, and pork from retail meat stores in the United States: risk assessment to consumers. *J Parasitol* 2005;**91**: 1082–93.
60. Dubey JP, Miller NL, Frenkel JK. The *Toxoplasma gondii* oocyst from cat feces. *J Exp Med* 1970;**132**:636–62.
61. Dubremetz JF. Rhopteries are major players in *Toxoplasma gondii* invasion and host cell interaction. *Cell Microbiol* 2007;**9**:841–8.
62. Dunn D, Newell ML, Gilbert R. Low risk of congenital toxoplasmosis in children born to women infected with human immunodeficiency virus. *Pediatr Infect Dis J* 1997;**16**:84.
63. Dunn D, Wallon M, Peyron F, et al. Mother-to-child transmission of toxoplasmosis: risk estimates for clinical counseling. *Lancet* 1999;**353**:1829.
64. Eichenwald HG. A study of congenital toxoplasmosis. In: Siim JC, editor. *Human toxoplasmosis*. Copenhagen: Munksgaard; 1960. p. 41–9.
65. Featherstone H. *A difference in the family*. New York: Penguin; 1987.
66. Filisetti D, Cocquerelle V, Pfaff A, et al. Placental testing for *Toxoplasma gondii* is not useful to diagnose congenital toxoplasmosis. *Pediatr Infect Dis J* 2010;**29**:665–7.
67. Foulon W, Naessens A, Ho-Yen D. Prevention of congenital toxoplasmosis. *J Perinat Med* 2000;**28**:337–45.
68. Frenkel JK. Toxoplasmosis. In: Marcial-Rojas RA, editor. *Pathology of protozoal and helminthic diseases*. Baltimore: Williams & Wilkins; 1971. p. 254–90.
69. Frenkel JK. Toxoplasmosis: a parasite life cycle, pathology, and immunology. In: Hammond DM, editor. *The coccidian*. Baltimore: University Park Press; 1973. p. 343–410.
70. Frenkel JK. Pathology and pathogenesis of congenital toxoplasmosis. *Bull N Y Acad Med* 1974;**50**:182–91.
71. Frenkel J, Dubey JP, Miller NL. *Toxoplasma gondii* in cats: fecal stage identified as coccidian oocysts. *Science* 1970;**167**:893–6.
72. Frenkel JK, Weber RW, Lunde MN. Acute toxoplasmosis: effective treatment with pyrimethamine, sulfadiazine, leucovorin calcium and yeast. *JAMA* 1960;**173**:1471–6.
73. Galanakis E, Manoura A, Antoniou M, et al. Outcome of toxoplasmosis acquired during pregnancy following treatment in both pregnancy and early infancy. *Fetal Diagn Ther* 2007;**22**:444–8.
74. Galli-Tsinopoulou A, Kyrgios I, Giannopoulou EZ, et al. Acquired toxoplasmosis accompanied by facial nerve palsy in an immunocompetent 5-year-old child. *J Child Neurol* 2010;**25**:1525–8.
75. Garcia AGP. Congenital toxoplasmosis in two successive sibs. *Arch Dis Child* 1979;**43**:705–9.
76. Garcia de la Fuente I, Ansari M, Rougemont AL, et al. Acute disseminated fatal toxoplasmosis after haploidentical stem cell transplantation despite atovaquone prophylaxis in a young man. *Pediatr Infect Dis J* 2010;**29**:1059–60.
77. Gazzinelli RT, Hayashi S, Wysocka M, et al. Role of IL-12 in the initiation of cell-mediated immunity by *Toxoplasma gondii* and its regulation by IL-10 and nitric oxide. *J Eukaryot Microbiol* 1994;**41**(Suppl):9S.
78. Gazzinelli RT, Oswald IP, James SL, et al. IL-10 inhibits parasite killing and nitrogen oxide production by IFN-γ–activated macrophages. *J Immunol* 1992;**148**:1792–6.
79. Gilbert R, Dezateux C. Newborn screening for congenital toxoplasmosis: feasible, but benefits are not established. *Arch Dis Child* 2006;**91**:629–31.
80. Gilbert R, Tan HK, Cliffe S, et al. Symptomatic toxoplasma infection due to congenital and postnatally acquired infection. *Arch Dis Child* 2006;**91**:495–8.
81. Gomez-Marin JE, delaTorre A. Positive benefit of postnatal treatment in congenital toxoplasmosis. *Arch Dis Child* 2007;**92**:88–9.
82. Gomez-Marin JE, Gonzalez MM, Montoya MT, et al. A newborn screening programme for congenital toxoplasmosis in the setting of a country with less income. *Arch Dis Child* 2007;**92**:88.
83. Gras L, Wallon M, Pollak A, et al. Association between prenatal treatment and clinical manifestations of congenital toxoplasmosis in infancy: a cohort study in 13 European centers. *Acta Paediatr* 2005;**94**:1721–31.
84. Grigg ME, Bonnefoy S, Hehl AB, et al. Success and virulence in *Toxoplasma* as the result of sexual recombination between two distinct ancestries. *Science* 2001;**294**:161–5.
85. Grigg ME, Ganatra J, Boothroyd JC, Margolis TP. Unusual abundance of atypical strains associated with ocular toxoplasmosis. *J Infect Dis* 2001;**184**:633–9.
86. Grover CM, Thulliez P, Remington JS, et al. Rapid prenatal diagnosis of congenital *Toxoplasma* infection using polymerase chain reaction and amniotic fluid. *J Clin Microbiol* 1990;**28**:2297–301.
87. Guerina NG, Hsu H-W, Meissner HC, et al. Neonatal serologic screening and early treatment for congenital *Toxoplasma gondii* infection. *N Engl J Med* 1994;**33**:1858–63.
88. Hakim FT, Gazzinelli RT, Denkers E, et al. $CD8^+$ T cells from mice vaccinated against *Toxoplasma gondii* are cytotoxic for parasite-infected or antigen-pulsed host cells. *J Immunol* 1991;**147**: 2310–16.
89. Hohlfeld P, Daffos T, Costa JM, et al. Prenatal diagnosis of congenital toxoplasmosis with a polymerase-chain reaction test on amniotic fluid. *N Engl J Med* 1994;**331**:695–9.
90. Hohlfeld P, Daffos F, Thulliez P, et al. Fetal toxoplasmosis: outcome of pregnancy and infant follow-up after in utero treatment. *J Pediatr* 1989;**115**:765–9.
91. Holfels E, McAuley J, Mack D, et al. In vitro effects of artemisinin ether, cycloguanil hydrochloride (alone and in combination with sulfadiazine), quinine sulfate, mefloquine, primaquine phosphate, trifluoperazine hydrochloride, and verapamil on *Toxoplasma gondii*. *Antimicrob Agents Chemother* 1994;**38**:1392–6.
92. Holland GN. Ocular toxoplasmosis: a global reassessment, part II: disease manifestations and management. *Am J Ophthalmol* 2004;**137**:1–17.
93. Huskinson-Mark J, Araujo FG, Remington JS. Evaluation of the effect of drugs on the cyst form of *Toxoplasma gondii*. *J Infect Dis* 1991;**164**:170–7.
94. Hutchison WM, Dunachie JF, Siim JC, et al. Coccidian-like nature of *Toxoplasma gondii*. *BMJ* 1970;**1**:142–4.
95. Ives NJ, Gazzard BG, Easterbrook PJ. The changing pattern of AIDS-defining illnesses with the introduction of highly active antiretroviral therapy (HAART) in a London clinic. *J Infect* 2001;**42**:134–9.
96. Janku J. Pathogenesa a pathologicka anatomie tak nazveneho vrozeneho kolobomu slute skvrny v oku normalne velikem a mikrophthalmickem s nalezem parazitu v sitnici. *Cas Lek Cesk* 1923; **60**:1021.
97. Jara M, Hsu HW, Eaton RB, Demaria A Jr. Epidemiology of congenital toxoplasmosis identified by population-based newborn screening in Massachusetts. *Pediatr Infect Dis J* 2001;**20**:1132.
98. Johnson A, McLeod R, Cesbron-Delauw MF, et al. *Vaccine development and technology to prevent toxoplasmosis*. Fontevraud, France: WHO Working Group; 1992. p. 1.
99. Jones JL, Dubey JP. Foodborne toxoplasmosis. *Clin Infect Dis* 2012;**55**:845–51.
100. Jones JL, Holland GN. Short report: annual burden of ocular toxoplasmosis in the United States. *Am J Trop Med Hyg* 2010; **82**:464–5.

101. Jones JL, Kruszon-Moran D, Sanders-Lewis K, Wilson M. *Toxoplasma gondii* infection in the United States, 1999-2004, decline from prior decade. *Am J Trop Med Hyg* 2007;**77**:405–10.
102. Jones JL, Kruszon-Moran D, Wilson M, et al. *Toxoplasma gondii* infection in the United States: seroprevalence and risk factors. *Am J Epidemiol* 2001;**154**:357–65.
103. Jones JL, Roberts JM. Toxoplasmosis hospitalizations in the United States, 2008, and trends, 1993-2008. *Clin Infect Dis* 2012; **54**:e58–61.
104. Jost C, Touafek F, Fekkar A, et al. Utility of immunoblotting for early diagnosis of toxoplasmosis seroconversion in pregnant women. *Clin Vaccine Immunol* 2011;**18**:1908–12.
105. Kasper LH, Khan IA, Ely KH, et al. Antigen-specific (P30) mouse $CD8^+$ T cells are cytotoxic against *Toxoplasma gondii*–infected peritoneal macrophages. *J Immunol* 1992;**148**:1493–8.
106. Kazdan JJ, McCulloch JC, Crawford JS. Uveitis in children. *Can Med Assoc J* 1967;**96**:385.
107. Khan A, Jordan C, Muccioli C, et al. Genetic divergence of *Toxoplasma gondii* strains associated with ocular toxoplasmosis, *Brazil Emerg Infect Dis* 2006;**12**:942–9.
108. Khan AA, Slifer T, Araujo FG, et al. Trovafloxacin is active against *Toxoplasma gondii*. *Antimicrob Agents Chemother* 1996;**40**:1855–9.
109. Khan IA, Ely KH, Kasper LH. A purified parasite antigen (p30) mediates $CD8^+$ T cell immunity against fatal *Toxoplasma gondii* infection in mice. *J Immunol* 1991;**147**:3501–6.
110. Kim K. Time to screen for congenital toxoplasmosis? *Clin Infect Dis* 2006;**42**:1395–7.
111. Kim K, Soldati D, Boothroyd JC. Gene replacement in *Toxoplasma gondii* with chloramphenicol acetyltransferase as selectable marker. *Science* 1993;**262**:911–14.
112. Koppe JG, Kloosterman GJ, de Roever-Bonnet H, et al. Toxoplasmosis and pregnancy, with a long-term follow-up of the children. *Eur J Obstet Gynecol Reprod Biol* 1974;**413**:101–10.
113. Koppe JG, Loewer-Sieger DH, de Roever-Bonnet H. Results of 20-year follow-up of congenital toxoplasmosis. *Lancet* 1986;**1**: 254–6.
114. Kraubig H. Preventive Behandlung der konatalen Toxoplasmose. In: Kirchoff H, Kraubig H, editors. *Toxoplasmose: Praktische Fragen und Ergebnisse*. Stuttgart: Georg Thieme Verlag; 1966.
115. Kusbeci OY, Miman O, Yaman M, et al. Could *Toxoplasma gondii* have any role in Alzheimer disease? *Alzheimer Dis Assoc Disord* 2011;**25**:1–3.
116. Labadie MD, Hazemann JJ. Apport des bilans de sante de l'enfant pour le depistage et l'etude epidemiologique de la toxoplasmose congenitale. *Ann Pediatr (Paris)* 1984;**31**:823.
117. Lago EG, Baldisserotto M, Hoefel Filho JR, et al. Agreement between ultrasonography and computed tomography in detecting intracranial calcifications in congenital toxoplasmosis. *Clin Radiol* 2007;**62**:1004–11.
118. Lau YL, Meganathan P, Sonaimuthu P, et al. Specific, sensitive, and rapid diagnosis of active toxoplasmosis by loop-mediated isothermal amplification method using blood samples from patients. *J Clin Microbiol* 2010;**48**:3698–702.
119. Leport C, Chakroun M, Matheron S, et al. Zidovudine efficacy and tolerance in 32 patients with cerebral toxoplasmosis in the acquired immunodeficiency syndrome. *Presse Med* 1988;**17**: 1813–14.
120. Lesle F, Touafek F, Fekkar A, et al. Discrepancies between a new highly sensitive *Toxoplasma gondii* ELISA assay and other reagants: interest of Toxo IgG Western blot. *Eur J Clin Microbiol Infect Dis* 2011;**30**:1207–12.
121. Levaditi C. Au sujet de certaines protozooses hereditaires humaines a localisations oculaires et nerveuses. *C R Soc Biol* 1928;**98**:297.
122. Liesenfeld O, Wong SY, Remington JS. Toxoplasmosis in the setting of AIDS. In: Merigan TC, Bartlett JG, Bolognesi D, editors. *Textbook of AIDS medicine*. Baltimore: Williams & Williams; 1999. p. 225–9.
123. Lim Z, Baker B, Zuckerman M, et al. Toxoplasmosis following alemtuzumab based allogeneic haematopoietic stem cell translplantation. *J Infect* 2007;**54**:e83–6.
124. Ling VJ, Lester D, Mortensen PB, et al. *Toxoplasma gondii* seropositivity and suicide rates in women. *J Nerv Ment Dis* 2011; **199**:440–4.
125. Luft BJ, Conley F, Remington JS. Outbreak of central-nervous-system toxoplasmosis in Western Europe and North America. *Lancet* 1983;**1**:781.
126. Luft BJ, Naot Y, Araujo FG, et al. Primary and reactivated *Toxoplasma* infection in patients with cardiac transplants: clinical spectrum and problems in diagnosis in a defined population. *Ann Intern Med* 1983;**99**:27.
127. Luft BJ, Remington JS. Acute *Toxoplasma* infection among family members of patients with acute lymphadenopathic toxoplasmosis. *Arch Intern Med* 1984;**144**:53–6.
128. Luft BJ, Remington JS. Toxoplasmic encephalitis in AIDS. *Clin Infect Dis* 1992;**15**:211–22.
129. Mack DG, Johnson JJ, Roberts F, et al. Murine and human MHC class II genes determine susceptibility to toxoplasmosis. *Int J Parasitol* 1999;**29**:1351–8.
130. Masur H, Jones TC, Lempert JA, et al. Outbreak of toxoplasmosis in a family and documentation of acquired retinochoroiditis. *Am J Med* 1978;**64**:396–402.
131. Matsuo Y, Takeishi S, Miyamoto T, et al. Toxoplasmosis encephalitis following severe graft-vs.-host disease after allogenic hematopoietic stem cell transplantation: 17 yr experience in Fukuoka BMT group. *Eur J Haematol* 2007;**79**:317–21.
132. McCabe RE, Brooks RG, Dorfman RF, et al. Clinical spectrum in 107 cases of toxoplasmic lymphadenopathy. *Rev Infect Dis* 1987;**9**:754.
133. McCabe R, Gibbons D, Brooks RG, et al. Agglutination test for diagnosis of toxoplasmosis in AIDS. *Lancet* 1983;**2**:680.
134. McCabe RE, Remington JS. Toxoplasmosis: the time has come. *N Engl J Med* 1988;**318**:313–15.
135. McGee T, Wolters C, Stein L, et al. Absence of sensorineural hearing abnormalities in treated infants with congenital toxoplasmosis. *Otolaryngol Head Neck Surg* 1992;**106**:75–80.
136. McLeod R, Beem MO, Estes RG. Lymphocyte anergy specific to *Toxoplasma gondii* antigens in a baby with congenital toxoplasmosis. *J Clin Lab Immunol* 1985;**17**:149–53.
137. McLeod R, Boyer KM, Karrison T, et al. Outcome of treatment for congenital toxoplasmosis, 1981–2004: the national collaborative Chicago-based, congenital toxoplasmosis study. *Clin Infect Dis* 2006;**42**:1383–94.
138. McLeod R, Boyer KM, Roizen N, et al. The child with congenital toxoplasmosis. *Curr Clin Top Infect Dis* 2000;**20**:189–208.
139. McLeod R, Brown C, Mack D. Immunogenetics influence outcome of *Toxoplasma gondii* infection. *J Immunol* 1989;**142**: 3247–55.
140. McLeod R, Frenkel JK, Estes RG, et al. Subcutaneous and intestinal vaccination with tachyzoites of *Toxoplasma gondii* and acquisition of immunity to peroral and congenital *Toxoplasma* challenge. *J Immunol* 1988;**140**:1632–7.
141. McLeod R, Khan AR, Noble GA, et al. Severe sulfadiazine hypersensitivity in a child with reactivated congenital toxoplasmic chorioretinitis. *Pediatr Infect Dis J* 2006;**25**:270–2.
142. McLeod R, Mack D. A new micromethod to study the effects of antimicrobial agents of *Toxoplasma gondii*: comparison of sulfadoxine and sulfadiazine individually and in combination with pyrimethamine and study of clindamycin, metronidazole, and cyclosporin A. *Antimicrob Agents Chemother* 1984;**26**:26–30.
143. McLeod R, Mack DG, Boyer KM, et al. Phenotypes and functions of lymphocytes in congenital toxoplasmosis. *J Lab Clin Med* 1990;**116**:623–35.
144. McLeod R, Mack D, Brown C. New advances in cellular and molecular biology of *Toxoplasma gondii*. *Exp Parasitol* 1991;**72**: 109–21.
145. McLeod R, Mack D, Foss R, et al. Levels of pyrimethamine in sera and cerebrospinal and ventricular fluids from infants treated for congenital toxoplasmosis. *Antimicrob Agents Chemother* 1992; **36**:1040–8.
146. Mets MB, Holfels EM, Boyer KM, et al. Eye manifestations of congenital toxoplasmosis. *Am J Ophthalmol* 1996;**122**:309–24.
147. Miller MJ, Remington JS. Toxoplasmosis in infants and children with HIV infection or AIDS. In: Pizzo PA, Wilfert CM, editors. *Pediatric AIDS: the challenge of HIV infection in infants, children and adolescents*. Baltimore: Williams & Wilkins; 1990. p. 299–307.
148. Mitchell CD, Erlich SS, Mastrucci MT, et al. Congenital toxoplasmosis occurring in three infants perinatally infected with the human immunodeficiency virus (HIV-1). *Pediatr Infect Dis J* 1990; **9**:512.
149. Mitchell W. Neurological and developmental effects of HIV and AIDS in children and adolescents. *Ment Retard Dev Disabil Res Rev* 2001;**7**:211–16.

150. Montoya JG. Laboratory diagnosis of *Toxoplasma gondii* infection and toxoplasmosis. *J Infect Dis* 2002;**185**(Suppl):73–82.
151. Montoya JG, Liesenfeld O. Toxoplasmosis. *Lancet* 2004;**363**: 1965–76.
152. Montoya JG, Remington JS. Studies on the serodiagnosis of toxoplasmic lymphadenitis. *Clin Infect Dis* 1995;**20**:781–9.
153. Montoya JG, Remington JS. Toxoplasmic chorioretinitis in the setting of acute acquired toxoplasmosis. *Clin Infect Dis* 1996;**23**: 277–82.
154. Morris JG, Greenspan A, Howell K, et al. Southeastern Center for Emerging Biologic Threats tabletop exercise: foodborne toxoplasmosis outbreak on college campuses. *Biosecur Bioterror* 2012;**10**:89–102.
155. Munoz-Zanzi CA, Fry P, Lesina B, Hill D. *Toxoplasma gondii* oocyst-specific antibodies and source of infection. *Emerg Infect Dis* 2010;**16**:1591–7.
156. Naot Y, Barnett EV, Remington JS. Method for avoiding false positive results occurring in immunoglobulin M enzyme-linked immunosorbent assays due to presence of both rheumatoid factor and antinuclear antibodies. *J Clin Microbiol* 1981;**14**: 73–8.
157. Naot Y, Desmonts G, Remington JS. IgM enzyme-linked immunosorbent assay test for the diagnosis of congenital *Toxoplasma* infection. *J Pediatr* 1981;**98**:32–6.
158. Naot Y, Remington JS. An enzyme-linked immunosorbent assay for detection of IgM antibodies to *Toxoplasma gondii*: use for diagnosis of acute acquired toxoplasmosis. *J Infect Dis* 1981;**142**: 757–66.
159. Nicolle C, Manceaux L. Sur une infection à corps de Leishman (ou organisme voisins) du gondi. *C R Acad Sci* 1908;**147**:763.
160. Nicolle C, Manceaux L. Sur une protozoaire nouveau du gondi. *C R Acad Sci* 1909;**148**:369.
161. O'Connor GR. Manifestations and management of ocular toxoplasmosis. *Bull N Y Acad Med* 1974;**50**:192.
162. O'Donohoe JM, Brueton MJ, Holliman RE. Concurrent congenital human immunodeficiency virus infection and toxoplasmosis. *Pediatr Infect Dis J* 1991;**10**:627.
163. Patel DV, Hofels E, Vogel N, et al. Resolution of intracerebral calcifications in children with treated congenital toxoplasmosis. *Radiology* 1996;**199**:433–40.
164. Peyron F, Garweg JG, Wallon M, et al. Long-term impact of treated congenital toxoplasmosis on quality of life and visual performance. *Pediatr Infect Dis J* 2011;**30**:597–600.
165. Pfefferkorn ER, Nothnagel RF, Borotz SE. Parasiticidal effect of clindamycin on *Toxoplasma gondii* grown in cultured cells and selection of a drug-resistant mutant. *Antimicrob Agents Chemother* 1992;**36**:1091–6.
166. Pinkerton H, Weinman D. *Toxoplasma* infection in man. *Arch Pathol* 1940;**30**:374.
167. Pinon JM, Chemla C, Villena I, et al. Early neonatal diagnosis of congenital toxoplasmosis: value of comparative enzyme linked immunofiltration assay immunological profiles and anti–*Toxoplasma gondii* immunoglobulin M (IgM) or IgA immunocapture and implications for postnatal therapeutic strategies. *J Clin Microbiol* 1996;**34**:579–83.
168. Pinon JM, Dumon H, Chemla C, et al. Strategy for diagnosis of congenital toxoplasmosis: evaluation of methods comparing mothers and newborns and standard methods for postnatal detection of immunoglobulin G, M, and A antibodies. *J Clin Microbiol* 2001;**39**:2267.
169. Rabilloud M, Wallon M, Peyron F. In utero and at birth diagnosis of congenital toxoplasmosis: use of likelihood ratios for clinical management. *Pediatr Infect Dis J* 2010;**29**:421–5.
170. Remington J, Eimstad W, Araujo F. Detection of immunoglobulin M antibodies with antigen-tagged latex particles in an immunosorbent assay. *J Clin Microbiol* 1983;**17**:939–41.
171. Remington JS, McLeod R. Toxoplasmosis. In: Braude AI, editor. *International textbook of medicine Medical microbiology and infectious disease*, vol. II. Philadelphia: WB Saunders; 1981. p. 1818.
172. Remington JS, McLeod R, Thulliez P, et al. Toxoplasmosis. In: Remington JS, Klein JO, Wilson CB, et al, editors. *Infectious diseases of the fetus and newborn*. 6th ed. Philadelphia: WB Saunders; 2006. p. 947–1092.
173. Robert-Gangneux F, Darde ML. Epidemiology of and diagnostic strategies for toxoplasmosis. *Clin Microbiol Rev* 2012;**25**:264–96.
174. Robert-Gangneux F, Dupretz P, Yvenou C, et al. Clinical relevance of placenta examination for the diagnosis of congenital toxoplasmosis. *Pediatr Infect Dis J* 2010;**29**:33–8.
175. Roberts CW, Roberts F, Lyons RE, et al. The shikimate pathway and its branches in apicomplexan parasites. *J Infect Dis* 2002; **185**(Suppl):25–36.
176. Roberts F, McLeod R. Pathogenesis of toxoplasmic retinochoroiditis. *Parasitol Today* 1999;**15**:51–7.
177. Roberts F, Mets MB, Ferguson DJP, et al. Histopathological features of ocular toxoplasmosis in the fetus and infant. *Arch Ophthalmol* 2001;**19**:51–8.
178. Roberts F, Roberts CW, Johnson JJ, et al. Evidence for the shikimate pathway in apicomplexan parasites. *Nature* 1998;**393**:801–5.
179. Roberts T, Frenkel JK. Estimating income losses and other preventable costs caused by congenital toxoplasmosis in people in the United States. *JAMA* 1990;**2**:249–57.
180. Roizen N, McLeod R, Boyer KM, et al. Impact of visual impairment on measures of cognitive function for children with congenital toxoplasmosis: implications for compensatory intervention strategies. *Pediatrics* 2006;**118**:379–90.
181. Roizen N, Swisher C, Stein MA, et al. Neurologic and developmental function in treated congenital toxoplasmosis. *Pediatrics* 1995;**95**:11–20.
182. Romand DS, Chosson M, Franck J, et al. Usefulness of quantitative polymerase chain reaction in amniotic fluid as early prognostic marker of fetal infection with *Toxoplasma gondii*. *Am J Gynecol* 2004;**90**:797–802.
183. Romand DS, Wallon M, Franck J, et al. Prenatal diagnosis using polymerase chain reaction on amniotic fluid for congenital toxoplasmosis. *Obstet Gynecol* 2001;**97**:296–300.
184. Ruskin J, Remington JS. Toxoplasmosis in the compromised host. *Ann Intern Med* 1976;**84**:193.
185. Sabin AB. Toxoplasmic encephalitis in children. *JAMA* 1941;**116**: 801–7.
186. Sabin AB, Feldman HA. Dyes as microchemical indicators of a new immunity phenomenon affecting a protozoon parasite (*Toxoplasma*). *Science* 1948;**108**:660.
187. Sacks JJ, Roberto RR, Brooks NF. Toxoplasmosis infection associated with raw goat's milk. *JAMA* 1982;**248**:1728–32.
188. Schlaegel TF. *Ocular toxoplasmosis and pars planitis*. New York: Grune & Stratton; 1978.
189. Schoondermark–Van de Ven E, Galama J, Camps W, et al. Pharmacokinetics of spiramycin in the rhesus monkey: transplacental passage and distribution in tissue in the fetus. *Antimicrob Agents Chemother* 1994;**38**:1922–9.
190. Schoondermark–Van de Ven E, Melchers W, Camps W, et al. Effectiveness of spiramycin for treatment of congenital *Toxoplasma gondii* infection in rhesus monkeys. *Antimicrob Agents Chemother* 1994;**38**:1930–6.
191. Shenep JL, Barenkamp SJ, Brammeier SA, et al. An outbreak of toxoplasmosis on an Illinois farm. *Pediatr Infect Dis* 1984;**3**: 518–22.
192. Sher A, Oswald IP, Hieny S, et al. *Toxoplasma gondii* induces a T-independent IFN-γ response in natural killer cells that requires both adherent accessory cells and tumor necrosis factor-α. *J Immunol* 1993;**150**:3982–9.
193. Sibley LD, Pfefferkorn ER, Boothroyd JC. Development of genetic systems for *Toxoplasma gondii*. *Parasitol Today* 1993;**9**: 392–5.
194. Smith KL, Wilson M, Hightower AL, et al. Prevalence of *Toxoplasma gondii* antibodies in U. S. military recruits in 1989: comparison with data published in 1965. *Clin Infect Dis* 1996;**23**: 1182–3.
195. Soheilian M, Sadoughi MM, Ghajarnia M, et al. Prospective randomized trial of trimethoprim/sulfamethoxazole versus pyrimethamine and sulfadiazine in the treatment of ocular toxoplasmosis. *Ophthalmology* 2005;**112**:1876–82.
196. Splendore A. Un nuovo protozoa parassita dei conigli: incontrato nelle lesioni anatomiche d'una malattia che ricorda in molti punti il Kalaazar dell'uomo. *Rev Soc Sci* 1908;**3**:109.
197. Stepick-Biek P, Thulliez P, Araujo FG, et al. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. *J Infect Dis* 1990;**162**:270–3.
198. Stillwaggon E, Carrier CS, Sautter M, McLeod R. Maternal serologic screening to prevent congenital toxoplasmosis: a decision-analytic economic model. *PLoS Negl Trop Dis* 2011;**5**:e1333–9.

199. Stray-Pedersen B. Infants potentially at risk for congenital toxoplasmosis: a prospective study. *Am J Dis Child* 1980;**134**:638–42.
200. Suzuki Y, Orellana MA, Schreiber RD, et al. Interferon-γ: the major mediator of resistance against *Toxoplasma gondii*. *Science* 1988;**240**:516–18.
201. Swisher CN, Boyer K, McLeod R. Congenital toxoplasmosis. The Toxoplasmosis Study Group. *Semin Pediatr Neurol* 1994;**1**: 4–25.
202. Tabbara K, O'Connor R. Treatment of ocular toxoplasmosis with clindamycin and sulfadiazine. *Ophthalmology* 1980;**87**:129.
203. Tan HK, Schmidt D, Stanford M, et al. Risk of visual impairment in children with congenital toxoplasmic retinochoroiditis. *Am J Ophthalmol* 2007;**144**:648–53.
204. Teutsch SM, Juranek DD, Sulzer A, et al. Epidemic toxoplasmosis associated with infected cats. *N Engl J Med* 1979;**300**:695–9.
205. The SYROCOT Study Group; Theibaut R, Leproust S, Chene G, Gilbert RE. Effectiveness of prenatal treatment for congenital toxoplasmosis: a meta-analysis of individual patients' data. *Lancet* 2007;**369**:115–22.
206. Thiebaut R, Leroy V, Alioum A, et al. Biases in observational studies of the effect of prenatal treatment for congenital toxoplasmosis. *Eur J Obstet Gynecol Reprod Biol* 2005;**124**:3–9.
207. Vasconcelos-Santos DV, Queiroz-Andrade GM. Geographic differences in outcomes of congenital toxoplasmosis. *Pediatr Infect Dis J* 2011;**30**:816–17.
208. Vietzke WM, Gelderman AH, Grimley PM, et al. Toxoplasmosis complicating malignancy. *Cancer* 1968;**21**:816.
209. Vogel N, Kirisits M, Michael E, et al. Congenital toxoplasmosis transmitted from an immunologically competent mother infected before conception. *Clin Infect Dis* 1996;**23**:1055–60.
210. Wallon M, Cogan G, Ecochard R, et al: Serological rebound in congenital toxoplasmosis: long-term follow-up of 133 children. *Eur J Pediatr* 2001;**160**:534–40.
211. Wallon M, Franck J, Thulliez P, et al. Accuracy of real-time polymerase chain reaction for *Toxoplasma gondii* in amniotic fluid. *Obstet Gynecol* 2010;**115**:727–33.
212. Wallon M, Kodjikian L, Binquet C, et al. Long-term ocular prognosis in 327 children with congenital toxoplasmosis. *Pediatrics* 2004;**113**:1567–72.
213. Walls KW, Remington JS. Evaluation of a commercial latex agglutination method for toxoplasmosis. *Diagn Microbiol Infect Dis* 1983;**1**:265–71.
214. Weiss L, Kim K, editors. *Toxoplasma gondii, the model apicomplexan parasite: perspectives and methodology*. London: Elsevier Academic Press; 2007.
215. Weiss LM, Harris C, Berger M, et al. Pyrimethamine concentrations in serum and cerebrospinal fluid during treatment of acute *Toxoplasma* encephalitis in patients with AIDS. *J Infect Dis* 1988; **157**:580–3.
216. Welch PC, Masur H, Jones TC, et al. Serologic diagnosis of acute lymphadenopathic toxoplasmosis. *J Infect Dis* 1981;**142**:256.
217. Wilson CB. Treatment of congenital toxoplasmosis during pregnancy. *J Pediatr* 1990;**116**:1003–4.
218. Wilson CB, Desmonts G, Couvreur J, et al. Lymphocyte transformation in the diagnosis of congenital *Toxoplasma* infection. *N Engl J Med* 1980;**302**:785–8.
219. Wilson CB, Remington JS. What can be done to prevent congenital toxoplasmosis? *Am J Obstet Gynecol* 1980;**138**:357–63.
220. Wilson CB, Remington JS, Stagno S, et al. Development of adverse sequelae in children born with subclinical congenital *Toxoplasma* infection. *Pediatrics* 1980;**66**:767–74.
221. Wilson M, Jones JL, McAuley JB. *Toxoplasma*. In: Murray PR, Baron EJ, Jorgenson JH, et al, editors. *Manual of clinical microbiology*. 8th ed. Washington, DC: ASM Press; 2006. p. 1970–80.
222. Wolf A, Cowen D. Granulomatous encephalomyelitis due to an Encephalitozoon (encephalitozoic encephalomyelitis): a new protozoan disease of man. *Bull Neurol Inst N Y* 1937;**6**:307.
223. Wong SY, Hajdu MP, Ramirez R, et al. The role of specific immunoglobulin E in the diagnosis of acute *Toxoplasma* infection and toxoplasmosis. *J Clin Microbiol* 1993;**31**:2952–9.
224. Wong SY, Remington JS. Toxoplasmosis in pregnancy. *Clin Infect Dis* 1994;**18**:853–62.
225. Yamada H, Nishikawa A, Yamamoto T, et al. Prospective study of congenital toxoplasmosis screening with use of IgG avidity and multiplex nested PCR methods. *J Clin Microbiol* 2011;**49**: 2552–6.
226. Zufferey J, Hohlfeld P, Bille J, et al. Value of the comparative enzyme-linked immunofiltration assay for early neonatal diagnosis of congenital *Toxoplasma* infection. *Pediatr Infect Dis J* 1999;**18**:971.

THE ORGANISM

Pneumocystis was originally identified in 1909 by Chagas, who believed it was a form of *Trypanosoma cruzi*.[9] It is now known to be a unique and ubiquitous pathogen of mammals. The first description of *Pneumocystis* infecting a human was reported in 1942.[76] The taxonomic placement of *Pneumocystis* has been difficult and varied over time. The organism has features of both protozoa and fungi. Protozoan features include morphology with three distinct forms, termed *cyst*, *sporozoite*, and *trophozoite*, sensitivity to antimicrobial agents with activity against protozoal organisms, and resistance to most antifungal drugs. Fungal features include the structure of the cyst wall, which contains chitin and β-1,3-glucan, and its genetic structure is more typical of that of fungi.[20,21]

Because *Pneumocystis* cannot be maintained in culture, definitive knowledge of its life cycle is limited. The thick-walled cyst is 5 to 8 μm in diameter and contains up to 8 μm sporozoites (or intracystic bodies), which on leaving the cyst become trophozoites (or tropic forms) varying in size from 1 to 4 μm (Fig. 224-1). There is some evidence for a sexual cycle in *Pneumocystis* based largely on the presence of homologs of genes involved in sexual cycles in fungi.

Pneumocystis strains or species have the remarkable biologic feature of only being able to infect a single mammalian species[26]; for example, organisms isolated from mice are incapable of infecting other mammals, even one as closely related as a rat. This restricted transmission is consistent with studies demonstrating unique phenotypes and genotypes of *Pneumocystis* isolated from each mammalian host studied to date.[28,30,58,69,84] The obvious implication of this biologic property is that the source of *Pneumocystis* causing PCP in humans must be other humans.

The host species specificity and restricted transmission have led to discussions regarding whether these features represent distinct species of *Pneumocystis*. Efforts have been made to adopt new nomenclature for *Pneumocystis*, primarily to distinguish between strains derived from humans and strains from lower mammals. In 1976, Frenkel suggested *P. jiroveci* n. sp. for isolates from humans.[23] During the ensuing three decades this term was not used. In 1988, Hughes and Gigliotti[37] proposed a nomenclature merely designating the host from which the organism was obtained (e.g., *P. carinii*, *humanus; P. carinii*, *rattus*). In 1994, Stringer and the *Pneumocystis* Workshop[70] used DNA sequence data to employ a "special forms" designation (e.g., *P. carinii* f. sp *hominis*). A subsequent system put forth in 2002 by Stringer and colleagues[71] is currently used by some investigators and designates the human form as *P. jiroveci* Frenkel. *P. jiroveci* is being increasingly used to designate *Pneumocystis* that infects humans. However, because issues have been raised regarding this new nomenclature, some authors use only the genus name when describing this organism.[25,32,43,71]

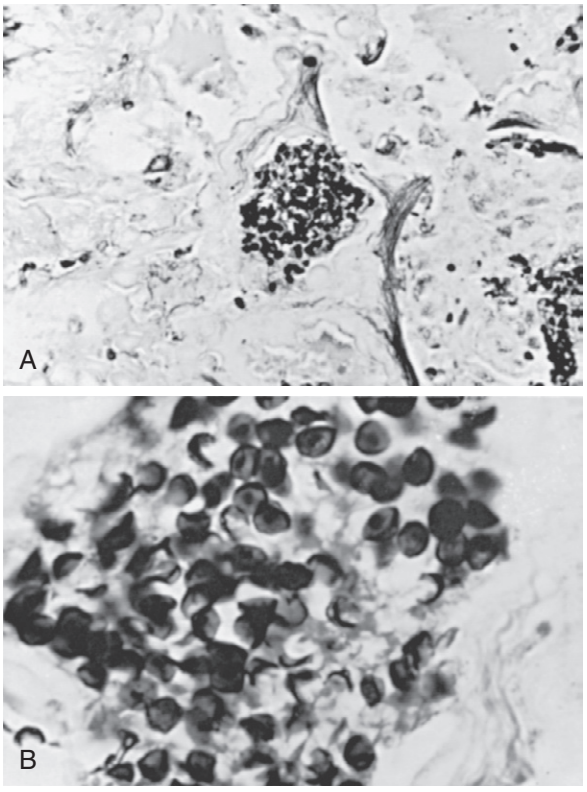

FIGURE 224-1 ■ Typical cyst forms shown by Gomori silver methenamine stain of lung tissue obtained by open lung biopsy from a 20-month-old child with severe combined immunodeficiency disease (**A,** 100×; **B,** 100×).

TRANSMISSION AND EPIDEMIOLOGY

Transmission

Pneumocystis is spread by the airborne route.[33] *Pneumocystis* has been isolated from a wide variety of animal species from all over the world. Because *Pneumocystis* organisms isolated from different mammalian hosts are morphologically quite similar, it was initially thought that PCP in humans was a zoonotic infection stemming from contact with infected animals. As noted earlier, it is now clear that transmission between two different mammalian species is highly unlikely. Longitudinal serologic studies starting just after birth demonstrate that most children are infected with *Pneumocystis* by 2 years of age.[77] These serologic data are substantiated by the finding of *Pneumocystis* in the lungs of many infants who have died of sudden infant death syndrome.[54,78] Infection of a normal host early in life could result in the establishment of a latent infection, as happens in the case of tuberculosis. This was believed to be the case with *Pneumocystis* where infection in the immunocompromised host was postulated to be the result of reactivation of a latent *Pneumocystis* infection.[13] However, under controlled experimental conditions, normal mice were shown to control *Pneumocystis* after infection in a typical pattern of infection, immune response, and resolution of infection.[3] Furthermore, latent infection does not occur after immune-mediated resolution of infection.[10] Once infected, but before

resolution of infection, immunologically normal mice can transmit *Pneumocystis* to another mouse.[29] If the recipient mouse is normal, a subclinical infection occurs. This cycle, if repeated, would serve to maintain *Pneumocystis* in the population. If instead the recipient mouse is immunocompromised, then full PCP results.

By using information gained from animal models and observational studies it is possible to propose a likely transmission cycle for *Pneumocystis*. Seronegative infants who are continuously introduced into the population present a large pool of susceptible humans that become transiently infected. Infection can be either asymptomatic or mildly symptomatic.[77] In either case, the infection is transmissible. Continued passage from normal host to normal host would serve as a reservoir of *Pneumocystis* that when transmitted to an immunocompromised individual results in life-threatening PCP. Consistent with this model, molecular studies suggest that a second case of PCP occurring more than 6 months after the first episode is most likely the result of reinfection with a new strain of *Pneumocystis*.[45] It is not known whether subclinical reinfection of adults can serve as another reservoir of *Pneumocystis*, but the finding of microscopically visible organisms rarely has been documented in adults.

Pneumocystis Pneumonia and AIDS

AIDS was brought to the attention of the medical community in the United States as a result of a small cluster of cases of PCP in previously healthy young men,[8] and PCP remains the most common AIDS-defining illness. It was reported in 39 percent of 2786 pediatric AIDS patients reported to the Centers for Disease Control and Prevention (CDC) through 1990 and in 75 percent of untreated adults with AIDS, and more than half of adults dying with AIDS have had at least one episode of PCP.[43,67] The key determinant in host defense against PCP is the $CD4^+$ T cell. Although the precise role of $CD4^+$ T cells in protection against PCP has not been defined, it is clear that any condition that interferes with $CD4^+$ T-cell number or function puts the patient at risk for developing PCP. Because the pathogenesis of AIDS involves the destruction of $CD4^+$ T cells, it is easy to identify at-risk patients simply by checking $CD4^+$ T-cell counts. Adults whose $CD4^+$ T-cell counts fall below 200/μL are at high risk to develop PCP. The determination of risk of PCP in children with AIDS is based on age because $CD4^+$ T-cell counts are very high at birth, fall rapidly during the first year of life, and then fall more slowly to reach adult levels around 6 years of age. Thus, children with AIDS are at high risk to develop PCP if they are younger than 1 year of age with $CD4^+$ T-cell counts less than 1500/μL; 1 to 5 years of age with counts less than 500/μL; and older than 5 years of age with $CD4^+$ T-cell counts less than 200/μL.

Pneumocystis Pneumonia Not Associated with AIDS

The other large group of patients at risk for PCP is those with congenital immunodeficiencies involving T- and B-cell function and those with medical conditions requiring immunosuppressive therapy, such as malignancies. For this latter group, it is important to remember that it is generally the therapy and not the underlying disease that puts the patient at risk to develop PCP. This is clearly demonstrated in children with acute lymphocytic leukemia when those receiving one, two, or three chemotherapeutic agents had an incidence of PCP between 2 and 5 percent whereas in those receiving four drugs the incidence was 22 percent.[29] Likewise, the addition of irradiation to a three-drug chemotherapeutic regimen increased the incidence of PCP to as high as 35 percent.[35] Thus, it is important to keep in mind that as therapies change, so, too, does the risk for developing PCP. For example, PCP was rarely seen in patients with Crohn disease receiving conventional immunosuppressive therapy. However, PCP is now a well-recognized complication of Crohn disease since the addition of anti-tumor necrosis factor (TNF) therapy to the treatment regimen.[44]

The incidence of PCP seems to be increasing, possibly owing to the increasing use of biologic response modifiers, such as anti-TNF agents, and newer more potent immunosuppressive drugs. Reports from cancer centers show that the incidence or prevalence or both of PCP has increased in this population.[58,85] Similar increases are reported among patients receiving organ transplants, those with autoimmune diseases, and those who have had a bone marrow transplant.[4,57,75]

In contrast to patients with AIDS, in which there is consensus on the guidelines for starting PCP prophylaxis, the risk for developing non–AIDS-associated PCP can be more difficult to predict. This is because the immunosuppressive regimen affects not only cell number but also cell function. Thus, a patient on chemotherapy may have a $CD4^+$ T-cell count above the risk threshold seen in AIDS patients, but if function is suppressed then the risk for PCP increases. Because T-cell function is not routinely measured in clinical laboratories, physicians must rely on cumulative clinical experience when judging the need for PCP prophylaxis.

IMMUNOPATHOGENESIS

Pneumocystis is the classic opportunistic pathogen: it is incapable of producing overt pneumonia in the immunologically normal host, yet in the immunocompromised host PCP is universally fatal if untreated. Even with treatment the mortality is high, ranging from 5 to 25 percent, depending on the patient population.

The occurrence of PCP in patients with AIDS and pure T-cell deficiency disease provides indirect evidence of the potential importance of cellular immunity in protecting the host against this opportunist. Direct evidence for the necessity of $CD4^+$ T cells comes from mouse studies showing that depletion of this cell population renders the mouse susceptible to PCP. Less well appreciated is the evidence for the importance of humoral immunity in protection against *Pneumocystis*. For example, PCP occurs in patients with agammaglobulinemia or hyper-IgM syndrome and mice with a variety of genetically engineered defects in antibody response pathways have been demonstrated to be susceptible to PCP.

The host response to *Pneumocystis* is likely the critical determinant of the severity of lung injury occurring as a result of PCP.[27] Three clinical observations support the importance of the inflammatory response in the outcome of PCP: (1) neutrophil number, but not organism counts, in lung secretions correlates with higher morbidity and mortality[50]; (2) the differential in mortality between AIDS-related and non–AIDS-related PCP[51]; and (3) the ability of PCP to trigger the immune reconstitution inflammatory syndrome (IRIS).[56]

In recent years, the application of molecular technology has provided a vast amount of information to aid in understanding the immunopathogenesis of PCP.[27] When *Pneumocystis* reaches the lungs of a normal host by the airborne route, the organism elicits what we have termed a *balanced immune response* and the subject remains asymptomatic or mildly symptomatic while the infection is cleared. Alternatively, if the host is severely immunocompromised owing to insufficient $CD4^+$ cells, a life-threatening pneumonitis develops, driven by $CD8^+$ T cells. Because $CD8^+$ T cells are a critical determinant of the lung injury, without controlling organism burden, we have termed this response *ineffective inflammation*. Finally, in the setting of return of $CD4^+$ T cells, such as after institution of treatment for human immunodeficiency virus (HIV) infection or during engraftment after bone marrow transplantation, one may see an exaggerated but effective response, as seen in IRIS, that clears the *Pneumocystis* but at the expense of significant lung injury.

Alveolar macrophages play a critical role in pulmonary host defense and contribute to both innate and adaptive immune responses. Alveolar macrophages have long been thought to serve as the effector cell for clearing *Pneumocystis* organisms from the lung, but these cells are not capable of controlling *Pneumocystis* infection in hosts with impaired $CD4^+$ T-cell function. A recent study utilizing an animal model of PCP has demonstrated that macrophage phagocytosis is the effector mechanism for the $CD4^+$ T-cell–mediated clearance of *Pneumocystis* organisms from the lung, indicating that the adaptive immune response must direct the macrophage-mediated clearance of this fungal pathogen.[80] In addition to host defense, macrophages also likely contribute to the immunopathogenesis of PCP. Macrophages are significant sources of proinflammatory cytokines, including TNF-α and interleukin-1 (IL-1), and *Pneumocystis* β-glucan is a potent stimulator of the alveolar macrophage cytokine response.[47] Therefore, in addition to their function in host defense, alveolar macrophages may also contribute to PCP-related lung injury through elaboration of inflammatory mediators.

The proinflammatory cytokines TNF-α and IL-1 are required for host defense against *Pneumocystis* infection[11,12] but also contribute to the immunopathology associated with PCP. These cytokines, acting through chemokine signals, promote neutrophil, lymphocyte, and monocyte recruitment to the lung, which may cause alveolar damage and diminished gas exchange, resulting in respiratory failure. Importantly, mice lacking TNF receptors are partially protected from PCP-related respiratory impairment and weight loss.[83] Other cytokines associated with T-helper cell type 1, 2, or 17 responses may also modulate host defense and PCP-related immunopathogenesis by regulating alveolar macrophage phenotype and activation state, but these issues need further clarification.

Surfactant phospholipids and apoproteins are critical to lung function and are adversely affected by *Pneumocystis* infection. Patients with PCP display dramatic alterations in surfactant function and composition,[65] which is likely an important determinant of disease severity. In vitro studies have determined that *Pneumocystis* organisms interact directly with pulmonary surfactant to reduce its surface tension–lowering properties.[81] Furthermore, studies in animal models have found that *Pneumocystis*-driven inflammation also contributes to surfactant dysfunction in the setting of PCP.[5,82] Anecdotal reports describe some benefit from the administration of exogenous surfactant to patients with PCP.[15]

PATHOLOGY

The pathologic findings of *Pneumocystis* infections are, with rare exceptions, limited to the lungs. Rarely, *Pneumocystis* organisms have been detected in the lymph nodes, spleen, liver, retina, bone marrow, gastrointestinal tract, pancreas, heart, adrenals, and peripheral blood.[73]

In the infantile "epidemic" form of disease, essentially all alveoli contain large numbers of organisms. Extensive interstitial plasma cell infiltrates distend the alveolar walls 5 to 20 times over their normal thickness, and almost no intra-alveolar fibrinous exudate is noted.[31]

The progression of disease in childhood and adult forms of PCP can be divided into three pathologic stages.[31,61] An initial stage is characterized by the presence of cysts and trophozoites attached, possibly by fibronectin, to the alveolar walls.[60] No septal inflammatory or cellular response is evident, and no clinical disease is associated with this stage. A second stage, which may or may not be associated with clinical signs and symptoms, is characterized by desquamation of alveolar cells and an increase in the number of cysts within alveolar macrophages. TNF may be a major mediator involved in the killing of *Pneumocystis* by activated alveolar macrophages and may be induced by oxidative stress in the alveoli.[59] The final stage is typified by extensive reactive and desquamative alveolitis manifested by marked cytoplasmic vacuolization of macrophages, mononuclear and plasma cell infiltrates within alveolar septa, and clusters of organisms located predominantly within macrophages in the lumen of alveoli. The histopathology of this final stage definitely is associated with clinical manifestations of pneumonitis.[61]

CLINICAL MANIFESTATIONS

Hypoxia is the hallmark of PCP. The natural course of *Pneumocystis* infection varies greatly and depends primarily on the status of host defenses in individual patients. The onset may be insidious, with a clinical course of 3 weeks or more, or be fulminant and rapidly progressive over a few days. For example, AIDS patients are probably

more immunocompromised than oncology patients at the point in time when they develop PCP, as evidenced by typically higher organism burdens, yet AIDS patients are more likely to have a longer history of symptoms and a lower mortality rate than non-AIDS patients.[51,72]

Symptoms in immunosuppressed children or adults without AIDS may be more abrupt in onset and rapidly progressive, but, even with these patients, the course varies greatly.[6,41] The mortality rate approaches 100 percent in untreated cases. In cases in children and adults, in contrast to infantile cases, fever generally is present and of high grade. It often precedes the onset of nonproductive cough, tachypnea, and severe dyspnea. Fever, tachypnea, and the radiographic appearance of pulmonary infiltrates, in that sequence, 1 to 21 days before diagnosis was established, occurred in a group of children with malignancies. The time of onset of clinical disease in non-AIDS, high-risk patients is unpredictable, but disease often occurs after discontinuance or a reduction in the dose of corticosteroid therapy.[6] Patients with severe combined immunodeficiency disease who receive a bone marrow transplant subsequently may contract fulminant PCP when apparent immunologic reconstitution has occurred.[6] These observations support the hypothesis that the clinical features early in the course of PCP are dependent to a large extent on the patient's ability to mount an inflammatory response.

Infants and children with AIDS may have a more insidious onset of PCP and therefore may come to medical attention later than non-AIDS patients. In this scenario, the patient is usually acutely ill with fever, cough, dyspnea, tachypnea, and an alveolar-arterial oxygen gradient greater than 30 mm Hg at the time of diagnosis.[14]

In children and adults with and without AIDS, physical examination at the time of initial evaluation may reveal tachypnea; nasal flaring; and intercostal, subcostal, or suprasternal retractions. An ashen color or cyanosis may be present or may develop rapidly. Auscultation of the chest frequently is characterized by a conspicuous absence of adventitious sounds despite the presence of rapid (80 to 100 breaths/min), shallow respirations. Scattered rales, rhonchi, or wheezes most often are detected later in the clinical course as resolution occurs. Aside from variable elevation in temperature, few other physical abnormalities are noted except those referable to pulmonary disease or secondary to the patient's underlying disease or treatment.[6,41]

Various radiographic abnormalities have been observed in documented cases of isolated PCP.[19,22] These variations result partly from observations at different stages in the course of disease. Bilateral diffuse parenchymal infiltrates (Fig. 224-2) occur most commonly, but no pattern is sufficiently specific either to exclude or to confirm a diagnosis of PCP. Although initially a reticulogranular interstitial process, *Pneumocystis* pneumonitis progresses to a predominantly alveolar process with coalescence and air bronchogram formation. Late in the course of the disease, lung fields may opacify completely. Unusual radiographic findings, including an asymmetric distribution, consolidated lobar infiltrates, pneumothorax and pneumomediastinum, localized parenchymal nodular densities, and pleural effusion, have been documented.[19] During treatment, radiographs show gradual clearing after a variable latent period, during which they may appear worse. Residual interstitial fibrosis occurs in a small percentage of patients.

DIAGNOSIS

The clinical features associated with PCP are not sufficiently specific to differentiate it from other opportunistic pulmonary infections. Mixed infections with viral, bacterial, fungal, or parasitic agents have been documented along with *Pneumocystis*. Implicit in these observations is the importance and urgency of establishing a definitive diagnosis before the institution of specific

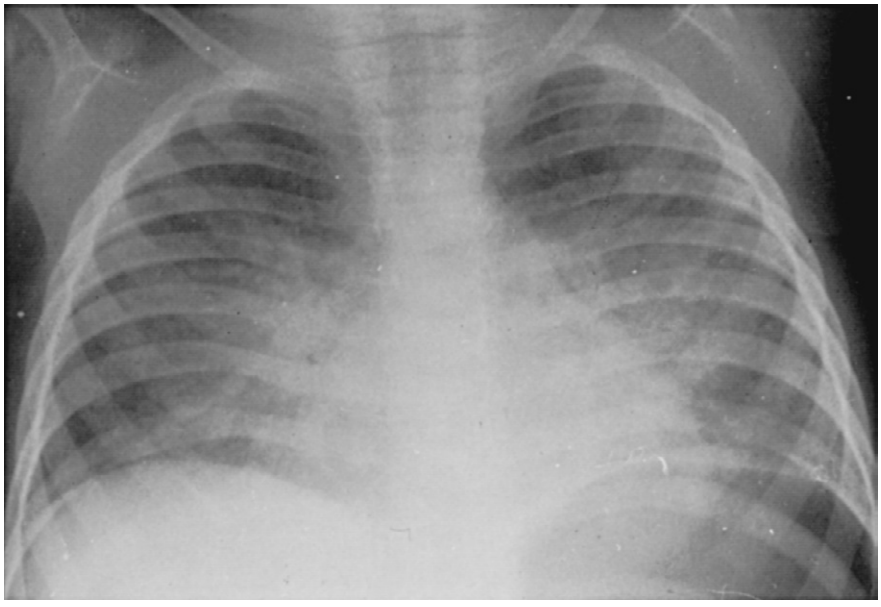

FIGURE 224-2 ■ Typical diffuse interstitial infiltrates seen in a 20-month-old child with severe combined immunodeficiency disease at the time of evaluation for clinical *Pneumocystis carinii* pneumonitis.

therapy. An etiologic diagnosis can be ascertained only by the demonstration of *Pneumocystis* organisms in lung tissue or respiratory secretions.

Numerous techniques have been used to obtain suitable material for diagnostic purposes. Specimens obtained by noninvasive methods, especially from induced sputum or tracheal aspirate, have been used to reveal *Pneumocystis*, and the sensitivity of these methods is 50 to 60 percent. Bronchoalveolar lavage is quite reliable with a sensitivity of 90 to 95 percent.[16] Other methods of obtaining a specimen, such as endobronchial brush biopsy, transbronchial lung biopsy, or percutaneous needle aspiration, have been used successfully to establish a diagnosis of PCP but are not commonly used since the advent of bronchoalveolar lavage.

If PCP remains a possibility after using the just-noted diagnostic methods, one should proceed to an invasive technique such as open-lung biopsy. Open-lung biopsy provides the most dependable specimen from which identification of the organism and the extent of the infection can be ascertained.[1] The chief disadvantage is the need for general anesthesia.

For diagnostic purposes, the methenamine silver nitrate method of Gomori and the less widely used, but more rapid, toluidine blue O and calcofluor white stains are most useful for showing cyst forms in tissue sections, aspirates, or imprints. For more detailed morphologic study of intracystic sporozoites and trophozoites, polychrome stains, including Giemsa, Wright, Gram, and methylene blue stains, are more suitable. In tissue sections, the Gomori stain in combination with hematoxylin and eosin staining allows study of the organism and host tissue. A direct immunofluorescent monoclonal antibody technique has become commercially available.[62] Serologic methods are not useful for diagnostic purposes.

There are a large number of studies describing detection of *Pneumocystis* DNA by polymerase chain reaction. Although this has proved to be a valuable laboratory technique, it has had limited use in clinical situations. Reported sensitivities are excellent, but specificities can be less than 85 to 90 percent.[2,74] At this time, polymerase chain reaction remains a research tool and is not widely used in general clinical practice.

Hematologic studies are of no diagnostic value, primarily because of baseline abnormalities reflecting the underlying disease of affected patients. Eosinophilia has been reported in isolated cases but usually is not present. Depressed serum total protein and albumin values frequently are noted because of the poor nutritional status of representative patients. Lactate dehydrogenase activity is increased, but it is not a specific marker for PCP and is of little diagnostic benefit.[79]

PROGNOSIS

Before the availability of specific therapeutic agents, the overall prognosis of patients with PCP was poor. Despite supportive care, almost 100 percent of infected patients with underlying neoplastic or immunodeficiency disorders died, whereas approximately 50 percent of infants in the European epidemics died as a result of this pulmonary infection. With modern medical management, the prognosis for patients with PCP depends to some extent on the underlying risk factor for PCP. For example, the mortality for PCP in cancer patients (25% to 40%) is four to five times higher than for those with AIDS (5% to 10%).[51] Patients who require mechanical ventilation have mortality rates in excess of 60 to 70 percent. A similar high mortality is seen in infants with PCP whose disease progresses and who then require mechanical ventilation.[66]

TREATMENT

Trimethoprim-sulfamethoxazole is the drug of choice for both the treatment and prevention of PCP.[36,46] A minimum of 2 weeks of therapy is recommended for non-AIDS patients; 3 weeks may be optimal for patients with AIDS. The CDC, the National Institutes of Health, and the Infectious Diseases Society of America have provided consensus guidelines for the treatment and prevention of PCP in immunosuppressed patients (Table 224-1).[7] Atovaquone, a hydroxynaphthoquinone approved by the U.S. Food and Drug Administration, is effective and has few side effects (rash, nausea, and diarrhea). It can be administered only by the oral route.[40] The sulfone dapsone has been evaluated in children[24] and may be used alone but is synergistic with trimethoprim[49] against *P. jiroveci*. Dapsone may cause rashes, neutropenia, thrombocytopenia, anemia, and methemoglobinemia. Clindamycin plus primaquine has also been used.[64]

Some studies in adults with AIDS and PCP suggest that the administration of corticosteroids early in the course of moderately severe pneumonitis reduces the occurrence of respiratory failure and improves oxygenation.[55] Other studies show no benefit from corticosteroids.[18] Limited studies of this supportive therapy have been reported to increase survival in children.[68] When used, a reasonable approach seems to be to withdraw corticosteroid therapy as soon as pulmonary function has become stabilized.

PREVENTION

PCP can be prevented in more than 95 percent of patients at high risk for acquiring the disease.[39,42] Patients in high-risk groups, such as patients with cancer, acquired and congenital immunodeficiency disorders, and organ or bone marrow transplants or those receiving intensive immunosuppressive therapy, should be placed on a chemoprophylaxis regimen throughout the risk period (see Table 224-1).

Because of the high risk for acquiring PCP during the first year of life, often before HIV infection is recognized, PCP prophylaxis should be initiated at 4 to 6 weeks of age in all infants born of HIV-infected women, regardless of $CD4^+$ lymphocyte cell counts. The use of chemoprophylaxis in this age group has been highly effective in HIV-infected infants.[63] When infants are shown not to be infected with HIV, prophylaxis may be discontinued. HIV-infected infants and infants with undetermined

TABLE 224-1 **Dosing Recommendations for the Prevention and Treatment of *Pneumocystis* Pneumonia**

Indication	First Choice	Alternative	Comments/Special Issues
Primary prophylaxis	Trimethoprim 5 mg/kg with sulfamethoxazole 25 mg/kg (TMP-SMX) daily generally divided into two doses. The total daily dose should not exceed 1600 mg SMX and 320 mg TMP. Several dosing schemes have been used successfully: given 3 days per week on consecutive days or on alternate days; given 2 days per week on consecutive days or on alternate days; or given every day.	Dapsone (children ≥1 mo), 2 mg/kg (maximum, 100 mg) orally daily or 4 mg/kg (maximum, 200 mg) orally weekly. Atovaquone (children aged 1 to 3 mo and >24 mo, 30 mg/kg orally daily; children aged 4 to 24 mo, 45 mg/kg orally daily) Aerosolized pentamidine (children aged ≥5 yr), 300 mg every month via Respirgard II nebulizer	*Primary prophylaxis indicated for:* All HIV-infected or HIV-indeterminate infants aged 4-6 wk to 12 mo regardless of CD4 count/percentage; HIV-infected children aged 1 to 5 yr with CD4 count <500 cells/mm^3 or CD4 percentage <15%; HIV-infected children aged 6 to 12 yr with CD4 count <200 cells/mm^3 or CD4 percentage <15%. *Criteria for discontinuing primary prophylaxis:* Do not discontinue in children aged <1 yr. After ≥6 mo of HAART and: Age 1 to 5 yr, CD4 percentage or count is ≥15% or ≥500 cells/mm^3 for >3 consecutive months *or* Age ≥6 yr, CD4 percentage or count is ≥15% or ≥200 cells/mm^3 for >3 consecutive months *Criteria for restarting primary prophylaxis:* Age 1 to 5 yr with CD4 percentage <15% or count <500 cells/mm^3 Age ≥6 yr with CD4 percentage <15% or count <200 cells/mm^3
Secondary prophylaxis (prior *Pneumocystis* pneumonia [PCP])	Same as for primary prophylaxis	Same as for primary prophylaxis	Secondary prophylaxis indicated for children with prior episode of PCP. *Criteria for discontinuing secondary prophylaxis:* same as for primary prophylaxis. *Criteria for restarting secondary prophylaxis:* same as for primary prophylaxis.
Treatment	TMP-SMX 15–20 mg/kg TMP *plus* 75-100 mg/kg SMX daily given IV orally in three to four divided doses given for 21 days (followed by prophylaxis dosing)	*If TMP-SMX intolerant or clinical treatment failure after 5 to 7 days of TMP-SMX therapy):* Pentamidine 4 mg/kg IV once daily is first choice alternative regimen (pentamidine may be changed to atovaquone after 7 to 10 days IV therapy) Atovaquone 30-40 mg/kg (maximum, 1500 mg/day) orally in two divided doses with food for patients from birth to 3 mo and ≥24 mo. For infants from 3 to 24 mo, an increased dose of 45 mg/kg daily in two divided doses with food is needed.	After acute pneumonitis resolved in mild-moderate disease, IV TMP-SMX may be changed to oral dosing. Dapsone 2 mg/kg orally once daily (maximum, 100 mg/day) *plus* trimethoprim 15 mg/kg/day orally divided into three doses has been used in adults but data in children are limited. Primaquine base 0.3 mg/kg orally once daily (maximum, 30 mg/day) *plus* clindamycin 10 mg/kg IV or by mouth (maximum 600 mg given IV and 300 to 450 mg given orally) every 6 hr has been used in adults, but data in children are not available. *Indications for corticosteroids:* Pao_2 <70 mm Hg at room air or alveolar-arterial oxygen gradient >35 mm Hg Prednisone dose: 1 mg/kg orally twice daily for 5 days, then 0.5-1 mg/kg orally twice daily for 5 days, then 0.5 mg/kg orally once daily for days 11 to 21. Chronic suppressive therapy (secondary prophylaxis) with TMP/SMX is recommended in children and adults after initial therapy.

status should be continued on prophylaxis during the first year of life. At 1 year of age and subsequently, the use of chemoprophylaxis is based on the $CD4^+$ lymphocyte count and other AIDS-defining features.

Trimethoprim-sulfamethoxazole given 3 days per week is the preferred drug for chemoprophylaxis. For patients unable to take trimethoprim-sulfamethoxazole, dapsone 2 mg/kg/day or 4 mg/kg/wk is suggested.[24] Studies in adults show that daily doses of dapsone[53] or one dose of dapsone per week[38] is effective prophylaxis.[52] An alternative to dapsone is aerosolized pentamidine 300 mg monthly via Respirgard II inhaler.[48] The dosage for aerosolized pentamidine is the same as the dosage for adults.[48] Atovaquone has been shown to be effective and safe in children.[17] A controlled study compared prophylaxis with atovaquone and azithromycin with trimethoprim-sulfamethoxazole in 366 high-risk, HIV-infected children.[34] The regimens were similar in effectiveness in prevention of bacterial infections, and PCP occurred with equal frequency.

Studies in adults and children show that PCP prophylaxis can be discontinued safely in patients responding to highly active antiretroviral therapy with a sustained increase in $CD4^+$ T lymphocytes to levels above the risk threshold for PCP (see Table 224-1).

Patients are protected from pneumonitis only while receiving chemoprophylaxis and become susceptible again when use of the drugs is discontinued. No vaccine is available.

NEW REFERENCES SINCE SIXTH EDITION

5. Atochina-Vasserman EN, Gow AJ, Abramova H, et al. Immune reconstitution during *Pneumocystis* lung infection: disruption of surfactant component expression and function by S-nitrosylation. *J Immunol* 2009;**182**:2277–87.
7. CDC Guidelines (in press).
56. Novak RM, Richardson JT, Buchacz K, et al; HIV Outpatient Study (HOPS) Investigators. Immune reconstitution inflammatory syndrome: incidence and implications for mortality. *AIDS* 2012; **26**(6):721–40.
72. Tasaka S, Tokuda H, Sakai F, et al. Comparison of clinical and radiological features of *Pneumocystis* pneumonia between malignancy cases and acquired immunodeficiency syndrome cases: a multicenter study. *Intern Med* 2010;**49**:273–81.
79. Vogel MN, Weissgerber P, Goeppert B, et al. Accuracy of serum LDH elevation for the diagnosis of *Pneumocystis jiroveci* pneumonia. *Swiss Med Wkly* 2011;**141**:w13184.
80. Wang J, Gigliotti F, Bahgwat SJ, et al. Immune modulation with sulfasalazine attenuates immunopathogenesis but enhances macrophage-mediated fungal clearance during *Pneumocystis* pneumonia. *PLoS Pathog* 2010;**6**(8):e1001058.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 2 *NEMATODES*

CHAPTER 225

Parasitic Nematode Infections

Peter J. Hotez

INTESTINAL NEMATODES

The three major intestinal nematodes of children, *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms, together have a substantial impact on the health and well-being of children living in less-developed nations of the world.[3] This "unholy trinity" of soil-transmitted helminths (STHs), or "geohelminths," deprives hundreds of millions of young children of their full intellectual and growth potential. Some estimates suggest that 2 billion people are infected with STHs, 300 million of whom have severe morbidity. An estimated 400 million schoolchildren are infected with STHs, although these estimates are undergoing revision through the activities of a new global helminth atlas.[4] Building on studies from the early part of the 20th century,[21,22] extensive new data from many different geographic regions confirm that chronic intestinal nematode infections acquired during childhood

REFERENCES

1. Ahmad A, Khan S, Soubani AO. The findings of surgical lung biopsy in HIV-positive patients in the era of highly active antiretroviral therapy. *Ann Thorac Surg* 2005;**79**:1862–5.
2. Alvares-Martinex MJ, Miro JM, Valls ME, et al. Sensitivity and specificity of nested and real-time PCR for the detection of *Pneumocystis jiroveci* in clinical specimens. *Diagn Microbiol Infect Dis* 2006;**56**(2):153–60.
3. An CL, Gigliotti F, Harmsen AG. Exposure of immunocompetent adult mice to *Pneumocystis carinii* f. sp. Muris by cohousing: growth of *P. carinii* f. sp. Muris and host immune response. *Infect Immun* 2003;**71**(4):2065–70.
4. Arend SM, Kroon FP, van't Wout JW. *Pneumocystis carinii* pneumonia in patients without AIDS, 1980 through 1993. *Arch Intern Med* 1995;**155**:2436–41.
5. Atochina-Vasserman EN, Gow AJ, Abramova H, et al. Immune reconstitution during *Pneumocystis* lung infection: disruption of surfactant component expression and function by S-nitrosylation. *J Immunol* 2009;**182**:2277–87.
6. Burke BA, Good RA. *Pneumocystis carinii* infection. *Medicine* 2009; **52**:23–51.
7. CDC Guidelines (in press).
8. Centers for Disease Control and Prevention. Twenty-five years of HIV/AIDS—United States, 1981-2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:585–9.
9. Chagas C. Nova trypanomiazaia humana. *Mem Inst Oswaldo Cruz* 1909;**1**:159.
10. Chen W, Gigliotti F, Harmsen AG. Latency is not an inevitable outcome of infection with *Pneumocystis carinii*. *Infect Immun* 1993; **61**(12):5406–9.
11. Chen W, Havell EA, Harmsen AG. Importance of endogenous tumor necrosis factor alpha and gamma interferon in host resistance against *Pneumocystis carinii* infection. *Infect Immun* 1992;**60**: 1279–84.
12. Chen W, Havell EA, Moldawer LL, et al. Interleukin 1: an important mediator of host resistance against *Pneumocystis carinii*. *J Exp Med* 1992;**176**:713–18.
13. Chusid MJ, Heyman KA. An outbreak of *Pneumocystis carinii* pneumonia at a pediatric hospital. *Pediatrics* 1978;**62**(6):1031–5.
14. Connor E, Bagarazzi M, McSherry G, et al. Clinical and laboratory correlates of *P. carinii* pneumonia in children infected with HIV. *JAMA* 1991;**265**:1693–7.
15. Creery WD, Hashmi A, Hutchinson JS, Singh RN. Surfactant therapy improves pulmonary function in infants with *Pneumocystis carinii* pneumonia and acquired immunodeficiency syndrome. *Pediatr Pulmonol* 1997;**24**:370–3.
16. Cruciani M, Marcati P, Malena M, et al. Meta-analysis of diagnostic procedures for *Pneumocystis carinii* pneumonia in HIV-1-infected patients. *Eur Respir J* 2002;**20**:982–9.
17. Dankner W, Yogev R, Hughes WT, et al. Phase II/III, randomized, double-blind trial to compare atovaquone plus azithromycin to trimethoprim-sulfamethoxazole in the prevention of multiple opportunistic infections in HIV-infected children. *Pediatr Res* 2000;**47**:260.
18. Delclaux C, Zahar J-R, Amraoui G, et al. Corticosteroids as adjunctive therapy for severe *Pneumocystis carinii* pneumonia in non–human immunodeficiency virus-infected patients: retrospective study of 31 patients. *Clin Infect Dis* 1999;**29**:670–2.
19. Doppman JL, Geelhoed GW, DeVita VT. Atypical radiographic features in *Pneumocystis carinii* pneumonia. *Radiology* 1975;**114**: 39–44.
20. Edman JC, Edman U, Cao M, et al. Isolation and expression of the *P. carinii* dihydrofolate reductase gene. Proc Natl. *Acad Sci U S A* 1989;**86**:8625.
21. Edman JC, Kovacs JA, Masur H, et al. Ribosomal RNA sequence shows *P. carinii* to be a member of the fungi. *Nature* 1988;**334**: 519–22.
22. Forest JV. Radiographic findings in *Pneumocystis carinii* pneumonia. *Raidology* 1972;**103**:539–44.
23. Frenkel JK. *Pneumocystis jiroveci* n. sp. from man: morphology, physiology and immunology in relation to pathology. *Natl Cancer Inst Monogr* 1976;**43**:13–30.
24. Gatti G, Loy A, Casazza R, et al. Pharmacokinetics of dapsone in human immunodeficiency vine-infected children. *Antimicrob Agents Chemother* 1995;**39**:1101–6.
25. Gigliotti F. *Pneumocystis carinii:* has the name really been changed? *Clin Infect Dis* 2005;**41**:1752–5.
26. Gigliotti F, Harmsen AG, Haidaris CG, Haidaris PJ. *Pneumocystis carinii* is not universally transmissible between mammalian species. *Infect Immun* 1993;**61**(7):2886–90.
27. Gigliotti F, Wright TW. Immunopathogenesis of *Pneumocystis carinii* pneumonia. *Exp Rev Mol Med* 2005;**7**(26):1–16.
28. Gigliotti F, Haidaris CG. Antigenic characterization of *Pneumocystis carinii*. *Semin Respir Infect* 1998;**13**(4):313–22.
29. Gigliotti F, Harmsen AG, Wright TW. Characterization of transmission of *Pneumocystis carinii* f. sp. Muris through immunocompetent BALB/c mice. *Infect Immun* 2003;**71**(7):3852–6.
30. Gigliotti F, Stokes DC, Cheatham AB, et al. Development of murine monoclonal antibodies to *Pneumocystis carinii*. *J Infect Dis* 1986;**154**:315–22.
31. Hughes WT. Current status of laboratory diagnosis of *Pneumocystis carinii* pneumonitis. *C R C Crit Rev Clin Lab Sci* 1975;**6**:145–70.
32. Hughes WT. *Pneumocystis carinii* vs. *Pneumocystis jiroveci:* another misnomer (response to Stringer, et al.). *Emerg Infect Dis* 2003;**9**: 276–9.
33. Hughes WT, Bartley DL, Smith BM. A natural source of infection due to *Pneumocystis carinii*. *J Infect Dis* 1983;**147**:595.
34. Hughes WT, Dankner WM, Yogev R, et al. Comparison of atovaquone and azithromycin with trimethoprim-sulfamethoxazole for the prevention of serious bacterial infections in children with HIV infection. *Clin Infect Dis* 2005;**40**:136–45.
35. Hughes WT, Feldman S, Aur RJ, et al. Intensity of immunosuppressive therapy and the incidence of *Pneumocystis carinii* pneumonitis. *Cancer* 1975;**36**:2004–9.
36. Hughes WT, Feldman S, Chaudhary S, et al. Comparison of pentamidine isethionate and trimethoprim-sulfamethoxazole in the treatment of *Pneumocystis carinii* pneumonitis. *J Pediatr* 1978;**92**: 285–91.
37. Hughes WT, Gigliotti F. Nomenclature for *Pneumocystis carinii*. *J Infect Dis* 1988;**157**:432–3.
38. Hughes WT, Kennedy W, Dugdale M, et al. Prevention of *P. carinii* pneumonitis in AIDS patients with weekly dapsone. *Lancet* 1990;**2**: 1066.
39. Hughes WT, Kuhn S, Chaudhary S, et al. Successful chemoprophylaxis for *Pneumocystis carinii* pneumonitis. *N Engl J Med* 1977; **297**:1419–26.
40. Hughes WT, Leoung G, Kramer F, et al. Comparison of atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis carinii* pneumonia in patients with AIDS. *N Engl J Med* 1993;**328**:1521–7.
41. Hughes WT, Price RA, Kim H, et al. *Pneumocystis carinii* pneumonitis in children with malignancies. *J Pediatr* 1973;**82**:404–15.
42. Hughes WT, Rivera GK, Schell MJ, et al. Successful intermittent chemoprophylaxis for *Pneumocystis carinii* pneumonitis. *N Engl J Med* 1987;**316**:1627–32.
43. Jones JL, Hanson DL, Dworkin MS, et al. Surveillance for AIDS-defining opportunistic illnesses, 1992-1997. *MMWR CDC Surveill Summ* 1999;**48**(2):1–22.
44. Kaur N, Mahl TC. *Pneumocystis jiroveci (carinii)* pneumonia after infliximab therapy: a review of 84 cases. *Dig Dis Sci* 2007;**52**(6): 1481–4.
45. Keely SP, Stringer JR, Baughman RP, et al. Genetic variation among *Pneumocystis carinii* hominis isolates in recurrent pneumocystosis. *J Infect Dis* 1995;**172**(2):595–8.
46. Lau WK, Young LS. Trimethoprim-sulfamethoxazole treatment of *Pneumocystis carinii* in adults. *N Engl J Med* 1976;**295**:716–18.
47. Lebron F, Vassallo R, Puri V, Limper AH. *Pneumocystis carinii* cell wall beta-glucans initiate macrophage inflammatory responses through NF-kappaB activation. *J Biol Chem* 2003;**278**:25001–8.
48. Leoung GS, Feigal DW, Montgomery AB, et al. Aerosolized pentamidine for prophylaxis against *P. carinii* pneumonia. *N Engl J Med* 1990;**323**:669–75.
49. Leoung GS, Mills J, Hopewell PC, et al. Dapsone-trimethoprim for *P. carinii* pneumonia in acquired immunodeficiency syndrome. *Ann Intern Med* 1988;**105**:45–8.
50. Limper AH, Offord KP, Smith TF, Martin WJ 2nd. *Pneumocystis carinii* pneumonia: differences in lung parasite number and inflammation in patients with and without AIDS. *Am Rev Respir Dis* 1989; **140**(5):1204–9.
51. Mansharamani NG, Garland R, Delaney D, Koziel H. Management and outcome patterns for adult *Pneumocystis carinii*

pneumonia, 1985-1995: comparison of HIV-associated cases to other immunocompromised states. *Chest* 2000;**118**(3):704–11.
52. McIntosh K, Cooper E, Xu J, et al. Toxicity and efficacy of daily vs. weekly dapsone for prevention of *Pneumocystis carinii* pneumonia in children infected with HIV. *Pediatr Infect Dis J* 1999;**18**:432–9.
53. Metroka CE, Jacobus D, Lewis N: *Successful chemoprophylaxis for Pneumocystis with dapsone or Bactrim*. Presented before the 5th International Conference on AIDS. Montreal, 1989.
54. Morgan DJ, Vargas SL, Reyes-Mugica M, et al. Identification of *Pneumocystis carinii* in the lungs of infants dying of sudden infant death syndrome. *Pediatr Infect Dis* 2001;**20**(3):306–9.
55. National Institutes of Health-University of California Expert Panel for Corticosteroids as Adjunctive Therapy for *Pneumocystis* Pneumonia: Consensus statement on the use of corticosteroids as adjunctive therapy for *Pneumocystis* pneumonia in the acquired immunodeficiency syndrome. *N Engl J Med* 1990;**323**:1500–4.
56. Novak RM, Richardson JT, Buchacz K, et al; HIV Outpatient Study (HOPS) Investigators. Immune reconstitution inflammatory syndrome: incidence and implications for mortality. *AIDS* 2012; **26**(6):721–40.
57. Nuesh R, Bellini C, Zimmerle W. *Pneumocystis carinii* pneumonia in human immunodeficiency virus (HIV)-positive and HIV-negative immunocompromised hosts. *Clin Infect Dis* 1999;**29**: 1519–23.
58. Pagano L, Fianchi L, Mele L, et al. *Pneumocystis carinii* pneumonia in patients with malignant hematological diseases: 10 years' experience of infection in GIMEMA centers. *Br J Haematol* 2002;**117**: 379–86.
59. Pesanti EL. Interaction of cytokines and alveolar cells with *Pneumocystis carinii* in vitro. *J Infect Dis* 1991;**163**:611–16.
60. Pottratz ST, Martin WJ 2nd. Role of fibronectin in *Pneumocystis carinii* attachment of cultured lung cells. *J Clin Invest* 1991;**85**: 351–6.
61. Price RA, Hughes WT. Histopathology of *Pneumocystis carinii* infestation and infection in malignant disease. *Hum Pathol* 1974; **5**(6):737–52.
62. Procop GW, Haddad S, Quinn J, et al. Detection of *Pneumocystis jiroveci* in respiratory specimens by four staining methods. *J Clin Microbiol* 2004;**42**:3333–5.
63. Rigaud M, Pollack M, Leibovitz E, et al. Efficacy of primary chemoprophylaxis against *Pneumocystis carinii* pneumonia during the first year of life in infants infected with human immunodeficiency virus type 1. *J Pediatr* 1994;**125**:476–80.
64. Ruf B, Rohde I, Phole HD. Efficacy of clindamycin/primaquine vs trimethoprim sulfamethoxazole in primary treatment of *Pneumocystis carinii* pneumonia. *Eur J Clin Microbiol Infect Dis* 1991; **10**:207–10.
65. Schmidt R, Markarat P, Ruppert C, et al. Pulmonary surfactant in patients with *Pneumocystis* pneumonia and acquired immunodeficiency syndrome. *Crit Care Med* 2006;**4**:2370–6.
66. Sheikh S, Bakshi S, Pahwa SG. Outcome and survival in HIV-infected infants with *Pneumocystis carinii* pneumonia and respiratory failure. *Pediatr AIDS HIV Infect* 1996;**7**:155–63.
67. Simonds RJ, Oxtoby MJ, Caldwell MB, et al. *Pneumocystis carinii* pneumonia among U.S. children with perinatally acquired HIV infection. *JAMA* 1993;**270**:470–3.
68. Sleasman JW, Hemenway C, Klein AS, et al. Corticosteroids improve survival of children with AIDS and *Pneumocystis carinii* pneumonia. *Am J Dis Child* 1993;**147**:30–4.
69. Smulian AG. *Pneumocystis carinii*: genetic diversity and cell biology. *Fungal Genet Biol* 2001;**34**(3):145–54.
70. Stringer JR; The Pneumocystis Workshop. Revised nomenclature for *Pneumocystis carinii*. *J Eukaryor Microbiol* 1994;**41**:121–2.
71. Stringer JR, Beard CB, Miller RF, Wakefield AE. A new name *(Pneumocystis jiroveci)* for *Pneumocystis* from humans. Emerg. *Infect Dis* 2002;**8**:891–6.
72. Tasaka S, Tokuda H, Sakai F, et al. Comparison of clinical and radiological features of *Pneumocystis* pneumonia between malignancy cases and acquired immunodeficiency syndrome cases: a multicenter study. *Intern Med* 2010;**49**:273–81.
73. Telzak EE, Cote RJ, Gold JWM, et al. Extrapulmonary *Pneumocystis carinii* infections. *Rev Infect Dis* 1990;**12**:380–6.
74. Torres J, Goldman M, Wheat LF, et al. Diagnosis of *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected patients with polymerase chain reaction: a blind comparison to standard methods. *Clin Infect Dis* 2000;**30**:141–5.
75. Torres HA, Chemaly RF, Storey R, et al. Influence of type of cancer and hematopoietic stem cell transplantation on clinical presentation of *Pneumocystis jiroveci* pneumonia in cancer patients. *Eur J Clin Microbiol Infect Dis* 2006;**25**:382–8.
76. Van der Meer G, Brug SL. Infection par *Pneumocystis* chez l'homme et chez les animaux, Ann Soc. *Belg Med Trop* 1942;**22**:301.
77. Vargas SL, Hughes WT, Santolaya ME, et al. Search for primary infection by *Pneumocystis carinii* in a cohort of normal, healthy infants. *Clin Infect Dis* 2001;**32**:855–61.
78. Vargas SL, Ponce CA, Hughes WT, et al. Association of primary *Pneumocystis carinii* infection and sudden infant death syndrome. *Clin Infect Dis* 1999;**29**:1489–93.
79. Vogel MN, Weissgerber P, Goeppert B, et al. Accuracy of serum LDH elevation for the diagnosis of *Pneumocystis jiroveci* pneumonia. *Swiss Med Wkly* 2011;**141**:w13184.
80. Wang J, Gigliotti F, Bahgwat SJ, et al. Immune modulation with sulfasalazine attenuates immunopathogenesis but enhances macrophage-mediated fungal clearance during *Pneumocystis* pneumonia. *PLoS Pathog* 2010;**6**(8):e1001058.
81. Wang Z, Foye A, Chang Y, et al. Inhibition of surfactant activity by *Pneumocystis carinii* organisms and components in vitro. *Am J Physiol Lung Cell Mol Physiol* 2005;**288**:L1124–31.
82. Wright TW, Notter RH, Wang Z, et al. Pulmonary inflammation disrupts surfactant function during *Pneumocystis carinii* pneumonia. *Infect Immun* 2001;**69**:758–64.
83. Wright TW, Pryhuber GS, Chess PR, et al. TNF receptor signaling contributes to chemokine secretion, inflammation, and respiratory deficits during *Pneumocystis* pneumonia. *J Immunol* 2004;**172**: 2511–21.
84. Wright TW, Simpson-Haidaris PJ, Gigliotti F, et al. Conserved sequence homology of cysteine-rich regions in genes encoding glycoprotein A in *Pneumocystis carinii* derived from different host species. *Infect Immun* 1994;**62**(5):1513–19.
85. Yale SH, Limper A. *Pneumocystis carinii* pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. *Mayo Clin Proc* 1996;**71**:5–13.

suppress cognitive and intellectual development[6,19,22] and impair physical growth and fitness.[7,24-26] Through these mechanisms the STH infections can actually cause poverty in the world's low- and middle-income countries.[11] Because many of these detrimental effects are reversible with the use of anthelmintic drugs to eliminate nematodes from the intestinal tract,[25,26] several investigators and international relief agencies have advocated the administration of benzimidazole anthelmintics (sometimes known as "deworming") as a cornerstone of public health programs directed at school-aged children.[3,5,20]

A 2001 World Health Assembly resolution urges its member states to "deworm" at least 75 percent and up to 100 percent of school-aged children at risk for acquiring STH infections through programs of mass drug administration (MDA).* Additional studies also point to the benefits of deworming preschool-aged children.[1] Globally, efforts are underway to link deworming for STH infections with MDA for other neglected tropical diseases, including lymphatic filariasis, onchocerciasis, and trachoma,[8,11,15] as well as linking deworming to the control of human immunodeficiency virus (HIV) infection and acquired immunodeficiency syndrome (AIDS), malaria, and tuberculosis.[13]

In some highly endemic areas, reinfection with *A. lumbricoides*, *T. trichiura*, and hookworms occurs within 6 months of receiving anthelmintic treatment.[2] Therefore, MDA is not always a practical means of control, unless the drugs are used frequently. Newer information suggests that the efficacy of single-dose anthelmintic drugs used for MDA may not be as high as believed previously.[17] Concern about the potential for emerging anthelmintic drug resistance also exists. Some researchers have pointed to the use of genetically engineered anthelmintic vaccines as one possible solution to the problem of worm reinfection and disease during childhood.[10] Still another public health concern about the chronic effects of STH infections is new evidence suggesting that they may promote the susceptibility to, or worsen the severity of, malaria and HIV infection/AIDS.[14] Interest has developed in examining the impact of large-scale deworming on these killer diseases.[13,18] New information also indicates the potential impact of parasitic infections, including STH infections in developed countries such as the United States and Europe.[8,12,23]

In addition to the chronic effects seen when children harbor numerous intestinal nematodes are the increasingly recognized unique neonatal and infantile syndromes that new information indicates may result from vertical transmission of the infective stages of some intestinal nematodes, probably in colostrum and breast milk.[3,9] The best documented example of vertical transmission in infants results in the "swollen belly syndrome" caused by *Strongyloides fuelleborni kellyi*, although other perinatal nematode infections probably also occur (see later discussion). Finally, children experience significant morbidity during zoonotic transmission of the infective stages of intestinal nematodes of companion animals such as dogs and cats. The resultant aberrant migration of these foreign nematode larvae (visceral larva migrans) has become a major pediatric public health problem in large urban areas of the United States and Europe.[12,16]

*www.who.int/wormcontrol.

ASCARIS LUMBRICOIDES

Ascariasis is one of the most prevalent infections in the world,[33,34] with an estimated 800 million cases in the developing world.[3] Accurate determinations based on 1,477,742 fecal examinations conducted during the 1990s indicated that more than 500 million cases of ascariasis occur in China alone[49]; however, revised estimates now suggest that those numbers have declined significantly, especially in economically developed regions of eastern China.[3] Currently, the greatest number of cases of ascariasis occur in Southeast Asia and the Indian subcontinent and sub-Saharan Africa.[3] Surveys conducted in the rural southern communities of the United States during the 1970s indicated that the prevalence of ascariasis was 20 to 67 percent.[28] Those rates most likely have declined significantly over the past few decades, however.[8,23]

Ascaris infection, when relatively light, is usually not apparent until the patient passes a worm through the rectum. In heavy infections, constitutional symptoms may occur during the early phase and intestinal malabsorption and obstruction may occur in the later phase. The infection is acquired by ingestion of the infective eggs, which hatch in the upper part of the small intestine and free the larvae. The larvae penetrate the intestinal wall, reach venules or lymphatics, and pass through the portal circulation to the liver, the right ventricle, and the lungs.

In the lungs, the larvae break out of the capillaries and begin ascending through the respiratory radicles until they reach the glottis; passing over the epiglottis, they enter the esophagus and are carried down to the small intestine, where they mature and become adult worms. The adult female *Ascaris* produces huge numbers of eggs—possibly 200,000 a day. To ensure adequate quantities of egg-requiring cholesterol, the parasite sequesters oxygen through a specially modified hemoglobin.[35] The entire cycle, beginning with the infective eggs and resulting in ovipositing females, lasts approximately 2 months. Infection is maintained in the community by the deposition of human stool in soil, which permits embryonated eggs to develop into the infective stage. This process takes approximately 2 weeks. The high prevalence of infection results not only from deficient sanitary facilities for disposal of human excreta but also from the deliberate use of human feces as fertilizer.

Epidemiology

In many regions of the developing world, *Ascaris* eggs are almost ubiquitous in the environment. All ages are affected by the parasite; young children, who are exposed more often to the contaminated soil, are affected most frequently. These children usually harbor greater numbers of adult worms in their intestine than do adults living under similar conditions.[24-26,32] High worm burdens in children also occur with *Trichuris* infections (see later discussion). Because the phenomenon of heavy *Ascaris*

and *Trichuris* worm burdens in childhood is found throughout most of the developing world, pediatric predisposition to "worminess" is widely thought to have a possible genetic or immunologic basis.[7]

Ascaris eggs are extremely hardy and resistant to extremes of temperature and desiccation, which may explain the common finding of high rates of ascariasis in impoverished urban environments (e.g., Guatemala City, Mexico City) and in rural environments. The eggs also are resistant to chemical disinfectants and are not destroyed readily by sewage treatment. In some areas, pigs may serve as a reservoir for zoonotic *Ascaris* infection.[27]

Pathophysiology

During the migratory phase of the infection, the larvae evoke an inflammatory response associated with eosinophilic infiltration. *Ascaris* antigens—so-called ABA-1 allergen—released during the molting of larvae evoke an immune response, and IgE antibodies directed against ABA-1 may be associated with resistance.[43] *Ascaris* larvae also release immunomodulatory glycosphingolipids that inhibit T-helper (Th) 1 responses.[3,32] Chronic ascariasis and trichuriasis may suppress host inflammation and even responses to vaccines by promoting a Th2-type cytokine profile.[46] There are also data to support a role for ascariasis in promoting susceptibility to malaria,[18] although not all studies confirm this finding. A controversial role of IgE anti-*Ascaris* antibodies is in protecting children against atopic states, including asthma.[42] During the intestinal stage of infection, symptoms derive primarily from the physical presence of the worms in the gut, from aberrant migration into other lumina, or from perforation into the peritoneum. As a protective mechanism for its own survival, *Ascaris* secretes peptides that block the action of pancreatic digestive enzymes (trypsin, chymotrypsin, elastase),[36,39] which may play a role in parasite-associated nutrient malabsorption, including the malabsorption of vitamin A.[31] Ascariasis also results in lactose intolerance.[31] Whether these phenomena provide the basis of observed *Ascaris*-associated physical growth retardation during childhood is unknown.[6,24-26,32,44]

Clinical Manifestations

The degree of disease induced by the migratory phase of *Ascaris* is related directly to the number of larvae migrating simultaneously. In light infections, this phase is typically unrecognized. Heavy infection, such as that induced in himself by Koino,[41] who swallowed 2000 infected eggs, may cause severe pneumonitis. In some regions of the world, pediatric *Ascaris* pneumonitis is seasonal.[3]

In the lumen of the intestine, *Ascaris* adult worms may become matted together and form a bolus large enough to cause intestinal obstruction, sometimes leading to bowel infarction and intestinal perforation. This is a particular problem in very young children because of the small luminal diameter of their intestines.[40] The incidence of this complication has been estimated at 2 per 1000 infected children per year.[28] Children with *Ascaris* obstruction exhibit a toxic appearance, often with signs and symptoms of peritonitis.[3] When recognized early, the obstruction can be treated with medical management, but in many cases surgical intervention is mandatory. Ascariasis has also been linked to chronic intussusception.[45]

Hepatobiliary and pancreatic ascariasis (HPA) results from blockage of the bile duct and pancreatic duct. Patients with HPA are subject to cholecystitis, acute cholangitis, "biliary colic," acute pancreatitis, or hepatic abscess.[40] In contrast to intestinal obstruction, HPA tends to be more common in adults, especially women.[40] Certain irritants, such as halogenated hydrocarbons (e.g., carbon tetrachloride and tetrachloroethylene, used in the past to treat certain hookworm infections), elevation of body temperature, and general anesthesia, have been known to precipitate aberrant migration. *Ascaris* screening by fecal examination should be considered before performing elective surgery on a child who might have immigrated recently from an impoverished area of the tropics.

Whatever the mechanism of interference by *Ascaris* with growth or nutrition, treatment with anthelmintics leads to substantial catch-up growth in previously parasitized children.[6,24-26,42,44] Ascariasis in pregnant women results in intrauterine growth retardation.[48] Most individuals with light infections rarely are symptomatic (they become aware of the parasites by passage of adult worms in stool or through regurgitating and vomiting the adult worms), although researchers have conjectured that even these individuals may exhibit subtle deficits in cognitive and intellectual development.[6]

Several cases of neonatal ascariasis have been described in the literature.[29,30] The mode of acquisition of these infections is unknown, but canine and feline ascarid infections commonly are acquired by a transplacental route, suggesting the possibility that this route also may occur in humans.

Diagnosis and Differential Diagnosis

The differential diagnosis of pneumonia caused by *Ascaris* suggests a parasitic etiology because of the peripheral eosinophilia. Any nematode with a migratory phase through the lungs can mimic this infection, however. *Ascaris* must be considered as a cause of intestinal obstruction in any geographic locale where its prevalence is high.

The diagnosis of intestinal ascariasis is established by identifying the characteristic ascarid eggs through microscopic examination of stool. Efforts have been made more recently to detect *Ascaris* metabolites in the urine by gas-liquid chromatography.[37,38] HPA is suspected in heavily infected children who have signs of biliary obstruction. Ultrasonography and endoscopic retrograde cholangiopancreatography are useful adjunctive diagnostic procedures for these conditions.[40]

Treatment

For an ordinary *Ascaris* infection, either albendazole administered at a fixed dose of 400 mg once or mebendazole administered at a fixed dose of 100 mg twice daily for 3 days is effective. A single fixed dose of 500 mg of mebendazole also may be effective, but it may be unsuitable for the treatment of other STH coinfections. As an alternative, pyrantel pamoate administered in a single dose of

11 mg/kg, not to exceed 1 g, is effective. In a systematic review it was shown that single-dose oral albendazole, mebendazole, and pyrantel pamoate result in ascariasis cure rates of 88, 95, and 88 percent respectively.[17] The benzimidazoles and pyrantel pamoate are not approved for children younger than 2 years old, and all the printed statements caution against the use of these drugs in the younger age group. An examination of the use of benzimidazoles in children 12 to 24 months old concluded, however, that these agents are probably safe and should be used if the circumstances warrant it.[44] In such cases, the dose of albendazole in these young children should be reduced to 200 mg.[3] The risk for treatment in this younger age group currently is undergoing more rigorous investigation because of mounting evidence that the growth and cognitive delay caused by STHs may be corrected with albendazole or mebendazole.[24-26,44,47] These drugs have a potential for embryotoxicity, however, so judicious use in young children is warranted. The World Health Organization is evaluating the use of benzimidazoles during pregnancy, particularly for use during the second and third trimesters.

In cases of intestinal obstruction, piperazine citrate may be effective because this drug paralyzes the myoneural junction of *Ascaris* and may result in relaxation of the matted bolus of worms. It is antagonistic to pyrantel pamoate, and these two drugs should not be administered together.

Management of intestinal obstruction or HPA often is surgical. For some patients, biliary decompression may be performed with endoscopic retrograde cholangiopancreatography.[40]

Prognosis

The prognosis is excellent in most cases of ascariasis. In patients with obstruction or perforation, the prognosis depends entirely on the speed of recognition and therapy.

Prevention

Ascaris infection could be eliminated entirely through proper disposal of human excreta. As an isolated means of health improvement in the world, this approach seldom is successful. Elimination of *Ascaris* from a community or a substantial reduction in the incidence of this infection usually occurs after general efforts in poverty reduction and improvements in the standard of living have been achieved. Periodic administration of community-wide therapy with anthelmintics has been effective in reducing worm burden as a short-term strategy. In the absence of aggressive sanitation and other control measures, however, re-infection can occur within 6 months after treatment in areas of high transmission,[2] although in areas of periodic therapy the intensity of infection is decreased, even if the prevalence is little affected.

TRICHURIS TRICHIURA

Trichuriasis has a prevalence approximating that of the other major intestinal nematode infections, or approximately 600 million cases worldwide,[1] but the infection usually is asymptomatic because in most cases it is light. Children with so-called asymptomatic light infection may have deficits in cognition, however.[6,19,64]

The infection is acquired by the ingestion of embryonated eggs acquired from the soil on hands or through contaminated food. The eggs hatch in the upper part of the small intestine, and the liberated larvae penetrate the villi. In contrast to the larvae of *Ascaris*, *Trichuris* larvae do not undergo extraintestinal migration but remain in situ for approximately 1 week, at which time they begin a progressive descent into the cecum and the colon. They mature there, and the attenuated anterior end of the adult worm embeds itself in the colonic mucosa. Creation of syncytial tunnels (derived from the columnar epithelium of the colon) is facilitated by the release of a parasite-derived, pore-forming protein.[54] The parasites derive their nourishment from these colonic mucosal tunnels. The entire cycle from the ingestion of embryonated ova to the development of sexually mature adults takes approximately 2 months. Persistence of the infection in a community depends on continual contamination of the soil with human feces.

Epidemiology

These parasites are found most commonly throughout the developing countries of the tropics and subtropics. The distribution of this worm often parallels that of *Ascaris*. In addition, as in the case of *Ascaris*, children with trichuriasis usually harbor greater numbers of worms than adults living under similar conditions.[51,52] Consequently, children have greater morbidity from trichuriasis than adults do. The mechanistic basis of added worminess in children is unknown, although researchers have observed that for all of the soil-transmitted helminthiases, including trichuriasis, infections are aggregated, with a few children having particularly heavy infections. These heavily infected children seem to have a genetic or immunologic predisposition to *Trichuris* infection.[51,52] Children also are the major source of *Trichuris* eggs in the environment, which when deposited in the soil become infective in approximately 1 month and remain viable for several months. They are killed by exposure to temperatures greater than 40° C within 1 hour. Freezing temperatures less than –8° C also destroy these eggs. Similar to ascarid eggs, they are resistant to chemical disinfectants.

Pathophysiology

At the site of attachment, the adult worm elicits characteristic changes in the colonic mucosa as noted earlier. Inflammatory cells also are found at these sites, but they do not seem to account entirely for the clinical resemblance of trichuriasis to some forms of inflammatory bowel disease.[51,60] Similar to many other intestinal worms, *Trichuris* is expelled through the action of the host's immune system. This expulsion results from a combined effect of antibody and lymphoid cells.[65] Regulation of *Trichuris* populations in the gut has been ascribed to a carefully orchestrated balance of host-derived cytokines.[55] Although anemia has been attributed to trichuriasis,[58] the

amount of blood loss caused by this parasite is much less than hookworm-associated blood loss and is insufficient to account for anemia.[59] One possibility is that *Trichuris*-induced anemia results from chronic inflammation, similar to the anemia of inflammatory bowel disease.[51,52]

Clinical Manifestations

Two major disease syndromes are caused by heavy *Trichuris* infection during childhood.[51,52] *Trichuris* dysentery syndrome (TDS) is associated with severe diarrhea with blood and mucus. It can also be associated with chronic iron-deficiency anemia,[56] although anemia should be considered first and foremost a hallmark of human hookworm infection. Children with TDS are anemic and frequently manifest growth retardation and failure to thrive.[53] Infants and toddlers with TDS are at risk for protracted tenesmus, which leads to rectal prolapse. *Trichuris* colitis is a more chronic manifestation of moderate to heavy infection that is characterized by a form of inflammatory bowel disease similar to what occurs in Crohn disease or ulcerative colitis. Children with this form of colitis also can have chronic malnutrition and short stature. Moderately and heavily infected (and possibly even lightly infected) children are at risk for having deficits in cognition and intellectual development.[64]

Diagnosis and Differential Diagnosis

The diagnosis is established by identification of the characteristic barrel-shaped eggs through microscopic examination of stool. Clinically, heavily infected children with TDS may resemble children with amebic or bacillary dysentery. Children with *Trichuris* colitis may have signs and symptoms that resemble other forms of inflammatory bowel disease. The erythrocyte sedimentation rate is not elevated in children with *Trichuris* colitis, however. Among other diagnostic modalities, *Trichuris* colitis has been detected by Doppler ultrasonography,[50] whereas the presence of adult worms can also be seen on colonoscopy.[63]

Treatment

In the past, when noxious or toxic drugs had to be used, only patients with heavy infection were treated. Currently, any patient with this infection can be treated by the administration of either albendazole, administered as a single fixed dose of 400 mg in MDA programs or on 3 successive days otherwise, or mebendazole 100 mg twice daily for 3 days.[61] A single dose of 500 mg of mebendazole also is used for MDA in some developing countries. A recent systematic review, however, has revealed that cure rates after single-dose oral albendazole and mebendazole are only 28 and 36 percent, respectively.[17] Recent studies indicate that the efficacy can be approved by using a double dose of albendazole or mebendazole given 8 hours apart.[62] A combination of albendazole and mebendazole was also effective,[62] as was adding ivermectin to either albendazole or mebendazole.[57] As noted earlier, the safety of the benzimidazoles in very young children has not been established, but this may not preclude its use in the practice of pediatrics.[39] As an alternative, the drug oxantel is effective for the treatment of trichuriasis. In some countries, oxantel is formulated with pyrantel pamoate.[51]

Prevention

As in the case of other nematodes, sanitary disposal of excreta—but more important, improvement in the standard of living—tends to reduce the incidence of infection. Frequent mass treatments with mebendazole reduce the worm burden in a community.

HOOKWORMS

Hookworm infection is one of the most important infections of adults and children in the developing world, with an estimated prevalence of approximately 600 million cases.[3,10] Hookworms exert their pathogenic effect by causing intestinal blood loss, which leads to iron-deficiency anemia.[76] By considering the average daily blood loss induced by each worm, a moderate hookworm infection comprising 25 adult hookworms produces 1 mL of blood loss per day, often sufficient blood loss to rob a child of his or her daily iron requirement.[73] Because loss of blood represents loss of erythrocytes *and* plasma, heavy infections also contribute to protein malnutrition.[78]

Two major species of hookworms infect the human intestine: *Ancylostoma duodenale* and *Necator americanus*, although *N. americanus* is found more commonly worldwide (responsible for more than 80 percent of the global hookworm burden), especially in sub-Saharan Africa, Southeast Asia, and the Americas.[76] Two other members of the genus *Ancylostoma*, *A. ceylanicum* and the dog hookworm *A. caninum*,[80] are much less frequent causes of intestinal pathology in humans.[76]

The infection is acquired either by exposure of skin to moist soil infested with the larvae of these worms (*A. duodenale* and *N. americanus*) or by ingestion of the infective larvae (*A. duodenale* only). The most propitious circumstances for infection are shady areas and sandy or loamy soil. Infection is particularly likely to occur early in the morning, when the ground is moist with dew, or after a rainfall. After the larvae enter the host, they initiate a developmental program that continues until they enter the intestine.[7,76] This process is coupled to the release of parasite-derived proteases and other virulence factors.[86] Larvae that enter through the skin are carried by the venous circulation to the right ventricle and there follow the route described for *Ascaris*, whereas larvae that are ingested may develop entirely within the gastrointestinal tract.

On reaching the small intestine, the larvae mature to adult worms, which become attached with their mouth parts to the intestinal mucosa. The worms sustain themselves by releasing hydrolytic enzymes that degrade the intestinal mucosa and then feed on cellular and connective tissue debris.[76] During this process, capillaries and arterioles are eroded and lacerated, with subsequent extravasation of blood. The adult worms also ingest hemoglobin in blood through the action of parasite gut

brush border proteases.[84] Anticoagulants that block the activity of host factor Xa and VIIa/tissue factor facilitate blood flow and are responsible for continued bleeding from the original site after the worm has moved to a new one.[72]

The entire cycle from penetration of the skin or ingestion of larvae to the development of mature worms usually takes 6 to 8 weeks. At that time, hookworm eggs appear in the feces. *A. duodenale* larvae also may undergo a period of developmental arrest within the human host that lasts weeks or months. Intestinal ancylostomiasis can occur up to 1 year (and possibly longer) after initial exposure to infective larvae has occurred.[81,82] Investigators have conjectured that the reservoir of arrested *A. duodenale* larvae enters the mammary glands and breast milk.[75] This sequence of events may account for cases of infantile ancylostomiasis noted in Africa, India, and China.[67,71,75] Humans are the major reservoir of these organisms, and this infection is maintained by continual contamination of soil by human feces.

Epidemiology

Although in ancient times these parasites had a worldwide distribution, they currently are most prevalent in areas of rural poverty in the tropics and subtropics. *A. duodenale* predominates in many parts of the Indian subcontinent and focal areas of China north of the Yangtze River.[7] *N. americanus* is the predominant hookworm in the world, especially in the Western Hemisphere, most of Africa, China south of the Yangtze River, Southeast Asia and Indonesia, and certain islands of the Pacific. *A. duodenale* also is a major parasite in Egypt and other parts of the Mediterranean region, Africa, and a few focal areas in South America, including Paraguay and northern Argentina. A small Central American focus also exists in southern Honduras and El Salvador, and one exists among aboriginal populations in Australia. This differential distribution is not always absolute, and small numbers of either parasite can be present where the other predominates. Mixed infections with both species are common. *A. ceylanicum* occurs in focally endemic areas of southern Asia,[76] whereas the dog hookworm *A. caninum* has been described as a cause of human eosinophilic enteritis in Australia and possibly elsewhere.[78]

Larvae survive in soil for 6 weeks. They are destroyed by drying, freezing temperatures, and heat greater than 45°C. Hookworm infection occurs in areas with high agricultural intensity and is not found frequently in urban areas, where *Ascaris* and *Trichuris* might predominate. Because shade and moisture are essential for survival at the infective larval stage, a not surprising finding is high rates of hookworm infection in families that harvest tea in India and Bangladesh, mulberry leaves (for the silkworm industry) in eastern China, sweet potatoes and corn in western China, coffee and bananas in Central and South America, and rubber in Africa.

Similar to other STHs, hookworm infections usually are aggregated such that most individuals harbor light worm burdens, whereas a substantial minority harbor moderate or heavy infections.[76,82] Even after receiving specific anthelmintic chemotherapy, moderately and heavily infected individuals seem to be predisposed to the reacquisition of heavy infection.[82] A predisposition to hookworm infection may have a genetic or immunologic basis.

In contrast to the other STHs, high rates of hookworm infection occur in adults and children. In some regions, the age-associated prevalence and intensity increase linearly, with the heaviest infections occurring in elderly populations.[74] Hookworm coinfections with strongyloidiasis,[66] schistosomiasis,[10,85] and malaria[68,69] have been described and are believed to be common in low- and middle-income countries.

Pathophysiology

During the migratory phase of the infection, the larvae evoke an inflammatory response associated with eosinophilic infiltration. Immune responses to the infection have been difficult to study in humans, but in an animal model a dog infected with *A. caninum* can be rendered immune to challenge infection by receiving repeated dosing with the infective larvae. This observation has provided a means of producing a live attenuated larval vaccine in the laboratory, but it is unsuitable for humans.

Hookworms feed on human blood and have the capacity to lyse red blood cells and degrade host hemoglobin.[79] The major source of injury to the host is loss of blood, with *A. duodenale* producing greater blood loss than *N. americanus*. Rarely, massive bleeding has been reported.[80] Iron-deficiency anemia results when iron loss exceeds the host's iron reserves. Because children and women of reproductive age generally have lower iron reserves than do other populations, they are at the highest risk for developing hookworm disease and anemia.[76] Recent systematic reviews confirm the association between anemia and hookworm infections in children and pregnant and nonpregnant adults.[70,83] Long-standing moderate and heavy infections result in the characteristic features of severe iron deficiency. Loss of protein contributes further to malnutrition. Together the iron and protein malnutrition result in impaired growth and physical fitness.[21,24-26,76] In some cases, hypoproteinemia can be corrected by a high-protein diet without deworming the patient,[80] although the World Food Program and other international agencies now frequently incorporate deworming into their nutritional supplementation programs. In addition to the physical deficits noted earlier, hookworms may affect cognitive development because iron is important for the development of dopaminergic neurons and for the biosynthesis of some neurotransmitters.[76] An experimental human hookworm vaccine for *N. americanus* infections is under development; the vaccine targets key parasite enzymes involved in blood digestion and heme detoxification.[10]

Clinical Manifestations

Penetration by larvae causes "ground itch" or "dew itch," a pruritus that occurs after walking in the morning dew. These cutaneous manifestations can occur on almost any part of the body. This phase of early infection is followed within days to weeks by a pneumonitis, which typically is less pronounced than *Ascaris* pneumonitis.

The acute intestinal phase in heavy infections is characterized by abdominal pain, diarrhea, nausea, and anorexia.[75] Eosinophilia occurs approximately at the onset of the intestinal phase. Subsequently, well-nourished individuals with light infections have mild gastrointestinal symptoms, but no evidence of anemia or malnutrition. At the other extreme, children with moderate hookworm infections can show signs and symptoms of iron-deficiency anemia, whereas heavily infected children can have hemoglobin values of 2 g/dL and edema or anasarca caused by hypoproteinemia. Infants with severe ancylostomiasis can exhibit failure to thrive, profound pallor, and melena.[67,71,75]

Deficits in physical and intellectual growth as a result of chronic hookworm infection in childhood have been reported since the early part of the 20th century.[6,7,21,22,76] Some recovery has been described after "deworming," although some deficits occurring in infancy may be irreversible.

Diagnosis and Differential Diagnosis

Hookworm pneumonitis resulting from the early migratory stages typically is nonspecific in its presentation and difficult to diagnose. Hookworm anemia is caused by blood loss; it must be distinguished from all other causes of intestinal loss of blood. In developing countries, where severe hookworm anemia is common, the probability of the occurrence of rarer causes of intestinal blood loss, such as Meckel diverticulum and polyps, is low. The opposite holds true for regions where hookworm infections are light or infrequent. In sub-Saharan Africa, the distribution of hookworm overlaps with other significant causes of so-called agricultural anemias, including malaria, schistosomiasis, malnutrition, and hemoglobin polymorphisms.[14]

The diagnosis is established by identifying the characteristic eggs by microscopic examination of stool. The eggs of *A. duodenale* and *N. americanus* cannot be distinguished from each other by morphologic criteria, but the worms can be differentiated by direct examination of the infective larvae or adults. One must assume that it is one or the other species on the basis of the geographic origin of the patient. Although this decision is not of paramount importance, it does have some therapeutic implications, including the possibility that arrested larvae of *A. duodenale* would become reactivated and either repopulate the intestine at some point after treatment or enter breast milk during or after parturition. Hookworms have been detected visually by endoscopy.[77]

Treatment

For *N. americanus* infection, the drug of choice is either albendazole, administered at a fixed dose of 400 mg, or mebendazole, given as 100 mg twice daily for 3 days. In settings of MDA for low-income countries, mebendazole is sometimes given as a single 500-mg dose. Pyrantel pamoate given as a dose of 11 mg/kg, not to exceed 1 g, for 3 days is a suitable alternative drug. A recent meta-analysis and systematic review found that the efficacy of single-dose oral albendazole for hookworm infection was 72 percent, whereas single-dose mebendazole only results in a cure rate of 15 percent and pyrantel pamoate in one of 31 percent.[17] Therefore, albendazole should be considered the first-line treatment of hookworm infection. In areas endemic for ancylostomiasis, the health care provider should be aware of the potential of arrested tissue larvae to repopulate the intestine or enter breast milk and infect infants during the perinatal period. Iron supplementation and transfusion are occasional important adjunctive therapies for severe hookworm disease.

Prevention

As in all cases of all STH infections, the sanitary control of the disposal of excreta represents an important public health control measure. Accomplishing effective sanitation in resource-poor settings has not been feasible in most of the developing world. The popular recommendation to wear shoes is naïve because *N. americanus* larvae also enter through the upper extremities, torso, and legs, and because *A. duodenale* also is orally infective. Studies to develop and test a first-generation recombinant hookworm vaccine are in progress.[10]

ENTEROBIUS VERMICULARIS

Pinworm infection (enterobiasis), or oxyuriasis (the older term), is one of the most frequent of all human helminth infections and one that is common in North America and Europe.[97] Some investigators believe that the overall prevalence of enterobiasis is declining in the United States, however. Most infected individuals are children, and the infection is found in all socioeconomic classes. It is acquired by the ingestion of infective eggs picked up on the perianal skin, in the air, or on bedclothes and underwear. Swallowed eggs, transmitted to the mouth by fingers or through inhalation, hatch in the duodenum, and the liberated larvae undergo additional maturational steps in the small intestine before reaching the cecum. There, the sexually mature worms copulate and proceed to the rectum and eventually to the perianal skin, where the gravid females lay eggs. The eggs become infective within 2 to 4 hours after deposition.

The entire cycle from ingestion of the egg to the egg-laying phase of the gravid female is 4 to 6 weeks. Rarely, a retrograde infection occurs in which eggs hatch on the anal mucosa and the larvae migrate up the bowel and mature to adult worms. Sexual transmission from oral-anal contact has also been reported.[87] Although enterobiasis is a human infection, anthropoid apes can be infected experimentally.

Epidemiology

The infection is worldwide in distribution, and children are infected most frequently. Communal living, especially assembling in school gymnasia and living in crowded households, promotes transmission of the infection. Adults tend to be infected through their contact with children; parents and teachers are the most vulnerable. Orphanages and daycare centers frequently are affected.[97]

Pathophysiology

No intestinal reactions occur during the migratory phase, and because no tissue migration takes place, no eosinophilia occurs to the degree seen with some other nematodes. Occasionally, hypersensitive individuals may have a slight increase in eosinophils. The deposited ova induce pruritus on the perianal skin. No evidence indicates that some individuals are more susceptible than others to this infection.

Enterobius occasionally has been found in vermiform appendixes removed at surgery.[88] A causal relationship to appendicitis has not been established, but some evidence points to the possibility that this worm induces granuloma formation and may cause obstruction of this vestigial structure.[89]

When the adult gravid female migrates along the perineal skin into the vagina, it may cause vulvitis as a reaction to the eggs deposited in that region.[91] Some investigators speculate that migrating pinworms may introduce bacteria into the lower urinary tract and result in urinary tract infection.[96] *E. vermicularis* has also been reported from the fallopian tube and as a cause of infertility.[99] Other aberrant infections, such as those causing hepatic granuloma and renal disease, occur more infrequently.[90,94]

Clinical Manifestations

Pruritus is the most common symptom; its intensity varies from mild perianal itching to acute, intractable pain. Secondary cellulitis also may occur in severely pruritic cases.[93] Perianal abscess has also been described.[95] Vaginal discharge and vulval itching are symptoms in the rare cases in which a worm has migrated into the vagina. Insomnia, restlessness, irritability, loss of appetite, loss of weight, and grinding of teeth all have been reported anecdotally in individuals with pinworm infection, but no evidence has shown that any of these symptoms is related causally to *Enterobius* infection. Enuresis also has been blamed on the pinworm, but one epidemiologic study failed to determine the causality.[98] An unusual form of eosinophilic ileocolitis resulting from massive infestation with many *E. vermicularis* larvae has been described in a homosexual man.[92] A variety of clinical manifestations resulting from ectopic infections, including appendicitis and infertility have been reported.[88,99]

Diagnosis

E. vermicularis eggs are identified readily by low-power microscopic examination of transparent adhesive tape previously applied to the perianal skin and then affixed to a microscope slide.

Treatment

Single-dose therapy with mebendazole (100 mg), albendazole (400 mg), or pyrantel pamoate (11 mg/kg, not to exceed 1 g) is effective. With the current availability of these three highly effective drugs, the intensive laundering of underwear and bed clothing, recommended in the older literature, no longer is necessary. Treating all members of the household is advisable, however, because they all must be presumed to be infected. Re-treatment in 2 or 3 weeks to destroy any adult worms that have hatched from the eggs swallowed at the time of initial therapy may be necessary. None of these drugs destroys the eggs.

One of the most important aspects of management of this infection is reassurance that in some areas its ubiquity virtually precludes effective eradication. Re-infection can be anticipated in any family infected with pinworms because of the high prevalence of this worm in the community. It also is important to reassure families that the presence of pinworms does not suggest poor hygienic standards in the family.

STRONGYLOIDES STERCORALIS AND *S. FUELLEBORNI KELLYI*

Strongyloides stercoralis and *S. fuelleborni kellyi* are among the most virulent helminthic pathogens of humans, although human strongyloidiasis is believed to be less prevalent than *Ascaris* or hookworm, with some estimates indicating 30 to 100 million cases worldwide.[123] *S. stercoralis* has the unusual ability to cause autoinfection, which can lead to hyperinfection and disseminated infection in immunocompromised hosts.* *S. fuelleborni kellyi* causes an aggressive infantile protein-losing enteropathy that leads to ascites and high mortality rates.[100,101]

S. stercoralis infection is acquired by the exposure of skin to infective larvae in the soil, much as in the case of hookworm infection. Similar circumstances that promote the survival of hookworm larvae in soil (i.e., moisture, sandy or loamy soil, and shade) promote the survival of *Strongyloides*. Larvae penetrate skin, facilitated by a potent histolytic protease that they secrete.[103,122] From the moment of penetration of the skin to the arrival of the worms in the intestine, the cycle commonly is thought to be similar to that of hookworms, although experimental evidence suggests that *S. stercoralis* also may explore routes of migration that bypass the lungs.[128] Within the intestine, the small adult worms do not attach to the mucosa as hookworms do but instead lie embedded in its folds. The cycle from penetration of skin to development of mature worms in the intestine is approximately 28 days.

No parasitic adult male worms exist to fertilize the eggs. Instead, the mature eggs may develop by parthenogenesis. In addition, in contrast to the eggs of other parasitic nematodes, these eggs usually are not found in feces but instead embryonate within the intestine and develop into larvae, which are deposited in soil with human stool. These so-called rhabditiform larvae must molt before they become infective.

This cycle has two variations. One permits the development of nonparasitic male and female adults in soil, which can maintain infestation of the soil for a certain period; this free-living phase sometimes is called the *heterogonic life cycle*. The second variation has much greater

*References 106, 110, 113-117, 119, 125, 126.

clinical relevance. Under certain conditions that are not well defined, the rhabditiform larvae molt to new infective larvae while still in the intestine. These new infective larvae can penetrate the intestine and set up a new cycle, commonly called *autoinfection* or the *autoinfective cycle*.[115] In this fashion, this nematode, in contrast to most other intestinal nematodes of humans, actually can increase in number without reinfection from the outside world. This phenomenon also is responsible for persistence of this infection for decades in an untreated host.[117] Some investigators think that low levels of autoinfection occur in most patients with strongyloidiasis.

When host defenses are impaired, especially through use of high-dose corticosteroids (discussed later), *S. stercoralis* can undergo multiple rounds of autoinfection, leading to the production of thousands to hundreds of thousands of adult parasites in the intestine. This phenomenon is known as *hyperinfection*.[104,110,116,125] One possible consequence of hyperinfection is disseminated infection, in which larval and adult worms are identified at extraintestinal sites.

S. fuelleborni kellyi larvae are passed to infants by ingestion in breast milk.[100,105] Transmammary infection by nematode larvae is an extremely common route of transmission in nonhuman nematode infections[20]; although not well studied, it probably also occurs commonly in humans.

Epidemiology

S. stercoralis infection has worldwide distribution, but it is most prevalent in tropical and subtropical regions.[123] In North America, strongyloidiasis is focally endemic in some parts of Appalachia[106] and is common in Southeast Asian immigrants.[118] In one study, 76.6 percent of Kampuchean immigrants and 55.6 percent of Laotian immigrants were seropositive for *S. stercoralis* infection.[118] Between 1991 and 2006 an estimated 347 deaths from strongyloidiasis occurred in the United States, mostly among older white males and those living in the southeastern states.[109] Strongyloidiasis also is endemic in Brazil and Jamaica and presumably elsewhere in Latin America, as well as in sub-Saharan Africa.[111,123,126] Because of the possibility of autoinfection and, by extension, infection through contamination of skin by infested feces, strongyloidiasis is highly prevalent in mental hospitals, prisons, and homes for mentally disabled children. Dogs and anthropoid apes may serve as animal reservoir hosts for *S. stercoralis*. *S. fuelleborni kellyi* infection is endemic in Papua New Guinea and parts of sub-Saharan Africa.[100,101,120]

Pathophysiology

During the migratory phase of the infection, the larvae of *S. stercoralis* evoke an inflammatory response associated with eosinophilic infiltration. The adult phase in the intestine, even in moderate infection, may be associated with an inflammatory reaction sufficient to be symptomatic. Some evidence suggests that *Strongyloides* induces a malabsorption syndrome, which has been treated effectively by deworming.[106,124] It also has been noted, however, that the diarrhea associated with strongyloidiasis in young children occurs more frequently in those with underlying malnutrition.[110] Young children with *S. stercoralis*–induced malabsorption experience stunted growth and failure to thrive.[106]

The deficits in host defense that promote hyperinfection and disseminated strongyloidiasis are not well understood. Although cell-mediated immune deficits, such as those occurring in immunosuppression, organ transplantation, severe malnutrition, and cytotoxic chemotherapy for neoplasms and collagen vascular disease, are associated with this phenomenon, certain established deficits in cell-mediated immunity, such as those in HIV infection, do not trigger hyperinfection.[115] Researchers have suggested that patients receiving large doses of corticosteroids are particularly susceptible to hyperinfection because the corticosteroids themselves function as direct signals or ligands for the parasite to undergo autoinfection.[115] Patients with human T-cell lymphotropic virus type I seem to be at high risk for acquiring opportunistic strongyloidiasis,[113,119] possibly because of a specific deficit in their effector IgE immune response.[126] The pathogenesis of the marked protein-losing enteropathy that leads to ascites in the swollen belly syndrome of *S. fuelleborni kellyi* infection has not been established.

Clinical Manifestations

During the migratory phase of larval strongyloidiasis, patients may be susceptible to the development of pneumonitis associated with eosinophilia. Larval migration through the skin can result in larva currens. Although most patients harboring *S. stercoralis* in their intestine are asymptomatic, patients with moderate or heavy infection classically have intense diarrhea productive of watery, mucous stool. Periods of alternating diarrhea and constipation may occur. Anorexia and cachexia, which lead to failure to thrive and other deficits in physical growth, are common features of pediatric strongyloidiasis.[106] Eosinophilia is also common, although among pediatric refugees this sign was not considered predictive for *S. stercoralis* infection.[112] Chronic strongyloidiasis has been linked to nephrotic syndrome.[108]

In disseminated strongyloidiasis caused by the hyperinfective cycle, larvae may invade all tissues, including the central nervous system (CNS). Because larvae penetrate the intestine, they may carry with them enteric flora and cause sepsis or meningoencephalitis.[104] Although diarrhea is the most commonly recognized consequence of *Strongyloides* infection, the hyperinfective cycle has the greatest portent for immunosuppressed patients. Infants with *S. fuelleborni kellyi* infection may manifest the swollen belly syndrome with marked abdominal ascites and pleural effusions that can be fatal. In older children *S. fuelleborni* kellyi infection can be associated with malnutrition.[120]

Diagnosis and Differential Diagnosis

Strongyloides pneumonitis can resemble the clinical manifestations associated with the lung migration of other nematode parasites, such as *Ascaris* and hookworm. The differential diagnosis of diarrhea must include causes of

chronic diarrheal disease. In some patients, eosinophilia is a presenting sign of strongyloidiasis, either in patients with diarrhea or even in asymptomatic immigrants from developing countries.

The diagnosis is established by identification of the characteristic larvae during microscopic examination of stool, which is not easy because rhabditiform larvae usually are not produced in abundance. Specific stool concentration techniques are available to increase the sensitivity of fecal examination, although they are not as effective as amplifying the heterogonic life cycle by the Baermann technique or by looking for characteristic larval tracks on nutrient agar plates.[129] The stool of all immunosuppressed individuals, including those given corticosteroids for any reason, who have ever been in a region where *Strongyloides* is found must be examined to rule out this infection. If routine stool examination results are negative, the stool should be processed as outlined earlier. In addition, examination of duodenal contents can be attempted by the string test (Enterotest). This examination only divulges the contents of the duodenum, however, and can miss the larvae in the lower part of the small intestine. An enzyme-linked immunosorbent assay (ELISA) for detection of *Strongyloides*-specific antibodies is available on a research basis.[118,126]

Children with the swollen belly syndrome from *S. fuelleborni kellyi* infection shed eggs rather than larvae in their feces. Large numbers of eggs are common findings in clinical cases.

Treatment

Previously, the drug of choice for *S. stercoralis* and *S. fuelleborni kellyi* infection was thiabendazole.[107] The drug has high toxicity, however, which frequently includes nausea, vomiting, and vertigo and sometimes requires interruption of therapy. Rarely, it induces leukopenia, rash, and Stevens-Johnson syndrome. Because the drug is detoxified in the liver, its dose may have to be reduced for patients with liver failure. Another benzimidazole used with increasing frequency is albendazole 400 mg twice daily for 7 days. Recent studies have shown that ivermectin is equivalent to thiabendazole in terms of its efficacy, but ivermectin has a much better safety profile.[102] Similarly, single and double doses of oral ivermectin are more effective than a 7-day course of high-dose albendazole for patients with *S. stercoralis* chronic infection.[130]

Ivermectin is considered the treatment of choice for strongyloidiasis. It is administered at a dose of 200 μg/kg/day for 2 days. Treatment with ivermectin gives a cure rate of 80 percent.[119,121] The safety of the drug in young children (<15 kg) and pregnant women remains to be established. Because the mortality rate of patients with disseminated strongyloidiasis remains high despite specific anthelmintic therapy, a common practice is to treat with a prolonged course or with a repeat course. In addition, several case reports have now described the use of veterinary parenteral formulations in patients too ill to take oral medications[121] or the use of ivermectin combined with albendazole.[124] Secondary bacterial complications, such as sepsis and meningitis, are common with disseminated strongyloidiasis, so judicious use of broad-spectrum antimicrobial agents frequently is indicated for this condition. As an additional supportive measure, patients who have hyperinfective strongyloidiasis and are receiving high-dose corticosteroid therapy probably benefit from steroid taper. Patients with transplants and cyclosporine immunosuppression may benefit from some of the direct helminthotoxic properties of this compound.[127]

Prognosis

The prognosis is excellent in patients who do not have disseminated infection and are treated promptly. Unrecognized disseminated infection can be lethal.

Prevention

Proper disposal of human excreta substantially reduces the prevalence of strongyloidiasis in any community. In closed institutions, where control of direct spread is not likely to be achieved, identification plus treatment of infected individuals is the only feasible control.[114]

ABERRANT INFECTIONS WITH INTESTINAL NEMATODES

TOXOCARA CANIS AND *T. CATI*

As indicated in the previous sections, the life cycles of the intestinal nematodes are adjusted precisely through evolutionary selection. In many instances, only one host in which the cycle can be completed is parasitized by the nematode. Infection of an unnatural host, in most cases, leads to complete failure of development and causes no disease. In a few instances, an infection may be established, but the cycle is not completed. Under such circumstances, the process of aberrant migration of larvae may be more pathogenic than steps in the natural cycle.

One of the most dramatic examples of an aberrant infection is visceral larva migrans, caused by infection with *Toxocara canis.* This roundworm causes intestinal infection in the dog, in which its cycle resembles that of *A. lumbricoides* in humans. Humans become infected by *Toxocara* through the ingestion of an embryonated egg, much as in human infection with *Ascaris.* Larvae hatch in the small intestine, penetrate the villi, and begin a migration that takes them through every organ and tissue of the body. Because they cannot mature, the larvae tend to migrate for months until they are overcome by the inflammatory reaction of the host and die. Although

larvae of other toxocarids such as *T. cati* and *Toxascaris leonina* have been suggested as possible causes of visceral larva migrans, they may be less important as zoonotic pathogens in humans.

Because population-based serologic testing is limited to only a handful of research studies,[131,143,151,152] the prevalence of toxocariasis in North America and elsewhere may be underestimated. According to some investigators, *T. canis* infection may have replaced *E. vermicularis* as the most common helminth parasite in the United States.[16]

Epidemiology

The prevalence of toxocariasis is difficult to assess because of the failure of establishing the diagnosis in many cases. The disease has been reported from many parts of the world, including temperate climates, and one can assume that it is found wherever humans and dogs coexist. High prevalence rates of the infection have been reported from Brazil, Africa, Eastern Europe, and the United States[8,12,16,137]; toxocariasis may represent one of the most common helminth infections worldwide.[16] Young children often come into contact with *T. canis* eggs while playing in sandboxes and on playgrounds that were contaminated by a family pet.[145,147] The level of contamination of public areas also is difficult to ascertain. In several studies, ova were present in 5 to 25 percent of soil samples obtained, and surveys of dogs in urban communities have shown the occurrence of frequent infections, particularly in puppies, which are infected almost universally with *T. canis* (canine infection occurs transplacentally).[140,141]

Subsequent dissemination of *T. canis* eggs in the environment probably is aided by migrating earthworms and other soil invertebrates.[136] The seroprevalence of toxocariasis in the United States is high, and the parasite should be considered an emerging pathogen in some poor rural and urban areas. In some groups of socioeconomically disadvantaged black children, the seroprevalence is 30 percent,[144,150] with even higher rates occurring among U.S. inner-city Hispanic children.[152] In the United States, the disease is linked to being poor and of African-American or Hispanic ethnicity.[8,16,138,155] It has been estimated that up to 2.8 million African Americans have toxocariasis, so this disease represents a major but neglected health disparity.[8] Toxocariasis is endemic to Puerto Rico. Major risk factors for acquiring toxocariasis include having a litter of puppies in the home and the habit of geophagia.[148] The latter risk factor probably accounts for the observed association between toxocariasis and elevated lead levels.[148] In Ireland, the prevalence of ophthalmologist-diagnosed ocular toxocariasis is 9.7 per 100,000, while in the United States it was determined that 68 patients were newly diagnosed with ocular toxocariasis between September 2009 and September 2010.[135,145]

The presence of a positive skin test for toxocariasis is associated with poliomyelitis statistically.[156] No direct causal relationship exists; possibly, the circumstances leading to ingestion of *Toxocara* ova also are conducive to ingestion of poliomyelitis virus. *Toxocara* and *Toxoplasma* coinfections have also been noted; possibly these are from *T. cati* infection reflecting concurrent exposure from cats.[146] Likewise, seizures and asthma are correlated with seropositivity for *Toxocara* antibodies, but a causal relationship has not been established.[16,131] Some investigators have postulated that toxocariasis may be an important cause of asthma and so-called idiopathic seizures in young children.[16,142,145]

Pathophysiology

The entire infection is restricted to the migratory phase and bears some clinical resemblance to the symptoms found during the early phases of *A. lumbricoides* infection. Symptoms are protean and depend on which organ or tissue is infected. For unknown reasons, visceral migration through the liver, lungs, and brain occurs more commonly in toddlers and children younger than 5 years old, whereas older children are more likely to exhibit only ocular involvement.[145,147] Epidemiologic evidence suggests that this infection produces two distinct syndromes, visceral and ocular, because involvement of one tends to occur in the absence of the other.[143] Visceral migration elicits eosinophilic granuloma formation in the target organs and leads to hepatitis, pneumonitis, or cerebritis. Larval migration in the retina results in ocular larva migrans, which includes granuloma formation in the retina.[132,139,153] The lesion can resemble retinoblastoma so that it often is confused with it. Endophthalmitis[139] or papillitis[136] also may develop. Invasion of other organs and tissues induces granuloma formation.

Clinical Manifestations

Most patients infected with *T. canis* are thought to be asymptomatic. Some of these individuals have isolated findings, including eosinophilia, or wheezing and asthma. The term *covert toxocariasis* is used by some investigators to describe these patients, who often are identified by their circulating anti–*T. canis* antibody titers. An association between asthma and covert toxocariasis has been well described in Europe, but as yet this association is unproven in North America.[152] Potentially, this association would represent an important avenue for investigation, given the high prevalence rates among African-American and Hispanic children who live in poverty in the United States and are considered at high risk for asthma.[16]

Visceral larva migrans, the extreme form of toxocariasis, typically occurs in a toddler with the symptoms and signs of a multisystemic disease. It is associated with fever, hepatosplenomegaly, lung infiltrates accompanied by wheezing, a high degree of eosinophilia (approaching 80%), and elevated immunoglobulin levels, particularly of the IgM class.[142,145,147,148] Seizures and neuropsychiatric disturbances also are common. In one case report, the child's major neurologic manifestation was a static encephalopathy.[142]

In contrast, ocular larva migrans is characterized by a unilateral vision deficit and, sometimes, strabismus. Ophthalmologic examination frequently reveals one or more posterior poles or peripheral pole granulomas.[139,147,153] More global eye inflammation also can occur (discussed

earlier). Children with ocular involvement usually have few, if any, systemic manifestations. Often, no laboratory abnormalities are detected.

Diagnosis and Differential Diagnosis

Visceral larva migrans must be distinguished from the migratory phase of the other nematode infections. Because of hepatosplenomegaly and hypereosinophilia, eosinophilic leukemia occasionally has been suspected, but it can be ruled out readily by examining bone marrow.

T. canis larvae can be identified in tissues in liver biopsy specimens, but the diagnostic yield is low. One must resort to indirect means and be aware that a multisystemic disease with elevated IgM and hypereosinophilia fits the diagnostic criteria. An ELISA available at the Centers for Disease Control and Prevention is highly specific and diagnostic.[147,150,151] However, the exact interpretation of being seropositive to *T. canis* is considered controversial, with some investigators of the opinion that this finding reflects previous exposure whereas others believe it indicates the presence of actively migrating larvae.

Ocular larva migrans usually is diagnosed by an experienced ophthalmologist who recognizes the characteristic granulomas and larval tracks on retinal examination. Presumably because of minimal antigen presentation by a few migrating larvae in the eye, often no measurable immune response occurs in this condition. For this reason, ELISA frequently is unreliable for establishing the diagnosis of ocular larva migrans.[144,147,148,150,151]

Treatment

Traditionally, treatment of visceral larva migrans was primarily symptomatic, especially because much of the morbidity is associated with immunopathologic responses against dying parasites. In the 1960s, thiabendazole and diethylcarbamazine were determined to be effective against migrating larvae.[149] Since then, new agents of the benzimidazole class have been claimed to be equally effective but associated with fewer drug toxicities.[145] In a comparative study with thiabendazole, albendazole 10 mg/kg/day in two divided doses for 5 days was shown to be well tolerated and less toxic.[154] Another benzimidazole, mebendazole, also may be effective when given in doses high enough to achieve significant extraintestinal levels.[133] Albendazole 400 mg twice daily for 5 days is the treatment of choice. Although anecdotal experience with albendazole and mebendazole overseas suggests that these drugs are safe in children,[134] the large doses required for the treatment of larva migrans may be associated with hepatic and other toxicities (including embryotoxicities), and they have not been approved for this purpose.

Treatment of ocular larva migrans often requires surgical management, particularly in cases associated with tractional retinal detachment.[139,153] Specific anthelmintic adjunctive chemotherapy seems to be beneficial in some cases.[140,145,153]

Prognosis

Except for patients in whom blindness develops as a consequence of retinal damage and a rare fatal case resulting from the intensity of the acute clinical reaction, most patients recover. The recovery phase may be slow, however, and may take 2 years.

Prevention

Theoretically, the disease can be prevented by elimination of dog feces from the human environment, but in practice that is no less difficult to achieve than control of human excrement disposal.

OTHER ABERRANT INFECTIONS WITH INTESTINAL NEMATODES

Baylisascaris procyonis, a parasite of raccoons, also can cause visceral larva migrans, as well as an emerging eosinophilic meningitis.[164] *Baylisascaris* infection occurs when humans accidentally ingest parasite eggs that are shed in barn lofts and attics accessible to raccoons.[148,162,163,166,168] In at least one reported human case, the infection was fatal in an infant.[166] Similar to *Toxocara* infection, *Baylisascaris* larvae within aberrant hosts cannot complete their cycle and continue their aimless migration through the tissues of these hosts. Baylisascariasis is probably more severe, owing to the propensity of the larval stages of the parasite to invade the CNS, however, along with the continued growth of the larvae during migration resulting in greater mechanical damage.[162,163] The lesions caused by the larvae are eosinophilic granulomas, which tend to be concentrated in the CNS and result in eosinophilic meningitis. The disease is characterized by a severe eosinophilic pleocytosis in the cerebrospinal fluid and nonspecific diffuse white matter lesions seen on magnetic resonance imaging.[164] Neither the frequency nor the range of severity of this infection in humans is known. Most human cases have been diagnosed at autopsy. Because baylisascariasis is so uncommon, no studies are available to evaluate different anthelmintic chemotherapy regimens systematically. Some clinicians have recommended prolonged treatments with high doses of albendazole.[162,163] Given the potential severity of infection, some investigators propose

administering postexposure prophylaxis after intimate contact with raccoon feces or soil contaminated with raccoon feces (albendazole 25 mg/kg/day orally for 20 days) as soon as possible after exposure. Prolonged albendazole treatment requires monitoring of blood cell counts and liver function.

Other, less severe aberrant infections of importance to humans are those caused by the dog hookworm, primarily *Ancylostoma braziliense*, but possibly also *Uncinaria stenocephala*.[75,162,165,176] Infection with the larvae of *A. braziliense* and *U. stenocephala* cannot be completed, and larvae remain viable and migrate in the skin (usually between the epidermis and dermis); hence the terms *cutaneous larva migrans* and *creeping eruption* are used. In North America, cutaneous larva migrans is common along the Gulf Coast and along the Atlantic seaboard.[76] It also is common in the Caribbean. Failure of these zoonotic hookworms to complete entry through the human skin may reflect differences in the hydrolytic enzymes released.[165] Infection is acquired in the same fashion as that of the human hookworms. Children who expose their whole bodies to contaminated soil may be infected at any site. Adults are most likely to have infection in the lower extremities, but plumbers in the tropics, who often must crawl beneath houses, acquire infection on the elbows and knees.

The interval from exposure to appearance of the first symptoms is approximately 2 weeks; papules 2 mm in diameter then begin to appear on the skin. Behind them usually are serpiginous, erythematous, intracutaneous tunnels. The entire area itches intensely. If left untreated, cutaneous larva migrans tends to last 2 months. Albendazole 400 mg daily for 3 days or ivermectin 200 µg/kg daily for 1 to 2 days is effective treatment when administered orally. Topical therapy with a 15 percent aqueous suspension of thiabendazole was successful in one reported series. Forty-seven of 50 patients achieved permanent cure in 2 weeks, and 2 more patients were cured after a third week of treatment.[179] Placebo-treated patients were used as controls in this study. In contrast to skin penetration of zoonotic hookworms, oral ingestion of the dog hookworm *A. caninum* results in an eosinophilic enteritis syndrome (see the earlier discussion on hookworm infection).

Rarer aberrant infections include those caused by various species of *Trichostrongylus*, *Oesophagostomum*, *Angiostrongylus*, *Capillaria*, and *Anisakis*. *Trichostrongylus* is a common parasite of many mammals, and it has been found in the small intestine of humans, mainly in Asia, Africa, and Australia. Ingestion of larvae leads to the development of adult worms in the small intestine. Whether this development results in any disease remains a moot point because infections tend to be mild and usually are associated with other helminth infections.[163] *Oesophagostomum bifurcum* is a common nematode of subhuman primates in Africa and has been reported to be a common intestinal nematode that causes nodular disease of the intestines in humans living in West Africa.[170,173] Human esophagostomiasis has been treated successfully with pyrantel pamoate.[170]

Land snails and slugs serve as intermediate hosts for *Angiostrongylus* spp. *A. cantonensis* is a cause of eosinophilic meningitis (neuroangiostrongyliasis) throughout eastern Asia and Hawaii[159,169,174,175]; *A. cantonensis* has also emerged in the mainland United States, especially on the Gulf Coast after importation of infected rats and African land snails on container ships.[161] *A. costaricensis* is a cause of mesenteric arteritis and abdominal pain in Central and South America and in Latin American immigrants to the United States.[167] The rat serves as the natural host of these parasites, which live either in the lung *(A. cantonensis)* or in mesenteric arteries *(A. costaricensis)*. The rat eats the infected mollusks and ingests the larvae, which migrate to their final destination. An incomplete infection develops in individuals who ingest either the mollusks or food contaminated by the mollusks. *A. cantonensis* infection usually is limited to the CNS and is manifested as eosinophilic meningitis.[159,169,174,175]

Signs and symptoms of neuroangiostrongyliasis include meningismus, severe headache, paresthesias, and, less commonly, cranial nerve palsies. No specific treatment is available (although anthelmintics are effective in some experimental animal models[159]), but the disease is self-limited and lasts no longer than 2 weeks. Symptomatic relief has been reported with the use of prednisone. Some investigators have reported success in treating neuroangiostrongyliasis with albendazole and prednisone, together with repeated spinal taps to decrease intracranial pressure.[164] In contrast, *A. costaricensis* infection typically is manifested as abdominal or right iliac fossa pain, fever, and eosinophilia. In children with this condition, appendicitis or Meckel diverticulum may be diagnosed.[167] High doses of mebendazole have been tried as therapy for this condition, as well as albendazole.

Capillaria philippinensis is a common parasite of water fowl in the Philippines.[158] The mode of transmission of this parasite to humans is unknown, but human cases have been reported in which 40,000 adult worms were found embedded in the crypts of the small intestine. *C. philippinensis*, similar to *S. stercoralis*, can undergo autoinfection and hyperinfection in humans.[158] No associated inflammatory reaction occurs, but flattening of villi, loss of epithelial surface area, and severe malabsorption have been reported.[158,160,178] In one series of 1000 cases of *C. philippinensis* infection, a mortality rate of 10 percent was reported.[160] Thiabendazole may be effective in shortening the course of the infection,[178] although albendazole has become the treatment of choice more recently.[158] Another member of the genus, *C. hepatica*, a rare zoonosis of humans, has been known to disseminate to the lungs, liver, and other viscera.[172]

Anisakis spp. are nematode parasites of marine mammals, with fish being intermediate hosts. When the infective larvae of the parasite are ingested as a result of eating raw or poorly cooked fish, they may become embedded in the gastric mucosa and cause eosinophilic granuloma.* In adults, the infection may resemble carcinoma of the stomach clinically and radiographically. Human anisakiasis occurs frequently in Japan, where raw marine fish are eaten commonly, and in Holland, where lightly pickled herring is considered a delicacy.

*References 76, 157, 171, 177, 180, 185.

FILARIAL PARASITES

Except for rare instances of zoonotic *Brugia* infection, the filarial worms parasitizing humans affect people within the geographic area almost entirely limited to the developing world, especially sub-Saharan Africa and India. Although accurate data are lacking, approximately 115 million people are infected with lymphatic filariasis and 26 million are infected with onchocerciasis.[183] The various human parasites in this category have certain characteristics in common. They all are spread by vectors, and the adults invade and occupy the lymphatics, skin, connective tissue, or blood. They produce live embryos called *microfilariae* that enter the bloodstream or skin, where they can survive for months or years without further development. The range of disease caused by these worms is wide; some produce no symptoms, whereas others can be responsible for severe clinical disorders.

The life cycles of the filarial worms are similar in that infections are acquired through an insect bite, during which transmission is effected by introduction of infective larvae onto the skin of the host from the mouth parts of the insect. The larvae enter the wound in the skin and make their way to the respective tissue, where they mature into adult worms. The adults mate and produce live microfilariae, which, in lymphatic filariasis, migrate to the blood through the walls of the lymphatics or through the thoracic duct. To complete the life cycle, the microfilariae are ingested by blood-sucking insects, in which they undergo metamorphosis through two larval stages until they reach the third, infective stage. The interval from the infective bite to the appearance of microfilariae in the blood of the host can be 6 to 12 months.

Mass drug administration with ivermectin alone (for onchocerciasis), or ivermectin together with albendazole (for lymphatic filariasis), or diethylcarbamazine with albendazole (also for lymphatic filariasis) can reduce the microfilarial load of human populations living in endemic regions.[184] For onchocerciasis, reductions in microfilarial loads lead to reductions in chronic morbidity, with marked improvement in the serious skin manifestations of the infection and the prevention of blindness. For lymphatic filariasis, widespread implementation of mass drug administration one day may reduce transmission to the point of elimination. Such programs are currently under way in 10 countries of sub-Saharan Africa. In Egypt, five rounds of mass drug distribution already may have effectively eliminated transmission.[185] Mass drug administrations provide the core activities of several international public and private partnerships, including the Global Alliance to Eliminate Lymphatic Filariasis, the African Programme for Onchocerciasis Control, and the Onchocerciasis Elimination Program for the Americas.[181,182]

LYMPHATIC FILARIASIS: *WUCHERERIA BANCROFTI* AND *BRUGIA* SPECIES

Epidemiology

Wuchereria bancrofti is prevalent primarily between the Tropic of Cancer and the Tropic of Capricorn but also is encountered north of the Tropic of Cancer in Africa. An estimated 115 million infections exist in 80 countries worldwide, with approximately half of the infected individuals living in sub-Saharan Africa.[183,185,191] In each of its geographic locales, it has a specific anopheline, culicine, or aedine mosquito vector. In the Caribbean area, South America, Asia, East and West Africa, and Papua New Guinea, the microfilariae of this worm exhibit nocturnal periodicity; in the South Pacific, their periodicity is diurnal. *W. bancrofti* has no animal hosts.

Brugia malayi occurs in India, Malaysia, and other parts of Southeast Asia. Some strains of *B. malayi* are associated with animal reservoirs, as are certain other members of the genus *Brugia*, such as *B. timori*. In the United States, zoonotic Brugia infections caused by *B. beaveri* and *B. lepori* may infect humans but cannot develop patent infections.[182,189,190]

Pathophysiology

The pathology of lymphatic filariasis is caused principally by the adult-stage parasite, the host response, and bacterial superinfection of tissues with compromised lymphatic function. Adult worms induce lymphatic dilation that results in lymphatic dysfunction, lymphedema, and a greater susceptibility to bacterial infection. The consequent inflammation, plus that caused by host responses to dying parasites, damages the delicate lymphatic vessels further and compromises lymphatic function further. When such processes occur in the lymphatic vessels of the scrotum, hydrocele develops. Microfilaremia often is "asymptomatic" but frequently is associated with immune complex nephritis; more rarely, the microfilariae can be the target of immunologic hyperresponsiveness and result in a severe "tropical pulmonary eosinophilia" syndrome.[187]

Clinical Manifestations

Asymptomatic microfilaremia often develops in children living in endemic areas. By using highly sensitive diagnostic tests, including antigen detection and ultrasound ("dancing filarial sign"), one third of children living in endemic areas can be seen to be infected before age 5 years. Damage to the lymphatics of these young children frequently is subclinical, however.[192] With repeated exposure, these children begin to have episodes of acute adenolymphangitis, sometimes associated with fever and lymphangitis. These "filarial fevers" may reflect either an inflammatory response to dying parasites (with "retrograde" lymphangitis) or bacterial superinfection.[181] Progression to lymphedema of the upper and lower extremities, the most common sequela of chronic lymphatic filariasis, is an uncommon occurrence in children until after the age of puberty. Arthritis has also been noted as an occult manifestation of lymphatic filariasis in India.[186] Benign lymphedema has been described in a few patients in the United States with zoonotic *Brugia* infection.[182,190] Chronic, recurrent eosinophilic pneumonitis associated with wheezing, cough, chest pain,

pulmonary infiltrations, and hypereosinophilia (tropical pulmonary eosinophilia) is an IgE-mediated hypersensitivity reaction to microfilariae trapped in the lungs and, if left untreated, leads to debilitating interstitial pneumonitis.[187]

Diagnosis and Differential Diagnosis

The differential diagnosis of lymphatic obstruction in children should rule out other more likely conditions before focusing on filariasis. In older children and adults, familial lymphedema (Milroy disease) can mimic filariasis.

In endemic regions, lymphatic filariasis typically is diagnosed on the basis of appropriate clinical findings in a region with known endemicity for the disease. In young children living in endemic areas, however, early infection is largely subclinical.[192] A definitive diagnosis of lymphatic filariasis still relies on detecting adult parasites in the lymphatics through ultrasonography or by recovering microfilariae from the blood. In view of the circadian periodicity of the appearance of numerous microfilariae in blood, a specimen should be collected at the appropriate time, in many regions of the world between 10 PM and 2 AM, for microscopic examination by a staining or concentration technique. Increasingly, an immunochromatographic card test that measures specific filarial antigen is being used, especially in amicrofilaremic individuals.[181]

Treatment

New agents or drug regimens are needed that would be more effective against the adult filarial worm and at the same time elicit minimal immunopathologic damage. Currently, the major drugs used are especially effective against microfilariae. Diethylcarbamazine is the treatment of choice and typically is given in a 12-day course (6 mg/kg/day in three doses) for a total dose of 72 mg/kg. In the United States, for patients with microfilariae in the blood it is common practice to administer first small doses of 1 to 2 mg/kg (maximum of 50 to 100 mg) before beginning this regimen. One regimen includes beginning 50 mg on day 1, followed by 50 mg three times a day on day 2, 100 mg three times a day on day 3, and 6 mg/kg/d in three doses on days 4 to 14. More recently, a single dose of 6 mg/kg has been recognized to produce a similar therapeutic result (presumably following the test doses as just outlined).[181] Diethylcarbamazine is available under an investigational new drug protocol.*

Side effects of specific antifilarial therapy include allergic and febrile reactions that are caused by the inflammatory response to dying microfilariae rather than by the drug itself and that occur primarily in patients with high levels of circulating microfilariae. These systemic manifestations can be treated symptomatically with antihistamines or corticosteroids. Some of the allergic manifestations also may be avoided by administering diethylcarbamazine in a graded, stepwise manner. For patients with *Loa loa* coinfections, the presence of microfilariae presents a particular problem because high levels of microfilaremia can lead to encephalopathy after treatment with antiparasitic drugs. Diethylcarbamazine also is the treatment of choice for tropical pulmonary eosinophilia, a syndrome caused by circulating microfilariae. Ivermectin also may have a role in the medical treatment of lymphatic filariasis because it is very effective in clearing circulating microfilariae.[188] Albendazole, in high doses, is effective in killing adult worms and, in lower doses, inhibits microfilarial production, but it is not registered for use in lymphatic filariasis in the United States.[185]

The difficulty in management of filariasis by drugs is that late symptoms, such as elephantiasis, do not abate. The main usefulness of chemotherapy is in cases recognized early, before the anatomic abnormalities develop. Hydrocele can be treated surgically. Of critical importance to the management of lymphedema and elephantiasis are attention to hygiene, wearing shoes to prevent injury, and reduction of lymphostasis with exercise and elevation of the lower extremity.

The more recent discovery that *W. bancrofti* harbors bacterial endosymbionts has led to efforts to use tetracycline and related antimicrobial agents as part of the therapeutic regimen for lymphatic filariasis.[190] The therapeutic use of such regimens is under active investigation. As of this writing, the Centers for Disease Control and Prevention (see earlier discussion) and the Clinical Center of the National Institutes of Health (Bethesda, Md.) have extensive experience and expertise in the management and treatment of human filarial infections, including *W. bancrofti*, *L. loa*, and *Onchocerca volvulus* infections.

Prevention

Prevention in the past depended principally on vector control, which was unsatisfactory, primarily because of the difficulty in developing effective insecticides that also would be nontoxic to the rest of the environment. Successful experience in China has shown that large-scale chemoprophylaxis approaches to prevention are possible in endemic countries. Currently, through the Global Alliance to Eliminate Lymphatic Filariasis, the international community is directing a large-scale lymphatic filariasis control campaign on this principle. Typically, this control is being accomplished through single-dose combination of albendazole 400 mg with either ivermectin 200 µg/kg or diethylcarbamazine 6 mg/kg. On this basis, lymphatic filariasis has been eliminated in more than 20 countries and long-term targets for the global elimination of this infection have been proposed.[183-185] The finding of extensive subclinical infection among children living in endemic regions highlights the importance of targeting this group for MDA as well as adults.[192]

LOA LOA

L. loa infection is limited to a small area of western and central Africa and is spread by *Chrysops* flies. Periodicity

*From the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, Ga., 30333; 404-639-3670 (evenings, weekends, or holidays: 770-488-7100).

in *L. loa* microfilariae is diurnal. The parasite elicits numerous allergic inflammatory responses that are most evident in expatriates.[193,194] Infection leads to high eosinophilia and recurrent angioedema, which when localized develop into painful, pruritic, subcutaneous swellings on the extremities and the face known as Calabar swellings. Rarely, some cases of lymphatic obstruction of the lower extremities and hydroceles have been reported.

The most dramatic manifestation of this infection is the occasional appearance of a migrating *L. loa* adult under the conjunctiva of the eye. It does not damage the eye and can be removed surgically. Treatment with diethylcarbamazine, as indicated earlier, effectively destroys the adults, but reactions may be more intense than in the treatment of *Wuchereria* and *Brugia* infections. Diethylcarbamazine should be administered in a gradual, stepwise manner as described earlier, particularly in patients with high levels of circulating microfilariae. The recommended dose is higher than that used for lymphatic filariasis (9 mg/kg/d in three doses for 12 days). Coadministration of corticosteroids often is required during treatment. Encephalopathy has been described in patients receiving diethylcarbamazine, especially when heavily infected.[193] Ivermectin elicits fewer inflammatory symptoms during treatment and may be less toxic in general, but encephalopathy still can occur in patients with heavy *Loa* infections.[195] Because of the risk for encephalopathy, patients with loasis should be treated in consultation with an expert in this infection. Approaches to treatment may include plasmapheresis or a single 50-mg dose of diethylcarbamazine as for lymphatic filariasis (see earlier), gradually building to the final dose of 9 mg/kg/day over 2 weeks.

ONCHOCERCA VOLVULUS

Onchocerca volvulus infection is acquired through the bite of a *Simulium* fly, which tends to breed along rivers and streams (hence the name of the disease "river blindness").

Epidemiology

Disease is limited to sub-Saharan Africa, where approximately 99 percent of the world's 26 million cases occur (approximately half of the cases occur in Nigeria and Congo),[183] and focal pockets in Central America (primarily Guatemala and Chiapas State, Mexico), and the northern parts of South America (near the border between Venezuela and Brazil and in Ecuador). In view of human dependence on water and the establishment of settlements along rivers, the frequency of infection tends to be high in areas where *Simulium* prevails. The development of hydroelectric power based on the construction of large dams can increase the breeding sites of *Simulium* and increase the incidence of *Onchocerca* infection.

Pathophysiology

Larvae deposited by a *Simulium* bite remain in the subcutaneous tissue and develop into adult worms there. Adult worms tend to become coiled, and worms of both sexes become enveloped by fibrous tissue and form nodules within which they reproduce. The larvae produced by fertilized females invade the skin, where they remain until they are picked up by a *Simulium* bite, or they die about 30 months later. In addition to the skin, microfilariae penetrate the eye and affect every layer from the conjunctiva to the optic nerve. In African onchocerciasis, chorioretinitis and optic atrophy occur commonly; in the Central American disease, iritis is the primary lesion.

The probability of the development of eye disease is related to the location of the adult worms. When the nodules are situated around the head, eye lesions are common; when they are in the lower parts of the body, eye lesions occur less frequently. In Africa, the nodules tend to be distributed primarily in the lower parts of the body, but because of the high prevalence of the infection, onchocercal blindness is common. In Central America, the lesions tend to be on the upper part of the body.

Clinical Manifestations

The appearance of the skin nodules and the presence of live microfilariae within the eye (readily seen with a slit-lamp ophthalmoscopic examination) are the manifestations of early and intermediate disease. Later, the eye involvement includes keratitis, iridocyclitis, chorioretinitis, and, eventually, blindness. Microfilariae in the skin cause an inflammatory reaction that includes acute papular onchodermatitis and chronic changes such as edema, lichenification, atrophy, and depigmentation.[191]

Diagnosis and Differential Diagnosis

Because the skin invasion is associated with itching, the pruritus of onchocercal infection must be differentiated from contact dermatitis, prickly heat, insect bites, and scabies. Onchocerciasis is identified by examination of a skin snip. Examination of sectioned and stained tissue or a stained impression smear reveals microfilariae.

Treatment

Surgical removal of all visible nodules may radically extirpate the source of new microfilariae that invade the eye. The routine use of nodulectomy is still controversial, however. Chemotherapy with ivermectin (single oral dose of 150 μg/kg administered every 6 to 12 months) until asymptomatic is the treatment of choice. Ivermectin reduces the number of microfilariae in the skin within days, and subsequently the number of microfilariae in the eye, and prevents the onset of blindness.[196] Ivermectin also can be used in mass treatment to diminish transmission by the vector and control the incidence of this infection.[183,184,196,197] Diethylcarbamazine is contraindicated for patients with onchocerciasis because it can lead to severe allergic symptoms, a condition sometimes known as the Mazzotti reaction.

Prevention

Today the mainstay of river blindness control is MDA with ivermectin, which has recently led to the elimination

of onchocerciasis in Mali and Senegal and will soon lead to elimination of the disease in the six remaining Latin American countries.[183]

MANSONELLA PERSTANS AND *MANSONELLA OZZARDI*

Neither *Mansonella perstans* nor *Mansonella ozzardi* is known to cause significant human pathology, but the microfilariae present in blood must be distinguished morphologically from the microfilariae of the other more pathogenic filariae. *M. ozzardi* is found throughout the Caribbean (especially Haiti) and Central America. It has been suggested as a cause of chronic arthritis in these regions. *M. perstans* also is found in Africa, where it has been identified as a cause of painless nodules in the conjunctiva and secondary eyelid swelling. For that reason, it sometimes is called the Kampala or Ugandan eye worm.[198] Albendazole or mebendazole is the treatment of choice for *M. perstans* infection.

DIROFILARIA IMMITIS

Dirofilaria immitis is a filarial worm commonly found in dogs, in which it occupies the right ventricle of the heart. The microfilariae produced circulate in blood and are transmitted to new animals through the bite of culicine mosquitoes. Fewer than 100 cases of human infection have been reported, none of them in children. Most human hosts infected with *Dirofilaria* were asymptomatic, but individuals who had symptoms complained of chest pain, wheezing, and cough. All infected individuals had coin lesions detected on pulmonary radiographs.[199,200] Human infection is transmitted through a mosquito bite. As with visceral larva migrans and the zoonotic *Brugia* infections, *D. immitis* cannot complete its life cycle in humans. No microfilariae of this worm have ever been shown in human peripheral blood.

All patients evaluated for pulmonary dirofilariasis have had mild peripheral eosinophilia, usually not exceeding 10 percent. Because the radiographic picture is not diagnostic, and in view of the potential seriousness of a coin lesion,[199] the lesion must be examined histologically. If a worm is found, the diagnosis of dirofilariasis can be made; if it is not found, the diagnosis is still tenable. In the presence of eosinophilia and pneumonitis, however, a whole range of other diagnostic possibilities must be considered, including eosinophilic pneumonia, polyarteritis nodosa, Wegener granulomatosis, and histiocytosis X. No treatment is necessary for this infection in humans.

DRACUNCULUS MEDINENSIS

Infection by *Dracunculus medinensis* (guinea worm) is limited largely to sub-Saharan Africa. As of this writing in 2011, only 1,008 cases are believed to remain worldwide, with almost all of the cases in Sudan or South Sudan. Ten or fewer cases remain in Mali, Chad, and Ethiopia, respectively.[201]

Because filtering of drinking water can prevent this infection effectively, authorities from the World Health Organization and the Carter Center are optimistic that this parasite will soon be eradicated through appropriate control measures.[183]

NEW REFERENCES SINCE THE SIXTH EDITION

Intestinal Nematodes

1. Albonico M, Allen H, Chitsulo L, et al. Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis* 2008;**26**:e126.
4. Brooker S, Hotez PJ, Bundy DA. The global atlas of helminth infection: mapping the way forward in neglected tropical disease control. *PLoS Negl Trop Dis* 2010;**4**:e779.
8. Hotez PJ. Neglected infections of poverty in the United States of America. *PLoS Negl Trop Dis* 2008;**2**:e256.
9. Hotez PJ. A plan to defeat neglected tropical diseases. *Sci Am* 2010;**302**:90–4.
10. Hotez PJ, Bethony JM, Diemert DJ, et al. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* 2010;**8**:814–26.
11. Hotez PJ, Fenwick A, Savioli L, et al. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet* 2009;**373**:1570–5.
12. Hotez PJ, Gurwith M. Europe's neglected infections of poverty. *Int J Infect Dis* 2011;**15**:e611–19.
13. Hotez PJ, Mistry N, Rubinstein J, et al. Integrating neglected tropical diseases into AIDS, tuberculosis, and malaria control. *N Engl J Med* 2011;**364**:2086–9.
16. Hotez PJ, Wilkins PP. Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance. *PLoS Negl Trop Dis* 2009;**3**:e400.
17. Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: a systematic review and meta-analysis. *JAMA* 2008;**299**:1937–48.
18. Kirwan P, Jackson AL, Asaolu SO, et al. Impact of repeated four-monthly anthelmintic treatment on *Plasmodium* infection in pre-school children: a double-blind placebo-controlled randomized trial. *BMC Infec Dis* 2010;**10**:277.
23. Starr MC, Montgomery SP. Soil-transmitted helminthiasis in the United States: a systematic review—1940-2010. *Am J Trop Med Hyg* 2011;**85**:680–4.

Ascaris lumbricoides

34. Dold C, Holland CV. *Ascaris* and ascariasis. *Microbes Infect* 2010;**13**:632–7.
45. Nikolic H, Palcevski G, Saina G, et al. Chronic intussusception in children caused by *Ascaris lumbricoides*. *Wien Klin Wochenschr* 2011;**123**:294–6.
46. Reina Ortiz M, Schreiber F, Benitez S, et al. Effects of chronic ascariasis and trichuriasis on cytokine production and gene expression in human blood: a cross-sectional study. *PLoS Negl Trop Dis* 2011;**5**:e1157.

Trichuris trichiura

50. Baldisserotto M. Trichuriasis colitis detected by Doppler sonography. *Pediatr Radiol* 2010;**40**(Suppl. 1):S95–7.
56. Khuroo MS, Khuroo MS, Khuroo NS. *Trichuris* dysentery syndrome: a common cause of chronic iron deficiency anemia in adults in an endemic area (with videos). *Gastrointest Endosc* 2009;**71**:200–4.
57. Knopp S, Mohammed KA, Speich B, et al. Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura:* a randomized controlled trial. *Clin Infect Dis* 2010;**51**:1420–8.
62. Namwanje H, Kabatereine NB, Olsen A. Efficacy of single and double doses of albendazole and mebendazole alone and in combination in the treatment of *Trichuris trichiura* in school-age children in Uganda. *Trans R Soc Trop Med Hyg* 2011;**105**:586–90.

63. Ok KS, Kim YS, Song JH, et al. *Trichuris trichiura* infection diagnosed by colonoscopy: case reports and review of literature. *Korean J Parasitol* 2009;**47**:275–80.

Hookworms

66. Becker SL, Sieto B, Silue KD. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis* 2011;**5**:e1292.
67. Bhatia V, Das MK, Kumar P, et al. Infantile hookworm disease. *Indian Pediatr* 2010;**47**:190–2.
70. Brooker S, Hotez PJ, Bundy DA. Hookworm-related anaemia among pregnant women: a systematic review. *PLoS Negl Trop Dis* 2008;**2**:e291.
71. Budhathoki S, Shah D, Bhurtyal KK. Hookworm causing melaena and severe anaemia in early infancy. *Ann Trop Paediatr* 2008;**28**:293–6.
77. Kalli T, Karamanolis G, Triantaafyllou K. Hookworm infection detected by capsule endoscopy in a young man with iron deficiency. *Clin Gastroenterol Hepatol* 2011;**9**:e33.
79. Ranjit N, Zhan B, Hamilton B, et al. Proteolytic degradation of hemoglobin in the intestine of the human hookworm *Necator americanus*. *J Infect Dis* 2009;**199**:904–12.
83. Smith JL, Brooker S. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. *Trop Med Int Health* 2010;**15**:776–95.
85. Yajima A, Gabrielli AF, Montresor A, et al. Moderate and high endemicity of schistosomiasis is a predictor of the endemicity of soil-transmitted helminthiasis: a systematic review. *Trans R Soc Trop Med Hyg* 2011;**105**:68–73.

Enterobius Vermicularis

87. Abdolrasouli A, Hart J. Oral-anal intercourse and sexual transmission of *Enterobius vermicularis*; do we need to screen for other intestinal parasites. *Int J STD AIDS* 2009;**20**:739.
88. Ariyarathenam AV, Nachimuthu S, Tang TY, et al. *Enterobius vermicularis* infestation of the appendix and management at the time of laparoscopic appendectomy: case series and literature review. *Int J Surg* 2010;**8**:466–9.
90. Cateau E, Yacoub M, Tavillien C, et al. *Enterobius vermicularis* in the kidney: an unusual location. *J Med Microbiol* 2010;**59**:860–1.
91. Konanahalli P, Menon P, Walsh MY, et al. *Enterobius vermicularis* (pinworm) infestation of the vulva: report of 2 cases of a pseudoneoplastic lesion mimicking squamous carcinoma. *Int J Gynecol Pathol* 2010;**29**:490–3.
95. Shelat VG, Ng SR, Sim R. Perianal abscess secondary to *Enterobius vermicularis* infestation. *Ann Acad Med Singapore* 2011;**40**:149.
99. Young C, Tataryn I, Kowalewska-Grochowska KT, et al. *Enterobius vermicularis* infection of the fallopian tube in an infertile female. *Pathol Res Pract* 2010;**206**:405–7.

Strongyloides stercoralis and *Strongyloides fuelleborni*

102. Bisoffi Z, Buonfrate D, Angheben A, et al. Randomized clinical trial of ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS Negl Trop Dis* 2011;**5**:e1254.
108. Copelovitch L, Sam OI O, Taraquinio S, et al. Childhood nephrotic syndrome in Cambodia: an association with gastrointestinal parasites. *J Pediatr* 2010;**156**:76–81.
109. Croker C, Reporter R, Redelings M, et al. Strongyloidiasis-related deaths in the United States 1991-2006. *Am J Trop Med Hyg* 2010;**83**:422–6.
111. Dada-Adegbola HO, Oluwatoba OA, et al. Strongyloidiasis: prevalence, risk factors, clinical and laboratory features among diarrhea patients in Ibadan Nigeria. *Afr J Med Med Sci* 2010;**39**:285–92.
112. Dawson-Hahn EE, Greenberg SL, Domachowske JB, et al. Eosinophilia and the seroprevalence of schistosomiasis and strongyloidiasis in newly arrived pediatric refugees: an examination of Centers for Disease Control and Prevention screening guidelines. *J Pediatr* 2010;**156**:1016–18.
123. Olsen A, van Lieshout L, Marti H, Polderman T, et al. The most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg* 2009;**103**:967–72.
130. Suputtamongkol Y, Premasathian N, Bhumimuang K, et al. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS Negl Trop Dis* 2011;**5**:e1044.

Toxocara canis

135. Centers for Disease Control and Prevention (CDC). Ocular toxocariasis—United States, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:734–6.
137. Colli CM, Rubinsky-Elefant G, Paludo ML, et al. Serological, clinical and epidemiological evaluation of toxocariasis in urban areas of south Brazil. *Rev Inst Med Trop Sao Paulo* 2010;**52**:69–74.
146. Jones JL, Kruszon-Moran D, Won K, et al. *Toxoplasma gondii* and Toxocara spp. co-infection. *Am J Trop Med Hyg* 2008;**78**:35–9.
155. Won KY, Kruszon-Moran D, Schantz PM, et al. National seroprevalence and risk factors for zoonotic *Toxocara* spp. infection. *Am J Trop Med Hyg* 2008;**79**:552–7.

Other Aberrant Infections with Intestinal Nematodes

161. Diaz JH. Helminthic eosinophilic meningitis: emerging zoonotic diseases in the South. *J La State Med Soc* 2008;**160**:333–42.
164. Graeff-Teixeira C, Aramburu da Silva AC, Yoshimura K. Update on eosinophilic meningoenceophalitis and its clinical relevance. *Clin Microbiol Rev* 2009;**22**:322–48.

Filarial Parasites

183. Hotez PJ. Enlarging the "audacious goal": elimination of the world's high prevalence neglected tropical diseases. *Vaccine* 2011;**29**(Suppl. 4):D104–10.
191. Taylor MJ, Hoerauf A, Bockarie M. Lymphatic filariasis and onchocerciasis. *Lancet* 2010;**376**:1175–85.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

Intestinal Nematodes

1. Albonico M, Allen H, Chitsulo L, et al. Controlling soil-transmitted helminthiasis in pre-school-age children through preventive chemotherapy. *PLoS Negl Trop Dis* 2008;**26**:e126.
2. Albonico M, Smith PG, Ercole E, et al. Rate of reinfection with intestinal nematodes after treatment of children with mebendazole or albendazole in a highly endemic area. *Trans R Soc Trop Med Hyg* 1995;**89**:538–41.
3. Bethony J, Brooker S, Albonico M, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *Lancet* 2006;**367**:1521–32.
4. Brooker S, Hotez PJ, Bundy DA. The global atlas of helminth infection: mapping the way forward in neglected tropical disease control. *PLoS Negl Trop Dis* 2010;**4**:e779.
5. Bundy DAP. New initiatives in the control of helminths. *Trans R Soc Trop Med Hyg* 1990;**84**:467–8.
6. Drake LJ, Jukes MCH, Sternberg RJ, et al. Geohelminth infections (ascariasis, trichuriasis, and hookworm): Cognitive and developmental impacts. *Semin Pediatr Infect Dis* 2000;**11**:245–51.
7. Hotez PJ. Pediatric geohelminth infections: Trichuriasis, ascariasis, and hookworm infections. *Semin Pediatr Infect Dis* 2000;**11**: 236–44.
8. Hotez PJ. Neglected infections of poverty in the United States of America. *PLoS Negl Trop Dis* 2008;**2**:e256.
9. Hotez PJ. A plan to defeat neglected tropical diseases. *Sci Am* 2010;**302**:90–4.
10. Hotez PJ, Bethony JM, Diemert DJ, et al. Developing vaccines to combat hookworm infection and intestinal schistosomiasis. *Nat Rev Microbiol* 2010;**8**:814–26.
11. Hotez PJ, Fenwick A, Savioli L, et al. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet* 2009;**373**: 1570–5.
12. Hotez PJ, Gurwith M. Europe's neglected infections of poverty. *Int J Infect Dis* 2011;**15**:e611–19.
13. Hotez PJ, Mistry N, Rubinstein J, et al. Integrating neglected tropical diseases into AIDS, tuberculosis, and malaria control. *N Engl J Med* 2011;**364**:2086–9.
14. Hotez PJ, Molyneux DH, Fenwick A, et al. Incorporating a rapid-impact package for neglected tropical diseases with programs for HIV/AIDS, tuberculosis, and malaria. *PLoS Med* 2006;**3**:e102.
15. Hotez PJ, Molyneux DH, Fenwick A. Control of neglected tropical diseases. *N Engl J Med* 2007;**357**:1018–27.
16. Hotez PJ, Wilkins PP. Toxocariasis: America's most common neglected infection of poverty and a helminthiasis of global importance. *PLoS Negl Trop Dis* 2009;**3**:e400.
17. Keiser J, Utzinger J. Efficacy of current drugs against soil-transmitted helminth infections: a systematic review and meta-analysis. *JAMA* 2008;**299**:1937–48.
18. Kirwan P, Jackson AL, Asaolu SO, et al. Impact of repeated four-monthly anthelmintic treatment on *Plasmodium* infection in pre-school children: a double-blind placebo-controlled randomized trial. *BMC Infec Dis* 2010;**10**:277.
19. Nokes C, Cooper ES, Robinson BA, et al. Geohelminth infection and academic assessment in Jamaican children. *Trans R Soc Trop Med Hyg* 1991;**85**:272–3.
20. Savioli L, Bundy D, Tomkins A. Intestinal parasitic infections: a soluble public health problem. *Trans R Soc Trop Med Hyg* 1992; **86**:353–4.
21. Smillie WG, Augustine DL. Hookworm infestation: the effect of varying intensities on the physical condition of school children. *Am J Dis Child* 1926;**31**:151–68.
22. Smillie WG, Spencer CR. Mental retardation in school children infested with hookworms. *J Educ Psychol* 1926;**17**:314–21.
23. Starr MC, Montgomery SP. Soil-transmitted helminthiasis in the United States: a systematic review—1940-2010. *Am J Trop Med Hyg* 2011;**85**:680–4.
24. Stephenson LS. Helminth parasites, a major factor in malnutrition. *World Health Forum* 1994;**15**:169–72.
25. Stephenson LS, Latham MC, Kinoti SN, et al. Improvements in physical fitness of Kenyan schoolboys infected with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* following a single dose of albendazole. *Trans R Soc Trop Med Hyg* 1990;**84**:277–82.
26. Stephenson LS, Latham MC, Kurz KM, et al. Treatment with a single dose of albendazole improves growth of Kenyan schoolchildren with hookworm, *Trichuris trichiura*, and *Ascaris lumbricoides* infections. *Am J Trop Med Hyg* 1989;**41**:78–87.

Ascaris lumbricoides

27. Anderson TJC. *Ascaris* infections in humans from North America: molecular evidence for cross-infection. *Parasitology* 1995;**110**: 215–19.
28. Blumenthal DS, Schultz MG. Incidence of intestinal obstruction in children infected with *Ascaris lumbricoides*. *Am J Trop Med Hyg* 1975;**24**:801–5.
29. Chu WG, Chen PM, Huang CC, et al. Neonatal ascariasis. *J Pediatr* 1972;**81**:783–5.
30. Costa-Macedo LM, Rey L. *Ascaris lumbricoides* in neonate: evidence of congenital transmission of intestinal nematodes. *Rev Inst Med Trop São Paulo* 1990;**32**:351–4.
31. Crompton DWT. Ascaris and ascariasis. *Adv Parasitol* 2001;**48**: 285–375.
32. Deehan MR, Goodridge HS, Blair D, et al. Immunomodulatory properties of *Ascaris suum* glycosphingolipids: phosphorylcholine and non–phosphorylcholine-dependent effects. *Parasite Immunol* 2002;**24**:463–9.
33. DeSilva NR, Chan MS, Bundy DA. Morbidity and mortality due to ascariasis: Re-estimation and sensitivity analysis of global numbers at risk. *Trop Med Int Health* 1997;**2**:519–28.
34. Dold C, Holland CV. *Ascaris* and ascariasis. *Microbes Infect* 2010; **13**:632–7.
35. Goldberg DE. Oxygen avid hemoglobin of ascariasis. *Chem Rev* 1999;**99**:3371–8.
36. Grasberger BL, Clore GM, Gronenborn AM. High resolution structure of *Ascaris* trypsin inhibitor in solution: direct evidence for a pH-induced conformational transition in the reactive site. *Structure* 1994;**2**:669–78.
37. Hall A, Romanova T. *Ascaris lumbricoides*: detecting its metabolites in the urine of the infected people using gas-liquid chromatography. *Exp Parasitol* 1990;**70**:35–42.
38. Haswell-Elkins MR, Kennedy MW, Maizels RM, et al. Detection of *Ascaris* metabolites in the urine by gas-liquid chromatography also correlates with the worm burden. *Parasite Immunol* 1989;**11**: 615–27.
39. Huang K, Strynadka NC, Bernard VD, et al. The molecular structure of the complex of *Ascaris* chymotrypsin/elastase inhibitor with porcine elastase. *Structure* 1994;**2**:679–89.
40. Khuroo MS, Zargar SA, Mahajan R. Hepatobiliary and pancreatic ascariasis in India. *Lancet* 1990;**335**:1503–6.
41. Koino S. Experimental infections on human body with ascarides. *Jpn Med World* 1922;**2**:317–20.
42. Lynch NR, Hagel IA, Palenque ME, et al. Relationship between helminthic infection and IgE response in atopic and nonatopic children in a tropical environment. *J Allergy Clin Immunol* 1998; **101**:217–21.
43. McSharry C, Xia Y, Holland CV, et al. Natural immunity to *Ascaris lumbricoides* associated with immunoglobulin E antibody to ABA-1 allergen and inflammation indicators in children. *Infect Immun* 1999;**67**:484–9.
44. Montresor A, Awasthi S, Crompton DW. Use of benzimidazoles in children younger than 24 months for the treatment of soil-transmitted helminthiases. *Acta Trop* 2003;**86**:221–32.
45. Nikolic H, Palcevski G, Saina G, et al. Chronic intussusception in children caused by *Ascaris lumbricoides*. *Wien Klin Wochenschr* 2011;**123**:294–6.
46. Reina Ortiz M, Schreiber F, Benitez S, et al. Effects of chronic ascariasis and trichuriasis on cytokine production and gene expression in human blood: a cross-sectional study. *PLoS Negl Trop Dis* 2011;**5**:e1157.
47. Tankhiwalle SR, Kukade AL, Sarmah HC, et al. Single dose therapy of ascariasis: a randomized comparison of mebendazole and pyrantel. *J Commun Dis* 1989;**21**:71–4.
48. Villar J, Klebanoff M, Kestler E. The effect on fetal growth of protozoan and helminthic infection during pregnancy. *Obstet Gynecol* 1989;**74**:915–29.
49. Xu L, Jian Z, Yu S, et al. Nationwide survey of the distribution of human parasites in China: infection with parasite species in human population. *Chin J Parasitol Parasitic Dis* 1995;**13**:1–7.

Trichuris trichiura

50. Baldisserotto M. Trichuriasis colitis detected by Doppler sonography. *Pediatr Radiol* 2010;**40**(Suppl. 1):S95–7.
51. Bundy DAP, Cooper ES. *Trichuris* and trichuriasis in humans. *Adv Parasitol* 1989;**28**:107–73.
52. Cooper ES, Bundy D. *Trichuris* is not trivial. *Parasitol Today* 1988;**4**:301–5.
53. Cooper ES, Bundy D, MacDonald T, et al. Growth suppression in the *Trichuris* dysentery syndrome. *Eur J Clin Nutr* 1990;**44**: 285–91.
54. Drake L, Korchev Y, Bashford L, et al. The major secreted product of the whipworm *Trichuris* is a pore-forming protein. *Proc R Soc Lond B* 1994;**257**:255–61.
55. Else KJ, Finkelman FD, Maliszewski CR, et al. Cytokine-mediated regulation of chronic intestinal helminth infection. *J Exp Med* 1994;**179**:347–51.
56. Khuroo MS, Khuroo MS, Khuroo NS. *Trichuris* dysentery syndrome: a common cause of chronic iron deficiency anemia in adults in an endemic area (with videos). *Gastrointest Endosc* 2009;**71**: 200–4.
57. Knopp S, Mohammed KA, Speich B, et al. Albendazole and mebendazole administered alone or in combination with ivermectin against *Trichuris trichiura:* a randomized controlled trial. *Clin Infect Dis* 2010;**51**:1420–8.
58. Layrisse M, Aparcedo L, Martinez Torres C, et al. Blood loss due to infection with *Trichuris trichiura*. *Am J Trop Med Hyg* 1967;**16**:613–16.
59. Lotero M, Tripathy K, Bolanos O. Gastrointestinal blood loss in *Trichuris* infection. *Am J Trop Med Hyg* 1974;**23**:1203–7.
60. MacDonald TT, Choy MY, Spencer J, et al. Histopathology of the caecum in children with *Trichuris* dysentery syndrome. *J Clin Pathol* 1991;**44**:194–9.
61. Maqbool S, Lawrence D, Katz M. Treatment of trichuriasis with a new drug, mebendazole. *J Pediatr* 1975;**86**:463–5.
62. Namwanje H, Kabatereine NB, Olsen A. Efficacy of single and double doses of albendazole and mebendazole alone and in combination in the treatment of *Trichuris trichiura* in school-age children in Uganda. *Trans R Soc Trop Med Hyg* 2011;**105**:586–90.
63. Ok KS, Kim YS, Song JH, et al. *Trichuris trichiura* infection diagnosed by colonoscopy: case reports and review of literature. *Korean J Parasitol* 2009;**47**:275–80.
64. Simeon DT, Grantham-McGregor SM, Wong MS. *Trichuris trichiura* infection and cognition in children: results of a randomized clinical trial. *Parasitology* 1995;**110**:457–64.
65. Wakelin D, Gelby RG. Immune expulsion of *Trichuris muris* from resistant mice: suppression by irradiation and restoration by transfer of lymphoid cells. *Parasitology* 1976;**72**:41–50.

Hookworms

66. Becker SL, Sieto B, Silue KD. Diagnosis, clinical features, and self-reported morbidity of *Strongyloides stercoralis* and hookworm infection in a co-endemic setting. *PLoS Negl Trop Dis* 2011;**5**: e1292.
67. Bhatia V, Das MK, Kumar P, et al. Infantile hookworm disease. *Indian Pediatr* 2010;**47**:190–2.
68. Brooker S, Akhwale W, Pullan R, et al. Epidemiology of *Plasmodium*-helminth coinfection in Africa: populations at risk, potential impact on anemia and prospects for combining control. *Am J Trop Med Hyg* 2007;**77**(6 Suppl):88–96.
69. Brooker S, Clements AC, Hotez PJ. The co-distribution of *Plasmodium falciparum* and hookworm among African schoolchildren. *Malaria J* 2006;**5**:99.
70. Brooker S, Hotez PJ, Bundy DA. Hookworm-related anaemia among pregnant women: a systematic review. *PLoS Negl Trop Dis* 2008;**2**:e291.
71. Budhathoki S, Shah D, Bhurtyal KK. Hookworm causing melaena and severe anaemia in early infancy. *Ann Trop Paediatr* 2008;**28**: 293–6.
72. Cappello M, Vlasuk GP, Bergum PW, et al. *Ancylostoma caninum* anticoagulant peptide (AcAP): a novel hookworm-derived inhibitor of human coagulation factor Xa. *Proc Natl Acad Sci U S A* 1995;**92**:6152–6.
73. Crompton DW. The public health importance of hookworm disease. *Parasitology* 2000;**121**(Suppl):S39–50.
74. Gandhi NS, Jizhang C, Khoshnood K, et al. Epidemiology of *Necator americanus* hookworm infections in Xiulongkan Village, Hainan Province, China: high prevalence and intensity among middle-aged and elderly residents. *J Parasitol* 2001;**87**:739–43.
75. Hotez PJ. Hookworm disease in children. *Pediatr Infect Dis J* 1989;**8**:516–20.
76. Hotez PJ, Brooker S, Bethony JM, et al. Hookworm infection. *N Engl J Med* 2004;**351**:799–807.
77. Kalli T, Karamanolis G, Triantaafyllou K. Hookworm infection detected by capsule endoscopy in a young man with iron deficiency. *Clin Gastroenterol Hepatol* 2011;**9**:e33.
78. Prociv P, Croese J. Human eosinophilic enteritis caused by dog hookworm, *Ancylostoma caninum*. *Lancet* 1990;**335**:1299–302.
79. Ranjit N, Zhan B, Hamilton B, et al. Proteolytic degradation of hemoglobin in the intestine of the human hookworm *Necator americanus*. *J Infect Dis* 2009;**199**:904–12.
80. Saraya AK, Tandon BN. Hookworm anemia and intestinal malabsorption associated with hookworm infestation. *Prog Drug Res* 1975;**19**:108–18.
81. Schad GA. Hypobiosis and related phenomena in hookworm infection. In: Warren KS, Schad GA, editors. *Hookworm disease, current status and future directions*. London: Taylor & Francis; 1990. p. 71–88.
82. Schad GA, Anderson RM. Predisposition to hookworm infection. *Science* 1985;**228**:1537–40.
83. Smith JL, Brooker S. Impact of hookworm infection and deworming on anaemia in non-pregnant populations: a systematic review. *Trop Med Int Health* 2010;**15**:776–95.
84. Williamson AL, Lecchi P, Turk BE, et al. A multi-enzyme cascade of hemoglobin proteolysis in the intestine of blood-feeding hookworms. *J Biol Chem* 2004;**279**:3590–7.
85. Yajima A, Gabrielli AF, Montresor A, et al. Moderate and high endemicity of schistosomiasis is a predictor of the endemicity of soil-transmitted helminthiasis: a systematic review. *Trans R Soc Trop Med Hyg* 2011;**105**:68–73.
86. Zhan B, Hotez PJ, Wng Y, et al. A developmentally regulated metalloprotease secreted by host-stimulated *Ancylostoma caninum* third-stage infective larvae is a member of the astacin family of proteases. *Mol Biochem Parasitol* 2002;**120**:291–6.

Enterobius vermicularis

87. Abdolrasouli A, Hart J. Oral-anal intercourse and sexual transmission of *Enterobius vermicularis;* do we need to screen for other intestinal parasites. *Int J STD AIDS* 2009;**20**:739.
88. Ariyarathenam AV, Nachimuthu S, Tang TY, et al. *Enterobius vermicularis* infestation of the appendix and management at the time of laparoscopic appendectomy: case series and literature review. *Int J Surg* 2010;**8**:466–9.
89. Bhaskaran CS, Devi ES, Rao KV. *Enterobius vermicularis* and vermiform appendix. *J Indian Med Assoc* 1975;**64**:334–6.
90. Cateau E, Yacoub M, Tavillien C, et al. *Enterobius vermicularis* in the kidney: an unusual location. *J Med Microbiol* 2010;**59**: 860–1.
91. Konanahalli P, Menon P, Walsh MY, et al. *Enterobius vermicularis* (pinworm) infestation of the vulva: report of 2 cases of a pseudoneoplastic lesion mimicking squamous carcinoma. *Int J Gynecol Pathol* 2010;**29**:490–3.
92. Liu LX, Chi J, Upton MP, et al. Eosinophilic colitis associated with larvae of the pinworm *Enterobius vermicularis*. *Lancet* 1995; **346**:410–12.
93. Mattia AR. Perianal mass and recurrent and cellulitis due to *Enterobius vermicularis*. *Am J Trop Med Hyg* 1992;**47**:811–15.
94. Mondou EN, Gnepp DR. Hepatic granuloma resulting from *Enterobius vermicularis*. *Am J Clin Pathol* 1989;**91**:97–100.
95. Shelat VG, Ng SR, Sim R. Perianal abscess secondary to *Enterobius vermicularis* infestation. *Ann Acad Med Singapore* 2011;**40**: 149.
96. Simon RD. Pinworm infestation and urinary tract infection in young girls. *Am J Dis Child* 1974;**128**:21–2.
97. Verumund SH. Pinworm *(Enterobius vermicularis)*. *Semin Pediatr Infect Dis* 2000;**11**:252–6.
98. Weller TH, Gorenson CW. Enterobiasis: its incidence and symptomatology in a group of 505 children. *N Engl J Med* 1941;**224**: 143–6.
99. Young C, Tataryn I, Kowalewska-Grochowska KT, et al. *Enterobius vermicularis* infection of the fallopian tube in an infertile female. *Pathol Res Pract* 2010;**206**:405–7.

Strongyloides stercoralis and Strongyloides fuelleborni

100. Ashford RW, Vince JD, Gratten MJ, et al. *Strongyloides* infection associated with acute infantile disease in Papua New Guinea. *Trans R Soc Trop Med Hyg* 1978;**72**:554.
101. Barnish G, Ashford RW. *Strongyloides* cf. *fuelleborni* and hookworm in Papua New Guinea: patterns of infection within the community. *Trans R Soc Trop Med Hyg* 1989;**83**:684–9.
102. Bisoffi Z, Buonfrate D, Angheben A, et al. Randomized clinical trial on ivermectin versus thiabendazole for the treatment of strongyloidiasis. *PLoS Negl Trop Dis* 2011;**5**:e1254.
103. Brindley PJ, Gam AA, McKerrow JH, et al. Ss40: The zinc endopeptidase secreted by infective larvae of *Strongyloides stercoralis*. *Exp Parasitol* 1995;**80**:1–7.
104. Brown HW, Perna VP. An overwhelming *Strongyloides* infection. *JAMA* 1958;**168**:1648–51.
105. Brown RC, Girardeau MHF. Transmammary passage of *Strongyloides* sp. larvae in the human host. *Am J Trop Med Hyg* 1977;**26**: 215–19.
106. Burke JA. Strongyloidiasis in childhood. *Am J Dis Child* 1978; **132**:1130–6.
107. Campbell WC, Cuckler A. Thiabendazole in the treatment and control of parasitic infection in man. *Tex Rep Biol Med* 1964; **27**:665–92.
108. Copelovitch L, Sam OI O, Taraquinio S, et al. Childhood nephrotic syndrome in Cambodia: an association with gastrointestinal parasites. *J Pediatr* 2010;**156**:76–81.
109. Croker C, Reporter R, Redelings M, et al. Strongyloidiasis-related deaths in the United States 1991-2006. *Am J Trop Med Hyg* 2010; **83**:422–6.
110. Dada-Adegbola HO, Bakare RA. Strongyloidiasis in children five years and below. *West Afr J Med* 2004;**23**:194–7.
111. Dada-Adegbola HO, Oluwatoba OA, Bakare RA. Strongyloidiasis: prevalence, risk factors, clinical and laboratory features among diarrhea patients in Ibadan Nigeria. *Afr J Med Med Sci* 2010; **39**:285–92.
112. Dawson-Hahn EE, Greenberg SL, Domachowske JB, et al. Eosinophilia and the seroprevalence of schistosomiasis and strongyloidiasis in newly arrived pediatric refugees: an examination of Centers for Disease Control and Prevention screening guidelines. *J Pediatr* 2010;**156**:1016–18.
113. Dixon AC, Yanaghihara ET, Kwock DW, et al. Strongyloidiasis associated with human T-cell lymphotropic virus type 1 infection in a nonendemic area. *West J Med* 1989;**151**:410–13.
114. Genta RM. Global prevalence of strongyloidiasis: critical review with epidemiologic insights into the prevention of disseminated disease. *Rev Infect Dis* 1989;**11**:755–67.
115. Genta RM. Dysregulation of strongyloidiasis: a new hypothesis. *Clin Microbiol Rev* 1992;**5**:345–55.
116. Genta RM, Miles P, Fields K. Opportunistic *Strongyloides stercoralis* infection in lymphoma patients: report of a case and review of the literature. *Cancer* 1989;**63**:1407–11.
117. Gill GV, Bell DR. Longstanding tropical infections amongst former war prisoners of the Japanese. *Lancet* 1982;**1**:958–9.
118. Gyorkos TW, Genta RM, Viens P, et al. Seroepidemiology of *Strongyloides* infection in the southeast Asian refugee population in Canada. *Am J Epidemiol* 1990;**132**:257–64.
119. Keiser PB, Nutman T. *Strongyloides stercoralis* in the immunocompromised population. *Clin Microbiol Rev* 2004;**17**:208–17.
120. King SE, Mascie-Taylor CG. *Strongyloides fuelleborni kellyi* and other intestinal helminths in children from Papua New Guinea: associations with nutritional status and socioeconomic factors. *PNG Med J* 2004;**47**:181–91.
121. Marty FM, Lowry CM, Rodriguez M, et al. Treatment of human disseminated strongyloidiasis with a parenteral veterinary formulation of ivermectin. *Clin Infect Dis* 2005;**41**:e5–8.
122. McKerrow JH, Brindley P, Brown M, et al. *Strongyloides stercoralis*: identification of a protease that facilitates penetration of skin by the infective larvae. *Exp Parasitol* 1990;**70**:134–43.
123. Olsen A, van Lieshout L, Marti H, et al. The most neglected of the neglected tropical diseases? *Trans R Soc Trop Med Hyg* 2009;**103**:967–72.
124. Paornusuriyasak P, Niticharoenpong K, Sakapibunnan A. Disseminated strongyloidiasis successfully treated with extended duration ivermectin combined with albendazole: a case report of intractable strongyloidiasis. *Southeast Asian J Trop Med Public Health* 2004;**35**:531–4.
125. Purtillo DT, Meyers WM, Connor DH. Fatal strongyloidiasis in immunosuppressed patients. *Am J Med* 1974;**56**:488–93.
126. Robinson RD, Lindo JF, Neva FA, et al. Immunoepidemiologic studies of *Strongyloides stercoralis* and human T lymphotropic virus type I infections in Jamaica. *J Infect Dis* 1994;**169**:692–6.
127. Schad GA. Cyclosporine may eliminate the threat of overwhelming strongyloidiasis in immunosuppressed patients. *J Infect Dis* 1986;**153**:178.
128. Schad GA, Aikens LM, Smith G. *Strongyloides stercoralis*: is there a canonical migratory route through the host? *J Parasitol* 1989; **75**:740–9.
129. Sukhavat K, Morakote N, Chaiwong P, et al. Comparative efficacy of four methods for the detection of *Strongyloides stercoralis* in human stool specimens. *Ann Trop Med Parasitol* 1994;**88**:95–6.
130. Suputtamongkol Y, Premasathian N, Bhumimuang K, et al. Efficacy and safety of single and double doses of ivermectin versus 7-day high dose albendazole for chronic strongyloidiasis. *PLoS Negl Trop Dis* 2011;**5**:e1044.

Aberrant Infections with Intestinal Nematodes

Toxocara canis

131. Arpino C, Gattinara GC, Piergili D, et al. *Toxocara* infection and epilepsy in children: a case-control study. *Epilepsia* 1990;**31**:33–6.
132. Ashton N. Larval granulomatosis of the retina due to *Toxocara*. *Br J Ophthalmol* 1960;**44**:129–48.
133. Bekhti A. Mebendazole in toxocariasis. *Ann Intern Med* 1984; **100**:463.
134. Biddulph J. Mebendazole and albendazole for infants. *Pediatr Infect Dis J* 1990;**9**:373.
135. Centers for Disease Control and Prevention (CDC). Ocular toxocariasis—United States, 2009-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:734–6.
136. Cianferoni A, Schneider L, Schantz PM, et al. Visceral larva migrans associated with earthworm ingestion: clinical evolution in an adolescent patient. *Pediatrics* 2006;**117**:e336–9.
137. Colli CM, Rubinsky-Elefant G, Paludo ML, et al. Serological, clinical and epidemiological evaluation of toxocariasis in urban areas of south Brazil. *Rev Inst Med Trop Sao Paulo* 2010;**52**: 69–74.
138. Congdon P, Lloyd P. *Toxocara* infection in the United States: the relevance of poverty, geography and demography as risk factors and implications for estimating county prevalence. *Int J Public Health* 2011;**56**:15–24.
139. Dinning WJ, Gillespie SH, Cooling RJ, et al. Toxocariasis: a practical approach to management of ocular disease. *Eye* 1988;**2**: 580–2.
140. Douglas JR, Baker NF. Some host-parasite relationships of canine helminths. In: McCauley JE, editor. *Host-parasite relationships. Proceedings of the 26th annual biological colloquium*. Corvallis: Oregon State University Press; 1966. p. 97–115.
141. Editorial. The public health significance of canine ascarid infections. *Vet Rec* 1976;**99**:37–8.
142. Fortenberry JD, Kenney RD, Younger J. Visceral larva migrans producing static encephalopathy in an infant. *Pediatr Infect Dis J* 1991;**10**:403–6.
143. Glickman LT, Schantz PM. Epidemiology and pathogenesis of zoonotic toxocariasis. *Epidemiol Rev* 1982;**10**:143–8.
144. Good B, Holland CV, Taylor MR, et al. Ocular toxocariasis in schoolchildren. *Clin Infect Dis* 2004;**39**:173–8.
145. Hotez PJ. Visceral and ocular larva migrans. *Semin Neurol* 1993;**13**:175–9.
146. Jones JL, Kruszon-Moran D, Won K, et al. *Toxoplasma gondii* and *Toxocara* spp. co-infection. *Am J Trop Med Hyg* 2008;**78**:35–9.
147. Kazacos KR. Visceral and ocular larva migrans. *Semin Vet Med Surg (Sm Anim)* 1991;**6**:227–35.
148. Marmor M, Glickman L, Shofer F, et al. *Toxocara canis* infection of children: epidemiologic and neuropsychologic findings. *Am J Public Health* 1987;**77**:554–9.
149. Nelson JD, McConnel TH, Moore DV. Thiabendazole therapy of visceral larva migrans: a case report. *Am J Trop Med Hyg* 1966;**15**:930–3.
150. Schantz PM. *Toxocara* larva migrans now. *Am J Trop Med Hyg* 1989;**41**(Suppl.):21–34.
151. Schantz PM, Meyer D, Glickman LT. Clinical, serologic, and epidemiologic characteristics of ocular toxocariasis. *Am J Trop Med Hyg* 1979;**28**:24–8.

152. Sharghi N, Schantz PM, Caramico L, et al. Environmental exposure to *Toxocara* as a possible risk factor for asthma: a clinic-based case-control study. *Clin Infect Dis* 2001;**32**:e111–16.
153. Small KW, McCuen BW, De Juan E, et al. Surgical management of retinal retraction caused by toxocariasis. *Am J Ophthalmol* 1989; **108**:10–14.
154. Sturchler D, Schubarth P, Fualzata M, et al. Thiabendazole vs. albendazole in treatment of toxocariasis: a clinical study. *Ann Trop Med Parasitol* 1989;**83**:473–8.
155. Won KY, Kruszon-Moran D, Schantz PM, et al. National seroprevalence and risk factors for zoonotic *Toxocara* spp. infection. *Am J Trop Med Hyg* 2008;**79**:552–7.
156. Woodruff AW, Bisseru B, Bowe JC. Infection with animal helminths as a factor in causing poliomyelitis and epilepsy. *BMJ* 1966;**5503**:1576–9.

Other Aberrant Infections with Intestinal Nematodes

157. Chitwood M. Nematodes of medical significance found in market fish. *Am J Trop Med Hyg* 1970;**19**:599–602.
158. Cross J. Intestinal capillariasis. *Clin Microbiol Rev* 1992;**5**:120–9.
159. Cuckler AC, Egerton JR, Alicata JE. Therapeutic effect of thiabendazole on *Angiostrongylus cantonensis* infections in rats. *J Parasitol* 1965;**51**:392–6.
160. Dauz V, Cabrera BD, Cancas B. Human intestinal capillariasis, part I: clinical features. *Acta Med Philipp* 1967;**4**:72–83.
161. Diaz JH. Helminthic eosinophilic meningitis: emerging zoonotic diseases in the South. *J La State Med Soc* 2008;**160**:333–42.
162. Gavin PJ, Kazacos KR, Shulman ST. Baylisascariasis. *Clin Microbiol Rev* 2005;**18**:703–18.
163. Gavin PJ, Shulman ST. Racoon roundworm *(Baylisascaris procyonis)*. *Pediatr Infect Dis J* 2005;**22**:651–2.
164. Graeff-Teixeira C, Aramburu da Silva AC, Yoshimura K. Update on eosinophilic meningoenceophalitis and its clinical relevance. *Clin Microbiol Rev* 2009;**22**:322–48.
165. Hotez PJ, Narasimhan S, Haggerty J, et al. Hyaluronidase from *Ancylostoma* hookworm larvae and its function as a virulence factor in cutaneous larva migrans. *Infect Immun* 1992;**60**:1018–23.
166. Huff DS, Neaffie RC, Binder MJ, et al. The first fatal *Baylisascaris* infection in humans: an infant with eosinophilic meningoencephalitis. *Pediatr Pathol* 1984;**2**:1–20.
167. Hurlbert TV, Larsen RA, Chandrasoma PT. Abdominal angiostrongyliasis mimicking acute appendicitis and Meckel's diverticulum: report of a case in the United States and review. *Clin Infect Dis* 1992;**14**:836–40.
168. Kazacos KR, Boyce WM. *Baylisascaris* larva migrans. *J Am Vet Med Assoc* 1990;**195**:894–903.
169. Koo J, Pien F, Kliks MM. *Angiostrongylus (Parastrongylus)* eosinophilic meningitis. *Rev Infect Dis* 1988;**10**:1155–62.
170. Krepel HP, Polderman AM. Egg production of *Oesophagostomum bifurcum*, a locally common parasite of humans in Togo. *Am J Trop Med Hyg* 1992;**46**:469–72.
171. McKerrow JH, Sakanari JA, Deardorff TL. Revenge of the "sushi parasite." *N Engl J Med* 1988;**319**:1228–9.
172. Otto GI, Berthrong M, Appleby RE, et al. Eosinophilia and hepatomegaly due to *Capillaria hepatica* infection. *Bull Johns Hopkins Hosp* 1954;**94**:319–36.
173. Polderman AM, Kepel HP, Baeta S, et al. Oesophagostomiasis, a common infection of man in northern Togo and Ghana. *Am J Trop Med Hyg* 1991;**44**:336–44.
174. Rosen L, Chappel R, Laquer GL, et al. Eosinophilic meningoencephalitis caused by a metastrongyloid lungworm of rats. *JAMA* 1962;**179**:620–4.
175. Rosen L, Loisen G, Laigret J, et al. Studies of eosinophilic meningitis, part III: epidemiologic and clinical observations on Pacific islands and the possible etiologic role of *Angiostrongylus cantonensis*. *Am J Epidemiol* 1967;**85**:17–24.
176. Schad GA. Hookworms: pets to humans. *Ann Intern Med* 1994; **120**:434–5.
177. Schantz PM. The dangers of eating raw fish. *N Engl J Med* 1989;**320**:1143–5.
178. Whalen GE, Rosenberg EB, Strickland GT, et al. Intestinal capillariasis: a new disease in man. *Lancet* 1969;**1**:13–16.
179. Whitting DA. The successful treatment of creeping eruption with topical thiabendazole. *S Afr Med J* 1976;**50**:253–5.
180. Wittner M, Turner JW, Jacquette G. Eustrongyloidiasis: a parasitic infection acquired by eating sushi. *N Engl J Med* 1989; **320**:1124–6.

Filarial Parasites

181. Addis DG, Dreyer G. Treatment of lymphatic filariasis. In: Nutman TB, editor. *Lymphatic filariasis*. London: Imperial College Press; 2000. p. 151–91.
182. Baird JK, Alpert LI, Friedman R. North American brugian filariasis: report of nine infections of humans. *Am J Trop Med Hyg* 1986; **35**:1205.
183. Hotez PJ. Englarging the "audacious goal": elimination of the world's high prevalence neglected tropical diseases. *Vaccine* 2011;**29**(Suppl. 4):D104–10.
184. Molyneux DH, Bradley M, Hoerauf A, et al. Mass drug treatment for lymphatic filariasis and onchocerciasis. *Trends Parasitol* 2003; **19**:516–22.
185. Ottesen EA. Lymphatic filariasis: treatment, control, and elimination. *Adv Parasitol* 2006;**61**:395–441.

Lymphatic Filariasis: Wuchereria bancrofti *and* Brugia *Species*

186. Chaturvedi P, Gawdi AM, Parkhe K, et al. Arthritis in children: an occult manifestation of Bancroftian filariasis. *Indian J Pediatr* 1993;**60**:803–7.
187. Ottesen EA, Nutman TB. Tropical pulmonary eosinophilia. *Annu Rev Med* 1992;**43**:417–24.
188. Ottesen EA, Vijayasekaran V, Kumaraswami V, et al. A controlled trial of ivermectin and diethylcarbamazine in lymphatic filariasis. *N Engl J Med* 1990;**322**:1113–17.
189. Simmons CF Jr, Winter HS, Berde C, et al. Zoonotic filariasis with lymphedema in an immunodeficient infant. *N Engl J Med* 1984;**310**:1243–5.
190. Taylor MD, Hoerauf A. A new approach to the treatment of filariasis. *Curr Opin Infect Dis* 2001;**14**:727–31.
191. Taylor MJ, Hoerauf A, Bockarie M. Lymphatic filariasis and onchocerciasis. *Lancet* 2010;**376**:1175–85.
192. Witt C, Ottesen EA. Lymphatic filariasis: an infection of childhood. *Trop Med Int Health* 2001;**6**:582–606.

Loa loa

193. Kilon AD, Massoughbodji A, Sadeler BC, et al. Loiasis in endemic and nonendemic populations: immunologically mediated differences in clinical presentation. *J Infect Dis* 1991;**163**:1318–25.
194. Nutman TB, Reese W, Poindexter RW, et al. Immunologic correlates of the hyperresponsive syndrome of loiasis. *J Infect Dis* 1988;**157**:544–50.
195. Twum-Danso NA. *Loa loa* encephalopathy temporally related to ivermectin administration reported from onchocerciasis mass treatment programs from 1989 to 2001: Implications for the future. *Filaria J* 2003;**2**(Suppl. 1):S7.

Onchocerca volvulus

196. Boatin BA, Richards FO Jr. Control of onchocerciasis. *Adv Parasitol* 2006;**61**:349–94.
197. Taylor HR, Pacque M, Munoz B, et al. Impact of mass treatment of onchocerciasis with ivermectin on the transmission of infection. *Science* 1990;**250**:116–18.

Mansonella perstans and Mansonella ozzardi

198. Baird JK, Neafie RC, Connor DH. Nodules in the conjunctive, bung-eye, and bulge-eye in Africa caused by *Mansonella perstans*. *Am J Trop Med Hyg* 1988;**38**:553–7.

Dirofilaria immitis

199. Bloc T, Glynn T, Hinshaw M. Human pulmonary dirofilariasis. *Indiana Med* 1990;**83**:24–7.
200. Dayal Y, Neafie RC. Human pulmonary dirofilariasis: a case report and review of the literature. *Am Rev Respir Dis* 1975;**112**: 437–43.

Dracunculus medinensis

201. World Health Organization. Monthly report on dracunculiasis cases, January-September 2011. *Weekly Epidemiol Rec* 2011;**86**: 541–56.

SUBSECTION 3 *CESTODES*

CHAPTER 226

Cestodes

A. Clinton White, Jr. • Philip R. Fischer • Pedro Legua

OVERVIEW OF CESTODES AND THEIR IMPORTANCE FOR CHILDREN

Cestodes are multicellular helminth parasites.[234] Children may harbor the adult worms (tapeworms) or larval stages (e.g., cysticercosis or hydatid disease). The adult tapeworms are segmented worms that contain a scolex (attachment organ); a neck region, where the segments are formed; and segments termed *proglottids*. The scolex contains either sucking grooves (*Diphyllobothrium* and *Spirometra*) or round suckers (other species). In some species (e.g., *Taenia solium*), the scolex is armed with rows of hooks, which also aids in attachment. The proglottids develop in the neck region and move distally as they are displaced by newer proglottids. Eventually, chains of proglottids form and compose the body of the adult worm. The external surface of the proglottid forms an absorptive surface that functions as the parasite gut. As the proglottids are displaced from the scolex, they gradually mature, with both male and female sexual organs developing in each proglottid. The terminal proglottids contain the uterus, which are full of ova. The ova of *Spirometra* and *Diphyllobothrium* are shed into water, where they infect a series of aquatic intermediate hosts. The ova of other species infect the immediate host after being ingested. Both larval and adult forms of *Hymenolepis nana* live in the human intestines, and the entire life cycle can take place in a single host. Humans are dead-end hosts for numerous zoonotic tapeworms, including *T. solium* (cysticercosis), *Echinococcus* spp. (hydatid disease), and *Spirometra* (sparganosis). The term *cysticercosis* (literally, "bladder-tail") refers to infection of tissues with an intermediate form of the parasite containing a cystic parasite with one (or a small number of) invaginated scolex. Human cysticercosis usually is caused by *T. solium*. However, human cysticercosis is also rarely caused by *Taenia crassiceps* or *Taenia multiceps*. *Hydatid disease* refers to infection of tissues with the intermediate form of the parasites of the genus *Echinococcus*, which form cystic lesions filled with innumerable protoscolices, each of which can turn into a tapeworm or another hydatid lesion, depending on the tissues. Infections with cestodes have been common human infections since antiquity and have been found in mummies.[20,101] Currently, approximately 200 million people worldwide are infected by cestodes.

TAENIA SAGINATA (BEEF TAPEWORM) INFECTION

The different life cycle forms and designation of species of the beef tapeworm were identified in the 18th and 19th centuries.[259] Although other species names have been used in the past, *Taenia saginata* is accepted now as an inclusive term for all of the species of tapeworms acquired from beef. In recent years, however, *Taenia asiatica* has been identified as a distinct species similar to *T. saginata* but acquired from pork. Differentiation of these species morphologically, however, is difficult, and identification of species is aided by DNA analysis.[37,101]

The Organism

The scolex of *T. saginata* measures 1 to 2 mm in diameter and has four muscular suckers but bears no hooks. The length of the parasite usually varies from 4 to 10 m but may reach 25 m. The size of the proglottids depends on their stage of development and their state of muscular relaxation. Gravid proglottids typically are 16 to 20 mm in length and 5 to 7 mm in width. The linear, central uterine stem has 12 to 30 main lateral branches on each side. The eggs measure 30 to 40 μm in diameter. The six-hooked embryo is surrounded by a brown, radially striated embryophore. When the bovine host ingests the ova, embryos hatch and burrow through the intestinal mucosa, where they gain access to the circulation. After lodging in capillaries, the embryo develops into a larval cysticercus. This cystic, ovoid structure measures 7 to 10 mm × 4 to 6 mm and contains an invaginated scolex.

Cattle are the principal intermediate hosts. Humans are the only definitive host. Ingestion of *T. saginata* eggs is of no particular danger to children because humans are not susceptible to development of the cysticercus stage.

Transmission

The life cycle of *T. saginata* requires transmission of the infection to the intermediate host and subsequently to humans, who in turn transmit the organism to other intermediate hosts. Contamination of pastures or feed lots with human feces or untreated sewage leads to bovine cysticercosis. When humans ingest raw or poorly cooked beef containing viable cysticerci, the scolex evaginates

from the cysticercus and attaches to the intestinal wall. Proglottids develop from the neck region and gradually enlarge to form the adult tapeworm. Gravid proglottids, which are at the distal end of the worm, appear only 84 to 120 days after infection.[236] Infection may persist for years. In most geographic areas, parasitism is with a single worm, but in highly endemic areas, multiple infections may be found.[236]

Ova may be found within feces. However, eggs are shed more frequently when gravid proglottids actively migrate from the anus. As these muscular proglottids crawl about, eggs are expressed from the anterior margin. The ova of *T. solium* and *T. saginata* are not distinguishable on morphologic grounds.

Epidemiology

Worldwide, 45 to 60 million people are thought to be infected with *T. saginata*. Infection is most important in tropical and subtropical cattle-raising areas of Africa, the Middle East, Europe, Asia, and South America.[37,130,143] The prevalence of human taeniasis caused by *T. saginata* is poorly understood. In general, the prevalence of human infections is less than 1 percent of stool examinations in all but highly endemic areas, but because of the poor sensitivity of the assays, this figure may understate the prevalence. In recent studies, prevalence rates of more than 20 percent have been noted in East Africa, Bali, and Tibet.[40] Although the incidence of taeniasis in the United States is low, transmission does occur. However, less than 0.1 percent of fecal specimens examined in state health laboratories were positive for *Taenia* eggs.[116] With socioeconomic changes, improvements in the cattle industry, and decreasing instances of ingestion of undercooked beef, the prevalence of intestinal taeniasis likely will continue to drop in much of the world.

Pathology and Pathogenesis

Little is known of the pathology or pathogenesis of intestinal taeniasis. Speculation about local mucosal trauma, irritation, production of toxic substances, or induction of clinically significant hypersensitivity has scant documentation. Rare, ectopic localization of worms or proglottids may stimulate local inflammatory reactions. Because adult worms "rob" the infected host of nutrients, malnutrition could result from the child's incomplete uptake of ingested food, but little evidence exists to support this possibility.

Clinical Manifestations

A variety of symptoms have been attributed to *T. saginata*, but the parasite infection likely is often merely coincidental to the concurrently observed symptoms in the child.[37] The most common complaint is the discomfort caused by the migration of gravid proglottids from the anus. The patient's awareness of the infection frequently causes a preoccupation with gastrointestinal function. Some studies of *T. saginata* infection revealed abdominal pain, nausea, weakness, and loss of weight as the most common symptoms recorded.[37,39,259] Alterations in appetite and bowel habits were reported inconsistently. Intestinal obstruction and symptoms related to ectopic localization of proglottids are extremely rare. Although taeniasis usually is not associated with significant eosinophilia, some patients occasionally have significant eosinophilia between the sixth and ninth weeks after the initial infection occurs. One study reported that by the time the patients first began passing proglottids, the eosinophil counts had returned to normal or nearly normal.[87] Another series of observations showed a syndrome of marked eosinophilia and severe colicky abdominal pain that occurred approximately 1 month before the patients passed *Taenia* proglottids.[9] A single experimental infection produced symptoms of nausea, headache, and disturbed sleep at approximately the time that gravid proglottids appeared in the stool, 84 days after infection. A 10.5 percent increase in the number of eosinophils and a 13 percent increase in the number of lymphocytes were noted.[236]

Diagnosis

The scolex of *T. saginata* lacks hooks and is differentiated easily from the scolex of *T. solium*, which bears a circle of hooks. However, the scolex usually is not recovered, even after successful treatment. Studies suggest that use of polyethylene glycol salts as a purgative may improve recovery of proglottids.[114] Eggs may be seen on routine fecal wet mounts, but the likelihood of their being detected is enhanced by concentration methods. Even then, ova are shed only intermittently, and the yield of a single stool examination is low. Cellulose tape swabs applied to the anal and perianal skin, as used for the diagnosis of *Enterobius* infection, have been found to be even more efficient than fecal concentration.[241]

The eggs of *T. saginata* and *T. solium* cannot be differentiated morphologically. Thus, examination of gravid proglottids is the usual method for determining species. The patient is instructed to collect proglottids in a vial of saline and to deliver the specimen to the laboratory as soon as possible. Fixatives such as alcohol and formalin tend to render the proglottids rigid and opaque. The proglottid should be compressed between two microscope slides. If the uterus contains enough ova, counting the main branches of the uterine stem is relatively easy. The uterus is more readily identified by injecting a small amount of India ink into the midportion. A count of 13 or fewer branches on one side of the stem is considered diagnostic for *T. solium*. If the count is 14 or more, the species is designated *T. saginata*. Because of morphologic variations, this method has been criticized, particularly when the decision is based on gravid proglottids with counts in the range of 12 to 15. Analysis of many specimens of *Taenia*, as identified by their scolex, showed that mature proglottids with fully developed sex organs could be differentiated by several characteristics. The most prominent features are a vaginal sphincter seen only in *T. saginata* and a third ovarian lobe that is present only in *T. solium*. To define these and other features, the proglottids require painstaking staining and clearing as well as examination by a skilled parasitologist.

Antigen detection tests are becoming increasingly useful for the identification of *T. saginata*. Tests for *T.*

solium currently are more specific than tests for *T. saginata*.[2] Polymerase chain reaction (PCR) testing has been useful in diagnosis of *T. saginata* in field situations.[37] On occasion, the diagnosis of tapeworm is established when larger portions of a worm appear as a ribbon-like defect in the barium column during contrast studies of the gastrointestinal tract. In plain films of the abdomen, a tapeworm may be seen as a linear density in a gas-filled loop of bowel.

Treatment

Praziquantel, a pyrazinoisoquinoline derivative, is the primary medication used to treat *T. saginata*. It is given as a single oral dose of 5 to 10 mg/kg. An alternative to praziquantel for treatment of intestinal taeniasis is niclosamide (marketed as Yomesan in parts of Europe but not consistently available in the United States), which is given as a single oral dose of 50 mg/kg (up to 2 g for large children or for adults). Nitazoxanide, a thiazolide agent approved for use in children with other parasitic infections, has been effective in treating taeniasis that has been incompletely responsive to both praziquantel and niclosamide.[130]

Because infection with *T. saginata* is not contagious, no routine follow-up is needed after treatment. If, however, proglottids are noted through the anus or in the stool more than a week after treatment is completed, subsequent therapy may be considered, perhaps with an alternative agent.

Prognosis

Symptoms, when present, may be annoying but do not usually alter health significantly. Treatment is highly effective, and the prognosis is excellent.

Prevention

T. saginata infection can be prevented by avoiding the ingestion of raw or undercooked beef. In addition, visual meat inspection is effective in detecting all but light infections. Cooking beef to a temperature of 56°C (133°F) kills cysticerci. Education of workers in the cattle-raising industry, coupled with detection and prompt treatment of human infection, may reduce the incidence of transmission. Vaccines to prevent bovine *T. saginata* cysticercosis are feasible, but development has been slowed by limited commercial interest.[138]

TAENIA ASIATICA (ASIAN PORK TAPEWORM)

T. asiatica was identified in East and Southeast Asia, where humans become infected by eating small cysts contained in raw porcine liver. The proglottids are morphologically similar to those of *T. saginata*, such that it was not recognized as a separate species until 1993.[37,109]

The Organism

Although adult tapeworm proglottids of *T. asiatica* are morphologically similar to those of *T. saginata*, the scolex contains a rostellum (like *T. solium*) but no hooks (like *T. saginata*). Proglottids usually contain 16 to 21 uterine branches, similar to *T. saginata*. The larval cysticercus stage is also similar to that of *T. saginata*, but the cysticerci generally are smaller. The cysticerci also are located primarily in the liver, not generally in muscle. *T. asiatica* cysticerci are found mainly in pigs but have been identified in cattle, goats, monkeys, and wild boar.[109]

Transmission

Pigs (and also cattle, wild boar, and goats) become infected by ingesting *T. asiatica* eggs, which hatch, releasing the larvae, which in turn invade and develop into the cysticercal stage primarily in the liver. Animals, including humans, become infected when they ingest cysticerci (usually in raw porcine liver) and then develop the intestinal adult forms of infection. An experimental human infection demonstrated that a period of 76 days transpired between ingestion of the cysticercus and the appearance of gravid proglottids.[61]

Epidemiology

Asian taeniasis has been identified in Taiwan, Korea, China, Philippines, Vietnam, Thailand, Indonesia, and Malaysia.[37,61] It may be that other parts of the Asia-Pacific region also are endemic for *T. asiatica* but that previous surveys incorrectly identified the offending organism as *T. saginata*. The prevalence of infection varies among regions, probably related to religious and cultural choices about food; 0 to 21 percent of people in various parts of Indonesia are infected.[254]

Pathology and Pathogenesis

Observations in humans and studies in mice suggest that the pathogenesis of infection by *T. asiatica* is similar to that of *T. saginata*.[110] No human cysticercal form has been identified.

Clinical Manifestations, Diagnosis, Treatment, and Prognosis

The clinical manifestations, diagnosis, treatment, and prognosis of *T. asiatica* have not been described completely, but they appear to be similar or identical to those of *T. saginata*.

Prevention

Human *T. asiatica* infection can be prevented by avoiding ingestion of undercooked liver and other viscera (especially porcine).

TAENIA SOLIUM

Cysticercosis was first described in ancient Greece, where cysticerci were noted in infected pork.[70] The life cycle was clarified by the 19th century. The main clinical manifestation results from infection of the central nervous system (CNS) and is termed *neurocysticercosis*. Large case

series describing most of the clinical manifestations were published in the early 20th century.[52] Neurocysticercosis, however, was diagnosed only rarely until the late 1970s. Subsequent advances in neuroimaging led to a dramatic increase in recognition.[191] Neurocysticercosis is now recognized as among the more common causes of neurologic disease worldwide.*

The Organism

T. solium, referred to as the pork tapeworm, takes two distinct forms in the human host: taeniasis (tapeworm infection of the gut lumen) and cysticercosis (infection of tissues with the larval cysticercus form).[70] Neurocysticercosis refers to cysticercosis involving the CNS (including the subarachnoid space, spinal cord, and eyes). Humans develop the adult tapeworm after ingesting undercooked pork containing the cysticercus. The scolex evaginates in the intestines, attaches, and develops chains of proglottids. The tapeworms can be several meters long. Eggs and gravid proglottids are shed intermittently into stool. The off-white proglottids appear as flattened segments 1 mm thick and up to 2 cm in length and 1 cm in width. Pigs are infected after ingesting eggs or proglottids from human fecal material. Once ingested, the larval oncospheres emerge from the eggs, penetrate the wall of the gut, enter the bloodstream, and migrate to the tissues, where they develop into cysticerci during a period of a few weeks.

Transmission

Field studies of both taeniasis and cysticercosis are associated with tight clustering.† Taeniasis is acquired by ingestion of undercooked pork and is closely tied to pig-raising areas in endemic countries. Most carriers are from families that raise pigs and butcher them informally in the household. Cysticercosis is acquired from the human tapeworm carriers, who not only shed eggs into the environment but also harbor eggs on their hands and fingernails. These eggs can then autoinfect the tapeworm carrier or infect other people. Thus, most transmission occurs among close contacts of tapeworm carriers. Human cysticercosis is not always acquired directly from pork, as illustrated by outbreaks noted among vegetarians in India and orthodox Jews in the United States.[207,217,251] In both cases, transmission has been associated with domestic servants who were tapeworm carriers, a factor also seen in urban areas lacking infected pigs.[103]

Epidemiology

The global prevalence of human cysticercosis is not clearly defined. Between 50 and 100 million people are thought to be infected with cysticercosis, but far fewer with intestinal taeniasis.[22,36,164] Nearly all areas where pigs are raised that have access to human fecal material are endemic for cysticercosis.[70] In endemic areas, pigs often are reared on small farms, where they are allowed to forage for food rather than being fed. Improved sanitation, meat inspection, and animal husbandry led to the eradication of cysticercosis from western Europe, which previously was highly endemic. However, similar approaches have not proved sustainable in poorer countries. Currently, porcine cysticercosis remains highly endemic in Latin America, sub-Saharan Africa, and South and Southeast Asia, as well as parts of Korea, China, Indonesia, and Papua New Guinea.[109,165]

In the United States, porcine cysticercosis is a very rare occurrence. In contrast, human neurocysticercosis is widespread among immigrants from endemic countries.* Limited transmission occurs in the United States, mainly linked to tapeworm carriers infected in endemic areas.[42,167,212,229]

Pathology and Pathogenesis

Cysticerci can develop in a wide range of tissues. In most tissues, the cysticerci cause few symptoms and their survival appears to be limited. For example, many of those infected have cigar-shaped calcifications noted on radiographs of skeletal muscle, despite the absence of musculoskeletal symptoms. By contrast, infection of the CNS often lasts for years and can cause severe symptoms.

Cysticerci mature to their full size (typically 10 to 20 mm in diameter) within a few weeks after infection occurs, but as illustrated in studies of British subjects returning from India and Hispanic immigrants to the United States, symptoms do not develop for several years.[50,52] This delay may reflect the parasites' complex array of molecules used to modulate the host inflammatory response.[70] When the parasites lose the ability to evade immune attack, the cysticerci are attacked by a granulomatous response with a mixed cell population, including lymphocytes and mononuclear cells, with variable numbers of eosinophils and neutrophils.† This granulomatous host response to the parasite, when it is present in the brain parenchyma, is thought to be the cause of seizures, the characteristic clinical feature of parenchymal neurocysticercosis.[232] During the course of several months, the inflamed parasite is invaded by inflammatory cells, the cyst cavity collapses, and the parasite material is replaced by the host granulomatous response.[96] The lesion eventually either resolves or is replaced by a small calcified granuloma. The calcified lesions contain a mixture of fibrosis and residual parasitic debris. An inflammatory response to persistent parasitic antigen may be the cause of recurrent seizures in patients with cerebral calcifications. Rarely, massive infection is accompanied by a diffuse cerebral edema.[186,240]

Extraparenchymal neurocysticercosis is associated with a poorer prognosis, largely because of production of hydrocephalus.[70,72] Parasites can cause obstructive hydrocephalus by lodging in the outflow tracks of the cerebral ventricles. Cysticerci in the basilar cisterns often are associated with chronic arachnoiditis. This basilar inflammation may lead to chronic meningitis (with headache and

*References 17, 22, 36, 70, 160, 164.
†References 74, 75, 133, 165, 168, 205.

*References 50, 169, 212, 213, 214, 227, 228, 246.
†References 96, 139, 188, 189, 192, 213

nuchal rigidity but usually without fever), vasculitis and strokes, or communicating hydrocephalus. Less frequently, cysticerci in fissures enlarge to 5 cm or larger (termed giant cysticerci). The parasites, along with accompanying cerebral edema, may cause a mass effect. In patients with multiple cysticerci, several forms of the disease can be present at the same time.

Clinical Manifestations

Taeniasis

Taeniasis typically causes few symptoms. Cases have been associated with vague abdominal complaints and occasionally pruritus. The most specific symptom is the passage of proglottids in the stool. The proglottids appear flattened (typically 0.3 to 0.5 cm wide × 1 to 2 cm long and 1 mm thick). Each segment has 13 or fewer uterine branches. Unlike *T. saginata*, the proglottids usually are single and seldom are noted to be motile.

Cysticercosis

The clinical manifestations of cysticercosis are extremely variable, depending on the location of the parasites and the host inflammatory response. Experts now group neurocysticercosis into a range of syndromes that differ in pathogenesis, clinical manifestations, prognosis, and management.*

Single Enhancing Lesions. The most common presentation in children with neurocysticercosis is with seizures and a single enhancing lesion on neuroimaging studies.† Onset typically occurs in those 5 to 40 years of age, but it has been noted in infants. Most cases are manifested with seizures, which can be isolated or recurrent. The seizures typically are focal with secondary generalization, although they may be focal or generalized.[220,251] Some children experience only severe headaches,[180] which can resemble either tension headaches or migraines. Imaging studies of children reveal either a focal area of enhancement or a ring of enhancing lesions, often with surrounding edema. Lesions typically are found within the cortex.

Multiple Cystic (Viable) Lesions. Similar to children with single enhancing lesions, patients with multiple parenchymal cysticerci also have seizures. The seizures more often are generalized or focal with secondary generalization but also may be focal.[26,49,82,222] Neuroimaging studies demonstrate edema, contrast enhancement, or both for one or more parasites, and symptoms are thought to result from the host inflammatory response. Noninflamed cysts cause few symptoms, even when they are numerous,[71] but they pose a risk for development of recurrent symptoms when they degenerate.

Other symptoms include headaches. Infected children may develop learning disabilities,[33,134,193,198,253] but in many cases they may be the result of either poorly controlled seizures or hydrocephalus.[235] An association of cysticercosis with depression and occasionally psychotic episodes also may exist.[67]

Cysticercal Encephalitis. Some children have diffuse cerebral edema from large numbers of inflamed cysticerci, termed *cysticercal encephalitis.* The clinical presentation includes signs or symptoms of raised intracranial pressure, seizures, and altered mental status.[186,240] Cysticercal encephalitis is thought to result from a brisk inflammatory response to a massive infection and is seen more frequently in children and women than in adult men. This pathogenesis is the direct result of the host inflammatory response, and the key to management is to address inflammatory and cerebral edema. Antiparasitic drugs are contraindicated because they can worsen the cerebral edema.

Parenchymal Calcifications. Resolution of neurocysticercosis can be associated with the formation of calcified granulomata within the brain parenchyma.[160] The calcifications appear as well-defined calcified nodules measuring 2 to 10 mm. The calcified lesions contain fibrotic reactions, parasite debris, and calcium deposits, with variable degrees of inflammation. Patients frequently present with seizures.[159-161,219] The most common imaging finding from neurocysticercosis in population-based studies in endemic villages is with focal calcifications.[48,65,155,184] Few patients will have focal abnormalities on electroencephalographic studies.[28] Among patients with seizures, calcified lesions are a risk factor for having recurrent seizures.[24,85,161,183] Patients with seizures and calcifications should be treated with antiepileptic therapy indefinitely. About half of patients with calcified neurocysticercosis and seizures have associated enhancement and edema on magnetic resonance imaging (MRI) studies.[161] Rather than revealing viable parasites, the enhancement may result from breakdown of the calcified granulomata, with release of antigen resulting in restimulation of the host inflammation.[95,161]

Ventricular Neurocysticercosis. Approximately 10 to 20 percent of adult patients with cysticercosis have cysticerci in the ventricles,* but the numbers often are lower in children.[202] Cysticerci are found in any of the ventricles, but symptoms are particularly associated with obstruction of the outflow of the third or fourth ventricle at the cerebral aqueduct or foramina of Luschka and Magendie. In contrast to parenchymal disease that largely results from the host inflammatory response, symptoms of ventricular disease result from mechanical obstruction typically caused by viable parasites.[119] Within the thin-walled viable cysticerci, the cyst fluid often is isodense with cerebrospinal fluid (CSF), rendering them difficult to detect. Computed tomography (CT) scanning may show only obstructive hydrocephalus or distortion of the shapes of the involved ventricle. Even on MRI, the findings may be subtle and can be missed by inexperienced observers unless the patients have concomitant parenchymal cysticerci.[98,263,265]

*References 70, 72, 73, 160, 162, 213.
†References 78, 197, 198, 202, 213, 218, 251.

*References 70,72,162,187,203,212.

Symptoms from ventricular neurocysticercosis usually result from raised intracranial pressure and include headache, nausea or vomiting, altered mental status, papilledema with visual changes, or dizziness. The onset is extremely variable from chronic intermittent headache to sudden loss of consciousness.

Subarachnoid Cysticercosis. Subarachnoid cysticercosis can also be quite variable. Small cysticerci in the gyri have a clinical presentation and prognosis similar to that of parenchymal cysts, although they are associated more often with CSF pleocytosis, may be slightly larger, and respond less well to antiparasitic drugs.[70,203] Cysticerci in the basilar cistern can cause arachnoiditis, leading to CSF outflow obstruction, communicating hydrocephalus, vasculitis, and strokes.[70,230] In the era before antiparasitic drugs were available, this form carried a high case-fatality rate.[230] By contrast, more recent case series have been characterized by low mortality rates.[36,43,162,187,212]

Most patients with cysticerci in the basilar cisterns are infected with large numbers of parasites, and they frequently have coexisting ventricular or parenchymal as well as subarachnoid disease. Symptomatic cysticerci in the basilar cisterns typically are accompanied by chronic arachnoiditis. This arachnoiditis may present as meningitis with headache, stiff neck, and CSF pleocytosis, but it usually is not accompanied by fever. Patients often develop communicating hydrocephalus (headaches, nausea, vomiting, and dizziness). Basilar cysticercosis frequently involves the basilar vasculature either by direct invasion or by inducing vasculitis. Thus, patients may present with cerebrovascular accidents, which may involve either small vessels with lacunar infarctions from vasculitis or large-vessel strokes (associated with invasion of the vessel wall by the parasite).[11,44,72] Some patients present with intracerebral hemorrhages.[252]

Giant Cysticerci. Cysticerci, termed giant cysticerci, can develop in the fissures (especially the sylvian fissure) and may enlarge to more than 5 cm in diameter.[175] In some cases, the cysticerci can lose the scolex and may grow as clusters of cyst walls (termed racemose cysticercosis). Giant cysticerci not only are associated with symptoms of parenchymal inflammation but also can cause mass effects such as midline shift. Frequently, giant cysticerci are accompanied by cysticerci in the parenchyma or basilar cisterns. Giant cysticerci are readily visualized by CT or MRI, but the accompanying basilar cysticerci may not be seen as easily.

Other Forms of Cysticercosis. Neurocysticercosis rarely is limited to the spine, but spinal disease is common with subarachnoid disease.[25] Most patients present with radicular pain and, less frequently, myelitis. In most cases, cysticerci are in the spinal fluid, but they also can be intramedullary cysticerci.[132] The intramedullary form typically presents with myelitis. Orbital involvement includes subconjunctival cysticerci and involvement of the extraocular muscles.[179,211] Intraocular disease may be subretinal, intravitreal, or within the anterior chamber. Skeletal muscle involvement typically is asymptomatic, but it may result in pseudohypertrophy or weakness with massive infection. Subcutaneous lesions typically present as one or more painless mobile cystic lesions. Subcutaneous disease is more commonly noted in Asia and Africa than in the Western Hemisphere.

Diagnosis

Taeniasis

Taeniasis has been diagnosed traditionally by examination of the stool for proglottids, ova, or both. The ova and proglottids are shed only intermittently, and collection of stool specimens from children can prove difficult. Furthermore, the sensitivity of stool microscopy for ova is only 26 percent,[4] and the ova of *T. solium* and *T. saginata* are indistinguishable, which limits the specificity of the assay. Identification of species requires collection of the proglottids, which can be facilitated by treating the patient with polyethylene glycol.[114] The proglottids of the two species can be distinguished by counting the number of uterine branches, which are lower for *T. solium*.

More sensitive and specific techniques are available only as research tools. Antigen-detection assays have a specificity of 99.2 percent and a sensitivity of 70 to 92 percent,[3,4,195] but most cannot distinguish *Taenia* spp. Enzyme-linked immunosorbent assay (ELISA) to identify serum antibodies to *T. solium* tapeworm-stage antigens may be more sensitive than are stool-based tests and seem to be specific for the *T. solium* tapeworm stage.[97,135] However, the antibody may persist after treatment. PCR also can be used to improve sensitivity and specificity.[148]

Neurocysticercosis

Establishing the diagnosis of neurocysticercosis is more difficult than with many other parasitic infections. The major clinical presentations are nonspecific, and the location of the parasites within the CNS usually precludes their being observed directly. Serologic tests using crude antigens (including a number of commercially available ELISAs) are plagued by poor sensitivity and specificity. However, computerized imaging methods (e.g., CT and MRI) have led to a dramatic increase in the ability to recognize cases and are now the mainstay of diagnosis (Figs. 226-1 to 226-3), although even these techniques cannot always distinguish neurocysticercosis from other neurologic processes.

An expert group proposed diagnostic criteria based on neuroimaging studies, serologic tests, clinical history, and exposure.[46] The presence of either a single absolute criterion or two major criteria along with two minor or epidemiologic criteria is considered diagnostic. One major criterion plus two other criteria or three minor criteria along with exposure define a probable diagnosis.

Direct visualization of the parasite on ophthalmoscopic examination or histology is considered diagnostic of neurocysticercosis.[46] However, biopsy or autopsy material demonstrating *T. solium* parasites rarely is available, and parasites seldom are visualized in the eye. The

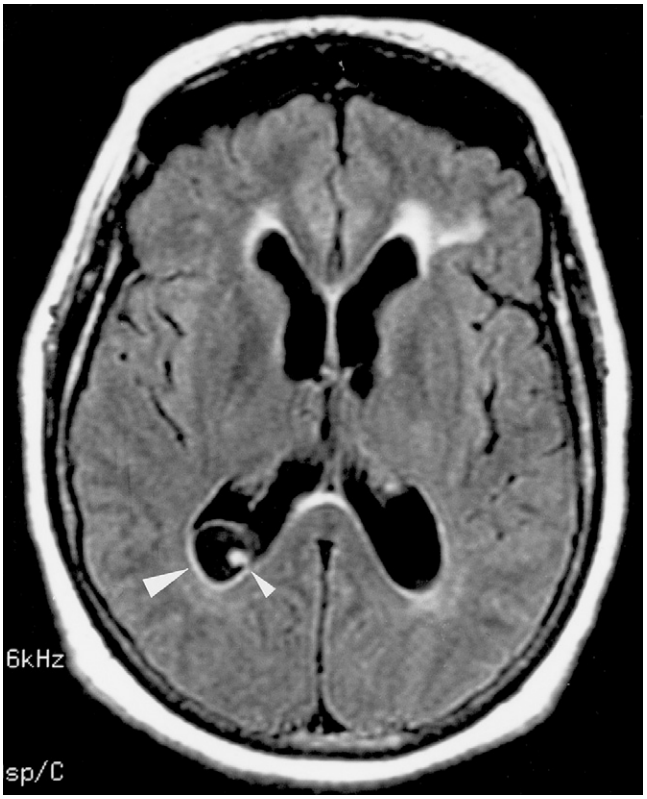

FIGURE 226-1 ■ Ventricular neurocysticercosis. (Courtesy C. Mark Mehringer, MD, Harbor-UCLA Medical Center.)

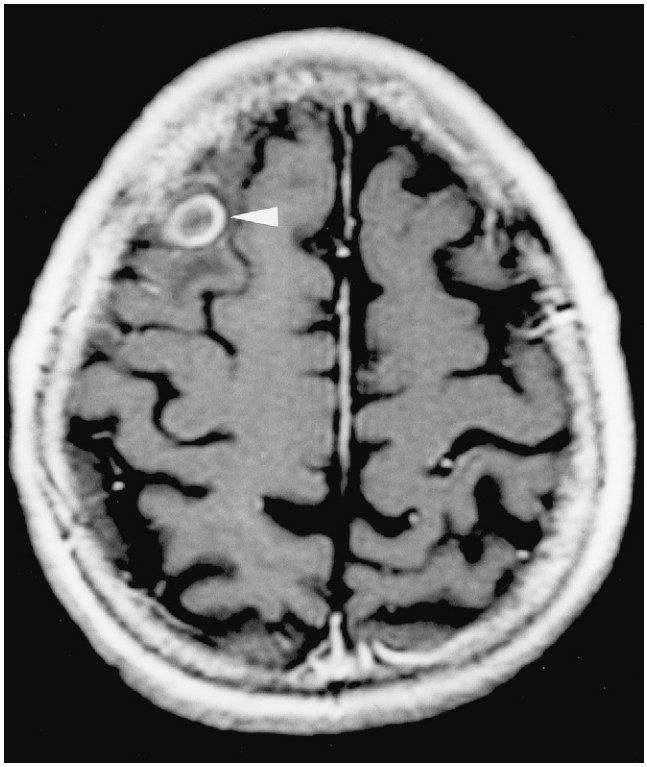

FIGURE 226-3 ■ Neurocysticercosis with parenchymal involvement. (Courtesy C. Mark Mehringer, MD, Harbor-UCLA Medical Center.)

FIGURE 226-2 ■ Neurocysticercosis with subarachnoid involvement. (Courtesy C. Mark Mehringer, MD, Harbor-UCLA Medical Center.)

neuroimaging pattern of a cystic lesion with a mural nodule measuring 1 to 3 mm (consistent with a scolex) is thought to be pathognomonic for cysticercosis.[263,264] However, rare cases have been reported in which similar findings have been noted in other diseases, and even in neurocysticercosis, the scolex cannot be visualized in most cases.

The main major diagnostic criterion for neurocysticercosis is one or more lesions on neuroimaging studies highly suggestive of neurocysticercosis.[45,46] Viable cysticerci appear on CT or MRI scans as rounded fluid collections typically 1 to 2 cm in diameter. The cyst fluid is isodense with CSF (hypodense in contrast to brain tissue on CT or T1 imaging). The scolex sometimes is visible as a small nodule found attached to the cyst wall. The wall of the cysticercus initially is isodense with the parenchyma and not easily visualized. On T2-weighted and fluid attenuated inversion recovery images, the fluid is seen as hyperintense. Inflamed cysticerci are characterized by perilesional edema and contrast enhancement.[232] Subsequently, the cyst fluid increases in density and becomes infiltrated by host inflammatory cells. The cyst cavity eventually collapses, forming a solid area of focal enhancement. At that point, the granulomatous inflammation either resolves or leads to the formation of a calcified nodule typically measuring 2 to 6 mm in diameter.

Although enhancing lesions are typical of neurocysticercosis, similar lesions can be noted with tuberculomas,

brain abscesses, and tumors. Rajshekhar and Chandy[182] noted that cysticercal lesions typically have a diameter smaller than 20 mm and rarely cause midline shift. A single, round, enhancing lesion less than 20 mm in diameter without midline shift on imaging studies in patients lacking signs or symptoms of systemic disease, focal neurologic deficits, or increased intracranial pressure was highly suggestive of neurocysticercosis in a prospective study of 401 patients in southern India.[184] However, the results are less specific in areas with a lower prevalence of cysticercosis. Spontaneous resolution or resolution achieved with antiparasitic drugs is another major criterion.

Serodiagnosis has proved problematic for cysticercosis. ELISAs employing unfractionated antigens, such as cyst fluid, have poor sensitivity and specificity.[176,185] The preferred serodiagnostic test is the enzyme-linked immunotransfer blot employing semipurified parasite glycoproteins.[247] A positive reaction to any one of seven glycoprotein bands is considered positive. ELISAs using recombinant versions of these antigens are being developed.[72] Studies have confirmed a specificity of nearly 100 percent in suspected cases, but the sensitivity is limited in subjects with either a single lesion or only calcified lesions.[89,176,208,258] For this assay, tests of serum are more sensitive than tests of CSF.[258] Assays to detect parasite antigens may prove to be important diagnostic studies.[62,77,79,81,171] Although several different antigen detection tests are in development, none is widely available commercially.

Minor criteria include (1) lesions on neuroimaging studies consistent with neurocysticercosis but less suggestive (e.g., isolated basilar meningitis, hydrocephalus without demonstration of cystic lesions, or filling defects in the spinal subarachnoid space without discrete cysticerci), (2) symptoms suggestive of seizures or hydrocephalus, and (3) cysticercosis outside the nervous system (e.g., cigar-shaped muscle calcifications or subcutaneous nodules).[46] Epidemiologic criteria include residence or prolonged visits to endemic areas and contact with a tapeworm carrier. Since the guidelines were published, publications have reported detection of parasite antigens in CSF, serum, and urine.[41,157,194,255] Currently, no commercial or well-standardized assays are available. Antigen is more frequently detected with viable cysticerci and with a larger parasite burden. They appear to be particularly useful for determining length of therapy in complicated subarachnoid cases. PCR assays have been reported on CSF, but these are also not well standardized.[151]

Numerous other tests may prove useful in excluding other illnesses but are not diagnostic of neurocysticercosis. These tests include blood counts, lumbar puncture for testing of CSF, and stool studies. Lumbar CSF frequently has elevated protein levels and may show hypoglycorrhachia. Cell counts are variable and may demonstrate a predominance of lymphocytes, neutrophils, or eosinophils.

Treatment

For patients with seizures caused by neurocysticercosis, initial management should focus on control of the seizures, which usually can be accomplished with a single antiepileptic drug. Most published studies describe use of phenytoin or carbamazepine. Newer agents (e.g., valproate, lamotrigine, levetiracetam, topiramate, oxcarbazepine, or clobazam) may be more effective, but they are not affordable in endemic areas.[118] Breakthrough seizures usually occur with subtherapeutic antiepileptic drug levels, often from poor adherence to medication therapies.[82] Seizure therapy can be tapered off for many patients after they have a seizure-free period if radiographic resolution of the lesion is seen. However, a substantial risk exists for recurrence of seizures if residual inflamed or calcified lesions are present.[26,82,161,183,251] Antiepileptic drugs should be continued indefinitely if calcifications develop.

Patients with obstructive hydrocephalus usually require surgery.[70,73,160,162,187] For patients with mild or intermittent symptoms (e.g., a cysticercus in the lateral ventricles not associated with midline shift), elective endoscopic removal of the cysticercus is the treatment of choice.[181,187] Cases with altered mental status or impending herniation should undergo emergency CSF diversion by a ventriculostomy or placement of a ventriculoperitoneal shunt. A high rate of shunt failure exists if shunting is not followed by administration of antiparasitic therapy or corticosteroids.[70,119,187,230] Patients with communicating hydrocephalus often require placement of a ventriculoperitoneal shunt.[119] In some patients, cysticerci and associated edema may lead to mass effects. If the symptoms are life-threatening or if the cysticercus is easily approachable (e.g., in the sylvian fissure), surgical decompression of the cysticercus is the preferred approach.

A consensus is emerging among experts about the proper role of antiparasitic drugs and corticosteroids in the management of neurocysticercosis.[70,161] Experts agree that neurocysticercosis represents a spectrum of diseases that differ in optimal management.

Single Enhancing Lesions

The most common presentation of children with neurocysticercosis is seizures and a single enhancing lesion. Seizures generally can be controlled with antiepileptic medications.[118,183,251] Controlled trials of antiparasitic drugs in patients with single enhancing lesions have demonstrated a favorable prognosis with symptomatic therapy. However, the length of time until radiologic resolution is achieved and the duration of time for risk for experiencing seizures appear to be somewhat shorter with antiparasitic drug treatment.[47,218,221,242] By contrast, these treatments do not appear to affect the formation of calcified lesions or development of chronic epilepsy. The role of steroids is better defined; several studies showed clear benefit in reducing seizure activity and faster resolution of the lesion on CT when steroids were used in conjunction with antiepileptic medications.[84,86,127,145,173] Overall, the best evidence points to a small benefit achieved from use of antiparasitic drugs (e.g., albendazole 15 mg/kg/day for 8 days) and a short course of corticosteroids (e.g., prednisone 1 mg/kg/day for 8 to 10 days). A single study suggested

that combinations of praziquantel 75 mg/kg/day and albendazole 15 mg/kg/day may be more effective in children with single enhancing lesions than albendazole alone.[117] The safety of this approach has been confirmed in a pharmacokinetics study in adults.[80]

Multiple Parenchymal Lesions

Patients with seizure activity with multiple cysticerci usually will have one or more lesions in the process of degenerating (e.g., edema or contrast enhancement on imaging studies). However, other cysticerci may be in the viable stage, posing a prolonged risk for having seizures. A placebo-controlled trial from Peru showed a modest reduction in the number of generalized seizures in patients treated with albendazole and corticosteroid,[82] but no decrease occurred in the development of chronic calcifications. A meta-analysis of trials also supported this approach.[47] One recent trial suggested a worse outcome with this treatment, an effect not seen in any other study. Overall, the current expert consensus is that patients with multiple parenchymal cysticerci usually should be treated with a course of an antiparasitic agent (albendazole 15 mg/kg/day in two daily doses for 8 days) and a simultaneous course of corticosteroids.[162] Data from a large clinical trial suggest that the combination of praziquantel and albendazole may be more effective than albendazole alone (H. Garcia, manuscript under review). Even so, symptomatic therapy (e.g., antiepileptic drugs) remains a key to management.

Cysticercal Encephalitis (Numerous Cysticerci with Cerebral Edema)

In this context of diffuse cerebral edema (cysticercal encephalitis), antiparasitic drugs are contraindicated because of their potential to exacerbate host inflammatory responses.[73,186,240] Most cases will resolve spontaneously with anti-inflammatory medications.

Calcified Parenchymal Cysticerci

Parenchymal brain calcifications can be seen in more than 10 percent of residents in some neurocysticercosis endemic areas.[160,184] These parenchymal calcifications are a significant risk factor for having continued seizure activity.[83,183,251] If a patient with a history of seizures has a calcified lesion, that patient should be treated with antiepileptic therapy indefinitely. Recurrent seizures may be due to concomitant mesiotemporal sclerosis.[250] Antiparasitic medications and anti-inflammatory drugs are not indicated. Surgical removal of the calcified focus has been used in some cases of intractable seizures.[257] By contrast, most cases can be managed with a single antiepileptic medication.

Ventricular Neurocysticercosis

Patients with obstructive hydrocephalus usually require surgery. For mild or intermittent symptoms (e.g., a cysticercus in the lateral ventricles not associated with midline shift), elective endoscopic removal of the cysticercus is the treatment of choice.* Cases with altered mental status or impending herniation should undergo emergency CSF diversion by a ventriculostomy or placement of a ventriculoperitoneal shunt. A high rate of shunt failure exists if shunting is not followed by administration of antiparasitic therapy. Cysticerci in the lateral and third ventricles usually can be removed through a rigid endoscope. A flexible endoscope is required to approach the fourth ventricle or basilar cisterns.[105,245,266] Many surgeons, however, prefer a suboccipital craniotomy for removal of cysticerci in the 4th ventricle. Endoscopic foraminotomy and third ventriculostomy often can be used to treat residual hydrocephalus. Antiparasitic medications should not be given before the procedure is performed because they can cause the cysticerci to be friable or to adhere to the ventricular wall. The cysticerci frequently rupture during removal, but this rupture has not been associated with any adverse effects. Endoscopic third ventriculostomy has been used as an alternative to shunting procedures.[104,128,177]

A ventriculoperitoneal shunt may be used to treat hydrocephalus. The rate of shunt failure is high in patients treated only with ventriculoperitoneal shunting. However, shunt failure appears to occur less frequently when patients receive adjunct therapy with corticosteroids and antiparasitic drugs. Although some patients have been treated with only chemotherapy and steroids, a substantial risk exists for development of acute hydrocephalus from cysticerci or accompanying inflammation causing obstruction of the foramina.

Subarachnoid Neurocysticercosis

Basilar subarachnoid cysticercosis is perhaps the most severe form of infection and carries risks for communicating hydrocephalus, vasculitis, and strokes.[70,72,160,162,187] Data on how best to treat this form are limited, but most experts agree that patients should be treated with antiparasitic drugs, corticosteroids, and, in most cases, CSF diversion procedures.[175] Most experts recommend treatment with albendazole, typically 15 mg/kg/day in divided doses for at least 28 days, but cure rarely is achieved with a single course of albendazole. Repeated courses, prolonged therapy, higher doses, switching to praziquantel, and combination of the two drugs have been tried in some cases.[146] In my experience, combination therapy seems to be more effective than albendazole alone. Improved responses with endoscopic debulking of some of the parasites are anecdotal,[94,162] and this approach has not yet been studied systematically.

Inflammation seems to drive much of the pathogenesis, and the residual symptoms may reflect a chronic inflammatory response to cysticercal antigens rather than viable cysticerci. Prednisone up to 60 mg/day or dexamethasone up to 24 mg per day should be used along with the antiparasitic drugs. After 2 to 4 weeks, the dose often can be tapered. In cases that require more prolonged steroid therapy, methotrexate has been used as a steroid-sparing agent.[152]

*References 73, 104, 160, 162, 178, 181, 187, 233.

Giant Subarachnoid Neurocysticercosis

Some cysticerci, particularly in the sylvian fissure or basilar cisterns, may enlarge to more than 5 cm in diameter, causing mass effect either directly or by surrounding edema. If mass effect cannot be reversed quickly by corticosteroids, surgical decompression may be required. In the absence of symptomatic mass effect, however, giant cysticerci can be treated similarly to other subarachnoid cysticerci.[162,175]

Other Forms of Neurocysticercosis

Cysticercosis of the eye, spine, or subcutaneous tissue generally is treated by surgical removal.[70] Anecdotes of medical therapy also exist.

Prognosis

The prognosis of neurocysticercosis is extremely variable, depending on the sort of infection. Patients with single enhancing lesions generally do well and often are seizure free and discontinue therapy within 1 to 2 years. Patients with calcified lesions may require chronic antiepileptic therapy. Patients with numerous subarachnoid cysticerci often require repeated courses of therapy and even repeated surgeries. However, with optimal management of hydrocephalus, cases are rarely fatal.

Prevention

Cysticercosis was eliminated from western Europe by improved sanitation, animal husbandry, and meat inspection.[76,92] For example, porcine cysticercosis can be prevented by not allowing pigs access to human feces.[249] However, confined pigs must be fed, which is not affordable for peasant farmers. Human infection with adult tapeworms also can be prevented by adequate cooking or freezing of infected pork, but meat inspection has not been successful in areas where pigs are slaughtered informally.[237] Mass chemotherapy of endemic populations for carriage of the human tapeworm or porcine cysticercosis has resulted in significant short-term decreases in prevalence,[3,49,92,204] but prevalence rates rapidly return unless mass treatment is continued. Development of an effective vaccine against cysticercosis may provide the best potential tool for eradication of the disease. Promising studies indicate that this will be possible for human and porcine cysticercosis.[66,88,93,112]

COENUROSIS (*TAENIA MULTICEPS*, OTHERS) AND CYSTICERCOSIS CAUSED BY *TAENIA CRASSICEPS*

Several species of *Taenia* have a larval stage called a *coenurus* that consists of a cystic membranous structure measuring up to 6 cm in diameter from which multiple scolices bud internally or externally.[96,107] On the basis of this morphologic appearance, the parasite was given the species name *multiceps*. Currently, the preferred genus name is *Taenia* because of the morphology of the adult worms and the inability to justify a genus designation based solely on the form of larvae. The following species generally are accepted for purposes of discussion. The adult tapeworm of *Taenia multiceps* is found in dogs, and the larval stages occur in herbivores. The larvae often develop in the CNS in sheep. In sheep-raising areas of Europe and Asia, the infection is still endemic in animals. *Taenia serialis* adult worms are found in dogs and other canids, but common intermediate hosts are rabbits, hares, and rodents. The coenurus develops in subcutaneous and intramuscular tissue. *T. serialis* has been reported from the United States, Canada, France, and Africa. *Taenia brauni* is the name given to the tapeworms of dogs, jackals, foxes, and genets found in tropical Africa. The larvae develop in gerbils and other rodents. Humans are a rare accidental host for the larval stages of these worms. Fewer than 100 human infections have been recorded. Most infections are from Africa, with many occurring in children. Giving a species designation based solely on the morphologic features of organisms recovered from human tissue is nearly impossible. The infection is presumed to be acquired by the ingestion of eggs excreted by the definitive hosts, usually dogs. Because of the subcutaneous and subconjunctival locations of cysts in infections in tropical Africa, some researchers have suggested that direct contact of the eggs on skin or the conjunctiva is a mode of transmission. A review of the six human infections in North America has been published.[96,107]

The clinical manifestations of coenurosis are related to the location of the parasite. Involvement of the CNS produces a spectrum of illness that resembles that of cysticercosis (discussed earlier), including meningeal reactions. Larvae have been seen in the subconjunctival and subretinal tissue, the extraocular muscles, the anterior chamber, and the vitreous. Subcutaneous and intramuscular lesions occur most commonly in the abdomen and chest wall. A definitive diagnosis is made by demonstrating the characteristic morphologic features of the larva recovered at surgery. The multiple scolices that bud from the delicate cyst membrane have double rows of hooklets of a typical shape, size, and number. In instances in which no scolices are found, differentiation of a coenurus from a cysticercus of *T. solium* is impossible. The diagnosis of intramuscular coenurosis has been made by examination of fine-needle aspirates.[96,107] Radiographic studies such as CT are useful in cerebral coenurosis but do not differentiate the parasite from other cystic lesions. The metabolic changes of magnetic resonance spectroscopy in an adult with cerebral coenurosis have been reported.[5] Treatment is surgical. Mortality rates in cerebral disease are high. Organisms in other locations, with the exception of subretinal lesions, are removed easily. A combination of praziquantel and a corticosteroid administered to a patient with a subretinal coenurus caused death of the parasite, which resulted in a severe inflammatory reaction, retinal detachment, and permanent loss of vision.[106] Severe reactions were reported in the two additional cases described in this review. Praziquantel should be used with great

caution, if at all, in cases of human coenurosis. Nonetheless, postoperative praziquantel and albendazole have been used without apparent complication.[12,60] Although the exact mode of infection in humans has not been identified, avoidance of close contact with dogs and dog excreta, which are the most likely sources of infection, is prudent.

T. crassiceps cysticerci usually are found in field mice, with the tapeworm found in foxes, other canids, and felines. There have been a few reported human cases with ocular disease or mass lesions in compromised hosts (e.g., patients with acquired immunodeficiency syndrome).* Experimental *T. crassiceps* infection induces a Th2 response that can also modulate immune responses to subsequent pathogenic challenges.[190] Fluid from *T. crassiceps* vesicles has been used to develop ELISA tests, but these tests are not specific for *T. crassiceps* infection and are rather used for seroprevalence studies of neurocysticercosis resulting from *T. solium* infection.[108,174] The diagnosis and management of *T. crassiceps* infection require surgery, but anthelmintic treatment might play a helpful role.[91]

DIPHYLLOBOTHRIUM SPECIES (FISH TAPEWORM)

Diphyllobothriasis can occur in children who ingest raw or undercooked fresh-water fish. The prevalence of infection increases with age in endemic areas. Nonspecific gastrointestinal complaints may occur in infected individuals, and megaloblastic anemia has resulted.[37,115,210,259]

Several species of *Diphyllobothrium* have been reported in humans. *Diphyllobothrium latum* was described first in 1592 from specimens found in Switzerland; it is now estimated to infect approximately 9,000,000 individuals worldwide, with most cases occurring in Europe.[56] *Diphyllobothrium pacificum* is a similar fish tapeworm found infecting humans in Japan and South America, and *Diphyllobothrium nihonkaiense* has been found in Pacific salmon (and, hence, in humans) in Japan and Europe.[37] The *Diphyllobothrium* spp. typically have fish-eating birds, marine mammals, bears, dogs, and foxes as definitive hosts, but children living in endemic areas may be infected when they eat raw or undercooked fish.

The Organism

The scolex of *D. latum* possesses neither discrete suckers nor hooks but has two deep grooves. The worm may be as long as 15 m. The gravid proglottids have a characteristic rosette-shaped central uterus. Eggs measure 55 to 61 μm by 37 to 56 μm and have light brown, operculated shells. The eggs are shed in an unembryonated state from a uterine pore into the fecal stream. The embryos develop within the eggs in fresh water. The ciliated embryos hatch and are ingested by copepods to develop further. After the fish have ingested the infected copepod, the larval stages develop into the plerocercoid stage in the muscles of fish. As the smaller fish are eaten by larger species, the larvae parasitize the muscles of the new host. Eventually, the larvae are found in fish that are sources of food for humans, such as species of pike, perch, turbot, lake trout, and whitefish. The plerocercoid larvae or spargana are white, ribbon-like worms approximately 5 cm in length.

Transmission

Children can be infected by ingesting the plerocercoid forms in raw or undercooked fish muscle that are infectious when ingested by people. Cooking and freezing kill the larvae. The cycle of infection is perpetuated by the discharge of untreated human sewage into fresh-water lakes and streams. Some fish-eating mammals also may serve as definitive hosts for *D. latum* and maintain the cycle of infection in the absence of humans. Because *D. latum* requires intermediate hosts, direct transmission cannot occur, and no techniques of isolation or special precautions are required for infected patients.

Epidemiology

The prevalence of infection increases with age in endemic areas. However, infections have been reported in children younger than 1 year of age.[37] The disease is worldwide in distribution, but higher endemicity is associated with eating fresh-water fish raw, lightly salted, or pickled without cooking. Infection occurs frequently in the Baltic countries and Russia; South America; Scandinavia; Switzerland and the adjacent lake regions of Italy, France, and Germany; the Danube River delta; the lake areas of the northern United States and river deltas of Alaska; and Canada. Pediatric infection by *D. latum* has been identified in school-age children in India, Taiwan, Korea, Argentina, and Hawaii. Salmonid fish (i.e., salmon, trout, whitefish) transmit *D. nihonkaiense* to humans in Japan. Infection of humans with a tapeworm parasite of seals, *D. pacificum*, has been reported from Peru.[115,210]

Pathology and Pathogenesis

Little is known about the direct effects of parasitization, with the exception of megaloblastic anemia.[7] The following factors may be significant: (1) the strains of *D. latum* found in Finland, where anemia occurs, absorb seven times more vitamin B_{12} than do strains from North America, where anemia has not been reported; (2) interference with absorption of vitamin B_{12} occurs in nonanemic carriers as well as in those with anemia, and deficient stores of vitamin B_{12} plus malabsorption of vitamin B_{12} may contribute to the anemic state; (3) dietary intake of vitamin B_{12} may be low in anemic patients, and oral vitamin B_{12} has been shown to cause reticulocytosis in the presence of the worm; (4) worms found in anemic patients take up more of an oral dose of vitamin B_{12} than do worms in asymptomatic carriers, and worms are attached more proximally in the small intestine in anemic patients;

*References 29, 32, 69, 91, 100, 144.

and (5) secretion of intrinsic factor is reduced in anemic patients.

Clinical Manifestations

Most infections are asymptomatic, but patients may notice proglottids or chains of segments in the stool. In Finland, comparison of nonanemic infected patients with uninfected controls revealed an increase in the symptoms of fatigue, weakness, craving for salt, lack of well-being, dizziness, and numbness of the extremities in the infected group.[199] Significant gastrointestinal symptoms occur infrequently. However, an experimental infection with seven larvae produced nausea, severe periumbilical pain, and marked weight loss. Episodes of intestinal obstruction associated with the vomiting of masses of tapeworms have been reported. Eosinophilia is an uncommon finding. Patients with *D. pacificum* reported abdominal pain, vomiting, diarrhea, and flatulence.[141] Megaloblastic anemia caused by *D. latum* infection is extremely rare outside Finland and neighboring endemic areas. Usually, it is seen in patients older than 50 years of age (and may occur in as many as 2 percent of adult tapeworm carriers), but it has been reported in children as young as 9 years.

Diagnosis

Identification of the characteristic eggs (oval, operculated, approximately 60 µm across) or proglottids provides the diagnosis. The parasite produces nearly 1 million eggs per day, and ova usually are detected easily by fecal examination.

Treatment and Prevention

Treatment with praziquantel (5 to 10 mg/kg as a single oral dose) is highly effective. An alternative medical treatment is niclosamide (50 mg/kg as a single oral dose). Tapeworm-related anemia is reversible by treatment of the infection, but the affected child should receive supplementation initially with vitamin B_{12}.

A significant reduction in the transmission of *D. latum* has been accomplished in many areas through the introduction of sewage treatment and targeted drug treatment of human infections. Raw and undercooked fish should not be consumed in areas where a possibility of larval infection exists. Larvae do not survive in fish that is either cooked for 5 minutes or more at temperatures of at least 55°C or frozen at temperatures of at least –10°C for 8 to 72 hours.[56]

DIPYLIDIUM CANINUM (DOG TAPEWORM)

Dipylidium caninum, the common tapeworm of dogs and cats, also infects infants and young children.[37,259] Transmission occurs through the accidental ingestion of infected fleas (*Ctenocephalides* spp.) or body lice (*Trichodectes* spp.). Although it usually is asymptomatic, infection may be associated with decreased appetite, abdominal discomfort, or, perhaps, diarrhea.

The Organism

D. caninum adult worms have a scolex with four cup-shaped suckers and a rostellum that bears one to seven rows of small hooks. The worm ranges from 10 to 50 cm in length. The gravid proglottids resemble cucumber seeds in size and shape. The proglottids have two pairs of sex organs, and a genital pore opens on each lateral margin. The eggs measure 35 to 65 µm in diameter and appear in packets of 5 to 30 enclosed in a membrane. The gravid proglottids are excreted in feces or migrate actively from the anus. The eggs are liberated as the proglottid disintegrates. Several species of flea serve as intermediate hosts. The larval flea ingests the egg of *D. caninum*. Development of the tapeworm's larval stages takes place as the flea metamorphoses into an adult. When the flea is ingested by the definitive host, the adult worm develops in the small intestine.

Transmission and Epidemiology

Humans acquire the infection by the accidental ingestion of infected fleas. Dogs may transfer infective larvae to humans by licking children after crushing fleas orally. Because of the motility of the proglottids, eggs may be disseminated widely in the environment of animal hosts. Fleas are so ubiquitous that transmission to the intermediate host is accomplished readily.

Infection of dogs and cats is a common occurrence worldwide. *D. caninum* also has been found in a variety of wild canines and felids. Reports of dipylidiasis in humans in the United States, however, are uncommon.[27,154]

Clinical Manifestations, Diagnosis, Prognosis, and Treatment

Although the incubation period in humans is unknown, infection has been seen in infants aged 5 weeks. Frequently, the only evidence of infection is the finding of proglottids in stool or in an infant's diapers. Varying degrees of abdominal pain, diarrhea, irritability, and even pruritus have been recorded in symptomatic children.

The characteristic proglottids must be differentiated from other parasites. A frequent error is to assume, from the parents' description, that the small, motile proglottids that migrate from the anus are pinworms. Proglottids also may be mistaken for fly larvae. Examination findings of stool specimens may be spuriously negative because proglottids tend to migrate from the fecal mass and disintegrate on the walls of the specimen container. Treatment with praziquantel 5 to 10 mg/kg as a single oral dose is effective. An alternative treatment is niclosamide 50 mg/kg as a single oral dose.

Infected dogs and cats also should be treated. Flea control requires the use of appropriate insecticides on pets. In addition, particular attention must be given to carpets and areas where pets sleep because these are the sites of development of the larval fleas.

HYMENOLEPIS NANA (DWARF TAPEWORM)

Infection with *Hymenolepis nana*, the dwarf tapeworm, occurs worldwide and probably is maintained by direct fecal-oral transmission from person to person.[37,259] Insects may serve as intermediate hosts. Infection may be asymptomatic, but gastrointestinal, neurologic, and allergic symptoms have been reported.

The Organism

Adult worms of *H. nana* measure 1 to 5 cm in length and are less than 1 mm in maximal width. The tiny scolex has four cup-shaped suckers and a rostellum with a circle of 20 to 30 small hooks. Gravid proglottids disintegrate within the intestine, and eggs are found in the feces. The oval eggs measure 30 to 50 μm in diameter and contain a six-hooked embryo within two envelopes. The space between the two envelopes contains filaments that extend from two small polar protrusions on the inner envelope. Larval stages develop in insect intermediate hosts after they have ingested the eggs. However, if the definitive host ingests eggs, larval stages develop in the intestinal villi in 4 to 5 days and then break into the lumen and develop into adult worms. Ova appear in feces 2 to 4 weeks after infection occurs. A morphologically identical organism found in rodents may be a separate species. The rodent strains are alternatively called *Hymenolepis fraterna* or *H. nana* var. *fraterna*.[37,143] The rodent strain occasionally infects humans.

Transmission

Human infection is acquired most commonly by the ingestion of eggs from the feces of infected persons. Humans serve as both intermediate and definitive hosts in this direct cycle. Poor hygienic habits, overcrowding, lack of running water, and any other factor that fosters fecal-oral transmission enhance transmission through this route. The rodent strains of *H. nana* are infectious for humans, and food contaminated by rodent feces is a possible source of infection. Eggs may be produced for periods longer than 1 year, but the possibility of recurrent autoinfection obscures determination of the duration of the initial infection. Internal autoinfection may occur when ova from gravid proglottids are exposed to appropriate conditions within the intestinal lumen. Hatching occurs, and the embryos penetrate the mucosa, undergo larval development, and eventually emerge as adult worms.

Epidemiology

H. nana is a common parasite of rats and mice worldwide, and 50 to 75 million people are thought to be infected.[37,259] The distribution of infection in humans is worldwide, with an increased prevalence in some urban areas. Stool surveys from endemic areas commonly show prevalence rates of 5 to 25 percent, which may approach 50 percent in children aged 4 to 10 years.[40]

Pathology and Pathogenesis

Little is known of the pathogenesis and pathology of *H. nana* infection in humans. The larval stage of *H. nana* is a cystlike structure called a *cysticercoid* that is approximately 250 μm in diameter. Local reactions consisting of mucosal inflammation or atrophy may occur at the site of attachment of the adult worms in the small intestine. Rare cases of disseminated infection in children with depressed cellular immunity have been reported.[140]

Clinical Manifestations

A wide variety of symptoms have been ascribed to *H. nana* infection, but few well-controlled clinical studies have been presented. Heavy burdens of worms have been associated with abdominal pain, diarrhea or loose stools, malnutrition, retardation of growth, and lethargy, whereas children with low burdens did not have significant symptoms.[30,31,223]

Diagnosis

Detection of the characteristic eggs in fecal material by examination of direct saline wet mounts or by concentration techniques usually is not difficult. However, a single examination is not always adequate to rule out infection.

Treatment and Prognosis

Praziquantel 25 mg/kg as a single oral dose is effective in treating *H. nana* infection. Nitazoxanide 100 mg by mouth twice daily for 3 days for children 1 to 3 years of age, 200 mg by mouth twice daily for 3 days for children 4 to 11 years of age, and 500 mg by mouth twice daily for 3 days for older children is a reasonable alternative therapy; efficacy is approximately 75 to 82 percent.[30,170] Family members should be examined and treated if they are infected. Infection with *H. nana* rarely is severe and responds well to appropriate treatment.

Prevention

Transmission of *H. nana* infection largely could be eliminated if proper methods of personal hygiene and disposal of human waste could be invoked throughout the world. Infected foodhandlers always should be treated and monitored to ensure that treatment has been successful. Stored food should be protected from rodents and insects.

HYMENOLEPIS DIMINUTA (RAT TAPEWORM)

Hymenolepis diminuta is a rat tapeworm that only rarely presents as a human infection. Adult worms are 20 to 90 cm long and the eggs are round.[37,259] Children become infected after ingesting larva-infected insects. Infection often is asymptomatic, but abdominal discomfort and pruritus have been reported. Treatment is with praziquantel 25 mg/kg in a single oral dose or niclosamide.

SPARGANOSIS (INTERMEDIATE-STAGE *SPIROMETRA* SPECIES INFECTION)

Sparganosis is a zoonotic infection seen most frequently in eastern Asia.[96,259] It occurs when humans are infected with a metacestode larval stage of *Spirometra* spp. The condition is also referred to as "plerocercoidosis" and is often caused by *Spirometra erinaceieuropaei* in Asia and *Spirometra mansonoides* in the Americas.[6]

The Organism

Evidence indicates that the amorphous, thin, ribbon-like *Spargana* organisms recovered from human infections belong to the genus *Spirometra*.[96,259] The adult tapeworms are found in dogs, cats, raccoons, and a variety of wild carnivores. Eggs passed in the feces of these definitive hosts hatch in fresh water. The embryo that emerges from the egg is ingested by a copepod, where it undergoes larval development as a procercoid. When a second intermediate host ingests the infected copepod, the larva develops into a sparganum (plerocercoid). The range of second intermediate hosts includes amphibians, reptiles, birds, and mammals. When the second intermediate host is eaten by the definitive host, the sparganum attaches to the intestinal mucosa and develops into the adult tapeworm. A rare form of sparganosis that buds, branches, and multiplies asexually to massive numbers in humans is called *Sparganum proliferum*.[158]

Transmission

In parts of Asia, application of poultices of amphibian or reptile flesh to wounds or sores is a common practice, and children can be infected when the *Spargana* organisms migrate from the poultice into human tissues. Human sparganosis in the United States often is associated with drinking well water or untreated surface water that could contain the minute copepods containing the procercoid form. Transmission by ingestion of the raw or inadequately cooked flesh of second intermediate hosts has been demonstrated experimentally in humans. Animal sources such as snakes, frogs, chickens, and pigs have been incriminated in human infection. Asymptomatic cats, especially those living outdoors, are infected in some areas.[99,111,200]

Epidemiology

Human infection is not uncommon in Thailand.[6] It is also widely reported from China, Korea, Japan, India, Indonesia, the Philippines, Australia, Africa, Italy, South America, and the former Soviet Union.[96,136,259,260] With changes in lifestyle and environmental control, the rate of sparganum seropositivity among patients suspected of having parasitic disease in South Korea dropped from 2.6 percent in 1993 to 1.6 percent in 2006.[131] However, the infection still seems to be a bit less uncommon in North Korea.[216] In the United States, almost all reports have come from the southern or southeastern states and from Puerto Rico or were reported in persons who had been in these areas, but one infected child was reported from southern California.[35]

Pathology, Pathogenesis, and Clinical Manifestations

Ocular sparganosis, usually acquired by contact, may involve conjunctival, retro-orbital, or palpebral tissues and cause conjunctivitis, periorbital and palpebral edema, exophthalmos, chemosis, and corneal ulceration.[96,259,260] Subcutaneous sparganosis is the most common form of the infection, with the trunk frequently being involved. The lesion usually is nodular. Tenderness and inflammation may be absent, intermittent, or constant. Some lesions are migratory. Eosinophils may or may not be present. Some infections have had an associated peripheral eosinophilia, but it is not a constant finding. Spargana have been identified in a wide range of other tissues. Sparganosis of the CNS may be associated with a variety of neurologic manifestations.[96,126,226,259,260] Brain imaging of patients with cerebral sparganosis shows edema and degeneration of cerebral white matter commonly with a "tunnel sign" of hypodensity and ring enhancement.[226] Sequential MRI imaging has revealed migration of larva between various regions of the brain in some children.[14,137] The disease caused by *Sparganum proliferum* consists of progressive replacement of host tissues by the multiplying organisms.

Diagnosis

The diagnosis may be suspected in persons with typical subcutaneous lesions and a suggestive epidemiologic background. Usually, however, the diagnosis is made during surgical removal of a painful or cosmetically disturbing lump without anticipation of the parasitic etiology. *Spargana* organisms vary considerably in width and length. They usually are several centimeters in length, whitish, and opaque, and they may have the grooved indentations on the anterior end that are precursors of the bothria, or suckers of the scolex, of the adult worm. Small portions of *Spargana* are difficult to distinguish grossly or microscopically from cysticerci and other less common larval cestodes. Neuroimaging may yield characteristic findings in cerebral sparganosis.[126,226] Performing an ELISA or serum and CSF antibody appears to be useful in endemic areas, but this test is not generally available.[259]

Treatment and Prevention

Surgical removal is the only known form of therapy. Stereotactic aspiration and microsurgery have been effective, but care to remove all larval material is essential.[51] Praziquantel has been used in some cases, but no convincing evidence of clinical activity exists. Filtration of water prevents the ingestion of copepods from wells or ponds. Proper cooking of meat eliminates second intermediate hosts as sources of infection. Educational effort should be made to warn of the dangers of applying raw flesh poultices in areas where it is a cultural practice.

ECHINOCOCCUS GRANULOSUS AND RELATED SPECIES (CYSTIC HYDATID DISEASE)

Humans may be infected with the larval stages of parasites of the genus *Echinococcus*.* Humans acquire the infection by the ingestion of ova from the feces of carnivorous definitive hosts. The disease in humans and intermediate hosts is hydatid disease and is characterized further according to the morphologic features of the larval stages: cystic echinococcosis caused by *Echinococcus granulosus* and related organisms, alveolar echinococcosis caused by *Echinococcus multilocularis*, and polycystic echinococcosis caused by *Echinococcus vogeli* or *Echinococcus oligarthrus*. Most cases in children present with cystic hydatid disease, which is the focus of this section of the chapter.†

The Organisms

Cystic hydatid disease is caused by *E. granulosus* and related species. On the basis of morphologic features, all cases initially were considered to belong to a single species. However, genetic studies have demonstrated 10 separate genotypes that infect different intermediate hosts.[58,113,206,238] Significantly different strains are found in sheep, Tasmanian sheep, cattle, pigs, horses, camels, and cervids (i.e., deer, reindeer, moose, elk). Most human infections are caused by the sheep strain. However, Tasmanian sheep, cattle, camel, and cervid strains also can cause human infection. The life cycles of the species of *Echinococcus* are similar, but the geographic distributions, types of hosts, and morphologic features of the parasites differ significantly. Adult tapeworms of the sheep strain are found in dogs. The cervid strain involves primarily wolves, but dogs also may be infected. The adult tapeworm is only 3 to 8 mm long and has two to five segments. Dogs often are hosts to thousands of adult worms. When sheep or humans ingest eggs from the feces of infected dogs, the embryos hatch in the intestine and burrow through the intestinal wall to gain access to the portal circulation. The embryos that survive are able to develop in many tissues, but they do so most commonly in the liver or lungs, where they become the cystic larval structures called *hydatid cysts*. A hydatid cyst is composed of an outer laminated, acellular membrane that is lined by a thin, cellular germinal membrane. Spherical structures called *brood capsules* grow from the germinal membrane. Protoscolices develop within the brood capsules. Each protoscolex has suckers and hooks and the potential to become the scolex of an adult worm if it is eaten by a dog. Compression and reaction from growth of the cyst produce a "pericyst" of compact, collagen-rich host tissue around its exterior. In older cysts, so-called daughter cysts may develop within the primary cyst cavity.

*References 16, 17, 38, 39, 58, 59, 113, 206.
†References 16, 38, 39, 58, 59, 113, 206.

Transmission

Humans acquire cystic hydatid disease by ingestion of ova shed by the definite host, typically dogs.[39,58,150,156,201,206] Close contact with dogs can result in infection because tapeworm eggs can be found on the dog's perianal hair, muzzle, and paws. Contaminated food, drink, or fomites (e.g., flies and other insects) may disseminate the eggs from dogs' feces. However, most cases occur in pastoral families and are associated with close contact with infected dogs.

Epidemiology

The sheep strain of *E. granulosus* is found worldwide in most areas where sheep are raised.* Studies of the global burden of disease and economic impact of cystic hydatid disease suggest that the impact is significantly underestimated in contrast to other illnesses.[21] The disease is endemic in the sheep-raising areas of South America, the Middle East, the Mediterranean basin, China, and the former Soviet Union.† One of the highest morbidity rates is seen in rural Africans in Kenya and Uganda, where people live in close association with their dogs. The custom of feeding sheep viscera to sheepdogs maintains foci of infection. In the United States, Basques in central California, Mormon ranchers in Utah, and Native Americans in Arizona and New Mexico have been infected. The cervid strain of *E. granulosus* is found in northern parts of the Western Hemisphere. In Alaska and Canada, human infection usually is limited to Eskimos and Native Americans who have working dogs that are fed moose and reindeer viscera.[239] The strain found in this region often is manifested as giant pulmonary cysts.

Pathology and Pathogenesis

In cystic hydatid disease, the pathologic process is related to compression or displacement of the host's tissue.[58,172,206] Cyst growth is variable but probably averages approximately 1 cm in diameter per year. Rates of up to 4 to 5 cm per year have been reported, but growth may be more rapid in the lung. The cyst may exceed 35 cm in diameter in the abdominal cavity. When rupture or leakage of a cyst occurs, an allergic reaction caused by the antigenic cyst contents may develop. Cysts may calcify after many years, which usually signifies death of the parasite. Hydatid cysts may form foci for secondary bacterial infection.

Clinical Manifestations

Most children with *E. granulosus* infection have a single unilocular cyst, but multiple cysts are seen in 15 to 30 percent of patients, usually in a single organ system.[58,172,206] The most common site of the cysts is in the liver. In clinical series, approximately one in five children with a pulmonary cyst also has a concurrent liver cyst, but the

*References 16, 39, 58, 113, 150, 156, 206.
†References 19, 39, 58, 113, 150, 156, 201, 206.

proportion outside the liver is lower in population-based screening.[49] Approximately 10 percent of cysts are found in sites other than the liver or lung, including the spleen, kidney, peritoneum, genitourinary tract, bone, muscles, heart, eye, and brain. The cervid strain of *E. granulosus* found in Canada and Alaska characteristically produces pulmonary cysts.[58,172,206] CNS hydatid disease occurs much more commonly in children than in adults.* Bone cysts, which may be seen in preschool children, occur most frequently in vertebral and long bones.[172,206] Unlike cysts in other sites, bone cysts characteristically are multilocular and contain little fluid. Eye, bone, and brain cysts typically are small when they are discovered, whereas cysts in other sites may exceed 35 cm in diameter before being detected. Cysts grow approximately 1 to 3 cm in diameter each year, but growth may be more rapid with pulmonary cysts. Complications usually occur only if cysts become large and include secondary bacterial infection of hydatid cysts and leakage or rupture with resultant hypotension, urticaria, and eosinophilia. Rupture may occur spontaneously or secondary to trauma or surgery, but it generally is considered to be an uncommon complication of hydatid disease in children.

The wide spectrum of symptoms in cystic hydatid disease depends on the number, size, and location of the cysts.[58,172,206] Slowly growing cysts in liver or lung often are asymptomatic for years. Symptomatic intrahepatic cysts cause constant or intermittent right upper quadrant or epigastric pain, enlargement of the liver, or nausea and vomiting. Unruptured cysts rarely cause fever. Rupture of a cyst may be precipitated by a traumatic event and may cause a range of symptoms, including fever, abdominal pain, hypotension, and allergic manifestations, including eosinophilia, urticaria, and anaphylaxis. Rupture into the biliary tract may cause cholangitis with fever and right upper quadrant pain. Some children with hydatid disease also have retarded growth patterns.[149]

Intact pulmonary cysts typically do not cause symptoms.[58,172,206,215,244] Rupture or leakage, however, may cause cough, chest pain, dyspnea, hemoptysis, and a salty taste. Fever may be present, but it is often a marker for secondary bacterial infection. As many as a third of pulmonary cysts rupture into the pleural space or into a bronchus. In the latter case, the patient may describe coughing up portions of the membranes, which may resemble grape skins, or noting a characteristic salty taste. Bone cysts are seen in patients with bone pain or pathologic fracture. Vertebral hydatid disease causes signs and symptoms of spinal cord and radicular compression; severe pain on palpation of the affected portion of the spine is characteristic. Of intracranial cysts, 50 to 75 percent are seen in children.[34,90,123,248] Increased intracranial pressure with headache, vomiting, and focal neurologic symptoms are common findings. Seizures also may occur.

Diagnosis

The diagnosis of cystic echinococcosis usually is suspected on the basis of clinical or radiologic findings plus a history of residence in an endemic area.[16-18] Physical examination rarely is definitive. Only half of patients with hepatic cysts have abnormal liver enzyme results. Eosinophilia typically is low grade or absent. The initial diagnosis of cystic hydatid disease often is based on imaging findings. An unruptured pulmonary hydatid cyst has a sharply demarcated, round or oval smooth border. In the lung, it has a homogeneous cannonball appearance and sometimes is surrounded by a layer of atelectatic lung.[58,172,206] After the cyst has ruptured into a bronchus, a crescent-shaped air layer may be seen that is virtually diagnostic. In addition to the arc of air between the parasite and the host cyst wall, air in the cyst lumen also may be present. The membrane of a collapsed cyst floating on the surface of the fluid in a ruptured pulmonary cyst has a characteristic "water lily" appearance.

Cysts in the liver usually are visualized easily and measured with ultrasonography, CT, or MRI. Ultrasound techniques are useful for defining most cysts within the abdomen and can differentiate fluid-filled cysts from solid tumors. Because ultrasonography is portable and relatively inexpensive, it has been the main technique used in epidemiologic studies and clinical cases in developing countries. A World Health Organization staging system has been developed to define management (Fig. 226-4).[8,196,256] Stage CL cysts are unilocular collections without a discrete wall and with uniform anechoic contents. This appearance is not specific for *Echinococcus*. Stage CE1 cysts have a dense wall and may have internal echoes. This so-called hydatid sand is caused by protoscolices within the cyst. CE2 cysts have internal septa or daughter cysts, forming honeycomb patterns, that are highly characteristic of hydatid cysts. CE3 cysts demonstrate detachment of the cyst wall or early collapse. CE4 cysts demonstrate inhomogeneous material instead of a cyst cavity. CE5 cysts demonstrate a thick rim of calcification. Radiographically apparent cyst wall calcification occurs only in liver or spleen cysts and generally takes more than 5 to 10 years to develop. Bone cysts typically produce radiolucencies without periosteal reaction. MRI offers little advantage over CT for the imaging of cysts except in the CNS and for demonstration of biliary fistulas.[102]

Fine-needle aspiration of cysts for establishment of the diagnosis can be dangerous if it is not performed carefully because of the risk for spillage of hydatid fluid, which can induce anaphylactic shock and possibly secondary cyst development. However, the percutaneous route has been used for both diagnosis and treatment, with few untoward events (see later discussion). The fluid obtained should be examined for evidence of protoscolices, hooks, or antigen.

Serologic tests can be useful in confirmation of the diagnosis.[58,172,206] Antibody tests using crude antigen of either *E. granulosus* or *E. multilocularis* tested by ELISA, indirect hemagglutination, and fluorescent antibody assays are positive in 50 to 90 percent of cases. False-negative results are more common in patients with pulmonary, brain, and splenic cysts. Because the tests available may cross-react with cysticercosis or other parasitic infections, positive results should be confirmed by a more specific test, either an immunoblot assay or a gel diffusion assay.

*References 34, 54, 55, 123, 153, 166.

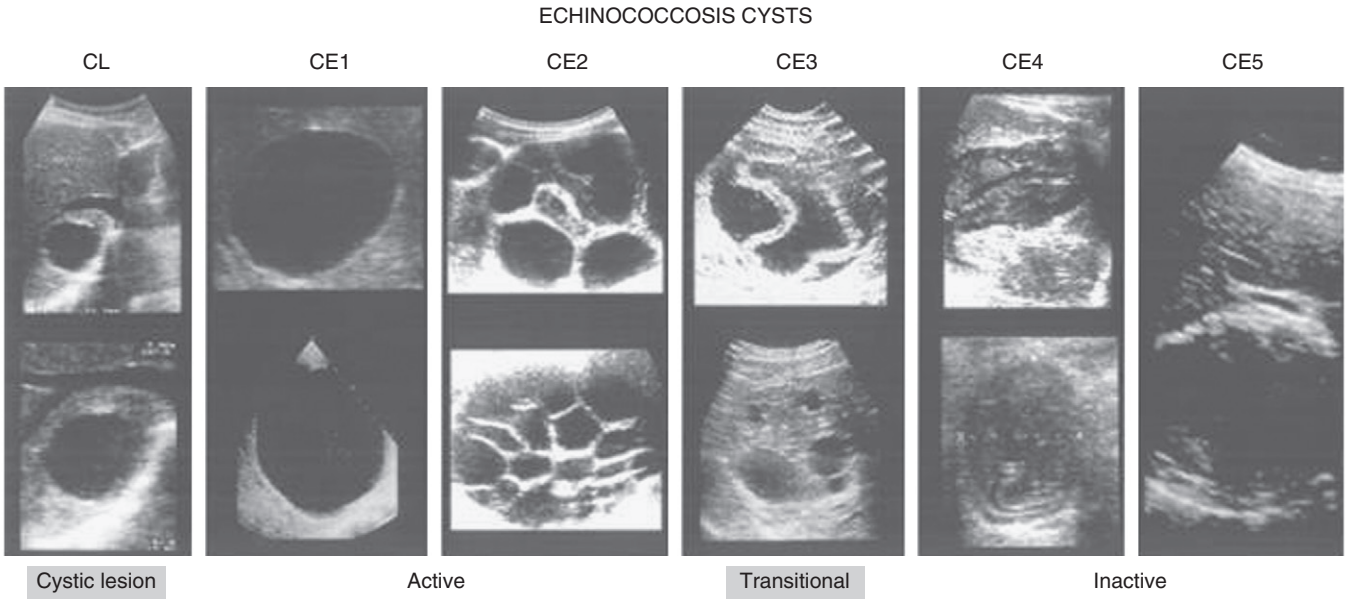

FIGURE 226-4 ■ Staging of echinococcosis. (From World Health Organization Informal Working Group on Echinococcosis: International classification of ultrasound images of cystic echinococcosis. Reproduced with the permission of the World Health Organization.)

Treatment and Prognosis

The treatment of cystic hydatid disease is in evolution.[17,18] Surgery was the traditional treatment of choice for nearly all cases of cystic hydatid disease, but that approach has given way gradually to a range of approaches, including surgery, antiparasitic chemotherapy, percutaneous drainage termed PAIR (*p*ercutaneous *a*spiration, *i*ntroduction of scolicide, and *r*easpiration), and "watch and wait."[18,58,206] Currently, expert opinion suggests that treatment should vary on the basis of the location of the cyst or cysts, the nature and size of the cysts, the condition of the patient, and the available expertise with surgery required by only a minority of cases. For example, in a prospective study, only 10 percent of children with hepatic cysts detected in a population-based ultrasound survey required surgery during the subsequent 5 years.[49,129]

The benzimidazoles albendazole and mebendazole have been used for chemotherapy.* Approximately a third of hepatic cysts will resolve in response to benzimidazoles, and 30 to 50 percent improve. Many of the residual lesions considered treatment failures were shown subsequently to be nonviable. The response to chemotherapy is better with smaller lesions. It is now thought to be the treatment of choice for small (<5 cm) CL, CE1, and perhaps CE3 lesions.[18,58,206] Chemotherapy also has proved to be successful for treatment of small pulmonary cysts.[53] However, a high failure rate exists with chemotherapy alone for larger lesions in either the liver or the lung.[18] Albendazole is thought to be more effective than mebendazole. Albendazole typically is dosed at 10 to 15 mg/kg/day in two daily doses continued for 3 to 6 months. Side effects may include neutropenia, elevated liver enzymes, and alopecia. Prospective studies of surgically removed specimens show that protoscolices can remain viable for as long as 3 months, which is the minimum duration of chemotherapy.[13,63] Treatment failures have been successfully managed by further chemotherapy, PAIR, or surgery. In some cases, PAIR may demonstrate that protoscolices no longer are viable, even with persistent lesions. Because chemotherapy can be associated with relapses or treatment failure, children should receive follow-up imaging studies for years after receiving chemotherapy. Chemotherapy also is an important adjunct to surgical therapy.

The PAIR technique offers a less invasive option than the traditional surgical approaches.* Results have been similar to those with surgery, but with fewer perioperative complications. PAIR is thought to be the treatment of choice for uncomplicated CL and CE1 lesions larger than 5 cm and CE3 lesions. CE2 lesions can be treated with a modification of PAIR that employs a larger bore catheter and drainage.[19,209] Although the data comparing different treatments are limited, PAIR was associated with similar efficacy and less morbidity than surgery in one randomized trial. PAIR should be performed after initiation of albendazole chemotherapy for at least 4 hours but preferably for several days. In a controlled trial, PAIR plus chemotherapy was superior to either chemotherapy or PAIR alone.[124,163] Aspiration generally should go through solid tissue (e.g., liver) rather than directly into the cyst (to limit spillage). The cyst fluid should be examined for protoscolices or hooks (to confirm the diagnosis). Before scolicidal agents are injected, the cyst fluid should be checked for bile staining (by dipstick or, preferably, by injection of contrast medium) because these agents can cause sclerosing cholangitis if they are injected into the biliary tract. Alcohol and hypertonic saline have been used as scolicidal agents in this procedure. Contraindications to PAIR include communication with the biliary tract, inaccessibility, superficial cysts, and cysts that are free in the abdomen, heart, brain, or spine. If the cyst is found after puncture to communicate with the biliary tract, contrast media can be used to further define

*References 58, 63, 68, 122, 129, 206, 231, 243.

*References 18, 19, 64, 125, 163, 206, 225, 261, 262

the communication. Contrast material also is mildly scolicidal.

Surgery has been the traditional approach to therapy for hydatid disease and remains the treatment of choice for complicated infections and some CE3 lesions.[18,58,172,206] The goal of surgery is to remove the entire cyst without spilling its contents. Rupture of the cyst contents at the time of surgery carries a risk for causing disseminated echinococcosis and a small risk for anaphylaxis occurring at the time of operation. Surgical approaches have included pericystectomy (removal of the entire cyst and a rim of surrounding tissues), resection of the involved organ, simple drainage, omentoplasty, and capitonnage. In general, the more radical procedures have been associated with fewer relapses, but at the cost of more perioperative complications. However, laparoscopic approaches have been reported recently to be associated with a high success rate and less morbidity.[23,57,142,262] Chemotherapy with albendazole with or without praziquantel is performed routinely.[10,63,206] With administration of chemotherapy, pressure within the cyst may be reduced, and removal of cyst membranes and contents will be facilitated. In addition, treatment decreases the likelihood of development of secondary cysts. With albendazole alone, cysts remain viable for as long as 3 months.[13] The preoperative administration of praziquantel combined with a benzimidazole has been suggested because of the scolicidal properties of praziquantel.[10] The practice of injecting the cyst with scolicidal drugs before removing it is associated with complications and is not thought to be necessary in the setting of preoperative and postoperative chemotherapy.[206]

Pulmonary cysts have been treated successfully surgically, medically, and with PAIR.* No controlled trials of these different strategies have been performed. However, retrospective series suggest that smaller cysts often respond to antiparasitic chemotherapy.[53] Larger cysts may be treated with surgery. The cervid strains of *E. granulosus* from Alaska and Canada do not produce anaphylaxis on rupture, and pulmonary cysts spontaneously resolve after evacuating into bronchi. If the cyst wall remains in place after the patient has undergone chemotherapy or PAIR, residual parasite material can serve as a nidus for bacterial superinfections.[1] Thus, many experts recommend against PAIR for pulmonary disease.[18]

The prognosis of hydatid disease varies widely from self-limited asymptomatic infection to fatal infection associated with rupture of the cyst. All of the modes of therapy are associated with relapse during the first few years of treatment. Thus, careful follow-up is essential. With appropriate therapy, however, few cases are fatal.

Prevention

Control programs for cystic hydatid disease have met with variable success.[38,39,206] *E. granulosus* was eliminated from Iceland by banning home slaughter of sheep. Banning of home slaughter, education, arecoline purging of dogs, and treatment of infected dogs with praziquantel led to eradication in New Zealand and Tasmania. However, these eradication programs required significant resources and have been less successful elsewhere. A recombinant vaccine has proved to be highly effective in field studies but awaits implementation.[39] Other tools in developmental stages include coproantigen tests to detect infected dogs and improved therapy for ovine infection.

OTHER *ECHINOCOCCUS* SPECIES

Echinococcus multilocularis is the cause of alveolar hydatid disease.[58,113,121,172,206] Humans are an incidental host in a life cycle that typically involves foxes as definitive hosts (but also wolves and occasionally domestic dogs) and rodents as intermediate hosts. The larval parasite does not form a large cystic structure but grows by progressive external budding. The laminated membrane and host pericyst are thin. The larval mass slowly enlarges and replaces liver tissue much as a malignant neoplasm does. *E. multilocularis* is endemic in arctic and alpine areas, including western China, central Europe, and scattered foci in North America. In recent years, disease is emerging in much of central Europe. The larvae may invade contiguous structures and rarely metastasize. Human infection almost invariably involves the liver, but it may spread to adjacent tissues or metastasize to the brain. Because the larval mass grows slowly, with a typical latent period of 5 to 15 years before clinical presentation, clinical disease is a rare finding in children. Diagnosis involves imaging studies with serologic confirmation. Management requires surgical resection along with prolonged chemotherapy. Comprehensive reviews of epidemiology, clinical manifestations, diagnosis, and management are available.*

E. vogeli is a parasite of bush dogs and feral dogs in South America.[206] The intermediate hosts are rodents (i.e., pacas, agoutis, spiny rats). When humans are infected, the germinal membrane grows externally to form additional cysts, and septa develop within the original cyst. This manifestation is the "polycystic" variety of hydatid disease. A rare polycystic hydatid disease in humans has been attributed to *E. oligarthrus*, a fourth species that is found as adult tapeworms in wild felids such as pumas and jaguars. The intermediate hosts are the same as those for *E. vogeli*. Most infections occur in Central and South America. Polycystic hydatid disease is limited to South and Central America, where fewer than 100 human infections have been reported. Initial management should include administration of albendazole.

NEW REFERENCES SINCE THE SIXTH EDITION

5. Ambekar S, Prasad C, Dwarakanath S, et al. MRS Findings in cerebral coenurosis due to *Taenia multiceps*. *J Neuroimaging* 2013;**23**:149–51.
6. Anantaphruti MT, Nawa Y, Vanvanitchai Y. Human sparganosis in Thailand: an overview. *Acta Trop* 2011;**118**:171–6.
16. Brunetti E, White AC Jr. Cestode infestations: hydatid disease and cysticercosis. *Infect Dis Clin North Am* 2012;**26**:421–35.
17. Brunetti E, Garcia HH, Junghanss T. Cystic echinococcosis: chronic, complex, and still neglected. *PLoS Negl Trop Dis* 2011;**5**:e1146.

*References 1, 18, 53, 120, 147, 224.

*References 15, 58, 113, 121, 172, 206.

18. Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010;**114**:1–16.
22. Budke CM, White AC Jr, Garcia HH. Zoonotic larval cestode infections: neglected, neglected tropical diseases? *PLoS Negl Trop Dis* 2009;**3**:e319.
24. Bustos JA, Montoya E, Garcia HH. Residual brain calcifications in neurocysticercosis. *Am J Trop Med Hyg* 2008;**78**:363.
25. Callacondo D, Garcia HH, Gonzales I, et al. High frequency of spinal involvement in patients with basal subarachnoid neurocysticercosis. *Neurology* 2012;**78**:1394–400.
33. Ciampi de Andrade D, Rodrigues CL, Abraham R, et al. Cognitive impairment and dementia in neurocysticercosis: a cross-sectional controlled study. *Neurology* 2010;**74**:1288–95.
36. Coyle CM, Mahanty S, Zunt JR, et al. Neurocysticercosis: neglected but not forgotten. *PLoS Negl Trop Dis* 2012;**6**:e1500.
41. Deckers N, Dorny P. Immunodiagnosis of *Taenia solium* taeniosis/cysticercosis. *Trends Parasitol* 2010;**26**:137–44.
45. Del Brutto OH. Diagnostic criteria for neurocysticercosis, revisited. *Pathog Glob Health* 2012;**106**:299–304.
51. Deng L, Xiong P, Qian S. Diagnosis and stereotactic aspiration treatment of cerebral sparganosis: summary of 11 cases. *J Neurosurg* 2011;**114**:1421–5.
54. Duishanbai S, Geng D, Liu C, et al. Treatment of intracranial hydatid cysts. *Chin Med J (Engl)* 2011;**124**:2954–8.
55. Duishanbai S, Jiafu D, Guo H, et al. Intracranial hydatid cyst in children: report of 30 cases. *Childs Nerv Syst* 2010;**26**:821–7.
60. El-On J, Shelef I, Cagnano E, et al. *Taenia multiceps:* a rare human cestode infection in Israel. *Vet Ital* 2008;**44**:621–31.
70. Garcia HH, Coyle CM, White AC Jr. Cysticercosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 815–23.
78. Garcia HH, Gonzalez AE, Rodriguez S, et al. Neurocysticercosis: unraveling the nature of the single cysticercal granuloma. *Neurology* 2010;**75**:654–8.
80. Garcia HH, Lescano AG, Lanchote VL, et al. Pharmacokinetics of combined treatment with praziquantel and albendazole in neurocysticercosis. *Br J Clin Pharmacol* 2011;**72**:77–84.
88. Gauci CG, Jayashi CM, Gonzalez AE, et al. Protection of pigs against *Taenia solium* cysticercosis by immunization with novel recombinant antigens. *Vaccine* 2012;**30**:3824–8.
91. Goesseringer N, Lindenblatt N, Mihic-Probst D, et al. Taenia crassiceps upper limb fasciitis in a patient with untreated acquired immunodeficiency syndrome and chronic hepatitis C infection: the role of surgical debridement. *J Plast Reconstr Aesthet Surg* 2011;**64**:e174–6.
99. Headley SA, Gillen MA, Sanches AW, et al. *Platynosomum fastosum*-induced chronic intrahepatic cholangitis and *Spirometra* spp. infections in feral cats from Grand Cayman. *J Helminthol* 2012;**86**:209–14.
102. Hosch W, Stojkovic M, Janisch T, et al. MR imaging for diagnosing cysto-biliary fistulas in cystic echinococcosis. *Eur J Radiol* 2008;**66**:262–7.
112. Jayashi CM, Kyngdon CT, Gauci CG, et al. Successful immunization of naturally reared pigs against porcine cysticercosis with a recombinant oncosphere antigen vaccine. *Vet Parasitol* 2012;**188**:261–7.
115. Jimenez JA, Rodriguez S, Gamboa R, et al. *Diphyllobothrium pacificum* infection is seldom associated with megaloblastic anemia. *Am J Trop Med Hyg* 2012;**87**:897–901.
117. Kaur S, Singhi P, Singhi S, et al. Combination therapy with albendazole and praziquantel versus albendazole alone in children with seizures and single lesion neurocysticercosis: a randomized, placebo-controlled double blind trial. *Pediatr Infect Dis J* 2009;**28**:403–6.
136. Li MW, Song HQ, Li C, et al. Sparganosis in mainland China. *Int J Infect Dis* 2011;**15**:e154–6.
137. Li YX, Ramsahye H, Yin B, et al. Migration: a notable feature of cerebral sparganosis on follow-up MR imaging. *AJNR Am J Neuroradiol* 2012.
151. Michelet L, Fleury A, Sciutto E, et al. Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid. *J Clin Microbiol* 2011;**49**:195–200.
153. Mohindra S, Savardekar A, Gupta R, et al. Varied types of intracranial hydatid cysts: radiological features and management techniques. *Acta Neurochir (Wien)* 2012;**154**:165–72.
159. Nash TE, Garcia HH. Diagnosis and treatment of neurocysticercosis. *Nat Rev Neurol* 2011;**7**:584–94.
161. Nash TE, Pretell EJ, Lescano AG, et al. Perilesional brain oedema and seizure activity in patients with calcified neurocysticercosis: a prospective cohort and nested case-control study. *Lancet Neurol* 2008;**7**:1099–105.
164. Ndimubanzi PC, Carabin H, Budke CM, et al. A systematic review of the frequency of neurocyticercosis with a focus on people with epilepsy. *PLoS Negl Trop Dis* 2010;**4**:e870.
165. Ngowi HA, Kassuku AA, Carabin H, et al. Spatial clustering of porcine cysticercosis in Mbulu district, northern Tanzania. *PLoS Negl Trop Dis* 2010;**4**:e652.
166. Nourbakhsh A, Vannemreddy P, Minagar A, et al. Hydatid disease of the central nervous system: a review of literature with an emphasis on Latin American countries. *Neurol Res* 2010;**32**:245–51.
167. O'Neal S, Noh J, Wilkins P, et al. *Taenia solium* tapeworm infection, Oregon, 2006-2009. *Emerg Infect Dis* 2011;**17**:1030–6.
168. O'Neal SE, Moyano LM, Ayvar V, et al. Geographic correlation between tapeworm carriers and heavily infected cysticercotic pigs. *PLoS Negl Trop Dis* 2012;**6**:e1953.
174. Prestes-Carneiro LE, Souza DH, Moreno GC, et al. Toxocariasis/cysticercosis seroprevalence in a long-term rural settlement, São Paulo, Brazil. *Parasitology* 2009;**136**:681–9.
181. Rajshekhar V. Surgical management of neurocysticercosis. *Int J Surg* 2010;**8**:100–4.
190. Reyes JL, Espinoza-Jimenez AF, Gonzalez MI, et al. *Taenia crassiceps* infection abrogates experimental autoimmune encephalomyelitis. *Cell Immunol* 2011;**267**:77–87.
192. Robinson P, Garza A, Weinstock J, et al. Substance P causes seizures in neurocysticercosis. *PLoS Pathog* 2012;**8**:e1002489.
193. Rodrigues CL, de Andrade DC, Livramento JA, et al. Spectrum of cognitive impairment in neurocysticercosis: differences according to disease phase. *Neurology* 2012;**78**:861–6.
194. Rodriguez S, Dorny P, Tsang VC, et al. Detection of *Taenia solium* antigens and anti-*T. solium* antibodies in paired serum and cerebrospinal fluid samples from patients with intraparenchymal or extraparenchymal neurocysticercosis. *J Infect Dis* 2009;**199**:1345–52.
200. Sabshin SJ, Levy JK, Tupler T, et al. Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. *J Am Vet Med Assoc* 2012;**241**:331–7.
206. Schantz P, Schantz PM, Kern P, et al. Echinococcosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 824–38.
210. Scholz T, Garcia HH, Kuchta R, et al. Update on the human broad tapeworm (genus *Diphyllobothrium*), including clinical relevance. *Clin Microbiol Rev* 2009;**22**:146–60, Table of Contents.
212. Serpa JA, White AC Jr. Neurocysticercosis in the United States. *Pathog Glob Health* 2012;**106**:256–60.
213. Serpa JA, Graviss EA, Kass JS, et al. Neurocysticercosis in Houston, Texas: an update. *Medicine (Baltimore)* 2011;**90**:81–6.
218. Singh G, Rajshekhar V, Murthy JM, et al. A diagnostic and therapeutic scheme for a solitary cysticercus granuloma. *Neurology* 2010;**75**:2236–45.
234. Tanowitz HB, Wittner M, White AC Jr. Introduction to tapeworm infections. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 813–14.
252. Viola GM, White AC, Serpa JA Jr. Hemorrhagic cerebrovascular events and neurocysticercosis: a case report and review of the literature. *Am J Trop Med Hyg* 2011;**84**:402–5.
253. Wallin MT, Pretell EJ, Bustos JA, et al. Cognitive changes and quality of life in neurocysticercosis: a longitudinal study. *PLoS Negl Trop Dis* 2012;**6**:e1493.
255. White AC Jr. New developments in the management of neurocysticercosis. *J Infect Dis* 2009;**199**:1261–2.
259. Wittner M, White AC Jr, Tanowitz HB. *Taenia* and other tapeworms. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 839–47.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Akhan O, Ozmen MN, Dincer A, et al. Percutaneous treatment of pulmonary hydatid cysts. *Cardiovasc Intervent Radiol* 1994;**17**: 271–5.
2. Allan JC, Craig PS. Coproantigens in taeniasis and echinococcosis. *Parasitol Int* 2006;**55**(Suppl.):S75–80.
3. Allan JC, Velasquez-Tohom M, Fletes C, et al. Mass chemotherapy for intestinal *Taenia solium* infection: effect on prevalence in humans and pigs. *Trans R Soc Trop Med Hyg* 1997;**91**:595–8.
4. Allan JC, Velasquez-Tohom M, Torres-Alvarez R, et al. Field trial of the coproantigen-based diagnosis of *Taenia solium* taeniasis by enzyme-linked immunosorbent assay. *Am J Trop Med Hyg* 1996; **54**:352–6.
5. Ambekar S, Prasad C, Dwarakanath S, et al. MRS findings in cerebral coenurosis due to *Taenia multiceps*. *J Neuroimaging* 2013; **23**:149–51.
6. Anantaphruti MT, Nawa Y, Vanvanitchai Y. Human sparganosis in Thailand: an overview. *Acta Trop* 2011;**118**:171–6.
7. Anonymous. Pathogenesis of the tapeworm anaemia. *Br Med J* 1976;**2**:1028.
8. Anonymous. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003;**85**:253–61.
9. Auche Y, Bernard JP, Faivre J. [Massive blood eosinophilia and taeniasis]. *Arch Fr Mal App Dig* 1971;**60**:491–2.
10. Ayles HM, Corbett EL, Taylor I, et al. A combined medical and surgical approach to hydatid disease: 12 years' experience at the Hospital for Tropical Diseases, London. *Ann R Coll Surg Engl* 2002;**84**:100–5.
11. Barinagarrementeria F, Cantu C. Frequency of cerebral arteritis in subarachnoid cysticercosis: an angiographic study. *Stroke* 1998; **29**:123–5.
12. Benifla M, Barrelly R, Shelef I, et al. Huge hemispheric intraparenchymal cyst caused by *Taenia multiceps* in a child. Case report. *J Neurosurg* 2007;**107**:511–14.
13. Bildik N, Cevik A, Altintas M, et al. Efficacy of preoperative albendazole use according to months in hydatid cyst of the liver. *J Clin Gastroenterol* 2007;**41**:312–16.
14. Bo G, Xuejian W. Neuroimaging and pathological findings in a child with cerebral sparganosis: case report. *J Neurosurg* 2006; **105**:470–2.
15. Bresson-Hadni S, Delabrousse E, Blagosklonov O, et al. Imaging aspects and non-surgical interventional treatment in human alveolar echinococcosis. *Parasitol Int* 2006;**55**(Suppl.):S267–72.
16. Brunetti E, Garcia HH, Junghanss T. Cystic echinococcosis: chronic, complex, and still neglected. *PLoS Negl Trop Dis* 2011;**5**: e1146.
17. Brunetti E, Kern P, Vuitton DA. Expert consensus for the diagnosis and treatment of cystic and alveolar echinococcosis in humans. *Acta Trop* 2010;**114**:1–16.
18. Brunetti E, Troia G, Garlaschelli AL, et al. Twenty years of percutaneous treatments for cystic echinococcosis: a preliminary assessment of their use and safety. *Parassitologia* 2004;**46**:367–70.
19. Brunetti E, White AC Jr. Cestode infestations: hydatid disease and cysticercosis. *Infect Dis Clin North Am* 2012;**26**:421–35.
20. Bruschi F, Masetti M, Locci MT, et al. Short report: cysticercosis in an Egyptian mummy of the late Ptolemaic period. *Am J Trop Med Hyg* 2006;**74**:598–9.
21. Budke CM, Deplazes P, Torgerson PR. Global socioeconomic impact of cystic echinococcosis. *Emerg Infect Dis* 2006;**12**:296–303.
22. Budke CM, White AC Jr, Garcia HH. Zoonotic larval cestode infections: neglected, neglected tropical diseases? *PLoS Negl Trop Dis* 2009;**3**:e319.
23. Busic Z, Lemac D, Stipancic I, et al. Surgical treatment of liver echinococcosis: the role of laparoscopy. *Acta Chir Belg* 2006;**106**: 688–91.
24. Bustos JA, Montoya E, Garcia HH. Residual brain calcifications in neurocysticercosis. *Am J Trop Med Hyg* 2008;**78**:363.
25. Callacondo D, Garcia HH, Gonzales I, et al. High frequency of spinal involvement in patients with basal subarachnoid neurocysticercosis. *Neurology* 2012;**78**:1394–400.
26. Carpio A, Hauser WA. Prognosis for seizure recurrence in patients with newly diagnosed neurocysticercosis. *Neurology* 2002;**59**: 1730–4.
27. Chappell CL, Enos JP, Penn HM. *Dipylidium caninum*, an underrecognized infection in infants and children. *Pediatr Infect Dis J* 1990;**9**:745–7.
28. Chayasirisobhon S, Menoni R, Chayasirisobhon W, et al. Correlation of electroencephalography and the active and inactive forms of neurocysticercosis. *Clin Electroencephalogr* 1999;**30**:9–11.
29. Chermette R, Bussieras J, Marionneau J, et al. [Invasive cysticercosis due to *Taenia crassiceps* in an AIDS patient]. *Bull Acad Natl Med* 1995;**179**:777–80; discussion 780–73.
30. Chero JC, Saito M, Bustos JA, et al. *Hymenolepis nana* infection: symptoms and response to nitazoxanide in field conditions. *Trans R Soc Trop Med Hyg* 2007;**101**:203–5.
31. Chitchang S, Piamjinda T, Yodmani B, et al. Relationship between severity of the symptom and the number of *Hymenolepis nana* after treatment. *J Med Assoc Thai* 1985;**68**:423–6.
32. Chuck RS, Olk RJ, Weil GJ, et al. Surgical removal of a subretinal proliferating cysticercus of *Taeniaeformis crassiceps*. *Arch Ophthalmol* 1997;**115**:562–3.
33. Ciampi de Andrade D, Rodrigues CL, Abraham R, et al. Cognitive impairment and dementia in neurocysticercosis: a cross-sectional controlled study. *Neurology* 2010;**74**:1288–95.
34. Ciurea AV, Fountas KN, Coman TC, et al. Long-term surgical outcome in patients with intracranial hydatid cyst. *Acta Neurochir (Wien)* 2006;**148**:421–6.
35. Corrall CJ 2nd, Appel BL. Sparganosis: a clinical and pathologic observation of the first observed case in a child. *Pediatr Infect Dis J* 1987;**6**:481–5.
36. Coyle CM, Mahanty S, Zunt JR, et al. Neurocysticercosis: neglected but not forgotten. *PLoS Negl Trop Dis* 2012;**6**:e1500.
37. Craig P, Ito A. Intestinal cestodes. *Curr Opin Infect Dis* 2007; **20**:524–32.
38. Craig PS, Larrieu E. Control of cystic echinococcosis/hydatidosis: 1863-2002. *Adv Parasitol* 2006;**61**:443–508.
39. Craig PS, McManus DP, Lightowlers MW, et al. Prevention and control of cystic echinococcosis. *Lancet Infect Dis* 2007;**7**:385–94.
40. Crompton DW. How much human helminthiasis is there in the world? *J Parasitol* 1999;**85**:397–403.
41. Deckers N, Dorny P. Immunodiagnosis of *Taenia solium* taeniosis/cysticercosis. *Trends Parasitol* 2010;**26**:137–44.
42. DeGiorgio C, Pietsch-Escueta S, Tsang V, et al. Sero-prevalence of *Taenia solium* cysticercosis and *Taenia solium* taeniasis in California, USA. *Acta Neurol Scand* 2005;**111**:84–8.
43. Del Brutto OH. Albendazole therapy for subarachnoid cysticerci: clinical and neuroimaging analysis of 17 patients. *J Neurol Neurosurg Psychiatry* 1997;**62**:659–61.
44. Del Brutto OH. Meningeal cysticercosis. In: Singh G, Prabhakar S, editors. *Taenia solium cysticercosis: from basic to clinical science*. Oxford: CABI; 2002. p. 177–88.
45. Del Brutto OH. Diagnostic criteria for neurocysticercosis, revisited. *Pathog Glob Health* 2012;**106**:299–304.
46. Del Brutto OH, Rajshekhar V, White AC Jr, et al. Proposed diagnostic criteria for neurocysticercosis. *Neurology* 2001;**57**: 177–83.
47. Del Brutto OH, Roos KL, Coffey CS, et al. Meta-analysis: Cysticidal drugs for neurocysticercosis: albendazole and praziquantel. *Ann Intern Med* 2006;**145**:43–51.
48. Del Brutto OH, Santibanez R, Idrovo L, et al. Epilepsy and neurocysticercosis in Atahualpa: a door-to-door survey in rural coastal Ecuador. *Epilepsia* 2005;**46**:583–7.
49. Del Carpio M, Mercapide CH, Salvitti JC, et al. Early diagnosis, treatment and follow-up of cystic echinococcosis in remote rural areas in Patagonia: impact of ultrasound training of non-specialists. *PLoS Negl Trop Dis* 2012;**6**:e1444.
50. del la Garza Y, Graviss EA, Daver NG, et al. Epidemiology of neurocysticercosis in Houston, Texas. *Am J Trop Med Hyg* 2005; **73**:766–70.
51. Deng L, Xiong P, Qian S. Diagnosis and stereotactic aspiration treatment of cerebral sparganosis: summary of 11 cases. *J Neurosurg* 2011;**114**:1421–5.
52. Dixon HBF, Lipscomb FM. *Cysticercosis: an analysis and follow-up of 450 cases*, vol. 299. London: Her Majesty's Stationary Service; 1961.
53. Dogru D, Kiper N, Ozcelik U, et al. Medical treatment of pulmonary hydatid disease: for which child? *Parasitol Int* 2005; **54**:135–8.

54. Duishanbai S, Geng D, Liu C, et al. Treatment of intracranial hydatid cysts. *Chin Med J (Engl)* 2011;**124**:2954–8.
55. Duishanbai S, Jiafu D, Guo H, et al. Intracranial hydatid cyst in children: report of 30 cases. *Childs Nerv Syst* 2010;**26**:821–7.
56. Dupouy-Camet J, Peduzzi R. Current situation of human diphyllobothriasis in Europe. *Euro Surveill* 2004;**9**:31–5.
57. Dziri C, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004;**28**:731–6.
58. Eckert J, Deplazes P. Biological, epidemiological, and clinical aspects of echinococcosis, a zoonosis of increasing concern. *Clin Microbiol Rev* 2004;**17**:107–35.
59. Eckert J, Gemmell MA, Meslin F-X, et al, editors. *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern*. Paris: World Organization for Animal Health; 2001.
60. El-On J, Shelef I, Cagnano E, et al. *Taenia multiceps:* a rare human cestode infection in Israel. *Vet Ital* 2008;**44**:621–31.
61. Eom KS. What is Asian *Taenia*? *Parasitol Int* 2006;**55**(Suppl.): S137–41.
62. Espindola NM, Vaz AJ, Pardini AX, et al. Excretory/secretory antigens (ES) from in-vitro cultures of *Taenia crassiceps* cysticerci, and use of an anti-ES monoclonal antibody for antigen detection in samples of cerebrospinal fluid from patients with neurocysticercosis. *Ann Trop Med Parasitol* 2002;**96**:361–8.
63. Falagas ME, Bliziotis IA. Albendazole for the treatment of human echinococcosis: a review of comparative clinical trials. *Am J Med Sci* 2007;**334**:171–9.
64. Filice C, Brunetti E, Bruno R, et al. Percutaneous drainage of echinococcal cysts (PAIR: puncture, aspiration, injection, reaspiration): results of a worldwide survey for assessment of its safety and efficacy. WHO-Informal Working Group on Echinococcosis-Pair Network. *Gut* 2000;**47**:156–7.
65. Fleury A, Gomez T, Alvarez I, et al. High prevalence of calcified silent neurocysticercosis in a rural village of Mexico. *Neuroepidemiology* 2003;**22**:139–45.
66. Flisser A, Gauci CG, Zoli A, et al. Induction of protection against porcine cysticercosis by vaccination with recombinant oncosphere antigens. *Infect Immun* 2004;**72**:5292–7.
67. Forlenza OV, Filho AH, Nobrega JP, et al. Psychiatric manifestations of neurocysticercosis: a study of 38 patients from a neurology clinic in Brazil. *J Neurol Neurosurg Psychiatry* 1997;**62**:612–16.
68. Franchi C, Di Vico B, Teggi A. Long-term evaluation of patients with hydatidosis treated with benzimidazole carbamates. *Clin Infect Dis* 1999;**29**:304–9.
69. Francois A, Favennec L, Cambon-Michot C, et al. *Taenia crassiceps* invasive cysticercosis: a new human pathogen in acquired immunodeficiency syndrome? *Am J Surg Pathol* 1998;**22**:488–92.
70. Garcia HH, Coyle CM, White AC Jr. Cysticercosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 815–23.
71. Garcia HH, Del Brutto OH. Heavy nonencephalitic cerebral cysticercosis in tapeworm carriers. The Cysticercosis Working Group in Peru. *Neurology* 1999;**53**:1582–4.
72. Garcia HH, Del Brutto OH, Nash TE, et al. New concepts in the diagnosis and management of neurocysticercosis *(Taenia solium)*. *Am J Trop Med Hyg* 2005;**72**:3–9.
73. Garcia HH, Evans CA, Nash TE, et al. Current consensus guidelines for treatment of neurocysticercosis. *Clin Microbiol Rev* 2002; **15**:747–56.
74. Garcia HH, Gilman RH, Gonzalez AE, et al. Human and porcine *Taenia solium* infection in a village in the highlands of Cusco, Peru. The Cysticercosis Working Group in Peru. *Acta Trop* 1999; **73**:31–6.
75. Garcia HH, Gilman RH, Gonzalez AE, et al. Hyperendemic human and porcine *Taenia solium* infection in Peru. *Am J Trop Med Hyg* 2003;**68**:268–75.
76. Garcia HH, Gonzalez AE, Del Brutto OH, et al. Strategies for the elimination of taeniasis/cysticercosis. *J Neurol Sci* 2007;**262**: 153–7.
77. Garcia HH, Gonzalez AE, Gilman RH, et al. Circulating parasite antigen in patients with hydrocephalus secondary to neurocysticercosis. *Am J Trop Med Hyg* 2002;**66**:427–30.
78. Garcia HH, Gonzalez AE, Rodriguez S, et al. Neurocysticercosis: unraveling the nature of the single cysticercal granuloma. *Neurology* 2010;**75**:654–8.
79. Garcia HH, Harrison LJ, Parkhouse RM, et al. A specific antigen-detection ELISA for the diagnosis of human neurocysticercosis. The Cysticercosis Working Group in Peru. *Trans R Soc Trop Med Hyg* 1998;**92**:411–14.
80. Garcia HH, Lescano AG, Lanchote VL, et al. Pharmacokinetics of combined treatment with praziquantel and albendazole in neurocysticercosis. *Br J Clin Pharmacol* 2011;**72**:77–84.
81. Garcia HH, Parkhouse RM, Gilman RH, et al. Serum antigen detection in the diagnosis, treatment, and follow-up of neurocysticercosis patients. *Trans R Soc Trop Med Hyg* 2000;**94**:673–6.
82. Garcia HH, Pretell EJ, Gilman RH, et al. A trial of antiparasitic treatment to reduce the rate of seizures due to cerebral cysticercosis. *N Engl J Med* 2004;**350**:249–58.
83. Garcia-Noval J, Moreno E, de Mata F, et al. An epidemiological study of epilepsy and epileptic seizures in two rural Guatemalan communities. *Ann Trop Med Parasitol* 2001;**95**:167–75.
84. Garg RK, Kar AM, Singh MK. Prednisolone-responsive headache in patients with solitary cysticercus granuloma and seizures. *Headache* 2004;**44**:365–9.
85. Garg RK, Karak B, Mohan Kar A. Neuroimaging abnormalities in Indian patients with uncontrolled partial seizures. *Seizure* 1998;**7**:497–500.
86. Garg RK, Potluri N, Kar AM, et al. Short course of prednisolone in patients with solitary cysticercus granuloma: a double blind placebo controlled study. *J Infect* 2006;**53**:65–9.
87. Garin JP, Mojon M. [Blood eosinophilia during taeniasis due to *T. saginata*]. *Lyon Med* 1972;**228**:339–43.
88. Gauci CG, Jayashi CM, Gonzalez AE, et al. Protection of pigs against *Taenia solium* cysticercosis by immunization with novel recombinant antigens. *Vaccine* 2012;**30**:3824–8.
89. Gekeler F, Eichenlaub S, Mendoza EG, et al. Sensitivity and specificity of ELISA and immunoblot for diagnosing neurocysticercosis. *Eur J Clin Microbiol Infect Dis* 2002;**21**:227–9.
90. Gezen F, Baysefer A, Koksel T, et al. Hydatid cysts of the brain. *Clin Infect Dis* 1995;**21**:938–42.
91. Goesseringer N, Lindenblatt N, Mihic-Probst D, et al. *Taenia crassiceps* upper limb fasciitis in a patient with untreated acquired immunodeficiency syndrome and chronic hepatitis C infection: the role of surgical debridement. *J Plast Reconstr Aesthet Surg* 2011;**64**:e174–6.
92. Gonzalez AE, Garcia HH, Gilman RH, et al. Control of *Taenia solium*. *Acta Trop* 2003;**87**:103–9.
93. Gonzalez AE, Gauci CG, Barber D, et al. Vaccination of pigs to control human neurocysticercosis. *Am J Trop Med Hyg* 2005;**72**: 837–9.
94. Gravori T, Steineke T, Bergsneider M. Endoscopic removal of cisternal neurocysticercal cysts. Technical note. *Neurosurg Focus* 2002;**12**:e7.
95. Gupta RK, Kumar R, Chawla S, et al. Demonstration of scolex within calcified cysticercus cyst: its possible role in the pathogenesis of perilesional edema. *Epilepsia* 2002;**43**:1502–8.
96. Gutierrez Y. Cysticercosis, coenurosis, and sparganosis. In: Gutierrez Y, editor. *Diagnostic pathology of parasitic infection with clinical correlation*. Oxford: Oxford University Press; 2000. p. 635–74.
97. Hancock K, Pattabhi S, Greene RM, et al. Characterization and cloning of GP50, a *Taenia solium* antigen diagnostic for cysticercosis. *Mol Biochem Parasitol* 2004;**133**:115–24.
98. Hauptman JS, Hinrichs C, Mele C, et al. Radiologic manifestations of intraventricular and subarachnoid racemose neurocysticercosis. *Emerg Radiol* 2005;**11**:153–7.
99. Headley SA, Gillen MA, Sanches AW, et al. Platynosomum fastosum-induced chronic intrahepatic cholangitis and *Spirometra* spp. infections in feral cats from Grand Cayman. *J Helminthol* 2012;**86**:209–14.
100. Heldwein K, Biedermann HG, Hamperl WD, et al. Subcutaneous *Taenia crassiceps* infection in a patient with non-Hodgkin's lymphoma. *Am J Trop Med Hyg* 2006;**75**:108–11.
101. Hoberg EP. Phylogeny of *Taenia:* species definitions and origins of human parasites. *Parasitol Int* 2006;**55**(Suppl.):S23–30.
102. Hosch W, Stojkovic M, Janisch T, et al. MR imaging for diagnosing cysto-biliary fistulas in cystic echinococcosis. *Eur J Radiol* 2008;**66**:262–7.
103. Huisa BN, Menacho LA, Rodriguez S, et al. Taeniasis and cysticercosis in housemaids working in affluent neighborhoods in Lima, Peru. *Am J Trop Med Hyg* 2005;**73**:496–500.

104. Husain M, Jha DK, Rastogi M, et al. Neuro-endoscopic management of intraventricular neurocysticercosis (NCC). *Acta Neurochir (Wien)* 2007;**149**:341–6.
105. Husain M, Rastogi M, Jha DK, et al. Endoscopic transaqueductal removal of fourth ventricular neurocysticercosis with an angiographic catheter. *Neurosurgery* 2007;**60**:249–53; discussion 254.
106. Ibechukwu BI, Onwukeme KE. Intraocular coenurosis: a case report. *Br J Ophthalmol* 1991;**75**:430–1.
107. Ing MB, Schantz PM, Turner JA. Human coenurosis in North America: case reports and review. *Clin Infect Dis* 1998;**27**:519–23.
108. Ishida MM, Almeida MS, Espindola NM, et al. Seroepidemiological study of human cysticercosis with blood samples collected on filter paper, in Lages, State of Santa Catarina, Brazil, 2004-2005. *Rev Soc Bras Med Trop* 2011;**44**:339–43.
109. Ito A, Nakao M, Wandra T. Human taeniasis and cysticercosis in Asia. *Lancet* 2003;**362**:1918–20.
110. Ito A, Nakaya K, Sako Y, et al. NOD-scid mouse as an experimental animal model for cysticercosis. *Southeast Asian J Trop Med Public Health* 2001;**32**(Suppl. 2):85–9.
111. Itoh N, Ikegami H, Takagi M, et al. Prevalence of intestinal parasites in private-household cats in Japan. *J Feline Med Surg* 2012;**14**:436–9.
112. Jayashi CM, Kyngdon CT, Gauci CG, et al. Successful immunization of naturally ñ reared pigs against porcine cysticercosis with a recombinant oncosphere antigen vaccine. *Vet Parasitol* 2012;**188**: 261–7.
113. Jenkins DJ, Romig T, Thompson RC. Emergence/re-emergence of *Echinococcus* spp.: a global update. *Int J Parasitol* 2005;**35**: 1205–19.
114. Jeri C, Gilman RH, Lescano AG, et al. Species identification after treatment for human taeniasis. *Lancet* 2004;**363**:949–50.
115. Jimenez JA, Rodriguez S, Gamboa R, et al. *Diphyllobothrium pacificum* infection is seldom associated with megaloblastic anemia. *Am J Trop Med Hyg* 2012;**87**:897–901.
116. Kappus KD, Lundgren RG Jr, Juranek DD, et al. Intestinal parasitism in the United States: update on a continuing problem. *Am J Trop Med Hyg* 1994;**50**:705–13.
117. Kaur S, Singhi P, Singhi S, et al. Combination therapy with albendazole and praziquantel versus albendazole alone in children with seizures and single lesion neurocysticercosis: a randomized, placebo-controlled double blind trial. *Pediatr Infect Dis J* 2009; **28**:403–6.
118. Kaushal S, Rani A, Chopra SC, et al. Safety and efficacy of clobazam versus phenytoin-sodium in the antiepileptic drug treatment of solitary cysticercus granulomas. *Neurol India* 2006;**54**:157–60.
119. Kelley R, Duong DH, Locke GE. Characteristics of ventricular shunt malfunctions among patients with neurocysticercosis. *Neurosurgery* 2002;**50**:757–61; discussion 761–2.
120. Keramidas D, Mavridis G, Soutis M, et al. Medical treatment of pulmonary hydatidosis: complications and surgical management. *Pediatr Surg Int* 2004;**19**:774–6.
121. Kern P, Bardonnet K, Renner E, et al. European echinococcosis registry: human alveolar echinococcosis, Europe, 1982-2000. *Emerg Infect Dis* 2003;**9**:343–9.
122. Keshmiri M, Baharvahdat H, Fattahi SH, et al. Albendazole versus placebo in treatment of echinococcosis. *Trans R Soc Trop Med Hyg* 2001;**95**:190–4.
123. Khaldi M, Mohamed S, Kallel J, et al. Brain hydatidosis: report on 117 cases. *Childs Nerv Syst* 2000;**16**:765–9.
124. Khuroo MS, Dar MY, Yattoo GN, et al. Percutaneous drainage versus albendazole therapy in hepatic hydatidosis: a prospective, randomized study. *Gastroenterology* 1993;**104**:1452–9.
125. Khuroo MS, Wani NA, Javid G, et al. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997;**337**:881–7.
126. Kim DG, Paek SH, Chang KH, et al. Cerebral sparganosis: clinical manifestations, treatment, and outcome. *J Neurosurg* 1996;**85**: 1066–71.
127. Kishore D, Misra S. Short course of oral prednisolone on disappearance of lesion and seizure recurrence in patients of solitary cysticercal granuloma with single small enhancing CT lesion: an open label randomized prospective study. *J Assoc Physicians India* 2007;**55**:419–24.
128. Lapergue B, Hosseini H, Liance M, et al. Hydrocephalus and racemose cysticercosis: surgical alternative by endoscopic third ventriculostomy. *Neurochirurgie* 2005;**51**:481–8.
129. Larrieu E, Del Carpio M, Salvitti JC, et al. Ultrasonographic diagnosis and medical treatment of human cystic echinococcosis in asymptomatic school age carriers: 5 years of follow-up. *Acta Trop* 2004;**91**:5–13.
130. Lateef M, Zargar SA, Khan AR, et al. Successful treatment of niclosamide- and praziquantel-resistant beef tapeworm infection with nitazoxanide. *Int J Infect Dis* 2008;**12**:80–2.
131. Lee MK, Hong SJ, Kim HR. Seroprevalence of tissue invading parasitic infections diagnosed by ELISA in Korea. *J Korean Med Sci* 2010;**25**:1272–6.
132. Leite CC, Jinkins JR, Escobar BE, et al. MR imaging of intramedullary and intradural-extramedullary spinal cysticercosis. *AJR Am J Roentgenol* 1997;**169**:1713–17.
133. Lescano AG, Garcia HH, Gilman RH, et al. Swine cysticercosis hotspots surrounding Taenia solium tapeworm carriers. *Am J Trop Med Hyg* 2007;**76**:376–83.
134. Levav M, Mirsky AF, Cruz ME, et al. Neurocysticercosis and performance on neuropsychologic tests: a family study in Ecuador. *Am J Trop Med Hyg* 1995;**53**:552–7.
135. Levine MZ, Calderon JC, Wilkins PP, et al. Characterization, cloning, and expression of two diagnostic antigens for Taenia solium tapeworm infection. *J Parasitol* 2004;**90**:631–8.
136. Li MW, Song HQ, Li C, et al. Sparganosis in mainland China. *Int J Infect Dis* 2011;**15**:e154–6.
137. Li YX, Ramsahye H, Yin B, et al. Migration: a notable feature of cerebral sparganosis on follow-up MR imaging. *AJNR Am J Neuroradiol* 2012.
138. Lightowlers MW. Cestode vaccines: origins, current status and future prospects. *Parasitology* 2006;**133**(Suppl.):S27–42.
139. Londono DP, Alvarez JI, Trujillo J, et al. The inflammatory cell infiltrates in porcine cysticercosis: immunohistochemical analysis during various stages of infection. *Vet Parasitol* 2002;**109**: 249–59.
140. Lucas SB, Hassounah OA, Doenhoff M, et al. Aberrant form of *Hymenolepis nana:* possible opportunistic infection in immunosuppressed patients. *Lancet* 1979;**2**:1372–3.
141. Lumbreras H, Terashima A, Alvarez H, et al. Single dose treatment with praziquantel (Cesol R, EmBay 8440) of human cestodiasis caused by *Diphyllobothrium pacificum*. *Tropenmed Parasitol* 1982;**33**:5–7.
142. Maazoun K, Mekki M, Chioukh FZ, et al. Laparoscopic treatment of hydatid cyst of the liver in children: a report on 34 cases. *J Pediatr Surg* 2007;**42**:1683–6.
143. Macnish MG, Morgan-Ryan UM, Monis PT, et al. A molecular phylogeny of nuclear and mitochondrial sequences in *Hymenolepis nana* (Cestoda) supports the existence of a cryptic species. *Parasitology* 2002;**125**:567–75.
144. Maillard H, Marionneau J, Prophette B, et al. *Taenia crassiceps* cysticercosis and AIDS. *AIDS* 1998;**12**:1551–2.
145. Mall RK, Agarwal A, Garg RK, et al. Short course of prednisolone in Indian patients with solitary cysticercus granuloma and new-onset seizures. *Epilepsia* 2003;**44**:1397–401.
146. Marquez-Caraveo C, Gongora-Rivera F, Santos Zambrano J, et al. Pre-treatment with corticosteroids and a single cycle of high dose albendazole for subarachnoidal cysticercosis. *J Neurol Neurosurg Psychiatry* 2004;**75**:938–9.
147. Mawhorter S, Temeck B, Chang R, et al. Nonsurgical therapy for pulmonary hydatid cyst disease. *Chest* 1997;**112**:1432–6.
148. Mayta H, Gilman RH, Prendergast E, et al. Nested PCR for specific diagnosis of *Taenia solium* taeniasis. *J Clin Microbiol* 2008; **46**:286–9.
149. McIntyre A. Hydatid disease in children in South Australia. *Med J Aust* 1971;**1**:1064–5.
150. McManus DP, Zhang W, Li J, et al. Echinococcosis. *Lancet* 2003;**362**:1295–304.
151. Michelet L, Fleury A, Sciutto E, et al. Human neurocysticercosis: comparison of different diagnostic tests using cerebrospinal fluid. *J Clin Microbiol* 2011;**49**:195–200.
152. Mitre E, Talaat KR, Sperling MR, et al. Methotrexate as a corticosteroid-sparing agent in complicated neurocysticercosis. *Clin Infect Dis* 2007;**44**:549–53.
153. Mohindra S, Savardekar A, Gupta R, et al. Varied types of intracranial hydatid cysts: radiological features and management techniques. *Acta Neurochir (Wien)* 2012;**154**:165–72.
154. Molina CP, Ogburn J, Adegboyega P. Infection by *Dipylidium caninum* in an infant. *Arch Pathol Lab Med* 2003;**127**:e157–9.

155. Montano SM, Villaran MV, Ylquimiche L, et al. Neurocysticercosis: association between seizures, serology, and brain CT in rural Peru. *Neurology* 2005;**65**:229–33.
156. Moro P, Schantz PM. Cystic echinococcosis in the Americas. *Parasitol Int* 2006;**55**(Suppl.):S181–6.
157. Mwape KE, Praet N, Benitez-Ortiz W, et al. Field evaluation of urine antigen detection for diagnosis of *Taenia solium* cysticercosis. *Trans R Soc Trop Med Hyg* 2011;**105**:574–8.
158. Nakamura T, Hara M, Matsuoka M, et al. Human proliferative sparganosis: a new Japanese case. *Am J Clin Pathol* 1990;**94**: 224–8.
159. Nash TE, Del Brutto OH, Butman JA, et al. Calcific neurocysticercosis and epileptogenesis. *Neurology* 2004;**62**:1934–8.
160. Nash TE, Garcia HH. Diagnosis and treatment of neurocysticercosis. *Nat Rev Neurol* 2011;**7**:584–94.
161. Nash TE, Pretell EJ, Lescano AG, et al. Perilesional brain oedema and seizure activity in patients with calcified neurocysticercosis: a prospective cohort and nested case-control study. *Lancet Neurol* 2008;**7**:1099–105.
162. Nash TE, Singh G, White AC, et al. Treatment of neurocysticercosis: current status and future research needs. *Neurology* 2006;**67**: 1120–7.
163. Nasseri Moghaddam S, Abrishami A, Malekzadeh R. Percutaneous needle aspiration, injection, and reaspiration with or without benzimidazole coverage for uncomplicated hepatic hydatid cysts. *Cochrane Database Syst Rev* 2006;(2):CD003623.
164. Ndimubanzi PC, Carabin H, Budke CM, et al. A systematic review of the frequency of neurocyticercosis with a focus on people with epilepsy. *PLoS Negl Trop Dis* 2010;**4**:e870.
165. Ngowi HA, Kassuku AA, Carabin H, et al. Spatial clustering of porcine cysticercosis in Mbulu district, northern Tanzania. *PLoS Negl Trop Dis* 2010;**4**:e652.
166. Nourbakhsh A, Vannemreddy P, Minagar A, et al. Hydatid disease of the central nervous system: a review of literature with an emphasis on Latin American countries. *Neurol Res* 2010;**32**: 245–51.
167. O'Neal S, Noh J, Wilkins P, et al. *Taenia solium* tapeworm infection, Oregon, 2006-2009. *Emerg Infect Dis* 2011;**17**:1030–6.
168. O'Neal SE, Moyano LM, Ayvar V, et al. Geographic correlation between tapeworm carriers and heavily infected cysticercotic pigs. *PLoS Negl Trop Dis* 2012;**6**:e1953.
169. Ong S, Talan DA, Moran GJ, et al. Neurocysticercosis in radiographically imaged seizure patients in U.S. emergency departments. *Emerg Infect Dis* 2002;**8**:608–13.
170. Ortiz JJ, Lopez Chegne N, Gargala G, et al. Comparative clinical studies of nitazoxanide, albendazole and praziquantel in the treatment of ascariasis, trichuriasis and hymenolepiasis in children from Peru. *Trans R Soc Trop Med Hyg* 2002;**96**:193–6.
171. Pardini AX, Vaz AJ, Dos Ramos Machado L, et al. Cysticercus antigens in cerebrospinal fluid samples from patients with neurocysticercosis. *J Clin Microbiol* 2001;**39**:3368–72.
172. Pawlowski ZS. Echinococcosis in humans: clinical aspects, diagnosis, and treatment. In: Eckert J, Gemmell MA, Meslin F-X, et al, editors. *WHO/OIE manual on echinococcosis in humans and animals: a public health problem of global concern*. Paris: World Organization for Animal Health; 2001. p. 20–71.
173. Prakash S, Garg RK, Kar AM, et al. Intravenous methyl prednisolone in patients with solitary cysticercus granuloma: a random evaluation. *Seizure* 2006;**15**:328–32.
174. Prestes-Carneiro LE, Souza DH, Moreno GC, et al. Toxocariasis/cysticercosis seroprevalence in a long-term rural settlement, São Paulo, Brazil. *Parasitology* 2009;**136**:681–9.
175. Proano JV, Madrazo I, Avelar F, et al. Medical treatment for neurocysticercosis characterized by giant subarachnoid cysts. *N Engl J Med* 2001;**345**:879–85.
176. Proano-Narvaez JV, Meza-Lucas A, Mata-Ruiz O, et al. Laboratory diagnosis of human neurocysticercosis: double-blind comparison of enzyme-linked immunosorbent assay and electroimmunotransfer blot assay. *J Clin Microbiol* 2002;**40**:2115–18.
177. Psarros TG, Coimbra C. Endoscopic third ventriculostomy for patients with hydrocephalus and fourth ventricular cysticercosis: a review of five cases. *Minim Invasive Neurosurg* 2004;**47**:346–9.
178. Psarros TG, Krumerman J, Coimbra C. Endoscopic management of supratentorial ventricular neurocysticercosis: case series and review of the literature. *Minim Invasive Neurosurg* 2003;**46**: 331–4.
179. Pushker N, Bajaj MS, Chandra M, et al. Ocular and orbital cysticercosis. *Acta Ophthalmol Scand* 2001;**79**:408–13.
180. Rajshekhar V. Severe episodic headache as the sole presenting ictal event in patients with a solitary cysticercus granuloma. *Acta Neurol Scand* 2000;**102**:44–6.
181. Rajshekhar V. Surgical management of neurocysticercosis. *Int J Surg* 2010;**8**:100–4.
182. Rajshekhar V, Chandy MJ. Validation of diagnostic criteria for solitary cerebral cysticercus granuloma in patients presenting with seizures. *Acta Neurol Scand* 1997;**96**:76–81.
183. Rajshekhar V, Jeyaseelan L. Seizure outcome in patients with a solitary cerebral cysticercus granuloma. *Neurology* 2004;**62**:2236–40.
184. Rajshekhar V, Raghava MV, Prabhakaran V, et al. Active epilepsy as an index of burden of neurocysticercosis in Vellore district, India. *Neurology* 2006;**67**:2135–9.
185. Ramos-Kuri M, Montoya RM, Padilla A, et al. Immunodiagnosis of neurocysticercosis: disappointing performance of serology (enzyme-linked immunosorbent assay) in an unbiased sample of neurological patients. *Arch Neurol* 1992;**49**:633–6.
186. Rangel R, Torres B, Del Bruto O, et al. Cysticercotic encephalitis: a severe form in young females. *Am J Trop Med Hyg* 1987;**36**: 387–92.
187. Rangel-Castilla L, Serpa JA, Gopinath SP, et al. Contemporary neurosurgical approaches to neurocysticercosis. *Am J Trop Med Hyg* 2009;**80**:373–8.
188. Restrepo BI, Alvarez JI, Castano JA, et al. Brain granulomas in neurocysticercosis patients are associated with a Th1 and Th2 profile. *Infect Immun* 2001;**69**:4554–60.
189. Restrepo BI, Llaguno P, Sandoval MA, et al. Analysis of immune lesions in neurocysticercosis patients: central nervous system response to helminth appears Th1-like instead of Th2. *J Neuroimmunol* 1998;**89**:64–72.
190. Reyes JL, Espinoza-Jimenez AF, Gonzalez MI, et al. *Taenia crassiceps* infection abrogates experimental autoimmune encephalomyelitis. *Cell Immunol* 2011;**267**:77–87.
191. Richards FO Jr, Schantz PM, Ruiz-Tiben E, et al. Cysticercosis in Los Angeles County. *JAMA* 1985;**254**:3444–8.
192. Robinson P, Garza A, Weinstock J, et al. Substance P causes seizures in neurocysticercosis. *PLoS Pathog* 2012;**8**:e1002489.
193. Rodrigues CL, de Andrade DC, Livramento JA, et al. Spectrum of cognitive impairment in neurocysticercosis: differences according to disease phase. *Neurology* 2012;**78**:861–6.
194. Rodriguez S, Dorny P, Tsang VC, et al. Detection of *Taenia solium* antigens and anti-*T. solium* antibodies in paired serum and cerebrospinal fluid samples from patients with intraparenchymal or extraparenchymal neurocysticercosis. *J Infect Dis* 2009;**199**: 1345–52.
195. Rodriguez-Canul R, Fraser A, Allan JC, et al. Epidemiological study of *Taenia solium* taeniasis/cysticercosis in a rural village in Yucatan state, Mexico. *Ann Trop Med Parasitol* 1999; **93**:57–67.
196. Rogan MT, Hai WY, Richardson R, et al. Hydatid cysts: does every picture tell a story? *Trends Parasitol* 2006;**22**:431–8.
197. Rosenfeld EA, Byrd SE, Shulman ST. Neurocysticercosis among children in Chicago. *Clin Infect Dis* 1996;**23**:262–8.
198. Ruiz-Garcia M, Gonzalez-Astiazaran A, Rueda-Franco F. Neurocysticercosis in children: clinical experience in 122 patients. *Childs Nerv Syst* 1997;**13**:608–12.
199. Saarni M, Nyberg W, Grasbeck R, et al. Symptoms in carriers of *Diphyllobothrium latum* and in non-infected controls. *Acta Med Scand* 1963;**173**:147–54.
200. Sabshin SJ, Levy JK, Tupler T, et al. Enteropathogens identified in cats entering a Florida animal shelter with normal feces or diarrhea. *J Am Vet Med Assoc* 2012;**241**:331–7.
201. Sadjjadi SM. Present situation of echinococcosis in the Middle East and Arabic North Africa. *Parasitol Int* 2006;**55**(Suppl.): S197–202.
202. Saenz B, Ruiz-Garcia M, Jimenez E, et al. Neurocysticercosis: clinical, radiologic, and inflammatory differences between children and adults. *Pediatr Infect Dis J* 2006;**25**:801–3.
203. Salgado P, Rojas R, Sotelo J. Cysticercosis: clinical classification based on imaging studies. *Arch Intern Med* 1997;**157**:1991–7.
204. Sarti E, Schantz PM, Avila G, et al. Mass treatment against human taeniasis for the control of cysticercosis: a population-based intervention study. *Trans R Soc Trop Med Hyg* 2000;**94**:85–9.

205. Sarti-Gutierrez EJ, Schantz PM, Lara-Aguilera R, et al. *Taenia solium* taeniasis and cysticercosis in a Mexican village. *Trop Med Parasitol* 1988;**39**:194–8.
206. Schantz P, Schantz PM, Kern P, et al. Echinococcosis. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 824–38.
207. Schantz PM, Moore AC, Munoz JL, et al. Neurocysticercosis in an Orthodox Jewish community in New York City. *N Engl J Med* 1992;**327**:692–5.
208. Schantz PM, Sarti E, Plancarte A, et al. Community-based epidemiological investigations of cysticercosis due to *Taenia solium:* comparison of serological screening tests and clinical findings in two populations in Mexico. *Clin Infect Dis* 1994;**18**:879–85.
209. Schipper HG, Lameris JS, van Delden OM, et al. Percutaneous evacuation (PEVAC) of multivesicular echinococcal cysts with or without cystobiliary fistulas which contain non-drainable material: first results of a modified PAIR method. *Gut* 2002;**50**:718–23.
210. Scholz T, Garcia HH, Kuchta R, et al. Update on the human broad tapeworm (genus *Diphyllobothrium*), including clinical relevance. *Clin Microbiol Rev* 2009;**22**:146–60, Table of Contents.
211. Sekhar GC, Lemke BN. Orbital cysticercosis. *Ophthalmology* 1997;**104**:1599–604.
212. Serpa JA, Graviss EA, Kass JS, et al. Neurocysticercosis in Houston, Texas: an update. *Medicine (Baltimore)* 2011;**90**:81–6.
213. Serpa JA, Moran A, Goodman JC, et al. Neurocysticercosis in the HIV era: a case report and review of the literature. *Am J Trop Med Hyg* 2007;**77**:113–17.
214. Serpa JA, White AC Jr. Neurocysticercosis in the United States. *Pathog Glob Health* 2012;**106**:256–60.
215. Shalabi RI, Ayed AK, Amin M. 15 Years in surgical management of pulmonary hydatidosis. *Ann Thorac Cardiovasc Surg* 2002;**8**: 131–4.
216. Shen C, Li S, Zheng S, et al. Tissue parasitic helminthiases are prevalent at Cheongjin, North Korea. *Korean J Parasitol* 2007;**45**: 139–44.
217. Singh G, Prabhakar S, Ito A, et al. Taenia solium taeniasis and cysticercosis in Asia. In: Singh G, Prabhakar S, editors. *Taenia solium cysticercosis: from basic to clinical science*. Oxford: CABI; 2002. p. 111–27.
218. Singh G, Rajshekhar V, Murthy JM, et al. A diagnostic and therapeutic scheme for a solitary cysticercus granuloma. *Neurology* 2010;**75**:2236–45.
219. Singh G, Sachdev MS, Tirath A, et al. Focal cortical-subcortical calcifications (FCSCs) and epilepsy in the Indian subcontinent. *Epilepsia* 2000;**41**:718–26.
220. Singh MK, Garg RK, Nath G, et al. Single small enhancing computed tomographic (CT) lesions in Indian patients with new-onset seizures: a prospective follow-up in 75 patients. *Seizure* 2001; **10**:573–8.
221. Singhi P, Jain V, Khandelwal N. Corticosteroids versus albendazole for treatment of single small enhancing computed tomographic lesions in children with neurocysticercosis. *J Child Neurol* 2004;**19**:323–7.
222. Singhi P, Ray M, Singhi S, et al. Clinical spectrum of 500 children with neurocysticercosis and response to albendazole therapy. *J Child Neurol* 2000;**15**:207–13.
223. Sirivichayakul C, Radomyos P, Praevanit R, et al. *Hymenolepis nana* infection in Thai children. *J Med Assoc Thai* 2000;**83**: 1035–8.
224. Sivrikoz MC, Tuncozgur B, Nacak I, et al. Surgical treatment of pulmonary hydatid cysts in pediatric patients. *Pediatr Pulmonol* 2003;**35**:87–90.
225. Smego RA Jr, Bhatti S, Khaliq AA, et al. Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. *Clin Infect Dis* 2003;**37**:1073–83.
226. Song T, Wang WS, Zhou BR, et al. CT and MR characteristics of cerebral sparganosis. *AJNR Am J Neuroradiol* 2007;**28**: 1700–5.
227. Sorvillo FJ, DeGiorgio C, Waterman SH. Deaths from cysticercosis, United States. *Emerg Infect Dis* 2007;**13**:230–5.
228. Sorvillo FJ, Portigal L, DeGiorgio C, et al. Cysticercosis-related deaths, California. *Emerg Infect Dis* 2004;**10**:465–9.
229. Sorvillo FJ, Waterman SH, Richards FO, et al. Cysticercosis surveillance: locally acquired and travel-related infections and detection of intestinal tapeworm carriers in Los Angeles County. *Am J Trop Med Hyg* 1992;**47**:365–71.
230. Sotelo J, Marin C. Hydrocephalus secondary to cysticercotic arachnoiditis: a long-term follow-up review of 92 cases. *J Neurosurg* 1987;**66**:686–9.
231. Stojkovic M, Zwahlen M, Teggi A, et al. Treatment response of cystic echinococcosis to benzimidazoles: a systematic review. *PLoS Negl Trop Dis* 2009;**3**:e524.
232. Stringer JL, Marks LM, White AC Jr, et al. Epileptogenic activity of granulomas associated with murine cysticercosis. *Exp Neurol* 2003;**183**:532–6.
233. Suri A, Goel RK, Ahmad FU, et al. Endoscopic excision of intraventricular neurocysticercosis in children: a series of six cases and review. *Childs Nerv Syst* 2008;**24**:281–5.
234. Tanowitz HB, Wittner M, White AC Jr. Introduction to tapeworm infections. In: Guerrant RL, Walker DH, Weller PF, et al, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 813–14.
235. Terra-Bustamante VC, Coimbra ER, Rezek KO, et al. Cognitive performance of patients with mesial temporal lobe epilepsy and incidental calcified neurocysticercosis. *J Neurol Neurosurg Psychiatry* 2005;**76**:1080–3.
236. Tesfa-Yohannes TM. Observations on self-induced *Taenia saginata* infection. *Ethiop Med J* 1990;**28**:91–3.
237. The Cysticercosis Working Group in Peru. The marketing of cysticercotic pigs in the Sierra of Peru. *Bull World Health Organ* 1993;**71**:223–8.
238. Thompson RC. The taxonomy, phylogeny and transmission of *Echinococcus*. *Exp Parasitol* 2008;**119**:439–46.
239. Thompson RC, Boxell AC, Ralston BJ, et al. Molecular and morphological characterization of *Echinococcus* in cervids from North America. *Parasitology* 2006;**132**:439–47.
240. Thomson AJG. Neurocysticercosis: experience at the teaching hospitals of the University of Cape Town. *S Afr Med J* 1993;**83**: 332–4.
241. Thornton H, Goldsmid JM. Cellophane tape as an aid to the detection of *Taenia saginata* eggs. *Cent Afr J Med* 1973;**19**: 149–51.
242. Thussu A, Chattopadhyay A, Sawhney IM, et al. Albendazole therapy for single small enhancing CT lesions (SSECTL) in the brain in epilepsy. *J Neurol Neurosurg Psychiatry* 2008;**79**:272–5.
243. Todorov T, Vutova K, Donev S, et al. The types and timing of the degenerative changes seen in the cysts during and after benzimidazole treatment of cystic echinococcosis. *Ann Trop Med Parasitol* 2005;**99**:649–59.
244. Tor M, Atasalihi A, Altuntas N, et al. Review of cases with cystic hydatid lung disease in a tertiary referral hospital located in an endemic region: a 10 years' experience. *Respiration* 2000;**67**: 539–42.
245. Torres-Corzo J, Rodriguez-della Vecchia R, Rangel-Castilla L. Bruns syndrome caused by intraventricular neurocysticercosis treated using flexible endoscopy. *J Neurosurg* 2006;**104**:746–8.
246. Townes JM, Hoffmann CJ, Kohn MA. Neurocysticercosis in Oregon, 1995-2000. *Emerg Infect Dis* 2004;**10**:508–10.
247. Tsang VC, Brand JA, Boyer AE. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis *(Taenia solium)*. *J Infect Dis* 1989;**159**:50–9.
248. Turgut M. Intracranial hydatidosis in Turkey: its clinical presentation, diagnostic studies, surgical management, and outcome: a review of 276 cases. *Neurosurg Rev* 2001;**24**:200–8.
249. Vazquez-Flores S, Ballesteros-Rodea G, Flisser A, et al. Hygiene and restraint of pigs is associated with absence of *Taenia solium* cysticercosis in a rural community of Mexico. *Salud Publica Mex* 2001;**43**:574–6.
250. Velasco TR, Zanello PA, Dalmagro CL, et al. Calcified cysticercotic lesions and intractable epilepsy: a cross sectional study of 512 patients. *J Neurol Neurosurg Psychiatry* 2006;**77**:485–8.
251. Verma A, Misra S. Outcome of short-term antiepileptic treatment in patients with solitary cerebral cysticercus granuloma. *Acta Neurol Scand* 2006;**113**:174–7.
252. Viola GM, White AC Jr, Serpa JA. Hemorrhagic cerebrovascular events and neurocysticercosis: a case report and review of the literature. *Am J Trop Med Hyg* 2011;**84**:402–5.
253. Wallin MT, Pretell EJ, Bustos JA, et al. Cognitive changes and quality of life in neurocysticercosis: a longitudinal study. *PLoS Negl Trop Dis* 2012;**6**:e1493.

254. Wandra T, Depary AA, Sutisna P, et al. Taeniasis and cysticercosis in Bali and North Sumatra, Indonesia. *Parasitol Int* 2006;**55**(Suppl.):S155–60.
255. White AC Jr. New developments in the management of neurocysticercosis. *J Infect Dis* 2009;**199**:1261–2.
256. WHO Informal Working Group on Echinococcosis. International classification of ultrasound images in cystic echinococcosis for application in clinical and field epidemiological settings. *Acta Trop* 2003;**85**:253–61.
257. Wichert-Ana L, Velasco TR, Terra-Bustamante VC, et al. Surgical treatment for mesial temporal lobe epilepsy in the presence of massive calcified neurocysticercosis. *Arch Neurol* 2004;**61**:1117–19.
258. Wilson M, Bryan RT, Fried JA, et al. Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. *J Infect Dis* 1991;**164**:1007–9.
259. Wittner M, White AC Jr, Tanowitz HB. *Taenia* and other tapeworms. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases: principles, pathogens, and practice*. Philadelphia: Churchill Livingstone; 2011. p. 839–47.
260. Wiwanitkit V. A review of human sparganosis in Thailand. *Int J Infect Dis* 2005;**9**:312–16.
261. World Health Organization Informal Working Group on Echinococcosis. *PAIR: puncture, aspiration, injection, re-aspiration: an option for the treatment of cystic echinococcosis*. Geneva: World Health Organization; 2001.
262. Yagci G, Ustunsoz B, Kaymakcioglu N, et al. Results of surgical, laparoscopic, and percutaneous treatment for hydatid disease of the liver: 10 years experience with 355 patients. *World J Surg* 2005;**29**:1670–9.
263. Zee CS, Go JL, Kim PE, et al. Imaging of neurocysticercosis. *Neuroimaging Clin N Am* 2000;**10**:391–407.
264. Zee CS, Segall HD, Boswell W, et al. MR imaging of neurocysticercosis. *J Comput Assist Tomogr* 1988;**12**:927–34.
265. Zee CS, Segall HD, Destian S, et al. MRI of intraventricular cysticercosis: surgical implications. *J Comput Assist Tomogr* 1993;**17**:932–9.
266. Zymberg ST, Paiva Neto MA, Gorgulho AA, et al. Endoscopic approach to fourth ventricle cysticercosis. *Arq Neuropsiquiatr* 2003;**61**:204–7.

SUBSECTION 4 *TREMATODES*

CHAPTER 227

Foodborne Trematodes

Miguel M. Cabada • Philip R. Fischer • A. Clinton White, Jr.

An estimated 56 million people are infected with foodborne trematodes, referred to as flukes. Almost 8 million have heavy infections.[33] Approximately 10 percent of the world's population is at risk for acquiring a trematode infection.

Flukes are flatworms (members of the phylum Platyhelminthes and of the subphylum Trematoda) (Figs. 227-1, 227-2, and 227-3).[48,66,88] The adult forms of foodborne trematodes are flat and leaf-shaped, with an oral and ventral sucker and a bifurcated, blind-ended gastrointestinal tract. Foodborne trematodes are hermaphroditic, producing operculated eggs that typically are passed in host feces. Eggs possess an operculum at one end, the opening through which a ciliated larva will hatch. Freshwater snails are intermediate hosts for all trematodes and are infected by either direct penetration by the ciliated larva or by ingestion of the unhatched egg. Complex larval development and multiplication occur within the snail host, with large numbers of larvae called *cercariae* ultimately produced. In most trematode life cycles, the cercariae emerge from the snails and swim about until they are ingested by or attach to the second intermediate host, which may be fish, crustaceans, mollusks, or aquatic vegetation. After attachment occurs, the larvae develop into metacercariae within protective cyst walls. Humans are typically infected by ingestion of the metacercariae. In Southeast Asia and the western Pacific region, foodborne trematodiasis has become an emerging public health problem, associated with the growth of aquaculture.[34,50] Many of the medically important flukes have a wide range of definitive hosts. Diagnosis of fluke infections typically is accomplished by examination of fecal specimens (or sputum in the case of *Paragonimus* spp.) for characteristic ova. Immunodiagnostic tests are available to aid in establishing the diagnosis of some flukes and are becoming the preferred method of diagnosis for certain species. Common liver, intestinal, and lung flukes are reviewed in this chapter.

LIVER FLUKES

CLONORCHIASIS AND OPISTHORCHIASIS

Three similar trematodes of the genus *Opisthorchis* infect the bile ducts of humans.[34,35,51,66] Although the name *Opisthorchis sinensis* is proper parasitologically, this organism is commonly referred to as *Clonorchis sinensis*, and the name of the infection, clonorchiasis, is well entrenched in the clinical literature. *Opisthorchis viverrini* and *Opisthorchis felineus* have similar life cycles and produce similar lesions and illnesses in humans.

The Organisms

The adult flukes measure 4 to 25 mm in length and 2 to 5 mm in breadth and have an anterior oral sucker. The life cycle involves primarily humans, but dogs and cats also act as definitive hosts. Fresh water snails and fish participate as first and second intermediate hosts. In the definitive hosts, parasites are found mainly in the intrahepatic biliary ducts and occasionally in the extrahepatic and pancreatic ducts. *Opisthorchis* spp. and *Clonorchis* spp. produce small operculated eggs (30×16 μm), which are nearly identical morphologically. The ova are shed into bile and excreted in feces. The snail host ingests the embryonated eggs, and after a complex cycle of asexual reproduction, free-swimming cercariae emerge 6 to 8 weeks later. The cercariae encyst under the scales or in the muscle of fresh-water fish as metacercariae. Humans and other definitive hosts are infected by ingestion of raw, inadequately cooked, or pickled fish infected with metacercariae. The larvae excyst in the intestine and migrate to the intrahepatic bile ducts through the ampulla of Vater. Juvenile flukes travel along the biliary tree against bile flow and attach to the bile duct epithelium with their suckers. Adult worms begin producing eggs approximately 4 weeks after ingestion and can survive for as long as 30 years.

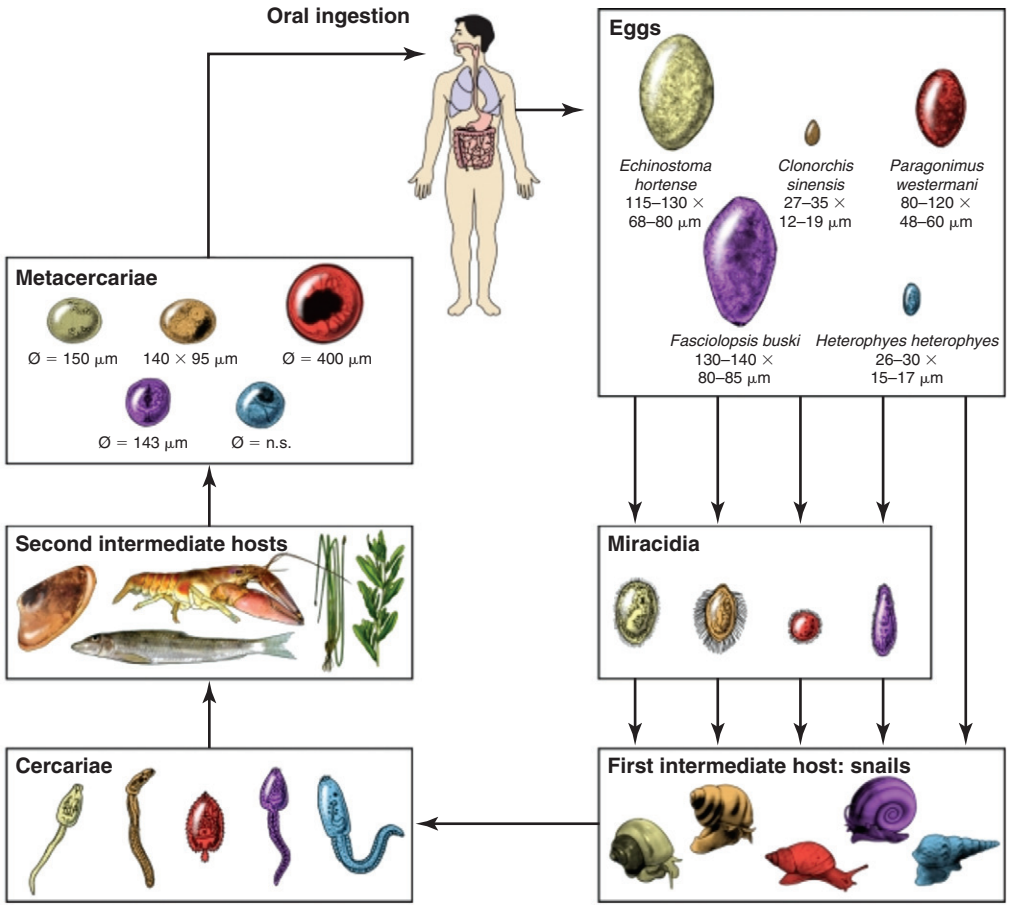

FIGURE 227-1 ■ Life cycle of foodborne trematodes including intestinal flukes (*Echinostoma spp, Fasciolopsis, Heterophyes*), a liver fluke (*O. sinensis*), and a lung fluke (*Paragonimus*). (See companion Expert Consult website for color version.) (From Keiser J, Utzinger J. Food-borne trematodiasis. *Clin Micro Rev* 2009;**22**:466–83.)

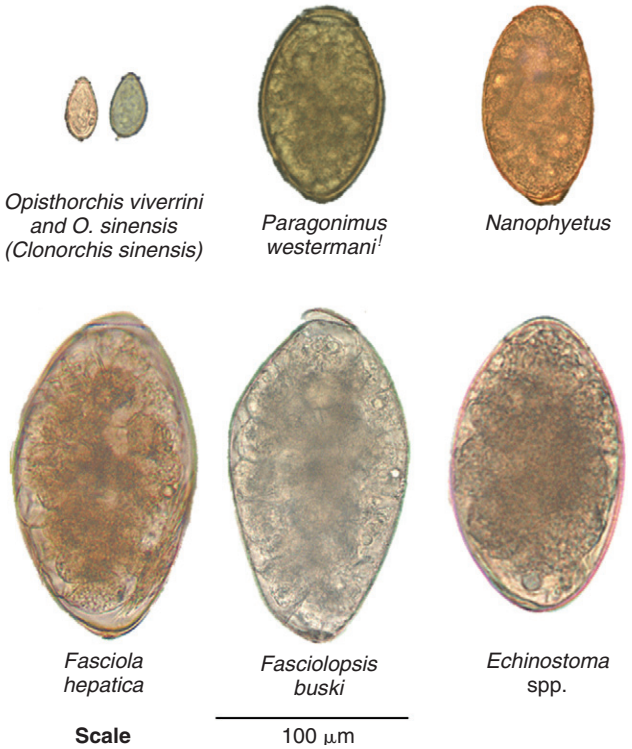

FIGURE 227-2 ■ Foodborne trematode ova found in human stool. (See companion Expert Consult website for color version.) (Modified from http://www.dpd.cdc.gov/dpdx/HTML/MorphologyTables.htm.)

Transmission and Epidemiology

Transmission is through the ingestion of raw or inadequately cooked fresh-water fish infected with metacercariae. Cats, dogs, civet cats, and other fish-eating mammals serve as definitive hosts in nature. The expansion of aquaculture in China and other Asian countries and poor sanitation practices allow the contact of human feces with snails in ponds where fish are raised. This situation increases the risk for parasite transmission and hinders control efforts.[31,38]

An estimated 600 million people are at risk for *O. sinensis* infection, 67 million for *O. viverrini*, and 12 million for *O. felineus*.[33] *O. sinensis* is endemic in China, Japan, Korea, Taiwan, and Vietnam. Some of the highest infection rates are reported in endemic areas of China (16%) and Vietnam (26%).[41] *O. viverrini* is endemic to Thailand, Lao People's Democratic Republic, Vietnam, and Cambodia. In northern Thailand, infection rates may reach 60 percent and it is estimated that 7 million residents are infected in that area.[84] *O. felineus* is endemic in central Siberia and in eastern and southeastern Europe. Sporadic infections with *O. felineus* have been reported from several Asian countries.

Eating habits and snail presence are the major determinants for the geographic distribution of the infection. The three species of *Opistochis* follow similar patterns of infection in most areas. The lowest prevalence and intensity of

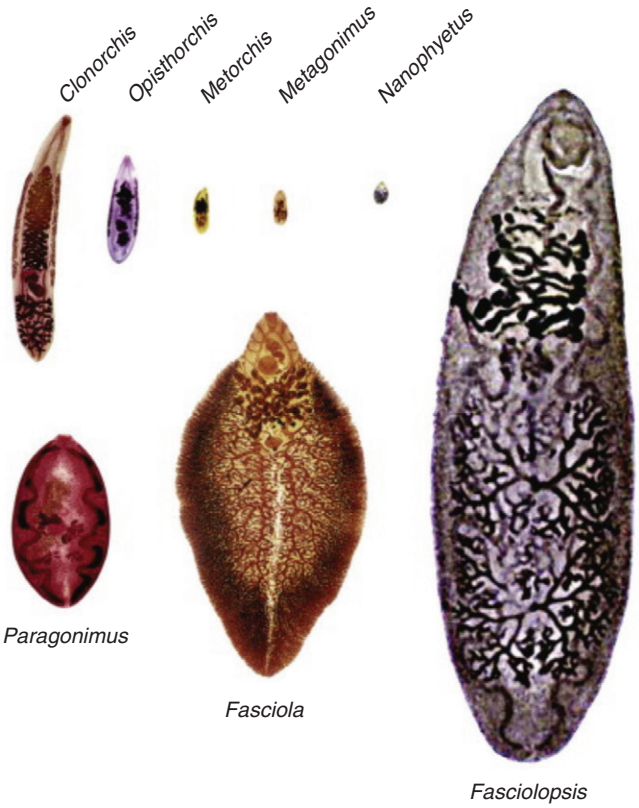

FIGURE 227-3 ■ Adult worms of foodborne trematodes. This is a 3-fold magnification of selected adult flukes illustrating their relative sizes. Actual lengths: *Nanophyetes salmincola* 0.8-2.5 mm, *Opisthorchis viverrini* 5-10 mm, *Paragoniums westermani* 7-16 mm, *Fasciola* 20-30 mm, and *Fasciolopsis* 20-75 mm. (See companion Expert Consult website for color version.) (From Mahanty S, MacLean JD, Cross JH. *Liver, Pathogens, and Practice,* 3rd ed. Philadelphia; Saunders: 2011, p 854–67.)

infection is found in the youngest age groups and in women; whereas intensity of egg excretion often increases with age and male gender.[80] The prevalence peaks in older persons, as shown in studies in China and Korea.[20,102]

Pathology and Pathogenesis

The epithelium of infected bile ducts reacts with desquamation, adenomatous hyperplasia, and metaplasia of goblet cells, accompanied by an increase in mucus production. The pathogenesis of the hepatobiliary damage may be due to the induction of an immunologic response in the host by mechanical action and secreted products from the flukes. Fibrosis and ductal dilation and obstruction are the consequence of the inflammatory reaction elicited by the parasites. Bile stasis in turn may lead to bacterial cholangitis and further inflammation in the bile ducts. The severity of the changes relates to the intensity of infection, host susceptibility, and number of reinfections. Chronic infection may lead to extensive periportal and periductal fibrosis, a process in which interleukin-6 mediated responses seemed to play a key role.[86,88,89]

The infection is a significant risk factor for cholangiocarcinoma in endemic areas.[87,88] Experiments in hamsters have shown that repeated infection with *O. viverrini* induces overproduction of nitric oxide, which may play a significant role in the development of cholangiocarcinoma through oxidative and nitrosyl DNA damage.[75] In addition, excretory and secretory products from *O. viverrini* induce proliferation of fibroblasts.[90] The excretory and secretory products induce the production of retinoblastoma and cyclin D1 proteins in the hamster model, both of which have been implicated in the mechanism of *Opisthorchis*-induced cholangiocarcinoma.[12] *O. viverrini* infections involve the gallbladder and may lead to complications such as gallbladder sludge, cholecystitis, and formation of gallstones.[67,68] The parasites may be found in the pancreatic ducts, where reactions of the epithelium are similar to those in the bile duct. Pancreatitis occurs infrequently in these liver fluke infections and is usually mild.

Clinical Manifestations

In endemic areas, infections may begin early in life and often are asymptomatic.[68,89] The occurrence of symptoms correlates with the intensity of infection. Patients with higher fluke burdens are more likely than those with lower burdens to have right upper quadrant pain, abdominal discomfort, and jaundice. They are also more likely to have abnormalities on ultrasound examination, such as intrahepatic bile duct dilation and periductal liver fibrosis.[80] Other findings may include weakness, malaise, and liver enlargement. A community-based study showed significant improvement of symptoms, laboratory test results, and ultrasound abnormalities after treatment of infected subjects.[76]

An acute febrile syndrome starting 2 to 3 weeks after exposure has been reported with *O. sinensis* and *O. felineus* infections.[35,88] Symptoms include fever, malaise, anorexia, diarrhea, and tender hepatomegaly. Eosinophilia, which is not as common in the chronic phase, is typically found during the acute illness.

Complications are more likely to occur in heavy infections. Relapsing cholangitis, cholecystitis, bilirubin gallstones, and pancreatitis all have been associated with infection.[68,80] Clonorchiasis has been cited as a cause of pancreatitis in individuals from endemic areas.[54] *O. viverrini* and *O. sinensis* are considered carcinogenic to humans and have been included in the World Health Organizations' list as group I carcinogens.[41] *O. viverrini* and *O. sinensis* are associated with cholangiocarcinoma in adults, and the former is presumed to be responsible for more than 60 percent of liver tumors in northeastern Thailand.[32]

Diagnosis

Symptoms of acute infection may develop 3 to 4 weeks before eggs appear in the stool. Immunologic tests are useful to establish the diagnosis during this phase. Antibody-based tests are in use in some endemic areas, but suffer from high rates of cross-reactions with antibodies to other parasites.[45,55,88] The sensitivity and specificity vary depending on the antigens used. ELISA using excretory and secretory products performs better than crude antigen enzyme-linked immunosorbent assay (ELISA), but cost precludes widespread use.[22,44] Demonstration of *O. viverrini* antigen in stools using a monoclonal antibody–based ELISA appeared to be specific and highly sensitive.[45,82,88]

In chronic infections, eggs are evident on fecal microscopy examination. The Kato Katz test is widely used but lacks sensitivity, especially in patients with very low parasite burdens. Concentration techniques such as the formalin-ether test are more sensitive than the Kato Katz.[6,34,35] Microscopy is useful in the clinical setting and in epidemiologic studies because it provides information regarding intensity of infection. Nonetheless, microscopic differentiation of *O. viverrini* and *O. sinensis* eggs is not easy. In addition, differentiation of these eggs from eggs of small heterophyid flukes is also difficult. Polymerase chain reaction (PCR)-based tests have shown promising results for the diagnosis of *O. Viverrini* and *O. sinensis*.[52,79] The loop-mediated isothermal amplification test, based on DNA polymerase with strand displacement at a constant temperature may be even more sensitive.[4,14,41,58]

Imaging studies may aid in diagnosis of these flukes. Ultrasound findings include intrahepatic bile duct dilation, increased periductal echogenicity, and gallbladder sludge.[23,68] Cholangiography often shows multiple cystic dilations of the ducts, elliptic or filamentous filling defects within the peripheral intrahepatic ducts, and intrahepatic duct haziness.[21] A combination of large cystic dilations and small cystic ectasias or mulberry-like dilations is considered diagnostic. The adult worms also may be visible on endoscopic retrograde cholangiopancreatography.

Treatment and Prognosis

All three *Opisthorchis* infections respond well to treatment with praziquantel.[33,35,63,88] The recommended dose is 25 mg/kg given three times for 2 days. A single dose of 40 mg/kg also can be used. At the recommended doses, praziquantel cures over 80 percent of infected people.[63] Side effects of headache and dizziness are common occurrences. Treatment may decrease the risk for development of cholangiocarcinoma in those infected. Preliminary studies suggest that there may be efficacy for tribendimidine.[85]

PREVENTION

The main barriers to prevention are deep-seated eating habits on raw or improperly cooked fish in endemic areas.[100] Thus, preventing human stool contamination of fish ponds and decreasing the snail population in these setting helps decreasing the prevalence of fishborne fluke infections. An approach in which infected human and other mammals are treated, sanitation is improved to avoid contamination of fishponds with raw human sewage, and health-promotion activities are carried out to decrease raw fish consumption is likely to have an impact on the incidence of infection.[83]

FASCIOLIASIS

Fascioliasis is a parasitic disease caused by the flukes *Fasciola hepatica* and *Fasciola gigantica*. With 91 million people at risk for acquiring infection, human fascioliasis is an important public health problem with an increasing incidence of human cases in 51 countries.[13,33,34,66,72] Fascioliasis is primarily a zoonosis of ungulates (e.g., cattle and sheep). Although humans are considered an incidental host, the epidemiology of the disease is driven by human infection in hyperendemic areas.

The Organisms

Fascioliasis is caused by two foodborne trematodes from the genus *Fasciola*, *F. hepatica* and *F. gigantica*. *F. hepatica* adult worms measure up to 30 mm in length and 13 mm in width. *F. gigantica* measures up to 75 mm.[66,88] During chronic infection adult parasites reside in the bile ducts, where eggs are produced. They reach the intestine through the bile and are excreted in feces. Eggs measure 130 μm in length and 90 μm in width and are indistinguishable from those of *F. buski*. After being incubated in water for several days, the ciliated miracidium hatches and penetrates the freshwater snail host, where it undergoes a complex cycle of asexual multiplication. Cercariae leave the snail and encyst on fresh-water vegetation, including watercress and alfalfa. These encysted metacercariae initiate infection when the mammalian host ingests the raw aquatic vegetation or water contaminated with metacercariae. The metacercariae excyst in the intestine. The larvae penetrate the intestinal wall and migrate from the peritoneal cavity to the liver. The developing worms penetrate the liver capsule and slowly burrow through the parenchyma to the bile ducts, where they mature into the adult worms. Adult *Fasciola* feed on liver cells and duct epithelium. The life span of the adult fluke in humans ranges from 9 to 13 years.

Transmission and Epidemiology

Epidemiologic studies suggest that human *Fasciola* infection is transmitted through ingestion of infected raw aquatic plants, such as watercress and alfalfa, and by drinking untreated water with metacercariae.[51,69,72] Herbivorous mammals, such as sheep, cattle, goats, and camelids, act as definitive hosts in nature. Fascioliasis has a worldwide distribution, with human infection reported from at least 51 countries in South America, the Caribbean, Africa, and Asia.[72] The number of human cases is estimated at over 17 million, with more than half of the burden of infection reported from the Andean region and the Caribbean.[33] More recent data suggest that those estimates may have significantly underestimated the burden of disease. For example, reports from Bolivia and Peru showed a prevalence as high as 70 percent in hyperendemic areas.[13] The highest fascioliasis prevalence in the Andean region is observed in children, whereas in the rest of the world the infection prevalence is more evenly distributed among different age groups. However, recent data suggest that fascioliasis is emerging as a serious public health problem in Vietnam, with thousands of cases diagnosed in recent years.[72,88,92] Similarly, outbreaks have occurred in Iran, with more than 17,000 clinical cases.[39,77,78]

Pathology and Pathogenesis

Fascioliasis has an acute phase caused by juvenile flukes that migrate through the liver parenchyma and a chronic phase caused by the adult flukes that start egg production in the biliary tract. The pathogenesis of acute fascioliasis is related to direct liver damage caused by the migrating flukes and to the inflammatory reaction triggered by the fluke antigens. Linear necrotic lesions containing eosinophils form as flukes progress through the liver parenchyma. Flukes that die before reaching the bile ducts may produce necrotic cavities that eventually evolve into fibrous scar tissue. During the chronic stage, adult worms in the bile ducts cause inflammation and adenomatous changes in biliary epithelium, leading to duct thickening and dilation. Ductal and periductal fibrosis occurs. The gallbladder and extrahepatic ducts may be invaded and undergo similar inflammatory and fibrotic reactions. Adult worms may migrate back into the liver parenchyma through eroded biliary epithelium and cause formation of abscesses. Liver cirrhosis has been described in severe forms of fascioliasis. The inflammation associated with infection is probably associated with the anemia and weight loss that occurs with fascioliasis.

Clinical Manifestations

It is estimated that 15 percent of fascioliasis cases have high-intensity infections. As with other helminthes, those with a heavy burden of parasites are more likely to be symptomatic.[34] Even those considered asymptomatic may suffer from anemia.[62] Thus, approximately 85 percent of *Fasciola* infections may remain subclinical and undiagnosed. Symptoms appear approximately 4 to 6 weeks after infection. The duration of this latent period depends on host response and the intensity of infection. The acute phase is characterized by right upper quadrant or generalized abdominal pain, tender hepatomegaly, fever, and anemia, typically associated with marked eosinophilia.[5,10,35,47,91] Sweating, dizziness, wheezing, and urticaria may occur. This stage lasts 1 to 3 months. The chronic form of the disease is associated with symptoms of biliary system obstruction. These symptoms frequently are identical to those of gallbladder disease, cholangitis, and pancreatitis caused by nonparasitic conditions. Elevation in alkaline phosphatase levels is a common manifestation.[5,10] Heavy and repeated infections eventually may cause liver damage sufficient to produce cirrhosis. Eosinophilia is not uncommon in chronic infections, but is usually low grade. Juvenile flukes that fail to migrate into the liver may wander about and cause ectopic fascioliasis. The most common ectopic localization of immature *F. hepatica* flukes is in the subcutaneous tissues of the thorax, back, and extremities but also has been observed in lungs, heart, brain, and intestinal wall.[88] Blancas-Torres reviewed a large series of fascioliasis patients (37 acute and 240 chronic) admitted to a hospital in Peru. A significant finding among these patients was the prevalence of weight loss (40%) and anemia (30%).[10] Severe acute disease may follow massive exposure and present with fever, eosinophilia, subcapsular hematoma, and tracks on imaging studies.[70]

Diagnosis

The acute manifestations of human fascioliasis may precede the appearance of eggs in the stool by 7 to 12 weeks. The triad of fever, abdominal pain, and eosinophilia combined with compatible exposures are helpful to suspect the diagnosis. Multiple immunodiagnostic tests are available to detect *Fasciola* during the acute phase of infection. Differences in sensitivity vary according to the antigens and antibodies used. The Fas2 ELISA test has a sensitivity of 92 percent and a specificity of 83 percent.[28] A commercially available sandwich ELISA using excretory or secretory antigens had a sensitivity and specificity over 95 percent when tested using serum of chronically infected individuals.[95] Serologic tests are also useful in the diagnosis of ectopic fascioliasis.

During chronic infection, *Fasciola* eggs may be detected in the stools. The Kato Katz and the rapid sedimentation tests are commonly used for microscopy diagnosis of fascioliasis.[6] These tests have low sensitivity, and testing multiple stool specimens is often required. False-positive stool examination results may occur if the patient recently ingested raw infected liver containing *Fasciola* eggs. Serum and stool antigen tests are available for diagnosis in veterinary medicine, and their use in humans is under study,[13] with pilot studies suggesting that they may be useful in human infections.[24,94] PCR-based tests are being developed for the detection and differentiation of *Fasciola spp.* PCR targeting the ITS-2 region of the ribosomal DNA gene has shown the highest sensitivity and specificity. A loop-mediated isothermal amplification test has been used to successfully detect *Fasciola* DNA with 10^4 times more sensitivity than conventional PCR in laboratory specimens. The loop-mediated isothermal amplification test could also differentiate *F. hepatica* from *F. gigantica*.[2]

Adult flukes may be seen on ultrasonography and appear as curvilinear lucent areas in the contrast medium in cholangiography. Endoscopic retrograde cholangiopancreatography may directly visualize the adult flukes, which are leaf shaped and bile stained. Eggs also may be identified in bile aspirates by microscopy. Radiologic imaging of the liver may demonstrate slowly evolving lesions, including track lines, small abscesses, and subcapsular hematomas.[16,27,47,56,71]

Treatment and Prognosis

Unlike other trematode infections, fascioliasis is relatively resistant to praziquantel. The drug of choice is triclabendazole at a dose of 10 mg/kg with a meal once or twice. The formulation manufactured for human use as Egaten is currently not available in the United States. Triclabendazole (Fasinex) has been used for many years by veterinarians for treatment of livestock in endemic areas. Anecdotally, it has been used safely in humans. However, resistance is emerging in isolates from livestock. Winkelhagen and colleagues[98] reported a possible case of triclabendazole resistance in an elderly sheep

farmer who failed several courses of treatment. Triclabendazole has been used successfully in a variety of open clinical trials in adults and children for both acute and chronic fascioliasis.[70,96] It is effective against both migrating flukes and established infections. Artemisinins have been used for the treatment of fascioliasis in two trials, with disappointing results.[49] Nonetheless, the combination of triclabendazole and artemisinins or ketoconazole showed promising results to overcome resistance in animal studies.[48] Nitazoxanide may be used to treat chronic fascioliasis.[29]

Prevention

Education and communication on safe growing of water plants for human consumption to avoid contamination with fecal material should be promoted.[72,88] Consumption of wild water plants in the field should be discouraged. Water purification systems should be implemented in highly endemic areas. The World Health Organization is promoting the use of mass treatment of children in endemic areas, but data are limited on safety and efficacy of this approach.[96,100]

LUNG FLUKE

PARAGONIMIASIS

The parasites of the genus *Paragonimus* are zoonotic parasites with felines, canines, and other carnivores as definitive hosts, snails as intermediate hosts, and crabs or crayfish as second intermediate hosts.[8,33,48,66,88] Over 40 species of *Paragonimus* have been identified, at least 8 of which have been recovered from humans. The species causing human disease vary by region.

The Organisms

The adult forms of *Paragonimus* form flattened leaf-life structures that measure about 7 to 16 mm long and 3.5 to 6 mm wide with anterior and ventral suckers.[8,66] The adult worms typically are located in the lungs of the definitive host. The ova are large (80 to 120 µm × 48 to 65 µm), yellow-brown in color, and contain a single operculum. Ova are shed into the bronchi and are either expectorated or swallowed and excreted in feces. After an incubation period of at least 2 weeks in fresh water, the free-living miracidia emerge and penetrate the snail intermediate host. After several weeks, the cercariae emerge and invade or are ingested by fresh-water crabs and crayfish, forming cystic metacercaria in the tissues. Humans and other mammalian hosts are infected by ingestion of undercooked crab or crayfish. Larvae released from the metacercaria penetrate the wall of the intestines and migrate through the diaphragm to reach the lungs. In some instances, the worms may lodge in ectopic sites within the abdomen, liver, subcutaneous tissue, or the central nervous system (CNS). The worms usually are found singly or in pairs within a capsule or cyst of reactive host tissue. Approximately 2 to 3 months are required from the time of ingestion until the worms are fully mature. Diploid forms reproduce sexually and triploid forms by parthenogenesis. In most infections, the worms die within 10 years; however, production of eggs for 20 years after the individual has left the endemic area has been reported.

In the past, most cases of paragonimiasis were attributed to infection with *P. westermani*, but molecular studies have revealed that more than nine different species of *Paragonimus* infect humans.[8,9,42,66] Molecular characterization has shown that typical pleuropulmonary infections in China, Japan, and Korea were largely due to *P. westermani*.[8] However, *P. skrjabini* (and the related *P. myazakii*) cause atypical clinical disease in those areas.[8,9,88] Although *P. westermani* is endemic in India and Southeast Asia, nearly all clinical isolates are *P. heterotremus*.[25,26,81,101] Endemic areas with *P. africanus* and *P. uterobilateralis* can be found in Central Africa (Cameroon and Nigeria)[3,74] and with *P. mexicanus* also the Pacific rim from Peru to Central America, with the major focus in Ecuador.[8,43] Endemic cases have also been increasingly recognized in Missouri and are due to *P. kellicotti*.[30]

Transmission and Epidemiology

The endemic areas for paragonimiasis are largely limited to areas where the ecology allows for infection of all of the hosts, including infection of fresh-water snails, spread to fresh-water crabs and crayfish, and ingestion of the crabs and crayfish by the definitive hosts.[8,66] Human infection is acquired by eating fresh-water crabs, crayfish, or shrimp that are raw, inadequately cooked, salted, or pickled. Recent cases acquired in the United States have been traced to ingestion of raw crayfish (*P. kellicotti*) or imported crabs (*P. westermani*).[11,57] A number of autochthonous cases have recently been described associated with freshwater canoe trips in which raw crayfish were ingested on a dare.[57] Infection has also been associated with ingestion of undercooked wild boar meat, with the boar serving as a paratenic host. There are also reports of infection from ingestion of water contaminated with metacercaria from crabs.

The global prevalence of human infection is estimated at over 20 million persons infected with 293 million at risk.[8,33,34] Overall, prevalence has been decreasing in much of east Asia such that disease is now rare in Japan, Korea, Taiwan, Philippines, and eastern China, which once were highly endemic.[8,34,60] Nevertheless, a recent national survey in China revealed a seroprevalence of paragonimiasis in 1.7 percent with disease found in 21 different provinces.[60] Disease appears to be increasing in some areas after construction of the Three Gorges Dam.[104] Paragonimiasis remains endemic in much of Southeast Asia (including Vietnam, Thailand, and the Lao People's Democratic Republic). For example, a recent study revealed seroprevalence ranging from 0 to 12.7 percent in parts of Vietnam[26] and disease is described in all countries of southeast Asia as well as eastern

India,[25,81] where more than half of children were seropositive in one focus. *Paragonimus* is endemic in central and western Africa, but most human cases are reported from Cameroon and Nigeria, with up to 14.8 percent of the population seropositive in some regions of southwestern Cameroon.[3,74] *Paragonimus mexicanus* is endemic in Latin America, with the primary focus in Ecuador and northern Peru, but case series have been reported from Colombia and Venezuela, in Central America, and as far north as Mexico.[8,15,43]

Pathology and Pathogenesis

Larvae are released after ingestion of ova. The larvae penetrate the duodenum and enter the abdominal cavity aided by excretory proteases.[8] Larvae may penetrate the diaphragm to the pleura soon thereafter or the larvae may linger in the abdominal cavity or liver. After penetration of the pleura, the larvae burrow into the lung parenchyma and lodge near larger bronchioles or bronchi surrounded by an exudate of neutrophils and eosinophils. In time, this inflammatory response around the parasite organizes into a fibrotic wall that may measure up to several millimeters thick. The cysts usually are 1 to 2 cm in diameter and often are filled with a brownish material that likely contains hematin. Ova are released via communications with bronchioles or bronchi into sputum. Complications of paragonimiasis include bronchiectasis, fibrosis, interstitial pneumonia, and secondary bacterial infections.

Parasites can also be found in ectopic locations such as the CNS or subcutaneously. Ectopic parasites are more common for species that are less well adapted to the human host, especially *P. skrjabini*. Up to 31 percent of cases may involve the CNS in some areas.[18,64] All areas of the brain and meninges are susceptible to invasion. The adult worms and the eggs cause areas of central necrosis and granuloma formation with dense collagenous walls surrounded by lymphocytes, plasma cells, eosinophils, and Charcot-Leyden crystals. The lesions vary in size and may be several centimeters in diameter and may appear cystic. Eventually, the wall may calcify. Spinal cord lesions are similar. The organisms can also cause a migratory subcutaneous nodule. Other sites at which worms may cause cysts or abscesses are the intestinal wall, mesentery, peritoneal cavity, liver, diaphragm, and myocardium.

Clinical Manifestations

Classical (Pleuropulmonary) Paragonimiasis

The classic presentation of paragonimiasis is with pleural or pulmonary disease.[33,35,66] Pulmonary disease is more common with species better adapted to the human host (e.g., *P. westermani*, *P. heterotremus*, or *P. africanus*)[25,26,60,74] than species less adapted to the human host (e.g., *P. skrjabini*).[8] Migration of the worms from the intestinal tract to the lungs does not usually cause symptoms, but has rarely been associated with fever, abdominal pain, diarrhea, and eosinophilia. However, if the parasites migrate across the liver, patients frequently present with abdominal pain and eosinophilia.[59] As the parasites enter the pleura, patients may present with fever, chest pain, dyspnea, cough, malaise, and night sweats. The pulmonary clinical manifestations are mainly a chronic cough. Cough may be dry or associated with sputum production, which may be described as mucoid, rust-colored or blood-streaked. Hemoptysis usually is intermittent and occasionally may be severe. Eosinophilia is a common finding in the early stages of infection but may return to normal over a period of months or years. Complications may include pneumothorax, pleural effusion, empyema, and pneumonia. The presence of hemoptysis and cavitary lung lesions can be confused with tuberculosis, but tuberculosis typically has more systemic symptoms (e.g., fevers, night sweats, and weight loss). However, systemic symptoms have been noted in most recent cases from North America.[57]

Nonclassic Presentations

Paragonimiasis may also infect other sites, including the subcutaneous tissues, brain, liver, abdomen, pericardium, and abdominal cavities. Extrathoracic disease is more common with parasites less adapted to the human host, especially *P. skrjabini*, but has been described from all endemic areas.[104]

Up to 31 percent of cases may involve the CNS.[18] Cerebral involvement is more common in children than adults. Most cases present with headache, nausea, and vomiting. Others may present with focal findings (e.g., aphasia, motor involvement, or sensory abnormalities). Patients also may have seizures, which are usually described as generalized. They may or may not have coexisting pleuropulmonary disease. Spinal cord lesions often are extradural and mimic mass lesions caused by tumors or infection.

Cutaneous paragonimiasis presents with subcutaneous nodules, which may be fixed or slowly migrate. Cutaneous nodules can account for up to 42 percent of cases in areas endemic for *P. skrjabini*, but are also noted in other areas. Worms recovered from these lesions are immature.

DIAGNOSIS

Most patients have pleuropulmonary symptoms, and initial evaluation includes a chest radiograph. Radiographs initially may be normal, but more typically demonstrate pleural effusions, nodules, or infiltrates.[25,40,46,53,74] Other radiographic findings include cavitations and fibrosis. Nodules and cysts can be found in any part of the lungs, but tend to localize in the periphery of the middle and lower lung fields. A common radiographic diagnostic feature is a "ring shadow." This finding represents the circular or oval thin-walled cyst with a crescent-shaped opacity along one side. Chest computed tomography (CT) findings vary by region (and thus likely by parasite species). Studies from Korea noted infiltrated and nodules in most cases.[53] Studies from Laos and India were more likely to show multiple cavitary lesions.[25,46] North American series noted more pleural effusions, pericardial involvement, and lymph nodes.[40]

Cerebral paragonimiasis may be seen as either an avascular mass or a hemorrhage on CT or magnetic resonance imaging.[1,18] Findings may include low-density, irregular shadows in most cases or, less frequently, hemorrhages. Either can be associated with edema and contrast enhancement. The ring enhancement may involve clusters of rings. In long-standing cerebral infections, the cystlike structures may calcify and be seen as a cluster of "soap bubbles." The individual oval or spherical bubbles may measure 2 to 40 mm in diameter, and a cluster of these structures may extend 10 cm.

Nearly all cases of paragonimiasis will present with eosinophilia. There can be some variability such that some authorities recommend repeating blood counts if eosinophilia is not present on the initial examination. Radiologic studies play a key role in diagnosis.

Pulmonary paragonimiasis is diagnosed definitively by finding the characteristic eggs in sputum or stool from a patient with symptoms suggesting the infection. Ova are more readily visualized with concentration or Ziehl-Nielsen staining.[84] Eggs are not present until 2 to 3 months after infection develops. Concentration techniques may help identify eggs in patients with light infections. Eggs have also been identified by percutaneous aspiration of lung nodules.[103] Several immunodiagnostic tests, including ELISA and immunoblotting have been developed, which may assist with confirming the presence of infections. Immunodiagnosis is especially useful in infections in which eggs are not demonstrated easily, such as in cerebral paragonimiasis. The immunoblot assay performed with a crude antigen extract of *P. westermani* has a sensitivity of 96 percent and specificity of 99 percent and is the serodiagnostic test of choice.[37] Antibody levels detected by enzyme immunoassay and immunoblot decline slowly after successful treatment, so antigen-detection systems may be a more useful approach to monitor treatment efficacy.[43] Tests that amplify parasite DNA, including loop-mediated amplification, may improve sensitivity, but are not widely available.[19]

Treatment and Prognosis

Praziquantel 75 mg/kg divided into three doses is given daily for 2 or 3 days.[33,35] Triclabendazole has been studied as an alternative treatment for humans infected with *Paragonimus* spp.[21] One study demonstrated 84 to 90 percent efficacy with single-day regimens of 1 or 2 doses of triclabendazole for the treatment of pulmonary paragonimiasis.[5]

The prognosis is good in most pulmonary infections, even if they go untreated, although symptoms may persist for many years. Treatment effectively resolves the pulmonary lesions and symptoms. The prognosis for CNS involvement depends on the location and extent of the lesions but is usually grave.

Prevention

The key in prevention is education of the population in endemic areas concerning the source of infection. Crabs and crayfish, prepared in a manner that transmits paragonimiasis, are delicacies in many parts of the world. However, education about preparation of these foods has led to dramatic reductions in the number of cases in Japan, Korea, the Philippines, and eastern China. Mass chemotherapy is being used to try to help prevent infections. Because paragonimiasis is a zoonosis, however, it is less amenable to control by mass treatment of human populations or improved sanitation.[100]

INTESTINAL FLUKES

Approximately 40 to 50 million people are thought to be infected by one or more of the 70 species and 14 families of intestinal flukes.[17,51,66] They are diverse morphologically, ranging in size from the *F. buski* (7.5 cm in length) to the tiny heterophyids (1 to 2.5 mm in length). Most cases cause few symptoms. However, heavy infections have been associated with a number of gastrointestinal symptoms. The most widely recognized organisms include *F. buski*, *Heterophyes* spp., *Metagonimus yokogawai*, and *Echinostoma* spp.

FASCIOLOPSIASIS

Life Cycle

Fasciolopsiasis is a foodborne intestinal zoonosis caused by *F. buski*, the giant intestinal fluke.[17,36,37,66,88] The adult fluke measures up to 7.5 cm in length and attaches to the duodenum and jejunum walls. The life span of adult *Fasciolopsis* is approximately 6 months. Egg production starts about 3 months after infection and these are 130 to 150 μm in length by 80 to 85 μm in breadth. A single parasite may excrete 25,000 eggs daily. The eggs are released in the feces, and after several weeks in fresh water, ciliated larvae hatch from the eggs and penetrate snails, where they reproduce asexually undergoing several larval stages. Cercariae emerge from the snail approximately 1 to 2 months later and encyst as metacercariae on a wide variety of aquatic vegetation and debris as well as on the water surface. Mammalian hosts are infected after ingestion of the metacercariae. Pigs are common reservoir hosts. Water chestnuts, caltrop, and water bamboo are common sources of human infection. Ingestion of larvae also can occur through drinking untreated water or handling water-derived plants (e.g., peeling contaminated water chestnuts with the teeth).

Epidemiology

Fasciolopsiasis occurs most often in Asia and the Indian subcontinent, with high prevalence rates recorded in

parts of south Asia and Thailand.[17,36,37,88,89] Pigs are the definitive host, and the prevalence of fasciolopsiasis is higher in areas where pigs are raised and aquatic plants are consumed. Fasciolopsiasis is most prevalent in school-age children, in whom high worm burdens are not uncommon findings.

Pathology

The parasites infect the duodenum and jejunum, but in heavy infections they can be observed in the stomach, ileum, and colon. Inflammation, ulceration, and small abscesses sometimes develop in the intestinal mucosa where the parasite is attached. Increased mucus secretion and mild bleeding can result. Light infections typically are asymptomatic. Massive infections can lead to intestinal obstruction and perforation. Complications such as malabsorption and protein-losing enteropathy, are common occurrences in heavy infections and can lead to generalized edema and ascites.

Clinical Manifestations

The severity of symptoms is correlated with the burden of parasites. Epigastric discomfort has been reported as early as 30 days after exposure. Diarrhea and abdominal pain may be intermittent and may occur separately. In heavy infections, nausea and vomiting may develop. Facial edema, anasarca, ileus, and intestinal perforation are encountered in severe infections. Eosinophilia with counts greater than 30 percent is not an uncommon occurrence. Leukocytosis and mild anemia may be noted.

In endemic areas, fasciolopsiasis contributes to malnutrition in children, especially when associated with other helminth infections. Fasciolopsiasis adds an additional burden to the host and in concert with other chronic helminth infections may be responsible for significant morbidity.

Diagnosis

The eggs of *Fasciolopsis* are demonstrated easily by routine fecal examination, but they are virtually indistinguishable from those of the liver fluke *F. hepatica* and the echinostomes. Epidemiologic information, such as travel history and exposure to sources of infection, may be helpful in determining the diagnosis. Because the life span of adult *Fasciolopsis* is short, anyone who has been out of an endemic area for longer than 9 months is not likely to have fasciolopsiasis. Incidentally, fasciolopsiasis can be diagnosed by upper endoscopy.

Treatment and Prognosis

Praziquantel 75 mg/kg divided into three doses given in 1 day is effective and safe. Infections treated early and most light infections, even when they are untreated, have an excellent prognosis. Heavy infections in children, especially when they are complicated by intestinal obstruction, edema, or concomitant secondary infections, portend a much poorer prognosis.

Prevention

Avoiding raw water-plants and untreated water may be a practical approach to prevent fasciolopsiasis. Cooking aquatic vegetation or immersing the plants or nuts briefly in boiling water usually is sufficient to prevent infection. Also, discouraging the use of human feces as fertilizer and promoting the institution of modern pig farming both can aid in the control of fasciolopsiasis. Successful efforts in health education or mass chemotherapy have reduced the transmission of this parasite in some endemic areas[88,97]; however, in areas where fasciolopsiasis was presumed controlled, such as Uttar Pradesh, reemerging infection has been reported.[7]

HETEROPHYIASIS

More than 11 different species of the family Heterophyidae have been reported to cause human infection.[17] *Heterophyes* spp., *Haplorchis* spp., and *M. yokogawai* are common human parasites, whereas other organisms are uncommon.

The Organism

Heterophyes spp., *Haplorchis* spp., and *M. yokogawai* adult worms attach to the mucosa of the small intestine. The parasites are minute, measuring 1 to 2.5 mm in length and less than 1 mm in width. They often burrow deeply into the mucosa. The eggs measure 26 to 30 μm in length and 15 to 17 μm in width. The eggs are nearly identical. Eggs excreted in the stool are fully embryonated. The snail intermediate host becomes infected by ingesting the trematode egg. After multiplication in the snail, cercariae emerge and encyst as metacercariae under the scales, skin, or in the muscle of a variety of fresh-water fish. After being ingested by the definitive host, the metacercariae are freed from their cysts and develop into adults in as little as 5 days.

Transmission and Epidemiology

Humans acquire the infection by eating fresh-water fish that is raw or inadequately cooked. Transmission may have a seasonal pattern, with the highest fish infection rates found during the summer.[61,93] Heterophyiasis occurs worldwide. Areas of endemic infection are Southeast Asia, the Middle East, the Nile Delta, China, Japan, Taiwan, the Philippines, and parts of the former Soviet Union. Many reservoir hosts, such as dogs, cats, and fish-eating birds, may play an important role in maintenance of infection in some endemic areas.

Pathology

The small heterophyid flukes cause variable degrees of inflammation in the intestinal wall.[17] Superficial inflammation and erosions are found at the sites of mucosal attachment. Eggs deposited beneath the mucosa cause eosinophilic granulomatous lesions. Eggs may invade the circulatory system through the intestinal capillaries or

lymphatics. Eggs may reach distant sites such as the heart, brain, and spinal cord, causing significant morbidity and even mortality. The embolized eggs induce a granulomatous response, with the eventual production of fibrosis in the infected areas. The complications of embolic heterophyiasis appear to be particularly frequent in the Philippines, where heart disease often is attributed to heterophyiasis.

Clinical Manifestations

Light infections without the deposition of ectopic eggs usually are asymptomatic, although eosinophilia may be noted. In heavier infections, epigastric pain, diarrhea, malabsorption, and weight loss may occur. Seizures may result from eggs carried to the brain. Congestive heart failure or arrhythmias may occur after cardiac involvement.

Diagnosis

Routine fecal microscopy may demonstrates eggs. Nonetheless, differentiating morphologically heterophyid eggs from those of the liver flukes may be difficult. Eggs recovered from patients who have been exposed more than 2 years before presentation are very likely to be from *Opisthorchis* or *Clonorchis*, because the life span of heterophyids is usually less than a year.

Treatment and Prognosis

Praziquantel 75 mg/kg/day in three divided doses for 1 day is the recommended treatment. With the exception of the complication of egg emboli to distant organs, the prognosis is excellent.

METORCHIASIS

M. conjunctus, endemic in North American; *M. bilis*, in Russia; and *M. orientalis*, in China and Korea, are members of the Opisthorchiidae family. They have a life cycle similar to that of the other opisthorchids. A wide variety of fish-eating mammals may serve as definitive hosts. This infection is a significant cause of morbidity and mortality in sled dogs in Canada. It is an occasional incidental finding on fecal examination in native communities in northern Canada. An outbreak of 19 cases of *Metorchis* infection was related to fish prepared raw as sashimi.[65] The epidemiology of metorchiasis outside North America seems to be far from an incidental illness. In areas where the epidemiology of *O. felineus* and *M. bilis* overlap, the difficulties differentiating *M. bilis* from *O. felineus* eggs have led to overdiagnosis of opisthorchiasis and the impression that it is much more common than metorchiasis. In Russia, serologic studies showed that metorchiasis is more common than previously recognized. In one study, 13 percent of subjects diagnosed as having opisthorchiasis had a positive ELISA for *M. bilis* and 37 percent had positive ELISA tests for both trematodes.[73] Similarly, national surveys in China have identify some foci of human *M. orientalis* infection in the Fujian province. The severity and duration of the symptoms probably correlate with parasite burden. The symptoms during acute infection are fatigue, upper abdominal tenderness, fever, epigastric abdominal pain, headache, weight loss, anorexia, nausea, diarrhea, vomiting, muscle pain, backache, cough, and rash.[65] The degree of eosinophilia and elevation in liver enzymes are proportional to the intensity of infection. Eggs are noted in stool 10 days after infection develops. Praziquantel is effective during acute metorchiasis and in the chronic phase.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdel Razek AA, Watcharakorn A, Castillo M. Parasitic diseases of the central nervous system. *Neuroimaging Clin N Am* 2011; **21**:815–41, viii.
2. Ai L, Li C, Elsheikha HM, et al. Rapid identification and differentiation of *Fasciola hepatica* and *Fasciola gigantica* by a loop-mediated isothermal amplification (LAMP) assay. *Vet Parasitol* 2010;**174**:228–33.
3. Aka NA, Adoubryn K, Rondelaud D, et al. Human paragonimiasis in Africa. *Ann Afr Med* 2008;**7**:153–62.
4. Arimatsu Y, Kaewkes S, Laha T, et al. Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). *Parasitol Int* 2012;**61**:178–82.
6. Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol* 2009;**25**:151–6.
7. Bhatti HS, Malla N, Mahajan RC, et al. Fasciolopslasis: a re-emerging infection in Azamgarh (Uttar Pradesh). *Indian J Pathol Microbiol* 2000;**43**:73–6.
8. Blair D, Agatsuma T, Wang W. Paragonimiasis. In: Murrell KD, Fried B, editor. *Food-borne parasitic zoonoses: fish and plant-borne parasites*, vol. 11. New York: Springer; 2007. p. 117–50.
9. Blair D, Chang Z, Chen M, et al. *Paragonimus skrjabini* Chen, 1959 (Digenea: Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. *Syst Parasitol* 2005;**60**:1–21.
10. Blancas-Torres GB, Terashima-Iwashita A, Maguines-Vargas C, et al. [Human fasciolasis and gastrointestinal compromise: study of 277 patients in the Cayetano Heredia National Hospital (1970-2002)]. *Rev Gastroenterol Peru* 2004;**24**:143–57.
11. Boland JM, Vaszar LT, Jones JL, et al. Pleuropulmonary infection by *Paragonimus westermani* in the United States: a rare cause of eosinophilic pneumonia after ingestion of live crabs. *Am J Surg Pathol* 2011;**35**:707–13.
12. Boonmars T, Wu Z, Boonjaruspinyo S, et al. Alterations of gene expression of RB pathway in *Opisthorchis viverrini* infection-induced cholangiocarcinoma. *Parasitol Res* 2009;**105**:1273–81.
13. Cabada MM, White AC. New developments in epidemiology, diagnosis, and treatment of fascioliasis. *Curr Opin Infect Dis* 2012;**25**:518–22.
14. Cai XQ, Xu MJ, Wang YH, et al. Sensitive and rapid detection of *Clonorchis sinensis* infection in fish by loop-mediated isothermal amplification (LAMP). *Parasitol Res* 2010;**106**:1379–83.
16. Cantisani V, Cantisani C, Mortelé K, et al. Diagnostic imaging in the study of human hepatobiliary fascioliasis. *Radiol Med* 2010;**115**:83–92.
17. Chai J-Y. Intestinal flukes, In: Murrell KD, Fried B, editors. *Parasitic zoonoses: fish and plant-borne parasites*, vol. 11. New York: Springer; 2007. p. 53–115.
18. Chen J, Chen Z, Lin J, et al. Cerebral paragonimiasis: a retrospective analysis of 89 cases. *Clin Neurol Neurosurg* 2012.
19. Chen MX, Ai L, Zhang RL, et al. Sensitive and rapid detection of *Paragonimus westermani* infection in humans and animals by loop-mediated isothermal amplification (LAMP). *Parasitol Res* 2011;**108**:1193–8.
20. Cho SH, Lee KY, Lee BC, et al. Prevalence of clonorchiasis in southern endemic areas of Korea in 2006. *Korean J Parasitol* 2008;**46**:133–7.
22. Choi MH, Park IC, Li S, Hong ST. Excretory-secretory antigen is better than crude antigen for the serodiagnosis of clonorchiasis by ELISA. *Korean J Parasitol* 2003;**41**:35–9.

24. Demerdash ZA, Diab TM, Aly IR, et al. Diagnostic efficacy of monoclonal antibody based sandwich enzyme linked immunosorbent assay (ELISA) for detection of *Fasciola gigantica* excretory/secretory antigens in both serum and stool. *Parasit Vectors* 2011;**4**:176.
25. Devi KR, Narain K, Bhattacharya S, et al. Pleuropulmonary paragonimiasis due to *Paragonimus heterotremus:* molecular diagnosis, prevalence of infection and clinicoradiological features in an endemic area of northeastern India. *Trans R Soc Trop Med Hyg* 2007;**101**:786–92.
26. Doanh PN, Dung DT, Thach DT, et al. Human paragonimiasis in Viet Nam: epidemiological survey and identification of the responsible species by DNA sequencing of eggs in patients' sputum. *Parasitol Int* 2011;**60**:534–7.
27. Dusak A, Onur MR, Cicek M, et al. Radiological imaging features of *Fasciola hepatica* infection: a pictorial review. *J Clin Imaging Sci* 2012;**2**:2.
28. Espinoza JR, Maco V, Marcos L, et al. Evaluation of Fas2-ELISA for the serological detection of *Fasciola hepatica* infection in humans. *Am J Trop Med Hyg* 2007;**76**:977–82.
30. Fischer PU, Curtis KC, Marcos LA, et al. Molecular characterization of the North American lung fluke *Paragonimus kellicotti* in Missouri and its development in Mongolian gerbils. *Am J Trop Med Hyg* 2011;**84**:1005–11.
31. Forrer A, Sayasone S, Vounatsou P, et al. Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in southern Lao PDR. *PLoS Negl Trop Dis* 2012;**6**:e1481.
32. Fried B, Reddy A, Mayer D. Helminths in human carcinogenesis. *Cancer Lett* 2011;**305**:239–49.
33. Fürst T, Duthaler U, Sripa B, et al. Trematode infections: liver and lung flukes. *Infect Dis Clin North Am* 2012;**26**:399–419.
34. Fürst T, Keiser J, Utzinger J. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:210–21.
35. Fürst T, Sayasone S, Odermatt P, et al. Manifestation, diagnosis, and management of foodborne trematodiasis. *BMJ* 2012;**344**:e4093.
36. Gilman RH, Mondal G, Maksud M, et al. Endemic focus of *Fasciolopsis buski* infection in Bangladesh. *Am J Trop Med Hyg* 1982;**31**:796–802.
37. Graczyk TK, Gilman RH, Fried B. Fasciolopsiasis: is it a controllable food-borne disease? *Parasitol Res* 2001;**87**:80–3.
38. Grundy-Warr C, Andrews RH, Sithithaworn P, et al. Raw attitudes, wetland cultures, life-cycles: socio-cultural dynamics relating to Opisthorchis viverrini in the Mekong Basin. *Parasitol Int* 2012;**61**:65–70.
39. Hatami H, Asmar M, Masoud J, et al. The first epidemic and new-emerging human fascioliasis in Kermanshah (Western Iran) and a ten-year follow up, 1998-2008. *Int J Prev Med* 2012;**3**:266–72.
40. Henry TS, Lane MA, Weil GJ, et al. Chest CT features of North American paragonimiasis. *AJR Am J Roentgenol* 2012;**198**:1076–83.
41. Hong ST, Fang Y. *Clonorchis sinensis* and clonorchiasis, an update. *Parasitol Int* 2012;**61**:17–24.
42. Iwagami M, Ho LY, Su K, et al. Molecular phylogeographic studies on *Paragonimus westermani* in Asia. *J Helminthol* 2000;**74**:315–22.
43. Iwagami M, Monroy C, Rosas MA, et al. A molecular phylogeographic study based on DNA sequences from individual metacercariae of *Paragonimus mexicanus* from Guatemala and Ecuador. *J Helminthol* 2003;**77**:33–8.
45. Johansen MV, Sithithaworn P, Bergquist R, et al. Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. *Adv Parasitol* 2010;**73**:171–95.
46. Kanpittaya J, Sawanyawisuth K, Vannavong A, et al. Different chest radiographic findings of pulmonary paragonimiasis in two endemic countries. *Am J Trop Med Hyg* 2010;**83**:924–6.
47. Kaya M, Beştaş R, Cetin S. Clinical presentation and management of *Fasciola hepatica* infection: single-center experience. *World J Gastroenterol* 2011;**17**:4899–904.
48. Keiser J, Duthaler U, Utzinger J. Update on the diagnosis and treatment of food-borne trematode infections. *Curr Opin Infect Dis* 2010;**23**:513–20.
49. Keiser J, Sayed H, el-Ghanam M, et al. Efficacy and safety of artemether in the treatment of chronic fascioliasis in Egypt: exploratory phase-2 trials. *PLoS Negl Trop Dis* 2011;**5**:e1285.
51. Keiser J, Utzinger J. Food-borne trematodiases. *Clin Microbiol Rev* 2009;**22**:466–83.
52. Kim EM, Verweij JJ, Jalili A, et al. Detection of Clonorchis sinensis in stool samples using real-time PCR. *Ann Trop Med Parasitol* 2009;**103**:513–18.
53. Kim TS, Han J, Shim SS, et al. Pleuropulmonary paragonimiasis: CT findings in 31 patients. *AJR Am J Roentgenol* 2005;**185**:616–21.
55. Kim YJ, Lee SM, Choi GE, et al. Performance of an enzyme-linked immunosorbent assay for detection of *Clonorchis sinensis* infestation in high- and low-risk groups. *J Clin Microbiol* 2010;**48**:2365–7.
56. Koç Z, Ulusan S, Tokmak N. Hepatobiliary fascioliasis: imaging characteristics with a new finding. *Diagn Interv Radiol* 2009;**15**:247–51.
57. Lane MA, Marcos LA, Onen NF, et al. *Paragonimus kellicotti* flukes in Missouri, USA. *Emerg Infect Dis* 2012;**18**:1263–7.
58. Le TH, Nguyen NT, Truong NH, et al. Development of mitochondrial loop-mediated isothermal amplification for detection of the small liver fluke *Opisthorchis viverrini* (Opisthorchiidae; Trematoda; Platyhelminthes). *J Clin Microbiol* 2012;**50**:1178–84.
59. Li XM, Yu JQ, Yang ZG, et al. Correlations between MDCT features and clinicopathological findings of hepatic paragonimiasis. *Eur J Radiol* 2012;**81**:e421–5.
60. Liu Q, Wei F, Liu W, et al. Paragonimiasis: an important food-borne zoonosis in China. *Trends Parasitol* 2008;**24**:318–23.
61. Lobna SM, Metawea YF, Elsheikha HM. Prevalence of heterophyiosis in tilapia fish and humans in Northern Egypt. *Parasitol Res* 2010;**107**:1029–34.
62. Lopez M, White AC, Cabada MM. Burden of *Fasciola hepatica* infection among children from Paucartambo in Cusco, Peru. *Am J Trop Med Hyg* 2012;**86**:481–5.
63. Lovis L, Mak TK, Phongluxa K, et al. Efficacy of Praziquantel against *Schistosoma mekongi* and *Opisthorchis viverrini:* a randomized, single-blinded dose-comparison trial. *PLoS Negl Trop Dis* 2012;**6**:e1726.
64. Lv S, Zhang Y, Steinmann P, et al. Helminth infections of the central nervous system occurring in Southeast Asia and the Far East. *Adv Parasitol* 2010;**72**:351–408.
66. Mahanty S, Maclean JD, Cross JH. Liver, lung, and intestinal fluke infections. In: Guerrant RL, Walker DH, Weller PF, editors. *Tropical infectious diseases*, 3rd ed. Edinburgh: Saunders; 2011. p. 854–67.
67. Mairiang E, Laha T, Bethony JM, et al. Ultrasonography assessment of hepatobiliary abnormalities in 3359 subjects with *Opisthorchis viverrini* infection in endemic areas of Thailand. *Parasitol Int* 2012;**61**:208–11.
70. Marcos LA, Tagle M, Terashima A, et al. Natural history, clinicoradiologic correlates, and response to triclabendazole in acute massive fascioliasis. *Am J Trop Med Hyg* 2008;**78**:222–7.
71. Marcos LA, Terashima A, Gotuzzo E. Update on hepatobiliary flukes: fascioliasis, opisthorchiasis and clonorchiasis. *Curr Opin Infect Dis* 2008;**21**:523–30.
72. Mas-Coma S, Valero MA, Bargues MD. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Adv Parasitol* 2009;**69**:41–146.
73. Mordvinov VA, Yurlova NI, Ogorodova LM, et al. *Opisthorchis felineus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. *Parasitol Int* 2012;**61**:25–31.
74. Nkouawa A, Okamoto M, Mabou AK, et al. Paragonimiasis in Cameroon: molecular identification, serodiagnosis and clinical manifestations. *Trans R Soc Trop Med Hyg* 2009;**103**:255–61.
77. Rokni MB. The present status of human helminthic diseases in Iran. *Ann Trop Med Parasitol* 2008;**102**:283–95.
78. Salahi-Moghaddam A, Habibi-Nokhandam M, Fuentes MV. Low-altitude outbreaks of human fascioliasis related with summer rainfall in Gilan province, Iran. *Geospat Health* 2011;**6**:133–6.
79. Sato M, Thaenkham U, Dekumyoy P, et al. Discrimination of *O. viverrini*, *C. sinensis*, *H. pumilio* and *H. taichui* using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. *Acta Trop* 2009;**109**:81–3.
80. Sayasone S, Rasphone O, Vanmany M, et al. Severe morbidity due to opisthorchis viverrini and schistosoma mekongi infection in Lao People's Democratic Republic. *Clin Infect Dis* 2012;**55**:e54–57.

81. Singh TS, Sugiyama H, Rangsiruji A. *Paragonimus* & paragonimiasis in India. *Indian J Med Res* 2012;**136**:192–204.
82. Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR, et al. Evaluation of a monoclonal antibody-based enzyme linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. *Am J Trop Med Hyg* 1995;**52**:521–4.
83. Sithithaworn P, Andrews RH, Nguyen VD, et al. The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitol Int* 2012;**61**:10–16.
84. Slesak G, Inthalad S, Basy P, et al. Ziehl-Neelsen staining technique can diagnose paragonimiasis. *PLoS Negl Trop Dis* 2011;**5**: e1048.
85. Soukhathammavong P, Odermatt P, Sayasone S, et al. Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel in patients with *Opisthorchis viverrini:* a randomised, exploratory, open-label, phase 2 trial. *Lancet Infect Dis* 2011;**11**:110–18.
86. Sripa B. Pathobiology of opisthorchiasis: an update. *Acta Trop* 2003;**88**:209–20.
87. Sripa B, Brindley PJ, Mulvenna J, et al. The tumorigenic liver fluke *Opisthorchis viverrini:* multiple pathways to cancer. *Trends Parasitol* 2012.
88. Sripa B, Kaewkes S, Intapan PM, et al. Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. *Adv Parasitol* 2010;**72**:305–50.
89. Sripa B, Thinkhamrop B, Mairiang E, et al. Elevated plasma IL-6 associates with increased risk of advanced fibrosis and cholangiocarcinoma in individuals infected by *Opisthorchis viverrini*. *PLoS Negl Trop Dis* 2012;**6**:e1654.
90. Thuwajit C, Thuwajit P, Kaewkes S, et al. Increased cell proliferation of mouse fibroblast NIH-3T3 in vitro induced by excretory/secretory product(s) from *Opisthorchis viverrini*. *Parasitology* 2004; **129**:455–64.
91. Torres GB, Iwashita AT, Vargas CM, et al. [Human fasciolasis and gastrointestinal compromise: study of 277 patients in the Cayetano Heredia National Hospital (1970-2002)]. *Rev Gastroenterol Peru* 2004;**24**:143–57.
92. Tran VH, Tran TK, Nguye HC, et al. Fascioliasis in Vietnam. *Southeast Asian J Trop Med Public Health* 2001;**32**(Suppl. 2): 48–50.
93. Trung Dung D, Van De N, Waikagul J, et al. Fishborne zoonotic intestinal trematodes, Vietnam. *Emerg Infect Dis* 2007;**13**:1828–33.
94. Ubeira FM, Muiño L, Valero MA, et al. MM3-ELISA detection of *Fasciola hepatica* coproantigens in preserved human stool samples. *Am J Trop Med Hyg* 2009;**81**:156–62.
95. Valero MA, Periago MV, Pérez-Crespo I, et al. Assessing the validity of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological situations. *Trop Med Int Health* 2012;**17**:630–6.
96. Villegas F, Angles R, Barrientos R, et al. Administration of triclabendazole is safe and effective in controlling fascioliasis in an endemic community of the bolivian altiplano. *PLoS Negl Trop Dis* 2012;**6**:e1720.
97. Weng YL, Zhuang ZL, Jiang HP, et al. [Studies on ecology of *Fasciolopsis buski* and control strategy of fasciolopsiasis]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 1989;**7**: 108–11.
98. Winkelhagen AJ, Mank T, de Vries PJ, et al. Apparent triclabendazole-resistant human Fasciola hepatica infection, the Netherlands. *Emerg Infect Dis* 2012;**18**:1028–9.
100. World Health Organization. *Report of the WHO expert consultation on foodborne trematode infections and taeniasis/cysticercosis*, Vientiane: La People's Democratic Republic; 2011. 12-6 October 2009.
101. Yahiro S, Habe S, Duong V, et al. Identification of the human paragonimiasis causative agent in Lao People's Democratic Republic. *J Parasitol* 2008;**94**:1176–7.
102. Yu SH, Kawanaka M, Li XM, et al. Epidemiological investigation on *Clonorchis sinensis* in human population in an area of South China. *Jpn J Infect Dis* 2003;**56**:168–71.
103. Zarrin-Khameh N, Citron DR, Stager CE, et al. Pulmonary paragonimiasis diagnosed by fine-needle aspiration biopsy. *J Clin Microbiol* 2008;**46**:2137–40.
104. Zhang XL, Wang Y, Wang GX, et al. Distribution and clinical features of Paragonimiasis *skrjabini* in Three Gorges Reservoir Region. *Parasitol Int* 2012;**61**:645–9.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 228

Schistosomiasis

Philip R. Fischer • A. Clinton White, Jr.

Approximately 230 million individuals are infected with parasites of the genus *Schistosoma*,[36,81,140,186,187] and another 700 million people are at risk for infection.[156,187,188] Many are unaware of their infection, although approximately 20 million people have severe disease with potentially fatal portal hypertension, urinary tract involvement, renal failure, and bladder cancer. Initial infection usually occurs during childhood. The prevalence typically peaks in the teen years, and, although most of the morbidity occurs in adults, symptomatic disease develops in millions of children.

There is historical evidence of schistosomiasis occurring as early as the second millennium BCE.[165,168] For example, *Schistosoma* eggs have been found in mummies from the 20th dynasty in ancient Egypt and in ancient China. In 1851, Theodor Bilharz identified the parasitic etiology of endemic hematuria. At the same time, the association between *Schistosoma* and Katayama fever was

REFERENCES

1. Abdel Razek AA, Watcharakorn A, Castillo M. Parasitic diseases of the central nervous system. *Neuroimaging Clin N Am* 2011; **21**:815–41, viii.
2. Ai L, Li C, Elsheikha HM, et al. Rapid identification and differentiation of *Fasciola hepatica* and *Fasciola gigantica* by a loop-mediated isothermal amplification (LAMP) assay. *Vet Parasitol* 2010;**174**:228–33.
3. Aka NA, Adoubryn K, Rondelaud D, et al. Human paragonimiasis in Africa. *Ann Afr Med* 2008;**7**:153–62.
4. Arimatsu Y, Kaewkes S, Laha T, et al. Rapid detection of *Opisthorchis viverrini* copro-DNA using loop-mediated isothermal amplification (LAMP). *Parasitol Int* 2012;**61**:178–82.
5. Arjona R, Riancho JA, Aguado JM, et al. Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. *Medicine (Baltimore)* 1995;**74**:13–23.
6. Bergquist R, Johansen MV, Utzinger J. Diagnostic dilemmas in helminthology: what tools to use and when? *Trends Parasitol* 2009;**25**:151–6.
7. Bhatti HS, Malla N, Mahajan RC, et al. Fasciolopslasis: a re-emerging infection in Azamgarh (Uttar Pradesh). *Indian J Pathol Microbiol* 2000;**43**:73–6.
8. Blair D, Agatsuma T, Wang W. Paragonimiasis. In: Murrell KD, Fried B, editors. *Food-borne parasitic zoonoses: fish and plant-borne parasites*, vol. 11. New York: Springer; 2007. p. 117–50.
9. Blair D, Chang Z, Chen M, et al. *Paragonimus skrjabini* Chen, 1959 (Digenea: Paragonimidae) and related species in eastern Asia: a combined molecular and morphological approach to identification and taxonomy. *Syst Parasitol* 2005;**60**:1–21.
10. Blancas-Torres GB, Terashima-Iwashita A, Maguines-Vargas C, et al. [Human fasciolasis and gastrointestinal compromise: study of 277 patients in the Cayetano Heredia National Hospital (1970-2002)]. *Rev Gastroenterol Peru* 2004;**24**:143–57.
11. Boland JM, Vaszar LT, Jones JL, et al. Pleuropulmonary infection by *Paragonimus westermani* in the United States: a rare cause of Eosinophilic pneumonia after ingestion of live crabs. *Am J Surg Pathol* 2011;**35**:707–13.
12. Boonmars T, Wu Z, Boonjaruspinyo S, et al. Alterations of gene expression of RB pathway in *Opisthorchis viverrini* infection-induced cholangiocarcinoma. *Parasitol Res* 2009;**105**:1273–81.
13. Cabada MM, White AC. New developments in epidemiology, diagnosis, and treatment of fascioliasis. *Curr Opin Infect Dis* 2012;**25**:518–22.
14. Cai XQ, Xu MJ, Wang YH, et al. Sensitive and rapid detection of Clonorchis sinensis infection in fish by loop-mediated isothermal amplification (LAMP). *Parasitol Res* 2010;**106**:1379–83.
15. Calvopiña M, Guderian RH, Paredes W, Cooper PJ. Comparison of two single-day regimens of triclabendazole for the treatment of human pulmonary paragonimiasis. *Trans R Soc Trop Med Hyg* 2003;**97**:451–4.
16. Cantisani V, Cantisani C, Mortelé K, et al. Diagnostic imaging in the study of human hepatobiliary fascioliasis. *Radiol Med* 2010; **115**:83–92.
17. Chai J-Y. Intestinal flukes. In: Murrell KD, Fried B, editors. Parasitic zoonoses: fish and plant-borne parasites, vol. 11. New York: Springer; 2007. p. 53–115.
18. Chen J, Chen Z, Lin J, et al. Cerebral paragonimiasis: a retrospective analysis of 89 cases. *Clin Neurol Neurosurg* 2012.
19. Chen MX, Ai L, Zhang RL, et al. Sensitive and rapid detection of *Paragonimus westermani* infection in humans and animals by loop-mediated isothermal amplification (LAMP). *Parasitol Res* 2011;**108**:1193–8.
20. Cho SH, Lee KY, Lee BC, et al. Prevalence of clonorchiasis in southern endemic areas of Korea in 2006. *Korean J Parasitol* 2008;**46**:133–7.
21. Choi BI, Han JK, Hong ST, et al. Clonorchiasis and cholangiocarcinoma: etiologic relationship and imaging diagnosis. *Clin Microbiol Rev* 2004;**17**:540–52.
22. Choi MH, Park IC, Li S, Hong ST. Excretory-secretory antigen is better than crude antigen for the serodiagnosis of clonorchiasis by ELISA. *Korean J Parasitol* 2003;**41**:35–9.
23. Choi MS, Choi D, Choi MH, et al. Correlation between sonographic findings and infection intensity in clonorchiasis. *Am J Trop Med Hyg* 2005;**73**:1139–44.
24. Demerdash ZA, Diab TM, Aly IR, et al. Diagnostic efficacy of monoclonal antibody based sandwich enzyme linked immunosorbent assay (ELISA) for detection of *Fasciola gigantica* excretory/secretory antigens in both serum and stool. *Parasit Vectors* 2011; **4**:176.
25. Devi KR, Narain K, Bhattacharya S, et al. Pleuropulmonary paragonimiasis due to *Paragonimus heterotremus:* molecular diagnosis, prevalence of infection and clinicoradiological features in an endemic area of northeastern India. *Trans R Soc Trop Med Hyg* 2007;**101**:786–92.
26. Doanh PN, Dung DT, Thach DT, et al. Human paragonimiasis in Viet Nam: epidemiological survey and identification of the responsible species by DNA sequencing of eggs in patients' sputum. *Parasitol Int* 2011;**60**:534–7.
27. Dusak A, Onur MR, Cicek M, et al. Radiological imaging features of *Fasciola hepatica* infection: a pictorial review. *J Clin Imaging Sci* 2012;**2**:2.
28. Espinoza JR, Maco V, Marcos L, et al. Evaluation of Fas2-ELISA for the serological detection of *Fasciola hepatica* infection in humans. *Am J Trop Med Hyg* 2007;**76**:977–82.
29. Favennec L, Jave Ortiz J, Gargala G, et al. Double-blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from northern Peru. *Aliment Pharmacol Ther* 2003;**17**:265–70.
30. Fischer PU, Curtis KC, Marcos LA, et al. Molecular characterization of the North American lung fluke *Paragonimus kellicotti* in Missouri and its development in Mongolian gerbils. *Am J Trop Med Hyg* 2011;**84**:1005–11.
31. Forrer A, Sayasone S, Vounatsou P, et al. Spatial distribution of, and risk factors for, *Opisthorchis viverrini* infection in southern Lao PDR. *PLoS Negl Trop Dis* 2012;**6**:e1481.
32. Fried B, Reddy A, Mayer D. Helminths in human carcinogenesis. *Cancer Lett* 2011;**305**:239–49.
33. Fürst T, Duthaler U, Sripa B, et al. Trematode infections: liver and lung flukes. *Infect Dis Clin North Am* 2012;**26**:399–419.
34. Fürst T, Keiser J, Utzinger J. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012;**12**:210–21.
35. Fürst T, Sayasone S, Odermatt P, et al. Manifestation, diagnosis, and management of foodborne trematodiasis. *BMJ* 2012;**344**: e4093.
36. Gilman RH, Mondal G, Maksud M, et al. Endemic focus of Fasciolopsis buski infection in Bangladesh. *Am J Trop Med Hyg* 1982;**31**:796–802.
37. Graczyk TK, Gilman RH, Fried B. Fasciolopsiasis: is it a controllable food-borne disease? *Parasitol Res* 2001;**87**:80–3.
38. Grundy-Warr C, Andrews RH, Sithithaworn P, et al. Raw attitudes, wetland cultures, life-cycles: socio-cultural dynamics relating to Opisthorchis viverrini in the Mekong Basin. *Parasitol Int* 2012;**61**:65–70.
39. Hatami H, Asmar M, Masoud J, et al. The First Epidemic and New-emerging Human Fascioliasis in Kermanshah (Western Iran) and a Ten-year Follow Up, 1998-2008. *Int J Prev Med* 2012;**3**:266–72.
40. Henry TS, Lane MA, Weil GJ, Bailey TC, Bhalla S. Chest CT features of North American paragonimiasis. *AJR Am J Roentgenol* 2012;**198**:1076–83.
41. Hong ST, Fang Y. Clonorchis sinensis and clonorchiasis, an update. *Parasitol Int* 2012;**61**:17–24.
42. Iwagami M, Ho LY, Su K, et al. Molecular phylogeographic studies on Paragonimus westermani in Asia. *J Helminthol* 2000; **74**:315–22.
43. Iwagami M, Monroy C, Rosas MA, et al. A molecular phylogeographic study based on DNA sequences from individual metacercariae of Paragonimus mexicanus from Guatemala and Ecuador. *J Helminthol* 2003;**77**:33–8.
44. Jamornthanyawat N. The diagnosis of human opisthorchiasis. *Southeast Asian J Trop Med Public Health* 2002;**33**(Suppl. 3): 86–91.
45. Johansen MV, Sithithaworn P, Bergquist R, Utzinger J. Towards improved diagnosis of zoonotic trematode infections in Southeast Asia. *Adv Parasitol* 2010;**73**:171–95.
46. Kanpittaya J, Sawanyawisuth K, Vannavong A, et al. Different chest radiographic findings of pulmonary paragonimiasis in two endemic countries. *Am J Trop Med Hyg* 2010;**83**:924–6.

47. Kaya M, Beştaş R, Cetin S. Clinical presentation and management of Fasciola hepatica infection: single-center experience. *World J Gastroenterol* 2011;**17**:4899–904.
48. Keiser J, Duthaler U, Utzinger J. Update on the diagnosis and treatment of food-borne trematode infections. *Curr Opin Infect Dis* 2010;**23**:513–20.
49. Keiser J, Sayed H, el-Ghanam M, et al. Efficacy and safety of artemether in the treatment of chronic fascioliasis in Egypt: exploratory phase-2 trials. *PLoS Negl Trop Dis* 2011;**5**:e1285.
50. Keiser J, Utzinger J. Emerging foodborne trematodiasis. *Emerg Infect Dis* 2005;**11**:1507–14.
51. Keiser J, Utzinger J. Food-borne trematodiases. *Clin Microbiol Rev* 2009;**22**:466–83.
52. Kim EM, Verweij JJ, Jalili A, et al. Detection of Clonorchis sinensis in stool samples using real-time PCR. *Ann Trop Med Parasitol* 2009;**103**:513–18.
53. Kim TS, Han J, Shim SS, et al. Pleuropulmonary paragonimiasis: CT findings in 31 patients. *AJR Am J Roentgenol* 2005;**185**: 616–21.
54. Kim YH. Pancreatitis in association with Clonorchis sinensis infestation: CT evaluation. *AJR Am J Roentgenol* 1999;**172**:1293–6.
55. Kim YJ, Lee SM, Choi GE, et al. Performance of an enzyme-linked immunosorbent assay for detection of Clonorchis sinensis infestation in high- and low-risk groups. *J Clin Microbiol* 2010; **48**:2365–7.
56. Koç Z, Ulusan S, Tokmak N. Hepatobiliary fascioliasis: imaging characteristics with a new finding. *Diagn Interv Radiol* 2009; **15**:247–51.
57. Lane MA, Marcos LA, Onen NF, et al. Paragonimus kellicotti Flukes in Missouri, USA. *Emerg Infect Dis* 2012;**18**:1263–7.
58. Le TH, Nguyen NT, Truong NH, De NV. Development of mitochondrial loop-mediated isothermal amplification for detection of the small liver fluke Opisthorchis viverrini (Opisthorchiidae; Trematoda; Platyhelminthes). *J Clin Microbiol* 2012;**50**: 1178–84.
59. Li XM, Yu JQ, Yang ZG, Chu ZG, Peng LQ, Kushwaha S. Correlations between MDCT features and clinicopathological findings of hepatic paragonimiasis. *Eur J Radiol* 2012;**81**:e421–5.
60. Liu Q, Wei F, Liu W, Yang S, Zhang X. Paragonimiasis: an important food-borne zoonosis in China. *Trends Parasitol* 2008;**24**: 318–23.
61. Lobna SM, Metawea YF, Elsheikha HM. Prevalence of heterophyiosis in Tilapia fish and humans in Northern Egypt. *Parasitol Res* 2010;**107**:1029–34.
62. Lopez M, White AC, Cabada MM. Burden of Fasciola hepatica Infection among children from Paucartambo in Cusco, Peru. *Am J Trop Med Hyg* 2012;**86**:481–5.
63. Lovis L, Mak TK, Phongluxa K, et al. Efficacy of Praziquantel against Schistosoma mekongi and Opisthorchis viverrini: A Randomized, Single-Blinded Dose-Comparison Trial. *PLoS Negl Trop Dis* 2012;**6**:e1726.
64. Lv S, Zhang Y, Steinmann P, et al. Helminth infections of the central nervous system occurring in Southeast Asia and the Far East. *Adv Parasitol* 2010;**72**:351–408.
65. MacLean JD, Arthur JR, Ward BJ, et al. Common-source outbreak of acute infection due to the North American liver fluke Metorchis conjunctus. *Lancet* 1996;**347**:154–8.
66. Mahanty S, Maclean JD, Cross JH. Liver, lung, and intestinal fluke infections. In: Guerrant RL, Walker DH, Weller PF, editors, *Tropical infectious diseases*, 3rd ed. Edinburgh: Saunders; 2011. p. 854–67.
67. Mairiang E, Mairiang P. Clinical manifestation of opisthorchiasis and treatment. *Acta Trop* 2003;**88**:221–7.
68. Mairiang E, Laha T, Bethony JM, et al. Ultrasonography assessment of hepatobiliary abnormalities in 3359 subjects with Opisthorchis viverrini infection in endemic areas of Thailand. *Parasitol Int* 2012;**61**:208–11.
69. Marcos L, Maco V, Samalvides F, et al. Risk factors for Fasciola hepatica infection in children: a case-control study. *Trans R Soc Trop Med Hyg* 2006;**100**:158–66.
70. Marcos LA, Terashima A, Gotuzzo E. Update on hepatobiliary flukes: fascioliasis, opisthorchiasis and clonorchiasis. *Curr Opin Infect Dis* 2008;**21**:523–30.
71. Marcos LA, Tagle M, Terashima A, et al. Natural history, clinicoradiologic correlates, and response to triclabendazole in acute massive fascioliasis. *Am J Trop Med Hyg* 2008;**78**:222–7.
72. Mas-Coma S, Valero MA, Bargues MD. Chapter 2. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Adv Parasitol* 2009;**69**:41–146.
73. Mordvinov VA, Yurlova NI, Ogorodova LM, et al. *Opisthorchis felineus* and *Metorchis bilis* are the main agents of liver fluke infection of humans in Russia. *Parasitol Int* 2012;**61**: 25–31.
74. Nkouawa A, Okamoto M, Mabou AK, et al. Paragonimiasis in Cameroon: molecular identification, serodiagnosis and clinical manifestations. *Trans R Soc Trop Med Hyg* 2009;**103**:255–61.
75. Pinlaor S, Ma N, Hiraku Y, et al. Repeated infection with Opisthorchis viverrini induces accumulation of 8-nitroguanine and 8-oxo-7,8-dihydro-2'-deoxyguanine in the bile duct of hamsters via inducible nitric oxide synthase. *Carcinogenesis* 2004;**25**:1535–42.
76. Pungpak S, Viravan C, Radomyos B, et al. Opisthorchis viverrini infection in Thailand: studies on the morbidity of the infection and resolution following praziquantel treatment. *Am J Trop Med Hyg* 1997;**56**:311–14.
77. Rokni MB. The present status of human helminthic diseases in Iran. *Ann Trop Med Parasitol* 2008;**102**:283–95.
78. Salahi-Moghaddam A, Habibi-Nokhandam M, Fuentes MV. Low-altitude outbreaks of human fascioliasis related with summer rainfall in Gilan province, Iran. *Geospat Health* 2011;**6**: 133–6.
79. Sato M, Thaenkham U, Dekumyoy P, Waikagul J. Discrimination of O. viverrini, C. sinensis, H. pumilio and H. taichui using nuclear DNA-based PCR targeting ribosomal DNA ITS regions. *Acta Trop* 2009;**109**:81–3.
80. Sayasone S, Rasphone O, Vanmany M, et al. Severe Morbidity Due to Opisthorchis viverrini and Schistosoma mekongi Infection in Lao People's Democratic Republic. *Clin Infect Dis* 2012;**55**: e54–7.
81. Singh TS, Sugiyama H, Rangsiruji A. *Paragonimus* & paragonimiasis in India. *Indian J Med Res* 2012;**136**:192–204.
82. Sirisinha S, Chawengkirttikul R, Haswell-Elkins MR, et al. Evaluation of a monoclonal antibody-based enzyme linked immunosorbent assay for the diagnosis of *Opisthorchis viverrini* infection in an endemic area. *Am J Trop Med Hyg* 1995;**52**:521–4.
83. Sithithaworn P, Andrews RH, Nguyen VD, et al. The current status of opisthorchiasis and clonorchiasis in the Mekong Basin. *Parasitol Int* 2012;**61**:10–16.
84. Slesak G, Inthalad S, Basy P, et al. Ziehl-Neelsen staining technique can diagnose paragonimiasis. *PLoS Negl Trop Dis* 2011;**5**: e1048.
85. Soukhathammavong P, Odermatt P, Sayasone S, et al. Efficacy and safety of mefloquine, artesunate, mefloquine-artesunate, tribendimidine, and praziquantel in patients with Opisthorchis viverrini: a randomised, exploratory, open-label, phase 2 trial. *Lancet Infect Dis* 2011;**11**:110–18.
86. Sripa B. Pathobiology of opisthorchiasis: an update. *Acta Trop* 2003;**88**:209–20.
87. Sripa B, Brindley PJ, Mulvenna J, et al. The tumorigenic liver fluke Opisthorchis viverrini—multiple pathways to cancer. *Trends Parasitol* 2012.
88. Sripa B, Kaewkes S, Intapan PM, et al. Food-borne trematodiases in Southeast Asia epidemiology, pathology, clinical manifestation and control. *Adv Parasitol* 2010;**72**:305–50.
89. Sripa B, Thinkhamrop B, Mairiang E, et al. Elevated plasma IL-6 associates with increased risk of advanced fibrosis and cholangiocarcinoma in individuals infected by Opisthorchis viverrini. *PLoS Negl Trop Dis* 2012;**6**:e1654.
90. Thuwajit C, Thuwajit P, Kaewkes S, et al. Increased cell proliferation of mouse fibroblast NIH-3T3 in vitro induced by excretory/secretory product(s) from Opisthorchis viverrini. *Parasitology* 2004;**129**:455–64.
91. Torres GB, Iwashita AT, Vargas CM, et al. [Human fasciolasis and gastrointestinal compromise: study of 277 patients in the Cayetano Heredia National Hospital (1970-2002)]. *Rev Gastroenterol Peru* 2004;**24**:143–57.
92. Tran VH, Tran TK, Nguye HC, Pham HD, Pham TH. Fascioliasis in Vietnam. *Southeast Asian J Trop Med Public Health* 2001; **32**(Suppl. 2):48–50.
93. Trung Dung D, Van De N, Waikagul J, et al. Fishborne zoonotic intestinal trematodes, Vietnam. *Emerg Infect Dis* 2007;**13**:1828–33.

94. Ubeira FM, Muiño L, Valero MA, et al. MM3-ELISA detection of Fasciola hepatica coproantigens in preserved human stool samples. *Am J Trop Med Hyg* 2009;**81**:156–62.
95. Valero MA, Periago MV, Pérez-Crespo I, et al. Assessing the validity of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological situations. *Trop Med Int Health* 2012;**17**:630–6.
96. Villegas F, Angles R, Barrientos R, et al. Administration of triclabendazole is safe and effective in controlling fascioliasis in an endemic community of the bolivian altiplano. *PLoS Negl Trop Dis* 2012;**6**:e1720.
97. Weng YL, Zhuang ZL, Jiang HP, Lin GR, Lin JJ. [Studies on ecology of Fasciolopsis buski and control strategy of fasciolopsiasis]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi* 1989;**7**:108–11.
98. Winkelhagen AJ, Mank T, de Vries PJ, Soetekouw R. Apparent triclabendazole-resistant human Fasciola hepatica infection, the Netherlands. *Emerg Infect Dis* 2012;**18**:1028–9.
99. Wiwanitkit V, Suwansaksri J, Chaiyakhun Y. High prevalence of Fasciolopsis buski in an endemic area of liver fluke infection in Thailand. *Med Gen Med* 2002;**4**:6.
100. World Health Organization. *Report of the WHO expert consultation on foodborne trematode infections and taeniasis/cysticercosis*, Vientiane: La People's Democratic Republic; 2011. 12-6 October 2009.
101. Yahiro S, Habe S, Duong V, et al. Identification of the human paragonimiasis causative agent in Lao People's Democratic Republic. *J Parasitol* 2008;**94**:1176–7.
102. Yu SH, Kawanaka M, Li XM, Xu LQ, Lan CG, Rui L. Epidemiological investigation on Clonorchis sinensis in human population in an area of South China. *Jpn J Infect Dis* 2003;**56**:168–71.
103. Zarrin-Khameh N, Citron DR, Stager CE, Laucirica R. Pulmonary paragonimiasis diagnosed by fine-needle aspiration biopsy. *J Clin Microbiol* 2008;**46**:2137–40.
104. Zhang XL, Wang Y, Wang GX, et al. Distribution and clinical features of Paragonimiasis skrjabini in Three Gorges Reservoir Region. *Parasitol Int* 2012;**61**:645–9.

recognized in Japan. The complete life cycle was identified during the first decades of the 20th century. Before the development of effective curative medication in the 1970s, schistosomiasis was considered the "most dreadful of the remaining plagues of Egypt."[81,82] Nonetheless, it had been reported in 1919 that "it is now quite within the bounds of possibility that the disease may be largely controlled"[139]; even in this new millennium, success with disease control and eradication is still quite limited.

EPIDEMIOLOGY

Schistosomiasis is caused by helminth parasites of the class Trematoda, which includes the flukes. Schistosomes differ from other human flukes in that they live within the vascular system and have separate male and female sexes. Humans are the definitive host for five species within the *Schistosoma* genus.[165,168] *Schistosoma haematobium* is the cause of urinary schistosomiasis. *Schistosoma japonicum* and *Schistosoma mansoni* are common causes of disease resulting from infection of the intestinal vasculature. *Schistosoma mekongi* and *Schistosoma intercalatum* also cause infection along the intestinal tract but are found only in small geographic areas. Other mammalian *Schistosoma* spp. occasionally infect humans.

Each species of *Schistosoma* uses fresh-water snails as obligate intermediate hosts,[165,168] and the geographic distribution of *Schistosoma* is limited by the habitat of these snails (Fig. 228-1). Oncomelania snails transmitting *S. japonicum* live in the moist soil along slow-flowing streams and irrigation canals, limited primarily to parts of China, the Philippines, and Indonesia. The *Bulinus* snails associated with *S. haematobium* and the *Biomphalaria* snails associated with *S. mansoni* live in shaded, slow-flowing, shallow water. *S. haematobium* is seen primarily in tropical Africa, along the Nile River, and in the Middle East. *S. mansoni* is found across tropical Africa, along the Atlantic coast of South America, and on some Caribbean islands. *Tricula*, the snail host for *S. mekongi*, is limited to the Mekong River in Laos and Cambodia. *S. intercalatum* and its snail host *Bulinus* spp. are found only in Cameroon and the Democratic Republic of Congo. Most human infections occur when children come into contact with fresh-water streams, rivers, or lakes inhabited by infected snails (Fig. 228-2).[168] Thus, for most of the world, transmission of disease occurs during routine activities such as obtaining water for household use, bathing, fishing, and irrigation. Humans are infected by the fork-tailed schistosomal cercariae. Cercariae penetrate the skin with the aid of secretory serine proteases.[105,149] During penetration, the parasites lose their tails and modify their tegument to form the schistosomula stage. Schistosomas enter the bloodstream and then migrate to the lungs. They move from the pulmonary arterial circulation to the venous circulation without exiting the vessels. The parasites then travel through the systemic circulation

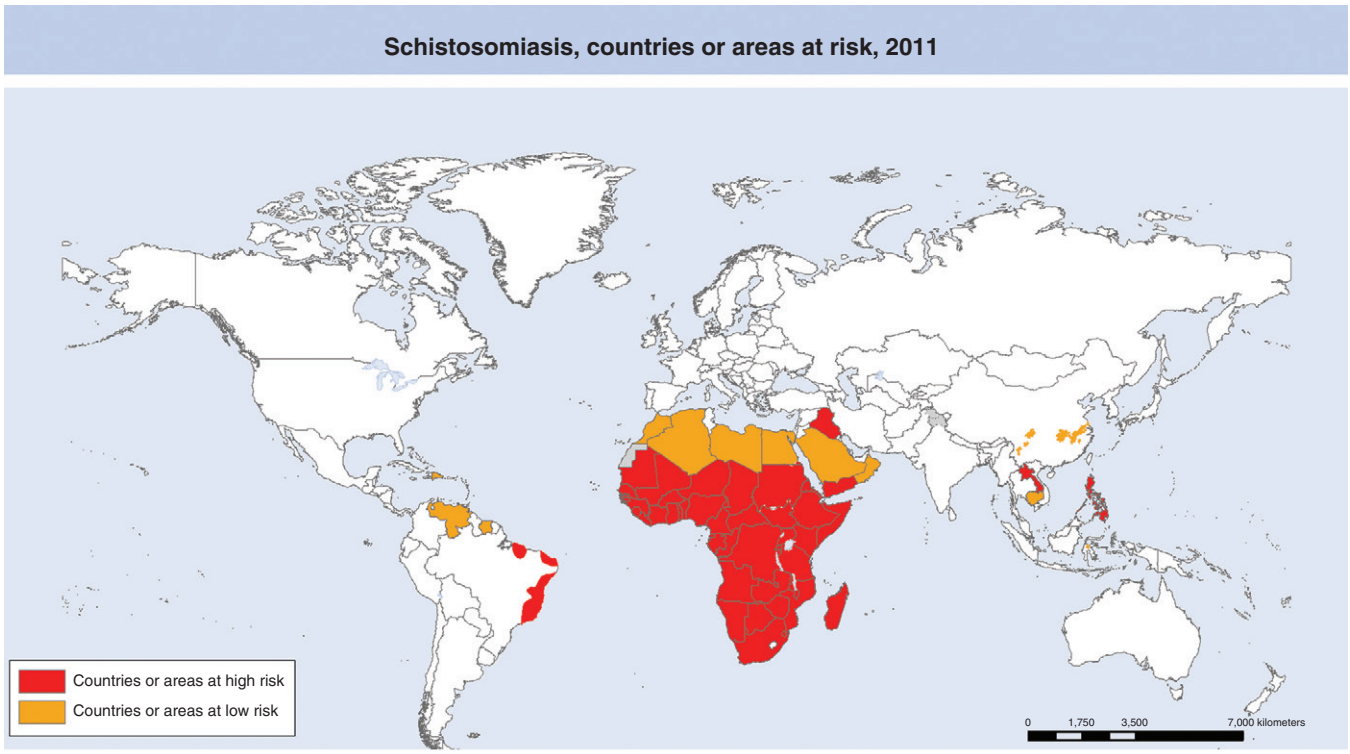

The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

World Health Organization

© WHO 2012. All rights reserved.

FIGURE 228-1 ■ Countries or areas at risk. (See companion Expert Consult website for color version.) (From World Health Organization 2012.)

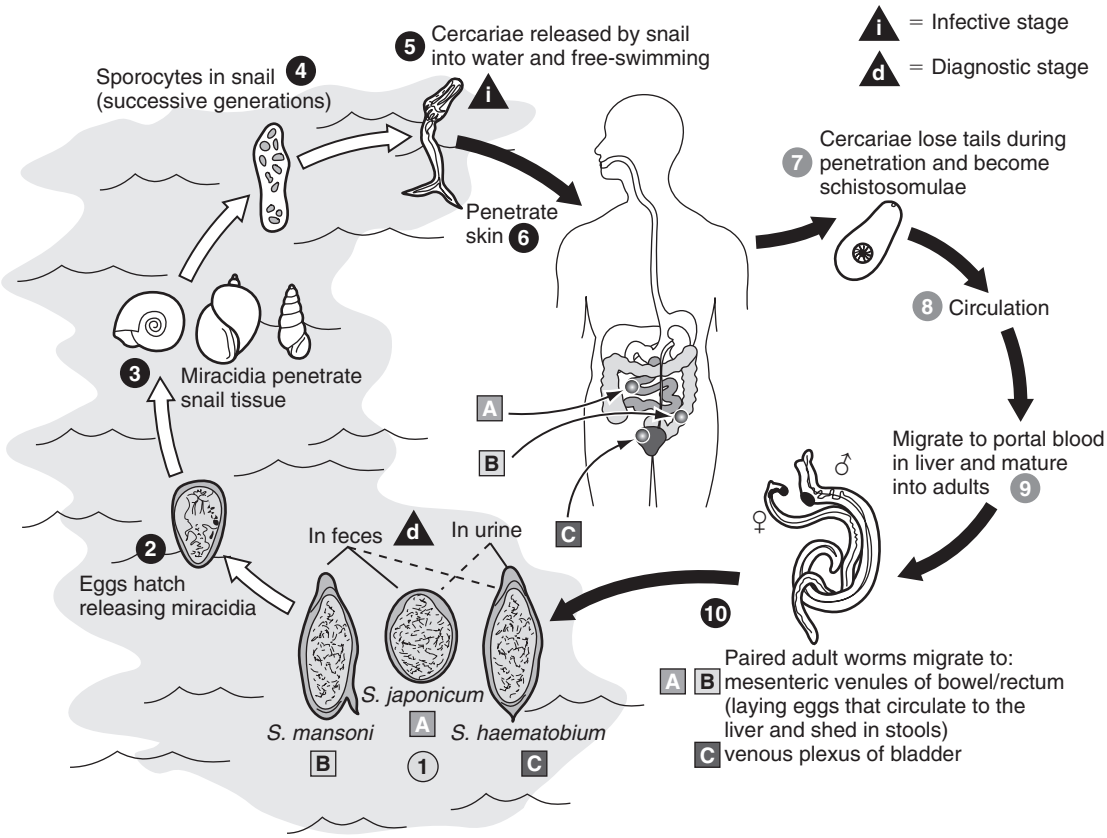

FIGURE 228-2. ■ **Life cycle of the human schistosome species.** (From Centers for Disease Control and Prevention: Schistosomiasis. Available at: http://www.dpd.cdc.gov/dpdx/HTML/Schistosomiasis.htm.)

until they reach splanchnic vessels and gain access to the portal system.

During the course of a few weeks, the schistosomas develop into mature male and female adults that mate continuously.[167,168] Adult worms are 1 to 3 cm in length and usually live for 3 to 7 years. *S. haematobium* localizes to the venules of the urinary bladder, *S. japonicum* goes to the superior mesenteric vessels, and *S. mansoni* goes to the inferior mesenteric vessels. The gravid females migrate upstream to lay eggs near the bladder or intestines. Each adult female can produce hundreds (in the case of African species) to thousands (for Asian species) of eggs per day.[69] Most of the eggs become embedded in the walls of the peripheral vessel where they were laid. However, some of them flow with the blood and can stimulate reactions and cause disease at other sites. A localized granulomatous response allows the eggs to pass through the wall of the bladder (for *S. haematobium*) or the intestine (for other species) to be released in urine or stool. Released eggs mature in a week's time and then hatch to release miracidia. The miracidia penetrate the snail to continue their life cycle and eventually develop into hundreds of cercariae.

Infection is seen in children as young as 6 months; exposure to cercariae often is greatest in boys between the ages of 5 and 10 years.[81,87,165] In lakeside Ugandan villages, however, nearly half of the children are infected during the first 3 years of life,[124] and a study in a western Kenya town on the shores of Lake Victoria revealed infection in 14 percent of 1 year olds, 63 percent of 5 year olds, and 90 percent of 10 year olds.[180] The peak intensity of infection, as measured by excretion of eggs, occurs in children as young as 8 to 12 years in heavily infected areas and takes place during the teenage years in more lightly infected areas. The intensity and the incidence of excretion of eggs decline after the adolescent years, and hormonal changes during puberty are associated with increased resistance to infection.[93]

PATHOGENESIS AND IMMUNITY

Within hours of penetration of the skin, local erythema and inflammation may result from the activity of parasite proteases.[104,148,172] In individuals previously sensitized, a delayed immune reaction may cause papular or vesicular lesions.[91,165] Infiltration of the epidermis and dermis with a mixture of mononuclear cells and eosinophils causes papules. This reaction (termed *swimmer's itch*) is more pronounced for avian schistosomes, which are unable to penetrate into the human circulation and die in situ. During the initial migration of schistosomes through the lungs, patients may develop fever, pneumonitis, pulmonary infiltrates, and eosinophilia. However, these reactions resolve spontaneously.

Acute schistosomiasis typically begins 2 to 12 weeks after exposure, at the time the female parasites begin to produce eggs.[49,142] This condition is recognized more commonly with *S. japonicum* than with *S. mansoni* and rarely is described with *S. haematobium*.[91,165] Patients characteristically have marked eosinophilia, along with immunoglobulin (Ig)E and IgG antibodies to the parasites, consistent with an active T_H2 response.[91,165] However, studies suggest that proinflammatory cytokines play an important role.[46,119] The antibodies react with egg antigens, which cross-react with antigens from the cercaria or schistosomula stages.[10,75,76] During early production of eggs, the quantity of antigen exceeds that of antibody, and soluble immune complexes are formed. Immune activation and immune complex deposition lead to the clinical manifestations, including fever, myalgia, urticarial rash, and bloody diarrhea. Pulmonary nodules and infiltrates may be noted during acute infection.[39,150] In addition, acute infection can be complicated by ectopic egg production. Patients with acute *S. mansoni* or *S. haematobium* infection occasionally can have neurologic involvement.[31,55,133] Interestingly, acute disease occurs rarely in endemic populations, perhaps because of modulation of the immune response from exposure to parasite antigens in utero.

Chronic Schistosomiasis

Despite the fact that the adult worms live within the bloodstream, they cause little host response. The parasite surface incorporates blood group glycoproteins and major histocompatibility antigens and down-regulates expression of its own surface proteins, which may help the adults evade immune system attack.[66,130,157]

Chronic infection is characterized by localized granulomas surrounding the parasite eggs.[91,140,165] Excretory products of the eggs (termed *soluble egg antigens*) are the major antigens. The egg antigens are cytotoxic to host cells, such that infection of mice lacking T cells results in increased localized necrosis surrounding the eggs; the granulomas help protect the host from the ravages of the eggs. Interleukin (IL)-17 mediates the formation of granulomas in the liver.[196] The granulomas also facilitate transit of the eggs into stool and urine, as illustrated by the fact that immunodeficient hosts tend to have lower rates of shedding of eggs despite having high concentrations of eggs in tissues.[48,85,121] High levels of vasoactive endothelial growth factor and angiogenesis are associated with initial granuloma formation.[154]

The granulomas also contribute to the pathologic process. They are composed of a mixture of eosinophils, lymphocytes, macrophages, and granulocytes.[19] Late-stage granulomas also contain fibroblasts, collagen, and giant cells. Initially, antigen-presenting cells stimulate $CD4^+$ T cells to produce IL-2, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ).[120] Early expression of TNF-α plays a key role in the initial formation of granulomas.[5] A subsequent shift occurs toward production of T_H2 cytokines (e.g., IL-4, IL-5, IL-10, and IL-13) along with antibody.[131] This event is accompanied by eosinophil recruitment. IL-4 and IL-13 also play a primary role in the development of hepatic granulomas, and IL-13 expression is linked closely to the development of fibrosis in murine models.[34,35] Subsequent immunomodulation, largely mediated by IL-10 and transforming growth factor-β (TGF-β), results in downsizing of the granuloma and suppression of the inflammation.* When granulomas are limited to the intestines, the cytokine response is dominated by anti-inflammatory cytokines (e.g., IL-10 and TGF-β), and patients have few symptoms.[41] By contrast, patients with granulomas in the liver are more likely to be symptomatic. They express more inflammatory cytokines, including IL-13 and TNF-α. TGF-β also is associated with fibrosis in primate models,[52] whereas T_H1 cytokines (e.g., IFN-γ and IL-12) suppress fibrosis.[21,189,195] Macrophages activated by Il-4 and IL-13 (alternative rather than classic IFN-γ–based activation) seem to protect mice against schistosomiasis-induced inflammation and fibrosis with the acute infection while later promoting fibrosis with chronic infection.[13]

IL-13 in particular stimulates the formation of fibrosis in the portal tracts,[34] probably through the induction of expression of fibrosis-associated genes.[108] Portal fibrosis, in turn, leads to the development of portal hypertension (i.e., hepatosplenomegaly, ascites, and esophageal varices).[91] Death from hepatic schistosomiasis typically results from gastrointestinal bleeding. Overt bleeding is an uncommon occurrence in children, but it can occur in adolescents and young adults. In patients with isolated schistosomiasis, hepatocellular function is surprisingly normal.[19] Patients are thought to tolerate even gastrointestinal bleeding better than do those with cirrhosis. By contrast, progressive hepatocellular damage and rapid progression to liver failure develop in patients coinfected with hepatitis viruses, particularly hepatitis C.[9,38,84] The immunomodulation that develops in response to schistosomiasis is thought to suppress immune control of the hepatitis virus. Whether schistosomiasis-associated immune changes alter susceptibility to or expression of human immunodeficiency virus is unknown.[179]

Interestingly, patients with schistosomiasis are less susceptible to allergies.[27,178] Evidence indicates that asthmatics with schistosomiasis produce lower levels of IL-5 and greater levels of IL-10 than do asthmatics without schistosomiasis, and it is this suppression of T_H2 responses that might modulate the expression of asthma and atopic disease in patients with schistosomiasis.[7] Alternatively, it is possible that a common genetic predisposition alters the risk for subsequent development of both atopic disease and schistosomiasis.[12,110] A population-based study in Zimbabwe identified a negative correlation between atopy and the intensity of schistosomal infection in a village with a high frequency of schistosomiasis but not in a village with lower infection frequency,[145] indicating the interrelatedness of schistosomal antigenic stimulation and the development of allergic disease.

Several means exist by which schistosomiasis might be related to anemia, and the exact pathologic mechanisms are not fully understood.[60] At least sometimes, however, the anemia is due to inflammation rather than iron deficiency.[96] Generalized undernutrition is associated with

*References 8, 52, 58, 77, 111, 119, 155, 183.

schistosomiasis; proinflammatory cytokines (IL-1β) and generalized inflammation (as manifested by elevated levels of C-reactive protein) are contributing factors.[43] Even without acute inflammation, schistosomal infection and periportal fibrosis are associated with reduced vitamin A status and high oxidative stress (increased serum hydroperoxides).[17] Schistosomiasis-affected children have lower levels of regulatory T cells with altered cytokine responses; this seems to provide some protection against concurrent malaria infection.[109] Similarly, parasite-induced changes in the T_H1/T_H2 balance are hypothesized to explain how young children with underlying schistosomiasis are less likely to develop malaria.[110]

Approximately 40,000,000 women of child-bearing age are infected with schistosomes. Although mechanisms are not clear, some evidence indicates that schistosomiasis during pregnancy is associated with decreased birth weight and a higher risk for preterm delivery.[61]

Urinary Schistosomiasis

Genital and urinary schistosomiasis also is the result of granuloma formation. Severity of disease correlates with increased production of TNF-α and decreased production of IL-10.[92] Direct inflammatory responses to eggs along the ureters or from obstruction to ureteral drainage caused by space-occupying granulomata in the bladder wall block ureteral emptying.[88,158] All tissue layers may be involved, but pathologic changes are usually most extensive in the submucosal layer.[86] Abnormal ureteral flow, dilatation, and hydronephrosis develop.[158] In some communities where *S. haematobium* is common, ureteral deformities demonstrable on intravenous pyelography are noted in half of affected children and hydronephrosis develops in more than 10 percent.[89] Eventually, scarring and calcifications form and may be associated with chronic hydronephrosis, even with resolved or light chronic infection.[92,158] The hydronephrosis and urinary stasis predispose patients to chronic bacteriuria and urinary tract infections.

In *S. mansoni* infection, immune complexes may mediate glomerulonephritis, as evidenced by the finding of schistosome-derived immune complex deposits in glomeruli.[14,160] Nephrotic syndrome has been reported in association with chronic schistosomiasis.[113,114] Amyloid deposits have been found in the kidneys of children with chronic schistosomiasis, and this condition probably is caused by immune responses that are altered by the chronic parasitic infection.[15,181]

Squamous cell carcinoma of the bladder is a common occurrence in areas of heavy infection with *S. haematobium*, but otherwise it is a rare form of bladder cancer.[92,158,165] The prolonged irritation of bladder epithelium by schistosome eggs and the resulting immune response are thought to trigger hyperplasia and subsequent malignant disease.[97] In an animal model, direct exposure to schistosomal antigens stimulates increased proliferation and decreased apoptosis of endothelial cells along with downregulation of tumor suppressor p27 and upregulation of antiapoptotic molecule Bcl-2.[23] This condition could be aggravated by urinary stasis in the bladder with secondary increases in pH that favor malignant transformation of epithelial cells. Carcinogenic tryptophan metabolites also have been postulated to link the nutritional activities of parasites near the bladder with subsequent carcinogenesis. Schistosoma-related bladder cancers have biomarker prevalence different from those of nonschistosomal bladder cancers, and this might have an impact on eventual treatment choices.[2]

CLINICAL MANIFESTATIONS

The main clinical manifestations of schistosomiasis vary with the stage of the parasite's life cycle and also differ among species. Many patients remain asymptomatic. Patients with asymptomatic schistosomiasis may be identified during community screening programs in an endemic area, through immigrant or returned traveler evaluations in a nonendemic area, or when diagnostic tests such as urine or stool microscopy are performed to evaluate other, seemingly unrelated symptoms. The minority of infected children, those with heavy infections, are most likely to develop early symptoms and also are at greatest risk for subsequent major health complications.

Cercarial Penetration

Within a few minutes of coming into contact with cercariae, some children develop pruritus. This response to initial contact with the parasite occurs more commonly in nonimmune visitors to endemic areas than it does in indigenous residents of endemic areas, but it seems to be more pronounced after repeated exposure to cercariae than on the first exposure. It may occur after contact with any of the human *Schistosoma* spp. but more commonly results after contact with the cercariae of avian schistosomes. An erythematous and sometimes papular rash may develop.[91,165] The rash usually subsides during a period of 2 to 10 days without scarring, regardless of whether specific treatment is given.

Acute Schistosomiasis (Katayama Fever)

Acute schistosomiasis, referred to as Katayama fever, typically begins 2 to 12 weeks after exposure, when the adult worms begin to produce eggs.[48,80,142] Clinical manifestations of acute schistosomiasis usually include high fever, chills, myalgia, headache, and a general ill appearance.[46,49] An urticarial rash, which may include giant urticaria, and diffuse lymphadenopathy may be seen.[138,198] Cough, rales, and pulmonary infiltrates may be noted, even in the absence of fever.[39,150] Gastrointestinal symptoms of anorexia, abdominal pain, and loose stools sometimes are observed. Bloody diarrhea may be seen acutely with heavy infections by *S. japonicum* and *S. mansoni*. Tender hepatomegaly and mild splenic enlargement develop in approximately 30 percent of children with Katayama fever. Some reports suggest that myelopathies are a common accompaniment of acute schistosomiasis.[55,133] Genital symptoms (primarily hematospermia) also are frequent initial symptoms.[40,136] Marked eosinophilia often is seen with acute schistosomiasis.

Urinary Schistosomiasis

Hematuria is the classic finding of *S. haematobium* infection.[25,47,88,165,185] In some highly endemic areas, for boys nearing puberty not to display this evidence of "male menstruation" is considered abnormal. Typically, hematuria results from the release of blood from irritated, inflamed areas around granulomas in the bladder wall as the bladder contracts during micturition.[158] Thus, the blood is most obvious at the end of the urine stream ("terminal hematuria"). The hematuria usually is not associated with pain or discomfort, but dysuria can be present.[1,25] With chronic infection in children who either were not treated initially or were reinfected, obstructive uropathy can develop. In community surveys in endemic areas, as many as 40 percent of children were found to have significant renal or ureteral abnormalities (or both).[1,73,182] Hematuria and dysuria occur more commonly in children than in adults, presumably because they have heavier parasite loads. However, hydronephrosis, which develops more slowly, is seen more often in adults.[88,89,118,158]

Bacterial urinary tract infection may coexist with urinary schistosomiasis, probably secondary to obstruction of urinary outflow.[158] Chronic infection can lead to renal failure. Obstructive uropathy, with or without incident or recurrent urinary tract infections, can lead to loss of renal function. Children with schistosomal infection also can have glomerulonephritis and nephrotic syndrome. Bladder cancer, when it occurs, typically develops in the setting of untreated, heavy chronic urinary schistosomiasis.

Genital Schistosomiasis

Studies have emphasized the genital tract as an important site of schistosomiasis in both men and women.[28,98,133,134] In women, egg granulomas may be found in the cervix, uterus, or fallopian tubes.[53,98,99,134] Cervical irritation and ulceration, vaginal bleeding, or ectopic pregnancy may develop. Genital schistosomiasis can be confused with cancer.[170] Adolescent boys may have involvement of the prostate and seminal vesicles.[98,136] Eggs were noted in semen from 43 percent of men in an area endemic for *S. haematobium*.[98] The main clinical finding is hematospermia,[98] which may occur with acute infection or during chronic disease. In Africa in particular, genital schistosomiasis may be a cofactor in transmission of human immunodeficiency virus infection.

Intestinal Disease

Most children with intestinal schistosomiasis do not have intestinal symptoms.[164,165] Thus, even finding eggs in stool does not mean that symptoms are related to the schistosomiasis.[71] Nonetheless, both the small and large intestines may be involved with schistosomal disease. Irritation of the bowel wall from inflammatory reactions induced by eggs may lead to diarrhea that sometimes contains blood or mucus.[32,33,164-166] Crampy abdominal pain and generalized malaise may occur. Endoscopy can reveal granular inflammation with hyperemic areas, ulceration, and hemorrhage. Polyps, which may develop around granulomas, may be identified by contrast radiography or endoscopy. Protein-losing enteropathy and blood loss can result in malnutrition and iron deficiency, especially in cases with significant small bowel disease. The role of schistosomiasis in malnutrition was illustrated by studies of mass chemotherapy, which resulted in improved nutrition and a lower prevalence of anemia.[11,125,171]

Hepatosplenic Disease (Hepatomegaly, Splenomegaly, and Portal Hypertension)

The life-threatening complications caused by *S. japonicum* and *S. mansoni* are the result of eggs that remain in the venous vasculature and migrate back to the liver.[19] Egg burden seems to be the greatest predictor of hepatic involvement with schistosomiasis, but some link with human leukocyte antigen type has been suggested.[115,116,152,184]

Children with compensated disease initially have few symptoms. These symptoms may include anorexia, malaise, and abdominal fullness.* Children have hepatomegaly even without significant portal hypertension. The hepatomegaly generally is firm and either minimally tender or nontender. Splenic enlargement usually is noted as well. Liver function is affected only late in the course of hepatic schistosomiasis, so jaundice and liver enzyme elevations are unusual findings.

As fibrosis develops around eggs in the liver, portal hypertension ensues. Liver function usually remains intact, but esophageal varices can cause death during the late adolescent and early adult years.[19,33,127] Ascites gradually develops during a period of years, and the spleen may become very large. Bleeding esophageal varices are a common cause of demise in individuals who had heavy schistosomal infections in childhood. Such patients may come to medical attention for melena or hematemesis. Initial episodes are well tolerated. However, with progressive liver decompensation, variceal bleeding can be fatal.

Children with severe hepatosplenic schistosomiasis do not grow as well as other children. Debate has ensued about whether this deficiency is due to a direct effect of the parasitic infection or poor nutritional intake caused by poor general health. Some recent data suggest that a decrease in insulin-like growth factor–1 activity is linked to hepatic schistosomiasis.[129] Extensive formation of hepatic granulomata may hinder the liver's structure and function in such a way that growth-promoting factors are not produced normally.

Pneumonitis and Cor Pulmonale

Sometimes, children initially have low-grade fever and cough as schistosome larvae migrate through their lungs. This condition can occur with an initial heavy infection (when eggs will not be detectable) or a heavy reinfection (when eggs will be detectable in urine or stool from the preceding underlying infection). This larval pneumonitis

*References 19, 33, 94, 127, 136, 185.

can be manifested on lung examination as basilar rales and wheezing.[39,150] Radiographs may show basilar mottling in the lung fields. Eosinophilia is a common occurrence with this type of pneumonitis. Resolution, even without treatment, usually occurs in 2 to 4 weeks. Similar symptoms also may be seen as a reactive pneumonitis when patients with heavy parasite burdens are treated.

In children with advanced hepatosplenic schistosomiasis and portal hypertension, eggs may bypass the liver and flow from the abdominal and pelvic veins into the small lung vessels. Localized granulomas form around eggs lodged in the lungs. Over time, children may be subject to fatigue, cough, and right-sided heart failure. Medical therapy stops the progress of disease and may decrease the ongoing inflammatory responses, but right-sided heart failure, once it is established, is not fully reversible.

Central Nervous System Involvement

Neurologic manifestations of schistosomiasis may be dramatic, even if not common.* Worms do not always follow the typical routes described for urinary and intestinal schistosomiasis. On occasion, worms migrate to cerebral blood vessels. Production of eggs in that location can cause seizures and headaches in children.[133,151] Sometimes, optic field defects and dysarthria are noted; these findings seem to result from localized space-occupying inflammatory reactions that develop around worms or eggs. Magnetic resonance imaging (MRI) evaluation reveals a mass or masses of multiple intensely enhancing nodules.[106] Spinal fluid pressure may be elevated, and both protein concentrations and lymphocyte counts in spinal fluid may be increased. This cerebral schistosomiasis occurs more commonly with *S. japonicum* infection[56] and is thought to develop in as many as 2 percent of infected children.

S. mansoni infection and, to a lesser extent, *S. haematobium* infection can produce eggs that embolize to the spinal cord. The eggs or associated granulomas can cause transverse myelitis. Paraplegia along with urinary and fecal incontinence often is the initial problem in children with spinal schistosomiasis.[55,151]

Chronic or Recurrent Salmonellosis

In endemic areas, some children have salmonellosis and schistosomiasis concurrently and experience chronic low-grade fever, fatigue, malaise, and poor growth.† Blood cultures frequently demonstrate *Salmonella* bacteremia, but stool may not contain these bacteria. Some children have chronic *Salmonella* bacteriuria. Sepsis and mortality are unusual findings despite these persistent bacterial infections. Relapse of the *Salmonella* infection occurs commonly unless the coexisting schistosomal infection is treated. The bacteria are thought to hide within the parasitic worms, where antibiotic penetration is poor, and thereby evade host defenses.

*References 29, 55, 56, 123, 133, 141, 151.
†References 65, 94, 95, 137, 147, 192.

DIAGNOSIS

A clinical diagnosis of schistosomiasis should be entertained in the presence of typical clinical features. These features might be as obvious as painless hematuria in a child in a region endemic for *S. haematobium*, or portal hypertension may be noted late in the second decade of life in an otherwise healthy adolescent from an area known to be endemic for *S. mansoni* or *S. japonicum*.[136,149] The other clinical findings described also should prompt the clinician to consider a diagnosis of schistosomiasis when the child has resided in or traveled through a *Schistosoma*-endemic area.[169] In traveling children, the index of suspicion must be particularly high because patients may be asymptomatic or have atypical symptoms, they may not have clear history of exposure to contaminated water, and parasitologic examination findings often are negative.[18,136]

Outside endemic areas, reinfection is not expected. Thus, a reasonable approach is to test all children, including asymptomatic children, who have returned or emigrated from an endemic area where they had potentially significant exposure to fresh water. Such testing would involve all travelers who swam in, waded in, or even touched suspicious water, and it could involve immigrants who have spent long periods in endemic areas. Certainly, any child outside an endemic area who has even a remote history of having had possible contact with cercariae and clinical findings suggestive of any form of schistosomiasis should be tested. Testing is appropriate because even late therapy can favorably alter the course and outcome of schistosomiasis. In some highly endemic areas, diagnostic testing is of limited feasibility, questionable reliability, or both, and curative medications are readily available. In such settings, establishing a proved diagnosis might be less necessary. For example, in a highly endemic setting, a urine paper strip test result positive for hematuria could provide sufficient suspicion of infection to warrant administration of specific antischistosomal therapy. In populations with known high rates of infection, mass treatment (without expensive diagnostic testing) is reasonable.

In established heavy infections, eggs usually are readily apparent on microscopic examination of urine or stool. For lighter infections, concentrating techniques are useful. Urine can be centrifuged and filtered to increase the yield of eggs. The result of urine examination is most likely to be positive when urine is voided at midday and when it is collected at the end of the urine stream. In the event of high suspicion and negative urine findings, bladder wall biopsy can be performed. The procedure usually is not necessary, however, because urine microscopy, especially with filtered or concentrated samples, generally is positive. Examination of multiple samples increases the yield.

The eggs of *S. haematobium* and *S. mansoni* are approximately 90 mm in diameter, whereas the eggs of *S. japonicum* and *S. mekongi* are somewhat smaller. As demonstrated in Figure 228-3, identification of species is aided by observation of the spine—small for *S. japonicum* and *S. mekongi*, at one end for *S. haematobium*, lateral for *S.*

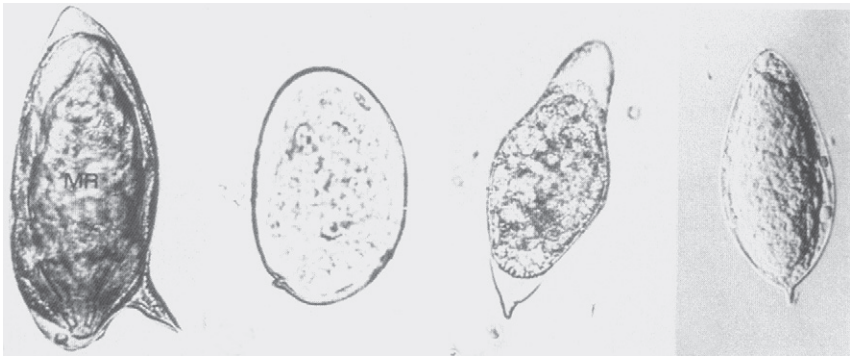

FIGURE 228-3 ■ Human schistosome eggs. **A,** *Schistosoma mansoni.* **B,** *Schistosoma japonicum.* **C,** *Schistosoma intercalatum.* **D,** *Schistosoma haematobium.* (From Mahmoud, A. A. F., editor. *Schistosomiasis.* London, Imperial College Press, 2001. Reprinted with permission.)

mansoni, and broad at each end for *S. intercalatum*. Eggs usually are detectable in voided urine. Plain radiographs might demonstrate some calcification in the bladder wall around chronic granulomas. Cystoscopy, if it is performed, reveals bladder wall hyperemia and, subsequently, nodular lesions and fibrosis that give rise to "sandy patches" on the bladder wall. Granulomas may protrude from the bladder wall into the lumen. Both edema and granulomas may be seen obstructing ureteral orifices.

In light infections, examination of stool or urine samples may not reveal ova because of intermittent or low-volume shedding. Thus, negative stool or urine test results do not adequately rule out intestinal schistosomiasis.[19,136] When stools repeatedly are negative for ova, rectal biopsy may be performed. Samples may be obtained by random punch biopsy, but the yield is increased when samples are taken from inflamed sites under direct visualization. Eggs are detected in unstained smears made by pressing the tissue sample between a coverslip and a glass slide, but eggs also can be seen with standard histologic stains.

Nonviable eggs may be excreted for months or years after therapy has been successful. With good medical treatment, eggs usually are not viable when they are passed more than a week after the initiation of therapy. With ineffective treatment or reinfection, however, viable eggs may continue to be passed. On microscopic examination, living ova contain transparent miracidia within the egg, which often are motile. For further documentation of their viability, the eggs may be hatched by placing them in fresh water exposed to light for 20 minutes; observation with a hand lens then can reveal swimming miracidia.

Serologic tests may be needed in acute infection, which may occur before the eggs are excreted or with eggs in ectopic locations.[72,174] For travelers with limited exposure and the likelihood of having either an acute or a limited chronic infection, serologic examination is the main diagnostic tool. A common screening serologic test is the Falcon assay screening test–enzyme-linked immunosorbent assay (FAST-ELISA) using *S. mansoni* adult worm microsomal antigen.[175] This test also cross reacts with *S. haematobium* but is less sensitive for *S. japonicum* or *S. mekongi* infection. Indirect hemagglutination tests are 70 to 80 percent sensitive for Katayama syndrome, and ELISA tests do not add to the diagnostic value when an indirect hemagglutination test has already been positive.[142] The species can be confirmed by use of an immunoblot assay (enzyme-linked immunotransfer blot).[174] An immunoblot assay also is available for *S. japonicum*. New tests hold promise in easily identifying incriminating schistosomal antibodies in serum[194] and urine.[51] Antigen-detection assays may be helpful.[3] In a small study of European travelers with acute schistosomiasis from Rwanda, serum DNA detection performed better than parasitologic and serologic tests in confirming the diagnosis.[37]

Ultrasonography is the main imaging procedure used in schistosomiasis.[73] It can document involvement of the portal and urinary tracts and, in selected cases, be diagnostic of schistosomiasis. Ultrasonography can identify bladder polyps, ureteral dilation, hydronephrosis, and calcifications within the urinary tract. When resources are available, an ultrasound evaluation of the urinary tract should be performed in any child found to be infected with *S. haematobium*. Ultrasonography can be readily implemented in clinical settings in developing countries to follow lesions of urinary schistosomiasis.[20] For children with persistent urinary symptoms (i.e., dysuria, suprapubic pain, and hematuria) despite having received good medical treatment, cystoscopy should be considered, in part to screen for bladder cancer. Other radiographs and tests of renal function and excretion generally are not indicated unless the ultrasound evaluation reveals significant urologic disease.

Portal ultrasonography is the imaging study of choice for hepatic involvement. The presence of periportal fibrosis with thickening of the portal tracts and vein walls (so-called clay pipestem or Symmer fibrosis) correlates with parasite burden and hepatic disease. In experienced hands, schistosomiasis can be distinguished reproducibly from cirrhosis and other chronic liver diseases.

MRI is helpful in diagnosing neuroschistosomiasis.[56] A variety of cerebral changes may be identified; although not specific for *S. japonicum* neuroschistosomiasis, large masses containing enhancing nodules may be seen in the brain.[56,106] Focal cerebral white matter hyperintensities and hyperintense basal ganglia may be seen in children with hepatosplenic schistosomiasis even when they do not have overt neurologic symptoms.[112] Spinal schistosomiasis, usually caused by *S. mansoni* or *S. haematobium*, can cause spinal pseudotumors with heterogeneous

enhancement as well as signal changes extending up and down the spinal cord from the thoracic level.[44,74]

TREATMENT

Optimal treatment of schistosomiasis varies with the stage of the disease. Cercarial dermatitis resolves spontaneously and requires no antiparasitic treatment. Oral antihistaminic agents, however, may be given for severe itching. Medical therapy for schistosomiasis caused by the human schistosomes is effective and well tolerated, without significant complications. No good medical reason exists to allow schistosomiasis to go untreated. The cost of therapy, however, is a limiting factor in resource-limited areas of the world. Several medications can be considered for curative treatment.

Praziquantel, a mixture of stereoisomers of pyrazinoisoquinoline ring structures, is a broad-spectrum oral anthelmintic agent that is effective against each of the five human schistosome species. The mechanism of action was thought to involve unmasking of parasite antigens with subsequent killing by the host immune response.[24] However, patients with acquired immunodeficiency syndrome respond well to praziquantel, raising questions about this hypothesis. Praziquantel is given in divided doses on a single day as full curative treatment. For infection with S. *haematobium*, *S. mansoni*, and *S. intercalatum*, the dose is 40 mg/kg in two divided doses given on the same day. For children infected by *S. japonicum* or *S. mekongi*, the recommended dose has been 60 mg/kg in three divided doses given on the same day.[4,6,143] Recent evidence, however, suggests that 40 mg/kg might be adequate for *S. japonicum* as well.[126] Heavily infected children sometimes experience nausea, vomiting, and abdominal cramping with treatment. Rare side effects, including headache, pruritus, bloody stools, and fever, are transient and resolve within 1 to 2 days after the initiation of treatment. Because praziquantel primarily kills mature adult worms,[68] repeat testing and/or a second course 6 to 12 weeks later is recommended for the treatment of acute infection.[141] In endemic area settings of school-based and community-based mass treatment, adding a second dose of praziquantel 2 to 8 weeks after the initial dose improves reduction in egg counts.[90] Praziquantel is effective in more than 90 percent of treated children. Some studies suggest that resistance may be emerging.[101,102] However, those studies were performed in areas with frequent reinfection and with high parasite burden. Thus, the poor response may be due to the fact that juvenile forms are not as susceptible to praziquantel. Recent work suggests that praziquantel is still effective in endemic areas,[153] and evidence is emerging that combining praziquantel with either artemether or artesunate might improve its efficacy.[107] Studies from nonendemic areas continue to demonstrate excellent efficacy.[185] In addition to curing infection, treatment with praziquantel is associated with improved growth and reduction in anemia.[42] In endemic areas with high rates of infection, school-based and community-based (when many children in the area do not regularly attend school) treatment strategies (without confirmatory diagnostic testing) are effective; cost issues, however, raise concerns of program sustainability.[62,90]

An alternative treatment effective only against *S. mansoni* is oxamniquine, a tetrahydroquinoline compound.[162] The mechanism by which this agent acts is unknown. The effective dose has varied with the geographic origin of the schistosomal infection, but some resistance has been reported. Current recommendations are that children treated with oxamniquine be given 40 to 60 mg/kg divided into two doses administered on 1 day or four separate doses given during the course of 2 days. Mild side effects include nausea, headache, and fever. Seizures are a rare side effect, so administration of this medication should be avoided in children with seizure disorders. Cure rates of more than 90 percent are reported with oxamniquine.

Metrifonate is an organophosphate compound that causes paralysis of the parasite. It is 70 to 80 percent effective against *S. haematobium* when used at a dosage of 10 mg/kg orally once every 2 weeks for 6 weeks. It does decrease the child's own plasma and erythrocyte cholinesterase activity, but actual cholinergic symptoms seldom occur. This medication is less expensive than the other options, but it generally is not used when praziquantel is available.

Medical therapy usually is effective for children with schistosomiasis,[144] even with advanced disease.[103,146,161,193] Clear improvement in the patient's disease burden occurs when treatment is given. Even established uropathy and portal hypertension often are reversible.[105,146,161] Surgical procedures usually are reserved for children with complications related to long-term infection, such as persistent portal hypertension. Propranolol prophylaxis and sclerotherapy or banding can reduce rebleeding from esophageal varices.[50] Some cases require surgical decompression of portal hypertension. Splenorenal shunts are associated with high rates of hepatic encephalopathy. Comparative studies suggest that in experienced hands, esophagogastric devascularization with splenectomy is the procedure of choice.[57,64]

Systemic steroids may be useful for severely ill children with Katayama fever or severe larval pneumonitis. Treatment of neuroschistosomiasis often involves a combination of corticosteroids and praziquantel, with surgery being reserved for those with medullary compression or hydrocephalus.[141] The role of steroids is significant[55,59] but controversial[30] and incompletely tested. Some experts suggest that glucocorticoids be started before the initiation of antiparasitic therapy, to prevent increased inflammatory responses triggered by dying worms.[141] Phenobarbital and phenytoin (but not levetiracetam) can alter metabolism of praziquantel; when used for neuroschistosomiasis-induced seizures, these medications might prompt upward adjustment of the praziquantel dose.[141] Corticosteroids have not been proved effective treatments in other forms of the disease.

PREVENTION

Schistosomiasis has been considered controllable or even eradicable for nearly 100 years.[139] Nonetheless, control is

still not quite within reach. Further multifaceted interventions are needed if we are to be rid of the scourge of schistosomiasis.[78,139]

Travelers and expatriates in endemic areas can prevent disease by limiting exposure to infectious fresh water. In endemic areas, they should avoid swimming in freshwater streams and lakes. In addition, the cercaria can be eliminated by chlorination or by allowing water to settle for 24 hours before bathing or washing.

The use of effective medical therapy coupled with improved urine and stool hygiene has the potential to eradicate schistosomiasis from endemic areas.[186] Elimination of the snails that serve as intermediate hosts also would be effective, but attempts at implementation of programs administering molluscicides have been unsuccessful to date. Historically, national control programs in China focusing on snail control, sanitation, and improved water supplies led to sustained reductions in infection rates, even apart from the influence of chemotherapy.[177,190] For specific populations, health education can lead to significant favorable changes in knowledge about the illness, water-exposure activities, and reinfection rates in school-age children.[70] Recently, control efforts have focused on mass chemotherapy, with particular emphasis on school-based therapy.[11,71,125,128,186] Similar treatment of preschoolers, especially combined with nonpharmacologic control efforts, is also effective.[122,163] Treatment programs in which the entire school-age population is treated, typically with praziquantel, have resulted in overall improvement in levels of nutrition and in a reduction in the prevalence of anemia. In areas where reinfection commonly occurs, however, the effects of mass treatment programs are of limited duration, and treatment must be repeated every 6 months to 1 year. Sadly, only about 10 percent of persons who could benefit from antischistosomiasis treatment are able to receive it each year.[78] Sustainability of national control programs and of favorable outcomes remains challenging in Africa.[63,83,54,79]

Several studies suggest that artemisinin derivatives can prevent development of infection and decrease the worm burden in those exposed to *S. mansoni* and *S. japonicum*.[100,107,140,176] With ongoing exposure to cercaria, prophylactic artemether or artesunate may be given as a 6 mg/kg dose every 1 to 2 weeks.[107] However, these medications are less effective for chemotherapy than is praziquantel,[22,45,141] probably because parasitologic activity is limited to the juvenile forms. Furthermore, the public health role of chemoprophylaxis in the prevention of infection has not been established.[141]

Immunization of experimental animals with irradiated cercariae can prevent experimental schistosomiasis.[159] Thus, development of a vaccine to prevent human schistosomiasis is theoretically possible. Several potentially protective antigens have been identified on schistosomes, and they are being used as targets for vaccine development. Animal studies with the Sm-p80 vaccine show promise with some favorable effects on worm reduction, worm egg production reduction, and decreased acute schistosomiasis.[156] Murine studies with Sm-TSP-2 show some efficacy, and chimerization with a hookworm vaccine antigen (Na-APR-1) increases the antischistosomal effectiveness of the vaccine.[132] Human studies are in progress with Sh-28-GST for *S. haematobium*[135] and Sm-14 for *S. mansoni*.[26,173] However, the correlates of protective immunity in humans are not well defined.[26,191] Vaccines related to several *S. japonicum* antigens also are being studied in animals, but efficacy is limited.[117,142,197] No vaccine is yet ready for clinical use.[16,67]

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abdulamir AS, Hafidh RR, Kadhim HS, et al. Tumor markers of bladder cancer: the schistosomal bladder tumors versus non-schistosomal bladder tumors. *J Exp Clin Cancer Res* 2009;**28**:27.
4. American Academy of Pediatrics. Drugs for parasitic infections. In: Pickering LK editor. *2012 red book: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 848–68.
13. Barron L, Wynn TA. Macrophage activation governs schistosomiasis-induced inflammation and fibrosis. *Eur J Immunol* 2011;**41**:2509–14.
20. Bonnard P, Boutouaba S, Diakhate I, et al. Learning curves of vesico-urinary ultrasonography in *Schistosoma haematobium* infection with WHO practical guide: a "simple to learn" examination. *Am J Trop Med Hyg* 2011;**85**:1071–4.
23. Botelho M, Ferreira AC, Oliveira MJ, et al. *Schistosoma haematobium* total antigen induces increased proliferation, migration and invasion, and decreases apoptosis of normal epithelial cells. *Int J Parasitol* 2009;**39**:1083–91.
27. Cardosa LS, Oliveira SC, Araujo MI. *Schistosoma mansoni* antigens as modulators of the allergic inflammatory response in asthma. *Endocr Metab Immune Disord Drug Targets* 2012;**12**:24–32.
29. Carod-Artal FJ. Neurological complications of *Schistosoma* infection. *Trans Roy Soc Trop Med Hyg* 2008;**102**:107–16.
30. Caumes E, Vidailhet M. Acute neuroschistosomiasis: a cerebral vasculitis to treat with corticosteroids not praziquantel. *J Trav Med* 2010;**17**:359–61.
37. Clerinx J, Bottieau E, Wichmann D, et al. Acute schistosomiasis in a cluster of travelers from Rwanda: diagnostic contribution of schistosome DNA detection in serum compared to parasitology and serology. *J Travel Med* 2011;**18**:367–72.
44. Crowell C, Kiruga JM, Figaji A, et al. Neuroschistosomiasis due to *Schistosoma haematobium* presenting as spinal cord tumor. *Pediatr Inf Dis J* 2011;**30**:1006–8.
51. Elhag SM, Abdelkareem EA, Yousif AS, et al. Detection of schistosomiasis antibodies in urine patients as a promising diagnostic marker. *Asian Pacific J Trop Med* 2011;**4**:773–7.
56. Ferrari TCA, Moreira PRR. Neuroschistosomiasis: clinical symptoms and pathogenesis. *Lancet* 2011;**10**:853–64.
68. Gryseels B. Schistosomiasis. *Infect Dis Clin North Am* 2012; **26**:383–97.
74. Henriques-Souza AMM, Valenca MM. Schistosomal myelopathy in childhood: findings of magnetic resonance imaging in 26 patients. *Pediatr Neurol* 2011;**45**:373–6.
78. Hotez P. Enlarging the "audacious goal": elimination of the world's high prevalence neglected tropical diseases. *Vaccine* 2011; **29**:D104–10.
79. Humphries D, Nguyen S, Boakye D, et al. The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Curr Opin Infect Dis* 2012;**25**:584–9.
86. Khalaf I, Shokeir A, Shalaby M. Urologic complications of genital schistosomiasis. *World J Urol* 2012;**30**:31–8.
90. King CH, Olbrych SK, Soon M, et al. Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high-risk communities in Africa: a systematic review. *PLoS Negl Trop Dis* 2011;**5**:e1321.
106. Liu H, Lim CCT, Feng X, et al. MRI in cerebral schistosomiasis: characteristic nodular enhancement in 33 patients. *Am J Radiol* 2008;**191**:582–8.
107. Liu R, Dong H, Guo Y, et al. Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasites Vectors* 2011;**4**:201.
108. Liu Y, Munker S, Mullenbach R, et al. IL-13 signaling in liver fibrosis. *Front Immunol* 2012;**3**:115.

109. Lyke KE, Dabo A, Arama C, et al. Reduced T regulatory cell response during acute *Plasmodium falciparum* infection in Malian children co-infected with *Schistosoma haematobium*. *PLoS One* 2012;**7**:e31647.
112. Manzella A, Borba-Filho P, Brandt CT, et al. Brain magnetic resonance imaging findings in young patients with hepatosplenic *Schistosomiasis mansoni* without overt symptoms. *Am J Trop Med Hyg* 2012;**86**:982–7.
122. Mutapi F, Rujeni N, Bourke C, et al. *Schistosoma haematobium* treatment in 1-5 year old children: safety and efficacy of the anthelminthic drug praziquantel. *PLoS Negl Trop Dis* 2011;**5**:e1143.
126. Olliaro PL, Vaillant MT, Belizario VJ, et al. A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs 60 mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. *PLoS Negl Trop Dis* 2011;**5**:e1165.
132. Pearson MS, Pickering DA, McSorley HJ, et al. Enhanced protective efficacy of a chimeric form of the schistosomiasis vaccine antigen Sm-TSP-2. *PLoS Negl Trop Dis* 2012;**6**:e1654.
135. Riveau G, Deplanque D, Femoue F, et al. Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. *PLoS Negl Trop Dis* 2012;**6**:e1704.
139. Rollinson D, Knopp S, Levitz S, et al. Time to set the agenda for schistosomiasis elimination. *Acta Tropica* in press. 2012.
141. Ross AG, McManus DP, Farrar J, et al. Neuroschistosomiasis. *J Neurol* 2012;**259**:22–32.
143. Ross AG, Vickers D, Olds GR, et al. Katayama syndrome. *Lancet* 2007;**7**:218–24.
144. Ruiz-Guevara R, de Noya BA, Valero SK, et al. Clinical and ultrasound findings before and after praziquantel treatment among Venezuelan schistosomiasis patients. *Rev Soc Bras Med Trop* 2007;**40**:505–11.
145. Rujeni N, Nausch N, Bourke CD, et al. Atopy is inversely related to schistosome infection intensity: a comparative study in Zimbabwean villages with distinct levels of *Schistosoma haematobium* infection. *Int Arch Allergy Immunol* 2012;**158**:288–98.
153. Seto EYW, Wong BK, Lu D, et al. Human schistosomiasis resistance to praziquantel in China: should be we worried? *Am J Trop Med Hyg* 2011;**85**:74–82.
154. Shariati F, Perez-Arrellano JL, Carranza C, et al. Evaluation of the role of angiogenic factors in the pathogenesis of schistosomiasis. *Exp Parasitol* 2011;**128**:44–9.
156. Siddiqui AA, Siddiqui BA, Ganley-Leal L. Schistosomiasis vaccines. *Hum Vaccines* 2011;**7**:1192–7.
159. Smythies LE, Betts C, Coulson PS, et al. Kinetics and mechanism of effector focus formation in the lungs of mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunol* 1996;**18**:359–69.
163. Stothard JR, Sousa-Figueiredo JC, Betson M, et al. Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children. *Parasitology* 2011;**138**:1593–606.
173. Tendler M, Simpson AJ. The biotechnology-value chain: development of Sm14 as a schistosomiasis vaccine. *Acta Trop* 2008;**108**:263–6.
180. Verani JR, Abudho B, Montgomery SP, et al. Schistosomiasis among young children in Usoma, Kenya. *Am J Trop Med Hyg* 2011;**84**:787–91.
187. World Health Organization. *Schistosomiasis. Fact sheet*. No. 115, January 2012, http://www.who.int/mediacentre/factsheets/fs115/en/index.html.
188. World Health Organization. Schistosomiasis: population requiring preventive chemotherapy and number of people treated in 2010. *Wkly Epidemiol Rec* 2012;**87**:37–44.
194. Yu L, Ding J, Wen L, et al. Development of a rapid dipstick with latex immunochromatographic assay (DLIA) for diagnosis of *Schistosomiasis japonica*. *Parasit Vectors* 2011;**4**:157.
196. Zhang Y, Chen L, Gao W, et al. IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in *Schistosoma japonicum* infected mice. *Eur J Immunol* 2012;**42**:1523–35.

The full reference list for this chapter is available at expertconsult.com.

SUBSECTION 5 *ARTHROPODS*

CHAPTER 229

Arthropods

Jan E. Drutz

Arthropods comprise nearly three fourths of the world's animal species. Although some arthropods are capable of producing damage to agriculture and homes and others serve as reservoirs, hosts, and vectors of human and animal pathogens, the majority have no significant impact in causing human disease.[71] However, among the more significant diseases transmitted to humans by arthropods are Chagas disease, malaria, dengue, and bubonic plague. In the United States, ticks transmit more vector-borne diseases caused by bacteria, rickettsia, viruses, and protozoa than do any other species.

REFERENCES

1. Abdel-Wahab MF, Esmat G, Ramzy I, et al. *Schistosoma haematobium* infection in Egyptian schoolchildren: demonstration of both hepatic and urinary tract morbidity by ultrasonography. *Trans R Soc Trop Med Hyg* 1992;**86**:406–9.
2. Abdulamir AS, Hafidh RR, Kadhim HS, et al. Tumor markers of bladder cancer: the schistosomal bladder tumors versus non-schistosomal bladder tumors. *J Exp Clin Cancer Res* 2009;**28**:27.
3. Al-Sherbiny MM, Osman AM, Hancock K, et al. Application of immunodiagnostic assays: detection of antibodies and circulating antigens in human schistosomiasis and correlation with clinical findings. *Am J Trop Med Hyg* 1999;**60**:960–6.
4. American Academy of Pediatrics. Drugs for parasitic infections. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2012. p. 848–68.
5. Amiri P, Locksley RM, Parslow TG, et al. Tumour necrosis factor alpha restores granulomas and induces parasite egg-laying in schistosome-infected SCID mice. *Nature* 1992;**356**:604–7.
6. Anonymous. Drugs for parasitic infections. *Med Lett Drugs Ther* 2002;**March**:1–12.
7. Araujo MI, de Carvalho EM. Human schistosomiasis decreases immune responses to allergens and clinical manifestations of asthma. *Chem Immunol Allergy* 2006;**90**:29–44.
8. Araujo MI, de Jesus AR, Bacellar O, et al. Evidence of a T helper type 2 activation in human schistosomiasis. *Eur J Immunol* 1996; **26**:1399–403.
9. Aquino RT, Chieffi PP, Catunda SM, et al. Hepatitis B and C virus markers among patients with hepatosplenic mansonic schistosomiasis. *Rev Inst Med Trop Sao Paulo* 2000;**42**:313–20.
10. Aronstein WS, Strand M. Gender-specific and pair-dependent glycoprotein antigens of *Schistosoma mansoni*. *J Parasitol* 1984;**70**: 545–757.
11. Assis A, Barreto ML, Prado MS, et al. *Schistosoma mansoni* infection and nutritional status in schoolchildren: a randomized, double-blind trial in northeastern Brazil. *Am J Clin Nutr* 1998; **68**:1247–53.
12. Barnes KC, Grant AV, Gao P. A review of the genetic epidemiology of resistance to parasitic disease and atopic asthma: common variants for common phenotypes? *Curr Opin Allergy Clin Immunol* 2005;**5**:379–85.
13. Barron L, Wynn TA. Macrophage activation governs schistosomiasis-induced inflammation and fibrosis. *Eur J Immunol* 2011;**41**:2509–14.
14. Barsoum RS. Schistosomal glomerulopathies. *Kidney Int* 1993; **44**:1–12.
15. Barsoum RS, Bassily S, Soliman MM, et al. Renal amyloidosis and schistosomiasis. *Trans R Soc Trop Med Hyg* 1979;**73**:367–74.
16. Bergquist R, Al-Sherbiny M, Barakat R, et al. Blueprint for schistosomiasis vaccine development. *Acta Trop* 2002;**82**:183–92.
17. Berhe N, Halvorsen BL, Gundersen TE, et al. Reduced serum concentrations of retinol and alpha-tocopherol and high concentrations of hydroperoxides are associated with community levels of S. mansoni infection and schistosomal periportal fibrosis in Ethiopian school children. *Am J Trop Med Hyg* 2007;**76**:943–9.
18. Bialek R, Knobloch J. Schistosomiasis in German children. *Eur J Pediatr* 2000;**159**:530–4.
19. Bica I, Hamer DH, Stadecker MJ. Hepatic schistosomiasis. *Infect Dis Clin North Am* 2000;**14**:583–604, viii.
20. Bonnard P, Boutouaba S, Diakhate I, et al. Learning curves of vesico-urinary ultrasonography in *Schistosoma haematobium* infection with WHO practical guide: a "simple to learn" examination. *Am J Trop Med Hyg* 2011;**85**:1071–4.
21. Boros DL, Whitfield JR. Enhanced Th1 and dampened Th2 responses synergize to inhibit acute granulomatous and fibrotic responses in murine schistosomiasis mansoni. *Infect Immun* 1999; **67**:1187–93.
22. Borrmann S, Szelak N, Faucher JF, et al. Artesunate and praziquantel for treatment of *Schistosoma haematobium* infections: a double-blind, randomized, placebo-controlled study. *J Infect Dis* 2001;**184**:1363–6.
23. Botelho M, Ferreira AC, Oliveira MJ, et al. *Schistosoma haematobium* total antigen induces increased proliferation, migration and invasion, and decreases apoptosis of normal epithelial cells. *Int J Parasitol* 2009;**39**:1083–91.
24. Brindley PJ, Sher A. The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *J Immunol* 1987;**139**:215–20.
25. Browning MD, Narooz SI, Strickland GT, et al. Clinical characteristics and response to therapy in Egyptian children infected with *Schistosoma haematobium*. *J Infect Dis* 1984;**149**:998–1004.
26. Capron A, Capron M, Dombrowicz D, Riveau G. Vaccine strategies against schistosomiasis: from concepts to clinical trials. *Int Arch Allergy Immunol* 2001;**124**:9–15.
27. Cardosa LS, Oliveira SC, Araujo MI. *Schistosoma mansoni* antigens as modulators of the allergic inflammatory response in asthma. *Endocr Metab Immune Disord Drug Targets* 2012;**12**:24–32.
28. Carey FM, Quah SP, Hedderwick S, et al. Genital schistosomiasis. *Int J STD AIDS* 2001;**12**:609–11.
29. Carod-Artal FJ. Neurological complications of *Schistosoma* infection. *Trans Roy Soc Trop Med Hyg* 2008;**102**:107–16.
30. Caumes E, Vidailhet M. Acute neuroschistosomiasis: a cerebral vasculitis to treat with corticosteroids not praziquantel. *J Trav Med* 2010;**17**:359–61.
31. Cetron MS, Chitsulo L, Sullivan JJ, et al. Schistosomiasis in Lake Malawi. *Lancet* 1996;**348**:1274–8.
32. Chen MC, Wang SC, Chang PY, et al. Granulomatous disease of the large intestine secondary to schistosome infestation: a study of 229 cases. *Chin Med J (Engl)* 1978;**4**:371–8.
33. Chen MG. *Schistosoma japonicum* and *S. japonicum*–like infections: clinical and pathological aspects. In: Jordan P, Webbe G, Sturrock RF, editors. *Human schistosomiasis*. Wallingford, UK: CAB International; 1993. p. 237–70.
34. Chiaramonte MG, Cheever AG, Malley JD. Studies of murine schistosomiasis reveal interleukin-13 blockade as a treatment for established and progressive liver fibrosis. *Hepatology* 2001;**34**: 273–82.
35. Chiaramonte MG, Schopf LR, Neben TY, et al. IL-13 is a key regulatory cytokine for Th2 cell–mediated pulmonary granuloma formation and IgE responses induced by *Schistosoma mansoni* eggs. *J Immunol* 1999;**162**:920–30.
36. Chitsulo L, Engels D, Montresor A, Savioli L. The global status of schistosomiasis and its control. *Acta Trop* 2000;**77**: 41–51.
37. Clerinx J, Bottieau E, Wichmann D, et al. Acute schistosomiasis in a cluster of travelers from Rwanda: diagnostic contribution of schistosome DNA detection in serum compared to parasitology and serology. *J Travel Med* 2011;**18**:367–72.
38. Conceição MJ, Argento CA, Chagas VL, et al. Prognosis of schistosomiasis mansoni patients infected with hepatitis B. *virus Mem Inst Oswaldo Cruz* 1998;**93**:255–8.
39. Cooke GS, Lalvani A, Gleesen FV, et al. Acute pulmonary schistosomiasis in travelers returning from Lake Malawi, sub-Saharan Africa. *Clin Infect Dis* 1999;**29**:836–9.
40. Corachan M, Valls ME, Gascon J, et al. Hematospermia: a new etiology of clinical interest. *Am J Trop Med Hyg* 1994;**50**: 580–4.
41. Correa-Oliveira R, Malaquias LC, Falcao PL, et al. Cytokines as determinants of resistance and pathology in human *Schistosoma mansoni* infection. *Braz J Med Biol Res* 1998;**31**:171–7.
42. Coutinho HM, Acosta LP, McGarvey ST, et al. Nutritional status improves after treatment of *Schistosoma japonicum*–infected children and adolescents. *J Nutr* 2006;**136**:183–8.
43. Coutinho HM, Leenstra T, Acosta LP, et al. Pro-inflammatory cytokines and C-reactive protein are associated with undernutrition in the context of *Schistosoma japonicum* infection. *Am J Trop Med Hyg* 2006;**75**:720–6.
44. Crowell C, Kiruga JM, Figaji A, et al. Neuroschistosomiasis due to *Schistosoma haematobium* presenting as spinal cord tumor. *Pediatr Inf Dis J* 2011;**30**:1006–8.
45. De Clercq D, Vecruysse J, Kongs A, et al. Efficacy of artesunate and praziquantel in *Schistosoma haematobium* infected schoolchildren. *Acta Trop* 2002;**82**:61–6.
46. de Jesus AR, Silva A, Santana LB, et al. Clinical and immunologic evaluation of 31 patients with acute schistosomiasis mansoni. *J Infect Dis* 2002;**185**:98–105.
47. Delegue P, Picquet M, Shaw DJ, et al. Morbidity induced by *Schistosoma haematobium* infections, as assessed by ultrasound before and after treatment with praziquantel, in a recently expanded focus (Senegal River basin). *Ann Trop Med Parasitol* 1998;**92**:775–83.

48. Doenhoff MJ, Hassounah O, Murare H, et al. The schistosome egg granuloma: immunopathology in the cause of host protection or parasite survival? *Trans R Soc Trop Med Hyg* 1986;**80**:503–14.
49. Doherty JF, Moody AH, Wright SG. Katayama fever: an acute manifestation of schistosomiasis. *BMJ* 1996;**313**:1071–2.
50. El Tourabi H, el Amin AA, Shaheen M, et al. Propranolol reduces mortality in patients with portal hypertension secondary to schistosomiasis. *Ann Trop Med Parasitol* 1994;**88**:493–500.
51. Elhag SM, Abdelkareem EA, Yousif AS, et al. Detection of schistosomiasis antibodies in urine patients as a promising diagnostic marker. *Asian Pacific J Trop Med* 2011;**4**:773–7.
52. Farah IO, Mola PW, Kariuki TM, et al. Repeated exposure reduces periportal fibrosis in *Schistosoma mansoni*–infected baboons: role of TGF-beta and IL-4. *J Immunol* 2000;**164**:5337–43.
53. Feldmeier H, Daccal RC, Martins MJ, et al. Genital manifestations of schistosomiasis mansoni in women: important but neglected. *Mem Inst Oswaldo Cruz* 1998;**93**:127–33.
54. Fenwick A, Webster JP. Schistosomiasis: challenges for control, treatment and drug resistance. *Curr Opin Infect Dis* 2006;**19**: 577–82.
55. Ferrari TC. Spinal cord schistosomiasis, a report of 2 cases and review emphasizing clinical aspects. *Medicine (Baltimore)* 1999;**78**: 176–90.
56. Ferrari TCA, Moreira PRR. Neuroschistosomiasis: clinical symptoms and pathogenesis. *Lancet* 2011;**10**:853–64.
57. Ferraz AA, Bacelar TS, Silveira MJ, et al. Surgical treatment of schistosomal portal hypertension. *Int Surg* 2001;**86**:1–8.
58. Flores-Villanueva PO, Zheng XX, Strom TB, et al. Recombinant IL-10 and IL-10/Fc treatment down-regulate egg antigen–specific delayed hypersensitivity reactions and egg granuloma formation in schistosomiasis. *J Immunol* 1996;**156**:3315–20.
59. Fowler R, Lee C, Keystone JS. The role of corticosteroids in the treatment of cerebral schistosomiasis caused by *Schistosoma mansoni:* case report and discussion. *Am J Trop Med Hyg* 1999; **61**:47–50.
60. Friedman JF, Kanzaria HK, McGarvey ST. Human schistosomiasis and anemia: the relationship and potential mechanisms. *Trends Parasitol* 2005;**21**:386–92.
61. Friedman JF, Mital P, Kansaria HK, et al. Schistosomiasis and pregnancy. *Trends Parasitol* 2007;**23**:159–64.
62. Gabrielli AF, Toure S, Sellin B, et al. A combined school- and community-based campaign targeting all school-age children of Burkina Faso against schistosomiasis and soil-transmitted helminthiasis: performance, financial costs and implications for sustainability. *Acta Trop* 2006;**99**:234–42.
63. Garba A, Toure S, Dembele R, et al. Implementation of national schistosomiasis control programmes in West Africa. *Trends Parasitol* 2006;**22**:322–6.
64. Gawish Y, El-Hammadi HA, Kotb M, et al. Devascularization procedure and DSRS: a controlled randomized trial on selected haemodynamic portal flow patterns in schistosomal portal hypertension with variceal bleeding. *Int Surg* 2000;**85**:325–30.
65. Gendrel D, Kombila M, Beaudoin-Leblevec G, et al. Nontyphoidal salmonellal septicemia in Gabonese children infected with *Schistosoma intercalatum*. *Clin Infect Dis* 1994;**18**:103–5.
66. Goldring OL, Clegg JA, Smithers SR, et al. Acquisition of human blood group antigens by *Schistosoma*. *Clin Exp Immunol* 1976;**26**:181–7.
67. Gryseels B. Schistosomiasis vaccines: a devil's advocate view. *Parasitol Today* 2000;**16**:46–8.
68. Gryseels B. Schistosomiasis. *Infect Dis Clin North Am* 2012;**26**: 383–97.
69. Gryseels B, Polman K, Clerinx J, et al. Human schistosomiasis. *Lancet* 2006;**368**:1106–8.
70. Guang-Han H, Jia H, Kuang-Yu S, et al. The role of health education and health promotion in the control of schistosomiasis: experiences from a 12-year intervention study in the Poyang Lake area. *Acta Trop* 2005;**96**:232–41.
71. Guyatt H, Gryseels B, Smith T, et al. Assessing the public health importance of *Schistosoma mansoni* in different endemic areas: attributable fraction estimates as an approach. *Am J Trop Med Hyg* 1995;**53**:660–7.
72. Hamilton JV, Klinkert M, Doenhoff MJ. Diagnosis of schistosomiasis: antibody detection, with notes on parasitological and antigen detection methods. *Parasitology* 1998;**117**(Suppl.):41–57.
73. Hatz CF. The use of ultrasound in schistosomiasis. *Adv Parasitol* 2001;**48**:225–84.
74. Henriques-Souza AMM, Valenca MM. Schistosomal myelopathy in childhood: findings of magnetic resonance imaging in 26 patients. *Pediatr Neurol* 2011;**45**:373–6.
75. Hiatt RA, Ottesen EA, Sotomayor ZR, et al. Serial observation of circulating immune complexes in patients with acute schistosomiasis. *J Infect Dis* 1980;**142**:665–70.
76. Hiatt RA, Sotomayor ZR, Sanchez G, et al. Factors in the pathogenesis of acute schistosomiasis mansoni. *J Infect Dis* 1979;**139**: 659–66.
77. Hoffmann KF, Cheever AW, Wynn TA. IL-10 and the dangers of immune polarization: excessive type 1 and type 2 cytokine responses induce distinct forms of lethal immunopathology in murine schistosomiasis. *J Immunol* 2000;**164**:6406–16.
78. Hotez P. Enlarging the "audacious goal": elimination of the world's high prevalence neglected tropical diseases. *Vaccine* 2011; **29**:D104–10.
79. Humphries D, Nguyen S, Boakye D, et al. The promise and pitfalls of mass drug administration to control intestinal helminth infections. *Curr Opin Infect Dis* 2012;**25**:584–9.
80. Istre GR, Fontaine RE, Tarr J, et al. Acute schistosomiasis among Americans rafting the Omo River, Ethiopia. *JAMA* 1984;**251**: 508–10.
81. Jordan P. From Katayama to the Dakhla Oasis: the beginning of epidemiology and control of bilharzia. *Acta Trop* 2000;**77**:9–40.
82. Jordan P, Webbe G. Epidemiology. In: Jordan P, Webbe G, Sturrock RF, editors. *Human schistosomiasis*. Wallingford, UK: CAB International; 1993. p. 87–158.
83. Kabatereine NB, Fleming FM, Nyandindi U, et al. The control of schistosomiasis and soil-transmitted helminthes in East Africa. *Trends Parasitol* 2006;**22**:332–9.
84. Kamal SM, Rasenack JW, Bianchi L, et al. Acute hepatitis C without and with schistosomiasis: correlation with hepatitis C–specific $CD4^+$ T-cell and cytokine response. *Gastroenterology* 2001;**121**:646–56.
85. Karanja DM, Colley DG, Nahlen BL, et al. Studies on schistosomiasis in western Kenya. I. Evidence for immune-facilitated excretion of schistosome eggs from patients with *Schistosoma mansoni* and human immunodeficiency virus coinfections. *Am J Trop Med Hyg* 1997;**56**:515–21.
86. Khalaf I, Shokeir A, Shalaby M. Urologic complications of genital schistosomiasis. *World J Urol* 2012;**30**:31–8.
87. King CH. Epidemiology of schistosomiasis: determinants of transmission of infection. In: Mahmoud AAF, editor. *Schistosomiasis*. London: Imperial College Press; 2001. p. 115–32.
88. King CH. Disease due to schistosomiasis haematobia. In: Mahmoud AAF, editor. *Schistosomiasis*. London: Imperial College Press; 2001. p. 265–95.
89. King CH, Keating CE, Muruka JF, et al. Urinary tract morbidity in schistosomiasis haematobia: associations with age and intensity of infection in an endemic area of Coast Province, Kenya. *Am J Trop Med Hyg* 1988;**39**:361–8.
90. King CH, Olbrych SK, Soon M, et al. Utility of repeated praziquantel dosing in the treatment of schistosomiasis in high-risk communities in Africa: a systematic review. *PLoS Negl Trop Dis* 2011; **5**:e1321.
91. King CL. Initiation and regulation of disease in schistosomiasis. In: Mahmoud AAF, editor. *Schistosomiasis*. London: Imperial College Press; 2001. p. 213–64.
92. King CL, Malhotra I, Mungai P, et al. *Schistosoma haematobium*–induced urinary tract morbidity correlates with increased tumor necrosis factor-alpha and diminished interleukin-10 production. *J Infect Dis* 2001;**184**:1176–82.
93. Kurtis JD, Friedman JF, Leenstra T, et al. Pubertal development predicts resistance to infection and reinfection with *Schistosoma japonicum*. *Clin Infect Dis* 2006;**42**:1692–8.
94. Lambertucci JR. *Schistosoma mansoni:* pathological and clinical aspects. In: Jordan P, Webbe G, Sturrock RF, editors. *Human schistosomiasis*. Wallingford, UK: CAB International; 1993. p. 195–235.
95. Lambertucci JR, Rayes AA, Gerspacher-Lara R. Salmonella–*S. mansoni* association in patients with acquired immunodeficiency syndrome. *Rev Inst Med Trop Sao Paulo* 1998;**40**:233–5.
96. Leenstra T, Acosta LP, Langdon GC, et al. *Schistosomiasis japonica*, anemia, and iron status in children, adolescents, and young adults in Leyte, Philippines. *Am J Clin Nutr* 2006;**83**:371–9.
97. Lemmer LB, Fripp PJ. Schistosomiasis and malignancy. *S Afr Med J* 1994;**84**:211–15.

98. Leutscher P, Ramarokoto CE, Reimert C, et al. Community-based study of genital schistosomiasis in men from Madagascar. *Lancet* 2000;**355**:117–18.
99. Leutscher P, Ravaoalimalala VE, Rabarisolo C, et al. Clinical findings in female genital schistosomiasis in Madagascar. *Trop Med Int Health* 1998;**3**:327–32.
100. Li S, Wu I, Liu Z, et al. Studies on prophylactic effect of artesunate on schistosomiasis japonica. *Chin Med J (Engl)* 1996;**109**: 848–53.
101. Liang YS, Coles GC, Doenhoff MJ. Detection of praziquantel resistance in schistosomes. *Trop Med Int Health* 2000;**5**:72.
102. Liang YS, Coles GC, Dai JR, et al. Biological characteristics of praziquantel-resistant and susceptible isolates of *Schistosoma mansoni*. *Ann Trop Med Parasitol*. 2001;**95**:715–23.
103. Liang YS, Dai JR, Ning A, et al. Susceptibility of *Schistosoma japonicum* to praziquantel in China. *Trop Med Int Health* 2001;**6**: 707–14.
104. Lim KC, Sun E, Bahgat M, et al. Blockage of skin invasion by schistosome cercariae by serine protease inhibitors. *Am J Trop Med Hyg* 1999;**60**:487–92.
105. Lischer GH, Sweat SD. 16-year-old boy with gross hematuria. *Mayo Clin Proc* 2002;**77**:475–8.
106. Liu H, Lim CCT, Feng X, et al. MRI in cerebral schistosomiasis: characteristic nodular enhancement in 33 patients. *Am J Radiol* 2008;**191**:582–8.
107. Liu R, Dong H, Guo Y, et al. Efficacy of praziquantel and artemisinin derivatives for the treatment and prevention of human schistosomiasis: a systematic review and meta-analysis. *Parasites Vectors* 2011;**4**:201.
108. Liu Y, Munker S, Mullenbach R, et al. IL-13 signaling in liver fibrosis. *Front Immunol* 2012;**3**:115.
109. Lyke KE, Dabo A, Arama C, et al. Reduced T regulatory cell response during acute *Plasmodium falciparum* infection in Malian children co-infected with *Schistosoma haematobium*. *PLoS One* 2012;**7**:e31647.
110. Lyke KE, Dicko A, Dabo A, et al. Association of *Schistosoma haematobium* infection with protection against acute *Plasmodium falciparum* malaria in Malian children. *Am J Trop Med Hyg* 2005; **73**:1124–30.
111. Malaquias LC, Falcao PL, Silveira AM, et al. Cytokine regulation of human immune response to *Schistosoma mansoni:* analysis of the role of IL-4, IL-5 and IL-10 on peripheral blood mononuclear cell responses. *Scand J Immunol* 1997;**46**:393–8.
112. Manzella A, Borba-Filho P, Brandt CT, et al. Brain magnetic resonance imaging findings in young patients with hepatosplenic schistosomiasis mansoni without overt symptoms. *Am J Trop Med Hyg* 2012;**86**:982–7.
113. Martinelli R, Noblat AC, Brito E, et al. *Schistosoma mansoni*–induced mesangiocapillary glomerulonephritis: influence of therapy. *Kidney Int* 1989;**35**:1227–33.
114. Martinelli R, Pereira LJ, Brito E, et al. Clinical course of focal segmental glomerulosclerosis associated with hepatosplenic schistosomiasis mansoni. *Nephron* 1995;**69**:131–4.
115. May J, Kremsner PG, Milovanovic D, et al. HLA-DP control of human *Schistosoma haematobium* infection. *Am J Trop Med Hyg* 1998;**59**:302–6.
116. McManus DP. The search for a vaccine against schistosomiasis—a difficult path but an achievable goal. *Immunol Rev* 1999;**171**: 149–61.
117. McManus DP, Ross AG, Williams GM, et al. HLA class II antigens positively and negatively associated with hepatosplenic schistosomiasis in a Chinese population. *Int J Parasitol* 2001;**31**: 674–80.
118. Medhat A, Zarzour A, Nafeh M, et al. Evaluation of ultrasonographic score for urinary bladder morbidity in *Schistosoma haematobium* infection. *Am J Trop Med Hyg* 1997;**57**:16–19.
119. Mola PW, Farah IO, Kariuki TM, et al. Cytokine control of the granulomatous response in *Schistosoma mansoni*–infected baboons: role of exposure and treatment. *Infect Immun* 1999;**67**:6565–71.
120. Montenegro SM, Miranda P, Mahanty S, et al. Cytokine production in acute versus chronic human schistosomiasis mansoni: the cross-regulatory role of interferon-gamma and interleukin-10 in the responses of peripheral blood mononuclear cells and splenocytes to parasite antigens. *J Infect Dis* 1999;**179**:1502–14.
121. Montes M, White AC Jr, Kontoyiannis DP. Symptoms of intestinal schistosomiasis presenting during treatment of large B cell lymphoma. *Am J Trop Med Hyg* 2004;**71**:552–3.
122. Mutapi F, Rujeni N, Bourke C, et al. *Schistosoma haematobium* treatment in 1-5 year old children: safety and efficacy of the anthelminthic drug praziquantel. *PLoS Negl Trop Dis* 2011;**5**: e1143.
123. Nascimento-Carvalho CM, Moreno-Carvalho OA. Neuroschistosomiasis due to *Schistosoma mansoni:* a review of pathogenesis, clinical syndromes and diagnostic approaches. *Rev Inst Med Trop Sao Paulo* 2005;**47**:179–84.
124. Odogwu SE, Ramamurthy NK, Kabatereine NB, et al. *Schistosoma mansoni* in infants (aged <3 years) along the Ugandan shoreline of Lake Victoria. *Ann Trop Med Parasitol* 2006;**100**:315–26.
125. Olds GR, King C, Hewlett J, et al. Double-blind placebo-controlled study of concurrent administration of albendazole and praziquantel in schoolchildren with schistosomiasis and geohelminths. *J Infect Dis* 1999;**179**:996–1002.
126. Olliaro PL, Vaillant MT, Belizario VJ, et al. A multicentre randomized controlled trial of the efficacy and safety of single-dose praziquantel at 40 mg/kg vs 60 mg/kg for treating intestinal schistosomiasis in the Philippines, Mauritania, Tanzania and Brazil. *PLoS Negl Trop Dis* 2011;**5**:e1165.
127. Olveda RM. Disease due to schistosomiasis japonicum. In Mahmoud AAF, editor. *Schistosomiasis*. London: Imperial College Press; 2001. p. 361–89.
128. Olveda RM, Daniel BL, Ramirez BD, et al. *Schistosomiasis japonica* in the Philippines: the long-term impact of population-based chemotherapy on infection, transmission, and morbidity. *J Infect Dis* 1996;**174**:163–72.
129. Orsini M, Rocha RS, Disch J, et al. The role of nutritional status and insulin-like growth factor in reduced physical growth in hepatosplenic *Schistosoma mansoni* infection. *Trans R Soc Trop Med Hyg* 2001;**95**:453–6.
130. Pearce EJ, Basch PF, Sher A. Evidence that the reduced surface antigenicity of developing *Schistosoma mansoni* schistosomula is due to antigen shedding rather than host molecule acquisition. *Parasite Immunol* 1986;**8**:79–94.
131. Pearce EJ, Caspar P, Grzych JM, et al. Downregulation of Th1 cytokine production accompanies induction of Th2 responses by a parasitic helminth, *Schistosoma mansoni*. *J Exp Med* 1991; **173**:159–66.
132. Pearson MS, Pickering DA, McSorley HJ, et al. Enhanced protective efficacy of a chimeric form of the schistosomiasis vaccine antigen Sm-TSP-2. *PLoS Negl Trop Dis* 2012;**6**:e1654.
133. Pittella JE. Neuroschistosomiasis. *Brain Pathol* 1997;**7**:649–62.
134. Poggensee G, Kiwelu I, Weger V, et al. Female genital schistosomiasis of the lower genital tract: prevalence and disease-associated morbidity in northern Tanzania. *J Infect Dis* 2000;**181**:1210–13.
135. Riveau G, Deplanque D, Femoue F, et al. Safety and immunogenicity of rSh28GST antigen in humans: phase 1 randomized clinical study of a vaccine candidate against urinary schistosomiasis. *PLoS Negl Trop Dis* 2012;**6**:e1704.
136. Roca C, Balanzo X, Gascom J, et al. Comparative, clinicoepidemiological study of *Schistosoma mansoni* infections in travelers and immigrants in Spain. *Eur J Clin Microbiol Infect Dis* 2002; **21**:219–23.
137. Rocha H, Kirk JW, Hearey CD Jr. Prolonged *Salmonella* bacteremia in patients with *Schistosoma mansoni* infection. *Arch Intern Med* 1971;**128**:254–7.
138. Rocha MO, Greco DB, Pedroso ER, et al. Secondary cutaneous manifestations of acute schistosomiasis mansoni. *Ann Trop Med Parasitol* 1995;**89**:425–30.
139. Rollinson D, Knopp S, Levitz S, et al. Time to set the agenda for schistosomiasis elimination. *Acta Tropica* in press. 2012.
140. Ross AG, Bartley PB, Sleigh AC, et al. Schistosomiasis. *N Engl J Med* 2002;**346**:1212–20.
141. Ross AG, McManus DP, Farrar J, et al. Neuroschistosomiasis. *J Neurol* 2012;**259**:22–32.
142. Ross AG, Sleigh AC, Li Y, et al. Schistosomiasis in the People's Republic of China: prospects and challenges for the 21st century. *Clin Microbiol Rev* 2001;**14**:270–95.
143. Ross AG, Vickers D, Olds GR, et al. Katayama syndrome. *Lancet* 2007;**7**:218–24.
144. Ruiz-Guevara R, de Noya BA, Valero SK, et al. Clinical and ultrasound findings before and after praziquantel treatment among Venezuelan schistosomiasis patients. *Rev Soc Bras Med Trop* 2007;**40**:505–11.
145. Rujeni N, Nausch N, Bourke CD, et al. Atopy is inversely related to schistosome infection intensity: a comparative study in

Zimbabwean villages with distinct levels of *Schistosoma haematobium* infection. *Int Arch Allergy Immunol* 2012;**158**:288–98.
146. Saconato H, Atallah A. Interventions for treating schistosomiasis mansoni. *Cochrane Database Syst Rev* 2000;(2):CD000528.
147. Salih SY, Subaa HA, Asha HA, et al. Salmonellosis complicating schistosomiasis in the Sudan. *J Trop Med Hyg* 1977;**80**:14–18.
148. Salter JP, Lim KC, Hansell E, et al. Schistosome invasion of human skin and degradation of dermal elastin are mediated by a single serine protease. *J Biol Chem* 2000;**275**:38667–73.
149. Samuel M, Misra D, Larcher V, et al. *Schistosoma haematobium* infection in children in Britain. *B J U Int* 2000;**85**:316–18.
150. Schwartz E, Roxenman J, Perelman M. Pulmonary manifestations of early schistosome infection among nonimmune travelers. *Am J Med* 2000;**109**:718–22.
151. Scrimgeour EM, Gajdusek DC. Involvement of the central nervous system in *Schistosoma mansoni* and *S. haematobium* infection: a review. *Brain* 1985;**108**:1023–38.
152. Secor WE, del Corral H, dos Reis MG, et al. Association of hepatosplenic schistosomiasis with HLA-DQB1*0201. *J Infect Dis* 1996;**174**:1131–5.
153. Seto EYW, Wong BK, Lu D, et al. Human schistosomiasis resistance to praziquantel in China: should be we worried? *Am J Trop Med Hyg* 2011;**85**:74–82.
154. Shariati F, Perez-Arrellano JL, Carranza C, et al. Evaluation of the role of angiogenic factors in the pathogenesis of schistosomiasis. *Exp Parasitol* 2011;**128**:44–9.
155. Sher A, Fiorentino D, Caspar P, et al. Production of IL-10 by $CD4^+$ T lymphocytes correlates with down-regulation of T1 cytokine synthesis in helminth infection. *J Immunol* 1991; **147**:2713–16.
156. Siddiqui AA, Siddiqui BA, Ganley-Leal L. Schistosomiasis vaccines. *Hum Vaccines* 2011;**7**:1192–7.
157. Simpson AJ, Singer D, McCutchan TF, et al. Evidence that schistosome MHC antigens are not synthesized by the parasite but are acquired from the host as intact glycoproteins. *J Immunol* 1983;**131**:962–5.
158. Smith JH, Christie JD. The pathobiology of *Schistosoma haematobium* infection in humans. *Hum Pathol* 1986;**17**:333–45.
159. Smythies LE, Betts C, Coulson PS, et al. Kinetics and mechanism of effector focus formation in the lungs of mice vaccinated with irradiated cercariae of *Schistosoma mansoni*. *Parasite Immunol* 1996;**18**:359–69.
160. Sobb MA, Moustafa FE, el-Housseini F, et al. Schistosomal specific nephropathy leading to end-stage renal failure. *Kidney Int* 1987;**31**:1006–11.
161. Squires N. Interventions for treating schistosomiasis haematobium. *Cochrane Database Syst Rev* 2000(2):CD000053.
162. Stelma FF, Sall S, Daff B, et al. Oxamniquine cures *Schistosoma mansoni* infection in a focus in which cure rates with praziquantel are unusually low. *J Infect Dis* 1997;**176**:304–7.
163. Stothard JR, Sousa-Figueiredo JC, Betson M, et al. Closing the praziquantel treatment gap: new steps in epidemiological monitoring and control of schistosomiasis in African infants and preschool-aged children. *Parasitology* 2011;**138**:1593–606.
164. Strickland GT. Gastrointestinal manifestations of schistosomiasis. *Gut* 1994;**35**:1334–7.
165. Strickland GT, Ramirez BL. Schistosomiasis. In: Strickland GT, editor. *Hunter's tropical medicine*. 8th ed. Philadelphia: W. B. Saunders; 1999. p. 804–32.
166. Strickland GT, Merritt W, El-Sahly A, et al. Clinical characteristics and response to therapy in Egyptian children heavily infected with *Schistosoma mansoni*. *J Infect Dis* 1982;**146**:20–9.
167. Sturrock RF. The parasites and their life cycles. In: Jordan P, Webbe G, Sturrock RF, editors. *Human schistosomiasis*. Wallingford, UK: CAB International; 1993.
168. Sturrock RF. The schistosomes and their intermediate hosts. In: Mahmoud AAF, editors. *Schistosomiasis*. London: Imperial College Press; 2001. p. 70–83.
169. Summer AP, Stauffer WM, Maroushek SR, et al. Hematuria in children due to schistosomiasis in a nonendemic setting. *Clin Pediatr* 2006;**45**:177–81.
170. Swai B, Poggensee G, Mtweve S, et al. Female genital schistosomiasis as an evidence of a neglected cause for reproductive ill-health: a retrospective histopathological study from Tanzania. *B M C Infect Dis* 2006;**6**:134.
171. Taylor M, Jinabbai CC, Couper I, et al. The effect of different anthelmintic treatment regimens combines with iron supplementation on the nutritional status of schoolchildren in KwaZulu-Natal, South Africa: a randomized controlled trial. *Trans R Soc Trop Med Hyg* 2001;**95**:211–16.
172. Teixeira MM, Doenhoff MJ, McNeice C, et al. Mechanisms of the inflammatory response induced by extracts of *Schistosoma mansoni* larvae in guinea pig skin. *J Immunol* 1993;**151**:5525–34.
173. Tendler M, Simpson AJ. The biotechnology-value chain: development of Sm14 as a schistosomiasis vaccine. *Acta Trop* 2008;**108**: 263–6.
174. Tsang VC, Wilkens PP. Immunodiagnosis of schistosomiasis. *Immunol Invest* 1997;**26**:175–88.
175. Tsang VCW, Wilkens PP. Immunodiagnosis of schistosomiasis: screen with FAST-ELISA and confirm with immunoblot. *Clin Lab Med* 1991;**11**:1029–39.
176. Utzinger J, N'Goran EK, N'Dri A, et al. Oral artemether for prevention of *Schistosoma mansoni* infection: randomised controlled trial. *Lancet* 2000;**355**:1320–5.
177. Utzinger J, Xiao-Nong Z, Ming-Gang C, et al. Conquering schistosomiasis in China: the long march. *Acta Trop* 2005;**96**:69–96.
178. van de Biggelaar AH, van Ree R, Rodrigues LC, et al. Decreased atopy in children infected with *Schistosoma haematobium:* a role for parasite-induced interleukin-10. *Lancet* 2000;**356**:1723–7.
179. Vennervald BJ, Dunne DW. Morbidity in schistosomiasis: an update. *Curr Opin Infect Dis* 2004;**17**:439–47.
180. Verani JR, Abudho B, Montgomery SP, et al. Schistosomiasis among young children in Usoma, Kenya. *Am J Trop Med Hyg* 2011;**84**:787–91.
181. Veress B, Musa AR, Osman H, et al. The nephritic syndrome in the Sudan with special reference to schistosomal nephropathy: a preliminary morphological study. *Ann Trop Med Parasitol* 1978;**72**:357–61.
182. Vester U, Kardorff R, Traore M, et al. Urinary tract morbidity due to *Schistosoma haematobium* infection in Mali. *Kidney Int* 1997; **52**:478–81.
183. Wahl SM, Frazier-Jessen M, Jin WW, et al. Cytokine regulation of schistosome-induced granuloma and fibrosis. *Kidney Int* 1997;**51**:1370–5.
184. Waine GJ, Ross AG, Williams FM, et al. HLA class II antigens are associated with resistance or susceptibility to hepatosplenic disease in a Chinese population infected with *Schistosoma japonicum*. *Int J Parasitol* 1998;**28**:537–42.
185. Whitty CJ, Mabey DC, Armstrong M, et al. Presentation and outcome of 1107 cases of schistosomiasis from Africa diagnosed in a nonendemic country. *Trans R Soc Trop Med Hyg* 2000;**94**: 531–4.
186. World Health Organization. *Report of the WHO informal consultation on schistosomiasis control*. Geneva: World Health Organization; 1998.
187. World Health Organization. *Schistosomiasis: fact sheet*. No. 115, January 2012, http://www.who.int/mediacentre/factsheets/fs115/en/index.html.
188. World Health Organization. Schistosomiasis: population requiring preventive chemotherapy and number of people treated in 2010. *Wkly Epidemiol Rec* 2012;**87**:37–44.
189. Wynn TA, Cheever AW, Jankovic D, et al. An IL-12–based vaccination method for preventing fibrosis induced by schistosome infection. *Nature* 1995;**376**:594–6.
190. Xiao-Nong Z, Li-Ying W, Ming-Gang C, et al. The public health significance and control of schistosomiasis in China: then and now. *Acta Trop* 2005;**96**:97–105.
191. Yang W, Jackson DC, Zeng Q, et al. Multi-epitope schistosome vaccine candidates tested for protective immunogenicity in mice. *Vaccine* 2000;**19**:103–13.
192. Young SW, Higashi G, Kamel R, et al. Interaction of salmonellae and schistosomes in host-parasite relations. *Trans R Soc Trop Med Hyg* 1973;**67**:797–802.
193. Yu DB, Li Y, Sleigh AC, et al. Efficacy of praziquantel against *Schistosoma japonicum:* field evaluation in an area with repeated chemotherapy compared with a newly identified endemic focus in Hunan China. *Trans R Soc Trop Med Hyg* 2001;**95**:537–41.
194. Yu L, Ding J, Wen L, et al. Development of a rapid dipstick with latex immunochromatographic assay (DLIA) for diagnosis of schistosomiasis japonica. *Parasit Vectors* 2011;**4**:157.

195. Zhang L, Mi J, Yu Y, et al. IFN-gamma gene therapy by intrasplenic hepatocyte transplantation: a novel strategy for reversing hepatic fibrosis in *Schistosoma japonicum*–infected mice. *Parasite Immunol* 2001;**23**:11–17.
196. Zhang Y, Chen L, Gao W, et al. IL-17 neutralization significantly ameliorates hepatic granulomatous inflammation and liver damage in *Schistosoma japonicum* infected mice. *Eur J Immunol* 2012; **42**:1523–35.
197. Zhong-Dao W, Zhi-Yue L, Xin-Bing Y. Development of a vaccine against *Schistosoma japonicum* in China: a review. *Acta Trop* 2005; **96**:106–16.
198. Zuidema PJ. The Katayama syndrome: an outbreak in Dutch tourists to the Omo National Park, Ethiopia. *Trop Geogr Med* 1981;**33**:30–5.

TICKS

Ticks, mites, spiders, and scorpions all belong to a class of arthropods known as Arachnida. Two of three families of ticks belonging to this class contain species capable of transmitting pathogens to humans. Ixodidae, the hard tick family, and Argasidae, the soft tick family, serve as both vectors and reservoirs for many rickettsiae (Table 229-1). By use of a fluorescent technique, these rickettsiae can be identified in the tick hemolymph.

Hard ticks are so named because of their scutum, a dorsal sclerotized shield. Owing to the limited scutum coverage of the female, she is more capable of becoming engorged with blood than is the male, a factor significant in the reproductive process. During feedings, these ticks remain attached to the host for hours or days at a time.[21,63] Three genera of Ixodidae are known to transmit disease to humans in the United States: *Amblyomma*, *Dermacentor*, and *Ixodes*.[62]

Soft ticks have no scutum and tend to live for long periods, often surviving for years without eating. Both the nymph form and the adult tick have a tendency to eat often but for brief periods often lasting less than 30 minutes.[21,63] Of the family Argasidae, only ticks of the genus *Ornithodoros* are known to transmit pathogens to humans in the United States.[62] Specifically, they are the vectors of tick-borne relapsing fever.

Local reaction from tick bites appears to be mediated by complement. The reaction from these bites may persist and subsequently develop into a so-called tick bite granuloma. Systemic reactions such as fever, chills, nausea, vomiting, abdominal pain, and headache can be associated with tick bites.

As noted, tick-borne diseases can result from infection with pathogens that include bacteria, rickettsia, viruses, and protozoa.[64] The major tick-borne diseases occurring in the United States include tick paralysis (discussed here), tularemia (see Chapter 132), tick-borne relapsing fever (Chapter 194), Lyme disease (Chapter 140), Colorado tick fever (Chapter 184), Rocky Mountain spotted fever (Chapter 194), ehrlichiosis (Chapter 194), and babesiosis (Chapter 218).

Tick-borne rickettsial diseases are clinically similar yet epidemiologically and etiologically distinct illnesses. In the United States, they include Rocky Mountain spotted fever, human monocytotropic (or monocytic) ehrlichiosis, human granulocytotropic (or granulocytic) anaplasmosis (formerly known as human granulocytotropic ehrlichiosis), *Ehrlichia ewingii* infection, and other emerging tick-borne rickettsial diseases.[13] Mediterranean spotted fever, a tick-borne disease caused by *Rickettsia conorii* (Chapter 194), is found almost exclusively in the Mediterranean area.

Within the past 10 years, five tick-borne diseases have been reported as occurring most often in the United States: Lyme disease, Rocky Mountain spotted fever, ehrlichiosis, anaplasmosis, and babesiosis. Lyme disease, babesiois, and anaplasmosis are all transmitted by the *Ixodes scapularis* tick. Among contributing factors possibly accounting for the prevalence of these diseases are an increase in the number of ticks, geographic spread, climatic change, improved physician awareness and reporting, and improved local and state surveillance.[24]

TABLE 229-1 **Human Infectious Diseases for Which Ticks are a Vector**

Disease	Agent
Relapsing fever	*Borrelia duttonii*
Q fever	*Coxiella burnetii*
Tularemia	*Francisella tularensis*
Queensland tick typhus	*Rickettsia australis*
Fièvre boutonneuse	*Rickettsia conorii*
Rocky Mountain spotted fever	*Rickettsia rickettsii*
Asian tick typhus	*Rickettsia sibirica*
Colorado tick fever	Arbovirus
Encephalitis	Arbovirus
Lyme disease	*Borrelia burgdorferi*
Human monocytic ehrlichiosis	*Ehrlichia chaffeensis*
Human granulocytic ehrlichiosis	*Ehrlichia* spp.
Babesiosis	*Babesia microti*

Tick Paralysis

Tick paralysis is a neurologic syndrome characterized primarily by an ascending flaccid paralysis. The etiologic agent is a potent neurotoxin produced by an attached, engorged tick.[32] A well-known disease of animals, it was reported to occur in humans in North America first in 1912.[67] Most cases have been reported in the Pacific Northwest and Rocky Mountain states, during spring and summer.[64] It occurs more frequently in children than in adults, especially in girls between 2 and 5 years of age. Patients present with paresthesias and leg weakness, but have no fever. Within 1 to 2 days, weakness in the legs ascends to the trunk musculature, resulting in an inability of the patient to sit or walk without assistance.[32]

Numerous genera of ticks are known to be associated with tick paralysis. In North America, *Dermacentor andersoni* (wood tick) and *Dermacentor variabilis* (dog tick) are the primary species responsible.[46] In Australia, the ticks implicated most commonly are *Ixodes holocyclus* and *Ixodes cornuatus*. Both male and female adults, as well as immature ticks, have been implicated as vectors for this disease.

Pathogenesis

Tick paralysis is thought to be caused by a neurotoxin (holocyclotoxin) produced in the tick's salivary glands.[44] It usually is released by gravid female ticks at the site of attachment, generally the scalp. The neurotoxin is a protein with temperature-dependent activity.[19] The exact mechanism and location of the toxin's action are not known but may include decreased entry of calcium into motor nerve terminals or interference with presynaptic excitation–secretion coupling, which leads to a reduction of acetylcholine release at the motor end plate.

Investigations in children with tick paralysis have implicated peripheral nerve dysfunction, attributable to somewhat site-specific diminished nerve conduction

velocities.[39,65] Compound muscle action potentials that are abnormally low or low-normal in amplitude when the patient has maximal neurologic deficits, generally are reversed rapidly once the tick is removed.[46] Swift and Ignacio[65] postulated that the major effect of the toxin is to prevent depolarization in the terminal portions of the motor neurons.

Clinical Manifestations

Onset of tick paralysis usually occurs 5 to 7 days after the female tick attaches to the skin. Until mating occurs with the male, engorgement of the female tick is relatively limited. Mating leads to rapid engorgement, fertilization of the eggs, and production of the neurotoxin. In turn, production of neurotoxin coincides with initial paralytic symptoms, and continual feeding accelerates production of toxin, accounting for rapid clinical deterioration. Once the female becomes engorged, she disengages from the skin to deposit her eggs.[32]

Ascending paralysis, along with hyporeflexia or areflexia, progresses to arms, and then to the bulbar structures involved in speech, swallowing, and facial expression.[32] Sensory function usually is spared, and the sensorium remains clear.[64] Alternatively, the disease can present as acute ataxia without muscle weakness.[47] Nystagmus, strabismus, and seizures may occur.[39] After tick removal, symptoms tend to resolve within several hours or days. Left untreated, tick paralysis can be fatal, with reported mortality rates of 10 to 12 percent.[59]

Routine laboratory tests are not helpful in establishing the diagnosis. The white blood cell count, urine analysis, cerebrospinal fluid, and erythrocyte sedimentation rate are usually normal.

Diagnosis

The diagnosis of tick paralysis can be established when the patient has the typical clinical picture and then improves rapidly after tick removal. A fine-toothed comb can aid in the detection of ticks attached to the scalp. The differential diagnosis of tick paralysis includes Guillain-Barré syndrome, botulism, poliomyelitis, myelitis, spinal cord neoplasm, syringomyelia, and porphyria. Botulism is characterized by slow, descending paralysis involving cranial nerves first, along with a usual extraocular palsy and large, poorly reactive pupils.[32]

The neurologic impairment caused by the *I. holocyclus* tick in Australia is more severe than that caused by *Dermacentor* spp. in North America. With exposure to *I. holocyclus* neurotoxin, the weakness and bulbar symptoms often intensify during the first 24 to 48 hours after tick removal. Clinical recovery is much slower than with *Dermacentor*-related paralysis. *I. holocyclus* antitoxin must be administered before removal of the ticks, and a longer observation period is required.[32]

Treatment

The earlier the tick is removed in the course of the illness, the more promptly the signs and symptoms will resolve. Forceps, not fingers, should be used to remove the tick. The tick's head should be gently but firmly grasped, and slow, reverse traction should be applied to remove all body parts. Recovery generally is complete within 1 to 5 days. In one reported case, weakness did not resolve for several months.[26] Intensive supportive care is required if the patient has cranial nerve dysfunction. Ineffective ventilation and ensuing respiratory failure require assisted ventilation.

MYIASIS

Myiasis is the invasion of a host's tissues by the larval stage (maggot) of nonbiting flies. Because of the immaturity of maggots, differentiating the various species is difficult. Myiasis can be classified according to the anatomic site of infestation (i.e., aural myiasis, ophthalmomyiasis, or cutaneous myiasis) or on the basis of the clinical syndrome (i.e., furuncular cutaneous myiasis, migratory cutaneous myiasis, or wound myiasis).[61] In the past, parasitic diseases were limited to their endemic areas. However, with the relative ease of worldwide travel, they are appearing with increasing frequency in the United States and other developed countries.[38]

Etiology

The true flies of the order *Diptera* undergo metamorphosis in four stages: egg, larva, pupa, and adult. To different degrees, some larvae of the suborder Cyclorrhapha have adapted to a parasitic relationship with humans. Some are classified as obligate, facultative, or accidental parasites in humans. In each case, the larval stage of the fly is able to invade the tissues of the host and progress in the stages of metamorphosis.

Epidemiology

The occurrence of human myiasis has been linked to humid and warm climates that favor the breeding of flies. Epizootics in livestock, marginal housing, poor disposal of refuse, and under-nutrition also are important factors in the development of human myiasis.[50] In the United States, myiasis has been reported both from flies native to North America and from the larvae of flies acquired during foreign travel. More than 50 species of flies have been reported to cause human myiasis.

Pathogenesis

The pathogenesis of human myiasis differs with the degree of parasitic adaptation by each fly. *Dermatobia hominis* (the human botfly) uses a bloodsucking insect as a vector to deposit its eggs on a warm-blooded host. The larvae emerge from the eggs and then penetrate the host's skin, frequently using the puncture site of the carrier insect. The larvae develop within the dermal layer of skin, which leads to a boil-like swelling. During this period, the human host develops clinical symptoms. *D. hominis* and *Cochliomyia hominivorax*, the primary screwworm, are examples causing obligate myiasis.[50] *C. hominivorax* can be responsible for aural or nasal myiasis.

The genus *Sarcophaga* (flesh flies) is capable of causing facultative myiasis. The adult fly is attracted to wounds or ulcers containing purulent and necrotic material. The adult fly deposits eggs in the open wound, where the larvae hatch.

Maggots seldom are found in the human intestinal or urinary tract.[61] Accidental myiasis can occur when humans ingest eggs or larvae and the larvae remain in the intestinal tract. Genitourinary myiasis is thought to occur by the deposition of eggs around the external urethral orifice. The larvae then may migrate into and up the urethra. Such situations should be called *pseudomyias*,[76] because the maggots are not living parasitically.

Clinical Manifestations

The lesions of cutaneous myiasis generally are located over the exposed area of the body. Early in the course of cutaneous myiasis, pruritus is the predominant symptom. As the larvae grow after the first week of infestation, a serous exudate may drain from the penetrating site. At this point, pain and pruritus are prominent symptoms and the lesion appears as a small furuncle (furuncular myiasis).[27] Tissue destruction by the larvae may continue, and secondary bacterial infection can occur. *Staphylococcus aureus*, group A streptococci, and gram-negative organisms have been isolated from infected cutaneous myiasis wounds.

Abdominal pain, diarrhea, and anal bleeding are the symptoms of intestinal myiasis, which is self-limited and may last 2 to 6 weeks. Larvae within the genitourinary tract may lead to proteinuria, dysuria, hematuria, and pyuria. Nasal myiasis can extend into bone, sinus cavities, and even the meninges.[4] Aural myiasis has been described in a child without underlying disease.[20] Ophthalmomyiasis is characterized by an acute catarrhal conjunctivitis.[27] Penetration into the brain has been associated with intracerebral hematomas.[45]

Diagnosis

A careful history of travel, occupation, and exposure is necessary to establish the diagnosis when the physician is confronted with unusual skin lesions that are pruritic and have not resolved with usual local care.[37,42] Myiasis is confirmed if larvae are demonstrated within the wound. A parasitologist or entomologist may be able to identify the species of larvae responsible.

Treatment

The removal of the larvae is necessary in any of the forms of myiasis. Endoscopic removal of nasal infestation is recommended. Surgical intervention may be required to expose the larvae in the wound. Forceps are used to pick out the larvae; the application of 5 percent chloroform in olive oil may facilitate removal. Occlusive coverings of the wound opening are helpful in extruding *Dermatobia* larvae because this tends to diminish the oxygen supply for the larvae. A thick layer of petrolatum jelly (Vaseline) effectively interrupts the airflow.[51] Local or systemic antibiotics may be required if secondary bacterial infection is present.

The prevention of human myiasis requires good wound care, adequate personal hygiene, screening to protect against flies, and the prevention of myiasis in domestic animals.

Mites

As mentioned earlier, mites belong to the same class of arthropods (Arachnida) as ticks, spiders, and scorpions. They, too, can be vectors for infectious agents. The house mite, *Liponyssoides sanguineus*, is the vector for rickettsialpox (Chapter 194) agent. Chiggers, which are larvae of mites (family Trombiculidae), transmit to humans the agent responsible for scrub typhus, *Rickettsia tsutsugamushi* (Chapter 194).

Some mites transmit no specific disease but can cause an annoying pruritic rash. In 2004, the microscopic itch mite *(Pyemotes herfsi)* was identified as a likely cause of an intensely pruritic skin reaction in individuals living in several Midwestern states, as well as Texas and Oklahoma. Skin lesions were papular and erythematous and occurred primarily on the face, neck, and limbs.[12]

Scabies, an extremely pruritic skin infestation, has been known for more than 2500 years. The etiologic agent for this condition, *Sarcoptes scabiei* var. hominis, is an obligate human parasite that burrows into the epidermis, no deeper than the stratum granulosum.[16] Scabies appears to have an increased incidence in 15-year cycles.[60] and is transmitted person to person through direct and usually prolonged contact.

Pathogenesis

Mites of all developmental stages tunnel into the stratum corneum and deposit feces (scybala) behind them. Characteristically, the gravid female lays eggs in the tunnels.[36] Clothing and bed linens are thought to be less important in the transmission of the *Sarcoptes* mite. Because scabies is transmitted by skin-to-skin contact, sexual transmission occurs commonly, as does nonsexual spread in family settings.[16] Epidemic outbreaks of scabies have been reported in hospitals and other institutions where people were living closely together and especially where poor sanitation predominated.[6]

Hypersensitivity of both immediate and delayed types has been implicated in the development of lesions other than burrows.[16] Papulovesicular scabies is characterized by perivascular lymphohistiocytic infiltrates with eosinophils. The papillary dermis is edematous. The histologic appearance of nodular scabies is one of a dense, superficial, and deep perivascular lymphohistiocytic infiltrate with many plasma cells and eosinophils. Various vascular changes also may be apparent.

Clinical Manifestations

Scabies is characterized by moderate to severe pruritus that starts several weeks to months after infestation, at which time the host has become hypersensitive to the mite or its products. Itching, most evident at night, is the primary symptom of infection. A papular or vesicular eruption with pustules and linear burrows occurs and

classically involves the webs between the fingers, flexures of the arms, axillae, and genital regions of adults. In infants and children, scabetic lesions more typically occur on the palms, soles, head, and neck in the form of vesicles, pustules, or nodules. In this age group, scabies often is not suspected because of the atypical skin lesions that result from vigorous scratching and secondary infection.[41]

Acute glomerulonephritis may be associated with pyoderma in scabies. A careful history may reveal that other family members or child caretakers have pruritus and skin lesions consistent with scabies. Examination of the skin of family members for signs of scabies frequently is helpful. Crusted scabies, previously known as Norwegian scabies, occurs in immunocompromised patients, especially those on immunosuppressive medications or those with human immunodeficiency virus (HIV) or human T-lymphotropic virus type 1 infection.[33] In this condition, mite replication is uncontrolled by the host's immune system.[22,33] It is a psoriasiform dermatitis, frequently associated with a generalized or localized nonspecific hyperkeratosis.[28,33]

Diagnosis

Definitive diagnosis can be made by microscopic identification of the mites, eggs, or mite feces.[40] To obtain this material, skin scrapings should be taken from papules or the end of a burrow or from underneath the surface of fingernails. In some cases, a variety of biopsy techniques can be useful.[9] The diagnosis should be considered when the physician is faced with an unusual papular or bullous rash. The differential diagnosis of scabies in children includes impetigo, atopic eczema, seborrheic or contact dermatitis, psoriasis, histiocytosis, and chickenpox.[40,41]

Treatment

The management of scabies involves the application of a topical scabicide to all areas of skin, including the face and scalp, with subsequent removal in 8 to 24 hours, depending on the product applied. An effective scabicide used in the past was gamma-hexachlorocyclohexane (lindane). It is no longer recommended for use in children, because of concerns regarding neurotoxicity.[22,48] Currently, effective topical scabicides licensed in the United States include 5 percent permethrin and 10 percent crotamiton. Although 5 percent malathion lotion is approved in the United States for treatment of head lice (see later discussion of head lice treatment), it has not received U.S. Food and Drug Administration (FDA) approval to be used as a scabicide.[36]

The agent of choice for infants and young children is 5 percent permethrin topical cream, applied in the evening to the entire head, neck, and body of the infant and left on overnight.[41] It is indicated and safe for use in newborns and young children, as well as pregnant and lactating women.[36] A second application generally is recommended 1 to 2 weeks after the first dose.[22,36] All family members should be treated simultaneously.[55] Antibiotics may be necessary if secondary bacterial infection is present.

The Centers for Disease Control and Prevention (CDC) has recommended the off-label use of oral ivermectin 200 μg/kg/dose given with food for the treatment of scabies, though the FDA has yet to grant such approval.[22] Ivermectin is considered to be highly efficacious as an oral therapy for scabies in a single dose for otherwise healthy or HIV-infected adults.[52] Some have recommended two doses, with the second dose given 1 to 2 weeks after the first dose.[22] It works against several different parasites by interrupting gamma-aminobutyric acid–induced neurotransmission.[16] The CDC recommends not using ivermectin in pregnant or lactating women and states that safety has not been determined in children who weigh less than 15 kg.

Articles of clothing and bed linens should be machine washed in hot water at 60° C (140° F). Insecticidal powder or aerosol should be reserved for materials that cannot be washed.[16] Pruritus often persists for some time after successful treatment with a scabicide and may be relieved by an oral antihistamine or mild to moderate topical steroids.[9,55] In hospitalized patients, contact isolation is recommended to lessen the potential for nosocomial transmission.

PEDICULOSIS

Arthropods of the order Anoplura (sucking lice) are important as vectors of rickettsial or spirochetal illnesses. The body louse, *Pediculus humanus humanus*, is the vector for epidemic typhus *(Rickettsia prowazekii)*, trench fever *(Bartonella quintana)*, and louse-borne relapsing fever *(Borrelia recurrentis)*. The body louse, head louse *(Pediculus humanus capitis)*, and crab louse *(Phthirus pubis)* all are capable of achieving human infestation. Pediculosis has been a problem for humans for more than 10,000 years.[16]

Pathogenesis

Head lice are the most common type of louse, affecting more than 100 million people worldwide each year.[17] Tiny, they measure no more than 1 to 4 mm in length. They have six legs, each with powerful claws allowing firm attachment. Mouth parts consist of stylets (retracted when not in use) modified for piercing and sucking. When they are not feeding, lice are translucent, with grayish-white bodies, but when engorged with blood, they become red. Each female is capable of laying as many as 300 eggs (nits) in a brief lifetime of 1 to 3 months. These eggs are less than 1 mm in diameter and hatch in 6 to 10 days, giving rise to nymphs. During a period of 10 days, the nymphs become adults. School children of all socioeconomic groups are affected, with head-to-head contact being the most common means of transmission.

Clinical Manifestations

Children or adults with head lice usually have white or opalescent nits attached to individual strands of hair. The lice themselves firmly adhere to the base of the hair shaft,

millimeters from the scalp. Once a louse pierces the skin, a poisonous salivary secretion is exuded, resulting in pruritic dermatitis. Itching is the primary symptom in acute cases.

Body lice occur when socioeconomic conditions are poor. Infestation occurs when people fail or are unable to wash their clothes regularly, such as those living in refugee camps. Intense itching is a major problem, with noticeable excoriations and occasional secondary infections.

Pubic lice are transmitted primarily through sexual contact, although not exclusively so. In children, pubic lice generally occur from nonsexually transmitted contact with an infected parent. Again, itching is a major presenting symptom. Pubic lice may become attached to other hairy areas of the body, such as the axillae of adolescents, the eyelashes of children, and the scalp hair of any age group.[16]

As mentioned, body lice can function as vectors for the transmission of several diseases. One of these is trench fever, caused by *B. quintana*. Although some patients may have no symptoms, others may have fever, myalgias, headache, meningoencephalitis, transient maculopapular rashes, or chronic adenopathies. A second potentially transmitted disease is epidemic typhus, caused by *R. prowazekii.* Signs or symptoms of this infection may include fever, pruritic rash, headache, and confusion. Relapsing fever caused by *B.* recurrentis is the third disease known to be transmitted by the body louse.[16]

Diagnosis

Head, body, and pubic lice are visibly recognizable. The nits of head lice are attached firmly to hair shafts and are distinguished readily from dandruff flakes, lint, and other debris, which can be easily removed from the hair. Viable nits, located close to the scalp, are oval and grayish-white to yellow-white. Empty nonviable nits, attached at some distance from the scalp, are almost completely clear. Although difficult to see with the naked eye, nits visibly fluoresce yellow-green under Wood's lamp. Polymerase chain reaction (PCR) helps identify host DNA from lice through their blood meal, providing valuable information for rape, homicide, and child abuse cases.[49]

The body louse has been demonstrated as the true vector of trench fever by using PCR to detect within them the *B. quintana* DNA from infected patients.[8] Detection of rickettsial DNA in body lice has confirmed the presence of human typhus.

Treatment

Head Lice

Numerous forms of treatment for the eradication of head lice have been used. Over the course of time, resistance developed to several of these forms of therapy. Gamma-hexachlorocyclohexane (lindane) shampoo was used almost exclusively until the early 1970s. Because of concern about potential neurotoxic effects for the patient, it no longer is recommended as a first-line medication for children.[48]

A systematic review (1995) concluded that sufficient evidence existed only for the use of a pyrethroid insecticide, 1 percent permethrin cream rinse.[17,68] This biodegradable and generally very safe product remains the recommended first-line therapy. It should be applied to wet hair and left in place for about 10 minutes before rinsing. Because of variable reports of head lice resistance to 1 percent permethrin, the current recommendation is two treatment applications 7 to 14 days apart, or even three treatments at 0, 7, and 15 days, based on the life cycle of head lice.[5] Some success has been reported with use of a 5 percent permethrin preparation and wearing a shower cap overnight.[57] To remove the firmly adherent nits, a fine-toothed stainless steel comb (LiceMeister) has been recommended.

Malathion lotion 0.5 percent, an organophosphate, has been an excellent alternative to the use of permethrin in areas where head lice-resistance has occurred. When applied to the hair and left in place for 8 to 12 hours, it provides residual protection. Caution should be taken because it is potentially flammable. Those treated should not be exposed to open flames or electric heat sources, including hair dryers and electric curlers.[1,5]

Cases of resistance to the malathion-containing formulation have been reported in Europe, but that product is different from the one licensed in the United States. The latter contains additional synergistic agents, including 78 percent isopropanol. Because it is hydrolyzed and detoxified by plasma carboxylesterases much more rapidly in mammals than in insects, it is considered safe. Although it is contraindicated for use in infants, the American Academy of Pediatrics considers it be safe for use in children 2 years of age and older.[1,5]

Ivermectin has been used when other medications have failed. A dose of 200 μg/kg given on days 1 and 8 appears to be effective. Concern exists when the drug is used in patients weighing less than 15 kg or those who are pregnant or breast-feeding.[10]

As a result of reports of difficult-to-treat head lice infestation, authors in one cluster-randomized, double-blind, double-dummy, controlled trial compared ivermectin 400 μg/kg to the application of 0.5 percent malathion lotion, each given on days 1 and 8. Ivermectin was reported to have superior efficacy. However, the malathion product was not the one licensed in the United States.[17]

In addition to malathion, two other topical pediculcides have received FDA approval for pediatric use. Labeled for use in children 6 months or older, benzyl alcohol should be applied to dry hair for at least 10 minutes. If live lice persist, a second application should be administered 7 days later. For children 4 years of age or older, spinosad should be applied in the same way as benzyl alcohol.

There have been other recommended approaches to the treatment of head lice, all with somewhat limited success. A short course of oral trimethoprim-sulfamethoxazole had been recommended in the past with the intent of destroying essential lice gut bacteria,

responsible for the production of vitamin B, essential for continued life. A nonchemical approach to treatment has been the application of a topical lotion (Cetaphil Skin Cleanser) to the hair, with subsequent use of a blow dryer to "shrinkwrap" and ultimately suffocate lice.[54] Although this approach may result in significant success, the original study was not sufficiently rigorous to prove its effectiveness.

The use of a high-volume, hot-air blow dryer alone has been reported to have killed 94 to 98 percent of lice eggs and 76 to 80 percent of hatched lice.[35] When examined 1 week later, virtually all of the patients were completely cured. Although promising, this approach necessitates further testing by independent investigators. If proven to be efficacious, it could potentially eliminate the use of chemicals, to which some lice have become resistant.

Body Lice

A general recommendation has been thorough washing of the body with soap and water followed by the application of a pyrethrin or pyrethroid or malathion for 8 to 24 hours. Again, caution should be taken in the use of these preparations in children. Decontamination of clothing and bed linens is essential by either washing them in hot soapy water at a temperature of 54° C (130° F) for 10 minutes or placing them in a hot clothes dryer for 20 minutes.

Pubic Lice

Treatment should be similar to that used for managing head lice. Clothes and bed linens should be decontaminated. In the case of children, parents also should be treated; and in the case of adolescents, sexual partners require treatment.

BED BUGS

Numerous recent reports have documented the reemergence of bed bugs *(Cimex lectularius)* as a cause of pruritic, erythematous papules.[14,66] Although they are known to harbor pathogens (e.g., plague and hepatitis B), bed bugs are considered incapable of transmitting those diseases to humans[43,69] and therefore are not thought to pose a medical threat. Secondary skin infections, however, can develop as a result of persistent scratching, and allergic reactions are a possibility.

The upsurge in bed bug infestation has been attributed to various factors, including increased international travel and transport, exchange of secondhand furniture, insecticide resistance, and changes in insecticide use.[23] The primary result of contending with these annoying insects has been both social and psychological. Hotels, department stores, apartment buildings, and individual home owners have encountered a significant economic loss in their efforts to eliminate these pests.

Pathogenesis and Clinical Manifestations

Bed bugs tend to be nocturnally active and are attracted to humans by arm body temperature and the exhalation of carbon dioxide. Most can be found within 5 feet of the bed. Survival depends on blood extracted from their respective hosts, which is achieved by piercing the skin with their beadlike proboscis containing a set of two hollow tubes. Online videos are available for viewing actual bed bug feeding.[25] Through one of these tubes, they inject saliva containing both anticoagulants and anesthetics; through the other one, blood is withdrawn.[66] Although they can live for up to 18 months without feeding, bedbugs typically seek blood every 5 to 10 days.

Feeding time generally takes place in the early morning hours, when the host is most calm and least likely to sense the presence of a crawling insect. Because bed bugs are opportunistic feeders, shift workers, who sleep during the day time, also are vulnerable.

Total feeding time can range from as short as 5 minutes to as long as 30 minutes, after which the insect abandons the skin surface and seeks seclusion among bed sheets, blankets, mattresses, floorboard cracks, or other crevices.[66] The host generally does not experience itching or skin irritation until several minutes or hours after the insect has fed. In some cases, no skin reaction occurs at all.

Diagnosis

Suspicion for the possible presence of bed bugs occurs when the host awakens with itching and visible bites not present before going to sleep. Clues to the presence of bed bugs include discrete reddish-brown bloodstains on sheets and mattresses, as well as flecks of excrement at the portals of hiding places.[64] Confirmation requires finding and identifying these bugs. Current approaches to early detection include the use of various lures or traps and training dogs to pick up the scent of certain bed bug pheromones.[23]

Treatment

Treatment of bites is relatively conservative, directed toward symptomatic relief of itching, irritation, and secondary infection. Antipruritic medication, either topical or oral preparations, may be considered. First-generation antihistamines can help alleviate the itching but may be sedating. Topical corticosteroids can help reduce the erythema and itching. Oral antibiotics alone are sufficient to treat secondary bacterial skin infections.

Eradication

Insecticides containing pyrethroids are used widely as a control measure; however, pyrethroid-resistant bed bug populations have been found in some states. Nonchemical methods to effectively control bed bugs include heating infested rooms to 48°C (118° F) for 1 hour or cooling rooms to –16° C (3° F) for 1 hour; encasing mattresses and box springs with bed bug–excluding covers

and vacuuming, steaming, laundering, and disposing of infested items. Any effective control measure for bed bugs requires support from all residents in affected buildings and ongoing monitoring for infestation from other housing units. In addition, multiple inspections and treatments frequently are needed.

In a relatively recent report, a total of 111 illnesses, representing seven different states, were associated with bed bug–related insecticide use. The majority of the illnesses were of low severity (81%). One fatality was confirmed. The most common factors contributing to illness were excessive insecticide applications, failure to wash or change pesticide-treated bedding, and inadequate notification of pesticide application.[15] To rid the living environment of bed bugs, a professional exterminator should be consulted.

SPIDERS

Of the thousands of species in the United States, only a few pose a threat to humans. Species of the genus *Loxosceles* are the spiders predominantly responsible for necrotic arachnidism in the United States. The two most common species are the black widow spider *(Loxosceles mactans)* and the brown recluse spider *(Loxosceles reclusa)*. Both prefer dark, undisturbed habitats, such as outdoor lavatories, woodpiles, underside of stones, or dark corners of garages and attics. The black widow has a characteristic red hourglass shape on its ventral surface. The brown recluse has a violin-shaped marking over its dorsal surface. In the Pacific Northwest area of the United States, the hobo spider *(Tegenaria agrestis)* produces an envenomation similar to that of the brown recluse spider.[11]

Pathogenesis

Venom from the black widow contains a neurotoxin (α-lactotoxin), not a tissue toxin. The main effect is at the presynaptic membrane of the neuromuscular junction. Venom from the brown recluse spider contains sphingomyelinase D, a phospholipase that induces dermal necrosis and also causes systemic effects through its interaction with red blood cells, platelets, and endothelium.[58] The necrosis caused by the venom depends on neutrophils, but the neutrophils are not activated by the venom itself. Rather, the venom is a potent stimulus for the inflammatory response of endothelial cells, which in turn activates the polymorphonuclear neutrophils to cause tissue destruction.[53]

Clinical Manifestations

The bite of the black widow spider generally is painless, but a target lesion may develop. Within 30 to 120 minutes, some patients complain of regional lymph node tenderness.[75] The primary symptom that follows the bite is muscle cramping, generally involving the abdomen, chest, or back, depending on the location of the bite. Autonomic symptoms, including profuse sweating, nausea, vomiting, and tachycardia, may occur.[58] Hypertension can be a significant problem. The degree of systemic symptoms depends on the amount of venom injected and the number of bites. Severe cases may progress to internal hemorrhage, paralysis, and, rarely, even death.

The brown recluse spider bite may produce a mild or sharp stinging sensation. After several hours, the pain intensifies and itching occurs.[2] Eventually, a blister with surrounding erythema forms at the site. In the course of time, the lesion becomes larger, and central necrosis develops. Systemic signs developing in the first 24 to 48 hours may include headache, fever, nausea, vomiting, and joint pain. Disseminated intravascular coagulation, multiorgan failure, and death have been reported in children.[34,72]

Like that of the brown recluse spider, the bite of the hobo spider may result in a central necrotic area. The most common systemic symptom is a severe headache. Protracted systemic effects, including aplastic anemia, intractable vomiting, and profuse secretory diarrhea, are rare occurrences but may be associated with death.[70]

Treatment

For the patient who has been bitten by a black widow spider, analgesia is the mainstay of care.[58] An antivenin is available, but only for the most severe cases of envenomation, unresponsive to other measures.[7]

For bites of brown recluse spiders, numerous treatments have been used, including hyperbaric oxygen therapy, early excision, antibiotics, dapsone, and corticosteroids. Careful supportive care, including monitoring of electrolytes and renal function, is required if symptoms are severe.[58] Systemic corticosteroid therapy may be of some benefit if the patient has considerable symptoms. Dapsone, an inhibitor of neutrophil function, appears to decrease the development of wound complications and subsequent need for surgical excision.[56]

SCORPIONS

Scorpions, like ticks, mites, and spiders, belong to the Arachnida class of arthropods.[30] The striped bark scorpion *(Centruroides vittatus)* is the most common type of scorpion found in the United States.[29] The most dangerous and perhaps the most lethal scorpion in this country is *Centruroides exilicauda* (former species name, *sculpturatus*), occasionally referred to as the bark scorpion.[31,58] It is indigenous to the desert Southwest, particularly Arizona. Depending on the species, scorpions tend to reside in cracks and crevices, in cupboards and closets, or under loose tree bark. They inject their venom when disturbed or threatened.

Pathogenesis and Clinical Manifestations

C. vittatus produces a neurotoxic venom stored in poison glands in the tip of the tail. The venom of these scorpions is deadly to insect prey and produces a painful sting for humans. For those allergic to the sting, an anaphylactic reaction may occur.[29] The venom from the *Vaejovis*

carolinianus (plain eastern stripeless scorpion) is considered to be mild, similar to that of a honeybee sting. Anecdotal reports indicate that on occasion, a severe reaction may occur.[30] The *C. exilicauda* scorpion produces a neurotoxic venom that can lead to cardiac failure, respiratory paralysis, agitation, paresthesias, hypersalivation, hypertension, and gastrointestinal symptoms. Although tachycardia and hypertension are frequent reactions, opposite effects are a possibility when the venom activates the parasympathetic nervous system.[56] The absence of local tissue reaction is helpful in distinguishing *C. exilicauda* stings from those of other scorpions found in Arizona.[31] Children and elderly adults are most at risk as a result of envenomation from *C. exilicauda*.[29]

Treatment

Most scorpion stings necessitate only nonprescription analgesic medication. For the management of *C. exilicauda* envenomation, atropine can be used to control hypersalivation, although it could be contraindicated when the sting has been produced by scorpions foreign to the United States. In some of those cases, the use of atropine can exacerbate an adrenergic toxicity reaction created by the sting of the scorpion. Treatment of *C. exilicauda* envenomation with an antivenin (available in Arizona only; not approved by the FDA) has been reported to provide relief from the neurotoxic effects. It has the unfortunate potential of causing both immediate and delayed hypersensitivity reactions.[31,58]

MURINE TYPHUS

Also known as endemic typhus fever, murine typhus is a worldwide flea-borne infection of humans. It is caused by *Rickettsial felis* and *Rickettsial typhi*.. The disease most commonly occurs among people in contact with rats or areas where rats live, but also occurs among people who live near or have contact with other small mammals (e.g., opossums and cats). Cases reported in the United States are usually confined to suburban areas of Texas and Southern California, as well as several Hawaii islands. The cat flea *Ctenocephalides felis* is considered to be the primary vector.[18] The disease is often difficult to diagnose, because of its nonspecific clinical presentation. Clinicians are led to believe that patients have other, more commonly seen vector-borne infections, such as dengue fever, ehrlichiosis, West Nile virus, and Rocky Mountain spotted fever, or non–vector-borne infections (e.g., bacterial or viral meningitis disease, Kawasaki disease, or secondary syphilis).[18]

Pathogenesis and Clinical Manifestations

Like other rickettsiae, *R. typhi* is a small (0.4 to 1.3 mm) gram-negative obligate intracellular bacterium depending on hematophagous arthropods (e.g., fleas and ticks) and mammals to maintain its life cycle. *R. typhi* multiplies in the epithelial cells of the flea midgut and subsequently are shed into the feces, which in turn are deposited on the host skin surface while the flea is feeding.[18] Humans and other mammals acquire the bacteria when the flea bites or feces are inoculated into the bite site.[3] *R. felis* has been identified as the cause of typhus-like illness in the United States (Texas), Mexico, France, and Brazil. Molecular studies have found that *R. felis* and *R. typhi* are indistinguishable serologically and require separate identification using PCR analysis.[18]

Following an incubation period of 1 to 2 weeks, the most common symptoms include 3 to 7 days of fever, headache, exanthema, and arthralgia. The exanthema can be variable from patient to patient. It can be macular or maculopapular and nonpruitic, beginning on the trunk, eventually spreading to the arms and legs, but sparing the palms and soles. It appears about 7 days after the onset of fever and remains unchanged for a period of 1 to 4 days.[18]

The mortality rate for murine typhus is low with use of appropriate antibiotics (1%) and about 4 percent without the use of antibiotics. In most cases, it will present as an acute, self-limited illness without complications. However, serious complications have been associated with acute infection, including endocarditis, splenic rupture, and aseptic meningitis.[18]

Diagnosis

Most rickettsial infections are diagnosed using serologic methods such as IFA, often considered to be the most ideal test. Unfortunately, serologic studies are rarely diagnostic when blood specimens are collected at the onset of symptoms. A convalescent-phase specimen, yielding a fourfold increase in titer between the acute- and convalescent phase samples, is usually required to confirm the diagnosis.[18,74]

Other available serologic assays include the indirect immunoperoxidase assay, latex agglutination, line blot, and Western immunoblot. Isolation of rickettsiae from blood cultures is rarely successful and usually necessitates the expertise of a specialized research laboratory. Diagnosis has been made by PCR with the use of peripheral blood, buffy coat, and plasma specimens and occasionally with fresh-frozen or paraffin-imbedded tissue specimens or with arthropod vectors acquired during ecologic surveillance (i.e., cat fleas).[18,74]

Treatment

Antimicrobial testing is not routinely performed on rickettsiae. As with other rickettsial diseases, the preferred antibiotic is a tetracycline, particularly doxycycline for children 9 years of age and older. The course of treatment is usually a week or less, limiting the risk for dental staining.[13,73]

NEW REFERENCES SINCE THE SIXTH EDITION

3. Azad AF. Epidemiology of murine typhus. *Ann Rev Entomol* 1990;**35**:553–69.
5. Bell EA. Pharmacotherapy update of head lice. *Infect Dis Child* 2011;**24**:26.

14. Centers for Disease Control and Prevention, Environmental Protection Agency. *Joint statement on bed bug control in the United States*. Atlanta: US Department of Health and Human Services; 2010.
15. Centers for Disease Control and Prevention. Acute illnesses associated with insecticides used to control bed bugs: seven states, 2003-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1269–74.
17. Chosidow O, Giraudeau B, Cottrell J, et al. Oral ivermectin versus malathion lotion for difficult-to-treat head lice. *N Engl J Med* 2010;**362**:896–905.
18. Civen R, Ngo V. Murine typhus: an unrecognized suburban vectorborne disease. *Clin Infect Dis* 2008;**46**:913–18.
22. Currie BJ, McCarthy JS. Permethrin and ivermectin for scabies. *N Engl J Med* 2010;**362**:717–25.
23. DeNyse A. Improved strategies necessary to combat bed bug resurgence. *Infect Dis Child* 2012;**25**:1, 10, 11.
24. DiMarcantonio T. Incidence rates of certain tick-borne diseases on the rise in US. *Infect Dis Child* 2012;**25**:9–13.
25. *Do My Own Pest Control*. http://www.DoMyOwnPestControl.com.
36. Golant AK, Levitt JO. Scabies: a review of diagnosis and management based on mite biology. *Pediatr Rev* 2012;**33**:e48–59.
51. Marty FM, Whiteside KR. Myiasis due to *Dermatobia hominis* (human botfly). *N Engl J Med* 2005;**352**:e21.
68. Vander Stichele RH, Dezeure EM, Bogaert MG. Systematic review of clinical efficacy of topical treatment for head lice. *BMJ* 1995; **311**:604–8.
73. Volovitz B, Shkap R, Amir J, et al. Absence of tooth staining with doscycycline treatment in young children. *Clin Pediatr* 2007;**46**: 121–6.
74. Walker DH, Bouyer DH. Rickettsia. In: Murray PR, Barron EJ, et al, editors. Manual of clinical biology, **vol. 1**. 8th ed. Washington, DC: American Society for Microbiology Press; 2003. p. 1005–104.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abramowicz M. Malathion for treatment of head lice. *Med Lett Drugs Ther* 1999;**41**:73–4.
2. Allen C. Arachnid envenomations. *Emerg Med Clin North Am* 1992;**10**:269–98.
3. Azad AF. Epidemiology of murine typhus. *Ann Rev Entomol* 1990; **35**:553–69.
4. Badia L, Lund V. Vile bodies: An endoscopic approach to nasal myiasis. *J Laryngol Otol* 1994;**108**:1083–5.
5. Bell EA. Pharmacotherapy update of head lice. *Infect Dis Child* 2011;**24**:26.
6. Bernstein B, Mihan R. Hospital epidemic of scabies. *J Pediatr* 1973;**83**:1086–7.
7. Bond GR. Snake, spider and scorpion envenomation in North America. *Pediatr Rev* 1999;**20**:147–50.
8. Brouqui D, Lascola B, Roux V, Raoult D. Chronic *Bartonella quintana* bacteremia in homeless patients. *N Engl J Med* 1999;**340**: 184–9.
9. Burgess I. *Sarcoptes scabiei* and scabies. *Adv Parasitol* 1994;**35**: 235–92.
10. Burkhart CG, Burkhart CN. Head lice therapies revisited. *Dermatol Online J* 2006;**12**:3.
11. Centers for Disease Control and Prevention. Necrotic arachnidism: Pacific Northwest, 1988-1996. *MMWR Morb Mortal Wkly Rep* 1996;**45**:433–6.
12. Centers for Disease Control and Prevention. Outbreak of pruritic rashes associated with mites: Kansas, 2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:952–5.
13. Centers for Disease Control and Prevention. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichiosis, and anaplasmosis—United States. *MMWR Recomm Rep* 2006;**55**(RR-4):1–29.
14. Centers for Disease Control and Prevention, Environmental Protection Agency. *Joint statement on bed bug control in the United States*. Atlanta: US Department of Health and Human Services; 2010.
15. Centers for Disease Control and Prevention. Acute illnesses associated with insecticides used to control bed bugs: seven states, 2003-2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1269–74.
16. Chosidow O. Scabies and pediculosis. *Lancet* 2000;**355**:819–26.
17. Chosidow O, Giraudeau B, Cottrell J, et al. Oral ivermectin versus malathion lotion for difficult-to-treat head lice. *N Engl J Med* 2010;**362**:896–905.
18. Civen R, Ngo V. Murine typhus: an unrecognized suburban vectorborne disease. *Clin Infect Dis* 2008;**46**:913–18.
19. Cooper BJ, Spence I. Temperature-dependent inhibition of evoked acetylcholine release in tick paralysis. *Nature* 1976;**263**:693–5.
20. Cunningham DG, Zonga JR. Myiasis of the external auditory canal. *J Pediatr* 1974;**84**:856–8.
21. Cupp EW. Biology of ticks. *Vet Clin North Am Small Anim Pract* 1991;**21**:1–26.
22. Currie BJ, McCarthy JS. Permethrin and ivermectin for scabies. *N Engl J Med* 2010;**362**:717–25.
23. DeNyse A. Improved strategies necessary to combat bed bug resurgence. *Infect Dis Child* 2012;**25**:1, 10, 11.
24. DiMarcantonio T. Incidence rates of certain tick-borne diseases on the rise in US. *Infect Dis Child* 2012;**25**:9–13.
25. *DoMyOwnPestContrl*. http://www.DoMyOwnPestControl.com.
26. Donat JR, Donat JF. Tick paralysis with persistent weakness and electromyographic abnormalities. *Arch Neurol* 1981;**38**:59–61.
27. Elgart ML. Flies and myiasis. *Dermatol Clin* 1990;**8**:237–44.
28. Elgart ML. Scabies. *Dermatol Clin* 1990;**8**:253–63.
29. Elston DM. What's eating you? Common striped bark scorpion *(Centruroides vittatus)*. *Cutis* 2006;**78**:233–4.
30. Elston DM. What's eating you? Plain eastern stripeless scorpion *(Vaejovis carolinianus)*. *Cutis* 2006;**78**:393–4.
31. Elston DM, Stockwell S. What's eating you? *Centruroides exilicauda*. *Cutis* 2002;**69**:19, 20.
32. Felz MW, Smith CD, Swift TR. A six-year-old girl with tick paralysis. N. *Engl J Med* 2000;**342**:90–4.
33. Fernandez N, Torres A, Ackerman AB. Pathologic findings in human scabies. *Arch Dermatol* 1977;**113**:320–4.
34. Ginsburg CM, Weinberg AG. Hemolytic anemia and multiorgan failure associated with localized cutaneous lesion. *J Pediatr* 1988; **112**:496–9.
35. Goates BM, Atkin JS, Wilding KG, et al. An effective nonchemical treatment for head lice: a lot of hot air. *Pediatrics* 2006;**118**: 1962–70.
36. Golant AK, Levitt JO. Scabies: a review of diagnosis and management based on mite biology. *Pediatr Rev* 2012;**33**:e48–59.
37. Guillozet N. Diagnosing myiasis. *JAMA* 1980;**244**:698–9.
38. Hall M, Wall R. Myiasis of humans and domestic animals. *Adv Parasitol* 1995;**35**:257–334.
39. Haller JS, Fabara JA. Tick paralysis: case report with emphasis on neurological toxicity. *Am J Dis Child* 1972;**124**:915–17.
40. Hurwitz S. Scabies in babies. *Am J Dis Child* 1973;**126**:226–8.
41. Hurwitz S, editor. Insect Bites and Parasitic Infestations. In: Hurwitz S, editor. *Clinical pediatric dermatology*. 2nd ed. Philadelphia: W. B. Saunders; 1993. p. 405–12.
42. Iannini PB, Brandt D, La Force FM. Furuncular myiasis. *JAMA* 1975;**233**:1375–6.
43. Jupp PR, Pircell M, Shapiro M, et al. Attempt to transmit hepatitis B virus to chimpanzees by arthropods. *S Afr Med J* 1991;**79**: 320–2.
44. Kaire GH. Isolation of tick paralysis toxin from *Ixodes holocyclus*. *Toxicon* 1996;**4**:91–7.
45. Kalelioglu M, Akturk G, Akturk F, et al. Intracerebral myiasis from *Hypoderma bovis* larva in a child. *J Neurosurg* 1989;**71**:929–31.
46. Kincaid JC. Tick bite paralysis. *Semin Neurol* 1990;**10**:32–4.
47. Lagos JC, Thies RE. Tick paralysis without muscle weakness. *Arch Neurol* 1969;**21**:471–4.
48. Lee B, Croth P. Scabies: Transcutaneous poisoning during treatment. *Pediatrics* 1977;**59**:643.
49. Lord WD, DiZinno JA, Wilson MR, et al. Isolation, amplification and sequencing of human mitochondrial DNA obtained from human crab louse, *Pthirus pubis* (L), blood meals. *J Forensic Sci* 1998;**43**:1097–100.
50. Macias EG, Graham AJ, Green M, et al. Cutaneous myiasis in South Texas. *N Engl J Med* 1973;**289**:1239–41.
51. Marty FM, Whiteside KR. Myiasis due to *Dermatobia hominis* (human botfly). *N Engl J Med* 2005;**352**:e21.
52. Meinking TL, Taplin D, Hermida JL, et al. The treatment of scabies with ivermectin. *N Engl J Med* 1995;**333**:26–30.
53. Patel KD, Modur V, Zimmerman GA, et al. The necrotic venom of the brown recluse spider induces dysregulated endothelial cell–dependent neutrophil activation: differential induction of GM-CSF, IL-8, and E-selectin expression. *J Clin Invest* 1994;**94**: 631–42.
54. Pearlman DL. A simple treatment for head lice: dry-on, suffocation-based pediculicide. *Pediatrics* 2004;**114**:e275–9.
55. Rasmussen JE. Scabies. *Pediatr Rev* 1994;**15**:110–14.
56. Rees RS, Altenbern DP, Lynch JB, et al. Brown recluse spider bites: a comparison of early surgical excision versus dapsone and delayed surgical excision. *Ann Surg* 1985;**202**:659–63.
57. Schachner LA. Treatment resistant head lice: alternative therapeutic approaches. *Pediatr Dermatol* 1997;**14**:409–10.
58. Schexnayder SM, Schexnayder RE. Bites, stings, and other painful things. *Pediatr Ann* 2000;**29**:354–8.
59. Schmitt N, Bowmer EJ, Gregson JD. Tick paralysis in British Columbia. *Can Med Assoc J* 1969;**100**:417–21.
60. Shaw PK, Juranek DD. Recent trends in scabies in the United States. *J Infect Dis* 1976;**134**:414–16.
61. Sherman RA. Wound myiasis in urban and suburban United States. *Arch Intern Med* 2000;**160**:2004–14.
62. Sonenshine DE, Azad AF. Ticks and mites in disease transmission. In: Strickland GT, editor. *Hunter's tropical medicine*. 7th ed. Philadelphia: W. B. Saunders; 1991. p. 971–81.
63. Sonenshine DE: *Biology of ticks*, **vol. 1**. New York: Oxford University Press; 1992.
64. Spach DH, Liles WC, Campbell GL, et al. Medical progress: tick-borne diseases in the United States. *N Engl J Med* 1993;**329**: 936–47.
65. Swift TR, Ignacio OJ. Tick paralysis: electrophysiologic studies. *Neurology* 1975;**25**:1130–3.
66. Ter Poorten MC, Prose NS. The return of the common bedbug. *Pediatr Dermatol* 2005;**22**:183–7.
67. Todd JL. Does a human tick-borne disease exist in British Columbia? *Can Med Assoc J* 1912;**2**:686.
68. Vander Stichele RH, Dezeure EM, Bogaert MG. Systematic review of clinical efficacy of topical treatment for head lice. *BMJ* 1995; **311**:604–8.

69. Vaughn JA, Azad AF. Patterns of erythrocyte digestion by blood-sucking insects: Constraints on vector competence. *J Med Entomol* 1993;**30**:214–16.
70. Vest DK. Protracted reactions following probable hobo spider *(Tegenaria agrestis)* envenomation. *Abstract Am Arachnol* 1993;**48**:10.
71. Vetter RS, Visscher PK. Bites and stings of medically important venomous arthropods. *Int J Dermatol* 1998;**37**:481–96.
72. Vorse H, Seccareccio P, Woodruff K, et al. Disseminated intravascular coagulopathy following fatal brown spider bite. *J Pediatr* 1972;**80**:1035–7.
73. Volovitz B, Shkap R, Amir J, et al. Absence of tooth staining with doscycycline treatment in young children. *Clin Pediatr* 2007;**46**: 121–6.
74. Walker DH, Bouyer DH. Rickettsia. In: Murray PR, Barron EJ, et al, editors. Manual of clinical biology, **vol. 1**. 8th ed. Washington, DC: American Society for Microbiology Press; 2003. p. 1005–104.
75. Walter FG, Bilden EF, Gibly RL. Envenomations. *Crit Care Clin* 1999;**15**:353–86.
76. Zumpt F. *Myiasis in man and animals in the Old World*. London: Butterworths; 1965.

SECTION **XXIII**

GLOBAL HEALTH

CHAPTER 230

Global Health

Holly Corwin • Gail J. Harrison

The keys to success as a global health professional are compassion, competence, critical thinking, and creative adaptability.
Chunhuei Chi

This chapter on Global Health will provide an introduction to the definition, evolution, disciplinary perspectives, interventions, and contemporary challenges associated with global health. For information on specific health conditions and infectious diseases of global concern, such as human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS), malaria, tuberculosis, parasites, or specific circumstances, such as international adoptions, the reader is referred to the individual chapters for each of these entities.

DEFINITION OF GLOBAL HEALTH

The term *global health* is widely used and very popular among academic institutions, humanitarian and government agencies, nongovernment organizations (NGOs), and nonprofit organizations around the world.[2,3] Global health is increasingly mentioned as an area of public health and medical professional interest, as well as a philanthropic goal, but is rarely defined by a definition agreed on by even the most influential health organizations.[2,3,20] Many organizations and institutions have used the term *global health* as a state of being or condition, as a goal, or as a field of study that relates to education, research, and practice. Uniformity of definition is desirable, because it sets the foundation for collaborative efforts of global purpose, clear objectives for action, and reliable instruments for measuring achievements. Fragments of public health and international health definitions, which evolved from hygiene and tropical medicine, have been used to assemble a concept of global health that is becoming universally accepted.[4-6] Global health often overlaps in the fundamental purpose and initiatives of both public and international health and can be seen as evolving from both disciplines. An understanding of the history and evolution of definitions of health, public health, and international health is helpful in understanding the overall objective of global health.[20]

The World Health Organization (WHO), one of the leading authorities for health, has defined health as "a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity."[7] This definition was the basis from which the WHO worked their evolving and emerging projects toward the goals to improve the overall health of the world. This definition is broad and all-inclusive and includes the larger picture of overall health for one person, or a whole community, and covers not only the administration of medical attention, but also prevention of disease through public health, policy, mental well-being, research, and many more factors influential in health.

The current public health concepts emerged in mid-19th–century Europe and the United States. During this time, medical advances in the cause, management, and prevention of infectious diseases and the growth of social reform movements helped establish the discipline of public health with four main concepts: (1) decision making based on data and evidence gathered through vital statistics, surveillance, outbreak investigations, and scientific method; (2) focus on populations or groups rather than individuals; (3) action rooted in social justice and equity; and (4) prevention of disease rather than treatment or cure of established disease. In 1988, the Institute of Medicine reconfirmed public health as a preventive discipline that employs a community approach and involves multiple partners working toward a common good of public health. The American Public Health Association (APHA) defines public health in contemporary terms as "the practice of preventing disease and promoting good health within groups of people, from small communities to entire countries" including the well-being of the population of a specific country or community within a country.[4] Public health practice emerged from the works of many pioneering scientists and physicians and the early discoveries about quarantine, sanitation, and housing; public knowledge on sexually transmitted infections and diseases; and vaccinations. Eventually, the practice of public health evolved from early efforts of individuals to organized efforts among societies and nations.

The field of international health was further developed from public health, hygiene, and tropical medicine disciplines, as medical missionaries, scientists, and other humanitarians traveled and shared their discoveries and new ideas. The specific focus of international health and international medicine currently lies in the differences among countries, peoples, and regions. International health applies the well-established public health principles of industrialized countries to assist the health

TABLE 230-1 **Comparison of Global, International, and Public Health**

Comparison Factors	Global Health	International Health	Public Health
Geographic reach	Focuses on issues that directly or indirectly affect health but that can transcend national boundaries.	Focuses on health issues of countries other than one's own, especially those of low and middle income.	Focuses on issues that affect the health of the population of a particular community or country.
Level of cooperation	Development and implementation of solutions often requires global cooperation.	Development and implementation of solutions usually requires binational cooperation.	Development and implementation of solutions does not usually require global cooperation.
Individual or populations	Embraces both prevention in population and clinical care of individuals.	Embraces both prevention in populations and clinical care of individual.	Mainly focused on prevention programs for populations.
Access to health	Health equity among nations and for all people is a major objective.	Seeks to help people of other nations.	Health equity within a nation or community is a major objective.
Range of disciplines	Highly interdisciplinary and multidisciplinary within and beyond health sciences.	Embraces a few disciplines but has not emphasized multidisciplinary.	Encourages multidisciplinary approaches, particularly within health sciences and with social sciences.

From Koplan JP, Bond TC, Merson MH, et al. Towards a common definition of global health. *Lancet* 2009;373:1993-1995.

problems and challenges of low-income populations in developing countries, through foreign aid activities and missionary work (Table 230-1). It often includes "diseases of the developing world" and "health work abroad," with a focus on infectious and tropical diseases, water and sanitation, malnutrition, and maternal–child health.[4-6] Previously, *international health* and *global health* were considered interchangeable terms, whereas now most experts recognize them as distinct disciplines and practices.[20]

Global health transcends the individual nations and borders and emphasizes worldwide improvement of health, reduction of disparities, and protection against threats to health of the people of all nations (see Table 230-1). Global health focuses on the similarities throughout the world, and the issues that cross nations.[20] It blurs the borders to create a sense of unity and understanding of how people, cultures, and the environment interact and affect each other. It emphasizes both the individual and the population and strives for equity among people of all nations. It also is highly interdisciplinary and multidisciplinary in its approach to problem solving and strives to provide creative and sustainable solutions for resource management in resource-poor regions (see Table 230-1).

Current global health issues involve not only the direct health impact, but also other disciplinary perspectives that indirectly influence health, such as climate change and natural disasters, economics, politics, conflict, war, migration, food crisis, energy crisis, and neglected diseases.[1] The political perspective of global health emphasizes the influences of war and economy on the health of the world. The educational and research perspectives of global health are evident when taught as curriculum in both medical and sociologic disciplines and are an educational goal of many institutions of higher learning. The epidemiologic perspective of global health identifies and follows trends of major global health threats and partners with the medical perspective to describe the pathologic factors, clinical manifestations, diagnosis, treatment, and prevention of these diseases. From an economic perspective, global health emphasizes cost-effectiveness and cost-benefit approaches for population health and includes the measurement of health burden, including life expectancy; adult, maternal, infant, and child mortality; disability; and quality of life.[11-13] Many experts also view global health from the ethical and societal perspective and include educational disparities, reproductive health, violence against women and children, and child labor and hawking laws as a global health concern.

HISTORY AND EVOLUTION OF GLOBAL HEALTH

The beginnings of the contemporary concepts surrounding global health evolved from European colonization and colonial enterprises. Colonialism affected the public health of the world at the time, through the introduction and rapid spread of non-native diseases, such as measles, smallpox, and malaria to previous nonimmune indigenous people in Saint Domingue, Haiti and the Americas. In addition, the extraction of wealth prevented the indigenous people from exiting the cycle of poverty and disease. Furthermore, colonization of Africa, India, and the Americas by British and other Europeans created urban centers and crowding that bred infectious diseases such as cholera, tuberculosis, and smallpox and fueled the HIV/AIDS epidemic. In contemporary terms, international spread of disease, such as influenza pandemics, through international travel, or, potentially, by bioterrorism is a global health concern.

Medicine underwent a dramatic change in the late 19th century with the discovery of the germ theory of

disease and the awareness of it in the public health consciousness.[4] Proponents of the germ theory included Louis Pasteur, the father of the germ theory and early vaccinology, Robert Koch, father of Koch's postulates to establish a list of criteria to determine the cause of a disease, and John Snow, the father of modern epidemiology, who discovered the transmissibility of cholera and its prevention. In 1902, a model international collaboration to fight yellow fever created the Pan American Health Organization. After World War I, the League of Nations Health Committee changed its focus on infectious diseases to include nutrition and maternal and infant health. In 1947, the WHO was created by the United Nations to serve as a single global entity charged with fostering collaboration among member nations to work toward the new definition of health as a state of well-being and not merely the absence of disease. During this time the social determinants of health began to coexist with the advent of health care delivery and treatment of infections and other diseases.

This history of global health also has included disease eradication efforts. Smallpox, the first global disease to be eradicated, was declared eradicated in 1980, through the successful efforts of the WHO Smallpox Eradication Campaign. The achievement of smallpox eradication resulted not only from the possession of an effective smallpox vaccine but also through global cooperation that used intensive surveillance, disease reporting, and aggressive focus on immunization in every local community. Malaria, a disease demonstrated first by Ronald Ross to be transmitted by mosquitoes, was targeted for eradication by the Malaria Eradication Campaign of the WHO, using basic strategies of personal protection, mosquito reduction, and treatment of malaria disease. Unfortunately, despite these effective means of prevention and treatment of malaria, malaria remains a global health problem because most rural or resource-poor communities have not adopted these preventive and curative health measures. This failure demonstrates the complexity of global health problems and highlights the interrelationships among health, infrastructure, culture, politics, and economics and emphasizes the importance of culturally sensitive programs. Other infectious diseases also now targeted for global eradication programs include polio, trachoma, tuberculosis, rabies, and HIV/AIDS.[30]

In the 1970s, a movement emphasizing primary health care in rural communities became increasingly popular. An example of such a movement was the "barefoot doctors" in China during the Cultural Revolution, who emphasized rural care and community-based health programs. In 1971, the NGO Doctors Without Borders was founded by French physicians who understood the importance of sociopolitical determinants of health. In 1978, the International Conference on Primary Health Care took place in Alma-Ata, the capital of the Soviet Republic of Kazakhstan, in the Soviet Union. From this conference emerged the Declaration of Alma-Ata, which stated that health was a fundamental human right and an important worldwide social goal and supported "Health for All by the Year 2000." It also emphasized the importance of culturally sensitive, local, community-based programs as opposed to top-down vertical programs that were previously used in the smallpox and malaria eradication campaigns.

From the broad concepts of primary health brought forth from the Declaration of Alma-Ata came selective and targeted goals for primary health care implementation. These programs included goals for measles and diphtheria-pertussis-tetanus vaccination, treatment of malaria, oral rehydration for diarrhea in children, encouragement of breast-feeding, and family planning. The goals, implementation, and success of targeted and focused strategies on specific disease prevention and treatment could also then be measured and assessed for political and philanthropic funding. Donations for global health were estimated to be over $10 billion dollars in 2000, and governments, agencies, companies, and individuals who fund global health initiatives now increasingly demand performance data in return for their financial aid.

Modern global health challenges include the ongoing balancing act between preventive public health initiatives and medical treatment interventions. A diagonal approach that synergistically incorporates both types of interventions is likely to be most successful. In addition, as industrialized development and economic progress spreads globally, the challenge will be to emphasize the importance of local cultural sensitivity and avoid resource exploitation that may occur at the expense of the health of the local people. Global health indicators to monitor changes in the health of various populations are being advocated for both developed and developing countries.[11-13] These indicators should be well defined and applied internationally in a uniform manner. They also should be valid, reliable, and readily interpretable measurements that can be feasibly gathered and affordably ascertained even in developing countries, and they should be useful to decision makers who can easily act on the indicators at the local, national, and international level. Health indicators in developed countries often reflect lifestyles and individual behavior, such as diet, exercise, and smoking, whereas health indicators in developing countries focus on morbidity, mortality, and severe disease precursors of both of these indicators.[21,22] Global health indicators are a contemporary challenge, especially in resource-poor areas with little or no access to electrical power and where records of birth, growth, lifestyle, medical illness, or death are not systematically recorded and kept.[21,22]

Global health communication, public health awareness, and humanitarian efforts are also now facilitated through Internet use, mobile cellular phones, mobile applications, and social media. Through the power of the periphery that social media and mobile cell phones provide, people are able to expand their community and access not only information but also health care and transportation. In addition, many global health care organizations use cellular phones in remote or resource-poor locations to collect research data for social health projects or instantaneously log information into surveillance systems for outbreak investigations.

SOCIAL DETERMINANTS OF HEALTH

The social determinants of health reflect where people are born, grow, live, work, and age and represent a powerful influence on the global health and the global disease burden of all nations.[35] Disease is often directly and indirectly influenced by other social, political, economic, and environmental factors, which in turn are responsible for ongoing health inequalities or acute and urgent health crises. These social determinants of health include broad and general political, socioeconomic, cultural, and environmental conditions; social and community networks; individual and family lifestyle factors; and individual characteristics such as age, gender, and genetic or constitutional factors (Table 230-2).[35]

TABLE 230-2 **Social, Environmental, and Individual Determinants of Health**

Socioeconomic, Political, and Cultural
Government
Politics
War
Refugees and displaced populations
Food security
Global connectivity through travel and Internet
Epidemics and pandemics both facilitated by travel and aborted by information exchange
Environmental
Global warming
Influences ecology and vector life cycles for hemorrhagic fevers
Natural disasters
Earthquakes, tsunami, hurricanes, volcanoes, floods, drought
Pollution
Industrial, fuel, wood-burning smoke
Water and sanitation
Agriculture and food production
Social and Community
Personal economic status
Housing or homelessness
Access to electrical power
Education opportunities, language, literacy
Child labor and child hawking practices
Transportation
Gender and age discrimination
Violence
Overcrowding
Access to health care and immunizations
Family and Individual Lifestyle
Employment or unemployment
Economic status
Chronic stress
Healthy food choices
Exercise
Smoking
Alcohol use or addiction
Drugs and addiction
Reproductive health and birth control
Individual Constitutional Factors
Prematurity and birth characteristics
Age
Gender
Physical health conditions
Sickle-cell hemoglobinopathy
Anemia
Malnutrition
Other chronic conditions
Mental health conditions

MILLENNIUM DEVELOPMENT GOALS FOR GLOBAL HEALTH

The Millennium Development Goals (MDGs) are eight broad global health goals established and agreed on by all U.N. Member States and 23 supporting organizations of the world during a U.N. summit in 2000 (Table 230-3).[31,32] The United Nations Millennium Declaration was adopted as a commitment to address the challenges facing human health globally and promote global development by improving social and economic conditions in the poorest countries, the "bottom billion," by the year 2015.[34,36,37] Goal four "to reduce child mortality" and goal five "to improve maternal health" specifically address pediatrics, and the sixth goal "to combat HIV-AIDS, malaria, and other diseases" specially addresses infectious diseases (Fig. 230-1).[19,25,30,32]

NEGLECTED TROPICAL DISEASES AND THE BOTTOM BILLION

The neglected tropical diseases (NTDs) refer to a group of 17 infections, 13 of which are bacterial and parasitic conditions, that are among the most common chronic infections and disabling conditions affecting the world's poorest and most disadvantaged people[9] (Tables 230-4 and 230-5). Whereas much global health attention has been paid to HIV/AIDS, malaria, and tuberculosis, as a result of the United Nations Millennium Declaration, the NTDs have not received as much attention or funding.[8,16,19] Nonetheless, eradication of NTDs would also support success toward the Declaration's global health goals. These NTDs are all infectious diseases that commonly infect infants, children, and adolescents.[14,17] Unlike acute infections, which often cause rapid mortality in developing countries, the NTD infections cause high morbidity but low mortality and result in chronic conditions that affect individuals for a lifetime. These affected individuals are often stigmatized or disfigured or unable to find work and income, further attributing to their cycle of poverty.

The NTDs are endemic in developing countries and commonly affect the "bottom billion," a term that refers to the poorest 2.7 billion people living in rural regions and poor urban areas, on less than U.S. $2.00 per day. By causing adverse effects on child development and learning, pregnancy outcome, and agricultural productivity, the NTDs are emerging as one of the most important groups of infections that trap the poorest people living in sub-Saharan Africa, Asia, and the Americas in a cycle of poverty and destitution. The NTDs both occur in the setting of poverty and promote and perpetuate

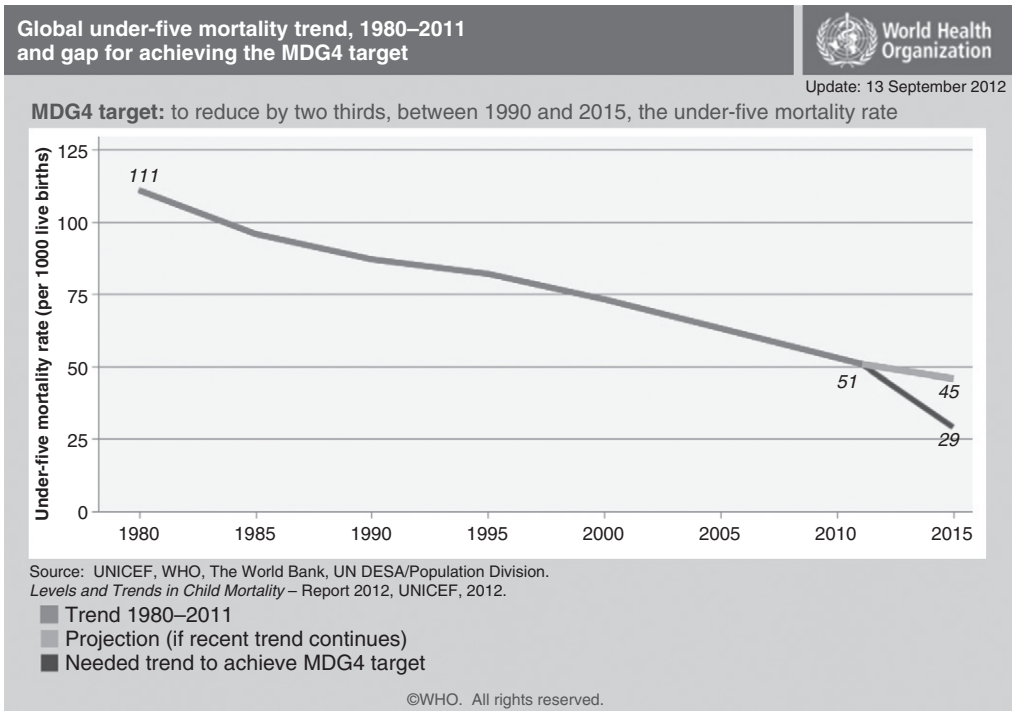

FIGURE 230-1 ■ Millennium Developmental Goal 4 to reduce child mortality. (From World Health Organization. Child health. Available at: http://www.who.int/gho/child_health/en/index.html.)

TABLE 230-3 Millennium Development Goals Adopted by World Leaders at the United Nations in 2000 To Be Achieved by 2015

1. Eradicate extreme poverty and hunger
   a. Halve the proportion of people living on less than U.S. $1 per day
   b. Achieve decent employment
   c. Halve the proportion of people suffering from hunger
2. Achieve universal primary education
   a. Allowing all children, regardless of gender, to complete a full course of primary schooling
3. Promote gender equality and empower women
   a. Eliminate gender disparity in primary and secondary education
4. Reduce child mortality
   a. Reduce the under 5 mortality rate by two thirds from the year 1990
5. Improve maternal health
   a. Reduce the maternal mortality rate by three fourths from the year 1990
   b. Achieve universal access to reproductive health
6. Combat HIV/AIDS, malaria, and other diseases
   a. Begin to reverse the spread of HIV/AIDS by 2015
   b. Achieve universal access to treatment of HIV/AIDS by 2010
   c. Begin to reverse the incidence of malaria and other major diseases by 2015
   d. Reduce tuberculosis worldwide
7. Ensure environmental sustainability
   a. Integrate the principles of sustainable development into country policy programs and reverse the loss of environmental resources
   b. Reduce biodiversity loss and achieve a significant reduction in the rate of loss
   c. Halve the proportion of the population without sustainable access to safe drinking water and basic sanitation
   d. Achieve a significant improvement in the lives of at least 100 million slum-dwellers
8. Develop a global partnership for development

TABLE 230-4 Neglected Tropical Diseases Caused by Bacteria or Parasites of Major Importance to Global Health

Ascariasis
Hookworm
Trichuriasis
Lymphatic filariasis
Onchocerciasis (river blindness)
Dracunculiasis (guinea worm disease)
Schistosomiasis
Chagas disease (American trypanosomiasis)
Human African trypanosomiasis (African sleeping sickness)
Leishmaniasis
Buruli ulcer (*Mycobacterium ulcerans* infection)
Leprosy (Hansen disease)
Trachoma

TABLE 230-5 Other Neglected Tropical Diseases of Importance to Global Health

Dengue fever
Cysticercosis
Echinococcosis
Foodborne trematode infections (i.e., clonorchiasis, fascioliasis, opisthorchiasis, paragonimiasis)
Yaws
Rabies
Leptospirosis
Strongyloidiasis
Scabies

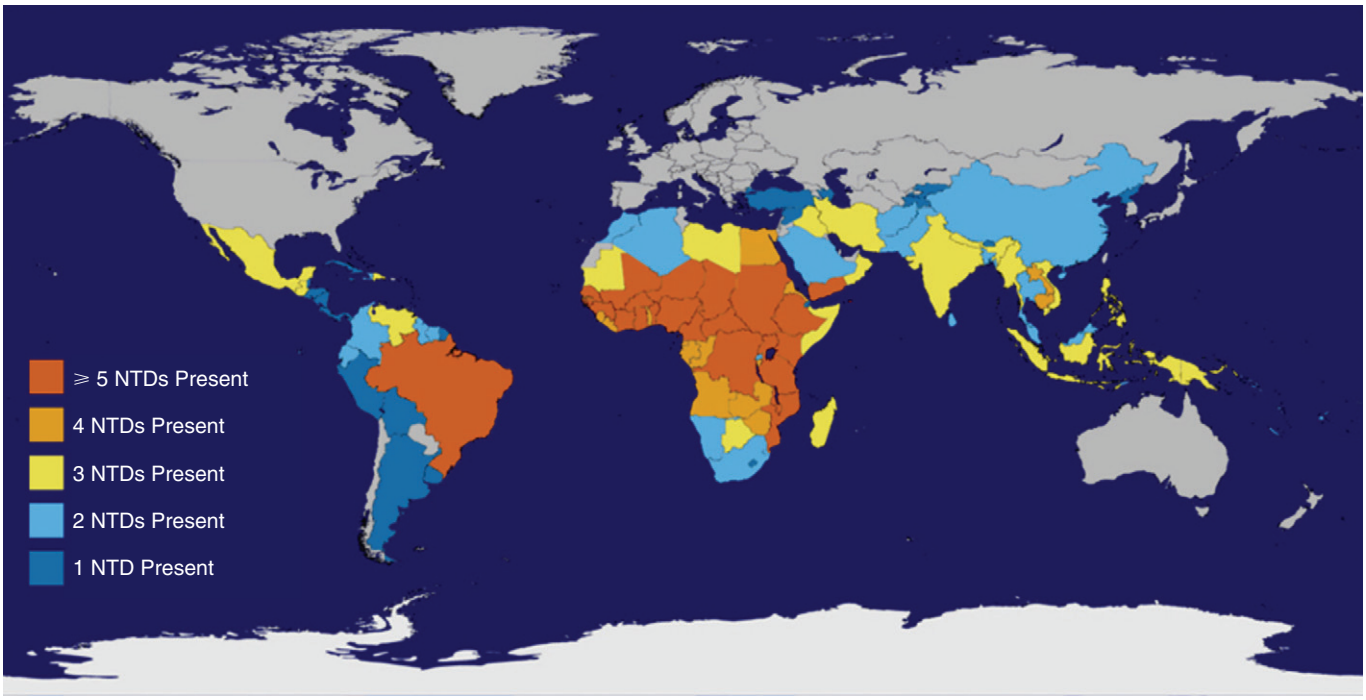

FIGURE 230-2 ■ Global map of six of the most common neglected tropical diseases burden (guinea worm disease, lymphatic filariasis, onchocerciasis, schistosomiasis, soil-transmitted helminths, and trachoma). (See companion Expert Consult website for color version.) (From Centers for Disease Control and Prevention. Neglected tropical diseases. Available at: http://www.cdc.gov/globalhealth/ntd/diseases/ntd-worldmap-static.html.)

poverty.[14-17] Caught in a poverty trap of dependence, debt, and disease, the bottom billion are directly affected by NTDs, economic failures, and lack of educational opportunities.[24] According to Paul Collier, director of the Centre for the Study of African Economies at Oxford University, these countries of the bottom billion have fallen into at least one of four possible traps: conflict, natural resources, landlocked next to bad neighboring countries, and bad governance in a small country.[24] This population lives within the rural regions and poor urban areas of roughly 58 countries, including Afghanistan, Angola, Azerbaijan, Benin, Bhutan, Bolivia, Burkina Faso, Burundi, Cambodia, Cameroon, Central African Republic, Chad, Comoros, Democratic Republic of the Congo, Republic of the Congo, Ivory Coast, Djibouti, Equatorial Guinea, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Guinea-Bissau, Guyana, Haiti, Kazakhstan, Kenya, North Korea, Kyrgyz Republic, Lao Peoples' Democratic Republic, Lesotho, Liberia, Madagascar, Malawi, Mali, Mauritania, Moldova, Mongolia, Mozambique, Myanmar, Nepal, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Somalia, Sudan, Tajikistan, Tanzania, Togo, Turkmenistan, Uganda, Uzbekistan, Yemen, Zambia, and Zimbabwe.

The majority of people in the bottom billion are affected by at least one of the NTDs, often leading to long-term sequelae and disability.[24] Seven NTDs are of particular significance because even though they have high prevalence the ability to control the infection does exist. These include the soil-transmitted helminthes (i.e., hookworm, ascariasis, trichuriasis), lymphatic filariasis, schistosomiasis, and diseases causing blindness—trachoma, and onchocerciasis (Figs. 230-2 to 230-4).

FIGURE 230-3 ■ Woman from Republic of Sierra Leone, West Africa with onchocerciasis or "river blindness," a parasitic disease caused by *Onchocerca volvulus* and spread by the black simulian fly, which breeds in fast-flowing rivers. It is a leading cause of blindness and endemic in sub-Saharan Africa, as well as in parts of Yemen and Central and South America. The social implications of the disease affect not only adult independence and productivity but also children, who are denied access to education because they must stay at home and act as caretakers for the blind older relatives. (See companion Expert Consult website for color version.) (Courtesy Olivier Asselin, Sabin Vaccine Institute, Baylor College of Medicine, and Texas Children's Hospital, Houston, Texas.)

These diseases occur primarily in rural areas and in some poor urban settings of low-income countries in sub-Saharan Africa, Asia, and Latin America. Although the NTDs can affect anyone at any age, the seven most common diseases listed earlier often infect children, leading to a lifetime of disfigurement and disability,

FIGURE 230-4 ■ Toddler from Republic of Sierra Leone, West Africa, with distended abdomen and malnutrition caused by soil-transmitted helminth parasitic disease, one of the neglected tropical diseases. Disabilities associated with this disease include anemia and growth and cognitive delays. (See companion Expert Consult website for color version.) (Courtesy Olivier Asselin, Sabin Vaccine Institute, Baylor College of Medicine, and Texas Children's Hospital, Houston, Texas.)

which in turn leaves them without the ability to work effectively and thus caught in the poverty trap. Along with childhood disfigurement, other issues affecting quality of life are impaired childhood growth and development, adverse outcomes of pregnancy, and reduced productive capacity. Seven of the NTDs (lymphatic filariasis, onchocerciasis, schistosomiasis, trachoma, and the soil-transmitted helminthes *Ascaris*, hookworm and, whipworm) are considered "tool ready" to implement massive drug administration (MDA), as well as diagnostic and follow-up surveillance strategies. These targeted MTDs are the focus of many of the contemporary global and health efforts. For example, since the beginning of the guinea worm eradication program, the number of cases of guinea worm disease has decreased from an estimated 3.5 million cases in 20 countries in 1986 to fewer than 2000 cases in 2010. Other NTDs (i.e., Buruli ulcer, Chagas disease, cysticercosis, dengue fever, echinococcosis, fascioliasis, human African trypanosomiasis, leishmaniasis, leprosy, rabies, and yaws) are considered "tool deficient" because they lack one or more of the components necessary to deliver an effective MDA campaign.

FUTURE DIRECTION OF GLOBAL HEALTH

The future directions of global health include collaborative progress by medical and public health teams, industry, world leaders, governments, NGOs, and private nonprofit philanthropy organizations toward the ambitious set of eight Millennium Development Goals to eliminate extreme poverty, hunger, and disease by the year 2015 (Figs. 230-5 through 230-10).[1]

In addition, advances in technology for diagnosis, treatment, and prevention of diseases of global importance, application of new technologies into global public health practice, education of new generations of global health scientists and global public health experts to develop and apply these new technologies to global health, and expansion of Internet and mobile applications to reach and educate people in developing world.[18]

An example of a rapidly emerging development in diagnostics in the field is "lab-on-a-chip" (LOC) technology, which may soon become an important part of efforts to improve global health, particularly through development of point-of-care testing devices. In poorly equipped clinics in resource-poor countries, treatable infectious diseases soon may be accurately diagnosed by clinicians using immunoassays and nucleic acid assays performed by microfluidic LOC technology, allowing appropriate treatments to be administered. For example, accurate diagnosis of HIV infection and measuring the number of CD^+ T lymphocytes, with resultant antiretroviral treatment, may soon be possible with LOC brought to resource-poor areas where most of the untested, untreated HIV-infected individuals reside.[8,25] For the chips to be used in areas with limited resources, many challenges must be overcome. In developed nations, the most highly valued traits for diagnostic tools include speed, sensitivity, and specificity; but in countries where the health care infrastructure is less well developed, attributes such as ease of use and shelf life must also be considered. If LOCs are to be used in the resource-poor areas of the developing world, their reagents must have a long shelf life at tropical room temperatures, as well as have low cost, scalability and recyclability.

Trainees in undergraduate and postgraduate courses in public health, medical schools, and medical residencies and fellowships, as well as students in schools for nursing and physician assistant training have a growing interest in understanding the major factors that influence global health of individuals and populations worldwide.[26-29] Most medical schools and public health institutions are developing global health essential core competencies to provide their students with basic understanding of the complexity of global health issues, especially in low-resource settings, and the control of diseases of importance to global health.[10,26] In addition to studies in the classroom, many institutions now provide practical experience in international settings and global health career path training (Fig. 230-11).[29] A new National School of Tropical Medicine (NSTM) was recently established in 2012 in Houston, Texas, providing a North American counterpart to the century-old Liverpool School of Tropical Medicine and the London School of Hygiene and Tropical Medicine.[15] The mission of the NSTM includes training global health scientists and global health care providers in the area of tropical medicine and to develop and deliver new generations of technologies in vaccines, drugs, and diagnostics for infections associated with

FIGURE 230-5 ■ **A,** River scene near the rural town of Doma in Nasarawa State of Nigeria, showing men, women, and children using river water for washing. The river also may be used to wash motorcycles and cars and even for drinking. Providing access for the world's people to safe and clean water for drinking and washing is one of the Millennium Developmental Goals for 2015. **B,** Orphan girl at the Kang'oma orphanage and daycare center near Mzuzu, Malawi takes a drink from a new water well and hand-pump built by U.N. volunteers in August 2012. The well provides access to clean water for drinking and washing and helps prevent diarrheal diseases, dehydration, trachoma, and other diseases. Malawi is one of the poorest host countries in the "bottom billion." To halve the number of people without access to clean water and sanitation is one of the Millennium Development Goals to be achieved by 2015. **(See companion Expert Consult website for color version.)** (**A,** Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011. **B,** Courtesy Byron McCord and Holly Corwin.)

FIGURE 230-6 ■ Children gathered in front of the medical and surgical cottage hospital in Doma, Nasarawa State, Nigeria. Many children are shown with water, melons, and cashews in baskets balanced on their head, which they sell to local people through street trading or hawking. This practice is common in Nigeria; although it allows a means to make an income for their family's survival, it also often keeps the children from attending school and receiving an education, perpetuating the cycle of poverty. To achieve universal primary education is one of the Millennium Development Goals for 2015. **(See companion Expert Consult website for color version.)** (Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011.)

FIGURE 230-7 ■ Surgical procedures performed in remote areas of the world often must be done under stark and challenging conditions. This 3-year-old boy from Doma, Nasarata State, Nigeria is having a right inguinal herniorrhaphy performed, under ketamine anesthesia, by local physicians in an operating room in Doma Hospital, Nasarata State, Nigeria. The procedure was successfully completed, he awakened, and received a post-operative intramuscular injection of an antibiotic to prevent infection. **(See companion Expert Consult website for color version.)** (Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011.)

FIGURE 230-8 ■ Evaluating and treating patients for infectious diseases in remote rural areas is challenging, because microbiologic cultures, laboratory tests, diagnostic imaging, biopsies, and intravenous infusions are not available. In this photograph, the author, using natural light provided through a window, explains the findings of a plain radiograph showing destructive bone changes of the proximial right femur, consistent with chronic osteomyelitis. The patient, an 11-year-old boy, had a 2-year history of painful limp, chronic draining fistulous tracts, and tenderness over the proximal femur. Lack of money and access to transportation precludes accessing medical care with specialists in an urban hospital. The child was provided with crutches to help ease pain and facilitate mobility, and a 3-month supply of oral antibiotics. He also was given a "written referral" to a specialist in the medical center in Abuja, Nigeria, but unless he is able to travel to the larger urban center, his next follow-up will be in 1 to 2 years at the next medical mission. (See companion Expert Consult website for color version.) (Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011.)

FIGURE 230-9 ■ Medical wards in hospitals in remote, resource-poor, rural areas of the "bottom billion" countries, are often stark, are maintained as well as possible with available resources, and lack modern equipment to which health care providers in resource-rich countries are accustomed. (See companion Expert Consult website for color version.) (Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011.)

FIGURE 230-10 ■ This 13-year-old Nigerian girl had a several-year history of increasing kyphosis. A soft, nontender mass was palpated along her spine. The presumptive diagnosis was Potts disease, a presentation of extrapulmonary tuberculosis that most commonly affects the lower thoracic and upper lumbar vertebrae of the spine. The sixth goal "to combat HIV-AIDS, malaria, and other diseases" of the Millennium Development Goals specifically addresses the health and economic impact of infectious diseases on global health. It includes programs specifically targeted to combat tuberculosis.[30] (See companion Expert Consult website for color version.) (Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011.)

FIGURE 230-11 ■ Dr. Gelane Workneh, *left,* works with residents and medical students as she make rounds in the pediatric ward at University of Gondar Hospital (October 11, 2011) in Ethiopia. Dr. Workneh is one of three doctors Texas Children's Hospital Global Health Corps physicians and the Baylor College of Medicine Pediatric AIDS Initiative provides to work with the Jewish Joint Distribution Committee and Gondar University College of Health Sciences in Gondar as part of a 3-year grant. These doctors work to expand pediatric medical capacity in Ethiopia by teaching medical students and interns from Gondar University and by training local health care professionals. Global health experiences have become an important part of medical education curriculum in many major medical centers. (See companion Expert Consult website for color version.) (Courtesy Smiley N. Pool and BIPAI, Houston, Texas. Copyright 2011.)

poverty and the poorest people in the lowest income countries of the world.[15]

The future of communication and information technology also includes global health. Social media provides unprecedented connectivity because even in the most resource-poor areas of the world, fragmented levels of power and Internet access are available (Fig. 230-12).[31] Social media are being used by philanthropic organizations, such as the Bill and Melinda Gates Foundation, to update their donors and partners on measureable markers of progress toward defined global health goals.[23] Mobile global health education applications, linking smart phones and pico projectors and other mobile technology, have the potential to be inexpensive, sustainable, ubiquitous, acceptable, and therefore effective in disseminating information on a global scale, linking resource-rich areas with remote, resource-poor areas of the world. In addition, mobile devices may be used for surveillance[33] of infectious diseases, as well as chronic conditions such as heart disease, hypertension, and diabetes. Mobile devices also may be used for global health surveys and health information systems management. Mobile technology is also ideal for providing rapid scientific, epidemiologic, and public health knowledge at the time of a disaster. Mobile technologies may soon revolutionize global health care because individuals who are patients in resource-poor remote areas also may own or borrow mobile phones, allowing communication to access health care when needed, and reducing mortality.[32] As an example of creative adaptability, in "light-poor" communities and villages who do not have reliable electrical power at night, the light provided by individual cell phones allows health care providers to assist in medical procedures, including deliveries of babies at night.[31]

FIGURE 230-12 ■ Internet access through personal computers, local "dot-com" Internet cafes, and handheld mobile devices has reached even the most remote areas of the world and provides creative opportunities to improve global health and awareness in these remote communities. (See companion Expert Consult website for color version.) (Courtesy Dr. Gail Harrison. Photograph taken during a medical mission cosponsored by Ntwatwa Global Services, Houston, Texas and the Onawa Medical Mission Project of Nasarawa State, Nigeria in December of 2011.)

REFERENCES

1. Alsan MM, Westerhaus M, Herce M, et al. Poverty, global health and infectious disease: Lessons from Haiti and Rwanda. *Infect Dis Clin North Am* 2011;**25**:611–22.
2. Bahl R, Martines J, Bhandari N, et al. Setting research priorities to reduce global mortality from preterm birth and low birth weight by 2015. *J Global Health* 2012;**2**:e010403.
3. Beaglehole R, Bonita R. What is global health? *Global Health Action* 2010;**3**:5142.
4. Benjamin G. Growth of international health: an analysis and history. *Monograph Am Public Health Assoc* 2003.
5. Bowen KJ, Friel S. Climate change adaptation: where does global health fit in the agenda? *Global Health* 2012;**8**:10.
6. Bozorgmehr K. Rethinking the "global" in global health. *Global Health* 2010;**6**:19.
7. Brown TM, Cueto M, Fee E. The world health organization and the transformation from international to global public health. *Am J Public Health* 2006;**96**:62–72.
8. Buck WC, Kabue MM, Kazembe PN, et al. Discontinuation of standard first-line antiretroviral therapy in a cohort of 1434 Malawian children. *J Int AIDS Soc* 2010;**13**:31.
9. Centers for Disease Control and Prevention. *The targeted neglected tropical diseases (NTDs)*. Factsheet. Available at: http://www.cdc.gov.
10. Chase J, Evert J. *Global health training in graduate medical education: a guidebook*. San Francisco: Global Health Consortium; 2011.
11. Denno D. Global child health. *Pediatr Rev* 2011;**32**:e25–38.
12. Dyar OJ, de Costa A. What is global health? *J Global Health* 2011;**1**:31–2. http://www.ghjournal.org/jgh-print/spring-2011-issue/what-is-global-health/.
13. Harvard School of Public Health. *Social threats and disparities*. Available at: http://www.hsph.harvard.edu/news/magazine/social-health-hazards/.
14. Hotez P. Control of neglected tropical diseases. *N Engl J Med* 2007;**357**:1018–26.
15. Hotez PJ. A national school of tropical medicine and neglected infections of poverty for North America. *PLoS Negl Trop Dis* 2010;**4**:e735.
16. Hotez PJ, Yamey G. The evolving scope of PLoS neglected tropical diseases. *PLoS Negl Trop Dis* 2009;**3**:e379. doi:10.1371/journal.pntd.0000379.
17. Hotez P, Fenwick A, Savioli L, et al. Rescuing the bottom billion through control of neglected tropical diseases. *Lancet* 2009;**373**:1570–5.
18. Hudacek DL, Kuruvilla S, Kim N, et al. Analyzing media coverage of the global fund diseases compared with lower funded diseases (childhood pneumonia, diarrhea, and measles). *PLoS ONE* 2011;**6**:e20438.
19. Kim MH, Saeed A, Buck WC, et al. The Tingathe Programme: a pilot intervention using community health workers to create a continuum of care in the prevention of mother to child transmission of HIV (PMTCT) cascade of services in Malawi. *J Int AIDS Soc* 2012;**15**(suppl. 2):17389.
20. Koplan JP, Bond TC, Merson MH, et al. Towards a common definition of global health. *Lancet* 2009;**373**:1993–5.
21. Larson C, Mercer A. *Global health indicators: an overview*. Available at: http://www.cmaj.ca/content/171/10/1199.full.
22. Larson K, Halfon N. Family income gradients in the health and health care access of US children. *J Matern Child Health* 2010;**14**:332–42.
23. Litzow JM, Bauchner H. The grand challenges of the Gates Foundation: what impact on global child health? *J R Soc Med* 2006;**99**:171–4.
24. Neglected Tropical Diseases. *The campaign trail*. Available at: http://wellcometrust.wordpress.com/2012/01/10/neglected-tropical-diseases-the-campaign-trail/.
25. Patel P, Davis S, Tolle M, et al. Prevalence of hepatitis B and hepatitis C coinfections in an adult HIV centre population in Gaborone, Botswana. *Am J Trop Hyg* 2011;**85**:390–4.
26. Redwood-Campbell L, Pakes B, Rouleau K, et al. Developing a curriculum framework for global health in family medicine: emerging principles, competencies, and educational approaches. *BMC Med Educ* 2011;**11**:1–8.
27. Rogers C. Australian Government AusAID Office of Development Effectiveness. *Bottom billion effectiveness summary*. Available at: http://www.ode.ausaid.gov.au/publications/documents/bottom-billion-issues-note.pdf.

28. Rudan I, Kapiriri L, Tomlinson M, et al. Evidence-based priority settings for health care and research: tools to support policy in maternal, neonatal, and child health in Africa. *PLoS Medicine* 2010;**7**:e1000308.
29. Scarlett HP, Nisbett RA, Stoler J, et al. South-to-north, cross-disciplinary training in global health practice: ten years of lessons learned from an infectious disease field course in Jamaica. *Am J Trop Med Hyg* 2011;**85**:397–404.
30. Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. *Clin Infect Dis* 2010;**50**(suppl. 3):S184–94.
31. United Nations. *United Nations Millennium Development Goals*. Available at: http://www.un.org/millenniumgoals/global.shtml.
32. United Nations. *Millennium Development Goals Indicators*. Available at: http://mdgs.un.org/unsd/mdg/default.aspx.
33. United Nations Health Alliance. *Improving child survival through the use of mobile technologies*. Available at: http://www.healthunbound.org/sites/default/files/uploads/UN_MHealth_OnePage_updated.pdf.
34. Vermund SH, Sahasrabuddhe VV, Khedkar S, et al. Building global health through a center-without-walls: the Vanderbilt Institute for Global Health. *Acad Med* 2008;**83**:154–64.
35. World Health Organization. *Social determinants of health. n.d.* Available at: http://www.who.int/social_determinants/en/.
36. World Health Organization. *Social determinants of health. Publications: Women and gender equality*. Available at: http://www.who.int/social_determinants/publications/womenandgender/en/index.html.
37. Yamey G. Scaling up global health interventions: a proposed framework for success. *PLoS Med* 2011;**8**:e1001049.

CHAPTER 231

International Travel Issues for Children

Michelle Weinberg • Nicholas Weinberg • Susan A. Maloney

During the past half-century, the number of persons traveling internationally has increased tremendously. Between 1980 and 2011, the number of international arrivals worldwide more than tripled, growing from 277 million to 983 million. In 2010, more than 28 million U.S. residents traveled overseas; of these residents, almost 23 million were traveling for leisure or visiting family and friends. Although the exact number of children traveling is unknown, 2.2 million (8%) of these U.S. travelers were adults and children traveling together and 3.1 million (11%) reported their occupation as students.[137] Among the more than 28 million U.S. resident travelers, 9.5 million (34%) visited Europe, 6.1 million (22%) visited the Caribbean, 5.6 million (20%) visited Asia, 4.2 million (15%) Central and South America, 1.6 million (6%) visited the Middle East, 840,000 (3%) visited Africa, and 560,000 (2%) visited Oceania.[136,157] Worldwide, Europe and Asia and the Pacific region were the fastest-growing destinations in terms of international tourist arrivals, and each grew more than 6 percent in 2011.

Study abroad by U.S. students enrolled in higher education has also tripled in the last 20 years; more than 270,000 U.S. students studied abroad during the 2009 to 2010 school year.[89] The United Kingdom, Italy, Spain, France, and China were the top host destinations; however, the number of students studying in Africa, Asia, and the Middle East increased by more than 8 percent each and nearly 14,000 students studied in China.[89]

International travelers may be exposed to a variety of health risks, depending on the travel destination, trip itinerary, duration of stay, planned travel activities (i.e., business vs. adventure), individual risk factors, and preventive measures taken. For persons from industrialized countries visiting developing countries, travel may expose them to a variety of infectious pathogens and environmental hazards, some of which they may rarely encounter at home. Despite the well-documented health risks associated with international travel, surveys have shown that many travelers do not seek pretravel health advice or obtain appropriate travel-related preventive medications or vaccinations. Surveys of U.S., European, and Australian travelers have shown that only 31 to 52.1 percent of the travelers sought travel advice before the trip. In an airport survey of 203 U.S. passengers traveling to high-risk, malaria-endemic areas, only 46 percent were carrying antimalarial medications with them[78,139,147] and few were vaccinated for their travel—only 11 percent for tetanus, 14 percent for hepatitis A, 13 percent for hepatitis B, and 5 percent for yellow fever.[78] Among 1704 respondents from the 2008 YouthStyles survey, an annual mail survey of demographics and health knowledge,

attitudes, and practices of youth 9 through 18 years of age, 131 (7.7%) traveled in the last 12 months; of these travelers, only 16.7 percent reported seeking pretravel medical care, mostly (84%) from a family doctor.[79] In a survey of 318 American students studying abroad, the primary source of travel health information was reported to be youth-oriented travel guidebooks (85%).[80]

Travelers who are visiting friends and relatives are at high risk for acquiring a variety of illnesses and are less likely to seek pretravel medical advice.[7-9,44,99] In a review of travelers seeking medical care for travel-related illnesses in the GeoSentinel Surveillance Network, only 16 percent of those who were immigrants and were visiting family and friends sought pretravel medical advice.[99] Systemic febrile illnesses (including malaria), nondiarrheal intestinal parasitic infections, respiratory syndromes, tuberculosis, and sexually transmitted diseases (STDs) were more commonly diagnosed among immigrants visiting friends and relatives than in other travelers, whereas acute diarrhea was less frequent. In contrast to other travelers, the immigrants who were visiting friends and relatives were more likely to travel to sub-Saharan Africa or Latin America and to take a trip lasting longer than 30 days.[99] In a review of travelers presenting at one GeoSentinel Surveillance Network clinic site, the proportion of travelers with malaria who had been visiting friends and relatives was eight times greater than among tourists (16.9% vs. 2.1%).[97]

PEDIATRIC TRAVELERS AND HEALTH RISKS ABROAD

Information is limited on the causes of serious morbidity and mortality among pediatric travelers, and efforts to collect these data should be undertaken to direct prevention measures so that morbidity and mortality rates in pediatric travelers can be decreased.[12,77,84,114,122] However, recent studies have identified several major categories of morbidity among pediatric travelers: diarrheal illnesses, respiratory diseases, dermatologic conditions, and systemic febrile illnesses. Herbinger and colleagues[82] reviewed data from 890 children younger than 20 years of age at an outpatient travel clinic at the University of Munich, Germany after travel to Africa (46%), Asia (35%), and Latin America (19%). The most frequent syndromes were acute diarrhea (25%, especially in children ages 0 to 4 years of age), dermatologic conditions (19%, especially in children ages 0 to 9 years), febrile and systemic diseases (20%), respiratory disorders (8%), chronic diarrhea (5%), and genitourinary disorders (3%). The 10 most frequently diagnosed infectious diseases were giardiasis (8%), schistosomiasis (4%), superinfected insect bites (4%), *Campylobacter* enteritis (4%), *Salmonella* enteritis (4%), cutaneous larva migrans (3%), amebiasis (3%), dengue fever (2%), mononucleosis (2%), and malaria (2%).[82] Hunziker and colleagues[85] reviewed data from 328 children ages 16 years and younger with travel-associated illness at the emergency room of the University of Zürich Children's Hospital. The main conditions were diarrheal illness (39%), respiratory (28.7%), and systemic febrile illness (13.4%). No deaths were reported. Among 36 children with more serious diseases requiring hospitalization, 12 (3.7% overall) had potentially serious diseases: malaria (n = 2), *Salmonella typhi* (n = 3), *Salmonella paratyphi* (n = 2), meningococcal meningitis (n = 1), tuberculosis (n = 2), visceral leishmania (n = 1), and hepatitis A (n = 1). Eleven of the 12 children with serious diseases were visiting friends and family or immigrant children.[85] Van Rijn and colleagues[140] conducted a prospective observational cohort study of 152 children and their parents traveling to the tropics and subtropics; 85 percent of all children reported some kind of aliment during travel, with a mean aliment rate of 7.0 ailments per person-month of travel. The most frequently reported ailments were skin problems, including insect bites, sunburn, and diarrhea.[140]

Other authors have reported substantial health risks for pediatric travelers from noninfectious causes such as injuries. Most deaths are associated with swimming or automobile accidents.[84,101] One study of British children drowning abroad while traveling showed that an average of eight British children drown each year abroad, primarily in swimming pools without adequate lifeguards.[64]

Although some data from adult travelers can be extrapolated to use in the management of pediatric travelers, infants and children have special vulnerabilities and needs when preparing for travel abroad. These needs include (1) up-to-date and appropriate vaccinations (at times through accelerated or altered routine childhood vaccine schedules and also including special travel-related vaccines); (2) appropriate malaria and other chemoprophylaxis regimens tailored for use in pediatric travelers; (3) prevention counseling, particularly in the areas of food and water safety, insect barriers, and injury prevention; and (4) anticipatory guidance for the management of potential illness and successful location of medical resources overseas. This chapter provides recommendations for conducting a pediatric pretravel assessment to ensure that the child has received the appropriate vaccination and chemoprophylaxis regimens, pretravel prevention counseling, and means of accessing additional sources of travel information about health risks and the availability of medical resources at specific travel destinations.

GENERAL APPROACH TO PRETRAVEL ASSESSMENT FOR CHILDREN

Before embarking on international travel, children should have a pretravel health assessment performed by a health care provider. The number of physicians who specialize in travel medicine for children is relatively limited, and many pretravel assessments are performed by general pediatricians, pediatricians who specialize in infectious diseases, or travel medicine specialists without specific pediatric training. The visit should be conducted with the goal of preventing travel-related illnesses. Depending on the destination and the vaccinations recommended or required, the assessment should be conducted optimally 1 to 6 months before travel. Because families may not be aware of the need for multiple vaccinations and medications, health care providers should be proactive and

routinely ask patients and their families if they are anticipating any international travel in the next several months, especially before holiday periods.

During the pretravel assessment, the provider should do the following:

1. Review the child's current and past medical history, including the status of routine childhood vaccinations.
2. Obtain specific details about the travel itinerary and planned activities. Review endemic diseases and recent outbreaks in travel destinations.
3. Administer indicated routine and travel-related vaccinations.
4. Prescribe antimalarial, other prophylactic or self-treatment medication (based on risk assessment from the medical history, travel itinerary, and planned activities).
5. Counsel about prevention of travel-related illnesses.
6. Provide guidance about seeking medical assistance for travel-related illness.

The age, immunization status, and medical history of the child are important information to have in conducting the pretravel assessment. The provider should consider three categories of vaccinations: routine childhood vaccinations, required travel-related vaccinations, and recommended travel-related vaccinations. Some routine vaccinations should be administered at an earlier age or after an accelerated schedule in preparation for international travel. In addition, the provider should review and administer travel-related vaccinations that are required for entry by various countries to prevent the importation of disease and development of outbreaks, as well as vaccinations recommended for the prevention of illness in the individual traveler.[32] Currently, only two vaccines—yellow fever and meningococcal—are required for entry by selected countries; however, these requirements may change in the future. In addition, some countries require documentation of human immunodeficiency virus (HIV) testing before allowing entry of long-term travelers (see discussion on international travel information resources). Guidelines for standard and accelerated schedules for routine childhood immunizations and for required and recommended travel-related vaccinations by specific travel destinations are summarized in this chapter in the section on vaccination for international travel. A review of medical conditions and contraindications is critical before vaccinations are administered or medications such as antimalarial chemoprophylaxis are prescribed.

The pretravel assessment should include a detailed review of the trip itinerary. The health care provider should ask about sources of water and food (homes vs. hotel restaurants), type of accommodations (e.g., camping, hotel chains, or residing with local families), amount and type of contact with the local population, and planned activities during travel (adventure travel with exposure to animals, water, high altitude, or any combination of these factors). Recommendations to prevent vector-borne diseases, including malaria, should be determined by a comprehensive review of the travel itinerary and planned activities. Guidance for determining appropriate regimens for antimalarial chemoprophylaxis in pediatric travelers is provided in this chapter in the discussion on malaria prevention. The risk for acquiring an infectious disease differs and is probably greater for an adolescent who is participating in a rural home-stay program for 3 months than for a 5-year-old child traveling with parents to a beach resort. Furthermore, expatriates who live abroad and foreign-born persons, especially immigrants, refugees, and their children visiting friends and relatives in their home country, have health risks different from those of short-term travelers who are visiting tourist destinations. These differential risk profiles should be taken into account during the pretravel assessment because such knowledge is essential for devising a specific pretravel management plan.

On the basis of risk assessment, preventive health counseling should include information about food and water safety, prevention of vector-borne illnesses, and other infectious and noninfectious illnesses, such as sexually transmitted infections and automobile accidents. General and specific prevention counseling recommendations are addressed later in the chapter. In addition, families should clarify with their health insurance company whether they are covered for health care provided abroad. If not, insurance can be purchased from several companies; such insurance may include coverage for health care providers in a specific country and airlift medical evacuation. If assistance for illness acquired abroad is needed, the U.S. embassy or consulate can provide names and addresses of English-speaking health care providers at the travel destination. This information can be obtained before departure by accessing embassy Internet sites or by calling the embassy. In addition, names of physicians abroad can be obtained from some worldwide directories (see the discussion on international travel information resources). Parents should be told to carry all health-related documents with them for easy retrieval, including written prescriptions for medications, health insurance information, and medical contacts abroad. Counseling on ensuring the use of sterile needles and the safety of the local blood supply also should be included in pretravel prevention counseling topics; blood screening and infection control practices may not be as stringent in some countries as in the United States.

Special consideration must be given to children who have chronic diseases. Children with medical conditions should take a summary of their medical history and treatment record. If the child has cardiac disease, the family should consider taking a copy of the child's recent electrocardiogram and echocardiography report. Children with diabetes who are traveling through more than one time zone should consult their pediatrician or endocrinologist for guidance on the need for altering insulin dosing. Prescription medications should be carried in original bottles, and a sufficient supply for the length of the planned trip and a few extra doses should be packed. Required medications should be carried on the plane rather than packed in luggage because bags may be lost or exposed to unfavorable environmental conditions. Families should be counseled about the potential hazards of obtaining medications in pharmacies in developing countries, where many medications can be purchased

over-the-counter without a prescription and where the names, content, and concentration of medications may be different from those of U.S. products. Parents carrying medical equipment (such as nebulizers) should remember to take adaptors for foreign electric current and bring a letter from a physician that documents the need for the equipment.[39] Similar documentation is encouraged for anaphylaxis kits or other medications that require syringes.

VACCINATION FOR INTERNATIONAL TRAVEL

Vaccination before international travel is among the most critical and complex components of the pretravel assessment for children. The pretravel assessment should include a review of both recommended routine childhood vaccinations and required and recommended travel-related vaccines. Vaccination recommendations change rapidly. Whereas this section provides guidance as of publication date, clinicians should obtain the most current recommendations available on the Centers for Disease Control and Prevention (CDC) website (see the discussion on international travel information resources).

Routine Childhood Vaccinations for Pediatric Travelers

For children who will be traveling internationally, having their routine immunizations brought up to date is essential because many vaccine-preventable diseases are more prevalent in developing countries than in the United States. In particular, children who have nonmedical exemptions to routine vaccinations may be at high risk for acquiring vaccine-preventable diseases, such as measles and rubella, that no longer are endemic in the United States but still occur frequently in other countries. The pretravel visit also may give the health care provider a unique opportunity to update the routine vaccination status of a patient who has fallen behind schedule. In certain cases, routine vaccinations may need to be accelerated to maximize protection, particularly against polio, diphtheria-tetanus-pertussis, varicella, and measles (e.g., measles vaccination is recommended for children 6 through 11 months of age).[40,121] Many vaccine series can be initiated as early as 6 weeks of age. The management plan for routine vaccinations will be determined by the travel destination and itinerary. For example, diphtheria and pertussis are prevalent in Eastern Europe and many developing countries, and measles is still endemic in the developing world and some European countries; therefore, if travel to these countries is planned, ensuring immunity is imperative, and accelerated schedules should be considered. In addition, parents should check their own immune status because traveling with children can increase their risk for exposure to measles and other vaccine-preventable diseases.[86,87]

Measles was declared eliminated in the United States in 2000. However, travelers to Europe, Asia, the Pacific, and Africa continue to be at risk. High two-dose mumps, measles, and rubella (MMR) vaccine coverage is essential for travelers to prevent measles importation and outbreaks in the United States. In 2011, 222 cases of measles were reported in the United States; these cases were associated with 17 outbreaks in the United States and represent an increase in contrast to a median of 60 (range, 37 to 140) cases and four (range, 2 to 10) outbreaks reported annually during 2001 to 2010.[55] Of the 222 cases, 200 (90%) were associated with importations from other countries, including 52 (26%) cases in U.S. residents returning from abroad. Of the 200 cases associated with importation, 72 (36%) were importations from other countries, 67 (30%) were linked epidemiologically to importations, 39 (20%) had virologic evidence (i.e., isolation of a viral genotype known to circulate in a country with measles) that suggested recent importation, and 22 (11%) were linked to cases with virologic evidence of recent importation. Among the 72 cases of measles importation, 52 were linked to U.S. residents who traveled abroad. Almost half (46%) of the 72 measles importations occurred among persons who acquired the disease in the World Health Organization (WHO) European Region, which reported more than 30,000 cases in 2011. The highest number of cases, 16 (22%) were associated with importation from India, followed by 13 (18%) associated with importation from France. Most cases (86%) occurred among persons who were unvaccinated or had unknown vaccination status.[55] In 2005, importation of measles by an unvaccinated U.S. traveler led to one of the largest outbreak of measles in the United States since 1996 and involved 34 confirmed cases. The index case was a 17-year-old student who had a nonmedical vaccination exemption and was working as a missionary in an orphanage in Romania, where a large measles outbreak subsequently was reported.[119] Numerous cases of serologically confirmed measles also have occurred among internationally adopted children and their new parents and siblings who had traveled to China to accompany them home. Data suggest that the exposure to measles occurred in China, probably at the orphanage, where an outbreak of measles was occurring.[36,37] The MMR vaccine is administered routinely to children starting at 12 months of age. However, the MMR vaccine should be administered to children 6 to 11 months of age who are traveling internationally. These children should be revaccinated with two doses of MMR vaccine—the first at ages 12 through 15 months and at least 4 weeks after the previous dose and the second at ages 4 through 6 years. Children older than 12 months of age who are traveling internationally should receive the second dose, as long as 4 weeks have passed since the previous dose; even if administered before 4 years of age, this second dose counts toward the routine vaccination and does not need to be repeated at a later age. Providers should remind their patients who plan to travel internationally of the increased risk for measles and potential exposures during bus, train, or air travel and at large international events or gatherings.

Hepatitis A is endemic in most of the world, and travelers are at risk in any area where sanitation is poor. In 2006, hepatitis A vaccination became part of routine

childhood recommendations in the United States for all children at 1 year of age (12 through 23 months), supplementing recommendations in 1999 for vaccination of children 2 to 18 years of age residing in states and communities with high incidence of disease and other high-risk groups. In addition, hepatitis A vaccine is now routinely recommended for all previously unvaccinated children aged 24 months or older for whom immunity is desired. Vaccination is recommended for pediatric travelers 1 year of age or older who will be visiting countries with intermediate to high endemicity, such as Mexico, Central and South America, Asia, Africa, and eastern Europe.[39,41,56] Hepatitis A vaccine is administered as a two-dose series. The first dose of hepatitis A vaccine should be administered at least 14 days before travel. Limited data are available about the timing of the appearance of neutralizing antibody. Among a sample of vaccinated persons, 54 to 62 percent were positive for neutralizing antibody 14 days after the first dose, and 94 to 100 percent were positive at 1 month.[41] The second dose can be administered 6 months after the first dose. Intramuscular immunoglobulin is recommended for immunoprophylaxis against hepatitis A in children younger than 1 year of age. In addition, for children 1 year of age or older who will be departing sooner than 2 weeks after the pretravel visit (and therefore may not have sufficient time for immunity to develop after hepatitis A vaccination), both immunoglobulin and vaccine can be given concurrently at different sites to ensure more immediate protection. Shortages of immunoglobulin have occurred in the past, so obtaining it for hepatitis A prophylaxis may be difficult.[56] Hepatitis A outbreaks also have occurred among adoptees and their contacts, leading to American Academy of Pediatrics and the Advisory Committee on Immunization Practices recommendations for hepatitis A vaccination for all household members and close contacts, including babysitters, when children are adopted from countries with high or intermediate rates of hepatitis A infection. All nonimmune, unvaccinated people who will have close exposure to international adoptees during the 60 days after their arrival (regardless of whether they travel) should receive hepatitis A immunization, ideally 2 or more weeks before arrival of the adopted child.[6,42]

Worldwide polio eradication efforts have decreased the number of countries where travelers are at risk for acquiring polio. In 2011, 650 cases of wild poliovirus were reported from 16 countries; however, wild poliovirus is considered endemic in only three countries: Afghanistan, Nigeria, and Pakistan. India, which was previously on the list of endemic countries considered to have never interrupted the transmission of wild poliovirus, was declared free of endemic transmission in February 2012, 1 year after the last reported case occurred in January 2011. Between January 2012 through June 2012, cases were reported in Chad, Kenya, Niger and Somalia. However, countries associated with imported cases are continuously changing.[35,123] To ensure protection, pediatric travelers visiting countries where polio is epidemic or still endemic should be fully immunized, and young adults ages 18 or older should receive one booster dose of inactivated polio vaccine (IPV) if traveling to areas with risk of transmission. Clinicians should obtain up-to-date information about areas of transmission from the WHO or CDC (see discussion on international travel information resources).

Respiratory tract infections caused by influenza are a major cause of illness among travelers.[98] Influenza can occur throughout the year in tropical countries. Peak influenza rates in temperate regions of the Southern Hemisphere occur from April through September. In North America, influenza vaccine may not be available during the summer months. All children 6 months of age and older should have routine influenza vaccination annually. Travelers to the Southern Hemisphere should be vaccinated by the spring, if possible. Influenza vaccinations should be considered for travelers who are at high risk for complications from influenza for travel to (1) the tropics any time of year, (2) the Southern Hemisphere from April through September, (3) any destination with large groups of tourists at any time of year, or (4) any destination where influenza outbreaks are occurring.[41] In 2009, pandemic H1N1 outbreak emerged in Mexico and spread quickly around the world through travel. Protection against H1N1 is included in seasonal influenza vaccine.[46,48]

Meningococcal disease occurs sporadically worldwide. Epidemic disease has been reported in India, Saudi Arabia, and sub-Saharan Africa; indeed, recurrent epidemics of meningococcal disease occur in sub-Saharan Africa, mainly from December to June (the dry season). Serogroup A is the most common cause of epidemics outside the United States, but serogroup C and other serogroups also have been associated with epidemics. Serogroup W-135 meningococcal infection has been reported in travelers returning from Saudi Arabia after visiting the Hajj.[136] Three meningococcal vaccines, a polysaccharide vaccine (MPSV4), and two conjugate vaccines (MCV4-D and MCV4-CRM), are available in the United States. Both conjugate vaccines are quadrivalent and effective against serogroups A, C, Y, and W-135. MCV4 (either MCV4-D or MCV4-CRM) is recommended for routine childhood vaccination in the United States for (1) all children at the 11- to 12-year-old visit, with a booster at 16 years of age, (2) unvaccinated adolescents at 13 through 18 years of age, with a booster at 16 through 18 years; and (3) two-dose primary series administered 2 months apart for children 2 through 54 years of age with persistent complement component deficiency or anatomic or functional asplenia and for adolescents with HIV infection, followed by a booster every 5 years. Meningococcal vaccination is recommended for pediatric travelers 9 months of age or older who are visiting sub-Saharan Africa during the dry season or any country that is hyperendemic or where an epidemic caused by a vaccine serogroup is occurring. MCV4-D is licensed for use for children starting at 9 months of age, and MCV4-CRM can be used for children starting at 2 years of age. For children 9 through 23 months of age, MCV4-D should be administered as two primary doses; the second dose is recommended 3 months after the first dose, but the minimal interval between the first and second doses is 8 weeks. For children 24 months to through 10 years of age, two doses of either MCV4-D or

MCV4-CRM should be administered at least 8 weeks apart. See Table 231-1 for additional information. For children traveling to areas with risk for transmission, revaccination with MCV4 is recommended after 3 years for children who were previously vaccinated at ages 9 months to 6 years. Revaccination with MCV4 is recommended after 5 years for people who were previously vaccinated at 7 years of age and older and every 5 years thereafter for people who are at continued risk. Although it is less immunogenic than when it is administered to older children and adults, MPSV4 also can be used in children as young as 3 months to provide protection against serogroup A, such as travelers to areas with an epidemic or during seasonal transmission. These children should have two doses, 3 months apart.[51-53,57,57,121]

Hepatitis B, *Haemophilus influenzae* type b, rotavirus, *Streptococcus pneumoniae*, varicella, and human papillomavirus are endemic in many developing countries, and vaccination should be ensured.[47] For persons 18 years of age and older, a combination hepatitis A and hepatitis B vaccine (Twinrix) can be administered to travelers who need both vaccines. It is administered as a standard three-dose schedule at 0, 1, and 6 months after the first dose or can be accelerated at 0, 7, or 21 to 30 days, followed by a booster dose at 12 months.[43] Travel in large groups on conveyances such as cruise ships can facilitate the transmission of infectious and vaccine-preventable diseases.[105] The CDC has provided consultation on managing outbreaks of varicella and meningococcal disease in pediatric travelers on international cruise ships (CDC, unpublished data, 2013). For detailed information about accelerating routine childhood vaccinations and indications for use in pediatric travelers, see Table 231-1.

Common Travel-Related Vaccines for Children

An important consideration in determining travel vaccination needs is "required" versus "recommended" vaccinations. The most recent travel requirements and recommendations for vaccinations can be obtained from the CDC Travelers' Health Internet site (see the discussion on international travel information resources). Few vaccinations are required. The United States does not require arriving travelers to have any vaccinations for entry or return to the United States. However, immigrants, refugees, asylum seekers, and internationally adopted children migrating to the United States for permanent residence have different requirements. Some other countries may require proof of vaccination against yellow fever for entry, especially if the traveler is arriving from a country where yellow fever is present. Yellow fever vaccine is available only from certified yellow fever vaccination centers; providers can contact their state public health department to locate certified centers in their areas. Saudi Arabia requires meningococcal vaccine for travelers visiting Mecca for the Hajj or for the Umrah.[28,38,53] Some countries previously have required cholera vaccination, but currently no countries require vaccination as a condition of entry.[156] However, non-U.S. customs officials who "unofficially" request proof of vaccination have been reported. Cholera vaccine is not currently available in the United States but is available in Europe and other countries. Cholera is rare in travelers, although it has been reported in humanitarian aid workers and more recently in persons visiting and working in Haiti following the earthquake in 2010. As more young adults participate in community service projects in at-risk countries, these travelers may benefit from counseling and medications for self-treatment to be used if signs and symptoms develop while abroad. Clinicians should check CDC Travelers' Health website (see resources) for updated country-specific recommendations.

Tables 231-1 and 231-2 provide general guidelines and indications for the use of routine and selected travel-related vaccines based on U.S. recommendations.[53,57,83,88-90,121] WHO recommendations may differ.[156] Depending on the complexity of the travel itinerary and the patient's medical history, the vaccination plan may need to be developed in consultation with a travel medicine or infectious disease specialist. Travel-related vaccine recommendations should be tailored carefully to the trip itinerary and based on the travel destination, season when the travel will occur, duration of the trip, and activities to be undertaken.

Yellow fever occurs year-round in the predominantly rural areas of sub-Saharan Africa and South America; however, outbreaks have been increasing in incidence, particularly in Africa.[154] Infection occurs primarily via the bite of an infected mosquito, usually *Aedes* or *Haemagogus* spp. Transmission occurs in a human-to-vector-to-human cycle with monkeys, mosquitoes, and humans in three patterns: sylvatic (jungle), intermediate (savannah), and urban transmission. The resurgence of yellow fever in urban areas, in association with *Aedes aegypti* mosquitoes, has raised concern about increased risk in Brazil and urban areas of Latin America.[53,141,142] Although a rare disease, yellow fever continues to be reported in travelers, particularly unvaccinated travelers, and is usually fatal. From 1970 through 2010, nine cases of yellow fever were reported in unvaccinated travelers from the United States and Europe who traveled to West Africa (five cases) or South America (four cases); eight (88%) of these travelers died. One case of yellow fever in a vaccinated traveler, who survived infection has been reported.[53,108] The risk for transmission is lower in South America than Africa because travelers are less likely to come in contact with monkeys in the forest canopy. Preventive measures taken against yellow fever should include the use of personal protection against mosquitoes and vaccination. As discussed previously, some countries require yellow fever vaccination for travelers arriving from endemic regions; current requirements and recommendations for vaccination based on travel destination can be obtained from the CDC Travelers' Health website. Yellow fever vaccine is largely considered to be a safe and effective vaccine. However, the vaccine has been found to be associated with an increased risk for development of yellow fever vaccine–associated neurologic disease (YEL-AND), which includes meningoencephalitis, Guillain-Barré syndrome, and other severe reactions in young infants.[121]

Text continued on p. 3106

TABLE 231-1 **Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers**

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
DTaP, DT	6 wk	5 doses at ages 2, 4, 6, 15-18 mo and 4-6 yr	5 doses MI from dose 1 to dose 2: 4 wk MI from dose 2 to dose 3: 4 wk MI from dose 3 to dose 4: 6 mo Minimum age for dose 4: 12 mo MI from dose 4 to dose 5: 6 mo Minimum recommended age for dose 5: 4-6 yr Dose 5 is not necessary if dose 4 was administered after the fourth birthday	Use for children 6 wk–7 yr of age. Use DT for children <7 yr of age for primary series (instead of DTaP) when pertussis vaccination is contraindicated. The fourth dose may be administered as early as age 12 mo, provided at least 6 mo have elapsed since the third dose.
Tdap	10-11 yr, depending on brand	One dose		Only 1 dose of Tdap is recommended. All subsequent doses of tetanus-diphtheria booster should be administered as Td. Tdap should be substituted for a single dose of Td in the catch-up series for children 7-10 yr. If additional doses are needed, use Td. Tdap can be administered regardless of the interval since the last tetanus and diphtheria toxoid–containing vaccine.
Td	7 years	1 booster dose every 10 yr Children ≥11 yr should receive 1 dose of Tdap, followed by booster doses of Td every 10 yr.	MI for booster for travel: 5 yr	Td should be used rather than Tdap if Tdap is not available and for: Anybody who has already received Tdap Additional doses for children 7-10 yr in the catch-up series (one dose of Tdap should be administered) See information for Tdap regarding timing of Td vs. Tdap.
Haemophilus influenzae type b (Hib)	6 weeks	4 doses at ages 2, 4, 6, and 12-15 mo *or* 3 doses at ages 2, 4, and 12-15 mo (PRP-OMP vaccines only) Unvaccinated child >15 mo: 1 dose	Accelerated schedule is available but varies by vaccine brand; see package insert for vaccine used. MI between doses: 4-8 wk	MI between doses depends on age. Children receiving the first dose of vaccine at age ≥7 mo require fewer doses to complete the series. See *MMWR Morb Mortal Wkly Rep* 2005;**54**:Q1-4, for indications. Vaccinate all children <5 yr of age and children >5 yr of age with special indications (e.g., asplenia, immunodeficiency).

Continued on following page

TABLE 231-1 **Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers** (Continued)

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
Hepatitis A	1 yr	2 doses at ages 12 and 18 mo	MI from dose 1 to dose 2: 6 mo	Recommended for all children at 1 yr (12-23 mo). Recommended for unvaccinated children ≥24 mo at high risk (see *MMWR Morb Mort Wkly Rep* 2006;**55**[RR-7]), and anyone ≥24 mo, previously unvaccinated, for whom immunity against hepatitis A virus infection is desired. Recommended for all persons ≥12 mo traveling to endemic areas, including Mexico, Central and South America, Asia (except Japan), Africa, and eastern Europe.§ Immunogenicity: limited data are available for the timing of the appearance of neutralizing antibody. Among a sample of vaccinated persons, 54%-62% were positive for neutralizing antibody 14 days after the first dose, and 94%-100% were positive at 1 mo.‡
Hepatitis B¶	Birth	3 doses at 0-2, 1-4, 6-18 mo For children not previously vaccinated: 3 doses at 0-, 1-, and 4-mo intervals For unvaccinated adolescents 11-15 yr: 2-dose vaccine series (only Recombivax HB, adult dose) at 0, 4-6 mo or 3 doses of other vaccine brands	3 doses (except 2 doses of Recombivax HB, adult dose for adolescents) MI from dose 1 to dose 2: 4 wk MI from dose 2 to dose 3: 8 wk MI from dose 1 to dose 3: 16 wk Minimum age for dose 3: 6 mo	Use for travelers to endemic areas, including Africa, Asia, Latin America, who plan to: Travel abroad for ≥3 mo Have intimate or sexual contact with local population Work in health care setting or may have contact with blood, blood products, or body fluids Count all doses; do not restart series even if time lapse between doses is greater than recommended interval. Accelerated schedule: 4 doses at 0, 7, 21-30 days and booster at 1 yr

Human papillomavirus vaccine (HPV)	9 yr For females and males 9-26 yr. Use HPV4 or HPV2 for females and HPV4 for males.	3 doses at 11-12 yr at 0-, 2-, and 6-mo intervals	Recommended interval dose 1 to dose 2: 1-2 mo MI dose 1 to dose 2: 4 wk Recommended interval dose 1 to dose 3: 6 mo Recommended interval dose 2 to dose 3: 4 mo MI dose 2 to dose 3: 3 mo	
Influenza, trivalent inactivated vaccine (TIV) or live attenuated vaccine (LAIV)	TIV: 6 mo; however, check product because minimum age varies by vaccine manufacturer LAIV: 2 years, approved for use only for persons 2-49 yr	Recommended annually for children ≥6 mo. LAIV should not be administered to some children with certain medical conditions. See *MMWR Recomm Rep* 2010;**59**(RR-08):1-62. Children aged 6 mo–8 yr of age who are receiving influenza vaccine for first time: 2 doses Children >8 yr and persons who previously received influenza vaccine: 1 dose	TIV: MI dose 1 to dose 2: 4 wk LAIV: MI dose 1 to dose 2: 6 wk	Influenza can occur throughout the year in tropical countries. Peak influenza in temperate regions of the Southern Hemisphere occurs from April through September. In North America, influenza vaccine may not be available during the summer months. Travelers to the Southern Hemisphere should be vaccinated by the spring, if possible. Consider for travelers who are at high risk for complications from influenza for travel to Tropics any time of year Southern Hemisphere from April through September Any destination with large groups of tourists at any time of year Any destination where influenza outbreaks are occurring
Measles, mumps, rubella (MMR)	6 mo	2 doses at ages 12-15 mo and 4-6 yr	Children 6-11 mo: 1 dose, then revaccinate at 12-15 mo and 4-6 yr of age Children >12 mon vaccinated with 1 prior dose: 1 additional dose at least 4 wk after dose 1 Unvaccinated children >12 mo: 2 doses separated by 4 wk MI between doses: 4 wk	Do not administer MMR for 3 mo after immunoglobulin for hepatitis A prophylaxis; see *MMWR Morb Mortal Wkly Rep* 2005;**54**:Q1-4, for deferral times for blood products and immunoglobulin administered for other indications. Defer immunoglobulin administration for 2 wk after MMR vaccination. Monovalent vaccines are available for measles, mumps, and rubella. Combination MMRV (measles-mumps-rubella-varicella vaccine) can be used for children 12 mo–12 yr but is associated with higher risk for fever and febrile seizures 5-12 days after the first dose among children 12-23 mo.

Continued on following page

TABLE 231-1 **Routine Childhood Vaccinations and Accelerated Schedules and Modifications for Pediatric Travelers** (Continued)

Vaccine	Minimum Age	Routine Schedule*	Accelerated Schedule and Modifications†	Indications for Use and Other Information‡
Meningococcal (quadrivalent A, C, Y, W135) Conjugate vaccine (MCV4) Polysaccharide vaccine (MPSV4)	MCV4-D (Menactra): 9 mo MCV4-CRM (Menveo): 2 yr MPSV4: 3 mo for serogroup A protection, standard recommended minimum age: 2 yr (see indications)	MCV4-D or MCV4-CRM: routine vaccination, 1 dose for children at age 11-12 yr and booster at 16 yr. Administer 1 dose at 13-8 yr of age if not previously vaccinated. MCV4-D: 2 doses for travelers to risk areas ideally at 9 and 12 mo or at least 8 wk apart for children 9-23 mo MCV4-D or MCV4-CRM: 2 doses for travelers to risk areas at least 8 wk apart for children 24 mo–10 yr.		Meningococcal vaccination is required for travelers to annual Hajj in Mecca, Saudi Arabia. Vaccination is recommended for travel to sub-Saharan Africa during dry season (December through June) or for travel to any countries where epidemic is occurring. Recommended for all children with certain medical conditions. See *MMWR Morb Mort Wkly Rep* 2011;**60**:72-6.
Pneumococcal conjugate: 7-valent (PCV7) and 13-valent (PCV13)	6 wk	4 doses at ages 2, 4, 6, and 12-15 mo 1 dose for all healthy children 24-59 mo who are not completely vaccinated for age 1 supplemental dose of PCV13 for certain children who received 1 dose of PCV7: (1) all children 14-59 mo and (2) children 60-71 mo with underlying medical conditions	MI between doses: 4-8 wk, depending on age Number of doses varies with age at first dose administered and current age.	MIs between doses depend on age. Children receiving the first dose of vaccine at ≥7 mo require fewer doses to complete the series. Pneumococcal polysaccharide vaccine (PPV23) is routinely recommended for high-risk persons aged ≥2 years. See *MMWR Morb Mortal Wkly Rep* 2010;**59**(RR 11).
Polio-inactivated poliovirus vaccine (IPV)	6 wk	4 doses at ages 2, 4, 6-18 mo and 4-6 yr	Previously unvaccinated children: 3 doses MI between doses: 4 wk Preferred interval between dose 2 and dose 3: 2 mo Minimum age for dose 2: 10 wk Minimum age for dose 3: 14 wk Minimum age for dose 4: 18 wk Previously unvaccinated children <4 yr at completion of dose 3: 4 doses Previously unvaccinated children ≥4 yr at completion of dose 3: 3 doses MI between doses: 4 wk If ≥4 doses are administered before 4 yr, an additional dose should be given at 4-6 yr. The final dose in the series should be administered on or after the 4th birthday and at least 6 mo after the previous dose.	Primary series is recommended for all travelers; booster dose for persons ≥18 yr is recommended for travelers to some areas of Africa, Asia and the Middle East; not routinely recommended for travel to Latin America. Single booster of IPV for previously vaccinated adults confers lifetime immunity. Oral poliovirus vaccine (OPV) is no longer recommended or available in the United States. For use in other countries, the World Health Organization recommends birth as the minimum age of administration. The MI between doses is 4 wk. OPV can be used for children younger than 6 wk traveling to areas with polio transmission.

Rotavirus (RV): RV1 (Rotarix) and RV5 (Rota Teq)	6 wk	RV1: 2 doses at ages 2, 4 mo RV5: 3 doses at 2, 4, and 6 mo	MI between doses: 4 wk; there is no maximum interval between doses. Minimum age for dose 2: 10 wk Minimum age for dose 3: 14 wk	Maximum age for first dose in series is 14 wk, 6 days; and 8 mo, 0 days for final dose in series Can be administered before, concurrently or after administration of immunoglobulin. See CDC website for information about risk for intussusception with rotavirus vaccine
Varicella	12 months	2 doses at 12-15 mo and 4-6 yr	MI between dose 1 and dose 2 for children <13 yr: 12 wk MI between dose 1 and dose 2 for persons ≥13 yr: 4 wk Minimum age for dose 2: 15 mo	Do not administer varicella vaccine for 3 mo after immunoglobulin for hepatitis A prophylaxis; see *MMWR Morb Mortal Wkly Rep* 2005;**54**:Q1-4, for deferral times for blood products and immunoglobulin administered for other indications. Defer immunoglobulin administration for 2 wk after varicella vaccination. Combination MMRV can be used for children 12 mo–12 yr but is associated with higher risk for fever and febrile seizures 5-12 days after the first dose among children 12-23 mo.

*Number of doses and recommended ages. Minimum age is the U.S. recommendation unless otherwise noted. Recommendations in the U.S. schedule may be different from those of the World Health Organization and other countries.

†Number of doses and MI between doses. *NOTE:* Interval represents time to dose.

‡Contraindications: Anaphylactic reaction to prior dose or to any vaccine component or moderate-to-severe acute illness is a contraindication for all vaccines.

§See MMWR Recomm Rep 2006;**55**(RR-7):1-23.

¶Combination hepatitis A–hepatitis B (Twinrix) is available for use in persons ≥18. The vaccine, which is composed of inactivated viral components, is administered intramuscularly. The schedule involves 3 doses at 0, 1, and 6 months. It can be administered at an accelerated schedule of 4 doses at 0, 7, and 21-30 days and 1 year.

DTap, Diphtheria and tetanus toxoids and acellular pertussis, vaccine; *DT,* diphtheria-tetanus (toxoid); *IPV,* inactivated poliomyelitis vaccine; *MI,* minimal interval; *MMR,* mumps, measles, rubella; *PRP-OMP,* PRP-outer membrane protein conjugate vaccine; *Tdap,* tetanus, diphtheria, and pertussis; *Td,* tetanus and diphtheria (toxoid).

TABLE 231-2 **Common Travel-Related Vaccines and Immunoglobulin for Children**

Vaccine*	Vaccine Type	Route	Minimum Age	Primary Series†	Booster or Revaccination‡	Accelerated Schedule	Protection After	General Indications for Use§
BCG	Live attenuated bacteria	ID, SC	Birth	1 dose	None	None	2 months after dose (WHO)	Consider for children <1 yr who will be long-term travelers residing in high-risk areas to protect against meningeal and miliary tuberculosis.
Immunoglobulin	Antibody from pooled human plasma	IM	Birth	1 dose	3-6 mo	None	Immediate	Use to prevent hepatitis A infection in children <1 yr who are traveling to risk areas or for persons departing within 4 wk. Defer MMR and varicella vaccination for 3 mo after IG administered for hepatitis A prevention; defer the administration of IG for hepatitis A prevention for 2 wk after MMR or varicella vaccination. Defer rotavirus vaccine (RV) administration for 6 wk if possible. However, if 6-wk deferral would cause the first dose of RV to be scheduled for age ≥13 wk, a shorter deferral interval should be used to ensure that the first dose of RV is administered no later than age 13 wk.
Japanese encephalitis (IXIARO)	Inactivated virus	SC	1 yr	2 doses on days 0 and 28	yearly Booster: 1 dose	None	7 days after dose 2	Recommended for travelers who plan to spend a month or longer in endemic areas during the JEV transmission season. Consider for short-term travelers (<1 mo) to endemic areas during JEV transmission season if they plan to travel outside of an urban areas and have an itinerary or activities that will increase their risk for JEV exposure; travelers to areas with an ongoing JE outbreak; and travelers to endemic areas who are uncertain of specific destinations, activities, or duration of travel. Not recommended for short-term travelers whose visit will be restricted to urban areas or times outside of a well-defined JEV transmission season.

								Licensed for use in persons ≥17 yr. Options for children <17 yr of age include off-label use of IXIARO, participation in a clinical trial or vaccination with an alternative JE vaccine overseas. See CDC website for further information. Clinicians should review updated data about risk areas because transmission varies by country and season. Destinations with transmission include to selected areas of Asia, Oceania (Australia and Papua New Guinea), Russia, especially long-term travelers to rural, agricultural endemic areas or epidemic regions during seasonal transmission (usually May to September). Mild adverse reactions (headache, myalgia, fatigue, influenza-like illness) reported in 13%-26% of vaccinees. There are no data on the use of IXIARO as a booster dose after a primary series with JE-VAX. Persons who received JE-VAX should receive the primary 2-dose series of IXIARO
Rabies Human diploid cell vaccine (HDCV) Purified chick-embryo cell culture vaccine (PCEC)	Inactivated virus; cell culture derived	IM	United States: None	3 doses at 0, 7, and 21 or 28 days	No boosters or serologic testing recommended for routine international travelers. Boosters and/or serologic testing recommended for high-risk groups. See CDC website for additional information.	None	14 days after dose 3	Use for travelers to rabies-endemic countries. Vaccination is recommended for long-term travel in rural areas or in areas with limited access to health care. Travelers with extensive unprotected outdoor, evening, and nighttime exposure in rural areas, such as backpacking, should consider vaccination even for trips of short duration. Widespread worldwide rabies distribution. See Health Information for Travelers for list of countries that have not reported cases of rabies recently.

Continued on following page

TABLE 231-2 **Common Travel-Related Vaccines and Immunoglobulin for Children** (Continued)

Vaccine*	Vaccine Type	Route	Minimum Age	Primary Series†	Booster or Revaccination‡	Accelerated Schedule	Protection After	General Indications for Use§
								Postexposure vaccination is required even for persons who receive vaccination before exposure. After exposure in vaccinated person: 2 doses at 0, 3 days (no rabies immunoglobulin required) After exposure in unvaccinated person: Rabies immunoglobulin (RIG) (20 IU/kg) plus vaccination with 4 doses at 0, 3, 7, and 14 days. Rabies immunoglobulin can be administered up to and including day 7 of the postexposure prophylaxis series.
Typhoid (ViCPS)	Capsular polysaccharide	IM	2 yr	1 dose	2 yr	None	14 days after dose	Use recommended for travelers to Africa, Asia, and Latin America for long-term stays, travel outside usual tourist destinations, or travelers who desire maximal protection. Patients should be counseled about protective efficacy of vaccine (approximately 50%-74% when studied in endemic areas) and importance of other preventive measures.
Typhoid oral (Ty21a)	Live attenuated bacteria	Oral	6 yr	4 doses at 0, 2, 4, 6 days	5 yr	None	7-10 days after last dose	See indications for typhoid ViCPS. Patients should be counseled about protective efficacy of vaccine (approximately 60%-85% when studied in endemic areas) and importance of other preventive measures.

								Must be swallowed (cannot be chewed) 1 hr before meal, and vaccine must be refrigerated until administration. Do not administer within 72 hr of any antibacterial agent, including doxycycline. Mefloquine, atovaquone-proguanil, and chloroquine can be administered concurrently. Contraindicated for immunocompromised patients
Yellow fever	Live attenuated virus	SC	6 mo	1 dose	10 yr	None	10 days after dose	Use for travelers to selected areas of Africa and South America. Yellow fever has not been reported in Asia; however, some countries require proof of vaccination if the traveler is arriving from a yellow fever–endemic country. Yellow fever vaccination for children 6-9 mo should be discussed with an expert to weigh risk for adverse events against risk for yellow fever. Reports of rare serious adverse events of multiorgan system failure after vaccination. Contraindicated for patients who are immunocompromised or have allergy to eggs.

*Cholera vaccine is not available in the United States. Tick-borne encephalitis vaccine is not available in the United States. It is administered as a 4-dose series at 0, 28-day, 42-day, and 1-yr intervals, with booster doses required every 3 to 5 yr. The vaccine is available in Europe.
†Number of doses and recommended ages and intervals. *NOTE:* interval represents time from dose.
‡Number of doses and recommended ages and intervals.
§Contraindications: Anaphylactic reaction to prior dose or to any vaccine component or moderate-to-severe acute illness is a contraindication for all vaccines.
BCG, Bacille Calmette-Guérin; *CDC,* Centers for Disease Control and Prevention; *ID,* intradermal; *IM,* intramuscular; *JE,* Japanese encephalovirus; *MMR,* measles, mumps, rubella; *SC,* subcutaneous; *WHO,* World Health Organization.

The incidence is 0.8/100,000 doses. The vaccine should not be used in children younger than 6 months of age. It should be used with caution in children 6 to 9 months of age after discussion with a travel medicine expert to weigh the risks and benefits.[53] Two cases of YEL-AND have been reported in children 6 to 8 months of age. Whenever possible, travel to yellow fever–endemic countries should be postponed for children younger than 9 months of age. Medical waivers can be given to children who are too young for vaccination and to those who have other contraindications to vaccination, such as immunodeficiency. Breast-feeding is a precaution for yellow fever vaccination. Three cases of transmission to infants have been associated with breast-feeding. None of the cases had permanent sequelae, but until more information is available, yellow fever vaccination should be avoided in breast-feeding women, if possible. Since 2001, yellow-fever vaccine-associated viscerotropic disease (YEL-AVD), life-threatening severe illness with major organ system failure similar to wild-type disease has been reported in more than 50 confirmed and suspected cases worldwide. The syndrome usually consists of fever, jaundice, and multiple organ system failure. The case/fatality ratio is 65 percent. The usual onset is 3 days (range, 1 to 8 days) after vaccination. The incidence of YEL-AVD cases in the United States is 0.4/100,000 doses of vaccine administered. The rate is higher in people aged 60 years of age and older, with a rate of 1.0/100,000 doses in people 60 to 69 years of age and 2.3/100,000 doses in people aged 70 years of age and older. The risk is thought to be approximately 1/200,000 to 300,000 doses in persons younger than 60 years and 1/40,000 to 50,000 doses in persons 60 years of age and older. The risk is greater in persons with a history of thymus disorder associated with abnormal immune cell function such as thymoma, myasthenia gravis, or DiGeorge syndrome.[58] Further studies are being conducted.[60,106,141] In the interim, the CDC has recommended that given the risk for serious illness and death from yellow fever,[101] evidence of increasing transmission of the disease,[128] and the known effectiveness of the vaccine, clinicians should continue to use yellow fever vaccine.[53] Health care providers should carefully review travel itineraries to ensure that only people traveling to areas endemic for yellow fever or areas where yellow fever activity is reported receive yellow fever vaccine.[26,29,53,156]

Japanese encephalitis is a viral infection transmitted by *Culex* mosquitoes that bite from dusk to dawn in an enzootic cycle between mosquitoes and vertebrate hosts, primarily pigs and wading birds. Japanese encephalitis is primarily a disease of childhood, with most cases occurring among children younger than 15 years of age. Disease develops in fewer than 1 percent of infected persons.[49] Japanese encephalitis occurs year-round in tropical regions and primarily from May through October in temperate zones. The risk is greatest for travelers to rural Asia, where the mosquito breeds in rice fields and other agricultural areas. Clinicians should consult the CDC Travelers Health website for the current list of areas with endemic and epidemic transmission. Japanese encephalitis is associated with high rates of case fatalities and severe neurologic sequelae, especially in young children and the elderly. Among an estimated 35,000 to 50,000 annual cases worldwide, approximately 20 to 30 percent of patients die, and 30 to 50 percent of survivors have neurologic or psychiatric sequelae.[49] However, Japanese encephalitis is rare among travelers, estimated at less than 1/1 million travelers. During 1973 to 2008, a total of 55 cases of travel-associated Japanese encephalitis among persons from nonendemic countries were reported in the literature. Five (9%) of the cases occurred in children 10 years of age or younger.[53] Expatriates and travelers who stay for prolonged periods in rural areas likely have a risk similar to that of the local population in affected areas (5 to 50/100,000/yr).[53] All travelers to risk areas should use personal protective measures, such as repellent and long-sleeve clothing, to minimize mosquito bites. Vaccination is recommended for travelers who plan to spend a month or longer in endemic areas during the Japanese encephalitis transmission season. Vaccination should be considered for travelers who will spend 30 days or longer in endemic areas during transmission season if they plan to travel outside of an urban area and have an itinerary or activities that will increase their risk for exposure; travelers to an area with an ongoing outbreak; and travelers to endemic areas who are uncertain of specific destinations, activities, or duration of travel. Consider vaccination for short-term travelers (<1 month) to endemic areas during transmission season if they plan to travel outside of an urban areas and have an itinerary or activities that will increase their risk for exposure. Vaccination is not generally recommended for short-term travelers (<30 days) whose visit will be restricted to urban areas or times outside of a well-defined transmission season.[53] Since 2009, one Japanese encephalitis vero culture–derived vaccine, IXIARO, has been licensed for use in children 17 years of age and older in the United States. IXIARO is administered as a two-dose series, with the second dose administered 28 days after the first dose. JE-Vax, an inactivated mouse brain–derived vaccine, was licensed in the United States in 1992 for use in travelers 1 year of age and older. In 2006, JE-Vax production was discontinued, and all remaining doses expired in May 2011. If the primary series of IXIARO was administered 1 year or more previously, a booster dose should be given before potential reexposure or if there is a continued risk for Japanese encephalitis virus infection. No data are available on the use of IXIARO as a booster dose after a primary series with JE-Vax. People who have received JE-Vax previously and require further vaccination against JEV should receive a two-dose primary series of IXIARO. Clinicians should consult the CDC website regarding options for younger children who would benefit from Japanese encephalitis virus vaccination. Other inactivated and live attenuated Japanese encephalitis vaccines are manufactured and used in Asia but are not licensed for use in the United States. Current options include participation in a clinical trial, off-label use of IXIARO, or vaccination in Asia with an alternative Japanese encephalitis vaccine.[53] See Table 231-2 for additional information.

Rabies virus, which causes a progressive fatal encephalomyelitis, occurs worldwide, except in Antarctica. In certain areas of the world, including (but not limited to)

parts of Africa, Asia, and Central and South America, canine rabies remains highly endemic. Rabies also occurs in other wild animals, including bats. The three-dose preexposure rabies vaccine should be considered for children visiting rabies-endemic countries for longer than 1 month, those undertaking extensive outdoor activities such as backpacking or camping in endemic countries, or children traveling to areas where access to health care is limited. Booster doses for persons who completed the three-dose preexposure vaccine series or the full postexposure prophylaxis are considered preimmunized and do not require routine boosters. Routine booster doses and/or serologic testing are recommended for certain high-risk persons working with rabies virus or persons, such as cavers, with frequent exposure to mammals, such as bats. To reduce the risk for acquiring rabies, children and their families should be counseled to stay away from stray dogs and other animals, especially if traveling to Latin America, Asia, or Africa.[53] Exposure is considered a medical emergency. Wound management should include thorough cleansing with soap, water, and povidone-iodine, if available, and suturing delay of a few days, if possible. Postexposure prophylaxis should be administered as soon as possible; travelers who received preexposure vaccination should receive two booster doses on days 0 and 3 after exposure, whereas persons who did not receive preexposure vaccination should receive rabies immunoglobulin and a series of four injections of rabies vaccine over 14 days.[45,50]

Typhoid infection is an acute, life-threatening illness caused by *Salmonella enterica* serotype Typhi, which is usually acquired through contaminated food and water. Vaccination is recommended for pediatric travelers visiting developing countries, especially for prolonged periods, or traveling outside the usual tourist destinations. Travelers visiting friends and relatives are at increased risk. Two vaccines are available in the United States: (1) an attenuated, live oral vaccine that can be administered to children 6 years of age and older and (2) an injectable Vi capsular polysaccharide vaccine that can be administered to children, starting at 2 years of age. The injectable vaccine should be completed 2 weeks or longer before travel, and boosters should be administered every 2 years The oral vaccine consists of four capsules, one taken every other day, and should be completed 1 week before travel. Boosters should be administered every 5 years. Parents should be cautioned, however, that vaccination is not 100 percent effective, and precautions regarding safe food and water should be followed.[53]

PREVENTION OF MALARIA

Epidemiology of Malaria

Malaria is a parasitic infection caused by one of four species of *Plasmodium: Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, or *Plasmodium malariae*. It is transmitted by the bite of an infective female *Anopheles* mosquito. Malaria is endemic in more than 100 countries on five continents; transmission occurs primarily in the tropic and subtropical regions of sub-Saharan Africa, Asia, Latin America, the Caribbean, the Middle East, and Oceania. Globally, malaria is responsible for 250 million clinical infections and nearly 1 million deaths annually, with most caused by *P. falciparum*, principally in young children and pregnant women.[150,153]

An estimated 10,000 to 30,000 travelers from industrialized countries are thought to contract malaria each year, but these numbers probably are underestimated because they do not include travelers in whom malaria was diagnosed and treated abroad.[91,93,103,149] The number of malaria cases reported in 2010 in the United States was the largest since 1980. In 2010, 1691 cases of malaria, including 9 fatal cases, were reported to CDC. All fatal cases were *P. falciparium*, and 7 were acquired in West Africa. Among the 898 cases in U.S. civilians for whom information on chemoprophylaxis use and travel area was known, only 45 (5%) reported that they had followed and adhered to a chemoprophylactic drug regimen recommended by CDC for the areas to which they had traveled. Among the 1573 cases among patients for whom age was known, 232 (15%) occurred in persons younger than 18 years of age. Among the 232 cases among persons younger than 18 years, 108 (47%) occurred among U.S. civilian children, 101 (44%) occurred among children of persons categorized as having a foreign resident status at the time their malaria infection was acquired, and 23 (10%) occurred among children of unknown resident status. Of the 108 cases among U.S. civilian children, 4 (4%) were younger than 24 months, 10 (9%) were 2 to 4 years, 54 (50%) were 5 to 12 years, and 40 (37%) were 13 to 17 years of age. Of these cases among U.S. civilian children for whom country of exposure was known 75 (75%) were attributable to travel to Africa. Among the 83 U.S. civilian children for whom reason for travel was known, 69 (83%) were visiting friends and relatives, 10 (12%) were traveling for educational purposes, 2 (2%) were traveling for missionary work, and 2 were either tourists or specified as other. Of the 85 children for whom chemoprophylaxis information was known, 26 (31%) were reported as having taken prophylaxis, of whom 16 (62%) had taken an appropriate regimen; however, only 5 (31%) of these patients reported adherence.[23]

Reviews of deaths attributed to malaria in the United States indicate that failure to take or to adhere to recommended antimalarial chemoprophylaxis, promptly seek medical case for posttravel illness, and promptly diagnose and treat suspected malaria contributed to fatal outcomes.[30,54,115] Retrospective reviews of malaria in children also have found that a substantial proportion of cases occurred in recent immigrants and the children of former immigrants who had traveled to visit their family's country of origin.[12,83,127] These data highlight the importance of pretravel assessment and counseling, particularly for foreign-born persons, who may assume they are protected by natural immunity or do not perceive the risk for acquisition of malaria by their children who accompany them on returning to visit their home country. The CDC recommends that prevention messages directed toward Africa-bound travelers, particularly those whose destination is West Africa, should be emphasized in early spring, accompanied with a reminder in late fall through early winter. Malaria prevention messages directed

toward Asia-bound travelers, specifically those bound for India, should be intensified in late spring.[54]

Preventing Malaria in Pediatric Travelers

The substantial proportion of U.S. civilian malaria cases reported in children younger than 18 years of age underscores the importance of pretravel counseling and strategies for malaria prevention. Young children and nonimmune persons of any age are at greater risk for development of severe complications from malaria. Prevention of malaria in pediatric travelers depends first on obtaining current and accurate information about the risk for acquiring malaria in proposed travel destinations and determining whether planned activities, such as rural versus urban travel, and the season of travel place the traveler at increased risk for exposure. Information on the geographic- and country-specific risks for acquiring malaria is available from multiple sources. Selected websites with information about the country-specific risk for acquiring malaria are listed in this chapter in the discussion on international travel information resources.

After a thorough assessment of the risk for acquiring malaria has been made, prevention strategies for pediatric travelers are twofold: personal protection measures against contact with mosquitoes and antimalarial chemoprophylaxis. The first mainstay of prevention of malaria is appropriate and effective use of personal protection measures. All travelers to malaria-endemic areas should be counseled on recommended measures to avoid bites from *Anopheles* mosquitoes, which typically are evening and nighttime feeders. Such measures include wearing clothing that reduces the amount of exposed skin (e.g., long-sleeved shirts, long pants tucked into socks, hats, stroller nets) and, whenever possible, remaining in well-screened or enclosed air-conditioned areas. Travelers staying overnight in facilities without air conditioning or screens should use insecticide-treated mosquito nets over the beds. Stroller nets can be used for children in strollers. Bed nets that have been treated with insecticide such as permethrin are more effective in preventing malaria than are untreated bed nets and are safe for children.[116] Travelers can purchase permethrin to spray on the bed nets or purchase pretreated (or impregnated) bed nets. Permethrin also can be sprayed on clothing, but it should not be applied directly to skin. Permethrin remains effective through multiple washings. Clothing and bed nets should be retreated according to the product label. Permethrin should not be applied to the skin. Although permethrin provides longer duration protection, recommended repellents that can be applied to skin (DEET [*N*,*N*-diethyl-*m*-toluamide], picaridin, oil of lemon eucalyptus [OLE], or *para*-menthane-3,8-diol (PMD, the synthesized version of OLE), and IR3535 can also be used on clothing and mosquito nets. During the evening, insecticide also can be sprayed inside rooms.

Another important focus of counseling for personal protection measures is appropriate use of insect repellent on exposed skin. The CDC recommends the use of repellent products with active ingredients registered with the U.S. Environmental Protection Agency (EPA). Products containing the following active ingredients provide long-lasting protection (DEET, picaridin (KBR 3023), IR3535, and OLE or PMD), the synthesized version of OLE. Note that "pure" oil of lemon eucalyptus (essential oil) is not the same product as OLE insect repellent. Some controversy has occurred regarding the recommended concentration of DEET for pediatric use. In 1998, the EPA conducted an extensive review of DEET safety. The agency concluded that no evidence indicates that DEET is toxic to infants or children. Additional evaluations have not demonstrated a link between seizures and topical use.[16,53]

Most repellents can be used on children older than 2 months of age, with the following considerations: (1) products containing OLE specify that they should not be used on children younger than 3 years; (2) repellent products must state any age restriction (if none is stated, the EPA has not required a restriction on the use of the product); and (3) many repellents contain DEET as the active ingredient. DEET formulations of up to 50 percent can be used for children older than 2 months. The concentration of DEET affects the duration of protection. Higher concentrations provide longer protection; however, the duration of protection reaches a plateau at approximately 30 to 50 percent. Therefore, most clinicians recommend products having up to 30 percent for children. The American Academy of Pediatrics recommends that 30 percent or lower DEET should be used on children over 2 months of age and repellents with DEET should not be used on infants younger than 2 months.[4] In a laboratory study, a product with 23.8 percent DEET provided an average of 5 hours of protection (range, 3 to 6 hours) and a product with 6.65 percent DEET provided an average of 2 hours of protection (range, 1.5 to 2.8 hours). Duration of protection may be affected by the environmental temperature, sweating, and wind conditions.[16,26,72,76] Products that contain repellents and sunscreen generally are not recommended because of the need to reapply sunscreen more frequently than repellent.[4,5,113] Mosquito coils should be used with extreme caution in the presence of children to avoid burns and inadvertent ingestion.[21]

Clinicians are encouraged to stress to parents and children alike the importance of using personal protection measures. Despite the demonstrated efficacy of these measures, studies have found that only 17 percent of adult travelers with malaria reported using insect protection methods and only 11 percent took the recommended chemoprophylaxis.[93]

The second mainstay of malaria prevention is chemoprophylaxis. Selection of the appropriate drug for antimalarial chemoprophylaxis must be based on numerous factors: the most recent information available about malaria in the proposed travel destinations; the trip itinerary; the age, weight, and medical history of the traveler; the personal preference for the frequency of dosing and the duration of chemoprophylaxis on return from the trip; and the cost of medication. These decisions can be challenging for primary care providers and clinicians with limited experience in infectious disease and travel medicine, so when in doubt, clinicians should seek the advice of a travel medicine or infectious disease expert. In

addition, the CDC provides resources with guidance on appropriate use of and recommended regimens for antimalarial chemoprophylaxis (listed in the section on international travel information resources).

Figure 231-1 outlines an algorithm for determining appropriate antimalarial chemoprophylaxis regimens for pediatric travelers. Because data on the distribution of drug-resistant malaria are evolving constantly, clinicians always should obtain the most recent information about the risk for malaria and zones of drug resistance from the CDC website or other sources of information before prescribing malaria chemoprophylaxis.[43,53,131]

The first decision point in selecting appropriate antimalarial chemoprophylaxis is whether travel is occurring in a region of chloroquine-sensitive or chloroquine-resistant malaria. For travel to areas with chloroquine-sensitive malaria, chloroquine is the drug of choice for antimalarial chemoprophylaxis. *P. ovale*, *P. malariae*, and most *P. vivax* are widely sensitive to chloroquine; however, chloroquine-resistant *P. vivax* is an emerging problem and has been reported from Guyana, Papua New Guinea, India, Burma (Myanmar), and areas of Indonesia.[51,93] In addition to chloroquine-resistant *P. vivax*, chloroquine-resistant *P. falciparum* has been reported from these areas, and consequently chloroquine would not be recommended as chemoprophylaxis for travelers to these regions.

If the traveler is visiting a region with chloroquine-resistant malaria, the next decision point is whether travel will include regions with chloroquine-resistant malaria only or both chloroquine- and mefloquine-resistant malaria. Chloroquine-resistant *P. falciparum* is widespread and exists in all malaria-endemic areas except Mexico, the Caribbean, Central America west of the former Panama Canal Zone, and some parts of the Middle East and China.[53,92] In some regions, *P. falciparum* may be resistant to both chloroquine and mefloquine; these areas currently are limited to the borders of Thailand with Myanmar (Burma) and Cambodia, the western provinces of Cambodia that border Thailand, and the eastern states of Myanmar (Burma) on the border between Burma and China, along the borders of Laos and Burma, the adjacent parts of the Thailand-Cambodia border, and southern Vietnam.[53,92] For travel to areas with chloroquine-resistant malaria, three current antimalarial chemoprophylaxis options are mefloquine (Lariam), atovaquone-proguanil (Malarone), and doxycycline. The CDC no longer recommends the use of chloroquine-proguanil for

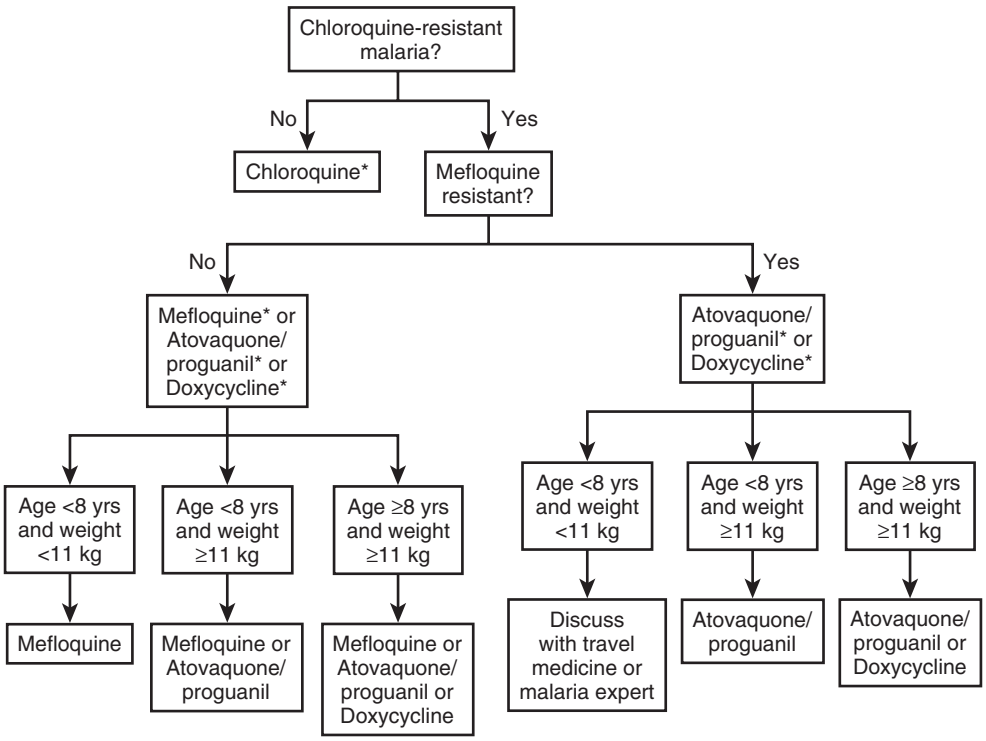

FIGURE 231-1 ■ Algorithm for determining appropriate antimalarial chemoprophylaxis regimens for pediatric travelers.

chemoprophylaxis in chloroquine-resistant areas. For travel to areas with chloroquine-resistant and mefloquine-resistant malaria, either atovaquone-proguanil or doxycycline can be used. Primaquine can be used as an option for primary prophylaxis in special circumstances. Clinicians should contact the CDC Malaria Hotline (770-488-7788 or 1-855-856-4713) for additional information. Primaquine also can be used for terminal prophylaxis to prevent relapses of *P. vivax* or *P. ovale*. In general, terminal prophylaxis is indicated only for persons who have had prolonged exposure to malaria-endemic areas (e.g., missionaries and expatriates).[53]

In evaluating options for antimalarial chemoprophylaxis, physicians should review each medication for contraindications and weight and age restrictions (Table 231-3). Pediatric doses of medications used for treatment should be calculated on the basis of body weight. Medications used for infants and young children are the same as those recommended for adults, except under the following circumstances: doxycycline should not be given to children younger than 8 years of age because of the risk for teeth staining, and atovaquone-proguanil should not be used for prophylaxis in children weighing less than 5 kg because of lack of data on safety and efficacy.

Chloroquine is relatively well tolerated in children. In the United States, chloroquine is available in tablet form; in Europe and other countries, it is also available as a syrup. Mefloquine can be used safely in children weighing less than 9 kg and may be useful for longer trips because it is administered once weekly.[104] However, it must be continued for 4 weeks after leaving the malarious area and no liquid preparation is available. Doses for children are one fourth, one half, and three fourths of a tablet, depending on weight. Mefloquine has been associated with rare but serious adverse reactions (e.g., psychoses and seizures) at prophylactic doses; these reactions are more frequent with higher doses used for treatment. Other side effects that have occurred in chemoprophylaxis studies include depression, anxiety disorder, mood changes, panic attacks, and other neuropsychiatric symptoms. Mefloquine is contraindicated in travelers with active depression, a recent history of depression, generalized anxiety disorder, psychosis, schizophrenia, other major psychiatric disorders, or seizures. It should be used with caution in people with a history of depression or psychiatric disorders. No data are available on the use of atovaquone-proguanil in children weighing less than 5 kg; however, studies are in progress. For children who weigh 5 kg or more and are at risk for acquiring chloroquine-resistant *P. falciparum* infection, atovaquone-proguanil can be advantageous for short trips because it is started 1 to 2 days before the trip commences and can be stopped 7 days after the trip. However, the patient or parent must remember to take or to administer the medication daily. It is available in pediatric tablet form. Doxycycline is contraindicated in children younger than 8 years because of concern about the propensity of tetracyclines to stain growing teeth or potentially affect developing bones. For older children, doxycycline must be administered daily and continued for 4 weeks after departing the malaria-endemic area.[53,92]

Chloroquine, mefloquine, and atovaquone-proguanil have a bitter taste. Before departure, pharmacists can be asked to pulverize tablets and prepare gelatin capsules with calculated pediatric doses. Mixing the powder in a small amount of food or drink can facilitate the administration of antimalarial drugs to infants and children. Additionally, any compounding pharmacy can alter the flavoring of malaria medication tablets so that they are more willingly ingested by children. Assistance with finding a compounding pharmacy is available on the International Academy of Compounding Pharmacists' website (http://www.iacprx.org). Because overdose of antimalarial drugs, particularly chloroquine, can be fatal, medication should be stored in childproof containers and kept out of the reach of infants and children.

The importance of determining appropriate antimalarial chemoprophylaxis regimens for travelers and counseling to improve compliance cannot be overemphasized. In retrospective reviews of pediatric malaria cases, between 75 and 100 percent of infected children had received inadequate or no chemoprophylaxis.[53,127,143] Finally, effective pretravel malaria prevention counseling includes anticipatory guidance for parents about recognition of and response to symptoms of malaria infection in young pediatric travelers. Parents should be counseled that although compliance with personal protection measures and chemoprophylaxis will decrease the risk, it cannot guarantee prevention of malaria infection. They should be instructed about the symptoms of malaria infection, such as fever, headache, vomiting, diarrhea, and myalgia, and be counseled to seek immediate medical attention if symptoms occur. Delay in recognition and treatment of malaria is associated directly with an increase in rates of morbidity and mortality; therefore, prompt and appropriate initiation of effective therapy is paramount.* Parents also should be advised that some types of malaria can become symptomatic several weeks to months after exposure and therefore prompt medical attention should be sought even if illness develops in a child months after international travel to malaria-endemic areas.

PREVENTION OF TRAVELER'S DIARRHEA IN CHILDREN

Epidemiology of Traveler's Diarrhea

One of the most difficult tasks faced by international travelers of any age is ensuring the safety of food and water. Traveler's diarrhea, caused by the ingestion of contaminated food and water, typically is defined as the occurrence of four or more unformed stools in a 24-hour period or three or more unformed stools in an 8-hour period with at least one of the following signs or symptoms: temperature higher than 38.0° C (100.4° F), abdominal cramping, nausea, vomiting, fecal urgency, tenesmus, or blood or mucus in stools.[68,70,120] Traveler's diarrhea affects approximately 20 to 50 percent of adult travelers and is the health problem most frequently

*References 53, 78, 91, 93, 134, 149.

TABLE 231-3 **Antimalarial Chemoprophylaxis Regimens for Pediatric Travelers**

Medication	Regimen	Dose	Contraindications and Precautions	Side Effects	General Indications and Information for Use
Chloroquine (Aralen)	Weekly starting 1-2 wk before trip Continue weekly during trip and for 4 wk after trip	5 mg base/kg (8.3 mg salt/kg) up to 300 mg base (500 mg salt) Tablets: 300 mg base (500 mg salt)	Prior retinal or visual field changes Psoriasis (may be exacerbated by chloroquine)	Gastrointestinal symptoms, seizures, rash headache, dizziness, pruritus (especially in dark-skinned persons), blurred vision, decreased hearing, tinnitus, retinal damage at high cumulative doses* High toxicity in overdoses, keep out of reach of children	Use only in areas of chloroquine-sensitive malaria Limited usefulness because of widespread chloroquine resistance Bitter taste
Mefloquine (Lariam)	Weekly starting 1 week before trip Continue weekly during trip and for 4 wk after trip	≤9 kg: 4.6 mg/kg base (5 mg/kg salt) 15-19 kg: ¼ tablet 20-30 kg: ½ tablet 31-45 kg: ¾ tablet ≥46 kg: 1 tablet Tablets: 228 mg base (250 mg salt)	Psychiatric conditions, cardiac conduction disorders, seizure disorders, persons with known hypersensitivity to mefloquine Should not take with quinine-like drugs	Gastrointestinal symptoms, dizziness, insomnia Occasional serious adverse effects: seizures, nightmares, depression, anxiety, psychosis, especially in persons with these preexisting medical conditions	Use in areas with chloroquine-resistant malaria Advantageous for long-term travelers because of weekly dosing and less costly than atovaquone-proguanil. Bitter taste
Atovaquone-proguanil (Malarone)	Daily starting 1-2 days before trip Continue daily during trip and for 7 days after trip	5-8 kg: 1/2 pediatric tablet (62.5 mg atovaquone/25 mg proguanil hydrochloride) 9-10 kg: 3/4 pediatric tablet 11-20 kg: pediatric tablet ≥40 kg: 1 adult tablet (250 mg atovaquone and 100 mg proguanil hydrochloride) Pediatric tablets: 62.5 mg atovaquone and 25 mg proguanil hydrochloride Adult tablets: 250 mg atovaquone and 100 mg proguanil hydrochloride	Contraindicated in severe renal failure Not recommended or use with caution for children <11 kg, pregnant or lactating women Do not take with tetracycline, metoclopramide, rifampin, or rifabutin (all reduce concentrations of atovaquone)	Gastrointestinal symptoms, headache, loss of appetite, dizziness, pruritus	Use in areas with chloroquine-resistant or mefloquine-resistant malaria. Advantageous for short-term travelers because prophylaxis can be stopped 1 week after leaving malaria area. Available in pediatric tablets. Take with food or milk.
Doxycycline	Daily starting 1-2 days before trip Continue daily during trip and for 4 wk after trip	2 mg/kg up to 100 mg daily Tablets: 50 mg, 100 mg	Do not use for children <8 yr, pregnant or lactating women Do not give within 3 hr of antacids, iron or Pepto-Bismol Do not administer oral typhoid vaccine within 72 hr of doxycycline	Gastrointestinal symptoms, photosensitivity, increased blood urea nitrogen level, hypersensitivity reactions, blood dyscrasias, vaginal candidiasis May decrease the effectiveness of oral contraceptives	Use in areas with chloroquine-resistant and mefloquine-resistant malaria (borders of Thailand with Myanmar (Burma) and Cambodia, the western provinces of Cambodia that border Thailand, and the eastern states of Myanmar (Burma), on the border between Burma and China, along the borders of Laos and Burma, the adjacent parts of the Thailand-Cambodia border, and in southern Vietnam. Take with food; taking with food or milk can decrease gastric irritation (absorption of doxycycline is not significantly decreased by simultaneous administration of milk or food).

*Despite the use of chloroquine as an antimalarial chemoprophylaxis agent for decades and the use of high-dose chloroquine for certain chronic diseases, the literature is inconclusive about the potential risk for retinopathy associated with long-term use of chloroquine for antimalarial prophylaxis. Retinopathy has rarely been reported in patients receiving weekly prophylaxis. Retinopathy appears to be related to dosage and accumulated dosage.

reported by travelers to developing countries.[67,68] It can occur any time during travel but typically occurs within the first week or two of the trip. The illness usually is self-limited, and for adults the average duration of illness has been reported to be 3 to 5 days.[70] Less information about traveler's diarrhea in children is known and the studies show conflicting data about whether certain age groups are more affected. In a recent prospective study among 152 children 2 to 18 years of age and their parents from the Netherlands, one third of the children experienced diarrhea during international travel and, of these, 30 percent graded their illness as moderate or severe. Of 328 children who sought care for travel-associated illness at the emergency room of the University of Zürich Children's Hospital, 129 (39%) had a diarrheal illness, including 3 with *Salmonella typhi* and 2 with *Salmonella paratyphi* infection. The proportion of children with diarrhea increased with age—33 percent in children age 0 to younger than 2 years, 39 percent in children age 2 to 5 years and younger, and 45 percent in children 6 to 16 years. Among the ill pediatric travelers visiting family and relatives, 41 percent had diarrhea.[85] In a study of 890 children younger than 20 years of age presenting to the outpatient travel clinic of the University of Munich after travel to the tropics and subtropics and their associates, 25 percent had acute diarrhea, the most frequent syndrome among all ill travelers. The most frequently diagnosed infectious enteric pathogens were giardiasis (8%), *Camplylobacter* enteritis (4%), *Salmonella* enteritis (4%) and amebiasis (3%).[82] A retrospective study of Swiss children who had visited the tropics or subtropics reported finding similar incidence rates of traveler's diarrhea in children—40.0, 8.5, 21.7, and 36.0 percent in children aged 0 to 2, 3 to 6, 7 to 14, and 15 years and older, respectively. In this study, the authors also found that small children (0 to 2 years old) most frequently were affected with traveler's diarrhea and that the clinical course tended to be more severe and prolonged than in older pediatric age groups. Overall, children were found to have longer lasting illness than that noted in adults, with an average duration of 11 days for all children combined and 29 days for small children.[122]

Enteric pathogens typically are isolated from approximately 50 to 75 percent of stool specimens from adult travelers with diarrhea; in the remainder, usually no pathogen is isolated. *Escherichia coli*, especially enterotoxigenic *E. coli*, is the most common overall cause of traveler's diarrhea (although the incidence can vary by destination), followed by *Campylobacter*, *Salmonella*, and *Shigella*. Other etiologic agents include pathogenic bacteria, such as *Aeromonas* and *Plesiomonas;* protozoa (e.g., *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium* spp., and *Cyclospora cayetanensis*); viruses, such as rotavirus and Norwalk-like viruses; and, rarely, helminths.[120] Numerous risk factors for traveler's diarrhea, including the consumption of certain high-risk foods (i.e., raw foods such as meats, seafood, and vegetables; unpasteurized dairy products; and ice and tap water) and travel to certain destinations, also have been identified.[133] Other authors previously categorized destinations by high, intermediate, and low levels of risk for acquiring traveler's diarrhea. Destinations generally considered to have the highest associated risk for contraction of traveler's diarrhea include most of Asia, Africa, Mexico and Central and South America; destinations with intermediate risk include Eastern Europe, South Africa and some of the Caribbean islands; and low-risk travel destinations include countries in North America, Northern and Western Europe, Australia, New Zealand, and Japan.[53] The location of food preparation also is a recognized risk factor for acquiring traveler's diarrhea, with a higher risk shown for travelers eating from street vendors and in local restaurants and a lower risk in luxury hotels and private homes. Although fewer data on traveler's diarrhea in children are available, children (especially toddlers) are probably more vulnerable to foodborne and waterborne pathogens for many reasons, including their propensity to touch multiple surfaces and to mouth objects. Of note, one study found that only 40 percent of parents said that they had practiced dietary prevention measures consistently and only 5 percent reported using oral rehydration solutions (the mainstay of treatment of diarrhea in children).[122]

Preventive Counseling for Traveler's Diarrhea

Counseling parents of pediatric travelers about appropriate preventive behavior to avoid traveler's diarrhea and anticipatory guidance to ensure successful management of diarrhea are important aspects of the pretravel assessment.

Food and Beverage Precautions

The most important aspect of preventive counseling for parents of pediatric travelers is appropriate food and beverage precautions. In addition to preventing the diarrheal diseases already listed, compliance with food and water precautions also will decrease the risk for contracting other foodborne diseases such as hepatitis A. Parents should be counseled that in areas where chlorinated tap water is not available or where hygiene and sanitation are poor, tap water (including ice cubes) should be considered contaminated and hence should be avoided. In addition, travelers also should be advised to avoid brushing their teeth with tap water. Breast-feeding, boiled or bottled water, and bottled carbonated beverages are recommended as typically safe beverages for children; powdered drink mixes made with boiled water (e.g., formula or tea) also generally can be considered safe. Parents should dry wet cans and bottles before opening and wipe drinking surfaces clean before serving.[90]

In areas where access to bottled water is poor, water may be boiled for 1 minute (or for 3 minutes at altitudes above 2000 m [6562 feet]). These procedures will kill bacterial, parasitic, and viral pathogens. Chemical disinfection with halogens (iodine or chlorine) is an alternative method for water treatment when the water cannot be boiled; however, some common waterborne parasites, such as *Cryptosporidium*, are poorly inactivated by iodine disinfection, even after extended contact time. Chlorine also can be used for chemical disinfection, but its

germicidal activity varies with pH, temperature, and organic content of the water; therefore, it can provide less consistent levels of disinfection in many types of water. Thus, chemical disinfection with iodine or chlorine should be supplemented with filtration to remove these organisms. However, chlorine dioxide, which is more potent than equivalent doses of chlorine, is effective against all waterborne pathogens, is simple to use and available in liquid or tablet form.[53] Tablets for water disinfection are available at pharmacies, sporting goods stores, and camping outfitters. Ultraviolet (UV) light is effective against all waterborne pathogens. Some portable devices are available; however, clear water is required to ensure that the UV rays can reach the pathogens in the water. Portable filters are available and provide various degrees of protection against microbes. Reverse-osmosis filters afford protection against viruses, bacteria, and protozoa, but they are large and expensive, and the small pores can be plugged by cloudy or muddy water. Microstrainer filters with pore sizes in the 0.1- to 0.3-μm range can remove bacteria and protozoa from drinking water, but they do not remove viruses. To kill viruses, parents using microstrainer filters should be advised to chemically disinfect water with iodine after filtration. Data are inadequate at present to evaluate the efficacy of specific brands or models of filters, and therefore the CDC cannot recommend specific brands or models of filters most likely to remove bacteria, viruses, and parasites.[53]

Parents of pediatric travelers also should be counseled on the importance of advance planning for food and beverage items, especially for infants and young children. Breast-feeding infants are considered relatively safe from contracting traveler's diarrhea because they are not exposed to local food and water; therefore, traveling mothers should be encouraged to continue breast-feeding for as long as practical. For infants receiving formula, formula concentrate and powdered forms are the most convenient for travel, but a clean water supply must be available or water must be boiled or chemically disinfected before preparation. For feeding toddlers and older children, the travel adage of "boil it, cook it, peel it, or forget it" applies. In general, unless beverages and food come from a can or can be completely cooked or peeled, they should be considered unsafe (including raw fruits and vegetables). Travelers should avoid unpasteurized dairy products, including cheese and ice cream. In addition, travelers should be advised to not consume raw seafood, shellfish, and meats. Freshly prepared steaming hot food, breads and cereals, hot pasta (including rice and noodles), and well-cooked meat or fish generally can be considered safe for consumption. Bringing crackers and peanut butter is a good suggestion for parents of hungry children when the availability of safe foods is uncertain. The incidence of traveler's diarrhea in children also can be reduced potentially by advising parents to encourage or supervise frequent hand washing, including the use of alcohol-based hand sanitizer, and to try to prevent children from placing objects in their mouths.

Some species of fish and shellfish can contain poisonous biotoxins, such as ciguatoxin and scombrotoxin, even when they are well cooked. Ciguatera poisoning is a potential risk in all subtropical and tropical regions of the West Indies and the Pacific and Indian oceans where the implicated fish are consumed; such fish include barracuda, red snapper, grouper, amberjack, and sea bass. Symptoms of ciguatera poisoning include gastroenteritis and neurologic manifestations such as dysesthesias and weakness. Scombroid poisoning occurs in tropical and temperate regions and is caused by high levels of histidine in the flesh of fish such as bluefin, yellowfin tuna, mackerel, bonito, mahi-mahi, herring, amberjack, and bluefish; if improperly refrigerated, histidine is converted to histamine, which can cause flushing, headache, nausea, vomiting, diarrhea, and urticaria.[53]

Managing Traveler's Diarrhea in Children

Adults traveling with children should be counseled about the signs and symptoms of dehydration and the proper use of WHO oral rehydration solution (ORS). Immediate medical attention is required for an infant or young child with diarrhea who has signs of moderate-to-severe dehydration, bloody diarrhea, temperature higher than 38.5° C (>101.5° F), or persistent vomiting. ORS should be provided to the infant by bottle or spoon while medical attention is being obtained.

Assessment and Treatment of Dehydration

The greatest risk to the infant with diarrhea and vomiting is dehydration. Fever or increased ambient temperature increases fluid losses and speeds dehydration. Parents should be advised that dehydration is best prevented and treated by use of ORS, in addition to the infant's usual food. Rice-based and other cereal-based ORS, in which complex carbohydrates are substituted for glucose, also are available and may be more acceptable to young children. Adults traveling with children should be counseled that sports drinks, which are designed to replace water and electrolytes lost through sweat, do not contain the same proportions of electrolytes as those of the solution recommended by WHO for rehydration during diarrheal illness.

ORS packets are available at stores or pharmacies in almost all developing countries. (See later information about ORS availability in the United States.) ORS is prepared by adding one packet to boiled or treated water. Travelers should be advised to check packet instructions carefully to ensure that the salts are added to the correct volume of water. ORS should be consumed or discarded within 12 hours if it is held at room temperature or 24 hours if it is kept refrigerated. A dehydrated child will drink ORS avidly; travelers should be advised to give it to the child as long as the dehydration persists. An infant or child who vomits the ORS usually will keep it down if it is offered by spoon, oral syringe, or straw in frequent small sips.[51,96,131]

Children weighing less than 10 kg who have mild-to-moderate dehydration should be administered 60 to 120 mL (2 to 4 oz) ORS for each diarrheal stool or vomiting episode. Children who weigh 10 kg should receive

120 to 240 mL (4 to 8 oz) of ORS for each diarrheal stool or vomiting episode. Severe dehydration is a medical emergency that usually requires administration of fluids by intravenous or intraosseous routes.[53,66,95,130]

A dehydrated child will usually drink ORS avidly; travelers should be advised to give it to the child as long as the dehydration persists. As dehydration lessens, the salty-tasting ORS solution may be refused, and another liquid can be offered. An infant or child who has been vomiting will usually keep ORS down if it is offered by spoon or oral syringe in small sips; these small amounts must be offered frequently, however, so the child can receive an adequate volume of ORS. Older children will often drink well by sipping through a straw. Severely dehydrated children, however, often will be unable to drink adequately. Severe dehydration is a medical emergency that usually requires administration of fluids by intravenous or intraosseous routes.

ORS packets are available in the United States from Jianas Brothers Packaging Company.* ORS packets may also be available at stores that sell outdoor recreation and camping supplies. In addition, Cera Products† markets a rice-based, rather than glucose-based, product.

Dietary Modification

Breast-fed infants should continue nursing on demand. Formula-fed infants should continue their usual formula during rehydration. They should receive a volume sufficient to satisfy energy and nutrient requirements. Lactose-free or lactose-reduced formulas usually are unnecessary. Dilution of formula may slow resolution of diarrhea and is not recommended. Older infants and children receiving semisolid or solid foods should continue to receive their usual diet during the illness. Recommended foods include starches, cereals, yogurt, fruits, and vegetables. Foods that are high in simple sugars, such as soft drinks, undiluted apple juice, gelatins, and presweetened cereals, can exacerbate diarrhea by osmotic effects and should be avoided. In addition, foods high in fat may not be tolerated because of their tendency to delay gastric emptying. The practice of withholding food for 24 hours or longer is inappropriate. Early feeding can decrease changes in intestinal permeability caused by infection, reduce illness duration, and improve nutritional outcome. Highly specific diets (e.g., the BRAT [bananas, rice, applesauce, and toast] diet) frequently have been recommended; however, similar to juice-centered and clear-fluid diets, such severely restrictive diets used for prolonged periods can result in malnutrition and should be avoided.[95]

Other Measures

Parents should be particularly careful to wash hands well after changing diapers of infants with diarrhea to avoid spreading infection to themselves and other family members.

Oral syringes available in most pharmacies for oral medications can be useful for the administration of ORS and can be included as part of the traveler's health kit for young children. Straws also can be useful for older children.

Bismuth subsalicylate (BSS), which is the active ingredient in Pepto-Bismol, can be used to prevent travelers' diarrhea in children 12 years of age and older. Studies from Mexico have shown that BSS taken daily as either 2 oz (60 mL) of liquid or two chewable tablets four times per day reduces the incidence of travelers' diarrhea from 40 percent to 14 percent. BSS can cause blackening of the tongue and stool and may cause nausea, constipation, and, rarely, tinnitus. BSS should be avoided by travelers with aspirin allergy, renal insufficiency, and gout and by those taking anticoagulants, probenecid, or methotrexate. In travelers taking aspirin or salicylates for other reasons, the use of BSS may result in salicylate toxicity. BSS is not generally recommended for children younger than 12 years of age; however, some clinicians use it off-label, with caution in certain circumstances. Because of the association between salicylates and Reye syndrome, BSS is not generally recommended to treat diarrhea in children younger than 12 years of age. However, some clinicians use it off-label, with caution in certain circumstances. Caution should be taken in administering BSS to children with viral infections, such as varicella or influenza, because of the risk for Reye syndrome. BSS is not recommended for children younger than 3 years of age. Studies have not established the safety of BSS use for periods longer than 3 weeks.[53] Insufficient data are available at this time to recommend the use of probiotics for the prevention of travelers' diarrhea in children.[53,69]

The use of antimotility agents (e.g., loperamide [Imodium] or dyphenoxylate and atropine [Lomotil]) in children younger than 2 years of age is not recommended. Because overdoses of these types of drugs can be fatal, they should be used with extreme caution in children. Side effects of these drugs in adults include opiate-induced ileus, drowsiness, and nausea. Lomotil has been associated with fatal overdoses and other severe complications, including coma and respiratory depression. Antinausea medications, such as promethazine and prochlorperazine, are not recommended routinely. They are contraindicated for use in children younger than 2 years. Fatal respiratory depression in children has been reported with use of promethazine. Children with an acute illness, including gastroenteritis and dehydration, are more susceptible than are adults to neuromuscular reactions, especially dystonias, associated with prochlorperazine. The extrapyramidal side effects associated with these medications can be confused with symptoms of other undiagnosed primary diseases associated with vomiting, such as Reye syndrome. These medications should not be prescribed routinely for empirical treatment of children with possible traveler's diarrhea. Adults traveling with children should be fully counseled about the indications, dosage, frequency, and possible side effects if these medications are prescribed.[53,95]

*Jianas Brothers Packaging Company: 816-421-2880; http://rehydrate.org/resources/jianas.htm.

†Cera Products: 843-842-2600 and 888-237-2598; http://www.ceraproductsinc.com.

Antibiotics

Prophylactic antibiotics are not recommended for most travelers. Many clinicians will prescribe antibiotics to be administered as empirical self-treatment; however, few data are available for empirical administration of antibiotics for traveler's diarrhea in children. The antimicrobial options for empirical treatment in children are limited. Trimethoprim-sulfamethoxazole was used previously for empirical treatment of traveler's diarrhea in children; however, its effectiveness has been reduced by widespread drug resistance and it is no longer routinely recommended. Fluoroquinolones are used frequently for the empirical treatment of traveler's diarrhea in adults. The use of fluoroquinolones generally is not recommended in children and adolescents younger than 18 years of age because of cartilage damage in animals tested.[63] In children, the American Academy of Pediatrics suggests some special circumstances for fluoroquinolone use, including the treatment of gastrointestinal infection caused by multidrug-resistant *Shigella* spp., *Salmonella* spp., *Vibrio cholerae*, or *Campylobacter jejuni*.[62] However, the routine use of fluoroquinolones for chemoprophylaxis or empirical treatment for traveler's diarrhea (TD) among young children is not recommended. For young adults ages 19 and older, the fluoroquinolones are generally prescribed as 1- to 3-day regimens. Clinicians should be aware that fluoroquinolone resistance in gastrointestinal organisms has been reported from some countries, particularly in Southeast Asia.

In some studies, azithromycin has been found to be as effective as fluoroquinolones in treating traveler's diarrhea in adults.[1] In practice, some clinicians prescribe azithromycin either as a single dose or at 10 mg/kg for 3 days for empirical treatment for children. Flavored oral suspension of azithromycin is available. The suspension does not require refrigeration; however, it should be used within 10 days of mixing. The unreconstituted form of azithromycin has a longer expiration period. In certain circumstances, the unreconstituted form can be provided with clear instructions for preparation and may be useful for children traveling for more than 10 days.

PREVENTION OF OTHER INFECTIOUS DISEASES IN PEDIATRIC TRAVELERS

Because the epidemiology of many diseases is evolving, prevention hinges on the clinician being knowledgeable about information on current outbreaks and risk in planned travel destinations, evaluating risk based on planned activities and the season of travel, and providing appropriate counseling and vaccination, if available. Information about specific infectious disease risks can be obtained from numerous sources listed in the section on international travel information resources. More detailed information about specific vaccinations is included in the section on vaccination for international travel.

A variety of pathogens increasingly are being recognized as emerging infectious diseases in travelers. In addition to malaria, other vector-borne infectious diseases are among the important diseases for consideration in travelers. Dengue is one of the most significant vector-borne viral infections worldwide. It is endemic in at least 100 countries in Asia, the Pacific, Africa, Latin America, and the Caribbean and occurs sporadically in the Middle East. Worldwide, an estimated 50 to 100 million cases of dengue fever occur annually, including 500,000 dengue hemorrhagic fever cases and 22,000 deaths, mostly among children.[15] Dengue fever has been reported among 2 to 14 percent of ill travelers.[10,74,117,145] Epidemics of dengue hemorrhagic fever, the more severe clinical form of dengue fever, occur every 3 to 5 years in Southeast Asia and are an emerging problem in Latin America.[118] Epidemics caused by all four serotypes (1 to 4) have become progressively more frequent and larger in the past 25 years. In addition, most tropical urban centers in endemic regions have multiple dengue virus serotypes co-circulating (hyperendemicity), which increases the risk for dengue transmission and the risk for dengue hemorrhagic fever.[52] Outbreaks of dengue fever also have occurred in Hawaii, Puerto Rico, the U.S. Virgin Islands, Samoa, Guam, and along the United States–Mexican border.[15,18,19,124] In a recent short report by Krishnan and associates,[96] eight children were diagnosed with dengue between 2007 and 2010 at Bronx-Lebanon Hospital Center in New York City. The cases had a median age of 13.6 years (range, 0.3 to 17.6 years). All of the children were visiting friends and relatives in Puerto Rico (12%) or the Dominican Republic (88%). Of the eight children, three (38%) developed complications, including dengue shock syndrome in one infant.[96] Dengue is transmitted primarily by day-biting *Aedes aegypti* mosquitoes, which breed in flower vases, barrels, and discarded tires that collect water. Transmission occurs in rural and urban areas, but the risk is greatest in urban areas. Prevention should focus on protection against mosquito bites. Travelers to areas of risk should be counseled to apply repellent during the day, even while visiting cities. No vaccine is available, and previous infection with one of the four serotypes does not protect against infection with another serotype. The risk for contracting dengue hemorrhagic fever increases after subsequent infection with a different serotype.[135]

Avian influenza A (H5N1) has become a panzoonotic infection affecting regions of Asia, Africa, and the Middle East. Between 2003 and July 6, 2012, the WHO reported 607 human cases and 358 deaths caused by avian influenza from 15 countries, primarily from Indonesia, Vietnam, Egypt, China, Cambodia, and Thailand. Most of these cases were associated with direct contact with infected poultry.[20,155] Risk to travelers is low. However, clinicians should obtain up-to-date information about affected countries and counsel travelers going to those areas. Persons visiting areas with reports of outbreaks of H5N1 among poultry or of human H5N1 cases can reduce their risk for infection by (1) avoiding direct contact with poultry, including touching well-appearing, sick, or dead chickens; (2) avoiding poultry farms and bird markets; (3) avoiding handling surfaces contaminated with poultry feces or secretions; (4) practicing frequent and careful hand washing; and (5) consuming all foods from poultry, including eggs and poultry blood, only if they have been thoroughly cooked. Because influenza

viruses are destroyed by heat, the cooking temperature for poultry meat should be 74° C (165° F).[20]

Chikungunya virus is an alphavirus that exists in tropical Africa and is transmitted by the bite of infected mosquitoes. Because chikungunya fever epidemics are sustained by human-mosquito-human transmission, the epidemic cycle is similar to those of dengue and urban yellow fever. Infection is associated with fever, headache, severe joint pain, and, in approximately half of the cases, a generalized maculopapular rash similar to that of dengue virus infection. Complications are more common in children younger than 1 year of age. Large outbreaks of chikungunya have been reported recently on several islands in the Indian Ocean and in India. During 2005 to 2006, 12 cases were reported in travelers who arrived in the United States from areas known to be epidemic or endemic for chikungunya virus.[39,75,132] No vaccines or specific preventive medications are available; therefore, prevention measures should focus on personal protection against mosquito bites and awareness of local epidemics.[39]

African trypanosomiasis (sleeping sickness), a parasitic infection transmitted by the bite of a tsetse fly, occasionally has been reported in travelers, including children. Infection can result in severe neurologic sequelae and is 100 percent fatal if untreated.[17] Infection is caused by two species of *Trypanosoma brucei: T. brucei rhodesiense*, which is found in eastern and southeastern Africa (primarily Tanzania, Uganda, Malawi, and Zambia) and *T. brucei gambiense*, which is found predominantly in central Africa and limited areas of West Africa. Most infections in U.S. residents are caused by *T. brucei rhodesiense* and occur among travelers to East Africa game parks. In 2001, significant increases in cases were reported among U.S. and European travelers to game parks in Tanzania and Kenya. Between 1967 and 2000, an imported case occurred on average every 1 to 2 years; however, in 2001, seven cases were reported in U.S. travelers.[11,34,70,71,110-113] Travelers should use insect repellent and long-sleeved clothing in neutral colors of medium-weight material, because tsetse flies are attracted to bright or dark clothes and they can bite through lightweight clothing.[53]

Schistosomiasis, another parasitic infection caused by flukes that live part of their life cycle in fresh-water snail hosts, affects more than 200 million people worldwide. Schistosomiasis has been reported in travelers to endemic areas of Africa, Asia, South America, and the Caribbean who participated in high-risk activities such as swimming and wading in fresh water.[53,58,59,63] Most travel-associated infections occur among travelers to sub-Saharan Africa. Because most acute infections are asymptomatic, preventive counseling is critical. While in schistosomiasis-endemic areas, children should not swim in fresh, unchlorinated water such as lakes or ponds.[53]

More than 9 million new tuberculosis (TB) cases and 2 million TB-related deaths occur annually worldwide. A rate of tuberculin skin test seroconversion of 1.8 percent was reported among travelers to areas endemic for TB.[61] Of 357 travelers to the Hajj for pilgrimage, 10 percent had evidence of seroconversion when tested with use of QuantiFERON, a whole-blood assay for TB antigens.[146] Multidrug-resistant TB has been increasing in incidence worldwide. Cases of extensively drug-resistant TB have been reported from several countries, including Estonia, Latvia, Lesotho, Peru, the Philippines, South Africa, Swaziland, and the United States.[151,152] Travelers who may have prolonged exposure to TB should have two-step tuberculin skin test (TST) or a single interferon-γ release assay (IGRA), either the QuantiFERON TB test (Gold or Gold In-Tube versions) or T-SPOT TB test, before leaving the United States. If the predeparture test is negative, a single TST or IGRA should be repeated 8 to 10 weeks after returning from travel. IGRA is preferred for children who have received BCG vaccine.[53] According to reports, U.S. children who had traveled to countries with a high prevalence of TB within the previous 12 months were 3.9 times more likely to have positive tuberculin skin test results than were children who lived in the same U.S. areas but had not traveled.[102] Bacille Calmette-Guérin (BCG) vaccine is a live vaccine prepared from attenuated strains of *Mycobacterium bovis;* BCG is used primarily in young infants to prevent disseminated and other forms of life-threatening disease caused by TB, such as tuberculous meningitis. BCG is recommended by the WHO for administration at birth. Vaccination of a young pediatric traveler (non–HIV infected and with a negative tuberculin skin test response) might be considered if long-term stay (e.g., children of missionaries or expatriates) is planned in a country with a high prevalence of TB, particularly multidrug-resistant or extensively drug-resistant TB, and prolonged contact with persons with active TB is thought to be a potential problem.[2,3,121] BCG vaccine can be obtained from the Canadian subdivisions of Sanofi Pasteur.*

Tick-borne encephalitis is transmitted primarily by the bite of *Ixodes* ticks. It also can be transmitted by the ingestion of unpasteurized dairy products from infected livestock. Transmission occurs during the summer months in western and central Europe, Scandinavia, and parts of the former Soviet Union. Russia has the highest incidence. Most cases occur from April through November, when ticks are most active.[53] Travelers to affected areas should use measures to avoid tick bites and avoid consuming unpasteurized dairy products. Persons who will be traveling for longer than 3 weeks in endemic rural areas or travelers who will be engaging in high-risk activities, such as camping, should be considered for vaccination. The vaccine is not available in the United States but can be obtained in Europe and Canada.[53]

Adventure travel can increase the risk for acquiring a variety of infectious diseases. Examples of recent outbreaks or cases of unusual pathogens affecting adventure travelers include fungal organisms (e.g., histoplasmosis and coccidioidomycosis), leptospirosis, and leishmaniasis. Histoplasmosis is a fungal infection acquired by the inhalation of spores, usually through exposure to bat, bird, or chicken droppings in barnyards and caves. The organism is endemic in the United States, Latin America, eastern Asia, parts of Europe, Africa, and Australia. Coccidioidomycosis, a fungal infection associated with the inhalation of organisms in soil from high-risk areas, is

*Sanofi Pasteur: http://www.sanofipasteur.ca.

endemic in the southwestern part of the United States and Latin America. Both infections can cause a spectrum of illness from asymptomatic infection to acute pulmonary infection to severe, disseminated disease, especially in immunocompromised persons. Several outbreaks of histoplasmosis have been reported in groups of U.S. visitors who entered a cave with bats in Costa Rica,[22] Ecuador,[138,153] Peru,[14] and Nicaragua.[144] More than 200 college students became infected with histoplasmosis during a spring break trip to Acapulco, Mexico.[34,120,156] Two outbreaks of coccidioidomycosis have been reported in youth missionary groups involved in construction work in Mexico.[13,25] Most of these fungal outbreaks have two common features: high-risk group activities and high attack rates, even in young, nonimmunocompromised individuals. Because no vaccine is available, prevention involves counseling travelers to avoid exposure or to use special masks for high-risk individuals who cannot avoid exposure.[23]

Leptospirosis is a zoonotic infection transmitted by exposure to water or soil contaminated with organisms excreted by domestic and wild animals. Outbreaks have been reported in white-water rafters in Costa Rica[24] and in athletes from 26 countries who participated in the Eco-Challenge multisport expedition race in Borneo, Malaysia, in 2000.[27,31] Because no vaccine against leptospirosis exists, persons engaging in high-risk activities with exposure to recreational water activities or exposure to contaminated surface waters and soil should be counseled to avoid exposure to water that may be contaminated or to wear protective clothing, especially footwear, and to cover cuts and abrasions with occlusive dressings. They should avoid contact with, submersion in, or swallowing potentially contaminated water. The CDC recommends that persons engaging in high-risk activities consider the use of doxycycline (200 mg orally, once per week), begun 1 to 2 days before exposure and continuing through the period of exposure for prophylaxis; doxycycline should not be used routinely for children younger than 8 years of age.[53] Travelers at increased risk for leptospirosis and in need of malaria chemoprophylaxis should consider using doxycycline for both indications.

Leishmaniasis, a parasitic infection transmitted by the bite of a sandfly, can lead to cutaneous or visceral infection. It has been reported in students who traveled to the rain forest in Costa Rica and other travelers.[81,83,109,148] The appropriate use of insect repellent and other personal protection measures against sandfly bites is the only prevention tool available.

Finally, studies of sexual practices among travelers have shown that 5 to 50 percent of short-term travelers engage in casual sex while abroad; 40 to 60 percent of long-term travelers have reported engaging in casual sex while abroad.[107,125,126] A study of British travelers reported that 18.6 percent had had new sexual partners, two thirds of those who were sexually active did not use condoms on every occasion, and 5.7 percent contracted STDs.[133] Adolescents should be counseled about the risks for and prevention of STDs, hepatitis B, and HIV infection associated with sexual contact, sharing needles, or receiving acupuncture or tattoos.

GENERAL TRAVEL HEALTH COUNSELING FOR CHILDREN

During the pretravel assessment, the clinician should provide the parents with general advice and preventive counseling to avoid health risks and injuries in children. Simple personal protection measures can prevent children from incurring many types of travel-associated injuries and conditions. One of the most common skin conditions reported by travelers is sunburn. To prevent sunburn and the subsequent risk for skin malignant neoplasms later in life, children should avoid sun exposure during peak hours and use sunscreen appropriate for the child's age. When sunscreen and insect repellent are used concomitantly, the sunscreen should be applied first. There is no evidence that air travel exacerbates the symptoms or complications associated with otitis media.[65] Travel to different time zones, "jet lag," and schedule disruptions can disturb sleep patterns in infants and children, as well as adults.[53]

Motor vehicle accidents, injuries, and drownings pose a great risk for morbidity and mortality among international travelers.[53,125] Vehicle-related accidents are the leading cause of death in children who travel. While traveling in automobiles and other vehicles, children less than 4 feet 9 inches in height should be restrained in age-appropriate car seats or booster seats, as described earlier. In general, children are safest traveling in the rear seat; no one should ever travel in the bed of a pickup truck. Families should be counseled that in many developing countries, cars may lack front or rear seatbelts. Parents traveling with infants or young children should be advised to bring their own child safety restraint seats for use during travel. Night driving can be more hazardous, especially outside urban areas in developing countries, and should be avoided.[53] Use of a helmet is imperative for bicycle travel. Fire injuries can be prevented by advising parents to inquire whether hotels have smoke detectors and sprinkler systems (they also may consider bringing their own smoke detector). Conditions at hotels and other lodging may not be as safe as those in the United States, and accommodations should be carefully inspected for exposed wiring, pest poisons, paint chips, or inadequate stairway or balcony railings.[53] Drowning is the second leading cause of death in young travelers. Children may not be familiar with hazards in the ocean or in rivers. Swimming pools may not have protective fencing to keep toddlers from falling into the pool. Close supervision of children around water is essential. Appropriate water-safety devices such as life vests may not be available abroad, and families should consider bringing these from home. Parents and children participating in boating activities should be counseled to be aware of weather conditions and forecasts, and parents should be advised that an adult should accompany children at all times when swimming. Personal floatation devices should be used when boating or water-skiing, regardless of the distance to be traveled or swimming ability.[129] Swimming in contaminated water can result in skin, eye, ear, and some intestinal infections; for prevention of infectious diseases, generally only pools that

contain chlorinated water can be considered safe. Parents also should be advised that swimming in fresh-water lakes and streams in developing countries carries a risk for contracting schistosomiasis, leptospirosis, and primary amebic meningoencephalitis.[53] In salt-water bodies, biting and stinging fish, corals, and jellyfish also can be a risk. In general, children should wear covered footwear rather than sandals, including when wading on reefs and swimming.[53,129] Children should avoid walking barefoot, particularly in rural areas, to prevent cutaneous larva migrans, hookworm, and *Strongyloides* infections. Children are more likely than adults to have contact with soil or sand, and therefore they may be exposed to diseases caused by infectious stages of parasites present in soil, including ascariasis, hookworm infestation, cutaneous or visceral larva migrans, trichuriasis, and strongyloidiasis. Children and infants should wear protective footwear and play on a sheet or towel rather than directly on the ground. Clothing should not be dried on the ground. When traveling in countries with a tropical climate, clothing or diapers dried in the open air should be ironed before use to prevent infestation with fly larvae. Parents of pediatric travelers, especially to areas endemic for rabies, should be reminded to warn children that they should not attempt to pet, handle, or feed domestic or wild animals (including monkeys). Travelers also should avoid snakes because most bites are the direct result of handling, harassing, or trying to kill snakes.[53]

Travelers to high-altitude destinations should be counseled about the risk and prevention of altitude illness, including acute mountain sickness, high-altitude cerebral edema, and high-altitude pulmonary edema. Altitude sickness generally occurs at 2500 to 2700 m (8000 to 9000 ft), and acclimatization generally takes 3 to 5 days. Susceptibility and resistance are genetic traits, and no screening tests are available to predict risk. Children are equally susceptible as adults. Travelers to high-altitude destinations should be advised to ascend gradually, if possible, and those travelers going to destinations higher than 8000 to 9000 ft should be advised to acclimization at 8000 to 9000 ft for a few days before moving to higher altitudes. For higher altitudes, travelers should move sleeping altitude no higher than 500 m (1600 ft) per day and to plan on spending an extra day for acclimatization for every 1000 m (3300 ft), if possible. For abrupt ascent, pediatric travelers may require acetazolamide, prescribed as 2.5 mg/kg/dose every 12 hours, up to the standard adult dose of 125 mg twice per day. Travelers should begin acetazolamide 1 day before ascent and continue for the first 2 days at altitude. Dexamethasone is generally reserved for treatment of altitude illness.[53]

THE PEDIATRIC TRAVELER'S HEALTH KIT

Children traveling to developing countries may have unique exposures, particularly if they are traveling to remote locations or will have close contact with other children. Depending on the travel itinerary, children may need prescriptions for malaria chemoprophylaxis, antibiotic for self-treatment of travelers' diarrhea, and medication to prevent high-altitude sickness. Consideration should be given to inclusion of the following medications as part of the traveler's health kit: rectal preparations (suppositories) of selected medications, such as acetaminophen; topical treatments for lice and scabies for pediatric travelers to developing countries who may have extensive contact with local children; and a topical antibiotic, such as mupirocin, for bacterial skin infections.[53,100] Children with history of anaphylaxis or serious allergic reactions should have a sufficient number of epinephrine pens and antihistamines in tablet or liquid form. Any medications purchased abroad should be obtained from a reliable pharmacy because of concern about counterfeit drugs, especially in developing countries. Adults accompanying these children and teenagers should consider obtaining basic first aid and safety training before departure. Although air travel is safe for healthy newborns, infants, and children, a few issues should be considered in preparation for travel. Children with chronic heart or lung problems may be at risk for hypoxia during flight, and a physician should be consulted before travel. Insurance coverage for illnesses and accidents while abroad should be verified before departure.[65] Consideration should be given to purchasing special travel insurance for airlifting or air ambulance to an area with adequate medical care.[53]

INTERNATIONAL TRAVEL INFORMATION RESOURCES

One of the most important functions a clinician can fulfill in preparing a pediatric patient and parents for international travel is to provide up-to-date and accurate travel health information and recommendations for preventing illness. Many varied sources of information are available, including software packages and databases designed specifically for use in travel medicine and pretravel care.[73,94] Increasingly, the Internet and computer-based travel resources are being used by practitioners and consumers alike because they provide current information to appropriately and effectively counsel and treat international travelers. Two reviews have provided comprehensive summaries of travel medicine resources.[73,94] Table 231-4 is a summary of selected travel health resources that can be useful for providing health care professionals and parents with essential information on health risks in specific travel destinations (including endemic or epidemic diseases) and current travel health recommendations (including immunizations and chemoprophylaxis). Travelers also should be aware of the need for health insurance and emergency evacuation coverage during travel and medical assistance in the event of a travel-related illness; a variety of companies offer these services, and travelers should investigate options before making any purchases. This summary is not meant to be comprehensive; because resources are constantly changing, clinicians are advised to review and to compare sites for availability and updated information and recommendations.

TABLE 231-4 **International Travelers' Health Information, Recommendations, and Outbreak Notices**

Resource	Contact Information	Information and Services Provided
Centers for Disease Control and Prevention (CDC) Travelers' Health Information Destination-Specific Information	http://wwwnc.cdc.gov/travel/	U.S. travelers' health recommendations by destination region and country, including malaria chemoprophylaxis and vaccinations; links to other sites, travel health warnings, advisories, and outbreak notices
CDC Health Information for International Travel (Yellow Book)	http://wwwnc.cdc.gov/travel/page/yellowbook-home-2012	General travelers' health information, region- and destination-specific recommendations, including malaria chemoprophylaxis and vaccinations
CDC Malaria Branch	http://wwwnc.cdc.gov/travel/diseases/malaria 770-488-7788 or 1-855-856-4713 (9:00 AM to 5:00 PM EST, Monday through Friday) 770-488-7100 (after hours, on weekends and holidays)	Information about malaria prophylaxis and treatment, intended for use by health care professionals
CDC *Morbidity and Mortality Weekly Report, Recommendations and Reports, Surveillance Summaries*	http://www.cdc.gov/mmwr	Reports on U.S. and international outbreak investigations, disease surveillance summaries, reports, and recommendations
CDC National Immunization Program	http://www.cdc.gov/vaccines/	Immunization information
U.S. Immunization Schedules	http://www.cdc.gov/vaccines/schedules/index.html	Immunization schedules for children, adolescents, and adults
Immunization Action Coalition	http://www.immunize.org	Immunization information and educational material for practitioners and parents
National Network for Immunization Information (NNii)	http://www.immunizationinfo.org	Information for public and health professionals about immunization Site includes immunization news, a vaccine information database, and a guide to evaluating vaccination information on the web
U.S. State Department	http://www.travel.state.gov	General information about travel, including safety, visa requirements, links to individual embassies and consulates
World Health Organization (WHO) Health Topics	http://www.who.int/topics/en/	Information and maps of health topics and diseases
WHO Yellow Book, International Travel and Health	http://www.who.int/ith/en/	General travelers' health recommendations; country-specific malarial risks and recommendations
WHO, Outbreak News	http://www.who.int/csr/don/en/	Notifications of recent infectious disease outbreaks
WHO, Weekly Epidemiological Record	http://www.who.int/wer/en/	Global disease surveillance and WHO program updates
Health Canada, Travel Health	http://www.phac-aspc.gc.ca/tmp-pmv/index-eng.php	Canadian recommendations for travelers' health; outbreak information
EuroSurveillance	http://www.eurosurveillance.org	Surveillance and outbreak information for European nations; weekly and monthly surveillance reports and outbreak notices
ProMED (Program for Monitoring Emerging Diseases)	http://www.promedmail.org	E-mail postings; verified and unverified reports on emerging diseases and outbreaks
University of Texas at Austin	http://www.lib.utexas.edu/maps	World maps online
American Society of Tropical Medicine and Hygiene (ASTMH)	http://www.astmh.org 847-480-9592	Directory of travel medicine clinics and practitioners certified by ASTMH
International Society of Travel Medicine (ISTM) NSF International	http://www.istm.org http://www.nsf.org/consumer	Directory of travel medicine clinics and practitioners with ISTM affiliation Information about water filters

NEW REFERENCES SINCE THE SIXTH EDITION

4. American Academy of Pediatrics. *Insect repellents*. n.d. Available at http://www.healthychildren.org.
5. American Academy of Pediatrics. *Sun safety*. n.d. Available at http://www.healthychildren.org.
6. American Academy of Pediatrics. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803–4.
11. Braakman HM, van de Molengraft FJ, Hubert WW, et al. Lethal African trypanosomiasis in a traveler: MRI and neuropathy. *Neurology* 2006;**66**:1094–6.

15. Centers for Disease Control and Prevention. *Dengue*. n.d. Available at http://www.cdc.gov/dengue.
17. Centers for Disease Control and Prevention. *Parasites-African trypanosomiasis*. n.d. Available at http://www.cdc.gov/sleepingsickness.
18. Centers for Disease Control and Prevention. *Statement regarding Rotarix and RotaTeq rotavirus vaccines and intussusception*. Available at http://www.cdc.gov/vaccines/vpd-vac/rotavirus/intussusception-studies-acip.htm.
41. Centers for Disease Control and Prevention. FDA approval of an alternate dosing schedule for a combined hepatitis A and B vaccine (Twinrix®). *MMWR Morb Mortal Wkly Rep* 2007;**56**:1057.
43. Centers for Disease Control and Prevention. Human rabies prevention: United States, 2008. Recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**(RR-03):1–28.
44. Centers for Disease Control and Prevention. Update: Novel influenza A (H1N1) virus infections—worldwide, May 6, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:453–8.
45. Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;**58**(RR-02):1–25.
46. Centers for Disease Control and Prevention. Swine influenza A (H1N1) infection in two children: Southern California, March–April 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**(15):400–2.
47. Centers for Disease Control and Prevention. Recommendations of the Advisory Committee on Immunization Practices (ACIP): Japanese encephalitis vaccines. *MMWR Recomm Rep* 2010; **59**(RR-01):1–27.
48. Centers for Disease Control and Prevention. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies. *MMWR Recomm Rec* 2010;**59**(RR-02):1–9.
49. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391–2.
50. Centers for Disease Control and Prevention. Updated recommendations for use of meningococcal conjugate vaccines: Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:72–6.
51. Centers for Disease Control and Prevention. *Health Information for International Travel 2012*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2012.
52. Centers for Disease Control and Prevention. Malaria Surveillance: United States, 2010. *MMWR Surveillance Summaries* 2012; **61**(SS-02):1–17.
54. Centers for Disease Control and Prevention. *Meningococcal vaccines: vaccine information sheet*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2012.
55. Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 1 through 18 years: United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012; **61**:1–5.
56. Centers for Disease Control and Prevention. *Yellow fever vaccine: vaccine information Sheet*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2012.
59. Centers for Disease Control and Prevention. Chikungunya fever diagnosed among international travelers: United States, 2005-2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1040–2.
61. Committee on Infectious Disease, American Academy of Pediatrics. The use of systemic fluoroquinolones. *Pediatrics* 2006;**118**: 1287–92.
68. DuPont HL, Ericsson CD, Farthing MJ, et al. Expert review of the evidence base for prevention of travelers' diarrhea. *J Travel Med* 2009;**16**:149–60.
76. Gibney KB, Fischer M, Prince HE, et al. Chikungunya fever in the United States: a fifteen year review of cases. *Clin Infect Dis* 2011;**52**:e121–6.
78. Hagmann S, Neugebauer R, Schwartz E, et al. Illness in children after international travel: analysis from the GeoSentinel Surveillance Network. *Pediatrics* 2010;**125**:e1072–e1080.
80. Han P, Balaban V, Marano C. Travel characteristics and risk-taking attitudes in youths traveling to nonindustrialized countries. *J Travel Med* 2010;**17**:316–21.
81. Hartjes L, Baumann L, Henriques J. Travel health risk perceptions and prevention behaviors of US study abroad students. *J Travel Med* 2009;**16**:338–43.
83. Herbinger KH, Drepup L, Alberer M, et al. Spectrum of imported infectious diseases among children and adolescents returning from the tropics and subtropics. *J Travel Med* 2012;**19**:150–7.
86. Hunziker T, Berger C, Staubli G, et al. Profile of travel-associated illness in children, Zürich, Switzerland. *J Travel Med* 2012;**19**: 158–62.
88. Immunization Action Coalition. *Summary of recommendations for adult immunization*. St. Paul, MN, 2012.
89. Immunization Action Coalition. *Summary of rules for childhood immunization*. St. Paul, MN, 2012.
90. Institute of International Education. *Open doors report 2011*. 2011. Available at http://www.iie.org/Research-and-Publications/Open-Doors/Data.
97. Krishnan N, Purswani M, Hagmann S. Severe dengue virus infection in pediatric travelers visiting friends and relatives after travel to the Caribbean. *Am J Trop Med Hyg* 2012;**86**:474–6.
101. Lederman E, Weld L, Elyazar I, et al. Dermatologic conditions of the ill returned traveler: an analysis from the GeoSentinel Surveillance Network. *Inter J Inf Dis* 2008;**12**:593–602.
114. Murphy ME, Montemarano AD, Debboun M, et al. The effect of sunscreen on the efficacy of insect repellent: a clinical trial. *J Am Acad Dermatol* 2000;**43**:219–22.
115. Neumann K. Pediatric travel medicine: where we are and where we hope to go. *J Travel Med* 2012;**19**:137–9.
122. Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *2012 Red book: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
124. Polio Global Eradication Initiative. *Infected Countries as of July 11, 2012*. Available at http://www.polioeradication.org.
133. Soumahoro MK, Fontenille D, Turbelin C, et al. Imported Chikungunya virus infection. *Emerg Infect Dis* 2010;**16**:162–3.
137. U.S. Department of Commerce. *Tourism Industries Office: Profile of U.S. resident travelers visiting overseas destinations: 2010 outbound. 2010. Trade Department, International Trade Administration*, http://tinet.ita.doc.gov/outreachpages/download_data_table/2010_Outbound_Profile.pdf1.
141. Van Rijn S, Driessen G, Overbosch D, et al. Travel-related morbidity in children: a prospective observational study. *J Travel Med* 2012;**19**:144–9.
151. World Health Organization. *Malaria*. n.d. Available at http://www.who.int.
156. World Health Organization. *Cumulative number of confirmed human cases for avian influenza A (H5N1) reported to WHO, 2003-2012*. Available at http://www.who.int/ith/en/.
157. World Health Organization. *International travel and health: vaccination requirements and health advice*. Geneva: World Health Organization; 2012. Available at http://www/who.int/ith/en/.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adachi J, Ericsson C, Jiang Z, et al. Azithromycin found to be comparable to levofloxacin for the treatment of US travelers with acute diarrhea acquired in Mexico. *Clin Infect Dis* 2003;**37**:1165–71.
2. Advisory Committee on Immunization Practices. The role of BCG vaccine in the prevention and control of tuberculosis in the United States. A joint statement by the Advisory Council for the Elimination of Tuberculosis and the MMWR. *Recomm Rep* 1996;**45**(RR-4):1–18.
3. Al-Jahdali H, Memish ZA, Menzies D. Tuberculosis associated with travel. *Int J Antimicrob Agents* 2003;**21**:125–30.
4. American Academy of Pediatrics. *Insect repellents*. n.d. Available at http://www.healthychildren.org.
5. American Academy of Pediatrics. *Sun safety*. n.d. Available at http://www.healthychildren.org.
6. American Academy of Pediatrics. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803–4.
7. Angell SY, Behrens RH. Risk assessment and disease prevention in travelers visiting friends and relatives. *Infect Dis Clin North Am* 2005;**19**:49–65.
8. Angell SY, Cetron MS. Health disparities among travelers visiting friends and relatives abroad. *Ann Intern Med* 2005;**142**:67–72.
9. Bacaner NM, Stauffer B, Boulware DR, et al. Travel medicine considerations for North American immigrants visiting friends and family. *JAMA* 2004;**291**:2856–64.
10. Bottieau E, Clerinx J, Schrooten W, et al. Etiology and outcome of fever after a stay in the tropics. *Arch Intern Med* 2006;**166**:1642–8.
11. Braakman HM, van de Molengraft FJ, Hubert WW, et al. Lethal African trypanosomiasis in a traveler: MRI and neuropathy. *Neurology* 2006;**66**:1094–6.
12. Brabin B, Ganley Y. Imported malaria in children in the UK. *Arch Dis Child* 1997;**77**:76–81.
13. Cairns L, Blythe D, Kao A, et al. Outbreak of coccidioidomycosis in Washington state residents returning from Mexico. *Clin Infect Dis* 2000;**30**:61–4.
14. Castellani-Pastoris M, Monaco R, Goldoni P, et al. Legionnaires' disease on a cruise ship linked to the water supply system: clinical and public health implications. *Clin Infect Dis* 1999;**28**:33–8.
15. Centers for Disease Control and Prevention. *Dengue*. N.d. Available at http://www.cdc.gov/dengue.
16. Centers for Disease Control and Prevention. *Insect repellent use and safety*. n.d. Available at http://www.cdc.gov/ncidod/dvbid/westnile/qa/insect_repellent.htm.
17. Centers for Disease Control and Prevention. *Parasites: African trypanosomiasis*. n.d. Available at http://www.cdc.gov/sleepingsickness.
18. Centers for Disease Control and Prevention. *Statement regarding Rotarix and RotaTeq rotavirus vaccines and intussusception*. n.d. Available at http://www.cdc.gov/vaccines/vpd-vac/rotavirus/intussusception-studies-acip.htm.
19. Centers for Disease Control and Prevention. *Travelers' health notice: dengue fever, Hawaii*. n.d. Available at http://www.cdc.gov/travel/other/dengue-hawaiioct2001.htm.
20. Centers for Disease Control and Prevention. *Travelers' health outbreak notice: human infection with avian influenza A (H5N1) virus*. n.d. Available at http://wwwn.cdc.gov/travel/contentAvianFluAsia.aspx.
21. Centers for Disease Control and Prevention. *Updated information regarding insect repellents*. n.d. Available at http://www.cdc.gov/ncidod/dvbid/westnile/RepellentUpdates.htm.
22. Centers for Disease Control and Prevention. Cave-associated histoplasmosis: Costa Rica. *MMWR Morb Mortal Wkly Rep* 1988;**37**:312–13.
23. Centers for Disease Control and Prevention. *Histoplasmosis: protecting workers at risk, revised guidelines for preventing histoplasmosis*. 2004. Available at http://www.cdc.gov/niosh/docs/2005-109.
24. Centers for Disease Control and Prevention. Outbreak of leptospirosis among white-water rafters: Costa Rica, 1996. *MMWR Morb Mortal Wkly Rep* 1997;**46**:577–9.
25. Centers for Disease Control and Prevention. Coccidioidomycosis in travelers returning from Mexico: Pennsylvania, 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:1004–6.
26. Centers for Disease Control and Prevention. Fatal yellow fever in a traveler returning from Venezuela, 1999. *MMWR Morb Mortal Wkly Rep* 2000;**49**:303–5.
27. Centers for Disease Control and Prevention. Outbreak of acute febrile illness among participants in Eco-Challenge-Sabah 2000: Malaysia, 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:816–17.
28. Centers for Disease Control and Prevention. Assessment of risk for meningococcal disease associated with the Hajj 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:221–2.
29. Centers for Disease Control and Prevention. Fever, jaundice, and multiple organ system failure associated with 17D–derived yellow fever vaccination, 1996-2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:643–5.
30. Centers for Disease Control and Prevention. Malaria deaths following inappropriate malaria chemoprophylaxis: United States, 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:597–9.
31. Centers for Disease Control and Prevention. Outbreak of acute febrile illness among athletes participating in Eco-Challenge-Sabah 2000: Borneo, Malaysia, 2000. *MMWR Morb Mortal Wkly Rep* 2001;**50**:21–4.
32. Centers for Disease Control and Prevention. Outbreak of acute respiratory febrile illness among college students: Acapulco, Mexico, March 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:261–2.
33. Centers for Disease Control and Prevention. Underdiagnosis of dengue: Laredo, Texas, 1999. *MMWR Morb Mortal Wkly Rep* 2001;**50**:57–9.
34. Centers for Disease Control and Prevention. Update: Outbreak of acute febrile respiratory illness among college students: Acapulco, Mexico, March 2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:359–60.
35. Centers for Disease Control and Prevention. Update: Outbreak of poliomyelitis: Dominican Republic and Haiti, 2000-2001. *MMWR Morb Mortal Wkly Rep* 2001;**50**:855–6.
36. Centers for Disease Control and Prevention. Measles among children adopted from China. *MMWR Morb Mortal Wkly Rep* 2004;**53**:459.
37. Centers for Disease Control and Prevention. Multistate investigation of measles among adoptees from China: April 9, 2004. *MMWR Morb Mortal Wkly Rep* 2004;**53**:309–10.
38. Centers for Disease Control and Prevention. *Health information for international travel 2005-2006*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2005.
39. Centers for Disease Control and Prevention. Chikungunya fever diagnosed among international travelers—United States, 2005-2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1040–2.
40. Centers for Disease Control and Prevention. General recommendations on immunization. *MMWR Recomm Rep* 2006;**55**(RR-15):1–56.
41. Centers for Disease Control and Prevention. Prevention and control of influenza. *MMWR Recomm Rep* 2006;**55**(RR-10):1–48.
42. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;**55**(RR-7):1–30.
43. Centers for Disease Control and Prevention. FDA approval of an alternate dosing schedule for a combined hepatitis A and B vaccine (Twinrix®). *MMWR Morb Mortal Wkly Rep* 2007;**56**:1057.
44. Centers for Disease Control and Prevention. *Health information for international travel 2007-2008*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2007.
45. Centers for Disease Control and Prevention. Human rabies prevention: United States, 2008. Recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**(RR-03):1–28.
46. Centers for Disease Control and Prevention. Novel influenza A (H1N1) virus infections: worldwide, May 6, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:453–8.
47. Centers for Disease Control and Prevention. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;**58**(RR-02):1–25.
48. Centers for Disease Control and Prevention. Swine influenza A (H1N1) infection in two children: Southern California, March–April 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:400–2.

49. Centers for Disease Control and Prevention. Recommendations of the Advisory Committee on Immunization Practices (ACIP): Japanese encephalitis vaccines. *MMWR Recomm Rep* 2010; **59**(RR-01):1–27.
50. Centers for Disease Control and Prevention. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies. *MMWR Recomm Rec* 2010;**59**(RR-02):1–9.
51. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391–2.
52. Centers for Disease Control and Prevention. Updated recommendations for use of meningococcal conjugate vaccines: Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:72–6.
53. Centers for Disease Control and Prevention. *Health information for international travel 2012*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2012.
54. Centers for Disease Control and Prevention. Malaria surveillance: United States, 2010. *MMWR Surveill Summ 2012* 2012; **61**(SS-02):1–17.
55. Centers for Disease Control and Prevention. Measles: United States. *MMWR Morb Mort Wkly Rep* 2012;**61**:253–7.
56. Centers for Disease Control and Prevention. *Meningococcal vaccines: vaccine information sheet*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2012.
57. Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 1 through 18 years: United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 1–5.
58. Centers for Disease Control and Prevention. *Yellow fever vaccine: vaccine information sheet*. Atlanta: U.S. Department of Health and Human Services, Public Health Service; 2012.
59. Cetron M, Chitsulo L, Sullivan J, et al. Schistosomiasis in Lake Malawi. *Lancet* 1996;**348**:1274–8.
60. Chen R, Penney D, Little D, et al. Hepatitis and death following vaccination with 17D-204 yellow fever vaccine. *Lancet* 2001;**358**: 121–2.
61. Cobelens FG, van Deutekom H, Draayer-Jansen IW, et al. Risk of infection with *Mycobacterium tuberculosis* in travelers to areas of high tuberculosis endemicity. *Lancet* 2000;**356**:461–5.
62. Committee on Infectious Disease, American Academy of Pediatrics. The use of systemic fluoroquinolones. *Pediatrics* 2006;**118**: 1287–92.
63. Cooke G, Lalvani A, Gleeson F, et al. Acute pulmonary schistosomiasis in travelers returning from Lake Malawi, sub-Saharan Africa. *Clin Infect Dis* 1999;**29**:836–9.
64. Cornall P, Howie S, Mughal A, et al. Drowning of British children abroad. *Child Care Health Dev* 2005;**31**:611–13.
65. Dawson A. Medical aspects of air travel. In: Dupont HL, Steffen R, editors. *Textbook of travel medicine and health*. 2nd ed. Hamilton, Ontario, Canada: B. C. Decker; 2001. p. 390–403.
66. Duggan C, Santosham M, Glass RI. The management of acute diarrhea in children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* 1992;**44**(RR-16):1–20.
67. Dupont H, Capsuto E. Persistent diarrhea in travelers. *Clin Infect Dis* 1996;**22**:124–8.
68. Dupont H, Ericsson C. Prevention and treatment of traveler's diarrhea. *N Engl J Med* 1993;**328**:1821–7.
69. DuPont HL, Ericsson CD, Farthing MJ, et al. Expert review of the evidence base for prevention of travelers' diarrhea. *J Travel Med* 2009;**16**:149–60.
70. Ericsson C, Dupont H. Travelers diarrhea: approaches to prevention and treatment. *Clin Infect Dis* 1993;**16**:616–24.
71. Faust SN, Woodrow CJ, Patel S, et al. Sleeping sickness in brothers in London. *Pediatr Infect Dis J* 2004;**23**:879–81.
72. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. *N Engl J Med* 2002;**347**:13–18.
73. Freedman D, Leuthold C. Information sources in travel medicine. In: Dupont HL, Steffen R, editors. *Textbook of travel medicine and health*. 2nd ed. Hamilton, Ontario, Canada: B. C. Decker; 2001. p. 37–45.
74. Freedman DO, Weld LH, Kozarsky PE, et al. Spectrum of disease and relation to place of exposure among ill returned travelers. *N Engl J Med* 2006;**354**:119–30.
75. Gibney KB, Fischer M, Prince HE, et al. Chikungunya fever in the United States: a fifteen year review of cases. *Clin Infect Dis* 2011;**52**:e121–6.
76. Greenberg A, Lobel H. Mortality from *Plasmodium falciparum* malaria in travelers from the United States. *Ann Intern Med* 1990;**113**:326–7.
77. Hagmann S, Neugebauer R, Schwartz E, et al. Illness in children after international travel: analysis from the GeoSentinel Surveillance Network. *Pediatrics* 2010;**125**:e1072–80.
78. Hamer DH, Connor BA. Travel health knowledge, attitudes and practices among United States travelers. *J Travel Med* 2004; **11**:23–6.
79. Han P, Balaban V, Marano C. Travel characteristics and risk-taking attitudes in youths traveling to nonindustrialized countries. *J Travel Med* 2010;**17**:316–21.
80. Hartjes L, Baumann L, Henriques J. Travel health risk perceptions and prevention behaviors of US study abroad students. *J Travel Med* 2009;**16**:338–43.
81. Hatchette T, Green P, Schlech W, et al. Lesion on the arm of a returning traveler. *Clin Infect Dis* 2001;**33**:815, 897–8.
82. Herbinger KH, Drepup L, Alberer M, et al. Spectrum of imported infectious diseases among children and adolescents returning from the tropics and subtropics. *J Travel Med* 2012;**19**:150–7.
83. Herwaldt B, Stokes S, Juranek D. American cutaneous leishmaniasis in US travelers. *Ann Intern Med* 1993;**118**:779–84.
84. Hostetter M. Epidemiology of travel-related morbidity and mortality in children. *Pediatr Rev* 1999;**20**:228–33.
85. Hunziker T, Berger C, Staubli G, et al. Profile of travel-associated illness in children, Zürich, Switzerland. *J Travel Med* 2012;**19**: 158–62.
86. Immunization Action Coalition. *Summary of recommendations for adult immunization*. St. Paul, Minn: The Committee; 2006.
87. Immunization Action Coalition. *Summary of recommendations for adult immunization*. St. Paul, Minn: The Committee; 2012.
88. Immunization Action Coalition. *Summary of rules for childhood immunization*. St. Paul, Minn: The Committee; 2012.
89. Institute of International Education. *Open doors report 2011*. Available at http://www.iie.org/Research-and-Publications/Open-Doors/Data.
90. Jenista J. The international child traveler. In: Jensen HB, Baltimore RS, editors. *Pediatric infectious diseases: principles and practice*. Norwalk, Conn: Appleton & Lange; 1995. p. 1509–23.
91. Kain K, Keystone J. Malaria in travelers: epidemiology, disease, and prevention. *Infect Dis Clin North Am* 1998;**12**:267–84.
92. Kain K, Shanks G, Keystone J. Malaria chemoprophylaxis in the age of drug resistance. I. Currently recommended drug regimens. *Clin Infect Dis* 2001;**33**:226–34.
93. Kain K, Harrington M, Tennyson S, et al. Imported malaria: prospective analysis of problems in diagnosis and management. *Clin Infect Dis* 1998;**27**:142–9.
94. Keystone J, Kozarsky P, Freedman D. Internet and computer-based resources for travel medicine practitioners. *Clin Infect Dis* 2001;**32**:757–65.
95. King CK, Glass R, Bresee JS, et al. Managing acute gastroenteritis among children: oral rehydration, maintenance, and nutritional therapy. *MMWR Recomm Rep* 2003;**52**(RR-16):1–16.
96. Krishnan N, Purswani M, Hagmann S. Severe dengue virus infection in pediatric travelers visiting friends and relatives after travel to the Caribbean. *Am J Trop Med Hyg* 2012;**86**:474–6.
97. Leder K, Black J, O'Brien D, et al. Malaria in travelers: a review of the GeoSentinel surveillance network. *Clin Infect Dis* 2004;**39**: 1104–12.
98. Leder K, Sundararajan V, Weld L, et al. Respiratory tract infections in travelers: a review of the GeoSentinel surveillance network. *Clin Infect Dis* 2003;**36**:399–406.
99. Leder K, Tong S, Weld L, et al. Illness in travelers visiting friends and relatives: a review of the GeoSentinel Surveillance Network. *Clin Infect Dis* 2006;**43**:1185–93.
100. Lederman E, Weld L, Elyazar I, et al. Dermatologic conditions of the ill returned traveler: an analysis from the GeoSentinel Surveillance Network. *Inter J Inf Dis* 2008;**12**:593–602.
101. Leggat PA, Fischer PR. Accidents and repatriation. *Travel Med Infect Dis* 2006;**4**:135–46.
102. Lobato M, Hopewell P. *Mycobacterium tuberculosis* infection after travel to or contact with visitors from countries with a high prevalence of tuberculosis. *Am J Respir Crit Care Med* 1998;**158**: 1871–5.

103. Lobel H, Kozarsky P. Update on prevention of malaria in travelers. *JAMA* 1997;**278**:1767–71.
104. Luxemberger C, Price R, Nosten F, et al. Mefloquine in infants and young children. *Ann Trop Paediatr* 1996;**16**:281–6.
105. Maloney S, Cetron M. Investigation and management of infectious diseases on international conveyances (airplanes and cruiseships). In: Dupont HL, Steffen R, editors. *Textbook of travel medicine and health*. 2nd ed. Hamilton, Ontario, Canada: B. C. Decker; 2001. p. 519–30.
106. Martin M, Tsai T, Cropp B, et al. Fever and multisystem organ failure associated with 17D-204 yellow fever vaccination: a report of four cases. *Lancet* 2001;**358**:98–104.
107. Matteelli A, Carosi G. Sexually transmitted diseases in travelers. *Clin Infect Dis* 2001;**32**:1063–7.
108. McFarland J, Baddour L, Nelson J, et al. Imported yellow fever in a United States citizen. *Clin Infect Dis* 1997;**25**:1143–7.
109. Melby P, Kreutzer R, McMahon-Pratt D, et al. Cutaneous leishmaniasis: review of 58 cases seen at the National Institutes of Health. *Clin Infect Dis* 1992;**15**:924–37.
110. Moore AC. African trypanosomiasis in U.S. travelers. Presented at the 50th Annual Meeting of the American Society of Tropical Medicine and Hygiene, Atlanta, November 13, 2001.
111. Moore AC, Ryan ET, Waldron MA. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Case 20-2002: a 37-year-old man with fever, hepatosplenomegaly, and a cutaneous foot lesion after a trip to Africa. *N Engl J Med* 2002;**346**:2069–76.
112. Moore DA, Edwards M, Escombe R, et al. African trypanosomiasis in travelers returning to the United Kingdom. *Emerg Infect Dis* 2002;**8**:74–6.
113. Murphy ME, Montemarano AD, Debboun M, et al. The effect of sunscreen on the efficacy of insect repellent: a clinical trial. *J Am Acad Dermatol* 2000;**43**:219–22.
114. Neumann K. Pediatric travel medicine: where we are and where we hope to go. *J Travel Med* **19**:137–9, 2012,
115. Newman RD, Parise ME. Malaria-related deaths among U.S. travelers, 1963 to 2001. *Ann Intern Med* 2004;**141**:547–55.
116. Nevill C, Some E, Mung'ala V, et al. Insecticide-treated bednets reduce mortality and severe morbidity from malaria among children on the Kenyan coast. *Trop Med Int Health* 1996;**1**:139–46.
117. O'Brien DP, Leder K, Matchett E, et al. Illness in returned travelers and immigrants/refugees: the 6-year experience of two Australian infectious disease units. *J Travel Med* 2006;**13**:145–52.
118. Pan American Health Organization. Reemergence of dengue in the Americans. *Epidemiol Bull Pan Am Health Organ* 1997;**18**:1–10.
119. Parker AA, Staggs W, Dayan GH, et al. Implications of a 2005 measles outbreak in Indiana for sustained elimination of measles in the United States. *N Engl J Med* 2006;**355**:447–55.
120. Peltola H, Gorbach S. Travelers' diarrhea: epidemiology and clinical aspects. In: Dupont HL, Steffen R, editors. *Textbook of travel medicine and health*. 2nd ed. Hamilton, Ontario, Canada: B. C. Decker; 2001. p. 151–9.
121. Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
122. Pitzinger B, Steffen R, Tschopp A. Incidence and clinical features of traveler's diarrhea in infants and children. *Pediatr Infect Dis J* 1991;**10**:719–23.
123. Polio Global Eradication Initiative. *Infected countries as of July 11, 2012*. Available at www.polioeradication.org.
124. Rawlings J, Hendricks K, Burgess C, et al. Dengue surveillance in Texas, 1995. *Am J Trop Med Hyg* 1998;**59**:95–9.
125. Reid D, Keystone J, Cossar J. Health risks abroad: general considerations. In: Dupont HL, Steffen R, editors. *Textbook of travel medicine and health*. 2nd ed. Hamilton, Ontario, Canada: B. C. Decker; 2001. p. 3–10.
126. Richens J. Sexually transmitted infections and HIV among travelers. *Travel Med Infect Dis* 2006;**4**:184–95.
127. Rivera-Matos I, Atkins J, Doerr C, et al. Pediatric malaria in Houston, Texas. *Am J Trop Med Hyg* 1997;**57**:560–3.
128. Robertson S, Hull B, Tomori O, et al. Yellow fever: a decade of reemergence. *JAMA* 1996;**276**:1157–62.
129. Ryan E, Kain K. Health advice and immunizations for travelers. *N Engl J Med* 2000;**342**:1716–25.
130. Sandhu BK. Practical guidelines for the management of gastroenteritis in children. *J Pediatr Gastroenterol Nutr* 2001;**33**(Suppl. 2): S36–9.
131. Shanks G, Kain K, Keystone J. Malaria chemoprophylaxis in the age of drug resistance. II. Drugs that may be available in the future. *Clin Infect Dis* 2001;**33**:381–5.
132. Soumahoro MK, Fontenille D, Turbelin C, et al. Imported chikungunya virus infection. *Emerg Infect Dis* 2010;**16**:162–3.
133. Steffen R. Epidemiologic studies of travelers' diarrhea, severe gastrointestinal infections, and cholera. *Rev Infect Dis* 1986; **8**(Suppl. 2):122–30.
134. Svenson J, MacLean J, Gyorkos T, et al. Imported malaria: clinical presentation and examination of symptomatic travelers. *Arch Intern Med* 1995;**155**:861–8.
135. Thein S, Aung M, Shwe T, et al. Risk factors in dengue shock syndrome. *Am J Trop Med Hyg* 1997;**56**:566–72.
136. U.S. Department of Commerce. *Tourism Industries Office: Travel statistics. Trade Department, International Trade Administration*, n.d. Available at http://www.tinet.ita.doc.gov.
137. U.S. Department of Commerce. *Tourism Industries Office: Profile of U.S. resident travelers visiting overseas destinations: 2010 outbound. 2010. Trade Department, International Trade Administration*, http://tinet.ita.doc.gov/outreachpages/download_data_table/2010_Outbound_Profile.pdf1.
138. Valdez H, Salata R. Bat-associated histoplasmosis in returning travelers: case presentation and description of a cluster. *J Travel Med* 1999;**6**:258–60.
139. Van Herck K, Castelli F, Zuckerman J, et al. Knowledge, attitudes and practices in travel-related infectious diseases: the European airport survey. *J Travel Med* 2004;**11**:3–8.
140. Van Rijn S, Driessen G, Overbosch D, et al. Travel-related morbidity in children: a prospective observational study. *J Travel Med* 2012;**19**:144–9.
141. Vasconcelos P, Luna E, Galler R, et al. Serious adverse events associated with yellow fever 17DD vaccine in Brazil: a report of two cases. *Lancet* 2001;**358**:9107.
142. Vasconcelos P, Travassos da Rosa A, Pinheiro F, et al. *Aedes aegypti*, dengue and re-urbanization of yellow fever in Brazil and other South American countries: past and present situation and future perspectives. *WHO Dengue Bull* 1999;**23**:55–6.
143. Viani R, Bromberg K. Pediatric imported malaria in New York: delayed diagnosis. *Clin Pediatr (Phila)* 1999;**38**:333–7.
144. Weinberg M, Weeks J, Lance-Parker S, et al. Severe histoplasmosis in travelers to Nicaragua. *Emerg Infect Dis* 2003;**9**:1322–5.
145. Wilder-Smith A, Schwartz E. Dengue in travelers. *N Engl J Med* 2005;**353**:924–32.
146. Wilder-Smith A, Foo W, Earnest A, et al. High risk of *Mycobacterium tuberculosis* infection during the Hajj pilgrimage. *Trop Med Int Health* 2005;**10**:336–9.
147. Wilder-Smith A, Khairullah NS, Song JH, et al. Travel knowledge, attitudes and practices among Australian travelers. *J Travel Med* 2004;**11**:9–15.
148. Wilson M, Lucchina L. Healthy student with a papule. *Clin Infect Dis* 2001;**33**:816, 899–900.
149. Winters R, Murray H. Malaria: the mime revisited: fifteen more years of experience at a New York City teaching hospital. *Am J Med* 1992;**93**:243–6.
150. World Health Organization. *Malaria*. n.d. Available at http://www.who.int.
151. World Health Organization. *Extensively drug-resistant tuberculosis (XDR-TB)*. Geneva, Switzerland. 2006. http://www.who.int/tb/challenges/mdr/xdr/en/index.html.
152. World Health Organization. Extensively drug-resistant tuberculosis (XDR-TB): recommendations for prevention and control. *Wkly Epidemiol Rec* 2006;**81**:430–2.
153. World Health Organization. *Roll back malaria. Fact sheet No. 94*. Geneva, Switzerland: World Health Organization; 2006.
154. World Health Organization. Yellow fever situation in Africa and South America, 2005. *Wkly Epidemiol Rec* 2006;**81**:317–24.
155. World Health Organization. *Cumulative number of confirmed human cases for avian influenza A (H5N1) reported to WHO, 2003-2012*. Available at http://www.who.int/influenza/human_animal_interface/EN_GIP_20121217CumulativeNumberH5N1cases.pdf.
156. World Health Organization. *International Travel and Health: Vaccination requirements and health advice*. Geneva: World Health Organization; 2012. Available at http://www/who.int/ith/en/.
157. World Tourism Organization. *World tourism highlights*, 2012. Madrid, Spain. Available at http://mkt.unwto.org/en/publication/unwto-tourism-highlights-2012-edition.

CHAPTER 232

Infectious Disease Considerations in International Adoptees and Refugees

Mary Allen Staat

Each year thousands of children immigrate to the United States to begin a new life (Fig. 232-1). The focus of this chapter will be on two groups of immigrants—refugees and internationally adopted children. Refugees are noncitizen immigrants who are unable or unwilling to return to their country of origin because of persecution or fear of persecution.[79] Internationally adopted children are immigrants that are classified as orphans; however, most of these children are not truly orphaned, but have been abandoned by or separated from both parents.[79] These children generally will be given U.S. citizenship as they arrive or shortly after they arrive in the United States to join their new families.

In 2010, a total of 73,293 refugees arrived in the United States.[79] Nearly 85 percent of these refugees came from just six countries: Iraq (25%), Burma (23%), Bhutan (17%), Somalia (7%), Cuba (7%), and Iran (5%). Although these are the most prevalent countries, refugees have come to the United States from countries all around the world, with the exception of northern Europe, Australia, New Zealand, and Canada. Of refugees immigrating to the United States in 2010, 36 percent were younger than 20 years of age and 48 percent were females. The ages of the children were fairly evenly distributed—younger than 5 years (26%), 5 to 9 years (26%), 10 to 14 years (24%), and 15 to 19 years (23%). Only 1 percent of refugee children were younger than 1 year of age.

Over the past 20 years, international adoption has been a popular way to build families.[80] Since 1999, more than 200,000 children have been internationally adopted to families in the United States. The peak of international adoptions was in 2004, with 22,990 children immigrating to the United States; 83 percent of children came from just five countries: China (31%), Russia (26%), Guatemala (14%), South Korea (8%), and Kazakhstan (4%).[80] However, since 2004, a dramatic decline has occurred in the number of adoptions and the countries where children are adopted from is far more diverse. In 2010, only 11,100 children were adopted internationally, with 83 percent of children coming from nine countries: China (30%), Ethiopia (23%), Russia (10%), South Korea (8%), the Ukraine (4%), Taiwan (3%), India (2%), Colombia (2%), and the Philippines (2%).[79] Several countries that once allowed adoption, such as Romania, Vietnam, Cambodia, and Guatemala, are now closed to adoptions or have dramatically decreased the number of international adoptions from their countries. For example, in 2008, the largest numbers of adopted children were from Guatemala, with 4123 adoptions. In contrast, after the United States stopped processing Guatemalan adoptions in 2010, only 49 children were adopted from Guatemala.[80] In addition, a change has been seen in the medical needs of internationally adopted children. Over the past few years, China has shifted to primarily referring children with special needs such as congenital heart disease, cleft lip and palate, limb deficiencies, anorectal malformations, and spina bifida. In previous years, the majority of children referred to families from China were healthy infants without known medical conditions.

In contrast to refugee children, internationally adopted children are more likely to be girls (56%), largely because of increased availability of girls from China, and are more likely to be younger, with 73 percent of children younger than 5 years of age and 21 percent younger than 1 year of age at the time of adoption.[79] However, the age and gender of adoptees also has changed over time. In 2004, 65 percent of adopted children were girls and 41 percent were younger than 1 year of age and 86 percent were younger than 5 years of age.[80] With the arrival of older children, health care providers need to be aware that they will have an increased risk for malnutrition, infectious disease exposures, and unmet medical needs, as well as a more difficult transition to joining their family in contrast to younger adoptees in the past.

Children available for international adoption generally have resided all or most of their lives in orphanages before coming to the United States. The exception is South Korea, where nearly all children are placed in foster care until their adoption. In more recent years, some children from China have also been placed with foster families until adopted.

Because refugees and internationally adopted children come from resource-poor countries, physicians and other health care providers need to be aware of the global prevalence of infectious diseases that are seen less commonly in native-born North Americans. Both refugees and internationally adopted children are at increased risk for common infectious diseases such as tuberculosis (TB), intestinal parasites, dermatologic infections, and infestations. In addition, in countries where resources are few

FIGURE 232-1 ■ A group of internationally adopted children. (See companion Expert Consult website for color version.)

and infrastructure limited for screening and prevention programs for hepatitis B, hepatitis C, human immunodeficiency virus (HIV), and syphilis, the prevalence is higher in contrast to the prevalence in the United States.

Although many similarities exist in the infectious disease considerations for refugees and internationally adopted children, several important differences also are found. Refugee and internationally adopted children differ in terms of the general medical screening they receive before their arrival to the United States.[4,8,14-16] Most refugees undergo organized screening evaluations before emigration visas are issued. Children have predeparture screening and a physical examination, and, depending on their country of departure, they may receive predeparture treatment for malaria, intestinal parasites, or tissue parasites. In contrast, only adoptees from certain countries with high rates of TB, such as China and Ethiopia, have been required to undergo this organized screening procedure for TB. Second, because medical screening for refugees is usually sponsored by responsible medical organizations, results of testing are typically accurate. In internationally adopted children, medical testing may be incomplete and is typically done shortly after birth or on entrance to the orphanage. However, families should expect to receive results for HIV, syphilis, and hepatitis B surface antigen testing with their referral information. For some countries, such as Ethiopia, HIV polymerase chain reaction (PCR) and hepatitis C test results also are provided. For Russian referrals, hepatitis C testing is often done as well. Although the reliability of testing done in the child's birth county has been a concern in the past, in recent years the testing has generally proven to be accurate when repeated in the United States. Third, preventive measures such as immunizations, vitamin supplementation, and dental care are provided for most refugee children while still in refugee camps; in adoptees, no standard approach is followed in the provision of these measures from country to country or even within a given country. Differences also exist in the types of infectious diseases and risks for acquiring these diseases in refugee children in contrast to internationally adopted children. Although both groups are susceptible to a variety of infectious agents, because the countries of origin and the living conditions differ, refugee children are more likely to have been exposed to infections such as typhoid fever, malaria, filariasis, flukes, and schistosomiasis, which are uncommonly seen in internationally adopted children from countries outside of Africa.

Health care professionals will inevitably have the opportunity to provide care for these children as they immigrate to the United States and should therefore be knowledgeable of the infectious disease issues they may encounter and the need for screening for infectious diseases in these populations.

OVERALL EVALUATION

Because of the predominance of infectious diseases in resource-poor countries, recommendations for screening tests in refugees and internationally adopted children have been weighted toward infectious disease processes and this will be the focus of this chapter. It is important, however, that the initial assessment include all aspects of general health, including evaluation of growth and development and vision, hearing, and dental evaluations.[6,8,14,15,84] For internationally adopted children, several others tests are recommended, including testing for blood lead concentration, complete blood cell count with red blood cell indices, and differential of white blood cells to evaluate for eosinophilia, glucose-6-phosphate dehydrogenase screening, hemoglobin electrophoresis, measurement of thyroid-stimulating hormone concentration, and examination for congenital anomalies (including fetal alcohol syndrome).[4] Similar to adoptees, a complete blood count and a lead level is recommended for refugees.[15] Additional tests, such as a urinalysis to determine if there is hematuria (for schistosomiasis) and screening for sexually transmitted infections are recommended. Mental health screening is encouraged if services are available.

Although many immigrant children may appear healthy on arrival, children should be evaluated by a health care professional within 2 weeks after arrival to ensure that their initial health care needs are addressed, initial screening is done and a plan for receiving preventive services such as immunizations are initiated. Table 232-1 outlines the infectious disease screening tests recommended for refugee and internationally adopted children with details for each condition outlined in the following discussion.

INFECTIOUS DISEASE SCREENING

Viral Hepatitis

Hepatitis A

Virtually all people residing in resource-poor countries have contracted hepatitis A by early adulthood and have hepatitis A immunoglobulin (Ig)G antibodies.[26] The prevalence of past hepatitis A infection varies by country and age. In a study examining the prevalence of acute and past hepatitis A infection in internationally adopted

TABLE 232-1 **Infectious Disease Screening for Refugee and Internationally Adopted Children**

Condition or Test	Refugee Children	Internationally Adopted Children
Hepatitis A Virus (Acute Infection)		
Hepatitis A IgM antibody	Not recommended	All
Hepatitis B Virus		
Hepatitis B surface antigen (HBsAg)	All	All*
Hepatitis B surface antibody (anti-HBs)	All	All*
Hepatitis B core antibody (anti-HBc)	All	All*
Hepatitis C Virus		
Hepatitis C antibody	Some	All*
Syphilis		
Nontreponemal test (RPR, VDRL, or ART)	≥15 years of age	All
Treponemal test (TPPA, MHA-TP, or FTA-ABS)	Not recommended	All
Human Immunodeficiency Virus		
Human immunodeficiency virus 1 and 2 antibody	≥13 years of age	All*
Recommended	<13 years of age	
Encouraged		
Tuberculosis		
Tuberculin skin test (TST)	All	All*
or		
Interferon-γ release assay	≥5 years of age	≥5 years of age
Intestinal Parasites		
Microscopic evaluation of stool for ova and parasites	All (2 specimens)†	All (3 specimens)
Giardia intestinalis and *Cryptosporidium* antigen	All (1 specimen)†	All (1 specimen)
Bacterial Enteric Infection		
In children with diarrhea-bacterial stool culture	All	All
Chagas (Endemic Areas)		
Trypanosoma cruzi serologic testing	All	All
Complete Blood Count	All	All
Eosinophilia with negative stool ova and parasite for helminths		
Strongyloides serologic testing	All	All
Schistosoma serologic testing (endemic countries)‡		
Lymphatic Filariasis (Endemic Areas)		
Filaria serology	All	≥2 years of age
Chagas (Endemic Areas)		
Trypanosoma cruzi serologic testing	All	All
Malaria Screening (Endemic Areas)		
Malaria polymerase chain reaction	All without pretreatment	Not recommended
Hematuria (Schistosomiasis)		
Urinalysis	All	Not recommended

*Consider reassessing 6 mo after arrival.
†All should be screened if there was no presumptive treatment before arrival to the United States; see guidelines for evaluation for intestinal parasites in refugees with presumptive treatment (http://cdc.gov/immigrantrefugeehealth/guidelines/domestic/intestinal-parasites-domestic.html).
‡Some experts recommend serologic testing regardless of whether eosinophilia is present.
ART, Automated reagin test; *FTA-ABS,* fluorescent treponemal antibody absorption; *MHA-TP,* microhemagglutination–*Treponema pallidum; RPR,* rapid plasma reagin; *TPPA, Treponema pallidum* particle agglutination; *VDRL,* Venereal Disease Research Laboratory.

children, 1 percent of children had acute hepatitis A infection (positive hepatitis A IgM) on arrival the United States and 29 percent had evidence of prior infection (positive total hepatitis A antibody).[1] In this study, the prevalence of prior infection increased with age; 13 percent of children younger than 2 years of age were immune in contrast to 80 percent of children 12 to 17 years of age. The highest region-specific prevalence was in Africa (72%), and the lowest was in the Asia and Pacific Rim Region (17%).

Given the high prevalence of hepatitis A virus (HAV) in these countries, there is an ongoing risk for infection of children while in their country of origin. In internationally adopted children, children may be incubating HAV infection at the time of adoption and could transmit the virus to their adoptive families and others while in country or after arrival to the United States. Several cases have been reported of HAV transmitted from internationally adopted children to family members and close contacts.[1,34,76,85] Therefore, adoptive parents and any accompanying family members should be immunized against hepatitis A or have documented immunity to HAV infection before international travel to receive their child.[7,25] It is also recommended that hepatitis A vaccine be administered before the arrival of the adoptee to all susceptible nontraveling people who plan to have close contact with an internationally adopted child from a country with high or intermediate hepatitis A endemicity.

In the United States, hepatitis A vaccine is recommended for all children 12 months of age and older.[23,28] Hepatitis A vaccine is not routinely administered to children or adults in resource-poor countries. Because the prevalence of past HAV infection is high in these countries, antibody testing can be done to determine HAV immunity. Hepatitis A antibody testing (total or IgG) should be considered in children older than 1 year of age for both refugees and internationally adopted children to determine the need for this vaccine.[3,4,15]

Infection with hepatitis A is often difficult to diagnose clinically in children; as many as 90 percent of children younger than 6 years of age and 50 to 60 percent of older children are anicteric.[23,26] Thus, most young children will not have signs or symptoms of hepatitis A, yet those who are infected may shed the virus for months. To identify hepatitis A infection in asymptomatic children, hepatitis A IgM antibody testing should be done.[4] When acute infection is found in a child, secondary cases can be prevented by providing hepatitis A vaccine to unvaccinated family members and close contacts of the case.[7,23,25] Although most children are asymptomatic, viral hepatitis should be considered in children with symptoms such as vomiting and loss of appetite, when accompanied by fever and an enlarged, tender liver even in the absence of jaundice.[4,23,26]

Hepatitis B

Routine screening for hepatitis B virus (HBV) infection is recommended for all refugees and internationally adopted children.[4,8,14,15] Most children who have hepatitis B infection and are carriers are asymptomatic. Therefore, screening is important to identify these children so appropriate management can be initiated and unvaccinated household contacts and caregivers can be vaccinated or be given hepatitis B Ig if they have had a significant exposure to blood of a hepatitis B carrier. The prevalence of hepatitis B infection is higher in resource-poor countries than in the United States. In Asia, Africa, and Central and South America, the prevalence of hepatitis B ranges from 2 to 7 percent in contrast to only 0.5 percent in the United States.[27] Several studies have shown rates of infection in refugee[12,17,49,53] and internationally adopted children* mirror the reported rates in their country of origin. Immigrant children have typically acquired HBV by vertical transmission, although bloodborne infection and horizontal transmission also have been implicated. In international adoptees, the highest reported rates were in Romanian adoptees from the early 1990s, with 53 percent having evidence of past or present infection and 20 percent with active infection.[39,45,46,62,64] More recent studies have consistently shown a lower range of prevalence (1% to 5%).† Several reasons explain the high prevalence of hepatitis B infection in Romanian adoptees. During that period, Romania had an overall high prevalence of hepatitis B and therefore many children in orphanages were infected with hepatitis B. Needles were commonly reused for immunizations and other injections. Transmission was facilitated through contaminated needles and close contact with children infected with hepatitis B. Hepatitis B vaccine was not used during that period. In addition, hepatitis B screening was not routinely done in children available for adoption; thus, many children arrived to the United States with unsuspected hepatitis B. In more recent years, hepatitis B vaccine in infants and screening for hepatitis B infection is routinely done and reported in the referral information. Thus, the diagnosis of hepatitis B infection is nearly always known before adoption and this condition is considered to be a special need.

Hepatitis B screening tests, including the hepatitis B surface antigen (HBsAg) and antibodies to surface (anti-HBs) and core (anti-HBc) antigens, are recommended in the medical evaluation of both internationally adopted children and refugees.[4,8,14,15] All three tests should be done to determine whether the child is immune as a result of immunization (anti-HBs positive only), has recovered from infection (anti-HBs and anti-HBc positive), or has acute or chronic hepatitis B (HBsAg and anti-HBc positive). Patterns of hepatitis B serology can be found in Chapter 156. In a child found to be HBsAg-positive, repeat testing should be done 6 months later. If the HBsAg persists for longer than 6 months, the child has chronic hepatitis B infection. If the child no longer has HBsAg and has developed anti-HBs, the child had acute infection and has recovered and is no longer able to transmit the virus to others. Children with acute or chronic hepatitis B (positive HBsAg) can transmit hepatitis B to others. A positive hepatitis B IgM differentiates acute hepatitis B from chronic hepatitis B. Children with acute or chronic hepatitis B should have additional testing

*References 2, 42, 43, 54, 58-60, 65, 73.
†References 2, 42, 43, 54, 56, 58, 60, 65, 73.

done, including testing for HBeAg, HBeAb, and serum hepatic enzyme levels. HBsAg-positive children should be followed closely by a hepatologist for management. Hepatitis D virus (HDV), which is seen only in patients with hepatitis B infection, occurs in North Africa, parts of South America, and the Mediterranean Basin.[4] Testing for HDV is not widely available, but is recommended in patients from these regions who are found to have hepatitis B infection; however, a positive test does not change clinical management. For children who initially test negative for HBsAg, HBsAb, and HBcAb, some experts recommend repeat testing for hepatitis B approximately 6 months after arrival to ensure the child was not infected just before arrival to the United States.[4] Children with acute or chronic hepatitis B should have serologic testing for hepatitis A to determine the need immunization with hepatitis A vaccine and for hepatitis C for overall management and treatment considerations.

Ideally, the family members of internationally adopted children should be vaccinated against hepatitis B before the child joins the family. If this has not been done, the household contacts of children with acute or chronic hepatitis B (HBsAg-positive) must be vaccinated promptly. Hepatitis B is easily transmitted to unvaccinated household contacts. Up to 20 percent of household contacts will become HBsAg-positive with 5 or more years of exposure within the home,[27,39] and transmission of hepatitis B from newly adopted children to their parents has been documented.[36,39,70]

Hepatitis C

Screening for hepatitis C is recommended for all internationally adopted children because of high rates of hepatitis C in the countries of origin of adoptees and because of the difficulty in determining risk factors for these children.[4] In refugees, testing is recommended if there is a history of specific risk factors such as current or former injection drug use, receipt of blood products, or residence in settings with a documented high prevalence of hepatitis C.[14,15] Data on the prevalence of hepatitis C in both refugee and internationally adopted children are limited. One study examining the prevalence of hepatitis C antibody in internationally adopted children found the prevalence to be less than 1 percent.[65] Antibody testing with an enzyme immunoassay (EIA) should be done with the initial screening. If the antibody is positive, PCR should be done to confirm infection. Maternal antibody may be present until a child is 18 months of age. Children found to have hepatitis C infection should be immunized for hepatitis A and B and should be referred to a hepatologist for further management (see Chapter 176).

Human Immunodeficiency Virus 1 and 2 Infections

In the past, as part of the required overseas medical assessment, HIV testing was required for all immigrants and refugees 15 years of age and older.[14] Children younger than 15 years of age were tested for HIV if history or examination raised concern about possible HIV infection (e.g., maternal history of HIV infection or history of rape or sexual assault). Since January 2010, HIV testing is no longer required for immigration medical assessment. The exception is that HIV testing still is recommended for people diagnosed with active TB. For refugees, HIV testing is recommended after arrival in the United States for persons 13 through 64 years of age and encouraged for those younger than 13 years of age (http://www.cdc.gov/immigrantrefugeehealth/guidelines/domestic/screening-hiv-infection-domestic.html).

The decision to screen children younger than 13 years of age depends on history and risk factors (e.g., receipt of blood products and maternal drug use), physical examination findings, and prevalence of HIV infection in the child's country of origin. If there is a suspicion of HIV infection, testing should be performed before administration of live vaccines.

In contrast, most internationally adopted children have been tested for antibodies to HIV-1 by enzyme immunoassay (EIA) in their birth country. In Ethiopia, an HIV-1 PCR test is also done. In children born to mothers with HIV and found to have antibodies to HIV in their country of origin, HIV-1 PCR testing is generally done. In more recent years, children known to be exposed to HIV have been given antiretroviral drugs. For internationally adopted children, on arrival to the United States, routine screening by EIA is recommended.[4]

Reports of HIV infection in internationally adopted children are rare. In the early 1990s, two cases of Romanian adoptees with HIV infection were reported.[44] Subsequently, Romania was found to have high rates of HIV in institutionalized children that were not felt to be maternally transimitted.[38] The high prevalence was found to be associated with transmission through contaminated needles and blood products. In a more recent case series, there have been no reports of HIV infection in adoptees.[65] In contrast to past years when children infected with HIV were not permitted to be adopted internationally, these children may now be adopted by families in the United States. Many children with HIV infection have joined their new families through adoption and they have generally been treated with antiretrovirals before arrival.

For internationally adopted children, HIV testing with an enzyme-linked immunosorbent assay (ELISA) is recommended shortly after their arrival. Positive or indeterminate results should be resolved with use of HIV DNA PCR to detect HIV-1 virus (see Chapter 191). If antibody testing by EIA is negative and clinical suspicion exists for HIV infection, HIV DNA PCR testing should be done. Retesting children older than 6 months of age after arrival to the United States should be considered.[4] Clinicians should be prepared to provide care and resources for children arriving to the United States with HIV infection.

Preadoptive testing for HIV-2 infection is not performed routinely. HIV-2 infection is prevalent in some African nations and is now recognized on several other continents; perinatal transmission appears to be limited. Symptoms suggestive of HIV infection with negative ELISA results for HIV-1 should prompt testing for HIV-2.[4]

Bacterial Infections

Syphilis

Refugees and internationally adopted children 15 years of age and older are routinely screened for syphilis before coming to the United States.[15] Immigrants with positive results are required to complete treatment before arriving to the United States. Screening of all refugees 15 years of age and older is recommended regardless of the overseas results.[14,15] Children who are younger than 15 years of age should be screened if there is a history of sexual activity or sexual assault or a history of maternal syphilis. In addition, all refugees immigrating from a country endemic for other treponemal infections such as yaws, bejel, or pinta should also be screened. Refugees of any age should be tested if there is clinical suspicion for syphilis.

Most internationally adopted children will have documentation of syphilis screening done in their birth country. For children with a history of syphilis or syphilis exposure, it is difficult to determine when the birth mother contracted syphilis and if she was treated and followed appropriately. In addition, in children with suspected syphilis exposure, details of the evaluation and prescribed treatment regimens are often incomplete or uninterpretable. Even though syphilis has rarely been reported in adoptees,* all internationally adopted children should be screened on arrival with both a nontreponemal test (rapid plasma reagin, Venereal Disease Research Laboratory, automated reagin test) and a treponemal test (microhemagglutination–*Treponema pallidum*, fluorescent treponemal antibody absorption, or *T. pallidum* particle agglutination) (see Chapter 143).[4] A positive treponemal test result warrants a complete and thorough evaluation to document the extent of the disease and to ensure adequate treatment regardless of a report of prior treatment. Because a positive treponemal test may be seen in pinta, bejel, yaws, and syphilis, it is important to consult with a specialist with expertise in these infections if the child is from a country where other treponemal infections are endemic.

Tuberculosis

TB is highly prevalent in the countries of origin for both refugees and internationally adopted children. Therefore, screening on arrival to the United States is recommended for both groups.[4,8,14,15] In 2010, 60.5 percent of all TB cases in the United States were among foreign-born people.[30] The rate of TB in foreign-born persons in the United States is 18.1/100,000; in U.S.-born persons, the rate is 1.6/100,000. In addition, in the United States in 2009, the majority (89.4%) of multidrug-resistant TB cases were in foreign-born persons. In children, 22 percent of TB cases were foreign-born. Case rates were significantly higher in foreign-born children (12.2/100,000) than in children born in the United States (1.1/100,000).[61]

Both refugees and internationally adopted children are at increased risk for TB infection. In immigrants and refugees, a high prevalence of latent tuberculosis infection (LTBI) was found in studies conducted in Minnesota (49%),[49] San Francisco (40%),[32] Buffalo (20%),[53] and Maine (35%).[37] A study in San Diego County found that 7 percent of immigrants screened had active pulmonary TB and 76 percent had LTBI.[50]

Overall, LTBI in internationally adopted children* appears to be lower than that seen in refugees.† This is likely due to the younger age and lower risk of living conditions of in this group of children. However, with the increased numbers of children coming from Ethiopia, a country with higher rates of tuberculosis, the rate of LTBI and tuberculosis in internationally adopted children is likely to increase. In two large studies in which all tuberculin skin tests (TSTs) were evaluated by a health care professional, 14 to 21 percent of international adoptees had LTBI.[52,78] The highest proportion with LTBI was seen in children from Russia and Eastern Europe (29%), and the lowest was in children adopted from China (10%). Of note, very few children from Africa were included in these studies. Children with initially negative TSTs had their TSTs repeated at least 3 months later, and 13 to 20 percent of the TSTs were positive, suggesting a high proportion of children initially had false positive TSTs. The authors postulate that this could be due to anergy, testing too soon after infection, or boosting from TB infection or BCG.

TB disease in international adoptees has rarely been reported.[31,46] In the first series of Korean adoptees, the overall prevalence was less than 1 percent, but two children had active TB and both had nonreactive TSTs.[46] One child had pneumonia caused by *Mycobacterium tuberculosis* and recovered. The other child had TB meningitis and died. These cases illustrate the importance of considering TB in the differential diagnosis even in children with negative TSTs. In the second case report,[31] a child with TB adopted from the Marshall Islands infected his female guardian and 20 percent of the contacts identified. On arrival in the United States, he had a TST done but the test was never read. He had cavitary TB but it was not diagnosed until his guardian developed TB.

In the past, refugees older than 15 years of age and children younger than 15 years with concern about TB exposure or disease were screened with a chest radiograph before arriving in the United States. If the chest radiograph was abnormal, microscopic evaluation of sputum or gastric aspirate for acid-fast bacilli is performed. Refugees who were sputum-positive are banned from entry until sputum smears are negative. In 2007, a major revision of the overseas TB screening requirements for immigrants and refugees bound for the United States was implemented that required TB screening for all people.[14] Information about the screening and implementation is available at the CDC's website (http://www.cdc.gov/ncidod/dq/panel_2007.htm). International adoptees from certain countries with high rates of TB (China and Ethiopia) now fall under the same

*References 2, 42, 54, 56, 58, 60, 65.

*References 2, 42, 43, 51, 52, 54, 56, 58, 60, 65, 78.
†References 8, 12, 32, 37, 49, 50, 53.

FIGURE 232-2 ■ Internationally adopted child from Guatemala having a tuberculin skin test placed. (See companion Expert Consult website for color version.)

requirements as other immigrants and refugees. For other adoptees, screening in the country of origin appears to be inconsistently done and generally is documented only in older children.

For both refugees and internationally adopted children, screening for TB should be done shortly after arrival to the United States.[4,8,14,15] This can be done at the initial assessment by placement of a TST (5 tuberculin units purified protein derivative) (Fig. 232-2). The test should be read 48 to 72 hours later by a health care professional. A reading of 10 mm or greater of induration is considered positive for both refugees and internationally adopted children arriving from a country endemic for TB. A reading of 5 mm or more is considered positive if there is a known contact with a person with active TB, an abnormal chest radiograph finding, signs or symptoms suggestive of TB, or evidence of immunosuppression. Children with a positive TST should have a thorough physical examination and chest radiograph performed to assess for *M. tuberculosis* disease. If the chest radiograph and physical examination are normal and the skin test is 10 mm or greater of induration, the diagnosis of LTBI is made and treatment with a 9-month course of isoniazid is begun. Similarly, a child with 5 to 9 mm of induration who has known exposure to someone with active TB or who is receiving immunosuppressive therapy or has an immunosuppressive condition, including HIV, with no evidence of disease, should receive isoniazid therapy as well. Retesting of TST-negative children 6 months or longer after arrival should be considered for all children given the high rate of false-negative TSTs shortly after arrival to the United States.[4,52,78]

Interferon-γ release assays (IGRAs), such as QuantiFERON Gold and T-SPOT.TB are available but are not currently recommended for children younger than 5 years of age, because of the lack of published data for younger children.[5] These assays use antigens that are secreted by *M. tuberculosis*–complex organisms but not by the Calmette-Guérin bacillus from the BCG vaccine. For TB disease, their sensitivity is comparable to that of the TST in children 5 years of age or older. For TB infection, the specificity of these tests is higher in contrast to that of TSTs but the sensitivity is not known. Logistical issues exist with the use of these tests because the test relies on stimulation of live blood mononuclear leukocytes with antigen, in vitro. In addition, these tests are more expensive than TSTs. For immunocompetent children 5 years of age and older, IGRAs can be used in place of a TST to confirm cases of TB or cases of LTBI. Similar to a positive TST, a child with a positive result from an IGRA should be considered infected with *M. tuberculosis* complex; however, a negative result from an IGRA cannot definitively rule out TB infection.

Although most refugees and internationally adopted children with *M. tuberculosis* infection have LTBI, disease should be considered in children with pneumonia or nonspecific symptoms such as fever, malaise, growth delay, weight loss, cough, night sweats, and chills. In children, pulmonary TB is the most common site of infection, accounting for 77 percent of cases, followed by lymphatic TB in 16 percent of children.[61] Chest radiographs may reveal adenopathy, segmental or lobar infiltrates, or pleural effusion. Cavitary lesions and miliary disease are less commonly seen. Extrapulmonary manifestations of the central nervous system, middle ear and mastoids, lymph nodes, bone, joints, and skin also can be seen. Every effort should be made to recover the organism in children with suspected disease to determine the susceptibilities of the organism. Many children arrive from countries in which drug-resistant *M. tuberculosis* is common. Gastric aspirates, bronchoscopy, or both, can be useful adjuncts in a child too young or too ill to expectorate sputum.

Initial management of active TB disease in refugees or internationally adopted children should include isoniazid, rifampin, pyrazinamide, and at least one other agent to ensure bactericidal coverage while culture results and susceptibility testing are pending (see Chapter 96).[5] The most recent resistant patterns from the child's country of origin should be considered along with any other epidemiologic information that can be obtained when making treatment decisions, especially if the cultures are negative.

Vaccination with BCG is common in most resource-poor countries. A history or evidence of BCG vaccine receipt is not a contraindication to the placement of a TST.[4,5] Studies have demonstrated that placement of a TST after BCG vaccination fails to elicit induration of 10 mm or greater.[48,67] Thus, the history of BCG vaccine should not affect the interpretation of purified protein derivative.

BCG vaccination can be recognized by a 2- to 4-mm scarification, often seen on the left deltoid. Documentation of vaccination should be made in the child's vaccine record, and the scar, if present, should be documented on the physical examination record. Occasionally, complications from BCG vaccination can occur. These may include enlargement of regional lymph nodes or a nodule or ulcer that may or may not be draining at the vaccination site (see Fig. 232-3). The lesion is granulomatous; culture of the lesion will yield *Mycobacterium bovis*. No consensus exists regarding the management of this condition. The outcome of these lesions varies. Some lesions resolve without treatment. Other lesions have been excised for diagnostic reasons, and others have been

BCG granuloma

FIGURE 232-3 ■ Internationally adopted child from Vietnam with a BCG granuloma on shoulder. (See companion Expert Consult website for color version.)

reported to require excision. Medical treatment with isoniazid, erythromycin, or clarithromycin has been used with success.[35,77]

Enteric Bacterial Infections

In contrast to intestinal parasites, bacterial enteric pathogens appear to be less common in immigrants and adoptees; however, most studies have not assessed systematically for bacterial causes. Children with bloody diarrhea or diarrhea associated with high fever should have stool examined for bacterial enteropathogens (e.g., *Salmonella*, *Shigella*, *Yersinia*, and *Campylobacter)* as part of the initial evaluation. Some experts also recommend obtaining stool cultures in newly arrived adoptees who have diarrhea.[4] However, if a bacterial pathogen is identified, treatment may not be indicated or needed if symptoms have resolved.

Intestinal Parasites

Intestinal parasites are common in both refugee* and internationally adopted† children (Table 232-2). Rates of infection vary by age and country of origin. Most children typically have not had testing before coming to the United States. However, presumptive treatment of refugees from certain regions before departure to the United States has been recommended since 1999, and screening strategies for these two groups are different.[16,75] Specifically, all refugees from the Middle East and South and Southeast Asia are recommended to receive a single dose of albendazole for round worms and ivermectin for *Strongyloides*. African refugees also should receive presumptive treatment for *Strongyloides*, and for those originating from areas endemic for schistosomiasis, presumptive treatment with praziquantel should be given. In African countries where Loa Loa is endemic, ivermectin should not be given for *Strongyloides* because treatment with this drug in the presence of Loa Loa has been associated with encephalopathy. Therefore in Loa Loa–endemic areas, 7 days of albendazole should be given. Additional details of the country-specific recommendations, timing, dosing, and duration of treatment can be found in the CDC's guidelines for refugee and immigrant care.[16]

In internationally adopted children, the reported prevalence of intestinal parasites has varied from 9 to 51 percent, depending on the age of the child and country of origin.* Older children and children originating from countries other than Korea have been found to be at

TABLE 232-2 **Pathogenic and Nonpathogenic Intestinal Parasites**

Parasite Type	Pathogens	Nonpathogens
Protozoa	*Giardia intestinalis*	*Endolimax nana*
	Entamoeba histolytica	*Entamoeba coli*
	*Dientamoeba fragilis**	*Entamoeba gingivalis*
	Balantidium coli	*Entamoeba hartmanni*
	*Blastocystis hominis**	*Entamoeba polecki*
	Isospora belli	*Iodamoeba butschlii*
	Cryptosporidium parvum	*Chilomastix mesnili*
	Cyclospora cayentensis	*Enteromonas hominis*
	Microsporidium spp.	*Retortamonas intestinalis*
		Trichomonas hominis
		Trichomonas tenax
Helminths		
Nematodes	*Ascaris lumbricoides* (roundworm)	
	Trichuris trichiura (whipworm)	
	Strongyloides stercoralis (threadworm)	
	Enterobius vermicularis (pinworm)	
	Necator americanus (hookworm)	
	Ancyclostoma duodenale (hookworm)	
Cestodes	*Hymenolepsis* spp.	
	Taenia saginata (beef tapeworm)	
	Taenia solium (pork tapeworm)	
	Schistosoma spp.	

*Controversy exists regarding the pathogenicity of this organism.

*References 9, 10, 12, 47, 49, 66, 75, 84.
†References 2, 43, 45, 54, 56, 58, 60, 65, 72.

*References 2, 43, 45, 54, 56, 58, 60, 65, 72.

highest risk for intestinal parasites. *Giardia intestinalis* has been the most commonly identified pathogen in all studies, with as many as 19 percent of children infected. Helminthic infections have been less frequently identified (<3%), with *Hymenolepsis* spp., *Ascaris lumbricoides*, and *Trichuris trichiura* most often reported. In the largest study, the overall prevalence of intestinal parasites in internationally adopted children was 29 percent.[72] In this study, the prevalence of intestinal parasites was similar in children with and without gastrointestinal symptoms and no significant difference was seen in malnourished children in contrast to children who were not malnourished.

Several studies report the prevalence of intestinal parasites in refugees, but few give pathogenic-specific data by age and country. In a large study of 26,956 refugees from Southeast Asia and Africa, the prevalence of intestinal parasites was examined.[75] The median age was 19 years, and 30 percent were children 2 to 14 years of age. For African refugees who were not pretreated, 7 percent had *Giardia* and 24 percent had at least one nematode, with *Trichuris* being most commonly identified. For Southeast Asian refugees, 12 percent had *Giardia* and 19 percent had at least one nematode, but hookworms were the most prevalent, at 11 percent. Children 2 to 14 years of age were significantly more likely than adults to have *Ascaris*, *Trichuris*, and *Giardia*, while hookworms were found more often in adults. In contrast, in another study, 17 percent of refugee children were found to have *Giardia* and 19 percent had helminthes, with hookworms most commonly seen.[9] This study found a high rate of hookworm in Bosnian refugees and found different rates of overall and pathogenic specific recovery by region. Interestingly, this study found that refugees who reported gastrointestinal symptoms were significantly less often infected in contrast to those without symptoms ($p < .001$).

Both refugees and adoptees should be screened for intestinal parasites on arrival to the United States.[4,8,14-16] The diagnosis for most intestinal parasites is done by examination of stool preserved in formalin and polyvinyl alcohol or sodium-acetate formalin for ova and parasite testing. Testing multiple stool specimens increases the sensitivity of microscopic examination for ova and parasites.[11,40,72] In one study in internationally adopted children, the sensitivity was 79 percent using one specimen and 92 percent for two specimens and a third specimen identified parasites in an additional 8 percent of children.[72] Examination by an experienced technologist is critical in identifying intestinal parasites. One stool specimen should also be evaluated for *G. intestinalis* and *Cryptosporidium parvum* using antigen testing.[4] Repeat stool samples are essential to assess the effectiveness of treatment for the identified parasite or determine the presence of new (or newly found) pathogens. If the child remains persistently symptomatic, additional stool sample examinations are warranted and testing for other parasites such as *Cyclospora* and *Strongyloides stercoralis* should be considered.

Tissue Parasites

In refugees or internationally adopted children who have negative stool ova and parasite examinations and in whom eosinophilia (absolute eosinophil count exceeding 450 cells/mm^3) is found on complete blood count, additional serologic testing should be done.[4,8,14-16,69] Serologic testing for *S. stercoralis* should be performed on all children with eosinophilia and no identified pathogen commonly associated with an increased eosinophil count, regardless of country of origin. Serologic testing for *Schistosoma* spp. should be performed in children with eosinophilia and no identified pathogen commonly associated with eosinophilia from Sub-Saharan Africa, Southeast Asia, or areas of Latin America where schistosomiasis is endemic.

Testing for filariasis should be done for children older than 2 years of age with eosinophilia who come from areas where filariasis is endemic.[4,14-16] Multiple parasites cause filariasis; therefore, if testing is positive, consultation with an infectious disease specialist with expertise in this disease should be done to ensure the correct treatment is given. Serologic testing for *Strongyloides*, *Schistosoma* spp., and filariasis is available through the CDC.

Chagas Disease (American Trypanosomiasis)

Chagas disease is endemic throughout much of Mexico, Central America, and South America (Chapter 221) and is caused by *Trypanosoma cruzi*. The risk for Chagas disease varies by region within countries with endemic infection. Although no studies have been done to assess the prevalence of Chagas disease in refugees or internationally adopted children, screening is warranted because treatment of infected children is highly effective in contrast to treatment in adults.[4,14-16] Children may become infected by vertical exposure or from vectors. Countries with endemic Chagas disease include Argentina, Belize, Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, Suriname, Uruguay, and Venezuela. Serologic testing should only be performed in children 12 months of age or older because of the potential presence of maternal antibody. Testing can be done through the CDC.

Malaria

For internationally adopted children, routine screening for malaria is not recommended.[4] In refugees who do not have a contraindication, predeparture presumptive antimalarial therapy with artesunate-combination therapy should be given to all sub-Saharan African refugees arriving from countries endemic for *Plasmodium falciparum*.[14,15] Refugees from these countries without presumptive treatment before departure, should have postarrival testing done using malaria PCR. If PCR is not available, traditional blood films or a rapid antigen test may be used, recognizing that these tests are much less sensitive, especially in persons who are asymptomatic. Alternatively, presumptive treatment can be provided instead of testing. Refugees from malaria-endemic regions outside sub-Saharan Africa do not require postarrival testing or treatment. However, any refugee or internationally adopted child from a malaria-endemic region with signs or symptoms of malaria should be thoroughly evaluated.

Other Testing

A complete blood count is recommended for refugees and internationally adopted children.[4,8,14,15] The hemoglobin and red blood cell indices may identify children with anemia, iron deficiency, hemoglobinopathies, or malaria. Low white blood cell counts and lymphopenia may indicate HIV infection or malnutrition. Eosinophilia is common among refugees and suggests parasitic infection.[8,14,69] The prevalence of eosinophilia has not been reported in internationally adopted children. For refugees, a urinalysis is recommended to assess for hematuria and pyuria.[8,14,15] Hematuria may indicate schistosomiasis, and pyuria may reflect a urinary tract infection or renal TB.

Elevated blood lead levels have been reported in internationally adopted children[18] and refugees,[22] and both groups should have a blood lead concentration as part of their initial evaluation.[4,14,15]

OTHER VACCINE PREVENTABLE DISEASES

In addition to hepatitis A and hepatitis B, other vaccine-preventable diseases have been transmitted or brought into the United States by immigrants. One case of pertussis in an internationally adopted child from Russia has been reported,[20] along with several cases of measles imported by refugees[29] and internationally adopted children.[19,21,24] Clinicians should be aware of the signs and symptoms of measles and do proper testing if measles is suspected.

Dermatologic Infections and Infestations

Lice, scabies, fungal infections, and molluscum contagiosum are common in both refugees and internationally adopted children. The prevalence of these conditions has not been well documented. The physical examination should include careful evaluation for these entities so that appropriate treatment is given.

IMMUNIZATION GUIDELINES

Making immunization recommendations can be challenging for clinicians providing care for immigrant children. Most refugees arrive in the United States without immunization records, and most international adoptees have some documentation of receipt of immunizations. Refugee and internationally adopted children have typically had the opportunity to have received BCG, diphtheria-tetanus-pertussis–containing vaccines, poliovirus vaccine, hepatitis B, and measles vaccines in their countries of origin. In internationally adopted children, there has been an increase in documentation of *Haemophilus influenzae* type b vaccine, especially for children from South Korea, Guatemala, India, and China. For measles vaccine, most children will have received and have documentation of a monovalent measles vaccine. Mumps and rubella vaccines in combination with measles vaccine as MMR have been documented for adoptees from South Korea and Guatemala or as monovalent vaccines in older children from Eastern Europe and Russia. Pneumococcal conjugate and varicella vaccines are not routinely given but have been documented in records for children from South Korea and Guatemala. Although children may have documentation of some vaccine-preventable diseases, a clinical diagnosis of measles, mumps, rubella, varicella, or hepatitis A from the child's country of origin should not be accepted as evidence of immunity.

The *Immigration and Nationality Act* (INA) of 1996 requires immigrant visa applicants to provide proof of vaccination with at least the first dose of vaccines recommended by the Advisory Committee on Immunization Practice (ACIP) before entry into the United States.[3,14,15] These regulations apply to most immigrant children entering the United States; however, internationally adopted children who are 10 years of age or younger may obtain an exemption from these requirements. Adoptive parents are required to sign a waiver indicating their intention to comply with the ACIP immunization requirements within 30 days after the child's arrival in the United States. Refugees are not required to meet immunization requirements of the INA at the time of initial entry into the United States, but they must show proof of immunization when they apply for permanent residency—typically 1 year after arrival. Information about immunization requirements for immigrants is available on the CDC's website (http://www.cdc.gov/ncezid/dgmq/).

People immunized in other countries, including refugees and internationally adopted children, should be immunized according to the United States' recommended schedule for healthy infants, children, and adolescents. Only written documentation should be accepted as evidence of previous immunization.* The written records may be considered valid if the vaccines, dates of administration, numbers of doses, intervals between doses, and age of the patient at the time of immunization are comparable to current U.S. or World Health Organization (WHO) schedules (http://apps.who.int/immunization_monitoring/en/globalsummary/countryprofileselect.cfm). Overall, most vaccines used around the world are produced with adequate quality-control standards and are generally considered reliable. Immunization is one of the most important measures used to prevent diseases in resource-poor countries, and immunization of children is a priority worldwide. However, some vaccines with inadequate potency have been produced in other countries. In addition, immunization records may be difficult to interpret or have dates that appear inaccurate, causing concern that the records may not be valid and accurately reflect protection. Therefore, for children who do not have a record or who have an unknown or uncertain immunization status, serologic testing and reimmunization are both reasonable approaches. If serologic testing is not or cannot be done, any immunizations that are not documented or do not adhere to the U.S. or WHO standards should be repeated.

*References 3, 4, 14, 16, 28, 63.

TABLE 232-3 **Serologic Testing for Verifying Immunization or Past Infection**

Age	Test	Protective Level
Children ≥5 Months of Age		
Diphtheria antibody (IgG)	ELISA	>0.10 IU/mL
Tetanus antibody (IgG)	ELISA	>0.10 IU/mL
Poliovirus antibody (serotypes 1–3)	Neutralizing Antibody	Detectable antibody
Hepatitis B surface antibody (IgG)	ELISA	≥10 mIU/mL
Haemophilus influenza type b, (IgG) polyribosylribitol phosphate antibody	ELISA	≥1.0 IU/mL
Children ≥12 Months of Age		
Rubeola (measles) antibody (IgG)	IFA or ELISA	*
Mumps antibody (IgG)	IFA or ELISA	*
Rubella antibody (IgG)	ELISA	*
Varicella antibody (IgG)	ELISA	*
Hepatitis A antibody (Total or IgG)	EIA	*

*Value for positive result varies by test and laboratory.

For children whose immunizations are not up-to-date, but who have a valid, written immunization record for the vaccines documented, vaccines as recommended for their age should be administered. For children without documentation of immunizations or who have a record that does not adhere to U.S. or WHO standards, a new vaccine schedule may be initiated or measurement of antibody concentrations to any or all of the following vaccine antigens can be done: diphtheria, tetanus, poliovirus (each serotype), measles, mumps, rubella, varicella, and hepatitis A (Table 232-3). Hepatitis B serology will be done to assess the child's hepatitis B status, which will also allow for determination of immunity. *Haemophilus influenzae* type b polyribosylribitol phosphate antibody also can be done; however, fewer data exist to support this testing to determine protection. Testing for measles, mumps, rubella, varicella, and hepatitis A should only be done in children older than 12 months of age because of the possible presence of maternal antibody. Because antibody testing for one vaccine may not be predictive for others, a combination of reimmunization and antibody testing also can be used. Serologic testing is not recommended for making vaccine decisions for pertussis, pneumococcus, or influenza. Because there are not reliable correlates of protection for pertussis, diphtheria and tetanus levels may be used to guide decision making. Age-appropriate immunization for conjugate pneumococcal and influenza vaccines is recommended.

Some caveats for interpreting antibody results should be reviewed. First, in general, antibody levels do not differentiate past disease from immunization. Measles, mumps, rubella, hepatitis A, varicella, and *H. influenzae* type b are prevalent in many of the countries of origin for refugees and internationally adopted children, so protective levels of antibody may be due to past immunization of infection. Poliovirus from oral poliovirus vaccine could be transmitted from another child, providing protection to children without documentation of poliovirus vaccine. For hepatitis B virus, however, serology will distinguish between past disease and immunization. The presence of anti-HBc with anti-HBs reflects past, recovered disease, whereas anti-HBs alone is due to immunization. Second, if protective antibody is found, additional age-appropriate immunizations should still be given for some vaccines. For example, if a 9-month-old infant has protective antibody for diphtheria and tetanus, the 15- and 18-month and 4- to 6-year DTaP doses should still be given. In contrast, if a 3-year-old child has protective antibody to measles, mumps and rubella, an additional MMR is not needed when the child is 4- to 6-years of age. Third, it is important to consider age, along with the antibody levels for diphtheria and tetanus. In a 3-year-old child with a protective level of 0.20 IU/mL for tetanus, a dose of DTaP should be given to boost the tetanus level and hopefully provide higher levels of protection for pertussis. Finally, if a child does not have protective antibody for measles, mumps, or varicella, two doses are needed. However, if measles and mumps antibody levels are protective and the rubella antibody is nonprotective, only one MMR is needed for rubella.

Several studies have been published examining antibody testing to verify the immunization status or immunity to vaccine-preventable diseases in internationally adopted children.* The results of these studies differ; some studies show inadequate protection, whereas others show good levels of protection. Differences in study design and laboratory methods likely account for the different results from the various studies. In the largest study to date, in internationally adopted children with three or more vaccine doses, overall protection was found to be high for diphtheria (85%), tetanus (95%), polio (93%), hepatitis B (77%), and *H. influenzae* type b (67%).[71] For children 12 months of age and older with more than one dose of measles, mumps, or rubella vaccines, 95, 72, and 94 percent, respectively, were immune. In contrast to children without documentation for a given vaccine antigen (i.e., no doses), a lower proportion of children had protective levels of antibody: diphtheria

*References 1, 13, 41, 55, 57-59, 65, 68, 71, 74, 81-83.

(69%), tetanus (76%), polio (83%), hepatitis B (42%), *H. influenzae* type b (67%), measles (58%), mumps (31%), or rubella (30%). However, many of these children were still protected, suggesting children either had a history of disease or had received vaccines and did not have documentation. In that same study, 33 percent of adoptees had protective levels of varicella antibody (with or without vaccine documentation); 83 percent of children with a history of varicella had protective antibody. Few data exist on verifying immunization status by antibody testing in refugees. In one study, screening for varicella antibody in individuals older than 5 years of age was found to be more cost-effective than providing immediate vaccine.[33]

CONCLUSION

Providing care for refugees and internationally adopted children can be rewarding. Screening for infectious diseases and determining immunization needs is critical not only for the health and prevention of disease in these immigrants but also for the prevention of disease in their families and communities.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdulla RY, Rice MA, Donauer S, et al. Hepatitis A in internationally adopted children: screening for acute and previous infection. *Pediatrics* 2010;**126**:e1039–44.
2. Albers LH, Johnson DE, Hostetter MK, et al. Health of children adopted from the former Soviet Union and Eastern Europe: comparison with preadoptive medical records. *JAMA* 1997;**278**: 922–4.
3. American Academy of Pediatrics. Immunization in special clinical circumstances: refugees and immigrants. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
4. American Academy of Pediatrics. Medical evaluation of internationally adopted children for infectious diseases. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
5. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
6. American Academy of Pediatrics, Committee on Community Health Services. Providing care for immigrant, homeless and migrant children. *Pediatrics* 2005;**115**;1095–100.
7. American Academy of Pediatrics, Committee on Infectious Diseases. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803–4.
8. Barnett ED. Infectious disease screening for refugees resettled in the United States. *Clin Infect Dis* 2004;**39**:833–41.
9. Benzeguir AK, Capraru T, Aust-Kettis A, et al. High frequency of gastrointestinal parasites in refugees and asylum seekers upon arrival in Sweden. *Scand J Infect Dis* 1999;**31**:79–82.
10. Buchwald D, Lam M, Hooton TM. Prevalence of intestinal parasites and association with symptoms in Southeast Asian refugees. *J Clin Pharm Ther* 1995;**20**:271–5.
11. Cartwright CP. Utility of multiple-stool-specimen ova and parasite examinations in a high-prevalence setting. *J Clin Microbiol* 1999; **37**:2408–11.
12. Catanzaro A, Moser RJ. Health status of refugees from Vietnam, Laos, and Cambodia. *JAMA* 1982;**247**:1303–8.
13. Cilleruelo MJ, de Ory F, Ruiz-Contreras J, et al. Internationally adopted children: what vaccines should they receive? *Vaccine* 2008; **26**:5784–90.
14. Centers for Disease Control and Prevention. *Guidelines for the US domestic medical examination for newly arriving refugees*. n.d. Available at: http://www.cdc.gov/immigrantrefugeehealth/guidelines/general-guidelines.html.
15. Centers for Disease Control and Prevention. *Medical examination of immigrants and refugees*. n.d. Available at: http://www.cdc.gov/immigrantrefugeehealth/exams/medical-examination.html.
16. Centers for Disease Control and Prevention. *Refugee health guidelines: intestinal parasites overseas recommendations*. n.d. Available at: http://www.cdc.gov/immigrantrefugeehealth/guidelines/domestic/intestinal-parasites-domestic.html.
17. Centers for Disease Control and Prevention. Screening for hepatitis B virus infection among refugees arriving to the United States, 1974-1991. *MMWR Morb Mortal Wkly Rep* 1991;**40**:784–6.
18. Centers for Disease Control and Prevention. Elevated blood lead levels among internationally adopted children: United States. *MMWR Morb Mortal Wkly Rep* 1998;**49**:97–100.
19. Centers for Disease Control and Prevention. Measles outbreak among internationally adopted children arriving in the United States, February-March 2001. *MMWR Morb Mortal Wkly Rep* 2001;**51**:1115–16.
20. Centers for Disease Control and Prevention. Pertussis in an infant adopted from Russia: May 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:394–5.
21. Centers for Disease Control and Prevention. Update-multistate investigation of measles among adoptees from China-April 16, 2004.California. *MMWR Morb Mortal Wkly Rep* 2004;**53**:323–4.
22. Centers for Disease Control and Prevention. Elevated blood lead levels in refugee children: New Hampshire, 2003-2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:42–6.
23. Centers for Disease Control and Prevention. Preventive of hepatitis A through active or passive immunization: recommendations of Advisory Committee of Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2006;**55**:1–23.
24. Centers for Disease Control and Prevention. Measles among adults associated with adoption in China-California, Wisconsin and Washington, July-August 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:144–6.
25. Centers for Disease Control and Prevention, Advisory Committee on Immunization Practice. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoption. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1006–7.
26. Centers for Disease Control and Prevention. *Traveler's health: yellow book. health information for international travel, 2010: hepatitis A*. 2010. Available at: http://www.cdc.gov/travel/content/yellowbook/home-2010.aspx.
27. Centers for Disease Control and Prevention. *Traveler's health: yellow book. health information for international travel, 2010; hepatitis B*. Available at: http://www.cdc.gov/travel/content/yellowbook/home-2010.aspx.
28. Centers for Disease Control and Prevention. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2011;**60**:1–61.
29. Centers for Disease Control and Prevention. Measles among U.S.-bound refugees from Malaysia-California, Maryland, North Carolina and Wisconsin, August–September 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1281–2.
30. Centers for Disease Control and Prevention. Trends in tuberculosis-United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011; **60**:333–7.
31. Curtis AB, Ridzon R, Vogel R, et al. Extensive transmission of *Mycobacterium tuberculosis* from a child. *N Engl J Med* 1999; **341**:1491–5.
32. DeRiemer K, Chin DP, Schecter GF, et al. Tuberculosis among immigrants and refugees. *Arch Intern Med* 1998;**158**:753–60.
33. Figueira M, Christiansen D, Barnett ED. Cost-effectiveness of sero-testing compared with universal immunization for varicella in refugee children from 6 geographic regions. *J Travel Med* 2003; **10**:203–13.
34. Fischer GE, Teshale EH, Miller C, et al. Hepatitis A among international adoptees and their contacts. *Clin Infect Dis* 2008;**47**: 812–14.
35. FitzGerald JM. Management of adverse reactions to bacille Calmette-Guérin vaccine. *Clin Infect Dis* 2000;**31**(Suppl. 3):S75–6.

36. Friede A, Harris JR, Kobayashi JM, et al. Transmission of hepatitis B virus from adopted Asian children to their American families. *Am J Public Health* 1988;**78**:26–9.
37. Hayes EB, Talbot SB, Matheson ES, et al. Health status of pediatric refugees in Portland, ME. *Arch Pediatr Adolesc Med* 1998;**152**: 564–8.
38. Hersh BS, Popovici F, Apertrei RC, et al. Acquired immunodeficiency syndrome in Romania. *Lancet* 1991;**338**:645–49
39. Hershow RC, Hadler SC, Kane MA. Adoption of children from countries with endemic hepatitis B: transmission risks and medical issues. *Pediatr Infect Dis J* 1987;**6**:431–7.
40. Hiatt RA, Markell EK, Ng E. How many stool examinations are necessary to detect pathogenic intestinal protozoa? *Am J Trop Med Hyg* 1995;**53**:36–9.
41. Hostetter MK, Johnson DE. Immunization status of adoptees from China, Russia, and Eastern Europe [abstract]. *Pediatr Res* 1998;**43**: 147A.
42. Hostetter MK, Iverson S, Thomas WE, et al. Prospective medical evaluation of internationally adopted children. *N Engl J Med* 1991;**325**:479–85.
43. Johnson DE, Miller LC, Iverson S, et al. The health of children adopted from Romania. *JAMA* 1992;**268**:3446–51.
44. Kurtz J. HIV infection and hepatitis B in adopted Romanian children [letter]. *Br Med J* 1991;**13**:741–9.
45. Lange WR, Warnock-Eckhart E. Selected infectious disease risks in international adoptees. *Pediatr Infect Dis J* 1987;**6**:447–50.
46. Lange WR, Warnock-Eckhart E, Bean ME. *Mycobacterium tuberculosis* infection in foreign born adoptees. *Pediatr Infect Dis J* 1989;**8**;625–9.
47. Lerman D, Barrett-Connor E, Norcross W. Intestinal parasites in asymptomatic adult Southeast Asian immigrants. *J Fam Pract* 1982;**15**:443–6.
48. Lifschitz M. The value of the tuberculin skin test as a screening test for tuberculosis among BCG-vaccinated children. *Pediatrics* 1965;**36**:624–7.
49. Lifson AR, Thai D, O'Fallon A, et al. Prevalence of tuberculosis, hepatitis B virus, and intestinal parasitic infections among refugees to Minnesota. *Public Health Rep* 2002;**117**:69–77.
50. LoBue PH, Moser KS. Screening of immigrants and refugees for pulmonary tuberculosis in San Diego County, California. *Chest* 2004;**126**;1777–82.
51. Mandalakas AM, Kirchner HL, Iverson S, et al. Predictors of *Mycobacterium tuberculosis* infection in international adoptees. *Pediatrics* 2007;**120**:e610–16.
52. Mandalakas AM, Kirchner HL, Zhu X, et al. Interpretation of repeat tuberculin skin testing in international adoptees. *Pediatr infect Dis J* 2008;**27**:913–19.
53. Meropol SB. Health status of pediatric refugees in Buffalo, NY. *Arch Pediatr Adolesc Med* 1995;**149**:887–92.
54. Miller L, Chan W, Comfort K, et al. Health of children adopted from Guatemala: comparison of orphanage and foster care. *Pediatrics* 2005;**115**:e710–17.
55. Miller LC. Internationally adopted children-immunization status [letter]. *Pediatrics* 1999;**103**:1078.
56. Miller LC, Hendrie NW. Health of children adopted from China. *Pediatrics* 2000;**105**:e76.
57. Miller LC, Comfort K, Kely N. Immunization status of internationally adopted children [Letter]. *Pediatrics* 2001;**108**:1050–1.
58. Miller LC, Tseng B, Tirella LG, et al. Health of children adopted from Ethiopia. *Matern Child Health J* 2008;**12**:599–605.
59. Murray DL. International adoptees and hepatitis B virus infection. *Am J Dis Child* 1990;**144**:523–4.
60. Murray TS, Groth ME, Weitzman C, et al. Epidemiology and management of infectious diseases in international adoptees. *Clin Microbiol Rev* 2005;**18**:510–20.
61. Nelson LJ, Schneider E, Wells CD, et al. Epidemiology of childhood tuberculosis in the United States, 1993-2001; the need for continued vigilance. *Pediatrics* 2004;**114**:333–41.
62. Patrascu IV, Constantinescu SN, Dublanchet A. HIV-1 infection in Romanian children. *Lancet* 1990;**335**:672.
63. Pickering LK, Baker CJ, Freed GL, et al. Immunization programs for infants, children, adolescents, and adults: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infec Dis* 2009;**49**:817–40.
64. Rudin CH, Berger R, Tobler R, et al. HIV-1, hepatitis (A, B, and C) and measles in Romanian children. *Lancet* 1990;**336**:1592.
65. Saiman L, Aronson J, Zhou J, et al. Prevalence of infectious diseases among internationally adopted children. *Pediatrics* 2001;**108**: 608–12.
66. Salas SD, Heifetz R, Barrett-Connor E. Intestinal parasites in Central American immigrants in the United States. *Arch Intern Med* 1990;**150**:1514–16.
67. Santiago EM, Lawson E, Gillenwater K, et al. A prospective study of Bacillus Calmette-Guérin scar formation and tuberculin skin test reactivity in infants in Lima, Peru. *Pediatrics* 2003;**112**:298–302.
68. Schulpen TW, van Seventer AH, Rumke HC, et al. Immunization status of children adopted from China [Letter]. *Lancet* 2001; **358**:2131–2.
69. Seybolt LM, Christiansen D, Barnett ED. Diagnostic evaluation of newly arrived asymptomatic refugees with eosinophilia. *Clin Infect Dis* 2006;**42**:36–9.
70. Sokal EM, Van Cullie O, Buts JP. Horizontal transmission of hepatitis B from children to adoptive parents [letter]. *Arch Dis Child* 1995;**72**:191.
71. Staat MA, Stadler LP, Donauer S, et al. Serologic testing to verify the immune status of internationally adopted children against vaccine preventable diseases. *Vaccine* 2010;**28**:7947–55.
72. Staat MA, Rice M, Donauer S, et al. Intestinal parasite screening in internationally adopted children: importance of testing multiple stool specimens. *Pediatrics* 2011;**128**:e613–22.
73. Stadler LP, Mezoff AG, Staat MA. Hepatitis B virus screening for internationally adopted children. *Pediatrics* 2008;**122**:223–8.
74. Stadler LP, Donauer S, Rice M, et al. Factors associated with protective antibody levels to vaccine preventable diseases in internationally adopted children. *Vaccine* 2010;**29**:95–103.
75. Swanson SJ, Phares CR, Mamo B, et al. Albendazole therapy and enteric parasites in United States-bound refugees. *N Engl J Med* 2012;**366**:1498–507.
76. Sweet K, Sutherland W, Ehresmann K, et al. Hepatitis A infections in recent international adoptees and their contacts in Minnesota, 2007-2009. *Pediatrics* 2011;**128**:e333–8.
77. Torres-Rojas JR, Rondon-Lugo A, Vidal R. Short course of clarithromycin in an immunocompetent patient with BCG-induced regional complications. *Dermatol Online J* 2002;**8**:6.
78. Trehan I, Meinzen-Derr JK, Jamison L, Staat MA. Tuberculosis screening in internationally adopted children: the need for initial and repeat testing. *Pediatrics* 2008;**122**:e7–14.
79. U.S. Department of Homeland. *Security yearbook of immigration statistics: 2010*. Washington, DC: U.S. Department of Homeland Security Office of Immigration Statistics; 2011. Available at: http://www.dhs.gov/xlibrary/assets/statistics/yearbook/2010/ois_yb_2010.pdf.
80. U.S. Department of State. *Immigrant visas issued to orphans coming into the US*. Available at: http://adoption.state.gov/about_us/statistics.php Accessed December 9, 2012.
81. van Schaik R, Wolfs TF, Geelen SP. Improved general health of international adoptees, but immunization status still insufficient. *Eur J Pediatr* 2009;**168**:1101–6.
82. Verla-Tebit E, Zhu X, Holsinger E, et al. Predictive value of immunization records and risk factors for immunization failure in internationally adopted children. *Arch Pediatr Adolesc Med* 2009;**163**: 473–9.
83. Viviano E, Cataldo F, Accomando S, et al. Immunization status of internationally adopted children in Italy. *Vaccine* 2006;**24**: 4138–43.
84. Walker PF, Jaranson J. Refugee and immigrant health care. *Med Clin North Am* 1999;**83**:1103–20.
85. Wilson ME, Kimble J. Post-travel hepatitis A: probable acquisition from an asymptomatic adopted child. *Clin Infec Dis* 2001;**33**: 1083–5.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdulla RY, Rice MA, Donauer S, et al. Hepatitis A in internationally adopted children: screening for acute and previous infection. *Pediatrics* 2010;**126**:e1039–44.
2. Albers LH, Johnson DE, Hostetter MK, et al. Health of children adopted from the former Soviet Union and Eastern Europe: comparison with preadoptive medical records. *JAMA* 1997;**278**: 922–4.
3. American Academy of Pediatrics. Immunization in special clinical circumstances: refugees and immigrants. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
4. American Academy of Pediatrics. Medical evaluation of internationally adopted children for infectious diseases. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
5. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book 2012: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
6. American Academy of Pediatrics, Committee on Community Health Services. Providing care for immigrant, homeless and migrant children. *Pediatrics* 2005;**115**;1095–100.
7. American Academy of Pediatrics, Committee on Infectious Diseases. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803–4.
8. Barnett ED. Infectious disease screening for refugees resettled in the United States. *Clin Infect Dis* 2004;**39**:833–41.
9. Benzeguir AK, Capraru T, Aust-Kettis A, et al. High frequency of gastrointestinal parasites in refugees and asylum seekers upon arrival in Sweden. *Scand J Infect Dis* 1999;**31**:79–82).
10. Buchwald D, Lam M, Hooton TM. Prevalence of intestinal parasites and association with symptoms in Southeast Asian refugees. *J Clin Pharm Ther* 1995;**20**:271–5.
11. Cartwright CP. Utility of multiple-stool-specimen ova and parasite examinations in a high-prevalence setting. *J Clin Microbiol* 1999;**37**: 2408–11.
12. Catanzaro A, Moser RJ. Health status of refugees from Vietnam, Laos, and Cambodia. *JAMA* 1982;**247**:1303–8.
13. Cilleruelo MJ, de Ory F, Ruiz-Contreras J, et al. Internationally adopted children: what vaccines should they receive? *Vaccine* 2008;**26**:5784–90.
14. Centers for Disease Control and Prevention. *Guidelines for the US domestic medical examination for newly arriving refugees*. n.d. Available at: http://www.cdc.gov/immigrantrefugeehealth/guidelines/general-guidelines.html.
15. Centers for Disease Control and Prevention. *Medical examination of immigrants and refugees*. n.d. Available at: http://www.cdc.gov/immigrantrefugeehealth/exams/medical-examination.html.
16. Centers for Disease Control and Prevention. *Refugee health guidelines: intestinal parasites overseas recommendations*. n.d. Available at: http://www.cdc.gov/immigrantrefugeehealth/guidelines/domestic/intestinal-parasites-domestic.html.
17. Centers for Disease Control and Prevention. Screening for hepatitis B virus infection among refugees arriving to the United States, 1974-1991. *MMWR Morb Mortal Wkly Rep* 1991;**40**: 784–6.
18. Centers for Disease Control and Prevention. Elevated blood lead levels among internationally adopted children: United States. *MMWR Morb Mortal Wkly Rep* 1998;**49**:97–100.
19. Centers for Disease Control and Prevention. Measles outbreak among internationally adopted children arriving in the United States, February-March 2001. *MMWR Morb Mortal Wkly Rep* 2001;**51**:1115–16.
20. Centers for Disease Control and Prevention. Pertussis in an infant adopted from Russia: May 2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:394–5.
21. Centers for Disease Control and Prevention. Update-multistate investigation of measles among adoptees from China-April 16, 2004. California. *MMWR Morb Mortal Wkly Rep* 2004;**53**:323–4.
22. Centers for Disease Control and Prevention. Elevated blood lead levels in refugee children: New Hampshire, 2003-2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:42–6.
23. Centers for Disease Control and Prevention. Preventive of hepatitis A through active or passive immunization: recommendations of Advisory Committee of Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2006;**55**:1–23.
24. Centers for Disease Control and Prevention. Measles among adults associated with adoption in China-California, Wisconsin and Washington, July-August 2006. *MMWR Morb Mortal Wkly Rep* 2007;**56**:144–6.
25. Centers for Disease Control and Prevention, Advisory Committee on Immunization Practice. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoption. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1006–7.
26. Centers for Disease Control and Prevention. *Traveler's health: yellow book. health information for international travel, 2010: hepatitis A*. 2010. Available at: http://www.cdc.gov/travel/content/yellowbook/home-2010.aspx.
27. Centers for Disease Control and Prevention. *Traveler's health: yellow book. health information for international travel, 2010; hepatitis B*. Available at: http://www.cdc.gov/travel/content/yellowbook/home-2010.aspx.
28. Centers for Disease Control and Prevention. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2011;**60**:1–61.
29. Centers for Disease Control and Prevention. Measles among U.S.-bound refugees from Malaysia-California, Maryland, North Carolina and Wisconsin, August–September 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1281–2.
30. Centers for Disease Control and Prevention. Trends in tuberculosis-United States, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 333–7.
31. Curtis AB, Ridzon R, Vogel R, et al. Extensive transmission of *Mycobacterium tuberculosis* from a child. *N Engl J Med* 1999;**341**: 1491–5.
32. DeRiemer K, Chin DP, Schecter GF, et al. Tuberculosis among immigrants and refugees. *Arch Intern Med* 1998;**158**:753–60.
33. Figueira M, Christiansen D, Barnett ED. Cost-effectiveness of sero-testing compared with universal immunization for varicella in refugee children from 6 geographic regions. *J Travel Med* 2003; **10**:203–13.
34. Fischer GE, Teshale EH, Miller C, et al. Hepatitis A among international adoptees and their contacts. *Clin Infect Dis* 2008;**47**: 812–14.
35. FitzGerald JM. Management of adverse reactions to bacille Calmette-Guérin vaccine. *Clin Infect Dis* 2000;**31**(Suppl. 3): S75–6.
36. Friede A, Harris JR, Kobayashi JM, et al. Transmission of hepatitis B virus from adopted Asian children to their American families. *Am J Public Health* 1988;**78**:26–9.
37. Hayes EB, Talbot SB, Matheson ES, et al. Health status of pediatric refugees in Portland, ME. *Arch Pediatr Adolesc Med* 1998;**152**: 564–8.
38. Hersh BS, Popovici F, Apertrei RC, et al. Acquired immunodeficiency syndrome in Romania. *Lancet* 1991;**338**:645–49
39. Hershow RC, Hadler SC, Kane MA. Adoption of children from countries with endemic hepatitis B: transmission risks and medical issues. *Pediatr Infect Dis J* 1987;**6**:431–7.
40. Hiatt RA, Markell EK, Ng E. How many stool examinations are necessary to detect pathogenic intestinal protozoa? *Am J Trop Med Hyg* 1995;**53**:36–9.
41. Hostetter MK, Johnson DE. Immunization status of adoptees from China, Russia, and Eastern Europe [abstract]. *Pediatr Res* 1998;**43**: 147A.
42. Hostetter MK, Iverson S, Thomas WE, et al. Prospective medical evaluation of internationally adopted children. *N Engl J Med* 1991;**325**:479–85.
43. Johnson DE, Miller LC, Iverson S, et al. The health of children adopted from Romania. *JAMA* 1992;**268**:3446–51.
44. Kurtz J. HIV infection and hepatitis B in adopted Romanian children [letter]. *Br Med J* 1991;**13**:741–9.
45. Lange WR, Warnock-Eckhart E. Selected infectious disease risks in international adoptees. *Pediatr Infect Dis J* 1987;**6**:447–50.
46. Lange WR, Warnock-Eckhart E, Bean ME. *Mycobacterium tuberculosis* infection in foreign born adoptees. *Pediatr Infect Dis J* 1989;**8**:625–9.

47. Lerman D, Barrett-Connor E, Norcross W. Intestinal parasites in asymptomatic adult Southeast Asian immigrants. *J Fam Pract* 1982;**15**:443–6.
48. Lifschitz M. The value of the tuberculin skin test as a screening test for tuberculosis among BCG-vaccinated children. *Pediatrics* 1965;**36**:624–7.
49. Lifson AR, Thai D, O'Fallon A, et al. Prevalence of tuberculosis, hepatitis B virus, and intestinal parasitic infections among refugees to Minnesota. *Public Health Rep* 2002;**117**:69–77.
50. LoBue PH, Moser KS. Screening of immigrants and refugees for pulmonary tuberculosis in San Diego County, California. *Chest* 2004;**126**;1777–82.
51. Mandalakas AM, Kirchner HL, Iverson S, et al. Predictors of *Mycobacterium tuberculosis* infection in international adoptees. *Pediatrics* 2007;**120**:e610–16.
52. Mandalakas AM, Kirchner HL, Zhu X, et al. Interpretation of repeat tuberculin skin testing in international adoptees. *Pediatr infect Dis J* 2008;**27**:913–19.
53. Meropol SB. Health status of pediatric refugees in Buffalo, NY. *Arch Pediatr Adolesc Med* 1995;**149**:887–92.
54. Miller L, Chan W, Comfort K, et al. Health of children adopted from Guatemala: comparison of orphanage and foster care. *Pediatrics* 2005;**115**:e710–17.
55. Miller LC. Internationally adopted children-immunization status [letter]. *Pediatrics* 1999;**103**:1078.
56. Miller LC, Hendrie NW. Health of children adopted from China. *Pediatrics* 2000;**105**:e76.
57. Miller LC, Comfort K, Kely N. Immunization status of internationally adopted children [Letter]. *Pediatrics* 2001;**108**:1050–1.
58. Miller LC, Tseng B, Tirella LG, et al. Health of children adopted from Ethiopia. *Matern Child Health J* 2008;**12**:599–605.
59. Murray DL. International adoptees and hepatitis B virus infection. *Am J Dis Child* 1990;**144**:523–4.
60. Murray TS, Groth ME, Weitzman C, et al. Epidemiology and management of infectious diseases in international adoptees. *Clin Microbiol Rev* 2005;**18**:510–20.
61. Nelson LJ, Schneider E, Wells CD, et al. Epidemiology of childhood tuberculosis in the United States, 1993-2001; the need for continued vigilance. *Pediatrics* 2004;**114**:333–41.
62. Patrascu IV, Constantinescu SN, Dublanchet A. HIV-1 infection in Romanian children. *Lancet* 1990;**335**:672.
63. Pickering LK, Baker CJ, Freed GL, et al. Immunization programs for infants, children, adolescents, and adults: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infec Dis* 2009;**49**:817–40.
64. Rudin CH, Berger R, Tobler R, et al. HIV-1, hepatitis (A, B, and C) and measles in Romanian children. *Lancet* 1990;**336**:1592.
65. Saiman L, Aronson J, Zhou J, et al. Prevalence of infectious diseases among internationally adopted children. *Pediatrics* 2001;**108**: 608–12.
66. Salas SD, Heifetz R, Barrett-Connor E. Intestinal parasites in Central American immigrants in the United States. *Arch Intern Med* 1990;**150**:1514–16.
67. Santiago EM, Lawson E, Gillenwater K, et al. A prospective study of Bacillus Calmette-Guérin scar formation and tuberculin skin test reactivity in infants in Lima, Peru. *Pediatrics* 2003; **112**;298–302.
68. Schulpen TW, van Seventer AH, Rumke HC, et al. Immunization status of children adopted from China [Letter]. *Lancet* 2001;**358**: 2131–2.
69. Seybolt LM, Christiansen D, Barnett ED. Diagnostic evaluation of newly arrived asymptomatic refugees with eosinophilia. *Clin Infect Dis* 2006;**42**:36–9.
70. Sokal EM, Van Cullie O, Buts JP. Horizontal transmission of hepatitis B from children to adoptive parents [letter]. *Arch Dis Child* 1995;**72**:191.
71. Staat MA, Stadler LP, Donauer S, et al. Serologic testing to verify the immune status of internationally adopted children against vaccine preventable diseases. *Vaccine* 2010;**28**:7947–55.
72. Staat MA, Rice M, Donauer S, et al. Intestinal parasite screening in internationally adopted children: importance of testing multiple stool specimens. *Pediatrics* 2011;**128**:e613–22.
73. Stadler LP, Mezoff AG, Staat MA. Hepatitis B virus screening for internationally adopted children. *Pediatrics* 2008;**122**:223–8.
74. Stadler LP, Donauer S, Rice M, et al. Factors associated with protective antibody levels to vaccine preventable diseases in internationally adopted children. *Vaccine* 2010;**29**:95–103.
75. Swanson SJ, Phares CR, Mamo B, et al. Albendazole therapy and enteric parasites in United States-bound refugees. *N Engl J Med* 2012;**366**:1498–507.
76. Sweet K, Sutherland W, Ehresmann K, et al. Hepatitis A infections in recent international adoptees and their contacts in Minnesota, 2007-2009. *Pediatrics* 2011;**128**:e333–8.
77. Torres-Rojas JR, Rondon-Lugo A, Vidal R. Short course of clarithromycin in an immunocompetent patient with BCG-induced regional complications. *Dermatol Online J* 2002;**8**:6.
78. Trehan I, Meinzen-Derr JK, Jamison L, Staat MA. Tuberculosis screening in internationally adopted children: the need for initial and repeat testing. *Pediatrics* 2008;**122**:e7–14.
79. U.S. Department of Homeland. *Security yearbook of immigration statistics: 2010*. Washington, DC: U.S. Department of Homeland Security Office of Immigration Statistics; 2011. Available at: http://www.dhs.gov/xlibrary/assets/statistics/yearbook/2010/ois_yb_2010.pdf.
80. U.S. Department of State. *Immigrant visas issued to orphans coming into the US*. Available at: http://adoption.state.gov/about_us/statistics.php Accessed December 9, 2012.
81. van Schaik R, Wolfs TF, Geelen SP. Improved general health of international adoptees, but immunization status still insufficient. *Eur J Pediatr* 2009;**168**:1101–6.
82. Verla-Tebit E, Zhu X, Holsinger E, et al. Predictive value of immunization records and risk factors for immunization failure in internationally adopted children. *Arch Pediatr Adolesc Med* 2009;**163**: 473–9.
83. Viviano E, Cataldo F, Accomando S, et al. Immunization status of internationally adopted children in Italy. *Vaccine* 2006;**24**:4138–43.
84. Walker PF, Jaranson J. Refugee and immigrant health care. *Med Clin North Am* 1999;**83**:1103–20.
85. Wilson ME, Kimble J. Post-travel hepatitis A: probable acquisition from an asymptomatic adopted child. *Clin Infec Dis* 2001;**33**:1083–5.

This page intentionally left blank

PART IV

THERAPEUTICS

This page intentionally left blank

CHAPTER 233

Antibiotic Resistance

Philip Toltzis • Jeffrey L. Blumer

Soil bacteria developed mechanisms of resistance to antibiotics millions of years ago as a means of protecting themselves against other antibiotic-producing microorganisms or their own antimicrobial products. The current crisis of antibiotic resistance, however, was prompted by the intense selective pressure posed by the worldwide use of antibiotics during the past half-century. The problem of antimicrobial resistance in humans has been exacerbated by the widespread administration of antibiotics to farm animals.[270,409,467] Resistant zoonotic bacteria selected by this practice contaminate the food supply and thereby spread to human consumers.

To understand antibiotic resistance fully, one must make a distinction between the in vitro phenomenon of resistance and the less frequently observed clinical consequences of this in vitro phenomenon. Much of the discussion of bacterial resistance to antibiotics derives from the apparent changes in susceptibility of pathogenic bacteria to the concentrations of antibiotic achieved in blood after standard antibiotic dosing.[177] These concentrations have been established as "breakpoints" based on an integration of data derived from both clinical and animal studies, along with the measurement of plasma and serum drug concentrations associated with successful outcomes. Unfortunately, such breakpoints are not absolute. Rather, they depend on an intricate interrelationship between the pharmacokinetics and pharmacodynamics of the drug in question.[63] As such, if one component of this complex interrelationship is changed, the apparent breakpoint distinguishing susceptibility from resistance possibly and most likely also will change. Consequently, in presenting information concerning the mechanisms of antibiotic resistance and their clinical contexts, one must remember that the discussion derives, in part, from certain artificial pretexts. Any changes in a drug-dosing paradigm (e.g., dose, infusion duration, formulation) may affect the clinical relevance of the in vitro observations dramatically.

Bacteria develop resistance to antimicrobial agents through three principal cellular mechanisms (Table 233-1). In some microorganisms, two or more mechanisms of resistance to a given agent exist simultaneously. Antibiotic-resistance genes may be encoded either on the bacterial chromosome or on extrachromosomal DNA. The spread of antibiotic resistance among bacteria has been facilitated by the inclusion of many resistance genes on transmissible extrachromosomal genetic elements. To date, three interrelated genetic elements have been implicated in the expression and spread of antibiotic resistance, namely, *plasmids*, *transposons*, and *integrons*.

Plasmids are composed of circular double-stranded extra-chromosomal DNA. Although plasmids characteristically use bacterial DNA polymerase to replicate, their replication occurs autonomously.[432] Many different plasmids exist in nature; they vary in size, in the complexity of their genetic machinery, in their host range and ability to conjugate, and in their stability within the bacterium. Minimal plasmids (replicons) contain genetic sequences encoding the site at which their replication begins and sequences that determine the copy number produced per cell. However, many more genetic elements can be inserted into the replicon. Indeed, plasmids range in size from 1 kb to more than 400 kb; additional genetic machinery is required for conjugation, and, therefore, conjugative plasmids are at least 30 kb.[392] Groups of plasmids containing homologous replicative sequences generally are unable to be maintained in the same bacterial cell. This property has been exploited to categorize plasmids into different "incompatibility groups."

Recognition of the importance of plasmids to human disease increased substantially when researchers discovered that these elements could incorporate one or more antibiotic-resistance genes. These so-called resistance plasmids are found in a wide variety of gram-positive and gram-negative bacteria and encode determinants conferring resistance to many antibiotics. Resistance-conferring sequences producing resistance to several drugs may be included on the same plasmid, and multiple, different plasmids expressing different determinants can be included in the same microorganism.[33,432] Several additional classes of plasmid-borne determinants besides antibiotic-resistance genes can confer selective advantage to the bacterium. These determinants include genes rendering resistance to environmental heavy metals (e.g., mercury, antimony, arsenic, and silver) and a variety of toxic organic compounds (e.g., camphor and toluene), genes leading to the synthesis of virulence factors such as adhesion ligands promoting bacterial attachment to epithelial surfaces and those encoding exotoxins, and genes encoding compounds enabling acquisition of chemicals necessary for bacterial survival (e.g., siderophores).[432] The coexistence of these genes on plasmids carrying antibiotic-resistance determinants serves to maintain the plasmid, even in the absence of antibiotic exposure.

Plasmids are transmitted from microorganism to microorganism both vertically and horizontally.[432] Vertical transmission occurs during bacterial division as the plasmids segregate to each daughter cell. Horizontal transmission is affected through conjugation, the transmission of the plasmid from one bacterium to another.

TABLE 233-1 Cellular Mechanisms of Antibiotic Resistance and Their Transfer

Mechanisms
Genes that encode enzymes that modify or degrade antibiotics
Genes that modify the molecular targets for antibiotics
Genes that code for changes in cell wall channels or active pumping mechanisms
Transfer
Plasmids: extrachromosomal genetic elements made of circular double-stranded DNA <10 to >400 kb pairs; autonomous, self-reproducing; may be conjugative or nonconjugative
Transposon: a mobile genetic element in the bacterial chromosome or plasmid that confers a recognizable phenotype characteristic; incapable of self-replication
Integrons: DNA sequences on bacterial chromosome or plasmid often linked to a resistance gene that facilitates recombination among nonhomologous DNA sequences

At a minimum, all conjugal plasmids encode a genetic sequence that serves as the origin of transmission, and most encode additional mobilization genes.[430] However, plasmids that are unable to conjugate on their own frequently are able to do so when a conjugal plasmid co-resides in the same cell.[432] Especially in gram-negative bacteria, the donor-recipient contact required for conjugation is effected through sex pili. Conjugation frequently is restricted to intragenus transfer, but conjugation across bacterial genera may occur, particularly in gram-negative organisms, and conjugation occasionally occurs across Gram-stain barriers.[265,430]

A second group of bacterial nucleic acid elements that carry antibiotic-resistance genes is termed *transposons*. Transposons move segments of nucleic acid from one location to another, such as from different sites on the bacterial chromosome or from chromosome to plasmid.[386,393] Unlike chromosomal and plasmid DNA, transposons do not replicate independently and therefore must be integrated into a molecule capable of replication to propagate. Antibiotic-resistance genes may be encoded on conjugative transposons, which, unlike nonconjugative transposons, are able to transfer from one bacterium to another.[393] Most antibiotic-resistant conjugative transposons characterized to date have been identified in gram-positive organisms, although transposons in *Bacteroides* have been well characterized and transposons in some facultative gram-negative bacteria have been discovered as well.[380] Conjugative transposons are very promiscuous in two senses. First, they move easily from one species of bacterium to another.[380,393] Second, the integration of transposons into other nucleic acid elements is relatively sequence nonspecific. Although a preference exists for integrating into host DNA sequences with long stretches rich in adenine and thymidine, integration of conjugative transposons targets no detectable consensus sequences.[380]

Intracellular shuttling of a transposon from DNA to DNA begins with the production of a staggered cut on either side of the transposon sequence to produce single-stranded overhangs. These overhang sequences (termed *coupling sequences*) join covalently to produce a circularized double-stranded intermediate that contains the non–base-paired coupling sequences derived from opposite ends of the transposon.[380,393] Integration of the circle into the target double-stranded DNA also results in flanking non–base-paired regions, which become complementary only after a round of replication occurs.[393] Conjugation of the transposon from cell to cell also begins with the circularized intermediate, which, after nicking at a presumed origin site, is transferred as a single strand from donor bacterium to recipient bacterium, whereupon it recircularizes. Complementary strands then are added to both single strands in the donor and the recipient, and integration into target DNA in both cells ensues.[380]

Excision and integration of transposons are catalyzed by a transposon-encoded integrase.[380,393] The efficiency of excision varies widely and accounts for the differences in rates of transfer of transposons. Conjugation of many transposons appears to be regulated.[380] Unlike some plasmids, conjugative transposons do not exclude entry of other transposons.[380] Indeed, some transposons promote the conjugation of other transposons as well as co-resident plasmids; the result is the dissemination of resistance determinants that are not encoded on the transposon itself.[393]

Integrons, a third type of bacterial DNA important in antibiotic resistance, are composed of segments of DNA that are able to capture and excise gene cassettes coding antibiotic-resistance genes. Integrons are unable to transfer from bacterium to bacterium themselves. To disseminate, the integron first must insert into a plasmid or transposon.[108,342,354] So-called superintegrons, carrying more than 50 gene cassettes, have been identified on the chromosomes of some nonpathogenic environmental bacteria.[264] Superintegrons possess many of the organizational features of antibiotic-resistance integrons and may have been their progenitors. Integrons contain an integrase that catalyzes the incorporation and excision of the cassette into the integron, as well as a promoter that mediates the expression of the resistance determinant, both situated 5′ to the cassette. The cassettes themselves are small segments usually encoding a single antibiotic-resistance determinant. Cassettes have been identified that confer resistance to a broad range of antibiotics, including the β-lactam agents, the aminoglycosides, fluoroquinolones, trimethoprim (TMP), and chloramphenicol.[108,342,354] At the 3′ end of each cassette is a variable region commonly termed the *attC site* or, alternatively, the *59-bp element*, although it varies in length from cassette to cassette from 57 bp to more than 100 bp.[264] This 3′ element contains the sequences recognized by the integrase for incorporation of the gene cassette into the integron.[264,354] Several classes of integrons have been defined, based on the sequence of the integrase and their preference for certain transposons. Most antibiotic-resistance integrons are derived from class 1.[264] A single integron may contain multiple antibiotic-resistance cassettes in tandem, all of which are controlled by the single integron promoter. Transcription of tandem cassettes may terminate prematurely, however, such that the cassettes closest

to the integron promoter are transcribed at the highest level.[342,354]

The consequences of mobile resistance genes on plasmids and transposons cannot be overstated. They have given bacteria a profound replicative advantage in the age of antibiotics by enabling the instant acquisition of multiple, ready-made antibiotic-resistance genes without requiring each organism to independently undergo the same or similar resistance-conferring mutations again and again.[264] Hence, bacteria may become resistant almost immediately after exposure to antibiotics and may efficiently transmit this resistance to bacteria co-residing in the same host and to bacteria colonizing or infecting multiple hosts in the same community.

RESISTANCE TO SPECIFIC ANTIBIOTICS

β-Lactam Antibiotics

The β-lactam antibiotics are a diverse, highly effective family of drugs with broad activity against a wide variety of hospital- and community-acquired pathogens. The β-lactams are composed of four subgroups, namely, the penicillins, the cephalosporins, the monobactams, and the carbapenems. Resistance to the β-lactams is mediated by one of two principal mechanisms: (1) production of enzymes capable of hydrolyzing the β-lactam ring and (2) alteration of the target bacterial molecules, the penicillin-binding proteins (PBPs).

β-Lactamase Production

Clinical Relevance. The explosive development in antimicrobial therapeutics since the mid-20th century has been driven, to a great extent, by the impact of β-lactamase activity on the response to antibiotic therapy in the clinical setting.[357] Shortly after penicillin became incorporated into the clinical armamentarium for the treatment of systemic infections, clinical failures were reported in patients treated for infections caused by *Staphylococcus aureus*. On further analysis, these failures were attributed to the presence of an enzyme, "penicillinase," elaborated and excreted by the bacteria, that inactivated the antibiotic before it could manifest its clinical effect. This finding led to the development of numerous new antistaphylococcal penicillins (i.e., the isoxazolyl penicillins) that were not substrates for this enzyme.

At the same time, a newer class of β-lactam agents, the cephalosporins, was developed. One of the criteria employed in moving candidate drugs from this class to the clinic was their resistance to inactivation by the penicillinase elaborated by staphylococci. Further development of all four of the subgroups in the β-lactam family (i.e., penicillins, cephalosporins, monobactams, and carbapenems) was influenced significantly by the recognition that the penicillinase elaborated by staphylococci was representative of a larger class of enzymes that were also present in gram-negative organisms. In this regard, history repeated itself. As gram-negative organisms such as *Escherichia coli* emerged as important clinical pathogens, their emerging resistance to aminopenicillins on the basis of β-lactamase elaboration spawned the development of a group of enzyme inhibitors including clavulanic acid, sulbactam, and tazobactam.[241,361] Moreover, even as it became necessary to expand the spectrum of both the penicillins and cephalosporins to include some of the more itinerant gram-negative organisms such as *Serratia* spp., *Enterobacter* spp., and even *Pseudomonas*, β-lactamase stability emerged as a necessary attribute for clinical success.[241,361] This expansion was accomplished either through the addition of one of the β-lactamase inhibitors to members of the carboxy and acylureido class of penicillins or through structural engineering such as that seen with the development of the second- and third-generation cephalosporins and carbapenems.

In pediatric practice, the importance of β-lactamase became apparent soon after the widespread use of antibiotics in hospitalized patients began. However, three sentinel clinical events were probably most responsible for establishing β-lactamase as a prominent pediatric clinical problem. In 1974, the first case of bacterial meningitis caused by ampicillin-resistant *Haemophilus influenzae* type b was reported.[296,429] This report resulted in marked changes in clinical practice. First, all infants and children with suspected bacterial meningitis were treated empirically with both ampicillin and chloramphenicol (a drug not susceptible to degradation by bacterial β-lactamase) instead of with ampicillin alone.[357] In addition, several newer antibiotics, particularly the second- and third-generation cephalosporins, were evaluated for their efficacy in this clinical setting. From these studies, ceftriaxone or cefotaxime emerged as the drug of choice for the empirical treatment of suspected or proven bacterial meningitis in children. This selection was based both on both greater potency against *H. influenzae* type b and β-lactamase stability.

The second circumstance resulting in the affirmation of β-lactamase as an important clinical problem in pediatrics was the recognition of *Moraxella catarrhalis*, a β-lactamase–producing organism, as the third leading cause of acute otitis media in infants and children, and the emergence of amoxicillin resistance among the nontypeable strains of *H. influenzae*. β-Lactamase production in *H. influenzae* was thought to be particularly associated with therapeutic failure, thus spawning the development of oral second- and third-generation cephalosporins and newer macrolides as therapeutic alternatives in childhood otitis media.

By the early 1980s, β-lactamase–producing bacteria garnered additional attention from pediatricians because of the role of these bacteria as nosocomial pathogens. These bacteria became prominent as children with severe but survivable conditions (e.g., hematologic malignancies and solid organ transplantation) required immunosuppressive therapy and prolonged hospitalization, both of which predisposed these children to acquisition of hospital-acquired infection. With each succeeding decade, the number and variety of β-lactamase–producing hospital-acquired bacteria increased. By the early 21st century, virtually all nosocomial *S. aureus*, coagulase-negative staphylococci, and enteric and nonenteric gram-negative pathogens elaborated β-lactamase. These organisms commonly encode multiple distinct

β-lactamases and express them simultaneously. Consequently, many bacterial infections currently acquired in the hospital are resistant to all β-lactam agents except for the higher-generation β-lactam– β-lactamase inhibitor combinations, the advanced-generation cephalosporins, or the carbapenems. In the most extreme cases, particularly in *Pseudomonas*, *Acinetobacter*, and carbapenem-resistant Enterbacteriaceae infections, the organisms are resistant to all available β-lactam drugs.[61,228,346]

Mechanism of Resistance. The β-lactamases constitute a broad array of enzymes that hydrolyze the β-lactam ring of selected antibiotics (Fig. 233-1). Most β-lactamases are structurally similar to the PBPs, and the consensus is that at least some of the β-lactamases were derived evolutionarily from them.[276] β-lactamases can be identified in both gram-positive and gram-negative bacteria. In gram-positive bacteria, the enzyme is secreted into the cell-free environment, whereas in gram-negative organisms, most of the enzyme is confined and concentrated in the periplasmic space between the cell wall and the outer membrane.

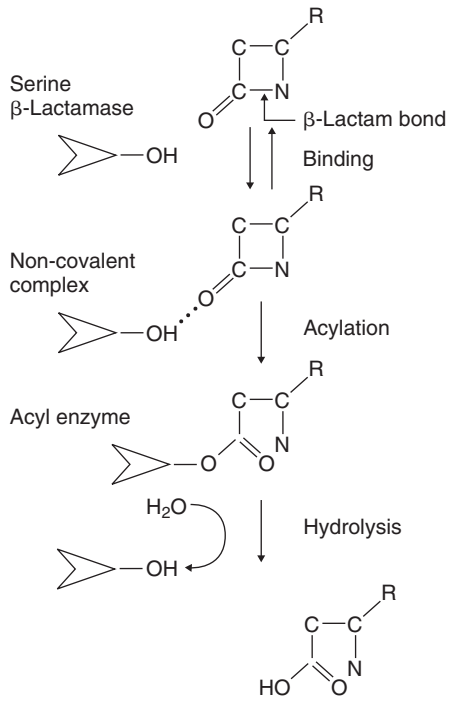

FIGURE 233-1 ■ Mode of action of β-lactamases (classes A, C, and D).

Nearly 1000 β-lactamases have been characterized,[38,185] and, doubtless, many more exist in nature. The β-lactamases have been categorized variously according to substrate preference, isoelectric focus, whether they are encoded on the bacterial chromosome or an episome, their ability to be inhibited by clavulanate, and their molecular structure. The molecular classification of Ambler[4] proposed in the 1980s is still widely employed. This classification divides the β-lactamases into four categories, A through D, although all but B, the metalloenzymes, have proved to have similar three-dimensional conformations.

The classification system of Bush, Jacoby, and Medeiros, proposed in 1995[39] and updated by Bush and Jacoby in 2010[38] divides these enzymes into three groups (with subcategories) and uses virtually all the properties listed earlier to discriminate one class of enzyme from the others. The properties of these groups are outlined in Table 233-2 and are described more fully here.

Group 1 AmpC β-Lactamases. The group 1 enzymes (also referred to as AmpC β-lactamases) hydrolyze virtually all β-lactam antibiotics except the carbapenems and are resistant to inhibition by clavulanate. These enzymes typically are chromosomally encoded on a sequence labeled *amp*C in a wide range of gram-negative bacteria; however, the AmpC β-lactamases are expressed in quantities sufficient to produce clinically important resistance only in selected species, including *Enterobacter cloacae*, *Serratia marcescens*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, and *Morganella morganii*. In the absence of drug exposure, expression of the group 1 AmpC β-lactamases in these species is repressed by an upstream sequence labeled *amp*R (Fig. 233-2).[276,334] However, exposure of the bacteria to a β-lactam leads to the production and intracellular incorporation of a bacterial cell wall metabolite that itself is processed by the enzyme AmpD.[180,239] In sufficient quantity, the AmpD substrate interacts with *amp*R, thus reversing the repression, and relevant quantities of the AmpC β-lactamase are thereby synthesized.

β-lactam antibiotics differ in their ability to induce AmpC β-lactamases by this mechanism. Ampicillin,

TABLE 233-2 **Properties of the Major Groups of β-Lactamases**

Group	1	2	3
Ambler class	C	A, D	B
Substrate preference	All but carbapenems	Variable*	Variable (carbapenems)
Chromosomal/plasmid	C	P	C/P
Distribution	Gram negative	Gram negative/positive	Gram negative
Clavulanate inhibition	No	Yes	No
Inducible†	Yes	No	No

*May be divided into subgroups including penicillinases, cephalosporinases, cloxacillinases, and carbapenemases. Enzymes frequently can hydrolyze more than one group of β-lactam.
†Indicates that normally repressed β-lactamase production can be induced by β-lactam exposure.
Data from Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995;**39**:1211-33.

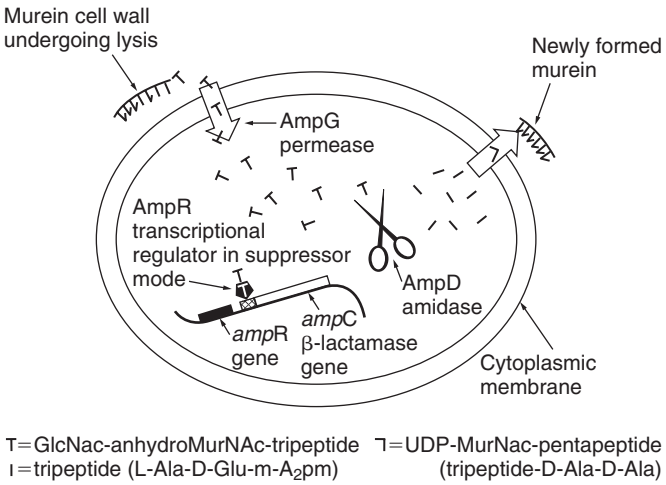

FIGURE 233-2 ■ A model for peptidoglycan recycling and β-lactamase induction in enterobacteria. The lytic transglycosylases in the periplasm cleave the bond between *N*-acetylglucosamine (GlcNac) and *N*-acetylmuramic acid (MurNac) and form the peptidoglycan degradation product, *N*-acetylglucosaminyl-1,6-anhydro-*N*-acetylmuramyl-L-alanyl-D-glutamyl-meso-diaminopimelic acid (T). The AmpG permease transports T into the cytoplasm. The AmpD amidase recognizes specifically substrate containing anhydromuramic acid and cleaves the bond between it and L-alanine, releasing the stem tripeptide, L-Ala-D Glu-m-A_2pm (I). Unprocessed T activates the AmpR transcriptional regulator, thereby inducing β-lactamase production. The tripeptide, I, conversely, recycles to form new peptidoglycan. By regulating the relative amounts of T versus I, AmpD may control peptidoglycan composition. The regulatory function of the AmpC β-lactamase, if any, is unknown.

cefoxitin, and imipenem are potent inducers, whereas most of the third-generation cephalosporins are weak. However, spontaneous mutations leading to a dysfunctional AmpD enzyme that occur at high frequency in nature result in permanent de-repression of the *amp*C gene, which, in turn, results in constitutive production of large quantities of the AmpC β-lactamase (see Fig. 233-2). De-repressed organisms are rendered highly resistant after this single mutational step. The third-generation cephalosporins are particularly prone to selecting such AmpD mutants, and the clinical emergence of resistant de-repressed isolates during the course of treatment with a third-generation cephalosporin has been documented.[55]

Group 2 β-Lactamases. The group 2 β-lactamases represent the largest and fastest growing group of β-lactamases owing largely to the proliferation of newer enzymes with broad substrate specificities.[38] The group 2 β-lactamases are encoded primarily on plasmids.[39,156,216,322,334] These plasmids commonly contain resistance determinants to other antibiotic classes as well. The plasmids are stable and frequently are maintained within the bacteria even in the absence of antibiotic pressure. Moreover, they can be transmitted by conjugation from organism to organism and across species, sometimes leading to a "plasmid outbreak" in a confined environment (e.g., a nursing home or intensive care unit [ICU]) that is caused by different species containing the same plasmid. The group 2 enzymes have substrate preferences that have become increasingly broad with the introduction of each new class of β-lactam antibiotic. Characteristically, the group 2 enzymes are inhibited by clavulanate and are found in both gram-positive and gram-negative bacteria (see Table 233-2).[39,216,276]

The prototypical group 2 β-lactamases are included in the TEM and SHV families of enzymes, although large numbers of group 2 enzymes not belonging to either of these families also have been characterized. TEM-1, first identified in Europe in 1963, hydrolyzed ampicillin. During the ensuing decades, the TEM sequence mutated in a stepwise fashion (with each new iteration labeled sequentially: TEM-2, TEM-3, and so on), resulting in the ability of each new enzyme to degrade an ever-increasing number of β-lactam antibiotics.[93,184,276] Many of these mutations widen a critical cavity, formed by the three-dimensional configuration of the molecule, containing a serine-active site at its bottom that is key to hydrolysis of the β-lactam.[127,185] Although these mutations render the enzymes kinetically less efficient than their parents, they allow the entry of the newer β-lactams with their bulky side chains into the cavity, thereby enabling the serine-active site at the bottom to reach the β-lactam ring.[93,276]

Extended-Spectrum β-Lactamases (ESBLs). Of increasing importance among the group 2 enzymes are the extended-spectrum β-lactamases (ESBLs). ESBL-producing organisms are distributed worldwide, but their incidence varies geographically.[332] Currently, bacteria elaborating ESBLs infect primarily critically ill, hospitalized patients,[323] although these enzymes are being reported in increasing numbers in the community.[58,281,340] Although they are molecularly diverse, ESBLs confer a common resistance phenotype, namely, reduced susceptibility to the third-generation cephalosporins, especially ceftazidime, and to aztreonam. Susceptibility to the carbapenems is preserved.[322] Common to other group 2 β-lactamases, hydrolytic activity typically is suppressed by the β-lactamase inhibitors. Most ESBLs are molecular descendants of TEM-1 and SHV-1 and are found in *E. coli*, *Klebsiella pneumoniae*, and to a lesser extent in *Proteus mirabilis.* Other families of ESBLs have been described. Among the more important ESBLs are the CTX-M enzymes, which are particularly active against cefotaxime, and some of the OXA-β-lactamases, the principal substrate of which is oxacillin but the spectrum of which has broadened to include the advanced-generation cephalosporins including cefepime.[38,322,332,340] Concomitant with their molecular evolution, ESBLs have been identified in an ever-widening range of bacteria, including *Pseudomonas*, *Salmonella*, and *Shigella* and *Enterobacter* spp.[322]

Although most ESBL-producing bacteria are overtly resistant, the third-generation cephalosporins have minimal inhibitory concentrations (MICs) against some ESBL-producing organisms that fall into the high end of the susceptible range. Consequently, the incidence of ESBL-producing bacteria is underestimated when conventional MIC breakpoints for resistance are used. Growing numbers of reports, however, indicate that infections caused by organisms expressing these "susceptible" MICs are clinically unresponsive to the third-generation cephalosporins.[352] Many hospital microbiology laboratories therefore screen all *E. coli*, *Klebsiella*, and

P. mirabilis clinical isolates for reduced susceptibility to both ceftazidime and cefotaxime and alert the clinician accordingly. Although the optimal therapy for infection caused by ESBL-producing, gram-negative bacteria is not established, many authorities recommend a carbapenem.[185,322,352]

Carbapenemases. The latter half of the 2000s witnessed the alarming spread of a new set of group 2 β-lactamases composed of carbapenemases, including the enzyme labeled "*Klebsiella pneumoniae* carbapenemase (KPC)"[28,30,167,407,474] and advanced-generation OXA β-lactamases that can hydrolyze carbapenems. This phenomenon has undermined the effectiveness of the class of antibiotics that had become the therapy of choice for previously encountered highly resistant organisms expressing Amp C β-lactamases and ESBLs.

KPC was first identified in an isolate of *K. pneumoniae* in the late 1990s from a patient in North Carolina.[28,167,407,474] Because of the presence of KPC on readily transmissible and promiscuous genetic elements, organisms expressing this enzyme spread rapidly up the American Atlantic Coast. By the mid 2000s, KPC-bearing *K. pneumoniae* had caused a city-wide epidemic among hospitalized patients in New York City[347]; soon thereafter, they were discovered in Europe, South America, the Middle East, and the Far East.[41,117,159,345,347] To date, infections by KPC-expressing organism have been identified primarily in critically ill adults in acute care hospitals[120,142,317] and residents of long-term care facilities,[52,328,473] without a significant representation in community-acquired disease. The organism has been reported only sporadically in children,[242] but their presence in the pediatric age group may be underappreciated. A survey of resistant organism-colonization in a long-term care facility for neurologically impaired children and young adults in Ohio, for example, revealed an unrecognized high prevalence of KPC colonization among the residents.[451]

Virtually all reports document that infection by a KPC-bearing bacterium is associated with increased risk for death, with crude mortality frequently around 50 percent.[242,410,441] In vitro data and clinical experience in adults have indicated that treatment with at least two agents is better than monotherapy, including regimens containing a carbapenem when the MIC is in the lower range.[188,349,441,484] Unfortunately, organisms expressing KPC are almost always multidrug resistant. Tigecycline and colistin are the antibiotics with the most consistent activity,[142] although some strains have emerged resistant to colistin.[24,290]

The KPCs are extraordinarily broad-spectrum enzymes, able to hydrolyze every class of β-lactam. Like other group 2 β-lactamases, the KPC enzymes have a serine-based active site for hydrolysis of the β-lactam ring. Their broad activity likely is the result of a more shallow position of the catalytic active site within the enzymatic cavity, rendering it more accessible to its antibiotic substrates.[347] However, unlike the other group 2 β-lactamases they are not sensitive to β-lactamase inhibitors; in fact, some data suggest that KPC enzymes hydrolyze inhibitors.[318,319]

The KPC family of enzymes is composed of multiple molecularly-similar homologues that have been numbered sequentially as they have been discovered and characterized.[214,347] The majority of disease to date has been caused by *K. pneumoniae* expressing KPC-2 and KPC-3. Dominant lineages of KPC-producing *K. pneumoniae* have been identified through multilocus sequence typing, a method that defines clonal relationships among bacteria by assessing polymorphisms within of a series of housekeeping genes; the sequence type ST 258 has been an especially prominent clone in the United States.[142,214] In any given geographical location, however, organisms expressing KPC can be found in a variety of *K. pneumoniae* sequence types and in species and genera of Enterobacteriaceae other than *K. pneumoniae*, indicating that KPCs have spread both by person-to-person transmission of dominant clones as well as by bacteria-to-bacteria transmission of enzyme-bearing plasmids.[214,230,261]

Newer OXA β-lactamases, particularly OXA-23 and OXA-48, also are able to degrade carbapenems. These enzymes are particularly prominent in *Acinetobacter*,[227] but they occasionally have been identified in other species.[38] Their activity is resistant to β-lactamase inhibitors. OXA-23 and OXA-48 are incorporated on a transmissible transposon, and they have been found in geographically diverse organisms.

Group 3 Metalloenzymes. The group 3 β-lactamases are composed of the metalloenzymes (see Table 233-2).[37,458] These β-lactamases are genetically heterogeneous and structurally dissimilar to those of groups 1 and 2. The metalloenzymes are not suppressed by β-lactamase inhibitors. The active site requires the participation of zinc; hence hydrolysis of the β-lactam ring is inhibited by chelators such as ethylene diaminetetraacetic acid (EDTA). The active site itself is composed of a wide plastic groove that can accommodate many β-lactam substrates. Some metalloenzymes are chromosomal (e.g., in *Stenotrophomonas*), but many have been identified on integrons that are inserted on transferable elements.[458] These latter metalloenzymes, which characteristically encode aminoglycoside-resistance determinants as well, are identified primarily in *Pseudomonas* and *Acinetobacter* spp. and selected Enterobacteriaceae. During the latter 1990s and early 2000s, organisms harboring transferable metallo-β-lactamases, particularly those from the IMP and VIM family of metalloenzymes, were identified in many countries in eastern Asia, Europe, and South America and in smaller numbers in the United States.[347] In 2008, a new plasmid-borne metallo-β-lactamase was identified in Western Europe labeled "New Delhi metallo-β-lactamase" (NDM) in patients who had previously been hospitalized in India.[223] Organisms harboring NDM subsequently were isolated throughout the Asian subcontinent and the United Kingdom. Bacteria expressing this metalloenzyme have been identified in patients in all age groups, including children, and in some instances have been the cause of community-associated infection.[223] The substrate preference of the metallo-β-lactamases varies, but most organisms expressing these enzymes have reduced susceptibility or overt

resistance to all the β-lactam antibiotics, including the carbapenems. Moreover, organisms encoding a metalloenzyme frequently express other chromosomal and plasmid-encoded β-lactamases as well, and this phenomenon, coupled with the coincident expression of resistance to other classes of antibiotic, often severely limits therapeutic options.

Although the properties summarized in Table 233-2 apply to most of the enzymes in each group, many exceptions have been noted. For example, enzymes that are genetically homologous to the group 2 family, typically plasmid-borne enzymes, have been identified on the bacterial chromosome in some isolates of *S. marcescens* and *E. cloacae*.[276] Conversely, group 1 AmpC enzymes, typically contained on the bacterial chromosome, have been identified on plasmids in genera such as *Klebsiella* and *Salmonella* that customarily do not express chromosomal *ampC* β-lactamase.[332-334] Some rare group 2–type enzymes are inducible.[276] TEM-type enzymes that are resistant to clavulanate have been identified (the so-called inhibitor-resistant TEMs [IRTs]).[47,207]

The degree of resistance exhibited by an organism encoding a β-lactamase frequently depends not only on the enzyme's in vitro substrate specificity and kinetics but also on the amount of enzyme produced. Hyperproduction of β-lactamase usually is the result of some alteration of a controlling sequence or an increase in number of copies of the β-lactamase gene within the bacterium.[126,315,479] High-level resistance results, and the susceptibility of the organism diminishes with increasing numbers of bacteria. Consequently, the organism may appear clinically unresponsive to a β-lactam antibiotic if hyperproduction occurs in the context of a high-inoculum disease. In some circumstances, hyperproduction operates in concert with other mechanisms to derive a resistant phenotype. Thus, some isolates of *E. cloacae* and *P. aeruginosa* have been rendered carbapenem resistant through a combination of hyperproduction of an AmpC β-lactamase, which normally is ineffective in hydrolyzing the carbapenems, and the simultaneous loss of the outer-membrane poron through which imipenem traverses to reach the PBP.[50,197,292,346]

Alteration of Penicillin-Binding Proteins

Clinical Relevance. Two species of clinical importance to pediatricians express resistance to β-lactam drugs through the alteration of PBPs, namely, penicillin-resistant *Streptococcus pneumoniae* and methicillin-resistant *Staphylococcus aureus* (MRSA). Indeed, not until penicillin-resistant *S. pneumoniae* was recognized did pediatricians begin to realize that alterations in the PBPs in the bacterial cell wall could pose a major clinical problem.[201,267] However, to date, the precise clinical importance of these organisms remains unclear.

Part of the problem in assessing the clinical importance of altered penicillin binding among pneumococci is that, rather than simply conferring penicillin resistance, the genes coding for this altered enzyme activity often co-segregate with genes coding for resistance to other antibiotics, such as TMP-sulfamethoxazole (TMP-SMX) and the macrolides.* Thus, unlike resistance associated with the elaboration of β-lactamase, in which simply selecting a different class of antibiotic often will provide a reasonable solution, resistance associated with alteration in PBPs is multifarious and, if clinically important, may require significant alterations in clinical practice to provide an effective antimicrobial regimen. The more limited menu of antibiotic choices for the treatment of infections caused by these organisms led to some interesting therapeutic conventions. For example, because the basis for this type of resistance is akin to altered enzyme affinity, investigators recognized in the mid 1990s that overcoming the apparent decrease in efficacy could be possible by simply increasing the dose of β-lactam employed.[201,268,269] Of the available agents, only amoxicillin had the requisite therapeutic index to support the increased dosing strategy. On this basis (almost exclusively), the high-dose amoxicillin regimen (80 to 100 mg/kg/day) that is currently recommended was adopted. Additionally, the perceived need for a drug effective against antibiotic-resistant *S. pneumoniae* led to the widespread use of the parenteral third-generation cephalosporin, ceftriaxone, in the setting of common upper and lower respiratory tract infections because of its in vitro activity against most of these organisms.

As the clinical impact of the emergence of the antibiotic-resistant *S. pneumoniae* has been examined more carefully in recent years, several trends have emerged.[201,478] In immunocompetent patients with pneumonia and most other non–central nervous system pneumococcal infections, no difference appears to exist in outcomes between patients infected with organisms that are susceptible to penicillin and those infected with organisms showing decreased susceptibility to penicillin.[314,331] Moreover, apparently no association exists between the intrinsic virulence of the organism and its antibiotic susceptibility. In contrast, in patients with pneumococcal meningitis caused by organisms with decreased penicillin susceptibility, there appears to be a delay in sterilization of cerebrospinal fluid in the presence of standard treatment.[247,477] For this reason, the current recommendation for empirical treatment of bacterial meningitis in infants and children consists of a combination of ceftriaxone or cefotaxime and vancomycin until the organism is identified and its antibiotic susceptibility is known.

S. pneumoniae remains the most prevalent bacterial cause of acute otitis media in infants and children. The clinical importance of the antibiotic susceptibility of these organisms in this clinical setting remains difficult to ascertain. The infection has a high rate of spontaneous resolution, and virtually all treatment is provided on an empirical basis. Consequently, any improved cure rate related to altered prescribing practices aimed at greater activity against organisms with decreased antibiotic susceptibility would be difficult to substantiate. Finally, the importance and virulence of these organisms in children who are either immunocompromised or immunosuppressed remain to be determined unequivocally.

*References 51, 75, 82, 83, 182, 358, 434.

The problem of penicillin-resistant *S. pneumoniae* was at least partially addressed by the introduction of the conjugated heptavalent pneumococcal vaccine (PCV7) in 2000. The serotypes included in the vaccine accounted for most penicillin-resistant pneumococci in the United States at that time.[435] Surveys conducted by the Centers for Disease Control and Prevention (CDC) indicated that the incidence of invasive *S. pneumoniae* infection caused by penicillin-resistant strains decreased by more than 50 percent in the years immediately following introduction of the vaccine.[224] The decrease was most pronounced in children younger than 2 years of age, but the herd immunity created by universal PCV7 immunization resulted in a reduction of pneumococcal infection in all age groups. This benefit was mitigated in part by increases in disease caused by resistant serotypes not included in the vaccine, most notably serotype 19A.[18,224] In response to this phenomenon, a 13-valent conjugated pneumococcal vaccine (PCV13) was licensed in the United States in 2010; it included all seven serotypes represented in PCV7, plus six additional serotypes, including 19A, that accounted for the majority of residual invasive and antibiotic-resistance pneumococcal infection encountered during the post-PCV7 era. Licensure of PCV13 was based on studies documenting immunogenicity rather than protective efficacy,[320,426] and hence the effectiveness of PCV13 in further reducing the circulation of antibiotic-resistant pneumococcal strains has yet to be established.

MRSA was isolated first from hospitalized adult patients in 1961 and became a prominent pathogen in critically ill patients during the ensuing decades. By the early 2000s, more than half of *S. aureus* infections in patients in ICUs in the United States were caused by MRSA.[293] Compared with those in adults, MRSA infections among hospitalized children occur relatively infrequently. In the mid 1990s, however, MRSA infections were identified among otherwise healthy children with no prior contact with hospitals or hospitalized patients.[157,175] These strains of community-associated MRSA (CA-MRSA) were molecularly distinct from MRSA infections previously identified in the hospital environment. By the mid-to-late 2000s, CA-MRSA had established a worldwide presence in both children and adults.[3,300] These organisms cause primarily skin and soft tissue infection but occasionally bone and joint infection, severe necrotizing pneumonia, and other invasive, deep tissue disease. CA-MRSA infections are especially common in populations inhabiting close quarters and engaging in skin-abrading activities, including athletes participating in competitive sports, military personnel, and prison populations.[461] Additionally, they increasingly are being identified in patients acquiring their infection in the hospital,[172,344] blurring the epidemiologic distinctions that had once characterized HA-MRSA and CA-MRSA lineages.

Most MRSA isolates, both hospital and community associated, are derived from a relatively small number of prominent, widely disseminated clones.[307] Several systems have been proposed to categorize the principal MRSA lineages. The system established by the CDC is based on *Sma*I macro-restriction patterns of bacterial DNA visualized after separation by pulse-field gel electrophoresis (PFGE). The PFGE types so defined have been labeled USA100 through USA1200.[79,178,272] Currently, the marked majority of MRSA strains derived from patients in the United States fall within one of these PFGE types.[79,272] Most American CA-MRSAs are included in the USA300 group, particularly those falling in the subgroup labeled USA300-114, with a minority derived from the USA400 and USA1100 groups.[213,300,395] HA-MRSA strains are derived from most of the remaining pulsed-field types. MRSA strains additionally have been categorized by multilocus sequence typing, a technique that distinguishes bacterial lineages based on polymorphisms found in a series of housekeeping genes, with the predominant American strain USA300-114 belonging to sequence type ST 8.[300] Elsewhere in the world, community-associated MRSA is represented by other PFGE-types and multilocus sequence types, although variants of the USA300 strain are common in Canada and Latin America.[300]

Mechanism of Resistance. β-Lactam antibiotics must bind to PBPs to confer their activity, and alteration of the structure of PBPs results in diminished affinity for drug and, consequently, antibiotic resistance. The PBPs are a heterogeneous group of compounds normally responsible for maintaining the peptidoglycan matrix supporting the bacterial cell wall. PBPs vary from species to species in number, size, copy number per cell, and affinity for β-lactam agents.[124,146,383] Typically, four to eight PBPs are present in any given bacterial isolate; they range in molecular weight from 35 to 120 kd.[124] PBPs possess two principal enzymatic activities. In general, the larger PBPs are responsible for peptidoglycan transpeptidase activity, whereas the smaller PBPs usually carry D,D-carboxypeptidase activity.[124] These activities constitute the final reactions in the formation of the peptidoglycan matrix and are responsible for the cross linkage that significantly adds to the tensile strength of the bacterial cell surface.

Examination of PBP-mediated β-lactam resistance in *S. pneumoniae* and *S. aureus* serves to exemplify some of the features of this mechanism of resistance. Detailed investigations indicate that penicillin resistance in pneumococcus is associated with alternations in PBP 1A, 1B, 2A, 2X, and 2B; cephalosporin resistance has been correlated with structural abnormalities in PBP 1A, 2A, and 2X.* Confirmation that the resistant phenotype is related directly to structural abnormalities in various pneumococcal PBPs has been achieved through transformation experiments.[15,57,135,289] In these studies, penicillin-susceptible strains are converted to penicillin-resistant strains by addition of amplified DNA encoding the putative resistant PBPs. Patterns of PBPs from resistant pneumococci vary considerably from geographic location to location, a finding indicating that PBP-related pneumococcal resistance probably arose independently in various parts of the world.[145]

PBPs from penicillin-resistant *S. pneumoniae* contain blocks of amino acid sequences that are remarkably

*References 15, 57, 135, 145, 147, 289.

divergent from those seen in susceptible pneumococci. These sequences are homologous to those encoding for PBPs from other streptococcal species (e.g., *Streptococcus oralis* and *Streptococcus mitis*), a finding suggesting that the resistant pneumococci contain mosaic PBPs that arose after transformation and recombination of homologous genes from closely related bacteria.[89,90,144,226] These sequences then developed point mutations conferring resistance. A similar pattern of "resistance blocks" of amino acids has been identified in the PBPs of penicillin-resistant *Neisseria gonorrhoeae*, which appear to have originated from other *Neisseria* spp.[146] The degree of resistance conferred by a single mutated PBP is relatively small.[124,135] Acquisition of multiple abnormal PBPs results in incremental resistance and, ultimately, an organism that can survive routine β-lactam therapy.

Whether associated with the hospital or the community, all isolates of MRSA contain the *mecA* gene, a 2130-bp chromosomal sequence encoding the PBP 2a, which, unlike the other staphylococcal PBPs, has low affinity for β-lactam antibiotics.[67,68] The *mecA* gene is carried on a mobile element designated the *staphylococcal cassette chromosome mec (SCCmec) sequence*.[486] All MRSA strains contain an SSC*mec* complex, but their size and organization vary, thus allowing them to be grouped into eight distinct SCC*mec* types, designated by roman numerals I through VIII.[178,306,486] Most hospital-acquired (HA)-MRSA strains contain SCC*mec* types I to III, whereas CA-MRSA strains characteristically harbor type IV.[178] In most HA-MRSA isolates, the SSC*mec* sequence contains resistance determinants additional to *mec*A, including those conferring resistance to clindamycin, the aminoglycosides, and the fluoroquinolones.[72,486] Hence, these organisms possess a selective advantage in ICUs, where exposure to multiple classes of antibiotics commonly occurs. The CA-MRSA SCC*mec* type IV encodes a smaller SCC*mec* sequence usually lacking the multiple antibiotic-resistance genes found in their hospital-associated counterparts.[72,486] Although a growing number of American strains of CA-MRSA are emerging resistant to fluoroquinolones through chromosomal mutations, to date only a small minority of CA-MRSA bacteria express resistance to other antibiotics.[271,431] On the other hand, USA300 CA-MRSAs express a number of virulence factors not common in other strains. Most notable among these is the Panton-Valentine leukocidin, possibly important for soft tissue breakdown and destruction, but a host of other virulence factors, as well as altered genetic elements controlling their expression, have been identified.[431] The smaller SCC*mec* sequences allow CA-MRSA to replicate more rapidly than does HA-MRSA,[305] because they can be maintained by the bacterium at a lower metabolic cost. The relatively small size of the CA-MRSA SCC*mec* sequences further enables efficient transfer to co-colonizing susceptible *S. aureus*.[486]

SCC*mec* elements likely originated in coagulase-negative staphylococci. Type IV SSC*mec* sequences, for example, have been identified in isolates of *Staphylococcus epidermidis* from the 1970s.[471] Additionally, sequences have been found in *S. epidermidis* and *Staphylococcus hominis* strains that contain the regulatory genes and insertion sequences of the SCC*mec* elements found in MRSA but that lack the *mecA* gene itself.[3]

Macrolides, Lincosamides, and Streptogramins

Clinical Relevance

The macrolides are compounds that contain 14-member (e.g., erythromycin and clarithromycin), 15-member (e.g., azithromycin), or 16-member (e.g., spiramycin) lactone rings. Their activity is directed primarily against gram-positive organisms and some gram-negative respiratory tract pathogens. In many bacteria, resistance to macrolides occurs concomitantly with that of the structurally unrelated lincosamides (including clindamycin) and streptogramin B families of antibiotics, two classes with activity also primarily against gram-positive bacteria. Because these three families of antibiotics interact competitively for ribosomal binding, they probably are associated with the same ribosomal site.

Resistance to the macrolides was noted in staphylococcal species soon after the introduction of erythromycin. More recently, however, macrolide resistance in streptococcal pathogens, particularly *S. pneumoniae* and *Streptococcus pyogenes*, emerged and assumed major clinical importance. Macrolide resistance among pneumococci is a result of both international dissemination of resistant clones and horizontal transmission of resistance determinants.[122,372] The late 20th century witnessed dramatic increases in the incidence of macrolide resistance in *S. pneumoniae* in many areas of the world.[158] In Southeast Asia, surveys indicated that nearly 40 percent of pneumococcal isolates were resistant to all macrolide antibiotics, and samplings of organisms from Europe and the Western Hemisphere showed similar but less extreme trends.[158,211] Perhaps most troubling, resistance to macrolides among pneumococci occurred particularly frequently in isolates coexpressing resistance to penicillin and other oral antibiotics.[83,176]

Similar to penicillin-resistant pneumococcus, the prevalence of macrolide-resistant *S. pneumoniae* has been reduced during the first decade of the 21st century by the introduction and widespread administration of the conjugated heptavalent pneumococcal vaccine, which includes serotypes that most commonly express both β-lactam and macrolide resistance.[435] Studies performed in Atlanta, Georgia, before release of the vaccine, for example, documented that 31 percent of pneumococcal isolates causing invasive disease were resistant to erythromycin.[121] Follow-up studies performed after the introduction of the vaccine recorded a drop in incidence of macrolide-resistant invasive pneumococcal disease by more than two thirds.[414] As in penicillin-resistant pneumococcus, this benefit has been partially offset by an increase in the incidence of invasive diseases by macrolide-resistant organisms derived from serotypes not included in the vaccine.[101]

Similar to *S. pneumoniae*, macrolide resistance in *S. pyogenes* has been unevenly distributed geographically.[206] By the start of the 21st century, most surveys in the Western Hemisphere, including the United States,

indicated a prevalence of resistance less than 10 percent,[136,313,362,427,465] although the citywide clonal outbreak of macrolide-resistant group A streptococcus experienced in Pittsburgh, Pennsylvania, in 2001 underscored the potential for this phenotype to spread rapidly within a community.[256] Macrolide resistance in *S. pyogenes* is, on average, more prevalent in Europe, but the prevalence there also varies substantially from area to area, ranging (by 2007) from 3 percent in Norway,[240] to 13 percent in Belgium,[248] to more than 20 percent in Spain and France.[22,330] Molecular analysis of macrolide-resistant group A streptococcal isolates from geographically diverse regions suggested that the resistance genes were acquired multiple times by many lineages, some of which then disseminated worldwide.[367]

The increase in macrolide resistance has occurred concomitant with increased worldwide consumption of these compounds for respiratory tract infections beginning in the 1980s,[16,21,56,176,199,394] a finding suggesting a biologic association between these two phenomena. A nationwide survey of macrolide susceptibility among pneumococcal isolates in the United States, for example, documented an increase in erythromycin resistance from 10.6 percent in 1995 to 20.4 percent in 1999, a time when the number of prescriptions for macrolides increased by 13 percent across the board and by 320 percent in pediatric patients.[176] Seppälä and colleagues[394] reported an increase in resistance among *S. pyogenes* isolates in Finland from 5 percent in 1988 to 13 percent in 1990 after national macrolide consumption doubled during the 1980s. In response, a national campaign to reduce macrolide prescribing practices in Finland was launched in the early 1990s, after which macrolide resistance fell by half.[394] A similar campaign in Taiwan yielded similar results.[171]

Although the epidemiologic trends in macrolide resistance are alarming, the clinical consequences of the increases in macrolide resistance among streptococci are largely unknown. Current convention employs an MIC of 4 μg/mL or greater to define resistance. The newer macrolide compounds, however, achieve tissue and intracellular concentrations that are much higher than this designation.[98,304] Italian investigators, for example, noted a very high incidence of erythromycin resistance among group A streptococcal throat isolates, but neither clinical nor microbiologic failures were clearly associated with macrolide treatment.[450] The macrolides do not distribute in high concentrations to the blood compartment, however, and several reports document poor outcomes when this class of drugs is used for bacteremic pneumonia caused by macrolide-resistant pneumococcus.[210,244]

Mechanism of Resistance

Two principal resistance phenotypes to macrolides and related antibiotics have been detected. The first, denoted the *MLS_B phenotype*, is characterized by resistance to all macrolides, regardless of the size of the ring, as well as to the lincosamides and the type B streptogramins. Most organisms expressing the MLS_B phenotype remain susceptible to the combination streptogramins (e.g., quinupristin-dalfopristin).[148,388] The second phenotype, denoted the *M phenotype*, is associated with resistance to the 14- and 15-member macrolides (including erythromycin, azithromycin, and clarithromycin) but not those with 16-member rings. The degree of resistance to the affected macrolides is lower, on average, compared with that seen with MLS_B resistance. Additionally, in organisms with the M phenotype, susceptibility to clindamycin is preserved.[262,419]

Most MLS_B resistance results from methylation of the 23S ribosomal RNA (rRNA) in the 50S subunit of the bacterial ribosome within the peptidyltransferase circle in domain V.[71,187,428,466] The methylases are encoded on transposons, with sequences found both on plasmids and in the bacterial chromosome.[129] Methylation of the 23S rRNA is encoded by a family of related genes labeled *erm* (*e*rythromycin *r*esistance *m*ethylases).[71,187,428,466] Historically, these methylases have been described and named in a haphazard fashion, although some investigators have proposed renaming many of these enzymes using a classification scheme based on genetic homology.[365]

Methylation is both inducible and constitutively expressed. Unlike many other inducible antibiotic-resistance factors, the mechanism of MLS_B resistance induction is at the level of translation, rather than transcription.[464] In the uninduced state, the leader sequence of the mRNA encoding ErmC, one of the methylases found in *S. aureus*, forms two stem loop structures that stall the ribosome's movement along the message, thereby preventing synthesis of the methylase enzyme. The attachment of erythromycin to the ribosome leads to a conformational alternation of this leader sequence that uncovers the appropriate ribosomal binding sites and allows the stalled ribosomes to proceed to translation.[464] Inducible macrolide resistance is detected in the clinical microbiology laboratory by the *double-disk D-test*. In this assay, an erythromycin disk and a clindamycin disk are placed side by side on a lawn of the test organism. Induction of clindamycin resistance in the presence of erythromycin is manifested by increased growth of bacteria on the erythromycin side of the clindamycin disk, producing a "D"-shaped zone of inhibition. Through the 1990s, greater numbers of *erm*-containing bacteria produced the rRNA methylase constitutively, a consequence of mutation of the leader sequence.[84,425]

In the mid 1990s, macrolide resistance mediated through a genetically transferable efflux pump was identified.[419] Most organisms expressing the M phenotype are resistant through this mechanism. Most macrolide-resistant pneumococci in North America express the macrolide efflux pump, and much of the expansion of macrolide resistance that occurred through the 1990s was the result of the appearance of organisms exhibiting this mechanism.[176,193,238,427] A family of related genes, termed *mef*, encodes the macrolide efflux pumps. The *mef* genes are included on transposons encoding associated functions, in particular an adenosine triphosphate (ATP)–binding protein.[121,122,129,262] As expected, ribosomes isolated from organisms resistant through *mef* genes bind to macrolide antibiotics as readily as do ribosomes from susceptible organisms. Radiolabeled erythromycin is excluded from the intracellular space in *mef*-positive

bacteria, a phenomenon that is reversed by compounds that poison ATP-dependent pumps.[419]

Mechanisms of macrolide resistance besides rRNA methylation and efflux pumps have been identified but to date are uncommon. Some organisms modify the antibiotic by hydrolysis, acetylation, phosphorylation, or esterification.[365] Some evidence indicates that some erythromycin resistance is caused by altered ribosomal proteins and results in the production of ribosomes with diminished affinity for drug.[53]

Trimethoprim and Sulfamethoxazole

Clinical Relevance

The combination antibiotic TMP-SMX inhibits bacterial folate synthesis at two successive steps. This mechanism accounted for its broad activity against a wide variety of gram-positive and gram-negative bacteria when it was first introduced in 1968. Despite the dual activity of this combination, resistance to TMP-SMX has risen steadily during the decades of its use and is now commonplace. Acquisition of resistance is potentiated by recent exposure to TMP-SMX.[102,413,476] When resistance occurs, the MIC expressed usually is very high,[173,174] and the affected organisms frequently are coresistant to multiple other classes of antibiotics. Indeed, TMP-SMX resistance is sufficiently widespread that it precludes the use of the drug as a first-line antibiotic in clinical circumstances in which it was formerly the agent of choice, namely, respiratory tract infections and *Shigella*-associated dysentery. Its utility in urinary tract infection (UTI) also may be limited when the community prevalence of TMP-SMX resistance is sufficiently high. Ironically, its continued activity against CA-MRSA has rejuvenated the use of TMP-SMX among many practitioners as the incidence of infections caused by CA-MRSA organisms has mushroomed.

Respiratory Tract Infections (Otitis Media and Pneumonia). The frequency of TMP-SMX resistance in organisms causing respiratory tract infections has increased significantly. Among *S. pneumoniae* organisms, resistance to TMP-SMX was identified during the 1990s in many geographic locations, with resistance detected in 12 percent to more than 50 percent of isolates.* A worldwide survey conducted in the late 1990s noted the highest incidence of TMP-SMX pneumococcal resistance in the Asian Pacific region and in Latin America.[158] In surveys of U.S. isolates, TMP-SMX resistance is the most common resistance phenotype found in *S. pneumoniae*,[468] although the incidence of resistance varies widely from region to region.[81] TMP-SMX resistance is strongly associated with resistance to other classes of antibiotics commonly used in respiratory tract infections, particularly penicillin and the macrolides. Among other organisms implicated in respiratory tract infections, TMP-SMX resistance also has been documented with increasing frequency in *H. influenzae*, to which resistance ranges between 7 percent in Finland[253] and more than 50 percent in Taiwan.[170] The susceptibility of *M. catarrhalis* to TMP-SMX varies little from region to region. Although most *M. catarrhalis* organisms remain susceptible to TMP-SMX, the MICs cluster near achievable respiratory tract concentrations.[182]

The clinical consequences of TMP-SMX resistance in respiratory tract pathogens are not well defined. Direct evidence of the association between isolation of a TMP-SMX–resistant organism from the middle ear and failure of therapy by TMP-SMX in acute otitis media, for example, is lacking, but such an association is assumed to exist. The issue of the clinical relevance of TMP-SMX resistance in respiratory tract infections is particularly pressing in developing countries, where World Health Organization (WHO) guidelines recommend TMP-SMX as a first-line agent for pneumonia. A comparison of amoxicillin and TMP-SMX for treating pneumonia in Pakistan demonstrated superiority of amoxicillin in producing clinical cure, but treatment failure was not associated with resistance in the infecting organism.[418] These data and others[208,353,369] support the continued use of TMP-SMX therapy of pneumonia in the developing world, at least at present.

Shigellosis. Resistance of *Shigella* spp. to TMP-SMX rose dramatically during the 1980s in many parts of the world, including Bangladesh,[20] Thailand,[160] Somalia,[44] Israel,[11] and Canada.[150] In these surveys, the incidence of disease from TMP-SMX–resistant *Shigella* increased twofold to threefold, to more than half the isolates; by 1998, more than 90 percent of *Shigella* organisms in Thailand,[160] as well as *Shigella* cultured from patients with acquired immunodeficiency syndrome (AIDS) in Kenya,[222] were resistant to TMP-SMX. Many of these *Shigella* organisms express extraordinarily high levels of resistance, with MICs to TMP exceeding 1000 μg/mL. In the United States, TMP-SMX resistance among *Shigella* isolates initially was confined to closed populations.[137] A more recent survey in Oregon, however, indicated that by the late 1990s the incidence of TMP-SMX resistance among *Shigella* isolates in that state approached 60 percent. Resistant isolates were found disproportionately frequently in migrant workers traveling from Mexico and other areas of Latin America,[359] a finding emphasizing the importance of geographic spread of resistance in modern society. Subsequent surveys performed by the CDC in the early 2000s revealed that TMP-SMX–resistant *Shigella* had spread nationwide, with the prevalence highest in the East and West.[401] The incidence of TMP-SMX resistance in *Shigella* mirrors the increased TMP-SMX resistance among other enteric species as well,[137,443] notably *Salmonella*.[1,137]

TMP-SMX resistance in *Shigella* is virtually always borne on conjugative plasmids, and frequently these plasmids contain resistance determinants to several other antibiotic classes.* Coresistance to four or more unrelated antibiotics, including β-lactams, chloramphenicol, tetracycline, and the aminoglycosides, is a common finding. Molecular analyses in a given geographic environment usually indicate fluid epidemiology, with

*References 83, 158, 170, 182, 253, 321, 417.

*References 44, 137, 222, 237, 401, 443.

multiple dominant chromosomal genotypes containing a variety of resistance plasmids.[29,237]

Urinary Tract Infections. Through the 1990s and 2000s, TMP-SMX resistance in urinary tract isolates of *E. coli*, the most prominent pathogen implicated in UTIs, rose to 15 to 25 percent in the developed world and to as high as 60 percent in nonindustrialized countries.* Studies in the 1980s indicated that TMP-SMX–resistant *E. coli* can be transmitted to household contacts.[371] Indeed, one analysis suggested that TMP-SMX–resistant urinary tract isolates from geographically disparate areas of the United States may have emanated from a single clone.[251] Although urinary concentrations of both TMP and SMX are severalfold higher than concomitant concentrations in serum, resistance to TMP-SMX nonetheless is associated with treatment failure in UTIs. Bacterial and clinical response rates to TMP-SMX are 80 to 95 percent in cystitis and pyelonephritis caused by susceptible organisms, but the response falls to approximately 50 percent when the infecting agent is resistant to TMP-SMX.[266,423] Considering all urinary tract pathogens together, however, the clinical and microbiologic response to TMP-SMX is nearly equivalent to comparator agents, and experts continue to recommend this drug as the first-line therapy for uncomplicated UTIs, based on its low cost.[104,166,460] Analyses have indicated that the total expense of treatment of UTIs with TMP-SMX begins to exceed that incurred by therapy with more expensive antibiotics, such as the fluoroquinolones, when the community TMP-SMX–resistance rates of urinary pathogens exceed approximately 20 percent, as a result of the additional expense of clinical treatment failure.[229,423] Alternatives to TMP-SMX also should be considered when the patient possesses risk factors for acquisition of infection with a TMP-SMX–resistant strain, namely, recent hospitalization, diabetes, or therapy with TMP-SMX within the previous 3 months.[166]

Community-Associated Methicillin-Resistant *Staphylococcus aureus*. Despite the explosive emergence of methicillin resistance in community-associated *S. aureus* infections in the latter half of the 1990s, these organisms have remained nearly uniformly susceptible to TMP-SMX.[31,200,285] Most infections caused by CA-MRSA are skin and soft tissue infections that can be treated without hospitalization, and TMP-SMX offers an inexpensive, oral therapeutic option in this setting. The clinical effectiveness of TMP-SMX in CA-MRSA infection relative to other oral agents is uncertain; indeed, whether any antibiotic is superior to incision and drainage is not clear.[279] Experiential reports, however, support the use of TMP-SMX as a principal or adjuvant therapy for CA-MRSA cellulitis.[422]

Mechanisms of Resistance

In susceptible bacteria, TMP-SMX inhibits sequential steps in the de novo synthesis of folate (Fig. 233-3). SMX inhibits the enzyme dihydropteroate synthetase (DHPS), which catalyzes the conversion of *para*-aminobenzoic acid to dihydrofolate. TMP inhibits the next enzyme in the pathway, namely, dihydrofolate reductase (DHFR), which catalyzes the conversion of dihydrofolate to tetrahydrofolate. The resulting depletion of folate within the bacterium interrupts the synthesis of critical cellular substrates, including purine nucleotides.[368] Eukaryotic DHFR is resistant to the activity of the sulfonamides, and eukaryotic cells do not express DHPS activity at all, thus accounting for the selective activity of these drugs against bacteria.[173,174]

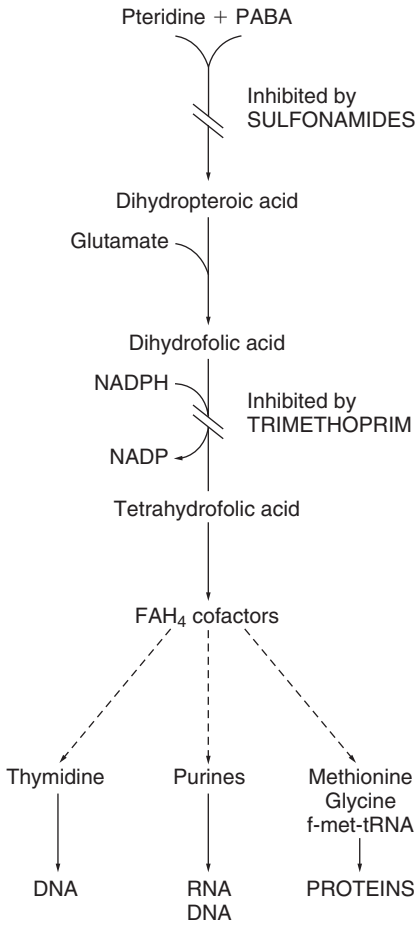

FIGURE 233-3 ■ Trimethoprim-sulfamethoxazole inhibition of de novo folate synthesis. NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced form of nicotinamide adenine dinucleotide phosphate; PABA, para-aminobenzoic acid.

The principal mechanism of resistance to TMP-SMX is alteration of the target enzymes. Altered genes for DHFR with reduced susceptibility to TMP have been identified on the chromosomes of intrinsically resistant organisms from a variety of species,[339] as well as on transferable elements. Many of the transferable resistance genes are encoded on transposons that can shuttle between plasmids and chromosomal DNA. Approximately 20 different transferable *dhfr* resistance genes have been identified.[174] Resistance usually is conferred by a single-point mutation that results in a disruption of the

*References 140, 141, 205, 251, 487, 488.

three-dimensional conformation of DHFR, thereby interrupting a hydrogen bond normally available for binding the antibiotic but leaving the affinity for its natural substrate intact.[66,174,339] These structural changes may occur in concert with mutations of controlling sequences and lead to overproduction of the altered DHFR.[339] Similarly, sulfonamide resistance usually is the result of mutated *dhps* genes. In TMP-SMX–resistant *Neisseria meningitidis*, the abnormal chromosomal *dhps* sequence is different compared with that found in susceptible isolates over a large portion of the gene, a finding implying that resistance resulted from a recombination event after horizontal transfer from an unrelated *Neisseria* spp.[350] Similar recombination events may account for sulfonamide resistance in pneumococcus. In other species, the abnormal *dhps* gene is the result of a single amino acid mutation, and the resistance gene is encoded on a transmissible transposon,[312] frequently linked to a *dhfr* resistance gene.[174]

Some organisms, particularly *P. aeruginosa*, are intrinsically resistant to TMP-SMX on the basis of the expression of multidrug efflux pumps.[217] The same pumps are responsible for resistance to a wide variety of other antibiotics, including fluoroquinolones, chloramphenicol, and β-lactams. Homologous pumps occasionally have been identified in other gram-negative species as well. Finally, in some isolates, the mechanism of resistance to TMP-SMX is not attributable to any of the previously listed mechanisms and remains undefined.[174,312]

Aminoglycosides

Clinical Relevance

The aminoglycosides are parenteral antibiotics that are employed primarily for serious infections in hospitalized patients. Resistance to aminoglycosides is encoded on transmissible elements that have disseminated throughout the world, both among gram-negative and gram-positive pathogens. Among gram-negative species, resistance to the aminoglycosides varies widely according to geographic location.[387,389,447] Surveys recording longitudinal trends in susceptibility patterns of hospital-acquired isolates between the 1990s and 2000s, however, documented that, relative to other agents, the increase in resistance to the aminoglycosides has been slow, and resistance has decreased in some areas.[19,112,203] Surveys of hospital isolates conducted throughout Europe and North America in the late 1990s and early 2000s indicated resistance rates of 1 to 15 percent among enteric bacilli and 10 to 50 percent among non–lactose fermenters.* In almost all cases, the incidence of resistance was lowest to amikacin.[112,299,387,389,447] Aminoglycoside resistance occurs with much higher frequency among gram-negative organisms with coexisting resistance to other antibiotics.[14,196,286] Not surprisingly, fecal colonization with aminoglycoside-resistant bacilli is an uncommon finding among healthy persons. Although resistance to some of the older aminoglycosides, such as streptomycin and kanamycin, has disseminated into the community, carriage of organisms resistant to gentamicin is a rare finding in ambulatory populations.[234,309,348]

Few data exist recording the endemic incidence of aminoglycoside resistance in gram-negative organisms specifically among hospitalized children. In a survey of pediatric patients in an ICU in Cleveland in the mid 1990s, approximately 10 percent of patients were colonized with a tobramycin-resistant, gram-negative bacillus before discharge.[436] A survey in the neonatal ICU in the same institution, where gentamicin frequently was employed, recorded an approximate 5 percent endemic incidence of colonization with gentamicin-resistant bacilli.[433] However, outbreaks caused by gram-negative rods resistant to aminoglycoside have been reported from numerous neonatal ICUs since the early 1980s.[59,191,273]

Although the aminoglycosides have no utility in the treatment of infections with gram-positive organisms when used alone, they improve bacterial killing in vitro when they are employed in combination with cell wall–active agents. For this reason, these drugs frequently are added to β-lactam agents or vancomycin in the treatment of difficult or persistent infections caused by *S. aureus*, coagulase-negative staphylococci, or enterococci. However, resistance to aminoglycosides has been documented in all these gram-positive microorganisms. Surveys in Europe indicated 20 to 30 percent resistance to aminoglycosides among hospital isolates of *S. aureus*.[387] The occurrence of aminoglycoside resistance is much higher among MRSA isolates than in bacteria that are methicillin susceptible.[70,107] As with gram-negative bacilli, aminoglycoside resistance among *S. aureus* isolates varies greatly from region to region,[387] a finding correlating largely with the incidence of MRSA. Aminoglycoside resistance in coagulase-negative staphylococci also is a common finding. As with *S. aureus*, resistance is much more frequent among organisms that are methicillin resistant (seen in more than half of the isolates tested from centers around the world), as opposed to the unusual hospital-acquired methicillin-susceptible, coagulase-negative staphylococci, for which resistance to aminoglycosides is recorded in fewer than 10 percent.[78,215,387]

Since the 1970s, proliferation of enterococci that express MICs to the aminoglycosides exceeding 2000 μg/mL has occurred, thus rendering the organisms resistant to all synergistic effects when the aminoglycosides are used with a cell wall–active agent.[96,325] Such high-level aminoglycoside resistance has been recorded in approximately 30 percent of enterococcal samples from hospitals throughout the world.[245,286,485] Organisms expressing high-level aminoglycoside resistance frequently test highly resistant to ampicillin as well.[325] High-level aminoglycoside resistance also is a common finding in vancomycin-resistant enterococci, especially those identified in the United States.[245]

Mechanism of Resistance

The aminoglycosides are hydrophilic sugars composed of one to four rings, with amino groups substituting some

*References 107, 196, 298, 387, 389, 449.

of the hydroxyl groups at sites that vary from drug to drug. The amino groups render the drugs polycationic and allow them to bind with high affinity to selected sequences of nucleic acid.[356] The antibacterial activity of these drugs results from their binding to specific sites on the 16S ribosomal RNA that, in turn, interferes with the recognition of transfer RNA during translation and the translocation of tRNA to the peptidyl-tRNA site. Three-dimensional studies of the aminoglycoside-rRNA interaction suggest that this interference is the result of conformational changes in the shape of the rRNA.[221] The affinity of the aminoglycosides to ribosomal RNA is 10-fold higher in prokaryotes than in eukaryotes.[355]

The principal mechanism of resistance to the aminoglycosides is through the expression of aminoglycoside-modifying enzymes (Table 233-3). Many of these enzymes are encoded on transposons that reside in plasmids along with other resistance determinates. Three groups of modifying enzymes have been identified, namely, acetyltransferases (AAC), nucleotidyltransferases (ANT), and phosphotransferases (APH), each of which adds its respective moieties to selected side groups of the aminoglycoside sugar rings.[221,396] Researchers have hypothesized that these genes originated from bacterial housekeeping enzymes and then expanded to include aminoglycosides among their substrates through mutational evolution.[396] Transcription of these resistance genes is constitutive. Most members of each group of modifying enzymes share structural homologies, with minor amino acid variations resulting in specific substrate profiles.[396] The enzymes are subdesignated by the site they modify and by the resistance profile they confer (Fig. 233-4). Thus, for example, AAC(6′)-II denotes an AAC that substitutes an acetyl group for the amino group at the 6′-site of the aminoglycoside, with a resulting resistance pattern II (including, in this case, resistance to gentamicin, tobramycin, and sisomicin, but not amikacin). At least one enzyme has been discovered that possesses both AAC and APH activity [designated AAC[6′]-Ie-APH[2″]-Ia].[54,215,396,485] The modifications conferred by these enzymes result in altered electrostatic qualities of the antibiotic and consequent reduced ability to interact with rRNA.

The pharmaceutical industry has altered the structure of the aminoglycoside compounds to render them poorer substrates for the modifying enzymes. This exercise has been most successful in the development of amikacin, a kanamycin derivative with a relatively long amino hydroxybutyrate added at the 1 position.[280] This substitution does not interfere with rRNA binding but protects the compound from alteration by many of the modifying enzymes. Consequently, organisms resistant to gentamicin, for example, may remain susceptible to amikacin. Not all positions are protected, however, and amikacin-resistant phenotypes are conferred by certain modifying enzymes, most notably AAC(6′)-I.[280]

Moderate-level resistance to the aminoglycosides has been achieved by some bacteria through decreased uptake of drug.[280] This mechanism has been noted most frequently in *Pseudomonas*. To date, modifications of the ribosomal binding sequences have been identified only rarely as a mechanism of resistance to the aminoglycosides.[280] The sequences involved in aminoglycoside binding likely are sufficiently critical to the survival of the organism that mutations within this site usually are fatal.[221]

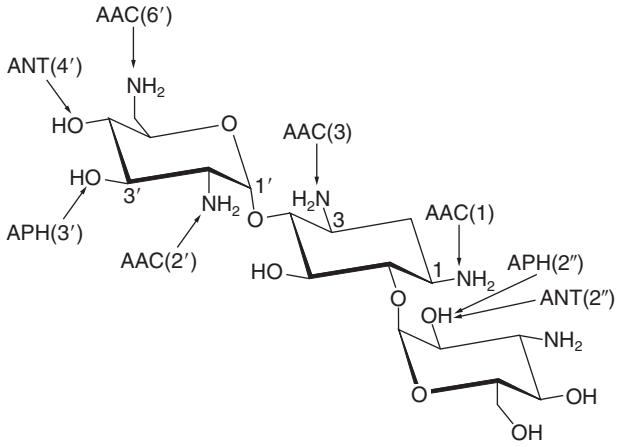

Kanamycin B

FIGURE 233-4 ■ Sites of modification on kanamycin B by various aminoglycoside-modifying enzymes. The arrows point to the sites of modification by the specific enzymes, namely, acetyltransferases (AAC), phosphotransferases (APH), and nucleotidyltransferases (ANT).

TABLE 233-3 **Aminoglycoside-Modifying Enzymes**

Drug	Aminoglycoside* Acetyltransferase Acetylation	Aminoglycoside* Nucleotidyltransferase Adenylation	Aminoglycoside* Phosphotransferase Phosphorylation
Amikacin	X	X	
Gentamicin	X	X	X
Kanamycin	X	X	X
Netilmicin	X		
Neomycin	X	X	X
Streptomycin		X	X
Tobramycin	X	X	X

*Numbers of susceptible sites on each molecule vary.
Adapted from Welton A, Neu HC, editors. *The aminoglycosides*. New York, Marcel Dekker, 1982.

Glycopeptides and Other Antibiotics Directed Against Gram-Positive Bacteria

Clinical Relevance

Vancomycin is one of two glycopeptides available for clinical use. The second, teicoplanin, is licensed in Europe but not the United States. Since the late 1980s, the prevalence of vancomycin resistance in organisms belonging to the genus *Enterococcus* (vancomycin-resistant enterococci [VRE]) has risen alarmingly. VRE frequently are highly resistant to many other antibiotics as well, and reported mortality from infection caused by these resistant organisms exceeds 30 percent.[76,287,399]

The appearance of VRE occurred coincident with an increased use of vancomycin in the United States and the use of the glycopeptide avoparcin as a growth promoter in animal feed in Europe, a practice banned by the European Union in 1997.[291] The incidence of VRE colonization and disease is much more prevalent in the United States than in other areas of the world,[77,245] but these organisms are present globally.[169,469] Ten to 20 percent of U.S. enterococcal isolates investigated in surveys in the late 1990s and early 2000s were resistant to vancomycin.[77,245] A disproportionate number of VRE in the United States are found in patients hospitalized in ICUs in large urban medical centers, especially among persons with prolonged hospitalizations and severe disease.[152,245,246,399] Additionally, a high incidence of VRE is seen among oncology patients,[95,260] transplant recipients,[94,275] and those with chronic renal failure who are receiving dialysis.[12,105,454] Some surveys have documented VRE among adults in chronic care facilities, who exported the organism into the community after acquisition in an acute care setting.[438] By contrast, the prevalence of VRE is relatively low in U.S. hospitals serving rural areas.[343,416]

Surveys of European centers also indicate a concentration of VRE in ICUs but in smaller numbers compared with the United States.[376,390] The occurrence of VRE in Europe, however, varies widely from country to country. In 2010, the proportion of blood isolates of *E. faecium* that were resistant to vancomycin ranged from 0 percent in Sweden and Finland to over 20 percent in Portugal, Greece, and Ireland.[45] VRE also have been reported in Asia and Latin America but at rates approximately half those found in the United States.[45]

The genetic machinery conferring vancomycin resistance in enterococci is complex, and resistance does not develop de novo by spontaneous mutation under antibiotic pressure.[291] The initial event in the appearance of VRE in a given unit is the importation of the resistant organism into the environment. The association between antecedent treatment with vancomycin and acquisition of VRE has been documented by many,[111,399] but not all, investigators.[42,86] Some researchers have established a stronger connection between the appearance of VRE and the use of extended-spectrum cephalosporins[26,311] and antibiotics with potent antianaerobic activity.[246] A study conducted in a Veteran's Affairs hospital reported that the stool density of VRE in patients who were treated with antianaerobic antibiotics increased exponentially, whereas the density decreased in those whose antibiotics had limited anaerobic activity.[85] The principal role of antecedent antibiotic exposure in VRE may be to open up ecologic niches in the gastrointestinal tract that allow a stable presence of VRE once the patient is exposed to the resistant organism.

Horizontal transmission of VRE is prominent in any closed environment and may outweigh antecedent antibiotic exposure as the principal determinant for acquisition.[26,152] Patient-to-patient spread in an ICU, presumably through the hands of caregivers, has been documented repeatedly, as has contamination of the immediate environment of the VRE-positive patient.[311,403] Indeed, perhaps the most significant risk factor for acquisition of VRE is the number of neighboring patients colonized with the organism at the same time.[13,26,65]

In many U.S. ICUs, multiple clones of VRE are present at any given moment, some imported into the unit and others spread after admission has occurred,[311] thus rendering control and containment very difficult to achieve. Extreme barrier isolation precautions, including surveillance for VRE on admission to a given unit, single-room isolation with dedicated medical instruments, and strict use of gowns and gloves, frequently are necessary to contain VRE once it has established an endemic presence, and even then these measures may not be entirely effective.[190,225,403]

With some exceptions,[134,274,440] to date VRE have been less a threat to pediatric patients than to adults, despite the wide use of vancomycin among hospitalized pediatric patients and their geographic proximity in many hospitals to sick adults colonized with the organism. Several outbreaks of VRE in the newborn nursery have been reported,[233,249] but they have resulted primarily from dissemination of a single clone within the unit and have been successfully eradicated with implementation of relatively simple barrier isolation and cohorting measures.

In the latter half of the 1990s, a second phenomenon involving reduced susceptibility to vancomycin was identified, namely, vancomycin-intermediate *S. aureus* (VISA).[110,405] These organisms express MICs to vancomycin in the intermediate range of approximately 8 μg/mL. Virtually all such isolates are coresistant to methicillin, but several have retained susceptibility to numerous other antimicrobial agents. To date, VISA isolates have been identified primarily in patients who have been treated with prolonged courses of vancomycin for MRSA infection. Disproportionate numbers of these patients have been undergoing dialysis.[406] Eradication of VISA has been difficult but achievable with high doses of vancomycin in combination with other antibiotics.[110,406] At present, infection with VISA is a rare occurrence, both in adults and in children.

In 2002, the first two cases of true vancomycin-resistant *S. aureus* (VRSA) in the United States were reported, one from Michigan and the other from Pennsylvania (MIC to vancomycin >128 μg/mL and =32 μg/mL, respectively).[444,445] Both patients were suffering from chronic foot ulcers from which the organisms were isolated. In both instances, the bacteria were susceptible to alternative agents, and the patients remained clinically stable. The potential for VRSA to emerge as a

major public health hazard is substantial, but subsequent cases have been rare.[446]

Mechanisms of Resistance

By 2012, eight genotypes for vancomycin resistance in enterococci had been defined, labeled VanA through VanE and VanG, VanL, VanM, and VanN, each associated with phenotypic nuances.[62,291] These genotypes differ in the species of enterococci affected, the relative resistance conferred to vancomycin and teicoplanin, and whether they are inducible or constitutively expressed (Table 233-4). The VanC phenotype is distinct from the others in that it is an intrinsically expressed, species-specific product of *Enterococcus gallinarum*, *Enterococcus casseliflavus*, and *Enterococcus flavescens.*[294] The other vancomycin genotypes are acquired, although several are not readily transferable (see Table 233-4). The marked majority of human isolates are derived from the VanA group.

In susceptible bacteria, the glycopeptides are large molecules that interfere with cell wall peptidoglycan synthesis at a point proximal to that targeted by the β-lactam antibiotics. Specifically, the synthesis of peptidoglycan in vancomycin-susceptible bacteria includes the incorporation of a D-alanyl-D-alanine dipeptide onto the carboxyl end of the growing glycopeptide chain to form a pentapeptide precursor. The pentapeptide is translocated from the cytoplasm to the outer surface of the bacteria.[62] The D-Ala-D-Ala carboxy-terminal end of the pentapeptide is critical for the continued synthesis of the peptidoglycan chain and, ultimately, for peptidoglycan cross linking and the formation of a stable cell wall. Vancomycin, which never enters the cell, binds to the D-Ala-D-Ala moiety of the pentapeptide through several hydrogen bonds along the outer surface of the cell wall,[17] thereby blocking synthesis of the remaining macromolecule.

The molecular basis for vancomycin resistance results from the complex, coordinated interactions of multiple genes usually encoded on a single genetic element. These interactions have been defined best for the VanA phenotype, although genetic homologues are found in organisms expressing the other vancomycin-resistant phenotypes. The genetic machinery for VanA resides on the 10,851-bp transposon Tn*1546.*[8,456] The molecules central to conferring the resistant phenotype are encoded by the contiguous genes labeled *vanH*, *vanA*, and *vanX*, all of which are essential for expression of vancomycin resistance. The *vanA* gene encodes a ligase that, unlike the native enterococcal ligase, produces an abnormal D-alanyl-D-lactate dimer over the normal D-alanyl-D-alanine.[34] This process results in the formation of the peptidoglycan precursor uridine diphosphate–MurNAc–L-Ala-D-Glu-L-Lys-D-Ala-D-Lac, the carboxy terminus of which has profoundly reduced affinity for vancomycin.[35,45] The homologous ligase in VanC-, VanE-, and VanG–expressing organisms synthesizes D-Ala-D-Ser (see Table 233-4) with similar consequences,[62,291] although the degree of resistance conferred by formation of D-Ala-D-Ser is less profound than that conferred by D -Ala-D-Lac. VanH catalyzes the reaction taking pyruvate to D-Lac and increases the availability of this substrate to VanA for production of the D-Ala-D-Lac dimer.[35] *vanX* and a second gene labeled *vanY* encode a cleaving enzyme for the D-Ala-D-Ala dipeptide, thus reducing the natural cellular substrate for the D-Ala-D-Ala adding enzyme and resulting in greater relative incorporation of D-Ala-D-Lac into the peptidoglycan precursor.[62,360]

The *vanS* and *vanR* genes included on the same vancomycin-resistance transposon regulate expression of resistance. The products of these genes are homologous to other compounds of so-called two-component regulatory systems.[442] Ultimately, the result is transcription of the resistance genes in the presence of vancomycin.[62] The functions of the last two gene products, VanY and VanZ, are not essential for expression of the resistant phenotype.[9,10]

TABLE 233-4 Types of Resistance to Glycopeptides in Enterococci

	Acquired Resistance								Intrinsic Resistance
	High Level		Variable	Moderate	Low Level				Low Level
Susceptibility	VanA	VanM	VanB	VanD	VanE	VanG	VanL	VanN	VanC1/C2/c3
Vancomycin	R	R	r-R	R	r	r	r	r	r
Teicoplanin	R	R	S	r-R	S	S	S	S	S
Transferability	+	+	+	–	–	+	–	+	–
Main enterococcal species	A/B*	A	A/B	A/B	B	B	B	A	G/D
Expression	I	?	I	C	I/C	I	I	C	C/I
Genetic location	Plasmid (Chr)	Plasmid (Chr)	Chr (plasmid)	Chr (plasmid)	Chr	Chr	?	Chr	Chr
Precursors end	D-ala-D-Lac	D-ala-D-Lac	D-ala-D-Lac	D-ala-D-Ser	D-ala-D-Ser	D-ala-D-Ser	D-ala-D-Ser	D-ala-D-Ser	D-ala-D-Ser

R, high level of resistance (MIC > 16 mg/L); r, low level of resistance (MIC = 8-16 mg/L); S, susceptible; A, *E. faecium;* B, *E. faecalis;* G, *E. gallinarium;* D, *E. casseliflavus;* I, inducible; C, constitutive; Chr, chromosome.

*Also other *Enterococcus* species.

From Cattoir V, Leclercq R. Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? J Antimicrob Chemother 2012 Dec 2. [Epub ahead of print].

Between 2002 and 2012, 12 adult patients were described who had fully vancomycin-resistant MRSA infections from *S. aureus* harboring the TN*1546* transposon found in VRE.[9,10,45,444-446] This finding indicated that true VRSA could result from the conjugal transfer of the vancomycin-resistance transposon from VRE to MRSA, a phenomenon that can be replicated in vitro.[301] By contrast, VISA isolates do not contain the resistance genes found in VRE; rather, these isolates acquire unusual extracellular material around the cell wall as detected by electron microscopy.[36,406] This layer represents accumulated peptidoglycan with reduced levels of cross linking, thereby exposing an excess of D-Ala-D-Ala moieties that bind to vancomycin before it can reach the cell wall.[457] Other vancomycin-resistance phenomena in *S. aureus* have been described and include those in which subpopulations express MICs twofold to eightfold higher than those in the parent population (termed *heteroresistance*),[110,198] as well as those in which *S. aureus* can be inhibited but not readily killed by vancomycin (termed *tolerance*).[263] Some investigations indicated that isolates of *S. aureus* expressing vancomycin heteroresistance develop into VISA with continued exposure to the drug.[457]

Resistance to Other Antibiotics Used for Gram-Positive Bacterial Infections. Two antibiotics introduced in the early 2000s, namely, linezolid and daptomycin, were developed to address the increasingly important problem of antibiotic-resistant, gram-positive pathogens, especially VRE and MRSA. In both instances, the antibiotic represented a novel class of molecules, in the hope that resistance would be slow to develop.

Linezolid, the first of the oxazolidinone antibiotics, has activity against all important human gram-positive pathogens, including MRSA, VISA, coagulase-negative staphylococci, and VRE. Gram-negative organisms are intrinsically resistant to linezolid, because they express efflux pumps that extrude the drug from the cytoplasm.[243] The molecular target of the oxazolidinones is domain V of the 23S rRNA of the 50S bacterial ribosomal subunit. Binding to this domain results in the inhibition of the initial steps of translation.[421] Although there is some overlap with the binding sites of other antibiotics whose activity is directed against the ribosome,[243] linezolid's binding site is unique to the oxazolidinones. Because the oxazolidinones are entirely synthetic, likely no significant natural resistance to this class of drugs existed before the introduction of linezolid.[277] To date, resistance to linezolid in vivo has been a rare occurrence. Multinational surveys conducted throughout the first decade of the 2000s have identified remarkably few linezolid-resistant organisms, despite the widespread use of the agent.[73,194,299] In most surveys, over 99 percent of *S. aureus* (including MRSA) and enterococci (including VRE), and more than 98 percent of coagulase-negative staphylococci, remain linezolid susceptible.[138,243,439,492] The few linezolid-resistant clinical isolates that have been encountered have been derived from enterococci, *S. aureus* and coagulase-negative staphylococci, usually after prolonged exposure to drug.[27,138,303,470] Three principal mechanisms of linezolid resistance have been defined. The majority of linezolid-resistant organisms possess a mutation in the gene encoding domain V of the 23S rRNA.[243,277] Most bacteria have multiple copy numbers of this gene. The degree of resistance is gene dose dependent, such that the MIC of linezolid usually remains in the susceptible range after a single mutation occurs but increases incrementally as each allele is affected.[277] In other linezolid-resistant organisms, the resistant phenotype is conferred through mutations of the ribosomal proteins L3 and L4.[243] Finally, ribosomal binding of linezolid may be inhibited by methylation of the rRNA; this mechanism is mediated through the plasmid-borne enzyme Cfr methylase.[243] Such methylation results in cross resistance to clindamycin and the streptogramin A antibiotics.[243] In some instances, Cfr-bearing plasmids include several other antibiotic resistance determinants as well.[133]

Daptomycin, a fermentation product of *Streptomyces roseoporus*, is a unique antimicrobial with activity against a full range of gram-positive bacteria. The compound is a cationic cyclic lipopeptide that interacts with the cell membrane in a manner similar to related cationic peptides that constitute part of the natural human host defense mechanisms against microbes. In the presence of calcium, this event disrupts the transmembrane electrical potential with a secondary interruption of ATP and macromolecular synthesis.[148,412] As with linezolid, laboratory surveys of large numbers of gram-positive clinical isolates from many areas of the world indicate that daptomycin resistance is rare.[373-375,439] Soon after daptomycin was introduced in the early 2000s, however, several cases were reported documenting the emergence of *S. aureus* and enterococci with reduced susceptibility to daptomycin during prolonged therapy.* In these cases, the infection emanated from a bony or endovascular focus into which penetration of the drug may have been limited. Daptomycin resistance has been linked to mutations in multiple genes that regulate the composition and integrity of the bacterial cell membrane.[114] The mutations implicated in daptomycin resistance differ in *S. aureus* and enterococcus, but in sum they result in alterations in the phospholipid content of the bacterial cell surface, changing its charge and thereby decreasing the affinity of the drug to its target.[7,209,282,370,437] Ultrastructural analysis of daptomycin-resistant isolates reveals a thickened envelope, and several reports have correlated vancomycin-intermediate resistance in *S. aureus* and reduced susceptibility to daptomycin.[7,64,448]

The cephalosporin ceftaroline was introduced into clinical practice in the latter part of the 2000s. In distinction to all prior cephalosporins, ceftaroline exhibits a high affinity for the penicillin-binding protein PBP2a responsible for conferring methicillin resistance in staphylococci,[382] rendering the drug active against MRSA. Additionally, it has activity against multidrug-resistant pneumococci as well as against staphylococci with intermediate resistance to vancomycin and reduced susceptibility to linezolid and daptomycin.[382] Its activity against enterococcus is variable.[382] Several surveys testing ceftaroline against a wide range of staphylococcal and pneumococcal clinical isolates in the late 2000s confirmed its

*References 7, 153, 209, 252, 258, 282.

nearly uniform in vitro activity.[99,100,106] Even early in ceftaroline's clinical history, however, a ceftaroline-resistant strain of MRSA was isolated and expressed a PBP2a molecule with reduced affinity to the drug.[278]

Quinolones

Clinical Relevance

The quinolone family of antibiotics possesses activity against a broad variety of microorganisms. As of the early 21st century, the quinolones had not been labeled for pediatric use. However, the safety profile in children of the more recently developed quinolones (i.e., the fluoroquinolones) is similar to that recorded in adults[123,189,384] and this class of antibiotics has been effectively employed in selected children with serious infections caused by bacteria resistant to alternative agents. The following subsections discuss clinical circumstances in which quinolone resistance is relevant to pediatric practice.

Cystic Fibrosis. The lungs of patients with cystic fibrosis (CF) are chronically infected early in life, primarily with *S. aureus* and later with *P. aeruginosa*. Exacerbations of pulmonary symptoms are treated effectively with antibiotics directed against these microorganisms. Ciprofloxacin represents the first oral agent with significant anti-*Pseudomonas* activity and manifests potency generally superior to that of the other fluoroquinolones.[337] As a result, experts in many centers treat patients with CF with ciprofloxacin, even when the recipients are younger than 18 years of age.[123] In most circumstances, pediatric patients with CF treated with ciprofloxacin have experienced improvement in their respiratory status.[385]

Pseudomonas surveys conducted with organisms derived from patients with CF as well as those without CF indicated susceptibility to ciprofloxacin in 50 to 90 percent of strains; CF isolates tended to express resistance more frequently than did other isolates.* Several investigators documented that decreased susceptibility of *Pseudomonas* lung isolates to ciprofloxacin occurs frequently during the course of ciprofloxacin treatment in patients with CF.[87,411] Indeed, high proportions of *Pseudomonas* strains infecting the lungs of patients with CF are "hypermutators," particularly prone to spontaneous mutation resulting from defects in genetic repair mechanisms,[308] that allow the ready emergence of antibiotic resistance. However, as a general rule, patients who improve clinically during therapy for an acute pulmonary exacerbation do not eradicate the infecting organisms, and the importance of emergence of resistant bacteria during therapy versus any of the commonly employed antibiotics is uncertain, as long as the infecting organisms were susceptible initially.[404] Moreover, frequently the *Pseudomonas* reverts back to a quinolone-susceptible phenotype several weeks after treatment with ciprofloxacin has been completed.[80,87] As a result, ciprofloxacin currently appears to be a useful option for patients with CF who have worsening pulmonary disease, if at least one of the organisms in the initial sputum culture is susceptible.

The usefulness of ciprofloxacin in the patient with CF who is infected with *S. aureus* also probably depends on the susceptibility of the pretreatment isolates. A worldwide survey conducted in the late 1990s indicated that most methicillin-susceptible strains of *S. aureus* also were susceptible to ciprofloxacin and other fluoroquinolones. However, the fluoroquinolone susceptibility among methicillin-resistant strains was less than 20 percent and susceptibility was extraordinarily low to ciprofloxacin in particular.[109,139,163]

Enteric Gram-Negative Bacilli: Uropathogens, Nosocomial Bacteria, and Agents of Bacterial Enteritis

Quinolone resistance in enteric gram-negative rods, particularly among uropathogens, has been an uncommon occurrence in North America. Studies of enteric urinary tract isolates in the United States and Canada documented susceptibility rates to ciprofloxacin of 95 percent or greater during the 1990s and 2000s among nonelderly patients,[487] a finding supporting the effectiveness of quinolones for community-acquired cystitis and uncomplicated pyelonephritis.* In contrast, in some regions of Europe, the incidence of quinolone resistance among uropathogens has increased as administration of this class of antibiotics has become more widespread.[118,131,168] A 1998 survey of urinary tract isolates from Latin America indicated an even more dramatic occurrence of quinolone resistance: 22 percent of *E. coli*, 30 percent of *P. mirabilis*, and more than 35 percent of *Enterobacter* were ciprofloxacin resistant.[118] Quinolone resistance in gram-negative urinary pathogens frequently is associated with resistance to other classes of antibiotics as well.[204] Consequently, several experts continue to recommend TMP-SMX over the fluoroquinolones as the first-line agent for uncomplicated UTIs, because clinical and microbiologic efficacy is nearly equivalent for the two classes of drugs and routine use of quinolone will needlessly promote quinolone resistance.[104,166,460]

The quinolones traditionally have been mainstays of treatment for nosocomial infections in adult patients with severe illness. During the late 1990s and early 2000s, however, quinolone resistance in nosocomial gram-negative pathogens increased in many areas of the world among both Enterobacteriaceae and non–lactose fermenters.[112,196,203,286,299] In several instances, quinolone resistance in gram-negative bacilli increased more rapidly than any other resistance phenotype.[112,196] Many of these hospital-acquired pathogens coexpress ESBLs and other resistance determinants.[6,32,324]

Antibiotic therapy, including treatment with quinolones, for bacterial enteritis usually is not indicated.[472] That said, with the exception of *Campylobacter*, most agents that cause bacterial enteritis have remained susceptible to the quinolones by in vitro testing,[130] although epidemic strains of both *Shigella* and *Salmonella*

*References 43, 109, 132, 155, 254, 379, 398.

*References 91, 104, 140, 141, 229, 266, 423.

expressing quinolone resistance have been identified in Asia.[116,327,424] *Salmonella typhimurium* DT104, which spread rapidly throughout Europe and the United States in the late 1990s, is resistant to nalidixic acid, but most isolates (except for some identified in the United Kingdom) are susceptible to the newer fluoroquinolones.[115,130,154,283] Similarly, surveys of other non-*typhi Salmonella* conducted in the early 2000s indicated that few were ciprofloxacin-resistant, although as many as one fifth of the strains had reduced susceptibility to the drug.[143,255,415]

In contrast to resistance among other enteritis-associated bacteria, quinolone resistance among *Campylobacter* isolates from many parts of the world has become prominent and high level.[160,186,335,475] *Campylobacter* can acquire quinolone resistance shortly after exposure to the antibiotic. Indeed, clinical relapse with quinolone-resistant organisms after therapy for *Campylobacter* enteritis is relatively common.[335,475] The introduction of quinolones into poultry feed for animals bred for food has exacerbated the spread of *Campylobacter* resistance in humans.[297,405] In chickens obtained from several different supermarkets in Minnesota in the mid 1990s, nearly 90 percent of samples were culture positive for *Campylobacter*; and approximately 20 percent of these samples were resistant to ciprofloxacin.[405] These circumstances prompted a U.S. Food and Drug Administration ruling withdrawing the use of quinolone in poultry in 2005. It is likely, however, that quinolone-resistant *Campylobacter* has found a stable ecologic niche in both animals and humans and will continue to be encountered despite this intervention.[297] Unlike most other bacteria, *Campylobacter* becomes highly resistant to the quinolones after a single mutation,[128,459] so most quinolone-resistant clinical isolates demonstrate MICs of 32 μg/mL or more, much higher than the conventional cutoff for defining quinolone resistance.

Community-Acquired Respiratory Tract Infections. The applicability of quinolones to respiratory tract infections has been shadowed by the marginal activity of the first modern quinolones, namely, ciprofloxacin, ofloxacin, and norfloxacin, against *S. pneumoniae*. MICs of these antibiotics against most clinical isolates of *S. pneumoniae* average 1 to 2 μg/mL, concentrations that are close to those achievable in bronchial secretions.[337] Several reports of clinical treatment failures were reported in which ciprofloxacin was administered in pneumococcal disease in the respiratory tract.[218,231,329] Moreover, even when *S. pneumoniae* was not the intended target of therapy, the wide use of the early quinolone agents in treating community-acquired infections during the 1990s resulted in the inadvertent exposure of respiratory tract colonizers, including *S. pneumoniae*, to these agents; the result was an even greater, albeit gradual, increase in the MICs of community-acquired *S. pneumoniae* isolates to ciprofloxacin.[48,103,192,378] By the mid 2000s, surveys recorded a prevalence of ciprofloxacin resistance (MIC ≥4 μg/mL) in *S. pneumoniae* of approximately 2.5 percent in the United States,[83,363] 4 percent in Canada,[2] and 15 percent in Italy.[74]

Most of the more recently introduced fluoroquinolones are substantially more potent against *S. pneumoniae* than is ciprofloxacin[220] and express MICs that are easily achieved in the respiratory tract. Usually, susceptibility is maintained, even in isolates resistant to the older quinolone agents.[195,337,364] However, MICs against the newer quinolones roughly correlate with those expressed against ciprofloxacin.[48,192,195,364] Consequently, clinically relevant resistance also has begun to emerge against these newer agents, particularly levofloxacin.[2,74,341,363] To date, susceptibility of other respiratory tract pathogens, including *H. influenzae*, *M. catarrhalis*,[23,181,337] and the bacterial agents causing atypical pneumonias, remains very good to all the fluoroquinolones, although exceptions, particularly in *Haemophilus* spp.,[295,483] have been reported.

Mechanisms of Resistance

The two principal molecular targets of the quinolone antibiotics are the topoisomerases DNA gyrase and topoisomerase IV. These enzymes mediate the three-dimensional topologic configuration of the bacterial duplex DNA ring during cell division and transcription and allow the repair and replication of nucleic acid and the separation of daughter chromosomes in the cramped intracellular environment to occur.[151,164] Several reactions have been identified that are mediated by these two bacterial DNA topoisomerases, including negative supercoiling and catenation.[161,420] Both DNA gyrase and topoisomerase IV are composed of two nonidentical subunits. In DNA gyrase, these subunits are labeled GyrA and GyrB, which are constructed into an A_2B_2 tetramer.[161,420] Quinolones bind to the GyrA subunit of the DNA gyrase-DNA-ATP complex and prevent further progress of the enzyme along the nucleic acid.[161] Bacterial cell death is enhanced by the release of toxic DNA ends from the quinolone-gyrase DNA complex.[151] Topoisomerase IV is composed of two equivalent subunits, labeled ParC and ParE, which share sequence homologies to GyrA and B, respectively.[162]

Quinolone resistance is conferred through several molecular mechanisms. Most organisms resistant to the fluoroquinolones demonstrate one or more mutations in DNA gyrase, topoisomerase IV, or both. Resistance to quinolones among gram-negative bacilli usually is mediated through mutations in DNA gyrase, whereas resistance in gram-positive organisms is the result of alterations of topoisomerase IV.[151,316,493] However, this pattern varies from organism to organism (e.g., the primary target in *S. pneumoniae* appears to be DNA gyrase) and from quinolone to quinolone.[151,162] In gram-negative bacilli, most first-step resistance-conferring mutations occur in *gyrA*, the gene encoding the GyrA subunit. These mutations are clustered in a segment termed the *quinolone-resistance determining region* (QRDR).[336,381,481,482] Hybrid DNA gyrase molecules composed of the A subunit from a resistant bacterial strain and the B subunit from a susceptible isolate confer quinolone resistance.[40] Within the QRDR, most mutations have involved the substitution of a serine for a bulkier, nonpolar amino acid at a single, specific site. This mutation, first identified in *E. coli*,[452,481] has been documented since in a variety of organisms.[69,179,219,284,351] Other amino acid substitutions near this site also have been described, with multiple-site mutations resulting in

increased quinolone resistance compared with a single mutation alone.[452] Mutations of the GyrB subunit, which result in a quinolone-resistant phenotype, also have been identified. In general, mutations affecting the GyrB subunit result in lower levels of resistance than those noted with GyrA.[482] As with GyrA mutations, those affecting GyrB preferentially localize to specific amino acids.[482]

In most quinolone-resistant, gram-positive organisms, mutations of topoisomerase IV occur at locations that are the equivalent to those noted in DNA gyrase. First-step mutations most frequently are noted in *parC*, which, like *gyrA*, contains a hot-spot segment around which most resistance-conferring mutations occur.[161,162,336] *GyrA* mutations in these organisms frequently are clinically silent unless they occur concomitantly with a mutation in *parC*.[161]

Three additional mechanisms of quinolone resistance, which may appear together and in conjunction with a topoisomerase mutation in the same cell, have been identified. Two of these additional mechanisms result in diminished intracellular accumulation of drug. The first mechanism involves decreased entry of antibiotic into the cell secondary to alterations in porin composition, a mutation that generally results in only moderate drug resistance and that affects individual quinolones differentially, depending on their hydrophilicity.[381] Particular attention has centered on alterations in outer member protein F (OmpF).[165,489] This mutation, identified principally in *E. coli*, frequently is associated with resistance to other classes of antibiotics as well, including chloramphenicol, tetracycline, and some of the β-lactams. The diminished expression of OmpF in these resistant isolates does not appear to result from mutations in the corresponding structural gene or its regulatory sequences. Rather, the decreased expression of this outer-membrane protein is mediated through the enhanced expression of the gene *micF*, which transcribes an antisense RNA complementary to the 5′ end of the *ompF* message,[5] thereby destabilizing its binding to the ribosome. Occasional mutants involving additional strains of Enterobacteriaceae have demonstrated other alterations in outer-membrane porin composition.[40]

In the second mechanism, many quinolone-resistant species mediate their resistance through increased drug efflux.[235,236,257] Both bacterial and eukaryotic cells normally encode several efflux pumps that provide protection from a range of potential exogenous toxins.[326] The overexpression of an efflux pump resulting from the mutation of a controlling sequence accounts for reduced susceptibility to quinolones in many clinical isolates.[183,257] Individual efflux pumps select substrates based on their physical chemical properties (e.g., hydrophobicity and net charge), rather than their gross structural similarities; therefore, the various quinolone compounds, which differ from one another regarding these properties, may be differentially susceptible to organisms expressing these pumps.[326]

The gene encoding the quinolone efflux pump in *S. aureus*, labeled *norA*,[480] has been cloned and sequenced. Transformation of susceptible bacteria with the putative gene results in a resistant phenotype, for which resistance can be abolished by the addition of inhibitors of energy-dependent cellular pumps.[480] This pump, however, appears to be able to transport only hydrophilic quinolones; intracellular accumulation of the hydrophobic drug sparfloxacin, for example, is unaffected.

A range of additional genes encoding efflux pumps capable of producing quinolone resistance has been identified in other species, such as *prmA* in *S. pneumoniae*,[336] *acrAB* in *E. coli*,[183,250] and the *mexAmexB-oprM* complex in *P. aeruginosa*.[259] This third complex gene encodes components that transport the drug through both the cell wall and across the periplasmic space, as well as an outer-membrane protein.[326] Most of these pumps are able to extrude a wide variety of exogenous substances, such as detergents, dyes, and other antibiotics, from the bacterial cytoplasm.[183] Therefore, reduced susceptibility to quinolones may be selected by exposures unrelated to the quinolones themselves.

The first decade of the 2000s has witnessed the rapid emergence of transmissible plasmid-mediated quinolone resistance, the third mechanism. All previously defined mechanisms of quinolone resistance were chromosomal. Organisms exhibiting the transmissible mechanism have been identified in Asia, Europe, and North America.[366] The products encoded by these plasmids have been labeled *Qnr proteins*, a group of molecules that bind to topoisomerase and protect it from the inhibitory effects of the quinolones.[302,366] Although all the Qnr molecules described to date belong to the so-called pentapeptide-repeat family of proteins, a finding suggesting similar three-dimensional conformations, distinct families of Qnr proteins with largely nonhomologous amino acid sequences have been characterized.[302,366] These families have been named QnrA, QnrB, and QnrS, with subtypes identified within each. The variety of plasmid and integron structures carrying Qnr proteins and the multiplicity of sequences of the proteins themselves support the notion that Qnr-mediated resistance arose through many independent molecular events in geographically distinct regions of the world. The plasmids encoding the Qnr proteins have been found in multiple species belonging to the Enterobacteriaceae, but to date they have not been identified in nonenteric bacteria such as *Pseudomonas* and *Acinetobacter*.[366] Qnr plasmids frequently carry other antibiotic-resistance genes, most notable those encoding ESBLs and the aminoglycoside-modifying gene *aac(6′)-Ib-cr*, a gene that is able to acetylate selected quinolones as well.[366] The plasmids also may carry resistance determinants against rifampin, tetracycline, sulfonamides, and chloramphenicol.[302]

Quinolone resistance occurs incrementally as the organism acquires a series of topoisomerase mutations, altered outer-membrane proteins, overexpressed efflux pumps, and Qnr proteins.[97] Thus, in gram-negative species, mutations of *gyrA* usually occur in first-step mutants producing relatively small increases in the MIC and frequently result in bacteria that still are susceptible to the antibiotic. Additional mutations to *gyrA*, or added mutations to *parC* or the overexpression of an efflux pump, which may in themselves confer undetectable or only minor resistance to the quinolones, result in substantial quinolone resistance when they operate in

concert.[162,381] Therefore, choosing a fluoroquinolone that can achieve a sustained concentration at the site of infection severalfold higher than the MIC may suppress the emergence of resistance, because this concentration is sufficient to suppress growth of both the parent organism and first-step mutants.[92,381] The propensity of a bacterial strain to emerge resistant to the quinolones can be quantified empirically by measuring the *mutant prevention concentration* (MPC), that is, the lowest concentration of quinolone required to suppress the emergence of resistant subpopulations among a high inoculum (10^{10}) of bacteria.[92,151] The acquisition of each resistance determinant results in an increase in MPC disproportionate to the increase in MIC, a finding indicating that ever-increasing concentrations of drug are required to halt the escalation of resistance as new resistance mechanisms are gained.[151,183]

Chloramphenicol

Clinical Relevance

In industrialized nations, chloramphenicol has been supplanted by other agents with similar or improved antibacterial potency and less toxicity. However, the antibiotic remains a staple for pediatric bacteremia and meningitis in the developing world because of its broad antibacterial activity, low cost, favorable achievable serum concentrations after oral or intramuscular administration, and excellent penetration across the blood-brain barrier. The incidence of resistance to chloramphenicol among the principal organisms causing bacteremia and meningitis in children, namely, *H. influenzae*, *S. pneumoniae*, *N. meningitidis*, and *Salmonella*, varies greatly by geographic location. Between the late 1980s and the early 2000s, surveys of *H. influenzae*, both type b and non–type b, were completed in several areas of the world. In vitro resistance to chloramphenicol ranged between 0 percent of isolates (from the Central African Republic and various locations in South America) and more than 50 percent (in India and Kenya).* Studies in 2001 and 2003 of *H. influenzae* isolates in Canada and the United States, where infection by these organisms is almost always treated with an alternative agent, indicated chloramphenicol resistance in fewer than 0.5 percent of tested bacteria.[158,490]

International surveys completed in the mid to late 1990s that measured in vitro susceptibility of *S. pneumoniae* to chloramphenicol indicated resistance exceeding 10 to 20 percent in many areas of the world.[182,232,310,369] Chloramphenicol resistance was recorded in approximately 10 percent of European isolates of *S. pneumoniae* and in 5 percent of isolates identified in the United States and Canada.[158] Resistance to chloramphenicol is seen more commonly and is of a higher grade in penicillin-resistant pneumococci than in isolates that are penicillin susceptible.[158] Even in penicillin-intermediate or penicillin-resistant strains that retain in vitro susceptibility to chloramphenicol, the clinical response to chloramphenicol in meningitis is poor. Some investigators have suggested that such bacteria express high minimal bactericidal concentrations to chloramphenicol; as a result, concentrations of drug achieved in the cerebrospinal fluid with routine dosing are lower than those required for good outcomes in central nervous system infections.[113]

Chloramphenicol retains excellent activity against most strains of *N. meningitidis*. However, investigators described meningococcal strains from Vietnam, Paris, and Australia that possessed high-level resistance to chloramphenicol.[119,400] Chloramphenicol resistance in *Salmonella* spp., a fourth group of organisms that causes disseminated disease and meningitis in pediatric patients, varies geographically. Resistance, with a prevalence frequently exceeding 50 percent, usually is detected in developing countries,[202] where chloramphenicol has been a readily available oral agent for typhoidal and nontyphoidal *Salmonella* infections for decades. In most *Salmonella* isolates, chloramphenicol resistance occurs coincident with resistance to multiple other antibiotics, particularly ampicillin, sulfonamides, tetracycline, and some of the older aminoglycosides. Chloramphenicol resistance has become increasingly common in industrialized nations as well.[60,408,462] In the United States, the strain *Salmonella typhimurium* DT104, which similarly coexpresses resistance to several classes of antibiotics including chloramphenicol, has become a widespread health hazard.[1,453]

Mechanisms of Resistance

The antibacterial activity of chloramphenicol is conferred by its interference with the peptidyl transferase region of the 50S prokaryotic ribosome. The principal mechanism of resistance is through the expression of the modifying enzyme chloramphenicol acetyltransferase (CAT), which catalyzes the acetylation of the C3-hydroxy group of the drug and thereby prevents its binding to the bacterial ribosome.[397] CATs actually represent a family of enzymes. Many CATs share chemical properties and structural homologies, especially around the active site.[397] CATs frequently reside on plasmids or transposons that carry other antibiotic-resistance determinants.[455] In *Salmonella* in particular, the chloramphenicol-resistance gene frequently is encoded on a class 1 integron incorporated in the so-called *Salmonella* Genomic Island 1 (SGI1), which includes multiple antibiotic-resistance cassettes.[88,288] SGI1 is transmissible and has been identified in many different *Salmonella* serovars.

Alternative mechanisms of resistance to chloramphenicol also have been identified in certain gram-negative species. In most of these organisms, resistance is linked to decreased intracellular accumulation of drug. In chloramphenicol-resistant *Pseudomonas*, this phenomenon has been associated with the overproduction of an approximately 50-kd outer-membrane protein,[236] which in the past led to the speculation that entry of drug into the bacterium was diminished by an abnormal porin. More recently, however, cell membrane proteins of this size have been identified as components of complex efflux apparatus that include both the pump itself and proteins that channel the extruded substance across the periplasmic space and through the outer membrane.[326] More

*References 46, 125, 191, 212, 310, 369, 377, 391, 402, 463.

direct evidence of such multidrug-resistant efflux pumps have been identified in chloramphenicol-resistant *E. coli*,[149] *Pseudomonas*,[236] *Stenotrophomonas*,[491] and *Salmonella*.[25,49,338] Usually, these pumps result in coresistance to multiple other antimicrobial agents, including tetracycline, sulfonamide, aminoglycosides, quinolones, and selected detergents.

NEW REFERENCES SINCE THE SIXTH EDITION

7. Arias CA, Panesso D, McGrath DM, et al. Genetic basis for in vivo daptomycin resistance in enterococci. *N Engl J Med* 2011; **365**:892–900.
24. Bogdanovich T, Adams-Haduch JM, Tian GB, et al. Colistin-resistant, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* belonging to the international epidemic clone ST258. *Clin Infect Dis* 2011;**53**:373–6.
38. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010;**54**:969–76.
41. Canton R, Akova M, Carmeli Y, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012;**18**:413–31.
45. Cattoir V, Leclercq R. Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? *J Antimicrob Chemother* 2012 Dec 2. [Epub ahead of print].
52. Chitnis AS, Caruthers PS, Rao AK, et al. Outbreak of carbapenem-resistant Enterobacteriaceae at a long-term acute care hospital: sustained reductions in transmission through active surveillance and targeted interventions. *Infect Control Hosp Epidemiol* 2012;**33**: 984–92.
99. Farrell DJ, Castanheira M, Mendes RE, et al. In vitro activity of ceftaroline against multidrug-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae:* a review of published studies and the AWARE Surveillance Program (2008-2010). *Clin Infect Dis* 2012;**55**(Suppl. 3):S206–14.
100. Farrell DJ, Flamm RK, Jones RN, et al. Spectrum and potency of ceftaroline tested against leading pathogens causing community-acquired respiratory tract infections in Europe (2010). *Diagn Microbiol Infect Dis* 2013;**75**:86–8.
106. Flamm RK, Sader HS, Farrell DJ, et al. Ceftaroline potency among 9 US Census regions: report from the 2010 AWARE Program. *Clin Infect Dis* 2012;**55**(Suppl. 3):S194–205.
117. Gales AC, Castanheira M, Jones RN, et al. Antimicrobial resistance among gram-negative bacilli isolated from Latin America: results from SENTRY Antimicrobial Surveillance Program (Latin America, 2008-2010). *Diagn Microbiol Infect Dis* 2012;**73**:354–60.
120. Gasink LB, Edelstein PH, Lautenbach E, et al. Risk factors and clinical impact of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. *Infect Control Hosp Epidemiol* 2009;**30**:1180–5.
133. Gopegui ER, Juan C, Zamorano L, et al. Transferable multidrug resistance plasmid carrying cfr associated with tet(L), ant(4′)-Ia, and dfrK genes from a clinical methicillin-resistant *Staphylococcus aureus* ST125 strain. *Antimicrob Agents Chemother* 2012;**56**: 2139–42.
138. Gu B, Kelesidis T, Tsiodras S, et al. The emerging problem of linezolid-resistant *Staphylococcus*. *J Antimicrob Chemother* 2013;**68**: 4–11.
142. Gupta N, Limbago BM, Patel JB, et al. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011;**53**:60–7.
159. Hoenigl M, Valentin T, Zarfel G, et al. Nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella oxytoca* in Austria. *Antimicrob Agents Chemother* 2012;**56**:2158–61.
172. Hulten KG, Kaplan SL, Lamberth LB, et al. Hospital-acquired *Staphylococcus aureus* infections at Texas Children's Hospital, 2001-2007. *Infect Control Hosp Epidemiol* 2010;**31**:183–90.
178. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother* 2009;**53**:4961–7.
188. Jernigan MG, Press EG, Nguyen MH, et al. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2012;**56**:3395–8.
209. Kelesidis T, Humphries R, Uslan DZ, et al. Daptomycin nonsusceptible enterococci: an emerging challenge for clinicians. *Clin Infect Dis* 2011;**52**:228–34.
214. Kitchel B, Rasheed JK, Patel JB, et al. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 2009;**53**:3365–70.
223. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;**10**:597–602.
227. Landman D, Babu E, Shah N, et al. Transmission of carbapenem-resistant pathogens in New York City hospitals: progress and frustration. *J Antimicrob Chemother* 2012;**67**:1427–31.
230. Leavitt A, Carmeli Y, Chmelnitsky I, et al. Molecular epidemiology, sequence types, and plasmid analyses of KPC-producing *Klebsiella pneumoniae* strains in Israel. *Antimicrob Agents Chemother* 2010;**54**:3002–6.
242. Logan LK. Carbapenem-resistant Enterobacteriaceae: an emerging problem in children. *Clin Infect Dis* 2012;**55**:852–9.
243. Long KS, Vester B. Resistance to linezolid caused by modifications at its binding site on the ribosome. *Antimicrob Agents Chemother* 2012;**56**:603–12.
261. Mathers AJ, Cox HL, Kitchel B, et al. Molecular dissection of an outbreak of carbapenem-resistant Enterobacteriaceae reveals Intergenus KPC carbapenemase transmission through a promiscuous plasmid. *MBio* 2011;**2**:e00204–11.
271. McDougal LK, Fosheim GE, Nicholson A, et al. Emergence of resistance among USA300 methicillin-resistant *Staphylococcus aureus* isolates causing invasive disease in the United States. *Antimicrob Agents Chemother* 2010;**54**:3804–11.
278. Mendes RE, Tsakris A, Sader HS, et al. Characterization of methicillin-resistant *Staphylococcus aureus* displaying increased MICs of ceftaroline. *J Antimicrob Chemother* 2012;**67**:1321–4.
282. Mishra NN, Bayer AS, Tran TT, et al. Daptomycin resistance in enterococci is associated with distinct alterations of cell membrane phospholipid content. *PLoS One* 2012;**7**:e43958.
290. Munoz-Price LS, De La Cuesta C, Adams S, et al. Successful eradication of a monoclonal strain of *Klebsiella pneumoniae* during a *K. pneumoniae* carbapenemase-producing *K. pneumoniae* outbreak in a surgical intensive care unit in Miami, Florida. *Infect Control Hosp Epidemiol* 2010;**31**:1074–7.
300. Nimmo GR. USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2012;**18**:725–34.
303. Ntokou E, Stathopoulos C, Kristo I, et al. Intensive care unit dissemination of multiple clones of linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium*. *J Antimicrob Chemother* 2012; **67**:1819–23.
317. Papadimitriou-Olivgeris M, Marangos M, Fligou F, et al. Risk factors for KPC-producing *Klebsiella pneumoniae* enteric colonization upon ICU admission. *J Antimicrob Chemother* 2012;**67**: 2976–81.
318. Papp-Wallace KM, Bethel CR, Distler AM, et al. Inhibitor resistance in the KPC-2 beta-lactamase, a preeminent property of this class A beta-lactamase. *Antimicrob Agents Chemother* 2010;**54**: 890–7.
319. Papp-Wallace KM, Taracila MA, Smith KM, et al. Understanding the molecular determinants of substrate and inhibitor specificities in the carbapenemase KPC-2: exploring the roles of Arg220 and Glu276. *Antimicrob Agents Chemother* 2012;**56**:4428–38.
328. Perez F, Endimiani A, Ray AJ, et al. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. *J Antimicrob Chemother* 2010;**65**:1807–18.
344. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis* 2008;**46**: 787–94.
345. Poulou A, Voulgari E, Vrioni G, et al. Imported *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* clones in a Greek hospital: impact of infection control measures for restraining their dissemination. *J Clin Microbiol* 2012;**50**:2618–23.

347. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007;**20**:440–58.
349. Qureshi ZA, Paterson DL, Potoski BA, et al. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae:* superiority of combination antimicrobial regimens. *Antimicrob Agents Chemother* 2012;**56**:2108–13.
370. Rubio A, Moore J, Varoglu M, et al. LC-MS/MS characterization of phospholipid content in daptomycin-susceptible and -resistant isolates of *Staphylococcus aureus* with mutations in mprF. *Mol Membr Biol* 2012;**29**:1–8.
373. Sader HS, Flamm RK, Farrell DJ, et al. Activity analyses of staphylococcal isolates from pediatric, adult, and elderly patients: AWARE Ceftaroline Surveillance Program. *Clin Infect Dis* 2012; **55**(Suppl. 3):S181–6.
382. Saravolatz LD, Stein GE, Johnson LB. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2011;**52**:1156–63.
410. Souli M, Galani I, Antoniadou A, et al. An outbreak of infection due to beta-lactamase *Klebsiella pneumoniae* carbapenemase 2–producing *K. pneumoniae* in a Greek university hospital: molecular characterization, epidemiology, and outcomes. *Clin Infect Dis* 2010;**50**:364–73.
431. Thurlow LR, Joshi GS, Richardson AR. Virulence strategies of the dominant USA300 lineage of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *FEMS Immunol Med Microbiol* 2012;**65**:5–22.
437. Tran TT, Panesso D, Gao H, et al. Whole-genome analysis of a daptomycin-susceptible *Enterococcus faecium* and its daptomycin-resistant variant arising during therapy. *Antimicrob Agents Chemother* 2013;**57**(1):261–8.
439. Tsai HY, Liao CH, Chen YH, et al. Trends in susceptibility of vancomycin-resistant *Enterococcus faecium* to tigecycline, daptomycin, and linezolid and molecular epidemiology of the isolates: results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) study, 2006 to 2010. *Antimicrob Agents Chemother* 2012; **56**:3402–5.
441. Tumbarello M, Viale P, Viscoli C, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase–producing *K. pneumoniae:* importance of combination therapy. *Clin Infect Dis* 2012;**55**:943–50.
448. van Hal SJ, Paterson DL, Gosbell IB. Emergence of daptomycin resistance following vancomycin-unresponsive *Staphylococcus aureus* bacteraemia in a daptomycin-naive patient–a review of the literature. *Eur J Clin Microbiol Infect Dis* 2011;**30**:603–10.
451. Viau RA, Hujer AM, Marshall SH, et al. "Silent" dissemination of *Klebsiella pneumoniae* isolates bearing *K. pneumoniae* carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. *Clin Infect Dis* 2012;**54**:1314–21.
473. Won SY, Munoz-Price LS, Lolans K, et al. Emergence and rapid regional spread of *Klebsiella pneumoniae* carbapenemase-producing Enterobacteriaceae. *Clin Infect Dis* 2011;**53**:532–40.
484. Zarkotou O, Pournaras S, Tselioti P, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clin Microbiol Infect* 2011;**17**:1798–803.
492. Zhao C, Sun H, Wang H, et al. Antimicrobial resistance trends among 5608 clinical gram-positive isolates in China: results from the Gram-Positive Cocci Resistance Surveillance program (2005-2010). *Diagn Microbiol Infect Dis* 2012;**73**:174–81.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Ackers ML, Puhr ND, Tauxe RV, et al. Laboratory-based surveillance of *Salmonella* serotype Typhi infections in the United States: antimicrobial resistance on the rise. *JAMA* 2000;**283**:2668–73.
2. Adam HJ, Schurek KN, Nichol KA, et al. Molecular characterization of increasing fluoroquinolone resistance in *Streptococcus pneumoniae* isolates in Canada, 1997 to 2005. *Antimicrob Agents Chemother* 2007;**51**:198–207.
3. Aires de Sousa M, de Lencastre H. Bridges from hospitals to the laboratory: genetic portraits of methicillin-resistant *Staphylococcus aureus* clones. *FEMS Immunol Med Microbiol* 2004;**40**:101–11.
4. Ambler RP. The structure of beta-lactamases. *Philos Trans R Soc Lond B Biol Sci* 1980;**289**:321–31.
5. Andersen J, Delihas N. micF RNA binds to the 5′ end of ompF mRNA and to a protein from *Escherichia coli*. *Biochemistry* 1990; **29**:9249–56.
6. Araque M, Velazco E. In vitro activity of fleroxacin against multiresistant gram-negative bacilli isolated from patients with nosocomial infections. *Intensive Care Med* 1998;**24**:839–44.
7. Arias CA, Panesso D, McGrath DM, et al. Genetic basis for in vivo daptomycin resistance in enterococci. *N Engl J Med* 2011; **365**:892–900.
8. Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob Agents Chemother* 1993;**37**: 1563–71.
9. Arthur M, Depardieu F, Molinas C, et al. The *vanZ* gene of Tn1546 from *Enterococcus faecium* BM4147 confers resistance to teicoplanin. *Gene* 1995;**154**:87–92.
10. Arthur M, Depardieu F, Snaith HA, et al. Contribution of VanY D,D-carboxypeptidase to glycopeptide resistance in *Enterococcus faecalis* by hydrolysis of peptidoglycan precursors. *Antimicrob Agents Chemother* 1994;**38**:1899–903.
11. Ashkenazi S, May-Zahav M, Sulkes J, et al. Increasing antimicrobial resistance of *Shigella* isolates in Israel during the period 1984 to 1992. *Antimicrob Agents Chemother* 1995;**39**:819–23.
12. Atta MG, Eustace JA, Song X, Perl TM, Scheel PJ Jr. Outpatient vancomycin use and vancomycin-resistant enterococcal colonization in maintenance dialysis patients. *Kidney Int* 2001;**59**:718–24.
13. Austin DJ, Bonten MJ, Weinstein RA, et al. Vancomycin-resistant enterococci in intensive-care hospital settings: transmission dynamics, persistence, and the impact of infection control programs. *Proc Natl Acad Sci U S A* 1999;**96**:6908–13.
14. Babini GS, Livermore DM. Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and Western Europe in 1997-1998. *J Antimicrob Chemother* 2000;**45**: 183–9.
15. Barcus VA, Ghanekar K, Yeo M, et al. Genetics of high level penicillin resistance in clinical isolates of *Streptococcus pneumoniae*. *FEMS Microbiol Lett* 1995;**126**:299–303.
16. Barkai G, Greenberg D, Givon-Lavi N, et al. Community prescribing and resistant *Streptococcus pneumoniae*. *Emerg Infect Dis* 2005;**11**:829–37.
17. Barna JC, Williams DH. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. *Annu Rev Microbiol* 1984;**38**:339–57.
18. Barricarte A, Castilla J, Gil-Setas A, et al. Effectiveness of the 7-valent pneumococcal conjugate vaccine: a population-based case-control study. *Clin Infect Dis* 2007;**44**:1436–41.
19. Barsic B, Tambic A, Santini M, et al. Antibiotic resistance among nosocomial isolates in a Croatian intensive care unit—results of a twelve-year focal surveillance of nosocomial infections. *J Chemother* 2004;**16**:273–81.
20. Bennish ML, Salam MA, Hossain MA, et al. Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983-1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. *Clin Infect Dis* 1992;**14**: 1055–60.
21. Bergman M, Huikko S, Huovinen P, et al. Macrolide and azithromycin use are linked to increased macrolide resistance in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2006;**50**:3646–50.
22. Bingen E, Bidet P, Mihaila-Amrouche L, et al. Emergence of macrolide-resistant *Streptococcus pyogenes* strains in French children. *Antimicrob Agents Chemother* 2004;**48**:3559–62.
23. Blondeau JM, Vaughan D, Laskowski R, et al. Susceptibility of Canadian isolates of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae* to oral antimicrobial agents. *Int J Antimicrob Agents* 2001;**17**:457–64.
24. Bogdanovich T, Adams-Haduch JM, Tian GB, et al. Colistin-resistant, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* belonging to the international epidemic clone ST258. *Clin Infect Dis* 2011;**53**:373–6.
25. Bolton LF, Kelley LC, Lee MD, et al. Detection of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. *J Clin Microbiol* 1999;**37**:1348–51.
26. Bonten MJ, Slaughter S, Ambergen AW, et al. The role of "colonization pressure" in the spread of vancomycin-resistant enterococci: an important infection control variable. *Arch Intern Med* 1998;**158**:1127–32.
27. Bourgeois-Nicolaos N, Massias L, Couson B, et al. Dose dependence of emergence of resistance to linezolid in *Enterococcus faecalis* in vivo. *J Infect Dis* 2007;**195**:1480–8.
28. Bradford PA, Bratu S, Urban C, et al. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Clin Infect Dis* 2004;**39**:55–60.
29. Bratoeva MP, John JF, Barg NL. Molecular epidemiology of trimethoprim-resistant *Shigella boydii* serotype 2 strains from Bulgaria. *J Clin Microbiol* 1992;**30**:1428–31.
30. Bratu S, Brooks S, Burney S, et al. Detection and spread of *Escherichia coli* possessing the plasmid-borne carbapenemase KPC-2 in Brooklyn, New York. *Clin Infect Dis* 2007;**44**:972–5.
31. Braun L, Craft D, Williams R, et al. Increasing clindamycin resistance among methicillin-resistant *Staphylococcus aureus* in 57 northeast United States military treatment facilities. *Pediatr Infect Dis J* 2005;**24**:622–6.
32. Brisse S, Milatovic D, Fluit AC, et al. Epidemiology of quinolone resistance of *Klebsiella pneumoniae* and *Klebsiella oxytoca* in Europe. *Eur J Clin Microbiol Infect Dis* 2000;**19**:64–8.
33. Brubaker RR. Mechanisms of bacterial virulence. *Annu Rev Microbiol* 1985;**39**:21–50.
34. Bugg TD, Dutka-Malen S, Arthur M, et al. Identification of vancomycin resistance protein VanA as a D-alanine:D-alanine ligase of altered substrate specificity. *Biochemistry* 1991;**30**: 2017–21.
35. Bugg TD, Wright GD, Dutka-Malen S, et al. Molecular basis for vancomycin resistance in Enterococcus faecium BM4147: biosynthesis of a depsipeptide peptidoglycan precursor by vancomycin resistance proteins VanH and VanA. *Biochemistry* 1991;**30**: 10408–15.
36. Burnie J, Matthews R, Jiman-Fatami A, et al. Analysis of 42 cases of septicemia caused by an epidemic strain of methicillin-resistant *Staphylococcus aureus:* evidence of resistance to vancomycin. *Clin Infect Dis* 2000;**31**:684–9.
37. Bush K. Metallo-beta-lactamases: a class apart. *Clin Infect Dis* 1998;**27**(Suppl. 1):S48–53.
38. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrob Agents Chemother* 2010;**54**:969–76.
39. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995;**39**:1211–33.
40. Cambau E, Gutmann L. Mechanisms of resistance to quinolones. *Drugs* 1993;**45**:15–23.
41. Canton R, Akova M, Carmeli Y, et al. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin Microbiol Infect* 2012;**18**:413–31.
42. Carmeli Y, Samore MH, Huskins C. The association between antecedent vancomycin treatment and hospital-acquired vancomycin-resistant enterococci: a meta-analysis. *Arch Intern Med* 1999;**159**:2461–8.
43. Carmeli Y, Troillet N, Eliopoulos GM, et al. Emergence of antibiotic-resistant *Pseudomonas aeruginosa:* comparison of risks associated with different antipseudomonal agents. *Antimicrob Agents Chemother* 1999;**43**:1379–82.
44. Casalino M, Nicoletti M, Salvia A, et al. Characterization of endemic *Shigella flexneri* strains in Somalia: antimicrobial resistance, plasmid profiles, and serotype correlation. *J Clin Microbiol* 1994;**32**:1179–83.
45. Cattoir V, Leclercq R. Twenty-five years of shared life with vancomycin-resistant enterococci: is it time to divorce? *J Antimicrob Chemother* 2012. [Epub ahead of print].

46. Cerquetti M, Cardines R, Giufre M, et al. Antimicrobial susceptibility of *Haemophilus influenzae* strains isolated from invasive disease in Italy. *J Antimicrob Chemother* 2004;**54**:1139–43.
47. Chaibi EB, Sirot D, Paul G, et al. Inhibitor-resistant TEM beta-lactamases: phenotypic, genetic and biochemical characteristics. *J Antimicrob Chemother* 1999;**43**:447–58.
48. Chen DK, McGeer A, de Azavedo JC, et al. Decreased susceptibility of *Streptococcus pneumoniae* to fluoroquinolones in Canada. Canadian Bacterial Surveillance Network. *N Engl J Med* 1999; **341**:233–9.
49. Chen S, Cui S, McDermott PF, et al. Contribution of target gene mutations and efflux to decreased susceptibility of *Salmonella enterica* serovar *typhimurium* to fluoroquinolones and other antimicrobials. *Antimicrob Agents Chemother* 2007;**51**:535–42.
50. Chevalier J, Pages JM, Eyraud A, et al. Membrane permeability modifications are involved in antibiotic resistance in *Klebsiella pneumoniae*. *Biochem Biophys Res Commun* 2000;**274**:496–9.
51. Chiba N, Kobayashi R, Hasegawa K, et al. Antibiotic susceptibility according to genotype of penicillin-binding protein and macrolide resistance genes, and serotype of *Streptococcus pneumoniae* isolates from community-acquired pneumonia in children. *J Antimicrob Chemother* 2005;**56**:756–60.
52. Chitnis AS, Caruthers PS, Rao AK, et al. Outbreak of carbapenem-resistant Enterobacteriaceae at a long-term acute care hospital: sustained reductions in transmission through active surveillance and targeted interventions. *Infect Control Hosp Epidemiol* 2012; **33**:984–92.
53. Chittum HS, Champney WS. Ribosomal protein gene sequence changes in erythromycin-resistant mutants of *Escherichia coli*. *J Bacteriol* 1994;**176**:6192–8.
54. Chow JW. Aminoglycoside resistance in enterococci. *Clin Infect Dis* 2000;**31**:586–9.
55. Chow JW, Fine MJ, Shlaes DM, et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy [see comments]. *Ann Intern Med* 1991;**115**:585–90.
56. Cizman M, Pokorn M, Seme K, et al. The relationship between trends in macrolide use and resistance to macrolides of common respiratory pathogens. *J Antimicrob Chemother* 2001;**47**:475–7.
57. Coffey TJ, Daniels M, McDougal LK, et al. Genetic analysis of clinical isolates of *Streptococcus pneumoniae* with high-level resistance to expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* 1995;**39**:1306–13.
58. Colodner R, Rock W, Chazan B, et al. Risk factors for the development of extended-spectrum beta-lactamase-producing bacteria in nonhospitalized patients. *Eur J Clin Microbiol Infect Dis* 2004;**23**:163–7.
59. Cook LN, Davis RS, Stover BH. Outbreak of amikacin-resistant Enterobacteriaceae in an intensive care nursery. *Pediatrics* 1980; **65**:264–8.
60. Cooke FJ, Day M, Wain J, et al. Cases of typhoid fever imported into England, Scotland and Wales (2000-2003). *Trans R Soc Trop Med Hyg* 2007;**101**:398–404.
61. Corbella X, Montero A, Pujol M, et al. Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant *Acinetobacter baumannii*. *J Clin Microbiol* 2000;**38**:4086–95.
62. Courvalin P. Vancomycin resistance in gram-positive cocci. *Clin Infect Dis* 2006;**42**(Suppl. 1):S25–34.
63. Craig WA. Basic pharmacodynamics of antibacterials with clinical applications to the use of beta-lactams, glycopeptides, and linezolid. *Infect Dis Clin North Am* 2003;**17**:479–501.
64. Cui L, Tominaga E, Neoh HM, et al. Correlation between reduced daptomycin susceptibility and vancomycin resistance in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006;**50**:1079–82.
65. D'Agata EM, Webb G, Horn M. A mathematical model quantifying the impact of antibiotic exposure and other interventions on the endemic prevalence of vancomycin-resistant enterococci. *J Infect Dis* 2005;**192**:2004–11.
66. Dale GE, Broger C, D'Arcy A, et al. A single amino acid substitution in *Staphylococcus aureus* dihydrofolate reductase determines trimethoprim resistance. *J Mol Biol* 1997;**266**:23–30.
67. de Jonge BL, Tomasz A. Abnormal peptidoglycan produced in a methicillin-resistant strain of *Staphylococcus aureus* grown in the presence of methicillin: functional role for penicillin-binding protein 2A in cell wall synthesis. *Antimicrob Agents Chemother* 1993;**37**:342–6.
68. de Lencastre H, de Jonge BL, Matthews PR, et al. Molecular aspects of methicillin resistance in *Staphylococcus aureus*. *J Antimicrob Chemother* 1994;**33**:7–24.
69. Deguchi T, Yasuda M, Asano M, et al. DNA gyrase mutations in quinolone-resistant clinical isolates of *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother* 1995;**39**:561–3.
70. Denis O, Deplano A, Nonhoff C, et al. National surveillance of methicillin-resistant *Staphylococcus aureus* in Belgian hospitals indicates rapid diversification of epidemic clones. *Antimicrob Agents Chemother* 2004;**48**:3625–9.
71. Depardieu F, Courvalin P. Mutation in 23S rRNA responsible for resistance to 16-membered macrolides and streptogramins in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2001;**45**: 319–23.
72. Deresinski S. Methicillin-resistant *Staphylococcus aureus:* an evolutionary, epidemiologic, and therapeutic odyssey. *Clin Infect Dis* 2005;**40**:562–73.
73. Deshpande LM, Fritsche TR, Moet GJ, et al. Antimicrobial resistance and molecular epidemiology of vancomycin-resistant enterococci from North America and Europe: a report from the SENTRY antimicrobial surveillance program. *Diagn Microbiol Infect Dis* 2007;**58**:163–70.
74. Deshpande LM, Sader HS, Debbia E, et al. Emergence and epidemiology of fluoroquinolone-resistant *Streptococcus pneumoniae* strains from Italy: report from the SENTRY Antimicrobial Surveillance Program (2001-2004). *Diagn Microbiol Infect Dis* 2006; **54**:157–64.
75. Dias R, Louro D, Canica M. Antimicrobial susceptibility of invasive *Streptococcus pneumoniae* isolates in Portugal over an 11-year period. *Antimicrob Agents Chemother* 2006;**50**:2098–105.
76. DiazGranados CA, Zimmer SM, Klein M, et al. Comparison of mortality associated with vancomycin-resistant and vancomycin-susceptible enterococcal bloodstream infections: a meta-analysis. *Clin Infect Dis* 2005;**41**:327–33.
77. Diekema DJ, BootsMiller BJ, Vaughn TE, et al. Antimicrobial resistance trends and outbreak frequency in United States hospitals. *Clin Infect Dis* 2004;**38**:78–85.
78. Diekema DJ, Pfaller MA, Schmitz FJ, et al. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 2001;**32**(Suppl. 2):S114–32.
79. Diep BA, Carleton HA, Chang RF, et al. Roles of 34 virulence genes in the evolution of hospital- and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *J Infect Dis* 2006;**193**:1495–503.
80. Diver JM, Schollaardt T, Rabin HR, et al. Persistence mechanisms in *Pseudomonas aeruginosa* from cystic fibrosis patients undergoing ciprofloxacin therapy. *Antimicrob Agents Chemother* 1991;**35**: 1538–46.
81. Doern GV, Heilmann KP, Huynh HK, et al. Antimicrobial resistance among clinical isolates of *Streptococcus pneumoniae* in the United States during 1999-2000, including a comparison of resistance rates since 1994-1995. *Antimicrob Agents Chemother* 2001; **45**:1721–9.
82. Doern GV, Pfaller MA, Kugler K, et al. Prevalence of antimicrobial resistance among respiratory tract isolates of *Streptococcus pneumoniae* in North America: 1997 results from the SENTRY antimicrobial surveillance program. *Clin Infect Dis* 1998;**27**: 764–70.
83. Doern GV, Richter SS, Miller A, et al. Antimicrobial resistance among *Streptococcus pneumoniae* in the United States: have we begun to turn the corner on resistance to certain antimicrobial classes? *Clin Infect Dis* 2005;**41**:139–48.
84. Doktor SZ, Shortridge V. Differences in the DNA sequences in the upstream attenuator region of erm(A) in clinical isolates of *Streptococcus pyogenes* and their correlation with macrolide/lincosamide resistance. *Antimicrob Agents Chemother* 2005;**49**: 3070–2.
85. Donskey CJ, Chowdhry TK, Hecker MT, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. *N Engl J Med* 2000;**343**:1925–32.

86. Donskey CJ, Schreiber JR, Jacobs MR, et al. A polyclonal outbreak of predominantly VanB vancomycin-resistant enterococci in northeast Ohio. Northeast Ohio Vancomycin-Resistant Enterococcus Surveillance Program. *Clin Infect Dis* 1999;**29**:573–9.
87. Dostal RE, Seale JP, Yan BJ. Resistance to ciprofloxacin of respiratory pathogens in patients with cystic fibrosis. *Med J Aust* 1992; **156**:20–4.
88. Doublet B, Weill FX, Fabre L, et al. Variant *Salmonella* genomic island 1 antibiotic resistance gene cluster containing a novel 3′-*N*-aminoglycoside acetyltransferase gene cassette, aac(3)-Id, in *Salmonella enterica* serovar Newport. *Antimicrob Agents Chemother* 2004;**48**:3806–12.
89. Dowson CG, Hutchison A, Brannigan JA, et al. Horizontal transfer of penicillin-binding protein genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. *Proc Natl Acad Sci U S A* 1989;**86**:8842–6.
90. Dowson CG, Hutchison A, Spratt BG. Extensive re-modelling of the transpeptidase domain of penicillin-binding protein 2B of a penicillin-resistant South African isolate of *Streptococcus pneumoniae*. *Mol Microbiol* 1989;**3**:95–102.
91. Drago L, De Vecchi E, Mombelli B, et al. Activity of levofloxacin and ciprofloxacin against urinary pathogens. *J Antimicrob Chemother* 2001;**48**:37–45.
92. Drusano GL, Louie A, Deziel M, et al. The crisis of resistance: identifying drug exposures to suppress amplification of resistant mutant subpopulations. *Clin Infect Dis* 2006;**42**:525–32.
93. Du Bois SK, Marriott MS, Amyes SG. TEM- and SHV-derived extended-spectrum beta-lactamases: relationship between selection, structure and function. *J Antimicrob Chemother* 1995;**35**: 7–22.
94. Dubberke ER, Hollands JM, Georgantopoulos P, et al. Vancomycin-resistant enterococcal bloodstream infections on a hematopoietic stem cell transplant unit: are the sick getting sicker? *Bone Marrow Transplant* 2006;**38**:813–19.
95. Edmond MB, Ober JF, Weinbaum DL, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. *Clin Infect Dis* 1995;**20**:1126–33.
96. Eliopoulos GM, Wennersten C, Zighelboim-Daum S, et al. High-level resistance to gentamicin in clinical isolates of *Streptococcus (Enterococcus) faecium*. *Antimicrob Agents Chemother* 1988; **32**:1528–32.
97. Everett MJ, Jin YF, Ricci V, et al. Contributions of individual mechanisms to fluoroquinolone resistance in 36 *Escherichia coli* strains isolated from humans and animals. *Antimicrob Agents Chemother* 1996;**40**:2380–6.
98. Facinelli B, Spinaci C, Magi G, et al. Association between erythromycin resistance and ability to enter human respiratory cells in group A streptococci. *Lancet* 2001;**358**:30–3.
99. Farrell DJ, Castanheira M, Mendes RE, et al. In vitro activity of ceftaroline against multidrug-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae:* a review of published studies and the AWARE Surveillance Program (2008-2010). *Clin Infect Dis* 2012; **55**(Suppl. 3):S206–14.
100. Farrell DJ, Flamm RK, Jones RN, et al. Spectrum and potency of ceftaroline tested against leading pathogens causing community-acquired respiratory tract infections in Europe (2010). *Diagn Microbiol Infect Dis* 2013;**75**:86–8.
101. Farrell DJ, Klugman KP, Pichichero M. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *Pediatr Infect Dis J* 2007;**26**:123–8.
102. Feikin DR, Dowell SF, Nwanyanwu OC, et al. Increased carriage of trimethoprim/sulfamethoxazole-resistant *Streptococcus pneumoniae* in Malawian children after treatment for malaria with sulfadoxine/pyrimethamine. *J Infect Dis* 2000;**181**:1501–5.
103. Felmingham D, Robbins MJ, Tesfaslasie Y, et al. Antimicrobial susceptibility of community-acquired lower respiratory tract bacterial pathogens isolated in the UK during the 1995-1996 cold season. *J Antimicrob Chemother* 1998;**41**:411–15.
104. Fihn SD. Clinical practice: acute uncomplicated urinary tract infection in women. *N Engl J Med* 2003;**349**:259–66.
105. Finelli L, Miller JT, Tokars JI, et al. National surveillance of dialysis-associated diseases in the United States, 2002. *Semin Dial* 2005;**18**:52–61.
106. Flamm RK, Sader HS, Farrell DJ, et al. Ceftaroline potency among 9 US Census regions: report from the 2010 AWARE Program. *Clin Infect Dis* 2012;**55**(Suppl. 3):S194–205.
107. Fluit AC, Jones ME, Schmitz FJ, et al. Antimicrobial susceptibility and frequency of occurrence of clinical blood isolates in Europe from the SENTRY antimicrobial surveillance program, 1997 and 1998. *Clin Infect Dis* 2000;**30**:454–60.
108. Fluit AC, Schmitz FJ. Class 1 integrons, gene cassettes, mobility, and epidemiology. *Eur J Clin Microbiol Infect Dis* 1999;**18**: 761–70.
109. Fluit AC, Verhoef J, Schmitz FJ. Antimicrobial resistance in European isolates of *Pseudomonas aeruginosa*. European SENTRY Participants. *Eur J Clin Microbiol Infect Dis* 2000;**19**:370–4.
110. Fridkin SK. Vancomycin-intermediate and -resistant *Staphylococcus aureus:* what the infectious disease specialist needs to know. *Clin Infect Dis* 2001;**32**:108–15.
111. Fridkin SK, Edwards JR, Courval JM, et al. The effect of vancomycin and third-generation cephalosporins on prevalence of vancomycin-resistant enterococci in 126 U.S. adult intensive care units. *Ann Intern Med* 2001;**135**:175–83.
112. Friedland I, Gallagher G, King T, et al. Antimicrobial susceptibility patterns in *Pseudomonas aeruginosa:* data from a multicenter Intensive Care Unit Surveillance Study (ISS) in the United States. *J Chemother* 2004;**16**:437–41.
113. Friedland IR, Klugman KP. Failure of chloramphenicol therapy in penicillin-resistant pneumococcal meningitis. *Lancet* 1992;**339**: 405–8.
114. Friedman L, Alder JD, Silverman JA. Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2006;**50**:2137–45.
115. Frost JA, Kelleher A, Rowe B. Increasing ciprofloxacin resistance in salmonellas in England and Wales 1991-1994. *J Antimicrob Chemother* 1996;**37**:85–91.
116. Gaind R, Paglietti B, Murgia M, et al. Molecular characterization of ciprofloxacin-resistant *Salmonella enterica* serovar Typhi and Paratyphi A causing enteric fever in India. *J Antimicrob Chemother* 2006;**58**:1139–44.
117. Gales AC, Castanheira M, Jones RN, et al. Antimicrobial resistance among gram-negative bacilli isolated from Latin America: results from SENTRY Antimicrobial Surveillance Program (Latin America, 2008-2010). *Diagn Microbiol Infect Dis* 2012;**73**:354–60.
118. Gales AC, Jones RN, Gordon KA, et al. Activity and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998). *J Antimicrob Chemother* 2000;**45**:295–303.
119. Galimand M, Gerbaud G, Guibourdenche M, et al. High-level chloramphenicol resistance in *Neisseria meningitidis*. *N Engl J Med* 1998;**339**:868–74.
120. Gasink LB, Edelstein PH, Lautenbach E, et al. Risk factors and clinical impact of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae*. *Infect Control Hosp Epidemiol* 2009;**30**:1180–5.
121. Gay K, Baughman W, Miller Y, et al. The emergence of *Streptococcus pneumoniae* resistant to macrolide antimicrobial agents: a 6-year population-based assessment. *J Infect Dis* 2000;**182**:1417–24.
122. Gay K, Stephens DS. Structure and dissemination of a chromosomal insertion element encoding macrolide efflux in *Streptococcus pneumoniae*. *J Infect Dis* 2001;**184**:56–65.
123. Gendrel D, Chalumeau M, Moulin F, et al. Fluoroquinolones in paediatrics: a risk for the patient or for the community? *Lancet Infect Dis* 2003;**3**:537–46.
124. Georgopapadakou NH. Penicillin-binding proteins and bacterial resistance to beta-lactams. *Antimicrob Agents Chemother* 1993;**37**: 2045–53.
125. Gessner BD, Sutanto A, Steinhoff M, et al. A population-based survey of *Haemophilus influenzae* type b nasopharyngeal carriage prevalence in Lombok Island, Indonesia. *Pediatr Infect Dis J* 1998;**17**:S179–82.
126. Gheorghiu R, Yuan M, Hall LM, et al. Bases of variation in resistance to beta-lactams in *Klebsiella oxytoca* isolates hyperproducing K1 beta-lactamase. *J Antimicrob Chemother* 1997;**40**:533–41.
127. Ghuysen JM. Serine beta-lactamases and penicillin-binding proteins. *Annu Rev Microbiol* 1991;**45**:37–67.
128. Gibreel A, Sjogren E, Kaijser B, et al. Rapid emergence of high-level resistance to quinolones in *Campylobacter jejuni* associated

with mutational changes in gyrA and parC. *Antimicrob Agents Chemother* 1998;**42**:3276–8.
129. Giovanetti E, Brenciani A, Lupidi R, et al. Presence of the *tet(O)* gene in erythromycin- and tetracycline-resistant strains of *Streptococcus pyogenes* and linkage with either the *mef(A)* or the *erm(A)* gene. *Antimicrob Agents Chemother* 2003;**47**:2844–9.
130. Glynn MK, Bopp C, Dewitt W, et al. Emergence of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 infections in the United States. *N Engl J Med* 1998;**338**:1333–8.
131. Goettsch W, van Pelt W, Nagelkerke N, et al. Increasing resistance to fluoroquinolones in *Escherichia coli* from urinary tract infections in the Netherlands. *J Antimicrob Chemother* 2000;**46**: 223–8.
132. Golini G, Favari F, Marchetti F, et al. Bacteriostatic and bactericidal activity of levofloxacin against clinical isolates from cystic fibrosis patients. *Eur J Clin Microbiol Infect Dis* 2004;**23**:798–800.
133. Gopegui ER, Juan C, Zamorano L, et al. Transferable multidrug resistance plasmid carrying cfr associated with *tet(L)*, *ant(4′)-Ia*, and *dfrK* genes from a clinical methicillin-resistant *Staphylococcus aureus* ST125 strain. *Antimicrob Agents Chemother* 2012;**56**: 2139–42.
134. Gray JW, George RH. Experience of vancomycin-resistant enterococci in a children's hospital. *J Hosp Infect* 2000;**45**:11–18.
135. Grebe T, Hakenbeck R. Penicillin-binding proteins 2b and 2x of *Streptococcus pneumoniae* are primary resistance determinants for different classes of beta-lactam antibiotics. *Antimicrob Agents Chemother* 1996;**40**:829–34.
136. Green MD, Beall B, Marcon MJ, et al. Multicentre surveillance of the prevalence and molecular epidemiology of macrolide resistance among pharyngeal isolates of group A streptococci in the USA. *J Antimicrob Chemother* 2006;**57**:1240–3.
137. Griffin PM, Tauxe RV, Redd SC, et al. Emergence of highly trimethoprim-sulfamethoxazole-resistant *Shigella* in a native American population: an epidemiologic study. *Am J Epidemiol* 1989;**129**:1042–51.
138. Gu B, Kelesidis T, Tsiodras S, et al. The emerging problem of linezolid-resistant *Staphylococcus*. *J Antimicrob Chemother* 2013;**68**: 4–11.
139. Guirao GY, Martinez Toldos MC, Mora Peris B, et al. Molecular diversity of quinolone resistance in genetically related clinical isolates of *Staphylococcus aureus* and susceptibility to newer quinolones. *J Antimicrob Chemother* 2001;**47**:157–61.
140. Gupta K, Hooton TM, Stamm WE. Increasing antimicrobial resistance and the management of uncomplicated community-acquired urinary tract infections. *Ann Intern Med* 2001;**135**: 41–50.
141. Gupta K, Sahm DF, Mayfield D, et al. Antimicrobial resistance among uropathogens that cause community-acquired urinary tract infections in women: a nationwide analysis. *Clin Infect Dis* 2001; **33**:89–94.
142. Gupta N, Limbago BM, Patel JB, et al. Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. *Clin Infect Dis* 2011;**53**:60–7.
143. Hakanen AJ, Kotilainen P, Pitkanen S, et al. Reduction in fluoroquinolone susceptibility among non-typhoidal strains of *Salmonella enterica* isolated from Finnish patients. *J Antimicrob Chemother* 2006;**57**:569–72.
144. Hakenbeck R, Balmelle N, Weber B, et al. Mosaic genes and mosaic chromosomes: intra- and interspecies genomic variation of *Streptococcus pneumoniae*. *Infect Immun* 2001;**69**:2477–86.
145. Hakenbeck R, Briese T, Chalkley L, et al. Antigenic variation of penicillin-binding proteins from penicillin-resistant clinical strains of *Streptococcus pneumoniae*. *J Infect Dis* 1991;**164**:313–19.
146. Hakenbeck R, Coyette J. Resistant penicillin-binding proteins. *Cell Mol Life Sci* 1998;**54**:332–40.
147. Hakenbeck R, Tarpay M, Tomasz A. Multiple changes of penicillin-binding proteins in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1980; **17**:364–71.
148. Hancock RE. Mechanisms of action of newer antibiotics for gram-positive pathogens. *Lancet Infect Dis* 2005;**5**:209–18.
149. Hansen LH, Jensen LB, Sorensen HI, et al. Substrate specificity of the OqxAB multidrug resistance pump in *Escherichia coli* and selected enteric bacteria. *J Antimicrob Chemother* 2007.
150. Harnett N. High level resistance to trimethoprim, cotrimoxazole and other antimicrobial agents among clinical isolates of *Shigella* species in Ontario, Canada—an update. *Epidemiol Infect* 1992;**109**: 463–72.
151. Hawkey PM. Mechanisms of quinolone action and microbial response. *J Antimicrob Chemother* 2003;**51**(Suppl. 1):29–35.
152. Hayden MK. Insights into the epidemiology and control of infection with vancomycin-resistant enterococci. *Clin Infect Dis* 2000;**31**:1058–65.
153. Hayden MK, Rezai K, Hayes RA, et al. Development of daptomycin resistance in vivo in methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 2005;**43**:5285–7.
154. Helms M, Ethelberg S, Molbak K. International *Salmonella typhimurium* DT104 infections, 1992-2001. *Emerg Infect Dis* 2005; **11**:859–67.
155. Henwood CJ, Livermore DM, James D, et al. Antimicrobial susceptibility of *Pseudomonas aeruginosa:* results of a UK survey and evaluation of the British Society for Antimicrobial Chemotherapy disc susceptibility test. *J Antimicrob Chemother* 2001;**47**:789–99.
156. Heritage J, M'Zali FH, Gascoyne-Binzi D, et al. Evolution and spread of SHV extended-spectrum beta-lactamases in gram- negative bacteria. *J Antimicrob Chemother* 1999;**44**:309–18.
157. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998;**279**:593–8.
158. Hoban DJ, Doern GV, Fluit AC, et al. Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 2001;**32**(Suppl. 2):S81–93.
159. Hoenigl M, Valentin T, Zarfel G, et al. Nosocomial outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella oxytoca* in Austria. *Antimicrob Agents Chemother* 2012;**56**:2158–61.
160. Hoge CW, Gambel JM, Srijan A, et al. Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clin Infect Dis* 1998;**26**:341–5.
161. Hooper DC. Bacterial topoisomerases, anti-topoisomerases, and anti-topoisomerase resistance. *Clin Infect Dis* 1998;**27**(Suppl. 1): S54–63.
162. Hooper DC. Mechanisms of action of antimicrobials: focus on fluoroquinolones. *Clin Infect Dis* 2001;**32**(Suppl. 1):S9–15.
163. Hooper DC. Fluoroquinolone resistance among gram-positive cocci. *Lancet Infect Dis* 2002;**2**:530–8.
164. Hooper DC. Quinolone mode of action—new aspects. *Drugs* 1993;**45**(Suppl. 3):8–14.
165. Hooper DC, Wolfson JS, Bozza MA, Ng EY. Genetics and regulation of outer membrane protein expression by quinolone resistance loci nfxB, nfxC, and cfxB. *Antimicrob Agents Chemother* 1992;**36**:1151–4.
166. Hooton TM, Besser R, Foxman B, et al. Acute uncomplicated cystitis in an era of increasing antibiotic resistance: a proposed approach to empirical therapy. *Clin Infect Dis* 2004;**39**:75–80.
167. Hossain A, Ferraro MJ, Pino RM, et al. Plasmid-mediated carbapenem-hydrolyzing enzyme KPC-2 in an *Enterobacter* sp. *Antimicrob Agents Chemother* 2004;**48**:4438–40.
168. Howard AJ, Magee JT, Fitzgerald KA, et al. Factors associated with antibiotic resistance in coliform organisms from community urinary tract infection in Wales. *J Antimicrob Chemother* 2001;**47**: 305–13.
169. Hsueh PR, Chen WH, Teng LJ, et al. Nosocomial infections due to methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci at a university hospital in Taiwan from 1991 to 2003: resistance trends, antibiotic usage and in vitro activities of newer antimicrobial agents. *Int J Antimicrob Agents* 2005;**26**: 43–9.
170. Hsueh PR, Liu YC, Shyr JM, et al. Multicenter surveillance of antimicrobial resistance of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in Taiwan during the 1998-1999 respiratory season. *Antimicrob Agents Chemother* 2000;**44**: 1342–5.
171. Hsueh PR, Shyr JM, Wu JJ. Changes in macrolide resistance among respiratory pathogens after decreased erythromycin consumption in Taiwan. *Clin Microbiol Infect* 2006;**12**:296–8.
172. Hulten KG, Kaplan SL, Lamberth LB, et al. Hospital-acquired *Staphylococcus aureus* infections at Texas Children's Hospital, 2001-2007. *Infect Control Hosp Epidemiol* 2010;**31**:183–90.
173. Huovinen P. Resistance to trimethoprim-sulfamethoxazole. *Clin Infect Dis* 2001;**32**:1608–14.

174. Huovinen P, Sundstrom L, Swedberg G, et al. Trimethoprim and sulfonamide resistance. *Antimicrob Agents Chemother* 1995;**39**: 279–89.
175. Hussain FM, Boyle-Vavra S, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus* colonization in healthy children attending an outpatient pediatric clinic. *Pediatr Infect Dis J* 2001;**20**:763–7.
176. Hyde TB, Gay K, Stephens DS, et al. Macrolide resistance among invasive *Streptococcus pneumoniae* isolates. *JAMA* 2001;**286**: 1857–62.
177. Inderlied C, Nash KA, et al. Antimycobacterial agents: in vitro susceptibility testing, spectra of activity, mechanisms of action and resistance and assays for activity in biological fluids. In: Lorian V, editor. *Antibiotics in laboratory medicine*. 4th ed. Baltimore: Williams & Wilkins; 1996. p. 127–75.
178. International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC). Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements. *Antimicrob Agents Chemother* 2009;**53**:4961–7.
179. Ito H, Yoshida H, Bogaki-Shonai M, et al. Quinolone resistance mutations in the DNA gyrase *gyrA* and *gyrB* genes of *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1994;**38**:2014–23.
180. Jacobs C, Joris B, Jamin M, et al. AmpD, essential for both beta-lactamase regulation and cell wall recycling, is a novel cytosolic *N*-acetylmuramyl-L-alanine amidase. *Mol Microbiol* 1995;**15**: 553–9.
181. Jacobs MR, Bajaksouzian S, Zilles A, et al. Susceptibilities of *Streptococcus pneumoniae* and *Haemophilus influenzae* to 10 oral antimicrobial agents based on pharmacodynamic parameters: 1997 U.S. Surveillance study. *Antimicrob Agents Chemother* 1999; **43**:1901–8.
182. Jacobs MR, Felmingham D, Appelbaum PC, et al. The Alexander Project 1998-2000: susceptibility of pathogens isolated from community-acquired respiratory tract infection to commonly used antimicrobial agents. *J Antimicrob Chemother* 2003;**52**:229–46.
183. Jacoby GA. Mechanisms of resistance to quinolones. *Clin Infect Dis* 2005;**41**(Suppl. 2):S120–6.
184. Jacoby GA. Beta-lactamase nomenclature. *Antimicrob Agents Chemother* 2006;**50**:1123–9.
185. Jacoby GA, Munoz-Price LS. The new beta-lactamases. *N Engl J Med* 2005;**352**:380–91.
186. Jain D, Sinha S, Prasad KN, et al. *Campylobacter* species and drug resistance in a north Indian rural community. *Trans R Soc Trop Med Hyg* 2005;**99**:207–14.
187. Jenssen WD, Thakker-Varia S, Dubin DT, et al. Prevalence of macrolides-lincosamides-streptogramin B resistance and *erm* gene classes among clinical strains of staphylococci and streptococci. *Antimicrob Agents Chemother* 1987;**31**:883–8.
188. Jernigan MG, Press EG, Nguyen MH, et al. The combination of doripenem and colistin is bactericidal and synergistic against colistin-resistant, carbapenemase-producing *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2012;**56**:3395–8.
189. Jick S. Ciprofloxacin safety in a pediatric population. *Pediatr Infect Dis J* 1997;**16**:130–3; discussion 133–4, 160–2.
190. Jochimsen EM, Fish L, Manning K, et al. Control of vancomycin-resistant enterococci at a community hospital: efficacy of patient and staff cohorting. *Infect Control Hosp Epidemiol* 1999;**20**:106–9.
191. John TJ, Cherian T, Raghupathy P. *Haemophilus influenzae* disease in children in India: a hospital perspective. *Pediatr Infect Dis J* 1998;**17**:S169–71.
192. Johnson AP, Warner M, George RC, et al. Activity of moxifloxacin against clinical isolates of *Streptococcus pneumoniae* from England and Wales. *J Antimicrob Chemother* 2001;**47**:411–15.
193. Johnston NJ, De Azavedo JC, Kellner JD, et al. Prevalence and characterization of the mechanisms of macrolide, lincosamide, and streptogramin resistance in isolates of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1998;**42**:2425–6.
194. Jones RN, Ross JE, Fritsche TR, et al. Oxazolidinone susceptibility patterns in 2004: report from the Zyvox Annual Appraisal of Potency and Spectrum (ZAAPS) Program assessing isolates from 16 nations. *J Antimicrob Chemother* 2006;**57**:279–87.
195. Jorgensen JH, Weigel LM, Swenson JM, et al. Activities of clinafloxacin, gatifloxacin, gemifloxacin, and trovafloxacin against recent clinical isolates of levofloxacin-resistant *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 2000;**44**:2962–8.
196. Jung R, Fish DN, Obritsch MD, et al. Surveillance of multi-drug resistant *Pseudomonas aeruginosa* in an urban tertiary-care teaching hospital. *J Hosp Infect* 2004;**57**:105–11.
197. Kaczmarek FM, Dib-Hajj F, Shang W, et al. High-level carbapenem resistance in a *Klebsiella pneumoniae* clinical isolate is due to the combination of bla(ACT-1) beta-lactamase production, porin OmpK35/36 insertional inactivation, and down-regulation of the phosphate transport porin phoe. *Antimicrob Agents Chemother* 2006;**50**:3396–406.
198. Kantzanou M, Tassios PT, Tseleni-Kotsovili A, et al. Reduced susceptibility to vancomycin of nosocomial isolates of methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 1999;**43**: 729–31.
199. Kaplan EL, Cornaglia G. Persistent macrolide resistance among group A streptococci: the lack of accomplishment after 4 decades. *Clin Infect Dis* 2005;**41**:609–11.
200. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
201. Kaplan SL, Mason EO Jr. Management of infections due to antibiotic-resistant *Streptococcus pneumoniae*. *Clin Microbiol Rev* 1998;**11**:628–44.
202. Kariuki S, Revathi G, Kariuki N, et al. Characterisation of community acquired non-typhoidal *Salmonella* from bacteraemia and diarrhoeal infections in children admitted to hospital in Nairobi, Kenya. *BMC Microbiol* 2006;**6**:101.
203. Karlowsky JA, Draghi DC, Jones ME, et al. Surveillance for antimicrobial susceptibility among clinical isolates of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from hospitalized patients in the United States, 1998 to 2001. *Antimicrob Agents Chemother* 2003;**47**:1681–8.
204. Karlowsky JA, Hoban DJ, Decorby MR, et al. Fluoroquinolone-resistant urinary isolates of *Escherichia coli* from outpatients are frequently multidrug resistant: results from the North American Urinary Tract Infection Collaborative Alliance-Quinolone Resistance study. *Antimicrob Agents Chemother* 2006;**50**:2251–4.
205. Karlowsky JA, Jones ME, Thornsberry C, et al. Trends in antimicrobial susceptibilities among Enterobacteriaceae isolated from hospitalized patients in the United States from 1998 to 2001. *Antimicrob Agents Chemother* 2003;**47**:1672–80.
206. Kataja J, Huovinen P, Seppala H. Erythromycin resistance genes in group A streptococci of different geographical origins. The Macrolide Resistance Study Group. *J Antimicrob Chemother* 2000;**46**:789–92.
207. Kaye KS, Gold HS, Schwaber MJ, et al. Variety of beta-lactamases produced by amoxicillin-clavulanate-resistant *Escherichia coli* isolated in the northeastern United States. *Antimicrob Agents Chemother* 2004;**48**:1520–5.
208. Keeley D, Nkrumah FK, Kapuyanyika C. Randomized trial of sulfamethoxazole + trimethoprim versus procaine penicillin for the outpatient treatment of childhood pneumonia in Zimbabwe. *Bull World Health Organ* 1990;**68**:185–92.
209. Kelesidis T, Humphries R, Uslan DZ, et al. Daptomycin nonsusceptible enterococci: an emerging challenge for clinicians. *Clin Infect Dis* 2011;**52**:228–34.
210. Kelley MA, Weber DJ, Gilligan P, et al. Breakthrough pneumococcal bacteremia in patients being treated with azithromycin and clarithromycin. *Clin Infect Dis* 2000;**31**:1008–11.
211. Kellner JD, Ford-Jones EL. *Streptococcus pneumoniae* carriage in children attending 59 Canadian child care centers. Toronto Child Care Centre Study Group. *Arch Pediatr Adolesc Med* 1999;**153**: 495–502.
212. Kim IS, Ki CS, Kim S, et al. Diversity of ampicillin resistance genes and antimicrobial susceptibility patterns in *Haemophilus influenzae* strains isolated in Korea. *Antimicrob Agents Chemother* 2007;**51**:453–60.
213. King MD, Humphrey BJ, Wang YF, et al. Emergence of community-acquired methicillin-resistant *Staphylococcus aureus* USA 300 clone as the predominant cause of skin and soft-tissue infections. *Ann Intern Med* 2006;**144**:309–17.
214. Kitchel B, Rasheed JK, Patel JB, et al. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob Agents Chemother* 2009;**53**:3365–70.
215. Klingenberg C, Sundsfjord A, Ronnestad A, et al. Phenotypic and genotypic aminoglycoside resistance in blood culture isolates of

coagulase-negative staphylococci from a single neonatal intensive care unit, 1989-2000. *J Antimicrob Chemother* 2004;**54**:889–96.
216. Knox JR. Extended-spectrum and inhibitor-resistant TEM-type beta-lactamases: mutations, specificity, and three-dimensional structure. *Antimicrob Agents Chemother* 1995;**39**:2593–601.
217. Kohler T, Kok M, Michea-Hamzehpour M, et al. Multidrug efflux in intrinsic resistance to trimethoprim and sulfamethoxazole in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1996;**40**: 2288–90.
218. Korner RJ, Reeves DS, MacGowan AP. Dangers of oral fluoroquinolone treatment in community acquired upper respiratory tract infections. *BMJ* 1994;**308**:191–2.
219. Korten V, Huang WM, Murray BE. Analysis by PCR and direct DNA sequencing of *gyrA* mutations associated with fluoroquinolone resistance in *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1994;**38**:2091–4.
220. Kosowska-Shick K, Credito K, Pankuch GA, et al. Antipneumococcal activity of DW-224a, a new quinolone, compared to those of eight other agents. *Antimicrob Agents Chemother* 2006;**50**: 2064–71.
221. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob Agents Chemother* 2000;**44**:3249–56.
222. Kruse H, Kariuki S, Soli N, et al. Multiresistant *Shigella* species from African AIDS patients: antibacterial resistance patterns and application of the E-test for determination of minimum inhibitory concentration. *Scand J Infect Dis* 1992;**24**:733–9.
223. Kumarasamy KK, Toleman MA, Walsh TR, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 2010;**10**:597–602.
224. Kyaw MH, Lynfield R, Schaffner W, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006;**354**:1455–63.
225. Lai KK, Kelley AL, Melvin ZS, et al. Failure to eradicate vancomycin-resistant enterococci in a university hospital and the cost of barrier precautions. *Infect Control Hosp Epidemiol* 1998; **19**:647–52.
226. Laible G, Spratt BG, Hakenbeck R. Interspecies recombinational events during the evolution of altered PBP 2x genes in penicillin-resistant clinical isolates of *Streptococcus pneumoniae*. *Mol Microbiol* 1991;**5**:1993–2002.
227. Landman D, Babu E, Shah N, et al. Transmission of carbapenem-resistant pathogens in New York City hospitals: progress and frustration. *J Antimicrob Chemother* 2012;**67**:1427–31.
228. Landman D, Quale JM, Mayorga D, et al. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY: the preantibiotic era has returned. *Arch Intern Med* 2002;**162**:1515–20.
229. Le TP, Miller LG. Empirical therapy for uncomplicated urinary tract infections in an era of increasing antimicrobial resistance: a decision and cost analysis. *Clin Infect Dis* 2001;**33**:615–21.
230. Leavitt A, Carmeli Y, Chmelnitsky I, et al. Molecular epidemiology, sequence types, and plasmid analyses of KPC-producing *Klebsiella pneumoniae* strains in Israel. *Antimicrob Agents Chemother* 2010;**54**:3002–6.
231. Lee BL, Padula AM, Kimbrough RC, et al. Infectious complications with respiratory pathogens despite ciprofloxacin therapy. *N Engl J Med* 1991;**325**:520–1.
232. Lee HJ, Park JY, Jang SH, et al. High incidence of resistance to multiple antimicrobials in clinical isolates of *Streptococcus pneumoniae* from a university hospital in Korea. *Clin Infect Dis* 1995; **20**:826–35.
233. Lee HK, Lee WG, Cho SR. Clinical and molecular biological analysis of a nosocomial outbreak of vancomycin-resistant enterococci in a neonatal intensive care unit. *Acta Paediatr* 1999;**88**: 651–4.
234. Lester SC, del Pilar Pla M, Wang F, et al. The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. *N Engl J Med* 1990;**323**:285–9.
235. Levy SB. Active efflux mechanisms for antimicrobial resistance. *Antimicrob Agents Chemother* 1992;**36**:695–703.
236. Li XZ, Livermore DM, Nikaido H. Role of efflux pump(s) in intrinsic resistance of *Pseudomonas aeruginosa:* resistance to tetracycline, chloramphenicol, and norfloxacin. *Antimicrob Agents Chemother* 1994;**38**:1732–41.
237. Lima AA, Sidrim JJ, Lima NL, et al. Molecular epidemiology of multiply antibiotic-resistant *Shigella flexneri* in Fortaleza, Brazil. *J Clin Microbiol* 1997;**35**:1061–5.
238. Lina G, Quaglia A, Reverdy ME, et al. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother* 1999; **43**:1062–6.
239. Lindberg F, Lindquist S, Normark S. Inactivation of the ampD gene causes semiconstitutive overproduction of the inducible *Citrobacter freundii* beta-lactamase. *J Bacteriol* 1987;**169**:1923–8.
240. Littauer P, Caugant DA, Sangvik M, et al. Macrolide-resistant *Streptococcus pyogenes* in Norway: population structure and resistance determinants. *Antimicrob Agents Chemother* 2006;**50**: 1896–9.
241. Livermore DM, et al. Beta-lactams: mode of action and mechanisms of bacterial resistance. In: Lorian V, editor. *Antibiotics in laboratory medicine*. 4th ed. Baltimore: Williams & Wilkins; 1996. p. 502–78.
242. Logan LK. Carbapenem-resistant Enterobacteriaceae: an emerging problem in children. *Clin Infect Dis* 2012;**55**:852–9.
243. Long KS, Vester B. Resistance to linezolid caused by modifications at its binding site on the ribosome. *Antimicrob Agents Chemother* 2012;**56**:603–12.
244. Lonks JR, Garau J, Gomez L, et al. Failure of macrolide antibiotic treatment in patients with bacteremia due to erythromycin-resistant *Streptococcus pneumoniae*. *Clin Infect Dis* 2002;**35**:556–64.
245. Low DE, Keller N, Barth A, et al. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. *Clin Infect Dis* 2001;**32**(Suppl. 2): S133–45.
246. Lucas GM, Lechtzin N, Puryear DW, et al. Vancomycin-resistant and vancomycin-susceptible enterococcal bacteremia: comparison of clinical features and outcomes. *Clin Infect Dis* 1998;**26**: 1127–33.
247. Lutsar I, McCracken GH Jr, Friedland IR. Antibiotic pharmacodynamics in cerebrospinal fluid. *Clin Infect Dis* 1998;**27**:1117–27, quiz 1128–9.
248. Malhotra-Kumar S, Lammens C, Chapelle S, et al. Macrolide- and telithromycin-resistant *Streptococcus pyogenes*, Belgium, 1999-2003. *Emerg Infect Dis* 2005;**11**:939–42.
249. Malik RK, Montecalvo MA, Reale MR, et al. Epidemiology and control of vancomycin-resistant enterococci in a regional neonatal intensive care unit. *Pediatr Infect Dis J* 1999;**18**:352–6.
250. Maneewannakul K, Levy SB. Identification for mar mutants among quinolone-resistant clinical isolates of *Escherichia coli*. *Antimicrob Agents Chemother* 1996;**40**:1695–8.
251. Manges AR, Johnson JR, Foxman B, et al. Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *N Engl J Med* 2001;**345**:1007–13.
252. Mangili A, Bica I, Snydman DR, et al. Daptomycin-resistant, methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 2005;**40**:1058–60.
253. Manninen R, Huovinen P, Nissinen A. Increasing antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in Finland. *J Antimicrob Chemother* 1997;**40**: 387–92.
254. Manno G, Cruciani M, Romano L, et al. Antimicrobial use and *Pseudomonas aeruginosa* susceptibility profile in a cystic fibrosis centre. *Int J Antimicrob Agents* 2005;**25**:193–7.
255. Marimon JM, Gomariz M, Zigorraga C, et al. Increasing prevalence of quinolone resistance in human nontyphoid *Salmonella* enterica isolates obtained in Spain from 1981 to 2003. *Antimicrob Agents Chemother* 2004;**48**:3789–93.
256. Martin JM, Green M, Barbadora KA, et al. Erythromycin-resistant group A streptococci in schoolchildren in Pittsburgh. *N Engl J Med* 2002;**346**:1200–6.
257. Martinez JL, Alonso A, Gomez-Gomez JM, et al. Quinolone resistance by mutations in chromosomal gyrase genes: just the tip of the iceberg? *J Antimicrob Chemother* 1998;**42**:683–8.
258. Marty FM, Yeh WW, Wennersten CB, et al. Emergence of a clinical daptomycin-resistant *Staphylococcus aureus* isolate during

treatment of methicillin-resistant *Staphylococcus aureus* bacteremia and osteomyelitis. *J Clin Microbiol* 2006;**44**:595–7.
259. Maseda H, Yoneyama H, Nakae T. Assignment of the substrate-selective subunits of the MexEF-OprN multidrug efflux pump of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2000;**44**: 658–64.
260. Matar MJ, Tarrand J, Raad I, Rolston KV. Colonization and infection with vancomycin-resistant *Enterococcus* among patients with cancer. *Am J Infect Control* 2006;**34**:534–6.
261. Mathers AJ, Cox HL, Kitchel B, et al. Molecular dissection of an outbreak of carbapenem-resistant Enterobacteriaceae reveals Intergenus KPC carbapenemase transmission through a promiscuous plasmid. *MBio* 2011;**2**:e00204–11.
262. Matsuoka M, Janosi L, Endou K, et al. Cloning and sequences of inducible and constitutive macrolide resistance genes in *Staphylococcus aureus* that correspond to an ABC transporter. *FEMS Microbiol Lett* 1999;**181**:91–100.
263. May J, Shannon K, King A, et al. Glycopeptide tolerance in *Staphylococcus aureus*. *J Antimicrob Chemother* 1998;**42**:189–97.
264. Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 2006;**4**:608–20.
265. Mazodier P, Davies J. Gene transfer between distantly related bacteria. *Annu Rev Genet* 1991;**25**:147–71.
266. McCarty JM, Richard G, Huck W, et al. A randomized trial of short-course ciprofloxacin, ofloxacin, or trimethoprim/sulfamethoxazole for the treatment of acute urinary tract infection in women. Ciprofloxacin Urinary Tract Infection Group. *Am J Med* 1999;**106**:292–9.
267. McCracken GH Jr. Emergence of resistant *Streptococcus pneumoniae:* a problem in pediatrics. *Pediatr Infect Dis J* 1995;**14**: 424–8.
268. McCracken GH Jr. Treatment of acute otitis media in an era of increasing microbial resistance. *Pediatr Infect Dis J* 1998;**17**:576–9; discussion 580.
269. McCracken GH Jr. Prescribing antimicrobial agents for treatment of acute otitis media. *Pediatr Infect Dis J* 1999;**18**: 1141–6.
270. McDonald LC, Rossiter S, Mackinson C, et al. Quinupristin-dalfopristin-resistant *Enterococcus faecium* on chicken and in human stool specimens. *N Engl J Med* 2001;**345**:1155–60.
271. McDougal LK, Fosheim GE, Nicholson A, et al. Emergence of resistance among USA300 methicillin-resistant *Staphylococcus aureus* isolates causing invasive disease in the United States. *Antimicrob Agents Chemother* 2010;**54**:3804–11.
272. McDougal LK, Steward CD, Killgore GE, et al. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J Clin Microbiol* 2003;**41**:5113–20.
273. McKee KT Jr, Cotton RB, Stratton CW, et al. Nursery epidemic due to multiply-resistant *Klebsiella pneumoniae:* epidemiologic setting and impact on perinatal health care delivery. *Infect Control* 1982;**3**:150–6.
274. McNeeley DF, Brown AE, Noel GJ, et al. An investigation of vancomycin-resistant *Enterococcus faecium* within the pediatric service of a large urban medical center. *Pediatr Infect Dis J* 1998; **17**:184–8.
275. McNeil SA, Malani PN, Chenoweth CE, et al. Vancomycin-resistant enterococcal colonization and infection in liver transplant candidates and recipients: a prospective surveillance study. *Clin Infect Dis* 2006;**42**:195–203.
276. Medeiros AA. Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics. *Clin Infect Dis* 1997;**24**(Suppl. 1):S19–45.
277. Meka VG, Gold HS. Antimicrobial resistance to linezolid. *Clin Infect Dis* 2004;**39**:1010–15.
278. Mendes RE, Tsakris A, Sader HS, et al. Characterization of methicillin-resistant *Staphylococcus aureus* displaying increased MICs of ceftaroline. *J Antimicrob Chemother* 2012;**67**:1321–4.
279. Miller LG, Quan C, Shay A, et al. A prospective investigation of outcomes after hospital discharge for endemic, community-acquired methicillin-resistant and -susceptible *Staphylococcus aureus* skin infection. *Clin Infect Dis* 2007;**44**:483–92.
280. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. *Antimicrob Agents Chemother* 1999; **43**:727–37.
281. Mirelis B, Navarro F, Miro E, et al. Community transmission of extended-spectrum beta-lactamase. *Emerg Infect Dis* 2003;**9**: 1024–5.
282. Mishra NN, Bayer AS, Tran TT, et al. Daptomycin resistance in enterococci is associated with distinct alterations of cell membrane phospholipid content. *PLoS One* 2012;**7**:e43958.
283. Molbak K, Baggesen DL, Aarestrup FM, et al. An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype typhimurium DT104. *N Engl J Med* 1999;**341**:1420–5.
284. Moore RA, Beckthold B, Wong S, et al. Nucleotide sequence of the *gyrA* gene and characterization of ciprofloxacin-resistant mutants of *Helicobacter pylori*. *Antimicrob Agents Chemother* 1995; **39**:107–11.
285. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med* 2006;**355**:666–74.
286. Morosini MI, Garcia-Castillo M, Coque TM, et al. Antibiotic coresistance in extended-spectrum-beta-lactamase-producing Enterobacteriaceae and in vitro activity of tigecycline. *Antimicrob Agents Chemother* 2006;**50**:2695–9.
287. Morris JG Jr, Shay DK, Hebden JN, et al. Enterococci resistant to multiple antimicrobial agents, including vancomycin: establishment of endemicity in a university medical center. *Ann Intern Med* 1995;**123**:250–9.
288. Mulvey MR, Boyd DA, Olson AB, et al. The genetics of *Salmonella* genomic island 1. *Microbes Infect* 2006;**8**:1915–22.
289. Munoz R, Dowson CG, Daniels M, et al. Genetics of resistance to third-generation cephalosporins in clinical isolates of *Streptococcus pneumoniae*. *Mol Microbiol* 1992;**6**:2461–5.
290. Munoz-Price LS, De La Cuesta C, Adams S, et al. Successful eradication of a monoclonal strain of *Klebsiella pneumoniae* during a *K. pneumoniae* carbapenemase-producing K. pneumoniae outbreak in a surgical intensive care unit in Miami, Florida. *Infect Control Hosp Epidemiol* 2010;**31**:1074–7.
291. Murray BE. Vancomycin-resistant enterococcal infections. *N Engl J Med* 2000;**342**:710–21.
292. Mussi MA, Limansky AS, Viale AM. Acquisition of resistance to carbapenems in multidrug-resistant clinical strains of *Acinetobacter baumannii:* natural insertional inactivation of a gene encoding a member of a novel family of beta-barrel outer membrane proteins. *Antimicrob Agents Chemother* 2005;**49**:1432–40.
293. National NSS. National Nosocomial Infections Surveillance (NNIS) System Report: data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* 2004;**32**: 470–85.
294. Navarro F, Courvalin P. Analysis of genes encoding D-alanine-D-alanine ligase–related enzymes in *Enterococcus casseliflavus* and *Enterococcus flavescens*. *Antimicrob Agents Chemother* 1994;**38**: 1788–93.
295. Nazir J, Urban C, Mariano N, et al. Quinolone-resistant *Haemophilus influenzae* in a long-term care facility: clinical and molecular epidemiology. *Clin Infect Dis* 2004;**38**:1564–9.
296. Nelson JD. Editorial: should ampicillin be abandoned for treatment of *Haemophilus influenzae* disease? *JAMA* 1974;**229**:322–4.
297. Nelson JM, Chiller TM, Powers JH, et al. Fluoroquinolone-resistant *Campylobacter* species and the withdrawal of fluoroquinolones from use in poultry: a public health success story. *Clin Infect Dis* 2007;**44**:977–80.
298. Neonakis I, Gikas A, Scoulica E, et al. Evolution of aminoglycoside resistance phenotypes of four gram-negative bacteria: an 8-year survey in a University Hospital in Greece. *Int J Antimicrob Agents* 2003;**22**:526–31.
299. Nicoletti G, Schito G, Fadda G, et al. Bacterial isolates from severe infections and their antibiotic susceptibility patterns in Italy: a nationwide study in the hospital setting. *J Chemother* 2006;**18**:589–602.
300. Nimmo GR. USA300 abroad: global spread of a virulent strain of community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Microbiol Infect* 2012;**18**:725–34.
301. Noble WC, Virani Z, Cree RG. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. *FEMS Microbiol Lett* 1992;**72**:195–8.
302. Nordmann P, Poirel L. Emergence of plasmid-mediated resistance to quinolones in Enterobacteriaceae. *J Antimicrob Chemother* 2005;**56**:463–9.

303. Ntokou E, Stathopoulos C, Kristo I, et al. Intensive care unit dissemination of multiple clones of linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium*. *J Antimicrob Chemother* 2012;**67**: 1819–23.
304. Nuermberger E, Bishai WR. The clinical significance of macrolide-resistant *Streptococcus pneumoniae:* it's all relative. *Clin Infect Dis* 2004;**38**:99–103.
305. Okuma K, Iwakawa K, Turnidge JD, et al. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. *J Clin Microbiol* 2002;**40**:4289–94.
306. Oliveira DC, de Lencastre H. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2002;**46**:2155–61.
307. Oliveira DC, Tomasz A, de Lencastre H. Secrets of success of a human pathogen: molecular evolution of pandemic clones of methicillin-resistant *Staphylococcus aureus*. *Lancet Infect Dis* 2002; **2**:180–9.
308. Oliver A, Canton R, Campo P, et al. High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection. *Science* 2000;**288**:1251–4.
309. Osterblad M, Hakanen A, Manninen R, et al. A between-species comparison of antimicrobial resistance in enterobacteria in fecal flora. *Antimicrob Agents Chemother* 2000;**44**:1479–84.
310. Ostroff SM, Harrison LH, Khallaf N, et al. Resistance patterns of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates recovered in Egypt from children with pneumonia. The Antimicrobial Resistance Surveillance Study Group. *Clin Infect Dis* 1996;**23**:1069–74.
311. Ostrowsky BE, Venkataraman L, D'Agata EM, et al. Vancomycin-resistant enterococci in intensive care units: high frequency of stool carriage during a non-outbreak period. *Arch Intern Med* 1999;**159**:1467–72.
312. Padayachee T, Klugman KP. Novel expansions of the gene encoding dihydropteroate synthase in trimethoprim-sulfamethoxazole-resistant *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1999;**43**:2225–30.
313. Palavecino EL, Riedel I, Berrios X, et al. Prevalence and mechanisms of macrolide resistance in *Streptococcus pyogenes* in Santiago, Chile. *Antimicrob Agents Chemother* 2001;**45**:339–41.
314. Pallares R, Linares J, Vadillo M, et al. Resistance to penicillin and cephalosporin and mortality from severe pneumococcal pneumonia in Barcelona, Spain. *N Engl J Med* 1995;**333**:474–80.
315. Palucha A, Mikiewicz B, Gniadkowski M. Diversification of *Escherichia coli* expressing an SHV-type extended-spectrum beta-lactamase (ESBL) during a hospital outbreak: emergence of an ESBL-hyperproducing strain resistant to expanded-spectrum cephalosporins. *Antimicrob Agents Chemother* 1999;**43**:393–6.
316. Pan XS, Fisher LM. DNA gyrase and topoisomerase IV are dual targets of clinafloxacin action in *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* 1998;**42**:2810–16.
317. Papadimitriou-Olivgeris M, Marangos M, Fligou F, et al. Risk factors for KPC-producing *Klebsiella pneumoniae* enteric colonization upon ICU admission. *J Antimicrob Chemother* 2012; **67**:2976–81.
318. Papp-Wallace KM, Bethel CR, Distler AM, et al. Inhibitor resistance in the KPC-2 beta-lactamase, a preeminent property of this class A beta-lactamase. *Antimicrob Agents Chemother* 2010;**54**: 890–7.
319. Papp-Wallace KM, Taracila MA, Smith KM, et al. Understanding the molecular determinants of substrate and inhibitor specificities in the carbapenemase KPC-2: exploring the roles of Arg220 and Glu276. *Antimicrob Agents Chemother* 2012;**56**: 4428–38.
320. Paradiso PR. Advances in pneumococcal disease prevention: 13-valent pneumococcal conjugate vaccine for infants and children. *Clin Infect Dis* 2011;**52**:1241–7.
321. Parry CM, Diep TS, Wain J, et al. Nasal carriage in Vietnamese children of *Streptococcus pneumoniae* resistant to multiple antimicrobial agents. *Antimicrob Agents Chemother* 2000;**44**:484–8.
322. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. *Clin Microbiol Rev* 2005;**18**:657–86.
323. Paterson DL, Ko WC, Von Gottberg A, et al. International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial Infections. *Ann Intern Med* 2004;**140**:26–32.
324. Paterson DL, Mulazimoglu L, Casellas JM, et al. Epidemiology of ciprofloxacin resistance and its relationship to extended-spectrum beta-lactamase production in *Klebsiella pneumoniae* isolates causing bacteremia. *Clin Infect Dis* 2000;**30**:473–8.
325. Patterson JE, Zervos MJ. High-level gentamicin resistance in *Enterococcus:* microbiology, genetic basis, and epidemiology. *Rev Infect Dis* 1990;**12**:644–52.
326. Paulsen IT, Brown MH, Skurray RA. Proton-dependent multidrug efflux systems. *Microbiol Rev* 1996;**60**:575–608.
327. Pazhani GP, Sarkar B, Ramamurthy T, et al. Clonal multidrug-resistant *Shigella dysenteriae* type 1 strains associated with epidemic and sporadic dysenteries in eastern India. *Antimicrob Agents Chemother* 2004;**48**:681–4.
328. Perez F, Endimiani A, Ray AJ, et al. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. *J Antimicrob Chemother* 2010;**65**:1807–18.
329. Perez-Trallero E, Garcia-Arenzana JM, Jimenez JA, et al. Therapeutic failure and selection of resistance to quinolones in a case of pneumococcal pneumonia treated with ciprofloxacin. *Eur J Clin Microbiol Infect Dis* 1990;**9**:905–6.
330. Perez-Trallero E, Montes M, Orden B, et al. Phenotypic and genotypic characterization of *Streptococcus pyogenes* isolates displaying the MLSB phenotype of macrolide resistance in Spain, 1999 to 2005. *Antimicrob Agents Chemother* 2007;**51**:1228–33.
331. Peterson LR. Penicillins for treatment of pneumococcal pneumonia: does in vitro resistance really matter? *Clin Infect Dis* 2006;**42**:224–33.
332. Pfaller MA, Segreti J. Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. *Clin Infect Dis* 2006;**42**(Suppl. 4):S153–63.
333. Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type beta-lactamases. *Antimicrob Agents Chemother* 2002;**46**:1–11.
334. Philippon A, Dusart J, Joris B, et al. The diversity, structure and regulation of beta-lactamases. *Cell Mol Life Sci* 1998;**54**:341–6.
335. Piddock LJ. Quinolone resistance and *Campylobacter* spp. *J Antimicrob Chemother* 1995;**36**:891–8.
336. Piddock LJ. Mechanisms of fluoroquinolone resistance: an update 1994-1998. *Drugs* 1999;**58**:11–18.
337. Piddock LJ, Johnson M, Ricci V, et al. Activities of new fluoroquinolones against fluoroquinolone-resistant pathogens of the lower respiratory tract. *Antimicrob Agents Chemother* 1998;**42**: 2956–60.
338. Piddock LJ, White DG, Gensberg K, et al. Evidence for an efflux pump mediating multiple antibiotic resistance in *Salmonella enterica* serovar Typhimurium. *Antimicrob Agents Chemother* 2000; **44**:3118–21.
339. Pikis A, Donkersloot JA, Rodriguez WJ, et al. A conservative amino acid mutation in the chromosome-encoded dihydrofolate reductase confers trimethoprim resistance in *Streptococcus pneumoniae*. *J Infect Dis* 1998;**178**:700–6.
340. Pitout JD, Nordmann P, Laupland KB, et al. Emergence of Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs) in the community. *J Antimicrob Chemother* 2005;**56**: 52–9.
341. Pletz MW, McGee L, Jorgensen J, et al. Levofloxacin-resistant invasive *Streptococcus pneumoniae* in the United States: evidence for clonal spread and the impact of conjugate pneumococcal vaccine. *Antimicrob Agents Chemother* 2004;**48**:3491–7.
342. Ploy MC, Lambert T, Couty JP, et al. Integrons: an antibiotic resistance gene capture and expression system. *Clin Chem Lab Med* 2000;**38**:483–7.
343. Polgreen PM, Beekmann SE, Chen YY, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* in a rural state. *Infect Control Hosp Epidemiol* 2006;**27**: 252–6.
344. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis* 2008;**46**: 787–94.
345. Poulou A, Voulgari E, Vrioni G, et al. Imported *Klebsiella pneumoniae* carbapenemase–producing *K. pneumoniae* clones in a Greek hospital: impact of infection control measures for restraining their dissemination. *J Clin Microbiol* 2012;**50**:2618–23.
346. Quale J, Bratu S, Gupta J, et al. Interplay of efflux system, ampC, and oprD expression in carbapenem resistance of *Pseudomonas*

aeruginosa clinical isolates. *Antimicrob Agents Chemother* 2006;**50**:1633–41.
347. Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. *Clin Microbiol Rev* 2007;**20**:440–58.
348. Quentin C, Arpin C, Dubois V, et al. Antibiotic resistance rates and phenotypes among isolates of Enterobacteriaceae in French extra-hospital practice. *Eur J Clin Microbiol Infect Dis* 2004;**23**:185–93.
349. Qureshi ZA, Paterson DL, Potoski BA, et al. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae:* superiority of combination antimicrobial regimens. *Antimicrob Agents Chemother* 2012;**56**:2108–13.
350. Radstrom P, Fermer C, Kristiansen BE, et al. Transformational exchanges in the dihydropteroate synthase gene of *Neisseria meningitidis:* a novel mechanism for acquisition of sulfonamide resistance. *J Bacteriol* 1992;**174**:6386–93.
351. Rahman M, Mauff G, Levy J, et al. Detection of 4-quinolone resistance mutation in *gyrA* gene of Shigella dysenteriae type 1 by PCR. *Antimicrob Agents Chemother* 1994;**38**:2488–91.
352. Ramphal R, Ambrose PG. Extended-spectrum beta-lactamases and clinical outcomes: current data. *Clin Infect Dis* 2006;**42**(Suppl. 4):S164–72.
353. Rasmussen ZA, Bari A, Qazi S, et al. Randomized controlled trial of standard versus double dose cotrimoxazole for childhood pneumonia in Pakistan. *Bull World Health Organ* 2005;**83**:10–19.
354. Recchia GD, Hall RM. Gene cassettes: a new class of mobile element. *Microbiology* 1995;**141**:3015–27.
355. Recht MI, Douthwaite S, Puglisi JD. Basis for prokaryotic specificity of action of aminoglycoside antibiotics. *Embo J* 1999;**18**:3133–8.
356. Recht MI, Fourmy D, Blanchard SC, et al. RNA sequence determinants for aminoglycoside binding to an A-site rRNA model oligonucleotide. *J Mol Biol* 1996;**262**:421–36.
357. Reed MD, Blumer JL. Anti-infective therapy. In: Jenson HB, Baltimore RS, editors. *Pediatric infectious diseases: principles and practice*. 2nd ed. Philadelphia: WB Saunders; 2002. p. 147–216.
358. Reinert RR, Reinert S, van der Linden M, et al. Antimicrobial susceptibility of *Streptococcus pneumoniae* in eight European countries from 2001 to 2003. *Antimicrob Agents Chemother* 2005;**49**:2903–13.
359. Replogle ML, Fleming DW, Cieslak PR. Emergence of antimicrobial-resistant shigellosis in Oregon. *Clin Infect Dis* 2000;**30**:515–19.
360. Reynolds PE, Depardieu F, Dutka-Malen S, et al. Glycopeptide resistance mediated by enterococcal transposon Tn1546 requires production of VanX for hydrolysis of D-alanyl-D-alanine. *Mol Microbiol* 1994;**13**:1065–70.
361. Rice L, et al. Genetic and biochemical mechanisms of bacterial resistance to antimicrobial agents. In: Lorian V, editor. *Anbiotics in laboratory medicine*. 4th ed. Baltimore: Williams & Wilkins; 1996. p. 453–501.
362. Richter SS, Heilmann KP, Beekmann SE, et al. Macrolide-resistant *Streptococcus pyogenes* in the United States, 2002-2003. *Clin Infect Dis* 2005;**41**:599–608.
363. Richter SS, Heilmann KP, Beekmann SE, et al. The molecular epidemiology of *Streptococcus pneumoniae* with quinolone resistance mutations. *Clin Infect Dis* 2005;**40**:225–35.
364. Rittenhouse S, McCloskey L, Broskey J, et al. In vitro antibacterial activity of gemifloxacin and comparator compounds against common respiratory pathogens. *J Antimicrob Chemother* 2000;**45**(Suppl. 1):23–7.
365. Roberts MC, Sutcliffe J, Courvalin P, et al. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999;**43**:2823–30.
366. Robicsek A, Jacoby GA, Hooper DC. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect Dis* 2006;**6**:629–40.
367. Robinson DA, Sutcliffe JA, Tewodros W, et al. Evolution and global dissemination of macrolide-resistant group A streptococci. *Antimicrob Agents Chemother* 2006;**50**:2903–11.
368. Roland S, Ferone R, Harvey RJ, et al. The characteristics and significance of sulfonamides as substrates for *Escherichia coli* dihydropteroate synthase. *J Biol Chem* 1979;**254**:10337–45.
369. Rowe AK, Deming MS, Schwartz B, et al. Antimicrobial resistance of nasopharyngeal isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* from children in the Central African Republic. *Pediatr Infect Dis J* 2000;**19**:438–44.
370. Rubio A, Moore J, Varoglu M, et al. LC-MS/MS characterization of phospholipid content in daptomycin-susceptible and -resistant isolates of *Staphylococcus aureus* with mutations in mprF. *Mol Membr Biol* 2012;**29**:1–8.
371. Rydberg J, Cederberg A. Intrafamilial spreading of *Escherichia coli* resistant to trimethoprim. *Scand J Infect Dis* 1986;**18**:457–60.
372. Sa-Leao R, Tomasz A, Sanches IS, et al. Carriage of internationally spread clones of *Streptococcus pneumoniae* with unusual drug resistance patterns in children attending day care centers in Lisbon, Portugal. *J Infect Dis* 2000;**182**:1153–60.
373. Sader HS, Flamm RK, Farrell DJ, et al. Activity analyses of staphylococcal isolates from pediatric, adult, and elderly patients: AWARE Ceftaroline Surveillance Program. *Clin Infect Dis* 2012;**55**(Suppl. 3):S181–6.
374. Sader HS, Fritsche TR, Jones RN. Antimicrobial activity of daptomycin tested against clinical strains of indicated species isolated in North American medical centers (2003). *Diagn Microbiol Infect Dis* 2005;**53**:329–32.
375. Sader HS, Fritsche TR, Streit JM, et al. Daptomycin in vitro activity tested against gram-positive strains collected from European and Latin American medical centers in 2003. *J Chemother* 2005;**17**:477–83.
376. Sader HS, Streit JM, Fritsche TR, et al. Antimicrobial susceptibility of gram-positive bacteria isolated from European medical centres: results of the Daptomycin Surveillance Programme (2002-2004). *Clin Microbiol Infect* 2006;**12**:844–52.
377. Saha SK, Baqui AH, Darmstadt GL, et al. Invasive *Haemophilus influenzae* type B diseases in Bangladesh, with increased resistance to antibiotics. *J Pediatr* 2005;**146**:227–33.
378. Sahm DF, Peterson DE, Critchley IA, Thornsberry C. Analysis of ciprofloxacin activity against *Streptococcus pneumoniae* after 10 years of use in the United States. *Antimicrob Agents Chemother* 2000;**44**:2521–4.
379. Saiman L, Mehar F, Niu WW, et al. Antibiotic susceptibility of multiply resistant *Pseudomonas aeruginosa* isolated from patients with cystic fibrosis, including candidates for transplantation. *Clin Infect Dis* 1996;**23**:532–7.
380. Salyers AA, Shoemaker NB, Stevens AM, et al. Conjugative transposons: an unusual and diverse set of integrated gene transfer elements. *Microbiol Rev* 1995;**59**:579–90.
381. Sanders CC. Mechanisms responsible for cross-resistance and dichotomous resistance among the quinolones. *Clin Infect Dis* 2001;**32**(Suppl. 1):S1–8.
382. Saravolatz LD, Stein GE, Johnson LB. Ceftaroline: a novel cephalosporin with activity against methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2011;**52**:1156–63.
383. Satta G, Cornaglia G, Mazzariol A, et al. Target for bacteriostatic and bactericidal activities of beta-lactam antibiotics against *Escherichia coli* resides in different penicillin-binding proteins. *Antimicrob Agents Chemother* 1995;**39**:812–18.
384. Schaad UB. Fluoroquinolone antibiotics in infants and children. *Infect Dis Clin North Am* 2005;**19**:617–28.
385. Schaad UB, Wedgwood J, Ruedeberg A, et al. Ciprofloxacin as antipseudomonal treatment in patients with cystic fibrosis. *Pediatr Infect Dis J* 1997;**16**:106–11; discussion 123–6.
386. Schmitt R. Molecular biology of transposable elements. *J Antimicrob Chemother* 1986;**18**(Suppl. C):25–34.
387. Schmitz FJ, Fluit AC, Gondolf M, et al. The prevalence of aminoglycoside resistance and corresponding resistance genes in clinical isolates of staphylococci from 19 European hospitals. *J Antimicrob Chemother* 1999;**43**:253–9.
388. Schmitz FJ, Sadurski R, Kray A, et al. Prevalence of macrolide-resistance genes in *Staphylococcus aureus* and *Enterococcus faecium* isolates from 24 European university hospitals. *J Antimicrob Chemother* 2000;**45**:891–4.
389. Schmitz FJ, Verhoef J, Fluit AC. Prevalence of aminoglycoside resistance in 20 European university hospitals participating in the European SENTRY Antimicrobial Surveillance Programme. *Eur J Clin Microbiol Infect Dis* 1999;**18**:414–21.
390. Schouten MA, Hoogkamp-Korstanje JA, Meis JF, et al. Prevalence of vancomycin-resistant enterococci in Europe. *Eur J Clin Microbiol Infect Dis* 2000;**19**:816–22.
391. Scott JA, Mwarumba S, Ngetsa C, et al. Progressive increase in antimicrobial resistance among invasive isolates of *Haemophilus*

influenzae obtained from children admitted to a hospital in Kilifi, Kenya, from 1994 to 2002. *Antimicrob Agents Chemother* 2005; **49**:3021–4.
392. Scott JR. Regulation of plasmid replication. *Microbiol Rev* 1984;**48**:1–23.
393. Scott JR, Churchward GG. Conjugative transposition. *Annu Rev Microbiol* 1995;**49**:367–97.
394. Seppala H, Klaukka T, Vuopio-Varkila J, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. *N Engl J Med* 1997;**337**: 441–6.
395. Seybold U, Kourbatova EV, Johnson JG, et al. Emergence of community-associated methicillin-resistant *Staphylococcus aureus* USA300 genotype as a major cause of health care-associated blood stream infections. *Clin Infect Dis* 2006;**42**:647–56.
396. Shaw KJ, Rather PN, Hare RS, et al. Molecular genetics of aminoglycoside resistance genes and familial relationships of the aminoglycoside-modifying enzymes. *Microbiol Rev* 1993;**57**: 138–63.
397. Shaw WV. Bacterial resistance to chloramphenicol. *Br Med Bull* 1984;**40**:36–41.
398. Shawar RM, MacLeod DL, Garber RL, et al. Activities of tobramycin and six other antibiotics against *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. *Antimicrob Agents Chemother* 1999;**43**:2877–80.
399. Shay DK, Maloney SA, Montecalvo M, et al. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. *J Infect Dis* 1995;**172**:993–1000.
400. Shultz TR, Tapsall JW, White PA, et al. Chloramphenicol-resistant *Neisseria meningitidis* containing catP isolated in Australia. *J Antimicrob Chemother* 2003;**52**:856–9.
401. Sivapalasingam S, Nelson JM, Joyce K, et al. High prevalence of antimicrobial resistance among *Shigella* isolates in the United States tested by the National Antimicrobial Resistance Monitoring System from 1999 to 2002. *Antimicrob Agents Chemother* 2006;**50**:49–54.
402. Skoczynska A, Kadlubowski M, Empel J, et al. Characteristics of *Haemophilus influenzae* type b responsible for meningitis in Poland from 1997 to 2004. *J Clin Microbiol* 2005;**43**:5665–9.
403. Slaughter S, Hayden MK, Nathan C, et al. A comparison of the effect of universal use of gloves and gowns with that of glove use alone on acquisition of vancomycin-resistant enterococci in a medical intensive care unit. *Ann Intern Med* 1996;**125**:448–56.
404. Smith AL, Fiel SB, Mayer-Hamblett N, et al. Susceptibility testing of *Pseudomonas aeruginosa* isolates and clinical response to parenteral antibiotic administration: lack of association in cystic fibrosis. *Chest* 2003;**123**:1495–502.
405. Smith KE, Besser JM, Hedberg CW, et al. Quinolone-resistant *Campylobacter jejuni* infections in Minnesota, 1992-1998. Investigation Team. *N Engl J Med* 1999;**340**:1525–32.
406. Smith TL, Pearson ML, Wilcox KR, et al. Emergence of vancomycin resistance in *Staphylococcus aureus*. Glycopeptide-Intermediate Staphylococcus aureus Working Group. *N Engl J Med* 1999;**340**:493–501.
407. Smith Moland E, Hanson ND, Herrera VL, et al. Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. *J Antimicrob Chemother* 2003;**51**: 711–14.
408. Soler P, Gonzalez-Sanz R, Bleda MJ, et al. Antimicrobial resistance in non-typhoidal *Salmonella* from human sources, Spain, 2001-2003. *J Antimicrob Chemother* 2006;**58**:310–14.
409. Sorensen TL, Blom M, Monnet DL, et al. Transient intestinal carriage after ingestion of antibiotic-resistant *Enterococcus faecium* from chicken and pork. *N Engl J Med* 2001;**345**:1161–6.
410. Souli M, Galani I, Antoniadou A, et al. An outbreak of infection due to beta-Lactamase *Klebsiella pneumoniae* carbapenemase 2–producing *K. pneumoniae* in a Greek university hospital: molecular characterization, epidemiology, and outcomes. *Clin Infect Dis* 2010;**50**:364–73.
411. Spencker FB, Staber L, Lietz T, et al. Development of resistance in *Pseudomonas aeruginosa* obtained from patients with cystic fibrosis at different times. *Clin Microbiol Infect* 2003;**9**:370–9.
412. Steenbergen JN, Alder J, Thorne GM, et al. Daptomycin: a lipopeptide antibiotic for the treatment of serious gram-positive infections. *J Antimicrob Chemother* 2005;**55**:283–8.
413. Steinke DT, Seaton RA, Phillips G, et al. Factors associated with trimethoprim-resistant bacteria isolated from urine samples. *J Antimicrob Chemother* 1999;**43**:841–3.
414. Stephens DS, Zughaier SM, Whitney CG, et al. Incidence of macrolide resistance in *Streptococcus pneumoniae* after introduction of the pneumococcal conjugate vaccine: population-based assessment. *Lancet* 2005;**365**:855–63.
415. Stevenson JE, Gay K, Barrett TJ, et al. Increase in nalidixic acid resistance among non-Typhi *Salmonella enterica* isolates in the United States from 1996 to 2003. *Antimicrob Agents Chemother* 2007;**51**:195–7.
416. Stevenson KB, Searle K, Stoddard GJ, et al. Methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci in rural communities, western United States. *Emerg Infect Dis* 2005;**11**: 895–903.
417. Stratchounski LS, Kretchikova OI, Kozlov RS, et al. Antimicrobial resistance of *Streptococcus pneumoniae* isolated from healthy children in day-care centers: results of a multicenter study in Russia. *Pediatr Infect Dis J* 2000;**19**:196–200.
418. Straus WL, Qazi SA, Kundi Z, et al. Antimicrobial resistance and clinical effectiveness of co-trimoxazole versus amoxycillin for pneumonia among children in Pakistan: randomised controlled trial. Pakistan Co-trimoxazole Study Group. *Lancet* 1998;**352**: 270–4.
419. Sutcliffe J, Tait-Kamradt A, Wondrack L. *Streptococcus pneumoniae* and *Streptococcus pyogenes* resistant to macrolides but sensitive to clindamycin: a common resistance pattern mediated by an efflux system. *Antimicrob Agents Chemother* 1996;**40**:1817–24.
420. Sutcliffe JA, Gootz TD, Barrett JF. Biochemical characteristics and physiological significance of major DNA topoisomerases. *Antimicrob Agents Chemother* 1989;**33**:2027–33.
421. Swaney SM, Aoki H, Ganoza MC, et al. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. *Antimicrob Agents Chemother* 1998;**42**:3251–5.
422. Szumowski JD, Cohen DE, Kanaya F, et al. Treatment and outcomes of infections by methicillin-resistant *Staphylococcus aureus* at an ambulatory clinic. *Antimicrob Agents Chemother* 2007;**51**: 423–8.
423. Talan DA, Stamm WE, Hooton TM, et al. Comparison of ciprofloxacin (7 days) and trimethoprim-sulfamethoxazole (14 days) for acute uncomplicated pyelonephritis pyelonephritis in women: a randomized trial. *JAMA* 2000;**283**:1583–90.
424. Talukder KA, Khajanchi BK, Islam MA, et al. Genetic relatedness of ciprofloxacin-resistant *Shigella dysenteriae* type 1 strains isolated in south Asia. *J Antimicrob Chemother* 2004;**54**:730–4.
425. Tamayo J, Perez-Trallero E, Gomez-Garces JL, et al. Resistance to macrolides, clindamycin and telithromycin in *Streptococcus pyogenes* isolated in Spain during 2004. *J Antimicrob Chemother* 2005;**56**:780–2.
426. Tan TQ. Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. *Clin Microbiol Rev* 2012;**25**:409–19.
427. Tanz RR, Shulman ST, Shortridge VD, et al. Community-based surveillance in the united states of macrolide-resistant pediatric pharyngeal group A streptococci during 3 respiratory disease seasons. *Clin Infect Dis* 2004;**39**:1794–801.
428. Thakker-Varia S, Jenssen WD, Moon-McDermott L, et al. Molecular epidemiology of macrolides-lincosamides-streptogramin B resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *Antimicrob Agents Chemother* 1987;**31**: 735–43.
429. Thomas WJ, McReynolds JW, Mock CR, et al. Letter: ampicillin-resistant *Haemophilus influenzae* meningitis. *Lancet* 1974;**1**:313.
430. Thompson R. R plasmid transfer. *J Antimicrob Chemother* 1986; **18**(Suppl. C):13–23.
431. Thurlow LR, Joshi GS, Richardson AR. Virulence strategies of the dominant USA300 lineage of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA). *FEMS Immunol Med Microbiol* 2012;**65**:5–22.
432. Timmis KN, Gonzalez-Carrero MI, Sekizaki T, et al. Biological activities specified by antibiotic resistance plasmids. *J Antimicrob Chemother* 1986;**18**(Suppl. C):1–12.
433. Toltzis P, Dul MJ, Hoyen C, et al. Molecular epidemiology of antibiotic-resistant gram-negative bacilli in a neonatal intensive care unit during a nonoutbreak period. *Pediatrics* 2001;**108**: 1143–8.

434. Toltzis P, Dul M, O'Riordan MA, et al. Impact of amoxicillin on pneumococcal colonization compared with other therapies for acute otitis media. *Pediatr Infect Dis J* 2005;**24**:24–8.
435. Toltzis P, Jacobs MR. The epidemiology of childhood pneumococcal disease in the United States in the era of conjugate vaccine use. *Infect Dis Clin North Am* 2005;**19**:629–45.
436. Toltzis P, Yamashita T, Vilt L, et al. Colonization with antibiotic-resistant gram-negative organisms in a pediatric intensive care unit. *Crit Care Med* 1997;**25**:538–44.
437. Tran TT, Panesso D, Gao H, et al. Whole-genome analysis of a daptomycin-susceptible *Enterococcus faecium* and its daptomycin-resistant variant arising during therapy. *Antimicrob Agents Chemother* 2013;**57**(1):261–8.
438. Trick WE, Kuehnert MJ, Quirk SB, et al. Regional dissemination of vancomycin-resistant enterococci resulting from interfacility transfer of colonized patients. *J Infect Dis* 1999;**180**:391–6.
439. Tsai HY, Liao CH, Chen YH, et al. Trends in susceptibility of vancomycin-resistant *Enterococcus faecium* to tigecycline, daptomycin, and linezolid and molecular epidemiology of the isolates: results from the Tigecycline In Vitro Surveillance in Taiwan (TIST) study, 2006 to 2010. *Antimicrob Agents Chemother* 2012;**56**:3402–5.
440. Tsiatis AC, Manes B, Calder C, et al. Incidence and clinical complications of vancomycin-resistant enterococcus in pediatric stem cell transplant patients. *Bone Marrow Transplant* 2004;**33**:937–41.
441. Tumbarello M, Viale P, Viscoli C, et al. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemase–producing *K. pneumoniae:* importance of combination therapy. *Clin Infect Dis* 2012;**55**:943–50.
442. Ulijasz AT, Weisblum B. Dissecting the VanRS signal transduction pathway with specific inhibitors. *J Bacteriol* 1999;**181**:627–31.
443. Urbina R, Prado V, Canelo E. Trimethoprim resistance in enterobacteria isolated in Chile. *J Antimicrob Chemother* 1989;**23**:143–9.
444. U.S. Public Health Service. Public health dispatch: vancomycin-resistant *Staphylococcus aureus*—Pennsylvania, 2002. *Morb Mortal Wkly Rpt* 2002;**51**:902.
445. U.S. Public Health Service. *Staphylococcus aureus* resistant to vancomycin—United States, 2002. *Morb Mortal Wkly Rpt* 2002;**51**:565–7.
446. U.S. Public Health Service. Brief report: vancomycin-resistant Staphylococcus aureus—New York, 2004. *Morb Mortal Wkly Rpt* 2004;**53**:322–3.
447. Van Eldere J. Multicentre surveillance of *Pseudomonas aeruginosa* susceptibility patterns in nosocomial infections. *J Antimicrob Chemother* 2003;**51**:347–52.
448. van Hal SJ, Paterson DL, Gosbell IB. Emergence of daptomycin resistance following vancomycin-unresponsive *Staphylococcus aureus* bacteraemia in a daptomycin-naive patient—a review of the literature. *Eur J Clin Microbiol Infect Dis* 2011;**30**:603–10.
449. Vanhoof R, Nyssen HJ, Van Bossuyt E, et al. Aminoglycoside resistance in gram-negative blood isolates from various hospitals in Belgium and the Grand Duchy of Luxembourg. Aminoglycoside Resistance Study Group. *J Antimicrob Chemother* 1999;**44**:483–8.
450. Varaldo PE, Debbia EA, Nicoletti G, et al. Nationwide survey in Italy of treatment of *Streptococcus pyogenes* pharyngitis in children: influence of macrolide resistance on clinical and microbiological outcomes. Artemis-Italy Study Group. *Clin Infect Dis* 1999;**29**:869–73.
451. Viau RA, Hujer AM, Marshall SH, et al. "Silent" dissemination of *Klebsiella pneumoniae* isolates bearing *K. pneumoniae* carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. *Clin Infect Dis* 2012;**54**:1314–21.
452. Vila J, Ruiz J, Marco F, et al. Association between double mutation in *gyrA* gene of ciprofloxacin-resistant clinical isolates of *Escherichia coli* and MICs. *Antimicrob Agents Chemother* 1994;**38**:2477–9.
453. Villar RG, Macek MD, Simons S, et al. Investigation of multidrug-resistant *Salmonella* serotype typhimurium DT104 infections linked to raw-milk cheese in Washington State. *JAMA* 1999;**281**:1811–16.
454. von Baum H, Schehl J, Geiss HK, et al. Prevalence of vancomycin-resistant enterococci among children with end-stage renal failure. Mid-European Pediatric Peritoneal Dialysis Study Group. *Clin Infect Dis* 1999;**29**:912–16.
455. Wain J, Kidgell C. The emergence of multidrug resistance to antimicrobial agents for the treatment of typhoid fever. *Trans R Soc Trop Med Hyg* 2004;**98**:423–30.
456. Walsh CT. Vancomycin resistance: decoding the molecular logic. *Science* 1993;**261**:308–9.
457. Walsh TR, Howe RA. The prevalence and mechanisms of vancomycin resistance in *Staphylococcus aureus*. *Annu Rev Microbiol* 2002;**56**:657–75.
458. Walsh TR, Toleman MA, Poirel L, et al. Metallo-beta-lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005;**18**:306–25.
459. Wang Y, Huang WM, Taylor DE. Cloning and nucleotide sequence of the *Campylobacter jejuni gyrA* gene and characterization of quinolone resistance mutations. *Antimicrob Agents Chemother* 1993;**37**:457–63.
460. Warren JW, Abrutyn E, Hebel JR, et al. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in women. Infectious Diseases Society of America (IDSA). *Clin Infect Dis* 1999;**29**:745–58.
461. Weber JT. Community-associated methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* 2005;**41**(Suppl. 4):S269–72.
462. Weill FX, Guesnier F, Guibert V, et al. Multidrug resistance in *Salmonella enterica* serotype Typhimurium from humans in France (1993 to 2003). *J Clin Microbiol* 2006;**44**:700–8.
463. Weinberg GA, Spitzer ED, Murray PR, et al. Antimicrobial susceptibility patterns of *Haemophilus* isolates from children in eleven developing nations. BOSTID Haemophilus Susceptibility Study Group. *Bull World Health Organ* 1990;**68**:179–84.
464. Weisblum B. Insights into erythromycin action from studies of its activity as inducer of resistance. *Antimicrob Agents Chemother* 1995;**39**:797–805.
465. Weiss K, De Azavedo J, Restieri C, et al. Phenotypic and genotypic characterization of macrolide-resistant group A *Streptococcus* strains in the province of Quebec, Canada. *J Antimicrob Chemother* 2001;**47**:345–8.
466. Westh H, Hougaard DM, Vuust J, et al. Prevalence of *erm* gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959 and 1988. *Antimicrob Agents Chemother* 1995;**39**:369–73.
467. White DG, Zhao S, Sudler R, et al. The isolation of antibiotic-resistant salmonella from retail ground meats. *N Engl J Med* 2001;**345**:1147–54.
468. Whitney CG, Farley MM, Hadler J, et al. Increasing prevalence of multidrug-resistant *Streptococcus pneumoniae* in the United States. *N Engl J Med* 2000;**343**:1917–24.
469. Willems RJ, Top J, van Santen M, et al. Global spread of vancomycin-resistant *Enterococcus faecium* from distinct nosocomial genetic complex. *Emerg Infect Dis* 2005;**11**:821–8.
470. Wilson P, Andrews JA, Charlesworth R, et al. Linezolid resistance in clinical isolates of *Staphylococcus aureus*. *J Antimicrob Chemother* 2003;**51**:186–8.
471. Wisplinghoff H, Rosato AE, Enright MC, et al. Related clones containing SCCmec type IV predominate among clinically significant *Staphylococcus epidermidis* isolates. *Antimicrob Agents Chemother* 2003;**47**:3574–9.
472. Wistrom J, Norrby SR. Fluoroquinolones and bacterial enteritis, when and for whom? *J Antimicrob Chemother* 1995;**36**:23–39.
473. Won SY, Munoz-Price LS, Lolans K, et al. Emergence and rapid regional spread of *Klebsiella pneumoniae* carbapenemase–producing Enterobacteriaceae. *Clin Infect Dis* 2011;**53**:532–40.
474. Woodford N, Tierno PM Jr, Young K, et al. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. *Antimicrob Agents Chemother* 2004;**48**:4793–9.
475. Wretlind B, Stromberg A, Ostlund L, et al. Rapid emergence of quinolone resistance in *Campylobacter jejuni* in patients treated with norfloxacin. *Scand J Infect Dis* 1992;**24**:685–6.
476. Wright SW, Wrenn KD, Haynes ML. Trimethoprim-sulfamethoxazole resistance among urinary coliform isolates. *J Gen Intern Med* 1999;**14**:606–9.
477. Wubbel L, McCracken GH Jr. Management of bacterial meningitis: 1998. *Pediatr Rev* 1998;**19**:78–84.
478. Wubbel L, Muniz L, Ahmed A, et al. Etiology and treatment of community-acquired pneumonia in ambulatory children. *Pediatr Infect Dis J* 1999;**18**:98–104.

479. Xiang X, Shannon K, French G. Mechanism and stability of hyperproduction of the extended-spectrum beta-lactamase SHV-5 in *Klebsiella pneumoniae*. *J Antimicrob Chemother* 1997; **40**:525–32.
480. Yoshida H, Bogaki M, Nakamura S, et al. Nucleotide sequence and characterization of the *Staphylococcus aureus norA* gene, which confers resistance to quinolones. *J Bacteriol* 1990;**172**: 6942–9.
481. Yoshida H, Bogaki M, Nakamura M, et al. Quinolone resistance–determining region in the DNA gyrase *gyrA* gene of *Escherichia coli*. *Antimicrob Agents Chemother* 1990;**34**:1271–2.
482. Yoshida H, Bogaki M, Nakamura M, et al. Quinolone resistance-determining region in the DNA gyrase *gyrB* gene of *Escherichia coli*. *Antimicrob Agents Chemother* 1991;**35**:1647–50.
483. Yoshizumi S, Takahashi Y, Watanabe Y, et al. In vitro antibacterial activities of new fluoroquinolones against clinical isolates of haemophilus influenzae with ciprofloxacin-resistance-associated alterations in GyrA and ParC. *Chemotherapy* 2004;**50**:265–75.
484. Zarkotou O, Pournaras S, Tselioti P, et al. Predictors of mortality in patients with bloodstream infections caused by KPC-producing *Klebsiella pneumoniae* and impact of appropriate antimicrobial treatment. *Clin Microbiol Infect* 2011;**17**:1798–803.
485. Zarrilli R, Tripodi MF, Di Popolo A, et al. Molecular epidemiology of high-level aminoglycoside-resistant enterococci isolated from patients in a university hospital in southern Italy. *J Antimicrob Chemother* 2005;**56**:827–35.
486. Zetola N, Francis JS, Nuermberger EL, et al. Community-acquired methicillin-resistant *Staphylococcus aureus:* an emerging threat. *Lancet Infect Dis* 2005;**5**:275–86.
487. Zhanel GG, Hisanaga TL, Laing NM, et al. Antibiotic resistance in *Escherichia coli* outpatient urinary isolates: final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int J Antimicrob Agents* 2006;**27**:468–75.
488. Zhanel GG, Karlowsky JA, Harding GK, et al. A Canadian national surveillance study of urinary tract isolates from outpatients: comparison of the activities of trimethoprim-sulfamethoxazole, ampicillin, mecillinam, nitrofurantoin, and ciprofloxacin. The Canadian Urinary Isolate Study Group. *Antimicrob Agents Chemother* 2000;**44**:1089–92.
489. Zhanel GG, Karlowsky JA, Saunders MH, et al. Development of multiple-antibiotic-resistant (Mar) mutants of *Pseudomonas aeruginosa* after serial exposure to fluoroquinolones. *Antimicrob Agents Chemother* 1995;**39**:489–95.
490. Zhanel GG, Palatnick L, Nichol KA, et al. Antimicrobial resistance in *Haemophilus influenzae* and *Moraxella catarrhalis* respiratory tract isolates: results of the Canadian Respiratory Organism Susceptibility Study, 1997 to 2002. *Antimicrob Agents Chemother* 2003;**47**:1875–81.
491. Zhang L, Li XZ, Poole K. Multiple antibiotic resistance in *Stenotrophomonas maltophilia:* involvement of a multidrug efflux system. *Antimicrob Agents Chemother* 2000;**44**:287–93.
492. Zhao C, Sun H, Wang H, et al. Antimicrobial resistance trends among 5608 clinical hram-positive isolates in China: results from the Gram-Positive Cocci Resistance Surveillance program (2005-2010). *Diagn Microbiol Infect Dis* 2012;**73**:174–81.
493. Zhao X, Xu C, Domagala J, et al. DNA topoisomerase targets of the fluoroquinolones: a strategy for avoiding bacterial resistance. *Proc Natl Acad Sci U S A* 1997;**94**:13991–6.

pharmacodynamics) and drug disposition (i.e., pharmacokinetics) is of critical importance in the selection and evaluation of any anti-infective drug regimen.

The *pharmacokinetic-pharmacodynamic interface* reflects an association between two determinants of drug effect—delivery of drug to the site of action and the intrinsic activity of a drug to alter cellular function once it reaches that site. In much of the contemporary literature in clinical pharmacology and therapeutics, *kinetics* generally is associated with a description of the rate processes associated with drug absorption, distribution, metabolism, and excretion (ADME). Although accurate in its definition concerning the movement of drugs into and out of the body, kinetics as a determinant of drug effect must be viewed in a much broader context, specifically, the sojourn and fate of a drug molecule in both the extracellular and intracellular milieu. Although understanding this facet of drug behavior admittedly is difficult because of a relative inability in the clinical context to track the intracellular fate of a drug in humans, achieving a high degree of control over anti-infective therapy by the application of well-characterized and understood kinetic principles is quite possible.

The focus of this chapter is on the pharmacologic considerations necessary for the prudent use of anti-infective agents in pediatric patients, specifically, the pharmacokinetic and pharmacodynamic principles that, if embraced, can be the key to optimizing drug treatment and maximizing therapeutic efficacy and safety. The pediatric patient presents a particular challenge to the clinician. Although pediatric practitioners generally recognize that "children are not just miniature adults," one may not appreciate that the dramatic changes associated with normal human growth and development can, and many times do, exert a profound influence on drug disposition, drug action, and, ultimately, therapeutic outcome.[113] Failure to compensate for developmental differences in pharmacokinetics in the context of therapeutic drug use in pediatrics can increase the risk of occurrence of adverse drug effects associated with either overdose (i.e., drug toxicity) or underdose (i.e., lack of efficacy).[78,113]

In the following sections of this chapter the basic principles are presented that are required to understand the pharmacokinetic-pharmacodynamic interface in pediatrics, namely, general principles in pharmacokinetics, the impact of development on drug disposition, the pharmacokinetic determinants of drug action, and methods to facilitate clinical integration of the pharmacokinetic and pharmacodynamic properties necessary to optimize anti-infective therapy.

PHARMACOKINETIC DETERMINANTS OF EXPOSURE

Implicit in the production of any pharmacologic effect is the association of a drug molecule with one or more receptors and the subsequent propagation of intracellular events that ultimately translate into drug action. In general terms, successful drug-receptor interactions are characterized by three principles: (1) *avidity*, the ability of a drug to combine with a receptor; (2) *affinity*, the physical combination of a drug with a receptor, and (3) *intrinsic activity*, the ability of the drug-receptor combination to generate one or more "impulses" or "signals" capable of activating biologic effector systems. The primary determinants of both avidity and affinity reside with the maintenance of structural specificity of both the drug and receptor to ensure that an association with one or more physicochemically distinct active sites can occur. Simply stated, a drug molecule must fit into a receptor as a key fits into a lock before intrinsic activity becomes a possibility. The receptor or receptors thus will function in the role of cellular targets for drug molecules when an effective combination is required for drug effect and, ultimately, therapeutic efficacy. The pharmacodynamic implications of these targets are discussed in the second half of this chapter and in other chapters in this textbook in the context of specific anti-infective agents.

The onset and offset of drug action are determined not only by receptor-modulated intrinsic activity but also by receptor occupancy. In almost all instances, these events are both concentration and time dependent. Hence, the disposition of a drug in the body (e.g., ADME) becomes the "driver" for its pharmacodynamics. This action is particularly true for anti-infective drugs when the therapeutic target, the infecting organism, must be exposed to a sufficient concentration of pharmacologically active (i.e., free) drug for a period sufficient for drug binding to critical cellular elements (e.g., penicillin-binding proteins [PBPs], intracellular enzymes) to occur and for subsequent disruption of normal cellular function (e.g., inhibition of protein biosynthesis, inhibition of cell wall synthesis) to result in cellular demise. Thus, the clinical determinants of drug efficacy (and safety), such as proper selection of both dose and dosing interval relative to the intrinsic sensitivity of the infecting pathogen or pathogens, and factors that determine delivery of the drug to the site or sites of infection embody the importance of pharmacokinetics in the selection and clinical use of anti-infective drugs.

BASIC TERMS

A complete discussion of pharmacokinetics is beyond the scope of this chapter because it would require a description of theory and a presentation of relatively sophisticated mathematic concepts. This information can be found in many excellent textbooks that provide both theoretical[77] and conceptual[113,190,237] presentations of pharmacokinetics and through Internet-based programmed instruction courses.[25] However, well within the scope of this chapter are a definition and glossary of pharmacokinetic terms sufficient to equip the reader with a conceptual, working knowledge of this pharmacotherapeutic tool. These definitions and concepts are presented as follows:

Absolute bioavailability (F) is the extent or fraction of drug absorbed after extravascular administration. It is determined by comparing the area under the plasma concentration versus time curve (AUC) after administration of an oral dose of a drug with the AUC after

administration of an intravenous dose (e.g., $F = AUC_{PO} \times dose_{IV}/AUC_{IV} \times dose_{PO}$).

Absorption of drugs describes the process of drug uptake from a site of extravascular administration (e.g., oral, intramuscular, subcutaneous, intraperitoneal, intraosseous, intratracheal, intravaginal, intraurethral, sublingual, buccal, rectal, and dermal) into the systemic circulation. Drug absorption is conceptualized most accurately by considering both rate (e.g., absorption half-life, time to peak concentration) and extent (e.g., bioavailability), either of which can be influenced by biopharmaceutical (e.g., drug formulation), physicochemical (e.g., pH, solubility, hydrophilicity and lipophilicity, protein binding, complexation characteristics with food or drugs), and physiologic factors (e.g., barrier integrity, motility, volume and pH of body fluids at the absorptive site, protein-binding capacity, degradation/biotransformation potential).

Area under the curve (AUC) is, conceptually, a measure of both the extent of drug absorbed and its persistence in the body. It is the integral of drug blood levels over time from zero to either a predetermined postdose time point (i.e., $AUC_{0\rightarrow tx}$) or extrapolated to infinity (i.e., $AUC_{0\rightarrow\infty}$) by using the apparent terminal elimination rate constant (similarly calculated from the observed plasma concentration versus time plot) (Fig. 234-1). AUC is therefore a pharmacokinetic parameter that is both time and concentration dependent.

Bioequivalence of a drug product is achieved if its extent and rate of absorption are not significantly different (i.e., within 80% to 125%) from those of a reference standard drug product when administered at the same molar dose. Bioequivalence is a pharmacokinetically determined parameter and does not entail a relative comparison of drug action or efficacy. Therefore, if a drug product (e.g., a generic drug) produces a rate and extent of absorption sufficiently similar to those of the reference formulation, the effect and toxicity profiles of the two drugs are assumed to be virtually identical.

Biopharmaceutics deals with the physical and chemical properties of the drug substance, the dosage form, and the body, as well as the biologic actions of a drug or drug product after administration. Biopharmaceutical considerations (e.g., rate and extent of the disintegration or dissolution of a dosage form, liberation of the active drug from a dosage form, solubility or binding of the drug at the site of absorption) can be rate limiting for drug absorption or bioavailability, or for both, and thus may limit the efficacy of drug therapy.

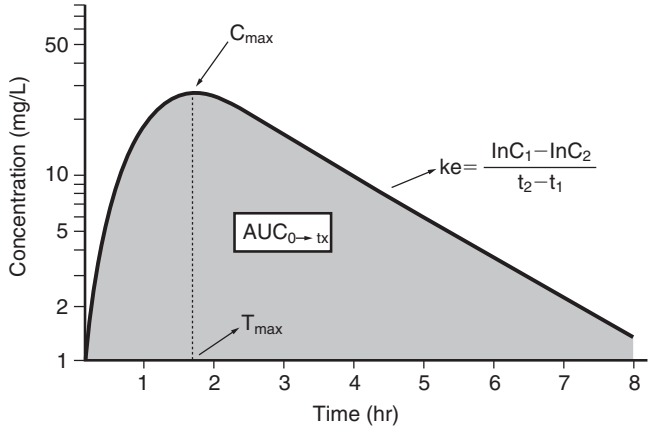

FIGURE 234-1 ■ Representative plasma concentration versus time curve. Illustrated are commonly referenced pharmacokinetic parameters, including AUC, area under the curve; C_{max}, maximal plasma concentration; ke, elimination rate constant; and T_{max}, time to achieve maximal plasma concentration.

Clearance (Cl) of a drug is represented conceptually by the volume of blood from which a certain amount of unmetabolized drug is removed (i.e., cleared) per unit of time by any and all pathways capable of drug removal (e.g., renal, hepatic, biliary, pulmonary, breast milk, sweat). In pharmacokinetics, clearance generally is represented as total-body (or plasma) clearance, renal clearance (Cl_{ren}), or nonrenal clearance (Cl_{nr}). Clearance is determined easily from knowledge of the drug dose, and AUC and can be calculated as follows:

$$Cl = Dose\ (mg/kg)/AUC\ (mg/L \times hr)$$

where AUC can represent either the $AUC_{0\rightarrow tx}$ for single-dose administration or the AUC from time zero to the end of the dosing interval at steady state (i.e., $AUCss_{0\rightarrow\tau}$). Calculation of renal clearance requires a complete, quantitative collection of urine (usually over the course of 24 hours) to determine the amount of drug excreted (Ae) unchanged. Nonrenal clearance generally is determined as the difference between total body clearance and renal clearance. For drug administration *by any extravascular route*, calculation of clearance yields an apparent value (e.g., Cl/F) in that it must be corrected for the extent of the drug dose absorbed (i.e., the bioavailability) from the site of administration.

A *compartment* in pharmacokinetics represents a *hypothetical space* into and out of which drug partitions as a function of time. Compartment *models* are used in pharmacokinetics to characterize the relationship between drug dose and concentration as a function of time. The most simple of all pharmacokinetic models is a *one-compartment open model* in which the only applicable rate processes represent drug ingress and egress from a single theoretical or central space. A *two-compartment model* has been used to characterize the disposition of many anti-infective agents in both pediatric and adult subjects. This particular model is composed of a central compartment and a peripheral compartment, both of which represent the various fluids and tissues where a drug may reside. Although a compartment often may not correspond to a true physiologic space that can be characterized by a specific volume of biologic fluid, compartment models can be used to conceptualize both drug distribution between physiologic spaces and elimination from the body. In the two-compartment model, for example, the central compartment often is used to represent a drug resident within the intravascular space and the highly exchangeable extracellular fluid of tissues and organs that are well perfused, whereas the peripheral compartment represents drug distribution to intracellular fluid and tissues and organs that are less well perfused and, in some instances, the association of the drug (e.g., binding) to specific tissues or tissue components. In all instances, compartmental models oversimplify the true processes of

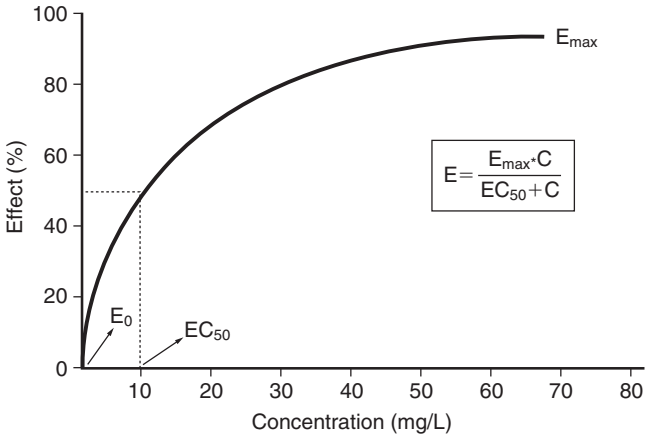

FIGURE 234-2 ■ Representative nonlinear concentration (C) versus effect profile. Emax, maximal effect; EC50, drug concentration for which the observed effect is 50 percent of the maximal effect.

ADME. However, they have been demonstrated repeatedly to be useful as a reliable means to model, and therefore predict, the relationship between drug dose and concentration in both plasma and tissue.

Disposition refers collectively to the processes of ADME, all of which occur simultaneously after administration of a drug, as opposed to being discrete pharmacologic events.

Dose-response curve is a graphic representation of the pharmacologic effect as a function of either the drug dose or the concentration of the drug. A log dose-response curve is often sigmoidal, whereas a cartesian concentration-effect curve is hyperbolic (Fig. 234-2). Theoretically, a drug concentration of zero indicates no drug effect (i.e., E_0). As a drug travels to and interacts with a receptor, the effect (E) increases in a concentration-dependent manner (i.e., E [(E_{max} C)/(E_50 + C)]) to a point at which a maximal effect (E_{max}) is attained and above which higher drug concentrations fail to enhance the effect. A pharmacologically important term that can be derived easily from a concentration-effect curve is the EC_{50}, or the drug concentration for which the observed effect is 50 percent of the E_{max}.

Elimination half-life of a drug is the time necessary to reduce the drug concentration (in blood serum or plasma) by 50 percent after absorption is complete and distribution between body compartments has attained equilibrium. Loss of drug from the body, as represented by the elimination half-life, reflects elimination of the administered parent drug molecule (i.e., not its metabolites) by metabolism, urinary excretion, or other pathways capable of resulting in elimination of drug. Accordingly, many individuals use elimination half-life as a surrogate indicator of drug clearance. Such a determination should be made cautiously because this particular pharmacokinetic parameter is dependent on both clearance and the apparent volume of distribution (VD), as illustrated by the following equation: $t_{1/2}$ elimination = [(0.693 × VD)/Cl], where $t_{1/2}$ is half-life. Practically speaking, the elimination half-life is an important pharmacokinetic parameter because it can be used to determine the period of time required for a drug dosing regimen to produce steady-state plasma concentrations (e.g., 5 × $t_{1/2}$ elimination) and the dosing interval required to produce a desired excursion (i.e., peak and trough) in plasma drug concentrations.

First-pass effect describes a phenomenon whereby drugs may be metabolized or chemically degraded (or both) after extravascular administration before they reach the systemic circulation. Specific examples include biotransformation of selected drugs (e.g., cytochrome CYP3A4 substrates) in the enterocyte and nonenzymatic hydrolysis of an active drug (e.g., aspirin) in the lumen of the gastrointestinal tract (e.g., stomach, intestine, rectum). Drugs subject to a first-pass effect generally have a reduced rate or extent of relative bioavailability, or both, when compared with that achieved by parenteral administration.

Protein binding is the phenomenon that occurs when a drug (or metabolite) combines with plasma or extracellular or tissue proteins to form a drug-protein complex. In general, drug-protein binding usually is nonspecific and depends on the drug's affinity for the protein molecule (i.e., binding site), the number of protein-binding sites, and the drug and protein concentrations. With few exceptions, drugs that are bound to proteins are pharmacologically inactive and cannot be metabolized or excreted readily. The pharmacokinetic consequences of drug-protein binding can influence the drug dose versus the plasma concentration versus the effect relationship. For example, drugs with extensive tissue binding have apparent volumes of distribution that are far in excess of the total-body water space and, in general, have relatively long elimination half-lives. Conditions in which intravascular proteins escape to extravascular sites (e.g., nephrotic syndrome, severe burns, ascites) can increase the apparent volume (and elimination half-life) of drugs that are extensively (i.e., >70%) bound to albumin.

Relative bioavailability reflects the extent of drug absorbed from one dosage form given by an extravascular route of administration in comparison to a dose of a "standard" drug formulation administered by the same route. Generally, it reflects the relative extent of systemic availability (F) and is calculated by comparing the AUC of the test regimen relative to the standard formulation (e.g., F = AUC_{test} × $dose_{standard}$/$AUC_{standard}$ × $dose_{test}$).

Steady state reflects the level of drug accumulation in the blood and tissue after multiple doses when input (i.e., the amount of drug placed into the systemic circulation) and output (i.e., drug clearance) are at equilibrium. When drugs are given at fixed doses and dosing intervals, the steady-state concentrations fluctuate between a maximum (C_{max}) and minimum (C_{min}) within a given dose interval that is identical between doses, provided that the size of the dose, method of administration, dosing interval, or drug pharmacokinetics (or any combination of these parameters) does not change. For drugs that follow first-order pharmacokinetics, steady-state plasma concentrations for a given dosing regimen are attained over a period of time that corresponds to four to five times the elimination half-life. In general, the pharmacokinetics of a drug at steady state provides the most accurate correlate for examining drug effect.

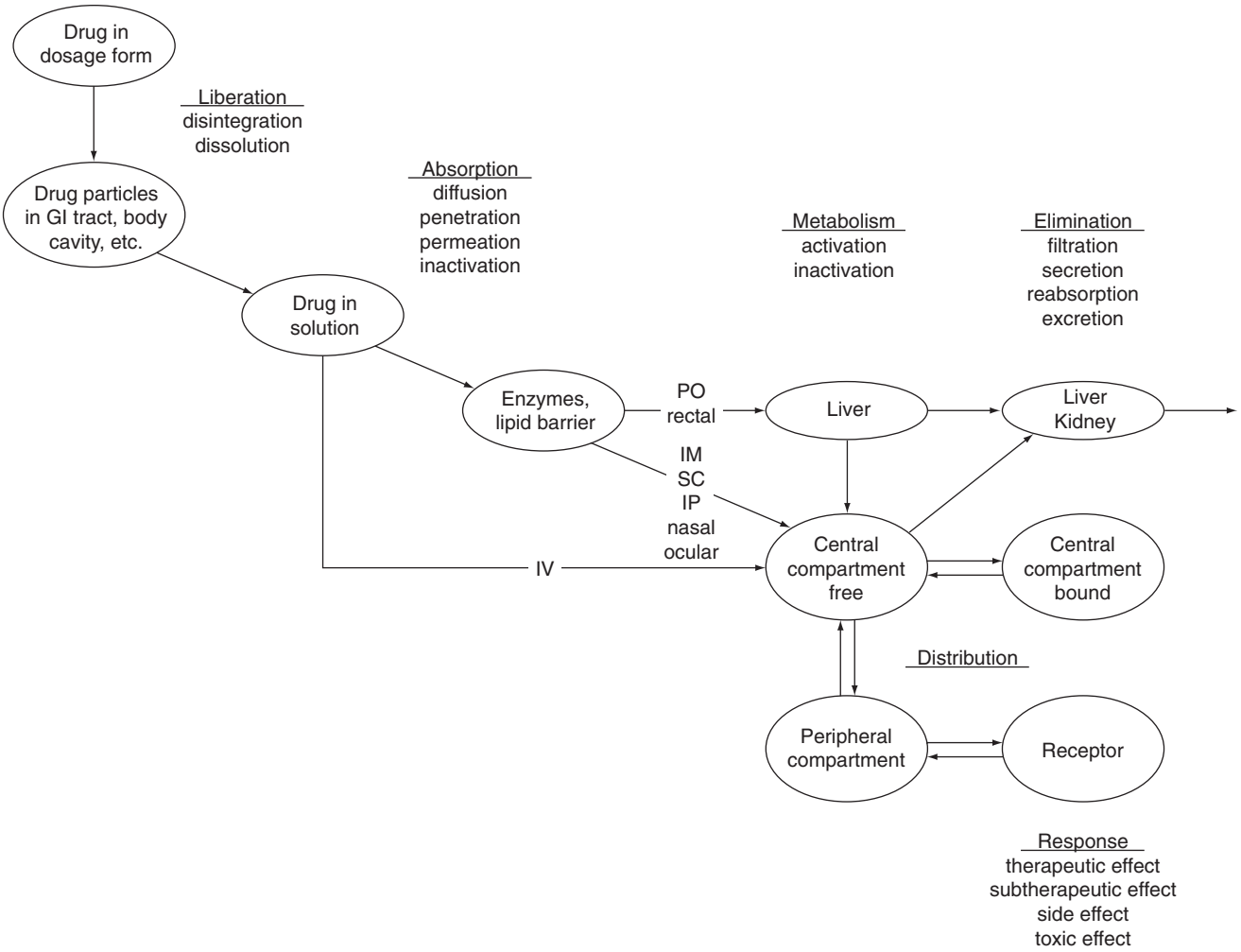

FIGURE 234-3 ■ Graphic representation of the pharmacokinetic-pharmacodynamic interface. GI, gastrointestinal; IM, intramuscular; IP, intraperitoneal; PO, oral; SC, subcutaneous.

Volume of distribution *(apparent volume of distribution)* (VD) represents a hypothetic volume of body fluid that would be required to dissolve the total amount of a drug at the same concentration as that found in the blood and is illustrated by the following equation:

$$VD = Dose/Cp^0$$

where Cp^0 represents the highest attainable plasma concentration after the administration of a single dose. As a proportionality constant, the VD is a determinant of plasma drug concentrations attained after administration of a given drug dose. For drugs that are not distributed extensively or that bind with great affinity to proteins and tissues, the apparent VD may correspond dimensionally to physiologic or anatomic body spaces (e.g., VD <0.1 L/kg approximates the intravascular space, 0.1 to 0.3 L/kg approximates the extracellular space, 0.6 to 0.7 L/kg approximates the total-body water space), which, when altered by disease or development, or by both, will influence the apparent VD and thus the achievable concentration for a given drug.

A working knowledge of these pharmacokinetic definitions allows one to have an understanding of the relationships among drug dose, concentration, and effect. The use of knowledge related to the physicochemical and pharmacologic properties of a drug, the impact of isolated or concurrent variables on drug disposition, and predictors of pharmacodynamics or drug response enables the clinician to optimize the selection of agents and dosing regimens and thus individualize drug therapy. To accomplish this goal, it is imperative that the relationship between drug pharmacokinetics and pharmacodynamics not be compartmentalized but rather, as illustrated in Figure 234-3, be conceptualized as multifactorial, in which the determinants of drug concentration and effect are dynamic and change as a function of a disease state and drug therapy.

IMPACT OF ONTOGENY ON PHARMACOKINETICS

Development represents a continuum of biologic events that enable adaptation, somatic growth, neurobehavioral maturation, and, eventually, reproduction. The impact of development on the pharmacokinetics of a given drug is

determined, to a great degree, by age-related changes in body composition and the acquisition of function in organs and organ systems that are important in determining drug metabolism and excretion. Although classifying pediatric patients on the basis of postnatal age (e.g., neonates, ≤1 month of age; infants, 1 to 24 months of age; children, 2 to 12 years of age; and adolescents, 12 to 18 years of age) often is convenient for providing drug therapy, recognizing that the changes in physiology are not linearly related to age and may not correspond to these age-defined breakpoints is important. In fact, the most dramatic changes in drug disposition occur during the first 18 months of life, when the acquisition of organ function is most dynamic. Additionally, the pharmacokinetics of a given drug may be altered in pediatric patients as a result of intrinsic (e.g., gender, genotype, ethnicity, inherited diseases) or extrinsic (e.g., acquired disease states, xenobiotic exposure, diet) factors that may occur during the first 2 decades of life.

Selection of an appropriate drug dose for a neonate, infant, child, or adolescent requires an understanding of the basic pharmacokinetic properties of a given compound and how the process of development affects each facet of drug disposition. Accordingly, conceptualizing pediatric pharmacokinetics by examining the impact of development on the physiologic variables that govern ADME is most useful.

Drug Absorption

The rate and extent of gastrointestinal absorption depend primarily on pH-dependent passive diffusion and motility of the stomach and small intestine, both of which control transit time. In full-term neonates, gastric pH ranges from 6 to 8 at birth and drops to 2 to 3 within the first few hours. After the first 24 hours of extrauterine life, gastric pH increases to 6 to 7 as a result of immaturity of the parietal cells. A relative state of achlorhydria remains until adult values and diurnal patterns of gastric pH are reached at 20 to 30 months of age. Although basal gastric acid output can be quite similar in neonates, young infants, and adults, stimulated acid output can be threefold to fourfold higher than that seen in adults.[53,54]

In neonates, gastrointestinal transit time is prolonged as a result of reduced motility and peristalsis. Gastric emptying is both irregular and erratic and only partially dependent on feeding. Gastric emptying rates approximate adult values by the time the infant reaches 6 to 8 months of age. During infancy, intestinal transit time generally is reduced relative to adult values because of increased intestinal motility.[113] In contrast to developmental changes in motility, histologic examination of the luminal absorptive surface suggests that the adult pattern of architecture (and hence absorptive surface area relative to body size) is present at birth.[82,233] In neonates and young infants, additional factors, including diminished splanchnic blood flow, immature biliary function, variable microbial colonization, and an apparent reduction in the activity of intestinal drug-metabolizing enzymes, may play a role in intestinal drug absorption.[41,183]

These developmental changes in gastrointestinal function and structure in the neonatal period and early infancy produce alterations in drug absorption that are quite predictable. In general, the oral bioavailability of acid-labile compounds (e.g., β-lactam antibiotics) is increased, whereas that of weak organic acids (e.g., phenobarbital, phenytoin) is decreased. For orally administered drugs with limited water solubility (e.g., phenytoin, carbamazepine), the rate of absorption (i.e., T_{max}) can be altered dramatically as a result of changes in gastrointestinal motility.[183] The pharmacokinetics of the antiviral agent pleconaril similarly provides an example of developmental differences in drug absorption. After administration of a 5-mg/kg dose, the AUC was much lower in neonates than either children or adults, thus demonstrating that the extent of pleconaril bioavailability in neonates was reduced. The reason for this difference may be attributed to the lipid-based pleconaril formulation and to developmental differences in the ability to absorb lipids as a consequence of biliary immaturity and reduced lipase secretion in neonates.[115]

In neonates and young infants, both rectal and percutaneous absorption is highly efficient for properly formulated drug products. The bioavailability of many drugs administered by the rectal route is increased as a result of not only efficient translocation across the rectal mucosa but also a reduced first-pass effect caused by the immaturity of a number of drug-metabolizing enzymes in the liver. Both the rate and extent of percutaneous drug absorption are increased because of the thinner and better hydrated stratum corneum in young infants. As a consequence, systemic toxicity can occur with the percutaneous application of some drugs (e.g., hexachlorophene) to seemingly small areas of skin during the first 8 to 12 months of life. In contrast to older infants and children, the rate of bioavailability for drugs administered by the intramuscular route may be altered (i.e., delayed T_{max}) more than the extent of absorption in a neonate. This developmental pharmacokinetic alteration is the consequence of relatively low muscular blood flow in the first few days of life, the relative inefficiency of muscular contractions (useful in dispersing an intramuscular drug dose), and an increased percentage of water per unit of muscle mass.[183]

Developmental differences in drug absorption among neonates, infants, and older children are summarized in Table 234-1. The data contained therein reflect developmental differences that may be expected to occur in healthy pediatric patients. Certain conditions or disease states that could modify the function or structure, or both, of the absorptive surface area, gastrointestinal motility, or systemic blood flow can further affect either the rate or the extent of absorption for extravascularly administered drugs in pediatric patients.

Distribution

Development is associated with marked changes in body composition as reflected by examination of total-body water, extracellular water, and stores of body fat (Fig. 234-4). The most dynamic changes occur in the first year of life, with the exception of total-body fat, which has a distinctly different pattern in male and female children. Furthermore, the adipose tissue of neonates may contain

TABLE 234-1 **Summary of Drug Absorption in Neonates, Infants, and Children***

	Neonates	Infants	Children
Physiologic Alteration			
Gastric emptying time	Irregular	Increased	Slightly increased
Gastric pH	>5	4 to 2	Normal (2-3)
Intestinal motility	Reduced	Increased	Slightly increased
Intestinal surface area	Reduced	Near adult	Adult pattern
Microbial colonization	Reduced	Near adult	Adult pattern
Biliary function	Immature	Near adult	Adult pattern
Muscular blood flow	Reduced	Increased	Adult pattern
Skin permeability	Increased	Increased	Near adult pattern
Possible Pharmacokinetic Consequences			
Oral absorption	Erratic: reduced	Increased rate	Near adult pattern
Intramuscular absorption	Variable	Increased	Adult pattern
Percutaneous absorption	Increased	Increased	Near adult pattern
Rectal absorption	Very efficient	Efficient	Near adult pattern
Presystemic clearance	Less than adult	Greater than adult	Greater than adult (increased rate)

*The direction of alteration is given relative to the expected normal adult pattern.
Modified from Ritschel WA, Kearns GL. Pediatric pharmacokinetics. In: Ritschel WA, Kearns GL, editors. *Handbook of basic pharmacokinetics.* 6th ed. Washington, D.C.: American Pharmaceutical Association; 2004, p. 227-40.

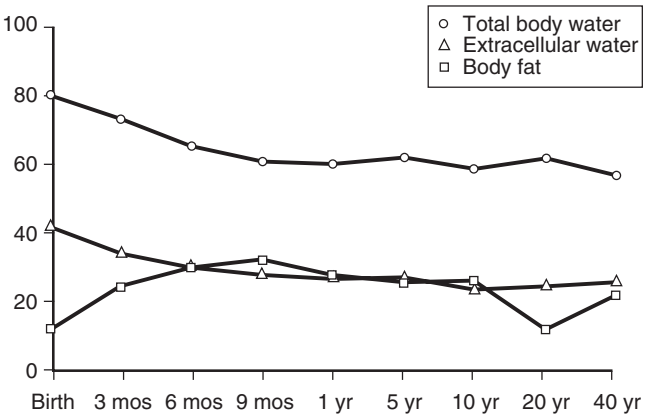

FIGURE 234-4 ■ Body composition reflected as a percentage (*y*-axis) of total-body mass for total-body water (TBW), extracellular water (ECW), and body fat as a function of age (*x*-axis). (From Ritschel WA, Kearns GL. Pediatric pharmacokinetics. In: Ritschel WA, Kearns GL, editors. *Handbook of basic pharmacokinetics.* 6th ed. Washington, D.C.: American Pharmaceutical Association; 2004, p. 227-40.)

as much as 57 percent water and 35 percent lipids, whereas values in adults approach 26.3 percent and 71.7 percent, respectively.[183]

In addition to age-related alterations in body composition, several physiologic changes that occur during the neonatal period are capable of altering the plasma protein binding of drugs (Table 234-2). In neonates, the free fraction of drugs that are bound extensively to circulating plasma proteins is increased markedly, largely because of lower concentrations of drug-binding proteins, reduced binding affinity of these proteins, the presence of a relatively acidic plasma pH, and endogenous competing ligands (e.g., bilirubin, free fatty acids). This consideration is exemplified by ceftriaxone, a weak acid that is approximately 95 percent bound to albumin in adults but only 70 percent bound in neonates and thus is capable of producing significant displacement of bilirubin.[92,147] The reduced plasma protein binding in combination with absolute and relative differences in the size of various body compartments (e.g., total-body water, extracellular fluid, composition of body tissues) frequently influences (i.e., increases) the apparent VD for many drugs and also their localization (i.e., both uptake and residence) in tissue, which, in turn, can alter their plasma elimination half-life.

Renal Excretion

The renal excretion of many drugs is directly proportional to age-dependent patterns in the acquisition of renal function, primarily glomerular filtration and active tubular secretion. Accordingly, developmental differences in renal function may serve as a major determinant of drug clearance in neonates and young infants for compounds that are not metabolized extensively.[183]

In preterm infants, renal function is reduced dramatically because of the continued development of functioning nephron units (i.e., nephrogenesis). In contrast, the acquisition of renal function in a term neonate represents, to a great degree, the recruitment of fully developed nephron units. In both term neonates and preterm infants who have birth weights greater than 1500 g, glomerular filtration rates increase dramatically during the first 2 weeks of postnatal life (Fig. 234-5). This particular dynamic change in function is a direct consequence of postnatal adaptations in the distribution of renal blood flow (i.e., medullary distribution to the corticomedullary border) and results in a dramatic recruitment of functioning nephron units.[113] In addition, a glomerular-tubular imbalance exists in which the maturation of the glomerular function is more advanced than is the proximal tubular

TABLE 234-2 **Plasma Protein Binding and Drug Distribution***

	Neonates	Infants	Children
Physiologic Alteration			
Plasma albumin	Reduced	Near normal	Near adult pattern
Fetal albumin	Present	Absent	Absent
Total protein	Reduced	Decreased	Near adult pattern
Serum bilirubin	Increased	Normal	Normal adult pattern
Serum free fatty acids	Increased	Normal	Normal adult pattern
Blood pH	7.1-7.3	7.4 (normal)	7.4 (normal)
Possible Pharmacokinetic Consequences			
Free fraction	Increased	Increased	Slightly increased
Apparent volume of distribution			
Hydrophilic drugs	Increased	Increased	Slightly increased
Hydrophobic drugs	Reduced	Reduced	Slightly decreased
Tissue-to-plasma ratio	Increased	Increased	Slightly increased

*The direction of alteration is given relative to the expected normal adult pattern.
Modified from Ritschel WA, Kearns GL. Pediatric pharmacokinetics. In: Ritschel WA, Kearns GL, editors. *Handbook of basic pharmacokinetics*. 6th ed. Washington, D.C.: American Pharmaceutical Association; 2004, p. 227-40.

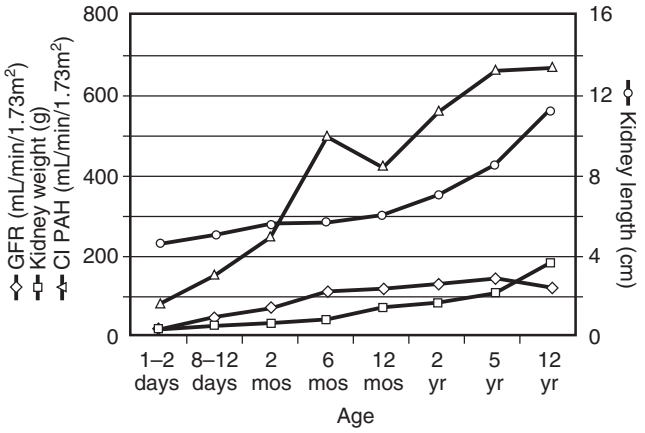

FIGURE 234-5 ■ Ontogeny of renal function. Cl, clearance; GFR, glomerular filtration rate; PAH, *p*-aminohippuric acid. (Modified from Papadopoulou ZL, Tina LU, Sandler P, et al. Size and function of the kidneys. In: Johnson TR, Moore WM, Jeffries JE, editors. *Children are different: developmental physiology*. 2nd ed. Columbus, OH: Ross Laboratories; 1978, p. 97-104.)

TABLE 234-3 **Impact of Ontogeny on the Elimination Half-Life of Drugs with Predominant Renal Routes of Excretion***

Drug	Cl (mL/hr/kg)	VD (mL/kg)	*Elimination $t_{1/2}$ (hr)*	
			NEONATE	ADULT
Gentamicin	35-72	350-500	4.4-11.4	2.5
Tobramycin	41-74	590-840	8.2-11.3	2.0
Amikacin	50	570	8.4	2.5
Cefotaxime	50-100	310-790	3.4-6.4	1.2
Ceftriaxone	44-60	530-610	7.7-8.4	6.5
Ceftazidime	31-42	292-363	5.0-8.7	1.8
Vancomycin	36-78	480-680	8.0-17	6.0

*Data represent average or a range of average values reported from individual studies and summarized by van den Anker and Kearns[213] (for preterm infants) and Ritschel and Kearns[175] for adults.
Cl, total plasma clearance; $t_{1/2}$, half-life; VD, apparent volume of distribution.

secretion. This imbalance may persist for up to 6 to 10 months of age, when both tubular function and glomerular function approach values approximately equal to those observed in healthy, young adults.[183]

The impact of development on each of the components of renal function can be characterized by a definable pattern during the first year of life (see Fig. 234-5).[167] Accordingly, the renal handling and, hence, excretion and elimination characteristics of virtually any drug in neonates, infants, and children can be predicted largely by considering the ontogeny of renal function and the specific pharmacologic characteristics of a given drug with regard to its renal excretion (e.g., routes of renal excretion, the percentage of a given dose excreted unchanged in urine) in adults. For antimicrobial agents, the impact of the ontogeny of the renal function on pharmacokinetics is reflected largely by alterations in the plasma drug clearance. As summarized by van den Anker and Kearns,[223] the elimination half-life of antimicrobial agents from different classes that share predominantly renal pathways of excretion is increased substantially in neonates and young infants (Table 234-3). This increase is not the case for antimicrobial and other agents that are not excreted primarily by the kidneys (e.g., ceftriaxone; see Table 234-3).

Metabolism

Simply stated, drug metabolism (or biotransformation) involves the modification of a drug molecule by one

or more enzymes such that the hydrophilicity of the product is increased relative to the parent drug, thus enhancing elimination and excretion. In most instances, drug metabolism creates products that are either less pharmacologically active than is the parent drug (e.g., desacetylcefotaxime) or devoid of pharmacologic activity altogether (e.g., cefotaxime lactone metabolites).[112] In other instances, metabolism can result in drug bioactivation and produce metabolites with pharmacologic activity from an inactive parent drug (i.e., prodrug) or generate metabolites that can result in cellular toxicity in the host (e.g., sulfamethoxazole's nitroso and hydroxylamine reactive metabolites, acetaminophen's NAPQI reactive metabolite).

Virtually every tissue has some ability to carry out drug biotransformation reactions. Although the liver is quantitatively the most important organ capable of drug metabolism, the small intestine, lungs, skin, and kidneys also have substantial drug biotransformation activities. In infants, children, and adolescents, developmental variations in drug metabolism have been associated with age, sex, maturation, and genetic constitution. For compounds that are extensively metabolized, developmental differences in drug metabolism can serve as the primary determinant for age-appropriate dose selection.[131] Much of the existing information regarding the impact of ontogeny on drug metabolism has been derived as a byproduct of pharmacokinetic investigations designed, in part, to determine whether age-dependent differences in drug disposition were evident.[113] The following paragraphs highlight important general issues regarding drug metabolism and its relationship with development.

PHASE I PATHWAYS

Phase I biotransformation reactions include oxidation, reduction, and hydrolysis reactions that, in general, introduce or unmask a functional group (e.g., hydroxyl, amine, sulfhydryl) that renders a drug more polar. Quantitatively, the P-450 cytochromes are the most important of the phase I enzymes and represent a superfamily of heme-containing proteins that catalyze the metabolism of many lipophilic endogenous substances (e.g., steroids, fatty acids and fat-soluble vitamins, prostaglandins, leukotrienes, and thromboxanes) and exogenous compounds. In humans, the P-450 cytochromes can be divided functionally into two distinct classes: steroidogenic enzymes expressed in specialized tissues, such as the adrenal glands, gonads, and placenta; and enzymes involved in the metabolism of drugs, pesticides, and environmental contaminants. P-450 cytochromes that have been identified as important in human drug metabolism are found predominantly in the *CYP1*, *CYP2*, and *CYP3* gene families.[131]

As reviewed by Leeder and Kearns,[131] considerable interindividual variability exists in the hepatic expression of P-450 enzymes, and, for a given individual, the pathway and rate of a compound's metabolic clearance constitute that individual's unique phenotype with respect to the forms and amounts of P-450 isoforms expressed. Of the P-450 cytochromes involved in drug biotransformation, CYP2C19, CYP2C9, and CYP2D6 are examples of isoforms that are polymorphically expressed in humans. Polymorphic expression of CYP1A2 and CYP3A4 has been reported in the literature but not definitively established with regard to functional consequences. Within a P-450 subfamily, several isoforms can exist (e.g., CYP3A4, CYP3A5, and CYP3A7), each of which can demonstrate substrate specificity and a distinct developmental pattern with regard to enzyme activity. In addition, certain P-450 cytochromes (e.g., CYP3A4) can work cooperatively with transporters (e.g., MDR-1 or P-glycoprotein) located in distinct cells or tissues to alter the availability of specific drugs to the systemic circulation (e.g., first-pass metabolism of CYP3A4 substrates in the enterocyte). Moreover, for certain polymorphically expressed P-450 cytochromes (e.g., CYP2C19, CYP2D6), enzyme activity has been shown to vary as a consequence of racial or ethnic origin[179] and, within a given phenotypic distribution (e.g., extensive metabolizers for CYP2D6), as a function of single nucleotide polymorphisms that exist in either the gene or its regulatory regions.[177] Finally, throughout development, the activities of the P-450 cytochromes can vary widely (e.g., 5- to 25-fold) among individuals of the same phenotype,[131] an important factor with respect to the ability for induction or inhibition and, thus, the prediction of drug-drug or drug-xenobiotic interactions in vivo.

PHASE II PATHWAYS

In general, phase II reactions involve the coupling of a drug or drug metabolite with an endogenous substance to enhance its hydrophilicity further and to facilitate drug excretion in either urine or bile. These reactions require the participation of specific transferase enzymes (e.g., epoxide hydrolase, glucuronosyltransferases, glutathione *S*-transferases, sulfotransferases, *N*-acetyltransferases, methyltransferases, transacylases) and high-energy, activated endogenous substances. Although most conjugation reactions result in drug detoxification, examples of bioactivation by phase II enzymes do exist.[182] Similar to what has been found for certain of the P-450 cytochromes, different isoforms of phase II enzymes also occur in humans. This finding is exemplified by the review of de Wildt and colleagues,[42] who described substrate specificity for 10 different glucuronosyltransferase isoforms in humans and known polymorphisms in 5 different isoforms of this enzyme. The impact of age on the disposition of several glucuronosyltransferase substrates (e.g., morphine, acetaminophen, zidovudine, chloramphenicol) suggests that isoform-specific, age-related differences in activity occur to a degree sufficient to produce profound effects on drug clearance that translate directly into age-specific differences in a drug dose.[42]

Normal growth and development can have a profound effect on the activity of drug-metabolizing enzymes. Failure to recognize important developmental differences in drug biotransformation and to appropriately compensate for them by individualizing drug dosing can lead to serious consequences. This is exemplified by the "gray baby syndrome" previously associated with

chloramphenicol, which is a life-threatening adverse drug reaction that was associated with supratherapeutic systemic drug exposure and, in some infants, produced cyanosis and cardiovascular collapse. The genesis of this particular toxicity resided with normal developmental reductions in the activity of the glucuronosyltransferase isoforms responsible for metabolizing chloramphenicol and the failure to appropriately adjust the dosing schedule to compensate for reductions in systemic clearance.[56,234] Traditionally, the impact of ontogeny for all enzymes was viewed as being extremely limited in neonates, rapidly increasing in the first year of life to levels in toddlers and older children that may exceed adult capacity, and declining to adult levels by the conclusion of puberty. Experimental and clinical data previously reviewed demonstrated that this theory is not accurate.[113,131] As illustrated by the summary data contained in Table 234-4, the impact of ontogeny on the activity of both phase I and phase II drug-metabolizing enzymes is very much a substrate- and isoform-specific event; development is one dynamic factor in a multitude of conditions (e.g., nutritional status, gender, diurnal variation, menstrual cycle, disease states/organ dysfunction, pregnancy, concomitant drug therapy) capable of altering the activity of drug-metabolizing enzymes and, thus, the clearance of drugs (and their metabolites) from plasma. Although discussion of the impact of ontogeny on the disposition (pharmacokinetics) of specific drugs is beyond the scope of this chapter, the clinician can use much of the information concerning specific enzymes provided in Table 234-4 as a tool to facilitate inquiry or clinical decision making, or both. It can be done simply by using readily available, updated information describing which enzymes are responsible for drug metabolism and then searching the published or unpublished (e.g., information available from pharmaceutical companies for drugs under development) literature for information describing the pharmacokinetics of the drug being considered.[59]

Clinicians must recognize that age-dependent differences in the activity of enzymes that catalyze drug biotransformation are not limited solely to P-450 cytochromes or the host of transferase enzymes responsible for phase II drug metabolism, and, in many cases, these differences represent a critical determinant for successful anti-infective drug therapy. This factor is exemplified by considering the example of two antimicrobial agents that have unique places in pediatric therapy: cefotaxime and linezolid. In the case of cefotaxime, non–P-450 enzymes (probably an esterase) capable of generating the active desacetylcefotaxime metabolite appear to be present and fully active by the third trimester of gestation.[117] Nonetheless, the elimination half-life of cefotaxime in neonates is approximately threefold greater (i.e., 3 to 4 hours) than that observed in older infants and children (i.e., 1 to 1.5 hours), a difference that permits extension of the dosing interval for the use of cefotaxime in neonates. The reasons for this developmental difference reside not with the enzymes responsible for generation of an active metabolite; instead, age-associated reductions in the activity of enzymes appear to be responsible for the generation of inactive metabolites of cefotaxime (e.g., cefotaxime lactone) and pathways involved in the renal clearance of desacetylcefotaxime.[116] Linezolid, an oxazolidinone antimicrobial approved in the United States for use in pediatric patients, undergoes extensive biotransformation in humans, not through a cytochrome P-450 enzyme but rather by nonselective chemical oxidation.[119] As demonstrated by Kearns and associates,[114] the mean plasma clearance of linezolid in children was

TABLE 234-4 **Developmental Patterns for the Ontogeny of Important Drug-Metabolizing Enzymes in Humans**

Enzyme(s)	Known Developmental Pattern
Phase I Enzymes	
CYP2D6	Low to absent in fetal liver but present at 1 wk of age; poor activity (i.e., 20% of adult) by 1 mo; adult competence by 1 to 3 yr of age
CYP2C9	Apparently absent in fetal liver; low activity in first 2 to 4 wk of life, with adult activity reached by approximately 6 mo; activity may exceed adult levels during childhood and declines to adult levels after conclusion of puberty
CYP1A2	Not present in appreciable levels in human fetal liver; adult levels reached by approximately 4 mo and exceeded in children at 1 to 2 yr of age; adult activity reached after puberty
CYP3A7	Fetal form of CYP3A that is functionally active (and inducible) during gestation; virtually disappears by 1 to 4 wk of postnatal life when CYP3A4 activity predominates but remains present in approximately 5% of individuals
CYP3A4	Extremely low activity at birth, reaching 30% to 40% of adult activity by 1 mo and full adult activity by 6 mo; may exceed adult activity between 1 and 4 yr of age and decrease to adult levels after puberty
Phase II Enzymes	
NAT2	Some fetal activity by 16 wk of gestation; poor activity between birth and 2 mo of age; adult phenotype distribution reached by 4 to 6 mo, with adult activity reached by 1 to 3 yr
TPMT	Fetal levels approximately 30% of adult values; in newborns, activity approximately 50% higher than in adults, with phenotype distribution approximating that of adults; exception is Korean children, in whom adult activity is seen by 7 to 9 yr of age
UGT	Ontogeny isoform specific; in general, adult activity reached by 6 to 24 mo of age
ST	Ontogeny isoform specific and appears faster than that for UGT; activity for some isoforms may exceed adult levels during infancy and early childhood

CYP, cytochrome P-450; NAT2, *N*-acetyltransferase-2; ST, sulfotransferase; TPMT, thiopurine methyltransferase; UGT, glucuronosyltransferase.

Modified from Leeder JS, Kearns GL. Pharmacogenetics in pediatrics: implications for practice. *Pediatr Clin North Am* 1997;**44**:55-7.

approximately threefold higher than that observed previously for adults, with the greatest increase noted in infants younger than 1 year. Given the mechanism of action for linezolid and properties that reflect time-dependent killing,[119] the clinical implications of the age-dependent pattern of increased plasma clearance for this drug in young infants and children suggest that for infections with selected pathogens that have relatively high (90%) minimal inhibitory concentration (MIC_{90}) values for the drug, shorter dosing intervals (e.g., every 8 hours) may be necessary to ensure sufficient exposure of the organism in blood and tissue through most of a dosing interval.[114]

PHARMACOKINETIC DETERMINANTS OF EFFECT

Collectively, the most important determinants of efficacy for anti-infective agents are the pharmacokinetic profile of the drug, the physicochemical and biochemical characteristics of the local environment (i.e., site of infection), and the susceptibility of the infecting organisms under local growth conditions. As previously reviewed by Barza,[14] the mechanisms and pharmacokinetics of drug transport to and accumulation at the site or sites of infection, as well as the subcellular localization of drugs, are critical to success and are poorly understood. In many circumstances, plasma drug concentrations may not be reflective of those in tissue. Pathophysiologic processes related to the host, the infection, and the physicochemical properties of the drug work in concert to regulate distribution into and retention of active drug at the site of infection. Because these considerations should be embraced by the clinician when a particular anti-infective drug is selected for treatment and its therapy is monitored for clinical evidence of success, some general examples are presented as follows.

In addition to age (as discussed earlier), many disease processes (e.g., trauma, malignancy, renal or hepatic disease) have an impact on both the quantity of circulating plasma proteins (e.g., albumin, α_1-acid glycoprotein) and their affinity to bind anti-infective agents. Given that only free drug is available to enter tissue, the degree of binding to protein components in the blood will affect tissue concentrations. Thus, despite the presence of total (i.e., free and bound) plasma drug concentrations well in excess of the MIC, these concentrations may not be predictive of concentrations of highly bound (i.e., >70%) drug at the site of infection. Properties of the capillary bed feeding the site of infection also dictate drug penetration. Highly vascularized tissue with a large ratio of capillary surface area to volume can be expected to accumulate higher drug concentrations than can tissue that is poorly vascularized, principally because of the rate and extent to which drug can be delivered to the site. As with protein binding, variability in capillary density may be a function of disease (e.g., severe atherosclerosis, diabetes), infection (e.g., abscess, cardiac vegetation), and age, and it should be expected to have an impact on drug delivery. Similarly, tissues with tight junctions and few fenestrations (e.g., eye, central nervous system [CNS]) afford lower tissue concentrations, given that drug entry is restricted to transport across the lipid bilayer of the endothelial cell (i.e., transcellular vs. paracellular transport). Here, adequate drug penetration is restricted to agents with a favorable lipid-water partition coefficient and ionization constant. As such, many infectious processes warrant direct instillation of antibiotic at the site of infection (e.g., intracisternal, intrathecal, intra-articular). Additionally, cellular transporters (e.g., P-glycoprotein at the blood-brain barrier, organic anion and cation transporters at the choroid plexus) capable of pumping drug from the cell can limit distribution and retention of drug at the tissue level. The pH at the site of infection (e.g., in tissue fluids, exudates, transudates) relative to the pH in plasma also can govern distribution of a drug. For example, in cases of bacterial meningitis, the pH that occurs in the CNS is lower relative to plasma. Weak acids (e.g., penicillin) are more highly ionized in plasma than in cerebrospinal fluid (CSF) and pass more readily from the CSF into plasma than in the reverse direction. Similarly, when the pH of the local environment drops in the presence of infection, as occurs in lung abscesses, aminoglycoside efficacy is reduced because of chemical inactivation of the drug by ionization and the formation of stable adducts with high DNA concentrations found in purulent secretions. Finally, the physicochemical association of an intact (i.e., unmetabolized) drug with a particular physiologic fluid as part of its normal excretion profile can serve to localize drug at the site of infection (e.g., accumulation of drugs excreted by the biliary route in patients with cholangitis and in the urine in patients with nephritis). It is important to recognize that such interactions modify the activity of antimicrobial agents. For example, in vitro data demonstrate that daptomycin interacts with pulmonary surfactant, which in turn decreases the activity of the antibiotic. Hence the drug is not currently indicated in the treatment of pulmonary infections.[203] When drug-tissue and drug-body fluid interactions occur, different approaches including dose modification or combination therapy may need to be considered to optimize therapy.

PHARMACODYNAMIC DETERMINANTS OF EFFECT

The desired effect of any drug is realized when sufficient concentrations are achieved and maintained at the active site for an adequate period. In many models of disease for which the treatment targets of modern chemotherapy remain fixed, the concentration-effect (i.e., pharmacodynamic) relationships can be described with relatively modest effort. In contrast, evaluating the concentration-effect relationship for antimicrobial agents can be anything but straightforward, given the dynamic nature of infection. The intended target of the anti-infective agent is in constant flux as the number of organisms changes, the quantity and affinity of target receptors evolve, and the contribution of the host response adapts accordingly during the course of infection. Moreover, antimicrobial agents demonstrate variable effects at different concentrations and under different physicochemical environments. In fact, evaluating whether an organism survives

in the presence of antibiotic is only one criterion by which to predict or define drug effect. Rather, a variety of effects related to antimicrobial concentration—from the extremes of complete eradication to complete survival—can be observed in the invading organism. The previous sections of this chapter review the principles of pharmacokinetics, specifically, factors that link dose to concentration. The sections that follow include a discussion of the pharmacodynamics of anti-infective therapy and the multitude of factors that link concentration with effect.

Effects Described by Pharmacokinetic Parameters and Conventional Susceptibility End Points

Among the many decision-making tools that clinicians have at their disposal to guide in the selection of antimicrobial therapy are qualitative (e.g., breakpoints) and quantitative (e.g., MIC, minimal bactericidal concentration [MBC]) estimates of susceptibility. Although numerous studies and years of clinical evidence support the correlation between qualitative end points and clinical outcome, their predictive power is diminished in the presence of immunocompromise, severe underlying disease, mixed infections, and infection with organisms demonstrating heterogeneic resistance patterns.[30,37,58,140,149] Quantitative end points provide a better assessment of dose-effect relationships, with antibiotic concentration serving as a surrogate for the dose. However, these data alone do not reveal the complete picture relative to the anticipated bacterial response because laboratory-based quantitative tests contain a notable number of artificial aspects. Factors appreciated to influence the activity of antimicrobial agents in vivo (e.g., protein binding, fluctuating drug concentrations, serial drug exposure, inoculum size, immune defense status, physicochemical environment, compound stability) essentially are neglected when determining MIC values. As such, agents with comparable MIC values in vitro may, in fact, demonstrate markedly different effect profiles in a patient.[1,29,66,107,124]

In an attempt to move beyond reliance on quantitative tests as the sole marker for predicting antimicrobial activity in vivo, the integration of susceptibility information with population- and patient-specific pharmacokinetic data continues to be explored. Surrogate end points, defined by a combination of pharmacokinetic parameters and quantitative susceptibility data, have been suggested to predict more reliably the efficacy of different antimicrobial agents. By far, pharmacodynamic determinants of antimicrobial effect most frequently link attainable drug concentrations or estimates of total-body exposure, or both, with in vitro estimates of pathogen sensitivity. For antimicrobial agents for which the mechanism of action is determined to be time dependent (concentration independent), the percentage of time that plasma or tissue concentrations remain above the MIC has been closely linked to therapeutic response (Fig. 234-6). By comparison, the ratio of C_{max} to MIC has demonstrated a similar relationship for drugs with concentration-dependent killing (see Fig. 234-6). Furthermore, the ratio of AUC to MIC, which reflects the concentration profile over the course of time, has been used with both classes of agents (see Fig. 234-6).

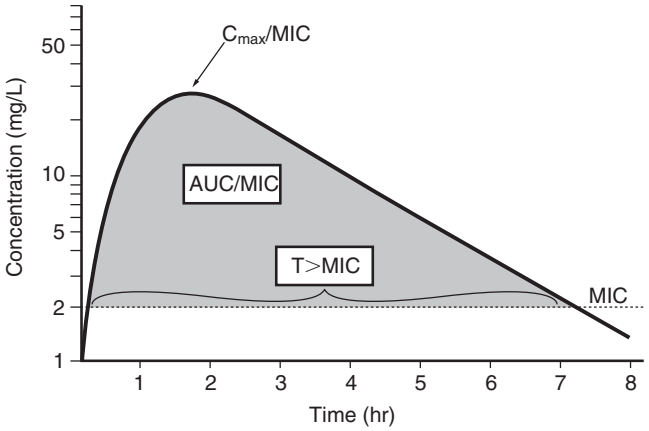

FIGURE 234-6 ■ Representative plasma concentration versus time curve illustrating commonly referenced pharmacodynamic parameters. AUC, area under the curve; C_{max}, maximal plasma concentration; MIC, minimal inhibitory concentration; T, time.

Evidence to support the application of specific pharmacodynamic surrogates is reviewed in the following paragraphs. However, a large degree of interdependence exists among these pharmacokinetic parameters. With few exceptions, when the dose of a drug is increased, C_{max} and the total AUC increase, and with no change in clearance, so, too, does the percentage of time that plasma drug concentrations remain above the MIC. Accordingly, making distinctions among the various pharmacokinetic parameters in an attempt to determine the optimal pharmacodynamic end point can be misleading. Given that many studies evaluate a limited number of doses or dosing intervals, restrict the number of pharmacokinetic parameters that are calculated, or neglect to perform multivariate analysis on the data, clear distinctions between parameters cannot always be made, and an appropriate degree of caution should be exercised when evaluating such data.[228]

Time Above the Minimal Inhibitory Concentration

Among the first class of antimicrobial agents to be discussed in the context of their concentration-effect relationships were the β-lactams, specifically penicillin. Although limited supporting evidence existed at the time, investigators were concerned with maintaining adequate blood levels of the drug in the host for a fixed period of time. This concern was reflected in their attempts to inhibit clearance pathways and formulate sustained-release preparations designed specifically for the purpose of extending the time spent above some minimal concentration (T > MIC).[17,186] As it turns out, in vitro studies support the relative concentration independence of penicillin and demonstrate that a maximal rate of killing can be observed that is quickly saturated at reasonably low multiples of the MIC (Fig. 234-7A). As concentrations increase above this maximal level,

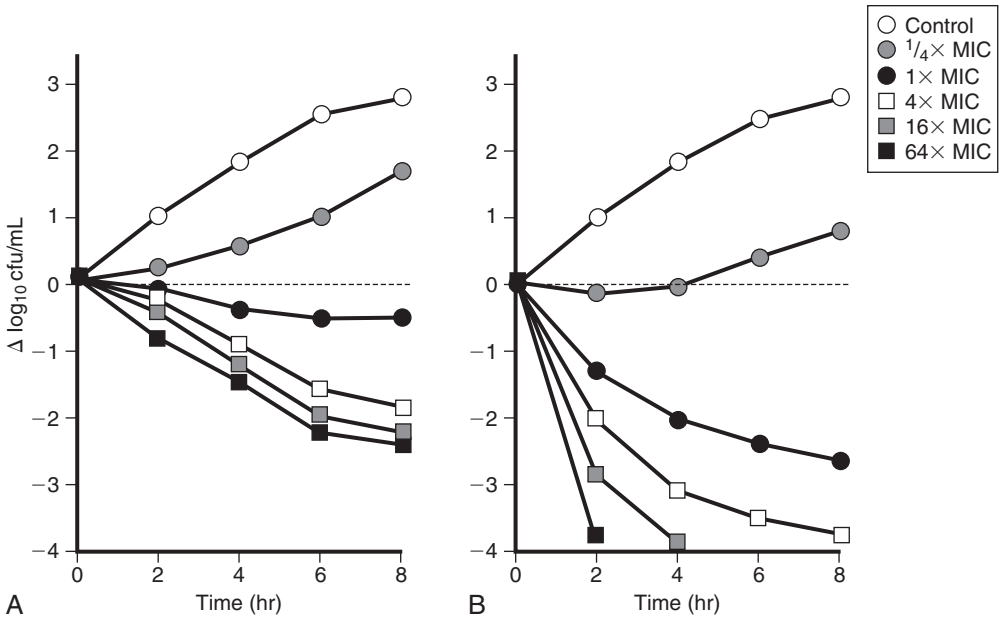

FIGURE 234-7 ■ Representative log-kill curves. These curves illustrate the response to concentration-independent **(A)** (time-dependent) and concentration-dependent **(B)** antimicrobial agents. MIC, minimal inhibitory concentration. (Modified from Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review. Scand J Infect Dis 1991;**74**[Suppl]:63-70.)

no faster rates of kill can be demonstrated.[34] Rather, penicillin displays time-dependent killing in which the duration of time spent above some minimal concentration (typically ±1 to 2 dilutions of the MIC or MBC) appears to be the most important determinant of activity.[49] As newer antimicrobial agents with putative time-dependent activity became available for evaluation, those with longer plasma and tissue terminal elimination half-lives demonstrated enhanced bactericidal activity when compared with similar drugs. Those that simply resulted in higher maximal peak concentrations, with no extension of the time above the MIC, produced minimal if any differences in effect.[10,83,193,235] This time-dependent activity has been observed in vitro with virtually all β-lactams (e.g., cephalosporins, carbapenems, monobactams) against both gram-positive and gram-negative organisms.[60,83,207,241] Similarly, other drug classes, namely, the macrolides, azalides, glycylcyclines, glycopeptides, ketolides, and oxazolidinones, appear to possess this same characteristic.[8,89,144,224,226]

When evaluating whether the time-dependent activity observed in vitro can be reproduced in vivo by evaluating intrinsic antibiotic activity in the host (through restriction of observations to animals in which the element of both humoral and cell-mediated immunity has been removed), the strongest relationship between antimicrobial concentration and effect for the aforementioned agents remains the time spent above the MIC.[187] Elegant studies evaluating extensive numbers of dosing regimens and drug-microbe combinations demonstrated that β-lactam and macrolide efficacy correlates best with the percentage of time spent above the MIC, irrespective of whether gram-positive or gram-negative pathogens are involved.[228] Given the same total daily dose, therapy administered at more frequent intervals is required for a successful outcome.[12,132] Even when the drug-microbe interaction is such that only bacteriostatic activity results, a regimen consisting of more frequent doses is able to maintain an environment of no net growth, whereas positive net growth is observed with larger doses administered less frequently.[75] From such data, the efficacy of time-dependent agents appears to be maximized by maintaining antibiotic concentrations above the MIC for as much of the dosing interval as possible.

When evaluating whether these agents obey the same relationship in the presence of a competent immune system, animal models similarly demonstrate a significant correlation between antimicrobial efficacy and the percentage of time spent above the MIC.[2,67] In contrast to immunocompromised animals, animals with a functional immune system do not require that virtually 100 percent of the dosing interval be spent above the MIC to achieve adequate antimicrobial activity. Although the bactericidal activity of penicillin ceases when concentrations fall below the MIC, the remaining organisms do not resume multiplication for many hours, well in excess of the time observed for the same organisms in vitro.[51,108] This difference may reflect the time required for the organism to recover from the initial antibiotic insult, coupled with the complementary activity of subinhibitory antibiotic concentrations and the immune system (these pharmacodynamic principles are explored later in further detail).[72] Irrespective of the mechanism by which the protracted antimicrobial effect occurs, the pharmacodynamic goals for time-dependent agents are not as stringent in vivo as they are in vitro. However, the persistent suppression of bacterial growth is not indefinite, and a maximal amount of time can be spent below the MIC before a reduction in efficacy becomes apparent. Thus, a maximal dosing interval for these agents appears to exist in immunocompetent hosts, beyond which a decrease in efficacy can be expected.[50,198]

Clinical studies further confirm that for the agents discussed earlier, time above the MIC is a suitable pharmacodynamic end point in patients. In clinical studies of upper respiratory tract infection, the highest bacterial eradication rates for the β-lactams are observed when the time above the MIC exceeds 40 to 50 percent of the dosing interval.[38] For cefuroxime, cure rates drop from greater than 90 percent to approximately 75 percent when the time above the MIC falls to less than 40 percent.[70,79] Patients infected with a susceptible organism show 92 percent efficacy with a continuous-infusion regimen of cefamandole versus 63 percent efficacy with intermittent administration of the agent. Finally, in neutropenic patients, an overall efficacy rate of 65 percent was observed after continuous infusion of cefamandole as opposed to 21 percent efficacy in patients receiving intermittent administration.[24] For macrolide-susceptible pathogens, cure rates approaching 100 percent were observed when concentrations remained above the MIC for greater than 80 percent of the dosing interval.[38] As the MIC increases such that the percentage of time spent above the MIC falls, so too do cure rates. Macrolide concentrations rarely exceed the MIC for *Haemophilus influenzae*, and cure rates are comparable to those seen in placebo-treated individuals.[38] A few studies appear to contradict the aforementioned data, findings indicating that little difference in clinical response is observed when the same total daily dose of selected agents is administered as a continuous infusion or with increased frequency versus less frequent intermittent administration; however, a prolonged postantibiotic effect (PAE) or protracted half-life, such that both of the regimens evaluated spend nearly 100 percent of the dosing interval above the MIC, may explain these discrepancies.[28,127,229]

A natural extension of the considerations just discussed relates to the utility of continuous infusions for antimicrobial agents that exhibit time-dependent killing, a topic that has historically been controversial. Theoretically, continuous drug administration would ensure that serum concentrations and potentially those at the site of action would never fall below the MIC. This would be of particular value for antimicrobial agents with a short half-life, in patients in whom drug exposure is limited based on increased volumes of distribution or clearance rates, and in patients who have a significant degree of immunocompromise. Although the benefits appear obvious, the clinical findings are equivocal, with data both supporting and refuting improvement in clinical outcomes related to continuous antimicrobial administration. Variability in antibiotic dosing, disease severity, causative bacterial pathogens, and susceptibility patterns all add to the complexity of accurately assessing prolonged infusions.[185] Moreover, challenges encountered with the administration of a continuous infusions of antimicrobial agents include obtaining adequate serum concentrations, drug stability, practicality of administration, and potential adverse reactions associated with prolonged drug exposure. Consequently, clinical guidelines for the application of continuous infusions are lacking and additional data are needed to better understand the potential advantages and disadvantages of continuous infusions, particularly in the setting of more highly resistant pathogens and in complex pediatric patient populations.

Concentration Above the Minimal Inhibitory Concentration

In contrast to the aforementioned agents, a smaller group of agents demonstrate concentration dependence, with the magnitude of antimicrobial effect increasing in direct proportion to increasing drug concentrations (see Fig. 234-7B).[16,146] The aminoglycoside and fluoroquinolone antibiotics are the prototypic drugs of this pharmacodynamic class; however, concentration-dependent activity also has been observed with the lipopeptides, metronidazole and the echinocandins.* Unlike the time-dependent agents, for which shortening the dosing interval results in increased efficacy, extending the dosing interval for concentration-dependent drugs affords an ability to increase the dose and results in a greater reduction in bacterial inoculum and, for some agents, a diminution in adaptive resistance.[39] Although highly variable, optimal ratios of peak plasma concentration to MIC that result in complete killing have been defined for many drug-microbe combinations. Similarly, ratios of C_{max} to MIC (C_{max}/MIC) can be defined, below which the organism is afforded the opportunity to regrow with increased selection of resistant subpopulations.[22,45,46]

Concentration-dependent killing for many of these agents has been demonstrated in both animal models and human clinical trials. In neutropenic mice with pseudomonal soft tissue infection, dosing schemes that deliver larger aminoglycoside doses less frequently result in greater killing than do schemes with the same total daily dose administered more frequently, even though all regimens achieved serum concentrations in excess of the MIC.[75] In patients receiving quinolone therapy for the treatment of respiratory tract, urinary tract, and skin and soft tissue infections, several pharmacodynamic parameters proved to correlate with clinical response; however, the best correlation was observed with the C_{max}/MIC ratio.[174] In clinical trials in which patients received aminoglycoside therapy for gram-negative infections, the strongest association between pharmacokinetic indices and clinical response was observed with the C_{max}/MIC ratio. In fact, the relationship between the C_{max}/MIC ratio and clinical response appeared to be linear over the range of clinically relevant plasma concentrations. No significant difference occurred in the time to achieve C_{max} between responders and nonresponders; therefore, the improved response rates in patients achieving higher peak plasma concentrations were not simply an artifact of drug accumulation as a result of longer duration of therapy.[154,155]

In addition to the absolute peak plasma concentration, the time that optimal peak levels are achieved appears to play a critical role in the response to concentration-dependent antimicrobial agents. A significant correlation with clinical improvement and reduction in mortality is observed if therapeutic peak plasma concentrations are reached early during the course of treatment.[156,159] The

*References 5, 40, 143, 176, 206, 225.

probable reason for this effect is that the response of the bacterial population to the same antibiotic concentration can change with subsequent doses. As alluded to earlier, subtherapeutic peak plasma concentrations serve to select out fewer susceptible variants within the population.[22,23,74] Based on these data, a key goal of therapy (i.e., in addition to proper drug selection) for concentration-dependent agents appears to be the attainment of high peak plasma concentrations early in therapy to afford rapid initial killing and minimize adaptive resistance.

Although prolonging the dosing interval affords the luxury of increasing the dose to optimize killing, the law of diminishing returns applies even for concentration-dependent agents, and it is possible to increase the dosing interval to excess and decrease efficacy. Despite the clear concentration dependence with escalating doses of fluoroquinolones, a simulated every-12-hour regimen was less effective than was a regimen using the same total daily dose administered every 8 hours.[15] Similarly, animal models of gram-negative lung and soft tissue infection suggest that delayed clearance of the infecting organism is observed in response to aminoglycoside and fluoroquinolone therapy when administered in protracted dosing regimens (e.g., dosing interval much greater than the half-life) as opposed to shorter dosing intervals.[132,158,187] Again, scattered studies appear to contradict these findings; however, they use agents with a longer half-life and therefore the dosing interval may, in fact, not exceed the time spent above the MIC plus the residual PAE.[43] Thus, despite their concentration dependence, an element of time appears to be involved in the response to these agents. Unless peak concentrations are achieved in excess of those necessary to obtain maximal kill rates and the dosing interval does not markedly exceed time above the MIC plus the PAE, the efficacy of such agents is not only dose dependent but also dose interval dependent.

Total-Body Exposure Above the Minimal Inhibitory Concentration

The AUC, a reflection of total-body exposure, is a pharmacokinetic parameter that integrates both time and drug concentration. Estimates of AUC reflect the magnitude of the dose received and the drug half-life relative to the dosing interval. Accordingly, researchers have proposed that the ratio of AUC to MIC (AUC/MIC ratio) may serve as a better surrogate of pharmacodynamic activity for both time- and concentration-dependent agents. In several studies, the AUC/MIC ratio serves as the best correlate of the reduction in number of organisms for aminoglycosides, quinolones, streptogramins, rifamycins, isoniazid, evernimicin, echinocandins, and azole antifungal agents.* Because of the interdependence between AUC and the pharmacokinetic parameters previously noted, studies also can be identified that demonstrate a relationship between clinical or microbiologic outcome and the AUC/MIC ratio for agents with time-dependent activity (e.g., the β- lactams and glycopeptides). Often, however, these studies fail to evaluate multiple pharmacokinetic parameters.[153,197]

**References 4-7, 44, 55, 109, 110, 168.

In an elegant study evaluating certain aminoglycoside dosing regimens against *Pseudomonas aeruginosa* and *Escherichia coli* infection, the log-normalized AUC provided the best correlation with efficacy. However, as was observed with concentration-dependent agents, when the dosing interval exceeded the time above the MIC plus the residual PAE, the best correlation with efficacy was the percentage of time spent above the MIC, thus reaffirming that the dosing interval can be too long with the aminoglycosides.[228] A meta-analysis of 19 studies evaluating eight quinolones and six different organisms in experimental endocarditis similarly demonstrated a correlation between the AUC/MIC and reduction in $\log_{10}$ colony-forming units (CFUs) per vegetation. However, given the relationship between the pharmacokinetic parameters, C_{max}/MIC and time greater than MIC also proved to correlate with a decrease in the size of the inoculum.[3] In human investigations, AUC/MIC appears to serve as the best predictor of clinical response, microbiologic response, and bacterial eradication for the fluoroquinolones. Nonetheless, C_{max}/MIC and time greater than MIC can be linked similarly to bacterial eradication for this class of agents.[63,64,170]

Clearly, the optimal AUC/MIC ratio will be different for different organisms despite sharing a similar quantitative susceptibility end point. Similarly, optimal AUC/MIC ratios are highly variable and depend on the antimicrobial agent being evaluated. For example, effective eradication of organisms implicated in nosocomial pneumonia is observed with an AUC/MIC ratio of 540 for cefmenoxime, 34 for tobramycin, and 23 for ciprofloxacin.[196] Consequently, attempts have been made to standardize this pharmacodynamic end point, and investigators have proposed a value of 125 as the cutoff below which a reduction in efficacy and increase in resistance may be expected.[100,195] However, optimal AUC/MIC ratios may differ, depending not only on a given organism and agent but also on the desired outcome and the disease state. A ratio greater than 125 resulted in bacterial eradication roughly 7 days after the initiation of β-lactam and quinolone therapy; however, an AUC/MIC ratio of 250 resulted in eradication of the pathogen within 1 to 2 days after initiating quinolone therapy.[195] For patients with acute exacerbations of chronic bronchitis who are receiving fluoroquinolones, an AUC/MIC ratio less than 276 was associated with longer time to clinical success, whereas a ratio greater than 576 was associated with a reduction in coughs per day and a ratio greater than 212 with decreasing days to a reduction in the volume of sputum.[151] In an animal model of bacterial endocarditis, significantly lower numbers of organism were found in the vegetation after 3 to 6 days of therapy when the AUC/MIC ratio exceeded 100.[3] Moreover, a value of 100 appeared to be the cutoff below which an increased risk of resistance was observed in a retrospective review of 107 acutely ill patients with lower respiratory tract infection.[218] Furthermore, in vitro models evaluating fluoroquinolone activity against *Streptococcus pneumoniae* demonstrated effective eradication with an AUC/MIC ratio between 30 and 65, much lower than the value of 125 reported as optimal for other pathogens.[126,136]

Although this hybrid parameter bridges concentration and time (i.e., extent of systemic drug exposure from a given dose) and has been demonstrated to correlate with efficacy for numerous drug classes, optimal criteria appear to vary with the organism, agent, disease state, and desired outcome. In addition, established relationships no longer may hold for some agents when a protracted dosing interval is used in therapy. As such, a general classification and standard outcome are not straightforward, and agents need to be evaluated with respect to known mechanisms of activity and the specific clinical and microbiologic situations in which they are used.

Optimal Surrogates for Drugs in Combination

Combination antimicrobial therapy is initiated in numerous circumstances: to ensure broad-spectrum coverage early in therapy, to treat polymicrobial infections, and to combat organisms that require multiple agents for effective eradication. Often, combination therapy entails the use of agents from different classes or agents that have different mechanisms of action (or both). This particular situation raises the question of how one applies the aforementioned pharmacodynamic principles to selecting or evaluating the efficacy of drug therapy when confronted with the combined use of agents that do not share similar pharmacodynamic targets. Although optimization of combination therapy may be possible by choosing the interval for one agent,[76] efficacy appears to be explained best when a combination of both pharmacodynamic properties is considered.[75,157]

Reasonable models of a concentration-effect relationship have been proposed for numerous agents alone and in combination; however, optimal ratios remain to be defined for many drug-microbe combinations. Moreover, it is probably more complex than simply defining the optimal ratio for specific combinations. Even though one can define the best correlate, killing rates, in fact, may vary with the site of infection because penetration of a drug into or clearance from tissue varies from site to site. For example, logAUC proves to be the best predictor for clearance of organisms from the lung regardless of the dosing interval used. In contrast, given faster drug clearance from the thigh, the time above the MIC was a more important predictor of eradication when a longer dosing interval was used, whereas the logAUC was the best predictor of clearance of organisms with the use of shorter dosing intervals.[133] Similarly, in animal models of bacterial meningitis, greater kill rates were observed as time greater than MBC increased. However, when concentrations remained above the MBC for the entire dosing interval, which may not be unexpected in situations in which the antibiotic half-life is longer in CSF than in plasma, kill rates were directly proportional to both peak/MBC and AUC/MBC ratios.[145] These data suggest that we may need to expand the characterization of pharmacodynamic surrogates to look at the pharmacokinetic-pharmacodynamic interface at the site of infection rather than simply defining dosing strategies based on in vitro susceptibility data and the disposition characteristics of drugs in plasma. Without question, many data need to be collected and verified in the human host. The maximal dose and dosing regimen ideally are selected so that the resultant pharmacokinetic profile affords optimal pharmacodynamic activity without causing unnecessary adverse events, therapeutic failure, or both, as may be observed with inappropriate dosing. Development of a successful therapeutic strategy requires the following: incorporation of knowledge regarding the infecting pathogen; the physicochemical, pharmacologic, and pharmacokinetic properties of the anti-infective agent; and specific factors of the host or disease that are capable of altering either drug disposition or action. Such an approach enables the clinician to make clearer distinctions among agents with similar susceptibility profiles yet different mechanistic or pharmacokinetic profiles and to select an optimal drug regimen that will be most likely to result in successful therapy.

Effect of Suprainhibitory Antimicrobial Concentrations (Eagle Effect, Paradoxical Zone Phenomenon, Concentration Quenching)

In simplest terms, one typically thinks that anti-infective activity increases with increasing antimicrobial concentration. More sophisticated models describe increasing activity with increasing drug concentration to a maximal effect until activity reaches a plateau and remains relatively constant despite further increases in drug concentration (see Fig. 234-7). However, for many antibiotics, an increase in drug concentration can, in fact, result in a reduction in antimicrobial activity (Fig. 234-8).[96] This paradoxical effect was observed as early as 1945 and described 3 years later by Eagle and Musselman, who reported the existence of antibiotic concentrations above the maximally effective concentration at which the killing rate of bacteria is paradoxically reduced.[52,122] This phenomenon has been described with a host of organisms and although originally observed with penicillin has been

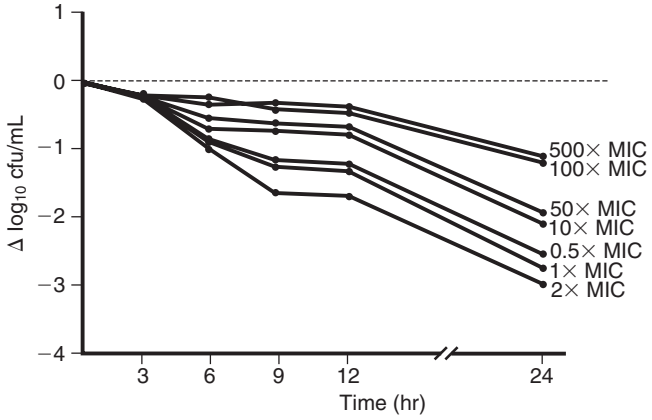

FIGURE 234-8 ■ Representative log-kill curve demonstrating the paradoxical effects that can be observed with suprainhibitory antimicrobial concentrations. MIC, minimal inhibitory concentration. (Modified from Holm SE, Odenholdt-Tornqvist I, Cars O. Paradoxical effects of antibiotics. *Scand J Infect Dis Suppl* 1991; **74**:113-7.)

confirmed with other β-lactams, glycopeptides, aminoglycosides, and fluoroquinolones.[142,161,171,213,238] Given the diversity of agents that demonstrate a paradoxical reduction in activity with increasing drug concentration, this phenomenon probably cannot be explained by a single mechanism; and although they are not definitively elucidated, numerous mechanisms have been proposed to explain these observations.

For certain β-lactam antimicrobials, induction of β-lactamase may be responsible for the paradoxical effects observed in selected organisms. In vitro experiments performed with *Proteus vulgaris* suggested that the greatest paradoxical response seen with β-lactams was observed with agents that demonstrated the highest β-lactamase–inducing capacity and the poorest stability to degradation by this enzyme. When a mutant strain of this organism that was unable to induce β-lactamase production was evaluated, no paradoxical effect was noted.[104] In animal models of peritoneal infection, antibiotics with strong dose-dependent induction of β-lactamase similarly demonstrated a paradoxical effect against β-lactamase–positive strains of *P. vulgaris.* However, no paradoxical effect was observed against β-lactamase–negative strains. Agents that were only weak inducers of β-lactamase demonstrated no evidence of a paradoxical effect, regardless of whether the *P. vulgaris* strains produced β-lactamase.[102]

Because this paradoxical effect can be demonstrated with the β-lactams against organisms that do not produce β-lactamase, other mechanisms must also be responsible. One such mechanism may involve dysregulation of the bacterial autolytic system, which often confers tolerance to an organism but also may uncover an Eagle-type effect.[91] A paradoxical reduction in antimicrobial activity at high doses may be related to differences in regulation or expression of these autolytic enzymes. In *Enterococcus* strains that fully express only one of two recognized autolytic enzymes, diminished or deficient production of the second enzyme resulted in a paradoxical response to penicillin. Strains in which both enzymes were produced remained susceptible to the antibiotic irrespective of concentration, thus suggesting that a proportional dose-response relationship may require the synergistic activity of both enzymes.[61,62] Further investigation into the paradoxical effect observed in tolerant organisms suggests that organisms in the exponential growth phase demonstrate this response more consistently than do those in the stationary phase.[172,175] Animal models of *Staphylococcus aureus* endocarditis provide in vivo confirmation of the paradoxical response observed with tolerant organisms in vitro. Administration of continuous-infusion cloxacillin to animals infected with a nontolerant strain produced a dose-proportional reduction in bacterial density (log_{10}CFUs/g) within the vegetation, whereas animals infected with a tolerant strain demonstrated a reduction and subsequent paradoxical increase in bacterial density with increasing dose. No statistical difference was observed in bacterial density within other organs or in overall survival, which raises questions regarding the in vivo significance of these observations.[230]

For antibiotics that demonstrate polyfunctional activity at supratherapeutic concentrations, a paradoxical response may occur as a result of secondary alterations in cellular functions that are requisite for antimicrobial activity. Despite a principal mechanism of action for the fluoroquinolones at the site of DNA gyrase, these agents appear to be bactericidal only in cells able to synthesize protein. As quinolone concentrations increase above those that are bactericidal, RNA synthesis and therefore protein synthesis are progressively inhibited, thus explaining the reduced rate of bactericidal activity observed at high quinolone concentrations.[184] Incompletely characterized genetic modifications also may confer a paradoxical response to antibiotics. β-Lactam resistance in methicillin-resistant *S. aureus* (MRSA) is mediated by derepressing the production of a low-affinity PBP. However, because the modified PBP serves as an inefficient transpeptidase, it is regarded as an internal selective pressure in the bacterium, and a countermutation has arisen. Although it is not completely understood, the phenotypic expression of this countermutation is manifested as an Eagle-type resistance to methicillin.[123,192] Finally, an observed paradoxical response may not be a result of the drug-microbe combination alone but a result of the chemical environment as well. A paradoxical effect with penicillin, in which both cell count and the rate of killing were affected, was demonstrated in *Streptococcus agalactiae* when the pH was reduced under in vitro conditions.[103]

Most studies investigating the Eagle effect have been conducted in vitro, and the clinical implications of the paradoxical phenomenon have yet to be fully elucidated. However, anecdotal evidence appears to suggest that the clinical efficacy of antimicrobial agents can be compromised by high-dose antibiotic therapy. Case reports of β-lactam–susceptible streptococcal endocarditis and staphylococcal pneumonia describe patients who failed to respond to high-dose β-lactam therapy (plasma concentrations >100 times the MIC in one case), with subsequent improvement in each case when the dose was lowered.[73,84,216] For many agents, it is clearly advantageous to establish high peak drug concentrations early, the benefits of which include increasing log kill, preventing the selection of resistant mutants, overcoming refractoriness, and enhancing penetration of tissue. However, the relative safety of many drug classes (e.g., the β-lactams) often results in the use of doses that may be higher than necessary. When clear evidence is lacking for enhanced bactericidal activity at higher concentrations and the potential for diminished efficacy exists, escalation of antibiotic doses should be considered judiciously.

Effects of Subinhibitory Antimicrobial Concentration

Individuals may be exposed to subtherapeutic antimicrobial concentrations for many reasons, including underdosing, poor compliance, and, perhaps most commonly, the inability of the pharmacokinetic profile of the agent in question to afford adequate penetration to the site of infection. Exposure of bacteria to antibiotic concentrations below the MIC can have variable effects, depending on the organism and the antimicrobial agent. In fact, the

effects observed with subinhibitory levels are not simply a milder extension of those observed at inhibitory concentrations but, instead, are qualitatively different from the effects observed at concentrations that equal or exceed the MIC.[138] Subinhibitory concentrations can have the following effects: enhance or impair opsonization and phagocytosis; affect the virulence of the bacteria by modifying the bacterial cell surface; alter adherence properties, antigen expression, or the excretion of enzymes and toxins (or any combination of these effects); and inhibit bacterial growth.[163,164,221] The result can be an increase or decrease in responsiveness to therapy, both of which are discussed later in this section. Before reviewing the current data, however, one should note that this discussion does not refer to subinhibitory concentrations occurring after initial suprainhibitory drug levels. These particular effects are distinguished as PAE and are addressed separately.

Even though antibiotic concentrations may fail to reach or exceed the MIC, these agents still can affect the growth of an invading organism adversely.[138,139,141] In fact, the term *minimal antibacterial concentration* (MAC), usually a fraction of the MIC, was coined to describe the lowest concentration of antibiotic required to produce either structural changes in the bacterium, a 1 $\log_{10}$ decrease in the number of organisms present, or a 10 percent delay in turbidimetric growth in vitro.[71,212] Rather than resulting in treatment failure, subinhibitory antimicrobial concentrations in concert with defenses in an immunocompetent host may be sufficient to clear the infection.[221] Many effects have been observed to occur as a result of exposure to subinhibitory antimicrobial concentrations, and they are mediated by a variable number of mechanisms.

Increased susceptibility to phagocytosis may be seen with subinhibitory antibiotic concentrations and can occur in many ways. Subinhibitory concentrations may induce morphologic changes in the bacterium such that its size and shape are altered markedly, and, as a result, the immune system is signaled to clear the bacterium.[11,212,231] However, subinhibitory concentrations of drugs that share a common intracellular target cannot be expected to have the same effects on bacterial morphology. Even though both penicillins and cephalosporins act through PBPs, subinhibitory concentrations of penicillins induce filaments in *Proteus mirabilis*, whereas cephalosporins induce the formation of globules.[139,141,216] A likely explanation lies in their different affinity for the various PBPs, whereby they can be expected to bind selectively at low concentrations and rather nonspecifically at therapeutic or supratherapeutic concentrations. The preferential binding of ceftibuten to PBP-3 of *E. coli* at subinhibitory concentrations results in filamentation because this PBP allows crosswall but not sidewall synthesis.[26] Similarly, subinhibitory concentrations of the same drug applied to different organisms can produce different morphologic changes. In *S. aureus*, subinhibitory concentrations of loracarbef induce no morphologic changes, whereas similar concentrations lead to elongated forms and short-chain forms in *E. coli* and elongated and filamentous forms in *H. influenzae*.[222]

Although size may be one factor stimulating enhanced clearance of the organism, increased uptake by the immune system can be demonstrated even for cells with little gross morphologic alteration. Subinhibitory antibiotic concentrations can impair the synthesis of antiphagocytic surface factors or liberate their release, thereby enhancing complement binding, opsonization, phagocytosis, and, ultimately, bacterial killing.[93,98,128] Production of the antiphagocytic A protein of *S. aureus* can be repressed by subinhibitory concentrations of selected protein synthesis inhibitors and fluoroquinolones.[148,205] Subinhibitory concentrations of β-lactam agents, but not vancomycin or protein synthesis inhibitors, increase the susceptibility of *S. agalactiae* to uptake by polymorphonuclear leukocytes (PMNs) because of the loss of antiphagocytic capsular material.[97] Carbapenems and cephalosporins at subinhibitory concentrations increase the serum sensitivity of K1-positive *E. coli* by reducing expression of the K1 capsular polysaccharide.[214]

Even drugs with no inherent bacteriostatic or bactericidal activity against a specific organism can induce sufficient changes in the cell surface to affect serum sensitivity. Pre-incubation of *P. aeruginosa* with subinhibitory concentrations of certain macrolides decreases cell surface hydrophobicity and enhances the sensitivity of the organism to human serum bactericidal activity.[217] Subinhibitory β-lactam concentrations are capable of sufficiently altering the peptidoglycan structure in MRSA to enhance susceptibility of the organism to the lysozyme present in sputum, even though the organism, because of altered PBP production, is no longer susceptible to the bactericidal activity of these agents.[101] Although most effects stimulating bacterial clearance by the host occur as a direct result of cellular changes in the organism, immune cells also can be altered by subinhibitory antibiotic concentrations. Erythromycin does not affect the generation of serum-derived leuko-attractants, nor does it increase PMN cell numbers, yet it appears capable of stimulating the migration of PMNs. Researchers suggested that this effect probably results from the ability of erythromycin to sustain cellular migration by reducing the level of leuko-attractant–induced auto-oxidation mediated by the myeloperoxidase–hydrogen peroxide–halide system.[57]

In addition to enhancing bacterial clearance, subinhibitory antibiotic concentrations also can impair bacterial virulence mechanisms. For many organisms, pili, fimbriae, and fimbriae-associated adhesins are responsible for the initial events surrounding adherence and colonization at the site of entry or infection.[194] Subinhibitory quinolone and clindamycin concentrations are capable of decreasing the adherence of nosocomial pathogens (e.g., *Staphylococcus epidermidis)* to the synthetic materials of implantable catheters.[31,202] Subinhibitory concentrations of penicillins, cephalosporins, azalides, and quinolones all have demonstrated the ability to decrease the formation of fimbriae or alter structures that mediate adherence. Specific examples include decreased adherence to oral and urinary tract epithelial cells for organisms such as *Porphyromonas gingivalis*, *E. coli*, *Salmonella typhimurium*, and *P. aeruginosa*.[26,137,205,215,231] Moreover, these effects do

not appear to be growth phase–specific for select drug-microbe combinations.[27]

Subtherapeutic antibiotic concentrations also can have an impact on virulence by downregulating bacterial toxin production. Subinhibitory concentrations of clindamycin can inhibit toxin production in *S. epidermidis* markedly without appreciably altering bacterial growth.[202] *Pseudomonas* exoenzymes that facilitate disease pathogenesis (e.g., exotoxin A, exoenzyme S, phospholipase C, total protease, elastase) are suppressed after exposure to subinhibitory concentrations of ciprofloxacin, tobramycin, and ceftazidime.[85] Finally, given the ability of subinhibitory fluoroquinolone concentrations to disrupt DNA structure sufficiently to interfere with effective mRNA production, continuous exposure of *S. aureus* to fluoroquinolones at subinhibitory concentrations decreases the production of nuclease and hemolytic α-toxin production.[205]

Although discerning which of these effects, alone or in combination, are responsible for the subinhibitory effects is difficult, one may see in vivo that the impact of the sum total of these effects can be observed at the level of the host. In a non-neutropenic rat model of *Klebsiella pneumoniae* pneumonia, a continuous infusion of ceftazidime resulting in steady-state concentrations of 0.06 mg/L protected all the animals from death, despite an MIC for the pathogen of 0.2 mg/L. The same could not be said, however, for immunocompromised animals, which required steady-state concentrations of 0.38 mg/L for adequate protection.[188,189] In a similar model of intraperitoneal *K. pneumoniae* infection, subinhibitory clindamycin concentrations resulted in an increase in bacterial clearance and a decrease in the $\log_{10}$CFUs in blood at 72 hours.[9] In humans, low-dose ampicillin (such that concentrations in urine were no greater than one-fourth to one-half times the MIC) combined with large water intake was demonstrated to clear documented *E. coli* urinary tract infection in 16 of 20 patients within 2 days of initiating therapy, whereas none of the 18 control subjects receiving the fluid intervention alone demonstrated resolution of the infection.[181] Despite the resistance of *Pseudomonas* in patients with cystic fibrosis, the fluoroquinolones remain capable of eliciting a significant reduction in sputum colony counts and improvement in clinical signs and symptoms.[200,204]

Subinhibitory antimicrobial concentrations can be as potentially disadvantageous to the host as they can be beneficial. These concentrations can impair the ability of the host to respond to the bacterial inoculum, can augment bacterial defenses, and can serve to inactivate the antibiotic. For organisms that are susceptible to β-lactamase induction, strong inducers can upregulate β-lactamase synthesis at concentrations below the MIC. Although β-lactamase induction typically is a reversible phenomenon, many antibiotics demonstrate the ability to select out genetically de-repressed mutants at subinhibitory concentrations.[201,209] Irrespective of whether this induction is phenotypic or genotypic, many antibiotics to which the organism originally may have been susceptible no longer retain their efficacy in light of β-lactamase production. This type of adaptive resistance, observed in response to sublethal antibiotic concentrations, also can be nonenzymatic. A single, one-time exposure of *P. aeruginosa* to subinhibitory concentrations of chlorhexidine results in unstable resistance, and repeated exposure leads to stable expression of altered cell surface macromolecules or efflux systems responsible for resistance.[219] In a clinical strain of this same organism exposed to subinhibitory concentrations of fluoroquinolones and carbapenems for 5 days, a 16- to 32-fold increase in MIC was observed in concert with an alteration in overall protein expression.[32]

Sublethal antibiotic concentrations also can stimulate the production of toxins that may be detrimental to the host. Exposure of enterohemorrhagic *E. coli* to subinhibitory concentrations of fluoroquinolones, cephalosporins, tetracyclines, and inhibitors of folic acid synthesis induces enhanced expression of a *Shiga*-like toxin (SLT-1), which is proposed to mediate events surrounding the development of hemolytic-uremic syndrome. Subinhibitory fluoroquinolone concentrations also can stimulate the production of verotoxins in this same organism.[232,239] Lincomycin and tetracycline at subinhibitory concentrations stimulate the production of heat-labile enterotoxin in enterotoxigenic *E. coli*, as well as *Vibrio cholerae* enterotoxin, even though these agents are principally protein synthesis inhibitors. Prior data suggest that the copy number of plasmids does not increase and proposed that the rate of enterotoxin liberation is enhanced as a result of antibiotic inhibition of the synthesis of proteins responsible for degrading the toxin.[135,240] The α-toxin of *S. aureus* is demonstrated to be hemolytic, dermonecrotic, and antichemotactic and appears to be upregulated in the presence of subinhibitory nafcillin concentrations, irrespective of whether the isolate is susceptible to the agent. Greater hemolytic activity and increased lethality of the broth filtrate in a murine intraperitoneal model are observed, and this activity is ablated when incubated with anti–α-toxin antibody.[120] Other β-lactams (with the exception of aztreonam), ofloxacin, and trimethoprim are similarly capable of inducing α-toxin expression at subinhibitory concentrations.[165]

The upregulation of protein expression by subinhibitory antibiotic concentrations not only is responsible for contributing to antibiotic and cellular destruction but also can enhance the binding of pathogens to host proteins and implantable devices. Subinhibitory concentrations of ciprofloxacin increase the transcription of fibronectin-binding proteins in *S. aureus* isolates and thereby result in greater adhesion to immobilized fibronectin and fibronectin-coated polymers, a particular concern with subcutaneously implanted devices that have polymer surfaces.[19,20] Subinhibitory concentrations of antibiotic also can alter the immunogenicity of the pathogen such that the host can no longer mount the same degree of immune response. *S. aureus* isolates coincubated with subinhibitory concentrations of oxacillin demonstrated a diminished capacity to stimulate proinflammatory cytokine production (e.g., tumor necrosis factor-α, interleukin [IL]-1β, and IL-6) in human monocytes. Given that activation of the complement system and cytokine release are stimulated by peptidoglycan

molecules of a certain size or tertiary structure, subinhibitory β-lactam concentrations probably distort the peptidoglycan structure sufficiently that the immune system no longer effectively responds to these immunostimulatory components.[199]

Unless unusual circumstances prevail, the goal of therapy probably will not be to target plasma concentrations below the MIC. However, given the reality of encountering this situation in the clinical setting, practitioners certainly need to be aware of the potential implications for therapeutic response associated with subinhibitory concentrations of antimicrobial agents.

Effects That Persist After Antimicrobial Exposure (Postantibiotic Effect, Postantibiotic Leukocyte Enhancement, and Post–β-Lactamase Inhibitor Effect)

Early investigations into antibiotic activity described a delay in the recovery and regrowth of penicillin-exposed staphylococci and streptococci when the drug was removed by enzymatic inactivation or the organism was removed to drug-free media.[18,48,169] In addition, in vivo evaluations of penicillin activity suggested that the effects of penicillin in an immunocompetent animal lasted well beyond the time when blood levels remained above quantifiable concentrations. Despite a half-life of minutes, many hours could elapse after the administration of a dose of penicillin before bacterial growth resumes.[51,108,198] The term *postantibiotic effect* was coined to describe the suppression of bacterial growth that persists after brief exposure of the organism to an antimicrobial agent.[36] Unlike the subinhibitory effects discussed earlier in the chapter, the PAE occurs as a result of residual antimicrobial activity after initial exposure at inhibitory concentrations, as opposed to the subinhibitory concentrations themselves. The PAEs described to date typically are reversible; and although the mechanisms have not been elucidated fully, they probably are caused by either nonlethal damage or persistence of antibiotic at the site of action. In the same vein as the PAEs, the term *post–β-lactamase inhibitor effect* (PLIE) was coined to describe the residual effects of β-lactamase inhibitors after exposure to inhibitory concentrations.[220] Similarly, one encounters the term *postantibiotic leukocyte enhancement* (PALE), which is used to account for a PAE in vivo that lasts longer than that observed in vitro and is defined as exposure to subinhibitory concentrations of an antibiotic that render the organism more susceptible to the phagocytic and bactericidal action of neutrophils.

The PAE depends on many factors, the most discernible being the drug-microbe combination.[106] Since the initial descriptions with penicillin were reported, similar observations have been noted for a broad range of organisms, and essentially all antibiotic classes demonstrate a PAE against select organisms.[36] Virtually all agents evaluated demonstrate a PAE against susceptible gram-positive cocci. Inhibitors of protein and nucleic acid synthesis produce longer-lasting PAEs in vitro than do cell wall–acting agents, with an average of 1 to 2 hours for β-lactams, 1 to 3 hours for fluoroquinolones, and 3 to 5 hours for protein synthesis inhibitors. For resistant gram-positive cocci, descriptions of a PAE are mixed. Against gram-negative bacilli, no appreciable PAE is observed for trimethoprim or the β-lactams in vitro, with the exception of the carbapenems, which demonstrate a PAE of 1 to 2 hours. The fluoroquinolones and protein synthesis inhibitors have PAEs of roughly 1 to 3 hours and 3 to 8 hours, respectively. Within this group of organisms, *P. aeruginosa* serves as an exception and the fluoroquinolones and protein synthesis inhibitors display slightly shorter PAEs—1 to 2 hours and 2 to 3 hours on average for the respective drug classes. Similar observations have been described for the gram-negative anaerobes, with little or no PAE seen with the β-lactams but a measurable PAE observed for protein synthesis inhibitors.[36]

Given that most drug classes demonstrate a PAE, multiple mechanisms must exist by which this effect arises. For the β-lactams, researchers proposed that the PAE corresponds to the time required for the organism to synthesize new PBPs. The initial high β-lactam concentrations are thought to bind irreversibly to and thus inactivate the PBPs. As such, cell multiplication subsequently is prolonged until a critical number of PBPs are resynthesized and cell division can resume.[221] Within the large class of β-lactam agents, however, further distinctions can be made based on the specific PBP bound and the subsequent morphologic alteration that is induced. Against *E. coli*, the longest-lasting PAEs are observed for agents that induce the formation of spheroplasts and the shortest for agents that induce the formation of filaments. On drug removal, filaments (which may contain a biomass corresponding to more than 20 bacteria) readily separate into individual bacteria, whereas spheroplasts require a longer period to resynthesize a normal cell wall and resume replication.[90] Analogous to the β-lactams, the time required for the resynthesis or recovery of ribosomal proteins is probably responsible for the PAEs observed after the administration of an aminoglycoside. Evaluation of the intracellular events taking place in *E. coli* during aminoglycoside-induced PAEs suggests that both DNA and RNA synthesis resume immediately after drug removal; however, synthesis of structural and functional protein does not resume for nearly 5 hours.[13,106] For the fluoroquinolones, the proposed mechanism behind the PAE is not as consistent with the antimicrobial activity of these agents. A progressive increase in ^{3}H-thymidine incorporation in *S. aureus* during the PAE suggests that DNA synthesis continues to occur, and, thus, the PAE may represent the time needed to repair the damage to DNA gyrase, the time required to reestablish the function of DNA gyrase after dissociation of the antibiotic from the enzyme, or the time required to synthesize new DNA gyrase.[80]

In addition to the specific drug-microbe combination, the magnitude and duration of drug exposure also have an impact on the PAE. Cell wall–acting agents (e.g., β-lactams and glycopeptides) demonstrate a concentration-dependent PAE. Increases in the concentration and duration of exposure (i.e., increasing AUC) prolong the PAE

in both gram-positive and gram-negative organisms to a point of maximal effect, typically 2 to 6 hours, although the time may be shorter for some members of this class. In fact, the PAE appears to be related to the log-normalized AUC in a sigmoidal manner.[35,90,125] Similarly, an increasing concentration of protein synthesis inhibitors is associated with progressive prolongation in PAE to a point of maximal effect. However, it can be difficult to establish with the aminoglycosides and fluoroquinolones because complete killing at supratherapeutic concentrations can obscure accurate determination of the PAE.[36,106] Bacteriostatic agents generally demonstrate concentration-independent PAEs such that increasing the duration of antibiotic exposure, but not the concentration, results in an increase in PAE to some maximal effect.[36]

As expected, the magnitude of the PAE also depends on whether antimicrobial agents are administered alone or in combination. Indifferent, additive, and synergistic PAEs all have been described with the use of combination therapy.[68,87,106] However, for some drug-microbe combinations, the magnitude of the PAE may differ with the phenotypic expression of resistance. For *Enterococcus faecalis* isolates that demonstrate only low-level resistance to streptomycin or gentamicin, the effect of a penicillin-aminoglycoside combination on the PAE is markedly synergistic. In contrast, for isolates with high-level resistance to the aminoglycosides, no synergistic PAE is observed.[236] Additionally, physicochemical factors can affect in vitro determination of PAEs and, although the in vivo relevance is questionable, some are worthy of consideration. In *S. aureus*, the PAEs of penicillin and gentamicin are markedly protracted at the slightly acidic pH 6 as compared with a physiologic pH of 7.4. Given that a relatively acidic pH can occur at the site of active infection, a disease-PAE interaction is possible. A similar pH-dependent effect on PAE is not observed with the fluoroquinolones or macrolides against *S. aureus*, nor is it noted for *E. coli* and *P. aeruginosa* with the β-lactams, fluoroquinolones, and macrolides.[69,86] An increase in PAE in vitro also is observed as temperature drops, a finding that raises the question whether the PAE is truly altered or the generation time is lengthened. Regardless, the temperatures evaluated were much lower than physiologic temperatures and probably have no clinical impact in the presence of infection. Finally, anaerobic conditions are observed to increase the PAE for ciprofloxacin/*E. coli* and gentamicin/*S. aureus* combinations, but not with other combinations.[69]

The attention paid to PAE of late highlights its significance, which lies in the flexibility that it affords to extend the dosing interval before reexposure to drug, essentially redosing, is necessary. Researchers suggested that agents with a small PAE require that a dosing interval be selected that will maintain concentrations above the MIC throughout most of the interval. Agents with a more protracted PAE can be given less often and thus at higher doses, thereby supporting the argument for once-daily dosing with drugs such as the aminoglycosides. However, many nuances can be identified between the in vitro conditions under which PAEs are determined and the in vivo conditions of infection such that the ultimate clinical (i.e., therapeutic) implications of these data have yet to be determined. In vitro, attempts are made to remove the drug completely (i.e., abruptly terminate drug exposure to the microbe) after initial exposure when determining the PAE. In contrast, drug concentrations fall in a more controlled, typically first-order fashion in vivo. The impact of this distinction on PAEs in patients remains unknown. Variable growth rates or metabolic states of the organism when growing in vivo similarly may have an impact on the PAE. In vitro, a prolonged PAE is observed when *Enterococcus faecium* is exposed to the combination of gentamicin and penicillin; yet, when the same drug concentrations and the same inoculum size are achieved in the vegetation of a murine aortic valve endocarditis model, no prolongation of PAE can be observed.[95] Similarly, a rat model of *P. aeruginosa* endocarditis failed to demonstrate a PAE in vivo for the combination of imipenem and gentamicin despite a PAE of nearly 5 hours determined in vitro; however, an observable PAE in vitro and in vivo was noted in the same model when ciprofloxacin was evaluated.[94,105]

Impact of Inoculum Size on the Concentration-Effect Relationship

The antimicrobial effect observed at any given drug concentration may vary with the number of organisms at the site of infection. Accordingly, the size of the inoculum can be a primary determinant of drug efficacy. Early investigations clearly demonstrated that the duration of the infection before treatment is a significant predictor of clinical response.[47] When therapy is delayed, presumably leading to a larger inoculum, the efficacy of that therapy often is affected adversely. In non-neutropenic rats, larger doses of antibiotic were needed to treat *K. pneumoniae*, whether administered by continuous or intermittent infusion, when the infection was allowed to progress 34 hours before therapy was initiated versus 5 hours.[188] Many potential mechanisms have been proposed to account for this event. Large inocula perhaps generate a higher local density of enzymes that effectively reduce active (i.e., functional) antibiotic concentrations at the site of infection. Large bacterial populations also are statistically more likely to contain resistant organisms that arise by spontaneous mutation and predominate within the population as a result of the selective pressure posed by the antibiotic. One such example has been observed in a murine model of group A streptococcal myositis, in which the activity of penicillin is compromised most severely in the presence of a large inoculum (10^8 to 10^9 CFUs/mL), with relatively little impact on the activity of clindamycin. Given that the isolate did not produce β-lactamase and any residual PAE was irrelevant because both regimens maintained drug concentrations above the MIC for the duration of the dosing interval, this effect of the inoculum was attributed in part to the selection of a cell wall–deficient mutant.[210] Clindamycin, however, is not devoid of susceptibility to the inoculum effect for all organisms; an increase in the size of the inoculum or an increase in the time until therapy resulted in a lower reduction in $\log_{10}$CFUs in a murine *Bacillus*

fragilis abscess model.[111] Another proposed mechanism for the effect of the inoculum lies in the finding that dense populations of organisms may grow at a slower rate or be metabolically inactive when compared with their less dense counterparts. An effect arising by this mechanism probably will not affect antimicrobial agents that are bactericidal against both rapidly growing and stationary-phase organisms (e.g., fluoroquinolones). However, for agents that exert their bactericidal effects primarily on rapidly dividing organisms (e.g., β-lactams), activity may be compromised in the presence of an established infection in which the bacterial population is large and as many as 90 percent of the organisms may be slowly dividing and, thus, metabolically inactive. For clinical isolates of group G streptococci, time-kill studies demonstrated rapid complete bacterial killing when the organism was primarily in log-phase growth, regardless of whether 10^4 or 10^7 organisms were involved. In contrast, when the organism was principally in the stationary phase, rapid and complete killing was observed only for the smaller inoculum (10^4 organisms), with no appreciable killing at 10^8 organisms. Furthermore, in patients with a protracted clinical course of infection or recalcitrant infection, therapy failed despite high drug doses and the presence of a susceptible isolate.[129]

Attempting to link multiple pharmacodynamic effects in evaluating the outcome of anti-infective therapy poses an arduous task from both theoretical and practical perspectives, and, hence, a complete discussion is not undertaken in this chapter. Nonetheless, the combination of such effects has received some attention in the literature and merits mention. Specifically, large inocula appear to be capable of modulating the PAE and subinhibitory effects observed with numerous agents. Exposure of *E. coli* to subinhibitory sparfloxacin concentrations produced a pronounced inoculum-dependent subinhibitory effect. At a dose that was 0.3 times the MIC, growth of the organism was delayed by 0.3, 0.9, and 3.3 hours with inocula of 10^8, 10^6, and 10^4 CFUs, respectively, with similar observations noted for other drug-microbe combinations.[35,162]

Concentration-Dependent Combination Effects (Synergy and Antagonism)

As discussed earlier and elsewhere in this text, antimicrobial combinations are used for many purposes: to prevent or delay the emergence of resistance, to enable dosage reduction and thereby minimize dose-related toxicities, and to treat polymicrobial infections. Combination therapy can be designed with the goal of synergistic or additive activity in mind, or, alternatively, these effects can occur without conscious consideration or forethought. Moreover, the undesirable outcomes of indifference or antagonism similarly can result through the combination of agents. Although a complete review of antagonism and synergy is beyond the scope of this section, we would be remiss not to mention concentration-dependent combination effects. We provide this brief discussion specifically to point out that a simple classification of drug combinations as synergistic, antagonist, and so forth is not always feasible. As discussed elsewhere, disagreement in the classification certainly can arise when different methods are used for evaluation.[21,160]

However, even when restricted to evaluations using the same experimental methods, certain combinations can be classified differently, depending on antibiotic concentration. Synergy is observed with cefoperazone and low imipenem concentrations against MRSA; however, higher concentrations of imipenem antagonize the activity of cefoperazone. Although antagonism with imipenem often is explained away as being β-lactamase mediated, the concentration-dependent antagonism observed remains unexplained by this mechanism because antagonism was shown for strains that lacked β-lactamase production.[178] The combination of penicillin and clindamycin at subinhibitory concentrations demonstrates synergy against group A β-hemolytic streptococci. At concentrations ranging from two to four times the MIC, the combination demonstrates antagonism, and at clinically relevant concentrations (≈100 times the MIC), indifference is observed, with the combination exhibiting no advantage over either agent alone.[211] Against isolates of vancomycin-intermediate *S. aureus*, antagonism is observed when methicillin and vancomycin are combined at subinhibitory concentrations. However, when the methicillin concentration exceeds the MIC, synergy is observed with the same combination. Although the mechanism behind this differential activity remains unclear, researchers proposed that the antagonism observed at lower methicillin concentrations results from an increase in the density of non–cross-linked D-alanyl-D-alanine side chains, the target site for vancomycin and a substrate for the PBPs. This increase would decrease the efficacy of methicillin as a PBP inhibitor by substrate competition.[99] For certain strains of methicillin-sensitive *S. aureus*, a combination of rifampin and methicillin below the MIC is synergistic, but above the MBC it is antagonistic. Similarly, a rifampin-vancomycin combination is synergistic at concentrations close to the MBC but indifferent as concentrations rise.[242] Furthermore, for some drug-microbe combinations, it is not the classification per se that is affected by the concentration but rather the magnitude of the effect observed. Against *E. faecium*, fixed piperacillin and variable teicoplanin concentrations are synergistic. However, the degree of synergy depends on the teicoplanin concentration; comparable synergistic activity occurs at teicoplanin concentrations of 2, 4, or 8 mg/L; reduced synergistic activity occurs at teicoplanin concentrations of 16 and 32 mg/L; and the greatest degree of synergy is observed at a concentration of 64 mg/L.[180]

Thus, the available data support the finding that, in many cases, the clinical assignment or "expectation" of synergy, additivity, or antagonism by specific combinations of anti-infective agents cannot be assumed simply by virtue of the drug class or drug-specific mechanisms of action, or both. With drug combinations for which the activity against an infecting pathogen is potentially concentration dependent, prediction of therapeutic outcome must entail some assessment, be it actual or theoretical (e.g., the use of pharmacokinetic modeling), of the time-dependent drug concentration profile at the site or sites of infection.

PHARMACOGENETIC DETERMINANTS OF EFFECT

Although this chapter is designed to detail the pharmacokinetic and pharmacodynamic determinants of anti-infective drug response, we would be remiss not to introduce the emerging role of pharmacogenetics as a predictor of drug response and a driver of anti-infective drug therapy. Although the application of molecular biology to infectious diseases traditionally has been limited to diagnostics (e.g., polymerase chain reaction–based assays for the detection of viral pathogens and slow-growing microorganisms) and disease monitoring (e.g., viral loads), molecular tools increasingly are finding application with identifying the likelihood of drug response a priori and determining optimal treatment strategies for infectious pathogens. Genotyping strategies are used to identify mutations that confer drug resistance and guide the selection of antiretroviral therapy in management of human immunodeficiency virus infection.[134,227] Similarly, viral genotyping appears to be among the most robust predictors of response to combination therapy in the treatment of hepatitis C virus,[130,191] and it has prompted similar investigations for hepatitis B virus.[88,118] Finally, commercially available molecular-based assays have been developed for the determination of methicillin resistance in *S. aureus*, multidrug resistance in *M. tuberculosis*, and vancomycin resistance in *Enterococcus* in clinical isolates. These kits appear to perform well when compared with conventional phenotype-based methods.[121,152,166] However, the degree of integration for these kits into routine clinical practice remains to be determined.

FURTHER CONSIDERATIONS

As denoted earlier in the chapter, there are numerous studies that have explored pharmacodynamic surrogates for agents active against invasive fungal pathogens. Given the marked mortality rates associated with invasive fungal disease and the increasing number of pediatric patients requiring antifungal therapy, a few caveats surrounding susceptibility testing should be addressed.[150,173] The Clinical and Laboratory Standards Institute (CLSI) has published guidelines for susceptibility testing of *Candida* spp.; however, standardizing and interpreting MIC results for filamentous fungi (molds) remains complex.[33] Standardizing inoculum concentrations across the wide variety of molds can be challenging, determining the MIC end point is difficult as a reduction of turbidity is not easily visualized with molds, and discriminating drug effects from drug-solvent combination effects can be misleading.[65] Thus, the correlation between drug exposure and treatment success in children remains unclear and is largely based on clinical observation of the patient. Further investigation in the pharmacokinetic-pharmacodynamic interface for antifungal agents is critical with the continued need to identify surrogate measures to allow for optimizing therapy.

CONCLUSIONS

With a recognized decline in the development of new antimicrobial therapies over the past 2 decades and the emergence of multidrug-resistant organisms (e.g. gram-negative organisms, *Mycobacterium tuberculosis*), a continued need persists for the re-purposing of older agents while awaiting the future development of newer therapies.[208] Unfortunately, there remains a relative paucity of pharmacokinetic-pharmacodynamic information for many of the currently available antimicrobial agents in children (e.g., antitubercular agents); and for older agents that continue to hold important places in pediatric therapeutics (e.g., metronidazole, clindamycin), complete profiles of their clinical pharmacology (i.e., absorption, biotransformation) in infants and children are lacking.[81] For both existing and emerging drug therapies, pharmacokinetics provides a powerful and valuable tool. The clinical scientist can use pharmacokinetics to characterize a given drug by providing a profile of its absorption, distribution, metabolism, and excretion in individuals with and without disease, during normal alterations of human physiology (e.g., pregnancy), as well as under conditions of normal altered organ function or body composition (e.g., growth and development, senescence). For the clinical practitioner, the tool of pharmacokinetics can provide a means to individualize drug therapy by characterizing the relationship between drug dose and resultant drug concentrations in plasma or other relevant biologic fluids (e.g., urine, CSF, synovial fluid, pleural fluid, peritoneal fluid). When linked with information regarding the pharmacodynamic behavior of the antibiotic, the susceptibility of the organism, and the status of the host, the application of pharmacokinetics affords the practitioner the ability to exercise some degree of adaptive control over drug therapy by selecting a drug and dosing regimen that have the greatest likelihood of producing both efficacy and safety.

NEW REFERENCES SINCE THE SIXTH EDITION

33. Clinical Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of yeasts; third informational supplement (M27-A3)*. Wayne, PA: CLSI; 2008.
81. Graham SM. Treatment of paediatric TB: revised WHO guidelines. *Paediatr Respir Rev* 2011;**12**:22–6.
173. Prasad PA, Coffin SE, Leckerman KH, et al. Pediatric antifungal utilization: new drugs, new trends. *Pediatr Infect Dis J* 2008;**27**:1083–8.
185. Roberts JA, Webb S, Paterson D, et al. A systematic review on clinical benefits of continuous administration of ß-lactam antibiotics. *Crit Care Med* 2009;**37**:2071–8.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Anderson ET, Young LS, Hewitt WL. Simultaneous antibiotic levels in "breakthrough" gram-negative rod bacteremia. *Am J Med* 1976;**61**:493–7.
2. Andes D, Craig WA. In vivo activities of amoxicillin and amoxicillin-clavulanate against *Streptococcus pneumoniae*: application to breakpoint determinations. *Antimicrob Agents Chemother* 1998;**42**:2375–9.
3. Andes D, Craig WA. Pharmacodynamics of fluoroquinolones in experimental models of endocarditis. *Clin Infect Dis* 1998;**27**: 47–50.
4. Andes D, Marchillo K, Conklin R, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. *Antimicrob Agents Chemother* 2004;**48**:137–42.
5. Andes D, Marchillo K, Lowther J, et al. In vivo pharmacodynamics of HMR 3270, a glucan synthase inhibitor, in a murine candidiasis model. *Antimicrob Agents Chemother* 2003;**47**:1187–92.
6. Andes D, Marchillo K, Stamstad T, Conklin R. In vivo pharmacodynamics of a new triazole, ravuconazole, in a murine candidiasis model. *Antimicrob Agents Chemother* 2003;**47**:1193–9.
7. Andes D, Marchillo K, Stamstad T, Conklin R. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. *Antimicrob Agents Chemother* 2003; **47**:3165–9.
8. Andes D, Van Ogtrop ML, Peng J, Craig WA. In vivo pharmacodynamics of a new oxazolidinone (linezolid). *Antimicrob Agents Chemother* 2002;**46**:3484–9.
9. Arbo A, Mancilla J, Alpuche C, Santos JI. In vitro and in vivo effects of subinhibitory concentrations of clindamycin on experimental *Klebsiella pneumoniae* sepsis. *Chemotherapy* 1990;**36**:337–44.
10. Azoulay-Dupuis E, Vallee E, Bedos JP, et al. Prophylactic and therapeutic activities of azithromycin in a mouse model of pneumococcal pneumonia. *Antimicrob Agents Chemother* 1991;**35**: 1024–8.
11. Baker PJ, Busby WF, Wilson ME. Subinhibitory concentrations of cefpodoxime alter membrane protein expression of *Actinobacillus actinomycetemcomitans* and enhance its susceptibility to killing by neutrophils. *Antimicrob Agents Chemother* 1995;**39**:406–12.
12. Bakker-Woudenberg IA, van Gerwen AL, Michel MF. Efficacy of antimicrobial therapy in experimental rat pneumonia: antibiotic treatment schedules in rats with impaired phagocytosis. *Infect Immun* 1979;**25**:376–87.
13. Barmada S, Kohlhepp S, Leggett J, et al. Correlation of tobramycin-induced inhibition of protein synthesis with postantibiotic effect in *Escherichia coli*. *Antimicrob Agents Chemother* 1993; **37**:2678–83.
14. Barza M. Challenges to antibiotic activity in tissue. *Clin Infect Dis* 1994;**19**:910–15.
15. Baurenfiend A. Questioning dosing regimens of ciprofloxacin. *J Antimicrob Chemother* 1993;**31**:789–98.
16. Begg EJ, Peddie BA, Chambers ST, Boswell DR. Comparison of gentamicin dosing regimens using an in vitro model. *J Antimicrob Chemother* 1992;**29**:427–33.
17. Beyer KH, Woodward R, Peters L, et al. The prolongation of penicillin retention in the body by means of *para*-aminohippuric acid. *Science* 1944;**100**:107–8.
18. Bigger JW. The bactericidal action of penicillin on *Staphylococcus pyogenes*. *Ir J Med Sci* 1944;**227**:533–68.
19. Bisognano C, Vaudaux PE, Lew DP, et al. Increased expression of fibronectin-binding proteins by fluoroquinolone-resistant *Staphylococcus aureus* exposed to subinhibitory levels of ciprofloxacin. *Antimicrob Agents Chemother* 1997;**41**:906–13.
20. Bisognano C, Vaudaux P, Rohner P, et al. Induction of fibronectin-binding proteins and increased adhesion of quinolone-resistant *Staphylococcus aureus* by subinhibitory levels of ciprofloxacin. *Antimicrob Agents Chemother* 2000;**44**:1428–37.
21. Blaser J. Interactions of antimicrobial combinations in vitro: the relativity of synergism. *Scand J Infect Dis Suppl* 1991;**74**: 71–9.
22. Blaser J, Stone BB, Groner MC, Zinner SH. Comparative study with enoxacin and netilmicin in a pharmacodynamic model to determine importance of ratio of antibiotic peak concentration to MIC for bactericidal activity and emergence of resistance. *Antimicrob Agents Chemother* 1987;**31**:1054–60.
23. Blaser J, Stone BB, Zinner SH. Efficacy of intermittent versus continuous administration of netilmicin in a two-compartment in vitro model. *Antimicrob Agents Chemother* 1985;**27**:343–9.
24. Bodey GP, Ketchel SJ, Rodriguez V. A randomized trial of carbenicillin plus cefamandole or tobramycin in the treatment of febrile episodes in cancer patients. *Am J Med* 1979;**67**:608–16.
25. Bourne D, editor. *A first course in pharmacokinetics and biopharmaceutics*. Multimedia Resources for Pharmaceutical Education, American Association of College of Pharmacy; 2001. Available at http://gaps.cpb.ouhsc.edu.
26. Braga PC, Piatti G. Kinetics of filamentation of *Escherichia coli* induced by different sub-MICs of ceftibuten at different times. *Chemotherapy* 1993;**39**:272–7.
27. Breines DM, Burnham JC. Modulation of *Escherichia coli* type 1 fimbrial expression and adherence to uroepithelial cells following exposure of logarithmic phase cells to quinolones at subinhibitory concentrations. *J Antimicrob Chemother* 1994;**34**:205–21.
28. Brewin A, Arango L, Hadley WK, Murray JF. High-dose penicillin therapy and pneumococcal pneumonia. *JAMA* 1974;**230**: 409–13.
29. Bryan CS, Reynolds KL, Brenner ER. Analysis of 1,186 episodes of gram-negative bacteremia in non-university hospitals: the effects of antimicrobial therapy. *Rev Infect Dis* 1983;**5**:629–38.
30. Chalmers JP, Tiller DJ. Effects of treatment on the mortality rate in septicemia. *BMJ* 1969;**2**:338–41.
31. Chaudhry AZ, Knapp CC, Sierra-Madero J, Washington JA. Antistaphylococcal activities of sparfloxacin (CI-978 AT-4140), ofloxacin, and ciprofloxacin. *Antimicrob Agents Chemother* 1990;**34**: 1843–5.
32. Cipriani P, Giordano A, Magni A, et al. Outer membrane alterations in *Pseudomonas aeruginosa* after five-day exposure to quinolones and carbapenems. *Drugs Exp Clin Res* 1995;**2**:139–44.
33. Clinical Laboratory Standards Institute. *Reference method for broth dilution antifungal susceptibility testing of yeasts; third informational supplement (M27-A3)*. Wayne, PA: CLSI; 2008.
34. Craig WA. Choosing an antibiotic on the basis of pharmacodynamics. *Ear Nose Throat J* 1998;**77**(Suppl.):7–11.
35. Craig WA, Ebert SC. Killing and regrowth of bacteria in vitro: a review. *Scand J Infect Dis* 1991;**74**(Suppl.):63–70.
36. Craig WA, Gudmundsson S. Postantibiotic effect. In: Lorian V, editor. *Antibiotics in laboratory medicine*. 4th ed. Baltimore: Williams & Wilkins; 1996. p. 296–329.
37. Craven DE, Kollisch NR, Hsieh CR, et al. Vancomycin treatment of bacteremia caused by oxacillin-resistant *Staphylococcus aureus*: comparison with beta-lactam antibiotic treatment of bacteremia caused by oxacillin-sensitive *Staphylococcus aureus*. *J Infect Dis* 1983;**147**:137–43.
38. Dagan R, Klugman KP, Craig WA, Baquero F. Evidence to support the rationale that bacterial eradication in respiratory tract infection is an important aim of antimicrobial therapy. *J Antimicrob Chemother* 2001;**47**:129–40.
39. Daikos GL, Jackson GG, Lolans VT, Livermore DM. Adaptive resistance to aminoglycoside antibiotics from first-exposure down-regulation. *J Infect Dis* 1990;**162**:414–20.
40. Daikos GL, Lolans VT, Jackson GG. First-exposure adaptive resistance to aminoglycoside antibiotics in vivo with meaning for optimal clinical use. *Antimicrob Agents Chemother* 1991;**35**: 117–23.
41. de Wildt SN, Kearns GL, Hop WC, et al. Pharmacokinetics and metabolism of oral midazolam in preterm infants. *Br J Clin Pharmacol* 2002;**53**:390–2.
42. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN. Glucuronidation in humans: pharmacogenetic and developmental aspects. *Clin Pharmacokinet* 1999;**36**:439–52.
43. Drusano GL, Johnson D, Rosen M, Standiford MC. Pharmacodynamics of a fluoroquinolone antimicrobial agent in a neutropenic rat model of *Pseudomonas* sepsis. *Antimicrob Agents Chemother* 1993;**37**:483–90.
44. Drusano GL, Preston SL, Hardalo C, et al. Use of preclinical data for selection of a phase II/III dose for evernimicin and identification of a preclinical MIC breakpoint. *Antimicrob Agents Chemother* 2001;**45**:13–22.
45. Dudley MN, Blaser J, Gilbert D, et al. Combination therapy with ciprofloxacin plus azlocillin against *Pseudomonas aeruginosa*: effect of simultaneous versus staggered administration in an in vitro model. *J Infect Dis* 1991;**164**:499–506.

46. Dudley MN, Mandler HD, Gilbert D, et al. Pharmacokinetics and pharmacodynamics of intravenous ciprofloxacin: studies in vivo and in an in vitro dynamic model. *Am J Med* 1987;**82**:363–8.
47. Eagle H. The effect of the size of inoculum and the age of the infection and the curative dose of penicillin in experimental infections with streptococci, pneumococci and *Treponema pallidum*. *J Exp Med* 1949;**90**:595–607.
48. Eagle H. The recovery of bacteria from the toxic effects of penicillin. *J Clin Invest* 1949;**28**:832–6.
49. Eagle H, Fleishman R, Levy M. "Continuous" vs. "discontinuous" therapy with penicillin: the effect of the interval between injections on therapeutic efficacy. *N Engl J Med* 1953;**248**:481–8.
50. Eagle H, Fleishman R, Levy M. On the duration of penicillin action in relation to its concentration in the serum. *J Lab Clin Med* 1953;**41**:122–32.
51. Eagle H, Fleischman R, Musselman AD. The bactericidal action of penicillin in vivo: the participation of the host, and slow recovery of the surviving organisms. *Ann Intern Med* 1950;**33**:544–71.
52. Eagle H, Musselman AD. The rate of bactericidal action of penicillin in vitro as a function of its concentration, and its paradoxically reduced activity at high concentration against certain organisms. *J Exp Med* 1948;**88**:99–131.
53. Euler A, Byrne W, Campbell M. Basal and pentagastrin-stimulated gastric acid secretory rates in normal children and those with peptic ulcer disease. *J Pediatr* 1983;**103**:766–8.
54. Euler A, Byrne W, Meis P, et al. Basal and pentagastrin stimulated acid secretion in newborn human infants. *Pediatr Res* 1979;**13**:36–7.
55. Fantin B, Leclercq R, Merle Y, et al. Critical influence of resistance to streptogramin B–type antibiotics on activity of RP 59500 (quinupristin-dalfopristin) in experimental endocarditis due to *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1995;**39**:400–5.
56. Feder HM. Chloramphenicol: what we have learned in the last decade. *South Med J* 1986;**79**:1129–34.
57. Fernandes AC, Anderson R, Theron AJ, et al. Enhancement of human polymorphonuclear leucocyte motility by erythromycin in vitro and in vivo. *S Afr Med J* 1984;**66**:173–7.
58. Flick MR, Cluff LE. *Pseudomonas* bacteremia: review of 108 cases. *Am J Med* 1976;**60**:501–8.
59. Flockhart DA. *Clinically used drugs metabolized by cytochrome P450*. Indiana University School of Medicine; 2003. Available at http://medicine.iupui.edu/flockhart.
60. Fluckiger U, Segessenmann C, Gerber AU: Integration of pharmacokinetics and pharmacodynamics of imipenem in a human-adapted mouse. *Antimicrob Agents Chemother* 1991;**35**:1905–10.
61. Fontana R, Amalfitano G, Rossi L, Staat G. Mechanisms of resistance to growth inhibition and killing by beta-lactam antibiotics in enterococci. *Clin Infect Dis* 1992;**15**:486–9.
62. Fontana R, Boaretti M, Grossato A, et al. Paradoxical response of *Enterococcus faecalis* to the bactericidal activity of penicillin is associated with reduced activity of one autolysin. *Antimicrob Agents Chemother* 1990;**34**:314–20.
63. Forrest A, Ballow CH, Nix DE, et al. Development of a population pharmacokinetic model and optimal sampling strategy for intravenous ciprofloxacin. *Antimicrob Agents Chemother* 1993;**37**:1065–72.
64. Forrest A, Nix DE, Ballow CH, et al. Pharmacodynamics of intravenous ciprofloxacin in seriously ill patients. *Antimicrob Agents Chemother* 1993;**37**:1073–81.
65. Fothergill AW, Rinaldi MG, Sutton DA. Antifungal susceptibility testing. *Infect Dis Clin North Am* 2006;**20**:699–709.
66. Freid MA, Vosti KL. The importance of underlying disease in patients with gram-negative bacteremia. *Arch Intern Med* 1968;**121**:418–23.
67. Frimodt-Moller N, Bentzon MW, Thomsen VF. Experimental infection with *Streptococcus pneumoniae* in mice: correlation of in vitro activity and pharmacokinetic parameters with in vivo effect for 14 cephalosporins. *J Infect Dis* 1986;**154**:511–17.
68. Fuursted K. Comparative killing activity and postantibiotic effect of streptomycin combined with ampicillin, ciprofloxacin, imipenem, piperacillin or vancomycin against strains of *Streptococcus faecalis* and *Streptococcus faecium*. *Chemotherapy* 1988;**34**:229–34.
69. Fuursted K. Postexposure factors influencing the duration of postantibiotic effect: significance of temperature, pH, cations, and oxygen tension. *Antimicrob Agents Chemother* 1997;**41**:1693–6.
70. Gehanno P, Lenoir G, Berche P. In vivo correlates for *Streptococcus pneumoniae* penicillin resistance in acute otitis media. *Antimicrob Agents Chemother* 1995;**39**:271–2.
71. Gemmell CG, Lorian V. Effects of low concentrations of antibiotics on bacterial ultrastructure, virulence and susceptibility to immunodefenses: clinical significance. In: Lorian V, editor. *Antibiotics in laboratory medicine*. 4th ed. Baltimore: Williams & Wilkins; 1996. p. 397–452.
72. Gengo FM, Mannion TW, Nightingale CH, Schentag JJ: Integration of pharmacokinetics and pharmacodynamics of methicillin in curative treatment of experimental endocarditis. *J Antimicrob Chemother* 1984;**14**:619–31.
73. George RH, Dyas A. Paradoxical effect of penicillin in vivo. *J Antimicrob Chemother* 1986;**17**:684–5.
74. Gerber AU, Craig WA. Aminoglycoside-selected subpopulations of *Pseudomonas aeruginosa*. *J Lab Clin Med* 1982;**100**:671–81.
75. Gerber AU, Craig WA, Brugger HP, et al. Impact of dosing intervals on activity of gentamicin and ticarcillin against *Pseudomonas aeruginosa* in granulocytopenic mice. *J Infect Dis* 1983;**147**:910–17.
76. Gerber AU, Kozac S, Segessenmann C, et al. Once daily versus thrice-daily administration of netilmicin in combination therapy of *Pseudomonas aeruginosa* infection in a man-adapted neutropenic animal model. *Eur J Clin Microbiol Infect Dis* 1989;**8**:233–7.
77. Gibaldi M, Perrier D, editors. *Pharmacokinetics*. 2nd ed. revised and expanded. New York: Marcel Dekker; 1982.
78. Gilman JT, Gal P. Pharmacokinetic and pharmacodynamic data collection in children and neonates: a quiet frontier. *Clin Pharmacokinet* 1992;**23**:1–9.
79. Ginsburg CM, McCraken GH Jr, Petruska M, Olson K. Pharmacokinetics and bactericidal activity of cefuroxime axetil. *Antimicrob Agents Chemother* 1985;**28**:504–7.
80. Gottfredsson M, Erlendsdottir H, Kolka R, Gudmundsson S. Metabolic and ultrastructural effects induced by ciprofloxacin in *Staphylococcus aureus* during the postantibiotic effect (PAE) phase. *Scand J Infect Dis Suppl* 1991;**74**:124–8.
81. Graham SM. Treatment of paediatric TB: revised WHO guidelines. *Paediatr Respir Rev* 2011;**12**:22–6.
82. Grand RJ, Watkins JG, Torti FM. Development of the human gastrointestinal tract. *Gastroenterology* 1976;**70**:790–810.
83. Grasso S, Meinardi G, de Carneri I, Taassia V. New in vitro models to study the effect of antibiotic concentration and rate of elimination on antibacterial activity. *Antimicrob Agents Chemother* 1978;**13**:570–6.
84. Griffiths LR, Green HT. Paradoxical effect of penicillin in-vivo. *J Antimicrob Chemother* 1985;**15**:507–8.
85. Grimwood K, To M, Rabin HR, Woods DE. Inhibition of *Pseudomonas aeruginosa* exoenzyme expression by subinhibitory antibiotic concentrations. *Antimicrob Agents Chemother* 1989;**33**:41–7.
86. Gudmundsson A, Erlendsdottir H, Gottfredsson M, Gudmundsson S. Impact of pH and cationic supplementation on in vitro postantibiotic effect. *Antimicrob Agents Chemother* 1991;**35**:2617–24.
87. Gudmundsson S, Erlensdottir H, Gottfredsson M, Gudmundsson A. The postantibiotic effect induced by antimicrobial combinations. *Scand J Infect Dis Suppl* 1991;**74**:80–93.
88. Guettouche T, Hnatyszyn HJ. Chronic hepatitis B and viral genotype: the clinical significance of determining HBV genotypes. *Antivir Ther* 2005;**10**:593–604.
89. Gustafsson I, Hjelm E, Cars O. In vitro pharmacodynamics of the new ketolides HMR 3004 and HMR 3647 (telithromycin) against *Chlamydia pneumoniae*. *Antimicrob Agents Chemother* 2000;**44**:1846–9.
90. Hanberger H, Nilsson LE, Maller R, Nilsson M. Pharmacodynamics of beta-lactam antibiotics on gram-negative bacteria: Initial killing, morphology and post-antibiotic effect. *Scand J Infect Dis Suppl* 1991;**74**:118–23.
91. Handwerger S, Tomasz A. Antibiotic tolerance among clinical isolates of bacteria. *Rev Infect Dis* 1985;**7**:368–86.
92. Hayton WL, Stoeckel K. Age-associated changes in ceftriaxone pharmacokinetics. *Clin Pharmacokinet* 1986;**11**:76–86.
93. Herrera-Insua I, Perez P, Ramos C, et al. Synergistic effect of azithromycin on the phagocytic killing of *Staphylococcus aureus* by human polymorphonuclear leukocytes. *Eur J Clin Microbiol Infect Dis* 1997;**16**:13–16.

94. Hessen MT, Pitsakis PG, Levison ME. Absence of a postantibiotic effect in experimental *Pseudomonas* endocarditis treated with imipenem with or without gentamicin. *J Infect Dis* 1988;**158**: 542–8.
95. Hessen MT, Pitsakis PG, Levison ME. Postantibiotic effect of penicillin plus gentamicin versus *Enterococcus faecalis* in vitro and in vivo. *Antimicrob Agents Chemother* 1989;**33**:608–11.
96. Holm SE, Odenholdt-Tornqvist I, Cars O. Paradoxical effects of antibiotics. *Scand J Infect Dis Suppl* 1991;**74**:113–17.
97. Horne D, Tomasz A. Hypersusceptibility of penicillin treated group B streptococci to bactericidal activity of human polymorphonuclear leukocytes. *Antimicrob Agents Chemother* 1981;**19**: 745–53.
98. Hostacka A. Changes in serum sensitivity and hydrophobicity in a clinical isolate of *Klebsiella pneumoniae* treated with subinhibitory concentrations of aminoglycosides. *Zentralbl Bakteriol* 1998;**288**: 519–26.
99. Howe RA, Wootton M, Bennett PM, et al. Interactions between methicillin and vancomycin in methicillin-resistant *Staphylococcus aureus* strains displaying different phenotypes of vancomycin susceptibility. *J Clin Microbiol* 1999;**37**:3068–71.
100. Hyatt JM, Nix DE, Schentag JJ. Pharmacokinetic and pharmacodynamic activities of ciprofloxacin against strains of *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* for which MICs are similar. *Antimicrob Agents Chemother* 1994;**38**: 2730–7.
101. Igarashi K, Matsuyama T. Alternative activity of beta-lactam antibiotics against methicillin- and cephem-resistant *Staphylococcus aureus* in the presence of respiratory tract mucus. *J Infect Dis* 1990;**161**:250–4.
102. Ikeda Y, Fukuoka Y, Motomura K, et al. Paradoxical activity of beta-lactam antibiotics against *Proteus vulgaris* in experimental infection in mice. *Antimicrob Agents Chemother* 1990;**34**:94–7.
103. Ikeda N, Hanaki H, Hiramatsu K, Kuwabara Y. In vitro susceptibility of *Streptococcus agalactiae* clinical isolates to beta-lactam antibiotics. *Kansenshogaku Zasshi* 1999;**73**:163–71.
104. Ikeda Y, Nishino T. Paradoxical antibacterial activities of beta-lactams against *Proteus vulgaris*: mechanism of the paradoxical effect. *Antimicrob Agents Chemother* 1988;**32**:1073–7.
105. Ingerman MJ, Pitsakis PG, Rosenberg AF, Levison ME. The importance of pharmacodynamics in determining the dosing interval in therapy for experimental *Pseudomonas* endocarditis in the rat. *J Infect Dis* 1986;**153**:707–14.
106. Isaksson B, Hanberger H, Maller R, et al. The postantibiotic effect of amikacin alone and in combination with piperacillin on gram-negative bacteria. *Scand J Infect Dis Suppl* 1991;**74**: 129–32.
107. Isenberg HD. Clinical evaluation of laboratory guidance to antibiotic therapy. *Health Lab Sci* 1967;**4**:164–80.
108. Jawetz E. Dynamics of the action of penicillin in experimental animals. *Arch Intern Med* 1946;**77**:1–15.
109. Jayaram R, Gaonkar S, Kaur P, et al. Pharmacokinetics-pharmacodynamics of rifampin in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother* 2003;**47**:2118–24.
110. Jayaram R, Shandil RK, Gaonkar S, et al. Isoniazid pharmacokinetics-pharmacodynamics in an aerosol infection model of tuberculosis. *Antimicrob Agents Chemother* 2004;**48**: 2951–7.
111. Joiner K, Lowe B, Dzink J, Bartlett JG. Comparative efficacy of 10 antimicrobial agents in experimental infection with *Bacteroides fragilis*. *J Infect Dis* 1982;**145**:561–8.
112. Kearns GL. Desacetylcefotaxime: clinical implications of an active metabolite in infants and children. *Drug Invest* 1992;**4**(Suppl. 2): 9–17.
113. Kearns GL. Impact of developmental pharmacology on pediatric study design: overcoming the challenges. *J Allergy Clin Immunol* 2000;**106**(Suppl.):128–38.
114. Kearns GL, Abdel-Rahman SM, Blumer JL, et al. Single dose pharmacokinetics of linezolid in infants and children. *Pediatr Infect Dis J* 2000;**19**:1178–84.
115. Kearns GL, Bradley JS, Jacobs RF, et al. Single dose pharmacokinetics of pleconaril in neonates. Pediatric Pharmacology Research Unit Network. *Pediatr Infect Dis J* 2002;**19**:833–9.
116. Kearns GL, Young RA. Pharmacokinetics of cefotaxime and desacetylcefotaxime in the young. *Diagn Microbiol Infect Dis* 1995; **22**:97–104.
117. Kearns GL, Young RA, Jacobs RF. Cefotaxime dosing in infants and children: pharmacokinetic and clinical rationale for an extended dosing interval. *Clin Pharmacokinet* 1992;**4**:284–97.
118. Keeffe EB, Dieterich DT, Han SH, et al. A treatment algorithm for the management of chronic hepatitis B virus infection in the United States: an update. *Clin Gastroenterol Hepatol* 2006;**4**: 936–62.
119. Kennedy MJ, Abdel-Rahman SM, Kearns GL. Oxazolidinones: clinical pharmacology and use in the treatment of infections caused by resistant gram-positive pathogens. *Semin Pediatr Infect Dis* 2001;**12**:186–99.
120. Kernodle DS, McGraw PA, Barg NL, et al. Growth of *Staphylococcus aureus* with nafcillin in vitro induces alpha-toxin production and increases the lethal activity of sterile broth filtrates in a murine model. *J Infect Dis* 1995;**172**:410–19.
121. Kilic A, Baysallar M, Bahar G, et al. Evaluation of the EVIGENE VRE detection kit for detection of *vanA* and *vanB* genes in vancomycin-resistant enterococci. *J Med Microbiol* 2005;**54**: 347–50.
122. Kirby WMM. Bacteriostatic and lytic actions of penicillin on sensitive and resistant staphylococci. *J Clin Invest* 1945;**24**: 165–9.
123. Kondo N, Kuwahara-Arai K, Kuroda-Murakami H, et al. Eagle-type methicillin resistance: new phenotype of high methicillin resistance under *mec* regulator gene control. *Antimicrob Agents Chemother* 2001;**45**:815–24.
124. Kreger BE, Craven DE, McCabe WR. Gram-negative bacteremia, part IV: re-evaluation of clinical features and treatment in 612 patients. *Am J Med* 1980;**68**:344–55.
125. Kuenzi B, Segessenmann C, Gerber AU. Postantibiotic effect of roxithromycin, erythromycin, and clindamycin against selected gram-positive bacteria and *Haemophilus influenzae*. *J Antimicrob Chemother* 1987;**20**(Suppl. B):39–46.
126. Lacy MK, Lu W, Xu X, et al. Pharmacodynamic comparisons of levofloxacin, ciprofloxacin and ampicillin against *Streptococcus pneumoniae* in an in vitro model of infection. *Antimicrob Agents Chemother* 1999;**43**:672–7.
127. Lagast H, Meunier-Carpienter F, Klastersky J. Treatment of gram-negative bacillary septicaemia with cefoperazine. *Eur J Clin Microbiol* 1983;**2**:554–8.
128. Lam C, Georgopoulos A, Laber G, Schultze E. Therapeutic relevance of penicillin-induced hypersensitivity of *Staphylococcus aureus* to killing by polymorphonuclear leukocytes. *Antimicrob Agents Chemother* 1984;**26**:149–54.
129. Lam K, Bayer AS. Serious infections due to group G streptococci: report of 15 cases with in vitro–in vivo correlations. *Am J Med* 1983;**75**:561–70.
130. Lee SS, Heathcote EJ, Reddy KR, et al. Prognostic factors and early predictability of sustained viral response with peginterferon alfa-2a (40 KD). *J Hepatol* 2002;**37**:500–6.
131. Leeder JS, Kearns GL: Pharmacogenetics in pediatrics: implications for practice. *Pediatr Clin North Am* 1997;**44**:55–7.
132. Leggett JE, Ebert S, Fantin B, Craig WA. Comparative dose-effect relations at several dosing intervals for beta-lactam, aminoglycoside and quinolone antibiotics against gram-negative bacilli in murine thigh-infection and pneumonitis models. *Scand J Infect Dis Suppl* 1991;**74**:179–84.
133. Leggett JE, Fantin B, Ebert S, et al. Comparative antibiotic dose-effect relations at several dosing intervals in murine pneumonitis and thigh infection models. *J Infect Dis* 1989;**159**:281–92.
134. Lengauer T, Sing T. Bioinformatics-assisted anti-HIV therapy. *Nat Rev Microbiol* 2006;**4**:790–7.
135. Levner M, Weiner FP, Rubin BA. Induction of *Escherichia coli* and *Vibrio cholerae* enterotoxins by an inhibitor of protein synthesis. *Infect Immun* 1977;**15**:132–7.
136. Lister PD, Sanders CC. Pharmacodynamics of levofloxacin and ciprofloxacin against *Streptococcus pneumoniae*. *J Antimicrob Chemother* 1999;**43**:79–86.
137. Lo Bue AM, Rossetti B, Cali G, et al. Antimicrobial interference of a subinhibitory concentration of azithromycin on fimbrial production of *Porphyromonas gingivalis*. *J Antimicrob Chemother* 1997;**40**:653–7.
138. Lorian V. Some effects of subinhibitory concentrations of antibiotics on bacteria. *Bull N Y Acad Med* 1975;**51**:1046–55.
139. Lorian V, Atkinson B. Abnormal forms of bacteria produced by antibiotics. *Am J Clin Pathol* 1975;**64**:678–88.

140. Lorian V, Burns L. Predictive value of susceptibility tests for the outcome of antibacterial therapy. *J Antimicrob Chemother* 1990; **25**:175–81.
141. Lorian V, Sabath LD. Penicillin and cephalosporin: differences in morphologic effects on *Proteus mirabilis*. *J Infect Dis* 1972; **125**:560–4.
142. Lorian V, Silletti RP, Biondo FX, De Freitas CC. Paradoxical effect of aminoglycoside antibiotics on the growth of gram-negative bacilli. *J Antimicrob Chemother* 1979;**5**:613–16.
143. Louie A, Deziel M, Liu W, et al. Pharmacodynamics of caspofungin in a murine model of systemic candidiasis: importance of persistence of caspofungin in tissues to understanding drug activity. *Antimicrob Agents Chemother* 2005;**49**:5058–68.
144. Lowdin E, Odenholt I, Cars O. In vitro studies of pharmacodynamic properties of vancomycin against *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Agents Chemother* 1998;**42**: 2739–44.
145. Lutsar I, Friedland IR, Wubbel L, et al. Pharmacodynamics of gatifloxacin in cerebrospinal fluid in experimental cephalosporin-resistant pneumococcal meningitis. *Antimicrob Agents Chemother* 1998;**42**:2650–5.
146. MacArthur RD, Lolans V, Zar F, Jackson GG. Biphasic, concentration-dependent and rate-limited, concentration independent bacterial killing by an aminoglycoside antibiotic. *J Infect Dis* 1984;**150**:778–9.
147. Martin E, Fanconi S, Kalin P, et al. Ceftriaxone-bilirubin-albumin interactions in the neonate: an in vivo study. *Eur J Pediatr* 1993;**152**:530–40.
148. Mascellino MT, De Vito ML, Maclean Feeney E, et al. Phagocytosis and killing of A-protein positive *Staphylococcus aureus* in the presence of low doses of antibiotics. *Drugs Exp Clin Res* 1989;**15**: 63–9.
149. McCabe WR, Jackson GG. Gram-negative bacteremia, part II: clinical and therapeutic observations. *Arch Intern Med* 1962; **110**:856–64.
150. McNeil MM, Nash SL, Hajjeh RA, et al. Trends in mortality due to invasive mycotic diseases in the United States, 1980–1997. *Clin Infect Dis* 2001;**33**:641–7.
151. Meinl B, Hyatt JM, Forrest A, et al. Pharmacokinetic/pharmacodynamic predictors of time to clinical resolution in patients with acute bacterial exacerbations of chronic bronchitis treated with a fluoroquinolone. *Int J Antimicrob Agents* 2000;**16**: 273–80.
152. Miotto P, Piana F, Penati V, et al. Use of genotype MTBDR assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* clinical strains isolated in Italy. *J Clin Microbiol* 2006;**44**:2485–91.
153. Moise PA, Forrest A, Bhavnani SM, et al. Area under the inhibitory curve and a pneumonia scoring system for predicting outcomes of vancomycin therapy for respiratory infections by *Staphylococcus aureus*. *Am J Health Syst Pharm* 2000;**57**(Suppl. 2): 4–9.
154. Moore RD, Lietman PS, Smith CR. Clinical response to aminoglycoside therapy: importance of the ratio of peak concentration to minimum inhibitory concentration. *J Infect Dis* 1987;**155**:93–9.
155. Moore RD, Smith CR, Lietman PS. Association of aminoglycoside plasma levels with therapeutic outcome in gram-negative pneumonia. *Am J Med* 1984;**77**:657–62.
156. Moore RD, Smith CR, Lietman PS. The association of aminoglycoside plasma levels with mortality in patients with gram negative bacteremia. *J Infect Dis* 1984;**149**:443–8.
157. Mouton JW, van Ogtrop ML, Andes D, Craig WA. Use of pharmacodynamic indices to predict efficacy of combination therapy in vivo. *Antimicrob Agents Chemother* 1999;**43**:2473–8.
158. Nishi T, Tsuchiya K. Experimental respiratory tract infection with *Klebsiella pneumoniae* DT-S in mice: chemotherapy with kanamycin. *Antimicrob Agents Chemother* 1980;**17**:494–505.
159. Noone P, Parsons TMS, Pattison JR, et al. Experience in monitoring gentamicin therapy during treatment of serious gram negative sepsis. *BMJ* 1974;**1**:477–81.
160. Norden CW, Wentzel H, Keleti E. Comparison of techniques for measurement of in vitro antibiotic synergism. *J Infect Dis* 1979;**140**:629–33.
161. Odenholt-Tornqvist I. Pharmacodynamics of beta-lactam antibiotics. *Scand J Infect Dis Suppl* 1989;**58**:1–55.
162. Odenholt-Tornqvist I, Bengtsson S. Postantibiotic effect, and postantibiotic effect of subinhibitory concentrations, of sparfloxacin on gram-negative bacteria. *Chemotherapy* 1994;**40**:30–6.
163. Odenholt-Tornqvist I, Lowdin E, Cars O. Pharmacodynamic effects of subinhibitory concentrations of β-lactam antibiotics in vitro. *Antimicrob Agents Chemother* 1991;**35**:1834–9.
164. Odenholt-Tornqvist I, Lowdin E, Cars O. Postantibiotic sub-MIC effect of vancomycin, roxithromycin, sparfloxacin and amikacin. *Antimicrob Agents Chemother* 1992;**36**:1852–8.
165. Ohlsen K, Ziebuhr W, Koller KP, et al. Effects of subinhibitory concentrations of antibiotics on alpha-toxin (HLA) gene expression of methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolates. *Antimicrob Agents Chemother* 1998;**42**:2817–23.
166. Otte KM, Jenner S, Wulffen HV. Identification of methicillin-resistant *Staphylococcus aureus* (MRSA): comparison of a new molecular genetic test kit (GenoType MRSA) with standard diagnostic methods. *Clin Lab* 2005;**51**:389–93.
167. Papadopoulou ZL, Tina LU, Sandler P, et al. Size and function of the kidneys. In: Johnson TR, Moore WM, Jeffries JE, editors. *Children are different: developmental physiology*. 2nd ed. Columbus, OH: Ross Laboratories; 1978. p. 97–104.
168. Pappas PG, Rotstein CM, Betts RF, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. *Clin Infect Dis* 2007;**45**:883–93.
169. Parker RF, Luse S. The action of penicillin on staphylococcus: further observations on the effect of a short exposure. *J Bacteriol* 1948;**56**:75–84.
170. Peloquin CA, Cumbo TJ, Nix DE, et al. Evaluation of intravenous ciprofloxacin in patients with nosocomial lower respiratory tract infections. *Arch Intern Med* 1989;**149**:2269–73.
171. Piddock LJ, Walters RN, Diver JM. Correlation of quinolone MIC and inhibition of DNA, RNA, and protein synthesis and induction of the SOS response in *Escherichia coli*. *Antimicrob Agents Chemother* 1990;**34**:2331–6.
172. Powley L, Meeson J, Greenwood D. Tolerance to penicillin in streptococci of viridans group. *J Clin Pathol* 1989;**42**:77–80.
173. Prasad PA, Coffin SE, Leckerman KH, et al. Pediatric antifungal utilization: new drugs, new trends. *Pediatr Infect Dis J* 2008;**27**: 1083–8.
174. Preston SL, Drusano GL, Berman AL, et al. Pharmacodynamics of levofloxacin: a new paradigm for early clinical trials. *JAMA* 1998;**279**:125–9.
175. Puntorieri M, Primavera A, Privitera O, et al. Observations on the tolerance and the paradoxical effect in enterococci. *J Chemother* 1994;**6**:377–82.
176. Queiroz MLS, Bathirunathan N, Mawer GE. Influence of dosage interval on the therapeutic response to gentamicin in mice infected with *Klebsiella pneumoniae*. *Chemotherapy* 1987;**33**:68–76.
177. Raimundo S, Fischer J, Eichelbaum M, et al. Elucidation of the genetic basis of the common "intermediate" metabolizer phenotype for drug oxidation by CYP2D6. *Pharmacogenomics* 2000;**10**: 577–81.
178. Rand KH, Brown P. Concentration-dependent synergy and antagonism between cefoperazone and imipenem against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1995; **39**:1173–7.
179. Raucy JL, Allen SW. Recent advances in P450 research. *Pharmacogenomics J* 2001;**1**:178–86.
180. Ravizzola G, Cabibbo E, Peroni L, et al. In vitro study of the synergy between β-lactam antibiotics and glycopeptides against enterococci. *J Antimicrob Chemother* 1997;**39**:461–70.
181. Redjeb SB, Slim A, Horchani A, et al. Effects of ten milligrams of ampicillin per day on urinary tract infections. *Antimicrob Agents Chemother* 1982;**22**:1084–6.
182. Riddick DS. Drug biotransformation. In: Kalant H, Roschlau WHE, editors. *Principles of medical pharmacology*. 6th ed. New York: Oxford University Press; 1998. p. 38–66.
183. Ritschel WA, Kearns GL. Pediatric pharmacokinetics. In: Ritschel WA, Kearns GL, editors. *Handbook of basic pharmacokinetics*. 6th ed. Washington, D.C. American Pharmaceutical Association; 2004. p. 227–40.
184. River JM, Wise R. Morphological and biochemical changes in *E. coli* after exposure to ciprofloxacin. *J Antimicrob Chemother* 1986;**18**(Suppl. D):31–41.

185. Roberts JA, Webb S, Paterson D, et al. A systematic review on clinical benefits of continuous administration of β-lactam antibiotics. *Crit Care Med* 2009;**37**:2071–8.
186. Romansky MJ, Rittman GE. A method of prolonging the action of penicillin. *Science* 1944;**100**:196–8.
187. Roosendaal R, Bakker-Woudenberg IA. Impact of the antibiotic dosage schedule on efficacy in experimental lung infections. *Scand J Infect Dis Suppl* 1991;**74**:155–62.
188. Roosendaal R, Bakker-Woudenberg IA, van den Berg JC, Michel MF. Therapeutic efficacy of continuous versus intermittent administration of ceftazidime in an experimental *Klebsiella pneumoniae* pneumonia in rats. *J Infect Dis* 1985;**152**:373–8.
189. Roosendaal R, Bakker-Woudenberg IA, van den Berghe-van Raffe M, Michel MF. Continuous vs. intermittent administration of ceftazidime in experimental *Klebsiella pneumoniae* pneumonia in normal and leukopenic rats. *Antimicrob Agents Chemother* 1986;**30**: 403–8.
190. Rowland M, Tozer TN, editors. *Clinical pharmacokinetics: concepts and applications*. 2nd ed. Philadelphia: Lea & Febiger; 1989.
191. Russo MW, Fried MW. Guidelines for stopping therapy in chronic hepatitis C. *Curr Gastroenterol Rep* 2004;**6**:17–21.
192. Ryffel C, Strassle A, Kayser FH, Berger-Bachi B. Mechanisms of heteroresistance in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1994;**38**:724–8.
193. Scaglione F, Demartini G, Dugnani S, Fraschini F. In vitro comparative dynamics of modified-release clarithromycin and of azithromycin. *Chemotherapy* 2000;**46**:342–52.
194. Schaeffer AJ, Amundsen SK, Schmidt LN. Adherence of *Escherichia coli* to human urinary tract epithelial cells. *Infect Immun* 1979;**24**:753–9.
195. Schentag JJ. Antimicrobial action and pharmacokinetics/pharmacodynamics: the use of AUIC to improve efficacy and avoid resistance. *J Chemother* 1999;**11**:426–39.
196. Schentag JJ, Nix DE, Adelman MH. Mathematical examination of dual individualization principles, part I: relationships between AUC above MIC and area under the inhibitory curve for cefmenoxime, ciprofloxacin and tobramycin. *Ann Pharmacother* 1991;**25**:1050–7.
197. Schentag JJ, Smith IL, Swanson DJ, et al. Role for individualization with cefmenoxime. *Am J Med* 1984;**77**(Suppl. 6A): 43–50.
198. Schmidt LH, Walley A, Larson RD. The influence of the dosage regimen on the therapeutic activity of penicillin G. *J Pharmacol Exp Ther* 1949;**96**:258–68.
199. Schmitz FJ, Verhoef J, Hadding U, et al. Reduction in cytokine release from human monocytes by modifications in cell wall structure of *Staphylococcus aureus* induced by subinhibitory concentrations of oxacillin. *J Med Microbiol* 1998;**47**:533–41.
200. Shalit I, Stutman HR, Marks MI, et al. Randomized study of two dosage regimens of ciprofloxacin for treating chronic bronchopulmonary infection in patients with cystic fibrosis. *Am J Med* 1987;**82**(Suppl. 4A):189–95.
201. Shannon K, Phillips I. The effects on β-lactam susceptibility of phenotypic induction and genotypic derepression of β-lactamase synthesis. *J Antimicrob Chemother* 1968;**18**(Suppl. E):15–22.
202. Shibl AM, Ramadan MA, Tawfik AF. Differential inhibition by clindamycin on slime formation, adherence to Teflon catheters and hemolysin production by *Staphylococcus epidermidis*. *J Chemother* 1994;**6**:107–10.
203. Silverman JA, Mortin LI, Vanpraagh AD, et al. Inhibition of daptomycin by pulmonary surfactant: in vitro modeling and clinical impact. *J Infect Dis* 2005;**191**:2149–52.
204. Smith AL. Antibiotic resistance is not relevant in infections in cystic fibrosis. *Pediatr Pulmonol* 1990;**8**(Suppl. 5):93.
205. Sonstein SA, Burnham JC. Effect of low concentrations of quinolone antibiotics on bacterial virulence mechanisms. *Diagn Microbiol Infect Dis* 1993;**16**:277–89.
206. Sorberg M, Hanberger H, Nilsson M, Nilsson LE. Pharmacodynamic effects of antibiotics and acid pump inhibitors on *Helicobacter pylori*. *Antimicrob Agents Chemother* 1997;**41**:2218–23.
207. Soriano F, Garcia-Corbeira P, Ponte C, et al. Correlation of pharmacodynamic parameters of five beta-lactam antibiotics with therapeutic efficacies in an animal model. *Antimicrob Agents Chemother* 1996;**40**:2686–90.
208. Spellberg B, Powers JH, Brass EP, et al. Trends in antimicrobial drug development: implications for the future. *Clin Infect Dis* 2004;**38**:1279–86.
209. Stapleton P, Shannon K, Phillips I. The ability of beta-lactam antibiotics to select mutants with derepressed beta-lactamase synthesis from *Citrobacter freundii*. *J Antimicrob Chemother* 1995; **36**:483–96.
210. Stevens DL, Gibbons AE, Bergstrom R, Winn V. The Eagle effect revisited: efficacy of clindamycin, erythromycin, and penicillin in the treatment of streptococcal myositis. *J Infect Dis* 1988;**158**: 23–8.
211. Stevens DL, Madaras-Kelly KJ, Richards DM. In vitro antimicrobial effects of various combinations of penicillin and clindamycin against four strains of *Streptococcus pyogenes*. *Antimicrob Agents Chemother* 1998;**42**:1266–8.
212. Stille W. Dose-activity relationships in chemotherapy. *Infection* 1980;**8**(Suppl. 1):14–20.
213. Stratton CW, Liu C, Ratner HB, Weeks LS. Bactericidal activity of daptomycin (LY146032) compared with those of ciprofloxacin, vancomycin, and ampicillin against enterococci as determined by kill-kinetic studies. *Antimicrob Agents Chemother* 1987;**31**: 1014–16.
214. Suerbaum S, Leying H, Meyer B, Opferkuch W. Influence of beta-lactam antibiotics on serum resistance of K1-positive blood culture isolates of *Escherichia coli*. *Antimicrob Agents Chemother* 1990;**34**:628–31.
215. Svanborg-Eden C, Sandberg T, Stenqvist K, Ahlstedt S. Decrease in adhesion of *Escherichia coli* to human urinary tract epithelial cells in vitro by subinhibitory concentrations of ampicillin. *Infection* 1978;**6**(Suppl. 1):121–3.
216. Svenungsson B, Kalin M, Lindgren LG. Therapeutic failure in pneumonia caused by a tolerant strain of *Staphylococcus aureus*. *Scand J Infect Dis* 1982;**14**:309–11.
217. Tateda K, Hirakata Y, Furuya N, et al. Effects of sub-MICs of erythromycin and other macrolide antibiotics on serum sensitivity of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1993;**37**: 675–80.
218. Thomas JK, Forrest A, Bhavnani SM, et al. Pharmacodynamic evaluation of factors associated with the development of bacterial resistance in acutely ill patients during therapy. *Antimicrob Agents Chemother* 1998;**42**:521–7.
219. Thomas L, Maillard JY, Lambert RJ, Russell AD: Development of resistance to chlorhexidine diacetate in *Pseudomonas aeruginosa* and the effect of a "residual" concentration. *J Hosp Infect* 2000; **46**:297–303.
220. Thorburn CE, Molesworth SJ, Sutherland R, Rittenhouse S. Postantibiotic and post–b-lactamase inhibitor effects of amoxicillin plus clavulanate. *Antimicrob Agents Chemother* 1996;**40**:2796–801.
221. Tornqvist IO, Holm SE, Cars O. Pharmacodynamic effects of subinhibitory antibiotic concentrations. *Scand J Infect Dis Suppl* 1991;**74**:94–101.
222. Tripodi MF, Adinolfi LE, Utili R, et al. Influence of subinhibitory concentrations of loracarbef (LY 163892) and daptomycin (LY 146032) on bacterial phagocytosis, killing and serum sensitivity. *J Antimicrob Chemother* 1990;**26**:491–501.
223. van den Anker JN, Kearns GL. Pharmacology of antimicrobial agents in preterm infants. In: Tibboel D, van der Voort E, editors. *Intensive care in childhood: a challenge to the future*. New York: Springer; 1996. p. 400–10.
224. van Ogtrop ML, Andes D, Stamstad TJ, et al. In vivo pharmacodynamic activities of two glycylcyclines (GAR-936 and WAY 152,288) against various gram-positive and gram-negative bacteria. *Antimicrob Agents Chemother* 2000;**44**:943–9.
225. Vance-Bryan K, Larson TA, Rotschafer JC, Toscano JP. Investigation of the early killing of *Staphylococcus aureus* by daptomycin by using an in vitro pharmacodynamic model. *Antimicrob Agents Chemother* 1992;**36**:2334–7.
226. Veber B, Vallee E, Desmonts JM, et al. Correlation between macrolide lung pharmacokinetics and therapeutic efficacy in a mouse model of pneumococcal pneumonia. *J Antimicrob Chemother* 1993; **32**:473–82.
227. Vercauteren J, Vandamme AM. Algorithms for the interpretation of HIV-1 genotypic drug resistance information. *Antiviral Res* 2006;**71**:335–42.

228. Vogelman B, Gudmundsson S, Leggett J, et al. Correlation of antimicrobial pharmacokinetic parameters with therapeutic efficacy in an animal model. *J Infect Dis* 1988;**158**:831–47.
229. Vogelman B, Gudmundsson S, Turnridge J, et al. In vivo postantibiotic effect in a thigh infection in neutropenic mice. *J Infect Dis* 1988;**157**:287–98.
230. Voorn GP, Thompson J, Goessens WH, et al. Paradoxical dose effect of continuously administered cloxacillin in treatment of tolerant *Staphylococcus aureus* endocarditis in rats. *J Antimicrob Chemother* 1994;**33**:585–93.
231. Vranes J, Zagar Z, Kurbel S. Influence of subinhibitory concentrations of ceftazidime, ciprofloxacin and azithromycin on the morphology and adherence of P-fimbriated *Escherichia coli*. *J Chemother* 1996;**8**:254–60.
232. Walterspiel JN, Ashkenazi S, Morrow AL, Cleary TG. Effect of subinhibitory concentrations of antibiotics on extracellular Shiga-like toxin I. *Infection* 1992;**20**:25–9.
233. Weaver LT, Austin S, Cole TJ. Small intestinal length: a factor essential for gut adaptation. *Gut* 1991;**32**:1321–3.
234. Weiss CF, Glazko AJ, Weston JK. Chloramphenicol in the newborn infant. *N Engl J Med* 1960;**262**:787–94.
235. White CA, Toothaker RD. Influence of ampicillin elimination half-life on in-vitro bactericidal effect. *J Antimicrob Chemother* 1985;**15**(Suppl. A):257–60.
236. Winstanley TG, Hastings JGM. Penicillin-aminoglycoside synergy and post-antibiotic effect for enterococci. *J Antimicrob Chemother* 1989;**23**:189–99.
237. Winter ME, Koda-Kimble MA, Young LY, editors. *Basic clinical pharmacokinetics*. 2nd ed. Vancouver, Wash: Applied Therapeutics; 1988.
238. Woolfrey BF, Enright MA. Ampicillin killing curve patterns for ampicillin-susceptible nontypeable *Haemophilus influenzae* strains by the agar dilution plate count method. *Antimicrob Agents Chemother* 1990;**34**:1079–87.
239. Yoh M, Frimpong EK, Voravuthikunchai SP, Honda T. Effect of subinhibitory concentrations of antimicrobial agents (quinolones and macrolide) on the production of verotoxin by enterohemorrhagic *Escherichia coli* O157:H7. *Can J Microbiol* 1999;**45**:732–9.
240. Yoh M, Yamamoto K, Honda T, et al. Effects of lincomycin and tetracycline on production and properties of enterotoxins of enterotoxigenic *Escherichia coli*. *Infect Immun* 1983;**42**:778–82.
241. Zinner SH, Dudley MN, Gilbert D, Bassignani M. Effect of dose and schedule on cefoperazone pharmacodynamics in an in vitro model of infection in a neutropenic host. *Am J Med* 1988;**85**(Suppl. 1A):56–8.
242. Zinner SH, Lagast H, Klastersky J. Antistaphylococcal activity of rifampin with other antibiotics. *J Infect Dis* 1981;**144**:365–75.

CHAPTER 235

Antibacterial Therapeutic Agents

Carla Garcia • George H. McCracken, Jr.

This review of the use of antimicrobial agents is divided into two sections: (1) the clinical pharmacology of currently available antibacterial drugs and (2) the various aspects of administration of antimicrobial agents to infants and children. The second section includes dosage schedules and routes, prophylactic use of antimicrobial agents, considerations in writing orders and prescriptions, and other aspects of administration. Drugs of value in the treatment of disease caused by viruses, fungi, mycobacteria, and parasites are discussed in other chapters dealing with these pathogens. For the most part, only antimicrobial agents approved for use in infants and children by the U.S. Food and Drug Administration (FDA) are discussed, although promising new agents that are likely to be approved are mentioned where relevant.

CLINICAL PHARMACOLOGY

The antibacterial agents of value in treating infectious diseases in infants and children may be classified into three major groups:

1. Agents that target cell wall or cell membrane: these agents include the following:
   - The β-lactams, including penicillins, cephalosporins, carbacephems, monobactams, and carbapenems
   - The glycopeptides (i.e., vancomycin)
   - The lipopeptides (i.e., daptomycin)
   - The polymyxin: colistin
2. Agents that interfere with protein synthesis, including the following:
   - Agents that bind the 30S subunit, including aminoglycosides, chloramphenicol, and tetracyclines
   - Agents that bind the 50S subunit, including macrolides, clindamycin, and linezolid
   - Other mechanisms: Rifamycins
3. Agents that target nucleic acids, including fluoroquinolones, sulfonamides, and metronidazole

The following properties that govern the use of each group of drugs in infants and children are considered: mechanism of action, mechanisms of resistance, in vitro efficacy, pharmacokinetics, therapeutic uses, available preparations, and side effects and toxicity.

AGENTS THAT TARGET THE CELL WALL

β-Lactams

Biochemical Structure

The β-lactams are a large group of compounds that have in common a four-membered β-lactam ring. The subclasses of β-lactams differ from one another with regard to their side chains and the presence of other ring structures: the penicillins contain a five-membered thiazolidine β-ring fused to the β-lactam ring,[173] the cephalosporins have a six-membered dihydrothiazine instead of the thiazolidine ring, and the carbacephems have a methylene group replacing the sulfur atom in the dihydrothiazine ring of the cephalosporin nucleus. The β-lactam ring is essential for antibacterial activity, whereas the side chains influence the pharmacologic properties of the β-lactam and the spectrum of antibacterial activity.

Mechanism of Action

Our understanding of the mechanism of action of the β-lactams has evolved over the past 3 decades. Previously, researchers thought that binding of β-lactam to a bacterial cell membrane–associated enzyme (transpeptidase) blocked the terminal step in synthesis of the peptidoglycan layer of the bacterial cell wall. Cell death would ensue because the weakened cell wall could not withstand the osmotic and mechanical pressure resulting from a growing bacterium.[303] More recent evidence suggested that it is a more complex process involving activation of endogenous autolytic systems[218,305] and inhibition of bacterial enzymes called the *penicillin binding proteins* (PBPs), which include transpeptidase, carboxypeptidase, and endopeptidase. These enzymes are located beneath the cell wall and are responsible for cell wall synthesis by achieving cross-linkage between peptide chains of peptidoglycans. PBPs are vital for cell division, cell shape, and structural integrity. Because the specific PBPs within each bacterial species and the affinity of each β-lactam antibiotic for a particular PBP differ, some β-lactams have better activity than others against particular bacteria. The various β-lactam antibiotics can have different morphologic effects on the same bacterial species; this property is thought to be related to specific functions of the PBP to which the β-lactam binds.[287] Some bacteria

have a deficiency in the system of autolytic enzymes that results in inhibition, but not killing of the bacteria by a β-lactam that otherwise would be bactericidal. This phenomenon is termed *tolerance* and is demonstrated in vitro by a minimal inhibitory concentration (MIC) in the susceptible range and a ratio of minimal bactericidal concentration (MBC) to MIC of 32 or greater.[133]

Lactam antibiotics are bactericidal against most susceptible bacteria. The nature of the bactericidal activity has been described as time-dependent, as opposed to the concentration-dependent bactericidal activity of aminoglycosides.[188] Bactericidal activity is thought to be optimal when the concentration of β-lactam antibiotic at the site of infection is 4 to 10 times greater than that of the MIC (or MBC) of the infecting organism. The rapidity and extent of killing are not increased when concentrations exceed that ratio. A more important determinant of bactericidal activity for β-lactams is the length of time during the dosing interval that the concentration of antibiotic exceeds the MIC (time > MIC) for the infecting organism. Optimal bacterial killing correlates with time greater than MIC that exceeds 40 to 50 percent of the dosing interval.[80]

Penicillins

Although first discovered by Fleming in the late 1920s, penicillin G was not available for general use in the United States for another 20 years. Since that time, numerous semisynthetic penicillins have been developed. The penicillins can be classified into four groups based on their antimicrobial activity, with some overlap (Table 235-1). The spectrum of activity among the compounds within each group usually is similar; the differences are attributable to their pharmacologic properties (Table 235-2).

Penicillin G and Penicillin V

Despite the more than 50 years that penicillin G has been in use, some bacteria continue to be exquisitely susceptible. Resistant strains of *Streptococcus pyogenes* (group A streptococcus) and *Streptococcus agalactiae* (group B streptococcus) have not emerged. Penicillin G remains the drug of choice for the treatment of disease caused by a wide variety of microorganisms (Table 235-3).

The mechanism by which most bacteria have acquired resistance to penicillin G is that of β-lactamase production; resistance caused by altered PBPs occurs less commonly. Most strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* produce penicillinase. *Neisseria gonorrhoeae* gradually developed resistance to penicillin G beginning in the late 1960s by various mechanisms, including plasmid-mediated production of a TEM1-like β-lactamase, as well as chromosomal mutations in at least four genes (*penA*, *penB*, *ponA*, and *mtrR*) that encode increased expression of an efflux pump and alterations in PBPs and porins.[238] Mechanisms of β-lactam resistance in *Bacteroides* spp. and other gram-negative anaerobes include production of class A lactamases (penicillinase or cephalosporinase) and class B metallo-β-lactamases that hydrolyze carbapenems, as well as alteration of PBPs.[17]

TABLE 235-1 **Classification Scheme for Penicillins**

Generic Name	Trade Name	Route
Natural Penicillins		
Penicillin G	Many	PO,* IM, IV
Penicillin V	Many	PO
Aminopenicillins		
Ampicillin	Many	PO, IM, IV
Amoxicillin	Many	PO
Amoxicillin/clavulanate	Augmentin	PO
Ampicillin/sulbactam	Unasyn	IM, IV
Bacampicillin	Spectrobid	PO
Penicillinase-Resistant Penicillins		
Cloxacillin	Cloxapen	PO
Dicloxacillin	Dycill, Dynapen, Pathocil	PO
Methicillin	Staphcillin	IM, IV
Nafcillin	Nafcil, Nallpen, Unipen	IM, IV, PO
Oxacillin	Bactocill, Prostaphlin	IM, IV, PO
Extended-Spectrum Penicillins		
Ticarcillin	Ticar	IM, IV
Ticarcillin/clavulate‡	Timentin	IV
Mezlocillin	Mezlin	IM, IV
Piperacillin†	Pipracil	IM, IV
Piperacillin/tazobactam†	Zosyn	IV

Modified from U.S. Dispensing Information. *Information for the health care professional.* Vol. 1, Thomson Micromedex, 2006.
*No longer available.
†Safety and efficacy have not been established for children younger than 2 months.
‡Safety and efficacy have not been established for children younger than 1 month.
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

Historically, penicillin has been the antibiotic of choice for treatment of *Neisseria meningitidis* infections, but strains with reduced susceptibility to penicillin have been reported in Europe, South America, Asia, Australia, and the United States, where approximately 15 percent of strains have alterations in PBP2 encoded by the *penA* gene that likely results from mosaic genes derived through transformation with DNA from commensal *Neisseria* spp. in the nasopharynx of colonized individuals. Although penicillin resistance resulting from production of β-lactamase has been reported, such strains are rare.[161]

Of particular concern is the global dissemination of strains of *Streptococcus pneumoniae* resistant to penicillin G as a result of altered PBPs. The first documented clinical case of infection caused by penicillin-resistant pneumococcus was reported in 1967 from Australia. Surveillance data in the United States estimated the average rate of penicillin nonsusceptibility to be 33 percent (high-level resistance, 16%), with the highest incidence of 44 percent reported in the south-central region.[156] However, since the introduction of the 7-valent

TABLE 235-2 **Pharmacokinetics of Penicillins**

Antibiotic	Oral Absorption (%)	Protein Binding (%)	Metabolized (%)	Urinary Recovery* (%)	Approximate Half-Life† (hr)
Natural Penicillins					
Penicillin G	—	60	20	20/60-90	0.5-0.7
Penicillin V	60-73	80	55	20-40	0.5-1
Aminopenicillins					
Ampicillin	35-50	20	10	40-45/75-90	1-1.5
Amoxicillin	75-90	20	10	60-75	1
Penicillinase-Resistant Penicillins					
Cloxacillin	50	95	20	30-60	0.5-1
Dicloxacillin	37-50	95-98	10	50-70	0.5-1
Methicillin	—	40	10	60-80	0.3-1
Nafcillin	Erratic	90	60-70	11-30	0.5-1.5
Oxacillin	30-35	90-94	45	55-60	0.4-0.7
Extended-Spectrum Penicillins					
Ticarcillin		45-60	15	60-80	1.0-1.2
Mezlocillin		16-42	20-30	55-60	0.8-1.1
Piperacillin		16	20-30	60-80	0.6-1.2

Modified from U.S. Dispensing Information. *Information for the health care professional.* Vol. 1, Thomson Micromedex, 2006.
*Urinary recovery after oral or parenteral administration.
†With normal renal function.

TABLE 235-3 **Microorganisms for Which Penicillin G or V Is the Drug of Choice**

Actinomyces israelii
Bacillus anthracis
Clostridium spp.
Corynebacterium diphtheriae
Erysipelothrix rhusiopathiae
Leptospira spp.
*Neisseria gonorrhoeae**
*Neisseria meningitidis**
Pasteurella multocida
Spirillum minus
*Staphylococcus aureus**,†
Streptobacillus moniliformis
Streptococcus groups A, B, C, D, G; viridans group†; anaerobic strains
Streptococcus pneumoniae†
Treponema pallidum

*Strains that do not produce lactamase.
†Only those without altered penicillin binding proteins.

pneumococcal conjugate vaccine (PCV7) in 2000, invasive pneumococcal disease in young children caused by vaccine serotypes decreased by approximately 90 percent. Conversely, invasive disease caused by nonvaccine serotypes increased.[181] The incidence of disease caused by 19A serotype increased, and these isolates are associated with β-lactam antibiotic resistance (including penicillin and third-generation cephalosporins).[143,295] The Active Bacterial Core Surveillance (ABC) report from 2010 showed penicillin nonsusceptibility of 10.6 percent and cefotaxime nonsusceptibility of 8.6 percent.[65] Currently, approximately 6 percent of pneumococci in the United States exhibit a multidrug-resistance phenotype defined as resistance to penicillin, trimethoprim-sulfamethoxazole (TMP-SMX), erythromycin, clindamycin, and tetracycline.[156] Although penicillin-resistant pneumococcal meningitis has developed in patients who were receiving vancomycin for pneumococcal sepsis,[69] no treatment failures have been documented when the appropriate dosage of vancomycin was administered. In 2010, a 13-valent pneumococcal vaccine (PCV13) that includes serotype 19A was implemented. Epidemiologic surveillance continues to establish changes in trends for invasive pneumococcal disease.

In 2008, the Clinical and Laboratory Standards Institute (CLSI; formerly the National Committee for Clinical Laboratory Standards) published new *S. pneumoniae* breakpoints for penicillin because of a reevaluation that showed clinical response to penicillin was being preserved in clinical studies of pneumococcal infection, despite reduced susceptibility in vitro. Under the former criteria, susceptible, intermediate, and resistant MIC breakpoints for penicillin were less than 0.06, 0.121, and greater than 2 μg/mL, respectively, for all pneumococcal isolates, regardless of clinical syndrome or route of penicillin administration. Those breakpoints remain unchanged for patients without meningitis who can be treated with oral penicillin. However, for patients without meningitis who are treated with intravenous penicillin, the new breakpoints are less than 2, 4, and greater than 8 μg/mL, respectively. Furthermore, isolates from patients with meningitis are now categorized as either susceptible or resistant, with intravenous penicillin breakpoints of less than 0.06 or greater than 0.12 μg/mL, respectively. Use of narrow-spectrum agents, such as penicillin, is encouraged to prevent the spread of antimicrobial-resistant *S. pneumoniae* and also the spread of methicillin-resistant *S. aureus* and *Clostridium difficile*,

which can result from use of broader spectrum antimicrobials.

Several oral and parenteral forms of penicillin G are available. Selection of a preparation is based on the pattern of antimicrobial activity, including the peak and duration of activity in serum and tissues, and factors that reflect absorption, distribution, and excretion of the drug. These characteristics of penicillins are as follows:

1. Aqueous (water-soluble) penicillin G produces high peak concentrations of antibacterial activity in serum within 30 minutes after intramuscular administration but is excreted rapidly; thus, the concentration in serum is low within 2 to 4 hours. If aqueous penicillin G is given by the intravenous route, the peak is higher and occurs earlier, and the duration of antibacterial activity in serum is shorter (≈2 hours). Aqueous penicillin G given intravenously is used for severe disease such as meningitis, complicated pneumonia, and endocarditis caused by susceptible pathogens. In such cases, the drug should be administered at frequent intervals, usually every 4 hours, until the infection has been controlled.
2. Procaine penicillin G given intramuscularly produces lower concentrations of serum antibacterial activity (≈10% to 30% of the peak concentration achieved by the same dosage of the aqueous form), but activity persists in serum for as long as 12 hours. Intramuscular administration of procaine penicillin G should be reserved for patients with mild-to-moderate disease who cannot tolerate oral preparations. A 10-day course of this agent may be used as an alternative to intravenously administered aqueous penicillin G for infants with documented or suspected congenital syphilis.[316]
3. Benzathine penicillin G given intramuscularly is a repository preparation that provides low concentrations of serum activity (≈1% to 2% of the peak concentration achieved by the same dosage of the aqueous form). After administration of this drug, concentrations of penicillin are measurable in serum for 3 weeks or more and in urine for several months. Pain at the site of injection is the major deterrent to widespread use of this unique antibiotic. A combination of the benzathine and procaine salts (900,000 and 300,000 U, respectively) is a less painful treatment and is comparable in efficacy to benzathine alone (1,200,000 U) for the treatment of streptococcal pharyngitis.[25] Benzathine penicillin G is appropriate for only highly sensitive organisms present in tissues that are well vascularized so that the drug can diffuse readily to the site of infection. Thus, benzathine penicillin G is suitable for treatment of children with group A streptococcal pharyngitis or impetigo and for prophylaxis of streptococcal infection in children who have had rheumatic carditis. Current recommendations by the Centers for Disease Control and Prevention (CDC)[316] for management of syphilis include the use of benzathine penicillin G for primary, secondary, and early latent syphilis (<1 year's duration). Benzathine penicillin G also is recommended for infants with suspected congenital syphilis who do not meet the criteria for therapy with a 10-day course of aqueous or procaine penicillin G and whose follow-up can be ensured for repeat serologic testing.
4. Oral preparations of buffered penicillin G (no longer available in the United States) and phenoxymethylpenicillin (penicillin V) are absorbed well from the gastrointestinal tract. The peak concentration of serum activity of penicillin V is approximately 40 percent, and that of buffered penicillin G is approximately 20 percent of the concentration achieved by the same dosage of aqueous penicillin G administered intramuscularly. Therefore, oral penicillins may be satisfactory for treating mild to moderately severe infections caused by susceptible organisms. Penicillin V and penicillin G have equivalent activity in vitro against gram-positive cocci, but penicillin V is less active than is penicillin G *against N. meningitidis*, *N. gonorrhoeae*, and susceptible strains of *Haemophilus influenzae*.[173] The benefit of treating streptococcal tonsillopharyngitis with a 10-day regimen of penicillin V for the prevention of acute rheumatic fever is based on indirect evidence derived from rates of pharyngeal eradication of *S. pyogenes* equivalent to those of injectable penicillin, the treatment with proven efficacy.[90,281]

All penicillins are excreted by both glomerular filtration and tubular secretion. The concomitant use of probenecid, a drug that blocks tubular secretion of organic acids, with a penicillin can produce higher peaks and more sustained concentrations of antimicrobial activity. Dosages and dosing intervals may need adjustment when penicillins are administered to persons with altered renal function.

Penicillinase-Resistant Penicillins

The semisynthetic penicillinase-resistant penicillins were developed in response to the emergence of penicillinase-producing staphylococci. The acyl side chain, by means of steric hindrance, prevents hydrolysis of the β-lactam ring by penicillinases. Most strains of *S. aureus* produce penicillinase, regardless of whether the infection is health care–associated or community acquired. In areas of low rates of resistance to β-lactams, penicillinase-resistant penicillins are the β-lactams of choice for the initial management of patients with suspected staphylococcal disease. However, alternative effective antimicrobials should be substituted or added if endemic rates of antibiotic resistance exceed 10 to 15 percent or if the severity of the disease warrants empirical treatment of methicillin-resistant *S. aureus* (MRSA).[123] With the exception of methicillin, these agents are active against streptococci and can be used for empirical treatment of infections commonly caused by both staphylococci and streptococci. Because these agents are less active than penicillin G against streptococci, penicillin G should be used instead of these agents if streptococci alone are isolated from culture. Penicillinase-resistant penicillins have no activity against gram-negative bacteria or enterococci.[203]

Methicillin was the first penicillinase-resistant penicillin to be introduced and was available in parenteral form only. Oxacillin and nafcillin are available in both parenteral and oral preparations. Cloxacillin and dicloxacillin are available in oral forms only and are absorbed more efficiently from the gastrointestinal tract than the other oral drugs. Differences among these five penicillins include routes of elimination, degree of binding to proteins and degradation by β-lactamases, and in vitro susceptibility.[229] However, all are effective in the treatment of susceptible strains of staphylococcal isolates, and clinical studies have shown them to be equivalent when used at appropriate dosage schedules. Suspension formulations are poorly tolerated because of their unpleasant taste. Disease caused by methicillin-resistant staphylococci was reported shortly after introduction of the drug in the 1960s. Resistance is caused by alterations in PBPs, rather than production of β-lactamase. The *mecA* gene encodes a new PBP2a that has low affinity for β-lactams, thereby resulting in resistance to most β-lactam antibiotics currently available, including penicillinase-resistant penicillins, cephalosporins (first to fourth generation), and carbapenems. The *mecA* gene is carried on one of four mobile genetic elements known as the staphylococcal chromosomal cassette (SCC)mec I to IV that differ in size and the presence of additional resistance genes. The mechanisms of resistance to other antibiotics are unrelated to PBPs and may be plasmid mediated or chromosomal.[200] SSCmec IV is traditionally found in community-acquired strains of MRSA (CAMRSA) that are not associated with multidrug resistance, whereas SSCmec I to III are found typically in health care–associated strains that are resistant to multiple antibiotics, including aminoglycosides, clindamycin, fluoroquinolones, fusidic acid, macrolides, rifampin, sulfonamides (TMP-SMX), and tetracyclines.[259] However, these distinguishing characteristics are tending to merge as *S. aureus* becomes increasingly resistant. Coagulase-negative staphylococci, including *S. epidermidis*, are residents of the normal microbial flora of the skin and are occasional contaminants of body fluid cultures. These organisms can be pathogens in certain settings, such as in neonates, or in infections of prosthetic devices, such as heart valves or cerebrospinal fluid (CSF) shunts. Most strains of coagulase-negative staphylococci produce a penicillinase that inactivates penicillin G, penicillin V, and ampicillin, and many strains have *mecA*-mediated altered PBP2a, leading to methicillin resistance. In addition, these methicillin-resistant, coagulase-negative staphylococci frequently are resistant to cephalosporins, erythromycin, and clindamycin. Vancomycin is the drug of choice for pathogenic coagulase-negative staphylococci and severe disease known or suspected to be caused by MRSA. Some experts advocate the addition of nafcillin or oxacillin for empirical therapy for suspected MRSA in patients who are severely ill, although toxicity may be increased with combination therapy.[109,123]

Aminopenicillins

The aminopenicillins are semisynthetic β-lactam antibiotics formed by the addition of an amino group to benzylpenicillin. Amoxicillin differs from ampicillin by the presence of a hydroxyl group on the phenyl side chain. The aminopenicillins were the first penicillins that had activity against some gram-negative organisms, including *H. influenzae*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella* spp., and *Shigella* spp., while retaining activity against penicillin-susceptible, gram-positive bacteria.[228]

In contrast to penicillin G, aminopenicillins are significantly more active against *Listeria monocytogenes*, slightly more active against enterococci, equally active against *Actinomyces*, *N. meningitidis*, and clostridial and corynebacteria species, and slightly less active against group A streptococci, group B streptococci, and pneumococci. Aminopenicillins are the drugs of choice for the treatment of infections caused by *L. monocytogenes* and enterococci. Other organisms susceptible in vitro to ampicillin and amoxicillin include non–penicillinase-producing strains of *H. influenzae*, *Moraxella catarrhalis*, *N. gonorrhoeae*, *S. aureus*, *E. coli*, *Salmonella*, and *Shigella*. A survey of health care–associated and community-acquired *H. influenzae* and *M. catarrhalis* strains isolated from the blood and respiratory tracts of children in North America documented ampicillin resistance rates of 34 percent and 99 percent, respectively.[160] The in vitro spectrum of activity for amoxicillin and ampicillin is identical, except amoxicillin is two times less active against *Shigella* and two to four times more active against enterococci and *Salmonella* than ampicillin.[228]

As seen with penicillin G, the primary means of acquired resistance to aminopenicillins is the production of β-lactamase. However, organisms that are resistant to penicillin G or to methicillin because of altered PBPs—*S. pneumoniae* and *S. aureus*, respectively—also are resistant to aminopenicillins. Lactamase-negative, ampicillin-resistant strains of *H. influenzae* have been identified in the United States much less frequently than in Japan (0.2% vs. 2.6%, respectively).[150]

The broad-spectrum bactericidal activity of ampicillin and amoxicillin provides the basis for the use of these drugs as empirical therapy for lower respiratory tract infections and acute otitis media, for which amoxicillin remains the drug of choice.[10] Ampicillin or penicillin G is the recommended initial empirical therapy in community-acquired pneumonia in a fully immunized infant or school-aged child admitted to a hospital ward if the local epidemiology reveals no substantial high-level resistance for invasive *S. pneumoniae*.[49] Aminopenicillins are indicated for treatment of susceptible strains of *Shigella*-associated or *Salmonella*-associated enteric infections as well as susceptible acute and chronic infections of the urinary tract. By bacteriologic and clinical measures, amoxicillin is significantly less effective than ampicillin for the treatment of shigellosis.[226]

Both drugs are available for oral administration; ampicillin alone is available in a parenteral form. Amoxicillin provides higher and more prolonged serum concentrations than those achieved with equivalent dosages of ampicillin; thus, amoxicillin can be given in lower dosage, two or three times per day, rather than four times, as required for ampicillin. An additional advantage of amoxicillin is that absorption is not altered when the antibiotic is administered with food, whereas absorption

of ampicillin is decreased significantly when it is given with food. Ampicillin is associated more frequently with diarrhea than is amoxicillin.

Extended-Spectrum Penicillins

Extended-spectrum penicillins are semisynthetic derivatives of ampicillin that have better activity against gram-negative organisms because of a higher affinity for PBPs and greater penetration through the gram-negative outer membrane. Carbenicillin and ticarcillin, the carboxypenicillins, have a carboxyl group replacing the amino group side chain of ampicillin, whereas the acylureidopenicillins have a ureido (urea) side chain (mezlocillin) or ureido and piperazine side chains (piperacillin). Their pharmacologic properties are similar and include susceptibility to hydrolysis by staphylococcal penicillinases and, to a lesser extent, the β-lactamases of gram-negative bacteria. They differ somewhat in their toxicities and spectrum of activity. An oral preparation, carbenicillin indanyl (Geocillin) is active against susceptible strains of *Pseudomonas aeruginosa*, but its production was discontinued in 2008.

The activity of carbenicillin is equivalent to or slightly less than that of ampicillin against non–β-lactamase-producing *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, *E. coli*, *P. mirabilis*, *Salmonella* spp., and *Shigella* spp. It is less active than ampicillin against group A streptococci, pneumococci, and enterococci. Its activity is variable against many *Bacteroides fragilis*, *Enterobacter*, and *Serratia* organisms, and it is not active against *Klebsiella*.

The activity of ticarcillin is similar to that of carbenicillin, but it is two to four times more active against some strains of *P. aeruginosa* and less active against gram-positive cocci. In addition, ticarcillin can be used for infections caused by susceptible strains of *Acinetobacter*. Because of its increased activity, ticarcillin may be used in smaller doses than needed with carbenicillin for the treatment of disease caused by gram-negative organisms.

Piperacillin and mezlocillin are parenteral penicillins with a spectrum of activity similar to that of ticarcillin but greater activity in vitro against some gram-negative bacilli and anaerobic bacteria. In vitro studies against *P. aeruginosa* demonstrated that piperacillin has activity that is four times that of mezlocillin and ticarcillin and about eight times that of carbenicillin, the clinical significance of which is unknown. Piperacillin and mezlocillin are more active in vitro than carbenicillin or ticarcillin against susceptible strains of *E. coli*, *Klebsiella*, *Enterobacter*, *Serratia*, and *B. fragilis*. Furthermore, piperacillin, mezlocillin, and ticarcillin are effective in the treatment of infections caused by susceptible strains of *Citrobacter*, indole-positive *Proteus*, and *Providencia*. Carbenicillin and ticarcillin are disodium salts and contain 4.7 mEq (108 mg) and 5.2 mEq (120 mg) of sodium per gram, respectively. The acylureidopenicillins are monosodium salts that have a lower sodium content (1.9 to 2.2 mEq) than the carboxypenicillins. The amount of sodium administered may be of concern when treating certain patients with renal or cardiac disease. Hypokalemia with metabolic alkalosis occasionally occurs with the administration of extended-spectrum penicillins, especially the carboxypenicillins. The penicillin acts as a nonreabsorbable anion in the distal renal tubules, where it affects normal hydrogen exchange and secondarily results in loss of potassium. These extended-spectrum penicillins can bind to platelet adenosine diphosphate receptors and thereby result in abnormal platelet aggregation and prolonged bleeding times. This dose-related phenomenon occurs more frequently with the administration of ticarcillin than with the acylureidopenicillins. The effects on platelet function may be a consideration when choosing empirical therapy for a thrombocytopenic patient with suspected gram-negative infection.[318]

When carboxypenicillins or ureidopenicillins have been used as single agents for empirical treatment of patients with severe gram-negative infections, fever and neutropenia, or polymicrobial intra-abdominal infections, bacterial resistance has developed in 10 to 15 percent of cases during treatment. Combinations of extended-spectrum penicillins with an aminoglycoside demonstrate synergistic in vitro inhibition of certain gram-negative bacteria, including *P. aeruginosa*. Based on in vitro data and clinical experience, extended-spectrum penicillins should be combined with an aminoglycoside in some patients (particularly if immunocompromised) with severe infections, in case bacterial resistance develops.[318] All of the extended-spectrum penicillins are available in parenteral formulations only.

β-Lactam-β-Lactamase Inhibitor Combinations

β-Lactamase inhibitors are compounds that have weak antibacterial activity but can bind irreversibly to the catalytic site of many β-lactamases and render them inactive. The inhibitors currently in use are clavulanic acid, sulbactam, and tazobactam, the latter of which is halogenated penicillanic acid derivative. All three inhibitors are identical in their mode of activity but differ to some degree in their potency and spectrum of enzyme inhibition. These agents primarily inhibit the plasmid-encoded β-lactamases and less frequently the chromosomally encoded β-lactamases. β-Lactamase inhibitors have been formulated in a fixed ratio with a β-lactam antibiotic. The spectrum of activity of each combination is determined primarily by the spectrum of activity of the β-lactam. However, many determinants of the inhibitor influence its activity, including its affinity for the β-lactamase and its ability to traverse the gram-negative cell wall to bind to periplasmic β-lactamases.[192] From a clinical perspective, all three β-lactamase inhibitors are considered therapeutically equivalent. The β-lactamase inhibitors are most effective against *S. aureus*, *H. influenzae*, *M. catarrhalis*, *Bacteroides* spp., *E. coli*, and other Enterobacteriaceae. The main indication for the use of these combination antimicrobial agents is for the treatment of health care–associated infections or infections caused by susceptible β-lactamase–producing pathogens.

Amoxicillin–Clavulanic Acid. Amoxicillin combined with potassium clavulanate was introduced in 1984 for oral administration. The pharmacokinetic properties of

the two drugs are similar; both are absorbed rapidly and are not affected when taken with meals. Gastrointestinal side effects, including nausea, vomiting, and diarrhea, occur more commonly with amoxicillin-clavulanate than with amoxicillin alone.[172]

The combination drug is equivalent to amoxicillin alone in activity against amoxicillin-susceptible organisms. The addition of clavulanic acid extends the activity of amoxicillin to include β-lactamase–producing strains of *S. aureus* (but not methicillin-resistant strains), *H. influenzae*, *M. catarrhalis*, *N. gonorrhoeae*, *E. coli*, *Proteus*, *Klebsiella*, *Providencia*, *Haemophilus ducreyi*, and some anaerobic bacteria, including *B. fragilis*. Some β-lactamase–producing, gram-negative bacilli are resistant to amoxicillin–clavulanic acid because of either hyperproduction of β-lactamase or production of a β-lactamase that is not susceptible to clavulanate. Amoxicillin-clavulanate is not active against penicillin-resistant *S. pneumoniae* or vancomycin-resistant enterococci.

Amoxicillin is considered the preferred therapy for low-risk children with uncomplicated, mild to moderately severe disease of the respiratory tract, including initial empirical therapy for otitis media. In children who are at risk for penicillin-nonsusceptible pneumococcal acute otitis media, a 90-mg/kg daily regimen in two divided doses is recommended. Alternative therapy, including amoxicillin-clavulanic acid, should be considered if a β-lactamase–producing organism is known or suspected to be the cause of the disease based on risk factors, treatment experience, or microbiologic results. In 2001, high-dose amoxicillin–clavulanic acid suspension (Augmentin ES600; 600 mg of amoxicillin/5 mL and 42.9 mg of clavulanic acid/5 mL) was approved for use in children in the United States. The recommended dosage of this formulation is 90 mg/kg/day divided every 12 hours for the treatment of high-risk patients with acute otitis media.[10] The high ratio (14:1) of amoxicillin to clavulanate optimizes the amoxicillin dose without intensifying the risks for undesirable adverse effects associated with the β-lactamase inhibitor. The combination drug is useful in areas where the proportion of β-lactamase–producing strains of *H. influenzae* is large (30%) and *M. catarrhalis* organisms (most of which are β-lactamase producers) are identified more frequently as pathogens in otitis media, sinusitis, and other respiratory tract infections. Amoxicillin-clavulanate also has been used successfully for the oral treatment of urinary tract, skin, and soft tissue infections, as well as for the treatment of human and animal bite wounds.[58]

Ampicillin-Sulbactam. Ampicillin combined with sulbactam as a parenteral β-lactam and β-lactamase inhibitor combination was approved in 1987. The spectrum of activity of ampicillin-sulbactam is similar to that of amoxicillin-clavulanate. It is most useful as monotherapy for potential polymicrobial intra-abdominal or gynecologic infections and is effective for treatment of soft tissue, urinary tract, and respiratory tract infections. Ampicillin-sulbactam has been shown to be safe and efficacious for the treatment of skin and skin structure infections caused by susceptible organisms in children aged 3 months to 12 years.[19] The combination of ampicillin-sulbactam and an aminoglycoside has efficacy equivalent to that of a combination of ampicillin, clindamycin, and an aminoglycoside for empirical treatment of intra-abdominal infections in children.[74] Ampicillin-sulbactam is potentially useful for multidrug-resistant health care–associated infections caused by *Acinetobacter baumannii-calcoaceticus* complex.[221] Carbapenems and third-generation cephalosporins are comparatively more active against members of the Enterobacteriaceae. Because ampicillin-sulbactam has no activity against certain strains of the Enterobacteriaceae that produce Bush group 1 β-lactamases and *P. aeruginosa*, it should not be used as empirical single-agent therapy for febrile neutropenic patients or severely ill patients with probable bacteremia.

Ticarcillin–Potassium Clavulanate. Ticarcillin combined with potassium clavulanate was approved for use in 1985. It extends the spectrum of activity of ticarcillin to include β-lactamase–producing strains of staphylococci (but not methicillin-resistant strains), *H. influenzae*, *M. catarrhalis*, *E. coli*, *Klebsiella*, *Proteus*, *Providencia*, *N. gonorrhoeae*, and *B. fragilis*.[71] Ticarcillin-clavulanate also often is active against multidrug-resistant *Stenotrophomonas maltophilia*. Enterococci are moderately resistant to this agent. Because clavulanic acid does not inhibit Bush group 1–inducible chromosomal β-lactamases, *Citrobacter*, *Enterobacter*, and *Serratia* spp., as well as derepressed mutant strains of *P. aeruginosa* that are resistant to ticarcillin because of inducible cephalosporinases, also are resistant to ticarcillin–potassium clavulanate. Ticarcillin–potassium clavulanate is approved for use in children older than 3 months of age for lower respiratory tract, skin and skin structure, urinary tract, bone and joint, and intra-abdominal infections caused by susceptible pathogens.[38] Treatment of febrile neutropenic adult patients with ticarcillin-clavulanate combined with an aminoglycoside has been shown to be effective.

Piperacillin-Tazobactam. Piperacillin combined with tazobactam was approved for use in adults in 1993 and was approved for pediatric use (2 months to 17 years of age) in the United States in 2007.[306] It extends the spectrum of activity of piperacillin to include β-lactamase–producing strains of oxacillin-susceptible *S. aureus*, many members of the Enterobacteriaceae, and virtually all gram-positive and gram-negative anaerobes; its spectrum of activity is superior to that of ceftazidime and other β-lactam and β-lactamase inhibitor combinations. It has greater activity than ticarcillin-clavulanate against gram-positive and gram-negative bacteria and equivalent activity against gram-positive bacteria in contrast to ampicillin-sulbactam. Tazobactam, however, does not increase the activity of piperacillin against bacteria with resistance that is mediated by altered cell wall permeability to piperacillin, including certain strains of *P. aeruginosa*. Penicillin-resistant pneumococci, methicillin-resistant staphylococci, *Corynebacterium jeikeium*, most *Enterococcus faecium* strains, and most gram-negative bacteria that produce Bush group 1 β-lactamases (*Citrobacter*, *Enterobacter*, *Morganella*, certain strains of *Pseudomonas*, and *Serratia* spp.) are resistant to piperacillin-tazobactam.[58]

Although studies have shown piperacillin-tazobactam to be safe and effective for the treatment of lower respiratory tract, intra-abdominal, pelvic, skin and skin structure, bone, and diabetes-related foot infections in adults, as well as for empirical treatment of febrile episodes in neutropenic adults,[45] the experience in children is limited. It is a potentially useful agent that has been administered for the treatment of serious or complicated polymicrobial infections in children, especially in febrile neutropenic children, as initial empirical treatment. This antimicrobial agent should be used in combination with an aminoglycoside for serious *P. aeruginosa* infections to prevent the development of resistance. Piperacillin-tazobactam (or ticarcillin-clavulanate) has been proposed as an alternative treatment to carbapenems for single-agent therapy in community-acquired intra-abdominal infections.[286] Clinicians also should be aware that administration of piperacillin-tazobactam can cause false-positive *Aspergillus galactomannan* test results for up to 5 days after cessation of treatment.[16] Monte Carlo simulations have predicted achieving more time above the MIC_{50} with prolonged infusions of piperacillin-tazobactam (i.e., 4 hours) in contrast to conventional dosing, for MIC range MIC 4-16 mg/L. Similar results have been observed with other β-lactams, including meropenem, doripenem, cefepime, and ceftazidime. Clinical studies have not been conclusive.[201]

Adverse Effects and Sensitization

The penicillins are unique among antimicrobial agents in having little dose-related toxicity (Table 235-4). Seizures may occur under circumstances that result in high concentrations of penicillin in nervous tissue: rapid intravenous infusion of single large doses, substantial dosages for prolonged periods in patients with impaired renal function, or high concentrations given by the intrathecal route. Confusion, dizziness, seizures, and psychosis caused by toxic concentrations of procaine have been associated with the administration of procaine penicillin G.[284] Nephritis has been associated with the administration of some penicillins, most frequently after the use of methicillin. Bleeding because of drug-induced platelet aggregation has been noted after the administration of carbenicillin and penicillin G.

Although toxicity may not be a significant concern with the penicillins, sensitization is an important factor.[191] Penicillins are haptens, that is, they are low-molecular-weight compounds too small to elicit an immune response alone, but when bound to a carrier molecule (e.g., host tissues or proteins), they are highly immunogenic in humans. The native penicillin molecule can bind to a protein, as can its major (benzylpenicilloyl) and minor (benzylpenicillin, benzylpenicilloate, benzylpenilloate) determinants. Four types of immune-mediated reactions can occur after the administration of a penicillin (or any drug or antigen): immediate hypersensitivity (immunoglobulin E [IgE]-mediated) reactions, cytotoxic antibody reactions, immune complex reactions (Arthus reaction), and delayed (cell-mediated) hypersensitivity. Allergic reactions also can be classified as (1) immediate (onset ≤1 hour), accelerated (1 to 72 hours), or delayed (>72 hours).

TABLE 235-4 **Adverse Reactions to Penicillins**

Type of Reaction	Frequency (%)	Most Frequent*
Electrolyte Disturbance		
Sodium overload	Variable	Ticar
Hypokalemia	Variable	Ticar
Hyperkalemia, acute	Rare	PCN G
Gastrointestinal		
Diarrhea	2-5	Amp
Enterocolitis	<1	Amp
Hematologic		
Hemolytic anemia	Rare	PCN G
Neutropenia	1-4	PCN G, Naf, Ox, Pip
Platelet dysfunction	3	Ticar
Hepatic		
Elevated AST	1-4	Ox, Naf
Neurologic		
Seizures	Rare	PCN G
Bizarre sensations	Rare	Procaine PCN
Renal		
Interstitial nephritis	1-2	Meth
Hemorrhagic cystitis	Rare	Meth
Allergic		
IgE-mediated	0.004-0.4	PCN G
Cytotoxic antibody	Rare	PCN G
Immune complexes	Rare	PCN G
Delayed hypersensitivity	4-8	Amp

Modified from Chambers HF, Neu HC. Penicillins. In: Mandell GL, Bennett JE, Dollin R, editors. *Mandell, Douglas and Bennett's principles and practice of infectious diseases.* 4th ed. New York, Churchill Livingstone, 1995.
*All reactions can occur with any penicillin.
Amp, Ampicillin; *AST,* aspartate transaminase; *Meth,* methicillin; *Naf,* nafcillin; *Ox,* oxacillin; *PCN G,* penicillin G; *Pip,* piperacillin; *Ticar,* ticarcillin.

Researchers have estimated that an immediate serious reaction occurs in 2/10,000 courses, and fatal reactions occur in 1/100,000 treatment courses.[27,301]

1. Type 1 immediate hypersensitivity reactions usually occur within 30 minutes after administration and are life-threatening events. The interaction of preformed mast cell–bound IgE antibody to the antigenic determinants of penicillin results in the release of mast cell mediators.[41] Clinical signs include hypotension or shock, urticaria, laryngeal edema, and bronchospasm. Acute anaphylaxis is a rare event after the administration of penicillin, but significant numbers of fatalities occur each year because of the extensive use of these drugs. Children are thought to have fewer systemic reactions than do adults, presumably because of fewer previous exposures to penicillin antigens. Oral preparations are less likely to result in an immediate reaction than are parenteral forms, perhaps because

antigens are altered in the gastrointestinal tract, absorption is slower, dosages are smaller, or a combination of these factors.

2. Type 2 cytotoxic antibody reactions can occur after passive absorption of the benzylpenicilloyl hapten by the membrane of circulating blood cells or by renal interstitial cells, especially when high dosages of penicillins are used for prolonged periods. IgM antibody, IgG antibody, or both antibodies to the benzylpenicilloyl antigen bind to the altered cell surface; complement can be activated, and damage to the cell ensues. Cytotoxic antibody reactions usually are manifested more than 72 hours after initiation of antibiotic therapy and include hemolytic anemia, leukopenia, thrombocytopenia, and drug-induced interstitial nephritis.
3. Type 3 immune complex (Arthus) reactions occur after the formation of immune complexes between soluble penicillin antigens and IgG and IgM antibodies. The complexes lodge in the skin, joints, kidneys, or other tissue sites. Complement activation occurs, and the clinical manifestations of serum sickness ensue—cutaneous symptoms (i.e., urticaria, maculopapular rash, and erythema multiforme), polyarthralgia, and fever. The onset typically is 7 to 21 days after initiation of penicillin therapy but can occur after use of the antibiotic has been discontinued. Although serum sickness has been associated with the administration of penicillins, it occurs more frequently after administration of cefaclor.
4. Type 4 delayed hypersensitivity reactions involve cellular rather than humoral immunity. On exposure to certain antibiotics, T lymphocytes become sensitized in a major histocompatibility restricted fashion and they mediate mild-to-severe reactions that include contact dermatitis, some maculopapular rashes, fixed drug eruption at sites of previous tinea infections, and toxic epidermal necrolysis. Diagnostic tools to detect an antibiotic that induces a type 4 reaction include the delayed reading (>6 hours) intradermal skin test, patch test, and in vitro lymphocyte transformation test. However, these tests have not been adequately standardized for routine use.[301]

Idiopathic reactions are those for which an immune-mediated mechanism has not been proved, but more recent data suggest that these reactions may represent a type 4 hypersensitivity reaction.[301] Included in this category are the morbilliform exanthems, erythema multiforme, photosensitivity reactions, exfoliative dermatitis, and pruritus. Approximately 4 percent of courses of penicillin and up to 9 percent of courses of ampicillin or amoxicillin therapy are associated with a maculopapular rash. In some of these patients, the rash is a manifestation of a primary viral infection such as infectious mononucleosis, for which the penicillin was prescribed inappropriately.

Identifying patients who will have a significant reaction if penicillin is administered remains difficult. Serologic assays (radioallergosorbent tests) for the detection of IgE antibodies to major and minor penicillin determinants are available but are time-consuming, expensive, and less sensitive than skin testing. Because the immediate reaction is mediated largely by IgE reagin or skin-sensitizing antibody, patients who are likely to respond subsequently with a life-threatening reaction can be identified by the use of intradermal tests with appropriate antigens. However, selecting the most appropriate antigens to be used for skin testing has been problematic because many different antigens may play roles in the allergic reaction. Penicillin is degraded to various clinical products. At least 10 metabolic breakdown products of the penicillin nucleus have been identified. Other potential antigens include macromolecular impurities present in solutions of the drug, high-molecular-weight penicillin polymers found in poorly buffered penicillin solutions standing for prolonged periods, side chains of the various penicillins, and the bacterial enzymes (amidases) used to prepare semisynthetic penicillins. Thus, investigators have had difficulty choosing sensitive and specific antigens to use for skin testing. When these products are used in penicillin skin testing, the predictive value of testing for drug-specific IgE antibodies is high. However, skin testing evaluates IgE-mediated allergy only. For antibiotics other than penicillin it is not clear if the appropriate antigenic determinants are being used, for which a negative test should be interpreted with caution.[128]

The most informative studies of skin test antigens have been those of Levine,[186] who identified two antigens, benzylpenicilloyl poly-L-lysine (PrePen) and a "minor determinant mixture," a preparation of a dilute solution of aqueous crystalline penicillin G that includes metabolic breakdown products. Only the PrePen reagent is available commercially in the United States. The CDC currently recommends the use of benzylpenicilloyl poly-L-lysine and, if available, a freshly prepared mixture of dilute minor determinant precursors, including benzylpenicillin G, benzylpenicilloate, and penicilloyl propylamine, as well as a positive control (histamine) and a negative control (diluent, phenol saline) after a protocol prepared by Beall.[27,316] If the full battery of minor antigens is not available, penicillin G should be used. An epicutaneous (prick) test is performed first. If the patient's history of penicillin allergy is that of a mild reaction, the skin test reagent can be used at full strength; however, if the previous history is suggestive of anaphylaxis, a 1:100 dilution of the reagent should be used for the first prick test, followed by a full-strength test if no reaction with the diluted reagent occurs. If the prick test result is negative, an intradermal test is performed using duplicate 0.02-mL volumes of antigen solutions, and the skin is observed for 20 minutes. A positive result is indicated by a wheal-and-flare reaction in 15 minutes that is more than 2 mm larger than the initial wheal; this result suggests a significant chance that a reaction will occur on subsequent administration of a penicillin. A negative result suggests that a significant allergic reaction will not take place. Although much effort has gone into clinical tests of these antigens, the predictive value of positive and negative results in children remains uncertain. Because of the risk for severe life-threatening reactions when a skin test is performed, having a physician present and resuscitation equipment and medications readily available is prudent.

At present, the physician must rely on the patient's history of an adverse reaction after administration of a penicillin to identify who is likely to be allergic. If the reaction appears to be related to the administration of a penicillin, the drug should be avoided for minor infections. More recent experience suggests that fewer than 10 percent of adults with a history of penicillin allergy have positive skin tests. Of those individuals with negative skin tests, IgE-mediated reactions occur in 4 percent or fewer if these patients receive penicillin, a rate that is similar to that in individuals with no history of penicillin allergy.[301] Because no proved alternative therapies to penicillin are available for treating patients with neurosyphilis, congenital syphilis, or syphilis in pregnancy, the CDC recommends skin testing and desensitization of patients considered to have reacted positively to the skin test antigens.[316] If a life-threatening infection should occur and penicillin is clearly the drug of choice, the physician may choose to administer the drug under carefully controlled conditions after desensitization. All penicillins are cross reactive with regard to sensitization; allergy to any one implies sensitization to all, although cross sensitivity is considerably less than 100 percent.

Cephalosporins

The cephalosporins have a broad range of activity that includes gram-positive cocci, gram-negative enteric bacilli, and anaerobic bacteria. Most cephalosporins are relatively resistant to hydrolysis by β-lactamases produced by *S. aureus*, but many are susceptible to gram-negative β-lactamases. Unlike other antimicrobial agents, this group has a high therapeutic-toxic index. For simplicity, the cephalosporins have been categorized as first-, second-, third-, and fourth-generation agents (Table 235-5), based on the pattern of in vitro activity. Newer generation cephalosporins have been evaluated recently, and in 2010 ceftaroline was approved by the FDA for use in adults with acute skin and soft tissue infections and community-acquired bacterial pneumonia. Ceftobiprole has not been approved for use in the United States, but is available in Canada.

Pharmacokinetics

The cephalosporins are available as parenteral and oral products (Table 235-6). Most of the parenteral drugs can be administered by the intravenous or intramuscular routes. Most of the oral products are absorbed well from the gastrointestinal tract. Esterification of the base compounds of cefuroxime and cefpodoxime is required to enhance gastrointestinal absorption. The presence of food does not alter absorption and, for some antibiotics, even can enhance absorption. Cephalosporins penetrate most tissues and body fluids well, except CSF. The first- and second-generation cephalosporins do not achieve therapeutic CSF concentrations. CSF penetration of third-generation drugs varies but is generally adequate for most major meningeal pathogens, except penicillin-nonsusceptible pneumococci, for which vancomycin is added in the initial empirical regimen.[177] Cefepime, a fourth-generation cephalosporin, reaches adequate CSF concentrations for the treatment of most common invasive pathogens. Because glomerular filtration and tubular secretion are the major modes of excretion, the urinary concentrations achieved are sufficient for the treatment of urinary tract infections. Ceftriaxone has dual excretion by the kidneys and the biliary tract and

TABLE 235-5 **Classification Scheme for Cephalosporins**

Generic Name	Trade Name	Route
First Generation		
Cephalexin[a]	Keflex, Keftab	PO
Cefadroxil	Duricef	PO
Cephradine	Velosef	PO
Cephalothin[b]		IM, IV
Cefazolin[a]	Ancef, Kefzol	IM, IV
Cephapirin[c]	Cefadyl	IM, IV
Second Generation		
Cefaclor[a]	Ceclor	PO
Cefuroxime axetil[c]	Ceftin	PO
Cefprozil[d]	Cefzil	PO
Ceftibuten[d]	Cedax	PO
Cefamandole[b]	Mandol	IM, IV
Cefonicid[e]	Monocid	IM, IV
Cefuroxime[c]	Zinacef, Kefurox	IM, IV
Cephamycins		
Cefoxitin	Mefoxin	IM, IV
Cefotetan[e]	Cefotan	IM, IV
Third Generation		
Cefixime[a]	Suprax	PO
Cefpodoxime proxetil[f]	Vantin	PO
Cefdinir[d]	Omnicef	PO
Cefditoren pivoxil[g]	Spectracef	PO
Ceftizoxime[d]	Cefizox	IM, IV
Cefotaxime	Claforan	IM, IV
Ceftriaxone	Rocephin	IM, IV
Ceftazidime	Fortax, Tazicef, Tazidime, Ceptaz	IM, IV
Cefoperazone[e]	Cefobid	IM, IV
Fourth Generation		
Cefepime[h]	Maxipime	IM, IV
Fifth Generation		
Ceftaroline[i]	Teflaro	IV

Modified from U.S. Dispensing Information. *Information for the health care professional.* Vol. 1, Thomson Micromedex, 2006.
[a]Safety and efficacy have not been determined for infants younger than 1 month.
[b]No longer available in the United States.
[c]Safety and efficacy have not been established for infants younger than 3 months.
[d]Safety and efficacy have not been determined for infants younger than 6 months.
[e]Not approved for use in children.
[f]Safety and efficacy have not been established for infants younger than 5 months.
[g]Safety and efficacy have not been established for children younger than 12 years.
[h]Safety and efficacy have not been established for infants younger than 2 months.
[i]Not FDA approved for younger than 18 years
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

TABLE 235-6 **Pharmacokinetics of Cephalosporins**

Antibiotic	Bioavailability[a] (%)	Protein Binding (%)	Metabolized (%)	Urinary Recovery (%/hr)	Approximate Half-Life[b] (hr)
First Generation					
Cephalexin	95	10-15	0	90/8	0.9-1.5
Cefadroxil	95	15-20	0	93/24	1.2-1.5
Cephradine	95	8-17	0	60-90/6	0.8-1.3
Cephalothin	—	70	20-30	60-70/6	0.5-1.0[c]
Cefazolin	—	85	0	70-86/24	1.4-2.0[c]
Cephapirin	—	44-50	40	70/6	0.5-0.8
Second Generation					
Cefaclor	95	25	0	60-85/8	0.6-0.9
Cefuroxime axetil	37/52	33-50	0[d]	50/12	1.2-1.9[c]
Cefprozil	95	36-45	0	60-70/8	1.8-2.1
Ceftibuten	75-90/<75[e]	65-77	10[f]	95/24	1.4-2.6
Cefonicid	—	>90	0	99/24	3.5-4.5
Cefuroxime	—	33-50	0	96/24	1.2-1.9[c]
Cefoxitin	—	70-80	<5	85/6	0.7-1.1[c]
Third Generation					
Cefixime	40-50	65	0	16/24	3-4
Cefpodoxime proxetil	50/>50	21-40	0[d]	29-33/12	2.1-2.8
Cefdinir	16-25[g]	60-70	0	12-18/1.7	1.5-1.7
Cefditoren pivoxil	13-19	88	0[d]	—	1.6
Ceftizoxime	—	30	0	70-100/24	1.4-1.7
Cefotaxime	—	30-50	30-50	15-25/6	1.0
Ceftriaxone	—	83-96	—	33-67[h]/24	4.3-8.7[i]
Ceftazidime	—	<10	0	80-90/24	1.4-2.0
Fourth Generation					
Cefepime	100	20	15	80-85/12	2.0

Modified from U.S. Dispensing Information. *Information for the health care professional.* Vol. 1, Thomson Micromedex, 2006.
[a]Fasting or nonfasting.
[b]Normal renal function.
[c]Elimination half-life prolonged in neonates.
[d]Prodrug rapidly metabolized to active drug; otherwise, no significant metabolism.
[e]Because the bioavailability of suspensions is decreased by food, ceftibuten should be administered at least 2 hours before or 1 hour after a meal.
[f]*Cis*-isomer converted to *trans*-isomer.
[g]Suspension formulation has greater bioavailability than the capsule formulation.
[h]Forty to 75 percent eliminated unchanged in bile.
[i]The half-life is prolonged after intramuscular administration.

achieves high concentrations in urine and bile. The presence of moderate-to-severe renal insufficiency may require adjustment of the dosage or dosing interval for all cephalosporins, except those agents with biliary excretion. Hepatic insufficiency can affect the metabolism of cephalosporins that undergo biliary excretion; however, adjustments in ceftriaxone dosage or dosing interval are required only in the presence of both hepatic and renal insufficiency.

Therapeutic advantages of cephalosporins include concentration-independent bactericidal activity, broad-spectrum antibacterial activity, lack of significant dose-related toxicity, and relative stability against staphylococcal β-lactamases. Certain cautions must be kept in mind when prescribing cephalosporins: none is effective against enterococci, methicillin-resistant staphylococci, *L. monocytogenes*, chlamydial species, and *C. difficile*, and resistance may develop rapidly in closed communities, such as neonatal or pediatric intensive care units, because of inducible chromosomal β-lactamases produced by gram-negative bacteria. The new-generation cephalosporin, ceftaroline, has minimal activity against *Enterococcus faecalis* and no activity against *E. faecium*.[29,288]

First-Generation Cephalosporins

First-generation cephalosporins are effective against gram-positive cocci, including β-lactamase–producing *S. aureus*, and they have variable activity against gram-negative enteric bacilli. Five first-generation cephalosporins currently are available for infants and children: the parenteral drugs cephapirin and cefazolin, the oral products cephalexin and cefadroxil, and cephradine.

Cephalothin is not available in the United States. Because cephapirin is painful in intramuscular injections, the intravenous route is preferred. Cefazolin produces higher concentrations in blood than do the other parenteral first-generation drugs. Cefadroxil can be administered in twice-daily doses because of its longer serum half-life. None of these agents will attain appreciable concentrations in the central nervous system (CNS).

These drugs are of value as alternatives to penicillin for disease caused by *S. aureus*, *S. pyogenes*, and susceptible *S. pneumoniae*, and they are active against some strains of community-acquired gram-negative enteric bacilli such as *E. coli*, *P. mirabilis*, and *Klebsiella pneumoniae*. The three oral preparations have comparable activity in vitro and in vivo. First-generation cephalosporins are valuable in children who have a history of nonanaphylactic allergy to penicillin. These drugs have been used to treat staphylococcal and streptococcal skin and skin structure infections, bone and joint infections, pharyngitis, and uncomplicated community-acquired urinary tract infections caused by susceptible bacteria. A meta-analysis revealed that many first-generation and other-generation cephalosporins (i.e., cephalexin, cefadroxil, cefuroxime, cefprozil, cefdinir, cefixime, cefpodoxime, and ceftibuten) have equivalent or superior bacteriologic and clinical cure rates in contrast to penicillin for treatment of group A streptococcal pharyngotonsillitis.[62] Experts disagree on the significance of the results of this meta-analysis. Cefadroxil is effective for the treatment of streptococcal pharyngitis in a once-daily dosage schedule. Cephalexin has been used for sequential parenteral-oral treatment of staphylococcal osteomyelitis and arthritis occurring after surgical intervention and an initial period of parenteral antibiotic therapy. Cefazolin and cefuroxime are the antibiotics of choice for perioperative prophylaxis in selected surgical procedures.[50] Because first-generation cephalosporins have minimal, if any, activity against *H. influenzae* and *M. catarrhalis* and inadequate activity against penicillin-resistant *S. pneumoniae*, they should not be used for empirical treatment of respiratory tract infections. First-generation cephalosporins should not be used for empirical therapy of suspected severe gram-negative health care–associated infections; a third- or fourth-generation cephalosporin in combination with an aminoglycoside, or other classes of antibiotics, should be selected for this purpose. The safety and efficacy of cefaclor, cefazolin or cephalexin, and cephapirin have not been established for infants younger than 1 and 3 months of age, respectively.

Second-Generation Cephalosporins

The second-generation cephalosporins that are approved for use in children consist of two parenteral drugs (cefamandole and cefuroxime) and three oral preparations (cefaclor, cefuroxime axetil, and cefprozil). Also classified with the second-generation cephalosporins are the cephamycins, of which only one, cefoxitin, is approved for use in children. When compared with the first-generation cephalosporins, the second-generation agents have similar or somewhat less activity against gram-positive cocci but better activity against *H. influenzae*, *M. catarrhalis*, *N. meningitidis*, *N. gonorrhoeae*, and some members of the Enterobacteriaceae. The cephamycins are more active than first- or other second-generation cephalosporins against gram-negative enteric bacteria and *B. fragilis*, but they have poor activity against gram-positive cocci.

Cefaclor. Cefaclor is more active than cephalexin against *H. influenzae*, *M. catarrhalis*, *E. coli*, and *P. mirabilis* and has activity against staphylococci similar to that of cephalexin. Cefaclor is an unstable compound and is destroyed within 2 hours in human plasma. It is susceptible in vitro to hydrolysis by the β-lactamases produced by approximately 14 percent of *H. influenzae* and 90 percent of *M. catarrhalis* isolates; presumed rates of resistance of *H. influenzae* are considerably higher when pharmacokinetic and pharmacodynamic parameters are taken into account.[150] Although cefaclor is effective therapy for susceptible bacteria causing otitis media, sinusitis, and mild-to-moderate cases of pneumonia, it is not the preferred empirical agent for these indications because it is not effective against penicillin-nonsusceptible strains of *S. pneumoniae*. The rare but potential risk for serum sickness, as well as the poor stability of this drug to some β-lactamases, has limited its use. The safety and efficacy of cefaclor have not been established for infants younger than 1 month of age, although the drug has been used successfully to treat neonatal otitis media.

Cefamandole. Cefamandole is active against gram-positive cocci, including β-lactamase–producing *S. aureus*, and some gram-negative enteric bacteria, and it was the first cephalosporin to be effective for infections caused by *H. influenzae*, including β-lactamase–producing strains. However, this agent is no longer available in the United States. Before the development of cefuroxime, cefamandole was used for respiratory tract, skin, and soft tissue infections in infants and children.[18] Once cefuroxime was approved for use, cefamandole seldom was used because, although its spectrum of activity was comparable with that of cefuroxime, some of its pharmacologic features made it less favorable. Cefamandole does not penetrate the CSF well, and because it contains a methylthiotetrazole side chain, altered hemostasis may occur. Clinical and microbiologic failure in cases of meningitis caused by *H. influenzae* (despite in vitro susceptibility and evidence of CSF penetration) has limited the use of cefamandole to disease in which the development of sepsis is not a concern. The safety and efficacy of this agent have not been established for infants younger than 1 month of age.

Cefoxitin. Cefoxitin has excellent activity against anaerobic organisms and is the most active cephalosporin against *B. fragilis*.[31] It has selective activity against gram-negative enteric bacilli, but *Enterobacter* or *Pseudomonas* spp. are inherently resistant. Cefoxitin is resistant to hydrolysis by the β-lactamases produced by gram-positive bacteria and some of the gram-negative β-lactamases. Because it is a potent inducer of Bush group 1 chromosomal β-lactamases, its indiscriminate use should be avoided. Cefoxitin has been shown to be effective for

infections involving facultative gram-negative bacilli and anaerobes, such as intra-abdominal, pelvic, and gynecologic infections. When combined with doxycycline, cefoxitin is effective for the treatment of pelvic inflammatory disease.[316] The use of β-lactam and β-lactamase inhibitor combinations or metronidazole, rather than cefoxitin, should be considered for empirical treatment of patients with life-threatening anaerobic infections because as many as 25 percent of *B. fragilis* strains can be resistant to cefoxitin.[5] Higher doses of cefoxitin have been associated with an increased incidence of eosinophilia and elevated aspartate aminotransferase (AST) concentration. A diminished zone of inhibition in cefoxitin disk diffusion assays are useful in the clinical microbiology laboratory to detect inducible or constitutive (stably derepressed) AmpC Bush group 1 β-lactamase production among strains of *Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella*, *Morganella*, *Providencia*, *Pseudomonas*, and *Serratia*. Identification of this phenotype in vitro is important because these strains have high-level resistance to many classes of β-lactam antibiotics that may not be detected by standard susceptibility assays. Fourth-generation cephalosporins and carbapenems are potentially effective antimicrobial agents for these multidrug-resistant pathogens.[158]

Cefprozil. Cefprozil has a structure similar to that of the first-generation cephalosporin cefadroxil. It is more active than the oral first-generation agents against *S. pyogenes*, *S. pneumoniae*, *Neisseria* spp., *H. influenzae*, *M. catarrhalis*, *E. coli*, *P. mirabilis*, *Klebsiella*, and, to a lesser extent, staphylococci. This drug is hydrolyzed by the β-lactamases produced by approximately 11 percent of *H. influenzae* and 91 percent of *M. catarrhalis* isolates; rates of resistance of *H. influenzae* are likely considerably higher when pharmacokinetic and pharmacodynamic parameters are taken into account.[150] The rate of resistance among *S. pneumoniae* isolates in the United States is approximately 20 percent.[156] Because of its relatively long serum half-life, cefprozil can be administered twice daily. Cefprozil is comparable to penicillin, cefaclor, and erythromycin in the treatment of pharyngitis,[206] and it is equivalent or superior to cefaclor and erythromycin in the treatment of mild-to-moderate skin and skin structure infections.[233] Cefprozil is approved for the treatment of acute otitis media, mild lower respiratory tract infections, acute sinusitis, and skin and skin structure infections. However, it is not the preferred antibiotic for these indications because it is unlikely to be effective in children with penicillin-nonsusceptible *S. pneumoniae*, *H. influenzae*, or *M. catarrhalis*, on the basis of unfavorable pharmacodynamic parameters.[307] Cefprozil is safe and effective as part of a parenteral-oral antibiotic regimen for the treatment of suppurative skeletal infections in children.[308] The safety and efficacy of cefprozil have not been established for infants younger than 6 months of age.

Cefuroxime. In contrast to first-generation cephalosporins, this parenteral second-generation cephalosporin is slightly less active against staphylococci but is more active against group A streptococci and pneumococci. Cefuroxime has excellent activity against many members of the Enterobacteriaceae.[230] Its stability to β-lactamases is greater than that of first-generation agents, and it is the only first- or second-generation antibiotic that achieves substantial CSF concentrations. However, cefuroxime is not recommended for treatment of proved or suspected bacterial meningitis because of suboptimal CSF pharmacologic characteristics, particularly in the context of infections caused by *H. influenzae* or nonsusceptible *S. pneumoniae*, for which third-generation cephalosporins (cefotaxime and ceftriaxone) are indicated.[280] Cefuroxime has been approved for the treatment of skin and skin structure infections, lower respiratory tract infections, bone and joint infections, uncomplicated gonorrhea, and uncomplicated urinary tract infections caused by susceptible bacteria. Cefuroxime is most useful for the treatment of infections sufficiently severe to warrant parenteral therapy, in which susceptible *S. aureus*, *S. pneumoniae*, *S. pyogenes*, encapsulated or nonencapsulated *H. influenzae*, and *M. catarrhalis* are probable pathogens, such as suppurative arthritis, orbital cellulitis, or severe pneumonia. Cefuroxime offers the advantage of single-drug therapy for these diseases. The safety and efficacy of cefuroxime have not been established for infants younger than 3 months of age.

Cefuroxime Axetil. Cefuroxime axetil is an oral form of cefuroxime with a similar spectrum of activity. It is an ester prodrug of cefuroxime that is metabolized to the active drug by intestinal esterases. Oral absorption is increased by the presence of food. When crushed, the tablet has a bitter taste that renders it unpalatable. The suspension has an unpleasant flavor that makes it difficult for some children to tolerate and therefore may limit adherence. The drug may be considered a suitable alternative to amoxicillin for the treatment of otitis media[10] and sinusitis when coverage must include β-lactamase–producing bacteria. Cefuroxime axetil has limited activity against penicillin-nonsusceptible pneumococci and *M. catarrhalis*, of which approximately 22 percent and 50 percent, respectively, of isolates are resistant.[150,156] This agent has been approved for the treatment of uncomplicated urinary tract infection, skin and soft tissue infection, acute bacterial maxillary sinusitis, and lower respiratory tract infection caused by susceptible bacteria. Cefuroxime axetil is an effective alternative to doxycycline and amoxicillin for the treatment of early Lyme disease.[317] The safety and efficacy of cefuroxime axetil have not been established for infants younger than 3 months of age.

Third-Generation Cephalosporins

Third-generation cephalosporins approved for use in children include the parenteral agents cefotaxime, ceftizoxime, ceftriaxone, and ceftazidime and the oral agents cefixime, cefpodoxime proxetil, ceftibuten, cefdinir, and cefditoren pivoxil. Third- and fourth-generation cephalosporins are the most potent cephalosporins against gram-negative enteric bacteria.[160] Most of them have excellent activity against *H. influenzae*, *M. catarrhalis*, *N. gonorrhoeae*, *N. meningitidis*, group A streptococci, and penicillin-susceptible pneumococci but relatively poor

activity against staphylococci. Ceftazidime is the only agent with activity against *P. aeruginosa*. A serious global health concern has emerged because members of the Enterobacteriaceae have developed increased resistance to third-generation cephalosporins on the basis of plasmid-mediated production of extended-spectrum and AmpC β-lactamases.[245] The parenteral cephalosporins provide high concentrations of drug in serum and adequate concentrations in CSF. Third-generation cephalosporins and aminoglycosides are synergistic in vitro against certain susceptible and resistant strains of *P. aeruginosa* as well as *Serratia marcescens* and other Enterobacteriaceae, including *Enterobacter cloacae*, *E. coli*, *K. pneumoniae*, and *P. mirabilis*.

In 2010, the CLSI released revised breakpoints for Enterobacteriaceae for third-generation cephalosporins and the first-generation cefazolin. The current MIC breakpoints in micrograms per milliliter are 1 and less for susceptible, 2 for intermediate, and 4 and greater for resistant organisms for cefazolin, cefotaxime, ceftizoxime, and ceftriaxone. For ceftazidime the MIC breakpoints are 4 and less, 8, and 16 or greater for susceptible, intermediate, and resistant organisms, respectively. Similarly, breakpoints for aztreonam (a monobactam) were revised. The revised breakpoints seek to eliminate the need to perform ESBL screen to direct patient care.[314]

Cefotaxime. Cefotaxime has excellent activity against group A streptococci, susceptible pneumococci, *H. influenzae*, *N. meningitidis*, and *N. gonorrhoeae*. Because cefotaxime can be hydrolyzed by AmpC Bush group 1–inducible chromosomal β-lactamases and by extended-spectrum β-lactamases (ESBLs), it is not active against strains of Enterobacteriaceae that produce these β-lactamases. Cefotaxime is metabolized in the liver to desacetyl cefotaxime, a less active metabolite that may act synergistically with cefotaxime. Although it is metabolized in the liver, cefotaxime is excreted by the kidneys. High serum, tissue, and CSF concentrations of cefotaxime can be achieved at the recommended dosages. The rapid development of resistance by colonizing gram-negative enteric bacilli when cefotaxime was used extensively for initial treatment of neonatal sepsis raised concern that extensive use of newer cephalosporins in the nursery or intensive care units could lead to more rapid emergence of drug-resistant bacteria than had been identified with the traditional regimens of a penicillin and an aminoglycoside. Because of its broad spectrum of activity against many of the common pathogens causing pediatric infections, cefotaxime is used widely for inpatient treatment of lower respiratory tract infections, urinary tract infections, sepsis, intra-abdominal infections, bone and joint infections, and meningitis or ventriculitis caused by susceptible organisms. Studies in neonates have raised concern that when cefotaxime is used routinely as part of an empirical regimen for neonatal sepsis, colonizing gram-negative enteric bacilli can rapidly develop resistance and certain neonates have adverse outcomes.[70,89] Therefore, it is prudent to reserve cefotaxime for appropriate clinical and microbiologic indications. In 2003, the CLSI published new breakpoints for defining the susceptibility of *S. pneumoniae* isolates to cefotaxime and ceftriaxone. The former breakpoints were based on attainable concentrations of these antibiotics in CSF and the level at which it was presumed that meningitis treatment failed because of elevated MICs. According to the former criteria, susceptible, intermediate, and resistant MIC breakpoints for cefotaxime and ceftriaxone were 0.5 μg/mL or less, 1 μg/mL, and 2 μg/mL or more, respectively, for pneumococci. Based on the newer criteria, the original breakpoints should be used for isolates from CSF in patients with suspected meningitis, but isolates causing nonmeningeal syndromes have breakpoints of 1 μg/mL or less, 2 μg/mL, and 4 μg/mL or more, respectively.[64]

Ceftizoxime. Ceftizoxime is a parenteral agent with a spectrum of activity similar to that of cefotaxime. Its safety and efficacy have not been established for infants younger than 6 months of age, and it rarely is used in pediatrics.

Ceftriaxone. The antibacterial spectrum of ceftriaxone is similar to that of cefotaxime, with activity against β-hemolytic streptococci (including groups A, B, C, F, and G), susceptible pneumococci, *H. influenzae*, *M. catarrhalis*, *Neisseria* spp., and many members of the Enterobacteriaceae. Surveillance data from pediatric patients in the United States revealed that no significant resistance has been reported against ceftriaxone among oxacillin-susceptible strains of *S. aureus*.[160] Nonetheless, this drug should not be used as empirical monotherapy for severe infections presumed to be caused by staphylococci, because it has minimal activity against MRSA and its efficacy for treatment of susceptible strains has not been established in children. Ceftriaxone is not active against most strains of *Pseudomonas*. It differs from cefotaxime in pharmacokinetic properties. Ceftriaxone undergoes extensive protein binding and has a long serum half-life. Because of its broad spectrum of activity against many of the pathogens that commonly cause sepsis, meningitis, and respiratory tract infections in older infants and children, along with its unique pharmacokinetic features, ceftriaxone has been used excessively for outpatient management of febrile infants and young children being evaluated for possible systemic bacterial infection. Single-dose therapy was approved for the treatment of acute otitis media in 1997; a 3-day regimen is recommended as alternative therapy for patients with refractory cases.[10,185]

Ceftriaxone is effective for various sexually transmitted diseases (STDs), including chancroid, proctitis, epididymitis (in combination with doxycycline), and different forms of gonococcal disease (e.g., neonatal ophthalmia); uncomplicated urethral, endocervical, rectal, or pharyngeal gonorrhea (in combination with doxycycline for treatment of possible coexisting chlamydial infection); and disseminated gonococcal infection, meningitis, or endocarditis.[316] Short-course ceftriaxone therapy for typhoid fever in children is comparable in effectiveness to chloramphenicol therapy.[216] However, duration of ceftriaxone therapy longer than 5 days has been associated with fewer confirmed bacteriologic relapses.[294] Ceftriaxone (or cefotaxime) is recommended for treatment of

acute Lyme disease with advanced heart block (14 to 21 days) or meningitis (14 to 28 days).[317] Although third-generation cephalosporins have been used for empirical treatment of health care–associated respiratory, wound, intra-abdominal, and urinary tract infections caused by gram-negative bacteria, administering an aminoglycoside instead of or in addition to a third-generation cephalosporin often is prudent because of the risk for encountering plasmid-mediated, ESBL resistance, or inducing the expression of chromosomally mediated AmpC Bush group 1 β-lactamases.

For infections requiring prolonged therapy (e.g., suppurative arthritis, osteomyelitis, and brain abscess) and caused by susceptible organisms, ceftriaxone therapy is cost-effective for use outside of the hospital. Once the acute signs of disease have diminished and the child remains in the hospital for only parenteral therapy, discharge and once-daily administration of ceftriaxone in the home or clinic can be considered.

Because of its extensive protein binding, ceftriaxone can displace bilirubin from albumin-binding sites, with the potential risk for inducing kernicterus, although to date this adverse clinical effect has not been reported. Cefotaxime is administered to neonates more often than ceftriaxone because considerably more information is available on its safety and pharmacokinetics. Ceftriaxone is excreted and concentrated in bile. Gallbladder "sludge," diagnosed by abdominal sonography (and not identifiable by other radiographic techniques), has been demonstrated in some patients who received ceftriaxone. The material appears to be a calcium–ceftriaxone complex and resolves on cessation of the drug. Most patients with ceftriaxone-associated sludge are asymptomatic, but occasionally patients have symptoms of gallbladder disease and incidents of acute cholecystitis have been reported in a few children.

Ceftazidime. In contrast to cefotaxime and ceftriaxone, ceftazidime has poor antibacterial activity against *S. aureus*, is less active against penicillin-susceptible *S. pneumoniae*, and is slightly less active against group A streptococci, but it is more active against *P. aeruginosa*. On a weight basis, ceftazidime is the most effective of all β-lactam antimicrobial agents in vitro against *P. aeruginosa*. It frequently is active in vitro against *P. aeruginosa* strains that are resistant to antipseudomonal penicillins. Resistance to ceftazidime can develop in gram-negative bacteria by the production of ESBLs or because of decreased bacterial cell permeability, as seen with *P. aeruginosa*, *Acinetobacter*, and some *Serratia* strains. Ceftazidime is indicated for use in the following circumstances: infections suspected to be caused by *Pseudomonas*, including acute exacerbations of chronic pulmonary infections in patients with cystic fibrosis, chronic suppurative otitis media, or malignant otitis externa; thermal injuries, puncture wound infections of the foot, and complicated health care–associated infections; and febrile illnesses in neutropenic patients with cancer or other immunocompromised individuals.

Cefixime. Cefixime has a vinyl group instead of a chlorine atom at position 3 of the cephem nucleus and an aminothiazole oxime group, rather than a phenyl glycine side chain. These biochemical changes result in potent gram-negative activity. Cefixime was introduced in 1989 as an oral third-generation cephalosporin with a broad spectrum of activity, including activity against group A streptococci, *H. influenzae*, *M. catarrhalis*, susceptible *S. pneumoniae*, and many members of the Enterobacteriaceae, including *Shigella*, *Salmonella*, *E. coli*, *Klebsiella*, and *P. mirabilis*.[24] Cefixime, like ceftibuten, is distinguished from other extended-spectrum oral cephalosporins by its lack of activity against *S. aureus*. It also is inactive against *Citrobacter freundii*, *Enterobacter*, *P. aeruginosa*, and *Serratia*. Although cefixime has in vitro activity against penicillin-susceptible pneumococci, lower bacteriologic cure rates occurred with cefixime than with amoxicillin when cefixime was used to treat children with pneumococcal acute otitis media.[142] Cefixime is resistant to degradation by certain β-lactamases. Administration of cefixime is facilitated by once-daily dosing,[100] pleasant taste, and stability of the suspension at room temperature. Cefixime is effective therapy for uncomplicated urinary tract infections caused by *E. coli* or *P. mirabilis*, for shigellosis, and for acute otitis media or sinusitis caused by *H. influenzae* or *M. catarrhalis*.[36] Some specialists use cefixime to treat uncomplicated gonococcal infections in children because the drug can be administered orally; however, no reports have been published concerning the safety or effectiveness of cefixime used for this purpose.[316] Clinical studies indicate that the rate of laboratory-confirmed eradication of *Shigella sonnei* in an epidemic setting is higher with a 5-day than with a 2-day cefixime regimen,[204] cefixime alone is equally effective as sequential therapy with intravenous and oral antibiotics in selected febrile infants with urinary tract infections,[34,138] and a 14-day cefixime regimen for treatment of multidrug-resistant *Salmonella typhi* septicemia in children has efficacy comparable to that of a 5-day course of ceftriaxone therapy.[119] Cefixime is not recommended for the treatment of infections frequently caused by staphylococci, such as skin or soft tissue infections, and it is not the most effective agent for the treatment of pneumococcal infections. The safety and efficacy of cefixime have not been established for infants younger than 1 month of age. Wyeth discontinued manufacturing cefixime in 2002, but the drug was reintroduced in 2004 as a generic formulation (Lupin Pharmaceuticals).

Cefpodoxime Proxetil. The antibacterial activity of cefpodoxime proxetil is similar to that of cefixime, with the exception of improved activity against staphylococci. Cefpodoxime is active against group A streptococci; susceptible pneumococci; β-lactamase–producing strains of *N. gonorrhoeae*, *H. influenzae*, and *M. catarrhalis;* oxacillin-susceptible *S. aureus;* and many members of the Enterobacteriaceae. It is not active against *Enterobacter*, *Pseudomonas*, *Serratia*, or *Morganella*. Cefpodoxime proxetil, the ester prodrug of cefpodoxime, is cleaved by intestinal esterases to the active drug. Oral absorption is increased by the presence of food. Because of its longer serum half-life, cefpodoxime proxetil can be administered in twice-daily dosing intervals. Cefpodoxime is hydrolyzed by some ESBLs. The unfavorable

palatability of cefpodoxime proxetil may adversely affect adherence.[289]

Treatment of group A streptococcal pharyngitis with a 5-day cefpodoxime proxetil regimen is equivalent to therapy with penicillin V and has been approved for this indication by the FDA.[82] A 5-day regimen is approved for the treatment of acute otitis media based on clinical outcomes that are comparable or superior to those with therapy with amoxicillin-clavulanate, cefaclor, or cefixime.[72] Cefpodoxime is not likely to be effective for the treatment of acute otitis media caused by penicillin-nonsusceptible pneumococci because of its unfavorable pharmacodynamic parameters.[312] It also has been approved for outpatient treatment of community-acquired respiratory tract infections, uncomplicated urinary tract infections, and mild skin and skin structure infections. The safety and efficacy of cefpodoxime proxetil have not been established for infants younger than 5 months of age.

Ceftibuten. Ceftibuten was approved by the FDA for pediatric use in 1995. The primary active component is formulated as a *cis*-isomer that is converted in serum to the less active *trans*-isomer. The chemical structure and spectrum of antimicrobial activity are similar to those of cefixime. Ceftibuten, like cefixime, is distinguished from other extended-spectrum oral cephalosporins by its lack of activity against *S. aureus*. Its antimicrobial activity against groups A, C, F, and G β-hemolytic streptococci is good, whereas its activity against groups B and D, as well as viridans streptococci, is poor. Ceftibuten exhibits MICs for penicillin-susceptible pneumococci in the susceptible range, whereas penicillin-nonsusceptible strains are ceftibuten-resistant. In contrast to other oral cephalosporins, ceftibuten is the most stable to hydrolysis by β-lactamases produced by Neisseriaceae, *Moraxella*, *Haemophilus*, and Enterobacteriaceae, including most strains of *E. coli* and *K. pneumoniae* that produce Ambler class A plasmid-mediated, ESBLs of the TEM, SHV, and OXA types. Ceftibuten is variably active against strains of *Citrobacter*, *Serratia*, and *Morganella*, but it is inactive against *Acinetobacter*, *Pseudomonas*, *Enterobacter*, *Bordetella*, and anaerobic species. It is also a weak inducer of AmpC Bush group 1 β-lactamases produced by *S. marcescens*, *E. cloacae*, and *Enterobacter aerogenes*.[129]

Ceftibuten has excellent bioavailability and a favorable pharmacokinetic profile that facilitates a once-daily dosing schedule.[23] Treatment regimens of 10 days have been approved for streptococcal tonsillopharyngitis and acute otitis media caused by *H. influenzae*, *M. catarrhalis*, or *S. pyogenes* because of superior clinical and microbiologic efficacy data for ceftibuten in comparison with penicillin V in children with streptococcal tonsillopharyngitis[248] and comparable clinical efficacy data for ceftibuten versus amoxicillin, amoxicillin-clavulanate, cefaclor, and cefprozil for the treatment of children with clinically defined acute otitis media with or without effusion. However, cumulative data from clinical trials indicate that microbiologic cure rates of pneumococcal otitis media are lower for ceftibuten than for comparable antimicrobial agents.[129] Because of the increasing prevalence of nonsusceptible *S. pneumoniae* isolates, ceftibuten has almost no place in the treatment of acute otitis media, especially in young or otherwise at-risk infants and children.

Ceftibuten also should be considered as alternative therapy for sinusitis caused by susceptible organisms. Although not approved for this indication, ceftibuten achieved clinical success equivalent to and bacterial eradication superior to those reported with trimethoprim-sulfamethoxazsole in the treatment of complicated or recurrent urinary tract infections in children.[22] Adverse reactions to ceftibuten, which are limited mainly to the gastrointestinal tract, occur in approximately 10 percent of children, a rate similar to comparable oral β-lactam antibiotics. The safety and efficacy of ceftibuten have not been established for infants younger than 6 months of age.

Cefdinir. Cefdinir was approved in 1999 for the treatment of acute otitis media in children. It has a broad spectrum of in vitro antimicrobial activity, favorable pharmacokinetics, a convenient once-daily or twice-daily dosage schedule, superior palatability, a low rate of adverse events, and proved clinical efficacy against common childhood pathogens.[140,174] In contrast to cephalexin, cefaclor, cefuroxime, cefixime, and cefpodoxime, cefdinir has equivalent or superior MICs for groups A, B, C, F, and G streptococci, viridans streptococci, and oxacillin-susceptible staphylococci. Its in vitro activity against penicillin-susceptible and penicillin-nonsusceptible strains of *S. pneumoniae* is equivalent to that of cefuroxime and cefpodoxime but superior to that of other oral cephalosporins. Cefixime and cefpodoxime have superior activity against *H. influenzae* (including β-lactamase–producing strains), whereas cefdinir and cefuroxime have comparable activity against this pathogen. The activity of cefdinir against *M. catarrhalis* is similar to that of cefixime, cefpodoxime, and cefuroxime but superior to that of earlier-generation cephalosporins. Cefdinir is stable to hydrolysis by many common Enterobacteriaceae-produced β-lactamases. However, activity against these pathogens is variable. Cefdinir is inactive against strains of enterococci, methicillin-resistant staphylococci, *Legionella*, *Listeria*, *Acinetobacter*, *Citrobacter*, *Enterobacter*, *P. aeruginosa*, *Serratia*, *Stenotrophomonas*, and most anaerobes.[130]

Based on comparative clinical and microbiologic efficacy trials, administration of cefdinir should be considered for children with (1) acute bacterial otitis media caused by β-lactamase–positive or β-lactamase–negative strains of *H. influenzae* and *M. catarrhalis* or penicillin-susceptible strains of *S. pneumoniae* as either a 5- or 10-day regimen; cefdinir has efficacy equivalent to that of cefuroxime and cefpodoxime but superior palatability and therefore should be considered as an alternative to other second-line regimens; however, use of amoxicillin-clavulanate or three single daily doses of parenteral ceftriaxone is recommended if penicillin-nonsusceptible pneumococci are suspected or proved[10,12,174]; (2) group A streptococcal tonsillopharyngitis in 5- or 10-day dosage schedules; (3) acute maxillary sinusitis caused by susceptible organisms; and (4) uncomplicated skin and skin structure infections caused by oxacillin-susceptible

S. aureus or group A streptococci.[159] Although cefdinir is not approved for treatment of urinary tract infections, microbiologic data support its potential utility for community-acquired uncomplicated urinary infections.[43] The safety and efficacy of cefdinir have not been established for infants younger than 6 months of age.

Cefditoren Pivoxil. Cefditoren pivoxil was approved by the FDA in 2002 for oral treatment of acute exacerbations of chronic bronchitis, pharyngitis, tonsillitis, and uncomplicated skin and soft tissue infections in adults and adolescents 12 years of age and older. Cefditoren is similar to cefdinir and cefpodoxime in its antibacterial activity. It is stable to hydrolysis by many common Enterobacteriaceae-produced β-lactamases. Cefditoren has the greatest in vitro antimicrobial activity of all oral cephalosporins against *S. pneumoniae*. The inactive metabolite pivalate is eliminated by the kidneys in combination with carnitine as pivaloylcarnitine. Although cefditoren transiently decreases serum concentrations of carnitine, the clinical significance of this is not clear, but no adverse effects have been reported.[205]

Fourth-Generation Cephalosporins

Cefepime. Cefepime, the prototypic agent of this class of antibiotics, was approved by the FDA in 1996. It is distinguished from other cephalosporins by the rapid penetration of outer-membrane porins into the periplasmic space of gram-negative bacteria, facilitated by its net neutral charge; enhanced stability against hydrolysis by inducible or constitutively expressed chromosomally mediated AmpC Bush group 1 β-lactamases and Ambler class A plasmid-mediated SHV- and TEM-type ESBLs; and increased binding affinity to multiple PBPs. These factors contribute to cefepime's expanded spectrum of activity and improved efficacy against gram-negative pathogens in contrast to third-generation cephalosporins.

The in vitro activity of cefepime encompasses a broad range of gram-positive and gram-negative organisms, including oxacillin-susceptible staphylococci; approximately 75 percent of penicillin-resistant strains of *S. pneumoniae;* viridans streptococci; most strains of Enterobacteriaceae, including, most notably, ESBL-producing strains of *E. coli* and *K. pneumoniae;* and AmpC-mediated resistant *Enterobacter* spp., and *Citrobacter* spp., as well as approximately 90 percent of *P. aeruginosa* isolates. Furthermore, bacteria in which resistance to third-generation cephalosporins develops by means of single-step mutations usually remain susceptible to cefepime. Clinical and laboratory data also indicate that selection of cefepime resistance, specifically among *Enterobacter* spp., and *P. aeruginosa*, is rare. As with other cephalosporins, cefepime is inactive against enterococci, methicillin-resistant staphylococci, *S. maltophilia*, and many anaerobic organisms.[160,170]

Cefepime is indicated for the parenteral treatment of lower respiratory tract, urinary tract, skin and skin structure, and intra-abdominal (in combination with anaerobic antibacterial agents) infections, as well as for empirical monotherapy in pediatric patients with cancer who have fever and neutropenia, for whom cefepime has been shown to be as effective as meropenem, piperacillin-tazobactam, or ceftazidime with or without an aminoglycoside. In comparative clinical and microbiologic efficacy trials, cefepime as a single antibacterial agent was equivalent to third-generation cephalosporins for the most common childhood pathogens. In addition, cefepime is as safe and well tolerated, as are other cephalosporin antibiotics.[47,223,279]

Cefepime should be reserved for complicated community-acquired, health care–associated, or polymicrobial infections, penicillin-resistant and third-generation cephalosporin–resistant pathogens, and patients with cystic fibrosis and *P. aeruginosa* lung infections. Cefepime concentrations in CSF reach approximately 3 to 6 μg/mL (9% of the peak plasma concentration) in children with bacterial meningitis. Therefore, achievable CSF concentrations exceed the MICs of common CNS pathogens by at least 10-fold, except for penicillin- and cephalosporin-nonsusceptible *S. pneumoniae*.[39] Currently, data are insufficient to recommend cefepime as single-agent therapy for meningitis. The safety and efficacy of cefepime have not been established for neonates, but data extrapolated from pharmacokinetic studies among preterm and term infants support a dose of 30 mg/kg every 12 hours for infants younger than 14 days of age, regardless of gestational age. This dosage should provide antibiotic exposure equivalent to or greater than the approved dose of 50 mg/kg every 8 to 12 hours in older infants and children.[61]

Fifth-Generation Cephalosporins

Ceftaroline. Ceftaroline fosamil is a recently approved broad-spectrum cephalosporin for the treatment of acute bacterial skin and skin structure infections and community-acquired pneumonia in adults. It received FDA approval in 2010. Ceftaroline has high affinity for PBPs 1, 2, and 3 for methicillin-susceptible strains of *S. aureus* (MSSA). It also contains a side chain with alkoxyimino group in the C7 acyl moiety that provides in vitro activity for MRSA with high affinity for the PBP2a and another side chain with a 2-thiazolythio spacer group modification that optimizes the anti–MRSA activity.[288] Ceftaroline also retains activity against isolates with reduced susceptibility to vancomycin (heterogeneous glycopeptide-intermediate *S. aureus* [hGISA], GISA, and glycopeptide-resistant *S. aureus* GRSA) or linezolid.[29,288] This antibiotic has in vitro activity against gram-positive organisms, including *S. aureus*, both MSSA and MRSA; coagulase-negative staphylococci; group A and group B β-hemolytic streptococci; and *S. pneumoniae*, including multidrug-resistant strains and modest activity against *E. faecalis*. Ceftaroline has no activity against *E. faecium* (MIC_{90} >1632 μg/mL). It has activity against penicillin-nonsusceptible viridans group streptococci. Ceftaroline is active against non-ESBL enteric gram-negative organisms, including *E. coli* and *K. pneumoniae*, respiratory gram-negative organisms (including *M. catarrhalis* and *H. influenzae* and β-lactamase–positive strains), and

N. gonorrhoeae. It has limited activity against nonfermenting gram-negative organisms, including *P. aeruginosa* and *A. baumannii* (with MIC >16 mg/L for both). Ceftaroline can induce expression of AmpC β-lactamases in a degree similar to that of ceftriaxone and cefotaxime based on in vitro induction assays.[288]

Ceftaroline fosamil is a prodrug that is converted into its active form by plasma phosphatases. It is administered only in the intravenous form, infused over 60 minutes in a twice-daily regimen. It is eliminated primarily by renal excretion with an approximate half-life of 3 hours. Dosage adjustment is required for moderate renal impairment and for patients receiving hemodialysis. Protein binding seems to be minimal (ranging from 1% to 19% at different concentrations).

Phase II and III clinical trials evaluated the effectivity of ceftaroline in contrast to the combination vancomycin and aztreonam for the treatment of complicated skin and skin structure infections, with similar clinical cure rates; the primary outcome was noninferiority of clinical cure rate. For patients with microbiologic data, eradication rates were also similar. The drug was well tolerated, and no safety concerns were reported. The most common adverse effects were diarrhea, nausea, headache, and insomnia. It may cause changes in urine color or smell. Ceftaroline did not cause QTc interval prolongation.[78,291]

Results from two randomized, double-blind, multicenter, phase III trials showed similar cure rates for patients with community-acquired pneumonia comparing ceftaroline and ceftriaxone (± clarithromycin).[104,196]

Adverse Effects

The cephalosporins, like the penicillins, are safe for children and have almost no dose-related toxicity. The most common reactions are local, including pain at the injection site or thrombophlebitis with parenteral administration and mild gastrointestinal complaints with oral dosing. Hypersensitivity reactions occur in approximately 1 to 3 percent of treatment courses and include morbilliform rash, urticaria, and pruritus. Drug fever has been associated with the administration of cephalosporins. Nonspecific antibiotic-associated diarrhea and, less commonly, *C. difficile* toxin–mediated colitis can occur after use of cephalosporin.

Other adverse effects are rare, and some are unique to one or a few cephalosporins. Physicians should be alert for uncommon reactions, including reversible neutropenia, which can occur after prolonged use of high-dosage cephalosporins, Coombs-positive hemolytic anemia, and bleeding. Altered hemostasis because of hypoprothrombinemia can result when using any cephalosporin that contains a methylthiotetrazole side chain (cefamandole, cefotetan, and moxalactam). These agents act as competitive inhibitors of vitamin K–dependent carboxylase, which converts clotting factors II, VII, IX, and X to their active forms. Gallbladder sludging, biliary pseudolithiasis, and symptomatic obstructive biliary disease rarely have been associated with the administration of ceftriaxone.[323]

The cephalosporins may produce allergic reactions similar to those caused by the penicillins. Cross sensitization exists among the cephalosporins, and allergy to one cephalosporin implies allergy to all. Various degrees of immunologic cross reaction of penicillins and cephalosporins have been demonstrated in vitro and in animal models.[244] Previously quoted studies suggest that the frequency of allergic reactions to cephalosporins ranges from 5.4 to 16.5 percent in adult patients with a history of penicillin allergy and 1.0 to 2.5 percent in those without such a history. The incidence of hypersensitivity to unrelated drugs is increased in some patients who are allergic to penicillin, a finding suggesting that excipients in antibiotic preparations may be responsible for these reactions.[276] Most patients who are thought to be allergic to penicillin receive cephalosporins without adverse reaction. Data indicate that the risk for an allergic reaction to a cephalosporin challenge among penicillin-allergic subjects with positive skin test responses is 4 percent. Therefore, the following options are available for individuals with positive skin test responses for penicillin hypersensitivity: administration of an alternative non–β-lactam antibiotic, administration of a cephalosporin by graded challenge, or desensitization to the cephalosporin. Individuals with a history of penicillin allergy who have negative penicillin skin test responses may receive cephalosporins because they are at no higher risk than the general population for experiencing allergic reactions.[190] In the absence of penicillin skin testing, a cephalosporin may be used with caution as an alternative to penicillin in children who have an ambiguous history of rash; however, cephalosporins should be avoided in patients with a known immediate or accelerated reaction to a penicillin. Currently, skin testing to evaluate for cephalosporin hypersensitivity is not possible because the potential cephalosporin haptens are unknown, and no standardized antigen exists.

Some products may cause unexpected reactions, such as bile sludge attributed to ceftriaxone and serum sickness–like disease described with cefaclor. A generalized pruritic rash, similar to erythema multiforme, developed in some children treated with cefaclor, along with fever, purpura, and arthritis with pain and swelling in the knees and ankles. The signs appeared 5 to 19 days after the start of therapy and generally disappeared within 4 to 5 days after discontinuing use of the drug. The children had no previous history of allergy to a penicillin or cephalosporin.[222] Levine[187] compared adverse reactions in children who received cefaclor (1017 patients and 2513 courses) or amoxicillin (1009 patients and 2358 courses). Serum sickness (defined as arthritis or arthralgia in addition to a rash or urticaria) or erythema multiforme occurred in 11 children (1.1%) who received cefaclor but in none of those who received amoxicillin. Studies suggest that serum sickness–like reactions to cefaclor are associated with lymphocyte sensitization.[168]

Carbacephems

Carbacephems have a carbon atom at position 1 of the dihydrothiazine ring (cephem nucleus), rather than a

sulfur atom. The only carbacephem currently available is loracarbef. Loracarbef is structurally similar to cefaclor but has greater chemical stability in solution. The antibacterial spectrum of loracarbef is similar to that of second-generation cephalosporins. In contrast to cefaclor, loracarbef has similar activity against penicillin-susceptible pneumococci, group A streptococci, and oxacillin-susceptible *S. aureus* and greater activity against *H. influenzae* and *M. catarrhalis*, including β-lactamase–producing strains.[94] Loracarbef is not active against *P. aeruginosa*, *Enterobacter*, *Citrobacter*, indole-positive *Proteus*, or *B. fragilis*.

Loracarbef is more stable than cefaclor to hydrolysis by lactamases but can be hydrolyzed by ESBLs. Loracarbef is available for oral administration only. It is absorbed rapidly and well; however, absorption is decreased when the agent is taken with food. Loracarbef has a longer serum half-life than some of the penicillins and can be given twice daily. It is excreted by the kidneys. Loracarbef is relatively well tolerated. Gastrointestinal complaints, including diarrhea, nausea, and vomiting, are the adverse effects most frequently reported, but they occur in fewer than 5 percent of treatment courses. The safety and efficacy of loracarbef have not been established for infants younger than 6 months of age.

Loracarbef can be used as an alternative to penicillin or erythromycin for the treatment of group A streptococcal pharyngitis.[93] The effectiveness of loracarbef is similar to that of amoxicillin and amoxicillin-clavulanate for the treatment of acute otitis media caused by susceptible bacteria.[107] Loracarbef is not effective against penicillin-nonsusceptible pneumococci; therefore, it is not considered appropriate for empirical therapy of acute otitis media.[10] For the treatment of mild-to-moderate skin and skin structure infections, the efficacy of loracarbef is comparable to that of cefaclor. Loracarbef has been approved for use in the treatment of acute bacterial sinusitis and uncomplicated urinary tract infections caused by susceptible bacteria.

Monobactams

Aztreonam is the prototype monobactam, a name that refers to the unique monocyclic nucleus. This drug has aerobic, gram-negative antibacterial activity similar to that of ceftazidime, but it has no significant gram-positive activity. Unlike most β-lactam antibiotics, aztreonam has the advantage of not inducing β-lactamase activity, and its molecular structure confers a high degree of stability to hydrolysis by β-lactamases. The mechanisms of antibiotic resistance of some gram-negative bacteria include expression of efflux pumps, reduced expression of outer-membrane porin proteins, and production of AmpC-type β-lactamases or ESBLs. Approximately 15 percent of Enterobacteriaceae in North America are resistant to aztreonam.[270] Although it is a β-lactam, aztreonam is weakly immunogenic and can be used in patients with minor forms of β-lactam allergy. Its use in pediatrics is indicated as a secondary agent for the intravenous treatment of lower respiratory tract, skin and soft tissue, urinary tract, and intra-abdominal infections caused by susceptible pathogens. Higher doses of aztreonam may be warranted for pediatric patients with cystic fibrosis. Aztreonam and aminoglycosides are synergistic in vitro against most strains of *P. aeruginosa*, many strains of Enterobacteriaceae, and other aerobic gram-negative bacilli. The safety and efficacy of aztreonam have not been established for infants younger than 9 months of age or for children with impaired renal function. However, aztreonam has been used with favorable outcomes in premature and term neonates, as well as in young infants. Updated breakpoints for enteric gram-negative organisms to aztreonam were released in 2010. The revised breakpoints include 4 or less, 8, and 16 or greater for susceptible, intermediate, and resistant organisms, respectively.[314] Aztreonam lysinate for inhalation is a novel monobactam formulation approved by the FDA in February 2010 for patients with cystic fibrosis who are colonized with *P. aeruginosa*. It has appeared to be safe and well tolerated in patients with cystic fibrosis. For children 7 years of age or older and adults, the dose recommended for inhaled aztreonam is 75 mg three times daily for 28 days.[320,250]

Carbapenems

Carbapenems differ from penicillin by virtue of the substitution of a sulfur atom by a carbon atom at position 1 of the β-lactam ring and possession of an unsaturated bond between carbon atoms at positions 2 and 3 in the structure. The carbapenem class of antimicrobial agents exhibits the broadest spectrum of activity of all β-lactam antibiotics; these agents are active against most clinically significant gram-positive and gram-negative pathogens, including anaerobic organisms. Because they are acid-labile in the stomach, they must be administered parenterally.[79] Three carbapenems, imipenem-cilastatin, meropenem, and ertapenem, are approved for use in children.

Imipenem-Cilastatin

Imipenem-cilastatin was the first carbapenem evaluated for the treatment of severe bacterial infections in children. Extensive hydrolysis of imipenem by dehydropeptidase I occurs in the proximal renal tubule and results in the production of a potentially nephrotoxic inactive metabolite. Consequently, imipenem must be administered with cilastatin, an inhibitor of dehydropeptidase I. Despite favorable in vitro efficacy against a broad spectrum of bacterial pathogens, the clinical suitability of this antibiotic is limited because of the drug's epileptogenic potential, especially in children with bacterial meningitis. Imipenem causes seizures presumably by acting as a competitive inhibitor of γ-aminobutyric acid, an inhibitory neurotransmitter.[79] Of concern is that imipenem acts as a strong inducer of production of ESBL and may select for β-lactam resistance.[84] Imipenem-cilastatin is indicated for children with severe non-CNS infections caused by susceptible organisms. The dose must be reduced for children with impaired renal function. With the advent of meropenem, imipenem-cilastatin has been used less frequently in pediatrics.

Meropenem

Meropenem was approved for use in children in 1996. It is structurally related to imipenem. However, it is more stable against degradation by renal dehydropeptidase I and therefore does not require coadministration with cilastatin. On the basis of its superior safety and efficacy, meropenem generally is considered the preferred carbapenem for treatment of childhood infections.

Mechanisms of Action. As with other β-lactam antibiotics, meropenem is bactericidal against susceptible bacteria because it inhibits bacterial cell wall synthesis. The *trans* configuration of the hydroxyethyl side chain and hydrogen atoms protect the parent β-lactam structure from inactivation by the most common β-lactamases, including almost all Bush groups 1 and 2 (Ambler classes A, C, and D) β-lactamase–producing organisms, including those that produce ESBLs (*Citrobacter*, *Enterobacter*, *E. coli*, *Klebsiella* spp., and *P. mirabilis*) or AmpC β-lactamases (*Citrobacter*, *Enterobacter*, *Pseudomonas*, and *Serratia*). In addition, the pyrrolidine side chain enhances the compound's antipseudomonal activity.[84]

Mechanisms of Resistance. Resistance may be intrinsic or acquired and is mediated by numerous mechanisms: (1) some strains of *P. aeruginosa* are deficient in the cell wall porin proteins (OprD) that usually facilitate intracellular penetration; (2) efflux pumps in some gram-negative bacteria are capable of excreting carbapenems; (3) *S. maltophilia* and some other gram-negative pathogens possess uncommon metallo-β-lactamases, the so-called carbapenemases (i.e., Bush groups 3a and 3b and Ambler class B), which form a diverse group of β-lactamases comprising the IMP and VIM families, and SPM1 and GIM1 enzymes; these carbapenemases have the ESBL and AmpC antibiotic substrate profile; (4) some gram-negative bacteria possess clavulanic acid–inhibited carbapenemases consisting of the *K. pneumoniae* carbapenemases (KPCs) (i.e., Bush group 2f and Ambler class A) or OXA enzymes (i.e., Bush group 2d and Ambler class D); and (5) MRSA strains and *E. faecium* have altered PBPs, which account for their inherent resistance. Furthermore, meropenem acts as a weak inducer of ESBLs.[46,84] At present, the risk that a single-step spontaneous mutation causes resistance among *Pseudomonas* spp. appears to be low.

In Vitro Activity. Meropenem has equivalent or slightly less in vitro potency than imipenem against gram-positive pathogens, but it is significantly more active against gram-negative organisms. The spectrum of activity of meropenem encompasses streptococci (excluding many strains of penicillin and cefotaxime-nonsusceptible *S. pneumoniae*), oxacillin-susceptible staphylococci, ampicillin-susceptible enterococci, *L. monocytogenes*, *H. influenzae*, *N. meningitidis*, Enterobacteriaceae, most strains of *P. aeruginosa*, and anaerobes (including β-lactamase–positive strains of *B. fragilis*). Pathogens resistant to meropenem are *Stenotrophomonas*, MRSA, *E. faecium*, approximately 10 percent of *Pseudomonas* strains, and most strains of penicillin and cefotaxime-nonsusceptible *S. pneumoniae*.[46,55,311] In vitro tests indicate that meropenem acts synergistically with aminoglycoside antibiotics against some isolates of *P. aeruginosa*.[147]

Pharmacokinetics. Meropenem exhibits nearly linear pharmacokinetics; increases in dosage result in approximately proportional increases in the peak plasma concentration and area under the plasma concentration–time curve (AUC).[37] Only 2 percent of the drug is bound to plasma proteins, and the drug is distributed widely into tissues and fluids.[79] The elimination half-life declines with increasing age (premature neonates, 2.9 hours; term neonates, 2 hours; children, 1.1 hours; adults, 1 hour) and is longer than that for imipenem. Because meropenem is cleared primarily by glomerular filtration, the dosage should be reduced in children with renal dysfunction.[46] Furthermore, meropenem is cleared efficiently by hemodialysis; therefore, it should be administered after the patient undergoes this procedure. Pharmacodynamic studies indicate that a favorable bacteriologic outcome is best predicted by the duration of the dosing interval for which the meropenem plasma concentration exceeds the MIC_{90} of the target pathogen.[79] Accordingly, a dose of 20 mg/kg every 8 hours achieves optimal plasma concentrations (50 to 60 μg/mL) for systemic infections that do not involve the CNS,[40] whereas a dose of 40 mg/kg every 8 hours is recommended for patients with cystic fibrosis because of accelerated drug excretion.[139] Penetration of meropenem through inflamed meninges is approximately 8 percent of the mean plasma concentration. Consequently, a dose of 40 mg/kg every 8 hours is required to achieve mean peak CSF concentrations of 0.9 to 6.5 μg/mL and ensure effective treatment of bacterial meningitis.[79,236] An administration schedule of every 12 hours probably is appropriate for neonates because of their immature renal function.[37]

Indications for Use. The FDA has approved meropenem as monotherapy for susceptible organisms causing intra-abdominal infections and bacterial meningitis in children older than 2 months of age. Meropenem should be reserved for treatment of cephalosporin-resistant nosocomial pathogens, severe polymicrobial infections with favorable susceptibility profiles, and infections that fail to respond to other antimicrobial agents. Meropenem monotherapy has clinical and microbiologic efficacy equivalent to that of third-generation cephalosporin-based regimens for septicemia and infections of the CNS, abdominal cavity, lower respiratory tract, urinary tract, and skin.[48,236] In addition, meropenem monotherapy has been shown to be equivalent to ceftazidime and amikacin and to piperacillin-tazobactam for empirical treatment of febrile children with neutropenia.[75,243] Clinical efficacy against cephalosporin-resistant pathogens also has been demonstrated in patients with cystic fibrosis.[139] In view of increasing resistance of pneumococci to β-lactam antibiotics, it is prudent to administer vancomycin with meropenem for empirical treatment of suspected bacterial meningitis until CSF microbiologic results are available. Combination therapy with aminoglycosides should be considered for suspected or proved

P. aeruginosa infections in view of emerging resistant strains and potential antibiotic synergy. The effectiveness of meropenem for neonatal infections has been assessed in small, noncomparative studies that demonstrated a favorable clinical response in neonates in whom previous conventional therapy had failed.[147]

Adverse Effects. Data from well-designed clinical trials indicate that meropenem has clinical and laboratory adverse event profiles that are equivalent to those of comparable antibiotic regimens. In contrast to treatment with imipenem-cilastatin, no increased risk for seizures exists in children with or without meningitis who are treated with meropenem,[176] presumably because meropenem has less affinity than imipenem for the γ-aminobutyric acid receptor. Meropenem can be given intravenously as small-volume bolus injections (over a course of 3 to 5 minutes) without inducing nausea or vomiting. The most common adverse reactions include diarrhea (4%), rash (2%), and vomiting (1%).[46]

β-Lactam Antibiotics Not Approved for Use in Children

Several β-lactam antibiotics available in the United States are not approved for use in children. These agents include cefonicid, a second-generation cephalosporin; cefotetan, a cephamycin; and cefoperazone, a third-generation cephalosporin. Several extended-spectrum cephalosporins and carbapenems that have activity against MRSA are undergoing clinical investigation, and the newer generation agent (ceftaroline) was approved for the treatment of acute bacterial skin and skin structure infections and community-acquired pneumonia in adults.

Ertapenem

Ertapenem is a newer, long-acting, parenteral carbapenem that initially was approved for use in adults in 2001. An indication for children was approved in 2005. However, clinical experience with this agent in children is limited. Ertapenem is distinctive because it is administered in a single daily dose in adolescents and adults and is stable against hydrolysis by many β-lactamases, except metallo-β-lactamases produced by certain bacterial nonfermenters, particularly *Pseudomonas* and *Acinetobacter*.[205] Apart from these organisms, its antimicrobial spectrum is similar to that of meropenem. Ertapenem has restricted activity against methicillin-resistant staphylococci, penicillin-nonsusceptible pneumococci, enterococci, *Pseudomonas* spp., and *Acinetobacter* spp.[169] The agent may be administered by intravenous or intramuscular routes. It exhibits nonlinear pharmacokinetics because of its concentration-dependent protein binding. The drug is excreted renally, and dosages need to be adjusted in patients with renal insufficiency.

Ertapenem is indicated for the treatment of patients 3 months of age and older with complicated intra-abdominal infections, complicated skin and soft tissue infections, community-acquired pneumonia, complicated urinary tract infections, and acute pelvic infections. Ertapenem is not recommended for the treatment of meningitis because drug concentrations in the CSF are subtherapeutic. The recommended dose in patients 3 months to 12 years of age is 15 mg/kg given parenterally twice daily, up to a maximum of 1 g/day. In older children and adults, the recommended dose is 1 g given once daily. The recommended duration is up to 14 days for intravenous administration. The most common adverse reactions, which include diarrhea, vomiting, and pain at the site of infusion, have rates comparable to those of other parenteral broad-spectrum β-lactam antimicrobials. Seizures have been reported in patients receiving ertapenem and may occur more commonly in individuals with underlying seizure disorders, brain lesions, or renal dysfunction.

In 2007, doripenem was approved for use in adults with complicated intra-abdominal infections and complicated urinary tract infections and is currently under review for treatment of hospital-acquired pneumonia, including ventilator-associated pneumonia, and catheter-related bacteremia. Doripenem has activity against gram-positive organisms, including MSSA, penicillin-susceptible and nonsusceptible *S. pneumoniae*, and *E. faecalis*. Activity against gram-negative bacteria is similar to that of meropenem and includes ESBL-producing strains. It also has good anaerobic coverage, including *B. fragilis*.[66,242]

Mechanisms of Resistance

Bacteria can acquire resistance to an antibiotic by at least four mechanisms: (1) alteration in the antimicrobial target, (2) decreased uptake of the antibiotic by expression of efflux pumps or by reduction of cell membrane permeability that is determined by porin channels, (3) production of an enzyme that inactivates the antibiotic, and (4) production of an alternative metabolic pathway that bypasses the action of the drug.[296] With respect to β-lactam antibiotics, resistance involves alterations in PBPs, leading to decreased affinity for the β-lactam (this mechanism of resistance is observed in methicillin-resistant strains of *S. aureus* and penicillin-resistant strains of *S. pneumoniae*), decreased permeability of the bacterial cell wall, resulting in diminished amounts of β-lactam reaching the PBPs, or production of β-lactamases that hydrolyze the β-lactam ring. Hydrolysis of β-lactams is the mechanism that is most significant clinically. Gram-positive bacteria excrete their β-lactamases outside the cell wall, whereas the β-lactamases of gram-negative bacteria remain in the periplasmic space. The spectrum of β-lactamase activity involves (1) narrow-spectrum penicillinases that preferentially hydrolyze penicillins; (2) broad-spectrum β-lactamases that hydrolyze penicillins and cephalosporins equally well; (3) cephalosporinases that preferentially hydrolyze cephalosporins and are resistant to inhibition by clavulanic acid; (4) and ESBLs that hydrolyze first-, second-, and third-generation cephalosporins but are susceptible to inhibition by clavulanic acid; and (5) carbapenemases that inactivate all β-lactams, including imipenem-cilastatin.[57]

Broad-spectrum β-lactamases were initially recognized in clinical isolates in the 1980s. Currently, the spread of ESBLs among enteric gram-negative organisms

in both hospital and community settings has become an important public health problem. Rates of ESBL production have been high in Latin America, Asia and the Pacific region, Europe, and North America. For Latin America, the rates of ESBL production for *E. coli* and *K. pneumoniae* were 13.5 and 44 percent, respectively, from 2004 to 2006.[257] β-Lactamases are classified on the basis of their (1) responses to β-lactamase inhibitors, (2) spectrum of antibiotic substrates (Bush-Jacoby-Medeiros classification, groups 1 to 4), or (3) primary molecular structure (i.e., Ambler classification: serine β-lactamases, which are class A, C, or D; and metallo-β-lactamases, which are class B). Bush group 2 corresponds to Ambler classes A and D, which are encoded frequently by genes carried on plasmids and easily spread among different species, whereas Bush groups 1 and 3 (Ambler classes C and B, respectively) are frequently encoded by chromosomal genes and therefore confined to certain species.[84,241] Chromosomally mediated, inducible Bush group 1 β-lactamases that are produced by certain species of *Citrobacter*, *Enterobacter*, *Morganella*, *Pseudomonas*, and *Serratia* are not inhibited by the β-lactamase inhibitors, and, consequently, they remain resistant to the accompanying β-lactam. However, *Bacteroides*, *Klebsiella*, *Legionella*, and *Moraxella* spp. produce chromosomally mediated β-lactamases that are effectively inactivated. Clavulanic acid and tazobactam are more potent inhibitors of β-lactamases. Furthermore, clavulanate induces Bush group 1 enzymes. Disks containing β-lactamase inhibitors can be used in the clinical microbiology laboratory to detect pathogens that produce Ambler class A ESBLs of the TEM and SHV types that may be overlooked otherwise.[58]

The ESBLs are classified in the Ambler class A and the functional (Bush group) 2be. ESBLs are present in various members of the Enterobacteriaceae family, particularly *E. coli* and *K. pneumoniae*, but they are also found in nonfermentative gram-negative bacteria, including *P. aeruginosa* and *A. baumannii*. Traditionally, β-lactamases derived mainly from the type A (Ambler) or 2be (Bush) groups, most commonly TEM (1 and 2) and SHV (1), derived by point mutations of the parent enzymes. These β-lactamases degrade penicillin, ampicillin, antipseudomonal penicillins, and narrow-spectrum cephalosporins (i.e., cefazolin, cephalothin, cefamandole, and cefuroxime). They are inhibited by clavulanic acid, and this allows for laboratory detection of these enzymes. Another type of class A ESBL is the expanded-spectrum CTXM, which is becoming more prevalent, particularly in *E. coli* and *K. pneumoniae*. It preferably hydrolyzes cefotaxime in contrast to ceftazidime and degrades aztreonam and the same products hydrolyzed by TEM and SHV; for some enzymes cefepime may be a substrate. More than 50 enzymes on the CTXM group have been identified and they can be divided into five groups on the basis of amino acid changes (i.e., CTXM1, CTXM2, CTXM8, CTXM9, and CTXM25). This enzyme has been disseminated on plasmids and it also inhibited by clavulanic acid. Carbapenemase of the type KPC (*Klebsiella pneumoniae* carbapenemases 1, 2, and 3) are also included on the Ambler class A β-lactamases and they have the same substrates hydrolyzed by CTXM as well as cephamycins and carbapenems. They are inhibited by amoxicillin-clavulanate. Ambler class B (Bush group 3) requires divalent cations, particularly Zn^{2+}, at the active site. These enzymes can be chromosomally encoded or transferred by plasmids. This group includes the IMP and VIM families, as well as GIM1 and SPM1 β-lactamase. These β-lactamases are included in the group of carbapenemases and degrade the same products hydrolyzed KPC. They are not inhibited by clavulanic acid. Ambler class C (Bush group 1) β-lactamases are encoded by chromosomes. They are referred as AmpC and degrade substrates of the TEM and SHV, oxyimino-cephalosporins (i.e., cefotaxime, cefpodoxime, ceftazidime, and ceftriaxone), monobactams, and cephamycins (i.e., cefotetan and cefoxitin). These enzymes are not inhibited by clavulanic acid. The OXA family β-lactamase (Ambler class D) has higher rates of hydrolysis for oxacillin in contrast to penicillin and also degrades substrates of CTXM. These enzymes are resistant to β-lactamase inhibitors. Plasmid-encoded OXA enzymes have been found in *P. aeruginosa*.[98,152,257]

Laboratory detection of ESBL includes phenotypic and genotypic tests. Phenotypic tests include screening tests by testing for resistance to cefpodoxime (hydrolyzed by all TEM, SHV, and CTXM ESBLs), cefotaxime, ceftazidime, ceftriaxone, and aztreonam. Then a confirmatory step is performed by demonstrating synergy between these agents and clavulanic acid (double-disc synergy test, combination disc method, or ESBL E tests). Genotypic tests consist of polymerase chain reaction amplification of specific genes.[98]

Treatment of ESBL-producing organisms represents a challenge. In vitro sensitivity may not correlate with effective treatment in vivo. For organisms producing TEM and SHV-type ESBL cefepime and piperacillin-tazobactam may appear susceptible in vitro but may not be susceptible if the inoculum is increased to 10^5 to 10^7 organisms. In these circumstances it may be difficult to ensure good concentrations of the antibiotic at the site of the infection (above the MIC). A carbapenem remains the antibiotic of choice for treatment of ESBL-producing organisms. AmpC-producing strains are typically susceptible to carbapenems; however, diminished porin expression can make it a carbapenem-resistant strain. Bacteria expressing IMP, VIM, or OXA-type carbapenemases usually remain susceptible to aztreonam. Care should be taken in selecting the correct antibiotic because resistance to fluoroquinolones and aminoglycosides can occur.[152] Promising alternatives include the recently approved tigecycline, a novel glycylcycline antibiotic (which shows in vitro activity against ESBL-producing *E. coli* and *K. pneumoniae*).

Vancomycin

Vancomycin, first isolated from *Amycolatopsis orientalis* (formerly called *Streptomyces*, then *Nocardia*) in soil samples from Borneo, is a high-molecular-weight, complex, soluble glycopeptide.[246] Because of a lack of adequate therapy for penicillinase-producing staphylococci, vancomycin was approved expeditiously by the

FDA in 1956 before exhaustive pharmacologic and toxicologic studies had been performed. Original preparations contained fermentation by-products that caused significant toxicity. Once the penicillinase-resistant penicillins were developed, vancomycin no longer was indispensable until the emergence of methicillin-resistant staphylococci in the late 1970s.

Mechanisms of Action

Vancomycin is slowly bactericidal against most susceptible gram-positive bacteria, except enterococci, for which it is bacteriostatic. It forms complexes with the D-alanyl-D-alanine portion of peptide precursor units and thus prevents polymerization of the phosphodisaccharide pentapeptide lipid complex and cross linking of peptidoglycan during the second stage of cell wall synthesis. Because the site of action of vancomycin is distinct from that of β-lactam antibiotics, no cross resistance among the drugs and no competitive inhibition exist. Like penicillin, vancomycin exerts its antimicrobial effect on bacteria in the active growth phase. Additionally, vancomycin alters cytoplasmic membrane permeability and impairs RNA synthesis. Animal infection models suggest that the drug exhibits time-dependent bactericidal activity against susceptible organisms, as well as a moderately long in vitro postantibiotic effect. The most important pharmacokinetic and pharmacodynamic parameter that correlates with antimicrobial efficacy is the ratio of the 24-hour AUC to the MIC (24-hour AUC/MIC).[80] A lag phase before the onset of rapid killing has been demonstrated in serum killing studies.[3]

Mechanisms of Resistance

Resistance can be categorized into three types: tolerance, acquired resistance, and inherent resistance. Some susceptible gram-positive bacteria, especially enterococci, are tolerant to the bactericidal activity of vancomycin; that is, vancomycin inhibits but does not kill the bacteria (MBC/MIC ratio >32). Staphylococci tolerant to vancomycin can have autolysin deficiencies. Although most resistance to vancomycin by gram-positive bacteria is acquired, three genera of gram-positive organisms are inherently resistant to vancomycin: *Erysipelothrix*, *Leuconostoc*, and *Pediococcus*. Five genes code for acquired vancomycin resistance in enterococci: *VanA*, *VanB*, *VanC*, *VanD*, and *VanE*.[220] The *VanA* gene that encodes the VanA phenotype (vancomycin MIC >256 and teicoplanin resistance) is on a plasmid that is easily transferable by conjugation to other enterococci. The VanB phenotype also is transferable and encodes vancomycin but not teicoplanin resistance. The presence of an inducer such as vancomycin or teicoplanin, another glycopeptide that has not been approved for use in the United States, activates the transcription of genes that encode ligases necessary for resistance to vancomycin. Some enzymes make cell wall precursors ending in D-alanyl-D-lactate located at the end of the pentapeptide side chain, to which vancomycin binds with very low affinity. Other enzymes inhibit or alter the synthesis of endogenous cell wall precursors ending in D-alanyl-D-alanine, to which vancomycin is unable to bind. The finding that vancomycin resistance genes can be transferred experimentally from vancomycin-resistant enterococci (VRE) to staphylococci highlights the potential risk for spread of resistance by means of naturally occurring plasmid or transposon-mediated conjugative systems.[220] Although vancomycin resistance can affect susceptibility to other investigational glycopeptides, no cross resistance exists between vancomycin and other unrelated antibiotics. Resistance to vancomycin rarely develops during appropriate therapy, perhaps because of its multiple mechanisms of action, but prolonged or indiscriminate use of the drug can contribute to selective pressure and result in the development of vancomycin-resistant enterococci colonizing the gut. Of great concern is the emergence of invasive strains of multidrug-resistant enterococci (≤14% of health care–associated enterococcal strains)[199] and staphylococci, for which only a few therapeutic options exist, including linezolid and other antimicrobials not approved for use in children such as quinupristin-dalfopristin, daptomycin, and tigecycline (a novel glycylcycline antibiotic). *VanC* genes are expressed constitutively in *Enterococcus gallinarum*, conferring low-level vancomycin resistance. This organism remains susceptible to β-lactams (ampicillin). The first strains of *S. aureus* with intermediate resistance to vancomycin/glycopeptide (VISA/GISA) were detected in the United States in 1997. Soon thereafter, three cases of vancomycin-resistant *S. aureus* were isolated. The mechanism for this high-level vancomycin resistance involves the horizontal transfer of a transposon containing *VanA* and associated genes from vancomycin-resistant enterococci.[259] Glycopeptide-resistant isolates were found to have thicker extracellular matrices, a characteristic that may cause vancomycin to become trapped in the outer layers of the cell wall and may limit access to the cytoplasmic membrane where the functional targets are located. Pneumococci tolerant to vancomycin were described first in 1999.[235] These strains escape lysis and killing by vancomycin because of an alteration in the regulation of autolysin. Their prevalence is 4 to 10 percent of nasopharyngeal and invasive isolates, and they are associated with increased mortality when etiologic in meningitis.[266] In 2006, the CLSI modified the MIC breakpoints for *S. aureus* to vancomycin: from 4 μg/mL or less to 2 μg/mL or less for "susceptible," from 816 μg/mL to 48 μg/mL for "intermediate," and from 32 μg/mL or greater to 16 μg/mL or greater for "resistant." This change was made to increase the detection of heterogeneously resistant isolates and based on clinical data showing less response to vancomycin when the MIC is 4 μg/mL or greater.[297] The CDC recommends that the use of vancomycin be restricted appropriately to reduce the risk for inducing vancomycin resistance.[63]

In Vitro Activity

The in vitro spectrum of activity for vancomycin is limited to gram-positive aerobic and anaerobic bacteria, with little, if any, activity against aerobic or anaerobic gram-negative bacilli, except *Chryseobacterium meningosepticum.* Group A streptococci, pneumococci, *Corynebacterium*

spp., and *C. difficile* are highly susceptible to vancomycin, whereas *L. monocytogenes*, microaerophilic and anaerobic streptococci, enterococci, coagulase-positive and coagulase-negative staphylococci, Bacillus anthracis and other spp., *Lactobacillus*, *Actinomyces*, and other *Clostridium* spp. have higher MIC values that are still in the susceptible range. Pathogens resistant to vancomycin include *Erysipelothrix* and *Leuconostoc* and *Pediococcus* spp., which can cause serious infections in immunocompromised patients. The bactericidal activity of vancomycin combined with gentamicin has been shown to be synergistic in vitro against strains of enterococci (provided there is no high-level gentamicin resistance; MIC >2000 μg/mL), nonenterococcal group D streptococci, viridans streptococci, and most methicillin-susceptible *S. aureus* and MRSA. Addition of rifampin, gentamicin, or both, results in improved cure rates for *S. epidermidis* prosthetic valve endocarditis.[199] Although vancomycin has activity against many gram-positive bacteria, it is not the most active agent for these organisms, but it is the drug of choice for multidrug-resistant bacteria such as methicillin-resistant staphylococci and highly penicillin-resistant and cephalosporin-resistant pneumococci.

Pharmacokinetics

Intravenous administration of vancomycin is preferred because intramuscular injection causes pain and tissue necrosis. Intravenous preparations must be diluted further in normal saline or dextrose solutions before beginning slow infusion. Vancomycin is approximately 55 percent bound to serum proteins and diffuses well into most body tissues, with adequate concentrations achieved in pericardial, pleural, ascitic, bone, and synovial fluids, but not in aqueous humor or bile. Vancomycin does not diffuse well into CSF in the absence of inflamed meninges, but CSF concentrations of 7 to 21 percent of concomitant serum levels can be achieved during therapy for meningitis when higher doses (15 mg/kg every 6 hours) are administered. The bone-to-serum ratio of vancomycin is 10 percent, but this figure increases up to 30 percent in infected bone. Vancomycin remains active at pH 6.5 to 8, and concentrations achieved in abscess fluid approximate serum levels.[199] Intrathecal or intraventricular administration has been used infrequently for CNS infections that are difficult to eradicate.[198] Vancomycin is not metabolized significantly and is excreted by glomerular filtration. The mean serum elimination half-life in adults with normal renal function is 6 hours. For children, it ranges from 5 to 10 hours in newborns and 4 hours in older infants to 2 to 3 hours in children. In anephric patients, the elimination half-life extends to 7 or more days. Hemodialysis removes small amounts of vancomycin, whereas peritoneal dialysis may reduce serum concentrations by approximately 40 percent. Nomograms and patient-individualized bayesian dosing regimens have been used for vancomycin dosing in renal failure in adults.[182,214]

Current recommendations for vancomycin monitoring in adult patients include checking trough concentrations rather than peak values as the most accurate method for vigilance of efficacy and toxicity. Trough values should be checked before the fourth dose of the antibiotic (steady state). Minimal concentration should be maintained above 10 mg/L, and target concentrations of 15 to 20 mg/mL are preferred for complicated infections secondary to *S. aureus* (bacteremia, endocarditis, osteomyelitis, meningitis, and hospital-acquired pneumonia).[268]

Indications for Use

Because of the potential for development of resistance, vancomycin should be reserved for patients with moderate-to-severe infections caused by vancomycin-susceptible bacteria that are resistant to other antibiotics. Vancomycin can be used for the treatment of infections caused by β-lactam–susceptible and vancomycin-susceptible bacteria in patients with hypersensitivity reactions to β-lactam antimicrobial agents. Empirical therapy for patients with ventricular shunt–related and catheter-associated infections frequently includes vancomycin to provide activity against coagulase-negative staphylococci. Vancomycin can be used in combination with gentamicin, rifampin, or both, for bactericidal synergistic activity to treat documented prosthetic device–related *S. epidermidis* infections, methicillin-resistant staphylococcal endocarditis, or endocarditis caused by high-level penicillin-resistant, aminoglycoside-susceptible strains of enterococci. *S. epidermidis* infections of long-term intravenous catheters usually can be cured without removal of the device.[199] In addition, vancomycin is the drug of choice in patients with immediate-type hypersensitivity to penicillins and other β-lactam antibiotics for prophylaxis (e.g., in gastrointestinal or genitourinary procedures) or treatment of endocarditis caused by streptococci, enterococci, and staphylococci.[20,83] Empirical treatment of bacterial meningitis suspected or proved to be caused by *S. pneumoniae* in children 1 month of age or older should consist of vancomycin in addition to a third-generation cephalosporin until susceptibility data are available; if cephalosporin resistance is documented, rifampin should be added to the regimen. However, vancomycin need not be used if compelling evidence implicates pathogens other than *S. pneumoniae*, such as the observation of gram-negative diplococci on a CSF smear. Vancomycin also is indicated for the treatment of infections with penicillin-resistant streptococci, *C. jeikeium*, and *Bacillus* spp., penicillin-resistant enterococci, as well as for bacteremia, pneumonia, cellulitis, and osteomyelitis caused by methicillin-resistant staphylococci. Some experts advocate the addition of nafcillin, oxacillin, gentamicin, or rifampin for optimal coverage of oxacillin-susceptible *S. aureus* in severely ill patients who are receiving empirical treatment with vancomycin because certain strains of staphylococci are tolerant to vancomycin.[123] Proof of enhanced efficacy with combination therapy in this setting is lacking. Although nonabsorbable oral vancomycin is effective therapy for *C. difficile* colitis, oral metronidazole represents first-line therapy; vancomycin should be given only for metronidazole treatment failures, to limit the development of vancomycin-resistant enterococci. Vancomycin is among

the agents of choice for the treatment of meningitis caused by *C. meningosepticum*.

Adverse Effects

The purification of vancomycin allegedly decreased the frequency of adverse reactions noted several decades ago. The most common adverse effect is "red man" or "red neck" syndrome or glycopeptide-induced anaphylactoid reaction manifested as flushing of the face and upper part of the trunk, pruritus during vancomycin infusion, angioedema, and, rarely, hypotension. Vancomycin directly causes release of histamine from mast cells by non–immune-mediated mechanisms. Because it is a dose- and rate-dependent reaction, administering doses less than 500 mg, prolonging the infusion period to at least 1 hour, or both, decreases the risk for occurrence. Pretreatment with histamine-1–receptor antagonists (i.e., diphenhydramine or hydroxyzine) prevents the development of this reaction, and symptoms usually resolve promptly after discontinuation of the infusion.

Controversy surrounds the issue of vancomycin-induced ototoxicity and nephrotoxicity.[54] Such toxicity has not been demonstrated in experimental animal models. Although numerous cases of toxicity in humans have been reported, the literature is difficult to interpret because of confounding variables, including recent or concurrent use of aminoglycosides or other ototoxic or nephrotoxic agents, lack of identification of antecedent otologic or renal disease, and inconsistencies in sampling methods when measuring serum vancomycin concentrations. Tinnitus and high-tone hearing loss have been associated with the administration of vancomycin, particularly when the peak serum levels exceed 50 µg/mL. If vancomycin is ototoxic, whether toxic peak or toxic trough serum concentrations are responsible is not clear. Several studies suggest an increased risk for nephrotoxicity when aminoglycosides are used concurrently with vancomycin, especially when they are administered for longer than 21 days.[120] Other adverse effects seen occasionally with the use of vancomycin include reversible neutropenia, thrombocytopenia, drug fever, and macular rash.

The usefulness of serum vancomycin concentration determinations is controversial because a definitive relationship between vancomycin concentration and either adverse effects or clinical outcome has not been proved.[59,61,213] Until the significance of vancomycin serum concentration determinations has been clarified, monitoring has been suggested for the following clinical situations only: (1) patients with altered renal function, including premature infants; (2) patients receiving larger than normal dosages, especially those with meningitis in whom adequate CSF values are necessary; (3) anephric patients undergoing hemodialysis (to avoid subtherapeutic vancomycin concentrations during prolonged dosing intervals); and (4) patients receiving concomitant therapy with nephrotoxic agents.[213] Because serum vancomycin values commonly are subtherapeutic in the first days of treatment for severe sepsis, presumably because of larger volumes of distribution of vancomycin in these ill children, we recommend an initial dosage of 60 mg/kg/day in four divided doses.

Daptomycin

Daptomycin is a cyclic lipopeptide derived from the fermentation of *Streptomyces roseosporus*. It has been approved for treatment of adults with complicated skin and soft tissue infections, endocarditis, and sepsis. It is a parenteral lipopeptide that disrupts cell membrane potential, and, unlike vancomycin, it is rapidly bactericidal against MRSA strains. Daptomycin's mechanism of action involves a calcium-dependent insertion into the bacterial cell membrane resulting in rapid depolarization with resulting cell death, without cell lysis, in a concentration-dependent activity. It is administered once daily. Pediatric experience is limited, with few case reports and case series published in children. A retrospective review of 16 pediatric cases with invasive gram-positive bacterial infections (MRSA, $n = 14$; MSSA, $n = 1$; and vancomycin-resistant *Enterococcus* [VRE], $n = 1$) showed clinical improvement after the addition of daptomycin to conventional therapy in patients with persistent bacteremia and severe clinical disease.[11] A pharmacokinetic analysis evaluated single-dose daptomycin for children 2 to 6 years of age. A dose of 8 to 10 mg/kg was shown to be well tolerated and reached appropriate systemic exposure, comparable to that in adults receiving doses of 4 to 6 mg/kg.[1] Daptomycin is active against *S. aureus* (including MRSA, MSSA, and GRSA), streptococci, and *Enterococcus* spp. This antibiotic is not effective in cases of pneumonia because it is inactivated by surfactant. Side effects of daptomycin include prolongation of prothrombin time and myopathy, with elevation of the serum creatinine kinase, which should be monitored weekly during treatment.

Telavancin

Other semisynthetic glycopeptides approved for adults include telavancin and dalbavancin. They may have some advantage over vancomycin and daptomycin in that resistance of MRSA is less likely because of their dual mechanisms of action. Telavancin was approved by the FDA in 2009 for treatment of complicated skin and skin structure infections caused by susceptible gram-positive bacteria in adults. It is a lipoglycopeptide that contains a hydrophobic side chain linked to the vancosamine sugar and a hydrophilic group on the 4 position of amino acid 7. The hydrophobic chain provides improved binding activity for D-alanine-D-alanine–containing peptidoglycan intermediates. Its dual mechanism of action includes binding the peptidoglycan precursor at the D-alanine-D-alanine terminus with subsequent inhibition of transglycosylation (peptidoglycan polymerization) and then inhibition of transpeptidation. The second mode of action includes binding to the cell membrane and triggering rapid concentration-dependent dissipation of cell membrane potential with leakage of cytoplasmic adenosine triphosphate (ATP) and potassium loss. Telavancin is active

against MSSA, MRSA, GISA, hGISA, coagulase-negative staphylococci, streptococci (including group A and group B β-hemolytic streptococci and *S. pneumoniae*), *Clostridium* spp., and *Actinomyces israelii*. It does not have activity against VRE (both VanA and VanB). It has high level of protein binding (~93%) and small volume of distribution. It is not inactivated by surfactant. Telavancin is mainly eliminated by renal excretion.[275]

COLISTIN (COLISTIMETHATE SODIUM: POLYMYXIN E)

Colistin is a cationic, cyclic polypeptide antibiotic that is structurally and pharmacologically related to polymyxin B. Colistin is less active in vitro and less toxic than is polymyxin B. Colistin was discovered in 1949 and is obtained from cultures of *Bacillus polymyxa* subsp. *colistinus*. The polymyxins have been used extensively for decades in topical ophthalmic and otic solutions. However, because of reports of common and serious nephrotoxicity and neurotoxicity, parenteral use of these drugs was abandoned in the early 1980s, except for treatment of multidrug-resistant, gram-negative bacterial infections in children and adults with cystic fibrosis. The emergence of gram-negative bacteria resistant to most classes of antibiotics and the lack of effective new antimicrobial agents led to the reconsideration of colistin as a valuable therapeutic option. Studies of patients who received intravenous polymyxins for the treatment of serious multidrug-resistant *P. aeruginosa* and *A. baumannii* infections, including pneumonia, bacteremia, and urinary tract infections, led to the conclusion that these antibiotics have acceptable effectiveness and considerably less toxicity than was reported previously.[167]

Mechanisms of Action

Colistimethate sodium is inactive until it is hydrolyzed to sulfomethylated derivatives and colistin, which acts as a cationic detergent with both lipophilic and lipophobic components. It damages the cytoplasmic membrane by displacing calcium and magnesium and binding to anionic lipopolysaccharide molecules and leads to permeability changes in the cell membrane, leakage of osmotically active intracellular metabolites and nucleosides, and cell death. Furthermore, colistin binds and neutralizes the lipid A portion of lipopolysaccharide in vitro; however, the clinical implications of this antiendotoxin activity are unknown.[99]

Mechanisms of Resistance

Gram-negative bacteria can develop resistance to colistin by means of inherited or acquired mechanisms. Inherited mutations occur infrequently and are not influenced by exposure to colistin. Acquired resistance rarely may be induced by prolonged exposure to the drug. Studies of polymyxin-resistant *P. aeruginosa* suggest that the development of resistance arises from modifications of the cell membrane, such as reductions in lipopolysaccharide, certain proteins or lipids, or magnesium or calcium. Currently, no evidence of efflux pump or enzyme-mediated resistance exists. Complete cross resistance occurs between colistin and polymyxin B, but not other antimicrobial agents.

In Vitro Activity

Colistin is highly active against many strains of aerobic gram-negative bacteria, including *Acinetobacter*, *Citrobacter*, *E. coli*, *Enterobacter*, *H. influenzae*, *Klebsiella*, *Morganella morganii*, *P. aeruginosa*, *Salmonella*, *Shigella*, and variable numbers of *Stenotrophomonas* strains. However, the antibiotic is inactive against most strains of *Brucella*, *Burkholderia cepacia*, *Edwardsiella*, *Proteus*, *Providencia*, *Serratia*, gram-negative cocci, gram-positive bacteria, and anaerobic bacteria. The CLSI defined bacterial resistance to colistin as an MIC of 4 μg/mL or greater. However, the sulfate form of colistin, which usually is used for in vitro testing, often is fourfold to eightfold more active than the sulfomethyl form, which is used in the parenteral formulation. Limited in vitro and clinical data suggest that synergism exists with colistin and other antimicrobial agents such as aminoglycosides, antipseudomonal β-lactams, fluoroquinolones, rifampin, and TMP-SMX.[99,167]

Pharmacokinetics

The pharmacologic evaluation of the polymyxins was performed decades ago. Consequently, pharmacokinetic and pharmacodynamic information is limited. Colistin is not absorbed from the gastrointestinal tract and must be given parenterally. The drug is distributed widely in the body, except for bones and synovial, pleural, and pericardial fluids. In a reported case of multidrug-resistant *A. baumannii* meningitis in an adolescent, parenterally administered colistin achieved a concentration in the CSF that was 25 percent of the serum concentration, and it cured the infection.[155] More than 50 percent of the agent binds to serum proteins. Colistimethate sodium and its metabolites are excreted mainly by the kidneys through glomerular filtration. Therefore, the dosage and frequency of administration should be decreased in children with renal impairment. Colistin is rapidly bactericidal against susceptible bacteria in a concentration-dependent manner, and it has a postantibiotic effect.[99,167] Considering these pharmacologic parameters, there may be a theoretical benefit in administering colistin less frequently with higher doses, but no clinical data validate this hypothesis.

Indications for Use

Colistin has been administered intravenously to infants and children of all ages for the treatment of acute or chronic infections caused by susceptible gram-negative organisms, particularly multidrug-resistant strains of *Pseudomonas* and *Acinetobacter* in patients with cystic fibrosis or complicated health care–associated infections. Colistin should be reserved for use when other more

effective and less toxic antimicrobial agents are contraindicated or ineffective. The dosage of colistimethate sodium is expressed in terms of colistin. The usual parenteral dosage for adults and children with normal renal function is 2.5 to 5 mg/kg (75,000 to 150,000 IU/kg)/day divided into two to four doses, depending on the severity of infection (maximum, 300 mg/day). Intramuscular administration is not recommended because it causes severe pain at the injection site. Experience with the use of colistimethate sodium administered by nebulization for multidrug-resistant, gram-negative respiratory infections in patients with cystic fibrosis is extensive. Successful use of colistimethate sodium by the intraventricular route also has been reported. Colistin sulfate in combination with neomycin sulfate and hydrocortisone acetate is available in the United States for otic use. Colistin sulfate for oral administration no longer is available in the United States. Polymyxin B sulfate is available for topical use.[99]

Adverse Effects

Nephrotoxicity and neurotoxicity are the most serious adverse effects and occur most frequently when the drug is administered in higher than recommended doses, in patients with renal insufficiency, or in combination with other nephrotoxic agents. Progressive azotemia or acute tubular necrosis may occur, but renal dysfunction usually is reversible when the antibiotic is discontinued. Renal function should be monitored closely during therapy, and colistin should be discontinued immediately if urine output diminishes or concentrations of blood urea nitrogen or creatinine increase. Transient effects on the nervous system, including dizziness, weakness, paresthesia, numbness, vertigo, ataxia, blurred vision, and slurred speech may occur, but these symptoms are reversible and may be alleviated by reducing the dosage of colistin. Coma, psychosis, seizures, and noncompetitive neuromuscular blockade causing apnea also have been reported and warrant prompt discontinuation of the drug.

AGENTS THAT TARGET PROTEIN SYNTHESIS

Aminoglycosides

Aminoglycosides are natural and semisynthetic compounds that consist of at least two amino sugars bound by a glycosidic linkage to a hexose nucleus, the aminocyclitol ring. A more appropriate name would be aminoglycosidic aminocyclitols. Streptomycin, isolated from *Streptomyces griseus*, was the first aminoglycoside and was available for use in 1944. Many aminoglycosides have been isolated or developed since that time. The suffix denotes the origin of the aminoglycoside—those ending with the suffix *mycin* were derived from *Streptomyces* spp., whereas those ending with *micin* were derived from *Micromonospora* spp. Currently, eight aminoglycosides are approved for use in the United States (Table 235-7), and several more are available in other countries. Despite the development of less toxic antibiotics with broad-spectrum activity, aminoglycosides continue to fulfill an essential role in the treatment of severe infections caused by aerobic gram-negative bacilli and enterococci.

TABLE 235-7 **Classification Scheme for Aminoglycosides**

Aminocyclitol Ring	Family	Member
Streptidine	Streptomycin	Streptomycin
2-Deoxystreptamine	Kanamycin	Kanamycin Amikacin Tobramycin
2-Deoxystreptamine	Gentamicin	Gentamicin Netilmicin
2-Deoxystreptamine	Neomycin	Neomycin Paromomycin

Mechanisms of Action

Against susceptible bacteria, aminoglycosides demonstrate rapid, concentration-dependent bactericidal activity.[97,310] They exert their effect by binding irreversibly to the 30S subunit of the bacterial ribosome, which results in inhibition of protein synthesis and induction of translational errors. Bacterial uptake of aminoglycosides can be facilitated by concomitant therapy with cell wall–active antibiotics such as β-lactams or vancomycin. Penetration of the outer membrane of gram-negative bacteria is mediated by a self-promoted uptake process involving aminoglycoside-induced disruption of magnesium bridges between adjacent lipopolysaccharide molecules. Passage through porin channels is unlikely because of the large size of aminoglycosides. Subsequent transport into cytoplasm and attachment to the 30S ribosomal subunit require two energy and oxygen-dependent steps, energy-dependent phases I and II (EDPI and EDPII), that use an electrochemical proton gradient. EDPI is inhibited by hyperosmolarity, low pH, and anaerobic conditions. Unlike other protein synthesis inhibitors, which usually are bacteriostatic, aminoglycosides are bactericidal. Binding of aminoglycosides to the 30S subunit does not prevent formation of the initiation complex of peptide synthesis. However, it disrupts elongation of the peptide chain by impairing the proofreading process, an impairment that results in translational inaccuracy. Aberrant protein products may be inserted in the cell membrane and cause altered permeability. The primed amino sugar and 2-deoxystreptamine groups are essential for the ribosome-specific activity of aminoglycosides.[211]

Mechanisms of Resistance

The prevalence of acquired aminoglycoside resistance is relatively low, and its development during therapy is an unusual event. Bacteria can acquire resistance to aminoglycosides because of alterations in the bacterial target, reduced bacterial cell permeability or uptake, or modification of the antibiotic by bacterial enzymes, the last being most significant clinically.[86] Mutations in the

aminoglycoside-binding site of the 30S ribosome have been associated with high-level resistance to streptomycin but not to other aminoglycosides, possibly because, unlike in streptomycin, they bind to multiple sites on the ribosome. Facultative aerobic bacteria causing infection in sites with reduced oxygen tension, anaerobic bacteria, and such fermentative bacteria as streptococci are inherently resistant to aminoglycosides because they are unable to generate an electrochemical proton gradient sufficient for aminoglycoside transport into the cytoplasm. Other bacteria can acquire resistance because of reduced permeability or lack of transport, as demonstrated in staphylococci, in which resistance to aminoglycosides quickly develops when monotherapy is administered. *P. aeruginosa*, other nonfermenting gram-negative bacilli, and, less frequently, members of the Enterobacteriaceae can acquire a moderate level of resistance to all aminoglycosides as a result of various mechanisms that interfere with uptake, cytoplasmic transport, or regulation of the anaerobic respiratory pathway. Plasmid-mediated production of aminoglycoside-modifying enzymes is the most common mechanism of acquired resistance. Many enzymes have different substrate specificities, several of which can be elaborated simultaneously in the same bacterium. These acetyltransferases, nucleotidyltransferases, and phosphotransferases interact with amino or hydroxyl groups on the aminoglycoside and modify the aminoglycoside so that it binds poorly to the 30S ribosome, thereby usually resulting in high-level resistance.[211] Gentamicin and tobramycin are each susceptible to at least five modifying enzymes, whereas amikacin possesses an aminohydroxybutyryl group that prevents enzymatic modification at multiple sites. Consequently, more than 80 percent of gentamicin-resistant strains of Enterobacteriaceae and 25 to 85 percent of gentamicin-resistant *P. aeruginosa* strains are susceptible to amikacin.[178]

In Vitro Activity

The in vitro antibacterial spectrum of aminoglycosides includes a wide range of aerobic gram-negative bacilli, many oxacillin-susceptible staphylococci, many enterococci, and some mycobacteria.[212] Some gram-negative bacilli, including *B. cepacia* and *S. maltophilia*, are consistently resistant to all aminoglycosides. The spectra of activity of gentamicin, tobramycin, and netilmicin are similar, and strains resistant to one usually are resistant to the others. The major advantage of tobramycin currently is its activity against some strains of *Acinetobacter* and indole-positive Proteae (*Morganella* spp., *Proteus* spp., *Providencia* spp.) that are resistant to gentamicin.[258] Because many aminoglycoside-modifying enzymes are active against gentamicin, tobramycin, and netilmicin but inactive against amikacin, amikacin frequently is prescribed for empirical treatment of nosocomial gram-negative bacillary infections. Amikacin also has activity against the *Mycobacterium avium* complex (MAC), some rapidly growing mycobacteria, and *Nocardia asteroides*. Netilmicin is less active than other aminoglycosides against *P. aeruginosa*, but it can have activity against some gentamicin-resistant, aerobic, gram-negative bacilli. Although gentamicin, tobramycin, amikacin, and netilmicin have similar spectra of activity, susceptibility testing is recommended because of geographic and interhospital variation in resistance patterns. Lack of activity against *P. aeruginosa*, *Klebsiella*, and *Serratia* has limited the use of kanamycin. Streptomycin is inactive against many gram-negative enteric bacilli but does have activity against *Francisella tularensis*, *Yersinia pestis*, and *Mycobacterium tuberculosis*.

Pharmacokinetics

Aminoglycosides have in common many pharmacokinetic characteristics. They are highly polar, water-soluble compounds that are positively charged cations at neutral pH. Their antibacterial activity is pH-dependent, with increased activity at higher pH. They are relatively resistant to degradation at various temperatures and pH values. After parenteral administration, aminoglycosides are distributed rapidly in extracellular body water, with slow accumulation in tissues. The volume of distribution is decreased (with respect to total body weight) in obese patients and is increased in patients with illnesses associated with edema, such as severe infections, burns, congestive heart failure, and ascites. With the exception of proximal renal tubular cells and possibly inner ear hair cells, penetration into other body compartments is impaired because of lipid insolubility, polycationic charge, and size of the aminoglycoside. Proximal renal tubular cells absorb aminoglycosides through carrier-mediated pinocytosis, and, as a result, renal cortical concentrations exceed those in plasma. Aminoglycosides do not penetrate the blood-brain barrier in the absence of meningeal inflammation, but with inflammation, approximately 20 to 25 percent of the serum concentration penetrates the CSF. These CSF concentrations are low in relation to the MIC of the meningeal pathogen for which it is given. Thus, because of this narrow therapeutic-toxic index, monotherapy for gram-negative bacillary meningitis with aminoglycosides is not recommended. Aminoglycosides are not metabolized and, after parenteral administration, are excreted unchanged in the kidney by glomerular filtration, with approximately 5 percent of excreted drug reabsorbed in the proximal tubular cells. Minimal amounts are excreted in saliva and feces. Urine concentrations exceed those in plasma by 25 to 100 times.

After intramuscular injection, aminoglycosides are absorbed completely, and peak serum concentrations are achieved within 90 minutes, except in some disease states that interfere with tissue perfusion, such as hypotension. When administered intravenously, the infusion should be given slowly over the course of 30 to 60 minutes to avoid the development of potential adverse effects. Peak serum concentrations are achieved within 30 to 60 minutes after infusion. Because of the polar nature of these drugs, absorption after oral administration is insignificant and inadequate to treat systemic infections, but aminoglycosides can accumulate in the presence of renal failure and result in concentrations sufficient to cause toxicity. Although aminoglycosides are nonirritating when they are instilled into pleural or peritoneal spaces, their absorption is rapid and can result in significant toxicity. In contrast, instillation into the lateral ventricles or

irrigation of the bladder has not been associated with significant systemic absorption. In contrast to parenteral administration, aerosol administration of aminoglycoside results in higher concentrations in bronchial secretions and less toxicity.[254] Indiscriminate use of topical aminoglycosides in the form of ointments, creams, or ophthalmic solutions may lead to the rapid development of aminoglycoside resistance, especially in immunocompromised patients.

Antimicrobial dosing in newborns and young infants differs from that in older children and adults because of developmental changes in renal function and increased total body water composition. Neonates and young infants have a larger volume of distribution and a reduced glomerular filtration rate; these differences are more pronounced in premature neonates with very low birthweight.[251] Therefore, twice-daily regimens have been the standard of care in newborn infants for years. Once-daily gentamicin dosing of 4 mg/kg in term neonates has been studied extensively and is used routinely in many nurseries; no differences in effectiveness or toxicity have been observed.[310] The aminoglycoside dosing schedule currently approved for use in older infants and children is a three-times-daily regimen. Because aminoglycosides demonstrate concentration-dependent bactericidal activity, higher peak serum concentrations result in more extensive and more rapid killing.[310] Aminoglycosides also demonstrate a postantibiotic effect against susceptible aerobic gram-negative bacilli in vitro and in vivo.[321] Furthermore, host leukocytes display enhanced phagocytosis of aminoglycoside-exposed bacteria in vitro, referred to as *postantibiotic leukocyte enhancement*.[106] The higher the peak serum concentration, the longer is the duration of the postantibiotic effect. In the presence of normal renal function, the serum concentration is low or undetectable before the administration of the next dose (trough value), but antibacterial activity persists because of the postantibiotic effect. These characteristics favor single-daily dosing. Numerous studies in adults have shown that once-daily dosing of aminoglycosides (i.e., amikacin, gentamicin, tobramycin, or netilmicin) is either equivalent or superior to multiple-daily dosing with regard to clinical and bacteriologic effectiveness, nephrotoxicity, ototoxicity, mortality, and cost-effectiveness.[310] The Hartford nomogram originally described by investigators from Hartford, Connecticut, commonly is used to guide extended-interval aminoglycoside dosing for adults. In adults, a single serum concentration of gentamicin or tobramycin is obtained 8 to 12 hours after the start of the infusion of the first dose and plotted on the nomogram to determine the appropriate dosing interval.[112] Similar nomograms for children have not been validated. Nonetheless, published guidelines support 7.5 mg/kg per dose of gentamicin for infants and children up to 10 years of age; for adolescents and adults, 6 to 7 mg/kg per dose is appropriate.[77,310] Extended-interval aminoglycoside dosing is contraindicated if the patient's volume status is altered (e.g., third spacing, thermal injury >20 percent total body surface area), optimal dosing regimen is predefined (e.g., in cystic fibrosis and endocarditis), renal function is impaired, or immune status is compromised.

Indications for Use

The major use of aminoglycosides in children (usually in combination with other antibacterial agents) is for serious infections caused by gram-negative enteric bacilli, including neonatal sepsis, sepsis in a child with malignant disease or an immunologic defect, abdominal and systemic infections associated with spillage of fecal contents into the peritoneum, endocarditis caused by certain susceptible bacteria, and complicated urinary tract infections. Because gentamicin is the least expensive aminoglycoside and the one with which physicians have the most experience, it often is considered the first-line agent for empirical treatment of suspected aerobic gram-negative bacillary infections in institutions with minimal background resistance. Combinations of an aminoglycoside and a cell wall–active antibiotic have been used for synergistic bactericidal activity. Gentamicin or streptomycin, in combination with penicillin G, ampicillin, or vancomycin, is recommended for the treatment of endocarditis caused by susceptible enterococci, viridans streptococci, or *Streptococcus bovis*. Administration of gentamicin for 3 to 5 days, in combination with an antistaphylococcal agent, should be considered for the treatment of staphylococcal endocarditis.[20] An aminoglycoside combined with an acylureidopenicillin, ceftazidime, cefepime, or a carbapenem is recommended for serious infections caused by *P. aeruginosa*. Amikacin often is used for empirical treatment of nosocomial aerobic, gram-negative, bacillary infections in patients in institutions having significant resistance to gentamicin and tobramycin. Streptomycin is indicated for use alone or in combination with other antibiotics for the treatment of tularemia and plague and in combination with other agents for the treatment of tuberculosis, brucellosis, and enterococcal endocarditis. For such purposes, gentamicin could be a more suitable agent for some of these conditions, such as brucellosis, tularemia, and plague. Paromomycin is too toxic for parenteral use, but when administered orally, it has been useful in the treatment of asymptomatic intestinal amebiasis, *Dientamoeba fragilis* infection, and *Giardia lamblia* infection during pregnancy. Tobramycin inhalation solution is approved by the FDA for twice-daily dosing in patients with cystic fibrosis.

Adverse Effects

Although aminoglycosides have intrinsic toxicity, allergic reactions are uncommon. All aminoglycosides can injure the proximal renal tubules, the cochlea, the vestibular apparatus, or a combination thereof and can cause neuromuscular blockade, but the risk varies with each agent. Because aminoglycosides do not induce a significant inflammatory response, pain at intramuscular injection sites and phlebitis at intravenous infusion sites are unusual. Hypersensitivity reactions and drug fever are rare events.

Many theories have been postulated regarding the mechanism of nephrotoxicity,[210] including inhibition of lysosomal phospholipases within the proximal renal tubules. Clinical findings include a mild, nonoliguric decrease in the glomerular filtration rate that typically is

reversible. In contrast to traditional dosing, once-daily dosing causes similar or lower rates of nephrotoxicity.[77,310] In humans, nephrotoxicity has been associated with prolonged duration of therapy at high dosages, previous aminoglycoside therapy, administration of drugs to critically ill patients with intravascular volume depletion or hyponatremia and to those with impaired kidney function, and concomitant administration of other potentially nephrotoxic agents such as amphotericin B and loop diuretics.[88]

The mechanism by which aminoglycosides cause vestibular and cochlear ototoxicity has not been elucidated fully. Cochlear damage is manifested as tinnitus or high-frequency hearing loss, whereas vestibular toxicity is associated with vertigo, nystagmus, and ataxia.[15] Damage can be unilateral or bilateral. Occasionally, the ototoxicity is reversible, but permanent damage occurs commonly. Mild cochlear damage may not be recognized because the high-frequency hearing range is affected first. Conventional audiograms that do not test high-frequency ranges may not detect cochlear injury. Delayed onset of high-frequency hearing loss has been observed in humans. Factors that increase the risk for development of aminoglycoside ototoxicity in humans include impaired renal function and prolonged duration of treatment. Although elevated serum peak and trough concentrations are thought to contribute to ototoxicity, a specific threshold for peak or trough concentrations has not been established. Neuromuscular blockade can occur after rapid intravenous infusion, after extensive peritoneal irrigation, or during routine parenteral aminoglycoside administration in patients with underlying conditions that affect the neuromuscular junction, such as myasthenia gravis and botulism, or during concomitant administration of agents that act on the neuromuscular junction, such as succinylcholine. Hypomagnesemia in the neonate may predispose to hearing loss.

To avoid toxicity and ensure therapeutic values, concentrations of aminoglycosides in serum should be monitored in all patients who have impaired renal function or who receive other nephrotoxic medications or prolonged treatment; such monitoring is particularly relevant to preterm, low-birth-weight infants. Monitoring also should be considered in obese and undernourished children, children with severe burns, and those with chronic disease, for which the volume of distribution of the drug can be altered (e.g., cystic fibrosis).

Macrolides

Erythromycin, isolated from *Streptomyces erythreus* found in a soil sample in the Philippines, was the first macrolide and was available for use in 1952. Many natural and semisynthetic erythromycin derivatives have been developed since then, three of which are approved for use in pediatrics: erythromycin, clarithromycin, and azithromycin. Macrolide antibiotics consist of a large lactone ring attached by a glycosidic bond to one or more amino or neutral sugar moieties. Erythromycin and clarithromycin have 14-member lactone rings, whereas azithromycin, an azalide antibiotic that is grouped with the macrolides, has a tertiary amino group inserted in its 15-member ring. In addition to similarities in chemical structure, these macrolides have similar antibacterial spectra, mechanisms of action, and mechanisms of resistance, but they differ in their pharmacokinetic characteristics. Ketolides are a new class of macrolides with in vitro activity against macrolide-resistant gram-positive organisms, including *S. pneumoniae*. Telithromycin received FDA approval in 2004 for use in adults, but use is limited to mild-to-moderate community-acquired pneumonia after increasing reports of hepatotoxicity.[325]

Mechanisms of Action

Macrolide antibiotics reversibly bind to the 50S ribosomal subunit and inhibit protein synthesis. Initial studies suggested that the antibacterial activity of erythromycin usually was bacteriostatic, but against some actively growing, susceptible bacteria, large concentrations of erythromycin were bactericidal.[132] Clarithromycin and azithromycin usually are bacteriostatic, but they are bactericidal against *S. pyogenes*, *S. pneumoniae*, and *H. influenzae*.[324] Macrolides cause dissociation of peptidyl-tRNA from bacterial ribosomes, which results in accumulation of intracellular peptidyl-tRNA and depletion of the free tRNA pool, with toxic effects for bacteria.[298] The specific target appears to be the 23S ribosomal RNA. Macrolides block the entrance to the tunnel in the 50S ribosomal subunit through which many, if not all, nascent peptide chains exit the ribosome. By blocking the exit tunnel, macrolides induce premature dissociation of peptidyl-tRNAs from the ribosome just after initiation of protein synthesis. Erythromycin interferes with binding to the 50S ribosome by chloramphenicol and clindamycin, thus suggesting common or overlapping binding sites for these agents.

Mechanisms of Resistance

Many gram-negative bacteria are inherently resistant to macrolides because of the relative impermeability of their outer membrane. Other bacteria can acquire resistance by production of enzymes that modify and inactivate the macrolide, active efflux of the antibiotic, and alteration of their ribosomal targets.[184] Two mechanisms by which bacteria can alter their ribosomes and acquire macrolide resistance have been identified. High-level resistance because of an altered protein component in domain V of the 50S ribosomal subunit has occurred after a one-step chromosomal mutation. Such resistance has been demonstrated in *M. avium*, *Helicobacter pylori*, *Treponema pallidum*, and *Propionibacterium* spp. Plasmid-mediated macrolide, lincosamide, and streptogramin B (MLS_B) resistance occurs when a single adenine residue within a conserved region of domain V of the nascent 23S rRNA component of the 50S ribosomal subunit is methylated. Such methylation results in an altered common target that confers cross resistance to macrolides (i.e., erythromycin and clarithromycin), azalides (i.e., azithromycin), lincosamides (i.e., clindamycin), and streptogramin B (i.e., quinupristin-dalfopristin).[184] The production of methylases is encoded by a class of genes referred to as *erm* (erythromycin ribosome methylation), nearly 40 of

which have been characterized in a wide range of microorganisms, including gram-positive species, spirochetes, and anaerobes. Twenty-one classes of *erm* genes with less than 80 percent sequence homology have been designated unique letters. Pathogenic bacteria are one of four major classes: bacteria in classes erm(A) and erm(C) typically are in methicillin-resistant or methicillin-susceptible strains of staphylococci, respectively; members of class erm(B) are mostly in streptococci and enterococci; and class erm(F) usually is associated with anaerobic pathogens.[263] These genes are exchanged easily among different strains by plasmids, transposons, or bacterial conjugation. Resistance mediated by erm can be constitutive or inducible. When bacteria with inducible MLS_B resistance are exposed to a macrolide inducer (i.e., erythromycin, clarithromycin, or azithromycin), inactive methylase mRNA undergoes rearrangements that permit ribosomes to translate the methylase coding sequence. The diversity of inducible macrolide-resistant genotypes results in complex phenotypes, particularly among streptococci and enterococci with *erm(B)* genes, whereas constitutive production of a methylase generally confers a predictable high-level cross resistance to all MLS_B antibiotics. Staphylococci that are resistant in vitro to 14- and 15-membered ring macrolides but susceptible to 16-membered ring macrolides, clindamycin, and streptogramins B should routinely undergo further testing with a double-disk diffusion test to screen for an erythromycin-induced, "D"-shaped zone of bacterial inhibition around a clindamycin disk. A positive D-test indicates that the *Staphylococcus* isolate has inducible MLS_B resistance and that use of clindamycin can result in clinical treatment failure, presumably related to selection of constitutive mutants in patients with severe infections and large bacterial inocula.[184] Similar inducible resistance phenotypes may be observed among various strains of streptococci for which the use of clindamycin should be discouraged.

Macrolide resistance associated with active efflux is caused by two classes of pumps: a plasmid-mediated, ATP-binding cassette (ABC) transporter encoded by the *msr(A)* gene and found in *Staphylococcus* spp., and a protein of the major facilitator superfamily found in streptococci and enterococci and encoded by the *mef(A)* gene. The MsrA pump confers a macrolide and streptogramin B (MS_B) pattern of resistance that can be distinguished from the MLS_B-inducible phenotype by the double-disk diffusion D-test. The Mef(A) pump affects only 14- and 15-membered ring macrolides (M phenotype).[184] Less commonly, bacteria produce enzymes that inactivate macrolides, including acetyltransferases, esterases, phosphotransferases, or glycosylases found in some strains of Enterobacteriaceae, but macrolides are not used to target these pathogens. Therefore, this mechanism of low-level resistance is not clinically important.[263] A causal association between erythromycin resistance and antibiotic use, probably mediated by selection pressure, was suggested by a nationwide epidemiologic study in Finland in which reduction of the use of erythromycin over the course of time was associated with a steady decline in erythromycin resistance among *S. pyogenes* isolates from throat swabs and pus specimens.[283] Conversely, increased consumption of macrolides in Spain since 1995 was related to an increase in macrolide resistance.[124] Pneumococcal resistance to macrolides has also increased to about 35 percent as reported in prospective multicenter monitoring studies. In the United States, 6 percent of the macrolide-resistant isolates exhibited low-level erythromycin resistance by expression of the *mef(A)* gene and about 20 percent expressed the *mef(A)* and the *erm(B)* gene, resulting in high-level resistance.[325,154]

In Vitro Activity

Erythromycin is effective in vitro against a diverse group of microorganisms, including *Bacteroides* spp., *Bordetella pertussis*, *Chlamydophila* spp., *Corynebacterium diphtheriae*, *H. pylori*, the bacterium of legionnaires' disease *(Legionella pneumophila)*, mycoplasmas (*Mycoplasma pneumoniae* and *Ureaplasma urealyticum*), spirochetes *(T. pallidum)*, and anaerobic and aerobic gram-positive cocci (*S. pneumoniae*, *S. pyogenes*, and penicillinase-producing and non–penicillinase-producing strains of oxacillin-susceptible *S. aureus*). Erythromycin is active against *Campylobacter jejuni*, *N. meningitidis*, *N. gonorrhoeae*, and, to a lesser extent, *H. influenzae*. Macrolide resistance among diverse pathogens is increasing worldwide, and the clinical consequences are topics of active research.[116,149,175]

The newer macrolides have spectra of activity similar to that of erythromycin. In contrast to erythromycin, clarithromycin has equivalent or greater activity against *M. catarrhalis*, *H. influenzae*, *M. pneumoniae*, *Chlamydia pneumoniae*, *Chlamydia trachomatis*, *L. pneumophila*, *U. urealyticum*, and *N. gonorrhoeae*. Clarithromycin is two to four times more active against most erythromycin-susceptible streptococci and staphylococci. However, pneumococci that are resistant to erythromycin (≤48% in certain regions[176]) also are resistant to clarithromycin and azithromycin; rates of macrolide resistance among pneumococci tend to correlate with rates of penicillin resistance.[324] Clarithromycin also has activity against organisms that are resistant to erythromycin, including *H. pylori*, *Toxoplasma gondii*, *Mycobacterium leprae*, MAC, and *Mycobacterium chelonae*.

In contrast to erythromycin, azithromycin has less activity against gram-positive bacteria, including *S. pneumoniae*, but has better activity against gram-negative bacteria, including *Vibrio cholerae* and some Enterobacteriaceae such as *Shigella* spp. The in vitro activity of azithromycin against *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila* is similar to that of erythromycin and clarithromycin. Azithromycin is more active than erythromycin against *C. trachomatis* and *U. urealyticum* and twofold to eightfold more active than erythromycin or clarithromycin against *M. catarrhalis*, *H. influenzae*,[21,208] *N. gonorrhoeae*, and *H. ducreyi*. Azithromycin has activity against *T. gondii* and, although less than that of clarithromycin, against MAC organisms. Azithromycin and clarithromycin are highly effective in vitro against *Borrelia burgdorferi*, *Bartonella henselae*, and *Bartonella quintana*.

Pharmacokinetics

The macrolides differ in pharmacokinetic properties (Table 235-8). Clarithromycin[131] and azithromycin[122] are gastric acid–stable and relatively well absorbed from the

TABLE 235-8 **Pharmacokinetics of Macrolide Antibiotics**

	Erythromycin	Clarithromycin*	Azithromycin†
Bioavailability (%)	30-65‡	55	37
Protein binding (%)	70-90	65-75	7-50§
Half-life (hr)	1.4-2	Nonlinear‖	11-14 (48-96)
C_{max} (μg/mL)¶	0.8-3‡	3-7 (1-2)	0.4 (0.25)
Elimination route			
Biliary excretion	Majority	Minimal	>50%
Renal excretion (%)	2-15	20-40 (10-15)	4.5

Modified from U.S. Dispensing Information. *Information for the health care professional.* Vol. 1, Thomson Micromedex, 2006.
*Values in parentheses are for the active metabolite 14-OH-clarithromycin.
†After one 500-mg dose; values in parentheses are at steady state after 500 mg × 1 day, then 250 mg/day (oral dosage forms).
‡Varies with the oral preparation and the presence of food.
§Protein binding varies with serum concentration.
‖Varies with dosing; 250 mg twice daily: clarithromycin = 3 to 4 hr, 14-OH-C = 5 to 6 hr; 500 mg twice daily; clarithromycin = 5 to 7 hours, 14-OH-C = 7 hr.
¶C_{max} = peak serum concentration.

gastrointestinal tract, whereas erythromycin is acid-labile and absorption varies with the oral preparation. Macrolides undergo metabolism by the hepatic microsomal cytochrome P (CYP)450 system. Erythromycin and clarithromycin, but not azithromycin, are inhibitors of the CYP450 enzyme system (CYP3A and CYP1A subclasses) and can interact with other drugs that are metabolized by this system.[239] Most of the metabolites are inactive, with the exception of 14-(R)-hydroxyclarithromycin, an active metabolite that can act additively or synergistically with clarithromycin. The lipophilic macrolides are distributed well in tissues and fluids, except CSF. High intracellular concentrations are achieved, but, with the exception of azithromycin, the macrolides rapidly diffuse out of cells when extracellular concentrations are low. Clarithromycin and azithromycin are transported actively into leukocytes and macrophages. High concentrations of clarithromycin are present in the nasal mucosa, tonsils, and pulmonary epithelial lining fluid and alveolar cells.[76] Tissue concentrations of clarithromycin and azithromycin exceed those found in plasma by 2 to 20 times and 10 to 100 times, respectively. The favorable cellular penetration probably contributes to the efficacy of clarithromycin and azithromycin in the treatment of intracellular pathogens. Furthermore, sustained intracellular concentrations of azithromycin facilitate short-course therapy for pharyngitis and acute otitis media and single-dose therapy for chlamydial STDs.[122] Isolated cases of intravascular bacterial infections developing during macrolide therapy for focal infections have been reported and evoke concern that, despite elevated tissue concentrations, low serum concentrations may not treat systemic infections consistently.[175] Erythromycin and azithromycin are eliminated primarily by biliary excretion, whereas clarithromycin is excreted predominantly by the kidneys. A reduction in the dosage of clarithromycin may be required in patients with moderate-to-severe renal insufficiency. Because of their longer serum half-life, azithromycin and clarithromycin can be administered in a once- and twice-daily regimen, respectively, in contrast to the three- or four-times-daily dosing necessary for erythromycin.

Because erythromycin base is unstable at the low pH of the stomach, better-absorbed products were prepared by the addition of protective enteric coating or by alteration of the chemical structure through the formation of salts and esters. Salt and ester derivatives include ethylsuccinate or propionate (esters), stearate (a salt), and estolate (salt of an ester). Estolate provides the highest concentration of antimicrobial activity in serum, but controversy continues about which preparation provides the most biologically active drug at the site of infection. Because the base is the active component, all erythromycin preparations must be hydrolyzed to the base after absorption. Formulations of erythromycin base include tablets, delayed-release tablets, and capsules. Erythromycin estolate is marketed in capsule form, suspension, and tablets. Erythromycin ethylsuccinate is available in suspension, tablets, and chewable tablets. Erythromycin stearate is in tablet form only in the United States. Parenteral preparations of erythromycin include the lactobionate and gluceptate derivatives. Intramuscular administration of these forms is painful and should be avoided. Clarithromycin can be taken without regard to food, whereas azithromycin should be taken at least 1 hour before or 2 hours after a meal. Clarithromycin is available in suspension (125 and 250 mg/5 mL) and in tablet (250 and 500 mg) forms. Azithromycin is manufactured in 250-mg capsules and as a suspension (100 and 200 mg/5 mL). Azithromycin for intravenous injection has not been approved for use in children younger than the age of 16 years, although pharmacokinetic data are available.[151]

Indications for Use

Erythromycin is approved for the treatment of chlamydial conjunctivitis, pneumonia and urethritis, mycoplasmal and *Legionella* pneumonia, group A streptococcal sinusitis and pharyngitis, mild pneumococcal pneumonia, uncomplicated skin and soft tissue infections caused by susceptible organisms, diphtheria, pertussis, erythrasma, listeriosis, and nongonococcal urethritis and as

second-line therapy for gonococcal urethritis. It also is approved for use as a preoperative bowel preparation, for penicillin-allergic persons as prophylaxis for bacterial endocarditis and rheumatic fever, and for the treatment of syphilis in nonpregnant individuals. In the context of increasing prevalence of erythromycin resistance among common pathogens, newer macrolides or other classes of antibacterial agents may be more effective for certain infections.

Clarithromycin is approved for treatment of bacterial exacerbations of bronchitis; streptococcal pharyngitis; mycoplasmal, chlamydial, and pneumococcal community-acquired pneumonia; acute maxillary sinusitis; and uncomplicated skin and soft tissue infections caused by susceptible bacteria. However, increasing rates of clarithromycin resistance among common pathogens of the respiratory tract and skin structures have been associated with treatment failure.[111,149] Clarithromycin and amoxicillin or metronidazole in combination with bismuth compounds, H_2-receptor antagonists, or proton pump inhibitors are recommended for the treatment of H. pylori–associated peptic ulcer disease. Clarithromycin also is approved for prophylaxis of disseminated MAC infections in children and adults with advanced human immunodeficiency virus (HIV) infection. Furthermore, clarithromycin, in combination with other antimycobacterials, is an effective therapeutic agent for disseminated MAC infections, but safety has not been established for this indication in children younger than 20 months of age. The safety and efficacy of clarithromycin have not been established for infants younger than 6 months of age.

In adults, azithromycin is approved for treatment of bacterial exacerbations of bronchitis; chlamydial and gonococcal cervicitis and urethritis; chancroid; streptococcal tonsillitis and pharyngitis; uncomplicated skin and soft tissue infections caused by susceptible bacteria; and acute otitis media and pneumonia caused by *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae*. It also is approved in adults for prophylaxis of disseminated MAC infection in patients with advanced HIV infection. Pediatric indications include group A streptococcal pharyngitis; acute otitis media caused by *H. influenzae*, *M. catarrhalis*, and *S. pneumoniae;* and mild community-acquired pneumonia caused by *S. pneumoniae*, *H. influenzae*, *M. pneumoniae*, or *C. pneumoniae.*

Five-day regimens have been approved for the treatment of pharyngitis (12 mg/kg/day) and otitis media (single dose of 10 mg/kg followed by 5 mg/kg/day). A randomized clinical trial comparing azithromycin with high-dose (90/6.4 mg/kg/day) amoxicillin-clavulanate for the treatment of acute otitis media revealed that the lactam–containing regimen achieved superior bacteriologic and clinical outcomes for all bacterial pathogens, including penicillin-resistant *S. pneumoniae* and *H. influenzae.*[137] Consequently, consensus guidelines do not recommend azithromycin or clarithromycin for empirical treatment of acute otitis media unless the patient has a type 1 hypersensitivity reaction to penicillins.[10] Shorter courses of azithromycin for uncomplicated group A streptococcal pharyngitis (20 mg/kg/day for 3 days)[73] or acute otitis media (single dose of 30 mg/kg)[13] have been found to be equally effective as standard therapy but have not been approved by the FDA. A clinical study suggested that a 5-day course of azithromycin for localized cervical lymphadenopathy caused by *B. henselae* (cat scratch disease) is beneficial.[26] The CDC recommends azithromycin for the treatment of chlamydial genital infections, nongonococcal urethritis, and chancroid (a single 20-mg/kg dose; maximum dose, 1 g)[316] and for prophylaxis of disseminated MAC infection in HIV-infected children.[163] The current American Thoracic Society and Infectious Disease Society of America guidelines for treatment and prevention of nontuberculous mycobacteria recommend a combination regimen including a macrolide (either clarithromycin or azithromycin) with ethambutol and rifampin for patients with nodular, bronchiectatic, or fibrocavitary lung disease caused by MAC.[126] Data supporting the use of azithromycin for treatment of ophthalmia neonatorum caused by *C. trachomatis* are limited to one small study in which a single oral dose of 20 mg/kg/day for 3 days appeared effective.[316]

Although the safety and efficacy of azithromycin and clarithromycin have not been determined for infants younger than 6 months of age (or for pregnant or lactating women), these antimicrobials are recommended as first-line agents for treatment and postexposure prophylaxis of pertussis in individuals older than the age of 1 month, based on their in vitro effectiveness, safety and efficacy in older children, and more convenient dosing schedule. Duration of therapy or prophylaxis for pertussis is 14 days for erythromycin (40 to 50 mg/kg/day in four divided doses), 7 days for clarithromycin (15 mg/kg/day in two divided doses), and 5 days for azithromycin (10 mg/kg as single daily doses for infants <6 months of age; 10 mg/kg as a single dose on day 1, then 5 mg/kg/day on days 2 to 5 for infants and children ≥6 months of age). Azithromycin is the preferred macrolide for postexposure prophylaxis and treatment of pertussis in neonates because it has fewer adverse effects in contrast to erythromycin and no association with infantile hypertrophic pyloric stenosis.[304] A multicenter study established that children with cystic fibrosis chronically infected with *P. aeruginosa* had significantly improved lung function and clinical end points associated with azithromycin treatment three times per week.[273] This effect may not persist. Studies in endemic regions showed that a single dose of azithromycin is as effective as standard therapy with erythromycin for treatment of cholera in children.[171] A 5-day course of azithromycin (20 mg/kg/day; maximum dose, 1 g) is an effective treatment for uncomplicated typhoid fever in children and adolescents.[113] One to six doses of azithromycin for trachoma have been shown to be equivalent to prolonged topical treatment with oxytetracycline polymyxin ointment.[87]

Adverse Effects

The macrolides usually are well tolerated and relatively safe. The most common adverse effect is gastrointestinal disturbance, which can occur with the administration of any macrolide but is associated most commonly with the use of erythromycin. It is a dose-related phenomenon. Because it acts as a motilin receptor agonist,

gastrointestinal symptoms (i.e., nausea, vomiting, diarrhea, flatulence, and abdominal cramps) can occur with orally or parenterally administered erythromycin. Enteric coating of erythromycin does not decrease the incidence. Rapid intravenous infusions of erythromycin can result in thrombophlebitis. Cholestatic hepatitis is an unusual, but serious, macrolide toxicity. It occurs more commonly in adults and possibly in pregnant women and is associated most frequently with the estolate preparation.[51] The onset typically begins approximately 16 days after initiation of therapy and is manifested as fever, pruritus, jaundice, elevated liver function tests, and, occasionally, rash, leukocytosis, and eosinophilia. Signs and symptoms resolve after discontinuation of the macrolide but recur with subsequent therapy. Transient hearing loss has been described after the administration of large dosages of erythromycin lactobionate. Torsade de pointes is an uncommon reaction to intravenous infusions of erythromycin. A sevenfold increase in the rate of infantile hypertrophic pyloric stenosis was temporally associated with and probably causally related to the prophylactic use of erythromycin in neonates during a pertussis outbreak in a community hospital.[141] Gastrointestinal disturbances occur less frequently with clarithromycin and azithromycin, principally because lower dosages are required for effective therapy. Postmarketing experience with the use of intravenous azithromycin reported two cases of infantile pyloric stenosis.

A common and potentially serious toxicity is that of drug interactions.[239] Macrolides are metabolized by hepatic microsomal CYP450 enzymes. Drug interactions can occur during concomitant therapy with two or more drugs that undergo hepatic microsomal P450 metabolism. One proposal for this drug interaction suggests that the macrolide is N-demethylated to a nitrosoalkane that interacts with and inactivates the microsomal enzyme. Toxicity can occur because of interference with metabolism and consequent accumulation of the second drug. The ability to inactivate the enzyme varies with each macrolide; erythromycin is a more potent inhibitor than is clarithromycin. Azithromycin has not been associated yet with nitrosoalkane formation and resulting drug interactions, but caution should be exercised with concomitant administration of warfarin, digitoxin, and antacids. Drugs with which macrolides can interact include astemizole, benzodiazepines, buspirone, carbamazepine, cimetidine, cisapride, cyclosporine, digoxin, methylprednisolone, rifampin, tacrolimus, terfenadine, theophylline, triazolam, verapamil, warfarin, zidovudine, and certain protease inhibitors, among others.[239]

Chloramphenicol

Chloramphenicol, originally derived from *Streptomyces venezuelae* obtained from soil near Caracas, Venezuela, in 1947, now is prepared synthetically. It is a chemically unique agent that contains an aromatic nitro group, an N-dichloroacetyl substituent, and two chiral centers. The availability of less toxic and equally or more effective agents has limited the usefulness of chloramphenicol in the United States.

Mechanisms of Action

Chloramphenicol reversibly binds to the 50S subunit of 70S bacterial ribosomes and thereby inhibits protein synthesis. The mechanism of action involves suppression of peptidyltransferase activity, with a resultant inability to form peptide bonds and elongate the peptide chain.[282,319] Because the ribosomal binding sites of chloramphenicol overlap with those of macrolides, clindamycin, and linezolid, concomitant use of these antibiotics can result in antagonism and should be avoided. Chloramphenicol usually is bacteriostatic, but it can be bactericidal when high concentrations are achieved against highly susceptible organisms such as meningococci and *H. influenzae*.

Mechanisms of Resistance

The most common mechanism of acquired resistance is plasmid-mediated production of chloramphenicol acetyltransferases (CATs), which acetylate chloramphenicol and render it unable to bind to the ribosomal target. Numerous CAT genes encode classic (type A) or novel (type B) CATs and may be associated with multidrug resistance phenotypes. This resistance has been documented in many different genera of bacteria, including *H. influenzae*, members of the Enterobacteriaceae, *Neisseria*, streptococci, and *S. aureus*. Less commonly, multiple specific or multidrug transporter mechanisms (efflux pumps) and chromosomal or plasmid-mediated alterations in permeability have resulted in chloramphenicol resistance in a wide range of bacteria, including *B. cepacia*, *E. coli*, *H. influenzae*, *P. aeruginosa*, and *S. typhi* (in the absence of OmpF protein). More recently described alterations in bacterial 23S rRNA have been associated with chloramphenicol resistance by inducing frameshift mutations and readthrough of stop codons.[302] In addition, isolated cases of resistance in *Bacillus subtilis* because of altered ribosomes and in anaerobes because of inactivation of chloramphenicol by nitroreduction have been reported.[282]

In Vitro Activity

Chloramphenicol has broad-spectrum activity against aerobic and anaerobic gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, spirochetes, and rickettsiae.[260] It is bactericidal against susceptible strains of *H. influenzae*, *N. meningitidis*, and penicillin-susceptible *S. pneumoniae*, whereas it is bacteriostatic against most other susceptible microorganisms.[253] Frequently susceptible aerobic gram-positive cocci include groups A and B β-hemolytic streptococci, viridans streptococci, and penicillin-susceptible pneumococci. Because penicillin-resistant pneumococci may be tolerant to chloramphenicol in vitro, meaning that concentrations of drug necessary to kill these pneumococci are approximately 30-fold higher than necessary to inhibit the organism, verifying chloramphenicol susceptibility by MBC testing is prudent, especially when treating meningitis.[114] Usually, chloramphenicol is active against oxacillin-susceptible *S. aureus*, but susceptibility patterns vary with the use of chloramphenicol, and more suitable alternatives for

therapy exist. Susceptible gram-positive bacilli include *Bacillus* spp., *L. monocytogenes*, *C. diphtheriae*, *Clostridium* spp., and *Eubacterium*. Most *N. meningitidis* and *N. gonorrhoeae* organisms are susceptible.[161] However, several high-level chloramphenicol-resistant strains of meningococcus belonging to serogroup B were isolated from the CSF of children in Vietnam.[115] The susceptibility of Enterobacteriaceae, including for *Salmonella* and *Shigella* spp., is variable. Other gram-negative bacilli frequently susceptible to chloramphenicol include *B. pertussis*, *B. cepacia*, *Brucella* spp., *C. jejuni*, *F. tularensis*, *H. influenzae*, *Pasteurella multocida*, *Pseudomonas pseudomallei*, *V. cholerae*, and *Y. pestis*. Virtually all obligate anaerobes are susceptible.

Pharmacokinetics

After oral administration, absorption of chloramphenicol is rapid and complete. Bioavailability is approximately 80 percent after oral administration, but it is only 70 percent after an intravenous dose because approximately 30 percent of the parenterally administered dose is excreted in urine before hydrolysis of the succinate ester to the active form. Intramuscular injection results in peak serum concentrations comparable to those achieved after intravenous infusion. Because of its lipid solubility, chloramphenicol diffuses rapidly and widely into tissues and fluids.[260] The highest concentrations are achieved in the liver and kidneys, with high concentrations present in urine, and therapeutic concentrations are achieved in aqueous and vitreous humor. CSF concentrations range from 21 to 50 percent and from 45 to 89 percent of serum values in the presence of uninflamed and inflamed meninges, respectively. Brain tissue concentrations exceed those in plasma. Chloramphenicol also is distributed into pleural, ascitic, and synovial fluids, as well as saliva and breast milk. Protein binding ranges from 32 percent in premature newborns to 50 to 60 percent in adults. Chloramphenicol palmitate and chloramphenicol sodium succinate are esterified prodrugs of chloramphenicol. Orally administered chloramphenicol palmitate is hydrolyzed to active drug by pancreatic esterases in the small intestine before absorption. After intravenous infusion, chloramphenicol sodium succinate is hydrolyzed rapidly to active drug in the kidneys, liver, and lungs. Ninety percent of active chloramphenicol is conjugated to the inactive glucuronide primarily by the liver. Immature metabolic function of the liver in the fetus and newborn results in inadequate conjugation of chloramphenicol, with subsequent accumulation of toxic concentrations of active drug. Peak serum concentrations in children after a dose of 25 mg/kg range from 19 to 28 μg/mL, whereas in adults receiving doses of 12.5 mg/kg, peak serum values of 11 to 18 μg/mL can be achieved. Although metabolized to inactive metabolites by the liver, chloramphenicol is excreted by the kidneys: 5 to 10 percent as active drug and 80 percent as inactive metabolites. The elimination half-life is significantly longer but variable, and serum concentrations are unpredictable in neonates.[260] Dosage adjustments should be considered in persons with severe hepatic insufficiency or combined hepatic and renal insufficiency and in patients receiving drugs that compete for hepatic CYP450 oxidases, such as phenytoin, phenobarbital, and rifampin. Chloramphenicol is not removed by peritoneal dialysis or hemodialysis, but charcoal hemoperfusion may lower serum concentrations.

Available formulations of chloramphenicol include chloramphenicol palmitate in an oral suspension of 150 mg base/5 mL, 250-mg capsules of chloramphenicol base, and chloramphenicol sodium succinate for parenteral use. Other formulations include 1 percent cream for topical use, 0.5 percent otic solution, 0.5 percent ophthalmic solution, and 1 percent ophthalmic ointment.

Indications for Use

Because of its low therapeutic-toxic index, use of chloramphenicol should be reserved for serious infections for which less toxic agents are ineffective or contraindicated. In some developed countries, chloramphenicol has been replaced by third-generation cephalosporins for the treatment of bacterial meningitis and by clindamycin or metronidazole for the treatment of anaerobic infections. Ceftriaxone is a safe and effective alternative to (1) chloramphenicol for the treatment of acute typhoid fever[216] and (2) long-acting oily chloramphenicol for epidemic meningococcal meningitis in sub-Saharan Africa.[225] Infections for which chloramphenicol may be indicated include pneumococcal, meningococcal, and *H. influenzae* meningitis in β-lactam–allergic individuals; brain abscesses caused by susceptible anaerobic bacteria resistant to other agents; acute typhoid fever; and rickettsial infections (i.e., typhus, Q fever, Rocky Mountain spotted fever). Doxycycline is preferred for treatment of rickettsial infections in children, even those younger than 9 years of age. Chloramphenicol is not indicated for the treatment of trivial infections, prophylaxis of infections, or treatment of typhoid carrier states.

Adverse Effects

Hematologic adverse events associated with the use of chloramphenicol include hemolytic anemia in patients with the Mediterranean type of glucose-6-phosphate dehydrogenase deficiency, reversible bone marrow suppression, and aplastic anemia. Reversible bone marrow suppression is a dose-related phenomenon. It usually occurs when serum concentrations exceed 25 μg/mL, as can be seen when administering large dosages, during prolonged therapy, or in patients with impaired liver function. Although mammalian cells contain 80S ribosomes, rather than the 70S ribosomes found in prokaryotes, mitochondria possess 70S ribosomes. A proposed mechanism of myelosuppression involves inhibition of mitochondrial protein synthesis in host bone marrow stem cells.[282] Dose-related bone marrow suppression is manifested by peripheral anemia with or without reticulocytopenia, leukopenia, and thrombocytopenia and bone marrow findings of increased cellularity, cytoplasmic vacuolization, and maturation arrest of erythroid and myeloid precursors. In contrast, aplastic anemia is a rare, often fatal idiosyncratic reaction that is unrelated to the dosage, duration, or route of therapy. The pathogenesis

is understood less well but possibly is related to DNA damage from toxic metabolites of chloramphenicol produced by nitroreduction.[319] The incidence ranges from 1/25,000 to 40,000 courses. Onset can begin during therapy but typically occurs weeks to months or, rarely, years after therapy has been discontinued. Manifestations include peripheral pancytopenia and hypoplastic or aplastic marrow.

Gray syndrome, or gray baby syndrome, is a rare but serious and potentially fatal adverse event that usually occurs in newborn and young infants, but has been described in older children and adults with hepatic insufficiency. Most often, this syndrome occurs when serum chloramphenicol concentrations exceed 40 μg/mL and is thought to be a result of inhibition of mitochondrial electron transport in liver, skeletal muscle, and myocardium. The onset typically begins 2 to 9 days after initiating therapy. Manifestations include hypothermia, tachypnea, blue-gray skin color (cyanosis), abdominal distention, emesis, unresponsiveness, and refractory metabolic acidosis that can progress to vasomotor collapse and death within 2 days.

Uncommon side effects include hypersensitivity reactions such as drug fever, rash, urticaria, anaphylaxis, and a Herxheimer-like reaction during therapy for syphilis, typhoid fever, and brucellosis; gastrointestinal symptoms, including nausea, emesis, diarrhea, and an unpleasant taste; and neurologic symptoms such as peripheral neuritis, headache, mental confusion, and optic neuritis, the last of which may not be entirely reversible.

Chloramphenicol can inhibit the metabolism of other drugs metabolized by the hepatic microsomal CYP450 system and thus result in the accumulation of alfentanil, barbiturates, cyclophosphamide, phenytoin, antidiabetic sulfonylureas (more common with chlorpropamide and tolbutamide than with glyburide and glipizide), and warfarin during concomitant therapy.[6] Because some agents such as rifampin, phenobarbital, and phenytoin are potent inducers of hepatic microsomal enzymes, when they are given concomitantly with chloramphenicol, metabolism is enhanced, and serum concentrations of active chloramphenicol are reduced. Other drug interactions include a reduction in the effectiveness of estrogen-containing oral contraceptives when used concurrently with chloramphenicol and a delay in the response to vitamin B_{12}, folic acid, and iron. A mild disulfiram-like reaction can occur when alcohol is ingested during chloramphenicol therapy. Both cimetidine and chloramphenicol have rare associations with aplastic anemia; a few reports of concomitant use resulting in aplastic anemia have been documented, thus suggesting a potential for additive or synergistic risk. Concomitant acetaminophen therapy has been the subject of controversy; some studies suggest that coadministration can prolong the elimination half-life of chloramphenicol, but other reports suggest no effect or enhanced metabolism of chloramphenicol. Lincosamides and macrolides can have an antagonistic effect when they are administered concurrently with chloramphenicol because of competition for ribosomal binding sites. Chloramphenicol is physically incompatible in solution with many drugs, including tetracyclines and vancomycin.

Because of considerable variability in serum chloramphenicol concentrations, the narrow therapeutic-toxic index, and the potential for drug interactions, monitoring serum concentrations of chloramphenicol and peripheral blood counts during therapy is prudent, particularly for neonates.

Tetracyclines

Chlortetracycline (Aureomycin) was the first natural tetracycline discovered when isolated from *Streptomyces aureofaciens* in 1948.[105] Many tetracyclines have been developed since then. Those currently marketed include the natural agents tetracycline, oxytetracycline, and demeclocycline and the semisynthetic agents doxycycline and minocycline. Their basic structure consists of a hydronaphthacene nucleus with four fused rings. They differ from each other biochemically by substituent variations at carbons 5, 6, or 7. Their mechanisms of action and mechanisms of resistance, as well as their spectra of activity, are similar, but the analogs differ in the degree of activity and pharmacokinetic properties. Tigecycline is a novel intravenously administered glycylcycline that is related structurally to minocycline. It is approved for use in adults with complicated skin and skin structure infections, community-acquired pneumonia, and complicated intra-abdominal infections. It is bacteriostatic, with improved activity against multidrug-resistant bacteria, and it has no cross resistance with other classes of antimicrobial agents. Tigecycline has activity against *S. aureus* (including MRSA), enterococci (including VRE), and multidrug-resistant gram-negative bacteria, including ESBL-producing enteric gram-negative organisms. It is also active against rapidly growing, nontuberculous mycobacteria. It is not recommended for serious infections caused by *P. aeruginosa*.[234]

Mechanisms of Action

Tetracyclines passively diffuse through outer-membrane porins and then traverse the cytoplasmic membrane by energy-dependent active transport. Tetracyclines are actively concentrated by most bacterial cells, but not by mammalian cells, a characteristic that explains the selective action of these drugs against bacteria. Tetracyclines are bacteriostatic agents that reversibly bind to the 30S subunit of 70S bacterial ribosomes and inhibit protein synthesis. Because attachment of aminoacyl-tRNA to the ribosome acceptor site is prevented, the bacteria are unable to add amino acids to the growing peptide chain.

Mechanisms of Resistance

In many bacteria, including members of the Enterobacteriaceae, *P. aeruginosa*, staphylococci, streptococci, and *Bacteroides*, resistance to tetracyclines has developed. Resistance in most bacteria results from the acquisition of new genes often associated with mobile elements such as plasmids or transposons, but it also can be chromosomally mediated. The genes encoding for acquired resistance are called tet (tetracycline)-resistance or otr

(oxytetracycline)-resistance determinants. Resistance to one tetracycline usually implies resistance to all; however, many tetracycline-resistant bacteria are susceptible to doxycycline, minocycline, or both. At least 38 acquired tetracycline and oxytetracycline resistance genes have been identified that mediate (1) energy-dependent efflux of tetracycline (the most common type of resistance mechanism), (2) protection of the ribosomes from the action of tetracycline, or (3) enzymatic inactivation of tetracycline.[262] Tet genes that encode membrane efflux proteins are different in gram-positive and gram-negative bacteria. Efflux has been found in a wide range of bacteria, including *Acinetobacter*, *Aeromonas*, *Chlamydia*, the Enterobacteriaceae, *Haemophilus*, *Moraxella*, *Neisseria*, *Pseudomonas*, staphylococci, *Stenotrophomonas*, *V. cholerae*, and others. Less commonly, ribosomal protection by a soluble ribosomal protection protein encoded by different tet and otr genes has been found in a wide variety of aerobic and anaerobic gram-positive and gram-negative bacteria. The precise mechanism by which ribosomal protection protein mediates resistance to tetracyclines is being investigated. Researchers have hypothesized that these protection determinants produce allosteric disruption of the primary tetracycline binding site that causes the tetracycline molecules to be released from the ribosome. Enzymatic inactivation of tetracycline, encoded for by other tet genes, has been demonstrated in in vitro studies of *Bacteroides*, and *E. coli*, but the clinical significance has not been established.[262] Susceptibility of staphylococci to this class of agents typically is tested using tetracycline. However, this approach may overestimate the prevalence of resistance to doxycycline and minocycline. Although the prevalence of tetracycline resistance remains relatively low among CAMRSA isolates, resistance in some strains has been associated with tetK, a finding indicating that doxycycline and minocycline may be effective treatment options. However, because data are sparse on clinical outcomes associated with the use of minocycline or doxycycline to treat infections caused by tetracycline-resistant *S. aureus* strains, these agents are not recommended.[123] Tigecycline overcomes two major resistance mechanisms, including drug efflux and protection of ribosomes.

In Vitro Activity

Tetracyclines are broad-spectrum antibiotics with activity against aerobic and anaerobic gram-positive and gram-negative bacteria, chlamydiae, mycoplasmas, rickettsiae, and spirochetes. Activity against gram-negative bacteria has been limited by the emergence of tetracycline-resistant strains. Most *P. aeruginosa* and many *Shigella* and *Salmonella* organisms are resistant. Penicillin-susceptible strains of *N. gonorrhoeae* and *N. meningitidis* usually are susceptible to tetracyclines, but penicillin-resistant strains of *N. gonorrhoeae* are not. Gram-negative organisms with continued susceptibility to tetracyclines include *Aeromonas hydrophila*, *Brucella*, *Campylobacter*, some *Haemophilus* organisms, *Helicobacter*, *P. multocida*, *Plesiomonas shigelloides*, and *Vibrio*. Tetracyclines are active against some gram-positive bacilli, including *A. israelii*, *B. anthracis*, many clostridia, *Listeria*, and *Nocardia*. Tetracyclines have excellent activity against *C. pneumoniae*, *M. pneumoniae*, and rickettsiae. Other organisms for which tetracyclines have activity include *Mycobacterium marinum* and *Borellia burgdorferi*. The more lipophilic agents doxycycline and minocycline generally are more active than the others. Minocycline has excellent activity against susceptible staphylococci, and both doxycycline and minocycline are more active than tetracycline against *S. aureus* and some streptococci, but they have no activity against enterococci and group B streptococci. Because of extensive plasmid-mediated resistance in pneumococci, therapy with tetracycline should be avoided. Doxycycline and minocycline are more active in vitro than the other agents against anaerobic bacteria, but alternative antimicrobials are preferred for anaerobic infections because more than 50 percent of *B. fragilis* isolates are resistant, and susceptibility among other anaerobes is highly variable.[261]

Pharmacokinetics

The five tetracycline compounds available for systemic use can be classified by duration of activity: tetracycline and oxytetracycline are short-acting agents, demeclocycline is intermediate acting, and doxycycline and minocycline are long-acting agents (Table 235-9). Oral

TABLE 235-9 **Pharmacokinetics of Tetracyclines**

Drug	Oral Absorption (%)	Protein Binding (%)	Primary Route of Excretion	Approximate Half-Life* (hr)
Short Acting				
Oxytetracycline	58	35	Renal	6-10
Tetracycline	75-77	65	Renal	6-11
Intermediate Acting				
Demeclocycline	66	91	Renal	10-17
Long Acting				
Doxycycline	90-100	93	Biliary	12-22
Minocycline	90-100	76	Biliary	11-23

Modified from U.S. Dispensing Information. *Information for the health care professional.* Vol. 1, Thomson Micromedex, 2006.
*Normal renal function.

administration is the preferred route because thrombophlebitis is associated with intravenous infusion and pain is associated with intramuscular injections. Oral absorption ranges from 58 percent for the short-acting agents to 100 percent for the long-acting ones. The presence of food decreases the absorption of demeclocycline, oxytetracycline, and tetracycline. Because tetracyclines form insoluble complexes in the gut with aluminum, calcium, iron, magnesium, zinc, and other bivalent and trivalent cations, coadministration with milk and other dairy products, antacids, calcium or iron supplements, cathartics, and other agents can reduce absorption and should be avoided. Differences in lipid solubility affect penetration of tissues by the various tetracyclines. All agents readily penetrate many tissues and fluids, among which are pleural, ascitic, and synovial fluids, sinus secretions, sputum, bone, teeth, and breast milk. Tetracyclines can cross the placenta. The highest concentrations are achieved in bile and can exceed serum concentrations by 5 to 20 times. Therapeutic concentrations of doxycycline can be achieved in tonsillar and pulmonary tissues, the eye, and the prostate, and high concentrations are achieved in myometrial and endometrial tissue and the kidney.[274] Minocycline penetrates into sputum, saliva, and tears, as well as into cells of the vestibular apparatus.[157] Minocycline is biotransformed to inactive metabolites by the liver; the other tetracyclines are not metabolized, although whether doxycycline is metabolized is not clear. Oxytetracycline, demeclocycline, and tetracycline are eliminated unchanged in urine. Approximately 10 percent of minocycline is excreted unchanged in urine, 20 to 35 percent is excreted unchanged in feces, and the remainder is excreted as inactive metabolites in urine and feces. Thirty to 40 percent of doxycycline is excreted in urine; the remainder is excreted by the biliary tract and by diffusion through the intestinal wall, where some of it is chelated and prevented from reabsorption and enterohepatic cycling. Dosing of short- and intermediate-acting tetracyclines must be adjusted for renal failure.

Systemic formulations available in the United States include tablets, capsules, delayed-release capsules, and suspensions for many of the tetracyclines, as well as parenteral preparations of doxycycline, minocycline, and oxytetracycline. Ophthalmic and topical preparations also are available. Tigecycline is available only as a parenteral formulation.

Indications for Use

With few exceptions, tetracyclines no longer are the drugs of choice for many infections because of the availability of cephalosporins and semisynthetic penicillins with equivalent or greater activity and less frequent side effects. As a result of the potential for dental toxicity, tetracyclines are not recommended for use in children younger than 9 years of age, except for specific infections for which alternative therapy is potentially more toxic, such as chloramphenicol for Rocky Mountain spotted fever and ehrlichiosis.[2] Approved indications in the United States include treatment of actinomycosis, anthrax, brucellosis, inclusion conjunctivitis, psittacosis, Q fever, rickettsialpox, Rocky Mountain spotted fever, typhus, relapsing fever, syphilis, trachoma, yaws, and Vincent necrotizing gingivostomatitis caused by *Fusobacterium*, as well as infections caused by *Bacteroides* spp., *Bartonella bacilliformis*, *Campylobacter fetus*, *F. tularensis*, *M. pneumoniae*, *V. cholerae*, *Y. pestis*, and others. Because of its excellent tissue penetration and spectrum of activity, doxycycline is the preferred tetracycline for treatment of atypical pneumonia caused by *M. pneumoniae* and *C. pneumoniae*, intra-abdominal or pelvic infections, and several STDs, including granuloma inguinale, chlamydial infections, *U. urealyticum* infection, nongonococcal urethritis, pelvic inflammatory disease (plus cefoxitin, ampicillin-sulbactam or single-dose ceftriaxone with or without metronidazole), acute epididymitis (plus ceftriaxone), proctitis (plus ceftriaxone), and prostatitis.[316] Doxycycline is an alternative drug for (nonpregnant) penicillin-allergic patients with primary, secondary, or late latent syphilis. Although minocycline is effective in eradicating nasopharyngeal carriage of meningococcus, its potential for vestibular toxicity precludes its use for this purpose. For prevention of Lyme disease after a recognized tick bite, a single dose of doxycycline may be offered to adults (200 mg dose) and to children older than 8 years of age (4 mg/kg, up to a maximum dose of 200 mg), provided the following criteria are met: (1) the attached tick can be identified reliably as an adult or nymphal *Ixodes scapularis* tick that is estimated to have been attached for more than 36 hours on the basis of the degree of engorgement of the tick, (2) prophylaxis can be started within 72 hours of tick removal, and (3) the local rate of infection of these ticks with *B. burgdorferi* is more than 20 percent. Doxycycline (4 mg/kg/day in two divided doses, up to 100 mg per dose) is indicated in children older than 8 years of age and adults for treatment of erythema migrans, isolated cranial nerve palsy, uncomplicated cardiac disease, and uncomplicated arthritis.[317] Topical tetracyclines have been used extensively for the treatment of acne vulgaris and other dermatologic illnesses.[145]

Adverse Effects

Gastrointestinal disturbances are the most common side effects associated with tetracycline and include anorexia, nausea, emesis, flatulence, and diarrhea. These symptoms occur less frequently with minocycline. Esophageal ulceration has been associated with the oral administration of tetracyclines.[108] All tetracyclines except doxycycline can cause a negative nitrogen balance, with an elevation in blood urea nitrogen that usually is not significant except in the presence of underlying renal insufficiency. Demeclocycline can cause nephrogenic diabetes insipidus and, for this reason, has been used for treatment of the syndrome of inappropriate antidiuretic hormone secretion. Rarely, tetracyclines have been associated with hepatic injury, as manifested by elevated transaminases and diffuse vacuolar fatty metamorphosis on biopsy, with or without pancreatitis. The effect is dose-related, and the risk is increased with pregnancy, malnutrition, and preexisting renal or hepatic disease and

in patients receiving other hepatotoxic agents. The use of tetracyclines in children younger than 9 years of age is relatively contraindicated because tetracyclines chelate with calcium and can be deposited in developing bones and teeth and lead to a transient decrease in bone growth, permanent tooth discoloration, and enamel hypoplasia. The risk that these side effects will occur with one course at an appropriate dosage is low; the degree of tooth discoloration is associated with the total dosage administered.[127] Minocycline has been associated with reversible vestibular toxicity. All tetracyclines have been noted to cause pseudotumor cerebri, a benign elevation in intracranial pressure that is reversible after discontinuation of the drug. Some data suggest that an increased risk may exist with the concurrent use of tetracycline and isotretinoin. Tetracyclines have been associated with an exaggerated sunburn reaction after sun exposure. This risk occurs most frequently with demeclocycline and rarely with minocycline. Hypersensitivity reactions occur infrequently and include morbilliform rash, urticaria, exfoliative dermatitis, and, rarely, anaphylaxis. Hyperpigmentation of the mucosal membranes, skin, and nails has been reported with the use of tetracycline, particularly minocycline.

Drug–drug interactions are numerous. A decrease in absorption of tetracyclines can occur with the coadministration of oral antacids, bismuth subsalicylate, iron, kaolin or pectin, zinc sulfate, and other divalent or trivalent cations that chelate the antibiotic. A reduced effect of tetracyclines because of increased metabolism is associated with the concomitant use of doxycycline with barbiturates, carbamazepine, phenytoin, rifampin, and alcohol in heavy drinkers. Coadministration with tetracyclines can increase the effect of oral anticoagulants, digoxin, lithium, and theophylline and can decrease the effect of oral contraceptive agents and oral iron. Tetracyclines can inhibit the in vitro bactericidal activity of penicillins and aminoglycosides. Other drug–drug interactions include benign intracranial hypertension with the concomitant administration of vitamin A, severe nephrotoxicity with the coadministration of methoxyflurane and tetracycline, and localized hemosiderosis with amitriptyline and minocycline.

Lincosamides

Lincosamide antibiotics consist of an amino acid linked to an amino sugar. Lincomycin, elaborated by *Streptomyces lincolnensis* variety lincolnensis, originally isolated from a soil sample near Lincoln, Nebraska, was the first lincosamide available for use. Clindamycin, a semisynthetic derivative of lincomycin produced by the substitution of a chlorine atom for a hydroxyl group at position 7, was available for use in the early 1970s. Because clindamycin has increased antibacterial activity and better oral absorption than lincomycin, lincomycin rarely is used.

Mechanisms of Action

Lincosamides bind to the 50S subunit of susceptible bacterial ribosomes close to the peptidyl transferase center and thereby inhibit protein synthesis. Data indicate that clindamycin causes dissociation of peptidyl-tRNAs from the ribosome, depletion of free tRNA pools, and prevention of the egress of nascent peptides through a tunnel in the 50S subunit.[298] Because the ribosomal binding sites for lincosamides overlap with those of chloramphenicol and erythromycin, concurrent use of these agents can result in antagonism and should be avoided. Lincosamides usually are bacteriostatic but can be bactericidal against highly susceptible microorganisms in the presence of high lincosamide concentrations.

Mechanisms of Resistance

Some bacteria, including members of the Enterobacteriaceae and *Pseudomonas* and *Acinetobacter* spp., are inherently resistant to lincosamides, most likely because of relative impermeability of the outer membrane of the cell wall. Acquired resistance can develop as a result of altered ribosomal targets and, less commonly, lincosamide inactivation, whereas resistance as a result of reduced lincosamide uptake has not been described. Two mechanisms by which bacteria can alter their ribosomes and acquire lincosamide resistance have been identified. High-level resistance because of an altered protein component of the 50S ribosomal subunit after a one-step chromosomal mutation confers resistance to erythromycin and often to the lincosamides. Plasmid-mediated MLS_B resistance occurs when adenine residues on the 23S RNA component of the 50S ribosomal subunit are methylated, and an altered target is created that confers cross resistance to macrolides, lincosamides, and streptogramin B. The production of ribosomal methylases is encoded by the erm class of genes.[263] This resistance can be constitutive or inducible and can be seen in staphylococci, streptococci, and *Bacteroides* spp. Erythromycin is the most potent inducer of MLS_B resistance in staphylococci, whereas any macrolide, lincosamide, or streptogramin B can be an inducer in streptococci. Some strains of *B. fragilis* can have inducible MLS_B resistance that is not detected easily by disk agar diffusion susceptibility testing[292] but is recognizable because of frequently concurrent high-level erythromycin resistance. A macrolide-spectrogramin-B pattern of resistance that is plasmid-mediated results in macrolide resistance as a result of active ATP-dependent efflux pumps encoded by the *mef(A)* and *msr(A)* genes. Isolates with these resistance genotypes are resistant to 14- and 15-membered ring macrolides and streptogramin B but not to clindamycin.[202] Rarely, staphylococci produce a plasmid-mediated, nonconjugative nucleotidyltransferase that inactivates lincosamides and results in high-level resistance to lincomycin and tolerance to clindamycin (MBC/MIC ratio >32).

In Vitro Activity

Both lincosamides are effective in vitro against gram-positive cocci, whereas clindamycin also is active against a wide range of anaerobic bacteria. Clindamycin is many times more active than lincomycin and is as active as or slightly more active than erythromycin against staphylococci, pneumococci, group A streptococci, and viridans

streptococci. Unlike erythromycin, the lincosamides do not have clinically significant activity against *H. influenzae*, *M. pneumoniae*, or *Neisseria* spp. Clindamycin is active against many gram-positive cocci, including penicillinase-producing and non–penicillinase-producing staphylococci and groups A, B, C, and G β-hemolytic streptococci and pneumococci, but it is not active against enterococci or most health care–associated methicillin-resistant staphylococci. Rates of in vitro inducible erm-mediated MLS_B resistance among CAMRSA isolates vary widely, ranging from 4.5 percent in Houston[166] to 94 percent in Chicago.[110] Presence of this type of resistance can be detected in erythromycin-resistant strains of *S. aureus* with the use of the double-disk diffusion assay (D-test), in which clindamycin- and erythromycin-impregnated disks are placed 15 to 20 mm apart on Müeller-Hinton agar that has been inoculated with a standardized (0.5 McFarland) suspension of *S. aureus.* The finding of a D-shaped blunting of the circular zone of inhibition around the clindamycin disk on the side closest to the erythromycin disk indicates the presence of in vitro–inducible MLS_B resistance. These isolates have a high rate of mutation to constitutive clindamycin resistance, a trait that would confer a selective advantage during clindamycin therapy. The use of clindamycin for D-test–positive *S. aureus* infections has been reported to result in treatment failure in a few patients. Therefore, alternative effective antimicrobial agents should be sought, or, if empirical clindamycin therapy has been initiated, response to therapy should be carefully monitored.[123,202] Erythromycin and lincosamide resistance in pneumococci and group A streptococci has been increasing. Surveillance studies revealed rates of clindamycin resistance among multidrug-resistant pneumococci to range from 12 to 19 percent in the United States and Europe, respectively.[156] Most facultative aerobic gram-negative bacilli, with the exception of *Campylobacter* spp. (including *C. jejuni* and *C. fetus*) and *H. pylori*, are inherently resistant to clindamycin. Anaerobes frequently susceptible to clindamycin include the anaerobic gram-negative bacilli *Bacteroides*, *Fusobacterium*, *Prevotella*, and *Porphyromonas* spp.; the non–spore-forming gram-positive bacilli *Propionibacterium*, *Eubacterium*, and *Actinomyces;* the anaerobic gram-positive cocci *Peptococcus*, *Peptostreptococcus*, and microaerophilic streptococci and many *Clostridium* organisms, excluding *C. difficile*, *Clostridium sporogenes*, and *Clostridium tertium*. Clindamycin resistance does occur, especially in *Fusobacterium varium*, the nonfragilis *Bacteroides* group, an increasing proportion of *B. fragilis* isolates (≤≈30% in some series),[5] and as many as 20 percent of anaerobic gram-positive cocci. When combined with other agents, clindamycin is active against certain other pathogens such as *Babesia*, *Plasmodium*, *Pneumocystis jiroveci*, and *T. gondii.*

Pharmacokinetics

Oral absorption of lincosamides occurs rapidly. The presence of food delays but does not decrease the absorption of clindamycin, but it does reduce the absorption of lincomycin. Concomitant administration of kaolin or attapulgite-containing antidiarrheal agents can decrease absorption; therefore, these agents should not be administered within 2 hours before or 3 to 4 hours after oral lincosamides are given. Lincosamides are distributed rapidly and widely to most tissues and fluids, including saliva, sputum, respiratory tissue, pleural fluid, soft tissues, bones and joints, brain, prostate, semen, appendix, and peritoneal fluid.[224,240] Lincosamides are transported actively into macrophages and polymorphonuclear leukocytes, and high concentrations are achieved in bile, urine, and bone. Penetration into CSF is limited, unless inflammation is present. In experimental pneumococcal meningitis, concentrations of clindamycin in CSF were approximately 10 percent of the corresponding serum values. Lincosamides are highly protein-bound, with values ranging from 70 to 75 percent for lincomycin and 92 to 94 percent for clindamycin. The inactive palmitate and phosphate esters are hydrolyzed in the liver to clindamycin, the active agent. Clindamycin undergoes hepatic biotransformation to active and inactive metabolites. Ten percent of absorbed lincomycin is excreted unchanged in urine, 3 percent is excreted unchanged in feces, and the remainder is excreted as inactive metabolites, primarily in the biliary system. Elimination is delayed in the presence of severe hepatic insufficiency alone or severe concurrent renal and hepatic impairment, and, therefore, adjustments in dosages may need to be made. Formulations of lincosamides available for use include lincomycin hydrochloride solution for parenteral use. Clindamycin phosphate, a water-soluble ester of clindamycin and phosphoric acid, is available for parenteral use. Oral formulations include clindamycin palmitate hydrochloride granules reconstituted to 75 mg base/5 mL and clindamycin hydrochloride capsules in 75-, 150-, and 300-mg of the base compound. Clindamycin also is available as a topical solution, gel, lotion, foam, pad, and vaginal cream and suppository.

Indications for Use

Lincomycin can be used for the treatment of serious infections caused by susceptible strains of staphylococci, pneumococci, other streptococci, or anaerobic organisms, but it rarely is used in pediatric patients. Clindamycin is effective against and has been approved for treatment of susceptible strains of staphylococcal bone and joint infections[102]; anaerobic pelvic infections, including pelvic inflammatory disease, nongonococcal tuboovarian abscess, and postsurgical vaginal cuff infections; anaerobic intra-abdominal infections, including peritonitis and abscesses; pneumonitis, empyema, and lung abscesses caused by anaerobes and as a second-line agent for infections caused by susceptible strains of pneumococci and staphylococci; anaerobic septicemia; and skin and soft tissue infections caused by anaerobes, susceptible strains of staphylococci, and streptococci. Clinical prediction rules relating to resistance among community-acquired, community-onset health care–associated and traditional nosocomial staphylococcal isolates are becoming less reliable because certain strains with distinctive genetic determinants of virulence (e.g., *pvl* genes) and antibiotic resistance (e.g., SSCmec) are tending to predominate.[144,217] Prevalences of constitutive and inducible

MLS_B resistance in community-associated *S. aureus* infections generally are increasing in the United States, although rates vary geographically and temporally as a function of predominant circulating clones.[67] Based on current experience, clindamycin should not be used for empirical therapy of suspected *S. aureus* infections if the local clindamycin resistance rate exceeds 10 to 15 percent. Conversely, clindamycin is effective in treating serious infections caused by clindamycin-susceptible (D-test–negative) CAMRSA infections, including bacteremia, osteomyelitis, suppurative arthritis, pleural empyema, and skin and soft tissue infections.[164,202] Clindamycin also is effective and has been approved as a topical agent for acne vulgaris. Unconfirmed retrospective data suggest that clindamycin in combination with a β-lactam antibiotic, with surgery if indicated, may be the most effective treatment of invasive *S. pyogenes* infections.[322] Other infections for which clindamycin may be effective but has not been approved for therapy include acute otitis media caused by penicillin-resistant pneumococci that has failed to respond to other antibacterial agents,[10] chronic suppurative otitis media or chronic sinusitis in which anaerobes may play a role, chronic pharyngeal carriers of group A streptococci,[293] odontogenic infections, toxoplasmosis of the CNS (in combination with pyrimethamine), uncomplicated *falciparum* malaria[179] or babesiosis (in combination with quinine), and mild-to-moderate *P. jiroveci* pneumonia in patients with acquired immunodeficiency syndrome (AIDS) (in combination with primaquine).[32] Because of poor penetration into CSF, this drug is not approved for the treatment of meningitis. Clindamycin is a third-line agent for endocarditis prophylaxis for upper respiratory tract, dental, or oral procedures in persons allergic to or intolerant of amoxicillin and erythromycin. Oral and intravenous formulations of clindamycin are approved for use in infants and children of all ages. The bitter taste of the oral suspension may limit compliance.

Adverse Effects

The most frequent side effects include generalized morbilliform-like rash and mild, self-limited diarrhea occurring in as many as 10 percent and in 2 to 20 percent of patients, respectively. Other gastrointestinal disturbances include anorexia, nausea, vomiting, flatulence, abdominal pain, and a metallic taste. Pseudomembranous colitis, a serious and sometimes fatal illness, is the adverse event posing the most concern. Most antibiotics have been associated with pseudomembranous colitis, but those most frequently implicated include ampicillin, lincosamides, and cephalosporins. The incidence of lincosamide-associated pseudomembranous colitis varies from 0.1 to 10 percent. Antibiotic-associated pseudomembranous colitis is caused by overgrowth of toxin-producing strains of *C. difficile;* at least two extracellular toxins are elaborated: toxin A, a potent enterotoxin; and toxin B, a cytotoxin.[52] The risk for developing disease is increased in elderly patients and those with chronic, debilitating conditions. Pseudomembranous colitis is unrelated to the total antibiotic dosage, duration of therapy, route of administration, or underlying disease. The onset most frequently occurs between days 4 and 9 of therapy, but signs and symptoms develop in one third of patients 2 to 10 weeks after discontinuation of the antibiotic. More than 80 percent of patients have fever, leukocytosis, crampy abdominal pain, and watery diarrhea, and 5 to 10 percent have bloody diarrhea. Sigmoidoscopic findings include plaquelike lesions on colonic or rectal mucosa consisting of polymorphonuclear leukocytes, chronic inflammatory cells, fibrin, and epithelial debris. Treatment includes prompt discontinuation of the antibiotic, avoidance of antiperistaltic agents, and oral administration of metronidazole. However, several factors that limit the effectiveness of metronidazole include occasional resistance of *C. difficile*, absence of convenient oral preparations for children, and complete absorption of the drug in the upper gastrointestinal tract so that bactericidal levels are achieved erratically in the lower gastrointestinal tract. Although this indication in children has not been approved by the FDA, metronidazole is considered the drug of choice. Use of a nonabsorbable oral vancomycin formulation is potentially effective. However, because of its expense, bad taste, associated relapse rate of 20 percent, and potential to induce resistance in enterococci, vancomycin should be reserved for children who fail to respond to metronidazole therapy. Potential alternative therapies for multiple recurrences of pseudomembranous colitis include probiotics, certain strains of yeast, solution of fresh stool from healthy donors, or cholestyramine.[52] An approved agent for traveler's diarrhea, rifaximin, has been shown to be effective in a few patients with recurrent or persistent *C. difficile* infection.

Other less common adverse events include hypersensitivity reactions such as urticarial rash, drug fever, and eosinophilia; transient neutropenia, agranulocytosis, or thrombocytopenia; and a mild, reversible elevation in hepatic transaminases. Caution must be exercised when administering lincosamides to newborns; fatal gasping syndromes have been described that possibly are related to the preservative benzyl alcohol.

Drug interactions include incompatibility with many agents in solution, including ampicillin, barbiturates, calcium gluconate, phenytoin, and magnesium sulfate, as well as interaction with hydrocarbon-containing inhalational anesthetics. Lincosamides are weak neuromuscular blockers,[285] but they can enhance neuromuscular blockade when they are administered concurrently with neuromuscular blockers. Chloramphenicol and macrolides can have an antagonistic effect when they are administered concurrently with lincosamides because of competition for ribosomal binding sites.

Linezolid

Linezolid is the first of a new class of oxazolidinone antibiotics that was approved for use in children by the FDA in 2002. Linezolid is a totally synthetic compound with favorable activity against multidrug-resistant, gram-positive bacteria.[92]

Mechanisms of Action

Linezolid inhibits an early stage of ribosomal protein synthesis by binding to a unique site, domain V on the bacterial 23S ribosomal RNA of the 50S subunit, and prevents formation of a functional 70S initiation complex. Inhibition of this process interferes with the bacterial translation process. Furthermore, linezolid causes ribosomes to shift reading frames and to read through stop codons, thereby affecting translational fidelity.[302]

Mechanisms of Resistance

Rarely, mutations conferring resistance have been reported in the peptidyl transferase region of the 23S rRNA genes. Cross resistance between linezolid and chloramphenicol is caused by a single nucleotide mutation in the peptidyl transferase center.[302] Cross resistance between linezolid and other classes of antibiotics that target the ribosome, such as aminoglycosides, macrolides, lincosamides, and streptogramins, rarely occurs because these agents target a distinct mechanism of protein elongation. Resistance to linezolid has been reported in as many as 4 percent of clinical isolates of enterococci, particularly *E. faecium*, *S. aureus*, and coagulase-negative staphylococci.[96] Predisposing factors include indwelling devices, undrained abscesses, and prolonged administration of linezolid, particularly if subtherapeutic antibiotic concentrations are achieved.[264]

In Vitro Activity

Time-kill studies demonstrated that linezolid is bacteriostatic against enterococci and staphylococci and bactericidal against most strains of streptococci. Linezolid is active against MRSA, coagulase-negative staphylococci, and vancomycin-resistant enterococci. Furthermore, linezolid has broad activity against *Nocardia* and rapidly growing mycobacteria, as well as slowly growing tuberculous and nontuberculous mycobacteria.[53]

Pharmacokinetics

Dosage adjustment is not necessary when switching from intravenous to oral formulations because the oral bioavailability is approximately 100 percent. Absorption is not significantly affected by food. The antibiotic is distributed to well-perfused tissues and has a relatively low protein binding (31%) that contributes to the high degree of penetration into bone, muscle, fat, alveolar cells, lung extracellular lining fluid, and CSF. Linezolid is metabolized primarily by oxidation and has no interaction with the CYP450 enzymes. The elimination half-life in children is less than 5 hours and is shorter than in adults. The disposition of linezolid varies as a function of age, with faster clearance and greater volume of distribution in children younger than 11 years of age. The AUC values for children are lower than those for adults, whereas preterm neonates (gestational age <34 weeks) within the first week of life have larger AUC values than many full-term neonates and older children. The pharmacokinetics of linezolid in adolescents 12 years of age and older is not significantly different from that of adults. Dosage adjustment is not necessary for patients with renal insufficiency or mild-to-moderate hepatic insufficiency.[162]

Indications for Use

The safety and effectiveness of linezolid have been established for pediatric patients from birth through 17 years of age. Intravenous and oral linezolid is indicated for treatment of children with (1) uncomplicated and complicated skin and skin structure infections caused by oxacillin-susceptible or oxacillin-resistant *S. aureus*, or by group A or group B β-hemolytic streptococci; (2) community- and health care–associated pneumonia caused by penicillin-susceptible strains of *S. pneumoniae* or oxacillin-susceptible or oxacillin-resistant *S. aureus;* and (3) infections caused by vancomycin-resistant enterococci.[165] The recommended dosage for newborns (≥34 weeks' postconceptual age) and children up to the age of 11 years is 10 mg/kg intravenously or orally every 8 hours for 10 to 14 days for pneumonia and complicated skin and skin structure infections and for 14 to 28 days for pneumonia or bacteremia caused by vancomycin-resistant enterococci. The 10-mg/kg dose is administered every 12 hours for 5- to 11-year-old children with uncomplicated skin and soft tissue infections and for premature neonates (gestational age <34 weeks) within the first week of life. The dose for adolescents older than 11 years and adults is 600 mg every 12 hours, with a 1200-mg daily limit. Linezolid offers a safe therapeutic alternative to vancomycin for multidrug-resistant, gram-positive bacteria and has the additional advantage of being available in an oral liquid preparation (100 mg/5 mL), which facilitates sequential intravenous and oral therapy.[91,153,162,165] Safety and efficacy of therapy for longer than 28 days have not been evaluated in controlled clinical trials. Currently, no indication exists for treatment of mycobacterial infections, although linezolid has been used successfully for certain nontuberculous infections.

Adverse Effects

Data from multiple trials indicate that linezolid is safe and well tolerated, with adverse events similar to those of comparative drugs. The most common drug-related adverse events are diarrhea, nausea, vomiting, rash, and headache.[272] However, dose and duration-dependent reversible myelosuppression, including mild-to-moderate anemia and thrombocytopenia and rarely neutropenia, have been reported in children treated with linezolid.[209,272] Therefore, weekly monitoring of complete blood counts is recommended in patients who receive linezolid for longer than 2 weeks, have preexisting myelosuppression, or receive other myelosuppressive antibiotics. Other rare adverse effects that warrant prompt evaluation include peripheral and optic neuropathy, lactic acidosis, and the serotonin syndrome in patients receiving concomitant selective serotonin reuptake inhibitors. The oral suspension formulation contains

phenylalanine, which is contraindicated in children with phenylketonuria.

Rifamycins

The rifamycins are a group of complex macrocyclic antibiotics that were isolated from *Streptomyces mediterranei* in the early 1960s. Rifampin (rifampicin) and rifabutin are structurally related, semisynthetic broad-spectrum antibiotics derived from rifamycin B and S, respectively. These agents avidly bind to the subunit of the DNA-dependent RNA polymerase, prevent attachment of the enzyme to DNA, and thus block initiation of RNA transcription and protein synthesis. Rifabutin was approved in 1992 for prevention of disseminated MAC disease in adults with advanced HIV infection. Single-point mutations that determine resistance develop rapidly when rifamycins are used alone. Therefore, these antibiotics should be administered in combination with other antimicrobial agents when indicated. Rifaximin also is a semisynthetic derivative of rifamycin that was approved by the FDA in 2004 for the treatment of individuals 12 years of age or older who have nondysenteric and afebrile traveler's diarrhea caused by noninvasive strains of *E. coli*.[4]

In Vitro Activity

Rifampin usually is bactericidal, but it may be bacteriostatic, depending on the organism and drug concentration. Rifamycins have excellent in vitro activity against many gram-positive cocci, including many methicillin-resistant staphylococci and penicillin-nonsusceptible *S. pneumoniae* (enterococci are notable exceptions); some gram-negative organisms, including meningococci, gonococci, and *H. influenzae; Legionella; C. trachomatis; T. gondii; M. tuberculosis;* and *M. leprae*. Rifampin is active against *Mycobacterium kansasii*, but not against most other species of atypical mycobacteria. In contrast, rifabutin is active against most atypical mycobacteria, except *M. chelonae*. Rifabutin also is more active than rifampin against rifampin-susceptible strains of *Mycobacterium tuberculosis* and is active against approximately one third of rifampin-resistant strains.[193,215] Resistance to rifampin is mediated by point mutations in the *rpoB* gene that encodes the subunit of the DNA-dependent RNA polymerase, as well as alterations in membrane permeability. Surveillance data documented that 3 percent of meningococcal isolates in the United States are resistant to rifampin.[167] Rifaximin has activity against most enteric bacterial pathogens, including *C. difficile*, *C. jejuni*, *E. coli*, *H. pylori*, *Salmonella* spp., *Shigella* spp., *V. cholera*, and *Yersinia enterocolitica*.[4]

Pharmacokinetics

Rifampin and rifabutin are highly lipid-soluble and highly protein-bound (72% to 89%) molecules. Consequently, they are well absorbed from the gastrointestinal tract and have excellent intracellular penetration. The drugs reach therapeutic concentrations in the CSF (≈10% to 20% of simultaneous serum concentrations), particularly in the presence of inflammation of the meninges. They are metabolized primarily by the liver through deacetylation, and 30 to 40 percent of the drug is excreted by the biliary system. In the presence of hepatic dysfunction, dosage adjustments are necessary, whereas no alteration in dosage is required for children with renal dysfunction. In adults, rifabutin has a significantly longer mean terminal half-life than rifampin (45 vs. 2 to 5 hours).[256] Rifampin, however, has a very long postantibiotic effect. Rifaximin is poorly absorbed, with a bioavailability of less than 0.4 percent. Therefore, dosage adjustment of rifaximin for hepatic or renal dysfunction is not necessary and the agent is unlikely to interact with medications metabolized by the CYP450 system.

Indications for Use

The only situation in which rifampin should be considered for use as a single antibacterial agent is for prophylaxis of infection, because resistance to rifampin can develop rapidly when this agent is used alone for therapeutic purposes. Prophylaxis with rifampin monotherapy is indicated to eradicate nasopharyngeal colonization in close contacts of individuals with infections caused by *H. influenzae* type b[125,219] and *N. meningitidis*,[30] as well as index cases with these infections, unless they were treated with third-generation cephalosporins.

The use of rifampin, combined with two or three other bactericidal antituberculosis agents for the treatment of active tuberculosis, as well as its combined use with clofazimine and dapsone for the treatment of leprosy, is considered an essential component of effective regimens. The effect of adding rifampin to certain antibiotics for treatment of other infections often is unpredictable and may result in synergistic, additive, indifferent, or antagonistic activity. In some patients, rifampin can be considered part of a combination regimen for the treatment of (1) meningitis caused by penicillin- and cephalosporin-resistant *S. pneumoniae* in combination with a third-generation cephalosporin and vancomycin; (2) shunt- or catheter-associated staphylococcal infections, often with removal of the foreign material; (3) *S. aureus* implant-related orthopedic infections in combination with an effective agent; (4) non–life-threatening susceptible MRSA skin and skin structure infections in combination with TMP-SMX with the theoretical benefit of eradicating MRSA carriage, considering that rifampin achieves high concentrations on mucosal surfaces; (5) prosthetic valve endocarditis caused by staphylococci; (6) complicated or severe *B. henselae* infection (cat-scratch disease); (7) brucellosis in combination with TMP-SMX, a tetracycline, or an aminoglycoside[197]; (8) MAC-associated disseminated disease or lymphadenitis, if not completely resectable, in combination with ethambutol and azithromycin or clarithromycin; (9) *L. pneumophila* pneumonia in combination with a macrolide if response to macrolides alone is poor; and (10) multidrug-resistant *S. maltophilia* in combination with colistin (i.e., colistimethate sodium or polymyxin E) based on favorable in vitro data.[167,193] Furthermore, rifampin and minocycline-impregnated central venous catheters have been used successfully in adults to prevent catheter colonization and bloodstream infection with gram-positive

and gram-negative pathogens, as well as with *Candida* spp. The American Academy of Pediatrics recommends selective use of these catheters in infants weighing more than 3 kg and children if the local catheter-related bloodstream infection rate exceeds 3.3/1000 catheter days and if the standard protective procedures already have been implemented.[85,237] In the last circumstance, surveillance for resistance is important. Many physicians use rifampin combined with antistaphylococcal agents for treatment of disseminated MRSA infection without evidence from controlled studies of its effectiveness.

Rifabutin is effective for the treatment of most mycobacterial species, *H. pylori*, and *T. gondii* in adults. Limited data are available for the treatment of MAC infections in children with HIV infection. Clinical studies in adults with advanced HIV infection indicate that administration of daily clarithromycin or weekly azithromycin is superior to rifabutin alone for prophylaxis of MAC infection.[215] The safety and efficacy of rifabutin for prophylaxis of MAC infection in children have not been established.[163]

Rifampin is available in capsule (150 and 300 mg) and intravenous formulations. Rifabutin is available only in capsule (150 mg) form. A suspension can be compounded by the pharmacy, or the capsule contents can be mixed with thick, sweet food for infants and young children. Absorption of rifampin is optimal if administered 1 hour before or 2 hours after a meal, whereas a high-fat meal decreases the rate, but not the total amount, of absorption of rifabutin. The use of fixed-dose combinations of rifampin, isoniazid, and pyrazinamide is not recommended for children. Although the safety and efficacy of rifapentine, a rifamycin antibiotic similar in structure and activity to rifampin and rifabutin, have not been established for children younger than 12 years of age, a pharmacokinetic study indicated that dosages in children need to be greater than those for adults to achieve comparable systemic exposure.[33] Combination treatment with rifapentine and isoniazid given once weekly for 3 months for the treatment of latent tuberculosis infection has recently been proposed for patients older than 12 years of age, with results similar to those of a 9-month regimen of daily isoniazid for the same indication.[290] The approved dosage of rifaximin for the treatment of traveler's diarrhea in adolescents and adults is 200 mg three times per day for 3 days. A dosage of 400 mg twice per day for 3 days apparently is equally effective and may be better tolerated by patients. Rifaximin should not be used to treat systemic infections or mucosally invasive enteric infections. Rifaximin is not indicated currently for the treatment of dysenteric diarrhea secondary to *Shigella*, *Salmonella*, or *Campylobacter* spp. infection or for the treatment of complicated diarrhea with fever, systemic toxicity, or bloody stools. The role of this agent for prophylaxis of traveler's diarrhea is being studied.[4,14]

Adverse Effects

Adverse events associated with rifampin include gastrointestinal disorders, rash, hepatotoxicity, hypersensitivity, and a flulike syndrome. Adverse reactions to rifabutin include gastrointestinal disorders, rash, leukopenia, neutropenia, and, rarely, uveitis, although safety data in children are limited. The parenteral form of rifampin is associated with thrombophlebitis. Routine determination of serum aminotransferase concentrations is not recommended for children receiving brief or prolonged courses of therapy, except for those with clinical evidence of hepatitis or severe or disseminated tuberculosis. In these situations, at least monthly measurements should be performed. In addition, patients should be warned that rifamycins discolor urine, tears, sweat, and feces and permanently stain soft contact lenses. Because rifamycins induce hepatic CYP450 enzyme activity, they may interact with other medications administered concurrently and cause decreased concentrations of some antiretroviral agents, particularly protease inhibitors, azole antifungal agents, barbiturates, oral contraceptives, clarithromycin, cyclosporine, digoxin, sulfonylureas, thyroxine, and warfarin (Coumadin). Conversely, rifamycin serum drug concentrations may increase if it is administered with other drugs that inhibit hepatic enzymes, and the dose of rifamycin antibiotic may need to be reduced by as much as 50 percent. Rifabutin induces hepatic enzymes to a lesser extent than does rifampin and therefore has less potential for drug interactions.[193,215] Rifaximin is well tolerated and has an excellent safety profile.

Antibiotics Interfering with Protein Synthesis Not Approved for Use in Children

Fidaxomicin is a macrocylic antibody isolated from the fermentation broth of *Dactylosporangium aurantiacum* that inhibits the bacterial sigma subunit involved in protein synthesis. It has a narrow spectrum of activity with bactericidal effect against gram-positive anaerobes, including *C. difficile*, and demonstrating activity for the NAP1/BI/027 strain. It received FDA approval in 2011 for treatment of *C. difficile*–associated diarrhea in adults. Fidaxomicin was shown to be noninferior to vancomycin for the treatment of *C. difficile* infection and was effective in preventing recurrences. It has minimal systemic absorption and appears to be well tolerated.[81,103,315]

AGENTS THAT TARGET NUCLEIC ACID

Fluoroquinolones

Fluoroquinolone antibiotics are derivatives of nalidixic acid. In contrast to nalidixic acid, fluoroquinolones have (1) a broader spectrum of activity that includes aerobic gram-negative enteric organisms, particularly Enterobacteriaceae, *Haemophilus* spp., *Neisseria* spp., *M. catarrhalis*, nonenteric gram-negative bacilli such as *P. aeruginosa*, oxacillin-susceptible staphylococci, some streptococci, bacteria associated with atypical pneumonia, certain genital pathogens, and certain strains of mycobacteria; (2) better penetration into tissues; (3) good intracellular penetration; and (4) rapid bactericidal activity.

Mechanisms of Action

Fluoroquinolones are the only class of antibacterial agents in clinical use that directly inhibit DNA synthesis. They bind to bacterial DNA gyrase and topoisomerase IV. Inhibition of bacterial gyrase causes relaxation of the supercoiled DNA that leads to termination of chromosomal replication and interference with cell division and gene expression. Inhibition of the activity of topoisomerase IV leads to separation of two united DNA molecules and subsequent interference with cellular replication.

Mechanisms of Resistance

A growing concern is that widespread use of fluoroquinolones among adult populations has been associated with resistance rates, especially among Enterobacteriaceae, that exceed 50 percent in some regions. Mechanisms of resistance include chromosomal mutations in genes that encode fluoroquinolone target enzymes (i.e., DNA gyrase and topoisomerase IV), efflux pumps, or porin (i.e., outer-membrane diffusion) channels. Newly identified plasmid-mediated resistance determinants in strains of *K. pneumoniae*, *E. coli*, *Enterobacter*, and other enteric bacteria include (1) Qnr, a protein that protects DNA gyrase from quinolone action and is associated with multidrug resistance and (2) a variant of the aminoglycoside acetyltransferase found in gram-negative bacteria that is capable of inactivating ciprofloxacin.[189,265] Fluoroquinolone-resistant strains of *N. gonorrhoeae* are common findings in many parts of the world and are spreading in the United States, especially in California and Hawaii. The CDC no longer recommends fluoroquinolones for treatment of gonorrhea in the United States.[316] Other bacteria with increasing rates of resistance include *S. pneumoniae* (≈3% to ciprofloxacin with a breakpoint 4 μg/mL or greater vs. no resistance to levofloxacin as of 2004 among invasive pediatric isolates in the United States[101]) and intestinal pathogens such as *Campylobacter jejuni*, *Shigella* spp., *Salmonella* spp., certain strains of *Klebsiella pneumoniae*, *E. coli*, *P. aeruginosa*, among others.[8]

In Vitro Activity

Levofloxacin and moxifloxacin have greater potency than ciprofloxacin against gram-positive cocci, and moxifloxacin has enhanced activity against anaerobic bacteria, although in some series, approximately 50 percent of invasive *Bacteroides* spp. are resistant to moxifloxacin.[121] Ciprofloxacin remains the most potent marketed fluoroquinolone against gram-negative bacteria, particularly against some strains of *P. aeruginosa*, *Providencia* spp., *Proteus* spp., and *S. marcescens*. In vitro studies of newer and investigational fluoroquinolones against penicillin and cephalosporin-resistant pneumococci indicate excellent activity, whereas activity of ciprofloxacin, norfloxacin, and ofloxacin is limited. Fluoroquinolone use for MRSA and oxacillin-resistant, coagulase-negative staphylococci is limited because resistant mutants can be selected relatively easily, thus leading to treatment failure and relapse. Constitutive high-level resistance exists among MRSA isolates from patients with CAMRSA infections in some areas of the United States. Although MICs tend to be lower for newer (e.g., moxifloxacin and gatifloxacin) in contrast to older (e.g., ciprofloxacin and levofloxacin) fluoroquinolones, genes conferring fluoroquinolone resistance in *S. aureus* confer resistance to the entire class of agents.[123] Activity against enterococci is marginal or absent. Fluoroquinolones are effective against atypical pneumonia pathogens (i.e., *Chlamydophila pneumoniae*, *L. pneumophila*, and *M. pneumoniae*) and certain genital pathogens (i.e., *C. trachomatis*, *U. urealyticum*, and *Mycoplasma hominis*). Ciprofloxacin, levofloxacin, and moxifloxacin are active in vitro against certain strains of *M. tuberculosis*, *Mycobacterium fortuitum*, *M. kansasii*, and *M. chelonae* and are less active against MAC. No quinolone antibiotic has been shown to be active against *T. pallidum*.

Pharmacokinetics

The fluoroquinolones generally have excellent bioavailability that is not substantially affected by food, with values of 70 percent for ciprofloxacin, 85 percent for moxifloxacin, and greater than 95 percent for ofloxacin and levofloxacin. These drugs have large volumes of distribution, and they concentrate in the kidneys and in urine (except moxifloxacin, which undergoes hepatic metabolism and biliary excretion). This class of agents has favorable pharmacokinetic properties in children, and their efficacy correlates best with the 24hour AUC/MIC ratio because of a combination of concentration-dependent killing and postantibiotic effect. These properties suggest that large doses given at relatively infrequent intervals would be most effective.[277] However, based on pharmacokinetic studies, doses for patients with cystic fibrosis should be higher and more frequent than for patients without cystic fibrosis, and the dosage should be decreased in proportion to increasing body weight. Fluoroquinolones can interact with a variety of other drugs, including caffeine, cyclosporine, dideoxyinosine, nonsteroidal antiinflammatory drugs, theophylline, and other agents that prolong the QT interval. Coadministration with antacids, ferrous sulfate, and multivitamins containing zinc can reduce oral bioavailability.

Indications for Use

Fluoroquinolones have been used extensively in adults for the treatment of skin and soft tissue, skeletal, and intra-abdominal infections, as well as infections of the urinogenital, gastrointestinal, and respiratory tracts. Fluoroquinolones commonly used in adults include the second-generation agents ciprofloxacin and levofloxacin and the fourth-generation agent moxifloxacin. Other agents with limited indications include enoxacin, ofloxacin, lomefloxacin, norfloxacin (second generation), and gemifloxacin (third generation). Four agents have been withdrawn from the market in the United States: gatifloxacin, grepafloxacin (causes cardiac toxicity), sparfloxacin, and trovafloxacin (causes hepatotoxicity). Ciprofloxacin, levofloxacin, and moxifloxacin are FDA approved

for the treatment of uncomplicated skin infections caused by susceptible organisms in adults. However, none of the fluoroquinolones are FDA approved for treatment of MRSA infections.

Use in pediatric patients has been limited because of the potential for inducing cartilage damage that leads to arthropathy, as demonstrated in juvenile animal studies. However, no convincing evidence exists that quinolone-induced arthropathy occurs in humans. Reversible arthralgia and tendinopathy have a temporal association with fluoroquinolone use. Fluoroquinolones are not approved for use in children and adolescents younger than 18 years, except in the following situations: (1) complicated urinary tract infections and pyelonephritis attributable to *E. coli* in children 1 year of age and older (approved by the FDA in 2004) and (2) exposure to aerosolized *B. anthracis* to prevent onset or progression of disease. However, these drugs have been used in certain clinical settings, such as cystic fibrosis, primarily because they are the only oral antibiotics with activity against *P. aeruginosa*. Furthermore, ciprofloxacin and levofloxacin are available in suspension formulations. In 2002 alone, approximately 500,000 prescriptions for fluoroquinolones were written in the United States for individuals younger than 18 years old.[8] These agents, particularly ciprofloxacin, have been used to treat exacerbations of chronic pseudomonal pulmonary infections in patients with cystic fibrosis; complicated urinary tract infections caused by multidrug-resistant bacteria; chronic suppurative otitis media associated with *P. aeruginosa;* osteochondritis attributable to *P. aeruginosa;* shigellosis, *C. jejuni,* and other bacterial causes of enteritis; multidrug-resistant typhoid fever; cholera; infections in neutropenic patients with cancer; in combination with other agents to treat multidrug-resistant mycobacterial disease; and multidrug-resistant gram-negative bacillary septicemia or meningitis (no controlled trials in children with meningitis have been reported using currently licensed agents).[8,60,271,278] Fluoroquinolones also effectively eradicate nasopharyngeal carriage of susceptible meningococci. One study showed that children with acute otitis media and otorrhea through tympanostomy tubes had better clinical outcomes with fewer adverse effects when they were treated with ciprofloxacin-dexamethasone otic suspension in contrast high-dose amoxicillin-clavulanic acid suspension.[95] Although gatifloxacin is not approved for use in children and currently is not available in the United States, noncontrolled studies confirmed that it is safe and effective for treatment of recurrent acute otitis media and acute otitis media when therapy with standard antimicrobials has failed.[247]

The current recommendation is that parenteral and oral fluoroquinolones should be reserved for situations in which no effective, safe, and nonrestricted alternative antimicrobial agents are available to treat complicated or multidrug-resistant bacterial infections. Appropriate uses should be limited to the following clinical situations: exposure to aerosolized *B. anthracis;* urinary tract infections caused by *P. aeruginosa* or other multidrug-resistant, gram-negative bacteria; chronic suppurative otitis media or malignant otitis externa caused by *P. aeruginosa;* acute or chronic osteomyelitis or osteochondritis caused by *P. aeruginosa;* exacerbations of pulmonary disease in patients with cystic fibrosis who have *P. aeruginosa* colonization and for whom oral therapy is appropriate; susceptible mycobacterial infections as part of a combination regimen; gram-negative bacterial infections in immunocompromised hosts in whom oral therapy is appropriate or resistance to alternative agents is present; gastrointestinal infections caused by multidrug-resistant *Shigella* spp., *Salmonella* spp., *V. cholera*, or *C. jejuni;* and proved bacterial septicemia or meningitis caused by multidrug-resistant pathogens that are susceptible only to fluoroquinolones, that have failed to respond to approved agents, or that require use of a fluoroquinolone because of life-threatening hypersensitivity to alternative agents.[8]

Adverse Effects

Adverse events associated with fluoroquinolone therapy occur in 3 to 17 percent of adult and pediatric patients and cause discontinuation of treatment in 1 to 2 percent. The most common reactions are gastrointestinal, minor CNS disorders, and allergic rashes.[277] Other infrequent adverse reactions include photosensitivity; disorders of glucose homeostasis; prolongation of the QT interval, with rare cases of torsade de pointes, particularly among patients with long QT syndrome; leukopenia; and anaphylactoid reactions. Reversible tendonitis and tendon rupture occur uncommonly in adults.

Sulfonamides

Sulfachrysoidine (Prontosil), discovered in the 1930s, was the first sulfonamide developed. Sulfonamides are broad-spectrum antimicrobial agents derived from sulfanilamide (*p*-aminobenzene sulfonamide) and are structural analogs of *p*-aminobenzoic acid (PABA); they compete with PABA and result in interference with nucleotide synthesis. Sulfanilamide was manipulated to form other compounds with expanded antimicrobial activity and reduced toxicity. Sulfonamides are distributed widely in fluids and tissues. The preparations currently available have greater solubility than earlier compounds and are less likely to cause crystalluria. Sulfonamides available for single-agent use include sulfacytine (Renoquid), sulfadiazine, sulfamethizole (Thiosulfil Forte), SMX (Gantanol), and sulfisoxazole (Gantrisin). Sulfonamide combinations, including TMP-SMX and erythromycin ethylsuccinate-sulfisoxazole acetyl (EES-SSX), are used frequently in pediatrics.

Trimethoprim-Sulfamethoxazole

TMP is a diaminopyrimidine antibiotic available for single-agent use. Combinations of TMP and sulfonamides were used in the late 1960s because of presumed synergistic antibacterial activity, although clinical evidence of synergy is equivocal. Because SMX has rates of absorption and elimination similar to those of TMP, it was the sulfonamide selected for combination. Iclaprim is a novel diaminopyrimidine with a broad spectrum of activity that includes TMP-resistant strains of *S. aureus*

(including MRSA and vancomycin-resistant *S. aureus*), *S. pneumoniae* (including penicillin-nonsusceptible strains), *P. jiroveci*, *Neisseria*, *M. catarrhalis*, and *C. pneumoniae*.[134] Iclaprim inhibits bacterial dihydrofolate reductase; it has been evaluated in clinical trials, including a multicenter, double-blind, phase II, randomized study comparing safety and efficacy with vancomycin but has not been approved by the FDA.[180]

Mechanisms of Action. SMX competitively inhibits dihydropteroate synthetase, the bacterial enzyme that assimilates PABA into dihydrofolic acid; such inhibition results in a reduction in dihydrofolic acid synthesis and therefore a reduction in the amount of tetrahydrofolic acid, a cofactor for nucleotide synthesis.[136] Only bacteria that must synthesize folic acid are potentially susceptible. SMX is bacteriostatic when used alone and can be inhibited by PABA and its derivatives (procaine and tetracaine). TMP reversibly binds and inhibits dihydrofolate reductase, an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid; this activity results in diminished amounts of folic acid, an essential cofactor in nucleic acid production.[56] TMP is bacteriostatic when used alone, but in combination with SMX against susceptible bacteria, bactericidal activity can be achieved by blockade of sequential steps in folic acid metabolism. The effect of sulfonamide on bacteria is circumvented in mammals, which obtain folate from food sources. The reaction inhibited by TMP is similar in bacteria and mammals but differs quantitatively in the extent of binding of the drug to the enzyme; mammalian dihydrofolate reductase is 60,000 times less sensitive to TMP than is the enzyme in susceptible bacteria.

Mechanisms of Resistance. Bacteria can be inherently resistant to either agent or can acquire resistance to TMP, SMX, or both agents. Resistance to SMX and other sulfonamides is associated with hyperproduction of PABA, as demonstrated in strains of *Neisseria* and staphylococci, or is caused by an altered dihydropteroate synthetase enzyme with lower affinity for sulfonamides, as found in *E. coli*, *N. meningitidis*, and *S. pneumoniae*.[300] Chromosomally mediated resistance to TMP on the basis of mutations in the *dhfr* gene has been observed in strains of *S. pneumoniae*, *H. influenzae*, *E. coli*, *Pediococcus*, *B. fragilis*, and *Nocardia* and *Clostridium* spp. Furthermore, *P. aeruginosa*, *K. pneumoniae*, and *S. marcescens* are inherently resistant to TMP because of cell wall impermeability. Acquired resistance to TMP can be plasmid-mediated or chromosomally mediated and occurs in members of the Enterobacteriaceae and in staphylococci and streptococci. Mechanisms of acquired TMP resistance include cell wall impermeability, thymine auxotrophy, resistant dihydrofolate reductase, and overproduction of dihydrofolate reductase, the most common being plasmid-mediated production of dihydrofolate reductases, which are encoded by at least 20 genes. Resistance to sulfonamides is encoded by the dhps family of genes that include the *folP* and *sulI a*nd *sulII* genes. The *sulI* gene usually is linked to other transferable resistance genes, often in the transposons that belong to the Tn21 family. Despite the reduced use of sulfonamides, the genetic determinants for sulfonamide resistance probably persist because of the efficient integron transfer mechanisms.[146] Bacterial resistance to both TMP and SMX can develop as a result of altered cell wall permeability or alternative metabolic pathways (e.g., thymine auxotrophy), whereby they obtain thymine or thymidine from the environment.[300]

In Vitro Activity. TMP-SMX has activity against a broad spectrum of gram-positive cocci and gram-negative enteric pathogens. TMP is more active than the sulfonamide, but the mixture is significantly more effective than either drug alone. Synergism is more likely to occur when the bacteria are susceptible to both drugs, but it can occur even when the bacteria are resistant to only one agent. Bacteria with potential susceptibility to the combination include *Aeromonas hydrophila*, *Brucella*, *B. cepacia*, *Campylobacter*, *E. coli*, *H. influenzae*, *M. catarrhalis*, some environmental mycobacteria, *N. gonorrhoeae*, *Nocardia*, *P. jiroveci*, *Proteus*, *Salmonella*, *S. marcescens*, *Shigella*, oxacillin-susceptible *S. aureus*, *S. maltophilia* (usually with a β-lactam–lactamase inhibitor combination), penicillin-susceptible *S. pneumoniae*, and *Yersinia enterocolitica*. However, high levels of resistance to TMP-SMX among strains of penicillin-nonsusceptible *S. pneumoniae*, methicillin-resistant staphylococci, *M. catarrhalis*, *H. influenzae*, *Neisseria*, *E. coli*, *Klebsiella*, *Salmonella*, *Shigella*, *Campylobacter*, *P. aeruginosa*, *Acinetobacter*, *Nocardia*, *Actinomyces*, *mycobacteria*, *Bacteroides*, *Clostridium*, and *C. pneumonia* limit the usefulness of this antibacterial agent.[146]

Pharmacokinetics. Optimal synergistic activity occurs when a 1:20 ratio of TMP and SMX serum concentrations is attained, as can be achieved after the administration of a fixed 1:5 ratio of TMP to SMX. Both agents are absorbed rapidly and fairly well when administered alone and in combination. Both penetrate most body fluids and tissues, although TMP frequently penetrates extravascular tissues to a greater degree than SMX. Both agents cross the placenta, are excreted in breast milk, and diffuse into pleural, peritoneal, and synovial fluid and CSF. Protein binding varies from 40 to 60 percent for TMP and from 60 to 70 percent for SMX. Both drugs are metabolized in the liver to inactive metabolites. The primary route of elimination is by the kidneys, with small amounts excreted in bile and feces. Dosage adjustments are required for renal impairment.

Available preparations include an oral suspension containing 40 mg of TMP and 200 mg of SMX per 5 mL, tablets containing 80 mg of TMP and 400 mg of SMX, double-strength tablets consisting of 160 mg of TMP and 800 mg of SMX, and a parenteral solution.

Indications for Use. TMP-SMX is approved for the treatment of acute exacerbations of chronic bronchitis in adults, enterocolitis caused by susceptible *Shigella* organisms, acute otitis media caused by susceptible strains of *H. influenzae* or pneumococcus, *P. jiroveci* pneumonia, traveler's diarrhea caused by *Shigella* and enterotoxigenic *E. coli*, and acute or chronic urinary tract infections. Although the combination is not approved for use and not usually prescribed as a first-line antibiotic, TMP-SMX has been effective therapy for typhoid fever, brucellosis,

nocardiosis, sinusitis, biliary tract infections, and bone and joint infections caused by susceptible organisms. Because early studies did not evaluate the effect of prolonged or recurrent therapy on somatic growth or bone marrow function in children, TMP-SMX was not approved for prophylaxis or prolonged treatment of otitis media. It is not recommended for treatment of group A streptococcal tonsillopharyngitis because it does not eradicate the organism or reliably prevent the nonsuppurative sequelae. TMP-SMX is not recommended as first-line monotherapy for community-acquired pneumonia, acute otitis media, or skin infections caused by *S. pyogenes* because of frequent resistance among the etiologic pathogens and absence of efficacy data. TMP-SMX is not FDA approved for the treatment of any form of staphylococcal infection. However, the medical literature contains reports of the successful use of TMP-SMX alone or in combination with one or more agents such as rifampin in the treatment of *S. aureus* infections, including MRSA.[123] TMP-SMX is recommended as an alternative agent to macrolides for treatment of pertussis in individuals 2 months of age or older.[304] TMP-SMX also is an important prophylactic agent that is indicated to prevent bacterial, *P. jiroveci*, and other infections in the following clinical settings: immunosuppressed oncology or posttransplant patients, individuals with HIV who meet clinical and hematologic criteria or certain infants who are exposed in utero to HIV, patients with urinary tract infections who are at risk for recurrent disease, and patients with chronic granulomatous disease or other congenital immunodeficiency syndromes.

Adverse Effects. Most of the side effects occurring during administration of TMP-SMX are caused by the sulfonamide. Gastrointestinal disturbances and hypersensitivity reactions are the adverse events most commonly observed. Anorexia, nausea, vomiting, diarrhea, drug eruption, and photosensitivity reactions can occur in 1 to 4 percent of patients. Hypersensitivity reactions that develop less frequently include erythema nodosum, erythema multiforme (including Stevens-Johnson syndrome), urticaria, anaphylaxis, and thyroid damage. Drug-induced hepatitis has been described but is an unusual event. CNS side effects include vertigo, ataxia, headache, and aseptic meningitis. TMP-SMX can affect renal function when it is administered to persons with underlying renal disease, but the side effect usually is reversible after discontinuation of therapy. Crystalluria occurred more commonly with earlier preparations because of low solubility. SMX has a greater tendency to cause crystalluria than other sulfonamides that are currently available, because of slower absorption and excretion. However, with adequate fluid intake, alkalinization of the urine usually is unnecessary. Interstitial nephritis and tubular necrosis seldom are associated with the use of TMP-SMX. Blood dyscrasias can be a limiting factor to the administration of TMP-SMX. Acute hemolytic anemia has been described after the use of TMP-SMX in patients with glucose-6-phosphate dehydrogenase deficiency. Although they are uncommon in patients with normal hematopoietic systems, aplastic anemia, agranulocytosis, leukopenia, and thrombocytopenia can occur. Prolonged use can result in megaloblastic anemia because of impaired folate usage. Administration of sulfonamide can trigger an acute attack of porphyria. Sulfonamides can displace bilirubin from albumin binding sites. In neonates, especially premature infants, the activity of sulfonamides can be increased as a result of reduced conjugation by the immature liver. Because of the increased risk for development of kernicterus from sulfonamide displacement of bilirubin, the use of sulfonamides during the last month of pregnancy and in the first 2 months of life is discouraged.

Drug–drug interactions with TMP-SMX are numerous. Sulfonamides can displace other drugs from albumin binding sites and thereby result in increased effective activity of the second drug, as can be seen with the concurrent administration of methotrexate, phenytoin, sulfonylurea hypoglycemic agents, thiazide diuretics, and warfarin. Drugs that, when coadministered, can displace sulfonamides from binding sites and lead to increased effective sulfonamide activity include indomethacin, probenecid, and salicylates. Agents that reduce the effect of sulfonamides include methenamine, which results in insoluble urinary precipitates of the sulfonamide, and derivatives of PABA. Sulfonamides are physically incompatible with many drugs, among them aminoglycosides, chloramphenicol, insulin, lincomycin, methicillin, tetracycline, and vancomycin.

Erythromycin Ethylsuccinate–Sulfisoxazole Acetyl

The combination of EES and SSX in a fixed ratio expands the spectrum of antibacterial activity. The mechanism of action, mechanisms of resistance, pharmacokinetics, and adverse events for EESSSX are the same as those for each individual drug. Because EESSSX is effective in vitro against common pathogens causing otitis media in children, it was approved for the treatment of acute otitis media.[42] However, it is not effective against many strains of pneumococci and no longer is recommended for empirical therapy of acute otitis media.[10] Although it is not approved for use, it can be effective therapy for sinusitis caused by *H. influenzae*, susceptible pneumococci, and *M. catarrhalis*. Albeit uncommon, its principal use today in pediatrics is for the treatment of acute otitis media caused by susceptible pathogens in patients with β-lactam hypersensitivity.

Metronidazole

Metronidazole is a synthetic imidazole available since 1959. It was first introduced for the treatment of *Trichomonas vaginalis*. Metronidazole has antiprotozoal and anaerobic activity.

Mechanisms of Action. Metronidazole enters the cells by passive diffusion, and the nitro side chain undergoes chemical reduction by the pyruvate-ferredoxin reductase complex into a toxic nitro radical that subsequently causes DNA destabilization and rupture with cell death. It has been postulated that metronidazole has anti-inflammatory and immunonomodulator effects by

decreasing neutrophil generation of hydrogen peroxide and hydroxyl radicals.

Mechanisms of Resistance. Bacteria become resistant to metronidazole by changes in the enzymes implicated in the activation of the antibiotic. Some bacteria contain *nim* genes that code for an inactivating enzyme that reduces the nitro group into an inactive amine. These genes may be present in plasmids and chromosomes.

Pharmacokinetics. Metronidazole has good oral absorption with a bioavailability of at least 90 percent. It is available in oral (i.e., tablets and capsules) and parenteral forms. It is also available for topical use (i.e., vaginal and rectal). It has good penetration and a low degree of protein binding (<20%), for which it has wide distribution, including good concentrations in CSF. Its half-life is approximately 8 hours. Metronidazole is eliminated mainly by renal clearance (60% to 80%), and 6 to 15 percent is eliminated unchanged in feces. It is cleared by hemodialysis but does not require dose adjustment with renal insufficiency. In cases of severe hepatic dysfunction, the dose should be adjusted because clearance can be diminished.

Indications for Use. Metronidazole is the treatment of choice for *T. vaginalis*, *Giardia duodenalis*, *Entamoeba histolytica*, bacterial vaginosis, and *C. difficile*–associated disease. Metronidazole has been widely used in pediatrics for the treatment of anaerobic infections, including intra-abdominal and head and neck infections (including brain abscess), despite its lack of FDA approval for these indications. For these infections a combination regimen should be used because metronidazole does not have aerobic activity. Metronidazole is used for the treatment of *H. pylori* ulcerative disease, in combination with other antibiotics and a proton pump inhibitor.[194] Safety and effectiveness of metronidazole in children have not been established, except for the treatment of amebiasis with oral metronidazole.

Adverse Effects. Gastrointestinal disturbances are the most frequent adverse effects, including nausea and diarrhea. Less frequently, dizziness, headache, loss of appetite, emesis, and abdominal pain have been reported. Other rare effects include taste changes, constipation, dry mouth, glossitis or stomatitis, headache, dark urine, pruritus, rash, insomnia, arthralgias, fever, and reversible hematologic disturbances, including leukopenia and thrombocytopenia. The parenteral form may cause phlebitis at the site of injection. With high doses, neurologic effects may be seen, including ataxia, confusion, and irritability.[44] The neurologic side effects require discontinuation of the drug. In older patients, the concomitant consumption of metronidazole and alcohol can produce a disulfiram effect.

SELECTED ASPECTS OF THE ADMINISTRATION OF ANTIMICROBIAL AGENTS

DOSAGE SCHEDULES FOR INFANTS AND CHILDREN

Dosage schedules of antimicrobial agents commercially available in the United States for infants (beyond the newborn period) and children are listed in Table 235-10. The list is subdivided into dosage schedules for mild-to-moderate and for severe disease. Oral regimens are used for mild-to-moderate infections caused by susceptible organisms in areas that are well vascularized and in which adequate concentrations of drug are achieved at the site of infection. Parenteral administration should be considered for severe infections, especially those caused by less susceptible organisms that produce disease in areas in which diffusion of drug is limited.

DOSAGE SCHEDULES FOR NEWBORN INFANTS

The clinical pharmacology of antimicrobial agents administered to newborn infants is unique and cannot be extrapolated from data derived from older children or adults. The physiologic and metabolic processes that affect the distribution, metabolism, and excretion of drugs undergo rapid changes during the child's first few weeks of life. The increased efficiency of kidney function after the infant's first 7 days requires an increase in dosage and a decrease in the interval between doses of penicillins and aminoglycosides for maintaining therapeutic concentrations of drug in blood and tissues. Thus, different dosage schedules are provided for the first week of life and for the subsequent weeks of the neonatal period (Tables 235-11 and 235-12). With survival of premature infants with very low birth-weights, more data are needed on the use of antimicrobial agents in these infants with immature metabolic and physiologic mechanisms.[251] Dosages of antibiotics in these preterm infants, especially those weighing less than 1000 g, are unclear for many agents, and measurement of serum concentrations should be considered for drugs such as vancomycin and the aminoglycosides.

SHOULD DOSAGES BE DETERMINED BY WEIGHT OR BY SURFACE AREA?

In most standard pediatric texts and in the package inserts prepared by manufacturers, dosages of antibiotics for children are based on body weight. Body surface area

TABLE 235-10 **Daily Dosage Schedules for Antimicrobial Agents in Pediatric Patients Beyond the Newborn Period**

Agent, Generic (Trade Name)	Route	Mild-to-Moderate Infections*	Severe Infections*
Penicillin G, crystalline (numerous)	IV, IM	100,000-250,000 U ÷ into four doses	250,000-450,000 U ÷ into six doses
Penicillin G, procaine (numerous)	IM	50,000 U once daily (congenital syphilis); 300,000 (<60 lb) or 600,000-1,000,000 U (≥60 lb) ÷ into one or two doses for group A streptococcal infections	Inappropriate
Penicillin G, benzathine (Bicillin)	IM	300,000-600,000 U (<60 lb)† or 900,000 U (≥60 lb)† for group A streptococcal infections; 50,000 U/kg (up to 2,400,000 U)† for syphilis	Inappropriate
Penicillin V, phenoxymethyl penicillin (numerous)	PO	25-50 mg ÷ into three or four doses	Inappropriate
Penicillinase-resistant Penicillins			
Cloxacillin (Cloxapen)	PO	50-100 mg ÷ into four doses	Inappropriate
Dicloxacillin (Dynapen, Dycill, Pathocil)	PO	25-50 mg ÷ into four doses	Inappropriate
Methicillin (Staphcillin)‡	IV, IM	100-200 mg ÷ into four doses	200-300 mg ÷ into four to six doses
Nafcillin (Nafcil, Unipen, Nallpen)	IV, IM	50-100 mg ÷ into four doses	100-200 mg ÷ into four to six doses
Oxacillin (Prostaphlin, Bactocill)	IV, IM	100-150 mg ÷ into four doses	150-200 mg ÷ into four to six doses
Aminopenicillins			
Amoxicillin (numerous)	PO	25-90 mg ÷ into two or three doses	Inappropriate
Amoxicillin + clavulanate (Augmentin)	PO	45-90 mg ÷ into two or three doses	Inappropriate
Ampicillin (numerous)	IV, IM	100-200 mg ÷ into four doses	200-400 mg ÷ into four to six doses
	PO	50-100 mg ÷ into 4 doses	Inappropriate
Ampicillin + sulbactam (Unasyn)	IV	100-200 mg of ampicillin ÷ into four doses	200-400 mg of ampicillin ÷ into four to six doses
Extended-Spectrum Penicillins			
Mezlocillin (Mezlin)	IV, IM	Inappropriate	200-300 mg ÷ into four to six doses
Piperacillin (Pipracil)	IV, IM	Inappropriate	200-300 mg ÷ into four to six doses
Piperacillin + tazobactam (Zosyn)	IV	Inappropriate	240-300 mg of piperacillin ÷ into three to four doses
Ticarcillin (Ticar)	IV, IM	100-200 mg ÷ into 4 doses	200-300 mg ÷ into four to six doses
Ticarcillin + clavulanate (Timentin)	IV	Inappropriate	200-300 mg ÷ into four to six doses
Monobactams			
Aztreonam (Azactam)	IV, IM	90 mg ÷ into three doses	120 mg ÷ into four doses
Cephalosporins			
Cefadroxil (Duricef)	PO	30 mg ÷ into two doses	Inappropriate
Cefazolin (Ancef, Kefzol)	IV, IM	50 mg ÷ into three doses	50-100 mg ÷ into three or four doses
Cephalexin (Keflex, Keftab)	PO	25-100 mg ÷ into four doses	Inappropriate
Cefaclor (Ceclor)	PO	20-40 mg ÷ into two or three doses	Inappropriate
Cefoxitin (Mefoxin)	IV	Inappropriate	80-160 mg ÷ into four to six doses
Cefprozil (Cefzil)	PO	15-30 mg ÷ into two doses	Inappropriate
Ceftibuten (Cedax)	PO	9 mg once daily	Inappropriate
Cefuroxime (Kefurox, Zinacef)	IV, IM	Inappropriate	100-150 mg ÷ into three or four doses
Cefuroxime axetil (Ceftin)	PO	20-30 mg ÷ into two doses	Inappropriate
Loracarbef (Lorabid)	PO	15-30 mg ÷ into two doses	Inappropriate
Cefdinir (Omnicef)	PO	14 mg ÷ into one or two doses	Inappropriate
Cefditoren (Spectracef)	PO	200-400 mg twice daily†,§	Inappropriate
Cefixime (Suprax)	PO	8 mg ÷ into one or two doses	Inappropriate
Cefotaxime (Claforan)	IV, IM	Inappropriate	100-180 mg ÷ into four to six doses
Cefpodoxime proxetil (Vantin)	PO	10 mg ÷ into two doses	Inappropriate
Ceftazidime (Fortaz, Tazicef, Tazidime, Ceptaz)	IV, IM	Inappropriate	100-150 mg ÷ into three doses

Continued on following page

TABLE 235-10 **Daily Dosage Schedules for Antimicrobial Agents in Pediatric Patients Beyond the Newborn Period** (Continued)

Agent, Generic (Trade Name)	Route	Mild-to-Moderate Infections*	Severe Infections*
Ceftriaxone (Rocephin)	IV, IM	Inappropriate	50-100 mg ÷ into one or two doses
Cefepime (Maxipime)	IV, IM	Inappropriate	100-150 mg ÷ into two or three doses
Carbapenems			
Ertapenem (Invanz)	IV, IM	Inappropriate	30 mg ÷ into two doses
Imipenem-cilastatin (Primaxin)	IV, IM	Inappropriate	60-100 mg ÷ into four doses
Meropenem (Merrem)	IV	Inappropriate	60-120 mg ÷ into three doses
Macrolides			
Azithromycin (Zithromax)	PO	10 mg on day 1, then 5 mg thereafter; 12 mg for pharyngitis	Inappropriate
Clarithromycin (Biaxin)	PO	15 mg ÷ into two doses	Inappropriate
Erythromycin base (numerous)	PO	30-50 mg ÷ into four doses	Inappropriate
Erythromycin estolate (Ilosone)	PO	30-50 mg ÷ into four doses	Inappropriate
Erythromycin ethylsuccinate (E.E.S., EryPed, Erythro)	PO	30-50 mg ÷ into four doses	Inappropriate
Erythromycin gluceptate (Ilotycin)	IV	Inappropriate	20-50 mg ÷ into four doses
Erythromycin lactobionate (Erythrocin)	IV	Inappropriate	20-50 mg ÷ into four doses
Erythromycin stearate (Erythrocin, Erythrocot, MyE)	PO	30-50 mg ÷ into four doses	Inappropriate
Lincosamides			
Clindamycin (Cleocin)	IV, IM	Inappropriate	20-40 mg ÷ into three or four doses
	PO	8-20 mg ÷ into three or four doses	Inappropriate
Vancomycin (Vancocin)	IV	Inappropriate	40-60 mg ÷ into four doses
Aminoglycosides			
Amikacin (Amikin)	IV, IM	Inappropriate	15-22.5 mg ÷ into three doses
Gentamicin (Garamycin)	IV, IM	Inappropriate	5-7.5 mg ÷ into three doses‖
Kanamycin (Kantrex)	IV, IM	Inappropriate	15-30 mg ÷ into three doses
Netilmicin (Netromycin)	IV, IM	Inappropriate	5-7.5 mg ÷ into three doses
Paramomycin (numerous)	PO	25-35 mg ÷ into three doses	Inappropriate
Streptomycin (numerous)	IM	Inappropriate	20-40 mg ÷ into one or two doses
Tobramycin (Nebcin)	IV, IM	Inappropriate	6-7.5 mg ÷ into three to four doses
Tetracyclines			
Doxycycline (numerous)	PO	2.2-4.4 mg ÷ into one or two doses	Inappropriate
Tetracycline (numerous)	PO	25-50 mg ÷ into four doses	Inappropriate
Chloramphenicol (Chloromycetin)	IV	Inappropriate	50-100 mg ÷ into four doses
	PO	Inappropriate	50-100 mg ÷ into four doses
Sulfonamides			
Erythromycin ethylsuccinatesulfisoxazole (Pediazole, Eryzole)	PO	50 mg erythromycin/150 mg sulfa ÷ into four doses	Inappropriate
Sulfadiazine (numerous)	PO	100-150 mg ÷ into four doses	Inappropriate
Sulfisoxazole (Gantrisin)	PO	150 mg ÷ into four doses	Inappropriate
Trimethoprim-sulfamethoxazole (Bactrim, Septra, Sulfatrim, Cotrim)	PO	8-12 mg trimethoprim/40-60 mg sulfamethoxazole ÷ into two doses	Inappropriate
	IV	Inappropriate	10-20 mg trimethoprim/50-100 mg sulfamethoxazole ÷ into four doses
Fluoroquinolones			
Ciprofloxacin (Cipro)	PO	20-30 mg ÷ into two doses	Inappropriate
	IV	Inappropriate	20-30 mg ÷ into two doses
Rifampin (Rifadin, Rimactane)	PO	10-20 mg ÷ into one or two doses	20 mg ÷ into two doses
	IV	Inappropriate	20 mg ÷ into two doses

TABLE 235-10 **Daily Dosage Schedules for Antimicrobial Agents in Pediatric Patients Beyond the Newborn Period** (Continued)

Agent, Generic (Trade Name)	Route	Mild-to-Moderate Infections*	Severe Infections*
Metronidazole (Flagyl)	PO	15-50 mg ÷ into three or four doses	Inappropriate
	IV	Inappropriate	30 mg ÷ into four doses
Linezolid (Zyvox)	PO	Inappropriate	20-30 mg ÷ into two or three doses
	IV	Inappropriate	20-30 mg ÷ into two or three doses
Quinupristin-dalfopristine (Synercid)	IV	Inappropriate	22.5 mg ÷ three doses
Colistimethate sodium (Colistin, ColyMycin M)	IV, IM	Inappropriate	2.5-5 mg ÷ into two to four doses

*Total daily dosage (per kilogram). For larger children, maximal dosages may apply.
†Total dose.
‡No longer available in the United States.
§Not approved for children younger than 12 years of age.
‖A dose of 4 mg/kg once daily is used commonly in neonates. A dose of 67.5 mg/kg once daily in older children is investigational.
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

TABLE 235-11 **Dosage Schedules for Antimicrobial Agents Used in Neonates**

		Dosage (mg/kg) and Interval of Administration				
		Weight <1200 g	***Weight 1200-2000 g***		***Weight >2000 g***	
Antibiotic	**Route**	**Age 0-4 wk**	**Age 0-7 Days**	**Age > 7 Days**	**Age 0-7 Days**	**Age > 7 Days**
Penicillin G, crystalline (U)	IV	25,000-50,000 q12h	25,000-50,000 q12h	25,000-50,000 q8h	25,000-50,000 q8h	25,000-50,000 q6h
Penicillin G, procaine (U)	IM		50,000 q24h	50,000 q24h	50,000 q24h	50,000 q24h
Penicillin G, benzathine (U)	IM		50,000 once	50,000 once	50,000 once	50,000 once
Penicillinase-resistant penicillins						
Oxacillin	IV, IM	25 q12h	25-50 q12h	25-50 q8h	25-50 q8h	25-50 q6h
Nafcillin	IV, IM	25 q12h	25-50 q12h	25-50 q8h	25-50 q8h	25-50 q6h
Broad-spectrum penicillins						
Ampicillin IV, IM						
Meningitis		50 q12h	50 q12h	50 q8h	50 q8h	50 q6h
Other infections		25 q12h	25 q12h	25 q8h	25 q8h	25 q6h
Ticarcillin	IV, IM	75 q12h	75 q12h	75 q8h	75 q8h	75 q6h
Meropenem	IV	20 q12h	20 q12h	20 q12h	20 q12h	20 q12h or q8h
Cephalosporins						
Cefazolin	IV, IM	20 q12h	20 q12h	20 q12h	20 q12h	20 q8h
Cefotaxime	IV, IM	50 q12h	50 q12h	50 q8h	50 q12h	50 q6h or q8h
Ceftriaxone I	V, IM	50 q24h	50 q24h	50 q24h	50 q24h	75 q24h
Ceftazidime	IV, IM	30 q12h	30 q12h	30 q12h	30 q12h	30 q12h
Cefepime	IV, IM	30 q12h	30 q12h	30 q12h	30 q12h	30 q12h to 50 q8h
Clindamycin	IV, IM	5 q12h	5 q12h	5 q8h	5 q8h	5 q6h
Erythromycin	PO	10 q12h	10 q12h	10 q8h	10 q12h	10 q6h or q8h

Modified from Sáez-Llorens X, McCracken GH, Jr. Clinical pharmacology of antibacterial agents. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant.* 4th ed. Philadelphia, W.B. Saunders, 1995, p. 1325.
IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

TABLE 235-12 **Dosage Schedule for Antibiotics Based on Postconceptual Age***

		Dosage (mg/kg) and Interval of Administration: Gestational Age Plus Weeks of Life			
Antibiotic	**Route**	**≤26**	**27-34**	**35-41**	**≥42**
Amikacin	IV, IM	7.5 q24h	7.5 q18h	7.5 q12h	7.5 q8h
Gentamicin	IV, IM	2.5 q24h	2.5 q18h	2.5 q12h†	2.5 q8h†
Tobramycin	IV, IM	2.5 q24h	2.5 q18h	2.5 q12h	2.5 q8h
Vancomycin	IV	10-15 q24h	10-15 q18h‡	10-15 q12h‡	10-15 q8h‡ or q6h

*Dosages should be adjusted according to serum drug concentrations.
†Single daily dosing is used commonly in neonates: 3-3.5 mg/kg (<35 wk) or 4 mg/kg (≥35 wk).
‡At 28 days of life, vancomycin is administered at 20 mg/kg per dose; the interval remains the same.
IM, Intramuscularly; *IV,* intravenously.

correlates more closely with extracellular fluid volume. Some investigators suggest that more predictable serum concentrations can be achieved by using calculations of dosages based on surface area than by using those based on weight.[135] This method may be more reliable for drugs that are distributed in extracellular fluid, such as aminoglycosides, especially when they are prescribed for obese or malnourished children. Currently, however, the convenience of calculating dosage on the basis of weight appears to be the more important consideration.

USE OF ORAL PREPARATIONS FOR SERIOUS INFECTIONS

Oral preparations of antimicrobial agents vary in their degree of absorption from individual to individual and within an individual, depending on the illness being treated and the formulation used. Because higher and more consistent serum concentrations of drug are achieved after parenteral administration, parenteral routes are preferable for serious infections. Sequential parenteral-oral antimicrobial therapy may be an option in patients with uncomplicated pneumonia, pyelonephritis, and intra-abdominal, skin and soft tissue, and suppurative skeletal infections.[207] Results of studies of orally administered antibiotics in children with skeletal infections indicate that this mode of administration can be used successfully for a portion of the therapeutic course.[227,299]

Specific guidelines for oral treatment of serious infections are recommended: (1) the patient should be able to swallow and retain the medication; (2) the dosage should be sufficiently large to provide adequate bactericidal concentrations of drug at the site of infection for at least 50 percent of the dosing interval for β-lactams; and (3) when possible, it is advisable to have the hospital laboratory determine serum antimicrobial concentrations. When this is not possible, careful followup is mandatory, and measurements of inflammatory indices, such as the erythrocyte sedimentation rate and C-reactive protein, are very useful in determining resolution of the infection.

Oral therapy can be considered for patients with osteomyelitis and suppurative arthritis only after an initial period of parenteral therapy (≥5 to 7 days), after results are available from cultures and susceptibility tests, and after the patient shows definite signs of resolution of inflammation. Oral therapy should be initiated before discharge from the hospital to ascertain compliance, determine serum antimicrobial concentrations when available, and observe for significant side effects that would preclude use of the oral antibiotic.

FOOD INTERFERES WITH THE ABSORPTION OF SOME ORAL ANTIBIOTICS

The absorption of some oral antimicrobial agents is decreased significantly when the drug is taken with food or near mealtime. These drugs include unbuffered penicillin G, penicillinase-resistant penicillins (i.e., nafcillin, oxacillin, cloxacillin, and dicloxacillin), ampicillin, and lincomycin. Dairy products and other foods or medications containing calcium or magnesium salts interfere with the absorption of tetracyclines. Absorption of penicillin V, buffered penicillin G, amoxicillin, cephalexin, cefaclor, chloramphenicol, erythromycin, and clindamycin is affected only slightly by food. When absorption is affected by the concurrent ingestion of food, antibiotics should be taken 1 or more hours before or 2 or more hours after meals. A dosage schedule of four times daily, rarely used for common infections, can be arranged for the drug to be given on arising, 1 hour before lunch and supper, and at bedtime. Most orally administered antibiotics can be administered twice or three times daily, a schedule that is accommodated easily by most parents.

INTRAVENOUS VERSUS INTRAMUSCULAR ADMINISTRATION

Although a brief period occurs when the serum antimicrobial concentration is higher after intravenous administration of an antimicrobial agent than after intramuscular administration, no therapeutic advantage of intravenous

as opposed to intramuscular administration has been demonstrated. Intravenous administration should be used if the patient is in shock or is suffering from a bleeding diathesis. When prolonged parenteral therapy is anticipated, the pain of injection and the small muscle mass of infants and young children preclude the intramuscular route and render intravenous therapy preferable. The physician must be alert for thrombophlebitis, which can result from prolonged intravenous administration, and for sterile abscesses, which can develop after intramuscular administration.

Chloramphenicol, erythromycin, linezolid, tetracyclines, and vancomycin should be administered intravenously rather than intramuscularly. Chloramphenicol was thought to be absorbed poorly from intramuscular sites, although more recent data suggested that such is not the case. Intramuscular injection of parenteral tetracyclines and erythromycin causes local irritation and pain, and intramuscular injection of vancomycin causes tissue necrosis. Care should be given to the administration of intramuscular injections.[28,195] Sites that minimize the risk for local neural, vascular, or tissue injury should be selected. The preferred site varies with the age of the child: the upper anterolateral aspect of the thigh in infants, the ventrogluteal area in children older than 2 years of age, and the deltoid area for older children. Inadvertent intra-arterial injection of benzathine penicillin G can cause tissue damage.

"PUSH" VERSUS "STEADY" OR "CONTINUOUS DRIP" INTRAVENOUS ADMINISTRATION

Antimicrobial agents can be administered intravenously by the "push" method, in which case the drug is infused in 5 to 15 minutes; by "steady drip" in 1 to 2 hours; or by "continuous drip," whereby the drug is given throughout the period of administration. The push method results in high antibacterial activity in serum for short periods, whereas the steady and continuous drip methods produce lower but more sustained activity. The risk for development of adverse effects influences whether an antimicrobial agent should be administered by push or by steady drip. Pharmacodynamic studies suggest optimization of bactericidal activity when aminoglycosides are given by push once daily because the activity of these agents is concentration-dependent. By contrast, β-lactams are given preferably in several doses or by a continuous drip to maintain concentrations of drug at the infection site that exceed the MIC of the pathogen for 50 percent or longer of the dosing interval (time-dependent pharmacodynamic principle). Rapid administration (<5 minutes) of large intravenous doses of penicillin should be avoided because of possible adverse CNS effects. Aminoglycosides given by the intravenous route should be infused in 20 to 60 minutes to obtain optimal peak concentrations. Antimicrobial activity, especially for penicillins, can deteriorate if drugs are kept in solution at room temperature for prolonged periods, as may occur with use of the continuous drip method. Fresh solutions of penicillins should be administered every 6 to 8 hours when the continuous drip method is used.

DIFFUSION OF ANTIMICROBIAL AGENTS ACROSS BIOLOGIC MEMBRANES

Diffusion of any drug across a biologic membrane depends on the molecular size of the drug, the degree of protein binding (only the unbound portion of the drug crosses), the degree of ionization at physiologic pH (only the unionized portion is available for equilibration), and solubility in lipids. Thus, the lipid solubility of the unionized and unbound fraction of an antimicrobial agent determines the capability of the drug to diffuse to the site of infection. Antibiotics usually are not distributed evenly throughout the body.[231,232]

Diffusion of antimicrobial agents from blood into a joint space, pleural and pericardial fluid, and middle ear fluid is relatively unimpeded, and high concentrations of many drugs are achieved in these sites after systemic administration. More than 60 percent of the peak serum concentration of various penicillins and cephalosporins is present in the inflamed joint space.[227] Loculations of fluid in the presence of fibrous adhesions may limit the passage of antimicrobial agents into infected areas.

Diffusion of antibiotics from blood into CSF or into the aqueous humor of the eye is limited. Drugs that are highly soluble in lipids, unionized, and minimally bound to proteins (e.g., chloramphenicol, isoniazid, rifampin, and sulfonamides) pass into CSF in high concentrations, even in the absence of inflammation, whereas drugs such as the macrolides diffuse into CSF little, if at all. Fluoroquinolones pass readily into the CSF space but do not have an indication for use for meningitis in children. Penicillins, cephalosporins, and aminoglycosides pass more effectively into CSF when the membrane is inflamed; variable, but often low, concentrations of drug in CSF can be present even in the early stages of meningitis. The β-lactams are pumped actively out of the CSF space by the choroid plexus, a process that is inhibited partially by inflammation.

DURATION OF THERAPY

Physicians must rely on empirically derived schedules of therapy for rapid and complete resolution of disease and minimal risk in terms of clinical or microbiologic failure or drug toxicity. Numerous studies evaluating the duration of therapy have been performed for streptococcal pharyngitis. The results are consistent in suggesting that the following are appropriate: 10 days of oral therapy with penicillins, cephalosporins, or macrolides; 5 days of azithromycin; or a single intramuscular dose of benzathine penicillin G. Opinions vary and data are conflicting regarding the duration of treatment for diseases such as osteomyelitis, suppurative arthritis, and infections of the

urinary tract. Radetsky[252] wrote an enlightening history of the recommendations for the duration of treatment in bacterial meningitis and pointed out: "Even in the absence of specific data certain numbers have an unaccountable power to satisfy and reassure. 7, 10, 14 and 21 days have consistently appeared. Even in the trials performed at the dawn of the antimicrobial era, these numbers were chosen."

DOSAGE SCHEDULES IN CHILDREN WITH RENAL OR HEPATIC INSUFFICIENCY

The kidneys are the major organs of excretion for most antimicrobial agents, including penicillins, cephalosporins, aminoglycosides, and tetracyclines (with the exception of doxycycline). Because impaired excretion can result in high and possibly toxic serum and tissue antimicrobial concentrations, alterations in dosage schedules should be considered in children with diminished renal function. Antibiotics that require careful dosage adjustment for renal impairment include aminoglycosides, ciprofloxacin, imipenem-cilastatin, meropenem, piperacillin, tetracyclines, ticarcillin, TMP-SMX, and vancomycin. Agents requiring dosage adjustments only when renal failure is severe include most penicillins, cephalosporins, and clindamycin. Drugs that are eliminated by nonrenal mechanisms and therefore do not require adjustment of the dosage schedule for renal impairment include chloramphenicol, cloxacillin, dicloxacillin, doxycycline, erythromycin (including the newer macrolides), metronidazole, linezolid, nafcillin, oxacillin, and rifampin.[313]

Dosage schedules for patients with renal insufficiency can be altered by administering the usual dosage for the initial dose and increasing the interval between doses or decreasing individual doses (or both, in the case of renal shutdown). Although numerous guidelines have been developed to assist the physician, these formulas have been generated from studies of adults with renal impairment, and pediatricians must be cautious in adapting the formulas for use in infants and young children.[313] Serum antimicrobial concentrations should be monitored when aminoglycosides, vancomycin, and other drugs of potential toxicity are administered to children with renal insufficiency. Serum specimens are obtained at the time of the anticipated peak and trough concentrations on the first day and repeated on subsequent days to ensure a safe and effective dosage schedule.

Hepatic disorders can alter plasma protein binding, tissue binding, hepatic metabolism, and the distribution of antimicrobials that are metabolized or excreted by the liver.[309] Few data exist regarding adjustment of dosage schedules for antibiotics that are metabolized by the liver in patients with hepatic insufficiency.[183] It would be prudent to avoid the use of tetracyclines and to exercise caution when prescribing chloramphenicol, clindamycin, metronidazole, macrolides, rifampin, and penicillinase-resistant penicillins to patients with underlying hepatic disease.

TOPICAL USE OF ANTIMICROBIAL AGENTS

Topical antimicrobial agents are used for a variety of indications: bacitracin or polymyxin ointments are available (in many cases without prescription) for first aid of minor cuts, abrasions, and burns; tetracycline, erythromycin, and clindamycin have been used for the treatment of pustular acne; and metronidazole is approved for the topical treatment of inflammatory lesions and erythema associated with rosacea. Erythromycin, chloramphenicol, sulfonamide, gentamicin, tobramycin, tetracycline, and a combination of TMP and polymyxin B ointment or drops are used for the treatment of conjunctivitis, styes, and other minor infections of the eye. Silver nitrate drops or erythromycin or tetracycline ointment is used for the prevention of gonococcal ophthalmia in newborn infants. A controlled trial involving newborn infants in Africa demonstrated equivalent or superior efficacy of a 2.5 percent ophthalmic solution of povidone-iodine in contrast to topical silver nitrate or erythromycin for prophylaxis against ophthalmia neonatorum caused by *C. trachomatis*, *N. gonorrhoeae*, staphylococci, or gram-negative bacteria.[148] Ofloxacin drops (Floxin Otic) are approved for the treatment of otitis externa, chronic suppurative otitis media, and acute otitis media in children with tympanostomy tubes.[35] Ciprofloxacin and dexamethasone otic solution (Ciprodex) is approved for children 6 months of age and older who have tympanostomy tubes, to treat acute otitis media caused by *S. aureus*, *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, and *P. aeruginosa*, as well as acute otitis externa caused by *S. aureus* and *P. aeruginosa*. Mupirocin is effective in vitro against most *S. aureus* (including methicillin-resistant strains) and group A streptococci and is approved for the treatment of impetigo. At present, approximately 10 percent of CAMRSA strains are resistant to mupirocin; in some areas, the rate is as high as 30 percent. Mupirocin applied to the anterior nares may be of value in eradicating nasal carriage of methicillin-resistant staphylococci. However, the few well-designed controlled studies that exist do not support the routine use of mupirocin, except possibly for patients undergoing dialysis, because prolonged use of mupirocin has been associated with resistance to this agent, and recolonization is a common occurrence.[68] Most antibiotics used topically, such as bacitracin, neomycin, and polymyxin B, are of limited use as systemic agents. Retapamulin ointment (Altabax), a pleuromutilin antibacterial, was approved by the FDA in 2007 for topical treatment of impetigo caused by *S. aureus* (methicillin-susceptible isolates only) or *S. pyogenes* in patients aged 9 months or older. The dosage is twice daily applications for 5 days.

Absorption after application to the conjunctivae or large areas of denuded skin can be significant, but application to normal skin does not result in detectable concentrations of antimicrobial activity in blood or urine. Sensitization does not appear to be an important problem with most topical antibiotics, although some patients with chronic dermatoses may react to certain agents such

as neomycin. Antimicrobial agents of value for systemic use should not be applied extensively to the surface of the body or used routinely in closed units (e.g., burn units) because of the risk for inducing resistance.

The Committee on Drugs of the American Academy of Pediatrics concluded that topical antimicrobial agents may prevent infection after minor cuts, abrasions, and burns, but in most instances, gentle cleansing of minor wounds and burns is sufficient antisepsis.[7] Systemic antibiotics, rather than topical drugs, are recommended for chronic pyoderma, including impetigo, especially when more than several lesions are present.

Intermittent administration of inhaled tobramycin (twice daily for 4 weeks followed by 4 drug-free weeks) is indicated for the management of children and adults with cystic fibrosis and *P. aeruginosa* infection. Clinical data from a large, randomized, placebo-controlled trial indicates that tobramycin treatment improves pulmonary function, decreases the density of *P. aeruginosa* in sputum, reduces the risk for hospitalization, and is well tolerated. The diminished microbial reduction during the third cycle of treatment was not explained by the development of resistance to tobramycin.[255]

The use of antimicrobial-coated central venous catheters is not accepted practice in pediatrics, but limited evidence supports its potential usefulness, particularly if rates of catheter-related bloodstream infections exceed 3.3/1000 catheter days and if standard protective procedures already have been implemented.[237] Rates of bacterial colonization and bloodstream infections associated with the use of central venous catheters in high-risk adult patients were found to be lower in patients whose catheters were impregnated with minocycline and rifampin than in those impregnated with chlorhexidine and silver sulfadiazine.[85]

CURRENT USE OF ANTIMICROBIAL AGENTS FOR PROPHYLAXIS

Chemoprophylaxis refers to the use of drugs to prevent infection. Antimicrobial treatment refers to the use of drugs after infection has taken place or when early signs of infectious disease are present or infection is suspected. The use of antimicrobial agents for prophylaxis has proved to be of value in many circumstances (Table 235-13) and currently is considered to be of probable value or is investigational for the prevention of infections in many other situations. Prophylaxis is of greatest value when the following criteria are met: use of a single drug with a narrow spectrum of activity, use of a drug with limited side effects or toxicity, and prevention of colonization by an organism of known susceptibility and one that is unlikely to become resistant during the period of drug use. Current recommendations from the American Academy of Pediatrics include the following circumstances: (1) site-related infections: otitis media, urinary tract infection, and endocarditis; (2) exposed host: *B. pertussis* exposure, *N. meningitidis* exposure, traveler's diarrhea, perinatal group B streptococci exposure, bite wound (i.e., human, animal, or reptile), infants born to HIV-infected mothers, close exposure to influenza in unimmunized hosts, susceptible contacts of index cases of invasive *H. influenzae* type b disease, and exposure to aerosolized spores of *B. anthracis;* (3) vulnerable host (pathogen): oncology patients (i.e., with *P. jirovecii* or fungi), HIV-infected children (i.e., with *P. jirovecii* or polysaccharide encapsulated bacteria), preterm neonates (i.e., with *Candida* sp.), anatomic or functional asplenia (i.e., with polysaccharide encapsulated bacteria), chronic granulomatous disease (i.e., with *S. aureus* and certain

TABLE 235-13 **Antimicrobial Prophylaxis in Children**

Prevention of Infection in Certain Patients	Antimicrobial Agent
Group A streptococcal infection in patients with a history of rheumatic fever	Benzathine penicillin G IM, penicillin V PO
Bacterial endocarditis in patients at risk during surgical procedures:	
Dental procedures, surgery on the upper respiratory tract	Amoxicillin PO; clindamycin, cephalexin, cefadroxil, azithromycin, or clarithromycin in penicillin-allergic patients
Gastrointestinal or genitourinary tract surgery or instrumentation	Amoxicillin PO, ampicillin IV, ampicillin plus gentamicin, or vancomycin plus gentamicin (for penicillin-allergic patients)
Neonatal sepsis caused by group B streptococcus	Penicillin (preferred) or ampicillin IM or IV (intrapartum)
Gonococcal ophthalmia in newborn infants	Silver nitrate or erythromycin ophthalmic ointment
Meningococcal disease in contacts	Rifampin, ceftriaxone
Haemophilus influenzae type b disease in contacts	Rifampin
Recurrent episodes of acute otitis media	Amoxicillin, sulfisoxazole
Postoperative infections	Penicillinase-resistant penicillins or first- or second-generation cephalosporins
Tuberculosis infections in close contacts	Isoniazid
Recurrent urinary tract infections	Trimethoprim-sulfamethoxazole, nitrofurantoin
Sepsis in patients with functional asplenia	Penicillin, amoxicillin, trimethoprim-sulfamethoxazole

IM, Intramuscularly; *IV,* intravenously; *PO,* orally.

other catalase-positive bacteria and fungi), congenital immunodeficiencies (i.e., with various pathogens), and rheumatic fever (i.e., with group A streptococci).[249]

USE OF ANTIMICROBIAL AGENTS FOR CHILDREN IN SCHOOL OR GROUP DAYCARE

Infants and children usually return to their school or daycare during a course of antimicrobial therapy. Because of problems with the administration of drugs outside the home, physicians should prescribe medications that are given infrequently, are relatively stable at ambient temperatures, and need only simple directions. Drugs that are administered in once- or twice-daily schedules are preferred. Chewable tablets, when available, may be of value in reducing the need for the school or daycare provider to measure specific amounts of liquid suspension and to refrigerate suspensions. Single-dose regimens, such as intramuscular benzathine penicillin G for group A streptococcal infections, may be advantageous. Guidelines for administration of medications in schools have been published by the Committee of School Health of the American Academy of Pediatrics and should be useful to the physician for prescribing drugs to children who attend school.[9]

RESTRICTION ON USE OF ANTIMICROBIAL AGENTS FOR INFANTS AND CHILDREN

Many antimicrobial agents are approved for use in adults but have not been approved by the FDA for use in infants and children. The reasons for lack of approval drugs with insufficient experience in children, such as tigecycline (Tygacil); agents with real or suspected toxicity in children (e.g., damage to articular cartilage in juvenile animals associated with administration of the fluoroquinolones) that should be used only when necessary; and antibiotics for which the manufacturers have chosen not to submit data on use in children to the FDA, such as metronidazole (Flagyl), piperacillin (Pipracil), and cefotetan (Cefotan). These last three agents appear to be safe and effective in infants and children as a result of use by pediatricians, but no well-controlled studies of their efficacy or safety in this age group have been performed. Although a drug that has been approved for adults may be used in children at the discretion of the physician, the prudent physician chooses to use such a drug only when it is uniquely appropriate for the infectious illness and records the basis for choice of the unapproved drug.

Seven antibiotics were approved for use in adults and have potential usefulness in the management of some pediatric patients with infections caused by multidrug-resistant pathogens. Daptomycin (Cubicin) is a cyclic lipopeptide that was approved by the FDA in 2003 for intravenous treatment of adults with complicated skin and skin structure infections caused by gram-positive organisms, including MRSA, in a single daily dose of 4 mg/kg. Daptomycin was approved in 2006 for adults with methicillin-susceptible or methicillin-resistant bacteremia or right-sided endocarditis in a dose of 6 mg/kg daily for a minimum of 2 to 6 weeks. It has been shown to be inferior to comparator agents for community-acquired pneumonia, likely as a result of inactivation of daptomycin in the presence of pulmonary surfactant.[269] Although experience with daptomycin is meager in pediatrics, the drug does appear to be effective in the setting of persistent bacteremia in children with disseminated MRSA infection. Dosages of 8 to 10 mg/kg daily are generally used in these patients.

Quinupristin-dalfopristin (Synercid), a semisynthetic streptogramin, has synergistic activity against the same organisms as does linezolid and has been used extensively in adults in Europe. The dosage for children is 7.5 mg/kg intravenously three times daily. The drug has been well tolerated by children; diarrhea is the most common adverse effect. Telithromycin (Ketek) was approved in 2004 for use in adults. It is a semisynthetic ketolide antibacterial that differs chemically from the macrolide group by lack of L-cladinose at position 3 of the erythronolide A ring. Telithromycin is indicated for enteral treatment of adults with mild-to-moderate community-acquired pneumonia caused by common respiratory tract pathogens, including multidrug-resistant *S. pneumoniae* and atypical bacteria. In 2007, the FDA removed two previously approved indications, namely, acute exacerbations of chronic bronchitis and acute bacterial sinusitis because telithromycin has been associated with severe liver toxicity in adults. Its use is contraindicated in patients with myasthenia gravis. Safety and efficacy have not been established in children.

Tigecycline (Tygacil) is a glycylcycline related to minocycline that was approved by the FDA in 2005 for intravenous use in the treatment of adults with complicated intra-abdominal infections and complicated skin and skin structure infections caused by aerobic and anaerobic gram-positive and gram-negative bacteria, including MRSA.

In 2007, doripenem was approved for use in adults with complicated intra-abdominal infections and complicated urinary tract infections. It has activity against MSSA, penicillin-susceptible and nonsusceptible *S. pneumoniae*, and *E. faecalis*. It also has activity against ESBL-producer gram-negative bacteria. Telavancin was approved by the FDA in 2009 for treatment of complicated skin and skin structure infections in adults and is active against resistant bacteria, including MRSA, GISA, and *S. pneumoniae*. Ceftaroline fosamil is a broad-spectrum cephalosporin that received FDA approval in 2010 for the treatment of acute bacterial skin and skin structure infections and community-acquired pneumonia in adults. It has activity against MRSA, GRSA, multidrug-resistant *S. pneumoniae*, and non-ESBL enteric gram-negative organisms, including *E. coli* and *K. pneumoniae*. Ceftaroline is currently being evaluated in children.

HOME INTRAVENOUS ANTIBIOTIC THERAPY

Home intravenous antibiotic therapy is now available in most communities and permits discharge from the hospital earlier than in the past. The safety, effectiveness, and cost-efficiency of such a program have been proved, and these factors are of particular value for children who require 4 to 6 weeks of therapy for osteomyelitis or suppurative arthritis or who have chronic disease that can be managed in the home, such as cystic fibrosis or malignant disease. In many cases, home care enables the patient to resume normal activities, including return to school. The following are factors that are necessary before consideration of home care:

1. Availability of a team that includes the physician, the pharmacist, a vendor who will supply the drug and supplies, and an intravenous specialty nurse
2. A disease that is stable and requires only continued antimicrobial therapy
3. Unavailability of a suitable oral antibacterial agent (see the earlier discussion on use of oral preparations for serious infections) and availability of a stable parenteral antibiotic with low toxicity that the patient can tolerate (as demonstrated in the hospital) and preferably with a long half-life to allow infrequent dosing
4. A member of the household who is able to administer the antibiotic and provide aseptic care of the venous access device
5. Appropriate follow-up that can be maintained for monitoring safety and effectiveness

If problems with venous access arise and ceftriaxone is appropriate therapy, the drug can be administered successfully at home by the intramuscular route once daily by a nurse.[117,267]

DRUG–DRUG INTERACTIONS

Drug–drug interactions can lead to therapeutic failure because of lack of effective activity of one or both drugs or serious adverse events resulting from toxic serum concentrations of one or both drugs.[118] Most children do not require daily medications for chronic diseases; thus, drug–drug interactions occur less commonly in pediatric than in geriatric patients, but the potential for interactions exists and must be considered when prescribing antibiotics. Because drug–drug interactions are not limited to prescription medications, inquiry into the use of over-the-counter medications should be made. Mechanisms for drug–drug interactions are classified as follows: physiochemical, in which one drug is physically incompatible in solution with another; pharmacokinetic, whereby one drug interferes with the absorption, distribution, metabolism, or excretion of the other; and pharmacodynamic, whereby one drug affects the activity of a second drug. Examples of each mechanism include inactivation of aminoglycosides by extended-spectrum penicillins, decreased absorption of tetracyclines with coadministration of antacids, antagonism of sulfonamide activity by procaine as a result of competition for PABA binding sites, and interference with the CYP450 oxidase enzyme system as observed with rifampin and macrolide drugs or with the azole antifungal agents and cyclosporine.

SUMMARY AND CONCLUSIONS

A summary of the information contained in this chapter is presented in the following questions that the physician must consider for appropriate use of antimicrobial agents in children:

1. Before the drug is administered:
   a. Have appropriate cultures been obtained for a specific microbiologic diagnosis?
   b. Has the patient received this drug or related compounds previously? If so, did the patient tolerate the drug? Were there any signs of toxicity or sensitization?
   c. Does the patient have a condition that requires exclusion of some drugs? For example, children with glucose-6-phosphate dehydrogenase deficiency may have induced hemolysis when a sulfonamide, nitrofurantoin, or primaquine is administered.
2. Factors to be considered when writing orders for the administration of antimicrobial agents in a hospital:
   a. If the drug is given by mouth, will coadministration with food interfere with absorption, or is diarrhea a risk?
   b. If a parenteral route is used, should the drug be administered by the intravenous or the intramuscular route?
   c. If the drug is administered by the intravenous route, is push, steady drip, or continuous drip preferred?
   d. Should the drug be instilled directly at the site of infection?
   e. Will the drug diffuse to the site of infection?
   f. Should incision and drainage of the infected area be performed before or after beginning therapy? Incision and drainage should be considered whenever a significant collection of pus is present. If a drainage procedure is performed, material should be obtained for culture and susceptibility testing.
   g. Does the patient have renal or hepatic insufficiency that requires alteration of the dosage schedule?
   h. Are any special precautions required for household contacts? Prophylaxis may be warranted in special circumstances of infection occurring in the household, daycare center, or nursery school.

3. Use of antimicrobial agents in children who are treated as outpatients:
   a. Have the names and functions of the drugs been communicated to the patient and the parent? Do any of the drugs prescribed interact with each other?
   b. Is the dosage schedule simple and satisfactory for the family circumstances (e.g., the child's school schedule or the schedule of the working parents)?
   c. Does the child have an adequate supply of the drug until it can be purchased? If not, the use of starter packages is of value. Administration of the first dose in the clinic is advantageous because it provides knowledge of acceptance and tolerability of the drug by the child.
   d. Are parents given instructions for reporting the clinical course by telephone? Is an appointment made for the next visit?
   e. Does the patient or parent know how to assess adequacy of response to the drug? Does the parent know how to take the child's temperature?
   f. Is the total amount of drug prescribed adequate for the course? Will refills of the prescription be needed?
   g. Is the drug provided in a convenient dosage form? Will the package be provided with an adequate means of measuring the drug? Does the agent require refrigeration?
   h. Has the patient or parent been informed of signs of side effects or toxicity?
   i. Are generic equivalents of the drug adequate?
   j. Will the patient be able to pay for the drug if it is purchased elsewhere (away from the clinic)? If applicable, will a third party pay for this prescription? (In some states, prescriptions by brand name may not be filled because reimbursement by the third-party payer, such as Medicaid, is insufficient.)
4. After the patient's course:
   a. How long should the patient take the drug?
   b. When should the initial choice of antimicrobial agents be reconsidered? When the results of cultures and appropriate susceptibility tests are available, should the initial choice be reevaluated and altered, if necessary?
   c. What studies should be performed to monitor the safety and adequacy of the regimen? Hematologic indices must be measured during the administration of certain antibiotics to detect any adverse reaction. Vigilance is important to detect symptoms or signs attributable to adverse reactions.
   d. Are repeat cultures necessary? In certain cases, the most appropriate criterion of efficacy is evaluation of the results of cultures.
   e. What clinical and laboratory signs of efficacy should be monitored? Signs may differ for different diseases and various drugs but should be considered by the physician when the course of therapy is designed.

TABLE 235-14 Factors Contributing to Antimicrobial Failure

Host Related
Foreign body present
Anatomic defect
Defect in immune response to infection
Poor absorption of enteral antibiotic
Disease Related
Antibiotic inappropriate for the disease
Ancillary therapy not instituted (e.g., surgical drainage)
Sequestered focus of infection (undetected or inaccessible)
Organism Related
Acquired resistance to an antimicrobial agent
Superinfection with resistant bacteria
Drug Related
Inadequate adherence
Improper dosage schedule: route, dose, or duration
Inadequate diffusion to the site of infection
Drug–drug interactions: antibiotic inactivation or antagonism
Deterioration of drug during storage

5. What factors should be considered if the patient fails to respond to the antimicrobial agent? If the patient does not respond appropriately to the course of therapy, various factors must be considered, including those related to the disease, host, drug, or organism (Table 235-14).
6. What steps can be taken to prevent reinfection? For example, in certain circumstances prophylactic antibiotics, immunizations, or surgical procedures may be warranted.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abdel-Rahman SM, Chandorkar G, Akins RL, et al. Single-dose pharmacokinetics and tolerability of daptomycin 8 to 10 mg/kg in children aged 2 to 6 years with suspected or proved gram-positive infections. *Pediatr Infect Dis J* 2011;**30**:712–14.
11. Ardura MI, Mejias A, Katz KS, et al. Daptomycin therapy for invasive gram-positive bacterial infections in children. *Pediatr Infect Dis J* 2007;**26**:1128–32.
14. Armstrong AW, Ulukan S, Weiner M, et al. A randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of rifaximin for the prevention of travelers' diarrhea in US military personnel deployed to Incirlik Air Base, Incirlik, Turkey. *J Travel Med* 2010;**17**:392–4.
29. Biek D, Critchley IA, Riccobene TA, et al. Ceftaroline fosamil: a novel broad-spectrum cephalosporin with expanded anti-Gram-positive activity. *J Antimicrob Chemother* 2010;**65**:9–16.
44. Bottenberg MM, Hegge KA, Eastman DY, et al. Metronidazole-induced encephalopathy: a case report and review of the literature. *J Clin Pharmacol* 2011;**51**:112–16.
48. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Disease Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011:e1–52.
65. Center for Disease Control and Prevention. 2012. Active bacterial core surveillance report, Emerging Infections Program Network, *Streptococcus pneumoniae*. Atlanta: CDC; 2010.

66. Chahine EB, Ferril MJ, Poulakos MN. Doripenem: a new carbapenem antibiotic. *Am J Health Syst Pharm* 2010;**67**: 2015–24.
78. Corrado ML. Integrated safety summary of CANVAS 1 and 2 trials: phase III, randomized, double-blind studies evaluating ceftaroline fosamil for the treatment of patients with complicated skin and skin structure infections. *J Antimicrob Chemother* 2010;**65**(Suppl. 4):iv6771.
81. Crook DW, Walker AS, Kean Y, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection: metaanalysis of pivotal randomized controlled trials. *Clin Infect Dis* 2012;**55**(Suppl. 2): S93–103.
99. Falagas ME, Karageorgopoulos DE. Extended-spectrum β–lactamase-producing organisms. *J Hosp Infect* 2009;**73**:345–54.
103. Figueroa I, Johnson S, Sambol SP, et al. Relapse versus reinfection: recurrent *Clostridium difficile* infection following treatment with fidaxomicin or vancomycin. *Clin Infect Dis* 2012;**55**(Suppl. 2): S104–9.
104. File TM, Low DE, Eckburg PB, et al. FOCUS 1: a randomized, double-blinded, multicentre, phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. *J Antimicrob Chemother* 2011;**66**(Suppl. 3): iii19–32.
126. Griffith DE, Aksamit T, Brown-Elliot BA, et al. Diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. An official ATS/IDSA statement. *Am J Respir Crit Care Med* 2007;**175**:367–416.
128. Gruchalla R. Understanding drug allergies. *J Allergy Clin Immunol* 2000;**105**:637–44.
143. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009;**360**:244–56.
152. Jacoby GA, MunozPrice LS. The new β-lactamases. *N Engl J Med* 2005;**352**:380–91.
154. Jenkins SG, Farrell DJ. Increase in pneumococcus macrolide resistance, United States. *Emerg Infect Dis* 2009;**15**:1260–4.
180. Krievins D, Brandt R, Hawser S, et al. Multicenter, randomized study of the efficacy and safety of intravenous iclaprim in complicated skin and skin structure infections. *Antimicrob Agents Chemother* 2009;**53**:2834–40.
194. Lofmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin Infect Dis* 2010;**50**:S16–23.
196. Low DE, File TM, Eckburg PB, et al. FOCUS 2: a randomized, double-blinded, multicentre, phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. *J Antimicrob Chemother* 2011;**66**(Suppl. 3): iii33–44.
201. MacGowan A. Revising beta-lactams PK/PD improves dosing of old antibiotics. *Curr Opin Pharmacol* 2011;**11**:470–6.
234. Noskin GA. Tigecycline: a new glycylcycline for treatment of serious infections. *Clin Infect Dis* 2005;**41**(Suppl. 5):S303–14.
242. Paterson DL, DePestel DD. Doripenem. *Clin Infect Dis* 2009;**49**:291–8.
249. Pickering LK, Baker CJ, Long SS, et al, editors. Antimicrobial prophylaxis. In: *Red book: American Academy of Pediatrics, 2012 report of the Committee on Infectious Diseases*. 29th ed, Elk Grove Village, Ill: American Academy of Pediatrics, 2012, p, 872.
250. Plosker GL. Aztreonam lysine for inhalation solution in cystic fibrosis. *Drugs* 2010;**70**:1843–55.
257. Reinert RR, Low DE, Rossi F, et al. Antimicrobial susceptibility among organisms from the Asia/Pacific rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. *J Antimicrob Chemother* 2007;**60**: 1018–29.
269. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am J Health Syst Pharm* 2009;**66**: 82–98.
275. Saravolatz LD, Stein GE, Johnson LB. Telavancin: a novel lipoglycopeptide. *Clin Infect Dis* 2009;**49**:1908–14.
286. Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intraabdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**: 133–64.
288. Steed ME, Rybak MJ. Ceftaroline: a new cephalosporin with activity against resistant grampositive pathogens. *Pharmacotherapy* 2010;**30**:375–89.
290. Sterling TR, Villarino ME, Borisov AS, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. *N Engl J Med* 2011;**365**:2155–66.
291. Talbot GH, Thye D, Das A, et al. Phase 2 study of ceftaroline versus standard therapy in treatment of complicated skin and skin structure infections. *Antimicrob Agents Chemother* 2007;**51**: 3612–16.
295. Techasaensiri C, Messina AF, Katz K, et al. Epidemiology and evolution of invasive pneumococcal disease caused by multidrug resistant serotypes of 19A in the 8 years after implementation of pneumococcal conjugate vaccine immunization in Dallas, Texas. *Pediatr Infect Dis J* 2010;**29**:294–300.
297. Tenover FC, Moellering RC Jr. The rationale for revising the clinical and laboratory standards institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clin Infect Dis* 2007;**44**:1208–2015.
306. Tornøe CW, Tworzyanski JJ, Imoisili MA, et al. Optimising piperacillin/tazobactam dosing in paediatrics. *Int J Antimicrob Agents* 2007;**30**:320–4.
314. Wang P, Hu F, Xiong Z, et al. Susceptibility of extended-spectrum beta-lactamase-producing Enterobacteriaceae according to the new CLSI breakpoints. *J Clin Microbiol* 2011;**49**:3127–31.
315. Whitman CB, Czosnowski QA. Fidaxomicin for the treatment of *Clostridium difficile* infections. *Ann Pharmacother* 2012;**46**: 219–28.
320. Zeitler K, Salvas B, Stevens V, et al. Aztreonam lysine for inhalation: new formulation of an old antibiotic. *Am J Health Syst Pharm* 2012;**60**:107–15.
325. Zuckerman JM, Qamar F, Bono BR. Macrolides, ketolides and glycylcyclines: azithromycin, clarithromycin, telithromycin, tigecycline. *Infect Dis Clin N Am* 2009;**23**:997–1026.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdel-Rahman SM, Chandorkar G, Akins RL, et al. Single-dose pharmacokinetics and tolerability of daptomycin 8 to 10 mg/kg in children aged 2 to 6 years with suspected or proved grampositive infections. *Pediatr Infect Dis J* 2011;**30**:13.
2. Abramson JS, Givner LB. Should tetracycline be contraindicated for therapy of presumed Rocky Mountain spotted fever in children less than 9 years of age? Editorial. *Pediatrics* 1990;**86**:123–4.
3. Ackerman BH, Vannier AM, Eudy EB. Analysis of vancomycin time-kill studies with *Staphylococcus* species by using a curve stripping program to describe the relationship between concentration and pharmacodynamic response. *Antimicrob Agents Chemother* 1992;**36**:1766–9.
4. Adachi JA, DuPont HL. Rifaximin: a novel nonabsorbed rifamycin for gastrointestinal disorders. *Clin Infect Dis* 2006;**42**:541–7.
5. Aldridge KE, Sanders CV. Susceptibility trending of blood isolates of the *Bacteroides fragilis* group over a 12-year period to clindamycin, ampicillin-sulbactam, cefoxitin, imipenem, and metronidazole. *Anaerobe* 2002;**8**:301–5.
6. Ambrose PJ. Clinical pharmacokinetics of chloramphenicol and chloramphenicol succinate. *Clin Pharmacokinet* 1984;**9**:222–38.
7. American Academy of Pediatrics Committee on Drugs. Topical antibiotics. *Pediatrics* 1977;**59**(Suppl.):1041–2.
8. American Academy of Pediatrics Committee on Infectious Diseases. The use of systemic fluoroquinolones. *Pediatrics* 2006;**118**:1287–92.
9. American Academy of Pediatrics Committee on School Health. Guidelines for the administration of medication in school. *Pediatrics* 2003;**112**:697–9.
10. American Academy of Pediatrics Subcommittee on Management of Acute Otitis Media. Diagnosis and management of acute otitis media. *Pediatrics* 2004;**113**:1451–65.
11. Ardura MI, Mejias A, Katz KS, et al. Daptomycin therapy for invasive gram-positive bacterial infections in children. *Pediatr Infect Dis J* 2007;**26**:1128–32.
12. Arguedas A, Dagan R, Leibovitz E, et al. A multicenter, open label, double tympanocentesis study of high dose cefdinir in children with acute otitis media at high risk of persistent or recurrent infection. *Pediatr Infect Dis J* 2006;**25**:211–18.
13. Arguedas A, Emparanza P, Schwartz RH, et al. A randomized, multicenter, double blind, double dummy trial of single dose azithromycin versus high dose amoxicillin for treatment of uncomplicated acute otitis media. *Pediatr Infect Dis J* 2005;**24**:153–61.
14. Armstrong AW, Ulukan S, Weiner M, et al. A randomized, double-blind, placebo-controlled study evaluating the efficacy and safety of rifaximin for the prevention of travelers' diarrhea in US military personnel deployed to Incirlik Air Base, Incirlik, Turkey. *J Travel Med* 2010;**17**:392–4.
15. Assael BM, Parini R, Rusconi F. Ototoxicity of aminoglycoside antibiotics in infants and children. *Pediatr Infect Dis J* 1982;**1**:357–65.
16. Aubry A, Porcher R, Bottero J, et al. Occurrence and kinetics of false-positive *Aspergillus galactomannan* test results following treatment with beta-lactam antibiotics in patients with hematological disorders. *J Clin Microbiol* 2006;**44**:389–94.
17. Ayala J, Quesada A, Vadillo S, et al. Penicillin-binding proteins of *Bacteroides fragilis* and their role in the resistance to imipenem of clinical isolates. *J Med Microbiol* 2005;**54**:1055–64.
18. Azimi PH. Clinical and laboratory investigation of cefamandole therapy of infections in infants and children. *J Infect Dis* 1978;**137**(Suppl.):S155–60.
19. Azimi PH, Barson WJ, Janner D, et al. Efficacy and safety of ampicillin/sulbactam and cefuroxime in the treatment of serious skin and skin structure infections in pediatric patients: UNASYN Pediatric Study Group. *Pediatr Infect Dis J* 1999;**18**:609–13.
20. Baddour LM, Wilson WR, Bayer AS, et al. Infective endocarditis: diagnosis, antimicrobial therapy, and management of complications—a statement for health care professionals from the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease, Council on Cardiovascular Disease in the Young, and the Councils on Clinical Cardiology, Stroke, and Cardiovascular Surgery and Anesthesia, American Heart Association, endorsed by the Infectious Diseases Society of America. *Circulation* 2005;**111**:e394–434.
21. Bahal N, Nahata MC. The new macrolide antibiotics: azithromycin, clarithromycin, dirithromycin, and roxithromycin. *Ann Pharmacother* 1992;**26**:46–55.
22. Banfi A, Gabriele G, Hill-Juarez JM, et al. Multinational comparative trial of ceftibuten and trimethoprim-sulfamethoxazole in the treatment of children with complicated or recurrent urinary tract infections: Ceftibuten Urinary Tract Infection International Study Group. *Pediatr Infect Dis J* 1993;**12**(Suppl.):S84–91.
23. Barr WH, Affrime M, Lin CC, et al. Pharmacokinetics of ceftibuten in children. *Pediatr Infect Dis J* 1995;**14**(Suppl.):S93–101.
24. Barry AL, Jones RN. Cefixime: spectrum of antibacterial activity against 16,016 clinical isolates. *Pediatr Infect Dis J* 1987;**6**:954–7.
25. Bass JW, Crast FW, Knowles CR, et al. Streptococcal pharyngitis in children: a comparison of four treatment schedules with intramuscular penicillin G benzathine. *JAMA* 1976;**235**:1112–16.
26. Bass JW, Freitas BC, Freitas AD, et al. Prospective randomized double blind placebo-controlled evaluation of azithromycin for treatment of catscratch disease. *Pediatr Infect Dis J* 1998;**17**:447–52.
27. Beall GN. Penicillins. In: Saxon, A., moderator: Immediate hypersensitivity reactions to beta-lactam antibiotics. *Ann Intern Med* 1987;**107**:204–15.
28. Bergeson PS, Singer SA, Kaplan AM. Intramuscular injections in children. *Pediatrics* 1982;**70**:944–8.
29. Biek D, Critchley IA, Riccobene TA, et al. Ceftaroline fosamil: a novel broad-spectrum cephalosporin with expanded anti-Gram positive activity. *J Antimicrob Chemother* 2010;**65**:9–16.
30. Bilukha OO, Rosenstein N. Prevention and control of meningococcal disease: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2005;**54**:121.
31. Birnbaum J, Stapley EO, Miller AK, et al. Cefoxitin, a semisynthetic cephamycin: a microbiological overview. *J Antimicrob Chemother* 1978;**4**:15–32.
32. Black JR, Feinberg J, Murphy RL, et al. Clindamycin and primaquine therapy for mild-to-moderate episodes of *Pneumocystis carinii* pneumonia in patients with AIDS: AIDS Clinical Trials Group 044. *Clin Infect Dis* 1994;**18**:905–13.
33. Blake MJ, Abdel-Rahman SM, Jacobs RF, et al. Pharmacokinetics of rifapentine in children. *Pediatr Infect Dis J* 2006;**25**:405–9.
34. Bloomfield P, Hodson EM, Craig JC. Antibiotics for acute pyelonephritis in children. *Cochrane Database Syst Rev* 2005;(1):CD003772.
35. Bluestone CD. Efficacy of ofloxacin and other oto-topical preparations for chronic suppurative otitis media in children. *Pediatr Infect Dis J* 2001;**20**:111–15.
36. Blumer JL. Cefixime. *Drug Ther* 1989;**19**:60–84.
37. Blumer JL. Pharmacokinetic determinants of carbapenem therapy in neonates and children. *Pediatr Infect Dis J* 1996;**15**:733–7.
38. Blumer JL. Ticarcillin/clavulanate for the treatment of serious infections in hospitalized pediatric patients. *Pediatr Infect Dis J* 1998;**17**:1211–15.
39. Blumer JL, Reed MD, Knupp C. Review of the pharmacokinetics of cefepime in children. *Pediatr Infect Dis J* 2001;**20**:341–6.
40. Blumer JL, Reed MD, Kearns GL, et al. Sequential, single-dose pharmacokinetic evaluation of meropenem in hospitalized infants and children. *Antimicrob Agents Chemother* 1995;**39**:1721–5.
41. Boguniewicz M, Leung DY. Hypersensitivity reactions to antibiotics commonly used in children. *Pediatr Infect Dis J* 1995;**14**:221–31.
42. Bonacorsi S, Bingen E. Bactericidal activity of erythromycin associated with sulphisoxazole against the infectious agents most frequently responsible for acute infantile otitis media [Letter]. *J Antimicrob Chemother* 1994;**33**:885–6.
43. Bonsu BK, Shuler L, Sawicki L, et al. Susceptibility of recent bacterial isolates to cefdinir and selected antibiotics among children with urinary tract infections. *Acad Emerg Med* 2006;**13**:76–81.
44. Bottenberg MM, Hegge KA, Eastman DY, et al. Metronidazole-induced encephalopathy: a case report and review of the literature. *J Clin Pharmacol* 2011;**51**:112–16.
45. Bow EJ, Rotstein C, Noskin GA, et al. A randomized, open-label, multicenter comparative study of the efficacy and safety of piperacillin-tazobactam and cefepime for the empirical treatment of febrile neutropenic episodes in patients with hematologic malignancies. *Clin Infect Dis* 2006;**43**:447–59.

46. Bradley JS. Meropenem: A new, extremely broad-spectrum beta-lactam antibiotic for serious infections in pediatrics. *Pediatr Infect Dis J* 1997;**16**:263–8.
47. Bradley JS, Arrieta A. Empiric use of cefepime in the treatment of lower respiratory tract infections in children. *Pediatr Infect Dis J* 2001;**20**:347–53.
48. Bradley JS, Faulkner KL, Klaugman KP. Efficacy, safety and tolerability of meropenem as empiric antibiotic therapy in hospitalized pediatric patients. *Pediatr Infect Dis J* 1996;**15**: 749–57.
49. Bradley JS, Byington CL, Shah SS, et al. The management of community-acquired pneumonia in infants and children older than 3 months of age: clinical practice guidelines by the Pediatric Infectious Disease Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2011:e1–52.
50. Bratzler DW, Houck PM. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Clin Infect Dis* 2004;**38**:1706–15.
51. Braun P. Hepatotoxicity of erythromycin. *J Infect Dis* 1969;**119**: 300–6.
52. Brook I. Pseudomembranous colitis in children. *J Gastroenterol Hepatol* 2005;**20**:182–6.
53. Brown-Elliott BA, Crist CJ, Mann LB, et al. In vitro activity of linezolid against slowly growing nontuberculous mycobacteria. *Antimicrob Agents Chemother* 2003;**47**:1736–8.
54. Brummett RE. Ototoxicity of vancomycin and analogues. *Otolaryngol Clin North Am* 1993;**26**:821–8.
55. Buckingham SC, Davis Y, English BK. Pneumococcal susceptibility to meropenem in a midsouth children's hospital. *South Med J* 2002;**95**:1293–6.
56. Burchall JJ. Mechanism of action of trimethoprim-sulfamethoxazole. II. *J Infect Dis* 1973;**128**(Suppl.):S437–41.
57. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for beta-lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother* 1995;**39**:1211–33.
58. Bush LM, Johnson CC. Ureidopenicillins and beta-lactam/beta-lactamase inhibitor combinations. *Infect Dis Clin North Am* 2000;**14**:409–33.
59. Cantu TG, Yamanaka Yuen NA, Lietman PS. Serum vancomycin concentrations: reappraisal of their clinical value. *Clin Infect Dis* 1994;**18**:533–43.
60. Cao XT, Kneen R, Nguyen TA, et al. A comparative study of ofloxacin and cefixime for treatment of typhoid fever in children: The Dong Nai Pediatric Center Typhoid Study Group. *Pediatr Infect Dis J* 1999;**18**:245–8.
61. Capparelli E, Hochwald C, Rasmussen M, et al. Population pharmacokinetics of cefepime in the neonate. *Antimicrob Agents Chemother* 2005;**49**:2760–6.
62. Casey JR, Pichichero ME. Metaanalysis of cephalosporin versus penicillin treatment of group A streptococcal tonsillopharyngitis in children. *Pediatrics* 2004;**113**:866–82.
63. Centers for Disease Control and Prevention. Preventing the spread of vancomycin resistance: a report from the Hospital Infection Control Practices Advisory Committee prepared by the Subcommittee on Prevention and Control of Antimicrobial-Resistant Microorganisms in Hospitals; comment period and public meeting—CDC. *Fed Regist* 1994;**59**:25758–63.
64. Centers for Disease Control and Prevention. Effect of new susceptibility breakpoints on reporting of resistance in *Streptococcus pneumoniae:* United States, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:152–4.
65. Center for Disease Control and Prevention. *2012. Active bacterial core surveillance Report, Emerging Infections Program Network*, Streptococcus pneumoniae. Atlanta: CDC; 2010.
66. Chahine EB, Ferril MJ, Poulakos MN. Doripenem: a new carbapenem antibiotic. *Am J Health Syst Pharm* 2010;**67**:2015–24.
67. Chavez-Bueno S, Bozdogan B, Katz K, et al. Inducible clindamycin resistance and molecular epidemiologic trends of pediatric community-acquired methicillin-resistant *Staphylococcus aureus* in Dallas, Texas. *Antimicrob Agents Chemother* 2005;**49**:2283–8.
68. Chen SF. Staphylococcus aureus decolonization. *Pediatr Infect Dis J* 2005;**24**:79–80.
69. Chesney PJ, Davis Y, English BK, et al. Occurrence of *Streptococcus pneumoniae* meningitis during vancomycin and cefotaxime therapy of septicemia in a patient with sickle cell disease. *Pediatr Infect Dis J* 1995;**14**:1013–15.
70. Clark RH, Bloom BT, Spitzer AR, et al. Empiric use of ampicillin and cefotaxime, compared with ampicillin and gentamicin, for neonates at risk for sepsis is associated with an increased risk of neonatal death. *Pediatrics* 2006;**117**:67–74.
71. Clarke AM, Zemcov SJ. Clavulanic acid in combination with ticarcillin: an in-vitro comparison with other beta-lactams. *J Antimicrob Chemother* 1984;**13**:121–8.
72. Cohen R. Clinical experience with cefpodoxime proxetil in acute otitis media. *Pediatr Infect Dis J* 1995;**14**(Suppl.):S12–18.
73. Cohen R, Reinert P, De La Rocque F, et al. Comparison of two dosages of azithromycin for three days versus penicillin V for ten days in acute group A streptococcal tonsillopharyngitis. *Pediatr Infect Dis J* 2002;**21**:297–303.
74. Collins MD, Dajani AS, Kim KS, et al. Comparison of ampicillin/sulbactam plus aminoglycoside vs. ampicillin plus clindamycin plus aminoglycoside in the treatment of intraabdominal infections in children: The Multicenter Group. *Pediatr Infect Dis J* 1998; **17**(Suppl.):S15–18.
75. Cometta A, Calandra T, Gaya H, et al. Monotherapy with meropenem versus combination therapy with ceftazidime plus amikacin as empiric therapy for fever in granulocytopenic patients with cancer. *Antimicrob Agents Chemother* 1996;**40**:1108–15.
76. Conte JE Jr, Golden JA, Duncan S, et al. Intrapulmonary pharmacokinetics of clarithromycin and of erythromycin. *Antimicrob Agents Chemother* 1995;**39**:334–8.
77. Contopoulos-Ioannidis DG, Giotis ND, Baliatsa DV, et al. Extended-interval aminoglycoside administration for children: a metaanalysis. *Pediatrics* 2004;**114**:e111–18.
78. Corrado ML. Integrated safety summary of CANVAS 1 and 2 trials: phase III, randomized, doubleblind studies evaluating ceftaroline fosamil for the treatment of patients with complicated skin and skin structure infections. *J Antimicrob Chemother* 2010; **65**(Suppl. 4):iv67–71.
79. Craig WA. The pharmacology of meropenem, a new carbapenem antibiotic. *Clin Infect Dis* 1997;**24**(Suppl. 2):S266–75.
80. Craig WA. Basic pharmacodynamics of antibacterials with clinical applications to the use of beta-lactams, glycopeptides, and linezolid. *Infect Dis Clin North Am* 2003;**17**:479–501.
81. Crook DW, Walker AS, Kean Y, et al. Fidaxomicin versus vancomycin for *Clostridium difficile* infection: metaanalysis of pivotal randomized controlled trials. *Clin Infect Dis* 2012;**55**(Suppl. 2): S93–103.
82. Dajani AS. Pharyngitis/tonsillitis: European and United States experience with cefpodoxime proxetil. *Pediatr Infect Dis J* 1995; **14**(Suppl.):S7–11.
83. Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis: recommendations by the American Heart Association. *Circulation* 1997;**96**:358–66.
84. Dalhoff A, Janjic N, Echols R. Redefining penems. *Biochem Pharmacol* 2006;**71**:1085–95.
85. Darouiche RO, Raad II, Heard SO, et al. A comparison of two antimicrobial-impregnated central venous catheters. *N Engl J Med* 1999;**340**:1–8.
86. Davies JE. Resistance to aminoglycosides: mechanisms and frequency. *Rev Infect Dis* 1983;**5**(Suppl. 2):S261–6.
87. Dawson CR, Schachter J, Sallam S, et al. A comparison of oral azithromycin with topical oxytetracycline/polymyxin for the treatment of trachoma in children. *Clin Infect Dis* 1997;**24**: 363–8.
88. De Broe ME, Giuliano RA, Verpooten GA. Choice of drug and dosage regimen: two important risk factors for aminoglycoside nephrotoxicity. *Am J Med* 1986;**80**:115–18.
89. de Man P, Verhoeven BA, Verbrugh HA, et al. An antibiotic policy to prevent emergence of resistant bacilli. *Lancet* 2000;**355**: 973–8.
90. Denny FW, Wannamaker LW, Brink WR, et al. Prevention of rheumatic fever: treatment of the preceding streptococcal infection. *J Am Med Assoc* 1950;**143**:151–3.
91. Deville JG, Adler S, Azimi PH, et al. Linezolid versus vancomycin in the treatment of known or suspected resistant grampositive infections in neonates. *Pediatr Infect Dis J* 2003;**22**(Suppl):S158–63.
92. Diekema DJ, Jones RN. Oxazolidinone antibiotics. *Lancet* 2001;**358**:1975–82.
93. Disney FA, Hanfling MJ, Hausinger SA. Loracarbef (LY163892) vs. penicillin VK in the treatment of streptococcal pharyngitis and tonsillitis. *Pediatr Infect Dis J* 1992;**11**(Suppl.):S20–6.

94. Doern G. In vitro activity of loracarbef and effects of susceptibility test methods. *Am J Med* 1992;**92**(Suppl.):S7–15.
95. Dohar J, Giles W, Roland P, et al. Topical ciprofloxacin/dexamethasone superior to oral amoxicillin/clavulanic acid in acute otitis media with otorrhea through tympanostomy tubes. *Pediatrics* 2006;**118**:e561–9.
96. Draghi DC, Sheehan DJ, Hogan P, et al. In vitro activity of linezolid against key grampositive organisms isolated in the united states: results of the LEADER 2004 surveillance program. *Antimicrob Agents Chemother* 2005;**49**:5024–32.
97. Drusano GL. Role of pharmacokinetics in the outcome of infections. *Antimicrob Agents Chemother* 1988;**32**:289–97.
98. Falagas ME, Karageorgopoulos DE. Extended-spectrum β-lactamase-producing organisms. *J Hosp Infect* 2009;**73**:345–54.
99. Falagas ME, Kasiakou SK. Colistin: The revival of polymyxins for the management of multidrug-resistant gram-negative bacterial infections. *Clin Infect Dis* 2005;**40**:1333–41.
100. Faulkner RD, Yacobi A, Barone JS, et al. Pharmacokinetic profile of cefixime in man. *Pediatr Infect Dis J* 1987;**6**:963–70.
101. Fedler KA, Biedenbach DJ, Jones RN. Assessment of pathogen frequency and resistance patterns among pediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on 3 continents. *Diagn Microbiol Infect Dis* 2006;**56**:427–36.
102. Feigin RD, Pickering LK, Anderson D, et al. Clindamycin treatment of osteomyelitis and septic arthritis in children. *Pediatrics* 1975;**55**:213–23.
103. Figueroa I, Johnson S, Sambol SP, et al. Relapse versus reinfection: recurrent clostridium difficile infection following treatment with fidaxomicin or vancomycin. *Clin Infect Dis* 2012;**55**(Suppl. 2):S104–109.
104. File TM, Low DE, Eckburg PB, et al. FOCUS 1: a randomized, double-blinded, multicentre, phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. *J Antimicrob Chemother* 2011;**66**(Suppl. 3):iii19–32.
105. Finland M. Twenty-fifth anniversary of the discovery of aureomycin: the place of the tetracyclines in antimicrobial therapy. *Clin Pharmacol Ther* 1974;**15**:3–8.
106. Fisman DN, Kaye KM. Once-daily dosing of aminoglycoside antibiotics. *Infect Dis Clin North Am* 2000;**14**:475–87.
107. Foshee WS, Qvarnberg Y. Comparative United States and European trials of loracarbef in the treatment of acute otitis media. *Pediatr Infect Dis J* 1992;**11**(Suppl.):S12–19.
108. Foster JA, Sylvia LM. Doxycycline-induced esophageal ulceration. *Ann Pharmacother* 1994;**28**:1185–7.
109. Fox PM, Lampen RJ, Stumpf KS, et al. Successful therapy of experimental endocarditis caused by vancomycin-resistant *Staphylococcus aureus* with a combination of vancomycin and beta-lactam antibiotics. *Antimicrob Agents Chemother* 2006;**50**:2951–6.
110. Frank AL, Marcinak JF, Mangat PD, et al. Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2002;**21**:530–4.
111. Freeman AF, Shulman ST. Macrolide resistance in group A *Streptococcus*. *Pediatr Infect Dis J* 2002;**21**:1158–60.
112. Freeman CD, Nicolau DP, Belliveau PP, et al. Once-daily dosing of aminoglycosides: review and recommendations for clinical practice. *J Antimicrob Chemother* 1997;**39**:677–86.
113. Frenck RW Jr, Mansour A, Nakhla I, et al. Short-course azithromycin for the treatment of uncomplicated typhoid fever in children and adolescents. *Clin Infect Dis* 2004;**38**:951–7.
114. Friedland IR, Shelton S, McCracken GH Jr. Chloramphenicol in penicillin-resistant pneumococcal meningitis [Letter]. *Lancet* 1993;**342**:240–1.
115. Galimand M, Gerbaud G, Guibourdenche M, et al. High-level chloramphenicol resistance in *Neisseria meningitidis*. *N Engl J Med* 1998;**339**:868–74.
116. Gibreel A, Taylor DE. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *J Antimicrob Chemother* 2006;**58**:243–55.
117. Gilbert DN, Dworkin RJ, Raber SR, et al. Drug therapy: outpatient parenteral antimicrobial-drug therapy. *N Engl J Med* 1997;**18**:829–38.
118. Gillum JG, Israel DS, Polk RE. Pharmacokinetic drug interactions with antimicrobial agents. *Clin Pharmacokinet* 1993;**25**:450–82.
119. Girgis NI, Sultan Y, Hammad O, et al. Comparison of the efficacy, safety and cost of cefixime, ceftriaxone and aztreonam in the treatment of multidrug-resistant *Salmonella typhi* septicemia in children. *Pediatr Infect Dis J* 1995;**14**:603–5.
120. Goetz MB, Sayers J. Nephrotoxicity of vancomycin and aminoglycoside therapy separately and in combination. *J Antimicrob Chemother* 1993;**32**:325–34. [Published erratum appears in J Antimicrob. *Chemother* 32:925, 1993.]
121. Golan Y, McDermott LA, Jacobus NV, et al. Emergence of fluoroquinolone resistance among *Bacteroides* species. *J Antimicrob Chemother* 2003;**52**:208–13.
122. Gordon EM, Blumer JL. Rationale for single and high dose treatment regimens with azithromycin. *Pediatr Infect Dis J* 2004;**23**(Suppl.):S102–7.
123. Gorwitz RJ, Jernigan DB, Powers JH, et al. *Strategies for clinical management of MRSA in the community: summary of an experts' meeting convened by the Centers for Disease Control and Prevention*. 2006. Available at http://www.cdc.gov/ncidod/dhqp/ar_mrsa_ca_prevention.html.
124. Granizo JJ, Aguilar L, Casal J, et al. *Streptococcus pyogenes* resistance to erythromycin in relation to macrolide consumption in Spain (1986-1997). *J Antimicrob Chemother* 2000;**46**:959–64.
125. Green M, Li KI, Wald ER, et al. Duration of rifampin chemoprophylaxis for contacts of patients infected with *Haemophilus influenzae* type B. *Antimicrob Agents Chemother* 1992;**36**:545–7.
126. Griffith DE, Aksamit T, Brown-Elliot BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;**175**:367–416.
127. Grossman ER, Walchek A, Freedman H. Tetracyclines and permanent teeth: the relation between dose and tooth color. *Pediatrics* 1971;**47**:567–70.
128. Gruchalla R. Understanding drug allergies. *J Allergy Clin Immunol* 2000;**105**:637–44.
129. Guay DR. Ceftibuten: a new expanded-spectrum oral cephalosporin. *Ann Pharmacother* 1997;**31**:1022–33.
130. Guay DR. Cefdinir: an advanced-generation, broad-spectrum oral cephalosporin. *Clin Ther* 2002;**24**:473–89.
131. Guay DR, Craft JC. Overview of the pharmacology of clarithromycin suspension in children and a comparison with that in adults. *Pediatr Infect Dis J* 1993;**12**(Suppl.):S106–11.
132. Haight TH, Finland M. Observations on mode of action of erythromycin. *Proc Soc Exp Biol Med* 1952;**81**:188–93.
133. Handwerger S, Tomasz A. Antibiotic tolerance among clinical isolates of bacteria. *Rev Infect Dis* 1985;**7**:368–86.
134. Hawser S, Lociuro S, Islam K. Dihydrofolate reductase inhibitors as antibacterial agents. *Biochem Pharmacol* 2006;**71**:941–8.
135. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. *J Pediatr* 1978;**93**:62–6.
136. Hitchings GH. Mechanism of action of trimethoprim-sulfamethoxazole. I. *J Infect Dis* 1973;**128**(Suppl.):S433–6.
137. Hoberman A, Dagan R, Leibovitz E, et al. Large dosage amoxicillin/clavulanate, compared with azithromycin, for the treatment of bacterial acute otitis media in children. *Pediatr Infect Dis J* 2005;**24**:525–32.
138. Hoberman A, Wald ER, Hickey RW, et al. Oral versus initial intravenous therapy for urinary tract infections in young febrile children. *Pediatrics* 1999;**104**:79–86.
139. Hoiby N, Ciofu O, Jensen T, et al. Use of carbapenems and other antibiotics for pulmonary infections in patients with cystic fibrosis. *Pediatr Infect Dis J* 1996;**15**:738–43.
140. Holas C, Chiu YL, Notario G, et al. A pooled analysis of seven randomized crossover studies of the palatability of cefdinir oral suspension versus amoxicillin/clavulanate potassium, cefprozil, azithromycin, and amoxicillin in children aged 4 to 8 years. *Clin Ther* 2005;**27**:1950–60.
141. Honein MA, Paulozzi LJ, Himelright IM, et al. Infantile hypertrophic pyloric stenosis after pertussis prophylaxis with erythromycin: a case review and cohort study. *Lancet* 1999;**354**:2101–5.
142. Howie VM, Owen MJ. Bacteriologic and clinical efficacy of cefixime compared with amoxicillin in acute otitis media. *Pediatr Infect Dis J* 1987;**6**:989–91.
143. Hsu HE, Shutt KA, Moore MR, et al. Effect of pneumococcal conjugate vaccine on pneumococcal meningitis. *N Engl J Med* 2009;**360**:244–56.

144. Hulten KG, Kaplan SL, Gonzalez BE, et al. Three-year surveillance of community onset health care–associated *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2006;**25**:349–53.
145. Humbert P, Treffel P, Chapuis JF, et al. The tetracyclines in dermatology. *J Am Acad Dermatol* 1991;**25**:691–7.
146. Huovinen P. Resistance to trimethoprim-sulfamethoxazole. *Clin Infect Dis* 2001;**32**:1608–14.
147. Hurst M, Lamb HM. Meropenem: a review of its use in patients in intensive care. *Drugs* 2000;**59**:653–80.
148. Isenberg SJ, Apt L, Wood M. A controlled trial of povidone-iodine as prophylaxis against ophthalmia neonatorum. *N Engl J Med* 1995;**332**:562–6.
149. Jacobs MR, Johnson CE. Macrolide resistance: an increasing concern for treatment failure in children. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S131–138.
150. Jacobs MR, Bajaksouzian S, Windau A, et al. Susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* to 17 oral antimicrobial agents based on pharmacodynamic parameters: 1998-2001 U S Surveillance Study. *Clin Lab Med* 2004;**24**:503–30.
151. Jacobs RF, Maples HD, Aranda JV, et al. Pharmacokinetics of intravenously administered azithromycin in pediatric patients. *Pediatr Infect Dis J* 2005;**24**:34–9.
152. Jacoby GA, Munoz-Price LS. The new β-lactamases. *N Engl J Med* 2005;**352**:380–91.
153. Jantausch BA, Deville J, Adler S, et al. Linezolid for the treatment of children with bacteremia or nosocomial pneumonia caused by resistant grampositive bacterial pathogens. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S164–71.
154. Jenkins SG, Farrell DJ. Increase in pneumococcus macrolide resistance, United States. *Emerg Infect Dis* 2009;**15**:1260–4.
155. Jimenez-Mejias ME, Pichardo-Guerrero C, Marquez-Rivas FJ, et al. Cerebrospinal fluid penetration and pharmacokinetic/pharmacodynamic parameters of intravenously administered colistin in a case of multidrug-resistant *Acinetobacter baumannii* meningitis. *Eur J Clin Microbiol Infect Dis* 2002;**21**:212–14.
156. Johnson DM, Stilwell MG, Fritsche TR, et al. Emergence of multidrug-resistant *Streptococcus pneumoniae:* report from the SENTRY Antimicrobial Surveillance Program (1999-2003). *Diagn Microbiol Infect Dis* 2006;**56**:69–74.
157. Jonas M, Cunha BA. Minocycline. *Ther Drug Monit* 1982;**4**:137–45.
158. Jones RN. Important and emerging beta-lactamase–mediated resistances in hospital-based pathogens: the Amp C enzymes. *Diagn Microbiol Infect Dis* 1998;**31**:461–6.
159. Jones RN, Sader HS. Update on the cefdinir spectrum and potency against pathogens isolated from uncomplicated skin and soft tissue infections in North America: are we evaluating the orally administered cephalosporins correctly? *Diagn Microbiol Infect Dis* 2006;**55**:351–6.
160. Jones RN, Sader HS, Fritsche TR, et al. Comparisons of parenteral broad-spectrum cephalosporins tested against bacterial isolates from pediatric patients: Report from the SENTRY Antimicrobial Surveillance Program (1998-2004). *Diagn Microbiol Infect Dis* 2006;**57**:109–16.
161. Jorgensen JH, Crawford SA, Fiebelkorn KR. Susceptibility of *Neisseria meningitidis* to 16 antimicrobial agents and characterization of resistance mechanisms affecting some agents. *J Clin Microbiol* 2005;**43**:3162–71.
162. Jungbluth GL, Welshman IR, Hopkins NK. Linezolid pharmacokinetics in pediatric patients: an overview. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S153–7.
163. Kaplan JE, Masur H, Holmes KK. Guidelines for preventing opportunistic infections among HIV-infected persons: 2002—recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. *MMWR Recomm Rep* 2002;**51**:152.
164. Kaplan SL. Treatment of community-associated methicillin-resistant *Staphylococcus aureus* infections. *Pediatr Infect Dis J* 2005;**24**:457–8.
165. Kaplan SL, Afghani B, Lopez P, et al. Linezolid for the treatment of methicillin-resistant *Staphylococcus aureus* infections in children. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S178–85.
166. Kaplan SL, Hulten KG, Gonzalez BE, et al. Three-year surveillance of community-acquired *Staphylococcus aureus* infections in children. *Clin Infect Dis* 2005;**40**:1785–91.
167. Kaye D. Current use for old antibacterial agents: polymyxins, rifampin, and aminoglycosides. *Infect Dis Clin North Am* 2004;**18**:669–89.
168. Kearns GL, Wheeler JG, Childress SH, et al. Serum sickness-like reactions to cefaclor: role of hepatic metabolism and individual susceptibility. *J Pediatr* 1994;**125**:805–11.
169. Keating GM, Perry CM. Ertapenem: a review of its use in the treatment of bacterial infections. *Drugs* 2005;**65**:2151–78.
170. Kessler RE. Cefepime microbiologic profile and update. *Pediatr Infect Dis J* 2001;**20**:335–40.
171. Khan WA, Saha D, Rahman A, et al. Comparison of single-dose azithromycin and 12-dose, 3-day erythromycin for childhood cholera: a randomised, double-blind trial. *Lancet* 2002;**360**:1722–7.
172. Klein JO. Amoxicillin/clavulanate for infections in infants and children: past, present and future. *Pediatr Infect Dis J* 2003;**22**:S139–148.
173. Klein JO, Finland M. The new penicillins. *N Engl J Med* 1963;**269**:1019–25.
174. Klein JO, McCracken GH Jr. Summary: role of a new oral cephalosporin, cefdinir, for therapy of infections of infants and children. *Pediatr Infect Dis J* 2000;**19**(Suppl.):S181–3.
175. Klugman KP, Lonks JR. Hidden epidemic of macrolide-resistant pneumococci. *Emerg Infect Dis* 2005;**11**:802–7.
176. Klugman KP, Dagan R;. Meropenem Meningitis Study Group: Randomized comparison of meropenem with cefotaxime for treatment of bacterial meningitis. *Antimicrob Agents Chemother* 1995;**39**:1140–6.
177. Klugman KP, Friedland IR, Bradley JS. Bactericidal activity against cephalosporin-resistant *Streptococcus pneumoniae* in cerebrospinal fluid of children with acute bacterial meningitis. *Antimicrob Agents Chemother* 1995;**39**:1988–92.
178. Kotra LP, Haddad J, Mobashery S. Aminoglycosides: perspectives on mechanisms of action and resistance and strategies to counter resistance. *Antimicrob Agents Chemother* 2000;**44**:3249–56.
179. Kremsner PG, Winkler S, Brandts C, et al. Clindamycin in combination with chloroquine or quinine is an effective therapy for uncomplicated *Plasmodium falciparum* malaria in children from Gabon. *J Infect Dis* 1994;**169**:467–70.
180. Krievins D, Brandt R, Hawser S, et al. Multicenter, randomized study of the efficacy and safety of intravenous iclaprim in complicated skin and skin structure infections. *Antimicrob Agents Chemother* 2009;**53**:2834–40.
181. Kyaw MH, Lynfield R, Schaffner W, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006;**354**:1455–63.
182. Leader WG, Chandler MH, Castiglia M. Pharmacokinetic optimisation of vancomycin therapy. *Clin Pharmacokinet* 1995;**28**:327–42.
183. Lebel MH. Pharmacology of antimicrobial agents in children with hepatic dysfunction. *Pediatr Infect Dis J* 1986;**5**:686–90.
184. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* 2002;**34**:482–92.
185. Leibovitz E, Piglansky L, Raiz S, et al. Bacteriologic efficacy of a three-day intramuscular ceftriaxone regimen in nonresponsive acute otitis media. *Pediatr Infect Dis J* 1998;**17**:1126–31.
186. Levine BB. Immunologic mechanisms of penicillin allergy: a haptenic model system for the study of allergic diseases of man. *N Engl J Med* 1966;**275**:1115–25.
187. Levine LR. Quantitative comparison of adverse reactions to cefaclor vs. amoxicillin in a surveillance study. *Pediatr Infect Dis J* 1985;**4**:358–61.
188. Levison ME. Pharmacodynamics of antimicrobial agents: bactericidal and postantibiotic effects. *Infect Dis Clin North Am* 1995;**9**:483–95.
189. Li XZ. Quinolone resistance in bacteria: emphasis on plasmid-mediated mechanisms. *Int J Antimicrob Agents* 2005;**25**:453–63.
190. Lieberman P, Kemp S, Oppenheimer J, et al. The diagnosis and management of anaphylaxis: an updated practice parameter. *J Allergy Clin Immunol* 2005;**115**(Suppl.):S483–523.
191. Lin RY. A perspective on penicillin allergy. *Arch Intern Med* 1992;**152**:930–7.
192. Livermore DM. Determinants of the activity of beta-lactamase inhibitor combinations. *J Antimicrob Chemother* 1993;**31**(Suppl. A):916–21.

193. Loeffler AM. Uses of rifampin for infections other than tuberculosis. *Pediatr Infect Dis J* 1999;**18**:631–2.
194. Löfmark S, Edlund C, Nord CE. Metronidazole is still the drug of choice for treatment of anaerobic infections. *Clin Infect Dis* 2010;**50**(Suppl. 1):S16–23.
195. Losek JD, Gyuro J. Pediatric intramuscular injections: do you know the procedure and complications? *Pediatr Emerg Care* 1992;**8**:79–81.
196. Low DE, File TM, Eckburg PB, et al. FOCUS 2: a randomized, doubleblinded, multicentre, phase III trial of the efficacy and safety of ceftaroline fosamil versus ceftriaxone in community-acquired pneumonia. *J Antimicrob Chemother* 2011;**66**(Suppl. 3): iii33–44.
197. Lubani MM, Dudin KI, Sharda DC, et al. A multicenter therapeutic study of 1100 children with brucellosis. *Pediatr Infect Dis J* 1989;**8**:75–8.
198. Luer MS, Hatton J. Vancomycin administration into the cerebrospinal fluid: a review. *Ann Pharmacother* 1993;**27**:912–21.
199. Lundstrom TS, Sobel JD. Antibiotics for grampositive bacterial infections: vancomycin, quinupristin-dalfopristin, linezolid, and daptomycin. *Infect Dis Clin North Am* 2004;**18**:651–68.
200. Lyon BR, Skurray R. Antimicrobial resistance of *Staphylococcus aureus:* genetic basis. *Microbiol Rev* 1987;**51**:88–134.
201. MacGowan A. Revising beta-lactams: PK/PD improves dosing of old antibiotics. *Curr Opin Pharmacol* 2011;**11**:470–6.
202. Marcinak JF, Frank AL. Epidemiology and treatment of community-associated methicillin-resistant *Staphylococcus aureus* in children. *Expert Rev Anti Infect Ther* 2006;**4**:91–100.
203. Marcy SM, Klein JO. The isoxazolyl penicillins: oxacillin, cloxacillin, and dicloxacillin. *Med Clin North Am* 1970;**54**:1127–43.
204. Martin JM, Pitetti R, Maffei F, et al. Treatment of shigellosis with cefixime: two days vs. five days. *Pediatr Infect Dis J* 2000;**19**: 522–6.
205. Martin SI, Kaye KM. Beta-lactam antibiotics: newer formulations and newer agents. *Infect Dis Clin North Am* 2004;**18**:603–19.
206. McCarty JM, Renteria A. Treatment of pharyngitis and tonsillitis with cefprozil: review of three multicenter trials. *Clin Infect Dis* 1992;**14**(Suppl. 2):S224–30.
207. McCracken GH Jr. New era for orally administered antibiotics: use of sequential parenteral-oral antibiotic therapy for serious infectious diseases of infants and children. *Pediatr Infect Dis J* 1987;**6**:951–3.
208. McCracken GH Jr. Microbiologic activity of the newer macrolide antibiotics. *Pediatr Infect Dis J* 1997;**16**:432–7.
209. Meissner HC, Townsend T, Wenman W, et al. Hematologic effects of linezolid in young children. *Pediatr Infect Dis J* 2003; **22**(Suppl.):S186–92.
210. Mingeot-Leclercq MP, Tulkens PM. Aminoglycosides: nephrotoxicity. *Antimicrob Agents Chemother* 1999;**43**:1003–12.
211. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. *Antimicrob Agents Chemother* 1999;**43**: 727–37.
212. Moellering RC Jr. In vitro antibacterial activity of the aminoglycoside antibiotics. *Rev Infect Dis* 1983;**5**(Suppl. 2):S212–31.
213. Moellering RC Jr. Monitoring serum vancomycin levels: Climbing the mountain because it is there? *Editorial Clin Infect Dis* 1994;**18**:544–6. [Published erratum appears in Clin. Infect. Dis. 19:379, 1994.]
214. Moellering RC Jr, Krogstad DJ, Greenblatt DJ. Vancomycin therapy in patients with impaired renal function: a nomogram for dosage. *Ann Intern Med* 1981;**94**:343–6.
215. Mofenson LM. Rifabutin. *Pediatr Infect Dis J* 1998;**17**:71–2.
216. Moosa A, Rubidge CJ. Once daily ceftriaxone vs. chloramphenicol for treatment of typhoid fever in children. *Pediatr Infect Dis J* 1989;**8**:696–9.
217. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med* 2006;**355**:666–74.
218. Moreillon P, Markiewicz Z, Nachman S, et al. Two bactericidal targets for penicillin in pneumococci: autolysis-dependent and autolysis-independent killing mechanisms. *Antimicrob Agents Chemother* 1990;**34**:33–9.
219. Murphy TV, Chrane DF, McCracken GH Jr, et al. Rifampin prophylaxis vs. placebo for household contacts of children with *Haemophilus influenzae* type b disease. *Am J Dis Child* 1983;**137**: 627–32.
220. Murray BE. Vancomycin-resistant enterococcal infections. *N Engl J Med* 2000;**342**:710–21.
221. Murray CK, Hospenthal DR. Treatment of multidrug resistant *Acinetobacter*. *Curr Opin Infect Dis* 2005;**18**:502–6.
222. Murray DL, Singer DA, Singer AB, et al. Cefaclor: a cluster of adverse reactions[Letter]. *N Engl J Med* 1980;**303**:1003.
223. Mustafa MM, Carlson L, Tkaczewski I, et al. Comparative study of cefepime versus ceftazidime in the empiric treatment of pediatric cancer patients with fever and neutropenia. *Pediatr Infect Dis J* 2001;**20**:366–73.
224. Nagar H, Berger SA, Hammar B, et al. Penetration of clindamycin and metronidazole into the appendix and peritoneal fluid in children. *Eur J Clin Pharmacol* 1989;**37**:209–10.
225. Nathan N, Borel T, Djibo A, et al. Ceftriaxone as effective as long-acting chloramphenicol in short-course treatment of meningococcal meningitis during epidemics: a randomised noninferiority study. *Lancet* 2005;**366**:308–13.
226. Nelson JD, Haltalin KC. Amoxicillin is less effective than ampicillin against Shigella in vitro and in vivo: relationship of efficacy to activity in serum. *J Infect Dis* 1974;**129**(Suppl.):S222–7.
227. Nelson JD, Howard JB, Shelton S. Oral antibiotic therapy for skeletal infections of children. I. Antibiotic concentrations in suppurative synovial fluid. *J Pediatr* 1978;**92**:131–4.
228. Neu HC. Antimicrobial activity and human pharmacology of amoxicillin. *J Infect Dis* 1974;**129**(Suppl.):S123–31.
229. Neu HC. Antistaphylococcal penicillins. *Med Clin North Am* 1982;**66**:5160.
230. Neu HC, Fu KP. Cefuroxime, a beta-lactamase-resistant cephalosporin with a broad spectrum of gram-positive and negative activity. *Antimicrob Agents Chemother* 1978;**13**:657–64.
231. Nix DE, Goodwin SD, Peloquin CA, et al. Antibiotic tissue penetration and its relevance: models of tissue penetration and their meaning. *Antimicrob Agents Chemother* 1991;**35**:1947–52.
232. Nix DE, Goodwin SD, Peloquin CA, et al. Antibiotic tissue penetration and its relevance: impact of tissue penetration on infection response. *Antimicrob Agents Chemother* 1991;**35**:1953–9.
233. Nolen TM. Clinical trials of cefprozil for treatment of skin and skin-structure infections: review. *Clin Infect Dis* 1992; **14**(Suppl. 2):S255–63.
234. Noskin GA. Tigecycline: a new glycylcycline for treatment of serious infections. *Clin Infect Dis* 2005;**41**(Suppl. 5):S303–14.
235. Novak R, Henriques B, Charpentier E, et al. Emergence of vancomycin tolerance in *Streptococcus pneumoniae*. *Nature* 1999;**399**: 590–3.
236. Odio CM, Puig JR, Feris JM, et al. Prospective, randomized, investigator-blinded study of the efficacy and safety of meropenem vs. cefotaxime therapy in bacterial meningitis in children. *Pediatr Infect Dis J* 1999;**18**:581–90.
237. O'Grady NP, Alexander M, Dellinger EP, et al. Guidelines for the prevention of intravascular catheter-related infections: Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2002; **51**:129.
238. Olesky M, Zhao S, Rosenberg RL, et al. Porin-mediated antibiotic resistance in *Neisseria gonorrhoeae:* ion, solute, and antibiotic permeation through PIB proteins with penB mutations. *J Bacteriol* 2006;**188**:2300–8.
239. Pai MP, Graci DM, Amsden GW. Macrolide drug interactions: an update. *Ann Pharmacother* 2000;**34**:495–513.
240. Panzer JD, Brown DC, Epstein WL, et al. Clindamycin levels in various body tissues and fluids. *J Clin Pharmacol New Drugs* 1972;**12**:259262.
241. Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. *Am J Med* 2006;**119**(Suppl.):S20–8.
242. Paterson DL, DePestel DD. Doripenem. *Clin Infect Dis* 2009; **49**:291–8.
243. Paul M, Fraser A, Leibovici L. Empirical antibiotic monotherapy for febrile neutropenia: systematic review and metaanalysis of randomized controlled trials—authors' response. *J Antimicrob Chemother* 2006;**58**:479–80.
244. Petz LD. Immunologic crossreactivity between penicillins and cephalosporins: a review. *J Infect Dis* 1978;**137**(Suppl.):S74–9.
245. Pfaller MA, Segreti J. Overview of the epidemiological profile and laboratory detection of extended-spectrum beta-lactamases. *Clin Infect Dis* 2006;**42**(Suppl. 4):S153–63.
246. Pfeiffer RR. Structural features of vancomycin. *Rev Infect Dis* 1981;**3**(Suppl.):S205–9.

247. Pichichero ME, Arguedas A, Dagan R, et al. Safety and efficacy of gatifloxacin therapy for children with recurrent acute otitis media (AOM) and/or AOM treatment failure. *Clin Infect Dis* 2005;**41**:470–8.
248. Pichichero ME, McLinn SE, Gooch WM, et al. Ceftibuten vs. penicillin V in group A beta-hemolytic streptococcal pharyngitis. *Pediatr Infect Dis J* 1995;**14**(Suppl.):S102–7.
249. Pickering LK, Baker CJ, Long SS, et al. Antimicrobial prophylaxis. In: *Red book: report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012, p. 872.
250. Plosker GL. Aztreonam lysine for inhalation solution in cystic fibrosis. *Drugs* 2010;**70**:1843–55.
251. Prober CG, Stevenson DK, Benitz WE. The use of antibiotics in neonates weighing less than 1200 grams. *Pediatr Infect Dis J* 1990;**9**:111–21.
252. Radetsky M. Duration of treatment in bacterial meningitis: a historical inquiry. *Pediatr Infect Dis J* 1990;**9**:29.
253. Rahal JJ Jr, Simberkoff MS. Bactericidal and bacteriostatic action of chloramphenicol against meningeal pathogens. *Antimicrob Agents Chemother* 1979;**16**:13–18.
254. Ramsey BW, Dorkin HL, Eisenberg JD, et al. Efficacy of aerosolized tobramycin in patients with cystic fibrosis. *N Engl J Med* 1993;**328**:1740–6.
255. Ramsey BW, Pepe MS, Quan JM, et al. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis: Cystic Fibrosis Inhaled Tobramycin Study Group. *N Engl J Med* 1999; **340**:23–30.
256. Reed MD, Blumer JL. Clinical pharmacology of antitubercular drugs. *Pediatr Clin North Am* 1983;**30**:177–93.
257. Reinert RR, Low DE, Rossi F, et al. Antimicrobial susceptibility among organisms from the Asia/Pacific rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. *J Antimicrob Chemother* 2007;**60**:1018–29.
258. Rhomberg PR, Jones RN. Contemporary activity of meropenem and comparator broad-spectrum agents: MYSTIC program report from the United States component (2005). *Diagn Microbiol Infect Dis* 2006;**57**:207–15.
259. Rice LB. Antimicrobial resistance in grampositive bacteria. *Am J Infect Control* 2006;**34**(Suppl.):S11–19.
260. Ristuccia AM. Chloramphenicol: clinical pharmacology in pediatrics. *Ther Drug Monit* 1985;**7**:159–67.
261. Roberts MC. Acquired tetracycline and/or macrolide-lincosamides-streptogramin resistance in anaerobes. *Anaerobe* 2003;**9**:63–9.
262. Roberts MC. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* 2005;**245**:195–203.
263. Roberts MC, Sutcliffe J, Courvalin P, et al. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999;**43**:2823–30.
264. Roberts SM, Freeman AF, Harrington SM, et al. Linezolid-resistant *Staphylococcus aureus* in two pediatric patients receiving low-dose linezolid therapy. *Pediatr Infect Dis J* 2006;**25**:562–4.
265. Robicsek A, Strahilevitz J, Jacoby GA, et al. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat Med* 2006;**12**:8388.
266. Rodriguez CA, Atkinson R, Bitar W, et al. Tolerance to vancomycin in pneumococci: detection with a molecular marker and assessment of clinical impact. *J Infect Dis* 2004;**190**:1481–7.
267. Russo TA, Cook S, Gorbach SL. Intramuscular ceftriaxone in home parenteral therapy. *Antimicrob Agents Chemother* 1988;**32**: 1439–40.
268. Rybak M, Lomaestro B, Rotschafer JC, et al. Therapeutic monitoring of vancomycin in adult patients: a consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. *Am J Health Syst Pharm* 2009;**66**: 82–98.
269. Rybak MJ. The efficacy and safety of daptomycin: first in a new class of antibiotics for grampositive bacteria. *Clin Microbiol Infect* 2006;**12**(Suppl. 1):24–32.
270. Sader HS, Fritsche TR, Jones RN. Potency and spectrum trends for cefepime tested against 65746 clinical bacterial isolates collected in North American medical centers: results from the SENTRY Antimicrobial Surveillance Program (1998-2003). *Diagn Microbiol Infect Dis* 2005;**52**:265–73.
271. Saha D, Khan WA, Karim MM, et al. Single-dose ciprofloxacin versus 12-dose erythromycin for childhood cholera: a randomised controlled trial. *Lancet* 2005;**366**:1085–93.
272. Saiman L, Goldfarb J, Kaplan SA, et al. Safety and tolerability of linezolid in children. *Pediatr Infect Dis J* 2003;**22**(Suppl.):S193–200.
273. Saiman L, Marshall BC, Mayer-Hamblett N, et al. Azithromycin in patients with cystic fibrosis chronically infected with *Pseudomonas aeruginosa:* a randomized controlled trial. *JAMA* 2003;**290**: 1749–56.
274. Saivin S, Houin G. Clinical pharmacokinetics of doxycycline and minocycline. *Clin Pharmacokinet* 1988;**15**:355–66.
275. Saravolatz LD, Stein GE, Johnson LB. Telavancin: A novel lipoglycopeptide. *Clin Infect Dis* 2009;**49**:19081914.
276. Saxon A. Immediate hypersensitivity reactions to lactam antibiotics. *Rev Infect Dis* 1983;**5**(Suppl.):S368–78.
277. Schaad UB. Pediatric use of quinolones. *Pediatr Infect Dis J* 1999;**18**:469–70.
278. Schaad UB, Abdus Salam M, Aujard Y, et al. Use of fluoroquinolones in pediatrics: consensus report of an International Society of Chemotherapy Commission. *Pediatr Infect Dis J* 1995;**14**: 1–9.
279. Schaad UB, Eskola J, Kafetzis D, et al. Cefepime vs. ceftazidime treatment of pyelonephritis: a European, randomized, controlled study of 300 pediatric cases. European Society for Pediatric Infectious Diseases (ESPID) Pyelonephritis Study Group. *Pediatr Infect Dis J* 1998;**17**:639–44.
280. Schaad UB, Suter S, Gianella-Borradori A, et al. A comparison of ceftriaxone and cefuroxime for the treatment of bacterial meningitis in children. *N Engl J Med* 1990;**322**:141–7.
281. Schwartz RH, Wientzen RL, Pedreira F, et al. Penicillin V for group A streptococcal pharyngotonsillitis: a randomized trial of seven vs ten days' therapy. *JAMA* 1981;**246**:1790–5.
282. Schwarz S, Kehrenberg C, Doublet B, et al. Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiol Rev* 2004;**28**:519–42.
283. Seppala H, Klaukka T, Vuopio-Varkila J, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland: Finnish Study Group for Antimicrobial Resistance. *N Engl J Med* 1997;**337**: 441–6.
284. Silber TJ, D'Angelo L. Psychosis and seizures following the injection of penicillin G procaine: Hoigne's syndrome. *Am J Dis Child* 1985;**139**:335–7.
285. Snavely SR, Hodges GR. The neurotoxicity of antibacterial agents. *Ann Intern Med* 1984;**101**:92–104.
286. Solomkin JS, Mazuski JE, Bradley JS, et al. Diagnosis and management of complicated intraabdominal infection in adults and children: guidelines by the Surgical Infection Society and the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**: 133–64.
287. Spratt BG. Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K12. *Proc Natl Acad Sci U S A* 1975;**72**:2999–3003.
288. Steed ME, Rybak MJ. Ceftaroline: a new cephalosporin with activity against resistant grampositive pathogens. *Pharmacotherapy* 2010;**30**:375–89.
289. Steele RW, Thomas MP, Begue RE. Compliance issues related to the selection of antibiotic suspensions for children. *Pediatr Infect Dis J* 2001;**20**:1–5.
290. Sterling TR, Villarino ME, Borisov AS, et al. Three months of rifapentine and isoniazid for latent tuberculosis infection. *N Engl J Med* 2011;**365**:2155–66.
291. Talbot GH, Thye D, Das A, et al. Phase 2 study of ceftaroline versus standard therapy in treatment of complicated skin and skin structure infections. *Antimicrob Agents Chemother* 2007;**51**: 3612–16.
292. Tally FP, Cuchural GJ Jr, Malamy MH. Mechanisms of resistance and resistance transfer in anaerobic bacteria: factors influencing antimicrobial therapy. *Rev Infect Dis* 1984;**6**(Suppl. 1):S260–9.
293. Tanz RR, Poncher JR, Corydon KE, et al. Clindamycin treatment of chronic pharyngeal carriage of group A streptococci. *J Pediatr* 1991;**119**:123128.
294. Tatli MM, Aktas G, Kosecik M, et al. Treatment of typhoid fever in children with a flexible-duration of ceftriaxone, compared with 14-day treatment with chloramphenicol. *Int J Antimicrob Agents* 2003;**21**:350–3.

295. Techasaensiri C, Messina AF, Katz K, et al. Epidemiology and evolution of invasive pneumococcal disease caused by multidrug-resistant serotypes of 19A in the 8 years after implementation of pneumococcal conjugate vaccine immunization in Dallas, Texas. *Pediatr Infect Dis J* 2010;**29**:294–300.
296. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. *Am J Med* 2006;**119**:S3–10.
297. Tenover FC, Moellering RC Jr. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. *Clin Infect Dis* 2007;**44**:1208–2015.
298. Tenson T, Lovmar M, Ehrenberg M. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *J Mol Biol* 2003; **330**:1005–14.
299. Tetzlaff TR, McCracken GH Jr, Nelson JD. Oral antibiotic therapy for skeletal infections of children. II. Therapy of osteomyelitis and suppurative arthritis. *J Pediatr* 1978;**92**:485–90.
300. Then RL. Mechanisms of resistance to trimethoprim, the sulfonamides, and trimethoprim-sulfamethoxazole. *Rev Infect Dis* 1982;**4**:261–9.
301. Thethi AK, Van Dellen RG. Dilemmas and controversies in penicillin allergy. *Immunol Allergy Clin North Am* 2004;**24**: 445–61.
302. Thompson J, O'Connor M, Mills JA, et al. The protein synthesis inhibitors, oxazolidinones and chloramphenicol, cause extensive translational inaccuracy in vivo. *J Mol Biol* 2002;**322**:273–9.
303. Tipper DJ. Mode of action of betalactam antibiotics. *Pharmacol Ther* 1985;**27**:1–35.
304. Tiwari T, Murphy TV, Moran J. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. *MMWR Recomm Rep* 2005;**54**:116.
305. Tomasz A. From penicillin-binding proteins to the lysis and death of bacteria: a 1979 view. *Rev Infect Dis* 1979;**1**:434–67.
306. Tornøe CW, Tworzyanski JJ, Imoisili MA, et al. Optimising piperacillin/tazobactam dosing in paediatrics. *Int J Antimicrob Agents* 2007;**30**:320–4.
307. Trujillo M, Correa N, Olsen K, et al. Cefprozil concentrations in middle ear fluid of children with acute otitis media. *Pediatr Infect Dis J* 2000;**19**:268–9.
308. Trujillo M, Ehrett S, Hoyt-Sehnert MJ, et al. Safety and efficacy of cefprozil as part of a parenteral-oral antibiotic regimen for the treatment of suppurative skeletal infections in children. *Clin Infect Dis* 1996;**23**:843.
309. Tschida SJ, Vance-Bryan K, Zaske DE. Antiinfective agents and hepatic disease. *Med Clin North Am* 1995;**79**:895–917.
310. Turnidge J. Pharmacodynamics and dosing of aminoglycosides. *Infect Dis Clin North Am* 2003;**17**:503–28.
311. Unal S, Garcia-Rodriguez JA. Activity of meropenem and comparators against *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated in the MYSTIC Program, 2002-2004. *Diagn Microbiol Infect Dis* 2005;**53**:265–71.
312. van Dyk JC, Terespolsky SA, Meyer CS, et al. Penetration of cefpodoxime into middle ear fluid in pediatric patients with acute otitis media. *Pediatr Infect Dis J* 1997;**16**:79–81.
313. Veltri MA, Neu AM, Fivush BA, et al. Drug dosing during intermittent hemodialysis and continuous renal replacement therapy: special considerations in pediatric patients. *Paediatr Drugs* 2004; **6**:4565.
314. Wang P, Hu F, Xiong Z, et al. Susceptibility of extended-spectrum beta-lactamase-producing Enterobacteriaceae according to the new CLSI breakpoints. *J Clin Microbiol* 2011;**49**:3127–31.
315. Whitman CB, Czosnowski QA. Fidaxomicin for the treatment of *Clostridium difficile* infections. *Ann Pharmacother* 2012;**46**:219–28.
316. Workowski KA, Berman SM. Sexually transmitted diseases treatment guidelines, 2006. *MMWR Recomm Rep* 2006;**55**:194.
317. Wormser GP, Dattwyler RJ, Shapiro ED, et al. The clinical assessment, treatment, and prevention of Lyme disease, human granulocytic anaplasmosis, and babesiosis: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2006;**43**:1089–134.
318. Wright AJ. The penicillins. *Mayo Clin Proc* 1999;**74**:290–307.
319. Yunis AA. Chloramphenicol: relation of structure to activity and toxicity. *Annu Rev Pharmacol Toxicol* 1988;**28**:83–100.
320. Zeitler K, Salvas B, Stevens V, et al. Aztreonam lysine for inhalation: new formulation of an old antibiotic. *Am J Health Syst Pharm* 2012;**60**:107–15.
321. Zhanel GG, Craig WA. Pharmacokinetic contributions to postantibiotic effects: focus on aminoglycosides. *Clin Pharmacokinet* 1994;**27**:377–92.
322. Zimbelman J, Palmer A, Todd J. Improved outcome of clindamycin compared with beta-lactam antibiotic treatment for invasive *Streptococcus pyogenes* infection. *Pediatr Infect Dis J* 1999;**18**:1096–100.
323. Zinberg J, Chernaik R, Coman E, et al. Reversible symptomatic biliary obstruction associated with ceftriaxone pseudolithiasis. *Am J Gastroenterol* 1991;**86**:1251–4.
324. Zuckerman JM. The newer macrolides: azithromycin and clarithromycin. *Infect Dis Clin North Am* 2000;**14**:449–62.
325. Zuckerman JM, Qamar F, Bono BR. Macrolides, ketolides and glycylcyclines: azithromycin, clarithromycin, telithromycin, tigecycline. *Infect Dis Clin N Am* 2009;**23**:997–1026.

CHAPTER 236

Antimicrobial Prophylaxis

Gary D. Overturf

Antimicrobial prophylaxis is the limited administration of an antimicrobial agent or agents before or immediately after exposure to an infectious agent, with the intent of preventing an infection. Prevention is always preferred, provided the means are available and the risk-benefit and cost-benefit ratios are acceptable. The focus of this chapter is on the prevention of morbidity and mortality from bacterial infections through the prophylactic, and often the empirical, use of antimicrobial agents. For many prophylactic regimens, their employment is justified on the severity of the disease; and in many instances, an evidence base supporting their use is meager or nonexistent, whereas for other regimens, such as those employed in surgery, substantial evidence of their efficacy exists for recommended regimens. Prophylaxis against viral infections such as human immunodeficiency virus and varicella, among others, will not be discussed but rather only prophylaxis against bacterial infections and some fungal (yeast) infections is addressed. For discussions of available prophylaxis against viral infections, please consult the chapters addressing specific viral agents.

GENERAL PRINCIPLES OF PROPHYLAXIS

Factors that influence the efficacy of prophylactic regimens are related to the potential pathogen, the prophylactic agent, the host, and the disease to be prevented (Table 236-1). In addition, the pharmacology, pharmacodynamics, and pharmacokinetics of the antimicrobial agents will affect the efficacy of prophylaxis and dictate the dosage and the timing of the administrations of the antibiotics and the duration of use. Failure to consider these factors will often lead to ineffective prophylaxis, overuse of antimicrobial agents, promotion of resistant microorganisms, economic waste, and risk for development of toxicity or other significant side effects.

The Bacterial Pathogen

Prophylaxis is theoretically more effective when a single pathogen is targeted. In general, the greater the number of targeted pathogens, the less effective, the more toxic, and the more expensive the regimen becomes. Ideally, prophylaxis should be administered at the time of exposure to the potential pathogen. If exposure is prolonged or continuous, prophylaxis becomes less effective and less desirable. Bacteria that are not endogenous to the host (i.e., not part of the host's normal flora) generally are targeted more effectively if the exposure is known and identified.

The Disease

The severity of the disease to be prevented is a major consideration. Potentially fatal infections such as meningococcemia or infections that result in high morbidity such as endocarditis or infections of surgically implanted devices are justifiably targeted. Prophylaxis usually is not required for minor illnesses (e.g., cuts, abrasions). The site of infection also is important. Adequate concentrations of antimicrobial agents are achieved readily in organs that are highly vascular and have no anatomic barriers, whereas infections in restricted anatomic compartments (e.g., middle ear) or those that involve prosthetic materials may require special consideration.

The Antimicrobial Agent

The most desirable prophylactic regimen is one in which a single, inexpensive antimicrobial drug is utilized with a narrow spectrum, which is easily administered and well tolerated with minimal side effects. The less frequently an agent is given, the more reliable the compliance of the patient to the prophylactic regimen.[74] When prophylaxis can be achieved effectively with a single administration of the antimicrobial agent, prophylaxis is likely to approach the ideal.

PROPHYLAXIS IN NEONATES

Ophthalmia Neonatorum

Prophylaxis recommendations target *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Ideally, prophylaxis should be directed at infants who are exposed to these two pathogens, but routine mass identification of such groups with certainty is problematic. Although routine prophylaxis has been discontinued in some countries (United Kingdom, Sweden),[37] topical antibiotic prophylaxis for ophthalmia neonatorum for these two agents continues to be recommended in the United States.[35,36]

In the past, topical 1 percent silver nitrate solutions, available in single-dose ampules, was the standard prophylaxis and tetracycline ophthalmic preparations were considered the alternative. Neither tetracycline nor silver nitrate is produced in the United States, whereas single-dose tubes of an ophthalmic ointment containing 0.5

TABLE 236-1 Factors Influencing Effective Prophylaxis

Single versus multiple potential pathogens
Time of exposure to the pathogen
Source of pathogens
Severity of the disease to be prevented
Targeted organs that could become infected
Spectrum of activity of the antimicrobial agent
Pharmacokinetics and the pharmacodynamics of the selected agent
Duration of chemoprophylaxis
Cost, toxicity, side effects, and acceptability of the agent
Likelihood and consequences of emerging resistance

percent erythromycin are provided. All three agents are effective prophylaxis for gonococcal ophthalmia neonatorum.[68] However, because silver nitrate frequently causes chemical conjunctivitis its use has been largely discontinued in most countries. Erythromycin and tetracycline ophthalmic ointments appear to be as effective as silver nitrate solution for routine prophylaxis of gonococcal ophthalmia,[87,139] but silver nitrate probably is the most reliably effective agent against penicillinase-producing *N. gonorrhoeae*. The effectiveness of erythromycin or tetracycline in the prevention of ophthalmia caused by penicillinase-producing *N. gonorrhoeae* has not been established. No topical regimen has proven efficacy against *Chlamydia* conjunctivitis or prevention of invasive *Chlamydia* infections of the neonate, such as lower respiratory tract infection.[18,38,69] Furthermore, topical regimens do not eliminate *C. trachomatis* from the nasopharynx and do not prevent pneumonia.

Prophylaxis should be administered as soon as possible after birth. Each eyelid should be wiped gently with sterile cotton before local prophylaxis is administered. Care must be exercised to ensure that the solution or ointment is in the conjunctival sac and that it is not flushed from the eye after instillation. Administration of ophthalmic prophylaxis is the law in most states. Most importantly, prophylaxis against *Chlamydia trachomatis* or gonorrhea infection should be provided if identified in pregnant women before delivery during routine screening. Appropriate regimens of ceftriaxone (25 to 50 mg/kg intramuscularly or intravenously not to exceed 125 mg in a single dose) for maternal infections with gonorrhea or chlamydia (azithromycin 1.0 g orally or alternative regimens of ampicillin or erythromycin) are reviewed in the periodic updates of the Centers for Disease Control and Prevention (CDC) sexually transmitted disease treatment guidelines.[35,36]

Group B Streptococcal Infections

Prophylaxis is recommended for the prevention of early-onset (onset within the first week of life) neonatal group B streptococcal infections.* No recommendations for prophylaxis against late-onset infections exist. The current indications for treatment of neonates and maternal carriers or those mothers at risk for transmission of group B streptococcal infections to their infants is reviewed in Chapter 83 and available from the CDC[34] and the American Academy of Pediatrics.[5]

Multiple regimens have been evaluated to reduce vertical transmission of group B streptococcus.[1] The antepartum use of oral antimicrobial agents to eradicate group B streptococcus colonization of mothers (antepartum chemoprophylaxis) have not been successful, even when sexual partners were treated concurrently.[105] Prophylaxis of neonates with penicillin G or ampicillin soon after birth (postnatal chemoprophylaxis) is ineffective in preventing early-onset group B streptococcal disease, primarily because in most patients infection occurs in utero and the infants are asymptomatic at or within a few hours after birth.

Therefore, current recommendations focus on treatment of all colonized women identified by recommended universal testing at 35 to 37 weeks' gestation.[5,34] American Academy of Pediatrics recommendations and CDC guidelines recommend that lower vaginal and anorectal (single swab) specimens for culture be obtained at 35 to 37 weeks' gestation, placed into selective broth medium, transported, and subcultured onto solid media.[8,32] Both hybridization assays and DNA amplification techniques for detection of group B streptococci also are available and serve as the only acceptable alternatives to culture.[16,73] Rapid nucleic amplification methods are available for testing women at the time of entry into labor and thus provide opportunities for treatment of women who may not have been tested at 35 to 37 weeks' gestation. Maternal group B streptococcus carriers identified ante partum or those without culture or identification at the time of delivery with rapid testing but with one or more risk factors (see Chapter 83) should be given intrapartum intravenous ampicillin (2 g initially, then 1 g every 4 hours) or penicillin G (5 million U initially, then 2.5 million U every 4 hours until delivery).[32] Penicillin-allergic women may be given clindamycin or erythromycin intravenously. Previous delivery of an infant with invasive group B streptococcal disease warrants intrapartum maternal chemoprophylaxis for each subsequent pregnancy, regardless of maternal colonization.

Intrapartum antibiotic prophylaxis prevents substantial numbers of, but not all, cases of early-onset neonatal group B streptococcal infection[75] and decreases the incidence of maternal group B streptococcal postpartum complications, including endometritis.[90,100] Since the institution of intrapartum antimicrobial prophylaxis, the rate of invasive group B streptococcal disease has declined by greater than 65 percent in prescreened mothers, and administration of intravenous ampicillin reduces the risk for development of early-onset group B streptococcal infection by 36 percent in infants born to women with premature rupture of membranes.[15] However, the decrease in the rate of group B streptococcal disease may been associated with an increased rate of disease caused by gram-negative pathogens.

Necrotizing Enterocolitis

Neonatal necrotizing enterocolitis (NEC) is a multifactorial disease, with bowel wall necrosis of variable length

*References 2, 8, 19, 105, 114, 117.

and depth being the characteristic common feature. Factors including ischemic stress, disruption of the bowel mucosal barrier, and resultant bacterial proliferation and invasion of the intestinal wall are part of the pathogenesis of NEC. Therefore, suppression of gastrointestinal flora with nonabsorbable oral antimicrobial agents has been used in an effort to prevent the development of NEC in premature infants. Administration of oral kanamycin or gentamicin prophylactically in the first few hours of life has resulted in contradictory data. Furthermore, selective overgrowth of resistant organisms in the bowel and significant systemic absorption of aminoglycosides from the injured mucosa are potential risk factors. Therefore, currently, oral aminoglycosides are not recommended for the routine prophylaxis or treatment of NEC. One report[104] suggests that oral vancomycin given for 48 hours before the introduction of oral feeding may be beneficial in preventing NEC. These observations on the use of antibiotics for prevention of NEC have not been confirmed, and such prophylaxis is not recommended. Lastly, orally administered probiotics have been suggested to alter the bowel flora and to reduce the incidence or severity of NEC. For instance, in recent studies, infants fed breast milk and products including *Lactobacillus acidophilus* and *Bifidobacterium infantis* had a reduced occurrence or reduced severity of NEC[92] and a meta-analysis or composite studies have supported the positive effects of probiotics in the prophylaxis against NEC.[82,93]

Intravascular Catheter Insertion

Infection with coagulase-negative staphylococci, primarily *Staphylococcus epidermidis*, is likely to occur in premature infants or infants who have indwelling vascular catheters. Low-grade sepsis is the most common clinical manifestation; however, meningitis, endocarditis, omphalitis, cellulitis, and other focal infections may occur. Two randomized trials of low-dose vancomycin added to total parenteral nutrition fluids (25 μg of vancomycin per milliliter of fluid) suggested that such a prophylactic regimen significantly reduces coagulase-negative staphylococcal infections in small premature infants in neonatal intensive care units.[78,127] Despite the possible effectiveness of vancomycin prophylaxis,[13] the American Academy of Pediatrics has not made a routine recommendation for low-dose vancomycin because of the potential promotion of emerging resistance in gram-positive pathogens. Although continuous administration of fluconazole has been studied in high-risk, low-birth-weight infants who are at risk for development of systemic infections caused by *Candida* spp., the American Academy of Pediatrics currently does not endorse the use of continuous fluconazole as an accepted prophylactic procedure. The recent interest in anti-infective agents such as ethanol as an antibiotic lock solution for treatment and/or elimination of colonization of chronic indwelling lines has been proposed as an acceptable alternative to antibiotics[107,115,122]; however, although these agents have been effective for treatment and/or elimination of potential pathogens in biofilms infecting lines, they remain only recommended for treatment of lines infected with organisms not responsive to recommended treatment.[106]

DISEASE-TARGETED PROPHYLAXIS

Rheumatic Fever

Group A streptococcal infections of the pharynx are the precipitating cause of rheumatic fever. Appropriate antibiotic treatment of streptococcal pharyngitis prevents the development of acute rheumatic fever in most cases.[47] Because at least one third of episodes of acute rheumatic fever result from inapparent streptococcal infections[41] and some symptomatic patients do not seek medical care, not all instances of rheumatic fever are preventable. Prevention of first attacks (primary prevention) is accomplished by proper identification, adequate antibiotic treatment, and eradication of this streptococcal infection. An individual who has suffered an attack of rheumatic fever is at very high risk for recurrence after subsequent group A streptococcal pharyngitis and needs continuous chemoprophylaxis to prevent such recurrence (secondary prevention).[43] The prophylaxis of rheumatic fever also is discussed in Chapter 29.

Primary Prevention

The primary treatment of episodic recurrent group A streptococcal infections is important in the prevention of rheumatic fever and recurrent episodes. The diagnosis and treatment of group A streptococcal infections are discussed in Chapter 82, and the prevention of rheumatic fever and its prophylaxis are discussed in Chapter 29. The acceptable regimens for treatment of acute group A streptococcal infections[4,10,62,136] are provided in Table 236-2. Even when treatment is started as long as 9 days after the onset of acute illness, penicillin may effectively prevent primary attacks of rheumatic fever.[30] Therefore, a brief delay (24 to 48 hours) for processing of the throat culture before initiation of antibiotic therapy does not increase the risk for development of rheumatic fever. However, therapy also shortens the duration of clinical symptoms, so initiation of therapy at the earliest possible time is favored.[14] Intramuscular benzathine penicillin G is preferred to oral penicillin, particularly for patients who are unlikely to complete a 10-day course of oral therapy and for patients with a personal or family history of rheumatic fever, rheumatic heart disease, or other factors that place them at substantial risk for development of rheumatic fever.[43]

Secondary Prevention

Continuous antibiotic administration is indicated in patients at risk for developing or recurrent disease after having infection with group A streptococcus or for those children with congenital or acquired cardiac disease who are at risk for development of endocarditis. Both the American Academy of Pediatrics and the American College of Cardiology provide recommendations for prophylaxis.

An individual in whom streptococcal pharyngitis develops after a previous attack of rheumatic fever is at high risk for having a recurrent attack of rheumatic fever, even after those infections that are asymptomatic or in

TABLE 236-2 **Prevention of Rheumatic Fever**

Agent	Dose	Mode	Duration
Primary Prevention			
Benzathine penicillin G	600,000 units for patients ≤27 kg 1.2 million units for patients >27 kg	Intramuscularly	Once
Penicillin V	Children: 250 mg 2-3 times daily (<60 lb) Children ≥ 60 lb or adults: 500 mg 2-3 times daily	Orally	10 days
For Individuals Allergic to Penicillin			
Erythromycin (Azithromycin for 5 days may be substituted; see package insert for dosage)	40 mg/kg/day divided 2-4 times daily (1 g maximum/day	Orally	10 days
Secondary Prevention			
Benzathine penicillin G	1.2 million units every 3-4 wk	Intramuscularly	See text
	or		
Penicillin V	250 mg twice daily	Orally	See text
	or		
Sulfadiazine	0.5 g once daily for patients ≤27 kg (60 lb) 1 g once daily for patients >27 kg (60 lb)	Orally	See text
For Individuals Allergic to Penicillin and Sulfadiazine			
Erythromycin	250 mg twice daily	Orally	See text

*Clarithromycin for 10 days or azithromycin for 5 days in recommended doses is also effective.
From Dajani A, Taubert K, Ferrieri P, et al. Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. *Pediatrics* 1995;**96**:758-764. Copyright 1995, American Academy of Pediatrics.

those symptomatic infections that are treated optimally. Therefore, prevention of recurrent rheumatic fever requires continuous antimicrobial prophylaxis rather than recognition and treatment of acute episodes of streptococcal pharyngitis.[43] Continuous prophylaxis is recommended for patients with a well-documented history of rheumatic fever (including cases manifested solely by Sydenham chorea) and for those with definite evidence of rheumatic heart disease. Such prophylaxis should be initiated as soon as acute rheumatic fever or rheumatic heart disease is diagnosed. A full therapeutic course of penicillin or other effective regimen should be given first to patients with acute rheumatic fever to eradicate residual group A streptococcus, even if a throat culture is negative at that time. Streptococcal infections occurring in family members of patients with rheumatic fever should be treated promptly.

An injection of 1.2 million units of benzathine penicillin G for patients weighing less than 27.3 kg (60 lb) every 4 weeks is the recommended regimen for secondary prevention in most circumstances in the United States (see Table 236-2). In countries where the incidence of rheumatic fever is particularly high, in special circumstances, or in certain high-risk individuals, such as patients with residual rheumatic carditis, administration of benzathine penicillin G every 3 weeks is justified and recommended.[94,95] Long-acting penicillin is of particular value in patients with a high risk for having a recurrence of rheumatic fever, especially those with rheumatic heart disease, in whom recurrence is very serious. The advantages of giving benzathine penicillin G must be weighed against the inconvenience to the patient and the pain of injection, which causes some individuals to discontinue prophylaxis.

Successful oral prophylaxis (penicillin V or sulfadiazine, see Table 236-2) depends primarily on the patient's adherence to prescribed regimens.[42] Patients need to be given careful and repeated instructions about the importance of prophylaxis. Most failures of prophylaxis occur in nonadherent patients. Even with optimal adherence of the patient, the risk for having a recurrence is higher in individuals receiving oral prophylaxis than in those receiving intramuscular benzathine penicillin G.[56] Oral agents are more appropriate for patients at lower risk for having rheumatic recurrence. Accordingly, some physicians elect to switch therapy to oral prophylaxis when patients have reached late adolescence or young adulthood and have remained free of rheumatic attacks for at least 5 years.

Duration of appropriate prophylaxis has been defined for those with rheumatic fever without carditis for 5 years after the last episode or until 21 years of age, whichever is longer, whereas for those with carditis prophylaxis should be at least 10 years or until age 21, whichever is longer.[17] For those with residual heart disease (e.g., persistent valvular disease), prophylaxis should be given at least 10 years after the last episode or until age 40 years, whichever is longer.[4,62]

Although sulfonamides are not effective in the eradication of group A streptococcus, they do prevent development of infection. Sulfonamide prophylaxis is contraindicated in late pregnancy because of transplacental passage of such drugs and potential competition with bilirubin for albumin-binding sites.

For patients who are allergic to penicillin and sulfisoxazole, erythromycin or other macrolide (e.g., azithromycin or clarithromycin) is recommended. No data have been published about the use of other penicillins, macrolides, or cephalosporins for the secondary prevention of rheumatic fever, but the American Academy of Pediatrics has stated that clarithromycin for 10 days or azithromycin for 5 days is adequate for treatment.

Bacterial Endocarditis

In 2007,[136] and again in 2008,[135] recommendations for antimicrobial prophylaxis were changed significantly from 1997 guidelines.[44] The rationale for these changes included three central concepts. First, endocarditis is much more likely to result from frequent exposure to random bacteremias associated with daily activities than from bacteremia caused by a dental, gastrointestinal tract, or genitourinary procedure. Second, prophylaxis may prevent an exceedingly small number of (if any) cases of endocarditis in individuals who undergo any procedure and the risk for adverse reactions to antibiotics probably exceeds the benefit in any form of prophylactic treatment. Third, maintenance of optimal oral health and hygiene may reduce the incidence of bacteremia from daily activities and is more important than prophylactic antibiotics for a dental procedure to reduce the risk for endocarditis.

Therefore, recommendations for the use of antibiotic prophylaxis after 2007 were limited to the highest risk cardiac conditions. Cardiac conditions associated with the highest risk are listed in Table 236-3. Prophylaxis is no longer provided to all subjects who have the highest lifetime risk for endocarditis. For patients with high-risk conditions listed in Table 236-3, all dental procedures that involve manipulation of gingival tissues or the periapical region of teeth or perforation of the oral mucosa are reasonable for administration of dental prophylaxis. Events and procedures such as routine anesthetic injections through noninfected tissues, taking dental radiographs, placement or removal of prosthodontic or orthodontic appliances, adjustment of orthodontic appliances, placement of orthodontic brackets, shedding of deciduous teeth, and bleeding from trauma to the lips or oral mucosa do not require prophylaxis. The recommendations for regimens for oral and parenteral antibiotics listed in Table 236-4 are for all dental procedures for which dental prophylaxis is reasonable for persons with high-risk conditions.

Recommendations for antimicrobial prophylaxis for respiratory procedures are only for procedures that involve the incision or incisional biopsy of the respiratory mucosa, such as tonsillectomy and adenoidectomy. Therefore, prophylaxis is not recommended for bronchoscopy unless an incision of the respiratory tract will be made. An antimicrobial regimen that includes coverage of viridans group streptococci[25] can be selected from Table 236-4.

When using prophylaxis for high-risk procedures of the gastrointestinal or genitourinary tract, consideration

TABLE 236-3 Cardiac Conditions Associated with the Highest Risk for Adverse Outcomes from Endocarditis for Which Prophylaxis with Dental Procedures is Reasonable

- Prosthetic cardiac valve or prosthetic material used for cardiac valve repair
- A history of previous infectious endocarditis
- Congenital heart disease (CHD)
  - Unrepaired cyanotic CHD, including palliative shunts and conduits
  - Completely repaired congenital heart defect with prosthetic material or device inserted by surgery or catheter intervention during the first 6 months after the procedure
  - Repaired CHD with residual defects at the site or adjacent to the site of a prosthetic patch or prosthetic device (which inhibit endothelialization)
- Cardiac transplantation recipients who develop valvulopathy

TABLE 236-4 Prophylactic Antimicrobial Regimens for Dental Procedures

		Regimen, Single Dose 30-60 Minutes Before Procedure	
Situation	**Agent**	**Adults**	**Children**
Oral	Amoxicillin	2 g	50 mg/kg
Unable to take oral medications	Ampicillin	2 g IV or IM	50 mg/kg IV or IM
	or		
	Cefazolin or ceftriaxone	1 g IV or IM	50 mg/kg IV or IM
Allergic to penicillins or ampicillin—oral	Cephalexin	2 g	50 mg/kg
	or		
	Clindamycin	600 mg	20 mg/kg
	or		
	Azithromycin or clarithromycin	500 mg	15 mg/kg
Allergic to penicillins or ampicillin and unable to take oral medications	Cefazolin or ceftriaxone	1 g IV or IM	50 mg/kg IV or IM
	or		
	Clindamycin	600 mg IV or IM	20 mg/kg IV or IM

IM, intramuscularly; IV, intravenously.

of the possible involvement of enterococcal organisms must be considered as well as the inclusion of mixed infections with aerobic and anaerobic gram-negative and gram-positive organisms.[15,52] However, only enterococci are frequent causes of endocarditis. In the revised recommendations of 2007, the administration of prophylactic antibiotics solely to prevent endocarditis is not recommended for patients who undergo esophagogastroduodenoscopy or colonoscopy, which is in contrast to previous recommendations made in 1997. For patients with infections of the gastrointestinal or genitourinary tract who may have intermittent or sustained enterococcal bacteremia or for those who receive antibiotic therapy to prevent wound infection or sepsis associated with a gastrointestinal or genitourinary tract procedure, it is reasonable to include an agent active against enterococci, such as penicillin, ampicillin, piperacillin, or vancomycin. Similarly for patients with high-risk conditions listed in Table 236-3, antibiotic therapy to eradicate the infection from the urine before surgery is reasonable for those who are scheduled for an elective cystoscopy or other urinary tract manipulation who have an enterococcal urinary tract infection or colonization. Amoxicillin or ampicillin is the preferred agent for enterococci, but vancomycin may be used in those intolerant of β-lactam antibiotics.

Incision of surgically scrubbed skin without an underlying or adjacent infection is not likely to cause bacteremia, and prophylaxis is not recommended. In contrast, procedures on infected skin, skin structure, or musculoskeletal tissues are likely to cause endocarditis. Therefore, for children with high-risk cardiac conditions, a therapeutic regimen administered for treatment of the infection should contain an agent active against staphylococci and β-hemolytic streptococci, such as an antistaphylococcal penicillin or cephalosporin. Vancomycin or clindamycin may be administered to children unable to tolerate β-lactam antibiotics or those who are known or suspected to be infected with a methicillin-resistant strain of staphylococcus (MRSA).

The 2007 changes in recommendations for prophylaxis for endocarditis represent substantial changes to all the recommendations before 1997. Therefore, a summary of those recommendations is listed in Table 236-5. In addition, the recommendations to withhold prophylaxis to subjects with a number of conditions have not changed, and those conditions or situations are listed in Table 236-6.

TABLE 236-5 Summary of Changes and New Recommendations for Antibiotic Prophylaxis for Bacterial Endocarditis*

Bacteremia resulting from daily activities is much more likely to cause endocarditis than bacteremia associated with a dental procedure.
Only an extremely small number of cases of endocarditis might be prevented by antibiotic prophylaxis even if prophylaxis were 100% effective.
Prophylaxis is now recommended only for high-risk procedures (see Table 236-3), and prophylaxis is no longer recommended for any other form of congenital heart disease except for those listed in Table 236-4.
Prophylaxis is reasonable only for dental procedures that involve the manipulation of the gingival tissues or periapical region of teeth or perforation of the oral mucosa, and only for those with very high-risk conditions.
Antibiotic prophylaxis is reasonable for procedures on respiratory tract or infected skin or skin structures or musculoskeletal tissues only for patients with underlying cardiac conditions with the highest risk for adverse outcomes from endocarditis.
Antibiotic prophylaxis solely to prevent endocarditis is no longer recommended for genitourinary or gastrointestinal tract procedures.
Conditions previously listed as not requiring prophylaxis (1997) continue to not require prophylaxis and now include vaginal delivery, hysterectomy, and tattooing, although body piercing for patients with high-risk conditions for endocarditis should not be performed.

*Summarized from Wilson W, Taubert KA, Gewitz PB, et al. Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation* 2007;**116**:1736–54.

Recurrent Otitis Media

Acute otitis media is one of the most common infections in infants and children and has a tendency to recur, particularly during the first few years of life. In addition to tympanostomy tube placement and adenoidectomy, antimicrobial prophylaxis is one of the options that may be considered for the management of recurrent otitis media.[29,51,113] Chemoprophylaxis of otitis media is discussed also in Chapter 16. Despite recommendations for the use of prophylaxis in some children with recurrent otitis media, these recommendations remain controversial and without a clear consensus for their effectiveness and safety.

Antimicrobial prophylaxis has been recommended for a child who has had three or more episodes of acute otitis media in 6 months or four episodes within a year, with the last episode occurring during the previous 6 months.[63,109] Patients most likely to benefit from prophylaxis include those younger than 2 years, those in out-of-home childcare, and Native American children.[83,109] Prophylaxis is directed against the most common potential pathogens that cause otitis media: *Streptococcus pneumoniae*, *Moraxella catarrhalis*, and nontypeable *Haemophilus influenzae*. Amoxicillin, at a dose of 20 mg/kg, or sulfisoxazole, at a dose of 50 mg/kg, may be given orally each evening for a period of 3 to 6 months or during the winter months. Although many other antimicrobial agents are used for the treatment of otitis media, only amoxicillin and sulfisoxazole currently are recommended as prophylactic agents because only these two agents have undergone critical analysis in prospective trials. Antimicrobial prophylaxis must be used with great caution and balanced against the potential for increasing the emergence of resistant organisms, particularly *S. pneumoniae*, and the possible occurrence of drug-associated toxicity (e.g., neutropenia, rash, or other). Other measures that may decrease the incidence of recurrent acute otitis

TABLE 236-6 Conditions for Which Antibiotics Should Not Be Used Solely for Endocarditis Prophylaxis

Cardiac Conditions

Isolated secundum atrial septal defects
Surgical repair of atrial septal defect, ventricular septal defect, or patent ductus arteriosus (without residual and beyond 6 months of age)
Previous coronary artery bypass graft surgery
Mitral valve prolapse without valvular regurgitation
Physiologic, functional, or innocent heart murmurs
Previous Kawasaki disease without valvular dysfunction
Previous rheumatic fever with valvular dysfunction
Cardiac pacemakers (intravascular and epicardial and implanted defibrillators)

Respiratory Tract

Endotracheal intubation
Bronchoscopy with a flexible bronchoscope, with or without biopsy
Tympanostomy tube insertion

Gastrointestinal Tract

Endoscopy with or without gastrointestinal biopsy

Genitourinary Tract

Vaginal hysterectomy
Vaginal delivery
Cesarean section
In uninfected tissue:
- Urethral catheterization
- Uterine dilation and curettage
- Therapeutic abortion
- Sterilization procedures
- Insertion or removal of intrauterine devices

Other

Cardiac catheterization, including balloon angioplasty
Implanted cardiac pacemakers, implanted defibrillators, and coronary stents
Incision or biopsy of surgically scrubbed skin
Circumcision

media include eliminating smoking in the home, reducing daycare attendance, eliminating pacifiers, and administering influenza and the heptavalent conjugated pneumococcal vaccines. If these measures do not prevent recurrent infections, referral to an otolaryngologist is recommended for evaluation and possible tympanostomy tube placement or adenoidectomy, or both procedures.

Recurrent Urinary Tract Infection

Urinary tract infection (UTI) occurs in approximately 5 percent of girls and 1 to 2 percent of boys.[142] Recurrent UTIs are noted in 30 to 50 percent of children with UTIs, with most recurrences taking place within 3 months after the initial episode. Eighty percent of recurrences are new infections caused by different colonic bacterial species that have become resistant to recently administered antibiotics. The recurrence rate is not altered by extending the duration of treatment.

Renal parenchymal infections and renal scarring are well-recognized complications of UTIs in children.[72,99,123] Parenchymal scarring is found in 10 to 15 percent of children with UTIs,[126,142] hypertension will develop in an estimated 10 percent of children with this complication, and renal insufficiency may develop in a smaller number.[76] Vesicoureteral reflux is noted in 30 to 50 percent of children with UTIs,[99] the frequency being related directly to the number of UTI episodes and inversely to age. Children with reflux have a much higher incidence (30% to 60%) of pyelonephritic scarring than do children without reflux. More than 90 percent of children with renal parenchymal scarring have had vesicoureteral reflux and a history of UTI.[126,142]

Children who have three or more UTIs in a 12-month period may benefit from suppressive antibiotic therapy for as long as 6 months to allow repair of intrinsic bladder defense mechanisms.[142] In children with anatomic defects or reflux, suppressive therapy may be needed for as long as the underlying defect exists.

Appropriate prophylactic agents should result in low serum but high urinary levels of the medication, have minimal effect on fecal flora, be well tolerated, and be inexpensive.[98] Methenamine mandelate (75 mg/kg divided every 12 hours) is a suitable agent for prophylaxis because it releases formaldehyde in an acid medium. A pH of 5.5 or lower must be maintained in the urine to obtain optimal results. Ascorbic acid or other acidifying agents should be used to achieve the desired urine acidity. Other useful agents for prophylaxis in children with normal renal function are trimethoprim-sulfamethoxazole (TMP-SMX), nitrofurantoin, and nalidixic acid.[23,55,70,99,120] TMP-SMX can be given at 2 mg of TMP and 10 mg of SMX per kilogram in a single daily dose or at 5 mg of TMP and 25 mg of SMX per kilogram twice a week. TMP has the additional unique characteristic of diffusing into vaginal and urethral fluids, thereby decreasing bacterial colonization with members of the Enterobacteriaceae and diminishing ascending reinfection.[70,128] Nitrofurantoin is recommended at 1 to 2 mg/kg, taken each night. It has been used effectively as prophylaxis for recurrent UTIs in infants and children. Pulmonary, neurologic, and hepatic adverse effects have been reported but are rare occurrences.[99] Nalidixic acid (not recommended for children younger than 3 months) is administered at 30 mg/kg divided every 12 hours. It is a bactericidal agent for most of the common gram-negative uropathogens. More recently, various cephalosporins and amoxicillin–clavulanic acid have been used as prophylactic agents with good results.[98] Prophylactic agents are best administered as a single dose at bedtime.

Prophylaxis for UTIs may reduce the incidence of UTIs by up to 50 percent in children with nocturnal continence. No studies of prophylaxis in children without nocturnal continence have shown efficacy, and prophylactic regimens employing broad-spectrum antibiotics (e.g., amoxicillin, cephalexin) are associated with the emergence of recurrent infections caused by antibiotic-resistant organisms such as *Pseudomonas aeruginosa*.

POSTEXPOSURE PROPHYLAXIS

Prophylaxis targeted against specific organisms after an individual is exposed is discussed in this section.

Pertussis

Prompt administration of erythromycin or other approved macrolide drug to those in close contact with a case of pertussis is effective in limiting secondary transmission. Close contacts are household members, attendees of childcare facilities, and other individuals who are in contact with the index infected individual for 4 hours or more a day. Chemoprophylaxis is recommended irrespective of age or vaccination status because immunity after receiving pertussis immunization is not absolute and may not prevent development of infection.[2] The recommended dose of erythromycin is 40 to 50 mg/kg/day (maximum, 2 g/day) to be given orally in four divided doses for 14 days. Both clarithromycin, 15 mg/kg, up to a maximum of 1.0 g, divided twice daily for 7 days, and azithromycin at standard doses (10 mg/kg the first day, up to a maximum of 500 mg, followed by 5 mg/kg, up to a maximum of 250 mg, each of the days 2 through 5) have been shown to be as effective as erythromycin and are much better tolerated than is erythromycin.[33] Individuals who are allergic to erythromycin or macrolides or those who cannot tolerate their side effects may be given TMP-SMX, although the efficacy of this regimen has not been documented. The dose is 8 mg/kg/day (TMP) and 40 mg/kg/day (SMX) orally in two divided doses for 14 days.[33]

Persons who have been in contact with an infected individual should be monitored closely for respiratory symptoms for 2 weeks after the last contact. The risk for contracting pertussis in adults providing medical care to children should be recognized. Symptoms may be mild and not readily recognized as pertussis; however, such individuals can transmit the infection.

Meningococcal Infections

Close contacts of patients with invasive disease caused by *Neisseria meningitidis* (meningococcemia, meningitis, or both) are at higher risk for acquisition of infection than is the general population. Secondary cases and outbreaks may occur in households, childcare centers, nursery schools, colleges, and military camps.[2] The attack rate for household contacts is 0.3 to 1.0 percent (300 to 1000 times the rate in the general population). Spread from patients to medical care providers occurs infrequently unless intimate contact (e.g., mouth-to-mouth resuscitation, intubation, suctioning) occurs. Respiratory tract cultures are not recommended and are not of value in deciding who should receive prophylaxis.[2]

Chemoprophylaxis should be administered as soon as possible, preferably within 24 hours of identification of the index case.[2] Treatment with penicillin G, ampicillin, or sulfonamides for meningococcal disease does not eradicate nasopharyngeal carriage of *N. meningitidis* reliably, whereas treatment with extended-spectrum cephalosporins (e.g., ceftriaxone and cefotaxime) does eliminate carriage. Therefore, antimicrobial chemoprophylaxis should be administered to the index patient before discharge from the hospital if the patient has been treated with the first three antibiotics.

The antibiotic of choice in most instances is rifampin. The recommended regimen is 10 mg/kg (maximum, 600 mg) every 12 hours for a total of four doses in 2 days. A liquid preparation can be formulated, or the powder can be mixed with applesauce or a similar vehicle. The rifampin prophylaxis regimen recommended for *Haemophilus influenzae* type b disease (see later) also is effective for meningococcal prophylaxis.

Rifampin prophylaxis has several shortcomings.[118] It fails to eradicate *N. meningitidis* in 10 to 20 percent of pharyngeal carriers.[102] It is not recommended for pregnant women. Side effects occur frequently and include headache, dizziness, gastrointestinal symptoms, discoloration of body secretions (saliva, tears, urine), staining of contact lenses, and hepatotoxicity. Finally, several studies have documented the emergence of resistant meningococcal strains after the administration of rifampin prophylaxis.[118]

If the meningococcal isolate is known to be susceptible to sulfonamides, sulfisoxazole may be recommended, but currently it is difficult to obtain. The dose is 500 mg/day for infants, 500 mg every 12 hours for children 1 to 12 years of age, and 1 g every 12 hours for children older than 12 years and adults. The duration of prophylaxis is 2 days.

During an outbreak, a single intramuscular injection of ceftriaxone was significantly more effective than rifampin in eradicating meningococci at 1 week (97% vs. 75%) and at 2 weeks (97% vs. 81%) after prophylaxis.[118] Ceftriaxone administered as a single intramuscular dose (125 mg for children younger than 15 years and 250 mg for adults) now is recommended as an acceptable alternative for prophylaxis. Ceftriaxone has the advantages of ease of administration, possibly greater efficacy, and safety in pregnancy. For high-risk contacts 18 years of age or older, a single 500-mg oral dose of ciprofloxacin is a third option for meningococcal prophylaxis.

Haemophilus Influenzae Type B Infections

The risk for development of secondary invasive disease with *H. influenzae* type b is age dependent.[12] The risk incidence of disease has declined dramatically with the routine use of *Haemophilus* conjugate vaccines. However, in the era when *H. influenzae* type b infections occurred frequently, household contacts younger than 1 year old had the highest risk (6%) for acquisition of secondary illness; the risk in children 4 years old or younger also was high (2.1%). Children older than age 6 years and adults are at little or no risk. The risk for children attending childcare centers may be increased but appears to be less than that for household contacts.[25,57,97,103,108] Exposed hospital personnel do not require antimicrobial prophylaxis. Data on the risk for spread with invasive disease due to other *Haemophilus* serotypes other than b, such as a or f, are unknown, and currently prophylaxis is not recommended for serotypes other than type b.

In addition to protecting vaccinated children against invasive disease, conjugate vaccines appear to decrease pharyngeal colonization, which further reduces *H.*

influenzae type b transmission to unvaccinated children. Prophylaxis currently is recommended for all household contacts, regardless of age, if at least one of the contacts is younger than 4 years and not immunized completely.[2] Complete immunization is defined as having received a conjugate vaccine: (1) at least one dose at 15 months or older, (2) two doses between 12 and 14 months of age, or (3) two or more doses before 12 months of age with a booster at 12 months or older.

Prophylaxis for nursery and daycare center contacts is less well defined, and definitive recommendations are lacking. In general, prophylaxis is recommended for childcare centers with the same regimen as that recommended for households if (1) the center is attended by unvaccinated or incompletely vaccinated children younger than 2 years of age where contact is 25 hours per week or more or (2) two or more cases of invasive *H. influenzae* type b disease occur among attendees within 60 days and unvaccinated or incompletely vaccinated children attend the facility.[2] In facilities where all contacts are older than 2 years or age, prophylaxis need not be given, regardless of vaccination status.

Rifampin in a single dose of 20 mg/kg/day (maximum, 600 mg) for 4 days effectively eliminates oropharyngeal carriage of *H. influenzae* type b in 95 percent of treated individuals.[2] This regimen has been shown to be effective in preventing secondary cases of invasive *H. influenzae* type b disease in household members, daycare settings, and classroom contacts.[12,25] Prophylaxis should be initiated as soon as possible because most secondary cases occur during the first week after identification of the index case.[12] The index patient also should receive rifampin prophylaxis, usually initiated during hospitalization and just before discharge.

If prophylaxis is given to limit secondary spread to a cohort (household or daycare), children vaccinated with any *H. influenzae* type b vaccine and unvaccinated susceptible children should receive prophylaxis.[2] Prophylaxis is not recommended for pregnant women.

Tuberculosis

The three goals of preventive therapy for tuberculosis are (1) to prevent asymptomatic (latent) infection from progressing to clinical (active) disease, (2) to prevent recurrence of past disease, and (3) to prevent initial infection post exposure to persons with active disease in individuals who have negative tuberculin skin test results. The first two goals are covered in detail elsewhere (see Chapter 96); prevention of initial infection post exposure is addressed here. Chemoprophylaxis is given in an attempt to prevent the establishment of infection, and the recipient is protected only as long as antituberculous therapy is continued.

In the United States, isoniazid administered for 9 months is the preferred drug for children for chemoprophylaxis against *Mycobacterium tuberculosis*, for susceptible children exposed to active tuberculosis, or for those with positive reactive skin tests or interferon-γ release assay (LTBI, latent tuberculosis infection). The recommended daily dose of INH is 10 to 15 mg/kg/day (maximum, 300 mg/day) to be given as a single dose. However, alternative regimens, including a 6-month regimen or daily or weekly isoniazid, are recommended for children who cannot comply with a 9-month regimen. For both the 9- and 6-month regimens, twice-weekly, directly observed therapy regimens are equal in efficacy to the daily regimens. Similarly, a daily or biweekly regimen of rifampin-pyrazinamide given for 2 weeks or rifampin given daily is recommended for children exposed to individuals with isoniazid-resistant isolates.[31]

Approximately 9 percent of isolates in the United States are resistant to isoniazid. However, most experts still recommend isoniazid to treat children exposed or reactive (LTBI), unless there is contact with a person with known isoniazid-resistant tuberculosis. If the source case is found to be have an isoniazid-resistant, rifampin-susceptible organism, isoniazid should be discontinued and rifampin (10 to 20 mg/kg, 600 mg maximum) should be given for a total of 6 months.[6] For those exposed to multidrug-resistant tuberculosis strains (defined as resistance to isoniazid and rifampin), neither the ideal regimen nor duration is known; however, drugs to consider include pyrazinamide, fluoroquinolones, and ethambutol depending on the susceptibility of the isolate, and individual consultation with a specialist in the treatment of tuberculosis is advisable.

Persons exposed to an infectious case of tuberculosis should undergo tuberculin skin testing, have a chest radiograph, and receive isoniazid prophylaxis or another appropriate and approved regimen as recommended earlier. If the tuberculin test result is negative and the chest radiograph is normal and the individual is not anergic, isoniazid or another appropriate regimen should be administered for 12 weeks and contact with the index patient should be broken. Isoniazid may be discontinued if the result of a repeated skin test or interferon-γ release assay after 12 weeks of prophylaxis remains negative. If the skin test result becomes positive, isoniazid is continued for a total of 9 months. Candidates for postexposure prophylaxis include persons with impaired immunity; household contacts, particularly children younger than 4 years old; recent contacts, especially human immunodeficiency virus–positive contacts; and persons known to be anergic from populations with a high prevalence of tuberculosis.

Management of a neonate whose mother or other household contact has tuberculosis should be based on individual considerations. In the case of an infant born to a mother (or where a household contact is present) when the chest radiograph is negative but the mother or contact has a positive tuberculin skin test or interferon-γ release assay, the mother and/or contact are candidates for treatment of latent tuberculosis infection, but the infant needs no special evaluation or therapy and the infant can breast-feed. If the mother or a household contact has suspected or active tuberculosis disease, this infection should be reported to the health department and appropriate therapy should be started immediately. If the mother has active disease, the infant should be evaluated for congenital infection and the infant should be separated from the mother and/or contact or until the mother or household contact is wearing a mask and receiving treatment. Once the infant is receiving isoniazid prophylaxis, separation is no longer necessary. Once the mother has received effective therapy, for at least 2 weeks, she may breast-feed the infant.[6]

HOST-TARGETED PROPHYLAXIS

Human and Animal Bites

Human and animal bites are relatively common occurrences. According to the CDC, more than 4.7 million animal bites occur annually in the United States and account for approximately 1 percent of pediatric emergency visits of all such visits.[5,119,133] Dog bites account for 80 to 90 percent of animal bites that require medical care.[24] The organisms most frequently isolated in human bites are *S. aureus*, γ-hemolytic streptococci, *Bacteroides* spp., *Eikenella corrodens*, and *Fusobacterium* spp.[129] In animal bites, *Pasteurella multocida*, *S. aureus*, and anaerobic cocci are the main pathogens.[130]

Data on the use of prophylactic antimicrobial agents after bites are sparse, and the role of prophylaxis in patients who seek medical care early for bite wounds is uncertain.[11,49,119,125] However, because these wounds usually are contaminated with potential pathogens, the administration of prophylaxis should be considered for patients who have the following risk factors: moderate or severe bite wounds, especially with edema or crush injury; puncture wounds, especially if there is penetration of bone, tendon sheath, or joints; a facial, hand or foot, or genital area bite; wounds in immunocompromised and asplenic children; or wounds with delayed presentation with signs of infection (e.g., delay of 18 hours or more between the time of injury and the time of initial physician assessment).* For many of these indications, the use of antibiotics is in effect early treatment rather than prophylaxis because contamination of the wound occurs at the time of the bite.

Because most human and animal bites result in polymicrobial aerobic and anaerobic infections, prophylaxis should target these organisms. For initial prophylaxis, amoxicillin-clavulanic acid (30 to 50 mg/kg/day) probably is optimal therapy.[20,24,55,125] Prophylaxis is recommended for 3 to 5 days. For children with penicillin allergy, an extended-spectrum cephalosporin or trimethoprim-sulfamethoxazole plus clindamycin has been recommended.[7] Although they are only moderately active against *P. multocida*, erythromycin 30 to 50 mg/kg/day and standard doses of clarithromycin or azithromycin are accepted alternatives in penicillin-allergic children, specifically when these agents are isolated. Other experts recommend clindamycin 30 mg/kg/day divided into three doses as an alternative in children allergic to penicillins or cephalosporins, as noted previously.

Asplenia

The spleen constitutes approximately 25 percent of the lymphoid mass. It filters blood at a rate of 150 mL/min and plays an important role in the primary defense against bacteria that gain access to the circulation.[131] The spleen has an active role in phagocytosis, is a major source of T lymphocytes, and produces immunoglobulin M antibodies, complement, opsonins, and tuftsin (a phagocytosis-promoting tetrapeptide).

Asplenia may be congenital or acquired. In the past, splenectomy was performed frequently and for a variety of indications; currently, a number of methods are available for splenic salvage after trauma. Overwhelming and often fatal septicemia and meningitis occur with increased frequency in asplenic individuals.[141] The frequency of sepsis is 60 times greater in children who undergo splenectomy than in normal children. The risk for sepsis or severe outcome from sepsis is greater in children who lose splenic function by disease or trauma before 2 years of age or in those who have congenital absence of the spleen. Numerous congenital cardiac conditions carry high risk for development of congenital asplenia. Fatality from sepsis in splenectomized individuals is 200 times more common than that in the normal population.[131] The risk for development of sepsis is greatest in patients who have undergone splenectomies for underlying immunologic or reticuloendothelial disorders, and the risk is lowest in children after splenectomy for trauma.[84] In all categories, the risk is highest in young infants and children, but it extends to teenagers and adults as well. The period of heightened susceptibility to infection is the initial 1 to 2 years after splenectomy; however, fulminant infection has been reported as long as 25 years after splenectomy.

S. pneumoniae is the most common cause of septicemia in splenectomized individuals. Despite prompt diagnosis and treatment, pneumococcal septicemia is associated with a fatality rate as high as 50 percent. Overall, 80 percent of post-splenectomy infections are caused by bacteria with capsular polysaccharides, particularly *S. pneumoniae* and *H. influenzae*.[124,131] Although *N. meningitidis* also has a polysaccharide capsule, lack of terminal components of complement constitute the greatest risk for development of infection and whether the incidence of sepsis is higher in persons with asplenia is unclear. However, because overwhelming meningococcal sepsis has been reported in patients with asplenia, it is reasonable to include these patients as an at-risk population for this pathogen.

To reduce the likelihood of serious infections after splenectomy, several measures are advisable. Splenectomy should be performed only when it is absolutely indicated. If possible, the best approach is to delay the surgical intervention until the child is 5 or 6 years of age. For children with congenital high-risk conditions, pneumococcal polysaccharide protein conjugate vaccines should be provided in the recommended schedules. Although multivalent pneumococcal polysaccharide vaccine provides incomplete protection for patients undergoing splenectomy, especially infants and young children, it should be administered to all patients who are older than 2 years, ideally 2 weeks before the splenectomy is performed.[127] Vaccination against *H. influenzae* type b and *N. meningitidis* types A, C, Y, and W-135 with the appropriate polysaccharide or polysaccharide-protein conjugate vaccine recommended for use in children should be provided.

For antibiotic prophylaxis, penicillin is the agent of choice. Penicillin V given twice daily (125 mg twice daily for children younger than 5 years; 250 mg twice daily for children older than 5 years) significantly decreases the frequency of invasive pneumococcal infection.

*References 11, 24, 48, 96, 119, 143.

Erythromycin and TMP-SMX are alternative options in patients with documented hypersensitivity to penicillin. The duration of prophylactic coverage remains controversial; current practice is to provide penicillin prophylaxis indefinitely in immunocompromised patients.[3,124]

Hemoglobinopathies

Functional asplenia is the primary reason for susceptibility to pneumococcal infection in children with sickle-cell anemia. Serum immunoglobulins are normal or increased in these children; however, they have a dysfunctional alternative complement pathway and decreased opsonic activity (which is mediated by both the alternative and the classic complement components) against *S. pneumoniae*. Leukocyte function also is defective in patients with sickle-cell anemia; intracellular production of hydrogen peroxide, respiratory stimulation, and hexose monophosphate shunt activity are inadequate during phagocytosis. In contrast, leukocytes from splenectomized patients without sickle-cell anemia exhibit normal phagocytic function accompanied by adequate metabolic stimulation. Immunologic dysfunction occurs less rapidly and less commonly in children with hemoglobin C sickle-cell anemia and hemoglobin C β-thalassemia.[88]

Patients with sickle-cell disease are at risk for development of overwhelming infection (septicemia and meningitis) by encapsulated bacteria, including *S. pneumoniae*, *H. influenzae* type b, and, rarely, *N. meningitidis*. *S. pneumoniae* is the most important and frequent cause of septicemia and meningitis in these patients.[137] The risk is particularly high in children younger than age 3 years.[60] A trend toward increased frequency of invasive disease in the first 2 to 5 years after splenectomy also has been noted. Unlike very young children, school-aged children appear to be less vulnerable to pneumococcal invasive infection, even though they remain functionally asplenic.[61,137,140]

The efficacy of penicillin prophylaxis in preventing pneumococcal infection in infants and young children with sickle-cell disease has been well documented in several reports.* The recommended dose of penicillin V is 125 mg twice daily in children younger than 5 years and 250 mg twice daily in children 3 years or older. Some physicians recommend use of amoxicillin 20 mg/kg/day or TMP-SMX 4 mg TMP plus 20 mg SMX per kilogram daily in children younger than age 5 years to include coverage against *Haemophilus* organisms, which are less likely to be a concern in children who are immunized adequately.

Because overwhelming infection can occur in infants as young as 3 months of age, detection of sickle-cell anemia should be accomplished in the neonatal period. Infants in whom sickle-cell anemia is diagnosed should start a prophylactic antibiotic regimen no later than 3 to 4 months of age.[61] The optimal duration of prophylaxis is not defined clearly, and the age at which prophylactic penicillin can be discontinued safely is determined arbitrarily.[3] A concern is that penicillin prophylaxis may decrease the development of natural immunity against pneumococcal infection in children receiving prophylaxis, thereby rendering them more susceptible to development of infection after prophylaxis is discontinued.[26] Another concern is the accelerated development of penicillin-resistant strains of *S. pneumoniae*.[54,137] A multicenter study by the Prophylactic Penicillin Study II group suggests that in children with sickle-cell anemia who have not had a previous severe pneumococcal infection or surgical splenectomy and are receiving comprehensive care, prophylaxis may be stopped safely at 5 years of age.[54] Continuous prophylaxis has limitations, and serious overwhelming infection can occur while patients are receiving prophylaxis. Patients or parents (or both) should be aware that any febrile illness is potentially serious, and immediate medical attention should be sought.[40,110]

Cerebrospinal Fluid Leakage

The value of antibiotic prophylaxis in patients with cerebrospinal fluid leakage has not been proven.[58,101] In the absence of meningeal inflammation, many antibiotics do not penetrate the blood-brain barrier and do not attain adequate levels in cerebrospinal fluid. Antibiotic prophylaxis often fails and frequently alters the normal flora of the respiratory tract, thereby resulting in colonization with resistant bacteria. Prophylaxis may be considered for a short duration while surgical repair is being planned.[101]

SURGICAL PROPHYLAXIS

The goal of prophylactic administration of antibiotics prior and/or during surgery is to reduce or eliminate postoperative morbidity, shorten hospitalization, and reduce the overall costs attributable to infection. Wounds can be classified as clean, clean-contaminated, contaminated, and dirty. Table 236-7 provides the conditions for these wound categories. Dirty wounds are those with purulence or abscess or preoperative perforation of gastrointestinal, oropharyngeal biliary or tracheobronchial tracts or penetrating trauma with environmental flora. The appropriate doses for adults or children (by weight) are provided in Table 236-8[21] and Table 236-9.[132]

An effective prophylactic regimen should be directed against the most likely infecting organisms but need not eradicate every potential pathogen. Therefore, for most procedures, the first-generation cephalosporin cefazolin, which is active against many streptococci and staphylococci, remains effective. When operations involve the bowel with exposure to aerobic and anaerobic bowl flora, then several agents including second-generation cephalosporins (cefoxitin, cefotetan) or ampicillin/sulbactam may suffice alone or combinations such as cefazolin with metronidazole may be substituted; Vancomycin can be used for prophylaxis when frequent infections with methicillin-resistant *S. aureus* are prominent, but it may not be more effective than cefazolin, and persistent use may lead to the emergence of vancomycin-resistant organisms. It is common practice to give antibiotics at the time of anesthesia induction, which should result in adequate tissue and serum levels at the time of incision and interoperative tissue manipulation. For most procedures lasting less than 4 hours, a single

*References 3, 53, 60, 61, 110, 140.

TABLE 236-7 **Surgical Procedures and Probable Pathogens**

Surgical Category	Most Likely Pathogens
Clean	
Neurosurgical	CNS, *Staphylococcus aureus*
Cardiovascular	CNS, *S. aureus*
Orthopedic	CNS, *S. aureus*
Clean-Contaminated	
Burn	Group A streptococci, *S. aureus,* GNB
Gastrointestinal	GNB, anaerobes, enterococci
Urogenital	GNB, enterococci
Respiratory	α-Hemolytic streptococci, anaerobes
Contaminated	
Ruptured viscera	GNB, anaerobes, enterococci
Traumatic wounds	*S. aureus,* group A streptococci, clostridia

CNS, coagulase-negative staphylococci; GNB, gram-negative bacilli.

dose is recommended. Published studies often use one or two additional doses after the completion of surgery, but most experts do not believe that these are necessary, and no data support the use of antibiotics beyond 48 hours.

General Surgical Procedures

Skin incision, organ manipulation, and surgical trauma increase the likelihood for development of local infection. Surgical procedures traditionally are classified as clean, clean-contaminated, and contaminated (see Table 236-7). A fourth category of dirty wounds are those known to be contaminated, where prior to surgery the skin or visceral space was compromised, introducing normal bowel or skin flora, or by contamination with a large number of environmental bacteria. Prophylactic antibiotics are effective in reducing postoperative infections after contaminated and clean-contaminated surgical procedures, whereas their efficacy is more controversial for clean surgical procedures. Clean surgical procedures generally carry a risk for development of postoperative wound infection that is less than 5 percent and, in many hospitals, less than 1 percent.

The critical period for development of infection is short, and optimal prophylaxis should be restricted to the perioperative period. Antibiotic prophylaxis of less than 24 hours' duration is effective both clinically and experimentally. Administration of antibiotics should be started at the time of induction of anesthesia or immediately before the surgical incision is made and discontinued within 24 hours.

Addressing all situations of surgical prophylaxis is beyond the scope of this chapter, and the reader is referred to several publications and reviews on the subject.* Nationwide standards and goals for surgical prophylaxis have been established.[22] The consensus of these statements is that effective surgical prophylaxis must include the first dose of antimicrobial agents within 60 minutes before the surgical procedure and that dosing should be discontinued within 24 hours after the end of surgery. These documents and others provide the routine basis and evidence base for all current guidelines on surgical prophylaxis, including the recommended regimens and dosing for cardiothoracic, vascular, and abdominal or colonic surgery as well as hip or knee arthroplasty, vaginal or abdominal hysterectomy, and neurosurgical procedures.[9]

Neurosurgical Procedures

The use of prophylactic antibiotics for clean neurosurgical procedures remains controversial.[59,66,101,113] However, because of the perceived benefits of prophylactic antibiotics in uncontrolled trials involving a large number of patients, of whom adults were the majority, and in view of the scarcity of definitive studies, current recommendations support the use of a short-course prophylactic regimen.[27,50,64,112,138] Prophylaxis for clean neurosurgical procedures is particularly valuable for high-risk groups (e.g., patients undergoing operative procedures in excess of 4 hours, operations in which craniotomies are performed, or patients with major underlying disease).[28,46,121]

Placement of cerebrospinal fluid shunts is one of the most common neurosurgical procedures in pediatric patients. An estimated 10,000 new shunt insertions and 6,000 revisions are performed annually in the United States. The frequency of shunt infection varies from 1.5 to 39 percent (average, 10% to 15%).[85,116] The major route of infection is colonization of the device or the operative wounds during placement.[64] Retrograde spread from the distal end of the catheter or hematogenous seeding accounts for some instances of infection.

Most infections are noted within 15 days to 2 months of shunt placement.[89,116] Commensal skin flora are the predominant pathogens. Coagulase-negative staphylococci are the most common pathogens and account for approximately 70 percent of shunt infections. *S. aureus* is less common. Gram-negative bacilli are the least common and often are the result of retrograde infection from the peritoneum.[85,116]

The role of prophylactic antibiotics for placement of cerebrospinal fluid shunts has been controversial.[65] A meta-analysis of 1359 patients in 12 randomized, controlled trials indicated that short-term perioperative antimicrobial prophylaxis at the time of placement of a cerebrospinal fluid shunt significantly decreases the risk for subsequent development of device-related infection.[89] Various antimicrobial regimens, including antistaphylococcal penicillins, cephalosporins, TMP-SMX, vancomycin, gentamicin, and combinations, were used in these trials. The choice of an appropriate prophylactic regimen in a particular setting should be based on the local epidemiology of suspected pathogens, local patterns of antimicrobial susceptibility, cost, and expected toxicity. The duration of perioperative prophylaxis should not exceed 48 hours.[81,89] A longer duration of prophylaxis increases cost and the risk for development of adverse reactions and promotes alteration of the normal flora and the emergence of resistant bacteria.

*References 45, 71, 77, 79, 111, 132, 134.

TABLE 236-8 **Suggested Initial Dose and Time to Redosing for Antimicrobials Commonly Used for Surgical Prophylaxis**

Antimicrobial	Half-Life Normal Renal Function (hr)	Half-Life End-Stage Renal Disease (hr)	Recommended Infusion Time (min)	Standard Intravenous Dose (g)	Weight-Based Dose Recommendation* (mg)	Recommended Redosing Interval† (hr)
Aztreonam	1.5-2	6	3-5‡	1-2	Maximum 2 g (adults)	3-5
Ciprofloxacin	3.5-5	5-9	60	400 mg	400 mg	4-10
Cefazolin	1.2-2.5	40-0	3-5‡ 15-60§	1-2	20-30 mg/kg 1 g < 80 kg; 2 g ≥ 80 kg 2 g ≥ 80 kg	2-5
Cefuroxime	1-2	15-2	3-5‡ 15-60§	1.5	50 mg/kg	3-4
Cefamandole	0.5-2.1	12.3-18‖	3-5‡ 15-60§	1		3-4
Cefoxitin	0.5-1.1	6.5-23	3-5‡ 15-60§	1-2	20-40 mg/kg	2-3
Cefotetan	2.8-4.6	13-25	3-5‡ 20-60§	1-2	20-40 mg/kg	3-6
Clindamycin	2-5.1	3.5-5.0¶	10-60 (Do not exceed 30 mg/min)	600-900 mg	<10 kg: at least 37.5 mg ≥10 kg: 3-6 mg/kg	3-6
Erythromycin base	0.8-3	5-6	NA	1 g orally 19, 18, 9 hr before surgery	9-13 mg/kg	NA
Gentamicin	2-3	50-70	30-60	1.5 mg/kg**	See footnote**	3-6
Neomycin	2-3 hours (3% absorbed under normal gastrointestinal conditions)	12-≥24	NA	1 g orally 19, 18, 9 hr before surgery	20 mg/kg	NA
Metronidazole	6-14	7-21 no change	30-60	0.5-1	15 mg/kg (adult) 7.5 mg/kg on subsequent doses	6-8
Vancomycin	4-6	44.1-406.4 (Cl_{cr} <10 mL/min)	1 g ≥ 60 min (use longer infusion time if dose < 1 g)	1.0	10-15 mg/kg (adult)	6-12

*Weight-based doses are primarily from published pediatric recommendations.
†For procedures of long duration, antimicrobials should be redosed at intervals of 1 to 2 times the half-life of the drug. The intervals in the table were calculated for patients with normal renal function.
‡Dose injected directly into vein or running intravenous fluids.
§Intermittent intravenous infusion.
‖In patients with a serum creatinine 5 to 9 mg/dL.
¶The half-life of clindamycin is the same or slightly increased in patients with end-stage renal disease compared with patients with normal renal function.
**If the patient's weight is 30% above their ideal body weight, dosing weight can be determined as follows: DW = IBW + 0.4 (total body weight-IBW).
DW, dosing weight; IBW, ideal body weight; NA, not applicable.
Modified from Bratzler DW, Houck M. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Clin Infect Dis* 2004;**38**:1706-15.

TABLE 236-9 Antimicrobial Prophylaxis for Surgery

Nature of Operation	Common Pathogens	Recommended Antimicrobials	Usual Adult Dosage[1]
Cardiac			
	Staphylococcus aureus, Staphylococcus epidermidis	cefazolin	1-2 g IV[2,3]
		OR cefuroxime	1.5 g IV[3]
		OR vancomycin[4]	1 g IV
Gastrointestinal			
Esophageal, gastroduodenal	Enteric gram-negative bacilli, gram-positive cocci	High-risk[5] only: cefazolin[6]	1-2 g IV[2]
Biliary tract	Enteric gram-negative bacilli, enterococci, clostridia	High-risk[7] only: cefazolin[6,8]	1-2 g IV[2]
Colorectal	Enteric gram-negative bacilli, anaerobes, enterococci	*Oral:* neomycin + erythromycin base[9] or metronidazole[9]	See footnote 9
		Parenteral: cefoxitin[6] or cefotetan[6]	1-2 g IV
		OR cefazolin[6] + metronidazole	1-2 g IV[2] + 0.5 g IV
		OR ampicillin/sulbactam[6,10]	3 g IV
Appendectomy, non-perforated[11]	Same as for colorectal	cefoxitin[6] or cefotetan[6]	1-2 g IV
		OR cefazolin[6] + metronidazole	1-2 g IV[2] + 0.5 g IV
Genitourinary			
Cystoscopy alone	Enteric gram-negative bacilli, enterococci	High-risk[12] only: ciprofloxacin[10]	500 mg PO or 400 mg IV
		OR trimethoprim-sulfamethoxazole	1 DS tablet
Cystoscopy with manipulation or upper tract instrumentation[13]	Enteric gram-negative bacilli, enterococci	ciprofloxacin[10]	500 mg PO or 400 mg IV
		OR trimethoprim-sulfamethoxazole	1 DS tablet
Open or laparoscopic surgery[14]	Enteric gram-negative bacilli, enterococci	cefazolin[6]	1-2 g IV[2]
Gynecologic and Obstetric			
Vaginal, abdominal or laparoscopic hysterectomy	Enteric gram-negative bacilli, anaerobes, Gp B strep, enterococci	cefazolin,[6] cefoxitin[6] or cefotetan[6]	1-2 g IV[2]
		OR ampicillin/sulbactam[6,10]	3 g IV
Cesarean section	same as for hysterectomy	cefazolin[6]	1-2 g IV[2]
Abortion, surgical	same as for hysterectomy	doxycycline	300 mg PO[15]
Head and Neck Surgery			
Incisions through oral or pharyngeal mucosa	Anaerobes, enteric gram-negative bacilli, *S. aureus*	clindamycin	600-900 mg IV
		OR cefazolin + metronidazole	1-2 g IV[2] + 0.5 g IV
		OR ampicillin/sulbactam[10]	3 g IV
Neurosurgery			
	S. aureus, S. epidermidis	cefazolin	1-2 g IV[2]
		OR vancomycin[4]	1 g IV
Ophthalmic			
	S. epidermidis, S. aureus, streptococci, enteric gram-negative bacilli, *Pseudomonas* spp.	gentamicin, tobramycin, ciprofloxacin, gatifloxacin levofloxacin, moxifloxacin, ofloxacin or neomycin-gramicidin-polymyxin B	multiple drops topically over 2 to 24 hours
		OR cefazolin	100 mg subconjunctivally
Orthopedic			
	S. aureus, S. epidermidis	cefazolin[16]	1-2 g IV[2]
		OR vancomycin[4,16]	1 g IV
Thoracic (Non-Cardiac)			
	S. aureus, S. epidermidis, streptococci, enteric gram-negative bacilli	cefazolin	1-2 g IV[2]
		OR ampicillin/sulbactam[10]	3 g IV
		OR vancomycin[4]	1 g IV

Continued on following page

TABLE 236-9 **Antimicrobial Prophylaxis for Surgery** (Continued)

Nature of Operation	Common Pathogens	Recommended Antimicrobials	Usual Adult Dosage[1]
Vascular			
Arterial surgery involving a prosthesis, the abdominal aorta, or a groin incision	*S. aureus, S. epidermidis,* enteric gram-negative bacilli	cefazolin OR vancomycin[4]	1-2 g IV[2] 1 g IV
Lower extremity amputation for ischemia	*S. aureus, S. epidermidis,* enteric gram-negative bacilli, clostridia	cefazolin OR vancomycin[4]	1-2 g IV[2] 1 g IV

1. Parenteral prophylactic antimicrobials can be given as a single IV dose begun within 60 minutes before the procedure. For prolonged procedures (>3 hours) or those with major blood loss, or in patients with extensive burns, additional intraoperative doses should be given at intervals 1-2 times the half-life of the drug (ampicillin/sulbactam q2 hours, cefazolin q4 hours, cefuroxime q4 hours, cefoxitin q2 hours, clindamycin q6 hours, vancomycin q12 hours) for the duration of the procedure in patients with normal renal function. If vancomycin or a fluoroquinolone is used, the infusion should be started within 60-120 minutes before the initial incision to have adequate tissue levels at the time of incision and to minimize the possibility of an infusion reaction close to the time of induction of anesthesia.
2. The recommended dose of cefazolin is 1 g for patients who weigh <80 kg and 2 g for those ≥80 kg. Morbidly obese patients may need higher doses.
3. Some experts recommend an additional dose when patients are removed from bypass during open-heart surgery.
4. Vancomycin can be used in hospitals in which methicillin-resistant *S.* aureus and *S.* epidermidis are a frequent cause of postoperative wound infection, in patients previously colonized with MRSA, or for those who are allergic to penicillins or cephalosporins. Rapid IV administration may cause hypotension, which could be especially dangerous during induction of anesthesia. Even when the drug is given over 60 minutes, hypotension may occur; treatment with diphenhydramine (*Benadryl*, and others) and further slowing of the infusion rate may be helpful. Some experts would give 15 mg/kg of vancomycin to patients weighing more than 75 kg, up to a maximum of 1.5 g, with a slower infusion rate (90 minutes for 1.5 g). For procedures in which enteric gram-negative bacilli are common pathogens, many experts would add another drug such as an aminoglycoside (gentamicin, tobramycin or amikacin), aztreonam or a fluoroquinolone.
5. Morbid obesity, GI obstruction, decreased gastric acidity or GI motility, gastric bleeding, malignancy or perforation, or immunosuppression.
6. For patients allergic to penicillins and cephalosporins, clindamycin or vancomycin with either gentamicin, ciprofloxacin, levofloxacin or aztreonam is a reasonable alternative. Fluoroquinolones should not be used for prophylaxis in cesarean section.
7. Age >70 years, acute cholecystitis, non-functioning gall bladder, obstructive jaundice or common bile duct stones.
8. Cefotetan, cefoxitin and ampicillin-sulbactam are reasonable alternatives.
9. In addition to mechanical bowel preparation, 1 g of neomycin plus 1 g of erythromycin at 1 PM, 2 PM and 11 PM or 2 g of neomycin plus 2 g of metronidazole at 7 PM and 11 PM the day before an 8 AM operation.
10. Due to increasing resistance of *E. coli* to fluoroquinolones and ampicillin/sulbactam, local sensitivity profiles should be reviewed prior to use.
11. For a ruptured viscus, therapy is often continued for about five days.
12. Urine culture positive or unavailable, preoperative catheter, transrectal prostatic biopsy, or placement of prosthetic material.
13. Shock wave lithotripsy, ureteroscopy.
14. Including percutaneous renal surgery, procedures with entry into the urinary tract, and those involving implantation of a prosthesis. If manipulation of bowel is involved, prophylaxis is given according to colorectal guidelines.
15. Divided into 100 mg before the procedure and 200 mg after.
16. If a tourniquet is to be used in the procedure, the entire dose of antibiotic must be infused prior to its inflation.

Cardiovascular Surgery

Infectious complications of cardiovascular surgery can be very serious and life-threatening, and antimicrobial prophylaxis is used commonly in most medical centers.[80,86] Most available data are based on reports from adult patients,[39,67] with little specific information available on prophylactic antibiotic use in pediatric patients. The goal of prophylactic therapy is prevention of wound infection, mediastinitis, and endocarditis. A survey of 43 North American academic centers with pediatric cardiovascular surgery programs indicated that all centers use prophylactic antibiotics for all operative procedures.[91] Monotherapy prophylaxis was used by 91 percent of respondents and consisted almost exclusively of a first- or second-generation cephalosporin. In 95 percent of centers, prophylaxis was started just before surgery or intraoperatively. Prophylaxis was continued for 48 hours or less in most (68%) instances. Prophylactic antibiotics often were continued while thoracostomy tubes, mediastinal tubes, or transthoracic vascular catheters were in place but usually not for endotracheal tubes, arterial or percutaneous central venous catheters, or temporary pacing wires.

NEW REFERENCES SINCE THE SIXTH EDITION

3. American Academy of Pediatrics. Children with asplenia or functional asplenia. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 84–6.
4. American Academy of Pediatrics. Group A streptococcal infections. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 625–7.
5. American Academy of Pediatrics. Group B streptococcal infections. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 628–34.
6. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 680–700.
7. American Academy of Pediatrics. Bite wounds. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*.

28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 187–91.
10. Armstrong C. AHA guidelines on prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. *Am Fam Physician* 2010;**81**:346–59.
21. Bratzler DW, Houck M. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Clin Infect Dis* 2004;**38**:1706–15.
34. Centers for Disease Control and Prevention. Prevention of group B streptococcal disease: revised guidelines from CDC 2010. *MMWR Recomm Rep* 2010;**59**(RR10):1–32.
35. Centers for Disease Control and Prevention. Ophthalmia neonatorum prophylaxis. In: Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):55.
36. Centers for Disease Control and Prevention. Ophthalmia caused by *C. trachomatis*. In: Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):47–9.
62. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. *Circulation* 2009;**119**:1541–51.
82. Kleigman RM, Willoughby RE. Prevention of enterocolitis with probiotics. *Pediatrics* 2005;**11**:171.
92. Lin HC, Hsu CH, Chen HL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter randomized controlled trial. *Pediatrics* 2008;**122**:693–700.
106. O'Grady NP, Alexander M, Burns LA, et al. *Guidelines for the prevention of intravascular catheter–related infections*. 2011. Available at http://www.cdc.gov/hicpac/BSI/06-bsi-background-info-2011.html.
107. Onland W, Shin C, Fustar S, et al. Ethanol lock technique for persistent bacteremia of long-term intravascular devices in pediatric patients. *Arch Pediatr Adolesc Med* 2006;**160**:1049–53.
115. Qu Y, Istivan TS, Daley AJ, et al. Comparison of various antimicrobial agents as catheter lock solutions: preference for ethanol in eradication of coagulase negative staphylococcal biofilms. *J Med Microbiol* 2009;**48**:442–50.
122. Sherertz RJ, Boger MS, Collins CA, et al. Comparative in vitro efficacies of various catheter lock solutions. *Antimicrob Agents Chemother* 2006;**50**:1865–8.
132. Treatment guidelines from the *Medical Letter:* Antimicrobial prophylaxis for surgery. *Med Lett* 2009;**7**:47–52.
135. Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis guidelines from the American Heart Association. *JADA* 2008;**139**:3S–24S.
136. Wilson W, Taubert KA, Gewitz PB, et al. Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation* 2007;**116**:1736–54.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 237

Outpatient Intravenous Antimicrobial Therapy for Serious Infections

John S. Bradley

Outpatient parenteral antimicrobial therapy (OPAT) is the means by which children can receive the benefits of parenteral (intravenous or intramuscular) therapy in an outpatient setting, usually the home, avoiding hospitalization with the associated exposure to nosocomial pathogens, inpatient costs, and psychological stress for child and family. The widespread acceptance of OPAT by physicians, families, and insurers has led to a tremendous increase in the outpatient treatment of serious infectious diseases.* The standard of care provided to children through OPAT should equal or surpass that provided to hospitalized children. National guidelines for OPAT in adults and children have been published by the Infectious Diseases Society of America and define both benefits and risks.[53] This chapter explains the basic concepts behind OPAT for children. The OPAT team, which includes the physician, OPAT coordinator, nurse, pharmacist, parent or caregiver, and third-party payer, needs to work closely to ensure a successful outcome for the child.

*References 4, 15, 16, 20, 23, 26, 33, 34, 36, 37, 41, 42, 44, 45, 48, 52.

EVALUATING A CHILD AND PARENTS FOR OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Broad criteria are used to determine the medical feasibility of using OPAT (Table 237-1). The most important

REFERENCES

1. Allen UD, Navas L, King SM. Effectiveness of intrapartum penicillin prophylaxis in preventing early-onset group B streptococcal infection: results of a meta-analysis. *Can Med Assoc J* 1993;**149**:1659–65.
2. American Academy of Pediatrics. *2006 Red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2006.
3. American Academy of Pediatrics. Children with asplenia or functional asplenia. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 84–6.
4. American Academy of Pediatrics. Group A streptococcal infections. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 625–7.
5. American Academy of Pediatrics. Group B streptococcal infections. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 628–34.
6. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 680–700.
7. American Academy of Pediatrics. Bite wounds. In: Pickering LK, editor. *Red book: 2009 report of the Committee on Infectious Diseases*. 28th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2009. p. 187–91.
8. American College of Obstetricians and Gynecologists. Group B streptococcal infections in pregnancy. *ACOG technical bulletin*. Washington, D.C.: American College of Obstetricians and Gynecologists; 1992.
9. Antimicrobial prophylaxis in surgery. *Med Lett Drugs Ther* 2006;**52**:82–8.
10. Armstrong C. AHA guidelines on prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. *Am Fam Physician* 2010;**81**:346–59.
11. Baker DM, Moore SE. Human bites in children: a six-year experience. *Am J Dis Child* 1987;**141**:1285–90.
12. Band JD, Fraser DW, Ajello G, et al. Prevention of *Haemophilus influenzae* type B disease. *JAMA* 1984;**251**:2381–6.
13. Barefield ES, Philips JB III. Vancomycin prophylaxis for coagulase-negative staphylococcal bacteremia. *J Pediatr* 1994;**125**:230–2.
14. Bass JW. Antibiotic management of group A streptococcal pharyngotonsillitis. *Pediatr Infect Dis J* 1991;**10**(Suppl.):43–9.
15. Benitz WE, Gould JB, Druzin ML. Antimicrobial prevention of early-onset group B streptococcal sepsis: estimates of risk reduction based on a critical literature review. *Pediatrics* 1999;**103**:1278.
16. Bergeron MG, Ke D, Menard C, et al. Rapid detection of group B streptococci in pregnant women at delivery. *N Engl J Med* 2000;**343**:175–9.
17. Berrios X, del Campo E, Guzman B, et al. Discontinuing rheumatic fever prophylaxis in selected adolescents and young adults. *Ann Intern Med* 1993;**118**:401–6.
18. Black-Payne C, Bocchini JA Jr, Cedotal C. Failure of erythromycin ointment for postnatal ocular prophylaxis of chlamydial conjunctivitis. *Pediatr Infect Dis J* 1989;**8**:491–8.
19. Boyer KM, Gotoff SP. Prevention of early-onset neonatal group B streptococcal disease with selective intrapartum chemoprophylaxis. *N Engl J Med* 1986;**314**:1665–9.
20. Brakenbury PH, Muwanga C. A comparative double blind study of amoxicillin/clavulanate vs. placebo in the prevention of infection after animal bites. *Arch Emerg Med* 1989;**6**:251–6.
21. Bratzler DW, Houck M. Antimicrobial prophylaxis for surgery: an advisory statement from the National Surgical Infection Prevention Project. *Clin Infect Dis* 2004;**38**:1706–15.
22. Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improved outcomes for patients having surgery. *Clin Infect Dis* 2006;**43**:322–30.
23. Brendstrup L, Hjelt K, Petersen KE, et al. Nitrofurantoin versus trimethoprim prophylaxis in recurrent urinary tract infection in children: a randomized, double blind study. *Acta Paediatr Scand* 1990;**79**:1225–34.
24. Brook I. Microbiology of human and animal bite wounds in children. *Pediatr Infect Dis J* 1987;**6**:29–32.
25. Broome CV, Mortimer EA, Katz SL, et al. Use of chemoprophylaxis to prevent the spread of *Hemophilus influenzae* B in day-care facilities. *N Engl J Med* 1987;**316**:1226–8.
26. Buchanan GR, Smith SJ. Pneumococcal septicemia despite pneumococcal vaccine and prescription of penicillin prophylaxis in children with sickle cell anemia. *Am J Dis Child* 1986;**140**:428–32.
27. Bullock R, Van Dellen JR, Ketelbey W, et al. A double-blind placebo-controlled trial of perioperative prophylactic antibiotics for elective neurosurgery. *J Neurosurg* 1988;**69**:687–91.
28. Byrne W, Euler A, Campbell M, et al. Bacteremia in children following upper gastrointestinal endoscopy or colonoscopy. *J Pediatr Gastroenterol Nutr* 1982;**1**:551–3.
29. Casselbrant ML, Kaleida PH, Rockette HE, et al. Efficacy of antimicrobial prophylaxis and of tympanostomy tube insertion for prevention of recurrent acute otitis media: results of a randomized clinical trial. *Pediatr Infect Dis J* 1992;**11**:278–86.
30. Catanzaro FJ, Stetson CA, Morris AJ, et al. Symposium on rheumatic fever and rheumatic heart disease. *Am J Med* 1954;**17**:749–56.
31. Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. *MMWR Recomm Rep* 2000;**49**(RR-6):1–54.
32. Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease. *MMWR Recomm Rep* 2002;**55**(RR-11):1–24.
33. Centers for Disease Control and Prevention. Antimicrobial agents for the treatment and postexposure prophylaxis of pertussis. *MMWR Recomm Rep* 2005;**54**(RR-14):1–16.
34. Centers for Disease Control and Prevention. Prevention of group B streptococcal disease: revised guidelines from CDC 2010. *MMWR Recomm Rep* 2010;**59**(RR10):1–32.
35. Centers for Disease Control and Prevention. Ophthalmia neonatorum prophylaxis. In: Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):55.
36. Centers for Disease Control and Prevention. Ophthalmia caused by *C. trachomatis*. In: Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12):47–9.
37. Chandler JW. Controversies in ocular prophylaxis of newborns. *Arch Ophthalmol* 1989;**107**:814–15.
38. Chen JY. Prophylaxis of ophthalmia neonatorum: comparison of silver nitrate, tetracycline, erythromycin and no prophylaxis. *Pediatr Infect Dis J* 1992;**11**:1026–30.
39. Conte JE Jr, Cohen SN, Roe BB, et al. Antibiotic prophylaxis and cardiac surgery: a prospective double-blind comparison of single-dose versus multiple-dose regimens. *Ann Intern Med* 1972;**76**:943–9.
40. Cummins D, Heuschkel R, Davies SC. Penicillin prophylaxis in children with sickle cell disease in Brent. *BMJ* 1991;**302**:989–90.
41. Dajani AS. Current status of nonsuppurative complications of group A streptococci. *Pediatr Infect Dis J* 1991;**10**(Suppl.):25–7.
42. Dajani AS. Adherence to physicians' instructions as a factor in managing streptococcal pharyngitis. *Pediatrics* 1996;**97**:976–80.
43. Dajani A, Taubert K, Ferrieri P, et al. Treatment of acute streptococcal pharyngitis and prevention of rheumatic fever: a statement for health professionals. *Pediatrics* 1995;**96**:758–64.
44. Dajani AS, Taubert KA, Wilson W, et al. Prevention of bacterial endocarditis: Recommendations by the American Heart Association. *JAMA* 1997;**277**:1794–801.
45. Dellinger EP, Gross PA, Barrett TL, et al. Quality standard for antimicrobial prophylaxis in surgical procedures. *Clin Infect Dis* 1994;**18**:422–7.
46. Dempsey R, Rapp RP, Young B, et al. Prophylactic parenteral antibiotics in clean neurosurgical procedures: a review. *J Neurosurg* 1988;**69**:52–7.
47. Denny FW, Wannamaker LW, Brink WR, et al. Prevention of rheumatic fever: treatment of the preceding streptococcal infection. *JAMA* 1950;**143**:151–3.
48. Dire DJ. Cat bite wounds: risk factors for infection. *Ann Emerg Med* 1991;**20**:973–9.
49. Dire DJ, Hogan DE, Walker JS. Prophylactic oral antibiotics for low-risk dog bite wounds. *Pediatr Emerg Care* 1992;**8**:194–9.

50. Djindjian M, Lepresel E, Homs JB. Antibiotic prophylaxis during prolonged clean neurosurgery. *J Neurosurg* 1990;**73**:383–6.
51. Dowell SF, Marcy SM, Phillips WR, et al. Otitis media—principles of judicious use of antimicrobial agents. *Pediatrics* 1998;**101**: 165–71.
52. El-Baba M, Tolia V, Lin C, et al. Absence of bacteremia after gastrointestinal procedures in children. *Gastrointest Endosc* 1996; **44**:378–81.
53. El-Hazmi MAF, Bahakim HM, Babikar MA, et al. Symptom-free intervals in sicklers: does pneumococcal vaccination and penicillin prophylaxis have a role? *J Trop Pediatr* 1990;**36**:56–62.
54. Falletta JM, Woods GM, Verter JI, et al. Discontinuing penicillin prophylaxis in children with sickle cell anemia. *J Pediatr* 1995; **127**:685–90.
55. Feder H Jr, Shanley JD, Barbera JA. Review of 59 patients hospitalized with animal bites. *Pediatr Infect Dis J* 1987;**6**:24–8.
56. Feinstein AR, Harrison FW, Epstein JA, et al. A controlled study of three methods of prophylaxis against streptococcal infection in a population of rheumatic children. *N Engl J Med* 1959;**260**: 697–702.
57. Fleming DW, Leibenhaut MH, Albanes D, et al. Secondary *Haemophilus influenzae* type B in day-care facilities. *JAMA* 1985;**254**: 509–14.
58. Frazee RC, Mucha P Jr, Farnell MB, et al. Meningitis after basilar skull fracture: does antibiotic prophylaxis help? *Postgrad Med* 1988;**83**:267–74.
59. Gardner BP, Gordon DS. Postoperative infection in shunts for hydrocephalus: are prophylactic antibiotics necessary? *BMJ* 1982; **284**:1914–15.
60. Gaston MH, Verter J. Sickle cell anaemia trial. *Stat Med* 1990; **9**:45–51.
61. Gaston MH, Verter JI, Woods G, et al. Prophylaxis with oral penicillin in children with sickle cell anemia. *N Engl J Med* 1986;**314**:1593–9.
62. Gerber MA, Baltimore RS, Eaton CB, et al. Prevention of rheumatic fever and diagnosis and treatment of acute streptococcal pharyngitis. *Circulation* 2009;**119**:1541–51.
63. Giebink GS. Preventing otitis media. *Ann Otol Rhinol Laryngol* 1994;**103**:20–3.
64. Guevara JA, Zuccaro G, Trevisan A, et al. Bacterial adhesion to cerebrospinal fluid shunts. *J Neurosurg* 1987;**67**:438–45.
65. Haines SJ. Efficacy of antibiotic prophylaxis in clean neurosurgical operations. *Neurosurgery* 1989;**24**:401–5.
66. Haines SJ, Goodman ML. Antibiotic prophylaxis of postoperative neurosurgical wound infection. *J Neurosurg* 1982;**56**:103–5.
67. Hall JC, Christiansen K, Carter MJ, et al. Antibiotic prophylaxis in cardiac operations. *Ann Thorac Surg* 1993;**56**:916–22.
68. Hammerschlag MR. Neonatal ocular prophylaxis. *Pediatr Infect Dis J* 1988;**7**:81–2.
69. Hammerschlag MR, Cummings C, Roblin P, et al. Efficacy of neonatal ocular prophylaxis for the prevention of chlamydial and gonococcal conjunctivitis. *N Engl J Med* 1989;**320**:769–72.
70. Hanson E, Hansson S, Jodol U. Trimethoprim-sulfadiazine prophylaxis in children with vesicoureteral reflux. *Scand J Infect Dis* 1989;**21**:201–4.
71. Hirschmann JV, Inui TS. Antimicrobial prophylaxis: a critique of recent trials. *Rev Infect Dis* 1980;**2**:1–23.
72. Holland NH, Jackson EC, Kazee M, et al. Relation of urinary tract infection and vesicoureteral reflux to scars: follow-up of thirty-eight patients. *J Pediatr* 1990;**116**(Suppl.):65–71.
73. Honest H, Sharma S, Khan KS. Rapid tests for group B *Streptococcus* colonization in laboring women: a systematic review. *Pediatrics* 2006;**117**:1055–66.
74. Hussar DA. Importance of patient compliance in effective antimicrobial therapy. *Pediatr Infect Dis J* 1987;**6**:971–5.
75. Jafari HS, Schuchat A, Hilsdon R, et al. Barriers to prevention of perinatal group B streptococcal disease. *Pediatr Infect Dis J* 1995;**14**:662–7.
76. Jodal U, Koskimies O, Hanson E, et al. Infection pattern in children with vesicoureteral reflux randomly allocated to operation or long-term antibacterial prophylaxis. *J Urol* 1992;**148**:1650–2.
77. Jones RN, Wojeski W, Bakke J, et al. Antibiotic prophylaxis of 1,036 patients undergoing elective surgical procedures: a prospective, randomized comparative trial of cefazolin, cefoxitin, and cefotaxime in a prepaid medical practice. *Am J Surg* 1987;**153**: 341–6.
78. Kacica MA, Horgan MJ, Ochoa L, et al. Prevention of gram-positive sepsis in neonates weighing less than 1500 grams. *J Pediatr* 1994;**125**:253–8.
79. Kaiser AB. Antimicrobial prophylaxis in surgery. *N Engl J Med* 1986;**315**:1129–38.
80. Kaiser AB, Petracek MR, Lea JW Jr, et al. Efficacy of cefazolin, cefamandole, and gentamicin as prophylactic agents in cardiac surgery. *Ann Surg* 1987;**206**:791–7.
81. Kestle JRW, Hoffman HJ, Soloniuk D, et al. A concerted effort to prevent shunt infection. *Childs Nerv Syst* 1993;**9**:163–5.
82. Kleigman RM, Willoughby RE. Prevention of enterocolitis with probiotics. *Pediatrics* 2005;**11**:171.
83. Klein JO. Preventing recurrent otitis: what role for antibiotics? *Contemp Pediatr* 1994;**11**:44–60.
84. Konradsen HB, Henrichsen J. Pneumococcal infections in splenectomized children are preventable. *Acta Paediatr Scand* 1991; **80**:423–7.
85. Kontny U, Hofling B, Gutjahr P, et al. CSF shunt infections in children. *Infection* 1993;**21**:89–92.
86. Kreter B, Woods M. Antibiotic prophylaxis for cardiothoracic operations: Meta-analysis of thirty years of clinical trials. *J Thorac Cardiovasc Surg* 1992;**104**:590–9.
87. Laga M, Plummer FA, Piot P, et al. Prophylaxis of gonococcal and chlamydial ophthalmia neonatorum: a comparison of silver nitrate and tetracycline. *N Engl J Med* 1988;**318**:653–7.
88. Lane PA, Rogers ZR, Woods GM, et al. Fatal pneumococcal septicemia in hemoglobin SC disease. *J Pediatr* 1994;**124**:859–62.
89. Langley JM, LeBlanc JC, Drake J, et al. Efficacy of antimicrobial prophylaxis in placement of cerebrospinal fluid shunts: meta-analysis. *Clin Infect Dis* 1993;**17**:98–103.
90. Larsen JW, Dooley SL. Group B streptococcal infections: an obstetrical viewpoint. *Pediatrics* 1993;**91**:148–9.
91. Lee KR, Ring JC, Leggiadro RJ. Prophylactic antibiotic use in pediatric cardiovascular surgery: a survey of current practice. *Pediatr Infect Dis J* 1995;**14**:267–9.
92. Lin HC, Hsu CH, Chen HL, et al. Oral probiotics prevent necrotizing enterocolitis in very low birth weight preterm infants: a multicenter randomized controlled trial. *Pediatrics* 2008;**122**:693–700.
93. Lin HC, Su BH, Chen AC, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birthweight infants. *Pediatrics* 2005;**115**:1–4.
94. Lue HC, Wu MH, Hsieh KH, et al. Rheumatic fever recurrences: controlled study of a 3-week versus 4-week benzathine penicillin prevention program. *J Pediatr* 1986;**108**:299–304.
95. Lue HC, Wu MH, Wang JK, et al. Long-term outcome of patients with rheumatic fever receiving benzathine penicillin G prophylaxis every three weeks versus every four weeks. *J Pediatr* 1994;**125**:812–16.
96. Maimaris C, Quinton DN. Dog-bite lacerations: a controlled trial of primary wound closure. *Arch Emerg Med* 1988;**5**:156–61.
97. Makintubee S, Istre GR, Ward JI. Transmission of invasive *Haemophilus influenzae* type B disease in day care settings. *J Pediatr* 1987;**111**:180–6.
98. Mangiarotti P, Pizzini C, Fanos V. Antibiotic prophylaxis in children with relapsing urinary tract infections: review. *J Chemother* 2000;**12**:115–23.
99. McCracken GH Jr. Options in antimicrobial management of urinary tract infections in infants and children. *Pediatr Infect Dis J* 1989;**8**:552–5.
100. Mohle-Boetani JC, Schuchat A, Plikaytis B, et al. Comparison of prevention strategies for neonatal group B streptococcal infection: a population-based economic analysis. *JAMA* 1993;**270**: 1442–8.
101. Mollman DH, Haines SJ. Risk factors for postoperative neurosurgical wound infection: a case-control study. *J Neurosurg* 1986;**64**: 902–6.
102. Munford RS, Vasconcelos ZJS, Phillips CJ, et al. Eradication of carriage of *Neisseria meningitidis* in families: a study in Brazil. *J Infect Dis* 1974;**129**:644–9.
103. Murphy TV, Clements JF, Breedlove JE, et al. Risk of subsequent disease among day-care contacts of patients with systemic *Hemophilus influenzae* type B disease. *N Engl J Med* 1987;**316**:5–10.
104. Ng PC, Dear PRF, Thomas DFM. Oral vancomycin in prevention of necrotising enterocolitis. *Arch Dis Child* 1988;**63**:1390–3.

105. Noya FJD, Baker CJ. Prevention of group B streptococcal infection. *Infect Dis Clin North Am* 1992;**6**:41–54.
106. O'Grady NP, Alexander M, Burns LA, et al. *Guidelines for the prevention of intravascular catheter–related infections*. 2011. Available at http://www.cdc.gov/hicpac/BSI/06-bsi-background-info-2011.html.
107. Onland W, Shin C, Fustar S, et al. Ethanol lock technique for persistent bacteremia of long-term intravascular devices in pediatric patient. *Arch Pediatr Adolesc Med* 2006;**160**:1049–53.
108. Osterholm MT, Pierson LM, White KE, et al. The risk of subsequent transmission of *Hemophilus influenzae* type B disease among children in day care. *N Engl J Med* 1987;**316**:1–5.
109. Paradise JL. Antimicrobial prophylaxis for recurrent acute otitis media. *Ann Otol Rhinol Laryngol* 1992;**155**:33–6.
110. Pegelow CH, Armstrong FD, Light S, et al. Experience with the use of prophylactic penicillin in children with sickle cell anemia. *J Pediatr* 1991;**118**:736–8.
111. Platt R. Methodologic aspects of clinical studies of perioperative antibiotic prophylaxis. *Rev Infect Dis* 1991;**13**(Suppl.):810–14.
112. Pons VG, Denlinger SL, Guglielmo BJ, et al. Ceftizoxime versus vancomycin and gentamicin in neurosurgical prophylaxis: a randomized, prospective, blinded clinical study. *Neurosurgery* 1993;**33**:416–23.
113. Principi N, Marchisio P, Massironi E, et al. Prophylaxis of recurrent acute otitis media and middle-ear effusion. *Am J Dis Child* 1989;**143**:1414–18.
114. Pylipow M, Gaddis M, Kinney JS. Selective intrapartum prophylaxis for group B *Streptococcus* colonization: management and outcome of newborns. *Pediatrics* 1994;**93**:631–5.
115. Qu Y, Istivan TS, Daley AJ, et al. Comparison of various antimicrobial agents as catheter lock solutions: preference for ethanol in eradication of coagulase negative staphylococcal biofilms. *J Med Microbiol* 2009;**48**:442–50.
116. Schoenbaum SC, Gardner P, Shillito J. Infections of cerebrospinal fluid shunts: epidemiology, clinical manifestations, and therapy. *J Infect Dis* 1975;**131**:543–51.
117. Schuchat A, Oxtoby M, Cochi S, et al. Population-based risk factors for neonatal group B streptococcal disease: results of a cohort study in metropolitan Atlanta. *J Infect Dis* 1990;**162**:672–7.
118. Schwartz B, Al-Ttobaiqi A, Al-Ruwais A. Comparative efficacy of ceftriaxone and rifampicin in eradicating pharyngeal carriage of group A *Neisseria meningitidis*. *Lancet* 1988;**1**:1239–42.
119. Schweich P, Fleisher G. Human bites in children. *Pediatr Emerg Care* 1985;**1**:51–3.
120. Shapiro ED. Infections of the urinary tract. *Pediatr Infect Dis J* 1992;**11**:165–8.
121. Shapiro M. Prophylaxis in otolaryngologic surgery and neurosurgery: a critical review. *Rev Infect Dis* 1991;**13**(Suppl. 10):858–68.
122. Sherertz RJ, Boger MS, Collins CA, et al. Comparative in vitro efficacies of various catheter lock solutions. *Antimicrob Agents Chemother* 2006;**50**:1865–8.
123. Shortliffe LM. The management of urinary tract infections in children without urinary tract abnormalities. *Urol Clin North Am* 1995;**22**:67–73.
124. Siddins M, Downie J, Wise K. Prophylaxis against postsplenectomy pneumococcal infection. *Aust N Z J Surg* 1990;**60**:183–7.
125. Skurka J, Willert C, Yogev R. Wound infection following dog bite despite prophylactic penicillin. *Infection* 1986;**14**:134–5.
126. Smellie JM, Normand ICS. Urinary infections in children, 1985. *Postgrad Med J* 1985;**61**:895–905.
127. Spafford PS, Sinkin RA, Cox C, et al. Prevention of central venous catheter–related coagulase-negative staphylococcal sepsis in neonates. *J Pediatr* 1994;**125**:259–63.
128. Stamay TA, Condy M, Mibara G. Prophylactic efficacy of nitrofurantoin macrocrystals and trimethoprim-sulfamethoxazole in urinary infections: biologic effect on the vaginal and rectal flora. *N Engl J Med* 1977;**296**:780–3.
129. Talan DA, Abrahamian FM, Moran GJ, et al. Clinical presentation and bacteriologic analysis of human bites in patients presenting to emergency departments. *Clin Infect Dis* 2003;**37**:1481–9.
130. Talan DA, Citron DM, Abrahamian FM, et al. Bacteriologic analysis of infected dog and cat bites. *N Engl J Med* 1999;**340**:85–92.
131. Terezhalmy GT, Hall EH. The asplenic patient: a consideration for antimicrobial prophylaxis. *Oral Surg* 1984;**57**:114–17.
132. Treatment guidelines from the *Medical Letter:* Antimicrobial prophylaxis for surgery. *Med Lett* 2009;**7**:47–52.
133. U.S. Public Health Service. Annual summary 1976. Publication No. CDC 77-8241. *MMWR Morb Mortal Wkly Rep* 1977;**25**:43.
134. Waldvogel FA, Vaudaux PE, Pittet D, et al. Session I. Theoretical and preclinical experimental bases of prophylaxis. Perioperative antibiotic prophylaxis of wound and foreign body infections: microbial factors affecting efficacy. *Rev Infect Dis* 1991;**13**(Suppl.):782–9.
135. Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis. Guidelines from the American Heart Association. *JADA* 2008;**139**:3S–24S.
136. Wilson W, Taubert KA, Gewitz PB, et al. Prevention of infective endocarditis: guidelines from the American Heart Association: a guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation* 2007;**116**:1736–54.
137. Wong WY, Overturf GD, Powars DR. Infection caused by *Streptococcus pneumoniae* in children with sickle cell disease: epidemiology, immunologic mechanisms, prophylaxis, and vaccination. *Clin Infect Dis* 1992;**14**:1124–36.
138. Young RF, Lawner PM. Perioperative antibiotic prophylaxis for prevention of postoperative neurosurgical infections: a randomized clinical trial. *J Neurosurg* 1987;**66**:701–5.
139. Zanoni D, Isenberg S, Apt L. A comparison of silver nitrate with erythromycin for prophylaxis against ophthalmia neonatorum. *Clin Pediatr (Phila)* 1992;**31**:295–8.
140. Zarkowsky HS, Gallagher D, Gill FM, et al. Bacteremia in sickle hemoglobinopathies. *J Pediatr* 1986;**109**:579–85.
141. Zarrabi MH, Rosner F. Serious infections in adults following splenectomy for trauma. *Arch Intern Med* 1984;**144**:1421–4.
142. Zelikovic I, Adelman RD, Nancarrow PA. Urinary tract infections in children: an update. *West J Med* 1992;**157**:554–61.
143. Zubowicz VN, Gravier M. Management of early human bites of the hand: a prospective randomized study. *Plast Reconstr Surg* 1991;**88**:111–14.

TABLE 237-1 **Medical and Social Criteria for Outpatient Parenteral Antimicrobial Therapy**

Medical Criteria for OPAT
Medical diagnosis of infection with a suspected or documented pathogen
Clinical response to anti-infective therapy (if therapy is started in the hospital)
No assessed need for urgent medical, surgical, or laboratory interventions to achieve a clinical response to anti-infective therapy
No significant risk for complications of the infection
No requirement for continuous skilled nursing care
Social Criteria for OPAT
Parents interested and motivated
Parents capable of assessing the child for complications of infection and therapy
24-hour telephone access to the OPAT team
Transportation for timely return to the clinic or hospital, if needed
Home environment acceptable and resources available
Parents documented to be capable of administering parenteral anti-infective agents, if required, before OPAT begins

OPAT, Outpatient parenteral antimicrobial therapy.

aspect in the assessment of a newborn, infant, or child for OPAT is a determination that ongoing parenteral therapy is required to treat the infection appropriately and that continuous skilled nursing observation and care are not required for management. Development of complications of the infection in the child should be considered highly unlikely by the time OPAT is undertaken; unskilled clinical observations by the parents or caregivers should be considered adequate for care. No additional significant risk should be placed on the child's recovery by enrollment in an OPAT program. Whenever possible, oral antibiotic therapy should be used instead of parenteral therapy. Recent options for oral therapy include agents such as the fluoroquinolones ciprofloxacin and levofloxacin, and an oxazolidinone, linezolid, that have excellent absorption and tissue exposure characteristics, but may be more costly or have some potential toxicities that may not be present with older generic antibiotics.

A child usually is prescribed an OPAT regimen from either a hospital setting after inpatient intravenous therapy or an outpatient setting such as a clinic or emergency department. For a child being discharged from the hospital, an unequivocal response to parenteral anti-infective therapy should be demonstrated. Most often, appropriate cultures will have directed the use of anti-infective therapy active against the isolated pathogens. For children with no positive cultures, the signs and symptoms of the infection should be resolving with empirical therapy, as demonstrated by decreasing fever and improving function. The child's infection should be judged by the clinician to be unlikely to progress or to result in complications. No further surgical or medical interventions should be anticipated during the remainder of the parenteral antimicrobial therapy course.

The child's clinical course during receipt of OPAT needs to be monitored to be certain that the anticipated response continues to occur. An outpatient visit usually is scheduled for a few days after discharge.

Before a child is enrolled in OPAT, an assessment of the parents or caregivers also is required (see Table 237-1). Because the parents are responsible for the nursing care of their child in the home, they must be willing and capable participants. Although most parents are very interested and highly motivated to return home with their child, not all are willing to perform or are capable of performing the required nursing functions. Most parents are capable of providing limited medical assessment of their children, but, unfortunately, some are not. In addition, the home environment should have the resources required for the proposed medical care. For example, intravenous therapy administered to a child through a tunneled, central catheter requires local sterile conditions for anti-infective drug infusions and dressing changes, which will require the availability of a clean room with running water, and a refrigerator if storage of antimicrobials and diluents is necessary. Less stringent criteria clearly would apply for a child visiting the outpatient clinic daily to receive intramuscular antibiotic injections. Communication between the parents and medical personnel is essential. For parents or caregivers, telephone access to nurses and clinicians is critical. In addition, the parents should be able to bring their child back to the hospital or clinic for reevaluation if complications arise either from the infection or from therapy. Hence, access to adequate transportation within a medically appropriate time frame is required. Families with no available transportation, families who live in rural areas located several hours' travel from a medical facility, and OPAT during the winter season in which weather conditions may severely limit travel, all should be considered before OPAT is started. In addition to observing their child and communicating information to medical providers, many parents may be asked to infuse anti-infective agents intravenously into their children. Doing so requires an additional level of skill in the parent or caregiver and assessment of parental competence for this function by the OPAT nursing and medical personnel.

THE OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY PROGRAM

The roles of physicians, OPAT coordinators, nurses, pharmacists, and parents or patients have been defined previously (Table 237-2).[53] Once the clinician considers that the child's condition is sufficiently stable to pursue intravenous outpatient therapy from a hospital ward, a clinic, or an emergency department setting, the coordinator of the outpatient treatment program is notified. This person is capable of assessing the feasibility of implementing OPAT for that particular child and family and assessing reimbursement by third party payers and obtaining authorizations, if needed. The OPAT coordinator interviews parents and medical staff to determine the parents' willingness and their abilities and to assess

TABLE 237-2 **Roles of Physician, Outpatient Parenteral Antimicrobial Therapy Coordinator, Nurse, Pharmacist, and Parent**

Role	Physician*	OPAT Coordinator*	Pediatric Nurse*	Pediatric Pharmacist*	Parent or Patient
Assess medical stability for home therapy	+	+	–	–	–
Assess family for capability for OPAT	+	+	+	–	–
Create and implement OPAT treatment plan	+	+	+	+	–
Order nursing visits	+				
Order clinic visits, laboratory tests, imaging examinations	+				
Educate parents about infection and complications	+	+	+	–	–
Educate parents about therapy and complications	+	+	+	+	–
Manage treatment	+	+	+	–	+
Monitor intravenous catheter status	+	–	+	–	+
Monitor clinical status	+	–	+	–	+
Monitor antimicrobial therapy	+	–	+	+	+
Prepare antimicrobial therapy	–	–	+	+	+
Availability for complications	+	+	+	+	+
Outcome assessment	+	+	+	+	+

*Certification or experience in pediatrics.
OPAT, Outpatient parenteral antimicrobial therapy.

the home environment. After the clinician prescribes the anti-infective agents, required nursing visits, ongoing laboratory and imaging tests, and follow-up clinic visits, the OPAT coordinator puts together the resources required for successful therapy. With knowledge of the parents' abilities and resources, the medication and equipment requirements, and the home nursing visits required, the coordinator makes the final determination about the feasibility of the program. As noted, the coordinator may need to obtain authorization from third-party payers; many insurance companies require a much greater financial contribution by families for the cost of care once a child is discharged, in contrast to their obligation for ongoing inpatient hospital charges. An example of an OPAT physician's order form that includes catheter management and medication orders, laboratory and imaging test orders, and clinic visits is shown in Figure 237-1. The coordinator of the OPAT program, whether it is clinic or hospital based, should ensure that open lines of communication exist among the clinician, the home visiting nurse, the pharmacist, and the parents. A defined, written plan for medical and nursing care should be established. All members of the OPAT team should be aware of their responsibilities.

OPAT may be provided by periodic visits to the clinic, emergency department, or day infusion center[20] or be provided in the home by visiting nurses and parents. For children receiving therapy at home, the frequency of the required home pediatric nursing visits needs to be individualized for each child and depends on many factors—the clinical course while the child is receiving therapy, the seriousness of the infection, the ability of the parents to effectively examine the child, and the risk for development of complications that can be related to either the infection or therapy. For most children, the frequency of home nursing visits will be greatest at the beginning of OPAT, often once daily. After treatment at home has been delivered as anticipated and recovery from infection is proceeding as expected, visits may be decreased to two or three times each week, particularly if the parents have demonstrated competency in providing nursing care, including managing an intravenous catheter, changing wound dressings, and providing clinical assessment. The home visiting nurse also may have the opportunity to establish ongoing assessment of the parents' ability to administer anti-infective agents in the home.

Physical assessment of neonates receiving OPAT may be particularly difficult for parents, and daily visits throughout the entire course of therapy by skilled pediatric home visiting nurses may be required. Daily visits also are important for children with less stable infections, such as central nervous system infections, because subtleties in the neurologic examination findings may not be appreciated by parents or caregivers and may provide clues to impending problems. Nursing aspects of pediatric OPAT have been outlined by successful programs.[26,33,49]

Children's

Children's Hospital - San Diego
3020 Children's Way
San Diego, California 92123-4282

Home Care Order Sheet for Infectious Diseases

Patient: _____

MR# _____

Account # _____

1. DISCHARGE DIAGNOSIS

2. NURSING REQUIREMENTS

a. Frequency of visits:

b. Complications to watch for (include with assessment at each visit):

c. Other nursing requirements

3. MEDICATIONS/ANTIBIOTICS (type, dose, dosing interval, duration):

a.

b.

c.

d. NS FLUSH _____ cc q _____ hrs e. Heparin flush _____ u/cc _____ cc ____ q hrs

4. LABORATORY TESTING:

☐ Via Venipuncture or ☐ Venous Access Device ☐ Other

a. Antibiotic levels

b. CBC

c. ESR, CRP

d. Chem 20

e. Cultures

5. OUTPATIENT VISITS (frequency while on therapy):

a. Infectious Disease Clinic (specify attending physician)

b. Other specialists

c. Primary care physician

6. OTHER TESTS TO BE SCHEDULED (imaging, etc.):

7. DC IV AND DC FROM HOMECARE WHEN THERAPY COMPLETE:

☐ YES ☐ RE-EVAL

8. ATTENDING PHYSICIAN

Dr. NOTIFIED OF PLAN BY

Signature _____ Date _____

NON STOCK / RBF 1898 (06/96) ORIGINAL - Chart CANARY - Home Care Agency PINK - Infectious Diseases GOLD - Primary Care Physician

FIGURE 237-1 ■ An example of a physician's order sheet for outpatient intravenous antimicrobial therapy (OPAT) for serious infections at Rady Children's Hospital San Diego, San Diego, California.

Pediatric competency in nursing is not standardized. Each state government in the United States licenses nurses on the basis of criteria specific for that state. However, just as for inpatient pediatric care, pediatric experience is an important qualification for the home visiting nurse caring for children. Unfortunately, home nursing agencies providing care to children are not required to have expertise in pediatric nursing. Therefore, considerable discrepancy exists in pediatric competency among nursing agencies. Given the importance of pediatric expertise in evaluation and management of infected children in the home, the home nursing agency contracted to provide care is expected to have personnel with training and experience equivalent to that of nurses providing care in a pediatric inpatient setting. The home nursing agency should be able to document proficiency in delivering care to newborns, infants, and children to the clinician and the OPAT coordinator.

In many states, only licensed nurses (registered nurses and licensed vocational nurses) may infuse anti-infective agents. However, most states have no rules prohibiting parents from administering drugs. Accordingly, parents may be expected to infuse medications to their children if licensed nurses are not deemed by the home care agency and the physician to be necessary to provide this function. The physician should be aware of the qualifications of the personnel responsible for the intravenous infusion of anti-infective agents.

The home visiting nurse should communicate with the physician about the medical status (e.g., the medical stability) of the child and plans for follow-up, before OPAT is started. Analogous to inpatient care, the nurses and physicians are expected to be on-call 24 hours each day.

Similarly, the pharmacist providing anti-infective therapy for OPAT should be familiar with the dosages and side effects of therapy provided to all pediatric age groups, from newborns to adolescents. Dosing errors in infants and children are not uncommon occurrences in health care facilities, given the broad range of doses administered.[29]

The parents should have written instructions for the tasks they are required to perform in the home, including information on sterile technique, administration of antimicrobials through sterile tubing and valves, flushing of catheters, and dressing changes. Their proficiency in management of the catheter and infusion of anti-infective agents should be demonstrated before the child is discharged from the hospital, emergency department, or clinic, because some parents or caregivers may not be capable of performing these tasks adequately. Telephone numbers should be provided for the 24-hour on-call nursing and physician personnel. A list of complications of the medications and information on how the parent should perform a clinical assessment of the child's infection should be provided, along with a set of parameters requiring immediate notification of medical personnel. Plans for disposal of needles, dressings, and other medical waste also should be made before the child is sent home.

Visits to clinicians (i.e., primary care providers, surgeons, subspecialists) are scheduled as necessary according to the clinical status of the child and the degree of expertise required by the examiner. If the child is stable in an outpatient setting, once-weekly visits to a physician usually are sufficient unless the home visiting nurse or parent observes problems requiring more immediate attention. For any high-risk, relatively unstable child, daily physician visits may be important, particularly if skilled pediatric home nursing is not available.

Although abuse of intravenous access seldom occurs, for an adolescent who may have a history of alcohol or drug dependence, the clinician should be particularly cautious in approving OPAT. For younger children with indwelling catheters, Münchausen syndrome by proxy may be a concern for infants and children who do not recover clinically as anticipated or have repeated, unanticipated complications.

INFECTIONS SUITABLE FOR OUTPATIENT PARENTERAL ANTIMICROBIAL THERAPY

Virtually any infection can be treated by OPAT at some point in the course of therapy by following the criteria listed in Table 237-1. For each child, a thorough assessment of the risks for development of complications of the infection must be made before outpatient therapy is considered. A partial list of infections treated with OPAT is given in Table 237-3; most of the studies are not prospective, randomized investigations comparing children receiving therapy in the hospital with those receiving therapy outside the hospital. The variety of infections that have been treated with OPAT at some point include bloodstream infections, such as bacteremia, catheter sepsis, and endocarditis; central nervous system infections, including meningitis, epidural abscess, subdural empyema, and brain abscess; osteomyelitis and septic arthritis; urinary tract infections; upper respiratory tract infections, including severe acute otitis media, chronic suppurative otitis, and mastoiditis; lower respiratory tract infections, including lung abscess and empyema; fever with or without neutropenia in low-risk immunocompromised children; neonatal bacterial sepsis; intra-abdominal infections; and postoperative wound infections.

DELIVERY OF ANTIMICROBIAL THERAPY

Selection of an Antimicrobial Agent

Selection of antimicrobial therapy for OPAT is similar to selection of therapy for any child in that the primary goal is to achieve clinical and microbiologic cure with the most efficacious, least toxic, and most cost-effective agents. Selection of an agent that is more convenient to administer but that may not be as effective is not appropriate. However, of the agents that demonstrate equivalent activity against the child's pathogen, the preferred agent is the one that is given least frequently, is nontoxic, and requires the least frequent monitoring for adverse events. Preferred agents for the most common community-acquired pathogens are given in Table 237-4. Almost any pathogen, community acquired or nosocomial, bacterial,

TABLE 237-3 Infections Treated with Outpatient Parenteral Antimicrobial Therapy Coordinator

Infection Treated	References	Design*
Collections of various infections	4, 6, 8, 18, 32, 49, 54	III
Appendicitis	3, 14, 47, 57	II
Catheter infection	30	III
Chronic suppurative otitis media	11	II
Cystic fibrosis	61	I
	9	II
	17, 24, 28, 33, 47, 50	III
Fever in immunocompromised children	37, 42, 59	I
	10, 42, 46	II
	26, 58	III
Meningitis	1, 2, 13	III
Mastoiditis	39	II
Neonatal infections	55	II
	5, 13	III
Osteoarticular infections	12, 35	III
Skin	7, 20	II
Urinary tract infection	16	II
	38	III
Peritonitis, peritoneal catheter related	31	III

**I,* Randomized, prospective, comparative trial; *II,* prospective evaluation; *III,* retrospectively reviewed clinical experience.
OPAT, Outpatient parenteral antimicrobial therapy.

fungal, or viral, can be treated in the home if the medical and social criteria for OPAT can be met.

In general, β-lactam agents (i.e., penicillins, cephalosporins, and carbapenems) require less frequent monitoring than do aminoglycosides (i.e., gentamicin, tobramycin, and amikacin), glycopeptides (i.e., vancomycin and teicoplanin), or oxazolidinones (e.g., linezolid). Agents that can be given intravenously during the course of a short period (5 to 15 minutes), such as β-lactam agents, are preferred to those that require up to an hour with each infusion (e.g., aminoglycosides and glycopeptides). Agents that can be given intramuscularly if intravenous access is temporarily lost are preferred to those that may be given only intravenously. Not only are these parameters designed to provide the optimal clinical outcome with minimal toxicity, they also are designed to facilitate therapy in the home. Dosing more frequently than every 8 hours poses a formidable challenge to many families, particularly those without extensive family support. Administration of two or three antibiotics that each require dosing three or four times each day is virtually impossible for most families to accomplish. These parameters are designed to maximize the potential for parents to be able to provide care at home with minimal risk. Although more frequent nursing visits may provide relief to parents, the charges for home nursing visits to administer therapy and to provide nursing care (e.g., wound dressing changes) are substantial; the charge for three nursing visits each day approaches the cost of a day in the hospital for a stable child, and most agencies do not have sufficient staff to provide more than one visit each day to a family.

TABLE 237-4 Selection of Antimicrobial Agents for Common Pathogens in Community-Acquired Infections

Pathogen	Antimicrobial Agent	Dosing Frequency (hr)	Toxicity
Gram-Positive Bacteria			
Streptococcus pneumoniae	Penicillin G*	6	
	Ampicillin*	6-8	
	Cefuroxime*	8	
	Cefotaxime*	6-8	
	Ceftriaxone*,†	24	
	Linezolid*	8-12	Neutropenia, thrombocytopenia
	Vancomycin (for penicillin and cephalosporin nonsusceptible strains)‡	8-12	Renal toxicity, ototoxicity
Staphylococcus aureus	Oxacillin*	6-8	
	Cefazolin†	8-12	
	Clindamycin*	8	Colitis
	Linezolid*	8-12	Neutropenia, thrombocytopenia
	Vancomycin‡	8-12	Renal toxicity, ototoxicity
Streptococcus pyogenes	See *S. pneumoniae*		
Enterococcus	Ampicillin plus gentamicin*,†	Ampicillin: 6-8 Gentamicin: 8	Renal toxicity, ototoxicity (gentamicin)
	Vancomycin plus gentamicin*,‡	Vancomycin: 8-12 Gentamicin: 8	Renal toxicity, ototoxicity (both gentamicin and vancomycin)

TABLE 237-4 **Selection of Antimicrobial Agents for Common Pathogens in Community-Acquired Infections** (Continued)

Pathogen	Antimicrobial Agent	Dosing Frequency (hr)	Toxicity
Gram-Negative Bacteria			
Haemophilus influenzae	Ampicillin*	6-8	
	Cefuroxime	8	
	Cefotaxime	8	
	Ceftriaxone†	24	
Escherichia coli	Ceftriaxone†	24	
	Ampicillin*	6-8	
	Cefotaxime‡	6-8	
	Gentamicin‡ or tobramycin	8-24	Renal toxicity, ototoxicity
	Amikacin‡	12-24	Renal toxicity, ototoxicity
Enterobacter, Serratia, Citrobacter (pathogens with inducible AmpC β-lactamases)	Cefepime†	8-12	
	Ertapenem†	12-24	
	Meropenem	8	
	Imipenem-cilastatin	6-8	
	Cefotaxime* or ceftriaxone plus an aminoglycoside‡ (gentamicin, tobramycin, amikacin)	Cefotaxime: 6-8 Ceftriaxone: 24 Gentamicin and tobramycin: 8-24 Amikacin: 24	Renal toxicity, ototoxicity (aminoglycoside)
	Ciprofloxacin§	12-24	Possible arthropathy
Pseudomonas aeruginosa	Meropenem†	8	
	Cefepime	8	
	Ceftazidime* plus an aminoglycoside‡	Ceftazidime: 8 Aminoglycoside: see above	Renal toxicity, ototoxicity (aminoglycoside)
	Imipenem-cilastatin	6-8	Central nervous system irritability in children with underlying central nervous system inflammation
	Ticarcillin-clavulanate* plus an aminoglycoside‡	Ticarcillin-clavulanate: 6 Aminoglycoside: see above	Renal toxicity, ototoxicity(aminoglycoside)
	Piperacillin-tazobactam* plus an aminoglycoside‡	Piperacillin-tazobactam: 6 Aminoglycoside: see above	Renal toxicity (aminoglycoside)
	Ciprofloxacin§	8-12	Possible arthropathy
Neisseria meningitidis	Ceftriaxone†	24	
	Penicillin G	6	
Fungal Pathogens			
Candida	Fluconazole IV†	24	
	Amphotericin B (lipid preparations are better tolerated)	24, or every other day; may return to infusion center for each injection to manage side effects	Fever, chills, anemia, hypokalemia, decreased glomerular function
	Caspofungin or micafungin	24	
Viral Pathogens			
Herpes simplex virus	Acyclovir	8	Neutropenia, renal toxicity
Cytomegalovirus	Ganciclovir	12-24	Neutropenia

*If organisms are documented to be susceptible.
†Preferred agents based on dosing frequency and side effects.
‡Requires monitoring of renal function and serum concentrations.
§Not indicated for therapy unless no other therapy options exist.

Depending on the agent used, monitoring for antimicrobial toxicity in the outpatient setting should occur with the same frequency as that in the inpatient setting. Monitoring of renal function and serum antibiotic concentrations is important for children being treated with aminoglycosides or vancomycin. For children receiving long-term β-lactam therapy, periodic monitoring of the peripheral white blood cell count and renal and hepatic function every 2 to 4 weeks may detect the uncommon but well-described antibiotic-mediated toxicities of this class of antibiotics before any clinical manifestations occur.

Delivery of Antimicrobial Agents

OPAT can be delivered by intramuscular or intravenous injection. In general, the need for more than two or three injections necessitates intravenous therapy. However, once-daily intramuscular therapy lasting 1 week or longer has not been associated with short-term complications in anecdotal reports.[5,6] Short polyethylene catheters normally used for inpatient intravenous therapy may be used for short-term therapy lasting less than 5 to 7 days.[20] On occasion, intravenous access will be lost and the child will need to be reevaluated for ongoing parenteral therapy; if clinical improvement exceeds expectations, an early switch to oral convalescent therapy may be possible. If not, intravenous access may need to be reestablished. It may be performed in the home but more often will require the child and parent to return to the clinic or hospital. An intramuscular injection or injections may be given temporarily in the home, clinic, or emergency department until the intravenous catheter can be replaced.

For therapy lasting longer than 5 to 7 days, central catheters are preferred. Peripherally inserted central catheters (PICC lines) are widely available, practical, and cost-effective and have become the intravenous access of choice for extended therapy.[24,34,52,54] These catheters may be inserted through many different peripheral sites (usually the antecubital vein) by physicians or nurses with training in the placement of central catheters. Sedation usually is required for younger infants and children. Alternatively, more traditional subcutaneously tunneled central catheters with one or more ports (e.g., Hickman or Groshong) may be placed by a surgeon in the operating room with the child under general anesthesia. These tunneled catheters, which also may be used for blood sampling, are preferred for children requiring several weeks of therapy with agents that require periodic blood tests for assessment of organ function and serum antibiotic concentrations. Each type of central catheter requires sterile techniques for accessing the catheter and close monitoring of the catheter exit site during administration of therapy. The home visiting nurse agency should have written protocols for the care and use of central catheters that are consistent with standards set by The Joint Commission (TJC) (formerly the Joint Commission on Accreditation of Healthcare Organizations [JCAHO]), which will provide a program for Home Care Accreditation in 2013.[50]

Anti-infective agents may be infused directly into the catheter by the caregiver by way of an antibiotic-containing syringe simply attached to the catheter hub ("IV push"), followed by flushing of the catheter with saline or a heparin-containing solution. This method is often used for β-lactam agents. Alternatively, particularly for agents that cannot be infused quickly, an intravenous drip system must be used, or the agent must be infused by a pump. Pumps come in three basic designs: those with no electronic or moving parts; syringe pumps, in which the syringe containing the anti-infective agent is placed in a motorized pump designed to administer the dose during the course of a certain period; and electronic programmable pumps, which may have a variety of pump mechanisms and are programmed to control infusions of one or more agents administered during the course of different periods.

The least expensive pumps are those with no moving parts. One type of pump requires injection of the antibiotic solution, under some pressure, into a thick elastomeric "balloon." The pressure used to inject the antibiotic into the balloon then pushes the antibiotic through an infusion rate–limiting valve into the intravenous line and subsequently into the child. Other types of pumps are designed to place the antibiotic solution into a bag that then is placed into a device in which spring-loaded plates press on the solution contained in the bag, again pushing the antibiotic into the child through an infusion rate–limiting valve. These pumps generally use disposable infusion containers and are adequate for treatment with a single antibiotic administered up to a few times each day.

New tools for OPAT are becoming available each year. Resource guides to the equipment used for OPAT are available.[40] Unfortunately, for children, most of the equipment and resources available are designed for adults.

OUTCOME ANALYSIS

The anticipated outcome in the treatment of an infection is clinical and microbiologic cure with no complications of therapy. Although the psychosocial benefits of OPAT for children from a secure home environment were the original impetus for the establishment of pediatric programs, the economic benefits of outpatient therapy have been easier to document for both adults and children, although current published data in children are sparse.* The cost of treatment is substantially less for outpatient therapy when neither hospital facilities nor 24-hour skilled nursing care is necessary. With shorter periods of hospitalization, the risk for acquiring a nosocomial viral or bacterial infection should diminish and further decrease the overall cost of care.

However, complications may occur in children receiving OPAT, just as they occur in inpatients.[12,19,20,27,32,43] The infection being treated may not be under control or may relapse while the child is being monitored by parents or home nursing personnel. Errors in antibiotic dosing and administration may occur,[29] particularly if parents are given the responsibility of preparing and administering antibiotics in the home. Complications of anti-infective therapy, including adverse drug reactions, may increase when the number of anti-infective agents and doses administered to a child increases. In one pediatric study of children receiving long-term therapy for osteomyelitis, 51 percent of courses in 54 children were associated with adverse drug events.[12] Catheter- and pump-related complications often arise during therapy, with 26 percent of 98 children in one study having catheter complications, most commonly occlusion or clotting and dislodgement, also related to longer duration of therapy.[24,27,43] Parents may not be compliant with clinic visits and may not be in the home when the home nursing agency has

*References 4, 7, 21, 22, 25, 28, 36, 56, 60-62.

scheduled a visit. Family members or neighbors other than those trained to care for the child may provide care without the knowledge of the home nursing agency or physician. The clinician may need to rehospitalize the child if doing so is required to complete parenteral therapy safely.

TJC publishes standards and reviews home care outcomes by evaluating specified parameters, including unscheduled inpatient admissions, early discontinuation of parenteral therapy, interruptions in parenteral therapy, catheter-related infections, and adverse drug reactions.[51]

Until recently, data on outcomes from various home nursing agencies were not made available to contracting physicians, hospitals, or payers. Outcome data now are required to be collected by home health organizations, which eventually will help standardize the assessment of OPAT programs. The physician or OPAT coordinator should request outcomes data from any home nursing agency proposing to care for a child by OPAT.

SUMMARY

Successful use of OPAT requires integrated delivery of care for children who are assessed to be at low risk for development of complications from their infections and therapy and do not require skilled nursing care. Parents fulfill the role of a pediatric nurse in the hospital and should be capable of providing a focused, limited assessment of their child and communicating any concerning changes in the child's medical condition or concerns regarding adverse drug reactions or catheter or pump malfunction to the appropriate medical personnel. The anti-infective agents, infusion equipment, and medical follow-up all should be designed for the outpatient setting compatible with the training and resources available to parents or caregivers in the home. Outcomes of pediatric OPAT should be equivalent to or better than those achieved in an inpatient setting.

NEW REFERENCES SINCE THE SIXTH EDITION

7. Brugha RE, Abrahamson E. Ambulatory intravenous antibiotic therapy for children with preseptal cellulitis. *Pediatr Emerg Care* 2012;**28**:226–8.
12. Faden D, Faden HS. The high rate of adverse drug events in children receiving prolonged outpatient parenteral antibiotic therapy for osteomyelitis. *Pediatr Infect Dis J* 2009;**28**:539–41.
15. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**52**:e56–93.
20. Gouin S, Chevalier I, Gauthier M, et al. Prospective evaluation of the management of moderate to severe cellulitis with parenteral antibiotics at a paediatric day treatment centre. *J Paediatr Child Health* 2008;**44**:214–18.
25. Heintz BH, Halilovic J, Christensen CL. Impact of a multidisciplinary team review of potential outpatient parenteral antimicrobial therapy prior to discharge from an academic medical center. *Ann Pharmacother* 2011;**45**:1329–37.
27. Hussain S, Gomez MM, Wludyka P, et al. Survival times and complications of catheters used for outpatient parenteral antibiotic therapy in children. *Clin Pediatr (Phila)* 2007;**46**:247–51.
51. Joint Commission. *2012 Comprehensive accreditation manual for home care (CAMHC)*. Oak Brook Terrace, Ill: TJC; 2012.
32. Le J, San Agustin M, Hernandez EA, et al. Complications associated with outpatient parenteral antibiotic therapy in children. *Clin Pediatr (Phila)* 2010;**49**:1038–43.
35. Maraqa NF, Rathore MH. Pediatric outpatient parenteral antimicrobial therapy: an update. *Adv Pediatr* 2010;**57**:219–45.
44. Rathore MH. The unique issues of outpatient parenteral antimicrobial therapy in children and adolescents. *Clin Infect Dis* 2010; **51**(Suppl. 2):S209–15.
54. Van Winkle P, Whiffen T, Liu IL. Experience using peripherally inserted central venous catheters for outpatient parenteral antibiotic therapy in children at a community hospital. *Pediatr Infect Dis J* 2008;**27**:1069–72.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 238

ANTIVIRAL AGENTS

Gail J. Harrison

HISTORY AND BACKGROUND

The age of modern antiviral therapy began in the early 1950s, when methisazone, a derivative of the thiosemicarbazones (early antituberculosis compounds), was found also to have activity against vaccinia and variola viruses.* In 1959, the first antiherpes compound, idoxuridine, was synthesized; in 1962, it was approved for the topical treatment of herpetic keratitis.[294,429] Shortly thereafter, in 1964, trifluridine also was used to treat herpetic

*References 38, 39, 215, 442, 536, 567.

REFERENCES

1. Al-Howasi M. The use of a modified out-patient infusion therapy for lowering the cost in Suleimania Children's Hospital. *J Kuwait Med Assoc* 1996;**28**:455–7.
2. Arditi M, Yogev R. Convalescent outpatient therapy for selected children with acute bacterial meningitis. *Semin Pediatr Infect Dis* 1990;**1**:404–10.
3. Bradley JS, Ching DK, Phillips SE. Outpatient therapy of serious pediatric infections with ceftriaxone. *Pediatr Infect Dis J* 1988;**7**: 160–4.
4. Bradley JS, Behrendt CE, Arrieta AC, et al. Convalescent phase outpatient parenteral antiinfective therapy for children with complicated appendicitis. *Pediatr Infect Dis J* 2001;**20**:19–24.
5. Bradley JS, Ching DL, Wilson TA, et al. Once-daily ceftriaxone to complete therapy of uncomplicated group B streptococcal infection in neonates: a preliminary report. *Clin Pediatr (Phila)* 1992;**31**: 274–8.
6. Bradley JS, Farhat C, Stamboulian D, et al. Ceftriaxone therapy of bacterial meningitis: Cerebrospinal fluid concentrations and bactericidal activity after intramuscular injection in children treated with dexamethasone. *Pediatr Infect Dis J* 1994;**13**:724–8.
7. Brugha RE, Abrahamson E. Ambulatory intravenous antibiotic therapy for children with preseptal cellulitis. *Pediatr Emerg Care* 2012;**28**:226–8.
8. Dagan R, Phillip M, Watemberg NM, et al. Outpatient treatment of serious community-acquired pediatric infections using once daily intramuscular ceftriaxone. *Pediatr Infect Dis J* 1987;**6**:1080–4.
9. Donati MA, Guenette G, Auerbach H. Prospective controlled study of home and hospital therapy of cystic fibrosis pulmonary disease. *J Pediatr* 1987;**111**:28–33.
10. Egerer G, Goldschmidt H, Muller I, et al. Ceftriaxone for the treatment of febrile episodes in nonneutropenic patients with hematooncological disease or HIV infection: comparison of outpatient and inpatient care. *Chemotherapy* 2001;**47**:219–25.
11. Esposito S, Noviello S, Ianniello F, et al. Ceftazidime for outpatient parenteral antibiotic therapy (OPAT) of chronic suppurative otitis media due to *Pseudomonas aeruginosa*. *J Chemother* 2000;**12**:88–93.
12. Faden D, Faden HS. The high rate of adverse drug events in children receiving prolonged outpatient parenteral antibiotic therapy for osteomyelitis. *Pediatr Infect Dis J* 2009;**28**:539–41.
13. Ferris EW. A neonatal home intravenous antibiotic therapy program. *J Intraven Nurs* 1990;**13**:383–7.
14. Fishman SJ, Pelosi L, Klavon SL, et al. Perforated appendicitis: prospective outcome analysis for 150 children. *J Pediatr Surg* 2000;**35**:923–6.
15. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011;**52**:e56–93.
16. Gauthier M, Chevalier I, Sterescu A, et al. Treatment of urinary tract infections among febrile young children with daily intravenous antibiotic therapy at a day treatment center. *Pediatrics* 2004;**114**:e469.
17. Gilbert J, Robinson T, Littlewood JM. Home intravenous antibiotic treatment in cystic fibrosis. *Arch Dis Child* 1988;**63**:512–17.
18. Goldenberg RI, Poretz DM, Eron LJ, et al. Intravenous antibiotic therapy in ambulatory pediatric patients. *Pediatr Infect Dis* 1984;**3**: 514–17.
19. Gomez M, Maraqa N, Alvarez A, et al. Complications of outpatient parenteral antibiotic therapy in childhood. *Pediatr Infect Dis J* 2001; **20**:541–3.
20. Gouin S, Chevalier I, Gauthier M, et al. Prospective evaluation of the management of moderate to severe cellulitis with parenteral antibiotics at a paediatric day treatment centre. *J Paediatr Child Health* 2008;**44**:214–18.
21. Grainger-Rousseau TJ, Segal R. Economic, clinical and psychosocial outcomes of home infusion therapy: a review of published studies. *Pharm Pract Manage Q* 1995;**15**:57–77.
22. Grizzard MB, Harris G, Karns H. Use of outpatient parenteral antibiotic therapy in a health maintenance organization. *Rev Infect Dis* 1991;**13**(Suppl. 2):S174.
23. Gutierrez K. Continuation of antibiotic therapy for serious bacterial infections outside of the hospital. *Pediatr Ann* 1996;**25**:639–45.
24. Hammond LJ, Caldwell S, Campbell PW. Cystic fibrosis, intravenous antibiotics, and home therapy. *J Pediatr Health Care* 1991;**5**: 24–30.
25. Heintz BH, Halilovic J, Christensen CL. Impact of a multidisciplinary team review of potential outpatient parenteral antimicrobial therapy prior to discharge from an academic medical center. *Ann Pharmacother* 2011;**45**:1329–37.
26. Hooker L, Kohler J. Safety, efficacy, and acceptability of home intravenous therapy administered by parents of pediatric oncology patients. *Med Pediatr Oncol* 1999;**32**:421–6.
27. Hussain S, Gomez MM, Wludyka P, et al. Survival times and complications of catheters used for outpatient parenteral antibiotic therapy in children. *Clin Pediatr (Phila)* 2007;**46**:247–51.
28. Kane RE, Jennison K, Wood C, et al. Cost savings and economic considerations using home intravenous antibiotic therapy for cystic fibrosis patients. *Pediatr Pulmonol* 1988;**4**:84–9.
29. Kaushal R, Bates DW, Landrigan C, et al. Medication errors and adverse drug events in pediatric inpatients. *JAMA* 2001;**285**: 2114–20.
30. Kinsey SE. Experience with teicoplanin in non-inpatient therapy in children with central line infections. *Eur J Haematol Suppl* 1998;**62**:11–14.
31. Kuizon B, Melocoton TL, Holloway M, et al. Infectious and catheter-related complications in pediatric patients treated with peritoneal dialysis at a single institution. *Pediatr Nephrol* 1995; **9**(Suppl.):S12–17.
32. Le J, San Agustin M, Hernandez EA, et al. Complications associated with outpatient parenteral antibiotic therapy in children. *Clin Pediatr (Phila)* 2010;**49**:1038–43.
33. Leaver J, Radivan F, Patel L, et al. Home intravenous antibiotic therapy: practical aspects in children. *J R Soc Med* 1997;**90**(Suppl. 31):26–33.
34. Maraqa NF, Rathore MH. Pediatric outpatient parenteral antimicrobial therapy: an update. *Adv Pediatr* 2010;**57**:219–45.
35. Maraqa NF, Gomez MM, Rathore MH. Outpatient parenteral antimicrobial therapy in osteoarticular infections in children. *J Pediatr Orthop* 2002;**22**:506–10.
36. Mullen CA, Petropoulos D, Roberts WM, et al. Economic and resource utilization analysis of outpatient management of fever and neutropenia in low-risk pediatric patients with cancer. *J Pediatr Hematol Oncol* 1999;**21**:212–18.
37. Mullen CA, Petropoulos D, Roberts WM, et al. Outpatient treatment of fever and neutropenia for low risk pediatric cancer patients. *Cancer* 1999;**86**:126–34.
38. Nelson DS, Gurr MB, Schunk JE. Management of febrile children with urinary tract infections. *Am J Emerg Med* 1998;**16**:643–7.
39. Niv A, Nash M, Peiser J, et al. Outpatient management of acute mastoiditis with periosteitis in children. *Int J Pediatr Otorhinolaryngol* 1998;**46**:9–13.
40. OPIT Source Book. *OutPatient infusion therapy source book*. Available at: http://www.opitsourcebook.com/.
41. Patrick CC, Shenep JL. Outpatient management of the febrile neutropenic child with cancer. *Adv Pediatr Infect Dis* 1999;**14**:29–47.
42. Petrilli AS, Dantas LS, Campos MC, et al. Oral ciprofloxacin vs. intravenous ceftriaxone administered in an outpatient setting for fever and neutropenia in low-risk pediatric oncology patients: randomized prospective trial. *Med Pediatr Oncol* 2000;**34**:87–91.
43. Porea TJ, Margolin JF, Chintagumpala MM. Radiological case of the month: pulmonary air embolus with home antibiotic infusion. *Arch Pediatr Adolesc Med* 2001;**155**:963–4.
44. Rathore MH. The unique issues of outpatient parenteral antimicrobial therapy in children and adolescents. *Clin Infect Dis* 2010;**51**(Suppl. 2):S209–15.
45. Rucker RW, Harrison GM. Outpatient intravenous medications in the management of cystic fibrosis. *Pediatrics* 1974;**54**:358–60.
46. Shemesh E, Yaniv I, Drucker M, et al. Home intravenous antibiotic treatment for febrile episodes in immune-compromised pediatric patients. *Med Pediatr Oncol* 1998;**30**:95–100.
47. Stovroff MC, Totten M, Glick PL. PIC lines save money and hasten discharge in the care of children with ruptured appendicitis. *J Pediatr Surg* 1994;**29**:245–7.
48. Strandvik B, Hjelte L, Malmborg AS, et al. Home intravenous antibiotic treatment of patients with cystic fibrosis. *Acta Paediatr* 1992;**81**:340–4.
49. Sudela KD. Nursing aspects of pediatric home infusion therapy for the treatment of serious infections. *Semin Pediatr Infect Dis* 1990;**1**: 306–17.
50. The Joint Commission. n.d. Available at http://www.jointcommission.org/Standards.

51. The Joint Commission. *2012 comprehensive accreditation manual for home care (CAMHC)*. Oak Brook Terrace, Ill: TJC; 2012.
52. Thiagarajan RR, Ramamoorthy C, Gettmann T, et al. Survey of the use of peripherally inserted central venous catheters in children. *Pediatrics* 1997;**99**:E4.
53. Tice AD, Rehm SJ, Dalovisio JR, et al. Practice guidelines for outpatient parenteral antimicrobial therapy. IDSA guidelines. *Clin Infect Dis* 2004;**38**:1651.
54. Van Winkle P, Whiffen T, Liu IL. Experience using peripherally inserted central venous catheters for outpatient parenteral antibiotic therapy in children at a community hospital. *Pediatr Infect Dis J* 2008;**27**:1069–72.
55. Wagner CL, Wagstaff P, Cox TH, et al. Early discharge with home antibiotic therapy in the treatment of neonatal infection. *J Perinatol* 2000;**20**:346–50.
56. Wai AO, Frighetto L, Marra CA, et al. Cost analysis of an adult outpatient parenteral antibiotic therapy (OPAT) programme: a Canadian teaching hospital and Ministry of Health perspective. *Pharmacoeconomics* 2000;**18**:451–7.
57. Warner BW, Kulick RM, Stoops MM, et al. An evidenced-based clinical pathway for acute appendicitis decreases hospital duration and cost. *J Pediatr Surg* 1998;**33**:1371–5.
58. Wiernikowski JT, Rothney M, Dawson S, et al. Evaluation of a home intravenous antibiotic program in pediatric oncology. *Am J Pediatr Hematol Oncol* 1991;**13**:144–7.
59. Wilimas JA, Flynn PM, Harris S, et al. A randomized study of outpatient treatment with ceftriaxone for selected febrile children with sickle cell disease. *N Engl J Med* 1993;**329**:472–6.
60. Williams DN, Bosch D, Boots J, et al. Safety, efficacy, and cost savings in an outpatient intravenous antibiotic program. *Clin Ther* 1993;**15**:169.
61. Wolter JM, Bowler SD, Nolan PJ, et al. Home intravenous therapy in cystic fibrosis: a prospective randomized trial examining clinical, quality of life and cost aspects. *Eur Respir J* 1997;**10**:896–900.
62. Woodin KA, Davis CJ. The economic and psychosocial impact of outpatient parenteral antibiotic therapy in pediatrics. *Semin Pediatr Infect Dis* 1990;**1**:419–28.

keratitis.[295,296] Also in 1964, the first description of vidarabine as an antiviral agent active against herpes simplex virus (HSV) was published, and amantadine was shown to have activity against influenza virus.[124,467] Two years later, amantadine was approved first for the prophylaxis and subsequently for the treatment of influenza A virus infection.[469] Shortly thereafter, in 1972, vidarabine was approved for the treatment of herpes encephalitis.[556] The year 1972 also marked the first description of ribavirin as a broad-spectrum antiviral agent, with activity against both DNA and RNA viruses, and in 1985, the aerosolized form of the drug was approved for the treatment of respiratory syncytial virus (RSV) bronchiolitis.[127,518] In 1977, acyclovir was reported to be a potent and selective inhibitor of the replication of both HSV and varicella-zoster virus (VZV) and boasted the added advantage of a favorable safety profile.[126,151,503] Subsequently, several structural analogues of acyclovir led to the development of antiviral agents with expanded antiviral spectra, such as ganciclovir, and agents with more favorable bioavailability, such as with the prodrugs valacyclovir and valganciclovir.[116,356] In the 1990s, the introduction of cidofovir, a broad-spectrum, long-lasting antiviral agent, and the neuraminidase inhibitors zanamivir and oseltamivir, which treat both influenza A and influenza B viruses, added new dimensions to antiviral therapy.[77,113,327,493] The research now being conducted no doubt will expand our antiviral armamentarium to combat viruses that may be used as biologic weapons as well as to prevent the emergence of resistant viruses and spawn creative strategies such as multidrug therapy and targeted delivery systems.[190,226]

Even though diseases caused by viruses (Table 238-1) now may be treated with a variety of antiviral agents, the close relationship between the viral replicative cycle and its host cell metabolism unfortunately has caused the development of safe and effective antiviral agents to lag behind the development of other antimicrobials, such as antibiotics and antifungals. Clinically successful antiviral agents target and inhibit virus-specific functions while keeping cellular toxicity to a minimum. In addition, as a further testament to the codependency between viruses and host cells, some antiviral agents actually require cellular metabolism for antiviral activity, such as the terminal phosphorylation of acyclovir monophosphate to the active triphosphate form.

Antiviral agents can be categorized as virucidals, antiviral chemotherapeutic agents or drugs, and immunomodulators. Virucidal agents inactivate the virus on contact and include detergents, solvents, and ultraviolet light. These agents are not useful for treatment of human viral disease because healthy tissue also is destroyed. They may, however, be used to inactivate viruses on the surface of the skin or inanimate objects. Antiviral treatments that

TABLE 238-1 **Virus-Associated Diseases That May Be Treated with Antiviral Agents That Are Currently Available or Under Clinical Development**

DNA Viruses

Herpes simplex virus
- Gingivostomatitis
- Keratoconjunctivitis
- Eczema herpeticum
- Whitlow
- Genital ulcers
- Esophagitis
- Hepatitis
- Encephalitis
- Aseptic meningitis
- Neonatal disease

Varicella-zoster virus
- Chickenpox
- Zoster
- Acute retinal necrosis
- Pneumonitis
- Hepatitis
- Cerebral vasculitis and stroke

Cytomegalovirus
- Retinitis
- Pneumonitis
- Esophagitis and colitis
- Fever and leukopenia syndrome
- Congenital disease

Epstein-Barr virus
- Mononucleosis syndrome
- Posttransplantation lymphoproliferative disease

Herpes B virus
- Encephalitis

Adenoviruses
- Disseminated disease
- Hemorrhagic cystitis
- Pneumonitis
- Colitis
- Conjunctivitis

Hepatitis B virus
- Acute and chronic hepatitis

Variola virus
- Smallpox

Vaccinia virus
- Vaccine-associated complications

Papillomaviruses
- Cutaneous and genital warts
- Laryngeal papillomatosis

Polyomaviruses (BK, JC, SV40)
- Hemorrhagic cystitis
- Progressive multifocal encephalopathy

RNA Viruses

Influenza viruses
- Influenza syndrome and complications

Parainfluenza viruses
- Laryngotracheobronchitis
- Pneumonitis

Respiratory syncytial virus
- Bronchiolitis
- Pneumonitis

Measles virus
- Measles syndrome
- Encephalitis
- Pneumonitis

Enteroviruses
- Aseptic meningitis and meningoencephalitis
- Myocarditis
- Neonatal disease

Arenavirus
- Lassa fever

Hepatitis C virus
- Acute and chronic hepatitis

physically destroy both virus and the tissues infected or transformed by them include cryotherapy, laser therapy, and podophyllin and are used primarily to treat recalcitrant or life-threatening warts on mucocutaneous or laryngotracheal tissue. The host immune response also is important and in many cases essential for recovery from viral disease or even maintenance of a latent or inactivated state of the virus. Therefore, successful antiviral treatment may necessitate relief from immunosuppression when it is feasible, such as for patients with Epstein-Barr virus (EBV)–induced lymphoproliferative disease who are undergoing cancer chemotherapy or organ transplantation. It also may include the use of biologic response modifiers, or immunomodulators, that manipulate the immune system to enhance its ability to contain viral infection. Examples of immunomodulators include immune globulin and monoclonal antibody preparations, cytokines such as interferons, and even novel approaches such as virus-specific cytotoxic T-cell lines designed to reconstitute host immunity.[21,35] In addition, supportive treatments including fluid resuscitation, blood product transfusions, mechanical ventilation, and extracorporeal membrane oxygenation are important for serious viral disease.[23,109] Finally, updated knowledge about specific antiviral therapy licensed for hepatitis viruses is reviewed in Chapters 47, 156, and 176, and antiretroviral therapy is reviewed in Chapter 191.

Antiviral chemotherapeutic agents usually inhibit virus-specific events (Fig. 238-1), such as adsorption or attachment to the host cell (pleconaril), penetration and uncoating of the viral genome (amantadine), viral gene expression and nucleic acid synthesis (acyclovir, ganciclovir), and even viral assembly of intact, infectious viral particles (interferons). Therefore, antiviral agents exhibit their "antiviral effect" primarily while viral replication is active at the host cell level. If the antiviral compound is withdrawn or discontinued, viral replication resumes. Furthermore, the currently available antiviral agents do not appear to eliminate viruses that are latent or in other dormant or nonreplicative states. The goal of antiviral therapy, for the most part, is to inhibit active viral replication to such a degree that the host immune response is able to contain or in some instances even to eliminate the infection.

Antiviral agents usually have a narrow range of activity that can be predicted by their molecular mechanism of

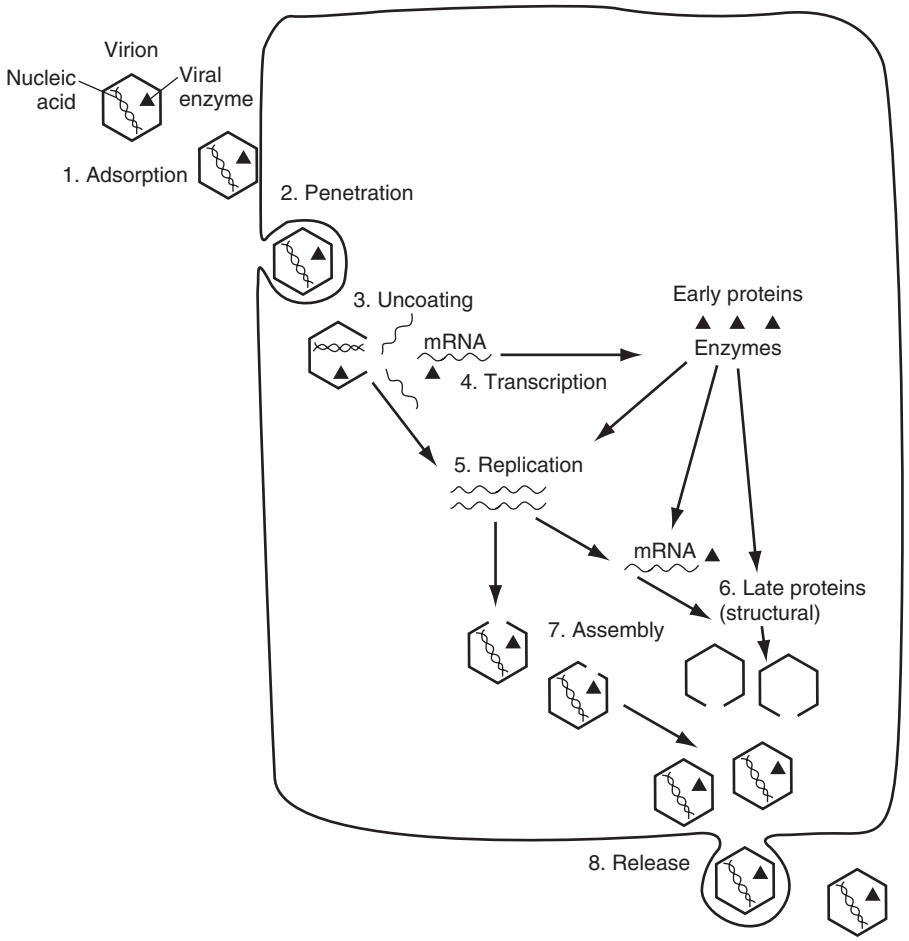

FIGURE 238-1 ■ Basic steps in intracellular (DNA) replication. *1,* Adsorption of virion to the cell membrane. *2,* Penetration. *3,* Uncoating, removal of the protein coat. *4,* Early gene expression, transcription of viral proteins. *5,* Replication, synthesis of DNA strands. *6,* Late gene expression, transcription of messenger RNA, and translation of late protein synthesis. *7,* Maturation and assembly of virions. *8,* Release.

action. For example, rimantadine and amantadine have high activity against the RNA-containing influenza A virus, limited activity against influenza B virus, and virtually no activity against the DNA herpesviruses,[113,124] whereas acyclovir, a deoxyguanosine analogue that requires monophosphorylation by the viral enzyme thymidine kinase (TK) for activation, has significant activity against DNA viruses (HSV), which carry TK, but no activity against RNA viruses such as influenza virus. Viruses infecting a host also may become resistant to a specific agent to which they originally were susceptible, usually by induced or selected mutations. Resistance is therefore most likely to occur in viruses that infect the host with a high viral load and that have a high intrinsic viral mutation rate as well as in viruses that infect hosts who are exposed to selective drug pressure during chronic, low-dose, or repeated treatment with an antiviral agent.[249] Both resistant and sensitive viruses are capable of causing serious disease, especially in an immunocompromised host. Currently, most antiviral agents are administered as single therapeutic agents. However, double and triple combination antiviral drug therapy, now a routine therapy in modern antiretroviral regimens, is also important in selected circumstances for treatment of other viral diseases in immunocompromised hosts or where presence or emergence of drug-resistant viruses is likely.[24,221,226,400,401] Combination strategy increases antiviral effectiveness, prevents the emergence of drug resistance, and allows the administration of lower, less toxic dosages.*

ANTIVIRAL AGENTS ACTIVE AGAINST RNA VIRUSES (Table 238-2)

Amantadine and Rimantadine

Spectrum of Activity

Amantadine (1-adamantanamine hydrochloride) and rimantadine (α-methyl-1-adamantane methylamine hydrochloride) are tricyclic amines with specific activity against influenza A viruses (Fig. 238-2). Mean inhibitory concentrations of 0.1 to 0.4 μg/mL for amantadine have been reported, and rimantadine is 4 to 10 times more active than amantadine against susceptible subtypes of influenza A virus.[72,224,422] Amantadine also has in vitro activity against rubella virus, but efficacy was not confirmed in animal models.[409,410,468,508] Much higher concentrations (10 to 50 μg/mL) appear to inhibit influenza B virus and parainfluenza viruses, but these high concentrations cannot be achieved safely in humans.[468] Research and development continue with designs of more adamantane-like inhibitors of the M2 proton channel against influenza A virus.[144,422] The adamantanes also have significant activity against hepatitis C virus (HCV) and rhabdoviruses (vesicular stomatitis virus and rabies virus).[480,490,560]

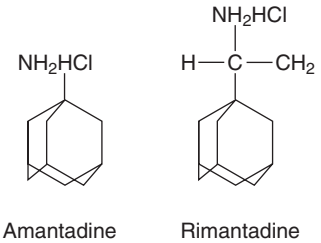

FIGURE 238-2 ■ Structure of amantadine and rimantadine.

Mechanism of Action and Resistance

The mechanism of action of both amantadine and rimantadine against influenza A virus appears to be primarily inhibition of the ion channel function of the M2 protein in the membrane of the virus, possibly affecting two stages of viral replication, uncoating and, to a lesser extent, assembly.[22,113,220,422] Amantadine and rimantadine resistance occurs when mutations cause an amino acid change in one of four critical sites (amino acids 26, 27, 30, and 31) in the transmembrane channel or domain of the M2 viral protein.[313] Such mutations occur frequently and rapidly during therapy because approximately 30 percent of adults and children who receive amantadine or rimantadine therapy for at least 5 days shed resistant influenza virus.[222,225] These resistant strains are genetically stable, may be transmitted to close contacts, and can produce disease.[44,222,579] Immunocompromised patients may shed drug-resistant influenza viruses for prolonged periods, and resistant strains can persist even after drug administration has been discontinued.[313] However, debate has begun among experts concerning whether patients in whom a resistant strain is identified should be isolated until viral shedding has ceased.[211] Adamantane-resistant strains of influenza A subtype H3N2 and subtype H1N12009 have produced community outbreaks and epidemics.[44,222,235,579] Some strains of highly pathogenic A(H5N1) avian influenza are susceptible to adamantanes, but most strains of A(H5N1) should be considered

TABLE 238-2 **Clinical Indications and Usual Treatment Dosages for Antiviral Agents Active Against RNA Viruses**

Agent	Indication	Dosage
Amantadine	Influenza A	2.2-4.4 mg/kg/dose PO bid (max, 100-200 mg daily)
Rimantadine	Influenza A	2.5 mg/kg/dose PO bid (max, 150 mg daily)
Oseltamivir	Influenza A and B	2 mg/kg/dose PO bid (max, 150 mg daily)
Zanamivir	Influenza A and B	5 mg/dose inhaled bid
Ribavirin	Respiratory syncytial virus	6 g/300 mL inhaled daily in an 18-hr period
		or
		2-6 g/100 mL inhaled in a 2-hr period tid
	Hepatitis C	600 mg PO bid (with interferon alfa)

*References 33, 43, 263, 504, 505.

resistant.[272] Strains of influenza A virus resistant to amantadine also are resistant to rimantadine, and vice versa, but they are usually susceptible to ribavirin and neuraminidase inhibitors. The mechanism of action against rabies virus appears to be by inhibition of later steps in replication, probably at the level of uncoating, when the virus is released into the intracytoplasmic compartment of infected cells.[520]

Pharmacokinetics

Amantadine is absorbed rapidly and nearly completely after oral administration, with peak plasma concentrations of 0.5 to 0.8 μg/mL attained within 2 hours of administration.[53,230] It is excreted almost unchanged in urine. Nasal and salivary secretions also contain high levels of amantadine, and the drug is present in cerebrospinal fluid (CSF) as well at approximately half the plasma level. Dose reductions, adjusted according to creatinine clearance, are required in patients with renal insufficiency and renal failure.[260,459,569] Rimantadine, on the other hand, is more slowly absorbed than amantadine, with peak plasma levels of 0.4 to 0.6 μg/mL achieved within 2 to 4 hours of administration.[14,230,393] Rimantadine is also present in high concentration in nasal secretions. It is excreted in urine, but only after extensive metabolism by the liver. Dose reduction is therefore recommended in patients with renal or hepatic disease.[79,561] Hemodialysis does not appear to clear either drug, so supplemental doses usually are not recommended after dialysis.[499] When they are administered to chronically ill, institutionalized elderly patients, dosages also may need to be adjusted.[385,416] In addition, aerosolized forms of amantadine and rimantadine have been evaluated in human volunteers.[228,234]

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Amantadine and rimantadine are equally effective for the treatment of disease caused by susceptible subtypes of influenza A virus.[113,313,561] If they are administered within the first 48 hours of symptoms, both agents significantly reduce the signs and symptoms of influenza in both adults and children by significantly shortening the duration of fever and respiratory and systemic symptoms by 1 day.* The duration and amount of viral shedding from the nasopharyngeal tract also are decreased, and otologic complications may be reduced.[139] Both agents are effective for prophylaxis against influenza A virus infection and disease, with approximately 50 percent overall effectiveness in preventing infection with the virus and approximately 60 to 70 percent overall effectiveness (up to 90% in some studies) in preventing clinical disease and, in individuals with breakthrough clinical disease, in reducing the severity of the disease.† The spontaneous emergence of resistant strains during rimantadine or amantadine therapy and subsequent transmission of these resistant strains may lessen the effectiveness of prophylaxis in some families and nursing homes.[222,362] Amantadine and rimantadine are both available as tablets (100 mg) and syrup (50 mg/5 mL) for oral administration.

Emergence of resistance to adamantanes has limited the use of amantadine and rimantadine as single agents for influenza treatment. Since 2005, all circulating H3N2 subtypes of influenza A virus have been resistant to amantadine and rimantadine. Also resistant to adamantanes is the 2009 pandemic subtype influenza A(H1N1)pdm2009, which replaced the previously susceptible seasonal H1N1 subtype when it emerged. Influenza B is intrinsically resistant to the adamantanes. Amantadine has also been included in treatment protocols for patients with rabies encephalitis.[560]

Dosage. The usual dose of amantadine for children is 4.4 to 8.8 mg/kg/day divided twice daily (many experts recommend 5 mg/kg/day), with a maximal recommended daily dose of 200 mg. The usual dose of rimantadine for children is 5 mg/kg/day divided twice daily, with a maximal recommended daily dose of 150 mg.[14,211,393,561] Older children and adolescents may take the usual adult dose for each drug, which is 200 mg/day given once daily or, for better tolerance, divided into two 100-mg doses daily.[14] Elderly patients should receive 100 mg daily or less if renal insufficiency is present. To be effective, these agents should be given as soon as possible after the onset of symptoms.[113,114] The duration of treatment of established influenza should be 3 to 7 days, or at least 2 days after the cessation of systemic signs and symptoms.[113] Prevention of influenza is accomplished best through annual vaccination; however, rimantadine and amantadine may be used to prevent or to lessen influenza symptoms in both adults and children.[101,113,115] Prophylaxis should be administered during the period of risk, whether exposure is in the family, community, or nosocomial setting.[183] In general, prophylaxis should continue for at least 10 days after a known exposure occurs in an unprotected individual.[183] For patients who receive the combination of inactivated vaccine and oral prophylaxis, rimantadine or amantadine should be continued for at least 2 to 4 weeks after vaccination, when maximal antibody response from the vaccine is expected to occur. For patients who are at high risk but did not receive the vaccine, oral prophylaxis should be continued for as long as the period of risk is anticipated and may be extended to the end of the community's influenza season. Doses of both drugs should be adjusted for patients with renal disease, and doses of rimantadine should be adjusted for patients with hepatic disease.[79,260,459,561] Prolonged treatment may be associated with side effects, especially in the elderly.[416] If side effects occur, the daily dose may be reduced to 100 mg and still be effective in most cases. The emergence of resistant strains during the period of drug administration may lessen the effectiveness of prophylaxis in some patients.[222,362] Combination with aerosolized ribavirin or the neuraminidase inhibitors may enhance the antiviral effect of amantadine or rimantadine against influenza A virus.[33,221,259,399,479]

An amantadine dose of 200 mg daily has been used to treat rabies encephalitis.[560]

*References 113, 129, 342, 537, 562, 573.

†References 113, 130, 137, 183, 278, 279, 386, 406, 451, 552, 580.

Adverse Effects. The adverse effects of both amantadine and rimantadine include central nervous system (CNS) effects such as anxiety, depression, insomnia, dizziness, and impaired concentration; in children, these side effects appear to be rare, but they may be manifested as behavioral changes.[101,113,137,496] Rimantadine reportedly has fewer CNS side effects than amantadine does.[113,137,229,380] Hallucinations, tremors, and seizures also may occur, especially in patients with renal failure who have high plasma levels of amantadine. In addition, rare cases of coma and fatal cardiac dysrhythmia have been reported.[423,459] A prudent procedure is to administer amantadine and rimantadine with caution to individuals being treated for seizures or neuropsychiatric disorders or to patients also receiving other medications that affect the nervous system, such as antihistamines.[380] Furthermore, the institutionalized elderly may have increased plasma levels and experience side effects more often than younger patients do.[416] On occasion, gastrointestinal symptoms such as nausea and vomiting may accompany the oral administration of these agents in all age groups. Most reported adverse effects of these two agents have been reversible, with significant long-term complications being exceedingly rare. Of note, amantadine also is used to treat parkinsonism and drug-induced extrapyramidal reactions.[469]

Neuraminidase Inhibitors (Zanamivir, Oseltamivir, Peramivir, and Laninamivir)

Spectrum of Activity

Zanamivir (5-[acetylamino]-4-[(aminoiminomethyl)-amino]-2,6-anhydro-3,4,5-trideoxy-D-glycero-D-galacto-non-2-enoic acid) and oseltamivir ([3*R*,4*R*,5*S*]-4-acetylamino-5-amino-3[1-ethylpropoxy]-1-cyclohexene-1-carboxylic acid ethyl ester phosphate [1:1]) are analogues of *N*-acetylneuraminic acid, the cell surface receptor for influenza viruses, and were designed to inhibit the viral neuraminidase of influenza viruses (Fig. 238-3). Oral oseltamivir and inhaled zanamivir are licensed in the United States. The cyclopentane peramivir ([1*S*,2*S*,3*S*,4*R*]-3-[(1*S*)-1-acetamido-2-ethyl-butyl]-4-(diaminomethylideneamino)-2-hydroxy-cyclopentane-1-carboxylic acid) and laninamivir ([4*S*,5*R*,6*R*]-acetamido-4-carbamimidamido-6-[(1*R*,2*R*)-3-hydroxy-2-methoxypropyl]-5-6-dihydro-4*H*-pyran-2-carboxylic acid) are newer neuraminidase inhibitors currently in clinical trials in the United States.

These four agents are potent and specific inhibitors of the neuraminidases of influenza A (seasonal H3N2, seasonal H1N1, H1N1pdm2009, and avian influenza viruses, including highly pathogenic H5N1 and H9N2) and influenza B viruses. Up to 90 percent of tested strains are inhibited at concentrations of 0.05 to 100 μmol/L.[200,304,539]

Peramivir has also shown efficacy for the treatment of influenza, especially H1N1pdm2009, in at least four efficacy trials.[50] Peramivir, intravenous form, is licensed in Japan and Korea. Peramivir was authorized for emergency use in the United States and other countries for treatment of adults and children with severe influenza during the 2009 H1N1 pandemic. It also was administered through emergency investigational new drug regulations[238] and appeared to be well tolerated and associated with recovery.[238] However, because peramivir under emergency use authorization was limited to critically ill patients for whom other therapy had failed or in whom oral drug absorption was unreliable or inhalation drug not feasible, the efficacy and safety of peramivir could not be adequately assessed.[255] Peramivir has not yet received Food and Drug Administration (FDA) approval in the United States and remains in phase III clinical trials.

Long-acting inhaled laninamivir octanoate is a new neuraminidase inhibitor, with activity against highly pathogenic avian influenza virus H5N1 in addition to influenza A (seasonal H3N2, seasonal H1N1, and H1N1pdm2009) and influenza B.[162,243,469,476] Laninamivir is licensed and available in Japan and in phase III clinical trials in the United States.[273,489,573] Influenza A (AH5N1, AH1N1) and B virus strains with reduced susceptibility to neuraminidase inhibitors (oseltamivir, zanamivir, and peramivir) have also been detected in hospitalized patients and community-acquired cases of influenza.*

Because influenza virus changes seasonally and antiviral resistance may evolve or emerge suddenly, any statements on presumed spectrum of antiviral activity of any neuraminidase inhibitor should be taken in the context of ongoing viral surveillance.[162,268,276,469,481]

These compounds also are capable of inhibiting neuraminidases from other pathogens and mammalian cells, but only at extremely high concentrations.

*References 34, 162, 243, 256, 268, 269, 270, 271, 273, 509, 521.

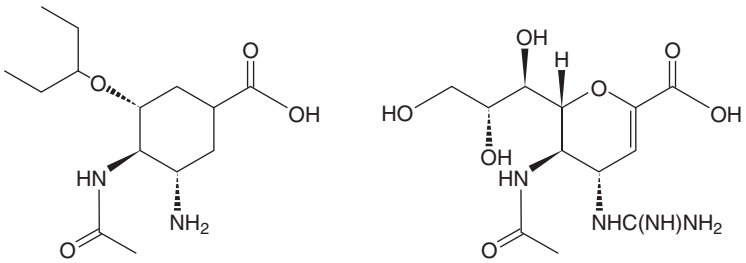

FIGURE 238-3 ■ Structure of the neuraminidase inhibitors oseltamivir and zanamivir.

Mechanism of Action and Resistance

Neuraminidase permits influenza virus to penetrate through the mucoproteins present in respiratory secretions to the surfaces of cells, and inhibition of this enzyme prevents viral access and infection of cells. Neuraminidase also destroys receptors recognized by viral hemagglutinin and is therefore necessary for optimal release of influenza virus particles from infected cells. Inhibition prevents cell-to-cell spread of virus within the respiratory tract and lessens the intensity of the initial infection.[105,113,304,395,539] Mutations that induce amino acid changes in viral neuraminidase alter enzyme stability or activity, mutations in hemagglutinin produce a reduced affinity for cell receptors, and mutations in both produce resistant influenza viruses.[113,202-205] Cross-resistance to both zanamivir and oseltamivir, as well as to oseltamivir and peramivir, also can occur, especially if the mutation is in the hemagglutinin portion.[375] Resistance occurs either by spontaneous mutation or by selection during antiviral treatment and has been documented by in vitro passage of the virus in increasing concentrations of the agents, in patients receiving these antiviral agents, and in newly emerged strains that acquire a change in neuraminidase through mutation or reassortment. In 2007, influenza A(H1N1) seasonal acquired a change in neuraminidase, designated H275Y, that resulted in oseltamivir resistance, but zanamivir susceptibility was retained. In 2009, oseltamivir-susceptible influenza A(H1N1) pdm2009 emerged, replacing the oseltamivir-resistant seasonal H1N1. Beginning in 2009, individual cases of oseltamivir resistance, usually in immunocompromised patients receiving treatment, as well as community clusters of oseltamivir-resistant influenza A(H1N1)pdm2009 began to be detected.[84,145] Sporadic oseltamivir resistance has also been detected in avian influenza A(H5N1). Clinical trials of oral oseltamivir for the treatment of naturally occurring influenza detected resistance in 1 to 2 percent of post-treatment isolates from adults and adolescents and in 5 to 8 percent of isolates from younger children.[113,204,260,576] Currently, most seasonal H1N1 strains circulating in 2008-2009 were resistant to oseltamivir, and the overall occurrence of influenza A H1N1 subtypes resistant to oseltamivir is estimated at a rate of 2.6 percent, with an association between oseltamivir resistance and pneumonia in four clinical studies.[34,235,270,273,529]

Peramivir resistance has also been documented in both influenza A, including pandemic A(H1N1)pdm2009, and influenza B.[413] A point mutation at H273Y in one influenza B isolate from a human patient conferred double resistance to both oseltamivir and peramivir.[243] Most peramivir- and oseltamivir-resistant influenza strains remain susceptible to zanamivir at this time.

Zanamivir resistance appears rare, documented in case reports of either influenza A or B virus infection in patients who were usually immunocompromised hosts receiving antiviral medications for prolonged periods.* Large randomized trials of healthy individuals have not documented zanamivir resistance, but individualized case reports and small case series have shown that zanamivir resistance has emerged.[202,227,231,236,382] In one study from Australia, analysis of 391 influenza A(H1N1) seasonal isolates documented zanamivir resistance in 9 (2.3%) isolates.[271] Influenza B viruses with mutations in the neuraminidase active site conferring resistance to oseltamivir, peramivir, and zanamivir have been reported from several geographic sites.[162,243,469,481] Overall, there are currently at least 50 different documented amino acid substitutions that have produced mutations conferring reduced antiviral susceptibility to oseltamivir, zanamivir, peramivir, or laninamivir in influenza A and B viruses.[402,403] Preliminary evidence suggested that some mutant resistant strains may have reduced infectivity or virulence in comparison to susceptible strains,[524] but resistant strains have caused outbreaks and epidemics.[235] Therefore, difference in influenza virulence cannot be predicted by the antiviral susceptibility pattern of the strain.

*References 202, 206, 227, 231, 236, 382, 529.

Pharmacokinetics

Zanamivir is administered as an inhaled dry powder and is deposited in the oropharynx and, to a lesser extent, in the tracheobronchial tree and lungs.[113] Approximately 4 to 17 percent of inhaled drug is absorbed systemically, and levels of 17 to 142 ng/mL can be detected in serum 1 to 2 hours after administration of a typical inhaled dose of 10 mg to adults.[80] Serum levels in pediatric patients who received an inhaled dose were extremely low (<10 ng/mL) or undetectable, which may be related to the ability of the patient to cooperate with the inhaled drug delivery system. The oral bioavailability of zanamivir is extremely low, with less than 5 percent of the administered dose absorbed, and therefore it is not administered in this form. Intravenous and intramuscular preparations of zanamivir also have been tested in clinical trials, but this form is not available clinically at this time.[78,80,180,235] Intravenous zanamivir provides a sustained antiviral effect.[175] It also penetrates lung tissue. Zanamivir administered intravenously at doses of 100 mg, 200 mg, and 600 mg provides concentrations in epithelial lung fluid 12 hours after dosing of 74, 146, and 419 ng/mL, respectively.[476] In contrast, inhaled zanamivir concentrations provide much higher levels of 396 to 891 ng/mL to the respiratory tract. Zanamivir is not metabolized, and approximately 90% of zanamivir is excreted unchanged in urine.[80]

Oseltamivir, in contrast, has good oral bioavailability. The prodrug compound oseltamivir phosphate is metabolized by hepatic esterases to the parent active compound oseltamivir carboxylate.[113] Approximately 75 to 80 percent of the administered dose is absorbed, and peak plasma concentrations are reached within 3 to 4 hours but may be delayed, but not reduced, if the drug is given with food. A typical 75-mg dose will provide plasma concentrations of 0.3 to 0.5 μg/mL. Both the prodrug and the active form are eliminated unchanged through the kidneys, and dosages should be adjusted for patients with renal insufficiency or renal failure. Whether the drug dosage should be adjusted for patients with hepatic disease or liver failure is unclear from the available evidence. Information from a small number of pediatric patients suggests that the drug may be eliminated faster

in younger children. Elderly, geriatric patients, however, appear to metabolize the drug similar to young adults.

Peramivir administered orally in doses ranging from 100 to 800 mg daily is not well absorbed and produces only modest antiviral effect.[37] Peramivir has been administered intravenously in doses of 200, 400, and 600 mg daily, or approximately 4 to 10 mg/kg once daily.[274] Plasma concentration of approximately 20 ng/mL within 24 hours of infusion has been documented, which is far in excess of the reported IC_{50} of peramivir against susceptible influenza strains. Pediatric dosing for peramivir suggests that 10 mg/kg daily produces adequate plasma levels and good antiviral effect with few adverse effects.[477] Pregnant or postpartum women may require higher doses on the basis of limited pharmacokinetic studies that have demonstrated increased plasma volume, increased volume of distribution of peramivir, and shorter half-life.[100] The half-life of peramivir varies between 7 and 20 hours in patients with normal renal function. Peramivir is excreted in the urine as the unchanged form, and dosage adjustments are needed in renal impairment. Patients with proven influenza infection show antiviral effects with decreased viral shedding between days 2 and 6 of treatment (7% to 20%), with clinical improvement and recovery.[477]

Laninamivir octanoate is the prodrug of the active compound laninamivir. It is administered as a single inhalation of either 20 mg or 40 mg to treat influenza infection. It is retained in the tissues and eliminated from the body very slowly, with a plasma half-life of 2 hours and lasting at least 3 days. Urinary excretion appears to occur. Clinical trials show that one inhalation of laninamivir is an effective and well-tolerated treatment for children and adults with seasonal influenza, including oseltamivir-sensitive and oseltamivir-resistant influenza A(H1N1) virus infection.[476,549]

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Zanamivir and oseltamivir have been shown to be effective in treating both influenza A and influenza B infection.[77,288,363,548,549] However, susceptibility of type B and H5NI and H1N1 subtypes of type A influenza may be reduced for oseltamivir.[520] When they are administered within the first 2 days of illness, they provide relief from symptoms up to 1.5 days earlier than placebo does and reduce the quantity and duration of viral shedding.* In some studies, the frequency of secondary complications, such as sinusitis, bronchitis, and otitis media with effusion (but not pneumonia), also was reduced.[113,544] Both antiviral agents are effective for the prophylaxis of influenza A and influenza B infection in both adults and children in family, community, transplant recipient, and nursing home situations.[276] They appear to be 30 to 50 percent effective in preventing viral infection and 64 to 84 percent effective in preventing disease.† However, up to 89 percent efficacy in preventing disease in families has been reported, and in elderly patients in nursing homes, 92 percent of cases of influenza may be prevented if the agents are administered to those who also have received influenza vaccine.[113,227,366] Oseltamivir may be administered to a patient of any age to treat influenza.

Peramivir may be administered intravenously, under investigational or emergency investigational new drug or clinical trials, to patients unable to tolerate oral oseltamivir or inhaled zanamivir.[347,451] The temporary emergency use authorization for peramivir for treatment of 2009 H1N1 influenza, granted by the FDA and managed through the Centers for Disease Control and Prevention, terminated in June 2010.[50,238] On the basis of results of clinical trials and experience with the emergency use authorization, 300- to 600-mg doses of peramivir appear to be effective for treatment of high-risk patients infected with susceptible influenza A or B virus strains.[50,238,316] Peramivir probably should not be used to treat patients with oseltamivir-resistant influenza infection (H275Y mutation), patients with mutations of neuraminidase E119D or R292K (which confer peramivir resistance), or patients suspected of having influenza caused by zanamivir resistance because it is likely not to be effective.

Laninamivir may be administered in some countries as a single inhalation to treat influenza A H1N1 and H5N1 infections in adults and children. A dose of laninamivir, 20 to 40 mg, administered in a single inhalation, is retained in the respiratory tissues for a prolonged time, providing very high levels, in excess of the IC_{50} of susceptible influenza strains. This high level may reduce the risk of emergence of resistant strains. Inhaled or intranasal laninamivir also enters the plasma, and renal excretion correlates with creatinine clearance. Pharmacokinetic studies suggest that dose adjustment may be necessary only in severe renal insufficiency.[274,549]

Intravenous zanamivir, which remains investigational at this time, appears effective in treating high-risk, critically ill patients, including immunocompromised patients, with oseltamivir- or peramivir-resistant influenza A infection.[145,175,189,276,413]

Dosage. Zanamivir is administered by oral inhalation with an inhalation device provided with the product. Each inhalation dose is 5 mg, and two inhalations twice daily for 5 days is the usual recommended dosage for all age groups. Use of this drug and device is dependent on the understanding and cooperation of the patient; therefore, it is not indicated for use in children younger than 7 years and may be of limited use in older children and adults who are unable to cooperate with the delivery system. No reduction in dosage is needed for patients with impaired renal function because very little of the inhaled drug is absorbed systemically.

Zanamivir has also been administered by nebulizer at a dosage of 16 to 32 mg (usually 24 mg) in 1 to 2 mL of sterile water every 6 hours for 5 to 10 days.[206]

Zanamivir has been administered intravenously in doses of 600 mg at a constant rate during 30 minutes, every 12 hours for 5 days, under investigational, compassionate use to individual patients and currently is being

*References 77, 113, 231, 313, 366, 382, 388, 426, 533, 553.
†References 113, 227, 233, 366, 387, 544.

evaluated in clinical trials (www.clinicaltrials.gov).[136,145,219] Pediatric dosing in the clinical trials is weight based and age adjusted. A dose of 4 to 10 mg/kg per dose every 12 hours has been evaluated.[144] Younger children appear to require higher doses; children who weigh less than 11 kg may be given up to 24 mg per dose if renal function is normal. Some institutions have administered up to 16 to 24 mg/kg per dose every 12 hours. All trials agree that a maximum of 600 mg/dose should be given twice daily, and this maximum dose should be used for children who are older than 13 years or weigh more than 37 kg and have normal renal function. Doses should be adjusted in renal impairment according to creatinine clearance.

Oseltamivir is administered orally and is available in 75-mg capsules and a 12-mg/mL suspension. The usual adult dosage is 75 mg twice daily for treatment and once or twice daily for as long as 6 weeks for prophylaxis. The pediatric dose is 2 mg/kg per dose given twice daily (4 mg/kg/day divided twice daily).[113] Double dosing of oral oseltamivir (up to 150 mg twice daily for adults and older children) was recommended for some patients during the 2009 H1N1 influenza A pandemic and may be used for treatment of severe seasonal or pandemic influenza or influenza caused by a strain thought to have reduced susceptibility to oseltamvir.[100]

Peramivir has been administered intravenously to adults in doses between 300 and 600 mg once daily, during a 60-minute infusion.[497,574] Weight-based dosing suggests a once-daily dose of approximately 4 to 10 mg/kg. Duration of treatment is recommended for 5 to 10 days.[274,275] Pediatric dosing, for ages 3 months to 15 years, has been studied. A once-daily dose of 10 mg/kg, with a maximum daily dose of 600 mg, appears to be clinically and virologically effective and safe in infants and children with influenza.[477,517] Neonates should be dosed at 6 mg/kg daily and infants at 8 to 10 mg/kg daily, according to FDA guidance (www.fda.gov). Dosage adjustments are needed in renal impairment. Patients with severe or end-stage renal disease should probably receive 100 mg daily and after each dialysis because peramivir is cleared by dialysis (www.fda.gov).

The dose of laninamivir is 20 to 40 mg, administered in a single inhalation to treat the entire illness. Dosage adjustments are necessary only in severe renal impairment.[274,516,549]

Adverse Effects. Both neuraminidase inhibitors are generally well tolerated, with few if any serious adverse effects observed in clinical trials. Zanamivir inhalation, however, may produce local irritation or bronchospasm in some patients, and oseltamivir has been associated with nausea and vomiting, which may be lessened by administration of the drug with food.[113] The most commonly observed side effect of inhaled laninamivir is gastrointestinal. The most common side effects of intravenous peramivir are nausea, vomiting, diarrhea, and neutropenia. Rarely, skin reactions and anaphylaxis have occurred with peramivir administration. Intravenous zanamivir appears well tolerated on the basis of limited clinical experience at this time.

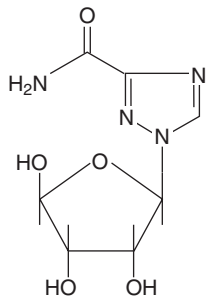

Ribavirin

FIGURE 238-4 ■ Structure of ribavirin.

Ribavirin

Spectrum of Activity

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a synthetic guanosine analogue with broad-spectrum antiviral activity (Fig. 238-4).[487] It inhibits the in vitro replication of a wide variety of RNA viruses, including orthomyxoviruses such as influenza A and B viruses; paramyxoviruses, such as RSV, parainfluenza viruses, and measles virus; arenaviruses, such as Lassa fever virus; bunyaviruses, which cause viral encephalitis; togaviruses, such as HCV; enteroviruses, such as enterovirus 71, polioviruses, and coxsackie B virus; rabies virus; and retroviruses, including human immunodeficiency virus (HIV) type 1.* Also inhibited by ribavirin are many DNA viruses, such as adenoviruses, hepatitis A virus, cytomegalovirus (CMV), and poxviruses (e.g., vaccinia virus).[138] It inhibits most orthomyxoviruses and paramyxoviruses at concentrations of 3 to 10 μg/mL and Lassa fever virus at 4 to 40 μmol/L.[266] Because most of its clinical use has been for the treatment of RNA viruses, ribavirin is included in this section of the chapter.

Mechanism of Action and Resistance

Ribavirin most likely exerts its antiviral effects by altering the cellular nucleotide pools and by interfering with viral mRNA formation.[71,160,511] The compound is phosphorylated by host cell enzymes to monophosphate, diphosphate, and triphosphate active forms, each with specific antiviral actions.[525] For example, ribavirin monophosphate competitively interferes with the synthesis of guanosine triphosphate and, subsequently, with viral nucleic acid synthesis. Ribavirin triphosphate inhibits the RNA polymerase of influenza virus and also interferes with the capping of its viral mRNA.[160,302,408,558,568] In addition, the diphosphate and triphosphate forms of ribavirin appear to inhibit the reverse transcriptase activity of HIV-1.[166] The mechanism of action of ribavirin against HCV and other viruses, however, is not well understood. Ribavirin also exerts an effect on the host immune system by inhibiting mast cell secretory responses and diminishing IgE responses.[443] Antiviral resistance is not well characterized

*References 160, 263, 302, 328, 330, 340, 408, 478, 560.

and appears to be a rare occurrence. No ribavirin-resistant RSV isolates have been identified during clinical trials, and information is scant regarding the resistance of other viruses to ribavirin.[213,350,464]

Pharmacokinetics

Ribavirin can be administered by aerosol, orally, and intravenously. Aerosol administration of ribavirin with a small-particle aerosol generator delivers very high concentrations (>1000 μg/mL) of the drug to the respiratory tract.[315] Aerosolized drug also is absorbed systemically, and peak plasma levels after 8 hours of continuous aerosol administration range from 0.5 to 2.2 μg/mL; after 20 hours, they reach 0.8 to 3.3 μg/mL. After oral administration, bioavailability is 33 to 45 percent, and peak plasma levels of 1.3 to 3.2 μg/mL have been observed 1 to 2 hours after administration. Intravenous administration of ribavirin produces 10-fold higher plasma levels in less than 1 hour than after the administration of equivalent doses by different routes.[414] Higher doses used to treat Lassa fever produce plasma levels of up to 24 μg/mL. Ribavirin also crosses the blood-brain barrier and is present in CSF at approximately 70 percent of the plasma level.[110,261] The drug is metabolized by the liver, and approximately 40 percent of the dose is excreted by the kidneys.[414] The triphosphate form also concentrates in erythrocytes at an erythrocyte-to-plasma ratio of 40:1 and is slowly eliminated with a half-life of 40 days or longer. Hemodialysis and hemofiltration do not remove significant amounts of ribavirin from the body.[319]

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Ribavirin aerosol is licensed for the treatment of RSV bronchiolitis and pneumonia in children.[210] Treatment shortens the duration of viral shedding and improves the fever, respiratory rate, arterial oxygen saturation, and lower respiratory tract signs on physical examination in severely ill patients who receive the antiviral agent early in the course of their illness.*

No clear benefit has been observed in patients with mild disease or in those who receive treatment late in the course of the disease. Studies of infants receiving mechanical ventilation have reported conflicting results in clinical benefit.[372,383,488] However, despite numerous clinical studies, indications for treatment remain controversial because efficacy has not been documented convincingly in all studies and the benefits observed generally have been mild and difficult to quantitate objectively.[210] Nonetheless, because antiviral treatment clearly appeared to provide benefit to some children, in 1996, the American Academy of Pediatrics recommended that ribavirin be considered for the treatment of hospitalized infants and children with severe lower respiratory tract infection caused by RSV. Currently, the American Academy of Pediatrics recommends that ribavirin may be considered for use in selected patients with documented, potentially life-threatening RSV infection.[9]

*References 201, 212, 240, 285, 372, 383, 430, 485, 488, 554.

Patients thought to potentially benefit the most from inhaled ribavirin treatment are those younger than 6 weeks; those with chronic illnesses, such as congenital heart disease and chronic lung disease; premature infants; immunosuppressed children with primary immune disorders or those undergoing marrow or solid-organ transplantation; and those with neurologic or neuromuscular disorders.[474] Treatment with ribavirin also appears to decrease nasopharyngeal secretion of RSV-specific IgE and IgA responses.[449] Long-term follow-up of treated patients suggests no adverse effects but possible improvement in pulmonary function.[240,285,346] Treatment may benefit immunocompromised children who have serious or prolonged RSV infection, such as those with congenital severe combined immunodeficiency.[369] Aerosolized or intravenous ribavirin, especially combined with intravenous immune globulin or RSV hyperimmune globulin, also may be of benefit to bone marrow transplant recipients and solid-organ transplant recipients with RSV lower tract disease.* Intravenous administration appears less costly than aerosolized treatments.[123,194,384] Anecdotal reports suggest that early administration of ribavirin to immunocompromised patients during upper respiratory tract disease may be more efficacious than supportive care alone in preventing progression to lower tract disease or pneumonia.[156,369] Some centers have used oral ribavirin therapy for RSV infection, instead of inhaled or intravenous administration. Small studies have shown similar therapeutic outcomes between oral and inhaled ribavirin therapy for RSV infection and lower respiratory tract disease after solid-organ transplantation.[339,417] Laboratory confirmation of RSV infection by viral culture or rapid diagnostic tests that detect viral antigen in respiratory secretions is recommended for all patients before treatment with ribavirin is initiated or continued beyond 12 to 24 hours. Ribavirin also is licensed in capsule form for the treatment of chronic hepatitis caused by HCV, and in combination with interferon alfa, it produces a sustained decrease in HCV RNA levels, improvement in systemic symptoms such as fatigue, reduction in transaminases, and improvement in liver histologic features. These clinical benefits have been observed during initial treatment in previously untreated patients, in individuals initially unresponsive to interferon alone, and in patients treated for relapse.† Treatment with high doses of orally or intravenously administered ribavirin significantly reduces mortality rates in patients with Lassa fever.[66,284,367] Ribavirin also appears to benefit patients suffering from other hemorrhagic fever syndromes, including Argentine, Sabiá, Bolivian, and Crimean-Congo hemorrhagic fevers, as well as those who have hemorrhagic fever with renal failure syndrome.[158,169,264,265,303] Ribavirin has also been used to treat La Crosse encephalitis, but clinical trials have not shown a clear therapeutic benefit.[370] In addition, ribavirin may benefit patients with other causes of viral encephalitis, including rabies, but clinical trials have not been performed.[81] Oral ribavirin also may be of benefit prophylactically in individuals who are exposed to

*References 123, 194, 346, 349, 384, 555.
†References 52, 55, 125, 134, 179, 262, 368, 436.

hemorrhagic fever syndromes, including Lassa fever.[257] Treatment of hantavirus pulmonary syndrome with intravenously administered ribavirin is being evaluated in clinical trials.

Ribavirin may benefit individuals with influenza A or B virus infection. In one randomized study of young adults suffering from influenza, aerosol treatment significantly reduced fever, systemic signs and symptoms, and virus titer in respiratory secretions.[487] Another study showed clinical benefit with high-dose oral ribavirin, but other studies have not confirmed a measurable clinical benefit or virologic response consistently with aerosolized or oral ribavirin.[47,314,467,487,507] Intravenous ribavirin also has been used anecdotally to treat serious, life-threatening influenza.[232,432] Early treatment with aerosolized or intravenous ribavirin in bone marrow and stem cell transplant recipients infected with parainfluenza virus may help prevent progression to lower tract disease and pneumonia or treat viremia and myocarditis, but controlled trials documenting efficacy and effect on overall mortality rates have not been published.[152,232,291,553] Ribavirin also has been used to treat measles pneumonia in immunocompetent and immunocompromised patients, with clinical benefit reported in some.[172,207,292] Ribavirin has also been included in treatment protocols for patients with rabies encephalitis.[353,560]

Clinical benefit, but not cure, also has been documented in case reports of children with subacute sclerosing panencephalitis caused by measles virus.[261,262,392,485] In these reports, repeated courses of large doses of ribavirin administered intravenously, combined with interferon alfa, provided serum and CSF levels that exceeded the minimal inhibitory concentrations needed to inhibit the virus in vitro and improved or stabilized seizures and neurologic status in treated patients.[261] Case reports and small series using ribavirin to treat serious adenoviral disease, including pneumonia and disseminated adenoviral disease, have been published, but no controlled trials documenting clinical benefit have been published.* Despite documented antiretroviral activity, treatment of HIV-infected patients with oral ribavirin has not shown clinical, virologic, or immunologic benefits consistently.[110,166,286]

Dosage. A number of different dosages and routes of administration have been used to administer ribavirin to a variety of patients experiencing various infections. In normal and immunocompromised patients of all ages, the usual dosage of ribavirin, when it is administered topically by aerosol to the respiratory tract for treatment of influenza and lower respiratory tract disease caused by RSV, parainfluenza virus, and measles virus, is 6 g of drug reconstituted in a final volume of 300 mL of sterile water (final concentration, 20 mg/mL); a small-particle aerosol generator (SPAG-2 unit) is used to administer the drug continuously during a period of 12 to 18 hours for 3 to 7 days.† The drug may be delivered to an infant oxygen hood or tent, by facemask, or through pressure- or volume-cycled ventilators, provided ventilatory pressure is monitored and the device is checked for precipitation, which may cause ventilator dysfunction.[178,407]

Intermittent high-dose therapy, with drug administered at 60 mg/mL for 2 hours three times daily for 5 days, also appears to be effective and may be better tolerated in some older patients.[155] Longer courses of aerosolized therapy for up to 14 days have been used in severely immunocompromised patients with prolonged viral shedding and severe disease.[338] Oral doses of up to 600 mg twice daily for 24 to 48 weeks, usually combined with interferon alfa, are used to treat hepatitis C in adults.* High doses of oral ribavirin also appear to be effective in treating influenza in normal hosts and RSV infection in immunocompromised hosts.[340,417,474] Even higher doses of oral ribavirin (2-g loading dose, followed by 2 g/day divided every 8 hours for 10 days) have been used for the treatment of Lassa fever and other hemorrhagic fever syndromes in adults.† The intravenous dose of ribavirin used to treat Lassa fever is a 2-g loading dose (25 to 33 mg/kg estimated pediatric dose), followed by 1-g doses (16 mg/kg estimated pediatric dose) every 6 hours for 4 days and then 500-mg doses (8 mg/kg estimated pediatric dose) every 8 hours for 3 days to complete a 7- to 10-day course of treatment.[284,367] This intravenous dosage regimen also has been used to treat measles pneumonia, severe influenza with complications, pneumonia caused by RSV and parainfluenza virus in immunocompromised patients, and disseminated adenovirus disease.[172,207,349,432] Continuous intravenous infusions of ribavirin have been used in patients with severe disease caused by influenza and parainfluenza viruses.[232] Doses used to treat subacute sclerosing panencephalitis in children have been considerably higher and administered for longer periods.[261,392] In one report, pediatric patients received interferon alfa combined with 10 mg/kg of ribavirin administered intravenously every 8 hours daily for 7 days. Escalating doses of 20 mg/kg and then 30 mg/kg given every 8 hours daily for 7 days at 7-day intervals then were administered.[261] The highest dose tolerated was given for 7 days, repeated in 7-day intervals, and administered for a period of 6 months.

Adverse Effects. Adverse effects of aerosolized ribavirin are unusual but include local irritation causing facial rash and conjunctivitis. Abnormalities in pulmonary function, including worsening of respiratory distress and bronchospasm, also have been reported.[193,213,483] In addition, one case of water intoxication associated with aerosolized ribavirin administration has been reported.[530] Older children, adolescents, and adults may be noncompliant with treatment or may even have anxiety attacks or experience other forms of psychological stress as they are being confined for continuous treatment for a 12- to 18-hour period. In these cases, intermittent, high-dose regimens may be better tolerated and might enhance compliance of the patient.[155] Precipitation of the drug in ventilator tubing systems and on filtering devices may

*References 31, 61, 86, 187, 361, 390.
†References 47, 152, 201, 212, 213, 292, 314, 338, 361, 372, 383, 390, 487, 488, 523, 555.

*References 52, 55, 125, 134, 258, 368, 436.
†References 158, 169, 257, 265, 284, 303, 367.

cause ventilator dysfunction and respiratory distress or failure in mechanically ventilated patients, especially those receiving high-dose intermittent administration, but such events may be minimized by frequent monitoring of ventilator pressure and changing of the filters.[178,372,383,407] Adverse hematologic effects, such as anemia, have not been documented consistently in patients who received aerosolized ribavirin. The environmental exposure to aerosolized ribavirin by health care workers providing direct care to a virus-infected patient may, in some instances, cause headache, conjunctivitis, contact lens damage, watery eyes, and bronchospasm in those with underlying reactive airway disease.[64,193,443,477]

These potential adverse effects are probably more likely to be seen in instances in which ribavirin is administered by hood, tent, or facemask than when it is administered to mechanically ventilated patients.[65] Environmental exposure of health care workers and family members may be reduced by providing routine patient care during periods when the aerosol generator has been turned off, by turning off the aerosol generator during periods of more urgent care, and by using protective equipment such as masks and goggles. Dose-related, reversible, usually mild anemia occurs frequently during and after long-term oral therapy and intravenous administration of ribavirin.[258,286,369] Rarely, severe anemia may occur and necessitate dose reduction or cessation of therapy.[370] Reversible increases in serum bilirubin, serum iron, and uric acid concentrations also have been documented during oral therapy. On rare occasion, intravenous administration of ribavirin has produced chills and rigors.[168,193]

Ribavirin has shown teratogenic, mutagenic, and embryotoxic effects in preclinical animal models.[247] Therefore, investigators recommend that exposure of pregnant women to ribavirin, either therapeutically or through environmental exposure, be minimized or prevented whenever possible or practical.

Pleconaril

Spectrum of Activity

Pleconaril (3-[3,5-dimethyl-4[[3-(3-methyl-5-isoxazolyl) propyl]oly]phenyl]-5-(trifluoromethyl)-1,2,4-oxadiazole) is an antiviral agent with significant and specific activity against picornaviruses, both enteroviruses and rhinoviruses (Fig. 238-5). In vitro, pleconaril inhibits the replication of most serotypes of enteroviruses at concentrations between 0.01 and 0.1 μg/mL. Echovirus 11, a commonly isolated enterovirus in the United States, appears to be the most sensitive enterovirus tested to date (inhibited at a mean concentration of 0.006 μg/mL), although resistant strains have been reported. Most rhinovirus serotypes are inhibited at slightly higher concentrations of 0.21 to 0.78 mg/mL but still within ranges safely achievable in patients.

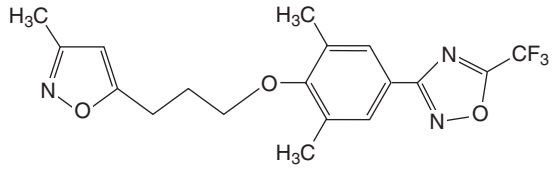

Pleconaril

FIGURE 238-5 ■ Structure of pleconaril.

Mechanism of Action and Resistance

Pleconaril binds efficiently to a specific hydrophobic pocket within the viral capsid of picornaviruses and exhibits an antiviral effect by interfering with uncoating, attachment, and cell-to-cell transmission of infectious viral particles.[450] The drug induces a more rigid structure within the viral capsid, which then interferes with viral uncoating and release of viral RNA.* Pleconaril also appears to change the conformation of the canyon floor of the virus capsid when it integrates with the underlying pocket, thereby interfering with attachment of the virus to host cell receptors.[420,421] Resistance of picornaviruses to pleconaril has been reported.

Pharmacokinetics

The pharmacokinetics of pleconaril varies with the age of the group tested. After oral administration of single doses of 200 to 400 mg to adults, peak plasma concentrations of 1.1 to 2.4 μg/mL are achieved in 1.5 to 5 hours, with an average elimination half-life of 25 hours.[2] Repeated dosing for a 7-day period increases plasma concentrations to 1.33 to 3.4 μg/mL. When pleconaril is taken with food, especially a meal high in fat, absorption is enhanced significantly.[1,298] Single oral doses of 5 mg/kg in children provide plasma concentrations of approximately 1.3 μg/mL with a more rapid elimination half-life of approximately 6 hours.[297] Neonates also absorb pleconaril well, but they do not exhibit dose-proportionate pharmacokinetics as adults do.[298] The drug also has a large volume of distribution and concentrates in tissues such as the liver, brain, and nasal epithelium, where viral replication and virus-induced disease are likely to occur.[298] Pleconaril appears to be excreted in feces and urine.

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Pleconaril currently is not licensed for therapeutic use, and it is not available at this time for compassionate use.[559] However, clinical trials continue to evaluate pleconaril for treatment of serious enteroviral disease as well as rhinovirus infection in neonates and children (www.clinicaltrials.gov). Pleconaril has shown efficacy in early clinical trials against picornavirus infections in adults, children, and neonates. In pretreated adults experimentally infected with coxsackie A21 virus, pleconaril significantly reduced viral titers as well as the clinical parameters of fever, nasal mucus production, and systemic symptoms compared with placebo.[223,450,465] In

*References 174, 371, 420, 421, 444, 450.

another trial, pleconaril resolved the symptoms of illness 2 days earlier than placebo did, and patients who received pleconaril did not require as much concomitant medication for symptomatic relief as did those who received placebo.[450] In two placebo-controlled trials in adults with aseptic meningitis caused by enterovirus, pleconaril treatment reduced the duration of headache and other symptoms of meningitis by 2 to 3 days and allowed treated patients to return to work and routine daily activities 2 days earlier than patients who received placebo.[450,465] In a placebo-controlled trial in pediatric patients with aseptic meningitis caused by enterovirus, pleconaril significantly improved illness scores and reduced the duration of headache in older children, but clinical efficacy was difficult to assess in infants and younger children.[3] Viral shedding from the throat also was reduced in the treatment group.[460]

In addition to treatment of the "common cold" and "viral meningitis," another potential use of pleconaril is for the treatment of serious, persistent, or life-threatening illness caused by enteroviruses. More than 90 patients with unusual or life-threatening infections with enterovirus have received pleconaril on a compassionate-use basis.[174,559] These patients included immunocompromised patients with chronic meningitis, neonates with disseminated disease, patients with myocarditis,[450,559] and individuals with poliomyelitis associated with vaccine or wild-type poliovirus.[445] In all groups, most of the patients showed clinical and virologic responses.

Dosage. Pleconaril is manufactured as a hard gelatin capsule and an oral solution. A nasal spray formulation has also been developed and tested in clinical trials against rhinovirus infection. The capsule contains 200 mg of drug, and doses used in adults and older children have been 200 to 400 mg every 8 hours for 7 days (21 doses). An oral solution also has been prepared for pediatric use. It contains 40 mg/mL of pleconaril, medium-chain triglycerides, two surfactants (Tween 80 and Arlacel), 1.5 percent saccharin, and cherry-peppermint flavoring. Doses of 5 mg/kg, 7.5 mg/kg, and 8 mg/kg of the oral solution administered every 8 to 12 hours for 7 days (14 to 21 doses) have been used in clinical trials of young children, infants, and neonates.[450] Oral pleconaril is generally well tolerated by all age groups.

Adverse Effects. Adverse effects associated with pleconaril treatment include nausea, diarrhea, stomach upset or discomfort, and headache. Serious toxicities have not been documented to date, and preclinical studies did not show teratogenicity or fetal toxicity.

ANTIVIRAL AGENTS ACTIVE AGAINST DNA VIRUSES (Table 238-3)

Acyclovir

Spectrum of Activity

Acyclovir, 9-[(2-hydroxyethoxy)methyl]-9*H*-guanine (also known as acycloguanosine), is an analogue of the nucleoside deoxyguanosine that has selective activity against the herpes family of viruses (Fig. 238-6).[120,151] It has the most potent antiviral activity against HSV type 1 and inhibits the virus at concentrations of 0.02 to 0.9 μg/mL.[542,557] Inhibitory concentrations of HSV type 2 and VZV are also high, at 0.3 to 2.2 μg/mL and 0.8 to 4.0 μg/mL, respectively.[51,503,542,557] In addition, EBV replication is inhibited by acyclovir at a mean concentration of 1.6 μg/mL, but the drug has no effect on cells latently infected with EBV.[103,241,519] CMV also is inhibited by very high concentrations of acyclovir (2 to 57 μg/mL).[51] Acyclovir has in vitro activity against simian herpesvirus B and hepatitis B virus (HBV).[46,260,542]

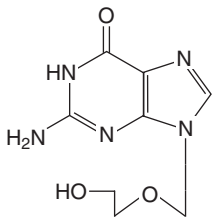

Acyclovir

FIGURE 238-6 ■ Structure of acyclovir.

Mechanism of Action and Resistance

Acyclovir is phosphorylated to acyclovir monophosphate by viral TK. Cellular enzymes then convert the monophosphate form to acyclovir triphosphate, the active form of the drug.[557] Acyclovir triphosphate is present in HSV-infected cells at 40 to 100 times the concentration found in uninfected cells, and it competitively inhibits viral DNA polymerase in infected cells. Acyclovir triphosphate also is incorporated into viral DNA and, because it lacks the 3′-hydroxyl group, acts as a chain terminator during viral DNA synthesis. The DNA polymerase enzymes of the various herpesviruses differ in their degree of inhibition by acyclovir triphosphate, and for EBV and CMV, which do not contain viral TK, this inhibition accounts for most of the antiviral effect of the drug.[131,181] Host cellular growth also can be inhibited by acyclovir, but only at levels thousands of times higher than those that inhibit viral replication. This relatively selective mechanism of antiviral action provides a uniquely high therapeutic index.

Three basic mechanisms of resistance have been identified for herpesviruses to become resistant to acyclovir: deficient TK activity caused by absent or low production of viral TK, altered TK activity caused by abnormal substrate specificity so that the enzyme is present and able to phosphorylate but just does not phosphorylate acyclovir, and altered DNA polymerase.[87] Most clinical isolates of HSV that are resistant to acyclovir have deficient TK activity, and most resistant VZV isolates have altered TK activity or altered DNA polymerase.[415,526] The prevalence of acyclovir-resistant HSV isolates in normal hosts is less than 1 percent, but it can be as high as 10 to 20 percent in immunocompromised hosts receiving acyclovir therapy

TABLE 238-3 **Clinical Indications and Usual Treatment Dosages for Antiviral Agents Active Against DNA Viruses**

Agent	Indication	Dosage
Acyclovir	HSV, mucocutaneous	5% ointment 6× daily
		15 mg/kg/dose PO 5× daily (max, 200 mg/dose)
		5-10 mg/kg/dose IV q8h
	HSV, encephalitis	10-20 mg/kg/dose IV q8h
	HSV, neonatal	20 mg/kg/dose IV q8h
	VZV, chickenpox and zoster	20 mg/kg/dose (max, 800 mg) PO q6h
		10-20 mg/kg/dose (500 mg/m^2/dose) IV q8h
Valacyclovir	HSV	500-1000 mg/dose PO bid
	VZV, zoster	1000 mg/dose PO bid
Penciclovir	HSV, mucocutaneous	1% cream q2h
Famciclovir	HSV, mucocutaneous	125-500 mg PO bid
	VZV, zoster	500 mg PO tid
Ganciclovir	CMV	5-6 mg/kg/dose IV q12h—induction
		5 mg/kg/dose qd 5 days/wk
		or
		1000 mg PO tid
		or
		500 mg PO 6× daily—maintenance
Valganciclovir	CMV	900 mg PO bid—induction
		900 mg PO qd—maintenance
		15 to 18 mg/kg/dose every 12 hours or BID
Foscarnet	CMV	60 mg/kg/dose IV q8h—induction
		90-120 mg/kg/dose daily—maintenance
Cidofovir	CMV	5 mg/kg/dose IV weekly
		or
		1 mg/kg/dose IV 3×/wk—induction
		5 mg/kg q2wk—maintenance

CMV, cytomegalovirus; HSV, herpes simplex virus; VZV, varicella-zoster virus.

for 2 weeks or longer.[96,119,157,186,244] Resistant strains of VZV, on the other hand, are unusual.[104] Resistant HSV and VZV isolates retain virulence and can cause disease, especially extensive mucocutaneous lesions in immunocompromised hosts, but invasive diseases such as keratitis, uveitis, meningoencephalitis, and pneumonia also have occurred with resistant strains of HSV.* Immunocompromised hosts may shed resistant strains of HSV for prolonged periods and may experience recurrent disease with either sensitive or resistant strains. Such mutants are also cross-resistant to other antiviral agents that require viral TK for phosphorylation and activation (e.g., penciclovir and ganciclovir), but they are inhibited by antiviral agents that have different mechanisms of action (e.g., foscarnet, cidofovir, and trifluridine).

Pharmacokinetics

Acyclovir is available for topical, oral, and intravenous administration. Systemic absorption of acyclovir after topical application to intact skin is minimal (<0.01 μg/mL), and in one study in which acyclovir ointment was applied to zoster lesions in immunocompromised patients, plasma concentrations of less than 0.1 to 0.78 μg/mL were observed, thus suggesting some degree of percutaneous absorption.[112,542] The bioavailability of oral acyclovir is low (15% to 21%), with peak plasma concentrations of 0.4 to 0.8 μg/mL observed after the intake of one 200-mg capsule and concentrations of up to 1.6 μg/mL observed after an 800-mg dose.[54] When an equivalent dose in liquid suspension is administered to children, slightly lower peak plasma levels of 1.0 μg/mL were observed.[518] Intravenous infusion of 5 mg/kg for 1 hour provides peak plasma levels of 9.8 μg/mL, whereas plasma levels of 20.7 μg/mL are produced after a 10-mg/kg infusion.[60] Most of the administered dose of acyclovir is excreted unchanged in urine.[542] The elimination half-life of acyclovir is 2.5 to 3 hours in adult patients with normal renal function, slightly longer (3.8 hours) in neonates, and up to 20 hours in anuric patients.[54,177,248] Therefore, dose adjustment, according to creatinine clearance, is needed in patients with renal insufficiency or renal failure.[331] Probenecid administration decreases renal clearance and prolongs the half-life of acyclovir. Acyclovir is distributed widely into a variety of body fluids,

*References 96, 157, 186, 244, 280, 532.

including saliva (13% of plasma levels), vaginal secretions (15% to 170% of plasma levels), zoster vesicular fluid (90% to 100% of plasma levels), aqueous humor (37% of plasma levels), breast milk (>300% of plasma levels), amniotic fluid and placenta (>200% of plasma levels), and CSF (50% of plasma levels).[177,267,268,310,332,378] More than half the drug is removed by hemodialysis but very little after peritoneal dialysis.[320,331]

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Topical acyclovir may decrease healing time and viral shedding slightly in mucocutaneous lesions of patients experiencing primary infection with HSV.[112] Little if any clinical benefit has been documented in patients with recurrent herpes simplex lesions; however, some patients report a soothing sensation after application of ointment to the lesions. Topical therapy also may produce a mild clinical benefit in immunocompromised patients with zoster lesions.[542] Oral acyclovir significantly reduces viral shedding, clinical symptoms, and time until lesion healing in normal and immunocompromised patients with a variety of mucocutaneous lesions (e.g., orolabial, pharyngeal, genital, rectal, gingivostomatitis, whitlow, skin) associated with primary HSV infection.[12,446,557] It also has been used to treat eye disease, including dendritic corneal ulcers.[108,239]

Oral acyclovir provides benefit to normal and immunocompromised children, adolescents, and adults with primary infection with VZV (varicella or chickenpox). Treatment reduces the duration of fever by 24 hours, the mean number of skin lesions, and the time to total crusting of lesions by 2 days. Postexposure prophylaxis with oral acyclovir also may reduce the risk of acquiring varicella by close and household contacts of patients with varicella. Administration of oral acyclovir during pregnancy is likely to clinically benefit a mother with varicella, but whether it reduces the risk of congenital or neonatal disease is not clear at this time. Recurrent HSV disease also is improved with oral acyclovir therapy. When it is administered during the prodrome or at the very first sign of lesions, treatment reduces viral shedding and time to lesion healing by 1.5 to 2 days.[437,543] Long-term, continuous suppressive therapy with oral acyclovir may be of benefit in some patients with frequent episodes of recurrent mucocutaneous disease, but asymptomatic shedding and person-to-person transmission still may occur.[195,447] Patients with recurrent skin disease, whitlow, or erythema multiforme may benefit from long-term suppressive therapy with oral acyclovir. Oral acyclovir suppression also reduces recurrences of genital herpes simplex during the last trimester of pregnancy and decreases the need for cesarean section delivery.[69,468,470-472] Short-term prophylaxis administered during a period of risk for recurrence (sunlight exposure, for example) may reduce the clinical recurrence of mucocutaneous disease. Oral acyclovir reduces virus shedding, time to skin lesion healing, and the duration of zoster-associated pain.[41,102] Treatment combined with steroids also appears to decrease the frequency and severity of postherpetic neuralgia in older patients and the elderly, but specific trials conducted in children have not been performed because children seldom experience postherpetic neuralgia. Oral acyclovir also may reduce recurrence of HSV and VZV infections in bone marrow and solid-organ transplant recipients. Its effect on CMV and EBV disease in these patients, however, is minimal, if any.[519] Continuous suppressive therapy with oral acyclovir reduces cutaneous recurrences after neonatal infection when it is administered after a 14- to 21-day course of intravenous therapy has been completed.[309] Whether long-term oral suppressive therapy in neonates also prevents CNS recurrence is unclear at this time. Bell palsy associated with HSV infection may be treated with a combination of acyclovir and prednisone.[5] EBV-associated diseases have been treated with acyclovir, with variable clinical benefit.[468,479] Oral hairy leukoplakia, however, appears to respond to oral acyclovir treatment.[439,534] Treatment of patients with infectious mononucleosis reduces viral shedding but does not alter the disease course.[494] No clinical benefit has been documented when acyclovir was used to treat chronic hepatitis B.[46]

Intravenous acyclovir is used to treat all severe or life-threatening diseases caused by HSV and VZV, including encephalitis, hepatitis, neonatal disease, acute retinal necrosis syndrome, mucocutaneous disease, and zoster, with or without visceral dissemination, in both normal and immunocompromised patients.* Pediatric patients suffering from severe herpetic gingivostomatitis may benefit as well.[12] Early clinical trials also showed efficacy in the treatment of primary genital HSV infection.[111] In addition, normal patients with complications of varicella, including pneumonitis, encephalitis, and hepatitis, should receive intravenous acyclovir.[208] Other diseases associated with VZV in which acyclovir treatment is beneficial include zoster ophthalmicus with complications such as keratitis, anterior uveitis, and contralateral hemiplegia as well as acute retinal necrosis syndrome.[412,492] Intravenous acyclovir is also highly effective in reducing the incidence of disease associated with HSV and VZV in marrow and solid-organ transplant recipients. Moreover, it may help reduce serious CMV disease in selected marrow and solid-organ transplant recipients, although it clearly is not as effective as ganciclovir or other antiviral agents with specific activity against CMV.[27,28,57,379,538] Treatment of human herpes infection with simian virus B requires high doses of intravenous acyclovir administered for a prolonged period, followed by suppressive therapy.[256] Despite in vitro activity, clinical benefit has not been documented in patients with hepatitis B.[46]

As of November 2012, a shortage of intravenous acyclovir related to manufacturing delays was announced. If the shortage is still in effect, or if another shortage occurs, ganciclovir, or foscarnet secondarily, is recommended for most patients who would ordinarily have received intravenous acyclovir (www.fda.gov/Drugs/DrugSafety/DrugShortages/ucm314739.htm). Cidofovir also has

*References 60, 208, 307, 312, 322, 412, 541, 572.

activity against HSV and VZV and may also be considered in selected patients.

Dosage. Topical acyclovir as a 5 percent ointment can be applied to local lesions every 3 to 4 hours five to six times daily for 7 days.[112] If the disease is unresponsive or severe, systemic therapy with oral or intravenous acyclovir should be initiated.

Oral acyclovir is formulated as 200-mg capsules, 800-mg tablets, and a 200-mg/5 mL banana-flavored suspension. The usual dose used to treat mucocutaneous lesions caused by primary infection with HSV in adults and adolescents is 200 mg administered five times daily for 10 days. The usual pediatric oral dose is 15 mg/kg per dose (maximum, 200 mg per dose) administered five times daily for 10 days. Recurrent HSV disease in adults and adolescents may be treated with a variety of different regimens: 800 mg three times daily for 2 days, 400 mg three times daily for 5 days, or 200 mg five times daily for 5 days.[294,295,543] Suppressive doses of 400 mg administered twice daily, often for many years, seem to be safe and effective.[195] This dose, administered to pregnant women with recent primary or recurrent genital herpes, also appears to decrease recurrences at or near delivery and to diminish the need for cesarean section delivery.[69,310,512] After intravenous therapy has been completed, neonatal suppressive therapy is recommended at a dose of 300 mg/m^2 per dose administered three times daily for 6 months.[309] Because neonatal prophylaxis decreases but does not eliminate the risk for CNS recurrence and because the emergence of resistant strains has been documented, all early cutaneous lesions that recur despite compliance with oral prophylaxis, as well as other signs and symptoms suggestive of recurrent visceral or CNS disease, should be evaluated carefully in young infants, and the need for systemic therapy should be considered.[309] Oral acyclovir must be administered in higher doses to treat varicella and other VZV-associated diseases.[557] Adults and adolescents require 800-mg doses administered five times daily for 7 to 10 days or until lesions have crusted over.[32,545] The pediatric dose of acyclovir to treat varicella or chickenpox is 20 mg/kg (up to 800 mg maximum) per dose (80 mg/kg/day) administered four times daily for 5 days or until lesions have crusted.[8,147]

The usual dose for intravenous administration of acyclovir to treat serious disease associated with HSV beyond the neonatal period is 5 to 10 mg/kg per dose administered as a 1-hour infusion every 8 hours.[428,542,557] Treatment for a duration of 7 days is usually sufficient for mucocutaneous disease, but it should be continued until all lesions are healed and followed by long-term suppression if the patient remains immunocompromised. Herpes encephalitis should be treated for at least 14 days or longer, depending on clinical response and clearance of HSV DNA from the CSF.[305,307,324,556] The recommended dose to treat all forms of neonatal HSV disease (skin, eye, and mouth disease; disseminated with visceral involvement; or encephalitis) is a high-dose regimen of 20 mg/kg per dose administered every 8 hours (60 mg/kg/day).[10] Duration of intravenous treatment is at least 14 days for skin, eye, and mouth disease and at least 21 days or until HSV DNA has cleared from the CSF, whichever is longer, for encephalitis and disseminated disease.[305,307] Intravenous acyclovir treatment for neonatal HSV disease should be followed by suppression treatment with oral acyclovir at 300 mg/m^2/dose every 8 hours until at least 6 months of age to reduce risks of recurrences and to improve neurodevelopmental outcome.[10] Clinical studies have documented that these higher doses are safe in neonates but may cause renal insufficiency in older children and adults. Treatment of VZV-associated disease requires higher doses of acyclovir: 500 mg/m^2 per dose (10 to 20 mg/kg per dose) administered every 8 hours (total, 1500 mg/m^2/day) for at least 5 to 7 days or until all lesions have been crusted over for 24 to 48 hours.[542,557] In all clinical indications, the dosage of intravenously administered acyclovir should be adjusted according to creatinine clearance in patients with renal insufficiency and renal failure.

Adverse Effects. Adverse effects of acyclovir are unusual.[300] Topical application of the ointment may produce pain or local irritation, usually caused by the polyethylene glycol base.[112,542] Hand washing must be performed after each local application to lesions to avoid autoinoculation or person-to-person transmission. Oral acyclovir may cause nausea and diarrhea, rash, or headache.[542] Long-term oral administration of acyclovir appears to be safe in adults but may be associated with neutropenia in 46 percent of infants who receive suppression after treatment of neonatal HSV infection.[309,381] Intravenously administered acyclovir is generally well tolerated. However, extravasation of the drug (pH 9 to 11) can cause inflammation, ulceration, and necrosis of surrounding tissue.[300,482,522] Phlebitis also can develop. Neurotoxicity occurs rarely, usually in patients with renal insufficiency who have high serum concentrations.[40,165,209,540] Neurotoxicity associated with acyclovir is characterized by lethargy, confusion, tremor, and rarely seizures and coma. Renal tubular damage, crystalline nephropathy, or interstitial nephritis may occur in approximately 5 percent of patients.[431,461] These renal complications are more likely to develop in patients who are not adequately hydrated or in whom the drug has been infused at a rate faster than the recommended infusion time of 1 hour. Keeping urine specific gravity below 1.010 is a reasonable guideline to ensure adequate hydration. No excess frequency of adverse events has been reported to date in pregnant women who receive acyclovir or in their fetuses or newborns.* Therefore, on the basis of available information, acyclovir seems to be safe when it is administered during pregnancy, especially during the last trimester, and does not appear to have any significant adverse effects.

Valacyclovir

Spectrum of Activity

Valacyclovir (L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9*H*-purin-9-yl)methoxy]ethyl ester monohydrochloride,

*References 69, 83, 310, 468, 472, 572.

also known as valacyclovir hydrochloride) is the hydrochloride salt of the L-valine ester of acyclovir.[42] It has essentially the same selective spectrum of antiviral activity against the herpesviruses as acyclovir does.[48]

Mechanism of Action and Resistance

Valacyclovir is converted rapidly to acyclovir and therefore has the same mechanisms of action and resistance as acyclovir does.[48] Viral isolates that are resistant to acyclovir will be resistant to valacyclovir.

Pharmacokinetics

The pharmacokinetics of valacyclovir have not been studied in the pediatric population, but information gained from adults can be applied, in many ways, to pediatric patients. After oral administration, valacyclovir is absorbed rapidly from the gastrointestinal tract. It is metabolized by first pass through the intestinal tract and also by the liver into acyclovir and L-valine.[48] It has the advantage over acyclovir of having 54 percent bioavailability (versus 20% for acyclovir), which is not altered by food.[498] A single dose of 1 g of valacyclovir administered to an adult produces plasma levels of 0.4 to 0.8 μg/mL. The half-life is 2.5 to 3 hours, similar to that of acyclovir, and is prolonged in patients with renal insufficiency or renal failure.[547,551] The drug is removed by hemodialysis. The rate but not the extent of conversion of valacyclovir to acyclovir is prolonged in patients with liver disease.

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Valacyclovir has been reported to be effective in treatment of primary and recurrent mucocutaneous disease caused by HSV-1, HSV-2, and VZV.* Randomized clinical trials conducted in adult patients showed valacyclovir to be superior to placebo and comparable to acyclovir in reducing the number and duration of lesions, pain, and time to healing in patients with primary and recurrent genital herpes and zoster.[56,438,565] It is also comparable to acyclovir for suppression of genital herpes recurrences. Furthermore, in a study of 27 CMV-seropositive heart transplant recipients, valacyclovir appeared to be equal or superior to oral acyclovir in preventing both laboratory and clinical parameters of CMV reactivation.[149] Because valacyclovir is converted to acyclovir, it is likely to be effective for all the clinical indications for which acyclovir has been shown to be effective.

Dosage. Valacyclovir is available only in 500-mg caplets; therefore, a pediatric liquid formulation is not available at this time. The usual dose is 1 g twice daily for 10 days for primary genital herpes and 500 mg twice daily for 3 to 5 days for recurrent disease.[48,56,337,465] A daily dose of 500 mg has been used safely for longer than a year to suppress recurrences of HSV disease.[357] The dose to treat VZV-associated disease is higher than that for HSV-associated disease. A dose of 1 g three times a day for at least 7 days is usually necessary to treat zoster, and therapy should be continued until the lesions have dried.[49] The dose of valacyclovir should be adjusted according to creatinine clearance in patients with renal insufficiency or renal failure.

Adverse Effects. The adverse effects of oral valacyclovir are similar to those of oral acyclovir and include gastrointestinal disturbances such as nausea, vomiting, and diarrhea.[48] Headache and behavioral changes also have been observed. Overdosage may produce precipitation of acyclovir in the renal tubules as well as CNS toxicity.

Penciclovir

Spectrum of Activity

Penciclovir (9-[4-hydroxy-3-hydroxymethylbut-1-yl] guanine) is an acyclic guanosine analogue with a selective spectrum of activity against the herpesviruses similar to that of acyclovir[63] (Fig. 238-7). This compound inhibits HSV-1 at very low concentrations (0.2 to 0.6 μg/mL) and HSV-2 at slightly higher concentrations (0.3 to 2.4 μg/mL).[25,63,196,550] At even higher concentrations (0.9 to 4.0 μg/mL), it inhibits VZV.[25,26] The compound has very little activity against CMV, and concentrations greater than 50 μg/mL are required to inhibit CMV. However, penciclovir does have activity against HBV.[107,318,475]

Mechanism of Action and Resistance

Like acyclovir, penciclovir is preferentially phosphorylated by viral TK in virus-infected cells to penciclovir monophosphate and by cellular enzymes to penciclovir triphosphate, which is the active form of the drug.[148] Penciclovir triphosphate competitively inhibits viral DNA polymerase. It is approximately 100 times less potent than acyclovir triphosphate in inhibiting viral DNA polymerase, but an antiviral effect is achieved because it is present in high concentrations for a prolonged period (7 to 20 hours) in virus-infected cells. However, unlike acyclovir, penciclovir is not a chain terminator during DNA synthesis. Penciclovir triphosphate also inhibits the DNA polymerase of HBV. Emergence of HSV strains resistant to penciclovir has been minimal.[335] Similar to HSV isolates resistant to acyclovir, isolates resistant to penciclovir may have viral TK gene mutations that produce mutants with deficient or altered TK activity, or they may have mutations in DNA polymerase

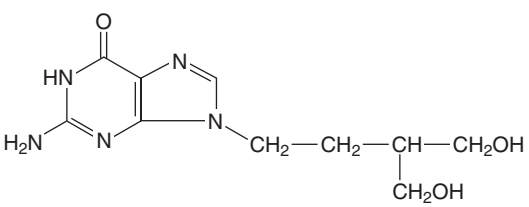

Penciclovir

FIGURE 238-7 ■ Structure of penciclovir.

*References 48, 49, 56, 337, 438, 465.

genes.[91] Strains of acyclovir-resistant HSV that are TK-deficient mutants also will be resistant to penciclovir, but strains of acyclovir-resistant HSV that are TK altered may be susceptible to penciclovir.[64,418]

Pharmacokinetics

Penciclovir is poorly absorbed after oral administration.[173] Adults who received a 10-mg/kg intravenous infusion of penciclovir had plasma levels of 12 μg/mL, but no pediatric information is available. Penciclovir 1 percent cream is not absorbed systemically. The systemic pharmacokinetics of penciclovir are discussed under famciclovir.

Clinical Indications, Dosage, and Adverse Effects

Penciclovir is available in the United States as a 1 percent cream in a propylene glycol base and is licensed for the treatment of herpes labialis lesions on the lips and face. Maximal clinical benefit occurs when the cream is applied to the lesions early, at the first clinical sign of disease, every 2 hours while awake for 3 to 5 days.[464] Application of penciclovir cream may be associated with local skin reactions, most likely caused by the propylene glycol base. An intravenous form of penciclovir has been tested in Europe, but it is not available in the United States. Penciclovir is not absorbed orally, but it is available as the oral prodrug famciclovir, which is converted rapidly to penciclovir. Dosages and systemic adverse effects of penciclovir are discussed under famciclovir.

Famciclovir

Spectrum of Activity

Famciclovir, 2-[(2-amino-9*H*-purin-9-yl)]-1,3-propanedial diacetate, is the prodrug of the antiviral agent penciclovir, which has selective activity against HSV-1, HSV-2, VZV, and HBV.

Mechanism of Action and Resistance

Famciclovir undergoes rapid transformation to penciclovir, which is monophosphorylated by viral TK to penciclovir monophosphate; the monophosphate, in turn, is converted by cellular enzymes to penciclovir triphosphate, the active form of the antiviral compound. Penciclovir then competitively inhibits viral DNA polymerase and, thereby, viral replication. The prolonged intracellular half-life observed with penciclovir also is observed with famciclovir. Penciclovir-resistant strains of HSV and VZV occur when mutations produce deficient or altered TK or altered DNA polymerase. Most TK-deficient acyclovir-resistant strains of HSV are also resistant to penciclovir.

Pharmacokinetics

Famciclovir is the diacetyl-6-deoxy analogue of the antiviral compound penciclovir. After oral administration, famciclovir is absorbed rapidly from the gastrointestinal tract with excellent (77%) bioavailability, and then it is metabolized in the liver by deacetylation and oxidation to form penciclovir. Single oral doses of 250 and 500 mg of famciclovir administered to adults produce peak plasma penciclovir levels of 1.6 to 1.9 μg/mL and 2.7 to 4.0 μg/mL, respectively. Administration of famciclovir with food reduces peak plasma concentrations by slowing the absorption time but does not alter the overall bioavailability of the drug. The half-life for penciclovir is 2 to 3 hours, after which it is excreted by filtration and active tubular secretion by the kidneys. Nonrenal clearance by fecal excretion also occurs for as much as one third of the oral dose. Excretion of penciclovir is decreased in patients with renal insufficiency and renal failure, and in patients with liver disease, peak plasma levels may be reduced.[58] Penciclovir is removed from the body by hemodialysis. After oral administration of famciclovir to rats, penciclovir is concentrated in breast milk; however, no studies have been performed in lactating mothers to confirm such concentration in humans.

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. In adult clinical trials, oral famciclovir was as effective as oral acyclovir and superior to placebo in treatment of recurrent mucocutaneous infections with HSV and VZV in both normal and immunocompromised patients.[99,358,452,535] When famciclovir was given early in the course of illness, time to lesion crusting and healing, duration of viral shedding, and length of acute pain all were shortened. In some studies, the duration of postherpetic neuralgia in elderly patients with zoster also was shortened.[535] Although to date it has not been studied specifically in clinical trials of patients with primary HSV and VZV infection, famciclovir probably also will provide clinical benefit in these conditions.[348] In addition, famciclovir is effective as suppressive therapy to reduce HSV recurrences and viral shedding.[133,377] Famciclovir also has been used to treat chronic HBV infection and for prophylaxis against recurrent HBV infection in liver transplant recipients.[214,358] No clinical trials in pediatric patients have been conducted.

Dosage. Famciclovir is available in 125-, 250-, and 500-mg tablets. An oral suspension for pediatric use is not available. The usual recommended oral dose of famciclovir to treat primary and recurrent mucocutaneous HSV disease is 125 mg twice daily for 5 days.[452] Dosages of suppressive therapy are slightly higher, 250 mg twice daily for as long as 1 year. Of note, clinical trials showed that single daily doses of 250 mg were not as effective as the twice-daily regimen in suppressing recurrences. For immunocompromised patients, especially those infected with HIV, even higher doses (500 mg twice daily for 7 days) may be necessary.[462] Treatment of VZV-associated disease, such as zoster, also requires higher doses (500 mg twice or three times daily for 10 days).[535] All dosage regimens should be reduced, according to creatinine clearance, in patients with renal insufficiency or renal failure.

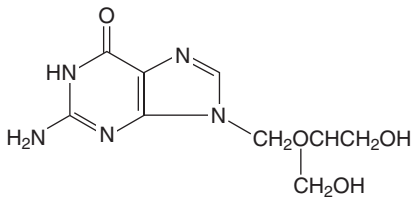

Ganciclovir

FIGURE 238-8 ■ Structure of ganciclovir.

Adverse Effects. Adverse effects of treatment with oral famciclovir are unusual and include gastrointestinal disturbances, such as nausea and diarrhea; rash; CNS complaints, such as confusion, hallucinations, and disorientation; neutropenia; and elevated liver transaminases.[99,122,457]

Ganciclovir

Spectrum of Activity

Ganciclovir, 9-(1,3-dihydroxy-2-propoxymethyl)guanine (also known as DHPG), is an analogue of deoxyguanosine, similar to acyclovir, yet different in that it has an additional hydroxymethyl group on the acyclic side chain[164,167,425,484] (Fig. 238-8). This antiviral compound has selective activity against the herpesviruses, with uniquely potent antiviral activity against CMV. Ganciclovir inhibits HSV-1 and HSV-2 at 0.05 to 0.6 μg/mL, VZV at 0.4 to 10 μg/mL, CMV at 0.02 to 3.4 μg/mL, and EBV at 1.5 μg/mL. Human herpesvirus types 6 (HHV-6), 7 (HHV-7), and 8 (HHV-8) also may be inhibited by ganciclovir.[299] It has in vitro activity against adenoviruses and HBV as well.[164,192,390,488] High concentrations (30 to >700 μg/mL) of ganciclovir will inhibit the growth of most uninfected mammalian cells; bone marrow–derived cells, however, appear to be uniquely sensitive and can be inhibited at much lower concentrations (<0.7 μg/mL).

Mechanism of Action and Resistance

Ganciclovir is monophosphorylated in infected cells by virus-induced enzymes: TK in HSV-infected cells and protein kinases that are encoded by the UL97 phosphotransferase gene in CMV-infected cells.[51,164,343,484] Cellular enzymes complete the phosphorylation to ganciclovir triphosphate, the active form of the compound, which is concentrated in infected cells. Ganciclovir triphosphate competitively inhibits the incorporation of deoxyguanosine triphosphate into viral DNA, where it slows and stops viral DNA chain elongation and produces short, noninfectious viral DNA fragments.[216] Ganciclovir also preferentially inhibits viral DNA polymerase.[351] Ganciclovir resistance has been detected in 8 to 38 percent of immunocompromised adult and pediatric patients who have received prolonged administration of ganciclovir, and such resistance is an important clinical problem.[93,142,191,466,495,531]

Patients may be infected with single or multiple strains of CMV, with both drug-sensitive and drug-resistant strains mixed in the population or in different body compartments.[159] Resistant strains may be induced or infect a patient primarily, emerge quickly after only weeks of therapy, or evolve more slowly and emerge sequentially after several months of antiviral prophylaxis or therapy.[94,159,277,448,557,566] These resistant mutants also retain virulence and are capable of producing serious and progressive disease.[59,159,277,455,491] Ganciclovir resistance in CMV strains occurs by at least two mechanisms: (1) point mutations or deletions in the UL97 gene that reduce intracellular phosphorylation of ganciclovir and (2) point mutations in the viral DNA polymerase UL54 (pol) gene that alter the function of the polymerase.[93,351,489,506] Most strains of CMV that are resistant to ganciclovir have UL97 mutations (most commonly at codons 460, 520, 594, and 595) and reduced phosphorylation, but they remain susceptible to foscarnet and cidofovir.[92,93,506] However, CMV strains that are resistant to ganciclovir because of a mutation in the UL54 DNA polymerase gene also may be resistant to foscarnet or cidofovir or to both.[351,458] Moreover, multiple highly resistant strains of CMV may occur if both UL97 phosphotransferase and UL54 DNA polymerase gene mutations are present.[94,489] Resistance conferred by UL54 mutations usually emerges after UL97 mutations have occurred.[191,489] Ganciclovir resistance in HSV occurs in TK-deficient, acyclovir-resistant strains of the virus.

Pharmacokinetics

Intravenous administration of a 5-mg/kg dose of ganciclovir in adults produces peak plasma levels of 8 to 11 μg/mL.[164,170] Similar pharmacokinetics in 10 pediatric patients aged 9 months to 12 years has been observed.[182] A study of the pharmacokinetics of ganciclovir in 27 neonates aged 2 to 49 days who were administered intravenous ganciclovir for treatment of congenital CMV disease showed a dose of 6 mg/kg to be the most appropriate dose for that age group, down to 32 weeks of gestation.[532,578] After intravenous administration, ganciclovir is distributed in CSF at 24 to 70 percent of plasma levels, and 38 percent of plasma levels enter brain tissue.[97,99] Drug levels in aqueous, vitreous, and subretinal fluid in the eye are comparable to serum levels, and even higher levels can be achieved with intravitreal implants.[19,170,321,360,391] Ganciclovir also accumulates in breast milk in animal models.[6] The plasma half-life is 2 to 4 hours in adults with normal renal function and longer than 24 hours in patients with renal insufficiency and renal failure.[494] The drug is eliminated by renal excretion, and dose reduction is required for patients with impaired creatinine clearance. Hydration will enhance elimination of the drug, and hemodialysis removes 60 percent of ganciclovir in plasma.[494] Ganciclovir also can be administered orally.[500] Its oral bioavailability is rather poor: 5 percent under fasting conditions and 6 to 9 percent if it is administered with food.[15,333] Oral doses of 1000 mg administered to adults every 8 hours produce plasma levels of 0.9 to 1.2 μg/mL.[15,460] The valine ester prodrug of ganciclovir, valganciclovir, has high oral bioavailability

and efficacy equal to that of intravenous ganciclovir for prevention and treatment of mild to moderate CMV disease in transplant recipients and treatment of congenitally infected newborns.[150,290,359]

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Ganciclovir is used for the treatment of established CMV disease; for early or preemptive therapy in immunocompromised patients with virologic markers of active infection that are predictive of CMV disease; and for prophylaxis of high-risk patients, such as those who are CMV seropositive or who have received transplants from CMV-seropositive donors.[76] Ganciclovir is licensed for treatment and chronic suppression of sight-threatening CMV retinitis in immunocompromised patients and for prevention of CMV disease in transplant recipients. Clinical trials have demonstrated efficacy in these conditions.* Treatment with ganciclovir also is beneficial in immunocompromised individuals with other forms of invasive CMV disease, including pneumonia, colitis, esophagitis, myocarditis, encephalitis, persistent fever and leukopenia syndrome, and viral sepsis syndrome.† Treatment of established CMV pneumonia in bone marrow and stem cell transplant recipients is difficult, and the disease may not respond to ganciclovir treatment, with or without immune modulators or globulins.[153] Most clinical trials have been performed in adults, but children and infants with CMV disease benefit from treatment as well. Ganciclovir treatment also has been evaluated in newborns congenitally infected with CMV with CNS involvement.[307,492,578] In addition, ganciclovir is used for preemptive therapy for patients (primarily recipients of solid-organ, marrow, and stem cell transplants) with virologic markers that are predictive of serious CMV disease.[68,198,241,502] Prophylaxis with ganciclovir to prevent CMV infection and disease in immunocompromised patients, including solid-organ and bone marrow transplant recipients and patients with acquired immunodeficiency syndrome (AIDS), also has been beneficial in most groups studied.‡ Prophylaxis reduces the risk for the acquisition of serious CMV disease or, in some groups, prolongs the incubation period, but it does not eliminate the risk.[146,197,506,563] Ganciclovir prophylaxis is also effective in preventing HSV infection in immunocompromised patients.[197] Ganciclovir appears to decrease HBV DNA levels and to improve hepatic enzymes in patients with posttransplantation infection or reactivation with HBV.[192] In addition, ganciclovir has been used to treat patients with serious disease associated with adenovirus infection, but clinical trials proving efficacy have not been performed.[528]

Dosage. Ganciclovir is supplied as a solution for intravenous infusion and as 250- and 500-mg capsules for oral administration. The intravenous infusions are delivered best in a concentration of 10 mg/mL or less and administered during a 1-hour period. Intravitreal implants are also available and are designed to slowly release relatively large doses of ganciclovir locally into the eye during a period of many months. Ganciclovir therapy for serious CMV disease usually is administered in two phases, induction and maintenance. The recommended dose for induction therapy for serious CMV disease in adults and children with normal renal function is 5 mg/kg per dose administered intravenously every 12 hours for 2 to 3 weeks. Successful induction therapy is accompanied by clinical and virologic response. Maintenance therapy at doses of 5 mg/kg/dose administered intravenously every 12 to 24 hours should be continued in severely immunocompromised patients who are at risk for relapse. Selected patients may receive oral ganciclovir for maintenance therapy. The usual recommended dose for adults is 1000 mg three times daily or, alternatively, 500 mg administered six times daily. The pediatric dose for maintenance oral therapy has not been established. The duration of maintenance therapy should be individualized for each patient, but such therapy usually lasts through the period of greatest risk, such as rejection or immune suppression, or may be lifelong, as in patients with AIDS. Some patients who experience CMV disease, such as solid-organ transplant recipients with minimal immune suppression and minimal or no rejection, will respond dramatically to induction therapy and will not require long-term maintenance therapy. A dose of 6 mg/kg administered every 12 hours for 6 weeks has been used to treat newborns with congenital CMV disease.[307,308,329,578] This dose appears to be adequate for term newborns and premature newborns as young as 32 weeks' gestation, provided renal function seems to be normal for age. The dose for extremely premature newborns is not known and should be individualized according to the clinical judgment of the infectious diseases specialist. Preemptive therapy, 5 mg/kg per dose administered every 12 hours, is initiated when virologic markers for active, invasive infection, such as positive cultures for CMV from bronchoalveolar lavage samples or the presence of CMV DNAemia or antigenemia, are identified by routine virologic surveillance.[301,506] After an induction period of 7 to 14 days, maintenance therapy usually is continued for 100 to 120 days after transplantation or longer if the patient remains at high risk for relapse of CMV disease. Prophylaxis with ganciclovir is administered immediately before transplantation and for a defined period after transplantation in patients who are at high risk, such as those who are CMV seropositive before transplantation and those who receive marrow or solid-organ transplants from a CMV-seropositive donor.*

Patients who do not respond to induction therapy or who relapse or progress during maintenance therapy with ganciclovir may have a resistant strain of CMV. The addition of another antiviral with different mechanisms of action, such as foscarnet or cidofovir, may be beneficial. If clinical or virologic responses still are not maintained, the possibility of a multiply resistant CMV strain should be considered. Patients with disseminated adenovirus

*References 68, 118, 146, 197, 198, 241, 254, 376, 466, 563.
†References 76, 97, 106, 135, 140, 153, 434, 435, 460, 462, 463.
‡References 68, 140, 146, 197, 502, 563, 564.

*References 67, 146, 184, 197, 462, 466, 563, 564.

disease have received doses of 5 mg/kg every 12 hours for 14 or more days, but clinical benefit has not been proved.[390]

Adverse Effects. Adverse effects of ganciclovir can be both local and systemic. Local reactions, such as phlebitis, irritation, blistering, and ulceration at or around the infusion site, can occur and usually are attributed to the alkaline pH (pH 11) of the intravenous solution. Local reactions can be minimized by paying careful attention to the infusion site or by administering the antiviral agent through a central venous catheter. The most common systemic toxicity associated with ganciclovir administration is dose-dependent, reversible neutropenia, which occurs in a third to a half of patients (adults, children, and newborns) who receive this antiviral for longer than 2 weeks.[118,164] Thrombocytopenia also can occur, most often in patients with AIDS who are receiving other antiviral agents, including antiretrovirals.[252] If the neutropenia is severe (absolute neutrophil count <500/mm^3), ganciclovir administration should be halted temporarily until the neutrophil count recovers. Ganciclovir then may be readministered, if it is still clinically indicated, at the same or half the original dose while the patient is carefully monitored for recurrence of the neutropenia. Some experts have used recombinant granulocyte-macrophage colony-stimulating factor successfully to treat ganciclovir-induced neutropenia.[218] Anemia associated with ganciclovir administration is an unusual occurrence. Other adverse effects associated with ganciclovir include CNS disturbances, such as headache, behavioral changes, psychosis, seizures, and coma; mild nephrotoxicity with azotemia; liver dysfunction with elevated transaminases; and rash. Ganciclovir also is mutagenic, carcinogenic, and immunosuppressive. In addition, preclinical animal studies showed reproductive toxicity, with teratogenicity, embryotoxicity, and testicular atrophy.[164] Long-term studies in children who received ganciclovir as newborns are being conducted to determine the long-term effects of ganciclovir administration, if any. Also, caretakers who prepare and administer ganciclovir to patients should take precautions to minimize direct exposure to the antiviral agent.

Valganciclovir

Spectrum of Activity

Valganciclovir (L-valine, 2-[(2-amino-1,6-dihydro-6-oxy-9*H*-purin-9-yl)methoxy]-3-hydroxypropyl ester) is a prodrug of ganciclovir and therefore has the same selective spectrum of activity as ganciclovir against herpesviruses, especially CMV, as well as limited activity against adenoviruses and HBV.

Mechanism of Action and Resistance

Valganciclovir is the L-valyl ester (prodrug) of ganciclovir.[359] It is metabolized rapidly in the body to ganciclovir. Ganciclovir then is monophosphorylated by viral protein kinase (coded for by UL97 genes) in CMV-infected cells and further phosphorylated to the active form ganciclovir triphosphate by cellular kinases. Viral DNA synthesis is inhibited by ganciclovir triphosphate (see ganciclovir for details). Isolates of CMV become resistant to valganciclovir by mutations in UL97, the viral kinase gene, or in UL54, the viral DNA polymerase gene. Mutations in UL97 confer resistance to ganciclovir and therefore to valganciclovir, whereas mutations in UL54 confer double or triple resistance to ganciclovir, foscarnet, and cidofovir. Mutations in both genes can produce highly resistant strains of CMV.

Pharmacokinetics

Valganciclovir is well absorbed after oral administration and is rapidly hydrolyzed in the intestine and liver to ganciclovir.[419] The bioavailability of valganciclovir is high, approximately 60 percent (vs. 6% to 9% for ganciclovir), and an oral dose of 900 mg administered to adults produces ganciclovir blood levels equivalent to a 5-mg/kg dose administered intravenously.[70,289,359] Absorption is enhanced significantly with the ingestion of food, so physicians recommend that valganciclovir be taken with food or meals. The drug is excreted by the kidneys, and renal insufficiency produces prolonged excretion and a longer half-life (see ganciclovir). Oral valganciclovir solution has been administered to neonates and infants up to 6 months of age, at a dosage of 16 mg/kg/dose (range of 15 to 18 mg/kg/dose) every 12 hours. Valganciclovir is 41% bioavailable and provides stable and constant plasma concentrations and antiviral effects comparable to intravenously administered ganciclovir.[310,472] Pharmacokinetic studies have shown stable and effective antiviral plasma concentrations (mean trough, 0.51 μg/mL; and mean 2-hour peak, 3.81 μg/mL).[345]

Clinical Indications, Dosage, and Adverse Effects

Valganciclovir is available in 450-mg tablets, and the usual adult dose is 900 mg (two 450-mg tablets) twice daily for 14 days (induction therapy), followed by a maintenance dose of 900 mg administered once daily.[359] An oral solution (50 mg/mL concentration) is also licensed and available.[306] Valganciclovir is licensed for induction and maintenance treatment of CMV retinitis in immunocompromised patients, but clinical trials have shown that valganciclovir is beneficial to a variety of patients with a number of different CMV infections.[359] Antiviral prophylaxis or preemptive treatment for CMV DNAemia with valganciclovir for 100 to 200 days after transplantation in high-risk transplant recipients significantly reduces the incidence of CMV disease and improves graft survival.[565] Treatment of newborns with symptomatic congenital CMV infection with oral valganciclovir (15-18 mg/kg/dose every 12 hours) appears beneficial, similar to intravenous ganciclovir benefits, providing improved head circumference, improved developmental milestones, and reduced risk of hearing loss and progression. A randomized clinical trial comparing 6 weeks versus 6 months of treatment in congenitally infected newborns has recently shown the longer 6-month duration of treatment to be more beneficial.[11] In some centers,

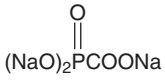

Foscarnet

FIGURE 238-9 ■ Structure of foscarnet.

treatment up to 12 months of age has shown benefit for prevention of hearing loss progression.[13] There are case reports of the use of valganciclovir or ganciclovir to treat newborns with asymptomatic congenital CMV with hearing loss, but conclusions about the efficacy of such treatment cannot be made from these reports at this time.[323,570]

As with ganciclovir, if a patient treated with valganciclovir experiences progression of disease or recurrence during maintenance therapy, a resistant strain of CMV should be considered a possibility and antiviral treatment adjusted accordingly. Adverse effects associated with the oral administration of valganciclovir include diarrhea and dose-dependent, reversible neutropenia.[359]

Foscarnet

Spectrum of Activity

Foscarnet (phosphonoformic acid or trisodium phosphonoformate hexahydrate) is a pyrophosphate analogue that selectively inhibits herpesviruses[405] (Fig. 238-9). It also has activity against HIV and HBV.[29,161] At concentrations of 100 to 300 µmol/L, CMV is inhibited; whereas slightly lower concentrations (80 to 200 µmol/L) inhibit HSV types 1 and 2, VZV, EBV, and HHV-8.[405] Concentrations between 20 and 200 µmol/L appear to inhibit HBV. Foscarnet also inhibits most but not all acyclovir-resistant HSV and VZV strains and most but not all ganciclovir-resistant CMV strains.[176,354,453] Combinations of ganciclovir and foscarnet are synergistic against CMV, and combinations of zidovudine and foscarnet appear to be synergistic against HIV.[161,354] At high concentrations (500 to 1000 µmol/L), foscarnet inhibits cellular DNA synthesis in uninfected cells.[95]

Mechanism of Action and Resistance

Foscarnet is not a nucleoside analogue, and it does not require phosphorylation or any other form of intracellular metabolism to be activated. Rather, it is a pyrophosphate analogue that directly inhibits viral and cellular DNA polymerase.[117,132] Selective viral inhibition is accomplished by noncompetitive and reversible blocking of the pyrophosphate binding site of the viral polymerase, in much lower concentrations than it inhibits cellular DNA polymerases. Because foscarnet does not require phosphorylation by viral TK or other kinases, it inhibits TK-deficient and altered strains of HSV and VZV that are resistant to acyclovir as well as UL97 phosphotransferase mutants of CMV that are resistant to ganciclovir. However, strains of herpesviruses of all types that are resistant to acyclovir or ganciclovir by mutation of the viral DNA polymerase gene are also resistant to foscarnet.[30,455]

Pharmacokinetics

Intravenous administration of 60 mg of foscarnet every 8 hours to adults produces plasma levels of 450 to 575 µmol/L; 90 mg administered every 12 hours produces plasma levels of 420 to 746 µmol/L.[132,453] CSF levels are usually approximately 60 percent of plasma levels, and vitreous concentrations in the eye are the same as or slightly higher than plasma levels.[237] Most (80%) of the dose of foscarnet is eliminated unmetabolized from the body through the kidneys, and plasma clearance decreases if renal function is impaired. The remaining 20 percent appears to be deposited in teeth and bone, where it accumulates and remains for months. The drug is removed by hemodialysis, but not appreciably by peritoneal dialysis.[7,352] Oral foscarnet has poor bioavailability (less than 10%) and causes diarrhea, and it is unlikely to be available for patient use.[404] Pharmacokinetic data in infants and children have not been published. However, preclinical studies showed that deposition of foscarnet in teeth and bones is greater in younger than in older animals.

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Foscarnet is licensed for both induction and maintenance treatment of CMV retinitis in immunocompromised patients and for treatment of mucocutaneous disease caused by acyclovir-resistant HSV.* Foscarnet also may be of benefit in patients with varicella or zoster caused by acyclovir-resistant VZV.[453] Because the combination of foscarnet and ganciclovir appears to be synergistic in vitro, immunocompromised patients who experience progression or relapse of CMV disease while receiving therapy with one or the other antiviral agent may benefit from combination therapy in some instances.[24,143,176,281] Foscarnet also may be used for the treatment or prophylaxis of serious or life-threatening CMV disease when ganciclovir is contraindicated or otherwise deemed clinically undesirable because of its myelosuppressive effects.[24,440,571] The antiretroviral properties of foscarnet, when it is combined with other antiviral agents, also may be beneficial in patients with HIV infection or AIDS.[45,283,411,513,514]

Dosage. Treatment with foscarnet usually is divided into two phases, induction and maintenance. The usual dosage of foscarnet for induction therapy is 60 mg/kg per dose administered intravenously every 8 hours for 3 weeks; maintenance therapy is 90 to 120 mg/kg/day administered indefinitely or through the period of risk.[293] The dose of foscarnet should be given slowly, during the course of 2 hours (or no faster than 1 mg/kg/min), to reduce renal toxicity. Creatinine clearance should be used to adjust dosage regimens in patients with renal insufficiency or renal failure. Published experience on the use of foscarnet in pediatric patients is limited, but undoubtedly, certain pediatric patients benefit from receiving

*References 132, 143, 281, 334, 352, 411, 455, 473, 474, 546.

foscarnet therapy.[546] In these cases, the same per-kilogram dosage regimens can be used for most patients. Prehydration with saline and the use of probenecid with each dose are recommended in some patients to reduce renal toxicity.

Adverse Effects. Foscarnet is associated with serious adverse effects and should be used only after thorough consideration of the risks and benefits involved.[95,132] Renal toxicity with azotemia, proteinuria, crystalluria, renal tubular acidosis or necrosis, and interstitial nephritis can occur in as many as a third of patients who receive foscarnet.[130,287] Renal toxicity generally occurs after the first week of therapy and usually is reversible. The risk for the development of renal toxicity is increased if the drug is given by rapid infusion or administered in high doses, if the patient is dehydrated, or if other nephrotoxic drugs are administered concomitantly with foscarnet.[396] Hydration, including saline loading, and administration of each dose during the course of at least 2 hours appear to reduce the risk for development of renal toxicity. Foscarnet binds divalent metal ions such as calcium in the body, and metabolic abnormalities, including hypocalcemia and hypercalcemia (total or ionized), hypophosphatemia and hyperphosphatemia, hypomagnesemia, and hypokalemia, may occur in approximately a third of patients who receive foscarnet.[282] Symptoms of these acute metabolic abnormalities include perioral tingling, numbness or paresthesias of the limbs, and, if severe, seizures, tetany, and cardiac dysrhythmias. Administration of the dose during the course of at least 2 hours also reduces the risk for development of metabolic abnormalities. Foscarnet also can be deposited and concentrate in bone, with as yet unclear long-term consequences.[132] CNS side effects also occur in approximately a fourth of patients and include headache, tremor, seizures, and behavioral changes. Abnormal liver function test results have been noted, and high urinary concentrations of foscarnet may produce painful genital ulcerations and rash in some patients.[242,492] Preclinical studies showed foscarnet to be mutagenic and associated with anomalies of skeletal development in young animals.[129]

Cidofovir

Spectrum of Activity

Cidofovir, (*S*)-1-3-hydroxy-2-[phosphonomethoxypropyl]) cytosine dihydrate (also known as HPMPC), is an acyclic phosphonate nucleotide analogue of deoxycytidine monophosphate with broad-spectrum in vitro antiviral activity against all DNA viruses, including herpesviruses (HSV types 1 and 2; CMV, EBV, and VZV; and HHV types 6, 7, and 8), adenoviruses, polyomaviruses (JC and BK viruses), papillomaviruses, and poxviruses (vaccinia, variola or smallpox, cowpox, monkeypox, camelpox, and molluscum contagiosum and orf viruses) (Fig. 238-10).* Cidofovir exhibits its most specific and potent antiviral activity against CMV (minimal inhibitory concentration, 0.25 μmol/L).[88,89,141,154] The compound also is very active against VZV (0.79 μmol/L), HSV-1 (12.7 μmol/L), and acyclovir-resistant, TK-deficient HSV-1 strains (6.24 μmol/L).[250,374,493] Cidofovir inhibits HSV-2 at concentrations of 31.7 μmol/L, adenoviruses at 10.8 μmol/L, and vaccinia virus at 12.7 μmol/L.[128,199,486] Strains of CMV UL97 mutants that are resistant to ganciclovir are inhibited by cidofovir, and cidofovir in combination with ganciclovir or foscarnet shows synergistic inhibition of CMV in vitro.[454]

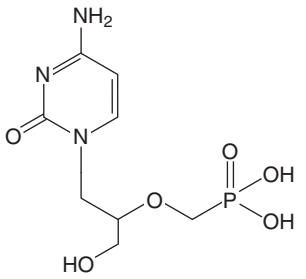

Cidofovir

FIGURE 238-10 ■ Structure of cidofovir.

Mechanisms of Action and Resistance

Cidofovir inhibits the replication of CMV and other viruses by selective inhibition of viral DNA polymerase.[89,250,374] The compound is phosphorylated by cellular enzymes to the active form cidofovir diphosphate. Cidofovir diphosphate inhibits both viral and cellular DNA polymerase; however, because the concentration necessary to inhibit cellular DNA synthesis is hundreds of times higher than that needed to inhibit viral DNA synthesis, cidofovir appears to selectively inhibit viral DNA synthesis at concentrations safely administered to humans. Cidofovir diphosphate has a long intracellular half-life that provides prolonged and persistent antiviral activity and allows infrequent dosing regimens in humans.[171,251,493] Because the compound does not require TK for initial phosphorylation, it is active against TK-deficient and TK-altered acyclovir-resistant HSV strains.[374] Resistance to cidofovir is unusual but can occur by mutations in viral DNA polymerase genes (codons 375 to 540 and possibly 978 to 988), most likely in patients who have received prolonged or repeated periods of treatment with ganciclovir or foscarnet. Strains of CMV that are resistant to cidofovir are also usually resistant to ganciclovir, and occasionally, triple mutants resistant to ganciclovir, foscarnet, and cidofovir occur.[141,527]

Pharmacokinetics

After an intravenous infusion of 5 mg/kg of cidofovir, peak plasma levels range from 11.6 to 26.1 μg/mL, with the latter occurring after administration of probenicid.[121,454,527] The plasma half-life is 2 to 3 hours, but the intracellular half-life is prolonged (between 17 and 65

*References 16, 88, 89, 128, 141, 154, 188, 199, 250, 374, 396, 397, 454, 482, 483, 486, 493, 536.

hours).[493] CSF penetration by cidofovir is not well studied, but in at least one patient, the drug did not appear to cross the blood-brain barrier in detectable amounts. After topical administration to the eye or intact skin, systemic absorption is low, with peak plasma levels usually less than 0.5 μg/mL.[326,327] However, patients with abraded or denuded skin may have significant absorption.[49] The systemic absorption that occurs after intralesional or subcutaneous administration is not well characterized at this time. Intravitreal administration produces sustained antiviral effects in animal models.[171] Cidofovir is not well absorbed orally, with less than 5 percent bioavailability. However, bioavailable alkoxyalkyl esters of cidofovir are in development and may lead to an oral compound in the near future.[301] Aerosolized cidofovir also is being studied in animal models and appears to deliver high concentrations of antiviral to the lungs.[67] Cidofovir is eliminated through the kidney by glomerular filtration and active tubular secretion, and more than 90 percent of the original dose can be recovered unchanged in urine.

Clinical Indications, Dosage, and Adverse Effects

Clinical Indications. Cidofovir is licensed for induction and maintenance treatment of CMV retinitis in immunocompromised adults with AIDS.[454,515] Clinical trials have shown that cidofovir significantly delays progression of CMV retinitis in previously untreated patients as well as in those who previously failed to respond to or were intolerant of foscarnet or ganciclovir therapy.* Cidofovir also has been shown to be effective for the treatment of CMV infection and disease in marrow and stem cell transplant recipients. In addition, it has been used, in selected patients, for preemptive treatment of CMV infection after marrow and stem cell transplantation.[85,344,424] One study also showed that cidofovir helped prevent posttransplantation CMV-associated atherosclerosis in rats.[73] Patients infected with CMV strains resistant to ganciclovir or foscarnet, or both, may benefit from receiving cidofovir treatment.[141] Cidofovir is used by clinicians to treat immunocompromised children with serious CMV disease despite the lack of published experience in children.[75,82]

Cidofovir has broad-spectrum antiviral activity, and published case reports show that it has been used to treat patients with serious infections caused by a wide variety of DNA viruses. For example, patients with acyclovir- and foscarnet-resistant HSV infection have been treated successfully with topical and systemic cidofovir.[74,317,325,327] It also may be effective in treatment of selected patients with acyclovir-resistant VZV infections.[486] Moreover, one report showed that treatment with cidofovir and anti-CD20 monoclonal antibody was associated with remission and regression of posttransplantation EBV-associated lymphoproliferative disease.[217] The use of cidofovir to treat patients with AIDS and HHV-8 viremia and disease also has been reported.[62,365] Human papillomavirus–induced epithelial cell proliferation may be responsive to treatment with topical and intralesional administration of cidofovir. Several small, uncontrolled case series have described the successful use of cidofovir to treat juvenile laryngeal papillomatosis, hypopharyngeal and esophageal papillomatous lesions, anogenital condylomas, and cervical intraepithelial neoplasia.* Furthermore, intravenous and intralesional therapy with cidofovir has been used to treat individual patients, including children, with refractory disseminated respiratory papillomatosis of the lung.[20,345,355] Human BK polyomavirus–associated acute hemorrhagic cystitis in immunocompromised patients has been treated with cidofovir, with varying results.[36,163] Case reports and small clinical trials evaluating cidofovir treatment in patients with progressive multifocal leukoencephalopathy who have AIDS or other immunocompromising conditions have not shown consistent benefit in survival or sustained improvement in neurologic status.† Successful cidofovir treatment of adenovirus disease in marrow and stem cell transplant recipients has been published in reports of case series, with the best results noted in patients in whom the disease was localized and treatment was initiated early.‡ Controlled clinical trials evaluating eye drops containing an investigational topical solution of cidofovir to treat patients with acute keratoconjunctivitis caused by adenovirus have not shown consistent benefit in the doses used.[245,246] Poxviruses are inhibited by cidofovir in concentrations safely achievable in humans, and at least one case report of successful treatment of orf (ecthyma contagiosum) has been published.[17,188] In addition, in vitro and animal model data suggest that cidofovir may be effective in the treatment and short-term, postexposure prophylaxis of smallpox and other related poxvirus infections in humans as well as in the treatment of complications that may occur after inoculation with smallpox (vaccinia-like) vaccine.§ However, no clinical trials in humans to evaluate the efficacy of cidofovir for the treatment of poxvirus infections have been published.[398]

Dosage. The usually recommended dose for induction therapy with cidofovir is 5 mg/kg given as an intravenous infusion during the course of 1 hour administered once weekly for 2 consecutive weeks. Some clinicians suggest a reduced dosage regimen of 1 mg/kg administered three times weekly to reduce the risk of renal toxicity.[253] Maintenance therapy usually is administered as 1-hour infusions of 5 mg/kg once every 2 weeks to complete a total of at least five doses. These doses should be decreased to 1 to 3 mg/kg if renal insufficiency is present and discontinued if significant elevation of serum creatinine concentration or proteinuria occurs. Probenecid should be administered orally with each dose of cidofovir, 3 hours before and then 2 and 8 hours after completion of the intravenous infusion. Prehydration with normal saline before each infusion also is recommended. Topical preparations of eye drops containing 0.2 to 1 percent cidofovir and creams containing 1 percent cidofovir are being

*References 326, 327, 344, 424, 427, 475.

*References 20, 90, 410, 470, 491, 575.
†References 18, 98, 185, 266, 389, 433, 456, 473.
‡References 61, 187, 253, 336, 364, 441.
§References 16, 88, 89, 128, 141, 454, 486.

investigated.[245,246] The usual concentration used for intralesional injection is 2.5 mg/mL.[310,355]

Adverse Effects. Cidofovir is nephrotoxic and produces clinically apparent proximal tubular dysfunction, including Fanconi syndrome and acute renal failure, in as many as half the patients who receive the drug.[373,454] Early laboratory signs of renal toxicity include proteinuria, glycosuria, azotemia, and metabolic acidosis. Therefore, patients receiving cidofovir should have a urinalysis and serum tests for renal function performed before taking each dose. The risk for development of renal toxicity can be reduced but not eliminated by probenecid and saline prehydration. Moreover, renal toxicity is more likely to occur if other nephrotoxic agents, such as aminoglycosides, amphotericin B, or foscarnet, are administered concurrently with cidofovir.[577] In clinical trials of patients with AIDS, administration of cidofovir also was associated with neutropenia. Ocular toxicity, including ocular hypotony (decreased intraocular pressure) and anterior uveitis and iritis, also has been reported in patients receiving cidofovir therapy.[4] Therefore, frequent monitoring by an ophthalmologist, including measurement of intraocular pressure, is recommended for patients receiving cidofovir therapy. Intravitreal administration of cidofovir has produced uveitis, vitreitis, reduced intraocular pressure, and loss of vision.[311,394] Local or topical treatment with cidofovir may produce local reactions in the skin.

NEW REFERENCES SINCE THE SIXTH EDITION

9. American Academy of Pediatrics, Committee on Infectious Diseases. Respiratory syncytial virus. In: Pickering L, Baker C, Kimberlin D, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 611.
10. American Academy of Pediatrics, Committee on Infectious Diseases. Herpes simplex virus infections. In: Pickering L, Baker C, Kimberlin D, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 402.
11. American Academy of Pediatrics, Committee on Infectious Diseases. Cytomegalovirus. In: Pickering L, Baker C, Kimberlin D, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 309.
13. Amir J, Wolf D, Levy I. Treatment of symptomatic congenital cytomegalovirus infection with intravenous ganciclovir followed by long-term oral valganciclovir. *Eur J Pediatr* 2010;**169**:1061–7.
17. Andrei G, Snoeck R. Cidofovir activity against poxvirus infections. *Viruses* 2010;**2**:2803–30.
22. Astrahan P, Arkin I. Resistance characteristics of influenza to amino-adamantyls. *Biochim Biophys Acta* 2011;**1808**:547–53.
23. Australia and New Zealand Extracorporeal Membrane Oxygenation (ANZ ECMO) Influenza Investigators; Davies A, Jones D, Bailey M, et al. Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) acute respiratory distress syndrome. *JAMA* 2009;**302**:1888–95.
24. Bacigalupo A, Boyd A, Slipper J, et al. Foscarnet in the management of cytomegalovirus infections in hematopoietic stem cell transplant patients. *Expert Rev Anti Infect Ther* 2012;**10**:1249–64.
33. Bantia A, Kellogg D, Parker C, Babu Y. Combination of peramivir and rimantadine demonstrate synergistic antiviral effects in sublethal influenza A (H3N2) virus mouse model. *Antiviral Res* 2010;**88**:276–80.
34. Baranovich T, Saito R, Suzuki Y, et al. Emergence of H274Y oseltamivir-resistant A(H1N1) influenza viruses in Japan during the 2008-2009 season. *J Clin Virol* 2010;**47**:23–8.
43. Beigel J, Bray M. Current and future antiviral therapy of severe seasonal and avian influenza. *Antiviral Res* 2008;**78**:91–102.
50. Bimkrant D, Cox E. The emergency use authorization for peramivir for treatment of 2009 H1N1 influenza. *N Engl J Med* 2011;**361**:2204–7.
60. Bomgaars L, Thompson P, Berg S, et al. Valacyclovir and acyclovir pharmacokinetics in immunocompromised children. *Pediatr Blood Cancer* 2008;**51**:504–10.
66. Branco L, Boisen M, Anderson K, et al. Lassa hemorrhagic fever in a later term pregnancy from northern Sierra Leone with a positive maternal outcome: case report. *Virol J* 2011;**8**:404.
84. Centers for Disease Control and Prevention. Oseltamivir-resistant novel influenza A(H1N1) virus infection in two immunosuppressed patients—Seattle, Washington 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:893–6.
100. Clay P, Adiga R, Taylor T, et al. Postpartum pharmacokinetics of peramivir in the treatment of 2009 H1N1 influenza: a case report. *Obstet Gynecol* 2011;**118**(Pt 2):463–7.
109. Combes A, Pellegrino V. Extracorporeal membrane oxygenation for 2009 influenza A (H1N1)–associated acute respiratory distress syndrome. *Semin Respir Crit Care Med* 2011;**32**:188–94.
123. Danziger-Isakov L, Arslan D, Sweet S, et al. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the International Pediatric Lung Transplant Collaborative. *Pediatr Transplant* 2012;**16**:638–44.
136. Cohna-Schwake C, Schweiger B, Felderhoff-Muser U, et al. Severe H1N1 infection in a pediatric liver transplant recipient treated with intravenous zanamivir: efficacy and complications. *Transplantation* 2010;**90**:223–4.
144. Du Q, Huang R, Wang S, Chou K. Designing inhibitors of M2 proton channel against H1N1 swine influenza virus. *PLoS One* 2010;**5**:e9388.
145. Dulek D, Williams J, Creech CB, et al. Use of intravenous zanamivir after development of oseltamivir resistance in a critically ill immunosuppressed child infected with 2009 pandemic influenza A (H1N1) virus. *Clin Infect Dis* 2010;**50**:1493–6.
150. Eid A, Razonable R. New developments in the management of cytomegalovirus infection after solid organ transplantation. *Drugs* 2010;**70**:965–81.
162. Escuret V, Frobert E, Bouscambert-Duchamp M, et al. Detection of human influenza A (H1N1) and B strains with reduced sensitivity to neuraminidase inhibitors. *J Clin Virol* 2008;**41**:25–8.
175. Fraaij P, van der Vries E, Beersma M, et al. Evaluation of the antiviral response to zanamivir administered intravenously for treatment of critically ill patients with pandemic influenza A (H1N1) infection. *J Infect Dis* 2011;**204**:777–82.
189. Ghosh S, Adams O, Schuster F, et al. Efficient control of pandemic 2009 H1N1 virus infection with intravenous zanamivir despite the lack of immune function. *Transpl Infect Dis* 2012;**14**:657–9.
219. Harter G, Zimmerman O, Mier L, et al. Intravenous zanamivir for patients with pneumonitis due to pandemic (H1N1) 2009 influenza virus. *Clin Infect Dis* 2010;**50**:1249–51.
238. Hernandez J, Adiga R, Armstrong R, et al. Clinical experience in adults and children treated with intravenous peramivir for 2009 influenza A (H1N1) under an Emergency IND program in the United States. *Clin Infect Dis* 2011;**52**:695–706.
242. Hick R, Prose N. Penile erosions associated with foscarnet therapy in a child. *Pediatr Dermatol* 2010;**27**:302–3.
243. Higgins R, Beniprashad M, Chong-King E, et al. Recovery of influenza B virus with the H273Y point mutation in the neuraminidase active site from a human patient. *J Clin Microbiol* 2012;**50**:2500–2.
255. Hollister A, Sheridan W. The emergency use authorization of peramivir IV: a view from the manufacturer. *Clin Pharmacol Ther* 2011;**89**:172–4.
259. Hoopes J, Driebe E, Kelley E, et al. Triple combination antiviral drug (TCAD) composed of amantadine, oseltamivir, and ribavirin impedes the selection of drug-resistant influenza A virus. *PloSD One* 2011;**6**:e29778.
268. Hurt A, Chotpitayasunondh T, Cox N, et al. Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. *Lancet Infect Dis* 2012;**12**:240–8.
269. Hurt A, Dent Y, Ernest J, et al. Oseltamivir-resistant influenza viruses circulating during the first year of the influenza A (H1N1) 2009 pandemic in the Asia-Pacific region, March 2009 to March 2010. *Euro Surveill* 2011;**16**:19770.

270. Hurt A, Ernest J, Deng Y, et al. Emergence and spread of oseltamivir-resistant A (H1N1) influenza viruses in Oceania, South East Asia, and South Africa. *Antiviral Res* 2009;**83**:90–3.
271. Hurt A, Holien J, Parker M, et al. Zanamivir-resistant influenza viruses with a novel neuraminidase mutation. *J Virol* 2009; **83**:10366–73.
273. Ikematsu H, Kawaii N. Laninamivir octanoate: a new long-acting neuraminidase inhibitor for the treatment of influenza. *Expert Rev Anti Infect Ther* 2011;**9**:851–7.
274. Ishizuka H, Yoshiva S, Yoshihara K, Okabe H. Assessment of the effects of renal impairment on the pharmacokinetic profile of laninamivir, a novel neuraminidase inhibitor, after a single inhaled dose of its Prodrug, CS-8958. *J Clin Pharmacol* 2011;**51**:243–51.
275. Ison M, Hui D, Clezy K, et al. A clinical trial of intravenous peramivir compared with oral oseltamivir for the treatment of seasonal influenza in hospitalized adults. *Antivir Ther* 2012 Oct 30. [Epub ahead of print].
276. Ison M, Szakaly P, Shapira M, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**:955–64.
288. Jefferson T, Jones M, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. *Cochrane Database Syst Rev* 2012;**1**:CD008965.
290. Kalil A, Freifeld A, Lyden E, Stoner J. Valganciclovir for cytomegalovirus prevention in solid organ transplant patients: an evidence-based reassessment of safety and efficacy. *PLoS One* 2009;**4**:e5512.
291. Kalimuddin S, Sessions O, Hou Y, et al. Successful clearance of human parainfluenza virus type 2 viraemia with intravenous ribavirin and immunoglobulin in a patient with acute myocarditis. *J Clin Virol* 2013;**56**:37–40.
316. Kohno S, Kida H, Mizuguchi M, et al. Intravenous peramivir for treatment of influenza A and B virus infection in high-risk patients. *Antimicrob Agents Chemother* 2011;**55**:2803–12.
323. Lackner A, Acham A, Albomo T. Effect on hearing of ganciclovir therapy for asymptomatic congenital cytomegalovirus infection: four to 10 year follow up. *J Laryngol Otol* 2009;**123**:391–6.
339. Li L, Avery R, Budey M, et al. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. *J Heart Lung Transplant* 2012;**31**:839–44.
340. Li Z, Li C, Ling P, et al. Ribavirin reduces mortality in enterovirus 71–infected mice by decreasing viral replication. *J Infect Dis* 2008;**197**:854–7.
345. Lombardi G, Garofoli F, Villani P, et al. Oral valganciclovir treatment in newborns with symptomatic congenital cytomegalovirus infection. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1465–70.
347. Louie J, Yang S, Yen C, et al. Use of intravenous peramivir for treatment of severe influenza A(H1N1)pdm09. *PLoS One* 2012;**7**:e40261.
353. Maier T, Schwarting A, Mauer D, et al. Management and outcomes after multiple corneal and solid organ transplantations from a donor infected with rabies virus. *Clin Infect Dis* 2010;**50**:1112–19.
364. Matthes-Martin S, Feuchtinger T, Shaw P, et al. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transpl Infect Dis* 2012;**14**:555–63.
370. McJunkin J, Nahata M, De Los Reyes E, et al. Safety and pharmacokinetics of ribavirin for the treatment of La Crosse encephalitis. *Pediatr Infect Dis J* 2011;**30**:860–5.
384. Molinos-Quintana A, Perez-de Soto C, Gomez-Rosa M, et al. Intravenous ribavirin for respiratory syncytial viral infections in pediatric hematopoietic SCT recipients. *Bone Marrow Transplant* 2013;**48**:265–8.
399. Nguyen J, Hoopes J, Smee D, et al. Triple combination of oseltamivir, amantadine, and ribavirin displays synergistic activity against multiple influenza virus strains in vitro. *Antimicrob Agents Chemother* 2009;**53**:4115–26.
400. Nguyen J, Hoopes J, Le M, et al. Triple combination of amantadine, ribavirin, and oseltamivir is highly active and synergistic against drug resistant influenza virus strains in vitro. *PLoS One* 2010;**5**:e9332.
401. Nguyen J, Smee D, Barnard D, et al. Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. *PLoS One* 2012;**7**:e31006.
402. Nguyen H, Fry A, Gubareva L. Neuraminidase inhibitor resistance in influenza viruses and laboratory testing methods. *Antivir Ther* 2012;**17**:159–73.
403. Nguyen H, Truillo A, Sheu T, et al. Analysis of influenza virus from patients clinically suspected of infection with an oseltamivir-resistant virus during the 2009 pandemic in the United States. *Antiviral Res* 2012;**93**:381–6.
413. Park K, Lee S, Choi S, et al. Successful salvage therapy with inhaled zanamivir in a patient with peramivir-resistant pandemic influenza A (H1N1) 2009 virus. *Scand J Infect Dis* 2011;**43**:151–5.
417. Pelaez A, Lyon G, Force S, et al. Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. *J Heart Lung Transplant* 2009;**28**:67–71.
422. Pielek R, Chou J. Flu channel drug resistance: a tale of two sites. *Protein Cell* 2010;**1**:246–58.
474. Shah J, Chemaly R. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011;**117**:2755–63.
476. Shelton M, Lovern M, Ng-Cashin J, et al. Zanamivir pharmacokinetics and pulmonary penetration into epithelial lining fluid following intravenous or oral inhaled administration to healthy adult subjects. *Antimicrob Agents Chemother* 2011;**55**:5178–84.
479. Simeonova L, GegovaG, Galabov A. Prophylactic and therapeutic combination effects of rimantadine and oseltamivir against influenza virus A (H3N2) infection in mice. *Antiviral Res* 2012;**95**:172–81.
481. Sleeman K, Sheu T, Moore Z, et al. Influenza B viruses with mutation in the neuraminidase active site, North Carolina, USA, 2010–2011. *Emerg Infect Dis* 2011;**17**:2043–6.
497. Sorbello A, Jones S, Carter W, et al. Emergency use authorization for intravenous peramivir: evaluation of safety in the treatment of hospitalized patients infected with 2009 H1N1 influenza A virus. *Clin Infect Dis* 2012;**55**:1–7.
509. Stephenson I, Democratis J, Lackenby A, et al. Neuraminidase inhibitor resistance after oseltamivir treatment of acute influenza A and B in children. *Clin Infect Dis* 2009;**48**:389–96.
512. Stronati M, Lombardi G, Garofoli F, et al. Pharmacokinetics, pharmacodynamics and clinical use of valganciclovir in newborns with symptomatic congenital cytomegalovirus infection. *Curr Drug Metab* 2013;**14**:208–15.
516. Sugaya M, Ohashi Y. Long-acting neuraminidase inhibitor laninamivir octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus infection. *Antimicrob Agents Chemother* 2010;**54**:2575–82.
517. Sugaya N, Kohno S, Ishibashi T, et al. Efficacy, safety, and pharmacokinetics of intravenous peramivir in children with 2009 pandemic H1N1 influenza A virus infection. *Antimicrob Agents Chemother* 2012;**56**:369–77.
529. Thorlund K, Awad T, Boivin G, Thabane L. Systemic review of influenza resistance to the neuraminidase inhibitors. *BMC Infect Dis* 2011;**11**:134.
548. Wang K, Shun-Shin M, Gill P, et al. Neuraminidase inhibitors for preventing and treating influenza in children (published trials only). *Cochrane Database Syst Rev* 2012;**4**:CD002744.
549. Wang K, Shun-Shin M, Gill P, et al. Neuraminidase inhibitors for preventing and treating influenza in children. *Cochrane Database Syst Rev* 2012;**1**:CD002744.
559. Wildenbeest J, van den Broek P, Benschop K, et al. Pleconaril revisited: clinical course of chronic enteroviral meningoencephalitis after treatment correlates with in vitro susceptibility. *Antivir Ther* 2012;**17**:459–66.
565. Witzke O, Hauser I, Bartels M, et al. Valganciclovir prophylaxis versus preemptive therapy in cytomegalovirus-positive renal allograft recipients: 1 year results of a randomized clinical trial. *Transplantation* 2012;**93**:61–8.
570. Yilmaz K, Cliftdogan D, Vardar G. Effect on hearing of oral valganciclovir for asymptomatic congenital cytomegalovirus infection. *J Trop Pediatr* 2011;**57**:132–4.
574. Yu Y, Garg S, Yu P, et al. Peramivir use for treatment of hospitalized patients with influenza A(H1N1)pdm09 under emergency use authorization, October 2009–June 2010. *Clin Infect Dis* 2012; **55**:8–15.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abdel-Rahman SM, Kearn GL. Single dose pharmacokinetics of pleconaril (VP63843) oral solution: effect of food. *Antimicrob Agents Chemother* 1998;**43**:2706–9.
2. Abdel-Rahman SM, Kearn GL. Single oral dose escalation pharmacokinetics of pleconaril (VP63843) capsules in adults. *J Clin Pharmacol* 1999;**39**:613–18.
3. Abzug MJ, Cloud G, Bradley J, et al. National Institute Allergy and Infectious Diseases Collaborative Antiviral Study Group. Double blind placebo-controlled trial of pleconaril in infants with enterovirus meningitis. *Pediatr Infect Dis J* 2003;**22**:335–41.
4. Accorinti M, Ciapparoni V, Pirraglia M, Pivetti-Pezzi P. Treatment of severe ocular hypotony in AIDS patients with cytomegalovirus retinitis and cidofovir-associated uveitis. *Ocul Immunol Inflamm* 2001;**9**:211–17.
5. Adour KK, Ruboyianes J, Von Doersten PG, et al. Bell's palsy treatment with acyclovir and prednisone compared with prednisone alone: a double-blind, randomized, controlled trial. *Ann Otol Rhinol Laryngol* 1996;**105**:371–8.
6. Alcorn J, McNamara P. Acyclovir, ganciclovir, and zidovudine transfer into rat milk. *Antimicrob Agents Chemother* 2002; **46**:1831–6.
7. Alexander A, Akers A, Matzke G, et al. Disposition of foscarnet during peritoneal dialysis. *Ann Pharmacol Ther* 1996;**30**:1106–9.
8. American Academy of Pediatrics, Committee on Infectious Diseases. The use of oral acyclovir on otherwise healthy children with varicella. *Pediatrics* 1993;**91**:674–6.
9. American Academy of Pediatrics, Committee on Infectious Diseases. Respiratory syncytial virus. In: Pickering L, Baker C, Kimberlin D, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 611.
10. American Academy of Pediatrics, Committee on Infectious Diseases. Herpes simplex virus infections. In: Pickering L, Baker C, Kimberlin D, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 402.
11. American Academy of Pediatrics, Committee on Infectious Diseases. Cytomegalovirus. In: Pickering L, Baker C, Kimberlin D, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 309.
12. Amir J, Harel L, Smetana Z, et al. Treatment of herpes simplex gingivostomatitis with acyclovir in children: a randomized, double blind, placebo-controlled study. *BMJ* 1997;**314**:1800–3.
13. Amir J, Wolf D, Levy I. Treatment of symptomatic congenital cytomegalovirus infection with intravenous ganciclovir followed by long-term oral valganciclovir. *Eur J Pediatr* 2010;**169**:1061–7.
14. Anderson EL, Van Voris LP, Bartram J, et al. Pharmacokinetics of a single dose of rimantadine in young adults and children. *Antimicrob Agents Chemother* 1987;**31**:1140–2.
15. Anderson RD, Griffy KG, Jung D, et al. Ganciclovir absolute bioavailability steady-state pharmacokinetics after oral administration of two 300 mg/d dosing regimens in human immunodeficiency virus– and cytomegalovirus-positive patients. *Clin Ther* 1995;**17**:435–2.
16. Andrei G, Snoeck R, Vandeputte M, De Clercq E. Activities of various compounds against murine and primate polyomaviruses. *Antimicrob Agents Chemother* 1997;**41**:587–93.
17. Andrei G, Snoeck R. Cidofovir activity against poxvirus infections. *Viruses* 2010;**2**:2803–30.
18. Angelini L, Pietrogrande M, Delle Piano M, et al. Progressive multifocal leukoencephalopathy in a child with hyperimmunoglobulin E recurrent infection syndrome and review of the literature. *Neuropediatrics* 2001;**32**:250–5.
19. Arevalo JF, Gonzalez C, Capparelli EV, et al. Intravitreous and plasma concentrations of ganciclovir and foscarnet after intravenous therapy in patients with AIDS and cytomegalovirus retinitis. *J Infect Dis* 1995;**172**:951–6.
20. Armbruster C, Kreuzer A, Vorbach H, et al. Successful treatment of severe respiratory papillomatosis with intravenous cidofovir and interferon alpha-2b. *Eur Respir J* 2001;**17**:830–1.
21. Arvin AM, Feldman S, Merigan TC. Human leukocyte interferon in the treatment of varicella in children with cancer: a preliminary controlled trial. *Antimicrob Agents Chemother* 1978;**13**:605–7.
22. Astrahan P, Arkin I. Resistance characteristics of influenza to amino-adamantyls. *Biochim Biophys Acta* 2011;**1808**:547–53.
23. Australia and New Zealand Extracorporeal Membrane Oxygenation (ANZ ECMO) Influenza Investigators; Davies A, Jones D, Bailey M, et al. Extracorporeal membrane oxygenation for 2009 influenza A(H1N1) acute respiratory distress syndrome. *JAMA* 2009;**302**:1888–95.
24. Bacigalupo A, Boyd A, Slipper J, et al. Foscarnet in the management of cytomegalovirus infections in hematopoietic stem cell transplant patients. *Expert Rev Anti Infect Ther* 2012;**10**:1249–64.
25. Bacon TH, Howard BA, Spender LC, Boyd MR. Activity of penciclovir in antiviral assays against herpes simplex virus. *J Antimicrob Chemother* 1996;**37**:303–13.
26. Bacon TH, Schimazi RF. An overview of the further evaluation of penciclovir against herpes simplex virus and varicella-zoster virus in cell culture highlighting contrasts with acyclovir. *Antiviral Chem Chemother* 1993;**4**:25–6.
27. Bailey AD, Seaberg EC, Porayko MD, et al. Prophylaxis of cytomegalovirus infection in liver transplantation: a randomized trial comparing a combination of ganciclovir and acyclovir to acyclovir. Liver Transplantation Database. *Transplantation* 1997;**64**:66–73.
28. Bailey TC, Ettinger NA, Storch GA, et al. Failure of high-dose oral acyclovir with or without immune globulin to prevent primary cytomegalovirus disease in recipients of solid organ transplants. *Am J Med* 1993;**95**:273–8.
29. Bain V, Daniels H, Chanas A, et al. Foscarnet therapy in chronic hepatitis B virus E antigen carriers. *J Med Virol* 1989;**29**:152–5.
30. Baldanti F, Underwood M, Stanat S, et al. Single amino acid changes in the DNA polymerase confer foscarnet resistance and slow growth phenotype, while mutations in the UL97-encoded phosphotransferase confer ganciclovir resistance in three double-resistant human cytomegalovirus strains recovered form patients with AIDS. *J Virol* 1996;**70**:1390–5.
31. Baldwin A, Kingman H, Darville M, et al. Outcome and clinical course of 100 patients with adenovirus infection following bone marrow transplantation. *Bone Marrow Transplant* 2000;**26**: 1333–8.
32. Balfour H, Rotbart HA, Feldman S, et al. Acyclovir treatment of varicella in otherwise healthy adolescents. The Collaborative Acyclovir Varicella Study Group. *J Pediatr* 1992;**120**:627–33.
33. Bantia A, Kellogg D, Parker C, Babu Y. Combination of peramivir and rimantadine demonstrate synergistic antiviral effects in sublethal influenza A (H3N2) virus mouse model. *Antiviral Res* 2010;**88**:276–80.
34. Baranovich T, Saito R, Suzuki Y, et al. Emergence of H274Y oseltamivir-resistant A(H1N1) influenza viruses in Japan during the 2008-2009 season. *J Clin Virol* 2010;**47**:23–8.
35. Baron S, Brunell PA, Grossbert SE. Mechanisms of action and pharmacology: the immune and interferon systems. In: Galasso GJ, Merigan TC, Buchanan RA, editors. *Antiviral agents and viral diseases in man*. New York: Raven Press; 1979. p. 151–208.
36. Barouch DH, Faquin WC, Chen Y, et al. BK virus–associated hemorrhagic cystitis in a human immunodeficiency virus–infected patient. *Clin Infect Dis* 2002;**35**:326–9.
37. Barroso L, Treanor J, Gubareva L, Hayden F. Efficacy and tolerability of the oral neuraminidase inhibitor peramivir in experimental human influenza: randomized, controlled trials for prophylaxis and treatment. *Antivir Ther* 2005;**19**:901–10.
38. Bauer DJ. The antiviral and synergistic actions of isatin thiosemicarbazone and certain phenoxypyrimidines in vaccinia infection in mice. *Br J Exp Pathol* 1955;**36**:105–14.
39. Bauer DJ, St. Vincent L, Kempe CH, Downie AW. Prophylactic treatment of smallpox contacts with *N*-methylisatin beta-thiosemicarbazone. *Lancet* 1963;**2**:494–6.
40. Bean B, Aeppli D. Adverse effects of high-dose intravenous acyclovir in ambulatory patients with acute herpes zoster. *J Infect Dis* 1985;**151**:262–5.
41. Bean B, Braun C, Balfour H. Acyclovir therapy for acute herpes zoster. *Lancet* 1982;**2**:118–21.
42. Beauchamp LM, Orr GF, deMiranda P, et al. Amino ester prodrugs of acyclovir. *Antiviral Chem Chemother* 1992;**3**:157–64.
43. Beigel J, Bray M. Current and future antiviral therapy of severe seasonal and avian influenza. *Antiviral Res* 2008;**78**:91–102.
44. Belshe RB, Burk B, Newman F, et al. Resistance of influenza A virus to amantadine and rimantadine: results of one decade of surveillance. *J Infect Dis* 1989;**159**:430–5.

45. Berghdal S, Jacobsen B, Moberg L, et al. Pronounced anti-HIV activity of foscarnet in patients without cytomegalovirus infection. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998;**18**:51–3.
46. Berk L, Schalm SW. Failure of acyclovir to enhance the antiviral effect of alpha lymphoblastoid interferon on HBe-seroconversion in chronic hepatitis B: a multi-center randomized controlled trial. *J Hepatol* 1992;**14**:305–9.
47. Bernstein DI, Reuman PD, Sherwood JR, et al. Ribavirin small particle aerosol treatment of influenza B virus infection. *Antimicrob Agents Chemother* 1988;**32**:761–4.
48. Beutner KR. Valacyclovir: a review of antiviral activity, pharmacokinetic properties, and clinical efficacy. *Antiviral Res* 1995;**28**:281–90.
49. Beutner KR, Friedman DJ, Forszpaniak C, et al. Valacyclovir compared with acyclovir for improved therapy for herpes zoster in immunocompetent adults. *Antimicrob Agents Chemother* 1995;**39**:1546–53.
50. Bimkrant D, Cox E. The emergency use authorization for peramivir for treatment of 2009 H1N1 influenza. *N Engl J Med* 2011;**361**:2204–7.
51. Biron KK, Stanat SC, Sorell JR, et al. Metabolic activation of the nucleoside analog 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy] methyl}guanine in human diploid fibroblasts infected with human cytomegalovirus. *Proc Natl Acad Sci U S A* 1985;**82**:2473–7.
52. Bizollon T, Palazzo U, Ducerf C, et al. Pilot study of the combination of interferon alpha and ribavirin as therapy of recurrent hepatitis C after liver transplantation. *Hepatology* 1997;**26**: 500–4.
53. Bleidner WE, Harmon JB, Hewes UE, et al. Absorption, distribution, and excretion of amantadine hydrochloride. *J Pharmacol Exp Ther* 1965;**150**:484–90.
54. Blum MR, Liao SH. Overview of acyclovir pharmacokinetic disposition in adults and children. *Am J Med* 1982;**73**:186–92.
55. Bodenheimer HJ, Lindsey KL, Davis GL, et al. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. *Hepatology* 1997;**26**:473–7.
56. Bodsworth NJ, Crooks RJ, Borelli S, et al. Valacyclovir versus acyclovir in patient initiated treatment of recurrent genital herpes: a randomized, double blind clinical trial. International Valacyclovir HSV Study Group. *Genitourin Med* 1997;**73**:110–16.
57. Boekth M, Gooley TA, Reusser P, et al. Failure of high-dose acyclovir to prevent cytomegalovirus disease after autologous marrow transplantation. *J Infect Dis* 1995;**172**:939–43.
58. Boike SC, Pue M, Audet PR, et al. Pharmacokinetics of famciclovir in subjects with chronic hepatitis disease. *J Clin Pharmacol* 1994;**34**:1199–207.
59. Boivin G, Chou S, Quirk M, et al. Detection of ganciclovir resistance mutations and quantitation of cytomegalovirus (CMV) DNA in leukocytes of patients with fatal disseminated CMV disease. *J Infect Dis* 1996;**173**:523–8.
60. Bomgaars L, Thompson P, Berg S, et al. Valacyclovir and acyclovir pharmacokinetics in immunocompromised children. *Pediatr Blood Cancer* 2008;**51**:504–10.
61. Bordigoni P, Carnet A, Vernard V, et al. Treatment of adenovirus infections in patients undergoing allogeneic hematopoietic stem cell transplantation. *Clin Infect Dis* 2001;**32**:1290–7.
62. Boulanger E, Agbalika F, Maarek O, et al. A clinical, molecular, and cytogenetic study of 12 cases of human herpesvirus 8 associated primary effusion lymphoma in HIV-infected patients. *Hematol J* 2001;**2**:172–9.
63. Boyd MR, Bacon TH, Sutton D, Cole M. Antiherpes activity of 9-(4-hydroxy-3-hydroxy-methylbut-1-yl) guanine (BRL 39123) in cell culture. *Antimicrob Agents Chemother* 1987;**31**:1238–42.
64. Boyd MR, Kern ER, Safrin S. Penciclovir: a review of its spectrum of activity, selectivity and cross-resistance pattern. *Antiviral Chem Chemother* 1993;**4**:3–11.
65. Bradley JS, Connor JD, Compogiannis LS, et al. Exposure of health care workers to ribavirin during therapy for respiratory syncytial virus infections. *Antimicrob Agents Chemother* 1990;**34**:668–70.
66. Branco L, Boisen M, Anderson K, et al. Lassa hemorrhagic fever in a later term pregnancy from northern Sierra Leone with a positive maternal outcome: case report. *Virol J* 2011;**8**:404.
67. Bray M, Martinez M, Kefauver D, et al. Treatment of aerosolized cowpox virus infection in mice with aerosolized cidofovir. *Antiviral Res* 2002;**54**:129–42.
68. Brennan D, Garlock K, Singer G, et al. Prophylactic oral ganciclovir compared with deferred therapy for control of cytomegalovirus in renal transplant recipients. *Transplantation* 1997; **64**:1843–6.
69. Brocklehurst P, Kinghorn G, Carney O, et al. A randomized placebo controlled trial of suppressive acyclovir in late pregnancy in women with recurrent genital herpes infection. *Br J Obstet Gynaecol* 1998;**105**:275–80.
70. Brown F, Banken L, Saywell K, Arum I. Pharmacokinetics of valganciclovir and ganciclovir following multiple oral dosages of valganciclovir in HIV- and CMV-seropositive volunteers. *Clin Pharmacokinet* 1999;**37**:167–96.
71. Browne MJ. Mechanism and specificity of action of ribavirin. *Antimicrob Agents Chemother* 1979;**15**:740–53.
72. Browne MJ, Moss MY, Boyd MR. Comparative activity of amantadine and ribavirin against influenza virus in vitro: possible clinical relevance. *Antimicrob Agents Chemother* 1983;**23**:503–5.
73. Bruning J, Persoons M, Lemstrom K, et al. Enhancement of transplantation-associated atherosclerosis by CMV, which can be prevented by antiviral therapy in the form of HPMPC. *Transpl Int* 1994;**7**(Suppl. 1):365–70.
74. Bryant P, Sasadeusz J, Carapetis J, et al. Successful treatment of foscarnet-resistant herpes simplex stomatitis with intravenous cidofovir in a child. *Pediatr Infect Dis J* 2001;**20**:1083–6.
75. Bueno J, Ramil C, Green M. Current management strategies for the prevention and treatment of cytomegalovirus infection in pediatric transplant recipients. *Paediatr Drugs* 2002;**4**:279–90.
76. Buhles WC Jr, Mastre BJ, Tinker AJ, et al. Ganciclovir treatment of life- or sight-threatening cytomegalovirus infection: experience in 314 immunocompromised patients. *Rev Infect Dis* 1988;**10**(Suppl. 3):495–506.
77. Calfree DP, Hayden FG. New approaches to influenza chemotherapy: neuraminidase inhibitors. *Drugs* 1998;**56**:537–53.
78. Calfree DP, Peng AW, Cass LM, et al. Safety and efficacy of intravenous zanamivir in preventing experimental human influenza A virus infection. *Antimicrob Agents Chemother* 1999; **43**:1616–20.
79. Capparelli EV, Stevens RC, Chow MS, et al. Rimantadine pharmacokinetics in healthy subjects and patients with end stage renal disease. *Clin Pharmacol Ther* 1988;**43**:536–41.
80. Cass LMR, Efthymiopoulos C, Bye A. Pharmacokinetics of zanamivir after intravenous, oral, inhaled or intranasal administration to healthy volunteers. *Clin Pharmacokinet* 1999;**36**(Suppl. 1): 1–11.
81. Cassidy LF, Patterson JL. Mechanism of La Crosse virus inhibition by ribavirin. *Antimicrob Agents Chemother* 1989;**33**:2009–11.
82. Castagnola E, Cristina E, Dallorso S, et al. Failure of cidofovir to reduce CMV-antigenemia in a child transplanted from a matched unrelated donor. *J Chemother* 2001;**13**:100–1.
83. Centers for Disease Control and Prevention. Pregnancy outcomes following systemic prenatal acyclovir exposure: June 1, 1984–June 30, 1993. *MMWR Morb Mortal Wkly Rep* 1993;**42**:806–9.
84. Centers for Disease Control and Prevention. Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients—Seattle, Washington 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:893–6.
85. Chakrabarti S, Collingham KE, Osman H, et al. Cidofovir primary preemptive therapy for post-transplant cytomegalovirus infections. *Transplantation* 2001;**28**:879–81.
86. Chakrabarti S, Mauther V, Osman H, et al. Adenovirus infections following allogeneic stem cell transplantation: incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood* 2002;**100**:1619–27.
87. Chatis PA, Crumpacker CS. Resistance of herpes viruses to antiviral drugs. *Antimicrob Agents Chemother* 1992;**36**:1589–95.
88. Cherrington JM, Fuller MD, Larry PD, et al. In vitro antiviral susceptibilities of isolates from CMV retinitis patients receiving first or second line cidofovir therapy: relationship to clinical outcome. *J Infect Dis* 1998;**178**:1821–5.
89. Cherrington J,M, Miner R, Hitchcock M, et al. Susceptibility of human cytomegalovirus to cidofovir is unchanged after limited in vivo exposure to various regimens of drug. *J Infect Dis* 1996;**173**:987–92.
90. Chhetri D, Blumin J, Shapiro N, Berke G. Office-based treatment of laryngeal papillomatosis with percutaneous injection of cidofovir. *Otolaryngol Head Neck Surg* 2002;**126**:642–8.

91. Chiou HC, Kumara K, Hu A, et al. Penciclovir-resistance mutations in the herpes simplex virus DNA polymerase gene. *Antiviral Chem Chemother* 1995;**6**:281–8.
92. Chou S, Erice A, Jordan MC, et al. Analysis of the UL97 phosphotransferase coding sequence in clinical cytomegalovirus isolates and identification of mutations conferring ganciclovir resistance. *J Infect Dis* 1995;**171**:576–83.
93. Chou S, Guentzel S, Michels KR, et al. Frequency of UL97 phosphotransferase mutations related to ganciclovir resistance in clinical cytomegalovirus isolates. *J Infect Dis* 1995;**172**:239–42.
94. Chou S, Marousek G, Guentzel S, et al. Evolution of mutations conferring multidrug resistance during prophylaxis and therapy for cytomegalovirus disease. *J Infect Dis* 1997;**176**:786–9.
95. Chrisp P, Clissold S. Foscarnet: a review of its antiviral activity, pharmacokinetic properties, and therapeutic use in immunocompromised patients with cytomegalovirus retinitis. *Drugs* 1991;**41**:104–29.
96. Christophers J, Clayton J, Craske J, et al. Survey of resistance of herpes simplex virus to acyclovir in Northwest England. *Antimicrob Agents Chemother* 1998;**42**:868–72.
97. Cinque P, Baldanti F, Vago L, et al. Ganciclovir therapy for cytomegalovirus (CMV) infection of the central nervous system in AIDS patients: monitoring by CMV DNA detection in cerebrospinal fluid. *J Infect Dis* 1995;**171**:1603–6.
98. Cinque P, Pierotti C, Vigano M, et al. The good and evil of HAART in HIV-related progressive multifocal leukoencephalopathy. *J Neurovirol* 2001;**7**:358–63.
99. Cirelli R, Herne K, McCrary M, et al. Famciclovir: review of clinical efficacy and safety. *Antiviral Res* 1996;**29**:141–5.
100. Clay P, Adiga R, Taylor T, et al. Postpartum pharmacokinetics of peramivir in the treatment of 2009 H1N1 influenza: a case report. *Obstet Gynecol* 2011;**118**(Pt 2):463–7.
101. Clover RD, Crawford SA, Abell TD, et al. Effectiveness of rimantadine prophylaxis of children within families. *Am J Dis Child* 1986;**140**:706–9.
102. Cobo LM, Foulks GN, Liesegang T, et al. Oral acyclovir in the treatment of acute herpes zoster ophthalmicus. *Ophthalmology* 1986;**93**:763–70.
103. Colby BM, Shaw JE, Elion GB, et al. Effect of acyclovir [9-(2-hydroxyethoxymethyl)guanine] on Epstein-Barr virus DNA replication. *J Virol* 1980;**34**:560–8.
104. Cole NL, Balfour H. Varicella-zoster virus does not become more resistant to acyclovir during therapy. *J Infect Dis* 1986; **153**:605–8.
105. Coleman PM. Influenza virus neuraminidase: structure, antibodies and inhibitors. *Protein Sci* 1994;**3**:1687–96.
106. Collaborative DHPG Treatment Study Group. Treatment of serious cytomegalovirus infections with 9-(1,3-dihydroxy-2-propoxymethyl)guanine in patients with AIDS and other immunodeficiencies. *N Engl J Med* 1986;**314**:801–5.
107. Colledge D, Locarnini S, Shaw T. Synergistic inhibition of hepadnaviral replication by lamivudine in combination with penciclovir in vitro. *Hepatology* 1997;**26**:216–25.
108. Collum LM, Benedict-Smith A, Hillary IB. Randomized double blind trial of acyclovir and idoxuridine in dendritic corneal ulceration. *Br J Ophthalmol* 1980;**64**:766–9.
109. Combes A, Pellegrino V. Extracorporeal membrane oxygenation for 2009 influenza A (H1N1)–associated acute respiratory distress syndrome. *Semin Respir Crit Care Med* 2011;**32**:188–94.
110. Connor ES, Morrison J, Lane J, et al. Safety, tolerance, and pharmacokinetics of systemic ribavirin in children with human immunodeficiency virus infection. *Antimicrob Agents Chemother* 1993;**37**:532–9.
111. Corey L, Fite KH, Benedetti JK, et al. Intravenous acyclovir for the treatment of primary genital herpes. *Ann Intern Med* 1983;**98**:914–21.
112. Corey L, Nahmias AJ, Guinan MD, et al. A trial of topical acyclovir in genital herpes simplex virus infections. *N Engl J Med* 1982;**306**:1313–19.
113. Couch RB. Prevention and treatment of influenza. *N Engl J Med* 2000;**343**:1778–87.
114. Couch RB, Jackson G. Antiviral agents in influenza: summary of Influenza Workshop VIII. *J Infect Dis* 1976;**134**:516–27.
115. Crawford SA, Clover RD, Abell TD, et al. Rimantadine prophylaxis in children: a followup study. *Pediatr Infect Dis J* 1988; **7**:379–83.
116. Crooks RJ, Murray A. Valacyclovir: a review of a promising new antiherpes agent. *Antiviral Chem Chemother* 1994;**5**(Suppl. 1):31–7.
117. Crumpacker C. Mechanism of action of foscarnet against viral polymerases. *Am J Med* 1992;**92**:S3-S7.
118. Crumpacker C. Ganciclovir. *N Engl J Med* 1996;**335**:721–9.
119. Crumpacker C, Schnipper LE, Marlowe SI, et al. Resistance to antiviral drugs of herpes simplex virus isolated from a patient treated with acyclovir. *N Engl J Med* 1979;**306**:343–5.
120. Crumpacker C, Schnipper LE, Zaia JA, et al. Growth inhibition by acycloguanosine of herpesviruses isolated from human infections. *Antimicrob Agents Chemother* 1979;**15**:642–5.
121. Cundy KC, Petty BG, Flaherty F, et al. Clinical pharmacokinetics of cidofovir in human immunodeficiency virus–infected patients. *Antimicrob Agents Chemother* 1995;**39**:1247–52.
122. Daniels S, Schentage JJ. Drug interaction studies and safety of famciclovir in healthy volunteers: a review. *Antiviral Chem Chemother* 1993;**4**:57–64.
123. Danziger-Isakov L, Arslan D, Sweet S, et al. RSV prevention and treatment in pediatric lung transplant patients: a survey of current practices among the International Pediatric Lung Transplant Collaborative. *Pediatr Transplant* 2012;**16**:638–44.
124. Davies WL, Grunert RR, Haff JW, et al. Antiviral activity of l-adamantanamine (amantadine). *Science* 1964;**144**:862–3.
125. Davis GI, Esteban-Mur R, Rustigi V, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;**339**:1493–9.
126. De Clercq E. Antivirals for the treatment of herpesvirus infections. *J Antimicrob Chemother* 1993;**32**(Suppl. A):121–32.
127. De Clercq E. Perspectives for the chemotherapy of respiratory syncytial virus (RSV) infections. *Int J Antimicrob Agents* 1996;**7**:193–202.
128. de Oliveira CB, Stevenson D, LaBree L, et al. Evaluation of cidofovir (HPMPC, GS-504) against adenovirus type 5 infection in vitro and in a New Zealand rabbit ocular model. *Antiviral Res* 1996;**31**:165–72.
129. Demicheli V, Jefferson T, Rivetti D, Deeks J. Prevention and early treatment of influenza in healthy adults. *Vaccine* 2000;**18**:957–1030.
130. Deray G, Martinez F, Katlama C, et al. Foscarnet nephrotoxicity: mechanism, incidence and prevention. *Am J Nephrol* 1989;**9**:316–21.
131. Derse D, Cheng YC, Furman PA, et al. Inhibition of purified human and herpes simplex virus–induced DNA polymerase by 9-(2-hydroxyethoxymethyl)guanine triphosphate: effects on primer template function. *J Biol Chem* 1981;**256**:11447–51.
132. DeTorres O. Focus on foscarnet: a pyrophosphate analog for use in CMV retinitis and other viral infections. *Hosp Formul* 1991;**26**:929–46.
133. Diaz-Mitoma F, Sibbald RG, Shafram SD. Oral famciclovir for the suppression of recurrent genital herpes: a randomized controlled trial. *JAMA* 1998;**200**:887–92.
134. DiBisceglie AM, Conjeevram HS, Friet MS, et al. Ribavirin as therapy for chronic hepatitis C: a randomized, double blind, placebo controlled trial. *Ann Intern Med* 1995;**123**:897–903.
135. Dietrick D, Kotler D, Busch D, et al. Ganciclovir treatment of cytomegalovirus colitis in AIDS: a randomized, double blind, placebo controlled multicenter study. *J Infect Dis* 1993;**167**:278–82.
136. Dohna-Schwake C, Schweiger B, Felderhoff-Muser U, et al. Severe H1N1 infection in a pediatric liver transplant recipient treated with intravenous zanamivir: efficacy and complications. *Transplantation* 2010;**90**:223–4.
137. Dolin R, Reichman RC, Madore HQ, et al. A controlled trial of amantadine and rimantadine in the prophylaxis of influenza A infection. *N Engl J Med* 1982;**307**:580–4.
138. Dowling JN, Postic B, Guevarra LO. Effect of ribavirin on murine cytomegalovirus infection. *Antimicrob Agents Chemother* 1976;**10**:809–13.
139. Doyle WJ, Skoner DP, Alper CM, et al. Effect of rimantadine treatment on clinical manifestation and otologic complications in adults experimentally infected with influenza A (H1N1) virus. *J Infect Dis* 1998;**177**:1260–5.
140. Drew WL. Cytomegalovirus infection in patients with AIDS. *Clin Infect Dis* 1992;**14**:608–15.

141. Drew WL. Treatment of drug-resistant CMV disease in HIV-infected patients. *Infect Med* 2000;**17**:737–44.
142. Drew WL, Miner RC, Busch DF, et al. Prevalence of resistance in patients receiving ganciclovir for serious cytomegalovirus infection. *J Infect Dis* 1991;**163**:716–19.
143. Drobyski W, Knox K, Carrigan D, et al. Foscarnet therapy of ganciclovir-resistant cytomegalovirus in marrow transplantation. *Transplantation* 1991;**52**:155–7.
144. Du Q, Huang R, Wang S, Chou K. Designing inhibitors of M2 proton channel against H1N1 swine influenza virus. *PLoS One* 2010;**5**:e9388.
145. Dulek D, Williams J, Creech CB, et al. Use of intravenous zanamivir after development of oseltamivir resistance in a critically ill immunosuppressed child infected with 2009 pandemic influenza A (H1N1) virus. *Clin Infect Dis* 2010;**50**:1493–6.
146. Duncan S, Paradis I, Dauber J, et al. Ganciclovir prophylaxis for cytomegalovirus infections in pulmonary allograft recipients. *Am Rev Respir Dis* 1992;**146**:1213–15.
147. Dunkle L, Arvin A, Whitley RJ, et al. A controlled trial of acyclovir for chickenpox in normal children. *N Engl J Med* 1991; **325**:1539–44.
148. Earnshaw DL, Bacon TH, Darlison SJ, et al. Mode of antiviral action of penciclovir in MRC-5 cells infected with herpes simplex virus type 1 (HSV-1), HSV-2, and varicella-zoster virus. *Antimicrob Agents Chemother* 1992;**36**:2747–57.
149. Egan JJ, Carroll KB, Yonan N, et al. Valacyclovir prevention of cytomegalovirus reactivation after heart transplantation: a randomized trial. *J Heart Lung Transplant* 2002;**21**:460–6.
150. Eid A, Razonable R. New developments in the management of cytomegalovirus infection after solid organ transplantation. *Drugs* 2010;**70**:965–81.
151. Elion GB, Furman PA, Fyfe JA, et al. Selectivity of action of an anti-herpetic agent, 9-(2-hydroxyethoxymethyl)guanine. *Proc Natl Acad Sci U S A* 1977;**74**:5716–20.
152. Elizaga J, Olavarria E, Apperley JF, et al. Parainfluenza virus 3 infection after stem cell transplant: relevance to outcome of rapid diagnosis and ribavirin treatment. *Clin Infect Dis* 2001;**32**:413–18.
153. Emanuel D, Cuningham I, Jules-Eluseek K, et al. Cytomegalovirus pneumonia after bone marrow transplantation successfully treated with the combination of ganciclovir and high-dose intravenous globulin. *Ann Intern Med* 1988;**109**:777–82.
154. Emery VC. Progress in understanding cytomegalovirus drug resistance. *J Clin Virol* 2001;**21**:223–8.
155. Englund JA, Piedra PA, Jefferson LS, et al. High dose, short duration ribavirin aerosol therapy in children with suspected respiratory syncytial virus infection. *J Pediatr* 1990;**117**:313–20.
156. Englund JA, Piedra PA, Whimbey E. Prevention and treatment of respiratory syncytial virus and parainfluenza viruses in immunocompromised patients. *Am J Med* 1997;**102**:61–70.
157. Englund JA, Zimmerman MD, Swierkosz EM, et al. Herpes simplex virus resistant to acyclovir: a study in a tertiary care center. *Ann Intern Med* 1990;**112**:416–22.
158. Enria DA, Maiziegui JI. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral Res* 1994;**23**:23–31.
159. Erice A, Chou S, Biron K, et al. Progressive disease due to ganciclovir-resistant cytomegalovirus in immunocompromised patients. *N Engl J Med* 1989;**320**:289–93.
160. Eriksson BE, Helgstrand NG, Johansson A, et al. Inhibition of influenza virus ribonucleic acid polymerase by ribavirin triphosphate. *Antimicrob Agents Chemother* 1977;**11**:946–51.
161. Eriksson B, Schinazi R. Combination of 3′-azido-3′-deoxythymidine (zidovudine) and phosphonoformate (foscarnet) against human immunodeficiency virus type 1 and cytomegalovirus replication in vitro. *Antimicrob Agents Chemother* 1989; **33**:663–9.
162. Escuret V, Frobert E, Bouscambert-Duchamp M, et al. Detection of human influenza A (H1N1) and B strains with reduced sensitivity to neuraminidase inhibitors. *J Clin Virol* 2008;**41**:25–8.
163. Faraci M, Cuzzubbo D, Lanino E, et al. Low dosage cidofovir without probenecid as treatment for BK virus hemorrhagic cystitis after hematopoietic stem cell transplant. *Pediatr Infect Dis J* 2009;**28**:55–7.
164. Faulds D, Heel R. Ganciclovir: a review of its antiviral activity, pharmacokinetic properties, and therapeutic efficacy in cytomegalovirus infections. *Drugs* 1990;**39**:597–638.
165. Feldman S, Rodman J, Gregory B. Excessive serum concentration of acyclovir and neurotoxicity. *J Infect Dis* 1986;**157**:385–8.
166. Fernandez-Larson R, Patterson JL. Ribavirin is an inhibitor of human immunodeficiency virus reverse transcriptase. *Mol Pharmacol* 1990;**38**:766–70.
167. Field AK, Davies ME, DeWitt C, et al. 9-{[2-hydroxy-1-(hydroxymethyl)ethoxy]methyl}guanine: a selective inhibitor of herpes group virus replication. *Proc Natl Acad Sci U S A* 1983; **80**:4139–43.
168. Fischer-Hoch SP, Gborie S, Parker L. Unexpected adverse reactions during a clinical trial in rural West Africa. *Antiviral Res* 1992;**19**:139–47.
169. Fischer-Hoch SP, Khan JA, Rehman S, et al. Crimean-Congo hemorrhagic fever treated with oral ribavirin. *Lancet* 1995;**346**:472–5.
170. Fletcher C, Sawchuk R, Chinnock B, et al. Human pharmacokinetics of the antiviral drug DHPG. *Clin Pharmacol Ther* 1986; **40**:281–6.
171. Flores-Aguilar M, Huang JS, Wiley CA, et al. Long-acting therapy of retinitis with (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine. *J Infect Dis* 1994;**169**:642–7.
172. Forni AL, Schluger NW, Roberts RB. Severe measles pneumonitis in adults: evaluation of clinical characteristics and therapy with intravenous ribavirin. *Clin Infect Dis* 1994;**19**:454–62.
173. Fowler SE, Pierce DM, Prince WT, et al. The tolerance to and pharmacokinetics of penciclovir (BRL39, 123A), a novel antiherpes agent, administered by intravenous infusion to healthy subjects. *Eur J Clin Pharmacol* 1992;**43**:513–16.
174. Fox MP, McKinlay MA, Diana GD, Dutko FJ. Binding affinities of structurally related human rhinovirus capsid-binding compounds are related to their activities against human rhinovirus type 14. *Antimicrob Agents Chemother* 1991;**35**:1040–7.
175. Fraaij P, van der Vries E, Beersma M, et al. Evaluation of the antiviral response to zanamivir administered intravenously for treatment of critically ill patients with pandemic influenza A (H1N1) infection. *J Infect Dis* 2011;**204**:777–82.
176. Freitas V, Fraser-Smith E, Matthews T. Increased efficacy of ganciclovir in combination with foscarnet against cytomegalovirus and herpes simplex virus type 2 in vitro and in vivo. *Antiviral Res* 1989;**12**:205–12.
177. Frenkel L, Brown ZA, Bryson YJ, et al. Pharmacokinetics of acyclovir in the term human pregnancy and neonate. *Am J Obstet Gynecol* 1991;**164**:569–76.
178. Frenkel LR, Wilson CW, Demers RR, et al. A technique for the administration of ribavirin to mechanically ventilated infants with severe respiratory syncytial virus infection. *Crit Care Med* 1987;**15**:1051–4.
179. Fried MW, Shiffman ML, Reddy R, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002;**347**:975–82.
180. Fritz RS, Hayden FG, Calfee DP, et al. Nasal cytokine and chemokine responses in experimental influenza A virus infection: results of a placebo-controlled trial of intravenous zanamivir treatment. *J Infect Dis* 1999;**180**:586–93.
181. Furman PA, St Clair MA, Spector T. Acyclovir triphosphate is a suicide inactivator of the herpes simplex virus DNA polymerase. *J Biol Chem* 1984;**359**:9575–9.
182. Gaines K, Jung D, Tan S-J. Final report of study GANS51788. An open label evaluation of the safety and pharmacokinetics of ganciclovir in children. *Clinical Research Report CL6791* 1994.
183. Galbraith AW, Oxford JS, Schild GC, et al. Study of l-amantanamine hydrochloride used prophylactically during the Hong Kong influenza epidemic in the family environment. *Bull World Health Organ* 1969;**41**:677–82.
184. Game E, Saliba F, Valdecassas G, et al. Randomized trial of efficacy and safety of oral ganciclovir in the prevention of cytomegalovirus disease in liver transplant recipients. *Lancet* 1997; **350**:1729–33.
185. Gasnault J, Kousignian P, Kahraman M, et al. Cidofovir in AIDS-associated progressive multifocal leukoencephalopathy: a monocenter observational study with clinical and JC viral load monitoring. *J Neurovirol* 2001;**7**:375–81.
186. Gaudreau A, Hill E, Balfour H, et al. Phenotypic and genotypic characterization of acyclovir-resistant herpes simplex viruses from immunocompromised patients. *J Infect Dis* 1998;**178**:297–303.

187. Gavin PJ, Katz BZ. Intravenous ribavirin treatment for severe adenovirus disease in immunocompromised children. *Pediatrics* 2002;**110**:e9–12.
188. Geerinck K, Lukito G, Snoeck R, et al. A case of human orf in an immunocompromised patient treated successfully with cidofovir cream. *J Med Virol* 2001;**64**:543–9.
189. Ghosh S, Adams O, Schuster F, et al. Efficient control of pandemic 2009 H1N1 virus infection with intravenous zanamivir despite the lack of immune function. *Transpl Infect Dis* 2012;**14**:657–9.
190. Gilbert BE, Knight V. Pulmonary delivery of antiviral drugs in liposome aerosols. *Semin Pediatr Infect Dis* 1996;**7**:148–54.
191. Gilbert C, Roy J, Belanger R, et al. Lack of emergence of cytomegalovirus UL97 mutations conferring ganciclovir (GCV) resistance following preemptive GCV therapy in allogeneic stem cell transplant recipients. *Antimicrob Agents Chemother* 2001;**215**: 3669–71.
192. Gish RG, Lau JY, Brooks L. Ganciclovir treatment of hepatitis B virus infection in liver transplant recipients. *Hepatology* 1996; **23**:1–7.
193. Glada JM, Ecobichon DJ. Evaluation of exposure of healthcare personnel to ribavirin. *J Toxicol Environ Health* 1989;**28**:1–12.
194. Glanville A, Scott A, Morton J, et al. Intravenous ribavirin is a safe and cost-effective treatment for respiratory syncytial virus infection after lung transplantation. *J Heart Lung Transplant* 2005;**24**:2114–19.
195. Goldberg LH, Kaufman R, Kurtz TO, et al. Long term suppression of recurrent genital herpes with acyclovir: a 5 year benchmark. Acyclovir Study Group. *Arch Dermatol* 1993;**129**:582–7.
196. Goldthorpe SE, Boud MR, Field HJ. Effects of penciclovir and famciclovir in a murine model of encephalitis induced by intranasal inoculation of herpes simplex virus type 1. *Antimicrob Agents Chemother* 1992;**3**:37–47.
197. Goodrich J, Bowden R, Fisher L, et al. Ganciclovir prophylaxis to prevent cytomegalovirus disease after allogeneic marrow transplant. *Ann Intern Med* 1993;**118**:173–8.
198. Goodrich J, Morci M, Gleaves C. Early treatment with ganciclovir to prevent cytomegalovirus disease after allogeneic bone marrow transplantation. *N Engl J Med* 1991;**325**:1601–7.
199. Gordon YJ, Ramanowski E, Araullo-Cruz T, et al. Inhibitory effect of (*S*)-HPMPC, (*S*)-HPMPA, and 2′-nor-cyclic GMP on clinical ocular adenoviral isolates is serotype-dependent in vitro. *Antiviral Res* 1991;**16**:11–16.
200. Govorkova E, Leneva I, Gooubeva O, et al. Comparison of efficacies of RWJ-270201, zanamivir, and oseltamivir against H5N1, H9N2, and other avian influenza viruses. *Antimicrob Agents Chemother* 2001;**45**:2723–32.
201. Groothius JR, Woodin KA, Katz R, et al. Early ribavirin treatment of respiratory syncytial viral infections in high risk children. *J Pediatr* 1990;**117**:792–8.
202. Gubareva LV, Matrosovich MN, Brenner MK, et al. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J Infect Dis* 1998;**178**:1257–62.
203. Gubareva LV, Robinson MJ, Bethall RC, Webster RG. Catalytic and framework mutations in the neuraminidase active site of influenza viruses that are resistant to 4-guanidino-Neu5AcZen. *J Virol* 1997;**71**:3385–90.
204. Gubareva LV, Webster RG, Hayden FG. Comparison of the activities of zanamivir, oseltamivir, and RWJ-270201 against clinical isolates of influenza virus and neuraminidase inhibitor-resistant variants. *Antimicrob Agents Chemother* 2001;**45**:3403–8.
205. Gubareva LV, Webster RG, Hayden FG. Detection of influenza virus resistance to neuraminidase inhibitors by an enzyme inhibition assay. *Antiviral Res* 2002;**53**:47–61.
206. Gubareva L, Mastrosovich M, Brenner M, et al. Evidence for zanamivir resistance in an immunocompromised child infected with influenza B virus. *J Infect Dis* 1998;**178**:1257–62.
207. Gururangan S, Stevens N, Morris D. Ribavirin response in measles pneumonia. *J Infect Dis* 1990;**20**:219–21.
208. Haake DA, Zakowski PC, Haake DL, Bryson YJ. Early treatment with acyclovir for varicella pneumonia in otherwise healthy adults: retrospective controlled study and review. *Rev Infect Dis* 1990;**12**:788–98.
209. Haefleri WE, Schonenberger RA, Weiss PP, et al. Acyclovir-induced neurotoxicity: concentration side effect relationship in acyclovir overdose. *Am J Med* 1993;**94**:212–15.
210. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001;**344**:1917–28.
211. Hall CB, Dolin R, Gala CL, et al. Children with influenza A infection: treatment with rimantadine. *Pediatrics* 1987;**80**: 275–82.
212. Hall CB, McBride JT, Gala CL, et al. Ribavirin treatment of respiratory syncytial viral infection in infants with underlying cardiopulmonary disease. *JAMA* 1985;**254**:3047–51.
213. Hall CB, McBride JT, Walsh EE, et al. Aerosolized ribavirin treatment of infants with respiratory syncytial viral infection: a randomized, double blind study. *N Engl J Med* 1983;**308**:1443–7.
214. Haller GW, Bechstein WO, Neuhaus R, et al. Famciclovir therapy for recurrent hepatitis B virus infection after liver transplantation. *Transpl Int* 1996;**9**(Suppl. 1):210–12.
215. Hamre D, Brownlea KA, Donovick R. Studies on the chemotherapy of vaccinia virus. II. The activity of some thiosemicarbazones. *J Immunol* 1951;**67**:305–12.
216. Hamzeh FM, Lietman PJ. Intranuclear accumulation of subgenomic noninfectious human cytomegalovirus DNA in infected cells in the presence of ganciclovir. *Antimicrob Agents Chemother* 1991;**35**:1818–23.
217. Hanel M, Fiedler F, Thorns C. Anti-CD20 monoclonal antibody (rituximab) and cidofovir as successful treatment of an EBV-associated lymphoma with CNS involvement. *Onkologie* 2001;**24**:491–4.
218. Hardy W. Combined ganciclovir and recombinant human granulocyte-macrophage colony-stimulating factor in the treatment of cytomegalovirus retinitis in AIDS patients. *J Acquir Immune Defic Syndr* 1991;**4**(Suppl.):22–8.
219. Harter G, Zimmerman O, Mier L, et al. Intravenous zanamivir for patients with pneumonitis due to pandemic (H1N1) 2009 influenza virus. *Clin Infect Dis* 2010;**50**:1249–51.
220. Hay AJ. The action of adamantanamines against influenza A viruses: inhibition of the M2 ion channel protein. *Semin Virol* 1992;**3**:21–30.
221. Hayden FG. Combined antiviral therapy for respiratory virus infections. *Antiviral Res* 1996;**29**:45–8.
222. Hayden FG, Belshe RB, Clover RD, et al. Emergence and apparent transmission of rimantadine-resistant influenza A virus in families. *N Engl J Med* 1989;**321**:1696–702.
223. Hayden FG, Coats T, Kim K, et al. Oral pleconaril treatment of picornavirus-associated viral respiratory illness in adults: efficacy and tolerability in phase II clinical trials. *Antiviral Ther* 2002;**7**:53–6.
224. Hayden FG, Cote KM, Douglas RG. Plaque inhibition assay for drug susceptibility testing of influenza viruses. *Antimicrob Agents Chemother* 1980;**17**:865–70.
225. Hayden FG, Couch RB. Clinical and epidemiological importance of influenza A virus resistant to amantadine and rimantadine. *Rev Med Virol* 1992;**2**:89–96.
226. Hayden FG, Douglas RG Jr, Simons R. Enhancement of activity against influenza viruses by combinations of antibacterial agents. *Antimicrob Agents Chemother* 1980;**18**:536–41.
227. Hayden FG, Gubareva LV, Monto AS, et al. Inhaled zanamivir for the prevention of influenza in families. Zanamivir Family Study Group. *N Engl J Med* 2000;**18**:1282–9.
228. Hayden FG, Hall WJ, Douglas RG Jr, et al. Amantadine aerosols in normal volunteers: pharmacology and safety testing. *Antimicrob Agents Chemother* 1979;**16**:644–50.
229. Hayden FG, Hoffman HE, Spyker DA. Differences in side effects of amantadine hydrochloride and rimantadine hydrochloride relate to differences in pharmacokinetics. *Antimicrob Agents Chemother* 1983;**23**:458–64.
230. Hayden FG, Minoha A, Spyker DA, et al. Comparative single dose pharmacokinetics of amantadine hydrochloride and rimantadine hydrochloride in young and elderly adults. *Antimicrob Agents Chemother* 1985;**28**:216–21.
231. Hayden FG, Osterhaus AD, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. *N Engl J Med* 1997;**337**:874–8.
232. Hayden FG, Sable CA, Connor JD, Lane J. Intravenous ribavirin by constant infusion for serious influenza and parainfluenza virus infection. *Antiviral Ther* 1996;**1**:51–6.
233. Hayden FG, Treanor JJ, Belts RF, et al. Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. *JAMA* 1996;**275**:295–9.

234. Hayden FG, Zlydnikov DM, Ijenko VI, et al. Comparative therapeutic effect of aerosolized and oral rimantadine HCl in experimental human influenza A virus infection. *Antiviral Res* 1982;**2**:142–53.
235. Hayden F. Development of new antiviral agents for influenza treatment: what does the future hold? *Clin Infect Dis* 2009;**48**(Supp. 1):S3–13.
236. Hedrick JA, Barzilai A, Behre U, et al. Zanamivir for treatment of symptomatic influenza A and B infection in children five to twelve years of age: a randomized controlled trial. *Pediatr Infect Dis J* 2000;**19**:410–17.
237. Hengge U, Brockmeyer N, Malessa R, et al. Foscarnet penetrates the blood brain barrier: rationale for therapy of cytomegalovirus encephalitis. *Antimicrob Agents Chemother* 1998;**37**:1010–14.
238. Hernandez J, Adiga R, Armstrong R, et al. Clinical experience in adults and children treated with intravenous peramivir for 2009 influenza A (H1N1) under an Emergency IND program in the United States. *Clin Infect Dis* 2011;**52**:695–706.
239. Herpetic Eye Disease Study Group. Acyclovir for the prevention of recurrent herpes simplex virus eye disease. *N Engl J Med* 1998;**339**:300–6.
240. Hiatt PW, Treece D, Morris L, et al. Longitudinal pulmonary function (PF) following treatment with ribavirin in infants hospitalized with RSV bronchiolitis. *Am J Respir Crit Care Med* 1993;**179**:209–13.
241. Hibberd P, Tolkoff-Rubin N, Conti D, et al. Pre-emptive ganciclovir therapy to prevent cytomegalovirus disease in cytomegalovirus antibody positive renal transplant recipients: a randomized controlled trial. *Ann Intern Med* 1995;**123**:18–26.
242. Hick R, Prose N. Penile erosions associated with foscarnet therapy in a child. *Pediatr Dermatol* 2010;**27**:302–3.
243. Higgins R, Beniprashad M, Chong-King E, et al. Recovery of influenza B virus with the H273Y point mutation in the neuraminidase active site from a human patient. *J Clin Microbiol* 2012;**50**:2500–2.
244. Hill EL, Hunter GA, Ellis MN, et al. In vitro and in vivo characterization of herpes simplex virus clinical isolates recovered from patients infected with human immunodeficiency virus. *Antimicrob Agents Chemother* 1991;**35**:2322–8.
245. Hillenkamp J, Reinhard T, Bohringer D, et al. Topical treatment of acute adenoviral keratoconjunctivitis with 0.2% cidofovir and 1% cyclosporin: a controlled clinical pilot study. *Arch Ophthalmol* 2001;**119**:1487–91.
246. Hillenkamp J, Reinhard T, Bohringer D, et al. The effects of cidofovir 1% with and without cyclosporin A 1% as a topical treatment of acute adenoviral keratoconjunctivitis: a controlled clinical pilot study. *Ophthalmology* 2002;**109**:845–50.
247. Hillyard IW. The preclinical toxicology and safety of ribavirin. In: Smith RA, Kirkpatrick W, editors. *Ribavirin: a broad spectrum antiviral agent*. New York: Academic Press; 1980. p. 59–60.
248. Hintz M, Connor JD, Spector SA, et al. Neonatal acyclovir pharmacokinetics in patients with herpes virus infections. *Am J Med* 1982;**73**:210–14.
249. Hirsch MS, Shooley RT. Resistance to antiviral drugs: the end of innocence. *N Engl J Med* 1989;**320**:313–14.
250. Hitchcock MJ, Jaffee AS, Martin JC, et al. Cidofovir: a new agent with potent anti-herpes activity. *Antiviral Chem Chemother* 1996;**7**:115–27.
251. Ho HT, Woods KL, Bronzon JJ, et al. Intracellular metabolism of the anti-herpes agent (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl]cytosine. *Mol Pharmacol* 1992;**41**:197–202.
252. Hochster H, Dietrich D, Bozzette S, et al. Toxicity of combined ganciclovir and zidovudine for cytomegalovirus disease associated with AIDS: an AIDS Clinical Trial Group Study. *Ann Intern Med* 1990;**113**:111–17.
253. Hoffman J, Shah A, Ross L, Kapoor N. Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2001;**7**:388–94.
254. Holland G, Sidikraro Y, Kreiger A, et al. Treatment of cytomegalovirus retinopathy with ganciclovir. *Ophthalmology* 1987;**94**:815–23.
255. Hollister A, Sheridan W. The emergency use authorization of peramivir IV: a view from the manufacturer. *Clin Pharmacol Ther* 2011;**89**:172–4.
256. Holmes GP, Chapman LE, Stewart JA, et al. Guidelines for the prevention and treatment of B-virus infections in exposed persons: the B Virus Working Group. *Clin Infect Dis* 1995;**20**:421–39.
257. Holmes GP, McCormick JB, Trock SC, et al. Lassa fever in the United States: investigation of a case and new guidelines for management. *N Engl J Med* 1990;**323**:1120–3.
258. Hoofnagle JH, Di Bisceglie AM. Drug therapy: the treatment of chronic viral hepatitis. *N Engl J Med* 1997;**336**:347–56.
259. Hoopes J, Driebe E, Kelley E, et al. Triple combination antiviral drug (TCAD) composed of amantadine, oseltamivir, and ribavirin impedes the selection of drug-resistant influenza A virus. *PloSD One* 2011;**6**:e29778.
260. Horadam VW, Sharp JG, Smilack JD, et al. Pharmacokinetics of amantadine hydrochloride in subjects with normal and impaired renal function. *Ann Intern Med* 1981;**94**:454–8.
261. Hosoya M, Shigeta S, Mori S, et al. High dose intravenous ribavirin therapy for subacute sclerosing panencephalitis. *Antimicrob Agents Chemother* 2001;**45**:943–5.
262. Hosoya M, Shigeta A, Nakamura K, De Clercq E. Inhibitory effect of selected antiviral compounds on measles (SSPE) virus replication in vitro. *Antiviral Res* 1989;**12**:87–98.
263. Hruska JF, Morrow PE, Suffin SC, et al. In vivo inhibition of respiratory syncytial virus by ribavirin. *Antimicrob Agents Chemother* 1982;**21**:125–30.
264. Huggins JW. Prospects for treatment of viral hemorrhagic fevers with ribavirin, a broad spectrum antiviral drug. *Rev Infect Dis* 1989;**11**(Suppl. 4):750–61.
265. Huggins JW, Hsiang CM, Cosgriff TM, et al. Prospective, double blind, concurrent, placebo controlled clinical trial of intravenous ribavirin therapy of hemorrhagic fever with renal syndrome. *J Infect Dis* 1991;**164**:1119–27.
266. Hugonenq C, Lethal V, Chambost H, et al. Progressive multifocal leukoencephalopathy revealing AIDS in a 13 year old girl. *Arch Pediatr* 2002;**9**:32–5.
267. Hung SO, Patterson A, Rees PJ. Pharmacokinetics of oral acyclovir (Zovirax) in the eye. *Br J Ophthalmol* 1984;**68**:192–5.
268. Hurt A, Chotpitayasunondh T, Cox N, et al. Antiviral resistance during the 2009 influenza A H1N1 pandemic: public health, laboratory, and clinical perspectives. *Lancet Infect Dis* 2012;**12**:240–8.
269. Hurt A, Dent Y, Ernest J, et al. Oseltamivir-resistant influenza viruses circulating during the first year of the influenza A (H1N1) 2009 pandemic in the Asia-Pacific region, March 2009 to March 2010. *Euro Surveill* 2011;**16**:19770.
270. Hurt A, Ernest J, Deng Y, et al. Emergence and spread of oseltamivir-resistant A (H1N1) influenza viruses in Oceania, South East Asia, and South Africa. *Antiviral Res* 2009;**83**:90–3.
271. Hurt A, Holien J, Parker M, et al. Zanamivir-resistant influenza viruses with a novel neuraminidase mutation. *J Virol* 2009; **83**:10366–73.
272. Hurt A, Selleck P, Komadina N, et al. Susceptibility of highly pathogenic A(H5N1) avian influenza viruses to the neuraminidase inhibitors and adamantanes. *Antiviral Res* 2007;**73**:228–31.
273. Ikematsu H, Kawaii N. Laninamivir octanoate: a new long-acting neuraminidase inhibitor for the treatment of influenza. *Expert Rev Anti Infect Ther* 2011;**9**:851–7.
274. Ishizuka H, Yoshiva S, Yoshihara K, Okabe H. Assessment of the effects of renal impairment on the pharmacokinetic profile of laninamivir, a novel neuraminidase inhibitor, after a single inhaled dose of its Prodrug, CS-8958. *J Clin Pharmacol* 2011;**51**:243–51.
275. Ison M, Hui D, Clezy K, et al. A clinical trial of intravenous peramivir compared with oral oseltamivir for the treatment of seasonal influenza in hospitalized adults. *Antivir Ther* 2012 Oct 30. [Epub ahead of print].
276. Ison M, Szakaly P, Shapira M, et al. Efficacy and safety of oral oseltamivir for influenza prophylaxis in transplant recipients. *Antivir Ther* 2012;**17**:955–64.
277. Jabs DA, Enger C, Dunn JP, et al. Cytomegalovirus retinitis and viral resistance: ganciclovir resistance. *J Infect Dis* 1998;**177**:770–3.
278. Jackson GG, Muldoon RC, Akers LW. Serologic evidence for prevention of influenzal infection in volunteers by an anti-influenzal drug, amantadine hydrochloride. *Antimicrob Agents Chemother* 1963;**3**:703–7.
279. Jackson GG, Stanley ED. Prevention and control of influenza by chemoprophylaxis and chemotherapy: prospects from examination of recent experience. *JAMA* 1976;**235**:2739–42.
280. Jacobsen MA, Berger TG, Fikrig S, et al. Acyclovir-resistant varicella-zoster virus infection after chronic oral acyclovir therapy in patients with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1990;**112**:187–91.

281. Jacobsen MA, Drew W, Feinberg J, et al. Foscarnet therapy for ganciclovir-resistant cytomegalovirus retinitis in patients with AIDS. *J Infect Dis* 1991;**163**:1318–51.
282. Jacobsen M, Gambertoglio J, Aweeka T, et al. Foscarnet-induced hypocalcemia and effects of foscarnet on calcium metabolism. *J Clin Endocrinol Metab* 1991;**72**:1130–5.
283. Jacobsen M, van der Hurst C, Causey D, et al. In vivo additive antiretroviral effect of combined zidovudine and foscarnet therapy for human immunodeficiency virus infection (ACTG Protocol 053). *J Infect Dis* 1991;**163**:1219–22.
284. Jahrling PB, Hesse RA, Eddy GA, et al. Lassa fever infection of rhesus monkeys: pathogenesis and treatment with ribavirin. *J Infect Dis* 1980;**141**:580–9.
285. Janai HK, Stutman HR, Zaleska M, et al. Ribavirin effect on pulmonary function in young infants with respiratory syncytial virus bronchiolitis. *Pediatr Infect Dis J* 1993;**12**:214–18.
286. Japour AJ, Lertora JJ, Meehan PM, et al. A phase-1 study of the safety, pharmacokinetics, and antiviral activity of combination didanosine and ribavirin in patients with HIV-1 disease. AIDS Clinical Trials Group 231 Protocol Team. *J Acquir Immune Defic Syndr Hum Retrovirol* 1996;**13**:235–46.
287. Jayaweera D. Minimizing the dosage-limiting toxicities of foscarnet induction therapy. *Drug Saf* 1997;**16**:258–66.
288. Jefferson T, Jones M, Doshi P, et al. Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children. *Cochrane Database Syst Rev* 2012;**1**:CD008965.
289. Jung D, Dorr A. Single-dose pharmacokinetics of valganciclovir in HIV- and CMV-seropositive subjects. *J Clin Pharmacol* 1999;**39**:800–4.
290. Kalil A, Freifeld A, Lyden E, Stoner J. Valganciclovir for cytomegalovirus prevention in solid organ transplant patients: an evidence-based reassessment of safety and efficacy. *PLoS One* 2009;**4**:e5512.
291. Kalimuddin S, Sessions O, Hou Y, et al. Successful clearance of human parainfluenza virus type 2 viraemia with intravenous ribavirin and immunoglobulin in a patient with acute myocarditis. *J Clin Virol* 2013;**56**:37–40.
292. Kaplan IJ, Daum RS, Smaron M, et al. Severe measles in immunocompromised patients. *JAMA* 1992;**267**:1237–41.
293. Katlama C, Dobin E, Caumes E, et al. Foscarnet induction therapy for cytomegalovirus retinitis in AIDS: comparison of twice-daily and three-times-daily regimens. *J Acquir Immune Defic Syndr* 1992;**5**(Suppl. 1):18–24.
294. Kaufman HE. Clinical cure of herpes simplex keratitis by 5-iodo-2′-deoxyuridine. *Proc Soc Exp Biol Med* 1962;**109**:251–2.
295. Kaufman HE, Heidelberger C. Therapeutic antiviral action of 5-trifluoromethyl-2′-deoxyuridine in herpes simplex keratitis. *Science* 1964;**145**:585–6.
296. Kaufman HE, Martola E, Dohlman C. Use of 5-iodo-2′-deoxyuridine (IDU) in treatment of herpes simplex keratitis. *Arch Ophthalmol* 1962;**68**:235–9.
297. Kearns GL, Abdel-Rahman SM, James LP, et al. Single-dose pharmacokinetics of a pleconaril (VP63843) oral solution in children and adolescents. *Antimicrob Agents Chemother* 1999;**43**:634–8.
298. Kearns GL, Bradley JS, Jacobs RF, et al. Single dose pharmacokinetics of pleconaril in neonates. *Pediatr Infect Dis J* 2000;**19**:833–9.
299. Kedes D, Ganem D. Sensitivity of sarcoma-associated herpesvirus replication to antiviral drugs: implications for potential therapy. *J Clin Invest* 1997;**99**:2082–6.
300. Keeney RE, Kirk LE, Bridgen D. Acyclovir tolerance in humans. *Am J Med* 1982;**73**:176–81.
301. Kern ER, Hartline C, Harden E, et al. Enhanced inhibition of orthopoxvirus replication in vitro by alkoxyalkylesters of cidofovir and cyclic cidofovir. *Antimicrob Agents Chemother* 2002;**46**:991–5.
302. Khare GP, Sidwell RW, Witkowski JT, et al. Suppression by 1-beta-d-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazol, ICN1229) of influenza virus–induced infections in mice. *Antimicrob Agents Chemother* 1973;**3**:517–22.
303. Kilgore PE, Ksiazek TG, Rollin PE, et al. Treatment of Bolivian hemorrhagic fever with intravenous ribavirin. *Clin Infect Dis* 1997;**24**:718–22.
304. Kim CU, Lew W, Williams MA, et al. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza activity. *J Am Chem Soc* 1997;**119**:681–90.
305. Kimberlin D, Lakeman F, Arvin A, et al. Application of the polymerase chain reaction to the diagnosis and management of neonatal herpes simplex virus disease. *J Infect Dis* 1996;**174**:1162–7.
306. Kimberlin DW, Acosta EP, Sánchez PJ, et al. Pharmacokinetic and pharmacodynamic assessment of oral valganciclovir in the treatment of symptomatic congenital cytomegalovirus disease. *J Infect Dis* 2008;**197**:836–45.
307. Kimberlin DW, Chin YY, Jacobs RF, et al. Safety and efficacy of high-dose intravenous acyclovir in the management of neonatal herpes simplex virus infections. *Pediatrics* 2001;**108**:230–8.
308. Kimberlin DW, Lin CY, Sanchez P, et al. Effect of ganciclovir therapy on hearing in symptomatic congenital cytomegalovirus involving the central nervous system: a randomized controlled trial. *J Pediatr* 2003;**43**:16–25.
309. Kimberlin DW, Powell D, Gruber W, et al. Administration of oral acyclovir, suppressive therapy after neonatal herpes simplex virus disease limited to the skin, eye and mouth: results of a phase I/II trial. *Pediatr Infect Dis J* 1996;**15**:247–54.
310. Kimberlin DW, Weller S, Whitley RJ, et al. Pharmacokinetics of oral valacyclovir and acyclovir in late pregnancy. *Am J Obstet Gynecol* 1998;**179**:846–51.
311. Kirsch LS, Arevalo J, Chavez D, et al. Intravitreal cidofovir (HPMPC) treatment of cytomegalovirus retinitis in patients with acquired immune deficiency syndrome. *Ophthalmology* 1995;**102**:702–9.
312. Klein NA, Mabie WC, Shaver DC, et al. Herpes simplex virus hepatitis in pregnancy: two patients successfully treated with acyclovir. *Gastroenterology* 1991;**100**:239–44.
313. Klimov AI, Rocha E, Hayden FG, et al. Prolonged shedding of amantadine-resistant influenza A viruses by immunodeficient patients: detection by polymerase chain reaction–restriction analysis. *J Infect Dis* 1995;**172**:1352–5.
314. Knight V, Gilbert BE. Ribavirin aerosol treatment of influenza. *Infect Dis Clin North Am* 1987;**1**:441–57.
315. Knight V, Yu CP, Gilbert BE, et al. Estimating the dosage of ribavirin aerosol according to age and other variables. *J Infect Dis* 1988;**158**:443–8.
316. Kohno S, Kida H, Mizuguchi M, et al. Intravenous peramivir for treatment of influenza A and B virus infection in high-risk patients. *Antimicrob Agents Chemother* 2011;**55**:2803–12.
317. Kopp T, Geusau A, Rieger A, Stinge G. Successful treatment of an acyclovir-resistant herpes simplex type 2 infection with cidofovir in an AIDS patient. *Br J Dermatol* 2002;**147**:134–8.
318. Korba BE, Boyd MR. Penciclovir is a selective inhibitor of hepatitis B virus replication in cultured human hepatoblastoma cells. *Antimicrob Agents Chemother* 1996;**40**:1282–4.
319. Kramer TH, Gaar GG, Ray CG, et al. Hemodialysis clearance of intravenously administered ribavirin. *Antimicrob Agents Chemother* 1990;**34**:489–90.
320. Krasny HC, Liao SH, deMiranda P, et al. Influence of hemodialysis on acyclovir pharmacokinetics in patients with chronic renal failure. *Am J Med* 1982;**73**:202–4.
321. Kupperman B, Quicenco J, Flores-Aguilar M, et al. Intravitreal ganciclovir concentration after intravenous administration in AIDS patients with cytomegalovirus retinitis: implications for therapy. *J Infect Dis* 1993;**168**:1506–9.
322. Kusne S, Schwartz M, Breinig MK, et al. Herpes simplex virus hepatitis after solid organ transplantation in adults. *J Infect Dis* 1991;**163**:1001–7.
323. Lackner A, Acham A, Albomo T. Effect on hearing of ganciclovir therapy for asymptomatic congenital cytomegalovirus infection: four to 10 year follow up. *J Laryngol Otol* 2009;**123**:391–6.
324. Lakeman F, Whitley R. Diagnosis of herpes simplex encephalitis: application of polymerase chain reaction to cerebrospinal fluid from brain-biopsied patients and correlation with disease. NIAID Collaborative Antiviral Study Group. *J Infect Dis* 1995;**171**:857–63.
325. Lalezari JP, Drew WL, Glutzer E, et al. Treatment with intravenous (*S*)-1-[3-hydroxy-2-(phosphonylmethoxy)propyl]-cytosine of acyclovir-resistant mucocutaneous infection with herpes simplex virus in patient with AIDS. *J Infect Dis* 1994;**170**:570–2.
326. Lalezari JP, Holland GN, Kramer F, et al. Randomized, controlled study of the safety and efficacy of intravenous cidofovir for the treatment of relapsing cytomegalovirus retinitis in patients with

AIDS. *J Acquir Immune Defic Syndr Hum Retrovirol* 1998; **17**:339–44.
327. Lalezari JP, Stagg R, Kuppermann B, et al. Intravenous cidofovir for peripheral cytomegalovirus retinitis in patients with AIDS. A randomized, controlled trial. *Ann Intern Med* 1997;**126**:257–63.
328. Larson EW, Stephen EC, Walker JS. Therapeutic effects of small-particle aerosols of ribavirin in parainfluenza (Sendai) virus infections in mice. *Antimicrob Agents Chemother* 1976;**10**:770–2.
329. Laskin OL, Cederberg D, Mills J, et al. Ganciclovir for the treatment and suppression of serious infections caused by cytomegalovirus. *Am J Med* 1987;**83**:201–7.
330. Laskin OL, Longstreth JA, Hart CC, et al. Ribavirin disposition in high-risk patients for acquired immunodeficiency syndrome. *Clin Pharmacol Ther* 1987;**41**:546–55.
331. Laskin OL, Longstreth JA, Shelton A, et al. Effect of renal failure on the pharmacokinetics of acyclovir. *Am J Med* 1982;**73**: 197–201.
332. Lau RJ, Emery MJ, Galinsky BE, et al. Unexpected accumulation of acyclovir in breast milk with estimate of infant exposure. *Obstet Gynecol* 1987;**69**:468–71.
333. Lavelle J, Fallansbee S, Trapnell CB, et al. Effect of food on the relative bioavailability of oral ganciclovir. *J Clin Pharmacol* 1996;**36**:238–41.
334. Lawell D, Rosenthal D, Aoki F, et al. Efficacy and safety of foscarnet for recurrent orolabial herpes: a multicentre randomized double-blind study. *CMAJ* 1988;**138**:329–33.
335. Leary JJ, Wittrock R, Sarisky R, et al. Susceptibilities of herpes simplex viruses to penciclovir and acyclovir in eight cell lines. *Antimicrob Agents Chemother* 2002;**46**:762–8.
336. Legrand F, Berrebi D, Houhou N, et al. Early diagnosis of adenovirus infection and treatment with cidofovir after bone marrow transplantation in children. *Bone Marrow Transplant* 2001; **27**:621–6.
337. Leone PA, Trottier S, Miller JM. Valacyclovir for episodic treatment of genital herpes: a shorter 3-day treatment course compared with 5-day treatment. *Clin Infect Dis* 2002;**34**:958–62.
338. Lewis VA, Champlin R, Englund J, et al. Respiratory disease due to parainfluenza virus in adult bone marrow transplant recipients. *Clin Infect Dis* 1996;**23**:1033–7.
339. Li L, Avery R, Budey M, et al. Oral versus inhaled ribavirin therapy for respiratory syncytial virus infection after lung transplantation. *J Heart Lung Transplant* 2012;**31**:839–44.
340. Li Z, Li C, Ling P, et al. Ribavirin reduces mortality in enterovirus 71–infected mice by decreasing viral replication. *J Infect Dis* 2008;**197**:854–7.
341. Lin JC, Smith MC, Cheng YC, et al. Epstein-Barr virus: inhibition of replication by three new drugs. *Science* 1983;**221**:578–9.
342. Little JW, Hall HJ, Douglas RG Jr. Amantadine effect on peripheral airways abnormalities in influenza. *Ann Intern Med* 1976;**85**:177–80.
343. Littler E, Stuart AD, Chee MS. Human cytomegalovirus UL97 open reading frame encodes a protein that phosphorylates the antiviral nucleoside analogue ganciclovir. *Nature* 1992; **358**:160–2.
344. Ljungman P, Deliliers G, Platzbecker V, et al. Cidofovir for cytomegalovirus infection and disease in allogeneic stem cell transplant recipients. The Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Blood* 2001;**97**:388–92.
345. Lombardi G, Garofoli F, Villani P, et al. Oral valganciclovir treatment in newborns with symptomatic congenital cytomegalovirus infection. *Eur J Clin Microbiol Infect Dis* 2009;**28**:1465–70.
346. Long CE, Voter KZ, Barker WH, et al. Long term followup of children hospitalized with respiratory syncytial virus lower respiratory tract infection and randomly treated with ribavirin or placebo. *Pediatr Infect Dis* 1997;**16**:1023–8.
347. Louie J, Yang S, Yen C, et al. Use of intravenous peramivir for treatment of severe influenza A(H1N1)pdm09. *PLoS One* 2012;**7**:e40261.
348. Loveless M, Sacks SL, Harris RJ. Famciclovir in the management of first episode genital herpes. *Infect Dis Clin Pract* 1997;**6**:512–16.
349. Lowensohn DM, Bowden RA, Mattson A, et al. Phase I study of intravenous ribavirin treatment of respiratory syncytial virus pneumonia after marrow transplantation. *Antimicrob Agents Chemother* 1996;**40**:2555–7.
350. Lugo RA, Nahata MC. Pathogenesis and treatment of bronchiolitis. *Clin Pharm* 1993;**12**:95–116.
351. Lurain NS, Thompson KD, Holes EW, et al. Point mutations in the DNA polymerase gene of human cytomegalovirus that result in resistance to antiviral agents. *J Virol* 1992;**66**:7146–52.
352. MacGregor R, Graziani A, Weiss R, et al. Successful foscarnet therapy for cytomegalovirus retinitis in an AIDS patient undergoing hemodialysis: rationale for empiric dosing and plasma level monitoring. *J Infect Dis* 1991;**164**:785–7.
353. Maier T, Schwarting A, Mauer D, et al. Management and outcomes after multiple corneal and solid organ transplantations from a donor infected with rabies virus. *Clin Infect Dis* 2010;**50**: 1112–19.
354. Manischewitz J, Quinnan G, Lane H, et al. Synergistic effect of ganciclovir and foscarnet on cytomegalovirus replication in vitro. *Antimicrob Agents Chemother* 1990;**34**:373–5.
355. Mandell DL, Arjmand EM, Kay D, et al. Intralesional cidofovir for pediatric recurrent respiratory papillomatosis. *Arch Otolaryngol Head Neck Surg* 2004;**130**:1319–23.
356. Markham A, Faulds D. Ganciclovir: an update of its therapeutic use in cytomegalovirus infection. *Drugs* 1994;**48**:455–84.
357. Marquaidt DL, Gruber HE, Walker LL. Ribavirin inhibits mast cell mediator release. *J Pharmacol Exp Ther* 1990;**240**:145–9.
358. Marques AR, Lau DT, McKenzie R, et al. Combination therapy with famciclovir and interferon-alpha for the treatment of chronic hepatitis B. *J Infect Dis* 1998;**178**:1483–7.
359. Martin DF, Sierra-Madero J, Walmsley S, et al. Controlled trial of valganciclovir as induction therapy for cytomegalovirus retinitis. *N Engl J Med* 2002;**346**:1119–26.
360. Marx JL, Kapusta M, Patel S, et al. Use of the ganciclovir implant in the treatment of recurrent cytomegalovirus retinitis. *Arch Ophthalmol* 1996;**114**:815–20.
361. Maslo C, Girard PM, Urban T, et al. Ribavirin therapy for adenovirus pneumonia in an AIDS patient. *Am J Respir Crit Care Med* 1997;**156**:911–14.
362. Mast EE, Harmon MW, Gravenstein S, et al. Emergence and possible transmission of amantadine-resistant viruses during nursing home outbreaks of influenza A (H3N2). *Am J Epidemiol* 1991;**13**:988–97.
363. Matheson NJ, Harnden AR, Perera R, et al. Neuraminidase inhibitors for preventing and treating influenza in children. *Cochrane Database Syst Rev* 2007;**1**:CD002744.
364. Matthes-Martin S, Feuchtinger T, Shaw P, et al. European guidelines for diagnosis and treatment of adenovirus infection in leukemia and stem cell transplantation: summary of ECIL-4 (2011). *Transpl Infect Dis* 2012;**14**:555–63.
365. Mazzi R, Parisi S, Sarmati L, et al. Efficacy of cidofovir on human herpesvirus 8 viraemia and Kaposi's sarcoma progression in two patients with AIDS. *AIDS* 2001;**15**:2061–2.
366. McClellan K, Perry CM. Oseltamivir: a review of its use in influenza. *Drugs* 2001;**61**:263–83.
367. McCormick JB, King IJ, Webb PA, et al. Lassa fever: effective therapy with ribavirin. *N Engl J Med* 1986;**314**:20–6.
368. McHutchison JG, Gordon SC, Schiff ER, et al. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998;**339**:1485–92.
369. McIntosh K, Kurachek SC, Cairns LM, et al. Treatment of respiratory syncytial viral infection in an immunodeficient infant with ribavirin aerosol. *Am J Dis Child* 1984;**138**:305–8.
370. McJunkin J, Nahata M, De Los Reyes E, et al. Safety and pharmacokinetics of ribavirin for the treatment of La Crosse encephalitis. *Pediatr Infect Dis J* 2011;**30**:860–5.
371. McKinlay MA, Pevear DC, Rossman MG. Treatment of the picornavirus common cold by inhibitors of viral uncoating and attachment. *Annu Rev Microbiol* 1992;**46**:635–54.
372. Meert KL, Sarnaik AP, Gelmini MI, et al. Aerosolized ribavirin in mechanically ventilated children with respiratory syncytial virus lower respiratory tract disease: a prospective, double-blind, randomized trial. *Crit Care Med* 1994;**22**:566–72.
373. Meier P, Dautheville-Gaibal S, Ronco P, Rossert J. Cidofovir-induced end-stage renal failure. *Nephrol Dial Transplant* 2002;**17**:148–9.
374. Mendel DB, Barkhimer DB, Chen MS. Biochemical basis for increased susceptibility to cidofovir of herpes simplex viruses with

altered or deficient thymidine kinase activity. *Antimicrob Agents Chemother* 1995;**39**:2120–2.
375. Mendel DB, Sidwell RW. Influenza virus resistance to neuraminidase inhibitors. *Drug Resist Updat* 1998;**1**:184–9.
376. Merigan TC, Renlund D, Keay S, et al. A controlled trial of ganciclovir to prevent cytomegalovirus disease after heart transplantation. *N Engl J Med* 1992;**326**:1182–6.
377. Mertz GJ, Loveless MO, Levin MJ, et al. Oral famciclovir for suppression of recurrent genital herpes simplex virus infection in women: a multicenter, double-blind placebo-controlled trial. Collaborative Famciclovir Genital Herpes Research Group. *Arch Intern Med* 1997;**157**:343–9.
378. Meyer LJ, Smith MP, Sheth N, et al. Acyclovir in human breast milk. *Am J Obstet Gynecol* 1988;**158**:586–8.
379. Meyers JD, Reed EC, Shepp DH, et al. Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N Engl J Med* 1988;**318**:70–5.
380. Millet VM, Dreisbach M, Bryson YJ. Double blind controlled study of central nervous system side effects of amantadine, rimantadine, and chlorpheniramine. *Antimicrob Agents Chemother* 1982;**21**:1–4.
381. Mindel A, Faherty A, Carney O, et al. Dosage and safety of long term suppressive acyclovir therapy for recurrent genital herpes. *Lancet* 1988;**1**:926–8.
382. MIST Study Group. Randomized trial of efficacy and safety of inhaled zanamivir in treatment of influenza A and B virus infections. *Lancet* 1998;**352**:1877–81.
383. Moler FW, Steinhart CM, Ohmit SE, Stidham GI. Effectiveness of ribavirin in otherwise well infants with respiratory syncytial virus associated respiratory failure. Pediatric Critical Care Study Group. *J Pediatr* 1996;**128**:422–8.
384. Molinos-Quintana A, Perez-de Soto C, Gomez-Rosa M, et al. Intravenous ribavirin for respiratory syncytial viral infections in pediatric hematopoietic SCT recipients. *Bone Marrow Transplant* 2013;**48**:265–8.
385. Montanaki C, Farrari P, Bavazzand A. Urinary excretion of amantadine by the elderly. *Eur J Clin Pharmacol* 1975;**8**:349–56.
386. Monto AS, Gunn RA, Bardyk MG. Prevention of Russian influenza by amantadine. *JAMA* 1979;**241**:1003–7.
387. Monto AS, Robinson DP, Herlocher ML, et al. Zanamivir in the prevention of influenza among healthy adults: a randomized controlled trial. *JAMA* 1999;**282**:31–5.
388. Monto AS, Webster A, Keene O. Randomized, placebo controlled studies of inhaled zanamivir in the treatment of influenza A and B: pooled efficacy analysis. *J Antimicrob Chemother* 1999;**44**(Suppl. B):23–9.
389. Morra C, Rajicic N, Barker D, et al. A pilot study of cidofovir for progressive multifocal leukoencephalopathy in AIDS. *AIDS* 2002;**16**:1791–7.
390. Munoz F, Demmler GJ. Disseminated adenovirus disease in immunocompromised and immunocompetent children. *Clin Infect Dis* 1998;**27**:1194–200.
391. Musch DC, Martin DF, Gordon J, et al. Treatment of cytomegalovirus retinitis with a sustained-release ganciclovir implant. The Ganciclovir Implant Study Group. *N Engl J Med* 1997;**337**: 83–90.
392. Mustafa MM, Witman SD, Wirick NJ, et al. Subacute measles encephalitis in the young immunocompromised host: report of two cases diagnosed by polymerase chain reaction and treated with ribavirin and review of the literature. *Clin Infect Dis* 1993;**16**:654–60.
393. Nahata MC, Brady MT. Serum concentrations and safety of rimantadine in pediatric patients. *Eur J Clin Pharmacol* 1986;**30**: 719–22.
394. Neau D, Renaud-Rougier MB, Viallard J, et al. Intravenous cidofovir-induced iritis. *Clin Infect Dis* 1999;**28**:156–7.
395. Nedyalkova MS, Hayden FG, Webster RG, Gubareva LV. Accumulation of defective neuraminidase (NA) genes by influenza A viruses in the presence of NA inhibitors as a marker of reduced dependence on NA. *J Infect Dis* 2002;**185**:591–8.
396. Nevarro J, Quereda C, Gallego N, et al. Nephrogenic diabetes insipidus and renal tubular acidosis secondary to foscarnet therapy. *Am J Kidney Dis* 1996;**27**:431–4.
397. Neyts J, De Clercq E. Efficacy of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)-purine for the treatment of lethal vaccinia virus infections in severe combined immune deficiency (SCID) mice. *J Med Virol* 1993;**41**:242–6.
398. Neyts J, De Clercq E. Effect of 5-iodo-2′-deoxyuridine on vaccinia virus (orthopoxvirus) infections in mice. *Antimicrob Agents Chemother* 2002;**46**:2842–7.
399. Nguyen J, Hoopes J, Smee D, et al. Triple combination of oseltamivir, amantadine, and ribavirin displays synergistic activity against multiple influenza virus strains in vitro. *Antimicrob Agents Chemother* 2009;**53**:4115–26.
400. Nguyen J, Hoopes J, Le M, et al. Triple combination of amantadine, ribavirin, and oseltamivir is highly active and synergistic against drug resistant influenza virus strains in vitro. *PLoS One* 2010;**5**:e9332.
401. Nguyen J, Smee D, Barnard D, et al. Efficacy of combined therapy with amantadine, oseltamivir, and ribavirin in vivo against susceptible and amantadine-resistant influenza A viruses. *PLoS One* 2012;**7**:e31006.
402. Nguyen H, Fry A, Gubareva L. Neuraminidase inhibitor resistance in influenza viruses and laboratory testing methods. *Antivir Ther* 2012;**17**:159–73.
403. Nguyen H, Truillo A, Sheu T, et al. Analysis of influenza virus from patients clinically suspected of infection with an oseltamivir-resistant virus during the 2009 pandemic in the United States. *Antiviral Res* 2012;**93**:381–6.
404. Noormohamed F, Youle M, Higgs C, et al. Pharmacokinetics and absolute bioavailability of oral foscarnet in human immunodeficiency virus positive patients. *Antimicrob Agents Chemother* 1998;**42**:293–7.
405. Oberg B. Antiviral effects of phosphonoformate (PFA, foscarnet sodium). *Pharmacol Ther* 1983;**19**:387–415.
406. O'Donoghue JM, Ray CG, Terry DW, et al. Prevention of nosocomial influenza infection with amantadine. *Am J Epidemiol* 1973;**97**:276–82.
407. Outwater KM, Meissner HC, Peterson MB. Ribavirin administration to infants receiving mechanical ventilation. *Am J Dis Child* 1988;**142**:512–15.
408. Oxford JS. Effects of 1-beta-d-ribofuranosyl-1,2,4-triazole-3-carboxamide on influenza virus replication and polypeptide synthesis. *J Antimicrob Agents Chemother* 1973;**1**(Suppl.):71–6.
409. Oxford JS, Schild GC. In vitro inhibition of rubella virus by 1-amantanamine hydrochloride. *Arch Ges Virusforsch* 1965;**17**: 313–29.
410. Oxford JS, Schild GC. The evaluation of antiviral compounds for rubella virus using organ cultures. *Arch Ges Virusforsch* 1967;**22**:349–56.
411. Palestine AG, Polis MA, DeSmet MD, et al. A randomized controlled trial of foscarnet in the treatment of cytomegalovirus retinitis in patients with AIDS. *Ann Intern Med* 1991;**115**:665–73.
412. Palsey DA, Sternberg PJ, Davis J, et al. Decrease in the risk of bilateral acute retinal necrosis by acyclovir therapy. *Am J Ophthalmol* 1991;**112**:250–5.
413. Park K, Lee S, Choi S, et al. Successful salvage therapy with inhaled zanamivir in a patient with peramivir-resistant pandemic influenza A (H1N1) 2009 virus. *Scand J Infect Dis* 2011;**43**: 151–5.
414. Paroni R, Borghi C, Sirtori CR, et al. Pharmacokinetics of ribavirin and urinary excretion of the major metabolite 1,2,4-triazole-3-carboxamide in normal volunteers. *Int J Clin Pharmacol Ther Toxicol* 1989;**27**:302–7.
415. Parris DS, Harrington JE. Herpes simplex virus variants resistant to high concentrations of acyclovir exist in clinical isolates. *Antimicrob Agents Chemother* 1982;**22**:71–7.
416. Patriarca PA, Kater NA, Kendeal AP, et al. Safety of prolonged administration of rimantadine hydrochloride in the prophylaxis of influenza A virus infection in nursing homes. *Antimicrob Agents Chemother* 1984;**26**:101–3.
417. Pelaez A, Lyon G, Force S, et al. Efficacy of oral ribavirin in lung transplant patients with respiratory syncytial virus lower respiratory tract infection. *J Heart Lung Transplant* 2009;**28**:67–71.
418. Pelosi E, Mulamba GB, Coen DM. Penciclovir and pathogenic phenotypes of drug resistant herpes simplex virus mutants. *Antiviral Res* 1998;**37**:17–28.
419. Pescovitz MD, Rabkin J, Merion R, et al. Valganciclovir results in improved oral absorption of ganciclovir in liver transplant recipients. *Antimicrob Agents Chemother* 2000;**44**:2811–15.

420. Pevear DC, Fancher MJ, Felock PJ, et al. Conformational changes in the floor of the human rhinovirus blocks adsorption to HeLa cell receptors. *J Virol* 1989;**63**:2002–7.
421. Pevear DC, Tull TM, Seipel ME, Broarke JM. Activity of pleconaril against enteroviruses. *Antimicrob Agents Chemother* 1999; **43**:2109–15.
422. Pielek R, Chou J. Flu channel drug resistance: a tale of two sites. *Protein Cell* 2010;**1**:246–58.
423. Pimentel L, Hughes B. Amantadine toxicity presenting with complex ventricular ectopy and hallucinations. *Pediatr Emerg Care* 1991;**7**:89–92.
424. Platzbecker U, Bandt U, Thiede C, et al. Successful preemptive cidofovir treatment for CMV antigenemia after dose reduced conditioning and allogeneic blood stem cell transplantation. *Transplantation* 2001;**15**:880–5.
425. Plotkin SA, Drew WL, Felsenstein D, et al. Sensitivity of clinical isolates of human cytomegalovirus to 9-(1,3-dihydroxy-2-propoxymethyl)guanine. *J Infect Dis* 1985;**152**:833–4.
426. Poehling KA, Edwards KM. Prevention, diagnosis, and treatment of influenza: current and future options. *Curr Opin Pediatr* 2001;**13**:60–4.
427. Polis M, Spooner K, Baird B, et al. Anticytomegaloviral activity and safety of cidofovir in patients with human immunodeficiency virus infection and cytomegalovirus viruria. *Antimicrob Agents Chemother* 1995;**39**:882–6.
428. Prober CG, Kirk LE, Keeney RE. Acyclovir therapy for chickenpox in immunosuppressed children: a collaborative study. *J Pediatr* 1982;**101**:622–5.
429. Prusoff WH. Synthesis and biological activities of iododeoxyuridine, an analogue of thymidine. *Biochim Biophys Acta* 1959; **32**:295–6.
430. Randolph AG, Wang E. Ribavirin therapy for respiratory syncytial virus lower respiratory tract infection: a systematic overview. *Arch Pediatr Adolesc Med* 1996;**150**:942–7.
431. Rashed A, Azadek B, Abu RH. Acyclovir induced acute tubulointerstitial nephritis. *Nephron* 1990;**56**:436–8.
432. Ray CG, Isenogle TB, Minnich LL, et al. The use of intravenous ribavirin to treat influenza-associated acute myocarditis. *J Infect Dis* 1989;**159**:829–36.
433. Razonable RR, Aksamit A, Wright A, Wilson J. Cidofovir treatment of progressive multifocal leukoencephalopathy in a patient receiving highly active antiretroviral therapy. *Mayo Clin Proc* 2001;**76**:1171–5.
434. Reed EC, Bowden R, Dandliker PS. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann Intern Med* 1988;**109**:783–8.
435. Reed E, Wolford S, Koppecky K, et al. Ganciclovir for the treatment of cytomegalovirus gastroenteritis in bone marrow transplant patients: A randomized, placebo-controlled trial. *Ann Intern Med* 1990;**112**:505–10.
436. Reichard O, Norkrans G, Fryden A, et al. Randomized, double-blind placebo controlled trial of interferon alpha-2b with and without ribavirin for chronic hepatitis C. *Lancet* 1998;**351**:83–7.
437. Reichman RC, Badger GJ, Mertz GJ, et al. Treatment of recurrent genital herpes simplex infections with oral acyclovir: a controlled trial. *JAMA* 1984;**251**:2103–7.
438. Reitano M, Tyring S, Lang W, et al. Valacyclovir for the suppression of recurrent genital herpes simplex virus infection: a large scale dose range finding study. *J Infect Dis* 1998;**178**:603–10.
439. Resnick L, Herbst JS, Abashi DV, et al. Regression of oral hairy leukoplakia after orally administered acyclovir therapy. *JAMA* 1988;**259**:384–8.
440. Reusser P, Gambertoglio J, Lilleby K, et al. Phase I/II trial of foscarnet for prevention of cytomegalovirus infection in autologous and allogeneic marrow transplant recipients. *J Infect Dis* 1992;**166**:473–9.
441. Ribaud P, Scieux D, Freymouth F, et al. Successful treatment of adenovirus disease with intravenous cidofovir in an unrelated stem cell transplant recipient. *Clin Infect Dis* 1999;**28**:690–1.
442. Ribeiro de Valle LA, Resposo de Melo P, de Salles Gomez LF, et al. Methisazone in prevention of variola minor among contacts. *Lancet* 1965;**2**:976–8.
443. Rodriguez WJ, Bui RH, Connor JD, et al. Environmental exposure of primary care personnel to ribavirin aerosol when supervising treatment of infants with respiratory syncytial virus infections. *Antimicrob Agents Chemother* 1987;**31**:1143–6.
444. Rombart B, Vrjsen R, Boeye A. Comparison of arildone and 3-methylquercatin as stabilizers of poliovirus. *Antiviral Res* 1985;**1**(Suppl.):67–73.
445. Romero JR, Gross T, Abromowitch M, Jung L. Pleconaril treatment of vaccine-acquired poliovirus [abstract]. *Pediatr Res* 1999;**45**:173.
446. Rompalo AM, Mertz GJ, Davis LG, et al. Oral acyclovir for treatment of first episode herpes simplex virus proctitis. *JAMA* 1988;**259**:2879–81.
447. Rooney JF, Felser JM, Ostrove JM, et al. Acquisition of genital herpes from an asymptomatic sexual partner. *N Engl J Med* 1986;**314**:1561–4.
448. Rosh HR, Benner KG, Flora KD, et al. Development of ganciclovir resistance during treatment of primary cytomegalovirus infection after liver transplantation. *Transplantation* 1996; **63**:476–8.
449. Rosner IK, Welliver RC, Edelson PJ, et al. Effect of ribavirin therapy on respiratory syncytial virus specific IgE and IgA responses after infection. *J Infect Dis* 1987;**155**:1043–7.
450. Rotbart HA. Pleconaril treatment of enterovirus and rhinovirus infections. *Infect Med* 2000;**17**:488–94.
451. Sabin AB. Amantadine hydrochloride: analysis of data related to its proposed use for prevention of A2 influenza virus disease in human beings. *JAMA* 1967;**200**:943–50.
452. Sacks SL, Aoki FY, Diaz-Mitoma F, et al. Patient-initiated, twice-daily oral famciclovir for early recurrent genital herpes: a randomized double blind multicenter trial. Canadian Famciclovir Study Group. *JAMA* 1996;**276**:44–9.
453. Safrin S, Berger T, Gilson I, et al. Foscarnet therapy in five patients with AIDS and acyclovir-resistant varicella-zoster virus infection. *Ann Intern Med* 1991;**115**:19–21.
454. Safrin S, Cherrington J, Jaffe HS. Clinical uses of cidofovir. *Rev Med Virol* 1997;**7**:145–56.
455. Safrin S, Kemmerly S, Plotkin B, et al. Foscarnet resistant herpes simplex virus infection in patients with AIDS. *J Infect Dis* 1994;**169**:193–6.
456. Salmaggi A, Maccagnano E, Castagna A, et al. Reversal of CSF positivity for JC virus genome by cidofovir in a patient with systemic lupus erythematosus and progressive multifocal leukoencephalopathy. *Neurol Sci* 2001;**22**:17–20.
457. Salzman R, Jurewicz R, Boon R. Safety of famciclovir in patients with herpes zoster and genital herpes. *Antimicrob Agents Chemother* 1994;**38**:2454–7.
458. Sarasini A, Baldanti F, Furione M, et al. Double resistance to ganciclovir and foscarnet of four human cytomegalovirus strains recovered from AIDS patients. *J Med Virol* 1995;**47**:237–44.
459. Sartori M, Pratt CM, Young JB. Malignant cardiac arrhythmia induced by amantadine poisoning. *Am J Med* 1984;**77**:388–91.
460. Sawyer MH, Saez-Llorenz Y, Luiz-Aviles C, et al. Oral pleconaril reduces the duration and severity of enteroviral meningitis in children [abstract]. *Pediatr Res* 1999;**45**:173.
461. Sawyer MH, Webb DE, Balow JE, et al. Acyclovir induced renal failure: clinical course and histology. *Am J Med* 1988;**84**: 1067–71.
462. Schacker T, Hu HL, Koelle DM, et al. Famciclovir for the suppression of symptomatic and asymptomatic herpes simplex virus reactivation in HIV infected persons. *Ann Intern Med* 1998; **128**:21–8.
463. Schaeffer HJ, Beauchamp L, DeMiranda P, et al. 9-(2-hydroxyethoxymethyl)guanine activity against viruses of the herpes group. *Nature* 1978;**272**:583–5.
464. Scheibel LM, Durbin RK, Stollar V. Sindbis virus mutants resistant to mycophenolic acid and ribavirin. *Virology* 1987;**158**:1–7.
465. Schiff GM, Sherwood JR. Clinical activity of pleconaril in an experimentally induced coxsackievirus A21 respiratory infection. *J Infect Dis* 2000;**181**:20–6.
466. Schmidt G, Horak D, Niland J, et al. A randomized controlled trial of prophylactic ganciclovir for cytomegalovirus pulmonary infection in recipients of allogeneic bone marrow transplants. The City of Hope–Stanford–Syntex CMV Study Group. *N Engl J Med* 1991;**324**:1005–11.
467. Schnabel FM Jr. The antiviral activity of 9-beta-d-arabinofuranosyladenine (ara-A). *Chemotherapy* 1968;**13**:321–38.

468. Schooley RT, Carey RW, Miller G, et al. Chronic Epstein-Barr virus infection associated with fever and interstitial pneumonitis: clinical and serologic features and response to antiviral chemotherapy. *Ann Intern Med* 1986;**104**:636–43.
469. Schwab RS, England AC Jr, Poskanzer DC, et al. Amantadine in the treatment of Parkinson's disease. *JAMA* 1969;**209**:1168–70.
470. Scott LL. Perinatal herpes: Current status and obstetric management strategies. *Pediatr Infect Dis J* 1995;**14**:827–32.
471. Scott LL. Prevention of perinatal herpes: prophylactic antiviral therapy. *Clin Obstet Gynecol* 1999;**42**:134–48.
472. Scott LL, Sanchez PJ, Jackson GL, et al. Acyclovir suppression to prevent cesarean delivery after first episode genital herpes. *Obstet Gynecol* 1996;**87**:69–73.
473. Segarra-Newnham M, Vodolo K. Use of cidofovir in progressive multifocal leukoencephalopathy. *Ann Pharmacol* 2001;**35**:741–4.
474. Shah J, Chemaly R. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011;**117**:2755–63.
475. Shaw T, Mok SS, Locarnini SA. Inhibition of hepatitis B virus DNA polymerase by enantiomers of penciclovir triphosphate and metabolic basis for selective inhibition of HBV replication by penciclovir. *Hepatology* 1996;**24**:996–1002.
476. Shelton M, Lovern M, Ng-Cashin J, et al. Zanamivir pharmacokinetics and pulmonary penetration into epithelial lining fluid following intravenous or oral inhaled administration to healthy adult subjects. *Antimicrob Agents Chemother* 2011;**55**:5178–84.
477. Shults RA, Baron S, Decker J, et al. Health care worker exposure to aerosolized ribavirin: biological and air monitoring. *J Occup Environ Med* 1996;**38**:257–63.
478. Sidwell RW, Huffmann JH, Khare GP, et al. Broad spectrum antiviral activity of virazole: 1-beta-d-ribofuranosyl-1,2,4-triazole-3-carboxamide. *Science* 1972;**17**:705–6.
479. Simeonova L, GegovaG, Galabov A. Prophylactic and therapeutic combination effects of rimantadine and oseltamivir against influenza virus A (H3N2) infection in mice. *Antiviral Res* 2012; **95**:172–81.
480. Singh N, Gayowski T, Wannstedt CF, et al. Pretransplant famciclovir as prophylaxis for hepatitis B virus recurrence after liver transplantation. *Transplantation* 1997;**63**:1415–19.
481. Sleeman K, Sheu T, Moore Z, et al. Influenza B viruses with mutation in the neuraminidase active site, North Carolina, USA, 2010–2011. *Emerg Infect Dis* 2011;**17**:2043–6.
482. Smee DF, Bailey KW, Sidwell RW. Treatment of lethal vaccinia virus respiratory infections in mice with cidofovir. *Antiviral Chem Chemother* 2001;**12**:71–6.
483. Smee DF, Bailey KW, Wong MH, Sidwell RW. Effects of cidofovir on the pathogenesis of a lethal vaccinia virus respiratory infection in mice. *Antiviral Res* 2001;**52**:55–62.
484. Smee DF, Martin JC, Verheyden TP, et al. Antiherpesvirus activity of the acyclic nucleoside 9-(1,3-dihydroxy-2-propoxymethyl) guanine. *Antimicrob Agents Chemother* 1983;**23**:676–82.
485. Smee DF, Matthews TR. Metabolism of ribavirin in respiratory syncytial virus infected and uninfected cells. *Antimicrob Agents Chemother* 1986;**30**:117–21.
486. Smee DF, Sidwell RW, Kefauver D, et al. Characterization of wild type and cidofovir resistant strains of camelpox, cowpox, monkeypox and vaccinia viruses. *Antimicrob Agents Chemother* 2002; **46**:1329–35.
487. Smith CB, Charette RP, Fox JP, et al. Double blind evaluation of ribavirin in naturally occurring influenza. In: Smith RA, Kilpatrick W, editors. *Ribavirin: a broad-spectrum antiviral agent*. New York: Academic Press; 1980. p. 147–64.
488. Smith DW, Frankel LR, Mathers LH, et al. A controlled trial of aerosolized ribavirin in infants receiving mechanical ventilation for severe respiratory syncytial virus infection. *N Engl J Med* 1991;**325**:24–9.
489. Smith IL, Cherrington JM, Jules RE, et al. High level resistance of cytomegalovirus to ganciclovir is associated with alterations in both the UL 97 and DNA polymerase genes. *J Infect Dis* 1997;**176**:69–77.
490. Smith JP. Treatment of chronic hepatitis C with amantadine. *Dig Dis Sci* 1997;**42**:1681–7.
491. Snoeck R, Andrei G, De Clercq E. Cidofovir in the treatment of HPV-associated lesions. *Verh K Acad Geneeskd Belg* 2001; **63**:93–120.
492. Snoeck R, Gerand M, Sadzot-Delvaux S, et al. Meningoradiculoneuritis due to acyclovir-resistant varicella-zoster virus in a patient with AIDS. *J Infect Dis* 1993;**168**:1330–1.
493. Soike KF, Huang JL, Zahang J-Y, et al. Evaluation of infrequent dosing regimens with (5)-1-[3-hydroxy-2-(phosphonylmethoxy) propyl]cytosine (5-HPMPC) in simian varicella infection in monkeys. *Antiviral Res* 1991;**16**:17–28.
494. Sommadassi JP, Bevan R, Ling T, et al. Clinical pharmacokinetics of ganciclovir in patients with normal and impaired renal function. *Rev Infect Dis* 1988;**10**(Suppl. 3):507–14.
495. Soo SK, Regan A, Cihlar T, et al. Cytomegalovirus ventriculoencephalitis in a bone marrow transplant recipient receiving antiviral maintenance: clinical and molecular evidence of drug resistance. *Clin Infect Dis* 2001;**33**:e105–8.
496. Soo W. Adverse effects of rimantadine: summary from clinical trials. *J Respir Dis* 1989;**10**(Suppl.):526–31.
497. Sorbello A, Jones S, Carter W, et al. Emergency use authorization for intravenous peramivir: evaluation of safety in the treatment of hospitalized patients infected with 2009 H1N1 influenza A virus. *Clin Infect Dis* 2012;**55**:1–7.
498. Soul-Lawton J, Seaber E, Oh N, et al. Absolute bioavailability and metabolic disposition of valacyclovir, the l-valyl ester of acyclovir, following oral administration to humans. *Antimicrob Agents Chemother* 1995;**39**:2759–64.
499. Soung LS, Ing TS, Daugirdas JT, et al. Amantadine hydrochloride pharmacokinetics in hemodialysis patients. *Ann Intern Med* 1980;**93**:46–9.
500. Spector SA, Busch D, Follanskee S, et al. Pharmacokinetic, safety, and antiviral profiles of oral ganciclovir in persons infected with human immunodeficiency virus: a phase I/II study. *J Infect Dis* 1995;**171**:1431–7.
501. Spector S, Hsia K, Wolf D, et al. Molecular detection of human cytomegalovirus and determination of genotypic ganciclovir resistance in clinical specimens. *Clin Infect Dis* 1995;**21**(Suppl. 2):170–3.
502. Spector SA, McKinley G, Lalezari J, et al. Oral ganciclovir for the prevention of cytomegalovirus disease in persons with AIDS. Roche Cooperative Oral Ganciclovir Study Group. *N Engl J Med* 1996;**334**:1491–7.
503. Spector SA, Weingeist T, Pollard RB, et al. A randomized, controlled study of intravenous ganciclovir therapy for cytomegalovirus peripheral retinitis in patients with AIDS. *J Infect Dis* 1993;**168**:557–63.
504. Spruance SL, Rea TL, Thorning C, et al. Penciclovir cream for the treatment of herpes simplex labialis: a randomized multicenter double blind placebo controlled trial. Topical Penciclovir Collaborative Study Group. *JAMA* 1997;**277**:1374–9.
505. Spruance SL, Tyring SK, Degregorio B, et al. A large scale, placebo controlled, dose ranging trial of peroral valacyclovir for episodic treatment of recurrent herpes genitalis. Valacyclovir Study Group. *Arch Intern Med* 1996;**156**:1729–35.
506. Stanat SC, Reardon JE, Erice A, et al. Ganciclovir-resistant cytomegalovirus clinical isolates: mode of resistance to ganciclovir. *Antimicrob Agents Chemother* 1991;**35**:2191–7.
507. Stein DS, Creticos CM, Jackson GG, et al. Oral ribavirin treatment of influenza A and B. *Antimicrob Agents Chemother* 1987;**31**:1285–7.
508. Stephenson JA, Artenstein MS, Parkman PD, et al. Effect of amantadine hydrochloride on rubella virus infection in the rhesus monkey. *Antimicrob Agents Chemother* 1965;**5**:548–52.
509. Stephenson I, Democratis J, Lackenby A, et al. Neuraminidase inhibitor resistance after oseltamivir treatment of acute influenza A and B in children. *Clin Infect Dis* 2009;**48**:389–96.
510. Stragier I, Snoeck R, De Clercq E, et al. Local treatment of HPV-induced skin lesions by cidofovir. *J Med Virol* 2002;**67**:241–5.
511. Streeter DG, Witkowski JT, Khare T, et al. Mechanism of action of 1-d-ribofuranosyl-1,2,3-triazole-3-carboxamide (Virazole), a new broad spectrum antiviral agent. *Proc Natl Acad Sci U S A* 1973;**70**:1174–8.
512. Stronati M, Lombardi G, Garofoli F, et al. Pharmacokinetics, pharmacodynamics and clinical use of valganciclovir in newborns with symptomatic congenital cytomegalovirus infection. *Curr Drug Metab* 2013;**14**:208–15.
513. Studies of Ocular Complications of AIDS Research Group in collaboration with the AIDS Clinical Trial Group. Mortality in

patients with the acquired immunodeficiency syndrome treated with either foscarnet or ganciclovir for cytomegalovirus retinitis. *N Engl J Med* 1992;**326**:213–20.
514. Studies of Ocular Complications of AIDS Research Group in collaboration with the AIDS Clinical Trials Group. Combination foscarnet and ganciclovir therapy versus monotherapy for the treatment of relapsed cytomegalovirus retinitis in patients with AIDS: the Cytomegalovirus Retreatment Trial. *Arch Ophthalmol* 1996;**114**:23–33.
515. Studies of Ocular Complications of AIDS Research Group. Parenteral cidofovir for cytomegalovirus retinitis in patients with AIDS: the HPMPC Peripheral Cytomegalovirus Retinitis Trial. A randomized controlled trial. *Ann Intern Med* 1997; **126**:264–74.
516. Sugaya M, Ohashi Y. Long-acting neuraminidase inhibitor laninamivir octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus infection. *Antimicrob Agents Chemother* 2010;**54**:2575–82.
517. Sugaya N, Kohno S, Ishibashi T, et al. Efficacy, safety, and pharmacokinetics of intravenous peramivir in children with 2009 pandemic H1N1 influenza A virus infection. *Antimicrob Agents Chemother* 2012;**56**:369–77.
518. Sullender WM, Arvin AM, Diaz PS. Pharmacokinetics of acyclovir suspension in infants and children. *Antimicrob Agents Chemother* 1987;**31**:1722–6.
519. Sullivan JL, Byron KS, Brewster FE, et al. Treatment of life threatening Epstein-Barr virus infection with acyclovir. *Am J Med* 1982;**73**:262–6.
520. Superti F, Seganti L, Pana A, Orsi N. Effect of amantadine on rhabdovirus infection. *Drugs Exp Clin Res* 1985;**11**:69–74.
521. Suzuki E, Ichihara K. The course of fever following influenza virus infection in children treated with oseltamivir. *J Med Virol* 2008;**80**:1065–71.
522. Sylvester RK, Ogden WB, Draxler CA, et al. Vesicular eruption: a local complication of concentrated acyclovir infusions. *JAMA* 1986;**255**:365–86.
523. Taber LH, Knight V, Gilbert BE, et al. Virazole aerosol treatment of bronchiolitis associated with respiratory tract infection in infants. *Pediatrics* 1983;**72**:613–18.
524. Tai CY, Escarpe PA, Sidwell RW, et al. Characterization of human influenza virus variants selected in vitro in the presence of the neuraminidase inhibitor 654071. *Antimicrob Agents Chemother* 1998;**42**:3224–41.
525. Takahashi TM, Hosoya K, Kimura K, et al. The cooperative effect of interferon and ribavirin on subacute sclerosing panencephalitis (SSPE) virus infections, in vitro and in vivo. *Antiviral Res* 1998;**37**:29–35.
526. Talarico CL, Phelps W, Biron K. Analysis of the thymidine kinase genes from acyclovir-resistant mutants of varicella-zoster virus isolated from patients with AIDS. *J Virol* 1993;**67**:1024–33.
527. Tatarowicz WA, Lurain NS, Thompson KD. A ganciclovir-resistant clinical isolate of human cytomegalovirus exhibiting cross-resistance to other DNA polymerase inhibitors. *J Infect Dis* 1992;**166**:904–7.
528. Taylor DL, Jeffries DJ, Taylor-Robinson D. The susceptibility of adenovirus infection to the anti-cytomegalovirus drug, ganciclovir (DHPG). *FEMS Microbiol Lett* 1988;**49**:337–41.
529. Thorlund K, Awad T, Boivin G, Thabane L. Systemic review of influenza resistance to the neuraminidase inhibitors. *BMC Infect Dis* 2011;**11**:134.
530. Titus BJ, Perez AF, Arcata BI. Water intoxication after nebulized ribavirin. *Lancet* 1995;**345**:1116.
531. Tokumoto JI, Hollander, H. Cytomegalovirus polyradiculopathy caused by a ganciclovir-resistant strain. *Clin Infect Dis* 1993;**17**:854–6.
532. Trang JM, Kidd L, Gruber W, et al; NIAID Collaborative Antiviral Study Group. Linear single-dose pharmacokinetics of ganciclovir in newborns with congenital cytomegalovirus infection. *Clin Pharmacol Ther* 1993;**53**:15–21.
533. Treanor JJ, Hayden FG, Vrooman PS, et al. Efficacy and safety of the oral neuraminidase inhibitor oseltamivir in treating acute influenza: a randomized controlled trial. *JAMA* 2000;**283**:1016–24.
534. Tynell E, Aurelius E, Brandell A, et al. Acyclovir and prednisolone treatment of acute infectious mononucleosis: a multicenter, double-blind placebo-controlled study. *J Infect Dis* 1996;**174**:324–31.
535. Tyring S, Barbarash RA, Nahlik JE, et al. Famciclovir for the treatment of acute herpes zoster: effects on acute disease and postherpetic neuralgia. *Ann Intern Med* 1995;**123**:89–96.
536. van Rooyen CE, Casey J, Lee SH, et al. Vaccinia gangrenosa and 1-methylisatin-3-thiosemicarbazone (methisazone). *Can Med Assoc J* 1967;**97**:160–5.
537. van Voris LP, Betts RF, Hayden FG, et al. Successful treatment of naturally occurring influenza A/USSR/77H1N1. *JAMA* 1981;**245**:1128–31.
538. Vasquez EM, Sanchez T, Pollak J, et al. High dose oral acyclovir prophylaxis for primary cytomegalovirus infection in seronegative renal allograft recipients. *Transplantation* 1993;**55**:448–50.
539. von Itzstein M, Wu WY, Kok GB, et al. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature* 1993;**36**:418–23.
540. Wade JC, Meyers JD. Neurologic symptoms associated with parenteral acyclovir treatment after marrow transplantation. *Ann Intern Med* 1983;**98**:921–5.
541. Wade JC, Newton B, McLaren C, et al. Intravenous acyclovir to treat mucocutaneous herpes simplex virus infection after marrow transplantation: a double blind trial. *Ann Intern Med* 1982;**96**:265–9.
542. Wagstaff AJ, Faulds D, Goa KC. Acyclovir: a reappraisal of its antiviral activity, pharmacokinetic properties, and therapeutic efficacy. *Drugs* 1994;**47**:153–205.
543. Wald A, Carrell D, Remington M, et al. Two day regimen of acyclovir for treatment of recurrent genital herpes simplex virus type 2 infection. *Clin Infect Dis* 2002;**34**:944–8.
544. Walker JB, Hussey EK, Treanor JJ. Effects of the neuraminidase inhibitor zanamivir on otologic manifestations of experimental human influenza. *J Infect Dis* 1997;**176**:1417–22.
545. Wallace MR, Bowler WA, Murray NB, et al. Treatment of adult varicella with oral acyclovir: a randomized, placebo controlled trial. *Ann Intern Med* 1992;**117**:358–63.
546. Walsh JE, Abinun M, Peiris JS, et al. Cytomegalovirus infection in severe combined immune deficiency: eradication with foscarnet. *Pediatr Infect Dis J* 1995;**14**:911–12.
547. Wang LH, Schultz M, Weller S, et al. Pharmacokinetics and safety of multiple dose valacyclovir in geriatric volunteers with and without concomitant diuretic therapy. *Antimicrob Agents Chemother* 1996;**40**:80–5.
548. Wang K, Shun-Shin M, Gill P, et al. Neuraminidase inhibitors for preventing and treating influenza in children (published trials only). *Cochrane Database Syst Rev* 2012;**4**:CD002744.
549. Wang K, Shun-Shin M, Gill P, et al. Neuraminidase inhibitors for preventing and treating influenza in children. *Cochrane Database Syst Rev* 2012;**1**:CD002744.
550. Weinberg A, Bate BJ, Masters H, et al. In vitro activities of penciclovir and acyclovir against herpes simplex virus types 1 and 2. *Antimicrob Agents Chemother* 1992;**36**:2037–8.
551. Weller S, Blum MR, Doucette M, et al. Pharmacokinetics of the acyclovir pro-drug valacyclovir after escalating single and multiple dose administration to normal volunteers. *Clin Pharmacol Ther* 1993;**54**:595–605.
552. Wendel HA, Snyder MT, Pell S. Trial of amantadine in epidemic influenza. *Clin Pharmacol Ther* 1966;**7**:38–43.
553. Wendt CH, Weisdorf DJ, Jordan MC, et al. Parainfluenza virus respiratory infection after bone marrow transplantation. *N Engl J Med* 1992;**326**:921–6.
554. Wheeler JG, Wofford J, Turner RB. Historical cohort evaluation of ribavirin efficacy in respiratory syncytial virus infection. *Pediatr Infect Dis J* 1993;**12**:209–13.
555. Whimbey E, Champlin RE, Englund JA, et al. Combination therapy with aerosolized ribavirin and intravenous immunoglobulin for respiratory syncytial virus disease in adult bone marrow transplant recipients. *Bone Marrow Transplant* 1995;**16**:393–9.
556. Whitley R, Alford C, Hirsch M, et al. Vidarabine versus acyclovir therapy in herpes simplex encephalitis. *N Engl J Med* 1986;**314**:144–9.
557. Whitley RJ, Gnann JJ. Acyclovir: a decade later. *N Engl J Med* 1992;**327**:782–9.
558. Whitley RJ, Hayden FG, Relsenger KS, et al. Oral oseltamivir treatment of influenza in children. *Pediatr Infect Dis J* 2001;**20**:127–33.
559. Wildenbeest J, van den Broek P, Benschop K, et al. Pleconaril revisited: clinical course of chronic enteroviral

meningoencephalitis after treatment correlates with in vitro susceptibility. *Antivir Ther* 2012;**17**:459–66.
560. Willoughby R, Tieves L, Hoffman G, et al. Survival after treatment of rabies with induction of coma. *N Engl J Med* 2005;**352**:2508–14.
561. Wills RJ, Belshe R, Tomlinsin D, et al. Pharmacokinetics of rimantadine hydrochloride in patients with chronic liver disease. *Clin Pharmacol Ther* 1987;**42**:449–54.
562. Wingfield WL, Pollack D, Grunert RR. Therapeutic efficacy of amantadine HCl and rimantadine in naturally occurring influenza A2 respiratory illness in man. *N Engl J Med* 1969;**281**:579–84.
563. Winston D, Ho W, Baroni K. Ganciclovir prophylaxis of cytomegalovirus infection and disease in allogeneic bone marrow transplant recipients: results of a placebo controlled, double blind trial. *Ann Intern Med* 1993;**118**:179–84.
564. Winston D, Wirin D, Shaked A, et al. Randomized comparison of ganciclovir and high-dose acyclovir for long term cytomegalovirus prophylaxis in liver transplant recipients. *Lancet* 1995; **346**:69–74.
565. Witzke O, Hauser I, Bartels M, et al. Valganciclovir prophylaxis versus preemptive therapy in cytomegalovirus-positive renal allograft recipients: 1 year results of a randomized clinical trial. *Transplantation* 2012;**93**:61–8.
566. Wolf DG, Yaniv I, Honigman A, et al. Early emergence of ganciclovir-resistant human cytomegalovirus strains in children with primary combined immunodeficiency. *J Infect Dis* 1998; **178**:535–8.
567. Woodson B, Joklik WK. The inhibition of vaccinia virus multiplication by isatin-3-thiosemicarbazone. *Proc Natl Acad Sci U S A* 1965;**54**:946–53.
568. Wray SK, Gilbert PE, Knight V. Effect of ribavirin triphosphate on primer generation and elongation during influenza virus transcription in vitro. *Antiviral Res* 1985;**5**:39–48.
569. Wu MJ, Ing TS, Soung LS, et al. Amantadine hydrochloride pharmacokinetics in patients with impaired renal function. *Clin Nephrol* 1982;**17**:19–23.
570. Yilmaz K, Cliftdogan D, Vardar G. Effect on hearing of oral valganciclovir for asymptomatic congenital cytomegalovirus infection. *J Trop Pediatr* 2011;**57**:132–4.
571. Youle M, Chanas A, Gazzard B. Treatment of acquired immune deficiency syndrome (AIDS)–related pneumonia with foscarnet: a double blind placebo controlled study. *J Infect Dis* 1990; **20**:41–50.
572. Young E, Chafizadeh E, Oliveira V, Genta R. Disseminated herpesvirus infection during pregnancy. *Clin Infect Dis* 1996;**22**:51–8.
573. Younsin SW, Betts RF, Roth FK, et al. Reduction in fever and symptoms in young adults with influenza A/Brazil/78H1N1 infection after treatment with aspirin or amantadine. *Antimicrob Agents Chemother* 1983;**23**:577–82.
574. Yu Y, Garg S, Yu P, et al. Peramivir use for treatment of hospitalized patients with influenza A(H1N1)pdm09 under emergency use authorization, October 2009–June 2010. *Clin Infect Dis* 2012; **55**:8–15.
575. Zabawski EJ. A review of topical and intralesional cidofovir. *Dermatol Online J* 2000;**6**:3–10.
576. Zambon M, Hayden FG. Position statement: global neuraminidase inhibitor susceptibility network. *Antiviral Res* 2001; **49**:147–56.
577. Zedtwitz-Liebenstein K, Prester E, Graninger W. Acute renal failure in a lung transplant patient after therapy with cidofovir. *Transplant Int* 2001;**14**:445–6.
578. Zhou XJ, Gruber W, Demmler G, et al. Population pharmacokinetics of ganciclovir in newborns with congenital cytomegalovirus infections. NIAID Collaborative Antiviral Study Group. *Antimicrob Agents Chemother* 1996;**40**:2202–5.
579. Ziegler T, Hamphill MI, Ziegler M-L, et al. Low incidence of rimantadine resistance in field isolates of influenza A virus. *J Infect Dis* 1999;**180**:935–9.
580. Zlydnikov DM, Kubar OI, Kovaleva RP. Study of rimantadine in the USSR: a review of the literature. *Rev Infect Dis* 1981;**3**:408–21.

CHAPTER 239

Antifungal Agents

Andreas H. Groll • Thomas J. Walsh

Invasive fungal infections are important causes of morbidity and mortality in children with severe underlying illnesses. These infections remain difficult to diagnose and can be rapidly fatal. As a consequence, early and appropriate antifungal chemotherapy is pivotal for successful management and survival. For more than two decades, options for antifungal chemotherapy were limited to amphotericin B deoxycholate (AmB-D) with or without the addition of flucytosine. The 1990s, however, witnessed major progress through the introduction of fluconazole and itraconazole and the development of less toxic formulations of amphotericin B. More recent advances include the advent of novel, potent, and broad-spectrum antifungal triazoles and the clinical development of echinocandins, an entirely new class of antifungal agents that target the fungal cell wall (Fig. 239-1). This chapter is devoted to the clinical pharmacology of systemic antifungal agents; emphasis is placed on pharmacokinetics, dosing, and safety in pediatric age groups.

AGENTS FOR TREATMENT OF INVASIVE MYCOSES

Polyene Antibiotics

Amphotericin B Deoxycholate

First isolated in the 1950s as a natural product of a soil actinomycete,[192] amphotericin B belongs to a family of approximately 200 polyene macrolide antibiotics and consists of seven conjugated double bonds, an internal ester, a free carboxyl group, and a glycoside side chain with a primary amino group (Fig. 239-2). The compound is amphoteric (capable of reacting as either an acid or a base), not orally or intramuscularly absorbed, and virtually insoluble in water. For parenteral use, amphotericin B has been solubilized with deoxycholate as a micellar suspension, and this formulation has been available for more than 50 years.[48]

Mechanism of Action. Amphotericin B, similar to other polyenes, acts primarily by binding to ergosterol, the principal sterol in the cell membrane of most fungi. This interaction with ergosterol results in the formation of ion channels, loss of protons and monovalent cations, depolarization, and concentration-dependent cell death. Although with less avidity, the compound also binds to cholesterol, the main sterol of mammalian cell membranes; this action accounts for most of the toxicities associated with this drug. A second mechanism of action of amphotericin B may involve oxidative damage of the cell through a cascade of oxidative reactions linked to its own oxidation, with formation of free radicals or an increase in membrane permeability. In addition to its antifungal activity, amphotericin B and its lipid formulations differentially augment innate host defense mechanisms against fungal pathogens.[75,142,208,510,511]

Antifungal Activity. Amphotericin B has a broad spectrum of antifungal activity that includes most fungi pathogenic in humans. True microbiologic resistance to antifungal polyenes has been associated with qualitative or quantitative differences in the sterol composition of the cell membrane, but this resistance also may be related to increased catalase activity with decreased susceptibility to oxidative damage.[208] Resistance to amphotericin B remains uncommon in *Candida* spp. other than *Candida lusitaniae*, *Candida guilliermondii*, and *Candida lipoyltica*. Some isolates of *Candida glabrata*, *Candida parapsilosis*, and *Candida tropicalis* also may be resistant to amphotericin B.[560,579,612] *Aspergillus terreus*[265,528] and some of the emerging pathogens such as *Trichosporon asahii*,[12,585,586] *Fusarium* spp.,[73,456] *Scedosporium apiospermum*,[541,588] *Scedosporium prolificans*,[57,338] and certain dematiaceous fungi[203] may be completely resistant to safely achievable serum concentrations of amphotericin B. Acquisition of secondary resistance is an uncommon occurrence and has not been a clinical problem.[207]

Pharmacodynamics. In time-kill studies, amphotericin B displays concentration-dependent fungicidal activity against susceptible *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*.[289,290,451] In addition to its concentration-dependent fungicidal dynamics, a prolonged postantifungal effect (PAFE) of amphotericin B of up to 12 hours' duration has been demonstrated in *C. albicans* and *C. neoformans*.[158,550] Studies in laboratory animals support the concentration-dependent kill kinetics of amphotericin B in vitro. In neutropenic pharmacokinetic and pharmacodynamic mouse models of disseminated candidiasis and pulmonary aspergillosis, peak plasma concentration (C_{max})/minimal inhibitory concentration (MIC) was the parameter that provided the best correlation with outcome as measured by the residual organismal burden in tissue.[26,610] These laboratory findings indicate that single daily doses rather than continuous infusion will be most effective. Therefore, the

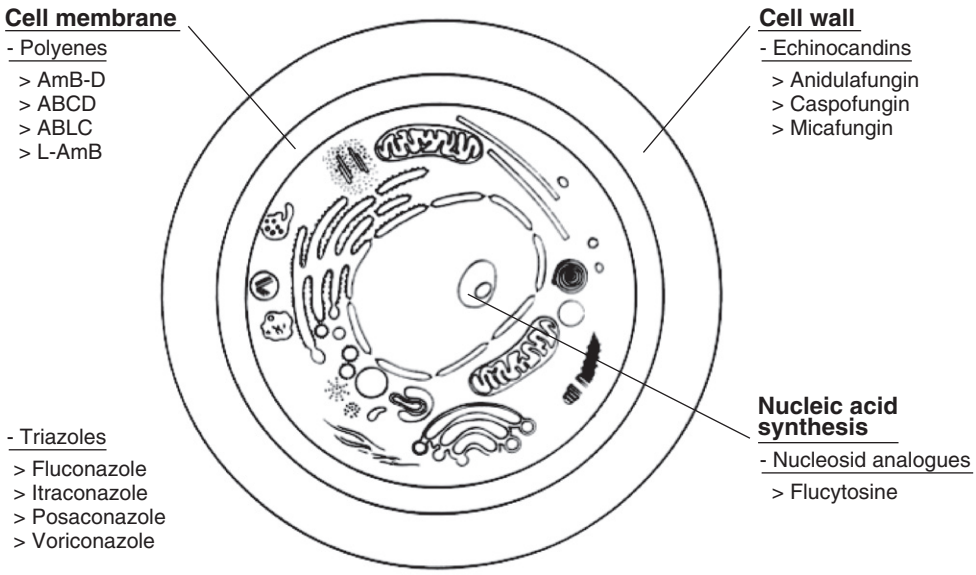

FIGURE 239-1 ■ Cellular targets of approved antifungal agents for treatment of invasive mycoses. (Modified from Groll AH, Piscitelli SC, Walsh TJ. Antifungal pharmacodynamics: concentration-effect relationships in vitro and in vivo. *Pharmacotherapy* 2001;21[suppl 8]:133-48.)

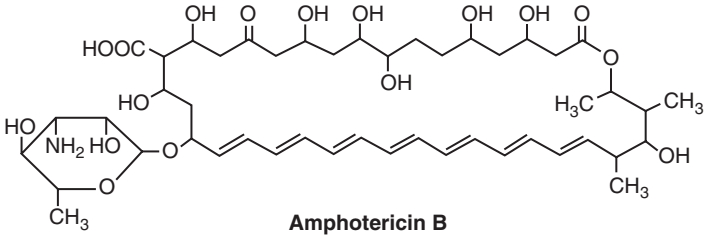

FIGURE 239-2 ■ Structural formula of amphotericin B.

dosage of amphotericin B should not be uncritically reduced, and infusion for durations longer than that recommended by the manufacturer should be avoided.

Pharmacokinetics. After intravenous administration, amphotericin B rapidly dissociates from its carrier and becomes highly protein-bound before distributing into tissues.[101] The disposition of the compound follows a three-compartment model, with rapid initial clearance from plasma followed by a biphasic pattern of elimination with a β half-life of 24 to 48 hours and a prolonged terminal (γ) half-life of 15 days or more.[36] Tissue levels of amphotericin B in laboratory animals are highest in liver, spleen, bone marrow, kidney, and lung; concentrations in body fluids other than plasma are generally low.[266,317] However, despite mostly undetectable concentrations in the cerebrospinal fluid (CSF) and comparatively low concentrations in brain tissue across all species, amphotericin B is effective in the treatment of fungal infections of the central nervous system (CNS). No metabolites of amphotericin B have been identified. Approximately 10 percent of parent compound is excreted into urine and bile, a finding suggesting that tissue accumulation accounts for most of the disposition of drug.[49,50,119,461] Accordingly, adjustment of dose is not necessary in patients with unrelated renal or hepatic dysfunction.

Reported pharmacokinetic data in pediatric age groups are characterized by a high interindividual variability, which may be related to differences in underlying diseases and modes of administration (Table 239-1).[40,55,297,389,522] However, infants and children appear to clear the drug from plasma more rapidly than do adults, as indicated by a negative correlation between age and clearance.[55,297,388] Because distribution into tissues appears to be the main route of clearance from plasma, the faster clearance in individuals of younger age may be explained by the larger relative volume of parenchymatous organs in contrast to that in adults.[379] Currently, dosage recommendations for all pediatric age groups do not differ from those in adult patients.

Adverse Effects. Infusion-related reactions and dose-limiting nephrotoxicity are major problems associated with the use of AmB-D and often limit successful therapy. Infusion-related reactions (i.e., fever, rigors, chills, myalgias, arthralgias, nausea, vomiting, and headaches) are thought to be mediated by the release of cytokines from monocytes in response to the drug.[30] These reactions can be noted in as many as 73 percent of patients prospectively monitored at the bedside.[578] In a prospective interventional study of pediatric patients with cancer, investigators observed fever, rigors, or both, associated

TABLE 239-1 **Pharmacokinetic Parameters of Amphotericin B Deoxycholate in Pediatric Patients***

Population/Reference	Dosage (mg/kg)	C_{max} (μg/mL)	$AUC_{0-\infty}$ (μg/mL/hr)	Vd_{ss} (L/kg)	Cl (L/hr/kg)	$t_{1/2}$ (hr)
Preterm neonates[522] (n = 5, 0.5-7.5 mo)	1/md	0.96	N/A	4.1	0.122	39
Preterm neonates[40] (n = 13, 0.06-1.8 mo)	0.5/md	0.96	N/A	1.5	0.036	14.8
Infants/children[297] (n = 13, 0.08-18 yr)	0.5/sd	1.5	N/A	0.37	0.026	9.9
Infants/children[55] (n = 12, 0.3-14 yr)	0.68/md	2.9	N/A	0.76	0.027	18.1
Infants/children[389] (n = 20, 2.2-14.3 yr)	0.98/sd	2.43	22.0	0.92	0.039	15.1
Children/adults[10] (n = 20, 4-66 yr)	1/md	2.9	36	1.1	0.028	39

*All values are given as means.
$AUC_{0-\infty}$, Area under the concentration versus time curve from time zero to infinity; *Cl*, plasma clearance; *C_{max}*, peak plasma concentration; *md*, multiple-dose data; *N/A*, not assessed; *sd*, single-dose data; *$t_{1/2}$*, elimination half-life; *Vd_{ss}*, apparent volume of distribution at steady state.

with the infusion of amphotericin B in 19/78 treatment courses (24%).[389] However, these characteristic adverse effects of amphotericin B are observed only rarely in the neonatal setting.[286] In clinical practice, infusion-related reactions associated with amphotericin B therapy may be blunted by slowing the infusion rate but often require premedication with acetaminophen 10 to 15 mg/kg, hydrocortisone 0.5 to 1 mg/kg, or meperidine 0.2 to 0.5 mg/kg.[579] Less common acute adverse effects are hypotension, hypertension, flushing, and vestibular disturbances; bronchospasm and true anaphylaxis are rare occurrences.[207] Cardiac arrhythmias and cardiac arrest resulting from acute potassium release may occur with rapid infusion (<60 minutes), especially if the patient has preexisting hyperkalemia or renal impairment.[85,197,373]

The hallmarks of amphotericin B–associated dose-limiting nephrotoxicity are azotemia, wasting of potassium, and wasting of magnesium. Renal tubular acidosis and impaired urinary concentration ability rarely are of clinical significance.[194,489] Relevant electrolyte wasting occurs in approximately 12 percent of prospectively monitored patients.[578] Hypokalemia can be quite refractory to replacement until hypomagnesemia is corrected.[489] Azotemia is a common occurrence. In a large prospective clinical trial, the baseline serum creatinine rose by more than 100 percent in 34 percent of 344 unstratified pediatric and adult patients receiving amphotericin B for empirical therapy of fever and neutropenia.[578] Azotemia can be exacerbated by concomitant nephrotoxic agents, in particular by cyclosporine and tacrolimus.[607] In series reporting safety data of amphotericin B 0.5 to 1 mg/kg in premature neonates, the incidence of azotemia ranged from zero to 15 percent,[84,190,286,319,322] a finding indicating that the compound is much better tolerated in this setting than reported early during its use.[39]

Nephrotoxicity associated with the use of amphotericin B has the potential to lead to renal failure and dialysis[614]; however, azotemia usually is reversible after discontinuation of the drug.[152,319,579] Avoiding concomitant nephrotoxic agents, appropriate hydration and normal saline loading (3 mEq Na^+/kg/day)[29,30,236] may greatly lessen the likelihood and severity of azotemia associated with amphotericin B therapy.

Other potentially relevant adverse effects of amphotericin B include demyelinizing encephalopathy[382] and normocytic, normochromic anemia associated with low erythropoietin levels after long-term administration.[120] Because amphotericin B deoxycholate is topically irritating, a central line should be used for infusion, and local instillation of amphotericin B should be considered only in conjunction with expert consultation.

Therapeutic Monitoring. Historically, 1-hour postinfusion plasma concentrations of twice the MIC of the fungal isolate have been proposed as targets for treatment of yeast infections.[150] However, monitoring of amphotericin B concentrations in plasma or CSF appears to be of little value because relationships between plasma and tissue concentrations and clinical efficacy or toxicity have not been adequately characterized.[207]

The toxicity of amphotericin B and the practice of normal saline loading warrant close monitoring of serum electrolytes, urea, and creatinine. The drug must not be infused in less than 60 minutes and only under particularly careful cardiac monitoring in newborns, as well as in patients with hyperkalemia and renal impairment, where arrhythmias resulting from acute potassium release may develop.[85,215]

Drug Interactions. Drug–drug interactions caused by shared metabolic pathways are unknown for amphotericin B. Hypokalemia may be aggravated by corticosteroids, cause rhabdomyolysis, and enhance the effects of nonpolarizing muscle relaxants. Similarly, hypomagnesemia may become especially profound in patients with cancer and platinum-associated nephropathy. Impairment of glomerular filtration by amphotericin B may enhance plasma levels and, thereby, toxicity of many renally cleared drugs, including aminoglycosides, vancomycin, fluorocytosine, and cyclosporine.[207] Finally, the

simultaneous infusion of granulocytes has been associated with acute pulmonary reactions[621] and therefore should be avoided.

Indications. With the exception of resource-limited settings, few indications are left for antifungal treatment of opportunistic mycoses with AmB-D. These indications include candidemia and acute tissue-invasive candidiasis, particularly in neonates, and induction therapy for cryptococcal meningitis; however, in the absence of adequate clinical trials, AmB-D is still a valid option for treatment of severe forms of the endemic mycoses (Tables 239-2 to 239-4). Depending on both the type of infection and the host, the recommended daily dosage ranges from 0.7 to 1 mg/kg/day administered over 2 to 4 hours as tolerated.[207]

For empirical antifungal therapy in the persistently febrile neutropenic host, the historical standard dosage has been 0.5 to 0.6 mg/kg/day.[162,441] Efficacy of prophylactic intravenous amphotericin B in the setting of anticancer therapy has not been documented,[209] and a large, randomized, multicenter study failed to show any preventive benefit of aerosolized amphotericin B in neutropenic patients at high risk for developing invasive mold infections.[498] However, aerosolized amphotericin B may have a preventive role in patients who have undergone lung transplantation.[145]

On principle, treatment should be started at the full target dose, with careful bedside monitoring during the first hour of infusion to allow for prompt intervention for infusion-related reactions.[579] With the exception of uncomplicated candidemia and induction therapy of cryptococcal meningoencephalitis, the duration of treatment is ill defined for most infections.

Amphotericin B Lipid Formulations

During the late 1990s, three novel formulations of amphotericin B were approved in the United States and most of Europe: AmB colloidal dispersion (ABCD, Amphocil, or Amphotec), AmB lipid complex (ABLC or Abelcet), and a small unilamellar liposomal formulation (L-AmB, AmBisome). Because of their reduced nephrotoxicity in contrast to AmB-D, these compounds allow for the safer delivery of higher dosages of the parent molecule (amphotericin B). However, data from animal models also suggest that higher dosages also are required for equivalent antifungal efficacy.[244,620]

Physicochemical Properties and Pharmacokinetics. The carriers of the lipid formulations are composed of biodegradable, amphiphilic bilayered membranes in which the hydrophilic heads of the lipid molecules face outward to shield the hydrophobic tails. The membranes may form either spherical vesicles termed liposomes or bilayered complexes or dispersions with no specific vesicular structure. Incorporated into these water-soluble carriers, amphotericin B becomes soluble in plasma and available for distribution. Each of the lipid formulations of amphotericin B possesses distinct physicochemical and pharmacokinetic properties. All three, however, preferentially distribute to organs of the mononuclear phagocytic system (MPS) and functionally spare the kidney. Although the micellar dispersion of ABCD behaves kinetically very similarly to AmB-D, the small unilamellar liposomal preparation has a prolonged circulation time in plasma, achieves strikingly high C_{max} and area under the concentration versus time curve (AUC) values, and is taken up only slowly by the MPS. In contrast, the

TABLE 239-2 **Principal Options for Medical Management of Invasive Infections by Opportunistic Yeast***

Fungal Disease	Management
Esophageal candidiasis	Fluconazole, itraconazole, voriconazole
	Caspofungin, micafungin
	Amphotericin B deoxycholate
Candidemia or invasive candidiasis	Fluconazole, voriconazole
	Caspofungin, micafungin
	Amphotericin B deoxycholate, liposomal amphotericin B
	Second-line options:
	One of the above with change in class Amphotericin B lipid complex
	Amphotericin B deoxycholate plus flucytosine
Cerebral cryptococcosis	Amphotericin B deoxycholate plus flucytosine, followed by fluconazole
	Second-line options: Fluconazole plus flucytosine; liposomal amphotericin B
Extracerebral manifestations	Fluconazole
	Amphotericin B deoxycholate ± flucytosine Liposomal amphotericin B

*In alphabetical order according to class (azoles/echinocandins/polyenes); for details and age-specific dosages, please refer to text.

TABLE 239-3 Principal Options for Medical Management of Invasive Infections by Opportunistic Molds

Fungal Disease	Management*
Invasive aspergillosis	Voriconazole
	Liposomal amphotericin B
	Second-line options:
	One of the above with change in class Posaconazole
	Caspofungin
	Amphotericin B lipid complex, amphotericin B colloidal dispersion, amphotericin B deoxycholate
	Liposomal amphotericin B plus caspofungin
	Voriconazole plus caspofungin
	Consolidation therapy:
	Itraconazole, posaconazole, voriconazole
Non-*Aspergillus* hyalohyphomycetes	Voriconazole, posaconazole
	Liposomal amphotericin B, amphotericin B lipid complex, amphotericin B deoxycholate
Mucormycosis	Liposomal amphotericin B, amphotericin B lipid complex
	Second-line or consolidation:
	Posaconazole
Dematiaceous molds	Voriconazole, posaconazole
	Liposomal amphotericin B, amphotericin B lipid complex, amphotericin B deoxycholate

*In alphabetical order according to class (azoles/echinocandins/polyenes); for details and age-specific dosages, please refer to text.

TABLE 239-4 Principal Options for Medical Management of Invasive Infections by Endemic Molds

Fungal Disease	Management*
Histoplasmosis	Liposomal amphotericin B followed by itraconazole
	Itraconazole†
	Secondary alternative options:
	Amphotericin B deoxycholate, amphotericin B lipid complex Fluconazole, posaconazole, voriconazole
Coccidioidomycosis	Amphotericin B deoxycholate, amphotericin B lipid complex, liposomal amphotericin B, followed by azoles
	Fluconazole‡
	Itraconazole†
	Secondary alternative options:
	Posaconazole, voriconazole
Blastomycosis	Amphotericin B deoxycholate, followed by itraconazole Liposomal amphotericin B or amphotericin B lipid complex, followed by itraconazole
	Itraconazole†
	Secondary alternative options: Voriconazole
Paracoccidioidomycosis	Amphotericin B deoxycholate, amphotericin B lipid complex, liposomal amphotericin B
	Itraconazole,† voriconazole
Penicilliosis	Amphotericin B deoxycholate
	Itraconazole,† voriconazole
Sporotrichosis	Amphotericin B deoxycholate, followed by itraconazole Liposomal amphotericin B or amphotericin B lipid complex, followed by itraconazole
	Itraconazole†
	Terbinafine (lymphocutaneous disease only)

*In alphabetical order according to class (polyenes/azoles); for details and age-specific dosages, please refer to text.
†Clinically stable patients with mild-to-moderate disease and no central nervous system involvement, or as consolidation or maintenance therapy.
‡Agent of first choice in (1) consolidation therapy of meningeal coccidioidomycosis, (2) Coccidioides meningitis, or (3) coccidioidomycosis in stable patients with mild-to-moderate disease or as consolidation or maintenance therapy.

TABLE 239-5 **Physicochemical Properties and Multiple-Dose Pharmacokinetic Parameters of the Four Amphotericin B Formulations***

Properties and Parameters	D-AmB	ABCD	ABLC	L-AmB
Lipids (molar ratio)	Deoxycholate	Cholesteryl sulfate	DMPC/DMPG (7:3)	HPC/CHOL/DSPG (2:1:0.8)
Mol% AmB	34%	50%	50%	10%
Lipid configuration	Micelles	Micelles	Membrane-like	SUVs
Diameter (μm)	0.05	0.12-0.14	1.6-11	0.08
Dosage (mg AmB/kg)	1	5	5	5
C_{max} (μg/mL)	2.9	3.1	1.7	58
$AUC_{0-\infty}$ (μg/mL/hr)	36	43	14	713
Vd_{ss} (L/kg)	1.1	4.3	131	0.22
Cl (L/hr/kg)	0.028	0.117	0.476	0.017

Modified from Groll AH, Muller FM, Piscitelli SC, et al. Lipid formulations of amphotericin B: clinical perspectives for the management of invasive fungal infections in children with cancer. *Klin Padiatr* 1998;210:264-273.

*Data represent mean values, stem from adult patients and were obtained after different rates of infusion.

$AUC_{0-\infty}$, Area under the concentration versus time curve from time zero to infinity; *CHOL,* cholesterol; *Cl,* plasma clearance; *C_{max}*, peak plasma concentration; *DMPC,* dimyristoyl phosphatidylcholine; *DMPG,* dimyristoyl phosphatidylglycerol; *DSPG,* distearoyl phosphatidylglycerol; *HPC,* hydrogenated phosphatidylcholine; *SUV,* small unilamellar vesicles; *Vd_{ss}*, apparent volume of distribution at steady state.

large, ribbon-like aggregates of ABLC are efficiently opsonized by plasma proteins and rapidly taken up by the MPS, thus resulting in lower peak plasma and AUC values (Table 239-5).[218,244] Whether and how the distinct physicochemical and pharmacokinetic features of each formulation translate into different pharmacodynamic properties in vivo are largely unknown. However, experimental head-to-head comparisons of all four formulations of amphotericin B against defined invasive mycoses suggest that important differences exist in antifungal efficacy depending on the agent, dose, type, and site of infection.[103,213,404]

Safety and Antifungal Efficacy. Safety and antifungal efficacy of ABCD, ABLC, and L-AmB were demonstrated in an array of phase II and III clinical trials in immunocompromised, mostly adult patients with a wide spectrum of underlying disorders.* The overall response rates in these trials ranged from 53 to 84 percent in patients with invasive candidiasis and 34 to 59 percent, respectively, in patients with presumed or documented invasive aspergillosis.[218,244] A few randomized, controlled trials were completed in which one of the newer formulations was compared with AmB-D. These studies consistently showed at least equivalent therapeutic efficacy and reduced nephrotoxicity of the investigated lipid formulation. Infusion-related side effects of fever, chills, and rigor were less frequent with L-AmB only.[17,578,606,608] Several individual cases of substernal chest discomfort, respiratory distress, and sharp flank pain may occur during infusion of L-AmB[273,465]; in comparative studies, hypoxic episodes associated with fever and chills occurred more frequently in ABCD recipients than in recipients of AmB-D.[74,606] Mild increases in serum bilirubin and alkaline phosphatase have been observed with all three formulations, as well as mild increases in serum transaminases with L-AmB. Nevertheless, no case of fatal liver disease has occurred.[218,244,620] An increased rate of hyperphosphatemia has been reported for L-AmB[291]; however, true hyperphosphatemia has to be carefully differentiated from pseudohyperphosphatemia that is due to interferences of L-AmB with assay systems for determination of serum phosphorous.[269]

Experience in Pediatric Patients. Considerable numbers of pediatric patients have been treated with ABCD, ABLC, or L-AmB on protocols in the clinical trials cited earlier.[524] Separately published pediatric data are discussed in the following subsections.

ABCD. ABCD is a complex of amphotericin B and sodium cholesteryl sulfate in an approximate 1:1 molar ratio that forms disklike colloidal structures on dissolution.[218] Estimated pharmacokinetic parameters in children are not significantly different from those in adult patients under conditions of steady state: mean AUC from 0 to 24 hours (AUC_{0-24}) is 7.10 mg/L/hr (normalized to a 1-mg/kg/day dose); mean volume of distribution (V_d) is 4.57 L/kg, and mean total clearance (Cl) is 0.144 L/hr/kg.[10]

A double-blind, randomized trial comparing ABCD (4 mg/kg/day) with AmB-D (0.8 mg/kg/day) for empirical antifungal therapy of febrile neutropenic patients that also included a larger number of pediatric patients found that ABCD was as effective but significantly less nephrotoxic than AmB-D.[606] Among children (0 to 15 years; mean, 8.8 years) with presumed or proved invasive fungal infections refractory to or intolerant of amphotericin B who were treated on five different open-label studies of ABCD with dosages that ranged from 0.8 to 7.5 mg/kg (mean, 4.5 mg/kg), 67 percent of patients reported infusion-related reactions, but nephrotoxicity, defined as an increase in serum creatinine to 2 times or more of the baseline value, was reported in only 12 percent.[484]

*References 110, 238, 312, 462, 471, 582, 607, 617.

The published experience in the neonatal setting is limited to 16 infants with very low birth-weight (779 ± 170 g; 25 ± 2 weeks) with invasive candidiasis and a serum creatinine concentration of 1.2 mg/dL or greater.[329] Infants received 3 mg/kg ABCD on day 1, followed by 5 mg/kg/day thereafter; a second agent was permitted for candidemia that persisted for 7 or more days. Of 14 evaluable patients, 13 cleared the organism after therapy with ABCD alone ($n = 8$) or in combination with another agent ($n = 5$), and overall survival was 75 percent.

These data overall indicate no fundamental differences in disposition, safety, and antifungal efficacy of ABCD in contrast to adult populations. The U.S. Food and Drug Administration (FDA)–approved indication is treatment of probable or proven invasive aspergillosis refractory to or intolerant of AmB-D, and the approved dosage is 3 to 4 mg/kg/day, administered over the course of 2 hours.

ABLC. ABLC is composed of dimyristoyl phosphatidylcholine/dimyristoyl phosphatidylglycerol (DMPC/DMPG) in a 1:1 molar ratio of lipid to amphotericin B and forms large, ribbon-like structures. The pharmacokinetic properties of ABLC were studied in whole blood in three pediatric patients with cancer who received the compound at 2.5 mg/kg over the course of 6 weeks for hepatosplenic candidiasis.[592] Steady state was achieved by day 7 of therapy; after the final dose, the mean $AUC_{0\text{-}24}$ was 11.9 ± 2.6 mg/L/hr, the mean C_{max} was 1.69 ± 0.75 mg/L, and clearance was 0.218 L/kg/hr. In the six patients evaluable for safety assessment, mean serum creatinine levels were stable at the end of therapy and at 1-month follow-up and no increase in hepatic transaminases occurred. Five of the patients had infusion-related reactions to the first dose, which were prospectively monitored without prior premedication; however, infusion-related adverse reactions were well controlled thereafter by conventional premedications. All evaluable patients responded to therapy.

Safety and antifungal efficacy of ABLC were studied in 111 treatment episodes in pediatric patients (21 days to 16 years of age) refractory to or intolerant of conventional antifungal agents through an open-label, emergency-use protocol in the United States.[590] ABLC was administered at a mean daily dosage of 4.85 mg/kg (range, 1.1 to 9.5 mg/kg/day) for a mean duration of 38.9 days (range, 1 to 198 days). The mean serum creatinine for the entire study population did not significantly change between baseline (1.23 ± 0.11 mg/dL) and cessation of ABLC therapy (1.32 ± 0.12 mg/dL) over 6 weeks. No significant differences were observed between baseline and end-of-therapy levels of serum potassium, magnesium, hepatic transaminases, alkaline phosphatase, and hemoglobin. Among 54 cases fulfilling criteria for evaluation of antifungal efficacy, a complete or partial therapeutic response was obtained in 38 patients (70%).

The safety and efficacy of ABLC also were assessed in 548 children and adolescents who were enrolled in the Collaborative Exchange of Antifungal Research registry. Most patients were either intolerant of or refractory to conventional antifungal therapy. Response data were evaluable for 255 of the 285 patients with documented single or multiple pathogens. A complete (cured) or partial (improved) response was achieved in 54.9 percent of patients. No significant difference was noted between the rates of new hemodialysis versus baseline hemodialysis. Elevations in serum creatinine of greater than 1.5 times baseline and greater than 2.5 times baseline values were seen in 24.8 and 8.8 percent of all patients, respectively. The overall response rate and safety profile in pediatric patients were consistent with earlier reported findings of smaller trials.[611]

Eleven infants aged 6 months and younger with candidemia were enrolled in the U.S. open-label, emergency-use protocol.[590] Of the 11 patients, 7 maintained a stable mean serum creatinine; in 4 patients, a rise in serum creatinine was observed, but in each case, the increase was less than 40 percent of the baseline value. No differences were observed between baseline and end-of-therapy mean bilirubin levels. Among the 8 evaluable infants, a complete response was observed in 6 (75%). A population pharmacokinetic study in 28 mostly premature neonates with invasive *Candida* infections demonstrated that the disposition of ABLC in neonates is similar to that observed in other age groups; weight was the only factor that influenced clearance.[622] The safety of ABLC in neonates is further supported by a retrospective case-control study in 35 infants with very low birth weight who received the compound for at least 2 weeks; treatment with ABLC was well tolerated and did not increase serum creatinine or decrease potassium.[37]

The current data suggest no fundamental differences in disposition, safety, and antifungal efficacy of ABLC in contrast to adults. The FDA-approved indication is treatment of invasive fungal infections refractory to or intolerant of AmB-D, and the approved dosage is 5 mg/kg/day administered over the course of 2 hours.

L-AmB. L-AmB consists of small, unilamellar spherical vesicles (true liposomes) composed of hydrogenated soy phosphatidylcholine and distearoyl phosphatidylglycerol stabilized by cholesterol and combined with amphotericin B in a 2:0.8:1:0.4 molar ratio.[218] The pharmacokinetic properties of L-AmB in pediatric patients beyond the neonatal period were investigated in a formal phase II, dose-escalation trial investigating dosages of 2.5, 5, and 7.5 mg/kg in immunocompromised patients, as well as by using a population-based approach. The results of these studies indicate that the disposition of L-AmB in pediatric patients is not substantially different from that in adults and that weight is a covariate that determines clearance and volume of distribution.[248,503] A pharmacokinetic pilot study was conducted in 12 children at risk for developing invasive fungal infections who received once-weekly, high-dose L-AmB (10 mg/kg over 2 hours) as prophylaxis. L-AmB was well tolerated and showed measurable amphotericin B plasma concentrations 7 days after the dose, a finding suggesting that once-weekly administration may provide useful protection against fungal infections.[360]

Many pediatric patients have been enrolled in clinical trials with L-AmB but have not been reported separately.[365,578] Two hundred four children (mean age, 7 years) with neutropenia and fever of unknown origin were randomized in an open-label, multicenter trial to receive conventional AmB-D at 1 mg/kg/day ($n = 63$), L-AmB at 1 mg/kg/day ($n = 70$), or L-AmB at 3 mg/kg/

day (n = 71) for empirical antifungal therapy.[445] Twenty-nine percent of patients treated with L-AmB 1, 39 percent of patients treated with L-AmB 3, and 54 percent of patients treated with AMBD experienced adverse effects (p = .01); nephrotoxicity, defined as 100 percent or more increase in serum creatinine from baseline, was noted in 8, 11, and 21 percent, respectively (not significant [NS]). Hypokalemia (<2.5 mmol/L) occurred in 10, 11, and 26 percent of patients, respectively (p = .02); increases in serum transaminase levels (≥110 U/L) occurred in 17, 23, and 17 percent, respectively (NS); and increases in serum bilirubin (≥35 μmol/L) occurred in 11, 12, and 10 percent of patients, respectively. L-AmB at either 1 or 3 mg/kg/day was significantly safer and at least equivalent to AMBD with regard to resolution of fever of unknown origin. L-AmB was well tolerated and effective in cohorts of immunocompromised children requiring antifungal therapy for proved or suspected infections, including patients with bone marrow transplants for primary immunodeficiencies[419] and patients with cancer.[463] A phase IV analysis of 141 courses of L-AmB administered for a mean of 17 days' duration at a mean maximum dosage of 2.5 mg/kg for various indications to pediatric patients with cancer and hematopoietic stem cell transplantation (HSCT) revealed a low rate of adverse events (4%), necessitating discontinuation. Whereas mean aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bilirubin values were slightly higher at the end of treatment (p = .01), bilirubin and creatinine values were not different from baseline.[294]

L-AmB 2.5 to 7 mg/kg/day was evaluated prospectively in 24 infants with very low birth-weight (mean birth-weight, 847 ± 244 g; mean gestational age, 26 weeks) with systemic candidiasis. The mean duration of therapy was 21 days; the cumulative L-AmB dose was 94 mg/kg. Fungal eradication was achieved in 92 percent of the episodes; 20 (83%) infants were considered clinically cured at the end of treatment. No major adverse effects were recorded. One infant developed increased bilirubin and hepatic transaminase levels during therapy. Four (17%) infants died; in 2 of them (8%), the cause of death was attributed directly to systemic candidiasis.[276] In a second study undertaken by the same investigators, high-dose (5 to 7 mg/kg/day) L-AmB was evaluated prospectively in 41 episodes of systemic candidiasis occurring in 37 neonates (36/37 were premature infants with very low birth-weights). Twenty-eight, 5, and 8 infants received 7, 6 to 6.5, and 5 mg/kg/day, respectively. Median duration of therapy was 18 days; median cumulative dose was 94 mg/kg. Fungal eradication was achieved in 39/41 (95%) episodes. Fungal eradication was achieved more rapidly in patients treated early with high doses and in patients who received high-dose L-AmB as first-line therapy.[275] Further prospective[32] and retrospective[347] cohort studies attest to the safety of L-AmB in infants with very low birth-weight. Of note, the higher mortality reported in a single center analysis for infants with invasive candidiasis treated with lipid formulations relative to conventional AmB-D or fluconazole[34] is likely due to differences in acuity of illnesses and comorbidities rather than pharmacokinetic considerations.

Current data indicate no substantial differences in pharmacokinetics and pharmacodynamics of L-AmB between pediatric and adult patients. The FDA-approved dosages are 3 mg/kg/day (empirical antifungal therapy in febrile neutropenic patients), 3 to 5 mg/kg/day (therapy of invasive infections intolerant or refractory to AmB-D), and 6 mg/kg/day for cryptococcal meningitis, administered over the course of 2 hours. In Europe, L-AmB is approved for first-line treatment of invasive opportunistic mycoses.

Indications. The lipid formulations of AmB represent an important therapeutic advance in the management of invasive opportunistic fungal infections in immunocompromised patients. All three compounds have less renal toxicity than conventional amphotericin B, as defined by development of azotemia; distal tubular toxicity also may be somewhat reduced. Infusion-related reactions of fever, chills, and rigor appear to occur substantially less frequently only with L-AmB, and no new toxicities have been noted. The available pharmacokinetic and safety data from children so far indicate no fundamental differences from data obtained in the adult population.

Therapeutically, the lipid formulations are at least as effective as is conventional amphotericin B for treatment of most opportunistic human mycoses, and these formulations can be effective if conventional amphotericin B has failed. The specific clinical indications of the lipid formulations are discussed in the respective fungal disease chapters and are summarized in Tables 239-2 to 239-4.

Flucytosine

Flucytosine (5-fluorocytosine [5-FC]) is a low-molecular-weight, water-soluble, synthetic fluorinated pyrimidine analog (Fig. 239-3). It is taken up into the fungal cell by the fungus-specific enzyme cytosine permease and is converted in the cytoplasm by cytosine deaminase to 5-fluorouracil, a potent antimetabolite that causes RNA miscoding and inhibits DNA synthesis at the level of thymidylate synthase (Fig. 239-4).[144] Flucytosine is relatively nontoxic to mammalian cells because of the absence or very low level of activity of cytosine deaminase. In the United States, flucytosine is available only as an oral formulation; an intravenous formulation is available outside the United States in select countries.

Antifungal Activity. The antifungal activity of flucytosine in vitro is essentially limited to *Candida* spp., *C. neoformans*, *Cryptococcus gattii*, *Saccharomyces cerevisiae*,

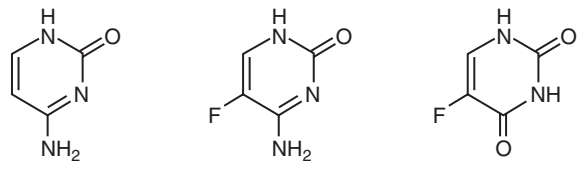

FIGURE 239-3 ■ Structural formulas of cytosine, flucytosine, and fluorouracil.

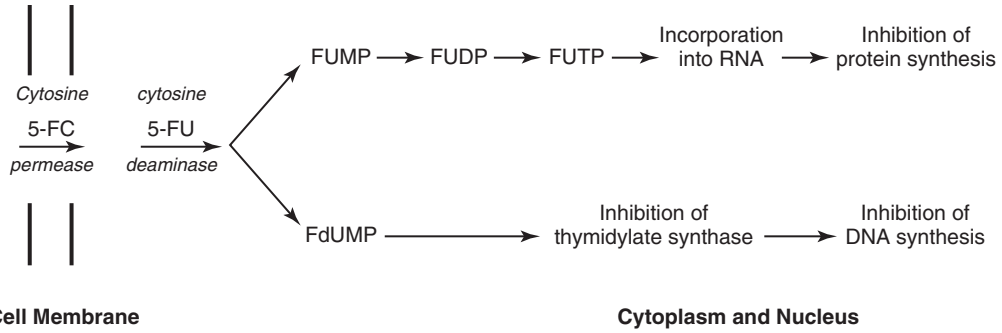

FIGURE 239-4 ■ Schematic of intracellular pathways and mechanism of action of flucytosine. *5-FC,* Flucytosine; *FdUMP,* 5-fluorodeoxyuridine monophosphate; *5-FU,* 5-fluorouracil; *FUDP,* 5-fluoridine diphosphate; *FUMP,* 5-fluoridine monophosphate; *FUTP,* 5-fluoridine triphosphate. (Modified from Groll AH, Piscitelli SC, Walsh TJ. Antifungal pharmacodynamics: concentration-effect relationships in vitro and in vivo. *Pharmacotherapy* 2001;21[suppl. 8]:133-48; and Vermes A, Guchelaar HJ, Dankert, J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother* 2000;46:171-9.)

Rhodotorula spp., and some dematiaceous molds.* Flucytosine generally is thought to have no or weak activity against *Aspergillus* spp. and other hyaline molds,[181,349] although this lack of activity in vitro may be pH-dependent[530] and does not correlate with the documented efficacy in animal models.[532] Notably, whereas *Candida krusei* appears to be less susceptible to flucytosine, the compound is highly active against *C. glabrata*.[144,170,433] Synergistic or additive effects in combination with amphotericin B have been observed against *Candida*[359,375] and *Aspergillus* spp.[531] and in combination with amphotericin B, fluconazole, voriconazole, and posaconazole against *C. neoformans*.[43,229,358,395,500] Combination with echinocandins was additive or indifferent in vitro against *Candida* spp.[278]

Two mechanisms of resistance have been reported: (1) mutations in enzymes necessary for cellular uptake and transport of flucytosine or its metabolism and (2) increased synthesis of pyrimidine that competes with the fluorinated antimetabolites of the compound.[139,567] In pretreatment isolates, intrinsically resistant strains have been found in 3 to 8 percent of *C. albicans*, in 0 to 8 percent of non-*albicans Candida* spp., and in 2 percent or fewer of *C. neoformans* isolates.[358,433] In an analysis of 8803 clinical isolates comprising 18 *Candida* spp. obtained from more than 200 medical centers worldwide, primary resistance to flucytosine was an uncommon occurrence among *Candida* spp. (95% sensitive, 2% intermediate, and 3% resistant), with the exception of *C. krusei* (5% sensitive, 67% intermediate, and 28% resistant).[433] Development of resistance to flucytosine can be observed during treatment,[170] and it is thought to be caused predominantly by selection of resistant clones.[567] As a consequence, flucytosine is rarely given alone but instead is given in combination with amphotericin B or fluconazole.

Pharmacodynamics. Time-kill assays against *Candida* spp. and *C. neoformans* demonstrated a predominantly concentration-independent fungistatic (99% reduction in colony-forming units) activity of flucytosine at concentrations exceeding the MIC of the investigated isolates.[325,556] A prolonged PAFE against these organisms was noted consistently that was dependent on concentration and duration of exposure and ranged from 0.8 to 10 hours.[367,491,492,626] Investigation of pharmacokinetic and pharmacodynamic relationships in a neutropenic mouse model of disseminated candidiasis that used the residual fungal burden in kidney tissue as the end-point of antifungal efficacy revealed that both the time above the MIC and the AUC/MIC ratio were important in predicting efficacy; maximal efficacy was observed when levels exceeded the MIC for only 20 to 25 percent of the 24-hour dosing interval.[19] In experiments that used a pharmacodynamic model of disseminated candidiasis and bridging to humans by population pharmacokinetics and Monte Carlo simulation, an in vivo drug exposure breakpoint for flucytosine was apparent when serum levels were greater than the MIC for 45 percent of the dosing interval. The Monte Carlo simulations suggested that, using a human dose of 100 mg/kg/day in four divided doses, flucytosine resistance was defined at an MIC of 32 mg/L. Target attainment rates after administration of 25, 50, and 100 mg/kg/day were similar.[253] These data collectively suggest that lower dosages or less frequent dosing may yield identical antifungal efficacy while further reducing potential toxicities of flucytosine that are thought to be concentration-dependent.[170]

Pharmacokinetics. Flucytosine is absorbed readily from the gastrointestinal tract, and oral bioavailability exceeds 80 percent (Table 239-6). C_{max} occurs 1 to 2 hours after administration. As a water-soluble compound, flucytosine has negligible protein binding (4%) and is widely distributed in the body, with a volume of distribution that approximates that of total body water. Mean CSF concentrations usually are 65 to 90 percent of simultaneous plasma concentrations. The drug penetrates well into peritoneal fluid, inflamed joints, and other fluid compartments, including the eye.[65,120,207]

In humans, less than 1 percent of a given dose of flucytosine is thought to undergo hepatic metabolism.[341] Some evidence suggests that bacteria of the gastrointestinal flora deaminate flucytosine to resorbable

*References 136, 170, 183, 246, 429, 433, 544.

TABLE 239-6 **Pharmacokinetics of Flucytosine in Adults**

Parameter or Characteristic	Value
Oral bioavailability	≥80%
C_{max}	50-120 μg/mL*
T_{max}	1-2 hr
Protein binding	4%
Vd_{ss}	0.6-0.7 L/kg
$t_{1/2}\beta$	3-6 hr
Clearance	≥95% renal
Unchanged drug in urine	≥95%
Relative cerebrospinal fluid levels	65%-90%

Modified from Groll AH, Piscitelli SC, Walsh TJ. Antifungal pharmacodynamics: concentration-effect relationships in vitro and in vivo. *Pharmacotherapy* 2001;21(suppl 8):133-48.
*At steady state in patients with cryptococcal meningitis receiving 4 × 2 g/day orally.
C_{max}, Peak plasma concentration; T_{max}, time until occurrence of peak plasma concentration; Vd_{ss}, volume of distribution at steady state.

5-fluorouracil,[231,342] a finding that may account for some of the toxicities observed after oral administration of the drug. Approximately 95 percent of a given dose of flucytosine is excreted into the urine in unchanged, active form by simple glomerular filtration.[207] Because the compound's elimination parallels the glomerular filtration rate, adjustment of the dosage is necessary in patients with impaired renal function.[122] In patients undergoing hemodialysis, a dose of 37.5 mg/kg is recommended after dialysis; in those undergoing hemofiltration, the dosage needs to be adjusted to the individual filtration rate.[264,315] In peritoneal dialysis, the compound can be administered systemically or intraperitoneally.[366] Although the data are limited, impaired liver function does not appear to alter the disposition of flucytosine.[120]

In infants and children, the pharmacokinetic properties of flucytosine have not been systematically characterized. However, because of very similar physicochemical and pharmacokinetic properties, developmental changes in disposition similar to those found with fluconazole can be anticipated. Indeed, a similarly marked interindividual variability in clearance and volume of distribution has been reported in neonates[40] that renders uniform dosing recommendations in this population impossible.

A starting dosage for both adults and children of 100 mg/kg daily divided into three or four doses is recommended currently. Monitoring of plasma concentrations is essential to adjust dosage to changing renal function and avoid toxicity. After oral administration, near peak levels 2 hours after dosing overlap with trough levels as patients reach steady state, and thus these levels are sufficient for therapeutic monitoring.[170] In practice, plasma levels between 40 (trough) and less than 100 mg/L (peak) correlate with antifungal efficacy and seldom are associated with hematologic adverse effects.[170,568] However, retrospective evaluations in the United Kingdom revealed that only a fraction of plasma levels ordered for therapeutic monitoring were in this concentration range.[420,520]

Adverse Effects. Common adverse effects associated with flucytosine that occur in 5 to 6 percent of patients include gastrointestinal intolerance and reversible elevations of hepatic transaminases and alkaline phosphatase. Rarer side effects are skin rashes, blood eosinophilia, and crystalluria.[207] Hematologic adverse effects have been reported in overall 6 percent of patients receiving oral flucytosine and may include neutropenia, thrombocytopenia, or pancytopenia. Notably, hematologic adverse effects occur less frequently if plasma levels of flucytosine do not exceed 100 mg/L.[170,207]

Drug Interactions. Orally administered, nonresorbable antibiotics and administration of aluminum-based or magnesium hydroxide–based antacids may delay but do not impair absorption of the compound from the gastrointestinal tract.[207] 5-FC undergoes only minor hepatic metabolism, and it is not known to interfere with the cytochrome P (CYP)450 enzyme system. However, any drug that can cause a reduction in the glomerular filtration rate may lead to increased flucytosine serum levels and thereby has the potential to enhance 5-FC–associated toxicity. This phenomenon almost invariably is encountered with concomitant administration of amphotericin B, but it can occur similarly with numerous antimicrobial agents, anticancer drugs, and cyclosporine, to name only the most common examples.[120] The anticancer drug cytosine arabinoside (Ara-C) competitively inhibits the action of 5-FC, and these drugs should not be given concomitantly.[88]

Clinical Indications. Because of the propensity of susceptible organisms to develop resistance in vitro,[442] 5-FC traditionally is not administered as a single agent. Ample laboratory and clinical experience exists regarding the combination of amphotericin B deoxycholate and 5-FC (see Table 239-2).[144,207,567] Randomized clinical trials established the use of amphotericin B in combination with 5-FC as standard for induction therapy of cryptococcal meningitis in non–human immunodeficiency virus (HIV)-infected and HIV-infected patients.[54,557] Indeed, a small but well-designed trial demonstrated that the clearance of cryptococci from the CSF was significantly faster with amphotericin B in combination with 5-FC than with amphotericin B alone, amphotericin B in combination with fluconazole, or triple therapy, a finding confirming that the combination is the most rapidly fungicidal regimen.[78]

Although no comparative trials have been performed, the cumulative clinical experience supports the combination of amphotericin B with 5-FC for the treatment of *Candida* infections involving deep tissues, particularly in critically ill patients and when non-*albicans* *Candida* spp. are involved.[170,255,350,534] This includes *Candida* meningitis, endophthalmitis, endocarditis, vasculitis, and peritonitis, as well as osteoarticular, renal, and disseminated candidiasis.[207,579]

The combination of 5-FC with fluconazole is an option for cryptococcal meningitis, when treatment with conventional or liposomal amphotericin B is not feasible.[314,368,369,399] This combination also may be useful as second-line therapy for individual patients with invasive *Candida* infections involving aqueous body compartments.

Antifungal Triazoles

The antifungal azoles are a class of synthetic compounds that have one or more azole rings and—attached to one of the nitrogen atoms—a more or less complex side chain. Whereas the imidazoles have two nitrogen atoms, the triazoles have three in the five-member ring. The triazole ring confers improved resistance to metabolic degradation, greater target specificity, and an expanded spectrum of activity.[198,199] The imidazoles miconazole and ketoconazole were the first azole compounds developed for systemic treatment of human mycoses. Severe toxicities associated with the drug carrier (miconazole) and erratic absorption and significant interference with the human CYP450 system (ketoconazole), however, limited their clinical usefulness.[207] The triazoles fluconazole and itraconazole (Fig. 239-5), in contrast, have become extremely useful components of the antifungal armamentarium. Overall, they are well tolerated and possess a broad spectrum of activity. Whereas fluconazole and itraconazole have been available since the 1990s, a new generation of antifungal triazoles has entered clinical practice more recently; these so-called second-generation triazoles include voriconazole and posaconazole (Fig. 239-6).[247]

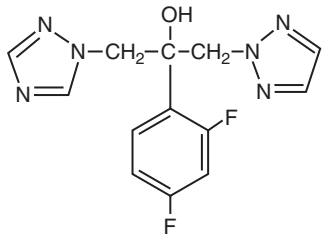

Fluconazole

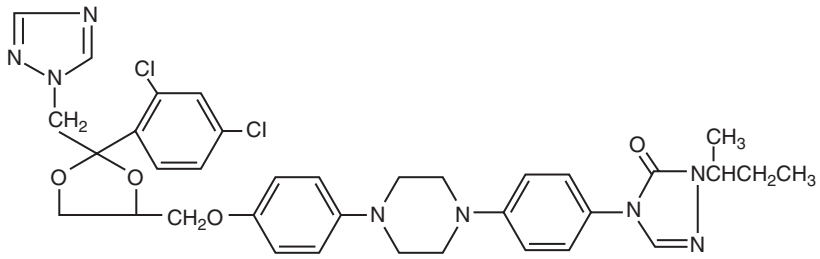

Itraconazole

FIGURE 239-5 ■ Structural formulas of first generation systemic antifungal triazoles: fluconazole and itraconazole.

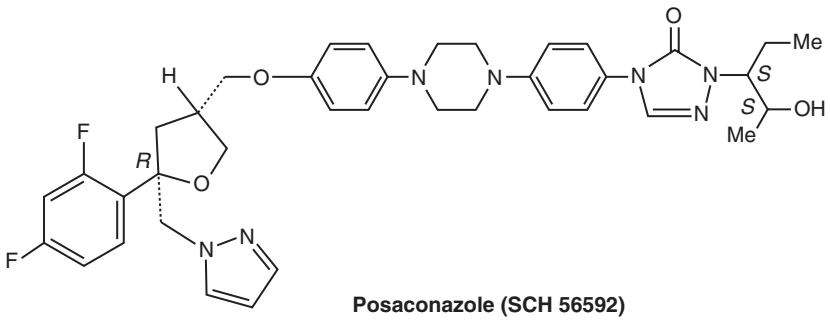

Posaconazole (SCH 56592)

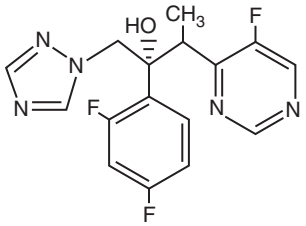

Voriconazole (UK 109496)

FIGURE 239-6 ■ Structural formulas of second generation systemic antifungal triazoles: posaconazole and voriconazole.

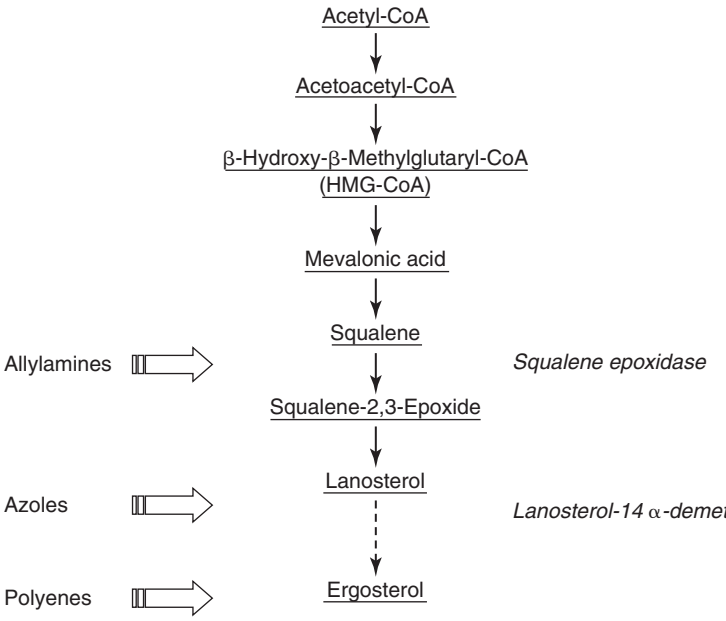

FIGURE 239-7 ■ Ergosterol biosynthesis and targets of current antifungal agents. *CoA,* Coenzyme A. (Modified from Groll AH, Walsh TJ. Uncommon opportunistic fungi: new nosocomial threats. *Clin Microbiol Infect* 2001;7[suppl 2]:8-24.)

Mechanism of Action. The antifungal azoles, as a class, target ergosterol biosynthesis by inhibiting the fungal CYP450-dependent enzyme lanosterol 14-α-demethylase. This inhibition interrupts the conversion of lanosterol to ergosterol and thus leads to accumulation of aberrant 14-α-methylsterols and depletion of ergosterol in the fungal cell membrane (Fig. 239-7). These effects alter cell membrane properties and function and, depending on organism and compound, may lead to cell death or inhibition of cell growth and replication. In addition, the azoles also inhibit CYP450-dependent enzymes of the fungal respiration chain, but the contribution of this action to their overall activity is unclear. Interaction with structurally similar mammalian CYP450-dependent enzyme systems is responsible for most toxicities and drug interactions of this class of compounds.[207,559]

Antifungal Activity. Fluconazole and itraconazole are active principally against dermatophytes, *Candida* spp., *C. neoformans*, *C. gattii*, *T. asahii*, and other uncommon yeast organisms, as well as against dimorphic fungi such as *Histoplasma capsulatum*, *Coccidioides* spp., *Blastomyces dermatitidis*, *Paracoccidioides brasiliensis*, and *Sporothrix schenkii*.[198,199,544] These drugs have less activity against *C. glabrata* and none against *C. krusei*.[207,459] Clinically useful activity against *Aspergillus* spp. and dematiaceous molds is restricted to itraconazole, and both itraconazole and fluconazole are considered inactive against *Fusarium* spp. and the Mucorales.[207] The second-generation triazoles voriconazole and posaconazole have enhanced target activity and are active against a wide spectrum of clinically important fungi, including *Candida* spp., *T. asahii*, *C. neoformans*, *Aspergillus* spp., *Fusarium* spp., and other hyaline molds, as well as dematiaceous and dimorphic molds.* In contrast to fluconazole and itraconazole, both agents are active against *C. glabrata* and *C. krusei;* posaconazole also is active against some genera of the order Mucorales, a feature that distinguishes it from all current azole compounds.[202]

Resistance. Several mechanisms of azole resistance in *Candida* spp. have been identified and include, but are not limited to, molecular alterations at the target binding site, increased target expression, and induction of cellular efflux pumps.[45,427,485,608] In contrast to pathogenic bacteria, genetic exchange mechanisms are largely unknown in fungi. Exposure-induced cumulative molecular events that lead to stable azole resistance have been reported; however, in the clinical setting, resistance is encountered most commonly in the form of a primarily resistant species or through selection of resistant subclones during exposure to azoles.[459,608]

Acquisition of microbiologic and clinical azole resistance was reported first in patients with chronic mucocutaneous candidiasis who were receiving long-term therapy with ketoconazole.[256] In the 1990s, before the advent of highly active antiretroviral therapy, azole-resistant oropharyngeal and esophageal candidiasis became a major clinical conundrum in patients with advanced HIV infection.[453,470] Emergence of *C. glabrata* and *C. krusei* infections in association with fluconazole prophylaxis has been observed in several bone marrow transplant centers[267,355,615,616] and in large cancer centers.[296] However, a large prospective series from Seattle showed an altogether low incidence of breakthrough candidemia (4.6%; in two thirds caused by *C. glabrata* or *C. krusei*) and a low attributable mortality (20%) in patients receiving fluconazole prophylaxis despite frequent colonization with fluconazole-resistant *Candida* spp.[355] Although cross-resistance of *Candida* spp. to antifungal azoles is a common occurrence,[46,193,383,422] it is not obligate; for example, patients with microbiologic and clinical fluconazole-resistant mucosal candidiasis may respond to itraconazole or second-generation triazoles.[235,477]

*References 6, 96, 99, 102, 207, 247.

Nonetheless, patients who have been exposed to azoles and who have breakthrough candidemia or an azole-resistant *Candida* isolate have a high likelihood of cross-resistance, and treatment with alternative agents is advised.[340,412] Acquired resistance to azoles has been documented in patients with *C. neoformans* meningitis[63,512,513] and increasing reports are being published of secondary azole resistance and azole cross resistance in filamentous fungi, especially *Aspergillus* spp.*

Fluconazole

Fluconazole is a synthetic, low-molecular-weight, water-soluble *bis*-triazole (see Fig. 239-5). In contrast to ketoconazole, the compound has similar potency at the target enzyme but is much more specific and therefore better tolerated. Fluconazole is active against *Candida* spp., *C. neoformans*, *C. gattii*, *T. asahii*, and endemic dimorphic fungi, but not against *Aspergillus* spp. and other hyaline or dematiaceous molds. Fluconazole has intermediate activity against *C. glabrata* and is inactive against *C. krusei*.[191,199,434,544]

Pharmacodynamics. Fluconazole generally is considered to be a fungistatic agent.[208] Time-kill assays performed over incubation periods of 24 to 48 hours in susceptible *Candida* spp. and *C. neoformans* showed fungistatic activity of fluconazole with variable concentration-related growth effects.[82,83,134,289,290] However, time-kill studies that used extended periods of incubation up to 14 days and nonproliferating growth conditions demonstrated direct fungicidal activity of fluconazole against *C. albicans*.[519] These observations raise the possibility that fluconazole may ultimately be able to eliminate *Candida* spp. without help from host defenses. In serum-free growth media, fluconazole displays no measurable PAFE against *C. albicans* and *C. neoformans*, but concentration-dependent PAFEs of 1 to 3.6 hours were observed in the presence of fresh serum. Finally, pretreatment of *C. albicans* with fluconazole increased its vulnerability to killing by polymorphonuclear leukocytes.[158,369]

In vivo pharmacodynamic studies of fluconazole in murine models of disseminated *C. albicans* candidiasis using the fungal burden in kidney tissue as an end point for antifungal efficacy collectively suggested that the AUC/MIC ratio is the pharmacodynamic parameter that best predicts antifungal efficacy of fluconazole[18,334,521] and that dosing regimens that produce prolonged sub-MIC concentrations are associated with development of resistance.[23] The linear pharmacokinetics and the available experimental and clinical data are in support of once-daily dosing regimens. Pharmacodynamic models and several animal studies demonstrate a correlation between the MIC of fluconazole and antifungal efficacy.† A more recent analysis of 1295 patient-episode-isolate events from 12 published clinical studies demonstrates success rates of 85 percent for those episodes in which the fluconazole MIC was 8 mg/L or less, 67 percent for those episodes in which the MIC was 16 to 32 mg/L, and 42 percent for those episodes with isolates that had an MIC of 64 mg/L or higher.[428] Of note, the additional consideration of wild-type MIC distributions and epidemiologic cutoff values has led to the proposition of species-specific clinical breakpoints that may be more sensitive to detect emerging resistance to common *Candida* species.[430]

Pharmacokinetics. Fluconazole is formulated for oral and parenteral use, and its disposition is independent of route and formulation. Fluconazole exhibits linear plasma pharmacokinetics that fit best into a two-compartment open model.[77]

Independent of food or intragastric pH, the oral bioavailability of fluconazole is greater than 90 percent. C_{max} occurs 1 to 2 hours after ingestion. Multiple dosing increases C_{max} approximately 2.5-fold. Steady state generally is reached within 4 to 7 days after once-daily dosing, but it can be achieved rapidly by doubling the dose on the first day.[191,199] Because of the free solubility in water of this drug, protein binding is low. Fluconazole distributes well into virtually all tissue sites and body fluids. The ratio of CSF and serum concentrations ranges from 0.5 to 0.9 during the dosing interval, and penetration into brain tissue and the different compartments of the eye is excellent. Fluconazole is relatively stable to metabolic conversion; more than 90 percent of a dose is excreted by the kidney, with approximately 80 percent recovered as unchanged, active drug and 11 percent recovered as inactive metabolites.[77,191,199] Because excretion of fluconazole parallels the glomerular filtration rate, the dosage must be adjusted in patients with renal impairment. A 50 percent reduction is required in patients with a creatinine clearance of 50 mL/minute or less, and a 75 percent reduction is required in patients with a creatinine clearance of less than 21 mL/min; the initial loading dose need not be adjusted.[59,199] Fluconazole is dialyzable; in patients undergoing hemodialysis, 100 percent of the target dose is given after each dialysis session. In continuous venovenous hemofiltration and hemodiafiltration, dosing at the higher end of the dosing range (i.e., 800 mg/day in adults) is suggested.[58,496] A dose of 150 mg in a single 2-L dialysate bag has been used for continuous ambulatory peritoneal dialysis.* Hepatic insufficiency per se does not require adjustments of dosing, but careful monitoring of additional hepatic toxicity is required.[472] Finally, infants requiring extracorporeal membrane oxygenation (ECMO) were shown to have a higher volume of distribution but similar clearance relative to controls not on ECMO and may require higher doses for treatment.[597]

The plasma pharmacokinetic properties of fluconazole in pediatric age groups reflect developmental changes in the volume of distribution and clearance that are characteristic for a highly water-soluble drug with minor metabolism and predominantly renal elimination (Table 239-7). Except for premature neonates, in whom clearance initially is decreased, pediatric patients tend to have an increased weight-normalized clearance rate from plasma that leads to a shorter half-life in contrast to that

*References 95, 129, 378, 427, 517, 561.
†References 14, 42, 411, 458, 467, 580.

*References 176, 191, 384, 396, 495, 541.

TABLE 239-7 **Pharmacokinetic Parameters of Fluconazole in Pediatric Patients***

Age Group	Vd_{ss} (L/kg)	Cl (L/hr/kg)	$t_{1/2}\beta$ (hr)
Preterm <1500 g			
Day 1	1.18	0.010	88
Day 6	1.84	0.019	67
Day 12	2.25	0.031	55
Term neonates	1.43	0.036	28
Infants >1-6 mo	1.02	0.037	19
Children, 5-15 yr	0.84	0.031	18
Adult volunteers	0.65	0.015	30

Data from references 76, 308, 320, 385, 490, and 501.
*Data represent mean values.
Cl, Total plasma clearance; $t_{1/2}\beta$, elimination half-life; Vd_{ss}, apparent volume of distribution at steady state.

in adults.* Whereas population-based pharmacokinetic studies do not exist for children, gestational age at birth, postnatal age, and creatinine level were identified as parameters of impact on clearance of fluconazole in premature infants.[573,574] Because exposure over the course of time appears to be the most predictive pharmacodynamic parameter,[18,334] fractionating the dose is not required in infants and children, despite the shorter half-life in these age groups.

Adverse Effects. In adults, fluconazole has been administered safely over prolonged periods at dosages of up to 1200 mg/day; dose-escalation to 1600 mg/day resulted mainly in increased hepatotoxicity, and dose-limiting neurotoxicity was observed at 2000 mg/day.[15] Compiled data from adult patients who received the drug at dosages of 100 to 400 mg/day over the course of at least 7 days indicate an overall incidence of at least possibly related adverse effects of 16 percent; significant adverse effects or laboratory abnormalities leading to the discontinuation of the drug were noted in overall 2.8 percent.[199] Nausea, vomiting, and other gastrointestinal symptoms are seen in fewer than 5 percent and skin rashes and headaches in fewer than 2 percent and usually reversible; asymptomatic hepatic transaminase elevations were reported in as many as 7 percent of adult patients.[105,199]

In pediatric patients of all age groups, at dosages up to 12 mg/kg/day, fluconazole generally is well tolerated. The most common reported side effects in pediatric patients include gastrointestinal disturbances (8%), increases in hepatic transaminases (5%), and skin reactions (1%); toxicity-related discontinuation of therapy with fluconazole occurs in approximately 3 percent of patients.[398] Severe side effects, including severe hepatotoxicity and exfoliative skin reactions, have been rarely reported in association with fluconazole therapy.[105,206]

Drug Interactions. Fluconazole undergoes minimal CYP450-mediated metabolism; it inhibits CYP3A4 and several other CYP450 isoforms in vitro and interacts with enzymes involved in glucuronidation, thus leading to certain significant drug–drug interactions.[105,205,222,440] Most important, concurrent therapy with cisapride, pimozide, quinidine, erythromycin, and newer antihistamines results in inhibition of the metabolic pathways of these drugs and potentially serious cardiac arrhythmias and is therefore strictly contraindicated.[207] By similar mechanisms, fluconazole can precipitate phenytoin toxicity,[371] may lead to increased plasma concentrations of cyclosporine and tacrolimus,[318,408] and may potentiate the effects of warfarin, sulfonylurea drugs, rifabutin (in particular, short-acting benzodiazepines and carbamazepine), and nifedipine.[295,318,403,482] Fluconazole also may interfere with the plasma clearance of cyclophosphamide, a widely used anticancer agent that must be metabolized by CYP450 enzymes to produce alkylating species. This interaction may potentially result in a relevant reduction in the therapeutic efficacy of cyclophosphamide.[625] Further potential interactions with anticancer drugs have been observed with busulfan[81] and all-*trans*-retinoic acid.[497]

Clinical Indications. The clinical indications for fluconazole are summarized in Tables 239-2, 239-4, 239-15, and 239-16. Fluconazole is highly effective against superficial infections caused by dermatophytes and *Pityrosporum* spp.,[173,225] and it has excellent activity in the treatment of mucosal candidiasis, including vaginal, oropharyngeal, esophageal, and chronic mucocutaneous candidiasis.[166,219,242,348]

Several controlled studies including both neutropenic and non-neutropenic adult patients indicated that intravenous fluconazole 400 to 800 mg/day is as effective as AmB-D 0.5 to 1 mg/kg/day against candidemia and other forms of invasive candidiasis, but it is better tolerated.* Fluconazole thus can be used for invasive *Candida* infections caused by susceptible organisms in patients who are in stable condition.[415,459] In patients who have received antifungal azoles for prophylaxis, however, breakthrough infections are likely to be caused by fluconazole-resistant *Candida* spp.,[308,355] and alternative agents are recommended. The recommended pediatric dosage range of fluconazole beyond the neonatal period is 6 to 12 mg/kg/day; in view of the faster clearance rate and the absence of population pharmacokinetics, however, 12 mg/kg/day may be the most appropriate dosage.[100,204]

Fluconazole is a useful agent for treatment of invasive *Candida* infections in the neonatal setting. In six published series including 10 or more patients with proved invasive *Candida* infections, treatment with fluconazole at a daily dosage of usually 5 to 6 mg/kg was successful in 83 to 97 percent, and crude mortality ranged from 10 to 33 percent. In none of the 125 patients was fluconazole discontinued because of toxicity.† For infants, population-based pharmacokinetics and Monte Carlo simulation showed that for the treatment of invasive candidiasis, a

*References 76, 308, 320, 385, 490, 501, 537.

*References 12, 16, 415, 435, 457, 459.
†References 64, 72, 148, 149, 163, 263, 575.

dose of at least 12 mg/kg/day in the first 90 days after birth is needed to achieve an AUC/MIC index greater than 50 for *Candida* spp. with MIC less than 8 μg/mL in 90 percent or more of infants at less than 30 weeks' gestation and 80 percent of infants at 30 to 40 weeks' gestation, respectively; according to the model, infants with serum creatinine of 1.3 mg/dL or greater have a slower clearance rate and dose adjustment is indicated if creatinine does not improve within 96 hours.[573,574] A loading dose of 25 mg/kg has been advocated for rapid attainment of target therapeutic drug concentrations.[438]

Fluconazole has been useful in the treatment of focal *Candida* urinary tract infections and uncomplicated funguria, and it has been used successfully in *Candida* peritonitis, endocarditis, osteomyelitis, meningitis, and endophthalmitis.[207,323] Further potential indications for fluconazole beyond the treatment of acute invasive *Candida* infections include consolidation therapy for chronic disseminated candidiasis[11,281] and cryptococcal meningitis.[476,557] High-dose fluconazole is an option for systemic infections caused by the yeast *T. asahii*.[13,203] Fluconazole is the current drug of choice for treatment of coccidioidal meningitis,[178,483] and it has proved effectiveness in nonmeningeal coccidioidal infections.[91,206] Fluconazole appears comparatively less active than itraconazole in the treatment of paracoccidioidomycosis, blastomycosis, histoplasmosis, and sporotrichosis.*

Fluconazole can prevent mucosal candidiasis in patients with HIV infection or cancer,[214,397,443] and it has proved efficacy in preventing invasive *Candida* infections in high-risk patients with acute leukemia or with bone marrow or liver transplants.[196,468,619] Fluconazole, given at 400 mg once daily to adults from the start of the conditioning regimen until day 75, can reduce the frequency of invasive *Candida* infections and lower mortality at day 110 after allogeneic bone marrow transplantation,[515] and it also may provide persistent protection against invasive candidiasis and *Candida*-related death. Fluconazole can decrease the frequency of severe, gut-related graft-versus-host disease (GVHD), and it has an independent overall survival benefit of 17 percent at 8 years after the end of treatment.[354] Fluconazole also has been shown to reduce the incidence of *Candida* infections in low-birth-weight infants.† Thus, fluconazole prophylaxis is a valid option for centers with a high frequency (>10%) of invasive *Candida* infections in low-birth-weight infants of less than 1000 g or in the setting of a nosocomial outbreak by a fluconazole-susceptible *Candida* spp. Population pharmacokinetics and Monte Carlo simulation support a dose of 3 or 6 mg/kg twice weekly during the first 42 days of life for early prevention of candidiasis in 23- to 29-week-old infants and a 6-mg/kg dose every 72 hours or 3 mg/kg daily for late prevention.[573,574]

Itraconazole

Itraconazole is a high-molecular-weight, highly lipophilic triazole (see Fig. 239-5). Structurally closely related to ketoconazole, it has a broader spectrum of antifungal activity that includes dermatophytes, *Candida* spp. (*C. krusei* excluded), *C. neoformans*, and *C. gattii*, *Aspergillus* spp., various dematiaceous molds, and the dimorphic fungi. Itraconazole binds more avidly to its fungal target than does ketoconazole, but more weakly to human CYP450, thus leading to comparatively fewer mechanism-associated adverse effects.[198]

Pharmacodynamics. In vitro, itraconazole exerts species- and strain-dependent fungistatic or fungicidal pharmacodynamics. Time-kill experiments in serum-free and serum-containing media demonstrated the concentration-independent, fungistatic activity of itraconazole against *Candida* spp. and *C. neoformans*.[82,83,176,626] However, against *Aspergillus* spp., itraconazole displayed time- and concentration-dependent fungicidal activity with 87 to greater than 97 percent killing within 24 hours of exposure to the drug.[342] Persistent effects have not been reported thus far.

The principal feasibility of a correlation between in vitro susceptibility and outcome was demonstrated in mice with experimental disseminated aspergillosis.[130] Relationships between drug concentrations and antifungal efficacy of itraconazole were assessed in a model of invasive pulmonary aspergillosis in methylprednisolone/cyclosporine-immunosuppressed rabbits. In this model, an inhibitory sigmoid maximum effect model predicted a significant pharmacodynamic relationship (r = .87, p <.001) between itraconazole concentrations in plasma and antifungal efficacy as a function of the burden of *A. fumigatus* in lung tissue.[56]

In patients, however, the main rationale for monitoring plasma levels has been the erratic oral bioavailability of itraconazole, particularly in neutropenic patients. Historically, the target plasma level for itraconazole has been estimated at 0.25 mg/L (high-performance liquid chromatography [HPLC]) at trough, based on the concentration that inhibits 90 percent of a large set of clinical isolates.[70,122] Later, the predictive value of threshold concentrations of prophylactic itraconazole in a large cohort in patients undergoing intensive chemotherapy for acute leukemia was found to be 0.5 mg/L at trough (HPLC) by means of multivariate logistic regression analysis.[188]

In a phase I and II clinical trial of oral cyclodextrin-itraconazole in HIV-infected children with oropharyngeal candidiasis, the relationships between pharmacodynamic parameters and therapeutic response as assessed by standardized scoring of mucosal disease after 14 days of therapy fitted to inhibitory maximum effect pharmacodynamic models. Best fits were observed: AUC, AUC/MIC, C_{max}, and C_{max}/MIC (r = .483 to .595; p <.01).[215]

Pharmacokinetics. Itraconazole is available as capsules and as oral solution in hydroxypropyl-β-cyclodextrin (HP-β-CD). Production of the parenteral solution[5] that also used HP-β-CD as solvent has been discontinued. Absorption of itraconazole from the capsule form depends on a low intragastric pH and is compromised in the fasting state, and it becomes erratic in patients with cancer and granulocytopenia or hypochlorhydria.[105,206,207] Absorption can be somewhat improved when the capsules

*References 135, 177, 228, 280, 282, 413, 414, 604.
†References 60, 232, 273, 283-285, 346, 490, 551.

are taken with food or an acidic beverage.[121,206] The oral solution of itraconazole in HP-β-CD has improved oral bioavailability that is further enhanced in the fasting state; however, this formulation may cause carrier-associated gastrointestinal intolerance.[46,460]

After oral administration, C_{max} is measured within 1 to 4 hours; following once-daily dosing, steady state is achieved after 7 to 14 days.[198,206,230] In adult patients with cancer who were receiving the standard regimen of 2.5 mg/kg of HP-β-CD itraconazole twice daily, mean trough levels were 0.8 mg/L under conditions of steady state[444]; systemic absorption of the carrier was negligible.[122] After administration of the parenteral solution, drug and carrier rapidly dissociated and followed their own disposition; after the recommended regimen of 200 mg twice daily for 2 days followed by 200 mg daily for 5 days, mean trough levels were 0.53 μg/mL. The carrier HP-β-CD was not significantly metabolized, and virtually 100 percent was eliminated from plasma within 24 hours in unchanged form through glomerular filtration.[206]

Itraconazole exhibits dose-dependent pharmacokinetics with nonlinear increases in the AUC with increasing and split dosages.[198,215,444] Itraconazole is highly (95%) protein-bound; only 0.2 percent circulates as free drug.[243] Although concentrations in nonproteinaceous body fluids are negligible, tissue concentrations in many organs, including the brain, may exceed corresponding plasma levels by 2 to 10 times.[121,243]

Itraconazole is metabolized extensively in the liver by numerous pathways to more than 30 metabolites and is excreted in metabolized form into bile and urine. The major metabolite, hydroxyitraconazole, possesses antifungal activity similar to that of itraconazole. It is eliminated more rapidly, but its plasma concentrations at steady state are 1.5 to 2 times higher than those of the parent compound.[122,206,504] The elimination half-life of the compound is 20 to 24 hours after single dosing and 35 to 40 hours under terms of steady state, a finding reflecting saturable excretion mechanisms.[330] The dosage of oral itraconazole does not need to be adjusted in patients with renal insufficiency or dialysis.[372] In patients with severe hepatic insufficiency, the elimination half-life of itraconazole can be prolonged and additional hepatic toxicity or possible drug interactions should be monitored carefully.

Several studies have investigated the pharmacokinetics of the oral HP-β-CD solution of itraconazole in pediatric patients (Table 239-8).[133,215,237,493] In 26 infants and children aged 6 months to 12 years with cancer ($n = 20$) or liver transplantation who received the compound at 5 mg/kg once daily, plasma concentrations were substantially lower than those reported in adult patients with cancer, particularly in children younger than 2 years of age.[133,444] In 16 neutropenic children (1.7 to 14.3 years of age) who received HP-β-CD itraconazole for antifungal prophylaxis in a split dosing regimen of 2.5 mg/kg twice daily, peak and trough levels of itraconazole were substantially higher; nonetheless, a similar trend toward lower plasma concentrations occurred in the group of children 5 years of age and younger.[493] In a cohort of 26 HIV-infected children and adolescents (1.25 to 18 years), HP-β-CD itraconazole was safe and effective for treatment of oropharyngeal candidiasis at dosages of 2.5 mg once per day or 2.5 mg twice daily given for at least 14 days.[215] Many (77%) of the patients were receiving concomitant therapy with protease inhibitors or clarithromycin, drugs that are strong inhibitors of the CYP3A4-dependent metabolism of itraconazole.[440] Peak and trough levels measured after administration of the split-dosage regimen were similar to those observed in patients with cancer who were receiving the same dosage regimen.[493] Of note, population-based pharmacokinetics in pediatric cystic fibrosis and allogeneic bone marrow

TABLE 239-8 **Pharmacokinetics of Itraconazole After Administration of Hydroxypropyl-β-Cyclodextrin Oral Solution to Immunocompromised Infants and Children***

	Children with Cancer/ Liver Transplant† (n = 8, 0.5-2 yr) 5 mg/kg daily × 14 days	Children with Cancer† (n = 7, 2-5 yr) 5 mg/kg daily × 14 days	Children with Cancer† (n = 11, 6-12 yr) 5 mg/kg daily × 14 days	Children with Cancer‡ (n = 9, 2-5 yr) 2.5 mg/kg bid × 14 days	Children with Cancer‡ (n = 6, 6-12 yr) 2.5 mg/kg bid × 14 days
Itraconazole					
C_{max} (μg/mL)	0.571 ± 0.416	0.534 ± 0.431	0.631 ± 0.358	1.024 ± 0.351	1.524 ± 0.770
T_{max} (hr)	1.9 ± 0.1	2.9 ± 2.5	3.1 ±2.1	N/A	N/A
C_{min} (μg/mL)	0.159 ± 0.218	0.179 ± 0.100	0.233 ± 0.14	0.711 ± 0.251	1.072 ± 0.408
$AUC_{0-\infty}$ (μg/mL/hr)	6.930 ± 5.83	7.33 ± 5.42	8.77 ± 5.05	N/A	N/A
$t_{1/2}\beta$ (hr)	47.4 ± 55.0	30.6 ±25.3	28.3 ± 9.6	N/A	N/A
Accumulation factor	6.2 ± 5.0	3.3±3.0	8.6 ± 7.4	N/A	N/A

*Pharmacokinetic parameters were obtained after daily dosing over 14 days. All values represent mean values ± SD.
†Data from de Repentigny L, Ratelle J, Leclerc JM, et al. Repeated-dose pharmacokinetics of an oral solution of itraconazole in infants and children. Antimicrob Agents Chemother 1998;42:404-8.
‡Data from Schmitt C, Perel Y, Harousseau J, et al. Pharmacokinetics of itraconazole oral solution in neutropenic children during long-term prophylaxis. Antimicrob Agents Chemother 2001;45:1561-4.
Accumulation factor, AUC_{0-24} day 14/$AUC_{0-\infty}$ day 1; $AUC_{0-\infty}$, area under the concentration versus time curve from zero to infinity; C_{max}, peak plasma levels; C_{min}, minimum plasma levels; N/A, not assessed; $t_{1/2}\beta$, elimination half-life; T_{max}, time until occurrence of peak plasma concentration.

transplant patients receiving the oral solution or the capsule formulation as antifungal prophylaxis, however, suggest a starting dose of 5 mg/kg twice daily.[237]

Despite the tremendous interpatient variability that results from both variable absorption and hepatic metabolism, the pharmacokinetic properties of itraconazole in pediatric patients appear not to be fundamentally different from those of adults. A starting dosage of 2.5 mg/kg twice daily can be advocated, based on the available pharmacokinetic data.[133,215,493] Trough levels should be monitored, and dosing should be adjusted to maintain plasma concentrations of the parent itraconazole of greater than 0.5 mg/L by HPLC.[595] Of note, itraconazole is not formally approved for pediatric age groups.

Adverse Effects. Itraconazole usually is well tolerated, with a pattern similar to that of fluconazole and a frequency of adverse effects approximately identical to that seen with fluconazole.[105] In 189 patients treated for systemic fungal infections at dosages of 50 to 400 mg/day for a median of 5 months, the rate of possibly or definitely related adverse effects was 39 percent.[549] Most of the observed reactions were transient and included nausea and vomiting (<10%), hypertriglyceridemia (9%), hypokalemia (6%), elevated hepatic transaminases (5%), rash or pruritus (2%), headaches or dizziness (<2%), and pedal edema (1%). Four percent of patients discontinued itraconazole treatment because of adverse effects. Gastrointestinal intolerance is the dose-limiting toxicity of the oral HP-β-CD formulation. In a comparative study in adult patients with acute leukemia, 46 percent of patients receiving a daily dose of 800 mg stopped treatment early because of severe nausea and vomiting. Crossover to the identical dose of the capsule formulation was well tolerated by all patients; patients receiving 400 mg/day of the solution had no gastrointestinal adverse effects.[187] Only a few cases of more severe hepatic injury or hepatitis have been described.[316] Itraconazole can have negative inotropic effects; because of a low but possible risk for cardiac toxicity, itraconazole should not be administered to patients with ventricular dysfunction.[7]

Cyclodextrin-itraconazole solution was safe and well tolerated for at least 14 days in reported phase I and II pharmacokinetic studies in immunocompromised pediatric patients.[133,215,493] Vomiting (12%), abnormal liver function tests (5%), and abdominal pain (3%) were the most common adverse effects considered definitely or possibly related to HP-β-CD itraconazole solution in an open study in 103 neutropenic pediatric patients with cancer who received the drug at 5 mg/kg daily or 2.5 mg/kg twice daily for antifungal prophylaxis for a median duration of 37 days; 18 percent of patients withdrew from the study because of adverse events.[168] In another report on pediatric patients with cancer who were receiving prophylactic oral itraconazole, adverse effects that led to the cessation of the itraconazole prophylaxis occurred in 11 percent of all 44 courses.[509]

Drug Interactions. In contrast to fluconazole, both the propensity for and the extent of drug–drug interactions are greater.[206,212] Itraconazole is a substrate of CYP3A4, but it also interacts with the heme moiety of CYP3A, thus resulting in noncompetitive inhibition of oxidative metabolism of many CYP3A substrates. An interaction also can result from inhibition of P-glycoprotein–mediated efflux; P-glycoprotein is extensively co-localized and exhibits overlapping substrate specificity with CYP3A.[205,222] Inhibition of hepatic CYP450 enzyme systems may lead to increased and potentially toxic concentrations of coadministered drugs. Most important, the coadministration of cisapride, pimozide, bepridil, mizolastine, terfenadine, astemizole, oral midazolam, quinidine, dofetilide, triazolam, sertindole, eletriptan, nisoldipine, and levacetylmethadol with itraconazole can lead to serious cardiac arrhythmias and is thus strictly contraindicated.[205,249,440] Similarly contraindicated is the coadministration of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA)-inhibitor cholesterol-lowering agents such as atorvastatin, lovastatin and simvastatin, which are associated with rhabdomyolysis,[205,394] and ergot alkaloids metabolized by CYP3A4, which may result in ergotism. Potentially toxic levels of the coadministered drug also can be reached when itraconazole is given along with phenytoin, carbamazepine, benzodiazepines, cyclosporine, tacrolimus, sirolimus, methylprednisolone, budesonide, digoxin, warfarin, sulfonylurea compounds, ritonavir, indinavir, haloperidol, clarithromycin, verapamil, felodipine, busulfan, and vinca alcaloids.* Increased metabolism of itraconazole resulting in decreased plasma levels can be induced by rifampin, rifabutin, isoniazid, carbamazepine, phenobarbital, and phenytoin.[205,440,548] As a consequence, patients who receive itraconazole along with one of the listed drugs should be followed closely and plasma concentrations of ideally both compounds, as well as hepatic function, should be monitored carefully.

Clinical Indications. The clinical indications for itraconazole are summarized in Tables 239-2 to 239-4, 239-15, and 239-16. Itraconazole is a useful agent for dermatophytic infections, pityriasis versicolor,[3,172,198,227] vaginal candidiasis,[523] and oropharyngeal and esophageal candidiasis.[1,133,215,477] Although the experience with itraconazole in the primary treatment of cryptococcal meningitis is scant, itraconazole has been used with success for long-term treatment of cryptococcal meningitis in patients with HIV infection.[476,557]

Itraconazole may be a second-line option for treatment of invasive *Aspergillus* infections, in particular as maintenance or consolidation therapy in non-neutropenic patients.[132,525] Beyond invasive aspergillosis, itraconazole may be useful in the management of infections by certain dematiaceous molds.[202,507] Itraconazole is a current treatment of choice for lymphocutaneous sporotrichosis[123,280,455] and non–life-threatening, nonmeningeal paracoccidioidomycosis, blastomycosis, and histoplasmosis in non-immunocompromised patients,† as well as induction and maintenance therapy of mild to moderate, nonmeningeal histoplasmosis in HIV-infected patients.[602,603,605] Although earlier uncontrolled clinical trials suggested a somewhat inferior efficacy against nonmeningeal and

*References 68, 205, 301, 403, 439, 480, 562.
†References 97, 137, 386, 392, 536, 605.

meningeal coccidioidomycosis in comparison with fluconazole,[200,546,547] a randomized, double-blind comparative study in patients with progressive, nonmeningeal coccidioidomycosis showed a trend toward slightly greater efficacy when either of the drugs were given at a daily dosage of 400 mg.[179] Nonetheless, amphotericin B remains the treatment of choice for endemic mycoses in most immunocompromised patients and for those with life-threatening infections.[207]

Prophylactic itraconazole may reduce the incidence of proved or suspected invasive fungal infections in patients with hematologic malignancies[364] and those who have undergone HSCT.[352,618] Efficacy in the prevention of invasive aspergillosis is supported by a large meta-analysis,[189] but not by a randomized, comparative trial. Finally, itraconazole was at least as effective as conventional amphotericin B and was superior with respect to its safety profile when it was investigated as empirical antifungal therapy in persistently neutropenic patients with cancer.[71]

Posaconazole

Posaconazole (see Fig. 239-6) is a novel lipophilic antifungal triazole with potent and broad-spectrum activity against opportunistic, endemic, and dermatophytic fungi in vitro. This activity extends to organisms that often are refractory to existing triazoles or amphotericin B or to echinocandins such as *C. glabrata*, *C. krusei*, *A. terreus*, and *Fusarium* spp. Posaconazole also possesses activity against some members of the order Mucorales both in vitro and in vivo, a feature that distinguishes it from all other available azoles.[161,202,479,538]

Pharmacodynamics. Posaconazole is considered fungistatic against *Candida* and fungicidal against *Aspergillus* spp.[202] In time-kill assays, posaconazole showed concentration- and time-dependent fungicidal activity against *A. fumigatus*.[216,343,344] In vivo, in a neutropenic kidney target murine model of *C. albicans* infection and in an inhalational murine model of invasive pulmonary aspergillosis, the AUC/MIC ratio was the parameter predictive of antifungal efficacy.[24,259] In a phase II clinical trial in patients with invasive aspergillosis, higher average plasma concentrations of posaconazole were associated with higher response rates.[589]

Pharmacokinetics. Posaconazole currently is available as oral suspension only and achieves optimal exposure when it is administered in two to four divided doses given with food or a nutritional supplement; common gastrointestinal comorbidities in leukemia- and hematopoietic stem cell transplant patients may negatively affect absorption.[305,306,307] The oral suspension has dose-proportional pharmacokinetics in the 50- and 800-mg dose range, with saturation of absorption occurring at doses higher than 800 mg; after repeat dosing, steady state is achieved after 7 to 10 days with a sixfold to eightfold accumulation of plasma concentrations[114] and marked accumulation within alveolar cells.[106] Posaconazole has a large volume of distribution in the order of 5 L/kg and a prolonged elimination half-life of approximately 20 hours.

TABLE 239-9 **Principal Pharmacokinetic Properties of Posaconazole and Voriconazole**

	Posaconazole	Voriconazole
Formulation	PO	PO/IV
Dose linearity	Yes	No
Oral bioavailability (%)	>50	>90
Protein binding (%)	>95	58
Volume of distribution (L/kg)	>5	2
Elimination half-life (hr)	25	6
Substrate/inhibitor of CYP450	3A4	3A4, 2C9, 2C19
Elimination through		
Feces (%/% metabolites)	77/-	<20/?
Urine (%/% metabolites)	14/14	80/78

Data from references 114, 115, 212, 304, 446, and 601.

Posaconazole is metabolized to a small extent by uridine diphosphate (UDP) glucuronidation; it is not significantly metabolized through the CYP450 enzyme system but is primarily excreted in unchanged form in the feces (Table 239-9).[202,304,601] Posaconazole is a substrate and inhibitor of P-glycoprotein. However, investigation of the relationship between human multidrug resistance gene *(MDR1)* mRNA expression and posaconazole exposure showed no correlation. Thus, the moderate variability in the compound's pharmacokinetics is unlikely to be caused by interindividual differences in P-glycoprotein expression.[115]

Adverse Effects. The overall safety of posaconazole was assessed in two open-label clinical trials of more than 400 patients with invasive fungal infections who received posaconazole suspension 800 mg/day in divided doses. Treatment-related adverse events occurred in 38 percent of patients (164/428); the most common were nausea (8%), vomiting (6%), headache (5%), abdominal pain (4%), and diarrhea (4%). Treatment-related abnormal liver function test results were observed in 3 percent of patients. No clinically significant differences occurred in mean QTc interval change from baseline. Serious adverse events considered possibly or probably related to posaconazole occurred in 35 (8%) patients. The most common severe adverse events were altered drug level, increased hepatic enzymes, nausea, rash, and vomiting (1% each). No significant trends related to age, sex, or race were observed, and no unique treatment-related adverse events were identified in patients during long-term exposure (>6 months) in contrast to those identified during shorter-duration therapy.[421,449] In two large, prospective, randomized comparative clinical trials investigating posaconazole for prevention of invasive fungal infections in high-risk patients with leukemia or HSCT, posaconazole was well tolerated, and the rate of study drug discontinuations in posaconazole-treated patients was not different from that in subjects in the control cohort receiving fluconazole or itraconazole.[111,552]

Drug Interactions. Posaconazole is not significantly metabolized through the CYP450 enzyme system; therefore, CYP450-mediated drug–drug interactions have limited potential to affect posaconazole pharmacokinetics.[304] Posaconazole is a potent inhibitor of cytochrome P3A4, but it has no effect on 1A2, 2C8, 2C9, 2D6, or 2E1 isoenzymes.[601] Concomitant administration of posaconazole with terfenadine, astemizole, cisapride, pimozide, halofantrine, and quinidine can lead to serious cardiac arrhythmias and is thus strictly contraindicated.[486,487] Similarly contraindicated is the coadministration of HMG CoA–inhibitor cholesterol-lowering agents such as atorvastatin, lovastatin, and simvastatin, which are associated with rhabdomyolysis, and ergot alkaloids metabolized by CYP3A4, which may result in ergotism. Potentially toxic levels of the coadministered drug can be reached when posaconazole is given along with phenytoin, carbamazepine, benzodiazepines, cyclosporine, tacrolimus, sirolimus, methylprednisolone, digoxin, warfarin, sulfonylurea compounds, ritonavir, indinavir, atazanavir, haloperidol, clarithromycin, verapamil, nifedipine, diltiazem, nisoldipine, rifabutin, and vinca alkaloids. Because of a relevant decrease in posaconazole concentrations, concomitant use with rifabutin, phenytoin, efavirenz, fosamprenavir, cimetidine, or esomeprazole should be avoided.[80,486,487]

Clinical Efficacy. Posaconazole has been targeted for prevention and treatment of serious infections caused by opportunistic and endemic fungal organisms. Posaconazole has demonstrated strong antifungal efficacy in phase II clinical trials in immunocompromised patients with primary or refractory oropharyngeal and esophageal candidiasis.[514,564] In a randomized, comparative phase III trial, posaconazole 100 mg daily (with day 1 dose 200 mg) was as effective as was fluconazole 100 mg daily (with day 1 dose 200 mg), in the primary treatment of HIV-associated oropharyngeal candidiasis: 155/169 (91.7%) patients receiving posaconazole in contrast to 148/160 (92.5%) of patients receiving fluconazole achieved a complete clinical response (cure). The relapse rate at day 42 was greater in fluconazole recipients than in posaconazole-treated patients (38.2% vs. 31.5%; $p = .038$).[565] Posaconazole 800 mg in divided doses also was investigated as salvage therapy in a large phase II study including 330 patients with invasive fungal infections intolerant to or refractory to standard therapies and a contemporaneous external control of 279 patients.[448] Most patients (86%) were refractory to previous therapy. Successful outcomes at the end of treatment in the posaconazole cohort and in the contemporaneous external control cohort were 42 versus 26 percent in aspergillosis (107 and 86 patients; separately published[589]), 39 versus 50 percent in fusariosis (18 vs. 4 patients), 56 versus 50 percent in zygomycoses (11 vs. 8 patients), 69 versus 43 percent (16 vs. 7 patients) in coccidioidomycosis, 52 versus 53 percent in candidiasis (23 vs. 30 patients), 48 versus 58 percent in cryptococcosis (31 vs. 64 patients), 81 versus 0 percent in chromoblastomycosis (11 vs. 2 patients), and 64 versus 60 percent in other invasive fungal infections (30 vs. 20 patients). In a subset analysis of 39 patients with mostly refractory proved or probable CNS infections, posaconazole proved efficacious against cryptococcal meningitis (success rate in 29 patients [48%]) and against infections caused by opportunistic or endemic molds (5/10 patients [50]).[439,448] These data are in agreement with the compound's extended spectrum of activity in vitro and demonstrate efficacy for treatment of refractory invasive opportunistic and endemic mycoses. A retrospective analysis of the manufacturer's compassionate-use program including 91 patients with proved or probable mucormycosis refractory or intolerant to prior antifungal therapy revealed a 60 percent success rate (complete and partial responses) at 12 weeks after initiation of therapy, thus providing first evidence for the clinical utility of posaconazole as second-line or consolidation therapy of mucormycosis.[554] Posaconazole has also been shown in a randomized, placebo- and active-controlled multicenter trial to be effective against toenail onychomycosis.[156]

Two pivotal preventive randomized phase III studies in high-risk patients with HSCT and GVHD[552] and acute leukemias[111] have been completed. In the first study, patients received either posaconazole 200 mg three times daily or fluconazole 400 mg daily, respectively, with the start of immunosuppression for a total of 16 weeks. Treatment with posaconazole led to a decreased incidence of invasive fungal infections at 16 weeks (5% vs. 9%; $p = .07$), with a statistically significant decrease in invasive *Aspergillus* infections (2% vs. 7%; $p = .006$). Seven days after end of treatment, fewer patients had invasive fungal disease (2% vs. 8%; $p = .004$) and fewer patients had invasive aspergillosis (1% vs. 6%; $p = .001$). No differences in overall mortality rates occurred at 12 weeks.[552] In the second study, patients received posaconazole 200 mg three times daily and either fluconazole 400 mg daily or itraconazole 200 mg twice daily, respectively. Treatment was started with each cycle after a drop of the absolute neutrophil count (ANC) to 500/μL or less for up to 12 weeks. Significantly fewer patients enrolled in the posaconazole arm of the trial developed an invasive fungal infection at day 7 after the end of treatment in contrast to the comparator arm (2% vs. 8%; $p = .01$); most important, treatment with posaconazole resulted in a significant decrease in the rate of invasive aspergillosis (1% vs. 7%; $p = .001$). At day +100 after randomization, the rate of invasive fungal infections was 5 and 11 percent ($p = .01$), respectively, and patients treated with posaconazole had a significantly improved survival probability ($p = .035$).[111] These two important studies demonstrate the preventive efficacy of posaconazole, in particular against invasive *Aspergillus* infections in high-risk patients, and a statistically significant survival benefit in patients with acute myeloblastic leukemia/myelodysplastic syndrome who are undergoing remission induction chemotherapy.

Approval Status and Dosing. In the United States, posaconazole is approved for treatment of oropharyngeal candidiasis, including disease refractory to itraconazole or fluconazole, and for prophylaxis of invasive *Candida* and *Aspergillus* infections in high-risk patients 13 years of age and older with HSCT and GVHD or those with hematologic malignancies and prolonged neutropenia. In the European Union, the compound also is approved for treatment of aspergillosis, fusariosis,

chromoblastomycosis, and coccidioidomycosis refractory to or intolerant of standard therapies in patients 18 years of age and older (see Tables 239-3, 239-4, and 239-16).

The recommended dose for primary treatment of oropharyngeal candidiasis is 100 mg/day (with day 1 dose 100 mg twice daily) and 400 mg twice daily for refractory disease; for prophylaxis of invasive *Candida* and *Aspergillus* infections, the recommended dose is 200 mg three times daily. The dose for salvage treatment is 400 mg twice daily given with food; for patients not tolerating solid food, a dose of 200 mg four times daily is recommended, preferentially together with a nutritional supplement.

Current data indicate no need for adjustments to dosage based on differences in age, gender, race,[488] or renal or hepatic function.[113,116] The pharmacokinetic properties of posaconazole in pediatric patients (<18 years of age) have not been systematically studied. Limited data obtained in 12 pediatric patients 8 years of age and older indicated no fundamental differences in trough plasma concentrations in contrast to that of adults.[307] A twice-daily dosing algorithm based on allometric scaling for posaconazole resulted in adequate exposure in children with chronic granulomatous disease.[599] Successful salvage treatment with posaconazole has been reported in 5/11 and 9/15 pediatric patients with invasive fungal infections, which appears similar to the outcome in the adult population.[67,321] A pediatric development program has been initiated by the manufacturer to define dosages, safety, and tolerance in pediatric patients beyond the neonatal period. In addition, a different oral formulation and an intravenous formulation are in advanced stages of development in the adult population.

Voriconazole

Voriconazole (see Fig. 239-6) is a synthetic antifungal triazole with activity against a wide spectrum of clinically important yeasts and molds, including *Candida* spp., *C. neoformans*, *Aspergillus*, and other hyaline molds, dematiaceous molds, and dimorphic molds, both in vitro and in animal models. A notable exemption is the Mucorales, against which voriconazole is intrinsically inactive.[212,271,272,380]

Pharmacodynamics. Against *Candida* spp. and *C. neoformans*, voriconazole exhibited non–concentration-dependent fungistatic activity; against *A. fumigatus*, voriconazole displayed time-dependent fungicidal activity.[288,342] A concentration-dependent PAFE of 0.2 to 4.1 hours has been observed in the presence of serum against *C. albicans*.[180] In a murine kidney target model of disseminated candidiasis, the AUC/MIC ratio was the pharmacodynamic parameter that correlated best with efficacy. Using 10 *C. albicans* isolates of different voriconazole susceptibilities, the free drug AUC/MIC ratios were similar to all the organisms studied and similar to those observed for other azoles.[25] The AUC/MIC ratio also correlates with antifungal activity in vitro against *A. fumigatus*.[268] Concentration-response relationships of voriconazole in patients were analyzed in a total of 825 subjects from nine previously published clinical trials using logistic regression and Monte Carlo simulation. Whereas logistic regression analysis showed a nonlinear concentration-response relationship with the probability lower at the extremes, Monte Carlo simulation revealed that a trough/MIC ratio of 2:5 was associated with a near-maximal probability of response and that this parameter can be used as the exposure target, on the basis of either an observed MIC or reported MIC_{90} values of the suspected fungal pathogen(s).[545]

Pharmacokinetics. Voriconazole is available in oral and intravenous formulations; oral bioavailability exceeds 90 percent in adults in the fasted state, but it is considerably lower (65%) in children.[577] In adults, the compound has nonlinear pharmacokinetics. Plasma protein binding is 58 percent, and the mean volume of distribution accounts for 2 L/kg. Tissue and CSF levels may exceed those of trough plasma levels severalfold. The plasma half-life is 6 hours, with elimination occurring primarily by oxidative hepatic metabolism to at least eight metabolites that are eliminated through the urine; less than 2 percent of a dose of voriconazole is excreted unchanged in urine. The major isoenzyme involved in voriconazole metabolism is CYP2C19, but CYP2C9 and CYP3A4 also contribute (see Table 239-9). In addition to considerable intrasubject variability, wide between-subject variability exists in the disposition of voriconazole that is in part related to a genetic CYP2C19 polymorphism.[212,377,437,446] Based on this variability, the significant correlations between exposure and effect[545] and significant effects of therapeutic drug monitoring on treatment responses,[418] therapeutic drug monitoring is advocated in patients with life-threatening invasive fungal infections with a target trough concentration of 1 or greater to 2 or greater mg/L of voriconazole.[540,545]

Safety. Voriconazole has an acceptable safety profile. The accrued clinical data indicate that side effects include four distinct clinical categories: transient liver enzyme abnormalities (10% to 20%), skin reactions (<10%), hallucinations or confusion (<10%), and transient, dose-related visual disturbances (altered or enhanced perception of light, blurred vision; 25% to 45%).[212] With regard to the visual disturbances, no morphologic correlation was noted in animal models and the underlying mechanism remains to be elucidated.[272] Drug-related adverse effects requiring the discontinuation of voriconazole were infrequent in comparative clinical trials and ranged from 2 to 13 percent.[9,239,587]

Serious hepatic reactions, including hepatic failure, have been observed during treatment with voriconazole; therefore, evaluation of liver function tests before and during treatment with voriconazole is advised. Azole therapy has been associated with QTc prolongation; for this reason, voriconazole should be administered with caution to patients with proarrhythmic conditions.[212] A possible link among long-term use of voriconazole, immunosuppression, and chronic phototoxicity with aggressive squamous cell carcinoma and melanoma has been reported in adult and pediatric patients.[117,370] As a consequence, the indication for further and prolonged voriconazole treatment in immunocompromised patients

who develop phototoxicity needs to be extremely carefully evaluated. Moreover, fluorosis and periostitis also have been reported during long-term voriconazole use.[600] Voriconazole is teratogenic in animals and may cause fetal harm when it is administered to a pregnant woman.[212]

Drug Interactions. Voriconazole is both substrate and inhibitor of CYP2C19, CYP2C9, and CYP3A4, and, therefore, numerous clinically relevant and potentially hazardous drug–drug interactions must be considered.[205,212] Voriconazole significantly increases exposure to cyclosporin, tacrolimus, benzodiazepines, methadone, fentanyl, alfentanil, Oxycodone, vinca alkaloids, the statins, omeprazole, warfarin, sulfonylurea drugs, phenytoin, protease inhibitors other than indinavir, and nonnucleoside reverse transcriptase inhibitors, thus requiring dosage adjustment or monitoring. Voriconazole exposure is significantly decreased by St. John's wort, phenytoin, ritonavir, efavirenz, rifabutin, carbamazepine, rifampin, and phenobarbital. Concurrent use of the last three enzyme inducers with voriconazole is contraindicated (risk for subtherapeutic levels of voriconazole), as is the concurrent use of terfenadine, astemizole, cisapride, quinidine, pimozide (risk of QTc prolongation resulting from increased exposure to these agents), ergotamine (risk for ergotism resulting from increased exposure), and sirolimus (increased exposure). Dosage adjustments of voriconazole are necessary when the drug must be used concurrently with phenytoin or rifabutin.[80,205,212,272,292]

Clinical Efficacy. Voriconazole has demonstrated excellent clinical efficacy in phase II and III clinical trials in patients with oropharyngeal candidiasis and esophageal candidiasis.[9,235] In salvage studies in patients with fungal infections refractory or intolerant to treatment, complete and partial responses were seen in 43 and 48 percent of patients with invasive aspergillosis,[131,423,584] in 45 to 52 percent of patients with invasive candidiasis,[423] in 30 to 63 percent of patients with scedosporiosis,[423,584] in 45 percent of patients with fusariosis,[423] and in 38 percent of patients with cryptococcosis.[423]

A large, multinational, randomized phase III clinical trial of voriconazole and amphotericin B deoxycholate followed by other licensed antifungal agents for primary therapy of invasive aspergillosis revealed superior outcomes in voriconazole-treated patients.[239] At week 12, successful outcomes were noted in 52.8 percent of the patients in the voriconazole group and in 31.6 percent of those in the amphotericin B group (absolute difference, 21.2 percentage points; 95% confidence interval (CI), 10.4 to 32.9). The survival rate at 12 weeks was 70.8 percent in the voriconazole group and 57.9 percent in the amphotericin B group (hazard ratio, 0.59; 95% CI, 0.40 to 0.88). Voriconazole-treated patients had significantly fewer severe drug-related adverse events. A randomized double-blind clinical trial comparing open-label voriconazole plus blinded anidulafungin or blinded placebo for treatment of proved or probable invasive aspergillosis in patients with hematologic malignancies or allogeneic HSCT has been completed. The preliminary results showed a statistical trend ($p = .07$) favoring the combination therapy with respect to the overall survival at 6 weeks, the primary end point of the study.[356] A randomized comparative study of voriconazole versus amphotericin B deoxycholate followed by fluconazole for treatment of candidemia in non-neutropenic patients showed similar response rates and end-of-treatment and similar survival rates at 3 months.[309] Twelve weeks after the end of treatment, successful outcomes were observed in 41 percent of patients in both treatment groups. Voriconazole cleared blood cultures as quickly as amphotericin B/fluconazole, and significantly fewer serious adverse events and cases of renal toxicity occurred. In a large, international collaborative study of voriconazole versus liposomal amphotericin B for empirical therapy, voriconazole did not meet the prespecified statistical end point for noninferiority in a composite end point but was associated with significantly fewer breakthrough invasive fungal infections, particularly those caused by invasive aspergillosis.[587] Finally, several reports also suggested the potential usefulness of voriconazole for treatment of infections by unusual hyaline and dematiaceous fungi,[423] as well as for treatment of cerebral mold infections.[499]

Voriconazole also has been investigated as primary antifungal prophylaxis.[351,613] A multicenter, randomized, double-blind trial compared voriconazole 200 mg twice daily to fluconazole 400 mg once daily in 600 adult allogeneic HSCT patients. There was no difference in the cumulative rates of proved, probable, and presumptive invasive fungal infections at 6 months (7.3% vs. 11.2% [$p = .12$]); however, a trend was noted toward fewer infections by *Aspergillus* (9% vs. 17%, $p = .09$). Fungal-free survival rates (78% and 75%, respectively) were similar, as were overall survival rates and rates of premature study drug withdrawal.[613] A second study compared voriconazole 200 mg twice daily to itraconazole 200 mg twice daily in 489 allogeneic HSCT recipients. Efficacy was assessed by a composite end point, including survival at 180 days after transplantation and no breakthrough infections and no discontinuation of the study drug for more than 14 days during the first scheduled 100 days. Using this end point, voriconazole was superior to itraconazole, with a 49.1 versus 34.5 percent success rate ($p = .0004$). However, the rate of proved and probable invasive fungal infections was low (3% vs. 6%).[351]

Approval Status and Dosing. Voriconazole is approved in the United States and the European Union for treatment of invasive aspergillosis, fusariosis, and scedosporiosis; treatment of esophageal candidiasis; and primary treatment of candidemia and certain forms of invasive candidiasis in non-neutropenic patients (see Tables 239-2 and 239-3). The recommended intravenous dosages for adults and adolescents (12 to 14 years and ≥50 kg; 15 to 17 years regardless of body weight) are 6 mg/kg twice daily on day 1, followed by 4 mg/kg twice daily. Oral doses in adults are 400 mg twice daily on day 1 (≥40 kg, 200 mg twice daily), followed by 200 mg twice daily (<40 kg, 100 mg twice daily). In patients with renal insufficiency, no adjustment of dosage is needed for the oral formulation; because of the renal clearance of the intravenous carrier, patients with a creatinine clearance of less than 50 mL/min should receive voriconazole by the oral route. In patients with mild-to-moderate hepatic

TABLE 239-10 Simulated Plasma Concentrations of Voriconazole After Multiple Doses of 3 and 4 mg/kg in Pediatric versus Adult Patients

	Value*			
	Pediatric Patients 2-11 yr		Adult Patients	
Parameter	3 mg/kg	4 mg/kg	3 mg/kg	4 mg/kg
AUC_{tau} (ng/hr/mL)	10,670	14,227	13,855	38,605
$C_{average}$ (ng/mL)	889	1,186	1,155	3,217

Modified from Walsh TJ, Karlsson MO, Driscoll T, et al. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. *Antimicrob Agents Chemother* 2004;48:2166-72.

*Data are reported as medians following 6 mg/kg every 12 hours on day 1 and maintenance dose 3.

Whereas in pediatric patients, an increase in dosage by a factor of 1.3 leads to proportional increase in the mean average plasma concentration ($C_{average}$) and in the mean area under the concentration versus time curve (AUC_{tau}), adult patients display a 2.8-fold, hyperproportional increase in exposure, indicating nonlinear disposition. As a consequence, pediatric patients given 4 mg/kg do not achieve the same exposure as adults given 4 mg/kg, the dosage that has led to the approval of voriconazole for first-line treatment of invasive aspergillosis.

function abnormalities, half of the daily maintenance dosage is recommended after the initial loading dose. Recommendations for severe liver failure are lacking.[212,272]

Whereas the disposition of voriconazole in infants younger than 2 years of age has not been systematically investigated,[138] initial pharmacokinetic studies in children 2 to 11 years of age revealed a faster weight-normalized clearance rate than in adults and lower exposure at similar dosages (Table 239-10).[583] This and several subsequent formal pharmacokinetic studies revealed that children need higher exposures relative to adults and that young adolescents 12 to 14 years of age with low body weight (<50 kg) appear to metabolize voriconazole more similarly to children.[147,146,279,577] As a consequence, an intravenous dosage of 8 mg/kg twice daily (with day 1 dose 9 mg/kg twice daily) and an oral dose of the suspension of 9 mg/kg twice daily has been adopted by the European Medicines Agency (EMA) for children 2 years of age to younger than 12 years and 12 to 14 years weighing less than 50 kg and is being further investigated in clinical phase II trials conducted by the manufacturer. Considering the significant relationships between exposure and outcome described earlier, therapeutic drug monitoring should be considered in pediatric patients with life-threatening invasive fungal infections.

Voriconazole has been administered safely and with success to children younger than 12 years of age who had no therapeutic alternative. Of 58 immunocompromised children with proved or probable invasive fungal infection refractory to or intolerant of conventional antifungal therapy, 26 patients (45%) had a complete or partial response and 4 patients (7%) discontinued therapy because of intolerance. A total of 23 patients had voriconazole-related adverse events, most commonly elevations in hepatic transaminases or bilirubin ($n = 8$), skin rash ($n = 8$), abnormal vision ($n = 3$), and photosensitivity reactions ($n = 3$).[584] A large retrospective postmarketing cohort study in 107 immunocompromised pediatric patients who received 252 courses of voriconazole for a median of 65 days (*range*, 1-1001) reported further safety data. Irrespective of causal relationships, increases in hepatic transaminases (53.5%), serum bilirubin (23.6%), and alkaline phosphatase (10.9%), skin eruptions (5.6%), and neurologic adverse events (4.8%) were mostly mild to moderate. Adverse events necessitating discontinuation of voriconazole occurred in 18 courses (7.1%). Treatment success was observed in 16/37 patients with proved, probable, or possible infections, and in 203/215 courses of empirical therapy or prophylaxis.[436] In other pediatric studies providing sufficient information, the rate of drug discontinuations because of adverse events was between 5 and 19 percent. Increases in hepatic transaminases were the most commonly observed adverse advents, followed by skin eruptions, and no consistent correlation between dose or exposure and adverse events have been established.[140] Voriconazole also has been used with success and acceptable tolerance in many patients with cystic fibrosis and allergic bronchopulmonary aspergillosis[245] and as antifungal prophylaxis in patients with hematologic malignancies or allogeneic HSCT.[345,374]

Echinocandin Lipopeptides

The echinocandins are a distinct class of semisynthetic amphiphilic lipopeptides that are composed of a cyclic hexapeptide core linked to a variably configured lipid side chain. The first compound of this class was cilofungin (LY 121019), an echinocandin B derivative with activity limited to *Candida* spp. However, clinical development was abandoned because of concerns regarding toxicity associated with the intravenous polyethylene glycol formulation vehicle.[185,207,475] Since the 1990s, a second generation of semisynthetic echinocandins with extended antifungal spectrum against *Candida* and *Aspergillus* spp., excellent safety profiles, and favorable pharmacokinetic characteristics has been developed: anidulafungin (Eraxis and Ecalta), caspofungin (Cancidas), and micafungin (Mycamine) (Fig. 239-8). With few exceptions, current data indicate that these agents are not fundamentally different with respect to spectrum, pharmacodynamics, pharmacokinetics, safety, and antifungal efficacy.

Mechanism of Action. The echinocandins act by noncompetitive inhibition of the synthesis of 1,3-β-D-glucan, a polysaccharide in the cell wall of many pathogenic fungi (Fig. 239-9). Together with chitin, the ropelike glucan fibrils are responsible for the cell wall's strength and shape. They are important in maintaining the osmotic integrity of the fungal cell and play a key role in cell division and cell growth.[126,127,234] The proposed molecular target of the echinocandins, glucan synthase, is a heteromeric enzyme complex composed of at least one large

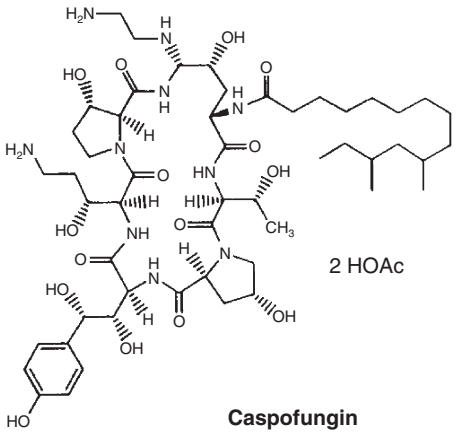

Caspofungin

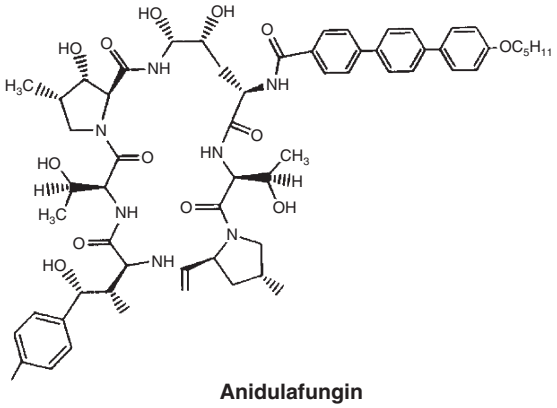

Anidulafungin

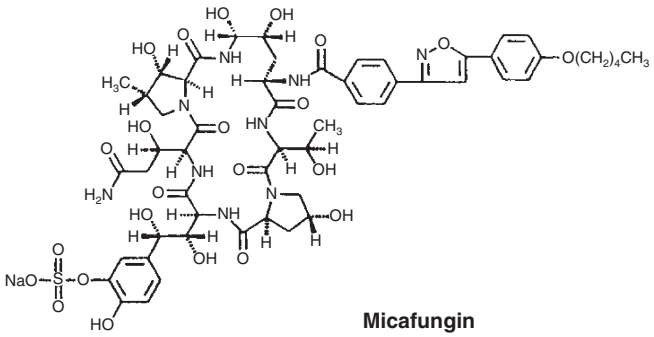

Micafungin

FIGURE 239-8 ■ Structural formulas of echinocandin lipopeptides: anidulafungin, caspofungin, and micafungin.

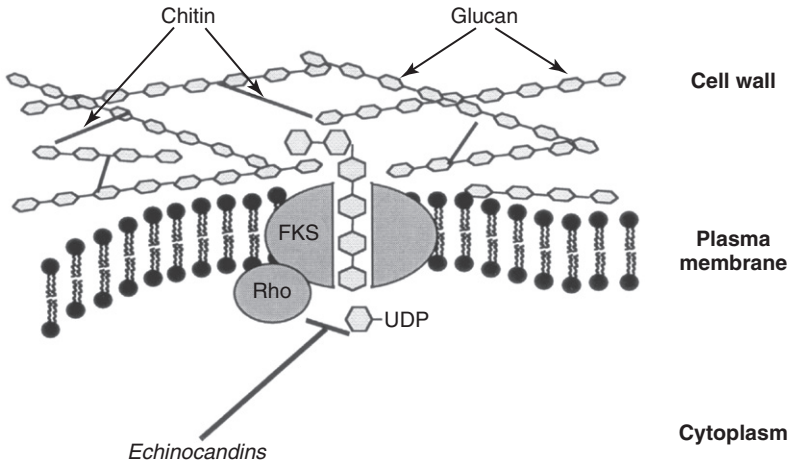

FIGURE 239-9 ■ Schematic of the proposed mechanism of action of echinocandin lipopeptides. Echinocandins inhibit the synthesis of cell wall 1,3-β-glucan at the level of the cell membrane. *FKS* is the proposed catalytic subunit, and *Rho* the proposed regulatory subunit of the glucan synthase complex. (Modified from Kurtz MB, Douglas CM. Lipopeptide inhibitors of fungal glucan synthase. *J Med Vet Mycol* 1997;35:79-86.)

integral membrane protein encoded by the *FKS* gene that binds the substrate (UDP-glucose), and one small regulatory subunit, Rho1p, a guanosine diphosphate (GTP)-binding protein; other, as-yet unidentified, components also may be involved.[310,535] Additional immunomodulatory effects through unmasking of (1→3)-β-D-glucan with proinflammatory host cytokine release have been described and may contribute to in vivo efficacy of the echinocandins.[324]

Antifungal Activity. The echinocandins have potent and broad-spectrum fungicidal in vitro activity against most *Candida* spp., including azole-resistant strains and biofilms, and potent inhibitory activity against *Aspergillus*

spp. Their antifungal efficacy against these organisms in vivo has been demonstrated in various animal models. The current echinocandins have variable activity against dematiaceous and endemic mold and are considered inactive against most Hyalohyphomycetes, Mucorales, *C. neoformans*, and *T. asahii*. All echinocandins have demonstrated preventive and therapeutic activity in animal models of *Pneumocystis jiroveci* (formerly *Pneumocystis carinii*) pneumonitis. Preclinical in vitro and in vivo studies consistently have shown no antagonism between echinocandins and other antifungal agents but have shown indifference, additivity, or synergy when tested against *Aspergillus* spp., *C. albicans*, and non-*albicans Candida* spp.*

Resistance. Whereas the echinocandins show no cross-resistance to amphotericin B, coresistance to both azoles and echinocandins in clinical isolates of *C. glabrata* has been described and is of concern.[431,629] Nevertheless, the frequency of resistance to echinocandins in otherwise susceptible fungal yeast species is low. Reduced echinocandin susceptibility may occur by three mechanisms: adaptive stress responses, which result in elevated cell wall chitin content and paradoxical growth *in vitro* at supraminimum inhibitory concentrations that greatly exceed those achieved in plasma (paradoxical effect); acquired *FKS* mutations, which confer reduced glucan synthase sensitivity, elevated MICs and clinical failure; and intrinsic *FKS* mutations. Intrinsic *FKS* mutations are naturally occurring mutations in the *C. parapsilosis* group and *C. guilliermondii* that confer elevated MIC levels but a lower level of reduced glucan synthase sensitivity in contrast to acquired *FKS* mutations. Of note, some *FKS* mutants have been shown to have significantly reduced fitness and virulence, which may contribute to the low incidence of echinocandin resistance reported in large surveillance studies.[62,310,417,424] New, species-specific clinical breakpoints that consider wild-type MICs, epidemiologic cutoff values, and pharmacokinetic and pharmacodynamic relationships have been proposed that are more sensitive to detect emerging resistance associated with FKS mutations and better able to predict risk for clinical failure.[424,432,466]

Relative to *Candida* spp., much less is currently known about echinocandin resistance among *Aspergillus* spp.; however, clinical breakthrough cases have been reported, and resistance has been confirmed in vivo.[182,258,427]

Pharmacodynamics. The echinocandins demonstrate a species-dependent mode of antifungal activity. Whole-cell in vitro assays reveal fungicidal activity against most *Candida* spp. but not against *Aspergillus* spp.[47,529] Microscopic examination of drug-exposed *A. fumigatus* shows a dose-dependent formation of microcolonies with progressively truncated, swollen hyphal elements that appear to be cell wall deficient but are able to regain their cell walls on subculture in the absence of drug.[143,311,400,454] These observations demonstrate differences in functional target sensitivity that are not fully understood. Time-kill studies in *Candida* spp. demonstrated predominantly concentration- and time-dependent fungicidal activity and rate-of-kill at concentrations greater than the MIC for all three compounds.[159,160,425] In addition, PAFEs between 5 and 12 hours at concentrations above the MIC have been demonstrated.[158,344]

In persistently neutropenic rabbit models, anidulafungin showed highly predictable concentration-effect relationships in experimental disseminated candidiasis; however, no concentration-effect relationships were observed in experimental pulmonary aspergillosis, despite full exploration of the dosage range.[217] Pharmacodynamic studies in murine kidney target models of disseminated candidiasis showed that the AUC/MIC ratio is the pharmacodynamic parameter that predicts efficacy of the echinocandins.[20,21,22,333] In a murine model of invasive pulmonary aspergillosis, the C_{max}/MEC ratio appeared to be the parameter most closely associated with efficacy.[609] Pharmacokinetic and pharmacodynamic correlations of all echinocandins have been explored in patients with esophageal and oropharyngeal candidiasis.[210] Using large datasets from two phase III clinical trials of micafungin for invasive candidiasis, a significant relationship between the AUC/MIC ratio of micafungin and mycologic response was found by population pharmacokinetics and regression analysis; Monte Carlo simulation revealed lower AUC/MIC target for *C. parapsilosis* relative to other *Candida* spp, supporting the concept of species-specific echinocandin susceptibility breakpoints.[22]

Pharmacokinetics. At present, all current echinocandins are available only for intravenous administration. They exhibit dose-proportional plasma pharmacokinetics with a triexponential elimination pattern. Their β half-life is between 10 and 15 hours, thus allowing for once-daily dosing without major accumulation after multiple dosing. All echinocandins are highly (≥95%) protein-bound and distribute into all major organ sites, including the brain; however, concentrations in uninfected CSF are low. Within the lung, anidulafungin and micafungin have been shown to predominantly concentrate in alveolar macrophages. The echinocandins are chemically degraded or metabolized by the liver and slowly excreted into urine and feces; only small fractions (≤2%) of a dose are excreted into urine in unchanged form (Table 239-11).[105,116,118,210,563,581] The current echinocandins are not dialyzable, and no adjustment in dose is required for patients with renal insufficiency or renal failure. Similarly, no adjustment in dose is necessary for patients with mild-to-moderate (all) or severe (anidulafungin, micafungin) hepatic failure. Whether the minor differences in individual pharmacokinetic parameters such as AUC, peak plasma levels, volume of distribution, and clearance of the echinocandins are of clinical significance remains to be elucidated.

Adverse Effects. At current dosages, anidulafungin, caspofungin, and micafungin generally are well tolerated. Fewer than 10 percent of patients enrolled in the various randomized comparative clinical trials discontinued echinocandin therapy because of drug-related adverse events.*

*References 161, 207, 214, 216, 424, 565.

*References 125, 303, 376, 452, 555, 591.

TABLE 239-11 **Principal Pharmacokinetic Properties of the Echinocandin Lipopeptides Caspofungin, Anidulafungin, and Micafungin**

Properties	Caspofungin	Anidulafungin	Micafungin
Formulation	IV	IV	IV
Dose linearity	Yes	Yes	Yes
Oral bioavailability (%)	N/A	N/A	N/A
Protein binding (%)	97	84	99
Volume of distribution (L/kg)	N/A	0.7-0.9	0.24
Elimination half-life (hr)	8-10	24	15
Substrate/inhibitor of CYP450	N/A	N/A	N/A
Routes of elimination	Degradation/ metabolization, urine > feces	Degradation only, feces	Metabolization, feces > urine

Data from references 201, 212, 221, 563.
CYP450, Cytochrome P450; *N/A,* not applicable.

Increased liver transaminases, gastrointestinal symptoms, skin rash, and headache probably or definitely associated with echinocandin treatment have been reported to occur at a frequency of less than 5 percent each. Like other basic polypeptides, the echinocandins have the theoretical potential to cause release of histamine.* Symptoms such as rash, facial swelling, pruritus, sensation of warmth, and anaphylactic reactions have been reported in isolated cases. Because of transient elevations of hepatic transaminases in interaction studies in healthy volunteers,[201] the concomitant use of caspofungin and cyclosporine currently is not recommended; clinical experience, however, indicates that both drugs can be given safely under careful monitoring.[211,353,542]

Drug Interactions. In vitro studies showed that anidulafungin does not significantly inhibit the activities of clinically important human CYP450 isoforms at clinically relevant concentrations and that it is not an inhibitor of P-glycoprotein. Interaction studies demonstrated that no adjustment of dosage of either drug is warranted when anidulafungin is coadministered with cyclosporine, tacrolimus, voriconazole, liposomal amphotericin B, or rifampin.[94]

Caspofungin is not a substrate of P-glycoprotein and is a poor substrate and a weak inhibitor of CYP450 enzymes.[38,623] Clinical studies demonstrated that the pharmacokinetic properties of caspofungin are not altered by itraconazole, amphotericin B, mycophenolate, nelfinavir, or tacrolimus. Caspofungin has no effect on the pharmacokinetics of itraconazole, amphotericin B, or the active metabolite of mycophenolate. Caspofungin can reduce the AUC of tacrolimus by approximately 20 percent, but it has no effect on cyclosporine levels. Inducers of drug clearance or mixed inducer and inhibitors, namely efavirenz, nelfinavir, nevirapine, phenytoin, rifampin, dexamethasone, and carbamazepine, may reduce caspofungin concentrations, and dose adjustment to a daily dose of 70 mg of caspofungin should be considered.[92,201,526]

*References 125, 303, 376, 452, 555, 591.

Micafungin is a weak inhibitor of CYP3A in vitro, but it is neither a P-glycoprotein substrate nor inhibitor in vitro. In clinical studies, micafungin had no effect on mycophenolate mofetil, cyclosporine, tacrolimus, prednisolone, fluconazole, and voriconazole pharmacokinetics, but it increased the AUC of sirolimus, nifedipine, and itraconazole by approximately 20 percent. Patients receiving sirolimus, nifedipine, or itraconazole in combination with micafungin should be monitored for toxicity of these drugs, and sirolimus, nifedipine, and itraconazole dosage should be reduced if necessary. Amphotericin B, mycophenolate mofetil, cyclosporine, tacrolimus, prednisolone, sirolimus, nifedipine, fluconazole, itraconazole, voriconazole, ritonavir, and rifampin were shown to have no effects on the pharmacokinetics of micafungin.[93,221]

Caspofungin

Clinical Efficacy. The clinical efficacy of caspofungin against *Candida* spp. was demonstrated first in phase II and III studies in immunocompromised patients with esophageal candidiasis.[27,570,571] A multicenter, randomized, double-blind phase III clinical trial investigated the efficacy of caspofungin for primary treatment of invasive *Candida* infections in 224 mostly non-neutropenic patients with AmB-D (0.6 to 1 mg/kg) as comparator agent. Among patients receiving at least one dose of the study drug, 73 percent of patients in the caspofungin cohort and 61.7 percent of patients in the AmB-D cohort had therapeutic success at the end of intravenous therapy. Among patients who received five or more doses, the response rates were 80.7 and 64.9 percent, respectively. No difference occurred in relapse or survival, but caspofungin was better tolerated.[376] Further large randomized clinical trials in patients with candidemia and other forms of invasive candidiasis showed similar outcomes of the standard dose of caspofungin relative to 100 and 150 mg daily of micafungin[416] and no general advantage of a high-dose (150 mg/day) caspofungin treatment regimen.[61] In deep-seated invasive candidiasis, including peritonitis, abdominal abscesses, chronic disseminated candidiasis, and arthritis, caspofungin was effective and safe at regular doses and up to 100 mg daily.[109]

A multicenter phase II salvage trial of caspofungin in 83 patients with definite or probable invasive aspergillosis revealed a complete or partial response in 45 percent of patients receiving at least one dose of caspofungin; in patients receiving the drug for more than 7 days, the response rate was 56 percent.[339] Rates of complete or partial responses in subsequent exploratory phase II clinical trials for primary treatment of proved or probable invasive aspergillosis appear to correlate with patients' comorbidities and range from 33[572] and 42 percent[240] to 54 percent.[112] Caspofungin in combination with a triazole or polyene has been shown to be safe and effective in phase II trials and is an option for salvage therapy of invasive aspergillosis.[87,220,336]

In a large, randomized, double-blind clinical trial including 1095 patients, caspofungin was as effective as liposomal amphotericin B for empirical antifungal therapy in persistently febrile granulocytopenic patients, but it was better tolerated. The proportion of patients who survived at least 7 days after therapy was greater in the caspofungin group (92.6% vs. 89.2%).[591] Finally, randomized prospective comparisons suggest that caspofungin may be useful for prophylaxis in high-risk patients with hematologic malignancies.[90,357]

Approval Status and Dosing. Caspofungin is licensed in both the United States and Europe in patients 3 months of age or older with candidemia and certain forms of invasive *Candida* infections, as second-line therapy for invasive aspergillosis, and for empirical antifungal therapy in granulocytopenic patients. In the United States, caspofungin also is approved for treatment of esophageal candidiasis (see Tables 239-2, 239-3, and 239-16).[92] The adult dosage regimen is 50 mg daily, with a single 70-mg loading dose on day 1, administered over 1 hour. No dosage adjustment is required in patients with renal insufficiency and mild hepatic insufficiency. In patients with moderate hepatic insufficiency (Child-Pugh category B), a maintenance dose of 35 mg/day is recommended after the loading dose of 70 mg. No recommendations exist for patients with severe hepatic insufficiency (Child-Pugh category C).[92,201]

In children and adolescents 2 to 17 years of age, the pharmacokinetics and safety of caspofungin were investigated using both a weight-based and a body surface area regimen. Whereas a maintenance dosage of 1 mg/kg/day achieved suboptimal exposure, dosing with 50 mg/m^2/day provided similar or slightly higher exposure relative to adults (Table 239-12).[576] In conjunction with pharmacokinetic data obtained in small children,[391] the dosage of 50 mg/m^2/day (with day 1 dose 70 mg/m^2/day and maximum daily dose of 70 mg) has been selected for pediatric patients between 3 months and 17 years of age. This dosing regimen has been confirmed by a population pharmacokinetic study including 124 pediatric patients 3 months to 17 years enrolled in four clinical trials.[326] In neonates up to 3 months of postnatal age, limited pharmacokinetic data obtained in a small cohort of patients suggest that a dosage of 25 mg/m^2/day results in similar bloodstream exposures as 50 mg/m^2/day in older patients.[402,481] The experience with anidulafungin and micafungin, however, suggests that higher doses will be needed to treat CNS infection.

Caspofungin appears to be well-tolerated in pediatric patients. In the previously mentioned phase I and II dose-finding studies, none of the patients developed a serious drug-related adverse event or discontinued because of toxicity.[391,481,576] In a phase III estimation trial with safety as the primary objective, 82 patients between the ages of 2 and 17 years with persistent fever and neutropenia were randomly assigned to receive caspofungin or liposomal amphotericin B 3 mg/kg daily in a 2:1 ratio. Rates of drug-related adverse events were similar (clinical 48.2% vs. 46.2%; laboratory 10.7% vs. 19.2%); serious drug-related adverse events occurred in 1.8 percent of caspofungin-treated patients and in 11.5 percent of amphotericin B-treated patients. Overall success rates were not different.[337] A prospective phase II trial in 49 patients with invasive *Candida* or *Aspergillus* infections revealed no serious drug-related adverse events and an overall response rate of 73 percent.[628] In the combined analysis of caspofungin safety in all five clinical registration trials involving 171 pediatric patients 1 week to 17 years of age, the most common drug-related adverse

TABLE 239-12 **Single-Dose Caspofungin Pharmacokinetics in Pediatric Versus Adult Patients***

	Children (2-11 yr)			
Dosage	**1 mg/kg**	**50 mg/m^2**	**Adolescents (12-17 yr) 50 mg/m^2**	**Adults 50 mg**
$AUC_{0\text{-}24\ hr}$ (μg/hr/mL)	41.5	96.4	77.6	70.6
C1 (μg/mL)	6.59	13.9	7.67	7.67
C24 (μg/mL)	0.45	1.09	1.35	1.35
$t_{1/2}\beta$ (hr)	7.2	7.6	11.7	11.7
Cl (mL/min/m^2)	8.57	7.78	6.07	6.07

Modified from Walsh TJ, Adamson PC, Seibel NL, et al. Pharmacokinetics, safety, and tolerability of caspofungin in children and adolescents. *Antimicrob Agents Chemother* 2005;49:4536-45.

*Least square means are reported for AUC, C1 (peak plasma concentration [C_{max}]), and C24 (minimum plasma concentration [C_{min}]), and harmonic means for $t_{1/2}\beta$. Data were obtained in groups of 6 to 10. Data in pediatric patients were compared with those obtained in 32 adult patients with mucosal candidiasis. In comparison with adult values, a 1-mg/kg dosage does not achieve the target trough concentration of caspofungin of 1 μg/mL and leads to a lower exposure as measured by the $AUC_{0\text{-}24\ hr}$; the dosage regimen of 50 mg/m^2 leads to similar or slightly higher trough concentrations and similar or slightly higher exposure ($AUC_{0\text{-}24\ hr}$).

AUC, Area under the concentration versus time curve from 0 to 24 hours; *Cl,* clearance; $t_{1/2}\beta$, elimination half-life.

events were fever (11.7%), increased alanine aminotransferase level (6.5%), and rash (4.7%); few events were serious (0.6%) or required treatment discontinuation (1.2%).[627] Favorable safety experiences also have been reported in immunocompromised pediatric patients who received the compound for various indications, mostly in combination with other antifungal agents,[141,171,211] and in neonates with refractory invasive candidiasis.[387,401,402]

Anidulafungin

Clinical Efficacy. The clinical efficacy of anidulafungin against *Candida* spp. was demonstrated in phase II or III studies in immunocompromised patients with esophageal candidiasis and candidemia. Anidulafungin had efficacy equivalent to that of fluconazole in esophageal candidiasis in a randomized, double-blind, international multicenter study; success was documented in 242/249 evaluable anidulafungin-treated patients (97.2%) and in 252/255 fluconazole-treated patients (98.8%). Adverse events leading to discontinuation were reported in 29 anidulafungin-treated patients (10%) versus 23 fluconazole-treated patients (8%).[302] In a dose-ranging study in 123 patients with invasive candidiasis randomized to anidulafungin 50, 75, or 100 mg once daily, success rates at end of therapy were 84, 90, and 89 percent, respectively.[303] A subsequent randomized, double-blind phase III noninferiority trial compared anidulafungin 100 mg once daily with fluconazole 400 mg once daily in a total of 245 mostly non-neutropenic patients with invasive candidiasis.[452] More patients receiving anidulafungin had clinical and microbiologic success at the end of intravenous therapy (75.6% vs. 60.2%); similar superiority was found at the 2- and 6-week follow-up after the end of all therapy (64.6% vs. 49.2% and 55.9% vs. 44.1%, respectively). The rate of death from all causes was 31 percent in the fluconazole group and 23 percent in the anidulafungin group (p = .13). Taken together, anidulafungin was shown to be noninferior to fluconazole on treatment of invasive candidiasis. Very recently, a randomized double-blind clinical trial comparing open-label voriconazole plus blinded anidulafungin or blinded placebo for treatment of proved or probable invasive aspergillosis in patients with hematologic malignancies or allogeneic HSCT has been completed. The results showed a statistical trend (p = .07) favoring the combination therapy with respect to the overall survival at 6 weeks, the primary end point of the study.[356] Because the full report has not been published and the data have not been reviewed by regulatory authorities, the clinical implications of this study are not clear at present.

Approval Status and Dosing. Anidulafungin is licensed in both the United States and Europe for patients 18 years of age or older for candidemia and certain forms of invasive *Candida* infections in non-neutropenic patients. In the United States, the compound is also licensed for esophageal candidiasis (see Table 239-2).[94] The recommended dose regimen consists of 100 mg (with day 1 dose 200 mg) for invasive candidiasis and 50 mg daily (with day 1 dose 100 mg), for esophageal candidiasis, administered at a rate of 1.1 mg/min or higher. No dosage adjustment is needed in patients with renal impairment, patients undergoing hemodialysis, and patients with hepatic impairment (Child-Pugh classes A, B, and C).[94,462]

A pediatric phase I and II multicenter study of the pharmacokinetics and safety of anidulafungin has been completed in 19 granulocytopenic children with cancer. Patients were divided into two age cohorts (2 to 11 and 12 to 17 years) and were enrolled into sequential groups to receive 0.75 or 1.5 mg/kg/day[51] (Table 239-13). No drug-related serious adverse events were recorded. Pharmacokinetic parameters were similar across age groups and dosage cohorts and similar relative to adult patients.

TABLE 239-13 **Single-Dose Anidulafungin Pharmacokinetics in Pediatric Versus Adult Patients***

	Pediatric Patients (2-17 yr)		***Adults***	
Dosage	**0.75 mg/kg**	**1.5 mg/kg**	**50 mg**	**100 mg**
C_{max} (μg/mL)	4.02	6.09	2.51	3.82
$AUC_{0\text{-}24\ hr}$ (μg/hr/mL)	48.0	9.7	53.3	104.8
$t_{1/2}\beta$ (hr)	20.8	19.5	39.3†	42.3†
Cl (L/hr/kg)	0.0175	0.0191	N/A	N/A
Vd_{ss} (L/kg)	0.45	0.49	0.72	0.78

Data from Benjamin DK Jr, Driscoll T, Seibel NL, et al. Safety and pharmacokinetics of intravenous anidulafungin in children with neutropenia at high risk for invasive fungal infections. *Antimicrob Agents Chemother* 2006;50:632-8; and Vasquez JA, Sobel JD: Anidulafungin: a novel echinocandin. *Clin Infect Dis* 2006;43:215-22.

*Pharmacokinetic parameters are expressed as mean values. Data were obtained in groups of six pediatric patients with compromised immunity and neutropenia per age group and dosage level and were compared with those obtained in 26 adult healthy volunteers.

†$t_{1/2}\gamma$.

AUC, Area under the concentration versus time curve from 0 to 24 hours; *Cl,* clearance; C_{max}, peak plasma concentration; *N/A,* not available; $t_{1/2}\beta$, elimination half-life; Vd_{ss}, volume of distribution at steady state.

Following single and multiple daily doses of 0.75 mg/kg and 1.5 mg/kg, plasma concentration data corresponded to those in adults following a daily 50 and 100 mg dose, respectively.[51] In infants and neonates at risk for invasive candidiasis receiving 1.5 mg/kg/day anidulafungin after a single loading dose of 3 mg/kg, drug exposure levels after multiple doses were similar between neonates and infants and similar to those in children receiving similar weight-based dosing and in adult patients receiving 100 mg/day. No drug-related serious adverse events were observed.[104] Bridging studies from laboratory animals, however, indicate that a regimen of 3 mg/kg, followed by 1.5 mg/kg/day, is suboptimal for treatment of *Candida* meningoencephalitis and that higher dosages will be required to treat this condition.[594] Although further data are not available at this point, the pediatric development program of the compound is in advanced stages.

Micafungin

Clinical Efficacy. Micafungin was studied in open-label dose-ranging trials of endoscopically proved esophageal candidiasis in patients with HIV infection.[426,527] A double-blind comparative study investigating 50, 100, and 150 mg/day versus fluconazole 200 mg/day for HIV-associated esophageal candidiasis showed similar endoscopic cure rates and safety profiles for micafungin at doses of 100 and 150 mg/day and fluconazole.[125] A further randomized, double-blind comparative trial in 523 patients 16 years of age and older with esophageal candidiasis investigated micafungin 150 mg/day versus fluconazole 200 mg/day.[124] For the primary end point of endoscopic cure, treatment difference was 0.3 percent (micafungin, 87.7%; fluconazole, 88.0%). A large randomized, double-blind noninferiority trial compared micafungin 100 mg once daily and liposomal amphotericin B 3 mg/kg once daily for first-line therapy of candidemia and other forms of invasive candidiasis in a total of 531 adult patients. The overall success rate in both treatment arms was similar (89.6% vs. 89.5%). No differences in survival were noted. Fewer severe treatment-related adverse events and adverse events that led to treatment discontinuation were observed in the micafungin arm.[312,471] In a second large, phase III, randomized clinical trial of a total of 595 patients with candidemia and other forms of invasive candidiasis, micafungin at 100 mg/day, micafungin at 150 mg/day, and caspofungin at 50 mg/day (with day 1 dose 70 mg) were equivalent in terms of efficacy, adverse events, and mortality.[151,416] A multinational, noncomparative open-label trial investigated micafungin for proved and probable invasive aspergillosis alone or in combination with another antifungal agent. A favorable response rate at the end of therapy was seen in 35.6 percent (80/225) of patients. Of those treated with only micafungin, favorable responses were seen in 6/12 (50%) of the primary and 9/22 (40.9%) of the salvage therapy group, with corresponding numbers in the combination treatment groups of 5/17 (29.4%) and 60/174 (34.5%) of the primary and salvage treatment groups, respectively.[128] Finally, micafungin 50 mg/day (<50 kg, 1 mg/kg) versus fluconazole 400 mg/day (<50 kg, 8 mg/kg) was investigated for prevention of invasive fungal infections in 882 patients undergoing HSCT. Prophylaxis was given from the start of the conditioning regimen until 5 days after engraftment. The overall success rate was significantly higher for patients randomized to receive micafungin (80.0% vs. 73.5%; $p = .03$). Drug-related adverse events were comparable.[555]

Approval Status and Dosing. In the United States, micafungin is licensed in adults for prevention of *Candida* infections in patients undergoing HSCT, for treatment of candidemia and defined forms of invasive candidiasis, and for treatment of esophageal candidiasis (see Table 239-2). In Europe, the compound is licensed for use in neonates, children, and adults for treatment of invasive candidiasis and as prophylaxis of *Candida* infections in patients with allogeneic HSCT and those with prolonged neutropenia; it is also licensed for treatment of esophageal candidiasis in individuals 16 years of age and older. The recommended dosage is 100 mg/day for invasive candidiasis (≤40 kg, 2 mg/kg), 150 mg/day for esophageal candidiasis (≤40 kg, 3 mg/kg), and 50 mg/day (≤40 kg, 1 mg/kg) for the preventive indication. Renal dysfunction, dialysis, or hepatic dysfunction does not alter the pharmacokinetics of micafungin.[93,233]

The pharmacokinetics and safety of micafungin have been studied in 70 febrile granulocytopenic children 2 to 17 years of age. Micafungin was well tolerated at dosages of 0.5 to 3 mg/kg/day; pharmacokinetics were linear and similar to those observed in adults (Table 239-14).[503] Population-based pharmacokinetic modeling of the data revealed that clearance in smaller children was higher than predicted on the basis of weight alone, requiring a relative dosage increase to ensure exposure comparable to that in older children; however, dosages of 2 mg/kg and 4 mg/kg in the children achieved exposures at least similar to 100 mg and 200 mg, respectively, in adults.[251] Of note, in the setting of prophylaxis, alternate dosing of micafungin (3 mg/kg) every other day provides drug exposure similar to standard dosing of 1 mg/kg given daily, and may provide an alternative for antifungal prophylaxis in high-risk patients.[361]

The pharmacokinetics in neonates were explored in a phase I single-dose, multicenter, open-label, sequential-dose trial of micafungin 0.75 mg/kg, 1.5 mg/kg, and 3 mg/kg in 18 premature infants weighing more than 1000 g. On average, half-life was shorter and clearance more rapid in contrast to older children and adults.[241] Population pharmacokinetics modeling of the neonatal pharmacokinetics data and Monte Carlo simulation suggest that a larger neonatal dose is required to produce drug exposure comparable to those predicted on the basis of weight in children and adults; additional population pharmokinetics based on open-label pharmokinetics studies with doses up to 15 mg/kg/day support a target dose of 10 mg/kg for neonates, particularly in view of the substantial risk for secondary brain involvement.[52,250,252,516] Although the treatment results in the pivotal candidemia study showed no signal for decreased efficacy at dosages between 2 and 4 mg/kg in this population,[447] we recommend a dose of at least 4 mg/kg in neonates with invasive candidiasis and a dose of 10 mg/kg in the case of CNS involvement.

TABLE 239-14 **Single-Dose Micafungin Pharmacokinetics in Pediatric Versus Adult Patients***

	Pediatric Patients (2-17 yr)			*Adults*	
Dosage	**1 mg/kg**	**2 mg/kg**	**4 mg**	**50 mg**	**100 mg**
C_{max} (μg/mL)	10.8	15.3	30.3	3.6	7.1
$AUC_{0\text{-}24\ hr}$ (μg/hr/mL)	40.3	83.0	191.4	33.9	59.9
$t_{1/2}\beta$ (hr)	12.5	13.2	11.6	12.5	13.0
Cl (L/hr/kg)	0.021	0.020	0.017	0.017†	0.018†
Vd_{ss} (L/kg)	0.33	0.31	0.28	0.31†	0.32†

Modified from Seibel NL, Schwartz C, Arrieta A, et al. Safety, tolerability, and pharmacokinetics of micafungin (FK463) in febrile neutropenic pediatric patients. *Antimicrob Agents Chemother* 2005;49:3317-24; and Groll AH, Stergiopoulou T, Roilides E, et al. Micafungin: pharmacology, experimental therapeutics and clinical applications. *Expert Opin Investig Drugs* 2005;14:489-5095.

*Pharmacokinetic parameters are expressed as mean values. Data were obtained in groups of 7 to 15 pediatric patients with compromised immunity and neutropenia per age group and dosage level and were compared with those obtained in cohorts of 8 to 9 adult patients with hematopoietic stem cell transplantation.

†Weight normalization calculated by assuming an average body weight of 70 kg.

AUC, Area under the concentration versus time curve from 0 to 24 hours; *Cl,* clearance; C_{max}, peak plasma concentration; $t_{1/2}\beta$, elimination half-life; Vd_{ss}, volume of distribution at steady state.

A noncomparative phase II study investigated the activity of micafungin alone or in combination with other agents in 58 pediatric patients with mostly refractory invasive aspergillosis. Most patients (n = 56) received micafungin in combination with another agent, primarily liposomal amphotericin B (n = 47). The mean daily dose was 2.0 ± 1.2 mg/kg/day, and the mean duration of dosing was 67 ± 85 days. Overall response was 26/58 (45%). Thirteen patients (22%) discontinued micafungin, primarily because of a progression of underlying disease or invasive aspergillosis.[167] The comparative safety of micafungin was investigated in the pediatric subpopulation of a double-blind, phase III trial in patients with invasive candidiasis or candidemia. Patients were randomized to receive intravenous micafungin 2 mg/kg/day or liposomal amphotericin B 3 mg/kg/day for a minimum of 14 days. There was no difference in the success rates (69.2% vs. 74.1%). The incidence of serious adverse events (3.8% vs. 9.3%) and the rate of patients discontinuing therapy because of an adverse event (3.8% vs. 16.7%) were lower in patients treated with micafungin.[447] In a pooled analysis of adverse events data from six clinical trials that included close to 300 patients, 26.7 percent of patients had a treatment-related adverse event and 2.4 percent had a treatment-related adverse event that led to treatment discontinuation. No trends were seen with respect to dose or duration of treatment, and the types and rates of events were similar to those observed in adults.[28,31]

In Europe, the Summary of Product Characteristics for micafungin recommends careful monitoring of liver function and early discontinuation in the presence of significant and persistent elevation of alanine aminotransferase and aspartate aminotransferase values. It also recommends that micafungin treatment be conducted on a careful risk-benefit assessment, particularly in patients with liver diseases or concomitant hepatotoxic-genotoxic therapies.[35] This recommendation is based on preclinical data from a high-dose, long-term exposure model in rats treated with micafungin for either 3 or 6 months (equivalent to 12.5% or 25% of the total life span of the rat, respectively). The observed development of foci of altered hepatocytes and liver tumors in this species was dependent on both dose and duration of micafungin treatment. The human relevance of this finding is presently not known.

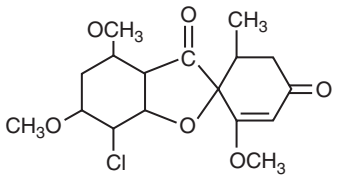

Griseofulvin

FIGURE 239-10 ■ Structural formula of griseofulvin.

AGENTS FOR SYSTEMIC TREATMENT OF MYCOSES OF THE SKIN AND ITS APPENDAGES

Griseofulvin

Griseofulvin (Fig. 239-10) was isolated originally in 1939 as a natural product of *Penicillium griseofulvum*.[410] However, only in the late 1950s was it reported to be effective as an antifungal agent.[184] Griseofulvin has been used since extensively for the systemic treatment of superficial dermatophyte infections in both adults and children.

Mechanism of Action. Griseofulvin interferes with fungal microtubule formation. It disrupts the cell's mitotic spindle formation and thus arrests the metaphase of cell division.[223] Several additional mechanisms of action have been proposed, including inhibition of nucleic acid synthesis, interference with the synthesis of cell wall chitin, and anti-inflammatory properties.[225] Griseofulvin is deposited in keratin precursor cells and produces an unfavorable environment for fungal invasion; infected skin, hair, and nails are replaced with tissue not infected by the dermatophyte.

Antifungal Activity. As evident from its mechanism of action, griseofulvin is a fungistatic compound. It is active against *Trichophyton*, *Microsporon*, and *Epidermophyton* spp. The drug has no activity against yeastlike organisms such as *Candida* spp., *Pityrosporum* spp., and *C. neoformans*. It also is inactive against opportunistic hyaline and dematiaceous molds and the dimorphic (endemic) molds.[164,172]

Pharmacokinetics. Griseofulvin is commercially available for oral administration only as griseofulvin microcrystalline and griseofulvin ultramicrocrystalline. Microcrystalline griseofulvin contains predominantly particles in the order of 4 μm in diameter, and ultramicrocrystalline griseofulvin contains predominantly particles of less than 1 μm in diameter. Griseofulvin is weakly water soluble and poorly absorbed from the gastrointestinal tract. In contrast to nonmicrocrystalline drug preparations, microcrystalline and ultramicrocrystalline formulations display enhanced absorption, particularly when polyethylene glycol is used as dispersion carrier in the ultramicrocrystalline formulations. Oral bioavailability of the microcrystalline formulation is variable and ranges from 25 to 70 percent; ultramicrocrystalline griseofulvin, in contrast, is almost completely absorbed.[66,225]

After oral administration, C_{max} occurs approximately 4 hours after dosing. Griseofulvin distributes to keratin precursor cells and is concentrated in skin, hair, nails, liver, adipose tissue, and skeletal muscles. In skin, over time, a concentration gradient is established, with the highest concentrations found in the outermost stratum corneum.[157,506] Non–protein-bound drug is carried in the extracellular fluid, in sweat, and through transepidermal fluid loss; in addition, reversible protein binding and high lipid solubility cause griseofulvin to partition into the stratum corneum, where its concentrations exceed that of serum. Within 48 to 72 hours after discontinuation of the drug, plasma concentrations of griseofulvin are markedly reduced, and the compound no longer is detectable in the stratum corneum.[66,225]

Griseofulvin is oxidatively demethylated and conjugated with glucuronic acid primarily in the liver; its major metabolite, 6-desmethylgriseofulvin, is microbiologically inactive.[327] The elimination of the compound from plasma is bi-exponential, with a terminal elimination half-life of 9 to 21 hours.[469] Approximately one third of a single dose of microcrystalline griseofulvin is excreted in feces, and 50 percent is excreted in urine within 5 days. In urine, the drug is excreted mainly as free and glucuronized 6-desmethylgriseofulvin. Unchanged drug in the urine accounts for less than 1 percent of the dose.[327,328]

Separate pharmacokinetic data for pediatric patients have not been published; however, the compound is approved for children older than 2 years of age. The recommended pediatric dosage of microcrystalline griseofulvin is 10 to 20 mg/kg/day, with a maximum of 1 g, administered in two divided doses; the recommended pediatric dosage of ultramicrocrystalline griseofulvin is 5 to 10 mg/kg/day (maximum, 750 mg), also given in two divided doses.[154,173]

Adverse Effects. Griseofulvin has an acceptable safety profile. More common adverse effects include headaches and a variety of gastrointestinal symptoms. Griseofulvin can cause photosensitivity and exacerbate lupus and porphyria. Cases of erythema multiforme–like reactions, toxic epidermal necrolysis, and a reaction resembling serum sickness have been reported. Proteinuria, nephrosis, hepatotoxicity, leukopenia, menstrual irregularities, and mental confusion have been reported rarely in association with griseofulvin therapy. Griseofulvin has been noted to produce estrogen-like effects in children and reversible diminution of hearing.[66,172,225] Griseofulvin is contraindicated in patients with porphyria or hepatocellular failure. The compound has been shown to be teratogenic in animals and should not be administered to pregnant women. Griseofulvin also has mutagenic and carcinogenic potential; the significance of these observations for humans, however, is unclear.[172,173]

Drug Interactions. Griseofulvin has been noted to enhance the clearance of oral contraceptives, cyclosporine, theophylline, aspirin, and warfarin. Concurrent use of phenobarbital may lead to decreased levels of griseofulvin. Finally, concurrent ingestion of alcohol may lead to a disulfiram-like reaction.[172,173]

Indications. Griseofulvin remains an option for the treatment of tinea capitis, particularly when caused by *M. canis*, and refractory tinea corporis (Table 239-15).[153,165, 502] For tinea capitis, 6 to 8 weeks of treatment usually are required.[154,257] The usual duration of therapy for refractory tinea corporis is 4 weeks.[154,174] Nail infections, which are increasingly observed in the pediatric population,[186] usually fail to respond to therapy with griseofulvin and are better treated with itraconazole or terbinafine. Because griseofulvin is not effective against other fungal infections (Table 239-16), the infecting organism always should be identified as a dermatophyte before therapy is initiated. In vitro resistance of dermatophytes to griseofulvin has been reported and may be the cause of therapeutic failure.[33]

Terbinafine

The allylamine terbinafine (Fig. 239-11) is a synthetic antifungal agent that is useful for topical and systemic (oral) treatment of superficial infections of the skin and its appendages by dermatophytes and yeasts, as well as possibly for treatment of cutaneous sporotrichosis.

TABLE 239-15 Options for Medical Management of Superficial Infections by Dermatophytes

Fungal Disease	Management*
Tinea capitis	Griseofulvin for a total of 6-8 wk
	Fluconazole, itraconazole for 4 wk
	Terbinafine for 4 wk
Tinea unguium	Itraconazole for 3-4 mo
	Terbinafine for 6 wk (fingernail) or 12 wk (toenail)
Tinea corporis, facialis, pedis	Topical antifungal azoles:
	Miconazole, clotrimazole, econazole, ketoconazole, sulconazole, oxiconazole bid for 2-4 wk
	Topical allyl/benzylamines, thiocarbamates:
	Terbinafine, naftifine, butenafine, tolnaftate daily to twice daily for 2-4 wk
	Other topical agents:
	Ciclopirox olamine bid for 2-4 wk
	Refractory infections/immunocompromised patients:
	Griseofulvin for 2-4 wk
	Fluconazole for 2-4 wk
	Itraconazole for 2-4 wk
	Terbinafine for 2-4 wk

Data from Friedlander SF, Suarez S. Pediatric antifungal therapy. *Dermatol Clin* 1998;16:527-37; and Howard RM, Frieden IJ. Dermatophyte infections in children. *Adv Pediatr Infect Dis* 1999;14:73-107.
*In alphabetical order according to class; for details and age-specific dosages, please refer to text.

TABLE 239-16 Options for Medical Management of Superficial Infections by Yeasts

Fungal Disease	Management*
Candida dermatitis	Topical antifungal azoles:
Tinea versicolor	Miconazole, sulconazole, econazole, oxiconazole, clotrimazole bid for 2-4 wk
	Topical polyenes:
	Amphotericin B or nystatin bid/qid for 2-4 wk
	Other topical agents:
	Ciclopirox olamine bid for 2-4 wk
	Refractory infections/immunocompromised patients:
	Fluconazole for 2-4 wk
	Itraconazole for 2-4 wk
Oropharyngeal candidiasis	Topical polyenes:
	Nystatin or amphotericin B four times daily for ≥2 wk
	Topical antifungal azoles:
	Clotrimazole lozenges five times daily for ≥2 wk
	Refractory infections/immunocompromised patients:
	Fluconazole for ≥2 wk
	Itraconazole for ≥2 wk
	Voriconazole
	Posaconazole
	Amphotericin B deoxycholate
	Echinocandin lipopeptides
Vulvovaginal candidiasis	Topical antifungal azoles:
	Miconazole, clotrimazole, butoconazole, terconazole, tioconazole qhs for ≥7 days
	Topical polyenes:
	Nystatin qhs for 14 days
	Systemic antifungal azoles:
	Fluconazole 150 mg PO × 1day/50 mg PO × 3days
	Refractory infections/immunocompromised patients:
	Fluconazole for ≥2 wk
	Itraconazole for ≥2 wk
	Amphotericin B deoxycholate

Data from Bennett JE. Antifungal agents. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases.* 4th ed. New York, Churchill Livingstone, 1995, p. 401-10; and Walsh TJ, Gonzalez C, Lyman CA, et al. Invasive fungal infections in children: recent advances in diagnosis and treatment. *Adv Pediatr Infect Dis* 1996;11:187-290.
*In alphabetical order according to class; for details and age-specific dosages, please refer to text.
PO, Orally; *qhs,* at bedtime.

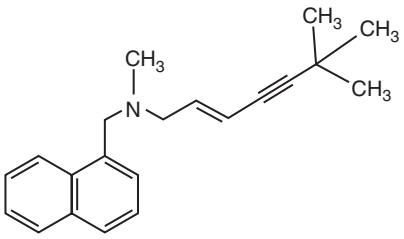

Terbinafine

FIGURE 239-11 ■ Structural formula of terbinafine.

Terbinafine acts by inhibiting the biosynthesis of fungal ergosterol at the level of squalene epoxidase (see Fig. 239-8), thus leading to depletion of ergosterol and accumulation of toxic squalenes in the fungal cell membrane.[41]

Antifungal Activity. Terbinafine has exceptionally potent and fungicidal in vitro activity against dermatophytes.[165] It also is highly active against *Aspergillus* spp., certain hyalohyphomycetes, dematiaceous and dimorphic fungi, and *P. jiroveci*. Its in vitro activity against yeasts appears more variable.[181,207,270,474] Comparative studies have indicated that terbinafine may be more active against *Aspergillus* spp. than itraconazole[505] or amphotericin B,[494] more active than itraconazole against *S. schenkii*,[293] and less or comparably active than the azoles against yeasts.[505] Synergy with triazoles against *C. albicans*,[169,598] *C. glabrata*,[44] *C. neoformans*,[169] *A. fumigatus*,[72,381] non-*Aspergillus* hyalohyphomycetes,[362,363,406,474] and Mucorales[195] has been reported. Although more variable, synergy with amphotericin B also has been demonstrated for filamentous and yeastlike fungi.[474] Terbinafine resistance in clinical dermatophyte isolates is a rare occurrence; only two cases have been documented in which the resistance mechanism was identified as a single amino acid substitution in squalene epoxidase.[407]

Pharmacokinetics. The pharmacokinetic properties of terbinafine in adults are well characterized.[41] Independent of food, oral bioavailability is 70 to 80 percent. Over the adult dosage range of 125 to 750 mg once daily, terbinafine displays linear plasma pharmacokinetics. C_{max} of 0.5 to 2.7 g/L is measured within 2 hours,[267,298,299] and steady state is reached after 10 to 14 days after only twofold accumulation.[41] As a lipophilic drug, terbinafine is strongly bound to plasma proteins. The compound is distributed extensively to tissues and accumulates throughout adipose tissues, dermis, epidermis, and nail. It exhibits a triphasic distribution pattern in plasma, with a terminal half-life up to 3 weeks; microbiologically active concentrations can be measured in plasma for weeks to months after the last dose, a finding consistent with a slow redistribution from peripheral tissue and adipose tissue sites.[41,299,390] Terbinafine undergoes extensive and complex hepatic biotransformation that involves at least seven CYP450 enzymes.[569] Fifteen metabolites have been identified, mainly in urine; none has been shown to be mycologically active.[299] Studies employing radiolabeled drug demonstrated that urinary excretion accounts for more than 70 percent and fecal elimination for 10 percent of radioactivity; the extent of enterohepatic recycling is unknown.[260,262] As a consequence of the compound's extensive hepatic metabolism and urinary excretion, caution is warranted in treating patients with severe hepatic and renal impairment (creatinine clearance ≤50 mL/min).[41]

The pharmacokinetic properties of terbinafine and five known major metabolites in plasma and urine were investigated carefully after single and repeated oral administration of 125 mg/day to 12 pediatric patients for up to 56 days (mean age, 8 years; age range, 5 to 11 years; weight range, 17 to 34 kg) (Table 239-17). No differences were found regarding the metabolism of terbinafine in contrast to that in healthy adults. Steady state was reached at least on day 21, and no further accumulation occurred between days 21 and 56.[260,393] Comparison of the kinetic parameters of terbinafine after single administration of 125 mg showed comparable C_{max} and T_{max} (time until occurrence of C_{max}) values and a 40 percent higher AUC; when dose was calculated as milligrams per kilogram or milligrams per square meter, children showed a lower AUC (range, 29% to 45%) in contrast to that in adults, a finding indicating a higher, weight-normalized volume of distribution into lipophilic tissue. Children had shorter β-phase elimination half-lives, but the γ-phase, terminal half-life determined after multiple dosing during washout was similar to that in adults. Thus, in children weighing 17 to 34 kg, a dose of 125 mg terbinafine yields pharmacokinetic properties similar to those in adults without drug accumulation, and use of a calculation of milligrams per kilogram or milligrams per square meter would lead to lower drug levels than those recorded in adults.[4,260,274,393] Exploration of lower doses (62.5 mg/day) in eight children weighing 19 to 35 kg revealed an approximate reduction in trough level of 50 percent, a finding indicating linearity of plasma pharmacokinetics in children.[274] Based on the experience with dosages of 10 mg/kg and less in adults and the described pharmacokinetic profile of the compound in children, a dose of 250 mg/day has been proposed for children weighing more than 40 kg, a dose of 125 mg/day for children weighing 20 to 40 kg, and 62.5 mg/day for children weighing less than 20 kg.[274]

Adverse Effects. In adults, terbinafine usually is well tolerated at doses of up to 500 mg/day, and it has a relatively low incidence of adverse effects. The primary adverse effects associated with terbinafine include gastrointestinal upsets, headache, and skin reactions in 2 to 7 percent of patients. Terbinafine can cause hepatitis and liver failure. Potentially severe hepatotoxicity is estimated to occur in 1/120,00 patients, and asymptomatic rises in liver enzyme activities are likely to occur at a frequency of 1/200. Although hepatotoxicity may occur in patients with or without chronic or active liver disease, the drug should not be administered in patients with an underlying liver problem and liver function tests results should be obtained before terbinafine is prescribed. Less common

TABLE 239-17 **Pharmacokinetic Parameters of Terbinafine in Children After a Single Dose of 125 mg Compared with Similar Parameters in Healthy Adults***

Parameters	Children with Tinea Capitis ($n = 12$)	Healthy Adults ($n = 16$)	Statistical Comparison
Age (yr)	8 ± 2	26 ± 4	—
Weight (kg)	26 ± 5	64 ± 6	—
C_{max} (μg/mL)	0.706 ± 0.277	0.565 ± 0.329	NS
T_{max} (hr)	2.1 ± 1.1	5 ± 0.7	NS
$AUC_{0-\infty}$ (μg/mL/hr)	2.967 ± 0.965	2.135 ± 1.131	$p < .05$
$t_{1/2}\beta$ (hr)	14.7 ± 4.3	27 ± 12	$p < .001$

Modified from Jones TC. Overview of the use of terbinafine (Lamisil) in children. *Br J Dermatol* 1995;132:683-9.
*Mean values ± SD
$AUC_{0-\infty}$, Area under the concentration versus time curve from zero to infinity; C_{max}, peak plasma concentration; *NS,* not significant; $t_{1/2}\beta$, elimination half-life; T_{max}, time until occurrence of peak plasma concentration.

significant adverse effects include mostly reversible loss of taste and/or smell; severe skin eruptions, including generalized erythematous pustulosis and drug-induced lupus erythematosus; Stevens-Johnson syndrome; blood dyscrasias; visual disturbances; and depressive symptoms.[2] No evidence indicates that these idiosyncratic effects are increasing in incidence with the increasing use of terbinafine.[409] Terbinafine has not been shown to be carcinogenic or mutagenic; its use is not recommended during pregnancy or in nursing mothers.

Several clinical studies have documented the safety of terbinafine in pediatric patients.* Terbinafine, administered for a median duration of 4 weeks (range, 1 to 28 weeks) was safe in children between 2 and 17 years of age who received the drug for various dermatophyte and yeast infections of the skin: of a total of 196 patients enrolled in six studies, 22 adverse events were observed in 15 patients. Adverse events probably associated with the use of terbinafine occurred in 6 of these patients (3%), but in none of these patients did terbinafine therapy have to be discontinued.[276] Terbinafine was similarly well tolerated in two double-blind, randomized trials in children with tinea capitis.[155,174,330]

Drug Interactions. Multiple CYP450 enzymes are involved in the metabolism of terbinafine. However, with the possible exception of CYP2D6 substrates, in vitro studies revealed little or no effect on the metabolism of many characteristic CYP substrates.[569] Inhibition of CYP2D6-mediated metabolism may be relevant with the concomitant use of tricyclic antidepressants, β blockers, selective serotonin reuptake inhibitors, and type B monoamine oxidase inhibitors.

In clinical interaction studies, no pharmacokinetic or pharmacodynamic interactions were observed with the concomitant administration of terfenadine,[464] midazolam,[8] and alfentanil.[478] Terbinafine can (1) reduce the clearance of theophylline[543]; (2) increase levels of nortryptiline,[558] amitryptiline,[89] desipramine,[335] imipramine,[533] and paroxetine[624]; (3) increase or reduce warfarin exposure[224,596]; and (4) reduce the trough cyclosporine concentration in patients with transplants.[331] Clearance of terbinafine may be decreased by cimetidine and increased by rifampin.[207]

Clinical Indications. Terbinafine is approved by the FDA in patients older than 18 years of age for the treatment of onychomycosis of toenails and fingernails caused by dermatophytes. It may be indicated for treatment of other superficial infections of the skin and its appendages caused by dermatophytes,[2,41,173] for cutaneous and lymphocutaneous sporotrichosis[98,260] subcutaneous phaeohyphomycosis[69,450] (see Tables 239-4 and 239-15), and, investigationally, for seborrhoic dermatitis.[566] Recommended durations of treatment for tinea capitis, tinea corporis, and tinea pedis, fingernail onychomycosis, and toenail onychomycosis in adults are 4, 2, 6, and 12 weeks, respectively.[274,502]

Several studies have explored the clinical efficacy of terbinafine in children and adolescents.* The overall mycologic and clinical efficacy for 152 children aged 2 to 17 years with various dermatophyte and yeast infections of the skin exceeded 95 percent.[274] In a prospective, randomized clinical trial in 210 children comparing treatment with 4 weeks of terbinafine versus 8 weeks of griseofulvin for *Trichophyton* tinea capitis, both regimens showed overall similar efficacy.[175] In a subsequent study of the duration of treatment in 176 patients, both 2 and 4 weeks of treatment were clinically superior to a 1-week regimen and had similar treatment efficacy, a finding suggesting that a 2-week course may be sufficient for patients with *Trichophyton* tinea capitis.[174] A similar study

*References 79, 86, 174, 175, 226, 274, 300, 330, 393, 508.

*References 79, 86, 175, 175, 274, 300, 330, 393, 508.

performed in 134 patients with *Microsporon* tinea capitis investigating 6, 8, 10, or 12 weeks of therapy demonstrated that 6 weeks of therapy were equivalent to griseofulvin.[330] Finally, a meta-analysis of six randomized, comparative clinical trials indicated that a 2- to 4-week course of terbinafine was at least as effective as a 6- to 8-week course of griseofulvin for the treatment of tinea capitis caused by *Trichophyton* spp.[165]

The broad-spectrum fungicidal in vitro activity, systemic availability, and lack of significant side effects suggested potential usefulness of terbinafine against deep-seated fungal infections. However, terbinafine was ineffective in animal models of pulmonary and disseminated aspergillosis,[287,495] systemic sporotrichosis,[277] cerebral phaeohyphomycosis, disseminated candidiasis, and pulmonary cryptococcosis,[473] findings explained by nonsaturable protein-binding kinetics.[495] Against experimental pulmonary pneumocystosis, terbinafine was active in some,[107,108] but not all,[593] models.

TOPICAL ANTIFUNGAL AGENTS

Apart from fungal keratitis, the use of topical antifungal agents is confined to superficial infections of the skin and mucosal surfaces. The decision to treat superficial infections of the skin and mucosal surfaces with a topical or systemic agent depends mainly on the site and extent of the infection. Immunocompromised children, however, usually require systemic therapies, as do patients with tinea capitis and onychomycosis (see Tables 239-15 and 239-16).[154]

Topical Therapeutics for Superficial Skin Infections

Dermatophytosis is caused by the filamentous fungi *Microsporon* spp., *Trichophyton* spp., and *Epidermophyton floccosum*. Many different agents and formulations are available for topical treatment of dermatophytic skin infections (i.e., tinea corporis, facialis, or pedis), including allylamines and azoles. Agents for treatment of *Candida* dermatitis and tinea (pityriasis) versicolor (caused by *Malassezia furfur* or *Malassezia pachydermatidis*) include various topical azoles and topical polyenes. Most topical agents are applied twice daily well beyond the clinical resolution of the infection. A detailed review of the pharmacologic properties of these topical drugs and of the treatment of cutaneous mycoses is beyond the scope of this chapter and can be found elsewhere.[154,173,257]

Topical Therapeutics For Mucosal Candidiasis

Agents for the topical treatment of vulvovaginal candidiasis include a large variety of antifungal azoles and the polyene nystatin.[53,518] Azole agents may be absorbed to a minor extent and potentially can interfere with the metabolism of concomitant drugs. For example, potentiation of the anticoagulatory effects of acenocoumarol was noted after vaginal administration of miconazole capsules to two postmenopausal patients[313] and after oral administration of miconazole gel in three elderly patients with oral candidiasis.[405]

Antifungal azoles such as clotrimazole and miconazole and antifungal polyenes such as amphotericin B and nystatin are effective in the treatment of oropharyngeal candidiasis. Many clinical trials evaluated the usefulness of these agents for prevention of fungal infections in immunocompromised patients with cancer or HSCT. Although most agents have documented efficacy in the prevention of oropharyngeal candidiasis, they are not effective in preventing invasive mycoses and in improving infection-related and overall mortality in this setting.*

FUTURE DIRECTIONS

Pediatric age groups display important differences in host biology, predisposing conditions, epidemiology, and presentation of fungal infections relative to the adult population. Since the late 1990s, major advances have been made in the field of medical mycology. Most importantly, an array of new antifungal agents has entered the clinical arena. Although the final pediatric approval of some of these agents remains to be established, the development of pediatric therapies has moved forward in an exemplary manner. Invasive fungal infections will remain important causes of morbidity and mortality in immunocompromised pediatric patients. The availability of alternative therapeutic options is an important advance; at the same time, however, antifungal therapy has become increasingly complex. In addition to information on prior antifungal therapies, microbiologic data, existing comorbidities, and concurrent medications, detailed knowledge of the available antifungal armamentarium and contemporary clinical trials is needed more than ever in the management of the individual patient.

NEW REFERENCES SINCE THE SIXTH EDITION

20. Andes D, Ambrose PG, Hammel JP, et al. Use of pharmacokinetic-pharmacodynamic analyses to optimize therapy with the systemic antifungal micafungin for invasive candidiasis or candidemia. *Antimicrob Agents Chemother* 2011;**55**:2113–21.
21. Andes D, Diekema DJ, Pfaller MA, et al. In vivo pharmacodynamic characterization of anidulafungin in a neutropenic murine candidiasis model. *Antimicrob Agents Chemother* 2008;**52**:539–50.
22. Andes DR, Diekema DJ, Pfaller MA, et al. In vivo pharmacodynamic target investigation for micafungin against *Candida albicans* and *C. glabrata* in a neutropenic murine candidiasis model. *Antimicrob Agents Chemother* 2008;**52**:3497–503.
31. Arrieta AC, Maddison P, Groll AH. Safety of micafungin in pediatric clinical trials. *Pediatr Infect Dis J* 2011;**30**:e97–102.
32. Arrieta AC, Shea K, Dhar V, et al. Once-weekly liposomal amphotericin B as *Candida* prophylaxis in very low birth weight premature infants: a prospective, randomized, open-label, placebo-controlled pilot study. *Clin Ther* 2010;**32**:265–71.
34. Ascher SB, Smith PB, Watt K, et al. Antifungal therapy and outcomes in infants with invasive *Candida* infections. *Pediatr Infect Dis J* 2012;**31**:439–43.
37. Auron A, Auron-Gomez M, Raina R, et al. Effect of amphotericin B lipid complex (ABLC) in very low birth weight infants. *Pediatr Nephrol* 2009;**24**:295–9.

*References 153, 209, 254, 332, 553, 539

52. Benjamin DK Jr, Smith PB, Arrieta A, et al. Safety and pharmacokinetics of repeat-dose micafungin in young infants. *Clin Pharmacol Ther* 2010;**87**:93–9.
61. Betts RF, Nucci M, Talwar D, et al. A Multicenter, double-blind trial of a high-dose caspofungin treatment regimen versus a standard caspofungin treatment regimen for adult patients with invasive candidiasis. *Clin Infect Dis* 2009;**48**:1676–84.
62. Beyda ND, Lewis RE, Garey KW. Echinocandin resistance in *Candida* species: mechanisms of reduced susceptibility and therapeutic approaches. *Ann Pharmacother* 2012;**46**:1086–96.
80. Brüggemann RJ, Alffenaar JW, Blijlevens NM, et al. Clinical relevance of the pharmacokinetic interactions of azole antifungal drugs with other coadministered agents. *Clin Infect Dis* 2009; **48**:1441–58.
90. Cattaneo C, Monte S, Algarotti A, et al. A randomized comparison of caspofungin versus antifungal prophylaxis according to investigator policy in acute leukaemia patients undergoing induction chemotherapy (PROFIL-C study). *J Antimicrob Chemother* 2011; **66**:2140–5.
93. Center for Drug Evaluation and Research. *Mycamine (micafungin sodium) for injection label information*. Washington, D.C.: U.S. Food and Drug Administration; 2011. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021506s011s012lbl.pdf.
104. Cohen-Wolkowiez M, Benjamin DK Jr, Piper L, et al. Safety and pharmacokinetics of multiple-dose anidulafungin in infants and neonates. *Clin Pharmacol Ther* 2011;**89**:702–7.
106. Conte JE Jr, Golden JA, Krishna G, et al. Intrapulmonary pharmacokinetics and pharmacodynamics of posaconazole at steady state in healthy subjects. *Antimicrob Agents Chemother* 2009;**53**: 703–7.
112. Cornely OA, Vehreschild JJ, Vehreschild MJ, et al. Phase II dose escalation study of caspofungin for invasive aspergillosis. *Antimicrob Agents Chemother* 2011;**55**:5798–803.
117. Cowen EW, Nguyen JC, Miller DD, et al. Chronic phototoxicity and aggressive squamous cell carcinoma of the skin in children and adults during treatment with voriconazole. *J Am Acad Dermatol* 2010;**62**:31–7.
118. Crandon JL, Banevicius MA, Fang AF, et al. Bronchopulmonary disposition of intravenous voriconazole and anidulafungin given in combination to healthy adults. *Antimicrob Agents Chemother* 2009;**53**:5102–7.
123. de Lima Barros MB, Schubach AO, de Vasconcellos Carvalhaes de Oliveira R, et al. Treatment of cutaneous sporotrichosis with itraconazole: study of 645 patients. *Clin Infect Dis* 2011; **52**:e200–6.
129. Denning DW, Park S, Lass-Florl C, et al. High-frequency triazole resistance found In nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin Infect Dis* 2011; **52**:1123–9.
138. Doby EH, Benjamin DK Jr, Blaschke AJ, et al. Therapeutic monitoring of voriconazole in children less than 3 years of age: a case report and summary of voriconazole concentrations for 10 children. *Pediatr Infect Dis J* 2012;**31**:632–5.
140. Dokos C, Pieper S, Lehrnbecher T, et al. Pharmacokinetics, safety and efficacy of voriconazole in pediatric patients: an update. *Curr Fungal Infect Reports* 2012;**6**:121–6.
141. Doring M, Hartmann U, Erbacher A, et al. Caspofungin as antifungal prophylaxis in pediatric patients undergoing allogeneic hematopoietic stem cell transplantation: a retrospective analysis. *BMC Infect Dis* 2012;**12**:151.
142. Dotis J, Simitsopoulou M, Dalakiouridou M, et al. Amphotericin B formulations variably enhance antifungal activity of human neutrophils and monocytes against Fusarium solani: comparison with *Aspergillus fumigatus*. *J Antimicrob Chemother* 2008;**61**: 810–17.
143. Driscoll TA, Yu LC, Frangoul H, et al. Pharmacokinetics and safety of intravenous voriconazole to oral switch in immunocompromised children compared to adults. *Antimicrob Agents Chemother* 2011;**55**:5770–9.
146. Driscoll TA, Yu LC, Frangoul HL, et al. Comparison of pharmacokinetics and safety of voriconazole intravenous-to-oral switch in immunocompromised adolescents and healthy adults. *Antimicrob Agents Chemother* 2011;**55**:5770–9.
152. Dutta A, Palazzi DL.Risk factors of amphotericin B toxicity in the nonneonatal pediatric population. *Pediatr Infect Dis J* 2012; **31**:910–14.
153. Dvorak CC, Fisher BT, Sung L, et al. Antifungal prophylaxis in pediatric hematology/oncology: new choices & new data. *Pediatr Blood Cancer* 2012;**59**:21–6.
155. Elewski BE, Cáceres HW, DeLeon L, et al. Terbinafine hydrochloride oral granules versus oral griseofulvin suspension in children with tinea capitis: results of two randomized, investigator-blinded, multicenter, international, controlled trials. *J Am Acad Dermatol* 2008;**59**:41–54.
156. Elewski B, Pollak R, Ashton S, et al. A randomized, placebo- and active-controlled, parallel-group, multicentre, investigator-blinded study of four treatment regimens of posaconazole in adults with toenail onychomycosis. *Br J Dermatol* 2012;**166**:389–98.
186. Ginter-Hanselmayer G, Weger W, Smolle J. Onychomycosis: a new emerging infectious disease in childhood population and adolescents—report on treatment experience with terbinafine and itraconazole in 36 patients. *J Eur Acad Dermatol Venereol* 2008; **22**:470–5.
204. Groll AH, Tragiannidis A. Update on antifungal agents for pediatric patients. *Clin Microbiol Infect* 2010;**16**:1343–53.
210. Groll AH, Schrey D, Walsh TJ. Echinocandins. In: Kauffman CA, Pappas PG, Sobel JD, Dismukes DE, editors. *Essentials of clinical mycology*. New York, NY: Oxford University Press; 2011. p. 95–112.
220. Groll AH, Silling G, Young C, et al. Randomized comparison of safety and pharmacokinetics of caspofungin, liposomal amphotericin B, and the combination of both in allogeneic hematopoietic stem cell recipients. *Antimicrob Agents Chemother* 2010; **54**:4143–9.
240. Herbrecht R, Maertens J, Baila L, et al. Caspofungin first-line therapy for invasive aspergillosis in allogeneic hematopoietic stem cell transplant patients: an European Organisation for Research and Treatment of Cancer study. *Bone Marrow Transplant* 2010;**45**:1227–33.
250. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous *Candida* meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008;**197**:163–71.
252. Hope WW, Smith PB, Arrieta A, et al. Population pharmacokinetics of micafungin in neonates and young infants. *Antimicrob Agents Chemother* 2010;**54**:2633–7.
258. Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus:* epidemiology and detection. *Med Mycol* 2011;**49**(Suppl. 1):S90–95.
259. Howard SJ, Lestner JM, Sharp A, et al. Pharmacokinetics and pharmacodynamics of posaconazole for invasive pulmonary aspergillosis: clinical implications for antifungal therapy. *Infect Dis* 2011;**203**:1324–32.
268. Jeans AR, Howard SJ, Al-Nakeeb Z, et al. Pharmacodynamics of voriconazole in a dynamic in vitro model of invasive pulmonary aspergillosis: implications for in vitro susceptibility breakpoints. *J Infect Dis* 2012;**206**:442–52.
269. Jensen GM, Bunch TH, Wolf S, et al. Erroneous determination of hyperphosphatemia ('pseudohyperphosphatemia') in sera of patients that have been treated with liposomal amphotericin B (AmBisome). *Clin Chim Acta* 2010;**411**:1900–5.
279. Karlsson M, Lutsar OI, Milligan PA. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.
285. Kaufman DA. "Getting to Zero": preventing invasive *Candida* infections and eliminating infection-related mortality and morbidity in extremely preterm infants. *Early Hum Dev* 2012;**88**(Suppl 2):S45–9.
291. Knoderer CA, Knoderer HM. Hyperphosphatemia in pediatric oncology patients receiving liposomal amphotericin B. *Pediatr Pharmacol Ther* 2011;**16**:87–91.
294. Kolve H, Ahlke E, Fegeler W, et al. Safety, tolerance and outcome of treatment with liposomal amphotericin B in paediatric patients with cancer or undergoing haematopoietic stem cell transplantation *J Antimicrob Chemother* 2009;**64**:383–7.
305. Krishna G, AbuTarif M, Xuan F, et al. Pharmacokinetics of oral posaconazole in neutropenic patients receiving chemotherapy for acute myelogenous leukemia or myelodysplastic syndrome. *Pharmacotherapy* 2008;**28**:1223–32.
319. Le J, Adler-Shohet FC, Nguyen C, et al. Nephrotoxicity associated with amphotericin B deoxycholate in neonates. *Pediatr Infect Dis J* 2009;**28**:1061–3.

321. Lehrnbecher T, Attarbaschi A, Duerken M, et al. Posaconazole salvage treatment in paediatric patients: a multicentre survey. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1043–5.
324. Leventakos K, Ben Ami R, Lewis RE, et al. Immunomodulating effects of antifungal therapy. *Curr Fungal Infect Rep* 2009; **3**:243–50.
326. Li CC, Sun P, Dong Y, et al. Population pharmacokinetics and pharmacodynamics of caspofungin in pediatric patients. *Antimicrob Agents Chemother* 2011;**55**:2098–105.
337. Maertens JA, Madero L, Reilly AF, et al. A randomized, double-blind, multicenter study of caspofungin versus liposomal amphotericin B for empiric antifungal therapy in pediatric patients with persistent fever and neutropenia. *Pediatr Infect Dis J* 2010;**29**: 415–20.
345. Mandhaniya S, Swaroop C, Thulkar S, et al. Oral voriconazole versus intravenous low dose amphotericin B for primary antifungal prophylaxis in pediatric acute leukemia induction: a prospective, randomized, clinical study. *J Pediatr Hematol Oncol* 2011;**33**: e333–41.
347. Manzoni P, Galletto P, Rizzollo S, et al. Liposomal amphotericin B does not induce nephrotoxicity or renal function impairment in premature neonates. *Early Hum Dev* 2012;**88**(Suppl. 2):S86–91.
351. Marks DI, Pagliuca A, Kibbler CC, et al.Voriconazole versus itraconazole for antifungal prophylaxis following allogeneic haematopoietic stem-cell transplantation, *Br J Haematol* 2011;**155**: 318–27.
356. Marr KAS, Rottinghaus H, Jagannatha S, et al. A randomised, double-blind study of combination antifungal therapy with voriconazole and anidalafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis. In: *Abstracts of the 2012 European Society for Clinical Microbiology and Infectious Diseases Meeting*. European Society for Clinical Microbiology and Infectious Diseases; Abstract LB2812 (2012).
361. Mehta PA, Vinks AA, Filipovich A, et al. Alternate-day micafungin antifungal prophylaxis in pediatric patients undergoing hematopoietic stem cell transplantation: a pharmacokinetic study. *Biol Blood Marrow Transplant* 2010;**16**:1458–62.
368. Milefchik E, Leal MA, Haubrich R, et al. Fluconazole alone or combined with flucytosine for the treatment of AIDS-associated cryptococcal meningitis. *Med Mycol* 2008;**46**: 393–5.
370. Miller DD, Cowen EW, Nguyen JC, et al. Melanoma associated with long-term voriconazole therapy: a new manifestation of chronic photosensitivity. *Arch Dermatol* 2010;**146**:300–4.
374. Molina JR, Serrano J, Sánchez-García J, et al. Voriconazole as primary antifungal prophylaxis in children undergoing allo-SCT. *Bone Marrow Transplant* 2012;**47**:562–7.
377. Moriyama B, Falade O, Leung J, et al. Prolonged half-life of voriconazole in a CYP2C19 homozygous poor metabolizer receiving vincristine chemotherapy: avoiding a serious adverse drug interaction. *Mycoses* 2011;**54**:e877–9.
378. Morio F, Aubin GG, Danner-Boucher I, et al. High prevalence of triazole resistance in *Aspergillus fumigatus*, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. *J Antimicrob Chemother* 2012;**67**:1870–3.
391. Neely M, Jafri HS, Seibel N, et al. Pharmacokinetics and safety of caspofungin in older infants and toddlers. *Antimicrob Agents Chemother* 2009;**53**:1450–6.
399. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin Infect Dis* 2010;**50**:338–44.
418. Park WB, Kim NH, Kim KH, et al. The effect of therapeutic drug monitoring on safety and efficacy of voriconazole in invasive fungal infections: a randomized controlled trial. *Clin Infect Dis* 2012;**55**:1080–7.
424. Perlin DS. Current perspectives on echinocandin class drugs. *Future Microbiol* 2011;**6**:441–57.
427. Pfaller MA. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *Am J Med* 2012;**125**(Suppl. 1):S3–13.
430. Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D; CLSI Subcommittee for Antifungal Susceptibility Testing. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and Candida: time for harmonization of CLSI and EUCAST broth microdilution methods. *Drug Resist Updat* 2010;**13**:180–95.
431. Pfaller MA, Castanheira M, Lockhart SR, et al. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. *J Clin Microbiol* 2012;**50**:1199–203.
432. Pfaller MA, Diekema DJ, Andes D, et al. CLSI Subcommittee for Antifungal Testing. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Updat* 2011;**14**:164–76.
436. Pieper S, Kolve H, Gumbinger HG, et al. Safety, tolerance and outcome of treatment with voriconazole in immunocompromised pediatric patients. *Mycoses* 2011;**54**(Suppl. 2):125.
437. Pieper S, Kolve H, Gumbinger HG, et al. Monitoring of voriconazole plasma concentrations in immunocompromised paediatric patients. *J Antimicrob Chemother* 2012;**67**:2717–24.
438. Piper L, Smith PB, Hornik CP, et al. Fluconazole loading dose pharmacokinetics and safety in infants. *Pediatr Infect Dis J* 2011;**30**:375–87.
447. Queiroz-Telles F, Berezin E, Leverger G, et al. Micafungin versus liposomal amphotericin B for pediatric patients with invasive candidiasis: substudy of a randomized double-blind trial. *Pediatr Infect Dis J* 2008;**27**:820–6.
466. Rodríguez-Tudela JL, Arendrup MC, Cuenca-Estrella M, et al. EUCAST breakpoints for antifungals. *Drug News Perspect* 2010; **23**:93–7.
481. Sáez-Llorens X, Macias M, Maiya P, et al. Pharmacokinetics and safety of caspofungin in neonates and infants less than 3 months of age. *Antimicrob Agents Chemother* 2009;**53**:869–75.
510. Simitsopoulou M, Roilides E, Georgiadou E, et al. Differential transcriptional profiles induced by amphotericin B formulations on human monocytes during response to hyphae of *Aspergillus fumigatus*. *Med Mycol* 2011;**49**:176–85.
511. Simitsopoulou M, Roilides E, Maloukou A, et al. Interaction of amphotericin B lipid formulations and triazoles with human polymorphonuclear leucocytes for antifungal activity against Zygomycetes. *Mycoses* 2008;**51**:147–54.
512. Sionov E, Chang YC, Garraffo HM, Kwon-Chung KJ. Heteroresistance to fluconazole in *Cryptococcus neoformans* is intrinsic and associated with virulence. *Antimicrob Agents Chemother* 2009;**53**:2804–15.
513. Sionov E, Lee H, Chang YC, Kwon-Chung KJ. *Cryptococcus neoformans* overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog* 2010;**6**:e1000848.
516. Smith PB, Walsh TJ, Hope W, et al. Pharmacokinetics of an elevated dosage of micafungin in premature neonates. *Pediatr Infect Dis J* 2009;**28**:412–15.
517. Snelders E, van der Lee HA, Kuijpers J, et al. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 2008;**5**:e219.
540. Tragiannidis A, Roilides E, Walsh TJ, et al. Invasive aspergillosis in children with acquired immunodeficiencies. *Clin Infect Dis* 2012;**54**:258–67.
540. Tragiannidis A, Dokos C, Lehrnbecher T, et al. Antifungal chemoprophylaxis in children and adolescents with haematological malignancies and following allogeneic haematopoietic stem cell transplantation: review of the literature and options for clinical practice. *Drugs* 2012;**72**:685–705.
545. Troke PF, Hockey HP, Hope WW. Observational study of the clinical efficacy of voriconazole and its relationship to plasma concentrations in patients. *Antimicrob Agents Chemother* 2011;**55**: 4782–8.
561. van der Linden JW, Snelders E, Kampinga GA, et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. *Emerg Infect Dis* 2011;**17**:1846–54.
572. Viscoli C, Herbrecht R, Akan H, et al. An EORTC Phase II study of caspofungin as first-line therapy of invasive aspergillosis in haematological patients. *J Antimicrob Chemother* 2009;**64**:1274–81.
573. Wade KC, Benjamin DK Jr, Kaufman DA, et al. Fluconazole dosing for the prevention or treatment of invasive candidiasis in young infants. *Pediatr Infect Dis J* 2009;**228**:717–23.
574. Wade KC, Wu D, Kaufman DA, et al. Population pharmacokinetics of fluconazole in young infants. *Antimicrob Agents Chemother* 2008;**52**:4043–9.

577. Walsh T, Driscoll T, Milligan P, et al. Pharmacokinetics, safety, and tolerability of voriconazole in immunocompromised children. *Antimicrob Agents Chemother* 2010;**48**:4116–23.
579. Walsh TJ, Goutelle S, Jelliffe RW, et al. Intrapulmonary pharmacokinetics and pharmacodynamics of micafungin in adult lung transplant patients. *Antimicrob Agents Chemother* 2010;**54**, 3451–9.
594. Warn PA, Livermore J, Howard S, et al. Anidulafungin for neonatal hematogenous *Candida* meningoencephalitis: identification of candidate regimens for humans using a translational pharmacological approach. *Antimicrob Agents Chemother* 2012;**56**:708–14.
597. Watt KM, Benjamin DK Jr, Cheifetz IM, et al. Pharmacokinetics and safety of fluconazole in young infants supported with extracorporeal membrane oxygenation. *Pediatr Infect Dis J* 2012;**31**:1042–7.
599. Welzen ME, Brüggemann RJ, Van Den Berg JM, et al. A twice daily posaconazole dosing algorithm for children with chronic granulomatous disease. *Pediatr Infect Dis J* 2011;**30**:794–7.
600. Wermers RA, Cooper K, Razonable RR, et al. Fluoride excess and periostitis in transplant patients receiving long-term voriconazole therapy. *Clin Infect Dis* 2011;**52**:604–11.
613. Wingard JR, Carter SL, Walsh TJ, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation, *Blood* 2010;**116**:5111–18.
627. Zaoutis T, Lehrnbecher T, Groll AH, et al. Safety experience with caspofungin in pediatric patients. *Pediatr Infect Dis J* 2009;**28**:1132–5.
628. Zaoutis TE, Jafri HS, Huang LM, et al. A prospective, multicenter study of caspofungin for the treatment of documented *Candida* or *Aspergillus* infections in pediatric patients. *Pediatrics* 2009;**123**:877–84.
629. Zimbeck AJ, Iqbal N, Ahlquist AM, et al. FKS mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from U.S. population-based surveillance. *Antimicrob Agents Chemother* 2010;**54**:5042–7.

The full reference list for this chapter is available at expertconsult.com.

The Medical Letter publications are protected by US and international copyright laws.
Forwarding, copying or any other distribution of this material is strictly prohibited. For further information call: 800-211-2769

CHAPTER 240

DRUGS FOR PARASITIC INFECTIONS

With increasing travel, immigration, use of immunosuppressive drugs and the spread of AIDS, physicians anywhere may see infections caused by parasites. The table below lists first-choice and alternative drugs for most parasitic infections. The table that begins on page 3351 summarizes the known prenatal risks of antiparasitic drugs. The brand names and manufacturers of the drugs are listed in the table on page 3354.

Infection		Drug	Adult dosage	Pediatric dosage
Acanthamoeba keratitis				
Drug of choice:		See footnote 1		
Amebiasis *(Entamoeba histolytica)*				
Asymptomatic				
Drug of choice:		Iodoquinol[2]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
	OR	Paromomycin[3]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
	OR	Diloxanide furoate[4*]	500 mg PO tid × 10 d	20 mg/kg/d PO in 3 doses × 10 d

FORWARDING OR COPYING IS A VIOLATION OF U.S. AND INTERNATIONAL COPYRIGHT LAWS

REFERENCES

1. Aanpreung P, Veerakul G. Itraconazole for treatment of oral candidosis in pediatric cancer patients. *J Med Assoc Thai* 1997;**80**:358–62.
2. Abdel-Rahman SM, Nahata MC. Oral terbinafine: a new antifungal agent. *Ann Pharmacother* 1977;**31**:445–56.
3. Abdel-Rahman SM, Powell DA, Nahata MC. Efficacy of itraconazole in children with *Trichophyton tonsurans* tinea capitis. *J Am Acad Dermatol* 1998;**38**:443–6.
4. Abdel-Rahman SM, Herron J, Fallon-Friedlander S, et al. Pharmacokinetics of terbinafine in young children treated for tinea capitis. *Pediatr Infect Dis J* 2005;**24**:886–91.
5. Abdel-Rahman SM, Jacobs RF, Massarella J, et al. Single-dose pharmacokinetics of intravenous itraconazole and hydroxypropyl-beta-cyclodextrin in infants, children, and adolescents. *Antimicrob Agents Chemother* 2007;**51**:2668–73.
6. Aguilar C, Pujol I, Sala J, et al. Antifungal susceptibilities of *Paecilomyces* species. *Antimicrob Agents Chemother* 1998;**42**:1601–4.
7. Ahmad SR, Singer SJ, Leissa BG. Congestive heart failure associated with itraconazole. *Lancet* 2001;**357**:1766–7.
8. Ahonen J, Olkkola KT, Neuvonen PJ. Effect of itraconazole and terbinafine on the pharmacokinetics and pharmacodynamics of midazolam in healthy volunteers. *Br J Clin Pharmacol* 1995;**40**:270–2.
9. Ally R, Schurmann D, Kreisel W, et al. A randomized, double-blind, double-dummy, multicenter trial of voriconazole and fluconazole in the treatment of esophageal candidiasis in immunocompromised patients. *Clin Infect Dis* 2001;**33**:1447–54.
10. Amantea MA, Bowden RA, Forrest A, et al. Population pharmacokinetics and renal function-sparing effects of amphotericin B colloidal dispersion in patients receiving bone marrow transplants. *Antimicrob Agents Chemother* 1995;**39**:2042–7.
11. Anaissie E, Bodey GP, Kantarjian H, et al. Fluconazole therapy for chronic disseminated candidiasis in patients with leukemia and prior amphotericin B therapy. *Am J Med* 1991;**91**:142–50.
12. Anaissie EJ, Darouiche RO, Abi-Said D, et al. Management of invasive candidal infections: results of a prospective, randomized, multicenter study of fluconazole versus amphotericin B and review of the literature. *Clin Infect Dis* 1996;**23**:964–72.
13. Anaissie E, Gokoslan A, Hachem R, et al. Azole therapy for trichosporonosis: clinical evaluation of eight patients, experimental therapy for murine infection, and review. *Clin Infect Dis* 1992;**15**:781–7.
14. Anaissie EJ, Karyotakis NC, Hachem R, et al. Correlation between in vitro and in vivo activity of antifungal agents against *Candida* species. *J Infect Dis* 1994;**170**:384–9.
15. Anaissie EJ, Kontoyiannis DP, Huls C, et al. Safety, plasma concentrations, and efficacy of high-dose fluconazole in invasive mold infections. *J Infect Dis* 1995;**172**:599–602.
16. Anaissie EJ, Vartivarian SE, Abi-Said D, et al. Fluconazole versus amphotericin B in the treatment of hematogenous candidiasis: a matched cohort study. *Am J Med* 1996;**101**:170–6.
17. Anaissie E, White M, Uzun O, et al. Amphotericin B lipid complex (ABLC) versus amphotericin B (AMB) for treatment of hematogenous and invasive candidiasis: a prospective, randomized, multicenter trial. Abstract LM 21. In: *Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 1995. p. 330.
18. Andes D, van Ogtrop M. Characterization and quantitation of the pharmacodynamics of fluconazole in a neutropenic murine disseminated candidiasis infection model. *Antimicrob Agents Chemother* 1999;**43**:2116–20.
19. Andes D, van Ogtrop M. In vivo characterization of the pharmacodynamics of flucytosine in a neutropenic murine disseminated candidiasis model. *Antimicrob Agents Chemother* 2000;**44**:938–42.
20. Andes D, Ambrose PG, Hammel JP, et al. Use of pharmacokinetic-pharmacodynamic analyses to optimize therapy with the systemic antifungal micafungin for invasive candidiasis or candidemia. *Antimicrob Agents Chemother* 2011;**55**:2113–21.
21. Andes D, Diekema DJ, Pfaller MA, et al. In vivo pharmacodynamic characterization of anidulafungin in a neutropenic murine candidiasis model. *Antimicrob Agents Chemother* 2008;**52**:539–50.
22. Andes DR, Diekema DJ, Pfaller MA, et al. In vivo pharmacodynamic target investigation for micafungin against *Candida albicans* and *C. glabrata* in a neutropenic murine candidiasis model. *Antimicrob Agents Chemother* 2008;**52**:3497–503.
23. Andes D, Lepak A, Nett J, et al. In vivo fluconazole pharmacodynamics and resistance development in a previously susceptible *Candida albicans* population examined by microbiologic and transcriptional profiling. *Antimicrob Agents Chemother* 2006;**50**:2384–94.
24. Andes D, Marchillo K, Conklin R, et al. Pharmacodynamics of a new triazole, posaconazole, in a murine model of disseminated candidiasis. *Antimicrob Agents Chemother* 2004;**48**:137–42.
25. Andes D, Marchillo K, Stamstad T, et al. In vivo pharmacokinetics and pharmacodynamics of a new triazole, voriconazole, in a murine candidiasis model. *Antimicrob Agents Chemother* 2003;**47**:3165–9.
26. Andes D, Stamsted T, Conklin R. Pharmacodynamics of amphotericin B in a neutropenic-mouse disseminated-candidiasis model. *Antimicrob Agents Chemother* 2001;**45**:922–6.
27. Arathoon EG, Gotuzzo E, Noriega LM, et al. Randomized, double-blind, multicenter study of caspofungin versus amphotericin B for treatment of oropharyngeal and esophageal candidiasis. *Antimicrob Agents Chemother* 2002;**46**:451–7.
28. Arieta A, Maddison P, Groll AH. Micafungin in pediatric patients: assessment of safety in clinical trials. *Pediatr Infect Dis J* 2011;**30**:e97–102.
29. Arning M, Scharf RE. Prevention of amphotericin B induced nephrotoxicity by loading with sodium-chloride: a report of 1291 days of treatment with amphotericin B without renal failure. *Klin Wochenschr* 1989;**67**:1020–8.
30. Arning M, Kliche KO, Heer-Sonderhoff AH, et al. Infusion-related toxicity of three different amphotericin B formulations and its relation to cytokine plasma levels. *Mycoses* 1995;**38**:459–65.
31. Arrieta AC, Maddison P, Groll AH. Safety of micafungin in pediatric clinical trials. *Pediatr Infect Dis J* 2011;**30**:e97–102.
32. Arrieta AC, Shea K, Dhar V, et al. Once-weekly liposomal amphotericin B as *Candida* prophylaxis in very low birth weight premature infants: a prospective, randomized, open-label, placebo-controlled pilot study. *Clin Ther* 2010;**32**:265–71.
33. Artis WM, Odle BM, Jones HE. Griseofulvin-resistant dermatophytosis correlates with in vitro resistance. *Arch Dermatol* 1981;**117**:16–19.
34. Ascher SB, Smith PB, Watt K, et al. Antifungal therapy and outcomes in infants with invasive Candida infections. *Pediatr Infect Dis J* 2012;**31**:439–43.
35. Astellas Pharma GmbH. *Mycamine: Summary of product characteristics*. Available at http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Product_Information/human/000734/WC500031075.pdf.
36. Atkinson AJ Jr, Bennett JE. Amphotericin B pharmacokinetics in humans. *Antimicrob Agents Chemother* 1978;**13**:271–6.
37. Auron A, Auron-Gomez M, Raina R, et al. Effect of amphotericin B lipid complex (ABLC) in very low birth weight infants. *Pediatr Nephrol* 2009;**24**:295–9.
38. Balani SK, Xu X, Arison BH, et al. Metabolites of caspofungin acetate, a potent antifungal agent, in human plasma and urine. *Drug Metab Dispos* 2000;**28**:1274–8.
39. Baley JE, Kliegman RM, Fanaroff AA. Disseminated fungal infections in very low-weight infants: therapeutic toxicity. *Pediatrics* 1984;**73**:153–7.
40. Baley JE, Meyers C, Kliegman RM, et al. Pharmacokinetics, outcome of treatment, and toxic effects of amphotericin B and 5-fluorocytosine in neonates. *J Pediatr* 1990;**116**:791–7.
41. Balfour JA, Faulds D. Terbinafine: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial mycoses. *Drugs* 1992;**43**:259–84.
42. Barchiesi F, Najvar LK, Luther MF, et al. Variation in fluconazole efficacy for *Candida albicans* strains sequentially isolated from oral cavities of patients with AIDS in an experimental murine candidiasis model. *Antimicrob Agents Chemother* 1996;**40**:1317–20.
43. Barchiesi F, Schimizzi AM, Najvar LK, et al. Interactions of posaconazole and flucytosine against *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 2001;**45**:1355–9.
44. Barchiesi F, Spreghini E, Maracci M, et al. In vitro activities of voriconazole in combination with three other antifungal agents against *Candida glabrata*. *Antimicrob Agents Chemother* 2004;**48**:3317–22.
45. Barker KS, Rogers PD. Recent insights into the mechanisms of antifungal resistance. *Curr Infect Dis Rep* 2006;**8**:449–56.

46. Barone JA, Moskovitz BL, Guarnieri J, et al. Enhanced bioavailability of itraconazole in hydroxypropyl-beta-cyclodextrin solution versus capsules in healthy volunteers. *Antimicrob Agents Chemother* 1998;**42**:1862–5.
47. Bartizal K, Gill CJ, Abruzzo GK, et al. In vitro preclinical evaluation studies with the echinocandin antifungal MK-0991 (L-743,872). *Antimicrob Agents Chemother* 1997;**41**:2326–32.
48. Barton E, Zinnes H, Moe RA, et al. Studies on a new solubilized preparation of amphotericin B. In: *Antibiotics annual, 1957–1958*. New York: Medical Encyclopedia; 1958. p. 53–7.
49. Bekersky I, Fielding RM, Dressler DE, et al. Pharmacokinetics, excretion, and mass balance of liposomal amphotericin B(AmBisome) and amphotericin B deoxycholate in humans. *Antimicrob Agents Chemother* 2002;**46**:828–33.
50. Bekersky I, Fielding RM, Dressler DE, et al. Plasma protein binding of amphotericin B and pharmacokinetics of bound versus unbound amphotericin B after administration of intravenous liposomal amphotericin B (AmBisome) and amphotericin B deoxycholate. *Antimicrob Agents Chemother* 2002;**46**:834–40.
51. Benjamin DK Jr, Driscoll T, Seibel NL, et al. Safety and pharmacokinetics of intravenous anidulafungin in children with neutropenia at high risk for invasive fungal infections. *Antimicrob Agents Chemother* 2006;**50**:632–8.
52. Benjamin DK Jr, Smith PB, Arrieta A, et al. Safety and pharmacokinetics of repeat-dose micafungin in young infants. *Clin Pharmacol Ther* **87**:93–9, 2010
53. Bennett JE. Antifungal agents. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 4th ed. New York: Churchill Livingstone; 1995. p. 401–10.
54. Bennett JE, Dismukes WE, Haywood M, et al. A comparison of amphotericin B alone and in combination with flucytosine in the treatment of cryptococcal meningitis. *N Engl J Med* 1979; **301**:126–31.
55. Benson JM, Nahata MC. Pharmacokinetics of amphotericin B in children. *Antimicrob Agents Chemother* 1989;**33**:1989–93.
56. Berenguer J, Ali NM, Allende MC, et al. Itraconazole for experimental pulmonary aspergillosis: comparison with amphotericin B, interaction with cyclosporin A, and correlation between therapeutic response and itraconazole concentrations in plasma. *Antimicrob Agents Chemother* 1994;**38**:1303–8.
57. Berenguer J, Rodriguez-Tudela JL, Richard C, et al. Deep infections caused by *Scedosporium prolificans*: a report on 16 cases in Spain and a review of the literature. *Scedosporium Prolificans* Spanish Study Group. *Medicine (Baltimore)* 1997;**76**:256–65.
58. Bergner R, Hoffmann M, Riedel KD, et al. Fluconazole dosing in continuous veno-venous haemofiltration (CVVHF): need for a high daily dose of 800 mg. *Nephrol Dial Transplant* 2006; **21**:1019–23.
59. Berl T, Wilner KD, Gardner M, et al. Pharmacokinetics of fluconazole in renal failure. *J Am Soc Nephrol* 1995;**6**:242–7.
60. Bertini G, Perugi S, Dani C, et al. Fluconazole prophylaxis prevents invasive fungal infection in high-risk, very low birth weight infants. *J Pediatr* 2005;**147**:162–5.
61. Betts RF, Nucci M, Talwar D, et al. A Multicenter, double-blind trial of a high-dose caspofungin treatment regimen versus a standard caspofungin treatment regimen for adult patients with invasive candidiasis. *Clin Infect Dis* 2009;**48**:1676–84.
62. Beyda ND, Lewis RE, Garey KW. Echinocandin resistance in *Candida* species: mechanisms of reduced susceptibility and therapeutic approaches. *Ann Pharmacother* **46**:1086–96, 2012
63. Bicanic T, Harrison T, Niepieklo A, et al. Symptomatic relapse of HIV-associated cryptococcal meningitis after initial fluconazole monotherapy: the role of fluconazole resistance and immune reconstitution. *Clin Infect Dis* **43**:1096–73, 2006
64. Bilgen H, Ozek E, Korten V, et al. Treatment of systemic neonatal candidiasis with fluconazole. *Infection* 1995;**23**:394.
65. Block ER, Bennett JE. Pharmacological studies with 5-fluorocytosine. *Antimicrob Agents Chemother* 1992;**1**:476–82.
66. Blumer JL. Pharmacologic basis for the treatment of tinea capitis. *Pediatr Infect Dis J* 1999;**18**:191–9.
67. Blumer J, Hare R, Krishna G, et al. Efficacy of posaconazole salvage therapy in pediatric subjects with invasive fungal infections. Abstract P-744. In: Proceedings of the 15th European Congress for Clinical Microbiology and Infectious Diseases. Munich, European Society for Clinical Microbiology and Infectious Diseases, 2005.
68. Bohme A, Ganser A, Hoelzer D. Aggravation of vincristine-induced neuro-toxicity by itraconazole in the treatment of adult ALL. *Ann Hematol* 1995;**71**:311–12.
69. Bonifaz A, Saul A, Paredes-Solis V, et al. Treatment of chromoblastomycosis with terbinafine: experience with four cases. *J Dermatolog Treat* 2005;**16**:47–51.
70. Boogaerts MA, Verhoef GE, Zachee P, et al. Antifungal prophylaxis with itraconazole in prolonged neutropenia: correlation with plasma levels. *Mycoses* 1989;**32**(Suppl. 1):103–8.
71. Boogaerts M, Winston DJ, Bow EJ, et al. Intravenous and oral itraconazole versus intravenous amphotericin B deoxycholate as empirical antifungal therapy for persistent fever in neutropenic patients with cancer who are receiving broad-spectrum antibacterial therapy: a randomized, controlled trial. Itraconazole Neutropenia Study Group. *Ann Intern Med* 2001;**135**:412–22.
72. Botas CM, Kurlat I, Young SM, et al. Disseminated candidal infections and intravenous hydrocortisone in preterm infants. *Pediatrics* 1995;**95**:883–7.
73. Boutati EI, Anaissie EJ. *Fusarium*, a significant emerging pathogen in patients with hematologic malignancy: ten years' experience at a cancer center and implications for management. *Blood* 1997;**90**:999–1008.
74. Bowden R, Chandrasekar P, White MH, et al. A double-blind, randomized, controlled trial of amphotericin B colloidal dispersion versus amphotericin B for treatment of invasive aspergillosis in immunocompromised patients. *Clin Infect Dis* 2002; **35**:359–66.
75. Brajtburg J, Powderly WG, Kobayashi GS, et al. Amphotericin B: Current understanding of mechanisms of action. *Antimicrob Agents Chemother* 1990;**34**:183–8.
76. Brammer KW, Coates PE. Pharmacokinetics of fluconazole in pediatric patients. *Eur J Clin Microbiol Infect Dis* 1994;**13**:325–9.
77. Brammer KW, Farrow PR, Faulkner JK. Pharmacokinetics and tissue penetration of fluconazole in humans. *Rev Infect Dis* 1990;**12**(Suppl. 3):318–26.
78. Brouwer AE, Rajanuwong A, Chierakul W, et al. Combination antifungal therapies for HIV-associated cryptococcal meningitis: a randomised trial. *Lancet* 2004;**363**:1764–7.
79. Bruckbauer HR, Hofmann H. Systemic antifungal treatment of children with terbinafine. *Dermatology* 1997;**195**:134–6.
80. Brüggemann RJ, Alffenaar JW, Blijlevens NM, et al. Clinical relevance of the pharmacokinetic interactions of azole antifungal drugs with other coadministered agents. *Clin Infect Dis* 2009; **48**:1441–58.
81. Buggia I, Zecca M, Alessandrino EP, et al. Itraconazole can increase systemic exposure to busulfan in patients given bone marrow transplantation: GITMO (Gruppo Italiano Trapianto di Midollo Osseo). *Anticancer Res* 1996;**16**:2083–8.
82. Burgess DS, Hastings RW. A comparison of dynamic characteristics of fluconazole, itraconazole, and amphotericin B against *Cryptococcus neoformans* using time-kill methodology. *Diagn Microbiol Infect Dis* 2000;**38**:87–93.
83. Burgess DS, Hastings RW, Summers KK, et al. Pharmacodynamics of fluconazole, itraconazole, and amphotericin B against *Candida albicans*. *Diagn Microbiol Infect Dis* 2000;**36**:13–18.
84. Butler KM, Rench MA, Baker CJ. Amphotericin B as a single agent in the treatment of systemic candidiasis in neonates. *Pediatr Infect Dis J* 1990;**9**:51–6.
85. Butler WT, Bennett JE, Alling DW, et al. Nephrotoxicity of amphotericin B: Early and late effects in 81 patients. *Ann Intern Med* 1964;**62**:175–87.
86. Caceres-Rios H, Rueda M, Ballona R, et al. Comparison of terbinafine and griseofulvin in the treatment of tinea capitis. *J Am Acad Dermatol* 2000;**42**:80–4.
87. Caillot D, Thiébaut A, Herbrecht R, et al. Liposomal amphotericin B in combination with caspofungin for invasive aspergillosis in patients with hematologic malignancies: a randomized pilot study (Combistrat trial). *Cancer* **110**:2740–6, 2007
88. Cartwright RY. Use of antibiotics: Antifungals. *Br Med J* 1978;**2**:101–11.
89. Castberg I, Helle J, Aamo TO. Prolonged pharmacokinetic drug interaction between terbinafine and amitriptyline. *Ther Drug Monit* 2005;**27**:680–2.
90. Cattaneo C, Monte S, Algarotti A, et al. A randomized comparison of caspofungin versus antifungal prophylaxis according to investigator policy in acute leukaemia patients undergoing induction

chemotherapy (PROFIL-C study). *J Antimicrob Chemother* **66**:2140–5, 2011
91. Catanzaro A, Galgiani JN, Levine BE, et al. Fluconazole in the treatment of chronic pulmonary and non-meningeal disseminated coccidioidomycosis. *Am J Med* 1995;**98**:249–56.
92. Center for Drug Evaluation and Research. *Cancidas (caspofungin acetate) for Injection Label Information* (June 2010). Washington, D.C.: U.S. Food and Drug Administration, Center for Drug Evaluation and Research. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2010/021227s028lbl.pdf.
93. Center for Drug Evaluation and Research. *Mycamine (micafungin sodium) for Injection Label Information* (August 2011). Washington, D.C.: U.S. Food and Drug Administration. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/021506s011s012lbl.pdf.
94. Center for Drug Evaluation and Research. *Eraxis (anidulafungin) for Injection Label Information* (June 2012). Washington, D.C.: U.S. Food and Drug Administration. Available at http://www.accessdata.fda.gov/drugsatfda_docs/label/2012/021632s011lbl.pdf.
95. Chandrasekar PH. Antifungal resistance in *Aspergillus*. *Med Mycol* 2005;**43**(Suppl. 1):S295–8.
96. Chan-Tack KM, Thio CL, Miller NS, et al. *Paecilomyces lilacinus* fungemia in an adult bone marrow transplant recipient. *Med Mycol* 1999;**37**:57–60.
97. Chapman SW, Bradsher RW Jr, Campbell GD, Jr, et al. Practice guidelines for the management of patients with blastomycosis: Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:679–83.
98. Chapman SW, Pappas P, Kauffmann C, et al. Comparative evaluation of the efficacy and safety of two doses of terbinafine (500 and 1000 mg day (-1)) in the treatment of cutaneous or lymphocutaneous sporotrichosis. *Mycoses* 2004;**47**:62–8.
99. Chiou CC, Groll AH, Walsh TJ. New drugs and novel targets for treatment of invasive fungal infections in patients with cancer. *Oncologist* 2000;**5**:120–35.
100. Chiou CC, Walsh TJ, Groll AH. Clinical pharmacology of antifungal agents in pediatric patients. *Expert Opin Pharmacother* 2007;**8**:2465–89.
101. Christiansen KJ, Bernard EM, Gold JWM, et al. Distribution and activity of amphotericin B in humans. *J Infect Dis* 1985; **152**:1037–43.
102. Clancy CJ, Nguyen MH. In vitro efficacy and fungicidal activity of voriconazole against *Aspergillus* and *Fusarium* species. *Eur J Clin Microbiol Infect Dis* 1998;**17**:573–5.
103. Clemons KV, Stevens DA. Comparison of Fungizone, Amphotec, AmBisome, and Abelcet for treatment of systemic murine cryptococcosis. *Antimicrob Agents Chemother* 1998;**42**:899–902.
104. Cohen-Wolkowiez M, Benjamin DK Jr, Piper L, et al. Safety and pharmacokinetics of multiple-dose anidulafungin in infants and neonates. *Clin Pharmacol Ther* 2011;**89**:702–7.
105. Como JA, Dismukes WE. Oral azole drugs as systemic antifungal therapy. *N Engl J Med* 1994;**330**:263–72.
106. Conte JE Jr, Golden JA, Krishna G, et al. Intrapulmonary pharmacokinetics and pharmacodynamics of posaconazole at steady state in healthy subjects. *Antimicrob Agents Chemother* **53**:703–7, 2009
107. Contini C, Colombo D, Cultrera R, et al. Employment of terbinafine against *Pneumocystis carinii* infection in rat models. *Br J Dermatol* 1996;**134**(Suppl. 46):30–2.
108. Contini C, Manganaro M, Romani R, et al. Activity of terbinafine against *Pneumocystis carinii* in vitro and its efficacy in the treatment of experimental pneumonia. *J Antimicrob Chemother* 1994;**34**: 727–35.
109. Cornely OA, Lasso M, Betts R, et al. Caspofungin for the treatment of less common forms of invasive candidiasis. *J Antimicrob Chemother* **60**:363–9,2007
110. Cornely OA, Maertens J, Bresnik M, et al. AmBiLoad Trial Study Group. Liposomal amphotericin B as initial therapy for invasive mold infection: a randomized trial comparing a high-loading dose regimen with standard dosing (AmBiLoad trial). *Clin Infect Dis* 2007;**44**:1289–97.
111. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med* 2007;**356**:348–59.
112. Cornely OA, Vehreschild JJ, Vehreschild MJ, et al. Phase II dose escalation study of caspofungin for invasive Aspergillosis. *Antimicrob Agents Chemother* 2011;**55**:5798–803.
113. Courtney R. Pharmacokinetics of posaconazole in patients with hepatic insufficiency. In: *Abstracts of the annual meeting of the American Academy for Pharmacological Sciences (AAPS)*. Abstract 3495. Washington, D.C.: American Academy for Pharmacological Sciences; 2000.
114. Courtney R, Pai S, Laughlin M, et al. Pharmacokinetics, safety, and tolerability of oral posaconazole administered in single and multiple doses in healthy adults. *Antimicrob Agents Chemother* 2003;**47**:2788–95.
115. Courtney R, Sansone A, Devlin D. P-glycoprotein expression and genotype: exploratory analysis of posaconazole in healthy volunteers. Abstract A-40. In: *Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2004.
116. Courtney R, Sansone A, Smith W, et al. Posaconazole pharmacokinetics, safety, and tolerability in subjects with varying degrees of chronic renal disease. *J Clin Pharmacol* 2005;**45**:185–92.
117. Cowen EW, Nguyen JC, Miller DD, et al. Chronic phototoxicity and aggressive squamous cell carcinoma of the skin in children and adults during treatment with voriconazole. *J Am Acad Dermatol* 2010;**62**:31–7.
118. Crandon JL, Banevicius MA, Fang AF, et al. Bronchopulmonary disposition of intravenous voriconazole and anidulafungin given in combination to healthy adults. *Antimicrob Agents Chemother* 2009;**53**:5102–7.
119. Craven PC, Ludden TM, Drutz DJ, et al. Excretion pathways of amphotericin B. *J Infect Dis* 1979;**140**:329–41.
120. Daneshmend TK, Warnock DW. Clinical pharmacokinetics of systemic antifungal drugs. *Clin Pharmacokinet* 1983;**8**:17–42.
121. De Beule K. Itraconazole: pharmacology, clinical experience and future development. *Int J Antimicrob Agents* 1996;**6**:175–81.
122. De Beule K, Van Gestel J. Pharmacology of itraconazole. *Drugs* 2001;**61**(Suppl. 1):27–37.
123. de Lima Barros MB, Schubach AO, de Vasconcellos Carvalhaes de Oliveira R, et al. Treatment of cutaneous sporotrichosis with itraconazole: study of 645 patients. *Clin Infect Dis* 2011;**52**: e200–6.
124. de Wet N, Bester AJ, Viljoen JJ, et al. A randomized, double blind, comparative trial of micafungin (FK463) vs. fluconazole for the treatment of oesophageal candidiasis. *Aliment Pharmacol Ther* 2005;**21**:899–907.
125. de Wet N, Llanos-Cuentas A, Suleiman J, et al. A randomized, double-blind, parallel-group, dose-response study of micafungin compared with fluconazole for the treatment of esophageal candidiasis in HIV-positive patients. *Clin Infect Dis* 2004;**39**:842–9.
126. Debono M, Gordee RS. Antibiotics that inhibit fungal cell wall development. *Annu Rev Microbiol* 1994;**48**:471–97.
127. Denning DW. Echinocandin antifungal drugs. *Lancet* 2003; **362**:1142–51.
128. Denning DW, Marr KA, Lau WM, et al. Micafungin (FK463), alone or in combination with other systemic antifungal agents, for the treatment of acute invasive aspergillosis. *J Infect* 2006;**53**: 337–49.
129. Denning DW, Park S, Lass-Florl C, et al. High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. *Clin Infect Dis* 2011;**52**: 1123–9.
130. Denning DW, Radford SA, Oakley KL, et al. Correlation between in vitro susceptibility testing to itraconazole and in vivo outcome of *Aspergillus fumigatus* infection. *J Antimicrob Chemother* 1997;**40**:401–14.
131. Denning DW, Ribaud P, Milpied N, et al. Efficacy and safety of voriconazole in the treatment of acute invasive aspergillosis. *Clin Infect Dis* 2002;**34**:563–71.
132. Denning DW, Tucker RM, Hanson LH, et al. Treatment of invasive aspergillosis with itraconazole. *Am J Med* 1989;**86**:791–800.
133. de Repentigny L, Ratelle J, Leclerc JM, et al. Repeated-dose pharmacokinetics of an oral solution of itraconazole in infants and children. *Antimicrob Agents Chemother* 1998;**42**:404–8.
134. Di Bonaventura G, Spedicato I, Picciani C, et al. In vitro pharmacodynamic characteristics of amphotericin B, caspofungin, fluconazole, and voriconazole against bloodstream isolates of

infrequent *Candida* species from patients with hematologic malignancies. *Antimicrob Agents Chemother* 2004;**48**:4453–6.
135. Diaz M, Negroni R, Montero-Gei F, et al. A Pan-American 5-year study of fluconazole therapy for deep mycoses in the immunocompetent host: Pan-American Study Group. *Clin Infect Dis* 1992;**14**(Suppl. 1):68–76.
136. Diekema DJ, Petroelje B, Messer SA, et al. Activities of available and investigational antifungal agents against *Rhodotorula* species. *J Clin Microbiol* 2005;**43**:476–8.
137. Dismukes WE, Bradsher RW, Cloud GC, et al. Itraconazole therapy for blastomycosis and histoplasmosis. *Am J Med* 1992;**93**:489–97.
138. Doby EH, Benjamin DK Jr, Blaschke AJ, et al. Therapeutic monitoring of voriconazole in children less than 3 years of age: a case report and summary of voriconazole concentrations for 10 children. *Pediatr Infect Dis J* 2012.
139. Dodgson AR, Dodgson KJ, Pujol C, et al. Clade-specific flucytosine resistance is due to a single nucleotide change in the FUR1 gene of *Candida albicans*. *Antimicrob Agents Chemother* 2004; **48**:2223–7.
140. Dokos C, Pieper S, Lehrnbecher T, et al. Pharmacokinetics, safety and efficacy of voriconazole in pediatric patients: an update. *Curr Fungal Infect Rep* **6**:121–6, 2012
141. Doring M, Hartmann U, Erbacher A, et al. Caspofungin as antifungal prophylaxis in pediatric patients undergoing allogeneic hematopoietic stem cell transplantation: a retrospective analysis. *BMC Infect Dis* 2012;**12**:151.
142. Dotis J, Simitsopoulou M, Dalakiouridou M, et al. Amphotericin B formulations variably enhance antifungal activity of human neutrophils and monocytes against *Fusarium solani:* comparison with *Aspergillus fumigatus*. *J Antimicrob Chemother* 2008;**61**:810–17.
143. Douglas CM. Understanding the microbiology of the *Aspergillus* cell wall and the efficacy of caspofungin. *Med Mycol* 2006; **44**(Suppl):95–9.
144. Drew RH, Perfect JR. Flucytosine. In: Yu VL, Merigan TC, Barriere S, editors. *Antimicrobial chemotherapy and vaccines*. Baltimore: Williams & Wilkins; 1998. p. 1170–84.
145. Drew RH, Dodds Ashley E, Benjamin DK, Jr, et al. Comparative safety of amphotericin B lipid complex and amphotericin B deoxycholate as aerosolized antifungal prophylaxis in lung-transplant recipients. *Transplantation* 2004;**77**:232–7.
146. Driscoll TA, Yu LC, Frangoul HL, et al. Comparison of pharmacokinetics and safety of voriconazole intravenous-to-oral switch in immunocompromised adolescents and healthy adults. *Antimicrob Agents Chemother* 2011;**55**:5770–9.
147. Driscoll TA, Yu LC, Frangoul H, et al. Pharmacokinetics and safety of intravenous voriconazole to oral switch in immunocompromised children compared to adults. *Antimicrob Agents Chemother* 2011 **55**:5770–9.
148. Driessen M, Ellis JB, Cooper PA, et al. Fluconazole vs. amphotericin B for the treatment of neonatal fungal septicemia: a prospective randomized trial. *Pediatr Infect Dis J* 1996;**15**:1107–12.
149. Driessen M, Ellis JB, Muwazi F, et al. The treatment of systemic candidiasis in neonates with oral fluconazole. *Ann Trop Paediatr* 1997;**7**:263–71.
150. Drutz DJ, Spickard A, Rogers DE, et al. Treatment of disseminated mycotic infections: a new approach to amphotericin B therapy. *Am J Med* 1968;**45**:405–18.
151. Dupont B, de Waele J, Betts R, et al. Micafungin vs. caspofungin in patients with invasive candidiasis or candidemia: A phase III, randomized double-blind parallel group, non-inferiority trial. Abstract O-140. In: *Abstracts of the 17th European Congress of Clinical Microbiology and Infectious Diseases*. Munich: European Society for Clinical Microbiology and Infectious Diseases; 2007.
152. Dutta A, Palazzi DL. Risk factors of amphotericin B toxicity in the nonneonatal pediatric population. *Pediatr Infect Dis J* **31**:910–14, 2012
153. Dvorak CC, Fisher BT, Sung L, et al. Antifungal prophylaxis in pediatric hematology/oncology: new choices & new data. *Pediatr Blood Cancer* 2012;**59**:21–6.
154. Elewski BE. Cutaneous mycoses in children. *Br J Dermatol* 1996;**134**(Suppl. 46):7–11.
155. Elewski BE, Cáceres HW, DeLeon L, et al. Terbinafine hydrochloride oral granules versus oral griseofulvin suspension in children with tinea capitis: results of two randomized, investigator-blinded, multicenter, international, controlled trials. *J Am Acad Dermatol* **59**:41–54, 2008
156. Elewski B, Pollak R, Ashton S, et al. A randomized, placebo- and active-controlled, parallel-group, multicentre, investigator-blinded study of four treatment regimens of posaconazole in adults with toenail onychomycosis. *Br J Dermatol* 2012;**166**:389–98.
157. Epstein WL, Shah VP, Riegelman S. Griseofulvin levels in stratum corneum. Study after oral administration in man. *Arch Dermatol* 1992;**106**:344–8.
158. Ernst EJ, Klepser ME, Pfaller MA. Postantifungal effects of echinocandin, azole, and polyene antifungal agents *Candida albicans* and *Cryptococcus neoformans*. *Antimicrob Agents Chemother* 2000; **44**:1108–11.
159. Ernst EJ, Klepser ME, Ernst ME, et al. In vitro pharmacodynamic properties of MK-0991 determined by time-kill methods. *Diagn Microbiol Infect Dis* 1999;**33**:75–80.
160. Ernst ME, Klepser ME, Wolfe EJ, et al. Antifungal dynamics of LY 303366, an investigational echinocandin B analog, against *Candida* ssp. *Diagn Microbiol Infect Dis* 1996;**26**:125–31.
161. Espinel-Ingroff A. Comparison of in vitro activities of the new triazole SCH56592 and the echinocandins MK-0991 (L-743,872) and LY303366 against opportunistic filamentous and dimorphic fungi and yeasts. *J Clin Microbiol* 1998;**36**:2950–6.
162. European Organisation for Research and Treatment of Cancer International Antimicrobial Therapy Cooperative Group. Empiric antifungal therapy in febrile granulocytopenic patients. *Am J Med* 1986;**86**:668–72.
163. Fasano C, O'Keeffe J, Gibbs D. Fluconazole treatment of neonates and infants with severe fungal infections not treatable with conventional agents. *Eur J Clin Microbiol Infect Dis* 1994; **13**:351–4.
164. Fernandez-Torres B, Carrillo AJ, Martin E, et al. In vitro activities of 10 antifungal drugs against 508 dermatophyte strains. *Antimicrob Agents Chemother* 2001;**45**:2524–8.
165. Fleece D, Gaughan JP, Aronoff SC. Griseofulvin versus terbinafine in the treatment of tinea capitis: a meta-analysis of randomized, clinical trials. *Pediatrics* 2004;**114**:1312–15.
166. Flynn P, Cunningham CK, Kerkering T, et al. Oropharyngeal candidiasis in immunocompromised children: a randomized, multicenter study of orally administered fluconazole suspension versus nystatin. *J Pediatr* 1995;**127**:322–8.
167. Flynn P, Seibel N, Arrieta A, et al. Treatment of invasive aspergillosis in pediatric patients with micafungin alone or in combination with other systemic antifungal agents. Abstract M-891. In: *Abstracts of the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2006, p. 407.
168. Foot A, Veys P, Gibson B. Itraconazole oral solution as antifungal prophylaxis in children undergoing stem cell transplantation or intensive chemotherapy for haematological disorders. *Bone Marrow Transplant* 1999;**24**:1089–93.
169. Fothergill AW, Leitner I, Meingassner JG, et al. Combination antifungal susceptibility testing of terbinafine and the triazoles fluconazole and itraconazole. Abstract E-53. In: *Abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 1996 p. 91.
170. Francis P, Walsh TJ. Evolving role of flucytosine in immunocompromised patients: new insights into safety, pharmacokinetics, and antifungal therapy. *Clin Infect Dis* 1992;**15**:1003–18.
171. Franklin JA, McCormick J, Flynn PM. Retrospective study of the safety of caspofungin in immunocompromised pediatric patients. *Pediatr Infect Dis J* 2003;**22**:747–9.
172. Friedlander SF. The evolving role of itraconazole, fluconazole and terbinafine in the treatment of tinea capitis. *Pediatr Infect Dis J* 1999;**18**:205–10.
173. Friedlander SF, Suarez S. Pediatric antifungal therapy. *Dermatol Clin* 1998;**16**:527–37.
174. Friedlander SF, Aly R, Krafchik B, et al. Terbinafine in the treatment of *Trichophyton* tinea capitis: a randomized, double-blind, parallel-group, duration-finding study. *Pediatrics* 2002; **109**:602–7.
175. Fuller LC, Smith CH, Cerio R, et al. A randomized comparison of 4 weeks of terbinafine vs. 8 weeks of griseofulvin for the treatment of tinea capitis. *Br J Dermatol* 2001;**144**:321–7.

176. Fung-Tomc JC, Huczko E, Minassian B, et al. In vitro activity of a new oral triazole, BMS-207147 (ER-30346). *Antimicrob Agents Chemother* 1998;**42**:313–18.
177. Galgiani JN, Ampel NM, Catanzaro A, et al. Practice guideline for the treatment of coccidioidomycosis: Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:658–61.
178. Galgiani JN, Catanzaro A, Cloud GA, et al. Fluconazole therapy for coccidioidal meningitis. *Ann Intern Med* 1993;**119**:28–35.
179. Galgiani JN, Catanzaro A, Cloud GA, et al. Comparison of oral fluconazole and itraconazole for progressive, nonmeningeal coccidioidomycosis: a randomized, double-blind trial. Mycoses Study Group. *Ann Intern Med* 2000;**133**:676–86.
180. Garcia MT, Llorente MT, Lima JE, et al. Activity of voriconazole: post-antifungal effect, effects of low concentrations and of pretreatment on the susceptibility of *Candida albicans* to leucocytes. *Scand J Infect Dis* 1999;**31**:501–4.
181. Garcia-Effron G, Gomez-Lopez A, Mellado E, et al. In vitro activity of terbinafine against medically important nondermatophyte species of filamentous fungi. *J Antimicrob Chemother* 2004;**53**:1086–9.
182. Gardiner RE, Souteropoulos P, Park S, et al. Characterization of *Aspergillus fumigatus* mutants with reduced susceptibility to caspofungin. *Med Mycol* 2005;**43**(Suppl. 1):S299–305.
183. Gehrt A, Peter J, Pizzo PA, et al. Effect of increasing inoculum sizes of pathogenic filamentous fungi on MICs of antifungal agents by broth microdilution method. *J Clin Microbiol* 1995;**33**:1302–7.
184. Gentles JC. Experimental ringworm in guinea pigs: oral treatment with griseofulvin. *Nature* 1958;**182**:476–7.
185. Georgopapadakou NH. Update on antifungals targeted to the cell wall: focus on beta-1,3-glucan synthase inhibitors. *Expert Opin Investig Drugs* 2001;**10**:269–80.
186. Ginter-Hanselmayer G, Weger W, Smolle J. Onychomycosis: a new emerging infectious disease in childhood population and adolescents: report on treatment experience with terbinafine and itraconazole in 36 patients. *J Eur Acad Dermatol Venereol* 2008;**22**:470–5.
187. Glasmacher A, Hahn C, Molitor E, et al. Itraconazole through concentrations in antifungal prophylaxis with six different dosing regimens using hydroxypropyl-beta-cyclodextrin oral solution or coated-pellet capsules. *Mycoses* 1999;**42**:591–600.
188. Glasmacher A, Hahn C, Molitor E, et al. Definition of itraconazole target concentration for antifungal prophylaxis. Abstract 700. In: *Abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2000. p. 363.
189. Glasmacher A, Prentice A, Gorschluter M, et al. Itraconazole prevents invasive fungal infections in neutropenic patients treated for hematologic malignancies: evidence from a meta-analysis of 3,597 patients. *J Clin Oncol* 2003;**21**:4615–26.
190. Glick C, Graves GR, Feldman S. Neonatal fungemia and amphotericin B. *South Med J* 1993;**86**:1368–71.
191. Goa KL, Barradell LB. Fluconazole: an update of its pharmacodynamic and pharmacokinetic properties and therapeutic use in major superficial and systemic mycoses in immunocompromised patients. *Drugs* 1995;**50**:658–90.
192. Gold W, Stout HA, Pagona IF, et al. Amphotericins A and B, antifungal antibiotics produced by a streptomycete. I. In vitro studies. In: *Antibiotics annual, 1955–1956*. New York: Medical Encyclopedia; 1955. p. 579–86.
193. Goldman M, Cloud GA, Smedema M, et al. Does long-term itraconazole prophylaxis result in in vitro azole resistance in mucosal *Candida albicans* isolates from persons with advanced human immunodeficiency virus infection? *Antimicrob Agents Chemother* 2000;**44**:1585–7.
194. Goldman RD, Koren G. Amphotericin B nephrotoxicity in children. *J Pediatr Hematol Oncol* 2004;**26**:421–6.
195. Gomez-Lopez A, Cuenca-Estrella M, Mellado E, et al. In vitro evaluation of combination of terbinafine with itraconazole or amphotericin B against Zygomycota. *Diagn Microbiol Infect Dis* 2003;**45**:199–202.
196. Goodman JL, Winston DJ, Greenfield RA, et al. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992;**326**:845–51.
197. Googe JH, Walterspiel JN. Arrhythmia caused by amphotericin B in a neonate. *Pediatr Infect Dis J* 1988;**7**:73.
198. Grant SM, Clissold SP. Itraconazole: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use in superficial and systemic mycoses. *Drugs* 1989;**37**:310–44.
199. Grant SM, Clissold SP. Fluconazole: a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in superficial and systemic mycoses. *Drugs* 1990;**39**:877–916.
200. Graybill JR, Stevens DA, Galgiani JN, et al. Itraconazole treatment of coccidioidomycosis. *Am J Med* 1990;**89**:282–90.
201. Groll AH, Walsh TJ. Caspofungin: Pharmacology, safety, and therapeutic potential in superficial and invasive fungal infections. *Expert Opin Investig Drugs* 2001;**10**:1545–58.
202. Groll AH, Walsh TJ. Posaconazole: clinical pharmacology and potential for management of fungal infections. *Expert Rev Anti Infect Ther* 2005;**3**:467–87.
203. Groll AH, Walsh TJ. Uncommon opportunistic fungi: new nosocomial threats. *Clin Microbiol Infect* 2001;**7**(Suppl. 2):8–24.
204. Groll AH, Tragiannidis A. Update on antifungal agents for pediatric patients. *Clin Microbiol Infect* 2010;**16**:1343–53.
205. Groll AH, Chiou CC, Walsh TJ. Antifungal drugs: drug interactions with antifungal azole derivatives. In: Aronson JK, van Boxtel CJ, editors. *Side effects of drugs, annual 24*. Amsterdam: Elsevier Science; 2001. p. 314–29.
206. Groll AH, Kolve H, Walsh TJ. Azoles. In: Yu VL, Edwards G, Mckinnon PS, et al., editors: *Antimicrobial chemotherapy and vaccines*. Vol. 2. 2nd ed. Pittsburgh, Pa: ESun Technologies; 2005. p. 609–36.
207. Groll AH, Piscitelli SC, Walsh TJ. Clinical pharmacology of systemic antifungal agents: a comprehensive review of agents in clinical use, current investigational compounds, and putative targets for antifungal drug development. *Adv Pharmacol* 1998;**44**:343–500.
208. Groll AH, Piscitelli SC, Walsh TJ. Antifungal pharmacodynamics: concentration-effect relationships in vitro and in vivo. *Pharmacotherapy* 2001;**21**(Suppl. 8):133–48.
209. Groll AH, Ritter J, Mueller FMC. Prevention of fungal infections in children and adolescents with cancer. *Klin Paediatr* 2001;**213**(Suppl. 1):A50–68.
210. Groll AH, Schrey D, Walsh TJ. Echinocandins. In: Kauffman CA, Pappas PG, Sobel JD, et al, editors. *Essentials of clinical mycology*. New York, NY: Oxford University Press; 2011. p. 95–112.
211. Groll AH, Attarbaschi A, Schuster FR, et al. Treatment with caspofungin in immunocompromised paediatric patients: a multicentre survey. *J Antimicrob Chemother* 2006;**57**:527–35.
212. Groll AH, Gea-Banacloche JC, Glasmacher A, et al. Clinical pharmacology of antifungal compounds. *Infect Dis Clin North Am* 2003;**17**:159–91.
213. Groll AH, Giri N, Petraitis V, et al. Comparative central nervous system distribution and antifungal activity of lipid formulations of amphotericin B in rabbits. *J Infect Dis* 2000;**182**:274–82.
214. Groll AH, Just-Nuebling G, Kurz M, et al. Fluconazole versus nystatin in the prevention of *Candida* infections in children and adolescents undergoing remission induction or consolidation chemotherapy for cancer. *J Antimicrob Chemother* 1997;**40**:855–62.
215. Groll AH, Mickiene D, McEvoy M, et al. Safety, pharmacokinetics and pharmacodynamics of cyclodextrin itraconazole in pediatric patients with oropharyngeal candidiasis. *Antimicrob Agents Chemother* 2002;**46**:2554–63.
216. Groll AH, Mickiene D, Petraitiene R, et al. Pharmacokinetics and pharmacodynamics of posaconazole (SCH 56592) in a neutropenic animal model of invasive pulmonary aspergillosis. Abstract 1675. In: *Abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2000. p. 385.
217. Groll AH, Mickiene D, Petraitiene R, et al. Pharmacokinetic and pharmacodynamic modeling of anidulafungin (LY303366): reappraisal of its efficacy in neutropenic animal models of opportunistic mycoses using optimal plasma sampling. *Antimicrob Agents Chemother* 2001;**45**:2845–55.
218. Groll AH, Muller FM, Piscitelli SC, et al. Lipid formulations of amphotericin B: clinical perspectives for the management of invasive fungal infections in children with cancer. *Klin Padiatr* 1998;**210**:264–73.
219. Groll A, Nowak-Goettl U, Wildfeuer A, et al. Fluconazole treatment of oropharyngeal candidosis in pediatric cancer patients with severe mucositis following antineoplastic chemotherapy. *Mycoses* 1992;**35**(Suppl.):35–40.

220. Groll AH, Silling G, Young C, et al. Randomized comparison of safety and pharmacokinetics of caspofungin, liposomal amphotericin B, and the combination of both in allogeneic hematopoietic stem cell recipients. *Antimicrob Agents Chemother* 2010; **54**:4143–9.
221. Groll AH, Stergiopoulou T, Roilides E, et al. Micafungin: pharmacology, experimental therapeutics and clinical applications. *Expert Opin Investig Drugs* 2005;**14**:489–509.
222. Gubbins PO, McConnell SA, Penzak SR. Antifungal agents. In: Piscitelli SC, Rodvold KA, editors: *Drug interactions in infectious diseases*. Totowa, NJ: Humana Press; 2001. p. 185–217.
223. Gull K, Trinci AP. Griseofulvin inhibits fungal mitosis. *Nature* 1973;**244**:292–4.
224. Gupta AK, Ross GS. Interaction between terbinafine and warfarin. *Dermatology* 1998;**196**:266–7.
225. Gupta AK, Sauder DN, Shear NH. Antifungal agents: an overview. *J Am Acad Dermatol* 1994;**30**:677–98, 911–933.
226. Gupta AK, Adamiak A, Cooper EA. The efficacy and safety of terbinafine in children. *J Eur Acad Dermatol Venereol* 2003; **17**:627–40.
227. Gupta AK, Nolting S, de Prost Y, et al. The use of itraconazole to treat cutaneous fungal infections in children. *Dermatology* 1999;**199**:248–52.
228. Hamill RJ, Thomas CJ, Dismukes WE. Fluconazole therapy for histoplasmosis: The National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Clin Infect Dis* 1996;**23**:996–1001.
229. Hamilton JD, Elliott DM. Combined activity of amphotericin B and 5-fluorocytosine against *Cryptococcus neoformans* in vitro and in vivo in mice. *J Infect Dis* 1975;**131**:129–37.
230. Hardin TC, Graybill JR, Fetchick R, et al. Pharmacokinetics of itraconazole following oral administration to normal volunteers. *Antimicrob Agents Chemother* 1988;**32**:1310–13.
231. Harris BE, Manning BW, Federle TW. Conversion of 5-fluorocytosine to 5-fluorouracil by human intestinal microflora. *Antimicrob Agents Chemother* 1986;**29**:44–8.
232. Healy CM, Baker CJ, Zaccaria E, et al. Impact of fluconazole prophylaxis on incidence and outcome of invasive candidiasis in a neonatal intensive care unit. *J Pediatr* 2005;**147**:166–71.
233. Hebert MF, Smith HE, Marbury TC, et al. Pharmacokinetics of micafungin in healthy volunteers, volunteers with moderate liver disease, and volunteers with renal dysfunction. *J Clin Pharmacol* 2005;**45**:1145–52.
234. Hector RF. Compounds active against cell walls of medically important fungi. *Clin Microbiol Rev* 1993;**6**:1–21.
235. Hegener P, Troke PF, Fakenheuer G, et al. Treatment of fluconazole-resistant candidiasis with voriconazole in patients with AIDS. *AIDS* 1998;**12**:2227–8.
236. Heidemann HT, Gerkens JF, Spickard WA, et al. Amphotericin B nephrotoxicity in humans decreased by salt repletion. *Am J Med* 1983;**75**:476–81.
237. Hennig S, Wainwright CE, Bell SC, et al. Population pharmacokinetics of itraconazole and its active metabolite hydroxy-itraconazole in paediatric cystic fibrosis and bone marrow transplant patients. *Clin Pharmacokinet* 2006;**45**:1099–114.
238. Herbrecht R. Safety of amphotericin B colloidal dispersion. *Eur J Clin Microbiol Infect Dis* 1997;**16**:74–80.
239. Herbrecht R, Denning DW, Patterson TF, et al. Voriconazole versus amphotericin B for primary therapy of invasive aspergillosis. *N Engl J Med* 2002;**347**:408–15.
240. Herbrecht R, Maertens J, Baila L, et al. Caspofungin first-line therapy for invasive aspergillosis in allogeneic hematopoietic stem cell transplant patients: an European Organisation for Research and Treatment of Cancer study. *Bone Marrow Transplant* 2010;**45**:1227–33.
241. Heresi GP, Gerstmann DR, Reed MD, et al. The pharmacokinetics and safety of micafungin, a novel echinocandin, in premature infants. *Pediatr Infect Dis J* 2006;**25**:1110–15.
242. Hernandez-Sempelayo T. Fluconazole vs. ketoconazole in the treatment of oropharyngeal candidiasis in HIV-infected children. *Eur J Clin Microbiol Infect Dis* 1994;**13**:340–4.
243. Heykants J, Michiels M, Meuldermans W, et al. The pharmacokinetics of itraconazole in animals and man: an overview. In: Fromtling RA, editor. *Recent trends in the discovery, development and evaluation of antifungal agents*. Barcelona: J. R. Prous; 1987. p. 223–49.
244. Hiemenz JW, Walsh TJ. Lipid formulations of amphotericin B: recent progress and future directions. *Clin Infect Dis* 1996;**22**(Suppl. 2):133–44.
245. Hilliard T, Edwards S, Buchdahl R, et al. Voriconazole therapy in children with cystic fibrosis. *J Cyst Fibros* 2005;**4**:215–20.
246. Hoban DJ, Zhanel GG, Karlowsky JA. In vitro susceptibilities of *Candida* and *Cryptococcus neoformans* isolates from blood cultures of neutropenic patients. *Antimicrob Agents Chemother* 1999; **43**:1463–4.
247. Hoffman HL, Ernst EJ, Klepser ME. Novel triazole antifungal agents. *Expert Opin Investig Drugs* 2000;**9**:593–605.
248. Hong Y, Shaw PJ, Nath CE, et al. Population pharmacokinetics of liposomal amphotericin B in pediatric patients with malignant diseases. *Antimicrob Agents Chemother* 2006;**50**:935–42.
249. Honig PK, Wortham DC, Hull R, et al. Itraconazole affects single-dose terfenadine pharmacokinetics and cardiac repolarization pharmacodynamics. *J Clin Pharmacol* 1993;**33**:1201–6.
250. Hope WW, Mickiene D, Petraitis V, et al. The pharmacokinetics and pharmacodynamics of micafungin in experimental hematogenous *Candida* meningoencephalitis: implications for echinocandin therapy in neonates. *J Infect Dis* 2008;**197**:163–71.
251. Hope WW, Seibel NL, Schwartz CL, et al. Population pharmacokinetics of micafungin in pediatric patients and implications for antifungal dosing. *Antimicrob Agents Chemother* 2007;**51**: 3714–19.
252. Hope WW, Smith PB, Arrieta A, et al. Population pharmacokinetics of micafungin in neonates and young infants. *Antimicrob Agents Chemother* 2010;**54**:2633–7.
253. Hope WW, Warn PA, Sharp A, et al. Derivation of an in vivo drug exposure breakpoint for flucytosine against *Candida albicans* and impact of the MIC, growth rate, and resistance genotype on the antifungal effect. *Antimicrob Agents Chemother* 2006;**50**:3680–8.
254. Hoppe JE. Treatment of oropharyngeal candidiasis and candidal diaper dermatitis in neonates and infants: review and reappraisal. *Pediatr Infect Dis J* 1997;**16**:885–94.
255. Horn R, Wong B, Kiehn TE, et al. Fungemia in a cancer hospital: changing frequency, earlier onset, and results of therapy. *Rev Infect Dis* 1985;**7**:646–55.
256. Horsburgh CR Jr, Kirkpatrick CH. Long-term therapy of chronic mucocutaneous candidiasis with ketoconazole: experience with twenty-one patients. *Am J Med* 1983;**74**:23–9.
257. Howard RM, Frieden IJ. Dermatophyte infections in children. *Adv Pediatr Infect Dis* 1999;**14**:73–107.
258. Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus:* epidemiology and detection. *Med Mycol* 2011;**49**(Suppl.1):S90–95.
259. Howard SJ, Lestner JM, Sharp A, et al. Pharmacokinetics and pharmacodynamics of posaconazole for invasive pulmonary aspergillosis: clinical implications for antifungal therapy. *Infect Dis* **203**:1324–32, 2011
260. Hull PR, Vismer HF. Treatment of cutaneous sporotrichosis with terbinafine. *J Dermatol* 1992;**126**(Suppl. 39):51–5.
261. Humbert H, Cabiac MD, Denouel J, et al. Pharmacokinetics of terbinafine and of its five main metabolites in plasma and urine, following a single oral dose in healthy subjects. *Biopharm Drug Dispos* 1995;**16**:685–94.
262. Humbert H, Denouel J, Cabiac MD, et al. Pharmacokinetics of terbinafine and five known metabolites in children, after oral administration. *Biopharm Drug Dispos* 1998;**19**:417–23.
263. Huttova M, Hartmanova I, Kralinsky K, et al. *Candida* fungemia in neonates treated with fluconazole: report of forty cases, including eight with meningitis. *Pediatr Infect Dis J* 1998;**17**:1012–15.
264. Ittel TH, Legler UF, Polak A, et al. 5-Fluorocytosine kinetics in patients with acute renal failure undergoing continuous hemofiltration. *Chemotherapy* 1987;**33**:77–84.
265. Iwen PC, Rupp ME, Langnas AN, et al. Invasive pulmonary aspergillosis due to *Aspergillus terreus:* 12-year experience and review of the literature. *Clin Infect Dis* 1998;**26**:1092–7.
266. Jagdis FA, Hoeprich PD, Lawrence RM, et al. Comparative pharmacology of amphotericin B and amphotericin B methyl ester in the non-human primate, *Macaca mulatta*. *Antimicrob Agents Chemother* 1977;**12**:582–90.
267. Jarque I, Saavedra S, Martin G, et al. Delay of onset of candidemia and emergence of *Candida krusei* fungemia in hematologic patients receiving prophylactic fluconazole. *Haematologica* 2000;**85**:441–3.

268. Jeans AR, Howard SJ, Al-Nakeeb Z, et al. Pharmacodynamics of voriconazole in a dynamic in vitro model of invasive pulmonary aspergillosis: implications for in vitro susceptibility breakpoints. *J Infect Dis* 2012;**206**:442–52.
269. Jensen GM, Bunch TH, Wolf S, et al. Erroneous determination of hyperphosphatemia ('pseudohyperphosphatemia') in sera of patients that have been treated with liposomal amphotericin B (AmBisome). *Clin Chim Acta* 2010;**411**:1900–5.
270. Jessup CJ, Ryder NS, Ghannoum MA. An evaluation of the in vitro activity of terbinafine. *Med Mycol* 2000;**38**:155–9.
271. Johnson EM, Szekely A, Warnock DW. In vitro activity of voriconazole, itraconazole and amphotericin B against filamentous fungi. *J Antimicrob Chemother* 1998;**42**:741–5.
272. Johnson LB, Kauffman CA. Voriconazole: A new triazole antifungal agent. *Clin Infect Dis* 2003;**36**:630–7.
273. Johnson MD, Drew RH, Perfect JR. Chest discomfort associated with liposomal amphotericin B: report of three cases and review of the literature. *Pharmacotherapy* 1998;**18**:1053–61.
274. Jones TC. Overview of the use of terbinafine (Lamisil) in children. *Br J Dermatol* 1995;**132**:683–9.
275. Juster-Reicher A, Flidel-Rimon O, Amitay M, et al. High-dose liposomal amphotericin B in the therapy of systemic candidiasis in neonates. *Eur J Clin Microbiol Infect Dis* 2003;**22**:603–7.
276. Juster-Reicher A, Leibovitz E, Linder N, et al. Liposomal amphotericin B (AmBisome) in the treatment of neonatal candidiasis in very low birth weight infants. *Infection* 2001;**28**:223–6.
277. Kan VL, Bennett JE. Efficacies of four antifungal agents in experimental murine sporotrichosis. *Antimicrob Agents Chemother* 1988;**32**:1619–23.
278. Karlowsky JA, Hoban DJ, Zhanel GG, et al. In vitro interactions of anidulafungin with azole antifungals, amphotericin B and 5-fluorocytosine against *Candida* species. *Int J Antimicrob Agents* 2006;**27**:174–7.
279. Karlsson MO, Lutsar I, Milligan PA. Population pharmacokinetic analysis of voriconazole plasma concentration data from pediatric studies. *Antimicrob Agents Chemother* 2009;**53**:935–44.
280. Kauffman CA, Hajjeh R, Chapman SW. Practice guidelines for the management of patients with sporotrichosis: for the Mycoses Study Group. Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:684–7.
281. Kauffman CA, Bradley SF, Ross SC, et al. Hepatosplenic candidiasis: successful treatment with fluconazole. *Am J Med* 1991; **91**:137–41.
282. Kauffman CA, Pappas PG, McKinsey DS, et al. Treatment of lymphocutaneous and visceral sporotrichosis with fluconazole. *Clin Infect Dis* 1996;**22**:46–50.
283. Kaufman D, Boyle R, Hazen KC, et al. Fluconazole prophylaxis against fungal colonization and infection in preterm infants. *N Engl J Med* 2001;**345**:1660–6.
284. Kaufman D, Boyle R, Hazen KC, et al. Twice weekly fluconazole prophylaxis for prevention of invasive *Candida* infection in high-risk infants of <1000 grams birth weight. *J Pediatr* 2005; **147**:172–9.
285. Kaufman DA. "Getting to Zero": preventing invasive *Candida* infections and eliminating infection-related mortality and morbidity in extremely preterm infants. *Early Hum Dev* 2012;**88**(Suppl 2):S45–9.
286. Kingo AR, Smyth JA, Waisman D. Lack of evidence of amphotericin B toxicity in very low birth weight infants treated for systemic candidiasis. *Pediatr Infect Dis J* 1997;**16**:1002–3.
287. Kirkpatrick WR, Vallor AC, McAtee RK, et al. Combination therapy with terbinafine and amphotericin B in a rabbit model of experimental invasive aspergillosis. *Antimicrob Agents Chemother* 2005;**49**:4751–3.
288. Klepser ME, Malone D, Lewis RE, et al. Evaluation of voriconazole pharmacodynamics using time-kill methodology. *Antimicrob Agents Chemother* 2000;**44**:1917–20.
289. Klepser ME, Wolfe EJ, Jones RN, et al. Antifungal pharmacodynamic characteristics of fluconazole and amphotericin B tested against *Candida albicans*. *Antimicrob Agents Chemother* 1997; **41**:1392–5.
290. Klepser ME, Wolfe EJ, Pfaller MA. Antifungal pharmacodynamic characteristics of fluconazole and amphotericin B against *Cryptococcus neoformans*. *J Antimicrob Chemother* 1998;**41**:397–401.
291. Knoderer CA, Knoderer HM. Hyperphosphatemia in pediatric oncology patients receiving liposomal amphotericin B. *Pediatr Pharmacol Ther* **16**:87–91, 2011
292. Kofla G, Ruhnke M. Voriconazole: review of a broad spectrum triazole antifungal agent. *Expert Opin Pharmacother* 2005; **6**:1215–29.
293. Kohler LM, Monteiro PC, Hahn RC, et al. In vitro susceptibilities of isolates of *Sporothrix schenckii* to itraconazole and terbinafine. *J Clin Microbiol* 2004;**42**:4319–20.
294. Kolve H, Ahlke E, Fegeler W, et al. Safety, tolerance and outcome of treatment with liposomal amphotericin B in paediatric patients with cancer or undergoing haematopoietic stem cell transplantation. *J Antimicrob Chemother* 2009;**64**:383–7
295. Konishi H, Morita K, Yamaji A. Effect of fluconazole on theophylline disposition in humans. *Eur J Clin Pharmacol* 1994;**46**:309–12.
296. Kontoyiannis DP, Reddy BT, Hanna H, et al. Breakthrough candidemia in patients with cancer differs from de novo candidemia in host factors and *Candida* species but not intensity. *Infect Control Hosp Epidemiol* 2002;**23**:542–5.
297. Koren G, Lau A, Klein J, et al. Pharmacokinetics and adverse effects of amphotericin B in infants and children. *J Pediatr* 1988;**113**:559–63.
298. Kovarik JM, Kirkesseli S, Humbert H, et al. Dose-proportional pharmacokinetics of terbinafine and its N-demethylated metabolite in healthy volunteers. *Br J Dermatol* 1992;**126**(Suppl. 39):8–13.
299. Kovarik JM, Mueller EA, Zehender H, et al. Multiple-dose pharmacokinetics and distribution in tissue of terbinafine and metabolites. *Antimicrob Agents Chemother* 1995;**39**:2738–41.
300. Krafchik B, Pelletier J. An open study of tinea capitis in 50 children treated with a 2-week course of oral terbinafine. *J Am Acad Dermatol* 1998;**41**:60–3.
301. Kramer MR, Marshall SE, Denning DW, et al. Cyclosporine and itraconazole interaction in heart and lung transplant recipients. *Ann Intern Med* 1990;**113**:327–9.
302. Krause DS, Reinhardt J, Vazquez JA, et al. Phase 2, randomized, dose-ranging study evaluating the safety and efficacy of anidulafungin in invasive candidiasis and candidemia: Anidulafungin Invasive Candidiasis Study Group. *Antimicrob Agents Chemother* 2004;**48**:2021–4.
303. Krause DS, Simjee AE, van Rensburg C, et al. A randomized, double-blind trial of anidulafungin versus fluconazole for the treatment of esophageal candidiasis. *Clin Infect Dis* 2004; **39**:770–5.
304. Krieter P, Flannery B, Musick T, et al. Disposition of posaconazole following single-dose oral administration in healthy subjects. *Antimicrob Agents Chemother* 2004;**48**:3543–51.
305. Krishna G, AbuTarif M, Xuan F, et al. Pharmacokinetics of oral posaconazole in neutropenic patients receiving chemotherapy for acute myelogenous leukemia or myelodysplastic syndrome. *Pharmacotherapy* 2008;**28**:1223–32.
306. Krishna G, Martinho M, Chandrasekar P, et al. Pharmacokinetics of oral posaconazole in allogeneic hematopoietic stem cell transplant recipients with graft-versus-host disease. *Pharmacotherapy* 2007;**27**:1627–36.
307. Krishna G, Sansone-Parsons A, Martinho M, et al. Posaconazole plasma concentrations in juvenile patients with invasive fungal infection. *Antimicrob Agents Chemother* 2007;**51**:812–18.
308. Krzeska I, Yeates RA, Pfaff G. Single dose intravenous pharmacokinetics of fluconazole in infants. *Drugs Exp Clin Res* 1993;**19**:267–71.
309. Kullberg BJ, Sobel JD, Ruhnke M, et al. Voriconazole versus a regimen of amphotericin B followed by fluconazole for candidaemia in non-neutropenic patients: a randomised non-inferiority trial. *Lancet* 2005;**366**:1435–42.
310. Kurtz MB, Douglas CM. Lipopeptide inhibitors of fungal glucan synthase. *J Med Vet Mycol* 1997;**35**:79–86.
311. Kurtz MB, Heath IB, Marrinan J, et al. Morphological effects of lipopeptides against *Aspergillus fumigatus* correlate with activities against (1,3)-beta-D-glucan synthase. *Antimicrob Agents Chemother* 1994;**38**:1480–9.
312. Kuse ER, Chetchotisakd P, da Cunha CA, et al. Micafungin versus liposomal amphotericin B for candidaemia and invasive candidosis: a phase III randomised double-blind trial. *Lancet* 2007;**369**: 1519–27.

313. Lansdorp D, Bressers HP, Dekens-Konter JA, et al. Potentiation of acenocoumarol during vaginal administration of miconazole. *Br J Clin Pharmacol* 1999;**47**:225–6.
314. Larsen RA, Bozette SA, Jones BE, et al. Fluconazole combined with flucytosine for treatment of cryptococcal meningitis in patients with AIDS. *Clin Infect Dis* 1994;**19**:741–5.
315. Lau AH, Kronfol NO. Elimination of flucytosine by continuous hemofiltration. *Am J Nephrol* 1995;**15**:327–31.
316. Lavrijsen APM, Balmus KJ, Nugteren-Huyning WM, et al. Hepatic injury associated with itraconazole. *Lancet* 1992; **340**:251–2.
317. Lawrence RM, Hoeprich PD, Jagdis FA, et al. Distribution of doubly radiolabelled amphotericin B methyl ester and amphotericin B in the non-human primate, *Macaca mulatta*. *J Antimicrob Chemother* 1980;**6**:241–9.
318. Lazar JD, Wilner KD. Drug interactions with fluconazole. *Rev Infect Dis* 1990;**12**(Suppl. 3):327–33.
319. Le J, Adler-Shohet FC, Nguyen C, et al. Nephrotoxicity associated with amphotericin B deoxycholate in neonates. *Pediatr Infect Dis J* 2009;**28**:1061–3.
320. Lee JW, Seibel NL, Amantea M, et al. Safety, tolerance, and pharmacokinetics of fluconazole in children with neoplastic diseases. *J Pediatr* 1992;**120**:987–93.
321. Lehrnbecher T, Attarbaschi A, Duerken M, et al. Posaconazole salvage treatment in paediatric patients: a multicentre survey. *Eur J Clin Microbiol Infect Dis* 2010;**29**:1043–5.
322. Leibovitz E, Iuster-Reicher A, Amitai M, et al. Systemic candidal infections associated with use of peripheral venous catheters in neonates: a 9-year experience. *Clin Infect Dis* 1992;**14**:485–91.
323. Leibovitz E, Rigaud M, Chandwani S, et al. Disseminated fungal infection in children with human immunodeficiency virus. *Pediatr Infect Dis J* 1991;**10**:888–94.
324. Leventakos K, Ben Ami R, Lewis RE, et al. Immunomodulating effects of antifungal therapy. *Curr Fungal Infect Rep* 2009; **3**:243–50.
325. Lewis RE, Klepser ME, Pfaller MA. In vitro pharmacodynamic characteristics of flucytosine determined by time-kill methods. *Diagn Microbiol Infect Dis* 2000;**36**:101–5.
326. Li CC, Sun P, Dong Y, et al. Population pharmacokinetics and pharmacodynamics of caspofungin in pediatric patients. *Antimicrob Agents Chemother* 2011;**55**:2098–2105.
327. Lin C, Symchowicz S. Absorption, distribution, metabolism, and excretion of griseofulvin in man and animals. *Drug Metab Rev* 1975;**4**:75–95.
328. Lin C, Magat J, Chang R, et al. Absorption, metabolism and excretion of ^{14}C-griseofulvin in man. *J Pharmacol Exp Ther* 1973;**187**:415–22.
329. Linder N, Klinger G, Shalit I, et al. Treatment of candidaemia in premature infants: comparison of three amphotericin B preparations. *J Antimicrob Chemother* 2003;**52**:663–7
330. Lipozencic J, Skerlev M, Orofino-Costa R, et al. A randomized, double-blind, parallel-group, duration-finding study of oral terbinafine and open-label, high-dose griseofulvin in children with tinea capitis due to *Microsporum* species: Tinea Capitis Study Group. *Br J Dermatol* 2002;**146**:816–23.
331. Lo ACY, Lui SL, Lo WK, et al. The interaction of terbinafine and cyclosporine A in renal transplant patients. *Br J Clin Pharmacol* 1997;**43**:340–1.
332. Lortholary O, Dupont B. Antifungal prophylaxis during neutropenia and immunodeficiency. *Clin Microbiol Rev* 1997; **10**:477–504.
333. Louie A, Deziel M, Liu W, et al. Pharmacodynamics of caspofungin in a murine model of systemic candidiasis: importance of persistence of caspofungin in tissues to understanding drug activity. *Antimicrob Agents Chemother* 2005;**49**:5058–68.
334. Louie A, Drusano GL, Banerjee P, et al. Pharmacodynamics of fluconazole in a murine model of systemic candidiasis. *Antimicrob Agents Chemother* 1998;**42**:1105–9.
335. Madani S, Barilla D, Cramer J, et al. Effect of terbinafine on the pharmacokinetics and pharmacodynamics of desipramine in healthy volunteers identified as cytochrome P450 2D6 (CYP2D6) extensive metabolizers. *J Clin Pharmacol* 2002;**42**:1211–18.
336. Maertens J, Glasmacher A, Herbrecht R, et al. Multicenter, noncomparative study of caspofungin in combination with other antifungals as salvage therapy in adults with invasive aspergillosis. *Cancer* 2006;**107**:2888–79.
337. Maertens JA, Madero L, Reilly AF, et al. A randomized, double-blind, multicenter study of caspofungin versus liposomal amphotericin B for empiric antifungal therapy in pediatric patients with persistent fever and neutropenia. *Pediatr Infect Dis J* 2010; **29**:415–20.
338. Maertens J, Lagrou K, Deweerdt H, et al. Disseminated infection by *Scedosporium prolificans*: an emerging fatality among haematology patients—case report and review. *Ann Hematol* 2000; **79**:340–4.
339. Maertens J, Raad I, Petrikkos G, et al. Efficacy and safety of caspofungin for treatment of invasive aspergillosis in patients refractory to or intolerant of conventional antifungal therapy. *Clin Infect Dis* 2004;**39**:1563–71.
340. Magill SS, Shields C, Sears CL, et al. Triazole cross-resistance among *Candida* spp.: case report—occurrence among bloodstream isolates, and implications for antifungal therapy. *J Clin Microbiol* 2006;**44**:529–35.
341. Malet-Martino MC, Martino R, deForni M, et al. Flucytosine conversion to fluorouracil in humans: does a correlation with gut flora status exist? A report of two cases using fluorine-19 magnetic resonance spectroscopy. *Infection* 1991;**19**:178–80.
342. Manavathu EK, Cutright JL, Chandrasekar PH. Organism-dependent fungicidal activities of azoles. *Antimicrob Agents Chemother* 1998;**42**:3018–21.
343. Manavathu EK, Cutright JL, Loebenberg D, et al. A comparative study of the in vitro susceptibilities of clinical and laboratory-selected resistant isolates of *Aspergillus* spp. to amphotericin B, itraconazole, voriconazole and posaconazole (SCH 56592). *J Antimicrob Chemother* 2000;**46**:229–34.
344. Manavathu EK, Ramesh MS, Baskaran I, et al. A comparative study of the post-antifungal effect (PAFE) of amphotericin B, triazoles and echinocandins on *Aspergillus fumigatus* and *Candida albicans*. *J Antimicrob Chemother* 2004;**53**:386–9.
345. Mandhaniya S, Swaroop C, Thulkar S, et al. Oral voriconazole versus intravenous low dose amphotericin B for primary antifungal prophylaxis in pediatric acute leukemia induction: a prospective, randomized, clinical study. *J Pediatr Hematol Oncol* 2011; **33**:e333–41.
346. Manzoni P, Arisio R, Mostert M, et al. Prophylactic fluconazole is effective in preventing fungal colonization and fungal systemic infections in preterm neonates: a single-center, 6-year, retrospective cohort study. *Pediatrics* 2006;**117**:e22–32.
347. Manzoni P, Galletto P, Rizzollo S, et al. Liposomal amphotericin B does not induce nephrotoxicity or renal function impairment in premature neonates. *Early Hum Dev* 2012;**88**(Suppl. 2):S86–91.
348. Marchisio P, Principi N. Treatment of oropharyngeal candidiasis in HIV-infected children with oral fluconazole. *Eur J Clin Microbiol Infect Dis* 1994;**13**:338–40.
349. Marco F, Pfaller MA, Messer SA, et al. Antifungal activity of a new triazole, voriconazole (UK-109,496), compared with three other antifungal agents tested against clinical isolates of filamentous fungi. *Med Mycol* 1998;**36**:433–6.
350. Marina NM, Flynn PM, Rivera GK, et al. *Candida tropicalis* and *Candida albicans* fungemia in children with leukemia. *Cancer* 1991;**68**:594–9.
351. Marks DI, Pagliuca A, Kibbler CC, et al.Voriconazole versus itraconazole for antifungal prophylaxis following allogeneic haematopoietic stem-cell transplantation, *Br J Haematol* 2011; **155**:318–27.
352. Marr KA, Crippa F, Leisenring W, et al. Itraconazole versus fluconazole for prevention of fungal infections in patients receiving allogeneic stem cell transplants. *Blood* 2004;**103**:1527–33.
353. Marr KA, Hachem R, Papanicolaou G, et al. Retrospective study of the hepatic safety profile of patients concomitantly treated with caspofungin and cyclosporin A. *Transpl Infect Dis* 2004;**6**:110–16.
354. Marr KA, Seidel K, Slavin MA, et al. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000;**96**:2055–61.
355. Marr KA, Seidel K, White TC, et al. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. *J Infect Dis* 2000;**181**:309–16.
356. Marr KAS. Rottinghaus H, Jagannatha S, et al. A randomised, double-blind study of combination antifungal therapy with

voriconazole and anidalafungin versus voriconazole monotherapy for primary treatment of invasive aspergillosis. In: *Abstracts of the 2012 European Society for Clinical Microbiology and Infectious Diseases meeting.* European Society for Clinical Microbiology and Infectious Diseases; Abstract LB2812 (2012).
357. Mattiuzzi GN, Alvarado G, Giles FJ, et al. Open-label, randomized comparison of itraconazole versus caspofungin for prophylaxis in patients with hematologic malignancies. *Antimicrob Agents Chemother* 2006;**50**:143–7.
358. Medoff G, Kobayashi GS. Strategies in the treatment of systemic fungal infections. *N Engl J Med* 1980;**302**:1451–5.
359. Medoff G, Comfort M, Kobayashi GS. Synergistic action of amphotericin B and 5-fluorocytosine against yeast-like organisms. *Proc Soc Exp Biol Med* 1971;**138**:571–574.
360. Mehta P, Vinks A, Filipovich A, et al. High-dose weekly AmBisome antifungal prophylaxis in pediatric patients undergoing hematopoietic stem cell transplantation: a pharmacokinetic study. *Biol Blood Marrow Transplant* 2006;**12**:235–40.
361. Mehta PA, Vinks AA, Filipovich A, et al. Alternate-day micafungin antifungal prophylaxis in pediatric patients undergoing hematopoietic stem cell transplantation: a pharmacokinetic study. *Biol Blood Marrow Transplant* 2010;**16**:1458–62.
362. Meletiadis J, Mouton JW, Meis JF, et al. In vitro drug interaction modeling of combinations of azoles with terbinafine against clinical *Scedosporium prolificans* isolates. *Antimicrob Agents Chemother* 2003;**47**:106–17.
363. Meletiadis J, Mouton JW, Rodriguez-Tudela JL, et al. In vitro interaction of terbinafine with itraconazole against clinical isolates of *Scedosporium prolificans*. *Antimicrob Agents Chemother* 2000; **44**:470–2.
364. Menichetti F, Del Favero A, Martino P, et al. Itraconazole oral solution as prophylaxis for fungal infections in neutropenic patients with hematologic malignancies: a randomized, placebo-controlled, double-blind, multicenter trial. GIMEMA Infection Program. Gruppo Italiano Malattie Ematologiche dell'. *Adulto Clin Infect Dis* 1999;**28**:250–5.
365. Meunier F, Prentice HG, Ringden O. Liposomal amphotericin B (AmBisome): safety data from a phase II/III clinical trial. *J Antimicrob Chemother* 1991;**28**(Suppl. B):83–91.
366. Michel C, Courdavault L, al Khayat R, et al. Fungal peritonitis in patients on peritoneal dialysis. *Am J Nephrol* 1994;**14**:113–20.
367. Mikami Y, Scalarone GM, Kurita N, et al. Synergistic postantifungal effect of flucytosine and fluconazole on *Candida albicans*. *J Med Vet Mycol* 1992;**30**:197–206.
368. Milefchik E, Leal MA, Haubrich R, et al. Fluconazole alone or combined with flucytosine for the treatment of AIDS-associated cryptococcal meningitis. *Med Mycol* 2008;**46**:393–5.
369. Minguez F, Chiu ML, Lima JE, et al. Activity of fluconazole: postantifungal effect, effects of low concentrations and of pretreatment on the susceptibility of *Candida albicans* to leucocytes. *J Antimicrob Chemother* 1994;**34**:93–100.
370. Miller DD, Cowen EW, Nguyen JC, et al. Melanoma associated with long-term voriconazole therapy: a new manifestation of chronic photosensitivity. *Arch Dermatol* 2010;**146**:300–4.
371. Mitchell AS, Holland JT. Fluconazole and phenytoin: a predictable inter-action. *Br Med J* 1989;**298**:1315.
372. Mohr JF, Finkel KW, Rex JH, et al. Pharmacokinetics of intravenous itraconazole in stable hemodialysis patients. *Antimicrob Agents Chemother* 2004;**48**:3151–3.
373. Mohr JF, Hall AC, Ericsson CD, et al. Fatal amphotericin B overdose due to administration of nonlipid formulation instead of lipid formulation. *Pharmacotherapy* 2005;**25**:426–8.
374. Molina JR, Serrano J, Sánchez-García J, et al. Voriconazole as primary antifungal prophylaxis in children undergoing allo-SCT. *Bone Marrow Transplant* 2012;**47**:562–7.
375. Montgomerie JZ, Edwards JE Jr, Guze LB. Synergism of amphotericin B and 5-fluorocytosine for *Candida* species. *J Infect Dis* 1975;**132**:82–6.
376. Mora-Duarte J, Betts R, Rotstein C, et al. Comparison of caspofungin and amphotericin B for invasive candidiasis. *N Engl J Med* 2002;**347**:2020–9.
377. Moriyama B, Falade O, Leung J, et al. Prolonged half-life of voriconazole in a CYP2C19 homozygous poor metabolizer receiving vincristine chemotherapy: avoiding a serious adverse drug interaction. *Mycoses* 2011;**54**:e877–879.
378. Morio F, Aubin GG, Danner-Boucher I, et al. High prevalence of triazole resistance in Aspergillus fumigatus, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. *J Antimicrob Chemother* 2012;**67**:1870–3.
379. Morselli PL. Clinical pharmacology of the perinatal period and early infancy. *Clin Pharmacokinet* 1989;**17**(Suppl. 1):13–28.
380. Mosquera J, Denning DW. Azole cross-resistance in *Aspergillus fumigatus*. *Antimicrob Agents Chemother* 2002;**46**:556–7.
381. Mosquera J, Sharp A, Moore CB, et al. In vitro interaction of terbinafine with itraconazole, fluconazole, amphotericin B and 5-flucytosine against *Aspergillus* spp. *J Antimicrob Chemother* 2002;**50**:189–94.
382. Mott SH, Packer RJ, Vezina LG, et al. Encephalopathy with parkinsonian features in children following bone marrow transplantation and high dose amphotericin B. *Ann Neurol* 1995;**37**:810–4.
383. Mueller FM, Weig M, Peter J, et al. Azole cross-resistance to ketoconazole, fluconazole, itraconazole and voriconazole in clinical *Candida albicans* isolates from HIV-infected children with oropharyngeal candidosis. *J Antimicrob Chemother* 2000;**46**:338–40.
384. Muhl E, Martens T, Iven H, et al. Influence of continuous veno-venous haemodiafiltration and continuous veno-venous haemofiltration on the pharmacokinetics of fluconazole. *Eur J Clin Pharmacol* 2000;**56**:671–8.
385. Nahata MC, Tallian KB, Force RW. Pharmacokinetics of fluconazole in young infants. *Eur J Drug Metab Pharmacokinet* 1999;**24**:155–7.
386. Naranjo MS, Trujillo M, Munera MI, et al. Treatment of paracoccidioidomycosis with itraconazole. *J Med Vet Mycol* 1990;**28**:67–76.
387. Natarajan G, Lulic-Botica M, Rongkavilit C, et al. Experience with caspofungin in the treatment of persistent fungemia in neonates. *J Perinatol* 2005;**25**:770–7.
388. Nath CE, McLachlan AJ, Shaw PJ, et al. Amphotericin B dose optimization in children with malignant diseases. *Chemotherapy* 2007;**53**:142–7.
389. Nath CE, Shaw PJ, Gunning R, et al. Amphotericin B in children with malignant disease: a comparison of the toxicities and pharmacokinetics of amphotericin B administered in dextrose versus lipid emulsion. *Antimicrob Agents Chemother* 1999;**43**:1417–23.
390. Nedelman JR, Gibiansky E, Robbins BA, et al. Pharmacokinetics and pharmacodynamics of multiple-dose terbinafine. *J Clin Pharmacol* 1996;**36**:452–61.
391. Neely M, Jafri HS, Seibel N, et al. Pharmacokinetics and safety of caspofungin in older infants and toddlers. *Antimicrob Agents Chemother* 2009;**53**:1450–6.
392. Negroni R, Palmieri O, Koren F, et al. Oral treatment of paracoccidioidomycosis and histoplasmosis with itraconazole in humans. *Rev Infect Dis* 1987;**9**(Suppl. 1):47–50.
393. Nejjam F, Zagula M, Cabiac MD, et al. Pilot study of terbinafine in children suffering from tinea capitis: evaluation of efficacy, safety and pharmacokinetics. *Br J Dermatol* 1995;**132**:98–105.
394. Neuvonen PJ, Jalava KM. Itraconazole drastically increases plasma concentrations of lovastatin and lovastatin acid. *Clin Pharmacol Ther* 1996;**60**:54–61.
395. Nguyen MH, Barchiesi F, McGough DA, et al. In vitro evaluation of combination of fluconazole and flucytosine against *Cryptococcus neoformans* var. *neoformans*. *Antimicrob Agents Chemother* 1995; **39**:1691–5.
396. Nicolau DP, Crowe H, Nightingale CH, et al. Effect of continuous arteriovenous hemofiltration on the pharmacokinetics of fluconazole. *Pharmacotherapy* 1994;**14**:502–5.
397. Ninane J, Gluckman E, Hann I, et al. A multicentre study of fluconazole versus oral polyenes in the prevention of fungal infection in children with hematological or oncological malignancies. *Eur J Clin Microbiol Infect Dis* 1994;**13**:330–7.
398. Novelli V, Holzel H. Safety and tolerability of fluconazole in children. *Antimicrob Agents Chemother* 1999;**43**:1955–60.
399. Nussbaum JC, Jackson A, Namarika D, et al. Combination flucytosine and high-dose fluconazole compared with fluconazole monotherapy for the treatment of cryptococcal meningitis: a randomized trial in Malawi. *Clin Infect Dis* 2010;**50**:338–44.
400. Oakley KL, Moore CB, Denning DW. In vitro activity of the echinocandin antifungal agent LY303,366 in comparison with itraconazole and amphotericin B against *Aspergillus* spp. *Antimicrob Agents Chemother* 1998;**42**:2726–30.

401. Odio CM, Araya R, Pinto LE, et al. Caspofungin therapy of neonates with invasive candidiasis. *Pediatr Infect Dis J* 2004; **23**:1093–7.
402. Odio CM, Pinto LE, Alfaro B, et al. Pharmacokinetics (PK) of caspofungin (CAS) in six premature neonates (PNN) with invasive candidiasis (IC) at a neonatal intensive care unit (NNICU). Abstract LB-16. In: *Abstracts of the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2005.
403. Olkkola KT, Ahonen J, Neuvonen PJ. The effects of the systemic antimycotics, itraconazole and fluconazole, on the pharmacokinetics and pharmacodynamics of intravenous and oral midazolam. *Anesth Analg* 1996;**82**:511–16.
404. Olson J, Satorius A, McAndrews B, et al. Treatment of systemic murine candidiasis with amphotericin B or different amphotericin B lipid formulations. Abstract B-11. In: *Abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 1997. p. 28.
405. Ortin M, Olalla JI, Muruzabal MJ, et al. Miconazole oral gel enhances acenocoumarol anticoagulant activity: a report of three cases. *Ann Pharmacother* 1999;**33**:175–7.
406. Ortoneda M, Capilla J, Javier Pastor F, et al. In vitro interactions of licensed and novel antifungal drugs against *Fusarium* spp. *Diagn Microbiol Infect Dis* 2004;**48**:69–71.
407. Osborne CS, Leitner I, Hofbauer B, et al. Biological, biochemical, and molecular characterization of a new clinical *Trichophyton rubrum* isolate resistant to terbinafine. *Antimicrob Agents Chemother* 2006;**50**:2234–6.
408. Osowski CL, Dix SP, Lin LS, et al. Evaluation of the drug interaction between intravenous high-dose fluconazole and cyclosporine or tacrolimus in bone marrow transplant patients. *Transplantation* 1996;**61**:1268–72.
409. O'Sullivan DP, Needham CA, Bangs A, et al. Postmarketing surveillance of oral terbinafine in the UK: report of a large cohort study. *Br J Clin Pharmacol* 1996;**42**:559–65.
410. Oxford AE, Raistrick H, Simonart P. Studies in the biochemistry of microorganisms: griseofulvin, C17H17O6Cl, a metabolic product of *Penicillium griseofulvum*. *Biochem J* 1939;**33**:240–8.
411. Pai MP, Turpin RS, Garey KW. Association of fluconazole area under the concentration-time curve/MIC and dose/MIC ratios with mortality in nonneutropenic patients with candidemia. *Antimicrob Agents Chemother* 2007;**51**:35–9.
412. Panackal AA, Gribskov JL, Staab JF, et al. Clinical significance of azole antifungal drug cross-resistance in *Candida glabrata*. *J Clin Microbiol* 2006;**44**:1740–3.
413. Pappas PG, Bradsher RW, Chapman SW, et al. Treatment of blastomycosis with fluconazole: a pilot study. *Clin Infect Dis* 1995;**20**:267–71.
414. Pappas PG, Bradsher RW, Kauffman CA, et al. Treatment of blastomycosis with higher doses of fluconazole: The National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Clin Infect Dis* 1997;**25**:200–5.
415. Pappas PG, Rex JH, Sobel JD, et al. Guidelines for treatment of candidiasis: Infectious Diseases Society of America. *Clin Infect Dis* 2004;**38**:161–89.
416. Pappas PG, Rotstein CM, Betts RF, et al. Micafungin versus caspofungin for treatment of candidemia and other forms of invasive candidiasis. *Clin Infect Dis* 2007;**45**:883–93.
417. Park S, Kelly R, Kahn JN, et al. Specific substitutions in the echinocandin target Fks1p account for reduced susceptibility of rare laboratory and clinical *Candida* sp. isolates. *Antimicrob Agents Chemother* 2005;**49**:3264–73.
418. Park WB, Kim NH, Kim KH, et al. The effect of therapeutic drug monitoring on safety and efficacy of voriconazole in invasive fungal infections: a randomized controlled trial. *Clin Infect Dis* 2012;**55**:1080–7.
419. Pasic S, Flannagan L, Cant AJ. Liposomal amphotericin B (AmBisome) is safe in bone marrow transplantation for primary immunodeficiency. *Bone Marrow Transplant* 1997;**19**:1229–32.
420. Pasqualotto AC, Howard SJ, Moore CB, et al. Flucytosine therapeutic monitoring: 15 years experience from the UK. *J Antimicrob Chemother* 2007;**59**:791–3.
421. Pedicone L, Ullmann AJ, Graybill J, et al. Comparison of posaconazole adverse event profiles in healthy volunteers and patients with invasive fungal infections. Abstract P-1106. In: *Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2004.
422. Pelletier R, Peter J, Antin C, et al. Emergence of resistance of *Candida albicans* to clotrimazole in human immunodeficiency virus–infected children: in vitro and clinical correlations. *J Clin Microbiol* 2000;**38**:1563–8.
423. Perfect JR, Marr KA, Walsh TJ, et al. Voriconazole treatment for less-common, emerging, or refractory fungal infections. *Clin Infect Dis* 2003;**36**:1122–31.
424. Perlin DS. Current perspectives on echinocandin class drugs. *Future Microbiol* 2011;**6**:441–57.
425. Petraitis V, Petraitiene R, Groll AH, et al. Comparative antifungal activity of the echinocandin micafungin against disseminated candidiasis and invasive pulmonary aspergillosis in persistently neutropenic rabbits. *Antimicrob Agents Chemother* 2002;**46**:1857–69.
426. Pettengell K, Mynhardt J, Kluyts T, et al. Successful treatment of oesophageal candidiasis by micafungin: a novel systemic antifungal agent. FK463 South African Study Group. *Aliment Pharmacol Ther* 2004;**20**:475–81.
427. Pfaller MA. Antifungal drug resistance: mechanisms, epidemiology, and consequences for treatment. *Am J Med* **125**(Suppl. 1):S3–13, 2012
428. Pfaller MA, Diekema DJ, Sheehan DJ. Interpretive breakpoints for fluconazole and *Candida* revisited: a blueprint for the future of antifungal susceptibility testing. *Clin Microbiol Rev* 2006;**19**:435–47.
429. Pfaller MA, Messer SA, Coffman S. In vitro susceptibilities of clinical yeast isolates to a new echinocandin derivative, LY303366, and other antifungal agents. *Antimicrob Agents Chemother* 1997;**41**:763–6.
430. Pfaller MA, Andes D, Diekema DJ, et al. CLSI Subcommittee for Antifungal Susceptibility Testing. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida:* time for harmonization of CLSI and EUCAST broth microdilution methods. *Drug Resist Updat* 2010;**13**:180–95.
431. Pfaller MA, Castanheira M, Lockhart SR, et al. Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of *Candida glabrata*. *J Clin Microbiol* 2012;**50**:1199–203.
432. Pfaller MA, Diekema DJ, Andes D, et al. CLSI Subcommittee for Antifungal Testing. Clinical breakpoints for the echinocandins and *Candida* revisited: integration of molecular, clinical, and microbiological data to arrive at species-specific interpretive criteria. *Drug Resist Updat* 2011;**14**:164–76.
433. Pfaller MA, Messer SA, Boyken L, et al. In vitro activities of 5-fluorocytosine against 8,803 clinical isolates of *Candida* spp.: Global assessment of primary resistance using National Committee for Clinical Laboratory Standards susceptibility testing methods. *Antimicrob Agents Chemother* 2002;**46**:3518–21.
434. Pfaller MA, Messer SA, Boyken L, et al. Cross-resistance between fluconazole and ravuconazole and the use of fluconazole as a surrogate marker to predict susceptibility and resistance to ravuconazole among 12,796 clinical isolates of *Candida* spp. *J Clin Microbiol* 2004;**42**:3137–41.
435. Phillips P, Shafran S, Garber G, et al. Multicenter randomized trial of fluconazole versus amphotericin B for treatment of candidemia in non-neutropenic patients: Canadian Candidemia Study Group. *Eur J Clin Microbiol Infect Dis* 1997;**16**:337–45.
436. Pieper S, Kolve H, Gumbinger HG, et al. Safety, tolerance and outcome of treatment with voriconazole in immunocompromised pediatric patients. *Mycoses* 2011;**54**(Suppl. 2):125.
437. Pieper S, Kolve H, Gumbinger HG, et al. Monitoring of voriconazole plasma concentrations in immunocompromised paediatric patients. *J Antimicrob Chemother* 2012;**67**:2717–24.
438. Piper L, Smith PB, Hornik CP, et al. Fluconazole loading dose pharmacokinetics and safety in infants. *Pediatr Infect Dis J* 2011; **30**:375–87.
439. Pitisuttithum P, Negroni R, Graybill JR, et al. Activity of posaconazole in the treatment of central nervous system fungal infections. *J Antimicrob Chemother* 2005;**56**:745–55.
440. Piscitelli SC, Gallicano KD. Interactions among drugs for HIV and opportunistic infections. *N Engl J Med* 2001;**344**:984–96.
441. Pizzo PA, Robichaud KJ, Gill FA, et al. Empiric antibiotic and antifungal therapy for cancer patients with prolonged fever and granulocytopenia. *Am J Med* 1982;**72**:101–11.

442. Polak A. Mode of action studies. In: Ryley JF, editor: *Handbook of experimental pharmacology*, Vol. 96. Berlin: Springer-Verlag; 1990. p. 153–82.
443. Powderly WG, Finkelstein D, Feinberg J, et al. A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with AIDS. *N Engl J Med* 1995;**332**:700–5.
444. Prentice AG, Warnock DW, Johnson SA, et al. Multiple dose pharmacokinetics of an oral solution of itraconazole in patients receiving chemotherapy for acute myeloid leukaemia. *J Antimicrob Chemother* 1995;**36**:657–63.
445. Prentice HG, Hann IM, Herbrecht R, et al. A randomized comparison of liposomal versus conventional amphotericin B for the treatment of pyrexia of unknown origin in neutropenic patients. *Br J Haematol* 1997;**98**:711–18.
446. Purkins L, Wood N, Ghahramani P, et al. Pharmacokinetics and safety of voriconazole following intravenous- to oral-dose escalation regimens. *Antimicrob Agents Chemother* 2002;**46**:2546–53.
447. Queiroz-Telles F, Berezin E, Leverger G, et al. Micafungin versus liposomal amphotericin B for pediatric patients with invasive candidiasis: substudy of a randomized double-blind trial. *Pediatr Infect Dis J* 2008;**27**:820–6.
448. Raad I, Chapman S, Bradsher R, et al. Posaconazole salvage therapy for invasive fungal infections. Abstract M-669. In: *Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology. 2004.
449. Raad I, Graybill JR, Bustamante AB, et al. Safety of long-term oral posaconazole use in the treatment of refractory invasive fungal infections. *Clin Infect Dis* 2006;**42**:1726–34.
450. Rallis E, Frangoulis E. Successful treatment of subcutaneous phaeohyphomycosis owing to *Exophiala jeanselmei* with oral terbinafine. *Int J Dermatol* 2005;**45**:1369–70.
451. Ralph ED, Khazindar AM, Barber KR, et al. Comparative in vitro effects of liposomal amphotericin B, amphotericin B-deoxycholate, and free amphotericin B against fungal strains determined by using MIC and minimal lethal concentration susceptibility studies and time-kill curves. *Antimicrob Agents Chemother* 1991;**35**:188–91.
452. Reboli AC, Rotstein C, Pappas PG, et al. Anidulafungin versus fluconazole for invasive candidiasis. *N Engl J Med* 2007; **356**:2472–82.
453. Redding S, Smith J, Farinacci G, et al. Resistance of *Candida albicans* to fluconazole during treatment of oropharyngeal candidiasis in a patient with AIDS: documentation by in vitro susceptibility testing and DNA subtype analysis. *Clin Infect Dis* 1994;**18**:240–2.
454. Rennie R, Sand C, Sherburne R. Electron microscopic evidence of the effect of LY303366 on *Aspergillus fumigatus*. Abstract p 451. In: *Abstracts of the 13th Meeting of the International Society for Human and Animal Mycology*. Parma, Italy: International Society for Human and Animal Mycology; 1997. p. 191.
455. Restrepo A, Robledo J, Gomez I, et al. Itraconazole therapy in lymphangitic and cutaneous sporotrichosis. *Arch Dermatol* 1986;**122**:413–17.
456. Reuben A, Anaissie E, Nelson PE, et al. Antifungal susceptibility of 44 clinical isolates of *Fusarium* species determined by using a broth microdilution method. *Antimicrob Agents Chemother* 1989;**33**:1647–9.
457. Rex JH, Bennett JE, Sugar AM, et al. A randomized trial comparing fluconazole with amphotericin B for the treatment of candidemia in patients without neutropenia. *N Engl J Med* 1994;**331**:1325–30.
458. Rex JH, Pfaller MA, Galgiani JN, et al. Development of interpretive breakpoints for antifungal susceptibility testing: conceptual framework and analysis of in vitro–in vivo correlation data for fluconazole, itraconazole, and *Candida* infections. Subcommittee on Antifungal Susceptibility Testing of the National Committee for Clinical Laboratory Standards. *Clin Infect Dis* 1997; **24**:235–47.
459. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. *Antimicrob Agents Chemother* 1995;**39**:1–8.
460. Reynes J, Bazin C, Ajana F, et al. Pharmacokinetics of itraconazole (oral solution) in two groups of human immunodeficiency virus–infected adults with oral candidiasis. *Antimicrob Agents Chemother* 1997;**41**:2554–8.
461. Reynolds ES, Tomkiewicz ZM, Dammin GJ. The renal lesion related to amphotericin B treatment for coccidioidomycosis. *Med Clin North Am* 1993;**47**:1149–54.
462. Ringden O. Clinical use of AmBisome with special emphasis on experience in children. *Bone Marrow Transplant* 1993;**12**(Suppl. 4):149–50.
463. Ringden O, Meunier F, Tollemar J, et al. Efficacy of amphotericin B encapsulated in liposome (AmBisome) in the treatment of invasive fungal infections in immunocompromised patients. *J Antimicrob Chemother* 1991;**28**(Suppl. B):73–82.
464. Robbins B, Chang CT, Cramer JA, et al. Safe coadministration of terbinafine and terfenadine: a placebo-controlled crossover study of pharmacokinetic and pharmacodynamic interactions in healthy volunteers. *Clin Pharmacol Ther* 1996;**59**:275–83.
465. Roden MM, Nelson LD, Knudsen TA, et al. Triad of acute infusion-related reactions associated with liposomal amphotericin B: analysis of clinical and epidemiological characteristics. *Clin Infect Dis* 2003;**36**:1213–20.
466. Rodríguez-Tudela JL, Arendrup MC, Cuenca-Estrella M, et al. EUCAST breakpoints for antifungals. *Drug News Perspect* 2010;**23**:93–7.
467. Rogers TE, Galgiani JN. Activity of fluconazole (UK 49,858) and ketoconazole against *Candida albicans* in vitro and in vivo. *Antimicrob Agents Chemother* 1986;**30**:418–22.
468. Rotstein C, Bow EJ, Laverdiere M, et al. Randomized placebo-controlled trial of fluconazole prophylaxis for neutropenic cancer patients: benefit based on purpose and intensity of cytotoxic therapy. The Canadian Fluconazole Prophylaxis Study Group. *Clin Infect Dis* 1999;**28**:331–40.
469. Rowland M, Riegelman S, Epstein WL. Absorption kinetics of griseofulvin in man. *J Pharm Sci* 1968;**57**:984–9.
470. Ruhnke M, Eigler A, Tennagen I, et al. Emergence of fluconazole-resistant strains of *Candida albicans* in patients with recurrent oropharyngeal candidosis and human immunodeficiency virus infection. *J Clin Microbiol* 1994;**32**:2092–8.
471. Ruhnke M, Kuse E, Chetchotisad H, et al. Comparison of micafungin and liposomal amphotericin B for invasive candidiasis. Abstract M-722c. In: *Abstracts of the 45th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2005.
472. Ruhnke M, Yeates RA, Pfaff G, et al. Single-dose pharmacokinetics of fluconazole in patients with liver cirrhosis. *J Antimicrob Chemother* 1995;**35**:641–7.
473. Ryder NS. Activity of terbinafine against serious fungal pathogens. *Mycoses* 1999;**42**(Suppl. 2):115–19.
474. Ryder NS, Leitner I. Synergistic interaction of terbinafine with triazoles or amphotericin B against *Aspergillus* species. *Med Mycol* 2001;**39**:91–5.
475. Ryley JF, McGregor S. A multiinfection model for antifungal screening in vivo. *J Antimicrob Chemother* 1988;**22**:353–8.
476. Saag MS, Cloud GA, Graybill JR, et al. A comparison of itraconazole versus fluconazole as maintenance therapy for AIDS-associated cryptococcal meningitis: National Institute of Allergy and Infectious Diseases Mycoses Study Group. *Clin Infect Dis* 1999;**28**:291–6.
477. Saag MS, Fessel WJ, Kaufman CA, et al. Treatment of fluconazole-refractory oropharyngeal candidiasis with itraconazole oral solution in HIV-positive patients. *AIDS Res Hum Retroviruses* 1999;**15**:1413–17.
478. Saari TI, Laine K, Leino K, et al. Voriconazole, but not terbinafine, markedly reduces alfentanil clearance and prolongs its half-life. *Clin Pharmacol Ther* 2006;**80**:502–8.
479. Sabatelli F, Patel R, Mann PA, et al. In vitro activities of posaconazole, fluconazole, itraconazole, voriconazole, and amphotericin B against a large collection of clinically important molds and yeasts. *Antimicrob Agents Chemother* 2006;**50**:2009–15.
480. Sachs MK, Blanchard LM, Green PJ. Interaction of itraconazole and digoxin. *Clin Infect Dis* 1993;**16**:400–3.
481. Sáez-Llorens X, Macias M, Maiya P, et al. Pharmacokinetics and safety of caspofungin in neonates and infants less than 3 months of age. *Antimicrob Agents Chemother* 2009;**53**:869–75.
482. Sahai J, Gallicano K, Pakuts A, et al. Effect of fluconazole on zidovudine pharmacokinetics in patients infected with human immunodeficiency virus. *J Infect Dis* 1994;**169**:1103–7.

483. Saitoh A, Homans J, Kovacs A. Fluconazole treatment of coccidioidal meningitis in children: two case reports and a review of the literature. *Pediatr Infect Dis J* 2000;**19**:1204–8.
484. Sandler ES, Mustafa MM, Tkaczewski I, et al. Use of amphotericin B colloidal dispersion in children. *J Pediatr Hematol Oncol* 2000;**22**:242–6.
485. Sanglard D, Odds FC. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. *Lancet Infect Dis* 2002;**2**:73–85.
486. Sansone A, Courtney R, Krishna G. Evaluation of the drug interaction potential of posaconazole: summary of 7 clinical pharmacology studies. Abstract P-1107. In: *Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2003.
487. Sansone A, Courtney R, Mellars L. Posaconazole has no clinically significant effect on QTc interval in healthy volunteers. Abstract A-1100. In: *Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2004.
488. Sansone-Parsons A, Krishna G, Simon J, et al. Effects of age, gender, and race/ethnicity on the pharmacokinetics of posaconazole in healthy volunteers. *Antimicrob Agents Chemother* 2007;**51**:495–502.
489. Sawaya BP, Briggs JP, Schnermann J. Amphotericin B nephrotoxicity: the adverse consequences of altered membrane properties. *J Am Soc Nephrol* 1995;**6**:154–64.
490. Saxen H, Hoppu K, Pohjavuori M. Pharmacokinetics of fluconazole in very low birth weight infants during the first two weeks of life. *Clin Pharmacol Ther* 1993;**54**:269–77.
491. Scalarone GM, Mikami Y, Kurita N, et al. Comparative studies on the postantifungal effect produced by the synergistic interaction of flucytosine and amphotericin B on *Candida albicans*. *Mycopathologia* 1992;**120**:133–8.
492. Scalarone GM, Mikami Y, Kurita N, et al. The postantifungal effect of 5-fluorocytosine on *Candida albicans*. *J Antimicrob Chemother* 1992;**29**:129–36.
493. Schmitt C, Perel Y, Harousseau J, et al. Pharmacokinetics of itraconazole oral solution in neutropenic children during long-term prophylaxis. *Antimicrob Agents Chemother* 2001;**45**:1561–4.
494. Schmitt HJ, Andrade J, Edwards F, et al. Inactivity of terbinafine in a rat model of pulmonary aspergillosis. *Eur J Clin Microbiol Infect Dis* 1990;**9**:832–5.
495. Schmitt HJ, Bernard EM, Andrade J, et al. MIC and fungicidal activity of terbinafine against clinical isolates of *Aspergillus* spp. *Antimicrob Agents Chemother* 1988;**32**:780–1.
496. Scholz J, Schulz M, Steinfath M, et al. Fluconazole is removed by continuous venovenous hemofiltration in a liver transplant patient. *J Mol Med* 1995;**73**:145–7.
497. Schwartz EL, Hallam S, Gallagher RE, et al. Inhibition of all-*trans*-retinoic acid metabolism by fluconazole in vitro and in patients with acute promyelocytic leukemia. *Biochem Pharmacol* 1995;**50**:923–8.
498. Schwartz S, Behre G, Heinemann V, et al. Amphotericin B inhalations as prophylaxis of invasive *Aspergillus* infections during prolonged neutropenia: results of a prospective randomized multicenter trial. *Blood* 1999;**93**:3654–61.
499. Schwartz S, Ruhnke M, Ribaud P, et al. Improved outcome in central nervous system aspergillosis, using voriconazole treatment. *Blood* 2005;**106**:2641–5.
500. Schwarz P, Dromer F, Lortholary O, et al. In vitro interaction of flucytosine with conventional and new antifungals against *Cryptococcus neoformans* clinical isolates. *Antimicrob Agents Chemother* 2003;**47**:3361–4.
501. Seay RE, Larson TA, Toscano JP, et al. Pharmacokinetics of fluconazole in immunocompromised children with leukemia or other hematologic diseases. *Pharmacotherapy* 1995;**15**:52–8.
502. Sethi A, Antaya R. Systemic antifungal therapy for cutaneous infections in children. *Pediatr Infect Dis J* 2006;**25**:643–4.
503. Seibel N, Walsh TJ. Safety, tolerance and pharmacokinetics of liposomal amphotericin B in children and adolescents. In: *Abstracts of Focus on Fungal Infections*. Alpharetta, Ga: Imedex; 1999, abstract 15.
504. Seibel NL, Schwartz C, Arrieta A, et al. Safety, tolerability, and pharmacokinetics of micafungin (FK463) in febrile neutropenic pediatric patients. *Antimicrob Agents Chemother* 2005;**49**:3317–24.
505. Shadomy S, Espinel-Ingroff A, Gebhart RJ. In-vitro studies with SF 86-327, a new orally active allylamine derivative. *Sabouraudia* 1985;**23**:125–32.
506. Shah VP, Riegelman S, Epstein WL. Determination of griseofulvin in skin, plasma, and sweat. *J Pharm Sci* 1972;**61**:634–6.
507. Sharkey PA, Graybill JR, Rinaldi MG, et al. Itraconazole treatment of phaeohyphomycosis. *J Am Acad Dermatol* 1990; **23**:577–86.
508. Silm H, Karelson M. Terbinafine: Efficacy and tolerability in young children with tinea capitis due to *Microsporum canis*. *J Eur Acad Dermatol Venereol* 2002;**16**:228–30.
509. Simon A, Besuden M, Vezmar S, et al. Itraconazole prophylaxis in pediatric cancer patients receiving conventional chemotherapy or autologous stem cell transplants. *Support Care Cancer* 2007; **15**:213–20.
510. Simitsopoulou M, Roilides E, Georgiadou E, et al. Differential transcriptional profiles induced by amphotericin B formulations on human monocytes during response to hyphae of *Aspergillus fumigatus*. *Med Mycol* 2011;**49**:176–85.
511. Simitsopoulou M, Roilides E, Maloukou A, et al. Interaction of amphotericin B lipid formulations and triazoles with human polymorphonuclear leucocytes for antifungal activity against Zygomycetes. *Mycoses* **51**:147–54, 2008
512. Sionov E, Chang YC, Garraffo HM, et al. Heteroresistance to fluconazole in *Cryptococcus neoformans* is intrinsic and associated with virulence. *Antimicrob Agents Chemother* 2009;**53**:2804–15.
513. Sionov E, Lee H, Chang YC, Kwon-Chung KJ. Cryptococcus neoformans overcomes stress of azole drugs by formation of disomy in specific multiple chromosomes. *PLoS Pathog* 2010; **6**:e1000848.
514. Skiest DJ, Vazquez JA, Anstead GM, et al. Posaconazole for the treatment of azole-refractory oropharyngeal and esophageal candidiasis in subjects with HIV infection. *Clin Infect Dis* 2007;**44**:607–14.
515. Slavin MA, Osborne B, Adams R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation: a prospective, randomized, double-blind study. *J Infect Dis* 1995;**171**:1545–52.
516. Smith PB, Walsh TJ, Hope W, et al. Pharmacokinetics of an elevated dosage of micafungin in premature neonates. *Pediatr Infect Dis J* 2009;**28**:412–15.
517. Snelders E, van der Lee HA, Kuijpers J, et al. Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. *PLoS Med* 2008;**5**:e219.
518. Sobel JD, Faro S, Force RW, et al. Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstet Gynecol* 1998;**178**:203–11.
519. Sohnle PG, Hahn BL, Erdmann MD. Effect of fluconazole on viability of *Candida albicans* over extended periods of time. *Antimicrob Agents Chemother* 1996;**40**:2622–5.
520. Soltani M, Tobin CM, Bowker KE, et al. Evidence of excessive concentrations of 5-flucytosine in children aged below 12 years: a 12-year review of serum concentrations from a UK clinical assay reference laboratory. *Int J Antimicrob Agents* 2006;**28**:574–7.
521. Sorensen K, Corcoran E, Chen S, et al. Pharmacodynamic assessment of efflux- and target based resistance to fluconazole on efficacy against C. *albicans* in a mouse kidney infection model. Abstract 1271. In: *Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 1999. p. 328.
522. Starke JR, Mason O, Kramer WG, et al. Pharmacokinetics of amphotericin B in infants and children. *J Infect Dis* 1987; **155**:766–74.
523. Stein GE, Mummaw N. Placebo-controlled trial of itraconazole for treatment of acute vaginal candidiasis. *Antimicrob Agents Chemother* 1993;**37**:89–92.
524. Steinbach WJ. Antifungal agents in children. *Pediatr Clin North Am* 2005;**52**:895–915.
525. Stevens DA, Lee JY. Analysis of compassionate use itraconazole therapy for invasive aspergillosis by the NIAID Mycoses Study Group criteria. *Arch Intern Med* 1997;**157**:1857–62.
526. Stone JA, McCrea J, Wickersham P, et al. A phase I study of caspofungin evaluating the potential for drug interactions with itraconazole, the effect of gender and the use of a loading dose regimen. Abstract 854. In: *Abstracts of the 40th Interscience*

Conference on Antimicrobial Agents and Chemotherapy. Washington, D.C.: American Society for Microbiology; 2000. p. 26.
527. Suleiman J, della Negra M, Llanos-Cuentas A. Open label study of micafungin in the treatment of esophageal candidiasis. Abstract M-722c. In: *Abstracts of the 42nd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2002.
528. Sutton DA, Sanche SE, Revankar SG, et al. In vitro amphotericin B resistance in clinical isolates of *Aspergillus terreus*, with a head-to-head comparison to voriconazole. *J Clin Microbiol* 1999; **37**:2343–5.
529. Tang TR, Parr R, Turner W, et al. LY-303366: A non-competitive inhibitor of (1,3)-b-D-glucan synthases from *Candida albicans* and *Aspergillus fumigatus*. Abstract 367. In: *Abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 1993.
530. Te Dorsthorst DT, Mouton JW, van den Beukel CJ, et al. Effect of pH on the in vitro activities of amphotericin B, itraconazole, and flucytosine against *Aspergillus* isolates. *Antimicrob Agents Chemother* 2004;**48**:3147–50.
531. Te Dorsthorst DT, Verweij PE, Meis JF, et al. In vitro interactions between amphotericin B, itraconazole, and flucytosine against 21 clinical *Aspergillus* isolates determined by two drug interaction models. *Antimicrob Agents Chemother* 2004;**48**:2007–13.
532. Te Dorsthorst DT, Verweij PE, Meis JF, et al. Efficacy and pharmacodynamics of flucytosine monotherapy in a nonneutropenic murine model of invasive aspergillosis. *Antimicrob Agents Chemother* 2005;**49**:4220–6.
533. Teitelbaum ML, Pearson VE. Imipramine toxicity and terbinafine. *Am J Psychiatry* 2001;**158**:2086.
534. Thaler M, Pastakia B, Shawker TH, et al. Hepatic candidiasis in cancer patients: the evolving picture of the syndrome. *Ann Intern Med* 1988;**108**:88–100.
535. Thompson JR, Douglas CM, Li W, et al. A glucan synthase FKS1 homolog in *Cryptococcus neoformans* is single copy and encodes an essential function. *J Bacteriol* 1999;**181**:444–53.
536. Tobon AM, Franco L, Espinal D, et al. Disseminated histoplasmosis in children: the role of itraconazole therapy. *Pediatr Infect Dis J* 1996;**15**:1002–8.
537. Toon S, Ross CE, Gokal R, et al. An assessment of the effects of impaired renal function and haemodialysis on the pharmacokinetics of fluconazole. *Br J Clin Pharmacol* 1990;**29**:221–6.
538. Torres HA, Hachem RY, Chemaly RF, et al. Posaconazole: a broad-spectrum triazole antifungal. *Lancet Infect Dis* 2005; **5**:775–85.
539. Tragiannidis A, Dokos C, Lehrnbecher T, et al. Antifungal chemoprophylaxis in children and adolescents with haematological malignancies and following allogeneic haematopoietic stem cell transplantation: review of the literature and options for clinical practice. *Drugs* 2012;**72**:685–705.
540. Tragiannidis A, Roilides E, Walsh TJ, et al. Invasive aspergillosis in children with acquired immunodeficiencies. *Clin Infect Dis* 2012;**54**:258–67.
541. Travis LB, Roberts GD, Wilson WR. Clinical significance of *Pseudallescheria boydii:* a review of 10 years' experience. *Mayo Clin Proc* 1985;**60**:531–7.
542. Trenschel R, Ditschkowski M, Elmaagacli AH, et al. Caspofungin as second-line therapy for fever of unknown origin or invasive fungal infection following allogeneic stem cell transplantation. *Bone Marrow Transplant* 2005;**35**:583–6.
543. Trepanier EF, Nafziger AN, Amsden GW. Effect of terbinafine on theophylline pharmacokinetics in healthy volunteers. *Antimicrob Agents Chemother* 1998;**42**:695–7.
544. Trilles L, Fernandez-Torres B, Lazera Mdos S, et al. In vitro antifungal susceptibility of *Cryptococcus gattii*. *J Clin Microbiol* 2004;**42**:4815–17.
545. Troke PF, Hockey HP, Hope WW. Observational study of the clinical efficacy of voriconazole and its relationship to plasma concentrations in patients. *Antimicrob Agents Chemother* 2011;**55**:4782–8.
546. Tucker RM, Denning DW, Arathoon EG, et al. Itraconazole therapy for nonmeningeal coccidioidomycosis: clinical and laboratory observations. *J Am Acad Dermatol* 1990;**23**:593–601.
547. Tucker RM, Denning DW, Dupont B, et al. Itraconazole therapy for chronic coccidioidal meningitis. *Ann Intern Med* 1990; **112**:108–12.
548. Tucker RM, Denning DW, Hanson LH, et al. Interaction of azoles with rifampin, phenytoin, and carbamazepine: in vitro and clinical observations. *Clin Infect Dis* 1992;**14**:165–74.
549. Tucker RM, Haq Y, Denning DW, et al. Adverse events associated with itraconazole in 189 patients on chronic therapy. *J Antimicrob Chemother* 1990;**26**:561–6.
550. Turnidge JD, Gudmundsson S, Vogelman B, et al. The postantibiotic effect of antifungal agents against common pathogenic yeasts. *J Antimicrob Chemother* 1994;**34**:83–92.
551. Uko S, Soghier LM, Vega M, et al. Targeted short-term fluconazole prophylaxis among very low birth weight and extremely low birth weight infants. *Pediatrics* 2006;**117**:1243–52.
552. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. *N Engl J Med* 2007;**356**:335–47.
553. Uzun O, Anaissie EJ. Antifungal prophylaxis in patients with hematologic malignancies: a reappraisal. *Blood* 1995;**86**:2063–72.
554. van Burik JA, Hare RS, Solomon HF, et al. Posaconazole is effective as salvage therapy in zygomycosis: a retrospective summary of 91 cases. *Clin Infect Dis* 2006;**42**:e61–5.
555. van Burik JA, Ratanatharathorn V, Stepan DE, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. *Clin Infect Dis* 2004;**39**:1407–16.
556. Van der Auwera P, Ceuppens AM, Heymans C, et al. In vitro evaluation of various antifungal agents alone and in combination by using an automatic turbidimetric system combined with viable count determinations. *Antimicrob Agents Chemother* 1986;**29**: 997–1004.
557. van der Horst CM, Saag MS, Cloud GA, et al. Treatment of cryptococcal meningitis associated with the acquired immunodeficiency syndrome. *N Engl J Med* 1997;**337**:15–21.
558. van der Kuy PH, Hooymans PM. Nortriptyline intoxication induced by terbinafine. *BMJ* 1998;**316**:441.
559. Vanden Bossche H, Koymans L. Cytochromes P450 in fungi. *Mycoses* 1998;**41**(Suppl. 1):32–8.
560. Vanden Bossche H, Dromer F, Improvisi I, et al. Antifungal drug resistance in pathogenic fungi. *Med Mycol* 1998;**36**(Suppl. 1):119–28.
561. van der Linden JW, Snelders E, Kampinga GA, et al. Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. *Emerg Infect Dis* 2011;**17**:1846–54.
562. Varhe A, Olkkola KT, Neuvonen PJ. Oral triazolam is potentially hazardous to patients receiving systemic antimycotics ketoconazole or itraconazole. *Clin Pharmacol Ther* 1994;**56**:601–7.
563. Vasquez JA, Sobel JD. Anidulafungin: a novel echinocandin. *Clin Infect Dis* 2006;**43**:215–22.
564. Vasquez JA, Northland R, Miller S. Posaconazole compared to fluconazole for oral candidiasis in HIV-positive patients. Abstract 1107. In: *Abstracts of the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 2000.
565. Vasquez JA, Skiest DJ, Nieto L, et al. A multicenter randomized trial evaluating posaconazole versus fluconazole for the treatment of oropharyngeal candidiasis in subjects with HIV/AIDS. *Clin Infect Dis* 2006;**42**:1179–86.
566. Vena GA, Micali G, Santoianni P, et al. Oral terbinafine in the treatment of multi-site seborrhoic dermatitis: a multicenter, double-blind placebo-controlled study. *Int J Immunopathol Pharmacol* 2005;**18**:745–53.
567. Vermes A, Guchelaar HJ, Dankert J. Flucytosine: a review of its pharmacology, clinical indications, pharmacokinetics, toxicity and drug interactions. *J Antimicrob Chemother* 2000;**46**:171–9.
568. Vermes A, van Der Sijs H, Guchelaar HJ. Flucytosine: correlation between toxicity and pharmacokinetic parameters. *Chemotherapy* 2000;**46**:86–94.
569. Vickers AE, Sinclair JR, Zollinger M, et al. Multiple cytochrome P-450s involved in the metabolism of terbinafine suggest a limited potential for drug-drug interactions. *Drug Metab Dispos* 1999; **27**:1029–38.
570. Villanueva A, Arathoon EG, Gotuzzo E, et al. A randomized double-blind study of caspofungin versus amphotericin for the treatment of candidal esophagitis. *Clin Infect Dis* 2001;**33**:1529–35.
571. Villanueva A, Gotuzzo E, Arathoon EG, et al. A randomized double-blind study of caspofungin versus fluconazole for the treatment of esophageal candidiasis. *Am J Med* 2002;**113**:294–9.

572. Viscoli C, Herbrecht R, Akan H, et al. An EORTC Phase II study of caspofungin as first-line therapy of invasive aspergillosis in haematological patients. *J Antimicrob Chemother* 2009;**64**: 1274–81.
573. Wade KC, Benjamin DK Jr, Kaufman DA, et al. Fluconazole dosing for the prevention or treatment of invasive candidiasis in young infants. *Pediatr Infect Dis J* 2009;**228**:717–23.
574. Wade KC, Wu D, Kaufman DA, et al. Population pharmacokinetics of fluconazole in young infants. *Antimicrob Agents Chemother* 2008;**52**:4043–9.
575. Wainer S, Cooper PA, Gouws H, et al. Prospective study of fluconazole therapy in systemic neonatal fungal infection. *Pediatr Infect Dis J* 1997;**16**:763–7.
576. Walsh TJ, Adamson PC, Seibel NL, et al. Pharmacokinetics, safety, and tolerability of caspofungin in children and adolescents. *Antimicrob Agents Chemother* 2005;**49**:4536–45.
577. Walsh T, Driscoll T, Milligan P, et al. Pharmacokinetics, safety, and tolerability of voriconazole in immunocompromised children. *Antimicrob Agents Chemother* 2010;**48**:4116–23.
578. Walsh TJ, Finberg RW, Arndt C, et al. Liposomal amphotericin B for empirical therapy in patients with persistent fever and neutropenia: National Institute of Allergy and Infectious Diseases Mycoses Study Group. *N Engl J Med* 1999;**340**:764–71.
579. Walsh TJ, Gonzalez C, Lyman CA, et al. Invasive fungal infections in children: recent advances in diagnosis and treatment. *Adv Pediatr Infect Dis* 1996;**11**:187–290.
580. Walsh TJ, Gonzalez CE, Piscitelli S, et al. Correlation between in vitro and in vivo antifungal activities in experimental fluconazole-resistant oropharyngeal and esophageal candidiasis. *J Clin Microbiol* 2000;**38**:2369–73.
581. Walsh TJ, Goutelle S, Jelliffe RW, et al. Intrapulmonary pharmacokinetics and pharmacodynamics of micafungin in adult lung transplant patients. *Antimicrob Agents Chemother* 2010;**54**:3451–9.
582. Walsh TJ, Hiemenz JW, Seibel N, et al. Amphotericin B lipid complex for invasive fungal infections: analysis of safety and efficacy in 556 cases. *Clin Infect Dis* 1998;**26**:1383–96.
583. Walsh TJ, Karlsson MO, Driscoll T, et al. Pharmacokinetics and safety of intravenous voriconazole in children after single- or multiple-dose administration. *Antimicrob Agents Chemother* 2004; **48**:2166–72.
584. Walsh TJ, Lutsar I, Driscoll T, et al. Voriconazole in the treatment of aspergillosis, scedosporiosis and other invasive fungal infections in children. *Pediatr Infect Dis J* 2002;**21**:240–8.
585. Walsh TJ, Melcher G, Rinaldi M, et al. *Trichosporon beigelii:* an emerging pathogen resistant to amphotericin B. *J Clin Microbiol* 1990;**28**:1616–22.
586. Walsh TJ, Newman KR, Moody M, et al. Trichosporonosis in patients with neoplastic disease. *Medicine (Baltimore)* 1986; **65**:268–79.
587. Walsh TJ, Pappas P, Winston DJ, et al. Voriconazole compared with liposomal amphotericin B for empirical antifungal therapy in patients with neutropenia and persistent fever. *N Engl J Med* 2002;**346**:225–34.
588. Walsh TJ, Peter J, McGough DA, et al. Activities of amphotericin B and antifungal azoles alone and in combination against *Pseudallescheria boydii*. *Antimicrob Agents Chemother* 1995;**39**:1361–4.
589. Walsh TJ, Raad I, Patterson TF, et al. Treatment of invasive aspergillosis with posaconazole in patients who are refractory to or intolerant of conventional therapy: An externally controlled trial. *Clin Infect Dis* 2007;**44**:2–12.
590. Walsh TJ, Seibel NL, Arndt C, et al. Amphotericin B lipid complex in pediatric patients with invasive fungal infections. *Pediatr Infect Dis J* 1999;**18**:702–8.
591. Walsh TJ, Teppler H, Donowitz GR, et al. Caspofungin versus liposomal amphotericin B for empirical antifungal therapy in patients with persistent fever and neutropenia. *N Engl J Med* 2004;**351**:1391–402.
592. Walsh TJ, Whitcomb P, Piscitelli S, et al. Safety, tolerance, and pharmacokinetics of amphotericin B lipid complex in children with hepatosplenic candidiasis. *Antimicrob Agents Chemother* 1997;**41**:1944–8.
593. Walzer PD, Ashbaugh A. Use of terbinafine in mouse and rat models of *Pneumocystis carinii* pneumonia. *Antimicrob Agents Chemother* 2002;**46**:514–16.
594. Warn PA, Livermore J, Howard S, et al. Anidulafungin for neonatal hematogenous *Candida* meningoencephalitis: identification of candidate regimens for humans using a translational pharmacological approach. *Antimicrob Agents Chemother* 2012; **56**:708–14.
595. Warnock DW, Turner A, Burke J. Comparison of high performance liquid chromatography and microbiological methods for determination of itraconazole. *J Antimicrob Chemother* 1986; **21**:93–100.
596. Warwick JA, Corrall RJ. Serious interaction between warfarin and oral terbinafine. *B M J* 1998;**316**:440.
597. Watt KM, Benjamin DK Jr, Cheifetz IM, et al. Pharmacokinetics and safety of fluconazole in young infants supported with extracorporeal membrane oxygenation. *Pediatr Infect Dis J* 2012;**31**: 1042–7.
598. Weig M, Muller FM. Synergism of voriconazole and terbinafine against *Candida albicans* isolates from human immunodeficiency virus–infected patients with oropharyngeal candidiasis. *Antimicrob Agents Chemother* 2001;**45**:966–8.
599. Welzen ME, Brüggemann RJ, Van Den Berg JM, et al. A twice daily posaconazole dosing algorithm for children with chronic granulomatous disease. *Pediatr Infect Dis J* 2011;**30**:794–7.
600. Wermers RA, Cooper K, Razonable RR, et al. Fluoride excess and periostitis in transplant patients receiving long-term voriconazole therapy. *Clin Infect Dis* 2011;**52**:604–11.
601. Wexler D, Courtney R, Richards W, et al. Effect of posaconazole on cytochrome P450 enzymes: a randomized, open-label, two-way crossover study. *Eur J Pharm Sci* 2004;**21**:645–53.
602. Wheat J, Hafner R, Korzun AH, et al. Itraconazole treatment of disseminated histoplasmosis in patients with the acquired immunodeficiency syndrome. *Am J Med* 1995;**98**:336–42.
603. Wheat J, Hafner R, Wulfsohn M, et al. Prevention of relapse of histoplasmosis with itraconazole in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1993;**118**:610–16.
604. Wheat J, McWhinney S, Hafner R, et al. Treatment of histoplasmosis with fluconazole in patients with acquired immunodeficiency syndrome: National Institute of Allergy and Infectious Diseases Acquired Immunodeficiency Syndrome Clinical Trials Group and Mycoses Study Group. *Am J Med* 1997;**103**:223–32.
605. Wheat J, Sarosi G, McKinsey D, et al. Practice guidelines for the management of patients with histoplasmosis: Infectious Diseases Society of America. *Clin Infect Dis* 2000;**30**:688–95.
606. White MH, Anaissie EJ, Kusne S, et al. Amphotericin B colloidal dispersion vs. amphotericin B as therapy for invasive aspergillosis. *Clin Infect Dis* 1997;**24**:635–42.
607. White MH, Bowden RA, Sandler ES, et al. Randomized, double-blind clinical trial of amphotericin B colloidal dispersion vs. amphotericin B in the empirical treatment of fever and neutropenia. *Clin Infect Dis* 1998;**27**:296–302.
608. White TC. Increased mRNA levels of ERG16, CDR, and MDR1 correlate with increases in azole resistance in *Candida albicans* isolates from a patient infected with human immunodeficiency virus. *Antimicrob Agents Chemother* 1997;**41**:1482–7.
609. Wiederhold NP, Kontoyiannis DP, Chi J, et al. Pharmacodynamics of caspofungin in a murine model of invasive pulmonary aspergillosis: evidence of concentration-dependent activity. *J Infect Dis* 2004;**190**:1464–71.
610. Wiederhold NP, Tam VH, Chi J, et al. Pharmacodynamic activity of amphotericin B deoxycholate is associated with peak plasma concentrations in a neutropenic murine model of invasive pulmonary aspergillosis. *Antimicrob Agents Chemother* 2006;**50**:469–73.
611. Wiley JM, Seibel NL, Walsh TJ. Efficacy and safety of amphotericin B lipid complex in 548 children and adolescents with invasive fungal infections. *Pediatr Infect Dis J* 2005;**24**:167–74.
612. Wingard JR. Infections due to resistant *Candida* species in patients with cancer who are receiving chemotherapy. *Clin Infect Dis* 1994;**19**(Suppl. 1):49–53.
613. Wingard JR, Carter SL, Walsh TJ, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood* 2010;**116**:5111–18.
614. Wingard JR, Kubilis P, Lee L, et al. Clinical significance of nephrotoxicity in patients treated with amphotericin B for suspected or proven aspergillosis. *Clin Infect Dis* 1999;**29**:1402–7.
615. Wingard JR, Merz WG, Rinaldi MG, et al. Increase in *Candida krusei* infection among patients with bone marrow transplantation and neutropenia treated prophylactically with fluconazole. *N Engl J Med* 1991;**325**:1274–7.

616. Wingard JR, Merz WG, Rinaldi MG, et al. Association of *Torulopsis glabrata* infections with fluconazole prophylaxis in neutropenic bone marrow transplant patients. *Antimicrob Agents Chemother* 1993;**37**:1847–9.
617. Wingard JR, White MH, Anaissie E, et al. L Amph/ABLC Collaborative Study Group. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. L Amph/ABLC Collaborative Study Group. *Clin Infect Dis* 2000;**31**:1155–63.
618. Winston DJ, Maziarz RT, Chandrasekar PH, et al. Intravenous and oral itraconazole versus intravenous and oral fluconazole for long-term antifungal prophylaxis in allogeneic hematopoietic stem-cell transplant recipients: a multicenter, randomized trial. *Ann Intern Med* 2003;**138**:705–13.
619. Winston DJ, Pakrasi A, Busuttil RW. Prophylactic fluconazole in liver transplant recipients: a randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 1999;**131**:729–37.
620. Wong-Beringer A, Jacobs RA, Guglielmo BJ. Lipid formulations of amphotericin B: clinical efficacy and toxicities. *Clin Infect Dis* 1997;**27**:603–18.
621. Wright DG, Robichaud KJ, Pizzo PA, et al. Lethal pulmonary reactions associated with the combined use of amphotericin B and leukocyte transfusions. *N Engl J Med* 1981;**304**:1185–9.
622. Wurthwein G, Groll AH, Hempel G, et al. Population pharmacokinetics of amphotericin B lipid complex in neonates. *Antimicrob Agents Chemother* 2005;**49**:5092–8.
623. Xu X, Deluna F, Nishime J, et al. Lack of metabolism-based drug interactions of MK-0991, a potent antifungal agent, in rats. *Pharm Res* 1997;**14**(Suppl. 11):558.
624. Yasui-Furukori N, Saito M, Inoue Y, et al. Terbinafine increases the plasma concentration of paroxetine after a single oral administration of paroxetine in healthy subjects. *Eur J Clin Pharmacol* 2007;**63**:51–6.
625. Yule SM, Walker D, Cole M, et al. The effect of fluconazole on cyclophosphamide metabolism in children. *Drug Metab Dispos* 1999;**27**:417–21.
626. Zhanel GG, Saunders DG, Hoban DJ, et al. Amphotericin B, azole, and 5-flucytosine pharmacodynamic parameters in the presence of human serum. Abstract 542. In: *Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. Washington, D.C.: American Society for Microbiology; 1999. p. 23.
627. Zaoutis T, Lehrnbecher T, Groll AH, et al. Safety experience with caspofungin in pediatric patients. *Pediatr Infect Dis J* 2009; **28**:1132–5.
628. Zaoutis TE, Jafri HS, Huang LM, et al. A prospective, multicenter study of caspofungin for the treatment of documented *Candida* or *Aspergillus* infections in pediatric patients. *Pediatrics* 2009; **123**:877–84.
629. Zimbeck AJ, Iqbal N, Ahlquist AM, et al. FKS mutations and elevated echinocandin MIC values among *Candida glabrata* isolates from U.S. population-based surveillance. *Antimicrob Agents Chemother* 2010;**54**:5042–7.

Infection		Drug	Adult dosage	Pediatric dosage
Mild to Moderate Intestinal Disease				
Drug of choice:[5]		Metronidazole	500-750 mg PO tid × 7-10 d	35-50 mg/kg/d PO in 3 doses × 7-10 d
	OR	Tinidazole[6]	2 g once PO daily × 3 d	≥3 yrs: 50 mg/kg/d (max. 2 g) PO in 1 dose × 3 d
		either followed by		
		Iodoquinol[2]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
	OR	Paromomycin[3*]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
Severe Intestinal and Extraintestinal Disease				
Drug of choice:		Metronidazole	750 mg PO tid × 7-10 d	35-50 mg/kg/d PO in 3 doses × 7-10 d
	OR	Tinidazole[6]	2 g once PO daily × 5 d	≥3 yrs: 50 mg/kg/d (max. 2 g) PO in 1 dose × 5 d
		either followed by		
		Iodoquinol[2]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
	OR	Paromomycin[3*]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
Amebic Meningoencephalitis, primary and granulomatous				
Naegleria fowleri				
Drug of choice:		Amphotericin B[7,8]	1.5 mg/kg/d IV in 2 doses × 3 d, then 1 mg/kg/d × 6 d plus 1.5 mg/d intrathecally × 2 d, then 1 mg/d every other day × 8 d	1.5 mg/kg/d IV in 2 doses × 3 d, then 1 mg/kg/d × 6 d plus 1.5 mg/d intrathecally × 2 d, then 1 mg/d every other day × 8 d
Acanthamoeba spp.				
Drug of choice:		See footnote 9		

*Availability problems. See table on page 3327.

1. Keratitis is typically associated with contact lens use (FR Carvalho et al, Cornea 2009; 28:516). Topical 0.02% chlorhexidine and polyhexamethylene biguanide (PHMB, 0.02%), either alone or in combination, have been used successfully in a large number of patients. Treatment with either chlorhexidine or PHMB is often combined with propamidine isethionate *(Brolene)* or hexamidine *(Desmodine).* None of these drugs is commercially available or approved for use in the US, but they can be obtained from compounding pharmacies (see footnote 4). Leiter's Park Avenue Pharmacy, San Jose, CA (800-292-6773; www.leiterrx.com) is a compounding pharmacy that specializes in ophthalmic drugs. Propamidine is available over the counter in the UK and Australia. Hexamidine is available in France. The combination of chlorhexidine, natamycin (pimaricin) and debridement also has been successful (K Kitagawa et al, Jpn J Ophthalmol 2003; 47:616), as has 0.1% sodium diclofenac (AL Agahan et al, Ann Acad Med Singapore 2009; 38: 175) in a small series of 3 patients. Debridement is most useful during the stage of corneal epithelial infection; keratoplasty in medically unresponsive keratitis was successful in 31 patients (AS Kitzmann et al, Ophthalmology 2009; 116: 864). Most cysts are resistant to neomycin; its use is no longer recommended. Azole antifungal drugs (ketoconazole, itraconazole) have been used as oral or topical adjuncts. Use of corticosteroids is controversial. Prolonged therapy (≥6 months) may be necessary (JK Dart et al, Am J Ophthalmol 2009; 148:487).
2. Iodoquinol should be taken after meals.
3. Paromomycin should be taken with a meal.
4. Not available commercially. It may be obtained through compounding pharmacies such as Expert Compounding Pharmacy, 6744 Balboa Blvd, Lake Balboa, CA 91406 (800-247-9767) or Medical Center Pharmacy, New Haven, CT (203-688-7064). Other compounding pharmacies may be found through the National Association of Compounding Pharmacies (800-687-7850) or the Professional Compounding Centers of America (800-331-2498, www.pccarx.com).
5. Nitazoxanide may be effective against a variety of protozoan and helminth infections (DA Bobak, Curr Infect Dis Rep 2006; 8:91; E Diaz et al, Am J Trop Med Hyg 2003; 68:384). It is effective against mild to moderate amebiasis, 500 mg bid × 3 d (JF Rossignol et al, Trans R Soc Trop Med Hyg 2007; 101:1025; AE Escobedo et al, Arch Dis Child 2009; 94:478). It is FDA-approved only for treatment of diarrhea caused by *Giardia* or *Cryptosporidium* (Med Lett Drugs Ther 2003; 45:29). Nitazoxanide is available in 500-mg tablets and an oral suspension; it should be taken with food.
6. A nitroimidazole similar to metronidazole, tinidazole appears to be as effective as metronidazole and better tolerated (Med Lett Drugs Ther 2004; 46:70). It should be taken with food to minimize GI adverse effects. For children and patients unable to take tablets, a pharmacist can crush the tablets and mix them with cherry syrup (*Humco*, and others). The syrup suspension is good for 7 days at room temperature and must be shaken before use (HB Fung and TL Doan, Clin Ther 2005; 27:1859). Ornidazole, a similar drug, is also used outside the US.

Infection		Drug	Adult dosage	Pediatric dosage
Balamuthia mandrillaris				
Drug of choice:		See footnote 10		
Sappinia diploidea				
Drug of choice:		See footnote 11		
Ancylostoma caninum (Eosinophilic enterocolitis)				
Drug of choice:		Albendazole[7,12]	400 mg PO once	400 mg PO once
	OR	Mebendazole	100 mg PO bid × 3 d	100 mg PO bid × 3 d
	OR	Endoscopic removal		
Ancylostoma duodenale, see HOOKWORM				
Angiostrongyliasis *(Angiostrongylus cantonensis, Angiostrongylus costaricensis)*				
Drug of choice:		See footnote 13		
Anisakiasis *(Anisakis* spp.**)**				
Treatment of choice:[14]	Surgical or endoscopic removal			
Ascariasis *(Ascaris lumbricoides,* roundworm)				
Drug of choice:[5]		Albendazole[7,12]	400 mg PO once	400 mg PO once
	OR	Mebendazole	100 mg bid PO × 3 d or 500 mg once	100 mg PO bid × 3 d or 500 mg once
	OR	Ivermectin[7,15]	150-200 mcg/kg PO once	150-200 mcg/kg PO once

7. Not FDA-approved for this indication.
8. A *Naegleria fowleri* infection was treated successfully in a 9-year old girl with combination of amphotericin B and miconazole (both drugs given intravenously and intrathecally) plus oral rifampin (JS Seidel et al NEJM 1982; 306:346). While amphotericin B and miconazole appear to have a synergistic effect, Medical Letter consultants believe the rifampin probably had no additional effect (GS Visvesvara et al, FEMS Immunol Med Microbiol 2007; 50:1). Parenteral miconazole is no longer available in the US. Azithromycin (changed to clarithromycin during therapy because of toxicity concerns and for better CNS penetration) has been used in multidrug combination regimens to treat *Balamuthia* infection. *In vitro*, azithromycin is more active than clarithromycin against *Naegleria*, so may be a better choice combined with amphotericin B for treatment of *Naegleria* (TR Deetz et al, Clin Infect Dis 2003; 37:1304; FL Schuster and GS Visvesvara, Drug Resistance Updates 2004; 7:41).Combinations of amphotericin B, ornidazole and rifampin (R Jain et al, Neurol India 2002; 50:470), amphotericin B, fluconazole (IV and PO) and rifampin (J Vargas-Zepeda et al, Arch Med Research 2005;36:83) and amphotericin B, chloramphicol and rifampacin have also been used (R Rai et al, Indian Pediatr 2008; 45:1004). Case reports of other successful therapy have been published (FL Schuster and GS Visvesvara, Int J Parasitol 2004; 34:1001).
9. Several patients with granulomatous amebic encephalitis (GAE) have been successfully treated with combinations of pentamidine, sulfadiazine, flucytosine, and either fluconazole or itraconazole (GS Visvesvara et al, FEMS Immunol Med Microbiol 2007; 50:1). GAE in an AIDS patient was treated successfully with sulfadiazine, pyrimethamine and fluconazole combined with surgical resection of the CNS lesion (M Seijo Martinez et al, J Clin Microbiol 2000; 38:3892). Chronic *Acanthamoeba* meningitis was successfully treated in 2 children with a combination of oral trimethoprim/sulfamethoxazole, rifampin and ketoconazole (T Singhal et al, Pediatr Infect Dis J 2001; 20:623). Disseminated cutaneous infection in an immunocompromised patient was treated successfully with IV pentamidine, topical chlorhexidine and 2% ketoconazole cream, followed by PO itraconazole (CA Slater et al, N Engl J Med 1994; 331:85) and with voriconazole and amphotericin B lipid complex (R Walia et al,Transplant Infect Dis 2007; 9:51). Other reports of successful therapy have been described (FL Schuster and GS Visvesvara, Drug Resistance Updates 2004; 7:41; AC Aichelburg et al, Emerg Infect Dis 2008; 14:1743). Susceptibility testing of *Acanthamoeba* isolates has shown differences in drug sensitivity between species and even among strains of a single species; antimicrobial susceptibility testing is advisable (FL Schuster and GS Visvesvara, Int J Parasitiol 2004; 34:1001).
10. *B. mandrillaris* is a free-living ameba that causes subacute to fatal granulomatous amebic encephalitis (GAE) and cutaneous disease (MMWR 2008; 57:768; FL Schuster et al, Clin Infect Dis 2009; 48:879). Three cases of *Balamuthia* encephalitis have been successfully treated with pentamidine, flucytosine, fluconazole and sulfadiazine plus either azithromycin or clarithromycin combined with surgical resection of the CNS lesion; in two cases flucytosine was given as well. Clarithromycin may have less toxicity and better penetration into CSF than azithromycin (TR Deetz et al, Clin Infect Dis 2003; 37:1304; S Jung et al, Arch Pathol Lab Med 2004; 128:466).
11. A free-living ameba that may rarely be pathogenic to humans (GS Visvesvara et al, FEMS Immunol Med Microbiol 2007; 50:1; F Marciano-Cabral, J Infect Dis 2009; 199: 1104). *S. diploidea* has been successfully treated with azithromycin, pentamidine, itraconazole and flucytosine combined with surgical resection of the CNS lesion (BB Gelman et al, J Neuropathol Exp Neurol 2003; 62:990).
12. Albendazole must be taken with food; a fatty meal increases oral bioavailability.
13. *A. cantonensis* causes predominantly neurotropic disease (QP Wang et al, Lancet Infect Dis 2008; 8:621). *A. costaricensis* causes gastrointestinal disease. Most patients infected with either species have a self-limited course and recover completely. Analgesics, corticosteroids and periodic removal of CSF can relieve symptoms from increased intracranial pressure (L Ramirez-Avila et al, Clin Infect Dis 2009; 48:322). Treatment of *A. cantonensis* is controversial and varies across endemic areas. No antihelminthic drug is proven to be effective and some patients have worsened with therapy. Mebendazole or albendazole each with or without a corticosteroid appear to shorten the course of infection (K Sawanyawisuth and K Sawanyawisuth, Trans R Soc Trop Med Hyg 2008; 102:990; V Chotmongkol et al. Am J Trop Med Hyg 2009; 81:443).

Infection		Drug	Adult dosage	Pediatric dosage
Babesiosis				
Drug of choice:[16]		Atovaquone[7,17]	750 mg PO bid × 7-10 d	40 mg/kg/d PO in 2 doses × 7-10 d
		plus azithromycin[7]	500-1000 mg PO on d1, then 250-1000 mg PO on d2-10	10 mg/kg (max 500 mg/dose) PO on d 1, then 5 mg/kg/d (max 250 mg dose) PO on d 2-10
	OR	Clindamycin[7,18]	300-600 mg IV qid or 600 mg PO tid × 7-10 d	20-40 mg/kg/d PO in 3 doses × 7-10 d
		plus quinine[7,19]	650 mg PO tid × 7-10 d	30 mg/kg/d PO in 3 doses × 7-10 d
Balamuthia mandrillaris, see AMEBIC MENINGOENCEPHALITIS, PRIMARY				
Balantidiasis *(Balantidium coli)*				
Drug of choice:		Tetracycline[7,20]	500 mg PO qid × 10 d	40 mg/kg/d (max. 2 g) PO in 4 doses × 10 d
Alternative:		Metronidazole[7]	500-750 mg PO tid × 5 d	35-50 mg/kg/d PO in 3 doses × 5 d
	OR	Iodoquinol[2,7]	650 mg PO tid × 20 d	30-40 mg/kg/d (max 2 g) PO in 3 doses × 20 d
Baylisascariasis *(Baylisascaris procyonis)*				
Drug of choice:		See footnote 21		
***Blastocystis* spp. infection**				
Drug of choice:		See footnote 22		
Capillariasis *(Capillaria philippinensis)*				
Drug of choice:		Mebendazole[7]	200 mg PO bid × 20 d	200 mg PO bid × 20 d
Alternative:		Albendazole[7,12]	400 mg PO daily × 10 d	400 mg PO daily × 10 d

14. Gastric anisakiasis can usually be diagnosed and treated by endoscopic removal of the worm. Enteric anisakiasis is more difficult to diagnose; it can be managed without worm removal as the worms eventually die. Surgery may be needed in the event of intestinal obstruction or peritonitis (A Repiso Ortega et al, Gastroenterol Hepatol 2003; 26:341; K Nakaji, Intern Med 2009; 48:573). Successful treatment of anisakiasis with albendazole 400 mg PO bid × 3-5 d has been reported, but diagnosis was presumptive (DA Moore et al, Lancet 2002; 360:54; E Pacios et al, Clin Infect Dis 2005; 41:1825).
15. Safety of ivermectin in young children (<15 kg) and pregnant women remains to be established. Ivermectin should be taken on an empty stomach with water (NM Fox, Curr Opin Infect Dis 2006; 19:588).
16. E Vannier et al, Infect Dis Clin North Am 2008; 22:469; GP Wormser et al, Clin Infect Dis 2006; 43:1089. *B. microti* is most common in the US. Most disease in Europe is attributed to *B. divergens* and is generally more severe. Several cases caused by various *B. divergens*-like agents have also been documented in the US (BL Herwaldt et al, Emerg Infect Dis 2004; 10:622). Exchange transfusion has been used in combination with drug treatment in severely ill patients and those with high (>10%) parasitemia. In non-immunosuppressed patients infected with *B. microti* who were not severely ill, combination therapy with atovaquone and azithromycin was as effective as clindamycin and quinine and better tolerated (PJ Krause et al, N Engl J Med 2000; 343:1454). Immunosuppressed patients and those with asplenia should be treated a minimum of 6 weeks and at least 2 weeks past the last positive smear. Resistance to azithromycin-atovaquone treatment has been reported in immunocompromised patients (GP Wormser et al. Clin Infect Dis 2010; 50:381). Some patients may be co-infected with the etiologic agents of Lyme disease and human granulocytic anaplasmosis.
17. Atovaquone is available in an oral suspension that should be taken with a meal to increase absorption.
18. Oral clindamycin should be taken with a full glass of water to minimize esophageal ulceration.
19. Quinine should be taken with or after a meal to decrease gastrointestinal adverse effects.
20. Use of tetracyclines is contraindicated in pregnancy and in children <8 years old. Tetracycline should be taken 1 hour before or 2 hours after meals and/or dairy products.
21. No drug has been demonstrated to be effective. Albendazole 25 mg/kg/d PO × 20 d started as soon as possible (up to 3 d after possible infection) might prevent clinical disease and is recommended for children with known exposure (ingestion of raccoon stool or contaminated soil) (WJ Murray and KR Kazacos, Clin Infect Dis 2004; 39:1484). Mebendazole, levamisole or ivermectin could be tried if albendazole is not available. Steroid therapy may be helpful, especially in eye and CNS infections (PJ Gavin et al, Clin Microbiol Rev 2005; 18:703). Ocular baylisascariasis has been treated successfully using laser photocoagulation therapy to destroy the intraretinal larvae (CA Garcia et al, Eye (Lond) 2004; 18:624).
22. Blastocystis has been reclassified as a fungus. Clinical significance of these organisms is controversial; metronidazole 750 mg PO tid × 10 d, iodoquinol 650 mg PO tid × 20 d or trimethoprim/sulfamethoxazole 1 DS tab PO bid × 7 d have been reported to be effective (KS Tan, Clin Microbiol Rev 2008; 21:639). Metronidazole resistance may be common in some areas (J Yakoob et al, Br J Biomed Sci 2004; 61:75). Nitazoxanide has been effective in clearing organisms and improving symptoms (E Diaz et al, Am J Trop Med Hyg 2003; 68:384; JF Rossignol, Clin Gastroenterol Hepatol 2005; 3:987).

Infection	Drug	Adult dosage	Pediatric dosage
Chagas' disease, see TRYPANOSOMIASIS			
Clonorchis sinensis, see FLUKE infection			
Cryptosporidiosis *(Cryptosporidium)*			
Non-HIV Infected			
Drug of choice:	Nitazoxanide[5]	500 mg PO bid × 3 d	1-3 yrs: 100 mg PO bid × 3 d 4-11 yrs: 200 mg PO bid × 3 d >12 yrs: 500 mg PO bid × 3 d
HIV Infected			
Drug of choice:	See footnote 23		
Cutaneous Larva Migrans (creeping eruption, dog and cat hookworm)			
Drug of choice:[24]	Albendazole[7,12]	400 mg PO daily × 3 d	400 mg PO daily × 3 d
OR	Ivermectin[7,15]	200 mcg/kg PO daily × 1-2 d	200 mcg/kg PO daily × 1-2 d
Cyclosporiasis *(Cyclospora cayetanensis)*			
Drug of choice:[25]	Trimethoprim/ sulfamethoxazole[7]	TMP 160 mg/SMX	TMP 10 mg/kg/SMX
		800 mg (1 DS tab) PO bid × 7-10 d	50 mg/kg/d PO in 2 doses × 7-10 d
Alternative:	Ciprofloxacin[7]	500 mg PO bid × 7 d	–
Cysticercosis, see TAPEWORM infection			
Cystoisosporiasis *(Cystoisospora belli,* formerly known as *Isospora)*			
Drug of choice:[26]	Trimethoprim-sulfamethoxazole[7]	TMP 160 mg/SMX 800 mg (1 DS tab) PO bid × 10 d	TMP 10 mg/kg/d/SMX 50 mg/kg/d PO in 2 doses × 10 d
Dientamoeba fragilis infection[27]			
Drug of choice:[28]	Iodoquinol[2,7]	650 mg PO tid × 20 d	30-40 mg/kg/d (max. 2 g) PO in 3 doses × 20 d
OR	Paromomycin[3,7*]	25-35 mg/kg/d PO in 3 doses × 7 d	25-35 mg/kg/d PO in 3 doses × 7 d
OR	Metronidazole[7]	500-750 mg PO tid × 10 d	35-50 mg/kg/d PO in 3 doses × 10 d
Diphyllobothrium latum, see TAPEWORM infection			
Dracunculus medinensis **(guinea worm) infection**			
Drug of choice:	See footnote 29		

23. No drug has proven efficacy against cryptosporidiosis in advanced AIDS (I Abubakar et al, Cochrane Database Syst Rev 2007; 1:CD004932). Potent antiretroviral therapy (ART) is the mainstay of treatment. Nitazoxanide, paromomycin, or a combination of paromomycin and azithromycin may be tried to decrease diarrhea and recalcitrant malabsorption of antimicrobial drugs, which can occur with chronic cryptosporidiosis (B Pantenburg et al, Expert Rev Anti InfectTher 2009; 7:385).
24. J Heukelbach and H Feldmeier, Lancet Infect Dis 2008; 8:302.
25. CA Warren, Curr Infect Dis Rep 2009; 11:108. In one study of HIV-infected patients with *Cyclospora* infection, ciprofloxacin treatment led to resolution in 87% of patients compared to 100% with TMP/SMX (RI Verdier et al, Ann Intern Med 2000; 132:885). HIV-infected patients may need higher dosage and long-term maintenance. Nitazoxanide (see also footnote 5) has also been used in a few patients (SM Zimmer et al, Clin Infect Dis 2007; 44:466; E Diaz et al, Am J Trop Med Hyg 2003; 68:384).
26. *Isospora belli* has been renamed and included the *Cystoisospora* genus. Usually a self-limited illness in immunocompetent patients. Immunosuppressed patients may need higher doses and longer duration (TMP/SMX qid for up to 3 to 4 weeks (Morbid Mortal Wkly Rep 2009; 58 RR4:1). They may require secondary prophylaxis (TMP/SMX DS tiw). In sulfa-allergic patients, pyrimethamine 50-75 mg daily in divided doses (plus leucovorin 10-25 mg/d) has been effective.
27. DJ Stark et al, Trends Parasitol 2006; 22:92; O Vandenberg et al, Pediatr Infect Dis J 2007; 26:88.
28. In one study, single-dose ornidazole, a nitroimidazole similar to metronidazole that is available in Europe, was effective and better tolerated than 5 days of metronidazole (O Kurt, Clin Microbiol Infect 2008; 14:601).
29. No drug is curative against *Dracunculus*. A program for monitoring local sources of drinking water to eliminate transmission has dramatically decreased the number of cases worldwide. The treatment of choice is slow extraction of worm combined with wound care and pain management (Morbid Mortal Wkly Rep 2009; 58:1123).
30. Since family members are usually infected, treatment of the entire household is recommended; retreatment after 14-21 d may be needed.
31. Pyrantel pamoate suspension can be mixed with milk or fruit juice.

Infection	Drug	Adult dosage	Pediatric dosage
Echinococcus, see TAPEWORM infection			
***Entamoeba histolytica*, see AMEBIASIS**			
***Enterobius** vermicularis* (pinworm) infection			
Drug of choice:[30]	Albendazole[7,12]	400 mg PO once; repeat in 2 wks	400 mg PO once; repeat in 2 wks
OR	Mebendazole	100 mg PO once; repeat in 2 wks	100 mg PO once; repeat in 2 wks
OR	Pyrantel pamoate[31*]	11 mg/kg base PO once (max. 1 g); repeat in 2 wks	11 mg/kg base PO once (max. 1 g); repeat in 2 wks
***Fasciola hepatica*,** see FLUKE infection			
Filariasis[32,33]			
Wuchereria bancrofti, Brugia malayi, Brugia timori			
Drug of choice:[34]	Diethylcarbamazine*	6 mg/kg/d PO in 3 doses × 12 d[35,36]	6 mg/kg/d PO in 3 doses × 12 d[35,36]
Loa loa			
Drug of choice:[37]	Diethylcarbamazine*	9 mg/kg/d PO in 3 doses × 12 d[35,36]	9 mg/kg/d PO in 3 doses × 12 d[35,36]
Mansonella ozzardi			
Drug of choice:	See footnote 38		
Mansonella perstans			
Drug of choice[39]	Albendazole[7,12]	400 mg PO bid × 10 d	400 mg PO bid × 10 d
OR	Mebendazole[7]	100 mg PO bid × 30 d	100 mg PO bid × 30 d
Mansonella streptocerca			
Drug of choice:[40]	Diethylcarbamazine*	6 mg/kg/d PO in 3 doses × 12 d[36]	6 mg/kg/d PO in 3 doses × 12 d[36]
OR	Ivermectin[7,15]	150 mcg/kg PO once	150 mcg/kg PO once
***Tropical Pulmonary Eosinophilia (TPE)*[41]**			
Drug of choice:	Diethylcarbamazine*	6 mg/kg/d in 3 doses × 12-21 d[36]	6 mg/kg/d in 3 doses × 12-21 d[36]
Onchocerca volvulus (River blindness)			
Drug of choice:	Ivermectin[15,42]	150 mcg/kg PO once, repeated every 6-12 mos until asymptomatic	150 mcg/kg PO once, repeated every 6-12 mos until asymptomatic

34. Most symptoms are caused by adult worm. A single-dose combination of albendazole (400 mg PO) with either ivermectin (200 mcg/kg PO) or diethylcarbamazine (6 mg/kg PO) is effective for reduction or suppression of *W. bancrofti* microfilaria; none of these drug combinations kills all the adult worms (D Addiss et al, Cochrane Database Syst Rev 2004; CD003753).
35. For patients with microfilaria in the blood, Medical Letter consultants start with a lower dosage and scale up: d1: 50 mg; d2: 50 mg tid; d3: 100 mg tid; d4-14: 6 mg/kg/d in 3 doses (for *Loa Loa* d4-14: 9 mg/kg/d in 3 doses). Multi-dose regimens have been shown to provide more rapid reduction in microfilaria than single-dose diethylcarbamazine, but microfilaria levels are similar 6-12 months after treatment (LD Andrade et al, Trans R Soc Trop Med Hyg 1995; 89:319; PE Simonsen et al, Am J Trop Med Hyg 1995; 53:267). A single dose of 6 mg/kg is used in endemic areas for mass treatment, but there are no studies directly comparing the efficacy of the single-dose regimen to a 12-day course. It should be used cautiously in geographic regions where *O. volvulus* coexists with other filariae. One review concluded that the 12-day regimen did not have a higher macrofilaricidal effect than single dose (A Hoerauf, Curr Opin Infect Dis 2008; 21: 673; J Figueredo-Silva et al, Trans R Soc Trop Med Hyg 1996; 90:192; J Noroes et al, Trans R Soc Trop Med Hyg 1997; 91:78).
32. Antihistamines or corticosteroids may be required to decrease allergic reactions to components of disintegrating microfilariae that result from treatment, especially in infection caused by *Loa loa*.
33. Endosymbiotic *Wolbachia* bacteria, which are present in most human filariae except *Loa loa*, are essential to filarial growth, development, embryogenesis and survival and represent an additional target for therapy. Doxycycline 100 or 200 mg/d PO × 6-8 wks in lymphatic filariasis, onchocerciasis, and *Mansonella perstans* has resulted in substantial loss of *Wolbachia* and decrease in both micro- and macrofilariae (MJ Bockarie et al, Expert Rev Anti Infect Ther 2009; 7:595; A Hoerauf Curr Opin Infect Dis 2008; 21:673;YI Coulibaly et al, N Engl J Med 2009; 361:1448). Use of tetracyclines is contraindicated in pregnancy and in children <8 yrs old.
36. Diethylcarbamazine should not be used for treatment of *Onchocerca volvulus* due to the risk of increased ocular side effects (including blindness) associated with rapid killing of the worms. It should be used cautiously in geographic regions where *O. volvulus* coexists with other filariae. Diethylcarbamazine is contraindicated during pregnancy. See also footnote 42.
37. In heavy infections with *Loa loa*, rapid killing of microfilariae can provoke encephalopathy. Apheresis has been reported to be effective in lowering microfilarial counts in patients heavily infected with *Loa loa* (EA Ottesen, Infect Dis Clin North Am 1993; 7:619). Albendazole may be useful for treatment of loiasis when diethylcarbamazine is ineffective or cannot be used, but repeated courses may be necessary (AD Klion et al, Clin Infect Dis 1999; 29:680; TE Tabi et al, Am J Trop Med Hyg 2004; 71:211). Ivermectin has also been used to reduce microfilaremia, but albendazole is preferred because of its slower onset of action and lower risk of precipitating encephalopathy (AD Klion et al, J Infect Dis 1993; 168:202; M Kombila et al, Am J Trop Med Hyg 1998; 58:458). Diethylcarbamazine, 300 mg PO once/wk, has been recommended for prevention of loiasis (TB Nutman et al, N Engl J Med 1988; 319:752).

Infection	Drug	Adult dosage	Pediatric dosage
Fluke, hermaphroditic, infection			
Clonorchis sinensis (Chinese liver fluke)[43]			
Drug of choice:	Praziquantel[44]	75 mg/kg/d PO in 3 doses × 2 d	75 mg/kg/d PO in 3 doses × 2 d
OR	Albendazole[7,12]	10 mg/kg/d PO × 7 d	10 mg/kg/d PO × 7 d
Fasciola Hepatica (sheep liver fluke)[43]			
Drug of choice:[45]	Triclabendazole*	10 mg/kg PO once or twice	10 mg/kg PO once or twice
Alternative:	Bithionol*	30-50 mg/kg on alternate days × 10-15 doses	30-50 mg/kg on alternate days × 10-15 doses
OR	Nitazoxanide[5,7]	500 mg PO bid × 7 d	1-3 yrs: 100 mg PO bid × 7 d 4-11 yrs: 200 mg PO bid × 7 d >12 yrs: 500 mg PO bid × 7 d
Fasciolopsis buski, Heterophyes heterophyes, Metagonimus yokogawai (intestinal flukes)			
Drug of choice:	Praziquantel[7,44]	75 mg/kg/d PO in 3 doses × 1 d	75 mg/kg/d PO in 3 doses × 1 d
Metorchis conjunctus *(North American liver fluke)*			
Drug of choice:	Praziquantel[7,44]	75 mg/kg/d PO in 3 doses × 1 d	75 mg/kg/d PO in 3 doses × 1 d
Nanophyetus salmincola			
Drug of choice:	Praziquantel[7,44]	60 mg/kg/d PO in 3 doses × 1 d	60 mg/kg/d PO in 3 doses × 1 d
Opisthorchis viverrini *(Southeast Asian liver fluke)*[43]			
Drug of choice:	Praziquantel[44]	75 mg/kg/d PO in 3 doses × 2 d	75 mg/kg/d PO in 3 doses × 2 d
Paragonimiasis (P.westermani, P. miyazaki, P. skrjabini, P. hueitungensis, P. heterotrema, P. utcerobilaterus, P. Africanus, P. Mexicanus, P. Kellicotti,) (lung fluke)			
Drug of choice:	Praziquantel[7,44]	75 mg/kg/d PO in 3 doses × 2 d	75 mg/kg/d PO in 3 doses × 2 d
Alternative:	Triclabendazole[46*]	10 mg/kg PO once or twice	10 mg/kg PO once or twice
	Bithionol*	30-50 mg/kg on alternate days × 10-15 doses	30-50 mg/kg on alternate days × 10-15 doses

38. Diethylcarbamazine has no effect. A single dose of ivermectin 200 mcg/kg PO reduces microfilaria densities and provides both short- and long-term reductions in *M. ozzardi* microfilaremia (AA Gonzalez et al, W Indian Med J 1999; 48:231).
39. One small study compared single-dose ivermectin to albendazole alone or the two together although the combination reduced microfilaremia 1 and 3 months post treatment, the effect was not significant at 6 and 12 months (SM Asio et al, Ann Trop Med Parasitol 2009; 103:31).
40. Diethylcarbamazine is potentially curative due to activity against both adult worms and microfilariae. Ivermectin is active only against microfilariae.
41. VK Vijayan, Curr Opin Pulm Med 2007; 13:428. Relapses occur and can be treated with a repeated course of diethylcarbamazine.
42. Diethylcarbamazine should not be used for treatment of this disease because rapid killing of the worms can lead to blindness. Periodic treatment with ivermectin (every 3-12 months), 150 mcg/kg PO, can prevent blindness due to ocular onchocerciasis (DN Udall, Clin Infect Dis 2007; 44:53). Skin reactions after ivermectin treatment are often reported in persons with high microfilarial skin densities. Ivermectin has been inadvertently given to pregnant women during mass treatment programs; the rates of congenital abnormalities were similar in treated and untreated women. Because of the high risk of blindness from onchocerciasis, the use of ivermectin after the first trimester is considered acceptable according to the WHO. Addition of 6-8 weeks of doxycycline to ivermectin is increasingly common. Doxycycline (100 mg/day PO for 6 weeks), followed by a single 150 mcg/kg PO dose of ivermectin, resulted in up to 19 months of amicrofilaridermia and 100% elimination of *Wolbachia* species (A Hoerauf et al, Lancet 2001; 357:1415).
43. LA Marcos, Curr Opin Infect Dis 2008; 21:523.
44. Praziquantel should be taken with liquids during a meal.
45. Unlike infections with other flukes, *Fasciola hepatica* infections may not respond to praziquantel. Triclabendazole (*Egaten*—Novartis) appears to be safe and effective, but data are limited (J Keiser et al, Expert Opin Investig Drugs 2005; 14:1513). It is available from Victoria Pharmacy, Zurich, Switzerland (www.pharmaworld.com; 011-4143-344-60-60) and should be given with food for better absorption. Nitazoxanide also appears to have efficacy in treating fascioliasis in adults and in children (L Favennec et al, Aliment Pharmacol Ther 2003; 17:265; JF Rossignol et al, Trans R Soc Trop Med Hyg 1998; 92:103; SM Kabil et al, Curr Ther Res 2000; 61:339).
46. J Keiser et al, Expert Opin Investig Drugs 2005; 14:1513. See footnote 45 for availability.

Infection		Drug	Adult dosage	Pediatric dosage
Giardiasis *(Giardia duodenalis)*				
Drug of choice:		Metronidazole[7]	250 mg PO tid × 5-7 d	15 mg/kg/d PO in 3 doses × 5-7 d
	OR	Tinidazole[6]	2 g PO once	≥3 yrs: 50 mg/kg PO once (max. 2 g)
	OR	Nitazoxanide[5]	500 mg PO bid × 3 d	1-3 yrs: 100 mg PO bid × 3 d 4-11 yrs: 200 mg PO bid × 3 d >12 yrs: 500 mg PO bid × 3 d
Alternative:[47]		Paromomycin[3,7,48*]	25-35 mg/kg/d PO in 3 doses × 5-10 d	25-35 mg/kg/d PO in 3 doses × 5-10 d
	OR	Furazolidone[*]	100 mg PO qid × 7-10 d	6 mg/kg/d PO in 4 doses × 7-10 d
	OR	Quinacrine[4,49*]	100 mg PO tid × 5 d	6 mg/kg/d PO in 3 doses × 5 d (max 300 mg/d)
Gnathostomiasis *(Gnathostoma spinigerum)*[50]				
Treatment of choice:		Albendazole[7,12]	400 mg PO bid × 21 d	400 mg PO bid × 21 d
	OR	Ivermectin[7,15]	200 mcg/kg/d PO × 2 d	200 mcg/kg/d PO × 2 d
		either		
	±	Surgical removal		
Gongylonemiasis *(Gongylonema* **sp.)**[51]				
Treatment of choice:		Surgical removal		
	OR	Albendazole[7,12]	400 mg/d PO × 3 d	400 mg/d PO × 3 d
Hookworm infection *(Ancylostoma duodenale, Necator americanus)*				
Drug of choice:		Albendazole[7,12]	400 mg PO once	400 mg PO once
	OR	Mebendazole	100 mg PO bid × 3 d or 500 mg once	100 mg PO bid × 3 d or 500 mg once
	OR	Pyrantel pamoate[7,31*]	11 mg/kg (max. 1 g) PO daily × 3 d	11 mg/kg (max. 1 g) PO daily × 3 d
Hydatid cyst, see TAPEWORM infection				
***Hymenolepis nana,* see TAPEWORM infection**				
***Isospora belli,* see** *Cystoisospora*				
Leishmaniasis				
Visceral[52,53]				
Drug of choice:		Liposomal amphotericin B[54]	3 mg/kg/d IV d 1-5, 14 and 21[55]	3 mg/kg/d IV d 1-5, 14 and 21[55]
	OR	Sodium stibogluconate[*]	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Meglumine antimonate[*]	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Miltefosine[56,57*]	2.5 mg/kg/d PO (max 150 mg/d) × 28 d	2.5 mg/kg/d PO (max 150 mg/d) × 28 d

47. Additional option: albendazole (400 mg/d PO × 5 d in adults and 10 mg/kg/d PO × 5 d in children) (K Yereli et al, Clin Microbiol Infect 2004; 10:527; O Karabay et al, World J Gastroenterol 2004; 10:1215). Refractory disease: standard doses of metronidazole plus quinacrine × 3 wks (TE Nash et al, Clin Infect Dis 2001; 33:22). In one study, nitazoxanide was used successfully in high doses (1.5 g PO bid × 30 d) to treat a case of *Giardia* resistant to metronidazole and albendazole (P Abboud et al, Clin Infect Dis 2001; 32:1792).
48. Poorly absorbed; may be useful for treatment of giardiasis in pregnancy.
49. Quinacrine should be taken with liquids after a meal. It is not available in the US but can be compounded by Gallipot Pharmacy (www.gallipot.com; 800-423-6967).
50. All patients should be treated with medication whether surgery is attempted or not. JS Herman and PL Chiodini, Clin Microbiol Rev 2009; 22:484; L Ramirez-Avila et al, Clin Infect Dis 2009; 48:322.
51. S Pasuralertsakul et al, Am Trop Med Parasitol 2008; 102:455; G Molavi et al, J Helminth 2006; 80:425.
52. To maximize effectiveness and minimize toxicity, the choice of drug, dosage and duration of therapy should be individualized based on the region of disease acquisition, likely infecting species, and host factors such as immune status (BL Herwaldt, Lancet 1999; 354:1191). Some of the listed drugs and regimens are effective only against certain *Leishmania* species/strains and only in certain areas of the world (J Arevalo et al, J Infect Dis 2007; 195:1846). Medical Letter consultants recommend consultation with physicians experienced in management of this disease.
53. Visceral infection is most commonly due to the Old World species *L. donovani* (kala-azar) and *L. infantum* (referred to as *L. chagasi* in the New World).

Infection		Drug	Adult dosage	Pediatric dosage
Alternative:	OR	Amphotericin B[7]	1 mg/kg IV daily × 15-20 d or every second day for up to 8 wks (total usually 15-20 mg/kg)	1 mg/kg IV daily × 15-20 d or every second day for up to 8 wks (total usually 15-20 mg/kg)
	OR	Paromomycin[3,7,58*] sulfate	15 mg/kg/d IM × 21 d	15 mg/kg/d IM × 21 d
Cutaneous[52,59]				
Drugs of choice:		Sodium stibogluconate[*]	20 mg Sb/kg/d IV or IM × 20 d	20 mg Sb/kg/d IV or IM × 20 d
	OR	Meglumine antimonate[*]	20 mg Sb/kg/d IV or IM × 20 d	20 mg Sb/kg/d IV or IM × 20 d
	OR	Miltefosine[56,60*]	2.5 mg/kg/d PO (max 150 mg/d) × 28 d	2.5 mg/kg/d PO (max 150 mg/d) × 28 d
Alternative:[61]		Paromomycin[3,7,58*]	Topically 2x/d × 10-20 d	Topically 2x/d × 10-20 d
	OR	Pentamidine[7]	2-3 mg/kg IV or IM daily or every second day × 4-7 doses[62]	2-3 mg/kg IV or IM daily or every second day × 4-7 doses[62]
Mucosal[52,63]				
Drug of choice:		Sodium stibogluconate[*]	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Meglumine antimonate[*]	20 mg Sb/kg/d IV or IM × 28 d	20 mg Sb/kg/d IV or IM × 28 d
	OR	Amphotericin B[7]	0.5-1 mg/kg IV daily or every second day for up to 8 wks	0.5-1 mg/kg IV daily or every second day for up to 8 wks
	OR	Miltefosine[56,64*]	2.5 mg/kg/d PO (max 150 mg/d) × 28 d	2.5 mg/kg/d PO (max 150 mg/d) × 28 d
Lice infestation *(Pediculus humanus, P. capitis, Phthirus pubis)*[65]				
Drug of choice:		Pyrethrins with piperonyl butoxide[66]	Topically, 2 × at least 7 d apart	Topically, 2 × at least 7 d apart
	OR	1% Permethrin[66]	Topically, 2 × at least 7 d apart	Topically, 2 × at least 7 d apart
	OR	5% Benzyl alcohol lotion[67]	Topically, 2 × at least 7 d apart	Topically, 2 × at least 7 d apart
	OR	0.5% Malathion[68]	Topically, 2 × at least 7 d apart	Topically, 2 × at least 7 d apart

54. Liposomal amphotericin B *(AmBisome)* is the only lipid formulation of amphotericin B FDA-approved for treatment of visceral leishmania, largely based on clinical trials in patients infected with *L. infantum* (A Meyerhoff, Clin Infect Dis 1999; 28:42). In one open-label study one 10 mg/kg dose of liposomal amphotericin B was as effective as 15 infusions of amphotericin B (1 mg/kg/d) on alternate days (S Sundar et al, N Engl J Med 2010; 362:504). It is the drug of choice for visceral leishmania in pregnancy. Two other amphotericin B lipid formulations, amphotericin B lipid complex *(Abelcet)* and amphotericin B cholesteryl sulfate *(Amphotec)* have been used, but are considered investigational for this condition and may not be as effective (C Bern et al, Clin Infect Dis 2006; 43:917).
55. The FDA-approved dosage regimen for immunocompromised patients (e.g., HIV infected) is 4 mg/kg/d IV on days 1-5, 10, 17, 24, 31 and 38. The relapse rate is high; maintenance therapy (secondary prevention) may be indicated, but there is no consensus as to dosage or duration.
56. Miltefosine *(Impavido)* is manufactured in 10- or 50-mg capsules by Paladin (Montreal, Canada) and is not available in the US. The drug is contraindicated in pregnancy; a negative pregnancy test before drug initiation and effective contraception during and for 2 months after treatment is recommended (HW Murray et al, Lancet 2005; 366:1561).
57. Miltefosine is effective for both antimony-sensitive and -resistant *L. donovani* (Indian).
58. Paromomycin IM has been effective against *Leishmania* in India; it has not yet been tested in South America or the Mediterranean and there are insufficient data to support its use in pregnancy (S Sundar et al, N Engl J Med 2007; 356:2571; S Sundar and J Chakravarty, Expert Opin Investig Drugs 2008; 17:787). One study in India used a 14-day course of paromomycin (S Sundar et al, Clin Infect Dis 2009; 49:914). Topical paromomycin should be used only in geographic regions where cutaneous leishmaniasis species have low potential for mucosal spread. A formulation of 15% paromomycin/12% methylbenzethonium chloride *(Leshcutan)* in soft white paraffin for topical use has been reported to be partially effective against cutaneous leishmaniasis due to *L. major* in Israel and *L. mexicana* and *L. (V.) braziliensis* in Guatemala, where mucosal spread is very rare (BA Arana et al, Am J Trop Med Hyg 2001; 65:466; DH Kim et al, PLoS Negl Trop Dis 2009; 3:e381). The methylbenzethonium is irritating to the skin; lesions may worsen before they improve.
59. Cutaneous infection is most commonly due to the Old World species *L. major* and *L. tropica* and the New World species *L. mexicana, L. (Vianna) braziliensis, L. (V.) panamensis* and others.
60. In a placebo-controlled trial in patients ≥12 years old, miltefosine was effective for treatment of cutaneous leishmaniasis due to *L.(V.) panamensis* in Colombia, but not *L.(V.) braziliensis* or *L. mexicana* in Guatemala (J Soto et al, Clin Infect Dis 2004; 38:1266). For forms of disease that require long periods of treatment, such as diffuse cutaneous leishmaniasis and post kala-azar dermal leishmaniasis, miltefosine might be a useful treatment (JJ Berman, Expert Opin Drug Metab Toxicol 2008; 4:1209).

Infection		Drug	Adult dosage	Pediatric dosage
Alternative:		Ivermectin[7,15,69]	200 or 400 mcg/kg PO	≥15 kg: 200 or 400 mcg/kg PO
Loa loa, see FILARIASIS				
Malaria, Treatment of *(Plasmodium falciparum,*[70] *P. vivax,*[71] *P. ovale, P. malariae*[72] and *P. knowlesi*[73]*)*				
Oral (Uncomplicated or mild infection)[74]				
P. falciparum or unidentified species[75] acquired in areas of chloroquine-resistant *P. falciparum*[70]				
Drug of choice:		Atovaquone/ proguanil[76]	4 adult tabs PO once/d or 2 adult tabs PO bid[77] × 3 d	<5 kg: not indicated
				5-8 kg: 2 peds tabs PO once/d × 3 d
				9-10 kg: 3 peds tabs PO once/d × 3 d
				11-20 kg: 1 adult tab PO once/d × 3 d
				21-30 kg: 2 adult tabs PO once/d × 3 d
				31-40 kg: 3 adult tabs PO once/d × 3 d
				>40 kg: 4 adult tabs PO once/d × 3 d[77]
	OR	Artemether/ lumefantrine[78,79]	6 doses over 3 d (4 tabs/ dose at 0, 8, 24, 36, 48 and 60 hours)	6 doses over 3 d at same intervals as adults;
				5-15 kg: 1 tab/dose
				≥15-25 kg: 2 tabs/dose
				≥25-35 kg: 3 tabs/dose
				≥35 kg: 4 tabs/dose
	OR	Quinine sulfate	650 mg PO q8h × 3 **or** 7 d[80]	30 mg/kg/d PO in 3 doses × 3 **or** 7 d[80]
		plus		
		doxycycline[7,20,81]	100 mg PO bid × 7 d	4 mg/kg/d PO in 2 doses × 7 d
		or plus		
		tetracycline[7,20]	250 mg PO qid × 7 d	25 mg/kg/d PO in 4 doses × 7 d
		or plus		
		clindamycin[7,18,82]	20 mg/kg/d PO in 3 doses × 7 d[83]	20 mg/kg/d PO in 3 doses × 7 d[83]

61. Although azole drugs (fluconazole, ketoconazole, itraconazole) have been used to treat cutaneous disease, they are not reliably effective and have very limited if any efficacy against mucosal disease (JA Blum and CS Hatz, J Travel Med 2009; 16:123). For treatment of *L. major* cutaneous lesions, a study in Saudi Arabia found that oral fluconazole, 200 mg once/d × 6 wks appeared to modestly accelerate the healing process (AA Alrajhi et al, N Engl J Med 2002; 346:891). Thermotherapy may be an option for some cases of cutaneous *L. tropica* infection (R Reithinger et al, Clin Infect Dis 2005; 40:1148). A device that generates focused and controlled heating of the skin is being marketed *(ThermoMed* - ThermoSurgery Technologies Inc., Phoenix, AZ, 602-264-7300; www.thermosurgery.com). In one small study after 12 months of followup localized thermal heat was as effective as 10 doses of sodium stibogluconate with less toxicity (NE Aronson et al, PLOS Negl Trop Dis 2010; 4:e628).
62. At this dosage pentamidine has been effective in Colombia predominantly against *L. (V.) panamensis* (J Soto-Mancipe et al, Clin Infect Dis 1993; 16:417; J Soto et al, Am J Trop Med Hyg 1994; 50:107). Activity against other species is not well established.
63. Mucosal infection (espundia) is most commonly due to New World species *L. (V.) braziliensis, L. (V.) panamensis,* or *L. (V.) guyanensis.*
64. Miltefosine has been effective for mucosal leishmania due to *L.(V.) braziliensis* in Bolivia (J Soto et al, Clin Infect Dis 2007; 44:350; J Soto et al, Am J Trop Med Hyg 2009; 81:387).
65. Pediculocides should not be used for infestations of the eyelashes. Such infestations are treated with petrolatum ointment applied 2-4x/d × 8-10 d. Oral TMP/SMX has also been used (TL Meinking and D Taplin, Curr Probl Dermatol 1996; 24:157). For pubic lice, treat with 5% permethrin or ivermectin as for scabies (see page 10). TMP/SMX has also been effective when used together with permethrin for head lice (RB Hipolito et al, Pediatrics 2001; 107:E30).
66. Permethrin and pyrethrin are pediculocidal; retreatment in 7-10 d is needed to eradicate the infestation. Some lice are resistant to pyrethrins and permethrin (TL Meinking et al, Arch Dermatol 2002; 138:220). Medical Letter consultants prefer pyrethrin products with a benzyl alcohol vehicle.
67. FDA-approved to treat head lice in 2009, benzyl alcohol prevents lice from closing their respiratory spiracles and the lotion vehicle then obstructs their airway causing them to asphyxiate. It is not ovicidal.Two applications at least 7 d apart are needed. Resistance, which is a problem with other drugs, is unlikely to develop (Med Lett Drugs Ther 2009; 51:57).
68. Malathion is both ovicidal and pediculocidal; 2 applications at least 7 d apart are generally necessary to kill all lice and nits.
69. Ivermectin is pediculocidal, but not ovicidal; more than one dose is generally necessary to eradicate the infestation (KN Jones and JC English 3rd, Clin Infect Dis 2003; 36:1355). The number of doses and interval between doses has not been established. In one study for treatment of head lice, 2 doses of ivermectin (400 mcg/kg) 7 days apart was more effective than treatment with topical malathion (O Chosidow et al, N Engl J Med 2010; 362:896). In one study for treatment of body lice, 3 doses of ivermectin (12 mg each) administered at 7 d intervals were effective (C Fouault et al, J Infect Dis 2006; 193:474).

Infection		Drug	Adult dosage	Pediatric dosage
Alternative:		Mefloquine[84,85]	750 mg PO followed 12 hrs later by 500 mg	15 mg/kg PO followed 12 hrs later by 10 mg/kg
	OR	Artesunate[78*]	4 mg/kg/d PO × 3 d	4 mg/kg/d PO × 3 d
		plus see footnote 86		
P. vivax acquired in areas of chloroquine-resistant *P. vivax*[71]				
Drug of choice:		Artemether/lumefantrine[78,79]	6 doses over 3 d (4 tabs/dose at 0, 8, 24, 36, 48 and 60 hours)	6 doses over 3 d at same intervals as adults;
				5-15 kg: 1 tab/dose
				≥15-25 kg: 2 tabs/dose
				≥25-35 kg: 3 tabs/dose
				≥35 kg: 4 tabs/dose
	OR	Atovaquone/proguanil[76]	4 adult tabs PO once/d or 2 adult tabs bid[77] × 3 d	<5 kg: not indicated
				5-8 kg: 2 peds tabs PO once/d × 3 d
				9-10 kg: 3 peds tabs PO once/d × 3 d
				11-20 kg: 1 adult tab PO once/d × 3 d
				21-30 kg: 2 adult tabs PO once/d × 3 d
				31-40 kg: 3 adult tabs PO once/d × 3 d
				>40 kg: 4 adult tabs PO once/d × 3 d[77]
	OR	Quinine sulfate	650 mg PO q8h × 3-7 d[80]	30 mg/kg/d PO in 3 doses × 3-7 d[80]
		plus		
		doxycycline[7,20,81]	100 mg PO bid × 7 d	4 mg/kg/d PO in 2 doses × 7 d
	ALL PLUS	primaquine phosphate[75,87]	30 mg base/d PO × 14 d	0.5 mg/kg/d PO × 14 d

70. Chloroquine-resistant *P. falciparum* occurs in all malarious areas except Central America (including Panama north and west of the Canal Zone), Mexico, Haiti, the Dominican Republic, Paraguay, northern Argentina, North and South Korea, Georgia, Armenia, most of rural China and some countries in the Middle East (chloroquine resistance has been reported in Yemen, Saudi Arabia and Iran). For treatment of multiple-drug-resistant *P. falciparum* in Southeast Asia, especially Thailand, where mefloquine resistance is frequent, atovaquone/proguanil, quinine plus either doxycycline or clindamycin, or artemether/lumefantrine may be used.
71. *P. vivax* with decreased susceptibility to chloroquine is a significant problem in Papua-New Guinea and Indonesia. There are also reports of resistance from Myanmar, Vietnam, Korea, India, the Solomon Islands, Vanuatu, Indonesia, Guyana, Brazil, Colombia and Peru (JK Baird, Clin Microbiol Rev 2009; 22:508).
72. Chloroquine-resistant *P. malariae* has been reported from Sumatra (JD Maguire et al, Lancet 2002; 360:58).
73. Human infection with the simian species, *P. knowlesi* has been reported in Malaysia where it was initially misdiagnosed as *P. malariae*. Additional cases have been reported from Thailand, Myanmar, Singapore, the Thai-Burma border, and the Philippines (J Cox-Singh et al, Clin Infect Dis 2008; 46:165; MMWR 2009; 58:229). Treatment with the usual antimalarials, such as chloroquine and atovaquone/proguanil appear to be effective.
74. Uncomplicated or mild malaria may be treated with oral drugs. Severe malaria (e.g. impaired consciousness, parasitemia >5%, shock, etc.) should be treated with parenteral drugs (KS Griffin et al, JAMA 2007; 297:2264).
75. Primaquine is given as part of primary treatment to prevent relapse after infection with *P. vivax* or *P. ovale*. Some experts also prescribe primaquine phosphate 30 mg base/d (0.6 mg base/kg/d for children) for 14 d after departure from areas where these species are endemic (Presumptive Anti-Relapse Therapy [PART], "terminal prophylaxis"). Since this is not always effective as prophylaxis (E Schwartz et al, N Engl J Med 2003; 349:1510), others prefer to rely on surveillance to detect cases when they occur, particularly when exposure was limited or doubtful. See also footnote 87.
76. Atovaquone/proguanil is available as a fixed-dose combination tablet: adult tablets (*Malarone*; atovaquone 250 mg/proguanil 100 mg) and pediatric tablets (*Malarone Pediatric*; atovaquone 62.5 mg/proguanil 25 mg). To enhance absorption and reduce nausea and vomiting, it should be taken with food or a milky drink. Safety in pregnancy is unknown; in a few small studies; outcomes were normal in women treated with the combination in the 2nd and 3rd trimester (AK Boggild et al, Am J Trop Med Hyg 2007; 76:208). The drug should not be given to patients with severe renal impairment (creatinine clearance <30 mL/min). There have been isolated case reports of resistance in *P. falciparum* in Africa, but Medical Letter consultants do not believe there is a high risk for acquisition of *Malarone*-resistant disease (E Schwartz et al, Clin Infect Dis 2003; 37:450; A Farnert et al, BMJ 2003; 326:628; S Kuhn et al, Am J Trop Med Hyg 2005; 72:407; CT Happi et al, Malaria Journal 2006; 5:82).
77. Although approved for once-daily dosing, Medical Letter consultants usually divide the dose in two to decrease nausea and vomiting.

Infection		Drug	Adult dosage	Pediatric dosage
Alternative:		Mefloquine[84]	750 mg PO followed 12 hrs later by 500 mg	15 mg/kg PO followed 12 hrs later by 10 mg/kg
		Chloroquine phosphate[88,89]	25 mg base/kg PO in 3 doses over 48 hrs	25 mg base/kg PO in 3 doses over 48 hrs
		plus		
		doxycycline[7,20,81]	100 mg PO bid × 7 d	4 mg/kg/d PO in 2 doses × 7 d
	ALL PLUS	primaquine phosphate[75,87]	30 mg base/d PO × 14 d	0.5 mg/kg/d PO × 14 d
All *Plasmodium* species except chloroquine-resistant *P. falciparum*[70] and chloroquine-resistant *P. vivax*[71]				
Drug of choice:[75]		Chloroquine phosphate[88]	1 g (600 mg base) PO, then 500 mg (300 mg base) 6 hrs later, then 500 mg (300 mg base) at 24 and 48 hrs	10 mg base/kg (max. 600 mg base) PO, then 5 mg base/kg 6 hrs later, then 5 mg base/kg at 24 and 48 hrs
Parenteral (Severe infection)[74]				
All *Plasmodium* species (Chloroquine-sensitive and resistant)				
Drug of choice:[75,90]		Quinidine gluconate[91]	10 mg/kg IV loading dose (max. 600 mg) in normal saline over 1-2 hrs, followed by continuous infusion of 0.02 mg/kg/min until PO therapy can be started	10 mg/kg IV loading dose (max. 600 mg) in normal saline over 1-2 hrs, followed by continuous infusion of 0.02 mg/kg/min until PO therapy can be started
	OR	Quinine dihydrochloride[91*]	20 mg/kg IV loading dose in 5% dextrose over 4 hrs, followed by 10 mg/kg over 2-4 hrs q8h (max. 1800 mg/d) until PO therapy can be started	20 mg/kg IV loading dose in 5% dextrose over 4 hrs, followed by 10 mg/kg over 2-4 hrs q8h (max. 1800 mg/d) until PO therapy can be started
	OR	Artesunate[78*]	2.4 mg/kg/dose IV × 3 d at 0, 12, 24, 48 and 72 hrs	2.4 mg/kg/dose IV × 3 d at 0, 12, 24, 48 and 72 hrs
		plus see footnote 86		

78. The artemisinin-derivatives, artemether and artesunate, are both frequently used globally in combination regimens to treat malaria. Both are available in oral, parenteral and rectal formulations, but manufacturing standards are not consistent (HA Karunajeewa et al, JAMA 2007; 297:2381; EA Ashley and NJ White, Curr Opin Infect Dis 2005; 18:531). Oral artesunate is not available in the US; the IV formulation is available through the CDC Malaria branch (M-F, 8am-4:30pm ET, 770-488-7788, or after hours, 770-488-7100) under an IND for patients with severe disease who do not have timely access, cannot tolerate, or fail to respond to IV quinidine (Med Lett DrugsTher 2008; 50:37).To avoid development of resistance, monotherapy should be avoided (PE Duffy and CH Sibley, Lancet 2005; 366:1908). Reduced susceptibility to artesunate characterized by slow parasitic clearance has been reported in Cambodia (WO Rogers et al, Malaria J 2009; 8:10; AM Dundorp et al, N Engl J Med 2009; 361:455). Based on the few studies available, artemesin have been relatively safe during pregnancy (I Adam et al, Am Trop Med Parisito 2009; 103; 205), but some experts would not prescribe them in the 1st trimester (RL Clark, Reprod Toxicol 2009; 28:285).
79. Artemether/lumefantrine is available as a fixed-dose combination tablet (*Coartem* in the US and in countries with endemic malaria, *Riamet* in Europe and countries without endemic malaria); each tablet contains artemether 20 mg and lumefantrine 120 mg. It is FDA-approved for treatment of uncomplicated malaria and should not be used for severe infection or for prophylaxis. It is contraindicated during the 1st trimester of pregnancy; safety during the 2nd and 3rd trimester is not known. The tablets should be taken with fatty food (tablets may be crushed and mixed with 1-2 tsp water, and taken with milk). Artemether/lumefantrine should not be used in patients with cardiac arrhythmias, bradycardia, severe cardiac disease or QT prolongation. Concomitant use of drugs that prolong the QT interval or are metabolized by CYP2D6 is contraindicated (Med Lett Drugs Ther 2009; 51:75).
80. Available in the US in a 324-mg capsule; 2 capsules suffice for adult dosage. In Southeast Asia, relative resistance to quinine has increased and treatment should be continued for 7 d. Quinine should be taken with or after meals to decrease gastrointestinal adverse effects. It is generally considered safe in pregnancy.
81. Doxycycline should be taken with adequate water to avoid esophageal irritation. It can be taken with food to minimize gastrointestinal adverse effects.
82. For use in pregnancy and in children <8 yrs.
83. B Lell and PG Kremsner, Antimicrob Agents Chemother 2002; 46:2315; M Ramharter et al, Clin Infect Dis 2005; 40:1777.
84. At this dosage, adverse effects include nausea, vomiting, diarrhea and dizziness. Disturbed sense of balance, toxic psychosis and seizures can also occur. Mefloquine should not be used for treatment of malaria in pregnancy unless there is not another treatment option (F Nosten et al, Curr Drug Saf 2006; 1:1). It should be avoided for treatment of malaria in persons with active depression or with a history of psychosis or seizures and should be used with caution in persons with any psychiatric illness. Mefloquine should not be used in patients with conduction abnormalities; it can be given to patients taking β-blockers if they do not have an underlying arrhythmia. Mefloquine should not be given together with quinine or quinidine, and caution is required in using quinine or quinidine to treat patients with malaria who have taken mefloquine for prophylaxis. Mefloquine should not be taken on an empty stomach; it should be taken with at least 8 oz of water.

Infection		Drug	Adult dosage	Pediatric dosage
Malaria, prevention of[92]				
All *Plasmodium* species in chloroquine-resistant areas[70-73]				
Drug of choice:[75]		Atovaquone/ proguanil[76]	1 adult tab/d[93]	5-8 kg: ½ peds tab/d[76,93]
				9-10 kg: ¾ peds tab/d[76,93]
				11-20 kg: 1 peds tab/d[76,93]
				21-30 kg: 2 peds tabs/ d[76,93]
				31-40 kg: 3 peds tabs/ d[76,93]
				>40kg: 1 adult tab/d[76,93]
	OR	OR Doxycycline[20,81]	100 mg PO daily[94]	2 mg/kg/d PO, up to 100 mg/d[94]
	OR	Mefloquine[85,95]	250 mg PO once/wk[96]	≤9 kg: 5 mg/kg salt once/ wk[96]
				9-19 kg: ¼ tab once/wk[96]
				>19-30 kg: ½ tab once/ wk[96]
				>31-45 kg: ¾ tab once/ wk[96]
				>45 kg: 1 tab once/wk[96]
Alternative:[97]		Primaquine[7,87] phosphate	30 mg base PO daily[98]	0.5 mg/kg base PO daily[98]
All Plasmodium species in chloroquine-sensitive areas[70-73]				
Drug of choice:[75,99]		Chloroquine phosphate[88,100]	500 mg (300 mg base) PO once/wk[101]	5 mg/kg base PO once/ wk, up to adult dose of 300 mg base[101]

85. *P. falciparum* with resistance to mefloquine is a significant problem in the malarious areas of Thailand and in areas of Myanmar and Cambodia that border on Thailand. It has also been reported on the borders between Myanmar and China, Laos and Myanmar, and in Southern Vietnam. In the US, a 250-mg tablet of mefloquine contains 228 mg mefloquine base. Outside the US, each 275-mg tablet contains 250 mg base.

86. Adults treated with artesunate should also receive oral treatment doses of either atovaquone/proguanil, doxycycline, clindamycin or mefloquine; children should take either atovaquone/proguanil, clindamycin or mefloquine (F Nosten et al, Lancet 2000; 356:297; M van Vugt, Clin Infect Dis 2002; 35:1498; F Smithuis et al, Trans R Soc Trop Med Hyg 2004; 98:182). If artesunate is given IV, oral medication should be started when the patient is able to tolerate it (SEAQUAMAT group, Lancet 2005; 366:717).

87. Primaquine phosphate can cause hemolytic anemia, especially in patients whose red cells are deficient in G-6-PD. This deficiency is most common in African, Asian and Mediterranean peoples. Patients should be screened for G-6-PD deficiency before treatment. Primaquine should not be used during pregnancy. It should be taken with food to minimize nausea and abdominal pain. Primaquine-tolerant *P. vivax* can be found globally. Relapses of primaquine-resistant strains may be retreated with 30 mg (base) × 28 d.

88. Chloroquine should be taken with food to decrease gastrointestinal adverse effects. If chloroquine phosphate is not available, hydroxychloroquine sulfate is as effective; 400 mg of hydroxychloroquine sulfate is equivalent to 500 mg of chloroquine phosphate.

89. Chloroquine combined with primaquine was effective in 85% of patients with *P. vivax* resistant to chloroquine and could be a reasonable choice in areas where other alternatives are not available (JK Baird et al, J Infect Dis 1995; 171:1678).

90. Exchange transfusion is controversial, but has been helpful for some patients with high-density (>10%) parasitemia, altered mental status, pulmonary edema or renal complications (PJ Van Genderen et al, Transfusion 2009; Nov 20 epub).

91. Continuous EKG, blood pressure and glucose monitoring are recommended. Quinine IV is not available in the US. Quinidine may have greater antimalarial activity than quinine. The loading dose should be decreased or omitted in patients who have received quinine or mefloquine. If more than 48 hours of parenteral treatment is required, the quinine or quinidine dose should be reduced by 30-50%. Intrarectal quinine has been tried for the treatment of cerebral malaria in children (J Achan et al, Clin Infect Dis 2007; 45:1446).

92. No drug guarantees protection against malaria. Travelers should be advised to seek medical attention if fever develops after they return. Insect repellents, insecticide-impregnated bed nets and proper clothing are important adjuncts for malaria prophylaxis (Treat Guidel Med Lett 2009; 7:83). Malaria in pregnancy is particularly serious for both mother and fetus; prophylaxis is indicated if exposure cannot be avoided.

93. Beginning 1-2 d before travel and continuing for the duration of stay and for 1 wk after leaving malarious zone. In one study of malaria prophylaxis, atovaquone/proguanil was better tolerated than mefloquine in nonimmune travelers (D Overbosch et al, Clin Infect Dis 2001; 33:1015). The protective efficacy of *Malarone* against *P. vivax* is variable ranging from 84% in Indonesian New Guinea (J Ling et al, Clin Infect Dis 2002; 35:825) to 100% in Colombia (J Soto et al, Am J Trop Med Hyg 2006; 75:430). Some Medical Letter consultants prefer alternate drugs if traveling to areas where *P. vivax* predominates.

Infection		Drug	Adult dosage	Pediatric dosage
Malaria, Prevention of relapses: *P. vivax* and *P. ovale*[75]				
Drug of choice:		Primaquine phosphate[87]	30 mg base/d PO × 14 d	0.5 mg base/kg/d PO × 14 d
Malaria, Self-Presumptive Treatment[102]				
Drug of Choice:		Atovaquone/ proguanil[7,76]	4 adult tabs once/d or 2 adult tabs bid × 3 d[77]	<5 kg: not indicated 5-8 kg: 2 peds tabs once/d × 3 d 9-10 kg: 3 peds tabs once/d × 3 d 11-20 kg: 1 adult tab once/d × 3 d 21-30 kg: 2 adult tabs once/d × 3 d 31-40 kg: 3 adult tabs once/d × 3 d >40 kg: 4 adult tabs once/d × 3 d[77]
	OR	Artemether/ lumefantrine[7,78,79]	6 doses over 3 d (4 tabs/ dose at 0, 8, 24, 36, 48 and 60 hours)	6 doses over 3 d at same intervals as adults; 5-15 kg: 1 tab/dose 15-25 kg: 2 tabs/dose 25-35 kg: 3 tabs/dose >35 kg: 4 tabs/dose
	OR	Quinine sulfate	650 mg PO q8h × 3 or 7 d[70]	30 mg/kg/d PO in 3 doses × 3 or 7 d[70]
		plus		
		doxycycline[7,20,81]	100 mg PO bid × 7 d	4 mg/kg/d PO in 2 doses × 7 d
	OR	Artesunate[78*]	4 mg/kg/d PO × 3 d	4 mg/kg/d PO ×3 d
		plus see footnote 86		
Microsporidiosis				
Ocular *(Encephalitozoon hellem, E. cuniculi, Vittaforma [Nosema] corneae)*				
Drug of choice:		Fumagillin[103*]		
		plus		
		albendazole[7,12]	400 mg PO bid	15 mg/kg/d in 2 doses (max 400 mg/dose)

94. Beginning 1-2 d before travel and continuing for the duration of stay and for 4 wks after leaving malarious zone. Doxycycline can cause gastrointestinal disturbances, vaginal moniliasis and photosensitivity reactions.
95. Mefloquine has not been approved for use during pregnancy. However, it has been reported to be safe for prophylactic use during the second and third trimester of pregnancy and possibly during early pregnancy as well (CDC Health Information for International Travel, 2010, page 141). Not recommended for use in travelers with active depression or with a history of psychosis or seizures and should be used with caution in persons with psychiatric illness. Mefloquine should not be used in patients with conduction abnormalities; it can be given to patients taking β-blockers if they do not have an underlying arrhythmia.
96. Beginning 1-2 wks before travel and continuing weekly for the duration of stay and for 4 wks after leaving malarious zone. Most adverse events occur within 3 doses. Some Medical Letter consultants favor starting mefloquine 3 weeks prior to travel and monitoring the patient for adverse events, this allows time to change to an alternative regimen if mefloquine is not tolerated. Mefloquine should not be taken on an empty stomach; it should be taken with at least 8 oz of water. For pediatric doses <½ tablet, it is advisable to have a pharmacist crush the tablet, estimate doses by weighing, and package them in gelatin capsules.There is no data for use in children <5 kg, but based on dosages in other weight groups, a dose of 5 mg/kg can be used.
97. The combination of weekly chloroquine (300 mg base) and daily proguanil (200 mg) is recommended by the World Health Organization (www.WHO.int) for use in selected areas; this combination is no longer recommended by the CDC. Proguanil (*Paludrine* – AstraZeneca, United Kingdom) is not available alone in the US but is widely available in Canada and Europe. Prophylaxis is recommended during exposure and for 4 weeks afterwards. Proguanil has been used in pregnancy without evidence of toxicity (PA Phillips-Howard and D Wood, Drug Saf 1996; 14:131).
98. Studies have shown that daily primaquine beginning 1 d before departure and continued until 3-7 d after leaving the malarious area provides effective prophylaxis against chloroquine-resistant *P. falciparum* (DR Hill et al, Am J Trop Med Hyg 2006; 75:402). Nausea and abdominal pain can be diminished by taking with food.
99. Alternatives for patients who are unable to take chloroquine include atovaquone/proguanil, mefloquine, doxycycline or primaquine dosed as for chloroquine-resistant areas.

Infection	Drug	Adult dosage	Pediatric dosage
Intestinal *(E. bieneusi, E. [Septata] intestinalis)*			
E. bieneusi			
Drug of choice:	Fumagillin[104*]	20 mg PO tid × 14 d	
E. intestinalis			
Drug of choice:	Albendazole[7,12]	400 mg PO bid × 21 d	15 mg/kg/d in 2 doses (max 400 mg/dose)
Disseminated *(E. hellem, E. cuniculi, E. intestinalis, Pleistophora sp., Trachipleistophora sp.* and Anncaliia *[Brachiola] vesicularum)*			
Drug of choice:[105]	Albendazole[7,12]	400 mg PO bid	15 mg/kg/d in 2 doses (max 400 mg/dose)
Mites, see SCABIES			
Moniliformis *moniliformis* infection			
Drug of choice:	Pyrantel pamoate[7,31*]	11 mg/kg PO once, repeat twice, 2 wks apart	11 mg/kg PO once, repeat twice, 2 wks apart
Naegleria **species,** see AMEBIC MENINGOENCEPHALITIS, PRIMARY			
Necator americanus, see HOOKWORM infection			
Oesophagostomum *bifurcum*			
Drug of choice:	See footnote 106		
Onchocerca volvulus, see FILARIASIS			
Opisthorchis viverrini, see FLUKE infection			
Paragonimus westermani, see FLUKE infection			
Pediculus capitis, humanus, Phthirus pubis, see LICE			
Pinworm, see ENTEROBIUS			
Pneumocystis Jirovecii (formerly *carinii*) pneumonia (PCP)[107]			
Moderate to Severe Disease[108]			
Drug of choice:	Trimethoprim/ sulfamethoxazole	TMP 15-20 mg/kg/d SMX 75-100 mg/kg/d PO or IV in 3 or 4 doses (change to PO after clinical improvement) × 21 d	TMP 15-20 mg/kg/d SMX 75-100 mg/kg/d PO or IV in 3 or 4 doses (change to PO after clinical improvement) × 21 d

100. Has been used extensively and safely for prophylaxis in pregnancy.
101. Beginning 1-2 wks before travel and continuing weekly for the duration of stay and for 4 wks after leaving malarious zone.
102. A traveler can be given a course of medication for presumptive self-treatment of febrile illness. The drug given for self-treatment should be different from that used for prophylaxis. This approach should be used only in very rare circumstances when a traveler would not be able to get medical care promptly.
103. CM Chan et al, Ophthalmology 2003; 110:1420. Ocular lesions due to *E. hellem* in HIV-infected patients have responded to fumagillin eyedrops prepared from *Fumidil-B* (bicyclohexyl ammonium fumagillin) used to control a microsporidial disease of honey bees (MJ Garvey et al, Ann Pharmacother 1995; 29:872), available from Leiter's Park Avenue Pharmacy (see footnote 1). For lesions due to *V. corneae*, topical therapy is generally not effective and keratoplasty may be required (RM Davis et al, Ophthalmology 1990; 97:953).
104. Oral fumagillin (*Flisint* – Sanofi-Aventis, France) has been effective in treating *E.* bieneusi in patients with HIV or solid organ transplants (J-M Molina et al, N Engl J Med 2002; 346:1963; F Lanternier et al, Transpl Infect Dis 2009; 11:83), but has been associated with thrombocytopenia and neutropenia. Potent anti retroviral therapy (ART) may lead to microbiologic and clinical response in HIV-infected patients with microsporidial diarrhea. Octreotide *(Sandostatin)* has provided symptomatic relief in some patients with large-volume diarrhea.
105. J-M Molina et al, J Infect Dis 1995; 171:245. There is no established treatment for *Pleistophora*. For disseminated disease due to *Trachipleistophora* or *Anncaliia*, itraconazole 400 mg PO once/d plus albendazole may also be tried (CM Coyle et al, N Engl J Med 2004; 351:42).
106. Albendazole or pyrantel pamoate may be effective (JB Ziem et al, Ann Trop Med Parasitol 2004; 98:385).

Infection		Drug	Adult dosage	Pediatric dosage
Alternative:		Pentamidine	3-4 mg/kg IV daily × 21 d	3-4 mg/kg IV daily × 21 d
	OR	Primaquine[7,87]	30 mg base PO daily × 21 d	0.3 mg/kg base PO (max. 30 mg) daily × 21 d
		plus		
		clindamycin[7,18]	600-900 mg IV tid or qid × 21 d, or 300-450 mg PO tid or qid × 21 d (change to PO after clinical improvement)	15-25 mg/kg IV tid or qid (max 600 mg/dose) × 21 d, or 10 mg/kg PO tid or qid (max 300-450 mg/dose) × 21 d (change to PO after clinical improvement)
Mild to Moderate Disease				
Drug of Choice:		Trimethoprim/ sulfamethoxazole	2 DS tablets (160 mg/800 mg) PO tid × 21 d	TMP 15-20 mg/kg/SMX 75-100 mg/kg/d PO in 3 or 4 doses × 21 d
Alternative:		Dapsone[7]	100 mg PO daily × 21 d	2 mg/kg/d (max. 100 mg) PO × 21 d
		plus		
		Trimethoprim[7]	15 mg/kg/d PO in 3 doses	15 mg/kg/d PO in 3 doses
	OR	Primaquine[7,87]	30 mg base PO daily × 21 d	0.3 mg/kg base PO daily (max. 30 mg) × 21 d
		plus		
		clindamycin[7,18]	300-450 mg PO tid or qid × 21 d	10 mg/kg PO tid or qid (max 300-450 mg/dose) × 21 d
	OR	Atovaquone17	750 mg PO bid × 21 d	1-3 mos: 30 mg/kg/d PO × 21 d 4-24 mos: 45 mg/kg/d PO × 21 d >24 mos: 30 mg/kg/d PO × 21 d
Primary and Secondary Prophylaxis[109]				
Drug of Choice:		Trimethoprim/ sulfamethox-azole	1 tab (SS or DS) daily or 1 DS tab PO 3 d/wk	TMP 150 mg/SMX 750 mg/m^2/d PO in 2 doses 3 d/wk
Alternative:		Dapsone[7]	50 mg PO bid or 100 mg PO daily	> 1 mos: 2 mg/kg/d (max. 100 mg) PO or 4 mg/kg (max. 200 mg) PO each wk
	OR	Dapsone[7]	50 mg PO daily or 200 mg PO each wk	
		plus pyrimeth-amine[110]	50 mg PO daily or 75 mg PO each wk	
	OR	Atovaquone[7,17]	1500 mg/d PO in 1 or 2 doses	1-3 mos: 30 mg/kg/d PO 4-24 mos: 45 mg/kg/d PO >24 mos: 30 mg/kg/d PO
	OR	Pentamidine	300 mg aerosol inhaled monthly via *Respirgard II* nebulizer	≥5 yrs: 300 mg inhaled monthly via *Respirgard II* nebulizer

107. Pneumocystis has been reclassified as a fungus.
108. In severe disease with room air $PO_2 \leq 70$ mmHg or Aa gradient ≥ 35 mmHg, prednisone or its IV equivalent should also be used. For adults: d 1-5: 40 mg PO bid; d 6-10: 40 mg PO daily; d 11-21: 20 mg PO daily. For children: d 1-5: 2 mg/kg/d PO in 2 doses; d 6-10: 1 mg/kg/d PO in 2 doses; d 11-21: 0.5 mg/kg/d PO daily (JE Kaplan et al, Morbid Mortal Wkly Rep 2009; 58(RR04):1; Morbid Mortal Wkly Rep 2009; 58(RR11):1).
109. Primary/secondary prophylaxis in patients with HIV can be discontinued after CD4 count increases to >200 × 10^6/L for >3 mos.
110. Plus leucovorin 25 mg with each dose of pyrimethamine. Pyrimethamine should be taken with food to minimize gastrointestinal adverse effects.

Infection	Drug	Adult dosage	Pediatric dosage
River Blindness, see FILARIASIS			
Roundworm, see ASCARIASIS			
Sappinia Diploidea, See AMEBIC MENINGOENCEPHALITIS, PRIMARY			
Sarcocystis spp. (intestinal and muscular), see footnote 111			
Scabies *(Sarcoptes scabiei)*[112]			
Drug of choice:	5% Permethrin	Topically, 2× at least 7 d apart	Topically, 2× at least 7 d apart
Alternative:[113]	Ivermectin[7,15]	200 mcg/kg PO 2× at least 7 d apart[114]	200 mcg/kg PO, 2× at least 7 d apart[114]
	10% Crotamiton	Topically overnight on days 1, 2, 3, 8	Topically overnight on days 1, 2, 3, 8
Schistosomiasis *(Bilharziasis)*			
S. haematobium			
Drug of choice:	Praziquantel[44,115]	40 mg/kg/d PO in 1 or 2 doses × 1 d	40 mg/kg/d PO in 2 doses × 1 d
S. intercalatum[116]			
Drug of Choice:	Praziquantel[44,115]	40 mg/kg/d PO in 1 or 2 doses × 1 d	40 mg/kg/d PO × 1 d
S. japonicum			
Drug of choice:	Praziquantel[44,115]	60 mg/kg/d PO in 2 or 3 doses × 1 d	60 mg/kg/d PO in 3 doses × 1 d
S. mansoni			
Drug of choice:	Praziquantel[44,115]	40 mg/kg/d PO in 1 or 2 doses × 1 d	40 mg/kg/d PO in 2 doses × 1 d
Alternative:	Oxamniquine[117*]	15 mg/kg PO once[118]	20 mg/kg/d PO in 2 doses × 1 d[118]
S. mekongi			
Drug of choice:	Praziquantel[44,115]	60 mg/kg/d PO in 2 or 3 doses × 1 d	60 mg/kg/d PO in 3 doses × 1 d
Sleeping Sickness, see TRYPANOSOMIASIS			
Strongyloidiasis *(Strongyloides stercoralis)*			
Drug of choice:[119]	Ivermectin[15]	200 mcg/kg/d PO × 2 d	200 mcg/kg/d PO × 2 d
Alternative:	Albendazole[7,12]	400 mg PO bid × 7 d	400 mg PO bid × 7 d
Tapeworm infection — ADULT (intestinal stage) *DIPHYLLOBOTHRIUM LATUM* (FISH), *TAENIA SAGINATA* (BEEF), *TAENIA SOLIUM* (PORK), *DIPYLIDIUM CANINUM* (DOG)			
Drug of choice:	Praziquantel[7,44]	5-10 mg/kg PO once	5-10 mg/kg PO once
Alternative:	Niclosamide[120*]	2 g PO once	50 mg/kg PO once

111. Sarcocystis in humans is acquired by ingesting sporocysts in infected meat, infections characterized by nausea, abdominal pain and diarrhea. Muscular infections are usually mild or subclinical (R Fayer, Clin Microbiol Rev 2004; 17:894). Albendazole was reported to be efficacious (MK Arness et al, Am J Trop Med Hyg 1999; 61:548).
112. TL Meinking et al, Infestations in LA Schachner and RA Hansen, eds. *Pediatric Dermatology*. 3rd ed. St Louis: Mosby; 2003, page 1291.
113. Lindane (γ-benzene hexachloride) should be reserved for treatment of patients who fail to respond to other drugs. The FDA has recommended it not be used for immunocompromised patients, young children, the elderly, pregnant and breast-feeding women, and patients weighing <50 kg.
114. BJ Currie and JS McCarthy, N Engl J Med 2010; 362:717. A second ivermectin dose taken 2 weeks later increased the cure rate to 95%, which is equivalent to that of 5% permethrin (V Usha et al, J Am Acad Dermatol 2000; 42:236). Ivermectin, either alone or in combination with a topical scabicide, is the drug of choice for crusted scabies in immunocompromised patients (P del Giudice, Curr Opin Infect Dis 2004; 15:123).
115. MJ Doenhoff et al, Curr Opin Infect Dis 2008; 21:659.
116. Geographically restricted to Central Western Africa and the island of São Tomé. Usually a disease of the lower GI tract; there are also case reports of complications including central nervous system, liver and cardiopulmonary involvement (A Murinello et al, GE—J Port Gastrenterol 2006; 13:97).

Infection	Drug	Adult dosage	Pediatric dosage
***HYMENOLEPIS NANA* (DWARF TAPEWORM)**			
Drug of choice:	Praziquantel[7,44]	25 mg/kg PO once	25 mg/kg PO once
Alternative:[121]	Niclosamide[120*]	2 g PO daily × 7 d	11-34 kg: 1 g PO on d 1 then 500 mg/d PO × 6 days > 34 kg: 1.5 g PO on d 1 then 1 g/d PO × 6 days
— LARVAL (tissue stage)			
***ECHINOCOCCUS GRANULOSUS* (hydatid cyst)**			
Drug of choice:[122]	Albendazole[12]	400 mg PO bid × 1-6 mos	15 mg/kg/d (max. 800 mg) PO in 2 doses × 1-6 mos
ECHINOCOCCUS MULTILOCULARIS			
Treatment of choice:	See footnote 123		
Taenia solium (Cysticercosis)			
Treatment of choice:	See footnote 124		
Alternative:	Albendazole[12]	400 mg PO bid × 8-30 d; can be repeated as necessary	15 mg/kg/d (max. 800 mg) PO in 2 doses × 8-30 d; can be repeated as necessary
OR	Praziquantel[7,44]	100 mg/kg/d PO in 3 doses × 1 day then 50 mg/kg/d in 3 doses × 29 days	100 mg/kg/d PO in 3 doses × 1 day then 50 mg/kg/d in 3 doses × 29 days
Toxocariasis, see VISCERAL LARVA MIGRANS			
Toxoplasmosis *(Toxoplasma gondii)*			
***CNS Disease*[125]**			
Drug of choice:	Pyrimethamine[126]	200 mg PO × 1 then 50-75 mg/d PO × 3-6 wks	2 mg/kg/d PO × 2 d, then 1 mg/kg/d (max. 25 mg/d) × 3-6 wks
	plus		
	sulfadiazine[127]	1-1.5 g PO qid × 3-6 wks	100-200 mg/kg/d PO × 3-6 wks
OR	**plus**		

117. Oxamniquine, which is not available in the US, is generally not as effective as praziquantel. It has been useful, however, in some areas in which praziquantel is less effective (ML Ferrari et al, Bull World Health Organ 2003; 81:190; A Harder, Parasitol Res 2002; 88:395). Oxamniquine is contraindicated in pregnancy. It should be taken after food.
118. In East Africa, the dose should be increased to 30 mg/kg PO, and in Egypt and South Africa to 30 mg/kg/d PO × 2 d. Some experts recommend 40-60 mg/kg PO over 2-3 d in all of Africa (KC Shekhar, Drugs 1991; 42:379).
119. In immunocompromised patients or disseminated disease, it may be necessary to prolong or repeat therapy, or to use other agents. Veterinary parenteral and enema formulations of ivermectin have been used in severely ill patients with hyperinfection who were unable to take or reliably absorb oral medications (FM Marty et al, Clin Infect Dis 2005; 41:e5; P Lichtenberger et al, Transpl Infect Dis 2009; 11:137). In disseminated strongyloidiasis, combination therapy with albendazole and ivermectin has been suggested (M Seqarra, Ann Pharmacother 2007; 41:1992).
120. Niclosamide must be thoroughly chewed or crushed and swallowed with a small amount of water.
121. Nitazoxanide may be an alternative (JJ Ortiz et al, Trans R Soc Trop Med Hyg 2002; 96:193; JC Chero et al, Trans R Soc Trop Med Hyg 2007; 101:203; E Diaz et al, Am J Trop Med Hyg 2003; 68:384).
122. Patients may benefit from surgical resection or percutaneous drainage of cysts. Praziquantel is useful preoperatively or in case of spillage of cyst contents during surgery. Percutaneous aspiration-injection-reaspiration (PAIR) with ultrasound guidance plus albendazole therapy has been effective for management of hepatic hydatid cyst disease (P Moro and PM Schantz, Int J Infect Dis 2009; 13:125.).
123. Surgical excision is the only reliable means of cure. Reports have suggested that in nonresectable cases use of albendazole (400 mg bid) can stabilize and sometimes cure infection (P Moro and PM Schantz, Int J Infect Dis 2009; 13:125).
124. Advances in neuroimaging using CT and MRI have facilitated the ability to make an accurate diagnosis (AC White Jr, J Infect Dis 2009; 199:1261). Initial therapy for patients with inflamed parenchymal cysticercosis should focus on symptomatic treatment with anti-seizure medication (S Sinha and BS Sharma, J Clin Neurosci 2009; 16:867). Patients with live parenchymal cysts who have seizures should be treated with albendazole together with steroids and an anti-seizure medication (HH Garcia et al, N Engl J Med 2004; 350:249). Patients with subarachnoid cysts or giant cysts in the fissures should be treated for at least 30 d (JV Proaño et al, N Engl J Med 2001; 345:879). Surgical intervention (especially neuroendoscopic removal) or CSF diversion followed by albendazole and steroids is indicated for obstructive hydocephalus. Arachnoiditis, vasculitis or cerebral edema is treated with albendazole or praziquantel plus prednisone (60 mg/d) or dexamethasone (4-6 mg/d). Any cysticercocidal drug may cause irreparable damage when used to treat ocular or spinal cysts, even when corticosteroids are used. An ophthalmic exam should always precede treatment to rule out intraocular cysts.

Infection		Drug	Adult dosage	Pediatric dosage
		clindamycin[7,128]	1.8-2.4 g/d IV or PO in 3 or 4 doses	5-7.5 mg/kg/d IV or PO in 3 or 4 doses (max 600 mg/dose)
	OR	**plus**		
		atovaquone[7,17,128]	1500 mg PO bid	1500 mg PO bid
Alternative:		Trimethoprim/ sulfamethoxazole[7]	15-20 mg/kg/SMX 75-100 mg/kg/d PO or IV in 3 or 4 doses	15-20 mg/kg/SMX 75-100 mg/kg/d PO or IV in 3 or 4 doses
Primary Infection in Pregnancy				
Treatment of choice:		See footnote 129		
Trichinellosis *(Trichinella spiralis)*				
Drug of choice:[130]		Steroids for severe symptoms, e.g.	prednisone 30-60 mg PO daily ×10-15 d	
		plus		
		Albendazole[7,12]	400 mg PO bid × 8-14 d	400 mg PO bid × 8-14 d
Alternative:		Mebendazole[7]	200-400 mg PO tid × 3 d, then 400-500 mg tid × 10 d	200-400 mg PO tid × 3 d, then 400-500 mg tid × 10 d
Trichomoniasis *(Trichomonas vaginalis)*				
Drug of choice:[131]		Metronidazole	2 g PO once	15 mg/kg/d PO in 3 doses × 7 d
	OR	Tinidazole[6]	2 g PO once	50 mg/kg once (max. 2 g)
Trichostrongylus infection				
Drug of choice:		Pyrantel pamoate[7,31*]	11 mg/kg base PO once (max. 1 g)	11 mg/kg PO once (max. 1 g)
Alternative:		Mebendazole[7]	100 mg PO bid × 3 d	100 mg PO bid × 3 d
	OR	Albendazole[7,12]	400 mg PO once	400 mg PO once
Trichuriasis (*Trichuris trichiura*, whipworm)				
Drug of choice:		Albendazole[7,12]	400 mg PO × 3 d	400 mg PO × 3 d
Alternative:		Mebendazole	100 mg PO bid × 3 d	100 mg PO bid × 3 d
	OR	Ivermectin[7,15]	200 mcg/kg/d PO × 3 d	200 mcg/kg/d PO × 3 d
Trypanosomiasis				
T. cruzi (American trypanosomiasis, Chagas' disease)[132]				
Drug of choice:		Nifurtimox*	8-10 mg/kg/d PO in 3-4 doses × 90-120 d	1-10 yrs: 15-20 mg/kg/d PO in 4 doses × 90-120 d 11-16 yrs: 12.5-15 mg/kg/d PO in 4 doses × 90-120 d
	OR	Benznidazole[133*]	5-7 mg/kg/d PO in 2 doses × 60-90 d	≤12 yrs: 10 mg/kg/d PO in 2 doses × 60-90 d >12 yrs: 5-7 mg/kg/d PO in 2 doses × 60-90 d

125. Treatment is followed by chronic suppression with lower dosage regimens of the same drugs. For primary prophylaxis in HIV patients with CD4 <100 × 10^6 cells/L, either trimethoprim-sulfamethoxazole, pyrimethamine with dapsone, or atovaquone with or without pyrimethamine can be used. Primary or secondary prophylaxis may be discontinued when the CD4 count increases to >200 × 10^6 cells/L for >3 mos (MMWR Morb Mortal Wkly Rep 2009; 58 [RR4]:1). In ocular toxoplasmosis with macular involvement, corticosteroids are recommended in addition to antiparasitic therapy (JG Montoya and O Liesenfeld, Lancet 2004; 363:1965).

126. Plus leucovorin 10-25 mg with each dose of pyrimethamine. Pyrimethamine should be taken with food to minimize gastrointestinal adverse effects.

127. Sulfadiazine should be taken on an empty stomach with adequate water.

128. Clindamycin has been used in combination with pyrimethamine to treat CNS toxoplasmosis in HIV infected patients who developed sulfonamide sensitivity while on sulfadiazine (G Beraud et al, Am J Trop Med Hygiene 2009; 80:583). Atovaquone has also been used to treat sulfonamide-intolerant patients (K Chirgwin et al, Clin Infect Dis 2002; 34:1243).

129. Women who develop toxoplasmosis during the first trimester of pregnancy should be treated with spiramycin (3-4 g/d). After the first trimester, if there is no documented transmission to the fetus, spiramycin can be continued until term. Spiramycin is not currently available in the US but can be obtained at no cost from Palo Alto Medical Foundation Toxoplasma Serology Laboratory (PAMF-TSL, 650-853-4828), US National Collaborative Treatment Trials Study (773-834-4152), or the FDA (301-796-1600). If transmission has occurred *in utero*, therapy with pyrimethamine and sulfadiazine should be started. Pyrimethamine is a potential teratogen and should be used only after the first trimester (JG Montoya and JS Remington, Clin Infect Dis 2008; 47:554). Congenitally infected new borns should be treated with pyrimethamine every 2 or 3 days and a sulfonamide daily for about one year (JS Remington and G Desmonts in JS Remington and JO Klein, eds, *Infectious Disease of the Fetus and Newborn Infant*, 6th ed, Philadelphia:Saunders, 2006, page 1038).

Infection		Drug	Adult dosage	Pediatric dosage
T. brucei gambiense (West African trypanosomiasis, sleeping sickness)[134]				
HEMOLYMPHATIC STAGE				
Drug of choice:[135]		Pentamidine[7]	4 mg/kg/d IM × 7 d	4 mg/kg/d IM × 7 d
Alternative:		Suramin*	100-200 mg (test dose) IV, then 1 g IV on days 1,3,7,14 and 21	20 mg/kg on d 1,3,7,14 and 21
LATE DISEASE WITH CNS INVOLVEMENT				
Drug of Choice:[136]		Eflornithine[137]*	400 mg/kg/d IV in 4 doses × 14 d	400 mg/kg/d IV in 4 doses × 14 d
	OR	Eflornithine[137]*	400 mg/kg IV in 2 doses × 7 d	
		plus		
		Nitfurtimox	15 mg/kg/d PO in 3 doses × 10 d	
	OR	Melarsoprol[138]	2.2 mg/kg/d IV × 10 d	2.2 mg/kg/d IV × 10 d
T. b. rhodesiense (East African trypanosomiasis, sleeping sickness)[134]				
HEMOLYMPHATIC STAGE				
Drug of choice:		Suramin*	100-200 mg (test dose) IV, then 1 g IV on days 1,3,7,14 and 21	20 mg/kg IV on d 1,3,7,14 and 21
LATE DISEASE WITH CNS INVOLVEMENT				
Drug of choice:		Melarsoprol[138]	2-3.6 mg/kg/d IV × 3 d; after 7 d 3.6 mg/kg/d × 3 d; repeat again after 7 d	2-3.6 mg/kg/d IV × 3 d; after 7 d 3.6 mg/kg/d × 3 d; repeat again after 7 d
Visceral Larva Migrans[139] *(Toxocariasis)*		Albendazole[7,12]	400 mg PO bid × 5 d	400 mg PO bid × 5 d
Drug of choice:	OR	Mebendazole[7]	100-200 mg PO bid × 5 d	100-200 mg PO bid × 5 d
Whipworm, see TRICHURIASIS				
Wuchereria bancrofti, see FILARIASIS				

130. B Gottstein et al. Clin Microbiol Rev 2009; 22:127.
131. Sexual partners should be treated simultaneously with same dosage. If treatment failure occurs and reinfection is excluded, treat with metronidazole 500 mg PO bid ×7 d, or tinidazole 2 g PO once (MMWR Morb Mortal Wkly Rep 2006; 55 [RR11]:1).
132. Treatment of chronic or indeterminate Chagas' disease with benznidazole has been associated with reduced progression and increased negative seroconversion (C Bern et al, JAMA 2007; 298:2171; JA Perez-Molina et al, J Antimicrob Chemother 2009; 64:1139).
133. Benznidazole should be taken with meals to minimize gastrointestinal adverse effects. It is contraindicated during pregnancy.
134. PG Kennedy, Ann Neurol 2008; 64:116.
135. Pentamidine and suramin have equal efficacy, but pentamidine is better tolerated.
136. In one study eflornithine for 7 days combined with nifurtimox × 10 d was more effective and less toxic than eflornithine × 14 d (G Priotto et al, Lancet 2009;374:56).
137. Eflornithine is highly effective in *T.b. gambiense*, but not in *T.b. rhodesiense* infections. In one study of treatment of CNS disease due to *T.b. gambiense*, there were fewer serious complications with eflornithine than with melarsoprol (PG Kennedy, Ann Neurol 2008; 64:116). Eflornithine is available in limited supply only from the WHO. It is contraindicated during pregnancy.
138. E Schmid et al, J Infect Dis 2005; 191:1922. Corticosteroids have been used to prevent arsenical encephalopathy (J Pepin et al, Trans R Soc Trop Med Hyg 1995;89:92). Up to 20% of patients with *T.b.gambiense* fail to respond to melarsoprol (MP Barrett, Lancet 1999; 353:1113). In one study, a combination of low-dose melarsoprol (1.2 mg/kg/d IV) and nifurtimox (7.5 mg/kg PO bid) × 10 d was more effective than standard-dose melarsoprol alone (S Bisser et al, J Infect Dis 2007; 195:322).
139. Optimum duration of therapy is not known; some Medical Letter consultants would treat × 20 d. For severe symptoms or eye involvement, corticosteroids can be used in addition (D Despommier, Clin Microbiol Rev 2003; 16:265).

PRINCIPAL ADVERSE EFFECTS OF ANTIPARASITIC DRUGS

Adverse effects of antiparasitic drugs vary with dosage, duration of administration, concomitant therapy, renal and hepatic function, immune competence, and the age of the patient. The principal adverse effects of antiparasitic agents are listed in the following table. The designation of adverse effects as "frequent," "occasional" or "rare" is based on published reports and on the experience of Medical Letter consultants. Information about adverse interactions between drugs, including probable mechanisms and recommendations for clinical management, are available in the Medical Letter Adverse Drug Interactions Program.

ALBENDAZOLE *(ALBENZA)*

Occasional: abdominal pain; reversible alopecia; increased serum transaminases
Rare: leukopenia; rash; renal toxicity

AMPHOTERICIN B DEOXYCHOLATE (*FUNGIZONE*, AND OTHERS)

Frequent: renal damage; hypokalemia; thrombophlebitis at site of peripheral vein infusion; anorexia; headache; nausea; weight loss; bone marrow suppression with reversible decline in hematocrit; chills, fever, vomiting during infusion, possibly with delirium, hypotension or hypertension, wheezing, and hypoxemia, especially in cardiac or pulmonary disease
Occasional: hypomagnesemia; normocytic, normochromic anemia
Rare: hemorrhagic gastroenteritis; blood dyscrasias; rash; blurred vision; peripheral neuropathy; convulsions; anaphylaxis; arrhythmias; acute liver failure; reversible nephrogenic diabetes insipidus; hearing loss; acute pulmonary edema; spinal cord damage with intrathecal use

AMPHOTERICIN B LIPID FORMULATIONS *(AMBISONE, ABELCET, AMPHOTEC)*

Similar to amphotericin B but generally better tolerated. Nephrotoxicity is less common and less severe with the lipid-based formulations. Acute infusion reactions are worse with *Amphotec*, less with *Abelcet* and least with *AmBisome*. Liver toxicity has been reported.

ARTEMETHER *(ARTENAM)*

Occasional: neurological toxicity; possible increase in length of coma; increased convulsions; prolongation of QTc interval

ARTEMETHER/LUMEFANTRINE *(COATEM, RIAMET)*

Frequent: abdominal pain; anorexia; headache; dizziness; diarrhea; vomiting; nausea; palpitations; arthralgia; myalgia; asthenia; fatigue; pruritus; rash; sleep disorder; cough
Occasional: somnolence; involuntary muscle contractions; paresthesia; hypoesthesia; abnormal gait; ataxia
Rare: Hypersensitivity

ARTESUNATE

Occasional: ataxia; slurred speech; neurological toxicity; possible increase in length of coma; increased convulsions; prolongation of QTc interval

ATOVAQUONE (*MEPRON*, *MALARONE* [WITH *PROGUANIL*])

Frequent: rash; nausea
Occasional: diarrhea; increased amino-transferases; cholestasis

AZITHROMYCIN (*ZITHROMAX*, AND OTHERS)

Occasional: nausea; diarrhea; abdominal pain; headache; dizziness; vaginitis
Rare: angioedema; cholestatic jaundice; photosensitivity; reversible dose-related hearing loss

BENZNIDAZOLE *(ROCHAGAN)*

Frequent: allergic rash; dose-dependent polyneuropathy; GI disturbance; psychic disturbances

BENZYL ALCOHOL *(ULESFIA LOTION)*

Frequent: eye irritation; contact dermatitis

BITHIONOL *(BITIN)*

Frequent: photosensitivity reactions; vomiting; diarrhea; abdominal pain; urticaria
Rare: leukopenia; toxic hepatitis

CHLOROQUINE HCL and CHLOROQUINE PHOSPHATE (*ARALEN*, AND OTHERS)

Occasional: pruritus; vomiting; headache; confusion; depigmentation of hair; skin eruptions; corneal opacity; weight loss; partial alopecia; extraocular muscle palsies; exacerbation of psoriasis, eczema, and other exfoliative dermatoses; myalgias; photophobia
Rare: irreversible retinal injury (especially when total dosage exceeds 100 grams); discoloration of nails and mucus membranes; nerve-type deafness; peripheral neuropathy and myopathy; heart block; blood dyscrasias; hematemesis

CLARITHROMYCIN (*BIAXIN*, AND OTHERS)

Occasional: nausea; diarrhea; abdominal pain; abnormal taste; headache; dizziness
Rare: reversible dose-related hearing loss; pseudomembranous colitis; pancreatitis; torsades de pointes

CLINDAMYCIN (*CLEOCIN*, AND OTHERS)

Frequent: diarrhea; allergic reactions
Occasional: pseudomembranous colitis, sometimes severe, can occur even with topical use
Rare: blood dyscrasias; esophageal ulceration; hepatotoxicity; arrhythmia due to QTc prolongation

CROTAMITON *(EURAX)*

Occasional: rash

DAPSONE

Frequent: rash; transient headache; GI irritation; anorexia; infectious mononucleosis-like syndrome
Occasional: cyanosis due to methemoglobinemia and sulfhemoglobinemia; other blood dyscrasias, including hemolytic anemia; nephrotic syndrome; liver damage; peripheral neuropathy; hypersensitivity reactions; increased risk of lepra reactions; insomnia; irritability; uncoordinated speech; agitation; acute psychosis
Rare: renal papillary necrosis; severe hypoalbuminemia; epidermal necrolysis; optic atrophy; agranulocytosis; neonatal hyperbilirubinemia after use in pregnancy

DIETHYLCARBAMAZINE CITRATE *(HETRAZAN)*

Frequent: allergic or febrile reactions, which may be severe, in patients with microfilaria in the blood or the skin; GI disturbance
Rare: encephalopathy

DILOXANIDE FUROATE *(FURAMIDE)*

Frequent: flatulence
Occasional: nausea; vomiting; diarrhea
Rare: diplopia; dizziness; urticaria; pruritus

EFLORNITHINE (DIFLUOROMETHYLORNITHINE, DFMO, *ORNIDYL)*

Frequent: anemia; leukopenia
Occasional: diarrhea; thrombocytopenia; seizures
Rare: hearing loss

FLUCONAZOLE (*DIFLUCAN*, AND OTHERS)

Occasional: nausea; vomiting; diarrhea; abdominal pain; headache; rash; increased aminotransferases
Rare: severe hepatic toxicity; exfoliative dermatitis; anaphylaxis; Stevens-Johnson syndrome; toxic epidermal necrolysis; hair loss

FLUCYTOSINE *(ANCOBON)*

Frequent: blood dyscrasias, including pancytopenia and fatal agranulocytosis; GI disturbance, including severe diarrhea and ulcerative colitis; rash; hepatic dysfunction
Occasional: confusion; hallucinations
Rare: anaphylaxis

FURAZOLIDONE *(FUROXONE)*

Frequent: nausea; vomiting
Occasional: allergic reactions, including pulmonary infiltration; hypotension; urticaria; fever; vesicular rash; hypoglycemia; headache
Rare: hemolytic anemia in G-6-PD deficiency and neonates; disulfiram-like reaction with alcohol; MAO-inhibitor interactions; polyneuritis

IODOQUINOL (*YODOXIN*, AND OTHERS)

Occasional: rash; acne; slight enlargement of the thyroid gland; nausea; diarrhea; cramps; anal pruritus

Rare: optic neuritis, atrophy and loss of vision; peripheral neuropathy after prolonged use in high dosage (for months); iodine sensitivity

ITRACONAZOLE (*SPORANOX*, AND OTHERS)

Occasional: nausea; epigastric pain; headache; dizziness; edema; hypokalemia; rash; hepatic toxicity
Rare: congestive heart failure

IVERMECTIN *(STROMECTOL)*

Occasional: Mazzotti-type reaction seen in onchocerciasis, including fever; pruritus; tender lymph nodes; headache; and joint and bone pain
Rare: hypotension

KETOCONAZOLE (*NIZORAL*, AND OTHERS)

Frequent: nausea; vomiting
Occasional: decreased testosterone synthesis; gynecomastia; oligospermia and impotence in men; abdomina pain; rash; hepatitis; pruritus; dizziness; constipation; diarrhea; fever and chills; photophobia; headache
Rare: fatal hepatic necrosis; liver injury with jaundice; transient elevated transaminase; severe epigastric burning and pain; may interfere with adrenal function; anaphylaxis

MALATHION *(OVIDE)*

Occasional: local irritation

MEBENDAZOLE *(VERMOX)*

Occasional: diarrhea; abdominal pain
Rare: leukopenia; agranulocytosis; hypospermia

MEFLOQUINE *(LARIAM)*

Frequent: vertigo; lightheadedness; nausea; other GI disturbances; nightmares; visual disturbances; headache; insomnia
Occasional: confusion
Rare: psychosis; hypotension; convulsions; coma; paresthesias

MEGLUMINE ANTIMONIATE *(GLUCANTIME)*

— Similar to sodium stibogluconate

MELARSOPROL *(MEL B)*

Frequent: myocardial damage; albuminuria; hypertension; colic; Herxheimer-type reaction; encephalopathy; vomiting; peripheral neuropathy
Rare: shock

METRONIDAZOLE (*FLAGYL*, AND OTHERS)

Frequent: nausea; headache; anorexia; metallic taste
Occasional: vomiting; diarrhea; insomnia; weakness; dry mouth; stomatitis; vertigo; tinnitus; paresthesias; rash; dark urine; urethral burning; disulfiram-like reaction with alcohol; candidiasis
Rare: pseudomembranous colitis; leukopenia; pancreatitis; seizures; peripheral neuropathy; encephalopathy; cerebellar syndrome with ataxia, dysarthria and MRI abnormalities

MICONAZOLE *(MONISTAT I.V.)*

Occasional: phlebitis; thrombocytosis; chills; intense, persistent pruritus; rash; vomiting; hyperlipidemia; dizziness; blurred vision; local burning and irritation with topical use
Rare: anemia; thrombocytopenia; hyponatremia; renal insufficiency; anaphylaxis; cardiac and respiratory arrest with initial dose

MILTEFOSINE *(IMPAVIDO)*

Frequent: nausea; vomiting; diarrhea; motion sickness; increased creatinine

NICLOSAMIDE *(NICLOCIDE)*

Occasional: nausea; abdominal pain

NITAZOXANIDE *(ALINIA)*

Occasional: GI disturbance; headache
Rare: yellow discoloration of sclera; allergic reactions; increased creatinine; dizziness; flatulence; malaise; salivary gland enlargement; discolored urine; anemia; leukoytosis

ORNIDAZOLE *(TIBERAL)*

Occasional: dizziness; headache; GI disturbance
Rare: reversible peripheral neuropathy

OXAMNIQUINE *(VANSIL)*

Occasional: headache; fever; dizziness; somnolence and insomnia; nausea; diarrhea; rash; increased aminotransferases; ECG changes; EEG changes; orange-red discoloration of urine
Rare: seizures; neuropsychiatric disturbances

PAROMOMYCIN (AMINOSIDINE; *HUMATIN*)

Frequent: GI disturbance with oral use
Rare: eighth-nerve damage (mainly auditory) and renal damage when aminosidine is given IV; vertigo; pancreatitis

PENTAMIDINE ISETHIONATE (*PENTAM 300*, *NEBUPENT*, AND OTHERS)

Frequent: hypotension; hypoglycemia often followed by diabetes mellitus; vomiting; blood dyscrasias; renal damage; pain at injection site; GI disturbance
Occasional: may aggravate diabetes; shock; hypocalcemia; liver damage; cardiotoxicity; delirium; rash
Rare: Herxheimer-type reaction; anaphylaxis; acute pancreatitis; hyperkalemia

PERMETHRIN (*NIX*, AND OTHERS)

Occasional: burning; stinging; numbness; increased pruritus; pain; edema; erythema; rash

PRAZIQUANTEL (BILTRICIDE)

Frequent: abdominal pain; diarrhea; malaise; headache; dizziness
Occasional: sedation; fever; sweating; nausea; eosinophilia
Rare: pruritus; rash; edema; hiccups

PRIMAQUINE PHOSPHATE

Frequent: hemolytic anemia in G-6-PD deficiency
Occasional: neutropenia; GI disturbance; methemoglobinemia
Rare: CNS symptoms; hypertension; arrhythmias

PROGUANIL (*PALUDRINE; MALARONE* [WITH ATOVAQUONE])

Occasional: oral ulceration; hair loss; scaling of palms and soles; urticaria
Rare: hematuria (with large doses); vomiting; abdominal pain; diarrhea (with large doses); thrombocytopenia

PYRANTEL PAMOATE (*ANTIMINTH*, AND OTHERS)

Occasional: GI disturbance; headache; dizziness; rash; fever

PYRETHRINS WITH PIPERONYL BUTOXIDE (*A-200*, AND OTHERS)

Occasional: allergic reactions

PYRIMETHAMINE *(DARAPRIM)*

Occasional: blood dyscrasias; folic acid deficiency
Rare: rash; vomiting; convulsions; shock; possibly pulmonary eosinophilia; fatal cutaneous reactions with pyrimethamine-sulfadoxine *(Fansidar)*

QUINACRINE

Frequent: disulfiram-like reaction with alcohol; nausea and vomiting; colors skin and urine yellow
Occasional: headache; dizziness
Rare: rash; fever; psychosis; extensive exfoliative dermatitis in patients with psoriasis

QUININE DIHYDROCHLORIDE AND QUININE SULFATE

Frequent: cinchonism (tinnitus, headache, nausea, abdominal pain, visual disturbance)
Occasional: deafness; hemolytic anemia; other blood dyscrasias; photosensitivity reactions; hypoglycemia; arrhythmias; hypotension; fever
Rare: blindness; sudden death if injected too rapidly; hypersensitivity reaction with TTP-HUS

SODIUM STIBOGLUCONATE *(PENTOSTAM)*

Frequent: myalgia and arthralgia (typically, large joint, may or may not be symmetric); malaise, fatigue and weakness; headache; anorexia; nausea; increased aminotransferases; increased amylase and lipase; T-wave flattening or inversion
Occasional: abdominal pain; liver damage; bradycardia; leukopenia; thrombocytopenia; rash; vomiting
Rare: diarrhea; pruritus; myocardial damage; hemolytic anemia; renal damage; shock; sudden death

SPIRAMYCIN *(ROVAMYCINE)*

Occasional: GI disturbance
Rare: allergic reactions

SULFONAMIDES

Frequent: allergic reactions (rash, photosensitivity, drug fever)
Occasional: kernicterus in newborn; renal damage; liver damage; Stevens-Johnson syndrome (particularly with long-acting sulfonamides); hemolytic anemia; other blood dyscrasias; vasculitis
Rare: transient acute myopia; pseudomembranous colitis; reversible infertility in men with sulfasalazine; CNS toxicity with trimethoprim-sulfamethoxazole in patients with AIDS

SURAMIN SODIUM

Frequent: vomiting; pruritus; urticaria; paresthesias; hyperesthesia of hands and feet; peripheral neuropathy; photophobia
Occasional: kidney damage; blood dyscrasias; shock; optic atrophy

TETRACYCLINES

(doxycycline—*Vibramycin*, and others; tetracycline hydrochloride—*Sumycin*, and others)
Frequent: GI disturbance; bone lesions and staining and deformity of teeth in children up to 8 years old, and in the newborn when given to pregnant women after the fourth month of pregnancy
Occasional: malabsorption; enterocolitis; photosensitivity reactions; increased azotemia with renal insufficiency (except doxycycline, but exacerbation of renal failure with doxycycline has been reported); hepatic injury; parenteral doses may cause serious liver damage, especially in pregnant women and patients with renal disease receiving 1 gram or more daily; esophageal ulcerations; cutaneous and mucosal hyperpigmentation
Rare: allergic reactions, including serum sickness and anaphylaxis; pseudomembranous colitis; blood dyscrasias; drug-induced lupus; autoimmune hepatitis; increased intracranial pressure; fixed-drug eruptions; transient acute myopia; blurred vision; diplopia; papilledema; photoonycholysis and onycholysis; aggravation of myasthenic symptoms with IV injection, reversed with calcium; possibly transient neuropathy; hemolytic anemia

TINIDAZOLE *(TINDAMAX)*

Occasional: metallic taste; GI symptoms; rash
Rare: weakness

TRIMETHOPRIM (*PROLOPRIM*, AND OTHERS)

Frequent: nausea and vomiting with high doses
Occasional: megaloblastic anemia; thrombocytopenia; neutropenia; rash; fixed drug eruption
Rare: pancytopenia; hyperkalemia

TRIMETHOPRIM/SULFAMETHOXAZOLE (*BACTRIM*, *SEPTRA*, AND OTHERS)

Frequent: rash; fever; nausea and vomiting
Occasional: hemolysis in G-6-PD deficiency; acute megaloblastic anemia; granulocytopenia; thrombocytopenia; pseudomembranous colitis; kernicterus in newborn; hyperkalemia
Rare: agranulocytosis; aplastic anemia; hepatotoxicity; Stevens-Johnson syndrome; aseptic meningitis; fever; confusion; depression; hallucinations; deterioration in renal disease; intrahepatic cholestasis; methemoglobinemia; pancreatitis; ataxia; CNS toxicity in patients with AIDS; renal tubular acidosis; hyperkalemia

SAFETY OF ANTIPARASITIC DRUGS IN PREGNANCY

Drug	Toxicity in Pregnancy	Recommendations	FDA
Albendazole (*Albenza*)	Teratogenic and embryotoxic in animals	Caution*	C
Amphotericin B (*Fungizone*, and others)	None known	Caution*	B
Amphotericin B liposomal *(AmBisome)*	None known	Caution*	B
Artemether/lumefantrine *(Coartem, Riamet)*[1]	Embryo-fetal loss in rats and and rabbits	Contraindicated during 1st trimester; caution 2nd and 3rd trimesters*	C
Artesunate[2]	Embryocidal and teratogenic in rats	Contraindicated during 1st trimester; caution 2nd and 3rd trimesters*	N/A
Atovaquone *(Mepron)*	Maternal and fetal toxicity in animals	Caution*	C
Atovaquone/proguanil *(Malarone)*[3]	Maternal and fetal toxicity in animals	Caution*	C

Drug	Toxicity in Pregnancy	Recommendations	FDA
Azithromycin (*Zithromax*, and others)	None known	Probably safe	B
Benznidazole *(Rochagan)*	Unknown	Contraindicated	N/A
Benzyl alcohol lotion *(Ulesfia Lotion)*	Unknown	Probably safe	B
Chloroquine (*Aralen*, and others)	None known with doses recommended for malaria prophylaxis	Probably safe in low doses	C
Clarithromycin (*Biaxin*, and others)	Teratogenic in animals	Contraindicated	C
Clindamycin (*Cleocin*, and others)[4]	None known	Caution*	B
Crotamiton *(Eurax)*	Unknown	Caution*	C
Dapsone	None known; carcinogenic in rats and mice; hemolytic reactions in neonates	Caution*, especially at term	C
Diethylcarbamazine (DEC; *Hetrazan*)	Not known; abortifacient in one study in rabbits	Contraindicated	N/A
Diloxanide *(Furamide)*	Safety not established	Caution*	N/A
Doxycycline (*Vibramycin*, and others)	Tooth discoloration and dysplasia inhibition of bone growth in fetus; hepatic toxicity and azotemia with IV use in pregnant patients with decreased renal function or with overdosage	Contraindicated	D
Eflornithine *(Ornidyl)*	Embryocidal in animals	Contraindicated	C
Fluconazole (*Diflucan*, and others)	Teratogenic	Contraindicated for high dose; caution* for single dose	C
Flucytosine *(Ancoban)*	Teratogenic in rats	Contraindicated	C
Furazolidone *(Furoxone)*	None known; carcinogenic in rodents; hemolysis with G-6-PD deficiency in newborn	Caution*; contraindicated at term	N/A
Hydroxychloroquine *(Plaquenil)*	None known with doses recommended for malaria prophylaxis	Probably safe in low doses	C
Itraconazole (*Sporanox*, and others)	Teratogenic and embryotoxic in rats	Caution*	C
Iodoquinol (*Yodoxin*, and others)	Unknown	Caution*	C
Ivermectin *(Stromectol)*[5]	Teratogenic in animals	Contraindicated	C
Ketoconazole (*Nizoral*, and others)	Teratogenic and embryotoxic in rats	Contraindicated; topical probably safe	C
Lindane	Absorbed from the skin; potential CNS toxicity in fetus	Contraindicated	C
Malathion, topical *(Ovide)*	None known	Probably safe	B
Mebendazole *(Vermox)*	Teratogenic and embryotoxic in rats	Caution*	C
Mefloquine *(Lariam)*[6]	Teratogenic in animals	Caution*	C
Meglumine *(Glucantine)*	Not known	Caution*	N/A
Metronidazole (*Flagyl*, and others)	None known—carcinogenic in rats and mice	Caution*	B
Miconazole *(Monistat i.v.)*	None known	Caution*	C
Miltefosine *(Impavido)*	Teratogenic in rats and induces abortions inanimals	Contraindicated; effective contraception must be used for 2 months after the last dose	N/A
Niclosamide *(Niclocide)*	Not absorbed; no known toxicity in fetus	Probably safe	B
Nitazoxanide *(Alinia)*	None known	Probably safe	B
Oxamniquine *(Vansil)*	Embryocidal in animals	Contraindicated	N/A
Paromomycin	Poorly absorbed; toxicity in fetus unknown	Oral capsules probably safe	C

Drug	Toxicity in Pregnancy	Recommendations	FDA
Pentamidine (*Pentam 300, NebuPent*, and others)	Safety not established	Caution*	C
Permethrin (*Nix*, and others)	Poorly absorbed; no known toxicity in fetus	Probably safe	B
Praziquantel *(Biltricide)*	None known	Caution	B
Primaquine	Hemolysis in G-6-PD deficiency	Contraindicated	C
Pyrantel pamoate (*Antiminth*, and others)	Absorbed in small amounts; no known toxicity in fetus	Probably safe	C
Pyrethrins and piperonyl butoxide (*A-200*, and others)	Poorly absorbed; no known toxicity in fetus	Probably safe	C
Pyrimethamine *(Daraprim)*[7]	Teratogenic in animals	Caution*; contraindicated during 1st trimester	C
Quinacrine *(Atabrine)*	Safety not established	Caution*	N/A
Quinidine	Large doses can cause abortion	Probably safe	C
Quinine *(Qualaquin)*	Large doses can cause abortion; auditory nerve hypoplasia, deafness in fetus; visual changes, limb anomalies, visceral defects also reported	Caution*	C
Sodium stibogluconate *(Pentostam)*	Not known	Caution*	N/A
Spiramycin *(Rovamycine)*[7]	None known	Probably safe	N/A
Sulfonamides	Teratogenic in some animal studies; hemolysis in newborn with G-6-PD deficiency; increased risk of kernicterus in newborn	Caution*; contraindicated at term	C
Suramin sodium *(Germanin)*	Teratogenic in mice	Caution*	N/A
Tetracycline (*Sumycin*, and others)	Tooth discoloration and dysplasia, inhibition of bone growth in fetus; hepatic toxicity and azotemia with IV use in pregnant patients with decreased renal function or with overdosage	Contraindicated	D
Tinidazole *(Tindamax)*	Increased fetal mortality in rats	Caution*	C
Trimethoprim	Folate antagonism; teratogenic in rats	Caution*	C
Trimethoprim-sulfamethoxazole (*Bactrim*, and others)	Same as sulfonamides and trimethoprim	Caution*; contraindicated at term	C

N/A = FDA pregnancy category not available
* Use only for strong clinical indication in absence of suitable alternative.
1. See also footnotes 78 and 79 on page 8.
2. See also footnote 78 on page 8.
3. See also footnote 76 on page 7.
4. See also footnote 82 on page 8.
5. See also footnote 42 on page 4.
6. See also footnotes 84 and 95 on pages 8 and 9.
7. See also footnote 129 on page 12.

MANUFACTURERS OF DRUGS USED TO TREAT PARASITIC INFECTIONS

A-200 (Hogil)—pyrethrins and piperonyl butoxide
albendazole—*Albenza* (GlaxoSmithKline)
Albenza (GlaxoSmithKline)—albendazole
Alinia (Romark)—nitazoxanide
AmBisome (Gilead)—amphotericin B, liposomal
amphotericin B—*Fungizone* (Apothecon), others
amphotericin B, liposomal—*AmBisome* (Gilead)
Ancobon (Valeant)—flucytosine
§ *Antiminth* (Pfizer)—pyrantel pamoate
• *Aralen* (sanofi-aventis)—chloroquine HCl and chloroquine phosphate
§ artemether—*Artenam* (Arenco, Belgium)
artemether/lumefantrine—*Coartem, Riamet* (Novartis)
§ *Artenam* (Arenco, Belgium)—artemether
• † artesunate—(Guilin No. 1 Factory, People's Republic of China)
atovaquone—*Mepron* (GlaxoSmithKline)
atovaquone/proguanil—*Malarone* (GlaxoSmithKline)
azithromycin—*Zithromax* (Pfizer), others
• *Bactrim* (AR Scientific)—TMP/Sulfa
Benzyl alcohol lotion—*Ulesfia* (Sciele)
§ benznidazole—*Rochagan* (Brazil)
• *Biaxin* (Abbott)—clarithromycin
Biltricide (Bayer)—praziquantel
† bithionol—*Bitin* (Tanabe, Japan)
† *Bitin* (Tanabe, Japan)—bithionol
§ *Brolene* (Aventis, Canada)—propamidine isethionate
chloroquine HCl and chloroquine phosphate—Aralen (sanofi-aventis), others
• clarithromycin—*Biaxin* (Abbott), others
• *Cleocin* (Pfizer)—clindamycin
clindamycin—*Cleocin* (Pfizer), others
Coartem (Novartis)—artemether/lumefantrine
crotamiton—*Eurax* (Ranbaxy)
dapsone—(Jacobus)
§ *Daraprim* (GlaxoSmithKline)—pyrimethamine USP
† diethylcarbamazine citrate (DEC)—*Hetrazan*
• *Diflucan* (Pfizer)—fluconazole
§ diloxanide furoate—*Furamide*
doxycycline—*Vibramycin* (Pfizer), others
§ eflornithine (Difluoromethylornithine, DFMO)—*Ornidyl* (Aventis)
§ *Egaten* (Novartis)—triclabendazole
Elimite (Allergan)—permethrin
Eurax (Ranbaxy)—crotamiton
• *Flagyl* (Pfizer)—metronidazole
§ *Flisint* (Sanofi-Aventis, France)—fumagillin
fluconazole—*Diflucan* (Pfizer), others
flucytosine—*Ancobon* (Valeant)
§ fumagillin—*Flisint* (Sanofi-Aventis, France)
• *Fungizone* (Apothecon)—amphotericin
§ *Furamide*—diloxanide furoate
§ furazolidone—*Furoxone* (Roberts)
§ *Furoxone* (Roberts)—furazolidone
† *Germanin* (Bayer, Germany)—suramin sodium
§ *Glucantime* (Aventis, France)—meglumine antimonate
† *Hetrazan*—diethylcarbamazine citrate (DEC)
§ *Impavido* (Paladin, Montreal, Canada)—miltefosine
iodoquinol—*Yodoxin* (Glenwood), others
itraconazole—*Sporanox* (Ortho-McNeil-Janssen), others
ivermectin—*Stromectol* (Merck)
ketoconazole—*Nizoral* (Janssen), others
† *Lampit* (Bayer, Germany)—nifurtimox
§ *Leshcutan* (Teva, Israel)—topical paromomycin
lumefantrine/artemether—*Coartem, Riamet* (Novartis)
Malarone (GlaxoSmithKline)—atovaquone/proguanil
malathion—*Ovide* (Taro)
mebendazole
mefloquine
§ meglumine antimonate—*Glucantime* (Aventis, France)
† melarsoprol—*Mel-B*
† *Mel-B*—melarsoprol
Mepron (GlaxoSmithKline)—atovaquone
metronidazole—*Flagyl* (Pfizer), others
§ miconazole—*Monistat i.v.*
§ miltefosine—*Impavido* (Paladin, Montreal, Canada)
§ *Monistat i.v.*—miconazole
NebuPent (Fujisawa)—pentamidine isethionate
Neutrexin (US Bioscience)—trimetrexate
§ niclosamide—*Yomesan* (Bayer, Germany)
† nifurtimox—*Lampit* (Bayer, Germany)
nitazoxanide—*Alinia* (Romark)
• *Nizoral* (Janssen)—ketoconazole
Nix (GlaxoSmithKline)—permethrin
§ ornidazole—*Tiberal* (Roche, France)
Ornidyl (Aventis)—eflornithine (Difluoromethylornithine, DFMO)
Ovide (Taro)—malathion
§ oxamniquine—*Vansil* (Pfizer)
§ *Paludrine* (AstraZeneca, United Kingdom)—proguanil
§ paromomycin—Oral generics; Leshcutan (Teva, Israel; (topical formulation not available in US)
Pentam 300 (Fujisawa)—pentamidine isethionate
pentamidine isethionate—*Pentam 300* (Fujisawa), *NebuPent* (Fujisawa)
† *Pentostam* (GlaxoSmithKline, United Kingdom)—sodium stibogluconate
permethrin—*Nix* (GlaxoSmithKline), *Elimite* (Allergan)
praziquantel—*Biltricide* (Bayer)
primaquine phosphate USP
§ proguanil—*Paludrine* (AstraZeneca, United Kingdom)
proguanil/atovaquone—*Malarone* (GlaxoSmithKline)
§ propamidine isethionate—*Brolene* (Aventis, Canada)
§ pyrantel pamoate—*Antiminth* (Pfizer)
pyrethrins and piperonyl butoxide—*A-200* (Hogil), others
§ pyrimethamine USP—*Daraprim* (GlaxoSmithKline)
Qualaquin—quinine sulfate (AR Scientific)
quinidine gluconate
§ quinine dihydrochloride
quinine sulfate—*Qualaquin* (AR Scientific)
Riamet (Novartis)—artemether/lumefantrine
• *Rifadin* (sanofi-aventis)—rifampin
rifampin—*Rifadin* (sanofi-aventis), others
§ *Rochagan* (Brazil)—benznidazole
• *Rovamycine* (Aventis)—spiramycin
† sodium stibogluconate—*Pentostam* (GlaxoSmithKline, United Kingdom)
• spiramycin—*Rovamycine* (Aventis)
• *Sporanox* (Ortho-McNeil-Janssen)—itraconazole
Stromectol (Merck)—ivermectin
sulfadiazine
† suramin sodium—*Germanin* (Bayer, Germany)
§ *Tiberal* (Roche, France)—ornidazole
Tindamax (Mission)—tinidazole
tinidazole—*Tindamax* (Mission)
TMP/Sulfa—*Bactrim* (AR Scientific), others
§ triclabendazole—*Egaten* (Novartis)
trimetrexate—*Neutrexin* (US Bioscience)
Ulesfia—benzyl alcohol
§ *Vansil* (Pfizer)—oxamniquine
• *Vibramycin* (Pfizer)—doxycycline
• *Yodoxin* (Glenwood)—iodoquinol
§ *Yomesan* (Bayer, Germany)—niclosamide
• *Zithromax* (Pfizer)—azithromycin

*Also available generically.
†Available from the CDC Drug Service, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; 404-639-3670 (evenings, weekends, or holidays: 770-488-7100).
§Not available in the US; may be available through a compounding pharmacy (see footnote 4).

CHAPTER 241

Immunomodulating Agents

Timothy R. La Pine • Harry R. Hill

Considerable clinical interest and basic science research into the functional mechanisms of the immune response and identification of the specific biologic factors that modulate this response have been generated lately. There are over tens of thousands of citations in the peer-reviewed medical literature on this topic. This research has established that a critical and delicate balance in the regulation of both cellular and humoral function is essential for complete immunologic response to invasive pathogens and that alteration of this regulation may have potential clinical significance. Attempts to augment immune function in the challenged host or during specific immunodeficiency states have focused on numerous modifiers of immune biologic response. These efforts, in combination with advances made in hybridoma, recombinant DNA technology, and proteomics, have resulted in numerous clinical trials conducted to explore the therapeutic utility of immunomodulating agents in the treatment of specific human disease states.[54] Biologic agents used to manipulate immune regulation for the prevention and management of infectious diseases in infants and children are discussed.

MONOCLONAL ANTIBODIES

The use of serum antibody therapy, in the late 1800s, was among the first clinical attempts to modulate the immune response in the treatment of human sepsis. By the middle 1930s, serum-based therapy was the standard of care for many infectious illnesses, particularly pneumonia. Controlled trials during that time demonstrated that the administration of type-specific pneumococcal serum reduced the mortality rate by 50 percent in patients with pneumococcal pneumonia.[49,50,84] With the introduction of antimicrobial pharmacologic agents in the 1940s, the use of serum antibody therapy for sepsis became less popular. The development of hybridoma technology by Kohler and Milstein[153] in 1975, however, provided the means to generate virtually unlimited amounts of monoclonal antibodies for potential clinical use. This technology in combination with the advances in recombinant DNA technology has allowed researchers to generate highly specific human monoclonal antibodies and to humanize murine monoclonal antibodies.[33,293] Monoclonal antibodies now are considered attractive molecules to be used potentially as antimicrobial and antiviral agents and immunomodulators, to deliver pharmacologic substances to sites of inflammation, or even to target certain cancerous tissues. This advance has led to the development of monoclonal antibody–based therapies to treat sepsis, septic shock, hemolytic-uremic syndrome, viral diseases, and a number of inflammatory diseases in infants, children, and adults.

A monoclonal antibody could, in theory, be generated that would alter the clinical course of any infectious or inflammatory disease state. This hypothesis has fostered numerous studies evaluating the use of monoclonal antibodies in a variety of clinical diseases, particularly those related to alloimmune and autoimmune phenomena.[62,75,113,185,231] The use of monoclonal antibodies to alter the pathogenesis of sepsis and septic shock also has received considerable experimental and clinical attention. These efforts have focused on two phases in the development of the septic shock syndrome: (1) to block bacteria and their components that induce shock and (2) to modify the release and action of the proinflammatory mediators that lead to septic shock.

Monoclonal antibody preparations to block bacteria or their components in the development of septic shock were initially employed in early studies using type-specific antisera against *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae*.[50] Attempts using monoclonal antibody therapy to alter the pathogenesis of bacterial sepsis leading to septic shock have focused on group B streptococcus (GBS) and *Escherichia coli*. These two bacteria are significant causes of neonatal morbidity and mortality. Infants usually acquire these bacterial infections from exposure in the birth canal; however, only a small number of the exposed infants actually develop bacterial sepsis. Factors predisposing infants to acquisition of infection with these organisms include prematurity, prolonged rupture of membranes, and maternal sepsis. Neonatal infections occur more commonly with bacteria that possess specific capsular polysaccharides (i.e., the type III polysaccharide of GBS or the K-1 capsule of *E. coli*). Bacteria bearing these capsules are able to avoid opsonization and subsequent phagocytic killing by polymorphonuclear leukocytes (PMNs). Neonates with deficiencies of type-specific antibodies to these bacterial capsular antigens due to prematurity, which contributes to decreased maternal transplacental antibody transport, or inadequate maternal stores are predisposed to acquisition of GBS and *E. coli* infections.[125] These observations led investigators to hypothesize that passive antibody administration may be beneficial in preventing or reducing neonatal morbidity and mortality observed with these bacterial infections.

Rebecca Lancefield in 1933 originally demonstrated a protective efficacy for GBS antibody therapy using rabbit

antisera. She established that this protective efficacy is type specific and classified three types of GBS strains. Antisera raised against type II or type III GBS did not protect against infection with type I bacteria. These experiments also suggested that antibodies to group B non–type-specific determinants (i.e., expressed on all GBS bacteria) are not protective.[93,169-171] Further experiments by Lancefield and coworkers with type I GBS demonstrated that antibodies to both carbohydrate and protein capsular components can be protective.[169,171] The first human studies to suggest that the administration of antibody to the infecting strain of bacteria could improve survival rates from early-onset GBS in infants occurred in the middle 1970s.[255-258] In these studies, fresh whole blood either containing or lacking opsonic antibody was administered to infants with early-onset GBS disease. All nine infants who demonstrated a rise in opsonic antibody after transfusion survived. In contrast, three of the six infants (50%) who received blood lacking antibody to their infection strain died.[254] Subsequent studies have suggested that intravenous immune globulins (IVIG) could offer some protection against GBS infections in experimental animal models.[87,130] Because of their lack of specificity and decreased opsonic activity, however, polyclonal-type IVIG antibody preparations have had only variable and limited ability to alter the course and outcome of GBS or other bacterial infections in clinical trials.[121]

The development of monoclonal antibodies to GBS type III occurred in the early 1980s.[255-258] The GBS type III–specific antibodies, which were of the immunoglobulin (Ig) M class, protected rats against intraperitoneal infection with homologous type GBS. Survival rates were 95 to 100 percent for rats protected by monoclonal antibody compared with 17 percent for unprotected rats. Protection was afforded even when therapy was delayed up to 24 hours after inoculation. Antibody administration resulted in the rapid accumulation of PMNs at the site of infection and prevented the depletion of bone marrow granulocyte stores commonly seen in animal models and human neonates with GBS infection.[256] Monoclonal IgG and IgA antibody preparations also have been generated to type III GBS.[23,212,255] A protective effect in animal models of GBS infection for all three immunoglobulin isotypes has been established.[257] In GBS infections, the ability of an antibody isotype preparation to activate the complement system is related directly to its protective and opsonic activity.[258] IgM is much more active in triggering complement than are monoclonal IgG and IgA preparations,[173,258] which may explain why monoclonal IgM is quantitatively more effective against GBS infections.[124,127,258] Although human monoclonal IgM antibodies to GBS are available and have demonstrated effectiveness in reducing mortality in animal models of GBS sepsis, they have yet to be tried clinically to prevent or to attenuate GBS sepsis and septic shock in human neonates.[234,258]

Concomitant with the studies of monoclonal antibody preparations for use in experimental models of GBS infections were similar studies using monoclonal preparations in *E. coli* sepsis. *E. coli* is an antigenically complex, gram-negative bacterium having more than 150 somatic (O) and 100 capsular (K) antigens. Several factors associated with *E. coli* have been implicated as contributing to the virulence of these organisms. Among these, the K-1 capsular polysaccharide and lipopolysaccharide (LPS) have received the most attention.[28] The K-1 capsular polysaccharide on *E. coli* as a contributing factor to the virulence of this organism was suggested first by Robbins and colleagues in 1974.[239] Subsequent studies established that *E. coli* strains that possess the K-1 capsular polysaccharide are resistant to opsonization through the alternative complement pathway.[30,263] Several investigators have shown that antibody preparations to the K-1 capsular polysaccharide are opsonic and protective against lethal *E. coli* bacteremia in animals.[29] Although protection has been demonstrated primarily with IgM antibody, polyclonal hyperimmune IgG has shown some protection in animal models of lethal *E. coli* sepsis.[233] Because the serum from neonates is deficient in opsonic activity for *E. coli*, some investigators suggested that the administration of antibody against *E. coli* K-1 would enhance neonatal resistance to infections with this organism.[31,60] Investigators since have developed murine and human monoclonal IgM antibodies to *E. coli* K-1 and demonstrated that these antibodies are opsonic and protective against lethal *E. coli* infections in animal models.[233] Staphylococci are a major cause of acquired infections in prematurely born infants. Antibiotic resistance among staphylococcal species in these immunocompromised patients is of significant concern. A human chimeric monoclonal antibody has been generated that is opsonic for *Staphylococcus epidermidis* and *Staphylococcus aureus.* In animal models of *S. epidermidis* and *S. aureus* sepsis, this monoclonal antibody enhanced bacterial clearance and significantly improved survival against both staphylococcal species. Clinical trials with this monoclonal antibody have been performed to determine the clinical usefulness of monoclonal antibody preparations in preventing nosocomial staphylococcal infections in high-risk preterm neonates.[88,282]

During the past several decades, the importance of pneumococcal vaccination for individuals at risk for acquiring infection has been underscored by the emergence of antibiotic resistance among pneumococcal strains and the increased prevalence of invasive pneumococcal disease in immunocompromised patients. Unfortunately, pure pneumococcal capsular polysaccharide vaccines are poorly immunogenic in many immunodeficient patients who are at risk for acquiring infection. This concern has led to the development of monoclonal antibodies against type-specific pneumococcal infections. A human monoclonal IgM antibody has been generated and shown to provide type-specific protection against lethal pneumococcal infections in mice, even in the presence of complement deficiency.[297] Thus, type-specific monoclonal antibody preparations likely could provide protection against pneumococcal infection in high-risk immunocompromised humans.

The use of monoclonal antibodies as a single-agent therapy to treat or to prevent bacterial sepsis has been limited to experimental animal models, primarily because of the narrow spectrum of monoclonal antibody therapy and the exceedingly large number of patients required to demonstrate the efficacy of human monoclonal antibody

therapy in clinical trials. The future clinical use of monoclonal antibodies directed at bacteria or their components in the treatment of sepsis requires several clinical considerations. First, because antibodies are more effective in preventing an infection than in treating an established infection, monoclonal antibody therapy is most useful when it is administered early in the course of a disease. Second, the successful implementation of monoclonal antibody therapy directed at a specific pathogen requires refinements in diagnostic laboratory methodology to hasten identification of pathogens, leading to early treatment. Third, monoclonal antibody therapy is pathogen specific, which is problematic in dealing with unknown infections early in their course and with mixed infections with multiple organisms or serotypes. For pathogens that are antigenically variable, use of monoclonal antibody cocktail preparations that combine monoclonal antibodies to common antigenic serotypes may be beneficial. Antibody cocktails also may be designed to include monoclonal antibodies of different isotypes (IgM, IgG, IgA) to further enhance their effectiveness. Fourth, combination therapy with monoclonal antibodies and antimicrobial chemotherapy agents may be an alternative means to treat bacteria and their components in the development of septic shock. Such therapies could potentially reduce the amount or duration of antibiotic treatment needed and reduce the number of antibiotic-resistant pathogens. Combination therapy also may be clinically beneficial in cases in which the antimicrobial agents are toxic themselves. Fifth, monoclonal antibodies may elicit self-neutralizing antibodies. Thus, the administration of a monoclonal antibody could promote the production of antibody against the monoclonal antibody itself, which would diminish the clinical response and potentially cause severe anaphylaxis in cases of antibody retreatment. Likewise, widespread use of monoclonal antibody therapy could select for antibody-resistant pathogens or other serotypes or even entirely different infecting organisms.[167]

Monoclonal antibodies directed against viral glycoprotein complexes or that immune modulate through other mechanisms are also being developed and evaluated for human use for a variety of viral diseases. Palivizumab, a humanized F protein monoclonal antibody, is routinely administered to preterm infants and other high-risk infants for immunoprophylaxis against respiratory syncytial virus (RSV) infection and disease.[95] It also is used for prophylaxis against RSV infection in older high-risk subjects, such as transplant recipients and children with cystic fibrosis.[239,240] In addition, palivizumab is administered in some patients to treat established RSV infections in immune compromised patients, while awaiting immune reconstitution. Recently, a recombinant human antirabies monoclonal antibody has been tested in India. It appears to be safe and equally efficacious than conventional serum-derived rabies immune globulin (RIG) and may soon replace the use of RIG for postexposure prophylaxis.[103,214,215] Monoclonal antibodies directed against vaccinia virus have been developed and tested in preclinical animal models as potential therapeutic antibodies for treatment of smallpox,[59] especially in viral glycoprotein complexes. The anti-angiogenic monoclonal antibody bevacizumab (Avastin) is also been used to treat recurrent respiratory papillomatosis.[19] Monoclonal antibodies directed against glycoprotein N of human cytomegalovirus (CMV) enhances neutralizing antibody activity in vitro and possibly in the future may be used to enhance natural humoral immunity response against human CMV.[157] In addition, postexposure immunoprophylaxis and treatment of alphavirus infections, such as Chikungunya virus, using human monoclonal antibodies are also being studied.[94]

Researchers have examined the possible blockade of the proinflammatory cascade that accompanies severe infections.[1,14,99,166] The experimental attempts using monoclonal antibodies to modify the release and action of the proinflammatory mediators that lead to septic shock have focused on the proinflammatory effects of the cytokine family, endotoxin, and the functions of neutrophils and complement. Eculizumab, a humanized monoclonal anti-C5 complement antibody that prevents the activation of terminal complement pathway, is now used as treatment in atypical hemolytic uremic syndrome.[271]

Treatment with monoclonal antibodies, such as rituximab, an antiCD20 therapeutic monoclonal antibody, and infliximab, an anti–tumor necrosis factor–α (TNF-α) monoclonal antibody, in patients with immune disorders increases the risk for certain infections, such as nontuberculous mycobacteria.[36,236]

THE CYTOKINES

Lymphokines and Monokines

The cytokines are a family of small soluble protein molecules responsible for cell-to-cell communication. They are produced by several cell types and play crucial roles in many biologic processes, including growth, inflammation, immunity, and hematopoiesis. During infection, genes for nearly all the cytokines are expressed. The biologic activity of the prototype cytokines, the lymphokines and monokines, include interleukins (ILs) and TNF-α; these molecules, along with the granulocyte colony-stimulating factors, have received considerable attention as potential immunomodulatory agents. In response to pathogen invasion, these cytokines perform a complex series of interactions to initiate a cascade of biologic events resulting in the propagation and subsequent regulation of the inflammatory response, leading to pathogen alienation while maintaining host preservation. Thus, the cytokine family, through a complex web of interactions, functions to initiate and then both to upregulate and downregulate the inflammatory response. On the basis of their roles of either upregulating or downregulating immune responsiveness, the cytokines generally and historically have been classified as either proinflammatory or anti-inflammatory molecules. Although many cytokines have the potential to perform dual functions, either directly or indirectly, their proinflammatory or anti-inflammatory properties are of considerable basic science and clinical therapeutic interest.*

*References 27, 51, 70, 91, 166, 174, 184, 226.

Some prominent proinflammatory cytokines and their cellular sources are listed in Table 241-1. TNF-α and IL-1 generally are considered to be prominent early proinflammatory mediators. They induce gene expression of other proinflammatory cytokines, including IL-6 and IL-8, leading to neutrophil activation, recruitment, and degranulation. TNF-α and IL-1 also activate a secondary cascade of inflammatory mediators, including arachidonic acid–derived prostaglandin I_2, thromboxane A_2, prostaglandin E_2, platelet-activating factor, and the complement system. The colony-stimulating factors and IL-3 also are proinflammatory cytokines that induce bone marrow stem cell production of granulocytes and monocytes in addition to activating neutrophils and inducing production of IL-1 and TNF-α.*

Prominent among the anti-inflammatory cytokines (Table 241-2) are IL-4, IL-10, IL-13, and transforming growth factor–β. These cytokines may block endotoxin induction of IL-1 and TNF-α and suppress lymphocyte and monocyte function. In addition, IL-1 receptor antagonist blocks the proinflammatory action of IL-1 by binding its receptor.†

Considerable experimental and clinical interest has focused on the proinflammatory cytokines TNF-α and IL-1 as important early mediators in the pathogenesis of sepsis and septic shock syndrome. The basis for the potential therapeutic utility of these two cytokines in human disease states involves two clinically distinct hypotheses: (1) excessive cytokine production results in host immune injury, leading to severe shock; and (2) deficient cytokine production renders a host susceptible to infection by invasive pathogens.[166]

The hypothesis that cytokine overproduction can lead to severe lethal shock is demonstrated in several animal models and is suggested in patients with overwhelming sepsis. The outer membranes of gram-negative bacteria contain lipopolysaccharides or endotoxin, which induce the early proinflammatory cytokines TNF-α and IL-1. Although these cytokines may protect the host from infection, if they are expressed in excessive amounts, their effects can result in multiple organ failure and death. During severe sepsis, the levels of both TNF-α and IL-1 increase proportionately with the degree of hypotension and organ failure. The combination of these two cytokines can result in synergism over their individual effects by severalfold, leading to lethal septic shock syndrome.[39,40] In animal models of shock and gram-negative sepsis, TNF-α levels rise rapidly after injection of bacteria or endotoxin, reaching peak concentrations at 60 to 90 minutes, whereas IL-1 levels rise more slowly, peaking at 180 minutes. A similar time course response has been observed in human subjects injected with endotoxin.[40,206] Children with septicemia and purpura fulminans and children with meningococcal disease demonstrated an association between morbidity and mortality and high serum levels of TNF-α and IL-1.[100,214] These studies and others implicate TNF-α and IL-1 as prominent modulators in the development of the septic shock syndrome and suggest that a potential therapeutic benefit may be obtained by inhibiting the production of these cytokines and reducing their proinflammatory effects.

Experimental attempts to attenuate the excessive proinflammatory cytokine activity of TNF-α and IL-1 have focused on (1) inhibiting release of endotoxin, (2) blocking endotoxin–target cell binding and preventing the transmembrane signaling mechanisms leading to production of TNF-α and IL-1, (3) controlling the synthesis of TNF-α and IL-1 by inhibiting or suppressing specific cytokine gene transcription and translation, (4) inhibiting release of TNF-α and IL-1, (5) administering TNF-α and IL-1 neutralizing antibodies and soluble receptors, (6) producing and administering TNF-α and IL-1 receptor antagonists that block specific cytokine binding to target cell receptors, and (7) blocking TNF-α or IL-1 intracellular transmembrane signaling mechanisms and preventing their action on target cells.[174]

The use of monoclonal anti-endotoxin antibodies to inhibit the binding of endotoxin to its target cells has received considerable attention. Numerous in vitro and in vivo animal experiments have suggested that blocking of endotoxin leads to improved survival by inhibiting proinflammatory cytokine production and expression.[216] The initial clinical studies in patients with gram-negative bacteremia treated with immunoglobulin preparations directed against endotoxin demonstrated a significant reduction in mortality rates.[15,248] Further studies suggested a reduction of septic shock in similarly treated high-risk surgical patients.[15] These observations led to the development of several clinical trials using human monoclonal anti-endotoxin antibodies. HA-1A is a human monoclonal antibody against the lipid A moiety of bacterial endotoxin. The mechanism of HA-1A action is to block endotoxin triggering of the intracellular events leading to proinflammatory cytokine synthesis. In placebo-controlled clinical trials of HA-1A, either HA-1A or placebo was infused during the course of 20 minutes to patients with severe sepsis. These patients also received cardiopulmonary support and antibacterial therapy. The etiologic agents of sepsis included *E. coli*, *Pseudomonas*, and *Klebsiella* and *Enterobacter* spp. The authors reported that HA-1A significantly reduced the incidence of mortality in adults with septic shock and gram-negative bacteremia. The mortality rate for those patients who were in severe shock before receiving HA-1A was reduced by 42 percent.[298] Although these early clinical studies using HA-1A were encouraging, a protective role for anti-endotoxin antibodies has not been established in subsequent clinical trials.[189] Anti-endotoxin therapy may be more effective if it is given earlier during the sepsis syndrome before the development of shock. Because bacterial lysis caused by antibiotics is an ongoing process and results in further release of endotoxin, multiple dosing of anti-endotoxin antibodies also may prove beneficial in treating sepsis. The future clinical use of anti-endotoxin antibodies may be in combination with other immunomodulation therapies, directed at simultaneously blocking several steps in both the propagation and action of proinflammatory cytokines.

Studies of cytokine inhibition have focused on controlling TNF-α and IL-1 with anti–TNF-α antibodies

*References 27, 51, 70, 91, 166, 174, 184.
†References 27, 51, 70, 91, 166, 174, 184.

TABLE 241-1 **Proinflammatory Cytokines**

Cytokine	Function	Predominant Cell Source
Tumor necrosis factor–α	Stimulates interleukin-6 and colony-stimulating factors Depresses erythropoiesis, stimulates interleukin-8 and interleukin-9 Promotes tumor necrosis and endotoxic shock	Monocytes and macrophages
Interleukin-1	Stimulates proliferation and differentiation of T and B lymphocytes Stimulates T lymphocytes to produce interleukin-2 Promotes colony-stimulating factor, interleukin-8 and interleukin-9 production, and endotoxic shock	Macrophages, astrocytes, monocytes, fibroblasts, keratinocytes, B cells, corneal epithelium, and other cell types
Interleukin-2	Stimulates growth of T lymphocytes Stimulates B-lymphocyte and monocyte differentiation Increases cytotoxicity of T lymphocytes and natural killer cells	Activated T lymphocytes
Interleukin-3	Multipotential hematopoietic cell growth factor Stimulates early B and T lymphocytes Mast cell growth factor	Activated T lymphocytes, natural killer cells
Interleukin-5	Stimulates eosinophil formation and differentiation Augments T lymphocyte cytotoxicity and proliferation of B lymphocytes	T lymphocytes, mast cells
Interleukin-7	Supports growth of pre-B lymphocytes Stimulates T lymphocytes	B lymphocytes, bone marrow fibroblasts, monocytes
Interleukin-8	Stimulates neutrophil, monocyte, and lymphocyte activation chemotaxis	Monocytes
Interleukin-9	Stimulates neutrophil, monocyte, and lymphocyte activation chemotaxis Stimulates erythroid progenitors, helper T-lymphocyte growth factor	T lymphocytes
Interleukin-11	T lymphocyte–dependent stimulator of B lymphocytes	Bone marrow fibroblasts
Interleukin-12	Stimulates helper T-lymphocyte differentiation and interleukin-2 production Stimulates interferon-γ production Increases cytotoxicity of natural killer cells	T and B lymphocytes, lymphoblastoid cells
Interleukin-14	Stimulates proliferation of activated B lymphocytes Inhibits immunoglobulin secretion from B lymphocytes	T lymphocytes
Interleukin-15	Stimulates T-lymphocyte function and proliferation Enhances natural killer cell function	Monocytes and macrophages
Interleukin-16	Promotes migration of T lymphocytes	T lymphocytes
Interleukin-17	Stimulates interleukin-6 and interleukin-8 production	T lymphocytes
Interleukin-18	Stimulates interferon-γ and tumor necrosis factor–α production	Macrophages, mononuclear cells, and dendritic cells
Granulocyte colony-stimulating factor	Stimulates neutrophil colony formation	Monocytes and fibroblasts
Granulocyte-macrophage colony-stimulating factor	Stimulates granulocyte and monocyte formation Induces tumor necrosis factor	T lymphocytes, natural killer cells, endothelial cells, fibroblasts, and keratinocytes
Macrophage colony-stimulating factor	Activates monocytes and granulocytes Stimulates macrophage colony formation Induces interleukin-1 and tumor necrosis factor	Fibroblasts, monocytes, and endothelial cells

directed at these specific cytokines and their receptors. Control of proinflammatory cytokine synthesis is specific for each individual cytokine and requires an understanding of the unique temporal relationships these molecules have during the propagation of inflammatory responses. A critical aspect of TNF-α and IL-1 gene expression in a variety of cell types has been the reported exquisite sensitivity these cytokines have to bacterial endotoxin.[51,70,166,174] Human blood monocytes synthesize TNF-α and IL-1 in the presence of endotoxin. In the absence of endotoxin, however, gene expression occurs, but protein translation does not take place.[249] Thus, these cells may be viewed as being primed for bacterial endotoxin exposure. TNF-α and IL-1 transcription is suppressed by the anti-inflammatory cytokines IL-10, IL-13, and transforming growth factor–β, but the clinical therapeutic benefits these cytokines have in treating septic shock remain to be defined.[53,117,208,250] Agents blocking the

TABLE 241-2 **Anti-inflammatory Cytokines**

Cytokine	Function	Predominant Cell Source
Interleukin-4	Stimulates proliferation of T and B lymphocytes and megakaryocytes A growth factor for mast cells and erythroid precursors	T lymphocytes
Interleukin-6	Blocks endotoxin induction of interleukin-1 and tumor necrosis factor B and T lymphocyte–stimulating activity	Monocytes, T and B lymphocytes, fibroblasts, epithelial and endothelial cells
Interleukin-10	Blocks production of interleukin-1 and tumor necrosis factor Inhibits primary allogeneic T-lymphocyte responses Inhibits interleukin-2, interleukin-8, and granulocyte-macrophage colony-stimulating factor	T and B lymphocytes, macrophages and monocytes
Interleukin-13	Blocks production of interleukin-1, tumor necrosis factor, and interleukin-8 Suppresses nitric oxide formation	T lymphocytes
Interleukin-1 receptor antagonist	Binds interleukin-1 receptors, blocking interleukin-1 effects	Monocytes and macrophages
Transforming growth factor–β	Reduces endotoxin-induced interleukin-1 and tumor necrosis factor production	Monocytes and macrophages

lipoxygenase pathway of arachidonate metabolism also have been implicated in the reduction of TNF-α and IL-1 synthesis, and corticosteroids have been shown to suppress both TNF-α and IL-1 transcription and synthesis, but only when they have been administered before transcription has been initiated.[89,247] The use of corticosteroids in infants and children with bacterial meningitis has demonstrated that treatment with a combination of dexamethasone and antibiotics results in lower cerebrospinal fluid levels of TNF-α than occurs with treatment with placebo and antibiotics.[214] In addition, patients treated with corticosteroids had fewer neurologic symptoms.[147] Subsequent multicenter trials, however, have failed to establish a protective effect of corticosteroid use in the treatment of children with meningitis.[277] One possible explanation for this discrepancy may be that corticosteroids almost exclusively suppress endotoxin-induced, proinflammatory cytokine gene transcription but have little or no effect on proinflammatory cytokine translation.[70,259] Thus, some investigators have suggested that the early administration of corticosteroids, before transcription has been initiated, would block cytokine synthesis. Timing of corticosteroid administration, therefore, may account for some of the clinical variability seen with its use in children with meningitis. Clinical trials are focusing on TNF-α and IL-1 synthesis with the administration of corticosteroids either before or while antibiotics are administered. Similarly, the temporal use of antibiotics, corticosteroids, and anti-endotoxin therapy may result in an additive therapeutic benefit.

Neutralizing monoclonal antibodies against murine or human TNF-α have been shown to decrease mortality rates in several experimental animal models of sepsis.[20,197,270] Studies with soluble TNF-α receptors or their immunoadhesin constructs also have demonstrated an immunoprotective effect of TNF-α blockade in animal models of endotoxemia or bacteremia.[18] Although anti–TNF-α antibodies are being used with caution in humans, anti–TNF-α treatment in a limited number of patients with established septic shock resulted in increased vascular hemodynamics and left ventricular stroke volume.[111] The in vitro use of free soluble TNF-α receptors to bind TNF-α results in a 10- to 50-fold increase in binding affinity over that observed with anti–TNF-α monoclonal antibodies.[112] Results from phase II clinical trials using soluble TNF-α receptors, however, have not shown improvement in survival rates; moreover, administration of high doses actually increased the incidence of mortality.[112,245]

Certain naturally occurring substances inhibit IL-1 synthesis and action, but they also have effects on many of the other cytokines. Specific inhibitors of IL-1, however, have been identified. Most prominent of these is the IL-1 receptor inhibitor that competes with the binding of IL-1 to its cell surface receptor. Recombinant IL-1 receptor antagonist (IL-1ra) that blocks IL-1 effects has been studied in various animal models.[4,224] For instance, IL-1ra prevents death from endotoxic shock in rabbits.[224] The therapeutic use of IL-1ra in treating septic shock in early phase II human clinical trials showed improved survival rates at 28 days.[90] The subsequent randomized phase III trials, however, failed to show improvement in patients with severe shock.[89] Further clinical studies are ongoing. IL-1ra also has been used in treatment trials of patients with acute myelogenous leukemia. The uncontrolled production of IL-1 by leukemic blasts has been proposed to result in the continued proliferation of these cells and the development of acute myelogenous leukemia. Studies have shown that IL-1ra blocks the spontaneous proliferation and production of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1, and IL-6 in the peripheral blood and bone marrow cells of these patients.[235] The potential clinical use of IL-1ra is being investigated in patients with psoriasis, rheumatoid arthritis, and myelogenous leukemia.[114] Similarly, combination therapy with the simultaneous blockade of both TNF-α and IL-1 is being investigated in endotoxin models of shock and a number of clinical disease states.[243]

Another approach to attenuate excessive proinflammatory cytokine responses is the use of pharmacologic agents. Salyer and colleagues[246] demonstrated that

pentoxifylline, a methylxanthine derivative that blocks TNF-α transcription and production, could override some of the effects of TNF-α on PMNs. In their study, the profound decrease in human PMN chemotactic ability caused by excessive TNF-α was restored to normal by treatment with pentoxifylline. The specific mechanisms of pentoxifylline's effect are not known, but it has been shown to restore PMN membrane fluidity inhibited by TNF-α that is critical for cell movement.[220,221] Furthermore, pentoxifylline can block PMN adhesion to endothelium and result in decreased PMN respiratory burst activity, which is thought to be responsible for the improved survival rates seen in pentoxifylline-treated animal models of endotoxin-induced septic shock.[225] Some of the clinical trials using pentoxifylline in surgical patients with early evidence of systemic inflammation have suggested a cardioprotective effect, although other studies have been less convincing.[82,262] Pentoxifylline also has been evaluated in preterm infants with sepsis.[176] A total of 78 infants with documented sepsis were randomly assigned to receive pentoxifylline (5 mg/kg/hr for 6 hours on 6 successive days) or placebo. Pentoxifylline-treated infants had significantly lower levels of TNF-α but not of IL-1. Only 1 of 40 infants in the pentoxifylline-treated group died, compared with 6 of 38 in the control group (p <.05). Amrinone, a phosphodiesterase inhibitor, also has been shown to reduce TNF-α levels in LPS-challenged mice.[101] Thus, these and other pharmacologic agents may be useful in preventing or reducing some of the adverse and even fatal effects that are mediated by proinflammatory cytokines.

IVIG may have a potential inhibitory effect on proinflammatory cytokine activity. Patients in the active phase of Kawasaki disease have increased levels of TNF-α and IL-1. These cytokines have been postulated to stimulate local inflammatory responses by regulating leukocyte adherence and activation, leading to the vascular damage that is a critical clinical aspect of this disease. Another suggestion is that the effects of IVIG therapy in Kawasaki disease, and perhaps in other diseases, may be results of the attenuating production of the proinflammatory cytokines TNF-α and IL-1.[6,11,124] Animal models of LPS-induced TNF-α and IL-1 synthesis demonstrate a suppression of mononuclear cell synthesis of these proinflammatory cytokines when they are treated with IVIG.[13] The peripheral blood mononuclear cell production of TNF-α and IL-1, however, in patients with Kawasaki disease receiving IVIG therapy showed decreased synthesis of IL-1 but not of TNF-α.[177,178] The role of IVIG as an immunomodulator has been suggested in an ever-growing number of clinical disease states because of its broad immunoregulatory potential.[11,120] Its specific role in arresting proinflammatory cytokine activity, either directly or indirectly, warrants further clinical investigation.)

The hypothesis that diminished levels of cytokines may render a host susceptible to development of infection was introduced by Weatherstone and Rich.[280] They suggested that the increased susceptibility to infection observed in premature neonates may be secondary to deficient proinflammatory cytokine production. In their study, they measured cord blood monocyte secretion of TNF-α and IL-1 with and without stimulation of LPS. IL-1 activity by LPS-stimulated preterm monocytes did not differ from that observed by LPS-stimulated adult monocytes. TNF-α activity, however, in the LPS-stimulated monocytes from preterm neonates was significantly lower than that in both stimulated and unstimulated adult monocytes. Thus, they concluded that diminished production of TNF-α may predispose the preterm infant to acquisition of infection. This finding has not been supported by the studies of infected animals, which show markedly elevated TNF-α levels, and subsequent studies in preterm infants have not established a deficiency in TNF-α.[287] Williams and colleagues[287] and Peat and coworkers[227] have, in contrast, shown that mononuclear cells from term neonates produced enhanced levels of TNF-α in response to group B streptococci or endotoxin. Ongoing studies are defining the role of cytokine production in neonatal and other infections.

The proinflammatory cytokine IL-2 acts on activated T cells and to some extent on B cells and natural killer (NK) cells and causes them to proliferate or differentiate. IL-2 is synthesized by both T cells and NK cells.[207,278] Decreased IL-2 production or IL-2 receptor expression has been noted in a number of clinical disease states, most notably in cases of severe combined immunodeficiency disease. Lesser degrees of abnormalities may occur in acquired immunodeficiency syndrome (AIDS), type 1 diabetes mellitus, systemic lupus erythematosus, and hypogammaglobulinemia.[166,174,184] The most intriguing potential for IL-2 in the treatment of disease involves its use in tumor therapy.[242] IL-2 currently is approved for the treatment of metastatic renal carcinoma. Use of IL-2–activated NK cells results in a decrease in tumor burden in approximately 20 percent of patients, although serious side effects occur. In addition to its potential role as an antitumor agent, IL-2 shares many of the same effects as interferon-γ (IFN-γ) and may, someday, function as a therapeutic agent in infection, autoimmunity, and immunodeficiency.[174,242]

Both IL-8 and IL-9 have been investigated in various human disease states, primarily because of their powerful role in stimulating neutrophil function, including activation, adhesion, and chemotaxis. Patients with cystic fibrosis, bronchiectasis, and chronic bronchitis have demonstrated elevated levels of IL-8 in their sputum.[237] The sputum from these patients is highly chemotactic to neutrophils, but with treatment with monoclonal antibodies to IL-8, this chemotactic effect was inhibited. Aerosolized IL-8 inhibitors have been used in patients with cystic fibrosis and have resulted in decreased inflammation in these patients.[199,237] IL-8 inhibitors are being evaluated in infants with bronchopulmonary dysplasia. Researchers have speculated that the persistently elevated levels of IL-8 in the tracheal fluid of ventilated preterm infants lead to neutrophil accumulation and the development of pulmonary fibrosis, which may be reduced by IL-8 inhibitors.[143,154] Similar studies are being conducted with IL-9 inhibitors. Thus, these cytokines may play an important role in the acute inflammatory response leading to chronic disease states, and blocking of this response may be of therapeutic benefit. The proinflammatory IL-5 has not been implicated in a specific human disease state, but

its strong B-cell proliferative effects suggest that it has a possible role in the pathogenesis of immunodeficiency, and its potent effects on eosinophil production, activation, and migration implicate its action in allergic responses.[37]

The proinflammatory activities of IL-12 and IL-18 are of current immunologic interest as well. IL-12 is an integral immune regulator that is produced primarily by macrophages and dendritic cells.[222] IL-12 has been shown to induce the production of IFN-γ by T cells and natural NK cells.[192,193,222] IL-18 is another recently described cytokine with many proinflammatory functions.[201] IL-18 was defined initially as IFN-γ–inducing factor.[41,71,175] IL-12 and IL-18, both alone and in synergy, regulate IFN-γ production in response to infection with intracellular parasites and bacteria and in certain autoimmune diseases.* The regulation of these cytokines may be therapeutically beneficial in certain disease states.[77,107,196] The other proinflammatory cytokines also are in various developmental stages of experimental investigation. As we learn more about their specific actions, we will better be able to determine their use as potential immunomodulating agents.

The anti-inflammatory cytokines have limited clinical use. IL-4 can block both IL-1 and TNF-α transcription.[117,250] IL-4 has been shown to inhibit human neutrophil adhesion to human endothelial cells while enhancing the adhesion of eosinophils.[211] These effects have not been implicated in specific human diseases, but an IL-4 action is suggested in allergic responses. Although IL-6 has both anti-inflammatory and proinflammatory effects, the levels of this cytokine were found to correlate with mortality rates in children with gram-negative and gram-positive sepsis, suggesting that monitoring of IL-6 levels may be of prognostic value.[274] Similarly, IL-10 has been shown to block production of IL-1, IL-6, IL-8, IL-12, and TNF-α as well as GM-CSF in animal models.[63,194] It also has effects on mast cells, T cells, and NK cells, and it inhibits primary allogeneic T-cell responses. Thus, IL-10 may have a potential role in treating acute and chronic inflammation and may be effective in suppressing transplant rejection.[16,63] Studies examining the safety and immunomodulatory effects of the intravenous injection of IL-10 in humans demonstrate that it is well tolerated and results in decreased production of both TNF-α and IL-1.[27] Additional clinical studies have been conducted on the potential immunoregulatory effects of the anti-inflammatory cytokines, which may indicate their therapeutic use in human disease states.

Interleukin-17 (IL-17) is a recently described proinflammatory cytokine implicated in the regulation of many inflammatory states. IL-17 is produced by several cell types including activated T-cell subsets, NK cells, and neutrophils.[73,186,265] IL-17 is involved in the regulation of inflammatory bowel disease and systemic lupus erythematosus (SLE).[156] IL-17 production is markedly deficient in autosomal dominant hyper-IgE (Job syndrome) patients who have neutrophil chemotactic defects and suffer recurrent bacterial and candida infections.[168] More recently, decreased intracellular and extracellular production of IL-17 by cord blood mononuclear cells suggests IL-17 may contribute to the human neonates' increased susceptibility to microbial infections.[47,48] Furthermore, the addition of IL-17 to cord blood mixed mononuclear cells significantly enhances production of the proinflammatory cytokines IL-6, TNF-α, IL-1β, and IL-8 in response to group B streptococci.[47,48] IL-23 promotes the development and survival of IL-17 producing cells. The IL-17/IL-23 axis has been implicated in mediating initial neutrophil responses during infection. Dysregulation of this pathway is suggested in autoimmune diseases, i.e., multiple sclerosis, Crohn disease, and rheumatoid arthritis.[73,74,264] IL-17/IL-23 levels are elevated in Lupus patients and correlate with symptomatology.182,[291,294] Current efforts are under investigation to modulate the IL-17/IL-23 axis to regulate autoimmunity and inflammation.[148]

The removal of cytokines from blood of septic patients has received recent attention. The continuous removal of cytokines in the systemic inflammatory response syndrome by hemofiltration and hemodialysis is currently being utilized.[291] Cytokine adsorption has also received recent attention. The use of synthetic polymer resins,[146] immobilized antibody systems,[221] and activated carbon[10] is under investigation. The polymyxin B filtration system has been shown to protect mice from the endotoxin-induced shock[35]and is under investigation in adult patients with gram-negative sepsis in intensive care units.[61] These efforts to reduce the proinflammatory cytokine load may have a role in the treatment of septic shock.

Colony-Stimulating Factors

Colony-stimulating factors are involved principally in the production of neutrophils and monocytes. They were discovered because of their ability to stimulate the formation of colonies of granulocytes and monocyte-macrophages in cultured bone marrow cells and were named according to the primary cell colony type that they elicited. Granulocyte-macrophage colony-stimulating factor (GM-CSF) induces peripheral blood macrophages and granulocytes. It also has other pleiotropic effects, including stimulation of precursors of megakaryocytes, mast cells, and eosinophils. In addition, GM-CSF has effects on neutrophil migration and phagocytosis. Granulocyte colony-stimulating factor (G-CSF) induces peripheral blood granulocytes. Its actions are on both the production and function of neutrophils, including migration, phagocytosis, and superoxide generation. Macrophage colony-stimulating factor (M-CSF) induces peripheral mononuclear phagocytes. IL-3 increases mast cell populations as well as the induction of granulocytes, macrophages, eosinophils, and megakaryocytes.*

Clinical studies with GM-CSF and G-CSF as adjuvant therapy have been performed in individuals with distinct lymphopoietic disorders (i.e., congenital agranulocytosis, cyclic neutropenia, Shwachman-Diamond syndrome) or those due to the consequences of cytotoxic

*References 71, 77, 107, 128, 134, 145, 196, 223, 260, 264, 265, 275, 281, 295.

*References 72, 73, 110, 164, 175, 183, 205.

chemotherapy, AIDS, and aplastic anemia. The colony-stimulating factors can stimulate granulocyte and monocyte populations in these individuals, but the response is restricted to the number of available stem cells. Colony-stimulating factor treatment can partially or completely reverse congenital neutropenia and has shown great promise in regeneration of lymphopoietic cells after administration of cytotoxic chemotherapy and high-dose chemotherapy followed by autologous bone marrow transplantation. Recombinant human GM-CSF (rhGM-CSF) also has been shown to be beneficial in patients with aplastic anemia.[9,10,272] Hammond and colleagues[115] demonstrated a dramatic increase in neutrophil counts in children with cyclic neutropenia treated with recombinant human G-CSF (rhG-CSF). Cyclic neutropenia is a rare disorder characterized by regular 21-day cyclic fluctuations in the number of blood neutrophils, monocytes, eosinophils, lymphocytes, platelets, and reticulocytes. Although the exact mechanism of this disorder is not known, it is attributed to a regulatory abnormality affecting proliferation of stem cells. These infants have recurrent aphthous stomatitis, pharyngitis, lymphadenopathy, fever, and numerous infections during the periods of neutropenia. The length of cycling in treated infants decreased from 21 days to 1 day, and the neutrophil turnover rate increased nearly fourfold, significantly reducing the frequency of infection. Recombinant G-CSF also has shown promise for infants and children with neutrophil production disorders. Infants with congenital agranulocytosis, Kostmann syndrome, and Shwachman-Diamond syndrome, disorders characterized by severe, persistent absolute neutropenia, show a dramatic increase in neutrophil count after treatment with rhG-CSF.[26] In addition, rhG-CSF and rhGM-CSF have been used in neutropenic patients with AIDS. Despite the concern that the effects of GM-CSF to activate macrophages may in turn promote replication of human immunodeficiency virus (HIV), current studies are defining the role of rhGM-CSF and rhG-CSF in patients with AIDS.[109,183]

Thus, colony-stimulating factors have emerged as significant modulators of human immune function and lymphopoiesis. Because the colony-stimulating factors can functionally activate mature granulocytes and monocytes, considerable attention has focused on their future role in treating individuals at risk for acquiring infection. English and colleagues[76] reported decreased GM-CSF production by neonatal T cells. Because a major factor contributing to the increased susceptibility of human neonates to severe infections is their inability to produce adequate numbers of neutrophils in response to bacterial infections, these neonates may benefit from colony-stimulating factor treatment during severe infection.[22] Similar treatment in immunologically stressed burn and trauma patients also may prove beneficial.[65,219]

INTERFERONS

Interferon-α and Interferon-β

The interferons are glycoproteins that were discovered because of their antiviral properties. They are known to possess antitumor and immunomodulatory activities in addition to their antiviral effects.[12] The interferons have been classified into three major groups: α, β, and γ. IFN-α and IFN-β were previously known as type I interferon and have similar protein structures and bind the same receptor. IFN-γ, formerly type II interferon, has a much different structure and its own receptor.[8] IFN-α is produced by leukocytes. The earliest demonstration of its clinical usefulness was in the treatment of AIDS-related Kaposi sarcoma. In these initial studies, a Kaposi sarcoma tumor response occurred in 30 to 50 percent of the patients treated with recombinant IFN-α.[108,161,209,213] Researchers have speculated that IFN-α exerts its antitumor effect by activating cytotoxic T cells. Since then, placebo-controlled clinical trials have shown that IFN-α also may have significant antiretroviral effect in HIV-infected patients.[159,172] Clinical trials have been conducted to determine the effect of early treatment with IFN-α in reducing progression of HIV disease and to determine the therapeutic effect of IFN-α in combination with other drugs in the treatment of HIV infection and HIV-related diseases.[158,160]

IFN-α also has been used in various parts of the world for the treatment of chronic myeloid leukemia, hairy-cell leukemia, basal cell carcinoma, multiple myeloma, hepatitis B and C, and condylomata acuminata (genital warts). In chronic myeloid leukemia, early treatment with IFN-α has been reported to elicit complete hematologic remission in more than 70 percent of the patients treated. Many of these patients had total elimination of the Philadelphia chromosome, which is a hallmark of the disease.[266-268] Complete to 80 percent remissions also have been reported in the treatment of hairy-cell leukemia.[232] Intralesional injection of IFN-α into basal cell carcinoma of the skin resulted in 81 percent tumor remission as determined by biopsy.[57,139] Despite the variability of the outcomes among reports on the use of IFN-α in the treatment of multiple myeloma, it has been approved as a therapy for these patients in a number of European countries.[195]

IFN-α also is used for the treatment of hepatitis B and C.[175] Clinical responses have been reported to be of long duration, often with complete loss of both hepatitis B surface antigen and evidence of viral replication. In chronic hepatitis C, complete responses to therapy determined by the decline of serum aspartate aminotransferase to normal levels have been observed in 50 to 70 percent of the patients treated. Although serum aspartate aminotransferase levels in half of the patients who had improved returned to pretreatment levels within 6 to 12 months after discontinuation of therapy, nearly 20 percent achieved sustained remission.[66,69,133,155,228] Studies also have shown that IFN-α is useful in treating condylomata acuminata caused by papillomaviruses.[34] Intralesional injections of IFN-α completely eliminated warts in more than 50 percent of the patients treated. IFN-α therapy also is suggested to reduce the number of lesions in juvenile laryngeal papillomatosis after systemic use.[190] Thus, IFN-α has therapeutic potential as both an antitumor and an antiviral agent.

IFN-β also has received attention as an antiviral and antitumor agent. Its clinical usefulness as a single-agent

therapy is suggested in relapsing multiple sclerosis. The mechanism of IFN-β therapeutic action in multiple sclerosis is unknown.[140] Ongoing studies are investigating IFN-β therapy in combination with IFN-α in the treatment of various malignant neoplasms.

Interferon-γ

IFN-γ is produced by $CD4^+$ and $CD8^+$ cells as well as by NK cells.[122] Investigators have reported that circulating mononuclear cells and T cells from neonates are markedly deficient in their ability to produce IFN-γ in response to a variety of stimuli, compared with adult cells.[182,252,253,288] Studies using recombinant IFN-γ have shown that preincubation of neonatal neutrophils, which are deficient in their chemotactic ability, with recombinant IFN-γ enhances their chemotactic response to a level equal to that of adult neutrophils.[108] Other studies have shown that neonatal mixed mononuclear cells are deficient in the production of the IFN-γ–stimulating cytokines IL-12 and IL-18 in response to GBS.[126,128,144] In vitro treatment with recombinant IL-12 and IL-18 can correct this defect in IFN-γ production in response to GBS. These findings suggest that a potential role exists for the regulation of IFN-γ in neonatal host defense.

Job syndrome was first described by Davis, Schaller, and Wedgwood[67] in 1965 in two patients with recurrent staphylococcal abscesses. Patients with this syndrome often develop chronic sinopulmonary infections and mucocutaneous *Candida* infections. Hill and coworkers[129] observed that the patients with Job syndrome also have a profound defect in neutrophil chemotactic responsiveness along with extreme hyperimmunoglobulin E. This defect in neutrophil chemotaxis is intermittent and occurs predominantly when the patient is symptomatic.[64,123,129] Because production of IFN-γ by mononuclear leukocytes in patients with hyperimmunoglobulin E is markedly deficient or absent, in vitro studies were conducted to determine the effect of recombinant IFN-γ on the chemotactic responsiveness of neutrophils from patients with this syndrome. After pretreatment with IFN-γ, the chemotactic response of the neutrophils from patients with Job syndrome increased significantly, with an average enhancement of 300 percent above baseline to levels not significantly different from those of matched healthy controls.[142,295] In four patients with Job syndrome, preliminary trials of IFN-γ therapy with hyperimmunoglobulin E suggested clinical benefit in three, with a significant decrease in eczema as well as in pulmonary symptoms and secretions.

Patients with chronic granulomatous disease (CGD) have an inherited deficiency in the proteins required for nicotinamide adenine dinucleotide phosphate oxidase activity. Phagocytes with this enzymatic defect are able to engulf bacteria but cannot generate the respiratory burst necessary to kill the organisms. Consequently, patients with CGD suffer severe chronic, recurrent life-threatening infections. The usefulness of treating CGD patients with IFN-γ was suggested by studies showing that this lymphokine can stimulate the respiratory burst of normal phagocytes. Results of studies by Ezekowitz and colleagues[80] and Sechler and coworkers[251] showed that when macrophages from patients with CGD were treated with IFN-γ in vitro, a respiratory burst occurred and superoxide anion was generated. Sechler and colleagues[251] further demonstrated a partial correction in neutrophils and monocytes from patients with CGD after subcutaneous treatment with recombinant IFN-γ. These initial results suggested that when it is administered in vivo to patients with CGD, recombinant IFN-γ could partially correct the defective ability of phagocytes to kill bacteria. Ezekowitz and coworkers[81,122] extended these findings in a double-blind placebo-controlled trial. They showed that recombinant IFN-γ significantly decreased the relative risk for development of serious infection in patients with CGD, but these studies failed to demonstrate an effect of IFN-γ on respiratory burst activity. This cytokine most likely affects the arginine–nitric oxide pathway in neutrophils and macrophages. Patients who received IFN-γ had a 70 percent reduction in the risk for development of serious infection compared with controls. Overall, IFN-γ decreases the risk for development of infection and the duration of hospitalization in patients with CGD. When it is administered with prophylactic antibiotics, an additive effect occurs, resulting in a nearly 20 percent increase in the infection-free rate of patients with CGD compared with IFN-γ alone.[81] IFN-γ was licensed by the U.S. Food and Drug Administration in December 1990 for the treatment of patients with CGD. The authors of the collaborative studies recommended its use with the addition of prophylactic antibiotics for treatment of patients diagnosed with CGD.[97]

TOLL-LIKE RECEPTORS

Toll-like receptors (TLRs) are pathogen pattern-recognition receptors central to innate immune responses.[141] TLRs are a family of type 1 integral membrane glycoproteins that detect pathogens and initiate cytokine production by activation of nuclear factor κB (NF-κB). The ligands recognized by TLRs consist of a wide range of evolutionarily conserved motifs, such as LPS, single- and double-stranded RNA, and zymosan. Collectively, these motifs are called pathogen-associated molecular patterns (PAMPs). TLR recognition of PAMPs contributes to the immediate response and broad specificity of innate immune recognition toward bacterial, viral, fungal, and parasitic pathogens. Twelve TLRs have been described in mammals, with at least 10 known in humans.[2,3,6]

The role of TLRs as immunomodulating agents is of contemporary interest. Amlie-Lefond and colleagues[5] described the potential use of TLR agonists as protective agents against biothreat pathogens. TLRs 1 to 10 in humans act to augment the immune response. TLR agonists can bolster this response and potentially offer protection against specific infections or diseases. Stimulation of TLR-7 and TLR-8 has shown antiviral and antineoplastic properties in humans. A drug known to do this, imiquimod (Aldara), has been shown to be beneficial against human papillomavirus, molluscum contagiosum, genital warts, and basal cell carcinoma.[5,58,269]

Immunomodulatory oligonucleotides stimulate Toll-like receptors and induce cytokine production. Immunomodulatory oligonucleotides offer potential clinical use for treatment of a number of inflammatory diseases, including severe acute respiratory syndrome, for which clinical trials are under way.[5] Similar use of immunomodulatory oligonucleotides in patients with renal carcinomas and HIV infection also is under investigation. Recent evidence suggests that TLR-3 activation can enhance innate immune response and enhances production of IFN-α.[162] These features render TLR-3 agonists attractive agents in the treatment of HIV-related infections and infections in certain immunocompromised patients.

TLR expression in human neonates is currently being defined.[244] TLR-2, TLR-4, and TLR-8 functions have been described in neonates. Researchers have speculated that deficiency of TLR activation may contribute to the neonate's increased susceptibility to infection.[276] The results of TLR expression in neonates have varied. In general, similar expression of TLRs 1 to 4 occurs in neonates and adults. Low-birth-weight or premature infants, however, demonstrate significantly decreased expression of TLR-4.[92,269] Most studies describe decreased cytokine production to TLR agonists in neonates compared with adults. Caron and colleagues[165] reported significantly lower LPS stimulation of TLR-4 in neonates compared with adults and speculated that this decreased stimulation may contribute to the neonate's increased susceptibility to *E. coli* and other gram-negative infections.[165] Similarly, Levy and colleagues[181] demonstrated that TLR-8 agonists are beneficial in activating costimulatory responses in neonatal antigen-presenting cells and suggested that this type of agent may be used adjuvantly to enhance the neonatal immune response.

NEUTROPHILS AND COMPLEMENT

Recruitment of neutrophils from the bloodstream to extravascular sites of inflammation is a critical event in host defense against bacterial infection and in the repair of tissue damage. Under certain circumstances, accumulation of neutrophils may contribute to vascular and tissue injury. Thus, the regulatory mechanisms involved in neutrophil activation, recruitment, and subsequent degranulation are of potential clinical significance. Neutrophil adherence to and migration through capillary endothelium is a critical early event in the acute inflammatory response. The adhesive interactions between leukocytes and endothelial cell surfaces are regulated by two novel families of glycoproteins: the integrins and the selectins. The β_2 integrins are membrane-bound glycoprotein receptors found on the surface of PMNs. The β_2 integrins CD11 and CD18 are required for adherence of PMNs to endothelial cell surfaces. The selectins also are membrane-bound glycoproteins that mediate neutrophil adhesion to endothelial cells. They include L-selectin, which is found on the surface of PMNs, and P-selectin and E-selectin, which are expressed on the surface of activated endothelial cells.[21,46,261,283,300]

The interaction between the β_2 integrins and the selectins serves to regulate PMN responses during inflammation. In general, the selectins P and E on the activated endothelial cell surface and L-selectin on the PMN cell surface function to facilitate PMN rolling and tethering to activated capillary endothelium. Once this tethering has occurred and the PMN itself is activated, the β_2 integrin CD11/CD18 receptors on the PMN form a tight adhesion with the endothelial cell surface, which facilitates PMN attachment and polarization, leading to migration.[21,46,261,300] Congenital β_2 integrin CD11/CD18 deficiency states have been described (leukocyte adhesion deficiency type I). These patients have profound PMN adhesion and motility defects and recurrent life-threatening infections along with delayed separation of the umbilical cord and juvenile periodontitis.[79,96] A second type, leukocyte adhesion deficiency type II, has been described as being due to a deficiency of sialyl Lewis^X, the PMN ligand for E-selectin on endothelial cells.[225,229] Researchers also have shown that the tethering of PMNs to P-selectin on activated endothelial cells is critical for PMN priming by platelet-activating factor and that monoclonal antibodies to P-selectin can block this response.[187] Monoclonal antibodies to P-selectin have been used in animal models of ischemia and reperfusion injury and have been shown to reduce significantly the severe edema and endothelial cell injury observed after reperfusion.[289] Similarly, monoclonal antibodies to P-selectin have resulted in significant endothelial cell preservation in animal models of lung injury and cardiac ischemia.[212,284] Monoclonal antibodies to E-selectin and L-selectin also are being tested in animal models.[200] With the rapid advancements occurring in identification of new molecules that influence endothelial cell–leukocyte interactions, we will gain greater understanding of the complexity of cellular communication during inflammation.

BACTERICIDAL/PERMEABILITY-INCREASING FACTOR AND DEFENSINS

In addition to their respiratory burst activity in antimicrobial defense, human neutrophils have been shown to contain a variety of granule-associated antibacterial proteins and peptides.[258] These include bactericidal/permeability-increasing protein (BPI) and the defensin family of peptides. BPI is a 55-kDa receptor present in neutrophil granules, which contain two domains. One of the domains binds with LPS to increase membrane permeability and the lysis of gram-negative bacteria, whereas the other promotes the opsonization of gram-negative bacteria.[131] Neutrophil BPI functions best in inflamed tissues, where it acts in concert with defensins and the membrane attack complex of complement to cause cell lysis.

Meningococcemia is a severe gram-negative infection that occurs predominantly in infants and young adults. Mortality rates of meningococcemia range from 10 to 20 percent.* Endotoxin levels may be profoundly elevated

*References 8, 32, 119, 285, 292, 299.

and correlate with the severity of the illness.[30] Recombinant BPI (rBPI) has been used as an adjunct to antimicrobial therapy in children with severe meningococcal sepsis[179]; 14 of 193 patients with severe meningococcal disease who received rBPI (2 mg/kg during 30 minutes) died, compared with 20 of 203 control infants, an insignificant difference. Among the surviving treated patients, however, a modest improvement was noted in long-term functional outcome, suggesting a possible beneficial effect in decreasing complications associated with septic shock.[102,179] Neonates have been shown to have reduced release and activity of BPI, which may contribute to their enhanced susceptibility to gram-negative bacterial infections.[180]

The defensins are strongly cationic, single-chain peptides contained in neutrophil primary, or azurophilic, granules with molecular weights between 3 and 4.5 kDa. Defensins comprise 50 percent of the protein content of the neutrophil primary granules.[98,296] These peptides possess broad antimicrobial activity against gram-positive and gram-negative bacteria, fungi, mycobacteria, and some viruses. The defensins create voltage-sensitive pores in microbial membranes, resulting in cell lysis. They are divided into α and β defensins. Humans have six human α defensins (HAD-1 to HAD-6) and two human β defensins (HBD-1 and HBD-2).

The α defensins, HAD-1 to HAD-6, are made by neutrophils and compose 30 to 50 percent of the primary granule content.[118] Defensins appear at the site of inflammation. They are present after neutrophil degranulation induced by LPS, IL-8, C5a, and other stimuli. They also are found on the epithelial surfaces of the bronchi and in bronchial lavage fluid of patients with various types of inflammatory lung disease. The antimicrobial activity of the defensins is inhibited by high salt content, which may be clinically important in the immune response of patients with cystic fibrosis.[105]

The β defensins are produced by epithelial cells of the respiratory and gastrointestinal tracts.[24,253] HBD-1 is expressed constitutively by epithelial cells in the bronchi and the intestine, whereas HBD-2 synthesis is upregulated by inflammatory stimuli, including LPS, TNF-α, bacterial infection, and injury. Thus, HBD-1 acts to kill organisms in the absence of inflammation, whereas HBD-2 acts primarily as part of the inflammatory process.

In inflammatory lung disease, such as chronic bronchitis and chronic obstructive lung disease, both α defensins HAD-1 to HAD-6 and β defensin HBD-2 may be increased significantly, perhaps contributing to airway inflammation.[296] There are no reported clinical disorders of defensin production. Potential roles for the defensins as immunoregulatory-antimicrobial agents are being investigated.

Endotoxin and other proinflammatory mediators activate the complement system and the chemotactic properties of C5a, which recruits neutrophils to sites of infection. Interruption of this process at various levels may be possible with use of monoclonal antibody strategies that counteract the effects of the complement leading to septic shock. Although monoclonal antibodies directed at the specific components of the complement cascade have not been tested in human clinical trials, the use of monoclonal antibodies to inhibit C5a in primates challenged with *E. coli* improved the survival rate and reduced the incidence of adult respiratory distress syndrome.[7,25]

PLATELET-ACTIVATING FACTOR

Platelet-activating factor (PAF) is a potent phospholipid inflammatory mediator with many biologic effects. Its synthesis is regulated by phospholipase A_2, an enzyme associated with the arachidonic acid pathway. PAF has a very short half-life in vivo because of its rapid degradation by PAF acetylhydrolase. PAF is synthesized by many cell types, including macrophages, neutrophils, platelets, eosinophils, endothelial cells, and hepatocytes.[286] Intravenous infusion of PAF into animals results in pulmonary hypertension, bronchoconstriction, neutropenia, thrombocytopenia, and ischemic bowel necrosis.[17,52,116] Production of PAF is stimulated in numerous clinical disease states, including hypoxia and ischemia, and after administration of biologic agents such as LPS, GM-CSF, TNF-α, IL-1, bradykinin, and thrombin.* Corticosteroids decrease levels of PAF by the induction of its natural inhibitor, PAF acetylhydrolase.[106] PAF has been shown to stimulate the production of many other mediators of inflammation, including TNF-α, complement breakdown products, oxygen radicals, catecholamines, prostaglandins, thromboxane, and the leukotrienes.† It also activates endothelial cells and neutrophils and monocytes, leading to their adherence and migration.[202] Thus, PAF is a ubiquitous phospholipid mediator with many biologic effects and interactions within the inflammatory cascade.

The regulation of PAF has been studied in numerous potential clinical disease states, including sepsis and septic shock.[135] In the clinical phase III trials in septic patients and patients in septic shock who received a PAF antagonist, a significant reduction in the mortality rate was noted.[68] The role of PAF, however, in the pathogenesis of necrotizing enterocolitis (NEC) has received considerable recent attention.[43,44] NEC often is a fatal gastrointestinal disease that predominantly affects premature infants. Exogenous administration of PAF into rat mesenteric circulation causes ischemic bowel necrosis and disease similar to that seen in neonatal NEC.[136] Endotoxin-induced intestinal injury is associated with increased PAF levels, and the infusion of high doses of endotoxin into animals produces a similar pathologic model of NEC, which can be prevented by administration of dexamethasone, PAF acetylhydrolase, or PAF receptor antagonists.[42,138] These animal studies suggest a link between PAF and its regulation and the development of NEC and implicate PAF as a potential endogenous inflammatory mediator in the pathogenesis of neonatal NEC.

Evidence in humans supports an association between PAF and human neonatal NEC. PAF levels are higher in infants with NEC compared with controls, and PAF

*References 83, 163, 203, 204, 230, 242, 290.
†References 85, 137, 163, 241, 273, 299.

acetylhydrolase activity is lower in infants with NEC.[45] PAF acetylhydrolase is suppressed with prematurity.[41] Because enteral feedings are necessary for the development of NEC, PAF levels were measured in feeding premature infants. In these studies, feedings alone increased circulating PAF levels but not PAF acetylhydrolase, and infants fed human breast milk had lower PAF levels and a lower incidence of NEC, suggesting a protective effect of human milk through PAF regulation.[191] Human milk is known to have a number of factors protective against infectious disease, including PAF acetylhydrolase.[38,104] Because PAF acetylhydrolase activity is present in human milk and absent in formulas, the suggestion has been made that the protective activity observed in human milk against the development of NEC may result from blocking of PAF-related inflammatory responses.[38] The modulation of the many interactions of PAF within the inflammatory cascade may have future clinical potential in regulating neonatal NEC and other inflammatory disease states.

NITRIC OXIDE

Nitric oxide is a membrane-permeable gas that functions in the regulation of vascular tone and in the inhibition of platelet aggregation and leukocyte adhesion. In addition, nitric oxide has been shown to have antitumor as well as antimicrobial activity. Under normal conditions, nitric oxide synthase induces endothelial cell production of nitric oxide. The signal transduction pathway for nitric oxide is linked to pathways involving vasodilation. Some evidence indicates that the L-arginine–nitric oxide pathway is activated in sepsis, in which the effects of nitric oxide on the vasculature are associated with the severe vascular failure observed during septic shock.* Thus, inhibition of production of nitric oxide has been proposed as a novel approach for the treatment of the severe hypotension associated with septic shock.[279]

The increased production of nitric oxide observed during septic shock may have several harmful effects. Nitric oxide may be largely responsible for sepsis-induced hypotension. In vitro studies implicate nitric oxide in sepsis-induced myocardial depression, although nitric oxide synthase inhibitors have not been shown to prevent endotoxin-induced myocardial depression in vivo. Nitric oxide also has direct cytotoxic effects, and its overproduction in septic shock can lead to tissue injury and organ failure.[55,86,150] In addition, in vitro experiments suggest that nitric oxide may enhance the release of proinflammatory cytokines during septic shock.[78,217] Production of nitric oxide may, however, have some beneficial effects during septic shock. It is implicated in maintaining visceral and other microvasculature blood flow, both as a counterregulatory mechanism to the vasoconstrictive mediators released during sepsis and by its ability to block platelet adhesion, reducing potential microvasculature stasis and thrombosis.[151,198] In addition, high levels of nitric oxide have antimicrobial activity and enhance LPS-induced production of cytokine,[164] although whether these levels of nitric oxide reflect actual physiologic states has yet to be determined.

Because hypotension during sepsis is an important predictor of organ injury and death, use of nitric oxide synthase inhibitors may improve survival in severe septic shock by increasing mean arterial pressure. Nitric oxide synthase inhibitors have been shown to restore vascular responsiveness to catecholamines in animal models of endotoxin-induced septic shock.[132] In addition, nitric oxide synthase inhibition has been shown to normalize mean arterial pressure in anesthetized animals challenged with endotoxin or TNF-α without causing hypertension.[150] These considerations have led to the use of nitric oxide synthase inhibitors to treat hypotension in patients with sepsis and in those receiving cytokine therapy for cancer.[149,188] Although these agents can alter mean arterial pressure, beneficial effects on clinical outcomes, including survival, are only suggested in human clinical trials.[217] Studies of endotoxin-challenged rats showed that partial nitric oxide synthase inhibition improved survival, whereas complete inhibition of nitric oxide production clearly is harmful, suggesting a beneficial effect with selective partial nitric oxide inhibition. Studies of nitric oxide inhibition that is more selective are being explored, and in the future such therapy may have clinical utility in the treatment of infectious disease states.

CONCLUSION

The initial attempts to augment immune function in the treatment of sepsis consisted of serum antibody therapy. The development of and refinements in hybridoma and recombinant technologies have provided the means to generate highly specific monoclonal antibodies. Although monoclonal antibodies directed toward bacteria and their components currently are not used during a course of sepsis, their future may be in combination with antimicrobial pharmacologic agents to treat infections with certain pathogens. The recent advances in basic science and clinical research also have demonstrated that the cytokine family plays many crucial roles in the pathogenesis of septic shock. The interaction among the proinflammatory cytokines initiates the development of a cascade of biologic events leading to the propagation and regulation of inflammation. Although the proinflammatory cytokines are effective in augmenting immune responses, their overexpression can lead to severe septic shock. The use of neutralizing monoclonal antibodies to endotoxin, anti–TNF-α antibodies, and soluble TNF-α and IL-1 receptors as well as IL-1 receptor antagonists in human clinical trials of septic shock, however, has shown limited therapeutic potential. The future clinical use of cytokine inhibition may include a combination of a number of both recombinant and pharmacologic agents temporally administered to regulate multiple proinflammatory cytokine–mediated steps in the development of septic shock. The hematopoietic growth factors have demonstrated considerable clinical effect, especially in individuals with distinct lymphopoietic disorders and in patients receiving immunosuppressive chemotherapy.

*References 55, 56, 152, 210, 217, 218.

The interferons α, β, and γ have received considerable attention during the past decade as potential immunomodulators. IFN-α has shown broad clinical application as an antitumor agent as well as an antiviral agent; IFN-β is being used with some success in patients with relapsing multiple sclerosis. The stimulatory effect of IFN-γ on human neutrophils is demonstrated in infants and children with specific neutrophil disorders and in neonatal sepsis. Toll-like receptor agonists are emerging as attractive therapeutic agents in a number of infectious diseases. Investigations defining the actions of the integrins and the selectins, bactericidal/permeability-increasing factor and defensins, and platelet-activating factor and nitric oxide may provide novel future clinical therapeutic approaches to attenuate acute inflammatory responses. As we learn more about the complexity of intracellular and extracellular interactions and the delicate balance these molecules have in regulating immune responses, we will be better able to implement their clinical use in regulating infectious disease states in infants and children.

NEW REFERENCES SINCE THE SIXTH EDITION

10. Ash S, Steczko J, Levy H, et al. Treatment of systemic inflammatory response syndrome by push-pull powdered sorbent pheresis: a phase I clinical trial. *Ther Apher* 2001;**5**:497–505.
19. Best S, Friedman D, Landau-Zemer T, et al. Safety and dosing of vevacizumab (Avastin) for the treatment of recurrent respiratory papillomatosis. *Ann Otol Laryngol* 2012;**121**:587–93.
36. Browne S, Zaman R, Sampaio E, et al. Anti-CD20 (rituximab) therapy for anti-IFN-gamma autoantibody-associated nontuberculous mycobacterial infection. *Blood* 2012;**119**:3922–39.
37. Bucklin S, Lake P, Logdberg L, et al. Therapeutic efficacy of a polymyxin B–dextran 7 conjugate in experimental model of endotoxemia. *Antimicrob Agents Chemother* 1995;**39**:1462–6.
47. Caron J, La Pine T, Augustine N, et al. Decreased intracellular and extracellular production of the T helper 1 cytokine IL-17 cord blood mononuclear cells may contribute to neonatal infections. *J Invest Med* 2012;**60**:235–6.
48. Caron J, La Pine TR, Augustine NH, et al. IL-17 induces proinflammatory cytokine production in cord blood mixed mononuclear cells in response to group B streptococcus. *J Invest Med* 2013;In review.
54. Cheung E, Beausoleil S, Zhang X, et al. A proteonomics approach for the identification and cloning of monoclonal antibodies from serum. *Nat Biotechnol* 2012;**30**:447–52.
59. Crickard L, Babas T, Seth S, et al. Protection of rabbits and immunodeficient mice against lethal poxvirus infections by human monoclonal antibodies. *PLoS One* 2012;**7**:e48706.
61. Cruz D, Antonelli M, Fumagalli R. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. *JAMA* 2009;**301**:2445–52.
73. Diversification of T-helper-cell lineages: finding the family root of IL-17 producing cells. *Nature Rev Immunol* 2006;**6**:329–33.
74. Edgerton C, Crispin C, Moratz C, et al. IL-17 producing $CD4^+$ T cells mediate accelerated ischemia/reperfusion-induced injury in autoimmunity-prone mice. *Clin Immunol* 2009;**130**:313–21.
94. Fric J, Bertin-Maghit S, Wang CI, et al. Use of human monoclonal antibodies to treat Chikungunya virus infection. *J Infect Dis* 2013;**207**:319–22.
95. Frogel M, Stewart D, Hoopes M, et al. A systematic review of compliance with palivizumab administration for RSV immunoprophylaxis. *J Manage Care Pharm* 2010;**16**:46–58.
103. Gogtay N, Thatte U, Kshirsager N, et al. Safety and pharmacokinetics of a human monoclonal antibody to rabies virus: a randomized, dose-escalation phase 1 study in adults. *Vaccine* 2012;**30**:7315–20.
146. Kellum A, Song M, Venkataraman R. Hemadsorption removes tumor necrosis factor, interleukin-6 and interleukin-10, reduces nuclear factor-kappaB DNA binding, and improves short-term survival in lethal endotoxemia. *Crit Care Med* 2004;**32**:801–5.
147. Kennedy WA, Hoyt MJ, McCracken GH Jr. The role of corticosteroid therapy in children with pneumococcal meningitis. *Am J Dis Child* 1991;**145**:1374.
148. Kikly K, Liu L, Sedgwick J. The IL-23/Th17 axis; therapeutic targets for autoimmune inflammation. *Curr Opin Immunol* 2006;**18**:670–5.
156. Korn T, Bettelli M, Oukka M, et al. IL-17 and Th17 cells. *Ann Rev Immunol* 2009;**27**:485–517.
157. Kropff B, Burkhardt C, Schott J, et al. Glycoprotein N of human cytomegalovirus protects the virus from neutralizing antibodies. *PLoS Pathog* 2012;**8**:e1002999.
168. La Pine TR, Hill HR. Hyperimmunoglobulin E syndrome. In: Rose BD, editor. *UptoDate*. Wellesley, MA: 2012. www.uptodate.com.
186. Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* 2012;**140**:845–58.
204. McKenzie B, Kastelein R, Cua D. Understanding the IL-23–IL-17 immune pathway. *Trends Immunol* 2006;**27**:17–23.
215. Nagarajan T, Rupprecht C, Dessain S, et al. Human monoclonal antibody and vaccine approaches to prevent human rabies. *Curr Top Microbiol Immunol* 2008;**317**:67–101.
221. Oda S, Hirasawa H, Shiga H, et al. Cytokine adsorptive property of various adsorbents in immunoadsorption columns and a newly developed adsorbent: an in vitro study. *Blood Purif* 2004;**22**:530–6.
236. Ramos J, Garcia-Sepulcre M, Rodriqguez J, et al. *Mycobacterium marinum* infection complicated by anti-tumor necrosis factor therapy. *J Med Microbiol* 2010;**59**:617–21.
240. Robinson K, Odelola O, Saldanha I, et al. Palivizumab for prophylaxis against respiratory syncytial virus infection in children with cystic fibrosis. *Cochrane Database Syst Rev* 2010;(2):CD007743.
265. Su D, Lu M, Shen M, et al. Roles of pro-and anti-inflammatory cytokines in the pathogenesis of SLE. *J Biomed Biotechnol* 2012;**2012**:347–414.
271. Tschumi S, Gugger M, Bucher B, et al. Eculizumab in atypical hemolytic uremic syndrome: long-term clinical course and histological findings. *Pediatr Nephrol* 2011;**26**:2085–8.
292. Wong C, Lit L, Tam L, et al. Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implication for TH176-mediated inflammation of autoimmunity. *Clin Immunol* 2008;**127**:385–93.
294. Yonekawa M. Cytokine and endotoxin removal in critically ill patients. *Ther Apher Dial* 2005;**9**:37–50.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abraham E. Why immunomodulatory therapies have not worked in sepsis. *Intensive Care Med* 1999;**25**:556–66.
2. Akira S, Takeda T. Toll-like receptor signaling. *Nat Rev Immunol* 2004;**4**:499–511.
3. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;**124**:783–801.
4. Alexander HR, Doherty GM, Buresh CM, et al. A recombinant human receptor antagonist to interleukin-1 improves survival after lethal endotoxemia in mice. *J Exp Med* 1991;**173**:1029.
5. Amlie-Lefond C, Paz DA, Connelly MP, et al. Innate immunity for biodefense: a strategy whose time has come. *J Allergy Clin Immunol* 2005;**116**:1334–42.
6. Andersson JP, Andersson UG. Human intravenous immunoglobulin modulates monokine production in vitro. *Immunology* 1990; **71**:372.
7. Andrews E, Feldhoff PA, Lassiter HA. Modulation of the complement system in the prevention and treatment of sepsis. *Semin Pediatr Infect Dis* 2001;**12**:54–63.
8. Anonymous. Serogroup B meningococcal disease: Oregon, 1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**:121–4.
9. Antman KS, Griffin JD, Elias A, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on chemotherapy-induced myelosuppression. *N Engl J Med* 1988; **319**:593.
10. Ash S, Steczko J, Levy H, et al. Treatment to systemic inflammatory response syndrome by push-pull powdered sorbent pheresis: a phase I clinical trial. *Ther Apher* 2001;**5**:497–505.
11. Ballow M. Mechanisms of action of intravenous immune serum globulin therapy. *Pediatr Infect Dis J* 1994;**13**:806.
12. Baron S, Tyring SK, Fleischmann WR, et al. The interferons: mechanism of action and clinical applications. *JAMA* 1991;**266**: 1375.
13. Basta M, Kirshborn P, Frank MM, et al. Mechanisms of therapeutic effects of high-dose intravenous immunoglobulin: attenuation of acute complement-dependent immune damage in a guinea pig model. *J Clin Invest* 1989;**84**:1974.
14. Baumgartner JD, Calandra T. Treatment of sepsis: past and future avenues. *Drugs* 1999;**57**:127–32.
15. Baumgartner JD, Glauser MP, McCutchan JA, et al. Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. *Lancet* 1985;**2**:59.
16. Berg DJ, Kühn R, Rajewsky K. Interleukin-10 is a central regulator of the response to LPS in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance. *J Clin Invest* 1995;**96**:2339.
17. Bernat A, Herbert JM, Salel V, et al. Protective effect of SR-27417, a novel PAF antagonist, on PAF- or endotoxin-induced hypotension in the rat and the guinea-pig. *J Lipid Mediat* 1992;**5**:41.
18. Bertini R, Delgado R, Faggioni R, et al. Urinary TNF-binding protein (TNF soluble receptor) protects mice against the lethal effect of TNF and endotoxin shock. *Eur Cytokine Network* 1993; **4**:39.
19. Best S, Friedman D, Landau-Zemer T, et al. Safety and dosing of vevacizumab (Avastin) for the treatment of recurrent respiratory papillomatosis. *Ann Otol Laryngol* 2012;**121**:587–93.
20. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor neurosis factor protects mice from lethal effect of endotoxin. *Science* 1985;**229**:869.
21. Bevilacqua MP, Nelson RM. Selectins. *J Clin Invest* 1993;**91**:379.
22. Bilgin K, Yaramis A, Haspolat K, et al. A randomized trial of granulocyte-macrophage colony-stimulating factor in neonates with sepsis and neutropenia. *Pediatrics* 2001;**107**:36–41.
23. Bohnsack JF, Hawley MM, Pritchard DG, et al. An IgA monoclonal antibody directed against type III antigen on group B streptococci acts as an opsonin. *J Immunol* 1989;**143**:3338–42.
24. Boman HG. Innate immunity and the normal microflora. *Immunol Rev* 2000;**173**:5–16.
25. Bone RC. Inhibitors of complement and neutrophils: a critical evaluation of their role in the treatment of sepsis. *Crit Care Med* 1992;**20**:891–8.
26. Bonilla MA, Gillio AP, Ruggeno M, et al. Effect of recombinant human granulocyte colony-stimulating factor on neutropenia in patients with congenital agranulocytosis. *N Engl J Med* 1989;**320**: 1574.
27. Borish L, Rosenwasser LJ. Update on cytokines. *J Allergy Clin Immunol* 1996;**97**:719.
28. Bortolussi R. Potential for intravenous gamma-globulin use in neonatal gram-negative infection: an overview. *Pediatr Infect Dis* 1986;**5**:S198–200.
29. Bortolussi R, Ferrieri P. Protection against *Escherichia coli* K1 infection in newborn rats by antibody to K1 capsular polysaccharide antigen. *Infect Immun* 1980;**28**:111–17.
30. Bortolussi R, Ferrieri P, Quie PG. Influence of growth temperature of *Escherichia coli* on K1 capsular antigen production and resistance to opsonization. *Infect Immun* 1983;**39**:1136–41.
31. Bortolussi R, Fischer GW. Opsonic and protective activity of immunoglobulin, modified immunoglobulin, and serum against neonatal *Escherichia coli* K1 infection. *Pediatr Res* 1986;**20**: 175–8.
32. Brandtzaeg P, Kierulf P, Gaustad P, et al. Plasma endotoxin as a predictor of multiple organ failure and death in systemic meningococcal disease. *J Infect Dis* 1989;**159**:195–204.
33. Breedveld FC. Therapeutic monoclonal antibodies. *Lancet* 2000; **355**:735–40.
34. Brodell RT. The use of natural alpha interferon in the treatment of condyloma acuminata. *Infect Med* 1996;**13**:56.
35. Broide DH, Paine MM, Firestein GS. Eosinophils express interleukin-5 and granulocyte-macrophage colony-stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* 1992;**90**:1414.
36. Browne S, Zaman R, Sampaio E, et al. Anti-CD20 (rituximab) therapy for anti-IFN-gamma autoantibody-associated nontuberculous mycobacterial infection. *Blood* 2012;**119**:3922–39.
37. Bucklin S, Lake P, Logdberg L, et al. Therapeutic efficacy of a polymyxin B–dextran 7 conjugate in experimental model of endotoxemia. *Antimicrob Agents Chemother* 1995;**39**:1462–6.
38. Buescher ES. Host defense mechanisms of human milk and their relations to enteric infections and necrotizing enterocolitis. *Clin Perinatol* 1994;**21**:247–62.
39. Calandra T, Baumgartner JD, Grau GE, et al. Prognostic values of tumor necrosis factor/cachectin, interleukin-1, interferon-alpha, interferon-gamma in the serum of patients with septic shock. *J Infect Dis* 1990;**161**:982.
40. Cannon JG, Tompkins RG, Gelfand JA, et al. Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. *J Infect Dis* 1990;**161**:79.
41. Caplan M, Hsueh W, Kelly A, et al. Serum PAF acetylhydrolase increases during neonatal maturation. *Prostaglandins* 1990;**39**: 705.
42. Caplan MS, Kelly A, Hsueh W. Endotoxin and hypoxia induced intestinal necrosis in rats: the role of platelet activating factor. *Pediatr Res* 1992;**31**:428.
43. Caplan MS, MacKendrick W. Inflammatory mediators and intestinal injury. *Clin Perinatol* 1994;**21**:235–46.
44. Caplan MS, Sun XM, Hsueh W. Hypoxia, PAF and necrotizing enterocolitis. *Lipids* 1991;**26**:1340.
45. Caplan MS, Sun XM, Hsueh W, et al. Role of platelet activating factor and tumor necrosis factor-alpha in neonatal necrotizing enterocolitis. *J Pediatr* 1990;**116**:960.
46. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. *Blood* 1994;**84**:2068.
47. Caron J, La Pine T, Augustine N, et al. Decreased intracellular and extra cellular production of the T helper 1 cytokine IL-17 cord blood mononuclear cells may contribute to neonatal infections. *J Invest Med* 2012;**60**:235–6.
48. Caron J, La Pine TR, Augustine NH, et al. IL-17 induces proinflammatory cytokine production in cord blood mixed mononuclear cells in response to group B streptococcus. *J Invest Med* 2013;In review.
49. Casadevall A, Scharff MD. Serum therapy revisited: animal models of infection and development of passive antibody therapy. *Antimicrob Agents Chemother* 1994;**38**:1695–702.
50. Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in infectious disease. *Clin Infect Dis* 1995;**21**:150–61.
51. Cerami A. Inflammatory cytokines. *Clin Immunol Pathol* 1992; **62**:53.
52. Chang SW, Feddersen CO, Henson PM, et al. Platelet activating factor mediates hemodynamic changes and lung injury in endotoxin-treated rats. *J Clin Invest* 1987;**79**:1498.

53. Chantry D, Turner M, Abney E, et al. Modulation of cytokine production by transforming growth factor-beta. *J Immunol* 1989; **142**:4295.
54. Cheung E, Beausoleil S, Zhang X, et al. A proteonomics approach for the identification and cloning of monoclonal antibodies from serum. *Nat Biotechnol* 2012;**30**:447–52.
55. Cobb JP, Cunnion RE, Danner RL. Nitric oxide as a target for therapy in septic shock. *Crit Care Med* 1993;**21**:1261.
56. Cobb JP, Natanson C, Hoffman WD, et al. *N*-Omega-amino-L-arginine, an inhibitor of nitric oxide synthase, raises vascular resistance but increases mortality rates in awake canines challenged with endotoxin. *J Exp Med* 1992;**176**:1175–82.
57. Cornell RC, Greenway HT, Tucker SB, et al. Intralesional interferon therapy for basal cell carcinoma. *J Am Acad Dermatol* 1990;**23**:694.
58. Cox JT, Petry KU, Rylander E, et al. Using imiquimod for genital warts in female patients. *J Womens Health (Larchmt)* 2004;**133**: 265–71.
59. Crickard L, Babas T, Seth S, et al. Protection of rabbits and immunodeficienty mice against lethal poxvirus infections by human monoclonal antibodies. *PLoS One* 2012;**7**:e48706.
60. Cross AS, Wooldridge WH, Zollinger WD. Monoclonal antibody 2-2-B kills K1-positive *Escherichia coli* in conjunction with cord blood neutrophils and sera, but not with spinal fluid. *Pediatr Res* 1984;**18**:770–2.
61. Cruz D, Antonelli M, Fumagalli R. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. *JAMA* 2009;**301**:2445–52.
62. Czuczman MS, Grillo-Lopez AJ, White CA, et al. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol* 1999;**17**:268–76.
63. D'Andrea A, Aste-Amezaga M, Vaiante NM, et al. Interleukin-10 (IL-10) inhibits human lymphocyte interferon-γ production by suppressing natural killer cell stimulatory factor IL-12 synthesis in accessory cells. *J Exp Med* 1993;**178**:1041.
64. Dahl MV, Greene WH Jr, Quie PG. Infection, dermatitis, increased IgE, and impaired neutrophil chemotaxis: a possible relationship. *Arch Dermatol* 1976;**112**:1387.
65. Dale DC. Potential role of colony-stimulating factors in the prevention and treatment of infectious diseases. *Clin Infect Dis* 1994;**18**:S180.
66. Davis GL, Balart LA, Shiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alpha: a multicenter randomized controlled trial. *N Engl J Med* 1989;**321**:1501.
67. Davis DS, Schaller J, Wedgwood RJ. Job's syndrome: recurrent "cold" staphylococcal abscesses. *Lancet* 1965;**1**:1013.
68. Dhainaut JF, Tenaillon A, Le Tulzo Y, et al. Platelet-activating factor receptor antagonist BN52021 in the treatment of severe sepsis: a randomized, double-blind, placebo-controlled, multicenter clinical trial. *Crit Care Med* 1994;**22**:1720.
69. DiBisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alpha therapy for chronic hepatitis C: a randomized, double-blind, placebo-controlled trial. *N Engl J Med* 1989;**321**:1506.
70. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and the treatment of the septic shock syndrome. *J Infect Dis* 1991;**163**:1177.
71. Dinarello CA. IL-18: a Th-1–inducing, proinflammatory cytokine and new member of the IL-1 family. *J Allergy Clin Immunol* 1999;**103**:11.
72. Donahue RE. Colony-stimulating factors: their biological activities and clinical promise. *Adv Vet Sci Comp Med* 1991;**36**:291.
73. Dong C. Diversification of T-helper-cell lineages: finding the family root of IL-17 producing cells. *Nature Rev Immunol* 2006;**6**: 329–33.
74. Edgerton C, Crispin C, Moratz C, et al. IL-17 producing $CD4^+$ T cells mediate accelerated ischemia/reperfusion-induced injury in autoimmunity-prone mice. *Clin Immunol* 2009;**130**:313–21.
75. Elliott MJ, Maini RN, Feldmann M, et al. Randomised double-blind comparison of chimeric monoclonal antibody to tumor necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet* 1994;**344**:1105–10.
76. English KB, Hammond WP, Lewis DB, et al. Decreased granulocyte-macrophage colony-stimulating factor production by human neonatal blood mononuclear cells and T cells. *Pediatr Res* 1992;**31**:211.
77. Esfandiari E, McInnes IB, Lindop G, et al. A proinflammatory role of IL-18 in the development of spontaneous autoimmune disease. *J Immunol* 2001;**167**:5338.
78. Estrada C, Gomez C, Martin C, et al. Nitric oxide mediates tumor necrosis factor alpha cytotoxicity in endothelial cells. *Biochem Biophys Res Commun* 1992;**186**:475.
79. Etzloni A, Frydman M, Pollack S, et al. Brief report: recurrent severe infections caused by a novel leukocyte adhesion deficiency. *N Engl J Med* 1992;**327**:1789.
80. Ezekowitz RA, Dinauer MC, Jaffe HS, et al. Partial correction of the phagocytic defect in patients with X-linked chronic granulomatous disease by subcutaneous interferon gamma. *N Engl J Med* 1988;**319**:146.
81. Ezekowitz RA; the International Collaborative Study Group to Assess the Efficiency of rIFN-gamma in CGD. Clinical efficacy of recombinant human interferon-gamma (rIFN-gamma) in chronic granulomatous disease (CGD). *Clin Res* 1990;**38**:465a.
82. Faist E, Schinkel C, Zimmer S. Update on the mechanisms of immune suppression of injury and immune modulation. *World J Surg* 1996;**20**:454.
83. Feuerstein G, Hallenbeck JM. Prostaglandins, leukotrienes and platelet activating factor in shock. *Annu Rev Pharmacol Toxicol* 1987;**27**:301.
84. Finberg SM. The therapy of (horse) serum reactions, general rules in the administration of therapeutic serums. *JAMA* 1936;**107**: 1717–19.
85. Fink MP. Platelet-activating factor, eicosanoids, and bradykinin as targets for adjuvant therapies for sepsis. *Semin Pediatr Infect Dis* 2001;**12**:30–41.
86. Finkel MS, Oddis CV, Jacob TD, et al. Negative inotropic effects of cytokines on the heart mediated by nitric oxide. *Science* 1992; **257**:387.
87. Fischer GW, Hemming VG, Hunter KW Jr, et al. Intravenous immunoglobulin in the treatment of neonatal sepsis: therapeutic strategies and laboratory studies. *Pediatr Infect Dis* 1986;**5**: S171–5.
88. Fischer G, Schuman R, Wilson S, et al. Monoclonal antibody enhances survival in neonatal models of lethal staphylococcal sepsis. *Eur Soc Paediatr Infect Dis* 1999;**17**:27.
89. Fisher CJ, Dhainautm J-FA, Opalm SM, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. *JAMA* 1994;**271**:1836.
90. Fisher CJ, Slotman GJ, Opal SM, et al. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open label, placebo controlled multicentric trial. *Crit Care Med* 1994;**22**:12.
91. Fisher CJ Jr, Zheng Y. Potential strategies for inflammatory mediator manipulation: retrospect and prospect. *World J Surg* 1996; **20**:447.
92. Forster-Waldl E, Sadeghi K, Tamandl D, et al. Monocyte Toll-like receptor 4 expression and LPS-induced cytokine production increase during gestational aging. *Pediatr Res* 2005;**58**:121–4.
93. Freimer EH, Lancefield RC. Type-specific polysaccharide antigens of group B streptococci. *J Hyg (Lond)* 1966;**64**:191–203.
94. Fric J, Bertin-Maghit S, Wang CI, et al. Use of human monoclonal antibodies to treat Chikungunya virus infection. *J Infect Dis* 2013;**207**:319–22.
95. Frogel M, Stewart D, Hoopes M, et al. A systematic review of compliance with palivizumab administration for RSV immunoprophylaxis. *J Manage Care Pharm* 2010;**16**:46–58.
96. Frydman M, Etzioni A, Eidlitz-Markus T, et al. Rambam-Hasharon syndrome of psychomotor retardation, short stature, defective neutrophil motility and Bombay phenotype. *Am J Med Genet* 1992;**44**:297.
97. Gallin JI. Interferon-gamma in the management of chronic granulomatous disease: the evolving use of biologicals in the treatment and prevention of infectious diseases. *Rev Infect Dis* 1991;**13**:973.
98. Ganz T, Weiss J. Antimicrobial peptides of phagocytes and epithelia. *Semin Hematol* 1997;**34**:343–54.
99. Gaur S, Kesarwala H, Gavai M, et al. Clinical immunology and infectious diseases. *Pediatr Clin North Am* 1994;**41**:745–82.
100. Girardin E, Grau GE, Dayer JM, et al. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. *N Engl J Med* 1988;**319**:397.
101. Giroir BP, Beutler B. Effect of amrinone on tumor necrosis factor production in endotoxic shock. *Circ Shock* 1992;**36**:200–7.

102. Giroir BP, Quint PA, Barton P, et al. Preliminary evaluation of recombinant amino-terminal fragment of human bactericidal/permeability-increasing protein in children with severe meningococcal sepsis. *Lancet* 1997;**350**:1439–43.
103. Gogtay N, Thatte U, Kshirsager N, et al. Safety and pharmacokinetics of a human monoclonal antibody to rabies virus: a randomized, dose-escalation phase 1 study in adults. *Vaccine* 2012;**30**:7315–20.
104. Goldman AS. The immune system of human milk: antimicrobial, antiinflammatory and immunomodulating properties. *Pediatr Infect Dis J* 1993;**12**:664.
105. Goldman MJ, Anderson GM, Stolzenberg ED, et al. Human β-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. *Cell* 1997;**88**:553–60.
106. Goppelt-Struebe M, Rehfeldt W. Glucocorticoids inhibit TNF alpha induced cytosolic phospholipase A_2 activity. *Biochim Biophys Acta* 1992;**1127**:163.
107. Gracie JA, Forsey RJ, Chan WL, et al. A proinflammatory role for IL-18 in rheumatoid arthritis. *J Clin Invest* 1999;**104**:1393.
108. Groopman JE, Gottlieb MS, Goodman J, et al. Recombinant alpha-2 interferon therapy for Kaposi's sarcoma associated with acquired immune deficiency syndrome. *Ann Intern Med* 1984;**100**:671.
109. Groopman JE, Mitsuyasu RT, DeLeo MJ, et al. Effect of recombinant human granulocyte-macrophage colony-stimulating factor on myelopoiesis in the acquired immunodeficiency syndrome. *N Engl J Med* 1987;**317**:593.
110. Grosh WW, Quesenberry PJ. Recombinant human hematopoietic growth factors in the treatment of cytopenias. *Clin Immunol Immunopathol* 1992;**62**:S25.
111. Guirao X, Lowry SF. Biological control of injury and inflammation: much more than too little or too late. *World J Surg* 1996;**20**:437.
112. Haak-Frendscho M, Marseters SA, Mordenti J, et al. Inhibition of TNF by a TNF receptor immunoadhesin: comparison to an anti-TNF monoclonal antibody. *J Immunol* 1994;**152**:1347.
113. Hamm CW, Heeschen C, Goldmann B, et al. Benefit of abciximab in patients with refractory unstable angina in relation to serum troponin T levels. c7E3 Fab Antiplatelet Therapy in Unstable Refractory Angina (CAPTURE) Study Investigators. *N Engl J Med* 1999;**340**:1623–9.
114. Hammerberg C, Arend WP, Fisher GJ, et al. Interleukin-1 receptor agonist in normal and psoriatic epidermis. *J Clin Invest* 1992;**90**:571.
115. Hammond WP 4th, Price TH, Souza LM, et al. Treatment of cyclic neutropenia with granulocyte colony-stimulating factor. *N Engl J Med* 1989;**320**:1306.
116. Hanahan DJ. Platelet activating factor: a biologically active phosphoglyceride. *Annu Rev Biochem* 1986;**55**:483.
117. Hart PH, Vitti GF, Burgess DR, et al. Potential antiinflammatory effects of interleukin-4: suppression of human monocyte tumor necrosis factor alpha, interleukin-1 and prostaglandin E_2. *Proc Natl Acad Sci U S A* 1984;**86**:3803.
118. Harwig SSL, Ganz T, Lehrer RI. Neutrophil defensins: purification, characterization and antimicrobial testing. *Methods Enzymol* 1994;**236**:160–72.
119. Havens PL, Garland JS, Brook MM, et al. Trends in mortality in children hospitalized with meningococcal infections, 1957 to 1987. *Pediatr Infect Dis J* 1989;**8**:8–11.
120. Hill HR. Intravenous immunoglobulin use in the neonate: role in prophylaxis and therapy of infection. *Pediatr Infect Dis J* 1993;**12**:549.
121. Hill HR. Additional confirmation of the lack of effect of intravenous immunoglobulin in the prevention of neonatal infection. *J Pediatr* 2000;**137**:595–7.
122. Hill HR, Augustine NH, Jaffe HS. Human recombinant interferon enhances neonatal polymorphonuclear leukocyte activation and movement and increases free intracellular calcium. *J Exp Med* 1991;**173**:767.
123. Hill HR, Estensen RD, Hogan NA, et al. Severe staphylococcal disease associated with allergic manifestations, hyperimmunoglobulin E, and defective neutrophil chemotaxis. *J Lab Clin Med* 1976;**88**:796.
124. Hill HR, Gonzales LA, Kelsey DK. The potential use of monoclonal antibodies as therapeutic modalities in neonatal infection. In: Ballow M, editor. *IVIG therapy today*. Totowa, NJ: Humana Press; 1992. p. 29–38.
125. Hill HR, Gonzales LA, Knappe WA, et al. Comparative protective activity of human monoclonal and hyperimmune polyclonal antibody against group B streptococci. *J Infect Dis* 1991;**163**:792–8.
126. Hill HR, Joyner JL, La Pine TR, et al. Defective production of IL-18 by cord blood cells in response to group B streptococci. *J Invest Med* 2001;**49**:72A.
127. Hill HR, Kelsey DK, Gonzales LA, et al. Monoclonal antibodies in the therapy of experimental neonatal group B streptococcal disease. *Clin Immunol Immunopathol* 1992;**62**:S87–91.
128. Hill HR, La Pine TR, Kwak SD, et al. Defective production of IL-18 and IL-12 by cord blood mononuclear cells influences the TH-1 interferon-gamma response to group B streptococci. *J Invest Med* 2002;**50**:50A.
129. Hill HR, Quie PG, Ochs HD, et al. Defect in neutrophil granulocyte chemotaxis in Job's syndrome of recurrent "cold" staphylococcal abscesses. *Lancet* 1974;**2**:617.
130. Hill HR, Shigeoka AO, Pincus S, et al. Intravenous IgG in combination with other modalities in the treatment of neonatal infection. *Pediatr Infect Dis* 1986;**5**:S180–4.
131. Hoffmann JA, Kafatos FC, Janeway CA Jr, et al. Phylogenetic perspectives in innate immunity. *Science* 1999;**284**:1313–18.
132. Hollenberg SM, Cunnion RE, Zimmerberg J: Nitric oxide synthase inhibition reverses arteriolar hyporesponsiveness to catecholamines in septic rats. *Am J Physiol* 1993;**264**:660.
133. Hoofnagle H. Chronic hepatitis B. *N Engl J Med* 1990;**323**:337.
134. Horwood NJ, Elliott J, Martin TJ, et al. IL-12 alone and in synergy and IL-18 inhibits osteoclast formation in vitro. *J Immunol* 2001;**166**:4915.
135. Hosford D, Koltai M, Braquet P. Platelet activating factor in shock, sepsis, and organ failure. In: Schlag G, Redl H, editors. *Pathophysiology of shock, sepsis and organ failure*. Heidelberg: Springer-Verlag; 1993. p. 502–17.
136. Hsueh W, Gonzalez-Crussi F, Arroyave JL. Platelet activating factor: an endogenous mediator for bowel necrosis in endotoxemia. *FASEB J* 1987;**1**:403.
137. Hsueh W, Gonzalez-Crussi F, Arroyave JL. Sequential release of leukotrienes and norepinephrine in rat bowel after platelet-activating factor. *Gastroenterology* 1988;**94**:1412.
138. Israel EJ, Schiffrin E, Carter EA, et al. Prevention of necrotizing enterocolitis in the rat with prenatal cortisone. *Gastroenterology* 1990;**99**:1333.
139. Itri LM. The interferons. *Cancer* 1992;**70**:940.
140. Jacobs LD, Cookfair DL, Rudick RA, et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann Neurol* 1996;**39**:285.
141. Janssens S, Beyeart R. Role of Toll-like receptors in pathogen recognition. *Clin Microbiol Rev* 2003;**16**:637–46.
142. Jeppson JD, Jaffe HS, Hill HR. Use of recombinant human interferon gamma to enhance neutrophil chemotactic responses in Job's syndrome of hyperimmunoglobulinemia E and recurrent infections. *J Pediatr* 1991;**118**:383.
143. Jones CA, Cayabyab RG, Kwong YC, et al. Undetectable interleukin IL-10 and persistent IL-8 expression early in hyaline membrane disease: a possible developmental basis for the predisposition to chronic lung inflammation in preterm newborns. *Pediatr Res* 1996;**39**:966.
144. Joyner JL, Augustine NH, Taylor KA, et al. Effects of group B streptococci on cord and adult mononuclear cell interleukin-12 and interferon-gamma mRNA accumulation and protein secretion. *J Infect Dis* 2000;**182**:74.
145. Kawakami K, Koguchi Y, Qureshi MH. IL-18 contributes to host resistance against infection with *Cryptococcus neoformans* in mice with defective IL-12 synthesis through induction of IFN-gamma production by NK cells. *J Immunol* 2000;**165**:941.
146. Kellum A, Song M, Venkataraman R. Hemadsorption removes tumor necrosis factor, interleukin-6 and interleukin-10, reduces nuclear factor-kappaB DNA binding, and improves short-term survival in lethal endotoxemia. *Crit Care Med* 2004;**32**:801–5.
147. Kennedy WA, Hoyt MJ, McCracken GH Jr. The role of corticosteroid therapy in children with pneumococcal meningitis. *Am J Dis Child* 1991;**145**:1374.

148. Kikly K, Liu L, Sedgwick J. The IL-23/Th17 axis; therapeutic targets for autoimmune inflammation. *Curr Opin Immunol* 2006; **18**:670–5.
149. Kilbourn RG, Griffith OW. Overproduction of nitric oxide in cytokine-mediated septic shock. *J Natl Cancer Inst* 1992;**84**:827.
150. Kilbourn RG, Gross SS, Jubran A, et al. N^G-Methyl-L-arginine inhibits tumor necrosis factor induced hypotension: implications for the involvement of nitric oxide. *Proc Natl Acad Sci U S A* 1990;**87**:3629.
151. Klabunde RE, Helgren MC. Cardiovascular actions of N^G-methyl-L-arginine are abolished in a canine shock model using high dose endotoxin. *Res Commun Chem Pathol Pharmacol* 1992; **78**:57.
152. Knowles RG, Moncada S. Nitric oxide synthases in mammals. *Biochem J* 1994;**298**:249–57.
153. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 1975;**256**:495–7.
154. Kotecha S, Wilson K, Wangoo A, et al. Increased interleukin IL-13 and IL-6 in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. *Pediatr Res* 1996; **40**:250.
155. Korenman J, Baker B, Waggoner J, et al. Long term remission of chronic hepatitis B after alpha-interferon therapy. *Ann Intern Med* 1991;**114**:629.
156. Korn T, Bettelli M, Oukka M, et al. IL-17 and Th17 cells. *Ann Rev Immunol* 2009;**27**:485–517.
157. Kropff B, Burkhardt C, Schott J, et al. Glycoprotein N of human cytomegalovirus protects the virus from neutralizing antibodies. *PLoS Pathog* 2012;**8**:e1002999.
158. Krown SE, Aeppli D, Balfour HH Jr. Phase II, randomized, open-label, community-based trial to compare the safety and activity of combination therapy with recombinant interferon-alpha2b and zidovudine versus zidovudine alone in patients with asymptomatic to mildly symptomatic HIV infection. HIV Protocol C91-253 Study Team. *J Acquir Immune Defic Syndr Hum Retrovirol* 1999; **20**:245–54.
159. Krown SE, Gold JWM, Niedzwiecki D. Interferon-alpha with zidovudine: safety, tolerance, and clinical and virological effect in patients with Kaposi sarcoma associated with the acquired immunodeficiency syndrome (AIDS). *Ann Intern Med* 1990;**112**:812.
160. Krown SE, Lee JY, Lin L, et al. Interferon-alpha2b with protease inhibitor-based antiretroviral therapy in patients with AIDS-associated Kaposi sarcoma: an AIDS malignancy consortium phase I trial. *J Acquir Immune Defic Syndr* 2006;**41**:149–53.
161. Krown SE, Real FX, Cunningham-Rundles S, et al. Preliminary observations on the effect of recombinant leukocyte A interferon in homosexual men with Kaposi's sarcoma. *N Engl J Med* 1983; **308**:1071.
162. Krug A, Rothenfusser S, Hornung V, et al. Identification of CpG oligonucleotide sequences with high induction of IFN-alpha/beta in plasmacytoid dendritic cells. *Eur J Immunol* 2001;**31**:2154–63.
163. Kubes P, Arfovs KE, Granger DN. Platelet-activating factor induced mucosal dysfunction—role of oxidants and granulocytes. *Am J Physiol* 1991;**260**:G965.
164. Kubes P, Suzuki M, Granger DN. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci U S A* 1991; **88**:4651.
165. La Pine TR, Augustine NH, Caron JE, et al. Toll-like receptors and tumor necrosis factor α production in neonatal host defense. *J Invest Med* 2007;**55**:S143.
166. La Pine TR, Hill HR. Immunomodifiers applicable to the prevention and management of infectious diseases in children. *Adv Pediatr Infect Dis* 1994;**9**:37–58.
167. La Pine TR, Hill HR. Monoclonal antibodies. *Semin Pediatr Infect Dis* 2001;**12**:64–70.
168. La Pine TR, Hill HR. Hyperimmunoglobulin E syndrome. In: Rose BD, editor. *UptoDate*. Wellesley, MA: 2012, www.uptodate.com.
169. Lancefield RC. A serologic differentiation of specific types of bovine hemolytic streptococci (group B). *J Exp Med* 1934;**59**: 441–9.
170. Lancefield RC. Two serological types of group B hemolytic streptococci with related, but not identical, type-specific substances. *J Exp Med* 1938;**67**:25–31.
171. Lancefield RC, McCarty M, Everly WN. Multiple mouse-protective antibodies directed against group B streptococci: special references to antibodies effective against protein antigens. *J Exp Med* 1975;**142**:165–74.
172. Lane HC, Davey V, Kovacs JA, et al. Interferon-alpha in patients with asymptomatic human immunodeficiency virus (HIV) infection: a randomized, placebo-controlled trial. *Ann Intern Med* 1990; **112**:805.
173. Lassiter HA, Robinson TW, Brown MS, et al. Effect of intravenous immunoglobulin G on the deposition of immunoglobulin G and C3 onto type III group B *Streptococcus* and *Escherichia coli* K1. *J Perinatol* 1996;**16**:346–51.
174. Lau AS. Cytokines in the pathogenesis and treatment of infectious diseases. *Adv Pediatr Infect Dis* 1994;**9**:211–36.
175. Lau AS, Lehman D, Geertsma FR, et al. Biology and therapeutic uses of myeloid hematopoietic growth factors and interferons. *Pediatr Infect Dis J* 1996;**15**:563.
176. Lauterbach R, Pawlik D, Kowalczyk D, et al. Effect of the immunomodulating agent, pentoxifylline, in the treatment of sepsis in prematurely delivered infants: a placebo-controlled, double-blind trial. *Crit Care Med* 1999;**27**:807–14.
177. Leung DY. The immunoregulatory effects of IVIG in Kawasaki disease and other autoimmune diseases. *Clin Rev Allergy* 1992;**10**: 93–104.
178. Leung DY, Cotran RS, Kurt-Jones E, et al. Endothelial cell activation and high interleukin-1 secretion in the pathogenesis of acute Kawasaki disease. *Lancet* 1989;**1**:1298.
179. Levin M, Quint PA, Goldstein B, et al. Recombinant bactericidal/permeability-increasing protein ($rBPI_{21}$) as adjunctive treatment for children with severe meningococcal sepsis: a randomized trial. RBPI21 Meningococcal Sepsis Study Group. *Lancet* 2000;**356**: 961–7.
180. Levy O, Martin S, Eichenwald E, et al. Impaired innate immunity in the newborn: newborn neutrophils are deficient in bactericidal/permeability-increasing protein. *Pediatrics* 1999;**104**:1327–33.
181. Levy O, Suter EE, Miller RL, Wessels MR. Unique efficacy of Toll-like receptor 8 agonists in activating human neonatal antigen-presenting cells. *Blood* 2006;**108**:1284–90.
182. Lewis DB, Weaver M, Prickett K, et al. Restricted production of IL-4 compared to IFN-gamma by human T cells during post natal development. *J Cell Biochem* 1989;**13**:233.
183. Lieschke GJ, Burgess AW. Granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor. *N Engl J Med* 1992;**327**:28.
184. Liles WC, Voorhis WC. Nomenclature and biological significance of cytokines involved in inflammation and host immune response. *J Infect Dis* 1995;**172**:1573.
185. Lipsky P, St. Clair EW, Furst D, et al. 54-Wk clinical and radiographic results from the ATTRACT trial: a phase III study of infliximab in patients with active RA despite methotrexate. *Arthritis Rheum* 1999;**42**:S401.
186. Littman DR, Rudensky AY. Th17 and regulatory T cells in mediating and restraining inflammation. *Cell* 2012;**140**:845–58.
187. Lorant DE, Topham MK, Whatley RE, et al. Inflammatory roles of P-selectin. *J Clin Invest* 1993;**92**:559.
188. Lorente JA, Landin L, De Pablo R, et al. L-Arginine pathway in the sepsis syndrome. *Crit Care Med* 1993;**21**:1287.
189. Luce JM. Introduction of new technology into critical care practice: a history of HA-1A human monoclonal antibody against endotoxin. *Crit Care Med* 1993;**21**:1233.
190. Lusk RP, McCabe BF, Mixon JH. Three-year experience of treating recurrent respiratory papilloma with interferon. *Ann Otol Rhinol Laryngol* 1987;**19**:158.
191. MacKendrick W, Hill N, Hsueh W, et al. Increase in plasma platelet-activating factor levels in enterally fed premature infants. *Biol Neonate* 1993;**64**:89.
192. Macatonia SE, Hsieh CS, Murphy KM, et al. Dendritic cells and macrophages are required for Th-1 development of $CD4^+$ T cells from αβ TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFN-γ production is IFN-γ–dependent. *Int Immunol* 1993;**5**:1119.
193. Magram J, Connaughton SE, Warrier RR, et al. IL-12–deficient mice are defective in IFN-γ production and type 1 cytokine responses. *Immunity* 1996;**4**:471.
194. de Malefyt R, Yssel H, Roncarolo MG, et al. Interleukin-10. *Curr Opin Immunol* 1992;**4**:314.
195. Mandelli F, Avvisati G, Amadori S, et al. Maintenance treatment with recombinant interferon alfa-2b in patients with multiple

myeloma responding to conventional induction chemotherapy. *N Engl J Med* 1990;**322**:1430.
196. Mastroeni P, Clare S, Khan S, et al. Interleukin 18 contributes to host resistance and gamma interferon production in mice infected with virulent *Salmonella typhimurium*. *Infect Immun* 1999; **67**:478.
197. Mathison JC, Wolfson E, Ulevitch RJ. Participation of tumor necrosis factor in the mediation of gram negative bacteria lipopolysaccharide-induced injury in rabbits. *J Clin Invest* 1988; **81**:1925.
198. May GR, Crook P, Moore PK, et al. The role of nitric oxide as an endogenous regulator of platelet neutrophil activation within the pulmonary circulation of the rabbit. *Br J Pharmacol* 1991; **102**:759.
199. McElvaney NG, Nakamura H, Birrer P, et al. Modulation of airway inflammation in cystic fibrosis: in vivo suppression of interleukin-8 levels on the respiratory epithelial surface by aerosolization of recombinant secretory leukoprotease inhibitor. *J Clin Invest* **90**:1296, 1992
200. McEver RP: *Selectins Curr Opin Immunol* 1994;**6**:75.
201. McInnes IB, Gracie JA, Leung BP, et al. Interleukin-18: a pleiotropic participant in chronic inflammation. *Immunol Today* 2000; **21**:312.
202. McIntyre TM, Zimmerman GA, Prescott SM. Leukotrienes C4 and D4 stimulate human endothelial cells to synthesize platelet activating factor and bind neutrophils. *Proc Natl Acad Sci U S A* 1986;**83**:2204.
203. McIntyre TM, Zimmerman GA, Satoh K, et al. Cultured endothelial cells synthesize both platelet-activating factor and prostacyclin in response to histamine, bradykinin, and adenosine triphosphate. *J Clin Invest* 1985;**76**:271.
204. McKenzie B, Kastelein R, Cua D. Understanding the IL-23-IL-17 immune pathway. *Trends inImmunol* 2006;**27**:17–23.
205. Metcalf D. Control of granulocytes and macrophages: molecular, cellular, and clinical aspects. *Science* 1991;**254**:529.
206. Michie HR, Manoque KR, Spriggs DR, et al. Detecting circulating tumor necrosis factor after endotoxin administration. *N Engl J Med* 1988;**318**:1481.
207. Minami Y, Kono T, Miyazaki T, et al. The IL-2 receptor complex: its structure, function, and target genes. *Annu Rev Immunol* 1993; **11**:245.
208. Minty A, Chalon P, Derocq JM, et al. Interleukin-13 is a new human lymphokine regulating inflammation and immune responses. *Nature* 1993;**362**:248.
209. Mitsuyasu RT. Use of recombinant interferons and hematopoietic growth factors in patients infected with human immunodeficiency virus. *Rev Infect Dis* 1991;**13**:979–84.
210. Moncada S, Palmer RM, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991;**43**: 109–42.
211. Moser R, Fehr J, Bruijnzeel PL. IL-4 controls the selective endothelium-driven transmigration of eosinophils from allergic individuals. *J Imunol* 1992;**149**:1432.
212. Mulligan MS, Polley MJ, Bayer RJ, et al. Neutrophil-dependent acute lung injury: requirement for P-selectin (GMP-140). *J Clin Invest* 1992;**90**:1600.
213. Murray HW. Interferon-gamma therapy in AIDS for mononuclear phagocyte activation. *Biotherapy* 1990;**2**:149.
214. Mustafa MM, Lebel MH, Ramilo O, et al. Correlation of interleukin-1 beta and cachectin concentration in cerebrospinal fluid and outcome from bacterial meningitis. *J Pediatr* 1989; **115**:208.
215. Nagarajan T, Rupprecht C, Dessain S, et al. Human monoclonal antibody and vaccine approaches to prevent human rabies. *Curr Top Microbiol Immunol* 2008;**317**:67–101.
216. Natanson C, Eichenholz PW, Danner R, et al. Endotoxin and tumor necrosis factor challenges in dogs stimulate the cardiovascular profile of human septic shock. *J Exp Med* 1989;**169**:823.
217. Natanson C, Hoffman WD, Suffredini AF. Selected treatment strategies for septic shock based on proposed mechanisms of pathogenesis. *Ann Intern Med* 1994;**120**:771.
218. Nathan CF, Hibbs JB Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr Opin Immunol* 1991;**3**:65.
219. Nelson S. Role of granulocyte colony-stimulating factor in the immune response to acute bacterial infection in the nonneutropenic host: an overview. *Clin Infect Dis* 1994;**18**:S197.
220. Newton JA, Ashwood ER, Yang KD, et al. Effect of pentoxifylline on developmental changes in neutrophil cell surface mobility and membrane fluidity. *J Cell Physiol* 1989;**140**:427.
221. Oda S, Hirasawa H, Shiga H, et al. Cytokine adsorptive property of various adsorbents in immunoadsorption columns and a newly developed adsorbent: an in vitro study. *Blood Purif* 2004;**22**: 530–6.
222. O'Garra A. Cytokines induce the development of functionally heterogeneous T helper cell subsets. *Immunity* 1998;**8**:275.
223. Ohkusu K, Yoshimoto T, Takeda K. Potentiality of interleukin-18 as a useful reagent for treatment and prevention of *Leishmania major* infection. *Infect Immun* 2000;**68**:2449.
224. Ohlsson K, Bjork P, Bergenfeldt M, et al. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature* 1990; **346**:550.
225. Oismüller C, Mayer N, Micksche M, et al. In-vivo modulation of human neutrophil function by pentoxifylline in patients with septic syndrome. *Shock* 1995;**4**:161.
226. Orlicek SL. Cytokine inhibitors for sepsis and septic shock. *Semin Pediatr Infect Dis* 2001;**12**:24–9.
227. Peat EB, Augustine NH, Drummond WK, et al. Effects of fibronectin and group B streptococci on tumor necrosis factor-α production by human culture-derived macrophages. *Immunology* 1995;**84**:440.
228. Perrillo RP. Treatment of chronic hepatitis B with interferon: experience in western countries. *Semin Liver Dis* 1989;**9**:240.
229. Phillips ML, Schwartz BR, Etzioni A, et al. Neutrophil adhesion in leukocyte adhesion deficiency syndrome type 2. *J Clin Invest* 1995;**96**:2898.
230. Prescott SM, Zimmerman GA, McIntyre TM. Human endothelial cells in culture produce platelet-activating factor (1-alkyl-22-acetyl-sn-glycero-3-phosphocholine) when stimulated with thrombin. *Proc Natl Acad Sci U S A* 1984;**81**:3534.
231. Present DH, Rutgeerts P, Targan S, et al. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999;**340**:1398–405.
232. Quesada JR, Reuben J, Manning JT, et al. Alpha interferon for induction of remission in hairy cell leukemia. *N Engl J Med* 1984;**310**:15.
233. Raff HV, Devereux D, Shuford W, et al. Human monoclonal antibody with protective activity for *Escherichia coli* K1 and *Neisseria meningitidis* group B infections. *J Infect Dis* 1988;**157**: 118–26.
234. Raff HV, Siscoe PJ, Wolff EA, et al. Human monoclonal antibodies to group B streptococcus: reactivity and in vivo protection against multiple serotypes. *J Exp Med* 1988;**168**:905–17.
235. Rambaldi A, Torcia M, Bettoni S, et al. Modulation of cell proliferation and cytokine production in acute myeloblastic leukemia by interleukin-1 receptor antagonists and lack of its expression by leukemic cells. *Blood* 1990;**76**:114a.
236. Ramos J, Garcia-Sepulcre M, Rodriqguez J, et al. *Mycobacterium marinum* infection complicated by anti-tumor necrosis factor therapy. *J Med Microbiol* 2010;**59**:617–21.
237. Richman-Eisenstat JB, Jorens PG, Hebert CA, et al. Interleukin-8: An important chemoattractant in the sputum of patients with chronic inflammatory airway diseases. *Am J Physiol* 1993;**264**: L413–L418.
238. Risso A. Leukocyte antimicrobial peptides: multifunctional effector molecules of innate immunity. *J Leukoc Biol* 2000;**68**:785–92.
239. Robbins JB, McCracken GH Jr, Gotschlich EC, et al. *Escherichia coli* K1 capsular polysaccharide associated with neonatal meningitis. *N Engl J Med* 1974;**290**:1216–20.
240. Robinson K, Odelola O, Saldanha I, et al. Palivizumab for prophylaxis against respiratory syncytial virus infection in children with cystic fibrosis. *Cochrane Database Syst Rev* 2010;(2):CD007743.
241. Rola-Pleszczynski M, Stankova J. Differentiation-dependent modulation of TNF production by PAF in human HL-60 myeloid leukemia cells. *J Leukoc Biol* 1992;**51**:609.
242. Rosenberg SA, Lotze MT, Muul LM, et al. A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high doses of interleukin-2 alone. *N Engl J Med* 1987;**316**:884.
243. Russell DA, Tucken KK, Chinookoswoung N, et al. Combined inhibition of interleukin-1 and tumor necrosis factor in rodent endotoxemia: improved survival and organ function. *J Infect Dis* 1995;**171**:1528.

244. Sadeghi K, Berger A, Langgartner M, et al. Immaturity of infection control in preterm and term newborns is associated with impaired Toll-like receptor signaling. *J Infect Dis* 2007;**195**:296–302.
245. Sadoff JC. Soluable TNF receptors. Presented at the Third International Congress of the Immune Consequences of Trauma, Shock and Sepsis: Mechanisms of Therapeutic Approaches. Munich, 1994.
246. Salyer JL, Bohnsack JF, Knape WA, et al. Mechanisms of tumor necrosis factor alpha alteration of PMN adhesion and migration. *Am J Pathol* 1990;**136**:831.
247. Schade UF, Burmeister I, Engel R. Increased 13-hydroxyoctadienoic acid content in lipopolysaccharide stimulated macrophages. *Biochem Biophys Res Commun* 1987;**147**:695.
248. Schedel I, Dreikhausen U, Nentwig B, et al. Treatment of gram-negative septic shock with an immunoglobulin preparation: a prospective, randomized clinical trial. *Crit Care Med* 1991;**19**:1104.
249. Schindler R, Clark BD, Dinarello CA. Dissociation between interleukin-1 beta mRNA and protein synthesis in peripheral blood mononuclear cells. *J Biol Chem* 1990;**265**:10232.
250. Schindler R, Mancilla J, Endres S, et al. Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* 1990;**75**:40.
251. Sechler JMG, Malech HL, White CJ, et al. Recombinant human interferon-gamma reconstitutes defective phagocyte function in patients with chronic granulomatous disease of childhood. *Proc Natl Acad Sci U S A* 1988;**85**:4874.
252. Seki H, Taga K, Matsoda N, et al. Phenotypic and functional characteristics of active suppressor cells against IFN-gamma production in PHA-stimulated cord blood lymphocytes. *J Immunol* 1986;**137**:3158.
253. Selsted ME, Ouellette AJ. Defensins in granules and nonphagocytic cells. *Trends Cell Biol* 1995;**5**:114–19.
254. Shigeoka AO, Hall RT, Hill HR. Blood-transfusion in group-B streptococcal sepsis. *Lancet* 1978;**1**:636–8.
255. Shigeoka AO, Jensen CL, Pincus SH, et al. Absolute requirement for complement in monoclonal IgM antibody–mediated protection against experimental infection with type III group B streptococci. *J Infect Dis* 1984;**150**:63–70.
256. Shigeoka AO, Pincus SH, Rote NS, et al. Protective efficacy of hybridoma type-specific antibody against experimental infection with group-B *Streptococcus*. *J Infect Dis* 1984;**149**:363–72.
257. Shigeoka AO, Pincus SH, Rote NS, et al. Monoclonal antibody preparations for immunotherapy of experimental GBS infection. *Antibiot Chemother* 1985;**35**:254–66.
258. Shyur SD, Raff HV, Bohnsack JF, et al. Comparison of the opsonic and complement triggering activity of human monoclonal IgG1 and IgM antibody against group B streptococci. *J Immunol* 1992;**148**:1879–84.
259. Sirko S, Weisman S, Dinarello CA. Transcription, translation and secretion of IL-1 and TNF: Effect of dual cyclooxygenase/5-lipooxygenase inhibitor. *Eur J Immunol* 1991;**21**:243.
260. Song C-H, Kim H-J, Park J-K, et al. Depressed interleukin-12 (IL-12), but not IL-18, production in response to a 30- or 32-kilodalton mycobacterial antigen in patients with active pulmonary tuberculosis. *Infect Immun* 2000;**68**:4477.
261. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte migration: the multistep paradigm. *Cell* 1994;**76**:301–14.
262. Staubach KH, Schroder J, Stuber F, et al. Effect of pentoxifylline in severe sepsis: results of a randomized, double-blind, placebo-controlled study. *Arch Surg* 1998;**133**:94–100.
263. Stevens P, Young LS, Adamu S. Opsonization of various (K) *E. coli* by the alternative complement pathway. *Immunology* 1983;**50**:497–502.
264. Stoll S, Jonuleit H, Schmitt E, et al. Production of functional IL-18 by different subtypes of murine and human dendritic cells (DC): DC-derived IL-18 enhances IL-12 dependent Th-1 development. *Eur J Immunol* 1998;**28**:3231.
265. Su D, Lu M, Shen M, et al. Roles of pro-and anti-inflammatory cytokines in the pathogenesis of SLE. *J Biomed Biotechnol* 2012;**2012**:347–414.
266. Talpaz M, Kantarjian H, McCredie K, et al. Hematologic remission and cytogenic improvement induced by recombinant human interferon alpha in chronic myelogenous leukemia. *N Engl J Med* 1986;**314**:1065.
267. Talpaz M, Kantarjian H, McCredie K, et al. Clinical investigation of human alpha interferon in chronic myelogenous leukemia. *Blood* 1987;**69**:1280.
268. Talpaz M, Kantarjian H, Kurzrock R, et al. Interferon alpha produces sustained cytogenic responses in chronic myelogenous leukemia. Philadelphia chromosome positive patients. *Ann Intern Med* 1991;**114**:532.
269. Theos AU, Cummins R, Silverger NB, et al. Effectiveness of imiquimod cream 5% for treating childhood molluscum contagiosum in a double-blind randomized pilot trial. *Cutis* 2004;**74**:134–8, 141–2.
270. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. *Nature* 1987;**330**:662.
271. Tschumi S, Gugger M, Bucher B, et al. Eculizumab in atypical hemolytic uremic syndrome: long-term clinical course and histological findings. *Pediatr Nephrol* 2011;**26**:2085–8.
272. Vadhan-Raj S, Buescher S, Broxmeyer HE, et al. Stimulation of myelopoiesis in patients with aplastic anemia by recombinant human granulocyte-macrophage colony-stimulating factor. *N Engl J Med* 1988;**319**:1628.
273. Valone FH, Ruis NM. Stimulation of tumor necrosis factor release by cytotoxic analogues of platelet-activating factor. *Immunology* 1992;**76**:24.
274. Van Deventer SJH, Buller HR, Sturk A, et al. Endotoxin induced chain reactions. *Circ Shock* 1995;**31**:246.
275. Vankayalapati R, Wizel B, Lakey DL, et al. T cells enhance production of IL-18 by monocytes in response to an intracellular pathogen. *J Immunol* 2001;**166**:6749.
276. Veimann D, Dubbel G, Schleifenbaum S, et al. Expression of Toll-like receptors in neonatal sepsis. *Pediatr Res* 2005;**58**:654–9.
277. Wald ER, Kaplan SL, Mason EO, et al. Dexamethasone therapy for children with bacterial meningitis. *Pediatrics* 1995;**95**:21.
278. Watson J, Mochizuki D. Interleukin 2: A class of T cell growth factors. *Immunol Rev* 1980;**51**:257.
279. Wearden ME. Nitric oxide synthase inhibitors for septic shock. *Semin Pediatr Infect Dis* 2001;**12**:42–5.
280. Weatherstone KB, Rich EA. Tumor necrosis factor/cachectin and interleukin-1 secretion by cord blood monocytes from premature infants. *Pediatr Res* 1989;**25**:342.
281. Wei XQ, Leung BP, Niedbala W, et al. Altered immune responses and susceptibility to *Leishmania major* and *Staphylococcus aureus* infection in IL-18–deficient mice. *J Immunol* 1999;**163**:2821.
282. Weisman LE, Wilson SR, Fischer GW. Intravenous immunoglobulin (IVIG) lots used in clinical trials to prevent late-onset infection in the high risk neonate contained variable *Staphylococcus epidermidis* antibody activity. *Pediatr Res* 1996;**139**:304A.
283. Welty SE. Inhibition of neutrophil adhesion and neutrophil function in sepsis. *Semin Pediatr Infect Dis* 2001;**12**:46–53.
284. Weyrich AS, Ma XY, Lefer DJ, et al. In vivo neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest* 1993;**91**:2620.
285. Whalen CM, Hockin JC, Ryan A, et al. The changing epidemiology of invasive meningococcal disease in Canada, 1985 through 1992: emergence of a virulent clone of *Neisseria meningitidis*. *JAMA* 1995;**273**:390–4.
286. Whatley RE, Zimmerman GA, McIntyre TM, et al. Production of platelet-activating factor by endothelial cells. *Semin Thromb Hemost* 1987;**13**:445.
287. Williams PA, Bohnsack JF, Augustine NH, et al. Production of tumor necrosis factor by human cells in vitro and in vivo induced by group B streptococci. *J Pediatr* 1993;**123**:292.
288. Wilson CB, Westall J, Johnston L, et al. Decreased production of interferon-gamma by human neonatal cells: intrinsic and regulatory deficiencies. *J Clin Invest* 1986;**77**:860.
289. Winn RK, Vedder NB, Paulson JC, et al. Monoclonal antibodies to P-selectin are effective in preventing reperfusion injury to rabbit ears. *Circulation* 1992;**86**:316.
290. Wirthmueller U, De Weck AL, Dahinden CA. Platelet-activating factor production in human neutrophils by sequential stimulation with granulocyte-macrophage colony-stimulating factor and chemotactic factors C5A or formyl-methionyl-leucyl-phenylalanine. *J Immunol* 1989;**142**:3213.
291. Wong C, Lit L, Tam L, et al. Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implication

for TH176-mediated inflammation of autoimmunity. *Clin Immunol* 2008;**127**:385–93.
292. Wong VK, Hitchcock W, Mason WH. Meningococcal infections in children: a review of 100 cases. *Pediatr Infect Dis* 1989;**8**: 224–7.
293. Wright A, Shin SU, Morrison SL. Genetically engineered antibodies: progress and prospects. *Crit Rev Immunol* 1972;**12**: 125–68.
294. Yonekawa M. Cytokine and endotoxin removal in critically ill patients. *Ther Apher Dial* 2005;**9**:37–50.
295. Yoshimoto T, Okamura HY, Tagawa I. Interleukin 18 together with interleukin 12 inhibits IgE production by induction of interferon-γ production from activated B cells. *Proc Natl Acad Sci U S A* 1997;**94**:3948.
296. Zhang P, Summer WR, Bagby GJ, et al. Innate immunity and pulmonary host defense. *Immunol Rev* 2000;**173**:39–51.
297. Zhong Z, Burns T, Chang Q, et al. Molecular and functional characteristics of a protective human monoclonal antibody to serotype 8 *Streptococcus pneumoniae* capsular polysaccharide. *Infect Immun* 1999;**67**:4119–27.
298. Ziegler EJ, Fischer CJ Jr, Sprung CL, et al. Treatment of gram-negative bacteremia in septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. *N Engl J Med* 1991;**324**:429.
299. Zimmerman GA, McIntyre TM, Prescott SM. Production of platelet-activating factor by human vascular endothelial cells: evidence for a requirement for specific agonists and modulation by prostacyclin. *Circulation* 1985;**72**:718.
300. Zimmerman GA, Prescott SM, McIntyre TM. Endothelial cell interactions with granulocytes: tethering and signaling molecules. *Immunol Today* 1992;**13**:93.

CHAPTER 242

Probiotics

Gail J. Harrison

This chapter is a general overview of the topic of probiotics, synbiotics, prebiotics, and postbiotics, and their application to prevention and treatment of disease in newborns, infants, and children.[4] The concept of probiotics may date back to the turn of the 20th century, in 1908, when Eli Metchnikoff observed that the longevity of Bulgarian peasants may have been linked to their diet, which included consumption of fermented milk. The term *probiotic* was probably first used scientifically in 1965 when Lilly and Stillwell described substances produced by one microorganism that stimulated the growth of another microorganism. *Lactobacillus rhamnosus* GG (LGG), derived from the *Lactobacillus* strain used for fermentation of dairy products, was likely the first probiotic used clinically, and remains the probiotic used most commonly in clinical studies and clinical medicine.

Increasing evidence from basic and translational research has shown that the timing and type of intestinal microflora colonization influences the development of immune function and is highly influenced by the infant's early diet and intestinal microbial environment.[38,39] Probiotic bacteria, postbiotic bacterial by-products, and dietary prebiotics likely exert positive effects on the development of the immune system during infancy and through competitive inhibition may prevent colonization of potential enteric pathogens. Colonization with nonbeneficial bacteria and exposure to antimicrobial agents in the newborn period may result in immune dysregulation and disorders of immune function. Disturbances in the gut microbial composition may play a role in the pathophysiology of a variety of disorders by modulating immune programming. The "hygiene hypothesis" suggests the reduction in general microbial exposure during early childhood may play an important role in modifying the early composition of gut microbial flora, with benefit to the host by reducing risk for infection and allergic disease. These research findings have led to clinical trials evaluating the role of these products in clinical medicine. The clinical effects of prebiotics and probiotics have varied in clinical trials, most likely because the influence of probiotics is a complex process, and varies with type, strain, dose, viability, timing, duration, adherence to regimen, other dietary restrictions, and method of administration evaluated for each different clinical condition within each clinical trial. Although not strictly antimicrobials in the classic sense, these biotic products may help provide additional defense against infectious diseases and are used widely in both developed and developing countries to improve the health and well-being of infants and children.

DEFINITIONS

Probiotics is a general term for different strains and species of microorganisms, usually commensal living bacteria of mammalian microbiota, with a wide range of clinical and immunologic uses.[4,12] The term is derived from Greek, meaning "for life." The United Nations Food and Agricultural Organization and the World Health Organization have defined probiotics as "live microorganisms, which, when administered in adequate amounts confer a health benefit on the host." Probiotics considered as oral supplements, additives, or food products are marketed as dietary supplements and do not require premarket review and approval by the U.S. Food and Drug Administration (FDA). However, probiotics or prebiotics that are marketed specifically for the treatment or prevention of a specific disease or condition are classified as biologic products and, thus, require FDA review and approval. The most commonly used probiotics include bacteria that are fermentative, anaerobic, and nonmotile and produce lactic acid. They are thought to generate small molecular metabolic by-products, such as short-chain fatty acids, sometimes called *postbiotics*, that may function as immune modulators. Probiotics are usually species of the bacteria *Lactobacillus*, *Bifodobacterium*, or *Streptococcus* or the yeast *Saccharomyces* (Table 242-1). Probiotic preparations may be administered as a single strain or a mixture of different types of bacteria or yeast.

Probiotics may influence immune function through their effects on local immunity and stabilization of intestinal barrier integrity.[17] Probiotics are generally regarded as safe for administration to otherwise normal infants, children, and even pregnant women, and they are available in many food products, such as live culture yogurt. Human breast milk also may naturally contain probiotics from maternal sources. Probiotics may be given separately like a medicine or supplement, mixed with or added to a naturally existing food, or even added to infant formula or human breast milk.[28] However, probiotics may stimulate growth of gut bacteria and cause deleterious metabolic activity, excessive immune stimulation, and even systemic infections. Therefore, preterm neonates, children with short bowel syndrome, immunocompromised patients, and other special hosts, should receive probiotics only after careful consideration and observation and careful weighing of potential benefits and risks.[38]

Prebiotics are nondigestible food ingredients added as supplements to food or infant formulas. Human breast milk contains many natural prebiotics.[12] Prebiotics used as supplements usually are oligosaccharides and include

TABLE 242-1 Commonly Used Probiotic Microorganisms for Prevention and Treatment of Disease

Bacteria

Lactobacillus
L. acidophilus
L. bulgaricus
L. caseii
L. fermentum
L. paracasei
L. plantarum
L. rhamnosus (GG)

Bifidobacterium
B. bifidum
B. breve
B. infantis
B. lactis
B. longum

Streptococcus
S. boulardii
S. salivarius
S. thermophilus

Lactococcus lactic

Enterococcus faecium SF68 (nonpathogenic)

***Escherichia coli* Nissle 1917 (nonpathogenic strain)**

Clostridium butyricum

Yeast
Saccharomyces boulardi

fructo-oligosaccharides (FOS), inulin (a group of FOS), galacto-oligosaccharides (GOS), soybean oligosaccharides, lactulose, and dietary fibers. They are a major substrate for bacteria and are designed to promote and stimulate the growth of beneficial, indigenous strains of bacteria, especially *Bifidobacterium* spp. The effects of prebiotics are potentially broader than a single probiotic strain of bacteria. The fermentation by-products of prebiotics also produce short-chain fatty acids that may have an anti-inflammatory effect and may directly affect the mucosal and systemic immune systems. A theoretical concern is that prebiotics may promote growth of potential pathogens or encourage growth of commensal bacteria that may translocate across the gut epithelial barrier in the preterm neonate with immature gut or in an immunocompromised host with altered immunity. In the otherwise normal older infant and child, prebiotics have not shown adverse effects, have appeared safe to administer, and have even been added to some infant formulas.[28]

Synbiotics are a combination of prebiotics and probiotics that may produce a synergistic beneficial effect.[12] The combination of both prebiotic dietary carbohydrates that escape digestion and probiotic bacteria appears to improve the survival of the probiotic bacteria in the gastrointestinal tract, and enhances their effect in certain circumstances.

USE OF PROBIOTICS AND PREBIOTICS FOR PREVENTION AND TREATMENT OF CLINICAL CONDITIONS

Gastrointestinal Disorders

Acute Infectious Diarrhea

Randomized clinical trials have shown benefit in giving probiotics, mixed with human or cow milk or formula or given as a separate oral supplement, to prevent acute gastroenteritis in healthy infants and children, especially if they attend group child-care settings, and especially if the cause of the gastroenteritis was rotavirus.[5,11,16,37] A variety of probiotic preparations have been studied and shown to be beneficial in preventing infectious viral diarrhea, including *L. rhamnosus* LGG, *Streptococcus thermophilus*, *Lactobacillus casei*, *Lactobacillus reuteri*, and *Bifidobacterium lactis*.[34,37,38] Randomized clinical trials have also shown probiotics have therapeutic benefit for treatment of acute infectious diarrhea, especially acute rotavirus diarrhea in otherwise healthy infants and children.[16,23] Probiotics, when administered early in the illness, reduce the number of diarrheal stools per day, shortens the duration of diarrhea by 1 day, and shortens the time for intravenous fluids needed for rehydration (Table 242-2).

Antibiotic-Associated Diarrhea

Antibiotic-associated diarrhea (AAD) or AAD associated with *Clostridium difficile* may occur in approximately one third of patients treated with antibiotics and may reduce compliance or adherence with antibiotic treatment regimens or cause significant morbidity. Randomized clinical trials have shown a prophylactic beneficial effect when a probiotic, when added to infant formula or taken as a separate supplement, was started at the same time antibiotic treatment was initiated.[3,14,18,19,20] The probiotics most commonly used to prevent AAD, including *C. difficile* AAD, include *L. rhamnosus* LGG, *B. lactis*, *S. thermophilus*, and *Saccharomyces boulardii*.[19,30,33,38] The clinical trial data to support the use of probiotics to treat AAD or AAD associated with *C. difficile* in children, however, are currently lacking, and the routine use of probiotics for treatment of AAD in children, at this time, is not routinely recommended.[19,34]

Gastritis Caused by Helicobacter pylori

Randomized clinical trials have shown clinical benefit for probiotics to improve eradication of *H. pylori* infection.[34] Therefore, it is often used as adjunctive therapy for *H. pylori*–associated gastritis in both adults and children.

Inflammatory Bowel Disease

Probiotics are commonly administered as adjunctive treatment for inflammatory bowel disease (IBD). The administration of probiotics in both adults and children with chronic ulcerative colitis and with pouchitis

TABLE 242-2 **Diseases in Infants and Children in Which Probiotics Have Been Used for Treatment or Prevention**

Diseases	Prevention	Treatment
Gastrointestinal Diseases		
Diarrhea		
Acute infectious diarrhea (especially rotavirus)	++	++
Traveler's diarrhea	+	?
Antibiotic-associated diarrhea	++	+
Clostridium difficile	+	?
Infantile colic	?	+
Inflammatory bowel disease		
Ulcerative colitis	?	+
Crohn disease	?	–
Helicobacter pylori gastritis	?	+
Hepatic encephalopathy	+	+
Irritable bowel syndrome	–	+
Constipation	–	–
Lactose intolerance	–	+
Pancreatitis	–	–
Allergic/Atopic Diseases	+	+
Upper Respiratory Tract Infections	+	–
Neonatal diseases		
Necrotizing enterocolitis	++	–
Neonatal sepsis	++	–
Cancer	?	?

+, Indicates limited evidence or conflicting evidence for benefit.
++, Indicates clear evidence for benefit.
– Indicates no clear evidence for benefit or possible deleterious effect.
? Indicates no studies conducted to date.

complicating ulcerative colitis have shown beneficial effects.[29] The probiotics most often used in clinical trials showing benefit for treatment of ulcerative colitis include *S. thermophilus* and *Lactobacillus* and *Bifidobacterium* spp., as well as *Escherichia coli* Nissle 1917. As yet, no proven benefit supporting probiotics for treatment of adults or children with Crohn disease has been demonstrated.[34]

Irritable Bowel Syndrome, Constipation, and Infant Colic

Probiotics have been shown to benefit children with irritable bowel syndrome by reducing abdominal distention and pain.[34,38] However, probiotics have not been shown to significantly improve functional constipation in children at this time. A positive therapeutic effect has also been shown when a probiotic containing *L. reuteri* was administered to infants with colic who were exclusively breast-fed. The benefit of probiotics to treat colic in formula-fed infants has not been convincingly demonstrated at this time.

Hepatic Encephalopathy

The potential benefits of probiotics administered to patients with hepatic encephalopathy from liver failure have been investigated in several clinical trials.[15,25] Probiotics appear to reduce plasma ammonia concentrations, but have not been shown consistently to be efficacious in improving clinically relevant outcomes, such as decreased incidence of sepsis, shorter duration of hospital stay, and improved mental functioning.

Allergic and Atopic Diseases

In one randomized clinical trial, a probiotic containing *L. rhamnosus* LGG, when administered to pregnant women in the final month of pregnancy and to lactating mothers and their breast-fed infants for 6 months after birth, significantly reduced the frequency of atopic eczema.[8,9,32,41] In one clinical trial, prebiotics containing oligosaccharides FOS and GOS administered to infants at risk for atopic diseases, a reduced incidence of eczema, recurrent wheezing, and allergic urticaria.[41] In addition, use of probiotic containing *Lactobacillus fermentum* administered to children with atopic dermatitis showed improvement in contrast to placebo. However, even though these findings were encouraging, they have not been confirmed in other clinical trials at this time. Therefore, experts differ in their recommendations regarding use of probiotics and prebiotics for treatment and prevention of allergic and atopic diseases.[38,41]

Upper and Lower Respiratory Tract Infections

Probiotic *L. rhamnosus* LGG, when administered to young children up to 6 years of age, may reduce the incidence of upper respiratory tract infections and the common cold, but has not been shown to be beneficial for prevention or treatment of established lower respiratory tract infections such as pneumonia in older children.[13,21] In one very recent study, synbiotics containing *Lactobacillus planatarum* and FOS oligosaccharides administered to neonates and young infants in developing countries reduced incidence of lower respiratory tract infections, including radiographically proved pneumonia requiring treatment with antibiotics.[31]

Neonatal Sepsis and Necrotizing Enterocolitis

Probiotics offer potential benefits for newborns, including premature infants.* Probiotic administration has been shown in randomized clinical trials to reduce incidence of necrotizing enterocolitis (NEC), as well as mortality from NEC in neonates, including preterm neonates.† A variety of different probiotics in single-organism formulations and combination mixtures have been evaluated, including *L. rhamnosus* LGG, *B. bifidus*, *Bifidobacterium*

*References 7, 26, 35, 36, 38, 40.
†References 1, 2, 6, 10, 22, 35.

breve, *Bifidobacterium infantis*, *S. thermophilus*, *L. acidophilus*, and *Saccharomyces*, with varying dosages, administered with human milk, formula, or a mixture of human milk and formula feeding regimens.[22,24] The clinical trial data to support benefit for probiotics to prevent late-onset neonatal sepsis is also emerging.[40] Earlier clinical trials did not convincingly show benefit for probiotics to prevent sepsis, but more recent clinical trials have shown a significant positive effect. In one recent report, a synbiotic preparation containing probiotic *L. plantarum* and prebiotic fructo-oligosaccharide, administered to neonates with birth-weight greater than 2000 g, significantly reduced the incidence of sepsis syndrome, culture-positive sepsis, and mortality associated with sepsis in neonates.[31] It also had a beneficial effect by reducing lower respiratory tract infection requiring antibiotic treatment in infants enrolled in the study.

Human Immunodeficiency Virus and Acquired Immunodeficiency Syndrome and Cancer

Probiotics and prebiotics also are being applied in resource-poor and disadvantaged countries to reduce duration of diarrhea, improve gut function, and prevent bacterial vaginosis.[7,27] In addition, some studies suggest probiotics may improve well-being in human immunodeficiency virus (HIV)-infected patients and even reduce the risk for HIV transmission.[27] However, challenges to scientific study and implementation of proved clinical efficacy in resource-poor settings in developing countries include lack of infrastructure and funding to execute appropriately designed clinical trials, as well as provisions to make clinically proved probiotic and prebiotic products available at affordable prices to patients in developing countries. Research interest in evaluating the ability of probiotics and prebiotics added to diet to prevent or reduce incidence of various cancers is evolving, but at this time, there is no proved benefit for probiotics or prebiotics to prevent or treat cancer.[38]

SAFETY OF PROBIOTICS AND PREBIOTICS IN INFANTS AND CHILDREN

Prebiotics and probiotics appear safe for healthy infants over 5 months of age and healthy children of all ages.[38] In developing countries, prebiotics, probiotics, and synbiotics offer great potential for prevention of serious and potentially fatal bacterial illnesses and diarrheal illnesses in infants and children. However, the safety of administering probiotic products containing live microorganisms in high-risk or special patient groups requires careful consideration and caution should be used when they are considered for treatment in special or immunocompromised hosts.[38] Probiotic sepsis and other serious infections caused by probiotic microorganisms, including neonatal sepsis and meningitis, have been documented in patients at risk, including immunocompromised patients, ill preterm and low-birth-weight neonates, and children with intestinal disorders, short gut, and indwelling catheters.[35,36] Therefore, the potential risk for probiotics and prebiotics must be carefully balanced with the potential benefit in these patient groups.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Alfaleh K, Anabrees J, Bassler D. Probiotics reduce the risk of necrotizing enterocolitis in preterm infants: a meta-analysis. *Neonatology* 2010;**97**:93–9.
2. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2011;(3):CD005496.
3. Davidson L, Hibberd P. Clostridium difficile *and probiotics*. UpToDate 2012. http://www.uptodate.com/contents/clostridium-difficile-and-probiotics.
4. deVrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol* 2008;**111**:1–66.
5. Dinleyici E, Eren M, Ozen M, et al. Effectiveness and safety of *Saccharomyces boulardii* for acute infectious diarrhea. *Expert Opin Biol Ther* 2012;**12**:395401.
6. Downard CD, Renaud E, St. Peter S, et al. Treatment of necrotizing enterocolitis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. *J Pediatr Surg* 2012;**47**:2111–22.
7. Enos M, Burton J, Dols J, et al. Probiotics and nutrients for the first 1000 days of life in the developing world. *Benef Microbes* 2013;**4**:3–16.
8. Foisy M, Boyle R, Chalmers J, et al. Overview of reviews: the prevention of eczema in infants and children—an overview of Cochrane and non-Cochrane reviews. *Evid Based Child Health* 2011;**6**:1322–39.
9. Foolad N, Brezinski E, Chase E, et al. Effect of nutrient supplementation on atopic dermatitis in children: a systematic review of probiotics, prebiotics, formula, and fatty acids. *Arch Dermatol Dec* 2012;**17**:1–6.
10. Ganguli K, Walker W. Probiotics in the prevention of necrotizing enterocolitis. *J Clin Gastroenterol* 2011;**45**(suppl):S133–8.
11. Guandalini S. Probiotics for prevention and treatment of diarrhea. *J Clin Gastroenterol* 2011;**45**(suppl):S149–53.
12. Gupta. V, Garg R. Probiotics. *Indian J Med Microbiol* 2009;**27**:202–9.
13. Hao Q, Lu Z, Dong B, et al. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev* 2011;(9):CD006895.
14. Hempel S, Newberry S, Maher W, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* 2012;**307**:1959–69.
15. Holte K, Krag A, Gluud L. Systematic review and meta-analysis of randomized trials on probiotics for hepatic encephalopathy. *Hepatol Res* 2012;**42**:1008–15.
16. Htwe K, Yee K, Tin M, Vandenplas Y. Effect of *Saccharomyces boulardii* in the treatment of acute watery diarrhea in Myanmar children: a randomized controlled trial. *Am J Trop Med Hyg* 2008;**78**:214–16.
17. Indrio F, Neu J. The intestinal microbiome of infants and the use of probiotics. *Curr Opin Pediatr* 2011;**23**:145–50.
18. Johnston B, Goldenberg J, Vandvik P, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2011;(11):CD004827.
19. Johnston B, Ma S, Goldenberg J, et al. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea: a systematic review and meta-analysis. *Ann Intern Med* 2012;**157**:878–88.
20. Johnston B, Supina A, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized, placebo-controlled trials. *CMAJ* 2006;**175**:377–83.
21. Kang E, Kim S, Hwang I, et al. The effect of probiotics on prevention of common cold: a meta-analysis of randomized controlled trial studies. *Korean J Fam Med* 2013;**34**:2–10.
22. Lin H, Su B, Chen A, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005;**115**:1–4.
23. Madsen K. The use of probiotics in gastrointestinal disease. *Can J Gastroenterol* 2001;**15**:817–22.

24. Martin C, Walker W. Probiotics: role in pathophysiology and prevention of necrotizing enterocolitis. *Semin Perinatol* 2008;**32**: 127–37.
25. McGee R, Bakens A, Wiley K, et al. Probiotics for patients with hepatic encephalopathy. *Cochrane Database Syst Rev* 2011;(11): CD008716.
26. Millar M, Wilks M, Fleming P, et al. Should the use of probiotics in the preterm be routine? *Arch Dis Child Fetal Neonatal Ed* 2012;**97**: F70–4.
27. Monachese M, Cunningham-Rundles S, Diaz M, et al. Probiotics and prebiotics to combat enteric infections and HIV in the developing world: a consensus report. *Gut Microbes* 2011;**2**:198–207.
28. Mugambi M, Musekiwa A, Lombard M, et al. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutr J* 2012;**11**:81–91.
29. Naidoo K, Gordon J, Fagbemi A, et al. Probiotics for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2011: CD007443.
30. Nelson R, Kelsey P, Leeman H et al. Antibiotic treatment for *Clostridium difficile*-associated diarrhea in adults. *Cochrane Database Syst Rev* 2011;(9):CD004610.
31. Panagrahi P, Parida S, Pradhan, et al. Effectiveness of *Lactobacillus plantarum* synbiotic treatment for preventing late onset sepsis in neonates. Pediatric Academic Societies Annual Meeting. Abstract presented in Platform Late Breaker Session. Washington, D.C, May 6, 2013.
32. Pelucchi C, Chatenoud L, Turati F, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. *Epidemiology* 2012;**22**:402–14.
33. Rohde C, Bartolini V, Jones N. The use of probiotics in the prevention and treatment of antibiotic-associated diarrhea with special interest in *Clostridium difficile*-associated diarrhea. *Nutr Clin Pract* 2009;**24**:33–40.
34. Sartor R. *Probiotics for gastrointestinal diseases*. UpToDate 2012. http://www.uptodate.com/contents/probiotics-for-gastrointestinal-diseases.
35. Schanler R. Probiotics and necrotizing enterocolitis in premature infants. *Arch Dis Child Fetal Neonatal Ed* 2006;**91**:F395–7.
36. Schanler R. *Prevention of necrotizing enterocolitis in newborns*. UpToDate 2012. http://www.uptodate.com/contents/prevention-of-necrotizing-enterocolitis-in-newborns.
37. Szajewska H, Mrukowicz J. Use of probiotics in children with acute diarrhea. *Paediatr Drugs* 2005;**7**:111–22.
38. Thomas D, Greer F, and Committee on Nutrition. Section on Gastroenterology, Hepatology, and Nutrition, American Academy of Pediatrics: Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**:1217–31.
39. Vandenplas Y, Veereman-Wauters G, DeGreef E, et al. Probiotics and prebiotics in prevention and treatment of diseases in infants and children. *J Pediatr (Rio J)* 2011;**87**:292–300.
40. Venkatesh M, Abrams S. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2010;(5):CD007137.
41. West C, Prescott S. *Prebiotics and probiotics for prevention of allergic disease*. UpToDate 2012. http://www.uptodate.com/contents/prebiotics-and-probiotics-for-prevention-of-allergic-disease.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alfaleh K, Anabrees J, Bassler D. Probiotics reduce the risk of necrotizing enterocolitis in preterm infants: a meta-analysis. *Neonatology* 2010;**97**:93–9.
2. Alfaleh K, Anabrees J, Bassler D, Al-Kharfi T. Probiotics for prevention of necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2011;(3):CD005496.
3. Davidson L, Hibberd P. Clostridium difficile *and probiotics*. UpToDate 2012. http://www.uptodate.com/contents/clostridium-difficile-and-probiotics.
4. deVrese M, Schrezenmeir J. Probiotics, prebiotics, and synbiotics. *Adv Biochem Eng Biotechnol* 2008;**111**:1–66.
5. Dinleyici E, Eren M, Ozen M, et al. Effectiveness and safety of *Saccharomyces boulardii* for acute infectious diarrhea. *Expert Opin Biol Ther* 2012;**12**:395401.
6. Downard CD, Renaud E, St. Peter S, et al. Treatment of necrotizing enterocolitis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. *J Pediatr Surg* 2012;**47**:2111–22.
7. Enos M, Burton J, Dols J, et al. Probiotics and nutrients for the first 1000 days of life in the developing world. *Benef Microbes* 2013;**4**:3–16.
8. Foisy M, Boyle R, Chalmers J, et al. Overview of reviews: the prevention of eczema in infants and children—an overview of Cochrane and non-Cochrane reviews. *Evid Based Child Health* 2011;**6**:1322–39.
9. Foolad N, Brezinski E, Chase E, et al. Effect of nutrient supplementation on atopic dermatitis in children: a systematic review of probiotics, prebiotics, formula, and fatty acids. *Arch Dermatol Dec* 2012;**17**:1–6.
10. Ganguli K, Walker W. Probiotics in the prevention of necrotizing enterocolitis. *J Clin Gastroenterol* 2011;**45**(suppl):S133–8.
11. Guandalini S. Probiotics for prevention and treatment of diarrhea. *J Clin Gastroenterol* 2011;**45**(suppl):S149–153.
12. Gupta. V, Garg R. Probiotics. *Indian J Med Microbiol* 2009;**27**:202–9.
13. Hao Q, Lu Z, Dong B, et al. Probiotics for preventing acute upper respiratory tract infections. *Cochrane Database Syst Rev* 2011;(9):CD006895.
14. Hempel S, Newberry S, Maher W, et al. Probiotics for the prevention and treatment of antibiotic-associated diarrhea: a systematic review and meta-analysis. *JAMA* 2012;**307**:1959–69.
15. Holte K, Krag A, Gluud L. Systematic review and meta-analysis of randomized trials on probiotics for hepatic encephalopathy. *Hepatol Res* 2012;**42**:1008–15.
16. Htwe K, Yee K, Tin M, Vandenplas Y. Effect of *Saccharomyces boulardii* in the treatment of acute watery diarrhea in Myanmar children: a randomized controlled trial. *Am J Trop Med Hyg* 2008;**78**:214–16.
17. Indrio F, Neu J. The intestinal microbiome of infants and the use of probiotics. *Curr Opin Pediatr* 2011;**23**:145–50.
18. Johnston B, Goldenberg J, Vandvik P, et al. Probiotics for the prevention of pediatric antibiotic-associated diarrhea. *Cochrane Database Syst Rev* 2011;(11):CD004827.
19. Johnston B, Ma S, Goldenberg J, et al. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea: a systematic review and meta-analysis. *Ann Intern Med* 2012;**157**:878–88.
20. Johnston B, Supina A, Vohra S. Probiotics for pediatric antibiotic-associated diarrhea: a meta-analysis of randomized, placebo-controlled trials. *CMAJ* 2006;**175**:377–83.
21. Kang E, Kim S, Hwang I, et al. The effect of probiotics on prevention of common cold: a meta-analysis of randomized controlled trial studies. *Korean J Fam Med* 2013;**34**:2–10.
22. Lin H, Su B, Chen A, et al. Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants. *Pediatrics* 2005;**115**:1–4.
23. Madsen K. The use of probiotics in gastrointestinal disease. *Can J Gastroenterol* 2001;**15**:817–22.
24. Martin C, Walker W. Probiotics: role in pathophysiology and prevention of necrotizing enterocolitis. *Semin Perinatol* 2008;**32**:127–37.
25. McGee R, Bakens A, Wiley K, et al. Probiotics for patients with hepatic encephalopathy. *Cochrane Database Syst Rev* 2011;(11):CD008716.
26. Millar M, Wilks M, Fleming P, et al. Should the use of probiotics in the preterm be routine? *Arch Dis Child Fetal Neonatal Ed* 2012;**97**:F70–4.
27. Monachese M, Cunningham-Rundles S, Diaz M, et al. Probiotics and prebiotics to combat enteric infections and HIV in the developing world: a consensus report. *Gut Microbes* 2011;**2**:198–207.
28. Mugambi M, Musekiwa A, Lombard M, et al. Synbiotics, probiotics or prebiotics in infant formula for full term infants: a systematic review. *Nutr J* 2012;**11**:81–91.
29. Naidoo K, Gordon J, Fagbemi A, et al. Probiotics for maintenance of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2011:CD007443.
30. Nelson R, Kelsey P, Leeman H, et al. Antibiotic treatment for *Clostridium difficile*-associated diarrhea in adults. *Cochrane Database Syst Rev* 2011;(9):CD004610.
31. Panagrahi P, Parida S, Pradhan, et al. Effectiveness of *Lactobacillus plantarum* synbiotic treatment for preventing late onset sepsis in neonates. Pediatric Academic Societies Annual Meeting. Abstract presented in Platform Late Breaker Session. Washington, D.C, May 6, 2013.
32. Pelucchi C, Chatenoud L, Turati F, et al. Probiotics supplementation during pregnancy or infancy for the prevention of atopic dermatitis: a meta-analysis. *Epidemiology* 2012;**22**:402–14.
33. Rohde C, Bartolini V, Jones N. The use of probiotics in the prevention and treatment of antibiotic-associated diarrhea with special interest in *Clostridium difficile*-associated diarrhea. *Nutr Clin Pract* 2009;**24**:33–40.
34. Sartor R. *Probiotics for gastrointestinal diseases*. UpToDate 2012. http://www.uptodate.com/contents/probiotics-for-gastrointestinal-diseases.
35. Schanler R. Probiotics and necrotizing enterocolitis in premature infants. *Arch Dis Child Fetal Neonatal Ed* 2006;**91**:F395–7.
36. Schanler R. *Prevention of necrotizing enterocolitis in newborns*. UpToDate 2012. http://www.uptodate.com/contents/prevention-of-necrotizing-enterocolitis-in-newborns.
37. Szajewska H, Mrukowicz J. Use of probiotics in children with acute diarrhea. *Paediatr Drugs* 2005;**7**:111–22.
38. Thomas D, Greer F, and Committee on Nutrition. Section on Gastroenterology, Hepatology, and Nutrition, American Academy of Pediatrics: Probiotics and prebiotics in pediatrics. *Pediatrics* 2010;**126**:1217–31.
39. Vandenplas Y, Veereman-Wauters G, DeGreef E, et al. Probiotics and prebiotics in prevention and treatment of diseases in infants and children. *J Pediatr (Rio J)* 2011;**87**:292–300.
40. Venkatesh M, Abrams S. Oral lactoferrin for the prevention of sepsis and necrotizing enterocolitis in preterm infants. *Cochrane Database Syst Rev* 2010;(5):CD007137.
41. West C, Prescott S. *Prebiotics and probiotics for prevention of allergic disease*. UpToDate 2012. http://www.uptodate.com/contents/prebiotics-and-probiotics-for-prevention-of-allergic-disease.

This page intentionally left blank

PART **V**

PREVENTION OF INFECTIOUS DISEASES

This page intentionally left blank

CHAPTER 243

Health Care–Associated Infections

W. Charles Huskins • Julia Shaklee Sammons • Susan E. Coffin

Health care–associated (nosocomial) infections are infections that occur as a consequence of health care. Many of these infections are associated with the use of modern invasive devices and procedures that breach normal host defenses to allow microbial invasion. Other infections are due to exposure to pathogens in the health care environment, which are transmitted from colonized or infected persons or from contaminated objects and surfaces, water, or air or occur in persons with compromised immune systems or who are particularly susceptible to infection, such as preterm infants. Finally, health care–associated infections may occur in persons delivering health care (i.e., occupationally-acquired infections in health care providers).

HISTORICAL ASPECTS

The history of health care–associated infections and their control is tightly linked to developments in institutional medical care.[595] Although not broadly recognized, efforts to study and prevent infection among hospitalized children have contributed significantly to the development of infection control and prevention efforts in general.

Semmelweis' classic studies of puerperal fever in the Vienna Lying-In Hospital in the mid 1800s provided insights into the etiology and prevention of perinatal infections in neonates, not just their afflicted mothers.[596] Children also suffered greatly from the epidemics of contagious diseases that spread through hospitals during the 18th and 19th centuries.[229] This problem lead to the opening of wards and entire hospitals designated for the treatment of patients with infectious diseases in the early 20th century.[595] By the mid-20th century, a number of interventions had been developed to minimize the spread of contagious diseases in hospitals, including use of quarantine areas for new admissions, confinement of each child in an individual cubicle, creation of cohorts for patients admitted during community epidemics, use of masks by persons caring for patients, exclusion of visitors, strict control of the health of nurses and physicians caring for the patients, and implementation of exhaust fans to prevent airborne transmission of measles and varicella.[68,83,281,391,404]

Outbreaks of *Staphylococcus aureus* infection in hospitalized infants had been documented in the late 1800s and early 1900s,[97,341] but the pandemic of *S. aureus* infections that plagued hospitals in the 1950s and 1960s drew special attention to the impact of infections caused by this organism. Outbreaks of staphylococcal disease were particularly devastating in neonatal nurseries, where epidemics caused by specific phage types caused substantial morbidity and mortality.[586] The seriousness of the hospital-acquired staphylococcal infection problem spawned more comprehensive efforts to document the impact and consequences of hospital-acquired infections[562-564] and served as the impetus to develop organized infection control programs, particularly in Great Britain (with its tradition of infection control sisters) and North America.

In 1970, a hospital-acquired infection surveillance and control program was established at Children's Hospital in Boston and data were reported to the nascent National Nosocomial Infections Study at the Centers for Disease Control (CDC).[234] The study emphasized the association of hospital-acquired infections with exposure to invasive devices and procedures. Additional epidemiologic studies of infections in pediatric patients cared for in intensive care units (ICUs) during the 1970s and early 1980s strengthened the association between the use of invasive devices and procedures and hospital-acquired infections.[252,287,298,417]

Advances in viral diagnostics in the 1970s led to greater appreciation of the importance of viruses as a significant cause of hospital-acquired infections, particularly in pediatric patients.[673,704] Spread of respiratory and gastrointestinal viruses, especially respiratory syncytial virus (RSV) and rotavirus, was documented to be a severe problem on pediatric wards.[185,270,447,569,707]

In the past 40 years we have witnessed additional important developments. Gram-positive bacteria, including coagulase-negative staphylococci, *S. aureus*, enterococci, and streptococci, have reemerged as significant pathogens in hospitalized patients.[219,260,319,628] Antimicrobial-resistant bacteria, such as methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), and gram-negative bacilli resistant to third-generation cephalosporins, aminoglycosides, carbapenems, and quinolones have become especially problematic.[293,348,543] Although MRSA has received the most attention in the media, gram-negative pathogens, such as carbapenemase-producing Enterobacteriaceae, *Burkholderia*, *Stenotrophomonas*, and *Acinetobacter* are even more problematic because they are resistant to most (or even all) available antibiotics and the pipeline of new active agents is virtually nonexistent.[73] Overutilization of antibiotics is in part responsible for the continued increase in *Clostridium difficile* infections,[120] but the clonal spread of more virulent strains of *C. difficile* (NAP-1) is a major problem for

both adults and children.[401,436,667] Multiple studies have documented an increase in *C. difficile* infection in hospitalized children over the past decade.[343,492,724] As increasing numbers of severely ill and immunosuppressed children are cared for in hospitals, the incidence of fungal infection has increased dramatically, especially infection caused by *Candida* and *Aspergillus*.[1,219,224,435] Health care–associated acquisition of common respiratory viruses, such as RSV, parainfluenza virus, and adenovirus, can cause severe, sometimes lethal pneumonia in these patients. Finally, the potential for health care–associated infection caused by bloodborne pathogens, such as the human immunodeficiency virus (HIV), hepatitis B, and hepatitis C, among patients as well as health care workers is well recognized.*

In the past decade, health care–associated infections have been viewed through the lens of efforts to improve the quality and safety of health care.[315,351] A majority of states require public reporting of institutional rates of health care–associated infections, and the Center for Medicare and Medicaid Services (CMS) requires facilities providing care for Medicare and Medicaid patients to report rates of health care–associated infections, with comparative data available for public viewing on the CMS Hospital Compare website.† These infections are broadly recognized as important contributors to the preventable harms that occur to patients as a consequence of health care, and a consortium of leading professional societies and governmental groups has issued a call to action for their elimination.[100] Supporting this goal is the fact that initiatives to reduce health care–associated infections have been among the most prominent of the patient safety success stories.[118,533,534] A variety of multicenter collaborative networks are actively working on prevention of health care–associated infections in children[302,452,592]; and, in 2011, a new national effort, the "Partnership for Patients" led by the U.S. Department of Health and Human Services, was launched with a goal of a 40 percent reduction in preventable harms, including health care–associated infections, by the end of 2013.[434] In addition, the U.S. Department of Health and Human Services has developed a "National Action Plan to Prevent Healthcare-Associated Infections: Roadmap to Elimination" that includes components for acute care hospitals, ambulatory surgical centers, end-stage renal disease facilities, and long-term care facilities.‡ Much of the information in the plan is relevant to the care of children in health care facilities, although it does not address pediatrics specifically.

Subsequent sections of this chapter expand on the themes just outlined by describing health care–associated infections due to spread of communicable infections in health care facilities, infections related to invasive devices and procedures, infections caused by special pathogens and occurring in special populations, as well as the components of programs to prevent these infections and to optimize antimicrobial stewardship.

*References 109, 114, 119, 265, 510, 663.
†www.hospitalcompare.hhs.gov.
‡www.hhs.gov/ash/initiatives/hai/infection.html.

HEALTH CARE–ASSOCIATED INFECTIONS DUE TO SPREAD OF COMMUNICABLE INFECTIONS IN HEALTH CARE FACILITIES

General Principles

Health care–associated infections that occur as a result of spread of communicable infections common in the community are a major concern for all facilities providing health care to children. Several principles regarding the epidemiology of these infections, modified from those initially published by Hall,[268] are summarized here. First, their appearance and spread on the wards parallels closely the disease activity in the community. Second, exposure to these pathogens generally results in infection in any host who lacks specific immunity; consequently, a susceptible child is at risk regardless of the nature or severity of his or her underlying disease or medical treatment. Third, these infections are often more severe in patients who have underlying diseases (e.g., prematurity, pulmonary or cardiac disease) or who are immunocompromised. Fourth, children hospitalized as a result of the community-acquired infections comprise the most important reservoir for microorganisms causing these infections, but mildly symptomatic or asymptomatically colonized adult caregivers and visitors may be important reservoirs for some agents (e.g., RSV, pertussis). Fifth, prevention is dependent primarily on the timely implementation of and compliance with isolation precautions designed to interrupt the modes of transmission of the microorganisms involved. In some situations, other interventions are also indicated, such as chemoprophylaxis for infants exposed to pertussis and immune globulin for high-risk individuals exposed to measles. Finally, unless postdischarge surveillance is performed, the frequency of these infections will be underestimated given short hospital stays and the fact that some infections (e.g., *C. difficile* infection) may be incubating at the time of discharge and manifest only after the child returns home.

Modes of Transmission

Airborne transmission involves spread of microorganisms by droplet nuclei, which are small particles (<5 μm) generated by the desiccation of larger respiratory droplets, especially those expelled by coughing. Because they are extremely light, droplet nuclei can travel over very long distances on air currents. Because they are so small, droplet nuclei can remain suspended in inhaled air, evading the mechanical host defenses of the upper respiratory tract, to reach the distal airways and alveoli.[464] Diseases spread by airborne transmission of respiratory droplet nuclei include measles, tuberculosis, smallpox, and, under certain circumstances, influenza and varicella.[158,381,466,550,699]

Droplet transmission involves transfer of microorganisms by large respiratory droplets, such as those generated by coughing or sneezing, which typically travel short distances in the air before settling. Important pathogens

spread by this route include *Bordetella pertussis*, *Neisseria meningitidis*, and group A streptococci.

Contact transmission is the principal mode of transmission for most health care–associated infections. Direct contact transmission involves physical contact between a person harboring the microorganism and a susceptible host, such as a caregiver with a herpetic whitlow who transmits the virus to a neonate. Indirect contact transmission involves transfer of microorganisms via an intermediary person, object, or surface. The hands of caregivers are a common source for indirect contact transmission,[77] but fomites and contaminated environmental surfaces are also important for certain pathogens (e.g., RSV, *C. difficile*, MRSA, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, norovirus).[507,695]

Endogenous infection (or autoinfection) is caused by a patient's own colonizing flora, usually as a consequence of host defenses that are compromised by severe underlying disease, immunosuppressive therapy, or invasive devices and procedures. Endogenous infections may be caused by microorganisms that are a part of the "normal" microbiome (e.g., coagulase-negative *Staphylococcus*) or that are acquired from exposure to the health care environment, usually by indirect contact transmission, and become persistent colonizers (e.g., VRE). These infections can be considered as a special case of contact transmission.

Common vehicle (common source) transmission involves the widespread dissemination of a microorganism to many persons via a contaminated item or substance. Examples include recent outbreaks of hepatitis B and C due to blood contamination of reused items[114,265] and outbreaks caused by nonenteric gram-negative bacilli, such as *P. aeruginosa*, that thrive in medications, solutions, or wet equipment and are relatively resistant to preservatives, antiseptics, and disinfectants.

Vector transmission of microorganisms either on (extrinsic) or within (intrinsic) insects is possible but rare in hospitals.[46,122,220]

Finally, some infections may be spread by more than one mode of transmission. For example, varicella zoster virus may be spread by airborne and contact transmission.

Standard and Transmission-Based Precautions

The Centers for Disease Control and Prevention's (CDC) Healthcare Infection Control Practices Advisory Committee (HICPAC) "Guideline for Isolation Precautions," describes strategies to prevent spread of communicable infections in health care facilities.[610] These strategies involve the use of Standard Precautions, which should be used for all hospitalized patients at all times, and Transmission-Based Precautions, which are used for patients with suspected or confirmed infections that may be transmissible to other patients or health care workers. Transmission-Based Precautions include Airborne Precautions, Droplet Precautions, and Contact Precautions, each of which is designed to interrupt one of the modes of transmission just described. The key components of Standard and Transmission-Based Precautions are outlined in Tables 243-1 and 243-2 and are described in more detail later in this chapter (see Infection Prevention and Control Programs in Health Care Facilities).

Respiratory Viruses

RSV is the most common health care–associated respiratory virus infection, especially among children younger than 2 years of age, because RSV infection accounts for a substantial number of hospital admissions in young children during epidemic periods.[78,269,375,601,681] In the absence of effective infection prevention and control programs, attack rates can be high because immunity after infection is short lived, inoculation of virus into the nose or eyes reliably leads to infection, infected children excrete high titers of virus in their copious secretions, and RSV survives for long periods in the environment.[267,269,271,274] Risk factors for health care–associated RSV infection are prematurity, heart and lung disease, immunocompromising conditions, and duration of hospitalization.*

The transmission of RSV can be markedly reduced by scrupulous attention to hand hygiene and the use of gloves and gowns when touching patients or their immediate inanimate environment.[261,379,676] Goggles and/or masks may be effective in further reducing the RSV attack rate in personnel.[7,232] However, it is unclear whether masks and eye protection are effective because they prevent direct deposition of respiratory droplets on the eyes or nose or merely because they reduce the likelihood that staff will rub their eyes or noses with contaminated hands. Covering only the nose and mouth with a mask is probably not very effective.[272] The HICPAC isolation guideline recommends use of Contact Precautions for care of hospitalized patients with RSV and does not require use of a mask.[610]

Some investigators have demonstrated that cohorting infected patients, combined with the use of barriers, can reduce spread of RSV.[189,356,415] However, it is not clear whether cohorting by clinical symptoms alone is effective or whether all admitted patients must be screened for RSV infection to identify children with minimal symptoms who may be excreting the virus. The added value of strict cohorting, as opposed to rigorous use of barrier precautions, has not been demonstrated. The role of palivizumab, either as routine seasonal prophylaxis or as postexposure prophylaxis for high-risk patients, has been examined in uncontrolled studies with generally positive but not conclusive results.[187,261,335,362,493]

Multidisciplinary programs to promote awareness of the potential for transmission of RSV and optimize the use of prevention measures, using all or various combinations of these strategies, have demonstrated effectiveness in reducing the incidence of health care–associated RSV infection.†

Health care–associated infections due to parainfluenza virus and human metapneumovirus are similar to RSV in their epidemiology and prevention.[269,459,479]

*References 273, 275, 276, 375, 412, 615, 681.
†References 14, 261, 332, 377, 379, 410.

TABLE 243-1 **Standard Precautions***

A. Hand Washing

Wash hands after touching blood, body fluids, secretions, excretions, and contaminated items, whether or not gloves are worn.

Wash hands immediately after gloves are removed, between patient contacts, and when otherwise indicated to avoid transfer of microorganisms to other patients or environments.

It may be necessary to wash hands between tasks and procedures on the same patient to prevent cross contamination of different body sites.

Use a plain (nonantimicrobial) soap for hand washing.

Use an antimicrobial agent or a waterless antiseptic agent for specific circumstances (e.g., control of outbreaks or hyperendemic infections), as defined by the infection control program.

B. Gloves

Wear gloves (clean nonsterile gloves are adequate) when touching blood, body fluids, secretions, excretions, and contaminated items.

Put on clean gloves just before touching mucous membranes and nonintact skin.

Change gloves between tasks and procedures on the same patient after contact with material that may contain a high concentration of microorganisms.

Remove gloves promptly after use, before touching noncontaminated items and environmental surfaces, and before going to another patient, and wash hands immediately to avoid transfer of microorganisms to other patients or environments.

C. Mask, Eye Protection, and Face Shield

Wear a mask and eye protection or a face shield to protect mucous membranes of the eyes, nose, and mouth during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, and excretions.

D. Gown

Wear a gown (a clean nonsterile gown is adequate) to protect skin and prevent soiling of clothing during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions.

Select a gown that is appropriate for the activity and amount of fluid likely to be encountered.

Remove a soiled gown as promptly as possible, and wash hands to avoid transfer of microorganisms to other patients or environments.

E. Patient Care Equipment

Handle used patient care equipment soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures, contamination of clothing, and transfer of microorganisms to other patients and environments.

Ensure that reusable equipment is not used for the care of another patient until it has been appropriately cleaned and reprocessed appropriately.

Ensure that single-use items are discarded properly.

F. Environmental Control

Ensure that the hospital has adequate procedures for the routine care, cleaning, and disinfection of environmental surfaces, beds, bedrails, bedside equipment, and other frequently touched surfaces, and ensure that these procedures are followed.

G. Linen

Handle, transport, and process used linen soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposure and contamination of clothing and that avoids transfer of microorganisms to other patients and environments.

H. Occupational Health and Bloodborne Pathogens

Take care to prevent injuries when using needles, scalpels, and other sharp instruments or devices; when handling sharp instruments after procedures; when cleaning used instruments; and when disposing of used needles.

Never recap used needles or otherwise manipulate them using both hands or any other technique that involves directing the point of a needle toward any part of the body; rather, use either a one-handed "scoop" technique or a mechanical device designed for holding the needle sheath.

Do not remove used needles from disposable syringes by hand, and do not bend, break, or otherwise manipulate used needles by hand.

Place used disposable needles and syringes, scalpel blades, and other sharp items in appropriate puncture-resistant containers, which are located as close as practical to the area in which the items were used, and place reusable syringes and needles in a puncture-resistant container for transport to the reprocessing area.

Use mouthpieces, resuscitation bags, or other ventilation devices as an alternative to mouth-to-mouth resuscitation methods in areas where the need for resuscitation is predictable.

I. Patient Placement

Place a patient who contaminates the environment or who does not (or cannot be expected to) assist in maintaining appropriate hygiene or environmental control in a private room.

If a private room is not available, consult with infection control professionals regarding patient placement or other alternatives.

*Standard Precautions apply to all patients regardless of their diagnosis or presumed infection status. Standard Precautions apply to any planned or potential contact with: (1) blood; (2) all body fluids, secretions, and excretions except sweat, regardless of whether they contain visible blood; (3) nonintact skin; and (4) mucous membranes.

From Siegel JD, Rhinehart E, Jackson M, et al. *Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings 2007.* Centers for Disease Control and Prevention, Healthcare Infection Control Practices Advisory Committee, 2007. Available at http://www.cdc.gov/hicpac/2007IP/2007isolationPrecautions.html. Accessed October 24, 2012.

Health care–associated influenza is a common cause of intercurrent fever in hospitalized children during epidemic periods,[270,681] and outbreaks in neonatal ICUs have been described.[156,471,686] Asthma was identified as a risk factor for health care–associated seasonal influenza.[378] Although the 2009-2010 pandemic of H1N1 influenza raised concerns about the potential for increased spread of influenza in health care facilities, the risk for transmission among hospitalized children appeared no different than for seasonal influenza,[209,504] perhaps because of

TABLE 243-2 Transmission-Based Precautions*

Airborne Precautions

A. Patient Placement

Place the patient in a private room that has (1) monitored negative air pressure in relation to the surrounding areas; (2) 6 to 12 air changes per hour; and (3) appropriate discharge of air outdoors or monitored high-efficiency filtration of room air before the air is circulated to other areas in the hospital.
Keep the room door closed and the patient in the room.
When a private room is not available, place the patient in a room with a patient who has active infection with the same microorganism, unless otherwise recommended, but with no other infection.
When a private room is not available and cohorting is not desirable, consultation with infection control professionals is advised before patient placement.

B. Respiratory Protection

Wear respiratory protection when entering the room of a patient with known or suspected infectious pulmonary tuberculosis.
Susceptible persons should not enter the room of patients known or suspected to have measles (rubeola) or varicella (chickenpox) if other immune caregivers are available.
If susceptible persons must enter the room of a patient known or suspected to have measles or varicella, they should wear respiratory protection.
Persons immune to measles or varicella need not wear respiratory protection.

C. Patient Transport

Limit the movement and transport of the patient from the room to essential purposes only.
If transport or movement is necessary, minimize patient's dispersal of droplet nuclei by placing a surgical mask on the patient, if possible.

D. Additional Precautions for Preventing Transmission of Tuberculosis

Consult the CDC "Guidelines for Preventing the Transmission of Tuberculosis in Health-Care Facilities" for additional prevention strategies.

Droplet Precautions

A. Patient Placement

Place the patient in a private room.
When a private room is not available, place the patient in a room with a patient(s) who has active infection with the same microorganism, but with no other infection.
When a private room is not available and cohorting is not achievable, maintain spatial separation of at least 3 feet between the infected patient and other patients and visitors.
Special air handling and ventilation are not necessary, and the door may remain open.

B. Mask

Wear a mask when working within 3 feet of the patient. (Logistically, some hospitals may want to implement the wearing of a mask to enter the room.)

C. Patient Transport

Limit the movement and transport of the patient from the room to essential purposes only.
If transport or movement is necessary, minimize patient dispersal of droplets by placing a surgical mask on the patient, if possible.

Contact Precautions

A. Patient Placement

Place the patient in a private room.
When a private room is not available, place the patient in a room with a patient(s) who has active infection with the same microorganism, but with no other infection.
When a private room is not available and cohorting is not achievable, consider the epidemiology of the microorganism and the patient population when determining patient placement; consultation with infection control professionals is advised before patient placement.

B. Gloves

Wear gloves (clean nonsterile gloves are adequate) when entering the room.
During the course of providing care for a patient, change gloves after having contact with infective material that may contain high concentrations of microorganisms (fecal material and wound drainage).
Remove gloves before leaving the patient's environment and wash hands immediately with an antimicrobial agent or a waterless antiseptic agent.
After glove removal and hand washing, ensure that hands do not touch potentially contaminated environmental surfaces or items in the patient's room to avoid transfer of microorganisms to other patients or environments.

C. Gown

Wear a gown (a clean, nonsterile gown is adequate) when entering the room if you anticipate that your clothing will have substantial contact with the patient, environmental surfaces, or items in the patient's room or if the patient is incontinent or has diarrhea, an ileostomy, a colostomy, or wound drainage not contained by a dressing.
Remove the gown before leaving the patient's environment.
After gown removal, ensure that clothing does not contact potentially contaminated environmental surfaces to avoid transfer of microorganisms to other patients or environments.

D. Patient Transport

Limit the movement and transport of the patient from the room to essential purposes only.
If the patient is transported out of the room, ensure that precautions are maintained to minimize the risk for transmission of microorganisms to other patients and contamination of environmental surfaces or equipment.

E. Patient Care Equipment

When possible, dedicate the use of noncritical patient care equipment to a single patient (or a cohort of patients infected or colonized with the pathogen requiring precautions) to avoid sharing between patients.
If use of common equipment or items is unavoidable, then adequately clean and disinfect them before use for another patient.

F. Additional Precautions for Preventing the Spread of Vancomycin Resistance

Consult the HICPAC report on preventing the spread of vancomycin resistance for additional prevention strategies.

*Transmission-Based Precautions are followed, when indicated, in addition to Standard Precautions.

From Siegel JD, Rhinehart E, Jackson M, et al. *Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings 2007.* Centers for Disease Control and Prevention, Healthcare Infection Control Practices Advisory Committee, 2007. Available at http://www.cdc.gov/hicpac/2007IP/2007isolationPrecautions.html. Accessed October 24, 2012.

heightened awareness and more rigorous use of barrier precautions.

The modes of transmission of influenza have not been precisely defined. Although direct, indirect, and droplet contact are likely to be important, the potential for airborne transmission and the relative contributions of these various modes of transmission in health care facilities, as well as potential differences in transmission of seasonal versus pandemic influenza, remain unclear.[101] For seasonal influenza, the CDC recommends use of Droplet Precautions, with use of respiratory protection equivalent to a fitted N95 filtering facepiece respirator or equivalent N95 respirator during aerosol-generating procedures.*

Annual influenza vaccination of high-risk individuals and health care workers is important to limit the impact of transmission in health care facilities.[117] Issues related to vaccination of health care workers against influenza are discussed later in this chapter (see Infection Prevention and Control Programs in Health Care Facilities). Antiviral medications are an adjunct to vaccination, both for treatment to reduce viral shedding and chemoprophylaxis to prevent infection after exposure.[214]

Health care–associated adenovirus infections occur sporadically throughout the year, and outbreaks of respiratory tract infection and pharyngoconjunctival fever among hospitalized children have been described.† Outbreaks in ICUs and units caring for immunocompromised patients have been associated with severe disease and substantial mortality.[432,619,708] In addition to direct and indirect contact, adenovirus may be spread by droplet contact, necessitating the use of Droplet and Contact Precautions.

Health care–associated coronavirus infections may be more common than recognized.[231] Rhinovirus infections are generally mild, although serious lower tract disease has been documented in children with underlying disorders.[130] Standard Precautions are sufficient as a control measure in most situations.

Pertussis

Outbreaks of pertussis in hospitals and chronic care institutions are well documented.‡ In many situations, health care workers were responsible for spreading pertussis to hospitalized children.[115,129,360,396,674] The beneficial impact, but high cost, of controlling the intrahospital spread of pertussis was illustrated during a large community-wide epidemic of pertussis in Cincinnati in 1993.[132]

Prevention of health care–associated pertussis is dependent on the appropriate isolation of children in whom the infection is suspected, use of Droplet Precautions, antimicrobial treatment of confirmed cases to minimize the potential for transmission, and chemoprophylaxis of exposed individuals.[18,610] Hospital staff who present with symptoms suggesting pertussis must be evaluated and treated promptly, and infected individuals should be excluded from work for at least the first 5 days of the recommended course of treatment. Health care workers should receive booster doses of pertussis vaccine (in the form of Tdap) to help prevent transmission in health care facilities.[117]

Gastrointestinal Viruses

Rotavirus is a common cause of endemic and epidemic health care–associated gastrointestinal virus infection.* The risk for health care–associated infection is closely associated with hospitalization during seasonal outbreaks, young age, and the length of hospitalization.[86,143,178,683] The risk for transmission from infected children is enhanced because infected children may shed the virus in their stool for many days after symptoms resolve, asymptomatically infected patients also may shed virus, and the virus can survive for extended periods on environmental surfaces.[29,143,233,582,712]

The primary mode of transmission is indirect contact via the contaminated hands of caregivers, and the scrupulous adherence to Contact Precautions and appropriate disinfection of environmental surfaces is critically important.[619,688] The use of rotavirus vaccine appears to have decreased the risk for health care–associated rotavirus concomitant with reductions in the numbers of children hospitalized due to community-acquired infection.[25]

Health care–associated norovirus infection—most often due to genogroup II.4 that can cause more severe disease—is a major problem, although wards with elderly populations have been affected more significantly than pediatric wards.[180,431] Contamination of environmental surfaces is particularly common and contributes to transmission.[465,507] A HICPAC isolation guideline recommends use of Contact Precautions and appropriate disinfection of environmental surfaces, with additional recommendations to be considered in outbreak situations.[411]

Other intestinal viruses have been documented to cause endemic and epidemic nosocomial gastrointestinal infection, including enteric adenoviruses,[155,354] caliciviruses,[632] astroviruses,[177,361] and toroviruses.[318] Contact Precautions should be used to prevent transmission of these enteric pathogens.[610]

Clostridium difficile

Toxin-producing *C. difficile* acquired in the community may be spread in health care settings, although this is difficult to distinguish from spread of *C. difficile* from reservoirs in the health care facility (i.e., from infected patients or the health care environment). *C. difficile* infection is discussed later in this chapter (see Health Care–Associated Infections Related to Special Pathogens).

Other Gastrointestinal Bacteria

Gastrointestinal bacterial pathogens, such as *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, *Escherichia coli*, and *Vibrio*

*Centers for Disease Control and Prevention: http://www.cdc.gov/flu/professionals/infectioncontrol/healthcaresettings.htm.
†References 213, 246, 475, 523, 530, 619, 643, 708.
‡References 115, 215, 360, 396, 602, 635, 656, 674.

*References 86, 155, 250, 546, 559, 622, 683.

cholerae, may cause outbreaks of health care–associated diarrhea in areas where gastrointestinal infections caused by these organisms are endemic.* However, they are so rare in modern health care facilities that, in the absence of an outbreak, the yield of routine stool cultures in the evaluation of health care–associated diarrhea is extremely low.[79,148,722]

Varicella Zoster Virus

Outbreaks of health care–associated varicella in hospitals have been documented in numerous reports.[228,264,381,588] These reports demonstrate conclusively that varicella can be transmitted by the airborne route, although spread via direct and droplet contact may well be more efficient. In addition, a study that used polymerase chain reaction (PCR) assay detected airborne virus in the hospital rooms of patients with active infection and in air samples obtained in the hallway just outside the patients' negative-pressure isolation rooms.[583]

Transmission of varicella can be minimized if infected patients are cared for using Airborne Precautions in single rooms with separate exhaust systems and negative air pressure relative to the hallway.[26,610] Contact Precautions to prevent direct and indirect contact transmission (gloves and gowns) are also important. Infected persons are infectious for 24 to 48 hours before distinctive symptoms and signs appear, so prompt recognition and isolation of patients and visitors who may be in the contagious phase of varicella is critical. Caregivers who are not immune to varicella should not care for such patients.

Options for postexposure prophylaxis, including passive immunization and use of varicella vaccine and acyclovir, are discussed in the American Academy of Pediatrics' *Red Book*.[19] Hospitalized exposed children should remain in isolation from day 8 to day 21 after exposure. If passive immunization is administered, isolation should be continued until day 28 after exposure.[19]

The use of varicella vaccine has diminished the risk for health care–associated varicella by decreasing the number of children hospitalized with varicella, reducing the size of the pool of susceptible children in hospital wards, and providing protective immunity to health care workers who did not have a history of prior infection.[117] However, incompletely vaccinated children and health care workers, as well as persons who develop breakthrough infection, pose a risk for transmission.

Cytomegalovirus

Two studies using molecular typing techniques have documented probable patient-to-patient spread of cytomegalovirus (CMV) in a neonatal ICU and a chronic care unit.[176,631] In both situations, the infected children had been in close proximity and were cared for by common caregivers for extended periods of time. Nonetheless, the risk for CMV transmission from infected patients to health care workers appears to be very low. A meta-analysis of a number of studies that have examined the risk for CMV among pediatric nurses indicates that CMV infection in this population occurs at a rate that is comparable to control populations (women of comparable age who are not nurses).[6] A subanalysis of these data suggests that nursery nurses may have a slightly higher rate of infection than control populations.[6] However, studies of CMV infection in nursery nurses that involved molecular typing of CMV isolates have shown that the infected nurses did not acquire CMV from the infants in their care[6] and presumably contracted their infections from children in their own households, through sexual contact, or from other community sources.

Given the frequency of asymptomatic CMV excretion in children (for example, approximately 1% of newborns excrete the virus), health care workers should assume that any child may be excreting virus and should practice hand hygiene and use of Standard Precautions as a part of routine patient care.[610] Additional interventions are not indicated, and pregnant staff need not be given special assignments.

Herpes Simplex Virus

Health care–associated transmission of herpes simplex virus (HSV) type 1 to neonates has been confirmed through studies using molecular typing.[277,395,579,678] Direct contact transmission from a hospital worker with herpes labialis and indirect contact transmission from one infected neonate to another were the most likely routes, although transmission from an asymptomatic parent or caregiver was possible. Health care–associated HSV infections among health care workers, patients, and family members have also been described in ICUs.[5,521] Herpetic whitlow has been observed among nurses, presumably as a result of direct transmission during suctioning of oral and respiratory secretions from infected patients.[5] The use of Standard Precautions, particularly the use of gloves during contact with oral and respiratory sections and hand hygiene, is adequate to prevent transmission of HSV.[510]

Measles, Mumps, and Rubella Viruses

Transmission of measles in health care facilities remains a threat, and hospitals have the potential to serve as niduses for community-wide infection.[72,116,127] Measles is transmitted by airborne droplet nuclei, as demonstrated by an outbreak in a pediatrician's office.[550]

Prevention of health care–associated measles hinges on the prompt recognition of infected or potentially infected patients and placement of these patients in isolation rooms with negative air pressure relative to the hallway.[610] Prophylactic vaccination of exposed susceptible individuals within 3 days of the exposure reliably prevents measles, whereas administration of immune globulin within 3 days attenuates disease but does not guarantee that the exposed individual will not develop contagious infection.[16] To avoid outbreaks of measles among health care workers, health care facilities should require new employees involved in patient care to provide evidence of immunity or appropriate vaccination or to

*References 48, 94, 99, 195, 244, 290, 334, 446, 545, 570, 597, 641, 677.

receive vaccination with either measles vaccine or MMR (measles, mumps, rubella) vaccine.[117,357]

Outbreaks of health care–associated mumps and rubella have been reported less commonly than measles.[291,527,642,709] The primary concern with regard to spread of both of these infections relates to potential complications in health care workers—mumps orchitis in adult males and rubella in pregnant staff. Information regarding the transmission of both of these viruses is limited, but it probably occurs primarily via droplet contact. Droplet Precautions should be observed during close contact with infected patients.[610] Transmission of mumps occurs before and within 5 days of parotitis onset, so isolation should be during this period.[364] Patients with congenital rubella syndrome may excrete large amounts of virus in their urine and respiratory secretions for extended periods. Consequently, gowns and gloves should be worn for contact with these patients for the first year of life unless nasopharyngeal and urine cultures are negative by 3 months of age.[610] There is no known effective postexposure prophylaxis for mumps or rubella-exposed individuals.

As with prevention of measles, new employees involved in patient care should provide evidence of immunity or appropriate vaccination or receive vaccination with MMR vaccine as a condition of employment.[117]

Parvovirus B19

The wide spectrum of disease caused by parvovirus B19, coupled with well-documented health care–associated outbreaks of infection, has fueled efforts to understand the transmission and control of this virus in hospitals. An experimental study of acute parvovirus B19 infection in normal adults detected virus in respiratory secretions during a period of viremia and systemic symptoms 6 to 13 days after inoculation.[28] Detectable virus in respiratory secretions disappeared as viremia and systemic symptoms diminished, and virus was not demonstrable in respiratory secretions when rash, arthralgias, and arthritis occurred several days later.[28] From studies of parvovirus B19 infection in health care facilities, it is apparent that the virus can be transmitted by acutely infected patients, probably owing to high levels of virus in their blood and respiratory sections.* The risk for transmission from chronically infected, immunocompromised patients is less clear; however, from one report it appears that these patients can transmit infection.[407]

The mode of transmission is not clear but probably involves direct or indirect contact with respiratory secretions or droplet transmission. Therefore, Droplet Precautions should be maintained during contact with infected patients, and gloves should be worn when handling fomites contaminated with respiratory secretions.[610]

Hepatitis A Virus

In the prevaccination era, hepatitis A virus caused rare but large epidemics in health care facilities.[37,192,347,486,693] Mildly symptomatic children who excrete virus in their stool can cause widespread transmission, and many secondary cases may occur before the infection is detected and contained.

Controlling a health care–associated hepatitis A outbreak is difficult and may require the assistance of the local health department. To limit the potential for indirect contact transmission, hospitalized infected children should be cohorted. Hand hygiene and use of barrier precautions should be emphasized to prevent direct and indirect contact transmission.[610] Postexposure prophylaxis, which may involve immune globulin or hepatitis A vaccination depending on age and underlying conditions, should be administered to all exposed, susceptible individuals.[17] Symptomatic health care providers should be furloughed until 1 week after the onset of symptomatic infection or until all susceptible persons have received immune globulin.[69]

Enteroviruses

Outbreaks of health care–associated enterovirus have been reported, most occurring in neonatal nurseries.*

Like for hepatitis A, hand hygiene and barrier precautions are of paramount importance to prevent transmission and cohorting of infected infants is prudent.[610] One report describes the value of early diagnosis using a PCR assay to successfully control measures.[34] The upper respiratory tract may be involved in acute enteroviral infection, but the use of masks by caregivers is not recommended.[610]

Tuberculosis

Health care–associated tuberculosis in pediatric patients occurs almost exclusively as a result of transmission of *Mycobacterium tuberculosis* from infected adults—parents, visitors, and health care workers—to other children and adults.† Probable transmission from infected children in health care settings has been described.[430,540] However, the risk for transmission from a child is very small because children infrequently have cavitary disease and consequently have fewer tubercle bacilli in their endobronchial secretions and because young children do not tend to generate aerosols of airborne droplet nuclei.[634] Nonetheless, transmission from children can occur as evidenced by extensive transmission from a 9-year-old child with bilateral cavitary disease.[158]

The risk for health care–associated tuberculosis in adult hospitals has been minimized by prompt recognition and treatment of pulmonary tuberculosis, adequate isolation of infectious patients in rooms with negative air pressure relative to the hallway, and proper use of personal protective equipment.[323] If rooms with appropriate ventilation are unavailable, alternative engineering solutions, such as well-placed and maintained ultraviolet lights, may be useful.[323] Personnel entering the rooms of infected patients should wear respiratory protection devices. Particulate respirators with a National Institute

*References 49, 207, 355, 407, 524, 549.

*References 41, 66, 125, 286, 305, 326, 346, 363, 541, 649.
†References 38, 243, 453, 485, 638, 700.

for Occupational Safety and Health certification of N95 or better satisfy the CDC specifications for these devices.[323] These devices must be fit tested on the individuals using them according to the standards of the Occupational Health and Safety Administration (OSHA).[323] Prompt identification of tuberculosis in parents, visitors, and health care workers in hospitals caring for pediatric patients is critical to reducing the risk for health care–associated tuberculosis in children and is cost saving compared with conducting postexposure follow-up investigations.[153] Health care workers should be screened yearly for evidence of tuberculosis.[323]

Invasive Bacterial Infections

Health care–associated transmission of *Neisseria meningitidis* is rare.[141,200,560] Nonetheless, *N. meningitidis* is spread by droplet transmission and health care workers should use Droplet Precautions during the care of patients with suspected invasive meningococcal disease until the patient has completed 24 hours of parenteral antimicrobial therapy.[610]

On the other hand, microbiology laboratory workers have developed fatal disease after working with cultures of this microorganism.[104,111] Clinical and research microbiologists who might be exposed routinely to isolates of *N. meningitidis* should receive meningococcal conjugate vaccine, as well as booster doses every 5 years if they remain at increased risk.[117]

Health care–associated transmission of *Haemophilus influenzae* and *Streptococcus pneumoniae* has been a rare phenomenon,* and the risk has likely been diminished substantially with vaccination.

Invasive bacterial infections in neonates, including infections caused by *S. aureus*, group B streptococci, *Citrobacter* spp., and *Enterobacter sakazakii*, are discussed later in this chapter (see Health Care–Associated Infections in Special Populations).

Ectoparasites

A large number of outbreaks of scabies have been reported from a variety of health care institutions. The presence of crusted scabies, which is associated with defects in cellular immunity, increases the risk for transmission dramatically because of the large number of mites in these lesions.[387] Although health care–associated transmission of pediculosis is possible, the direct or indirect contact necessary to spread this infection (i.e., head-to-head contact or sharing of combs) is less likely in medical settings than at home.

Intestinal Helminths

Person-to-person transmission of *Enterobius vermicularis* (pinworm), *Strongyloides stercoralis*, *Hymenolepis nana*, and *Taenia solium* is possible because these microorganisms do not require an intermediate host and because the eggs or larvae excreted in the stool are infectious.[386] However, evidence for health care–associated transmission of these microorganisms is limited.[386]

HEALTH CARE–ASSOCIATED INFECTIONS RELATED TO INVASIVE DEVICES AND PROCEDURES

Health care–associated infections can occur from contact with intravascular catheters and infusions, respiratory therapy, instrumentation of the urinary tract, and surgical procedures. Children are at risk for other health care–associated infections related to medical treatments, including infections associated with receipt of blood products. To learn more about these infections, the reader is referred to the chapters in this text related to common bloodborne pathogens.

Infections Related to Intravascular Catheters and Infusions

Infections related to intravascular catheters may be local or systemic. Local infections may involve the catheter exit site, subcutaneous track of tunneled catheters, or subcutaneous pocket of an implanted catheter.[443,494] Phlebitis is a common local complication but is usually due to chemical or mechanical irritation and is less common in children than adults.[238] Suppurative thrombophlebitis is rare in children.[340] Bloodstream infections related to intravascular catheters can have serious complications, including development of endocarditis, septic thrombophlebitis, and infection at distal sites due to hematogenous seeding, and independently increase the length of stay and hospital cost.[202,443,494]

Clinical and surveillance definitions for bloodstream infections related to central venous catheters differ, although they are often used interchangeably (Tables 243-3 and 243-4).[300,443] *Catheter-related bloodstream infections* are clinical events that require investigation to establish the catheter as the definitive source of infection (i.e., through quantitative blood cultures, differential time to positivity, or culture of the catheter itself, if it is removed) (see Table 243-3).[443] For surveillance purposes, the term *central line–associated bloodstream infection* (CLABSI) is used by the CDC's National Healthcare Safety Network (NHSN) to capture primary bloodstream infections in patients with central lines (see Table 243-4).[300] This definition is simpler for surveillance purposes. There is growing concern that NHSN definitions may overestimate catheter-related infections in some patients with impaired mucosal integrity in which bacterial translocation may be an important component in the pathogenesis of bloodstream infections,[222] and efforts to develop modified definitions for this subgroup of patients are underway.[184] Readers should refer to the NSHN website for the latest updates in NHSN definitions and methodology.* Although the focus in this chapter is primarily on clinical events related to catheters, most epidemiologic

*References 27, 39, 45, 133, 166, 171, 226, 303, 424, 438, 449, 491, 518, 623, 703.

*Centers for Disease Control and Prevention, National Healthcare Safety Network: http://www.cdc.gov/nhsn.

TABLE 243-3 Clinical Definitions for Intravascular Catheter-Related Infections

Infection	Diagnostic Criteria
Catheter colonization	Significant growth of ≥1 microorganism (>15 CFU) from the catheter tip, subcutaneous catheter segment, or catheter hub
Local infection	
Phlebitis	Induration, erythema, warmth, or pain/tenderness along the tract of a catheterized or recently catheterized vein
Exit site infection	Erythema, induration, or tenderness within 2 cm of the catheter exit site, with or without concomitant BSI
Tunnel infection	Tenderness, erythema, or site induration >2 cm from the catheter site along the subcutaneous tract of a tunneled (e.g., Hickman or Broviac) catheter, with or without concomitant BSI
Pocket infection	Purulent fluid in the subcutaneous pocket of a totally implanted intravascular catheter; often associated with spontaneous rupture and drainage or necrosis of the overlaying skin, with or without concomitant BSI
Bloodstream infection	
Infusate-related	Concordant growth of the same organism from the infusate and blood cultures (preferably percutaneously drawn) with no other identifiable source of infection
Catheter-related	Bacteremia/fungemia in a patient with an intravascular catheter with at least one positive blood culture obtained from a peripheral vein, clinical manifestations of infections (i.e., fever, chills, and/or hypotension) and no apparent source for the BSI except the catheter. One of the following should be present: a positive semiquantitative (>15 CFU/catheter segment) or quantitative (>10^2 CFU/catheter segment catheter) culture whereby the same organism (species and antibiogram) is isolated from the catheter segment and peripheral blood; simultaneous quantitative blood cultures with a >3:1 CFU/mL ratio of blood (catheter vs. peripheral); differential period of catheter culture versus peripheral blood culture positivity of >2 hr.

BSI, bloodstream infection; CFU, colony-forming unit.
Modified from Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1-45.

TABLE 243-4 National Healthcare Safety Network (NHSN) Surveillance Definitions for Infections Associated with Intravascular Catheters

Infection	Diagnostic Criteria
Central line associated	Central line was in place at the time of, or within 48 hours before, onset of event.
Primary bloodstream infections	Laboratory-confirmed bloodstream infections that are not secondary to an infection meeting criteria at another body site
Laboratory-confirmed bloodstream infections	One of the following criteria must be met: *Recognized pathogen* cultured from *one* or more blood cultures *and* Organism cultured from blood is *not* related to an infection at another site *or* Common commensal organism* is cultured from two or more blood cultures drawn on separate occasions *and* Patient† has at least one of the following signs or symptoms: fever (>38° C), chills, or hypotension *and* Signs and symptoms and positive laboratory results are not related to an infection at another site

*Diphtheroids (*Corynebacterium* spp. not *C. diphtheriae*), *Bacillus* spp. (not *B. anthracis*), *Propionibacterium* spp., coagulase-negative staphylococci (including *S. epidermidis*), viridans group streptococci, *Aerococcus* spp., and *Micrococcus* spp.
†For patients ≤1 year of age, hypothermia (<36° C), apnea, or bradycardia also should be considered.
See the NHSN website for the latest updates in NHSN definitions and methodology (www.cdc.gov/nhsn).
Modified from Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008:**36**:309-32.

data on bloodstream infections come from the use of surveillance definitions.

The vast majority of endemic catheter-associated bloodstream infections in children are associated with central venous catheters.[418] Peripheral venous catheters, especially catheters made with modern, nonthrombogenic materials, are rarely associated with bloodstream infections,[238,239,418,605] although presumably few data are systematically collected and the risk may be underestimated.[237,668] Peripheral arterial catheters have historically been associated with lower rates of infection compared with central venous catheters[193,230]; however, recent evidence among adult ICU patients suggests that rates of colonization and infection of these catheters may be

TABLE 243-5 Pooled Means of Central Line–Associated Bloodstream Infection Rates by Pediatric Unit Reported to National Healthcare Safety Network (NHSN)*

Type of Unit	Number of Units Reporting	Bloodstream Infection Rates†
Pediatric ICUs		
Medical	24	1.9
Medical/Surgical	204	1.8
Cardiothoracic	30	2.1
Step-down	11	1.0
Pediatric Inpatient Wards		
Medical	29	1.2
Medical/Surgical	187	1.5
Orthopedic	6	1.6
Rehabilitation	12	2.8
Surgical	8	1.3
Bone Marrow Transplant		
Temporary line	7	2.1
Permanent line	8	3.2
Hematology/Oncology		
Temporary line	30	2.8
Permanent line	31	1.7

*Data from January-December 2010.
†Central line–associated bloodstream infections/1000 central-line days.
Modified from Dudeck MA, Horan TC, Peterson KD, et al. National Healthcare Safety Network (NHSN) Report, data summary for 2010, device-associated module. *Am J Infect Control* 2011;**39**:798-816.

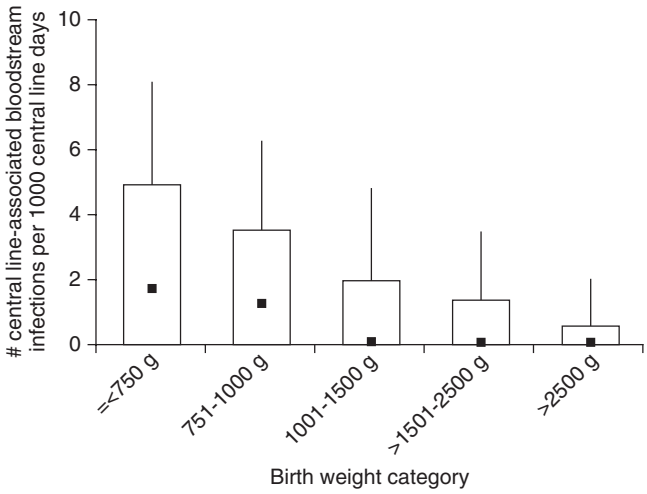

FIGURE 243-1 ■ Distribution of central line–associated bloodstream infection rates in level III neonatal intensive care units reported to the National Healthcare Safety Network (NHSN). Boxplots of the distribution of central line–associated bloodstream infection rates in neonatal intensive care units indicate the following: the solid square (■) represents the 50th percentile (median). The upper bound of the open vertical rectangle (□) represents the 75th percentile. The upper bound of the line extending above the open vertical rectangle represents the 90th percentile. The number of participating neonatal intensive care units in each of the birthweight classes ranged from 226 to 245. (Modified from Dudeck MA, Horan TC, Peterson KD, et al. National Healthcare Safety Network [NHSN] Report, data summary for 2010, device-associated module. *Am J Infect Control* 2011;**39**:798-816.)

similar to those observed for central lines.[350,406] The risk for bloodstream infection associated with peripheral arterial catheters appears to be highest in patients with femorally inserted arterial catheters.[206,403]

Given that the majority of infections occur in patients with central lines, the remainder of this section focuses on central venous catheters. National estimates of CLABSI rates are available through the CDC's NHSN during 2010. A recent NHSN report highlights data from 2473 hospitals from 49 states and the District of Columbia, of which 51 (2%) were dedicated children's hospitals.[194] Rates of bloodstream infection associated with central venous catheters in pediatric ICUs and inpatient wards and neonatal ICUs are displayed in Table 243-5 and Figure 243-1, respectively. Rates of infection are highest among children in specialty care areas, including hematology/oncology and bone marrow transplant, as well as pediatric rehabilitation.[194] Catheter-associated infections are also common among children with intestinal failure (or short-gut syndrome) who depend on parenteral nutrition. Among premature infants, infection rates are inversely proportional to birth weight. These data do not distinguish rates of infection associated with specific catheter types nor whether a specific catheter is the source of infection in patients who have multiple catheters in place concurrently.

The most commonly reported pathogens in nosocomial bloodstream infections include coagulase-negative staphylococci, *S. aureus*, enterococci, and *Candida* spp., although infections with gram-negative organisms may be increasing in some populations.[93,427,715] Pathogens associated with catheter infections in pediatric ICUs are available from 29 hospitals participating in the Children's Hospital Association Quality Transformation Network and are displayed in Table 243-6.[484] In this population, the majority of infections were caused by a single organism; gram-positive cocci were isolated most commonly, especially coagulase-negative staphylococci or enterococci. Of the *S. aureus* isolates, MRSA comprised nearly 50 percent. Gram-negative bacilli were identified in one third of cases and *Candida* spp. were found in 15 percent of cases. Antimicrobial resistance is a concern for all common pathogens isolated in bloodstream infections, including *S. aureus* and gram-negative bacilli.[92] Bloodstream infections due to VRE may be more prevalent in high-risk groups such as pediatric oncology patients and should be considered when selecting empirical therapy for bloodstream infections based on local epidemiology.[337] Nontuberculous mycobacteria and *Nocardia* spp. may also cause bloodstream infection.[10]

There are four major mechanisms for contamination of intravascular catheters: (1) migration of microorganisms colonizing the skin at the insertion site along the external catheter surface and into the blood vessel (extraluminal contamination); (2) contamination of the catheter or catheter hub through contact with hands of

TABLE 243-6 Central Line–Associated Bloodstream Infection Blood Culture Isolates in 29 Pediatric Intensive Care Units Participating in the Children's Hospital Association Quality Transformation Network Collaborative

	%	Number
All central line infections	100	308
1 organism	90.9	280
2 organisms	8.1	25
3 organisms	1.0	3
No pathogen info (excluded below)	—	6
Gram-positive cocci	46.6	158
Staphylococcus	24.2	82
Coagulase-negative	16.8	57
S. aureus	5.0	17
S. aureus, MRSA	2.4	8
Enterococcus	20.4	69
E. faecalis	12.4	42
E. faecium	5.3	18
E. faecalis/ faecium, VRE	1.5	5
Species NOS	1.0	4
Streptococcus species	2.1	7
Gram-negative bacilli	35.4	120
Enterobacter	11.8	40
E. cloacae	8.0	27
E. aerogenes	2.7	9
Species NOS	1.2	4
Klebsiella	9.1	31
K. pneumoniae	7.4	25
K. oxytoca or *ozaenae*	2.0	6
Pseudomonas	4.1	14
P. aeruginosa	3.5	12
Species NOS	0.6	2
Serratia marcescens	3.8	13
Stenotrophomonas maltophilia	2.4	8
Escherichia coli	2.1	7
Acinetobacter species NOS	0.6	2
Bacteroides species NOS	0.6	2
Citrobacter freundii	0.6	2
Alcaligenes xylosoxidans	0.3	1
Fungi	15.9	54
Candida	15.0	51
C. parapsilosis	7.4	25
C. albicans	6.5	22
C. tropicalis	1.2	4
Yeast NOS	1.0	3
Gram-positive bacilli	2.1	7
Bacillus cereus	0.9	3
Lactobacillus species NOS	1.2	4
Corynebacterium species NOS	0.0	0

MRSA, methicillin-resistant *Staphylococcus aureus*; NOS, not otherwise specified; VRE, vancomycin-resistant enterococci.
Modified from Niedner MF, Huskins WC, Colantuoni E, et al. Epidemiology of central line associated bloodstream infections in the pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:1200-8.

health care workers (intraluminal contamination); (3) hematogenous seeding from another focus of infection; and (4) contamination of the infusate.[150,151,494,495] The first two mechanisms are most important and differ in their contributions to infection depending on the duration of catheterization.[536] Extraluminal contamination predominates in the first 10 days (likely related to contamination during insertion), whereas the potential for intraluminal contamination increases with duration of catheterization and is most common after 30 days (most likely due to contamination during routine catheter maintenance and use).[536] Contamination of infusates is relatively uncommon; however, a number of epidemics have been associated with contaminated intravenous infusions,[397,420,603] medications,[52,259,420,506] and blood products.[108]

Additional pathogenic determinants for bloodstream infection include catheter material, host factors leading to the formation of a fibrin sheath,[442] as well as intrinsic virulence of the microorganism. Catheters made of polyethylene or stiff plastics like polyvinyl chloride are less resistant to colonization in vitro and may lead to more blood vessel trauma than catheters made of polyurethane. Surface irregularities and the thrombogenicity of catheter material may also contribute to colonization and infection. Adherence properties of microorganisms are also critical. Production of an extracellular polymeric substance by organisms such as coagulase-negative staphylococci, *S. aureus*, and *P. aeruginosa* leads to formation of a microbial biofilm in which microorganisms can become embedded and proliferate relatively protected from host defenses and antimicrobial agents.[4,191,307,439] *Candida* spp. also routinely produce biofilms, particularly in the presence of glucose-containing fluids.[80]

The principal risk factors for catheter-related bloodstream infection include heavy colonization of the skin at the catheter exit site[150,151,495,537] or catheter hub,[170,394] longer duration of insertion (>3 days),[152,238,239] use of catheters with multiple lumens as opposed to single lumens,[135] and the presence of a mural or atrial thrombus.[150,151,495] Both the level and composition of nurse staffing have also been associated with bloodstream infections in adult surgical ICUs: low nurse-to-patient and regular nurse (as opposed to pool nurse)-to-patient ratios have been associated with a higher risk for bloodstream infection.[225,558]

Dedicated pediatric studies are limited; however, age younger than 2 years, duration of catheterization (i.e., presence of a catheter >7 days), and receipt of parenteral nutrition or blood transfusions have been independently associated with catheter-associated bloodstream infection in pediatric ICU patients.[173,201,484,719] A recent study of children at a tertiary care center found that catheter breakage was associated with significantly increased risk for bloodstream infection within 30 days of repair.[408] Additional novel risk factors identified in one study include presence of a gastrostomy tube, nonoperative cardiovascular disease, and ICU catheter placement.[719] Among pediatric oncology patients, placement of the catheter within 1 month and frequent platelet transfusions have been associated with increased risk for bloodstream infection.[337] Infusion of parenteral nutrition with lipids increases the risk for infection

with coagulase-negative staphylococci and *Candida* in neonates.[35,85,223,574]

Among central venous catheters, nontunneled (percutaneously inserted) catheters account for the majority of catheter-associated bloodstream infections whereas totally implantable catheters carry the lowest risk,* although these comparisons may be confounded by a number of factors, including age and underlying severity of illness.[35,85,313,711,718] Tunneled catheters carry a lower infection risk compared with nontunneled catheters, because presence of a cuff prevents migration of microorganisms into the catheter tract. Peripherally inserted central venous catheters (PICCs) are used commonly in pediatric patients and have been associated with fewer bloodstream infections than other central venous catheters[389]; however, duration of catheter dwell was shown to increase risk for PICC-associated infection among neonates, with a significant daily increase in risk after 35 days.[454,598] Rates of infection are similar between catheters placed in the umbilical vein versus artery.[371]

Catheter insertion site influences infection risk in adults, likely related to the density of local skin microorganisms, although comparisons of infection rates between nontunneled catheters placed in the subclavian, internal jugular, and femoral vein have only been made using observational data. A recent meta-analysis showed that catheters inserted into the internal jugular vein carry a higher risk for colonization and infection as compared with subclavian catheters, whereas femorally inserted catheters carry the highest risk, although the authors suggest that interpretation may be limited by study heterogeneity and limitations in study design.[511] In a Cochrane Review it was concluded that subclavian catheters were associated with less colonization and fewer thrombotic complications than femoral catheters in short-term catheterization but no difference was found between subclavian and internal jugular venous catheters.[56] Of note, femoral catheters have not been associated with higher colonization rates and infection in children, but further study may be warranted.[484,552]

To differentiate bloodstream infections that arise from the catheter as opposed to another site, paired blood cultures should be drawn from both the catheter and a peripheral vein before starting therapy.[443] If a peripheral sample cannot be obtained, at least two samples should be drawn through different catheter lumens. If the catheter is removed owing to suspected infection, catheter tip culture is recommended to determine whether it is colonized. Semiquantitative (roll-plate) or quantitative catheter culture techniques (luminal flushing or sonication) are the most reliable modalities and are preferred over qualitative broth cultures.[423,537,571,604,611]

Clinical criteria for definitive diagnosis of catheter-related bloodstream infections are displayed in Table 243-3 and require positive percutaneous culture results with concordant microbial growth from the catheter tip or catheter-drawn cultures that meet criteria for quantitative culture or differential time-to-positivity.[443] Quantitative blood cultures are the most accurate; however, the differential time-to-positivity technique can be almost as sensitive (provided the same volume of blood is used to inoculate both blood culture bottles) and is less labor intensive.[571] Because peripheral blood cultures are drawn less frequently in children, owing to difficulty in obtaining peripheral specimens or concerns about drawing large blood volumes, the catheter is often implicated presumptively if no other source of infection is identified: this assumption has implications for management (including catheter removal) and is discussed further later.[443]

Rapid diagnostic techniques have also been developed. The acridine-orange leukocyte cytospin test rapidly detects microorganisms in hub blood without catheter removal. It is nearly as sensitive as both the paired quantitative blood culture and differential time to positivity techniques.[566,571] Emerging molecular techniques include fluorescence in situ hybridization[358] and PCR,[692] although these methods are not routinely available in most clinical laboratories.

Empirical antimicrobial therapy for children with suspected bloodstream infection is similar to that for adults: the choice of therapy should be based on severity of illness, the nature and severity of underlying conditions, and the relative frequency and susceptibility patterns of local pathogens causing this infection. The clinical practice guideline cited previously contains detailed recommendations for management.[443] In most cases, empirical therapy should include coverage of both gram-positive and gram-negative bacteria. Owing to the increased prevalence of MRSA across institutions, vancomycin is now often included. Empirical combination therapy for multidrug-resistant gram-negative bacilli, such as *P. aeruginosa*, should be added for patients who are severely ill, neutropenic, or known to be colonized with such organisms. Empirical antifungal therapy may be considered in septic patients with risk factors for candidemia, including receipt of parenteral nutrition, prolonged use of broad-spectrum antibiotics, hematologic malignancy or receipt of bone marrow or solid-organ transplant, or known colonization with *Candida* spp. Unlike adult recommendations, empirical antifungal coverage is not recommended for children with femoral catheters when bloodstream infection is suspected.[443] Once culture information is available, treatment can be tailored or discontinued as appropriate.

There is substantial evidence that most uncomplicated bloodstream infections in children can be treated effectively without catheter removal.[216,443,711] However, catheter removal should be strongly considered with difficult-to-treat bacteria, such as *S. aureus*, *P. aeruginosa*, and mycobacteria.[443] In particular, treatment of catheter-associated fungemia without catheter removal has a relatively low success rate and may be associated with higher mortality.[165,444] Although a recent retrospective study among adults with catheter-associated candidemia found no difference in outcomes between early and late catheter removal, it did not address long-term outcomes such as recurrence.[490] This finding will require further consideration in future practice guidelines. Catheters should be removed immediately in patients with severe sepsis, embolic phenomena, septic thrombophlebitis, or endocarditis or if infection continues despite more than 72 hours of targeted therapy.

*References 304, 367, 455, 599, 636, 711, 718.

The bloodstream infection management guideline also reviews the use of antibiotic lock therapy in the treatment of catheter-related bacteremia when catheter salvage is the goal.[443] The concept behind lock therapy is that organisms embedded in biofilms on intraluminal surfaces of long-term catheters may be killed more effectively by antibiotic concentrations 100 to 1000 times higher than standard concentrations of therapeutic parenteral antibiotics.[443] Antibiotic lock solutions are typically mixed with heparin in sufficient volumes to fill the lumen, allowed to dwell, and then removed. This approach is currently recommended in conjunction with systemic therapy for select organisms. In particular, lock therapy is not recommended for infections due to *S. aureus* and *Candida* spp. unless unusual extenuating circumstances are identified.[443] Use of adjunctive urokinase or other thrombolytic agents is not recommended.[33,366,443]

Duration of therapy varies by pathogen type but is typically 7 to 14 days.[443] With catheter removal, shorter courses (i.e., 5 to 7 days) may be appropriate for uncomplicated infections with less virulent organisms such as coagulase-negative staphylococci. Persistently positive blood cultures despite targeted therapy or recrudescence of infection shortly thereafter should prompt catheter removal. Catheter removal from patients with persistent fever but no positive culture results and no evidence of infection elsewhere is controversial and should be approached on a case-by-case basis.

Some success has been reported in treating uncomplicated exit site infections with the catheter in place. A trial of combined local and systemic antimicrobial treatment is warranted before a decision to remove the catheter is made. In contrast, treatment outcomes of tunnel or pocket infections are generally poor if the catheter is retained.[443,711]

A guideline for the prevention of infections related to intravascular catheters has been published.[494] In addition, several reviews have focused on novel technologies in the prevention of these infections.[150,292,537] Key preventive measures are emphasized here and focus on aspects of insertion and maintenance.

In general, use of intravascular catheters and infusions should be limited to only when necessary. Reducing exposure to central venous catheters is one of the simplest ways to reduce the risk for bloodstream infections. Several reports have highlighted the significant impact of preventive measures during catheter insertion in reducing bloodstream infections in adults, often including "bundles" of practices.[53,534,691] These interventions include performance of hand hygiene and use of effective skin antiseptic (chlorhexidine-based agents are more effective than povidone-iodine or alcohol-based agents but are not approved by the U.S. Food and Drug Administration (FDA) for use in infants < 2 months of age)[124,150,237,421] in addition to maximal sterile barrier precautions (e.g., sterile gowns, gloves, and drapes).[538] The catheter site should be covered with either sterile gauze or a sterile, transparent semi-permeable dressing.[494] If sterile gauze dressing is applied, it must be removed or changed within 48 hours. Routine application of topical antimicrobial agents is not recommended.[150,494,495]

Ongoing preventive measures during catheter maintenance are also critical. A pediatric quality improvement collaborative revealed that maximizing compliance with insertion bundles was not sufficient to reduce bloodstream infection rates in pediatric ICUs, underscoring the importance of scrupulous practices related to routine catheter use and maintenance.[322,331,452,593,714] Many improvement initiatives over the past decade have advocated "bundling" of preventive practices—simultaneous implementation of multiple preventive practices to achieve maximal reductions in infections. These bundled preventive measures include adherence to aseptic technique during dressing changes as well as attention to dressing integrity. Several studies have evaluated the efficacy of chlorhexidine-impregnated sponge dressings, including a neonatal study.* In the largest, multicenter randomized clinical trial to date comparing chlorhexidine-impregnated sponges to standard dressings in adult ICU patients, rates of catheter-related infections were reduced even when background rates were low.[664] In the neonatal study, colonization rates were also reduced, but 15 percent of neonates weighing less than 1000 g developed contact dermatitis.[236] Current guidelines recommend chlorhexidine-impregnated sponges in patients aged older than 2 months if infection rates are high despite adherence to basic practices.[494] Daily bathing with 2 percent chlorhexidine cloths has also been shown to decrease rates of bloodstream infection in adult ICU patients.[186,461,472,529] Dedicated studies evaluating chlorhexidine bathing in pediatric ICU patients are pending.

The bloodstream infection prevention guideline also highlights the importance of minimizing catheter manipulation and provides detailed guidance around maintaining closed systems and when to change infusion sets.[494] Minimizing the frequency of catheter access is also a common preventive strategy at many hospitals, although there are few data to support this practice.

For patients with short-term devices, use of catheters impregnated with chlorhexidine/silver sulfadiazine (coating the external surface only) and minocycline/rifampin (coating both the internal and external surfaces) significantly reduces rates of bloodstream infection compared with use of uncoated catheters.[150,494,495,537] Although the majority of studies have been in adults, two nonrandomized studies of impregnated catheters in pediatric ICU patients suggested a decreased risk for infection.[62,126] In a head-to-head adult trial, minocycline/rifampin-impregnated catheters were associated with lower rates of infection than those impregnated with chlorhexidine/silver sulfadiazine.[164] Second-generation chlorhexidine/silver sulfadiazine–impregnated catheters (with higher levels of antiseptic and coating of both internal and external surfaces)[565] and minocycline/rifampin plus chlorhexidine–impregnated catheters have also been developed[539]; however, there are no published comparative studies using these catheters. The long-term impact of these catheters on antimicrobial resistance has not been evaluated, although prospective studies suggest that this risk is likely low.[494,580] In general, these catheters

*References 98, 236, 278, 390, 419, 664.

should be considered only after bundled standard procedures have been reliably implemented and infection rates remain elevated.

Multiple controlled studies have evaluated the use of antibiotic or antiseptic locks for the prevention of catheter-related bloodstream infections.[494] The majority of these studies have been performed in high-risk patient populations, including neonates and children with cancer or intestinal failure.[235,494,501] Multiple antibiotic lock solutions have been evaluated. A meta-analysis evaluating the use of antibiotic locks in hemodialysis patients found that antibiotic lock therapy significantly reduced catheter-related infections: they found that only four patients would need to be treated with lock therapy to prevent one infection.[720] Another recent meta-analysis compared the pooled effectiveness of ethanol with heparin locks in the reduction of bloodstream infection in children with chronic parenteral nutrition dependence and found that ethanol locks significantly reduced rates of both catheter-related infection and catheter replacement.[501] Current guidelines recommend prophylactic lock solutions in patients with long-term catheters with a history of multiple bloodstream infections despite maximal adherence to aseptic technique.[494]

Infections Related to Respiratory Therapy

Most infections related to respiratory therapy are associated with mechanical ventilation. Infections related to the use of other respiratory equipment, such as small-volume medication nebulizers, have also been described, but usually in the setting of an epidemic that was related to the use of contaminated equipment.[149,429,458] Here the discussion is focused primarily on ventilator-associated events or complications and conditions related to the use of mechanical ventilation. Major infections include tracheitis, tracheobronchitis, and pneumonia. In addition, mechanical ventilation may also increase the risk for sinusitis and otitis media in nasally intubated patients, because nasotracheal tubes interfere with normal drainage of the sinus ostia and eustachian tubes.[54,262] Of these syndromes, the vast majority of clinical and surveillance data exist for ventilator-associated pneumonia.

Among adults, ventilator-associated pneumonia has been reported in 9 to 27 percent of intubated patients and is associated with increased length of stay, hospital costs, and mortality.[496] Rates of ventilator-associated pneumonia among children in pediatric and neonatal ICUs are available from the CDC's NHSN report from 2010.[194] Ventilator-associated pneumonia comprised approximately 10 percent of all pediatric device-related infections reported to the NHSN; rates of pneumonia were generally lower in pediatric compared with adult ICUs. Similar to catheter-associated bloodstream infections, infection rates among neonates are inversely proportional to birth weight.[194] Aside from these reports of the rates of infection, dedicated studies of ventilator-associated pneumonia in infants and children are limited, although several reviews have summarized the existing pediatric literature.[218,532,682,717] The majority of information discussed below draws from studies in adults.

Most cases of ventilator-associated pneumonia are bacterial in origin and are polymicrobial. In general, the duration of mechanical ventilation prior to symptom onset is an important determinant of the most likely pathogens: "early" pneumonia (<4 days) is more likely due to antibiotic sensitive community pathogens, such as *S. pneumoniae* and *Haemophilus* spp., whereas "late" infections (>4 days) are more often related to antibiotic-resistant organisms.[22,309,494] This difference is thought to be related to the frequency of exposure to prior antimicrobial therapy as well as the local epidemiology of organisms prevalent in the ICU; the distinction may be less meaningful for patients who are frequently admitted to health care settings. Major pathogens include both gram-positive and gram-negative bacteria; *S. aureus* (including MRSA) and *P. aeruginosa* are isolated most frequently.[13,260,309] Other pathogens include *Streptococcus* spp., Enterobacteriaceae, and *Acinetobacter* spp.[242,309,554] Anaerobic bacteria are uncommonly implicated[309] but may play a role in polymicrobial infections, particularly when pneumonia is due to aspiration. Nosocomial viruses and fungi are rare causes of pneumonia in immunocompetent hosts. Although *Candida* spp. are isolated in some cases from samples obtained from the lower respiratory tract, they rarely, if ever, are the primary cause of pneumonia.[22]

Ventilator-associated pneumonia occurs when bacteria invade the pulmonary parenchyma of a patient receiving mechanical ventilation.[139] This process is directly linked to aspiration of oropharyngeal or gastric secretions that harbor potentially pathogenic organisms.[22,309] Colonization with pathogenic bacteria is facilitated by a number of factors, including enhanced bacterial adherence to mucosal surfaces in severely ill or debilitated patients, bacterial overgrowth of the stomach when acid-suppressing medications are used, as well as reductions in microbial diversity and pathogen selection due to antibiotic exposure.[496,647] The presence of an endotracheal tube also interferes with normal protective airway reflexes and prevents effective coughing. Subsequently, contaminated secretions pool and leak around the endotracheal tube; this process is exacerbated during changes in position or deflation of the subglottic cuff of the endotracheal tube cuff. Bacterial biofilms also form on the inner surface of the tube, providing a direct route for microbial invasion into the lower airway.[496]

Regurgitation of bacteria from the stomach into the pharynx may provide another route for bacteria into the airway; normal gastric pH prevents heavy contamination of stomach contents, but bacteria proliferate when stomach acid is neutralized by antacids or histamine-2 (H_2) blockers. Still, the degree to which gastric colonization contributes to risk for pneumonia is unclear.[647] Other potential pathways for infection include use of contaminated equipment or medications, often in outbreak settings,[405] and bacteremia with hematogenous spread.[22]

Once pathogens enter the lower airway, host defenses are important in determining whether infection results. Diseases that compromise mucociliary clearance or the ability of the immune system to contain and inactivate pathogens dramatically increase the risk for infection.

Risk factors for ventilator-associated pneumonia in adults can be classified into several categories: (1) host factors that increase susceptibility to pneumonia, such as advanced age, underlying chronic lung disease, and immunosuppression; (2) treatment factors that enhance colonization of the oropharynx or stomach with pathogenic bacteria, including administration of antimicrobials or agents that increase gastric pH; (3) factors that promote reflux of gastric contents and aspiration into the lower airway, such as depressed mental status, supine positioning, nasogastric tubes, and enteral feeding; (4) conditions that increase duration of ventilation; and (5) factors that hinder adequate pulmonary toilet, such as thoracic or abdominal surgery and immobilization.[647]

Although dedicated pediatric studies are limited, it is likely that many of these adult risk factors are also important in children. Prospective studies of critically ill children have found that the presence of a genetic syndrome, postsurgical admission diagnosis, prior antibiotic therapy, use of narcotic medications, presence of enteral feeds, reintubation, and transport out of the ICU were independently associated with ventilator-associated pneumonia.[13,203,633] Trauma,[648] female gender,[633] and gastroesophageal reflux disease[2] have also been associated with ventilator-associated pneumonia in children in single studies.

The diagnosis of ventilator-associated pneumonia can be challenging. Clinical diagnosis requires evaluation of a combination of clinical, radiographic, and microbiologic data. Relevant clinical findings include fever, leukocytosis or leukopenia, purulent secretions, and worsening gas exchange. Auscultation is often hindered by sounds of the ventilation system itself (particularly with high-frequency ventilation); in addition, a variety of underlying pulmonary diseases and conditions (e.g., fluid overload) may produce sounds indistinguishable from those present in pneumonia. Radiographic findings include new or progressive pulmonary infiltrates, cavitation, air bronchograms, or pneumatoceles. Air bronchograms are the most sensitive for pneumonia[682]; however, chest radiograph findings have only limited specificity, because significant overlap exists between pneumonia and other pulmonary conditions.

Microbiologic data are important both in diagnosis and in guiding subsequent treatment. Samples of lower respiratory tract secretions should be obtained on all patients with suspected ventilator-associated pneumonia in addition to blood cultures. A variety of invasive and noninvasive methods have been evaluated in obtaining respiratory specimens, although the optimal diagnostic method remains unclear.[88,606,661] A recent meta-analysis found no difference in quantitative versus qualitative respiratory cultures (nor in invasive vs. noninvasive techniques) in reducing mortality, time in ICU or on mechanical ventilation, or rates of antibiotic change in adult patients with ventilator-associated pneumonia.[58] Lung biopsy and bronchoscopic bronchoalveolar lavage are the gold standards for diagnosis but are not routinely used in immunocompetent children. Tracheal aspirates are obtained most commonly but may be misleading in an asymptomatic patient, because endotracheal tubes are often colonized with potential pathogens.[251] Gram stains are helpful in ruling out pneumonia when negative but have very poor positive predictive value,[497] because localized irritation or superficial infection of the trachea may produce purulent secretions.

Significant controversy exists around surveillance definitions; as of 2012, NHSN[300] and the American Thoracic Society/Infectious Diseases Society of America (IDSA)[22] definitions have differing timelines to meet criteria, making institutional comparisons difficult. A streamlined definition for ventilator-associated pneumonia has been evaluated that has produced rapid and reliable results with similar ability to predict patient outcomes.[349] The CDC plans to issue a revised definition for adults older than 18 years in January 2013 that will include any ventilator-associated event (not limited to pneumonia), require a minimum period of time on the ventilator, focus on objective clinical data, and does not include radiographic findings.

Empirical therapy for ventilator-associated pneumonia should be based on the duration of intubation and hospitalization, prior or current antibiotic therapy, severity of clinical disease (as well as the nature and severity of underlying conditions), and knowledge of the relative frequency and susceptibility patterns of local pathogens. Several adult studies have shown that delayed initiation or inappropriate antibiotic therapy is associated with poor outcomes.[22] Initial broad-spectrum therapy with adequate coverage of gram-negative bacilli, including *P. aeruginosa*, and possibly MRSA as guided by local epidemiology, is generally appropriate and may be narrowed based on subsequent culture data and clinical and radiographic findings.[22]

Prolonged treatment of patients in whom the diagnosis is questionable should be avoided because this practice may lead to endotracheal colonization with antimicrobial resistant bacteria. Treatment duration for ventilator-associated pneumonia has not been evaluated in children. As in adults, uncomplicated cases may be treated with 7 to 10 days of therapy. For complicated cases, or those with necrotizing pneumonia, at least 14 days of therapy should be administered. Shorter courses (<7 days) may be appropriate for children with ventilator-associated tracheitis without evidence of pneumonia.[655] Nasotracheally intubated patients with otitis media or sinusitis should have the nasotracheal tube changed to an orotracheal tube. Short-term treatment with a decongestant and sinus lavage may also be beneficial in sinusitis treatment.

Comprehensive guidelines for prevention of health care–associated[647] and ventilator-associated pneumonia[139] are available. Key strategies include three major themes: (1) reducing aspiration of secretions, (2) reducing colonization of the aerodigestive tract, and (3) preventing use of contaminated equipment.[139] Many strategies are implemented as practice "bundles," aimed at producing more robust results when combined.[65,157,496]

General strategies for prevention include limiting the duration of ventilation and using noninvasive approaches whenever possible. Daily assessment of readiness to wean and the use of weaning protocols may also be effective, in conjunction with daily interruption of sedation.[139,665] Although a randomized clinical trial among ventilated

children failed to demonstrate a reduction in time to extubation with the use of a volume support weaning protocol, the majority of children included in the study were weaned from ventilator support in less than 2 days.[544] Hand hygiene and use of gloves during contact with objects or surfaces contaminated with respiratory secretions are also important.

Aspiration can be minimized by maintaining patients in a semirecumbent position with elevation of the head of the bed to 45 degrees, although this position may be difficult to achieve consistently.[139] Novel approaches to positioning have gained recent attention. A clinical trial involving 60 intubated infants found that lateral positioning (keeping the tube at or below the horizontal plane) was associated with a significantly decreased frequency of positive tracheal cultures compared with patients who were supine.[15] Additional, larger randomized studies in adults are underway.[496]

Keeping the endotracheal tube free of secretions is also important in preventing aspiration. Use of a cuffed endotracheal tube with subglottic or in-line suctioning is recommended in adults; however, cuffed tubes are not used routinely in all children, especially infants, and in-line suctioning has not been studied in children. Subglottic suctioning has been evaluated in a recent meta-analysis of adult patients and found to reduce significantly the incidence of ventilator-associated pneumonia.[689] Suctioning should be performed by gently using a sterile, single-use catheter, and sterile fluids should be used to loosen secretions and clear the suction catheter. A novel approach to removing secretions from the interior lumen of the endotracheal tube (called the mucus shaver) has been evaluated in a small randomized clinical trial in adults and found to significantly reduce colonization of the tube.[56] If this finding is reproduced in larger studies, this device may enhance our ability to keep endotracheal tubes free from secretions and heavily colonized biofilms.[496] Other measures to reduce aspiration include avoiding unplanned extubation and reintubation and avoiding gastric overdistention.

Strategies to reduce colonization of the aerodigestive tract include regular oral care with antiseptics. In particular, a recent meta-analysis showed that use of an oral chlorhexidine rinse significantly reduces rates of ventilator-associated pneumonia in adults.[368] Avoiding acid suppression in patients who are not at high risk for developing stress ulcers or gastritis is another strategy but has yielded inconsistent results.[139,647] In particular, a retrospective study of ventilated children found that a strategy used to prevent gastrointestinal bleeding was not associated with risk for ventilator-associated pneumonia.[402]

Prophylactic antibiotic regimens have also been used to modulate oropharyngeal colonization.[139,647] Specifically, selective decontamination of the digestive tract uses a combination of nonabsorbable oral antimicrobial agents, systemic cephalosporins, and avoidance of antibiotics with anti-anaerobic activity to prevent secondary bacterial colonization, preemptively treat possible respiratory tract infection with commensal organisms, and preserve normal intestinal microbiota, respectively.[174] Selective oropharyngeal decontamination is similar but includes application of topical oropharyngeal antimicrobial agents only. Selective decontamination of the digestive tract[172,174,359,613] has been shown to reduce mortality among adult ICU patients in randomized clinical trials, including a systematic review, but is not recommended routinely owing to increased selective pressure on antibiotic-resistant organisms in areas with high levels of endemic multiple-drug resistance.[139,647]

To minimize contamination of respiratory equipment, sterile water should be used to rinse reusable devices. Tubing should be maintained in a dependent position relative to the endotracheal tube, and condensate should be routinely discarded; circuits should remain closed during condensate removal. The ventilatory circuit should be changed only when visibly soiled or malfunctioning. All respiratory equipment should be stored and disinfected properly.[139] Items with direct or indirect contact with mucous membranes or respiratory secretions should be cleaned thoroughly and either sterilized or disinfected in a manner consistent with high-level disinfection.

Infections Related to Instrumentation of the Urinary Tract

Urinary tract infections are the most common health care–associated infections in hospitalized adults worldwide.[299] In fact, catheter-associated urinary tract infections accounted for approximately 40 percent of all device-related infections reported to the NHSN in 2010.[194] In contrast, catheter-associated urinary tract infections are a much smaller proportion (approximately 12%) of device-related health care–associated infections in hospitalized children.[194,219,319,373,704] This difference is largely explained by differences in urinary tract catheterization between adults and children. Still, the infectious risk associated with the use of indwelling urinary catheters is similar in adults and children; pooled means of catheter-associated urinary tract infections are similar between adult and pediatric units reporting to the CDC's NHSN in 2010.[194]

Although diagnostic criteria for catheter-associated urinary tract infection vary, and are often reported differently between studies, surveillance definitions have been established.[300] Urinary tract infections are catheter associated if the patient had an indwelling catheter in place at the time of or within 48 hours of onset. Symptomatic urinary tract infection is defined as the presence of signs or symptoms of infection (e.g., fever, urgency, frequency, dysuria, or suprapubic tenderness) and a quantitative urine culture with 10^5 or more colony-forming units (CFU) per milliliter of no more than two different species.[300] Lower colony counts ($\geq 10^3$ CFU/mL) are accepted with evidence of pyuria and organisms on Gram stain in a symptomatic patient. Asymptomatic bacteriuria or candiduria is defined as the presence of a quantitative urine culture with 10^5 CFU/mL or more of no more than two different species in a patient without signs or symptoms referable to the urinary tract.[299]

Urinary tract infections in patients with short-term indwelling catheters (<30 days) are usually caused by single organisms while infections in patients with

long-term catheters (>30 days) are more often polymicrobial.[299] *E. coli* is the most frequently isolated species in both children and adults but comprises less than one third of isolates.[2,299,502] Other gram-negative bacteria, including Enterobacteriaceae and *P. aeruginosa*, and *Enterococcus* spp. and various *Candida* spp. are also isolated.* In a single-center pediatric study, recent antibiotic exposure within the prior 2 months was more common in children with infections with *P. aeruginosa* as compared with children with infection due to *E. coli*.[67] The percentage of urinary tract infections attributed to coagulase-negative staphylococci has been reported in excess of 30 percent in neonatal ICU patients.[168,398]

Microbial colonization of the urine is the first step in the pathogenesis of urinary tract infection; the urinary catheter facilitates this by perturbing host defenses and providing uropathogens easier access into the bladder. Still, infection is not an inevitable result of bladder colonization, and many chronically catheterized patients may have evidence of urine colonization for long periods without clinical evidence of infection. Pathogens may be introduced at catheter insertion, by ascension along the external catheter surface, or intraluminally through contamination of the collecting tube or drainage bag. A detailed culturing study of catheterized patients suggests that roughly two thirds of infections are caused by extraluminal spread of microorganisms along the external catheter surface while the other one third are intraluminally acquired.[651] Additional support for the extraluminal route comes from a number of studies in catheterized adults correlating microorganisms colonizing the urethral meatus with those most frequently causing infection.[91]

After catheter insertion, adherence of microbes along the internal and external surfaces of the catheter leads to colonization and facilitates the rapid formation of biofilms composed of host proteins and microbial exopolysaccharides. These biofilms become polymicrobic and migrate to the bladder within 1 to 3 days.[299,316,576] Movement of microorganisms into the bladder is facilitated by obstruction of urine flow and reflux of urine into the bladder (i.e., raising the collection system above the level of the bladder), but many bacteria can migrate upstream even in properly maintained systems. Catheter encrustations may be formed by urease-splitting organisms within biofilms, such as *Proteus* and *Providencia* spp., serving as additional sites for microbial proliferation and attachment.[299] Once introduced in the bladder, microorganisms multiply in the small, but persistent, reservoir of urine that is incompletely drained by the catheter.[91]

The incidence of catheter-associated bacteriuria is reported between 3 and 8 percent per day; duration of catheterization is the most important risk factor.[299] Other risk factors for catheter-associated bacteriuria identified in adults include female sex, catheter insertion outside the operating room, catheter care violations, older age, and rapidly fatal underlying illness.[299] The epidemiology of bacteriuria and urinary tract infections has also been evaluated in children, although most studies are dated.† In a large prospective study of 525 hospitalized children with urinary catheters, bacteriuria was identified in 11 percent of all catheterized patients, of which 78 percent were symptomatic; median duration of catheterization preceding bacteriuria was 7 days (range, 2 to 77 days).[399]

There are no published data regarding rates of urinary tract infection associated with different types of indwelling catheters (e.g., urethral, suprapubic, ureteral, nephrostomy) in children, although anecdotal evidence suggests that bacteriuria and candiduria are more common in catheters that remain in place for long periods regardless of catheter type. Bacterial colonization of urinary stents is common in children, although its clinical significance remains unclear.[51,672] Urinary tract infections are also very common after renal transplantation, occurring in up to 40 percent of children.[205,210,612,680] Age younger than 5 years, lower urinary tract abnormality, and prior history of urinary tract infection or vesicoureteral reflux before transplant have been associated with increased infection risk in these children.[205,210] Data regarding the incidence of infection after other types of urinary tract instrumentation in children are not available.

The diagnosis of symptomatic catheter-associated urinary tract infection is often challenging because many catheterized patients do not manifest symptoms such as urgency, frequency, or dysuria. Consequently, fever is the most commonly identified sign of infection in these patients, emphasizing the importance of a quantitative urine culture in the evaluation of a new fever in a catheterized child. Pyuria is a reasonably good predictor of the presence of significant concentrations of gram-negative rods in the urine of catheterized patients but is a poor predictor for the presence of significant concentrations of enterococci or *Candida*.[652] Still, the presence of pyuria alone is not diagnostic for infection in a catheterized patient, particularly those with long-term indwelling catheters.[299] Conversely, the absence of pyuria in a symptomatic catheterized patient suggests an alternative diagnosis.

Definitive diagnosis of catheter-associated urinary tract infections depends heavily on quantitative urine culture results (see earlier discussion). In patients with short-term catheters, specimens should be collected from the aspiration port using aseptic technique. With long-term catheters, removing the catheter and collecting a specimen from a freshly placed catheter before initiating therapy is preferred.[299] Specimens should not be collected from the drainage bag.

Empirical antimicrobial therapy for urinary tract infection should be based on the severity of clinical disease, the nature and severity of underlying conditions, and knowledge of the relative frequency and susceptibility patterns of local pathogens. Parenteral therapy is typically used until excluding secondary bacteremia. Therapy with a broad-spectrum agent targeting gram-negative bacilli, potentially including *P. aeruginosa*, and enterococci is generally appropriate. Antifungal therapy can usually be withheld, unless yeast elements are identified on urinalysis or urine Gram stain. Based on the patient's clinical status and culture results, the treatment regimen can be tailored accordingly or discontinued.

*References 2, 168, 299, 398, 399, 502.
†References 63, 67, 168, 373, 398, 399, 502.

Seven days of antimicrobial therapy is generally recommended for treatment of most catheter-associated urinary tract infections; longer courses (10 to 14 days) are used for those with a delayed response or with evidence of pyelonephritis.[299] If the infection is uncomplicated and the child has responded clinically, oral therapy can be used to complete the course. Whenever possible, indwelling urinary catheters should be removed. Infections involving nephrostomy or suprapubic catheters often recur if these catheters must remain in place. In cases in which nephrostomy tubes must be used for extended periods, suppressive antimicrobial therapy may be considered after treatment is completed, although there is a risk for secondary infection with resistant bacteria or yeast with this approach.

Guidelines for the prevention of catheter-associated urinary tract infections have been published and the key features are summarized here.[256,299] Reducing unnecessary use of urinary catheters, including leaving them in place only as long as needed, is the most important and effective preventative measure. Simple interventions, including nurse-based or electronic physician reminder systems to reduce inappropriate catheterization, have been shown to reduce significantly the rates of catheter-associated urinary tract infections in both children and adults.[87,168,441]

Urinary catheters should be inserted by trained personnel using aseptic technique. After insertion, contamination of the interior surfaces of the catheter and collection system can be minimized by maintaining a closed system (i.e., avoiding disruption of the connection between the catheter and the collection system) and ensuring that the drainage bag and connection tube are kept below the level of the bladder. Antimicrobial (silver alloy or antibiotic)-coated catheters have been shown to reduce or delay the onset of catheter-associated bacteriuria in adult patients with short-term catheters, but it remains unclear whether the use of these catheters reduces the incidence of symptomatic infection, particularly in patients with long-term catheters.[299] There have been no studies of novel urinary catheters in children.

Although systemic antibiotics have been shown to reduce or delay the onset of catheter-associated bacteriuria or infection in many adult studies,[91] their use at the time of catheter insertion, removal, or replacement is not routinely recommended, owing to the potential for adverse effects and the development of antimicrobial resistance.[299] A wide variety of other interventions have been studied, primarily in adults and most with limited or variable benefit.[91,299,422] At present, the routine use of methenamine salts or cranberry products is not recommended for the prevention of catheter-associated urinary tract infections.

Infections Related to Surgical Procedures

Patients undergoing surgical procedures are at risk for a variety of health care–associated infections, including bacteremia, pneumonia, and urinary tract infection (often related to the presence of other medical devices), but surgical site infections are most common.[61,528] A number of reports describing the epidemiology of surgical site infections in general pediatric surgery patients have been published, although most are dated.* More recent analyses of large cohorts of pediatric surgical patients have shown rates of surgical site infections between 1.3 and 6.6 percent after surgery.[468,548,671] Surgical site infections have a significant impact on patient outcomes, including increased duration of hospitalization, health care costs, and increased risk for death.[24]

Consensus criteria for surgical site infections are available from the CDC's NHSN.[300] Surgical site infections are subcategorized into either incisional (superficial or deep) or organ/space infections and are summarized in Table 243-7. Surveillance definitions require that infections arise within 30 days of surgery, unless an implant is left in place, in which case infections may be identified for up to 1 year. Implants are defined as non–human-derived objects (e.g., prosthetic valves) that are permanently placed and not routinely manipulated for diagnostic or therapeutic purposes.[300] Stitch abscesses are not considered surgical site infections.

Traditionally, the NHSN has provided simple risk adjustment measures to account for differences in case mix and allow for meaningful comparisons between surgeons or hospitals. Previously, three equally weighted factors were included: (1) composite measure of severity of illness (the American Society of Anesthesiology [ASA] score); (2) wound classification I to IV (e.g., clean, clean-contaminated, contaminated, and dirty-infected); and (3) procedure duration.[154] Recently, the NHSN has developed procedure-specific risk models for each procedure category reported to the NHSN, which incorporate existing patient and hospital-specific data elements. These models have been shown to improve predictive performance compared with traditional risk stratification and are intended to facilitate improved external benchmarking.[467]

Among pediatric studies, independent risk factors for infection include longer surgery duration[61,167,169,190]; younger age[90,190,414,600]; nonwhite race[90]; prolonged preoperative hospitalization[167,169,190]; emergency surgery[60,190,666]; longer length of incision[167,190]; procedure performed by a trainee[666]; and presence of underlying diseases.[60] Potentially modifiable risk factors identified in a recent study included postoperative ICU admission, urinary catheter placement, and use of an implantable device.[90]

Additional data exist for specific surgery types. In particular, children undergoing cardiovascular surgery are at high risk for infection owing to the complexity of congenital heart disease and the aggressive medical management required to support them through recovery. A prospective study of over 300 children undergoing cardiovascular surgery found an infection rate of 7.1 percent; infection rates were higher among patients with an open sternotomy after surgery and among those with increased severity of illness (i.e., higher Pediatric Risk of Mortality [PRISM] score).[528] Subsequent studies have identified prolonged duration of surgery,[12,330,476] younger age[12,50,330,476] (specifically, age <30 days[629]), cyanotic heart

*References 60, 167, 169, 190, 414, 600.

TABLE 243-7 National Healthcare Safety Network Surveillance Definitions for Surgical Site Infections (SSI)*

Incisional SSI†

Superficial: Infection involves skin or subcutaneous tissue of the incision *and* patient has at least *one* of the following:
- Purulent drainage from superficial incision
- Organisms isolated from an aseptically obtained culture of fluid or tissue from superficial incision
- At least *one* sign or symptom of infection (pain/tenderness, localized swelling, redness or heat) *plus* superficial incision is deliberately opened by surgeon *and* is either culture positive or not cultured
- Diagnosis of superficial incisional SSI by surgeon or attending physician

Deep: Infection involves deep soft tissues (e.g., fascial, muscle layers) of the incision *and* patient has at least *one* of the following:
- Purulent drainage from the deep incision (excluding organ/space component)
- Deep incision spontaneously dehisces or is deliberately opened by a surgeon *and* is culture positive or not cultured *and* patient has at least one sign or symptom (fever >38° C, localized pain/tenderness)
- Abscess or other evidence of infection involving deep incision found on direct examination, reoperation, or by histopathology for radiologic examination
- Diagnosis of deep incisional SSI by surgeon or attending physician

Organ/Space SSI

Infection involves any part of the body (excluding the skin incision, fascia, or muscle layers) that is opened or manipulated during the operative procedure *and* patient has at least *one* of the following:
- Purulent drainage from a drain that is placed through a stab wound into the organ/space
- Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space
- Abscess or other evidence of infection involving organ/space found on direct examination, reoperation or by histopathology for radiologic examination
- Diagnosis of organ/space SSI by surgeon or attending physician

*For all classifications, infection is defined as occurring within 30 days of the operative procedure if no implant in place or within 1 year if an implant is in place and the infection is related to the procedure.

†Incisional infections may be primary (related to the primary incision made by the surgeon) or secondary (related to a secondary incision, e.g., donor site for coronary artery bypass graft).

See the NHSN website for the latest updates in NHSN definitions and methodology (www.cdc.gov/nhsn).

Modified from Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**:309-32.

disease,[50] undergoing more than one cardiothoracic procedure,[297] having a preoperative medical device,[629] and parenteral nutrition as independent risk factors.[629]

Infection rates after cerebrospinal fluid shunting procedures vary widely.[372,499] A recent analysis of shunt infection rates across 41 free-standing children's hospitals reported infections in more than 11 percent of patients undergoing shunt procedures over a 24-month period, with adjusted hospital level rates between 1.4 and 5.3 percent per procedure.[616] Shunt infections rates are higher among those with perioperative cerebrospinal fluid leak,[321] history of prior shunting procedure,[499] and myelomeningocele.[499] Additional risk factors include younger age and shunt revision.[617]

Surgical site infections are also common in orthopedic patients, particularly after revision surgery, posterior spinal fusion, or the placement of implants.[621] A recent study evaluating rates of infection after posterior spinal fusion at a tertiary care children's hospital reported mean annual infection rates of 4.4 percent.[393] An ASA score greater than 2, obesity, and antibiotic prophylaxis with clindamycin were independent risk factors for infection; of note, the majority of pathogens isolated were gram-negative bacilli.

The most recent national surveillance data on the microbiology of surgical site infections among children in pediatric ICUs identified gram-positive cocci in nearly half of all isolates[554]; *S. aureus* was the most common, but coagulase-negative staphylococci and *Enterococcus* spp. were isolated frequently. Gram-negative bacilli comprised 40 percent of isolates. Anaerobes, including *Bacteroides fragilis*, are uncommonly isolated, but anaerobic cultures may be infrequently performed. *Candida* spp. are isolated from a minority (4%) of patients. This distribution of microorganisms is similar to findings from several studies of surgical site infections in general pediatric and cardiovascular surgery patients*; however, a recent retrospective study of surgical site infections at a pediatric tertiary care center reported that 21 percent of culture-positive infections were polymicrobial.[90] Gram-positive bacteria are more commonly isolated in cerebrospinal fluid shunt infections[499,670]; *Propionibacterium acnes* is among those isolated but typically produces milder symptoms and requires longer incubation times for isolation.[31]

The pathogenesis of surgical site infection involves a complex relationship between microbial characteristics, host factors, and the nature of the surgical procedure. The greatest risk for microbial contamination of the surgical site occurs while the wound is open. If the incision is closed primarily, subsequent contamination from external sources is uncommon. The vast majority of contamination arises from endogenous flora at or near the surgical site, including microorganisms colonizing the skin, mucous membranes, and hollow viscera.[425] Postoperative hematogenous seeding from endogenous flora at remote sites can occur but is uncommon. Exogenous contamination from colonized or infected surgical personnel, surgical instruments, or the operating room environment are infrequently implicated as causes of infection.[36,255,557] Unusual environmental pathogens, including airborne fungal spores, are occasionally implicated.[555]

Even with careful skin preparation and expert technique, microbial contamination is virtually universal. Although skin organisms are a common cause of infection, a significant number of these organisms reside in skin appendages, such as sebaceous glands and hair

*References 61, 90, 167, 198, 528, 600.

follicles, which are not exposed to topical antiseptics.[425] Thus, careful surgical technique is required to avoid conditions that favor microbial growth postoperatively. These conditions include damaged or devascularized tissue resulting from rough handling or overuse of electrocautery, as well as hematomas and seromas, which provide optimal bacterial growth conditions. Burden of inoculation is also critical, and required inoculums for infection may be lower in the presence of foreign bodies or material.[24]

Surgical site infections rarely occur within 48 hours of surgery, except in cases of infection with *S. pyogenes* or *Clostridium* spp.; therefore, fever within 48 hours of surgery is unlikely to be related to the surgical site.[637] Once identified, management of surgical site infections may require a combination of surgical débridement and antimicrobial therapy, depending on the location and extent of infection. Superficial incisional infections may be treated topically or with oral antibiotics alone, whereas deep incisional and organ/space infections almost universally require débridement and drainage; contemporary interventional radiology techniques have revolutionized this process. In some cases, fluid collections may be inaccessible or attempts at drainage may compromise the patient's condition. In these cases, antibiotics may be tried alone, although therapy should be chosen carefully and clinical response monitored closely.

The presence of prosthetic or foreign material complicates management. In particular, shunt infections are unlikely to resolve without shunt removal.[721] In some special cases, antimicrobial therapy can successfully suppress infection with foreign material while postoperative healing takes place (i.e., plates and screws to stabilize bones after orthopedic procedures). However, device removal is typically recommended to achieve cure. The likelihood of salvage of an infected prosthesis improves if the infection is detected within 3 weeks of implantation, the implant remains stable and the surrounding tissue is healthy, and the patient receives appropriate systemic antimicrobial therapy.[725] With prosthetic joint infections, most patients receive 6 to 8 weeks of intravenous antimicrobial therapy.[725]

Guidelines for the prevention of surgical site infections are available[81,425] and have been recently updated.[11] Similar to efforts targeting other device-related infections, many of these practices have been "bundled" to reduce surgical site infections in many hospitals with good success.[568] Key preventive measures aimed at minimizing microbial contamination of the surgical site are summarized next.

Preoperative interventions can impact both the nature and degree of microbial contamination. In general, minimizing preoperative hospitalization can prevent colonization with hospital-acquired pathogens. In addition, identifying and treating preexisting infections at remote sites is recommended. The use of preoperative bathing to reduce the number of bacteria colonizing the skin before surgery is widespread, although its effect on reducing infection is unclear. Preoperative showering with chlorhexidine has been shown to reduce the number of bacteria at the incision site better than povidone-iodine or soap and water[11,425]; in addition, preoperative chlorhexidine shampoo was found to be superior to iodophor products in reducing the number of scalp microorganisms prior to neurosurgical procedures in children.[380] More substantial reduction of bacteria is seen with multiple showers or with showers immediately preceding surgery, suggesting that timing may be important.[11] Still, there is no definitive evidence that this practice reduces rates of infection.[11,425] A meta-analysis evaluating the effect of preoperative chlorhexidine bathing compared with other wash products found no clear benefit in reducing infection risk.[698]

The preoperative use of intranasal mupirocin to reduce infections has also been studied. The largest study to date was a randomized controlled trial among adult surgical patients undergoing a variety of procedures.[520] Although intranasal mupirocin was not associated with a reduction in surgical site infections compared with placebo, it significantly reduced the rate of all nosocomial *S. aureus* infections among patients who were *S. aureus* nasal carriers. A dedicated meta-analysis evaluating the effect of intranasal mupirocin in reducing *S. aureus* infections in surgical patients with nasal carriage also found a significant reduction in all *S. aureus* infections postoperatively; however, their results were heavily influenced by the large study cited previously.[679] Mupirocin resistance was infrequently reported among the studies included in the meta-analysis but has been reported after widespread use of nasal mupirocin in other studies.[451] Screening of patients for colonization is not universally recommended but may be considered in high-risk groups, such as those undergoing placement of prosthetic devices in regions where the community prevalence of MRSA is high.

Although removing hair appears to provide a "cleaner" operative site, current evidence suggests that shaving is associated with higher rates of infection, presumably owing to microscopic skin trauma and liberation of resident skin flora from deep skin structures.[11] If hair removal is necessary, current evidence suggests that clippers should be used over razors.[657] If required, most recommend hair removal immediately before surgery; however, a meta-analysis found no difference in infection rates between hair removal one day before or the day of surgery[657]; however, the authors note that studies were small and more research is needed.

Perioperatively, the use of antimicrobial prophylaxis is among the most important measures to prevent infection.[11] Numerous studies have examined the effectiveness of antimicrobial prophylaxis, although few have included children. A comprehensive "meta-analysis of meta-analyses" included 250 clinical trials with over 4800 patients to determine whether antibiotic prophylaxis is effective in preventing surgical site infections across a variety of procedures and found that perioperative antibiotics significantly reduced infection risk across 23 surgery types evaluated.[74] In addition, the protective effect was sustained across different levels of surgery cleanliness.[74] The most effective time for antibiotic administration is within 30 minutes of skin incision, except for agents such as vancomycin that require longer infusion times and therefore should be administered between 1 and 2 hours before surgery.[11] If prophylaxis is given outside this period, effectiveness is diminished and

has virtually no effect more than 6 hours after the incision.[136] If surgery is prolonged, re-dosing should be performed after two half-lives of the administered agent have elapsed. Antimicrobial agents should be stopped within 24 hours after the procedure.

A variety of intraoperative practices and procedures are used to prevent contamination of the surgical site, including use of antiseptics such as alcohols, iodophors, and chlorhexidine.[425] The surgical hand scrub by surgical personnel reduces the number of bacteria on the hands and the potential for contamination through visible or microscopic breaks in surgical gloves. A systematic review found that alcohol rub was as effective as aqueous scrubbing; however, meta-analysis was precluded by major differences between studies.[658] Skin antisepsis also reduces the number of viable bacteria at the surgical site. Current evidence from randomized clinical trials and meta-analyses suggests that use of chlorhexidine is superior to that of iodophors in preventing surgical site infections and results in cost savings.[163,382] However, a follow-up observational study evaluating use of povidone-iodine for disinfection found that residual bacteria left behind at the incision site were not associated with increased risk for infection.[669] Barrier precautions, including masks, caps, and gowns, are a major part of practice and provide protection for surgical personnel against blood and body fluid exposures, although their impact on infection risk is unclear. Operating room traffic should also be minimized, because activity increases bacterial counts in the air; however, the effect on infection risk is likely small. Bacterial counts can also be minimized by regularly servicing operating room ventilation systems and maintaining adequate ventilation parameters.[21]

Proper surgical technique is critical in preventing infection and cannot be overridden by any other measure.[11] Closed-suction drainage systems are frequently used in orthopedic surgeries to drain fluids from the surgical wound and reduce occurrence of hematoma; however, there is insufficient evidence from randomized trials to routinely recommend this practice.[512] A number of studies have demonstrated that confidential feedback of surgeon-specific rates of infection to individual surgeons allows them to adjust their practices accordingly and reduces overall infection rates.[662]

Other interventions evaluated in adult patients include glycemic control, maintenance of normothermia, and supplemental oxygen. These measures have not been investigated in pediatric surgical patients. In particular, tight glycemic control may have untoward consequences in special populations of children, particularly infants, and is in need of further study.

HEALTH CARE–ASSOCIATED INFECTIONS CAUSED BY SPECIAL PATHOGENS

Methicillin-Resistant *Staphylococcus aureus*

Methicillin-resistant *S. aureus* (MRSA) has long been recognized as a nosocomial pathogen and accounted for over 50 percent of *S. aureus* health care–associated infections in adult patients reported to the CDC in the 1990s.[477] In contrast, point prevalence surveys performed in the late 1990s by the Pediatric Prevention Network documented that only 2 percent of pediatric health care–associated infections were due to MRSA.[260,628] The emergence of novel strains of MRSA associated with community-onset infections has changed the epidemiology of health care–associated MRSA infections. The prevalence of MRSA in pediatric health care–associated infections has risen dramatically; over one fourth of all *S. aureus* infections in neonatal ICUs are due to methicillin-resistant strains.[385] Evidence from multiple adult and pediatric studies has demonstrated that community-associated MRSA strains (as defined by the unique mobile genetic element, the SCC*mec*IV cassette) have now become endemic in many institutions.[329,428]

This changing epidemiology has had an unfortunate impact on patients. As compared with patients with health care–associated infections due to methicillin-sensitive strains of *S. aureus*, patients with similar infections due to MRSA often have worse outcomes with increased rates of death, longer durations of hospitalization, and higher total hospital costs.[144,145,204] Risk factors for MRSA health care–associated infections include colonization pressure (the proportion of patients with MRSA carriage) within a given unit or institution, exposure to broad-spectrum antibiotics, prolonged hospital stay, and use of indwelling medical devices.

Prevention of health care–associated MRSA infections has been the topic of several recent guidelines from experts in the fields of infectious diseases and hospital epidemiology.[96,314,473,609] Key prevention principles rest on (1) identifying reservoirs of the organism; (2) strict adherence to hand hygiene to interrupt transmission via transient carriage on the hands of caregivers and visitors; (3) cohorting to prevent contamination of the environment of a noncolonized patient; (4) decontaminating the patient care environment and using dedicated patient equipment; and (5) antimicrobial stewardship.[96]

Over the past decade, several areas of controversy have emerged in the discussion of how to prevent MRSA health care–associated infections. A large cluster-randomized trial performed in adult ICUs, the STAR*ICU trial, examined the impact of active surveillance cultures (MRSA screening by culture or PCR of specimens obtained by nasal swabs from all newly admitted patients) and expanded barrier precautions (contact precautions for MRSA colonized or infected patients and universal gloving for all other patients).[311] In this study, active surveillance for MRSA and barrier precautions did not reduce the incidence of MRSA acquisition, although adherence to the study interventions was suboptimal. Different results were reported from an observational study that assessed the impact of an "MRSA bundle" (composed of active surveillance, hand hygiene, contact precautions for MRSA colonized or infected patients) on the rates of health care–associated MRSA infections in Veterans Affairs hospitals.[317] This intervention was associated with 62 percent and 45 percent reductions in the rates of MRSA health care–associated infections in ICUs and non-ICUs, respectively. Although these two studies

have seemingly conflicting findings, differences in their design, the nature of the interventions tested, and the outcomes measures make it difficult, and potentially misleading, to compare them directly.

Another issue that has stimulated debate is the role of decolonization as a strategy to prevent health care–associated MRSA infection. Decolonization therapy has been demonstrated in multiple studies to reduce the risk for MRSA transmission (both endemic transmission and associated with outbreaks). In 2008, the Society for Healthcare Epidemiology of America recommended that this strategy be considered for patient populations or locations where the rates of MRSA health care–associated infections remain unacceptably high despite adoption of other preventive strategies.[96] To date, MRSA decolonization has not been adopted as part of routine infection prevention practices in many institutions. MRSA decolonization has also helped control outbreaks in which colonized health care workers were epidemiologically linked to MRSA transmission. In these reports, various regimens that included intranasal application of mupirocin ointment and/or the use of antiseptic baths were associated with interrupted transmission of MRSA. Although decolonization therapy can reduce the transmission of MRSA within the health care setting, multiple studies have demonstrated that a large proportion of patients and health care workers who undergo decolonization become recolonized.[188]

A recently published systematic review examined the ability of various strategies to eradicate MRSA. Intranasal mupirocin, administered daily for 4 to 7 days, had the greatest likelihood of successful eradication of MRSA carriage: approximately 90 percent probability of immediate eradication with approximately 60 percent probability of persistent eradication (although duration of follow-up assessments ranged greatly).[23] Daily bathing with chlorhexidine has also been shown to reduce the risk for MRSA colonization.[333] Factors that have been associated with failure of the ability of topical treatments to eradicate MRSA carriage include the presence of wounds, eczema, indwelling medical devices, and mupirocin-resistant MRSA.[279,618] Numerous different regimens of oral antibiotics have also been studied, in isolation or as part of a regimen that includes topical decolonization. The pooled risk for treatment failure for systemic treatment with oral antibiotics approached 50 percent, although the variation in the interventions evaluated suggest these findings may require additional study. Of note, however, was the observed rate of 9 percent drug resistance that developed in patients who received oral antibiotics.[23]

Vancomycin-Resistant Enterococci

In contrast to methicillin-resistant strains of *S. aureus*, VRE are virtually exclusively found associated with health care exposure. Patients infected with VRE infections or found to have colonization with VRE typically have identifiable exposure to high-risk health care settings, devices, or procedures. Vancomycin resistance in *Enterococcus faecium* or *Enterococcus faecalis* arises most frequently from acquisition of a mobile genetic element that carries with it the *vanA* or *vanB* resistance gene. Although exposure to vancomycin provides selection pressure that encourages the expansion of VRE in a colonized host, it does not induce a vancomycin-sensitive strain of *Enterococcus* to become resistant. However, antibiotic exposure has been repeatedly associated with an increased risk for colonization and infection with VRE. First, in a previously uncolonized patient, antibiotic use reduces populations of commensal flora, thereby lessening colonization resistance and enhancing the capacity of VRE to establish colonization in a patient who is exposed to VRE. In a patient who is already colonized with VRE, antibiotic exposure will reduce other flora and thus allow the expansion of the organism.[473] In hospitalized children, vancomycin exposure, as well as mechanical ventilation, immunosuppression, and older age were all found to be independently associated with bacteremia due to VRE, as compared with vancomycin-sensitive *Enterococcus*.[266] Three major sources have been identified as important in the transmission of VRE to patients: health care workers' hands, shared patient equipment, and the health care environment. Several observations support the importance of contaminated health care workers' hands in the transmission of VRE. First, VRE can easily be recovered from the hands and gloves of caregivers of VRE-positive patients.[488] VRE has been recovered from computer keyboards used exclusively by health care workers, again implying that hand carriage of VRE can spread the organism beyond the environment of a colonized patient.[294] Finally, having a health care worker who is simultaneously providing care to both a VRE-positive and a VRE-negative patient has been demonstrated to be a risk factor for VRE acquisition.[76] Many studies have demonstrated that VRE can persist on inanimate objects for long periods of time.[478] Thus, transmission can easily occur when a patient comes into contact with inadequately decontaminated shared patient equipment (e.g., pulse oximeters and rectal thermometers). Similarly, inadequate cleaning and disinfection of both outpatient and inpatient health care settings has been implicated in various VRE outbreaks.[75]

Prevention of transmission of VRE relies on many of the same principles used to prevent transmission of other multidrug resistant organisms within the health care setting. Similar to preventing MRSA transmission (see earlier discussion), strategies for interrupting patient-to-patient transmission of VRE rely on interrupting indirect transmission of the organism via health care workers' hands, shared patient equipment, or the health care environment. As described in the HICPAC "Management of Multidrug-Resistant Organisms in Healthcare Settings, 2006,"[609] a comprehensive approach to the control of epidemiologically important organisms, including VRE, should include the following elements: active surveillance for infection, application of standard and transmission-based isolation precautions, disinfection of the patient care environment, and judicious use of antibiotics.[609] Several studies have demonstrated the frequency of contamination of health care workers' hands or gloves after providing care for a VRE-colonized patient.[70,660] Hand cultures performed after glove removal have found that hands can become contaminated with VRE even though

gloves have been worn, presumably via unrecognized inoculation of the hand during the process of glove removal.[660] Additionally, VRE has been recovered from health care workers' hands when they only touched the environment or equipment of a VRE-colonized patient.[70] These observations support the use of gloves when care is provided to a known VRE-colonized patient and underline the importance of hand hygiene after glove removal. Transmission-based precautions (to prevent patient-to-patient spread of VRE) have also been demonstrated to reduce the prevalence of VRE among hospitalized patients.[505,644]

As with MRSA, the role of active surveillance cultures to identify the reservoir of VRE (colonized patients who have not been previously identified) has been an ongoing topic of debate. The STAR*ICU trial, described earlier, examined the capacity of active surveillance cultures of adults who were newly admitted to an ICU and expanded barrier precautions to reduce the rate of new acquisition of VRE.[311] Although these measures were not associated with reduced incidence of VRE in the intervention units, adherence to the intervention was incomplete. Thus, it is unclear whether active surveillance for VRE by obtaining cultures on admission and/or universal gloving, as compared with contact precautions used for known VRE-positive patients, can achieve greater reductions in the transmission of VRE within the health care setting.

As described previously, antibiotic exposure does not induce resistance to vancomycin in enterococcal strains that are vancomycin sensitive. However, antibiotic exposure has been associated with an increased risk for colonization or infection with VRE. To date, it remains unclear whether alterations in the agents or duration of antibiotic therapy can result in reduced prevalence of VRE. However, most experts agree that limiting the spectrum and duration of antibiotic therapy to that which is required based on the known or suspected pathogens may have a beneficial impact on the spread of many multidrug-resistant organisms through lessening of selection pressure.[609]

Multidrug-Resistant Gram-Negative Bacilli

Multidrug-resistant gram-negative bacilli (MDR-GNB) have emerged as an important cause of health care–associated infections and accounted for up to 30 percent of infections reported to the NHSN in 2006 through 2007.[293] Although the majority of these reports have come from adult institutions, pediatric health care–associated infections due to extended-spectrum β-lactamase (ESBL)–producing *E. coli* and *Klebsiella* spp., multidrug-resistant *Acinetobacter baumanii* and *P. aeruginosa*, and carbepenemase-producing Enterobacteriaceae have been reported as causes of pediatric CLABSI, catheter-associated urinary tract infections, and ventilator-associated pneumonia.[293] In the United States, outbreaks of MDR-GNB have been reported within neonatal ICUs,[654] pediatric long-term care facilities,[685] and cystic fibrosis centers.[128] Point prevalence surveys conducted by the Pediatric Prevention Network in eight U.S. pediatric ICUs and seven U.S. neonatal ICUs in 2000 found the prevalence of colonization with ESBL-producing gram-negative bacilli was less than 3 percent.[260,628] A more recent report describes the prevalence of endemic carriage of MDR-GNB in a single academic pediatric ICU. MDR-GNB (defined in this study as resistance to three or more classes of antibiotics) were carried by 3.6 percent of patients from whom active surveillance cultures were obtained.[726] The most prevalent species were *E. coli*, *P. aeruginosa*, and *Klebsiella* spp.[726] Of note, molecular analysis demonstrated very little horizontal transmission of these organisms, perhaps related to the design of the unit (all single patient rooms) and/or excellent adherence to infection prevention practices. In June 2010, the Clinical and Laboratory Standards Institute lowered the minimum inhibitory concentration breakpoints for cephalosporins and carbapenems for Enterobacteriaceae.[138] This change will undoubtedly have a marked impact on the proportion of gram-negative health care–associated infections that are caused by MDR-GNB.

Similar to other epidemiologically important drug-resistant pathogens (see earlier sections on MRSA and VRE), MDR-GNB have frequently been isolated from the environment and equipment surrounding a colonized or infected patient. Recent studies have demonstrated that these organisms are often transferred after patient contact to health care workers' gloves, gowns, and even hands after glove removal.[463] Although fewer studies have examined risk factors for endemic transmission or infection with MDR-GNB than for MRSA and VRE, outbreak investigations have frequently implicated contaminated respiratory therapy equipment (including mechanical ventilators), inappropriate use of multiuse vials of medications or diluents and urinary catheters, and inadequately cleaned patient environment as reservoirs of these organisms.[519] Additional studies have implicated exposure to broad-spectrum antibiotics, especially fluoroquinolones, with an increased risk for ESBL-producing GNB.[547,705]

The same prevention and control measures used to limit transmission of resistant gram-positive organisms within the health care setting are presumed to be effective in preventing health care–associated infections due to MDR-GNB (see earlier discussions of MRSA and VRE).[609] Hand hygiene remains the cornerstone of practices that will prevent the transmission of MDR-GNB from patient to patient. Additional measures include identifying and controlling the reservoir of these organisms, typically through the use of Standard and Contact Precautions, effective cleaning and disinfection of the shared patient equipment and the health care environment, and antimicrobial stewardship. However, active surveillance cultures for MDR-GNB are not routinely performed. Therefore, most infection prevention programs continue to rely on clinical cultures to identify patients who carry a resistant gram-negative organism. The duration of carriage of these organisms is not known but is presumed to be prolonged. Therefore, contact precautions are typically continued for patients with MDR-GNB, at least until the time of hospital discharge.[426]

Clostridium difficile

Although toxin-producing *C. difficile* is a well-recognized cause of antimicrobial-associated diarrhea in adults, we have a limited understanding of the epidemiology of *C. difficile* disease in hospitalized children. However, national data suggest that the incidence of *C. difficile* disease in patients admitted to children's hospitals is rising. In 2001 the incidence of disease was 4.4 cases per 10,000 patient-days, whereas the incidence of disease rose to 6.5 cases per 10,000 patient-days in 2006.[343]

Studies conducted in the 1980s illustrated that toxin-producing *C. difficile* can often be found in the stools of neonates and young infants.[137] Because *C. difficile* is seldom found in the stool of healthy women and clusters of colonized infants can often be detected in nurseries,[137] health care–associated transmission, as opposed to vertical transmission, is presumed to play a role in the acquisition of this microorganism by neonates developing symptomatic *C. difficile*–associated diarrhea, and some children may develop severe disease, including pseudomembranous colitis.* Disease usually occurs in association with antimicrobial therapy, and several reports have described the occurrence of the disease in children with cancer.[89,103,535,727] A prospective study of health care–associated diarrhea in hospitalized children and adolescents found that, as a group, viral causes were most common; however, *C. difficile*–associated diarrhea was the most common single cause and was more common in older children.[374]

An epidemic strain of *C. difficile* (NAP-1) emerged and disseminated in Canada and the United States during the mid 2000s that involved patients in the community and health care facilities.[401,436] This strain has caused severe disease in populations thought to be at low risk, including peripartum women and children[112]; in a study conducted in two urban areas, approximately 20 percent of the *C. difficile* isolates were the NAP-1 strain.[667]

Several studies have examined risk factors for *C. difficile* disease in hospitalized children. Solid organ transplant, presence of a gastrostomy tube, and exposure to fluoroquinolones were all independent predictors of *C. difficile* infection in children.[581] Another study examined risk factors for severe disease among hospitalized children and found that exposure to three or more classes of antibiotics was the only independent risk factor for severe disease in a cohort of hospitalized children.[342] Pediatric patients with inflammatory bowel disease have also been found to have an increased risk for recurrent *C. difficile* infection.[338] Two adult studies have explored the relationship of humoral immunity and *C. difficile*–associated diarrhea and found that patients who had serum antibodies against *C. difficile* toxin A were less like to develop disease when colonized or develop recurrent disease.[365] The implications of these findings on the epidemiology of this disease in children have not been investigated.

C. difficile can be found on the hands of personnel and in the patient's immediate environment,[248] where *C. difficile* spores can survive for prolonged periods and are relatively resistant to disinfectants. Therefore, direct or indirect contact is responsible for the spread of this microorganism from patient to patient. Barriers (gowns and gloves) and hand hygiene may reduce transmission. Vigorous environmental cleaning is important because *C. difficile* spores can heavily contaminate the environment and survive for long periods. Conventional cleaning agents may not be sporicidal, and some authorities recommend use of dilute bleach to disinfect rooms of patients with *C. difficile* infection, particularly while they have diarrhea.[248]

Unusual Pathogens Including Agents of Biologic Warfare

A variety of unusual, potentially fatal infections may be transmitted in health care settings. International air travel has increased the mobility of the world's population, making it possible for persons to travel long distances during the incubation period of infections acquired in remote settings.[594] In addition, some of the potential agents of bioterrorism are highly transmissible (e.g., smallpox virus, *Yersinia pestis*, hemorrhagic fever viruses) and could result in secondary health care–associated cases after a primary bioterrorism attack.* A high index of suspicion for these infections and prompt institution of appropriate isolation precautions are necessary to reduce the risk for health care–associated transmission.

Smallpox is the most feared of the potential agents of biological warfare because it can be transmitted via droplet nuclei over large distances, the case-fatality ratio is approximately 30 percent, there is no effective treatment, and much of the world's population is either nonimmune or was immunized in the distant past.[288] Even a very limited number of cases of smallpox would be a global public health emergency, and detailed plans for containment would be needed. Hospitalized patients with smallpox would require airborne and contact isolation precautions, and health care workers caring for these patients must be vaccinated.[288,645]

Plague is endemic in portions of the western United States and in other countries. No person-to-person transmission of plague has been identified in the United States for more than a half century,[312] but an outbreak in India illustrated the potential for importation of plague to other countries.[105] Droplet transmission of *Y. pestis* is well documented from persons with pulmonary involvement. Health care workers should wear masks during the care of patients with signs or symptoms of pulmonary involvement.[312] Tetracycline, doxycycline, sulfonamides, chloramphenicol, and perhaps fluoroquinolones can be used for prophylaxis of contacts.[312]

Ebola, Marburg, Lassa, and Crimean-Congo viruses and the New World arenaviruses have been spread in hospitals from close contact with infectious body fluids or reuse of contaminated needles.† Health care–associated

*References 89, 103, 212, 280, 437, 500, 503, 535, 727.

*References 32, 71, 179, 288, 312, 462.
†References 43, 71, 336, 339, 460, 522, 553, 620, 646, 694.

transmission of hantavirus in the United States has not been reported but has occurred with Andes virus, another cause of hantavirus pulmonary syndrome.[508] Many of the hemorrhagic fever viruses have been proposed as potential biologic weapons.[71] Of these, only Ebola, Marburg, and Lassa viruses and the New World arenaviruses are spread from person to person.[71] The bulk of evidence suggests that airborne transmission of these viruses does not occur in clinical settings, although there are insufficient epidemiologic data to exclude this possibility.[106] In 2008, the World Health Organization (WHO) issued interim recommendations for infection control for care of patients with suspected hemorrhagic fever.[716] Key recommendations include (1) limiting nonessential health care providers and visitors; (2) use of full, fluid-impervious barrier precautions, including facial protection (medical mask and goggles or eye shield); and (3) limiting unnecessary procedures and use of needles and sharp instruments.

HEALTH CARE–ASSOCIATED INFECTIONS IN SPECIAL POPULATIONS

Neonates

Most healthy term neonates are born sterile but quickly become colonized with microorganisms derived from their mothers and the immediate environment. Predominant colonizers are coagulase-negative staphylococci on the skin and umbilicus and in the nose and α-hemolytic streptococci in the mouth. Acquisition of intestinal flora is more complex. Lactobacilli predominate in breast-fed babies, whereas more "adult" flora composed of *Bacteroides* spp., other anaerobes, and *E. coli* colonizes formula-fed babies.[252] These commensals help inhibit colonization with pathogenic microorganisms. Because pathogens can spread quickly in crowded nurseries, rooming-in and early discharge reduce the risk for colonization of normal neonates.[284,413]

The timing and nature of colonization of premature and full-term infants requiring care in neonatal ICUs is substantially different than for healthy term infants. First, colonization of these infants is substantially delayed, perhaps owing to limited contact with their mothers, delayed enteral feeding, and administration of parenteral antimicrobial agents.[249,252] When colonization does occur, the type of organisms and diversity of strains is also different than those found in healthy term infants. Although coagulase-negative staphylococci colonize the skin, umbilicus, and nose of both term and preterm infants,[252] molecular typing studies have demonstrated that particular strains of coagulase-negative staphylococci can persist in neonatal ICUs over extended periods, being transmitted on the hands of caregivers and causing bloodstream infections in some infants.[308,409,517] Hospitalized neonates, as compared with healthy term infants, often have a larger proportion of intestinal flora composed of aerobic gram-negative bacilli, including *E. coli*, *Klebsiella* spp., *Enterobacter* spp., *P. aeruginosa*, *S. marcescens*, and *Citrobacter* spp.[249,252] Antimicrobial resistant strains of these bacteria are a particular problem in many neonatal ICUs. Additionally, *Enterococcus* and *Candida* can become established on the skin and umbilicus and in the gut.[252] Because these potentially pathogenic bacteria can reach concentrations of 10^6 to 10^8 CFU/g in stool, it is not surprising that the hands of caregivers are frequently contaminated and create an important route of transmission if hand hygiene practices are lax. Unfortunately, the medical devices that help sustain these infants, such as intravascular catheters and mechanical ventilation, as well as immature skin and surgical wounds, provide portals of entry for these microorganisms to invade sterile sites and cause infection.

S. aureus remains a significant pathogen in neonates; it may colonize a variety of sites, particularly the umbilicus and nose.[252] Staphylococcal skin and soft tissue infections, including superficial skin infections, mastitis, and omphalitis, are the most common types of neonatal *S. aureus* infection. Risk factors for neonatal colonization with *S. aureus* include maternal colonization, anogenital carriage, prematurity, low birth weight, and exposure to medical devices.[324,344] Direct contact with colonized caregivers is the predominant mode of transmission. Environmental contamination, with transmission via the hands of caregivers of fomites, has also been identified as a risk factor for MRSA acquisition.[59] Indirect contact transmission from infected or colonized infants and droplet contact transmission from infants with coexisting viral respiratory tract infection (so-called cloud babies) are uncommon. Colonization of the skin, nose, umbilicus, or rectum precedes infection,[306] but there is poor correlation between rates of colonization and infection. Typically, the number of colonized infants far exceeds the number of infected infants; conversely, outbreaks of staphylococcal infection can occur in nurseries with low colonization rates. For this reason and because of cost considerations, surveillance cultures to detect colonized infants are not routinely recommended except under outbreak conditions.

MRSA has been a sporadic but recurrent problem in many neonatal ICUs for many years. However, infants in neonatal ICUs as well as otherwise healthy neonates are being affected by broader trends in the incidence of MRSA infection (see Health Care–Associated Infections Caused by Special Pathogens). Outbreaks of MRSA in neonatal ICUs have become more common, even involving multiple separate units in the same city.[247] Data from the CDC demonstrated that approximately one fourth of all *S. aureus* infections in hospitalized neonates were attributable to MRSA.[385] Risk factors for these infections include cesarean delivery (likely due to the longer postnatal hospital stay), male sex, improper infection control practices associated with circumcision, and contact with an infected health care worker.[57,113,483] Management of these outbreaks includes emphasis on standard infection control practices, such as hand hygiene, use of isolation precautions, and proper disinfection/sterilization of patient care equipment. A recent report demonstrated the capacity of whole genome sequencing to aid in the identification and management of an outbreak of MRSA in a neonatal ICU when multiple MRSA strains are present.[353] Whereas screening for colonization during the course of an outbreak is clearly useful to identify

asymptomatically colonized individuals, the role of routine screening of infants—and potentially health care workers—is less clear.[113,247]

The use of hexachlorophene (3%) for bathing is effective in reducing colonization,[254] but this agent was found to cause cystic degenerative changes in the white matter of premature infants,[531,607,608] although there was no evidence that this agent posed a hazard to full-term infants. A warning against the use of hexachlorophene was issued by the FDA in 1972.[254] Chlorhexidine is a reasonable alternative because it has good antistaphylococcal activity, and studies in neonates have demonstrated negligible absorption after bathing or cord care and no recognized toxicity. Although chlorhexidine is not currently approved by the FDA for use in infants younger than 2 months old,[8,146,328] a nationwide survey of 90 academic neonatal ICUs performed in 2009 revealed that chlorhexidine was used by over 60 percent of units. The most common indications for use included skin antisepsis at the time of dressing change and catheter insertion.[653] Use of iodophors may cause adsorption of iodine, and alcohols may cause chemical burns, so these agents should not be used for bathing. A variety of agents have been used for cord care, including triple dye (an aqueous mixture of brilliant green, proflavine hemisulfate, and crystal violet), alcohol, bacitracin, chlorhexidine, and mupirocin, although extensive efficacy and safety data are not available for any of these compounds.[254]

Fifty to seventy-five percent of women with group B streptococcal vaginal colonization will transmit this microorganism to their neonates in the absence of chemoprophylaxis, although only 1 to 2 percent of colonized infants will become infected. The CDC defines these and other infections transmitted via the birth canal as nosocomial infections.[240,242] The logic behind this designation has not been explicitly stated, but because these infections occur after an event (i.e., delivery) usually associated with medical care it is reasonable to consider them health care–associated infections. However, the impact of conventional infection control interventions on early-onset (i.e., within the first 7 days of life) invasive group B streptococcal infection is likely to be limited. Intrapartum prophylaxis of women is a far more effective intervention to reduce infection rates in neonates.[107,591] Group B streptococci can be associated with health care–associated infection not just perinatal infection. Outbreaks of group B streptococcal infection due to indirect contact transmission in nurseries have been well documented.[196,253,489]

Preterm infants are particularly susceptible to infection caused by a variety of yeasts and, to a lesser extent, filamentous fungi; *Candida* spp. are the most common fungal pathogens to infect hospitalized neonates.[30] Infants likely become colonized in gastrointestinal tracts due to carriage of *Candida*, especially *C. parapsilosis*, on the hands of health care workers.[573] Cutaneous colonization may also lead to invasive infection.[561] Risk factors for colonization with *Candida* include treatment with H_2 blockers or third-generation cephalosporins and delayed enteral feeding.[573] Risk factors for bloodstream infection and the use of prophylactic fluconazole have been discussed previously (see Infections Related to Intravascular Catheters and Infusions). Risk factors for *Malassezia furfur* infection are similar to those for *Candida*, but the association with lipid emulsion is particularly strong because this organism is obligatorily lipophilic.[159,400,556] Infections caused by filamentous fungi are rare and are usually associated with contaminated devices or practices that facilitate cutaneous invasion.[456,561]

Citrobacter koseri (previously classified as *C. diversus*) has been responsible for a number of outbreaks of health care–associated infection in neonates.[392,513,713] Although most infants colonized with this microorganism do not develop clinical disease, infection almost always results in meningitis, owing to the particular neurotropism of this bacterium, and is usually accompanied by formation of one or more brain abscesses. Sporadic outbreaks of *Citrobacter* infection have been reported,[392,513,713] and several published outbreak investigations have implicated transmission of this bacterium from the hands of caregivers to neonatal patients.[513,713] Additionally, molecular techniques have demonstrated that *C. koseri* also can be acquired perinatally by vertical transmission.[283]

Enterobacter sakazakii is a rare cause of health care–associated infection in neonates.[639] Similar to infections caused by *C. koseri*, infections caused by this bacterium are usually severe, often involving meningitis and brain abscess.* Outbreaks of *E. sakazakii* infection have implicated a variety of products,[110,370] including intrinsically contaminated powdered milk formulas.[64,110,474,614,675] Because powdered formulas are not marketed as sterile products, feeding practices in neonatal ICUs need to balance the relative nutritional benefits of particular products against the small but real risk for infection with *E. sakazakii* and other potential pathogens.

New obstetric practices, such as water birth, may pose infectious threats to infants, as demonstrated by the report of a case of *Legionella* pneumonia in an infant after a prolonged delivery in a pool with contaminated water.[221] Similarly, the emerging field of fetal surgery may also introduce the new condition of a surgical site infection in an unborn child.

Hand hygiene is the most effective intervention to prevent spread of pathogenic microorganisms.[77] However, the hands of some staff may remain colonized with these microorganisms for prolonged periods despite scrupulous hand cleansing[252] In addition, use of artificial nails or nail wraps has been associated with carriage of *P. aeruginosa* on the hands of health care workers in a neonatal ICU.[217] Therefore, healthcare workers should not be allowed to wear artificial nails or nail wraps when providing hands-on patient care.[572] Alcohol-based hand rubs are the most effective agents in reducing hand colonization with pathogenic bacteria and are the preferred method for hand hygiene when hands are not visibly soiled.[77]

Preceding sections contain specific recommendations for the prevention of health care–associated infections caused by community pathogens and infections related to the use of invasive devices. Other general references regarding the prevention of infections in normal nurseries and neonatal ICUs have been published, including recommendations regarding the design of facilities,

*References 64, 110, 134, 370, 614, 675.

appropriate staffing, and general infection prevention procedures.[20,140]

Several interventions designed specifically to prevent health care–associated infection in high-risk infants deserve additional comment.

The Vermont Oxford Network Evidence-Based Quality Improvement Collaborative for Neonatology is a large multicenter network of neonatal units organized for the purpose of implementing evidenced-based interventions to improve the quality of care of neonates.*[302] Data from a group of 669 neonatal ICUs spanning the years 2000 through 2009 describe a significant reduction in late bacterial infections; in 2000, 21.1 percent of surviving infants weighing 501 to 1500 g experienced a late bacterial or fungal infection, whereas the rate of late infections was 15.0 percent in similar infants in 2009.[301]

Many investigators have purposefully colonized infants in neonatal ICUs with nonpathogenic microorganisms, including viridans streptococci and *Lactobacillus*, to "interfere" with colonization by potential pathogens.[254,448,551] Some of the studies using viridans streptococci demonstrated favorable results, but this approach has not been pursued, in part owing to concern about the potential for adverse events.[254] Studies using *Lactobacillus* have demonstrated little or no beneficial effect.[448,551]

Infants of extremely low birth weight have immature, fragile skin that is predisposed to drying and cracking and may not be an adequate barrier to prevent invasion by bacteria colonizing the skin. For this reason, products containing emollients that include fatty acids have been developed to reduce transepidermal fluid loss and improve skin barrier function in very-low-birth-weight infants. A randomized controlled trial of the effect of an emollient ointment (Aquaphor) in infants with a birth weight of 501 to 1000 g and a gestational age of 30 weeks or younger was conducted in 53 neonatal ICUs participating in the Vermont Oxford Network.[199] The study found that treated infants had better skin condition on days 1 to 14 of life and less skin injury on days 15 to 28 of life. However, the treatment group had a higher rate of late-onset bacterial sepsis, with the majority of excess cases occurring in infants 501 to 750 g. There was no effect on mortality. In contrast, two randomized controlled trials conducted in Bangladesh found that sunflower seed oil reduced the rate of late-onset infection and mortality in preterm infants with a gestational age of 33 weeks or younger.[160,162] Interestingly, the second of these trials included Aquaphor as a comparator treatment and found a similar decrease in mortality associated with application of this agent. An additional study conducted during the second trial provided indirect evidence that the treatment effect was likely mediated by decreased passage of skin pathogens into the bloodstream.[161]

Because premature infants lack sufficient levels of opsonizing antibodies, multiple trials have examined the efficacy of intravenous immunoglobulin in preventing infection in premature infants.[42,208,345,416,702] Only one of these trials demonstrated any benefit in reducing overall infection rates.[42] A large trial that randomized almost 3500 infants with suspected or proven infection found therapy with intravenous immunoglobulin had no impact on death or major disability.[84] An evaluation of commercially available intravenous immunoglobulin preparations found a large degree of variability in opsonic activity against common neonatal pathogens between individual lots produced by the same manufacturer as well as preparations from differing various manufacturers.[701] The impact of high-titer, pathogen-specific antibody preparations in the prevention of invasive infection by specific neonatal bacterial pathogens remains unclear.

Because preterm infants have limited neutrophil pools as well as limited neutrophil function, several randomized, controlled studies have examined the prophylactic and therapeutic effect of granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) on infections in preterm infants. In all of these studies, infants receiving these agents had higher peripheral blood neutrophil counts than infants receiving placebo. However, the evidence of the beneficial effects of treatment with these agents is limited.[95,102,457]

Leukocytes present in stored blood products may have adverse effects on immune function, especially in preterm infants. However, a study examining the effect of universal prestorage red blood cell leukoreduction program found no effect on bloodstream infection and mortality but was associated with improvements in other clinical outcomes.[211] Use of postnatal corticosteroids to prevent or treat chronic lung disease in preterm infants has been associated with higher rates of health care–associated infection,[640] as well as other short- and long-term adverse outcomes.[142]

Immunocompromised Children

Children with congenital or acquired immunocompromising conditions are at high risk for health care–associated infections. The risk for these infections is related to host factors, such as the nature and severity of immunocompromise, use of indwelling medical devices, and frequency and nature of exposure to the health care environment.

The epidemiology of and risk factors associated with health care–associated infections in immunocompromised children have been best characterized for children with oncologic conditions. Investigators from St. Jude Children's Research Hospital have described the changes in the incidence of health care–associated infections among their patients hospitalized from 1983 through 2008.[435] In 2008, the overall infection rate was 2.0 per 100 patient discharges; CLABSI comprised approximately one third of all identified health care–associated infections. Neutropenia was identified as a risk factor associated with health care–associated infections; the risk for health care–associated infection was threefold greater among patients with a nadir absolute neutrophil count of less than 100 cells/mm^3 as compared with patients with less profound neutropenia. A single-center study also found that platelet transfusion and recent catheter placement were risk factors for CLABSI in pediatric oncology patients.[337]

*Vermont Oxford Network Evidence-Based Quality Improvement Collaborative for Neonatology: http://www.nicq.org.

The risk for health care–associated infections in children undergoing bone marrow or solid organ transplantation can be influenced by factors related to the patient's underlying health status, microbiologic characteristics of the donor and recipient of the graft, the duration and nature of immunosuppression required for successful engraftment of the transplanted organ, and the duration and nature of exposure to medical devices and the health care setting.[337] Solid organ transplant recipients are at risk for bacterial and fungal infections associated with the use of medical devices or in the surgical wound. Severe manifestations of common childhood viruses, such as CMV and RSV, also can pose a threat to a child who has recently undergone organ transplantation.

For immunocompromised children with indwelling medical devices or who undergo surgery or invasive procedures, the same strategies described earlier should be employed to prevent health care–associated infections. For children with nonpermanent immunocompromising conditions (e.g., those receiving chemotherapy or initiating antiretroviral therapy), all elective surgeries and invasive procedures should be deferred until their immunocompromised state has resolved.

The increased susceptibility to opportunistic pathogens necessitates heightened infection prevention measures for some children with the most profound degree of immunosuppression. Numerous outbreaks due to low-virulence organisms commonly found in the environment, such as mold infections related to construction in or near a health care facility have been reported.[687] Because airborne fungal spores can enter the health care environment, the CDC recommends placement of the most severely immunocompromised patients, such as neutropenic patients undergoing allogeneic stem cell transplantation, in an environment with added engineering measures to provide additional protection (i.e., a protective environment). Critical components of a protective environment include (1) positive-pressure patient care environment; (2) high-efficiency particulate air filtering; (3) selective air-handling units; and (4) high numbers of air exchanges per hour (>12 per hour).[594] Despite the broad adoption of these recommendations by many centers that care for high-risk oncology patients in the United States, relatively few studies have demonstrated the effectiveness of these measures. A recently published meta-analysis was unable to demonstrate that measures to control the air quality reduced the risk for invasive mold infections,[590] and a systematic review described an association between high-efficiency particulate air filtration and a reduction in fungal infections only when their analysis was limited to nonrandomized studies.[197]

Children with Burns

Information regarding the epidemiology of health care–associated infections in pediatric burn patients has been described in four reports,[147,241,589,696] including the experience of a single pediatric burn facility that performed prospective surveillance using modified CDC definitions.[696] A total of 14 percent of patients experienced an infection that was associated with an incidence density of 16 infections per 1000 patient-days.[696] Infection of the burn wound was observed in 10 percent of patients for an incidence density of 5.6 burn infections per 1000 patient-days. In this report, the rates of device-associated infections were 4.9 bloodstream infections per 1000 central venous catheter-days, 11.4 cases of ventilator pneumonia per 1000 ventilator-days, and 13.2 urinary tract infections per 1000 urinary catheter-days. These rates are higher than the most recently published pooled means for adult burn units.[194] Although comparable data are not available for dedicated pediatric burn units, it is reasonable to presume that similar reductions in health care–associated infections have also been achieved in pediatric burn centers. New definitions for burn infection were developed and endorsed by a consensus conference convened by the American Burn Association[257] and have been incorporated into NHSN definitions.[300]

The risk and microbiology of burn infections are related to the size of the burn wound and the time of onset of the infection. Infections in burn patients can arise from either endogenous organisms (often organisms colonizing the wound) or exogenous organisms. Colonization of a burn typically proceeds from gram-positive organisms, antibiotic-susceptible gram-negative organisms, and finally fungal and antibiotic-resistant gram-negative organisms.[542] Gram-positive cocci, including methicillin-susceptible and methicillin-resistant *S. aureus*, were the most common cause of burn infection in patients with relatively small burns (<30% of body surface area), whereas gram-negative bacteria (especially *P. aeruginosa*) were more common causes of infection in patients with extensive burns (>30% of body surface area).

Interventions to reduce the incidence of health care–associated infections in burn patients include the use of barrier techniques to reduce cross colonization of patients, measures to prevent cross colonization of patients during hydrotherapy treatments, topical antibiotics to retard growth of microorganisms in the burn wound, appropriate use of systemic antibiotics, and early excision and closure of the burn wound. The success of the Shriners Burns Hospital in Boston in controlling infections in severely burned children using these methods and "bacteria-controlled nursing units" (a laminar airflow unit providing controlled temperature and humidity combined with barrier precautions) has been described.[697] Finally, a randomized, controlled trial of selective decontamination of the digestive tract using polymixin E, tobramycin, and amphotericin B in severely burned children found no evidence of benefit.[44]

Children with Cystic Fibrosis

In 2003, Saiman and colleagues reviewed the epidemiology of important pathogens in persons with cystic fibrosis in a document summarizing the conclusions of an international consensus conference convened to develop evidence-based guidelines for standardized microbiologic and infection control practices for persons with cystic fibrosis.[575] They concluded that there was substantial evidence for transmission of *Burkholderia cepacia* complex, *P. aeruginosa*, and MRSA among persons with cystic fibrosis in health care settings, but that there was insufficient evidence to determine whether *Stenotrophomonas*

maltophilia, *Achromobacter xylosoxidans*, nontuberculous mycobacteria, or filamentous fungi could be transmitted from person to person. At the time of this writing, an expert panel is drafting revised infection control recommendations for patients with cystic fibrosis.

The recommendations from the 2003 consensus guideline emphasize standard infection control practices in the care of patients with cystic fibrosis, including appropriate hand hygiene and use of barriers by health care workers, proper use of isolation precautions, and effective procedures for cleaning and disinfecting/sterilizing reused equipment contaminated with respiratory secretions.[575]

In inpatient settings, the 2003 guideline recommends contact isolation and a private room for patients who are colonized or infected with MRSA, *B. cepacia* complex, and multidrug-resistant *P. aeruginosa*. Patients who are not colonized with these bacteria may share a room with a patient who does not have cystic fibrosis and who is at low risk for infection with these bacteria. All patients, whether colonized with these bacteria or not, should avoid contact with other patients with cystic fibrosis.

The 2003 guideline makes no recommendation regarding the use of masks by patients with cystic fibrosis either in inpatient or outpatient settings owing to lack of sufficient evidence for or against the efficacy of this practice.[575] However, a survey of 158 cystic fibrosis centers performed in 2004 found that many of the responding institutions had policies that advocated the use of masks when a patient was out of their hospital room (69%) or in the clinic waiting area (35%).[723]

In ambulatory settings, the 2003 guideline recommends that patients with cystic fibrosis who are colonized or infected with *B. cepacia* complex should be segregated from other cystic fibrosis patients and other patients colonized or infected with *B. cepacia* (to avoid replacement of one strain with another), their visits should be scheduled at the end of the clinic session or on another day, and they should be placed in an examination room immediately on arrival to the clinic instead of sitting with other patients in the waiting room.[575] Patients colonized or infected with multidrug-resistant *P. aeruginosa* should also be placed in an examination room immediately. For other patients with cystic fibrosis, the 2003 guideline recommends a variety of clinic logistics to minimize the time patients spend in waiting areas and emphasizes education of patients regarding appropriate waiting area behaviors to minimize contact among patients (e.g., discouraging handshakes and physical contact among patients, maintaining at least 3 feet between patients, avoiding contact with common-use items). Finally, all patients should receive annual influenza vaccination and age-appropriate pneumococcal vaccination.

Children Undergoing Dialysis

Children with chronic renal failure who are undergoing either hemodialysis or peritoneal dialysis have multiple risk factors for health care–associated infections. First, renal failure is associated with impaired immune function, including phagocyte dysfunction due to uremia. Children with renal failure often require frequent contacts with the health care system, including hospitalization, further increasing the risk for acquiring multidrug-resistant organisms. Finally, the indwelling medical devices required for dialysis pose additional risks. Studies of adult patients with end-stage renal disease have demonstrated an increased risk for infection associated with the presence of an immunocompromised condition (related to either an underlying disease process or use of immunosuppressing therapies), anemia, or iron overload.[40]

Infection of dialysis access devices, whether for hemodialysis or peritoneal dialysis, remains the most common type of health care–associated infection experienced by patients with end-stage renal disease.[40] In hemodialysis patients, the risk for bacteremia is influenced by the type of vascular access device in use. Arteriovenous fistulas are associated with the lowest risk for bacteremia, in contrast to nontunneled dialysis catheters, in which the risk for bacteremia has been reported to approach 22.5 infections per 1000 dialysis procedures, which is 7- to 10-fold higher than the rate of infections reported for patients undergoing dialysis via an arteriovenous fistula during the same time period.[295,659] In addition, adult studies have demonstrated that comorbid conditions (particularly diabetes mellitus and hypertension) are independently associated with an increased risk for bacteremia in patients undergoing hemodialysis.[384] Gram-positive organisms are the most common cause of bacteremia in patients undergoing hemodialysis; *S. aureus* and coagulase-negative staphylococci are the most frequently isolated pathogens.

Many of the same practices used to prevent catheter-associated bloodstream infections in patients with conventional central venous catheters are considered standard of care for patients with hemodialysis catheters. However, several important differences should be noted. In contrast to exit sites for conventional venous catheters, current guidelines recommend that antiseptic or antibacterial ointment (either povidone-iodine or bacitracin/gramicidin/polymyxin B) be placed at the exit site of hemodialysis catheters after insertion and after each dialysis session provided the catheter material is compatible with this substance.[494] Recent efforts to increase the use of arteriovenous fistulas and improve the access techniques have resulted in substantial reductions in the rate of bacteremia in adult dialysis patients. For hemodialysis patients with a history of multiple episodes of bacteremia, several meta-analyses[369,720] and a recent randomized trial have demonstrated that use of antibiotic or antiseptic locks between dialysis sessions were associated with a reduced risk for subsequent bacteremia.[630] Although few data are available from studies of pediatric patients, application of these same strategies to pediatric patients undergoing hemodialysis patients is also likely to reduce the risk for bacteremia in this patient population.

Peritonitis is the most common health care–associated infection in patients undergoing either intermittent or continuous peritoneal dialysis. In addition to raising the risk for infection-related death, peritonitis poses long-term risks to patients who require chronic dialysis;

repeated or protracted episodes of inflammation of the peritoneum can result in permanent fibrosis of the peritoneal membrane and render peritoneal dialysis ineffective. Recent pediatric data suggest gram-positive organisms account for 44 percent of cases of culture-positive peritonitis; approximately half of the gram-positive isolates were *S. aureus*, whereas the remaining isolates were coagulase-negative staphylococci. Approximately 50 percent of pediatric peritonitis in this population is due to gram-negative organisms, whereas less than 5 percent of episodes are due to fungi.[123]

Using data from the International Pediatric Peritoneal Dialysis Registry, the International Society for Peritoneal Dialysis has published guidelines for the treatment and prevention of peritonitis in pediatric patients.[690] These guidelines recommend that antibiotics be administered via the intraperitoneal route for the treatment of peritonitis. Empirical antibiotics should be selected based on available data on local resistance patterns of likely infecting organisms. Vancomycin is not routinely recommended for empirical therapy unless the institution's rate of MRSA exceeds 10 percent or the patient has a history of MRSA colonization or infection.[690] Definitive therapy should be tailored once the organism has been isolated and susceptibility data are available. The recommended duration of therapy varies based on the infecting organism but ranges from 2 to 3 weeks for most bacterial pathogens. Replacement of the peritoneal catheter is recommended for cases of refractory or relapsing bacterial peritonitis, in the setting of a tunnel infection, and for fungal peritonitis.

Prevention of health care–associated infection in children undergoing peritoneal dialysis rests on the same principles used to prevent intravascular infections: scrupulous adherence to aseptic technique during catheter insertion and access, and close attention to the care of the catheter exit site. Please refer to the recently published pediatric peritoneal dialysis guidelines for detailed recommendations.[690]

Children in Long-Term Care Facilities

In the preceding sections we have described a variety of microorganisms that have caused disease in long-term care facilities for children, including various viral infections and enteric pathogens that typically cause community-onset infections. Specific interventions to prevent these infections have been discussed previously.

Several reports have described the burden and spectrum of health care–associated infections among children in long-term care facilities. A single-center study, performed in 2009, reported the incidence of health care–associated infections among residents of a 109-bed pediatric long-term care facility as 6.01 infections per 1000 resident-days.[3] A prospective, longitudinal study of four pediatric facilities described the spectrum of endemic nosocomial infections in this population over a 2-year period in the 1990s.[684] The cumulative incidence of infection was 40 percent; upper respiratory tract infections and urinary tract infections were the most common types of infection and accounted for 37 percent and 31 percent of infections, respectively. In this report, nearly 80 percent of the urinary tract infections occurred in a small group of patients with functional and/or anatomic genitourinary abnormalities related to neural tube defects or neuromuscular disorders. This study, however, did not differentiate between symptomatic infections and asymptomatic bacteriuria. A more recent report described the burden of device-related infections in this population.[282] The incidence of catheter-associated urinary tract infection was more than twice as frequent as in pediatric ICUs reporting data to the National Nosocomial Infections Surveillance System, whereas the incidence of ventilator-associated pneumonia and central venous catheter-associated bloodstream infections were equivalent or slightly lower than that reported in pediatric ICUs. Other findings from these studies include the relatively high frequency of upper and lower respiratory tract infections among young children with tracheostomies and that skin infections accounted for 16 percent of infections (however, the specific percentage of decubitus ulcers was not reported). Gastrointestinal infections were remarkably uncommon; in contrast to adult facilities where infections due to *C. difficile* and outbreaks of norovirus are relatively common, these authors reported that gastrointestinal infections comprised only 4 percent of all infections. However, it is unclear whether detection bias may have led to underrecognition of these types of infections. Because of the heterogeneity in the populations of patients served and the ongoing improvement efforts underway at virtually all sites where health care is provided, readers would be advised to exercise caution when selecting a benchmark to which to compare their facility's rates of health care–associated infections.

Guidelines for the infrastructure and essential activities of infection control programs and key prevention practices in long-term care facilities have been published, although these documents do not deal with issues specific to the care of children.[227,624,625]

Key prevention measures are effective hand hygiene, appropriate use of barriers and isolation precautions, proper care of patients with indwelling devices (e.g., tracheostomies) or who require invasive procedures (e.g., bladder catheterization), prevention of decubitus ulcers, age-appropriate immunization (including yearly influenza vaccine), and early detection and control of outbreaks of infection.

INFECTION PREVENTION AND CONTROL PROGRAMS IN HEALTH CARE FACILITIES

This section highlights key elements of a comprehensive program to prevent and control health care–associated infections in health care facilities that provide inpatient care for pediatric patients. A detailed description of the development, implementation, and maintenance of an effective program is beyond the scope of this chapter, and the reader is referred to several excellent and authoritative references on this topic.[320,376,433,706]

Many external groups and organizations have significant influence on infection prevention and control programs in health care facilities; their activities are outlined in Table 243-8.

Organization and Activities of Health Care Infection Control Programs

In 1998, a consensus panel of national experts published a report on the requirements for infrastructure and activities of health-care–associated infection prevention and control programs.[587] The specific recommendations of the panel are listed in Table 243-9, accompanied by an indication of the strength of the recommendation, and are described in more detail in the report.[587] A similar document outlining the elements of infection prevention and control in long-term care facilities has also been published.[624] These publications do not deal specifically with pediatric issues, although the recommendations are generally applicable to facilities providing care to children.

Surveillance Strategies

Surveillance of health care–associated infections is necessary to understand the specific infection problems of individual facilities, to provide a means of evaluating the effectiveness of interventions to reduce the frequency of these infections, and to facilitate detection of outbreaks of infection. Infection prevention and control programs should have a surveillance plan for their health care facility that is based on the prioritized risks associated with care provided to patients in their facility and sound epidemiologic principles.[383]

The CDC NHSN surveillance methodology has become the de facto standard for surveillance. Although not without limitations, this methodology is well defined and time tested and allows institutions to benchmark themselves to other institutions through periodic data summaries from the NHSN as a whole.[194] In addition, NHSN methodology is used extensively by states and by the Center for Medicare and Medicaid Services for public reporting of rates of health care–associated infections (see Table 243-8). The NHSN has published definitions for health care–associated infections[300] with detailed instructions on the application of its methods.* Readers should refer to the NHSN website for the latest updates in NHSN definitions and methodology.†

Surveillance should also examine adherence to processes that are tightly linked to outcomes. As examples, facilities should monitor adherence with recommendations for hand hygiene and transmission-based isolation precautions,[82,584] "all-or-none" use of intervention bundles to prevent CLABSI and ventilator-associated pneumonia,[65,452,487,534] and administration of perioperative antimicrobial prophylaxis for prevention of surgical site infections.[81] These process measures can be used to guide and monitor process improvement efforts.

Surveillance and reporting of communicable infections to state departments of health is a duty of infection prevention and control programs and often done in collaboration with their microbiology laboratory.

Outbreak Investigation

Because outbreaks of health care–associated infections are often associated with significant patient morbidity and mortality, clusters of infection should be investigated promptly. A single, highly unusual infection, such as a postoperative group A streptococcal infection, is sufficient cause for an investigation. Clusters of health care–associated infections caused by an uncommon microorganism, a common microorganism with an unusual antimicrobial susceptibility pattern, or a series of infections at the same anatomic site (i.e., an outbreak of diarrhea) also are indications for an investigation. However, some outbreaks are more difficult to recognize because they occur intermittently, involve multiple microorganisms, or involve infection at various anatomic sites.

Table 243-10 provides an outline of the steps involved in investigating a cluster of health care–associated infections. Detailed discussions of the methodology for outbreak investigation also are found in other references.[47,320,376,433,706] Published outbreak investigations are also an invaluable resource because they provide insight into potential causes. For example, an outbreak caused by *Stenotrophomonas (Xanthomonas) maltophilia*, *Burkholderia cepacia*, or *Pseudomonas* spp. should suggest the possibility of a common source outbreak owing to a contaminated solution or medication or an inadequately disinfected piece of equipment. On the other hand, it is hazardous to jump to conclusions regarding the cause of a particular cluster of infections based on the results of a prior investigation. Careful investigation is necessary to establish or refute epidemiologic links between cases and potential causes.

Molecular genotyping techniques are an important adjunct to traditional epidemiologic methods (e.g., cohort or case-control studies) and can serve as a powerful tool to confirm or refute a particular source or mode of transmission of a particular microorganism. Two reports have described the use of whole-genome mapping in outbreak investigations, one involving MRSA infections in a neonatal ICU and the other involving carbapenemase-resistant *Klebsiella pneumoniae* infections in a specialty hospital.[353,626] In both cases, whole-genome mapping provided important additional insight into the spread of these organisms.

Policies and Procedures

Policies and procedures are necessary to optimize and standardize hospital routines and patient care practices. Certain generic policies and procedures apply to all departments, such as those for hand hygiene, isolation

*Centers for Disease Control and Prevention, National Healthcare Safety Network: http://www.cdc.gov/nhsn/TOC_manual.html.

†Centers for Disease Control and Prevention, National Healthcare Safety Network: http://www.cdc.gov/nhsn.

TABLE 243-8 **External Groups and Organizations Influencing Health Care–Associated Infection (HAI) Prevention and Control Programs**

Group/Organization	Activities
Division of Healthcare Quality Promotion (DHQP), National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention (CDC)	Provides data and guidance on prevention of HAIs; antimicrobial resistance; adverse drug events; blood, organ and tissue safety; and immunization safety; and other related adverse events or medical errors in health care affecting patients and health care personnel
	Investigates and responds to emerging infections and related adverse events among patients and health care personnel
	Conducts epidemiologic, and basic and applied laboratory research to identify new strategies to prevent HAIs, antimicrobial resistance, and related adverse events or medical errors
	Serves as the national reference laboratory for the identification and antimicrobial susceptibility testing of pathogens that cause HAIs
National Healthcare Safety Network (NHSN)	Collects and reports data on the incidence of HAIs, antimicrobial use and resistance, and process measures tightly linked with prevention of HAIs
Healthcare Infection Control Practices Advisory Committee (HICPAC)	Provides guidance to DHQP regarding surveillance, prevention, and control of HAIs; publishes evidence-based guidelines for HAI prevention
United States Department of Health and Human Services	Develops the "National Action Plan to Prevent Healthcare-Associated Infections: Roadmap to Elimination" that includes components for acute care hospitals, ambulatory surgical centers, end-stage renal disease facilities, and long-term care facilities
Center for Medicare and Medicaid Services	Requires reporting of some HAIs by facilities providing care to Medicare and Medicaid beneficiaries
	Leads the "Partnership for Patients," which has a goal of a 40% reduction in preventable harms, including HAIs, by the end of 2013
State health departments	Require public reporting of institutional rates of HAIs, with specifics varying by state
Professional societies	Advocate for public policy related to prevention of HAIs
Society of Healthcare Epidemiology of America (SHEA)	Develop and collaborate on publication of evidence-based guidelines for HAI prevention
Association for Professionals in Infection Control and Epidemiology (APIC)	Provide training for health care epidemiologists and infection preventionists
Infectious Diseases Society of America (IDSA)	Sponsor scientific meetings on HAI prevention
	Publish journals including articles on HAI prevention
The Joint Commission	Provides accreditation standards for infection prevention and control programs; promulgates National Patient Safety Goals, which includes goals related to prevention of HAIs
National Quality Forum	Selects measures of health care quality
United States Department of Labor's Occupational Safety and Health Administration (OSHA)	Issues regulations to minimize occupational hazards to health care workers, including exposure to bloodborne pathogens and tuberculosis
Institute of Medicine	Publishes scientific reports related to improving patient safety and reducing HAIs
Institute for Healthcare Improvement (IHI)	Provides guidance documents on performance improvement regarding patient safety, including preventing HAIs
	Conducts campaigns to improve patient safety
	Provides training for patient safety officers
Children's Hospital Association	Coordinates performance improvement collaboratives to reduce HAIs
Vermont Oxford Network	Collects and reports outcomes of neonatal care, including late-onset sepsis
	Coordinates improvement collaboratives to improve outcomes, including reducing late-onset sepsis
National Surgical Quality Improvement Project	Coordinates an outcomes-based program to measure and improve the quality of surgical care, including prevention of surgical site infections
Consumers Union	Publishes reports and ranking of hospitals with respect to HAI prevention
	Advocates for patient safety

TABLE 243-9 **Consensus Panel Recommendations Regarding the Requirements for Infrastructure and Essential Activities of Infection Control and Epidemiology in Hospitals**

Recommendation	Category*
Critical Data and Information	
Surveillance of nosocomial infections must be performed.	I
Surveillance data must be analyzed appropriately and used to monitor and improve infection control and health care outcomes.	I
Clinical performance and assessment indicators used to support external comparative measurements should meet the criteria delineated by SHEA and APIC.	II
Policies and Procedures	
Written infection prevention and control policies and procedures must be established, implemented, and updated periodically.	II and III
Policies and procedures should be monitored periodically for performance.	II and III
Regulations, Guidelines, and Accreditation Requirements	
Health care facilities should use infection control personnel to assist in maintaining compliance with relevant regulatory and accreditation requirements.	II
Infection control personnel should have appropriate access to medical or other relevant records and to staff members who can provide information on the adequacy of the institution's compliance with regard to regulations, standards, and guidelines.	II
The infection control program should collaborate with, and provide liaison to, appropriate local and state health departments for reporting of communicable diseases and related conditions and to assist with the control of infectious diseases.	II and III
Employee Health	
The infection control program personnel should work collaboratively with the facility's employee health program personnel.	II
At the time of employment, all facility personnel should be evaluated by the employee health program for conditions relating to communicable diseases.	II and III
Appropriate employees or other health care workers should have periodic medical evaluations to assess for new conditions related to infectious diseases that may have an impact on patient care, the employee, or other health care workers, which should include review of immunization and tuberculosis skin test status, if appropriate.	II and III
Employees must be offered appropriate immunizations for communicable diseases.	I and III
The employee health program should develop policies and procedures for the evaluation of ill employees, including assessment of disease communicability, indications for work restrictions, and management of employees who have been exposed to infectious diseases, including postexposure prophylaxis and work restrictions.	I
Prevention of Transmission of Infectious Diseases	
All health care facilities must have the capacity to identify the occurrence of outbreaks or clusters of infectious diseases.	I
All health care facilities must have access to the services of personnel trained and experienced in conducting outbreak investigations.	II
When an outbreak occurs, the infection control team must have adequate resources and the authority to ensure a comprehensive and timely investigation and the implementation of appropriate control measures.	II
Education and Training of Health Care Workers	
Health care facilities must provide ongoing educational programs in infection prevention and control to health care workers.	II and III
Educational programs should be evaluated periodically for effectiveness, and attendance should be monitored.	II and III
Personnel	
The personnel and supporting resources, including secretarial services, available to the hospital epidemiology and infection control program should be proportional to the size, complexity, and estimated risk for the population served by the institution.	II
All hospitals should have the continuing services of a trained hospital epidemiologist(s) and infection control professional(s).	I
Infection control professionals should be encouraged to obtain certification in infection control.	II
Health care facilities should provide or make available, in a timely fashion, sufficient office space, equipment, statistical and computer support, and clinical microbiology and pathology laboratory services to support the surveillance, prevention, and control program of the institution.	II

*I, strongly recommended for implementation based on evidence from at least one properly randomized, controlled trial, or evidence from at least one well-designed clinical trial without randomization, or evidence from cohort or case-control analytic studies (preferably from more than one center) or evidence from multiple time-series studies; II, recommended for implementation based on published clinical experience or descriptive studies, or reports of expert committees, or opinions of respected authorities; III, recommended when required by government rules or regulations

APIC, Association of Professionals in Infection Control and Epidemiology; SHEA, Society for Healthcare Epidemiology of America.

From Scheckler WE, Brimhall D, Buck AS, et al. Requirements for infrastructure and essential activities of infection control and epidemiology in hospitals: a consensus panel report. *Infect Control Hosp Epidemiol* 1998;**19**:114-24.

TABLE 243-10 Approach to the Investigation of Clusters of Health Care–Associated Infections

1. Confirm the diagnosis.
2. Make a case definition.*
3. Search for additional cases.
4. Plot the epidemic curve.
5. Compare pre-epidemic rates to current rates using statistical tests to prove that an epidemic exists.
6. Perform a literature review.
7. Open lines of communication with leaders of relevant departments, the microbiology laboratory, and the hospital administration.
8. Keep detailed records of events and conversations.
9. Review charts of all cases and compile a line listing of relevant information.
10. Formulate a hypothesis about a likely reservoir and mode of transmission.*
11. Institute temporary control measures.
12. Perform a case control study to develop epidemiologic evidence to support or refute the hypotheses.
13. Update control measures.
14. Document the reservoir and mode of transmission microbiologically. Confirm the relatedness of isolates using molecular genotyping techniques if necessary.*
15. Document the efficacy of control measures.
16. Write a report and distribute it to appropriate individuals.
17. Change policies and procedures if necessary.

*Molecular genotyping techniques may be employed early in the course of the investigation to evaluate the relatedness of pathogens associated with the cluster, reservoirs, and modes of transmission.

precautions, prevention of transmission of infectious diseases from and to visitors and health care workers, reprocessing of reusable patient care items, and disposal of medical waste. Other policies and procedures should be tailored to the potential infection risks relevant to specific departmental activities. Individual guidelines on various topics published by the HICPAC,* a compendium of guidelines on the prevention of health care–associated infections in acute care hospitals published by the Society for Healthcare Epidemiology of America (SHEA,)† and guidelines published by other professional societies (see Table 243-8) serve as useful references for these efforts.

However, merely developing policies, procedures, and standardized practices is insufficient. Indeed, recent achievements in prevention of health care–associated infections have been due primarily to improvement in reliably incorporating evidence-based practices as a part of routine patient care.[65,452] Effective leadership and application of principles and practices of implementation and improvement science are critical to the success of these efforts.[310,577,578]

*Centers for Disease Control and Prevention, Healthcare Infection Control Practices Advisory Committee: http://www.cdc.gov/hicpac/pubs.html.

†Society for Healthcare Epidemiology of America: http://www.shea-online.org/GuidelinesResources/CompendiumofStrategiestoPreventHAIs.aspx.

Hand Hygiene

Proper hand hygiene, which includes both hand washing using soap and water and hand antisepsis using a waterless, alcohol-based agent, reduces carriage of potential pathogens on the hands of health care workers.[77,525] Hand hygiene is widely regarded as the quintessential infection prevention measure.

Indications and procedures for hand hygiene are reviewed in detail in both a HICPAC guideline and a guidance document produced under the auspices of the WHO.[77,585] The WHO approach was developed as a "user-centric" model and has five "moments" for hand hygiene: before patient contact; before performing an aseptic task; after actual or potential contact with body fluids; after contact with the patient or his or her immediate surroundings; and after contact with the patient care environment outside the immediate surroundings.[585] Hand washing with soap and water must be performed when hands are visibly soiled to remove organic material and associated microorganisms. When hands are not visibly soiled, hand antisepsis using an alcohol-based, waterless product is preferred because it reduces the number of pathogenic bacteria that transiently colonize the hands more effectively than hand washing.[77,526] Hand hygiene is also necessary after removing gloves—an indication that many health care professionals fail to fully appreciate—because gloves may have macroscopic and microscopic holes that lead to hand contamination and because hands may be contaminated in the process of removing soiled gloves.[352]

The WHO has also provided a strategy for measurement of adherence with the five moments and behavior change strategies to improve adherence.[584,710]

Persons wearing artificial nails are more likely to harbor pathogenic gram-negative bacilli and yeast on their fingertips and these organisms are less effectively cleared on artificial nails by use of an alcohol-based agent than on natural nails.[285,440] Use of artificial nails or nail wraps was associated with carriage of *P. aeruginosa* on the hands of health care workers and infection among high-risk infants in a neonatal ICU.[217,263] For these reasons, the HICPAC hand hygiene guideline recommends that health care workers not wear artificial fingernails or extenders when providing patient care and that natural nails should be kept less than ¼ inch long.[77]

Isolation Precautions

Proper utilization of isolation precautions is important for all health care facilities but is especially critical in facilities caring for pediatric patients. Children hospitalized as the result of infections acquired in the community represent a substantial proportion of pediatric admissions, and transmission of these infections to other susceptible patients is a significant problem (see earlier discussion under Health-Care–Associated Infections Due to Spread of Communicable Infections Common in Health Care Facilities). Effective use of appropriate isolation precautions markedly reduces transmission of these agents.

The HICPAC isolation guideline recommends use of two tiers of precautions, Standard Precautions and Transmission-Based Precautions.[609,610]

Standard Precautions

Standard Precautions synthesizes the goals of protecting health care workers from bloodborne pathogens and protecting health care workers and patients from transmission of microorganisms from moist body substances.[610] Standard Precautions apply to any planned or potential contact with blood; all body fluids, secretions, and excretions except sweat, regardless of whether they contain visible blood; nonintact skin; mucous membranes; and supplies, equipment, or surfaces contaminated with these substances (see Table 243-1). Standard Precautions should be used in the care of all patients at all times regardless of their diagnosis or presumed infection status.[610]

Transmission-Based Precautions

Other precautions are needed to prevent transmission of contagious diseases (e.g., varicella, measles, tuberculosis, and pertussis) and other epidemiologically important microorganisms (e.g., multidrug-resistant microorganisms, *C. difficile*) from infected or colonized patients. Transmission-Based Precautions are designed to provide the necessary measures, in addition to those already specified by Standard Precautions, to interrupt known modes of transmission of these microorganisms.[610] There are three types of Transmission-Based Precautions: Airborne, Droplet, and Contact Precautions (see Table 243-2).

Airborne Precautions are designed to prevent transmission of microorganisms spread by droplet nuclei (e.g., measles, varicella, tuberculosis), which can be carried on air currents over substantial distances.[610] Special air handling and ventilation are required.[610]

Droplet Precautions are designed to prevent transmission of microorganisms spread by large respiratory droplets that only travel short distances before settling.[610] Special air handling and ventilation are not required.

Contact Precautions are designed to prevent transmission of microorganisms spread by direct and indirect contact.[610] Some infections are spread by more than one mode of transmission, so precautions systems may need to be combined for these infections (i.e., varicella requires Airborne Precautions and Contact Precautions).

Patients infected or colonized with more than one microorganism also may require a combination of precautions systems (i.e., a patient with active pulmonary tuberculosis and *C. difficile* enterocolitis requires both Airborne Precautions and Contact Precautions).

The HICPAC guideline lists clinical syndromes and conditions warranting empirical use of Transmission-Based Precautions, in addition to Standard Precautions, to prevent transmission of epidemiologically important pathogens until infection with these microorganisms is excluded.[609] To ensure that appropriate empirical precautions are implemented promptly, hospitals must have systems in place to evaluate patients for these infections as a part of their routine preadmission and admission care.

Visitors

Visitors with communicable diseases can inadvertently expose hospitalized patients and health care workers unless procedures to identify and exclude them are in place. Varicella, measles, and tuberculosis are the most problematic infections because these diseases are spread by airborne transmission, enabling infected visitors to expose a large number of individuals in a short time period. In addition, visitors with pertussis, viral respiratory and gastrointestinal infections, parvovirus B19 infection, rubella, and mumps can pose a significant hazard to patients and health care workers with whom they have close contact.

Procedures to identify potentially infected visitors should include visiting children since they are the most likely persons to be infected with these agents. Adult visitors and parents or guardians of visiting children should be asked a set of screening questions regarding the presence of fever, rash, and respiratory and gastrointestinal symptoms, as well as any recent exposure to other children with chickenpox, measles, or whooping cough. Persons without any significant symptoms or exposures should be allowed to visit with no restrictions. Persons who are otherwise well but have a history of a recent minor illness may be allowed to visit but potentially with restrictions, such as no visits by child visitors to the activity room and no sharing of food, drinks, or toys. Persons with fever, symptoms consistent with active respiratory or gastrointestinal infections, or rash consistent with a viral exanthem should not be allowed to visit. Persons with significant exposures to chickenpox or measles who are susceptible to these infections and persons with exposure to pertussis who may be in the incubation period of the disease should also not be allowed to visit. This screening process should be repeated each day that the person visits the hospital. This procedure does not guarantee that persons may not be in the incubation phase of a contagious disease at the time of their visit (varicella is an obvious example) but can be useful in limiting potential exposures.

Occupational Health

Health care workers require protection from the significant infectious risks inherent in patient care; conversely, patients and other health care workers need to be protected from exposure to health care workers with communicable diseases. Integrating management and prevention strategies to accomplish these two goals requires close collaboration between hospital infection prevention and control programs and employee health departments. A HICPAC guideline for infection control in health care workers contains comprehensive recommendations for evaluation of illnesses, postexposure evaluation and management, and prevention of occupationally-acquired infections in health care workers.[69]

Evaluation of Ill Health Care Workers

Hospital staff and volunteers with symptoms such as persistent fever, conjunctivitis, skin lesions or rashes, diarrhea, and persistent cough should be evaluated for the presence of a communicable disease. Possible cases of varicella, herpes zoster on an exposed area of the body, herpetic whitlow, adenoviral conjunctivitis, measles, mumps, rubella, pertussis, staphylococcal skin infection, enteric infection in a food service worker, and active pulmonary tuberculosis should be investigated promptly and confirmed with laboratory tests if necessary. Although many health care workers will choose to have these problems evaluated by their primary care provider, employee health departments have an interest in completing these assessments, especially if there is a question of whether the condition was acquired in the workplace, requires a furlough from work, or may have exposed patients or other health care workers. Infection prevention and control staff members can provide assistance in these evaluations as needed and should be kept abreast of the results.

Postexposure Evaluation and Management of Health Care Workers

A structured approach to the assessment and management of exposures of health care workers to patients with infectious diseases is critical to promptly provide postexposure prophylaxis, if indicated, and allay anxiety, while avoiding unnecessary interventions and loss of workdays. The first step is to develop criteria for assessing the nature of the exposure, because many reported encounters do not pose a significant risk for infection. Some exposures (e.g., varicella, hepatitis B) may require an assessment of the susceptibility of the health care worker to infection, and procedures should describe the indications for laboratory tests as well as the interpretation of results. Postexposure prophylaxis regimens for various exposures are discussed in a HICPAC guideline,[69] and those for bloodborne infections are available in various other guidelines.[109,510] Additional information for postexposure prophylaxis can be obtained by searching the Centers for Disease Control and Prevention website.* Counseling regarding the risks and consequences of the exposure is an important component of this service.

Prevention of Occupationally-Acquired Infections in Health Care Workers

Because vaccination against infectious diseases is a highly cost-effective prevention strategy, hospitals should offer vaccinations free of charge. Vaccination against measles is so effective that ensuring immunity to measles (either as the result of immunity or vaccination) among hospital staff is widely regarded as an appropriate quality of care indicator for occupational health programs.[357] Because the MMR vaccine is readily available, immunity against mumps and rubella can be ensured in an analogous fashion.

Yearly influenza vaccination of health care workers is recommended by the Immunization Practices Advisory Committee, although vaccination rates of health care workers are suboptimal.[121] Interventions to improve vaccination rates are provision of vaccine free of charge, enhanced access to vaccination, reminders and/or incentives, active management of the vaccination program, requiring active declination, and mandatory immunization policies.[296] Mandatory immunization policies are most effective[327,450]; however, despite endorsement by the American Academy of Pediatrics,[55] SHEA,[650] IDSA, and the National Patient Safety Foundation, the approach remains controversial.[327]

Given the significant occupational risk for hepatitis B infection in hospitals, hepatitis B vaccination should be offered routinely to health care workers. The OSHA bloodborne pathogens standard requires hospitals to offer hepatitis B vaccination free of charge; workers who do not wish to be vaccinated must sign a specific "informed refusal." Other vaccines, such as the varicella vaccine and acellular pertussis vaccine, can help eliminate or at least drastically reduce occupational acquisition of these diseases as well.

In addition to hepatitis B vaccination, the OSHA bloodborne pathogens standard mandates other specific prevention measures, including (1) the development of an exposure control plan that identifies employees with occupational risk for exposure to bloodborne pathogens; (2) annual training for these individuals regarding the risk for bloodborne infection and prevention measures; (3) provision of personal protective clothing and equipment; (4) work practice controls, including equipment and procedures for the safe handling and disposal of needles and sharp instruments; and (5) procedures for identification, transportation, storage, and disposal of contaminated items and waste.[498]

The CDC has also published a detailed guideline for prevention and control measures to prevent transmission of *M. tuberculosis* to health care workers.[323]

Reprocessing of Reusable Patient Care Items

A large number of health care–associated infection outbreaks have been related to use of contaminated equipment. Consequently, hospital infection prevention and control programs must work closely with all hospital departments reprocessing reusable patient care items to ensure proper selection, implementation, and quality monitoring of reprocessing methods.[567]

Education and Training of Heath Care Workers

Providing education and training in both general principles and specific aspects of hospital infection prevention and control is one of the primary responsibilities of program staff. Staff members need to be familiar with principles of adult learning, assessing the educational needs of the audience, defining learning objectives,

*Centers for Disease Control and Prevention: http://www.cdc.gov.

determining optimal instructional formats, using effective teaching and communication skills, and weighing the merits of various educational tools.

Product Evaluation

A large number of new medical products are introduced to the health care market every year. Although some of these products have the potential to reduce infectious risks, data to substantiate the safety and efficacy claims of manufacturers often are limited. Many devices marketed to hospitals caring for children have never actually been tested in children. Because these products often are substantially more expensive than existing products there must be a compelling rationale for their use. Infection prevention and control staff members can provide valuable assistance to hospital committees assessing the potential benefits of new products and, when appropriate, can design and conduct appropriate product evaluations trials.

ANTIMICROBIAL STEWARDSHIP PROGRAMS IN HEALTH CARE FACILITIES

The rapid emergence of pathogens resistant to multiple antimicrobial agents (e.g., VRE, ESBL-producing gram-negative bacteria), and the widespread dissemination of these resistant microorganisms, constitute an unprecedented crisis for hospitals worldwide.[73] The mechanisms involved in the emergence and spread of antimicrobial resistance are complex but are no doubt facilitated by intense selective pressure caused by overuse and misuse of antimicrobial agents in hospitals, particularly newer, broad-spectrum agents.

Data from a handful of studies demonstrate that a sizable proportion of hospitalized children—indeed, a substantial majority in some settings, such as ICUs and transplant units—receive empirical, targeted or prophylactic treatment with antimicrobial agents.[245,258,388,509] In one multicenter study, hospitals that used higher volumes of antimicrobial agents were also more likely to use broad-spectrum agents.[245] In another, use of carbapenems had increased substantially from 2002 to 2007.[509] Two studies have examined the appropriateness of therapy and found that inappropriate use of antimicrobial agents is common.[388,515]

In 2007, IDSA and SHEA collaborated to publish a guideline for institutional antimicrobial stewardship programs.[175] Although the guideline found substantial evidence that interventions to improve antimicrobial use decreased costs and improved patient safety (i.e., by reducing errors in prescription of agents to patients with allergies and improving appropriate dosing), the evidence that interventions were effective in improving patient outcomes or controlling antimicrobial resistance was relatively weak. Nonetheless, for the reasons just noted, the rationale for enhanced efforts to improve antimicrobial stewardship remains strong and a survey of infectious diseases specialists participating in the Emerging Infection Network (EIN) indicated that a majority of U.S. facilities have or are planning to establish an antimicrobial stewardship program, although financial support for these programs remains a key barrier.[325] An earlier EIN survey of pediatric infectious diseases specialists yielded similar findings.[289]

In 2012, the IDSA, SHEA, and the Pediatric Infectious Diseases Society (PIDS) collaborated to publish a policy statement on antimicrobial stewardship.[627] The recommendations contained in the statement, which are summarized in Table 243-11, address the leadership and composition of antimicrobial stewardship programs, the activities of these programs, and suggested evaluation approaches. More detail is presented in a recent review of antimicrobial stewardship in health care facilities providing care to children.[480] In addition, a growing literature is expanding the evidence base for interventions to improve antimicrobial stewardship in general and for specific antimicrobial agents that are used commonly (e.g., vancomycin).*

TABLE 243-11 **Minimum Requirements for Antimicrobial Stewardship Programs**

Recommendation
Leadership and composition of program:
Create a multidisciplinary interprofessional team with training in antimicrobial stewardship that includes a physician, a pharmacist, a clinical microbiologist, and an infection preventionist.
Antimicrobial stewardship activities:
Limit the formulary to include nonduplicative antibiotics with demonstrated clinical need.
Develop institutional guidelines for the management of common infection syndromes.
Provide prescribers with a facility-specific antibiogram indicating the rates of relevant antibiotic susceptibilities for key pathogens.
Implement interventions to reduce use of multidrug regimens with unnecessarily redundant antimicrobial spectra, antibiotic therapy for the management of nonbacterial syndromes or cultures that represent contamination or routine colonization, empiric regimens that are either inadequately or excessively broad spectrum for infection syndromes, regimens that do not adequately treat infections caused by culture-confirmed pathogens.
Evaluation
Monitor antimicrobial use at the institutional level for internal and external benchmarking and to gauge the effect of interventions to improve antimicrobial use.

Adapted from Society for Healthcare Epidemiology of America; Infectious Diseases Society of America; Pediatric Infectious Diseases Society. Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). *Infect Control Hosp Epidemiol* 2012;**33**:322-7.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abdel-Gawad TA, El-Hodhod MA, Ibrahim HM, et al. Gastroesophageal reflux in mechanically ventilated pediatric patients and

*References 9, 131, 181-183, 445, 469, 470, 480-482, 514, 516.

its relation to ventilator-associated pneumonia. *Crit Care* 2009; **13**:R164.
3. Abdolahi A, Fisher SG, Aquino C, et al. Nosocomial infections in a pediatric residential care facility. *Am J Infect Control* 2012;**40**: 502–6.
9. Agwu AL, Lee CK, Jain SK, et al. A World Wide Web–based antimicrobial stewardship program improves efficiency, communication, and user satisfaction and reduces cost in a tertiary care pediatric medical center. *Clin Infect Dis* 2008;**47**:747–53.
10. Al Akhrass F, Hachem R, Mohamed JA, et al. Central venous catheter-associated *Nocardia* bacteremia in cancer patients. *Emerg Infect Dis* 2011;**17**:1651–8.
11. Alexander JW, Solomkin JS, Edwards MJ. Updated recommendations for control of surgical site infections. *Ann Surg* 2011;**253**: 1082–93.
15. Aly H, Badawy M, El-Kholy A, et al. Randomized, controlled trial on tracheal colonization of ventilated infants: can gravity prevent ventilator-associated pneumonia? *Pediatrics* 2008;**122**:770–4.
16. American Academy of Pediatrics. Measles. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 489–99.
17. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 361–9.
18. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 553–66.
19. American Academy of Pediatrics. Varicella-zoster infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 774–89.
23. Ammerlaan HS, Kluytmans JA, Wertheim HF, et al. Eradication of methicillin-resistant *Staphylococcus aureus* carriage: a systematic review. *Clin Infect Dis* 2009;**48**:922–30.
24. Anderson DJ. Surgical site infections. *Infect Dis Clin North Am* 2011;**25**:135–53.
25. Anderson EJ, Rupp A, Shulman ST, et al. Impact of rotavirus vaccination on hospital-acquired rotavirus gastroenteritis in children. *Pediatrics* 2011;**127**:e264–70.
30. Arendrup MC, Fisher BT, Zaoutis TE. Invasive fungal infections in the paediatric and neonatal population: diagnostics and management issues. *Clin Microbiol Infect* 2009;**15**:613–24.
31. Arnell K, Cesarini K, Lagerqvist-Widh A, et al. Cerebrospinal fluid shunt infections in children over a 13-year period: anaerobic cultures and comparison of clinical signs of infection with *Propionibacterium acnes* and with other bacteria. *J Neurosurg Pediatr* 2008;**1**:366–72.
40. Bagdasarian N, Heung M, Malani PN. Infectious complications of dialysis access devices. *Infect Dis Clin North Am* 2012;**26**: 127–41.
51. Ben-Meir D, Golan S, Ehrlich Y, et al. Characteristics and clinical significance of bacterial colonization of ureteral double-J stents in children. *J Pediatr Urol* 2009;**5**:355–8.
55. Bernstein HH, Starke JR. Policy statement—recommendation for mandatory influenza immunization of all health care personnel. *Pediatrics* 2010;**126**:809–15.
56. Berra L, Coppadoro A, Bittner EA, et al. A clinical assessment of the Mucus Shaver: a device to keep the endotracheal tube free from secretions. *Crit Care Med* 2012;**40**:119–24.
58. Berton DC, Kalil AC, Teixeira PJ. Quantitative versus qualitative cultures of respiratory secretions for clinical outcomes in patients with ventilator-associated pneumonia. *Cochrane Database Syst Rev* 2012;**1**:CD006482.
63. Bi XC, Zhang B, Ye YK, et al. Pathogen incidence and antibiotic resistance patterns of catheter-associated urinary tract infection in children. *J Chemother* 2009;**21**:661–5.
65. Bigham MT, Amato R, Bondurrant P, et al. Ventilator-associated pneumonia in the pediatric intensive care unit: characterizing the problem and implementing a sustainable solution. *J Pediatr* 2009; **154**:582–7, e582.
67. Bitsori M, Maraki S, Koukouraki S, et al. *Pseudomonas aeruginosa* urinary tract infection in children: risk factors and outcomes. *J Urol* 2012;**187**:260–4.
72. Botelho-Nevers E, Gautret P, Biellik R, et al. Nosocomial transmission of measles: an updated review. *Vaccine* 2012;**30**:3996–4001.
73. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:1–12.
74. Bowater RJ, Stirling SA, Lilford RJ. Is antibiotic prophylaxis in surgery a generally effective intervention? Testing a generic hypothesis over a set of meta-analyses. *Ann Surg* 2009;**249**: 551–6.
82. Braun BI, Kusek L, Larson E. Measuring adherence to hand hygiene guidelines: a field survey for examples of effective practices. *Am J Infect Control* 2009;**37**:282–8.
84. Brocklehurst P, Farrell B, King A, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011;**365**: 1201–11.
86. Bruijning-Verhagen P, Quach C, Bonten M. Nosocomial rotavirus infections: a meta-analysis. *Pediatrics* 2012;**129**:e1011–19.
87. Bruminhent J, Keegan M, Lakhani A, et al. Effectiveness of a simple intervention for prevention of catheter-associated urinary tract infections in a community teaching hospital. *Am J Infect Control* 2010;**38**:689–93.
90. Bucher BT, Guth RM, Elward AM, et al. Risk factors and outcomes of surgical site infection in children. *J Am Coll Surg* 2011;**212**:1033–8, e1031.
92. Burton DC, Edwards JR, Horan TC, et al. Methicillin-resistant *Staphylococcus aureus* central line–associated bloodstream infections in US intensive care units, 1997-2007. *JAMA* 2009;**301**:727–36.
96. Calfee DP, Salgado CD, Classen D, et al. Strategies to prevent transmission of methicillin-resistant *Staphylococcus aureus* in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**(Suppl. 1): S62–80.
98. Camins BC, Richmond AM, Dyer KL, et al. A crossover intervention trial evaluating the efficacy of a chlorhexidine-impregnated sponge in reducing catheter-related bloodstream infections among patients undergoing hemodialysis. *Infect Control Hosp Epidemiol* 2010;**31**:1118–23.
100. Cardo D, Dennehy PH, Halverson P, et al. Moving toward elimination of healthcare-associated infections: a call to action. *Am J Infect Control* 2010;**38**:671–5.
101. Carlson AL, Budd AP, Perl TM. Control of influenza in healthcare settings: early lessons from the 2009 pandemic. *Curr Opin Infect Dis* 2010;**23**:293–9.
114. Centers for Disease Control and Prevention. Acute hepatitis C virus infections attributed to unsafe injection practices at an endoscopy clinic—Nevada, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:513–17.
115. Centers for Disease Control and Prevention. Hospital-acquired pertussis among newborns—Texas, 2004. *MMWR Morb Mortal Wkly Rep* 2008;**57**:600–3.
116. Centers for Disease Control and Prevention. Outbreak of measles—San Diego, California, January-February 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:203–6.
117. Centers for Disease Control and Prevention. Immunization of health-care personnel: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**:1–45.
118. Centers for Disease Control and Prevention. Vital signs: central line-associated blood stream infections—United States, 2001, 2008, and 2009. *MMWR Morb Mortal Wkly Rep* 2011;**60**:243–8.
119. Centers for Disease Control and Prevention. Updated CDC recommendations for the management of hepatitis B virus–infected health-care providers and students. *MMWR Recomm Rep* 2012; **61**:1–12.
120. Centers for Disease Control and Prevention. Vital signs: preventing *Clostridium difficile* infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:157–62.
121. Centers for Disease Control and Prevention. Influenza vaccination coverage among health-care personnel—2011-12 influenza season, United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 753–7.
123. Chadha V, Schaefer FS, Warady BA. Dialysis-associated peritonitis in children. *Pediatr Nephrol* 2010;**25**:425–40.
127. Chen SY, Anderson S, Kutty PK, et al. Health care–associated measles outbreak in the United States after an importation: challenges and economic impact. *J Infect Dis* 2011;**203**:1517–25.

138. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Wayne, Pa: Clinical and Laboratory Standards Institute; 2010.
139. Coffin SE, Klompas M, Classen D, et al. Strategies to prevent ventilator-associated pneumonia in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**(Suppl. 1):S31–40.
140. Coffin SE, Zaoutis TE. Healthcare-associated infections in the nursery. In: Remington JS, Klein JO, Wilson CB, et al, editors. *Infectious diseases of the fetus and newborn infant*. St. Louis: Elsevier; 2011. p. 1126–43.
147. Cox SG, Rode H, Darani AN, et al. Thermal injury within the first 4 months of life. *Burns* 2011;**37**:828–34.
153. Cruz AT, Medina D, Whaley EM, et al. Tuberculosis among families of children with suspected tuberculosis and employees at a children's hospital. *Infect Control Hosp Epidemiol* 2011;**32**:188–90.
155. Cunliffe NA, Booth JA, Elliot C, et al. Healthcare-associated viral gastroenteritis among children in a large pediatric hospital, United Kingdom. *Emerg Infect Dis* 2010;**16**:55–62.
163. Darouiche RO, Wall MJ Jr, Itani KM, et al. Chlorhexidine-alcohol versus povidone-iodine for surgical-site antisepsis. *N Engl J Med* 2010;**362**:18–26.
173. de Mello MJ, de Albuquerque Mde F, Ximenes RA, et al. Factors associated with time to acquisition of bloodstream infection in a pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2010; **31**:249–55.
174. de Smet AM, Kluytmans JA, Cooper BS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med* 2009;**360**:20–31.
180. Desai R, Hembree CD, Handel A, et al. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. *Clin Infect Dis* 2012;**55**:189–93.
181. Di Pentima MC, Chan S. Impact of antimicrobial stewardship program on vancomycin use in a pediatric teaching hospital. *Pediatr Infect Dis J* 2010;**29**:707–11.
182. Di Pentima MC, Chan S, Eppes SC, et al. Antimicrobial prescription errors in hospitalized children: role of antimicrobial stewardship program in detection and intervention. *Clin Pediatr* 2009;**48**: 505–12.
183. Di Pentima MC, Chan S, Hossain J. Benefits of a pediatric antimicrobial stewardship program at a children's hospital. *Pediatrics* 2011;**128**:1062–70.
184. Digiorgio MJ, Fatica C, Oden M, et al. Development of a modified surveillance definition of central line-associated bloodstream infections for patients with hematologic malignancies. *Infect Control Hosp Epidemiol* 2012;**33**:865–8.
186. Dixon JM, Carver RL. Daily chlorohexidine gluconate bathing with impregnated cloths results in statistically significant reduction in central line-associated bloodstream infections. *Am J Infect Control* 2010;**38**:817–21.
187. Dizdar EA, Aydemir C, Erdeve O, et al. Respiratory syncytial virus outbreak defined by rapid screening in a neonatal intensive care unit. *J Hosp Infect* 2010;**75**:292–4.
194. Dudeck MA, Horan TC, Peterson KD, et al. National Healthcare Safety Network (NHSN) Report, data summary for 2010, device-associated module. *Am J Infect Control* 2011;**39**:798–816.
205. Esezobor CI, Nourse P, Gajjar P. Urinary tract infection following kidney transplantation: frequency, risk factors and graft function. *Pediatr Nephrol* 2012;**27**:651–7.
206. Esteve F, Pujol M, Perez XL, et al. Bacteremia related with arterial catheter in critically ill patients. *J Infect* 2011;**63**:139–43.
209. Fanella ST, Pinto MA, Bridger NA, et al. Pandemic (H1N1) 2009 influenza in hospitalized children in Manitoba: nosocomial transmission and lessons learned from the first wave. *Infect Control Hosp Epidemiol* 2011;**32**:435–43.
210. Feber J, Spatenka J, Seeman T, et al. Urinary tract infections in pediatric renal transplant recipients—a two center risk factors study. *Pediatr Transplant* 2009;**13**:881–6.
214. Fiore AE, Fry A, Shay D, et al. Antiviral agents for the treatment and chemoprophylaxis against influenza: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2011;**60**(RR01):1–24.
222. Fraser TG, Gordon SM. CLABSI rates in immunocompromised patients: a valuable patient centered outcome? *Clin Infect Dis* 2011; **52**:1446–50.
233. Gallimore CI, Taylor C, Gennery AR, et al. Contamination of the hospital environment with gastroenteric viruses: comparison of two pediatric wards over a winter season. *J Clin Microbiol* 2008; **46**:3112–15.
245. Gerber JS, Newland JG, Coffin SE, et al. Variability in antibiotic use at children's hospitals. *Pediatrics* 2010;**126**:1067–73.
256. Gould CV, Umscheid CA, Agarwal RK, et al. Guideline for prevention of catheter-associated urinary tract infections 2009. *Infect Control Hosp Epidemiol* 2010;**31**:319–26.
261. Groothuis J, Bauman J, Malinoski F, et al. Strategies for prevention of RSV nosocomial infection. *J Perinatol* 2008;**28**:319–23.
265. Gutelius B, Perz JF, Parker MM, et al. Multiple clusters of hepatitis virus infections associated with anesthesia for outpatient endoscopy procedures. *Gastroenterology* 2010;**139**:163–70.
266. Haas EJ, Zaoutis TE, Prasad P, et al. Risk factors and outcomes for vancomycin-resistant enterococcus bloodstream infection in children. *Infect Control Hosp Epidemiol* 2010 Aug 31. [Epub ahead of print].
289. Hersh AL, Beekmann SE, Polgreen PM, et al. Antimicrobial stewardship programs in pediatrics. *Infect Control Hosp Epidemiol* 2009; **30**:1211–17.
292. Hewlett AL, Rupp ME. New developments in the prevention of intravascular catheter associated infections. *Infect Dis Clin North Am* 2012;**26**:1–11.
293. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol* 2008;**29**:996–1011.
296. Hollmeyer H, Hayden F, Mounts A, et al. Review: interventions to increase influenza vaccination among healthcare workers in hospitals. *Influenza Other Respi Viruses* 2012 Sep 18. [Epub ahead of print].
297. Holzmann-Pazgal G, Hopkins-Broyles D, Recktenwald A, et al. Case-control study of pediatric cardiothoracic surgical site infections. *Infect Control Hosp Epidemiol* 2008;**29**:76–9.
299. Hooton TM, Bradley SF, Cardenas DD, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**: 625–63.
300. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care–associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**:309–32.
301. Horbar JD, Carpenter JH, Badger GJ, et al. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. *Pediatrics* 2012;**129**:1019–26.
302. Horbar JD, Soll RF, Edwards WH. The Vermont Oxford Network: a community of practice. *Clin Perinatol* 2010;**37**: 29–47.
305. Huang FL, Chen CH, Huang SK, et al. An outbreak of enterovirus 71 in a nursery. *Scand J Infect Dis* 2010;**42**:609–12.
309. Hunter JD. Ventilator associated pneumonia. *BMJ* 2012;**344**: e3325.
310. Huskins WC. Quality improvement interventions to prevent healthcare-associated infections in neonates and children. *Curr Opin Pediatr* 2012;**24**:103–12.
311. Huskins WC, Huckabee CM, O'Grady NP, et al. Intervention to reduce transmission of resistant bacteria in intensive care. *N Engl J Med* 2011;**364**:1407–18.
314. Institute for Healthcare Improvement. *How-to-guide: reduce methicillin-resistant* Staphylococcus aureus *(MRSA) infection*. Institute for Healthcare Improvement. 2011. Available at http://www.ihi.org/knowledge/Pages/Tools/HowtoGuideReduceMRSAInfection.aspx. Accessed August 30, 2012.
315. Institute of Medicine. *Crossing the quality chasm: a new health system for the 21st century*. Washington, D.C.: National Academies Press; 2001.
316. Jacobsen SM, Stickler DJ, Mobley HL, et al. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev* 2008;**21**:26–59.
317. Jain R, Kralovic SM, Evans ME, et al. Veterans Affairs initiative to prevent methicillin-resistant *Staphylococcus aureus* infections. *N Engl J Med* 2011;**364**:1419–30.
321. Jeelani NU, Kulkarni AV, Desilva P, et al. Postoperative cerebrospinal fluid wound leakage as a predictor of shunt infection: a

prospective analysis of 205 cases. Clinical article. *J Neurosurg Pediatr* 2009;**4**:166–9.
322. Jeffries HE, Mason W, Brewer M, et al. Prevention of central venous catheter–associated bloodstream infections in pediatric intensive care units: a performance improvement collaborative. *Infect Control Hosp Epidemiol* 2009;**30**:645–51.
324. Jimenez-Truque N, Tedeschi S, Saye EJ, et al. Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Pediatrics* 2012;**129**:e1252–9.
325. Johannsson B, Beekmann SE, Srinivasan A, et al. Improving antimicrobial stewardship: the evolution of programmatic strategies and barriers. *Infect Control Hosp Epidemiol* 2011;**32**:367–74.
327. Johnson JG, Talbot TR. New approaches for influenza vaccination of healthcare workers. *Curr Opin Infect Dis* 2011;**24**:363–9.
331. Kaplan HC, Lannon C, Walsh MC, et al. Ohio statewide quality-improvement collaborative to reduce late-onset sepsis in preterm infants. *Pediatrics* 2011;**127**:427–35.
333. Karki S, Cheng AC. Impact of non-rinse skin cleansing with chlorhexidine gluconate on prevention of healthcare-associated infections and colonization with multi-resistant organisms: a systematic review. *J Hosp Infect* 2012;**82**:71–84.
335. Katz BZ, Sullivan C. Respiratory syncytial virus prophylaxis in a tertiary care neonatal intensive care unit. *Pediat Infect Dis J* 2009;**28**:842–4.
337. Kelly M, Conway M, Wirth K, et al. Moving CLABSI prevention beyond the intensive care unit: risk factors in pediatric oncology patients. *Infect Control Hosp Epidemiol* 2011;**32**:1079–85.
338. Kelsen JR, Kim J, Latta D, et al. Recurrence rate of *Clostridium difficile* infection in hospitalized pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011;**17**:50–5.
342. Kim J, Shaklee JF, Smathers S, et al. Risk factors and outcomes associated with severe *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2012;**31**:134–8.
343. Kim J, Smathers SA, Prasad P, et al. Epidemiological features of *Clostridium difficile*-associated disease among inpatients at children's hospitals in the United States, 2001-2006. *Pediatrics* 2008;**122**:1266–70.
349. Klompas M, Kleinman K, Khan Y, et al. Rapid and reproducible surveillance for ventilator-associated pneumonia. *Clin Infect Dis* 2012;**54**:370–7.
350. Koh DB, Gowardman JR, Rickard CM, et al. Prospective study of peripheral arterial catheter infection and comparison with concurrently sited central venous catheters. *Crit Care Med* 2008;**36**:397–402.
353. Koser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N Engl J Med* 2012;**366**:2267–75.
358. Krause R, Salzer HF, Honigl M, et al. Comparison of fluorescence in situ hybridisation using peptide nucleic acid probes, Gram stain/acridine orange leukocyte cytospin and differential time to positivity methods for detection of catheter-related bloodstream infection in patients after haematopoietic stem cell transplantation. *Clin Microbiol Infect* 2010;**16**:1591–3.
362. Kurz H, Herbich K, Janata O, et al. Experience with the use of palivizumab together with infection control measures to prevent respiratory syncytial virus outbreaks in neonatal intensive care units. *J Hosp Infect* 2008;**70**:246–52.
363. Kusuhara K, Saito M, Sasaki Y, et al. An echovirus type 18 outbreak in a neonatal intensive care unit. *Eur J Pediatr* 2008;**167**:587–9.
364. Kutty PK, Kyaw MH, Dayan GH, et al. Guidance for isolation precautions for mumps in the United States: a review of the scientific basis for policy change. *Clin Infect Dis* 2010;**50**:1619–28.
368. Labeau SO, Van de Vyver K, Brusselaers N, et al. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. *Lancet Infect Dis* 2011;**11**:845–54.
369. Labriola L, Crott R, Jadoul M. Preventing haemodialysis catheter-related bacteraemia with an antimicrobial lock solution: a meta-analysis of prospective randomized trials. *Nephrol Dial Transplant* 2008;**23**:1666–72.
372. Langley JM, Gravel D, Moore D, et al. Study of cerebrospinal fluid shunt-associated infections in the first year following placement, by the Canadian Nosocomial Infection Surveillance Program. *Infect Control Hosp Epidemiol* 2009;**30**:285–8.
376. Lautenbach E, Woeltje KF, Malani PN. *Practical healthcare epidemiology*. Chicago: University of Chicago Press; 2010.
377. Lavergne V, Ghannoum M, Weiss K, et al. Successful prevention of respiratory syncytial virus nosocomial transmission following an enhanced seasonal infection control program. *Bone Marrow Transplant* 2011;**46**:137–42.
378. Leckerman KH, Sherman E, Knorr J, et al. Risk factors for healthcare-associated, laboratory-confirmed influenza in hospitalized pediatric patients: a case-control study. *Infect Control Hosp Epidemiol* 2010;**31**:421–4.
382. Lee I, Agarwal RK, Lee BY, et al. Systematic review and cost analysis comparing use of chlorhexidine with use of iodine for preoperative skin antisepsis to prevent surgical site infection. *Infect Control Hosp Epidemiol* 2010;**31**:1219–29.
384. Lemaire X, Morena M, Leray-Moragues H, et al. Analysis of risk factors for catheter-related bacteremia in 2000 permanent dual catheters for hemodialysis. *Blood Purif* 2009;**28**:21–8.
385. Lessa FC, Edwards JR, Fridkin SK, et al. Trends in incidence of late-onset methicillin-resistant *Staphylococcus aureus* infection in neonatal intensive care units: data from the National Nosocomial Infections Surveillance System, 1995-2004. *Pediatr Infect Dis J* 2009;**28**:577–81.
388. Levy ER, Swami S, Dubois SG, et al. Rates and appropriateness of antimicrobial prescribing at an academic children's hospital, 2007-2010. *Infect Control Hosp Epidemiol* 2012;**33**:346–53.
389. Levy I, Bendet M, Samra Z, et al. Infectious complications of peripherally inserted central venous catheters in children. *Pediatr Infect Dis J* 2010;**29**:426–9.
393. Linam WM, Margolis PA, Staat MA, et al. Risk factors associated with surgical site infection after pediatric posterior spinal fusion procedure. *Infect Control Hosp Epidemiol* 2009;**30**:109–16.
405. Lucero CA, Cohen AL, Trevino I, et al. Outbreak of *Burkholderia cepacia* complex among ventilated pediatric patients linked to hospital sinks. *Am J Infect Control* 2011;**39**:775–8.
406. Lucet JC, Bouadma L, Zahar JR, et al. Infectious risk associated with arterial catheters compared with central venous catheters. *Crit Care Med* 2010;**38**:1030–5.
408. Lundgren IS, Zhou C, Malone FR, et al. Central venous catheter repair is associated with an increased risk of bacteremia and central line-associated bloodstream infection in pediatric patients. *Pediatr Infect Dis J* 2012;**31**:337–40.
411. MacCannell T, Umscheid CA, Agarwal RK, et al. Guideline for the prevention and control of norovirus gastroenteritis outbreaks in healthcare settings. *Infect Control Hosp Epidemiol* 2011;**32**:939–69.
426. Maragakis LL. Recognition and prevention of multidrug-resistant gram-negative bacteria in the intensive care unit. *Crit Care Med* 2010;**38**:S345–51.
427. Marcos M, Soriano A, Inurrieta A, et al. Changing epidemiology of central venous catheter-related bloodstream infections: increasing prevalence of gram-negative pathogens. *J Antimicrob Chemother* 2011;**66**:2119–25.
431. Matthews JE, Dickey BW, Miller RD, et al. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. *Epidemiol Infect* 2012;**140**:1161–72.
432. Mattner F, Sykora KW, Meissner B, et al. An adenovirus type F41 outbreak in a pediatric bone marrow transplant unit: analysis of clinical impact and preventive strategies. *Pediatr Infect Dis J* 2008;**27**:419–24.
433. Mayhall CG. *Hospital epidemiology and infection control*. Philadelphia: Lippincott Williams & Wilkins; 2011.
434. McCannon J, Berwick DM. A new frontier in patient safety. *JAMA* 2011;**305**:2221–2.
435. McCullers JA, Williams BF, Wu S, et al. Health care–associated infections at a children's cancer hospital, 1983-2008. *J Pediatr Infect Dis Soc* 2012;**1**:26–34.
441. Meddings J, Rogers MA, Macy M, et al. Systematic review and meta-analysis: reminder systems to reduce catheter-associated urinary tract infections and urinary catheter use in hospitalized patients. *Clin Infect Dis* 2010;**51**:550–60.
443. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.
445. Metjian TA, Prasad PA, Kogon A, et al. Evaluation of an antimicrobial stewardship program at a pediatric teaching hospital. *Pediatr Infect Dis J* 2008;**27**:106–11.

452. Miller MR, Griswold M, Harris JM 2nd, et al. Decreasing PICU catheter-associated bloodstream infections: NACHRI's quality transformation efforts. *Pediatrics* 2010;**125**:206–13.
453. Millership SE, Anderson C, Cummins AJ, et al. The risk to infants from nosocomial exposure to tuberculosis. *Pediatr Infect Dis J* 2009;**28**:915–16.
454. Milstone AM, Sengupta A. Do prolonged peripherally inserted central venous catheter dwell times increase the risk of bloodstream infection? *Infect Control Hosp Epidemiol* 2010;**31**:1184–7.
461. Montecalvo MA, McKenna D, Yarrish R, et al. Chlorhexidine bathing to reduce central venous catheter-associated bloodstream infection: impact and sustainability. *Am J Med* 2012;**125**:505–11.
463. Morgan DJ, Rogawski E, Thom KA, et al. Transfer of multidrug-resistant bacteria to healthcare workers' gloves and gowns after patient contact increases with environmental contamination. *Crit Care Med* 2012;**40**:1045–51.
465. Morter S, Bennet G, Fish J, et al. Norovirus in the hospital setting: virus introduction and spread within the hospital environment. *J Hosp Infect* 2011;**77**:106–12.
467. Mu Y, Edwards JR, Horan TC, et al. Improving risk-adjusted measures of surgical site infection for the national healthcare safety network. *Infect Control Hosp Epidemiol* 2011;**32**:970–86.
472. Munoz-Price LS, Hota B, Stemer A, et al. Prevention of bloodstream infections by use of daily chlorhexidine baths for patients at a long-term acute care hospital. *Infect Control Hosp Epidemiol* 2009;**30**:1031–5.
479. Neu N, Plaskett T, Hutcheon G, et al. Epidemiology of human metapneumovirus in a pediatric long-term care facility. *Infect Control Hosp Epidemiol* 2012;**33**:545–50.
480. Newland JG, Banerjee R, Gerber JS, et al. Antimicrobial stewardship in pediatric care: strategies and future directions. *Pharmacotherapy* 2012;**32**:735–43.
481. Newland JG, Stach LM, De Lurgio SA, et al. Impact of a prospective-audit-with-feedback antimicrobial stewardship program at a children's hospital. *J Ped Infect Dis*, 2012;**3**:179–86.
482. Newman RE, Hedican EB, Herigon JC, et al. Impact of a guideline on management of children hospitalized with community-acquired pneumonia. *Pediatrics* 2012;**129**:e597–604.
484. Niedner MF, Huskins WC, Colantuoni E, et al. Epidemiology of central line-associated bloodstream infections in the pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:1200–8.
490. Nucci M, Anaissie E, Betts RF, et al. Early removal of central venous catheter in patients with candidemia does not improve outcome: analysis of 842 patients from 2 randomized clinical trials. *Clin Infect Dis* 2010;**51**:295–303.
492. Nylund CM, Goudie A, Garza JM, et al. *Clostridium difficile* infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011;**165**:451–7.
493. O'Connell K, Boo TW, Keady D, et al. Use of palivizumab and infection control measures to control an outbreak of respiratory syncytial virus in a neonatal intensive care unit confirmed by real-time polymerase chain reaction. *J Hosp Infect* 2011;**77**:338–42.
494. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis* 2011;**52**:e162–93.
496. O'Grady NP, Murray PR, Ames N. Preventing ventilator-associated pneumonia: does the evidence support the practice? *JAMA* 2012;**307**:2534–9.
497. O'Horo JC, Thompson D, Safdar N. Is the gram stain useful in the microbiologic diagnosis of VAP? A Meta-analysis. *Clin Infect Dis* 2012;**55**:551–61.
501. Oliveira C, Nasr A, Brindle M, et al. Ethanol locks to prevent catheter-related bloodstream infections in parenteral nutrition: a meta-analysis. *Pediatrics* 2012;**129**:318–29.
504. Ostovar GA, Kohn N, Yu KO, et al. Nosocomial influenza in a pediatric hospital: comparison of rates of seasonal and pandemic 2009 influenza A/H1N1 infection. *Infect Control Hosp Epidemiol* 2012;**33**:292–4.
507. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol* 2011;**32**:687–99.
511. Parienti JJ, du Cheyron D, Timsit JF, et al. Meta-analysis of subclavian insertion and nontunneled central venous catheter-associated infection risk reduction in critically ill adults. *Crit Care Med* 2012;**40**:1627–34.
515. Patel SJ, Oshodi A, Prasad P, et al. Antibiotic use in neonatal intensive care units and adherence with Centers for Disease Control and Prevention 12 Step Campaign to Prevent Antimicrobial Resistance. *Pediatr Infect Dis J* 2009;**28**:1047–51.
516. Patel SJ, Saiman L, Duchon JM, et al. Development of an antimicrobial stewardship intervention using a model of actionable feedback. *Interdiscip Perspect Infect Dis* 2012;**2012**:150367.
519. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med* 2010;**362**:1804–13.
529. Popovich KJ, Hota B, Hayes R, et al. Effectiveness of routine patient cleansing with chlorhexidine gluconate for infection prevention in the medical intensive care unit. *Infect Control Hosp Epidemiol* 2009;**30**:959–63.
533. Pronovost PJ, Marsteller JA, Goeschel CA. Preventing bloodstream infections: a measurable national success story in quality improvement. *Health Aff (Millwood)* 2011;**30**:628–34.
539. Raad I, Mohamed JA, Reitzel RA, et al. Improved antibiotic-impregnated catheters with extended-spectrum activity against resistant bacteria and fungi. *Antimicrob Agents Chemother* 2012;**56**:935–41.
542. Rafla K, Tredget EE. Infection control in the burn unit. *Burns* 2011;**37**:5–15.
543. Ramsey AM, Zilberberg MD. Secular trends of hospitalization with vancomycin-resistant enterococcus infection in the United States, 2000-2006. *Infect Control Hosp Epidemiol* 2009;**30**:184–6.
547. Rattanaumpawan P, Tolomeo P, Bilker WB, et al. Risk factors for fluoroquinolone resistance in gram-negative bacilli causing healthcare-acquired urinary tract infections. *J Hosp Infect* 2010;**76**:324–7.
548. Raval MV, Dillon PW, Bruny JL, et al. American College of Surgeons National Surgical Quality Improvement Program Pediatric: a phase 1 report. *J Am Coll Surg* 2011;**212**:1–11.
552. Reyes JA, Habash ML, Taylor RP. Femoral central venous catheters are not associated with higher rates of infection in the pediatric critical care population. *Am J Infect Control* 2012;**40**:43–7.
567. Rutala WA, Weber DJ, Healthcare Infection Control Practices Advisory Committee. *Guideline for disinfection and sterilization in healthcare facilities, 2008*. Centers for Disease Control and Prevention; 2008. Available at http://www.cdc.gov/hicpac/pdf/Disinfection_Sterilization/Pages1_2Disinfection_Nov_2008. Accessed October 23, 2012.
568. Ryckman FC, Schoettker PJ, Hays KR, et al. Reducing surgical site infections at a pediatric academic medical center. *Jt Comm J Qual Patient Saf* 2009;**35**:192–8.
577. Saint S, Howell JD, Krein SL. Implementation science: how to jump-start infection prevention. *Infect Control Hosp Epidemiol* 2010;**31**(Suppl. 1):S14–17.
578. Saint S, Kowalski CP, Banaszak-Holl J, et al. The importance of leadership in preventing healthcare-associated infection: results of a multisite qualitative study. *Infect Control Hosp Epidemiol* 2010;**31**:901–7.
581. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2011;**30**:580–4.
584. Sax H, Allegranzi B, Chraiti MN, et al. The World Health Organization hand hygiene observation method. *Am J Infect Control* 2009;**37**:827–34.
590. Schlesinger A, Paul M, Gafter-Gvili A, et al. Infection-control interventions for cancer patients after chemotherapy: a systematic review and meta-analysis. *Lancet Infect Dis* 2009;**9**:97–107.
592. Schulman J, Stricof RL, Stevens TP, et al. Development of a statewide collaborative to decrease NICU central line-associated bloodstream infections. *J Perinatol* 2009;**29**:591–9.
593. Schulman J, Stricof RL, Stevens TP, et al. Statewide NICU central-line-associated bloodstream infection rates decline after bundles and checklists. *Pediatrics* 2011;**127**:436–44.
598. Sengupta A, Lehmann C, Diener-West M, et al. Catheter duration and risk of CLA-BSI in neonates with PICCs. *Pediatrics* 2010;**125**:648–53.
615. Simon A, Muller A, Khurana K, et al. Nosocomial infection: a risk factor for a complicated course in children with respiratory syncytial virus infection–results from a prospective multicenter German surveillance study. *Int J Hyg Environ Health* 2008;**211**:241–50.
616. Simon TD, Hall M, Riva-Cambrin J, et al. Infection rates following initial cerebrospinal fluid shunt placement across pediatric

hospitals in the United States. Clinical article. *J Neurosurg Pediatr* 2009;**4**:156–65.
617. Simon TD, Whitlock KB, Riva-Cambrin J, et al. Revision surgeries are associated with significant increased risk of subsequent cerebrospinal fluid shunt infection. *Pediatr Infect Dis J* 2012; **31**:551–6.
621. Smith JS, Shaffrey CI, Sansur CA, et al. Rates of infection after spine surgery based on 108,419 procedures: a report from the Scoliosis Research Society Morbidity and Mortality Committee. *Spine (Phila Pa 1976)* 2011;**36**:556–63.
622. Smith MJ, Clark HF, Lawley D, et al. The clinical and molecular epidemiology of community- and healthcare-acquired rotavirus gastroenteritis. *Pediatr Infect Dis J* 2008;**27**:54–8.
624. Smith PW, Bennett G, Bradley S, et al. SHEA/APIC guideline: infection prevention and control in the long-term care facility, July 2008. *Infect Control Hosp Epidemiol* 2008;**29**:785–814.
626. Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci Translation Med* 2012;**4**:148ra116.
627. Society for Healthcare Epidemiology of America (SHEA), Infectious Diseases Society of America (IDSA), Pediatric Infectious Diseases Society (PIDS): Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). *Infect Control Hosp Epidemiol* 2012;**33**:322–7.
629. Sohn AH, Schwartz JM, Yang KY, et al. Risk factors and risk adjustment for surgical site infections in pediatric cardiothoracic surgery patients. *Am J Infect Control* 2010;**38**:706–10.
630. Solomon LR, Cheesbrough JS, Ebah L, et al. A randomized double-blind controlled trial of taurolidine-citrate catheter locks for the prevention of bacteremia in patients treated with hemodialysis. *Am J Kidney Dis* 2010;**55**:1060–8.
633. Srinivasan R, Asselin J, Gildengorin G, et al. A prospective study of ventilator-associated pneumonia in children. *Pediatrics* 2009;**123**:1108–15.
648. Taira BR, Fenton KE, Lee TK, et al. Ventilator-associated pneumonia in pediatric trauma patients. *Pediatr Crit Care Med* 2009;**10**:491–4.
650. Talbot TR, Babcock H, Caplan AL, et al. Revised SHEA position paper: influenza vaccination of healthcare personnel. *Infect Control Hosp Epidemiol* 2010;**31**:987–95.
653. Tamma PD, Aucott SW, Milstone AM. Chlorhexidine use in the neonatal intensive care unit: results from a national survey. *Infect Control Hosp Epidemiol* 2010;**31**:846–9.
654. Tamma PD, Savard P, Pal T, et al. An outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2012;**33**: 631–4.
655. Tamma PD, Turnbull AE, Milstone AM, et al. Ventilator-associated tracheitis in children: does antibiotic duration matter? *Clin Infect Dis* 2011;**52**:1324–31.
657. Tanner J, Norrie P, Melen K. Preoperative hair removal to reduce surgical site infection. *Cochrane Database Syst Rev* 2011;**11**: CD004122.
658. Tanner J, Swarbrook S, Stuart J. Surgical hand antisepsis to reduce surgical site infection. *Cochrane Database Syst Rev* 2008;**1**: CD004288.
663. Thompson ND, Perz JF, Moorman AC, et al. Nonhospital health care–associated hepatitis B and C virus transmission: United States, 1998-2008. *Ann Intern Med* 2009;**150**:33–9.
664. Timsit JF, Schwebel C, Bouadma L, et al. Chlorhexidine-impregnated sponges and less frequent dressing changes for prevention of catheter-related infections in critically ill adults: a randomized controlled trial. *JAMA* 2009;**301**:1231–41.
666. Togo A, Coulibaly Y, Dembele BT, et al. Risk factors for surgical site infection in children at the teaching hospital Gabriel Toure, Bamako. *J Hosp Infect* 2011;**79**:371–2.
667. Toltzis P, Kim J, Dul M, et al. Presence of the epidemic North American Pulsed Field type 1 *Clostridium difficile* strain in hospitalized children. *J Pediatr* 2009;**154**:607–8.
668. Trinh TT, Chan PA, Edwards O, et al. Peripheral venous catheter–related *Staphylococcus aureus* bacteremia. *Infect Control Hosp Epidemiol* 2011;**32**:579–83.
669. Tschudin-Sutter S, Frei R, Egli-Gany D, et al. No risk of surgical site infections from residual bacteria after disinfection with povidone-iodine-alcohol in 1014 cases: a prospective observational study. *Ann Surg* 2012;**255**:565–9.
670. Tuan TJ, Thorell EA, Hamblett NM, et al. Treatment and microbiology of repeated cerebrospinal fluid shunt infections in children. *Pediatr Infect Dis J* 2011;**30**:731–5.
672. Uvin P, Van Baelen A, Verhaegen J, et al. Ureteral stents do not cause bacterial infections in children after ureteral reimplantation. *Urology* 2011;**78**:154–8.
679. van Rijen MM, Bonten M, Wenzel RP, et al. Intranasal mupirocin for reduction of *Staphylococcus aureus* infections in surgical patients with nasal carriage: a systematic review. *J Antimicrob Chemother* 2008;**61**:254–61.
680. Vasudevan A, Iyengar A, Jose B, et al. Pediatric renal transplantation: a single-center experience. *Transplant Proc* 2008;**40**:1095–8.
681. Vayalumkal JV, Gravel D, Moore D, et al. Surveillance for healthcare-acquired febrile respiratory infection in pediatric hospitals participating in the Canadian Nosocomial Infection Surveillance Program. *Infect Control Hosp Epidemiol* 2009;**30**:652–8.
682. Venkatachalam V, Hendley JO, Willson DF. The diagnostic dilemma of ventilator-associated pneumonia in critically ill children. *Pediatr Crit Care Med* 2011;**12**:286–96.
683. Verhagen P, Moore D, Manges A, et al. Nosocomial rotavirus gastroenteritis in a Canadian paediatric hospital: incidence, disease burden and patients affected. *J Hosp Infect* 2011;**79**:59–63.
685. Viau RA, Hujer AM, Marshall SH, et al. "Silent" dissemination of *Klebsiella pneumoniae* isolates bearing *K. pneumoniae* carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. *Clin Infect Dis* 2012;**54**:1314–21.
686. Vij NK, Stryker CC, Esper FP, et al. Influenza A/H1N1/09-10 infections in a NICU during the 2009-10 H1N1 pandemic. *Pediatrics* 2011;**128**:e1297–301.
688. Waisbourd-Zinman O, Ben-Ziony S, Solter E, et al. The percentage of nosocomial-related out of total hospitalizations for rotavirus gastroenteritis and its association with hand hygiene compliance. *Am J Infect Control* 2011;**39**:166–8.
689. Wang F, Bo L, Tang L, et al. Subglottic secretion drainage for preventing ventilator-associated pneumonia: an updated meta-analysis of randomized controlled trials. *J Trauma Acute Care Surg* 2012;**72**:1276–85.
690. Warady BA, Bakkaloglu S, Newland J, et al. Consensus guidelines for the prevention and treatment of catheter-related infections and peritonitis in pediatric patients receiving peritoneal dialysis: 2012 update. *Perit Dial Int* 2012;**32**(Suppl. 2):S32–86.
695. Weber DJ, Rutala WA, Miller MB, et al. Role of hospital surfaces in the transmission of emerging health care–associated pathogens: norovirus, *Clostridium difficile*, and *Acinetobacter* species. *Am J Infect Control* 2010;**38**:S25–33.
705. Wener KM, Schechner V, Gold HS, et al. Treatment with fluoroquinolones or with beta-lactam-beta-lactamase inhibitor combinations is a risk factor for isolation of extended-spectrum-beta-lactamase-producing *Klebsiella* species in hospitalized patients. *Antimicrob Agents Chemother* 2010;**54**:2010–16.
714. Wirtschafter DD, Powers RJ, Pettit JS, et al. Nosocomial infection reduction in VLBW infants with a statewide quality-improvement model. *Pediatrics* 2011;**127**:419–26.
716. World Health Organization. *Interim Infection Control Recommendations for Care of Patients with Suspected or Confirmed Filovirus (Ebola, Marburg) Haemorrhagic Fever*. 2008. http://www.who.int/csr/bioriskreduction/filovirus_infection_control/en/index.html
720. Yahav D, Rozen-Zvi B, Gafter-Gvili A, et al. Antimicrobial lock solutions for the prevention of infections associated with intravascular catheters in patients undergoing hemodialysis: systematic review and meta-analysis of randomized, controlled trials. *Clin Infect Dis* 2008;**47**:83–93.
723. Zhou J, Garber E, Saiman L. Survey of infection control policies for patients with cystic fibrosis in the United States. *Am J Infect Control* 2008;**36**:220–2.
724. Zilberberg MD, Shorr AF, Kollef MH. Increase in *Clostridium difficile*-related hospitalizations among infants in the United States, 2000-2005. *Pediatr Inf Dis J* 2008;**27**:1111–13.
726. Zoltanski J, Dul M, O'Riordan MA, et al. Low frequency of endemic patient-to-patient transmission of antibiotic-resistant gram-negative bacilli in a pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:915–17.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abbasi S, Shenep JL, Hughes WT, et al. Aspergillosis in children with cancer: A 34-year experience. *Clin Infect Dis* 1999;**29**: 1210–19.
2. Abdel-Gawad TA, El-Hodhod MA, Ibrahim HM, et al. Gastro-esophageal reflux in mechanically ventilated pediatric patients and its relation to ventilator-associated pneumonia. *Crit Care* 2009;**13**: R164.
3. Abdolahi A, Fisher SG, Aquino C, et al. Nosocomial infections in a pediatric residential care facility. *Am J Infect Control* 2012;**40**: 502–6.
4. Adam B, Baillie GS, Douglas LJ. Mixed species biofilms of *Candida albicans* and *Staphylococcus epidermidis*. *J Med Microbiol* 2002;**51**:344–9.
5. Adams G, Stover BH, Keenlyside RA, et al. Nosocomial herpetic infections in a pediatric intensive care unit. *Am J Epidemiol* 1981; **113**:126–32.
6. Adler SP. Hospital transmission of cytomegalovirus. *Infect Agents Dis* 1992;**1**:43–9.
7. Agah R, Cherry JD, Garakian AJ, et al. Respiratory syncytial virus infection rate in personnel caring for children with RSV infections: routine isolation procedure vs routine procedure supplemented by use of masks and goggles. *Am J Dis Child* 1987;**141**: 695–7.
8. Aggett PJ, Cooper LV, Ellis SH, et al. Percutaneous absorption of chlorhexidine in neonatal cord care. *Arch Dis Child* 1981;**56**: 878–80.
9. Agwu AL, Lee CK, Jain SK, et al. A World Wide Web–based antimicrobial stewardship program improves efficiency, communication, and user satisfaction and reduces cost in a tertiary care pediatric medical center. *Clin Infect Dis* 2008;**47**:747–53.
10. Al Akhrass F, Hachem R, Mohamed JA, et al. Central venous catheter–associated *Nocardia* bacteremia in cancer patients. *Emerg Infect Dis* 2011;**17**:1651–8.
11. Alexander JW, Solomkin JS, Edwards MJ. Updated recommendations for control of surgical site infections. *Ann Surg* 2011;**253**: 1082–93.
12. Allpress AL, Rosenthal GL, Goodrich KM, et al. Risk factors for surgical site infections after pediatric cardiovascular surgery. *Pediatr Infect Dis J* 2004;**23**:231–4.
13. Almuneef M, Memish ZA, Balkhy HH, et al. Ventilator-associated pneumonia in a pediatric intensive care unit in Saudi Arabia: a 30-month prospective surveillance. *Infect Control Hosp Epidemiol* 2004;**25**:753–8.
14. Altman CA, Englund JA, Demmler G, et al. Respiratory syncytial virus in patients with congenital heart disease: a contemporary look at epidemiology and success of preoperative screening. *Pediatr Cardiol* 2000;**21**:433–8.
15. Aly H, Badawy M, El-Kholy A, et al. Randomized, controlled trial on tracheal colonization of ventilated infants: can gravity prevent ventilator-associated pneumonia? *Pediatrics* 2008;**122**:770–4.
16. American Academy of Pediatrics. Measles. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 489–99.
17. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 361–9.
18. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 553–66.
19. American Academy of Pediatrics. Varicella-zoster infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 774–89.
20. American Academy of Pediatrics, American College of Obstetricians and Gynecologists. *Guidelines for perinatal care*. Elk Grove Village, Ill.: American Academy of Pediatrics; 1997.
21. American Institute of Architects, Academy of Architecture for Health. *Guidelines for design and construction of hospital and health care facilities*. Washington, D.C.: American Institute of Architects Press; 1996.
22. American Thoracic Society, Infectious Diseases Society of America. Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. *Am J Respir Crit Care Med* 2005;**171**:388–416.
23. Ammerlaan HS, Kluytmans JA, Wertheim HF, et al. Eradication of methicillin-resistant *Staphylococcus aureus* carriage: a systematic review. *Clin Infect Dis* 2009;**48**:922–30.
24. Anderson DJ. Surgical site infections. *Infect Dis Clin North Am* 2011;**25**:135–53.
25. Anderson EJ, Rupp A, Shulman ST, et al. Impact of rotavirus vaccination on hospital-acquired rotavirus gastroenteritis in children. *Pediatrics* 2011;**127**:e264–70.
26. Anderson JD, Bonner M, Scheifele DW, et al. Lack of nosocomial spread of varicella in a pediatric hospital with negative pressure ventilated patient rooms. *Infect Control* 1985;**6**:120–1.
27. Anderson JR, Smith MD, Kibbler CC, et al. A nosocomial outbreak due to non-encapsulated *Haemophilus influenzae:* analysis of plasmids coding for antibiotic resistance. *J Hosp Infect* 1994;**27**: 17–27.
28. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. *J Infect Dis* 1985;**152**:257–65.
29. Ansari SA, Sattar SA, Springthorpe VS, et al. Rotavirus survival on human hands and transfer of infectious virus to animate and nonporous inanimate surfaces. *J Clin Microbiol* 1988;**26**:1513–18.
30. Arendrup MC, Fisher BT, Zaoutis TE. Invasive fungal infections in the paediatric and neonatal population: diagnostics and management issues. *Clin Microbiol Infect* 2009;**15**:613–24.
31. Arnell K, Cesarini K, Lagerqvist-Widh A, et al. Cerebrospinal fluid shunt infections in children over a 13-year period: anaerobic cultures and comparison of clinical signs of infection with *Propionibacterium acnes* and with other bacteria. *J Neurosurg Pediatr* 2008;**1**:366–72.
32. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA* 2001;**285**:1059–70.
33. Atkinson JB, Chamberlin K, Boody BA. A prospective randomized trial of urokinase as an adjuvant in the treatment of proven Hickman catheter sepsis. *J Pediatr Surg* 1998;**33**:714–16.
34. Austin BJ, Croxson MC, Powell KF, et al. The successful containment of coxsackie B4 infection in a neonatal unit. *J Paediatr Child Health* 1999;**35**:102–4.
35. Avila-Figueroa C, Goldmann DA, Richardson DK, et al. Intravenous lipid emulsions are the major determinant of coagulase-negative staphylococcal bacteremia in very low birth weight newborns. *Pediatr Infect Dis J* 1998;**17**:10–17.
36. Ayliffe GA. Role of the environment of the operating suite in surgical wound infection. *Rev Infect Dis* 1991;**13**:S800–4.
37. Azimi PH, Roberto RR, Guralnik J, et al. Transfusion-acquired hepatitis A in a premature infant with secondary nosocomial spread in an intensive care nursery. *Am J Dis Child* 1986;**140**: 23–7.
38. Aznar J, Safi H, Romero J, et al. Nosocomial transmission of tuberculosis infection in pediatrics wards. *Pediatr Infect Dis J* 1995;**14**:44–8.
39. Bachrach S. An outbreak of *Haemophilus influenzae* type b bacteraemia in an intermediate care hospital for children. *J Hosp Infect* 1988;**11**:121–6.
40. Bagdasarian N, Heung M, Malani PN. Infectious complications of dialysis access devices. *Infect Dis Clin North Am* 2012;**26**: 127–41.
41. Bailly JL, Beguet A, Chambon M, et al. Nosocomial transmission of echovirus 30: molecular evidence by phylogenetic analysis of the VP1 encoding sequence. *J Clin Microbiol* 2000;**38**:2889–92.
42. Baker CJ, Melish ME, Hall RT, et al. Intravenous immune globulin for the prevention of nosocomial infection in low-birth-weight neonates. *N Engl J Med* 1992;**327**:213–19.
43. Baron RC, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull WHO* 1983;**61**:997–1003.
44. Barret JP, Jeschke MG, Herndon DN. Selective decontamination of the digestive tract in severely burned pediatric patients. *Burns* 2001;**27**:439–45.
45. Barton LL, Granoff DM, Barenkamp SJ. Nosocomial spread of *Haemophilus influenzae* type b infection documented by outer membrane protein subtype analysis. *J Pediatr* 1983;**102**:820–4.

46. Beatson SH. Pharaoh's ants as pathogen vectors in hospitals. *Lancet* 1972;**1**:425–7.
47. Beck-Sague C, Jarvis WR, Martone WJ. Outbreak investigations. *Infect Control Hosp Epidemiol* 1997;**18**:138–45.
48. Beers LM, Burke TL, Martin DB. Shigellosis occurring in newborn nursery staff. *Infect Control Hosp Epidemiol* 1989;**10**: 147–9.
49. Bell LM, Naides SJ, Stoffman P, et al. Human parvovirus B19 infection among hospital staff members after contact with infected patients. *N Engl J Med* 1989;**321**:485–91.
50. Ben-Ami E, Levy I, Katz J, et al. Risk factors for sternal wound infection in children undergoing cardiac surgery: a case-control study. *J Hosp Infect* 2008;**70**:335–40.
51. Ben-Meir D, Golan S, Ehrlich Y, et al. Characteristics and clinical significance of bacterial colonization of ureteral double-J stents in children. *J Pediatr Urol* 2009;**5**:355–8.
52. Bennett SN, McNeil MM, Bland LA, et al. Postoperative infections traced to contamination of an intravenous anesthetic, propofol. *N Engl J Med* 1995;**333**:147–54.
53. Berenholtz SM, Pronovost PJ, Lipsett PA, et al. Eliminating catheter-related bloodstream infections in the intensive care unit. *Crit Care Med* 2004;**32**:2014–20.
54. Berman SA, Balkany TJ, Simmons MA. Otitis media in the neonatal intensive care unit. *Pediatrics* 1978;**62**:198–201.
55. Bernstein HH, Starke JR. Policy statement—recommendation for mandatory influenza immunization of all health care personnel. *Pediatrics* 2010;**126**:809–15.
56. Berra L, Coppadoro A, Bittner EA, et al. A clinical assessment of the Mucus Shaver: a device to keep the endotracheal tube free from secretions. *Crit Care Med* 2012;**40**:119–24.
57. Bertin ML, Vinski J, Schmitt S, et al. Outbreak of methicillin-resistant *Staphylococcus aureus* colonization and infection in a neonatal intensive care unit epidemiologically linked to a healthcare worker with chronic otitis. *Infect Control Hosp Epidemiol* 2006;**27**: 581–5.
58. Berton DC, Kalil AC, Teixeira PJ. Quantitative versus qualitative cultures of respiratory secretions for clinical outcomes in patients with ventilator-associated pneumonia. *Cochrane Database Syst Rev* 2012;**1**:CD006482.
59. Bhalla A, Pultz NJ, Gries DM, et al. Acquisition of nosocomial pathogens on hands after contact with environmental surfaces near hospitalized patients. *Infect Control Hosp Epidemiol* 2004;**25**: 164–7.
60. Bhattacharyya N, Kosloske AM. Postoperative wound infection in pediatric surgical patients: a study of 676 infants and children. *J Pediatr Surg* 1990;**25**:125–9.
61. Bhattacharyya N, Kosloske AM, Macarthur C. Nosocomial infection in pediatric surgical patients: a study of 608 infants and children. *J Pediatr Surg* 1993;**28**:338–43.
62. Bhutta A, Gilliam C, Honeycutt M, et al. Reduction of bloodstream infections associated with catheters in paediatric intensive care unit: stepwise approach. *BMJ* 2007;**334**:362–5.
63. Bi XC, Zhang B, Ye YK, et al. Pathogen incidence and antibiotic resistance patterns of catheter-associated urinary tract infection in children. *J Chemother* 2009;**21**:661–5.
64. Biering G, Karlsson S, Clark NC, et al. Three cases of neonatal meningitis caused by *Enterobacter sakazakii* in powdered milk. *J Clin Microbiol* 1989;**27**:2054–6.
65. Bigham MT, Amato R, Bondurrant P, et al. Ventilator-associated pneumonia in the pediatric intensive care unit: characterizing the problem and implementing a sustainable solution. *J Pediatr* 2009;**154**:582–7, e582.
66. Birenbaum E, Handsher R, Kuint J, et al. Echovirus type 22 outbreak associated with gastro-intestinal disease in a neonatal intensive care unit. *Am J Perinatol* 1997;**14**:469–73.
67. Bitsori M, Maraki S, Koukouraki S, et al. *Pseudomonas aeruginosa* urinary tract infection in children: risk factors and outcomes. *J Urol* 2012;**187**:260–4.
68. Blatt ML. Cross-infection: its prevention in a children's hospital. *Illinois Med J* 1936;**70**:483–7.
69. Bolyard EA, Tablan OC, Williams WW, et al. Guideline for infection control in healthcare personnel, 1998. *Infect Control Hosp Epidemiol* 1998;**19**:407–63.
70. Bonten MJ, Hayden MK, Nathan C, et al. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. *Lancet* 1996;**348**:1615–19.
71. Borio L, Inglesby T, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons: medical and public health management. *JAMA* 2002;**287**:2391–405.
72. Botelho-Nevers E, Gautret P, Biellik R, et al. Nosocomial transmission of measles: an updated review. *Vaccine* 2012;**30**:3996–4001.
73. Boucher HW, Talbot GH, Bradley JS, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**:1–12.
74. Bowater RJ, Stirling SA, Lilford RJ. Is antibiotic prophylaxis in surgery a generally effective intervention? Testing a generic hypothesis over a set of meta-analyses. *Ann Surg* 2009;**249**: 551–6.
75. Boyce JM, Mermel LA, Zervos MJ, et al. Controlling vancomycin-resistant enterococci. *Infect Control Hosp Epidemiol* 1995;**16**: 634–7.
76. Boyce JM, Opal SM, Chow JW, et al. Outbreak of multidrug-resistant *Enterococcus faecium* with transferable vanB class vancomycin resistance. *J Clin Microbiol* 1994;**32**:1148–53.
77. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings: recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. *MMWR Recomm Rep* 2002;**51**: 1–45.
78. Boyce TG, Mellen BG, Mitchel EF Jr, et al. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. *J Pediatr* 2000;**137**:865–70.
79. Brady MT, Pacini DL, Budde CT, et al. Diagnostic studies of nosocomial diarrhea in children: assessing their use and value. *Am J Infect Control* 1989;**17**:77–82.
80. Branchini ML, Pfaller MA, Rhine-Chalberg J, et al. Genotypic variation and slime production among blood and catheter isolates of *Candida parapsilosis*. *J Clin Microbiol* 1994;**32**:452–6.
81. Bratzler DW, Hunt DR. The surgical infection prevention and surgical care improvement projects: national initiatives to improve outcomes for patients having surgery. *Clin Infect Dis* 2006;**43**: 322–30.
82. Braun BI, Kusek L, Larson E. Measuring adherence to hand hygiene guidelines: a field survey for examples of effective practices. *Am J Infect Control* 2009;**37**:282–8.
83. Brennemann J. The infant ward. *Am J Dis Child* 1932;**43**: 577–84.
84. Brocklehurst P, Farrell B, King A, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011;**365**: 1201–11.
85. Brodie SB, Sands KE, Gray JE, et al. Occurrence of nosocomial bloodstream infections in six neonatal intensive care units. *Pediatr Infect Dis J* 2000;**19**:56–65.
86. Bruijning-Verhagen P, Quach C, Bonten M. Nosocomial rotavirus infections: a meta-analysis. *Pediatrics* 2012;**129**:e1011–19.
87. Bruminhent J, Keegan M, Lakhani A, et al. Effectiveness of a simple intervention for prevention of catheter-associated urinary tract infections in a community teaching hospital. *Am J Infect Control* 2010;**38**:689–93.
88. Brun-Buisson C, Fartoukh M, Lechapt E, et al. Contribution of blinded, protected quantitative specimens to the diagnostic and therapeutic management of ventilator-associated pneumonia. *Chest* 2005;**128**:533–44.
89. Brunetto AL, Pearson AD, Craft AW, et al. *Clostridium difficile* in an oncology unit. *Arch Dis Child* 1988;**63**:979–81.
90. Bucher BT, Guth RM, Elward AM, et al. Risk factors and outcomes of surgical site infection in children. *J Am Coll Surg* 2011;**212**:1033–8, e1031.
91. Burke JP, Zavasky DM. Nosocomial urinary tract infections. In: *Mayhall CG: Hospital epidemiology and infection control*. Philadelphia: Lippincott Williams & Wilkins; 1999. p. 173–87.
92. Burton DC, Edwards JR, Horan TC, et al. Methicillin-resistant *Staphylococcus aureus* central line–associated bloodstream infections in US intensive care units, 1997-2007. *JAMA* 2009;**301**:727–36.
93. Burwell LA, Kaufman D, Blakely J, et al. Antifungal prophylaxis to prevent neonatal candidiasis: a survey of perinatal physician practices. *Pediatrics* 2006;**118**:e1019–26.
94. Butzler JP, Goossens H. *Campylobacter jejun*i infection as a hospital problem: an overview. *J Hosp Infect* 1988;**11**:374–7.
95. Cairo MS, Agosti J, Ellis R, et al. A randomized, double-blind, placebo-controlled trial of prophylactic recombinant human

granulocyte-macrophage colony-stimulating factor to reduce nosocomial infections in very low birth weight neonates. *J Pediatr* 1999;**134**:64–70.
96. Calfee DP, Salgado CD, Classen D, et al. Strategies to prevent transmission of methicillin-resistant *Staphylococcus aureus* in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**(Suppl. 1): S62–80.
97. Call EL. An epidemic of pemphigus neonatorum. *Am J Obstet* 1904;**50**:473.
98. Camins BC, Richmond AM, Dyer KL, et al. A crossover intervention trial evaluating the efficacy of a chlorhexidine-impregnated sponge in reducing catheter-related bloodstream infections among patients undergoing hemodialysis. *Infect Control Hosp Epidemiol* 2010;**31**:1118–23.
99. Cannon CG, Linnemann C Jr. *Yersinia enterocolitica* infections in hospitalized patients: the problem of hospital-acquired infections. *Infect Control Hosp Epidemiol* 1992;**13**:139–43.
100. Cardo D, Dennehy PH, Halverson P, et al. Moving toward elimination of healthcare-associated infections: a call to action. *Am J Infect Control* 2010;**38**:671–5.
101. Carlson AL, Budd AP, Perl TM. Control of influenza in healthcare settings: early lessons from the 2009 pandemic. *Curr Opin Infect Dis* 2010;**23**:293–9.
102. Carr R, Modi N, Dore CJ, et al. A randomized, controlled trial of prophylactic granulocyte-macrophage colony-stimulating factor in human newborns less than 32 weeks gestation. *Pediatrics* 1999;**103**:796–802.
103. Cartwright CP, Stock F, Beekmann SE, et al. PCR amplification of rRNA intergenic spacer regions as a method for epidemiologic typing of *Clostridium difficile*. *J Clin Microbiol* 1995;**33**:184–7.
104. Centers for Disease Control. Laboratory-acquired meningococcemia—California and Massachusetts. *MMWR Morb Mortal Wkly Rep* 1991;**40**:46–7, 55.
105. Centers for Disease Control and Prevention. Update: human plague—India, 1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**: 722–3.
106. Centers for Disease Control and Prevention. Update: management of patients with suspected viral hemorrhagic fever—United States. *MMWR Morb Mortal Wkly Rep* 1995;**44**:475–9.
107. Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease: a public health perspective. *MMWR Morb Mortal Wkly Rep* 1996;**45**(No. RR-7):1–31.
108. Centers for Disease Control and Prevention. Red blood cell transfusions contaminated with *Yersinia enterocolitica*—United States, 1991-1996, and initiation of a national study to detect bacteria-associated transfusion reactions. *MMWR Morb Mortal Wkly Rep* 1997;**46**:553–5.
109. Centers for Disease Control and Prevention. Updated U.S. Public Health Service guidelines for the management of occupational exposures to HBV, HCV, and HIV and recommendations for postexposure prophylaxis. *MMWR Recomm Rep* 2001;**50**:1–52.
110. Centers for Disease Control and Prevention. *Enterobacter sakazakii* infections associated with the use of powdered infant formula—Tennessee, 2001. *MMWR Morb Mortal Wkly Rep* 2002;**51**:297–300.
111. Centers for Disease Control and Prevention. Laboratory-acquired meningococcal disease—United States, 2000. *MMWR Morb Mortal Wkly Rep* 2002;**51**:141–4.
112. Centers for Disease Control and Prevention. Severe *Clostridium difficile*–associated disease in populations previously at low risk—four states, 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**: 1201–5.
113. Centers for Disease Control and Prevention. Community-associated methicillin-resistant *Staphylococcus aureus* infection among healthy newborns—Chicago and Los Angeles County, 2004. *MMWR Morb Mortal Wkly Rep* 2006;**55**:329–32.
114. Centers for Disease Control and Prevention. Acute hepatitis C virus infections attributed to unsafe injection practices at an endoscopy clinic—Nevada, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:513–17.
115. Centers for Disease Control and Prevention. Hospital-acquired pertussis among newborns—Texas, 2004. *MMWR Morb Mortal Wkly Rep* 2008;**57**:600–3.
116. Centers for Disease Control and Prevention. Outbreak of measles—San Diego, California, January-February 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:203–6.
117. Centers for Disease Control and Prevention. Immunization of health-care personnel: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**:1–45.
118. Centers for Disease Control and Prevention. Vital signs: central line–associated blood stream infections—United States, 2001, 2008, and 2009. *MMWR Morb Mortal Wkly Rep* 2011;**60**:243–8.
119. Centers for Disease Control and Prevention. Updated CDC recommendations for the management of hepatitis B virus-infected health-care providers and students. *MMWR Recomm Rep* 2012;**61**: 1–12.
120. Centers for Disease Control and Prevention. Vital signs: preventing *Clostridium difficile* infections. *MMWR Morb Mortal Wkly Rep* 2012;**61**:157–62.
121. Centers for Disease Control and Prevention. Influenza vaccination coverage among health-care personnel—2011-12 influenza season, United States. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 753–7.
122. Chadee DD, Le Maitre A. Ants: potential mechanical vectors of hospital infections in Trinidad. *Trans R Soc Trop Med Hyg* 1990; **84**:297.
123. Chadha V, Schaefer FS, Warady BA. Dialysis-associated peritonitis in children. *Pediatr Nephrol* 2010;**25**:425–40.
124. Chaiyakunapruk N, Veenstra DL, Lipsky BA, et al. Chlorhexidine compared with povidone-iodine solution for vascular catheter-site care: a meta-analysis. *Ann Intern Med* 2002;**136**:792–801.
125. Chambon M, Bailly JL, Beguet A, et al. An outbreak due to echovirus type 30 in a neonatal unit in France in 1997: usefulness of PCR diagnosis. *J Hosp Infect* 1999;**43**:63–8.
126. Chelliah A, Heydon KH, Zaoutis TE, et al. Observational trial of antibiotic-coated central venous catheters in critically ill pediatric patients. *Pediatr Infect Dis J* 2007;**26**:816–20.
127. Chen SY, Anderson S, Kutty PK, et al. Health care–associated measles outbreak in the United States after an importation: challenges and economic impact. *J Infect Dis* 2011;**203**:1517–25.
128. Cheng K, Smyth RL, Govan JR, et al. Spread of beta-lactam-resistant *Pseudomonas aeruginosa* in a cystic fibrosis clinic. *Lancet* 1996;**348**:639–42.
129. Cherry JD. Nosocomial pertussis in the nineties. *Infect Control Hospital Epidemiol* 1995;**16**:553–5.
130. Chidekel AS, Rosen CL, Bazzy AR. Rhinovirus infection associated with serious lower respiratory illness in patients with bronchopulmonary dysplasia. *Pediatr Infect Dis J* 1997;**16**:43–7.
131. Chiu CH, Michelow IC, Cronin J, et al. Effectiveness of a guideline to reduce vancomycin use in the neonatal intensive care unit. *Pediatr Infect Dis J* 2011;**30**:273–8.
132. Christie CDC, Glover AM, Willke MJ, et al. Containment of pertussis in the regional pediatric hospital during the Greater Cincinnati epidemic of 1993. *Infect Control Hospital Epidemiol* 1995;**16**:556–63.
133. Cimolai N, Cogswell A, Hunter R. Nosocomial transmission of penicillin-resistant *Streptococcus pneumoniae*. *Pediatr Pulmonol* 1999;**27**:432–4.
134. Clark NC, Hill BC, O'Hara CM, et al. Epidemiologic typing of *Enterobacter sakazakii* in two neonatal nosocomial outbreaks. *Diagn Microbiol Infect Dis* 1990;**13**:467–72.
135. Clark-Christoff N, Watters VA, Sparks W, et al. Use of triple-lumen subclavian catheters for administration of total parenteral nutrition. *JPEN J Parenter Enter Nutr* 1992;**16**:403–7.
136. Classen DC, Evans RS, Pestotnik SL, et al. The timing of prophylactic administration of antibiotics and the risk of surgical-wound infection. *N Engl J Med* 1992;**326**:281–6.
137. Cleary TG, Guerrant RL, Pickering LK. Microorganisms responsible for neonatal diarrhea. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus & newborn infant*. Philadelphia: WB Saunders; 2001. p. 1249–326.
138. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*. Wayne, Pa: Clinical and Laboratory Standards Institute; 2010.
139. Coffin SE, Klompas M, Classen D, et al. Strategies to prevent ventilator-associated pneumonia in acute care hospitals. *Infect Control Hosp Epidemiol* 2008;**29**(Suppl. 1):S31–40.
140. Coffin SE, Zaoutis TE. Healthcare-associated infections in the nursery. In: Remington JS, Klein JO, Wilson CB, et al, editors. *Infectious diseases of the fetus and newborn infant*. St. Louis: Elsevier; 2011. p. 1126–43.

141. Cohen MS, Steere AC, Baltimore R, et al. Possible nosocomial transmission of group Y *Neisseria meningitidis* among oncology patients. *Ann Intern Med* 1979;**91**:7–12.
142. Committee on the Fetus and Newborn, American Academy of Pediatrics, Canadian Paediatric Society. Postnatal corticosteroids to treat or prevent chronic lung disease in preterm infants. *Pediatrics* 2002;**109**:330–8.
143. Cone R, Mohan K, Thouless M, et al. Nosocomial transmission of rotavirus infection. *Pediatr Infect Dis J* 1988;**7**:103–9.
144. Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. *Clin Infect Dis* 2003;**36**: 1433–7.
145. Cosgrove SE, Qi Y, Kaye KS, et al. The impact of methicillin resistance in *Staphylococcus aureus* bacteremia on patient outcomes: mortality, length of stay, and hospital charges. *Infect Control Hosp Epidemiol* 2005;**26**:166–74.
146. Cowen J, Ellis SH, McAinsh J. Absorption of chlorhexidine from the intact skin of newborn infants. *Arch Dis Child* 1979;**54**: 379–83.
147. Cox SG, Rode H, Darani AN, et al. Thermal injury within the first 4 months of life. *Burns* 2011;**37**:828–34.
148. Craven D, Brick D, Morrisey A, et al. Low yield of bacterial stool culture in children with nosocomial diarrhea. *Pediatr Infect Dis J* 1998;**17**:1040–4.
149. Craven DE, Lichtenberg DA, Goularte TA, et al. Contaminated medication nebulizers in mechanical ventilator circuits: source of bacterial aerosols. *Am J Med* 1984;**77**:834–8.
150. Crnich CJ, Maki DG. The promise of novel technology for the prevention of intravascular device-related bloodstream infection, part I: pathogenesis and short-term devices. *Clin Infect Dis* 2002;**34**:1232–42.
151. Crnich CJ, Maki DG. The promise of novel technology for the prevention of intravascular device-related bloodstream infection, part II: long-term devices. *Clin Infect Dis* 2002;**34**:1362–8.
152. Cronin WA, Germanson TP, Donowitz LG. Intravascular catheter colonization and related bloodstream infection in critically ill neonates. *Infect Control Hosp Epidemiol* 1990;**11**:301–8.
153. Cruz AT, Medina D, Whaley EM, et al. Tuberculosis among families of children with suspected tuberculosis and employees at a children's hospital. *Infect Control Hosp Epidemiol* 2011;**32**:188–90.
154. Culver DH, Horan TC, Gaynes RP, et al. Surgical wound infection rates by wound class, operative procedure, and patient risk index. *Am J Med* 1991;**91**:S152–7.
155. Cunliffe NA, Booth JA, Elliot C, et al. Healthcare-associated viral gastroenteritis among children in a large pediatric hospital, United Kingdom. *Emerg Infect Dis* 2010;**16**:55–62.
156. Cunney RJ, Bialachowski A, Thornley D, et al. An outbreak of influenza A in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2000;**21**:449–54.
157. Curley MA, Schwalenstocker E, Deshpande JK, et al. Tailoring the Institute for Health Care Improvement 100,000 Lives Campaign to pediatric settings: the example of ventilator-associated pneumonia. *Pediatr Clin North Am* 2006;**53**:1231–51.
158. Curtis AB, Ridzon R, Vogel R, et al. Extensive transmission of *Mycobacterium tuberculosis* from a child. *N Engl J Med* 1999;**341**: 1491–5.
159. Dankner WM, Spector SA, Fierer J, et al. *Malassezia* fungemia in neonates and adults: complication of hyperalimentation. *Rev Infect Dis* 1987;**9**:743–53.
160. Darmstadt GL, Saha SK, Ahmed AS, et al. Effect of topical treatment with skin barrier–enhancing emollients on nosocomial infections in preterm infants in Bangladesh: a randomised controlled trial. *Lancet* 2005;**365**:1039–45.
161. Darmstadt GL, Saha SK, Ahmed AS, et al. Effect of topical emollient treatment of preterm neonates in Bangladesh on invasion of pathogens into the bloodstream. *Pediatr Res* 2007;**61**:588–93.
162. Darmstadt GL, Saha SK, Ahmed AS, et al. Effect of skin barrier therapy on neonatal mortality rates in preterm infants in Bangladesh: a randomized, controlled, clinical trial. *Pediatrics* 2008;**121**: 522–9.
163. Darouiche RO, Wall MJ Jr, Itani KM, et al. Chlorhexidine-alcohol versus povidone-iodine for surgical-site antisepsis. *N Engl J Med* 2010;**362**:18–26.
164. Darouiche RO, Raad II, Heard SO, et al. A comparison of two antimicrobial-impregnated central venous catheters. *N Engl J Med* 1999;**340**:1–8.
165. Dato VM, Dajani AS. Candidemia in children with central venous catheters: role of catheter removal and amphotericin B therapy. *Pediatr Infect Dis J* 1990;**9**:309–14.
166. Daum RS, Nachman JP, Leitch CD, et al. Nosocomial epiglottitis associated with penicillin- and cephalosporin-resistant *Streptococcus pneumoniae* bacteremia. *J Clin Microbiol* 1994;**32**:246–8.
167. Davenport M, Doig CM. Wound infection in pediatric surgery: a study in 1,094 neonates. *J Pediatr Surg* 1993;**28**:26–30.
168. Davies HD, Jones EL, Sheng RY, et al. Nosocomial urinary tract infections at a pediatric hospital. *Pediatr Infect Dis J* 1992;**11**: 349–54.
169. Davis SD, Sobocinski K, Hoffman RG, et al. Postoperative wound infections in a children's hospital. *Pediatr Infect Dis J* 1984;**3**: 114–16.
170. de Cicco M, Panarello G, Chiaradia V, et al. Source and route of microbial colonisation of parenteral nutrition catheters. *Lancet* 1989;**2**:1258–61.
171. de Galan BE, van Tilburg PM, Sluijter M, et al. Hospital-related outbreak of infection with multidrug-resistant *Streptococcus pneumoniae* in the Netherlands. *J Hosp Infect* 1999;**42**:185–92.
172. de Jonge E, Schultz MJ, Spanjaard L, et al. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet* 2003;**362**:1011–16.
173. de Mello MJ, de Albuquerque Mde F, Ximenes RA, et al. Factors associated with time to acquisition of bloodstream infection in a pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2010; **31**:249–55.
174. de Smet AM, Kluytmans JA, Cooper BS, et al. Decontamination of the digestive tract and oropharynx in ICU patients. *N Engl J Med* 2009;**360**:20–31.
175. Dellit TH, Owens RC, McGowan JE Jr, et al. Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. *Clin Infect Dis* 2007;**44**: 159–77.
176. Demmler GJ, Yow MD, Spector SA, et al. Nosocomial cytomegalovirus infections within two hospitals caring for infants and children. *J Infect Dis* 1987;**156**:9–16.
177. Dennehy PH, Nelson SM, Spangenberger S, et al. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. *J Infect Dis* 2001;**184**: 10–15.
178. Dennehy PH, Peter G. Risk factors associated with nosocomial rotavirus infection. *Am J Dis Child* 1985;**139**:935–9.
179. Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA* 2001;**285**:2763–73.
180. Desai R, Hembree CD, Handel A, et al. Severe outcomes are associated with genogroup 2 genotype 4 norovirus outbreaks: a systematic literature review. *Clin Infect Dis* 2012;**55**:189–93.
181. Di Pentima MC, Chan S. Impact of antimicrobial stewardship program on vancomycin use in a pediatric teaching hospital. *Pediatr Infect Dis J* 2010;**29**:707–11.
182. Di Pentima MC, Chan S, Eppes SC, et al. Antimicrobial prescription errors in hospitalized children: role of antimicrobial stewardship program in detection and intervention. *Clin Pediatr* 2009; **48**:505–12.
183. Di Pentima MC, Chan S, Hossain J. Benefits of a pediatric antimicrobial stewardship program at a children's hospital. *Pediatrics* 2011;**128**:1062–70.
184. Digiorgio MJ, Fatica C, Oden M, et al. Development of a modified surveillance definition of central line–associated bloodstream infections for patients with hematologic malignancies. *Infect Control Hosp Epidemiol* 2012;**33**:865–8.
185. Ditchburn RK, McQuillin J, Gardner PS, et al. Respiratory syncytial virus in hospital cross-infection. *BMJ* 1971;**3**:671–3.
186. Dixon JM, Carver RL. Daily chlorohexidine gluconate bathing with impregnated cloths results in statistically significant reduction in central line–associated bloodstream infections. *Am J Infect Control* 2010;**38**:817–21.
187. Dizdar EA, Aydemir C, Erdeve O, et al. Respiratory syncytial virus outbreak defined by rapid screening in a neonatal intensive care unit. *J Hosp Infect* 2010;**75**:292–4.
188. Doebbeling BN, Reagan DR, Pfaller MA, et al. Long-term efficacy of intranasal mupirocin ointment: a prospective cohort study

of *Staphylococcus aureus* carriage. *Arch Intern Med* 1994;**154**: 1505–8.
189. Doherty JA, Brookfield DS, Gray J, et al. Cohorting of infants with respiratory syncytial virus. *J Hosp Infect* 1998;**38**:203–6.
190. Doig CM, Wilkinson AW. Wound infection in a children's hospital. *Br J Surg* 1976;**63**:647–50.
191. Donlan RM. Biofilms: microbial life on surfaces. *Emerg Infect Dis* 2002;**8**:881–90.
192. Drusin LM, Sohmer M, Groshen SL, et al. Nosocomial hepatitis A infection in a paediatric intensive care unit. *Arch Dis Child* 1987;**62**:690–5.
193. Ducharme FM, Gauthier M, Lacroix J, et al. Incidence of infection related to arterial catheterization in children: a prospective study. *Crit Care Med* 1988;**16**:272–6.
194. Dudeck MA, Horan TC, Peterson KD, et al. National Healthcare Safety Network (NHSN) Report, data summary for 2010, device-associated module. *Am J Infect Control* 2011;**39**:798–816.
195. DuPont HL. Nosocomial salmonellosis and shigellosis. *Infect Control Hosp Epidemiol* 1991;**12**:707–9.
196. Easmon CS, Hastings MJ, Clare AJ, et al. Nosocomial transmission of group B streptococci. *BMJ Clin Res Ed* 1981;**283**: 459–61.
197. Eckmanns T, Ruden H, Gastmeier P. The influence of high-efficiency particulate air filtration on mortality and fungal infection among highly immunosuppressed patients: a systematic review. *J Infect Dis* 2006;**193**:1408–18.
198. Edwards MS, Baker CJ. Median sternotomy wound infections in children. *Pediatr Infect Dis J* 1983;**2**:105–9.
199. Edwards WH, Conner JM, Soll RF. The effect of prophylactic ointment therapy on nosocomial sepsis rates and skin integrity in infants with birth weights of 501 to 1000 g. *Pediatrics* 2004; **113**:1195–203.
200. Elias J, Claus H, Frosch M, et al. Evidence for indirect nosocomial transmission of Neisseria meningitidis resulting in two cases of invasive meningococcal disease. *J Clin Microbiol* 2006;**44**: 4276–8.
201. Elward AM, Fraser VJ. Risk factors for nosocomial primary bloodstream infection in pediatric intensive care unit patients: a 2-year prospective cohort study. *Infect Control Hosp Epidemiol* 2006;**27**: 553–60.
202. Elward AM, Hollenbeak CS, Warren DK, et al. Attributable cost of nosocomial primary bloodstream infection in pediatric intensive care unit patients. *Pediatrics* 2005;**115**:868–72.
203. Elward AM, Warren DK, Fraser VJ. Ventilator-associated pneumonia in pediatric intensive care unit patients: risk factors and outcomes. *Pediatrics* 2002;**109**:758–64.
204. Engemann JJ, Carmeli Y, Cosgrove SE, et al. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with *Staphylococcus aureus* surgical site infection. *Clin Infect Dis* 2003;**36**:592–8.
205. Esezobor CI, Nourse P, Gajjar P. Urinary tract infection following kidney transplantation: frequency, risk factors and graft function. *Pediatr Nephrol* 2012;**27**:651–7.
206. Esteve F, Pujol M, Perez XL, et al. Bacteremia related with arterial catheter in critically ill patients. *J Infect* 2011;**63**:139–43.
207. Evans JPM, Rossiter MA, Kumaran TO, et al. Human parvovirus aplasia: case due to cross infection in a ward. *BMJ* 1984; **288**:681.
208. Fanaroff AA, Korones SB, Wright LL, et al. A controlled trial of intravenous immune globulin to reduce nosocomial infections in very-low-birth-weight infants. *N Engl J Med* 1994;**330**: 1107–13.
209. Fanella ST, Pinto MA, Bridger NA, et al. Pandemic (H1N1) 2009 influenza in hospitalized children in Manitoba: nosocomial transmission and lessons learned from the first wave. *Infect Control Hosp Epidemiol* 2011;**32**:435–43.
210. Feber J, Spatenka J, Seeman T, et al. Urinary tract infections in pediatric renal transplant recipients—a two center risk factors study. *Pediatr Transplant* 2009;**13**:881–6.
211. Fergusson D, Hebert PC, Lee SK, et al. Clinical outcomes following institution of universal leukoreduction of blood transfusions for premature infants [see comment]. *JAMA* 2003;**289**: 1950–6.
212. Ferroni A, Merckx J, Ancelle T, et al. Nosocomial outbreak of *Clostridium difficile* diarrhea in a pediatric service. *Eur J Clin Microbiol Infect Dis* 1997;**16**:928–33.
213. Finn A, Anday E, Talbot GH. An epidemic of adenovirus 7a infection in a neonatal nursery: course, morbidity, and management. *Infect Control Hosp Epidemiol* 1988;**9**:398–404.
214. Fiore AE, Fry A, Shay D, et al. Antiviral agents for the treatment and chemoprophylaxis against influenza: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2011;**60**(RR01):1–24.
215. Fisher MC, Long SS, McGowan KL, et al. Outbreak of pertussis in a residential facility for handicapped people. *J Pediatr* 1989; **114**:934–9.
216. Flynn PM, Shenep JL, Stokes DC, et al. In situ management of confirmed central venous catheter-related bacteremia. *Pediatr Infect Dis J* 1987;**6**:729–34.
217. Foca M, Jakob K, Whittier S, et al. Endemic *Pseudomonas aeruginosa* infection in a neonatal intensive care unit. *N Engl J Med* 2000;**343**:695–700.
218. Foglia E, Meier MD, Elward A. Ventilator-associated pneumonia in neonatal and pediatric intensive care unit patients. *Clin Microbiol Rev* 2007;**20**:409–25, table of contents.
219. Ford-Jones EL, Mindorff CM, Langley JM, et al. Epidemiologic study of 4684 hospital-acquired infections in pediatric patients. *Pediatr Infect Dis J* 1989;**8**:668–75.
220. Fotedar R, Banerjee U, Singh S, et al. The housefly *(Musca domestica)* as a carrier of pathogenic microorganisms in a hospital environment. *J Hosp Infect* 1992;**20**:209–15.
221. Franzin L, Scolfaro C, Cabodi D, et al. *Legionella pneumophila* pneumonia in a newborn after water birth: a new mode of transmission. *Clin Infect Dis* 2001;**33**:e103–4.
222. Fraser TG, Gordon SM. CLABSI rates in immunocompromised patients: a valuable patient centered outcome? *Clin Infect Dis* 2011;**52**:1446–50.
223. Freeman J, Goldmann DA, Smith NE, et al. Association of intravenous lipid emulsion and coagulase-negative staphylococcal bacteremia in neonatal intensive care units. *N Engl J Med* 1990; **323**:301–8.
224. Fridkin SK. The changing face of fungal infections in health care settings. *Clin Infect Dis* 2005;**41**:1455–60.
225. Fridkin SK, Pear SM, Williamson TH, et al. The role of understaffing in central venous catheter-associated bloodstream infections. *Infect Control Hosp Epidemiol* 1996;**17**:150–8.
226. Friedland IR, Klugman KP. Antibiotic-resistant pneumococcal disease in South African children. *Am J Dis Child* 1992;**146**: 920–3.
227. Friedman C, Barnette M, Buck AS, et al. Requirements for infrastructure and essential activities of infection control and epidemiology in out-of-hospital settings: a consensus panel report. Association for Professionals in Infection Control and Epidemiology and Society for Healthcare Epidemiology of America. *Infect Control Hosp Epidemiol* 1999;**20**:695–705.
228. Friedman CA, Temple DM, Robbins KK, et al. Outbreak and control of varicella in a neonatal intensive care unit. *Pediatr Infect Dis J* 1994;**13**:152–4.
229. Frölich T. Infections nosocomiales dans les crèches et hôpitaux pour enfants. *Acta Pediatr* 1935;**17**:18–23.
230. Furfaro S, Gauthier M, Lacroix J, et al. Arterial catheter-related infections in children. A 1-year cohort analysis. *Am J Dis Child* 1991;**145**:1037–43.
231. Gagneur A, Vallet S, Talbot PJ, et al. Outbreaks of human coronavirus in a pediatric and neonatal intensive care unit. *Eur J Pediatr* 2008;**167**:1427–34.
232. Gala CL, Hall CB, Schnabel KC, et al. The use of eye-nose goggles to control nosocomial respiratory syncytial virus infection. *JAMA* 1986;**256**:2706–8.
233. Gallimore CI, Taylor C, Gennery AR, et al. Contamination of the hospital environment with gastroenteric viruses: comparison of two pediatric wards over a winter season. *J Clin Microbiol* 2008;**46**:3112–15.
234. Gardner P, Carles DG. Infections acquired in a pediatric hospital. *J Pediatr* 1972;**81**:1205–10.
235. Garland JS, Alex CP, Henrickson KJ, et al. A vancomycin-heparin lock solution for prevention of nosocomial bloodstream infection in critically ill neonates with peripherally inserted central venous catheters: a prospective, randomized trial. *Pediatrics* 2005;**116**: e198–205.
236. Garland JS, Alex CP, Mueller CD, et al. A randomized trial comparing povidone-iodine to a chlorhexidine gluconate–impregnated

dressing for prevention of central venous catheter infections in neonates. *Pediatrics* 2001;**107**:1431–6.
237. Garland JS, Buck RK, Maloney P, et al. Comparison of 10% povidone-iodine and 0.5% chlorhexidine gluconate for the prevention of peripheral intravenous catheter colonization in neonates: a prospective trial. *Pediatr Infect Dis J* 1995;**14**:510–16.
238. Garland JS, Dunne WM Jr, Havens P, et al. Peripheral intravenous catheter complications in critically ill children: a prospective study. *Pediatrics* 1992;**89**:1145–50.
239. Garland JS, Nelson DB, Cheah T, et al. Infectious complications during peripheral intravenous therapy with Teflon catheters: a prospective study. *Pediatr Infect Dis J* 1987;**6**:918–21.
240. Garner JS, Jarvis WR, Emori TG, et al. CDC definitions for nosocomial infections, 1988. *Am J Infect Control* 1988;**16**: 128–40.
241. Gastmeier P, Weigt O, Sohr D, et al. Comparison of hospital-acquired infection rates in paediatric burn patients. *J Hosp Infect* 2002;**52**:161–5.
242. Gaynes RP, Edwards JR, Jarvis WR, et al. Nosocomial infections among neonates in high-risk nurseries in the United States. *Pediatrics* 1996;**98**:357–61.
243. George RH, Gully PR, Gill ON, et al. An outbreak of tuberculosis in a children's hospital. *J Hosp Infect* 1986;**8**:129–42.
244. Gerards LJ, Hennekam RC, von Dijk WC, et al. An outbreak of gastroenteritis due to *Escherichia coli* 0142:H6 in a neonatal department. *J Hosp Infect* 1984;**5**:283–8.
245. Gerber JS, Newland JG, Coffin SE, et al. Variability in antibiotic use at children's hospitals. *Pediatrics* 2010;**126**:1067–73.
246. Gerber SI, Erdman DD, Pur SL, et al. Outbreak of adenovirus genome type 7d2 infection in a pediatric chronic-care facility and tertiary-care hospital. *Clin Infect Dis* 2001;**32**:694–700.
247. Gerber SI, Jones RC, Scott MV, et al. Management of outbreaks of methicillin-resistant *Staphylococcus aureus* infection in the neonatal intensive care unit: a consensus statement. *Infect Control Hosp Epidemiol* 2006;**27**:139–45.
248. Gerding DN, Muto CA, Owens RC Jr. Treatment of *Clostridium difficile* infection. *Clin Infect Dis* 2008;**46**(Suppl. 1):S32–42.
249. Gewolb IH, Schwalbe RS, Taciak VL, et al. Stool microflora in extremely low birthweight infants. *Arch Dis Child Fetal Neonatal Ed* 1999;**80**:F167–73.
250. Gianino P, Mastretta E, Longo P, et al. Incidence of nosocomial rotavirus infections, symptomatic and asymptomatic, in breast-fed and non-breast-fed infants. *J Hosp Infect* 2002;**50**: 13–17.
251. Golden SE, Shehab ZM, Bjelland JC, et al. Microbiology of endotracheal aspirates in intubated pediatric intensive care unit patients: correlations with radiographic findings. *Pediatr Infect Dis J* 1987;**6**:665–9.
252. Goldmann DA. Bacterial colonization and infection in the neonate. *Am J Med* 1981;**70**:417–22.
253. Goldmann DA. Strategies for preventing neonatal group B streptococcal disease. *Infect Control* 1986;**7**:137–9, 143.
254. Goldmann DA. Prevention and management of neonatal infections. *Infect Dis Clin North Am* 1989;**3**:779–813.
255. Goldmann DA, Breton SJ. Group C streptococcal surgical wound infections transmitted by an anorectal and nasal carrier. *Pediatrics* 1978;**61**:235–7.
256. Gould CV, Umscheid CA, Agarwal RK, et al. Guideline for prevention of catheter-associated urinary tract infections 2009. *Infect Control Hosp Epidemiol* 2010;**31**:319–26.
257. Greenhalgh DG, Saffle JR, Holmes JH, et al. American Burn Association consensus conference to define sepsis and infection in burns. *J Burn Care Res* 2007;**28**:776–90.
258. Grohskopf LA, Huskins WC, Sinkowitz-Cochran RL, et al. Use of antimicrobial agents in United States neonatal and pediatric intensive care patients. *Pediatr Infect Dis J* 2005;**24**:766–73.
259. Grohskopf LA, Roth VR, Feikin DR, et al. *Serratia liquefaciens* bloodstream infections from contamination of epoetin alfa at a hemodialysis center. *N Engl J Med* 2001;**344**:1491–7.
260. Grohskopf LA, Sinkowitz-Cochran RL, Garrett DO, et al. A national point-prevalence survey of pediatric intensive care unit-acquired infections in the United States. *J Pediatr* 2002;**140**: 432–8.
261. Groothuis J, Bauman J, Malinoski F, et al. Strategies for prevention of RSV nosocomial infection. *J Perinatol* 2008;**28**:319–23.
262. Guerin JM, Lustman C, Meyer P, et al. Nosocomial sinusitis in pediatric intensive care patients. *Crit Care Med* 1990;**18**:902.
263. Gupta A, Della-Latta P, Todd B, et al. Outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit linked to artificial nails. *Infect Control Hosp Epidemiol* 2004;**25**:210–15.
264. Gustafson TL, Lavely GB, Brawner E Jr, et al. An outbreak of airborne nosocomial varicella. *Pediatrics* 1982;**70**:550–6.
265. Gutelius B, Perz JF, Parker MM, et al. Multiple clusters of hepatitis virus infections associated with anesthesia for outpatient endoscopy procedures. *Gastroenterology* 2010;**139**:163–70.
266. Haas EJ, Zaoutis TE, Prasad P, et al. Risk factors and outcomes for vancomycin-resistant enterococcus bloodstream infection in children. *Infect Control Hosp Epidemiol* 2010 Aug 31. [Epub ahead of print].
267. Hall CB. Nosocomial respiratory syncytial virus infections: the "Cold War" has not ended. *Clin Infect Dis* 2000;**31**:590–6.
268. Hall CB. Nosocomial viral respiratory infections: perennial weeds on pediatric wards. *Am J Med* 1981;**70**:670–6.
269. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med* 2001;**344**:1917–28.
270. Hall CB, Douglas R Jr. Nosocomial influenza infection as a cause of intercurrent fevers in infants. *Pediatrics* 1975;**55**:673–7.
271. Hall CB, Douglas R Jr. Modes of transmission of respiratory syncytial virus. *J Pediatr* 1981;**99**:100–3.
272. Hall CB, Douglas R Jr. Nosocomial respiratory syncytial viral infections: should gowns and masks be used? *Am J Dis Child* 1981;**135**:512–15.
273. Hall CB, Douglas R Jr, Geiman JM, et al. Nosocomial respiratory syncytial virus infections. *N Engl J Med* 1975;**293**:1343–6.
274. Hall CB, Douglas R Jr, Geiman JM. Possible transmission by fomites of respiratory syncytial virus. *J Infect Dis* 1980;**141**:98–102.
275. Hall CB, Kopelman AE, Douglas R Jr, et al. Neonatal respiratory syncytial virus infection. *N Engl J Med* 1979;**300**:393–6.
276. Hall CB, Powell KR, MacDonald NE, et al. Respiratory syncytial viral infection in children with compromised immune function. *N Engl J Med* 1986;**315**:77–81.
277. Hammerberg O, Watts J, Chernesky M, et al. An outbreak of herpes simplex virus type 1 in an intensive care nursery. *Pediat Infect Dis J* 1983;**2**:290–4.
278. Hanazaki K, Shingu K, Adachi W, et al. Chlorhexidine dressing for reduction in microbial colonization of the skin with central venous catheters: a prospective randomized controlled trial. *J Hosp Infect* 1999;**42**:165–8.
279. Harbarth S, Dharan S, Liassine N, et al. Randomized, placebo-controlled, double-blind trial to evaluate the efficacy of mupirocin for eradicating carriage of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1999;**43**:1412–16.
280. Harmon T, Burkhart G, Applebaum H. Perforated pseudomembranous colitis in the breast-fed infant. *J Pediatr Surg* 1992;**27**: 744–6.
281. Harries EHR. Infection and its control in children's wards. *Lancet* 1935;**2**:173–8.
282. Harris JA. Infection control in pediatric extended care facilities. *Infect Control Hosp Epidemiol* 2006;**27**:598–603.
283. Harvey BS, Koeuth T, Versalovic J, et al. Vertical transmission of *Citrobacter diversus* documented by DNA fingerprinting. *Infect Control Hosp Epidemiol* 1995;**16**:564–9.
284. Hedberg K, Ristinen TL, Soler JT, et al. Outbreak of erythromycin-resistant staphylococcal conjunctivitis in a newborn nursery. *Pediatr Infect Dis J* 1990;**9**:268–73.
285. Hedderwick SA, McNeil SA, Lyons MJ, et al. Pathogenic organisms associated with artificial fingernails worn by healthcare workers. *Infect Control Hosp Epidemiol* 2000;**21**:505–9.
286. Helin I, Widell A, Borulf S, et al. Outbreak of coxsackievirus A-14 meningitis among newborns in a maternity hospital ward. *Acta Paediatr Scand* 1987;**76**:234–8.
287. Hemming VG, Overall JC Jr, Britt MR. Nosocomial infections in a newborn intensive-care unit: results of forty-one months of surveillance. *N Engl J Med* 1976;**294**:1310–16.
288. Henderson DA, Inglesby TV, Bartlett JG, et al. Smallpox as a biological weapon: medical and public health management. *JAMA* 1999;**281**:2127–37.
289. Hersh AL, Beekmann SE, Polgreen PM, et al. Antimicrobial stewardship programs in pediatrics. *Infect Control Hosp Epidemiol* 2009; **30**:1211–17.

290. Hershkowici S, Barak M, Cohen A, et al. An outbreak of *Campylobacter jejuni* infection in a neonatal intensive care unit. *J Hosp Infect* 1987;**9**:54–9.
291. Heseltine PN, Ripper M, Wohlford P. Nosocomial rubella—consequences of an outbreak and efficacy of a mandatory immunization program. *Infect Control* 1985;**6**:371–4.
292. Hewlett AL, Rupp ME. New developments in the prevention of intravascular catheter associated infections. *Infect Dis Clin North Am* 2012;**26**:1–11.
293. Hidron AI, Edwards JR, Patel J, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. *Infect Control Hosp Epidemiol* 2008;**29**:996–1011.
294. Hochmuth P, Magnuson J, Owens K. Survival of vancomycin resistant *Enterococcus faecium* on acrylic nails, bed linen, and plastic keyboard covers. *Am J Infect Control* 2005;**33**:e32.
295. Hoen B, Paul-Dauphin A, Hestin D, et al. EPIBACDIAL: a multicenter prospective study of risk factors for bacteremia in chronic hemodialysis patients. *J Am Soc Nephrol* 1998;**9**:869–76.
296. Hollmeyer H, Hayden F, Mounts A, et al. Review: interventions to increase influenza vaccination among healthcare workers in hospitals. *Influenza Other Respi Viruses* 2012 Sep 18. [Epub ahead of print].
297. Holzmann-Pazgal G, Hopkins-Broyles D, Recktenwald A, et al. Case-control study of pediatric cardiothoracic surgical site infections. *Infect Control Hosp Epidemiol* 2008;**29**:76–9.
298. Hoogkamp-Korstanje JA, Cats B, Senders RC, et al. Analysis of bacterial infections in a neonatal intensive care unit. *J Hosp Infect* 1982;**3**:275–84.
299. Hooton TM, Bradley SF, Cardenas DD, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 International Clinical Practice Guidelines from the Infectious Diseases Society of America. *Clin Infect Dis* 2010;**50**:625–63.
300. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* 2008;**36**:309–32.
301. Horbar JD, Carpenter JH, Badger GJ, et al. Mortality and neonatal morbidity among infants 501 to 1500 grams from 2000 to 2009. *Pediatrics* 2012;**129**:1019–26.
302. Horbar JD, Soll RF, Edwards WH. The Vermont Oxford Network: a community of practice. *Clin Perinatol* 2010;**37**:29–47.
303. Howard AJ. Nosocomial spread of *Haemophilus influenzae*. *J Hosp Infect* 1991;**19**:1–3.
304. Hruszkewycz V, Holtrop PC, Batton DG, et al. Complications associated with central venous catheters inserted in critically ill neonates. *Infect Control Hosp Epidemiol* 1991;**12**:544–8.
305. Huang FL, Chen CH, Huang SK, et al. An outbreak of enterovirus 71 in a nursery. *Scand J Infect Dis* 2010;**42**:609–12.
306. Huang YC, Chou YH, Su LH, et al. Methicillin-resistant *Staphylococcus aureus* colonization and its association with infection among infants hospitalized in neonatal intensive care units. *Pediatrics* 2006;**118**:469–74.
307. Huebner J, Goldmann DA. Coagulase-negative staphylococci: role as pathogens. *Annu Rev Med* 1999;**50**:223–36.
308. Huebner J, Pier GB, Maslow JN, et al. Endemic nosocomial transmission of *Staphylococcus epidermidis* bacteremia isolates in a neonatal intensive care unit over 10 years. *J Infect Dis* 1994;**169**:526–31.
309. Hunter JD. Ventilator associated pneumonia. *BMJ* 2012;**344**:e3325.
310. Huskins WC. Quality improvement interventions to prevent healthcare-associated infections in neonates and children. *Curr Opin Pediatr* 2012;**24**:103–12.
311. Huskins WC, Huckabee CM, O'Grady NP, et al. Intervention to reduce transmission of resistant bacteria in intensive care. *N Engl J Med* 2011;**364**:1407–18.
312. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. *JAMA* 2000;**283**:2281–90.
313. Ingram J, Weitzman S, Greenberg ML, et al. Complications of indwelling venous access lines in the pediatric hematology patient: a prospective comparison of external venous catheters and subcutaneous ports. *Am J Pediatr Hematol Oncol* 1991;**13**:130–6.
314. Institute for Healthcare Improvement. *How-to-guide: reduce methicillin-resistant Staphylococcus aureus (MRSA) infection*. Institute for Healthcare Improvement. 2011. Available at http://www.ihi.org/knowledge/Pages/Tools/HowtoGuideReduceMRSAInfection.aspx. Accessed August 30, 2012.
315. Institute of Medicine. *Crossing the quality chasm: a new health system for the 21st century*. Washington, D.C.: National Academies Press; 2001.
316. Jacobsen SM, Stickler DJ, Mobley HL, et al. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev* 2008;**21**:26–59.
317. Jain R, Kralovic SM, Evans ME, et al. Veterans Affairs initiative to prevent methicillin-resistant *Staphylococcus aureus* infections. *N Engl J Med* 2011;**364**:1419–30.
318. Jamieson FB, Wang EE, Bain C, et al. Human torovirus: a new nosocomial gastrointestinal pathogen. *J Infect Dis* 1998;**178**:1263–9.
319. Jarvis WR. Epidemiology of nosocomial infections in pediatric patients. *Pediatr Infect Dis J* 1987;**6**:344–51.
320. Jarvis WR, editor. *Bennett and Brachman's hospital infections*. Philadelphia: Lippincott Williams & Wilkins; 2007.
321. Jeelani NU, Kulkarni AV, Desilva P, et al. Postoperative cerebrospinal fluid wound leakage as a predictor of shunt infection: a prospective analysis of 205 cases. Clinical article. *J Neurosurg Pediatr* 2009;**4**:166–9.
322. Jeffries HE, Mason W, Brewer M, et al. Prevention of central venous catheter–associated bloodstream infections in pediatric intensive care units: a performance improvement collaborative. *Infect Control Hosp Epidemiol* 2009;**30**:645–51.
323. Jensen PA, Lambert LA, Iademarco MF, et al. Guidelines for preventing the transmission of *Mycobacterium tuberculosis* in healthcare settings, 2005. *MMWR Recomm Rep* 2005;**54**:1–141.
324. Jimenez-Truque N, Tedeschi S, Saye EJ, et al. Relationship between maternal and neonatal *Staphylococcus aureus* colonization. *Pediatrics* 2012;**129**:e1252–9.
325. Johannsson B, Beekmann SE, Srinivasan A, et al. Improving antimicrobial stewardship: the evolution of programmatic strategies and barriers. *Infect Control Hosp Epidemiol* 2011;**32**:367–74.
326. Johnson I, Hammond GW, Verma MR. Nosocomial coxsackie B4 virus infections in two chronic-care pediatric neurological wards. *J Infect Dis* 1985;**151**:1153–6.
327. Johnson JG, Talbot TR. New approaches for influenza vaccination of healthcare workers. *Curr Opin Infect Dis* 2011;**24**:363–9.
328. Johnsson J, Seeberg S, Kjellmer I. Blood concentrations of chlorhexidine in neonates undergoing routine cord care with 4% chlorhexidine gluconate solution. *Acta Paediatr Scand* 1987;**76**:675–6.
329. Jungk J, Como-Sabetti K, Stinchfield P, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* at a pediatric healthcare system, 1991-2003. *Pediatr Infect Dis J* 2007;**26**:339–44.
330. Kagen J, Bilker WB, Lautenbach E, et al. Risk adjustment for surgical site infection after median sternotomy in children. *Infect Control Hosp Epidemiol* 2007;**28**:398–405.
331. Kaplan HC, Lannon C, Walsh MC, et al. Ohio statewide quality-improvement collaborative to reduce late-onset sepsis in preterm infants. *Pediatrics* 2011;**127**:427–35.
332. Karanfil LV, Conlon M, Lykens K, et al. Reducing the rate of nosocomially transmitted respiratory syncytial virus. *Am J Infect Control* 1999;**27**:91–6.
333. Karki S, Cheng AC. Impact of non-rinse skin cleansing with chlorhexidine gluconate on prevention of healthcare-associated infections and colonization with multi-resistant organisms: a systematic review. *J Hosp Infect* 2012;**82**:71–84.
334. Karmali MA, Norrish B, Lior H, et al. *Campylobacter* enterocolitis in a neonatal nursery. *J Infect Dis* 1984;**149**:874–7.
335. Katz BZ, Sullivan C. Respiratory syncytial virus prophylaxis in a tertiary care neonatal intensive care unit. *Pediat Infect Dis J* 2009;**28**:842–4.
336. Keane E, Gilles HM. Lassa fever in Panguma Hospital, Sierra Leone, 1973-6. *BMJ* 1977;**1**:1399–402.
337. Kelly M, Conway M, Wirth K, et al. Moving CLABSI prevention beyond the intensive care unit: risk factors in pediatric oncology patients. *Infect Control Hosp Epidemiol* 2011;**32**:1079–85.
338. Kelsen JR, Kim J, Latta D, et al. Recurrence rate of clostridium difficile infection in hospitalized pediatric patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011;**17**:50–5.

339. Kerstiens B, Matthys F. Interventions to control virus transmission during an outbreak of Ebola hemorrhagic fever: experience from Kikwit, Democratic Republic of the Congo, 1995. *J Infect Dis* 1999;**179**(Suppl. 1):S263–7.
340. Khan EA, Correa AG, Baker CJ. Suppurative thrombophlebitis in children: a ten-year experience. *Pediatr Infect Dis J* 1997;**16**: 63–7.
341. Kilham EB. An epidemic of pemphigus neonatorum. *Am J Obstet* 1889;**22**:1039.
342. Kim J, Shaklee JF, Smathers S, et al. Risk factors and outcomes associated with severe clostridium difficile infection in children. *Pediatr Infect Dis J* 2012;**31**:134–8.
343. Kim J, Smathers SA, Prasad P, et al. Epidemiological features of *Clostridium difficile*–associated disease among inpatients at children's hospitals in the United States, 2001-2006. *Pediatrics* 2008; **122**:1266–70.
344. Kim YH, Chang SS, Kim YS, et al. Clinical outcomes in methicillin-resistant *Staphylococcus aureus*–colonized neonates in the neonatal intensive care unit. *Neonatology* 2007;**91**:241–7.
345. Kinney J, Mundorf L, Gleason C, et al. Efficacy and pharmacokinetics of intravenous immune globulin administration to high-risk neonates. *Am J Dis Child* 1991;**145**:1233–8.
346. Kinney JS, McCray E, Kaplan JE, et al. Risk factors associated with echovirus 11 infection in a hospital nursery. *Pediatr Infect Dis J* 1986;**5**:192–7.
347. Klein BS, Michaels JA, Rytel MW, et al. Nosocomial hepatitis A: a multinursery outbreak in Wisconsin. *JAMA* 1984;**252**: 2716–21.
348. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant *Staphylococcus aureus* infections in the United States. *JAMA* 2007;**298**:1763–71.
349. Klompas M, Kleinman K, Khan Y, et al. Rapid and reproducible surveillance for ventilator-associated pneumonia. *Clin Infect Dis* 2012;**54**:370–7.
350. Koh DB, Gowardman JR, Rickard CM, et al. Prospective study of peripheral arterial catheter infection and comparison with concurrently sited central venous catheters. *Crit Care Med* 2008;**36**: 397–402.
351. Kohn LT, Corrigan JM, Donaldson MS. *To err is human: building a safer health system*. Washington, D.C.: National Academies Press; 2000.
352. Korniewicz DM, Kirwin M, Cresci K, et al. Leakage of latex and vinyl exam gloves in high and low risk clinical settings. *Am Indust Hyg Assoc J* 1993;**54**:22–6.
353. Koser CU, Holden MT, Ellington MJ, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. *N Engl J Med* 2012;**366**:2267–75.
354. Kotloff KL, Losonsky GA, Morris J Jr, et al. Enteric adenovirus infection and childhood diarrhea: an epidemiologic study in three clinical settings. *Pediatrics* 1989;**84**:219–25.
355. Koziol DE, Kurtzman G, Ayub J, et al. Nosocomial human parvovirus B19 infection: lack of transmission from a chronically infected patient to hospital staff. *Infect Control Hosp Epidemiol* 1992;**13**:343–8.
356. Krasinski K, LaCouture R, Holzman RS, et al. Screening for respiratory syncytial virus and assignment to a cohort at admission to reduce nosocomial transmission. *J Pediatr* 1990;**116**:894–8.
357. Krause PJ, Gross PA, Barrett TL, et al. Quality standard for assurance of measles immunity among health care workers. *Infect Control Hosp Epidemiol* 1994;**15**:193–9.
358. Krause R, Salzer HF, Honigl M, et al. Comparison of fluorescence in situ hybridisation using peptide nucleic acid probes, Gram stain/acridine orange leukocyte cytospin and differential time to positivity methods for detection of catheter-related bloodstream infection in patients after haematopoietic stem cell transplantation. *Clin Microbiol Infect* 2010;**16**:1591–3.
359. Krueger WA, Lenhart FP, Neeser G, et al. Influence of combined intravenous and topical antibiotic prophylaxis on the incidence of infections, organ dysfunctions, and mortality in critically ill surgical patients: a prospective, stratified, randomized, double-blind, placebo-controlled clinical trial. *Am J Respir Crit Care Med* 2002;**166**:1029–37.
360. Kurt TL, Yeager AS, Guenette S, et al. Spread of pertussis by hospital staff. *JAMA* 1972;**221**:264–7.
361. Kurtz JB, Lee TW, Pickering D. Astrovirus associated gastroenteritis in a children's ward. *J Clinic Pathol* 1977;**30**:948–52.
362. Kurz H, Herbich K, Janata O, et al. Experience with the use of palivizumab together with infection control measures to prevent respiratory syncytial virus outbreaks in neonatal intensive care units. *J Hosp Infect* 2008;**70**:246–52.
363. Kusuhara K, Saito M, Sasaki Y, et al. An echovirus type 18 outbreak in a neonatal intensive care unit. *Eur J Pediatr* 2008;**167**: 587–9.
364. Kutty PK, Kyaw MH, Dayan GH, et al. Guidance for isolation precautions for mumps in the United States: a review of the scientific basis for policy change. *Clin Infect Dis* 2010;**50**:1619–28.
365. Kyne L, Warny M, Qamar A, et al. Association between antibody response to toxin A and protection against recurrent *Clostridium difficile* diarrhoea. *Lancet* 2001;**357**:189–93.
366. La Quaglia MP, Caldwell C, Lucas A, et al. A prospective randomized double-blind trial of bolus urokinase in the treatment of established Hickman catheter sepsis in children. *J Pediatr Surg* 1994;**29**:742–5.
367. La Quaglia MP, Lucas A, Thaler HT, et al. A prospective analysis of vascular access device-related infections in children. *J Pediatr Surg* 1992;**27**:840–2.
368. Labeau SO, Van de Vyver K, Brusselaers N, et al. Prevention of ventilator-associated pneumonia with oral antiseptics: a systematic review and meta-analysis. *Lancet Infect Dis* 2011;**11**:845–54.
369. Labriola L, Crott R, Jadoul M. Preventing haemodialysis catheter-related bacteraemia with an antimicrobial lock solution: a meta-analysis of prospective randomized trials. *Nephrol Dial Transplant* 2008;**23**:1666–72.
370. Lai KK. *Enterobacter sakazakii* infections among neonates, infants, children, and adults: case reports and a review of the literature. *Medicine (Baltimore)* 2001;**80**:113–22.
371. Landers S, Moise AA, Fraley JK, et al. Factors associated with umbilical catheter–related sepsis in neonates. *Am J Dis Child* 1991;**145**:675–80.
372. Langley JM, Gravel D, Moore D, et al. Study of cerebrospinal fluid shunt–associated infections in the first year following placement, by the Canadian Nosocomial Infection Surveillance Program. *Infect Control Hosp Epidemiol* 2009;**30**:285–8.
373. Langley JM, Hanakowski M, Leblanc JC. Unique epidemiology of nosocomial urinary tract infection in children. *Am J Infect Control* 2001;**29**:94–8.
374. Langley JM, LeBlanc JC, Hanakowski M, et al. The role of *Clostridium difficile* and viruses as causes of nosocomial diarrhea in children. *Infect Control Hosp Epidemiol* 2002;**23**:660–4.
375. Langley JM, LeBlanc JC, Wang EE, et al. Nosocomial respiratory syncytial virus infection in Canadian pediatric hospitals: a Pediatric Investigators Collaborative Network on Infections in Canada Study. *Pediatrics* 1997;**100**:943–6.
376. Lautenbach E, Woeltje KF, Malani PN. *Practical healthcare epidemiology*. Chicago: University of Chicago Press; 2010.
377. Lavergne V, Ghannoum M, Weiss K, et al. Successful prevention of respiratory syncytial virus nosocomial transmission following an enhanced seasonal infection control program. *Bone Marrow Transplant* 2011;**46**:137–42.
378. Leckerman KH, Sherman E, Knorr J, et al. Risk factors for healthcare-associated, laboratory-confirmed influenza in hospitalized pediatric patients: a case-control study. *Infect Control Hosp Epidemiol* 2010;**31**:421–4.
379. Leclair JM, Freeman J, Sullivan BF, et al. Prevention of nosocomial respiratory syncytial virus infections through compliance with glove and gown isolation precautions. *N Engl J Med* 1987; **317**:329–34.
380. Leclair JM, Winston KR, Sullivan BF, et al. Effect of preoperative shampoos with chlorhexidine or iodophor on emergence of resident scalp flora in neurosurgery. *Infect Control* 1988;**9**:8–12.
381. Leclair JM, Zaia JA, Levin MJ, et al. Airborne transmission of chickenpox in a hospital. *N Engl J Med* 1980;**302**:450–3.
382. Lee I, Agarwal RK, Lee BY, et al. Systematic review and cost analysis comparing use of chlorhexidine with use of iodine for preoperative skin antisepsis to prevent surgical site infection. *Infect Control Hosp Epidemiol* 2010;**31**:1219–29.
383. Lee TB, Baker OG, Lee JT, et al. Recommended practices for surveillance. *Am J Infect Control* 1998;**26**:277–88.
384. Lemaire X, Morena M, Leray-Moragues H, et al. Analysis of risk factors for catheter-related bacteremia in 2000 permanent dual catheters for hemodialysis. *Blood Purif* 2009;**28**:21–8.

385. Lessa FC, Edwards JR, Fridkin SK, et al. Trends in incidence of late-onset methicillin-resistant *Staphylococcus aureus* infection in neonatal intensive care units: data from the National Nosocomial Infections Surveillance System, 1995-2004. *Pediatr Infect Dis J* 2009;**28**:577–81.
386. Lettau LA. Nosocomial transmission and infection control aspects of parasitic and ectoparasitic diseases, part I: introduction/enteric parasites. *Infect Control Hosp Epidemiol* 1991;**12**:59–65.
387. Lettau LA. Nosocomial transmission and infection control aspects of parasitic and ectoparasitic diseases, part III: ectoparasites/summary and conclusions. *Infect Control Hosp Epidemiol* 1991;**12**:179–85.
388. Levy ER, Swami S, Dubois SG, et al. Rates and appropriateness of antimicrobial prescribing at an academic children's hospital, 2007-2010. *Infect Control Hosp Epidemiol* 2012;**33**:346–53.
389. Levy I, Bendet M, Samra Z, et al. Infectious complications of peripherally inserted central venous catheters in children. *Pediatr Infect Dis J* 2010;**29**:426–9.
390. Levy I, Katz J, Solter E, et al. Chlorhexidine-impregnated dressing for prevention of colonization of central venous catheters in infants and children: a randomized controlled study. *Pediatr Infect Dis J* 2005;**24**:676–9.
391. Lichtenstein A. Nosocomial infections in children's hospitals and institutions: our means for combating these infections. *Acta Paediat* 1935;**17**:36–49.
392. Lin FC, Devoe WF, Morrison C, et al. Outbreak of neonatal *Citrobacter diversus* meningitis in a suburban hospital. *Pediatr Infect Dis J* 1987;**6**:50–5.
393. Linam WM, Margolis PA, Staat MA, et al. Risk factors associated with surgical site infection after pediatric posterior spinal fusion procedure. *Infect Control Hosp Epidemiol* 2009;**30**:109–16.
394. Linares J, Sitges-Serra A, Garau J, et al. Pathogenesis of catheter sepsis: a prospective study with quantitative and semiquantitative cultures of catheter hub and segments. *J Clin Microbiol* 1985;**21**:357–60.
395. Linnemann CC Jr, Buchman TG, Light IJ, et al. Transmission of herpes-simplex virus type 1 in a nursery for the newborn: identification of viral isolates by DNA "fingerprinting." *Lancet* 1978;**1**:964–6.
396. Linnemann CC Jr, Ramundo N, Perlstein PH, et al. Use of pertussis vaccine in an epidemic involving hospital staff. *Lancet* 1975;**2**:540–3.
397. Liston TE, Levine PL, Anderson C. Polymicrobial bacteremia due to Polle syndrome: the child abuse variant of Munchausen by proxy. *Pediatrics* 1983;**72**:211–13.
398. Lohr JA, Donowitz LG, Sadler JE. Hospital-acquired urinary tract infection. *Pediatrics* 1989;**83**:193–9.
399. Lohr JA, Downs SM, Dudley S, et al. Hospital-acquired urinary tract infections in the pediatric patient: a prospective study. *Pediatr Infect Dis J* 1994;**13**:8–12.
400. Long JG, Keyserling HL. Catheter-related infection in infants due to an unusual lipophilic yeast–*Malassezia furfur*. *Pediatrics* 1985;**76**:896–900.
401. Loo VG, Poirier L, Miller MA, et al. A predominantly clonal multi-institutional outbreak of *Clostridium difficile*–associated diarrhea with high morbidity and mortality. *N Engl J Med* 2005;**353**:2442–9.
402. Lopriore E, Markhorst DG, Gemke RJ. Ventilator-associated pneumonia and upper airway colonisation with gram-negative bacilli: the role of stress ulcer prophylaxis in children. *Intensive Care Med* 2002;**28**:763–7.
403. Lorente L, Jimenez A, Jimenez JJ, et al. Higher arterial catheter–related infection rates in femoral than in dorsalis pedis access. *J Hosp Infect* 2010;**74**:365–9.
404. Lövegren E. Infection risks in nursing institutions for infants and young children and measures for their prevention. *Acta Paediatr* 1935;**17**:50–5.
405. Lucero CA, Cohen AL, Trevino I, et al. Outbreak of *Burkholderia cepacia* complex among ventilated pediatric patients linked to hospital sinks. *Am J Infect Control* 2011;**39**:775–8.
406. Lucet JC, Bouadma L, Zahar JR, et al. Infectious risk associated with arterial catheters compared with central venous catheters. *Crit Care Med* 2010;**38**:1030–5.
407. Lui SL, Luk WK, Cheung CY, et al. Nosocomial outbreak of parvovirus B19 infection in a renal transplant unit. *Transplantation* 2001;**71**:59–64.
408. Lundgren IS, Zhou C, Malone FR, et al. Central venous catheter repair is associated with an increased risk of bacteremia and central line–associated bloodstream infection in pediatric patients. *Pediatr Infect Dis J* 2012;**31**:337–40.
409. Lyytikainen O, Saxen H, Ryhanen R, et al. Persistence of a multiresistant clone of *Staphylococcus epidermidis* in a neonatal intensive-care unit for a four-year period. *Clin Infect Dis* 1995;**20**:24–9.
410. Macartney KK, Gorelick MH, Manning ML, et al. Nosocomial respiratory syncytial virus infections: the cost-effectiveness and cost-benefit of infection control. *Pediatrics* 2000;**106**:520–6.
411. MacCannell T, Umscheid CA, Agarwal RK, et al. Guideline for the prevention and control of norovirus gastroenteritis outbreaks in healthcare settings. *Infect Control Hosp Epidemiol* 2011;**32**:939–69.
412. MacDonald NE, Hall CB, Suffin SC, et al. Respiratory syncytial viral infection in infants with congenital heart disease. *N Engl J Med* 1982;**307**:397–400.
413. Mackenzie A, Johnson W, Heyes B, et al. A prolonged outbreak of exfoliative toxin A–producing *Staphylococcus aureus* in a newborn nursery. *Diagn Microbiol Infect Dis* 1995;**21**:69–75.
414. Madden NP, Levinsky RJ, Bayston R, et al. Surgery, sepsis, and nonspecific immune function in neonates. *J Pediatr Surg* 1989;**24**:562–6.
415. Madge P, Paton JY, McColl JH, et al. Prospective controlled study of four infection-control procedures to prevent nosocomial infection with respiratory syncytial virus. *Lancet* 1992;**340**:1079–83.
416. Magny JF, Bremard-Oury C, Brault D, et al. Intravenous immunoglobulin therapy for prevention of infection in high-risk premature infants: report of a multicenter, double-blind study. *Pediatrics* 1991;**88**:437–43.
417. Maguire GC, Nordin J, Myers MG, et al. Infections acquired by young infants. *Am J Dis Child* 1981;**135**:693–8.
418. Maki DG, Kluger DM, Crnich CJ. The risk of bloodstream infection in adults with different intravascular devices: a systematic review of 200 published prospective studies. *Mayo Clin Proc* 2006;**81**:1159–71.
419. Maki DG, Mermel LA, Kluger DM, et al. The efficacy of a chlorhexidine-impregnated sponge for the prevention of intravascular catheter-related infection—a prospective, randomized, controlled, multicenter trial [abstract 1430]. Presented before the 40th Interscience Conference on Antimicrobial Agents and Chemotherapy; 2000. p. 422.
420. Maki DG, Rhame FS, Mackel DC, et al. Nationwide epidemic of septicemia caused by contaminated intravenous products, part I: epidemiologic and clinical features. *Am J Med* 1976;**60**:471–85.
421. Maki DG, Ringer M, Alvarado CJ. Prospective randomised trial of povidone-iodine, alcohol, and chlorhexidine for prevention of infection associated with central venous and arterial catheters. *Lancet* 1991;**338**:339–43.
422. Maki DG, Tambyah PA. Engineering out the risk for infection with urinary catheters. *Emerg Infect Dis* 2001;**7**:342–7.
423. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. *N Engl J Med* 1977;**296**:1305–9.
424. Mandigers CM, Diepersloot RJ, Dessens M, et al. A hospital outbreak of penicillin-resistant pneumococci in The Netherlands. *Eur Respir J* 1994;**7**:1635–9.
425. Mangram AJ, Horan TC, Pearson ML, et al. Guideline for prevention of surgical site infection, 1999. *Am J Infect Control* 1999;**27**:97–132.
426. Maragakis LL. Recognition and prevention of multidrug-resistant Gram-negative bacteria in the intensive care unit. *Crit Care Med* 2010;**38**:S345–51.
427. Marcos M, Soriano A, Inurrieta A, et al. Changing epidemiology of central venous catheter-related bloodstream infections: increasing prevalence of gram-negative pathogens. *J Antimicrob Chemother* 2011;**66**:2119–25.
428. Maree CL, Daum RS, Boyle-Vavra S, et al. Community-associated methicillin-resistant *Staphylococcus aureus* isolates causing healthcare-associated infections. *Emerg Infect Dis* 2007;**13**:236–42.
429. Mastro TD, Fields BS, Breiman RF, et al. Nosocomial legionnaires' disease and use of medication nebulizers. *J Infect Dis* 1991;**163**:667–71.

430. Matlow AG, Harrison A, Monteath A, et al. Nosocomial transmission of tuberculosis (TB) associated with care of an infant with peritoneal TB. *Infect Control Hosp Epidemiol* 2000;**21**:222–3.
431. Matthews JE, Dickey BW, Miller RD, et al. The epidemiology of published norovirus outbreaks: a review of risk factors associated with attack rate and genogroup. *Epidemiol Infect* 2012;**140**: 1161–72.
432. Mattner F, Sykora KW, Meissner B, et al. An adenovirus type F41 outbreak in a pediatric bone marrow transplant unit: analysis of clinical impact and preventive strategies. *Pediatr Infect Dis J* 2008;**27**:419–24.
433. Mayhall CG. *Hospital epidemiology and infection control*. Philadelphia: Lippincott Williams & Wilkins; 2011.
434. McCannon J, Berwick DM. A new frontier in patient safety. *JAMA* 2011;**305**:2221–2.
435. McCullers JA, Williams BF, Wu S, et al. Health care–associated infections at a children's cancer hospital, 1983-2008. *J Pediatr Infect Dis Soc* 2012;**1**:26–34.
436. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene–variant strain of *Clostridium difficile*. *N Engl J Med* 2005;**353**:2433–41.
437. McFarland LV, Brandmarker SA, Guandalini S. Pediatric *Clostridium difficile:* a phantom menace or clinical reality? *J Pediatr Gastroenterol Nutr* 2000;**31**:220–31.
438. McGechie PB. Nosocomial bacteraemia in hospital staff caused by *Haemophilus influenzae* type b. *J Hosp Infect* 1992;**21**:159–60.
439. McKenney D, Hubner J, Muller E, et al. The ica locus of *Staphylococcus epidermidis* encodes production of the capsular polysaccharide/adhesin. *Infect Immun* 1998;**66**:4711–20.
440. McNeil SA, Foster CL, Hedderwick SA, et al. Effect of hand cleansing with antimicrobial soap or alcohol-based gel on microbial colonization of artificial fingernails worn by health care workers. *Clin Infect Dis* 2001;**32**:367–72.
441. Meddings J, Rogers MA, Macy M, et al. Systematic review and meta-analysis: reminder systems to reduce catheter-associated urinary tract infections and urinary catheter use in hospitalized patients. *Clin Infect Dis* 2010;**51**:550–60.
442. Mehall JR, Saltzman DA, Jackson RJ, et al. Fibrin sheath enhances central venous catheter infection. *Crit Care Med* 2002;**30**: 908–12.
443. Mermel LA, Allon M, Bouza E, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:1–45.
444. Mermel LA, Farr BM, Sherertz RJ, et al. Guidelines for the management of intravascular catheter-related infections. *Clin Infect Dis* 2001;**32**:1249–72.
445. Metjian TA, Prasad PA, Kogon A, et al. Evaluation of an antimicrobial stewardship program at a pediatric teaching hospital. *Pediatr Infect Dis J* 2008;**27**:106–11.
446. Mhalu FS, Mtango FD, Msengi AE. Hospital outbreaks of cholera transmitted through close person-to-person contact. *Lancet* 1984; **2**:82–4.
447. Middleton PJ, Szymanski MT, Petric M. Viruses associated with acute gastroenteritis in young children. *Am J Dis Child* 1977; **131**:733–7.
448. Millar MR, Bacon C, Smith SL, et al. Enteral feeding of premature infants with *Lactobacillus* GG. *Arch Dis Child* 1993;**69**:483–7.
449. Millar MR, Brown NM, Tobin GW, et al. Outbreak of infection with penicillin-resistant *Streptococcus pneumoniae* in a hospital for the elderly. *J Hosp Infect* 1994;**27**:99–104.
450. Miller BL, Ahmed F, Lindley MC, et al. Increases in vaccination coverage of healthcare personnel following institutional requirements for influenza vaccination: a national survey of U.S. hospitals. *Vaccine* 2011;**29**:9398–403.
451. Miller MA, Dascal A, Portnoy J, et al. Development of mupirocin resistance among methicillin-resistant *Staphylococcus aureus* after widespread use of nasal mupirocin ointment. *Infect Control Hosp Epidemiol* 1996;**17**:811–13.
452. Miller MR, Griswold M, Harris JM 2nd, et al. Decreasing PICU catheter–associated bloodstream infections: NACHRI's quality transformation efforts. *Pediatrics* 2010;**125**:206–13.
453. Millership SE, Anderson C, Cummins AJ, et al. The risk to infants from nosocomial exposure to tuberculosis. *Pediatr Infect Dis J* 2009;**28**:915–16.
454. Milstone AM, Sengupta A. Do prolonged peripherally inserted central venous catheter dwell times increase the risk of bloodstream infection? *Infect Control Hosp Epidemiol* 2010;**31**:1184–7.
455. Mirro J Jr, Rao BN, Kumar M, et al. A comparison of placement techniques and complications of externalized catheters and implantable port use in children with cancer. *J Pediatr Surg* 1990;**25**:120–4.
456. Mitchell SJ, Gray J, Morgan ME, et al. Nosocomial infection with *Rhizopus microsporus* in preterm infants: association with wooden tongue depressors. *Lancet* 1996;**348**:441–3.
457. Miura E, Procianoy RS, Bittar C, et al. A randomized, double-masked, placebo-controlled trial of recombinant granulocyte colony–stimulating factor administration to preterm infants with the clinical diagnosis of early-onset sepsis. *Pediatrics* 2001;**107**: 30–5.
458. Moffet HL, Allan D. Colonization of infants exposed to bacterially contaminated mists. *Am J Dis Child* 1967;**114**:21–5.
459. Moisiuk SE, Robson D, Klass L, et al. Outbreak of parainfluenza virus type 3 in an intermediate care neonatal nursery. *Pediatr Infect Dis J* 1998;**17**:49–53.
460. Monath TP, Mertens PE, Patton R, et al. A hospital epidemic of Lassa fever in Zorzor, Liberia, March-April 1972. *Am J Trop Med Hyg* 1973;**22**:773–9.
461. Montecalvo MA, McKenna D, Yarrish R, et al. Chlorhexidine bathing to reduce central venous catheter–associated bloodstream infection: impact and sustainability. *Am J Med* 2012;**125**:505–11.
462. Moran GJ, Talan DA, Abrahamian FM. Biological terrorism. *Infect Dis Clin North Am* 2008;**22**:145–87.
463. Morgan DJ, Rogawski E, Thom KA, et al. Transfer of multidrug-resistant bacteria to healthcare workers' gloves and gowns after patient contact increases with environmental contamination. *Crit Care Med* 2012;**40**:1045–51.
464. Morrow PE. Physics of airborne particles and their deposition in the lung. *Ann N Y Acad Sci* 1980;**353**:71–80.
465. Morter S, Bennet G, Fish J, et al. Norovirus in the hospital setting: virus introduction and spread within the hospital environment. *J Hosp Infect* 2011;**77**:106–12.
466. Moser MR, Bender TR, Margolis HS, et al. An outbreak of influenza aboard a commercial airliner. *Am J Epidemiol* 1979;**110**: 1–6.
467. Mu Y, Edwards JR, Horan TC, et al. Improving risk-adjusted measures of surgical site infection for the national healthcare safety network. *Infect Control Hosp Epidemiol* 2011;**32**:970–86.
468. Muhlemann K, Franzini C, Aebi C, et al. Prevalence of nosocomial infections in Swiss children's hospitals. *Infect Control Hosp Epidemiol* 2004;**25**:765–71.
469. Mullett CJ, Evans RS, Christenson JC, et al. Development and impact of a computerized pediatric antiinfective decision support program. *Pediatrics* 2001;**108**:E75.
470. Mullett CJ, Thomas JG, Smith CL, et al. Computerized antimicrobial decision support: an offline evaluation of a database-driven empiric antimicrobial guidance program in hospitalized patients with a bloodstream infection. *Int J Med Inform* 2004;**73**:455–60.
471. Munoz FM, Campbell JR, Atmar RL, et al. Influenza A virus outbreak in a neonatal intensive care unit. *Pediatr Infect Dis J* 1999;**18**:811–15.
472. Munoz-Price LS, Hota B, Stemer A, et al. Prevention of bloodstream infections by use of daily chlorhexidine baths for patients at a long-term acute care hospital. *Infect Control Hosp Epidemiol* 2009;**30**:1031–5.
473. Muto CA, Jernigan JA, Ostrowsky BE, et al. SHEA guideline for preventing nosocomial transmission of multidrug-resistant strains of *Staphylococcus aureus* and *Enterococcus*. *Infect Control Hosp Epidemiol* 2003;**24**:362–86.
474. Muytjens HL, Roelofs-Willemse H, Jaspar GH. Quality of powdered substitutes for breast milk with regard to members of the family Enterobacteriaceae. *J Clin Microbiol* 1988;**26**:743–6.
475. Nakayama M, Miyazaki C, Ueda K, et al. Pharyngoconjunctival fever caused by adenovirus type 11. *Pediatr Infect Dis J* 1992; **11**:6–9.
476. Nateghian A, Taylor G, Robinson JL. Risk factors for surgical site infections following open-heart surgery in a Canadian pediatric population. *Am J Infect Control* 2004;**32**:397–401.
477. National Nosocomial Infections Surveillance (NNIS) System. National Nosocomial Infections Surveillance (NNIS) System

Report, data summary from January 1992 through June 2004, issued October 2004. *Am J Infect Control* 2004;**32**:470–85.
478. Neely AN, Maley MP. Survival of enterococci and staphylococci on hospital fabrics and plastic. *J Clin Microbiol* 2000;**38**:724–6.
479. Neu N, Plaskett T, Hutcheon G, et al. Epidemiology of human metapneumovirus in a pediatric long-term care facility. *Infect Control Hosp Epidemiol* 2012;**33**:545–50.
480. Newland JG, Banerjee R, Gerber JS, et al. Antimicrobial stewardship in pediatric care: strategies and future directions. *Pharmacotherapy* 2012;**32**:735–43.
481. Newland JG, Stach LM, De Lurgio SA, et al. Impact of a prospective-audit-with-feedback antimicrobial stewardship program at a children's hospital. *J Ped Infect Dis*, 2012;**3**:179–86.
482. Newman RE, Hedican EB, Herigon JC, et al. Impact of a guideline on management of children hospitalized with community-acquired pneumonia. *Pediatrics* 2012;**129**:e597–604.
483. Nguyen DM, Bancroft E, Mascola L, et al. Risk factors for neonatal methicillin-resistant *Staphylococcus aureus* infection in a well-infant nursery. *Infect Control Hosp Epidemiol* 2007;**28**:406–11.
484. Niedner MF, Huskins WC, Colantuoni E, et al. Epidemiology of central line–associated bloodstream infections in the pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:1200–8.
485. Nivin B, Nicholas P, Gayer M, et al. A continuing outbreak of multidrug-resistant tuberculosis, with transmission in a hospital nursery. *Clin Infect Dis* 1998;**26**:303–7.
486. Noble RC, Kane MA, Reeves SA, et al. Posttransfusion hepatitis A in a neonatal intensive care unit. *JAMA* 1984;**252**:2711–15.
487. Nolan T, Berwick DM. All-or-none measurement raises the bar on performance. *JAMA* 2006;**295**:1168–70.
488. Noskin GA, Stosor V, Cooper I, et al. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. *Infect Control Hosp Epidemiol* 1995;**16**:577–81.
489. Noya FJ, Rench MA, Metzger TG, et al. Unusual occurrence of an epidemic of type Ib/c group B streptococcal sepsis in a neonatal intensive care unit. *J Infect Dis* 1987;**155**:1135–44.
490. Nucci M, Anaissie E, Betts RF, et al. Early removal of central venous catheter in patients with candidemia does not improve outcome: analysis of 842 patients from 2 randomized clinical trials. *Clin Infect Dis* 2010;**51**:295–303.
491. Nuorti JP, Butler JC, Crutcher JM, et al. An outbreak of multidrug-resistant pneumococcal pneumonia and bacteremia among unvaccinated nursing home residents. *N Engl J Med* 1998;**338**:1861–8.
492. Nylund CM, Goudie A, Garza JM, et al. *Clostridium difficile* infection in hospitalized children in the United States. *Arch Pediatr Adolesc Med* 2011;**165**:451–7.
493. O'Connell K, Boo TW, Keady D, et al. Use of palivizumab and infection control measures to control an outbreak of respiratory syncytial virus in a neonatal intensive care unit confirmed by real-time polymerase chain reaction. *J Hosp Infect* 2011;**77**:338–42.
494. O'Grady NP, Alexander M, Burns LA, et al. Guidelines for the prevention of intravascular catheter-related infections. *Clin Infect Dis* 2011;**52**:e162–93.
495. O'Grady NP, Alexander M, Dellinger EP, et al. Guidelines for the prevention of intravascular catheter-related infections. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2002;**51**:1–29.
496. O'Grady NP, Murray PR, Ames N. Preventing ventilator-associated pneumonia: does the evidence support the practice? *JAMA* 2012;**307**:2534–9.
497. O'Horo JC, Thompson D, Safdar N. Is the Gram stain useful in the microbiologic diagnosis of VAP? A meta-analysis. *Clin Infect Dis* 2012;**55**:551–61.
498. Occupational Safety and Health Administration, Department of Labor. Occupational exposure to bloodborne pathogens: Final rule. *Fed Regist* 1991;**56**:64175–82.
499. Odio C, McCracken GH Jr, Nelson JD. CSF shunt infections in pediatrics: a seven-year experience. *Am J Dis Child* 1984;**138**:1103–8.
500. Oguz F, Uysal G, Dasdemir S, et al. The role of *Clostridium difficile* in childhood nosocomial diarrhea. *Scand J Infect Dis* 2001;**33**:731–3.
501. Oliveira C, Nasr A, Brindle M, et al. Ethanol locks to prevent catheter-related bloodstream infections in parenteral nutrition: a meta-analysis. *Pediatrics* 2012;**129**:318–29.
502. Orrett FA, Brooks PJ, Richardson EG, et al. Paediatric nosocomial urinary tract infection at a regional hospital. *Int Urol Nephrol* 1999;**31**:173–9.
503. Oskarsdottir S, Mellander L, Marky I, et al. *Clostridium difficile* in children with malignant disease. *Pediatr Hematol Oncol* 1991;**8**:269–72.
504. Ostovar GA, Kohn N, Yu KO, et al. Nosocomial influenza in a pediatric hospital: comparison of rates of seasonal and pandemic 2009 influenza A/H1N1 infection. *Infect Control Hosp Epidemiol* 2012;**33**:292–4.
505. Ostrowsky BE, Trick WE, Sohn AH, et al. Control of vancomycin-resistant enterococcus in health care facilities in a region. *N Engl J Med* 2001;**344**:1427–33.
506. Ostrowsky BE, Whitener C, Bredenberg HK, et al. *Serratia marcescens* bacteremia traced to an infused narcotic. *N Engl J Med* 2002;**346**:1529–37.
507. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol* 2011;**32**:687–99.
508. Padula PJ, Edelstein A, Miguel SD, et al. Hantavirus pulmonary syndrome outbreak in Argentina: molecular evidence for person-to-person transmission of Andes virus. *Virology* 1998;**241**:323–30.
509. Pakyz AL, Gurgle HE, Ibrahim OM, et al. Trends in antibacterial use in hospitalized pediatric patients in United States academic health centers. *Infect Control Hosp Epidemiol* 2009;**30**:600–3.
510. Panlilio AL, Cardo DM, Grohskopf LA, et al. Updated U.S. Public Health Service guidelines for the management of occupational exposures to HIV and recommendations for postexposure prophylaxis. *MMWR Recomm Rep* 2005;**54**:1–17.
511. Parienti JJ, du Cheyron D, Timsit JF, et al. Meta-analysis of subclavian insertion and nontunneled central venous catheter–associated infection risk reduction in critically ill adults. *Crit Care Med* 2012;**40**:1627–34.
512. Parker MJ, Livingstone V, Clifton R, et al. Closed suction surgical wound drainage after orthopaedic surgery. *Cochrane Database Syst Rev* 2007;**3**:CD001825.
513. Parry MF, Hutchinson JH, Brown NA, et al. Gram-negative sepsis in neonates: a nursery outbreak due to hand carriage of *Citrobacter diversus*. *Pediatrics* 1980;**65**:1105–9.
514. Patel SJ, Larson EL, Kubin CJ, et al. A review of antimicrobial control strategies in hospitalized and ambulatory pediatric populations. *Pediatr Infect Dis J* 2007;**26**:531–7.
515. Patel SJ, Oshodi A, Prasad P, et al. Antibiotic use in neonatal intensive care units and adherence with Centers for Disease Control and Prevention 12 Step Campaign to Prevent Antimicrobial Resistance. *Pediatr Infect Dis J* 2009;**28**:1047–51.
516. Patel SJ, Saiman L, Duchon JM, et al. Development of an antimicrobial stewardship intervention using a model of actionable feedback. *Interdiscip Perspect Infect Dis* 2012;**2012**:150367.
517. Patrick CH, John JF, Levkoff AH, et al. Relatedness of strains of methicillin-resistant coagulase-negative *Staphylococcus* colonizing hospital personnel and producing bacteremias in a neonatal intensive care unit. *Pediatr Infect Dis J* 1992;**11**:935–40.
518. Patterson JE, Madden GM, Krisiunas EP, et al. A nosocomial outbreak of ampicillin-resistant *Haemophilus influenzae* type b in a geriatric unit. *J Infect Dis* 1988;**157**:1002–7.
519. Peleg AY, Hooper DC. Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med* 2010;**362**:1804–13.
520. Perl TM, Cullen JJ, Wenzel RP, et al. Intranasal mupirocin to prevent postoperative *Staphylococcus aureus* infections. *N Engl J Med* 2002;**346**:1871–7.
521. Perl TM, Haugen TH, Pfaller MA, et al. Transmission of herpes simplex virus type 1 infection in an intensive care unit. *Ann Intern Med* 1992;**117**:584–6.
522. Peters CJ, Kuehne RW, Mercado RR, et al. Hemorrhagic fever in Cochabamba, Bolivia, 1971. *Am J Epidemiol* 1974;**99**:425–33.
523. Piedra PA, Kasel JA, Norton HJ, et al. Description of an adenovirus type 8 outbreak in hospitalized neonates born prematurely. *Pediatr Infect Dis J* 1992;**11**:460–5.
524. Pillay D, Patou G, Hurt S, et al. Parvovirus B19 outbreak in a children's ward. *Lancet* 1992;**339**:107–9.
525. Pittet D, Allegranzi B, Sax H, et al. Evidence-based model for hand transmission during patient care and the role of improved practices. *Lancet* 2006;**6**:641–52.

526. Pittet D, Dharan S, Touveneau S, et al. Bacterial contamination of the hands of hospital staff during routine patient care. *Arch Intern Med* 1999;**159**:821–6.
527. Polk BF, White JA, DeGirolami PC, et al. An outbreak of rubella among hospital personnel. *N Engl J Med* 1980;**303**:541–5.
528. Pollock EM, Ford-Jones EL, Rebeyka I, et al. Early nosocomial infections in pediatric cardiovascular surgery patients. *Crit Care Med* 1990;**18**:378–84.
529. Popovich KJ, Hota B, Hayes R, et al. Effectiveness of routine patient cleansing with chlorhexidine gluconate for infection prevention in the medical intensive care unit. *Infect Control Hosp Epidemiol* 2009;**30**:959–63.
530. Porter JD, Teter M, Traister V, et al. Outbreak of adenoviral infections in a long-term paediatric facility, New Jersey, 1986/87. *J Hosp Infect* 1991;**18**:201–10.
531. Powell H, Swarner O, Gluck L, et al. Hexachlorophene myelinopathy in premature infants. *J Pediatr* 1973;**82**:976–81.
532. Principi N, Esposito S. Ventilator-associated pneumonia (VAP) in pediatric intensive care units. *Pediatr Infect Dis J* 2007;**26**:841–3; discussion 843–4.
533. Pronovost PJ, Marsteller JA, Goeschel CA. Preventing bloodstream infections: a measurable national success story in quality improvement. *Health Aff (Millwood)* 2011;**30**:628–34.
534. Pronovost P, Needham D, Berenholtz S, et al. An intervention to decrease catheter-related bloodstream infections in the ICU. *N Engl J Med* 2006;**355**:2725–32.
535. Qualman SJ, Petric M, Karmali MA, et al. *Clostridium difficile* invasion and toxin circulation in fatal pediatric pseudomembranous colitis. *Am J Clin Pathol* 1990;**94**:410–16.
536. Raad II, Costerton W, Sabharwal U, et al. Ultrastructural analysis of indwelling vascular catheters: a quantitative relationship between luminal colonization and duration of placement. *J Infect Dis* 1993;**168**:400–7.
537. Raad II, Hanna H, Maki D. Intravascular catheter-related infections: advances in diagnosis, prevention, and management. *Lancet Infect Dis* 2007;**7**:645–57.
538. Raad II, Hohn DC, Gilbreath BJ, et al. Prevention of central venous catheter–related infections by using maximal sterile barrier precautions during insertion. *Infect Control Hosp Epidemiol* 1994; **15**:231–8.
539. Raad II, Mohamed JA, Reitzel RA, et al. Improved antibiotic-impregnated catheters with extended-spectrum activity against resistant bacteria and fungi. *Antimicrob Agents Chemother* 2012;**56**: 935–41.
540. Rabalais G, Adams G, Stover B. PPD skin test conversion in health-care workers after exposure to *Mycobacterium tuberculosis* infection in infants. *Lancet* 1991;**338**:826.
541. Rabkin CS, Telzak EE, Ho MS, et al. Outbreak of echovirus 11 infection in hospitalized neonates. *Pediatr Infect Dis J* 1988;**7**: 186–90.
542. Rafla K, Tredget EE. Infection control in the burn unit. *Burns* 2011;**37**:5–15.
543. Ramsey AM, Zilberberg MD. Secular trends of hospitalization with vancomycin-resistant *Enterococcus* infection in the United States, 2000-2006. *Infect Control Hosp Epidemiol* 2009;**30**:184–6.
544. Randolph AG, Wypij D, Venkataraman ST, et al. Effect of mechanical ventilator weaning protocols on respiratory outcomes in infants and children: a randomized controlled trial. *JAMA* 2002;**288**:2561–8.
545. Ratnam S, Mercer E, Picco B, et al. A nosocomial outbreak of diarrheal disease due to *Yersinia enterocolitica* serotype 0:5, biotype 1. *J Infect Dis* 1982;**145**:242–7.
546. Ratner AJ, Neu N, Jakob K, et al. Nosocomial rotavirus in a pediatric hospital. *Infect Control Hosp Epidemiol* 2001;**22**:299–301.
547. Rattanaumpawan P, Tolomeo P, Bilker WB, et al. Risk factors for fluoroquinolone resistance in gram-negative bacilli causing healthcare-acquired urinary tract infections. *J Hosp Infect* 2010;**76**: 324–7.
548. Raval MV, Dillon PW, Bruny JL, et al. American College of Surgeons National Surgical Quality Improvement Program Pediatric: a phase 1 report. *J Am Coll Surg* 2011;**212**:1–11.
549. Ray SM, Erdman DD, Berschling JD, et al. Nosocomial exposure to parvovirus B19: low risk of transmission to healthcare workers. *Infect Control Hosp Epidemiol* 1997;**18**:109–14.
550. Remington PL, Hall WN, Davis IH, et al. Airborne transmission of measles in a physician's office. *JAMA* 1985;**253**:1574–7.
551. Reuman PD, Duckworth DH, Smith KL, et al. Lack of effect of *Lactobacillus* on gastrointestinal bacterial colonization in premature infants. *Pediatr Infect Dis* 1986;**5**:663–8.
552. Reyes JA, Habash ML, Taylor RP. Femoral central venous catheters are not associated with higher rates of infection in the pediatric critical care population. *Am J Infect Control* 2012;**40**:43–7.
553. Richards GA, Murphy S, Jobson R, et al. Unexpected Ebola virus in a tertiary setting: clinical and epidemiologic aspects. *Crit Care Med* 2000;**28**:240–4.
554. Richards MJ, Edwards JR, Culver DH, et al. Nosocomial infections in pediatric intensive care units in the United States. *Pediatrics* 1999;**103**:e39.
555. Richet HM, Craven PC, Brown JM, et al. A cluster of *Rhodococcus (Gordona) bronchialis* sternal-wound infections after coronary-artery bypass surgery. *N Engl J Med* 1991;**324**:104–9.
556. Richet HM, McNeil MM, Edwards MC, et al. Cluster of *Malassezia furfur* pulmonary infections in infants in a neonatal intensive-care unit. *J Clin Microbiol* 1989;**27**:1197–200.
557. Richman DD, Breton SJ, Goldman DA. Scarlet fever and group A streptococcal surgical wound infection traced to an anal carrier. *J Pediatr* 1977;**90**:387–90.
558. Robert J, Fridkin SK, Blumberg HM, et al. The influence of the composition of the nursing staff on primary bloodstream infection rates in a surgical intensive care unit. *Infect Control Hosp Epidemiol* 2000;**21**:12–17.
559. Rogers M, Weinstock DM, Eagan J, et al. Rotavirus outbreak on a pediatric oncology floor: possible association with toys. *Am J Infect Control* 2000;**28**:378–80.
560. Rose HD, Lenz IE, Sheth NK. Meningococcal pneumonia. A source of nosocomial infection. *Arch Intern Med* 1981;**141**: 575–7.
561. Rowen JL, Atkins JT, Levy ML, et al. Invasive fungal dermatitis in the < or = 1000-gram neonate. *Pediatrics* 1995;**95**:682–7.
562. Roy TE, McDonald S, Patrick ML, et al. A survey of hospital infection in a pediatric hospital, part I: description of hospital, organization of survey, population studied and some general findings. *Can Med Assoc J* 1962;**87**:531–8.
563. Roy TE, McDonald S, Patrick ML, et al. A survey of hospital infection in a pediatric hospital, part II: the distribution of hospital infections in different areas of the hospital, postoperative wound infections and the consequences of infection. *Can Med Assoc J* 1962;**87**:592–9.
564. Roy TE, McDonald S, Patrick ML, et al. A survey of hospital infection in a pediatric hospital, part III: staphylococcal infections, notes on antibiotic sensitivity of the staphylococci from postoperative wound and other infections, discussion and summary. *Can Med Assoc J* 1962;**87**:656–60.
565. Rupp ME, Lisco SJ, Lipsett PA, et al. Effect of a second-generation venous catheter impregnated with chlorhexidine and silver sulfadiazine on central catheter-related infections: a randomized, controlled trial. *Ann Intern Med* 2005;**143**:570–80.
566. Rushforth JA, Hoy CM, Kite P, et al. Rapid diagnosis of central venous catheter sepsis. *Lancet* 1993;**342**:402–3.
567. Rutala WA, Weber DJ, Healthcare Infection Control Practices Advisory Committee. *Guideline for disinfection and sterilization in healthcare facilities, 2008*. Centers for Disease Control and Prevention; 2008. Available at http://www.cdc.gov/hicpac/pdf/Disinfection_Sterilization/Pages1_2Disinfection_Nov_2008.pdf. Accessed October 23, 2012.
568. Ryckman FC, Schoettker PJ, Hays KR, et al. Reducing surgical site infections at a pediatric academic medical center. *Jt Comm J Qual Patient Saf* 2009;**35**:192–8.
569. Ryder RW, McGowan JE, Hatch MH, et al. Reovirus-like agent as a cause of nosocomial diarrhea in infants. *J Pediatr* 1977;**90**: 698–702.
570. Ryder RW, Rahman AS, Alim AR, et al. An outbreak of nosocomial cholera in a rural Bangladesh hospital. *J Hosp Infect* 1986;**8**: 275–82.
571. Safdar N, Fine JP, Maki DG. Meta-analysis: methods for diagnosing intravascular device-related bloodstream infection. *Ann Intern Med* 2005;**142**:451–66.
572. Saiman L, Lerner A, Saal L, et al. Banning artificial nails from health care settings. *Am J Infect Control* 2002;**30**:252–4.
573. Saiman L, Ludington E, Dawson JD, et al. Risk factors for *Candida* species colonization of neonatal intensive care unit patients. *Pediatr Infect Dis J* 2001;**20**:1119–24.

574. Saiman L, Ludington E, Pfaller M, et al. Risk factors for candidemia in neonatal intensive care unit patients. *Pediatr Infect Dis J* 2000;**19**:319–24.
575. Saiman L, Siegel J. Infection control recommendations for patients with cystic fibrosis: microbiology, important pathogens, and infection control practices to prevent patient-to-patient transmission. *Infect Control Hospital Epidemiol* 2003;**24**:S6–52.
576. Saint S, Chenoweth CE. Biofilms and catheter-associated urinary tract infections. *Infect Dis Clin North Am* 2003;**17**:411–32.
577. Saint S, Howell JD, Krein SL. Implementation science: how to jump-start infection prevention. *Infect Control Hosp Epidemiol* 2010;**31**(Suppl. 1):S14–17.
578. Saint S, Kowalski CP, Banaszak-Holl J, et al. The importance of leadership in preventing healthcare-associated infection: results of a multisite qualitative study. *Infect Control Hosp Epidemiol* 2010; **31**:901–7.
579. Sakaoka H, Saheki Y, Uzuki K, et al. Two outbreaks of herpes simplex virus type 1 nosocomial infection among newborns. *J Clin Microbiol* 1986;**24**:36–40.
580. Sampath LA, Tambe SM, Modak SM. In vitro and in vivo efficacy of catheters impregnated with antiseptics or antibiotics: evaluation of the risk of bacterial resistance to the antimicrobials in the catheters. *Infect Control Hosp Epidemiol* 2001;**22**:640–6.
581. Sandora TJ, Fung M, Flaherty K, et al. Epidemiology and risk factors for *Clostridium difficile* infection in children. *Pediatr Infect Dis J* 2011;**30**:580–4.
582. Sattar SA, Lloyd-Evans N, Springthorpe VS, et al. Institutional outbreaks of rotavirus diarrhoea: potential role of fomites and environmental surfaces as vehicles for virus transmission. *J Hyg (Lond)* 1986;**96**:277–89.
583. Sawyer MH, Chamberlin CJ, Wu YN, et al. Detection of varicella-zoster virus DNA in air samples from hospital rooms. *J Infect Dis* 1994;**169**:91–4.
584. Sax H, Allegranzi B, Chraiti MN, et al. The World Health Organization hand hygiene observation method. *Am J Infect Control* 2009;**37**:827–34.
585. Sax H, Allegranzi B, Uckay I, et al. "My five moments for hand hygiene": a user-centred design approach to understand, train, monitor and report hand hygiene. *J Hosp Infect* 2007;**67**:9–21.
586. Schaffer TE, Sylvester RF Jr, Baldwin JN. Staphylococcal infections in newborn infants, part II: report of 19 epidemics caused by an identical strain of *Staphylococcus pyogenes*. *Am J Public Health* 1957;**47**:990–1008.
587. Scheckler WE, Brimhall D, Buck AS, et al. Requirements for infrastructure and essential activities of infection control and epidemiology in hospitals: a consensus panel report. *Infect Control Hosp Epidemiol* 1998;**19**:114–24.
588. Scheifele D, Bonner M. Airborne transmission of chickenpox. *N Engl J Med* 1980;**303**:281–2.
589. Schlager T, Sadler J, Weber D, et al. Hospital-acquired infections in pediatric burn patients. *South Med J* 1994;**87**:481–4.
590. Schlesinger A, Paul M, Gafter-Gvili A, et al. Infection-control interventions for cancer patients after chemotherapy: a systematic review and meta-analysis. *Lancet Infect Dis* 2009;**9**:97–107.
591. Schrag SJ, Zywicki S, Farley MM, et al. Group B streptococcal disease in the era of intrapartum antibiotic prophylaxis. *N Engl J Med* 2000;**342**:15–20.
592. Schulman J, Stricof RL, Stevens TP, et al. Development of a statewide collaborative to decrease NICU central line–associated bloodstream infections. *J Perinatol* 2009;**29**:591–9.
593. Schulman J, Stricof RL, Stevens TP, et al. Statewide NICU central-line–associated bloodstream infection rates decline after bundles and checklists. *Pediatrics* 2011;**127**:436–44.
594. Sehulster LM, Chinn RYW, Arduino MJ, et al. *Guidelines for environmental infection control in health-care facilities*. Recommendations from CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC). Chicago: American Society for Healthcare Engineering/American Hospital Association; 2004.
595. Selwyn S. Hospital infection: the first 2500 years. *J Hosp Infect* 1991;**18**:5–64.
596. Semmelweis IF. *The etiology, the concept and the prophylaxis of childbed fever*. Ann Arbor, Mi: University of Michigan: Classics of Medicine Library; 1981.
597. Senerwa D, Olsvik O, Mutanda LN, et al. Enteropathogenic *Escherichia coli* serotype O111:HNT isolated from preterm neonates in Nairobi, Kenya. *J Clin Microbiol* 1989;**27**:1307–11.
598. Sengupta A, Lehmann C, Diener-West M, et al. Catheter duration and risk of CLA-BSI in neonates with PICCs. *Pediatrics* 2010; **125**:648–53.
599. Severien C, Nelson JD. Frequency of infections associated with implanted systems vs cuffed, tunneled Silastic venous catheters in patients with acute leukemia. *Am J Dis Child* 1991;**145**:1433–8.
600. Sharma LK, Sharma PK. Postoperative wound infection in a pediatric surgical service. *J Pediatr Surg* 1986;**21**:889–91.
601. Shay DK, Holman RC, Newman RD, et al. Bronchiolitis-associated hospitalizations among US children, 1980-1996. *JAMA* 1999;**282**:1440–6.
602. Shefer A, Dales L, Nelson M, et al. Use and safety of acellular pertussis vaccine among adult hospital staff during an outbreak of pertussis. *J Infect Dis* 1995;**171**:1053–6.
603. Sherertz RJ, Gledhill KS, Hampton KD, et al. Outbreak of *Candida* bloodstream infections associated with retrograde medication administration in a neonatal intensive care unit. *J Pediatr* 1992;**120**:455–61.
604. Sherertz RJ, Heard SO, Raad II. Diagnosis of triple-lumen catheter infection: comparison of roll plate, sonication, and flushing methodologies. *J Clin Microbiol* 1997;**35**:641–6.
605. Shimandle RB, Johnson D, Baker M, et al. Safety of peripheral intravenous catheters in children. *Infect Control Hosp Epidemiol* 1999;**20**:736–40.
606. Shorr AF, Sherner JH, Jackson WL, et al. Invasive approaches to the diagnosis of ventilator-associated pneumonia: a meta-analysis. *Crit Care Med* 2005:46–53.
607. Shuman RM, Leech RW, Alvord EC Jr. Neurotoxicity of hexachlorophene in the human, part I: a clinicopathologic study of 248 children. *Pediatrics* 1974;**54**:689–95.
608. Shuman RM, Leech RW, Alvord EC Jr. Neurotoxicity of hexachlorophene in humans, part II: a clinicopathological study of 46 premature infants. *Arch Neurol* 1975;**32**:320–5.
609. Siegel JD, Rhinehart E, Jackson M, et al. *Management of multidrug-resistant organisms in healthcare settings, 2006*. Centers for Disease Control and Prevention Healthcare Infection Control Practices Advisory Committee; 2006. Available at http://www.cdc.gov/ncidod/dhqp/pdf/ar/mdroGuideline2006.pdf. Accessed April 25, 2007.
610. Siegel JD, Rhinehart E, Jackson M, et al. *Guideline for isolation precautions: preventing transmission of infectious agents in healthcare settings 2007*. Centers for Disease Control and Prevention; 2007. Available at http://www.cdc.gov/ncidod/dhqp/pdf/guidelines/Isolation2007.pdf. Accessed October 24, 2012.
611. Siegman-Igra Y, Anglim AM, Shapiro DE, et al. Diagnosis of vascular catheter-related bloodstream infection: a meta-analysis. *J Clin Microbiol* 1997;**35**:928–36.
612. Silva A, Rodig N, Passerotti CP, et al. Risk factors for urinary tract infection after renal transplantation and its impact on graft function in children and young adults. *J Urol* 2010;**184**:1462–7.
613. Silvestri L, van Saene HK, Thomann C, et al. Selective decontamination of the digestive tract reduces pneumonia and mortality without resistance emerging. *Am J Infect Control* 2007;**35**: 354–7.
614. Simmons BP, Gelfand MS, Haas M, et al. *Enterobacter sakazakii* infections in neonates associated with intrinsic contamination of a powdered infant formula. *Infect Control Hosp Epidemiol* 1989;**10**: 398–401.
615. Simon A, Muller A, Khurana K, et al. Nosocomial infection: a risk factor for a complicated course in children with respiratory syncytial virus infection—results from a prospective multicenter German surveillance study. *Int J Hyg Environ Health* 2008;**211**: 241–50.
616. Simon TD, Hall M, Riva-Cambrin J, et al. Infection rates following initial cerebrospinal fluid shunt placement across pediatric hospitals in the United States. Clinical article. *J Neurosurg Pediatr* 2009;**4**:156–65.
617. Simon TD, Whitlock KB, Riva-Cambrin J, et al. Revision surgeries are associated with significant increased risk of subsequent cerebrospinal fluid shunt infection. *Pediatr Infect Dis J* 2012;**31**: 551–6.
618. Simor AE, Phillips E, McGeer A, et al. Randomized controlled trial of chlorhexidine gluconate for washing, intranasal mupirocin, and rifampin and doxycycline versus no treatment for the eradication of methicillin-resistant *Staphylococcus aureus* colonization. *Clin Infect Dis* 2007;**44**:178–85.

619. Singh-Naz N, Brown M, Ganeshananthan M. Nosocomial adenovirus infection: molecular epidemiology of an outbreak. *Pediatr Infect Dis J* 1993;**12**:922–5.
620. Smith DH, Johnson BK, Isaacson M, et al. Marburg-virus disease in Kenya. *Lancet* 1982;**1**:816–20.
621. Smith JS, Shaffrey CI, Sansur CA, et al. Rates of infection after spine surgery based on 108,419 procedures: a report from the Scoliosis Research Society Morbidity and Mortality Committee. *Spine (Phila Pa 1976)* 2011;**36**:556–63.
622. Smith MJ, Clark HF, Lawley D, et al. The clinical and molecular epidemiology of community- and healthcare-acquired rotavirus gastroenteritis. *Pediatr Infect Dis J* 2008;**27**:54–8.
623. Smith PF, Stricof RL, Shayegani M, et al. Cluster of *Haemophilus influenzae* type b infections in adults. *JAMA* 1988;**260**:1446–9.
624. Smith PW, Bennett G, Bradley S, et al. SHEA/APIC guideline: infection prevention and control in the long-term care facility, July 2008. *Infect Control Hosp Epidemiol* 2008;**29**:785–814.
625. Smith PW, Rusnak PG. Infection prevention and control in the long-term-care facility. *Infect Control Hosp Epidemiol* 1997;**18**:831–49.
626. Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci Translation Med* 2012;**4**:148ra116.
627. Society for Healthcare Epidemiology of America (SHEA), Infectious Diseases Society of America (IDSA), Pediatric Infectious Diseases Society (PIDS). Policy statement on antimicrobial stewardship by the Society for Healthcare Epidemiology of America (SHEA), the Infectious Diseases Society of America (IDSA), and the Pediatric Infectious Diseases Society (PIDS). *Infect Control Hosp Epidemiol* 2012;**33**:322–7.
628. Sohn AH, Garrett DO, Sinkowitz-Cochran RL, et al. Prevalence of nosocomial infections in neonatal intensive care unit patients: results from the first national point-prevalence survey. *J Pediatr* 2001;**139**:821–7.
629. Sohn AH, Schwartz JM, Yang KY, et al. Risk factors and risk adjustment for surgical site infections in pediatric cardiothoracic surgery patients. *Am J Infect Control* 2010;**38**:706–10.
630. Solomon LR, Cheesbrough JS, Ebah L, et al. A randomized double-blind controlled trial of taurolidine-citrate catheter locks for the prevention of bacteremia in patients treated with hemodialysis. *Am J Kidney Dis* 2010;**55**:1060–8.
631. Spector SA. Transmission of cytomegalovirus among infants in hospital documented by restriction-endonuclease-digestion analyses. *Lancet* 1983;**1**:378–81.
632. Spratt HC, Marks MI, Gomersall M, et al. Nosocomial infantile gastroenteritis associated with minirotavirus and calicivirus. *J Pediatr* 1978;**93**:922–6.
633. Srinivasan R, Asselin J, Gildengorin G, et al. A prospective study of ventilator-associated pneumonia in children. *Pediatrics* 2009;**123**:1108–15.
634. Starke JR. Tuberculosis in children. *Curr Opin Pediatr* 1995;**7**:268–77.
635. Steketee RW, Burstyn DG, Wassilak SG, et al. A comparison of laboratory and clinical methods for diagnosing pertussis in an outbreak in a facility for the developmentally disabled. *J Infect Dis* 1988;**157**:441–9.
636. Stenzel JP, Green TP, Fuhrman BP, et al. Percutaneous femoral venous catheterizations: a prospective study of complications. *J Pediatr* 1989;**114**:411–15.
637. Stevens DL, Bisno AL, Chambers HF, et al. Practice guidelines for the diagnosis and management of skin and soft-tissue infections. *Clin Infect Dis* 2005;**41**:1373–406.
638. Stewart CJ. Tuberculosis infection in a paediatric department. *BMJ* 1976;**1**:30–2.
639. Stoll BJ, Hansen N, Fanaroff AA, et al. *Enterobacter sakazakii* is a rare cause of neonatal septicemia or meningitis in VLBW infants. *J Pediatr* 2004;**144**:821–3.
640. Stoll BJ, Temprosa M, Tyson JE, et al. Dexamethasone therapy increases infection in very low birth weight infants. *Pediatrics* 1999;**104**:e63.
641. Stone A, Shaffer M, Sautter RL. *Salmonella poona* infection and surveillance in a neonatal nursery. *Am J Infect Control* 1993;**21**:270–3.
642. Strassburg MA, Imagawa DT, Fannin SL, et al. Rubella outbreak among hospital employees. *Obstet Gynecol* 1981;**57**:283–8.
643. Straube RC, Thompson MA, Van Dyke RB, et al. Adenovirus type 7b in a children's hospital. *J Infect Dis* 1983;**147**:814–19.
644. Strausbaugh LJ, Siegel JD, Weinstein RA. Preventing transmission of multidrug-resistant bacteria in health care settings: a tale of 2 guidelines. *Clin Infect Dis* 2006;**42**:828–35.
645. Strikas RA, Neff LJ, Rotz L, et al. US Civilian Smallpox Preparedness and Response Program, 2003. *Clin Infect Dis* 2008;**46**(Suppl. 3):S157–67.
646. Suleiman MN, Muscat-Baron JM, Harries JR, et al. Congo/Crimean haemorrhagic fever in Dubai: an outbreak at the Rashid Hospital. *Lancet* 1980;**2**:939–41.
647. Tablan OC, Anderson LJ, Besser R, et al. *Guidelines for preventing health-care–associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee*. Centers for Disease Control and Prevention Healthcare Infection Control Practices Advisory Committee; 2004. Available at http://www.cdc.gov/ncidod/dhqp/pdf/guidelines/CDCpneumo_guidelines.pdf. Accessed April 25, 2007.
648. Taira BR, Fenton KE, Lee TK, et al. Ventilator-associated pneumonia in pediatric trauma patients. *Pediatr Crit Care Med* 2009;**10**:491–4.
649. Takami T, Sonodat S, Houjyo H, et al. Diagnosis of horizontal enterovirus infections in neonates by nested PCR and direct sequence analysis. *J Hosp Infect* 2000;**45**:283–7.
650. Talbot TR, Babcock H, Caplan AL, et al. Revised SHEA position paper: influenza vaccination of healthcare personnel. *Infection control and hospital epidemiology: the official journal of the Society of Hospital Epidemiologists of America* 2010;**31**:987–95.
651. Tambyah PA, Halvorson KT, Maki DG. A prospective study of pathogenesis of catheter-associated urinary tract infections. *Mayo Clin Proc* 1999;**74**:131–6.
652. Tambyah PA, Maki DG. The relationship between pyuria and infection in patients with indwelling urinary catheters: a prospective study of 761 patients. *Arch Intern Med* 2000;**160**:673–7.
653. Tamma PD, Aucott SW, Milstone AM. Chlorhexidine use in the neonatal intensive care unit: results from a national survey. *Infect Control Hosp Epidemiol* 2010;**31**:846–9.
654. Tamma PD, Savard P, Pal T, et al. An outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive care unit. *Infect Control Hosp Epidemiol* 2012;**33**:631–4.
655. Tamma PD, Turnbull AE, Milstone AM, et al. Ventilator-associated tracheitis in children: does antibiotic duration matter? *Clin Infect Dis* 2011;**52**:1324–31.
656. Tanaka Y, Fujinaga K, Goto A, et al. Outbreak of pertussis in a residential facility for handicapped people. *Dev Biol Stand* 1991;**73**:329–32.
657. Tanner J, Norrie P, Melen K. Preoperative hair removal to reduce surgical site infection. *Cochrane Database Syst Rev* 2011;**11**:CD004122.
658. Tanner J, Swarbrook S, Stuart J. Surgical hand antisepsis to reduce surgical site infection. *Cochrane Database Syst Rev* 2008;**1**:CD004288.
659. Taylor G, Gravel D, Johnston L, et al. Prospective surveillance for primary bloodstream infections occurring in Canadian hemodialysis units. *Infect Control Hosp Epidemiol* 2002;**23**:716–20.
660. Tenorio AR, Badri SM, Sahgal NB, et al. Effectiveness of gloves in the prevention of hand carriage of vancomycin-resistant enterococcus species by health care workers after patient care. *Clin Infect Dis* 2001;**32**:826–9.
661. The Canadian Critical Care Trials Group. A randomized trial of diagnostic techniques for ventilator-associated pneumonia. *N Engl J Med* 2006;**355**:2619–30.
662. The Society for Hospital Epidemiology of America, the Association for Practitioners in Infection Control, the Centers for Disease Control, et al. Consensus paper on the surveillance of surgical wound infections. *Infect Control Hosp Epidemiol* 1992;**13**:599–605.
663. Thompson ND, Perz JF, Moorman AC, et al. Nonhospital health care–associated hepatitis B and C virus transmission: United States, 1998-2008. *Ann Intern Med* 2009;**150**:33–9.
664. Timsit JF, Schwebel C, Bouadma L, et al. Chlorhexidine-impregnated sponges and less frequent dressing changes for prevention of catheter-related infections in critically ill adults: a randomized controlled trial. *JAMA* 2009;**301**:1231–41.
665. Tobin MJ. Advances in mechanical ventilation. *N Engl J Med* 2001;**344**:1986–96.

666. Togo A, Coulibaly Y, Dembele BT, et al. Risk factors for surgical site infection in children at the teaching hospital Gabriel Toure, Bamako. *J Hosp Infect* 2011;**79**:371–2.
667. Toltzis P, Kim J, Dul M, et al. Presence of the epidemic North American Pulsed Field type 1 *Clostridium difficile* strain in hospitalized children. *J Pediatr* 2009;**154**:607–8.
668. Trinh TT, Chan PA, Edwards O, et al. Peripheral venous catheter-related *Staphylococcus aureus* bacteremia. *Infect Control Hosp Epidemiol* 2011;**32**:579–83.
669. Tschudin-Sutter S, Frei R, Egli-Gany D, et al. No risk of surgical site infections from residual bacteria after disinfection with povidone-iodine-alcohol in 1014 cases: a prospective observational study. *Ann Surg* 2012;**255**:565–9.
670. Tuan TJ, Thorell EA, Hamblett NM, et al. Treatment and microbiology of repeated cerebrospinal fluid shunt infections in children. *Pediatr Infect Dis J* 2011;**30**:731–5.
671. Uludag O, Rieu P, Niessen M, et al. Incidence of surgical site infections in pediatric patients: a 3-month prospective study in an academic pediatric surgical unit. *Pediatr Surg Int* 2000;**16**: 417–20.
672. Uvin P, Van Baelen A, Verhaegen J, et al. Ureteral stents do not cause bacterial infections in children after ureteral reimplantation. *Urology* 2011;**78**:154–8.
673. Valenti WM, Menegus MA, Hall CB, et al. Nosocomial viral infections, part I: epidemiology and significance. *Infect Control* 1980;**1**:33–7.
674. Valenti WM, Pincus PH, Messner MK. Nosocomial pertussis: possible spread by a hospital visitor. *Am J Dis Child* 1980;**134**: 520–1.
675. van Acker J, de Smet F, Muyldermans G, et al. Outbreak of necrotizing enterocolitis associated with *Enterobacter sakazakii* in powdered milk formula. *J Clin Microbiol* 2001;**39**:293–7.
676. van de Pol AC, Rossen JW, Wolfs TF, et al. Transmission of respiratory syncytial virus at the paediatric intensive-care unit: a prospective study using real-time PCR. *Clin Microbiol Infect* 2010;**16**:488–90.
677. van Dijk WC, van der Straaten PJ. An outbreak of *Campylobacter jejuni* infection in a neonatal intensive care unit. *J Hosp Infect* 1988;**11**:91–2.
678. Van Dyke RB, Spector SA. Transmission of herpes simplex virus type 1 to a newborn infant during endotracheal suctioning for meconium aspiration. *Pediatr Infect Dis J* 1984;**3**:153–6.
679. van Rijen MM, Bonten M, Wenzel RP, et al. Intranasal mupirocin for reduction of *Staphylococcus aureus* infections in surgical patients with nasal carriage: a systematic review. *J Antimicrob Chemother* 2008;**61**:254–61.
680. Vasudevan A, Iyengar A, Jose B, et al. Pediatric renal transplantation: a single-center experience. *Transplant Proc* 2008;**40**: 1095–8.
681. Vayalumkal JV, Gravel D, Moore D, et al. Surveillance for healthcare-acquired febrile respiratory infection in pediatric hospitals participating in the Canadian Nosocomial Infection Surveillance Program. *Infect Control Hosp Epidemiol* 2009;**30**: 652–8.
682. Venkatachalam V, Hendley JO, Willson DF. The diagnostic dilemma of ventilator-associated pneumonia in critically ill children. *Pediatr Crit Care Med* 2011;**12**:286–96.
683. Verhagen P, Moore D, Manges A, et al. Nosocomial rotavirus gastroenteritis in a Canadian paediatric hospital: incidence, disease burden and patients affected. *J Hosp Infect* 2011;**79**:59–63.
684. Vermaat JH, Rosebrugh E, Ford-Jones EL, et al. An epidemiologic study of nosocomial infections in a pediatric long-term care facility. *Am J Infect Control* 1993;**21**:183–8.
685. Viau RA, Hujer AM, Marshall SH, et al. "Silent" dissemination of *Klebsiella pneumoniae* isolates bearing *K. pneumoniae* carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. *Clin Infect Dis* 2012;**54**:1314–21.
686. Vij NK, Stryker CC, Esper FP, et al. Influenza A/H1N1/09-10 infections in a NICU during the 2009-10 H1N1 pandemic. *Pediatrics* 2011;**128**:e1297–301.
687. Vonberg RP, Gastmeier P. Nosocomial aspergillosis in outbreak settings. *J Hosp Infect* 2006;**63**:246–54.
688. Waisbourd-Zinman O, Ben-Ziony S, Solter E, et al. The percentage of nosocomial-related out of total hospitalizations for rotavirus gastroenteritis and its association with hand hygiene compliance. *Am J Infect Control* 2011;**39**:166–8.
689. Wang F, Bo L, Tang L, et al. Subglottic secretion drainage for preventing ventilator-associated pneumonia: an updated meta-analysis of randomized controlled trials. *J Trauma Acute Care Surg* 2012;**72**:1276–85.
690. Warady BA, Bakkaloglu S, Newland J, et al. Consensus guidelines for the prevention and treatment of catheter-related infections and peritonitis in pediatric patients receiving peritoneal dialysis: 2012 update. *Perit Dial Int* 2012;**32**(Suppl. 2):S32–86.
691. Warren DK, Cosgrove SE, Diekema DJ, et al. A multicenter intervention to prevent catheter-associated bloodstream infections. *Infect Control Hosp Epidemiol* 2006;**27**:662–9.
692. Warwick S, Wilks M, Hennessy E, et al. Use of quantitative 16S ribosomal DNA detection for diagnosis of central vascular catheter-associated bacterial infection. *J Clin Microbiol* 2004;**42**: 1402–8.
693. Watson JC, Fleming DW, Borella AJ, et al. Vertical transmission of hepatitis A resulting in an outbreak in a neonatal intensive care unit. *J Infect Dis* 1993;**167**:567–71.
694. Weber DJ, Rutala WA. Risks and prevention of nosocomial transmission of rare zoonotic diseases. *Clin Infect Dis* 2001;**32**: 446–56.
695. Weber DJ, Rutala WA, Miller MB, et al. Role of hospital surfaces in the transmission of emerging health care–associated pathogens: norovirus, *Clostridium difficile*, and Acinetobacter species. *Am J Infect Control* 2010;**38**:S25–33.
696. Weber JM, Sheridan RL, Pasternack MS, et al. Nosocomial infections in pediatric patients with burns. *Am J Infect Control* 1997; **25**:195–201.
697. Weber JM, Sheridan RL, Schulz JT, et al. Effectiveness of bacteria-controlled nursing units in preventing cross-colonization with resistant bacteria in severely burned children. *Infect Control Hosp Epidemiol* 2002;**23**:549–51.
698. Webster J, Osborne S. Preoperative bathing or showering with skin antiseptics to prevent surgical site infection. *Cochrane Database Syst Rev* 2007;**9**:CD004985.
699. Wehrle PF, Posch J, Richter KH, et al. An airborne outbreak of smallpox in a German hospital and its significance with respect to other recent outbreaks in Europe. *Bull World Health Organ* 1970;**43**:669–79.
700. Weinstein JW, Barrett CR, Baltimore RS, et al. Nosocomial transmission of tuberculosis from a hospital visitor on a pediatrics ward. *Pediatr Infect Dis J* 1995;**14**:232–4.
701. Weisman LE, Cruess DF, Fischer GW. Opsonic activity of commercially available standard intravenous immunoglobulin preparations. *Pediatr Infect Dis J* 1994;**13**:1122–5.
702. Weisman LE, Stoll BJ, Kueser TJ, et al. Intravenous immune globulin prophylaxis of late-onset sepsis in premature neonates. *J Pediatr* 1994;**125**:922–30.
703. Weiss K, Restieri C, Gauthier R, et al. A nosocomial outbreak of fluoroquinolone-resistant *Streptococcus pneumoniae*. *Clin Infect Dis* 2001;**33**:517–22.
704. Welliver RC, McLaughlin S. Unique epidemiology of nosocomial infection in a children's hospital. *Am J Dis Child* 1984;**138**: 131–5.
705. Wener KM, Schechner V, Gold HS, et al. Treatment with fluoroquinolones or with beta-lactam-beta-lactamase inhibitor combinations is a risk factor for isolation of extended-spectrum-beta-lactamase-producing *Klebsiella* species in hospitalized patients. *Antimicrob Agents Chemother* 2010;**54**:2010–16.
706. Wenzel RP. *Prevention and control of nosocomial infections*. Philadelphia: Lippincott Williams & Wilkins; 2003.
707. Wenzel RP, Deal EC, Hendley JO. Hospital-acquired viral respiratory illness on a pediatric ward. *Pediatrics* 1977;**60**:367–71.
708. Wesley AG, Pather M, Tait D. Nosocomial adenovirus infection in a paediatric respiratory unit. *J Hosp Infect* 1993;**25**: 183–90.
709. Wharton M, Cochi SL, Hutcheson RH, et al. Mumps transmission in hospitals. *Arch Intern Med* 1990;**150**:47–9.
710. Whitby M, Pessoa-Silva CL, McLaws ML, et al. Behavioural considerations for hand hygiene practices: the basic building blocks. *J Hosp Infect* 2007;**65**:1–8.
711. Wiener ES. Catheter sepsis: the central venous line achilles' heel. *Semin Pediatr Surg* 1995;**4**:207–14.
712. Wilde J, Yolken R, Willoughby R, et al. Improved detection of rotavirus shedding by polymerase chain reaction. *Lancet* 1991; **337**:323–6.

713. Williams WW, Mariano J, Spurrier M, et al. Nosocomial meningitis due to *Citrobacter diversus* in neonates: new aspects of the epidemiology. *J Infect Dis* 1984;**150**:229–35.
714. Wirtschafter DD, Powers RJ, Pettit JS, et al. Nosocomial infection reduction in VLBW infants with a statewide quality-improvement model. *Pediatrics* 2011;**127**:419–26.
715. Wisplinghoff H, Bischoff T, Tallent SM, et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; **39**:309–17.
716. World Health Organization. *Interim Infection Control Recommendations for Care of Patients with Suspected or Confirmed Filovirus (Ebola, Marburg) Haemorrhagic Fever*. 2008. http://www.who.int/csr/bioriskreduction/filovirus_infection_control/en/index.html
717. Wright ML, Romano MJ. Ventilator-associated pneumonia in children. *Semin Pediatr Infect Dis* 2006;**17**:58–64.
718. Wurzel CL, Halom K, Feldman JG, et al. Infection rates of Broviac-Hickman catheters and implantable venous devices. *Am J Dis Child* 1988;**142**:536–40.
719. Wylie MC, Graham DA, Potter-Bynoe G, et al. Risk factors for central line-associated bloodstream infection in pediatric intensive care units. *Infect Control Hosp Epidemiol* 2010;**31**:1049–56.
720. Yahav D, Rozen-Zvi B, Gafter-Gvili A, et al. Antimicrobial lock solutions for the prevention of infections associated with intravascular catheters in patients undergoing hemodialysis: systematic review and meta-analysis of randomized, controlled trials. *Clin Infect Dis* 2008;**47**:83–93.
721. Yogev R. Cerebrospinal fluid shunt infections: a personal view. *Pediatr Infect Dis J* 1985;**4**:113–18.
722. Zaidi AK, Macone A, Goldmann AD. Impact of simple screening criteria on utilization of low-yield bacterial stool cultures in a Children's Hospital. *Pediatrics* 1999;**103**:1189–92.
723. Zhou J, Garber E, Saiman L. Survey of infection control policies for patients with cystic fibrosis in the United States. *Am J Infect Control* 2008;**36**:220–2.
724. Zilberberg MD, Shorr AF, Kollef MH. Increase in *Clostridium difficile*-related hospitalizations among infants in the United States, 2000-2005. *Pediatr Inf Dis J* 2008;**27**:1111–13.
725. Zimmerli W, Trampuz A, Ochsner PE. Prosthetic-joint infections. *N Engl J Med* 2004;**351**:1645–54.
726. Zoltanski J, Dul M, O'Riordan MA, et al. Low frequency of endemic patient-to-patient transmission of antibiotic-resistant gram-negative bacilli in a pediatric intensive care unit. *Infect Control Hosp Epidemiol* 2011;**32**:915–17.
727. Zwiener RJ, Belknap WM, Quan R. Severe pseudomembranous enterocolitis in a child: case report and literature review. *Pediatr Infect Dis J* 1989;**8**:876–82.

CHAPTER 244

Active Immunizing Agents

Penelope H. Dennehy • Ian C. Michelow • Michael P. Koster • Nicole E. Alexander-Scott

Prevention of infectious diseases in children by immunization is one of the outstanding accomplishments of medical science. Children enjoy better health today because of effective immunization programs, which in many countries have markedly diminished the morbidity and mortality of once common contagious diseases. The striking decline in the United States in vaccine-preventable childhood diseases is demonstrated in Table 244-1.[166,311] Immunization programs have led to the global eradication of smallpox, elimination of measles and poliomyelitis in regions of the world, and substantial reductions in the morbidity and mortality attributed to diphtheria, tetanus, and pertussis.[187] The World Health Organization (WHO) estimated that 2 million child deaths were prevented by vaccinations in 2003.

To achieve this progress in child health, scientific technology and medical practice have combined efforts to (1) understand the biology of causal infectious agents, (2) purify these agents and in some cases their components, (3) develop and test safe and effective vaccines, (4) manufacture and administer these vaccines to appropriate segments of the population, (5) develop appropriate indications and implement schedules for immunizations, and (6) identify necessary contraindications.

Infectious diseases can be prevented through immunization by the following means: (1) stimulating an active immunologic defense (e.g., from humoral antibody) through the administration of antigens, usually before natural exposure to an infectious agent (i.e., active immunization), or (2) temporarily supplying preformed human or animal antibody to persons before or soon after exposure to certain infectious agents (i.e., passive immunization). Active immunizations, including the currently available vaccines, are discussed in this chapter. Major vaccines (i.e., those discussed in this chapter) and their composition and routes of administration are listed in Table 244-2.

ACTIVE IMMUNOPROPHYLAXIS: CONSIDERATIONS AND RECOMMENDATIONS

Vaccines

An ideal immunizing agent should include the following characteristics: (1) the agent should be easy to produce in well-standardized preparations that are readily quantifiable and stable in immunobiologic potency, (2) it should be easy to administer, (3) it should not produce disease in the recipient or susceptible contacts, (4) it should induce long-lasting (ideally permanent) immunity that is measurable by available and inexpensive techniques, (5) it should be free of contaminating and potentially toxic substances, and (6) adverse reactions should be minimal and minor in consequence. All these objectives rarely, if ever, are met with the currently available vaccines because they are neither completely safe nor completely effective. Partial immunity or undesirable side effects or reactions, or both, including rare severe reactions, can occur. Nonetheless, vaccines in current use are highly effective and very safe.

All active immunizing agents (vaccines) contain one or more antigens that stimulate a protective immunologic response. Some are live attenuated viruses or bacteria; other vaccines consist of killed microorganisms or contain inactivated components such as exotoxins (i.e., toxoids). In some vaccines, the antigen is a highly defined, single constituent, such as *Haemophilus influenzae* type b polysaccharide, whereas in others the antigen component is less well defined (e.g., live viruses or whole-cell pertussis vaccines composed of killed *Bordetella pertussis* organisms). Immunizing agents are administered in suspending fluids such as sterile water, saline solution, or complex tissue culture fluid that can contain proteins or other constituents derived from the medium from which the vaccine was produced (e.g., serum proteins, egg antigens, or other tissue culture–derived antigens). With some vaccines, certain preservatives, stabilizers, or antibiotics are added; in some cases, these additives can result in hypersensitivity reactions. To enhance immunogenicity, particularly for vaccines containing inactivated microorganisms or their extracted components, adjuvants such as aluminum compounds may be added.

Immunization Schedules

The age and timing of immunization are critical for the success of vaccination. The schedule by which a vaccine is provided is based on multiple factors, including the epidemiology of naturally occurring disease, the age-specific risk for complications caused by the natural disease, the anticipated immunologic response of the host to the antigens, the duration of immunity that can be induced, and, often, the recommended ages for routine health care visits. In general, vaccines are recommended at the youngest age at which significant risk for the natural disease and its complications exist and at which a protective immunologic response to the vaccine will occur. An example is measles vaccine, which in the United

TABLE 244-1 Reduction in Morbidity of Some Vaccine-Preventable Diseases in the United States

	Maximum Cases (Yr)	2011	Percent Decrease (%)
Diphtheria	206,939 (1921)	0	100
Pertussis	265,269 (1934)	18,719	93
Tetanus	1,314 (1922-1926)	36	97
Poliomyelitis, paralytic	21,269 (1952)	0	100
Measles	894,134 (1941)	222	>99
Mumps	152,209 (1968)	404	>99
Rubella	57,686 (1969)	4	>99
Congenital rubella syndrome	20,000 (1964-1965)	0	100
Haemophilus influenzae type b	20,000 (before 1987)	14	>99
Invasive pneumococcal disease (>5 yr)	15,933 (2000)	1,459	91
Hepatitis B	21,102 (1990)	2,903	86
Varicella*	158,364 (1992)	14,513	91

*Data from <40 states.

Modified from Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2010. MMWR Morb Mortal Wkly Rep 2012;59:1-111.

TABLE 244-2 Available Vaccines in the United States for Use in Children and Their Routes of Administration*

Vaccine	Type	Recommended Route
BCG	Live bacteria	ID (preferred) or SC
Diphtheria-tetanus (DT, Td)	Toxoids	IM
DTaP	Toxoids and inactivated bacterial components	IM
DTaP-hepatitis B- IPV (combination)	See DTaP, hepatitis B, and IPV	IM
DTaP-IPV/Hib (combination)	See DTaP, IPV, and Hib	IM
DTaP-IPV (combination)	See DTaP and IPV	IM
Hepatitis A	Inactivated virus	IM
Hepatitis B	Recombinant viral antigen	IM
Hepatitis A- hepatitis B (combination)	See hepatitis A and hepatitis B	IM
Hib	Bacterial polysaccharide-protein conjugate	IM
Hib-hepatitis B (combination)	See Hib and hepatitis B	IM
Human papilloma virus (HPV2 and HPV4)	Recombinant viral antigens	IM
Influenza (TIV)	Inactivated viral components	IM
Influenza (LAIV)	Live-attenuated viruses	Intranasal
Japanese encephalitis	Inactivated virus	SC
Meningococcal polysaccharide (MPSV4)	Bacterial polysaccharide	SC
Meningococcal conjugate (MCV4)	Bacterial polysaccharide-protein conjugate	IM
MenCY-Hib (combination)	Bacterial polysaccharide-protein conjugates	
MMR	Live-attenuated viruses	SC
MMRV	Live-attenuated viruses	SC
Pneumococcal polysaccharide (PPSV)	Bacterial polysaccharide	IM or SC
Pneumococcal conjugate (PCV13)	Bacterial polysaccharide-protein conjugate	IM
Poliovirus (IPV)	Inactivated viruses	SC
Rabies	Inactivated virus	IM
Rotavirus (RV1 and RV5)	Live-attenuated viruses	oral
Tdap	Toxoids and inactivated bacterial components	IM
Typhoid	Bacterial capsular polysaccharide	IM
Typhoid	Live-attenuated bacteria	Oral
Varicella	Live-attenuated virus	SC
Yellow fever	Live-attenuated virus	SC

*Only major childhood vaccines and selective others are included.

BCG, bacille Calmette-Guérin (tuberculosis); DT, diphtheria-tetanus toxoids (for children <7 yr); DTaP, diphtheria-tetanus toxoids–acellular pertussis vaccine (for children <7 yr); Hib, *Haemophilus influenzae* type b conjugate vaccine; ID, intradermal; IM, intramuscular; IPV, inactivated poliovirus vaccine; MMR, live measles-mumps-rubella viruses vaccine; MMRV, live measles-mumps-rubella-varicella viruses vaccine; SC, subcutaneous; Td, tetanus-diphtheria toxoid (for children ≥7 yr and adults); Tdap, diphtheria–reduced tetanus toxoids–acellular pertussis vaccine (for children ≥10 yr and adults).

States is recommended routinely at 12 to 15 months of age because many children have residual, transplacentally acquired maternal measles serum antibody in the first year of life that will interfere with the antibody response. However, during measles outbreaks or for travel of infants to measles endemic areas, measles vaccination is recommended for infants as young as 6 months because the risk for complications with measles is high in children younger than 1 year.[20,133] These infants should be vaccinated again at 12 to 15 months of age. Similarly, in countries where measles causes significant morbidity and mortality in infants younger than 9 months, the Global Advisory Group of the WHO's Expanded Programme on Immunization (EPI) has recommended giving measles vaccine to infants as young as 6 months.[293]

The recommended doses of vaccine are determined by the number necessary to achieve a uniform and predictable immunologic response and to sustain protection. Some immunizing agents require the administration of more than one dose for development of an adequate antibody response and require a booster dose to maintain protection; examples are pertussis, diphtheria, and tetanus vaccines. Intervals between doses are based on the kinetics of primary and secondary antibody responses.

Route of Administration

An example of the effect of the route of administration on immunologic response is provided by poliomyelitis vaccines. Inactivated poliovirus vaccines given intramuscularly induce systemic immunity through serum antibody production; however, they do not consistently evoke local secretory immunoglobulin A (IgA) antibodies in the intestinal tract and thereby effectively prevent subsequent transmission of wild-type virus. Because live attenuated oral polio vaccine (OPV) induces optimal intestinal as well as systemic antibody, it was the preferred vaccine for routine immunization of children in the United States against poliomyelitis for 3 decades and remains the recommended vaccine by the WHO for global eradication.[503,579] Vaccines containing adjuvants must be injected deep into the muscle mass because if they are administered subcutaneously or intradermally, they can cause local irritation, inflammation, granuloma formation, or necrosis.[9,358]

Injectable vaccines should be administered in areas unlikely to cause local neural, vascular, or tissue injury (Table 244-3).[9,358] Although the upper, outer quadrant of the buttocks has been used as a frequent site of immunization, this area ordinarily should not be used because the gluteal region consists mostly of fat in young children and because of potential injury to the sciatic nerve. Ideally, intramuscular injections should be given in the anterolateral aspect of the upper part of the thigh or the deltoid muscle of the upper part of the arm. The anterolateral aspect of the thigh is preferred for infants because of its muscle mass relative to other sites. For older children, the deltoid muscle usually is sufficiently large for intramuscular injection. The incidence of significant pain in 18-month-old children injected intramuscularly in the thigh is greater than that after deltoid injections and can result in transient limping.[326] The deltoid generally is the preferred site for intramuscular administration of vaccines in children 18 months and older, although some physicians prefer the anterolateral aspect of the thigh for toddlers.[9,358] Subcutaneous inoculations also usually should be given in the thigh of infants and the deltoid area of older children. Intradermal vaccines generally should be administered on the volar aspect of the forearm.

Recommended routes for administration of vaccines are provided in their package inserts and are summarized in recommendations for immunizations by the Committee on Infectious Diseases of the American Academy of Pediatrics (AAP)[9] and the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC).[9]

TABLE 244-3 **Site and Needle Length by Age for Intramuscular Immunization**

Age Group	Needle Length, inches (mm)	Suggested Injection Site
Neonate (preterm and term) and infants <1 mo of age	⅝ (16)	Anterolateral thigh muscle
Term infant, age 1-12 mo	1 (25)	Anterolateral thigh muscle
Toddlers and children	⅝-1 (16-25)	Deltoid muscle of the arm
	1-1¼ (25-32)	Anterolateral thigh muscle
Adolescents and young adults		
Female and male, <60 kg	1 (25)	Deltoid muscle of the arm
Female, 60-70 kg	1 (25)	Deltoid muscle of the arm
Female, 70-90 kg	1 (25)-1½ (38)	Deltoid muscle of the arm
Female, >90 kg	1½ (38)	Deltoid muscle of the arm
Male, 70-118 kg	1 (25)-1½ (38)	Deltoid muscle of the arm
Male, >118 kg	1½ (38)	Deltoid muscle of the arm

Modified from American Academy of Pediatrics. Active immunization. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 1-56.

Vaccine Dose

The recommended dose of each immunizing agent is derived from theoretical considerations and vaccine trials. Because inactivated immunizing agents cannot replicate in the host, these vaccines must contain an adequate antigenic mass to stimulate the desired immunologic response. Long-lasting immunity with such vaccines requires repeated doses. Exceeding the recommended dose can be hazardous because of excessive local or systemic concentrations of immunizing agents, whereas administration of doses smaller than those recommended may result in inadequate response and protection.[9,358]

Lapsed Immunizations

In general, intervals between multiple doses of an antigen longer than those recommended do not affect the antibody responses achieved, provided the immunization series is completed. Thus, restarting the series after interruption of the vaccine schedule or giving additional doses is not necessary.

Simultaneous Administration of Multiple Vaccines

Because most vaccines can be given simultaneously without impairment of effectiveness or safety, multiple vaccines are given to children concurrently.[348] Simultaneous administration of vaccines is particularly important for inadequately immunized children whose return for further immunization is doubtful or for patients with imminent travel plans. An inactivated vaccine and a live virus vaccine can be administered simultaneously at different sites without interference with the immune response. Exceptions are yellow fever and cholera vaccines because if they are administered simultaneously, antibody responses are diminished. If possible, administration of these vaccines should be separated by at least an interval of 3 weeks.[358]

In the case of live virus vaccines, the immune response to one live virus vaccine can be impaired if it is given within 4 weeks of another.[164,358] Thus, parenteral live virus vaccines not administered on the same day should be given at least 4 weeks apart. This consideration is the basis of the recommended minimal interval of 1 month (defined as 4 weeks) between doses of measles vaccine, such as would be the case for a previously unimmunized person who is entering college. Guidelines for spacing live and killed antigen vaccines are given in Table 244-4.

TABLE 244-4 Guidelines for Spacing the Administration of Live and Inactivated Antigens

Antigen Combination	Recommended Minimum Interval Between Doses
Two or more inactivated*	None; may be administered simultaneously or at any interval between doses
Inactivated plus live	None; may be administered simultaneously or at any interval between doses
Two or more live parenteral†	28-day minimum interval if not administered simultaneously

*If simultaneous administration of diphtheria–reduced tetanus toxoids–acellular pertussis vaccine (Tdap) and meningococcal polysaccharide–protein conjugate vaccine (MCV4) is not feasible (i.e., a vaccine is not available), the vaccines should be separated by at least 28 days.

†Some live oral vaccines (e.g., Ty21a typhoid vaccine, oral poliovirus vaccine, rotavirus vaccine) can be administered simultaneously or at any interval before or after inactivated or live parenteral vaccines.

Record Keeping, Patient Information, Informed Consent, and Reporting

Accurate record keeping by physicians is required, and parents (or patients) should keep up-to-date immunization records for their children. The 1986 National Childhood Vaccine Injury Act (NCVIA) requires that for routinely recommended childhood vaccines, health care providers record in the child's permanent medical record the date of administration of the vaccine, manufacturer, lot number, and the name of the health care provider administering the vaccines.[9]

As a general principle, all children and their parents or caregivers should be informed about the benefits and risks of any vaccines to be administered. For vaccines currently specified in the NCVIA, Vaccine Information Statements (VISs) have been prepared by the CDC and must be used by vaccine administrators.

Informed consent should be obtained before the administration of vaccines. Some physicians and other health care providers may choose to obtain the parent's signature, but current law does not require written consent. An appropriate alternative to written consent is to note in the patient's record that the VISs have been provided and discussed with the parent, patient, or legal guardian.[9]

To increase knowledge about adverse reactions, all temporally associated events severe enough to require the patient to seek medical attention should be reported to the Vaccine Adverse Events Reporting System (VAERS).* Health care providers who administer vaccines are required in the United States to report to the VAERS specific adverse events in recipients of the vaccines covered by the NCVIA.† This system for reporting adverse events associated with vaccination was established by the U.S. Department of Health and Human Services to foster recognition of vaccine-related reactions and further study, as indicated, to establish possible causation.

The decrease in the occurrence of vaccine-preventable infectious diseases has resulted in a greater number of adverse events temporally related to immunization than cases of disease. Although in some cases, such as vaccine-associated paralytic poliomyelitis, vaccine has been established to be the cause; in other circumstances, such as brain damage alleged to be attributed to whole-cell pertussis vaccine, causation by vaccine has not been proved.[47]

Increased public visibility of vaccine reactions contributed to a marked increase in vaccine litigation in the 1980s as compensation was sought through the judicial system by those alleged to have suffered serious vaccine-related sequelae. A marked increase in manufacturers' actual and anticipated liability costs and subsequent escalating increases in the price of vaccines occurred concomitantly. These and other developments, such as threats to the vaccine supply, concerns by parents about

*VAERS forms can be obtained by calling 800-822-7967 or by accessing the web site at http://vaers.hhs.gov.

†Health Resources and Services Administration: http://www.hrsa.gov/vaccinecompensation/vaccinetable.html.

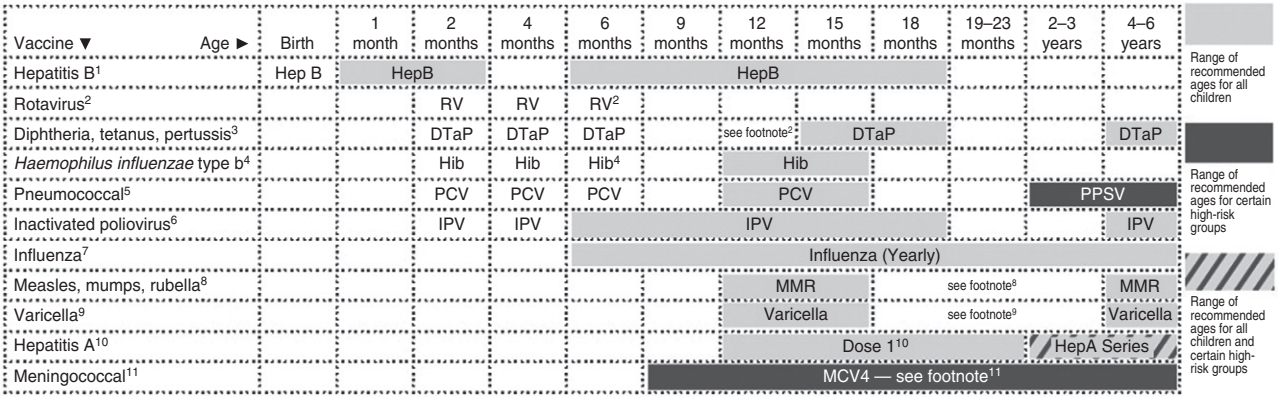

FIGURE 244-1 ■ Recommended immunization schedule for persons aged birth to 6 years: United States. (From Centers for Disease Control and Prevention. *Immunization schedules.* Available at http://www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html.)

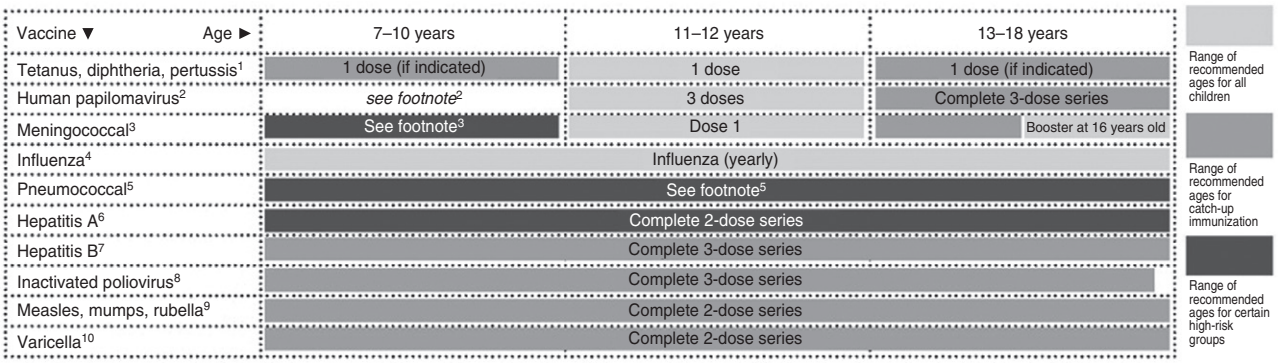

FIGURE 244-2 ■ Recommended immunization schedule for persons aged 10 to 18 years: United States. (From Centers for Disease Control and Prevention. *Immunization schedules.* Available at http://www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html.)

vaccine safety, and recognition of the benefits derived from improved coordination and planning of vaccine programs, led to passage of the 1986 NCVIA and the National Vaccine Injury Compensation Program, a no-fault system to compensate victims of certain presumed vaccine-related events.

The Department of Health and Human Services administers the compensation program. Decisions on compensation are made by the U.S. Court of Federal Claims and are based on the Vaccine Injury Table, which was revised since passage of the original legislation in response to new findings and analysis by the several Institute of Medicine (IOM) committees. Compensation for injuries occurring after the program's effective date of October 1, 1988, is provided by excise taxes on each vaccine. The program has been successful in reducing vaccine-related litigation, stabilizing vaccine prices, and creating a favorable environment for the introduction of new vaccines.[252]

Vaccine Recommendations and Schedules

In developing recommendations for immunization, multiple factors are considered and include the vaccine's characteristics, scientific knowledge about the principles of immunization, assessment of the benefits of the vaccine, the risk for development of the disease and its complications, vaccine costs, and the risk for having adverse reactions. Changes in relative benefits and risks necessitate continued review of recommendations. In the United States, recommendations for immunization of infants and children are made by two different committees, the ACIP of the CDC and the AAP Committee on Infectious Diseases. These committees work closely together, and, in most circumstances, their recommendations are similar. These two committees and the American Academy of Family Practice (AAFP) issue a single vaccine schedule each year. The 2012 schedule for routine administration of childhood vaccines is given in Figures 244-1 through 244-3.

A major change in 1996 was the establishment of a routine preadolescent immunization visit at 11 to 12 years of age.[124] The dose of adult tetanus-diphtheria-acellular pertussis (Tdap) vaccine should be given at that time, as should a dose of the meningococcal conjugate vaccine and the first dose of human papillomavirus (HPV) vaccine.[159] In addition, at this preadolescent visit, children not previously vaccinated with hepatitis B, varicella, or the second dose of measles-containing vaccine (or any combination of these vaccines) should be given the necessary immunizations and scheduled for future visits to receive any vaccines not administered during this visit.

Other countries have similar national mechanisms for formulating immunization schedules and recommendations that are based on the local epidemiology of diseases

Vaccine	Minimum Age for Dose 1	Minimum Interval Between Doses			
		Dose 1 to dose 2	Dose 2 to dose 3	Dose 3 to dose 4	Dose 4 to dose 5
Persons aged 4 months through 6 years					
Hepatitis B	Birth	4 weeks	8 weeks and at least 16 weeks after first dose; minimum age for the final dose is 24 weeks		
Rotavirus[1]	6 weeks	4 weeks	4 weeks[1]		
Diphtheria, tetanus, pertussis[2]	6 weeks	4 weeks	4 weeks	6 months	6 months[2]
Haemophilus influenzae type b[3]	6 weeks	4 weeks if first dose administered at younger than age 12 months 8 weeks (as final dose) if first dose administered at age 12–14 months No further doses needed if first dose administered at age 15 months or older	4 weeks[3] if current age is younger than 12 months 8 weeks (as final dose)[3] if current age is 12 months or older and first dose administered at younger than age 12 months and second dose administered at younger than 15 months No further doses needed if previous dose administered at age 15 months or older	8 weeks (as final dose) This dose only necessary for children aged 12 months through 59 months who received 3 doses before age 12 months	
Pneumococcal[4]	6 weeks	4 weeks if first dose administered at younger than age 12 months 8 weeks (as final dose for healthy children) if first dose administered at age 12 months or older or current age 24 through 59 months No further doses needed for healthy children if first dose administered at age 24 months or older	4 weeks if current age is younger than 12 months 8 weeks (as final dose for healthy children) if current age is 12 months or older No further doses needed for healthy children if previous dose administered at age 24 months or older	8 weeks (as final dose) This dose only necessary for children aged 12 months through 59 months who received 3 doses before age 12 months or for children at high risk who received 3 doses at any age	
Inactivated poliovirus[5]	6 weeks	4 weeks	4 weeks	6 months[5] minimum age 4 years for final dose	
Meningococcal[6]	9 months	8 weeks[6]			
Measles, mumps, rubella[7]	12 months	4 weeks			
Varicella[8]	12 months	3 months			
Hepatitis A	12 months	6 months			
Persons aged 7 through 6 years					
Tetanus, diphtheria/tetanus, diphtheria, pertussis[9]	7 years[9]	4 weeks	4 weeks if first dose administered at younger than 12 months 6 months if first dose administered at 12 months or older	6 months if first dose administered at younger than age 12 months	
Human papiliomavirus[10]	9 years	Routine dosing intervals are recommended[10]			
Hepatitis A	12 months	6 months			
Hepatitis B	Birth	4 weeks	8 weeks (and at least 16 weeks after first dose)		
Inactivated poliovirus[5]	6 weeks	4 weeks	4 weeks[5]	6 months[5]	
Meningococcal[6]	9 months	8 weeks[6]			
Measles, mumps, rubella[7]	12 months	4 weeks			
Varicella[8]	12 months	3 months if person is younger than age 13 years 4 weeks if person is aged 13 years or older			

FIGURE 244-3 ■ Catch-up immunization schedule for persons aged 4 months to 18 years who start late or who are more than 1 month behind. (From Centers for Disease Control and Prevention. *Immunization schedules.* Available at http://www.cdc.gov/vaccines/schedules/hcp/child-adolescent.html.)

and available vaccines. In developing countries, practices are guided by the WHO's EPI.[578] Current recommendations are listed in Table 244-5.

As new vaccines and scientific knowledge become available, vaccine recommendations and schedules are modified and changed. Examples of changes in the past decade include the use of pneumococcal conjugate vaccine beginning at 2 months of age, universal toddler hepatitis A immunization, recommendations for administration of a second dose of varicella vaccine, and the introduction of acellular pertussis vaccines for adolescents and adults.

Implementation of Vaccine Programs

In addition to the availability of safe and effective vaccines and appropriate schedules for their use, effective means of implementation and delivery are necessary for the success of vaccine programs. In the United States, high rates of immunization in school-aged children have been achieved, in part because of public health programs for vaccine administration, government support for vaccine purchase, and state laws requiring immunization for school entry. In contrast to rates of approximately 95 percent or higher in school-aged children, immunization rates in infants and young children in the 1980s were significantly lower.[591] In a survey of 21 primarily urban areas throughout the United States, 11 to 58 percent (median, 44%) of children who entered school in 1991 and 1992 were fully vaccinated by their second birthday. Failure to immunize young children was a major factor in the outbreaks of measles in major urban areas in the United States in 1989 through 1991.[525]

This epidemic and the recognition of low immunization rates prompted a national campaign to achieve the U.S. Public Health Service's goal of a 90 percent vaccine coverage rate in children by the time they were 2 years of age. Initiatives have included improved access to vaccines, education of health care providers in the community, and the development of standards for pediatric immunization practice.[432] These standards have been endorsed by the AAP, AAFP, and other major professional organizations and serve as guidelines to be followed for improving the delivery of vaccines. They include evaluation of the immunization status of patients at all medical visits, the use of valid contraindications, simultaneous

TABLE 244-5 **Schedule of the Expanded Programme on Immunization of the World Health Organization***

		Hepatitis B	
Age	**Vaccines†**	**Schedule A**	**Schedule B**
Birth	BCG. OPV-0	HB-1	HB-1
6 wk	DTP-1, OPV-1, Hib-1, PCV-1, RV-1	HB-2	HB-2
10 wk	DTP-2, OPV-2, Hib-2, PCV-2, RV-2		HB-3
14 wk	DTP-3, OPV-3, Hib-3, PCV-3, RV-3	HB-3	HB-4
9 mo	Measles, yellow fever‡		

*Modifications may be made by the ministries of health in individual countries on the basis of local conditions.
†Hib vaccines are in use in some developing countries. Vaccines for PCV and RV are not yet used in the national immunization program in most developing countries.
‡In countries where yellow fever poses a risk.
BCG, bacille Calmette-Guérin; DTP, diphtheria–tetanus–whole cell pertussis; HB, hepatitis B; OPV, oral poliovirus; Hib, *Haemophilus influenzae* type b; PCV, pneumococcal conjugate vaccine; RV, rotavirus vaccine.

administration of all indicated vaccines, and routine audit of the immunization status of patients by providers. These and other initiatives have resulted in increasing immunization rates of young children. According to the National Immunization Survey, coverage rates in children 19 to 35 months of age in 2011 were 74 percent for completion of the four doses of tetanus-diphtheria-pertussis/diphtheria-tetanus toxoid-acellular pertussis/diphtheria-tetanus (DTP/DTaP/DT), three of poliovirus vaccine, and one dose of measles-mumps-rubella (MMR) vaccine, three doses of hepatitis B vaccine, one dose of varicella vaccine, and four doses of pneumococcal conjugate vaccine. *H. influenzae* type b vaccine is excluded because of nationwide shortages.[134] Vaccination coverage rates in the United States for preschool-aged children consistently have been greater than 70 percent since 2009.

Vaccine Contraindications, Precautions, and Use in Special Circumstances

Recommendations for the use of specific vaccines include contraindications and use in special circumstances, such as immunocompromised patients (from underlying disease or therapy, such as high-dose corticosteroids) and pregnancy.[18,358] Established, generic contraindications are moderate or severe illness, a previous anaphylactic reaction to the specific vaccine, and a severe hypersensitivity reaction, such as anaphylaxis, to a vaccine constituent.

The decision to defer immunization in a febrile child should be based on the physician's assessment of the severity of the illness rather than the degree of fever. Children with minor illness and low-grade fever generally should be vaccinated, especially if a child is unlikely to return promptly for the deferred immunization.

Administration of live virus vaccines such as varicella and MMR vaccines generally is contraindicated in patients with altered immunity. However, the morbidity and mortality rates of measles and the lack of complications from vaccination of children infected with human immunodeficiency virus (HIV) have led to recommendations that these children, unless significantly immunocompromised, receive the MMR vaccine.[48,132,560]

Because of a theoretical risk to the developing fetus, administration of live virus vaccines in most cases is not recommended for pregnant women.[18,358] However, inadvertent administration of vaccine is not necessarily a reason for termination of the pregnancy, and some live virus vaccines, such as those for yellow fever and poliomyelitis, can be given safely to pregnant women. Inactivated bacterial and viral vaccines such as tetanus toxoids, hepatitis B, and influenza vaccine, which are composed of antigenic components or killed organisms, can and should be given during pregnancy if indicated.

In some recipients, vaccines can cause severe reactions, which may be a contraindication or precaution to subsequent administration of the specific vaccine. An example is a child in whom a fever of 40.5°C (105°F) or higher develops after receiving DTaP vaccine, for whom administration of further doses of pertussis-containing vaccine is not indicated in most cases. This recommendation is based on the unproven but reasonable presumption that children who experience adverse reactions after receiving pertussis immunization are at risk for having similar reactions of equal or greater magnitude on subsequent immunization.[192]

Anaphylactic reactions caused by allergenic components of a vaccine, such as gelatin or egg protein (in vaccine prepared in embryonated chicken eggs), have occurred in rare cases. Vaccines posing a potential risk for egg-sensitive persons include those against measles, mumps, inactivated influenza, and yellow fever. Before administering vaccines to persons with possible hypersensitivity to vaccine constituents, physicians should review current recommendations for these vaccines. In other circumstances, specific immunizations may be contraindicated because of previous reactions and the child's past history, such as with DTaP (e.g., evolving neurologic disorders) and MMR (e.g., immune thrombocytopenia occurring in temporal association with vaccination).[358]

Misconceptions

Appropriate and safe use of vaccines requires knowledge of the patient's relevant medical history, adverse reactions associated with previous receipt of vaccines, and specific indications and contraindications. Without this information, vaccines may be administered inadvertently or not given in circumstances in which immunization is indicated, thereby resulting in missed opportunities for receiving the recommended immunization and susceptibility of the child to a preventable disease. Examples of common misconceptions concerning contraindication to vaccines are given in Table 244-6.

TABLE 244-6 Misconceptions Concerning Vaccine Contraindications

- Mild acute illness with low-grade fever or mild diarrheal illness in an otherwise well child
- Current antimicrobial therapy or the convalescent phase of illness
- Reaction to previous vaccine dose that involved only soreness, redness, or swelling in the immediate vicinity of the vaccination site or temperature of less than 40.5°C (105°F)
- Prematurity. The appropriate age for initiating most immunization in the prematurely born infant is the usual recommended chronologic age. Vaccine doses should not be reduced for preterm infants.
- Pregnancy of mother or other household contact. Vaccine viruses in MMR vaccine are not transmitted by vaccine recipients. Although varicella vaccine and influenza vaccine viruses have been transmitted by a healthy vaccine recipient to contacts, the frequency is rare, only mild or asymptomatic infection has been reported, and use of vaccine is not contraindicated by pregnancy of either the child's mother or other household contacts.
- Recent exposure to an infectious disease
- Breast-feeding. The only vaccine virus that has been isolated from breast milk is rubella vaccine virus. No evidence indicates that breast milk from women immunized against rubella is harmful to infants.
- A history of nonspecific allergies or relatives with allergies
- Allergies to penicillin or any other antibiotic, except anaphylactic reactions to neomycin or streptomycin. These reactions occur rarely, if ever. None of the vaccines licensed in the United States contains penicillin.
- Allergies to duck meat or duck feathers. No vaccine available in the United States is produced in substrates containing duck antigens.
- Family history of convulsions in persons considered for pertussis or measles vaccination
- Family history of sudden infant death syndrome in children considered for DTaP vaccination
- Family history of an adverse event, unrelated to immunosuppression after vaccination
- Malnutrition

DTaP, diphtheria-tetanus-acellular pertussis; MMR, measles-mumps-rubella.

Modified from Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.

International Travel

Foreign travel often is an indication for giving vaccines not routinely administered to children.[18] The risk for exposure to certain vaccine-preventable diseases may be increased relative to that in the United States, and travelers may be exposed to infections that are uncommon or do not occur in the United States. Examples include vaccines against hepatitis A, typhoid fever, yellow fever, and Japanese encephalitis (JE) (Table 244-7), depending on the location and circumstances of the person's visit. Some countries may require yellow fever vaccination for entry. The second dose of measles vaccine should be given to children and adolescents who have received only one dose (provided 4 weeks or more have elapsed since administration of the first dose), irrespective of age, because the risk for exposure to cases of measles may be substantial in some foreign countries. In addition, children and adolescents should have received all vaccines routinely recommended for their age. Information on vaccine requirements for international travel to different countries is provided in a semiannually revised publication by the CDC entitled *Health Information for International Travel*.[383]* Information also is available from the CDC International Travelers Hotline† or on the Internet at the CDC Traveler's Health website.‡

Vaccine Safety

Immunizations are among the most cost-effective and widely used public health interventions. Public health recommendations for vaccine programs and practices represent a dynamic balancing of risks and benefits. Vaccine safety or monitoring of risk is necessary to weigh this balance accurately and adjust vaccination policies accordingly.

No vaccine is perfectly safe or effective. As the incidence of vaccine-preventable diseases is reduced, public concern refocuses from the risk for contracting disease to the health risks associated with vaccines. A higher standard of safety generally is expected of vaccines than of other medical interventions because, in contrast to most pharmaceutical products that are administered to ill persons for curative purposes, vaccines generally are given to healthy persons to prevent the acquisition of disease. Public tolerance of adverse reactions related to products given to healthy persons, especially healthy infants, is substantially lower than that to products administered to persons who are already ill. This lower tolerance of risk for vaccines necessitates investigating possible causes of rare adverse events after administration of vaccinations more aggressively than would be acceptable for other pharmaceutical products. Because vaccination is such a common event, any health problem that occurs after immunization may be attributed to the vaccine. Health effects reported as being associated with vaccines may be true adverse reactions or may be associated with vaccination only by coincidence. Because a temporal relationship alone does not necessarily indicate causation, cause-and-effect relationships often are impossible to establish. Epidemiologic and related studies must be performed to ascertain the incidence and nature of adverse reactions to vaccines, and such studies are important in ensuring a scientific rationale for recommendations for vaccine use and optimal public and professional acceptance of vaccines.

The topic of vaccine safety became prominent during the mid 1970s with increases in the number of lawsuits filed on behalf of patients presumably injured by the DTP vaccine.[261] Legal decisions were made and damages awarded despite the lack of scientific evidence to support claims of vaccine injury.[261] As a result of the liability, prices soared, and several manufacturers halted production. A shortage of vaccines resulted, and public health

*Available online from the http://wwwnc.cdc.gov/travel/page/yellowbook-2012-home.htm.

†800-CDC-INFO (800-232-4636).

‡http://wwwnc.cdc.gov/travel/.

TABLE 244-7 **Recommended Immunizations for Travelers to Developing Countries**

Immunizations	Length of Travel		
	Brief (<2 wk)	Intermediate (2 wk to 3 mo)	Long-Term Residential (>3 mo)
Review and complete age-appropriate childhood schedule.	+	+	+
• DTaP, poliovirus, pneumococcal, and *Haemophilus influenzae* type b vaccines may be given at 4-week intervals if necessary to complete the recommended schedule before departure.			
• Measles: Two additional doses given if < age 12 mo at first dose			
• Varicella			
• Hepatitis A*			
• Hepatitis B†			
Vaccines against the following diseases should be considered depending on the geographic area and circumstances of the visit:			
• Japanese encephalitis‡	±	±	+
• Meningococcal disease§	±	±	±
• Rabies‖	±	+	+
• Typhoid fever¶	±	+	+
• Yellow fever**	+	+	+

*Indicated for travelers to areas with intermediate or high endemic rates of HAV infection.
†If insufficient time to complete 6-month primary series, accelerated series can be given.
‡For regions with endemic infection. For high-risk activities in areas experiencing outbreaks, vaccine is recommended, even for brief travel.
§Recommended for regions of Africa with endemic infection and during local epidemics and required for travel to Saudi Arabia for the Hajj.
‖Indicated for persons with high risk for animal exposure (especially to dogs) and for travelers to countries with endemic infection.
¶Indicated for travelers who will consume food and liquids in areas of poor sanitation.
**For regions with endemic infection.
DTaP, diphtheria–tetanus–acellular pertussis.
Modified from American Academy of Pediatrics. *Immunizations in special clinical circumstances.* In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012, p. 103-9 and Centers for Disease Control and Prevention. *Health information for international travel, 2012: international travel with infants and young children.* Available at http://www.nc.cdc.gov/travel/yellowbook/2012/chapter-7-international-travel-infants-children/vaccine-recommendations-for-infants-and-children.htm.

officials became concerned about the return of epidemic diseases. To reduce liability and respond to public health concerns, Congress passed the NCVIA in 1986.

The NCVIA mandates that all health care providers report certain adverse events that occur after the administration of vaccination with routinely recommended childhood vaccines. As a result, VAERS was established by the U.S. Food and Drug Administration (FDA) and the CDC in 1990. VAERS provides a mechanism for the collection and analysis of adverse events associated with vaccines currently licensed in the United States. Adverse events are defined as health effects occurring after immunization that may or may not be related to the vaccine. VAERS data are monitored continually to detect previously unknown adverse events or increases in known adverse events.[191]

The gaps that exist in the scientific knowledge of rare vaccine adverse events prompted the CDC to develop the Vaccine Safety Datalink (VSD) project.[189] This project involved forming partnerships with four large health maintenance organizations to monitor vaccine safety continually. VSD is an example of a large, linked database and includes information on more than 6 million persons. The VSD project allows for planned vaccine safety studies, as well as timely investigations of hypotheses.

In the late 1990s, reports in the lay press questioned the safety of routine immunizations and alarmed parents with unsupported accounts of the dangers of vaccines. One major national television network broadcast a feature piece that linked diabetes to childhood immunizations. In the United Kingdom, a report linked receipt of measles vaccine to the development of autism, and in France, hepatitis vaccine was reported to cause multiple sclerosis (MS).

Vaccination and Diabetes. Classen and Classen suggested that certain vaccines, if given at birth, may decrease the occurrence of type 1 diabetes, whereas if initial vaccination is performed after children are 2 months of age, the occurrence of diabetes increases.[196] Other researchers have not found an increased risk for diabetes associated with vaccination.[319] The Classen theory is based on results from experiments in laboratory animals, as well as comparisons of rates of diabetes among countries with different immunization schedules.[196] Applying findings from laboratory animals to humans is fraught with uncertainties. Comparison of diabetes rates among countries with different vaccination policies also provides weak evidence because many factors, including vaccination schedules, may differ by country. In addition, other factors, including genetic predisposition and numerous possible environmental exposures unrelated to the administration of vaccines, may influence the development of diabetes.[319]

The most rigorous epidemiologic study of infant vaccinations and type 1 diabetes to date found that measles vaccine was associated with a decreased risk and no association was found with bacille Calmette-Guérin (BCG), smallpox, tetanus, pertussis, rubella, or mumps vaccine.[82] In a large clinical trial of the *Haemophilus influenzae* type b (Hib) vaccine conducted in Finland, no statistically significant association was found between the receipt of the vaccine and the development of type 1 diabetes during 10 years of follow-up.[338] The IOM Immunization Safety Committee reviewed the clinical and epidemiologic literature in 2002 and concluded that multiple immunizations do not lead to a risk for development of type 1 diabetes.[319]

Measles Vaccine and Autism. The causes of autism are unknown. Most experts agree, however, that autism is a condition that begins before birth.[478] Genetic factors seem to play a major role in the development of autism. Autism usually is diagnosed in children when they are 18 to 30 months old, shortly after they have received many of the recommended vaccinations. Because of this coincidence in timing, some parents of children with autism believe that an immunization may have caused their child's condition.

A 1998 report published in *The Lancet* of 12 patients who had inflammatory bowel disease and autism raised the hypothesis that a link could exist between the MMR vaccine and autism.[555] The authors of the report speculated that MMR vaccine was the possible cause of bowel problems, with the resultant malabsorption of essential vitamins and nutrients leading to autism.

Epidemiologic studies in both the United States and Great Britain do not support a causal association between MMR vaccine and inflammatory bowel disease,[226,425] nor do epidemiologic studies support a causal association between MMR vaccine (and other measles-containing vaccines) and autism.[233,234] Expert panels convened by both the IOM and the AAP examined the available data on autism and MMR vaccine. Both panels concluded that the available evidence does not support the hypothesis that MMR vaccine causes autism or associated disorders or inflammatory bowel disease.[295,322]

Hepatitis B Vaccine and Multiple Sclerosis. Reports of MS developing after receipt of hepatitis B vaccine led to the concern that immunization with hepatitis B vaccine may precipitate the onset of MS or lead to relapses. Since licensure, the safety of the hepatitis B vaccine has continued to be monitored. The several studies conducted did not find a scientific association between hepatitis B vaccination and the development of severe neurologic adverse events such as optic neuritis and Guillain-Barré syndrome. Two large epidemiologic studies examined the risk for an association between vaccines and MS. The first, a case-control study in a large cohort of nurses, demonstrated no significant association between hepatitis B vaccination and development of MS.[59] A French study of patients with MS found that vaccination did not appear to increase the short-term risk for relapse.[211]

The Immunization Safety Review Committee of the IOM reviewed all available data and issued a report in 2004. The committee concluded that the evidence did not support a causal relationship between hepatitis B vaccine administered to adults and onset or relapse of MS.[324]

Thimerosal-Containing Vaccines and Neurodevelopmental Disorders. Thimerosal is a mercury-containing preservative that has been used as an additive to biologics and vaccines since the 1930s because it is effective in preventing bacterial and fungal contamination, particularly in multidose containers. The FDA Modernization Act of 1997 required a review and assessment of the risk for all mercury-containing pharmaceuticals. Assessment of vaccines led to the recognition that, during the first 6 months of life, some children could be exposed to a cumulative quantity of mercury that exceeds one of the federal guidelines on methylmercury. However, a significant safety margin is incorporated into all the acceptable mercury exposure limits, and no data determined or evidence was found that any harm was caused by the level of exposure with the existing immunization schedules. In addition, the risk posed by exposure to thimerosal, which contains ethylmercury, not methylmercury, on which the federal guidelines were based, is unknown. However, to avoid any potential risk, the Public Health Service, the AAP, and vaccine manufacturers agreed in July 1999 that thimerosal-containing vaccines should be removed as soon as possible. As of March 2001, thimerosal no longer was used as a preservative in any of the pediatric vaccines given to children 6 years old or younger in the United States.[173]

A possible association between thimerosal-containing vaccines and increasing diagnoses of autism and autistic spectrum disorders has been investigated in studies in the United States and elsewhere. The Immunization Safety Review Committee of the IOM reviewed all available data and, in October of 2001, issued a report stating that the evidence is inadequate to accept or reject a causal relationship between exposure to thimerosal-containing vaccines and the neurodevelopmental disorders of autism, attention-deficit/hyperactivity disorder (ADHD), and speech and language delay. In addition, the report concluded that the hypothesis that exposure to thimerosal could be associated with the development of neurodevelopmental disorders, although not established, is biologically plausible.[323] The IOM committee recommended that thimerosal-free DTaP, hepatitis B, and Hib vaccines be used for infants, children, and pregnant women, provided an adequate supply is available.[323]

Since the IOM report, five large studies have compared the risk for autism in children who received vaccines containing thimerosal with that in children who received vaccines without thimerosal.[52,260,306,316,543] The studies found the incidence of autism to be the same in both groups.

Reference Sources

Several comprehensive sources of information about pediatric vaccines are available. The AAP publishes *The Red Book: Report of the Committee on Infectious Diseases* every 3 years. The next edition will be published in

2015. In the interval between editions, the AAP publishes recommendations in its newsletter *AAP News* and subsequently in *Pediatrics*. The ACIP issues vaccine recommendations and relevant information in *Morbidity and Mortality Weekly Report*. Manufacturers provide product information for each vaccine in the FDA-approved package inserts.

VACCINES RECOMMENDED FOR ROUTINE ADMINISTRATION

Diphtheria Toxoid

The introduction of diphtheria toxoid–containing vaccine in the 1940s led to a dramatic reduction in the incidence of diphtheria in the United States. From 1980 through 2004, 57 cases of diphtheria were reported in the United States, an average of 2 to 3 per year.[357] Only 5 cases have been reported since 2000. A confirmed case has not been reported in the United States since 2003.[12] However, diphtheria is still a potentially significant public health problem. Serologic surveys in the United States and England have suggested that many adults are not immune.[213,357,388] Although a study published in 1996 demonstrated that most adults in the United States do have protective concentrations of serum antitoxin,[288] more recent data obtained from a national population-based serosurvey published in 2002 revealed that the prevalence of immunity to diphtheria, as determined by the level of diphtheria antitoxin, progressively decreased from 91 percent among children 6 to 11 years of age to approximately 30 percent in those 60 to 69 years of age.[409] Of the reported cases with known patient age since 1980, 58 percent were in persons 20 years of age or older and 44 percent of cases were among persons 40 years of age or older. Most cases have occurred in unimmunized or inadequately immunized persons. The current age distribution of cases corroborates the finding of inadequate levels of circulating antitoxin in many adults. In addition, adequate immunization has not eliminated the potential for transmission of *Corynebacterium diphtheriae* completely because immunization does not prevent carriage of *C. diphtheriae* in the nasopharynx or on the skin.[86,357]

As a result of inadequate immunity in adults as well as in infants and children, an epidemic of diphtheria occurred in the 1990s throughout the former Soviet Union including Russia, Ukraine, and the central Asian republics, with case-fatality rates ranging from 3 to 23 percent.[262,299] In addition, diphtheria continues to be a significant cause of morbidity and mortality in developing countries,[262] and, therefore, it remains a source of possible exposure during travel to endemic countries. Because humans are the only known reservoir for *C. diphtheriae*, universal immunization with a diphtheria toxoid–containing vaccine is the only effective control measure.

Preparations

Diphtheria toxoid is produced by growing toxigenic *C. diphtheriae* in liquid medium. The filtrate is incubated with formaldehyde to convert toxin to toxoid and then is adsorbed onto an aluminum salt adjuvant.

Diphtheria toxoid is available in combination with DTaP for routine immunization of infants and children younger than 7 years of age and for adolescents and adults aged 11 to 64 years as a single booster (Tdap). Two brands of Tdap are available: Boostrix (approved for persons 10 years of age and older) and Adacel (approved for persons 11 to 64 years of age). DTaP and Tdap vaccines do not contain thimerosal as a preservative. Diphtheria toxoid also is available as a DTaP-inactivated poliovirus (IPV)-hepatitis B combination, a DTaP-inactivated poliovirus (IPV)-Hib conjugate combination, and in combination with tetanus toxoid (DT and Td) alone for use when pertussis vaccination is contraindicated. No diphtheria-only vaccine is available. Hib, pneumococcal, and meningococcal conjugate vaccines containing diphtheria toxoid or CRM_{197} protein, a nontoxic variant of diphtheria toxin, are not substitutes for diphtheria toxoid immunization.[12]

Pediatric formulations (DT and DTaP) contain a similar amount of tetanus toxoid as adult Td, but they contain three to four times as much diphtheria toxoid. The concentration of diphtheria toxoid (D) for children younger than 7 years of age per 0.5 mL intramuscular dose of DTaP or DT vaccine is 6.7 to 25 limit of flocculation units (Lf), depending on the vaccine manufacturer. Vaccines approved for children 7 years or older and adults (Tdap and Td) contain only a fraction of the diphtheria toxoid (d, 2 to 2.5 Lf) because of adverse reactions related to dose and age.[12,86,357]

Immunogenicity

After a primary series of three properly spaced diphtheria toxoid doses in adults or four doses in infants, a protective level of antitoxin (defined as ≥0.1 international unit [IU] of antitoxin/mL) is reached in more than 95 percent of vaccinees. Diphtheria toxoid has been estimated to have a clinical efficacy of 97 percent. Immune response to diphtheria toxoid was measured after administration of each adult Tdap vaccine and compared with that elaborated after Td vaccine. Seroprotective anti-diphtheria levels, defined as a titer of 0.1 IU/mL or more, and booster response rates to diphtheria were determined to be noninferior after administration of Tdap as compared with Td vaccination.[357]

Adverse Events

Other than local reactions of pain and swelling at the site of vaccine injection, immunization does not cause significant adverse events. These local reactions have been attributed to hypersensitivity reactions in response to the pertussis component and are not a contraindication for administration of further vaccination if otherwise indicated.

Regarding adverse reactions reported after administration of Tdap, vaccination with Boostrix was associated with a statistically higher rate of moderate to severe headache compared with Td, and vaccination with Adacel was associated with higher rates of mild injection site pain

and low-grade fever compared with the Td vaccine. No serious adverse events have been reported for Boostrix. There have been two reports of serious adverse events, both characterized as neuropathic reactions, in adults, possibly related to having received Adacel (none reported in adolescents); and in both cases, symptoms resolved completely within several days.[86]

Indications

Primary immunization consists of five doses of diphtheria toxoid, provided as DTaP or as DT if pertussis is contraindicated.[12,86,357] The first three doses are administered routinely to children who are 2, 4, and 6 months of age. A fourth dose is administered 6 to 12 months after the third dose, between 12 to 18 months of age, to maintain adequate antibody concentrations for the ensuing preschool years. For those not immunized in infancy, the initial dose is followed by two doses given 2 and 8 to 14 months later. A single booster dose given when the child is 4 to 6 years of age, before school entry, is indicated unless the preceding dose was given after the fourth birthday. Interruption of the recommended schedule or delay in administering subsequent doses during primary immunization does not reduce immunity or necessitate restarting the series.

In 2005, the FDA licensed two vaccines containing tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis antigens (Tdap) for routine use in adolescents and adults. Tdap is recommended only for a single dose across all age groups, including as a one-time only substitute for the next scheduled or recommended Td booster in those individuals who previously completed their primary vaccination series with DTP or DTaP. The preferred age for Tdap vaccination is 11 to 12 years. Adolescents aged 11 to 18 years who completed their primary series with DTP or DTaP are encouraged to receive Tdap. Based on additional data on the safety of administering Tdap less than 5 years after Td, pertussis vaccination, when indicated, should not be delayed, and Tdap should be administered regardless of interval since the last tetanus or diphtheria toxoid-containing vaccine. Although longer intervals between Td and Tdap could decrease the occurrence of local reactions, the benefits of protection against pertussis outweigh the potential risk for adverse events.[182] Every 10 years thereafter, tetanus booster should be provided by Td. Adolescents between the ages of 11 and 18 years who have never been vaccinated against tetanus, diphtheria, or pertussis initially should receive Tdap followed by Td for the subsequent two doses at 4 weeks or more and 6 to 12 months following.

No pertussis-containing vaccine has been FDA approved for children between the ages of 7 and 10 years. If pertussis vaccine is not contraindicated, children aged 7 through 10 years who are not fully vaccinated (have not received five doses of DTaP or four doses of DTaP if the fourth dose was administered on or after the fourth birthday) should receive a single dose of Tdap to provide protection against pertussis. If additional doses of tetanus and diphtheria toxoid–containing vaccines are needed for children aged 7 through 10 years, after Tdap vaccine is substituted as the first dose, Td vaccine should be the subsequent two doses at 4 weeks or more and 6 to 12 months following if indicated for the catch-up series.[12,182] Tdap is recommended in this age group because of its reduced antigen content compared with DTaP, resulting in reduced reactogenicity.[182]

After exposure to a case of strongly suspected or proven diphtheria, asymptomatic previously immunized persons should receive a booster of an age-appropriate diphtheria toxoid-containing vaccine if they have not received a booster dose of a diphtheria toxoid-containing vaccine within 5 years. Tdap (in persons 10 years of age and older) is preferred over Td, if the person previously did not receive pertussis booster vaccine.[12] If not previously immunized, carriers should receive age-appropriate diphtheria toxoid–containing vaccine (DTaP [or DT], Tdap, or Td) as soon as identified and should complete the entire series.

Patients recovering from diphtheria infection should be immunized because infection does not confer immunity.

Precautions and Contraindications

The only contraindication to diphtheria toxoid is a history of severe hypersensitivity developing after receipt of a previous vaccine dose. Vaccination with either diphtheria or tetanus toxoid is not known to be associated with an increased risk for having convulsions. Local reactions alone do not preclude continued use.

Haemophilus influenzae Type B Vaccine

Before the introduction of routine infant and childhood vaccination against *Haemophilus influenzae* type b, this pathogen was the major cause of invasive bacterial infections in young children in the United States. It was the most common cause of bacterial meningitis and epiglottitis and a significant cause of septic arthritis, osteomyelitis, septicemia, occult febrile bacteremia, pneumonia, pericarditis, and cellulitis in children younger than 5 years, in whom it caused an estimated 12,000 cases of meningitis and 8,000 additional cases of invasive *H. influenzae* type b disease annually.[201] The cumulative risk for *H. influenzae* type b disease was approximately 1 in every 200 U.S. children in the first 5 years of life, with the peak incidence of *H. influenzae* type b meningitis occurring in infants between 6 and 12 months of age. In high-risk populations, such as American Indians, rates of disease in the absence of immunizations were higher, and a greater proportion of cases of meningitis occurred in the first year of life than in non–high-risk populations.[201,308,567]

In 2006, the WHO estimated that at least 3 million cases of serious disease and approximately 386,000 deaths were attributable to *H. influenzae* type b annually. These figures were substantiated by a systematic study on global burden of disease.[561] As of 2011, 171 of the 193 WHO Member States (89 percent) had adopted the vaccine in their routine immunization programs. As a consequence, invasive *H. influenzae* type b disease has been virtually eliminated in many industrialized countries and its

incidence is dramatically reduced in many parts of the developing world. However, the WHO estimates that only 42 percent of the eligible population is vaccinated against *H. influenzae* type b in developing countries, and only 8 percent in the poorest countries.[426] *H. influenzae* type b remains the most common cause of nonepidemic bacterial meningitis in unvaccinated children younger than 1 year of age. Mortality rates for *H. influenzae* type b meningitis treated with appropriate antibiotics range from 3 to 20 percent, and 30 to 40 percent of survivors suffer severe neurologic sequelae.[582]

Because most cases of *H. influenzae* type b disease occur in infancy, vaccines that induce protection before the age of 6 months are necessary for effective control of *H. influenzae* type b disease. The *H. influenzae* type b polysaccharide capsule is the major virulence factor, and antibodies directed against the polysaccharide antigens are protective. Capsular type b is responsible for more than 90 percent of systemic *H. influenzae* infections. However, purified polysaccharide vaccines induce primarily a T-lymphocyte–independent IgM antibody response that is poorly immunogenic, particularly in children younger than 18 months who lack immunologic memory. Covalent linkage of the purified capsular polysaccharide, polyribosylribitol phosphate (PRP), to a protein carrier creates a conjugate glycoprotein that is T-lymphocyte dependent and elicits protective antibody in infants and young children and significantly greater concentrations of circulating anti-PRP at all ages than does the unconjugated polysaccharide. T-cell–dependent antigens involve helper T-lymphocyte activation of a B-cell humoral antibody response. T-cell–dependent antigens also are able to prime for a booster response.

The introduction of Hib conjugate vaccines in the United States, first in children at least 18 months of age in 1987 and for routine infant immunization in 1991, decreased the incidence of meningitis and bacteremia caused by *H. influenzae* type b dramatically. As of 2000, the incidence of invasive *H. influenzae* type b disease in the United States had decreased by 99 percent since the pre-vaccine era, with an incidence rate of less than 1 case per 100,000 children younger than 5 years old.[149] The remarkably rapid reduction in incidence of disease was partly the result of the ability of the vaccine to reduce asymptomatic nasopharyngeal carriage of the organism, an action that had the indirect effect of reducing exposure and infection in those not immunized (herd immunity).[63,303]

Preparations

Two single-antigen Hib conjugate vaccine products and three combination vaccine products that contain Hib conjugate are available in the United States. All Hib vaccines contain PRP (polyribosyl-ribitol-phosphate), the organism's polysaccharide capsular antigen. The two currently licensed single-antigen Hib conjugate vaccines, PRP-OMP (PedvaxHIB) and PRP-T (ActHIB), are approved for use in early infancy (Table 244-8). Another PRP-T product (Hiberix) is approved only for the booster immunization in children from 15 months to 4 years of age. Each conjugate vaccine is chemically and immunologically unique. Both PRP preparations are composed of the type b PRP antigen conjugated to a protein and differ in the protein carrier, size of the saccharide component, and the chemical linkage. The carrier proteins for PRP-OMP and PRP-T are an outer membrane protein from *Neisseria meningitidis* and tetanus toxoid, respectively. Since July 2000, the entire Hib vaccine supply for the United States has been thimerosal free. The dose of each Hib conjugate vaccine is 0.5 mL, given intramuscularly.

Three licensed combination vaccines containing Hib are as follows: (1) DTaP/PRP-T (TriHIBit), which is the combination of Hib with DTaP approved for the fourth dose only; (2) PRP-OMP/Hep B (Comvax), the bivalent Hib and recombinant hepatitis B virus (HBV) vaccine approved for use in children aged 6 weeks to 15 months who were born to hepatitis B surface antigen (HBsAg)–negative mothers, and (3) DTaP/IPV/PRP-T(Pentacel), which is approved for immunization at 2, 4, 6, and 15 to 18 months of age. In June 2012, a combination conjugate vaccine against *H. influenzae* type b (PRP-T) and meningococcus serogroups C and Y (HibMenCY-TT, MenHibrix) was approved for children 6 weeks to 18 months. Recommendations for use of this vaccine are being considered.

Immunogenicity and Efficacy

Placebo-controlled field trials of Hib conjugate vaccines in infants in the United States demonstrated nearly 100

TABLE 244-8 ***Haemophilus influenzae* Type b Conjugate Vaccines**

Manufacturer	Abbreviation (Trade Name)	Carrier Protein
Merck & Co.	PRP-OMP*(PedvaxHIB)	OMP (an outer-membrane protein complex of *Neisseria meningitidis*)
Sanofi Pasteur	PRP-T† (ActHIB)	Tetanus toxoid
GlaxoSmithKline	PRP-T‡ (Hiberix)	Tetanus toxoid

*PRP-OMP is also available as a combination vaccine with hepatitis B (COMVAX).
†PRP-T may be reconstituted with diphtheria–tetanus–acellular pertussis (DTaP) (Tripedia; Sanofi Pasteur) to form TriHIBit. TriHIBit is approved only for the fourth dose of the DTaP and *Haemophilus influenzae* type b (Hib) series. Other licensed formulations of DTaP have not been approved by the U.S. Food and Drug Administration for reconstitution and may not be used for this purpose. DTaP/IPV/PRP-T (Pentacel) is approved for the primary vaccine series in infants.
‡Hiberix is approved only for the fourth dose of the Hib series.
Modified from Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.

percent protection and provided the basis for the initial approval of these vaccines for use in this country. In a Navajo population of infants at high risk for acquiring *H. influenzae* type b disease who were vaccinated at 2 and 4 months of age with either PRP-OMP or placebo, vaccine efficacy was 100 percent at 1 year of age and 93 percent in total.[491] Licensure of PRP-T was based on immunogenicity in a three-dose schedule that was comparable to that of the other two products. In addition, an efficacy trial in Great Britain and the lack of cases in trials indicate an efficacy of PRP-T comparable to that of PRP-OMP.[83,491,537] Although the data are limited, some evidence suggests that administration of the first dose of the Hib vaccine before the child reaches the age of 6 weeks may result in immunologic tolerance to the *H. influenzae* type b antigen and reduce the immune response to subsequent doses.[410] A PRP antibody concentration of greater than 0.15 μg/mL is considered to indicate short-term protection; concentrations greater than 1.0 μg/mL 1 month after the completion of primary immunizations are considered to be markers of long-term protective immunity against invasive *H. influenzae* type b disease. Children who have invasive *H. influenzae* type b infection before 24 months of age may remain at risk for another *H. influenzae* type b infection and they should be immunized according to the age-appropriate schedule for unimmunized children.[13] Children who develop invasive disease despite two to three Hib vaccines as well as those with recurrent invasive *H. influenzae* type b warrant an immunologic evaluation. Nontypeable *H. influenzae* lacks a polysaccharide capsule that renders the Hib vaccine ineffective against this cause of acute otitis media (AOM), acute sinusitis, bronchitis, and community-acquired pneumonia.

Adverse Events

Hib vaccines are well tolerated. Local reactions occur in approximately 25 percent of recipients but typically are mild and last less than 24 hours.[228,567] Systemic reactions, such as fever and irritability, are infrequent occurrences. When conjugate vaccines are administered concurrently with DTaP vaccine, the incidence of systemic reactions is similar to that observed when only DTaP is given.[228]

Indications

Routine vaccination against *H. influenzae* type b disease is recommended for all children beginning at approximately 2 months of age.[13,121,155] Four vaccines, PRP-T (ActHIB), PRP-OMP (PedvaxHIB), PRP-OMP/hepatitis B (COMVAX), and DTaP/IPV/PRP-T (Pentacel), are licensed in the United States for use in infants. One of these products, PRP-T, can be given with DTaP (Hiberix), mixed in the same syringe for the fourth dose only.

Two or three doses, depending on the product, given at 2-month intervals (optimal) are indicated by the time that the child is 6 months of age. The recommended age to initiate the primary series is 2 months, with a minimum age of 6 weeks. The minimum interval between doses is 4 weeks, with at least 8 weeks separating the last dose in the primary series and the booster. Excellent immune responses have been achieved when vaccines from different manufacturers have been interchanged in the primary series.[51,76,281] PRP-T and PRP-OMP are considered interchangeable for primary as well as booster vaccinations. If more than a single brand of vaccine is used, the child should receive a three-dose primary series. A final dose of any product, irrespective of the previous vaccines received, is acceptable when the child is 12 to 15 months of age for completion of the Hib vaccine immunization schedule. When feasible, the conjugate vaccine product used for the first dose should be used for subsequent doses in children younger than 12 months.

For children in whom immunization for *H. influenzae* type b infection has not been initiated by the time that they reach 7 months of age, the recommended schedules differ according to the child's age and the choice of conjugate vaccine.[32,121] Previously unimmunized children between 15 and 59 months should be immunized with at least a single dose of any licensed conjugate Hib vaccine. For previously unimmunized children 5 years or older, immunization is indicated only if they have an underlying condition predisposing to *H. influenzae* type b disease, such as asplenia, sickle cell disease, immunoglobulin deficiency, or stem cell transplant, or are immunosuppressed because of chemotherapy or HIV infection. More than one dose may be recommended for this population.[13]

Precautions and Contraindications

Conjugate vaccines that contain either diphtheria toxoid or protein should not be considered as an immunizing agent against diphtheria; no changes in the schedule for administering DTP are recommended. A conjugate vaccine that contains meningococcal protein should not be considered as an immunizing agent against meningococcal disease.

Hepatitis A Vaccine

The occurrence of hepatitis A is highest in developing countries and reflects the primary route of transmission, fecal-oral, person-to-person spread. In the United States, before vaccine became available, hepatitis A was a common infection and caused substantial morbidity with significant associated costs.[175,353] In 1995, the hepatitis A vaccine was licensed by the FDA, and during the decade that followed the rate of hepatitis A infection in the United States dramatically declined, reaching an all-time low of 5683 cases in 2004.[255]

In the pre-vaccine era, the incidence of hepatitis A varied considerably in different populations.[255,516] A shift occurred in the epidemiology of hepatitis A in the United States after the introduction of hepatitis A vaccine. Disease in the United States most commonly occurred in children 5 to 14 years of age.[255] After vaccines were licensed, rates of infection among children declined more rapidly than did rates among adults, thus resulting in similar rates among all age groups. Historically, rates of infection were highest among Alaska Natives and American Indians, and most cases occurred in a small number of states and counties in the western and southwestern

regions of the country. During the decade after vaccine was licensed, age-specific, racial, and geographic disparities narrowed considerably.[255]

Individuals at risk for acquiring hepatitis A infections include close contacts of persons infected with hepatitis A virus (HAV), travelers to developing countries, those who engage in homosexual and bisexual activity, and injecting drug users. However, in approximately 50 percent of reported cases, no risk factor is identified.[14] These infections likely are attributable to fecal-oral spread from asymptomatic contacts. Because children frequently have asymptomatic infections and may shed virus for prolonged periods, they play an important role in transmission of HAV. Children and infants shed virus for longer periods than adults do, as long as several months after the onset of clinical illness. In one study involving adults without an identified source of infection, 52 percent of their households included a child younger than 6 years.[255] Therefore, it has long been recognized that the control and ultimate elimination of hepatitis A by active immunization would be best achieved through universal childhood immunization.[539] However, it was not until 2005 that hepatitis A vaccine was licensed for use in children from 12 to 23 months of age. Before then, it had been restricted to children older than 2 years old and consequently could not be incorporated readily into routine infant vaccinations. In May 2006, the ACIP recommended including hepatitis A vaccine in the routine infant immunization schedule.[255]

Before vaccine was available, community-wide outbreaks, recurring every 3 to 10 years in high-risk communities, accounted for much of the occurrence of hepatitis A infection and disease. Outbreaks among children attending daycare and daycare staff are common occurrences and have been associated with community outbreaks.[292] However, the prevalence of hepatitis A infection in daycare center staff and in children and adolescents who previously attended daycare is not increased, a finding suggesting that infections within daycare settings most commonly reflect transmission within the community that extends to these settings.[292] Transmission of HAV also can occur in institutions for the developmentally disabled and in neonatal intensive care units. Transmissions from hospitalized patients to health care professionals have been reported.[14] In addition to these examples of direct person-to-person transmission, infection can be acquired by the ingestion of contaminated food or water.

Preparations

Both inactivated and attenuated HAV vaccines have been developed.[216] However, only inactivated vaccines are licensed in the United States. Inactivated HAV vaccine is prepared by methods similar to those used for inactivated poliomyelitis vaccine. Virus is propagated in human diploid fibroblast cell cultures, formalin inactivated, and adsorbed to aluminum hydroxide adjuvant. Two inactivated HAV vaccines are licensed in the United States: Havrix (SmithKline Beecham Biologicals) and Vaqta (Merck & Co.). Both Vaqta and Havrix have two formulations, an adult and a pediatric product with different antigen content (Table 244-9). The pediatric formulation is indicated for persons aged 12 months to 18 years of age. The vaccines can be used interchangeably.[89] Limited data indicate that hepatitis A vaccine may be administered simultaneously with other vaccinations.[14] A combination HAV/hepatitis B vaccine (Twinrix) is licensed in the United States for persons 18 years of age and older. All HAV-containing vaccines are administered intramuscularly.

Immunogenicity and Efficacy

Inactivated hepatitis A vaccine is highly immunogenic. After receiving a single dose, 95 percent of children and nearly all adults seroconvert within 1 month.[14,255] After receipt of a second dose in children, seroconversion approximates 100 percent. Hepatitis A vaccine is immunogenic in children younger than 2 years old who do not have passively acquired maternal antibody.[462,531] In two large clinical trials of inactivated hepatitis A vaccine in children older than 2 years, protective efficacy was greater than 90 percent.[318,569] In a double-blind, placebo-controlled, randomized study in Thailand involving approximately 34,000 vaccinees, the protective efficacy against clinical hepatitis A was 94 percent after administration of two doses given 1 month apart; it was 100

TABLE 244-9 **Recommended Doses and Schedules for Inactivated Hepatitis A Vaccines**

Age (yr)	Vaccine*	Hepatitis A Antigen Dose	Volume per Dose (mL)	No. of Doses	Schedule
1-18	Havrix	720 ELU	0.5	2	Initial and 6-12 mo later
1-18	Vaqta	25 U†	0.5	2	Initial and 6-18 mo later
≥19	Havrix	1440 ELU	1.0	2	Initial and 6-12 mo later
≥19	Vaqta	50 U†	1.0	2	Initial and 6-18 mo later
≥18	Twinrix‡	720 ELU	1.0	3	Initial and 1 and 6 mo later

*Havrix and Twinrix are manufactured by GlaxoSmithKline Biologicals; Vaqta is manufactured and distributed by Merck & Co.
†Antigen units (each unit is equivalent to ≈1 μg of viral protein).
‡A combination of hepatitis B (Engerix-B, 20 μg) and hepatitis A (Havrix, 720 ELU) vaccine (Twinrix) is licensed for use in persons 18 years of age and older in a three-dose schedule. Havrix 360 ELU in single-dose vials is licensed in the United States but no longer is available.
ELU, enzyme-linked immunosorbent assay units.
Modified from Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.

percent after subsequent administration of a 12-month booster dose.[318]

Vaccination also has been demonstrated to be effective in controlling outbreaks in communities with high rates of disease.[255] For example, in a New York State community in which HAV is highly endemic in children, a single dose of vaccine was 100 percent effective beginning 3 weeks after immunization in preventing symptomatic disease.[569] Moreover, observations from countries where routine hepatitis A vaccination of infants or children has been implemented suggest a strong herd immunity effect.[219,235] The duration of protection after vaccination is likely to be prolonged. Protective antibody levels of anti-HAV were observed in 99 percent of children evaluated 5 to 6 years after receiving Vaqta.[568] Kinetic models of antibody decline indicate that protective levels of antibody could be present for more than 25 years in adults and up to 20 years in children.[538,540] No data are available currently to determine whether and when children will need a hepatitis A booster vaccine. In an ongoing study designed to address the need for a booster dose, no cases of hepatitis A among children 9 years after initial vaccination have been reported.[570]

Reduced vaccine immunogenicity has been observed in infants with passively acquired anti–hepatitis A antibody who are administered hepatitis A vaccines.[217,373,376] Studies demonstrated that, despite the presence of lower antibody levels in infants born to anti–hepatitis A–positive mothers, most infants with passively acquired antibody had an anamnestic response to a booster dose 1 to 6 years later.[217,254,336] In most infants, maternally acquired anti–hepatitis A antibody declines to undetectable levels by the time the child reaches 12 months of age.[377] Hepatitis A vaccine is highly immunogenic for all infants when administered when they are older than 1 year of age, irrespective of maternal antibody status.[69,217]

Concurrent administration of immune globulin and vaccine inhibits the peak serum antibody concentration achieved but not the rate of seroconversion.[553] Because antibody concentrations reach much higher than protective levels, this inhibition is not considered clinically significant and supports passive-active immunoprophylaxis when indicated.

Adverse Events

Except for rare reports of anaphylaxis and anaphylactoid reaction in adults in Europe and Asia, serious reactions to inactivated HAV vaccine have not been reported.[254] Pain, tenderness, and infection at the injection site can occur.[318]

Indications

HAV immunization is recommended routinely for children 12 through 23 months of age, for persons who are at increased risk for infection, for persons who are at increased risk for severe manifestations of hepatitis A if infected, and for any person who wants to obtain immunity.[14,33,175,184,255]

All children in the United States should receive hepatitis A vaccine routinely according to the licensed two-dose schedule, with the initial dose administered at age 1 year (12 to 23 months) and the second dose 6 to 12 months later. Children who are not immunized by 2 years of age can be immunized at subsequent visits.

In addition, hepatitis A vaccination is recommended routinely for the following persons who are at increased risk for acquiring infection[14,33,255]:

- All susceptible persons traveling to or working in countries that have a high or intermediate hepatitis A endemicity; these persons should be vaccinated or receive immune globulin before departure.[175] HAV vaccine at the age-appropriate dose is preferred to immune globulin. The first dose of HAV vaccine should be administered as soon as travel is considered. One dose of single-antigen vaccine administered at any time before departure can provide adequate protection for most healthy persons. Travelers who are younger than 12 months of age should receive a single dose of immune globulin (0.02 mL/kg), which provides effective protection for up to 3 months.
- Susceptible household members and other close personal contacts (e.g., regular babysitter) of international adoptees newly arriving from countries with high or intermediate hepatitis A endemicity.[43,184] Data from a study conducted at 3 adoption clinics in the United States indicate that 1 to 6 percent of newly arrived international adoptees have acute HAV infection. The risk for HAV infection among close personal contacts of international adoptees is estimated at 106 (range, 90 to 819) per 100,000 household contacts of international adoptees within the first 60 days of their arrival in the United States. Therefore, HAV vaccine should be administered to all previously unvaccinated persons who anticipate close personal contact (e.g., household contact or regular babysitting) with an international adoptee from a country with high or intermediate endemicity during the first 60 days after arrival of the adoptee in the United States. The first dose of the two-dose HAV vaccine series should be administered as soon as adoption is planned, ideally two or more weeks before the arrival of the adoptee.
- Men who have sex with men. Outbreaks of hepatitis A among men who have sex with men have been reported often, including in urban areas in the United States, Canada, and Australia. Therefore, men (adolescents and adults) who have sex with men should be immunized.
- Users of injection and noninjection drugs. Periodic outbreaks among injection and noninjection drug users have been reported in many parts of the United States and in Europe. Adolescents and adults who use illegal drugs should be immunized.
- Patients with clotting-factor disorders. Reported outbreaks of hepatitis A in patients with hemophilia receiving solvent-detergent–treated factor VIII and factor IX concentrates were identified during the 1990s, primarily in Europe, although one case was reported in the United States. Therefore, susceptible patients with chronic clotting disorders who

receive clotting-factor concentrates should be immunized.

- Persons at risk for occupational exposure (e.g., handlers of nonhuman primates and persons working with HAV in a research laboratory setting). Outbreaks of hepatitis A have been reported among persons working with nonhuman primates. These infected primates were born in the wild and were not primates that had been born and raised in captivity. Persons working with HAV-infected primates or with HAV in a research laboratory setting should be immunized.
- Persons with chronic liver disease. Because persons with chronic liver disease are at increased risk for fulminant hepatitis A, susceptible patients with chronic liver disease should be immunized. Susceptible persons who are awaiting or have received liver transplants should be immunized.

Although the ACIP guidelines do not recommend routine vaccination of food handlers, consideration may be given to vaccination of these workers in areas where state and local health authorities or private employers determine that vaccination is cost effective.

Postexposure Prophylaxis

Persons who have been exposed to HAV and previously have not received HAV vaccine should receive a single dose of single-antigen HAV vaccine or immune globulin as soon as possible (see Table 244-10 for prophylaxis guidance and dosages).[175] The efficacy of immune globulin or vaccine for postexposure prophylaxis when administered more than 2 weeks after exposure has not been established. Information about the relative efficacy of vaccine compared with immune globulin postexposure is limited, and no data are available for persons older than 40 years of age or persons with underlying medical conditions.

All previously unimmunized persons with close personal contact with a person with serologically confirmed HAV infection, such as household and sexual contacts, should receive vaccine or immune globulin within 2 weeks after the most recent exposure. Serologic testing of contacts is not recommended, because testing adds unnecessary cost and may delay administration of postexposure prophylaxis.

Outbreaks of HAV infection at child care centers have been recognized since the 1970s, but their frequency has decreased as HAV immunization rates in children have increased and as hepatitis A incidence among children has declined. HAV vaccine or immune globulin should be administered to all previously unimmunized staff members and attendees of child care centers or homes if (1) one or more cases of hepatitis A are recognized in children or staff members or (2) cases are recognized in two or more households of center attendees. In centers that provide care only to children who do not wear diapers, vaccine or immune globulin need be given only to classroom contacts of an index-case patient. When an outbreak occurs (i.e., hepatitis A cases in two or more families), vaccine or immune globulin also should be considered for members of households that have children (center attendees) in diapers. Children and adults with hepatitis A should be excluded from the center until 1 week after onset of illness, until the postexposure prophylaxis program has been completed in the center, or until directed by the health department. Although precise data concerning the onset of protection after postexposure prophylaxis are not available, allowing prophylaxis recipients to return to the child care center setting immediately after receipt of the vaccine or immune globulin dose seems reasonable.

Schoolroom exposure generally does not pose an appreciable risk for infection, and postexposure prophylaxis is not indicated when a single case occurs and the source of infection is outside the school. However, HAV vaccine or immune globulin could be used for unimmunized persons who have close contact with the index patient if transmission within the school setting is documented.

Usually, health care–associated HAV in hospital personnel has occurred through spread from patients with acute HAV infection in whom the diagnosis was not recognized. Careful hygienic practices should be emphasized when a patient with jaundice or known or suspected hepatitis A is admitted to the hospital. When outbreaks occur, HAV vaccine or immune globulin is recommended for persons in close contact with infected patients. There

TABLE 244-10 **Recommendations for Postexposure Immunoprophylaxis of Hepatitis A Virus**

Time Since Exposure	Age of Patient	Recommended Prophylaxis
≤2 wk	<12 mo	IG, 0.02 mL/kg
	12 mo through 40 yr	HAV vaccine*
	≥41 yr	IG, 0.02 mL/kg but HAV vaccine can be used if IG is unavailable
	Persons of any age who are immunocompromised or have chronic liver disease	IG, 0.02 mL/kg
≥2 wk	<12 mo	No prophylaxis
	≥12 mo	No prophylaxis but HAV vaccine may be indicated for ongoing exposure

*Dosage and schedule of HAV vaccine as recommended according to age in Table 244-9.
IG, immune globulin.
Modified from Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.

is no recommendation for routine preexposure use of HAV vaccine for hospital personnel.

If a food handler is diagnosed with hepatitis A, HAV vaccine or immune globulin should be provided to other food handlers at the same establishment. Food handlers with acute HAV infection should be excluded for 1 week after onset of illness. Because common-source transmission to patrons is unlikely, postexposure prophylaxis with HAV vaccine or immune globulin typically is not indicated but may be considered if the food handler directly handled food during the time when the food handler likely was infectious and had diarrhea or poor hygiene practices and if prophylaxis can be provided within 2 weeks of exposure. Routine HAV immunization of food handlers is not recommended.

Postexposure prophylaxis usually is not recommended, because these outbreaks commonly are recognized too late for prophylaxis to be effective in preventing HAV infection in exposed persons. HAV vaccine or immune globulin can be considered if it can be administered to exposed persons within 2 weeks of an exposure to the HAV-contaminated water or food.

Precautions and Contraindications

Hepatitis A vaccine should not be administered to persons with a hypersensitivity reaction to any of the vaccine components, such as alum or, in the case of Havrix, phenoxyethanol.[33] Safety data in pregnant women are not available, but the risk is considered to be low or nonexistent because the vaccine contains inactivated, purified viral proteins.

Hepatitis B Vaccine

HBV infection is a leading cause of acute hepatitis and a major public health problem of global importance. Its incidence is especially high in many Asian and African countries. Individuals with chronic infection are at risk for the development of chronic hepatitis, cirrhosis, and primary hepatocellular carcinoma. Rates of new infection are highest among adults, but chronic infection is more likely to occur in individuals infected as infants or children. These persons not only are at increased risk for chronic and malignant liver disease but also, as chronic carriers, serve as the reservoir for transmission of HBV.

The initial strategies for prevention of hepatitis B through vaccination reflect the varying epidemiology of HBV infection in different areas of the world.[96] In the United States, for example, infection is of comparatively low endemicity and occurs primarily in adolescents and adults. The risk for acquiring infection, however, is much greater in certain populations. Examples include those born and living in areas or among groups in which HBV is highly endemic and those with lifestyles predisposing to the acquisition of HBV, such as male homosexual activity, intravenous drug abuse, and promiscuous heterosexual activity.[6] In contrast, in geographic areas in which HBV infection is highly endemic, infection usually is acquired at birth or during childhood, a pattern resulting in the recommendation for universal vaccination of infants.

In 1991, the CDC initiated a comprehensive hepatitis B vaccination strategy to eliminate transmission of HBV in the United States.[96] Critical elements of this strategy included preventing perinatal transmission of HBV by identifying and providing immunoprophylaxis to infants of HBsAg-positive mothers and universal hepatitis B vaccination of infants to interrupt transmission and prevent future infection. The advantages of this approach included the existence of effective programs of routine childhood immunization, protection without any need to identify specific risk factors, and protection before significant exposure occurred. In addition, the positive effects of universal infant immunization had been observed in Taiwan, where the strategy of universal infant immunization already was being used. In Taiwan, the overall prevalence rate of HBV for children aged 1 to 10 years decreased from 9.8 percent in 1984 to 1.3 percent in 1994.[188]

In 1994, the ACIP expanded the recommendations to include previously unvaccinated children 11 to 12 years old.[172] In October 1997, these recommendations were expanded still further to include all unvaccinated children from birth to 18 years old and made hepatitis B vaccine available through the Vaccines for Children (VFC) program for persons from birth to 18 years of age who are eligible for this program.[178] The goal of the 1997 recommendations was to increase access to hepatitis B vaccine by encouraging the vaccination of previously unvaccinated children and adolescents from birth to 18 years old whenever they are seen for routine medical visits.[125] This expansion of the recommended age group for receiving vaccination and for VFC eligibility simplifies previous recommendations and the eligibility criteria for receiving VFC vaccine. The other ACIP priorities for giving hepatitis B vaccination to children remained unchanged and included the following groups: all infants; children in populations at high risk for acquiring HBV infection, such as Alaska Natives, Pacific Islanders, and children who reside in households of first-generation immigrants from countries where HBV infection is moderately or highly endemic; previously unvaccinated children 11 to 12 years old; and older adolescents and adults in defined at-risk groups.[96,172]

Between 1990 and 2004, the incidence of HBV infection in the United States declined by 75 percent. The decline was greatest among children and adolescents. Coincident with the decline in HBV incidence, vaccine coverage for children aged 19 to 35 months increased from 16 to 92 percent from 1991 to 2004.[135] Among adolescents 13 to 15 years old, according to CDC data, vaccine coverage increased from zero to 74 percent between 1993 and 2004. Clearly, since its inception in 1991, many aspects of the national immunization strategy to eliminate HBV transmission have been implemented with great success, especially the routine vaccination of all infants. Nonetheless, many challenges remain.

Despite the overall decline in hepatitis B incidence and the recommendations for routine vaccination of infants and children, increasing proportions of new HBV infections in the United States occur among adolescents and adults who have defined risk factors, including persons with multiple sex partners (more than one partner during

the preceding 6 months), men who have sex with men, and injecting drug users.[384] The primary means of preventing these infections is to identify settings such as correctional facilities, sexually transmitted disease clinics, and drug treatment centers where adolescents and adults with high-risk drug and sexual practices can be routinely accessed and vaccinated. In correctional facilities where previously unvaccinated inmates are offered HBV vaccination, 60 to 80 percent of inmates offered accepted vaccination.[566]

Even though universal screening of all pregnant women has been widely adopted across the United States, fewer than half of all HBsAg-positive pregnant women are identified through prenatal screening.[251] These women with unknown HBsAg status are not screened consistently, even when they are hospitalized during labor and delivery; consequently, their infants do not receive appropriate postexposure prophylaxis.[526] Furthermore, the birth dose of HBV vaccine, which could serve as a safety net for infants born to mothers for whom testing either was not performed or not performed correctly, was administered to only 45 percent of infants born in the United States in 2004, less than the rate of 54 percent seen before July of 1999, when recommendations were made to suspend the birth dose of hepatitis B vaccine temporarily until a thimerosal-free vaccine became available.[382]

To enhance existing strategies aimed at prevention of perinatal HBV transmission, the ACIP recommended in December of 2005 that all delivery hospitals institute specific policies and procedures to improve identification of infants born to HBsAg-positive mothers and mothers with unknown HBsAg status. These recommendations were made to ensure administration of appropriate postexposure prophylaxis and a birth dose of HBV vaccine to all medically stable infants. This statement also updated recommendations to improve vaccination coverage of adolescents and children by implementing immunization record reviews for all children 11 to 12 years old and children younger than 19 years of age who were born in countries with high or intermediate HBV endemicity and vaccinating all unvaccinated adolescents in settings that provide health care services to this age group.[399]

Preparations

Hepatitis B vaccines consisting of inactivated purified HBsAg derived from chronic hepatitis B plasma were introduced first in the early 1980s. In the late 1980s, two recombinant vaccines (Recombivax HB, Merck & Co.; and Engerix-B, SmithKline Beecham Biologicals) were licensed in the United States and currently are available in both single-antigen and combined formulations. Only the recombinant vaccines are available in the United States, but plasma-derived vaccines are widely used in other areas of the world. The recombinant vaccines contain 5 to 40 μg/mL of HBsAg protein. Pediatric formulations contain no thimerosal or only trace amounts. Vaccine is administered intramuscularly in the anterolateral thigh or deltoid area, depending on the age and size of the recipient. Administration in the buttocks or intradermally has been associated with decreased immunogenicity and is not recommended at any age.

Immunogenicity and Efficacy

The recommended series of three doses of vaccine induces a protective antibody response in more than 95 percent of infants, children, adolescents, and adults younger than 40 years.[16,399] In field trials, efficacy has been 80 to 95 percent and generally correlates with immunogenicity. Protection against disease is virtually 100 percent for persons who develop adequate serum antibody concentrations (anti-HBs ≥10 mIU/mL) after receiving vaccination. Adults older than 40 years of age and immunosuppressed persons are less likely to develop protective anti-HBs concentrations.

Hepatitis B vaccine is highly immunogenic. Therefore, postvaccination serologic testing is not indicated, with the exception of infants born to HBsAg-positive women, persons with ongoing occupational exposure to blood, and persons with immunosuppressive conditions. Active immunization in combination with passive immunoprophylaxis with hepatitis B immune globulin (HBIG) administered within 12 to 24 hours after birth to infants born to chronically infected mothers is more than 90 percent effective in preventing transmission of HBV to the infant. Active postexposure vaccination without HBIG administered soon after birth has been shown to be effective in preventing perinatal transmission and is used in areas where the use of HBIG is impractical.[282]

Vaccine-induced protection against symptomatic infection in a normal host is prolonged and correlates with immunologic memory, which has been demonstrated in immunized children and adults for at least 12 years after vaccination. Children immunized at birth are protected for at least 10 years. Thus, a need for routine booster doses has not been demonstrated.

Vaccine failures related to HBV variants with mutations in the S gene leading to conformational changes in the HBsAg protein, the major target for neutralizing anti-HBs antibody, have occurred in fully vaccinated children and perinatally exposed infants who received appropriate active and passive postexposure prophylaxis and in many cases had protective anti-HBs antibody levels.[315] Although no evidence to date establishes that these escape mutations pose a threat to the effectiveness of current hepatitis B vaccine programs, future surveillance to detect emergence of HBV variants in immunized populations is warranted.[399,595]

Adverse Events

Other than soreness at the injection site, reactions to hepatitis B vaccine rarely occur. Postvaccination surveillance performed after licensure of the plasma-derived vaccine indicated a possible association between Guillain-Barré syndrome and receipt of the first vaccine dose, but no evidence indicates an association of Guillain-Barré syndrome with recombinant vaccine.[399,400] Anaphylaxis has been estimated to occur in 1 in 600,000 doses distributed.[399,400] Several nonfatal cases have been reported in children.

Indications

Routine immunization with hepatitis B vaccine is recommended for all infants in the United States and should be completed by 6 to 18 months of age for all infants born to HBsAg-negative mothers.[15,399] Delivery hospitals should develop policies that ensure administration of a birth dose for all infants weighing 2000 g or more at birth unless a physician's order to defer immunization is in place and the serologic status of the mother is in the infant's medical record. Administering the first dose of hepatitis B vaccine soon after birth should minimize the risk for development of infection because of errors in maternal HBsAg testing or reporting or from exposure to persons with chronic HBV infection in the household and can increase the likelihood of completing the vaccine series. For infants weighing less than 2 kg who are born to HBsAg-negative mothers, initiation of vaccine should begin starting at 1 month of chronologic age. Administration of the second dose of vaccine is recommended 1 to 2 months after administration of the first dose, followed by a third dose when the infant is 6 to 18 months of age.

Only single-antigen hepatitis B vaccine can be used for doses given to infants between birth and 6 weeks of age. Single-antigen or combination vaccine may be used to complete the series; four doses of vaccine may be administered if a birth dose is given and a combination vaccine containing a hepatitis B component is used to complete the series.

Routine screening of all pregnant women for HBsAg is recommended because of the necessity for administering a birth dose of vaccine and HBIG.[16,399] Infants born to HBsAg-positive mothers, including pre-term and low-birth-weight infants, should be given HBIG (0.5 mL) within 12 hours of birth at a separate injection site, as well as the birth dose of HBV vaccine.[16,399] If a pre-term infant weighing less than 2000 g is born to an HBsAg-positive mother, the birth dose of hepatitis B vaccine should not be counted toward completion of the vaccine series and three additional doses of hepatitis B vaccine should be administered beginning when the child is 1 month of age. In addition to receiving HBIG and the hepatitis B vaccine series, infants of HBsAg-positive mothers should be tested for HBsAg and antibody to HBsAg (anti-HBs) at 9 to 15 months of age to identify those with chronic HBV infection or those who may require re-vaccination.[399]

A woman whose HBsAg status is unknown at delivery should undergo blood testing as soon as possible to determine her HBsAg status. The infant should receive the first dose of hepatitis B vaccine within 12 hours. If the woman is found to be HBsAg-positive, her infant should receive HBIG as soon as possible within 7 days. In populations where HBsAg testing of pregnant women is not feasible, all infants should receive hepatitis B vaccine at birth and 2 months and complete the series by the time they reach 6 months of age.

Hepatitis B vaccination is recommended for all children and adolescents younger than 19 years old in the United States. Children who have not been immunized previously may begin the vaccine series during any visit. All children aged 11 to 12 years old should have a review of their immunization records and receive the complete hepatitis B vaccine series if they have not been vaccinated previously. All children and adolescents younger than 19 years old who were born in Asia, the Pacific Islands, Africa, or other intermediate- or high-endemic countries or have at least one parent who was born in one of these areas should have a review of their immunization records and complete the hepatitis B vaccine series if they were not vaccinated previously.[399]

Vaccination also is recommended for those with one or more of the following risk factors for acquiring HBV infection[16]:

- Sexually active adolescents and adults who have a recently acquired sexually transmitted disease, are seeking evaluation or treatment for a sexually transmitted infection, are identified as prostitutes, or have had one or more sex partners in the previous 6 months
- Sexually active men who have sex with men
- Household contacts or sexual partners of HBsAg-positive persons
- Injecting drug users
- Persons at occupational risk for acquiring infection through exposure to blood or blood-contaminated body fluids, such as health care workers and public safety workers
- Residents and staff of institutions for the developmentally disabled
- Patients undergoing hemodialysis (vaccination of those with early renal failure is encouraged before they require hemodialysis)
- Patients with human immunodeficiency virus (HIV) infection, chronic liver disease, and diabetes mellitus
- Members of households with international adoptees who are HBsAg positive
- Travelers, especially children, to areas with high and intermediate rates of infection with HBV who have close contact with the local population or are likely to have contact with blood, such as in a medical setting, or sexual contact with residents
- Inmates in long-term correctional facilities

In addition to active and passive immunoprophylaxis of infants born to HBsAg-positive mothers, post-exposure prophylaxis is recommended in the following circumstances:

- Sexual partner of an HBsAg-positive person. A single dose of HBIG within 14 days of the last sexual contact and initiation of the three-dose hepatitis B vaccination is recommended for susceptible persons.
- Household exposure or close contact of an unvaccinated infant younger than 12 months old to a primary caregiver who has acute or chronic hepatitis B infection. Infants in this circumstance should receive HBIG, in addition to being vaccinated against hepatitis B. If, at the time of exposure only one dose of vaccine has been administered, the second dose should be administered if the interval is appropriate or HBIG should be administered if immunization is not yet due. If the infant has been immunized fully or has received at least two doses

of vaccine, the infant should be presumed protected and HBIG is not required.

- Accidental percutaneous or per mucosal exposure of a susceptible person to HBsAg-positive blood. This indication is exemplified most by a needle stick or other accident involving blood in a hospital. Another example is an injury to a susceptible person caused by the bite of an HBsAg-positive child. For this indication, HBIG as well as vaccine is given. Recommendations in these circumstances are complex and based on the availability of the blood source for HBsAg testing and the hepatitis B vaccination status of the exposed person.

Booster doses are not recommended except in the case of patients undergoing hemodialysis and possibly other immunocompromised patients, in whom annual antibody testing should assess the need. An additional dose is indicated for those whose serum anti-HBsAg concentration is less than 10 mIU/mL.

The current recommended doses and schedule for the use of hepatitis B vaccines licensed in the United States in infants and other age groups are shown in Table 244-11 (see elsewhere for more information[15,399,400]). For completing the hepatitis B vaccine series and achieving complete vaccination for hepatitis B, the two licensed hepatitis B vaccines are interchangeable when administered in doses recommended by the manufacturers.[16,399] In September of 1999, the FDA approved an optional two-dose schedule of Recombivax HB for vaccination of adolescents 11 to 15 years of age. The ACIP recommended that this schedule be included in the VFC program in February 2000.[136] With the two-dose schedule, the adult dose of Recombivax HB is administered to adolescents 11 to 15 years old, with the second dose given 4 to 6 months after the first dose. In immunogenicity studies in adolescents 11 to 15 years of age, antibody concentrations and seroprotection rates were similar with the two-dose schedule and the currently licensed three-dose schedule. Follow-up data collected during the course of 2 years indicate that the rate of decline in concentration of antibody for the two-dose schedule was similar to that for the three-dose schedule. No data are available to assess long-term protection or immune memory after vaccination with the two-dose schedule, and whether booster doses of vaccine will be required is not known. Children and adolescents who have begun vaccination with a dose of the pediatric formulation should complete the three-dose series with this dose. Similarly, if the formulation administered to an adolescent at the start of a series is not known, the series should be completed with the three-dose schedule.

A combination of hepatitis B (Engerix-B, 20 μg) and hepatitis A (Havrix, 720 enzyme-linked immunosorbent assay units [ELU]) vaccine (called Twinrix) is licensed for use in persons 18 years of age and older in a three-dose schedule administered at birth, 1 month, and 6 or more months later. Alternately, a four-dose schedule at birth and days 7 and then 21 to 30 followed by a booster dose at 12 months was approved in 2007 and may be used.[16]

In December of 2006, the ACIP approved recommendations for hepatitis vaccine use in adults.[400] In settings in which high proportions of adults have risks for acquiring HBV infection, the ACIP recommends universal hepatitis B vaccination for all unvaccinated adults. These settings include (1) sexually transmitted infection treatment facilities; (2) HIV testing and treatment facilities; (3) facilities providing drug abuse treatment and prevention services; (4) health care settings targeting services to injection drug users; (5) correctional facilities; (6) health care settings serving men who have sex with men; (7) chronic hemodialysis facilities and end-stage renal disease programs; and (8) institutions and nonresidential care facilities for persons with developmental disabilities. In other primary care and specialty medical settings in

TABLE 244-11 **Recommended Doses and Schedules of Hepatitis B Virus Vaccines in the United States**

Group	Recombivax HB Dose μg (mL)	Engerix-B Dose μg (mL)
Infants of HBsAg-negative mothers, children, and adolescents <20 yr	5 (0.5)	10 (0.5)
Infants of HBsAg-positive mothers (HBIG also recommended)	5 (0.5)	10 (0.5)
Adolescents	10 (0.5)*	10 (0.5)†
Adults (≥20 yr)	10 (1.0)	20 (1.0)
Patients undergoing dialysis and other immunocompromised persons		
<20 yr‡	5 (0.5)	10 (0.5)
≥20 yr	40 (1.0)§	40 (2.0)‖

*Adult formulation administered on a two-dose schedule at 0 months and then 4 to 6 months later, licensed for adolescents 11 through 15 years of age.[11]

†Pediatric formulation licensed for use in a three-dose schedule at 0-, 12-, and 24-month schedule for children 5 through 16 years of age, and a 0-, 1-, and 6-month schedule for adolescents 11 through 16 years of age. Also available as a four-dose schedule at 0, 1, 2, and 12 months for all age groups.[11]

‡Higher doses may be more immunogenic, but no specific recommendations have been made.

§Special formulation for patients administered for patients undergoing dialysis.

‖Two 1.0-mL doses administered at one site in a four-dose schedule at 0, 1, 2, and 6 months.

HBIG, hepatitis B immune globulin; HBsAg, hepatitis B surface antigen.

Data from references 16, 399, and 400.

which adults at risk for developing HBV infection receive care, the ACIP recommends that health care providers should inform all patients about the health benefits of vaccination, including risks for acquiring HBV infection, and persons for whom vaccination is recommended and should vaccinate adults who report having risks for developing HBV infection and any adults requesting protection from HBV infection. To promote vaccination in all settings, health care providers should implement standing orders to identify adults recommended for hepatitis B vaccination and administer vaccination as part of routine clinical services, not require acknowledgment of an HBV infection risk factor for adults to receive vaccine, and use available reimbursement mechanisms to remove financial barriers to receiving hepatitis B vaccination. In 2011, the ACIP recommended that all previously unvaccinated adults aged 19 through 59 years with diabetes mellitus (type 1 and type 2) be vaccinated against hepatitis B as soon as possible after a diagnosis of diabetes is made. Unimmunized adults with diabetes mellitus who are 60 years of age or older may be vaccinated at the discretion of the treating clinician after assessing their risk.[186]

Contraindications

The only contraindication to receiving HBV vaccination is a history of anaphylaxis to a previous dose of vaccine. Although data on the safety of HBV vaccines are not available for pregnant women, these vaccines contain only HBsAg and not live virus and should not be deleterious to the developing fetus. Because HBV infection during pregnancy can result in transmission to the neonate, susceptible women at increased risk for acquiring infection should be vaccinated during pregnancy. Inadvertent vaccination of HBsAg-positive persons has no deleterious effects.

Human Papillomavirus Vaccine

Genital HPV infection is thought to be the most common sexually transmitted viral infection, accounting for 6.2 million new cases annually in the United States.[94] The estimated prevalence of HPV infection ranges from 20 to 40 percent among sexually active 20-year-old women.[381] HPV typically infects girls and women soon after sexual exposure.[207,573] Median time from first intercourse to first detection of HPV is only 3 months.[207] The lifetime risk for women to acquire one or more genital HPV infections exceeds 75 percent.[94,381]

More than 40 HPV types can infect the genital tract and are classified on the basis of their association with cervical cancer.[429] The "low-risk," nononcogenic HPV types (e.g., types 6 and 11) are associated with anogenital warts, mild cervical dysplasia, and recurrent respiratory papillomatosis. The "high-risk" oncogenic HPV types are causally linked to several human cancers in both women and men, including cancers of the cervix, vulva, vagina, anus, and penis and a subset of head and neck cancers.[381] Infection with HPV causes virtually all cases of cervical cancer, the second most common cause of death from cancer among women globally, surpassed only by breast cancer.[381] The development of cervical cancer often occurs decades after initial infection. HPV types 16 and 18 account for 70 percent of cervical cancers.[199] A variety of other types account for the remaining 30 percent of cervical cancers, and these types vary in their distribution globally.

Although the incidence of HPV is high, most infections clear without intervention. Seventy percent of new infections clear within 1 year, and 91 percent clear within 2 years.[199] Persistent infection with high-risk HPV types, defined as detection of the same viral type at two or more visits 6 months apart, is the most important risk factor for development of cervical cancer precursor lesions. Studies have demonstrated that persistent infection with a high-risk HPV type is associated with a greater than 10-fold risk for high-grade cervical cancer precursors.[312,420]

A first generation of prophylactic HPV subunit vaccines has been developed.[355] These vaccines are expected to have a significant impact on the incidence of HPV-related cervical and other anogenital cancers in women. Incidence of recurrent respiratory papillomatosis also is likely to decrease as a result of widespread vaccination.

Preparations

In 2006, the FDA licensed the first HPV vaccine for use in girls and women 9 through 26 years of age in the United States, and this was done for use in males 9 through 26 years of age in 2009.[17,157,394] This quadrivalent vaccine (GARDASIL, Merck & Co.) contains HPV types 6, 11, 16, and 18 virus-like particles (VLPs) and protects against four HPV types, which together cause 70 percent of cervical cancers (HPV types 18 and 16) and 90 percent of genital warts (HPV types 6 and 11).

The quadrivalent HPV vaccine (HPV4) is prepared from the highly purified VLPs of the major capsid (L1) protein of HPV types 6, 11, 16, and 18. The L1 proteins are produced by *Saccharomyces cerevisiae* and self-assemble into VLPs. The VLPs mimic the HPV virus, but they contain no viral DNA. Each 0.5-mL dose contains 20 µg HPV 6 L1 protein, 40 µg HPV 11 L1 protein, 40 µg HPV 16 L1 protein, and 20 µg HPV 18 L1 protein.

A bivalent vaccine (CERVARIX, GlaxoSmithKline) which is directed against two oncogenic HPV types (16 and 18) was licensed for use in females 10 through 25 years of age in 2009 and not licensed for males.[17,118] The quadrivalent and bivalent HPV vaccines use alum as an adjuvant and do not contain thimerosal or antibiotics. The vaccines should be stored at 2°C to 8°C (36°F to 46°F) and not frozen.[118]

Primary vaccination dosing schedules with the quadrivalent and bivalent HPV vaccine are the same and consist of three 0.5-mL doses administered intramuscularly. The second and third doses should be administered 2 and 6 months after administration of the first dose.

Immunogenicity and Efficacy

Early data from randomized controlled trials have shown consistently that prophylactic HPV VLP vaccines are immunogenic and efficacious in preventing infection and

lesions caused by the targeted HPV types.[301,355,381,411,514] Vaccine efficacy seems to be mediated by a type-specific humoral immune response. Evaluations of the quadrivalent HPV4 vaccine have demonstrated that vaccinated individuals develop high antibody titers to the respective HPV types that exceed the antibody titers seen with natural HPV infection.[514] However, the level of antibody that confers a protective response is unknown.[514] For the quadrivalent HPV VLP vaccine, an antibody response significantly greater than placebo was sustained for at least 3 years without booster doses.[301,551] Although vaccine efficacy studies have been done only in adolescents ages 13 years and older, immunogenicity bridging studies have been performed in children as young as 9 years of age. Antibody titers to HPV types 6, 11, 16, and 18 were found to be higher in younger adolescents than in young adults. From these data, investigators inferred that protection exists against cervical cancer, cancer precursor lesions, and genital warts, even for young adolescent girls who receive the quadrivalent vaccine.

The results from four randomized controlled trials repeatedly demonstrate that a regimen of three intramuscular injections of HPV VLP vaccine provides high-level protection from infection and lesions caused by targeted HPV types.[355,381] Efficacy from a combined analysis of quadrivalent HPV vaccine efficacy from both phase II and phase III clinical trials is 100 percent against precancerous lesions (carcinoma in situ II/III or adenoma in situ) caused by HPV type 16 or 18 and is 99 percent against development of genital warts.[411] It is not known whether HPV vaccines provide long-term protection and whether booster doses will be necessary. The quadrivalent HPV4 vaccine has not been found to have efficacy against existing disease or infection caused by HPV.

Bivalent vaccine (HPV2) efficacy was evaluated in two randomized, double-blind, controlled clinical trials in females aged 15 through 25 years. In the analysis, 99 percent or more of participants developed an HPV 16 and 18 antibody response 1 month after completing the three-dose series. In addition, after concomitant administration of HPV2 with tetanus toxoid, diphtheria toxoid, and acellular pertussis vaccine and/or with meningococcal conjugate vaccine in females aged 11 through 18 years compared with those after administration at separate visits, the antibody responses for bivalent vaccine antigens were noninferior.[118]

HPV4 and HPV2 vaccines are highly effective in preventing HPV 16- and 18-related cervical precancerous lesions in females. The quadrivalent vaccine is highly effective in preventing HPV 6- and 11-related genital warts in females and males, and HPV4 vaccine also has been shown to be effective in preventing anal precancerous lesions in males. Vaccines offer no protection against progression of infection to disease from HPV acquired before immunization.[17]

Adverse Events

HPV vaccines appear to be generally safe and well tolerated. The quadrivalent HPV vaccine has been associated with minimal adverse reactions.[240] The most common are mild fever (<38°C [100.4°F]) and local injection site reactions such as pain, redness, pruritus, or swelling. Additional data on vaccine safety, including data on pregnancy and fetal and infant outcomes, are being collected in ongoing trials and will continue to be collected in post-licensure studies.

In analyses of local and general adverse events after bivalent HPV2 vaccine administration versus control vaccines, 92 percent reported injection-site pain; 48 percent, redness; and 44 percent, swelling in the HPV2 group. These findings compared with 64 to 87 percent, 24 to 28 percent, and 17 to 21 percent in the control groups for the same findings. Fatigue, headache, and myalgia were the most common general symptoms noted.[118]

Indications

The ACIP issued recommendations for the use of the quadrivalent HPV vaccine in March 2007 and for the bivalent HPV2 vaccine in May 2010.[118,119,157,394] Routine vaccination with three doses of either vaccine is recommended for girls 11 to 12 years of age. The vaccination series can be started in girls as young as 9 years of age, with physician and parental discretion. Catch-up vaccination is recommended for girls and women 13 to 26 years old who have not been vaccinated previously or who have not completed the full vaccination series. Only HPV4 vaccine is recommended for routine immunization of males at 11 or 12 years of age (the series can be started at 9 years of age) and also is recommended for males 13 through 21 years of age not previously immunized. Males 22 through 26 years of age may be immunized upon request. Vaccine is recommended for men who have sex with men and males who are immunocompromised (including those with HIV infection) through 26 years of age. Ideally, vaccination should be administered before potential exposure to HPV through sexual contact occurs. Neither quadrivalent or bivalent vaccines are licensed for use in persons older than 26 years of age.[17]

Vaccine is administered in a three-dose schedule, and the second and third doses should be administered 2 and 6 months after the first dose. Immunogenicity data allow for some degree of flexibility in the dosing schedule, which is often an important consideration in providing multidose vaccines to adolescents for whom contact with health providers can be sporadic. For both vaccines, the minimum interval from dose 1 to dose 2 is 4 weeks. The minimum interval from dose 2 to dose 3 should not be less than 12 weeks.

HPV vaccine can be administered at the same visit when other age-appropriate vaccines, such as Tdap, Td, and MCV4 (meningococcal), are provided. Women who have received an HPV vaccine must continue to have regular Papanicolaou test screening. HPV vaccines do not provide protection against all HPV types associated with development of cancer, and vaccines do not alter the progression of HPV infection acquired before immunization.

Persons through 26 years of age with evidence of current HPV infection, such as cervical dysplasia, a positive HPV DNA test result, or anogenital warts or a history of anogenital warts, should receive HPV

immunization because infection with all vaccine HPV types is unlikely and the vaccine could provide protection against HPV infection with types not already acquired.[17] Vaccine recipients should be advised that data from clinical trials do not indicate the vaccine will have any therapeutic effect on existing abnormalities found by Papanicolaou testing, on HPV infection, or on genital warts. However, HPV vaccination would provide protection against infection with vaccine HPV types not already acquired.

Lactating women can receive quadrivalent HPV vaccine, but the bivalent vaccine has not been studied in lactating women. Patients of the appropriate age who are immunocompromised from either disease or medication can receive HPV vaccine. However, the immune response to vaccination and vaccine effectiveness may be less than in those who are immunocompetent. HPV vaccine falls into pregnancy category B and is not recommended for use in pregnancy.[118] If an adolescent becomes pregnant during vaccination, subsequent doses should be deferred until after parturition. The vaccine has not been associated causally with adverse outcomes of pregnancy or adverse events to the developing fetus. However, data on vaccination during pregnancy are limited at this time.

Contraindications

Quadrivalent HPV4 vaccine is contraindicated for persons with a history of immediate hypersensitivity to yeast or to any vaccine component. Bivalent HPV2 vaccine is contraindicated for persons with a history of immediate hypersensitivity to any vaccine component. HPV2 vaccine in the prefilled syringe formulation should not be administered to latex-sensitive individuals, because the rubber stopper contains latex.[17]

Influenza Vaccine

Influenza virus infection continues to cause significant morbidity and mortality despite the availability of effective vaccines and antiviral therapy for prevention and treatment of influenza. In the United States, epidemics of influenza typically occur during the winter months and have been associated with an average of approximately 36,000 deaths per year in the United States.[529]

Influenza viruses cause disease among all age groups.[274,275,424] Rates of infection are highest among children, but rates of serious illness and death are highest among persons aged 65 years and older, children aged 2 years and younger, and persons of any age who have medical conditions that place them at increased risk for developing complications from influenza.[64,65,273,424] The impact of influenza on both normal children and those with underlying high-risk conditions is appreciable. Attack rates in normal children have been estimated at 10 to 40 percent each year, and approximately 1 percent of these influenza infections result in hospitalization.[276,467]

A major difficulty in the development and provision of satisfactory immunizing agents for the prevention of influenza is the antigenic variation in these viruses. Periodic minor antigenic changes in influenza A or B virus are the major factors in the continuing occurrence of yearly influenza. Although outbreaks generally are limited in magnitude, the resulting morbidity and mortality remain discouragingly high. Major antigenic changes in influenza A virus, as occurred in 1957 to 1958 (Asian strain), in 1968 to 1969 (Hong Kong strain), and again in 2009 (pH1N1) account for the pandemic spread of disease associated with greater overall morbidity and mortality. The CDC estimates that in the 2009 pandemic the H1N1 influenza virus caused more than 60 million Americans to become ill and led to more than 270,000 hospitalizations and 12,500 deaths.[332] Ninety percent of hospitalizations and deaths occurred in persons younger than 65 years of age. With typical seasonal influenza, approximately 90 percent of deaths occur in persons older than 65 years.

Preparations

Two types of influenza vaccines are licensed for use in the United States: inactivated trivalent influenza vaccine (TIV) and live attenuated influenza vaccine (LAIV).[19,44,144] These multivalent vaccines contain three virus strains (usually two type A and one type B), with the composition changed periodically in anticipation of the prevalent influenza strains expected to circulate in the United States in the following winter. Both vaccines are prepared from virus grown in the allantoic sac of the chick embryo. All vaccines contain the same antigenic composition, and there is no preferential recommendation for any of the formulations of TIV or LAIV.

TIV is an inactivated vaccine that contains no live virus. TIV distributed in the United States consists of either subvirion vaccine, prepared by disrupting the lipid-containing membrane of the virus, or purified surface-antigen vaccine. TIV formulations are now available for intramuscular (IM) and intradermal (ID) use.

The IM formulation of TIV is licensed and recommended for children aged 6 months or older and adults, including persons with and without chronic medical conditions. Vaccine is available in both pediatric (0.25-mL dose) and adult (0.5-mL dose) formulations. TIV is administered intramuscularly into the anterolateral thigh of infants and young children and into the deltoid muscle of older children and adults. The volume of vaccine is age dependent; infants and toddlers 6 months through 35 months of age should receive a dose of 0.25 mL, and all individuals 3 years (36 months) and older should receive 0.5 mL per dose. Children younger than 9 years of age who are being immunized against influenza for the first time should receive two doses of TIV given 4 weeks apart, before the start of the influenza season. The IM formulation of TIV is shipped and stored at 2° C to 8° C (35° F to 46° F).

The ID formulation of TIV is licensed for the 2012-2013 influenza season for use in individuals aged 18 through 64 years. It is administered intradermally preferably over the deltoid muscle and only by using the device included in the vaccine package. Vaccine is supplied in a single-dose, prefilled microinjection system (0.1 mL). The ID formulation of TIV is shipped and stored at 2° C to 8° C (35° F to 46° F).

Multiple manufacturers produce inactivated influenza vaccine each year for the U.S. market. Vaccines are available in multiple presentations (single-dose syringes and vials, multidose vials) and in preservative-free formulations. Approved age indications vary by manufacturer and product. Clinicians should obtain inactivated influenza vaccine appropriate for the age groups they plan to vaccinate. The ACIP does not recommend use of influenza vaccine outside the vaccine's FDA-approved age indication. Tables listing each year's influenza vaccines are available in the annual ACIP and American Academy of Pediatrics influenza statements and on the CDC influenza website.* Package inserts should be consulted for recommended age groups and possible contraindications for each vaccine in addition to information regarding additional components of various vaccine formulations.

LAIV is cold adapted, developed by passaging the viruses at successively lower temperatures in tissue culture, so that replication occurs only in the upper respiratory tract.[73] LAIV is administered intranasally. The cold-adapted formulation that is licensed in the United States must be stored at 2° to 8° C (35° F to 46° F). When the vaccine is warmed to room temperature for intended use, it must be used within 30 minutes.

LAIV is licensed by the FDA for healthy individuals aged 2 through 49 years. It is not recommended for those who have a history of asthma or other high-risk medical conditions associated with an increased risk for complications from influenza. LAIV is administered intranasally in a prefilled, single-use sprayer containing 0.2 mL of vaccine. A removable dose-divider clip is attached to the sprayer to administer 0.1 mL separately into each nostril. Children younger than 9 years of age being immunized against influenza for the first time should receive two doses of LAIV given 4 weeks apart, before the start of the influenza season. LAIV formulation currently licensed in the United States must be shipped and stored at 2° C to 8° C (35° F to 46° F)

All currently available influenza vaccines are trivalent and contain A(H1N1), A(H3N2), and B viral antigens. There are two antigenically distinct lineages of influenza B viruses referred to as Victoria and Yamagata lineages.[406] Immunization against B virus strains of one lineage provides limited cross protection against strains in the other lineage.[72] Because of this and the difficulty of predicting which B virus lineage will predominate during a given season, inclusion of a second influenza B vaccine virus strain in seasonal influenza vaccines has been proposed. In February 2012, FDA approved a new seasonal quadrivalent LAIV, FluMist Quadrivalent (MedImmune). This vaccine currently is anticipated to be available for the 2013-14 influenza season, at which time it is expected to replace the currently available seasonal trivalent FluMist formulation. Inactivated quadrivalent influenza vaccines currently are in development.

Immunogenicity and Efficacy

Children aged 6 months and older typically have protective levels of anti-influenza antibody against specific influenza virus strains after receiving the recommended number of doses of seasonal inactivated influenza vaccine.* Immunogenicity studies using the influenza A (H1N1) 2009 monovalent vaccine indicated that more than 90 percent of children aged 9 years or older responded to a single dose with anti-influenza antibody levels that are considered to be protective. Young children had inconsistent responses to a single dose of the influenza A (H1N1) 2009 monovalent vaccine across studies, with 20 percent of children aged 6 to 35 months responding to a single dose with protective anti-influenza antibody levels. However, in all studies, 80 to 95 percent of vaccinated infants, children, and adolescents developed protective anti-influenza antibody levels to the 2009 H1N1 influenza virus after two doses.[55,439,464]

In most seasons, one or more seasonal vaccine antigens are changed compared with the previous season. In consecutive years when vaccine antigens change, children aged younger than 9 years who received only one dose of vaccine in their first year of vaccination are less likely to have protective antibody responses when administered only a single dose during their second year of vaccination compared with children who received two doses in their first year of vaccination.[248,435,556]

When the vaccine antigens do not change from one season to the next, priming children aged 6 to 23 months with a single dose of vaccine in the spring followed by a dose in the fall results in similar antibody responses compared with a regimen of two doses in the fall.[247] However, one study conducted during a season when the vaccine antigens did not change compared with the previous season estimated 62 percent effectiveness against influenza-like illness (ILI) for healthy children who had received only one dose in the previous influenza season and only one dose in the study season compared with 82 percent for those who received two doses separated by 4 weeks or more during the study season.[5]

The antibody response among children at higher risk for influenza-related complications (e.g., children with chronic medical conditions) might be lower than those reported typically among healthy children.[70,285] However, antibody responses among children with asthma are similar to those of healthy children and are not substantially altered during asthma exacerbations requiring short-term prednisone treatment.[450]

Vaccine effectiveness studies also have indicated that two doses are needed to provide adequate protection during the first season that young children are vaccinated. Among children aged younger than 5 years who have never received influenza vaccine previously or who received only one dose of influenza vaccine in their first year of vaccination, vaccine effectiveness is lower compared with children who received two doses in their first year of being vaccinated. Two large retrospective studies of young children who had received only one dose of TIV in their first year of being vaccinated determined that no decrease was observed in ILI-related office visits compared with unvaccinated children.[5,474] Similar results were reported in a case-control study of children aged 6 to 59 months in which laboratory-confirmed influenza was the

*Centers for Disease Control and Prevention: http://www.cdc.gov/flu/.

*References 224, 279, 367, 433, 585, 586.

outcome measured.[505] These results, along with the immunogenicity data indicating that antibody responses are substantially higher when young children are given two doses, are the basis for the recommendation that all children aged 6 months to 8 years who are being vaccinated for the first time should receive two vaccine doses separated by 4 weeks or more.

Estimates of vaccine efficacy or effectiveness among children aged 6 months or older have varied by season and study design. In a randomized trial conducted during five influenza seasons (1985-1990) in the United States among children aged 1 to 15 years, annual vaccination reduced laboratory-confirmed influenza A substantially (77% to 91%).[434] A limited 1-year placebo-controlled study reported vaccine efficacy against laboratory-confirmed influenza illness of 56 percent among healthy children aged 3 to 9 years and 100 percent among healthy children and adolescents aged 10 to 18 years.[200] A randomized, double-blind, placebo-controlled trial conducted during two influenza seasons among children aged 6 to 24 months indicated that efficacy was 66 percent against culture-confirmed influenza illness during the 1999-2000 influenza season but did not reduce culture-confirmed influenza illness substantially during the 2000-2001 influenza season.[313] A case-control study conducted during the 2003-2004 season indicated vaccine effectiveness of 49 percent against laboratory-confirmed influenza.[505] An observational study among children aged 6 to 59 months with laboratory-confirmed influenza compared with children who tested negative for influenza reported vaccine effectiveness of 44 percent in the 2003-2004 influenza season and 57 percent during the 2004-2005 season.[245] Partial vaccination (only one dose for children being vaccinated for the first time) was not effective in either study. During an influenza season (2003-2004) with a suboptimal vaccine match, a retrospective cohort study conducted among approximately 30,000 children aged 6 months to 8 years indicated vaccine effectiveness of 51 percent against medically attended, clinically diagnosed pneumonia or influenza (i.e., no laboratory confirmation of influenza) among fully vaccinated children and 49 percent among approximately 5,000 children aged 6 to 23 months.[474] Another retrospective cohort study of similar size conducted during the same influenza season in Denver but limited to healthy children aged 6 to 21 months estimated clinical effectiveness of 2 TIV doses to be 87 percent against pneumonia or influenza-related office visits.[5]

Among children, TIV effectiveness might increase with age.[434,589] A systematic review of published studies estimated vaccine effectiveness at 59 percent for children aged 2 years or older but concluded that additional evidence was needed to demonstrate effectiveness among children aged 6 months to 2 years.[331]

Because of the recognized influenza-related disease burden among children with other chronic diseases or immunosuppression and the long-standing recommendation for vaccination of these children, randomized placebo-controlled studies to study efficacy in high-risk children are needed but are currently unavailable. In a nonrandomized controlled trial among children aged 2 to 6 years and 7 to 14 years who had asthma, vaccine efficacy was 54 percent and 78 percent against laboratory-confirmed influenza type A infection and 22 percent and 60 percent against laboratory-confirmed influenza type B infection, respectively. Vaccinated children aged 2 to 6 years with asthma did not have substantially fewer type B influenza virus infections compared with the control group in this study.[519] The association between vaccination and prevention of asthma exacerbations is unclear. Vaccination was demonstrated to provide protection against asthma exacerbations in some studies.[356,442]

TIV has been demonstrated to reduce AOM in some studies. Two studies have reported that TIV decreases the risk for influenza-related otitis media by approximately 30 percent among children with mean ages of 20 and 27 months, respectively.[197,304] However, a large study conducted among children with a mean age of 14 months indicated that TIV was not effective against AOM.[313] Influenza vaccine effectiveness against a nonspecific clinical outcome such as AOM, which is caused by a variety of pathogens and is not typically diagnosed using influenza virus culture, is expected to be relatively low.

Adverse Events

TIV is an inactivated vaccine that contains killed viruses and therefore cannot produce signs or symptoms of influenza caused by active virus infection. The most common symptoms associated with TIV administration are soreness at the injection site and fever. Fever, usually occurring 6 to 24 hours after immunization, affects 10 to 35 percent of children younger than 2 years old. Mild systemic symptoms, such as nausea, lethargy, headache, muscle aches, and chills, also can occur after receipt of TIV.

An increased risk for febrile seizures after TIV administration had not been observed in the United States prior to the 2010-2011 influenza season.[284,296] During the 2010-2011 influenza season, the CDC and the FDA conducted enhanced monitoring for febrile seizures after influenza vaccination because of reports of an increased risk for fever and febrile seizures in young children in Australia associated with a 2010 Southern Hemisphere vaccine produced by CSL Biotherapies (up to nine febrile seizures per 1000 doses). Because of the findings in Australia, ACIP does not recommend the U.S.-licensed CSL Biotherapies' TIV, Afluria, for children aged younger than 9 years.[177]

Surveillance for U.S.-licensed influenza vaccines during the 2010-2011 season subsequently detected safety signals for febrile seizures in young children after TIV administration.[372,533] Further assessment determined that the increased risk was in children aged 6 months through 4 years on the day of vaccination to the day after (the 0- to 1-day risk window). The risk was higher when children received concomitant PCV13 and peaked at approximately age 16 months.[533] No increased risk was observed in children aged 5 years or older after TIV or in children of any age after LAIV. The magnitude of the increased risk for febrile seizures in young children in the United States (<1/1000 children vaccinated) was substantially lower than the risk observed in Australia in 2010.

After evaluating the data on febrile seizures from the 2010-2011 influenza season and taking into consideration benefits and risks of vaccination, no policy change was recommended for use of TIV or PCV13 for the 2011-2012 season. According to the CDC, surveillance data on febrile seizures in young children after administration of influenza vaccine for the 2011-2012 influenza season (same vaccine formulation as 2010-2011) were consistent with those from the 2010-2011 influenza season. No changes in the use of TIV or PCV13 are recommended for the 2012-2013 influenza season.

LAIV generally is well tolerated and may produce mild signs or symptoms related to influenza virus infection that include headache and runny nose or nasal congestion in vaccinees. Transmission of LAIV strains to unimmunized contacts has been documented only once in pre-licensure studies. The proposed explanation for the uncommon occurrence of transmission is that the vaccine virus is shed for a shorter duration and in a much smaller quantity than are wild-type strains.

Indications

Routine annual influenza vaccination is recommended for all persons aged 6 months or older. To permit time for production of protective antibody levels,[87,286] vaccination optimally should occur before onset of influenza activity in the community. Therefore, vaccination providers should offer vaccination as soon as vaccine is available. Vaccination should be offered throughout the influenza season (i.e., as long as influenza viruses are circulating in the community).[44,144]

Healthy children aged 2 years or older can receive either TIV or LAIV. Particular focus should be on the administration of TIV for all children and adolescents who have underlying medical conditions associated with an increased risk for complications from influenza, including the following:

- Asthma or other chronic pulmonary diseases, including cystic fibrosis
- Hemodynamically significant cardiac disease
- Immunosuppressive disorders or therapy
- HIV infection
- Sickle cell anemia and other hemoglobinopathies
- Diseases that require long-term aspirin therapy, including juvenile idiopathic arthritis or Kawasaki disease
- Chronic renal dysfunction
- Chronic metabolic disease, including diabetes mellitus
- Any condition that can compromise respiratory function or handling of secretions or can increase the risk for aspiration, such as neurodevelopmental disorders, spinal cord injuries, seizure disorders, or neuromuscular abnormalities

Although universal immunization for all individuals aged 6 months or older is recommended, particular immunization efforts, with either TIV or LAIV, should be made for the following groups to prevent transmission of influenza to those at risk, unless contraindicated:

- Household contacts and out-of-home care providers of children younger than age 5 years and at-risk children of all ages (healthy contacts 2 to 49 years of age can receive either TIV or LAIV).
- Any female who is pregnant, is considering pregnancy, has just delivered, or is breastfeeding during the influenza season (TIV only). Studies have shown that infants born to immunized women have better influenza-related health outcomes. However, the estimated median seasonal vaccination coverage among women with a live birth was only 47 percent during the 2009-2010 influenza season, even though both pregnant women and their infants are at higher risk for complications. In addition, data from some studies suggest that influenza vaccination in pregnancy may decrease the risk for preterm birth.
- Health care personnel or health care volunteers. Despite the recent AAP recommendation for mandatory influenza immunization for all health care personnel,[75] many remain unvaccinated. As of November 2011, the CDC estimated that only 63 percent of health care personnel received the seasonal influenza vaccine. The AAP recommends mandatory vaccination of health care personnel because they frequently come into contact in their clinical settings with patients at high risk for influenza illness.
- Close contacts of immunosuppressed persons

Previously unimmunized children between 6 months to 9 years of age should receive two doses of influenza vaccine before the onset of influenza season. Available data suggest that children younger than 9 years who did not receive the second dose of influenza vaccine in the initial year that influenza vaccine was given may not be adequately protected the next influenza season with only one dose. In this group, levels of protection can be suboptimal, especially if the antigenic specificity of the predominant strains has changed from the previous year. Thus, the AAP recommends that two doses be given to these children the following influenza season.[44] This recommendation applies only to the influenza season that follows the first year that a child younger than 9 years old receives influenza vaccine (Fig. 244-4).

Precautions and Contraindications

TIV is contraindicated in infants younger than 6 months and children who have a moderate to severe febrile illness, on the basis of clinical judgment of the pediatrician. Persons with acute febrile illness usually should not be vaccinated until their symptoms have abated. However, minor illnesses with or without fever do not contraindicate the use of influenza vaccine, particularly in children with mild upper respiratory tract infection or allergic rhinitis.[44,144]

Pregnancy is not a contraindication to influenza vaccine administration, and vaccination with TIV is advised for pregnant girls and women who have an underlying high-risk condition. LAIV is contraindicated in any female who is pregnant or considering pregnancy.

Children should not be vaccinated with LAIV if they are younger than 2 years, have a moderate to severe febrile illness, are receiving aspirin or other salicylates,

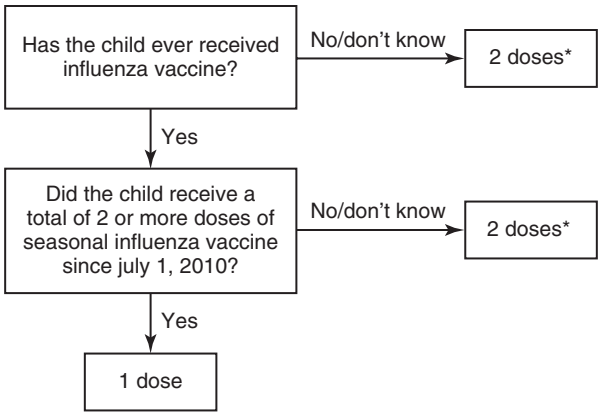

FIGURE 244-4 ■ Influenza vaccine dosing algorithm for children aged 6 months through 8 years—Advisory Committee on Immunization Practices, United States, 2012-2013 influenza season.
*Doses should be administered at least 4 weeks apart.
†For simplicity, this algorithm takes into consideration only doses of seasonal influenza vaccine received since July 1, 2010. As an alternative approach in settings where vaccination history from before July 1, 2010, is available, if a child aged 6 months through 8 years is known to have received at least two seasonal influenza vaccines during any previous season, and at least one dose of a 2009(H1N1)-containing vaccine (i.e., either 2010-2011 or 2011-2012 seasonal vaccine or the monovalent 2009[H1N1] vaccine), then the child needs only one dose for 2012-2013. Using this approach, children aged 6 months through 8 years need only one dose of vaccine in 2012-2013 if they have received any of the following: (1) two or more doses of seasonal influenza vaccine since July 1, 2010; (2) two or more doses of seasonal influenza vaccine before July 1, 2010, and one or more doses of monovalent 2009 (H1N1) vaccine; or (3) one or more doses of seasonal influenza vaccine before July 1, 2010, and one or more doses of seasonal influenza vaccine since July 1, 2010. Children for whom one of these conditions is not met require two doses in 2012-2013. (From Centers for Disease Control and Prevention. *Immunization schedules.* Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6132a3.htm.)

have known or suspected immune deficiency disease, are receiving immunosuppressive or immunomodulatory therapies, or have a chronic underlying medical condition, including metabolic disease, diabetes mellitus, asthma, other chronic disorders of the pulmonary or cardiovascular system, renal dysfunction, or hemoglobinopathies. Children should not be given LAIV if they have received other live virus vaccines within the past 4 weeks; however, other live virus vaccines can be given on the same day as LAIV. Children with any condition that can compromise respiratory function or handling of secretions or can increase the risk for aspiration, such as neurodevelopmental disorders, spinal cord injuries, seizure disorders, or neuromuscular abnormalities should not receive LAIV.

Children aged 2 through 4 years with a history of recurrent wheezing or a medically attended wheezing episode in the previous 12 months should not receive LAIV because of the potential for increased wheezing after immunization. In this age range, many children have a history of wheezing with respiratory tract illnesses and are eventually diagnosed with asthma. Therefore, when offering LAIV to children 24 through 59 months of age, the health care provider should screen them by asking the parent/guardian the question, "In the previous 12 months, has a health care professional ever told you that your child had wheezing?" If a parent answers "yes" to this question, LAIV is not recommended for the child. TIV would be recommended for the child to whom LAIV is not given.

Children taking an influenza antiviral medication should not receive LAIV until 48 hours after stopping the influenza antiviral therapy. If a child recently received LAIV but has an influenza illness for which antiviral agents are appropriate, the antiviral agents should be given. Reimmunization may be indicated because of the potential effects of antiviral medications on LAIV replication and immunogenicity.

Precaution also should be taken when considering LAIV administration to persons with minor acute illness, such as a mild upper respiratory tract infection with or without fever. Although the vaccine most likely can be given in this case, LAIV should be deferred temporarily until the congestion-inducing illness is resolved if nasal congestion will impede the delivery of the vaccine to the nasopharyngeal mucosa.

Available data indicate a very low risk for transmission of the virus in both children and adults vaccinated with LAIV. LAIV or TIV can be used to prevent influenza in those who are in close contact with most immunosuppressed individuals. Persons who are in contact with severely immunosuppressed individuals, such as those receiving care in a protective environment after undergoing hematopoietic stem cell transplantation, should not receive LAIV. For such individuals, TIV is recommended.

Severe allergic and anaphylactic reactions can occur in response to a number of influenza vaccine components, but such reactions are rare. All currently available influenza vaccines are prepared by means of inoculation of virus into chicken eggs. The use of influenza vaccines for persons with a history of egg allergy has been reviewed recently by the ACIP.[145] For the 2011-2012 influenza season, ACIP recommended that persons with egg allergy who report only hives after egg exposure should receive TIV, with several additional safety measures. Recent examination of VAERS data indicated no disproportionate reporting of allergy or anaphylaxis after influenza vaccination during the 2011-2012 season.[3] For the 2012-2013 influenza season, the ACIP recommended the following:

1. Persons with a history of egg allergy who have experienced only hives after exposure to egg should receive influenza vaccine, with the following additional safety measures (Fig. 244-5):
   a. Because studies published to date involved use of TIV, TIV rather than LAIV should be used.[342]
   b. Vaccine should be administered by a health care provider who is familiar with the potential manifestations of egg allergy.
   c. Vaccine recipients should be observed for at least 30 minutes for signs of a reaction after administration of each vaccine dose.[342]

   Other measures, such as dividing and administering the vaccine by a two-step approach and skin testing with vaccine, are not necessary.[342]

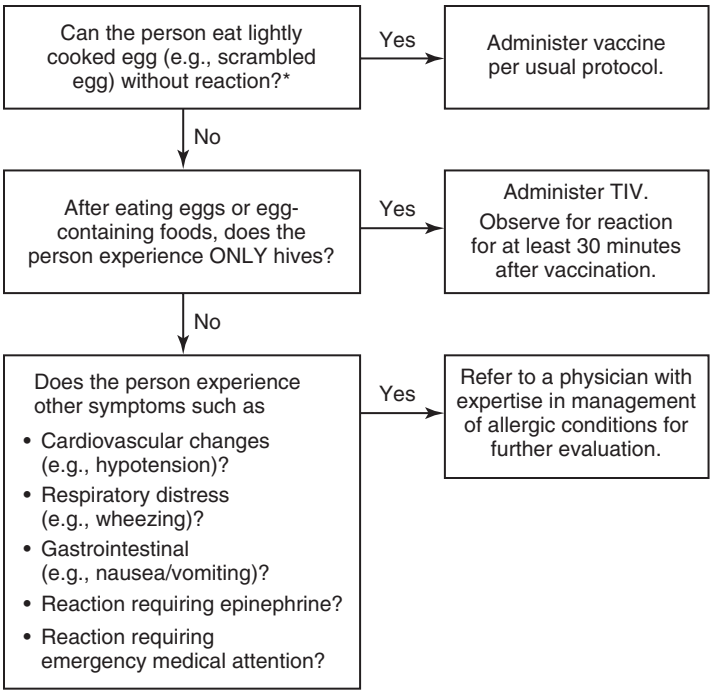

FIGURE 244-5 ■ Recommendations regarding influenza vaccination for persons who report allergy to eggs—Advisory Committee on Immunization Practices, United States, 2012-2013 influenza season. Persons with a history of egg allergy who have experienced only hives after exposure to egg should receive influenza vaccine, with the use of additional safety measures.
TIV, trivalent inactivated vaccine.
*Persons with egg allergy might tolerate egg in baked products (e.g., bread or cake). Tolerance to egg-containing foods does not exclude the possibility of egg allergy. (From Centers for Disease Control and Prevention. *Immunization schedules*. Available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6132a3.htm.)

2. Persons who report having had reactions to egg involving such symptoms as angioedema, respiratory distress, lightheadedness, or recurrent emesis or who required epinephrine or another emergency medical intervention, particularly those that occurred immediately or within a short time (minutes to hours) after egg exposure, are more likely to have a serious systemic or anaphylactic reaction on reexposure to egg proteins. Before receipt of vaccine, such persons should be referred to a physician with expertise in the management of allergic conditions for further risk assessment.

Measles Vaccine

Since the introduction of both an inactivated and a live virus, attenuated measles vaccine (Edmonston B strain) in the United States in 1963, the reported incidence of measles has decreased by more than 99 percent. Although the incidence of measles has declined in all age groups, the decline has been greatest in children aged 5 to 14 years.

Measles was targeted for elimination in the United States by 1982. Efforts to eliminate measles were not successful as a result of two factors. The first was vaccine failure. In the late 1980s, outbreaks occurred in older children in schools in which immunization rates usually were greater than 95 percent.[290,396,437] Attack rates were 1 to 5 percent because of the accumulation of measles-susceptible individuals from vaccine failure. The recurrent measles outbreaks among vaccinated school-aged children in the mid 1980s prompted both the ACIP and the AAP in 1989 to recommend that all children be given two doses of measles-containing vaccine, preferably as MMR.[34,97] Although administration of the second dose originally was recommended either at entry to primary school (ACIP) or middle/secondary school (AAP), the ACIP, the AAP, and the AAFP now recommend that a child be given the second dose at age 4 to 6 years rather than delaying it until the child is 11 to 12 years old.* The major benefit of administering the second dose is a reduction in the proportion of persons who remain susceptible because of primary vaccine failure. Waning immunity is not a major cause of vaccine failure and has little influence on transmission of measles, and revaccination of children who have low concentrations of measles antibody produces only a transient rise in antibody concentration.[392,395,401,448,557]

The second factor leading to outbreaks of measles was the failure to implement current immunization strategies, especially in the inner cities, where a high proportion of preschool-aged children had not been vaccinated. From 1989 through 1991, the proportion of unvaccinated persons with measles increased, as reflected by outbreaks among unvaccinated inner-city preschool-aged children. Multiple barriers to providing timely immunization to these children were identified during investigation of the measles resurgence that occurred between 1989 and 1991. Reported cases of measles declined rapidly after the 1989 to 1991 resurgence because of intensive efforts to vaccinate preschool-aged children. Measles vaccination levels among 2-year-old children increased from 70 percent in 1990 to 91 percent in 1997. From 2001-2008, a median of 56 measles cases were reported to the CDC annually.[451]

In 2000, the United States achieved measles elimination (defined as interruption of year-round endemic measles transmission).[339] However, importations of

*References 133, 392, 395, 398, 401, 448, 557.

measles into the United States continue to occur, posing risks for measles outbreaks and sustained measles transmission. Most cases are now imported from other countries or linked to imported cases. Most imported cases originate in Asia and Europe and occur among both U.S. citizens traveling abroad and persons visiting the United States from other countries. Since 1993, the largest outbreaks of measles in the United States have occurred in populations that refuse vaccination for religious or personal belief reasons. Most outbreaks have involved limited spread from measles imported from outside the United States.

During 2011, 222 cases of measles and 17 outbreaks were reported in the United States, an increase compared with cases and outbreaks reported during 2001 through 2010. Importations accounted for 32 percent of cases. Among patients who were U.S. residents, 85 percent were unvaccinated or had unknown vaccination status and were eligible for measles, mumps, and rubella vaccination.[131] The increased numbers of outbreaks and measles importations into the United States underscore the ongoing risk for measles among unvaccinated persons and the importance of vaccination against measles.

The recommended age for receiving routine vaccination with measles vaccine has been lowered in the past decade from 15 months to 12 to 15 months of age.[120] The decision to lower the age for receiving routine primary vaccination was based on the observation that most children are susceptible to measles by the time that they reach 12 months of age because of waning transplacental immunity.[385,393] Most mothers now have vaccine-induced immunity rather than immunity conferred by infection with wild virus. Antibody concentrations induced by measles vaccination generally are lower than those induced by natural measles. Therefore, measles-specific antibodies acquired transplacentally are lower in infants of vaccinated mothers, and these infants therefore are susceptible at an earlier age.

Preparations

The live measles virus vaccine (Moraten strain) available in the United States is prepared in chick fibroblast cell culture. Each dose of vaccine contains neomycin, sorbitol, and hydrolyzed gelatin as a stabilizer. Preparations include measles-mumps-rubella (MMR) and measles-mumps-rubella-varicella (MMRV) vaccines. MMRV vaccine was licensed in the United States in September 2005 and may be used instead of MMR and varicella vaccine to implement the recommended two-dose vaccine schedule for prevention of measles, mumps, rubella, and varicella among children aged 12 months to 12 years. Single-component measles vaccine is not available in the United States.

Inadequate protection against measles can result from the administration of improperly stored vaccine. Before reconstitution, measles vaccine must be stored at a temperature between 2°C and 8°C (35.6°F to 46.4°F) or colder and must be protected from light, which may inactivate the virus. Reconstituted vaccine should be stored in a refrigerator and discarded if not used within 8 hours.

Immunogenicity and Efficacy

Immunization produces a mild or inapparent, noncommunicable infection. Measles antibodies develop in approximately 95 percent of children vaccinated at 12 months of age and in 98 percent of children vaccinated at 15 months of age.[393] Studies indicate that serologic evidence of measles immunity develops in more than 99 percent of persons who receive two doses of measles vaccine, separated by at least 1 month, on or after their first birthday.[193,205] Although vaccine-induced antibody titers are lower than those after natural disease, persistence of protective titers for as long as 16 years after administration of vaccine has been demonstrated.[362,395] Most vaccinated persons who appear to lose antibody have an anamnestic response after revaccination, thus indicating that they most likely are still immune.[445] A small percentage of vaccinated individuals may lose protection after several years as a result of secondary vaccine failure.[401,594]

Adverse Events

Vaccine-associated symptoms, consisting of fever higher than 39.4°C (102.9°F) occurring 5 to 10 days after immunization or transient rash, develop in 5 to 18 percent of recipients.[370,457] Serious complications related to vaccine use occur far less frequently than after natural measles.[454]

Thrombocytopenia occurs at a rate of 1 case for every 30,000 to 40,000 doses distributed. Based on data from Sweden and Finland, the IOM concluded that a causal association exists between MMR and thrombocytopenia.[321] The decrease in platelet count presumably is caused by the measles component and usually is not clinically apparent. However, thrombocytopenic purpura occurring after vaccination has been reported.

Central nervous system disease, specifically encephalitis or encephalopathy, is reported at a rate of less than 1 case per 1 million doses of vaccine administered. Because the incidence of encephalitis or encephalopathy after the administration of measles vaccination to healthy children is lower than the observed incidence of encephalitis of unknown origin, some or most of the reported severe neurologic disorders may be only temporally, rather than causally, related to measles immunization. The risk for subacute sclerosing panencephalitis (SSPE) developing in vaccinated children is extremely low and is estimated to be approximately one twelfth the risk for SSPE developing after a case of natural measles (0.7 SSPE cases per million vaccine doses versus 8.5 cases per million natural measles infections).[80,102,321] Whether measles vaccine causes SSPE is unclear. Some cases of SSPE occur in children with no history of having measles or measles vaccination.[21]

Reactions to measles vaccine are not age related and occur only in susceptible vaccinees. After revaccination, reactions should be expected only in those who failed to respond to the first immunization.

To date, no convincing evidence establishes that any vaccine causes autism or autistic spectrum disorder. Concern has been raised about a possible relation between

MMR vaccine and autism by some parents of children with autism. Symptoms of autism often are noticed by parents during the second year of life and may manifest after administration of MMR by weeks or months. Two independent nongovernmental groups, the IOM and the AAP, have reviewed the evidence regarding a potential link between autism and MMR vaccine.[295,322,325] Both groups independently concluded that available evidence does not support an association and that the United States should continue its current MMR vaccination policy.

Indications

Unless otherwise contraindicated, measles vaccine is indicated for persons susceptible to measles.[21,133] The recommended age for receiving the first dose of measles vaccine is 12 to 15 months. In high-risk areas, such as those with recurrent measles transmission, the initial dose should be administered when the child reaches 12 months of age. The second dose is given routinely at 4 to 6 years and no later than 11 to 12 years of age. The minimal interval between the two doses is 4 weeks.

Adults born before 1957 generally may be considered immune to measles because of previous natural infection. Those born after 1956 and in whom immunoprophylaxis is indicated should receive two doses of vaccine.

During outbreaks, when the likelihood of exposure to measles is high, measles vaccine should be given to infants as young as 6 months. Seroconversion rates to vaccine are significantly less in children vaccinated before reaching 1 year of age than those in older children. Children immunized before their first birthday then should be revaccinated with MMR at 12 to 15 months of age, with a third dose given according to local policy.

Measles remains endemic in many areas of the world. Although vaccination against measles is not a requirement for entry into any country, susceptible children, adolescents, and adults born after 1956 should be offered measles vaccination (usually as MMR) before embarking on international travel. Infants 6 months or older who are traveling to areas where measles is endemic or epidemic should be vaccinated before departure and revaccinated at 12 to 15 months. Vaccination of infants younger than 6 months is not necessary because most young infants are protected by maternally derived antibodies.

Exposure of susceptible individuals to measles is not a contraindication to administering vaccination; vaccine given within 72 hours of exposure may provide protection. If exposure does not result in infection, immunization will protect against future infection.

MMRV vaccine may be used instead of MMR and varicella vaccine to implement the recommended two-dose vaccine schedule among children aged 12 months to 12 years. At the time of its licensure, use of MMRV vaccine was preferred for both the first and second doses over separate injections of equivalent component vaccines (MMR vaccine and varicella vaccine), which was consistent with the ACIP's 2006 general recommendations on use of combination vaccines.[359]

In February 2008, on the basis of preliminary data from two studies conducted post licensure that suggested an increased risk for febrile seizures 5 to 12 days after vaccination among children aged 12 to 23 months who had received the first dose of MMRV vaccine compared with children the same age who had received the first dose of MMR vaccine and varicella vaccine administered as separate injections at the same visit, ACIP issued updated recommendations regarding MMRV vaccine use.[176] These updated recommendations expressed no preference for use of MMRV vaccine over separate injections of equivalent component vaccines for both the first and second doses.

The final results of the two post-licensure studies indicated that among children aged 12 to 23 months, one additional febrile seizure occurred 5 to 12 days after vaccination per 2300 to 2600 children who had received the first dose of MMRV vaccine compared with children who had received the first dose of MMR vaccine and varicella vaccine administered as separate injections at the same visit.[349] Data from post-licensure studies do not suggest that children aged 4 to 6 years who received the second dose of MMRV vaccine had an increased risk for febrile seizures after vaccination compared with children the same age who received MMR vaccine and varicella vaccine administered as separate injections at the same visit.[350]

In June 2009, after consideration of the post-licensure data and other evidence, the ACIP adopted new recommendations regarding use of MMRV vaccine for the first and second doses and identified a personal or family (i.e., sibling or parent) history of seizure as a precaution for use of MMRV vaccine.[390] For the first dose of measles, mumps, rubella, and varicella vaccines at age 12 to 47 months, either MMR vaccine and varicella vaccine or MMRV vaccine may be used. Providers who are considering administering MMRV vaccine should discuss the benefits and risks of both vaccination options with the parents or caregivers. Unless the parent or caregiver expresses a preference for MMRV vaccine, the CDC recommends that MMR vaccine and varicella vaccine should be administered for the first dose in this age group. For the second dose of measles, mumps, rubella, and varicella vaccines at any age (15 months to 12 years) and for the first dose at age older than 48 months, use of MMRV vaccine generally is preferred over separate injections of its equivalent component vaccines (i.e., MMR vaccine and varicella vaccine). This recommendation is consistent with the ACIP's 2011 general recommendations regarding use of combination vaccines, which state that use of a combination vaccine generally is preferred over its equivalent component vaccines.[358]

Precautions and Contraindications

Immunocompromised patients with conditions such as lymphoreticular or other generalized malignant disease and primary or secondary immunodeficiency states should not be given live virus, attenuated measles vaccine.[21,133] After cessation of their chemotherapy, these individuals generally should not receive measles vaccine for at least 3 months. However, because the intensity and type of immunosuppressive therapy, radiation therapy, underlying disease, and other factors determine when

immunologic responsiveness will be restored, arriving at a definitive recommendation for an interval after cessation of immunosuppressive therapy when measles vaccine can be safely and effectively administered often is not possible.

An exception to the contraindication of administering measles vaccine to immunocompromised patients is asymptomatic HIV-infected patients, for whom measles vaccination, given as MMR at 12 to 15 months of age, is recommended. The need to protect HIV-infected persons who are at increased risk for having severe complications if infected with measles has been balanced against the risk for having adverse reactions. Measles vaccine is not recommended for HIV-infected persons with evidence of severe immunosuppression. A case of progressive measles pneumonitis occurred in a person with acquired immunodeficiency syndrome (AIDS) and severe immunosuppression to whom MMR vaccine was administered,[132] and morbidity related to measles vaccination has been reported in persons with severe immunosuppression unrelated to HIV infection. In addition, the antibody response to measles vaccine in severely immunocompromised HIV-infected persons is diminished.[57,71,402,418,421] In the United States, the incidence of measles currently is very low. Among HIV-infected persons who do not have evidence of severe immunosuppression, no serious or unusual adverse events have been reported after receiving measles vaccination.[407,443,449,512] Therefore, MMR vaccination is recommended for all asymptomatic HIV-infected persons who do not have evidence of severe immunosuppression and for whom measles vaccination would otherwise be indicated. MMR vaccination also should be considered for all symptomatic HIV-infected persons who do not have evidence of severe immunosuppression. Testing asymptomatic persons for HIV is not necessary before administering MMR.[48]

Systemically absorbed corticosteroids can suppress the immune system of an otherwise healthy person. However, neither the minimal dose nor the duration of therapy sufficient to cause immune suppression is well defined. Although the immunosuppressive effects of corticosteroid treatment vary, many clinicians consider that a corticosteroid dose equivalent to or greater than a prednisone dose of 2 mg/kg/day or a total of 20 mg/day is sufficiently immunosuppressive to raise concern about the safety of administering live virus vaccines. Persons who have received systemic corticosteroids in doses of 2 mg/kg/day or 20 mg daily or on alternate days for an interval of 14 days or longer should avoid receiving vaccination with MMR for at least 1 month after cessation of corticosteroid therapy.[133] Persons who have received prolonged or extensive topical, aerosolized, or other local corticosteroid therapy that causes clinical or laboratory evidence of systemic immunosuppression also should avoid receiving vaccination with MMR for at least 1 month after cessation of therapy.[133]

The live attenuated measles virus vaccine used for immunization is not communicable. Therefore, contacts of immunocompromised patients should be vaccinated to prevent the spread of natural measles to such patients.

Although no direct evidence has demonstrated that measles vaccine is harmful to a pregnant woman or her fetus, the vaccine should not be administered to women known to be pregnant or who are considering becoming pregnant because of the theoretical risk for fetal infection associated with a live virus vaccine. Women vaccinated with MMR should avoid conception for 28 days after being vaccinated.[138]

Because measles vaccination may diminish cutaneous manifestations of cell-mediated immunity temporarily, a tuberculin test performed several days to 6 weeks after receiving immunization can yield a false-negative result. Although natural measles infection can exacerbate tuberculosis, no evidence indicates that measles vaccination is associated with such an effect. Therefore, tuberculin skin testing is not a prerequisite for administering measles immunization. If a tuberculin test is indicated, it should be performed on the day of immunization or postponed for 4 to 6 weeks because measles vaccination may suppress tuberculin reactivity temporarily.

In persons allergic to eggs, the risk for having serious allergic reactions such as anaphylaxis after receiving MMR vaccine is extremely low, and skin testing with vaccine is not predictive of allergic reaction to vaccination.[21,330,344] Therefore, obtaining a skin test is not required before administering MMR to persons who are allergic to eggs. Similarly, the administration of gradually increasing doses of vaccine is not required.[133] Data indicate that most anaphylactic reactions to measles- and mumps-containing vaccines are not associated with hypersensitivity to egg antigens but to other components of the vaccines such as the gelatin stabilizer.[283,305,343,369]

Children with a previous history of thrombocytopenic purpura or thrombocytopenia may be at risk for developing clinically significant thrombocytopenia after receiving immunization with MMR.[68,321] The decision to vaccinate should be based on the benefits of immunity to measles, mumps, and rubella and the risk for recurrence or exacerbation of the thrombocytopenia after receiving vaccination or from natural infection with measles or rubella. For children in whom thrombocytopenia develops in the month after receiving a dose of measles-containing vaccine, withholding the second dose of measles vaccine is prudent if the incidence of measles remains low.

Receipt of antibody-containing blood products (whole blood, plasma, or parenteral immune globulin) may interfere with seroconversion to measles vaccine. High doses of immune globulin preparations can inhibit the immune response to measles vaccine for 3 or more months, depending on the dosage.[506] The length of time that such passively acquired antibody persists depends on the concentration and quantity of the blood product received (Table 244-12).[21,359]

As with any condition that induces fever during the second year of life, children predisposed to having febrile seizures may experience seizures after receiving measles vaccination. Most convulsions that develop after receiving measles immunization are simple febrile seizures and occur in children without known risk factors. Febrile seizures that occur after the administration of vaccinations do not increase the risk for the subsequent development of epilepsy or other neurologic disorders.[66] An increased risk for having seizures after receiving measles

TABLE 244-12 **Suggested Intervals Between Immune Globulin Administration and Measles or Varicella Vaccination**

Product/Indication	Route	Dose		Interval (mo)*
		U or mL	mg/kg	
RSV monoclonal antibody (Synagis)	IM		15 (monoclonal)	None
Tetanus (as TIG)	IM	250 U	10	3
Hepatitis A prophylaxis (as IG)				
Contact prophylaxis	IM	0.02 mL/kg	3.3	3
International travel	IM	0.06 mL/kg	10	3
Hepatitis B prophylaxis (as HBIG)	IM	0.06 mL/kg	10	3
Rabies prophylaxis (as RIG)	IM	20 IU/kg	22	4
Varicella prophylaxis (as VariZIG)	IM	125 U/10 kg (maximum, 625 U)	20-40	5
Measles prophylaxis (as IG)				
Standard	IM	0.25 mL/kg	40	5
Immunocompromised contact	IM	0.50 mL/kg	80	6
Blood transfusion				
Washed RBCs	IV	10 mL/kg	Negligible	None
RBCs, adenine-saline added	IV	10 mL/kg	10	3
Packed RBCs	IV	10 mL/kg	20-60	5
Whole blood	IV	10 mL/kg	80-100	6
Plasma/platelet products	IV	10 mL/kg	160	7
CMV immune globulin	IV	3 mL/kg	150	6
IGIV				
Replacement therapy for immune deficiencies	IV		400	8
Therapy for ITP	IV		400	8
Therapy for ITP	IV		1000	10
Therapy for ITP or Kawasaki disease	IV		1600-2000	11

*These intervals should provide sufficient time for decreases in passive antibodies in all children to allow for an adequate response to measles or varicella vaccine. Physicians should not assume that children are fully protected against measles during these intervals. Additional doses of immune globulin or measles vaccine may be indicated after exposure to measles.

CMV, cytomegalovirus; HBIG, hepatitis B immune globulin; IG, immune globulin; IM, intramuscular; ITP, immune thrombocytopenic purpura; IV, intravenous; IGIV intravenous immune globulin; RIG, rabies immune globulin; RBCs, red blood cells; RSV, respiratory syncytial virus; TIG, tetanus immune globulin; VariZIG, varicella-zoster immune globulin.

Modified from Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.

vaccination may occur in children with a previous history of convulsions or those with a history of convulsions in first-degree family members.[550] Although the exact risk cannot be determined, it appears to be low. The recommendation to immunize children with a personal history of seizures or those with a history of seizures in first-degree family members is based on factors indicating that the benefits greatly outweigh the risks. Prophylactic use of anticonvulsants usually is not feasible because therapeutic concentrations of many currently prescribed anticonvulsants are not achieved for some time after the initiation of therapy.

Meningococcal Vaccine

Neisseria meningitidis has become a leading cause of bacterial meningitis in the United States after dramatic reductions in the incidence of *Streptococcus pneumoniae* and *H. influenzae* type b infections after the introduction of conjugate vaccines.[494,572] Meningococcal disease is fatal in 10 to 14 percent of cases. Of patients who recover, 11 to 19 percent have permanent hearing loss, mental retardation, loss of limbs, or other serious sequelae.

Disease most often occurs in children 2 years of age or younger; the peak incidence occurs in children younger than 1 year of age. Another peak occurs in adolescents and young adults 16 through 21 years of age. Historically, freshman college students who lived in dormitories and military recruits in boot camp had a higher rate of disease compared with persons who are the same age and who are not living in such accommodations. Close contacts of patients with meningococcal disease are at increased risk for becoming infected. Patients with persistent complement component deficiencies (e.g., C5 to C9, properdin, or factor H or factor D deficiencies) or anatomic or functional asplenia are at increased risk for invasive and recurrent meningococcal disease. Patients are considered capable of transmitting the organism for up to 24 hours after initiation of effective antimicrobial treatment. Asymptomatic colonization of the upper respiratory tract provides the source from which the organism is spread. Transmission occurs from person-to-person through

droplets from the respiratory tract and requires close contact.

The incidence of meningococcal disease varies over time and by age and location. During the past 60 years, the annual incidence of meningococcal disease in the United States has varied from 0.5 to 1.5 cases per 100,000 population. Incidence cycles have occurred over multiple years. Since the early 2000s, annual incidence rates have decreased and are sustained. The reasons for this decrease, which preceded introduction of meningococcal polysaccharide-protein conjugate vaccine into the immunization schedule, are not known but may be related to immunity of the population to circulating meningococcal strains and to the changes in behavioral risk factors (e.g., smoking).

Distribution of meningococcal serogroups in the United States has shifted in the past two decades. Serogroups B, C, and Y each account for approximately 30 percent of reported cases, but serogroup distribution varies by age, location, and time. Approximately three fourths of cases among adolescents and young adults are caused by serogroups C, Y, or W135 and potentially are preventable with available vaccines. In infants, 50 to 60 percent of cases are caused by serogroup B and are not preventable with vaccines available in the United States.

In other parts of the world, the number of cases is much higher.[110] In the sub-Saharan African "meningitis belt," which extends from Mali to Ethiopia, peaks of serogroup A meningococcal disease occur regularly during the dry season. In addition, major epidemics occur every 8 to 12 years. The last epidemic during 1996 to 1997 resulted in 213,658 cases, with 21,830 deaths. Meningococcal disease continues to cause epidemics outside the meningitis belt, including recent epidemics in the Great Lakes of Africa. Serogroup W135 has also emerged as an epidemic strain causing disease in Saudi Arabia in association with the Hajj pilgrimage in 2000, in Burkina Faso in 2002, and in several African countries in 2003 and 2004.

Preparations

Three meningococcal vaccines are licensed in the United States for use in children and adults against serotypes A, C, Y, and W135.

Quadrivalent meningococcal polysaccharide vaccine (MPSV4) was licensed in 1981 for use in children 2 years of age and older. Each vaccine dose contains 50 mg of each of the four purified bacterial capsular polysaccharides.[146] MPSV4 is available both as a single dose that is thimerosal free and in 10-dose vials that contain 25 µg of thimerosal/0.5 mL; 50-dose vials are no longer are available. MPSV4 is administered subcutaneously as a single 0.5-mL dose and can be given concurrently with other vaccines but at different anatomic sites.

Two meningococcal conjugate vaccines (MenACWY-D [Menactra, Sanofi Pasteur] and MenACWY-CRM [Menveo, Novartis Vaccines]) are licensed for use in persons 2 through 55 years of age. MenACWY-D is also licensed for infants as a two-dose primary series, 3 months apart, among children 9 through 23 months of age. Both meningococcal conjugate vaccines are administered intramuscularly as a single 0.5-mL dose and can be given concurrently with other recommended vaccines.

MenACWY-D is a liquid solution supplied in 0.5-mL single-dose vials. Each dose contains 4 µg each of the four capsular polysaccharides conjugated to 48 µg of diphtheria toxoid. The vaccine should be stored at 2°C to 8°C (35°F to 46°F).

MenACWY-CRM consists of two components: (1) 10 µg of lyophilized meningococcal serogroup A capsular polysaccharide conjugated to CRM197 (MenA) and (2) 5 µg each of capsular polysaccharide of serogroup C, Y, and W135 conjugated to CRM197 in 0.5 mL of phosphate buffered saline, which is used to reconstitute the lyophilized MenA component before injection. The vaccine should be stored at 2°C to 8°C (35°F to 46°F) prior to reconstitution. The reconstituted vaccine should be used immediately but may be held at or below 25°C (77°F) for up to 8 hours.

In June 2012, a combination conjugate vaccine against *H. influenzae* type b (PRP-T) and meningococcus serogroups C and Y (HibMenCY-TT, MenHibrix) was approved for children 6 weeks to 18 months. Recommendations for use of this vaccine are being considered.

No vaccine is available in the United States for prevention of serogroup B meningococcal disease, but clinical trials of several serogroup B vaccines are underway.

Immunogenicity and Efficacy

Polysaccharide Vaccine. Protective antibody concentrations are achieved within 10 to 14 days of administration of MPSV4. The vaccine elicits protective levels of bactericidal antibody to all four serogroups in more than 97 percent of recipients as measured at 28 days after vaccination. The antibody responses to each of the four polysaccharides in the polysaccharide vaccine are serogroup specific and independent. Group A polysaccharide induces antibody in some children as young as 3 months, although a response comparable to that in adults is not achieved until the child is 4 to 5 years of age.[459] The serum antibody response to serogroup C is age dependent, with a poor response in children younger than 2 years.[278] Serum concentrations of antibodies against group A and C polysaccharides decrease markedly during the first 3 years after receipt of a single dose of vaccine. The decrease in antibody occurs more rapidly in infants and young children than in adults.[341,590]

Field trials of A and C meningococcal vaccines in Europe and Africa have demonstrated efficacy rates against serogroup A of 85 to 95 percent 1 year after vaccination.[459,554] After 3 years, efficacy rates were 67 percent in older children but only 10 percent in children younger than 4 years old at the time of immunization with serogroup A vaccine.[471] In an epidemic, serogroup C vaccine demonstrated clinical efficacy rates similar to those of the serogroup A vaccine.[524]

Serogroup Y and W135 antigens are immunogenic and safe in children more than 2 years of age. However, clinical efficacy has not been demonstrated as yet for

these preparations.[7,56,552] Persons with deficiencies of the terminal components of serum complement and those with anatomic or functional asplenia have antibody responses to quadrivalent meningococcal vaccines consistent with protection.[483,485] However, the clinical efficacy of vaccination has not been evaluated in these persons.

Conjugate Vaccine. Protective antibody concentrations are achieved within 8 days after administration of MenACWY-D.[78] In studies conducted among persons aged 11 to 55 years comparing the immunogenicity of conjugate vaccine with that of the polysaccharide vaccine at 28 days after vaccination, the percentage of subjects achieving at least a fourfold increase in bactericidal titer for each serogroup was higher in the MPSV4 group than in the MenACWY-D group for persons older than 18 years of age. Nonetheless, the criteria for demonstrating immunologic noninferiority to MPSV4 were still achieved. The percentage of subjects with at least a fourfold rise was highest for serogroup W135 and lowest for serogroup Y. The percentage of subjects achieving a protective level of bactericidal antibody was greater than 97 percent for all serogroups in both MenACWY-D and MPSV4 groups.

Response to revaccination with MenACWY-D was assessed by administering MenACWY-D to subjects previously vaccinated with MPSV4 or MenACWY-D and to vaccine-naïve control subjects. All subjects in all three groups achieved protective bactericidal antibody titers at both 8 and 28 days after receiving MenACWY-D. Subjects initially primed with MenACWY-D achieved higher bactericidal antibody concentrations than those of naïve control subjects for all serogroups except A. In contrast, bactericidal antibody titers of those primed with MPSV4 were lower than those of vaccine-naïve control subjects on both days 8 and 28 for all serogroups.

In study participants aged 11 to 18 years, noninferiority of MenACWY-CRM to MenACWY-D was demonstrated for all four serogroups.[130] The proportions of subjects with seroresponse were statistically higher for serogroups A, W, and Y in the MenACWY-CRM group, compared with the MenACWY-D group. The clinical relevance of higher postvaccination immune responses is not known.

The immunogenicity of MenACWY-CRM in children aged 2 through 10 years was evaluated in a multicenter, randomized controlled trial.[294] After a single MenACWY-CRM dose, seroresponses to group C, Y, and W135 in children aged 2 through 5 years and 6 through 10 years were noninferior to responses after a single MenACWY-D dose.

MPSV4 is to be administered subcutaneously, whereas MCV4 is to be administered intramuscularly. More than 100 persons have inadvertently received the MCV4 vaccine by the subcutaneous route. For a subset of these individuals, the CDC determined that, although the serologic responses were lower after MCV4 was administered subcutaneously compared with intramuscularly, the proportions of individuals who achieved antibody levels thought to be protective were similar. Therefore, the CDC did not recommend that those who had received MCV4 needed to be reimmunized.[127]

Adverse Events

MPSV4 has been used extensively in mass-immunization programs as well as in the military and among international travelers. Adverse reactions to MPSV4 generally are mild; the most frequent reactions are pain and redness at the injection site that last for 1 or 2 days.[62] Estimates of the incidence of such local reactions have varied, ranging from 4 to 56 percent. Transient fever occurs in as many as 5 percent of vaccine recipients in some studies but is less common in older children and adults.

Among adolescents 11 to 18 years of age, safety of administering MCV4 and MPSV4 was assessed in two randomized, controlled trials. Common adverse reactions after MPSV4 and MCV4 immunization include localized pain, headache, and fatigue, all of which are mild and last for 1 to 2 days. Pain, induration, swelling, and redness at the injection site are slightly greater after administration of MCV4 compared with MPSV4. The frequency of local adverse reactions reported after MCV4 administration was similar to that reported after Td administration, which, like MCV4, is given intramuscularly. Fever is reported by 2 to 5 percent of adolescents who receive either MPSV4 or MCV4.[78]

Indications

Routine childhood immunization with meningococcal conjugate vaccines is not recommended for children 9 months through 10 years of age, because the infection rate is low in this age group; the immune response is less robust than in older children, adolescents, and adults; and duration of immunity is unknown.[35,154,161,181] However, a meningococcal conjugate vaccine is recommended for children and adolescents who are in high-risk groups as a two-dose series at 9 months through 55 years of age.[130,154]

Recommendations for use of a meningococcal conjugate vaccine are as follows:

- Adolescents should be immunized routinely at the 11- through 12-year of age health care visit, when immunization status and other preventive health services can be addressed. A booster dose at 16 years of age is recommended for adolescents immunized at 11 through 12 years of age.
- Adolescents 13 through 18 years of age should be immunized routinely with a meningococcal conjugate vaccine if not previously immunized. Adolescents who receive the first dose at 13 through 15 years of age should receive a one-time booster dose at 16 through 18 years of age.
- Adolescents who receive their first dose of meningococcal conjugate vaccine at or after 16 years of age do not need a booster dose unless they have risk factors.
- Persons at increased risk for invasive meningococcal disease should be immunized with meningococcal conjugate vaccine beginning at 9 months of age. Persons at increased risk include:
  - Children 9 months of age and older, including adults who have a persistent complement component deficiency (C5 to C9, properdin, factor H, or factor D).

  - Children 24 months of age or older including adults who have anatomic or functional asplenia. Because of high risk for invasive pneumococcal disease, children with functional or anatomic asplenia should not be immunized with MCV4-D (Menactra) before 2 years of age to avoid interference with the immune response to the PCV series.
  - Children with persistent complement component deficiencies who are vaccinated at 9 through 23 months of age should receive a two-dose primary series at least 8 weeks apart.
  - Children 9 through 23 months of age who travel to or reside in countries in which meningococcal disease is hyperendemic or epidemic should receive a two-dose primary series at least 8 weeks apart. Children 2 through 10 years of age who travel to or reside in countries in which meningococcal disease is hyperendemic or epidemic should receive one dose. Children who remain at increased risk should receive a booster dose 3 years later if the primary dose was given from 9 months through 6 years of age and 5 years after the last dose if the previous dose was given at 7 years of age or older.*
  - Military recruits.
- Because persons with HIV infection are likely to be at higher risk for meningococcal disease, although not to the extent that they are at risk for invasive *S. pneumoniae* infection, they may be immunized with a meningococcal conjugate vaccine (MCV4). Persons with HIV infection who are 2 years of age or older should receive a 2-dose primary series at least 8 weeks apart.
- Persons, including parents of children who wish to decrease their risk for meningococcal disease, may elect to receive MCV4 if they are 9 months of age or older.
- For control of meningococcal outbreaks caused by vaccine-preventable serogroups (A, C, Y, or W135), MCV4 should be used for persons 9 months through 55 years of age. MPSV4 should be used for persons older than 55 years of age in outbreak situations.
- Immunization with MCV4 is preferred for children previously immunized with MPSV4 if at least 3 years have elapsed since receiving MPSV4.

Reimmunization

Children previously immunized with either MCV4 or MPSV4 who are at prolonged increased risk for meningococcal disease should be reimmunized with MCV4. In children 2 through 6 years of age, MCV4 should be given as a booster dose 3 years after initial immunization, because concentrations of bactericidal antibodies against serogroups in the vaccine after the first dose are unlikely to provide continued protection for these children at high risk. In children 7 through 10 years of age and in adolescents who have received either MCV4 or MSPV4, a booster dose of an MCV4 is recommended 5 years after the first dose. For children recommended to receive a two-dose primary series, a booster dose of an MCV4 is recommended 5 years after the primary series regardless of the child's age when the primary series was given.

Postexposure Immunoprophylaxis

Because secondary cases can occur several weeks or more after onset of disease in the index case, meningococcal vaccine is an adjunct to chemoprophylaxis when an outbreak is caused by a serogroup prevented by a meningococcal vaccine. For control of meningococcal outbreaks caused by vaccine-preventable serogroups (A, C, Y, and W135), the preferred vaccine in adults and children 2 years of age and older is a meningococcal conjugate vaccine.

Precautions and Contraindications

Because of theoretical considerations, meningococcal polysaccharide vaccines should not be administered to pregnant women unless the risk for acquiring disease is substantial. However, evaluation of pregnant women immunized during an epidemic in Brazil demonstrated no adverse effects.[405]

Immunization with MCV4 is contraindicated among persons known to have hypersensitivity to any component of the vaccine, including diphtheria toxoid, and to dry, natural rubber latex, which is used in the vial stopper. Because of the temporal association between MCV4 and Guillain-Barré syndrome, MCV4 should not be given to adolescents or adults with a history of Guillain-Barré syndrome who are not in a high-risk group for developing invasive meningococcal disease.

Mumps Vaccine

Live virus mumps vaccine became available in the United States in 1967 and was recommended for routine use in 1977. After vaccine licensure, the incidence of reported mumps cases decreased rapidly. A relative resurgence of mumps occurred in 1986 and 1987. In 1989, the ACIP and AAP implemented a two-dose combined MMR schedule given either at 4 to 6 or at 11 to 12 years of age. After implementation of the two-dose MMR vaccination requirement, the incidence of mumps disease decreased and reached a record low of 258 cases in 2004.[165] Before vaccine licensure in 1967 and during the early years of vaccine use, most reported cases occurred in the 5- to 9-year age group; 90 percent of cases occurred among children 15 years of age and younger. In the late 1980s, a shift toward older children occurred. Since 1990, persons age 15 years and older have accounted for 30 to 40 percent of cases per year.

Between January and October of 2006, more than 5700 confirmed or probable mumps cases were reported, predominantly among patients aged 18 to 24 years, many

*CDC Travelers' Health Hotline 877-FYI-TRIP or online at www.cdc.gov/travel.

of whom were college students. Twelve Midwestern states were most affected, but sporadic cases were documented in many other states, mostly related to travel from primary outbreak areas. Nearly half of all cases occurred in Iowa, where the resurgence began.[108] The 2006 outbreak underscored limitations in the 1998 recommendations relating to prevention of mumps transmission in health care and other settings with high risk for mumps transmission. After reviewing data from this outbreak and previous evidence on mumps vaccine effectiveness and transmission, the ACIP issued updated recommendations for mumps vaccination.[139] In the following 2 years, the number of reported cases returned to usual levels and outbreaks involved fewer than 20 cases.

Another outbreak in 2009-2010 affected more than 3500 persons, primarily members of traditional observant communities in New York and New Jersey.[174] Because two doses of mumps-containing vaccine are not 100 percent effective, in settings of high immunization coverage such as the United States, most mumps cases likely will occur in persons who have received two doses.

Preparations

The live attenuated mumps virus vaccine (Jeryl Lynn strain) in current use in the United States is prepared in chick embryo cell culture and is available in combination as MMR and MMRV. The AAP and ACIP recommend that combined MMR vaccine be used when any of the individual components is indicated and MMRV is recommended if the vaccinee is 12 months through 12 years of age.

Each dose of vaccine contains neomycin, sorbitol, and hydrolyzed gelatin as stabilizers. Before reconstitution, MMR vaccine must be stored at 2° C to 8° C (35.6° F to 46.4° F) or colder and protected from light to avoid inactivation. After reconstitution, the vaccine should be used within 8 hours or discarded.

Immunogenicity and Efficacy

The vaccine induces an asymptomatic, noncommunicable infection. More than 97 percent of susceptible recipients develop protective antibody titers, albeit lower than those after natural infection.[565] Post-licensure studies in the United States demonstrated that one dose of mumps vaccine was 78 to 91 percent effective in preventing clinical mumps with parotitis.[466] Studies of vaccine effectiveness during outbreaks suggested substantially higher levels of protection with a second dose of MMR.[85,300,458]

The effectiveness of the mumps component of MMR vaccine was estimated during the large outbreak in the United Kingdom in 2004 to 2005. Vaccine effectiveness was 88 percent for one dose and 95 percent for two doses. The effectiveness of one dose declined from 96 percent in 2-year olds to 66 percent in 11- to 12-year olds, and the effectiveness of two doses declined from 99 percent in 5- to 6-year olds to 86 percent in 11- to 12-year olds.[203] The duration of vaccine immunity is unknown, but serologic data showed persistence of antibody for more than 30 years.[563] However, data from more recent studies suggested that waning immunity may contribute to mumps outbreaks in older vaccinated populations.[203]

Adverse Events

The use of mumps vaccine is associated with very few side effects. Parotitis and fever have been reported rarely. Hypersensitivity reactions, including rash, pruritus, and purpura, have been associated temporally with vaccination, but they are transient and generally mild. Administration of MMR is not harmful if it is given to an individual already immune to one or more of the viruses.[22,133]

The frequency of reported central nervous system dysfunction after vaccination is not greater than the observed background rate in unimmunized persons.[133] The IOM concluded that evidence is inadequate to establish a causal relationship between the Jeryl Lynn strain of mumps vaccine used in the United States and aseptic meningitis, encephalitis, or sensorineural deafness.[321]

Indications

Routine active immunization as MMR in children 12 to 15 months of age is recommended.[22,108,133] Most children will receive a second dose of mumps vaccine in childhood as a result of the recommendation for routine measles revaccination with MMR. Susceptible older children, adolescents, and adults also should be vaccinated against mumps.

Evidence of immunity through documentation of vaccination is now defined as one dose of live mumps vaccine for preschool-aged children and adults not at high risk for exposure and infection and two doses of live mumps vaccine for school-aged children (i.e., grades kindergarten to 12) and adults at high risk for exposure and development of infection (i.e., health care workers, international travelers, and students at post–high school education institutions). Additional recommendations for outbreak control include administering a second dose of MMR for preschool-aged children and adults not at high risk for exposure and acquisition of infection if these persons are part of a group that is experiencing an outbreak.[108] To ensure high levels of immunity, especially among groups at high risk for exposure and development of infection, every opportunity should be used to provide the first or second dose of MMR vaccine to those without adequate evidence of immunity (e.g., documentation of vaccination).

Mumps remains endemic throughout most of the world. Although vaccination against mumps is not a requirement for entry into any country, susceptible children, adolescents, and adults born after 1956 should be offered mumps vaccination, usually as MMR, before engaging in international travel.

Mumps vaccine is of no proven value in the prevention of disease in susceptible individuals after exposure to mumps, probably because the time required to develop protective antibody titers after immunization exceeds the incubation period of clinical mumps. However, if the exposure does not result in infection, the vaccine confers subsequent immunity.

Precautions and Contraindications

Among persons who are allergic to eggs, the risk for having serious allergic reactions such as anaphylaxis after receiving MMR is extremely low and skin testing with vaccine is not predictive of allergic reaction to vaccination.[22,330,344] Therefore, performing skin testing is not required before administering MMR to persons who are allergic to eggs. Similarly, the administration of gradually increasing doses of vaccine is not required.[133] Data indicate that most anaphylactic reactions to measles- and mumps-containing vaccines are not associated with hypersensitivity to egg antigens but rather with hypersensitivity to other components of the vaccines, such as the gelatin stabilizer.[283,305,343,369]

Because of the theoretical risk for fetal damage, mumps vaccine should not be administered to women known to be pregnant or who are considering becoming pregnant. Women vaccinated with MMR should avoid conception for 28 days after vaccination.[138]

Lymphoreticular or other generalized malignancy and primary or secondary immunodeficiency states represent contraindications to the use of mumps vaccine. Exceptions are children with HIV infection who are immunized against measles with MMR (see Measles Vaccine). Because infection after vaccination is noncommunicable, susceptible close contacts of immunosuppressed patients should be vaccinated to avoid exposure to mumps in such patients.

After cessation of immunosuppressive therapy, live virus mumps vaccine generally is withheld for at least 3 months. Because the intensity and type of immunosuppressive therapy, radiation therapy, underlying disease, and other factors determine when immunologic responsiveness will be restored, making a definitive recommendation for an interval after cessation of immunosuppressive therapy when mumps vaccine can be safely and effectively administered often is not possible.

The effect of immune globulin preparations on the response to mumps vaccine is unknown. High doses of immune globulin preparations can inhibit the immune response to measles vaccine for 3 or more months, depending on the dosage.[22] If mumps vaccine is given as the MMR vaccine, the recommendations for measles vaccine should be followed (see Table 244-12).

Administration of mumps vaccine should be avoided if the individual is receiving immunosuppressive dosages of systemic corticosteroids. The effects of corticosteroids vary, but many clinicians consider that a dose equivalent to either 2 mg/kg/day or 20 mg/day of prednisone is sufficiently immunosuppressive to raise concern about the safety of vaccination with live virus vaccines.

Pertussis Vaccine

Pertussis (whooping cough), caused by the fastidious, gram-negative, pleomorphic bacillus *B. pertussis*, continues to produce significant morbidity and mortality worldwide among young children.[428] In the absence of vaccination, the WHO estimated that approximately 1 million deaths would have occurred from the disease and its complications. In the United States, the number of cases has been reduced by approximately 95 percent during the vaccine era. Despite an effective vaccine, however, pertussis continues to occur in the United States in all age groups. Since the historic low point achieved in the 1980s, the incidence of pertussis has increased steadily. Surveillance data collected by the CDC's National Immunization Program for the periods from 1994 through 1996 and 1997 through 2000 demonstrated that the incidence of pertussis increased 60 percent in adolescents and adults and 11 percent in infants younger than 6 months.[141] Data from the National Notifiable Disease Surveillance System reveal an escalation in the increase in all age groups between 2001 and 2004.[86] Incidence rates among the pediatric population peak in infants younger than 6 months of age and again during adolescence between 11 and 18 years of age.[39] The increase in the number of cases among young infants suggests that a true increase in pertussis circulation has occurred. The number of cases in children old enough to receive vaccine has remained stable. The likely reason that pertussis remains endemic in the United States despite high vaccination rates is that neither infection nor immunization provides life-long immunity. Humans are the only known reservoir for pertussis; immunity wanes after 5 to 10 years, thus maintaining a population of susceptible hosts for the continued circulation of bacteria.

The experiences of countries where rates of pertussis vaccination have markedly declined provide strong support for continuing routine immunization of infants and young children.[192] In the United Kingdom, as a result of adverse publicity about pertussis vaccination, a decrease in immunization rates in 2-year-old children from 77 percent in 1974 to 30 percent in 1978 was followed by an epidemic of 102,500 cases of pertussis. A similar experience occurred in Japan, which reported 13,105 cases and 41 deaths in 1979 after routine immunization had been suspended temporarily in 1975.

In the United States during the 1980s, publicity about alleged serious reactions to pertussis vaccine generated public controversy about the risk for receiving pertussis vaccine that resulted in costly litigation and escalating vaccine costs and also jeopardized vaccine supply and development.[427,460] The experience in countries such as England and Japan, the severity of pertussis in young infants, and the usually benign or self-limited sequelae of pertussis vaccination clearly justify continuing routine childhood immunization. Several risk-to-benefit analyses provided additional evidence in support of vaccination.[192,310]

Effective primary preventive programs necessitate immunizing young infants, usually beginning at 2 months of age, because the morbidity and mortality of pertussis are greatest in infants, especially those younger than 6 months.[142,192] Approximately 26 percent of reported cases in the United States occur in infants younger than 6 months. During the period from 1997 through 2000, the case-fatality rate for infants younger than 6 months was 0.8 percent; 63 percent of these infants were hospitalized with frequent complications, including pneumonia (11.8 percent), seizures (1.4 percent), and encephalopathy (0.2 percent).[141] From 2000 through 2004, 100

pertussis-related deaths were reported; 90 percent were among infants younger than 4 months of age and 76 percent occurred in infants younger than 2 months of age.[86] Maintaining high rates of immunization in children beyond infancy, with booster immunization for adolescents and adults, may reduce the risk for infection in infants by decreasing the incidence of infection in older family members and the resultant transmission of *B. pertussis* within the household. Vaccination, including boosters, is essential because the disease is highly infectious and transmission often occurs before adults seek medical care.

Preparations

Whole-cell pertussis vaccine was introduced first in the United States in the 1920s. Not until the 1940s to 1950s, however, did pediatric whole-cell pertussis vaccine became routinely recommended for children. This vaccine, unavailable in the United States since 2002, consisted of a suspension of inactivated *B. pertussis* combined with diphtheria and tetanus toxoids (DTP).[86] To reduce the incidence of local and systemic reactions caused by whole-cell vaccines, less reactogenic pediatric acellular vaccines composed of one or more purified components of *B. pertussis* combined with diphtheria and tetanus toxoids (DTaP) were developed. Initially licensed in 1991 for use as the fourth or fifth dose in the series, DTaP was licensed in 1997 for all five doses in the series. Numerous acellular vaccines formulated from the different components have been tested in children. All currently U.S. licensed vaccines contain detoxified/inactivated pertussis toxin (i.e., pertussis toxoid) and filamentous hemagglutinin.[227] In addition, most vaccines include one or both of the following *B. pertussis* antigens: pertactin (PRN, a 69-kDa outer-membrane protein) and fimbriae proteins (FIM, agglutinogens). The various vaccines differ in the amount of each component. No pertussis-only vaccine is available.

From 2001 until 2008, numerous changes were made to the list of licensed acellular pertussis–containing vaccines available in the United States. Three acellular vaccines currently are available for use in the primary vaccination series for children younger than 7 years of age (Table 244-13). Tripedia (Sanofi Pasteur), Daptacel (Sanofi Pasteur), and Infanrix (GlaxoSmithKline Biologicals) are licensed for the five-dose DTaP vaccination series. Pediarix (GSK), which combines DTaP with IPV and HBV vaccines, was licensed in 2002 for the first three doses in the DTaP series for use in children 6 weeks to 6 years old and born to mothers known to be HBsAg negative. This vaccine is not approved for the first HBV vaccine, for the fourth or fifth DTaP dose, or for infants born to women known to be HBsAg positive or whose hepatitis B status is unknown. TriHIBit (Sanofi Pasteur), the combination of Tripedia and ActHIB (Hib conjugate vaccine), was licensed by the FDA in 2001 for use as the fourth booster dose given when the infant is 15 to 18 months of age and not for use as part of the initial three-dose series. Pentacel (Sanofi Pasteur), which combines DTaP with IPV and Hib (PRP-T) vaccines, was licensed in 2008 for the first four doses in the DTaP series in children 6 weeks to 6 years of age. Kinrix (GSK), a combination of DTaP with IPV, was also licensed in 2008 for use for the fifth dose of DTaP in children 4 to 6 years of age.

For enhanced control of pertussis, routine revaccination of adolescents and adults with an acellular pertussis vaccine is now recommended to minimize the morbidity associated with infection in these age groups and to reduce the reservoir of infection. In 2005, the FDA licensed two Tdap vaccines (see Table 244-13); Boostrix (GSK) is approved for persons aged 10 years and older, and Adacel (Sanofi Pasteur) is approved for adolescents and adults aged 11 to 64 years. Both Tdap vaccines contain the same pertussis antigen components as are in the pediatric vaccines (some in reduced quantities). The tetanus and diphtheria components are the same as for the current adult formations of Td, with reduced diphtheria content as recommended for use in persons 7 years of age and older.

The ACIP recommends that, whenever feasible, the same DTaP vaccine product be used for all doses of the vaccination series. If the vaccine provider does not know or does not have available the type of DTaP previously administered, any of the available licensed DTaP vaccines may be used to complete the vaccination series.[185]

Immunogenicity

The Multicenter Acellular Pertussis Trial evaluated the immunogenicity and safety of 13 different acellular pertussis–containing vaccines as compared with whole-cell DTP administered to infants at 2, 4, and 6 months

TABLE 244-13 **Acellular Pertussis Vaccines Licensed for Use in the United States**

Trade Name	Vaccine Manufacturer	No. of Pertussis Antigens	Antigenic Content	Dose Series Approved
DTaP Vaccines for Children <7 Yr				
Tripedia	Sanofi Pasteur	2	PT, FHA	5
Infanrix	GlaxoSmithKline Biologicals	3	PT, FHA, PE	5
DAPTACEL	Sanofi Pasteur	5	PT, FHA, PE, 2 types of FIM	5
Tdap Vaccines for Adolescents				
BOOSTRIX	GlaxoSmithKline Biologicals	3	PT, FHA, PE	1
ADACEL	Sanofi Pasteur	5	PT, FHA, PE, 2 types of FIM	1

DTaP, diphtheria–tetanus–acellular pertussis; FHA, filamentous hemagglutinin; FIM, fimbriae; PE, pertactin; PT, pertussis toxin; Tdap, tetanus–diphtheria–acellular pertussis.

of age in the United States.[227] Serologic correlates of immunity to diphtheria and tetanus toxoids, defined as antibody levels 0.1 IU/mL or greater, were achieved after vaccination with Tripedia and Infanrix and were comparable with those achieved with DTP vaccination. Pertussis immunity, determined by at least a fourfold rise in antibody titer to pertussis toxin and filamentous hemagglutinin, was equal to or greater than with DTP, after immunization with either of the two DTaP vaccines.[244]

Efficacy

For both whole-cell and acellular vaccines, serologic correlates of immunogenicity have not been established for assessing efficacy. As a result, field and other epidemiologic studies must be performed to demonstrate efficacy. Studies in the United States of household contacts exposed to pertussis indicate that the efficacy of whole-cell vaccine was 80 percent or greater.[142,192,444] Studies reporting lower rates of vaccine efficacy often reflect the use of different criteria for the diagnosis of pertussis and lesser effectiveness of the vaccine in protecting against mild infection than against severe disease.[253] Vaccine-induced immunity persists for at least 3 years and subsequently diminishes with time. Pertussis in individuals previously vaccinated is less severe and is associated with fewer complications than in unvaccinated persons.

In studies of the efficacy of eight acellular pertussis vaccines in infants, rates of prevention of pertussis ranged from 58 to 93 percent.[465] Comparing efficacy among the different products, however, often is not possible because of differences in study design, vaccine schedule (specifically, the number of doses and age of administration), case definitions of pertussis, and other confounding variables. In general, these acellular vaccines appear to be similar in efficacy to most whole-cell vaccines. Whereas in two large trials in Sweden and Italy several acellular vaccines demonstrated substantially greater efficacy than noted in the one U.S. whole-cell vaccine previously approved, other whole-cell vaccines studied appeared to be slightly more effective than were acellular vaccines in other trials.[243] In addition, the vaccines in these Swedish and Italian trials were given in a three-dose schedule, in contrast to the four-dose primary schedule for vaccination of young children in the United States.

Adverse Events

Local and febrile reactions to whole-cell vaccines occur in more than half of DTP recipients.[202] These manifestations usually develop within the first 24 hours and are brief in duration. More serious reactions to whole-cell vaccines are uncommon. Such reactions include prolonged crying for 3 hours or longer occurring within the first 48 hours of receiving vaccination (1 to 3 percent of DTP recipients) and a temperature of 40.5° C or greater (≥104.9° F) within 48 hours (0.3%); a hypotonic-hyporesponsive episode described as collapse or shock-like state within 48 hours and seizure within 3 days of vaccination each was estimated to occur once per 1750 doses.[202] Episodes of inconsolable crying, high fever, and hypotonic-hyporesponsive episodes after receipt of DTP vaccine resolved without sequelae. Most post-DTP seizures occurring within 48 hours of receipt of vaccine are brief, self-limited, and generalized and occur in association with fever. These seizures have not been demonstrated to result in the subsequent development of epilepsy or other neurologic sequelae. Predisposing factors include an underlying convulsive disorder, a personal history of previous convulsion, and a family history of convulsions.[180]

The incidence of local and febrile reactions after the administration of acellular vaccines is significantly lower.[227,465] Comparison of rates of adverse events with different acellular vaccines compared with whole-cell vaccines demonstrated similar safety profiles for each of these vaccines.[195,493] The most common adverse events include injection site reactions (erythema, induration, tenderness) and the following mild system symptoms: slight to moderate fever, drowsiness, irritability, and loss of appetite. Rates of local reactions increase with each subsequent dose of DTaP vaccine.[463,530] Booster doses of acellular pertussis vaccine may be associated with extensive local swelling (i.e., limb swelling), especially with vaccines having a high diphtheria content.[472] The pathogenesis of this reaction is not understood but spontaneously resolves without sequelae and is not associated with an increased risk for similar adverse events occurring after receipt of the fifth dose.[23] Severe reactions to acellular vaccines such as prolonged crying of 3 hours or more, temperature greater than or equal to 40.5° C (104.9° F), hypotonic-hyporesponsive episodes, and seizures are rare.* As with local and febrile reactions, their occurrence with acellular pertussis vaccination is significantly less frequent than that after whole-cell vaccination.

Syncope can occur after immunization with Tdap, is more common among adolescents and young adults, and can result in serious injury if a vaccine recipient falls. Vaccinees should be seated and observed for 15 minutes after immunization. If syncope occurs, patients should be observed until symptoms resolve.[23]

Serious Neurologic Illness. The National Childhood Encephalopathy Study (NCES), a large case-control study from Great Britain that was published in 1985, estimated that the occurrence of acute neurologic illness resulting in hospitalization was 1 in 140,000 DTP vaccinations.[416] In a 10-year follow-up study published in 1993, neurologic sequelae were found to be common occurrences, but no more so than in children with unrelated, acute neurologic illness in infancy,[415] and reviews of the data have disputed the conclusion that pertussis vaccine can cause neurologic sequelae.[47,180,518] More recent data from Canada evaluating more than 12,000 pediatric hospitalizations for serious neurologic illness between 1993 and 2002 found no association after administration of more than 6.5 million doses between development of encephalopathy and receipt of DTaP vaccination.[24] Taken together, the data do not support a role for whole-cell pertussis vaccine in causing brain damage.[95]

*References 195, 227, 280, 290, 465, 493, 532.

Indications

Vaccination against pertussis with DTaP is recommended routinely for children at 2, 4, and 6 months of age, followed by a fourth dose at 12 to 18 months of age and a fifth dose at 4 to 6 years of age.[24,39,86] Immunization can be started when the child is as young as 6 weeks of age if pertussis is prevalent in the community. The interval between administrations of the three doses of the initial series can be as short as 4 weeks. The AAP and ACIP recommend exclusive use of acellular pertussis vaccines for all doses of the pertussis vaccine series.[24,39,86] DTP is no longer available in the United States. However, in many countries, including several in Europe as well as in developing countries, whole-cell vaccine remains the recommended product.

A single dose of Tdap is recommended universally for persons 11 years of age and older, including adults, in place of the initial Td booster vaccine. The preferred schedule is to administer Tdap at the 11- or 12- year-old preventive visit, for those who have completed their primary DTP/DTaP series.[24,39,86] Adolescents and adults 11 years of age and older who received Td but not Tdap should receive a single dose of Tdap to provide protection against pertussis. Tdap can be administered regardless of time since receipt of last tetanus- or diphtheria-containing vaccine.[23] Adolescents between the ages of 11 and 18 years who have never been vaccinated against tetanus, diphtheria, or pertussis should initially receive a single dose of Tdap followed by Td for the subsequent two doses at 4 weeks or more and 6 to 12 months later. No pertussis-containing vaccine is FDA approved for children between the ages of 7 and 10 years. If pertussis vaccine is not contraindicated, children aged 7 through 10 years who are not fully vaccinated (have not received five doses of DTaP or four doses of DTaP if the fourth dose was administered on or after the fourth birthday) should receive a single dose of Tdap to provide protection against pertussis. If additional doses of tetanus and diphtheria toxoid–containing vaccines are needed for children aged 7 through 10 years, Tdap vaccine is substituted as the first dose. The subsequent two doses should be Td vaccine at 4 weeks or more and 6 to 12 months after the previous dose if indicated for the catch-up series.[9,86]

Women should receive Tdap before becoming pregnant. Women who have not been immunized previously with Tdap should receive Tdap during pregnancy, preferably during the third or late-second trimester (after 20 weeks' gestation); or if it is not administered during pregnancy, Tdap should be administered in the immediate postpartum period.[183] Every 10 years thereafter, booster vaccination should be provided by Td.

As further protection against transmission of pertussis to infants who are at greatest risk for pertussis-related morbidity or mortality compared with other age groups, the cocoon strategy is recommended to immunize mothers, families, and close contacts against pertussis to decrease their likelihood of acquisition and subsequent transmission of *B. pertussis* to young infants. Adolescents and adults (e.g., parents, siblings, grandparents, child-care providers, and health care personnel) who have or anticipate having close contact with an infant younger than 12 months of age should receive a single dose of Tdap to protect against pertussis if they have not previously received Tdap. Ideally, these persons should receive Tdap at least 2 weeks before beginning close contact with the infant.[23,183]

Contraindications and Precautions

The contraindications and precautions for administering pertussis vaccine are based on adverse reactions associated with whole-cell vaccine. Although reactions occurring after the administration of DTaP are much less common than are those associated with DTP, at present the contraindications and precautions for DTaP are the same.[24,39,86] Adverse events temporally related to pertussis immunization that contraindicate further administration of DTaP are as follows:

- An immediate anaphylactic reaction. Subsequent immunization with any of the three components of the vaccine should be avoided.
- Encephalopathy occurring within 7 days. Children who experienced encephalopathy within 7 days after administration of a previous dose of diphtheria and tetanus toxoids and pertussis vaccine (DTP, DTaP, or Tdap) not attributable to another identifiable cause should not receive additional doses of a vaccine that contains pertussis. Encephalopathy in this context is defined as a severe, acute, central nervous system disorder (e.g., coma or prolonged seizures) unexplained by another cause and may be manifested by major alterations in consciousness or by generalized or focal seizures that persist for more than a few hours without recovery within 24 hours. Additional doses of DT (or Td) vaccine should be substituted for any pertussis-containing vaccine.

Post-vaccination reactions constituting precautions are as follows:

- Presence of a moderate or severe acute illness with or without a fever
- Guillain-Barré syndrome within 6 weeks after a previous dose of tetanus toxoid containing vaccine
- A convulsion, with or without fever, occurring within 3 days of receiving DTP or DTaP vaccination
- Persistent, severe, inconsolable screaming or crying for 3 hours or more within 48 hours of DTP or DTaP vaccination
- Hypotonic-hyporesponsive episode occurring within 48 hours of DTP or DTaP vaccination
- Temperature of 40.5° C (104.9° F) or greater that is unexplained by another cause and occurs within 48 hours of DTP or DTaP vaccination

With these adverse events occurring in temporal association with DTaP vaccination, the decision to administer additional doses of pertussis vaccine should be considered carefully. Children for whom vaccination is deferred because of moderate or severe acute illness should be vaccinated when their condition improves. In circumstances such as a pertussis outbreak in which the potential benefits of pertussis immunization outweigh the possible risks, vaccination is indicated, particularly because these events have not been proved to cause permanent sequelae.

In addition, the risk for these reactions occurring after receipt of DTaP is substantially lower than after receipt of DTP.

In children with an evolving neurologic disorder in the first year of life, pertussis immunization should be deferred until the nature and cause of the disorder have been established. A personal history of having a stable neurologic condition (previous convulsion unrelated to DTaP vaccination, a history of seizure disorder, well-controlled seizures, cerebral palsy) or a family history of convulsions is not a contraindication, and pertussis immunization should be administered on schedule.

As noted earlier, certain conditions after DTaP vaccine, such as temperature of 40.5 C (104.9° F) or higher, collapse or shock-like state, persistent crying, or convulsions with or without fever are a precaution to subsequent doses of DTaP. However, occurrence of one of these adverse reactions after DTaP vaccine in childhood is not a contraindication or precaution to administration of Tdap to an adolescent or adult. A history of severe Arthus hypersensitivity reaction after a previous dose of a tetanus or diphtheria toxoid–containing vaccine administered less than 10 years previously should lead to deferral of Tdap or Td immunization for 10 years after administration of the tetanus or diphtheria toxoid–containing vaccine.[23,86]

Pneumococcal Vaccine

S. pneumoniae is a leading bacterial pathogen, especially among young children, elderly persons, and persons with predisposing conditions. In children, it is the most common cause of otitis media, occult bacteremia, and bacterial pneumonia requiring hospitalization. After the widespread introduction of conjugate Hib vaccination and subsequent marked decline in occurrence of *H. influenzae* type b meningitis, *S. pneumoniae* became a leading cause of bacterial meningitis in children in the United States. In some populations, such as Alaska Natives, the incidence of bacteremia is markedly higher than that reported in other geographic areas of the United States.[225] Groups considered at high risk for invasive pneumococcal disease (IPD) include children with sickle-cell disease, asplenia, Hodgkin disease, congenital humoral immunodeficiency, HIV infection, and nephrotic syndrome, as well as recipients of organ transplants. Children who have received cochlear implants are at particular risk for developing meningitis.[143,470] Other chronic diseases associated with an increased risk for development of severe pneumococcal disease include chronic cardiovascular and pulmonary diseases, diabetes mellitus, and renal failure. The role of these chronic diseases in predisposing individuals to the development of pneumococcal infection, however, has been demonstrated primarily in adults. Mortality rates are highest in those who have bacteremia or meningitis, elderly persons, and patients with impaired humoral immunity or certain chronic diseases.

The purified polysaccharide vaccine has been effective in reducing severe disease in the adult population,[90] but it has had little impact in young children because the vaccine is not immunogenic in children younger than 2 years old.[25] In addition, the polysaccharide vaccine has not been effective in preventing otitis media caused by *S. pneumoniae*.[25]

Several factors have made the development of new preventive strategies for pneumococcal disease a high priority.[210] Morbidity and mortality rates of pneumococcal infection appear to be particularly high in developing countries. The increasing incidence of antimicrobial-resistant pneumococci further underscores the need for developing effective pneumococcal vaccines for young children.[84] Resistance of *S. pneumoniae* to multiple antibiotics has increased rapidly in the United States and even more rapidly in other parts of the world.[337] Children younger than 2 years of age have the highest rate of IPD but do not develop an effective antibody response to polysaccharide vaccine. In addition, children 2 to 5 years of age may have relatively poor responses to serotypes 6B, 14, 19F, and 23F, which are common causes of pediatric infections and the most prevalent penicillin-resistant serotypes.

These factors prompted the development of conjugated polysaccharide–protein vaccines, two of which have been licensed in the United States. These vaccines are similar in design to the licensed Hib conjugate vaccines. Because of the large number of serotypes of *S. pneumoniae* that cause disease, development of these conjugate pneumococcal vaccines has been more difficult than has the development of similar vaccines for *H. influenzae* type b. Each pneumococcal antigen must be coupled to a protein carrier, and the vaccine must be prepared to ensure that antigen is sufficient to induce an immune response but insufficient to elicit an adverse reaction.

One potential problem with conjugate pneumococcal vaccines is the need to immunize against many different serotypes of pneumococci. Because of local reactions to the protein component, conjugate vaccines that contain all implicated serotypes have proven difficult to produce. Initial decisions for serotype inclusion need to be carefully planned for specific groups. For instance, vaccine containing types 4, 6B, 9V, 14, 18C, 19F, and 23F (PCV7) would be necessary for prevention of otitis media in the United States whereas types 1, 2, and 5 would need to be added to prevent pneumonia in developing countries. Compounding this, the use of conjugate vaccines against limited serotypes has led to emergence of pneumococcal serotypes that were previously less common but have proven equally concerning for IPD and emerging resistance (e.g., 19A).[92,128,327] Specifically, emergence of replacement serotypes led to the expansion of the previous vaccine to the 13-valent pneumococcal conjugate vaccine (PCV13) in the United States. Routine infant immunization using these conjugate vaccines has led to significant reductions in the instances of *S. pneumoniae* disease. Since the widespread introduction of PCV7 in infants in 2000, rates of IPD have decreased markedly in the United States. The incidence of all IPD has decreased by 80 percent for children younger than 2 years of age and by up to 90 percent for infections caused by vaccine and vaccine-related serotypes.[25,128,572] An increase in nonvaccine serotype disease has occurred, but it is small compared with the overall reductions in vaccine-type disease.[50,92,116,128] A decline in the incidence of vaccine-type disease in unvaccinated children and adults has

occurred since the introduction of routine vaccination for infants in the United States.[116] In addition, rates of IPD in infants younger than 2 months of age have decreased significantly, thus providing evidence that vaccinating children aged 2 to 23 months has led to changes in pneumococcal carriage in infants too young to receive PCV7.[468] Herd immunity is estimated to prevent twice as many cases as do the direct effects of vaccination alone, and a similar benefit is anticipated with new serotypes included in PCV13.[116,128] Although the exact mechanism of herd immunity is uncertain, one hypothesis is that vaccinated children are less likely to have nasal carriage of pneumococcus and hence less pneumococcal transmission to their contacts.[218] Use of conjugate vaccines has been demonstrated to reduce nasopharyngeal carriage of vaccine serotypes with both PCV7 and PCV13.[204,220,221] Pneumococcal conjugate vaccination also has led to increased nasopharyngeal colonization of children with nonvaccine serotypes.[268,414] This underscores the need for ongoing surveillance of emerging nonvaccine serotypes with respect to nasopharyngeal colonization, antibiotic nonsusceptibility, as well as rates of IPD.[511]

Preparations

Two pneumococcal vaccines are available for use in children in the United States. The first is a 13-valent vaccine (PCV13) licensed in 2010 (Prevnar, Wyeth Vaccines), containing the capsular polysaccharides from serotypes contained in the previous PCV7 (4, 6B, 9V, 14, 18C, 19F, and 23F) and six additional serotypes (1, 3, 5, 6A, 7F, and 19A) conjugated to mutant diphtheria toxin (CRM_{197}).[25] Serotypes included in PCV7 and potentially cross-reactive serotypes (i.e., 6A, 9A, 9L, 18B, and 18F) accounted for 86 percent of cases of bacteremia, 83 percent of cases of meningitis, and 65 percent of AOM cases occurring in children younger than 6 years in the United States during the period 1978 to 1994.[440] In 2007, the Active Bacterial Core surveillance estimated 4600 cases of IPD in children younger than age 5 in the United States, with 2900 attributable to serotypes that would be covered by PCV13 versus 60 cases covered by PCV7.[128] The second vaccine (PPV23 [Pneumovax]) is composed of purified, capsular polysaccharide antigens of 23 pneumococcal serotypes. Although 90 different serotypes have been identified, vaccine serotypes in the 23-valent vaccine are responsible for 85 to 90 percent of adult infections and nearly 100 percent of invasive disease and 85 percent of otitis media cases in children.[98,269] Each vaccine is recommended in a dose of 0.5 mL to be given intramuscularly.

Immunogenicity and Efficacy

The immunogenicity of the conjugate polysaccharide vaccine appears to be determined by the pneumococcal polysaccharide serotype, rather than by the carrier protein. Some serotypes (14, 18C, and 19F) are excellent immunogens in that they elicit antibody protection after a single dose, whereas others (6B and 23F) require three doses of vaccine.[249] Conjugate vaccine elicits immunologic memory.[249] The antibody concentrations achieved after the initial series of three doses usually are sustained for only a few months and then decline to nearly preimmunization levels. A dose of pneumococcal vaccine, either polysaccharide or conjugate, given in the second year of life elicits an amnestic-type response.

Pre-licensure clinical efficacy of PCV7 was studied in a large, prospective, placebo-controlled efficacy trial in northern California involving 38,000 children. The vaccine was 89 percent effective in preventing invasive disease caused by any pneumococcal serotype and 97 percent effective against disease caused by the seven vaccine serotypes.[79] For noninvasive disease, a decrease of 7 percent in the number of cases of otitis media and 23 percent in doctor visits for recurrent otitis (six or more visits per year) occurred in vaccinated children. The study also demonstrated an 11 percent decrease in the number of clinical cases of pneumonia and efficacy of 26 percent against a first episode of radiograph-confirmed pneumonia in vaccines.[298,504] Pre-licensure immunogenicity of PCV13 was studied in a randomized, double-blind, active-controlled trial in which 663 U.S. infants received at least one dose of PCV7 or PCV13. The study was powered for noninferiority of immunoglobulin antibody response measured by enzyme immunoassay and also included subset analysis of functional antibody responses measured by opsonophagocytosis assay (OPA).[45,587] The noninferiority criterion (immunoglobulin geometric mean concentrations) was met for all shared serotypes; although slightly lower in the PCV13 group the functional OPA response was similar, suggesting PCV13 has similar efficacy as PCV7 against shared serotypes. After a fourth dose of PCV13, the additional six serotypes also produced a functional OPA response in 97 to 100 percent.

The ability of PCV7 to protect children against AOM also was evaluated in an efficacy trial conducted in Finland.[250] For prevention of AOM caused by pneumococci of any serotype, efficacy was estimated to be 34 percent, whereas efficacy against AOM irrespective of etiology was 6 percent. Efficacy against AOM caused by vaccine-related serotypes was 57 percent, but an increase of 33 percent in the rate of AOM episodes caused by non-vaccine serotypes occurred in the group receiving the heptavalent vaccine in comparison with controls. However, in spite of the increase in disease caused by nonvaccine serotypes, the net effect on pneumococcal AOM was a reduction of 34 percent.

Randomized controlled trials conducted in African children using a nine-valent pneumococcal conjugate vaccine demonstrated efficacy in the prevention of radiologically confirmed pneumonia and vaccine-type IPD.[215,352] The vaccine was efficacious in preventing vaccine-type invasive disease in HIV-infected children.[352] One of the trials demonstrated a reduction in all-cause mortality among vaccine recipients.[215] These data have presented a compelling case for extending the benefits of pneumococcal conjugate vaccination to developing countries.

Vaccination with purified polysaccharide vaccine results in serologic type-specific antibody in most healthy adults and older children. Immunocompromised patients may respond less well. In children younger than 2 years

old, antibody response is poor to most serotypes, including those most likely to cause infection, such as types 6A and 14.[98,147,269] Patients with AIDS have impaired antibody responses to vaccination, but asymptomatic HIV-infected adults do respond.[98,147]

Vaccine efficacy in preventing serious pneumococcal infection has been demonstrated for the purified polysaccharide vaccine primarily in immunocompetent adults, including elderly persons and those with chronic diseases such as chronic pulmonary and cardiac disorders and diabetes mellitus, which predispose these patients to development of pneumococcal infections. Efficacy against vaccine serotypes ranges from 61 to 75 percent in adults.[90,499] Investigations in adults in whom vaccine protection against pneumococcal infection has been substantially less have been criticized for methodologic problems.[98,147] Efficacy in the limited studies of children has been consistent with that in adults. In children with sickle-cell disease or anatomic asplenia, an octavalent vaccine was highly effective in preventing bacteremic infection.[49]

Adverse Events

Pneumococcal conjugate vaccines appear to be safe, and in 13 clinical trials the safety profile of PCV13 was comparable to that of PCV7.[25,45,440] The reactions most commonly reported have been local reactions at the injection site, but they occur at a lower frequency than do local reactions with other childhood vaccines such as DTP.[79]

Local reactions at the injection site, such as erythema and pain, are reported in approximately 50 percent of recipients of the purified polysaccharide vaccine.[25] However, more severe local and systemic reactions, such as fever and myalgia, are rare events that occur in less than 1 percent of vaccine recipients. Severe systemic reactions such as anaphylaxis rarely have been reported.[575] In adults who were revaccinated within 1 to 2 years in early studies, local reactions occurred more commonly than did those after initial immunization.[98,147] However, subsequent investigations, including studies in children, indicated no increase in the incidence or severity of local or systemic reactions on revaccination after longer intervals.[354,473]

Indications

Recommendations by the AAP and the ACIP for the use of PCV13 have been issued.[45,440] The AAP and ACIP recommend universal use of PCV13 in children 59 months and younger. For children in whom no prior PCV7 or PCV13 has been administered before they reach 7 months of age, four doses of PCV13 are recommended. Routinely these should be administered at 2, 4, 6, and 12 to 15 months of age. For children younger than 24 months who have not received their recommended four doses of PCV7, a dose of PCV13 should be administered at the next scheduled vaccine; the total number of doses differs based on the child's age (see elsewhere for further information[25,45,440]). A single supplemental dose of PCV13 is recommended for all children 14 months to 59 months who have previously received all four doses of PCV7. In addition, a single dose of PCV13 is recommended for children 60 months to 18 years who are fully immunized with PCV7 and have underlying medical conditions increasing their risk for IPD (see following list). If not previously administered, these children also should receive the 23-valent polysaccharide vaccine (PPV23) to expand serotype coverage.

The AAP recommends PPV23 for children 24 months or older and PCV13 for all children 59 months and older who belong to one or more of the following risk groups:

- Immunocompetent children with chronic heart disease, chronic lung disease, diabetes mellitus, cerebrospinal fluid leaks, cochlear implants
- Children with functional or anatomic asplenia, including sickle cell disease or other hemoglobinopathies, congenital or acquired asplenia, or splenic dysfunction
- Children with immunocompromising conditions, including HIV infection, chronic renal failure and nephrotic syndrome, diseases associated with treatment with immunosuppressive drugs or radiation therapy, including malignant neoplasms, leukemias, lymphomas, and Hodgkin disease, or solid organ transplantation and congenital immunodeficiency

For previously vaccinated children 18 years old or younger who are at high risk for acquiring severe pneumococcal infection, revaccination with PPV23 after 5 years is recommended.[45,440] Such children include those who have functional (e.g., sickle-cell disease) or anatomic asplenia, children and adolescents with HIV infection, and those who have a rapid antibody decline (e.g., nephrotic syndrome, renal failure, or organ transplantation).[270] Revaccination with PPV23 should be considered for high-risk older children and adults who were vaccinated 6 years or more previously, but no more than two total PPV23 doses are necessary and the interval between polysaccharide and conjugate vaccines should be at least 8 weeks.

Contraindications

No contraindications to initial vaccination exist.[45,440] The safety of pneumococcal vaccine in pregnant women has not been evaluated, but adverse consequences to the fetus have not been observed in neonates whose mothers were vaccinated inadvertently during pregnancy. Ideally, women at high risk for acquiring pneumococcal disease should be vaccinated before pregnancy. In persons who have had a severe reaction, such as anaphylaxis or a localized, severe hypersensitivity response, revaccination should be avoided.

Poliomyelitis Vaccine

The widespread implementation of poliovirus vaccine programs resulted in a dramatic reduction in the incidence of paralytic poliomyelitis throughout the world. In contrast to the pre-vaccine era, when more than 18,000 cases of paralytic disease occurred in the United States annually, the last known case in this country caused by indigenous wild-type virus occurred in 1979.[171] Other than rare imported cases, the only cases of paralytic

poliomyelitis in the United States since then have been vaccine related. The effectiveness of polio vaccination led the WHO to launch the Global Polio Eradication Initiative in 1988, when approximately 350,000 persons succumbed to polio each year. The initiative initially made rapid gains; the number of new wild poliovirus cases decreased by 99 percent between 1988 and 2005. The last case of paralytic poliomyelitis caused by serotype 2 wild poliovirus was detected in 1999. Currently, all nations in the Western Hemisphere, the Pacific region (including China), and Europe are free of poliomyelitis.[109,150,475] However, incidence rates have plateaued since 2001, with 1,000 to 2,000 new cases annually because of regional conflicts, natural disasters, and poor health care infrastructure. All the recently reported importation-associated outbreaks of polio in eight previously polio-free countries originated from the largest remaining reservoirs of indigenous virus in Nigeria, Pakistan, Afghanistan, and India. Three countries with imported virus (Angola, Chad, and the Democratic Republic of Congo) have reestablished transmission. By 2009, sustained investments in innovative technologies and community outreach resulted in a new effective bivalent oral poliovirus vaccine (OPV)[522] and targeted supplemental immunization campaigns. Since that time, new polio cases have decreased by 95 percent in northern Nigeria, India has been declared polio free as of February 2012, and many countries where poliovirus had been reintroduced have again become polio free.[60] The hope is, that with fortified efforts, the "end game" for complete global eradication of wild-type polio is within reach.[60,151]

The elimination of poliovirus infection has been achieved primarily through the use of OPV (Sabin strain). This product had been the vaccine of choice for children in the United States since the early 1960s because it induced optimal intestinal immunity, was painless to administer, and secondarily immunized some contacts by fecal-oral spread of the vaccine virus and thus contributed to the immunity of the population.[99] Because 8 to 10 cases of vaccine-associated paralytic polio occurred annually and the risk for exposure to wild-type poliovirus had been markedly reduced or eliminated in the United States, expanded use of inactivated poliovirus vaccine (IPV, Salk strain) was recommended by the CDC and the AAP beginning in 1997.[37] In 1999, as a result of progress in the global eradication of poliomyelitis, the need for further reduction in the risk for acquiring vaccine-associated paralytic polio, and the acceptance of IPV by parents and physicians,[126] IPV was recommended for the first two doses of poliovirus vaccine for routine childhood vaccination.[38,156] To eliminate the risk for vaccine-associated paralytic polio completely, in January of 2000 an all-IPV schedule was recommended for routine childhood vaccination in the United States.[40,156,158]

Since 2000, outbreaks of paralytic poliomyelitis caused by neurovirulent polioviruses that are derived from Sabin OPV vaccine strain have occurred among underimmunized children living in certain economically deprived regions. The low immunization rates in these areas have permitted these viruses, called vaccine-derived polioviruses (VDPVs), to circulate for long periods of time and, by continuous mutation, to acquire biologic properties that are indistinguishable from naturally occurring wild polioviruses. Outbreaks associated with circulating VDPVs have occurred in Hispaniola (2000 through 2001), the Philippines (2001), and Madagascar (2001 through 2002).[105,140,345,580] Retrospective studies also detected the circulation of endemic VDPV in Egypt (1988 through 1993) and the likely localized spread of OPV-derived virus in Belarus (1965 through 1966).[112,346] Gaps in OPV coverage and the previous eradication of the corresponding serotype of indigenous wild poliovirus were the critical risk factors for all VDPV outbreaks.[346] The VDPV outbreaks were stopped by mass immunization campaigns using OPV. The potential risk for emergence of VDPV has increased dramatically in recent years as wild poliovirus circulation has ceased in most of the world. The risk appears to be highest for the type 2 OPV strain, which was found in eight of nine countries with VDPV outbreaks between 2008 and 2010, because of its greater tendency to spread to contacts.[169,170] The emergence of VDPVs underscores the critical importance of eliminating the last pockets of wild poliovirus circulation, maintaining universally high levels of polio vaccine coverage (>90% of young children), stopping OPV use as soon as it is safely possible to do so, and continuing sensitive poliovirus surveillance into the foreseeable future. Particular attention must be given to areas where the risks for wild poliovirus circulation have been highest and where the highest rates of polio vaccine coverage must be maintained to suppress VDPV emergence.

Preparations

Trivalent IPV is the only vaccine available for routine infant and childhood immunization in the United States. IPV is prepared by inactivation of naturally occurring polioviruses by treatment with dilute formalin.[156] Four trivalent IPV vaccines are now available in the United States: IPOL (Aventis Pasteur), Pediarix (in combination with DTaP vaccine and HBV vaccine, GlaxoSmithKline), Pentacel (in combination with DTaP vaccine and Hib vaccine, Sanofi Pasteur), and Kinrix (in combination with DTaP vaccine, GSK). These vaccines are produced in monkey kidney (Vero) cells, and each contains 40-, 8-, and 32-D-antigen units of poliovirus types 1, 2, and 3, respectively. OPV is no longer available in the United States.

The trivalent OPV formulations available globally contain approximately $10^{6.5}$ 50 percent tissue culture infectious doses ($TCID_{50}$), $10^{5.5}$ $TCID_{50}$, and $10^{6.2}$ $TCID_{50}$ of poliovirus types 1, 2, and 3, respectively. The unequal contribution of each type to the trivalent preparation represents a "balanced" formulation designed to account for the more efficient replication of type 2 OPV virus in the gastrointestinal tract; if type 2 were given in equal concentrations with types 1 and 3, it would interfere with the replication of the two latter types. The live oral OPV strains originally were attenuated by passage in monkeys and in monkey kidney cell culture.

Trivalent IPV is now the preferred vaccine for other developed countries. However, because OPV maintains certain advantages for children in developing countries,

including cost, ease of administration, and transmission of vaccine virus to unimmunized contacts, it is the vaccine currently recommended by the WHO EPI.[579] The trivalent form of OPV has been the mainstay of EPI and the WHO Polio Eradication Program for many years. A new bivalent OPV vaccine (serotypes 1 and 3) has been available since 2009 and may replace the trivalent vaccine given that poliovirus type 2 is no longer in circulation.[151,522] Monovalent OPV vaccines have been introduced for outbreak control in certain settings.

Immunogenicity and Efficacy

Neutralizing antibodies are detectable to all three poliovirus types in 99 percent of recipients of IPV after two doses and in 100 percent after the third dose.[403,509] IPV-immunized children experience a large boost in antibody titer after receiving the third dose.[475] Detectable antibody persists at protective levels for at least 5 years, although geometric mean titers decline considerably.[523] However, few data are available regarding vaccine efficacy of the currently licensed "enhanced-potency" IPV formulations. A case-control study in Senegal indicated lower than expected protection rates of 36 and 89 percent for recipients of one and two doses, respectively.[476] However, because most IPV recipients experience a substantial boost in antibody titer after receiving a third dose, efficacy after three IPV doses likely would be quite high.

An optimal immune response to trivalent OPV requires multiple doses. In developed countries, three doses given at least 2 months apart are sufficient, with antibody prevalence to all three types being approximately 96 percent after the third dose.[403] Detectable serum antibody to all three types persists in 84 to 98 percent of vaccinees 5 years after receiving primary immunization.[361]

OPV efficacy was directly evaluated during a type 1 poliovirus outbreak in Taiwan in the early 1980s. During this outbreak, vaccine efficacy was estimated to be 82, 96, and 98 percent for one, two, and three or more doses, respectively.[347] In tropical countries, the series of OPV at 6, 10, and 14 weeks of age recommended by the WHO EPI fails to produce active immunity in a significant proportion of infants. Low seroconversion rates have been documented in many locations, averaging 73, 90, and 70 percent for types 1, 2, and 3, respectively.[455] Diarrheal disease at the time of immunization is a major factor.[431] The impact of diarrhea on seroconversion persists despite the administration of three or four OPV doses.

The monovalent OPV vaccines have higher type-specific seroconversion rates than does the trivalent vaccine and thus may be more effective when used in response to an outbreak caused by one poliovirus type.[93]

Adverse Events

No serious adverse events have been associated with use of the currently available IPV vaccine.[26,40,156] Because IPV vaccine contains trace amounts of streptomycin, neomycin, and polymyxin B, allergic reactions are possible in recipients with hypersensitivity to one or more of these antibiotics.

The OPV vaccine can cause vaccine-associated paralytic polio, the overall risk for which is approximately one case per 2.4 million doses distributed. The rate after the first dose is approximately one case per 750,000 doses, including vaccine recipient and contact cases. Chronic VDPV excretion by persons with severe primary B-cell immune defects is a rare complication of OPV use and it may cause fatal disease.

Indications

The polio vaccination series in the United States consists of four doses of IPV administered subcutaneously or intramuscularly.[26,40,156] The first and second doses are administered at 2 and 4 months of age, respectively. The third dose is usually given at 6 to 18 months of age, and a fourth dose is given routinely at 4 to 6 years of age, before school entry. Administration of a fourth dose is not necessary if the third dose was given on or after the child's fourth birthday. IPV is a component of Pediarix, Pentacel, and Kinrix. Pentacel may be used in children from 6 months to 18 months of age, and Kinrix may be used for children 4 to 6 years of age.

The ACIP and AAP continue to support the global eradication initiative and use of OPV as the vaccine of choice to eradicate wild-type polio in endemic areas and during epidemics. In the immunization schedule of the EPI of the WHO, doses of OPV are recommended at birth and when the child is 6, 10, and 14 weeks of age.[9] In geographic areas with endemic polio, a dose may be given when the neonate is discharged from the hospital. Supplementary doses often are given during mass community programs in these areas. Breast-feeding does not interfere with successful immunization with OPV.

Routine poliovirus vaccination is not necessary in adults residing in the United States. Such individuals are at minimal risk for exposure, and most are adequately protected because of vaccination during childhood. However, vaccination is recommended for individuals who have increased risk for exposure, including persons traveling to countries where poliomyelitis is epidemic or endemic, members of communities of specific population groups experiencing wild-type poliovirus disease, health care workers in close contact with patients who may be excreting wild-type poliovirus, and laboratory workers in contact with specimens that may contain wild-type poliovirus. Previously immunized adults who are at increased risk for exposure to poliomyelitis, such as those traveling to countries where poliomyelitis is still endemic, should receive a single dose of IPV. Available data do not indicate the need for more than a single lifetime booster dose of IPV. Adults who are unvaccinated or whose vaccination status is not documented should receive a primary vaccination series with IPV. This consists of two doses of IPV at 4- to 8-week intervals and a third dose 6 to 12 months after the second dose.

Precautions and Contraindications

IPV is contraindicated in persons who have experienced an anaphylactic reaction after receiving a previous dose of IPV or an anaphylactic reaction to one of the

antibiotics in the vaccine preparation (i.e., streptomycin, polymyxin B, or neomycin).[26,40,156] Poliomyelitis vaccination generally is contraindicated in pregnant women because of the theoretical risk for harm to the fetus. However, no deleterious effects from IPV administered during pregnancy have been demonstrated; and if immediate protection against poliomyelitis is needed, IPV may be given.

Rotavirus Vaccine

Rotavirus, a major cause of childhood morbidity and mortality, causes an annual 440,000 deaths or approximately 5 percent of all childhood deaths worldwide. Rotavirus is also a major cause of acute gastroenteritis in the United States. In their first 5 years of life, 4 of 5 children in the United States will develop rotavirus gastroenteritis, 1 in 7 will require a clinic or emergency department visit, 1 in 70 will be hospitalized, and 1 in 200,000 will die of this disease.[257,272,386,534]

Rotavirus gastroenteritis is preventable with live oral rotavirus vaccines. In 1998, the only approved rotavirus vaccine, a tetravalent rhesus-based rotavirus vaccine (RRV-TV, Rotashield, Wyeth Laboratories), was withdrawn from the market in the United States because of an association with intussusception among vaccinated infants.[430] In 2006, a bovine-based pentavalent rotavirus vaccine (RotaTeq, Merck & Co.) was licensed by the FDA for use in infants in the United States. Both the ACIP of the CDC and the AAP have recommended universal vaccination of U.S. infants against rotavirus.[41,212] In 2008 a live attenuated human rotavirus vaccine (Rotarix, GlaxoSmithKline) was licensed in the United States.

The epidemiology of rotavirus disease in the United States has changed dramatically since rotavirus vaccines became available in 2006. The rotavirus season now is shorter and relatively delayed, peaking in late spring, and the overall burden of rotavirus disease has declined dramatically.[115] In the first 2 years after the RV5 vaccine became available, emergency department visits and hospitalizations for rotavirus decreased by 85 percent, such that in the 2008 season there were an estimated 40,000 to 60,000 fewer gastroenteritis hospitalizations among children younger than 5 years of age compared with prevaccine seasons.[160] There also were substantial reductions in office visits for gastroenteritis during this time period.

Preparations

There are currently two rotavirus vaccines licensed for use in the United States. The pentavalent rotavirus vaccine (RV5) is an oral vaccine that contains five live reassortant rotaviruses. The rotavirus parent strains of the reassortants were isolated from human and bovine hosts. Four reassortant rotaviruses express one of the outer capsid proteins (G1, G2, G3, or G4) from the human rotavirus parent strain and the attachment protein (P7[5]) from the bovine rotavirus parent strain. The fifth reassortant virus expresses the attachment protein (P1A[8]) from the human rotavirus parent strain and the outer capsid protein G6 from the bovine rotavirus parent strain. The reassortants are propagated in Vero cells using standard tissue culture techniques.

RV5 vaccine is provided in a squeezable plastic dosing tube with a twist-off cap designed to allow for the vaccine to be administered directly to infants by mouth. Each tube contains a single 2-mL dose of the vaccine as a liquid buffered-stabilized solution.

The human rotavirus vaccine (RV1) is a G1 P1A[8] virus attenuated by passage in cell culture. RV1 is provided as a lyophilized powder that is reconstituted with a supplied diluent before administration.

Both rotavirus vaccines must be stored at refrigerator temperatures (2° C to 8° C [35° F to 46° F]) and protected from light. RV1 diluent may be stored at room temperature. The vaccines must not be frozen. The shelf life of properly stored vaccine is 24 months. RV5 should be administered as soon as possible after being removed from refrigeration. RV1 should be administered within 24 hours of reconstitution. Reconstituted RV1 may be stored at refrigerator or room temperature.

No studies have addressed the interchangeability of the rotavirus vaccines.

Immunogenicity and Efficacy

The immune correlates of protection from rotavirus infection and disease are not fully understood. In a large phase III clinical trial of RV5, an increase in titer of rotavirus group-specific serum IgA antibodies was used as one of the measures of the immunogenicity. Serum samples were obtained from a subset of study participants before they were immunized and approximately 2 weeks after they received the third dose; seroconversion was defined as a threefold or greater increase in antibody titer from baseline. Seroconversion rates for IgA antibody to rotavirus were 95 percent among vaccine recipients versus 14 percent in recipients of the placebo.[548]

The efficacy of RV5 was evaluated in two phase III trials.[81,548] In these trials, the efficacy of RV5 after completion of a three-dose regimen against rotavirus gastroenteritis of any severity was 74 percent and against severe rotavirus gastroenteritis it was 98 percent. Efficacy was observed against all G1 to G4 and G9 serotypes, but relatively few non-G1 rotavirus cases were reported. RV5 reduced the incidence of office visits by 86 percent, emergency department visits by 94 percent, and hospitalizations for rotavirus gastroenteritis by 96 percent. The efficacy of RV5 in the second rotavirus season after immunization was 63 percent against rotavirus gastroenteritis of any severity and 88 percent against severe rotavirus gastroenteritis. Neither breast-feeding nor concurrent administration of other childhood vaccines appears to diminish the efficacy of a three-dose series of RV5. The efficacy of fewer than three doses is not known.

Phase III clinical trials of RV1 efficacy have involved more than 21,000 infants 6 through 12 weeks of age, primarily in two studies in Latin America and Europe.[486,546] After completion of a two-dose RV1 regimen, the efficacy of rotavirus vaccine against severe rotavirus gastroenteritis (Latin America study) was 85 percent, and against any rotavirus gastroenteritis (Europe study) it was 87 percent.

RV1 reduced hospitalization for rotavirus gastroenteritis by 85 to 100 percent (depending on the study). The efficacy of fewer than two doses is not known.

Adverse Events

Safety with respect to intussusception was evaluated in 71,725 subjects enrolled in phase III efficacy trials of RV5.[81,548] For the prespecified 42-day postimmunization end point, six cases of intussusception were observed in the RV5 group versus five cases of intussusception in the placebo group (multiplicity-adjusted relative risk, 1.6). The data did not suggest an increased risk for development of intussusception relative to placebo. Among vaccine recipients, no confirmed cases of intussusception occurred within the 42-day period after administration of the first dose, which was the period of highest risk for the previously licensed RRV-TV vaccine. In addition, no evidence of clustering of cases of intussusception was observed within a 7- or 14-day window after immunization for any dose. For the 1-year follow-up period after administration of the first dose, 13 cases of intussusception were observed in the RV5 group versus 15 cases of intussusception in the placebo group (multiplicity-adjusted relative risk, 0.9).

Some studies performed outside the United States have detected a low level of increased risk for intussusception after rotavirus immunization shortly after the first dose. The level of risk observed in these postmarketing studies is substantially lower than the risk for intussusception after immunization with RotaShield, the previous rotavirus vaccine.[91,453,542] Although an increased risk for intussusception from rotavirus vaccine has not been documented in the United States, data currently available cannot exclude a risk as low as that detected in other locations.

Both vaccines are well tolerated and have a low reactogenicity profile when given alone. They do not cause clinically significant increases in reactogenicity when coadministered with other routine childhood vaccines.[229,480] For both vaccines, the incidence of fever, vomiting, diarrhea, and irritability were measured in the clinical trials. For RV5, no significant difference versus placebo was observed in the incidence of fever or severe fever and irritability or severe irritability. A 3 percent increase in the incidence of diarrhea and vomiting was observed with RV5, but these symptoms were mild and did not require treatment.[81,231,548] For RV1, no difference versus placebo was observed in the incidence of diarrhea, fever or severe fever, vomiting or severe vomiting, and irritability or severe irritability within 14 days of immunization with any dose.[230,461,545,547] There was no statistically significant increased risk for death or other serious adverse events noted with either vaccine compared with placebo.

Vaccine virus shedding in stool has been documented with both rotavirus vaccines. Rotavirus shedding occurs in approximately 9 percent of RV5 recipients after the first dose but rarely after subsequent doses.[231] No cases of transmission of RV5 have been documented. Shedding and transmission are not considered significant safety concerns because of the attenuated nature of the rotavirus vaccine strains. For RV1, live rotavirus shedding in stool occurs in approximately 25 percent of recipients, with peak excretion occurring around day 7 after the first dose. Transmission of virus has not been evaluated. There have been a few cases of documented transmission to contacts. The rate of transmission is unknown, but there are no known cases of patients who have developed symptoms of rotavirus gastroenteritis.[461]

In 2010, porcine circovirus or porcine circovirus DNA was detected in both rotavirus vaccines.[271,363,404] There is no evidence that this virus is a safety risk or causes illness in humans.

Indications

Infants should receive three doses of RV5 administered orally at 2, 4, and 6 months of age or two doses of the RV1 at 2 and 4 months of age.[41,212] The first dose should be administered when the child is between 6 and 14 weeks of age (i.e., on or before 14 weeks, 0 days of age). Subsequent doses should be administered at a minimum of 4-week intervals, and all doses of vaccine should be administered by the time the child is 32 weeks of age (i.e., on or before 32 weeks, 0 days).

Immunization should not be initiated for infants older than 14 weeks because of insufficient data on safety of the first dose of rotavirus vaccine in older infants. Vaccine should not be administered after 8 months of age because of insufficient data on the safety and efficacy of rotavirus vaccine in infants after this age. For infants in whom the first dose of rotavirus vaccine is inadvertently administered off-label at 15 weeks or older, the rest of the rotavirus immunization series should be completed as per the schedule defined earlier, because timing of the first dose should not affect the safety and efficacy of the second and third doses.

The rotavirus vaccine series should be completed with the same product whenever possible. However, vaccination should not be deferred if the product used for a prior dose or doses is not available or is not known. In this situation, the provider should continue or complete the series with the product that is available. If any dose in the series was RV5 (RotaTeq) or the vaccine brand used for any prior dose in the series is not known, a total of three doses of rotavirus vaccine should be administered.

Infants documented to have had rotavirus gastroenteritis before receiving the full course of rotavirus immunizations should still start or complete the three-dose schedule because the initial infection frequently provides only partial immunity.

Infants who are being breast-fed can receive rotavirus vaccine. Like other childhood vaccines, rotavirus vaccine can be administered to infants with transient, mild illnesses, with or without low-grade fever.[358] Rotavirus vaccine can be administered together with DTaP, Hib, IPV, hepatitis B, and pneumococcal conjugate vaccines.

The AAP and ACIP support immunization of preterm infants under the following conditions: the infant is at least 6 weeks of age, the infant is clinically stable, and the first dose of vaccine is given at the time of discharge or after the infant has been discharged from the hospital nursery.

Infants living in households with persons who have or are suspected of having an immunodeficiency disorder or impaired immune status or with a pregnant woman can be immunized. To minimize potential virus transmission, all members of the household should employ measures such as good hand washing after contact with the feces of the immunized infant (e.g., after changing a diaper) for at least 1 week after the first dose of rotavirus vaccine has been given.

An infant who regurgitates, spits out, or vomits during or after receiving a dose of rotavirus vaccine should not have that dose readministered. The infant can receive the remaining recommended doses of rotavirus vaccine at appropriate intervals. If a recently immunized child is hospitalized for any reason, no precautions other than standard precautions need be taken to prevent the spread of vaccine virus in the hospital setting.

Contraindications and Precautions

Rotavirus vaccine should not be administered to infants who have severe hypersensitivity to any component of the vaccine or those individuals who have experienced a serious allergic reaction to a previous dose of rotavirus vaccine.[41,212] The RV1 oral applicator contains latex rubber. RV5 may be preferred for children at high risk for acquiring latex sensitization.

Practitioners should consider the potential risks and benefits of administering rotavirus vaccine to infants with known or suspected altered immunocompetence. Children and adults who are immunocompromised because of congenital immunodeficiency, bone marrow transplantation, or solid organ transplantation sometimes experience severe, prolonged, and even fatal rotavirus gastroenteritis. However, no safety or efficacy data are available for the administration of rotavirus vaccine to infants who are potentially immunocompromised with the exception of infants with severe combined immune deficiency (SCID). SCID is a contraindication for use of both rotavirus vaccines.[106] Gastroenteritis, including severe diarrhea and prolonged shedding of vaccine virus, have been reported in infants who were administered live, oral rotavirus vaccines and later identified as having SCID.[61]

Rotavirus vaccine should not be administered to infants with acute, moderate to severe gastroenteritis until the condition improves. Rotavirus vaccine has not been studied among infants with concurrent acute gastroenteritis, among whom its immunogenicity and efficacy theoretically can be compromised. Infants with moderate to severe illness should be immunized as soon as they have recovered from the acute phase of the illness.

Infants with preexisting chronic gastrointestinal conditions and who are not undergoing immunosuppressive therapy should benefit from rotavirus vaccine immunization, and the benefits outweigh the theoretical risks. However, the safety and efficacy of rotavirus vaccine have not been established for infants with these preexisting conditions (e.g., congenital malabsorption syndromes, Hirschsprung disease, short-gut syndrome, or persistent vomiting of unknown cause).

A history of intussusception is a contraindication for use of both rotavirus vaccines. Some data suggest that infants with a history of intussusception may be at higher risk for having a repeat episode than are other infants.

Rubella Vaccine

Rubella is a viral disease that usually is manifested as a mild febrile rash illness in adults and children; however, 20 to 50 percent of infected persons are asymptomatic. Rubella can have severe adverse effects on the fetuses of pregnant women who contract the disease during the first trimester of pregnancy; it causes a wide range of congenital defects known as congenital rubella syndrome. The primary objective of the rubella vaccination program is to prevent intrauterine rubella infection. The primary strategies for rubella control in the United States are universal childhood vaccination, prenatal screening of pregnant women for rubella immunity, and vaccination of rubella-susceptible women postpartum.

In the pre-vaccine era, epidemics of rubella occurred every 6 to 9 years, with the last major epidemic in the United States taking place in 1964 through 1965. The incidence of reported cases of rubella fell sharply after routine rubella immunization of young children was initiated in the United States in 1969. From the estimated 2 million cases per year in the pre-vaccine era, fewer than 1000 cases were reported in 1983. A moderate resurgence of rubella occurred in 1990 through 1991, primarily due to outbreaks in California (1990) and among the Amish in Pennsylvania (1991). Since the mid 1990s, most reported cases of rubella have occurred among foreign-born young adults (particularly from Latin America) who were born in countries without routine rubella immunization programs.[222,469] In 2003, a record low annual total of seven cases was reported.

In October 2004, 35 years after initiation of the rubella immunization program, an independent panel of international experts was convened by the CDC to assess progress toward elimination of rubella and congenital rubella syndrome. Based on data showing fewer than 25 cases of rubella reported each year since 2001, at least 95 percent vaccination coverage among school-aged children, an estimated 91 percent population immunity, adequate surveillance to detect rubella outbreaks, and a pattern of virus genotypes consistent with virus originating in other parts of the world, panel members concluded unanimously that rubella no longer is endemic in the United States.[117]

Rubella continues to be endemic in many parts of the world. Internationally imported rubella cases may give rise to indigenous transmission. The United States will need to continue its vigilance against rubella and congenital rubella syndrome by the following means: (1) maintaining high vaccination rates among children; (2) ensuring vaccination among women of child-bearing age, especially women born outside the United States; (3) continuing surveillance of both rubella and congenital rubella syndrome; and (4) responding rapidly to any outbreak.[117]

Preparations

Since 1979, RA 27/3 (rubella abortus, 27th specimen/third extract) vaccine, prepared in human diploid tissue culture, has been the only vaccine available in the United States; it replaced the earlier HPV-77 and Cendehill vaccines. RA 27/3 induces higher antibody titers and more closely parallels the immune response after natural infection than did previous vaccines.[371,446] Two rubella-containing vaccines, MMR and MMRV, are available. Both vaccines should be kept at 2°C to 8°C (35.6°F to 46.4°F) or colder during storage and should be protected from light to avoid inactivation of the virus. Once reconstituted, the vaccine should be used within 8 hours.

Immunogenicity and Efficacy

At least 98 percent of susceptible vaccinees aged 12 months or older develop antibody titers that are protective.[417] Vaccine-induced rubella antibodies have persisted in more than 90 percent of vaccinees aged 16 years old after receiving the RA 27/3 vaccine.[194] Lifelong protection against clinical reinfection, asymptomatic viremia, or both, usually results from a single dose of vaccine given early in childhood.

In some cases, vaccinees exposed to natural rubella developed a rise in antibody titer unassociated with clinical symptoms. Reinfection is associated only rarely with viremia. Significant pharyngeal shedding also is observed infrequently. Person-to-person transmission, however, has not been reported. Reinfection caused by wild-type rubella virus also may be observed in individuals with previous natural rubella. The risk for CRS developing from rubella reinfection during pregnancy is extremely low.[477]

Adverse Events

Rubella vaccines generally are well tolerated. The most frequent complaints after vaccination are fever, lymphadenopathy, or rash, which occur in 5 to 15 percent of children 5 to 12 days after receiving vaccination.[27] Transient peripheral neuritis (paresthesia and pain in the arms and legs) has been observed uncommonly, primarily in older age groups.[492]

Approximately 3 percent of children have transient joint manifestations, including arthralgia and, less commonly, arthritis 1 to 3 weeks after being immunized. Although 25 percent of women report having joint pain after being vaccinated, arthritis with objective clinical findings lasting less than 10 days occurs in 13 to 15 percent. Cases of persistent or recurrent joint symptoms have been reported but are rare events. In 1992, the IOM reviewed the existing data on rubella and adverse joint events and concluded that the evidence available was consistent with a causal relationship between rubella vaccination and chronic arthritis in women, although data on current vaccine strains are limited.[314] The incidence of joint manifestations after immunization is lower than that after natural infection at the corresponding age.

Rubella revaccination is well tolerated, even among college-aged and older vaccinees, and is associated with a much lower incidence of adverse reactions than is primary rubella immunization of young adult populations. Reported rates of joint-related complaints of 4 to 18 percent after revaccination are lower than those reported after primary vaccination.[190,496]

Indications

Live virus rubella vaccine generally is recommended for all children aged 12 months or older; it is given as MMR or MMRV when children are 12 to 15 months of age.[27,114,133] A second dose of rubella vaccine administered as MMR or MMRV is given at the time they enter school, usually 4 to 6 years of age, according to recommendations for routine measles immunization. The vaccine should be provided to previously unimmunized preschool-aged children or older schoolchildren despite a history of having clinical rubella, unless serologic tests confirm immunity.

Emphasis should be placed on the immunization of the postpubertal male and female population, especially college students and those in the military. Rubella vaccine also should be administered to adolescent girls and women of child-bearing age who lack a history of previous vaccination. Other opportunities for immunization include premarital screening, routine gynecologic examinations, visits for neonatal and well-child care, or other medical visits. The immediate postpartum period also is an excellent time for giving immunizations. Rubella vaccine may be given after administration of anti-Rho(D) immune globulin, but serologic testing to determine whether seroconversion has occurred should be performed at least 8 weeks after vaccination. When practical, potential vaccinees may be screened for susceptibility. However, vaccination of girls and women of child-bearing age is justifiable and may be preferable, without previous serologic testing in women not known to be pregnant.

Adults in the United States who were born in countries where rubella vaccination was not offered are at higher risk for contracting rubella and having infants with CRS. Health care practitioners who treat foreign-born adults should document the rubella immunity of these patients with a written record of rubella-containing vaccine or by serologic testing. Susceptible adults, especially women of child-bearing age, should be vaccinated. During rubella outbreaks, all susceptible persons who have no contraindications to rubella vaccine should be identified and vaccinated.

Precautions and Contraindications

Specific contraindications to administration of live rubella vaccine include the following: (1) pregnancy; (2) severe febrile illness; (3) known history of anaphylactic reaction to rubella vaccine, gelatin, or neomycin, which are contained in the vaccine; and (4) immunodeficiency conditions (i.e., malignancy, primary immunodeficiency disease, immunosuppressive or corticosteroid therapy, and radiation therapy).[27,114,133]

Persons with mild immunosuppression, such as those with asymptomatic HIV infection or those taking short-term or low-dose corticosteroids, may be vaccinated.

Postpubertal women of child-bearing age who are known to be pregnant or who are attempting to become pregnant should not be vaccinated. Vaccinated women should be counseled about the need to avoid pregnancy for 28 days after receiving vaccination.[162] Although pregnancy is a contraindication to administering rubella vaccination, the maximal theoretical risk to the fetus is estimated to be 1.6 percent. From 1979 until 1989, the CDC registered 321 susceptible women who inadvertently had received RA 27/3 rubella vaccine within 3 months before or after conception and carried their pregnancies to term. None of their infants had defects compatible with CRS, although 2 percent had serologic evidence of intrauterine infection.[101] Because rubella virus has been isolated from the products of conception of women vaccinated during pregnancy, continued caution with respect to vaccination during pregnancy is advised. However, the evidence available indicates that rubella vaccination inadvertently given during pregnancy ordinarily does not represent a reason to consider interruption of pregnancy.

Although vaccine virus may be isolated from the pharynx, vaccinees do not transmit rubella to others, except in the case of a vaccinated breast-feeding mother. In this situation, the infant may be infected through breast milk and a mild rash illness may develop, but serious adverse effects have not been noted. Infants infected through breast-feeding respond normally to rubella vaccination at 15 months of age. Breast-feeding is not a contraindication to receiving rubella vaccination.

Concern about potential transmission of disease from immunized children to susceptible contacts, including pregnant women, has not been supported by studies of susceptible household contacts. Therefore, susceptible children whose household contacts are pregnant may be vaccinated.

Persons with a history of thrombocytopenia may experience thrombocytopenia after receipt of MMR vaccine. The decision to vaccinate should depend on the benefits of immunity versus the risk for recurrence or exacerbation of thrombocytopenia, either after vaccination or during natural infection with measles or rubella.

Rubella vaccine should not be given during an interval beginning 2 weeks before and extending 3 months after the administration of immune globulin or blood transfusion. Because rubella vaccine usually is given as MMR or MMRV, and evidence suggests that high doses of immune globulin preparations can inhibit the immune response to measles vaccine for 3 or more months, depending on the dosage, rubella vaccination with MMR or MMRV necessitates deferral for longer periods (see Measles Vaccine).[27,133,506]

Tetanus Toxoid

The efficacy of active immunization against tetanus was demonstrated most dramatically in military personnel during World War II, when tetanus toxoid virtually eliminated tetanus in injured servicemen. Since the 1940s, routine immunization of civilians in this country with tetanus toxoid has been successful in nearly eliminating tetanus. In almost all cases, disease has been reported in unimmunized or inadequately immunized individuals.[104] The primary three-dose series of a tetanus toxoid-containing vaccine confers protective immunity for 10 years or longer.[86,357]

Without a tetanus booster, immunity wanes over time. The potential for occurrence of tetanus is indicated by the significant number of adults in the United States who lack protective concentrations of serum antibody.[264] Although the incidence of disease is exceptionally low (a total of 624 cases of tetanus were reported in the United States between 1990 and 2004), the case-fatality rate was 18 percent.[452,513]

Neonatal tetanus also has been nearly eliminated in the United States. However, it is a leading cause of morbidity in neonates in developing countries. As a result, global elimination of neonatal tetanus remains a goal of the WHO.[152]

Preparations

Tetanus toxoid is prepared by formaldehyde treatment of *Clostridium tetani* toxin.[86,357] It has been prepared in both fluid and aluminum salt-adsorbed preparations, but in the United States only the latter is available. Fluid toxoid preparations result in a significantly shorter duration of immunity than that induced by aluminum-adsorbed antigens; therefore, adsorbed antigens are recommended.

Tetanus toxoid is available in combination with diphtheria toxoid and acellular pertussis vaccine (DTaP) for routine administration to infants and children younger than 7 years of age and for adolescents and adults aged 11 years and older as a single booster (Tdap). For all persons in whom pertussis vaccine is contraindicated, tetanus toxoid is combined with diphtheria toxoid as either DT or Td (see Diphtheria Toxoid). The first two Tdap vaccines approved for use in adolescents and adults in the United States, Boostrix (GlaxoSmithKline Biologicals) and Adacel (Sanofi Pasteur), were licensed in 2005. Preparations of DT, Td, and Tdap are identical in the amounts of tetanus toxoid they contain, but they differ in the quantity of diphtheria toxoid. The dose of Tdap is the same as that of DT or Td, 0.5 mL administered intramuscularly.

Immunogenicity and Efficacy

Adequate primary immunization provides sufficient protective titers of antitoxin for at least 10 years and ensures prompt, anamnestic responses to subsequent booster injections. Immune response to tetanus toxoid was measured after administration of each adult Tdap vaccine and compared with that elaborated after Td vaccine. Seroprotective anti-tetanus antibody concentrations, defined as a titer 0.1 IU/mL or greater, and booster response rates to tetanus were determined to be noninferior after vaccination with Tdap as compared with Td vaccination.[86,357]

Adverse Events

Local reactions of pain, swelling, and induration can occur, but these reactions in children usually are attributable to the pertussis component contained in the vaccine rather than to response to tetanus toxoid.[29] Hypersensitivity reactions can occur in adolescents and adults but very rarely are severe. Neurologic reactions occurring after the administration of tetanus toxoid are rare events. Such reactions include brachial neuritis and Guillain-Barré syndrome.

Regarding adverse reactions reported after Tdap, vaccination with Boostrix was associated with a statistically higher rate of moderate to severe headache compared with Td and that with Adacel was associated with higher rates of mild injection site pain and low-grade fever compared with the Td vaccine. No serious adverse events have been reported for Boostrix. Two cases of serious adverse advents, both characterized as neuropathic reactions, in adults possibly related to having received Adacel (none reported in adolescents), have been reported, and in both cases symptoms resolved completely within several days.[86,357]

Indications

Preexposure. For primary immunization, doses of tetanus toxoid, provided as DTaP, should be administered when the child is 2, 4, and 6 months of age.[29,39,86] A fourth dose is given 6 to 12 months after the third dose (i.e., at 12 to 18 months of age) to maintain adequate serum antibody concentrations for the ensuing preschool years. For children younger than 7 years of age not immunized in infancy, DTaP is given at birth (initial dose), 2, 4, and 10 to 16 months later, followed by a single booster dose at age 4 to 6 years, just before school entry.

In 2005, the FDA licensed two Tdap vaccines for routine use in adolescents and adults, Adacel (Sanofi Pasteur, approved for persons 11 to 64 years of age) and Boostrix (GlaxoSmithKline Biologicals, approved for adolescents 10 years of age and older). These vaccines are approved for one-time use only in those individuals who have previously completed their primary vaccination series with DTP or DTaP, as a substitute for their next Td booster.[182] The preferred age for Tdap vaccination is 11 to 12 years. Catch-up vaccination with Tdap is encouraged for adolescents 13 to 18 years old who completed their primary series with DTP or DTaP and have not received a Td or Tdap booster. Adolescents 11 through 18 years of age who received Td but not Tdap are encouraged to receive a single dose of Tdap to provide protection against pertussis if they have completed the recommended childhood DTP/DTaP immunization series. Tdap should be administered regardless of interval since the last tetanus or diphtheria toxoid-containing vaccine. Although longer intervals between Td and Tdap could decrease the occurrence of local reactions, the benefits of protection against pertussis outweigh the potential risk for adverse events.[29,182] Every 10 years thereafter, tetanus booster should be provided by Td. Adolescents between the ages of 11 and 18 years who have never been vaccinated against tetanus, diphtheria, or pertussis should receive Tdap initially, followed by Td for the subsequent two doses 4 or more weeks and then 6 to 12 months later. For children between the ages of 7 and 10 years who have not been vaccinated previously, Tdap vaccine should substitute an initial dose of Td in the catch-up series, followed by two subsequent doses of Td vaccine at 4 or more weeks, and 6 to 12 months after the previous dose.[29,182] Interruption of the recommended schedule or delay in administering subsequent doses during primary immunization does not reduce immunity.

Antepartum. In areas of the world where the risk for acquiring neonatal tetanus is significant, previously unimmunized, pregnant women should receive two antepartum doses of a tetanus toxoid-containing vaccine, properly spaced at least 4 weeks apart, and the second dose should be given at least 2 weeks before delivery and should complete the three-dose series subsequently.[277,357] Tdap should be substituted for the first Td dose if Tdap has not been administered previously. Immunization with Tdap is recommended during pregnancy, preferably at 20 weeks' gestation or later, if Tdap has not been administered previously. Women immunized more than 10 years previously should receive a booster dose with Td or Tdap if Tdap has not been administered previously.[29,182] If vaccination is not provided before delivery or during pregnancy, women who have not received Tdap vaccine previously should receive a single dose of Tdap in the immediate postpartum period, preferably before being discharged from their hospital or birthing center.

Postexposure Wound Management. The potential need for immunoprophylaxis is an integral aspect of wound management at the time of trauma or injury. The recommended use of tetanus toxoid in addition to tetanus immune globulin at the time of injury is given in Table 244-14. To ensure adequate immunity, children and adults receiving tetanus toxoid for wound management should be given age-appropriate preparations of vaccines containing diphtheria and pertussis, unless contraindicated, as well as tetanus toxoid. Tdap is preferred over Td if the person has not received Tdap previously.[29,182] Specific recommendations depend on the individual's immunization status, the nature of the wound, and the duration of time between when the injury occurred and evaluation and treatment were undertaken. After prophylaxis is provided, primary immunization should be completed subsequently in those lacking the recommended number of doses. This conservative approach to the frequent administration of booster doses of tetanus toxoid in wound management for previously immunized persons is supported by the prolonged immunity from tetanus vaccination and the increased incidence of hypersensitivity reactions associated with receipt of frequent booster injections.[456] Patients convalescing from tetanus infection should complete active immunization because infection often does not confer immunity.

Precautions and Contraindications

A history of having an immediate, severe hypersensitivity reaction to tetanus toxoid-containing preparations that is

TABLE 244-14 **Recommended Tetanus Prophylaxis in Wound Management**

History of Tetanus Toxoid (No. of Doses)	*Clean, Minor Wounds*		*All Other Wounds**	
	Td or Tdap†	TIG	Td or Tdap†	TIG
Fewer than three or unknown	Yes	No	Yes	Yes
Three or more	No‡	No	No§	No

*Such as, but not limited to, the following: wounds contaminated with dirt, feces, soil, and saliva; puncture wounds; avulsions; and wounds resulting from missiles, crushing, burns, and frostbite.
†For children <7 years of age; diphtheria–tetanus–acellular pertussis (DTaP) or diphtheria-tetanus (DT) (depending on vaccine status of patient) is preferred to tetanus toxoid (TT) alone. Tdap is preferred over Td for underimmunized children ≥7 years of age who have not received Tdap previously.[22] Td is preferred to TT for adolescents who received Tdap previously or when Tdap is not available.
‡Yes, 10 years or more since last tetanus-containing vaccine dose.
§Yes, 5 years or more since last tetanus-containing vaccine dose. More frequent boosters are not needed and can accentuate side effects.
Td, tetanus-diphtheria; Tdap, tetanus–diphtheria–acellular pertussis; TIG, tetanus immune globulin.

severe or anaphylactic in type is a contraindication to receiving further vaccination.[29,39,86,357] Persons who experience Arthus-type hypersensitivity reactions after receiving tetanus toxoid or temperature greater than 39.4°C (103°F) after a previous dose of a tetanus toxoid–containing preparation usually have high serum tetanus antitoxin concentrations and should not be given doses of Td more frequently than every 10 years, even if they have a tetanus-prone wound.[29] If an anaphylactic reaction to a previous dose of tetanus toxoid is suspected, intradermal skin testing may be helpful in determining whether to discontinue tetanus toxoid vaccination.[329] Because tetanus toxoid administration has been associated with recurrence of Guillain-Barré syndrome in rare cases,[320] the decision to give additional doses in persons with a previous history of this syndrome within 6 weeks after receipt of tetanus toxoid should be based on consideration of the benefit of revaccination and the comparative risk for having a recurrence of Guillain-Barré syndrome.[179] No physician-diagnosed cases of anaphylaxis or Arthus reactions or Guillain-Barré syndrome in any adolescent or adult following either Tdap vaccine have been reported.

Varicella Vaccine

In the pre-vaccine era, varicella was endemic in the United States and virtually all persons acquired varicella by the time they reached adulthood. Varicella infection was responsible for an estimated 4 million cases, 11,000 hospitalizations, and 100 deaths each year in the United States.[148] Approximately 90 percent of cases occurred in children, with the highest incidence in children 1 to 6 years old.

After introduction and routine use of varicella vaccine in 1995 the incidence of varicella fell by 90 percent, mortality from varicella declined by 66 percent, and rates of hospitalization for varicella decreased by 80 percent.[289,436,593] However, a high frequency of breakthrough varicella (chickenpox occurring in a previously vaccinated person) in immunized children and continuing outbreaks of varicella in schools and in daycare centers occurred, despite high rates of vaccination.[265] In addition, studies showed that over time the vaccine's effectiveness was less than 90 percent,[497] and in one study of healthy children the rate of seroconversion after one dose of the vaccine was only 76 percent.[412] Therefore, in June 2006, the CDC and the AAP recommended a routine two-dose varicella vaccination policy for children (first dose at 12 to 15 months, second dose at 4 to 6 years) and catch-up vaccinations for children, adolescents, and adults who previously had received only one dose.[42,391]

Since recommendation of a routine second dose of vaccine in 2006, the incidence of varicella has declined further in children. Data show that administration of two doses of varicella vaccine is associated with higher antibody titers (and presumably better protection from varicella).[365] Results from a controlled study of the effectiveness of two doses of varicella vaccine indicate that administration of two doses was highly effective in preventing varicella in the first 2.5 years after implementation of the two-dose schedule to prevent disease.[500] Odds of developing varicella were 95 percent lower for children who received two doses compared with one dose of varicella vaccine.

Preparations

Three varicella-containing vaccines are now approved for use in the United States: varicella vaccine (Varivax), combination MMRV vaccine (ProQuad), and herpes zoster vaccine (Zostavax). Varicella vaccine was licensed in the United States in 1995. It is a preparation of the Oka strain of varicella-zoster virus (VZV) obtained from the vesicle fluid of a healthy child with varicella that has been attenuated by serial propagation in human embryo lung fibroblasts, guinea pig embryonic cells, and human diploid cell cultures. The vaccine contains trace amounts of neomycin, fetal bovine serum, sucrose, residual components of human diploid (MRC-5) cells, and gelatin. The vaccine does not contain preservatives.

Varicella vaccine is lyophilized and stored frozen at –15°C or colder until reconstituted. Any freezer that reliably maintains an average temperature of –15°C and has a separate sealed freezer door is acceptable for storing vaccine. The vaccine also may be stored at refrigerator temperature (2°C to 8°C) for as long as 72 hours before reconstitution. Vaccine stored at 2°C to 8°C that is not used within 72 hours should be discarded. Reconstituted vaccine should be stored at room temperature and discarded if it is not used within 30 minutes.

In September 2005, a combined live attenuated MMRV vaccine was licensed for use in persons aged 12 months through 12 years.[137] The attenuated measles, mumps, and rubella vaccine viruses in MMRV are identical and of equal titer to those in the MMR vaccine. The

titer of Oka/Merck VZV is higher in MMRV vaccine than in single-antigen varicella vaccine. Each 0.5-mL dose contains a small quantity of hydrolyzed gelatin, human albumin, residual components of MRC-5 cells, neomycin, bovine calf serum, and other buffer and media ingredients. Unlike single-antigen varicella vaccine, MMRV vaccine cannot be stored at refrigerator temperature. MMRV vaccine must be stored frozen at an average temperature of −15°C or lower for up to 18 months. Once reconstituted, the vaccine should be used immediately to minimize loss of potency and should be discarded if it is not used within 30 minutes. The diluent should be stored separately at room temperature or in the refrigerator. MMRV vaccine contains no preservative. In May 2006, the FDA approved herpes zoster vaccine for use in persons 60 years of age and older. No indications for use of this vaccine in children exist.

Immunogenicity and Efficacy

Varicella vaccine is highly immunogenic in susceptible children. Seroconversion has occurred in more than 96 percent of children aged 12 months to 12 years after one dose of vaccine.[571] Preexisting antibody, if present at 12 months of age, does not appear to interfere with antibody response. As with other viral vaccines, the antibody response after immunization is lower than that from natural disease. Adolescents and adults have age-related decreases in the ability to develop a primary response to varicella virus.[267] Seroconversion rates of 78 to 82 percent after one dose and 99 percent after two doses have been reported in those older than 12 years.[267,571]

In studies in the United States and Japan, serum antibodies to varicella have been detected for as long as 10 to 20 years after immunization in more than 95 percent of immunized children.[58,333] Antibody concentrations have persisted for at least 1 year in 97 percent of adults and adolescents who were administered two doses of vaccine 4 to 8 weeks apart.[267] Cell-mediated immunity to VZV has been detected in 87 percent of children and 94 percent of adults 5 years after vaccination.[592]

MMRV vaccine was licensed on the basis of equivalence of immunogenicity of the antigenic components, rather than clinical efficacy. Clinical studies involving healthy children aged 12 to 23 months of age indicated that those who received a single dose of MMRV vaccine developed levels of antibody to measles, mumps, rubella, and varicella similar to those of children who received MMR and varicella vaccines concomitantly at separate injection sites.[364]

Varicella vaccine has been demonstrated to be highly effective in preventing varicella in children and in reducing the severity of infection if they do become infected. In pre-licensure clinical trials, vaccine was 70 to 90 percent effective in preventing varicella and more than 95 percent effective in preventing severe disease.[366,564] Several post-licensure studies have shown similar results, with vaccine effectiveness ranging from 83 to 100 percent in preventing varicella and 87 to 100 percent in preventing severe disease.[198,328,541] In follow-up studies, chickenpox has developed in 0.2 to 2.3 percent of vaccinated children per year after exposure to wild-type varicella virus, a rate that does not seem to increase with length of time after immunization.[549] These vaccine failure cases are mild, with fewer skin lesions, lower rates of fever, and faster recovery, and they are less contagious than are moderate to severe cases of varicella.[74,498,549,559] These infections have been termed *breakthrough varicella*.

Although findings of some studies have suggested otherwise, most investigations have not identified time since vaccination as a risk factor for breakthrough varicella. Some, but not all, investigations have identified the presence of asthma, use of corticosteroids, and vaccination at younger than 15 months of age as risk factors for breakthrough varicella.[242,263,328,535,544] Breakthrough varicella infection could be a result of several factors, including interference of vaccine virus replication by circulating antibody, impotent vaccine resulting from storage or handling errors, or inaccurate record keeping. Interference from live viral vaccine administered before varicella vaccine could also reduce vaccine effectiveness. A study of 115,000 children in two health maintenance organizations during 1995 to 1999 found that children who received varicella vaccine less than 30 days after MMR vaccination had a 2.5-fold increased risk for breakthrough varicella compared with those who received varicella vaccine before, simultaneously with, or more than 30 days after receiving MMR.[544] Inactivated vaccines (DTaP, Hib, IPV, and hepatitis B) and OPV did not increase the risk for breakthrough varicella if they were administered less than 30 days before administration of varicella vaccine.

In adults and adolescents who have seroconverted, varicella vaccine provides protective efficacy rates of approximately 70 percent after household exposure. In the remaining 30 percent, attenuated disease with fewer skin lesions and little or no systemic toxicity develops, as in children.[267]

Adverse Events

Varicella vaccine produces relatively few adverse reactions.[501,574] The most common adverse reactions that occur after receipt of varicella vaccine are local reactions, such as pain, soreness, erythema, and swelling. Based on information from the manufacturer's clinical trials of varicella vaccine, local reactions after receiving the first dose are reported by 19 percent of children and by 24 percent of adolescents and adults.[501,574] These local adverse reactions generally are mild and self-limited.

A varicella-like rash at the site of injection is reported by 3 percent of children and by 1 percent of adolescents and adults after receipt of the second dose. In both circumstances, a median of two lesions has been present. These lesions generally occur within 2 weeks and usually are maculopapular rather than vesicular.[501,574]

In post-licensure studies, the adverse event most frequently reported is a mild vesicular rash that occurs in approximately 5 percent of vaccines.[148,266] Most of these generalized rashes occur within 3 weeks, and most are maculopapular. In one study, vesicular rashes that occurred within 2 weeks of vaccination were more likely to be caused by wild-type varicella whereas rashes that

occurred more than 2 weeks after vaccination were more likely to be caused by the Oka vaccine strain.[501]

Systemic reactions are not common. Fever within 42 days of vaccination is reported by 15 percent of children and 10 percent of adolescents and adults. Most of these episodes of fever have been attributed to concurrent illness rather than to the vaccine.

Clinical trials of MMRV that compared events that occurred within 42 days of receiving either MMRV or MMR and varicella vaccine separately in different anatomic sites found the frequencies of local reactions and generalized varicella-like rash similar to those described for varicella vaccine.[364] A temperature of 38.9° C (102° F) or higher within 42 days of vaccination was more common in the MMRV group (22%) than in the group that received MMR and varicella vaccine at different sites (15%). A measles-like rash also occurred more frequently in MMRV recipients (3%) than in the group receiving separate injections (2%). Both fever and measles-like rash usually occurred 5 to 12 days after receipt of vaccination. In a post-licensure study, an increased risk for seizure was seen in children 12 to 23 months of age receiving MMRV vaccine when compared with those who received MMR and varicella vaccines given separately in the 7- to 10-day post-vaccination period.[349]

Varicella vaccine is a live virus vaccine and may result in a latent infection, similar to that caused by wild-type varicella virus. Consequently, zoster caused by the vaccine virus has been reported, mostly among vaccinated children. Based on reports to VAERS, the rate of herpes zoster after varicella vaccination is 2.6 per 100,000 vaccine doses distributed.[148] The incidence of herpes zoster after natural varicella infection in healthy persons younger than 20 years is 68 per 100,000 person-years,[287] and, for all ages, it is 215 per 100,000 person-years.[236] However, these rates should be compared cautiously because the latter rates are based on populations monitored for longer periods than the vaccinees were monitored. Cases of herpes zoster have been confirmed by polymerase chain reaction (PCR) to be caused by both vaccine virus and wild-type virus, a finding thus suggesting that some herpes zoster cases in vaccinees may result from antecedent natural varicella infection.[148,297] The majority of cases of herpes zoster that occur after administration of vaccine have been mild and have not been associated with complications such as postherpetic neuralgia.

Transmission of the vaccine virus occurs rarely and most often from immunocompromised vaccinees. Of the 15 million doses of varicella vaccine distributed, on only three occasions has transmission from immunocompetent persons been documented by PCR analysis.[368,490] All three cases resulted in mild disease without complications. In one case, a child 12 months old transmitted the vaccine virus to his pregnant mother.[379,490] The mother elected to terminate the pregnancy, but fetal tissue tested by PCR was negative for varicella vaccine virus. The other two documented cases involved transmission from healthy children aged 1 year to a healthy sibling aged 4½ months and to a healthy father.[148] Transmission also has occurred from a person with herpes zoster caused by vaccine strain virus.[88] Transmission has not been documented in the absence of a vesicular rash after vaccination. No evidence indicates reversion to virulence of the vaccine strain during transmission; siblings of leukemic vaccine recipients who acquired vaccine virus had mild rash in 75 percent of cases and symptomless seroconversion in 25 percent.[54]

Indications

Both monovalent varicella vaccine and MMRV have been licensed for use for healthy children 12 months through 12 years of age.* Children in this age group should receive two 0.5-mL doses of varicella vaccine administered subcutaneously, separated by at least 3 months. The recommendation for at least a 3-month interval between doses is based on the design of the studies evaluating two doses in this age group; if the second dose inadvertently is administered between 28 days and 3 months after the first dose, the second dose does not need to be repeated.

All healthy children routinely should receive the first dose of varicella-containing vaccine at 12 through 15 months of age. The second dose of vaccine is recommended routinely when children are 4 through 6 years of age (i.e., before a child enters kindergarten or first grade) but can be administered at an earlier age. Because of the minimal potential for increased febrile seizures after the first dose of MMRV vaccine in children 12 through 15 months of age, the American Academy of Pediatrics and the ACIP recommend a choice of either MMR plus monovalent varicella vaccine or MMRV for toddlers receiving their first immunization of this kind.[36,390] Parents should be counseled about the rare possibility of their child developing a febrile seizure 1 to 2 weeks after immunization with MMRV for the first immunizing dose. For the second dose at 4 through 6 years of age, MMRV generally is preferred over MMR plus monovalent varicella to minimize the number of injections. Varicella vaccine should be administered to all children in this age range unless there is evidence of immunity to varicella or a contraindication to administration of the vaccine. A catch-up second dose of varicella vaccine should be offered to all children 7 years of age and older who have received only one dose. A routine health maintenance visit at 11 through 12 years of age is recommended for all adolescents to evaluate immunization status and administer necessary vaccines, including the varicella vaccine.

Individuals 13 years of age or older without evidence of immunity should receive two 0.5-mL doses of varicella vaccine, separated by at least 28 days. The recommendation for at least a 28-day interval between doses is based on the design of the studies evaluating two doses in this age group. For persons who previously received only one dose of varicella vaccine, a second dose is necessary. Only monovalent varicella vaccine is licensed for use in this age group.

Women should be assessed prenatally for evidence of varicella immunity. On completion or termination of their pregnancies, women who do not have evidence of varicella immunity should receive the first dose of

*References 31, 36, 42, 176, 390, 391.

varicella vaccine before discharge from the health care facility. The second dose should be administered 4 to 8 weeks later (at the postpartum or other health care visit). To ensure administration of varicella vaccine, standing orders are recommended for health care settings where completion or termination of pregnancy occurs.

Postexposure Prophylaxis. Data from the United States and Japan in a variety of settings indicate that varicella vaccine is 70 to 100 percent effective in preventing illness or modifying the severity of illness if it is used within 3 days, and possibly up to 5 days, after exposure.[489,558] The ACIP recommends administration of varicella vaccine to persons without evidence of immunity 12 months of age or older, including adults, as soon as possible within 72 hours and possibly up to 120 hours after varicella exposure.[391] Varicella vaccine may prevent or modify disease and should be considered in these circumstances if there are no contraindications to its use. A second dose should be given at the age-appropriate interval after the first dose. Physicians should advise parents and their children that the vaccine may not protect against disease in all cases, because some children may have been exposed at the same time as the index case. However, if exposure to varicella does not cause infection, postexposure immunization with varicella vaccine will result in protection against subsequent exposure. There is no evidence that administration of varicella vaccine during the presymptomatic or prodromal stage of illness increases the risk for vaccine-associated adverse events or more severe natural disease.

In 2006, the ACIP approved a revised definition for evidence of immunity to varicella.[391] Revised criteria for evidence of immunity to varicella includes any of the following:

- Documentation of age-appropriate vaccination
- Preschool-aged children 12 months of age and older: one dose
- School-age children, adolescents, and adults: two doses
- Laboratory evidence of immunity or laboratory confirmation of disease
- Birth in the United States before 1980
- Diagnosis of varicella or verification of history of varicella disease by a health care provider
- History of herpes zoster based on health care provider diagnosis.

Precautions and Contraindications

Varicella-containing vaccines are contraindicated in the following situations: (1) pregnancy, (2) severe febrile illness, (3) known history of anaphylactic reaction to vaccine components, and (4) immunodeficiency states (malignancy, primary immunodeficiency disease, immunosuppressive or corticosteroid therapy, and radiation therapy).*

Varicella vaccine should not be administered to persons who have had an anaphylactic-type reaction to any component of the vaccine, including gelatin and neomycin. Most persons with allergy to neomycin have resulting contact dermatitis, a reaction that is not a contraindication to immunization. Monovalent varicella vaccine does not contain preservatives or egg protein, and although the measles and mumps vaccines included in MMRV vaccine are produced in chick embryo culture, the amounts of egg cross-reacting proteins are not significant. Therefore, children with egg allergy routinely may be given MMRV without previous skin testing.

Varicella vaccine should not be administered routinely to children who have congenital or acquired T-lymphocyte immunodeficiency, including persons with leukemia, lymphoma, and other malignant neoplasms affecting the bone marrow or lymphatic systems, as well as children receiving long-term immunosuppressive therapy. An exception includes certain children infected with HIV, as discussed later. Children with impaired humoral immunity may be immunized.

Immunodeficiency should be excluded before immunization in children with a family history of hereditary immunodeficiency. The presence of an immunodeficient or HIV-seropositive household family member does not contraindicate vaccine use in other family members.

In persons with possible altered immunity, immunization against chickenpox should utilize only monovalent varicella vaccine. The Oka vaccine strain remains susceptible to acyclovir; and if a high-risk patient develops vaccine-related varicella, then acyclovir should be used as treatment.

Before routine immunization of healthy children against varicella was instituted in the United States in 1995, many young children with leukemia were susceptible to chickenpox. Considering the variability in intensity of chemotherapy regimens and the current decreasing incidence of varicella in the United States, these high-risk children should not be immunized routinely. Immunization of leukemic children, if susceptible, without evidence of immunity in remission should be undertaken only with expert guidance and with availability of antiviral therapy, should complications occur.

Live-virus vaccines usually are withheld for an interval of at least 3 months after immunosuppressive cancer chemotherapy has been discontinued. However, the interval until immune reconstruction varies with the intensity and type of immunosuppressive therapy, radiation therapy, underlying disease, and other factors. Therefore, it often is not possible to make a definitive recommendation for an interval after cessation of immunosuppressive therapy when live-virus vaccines can be administered safely and effectively.

Screening for HIV infection is not indicated before routine VZV immunization. Varicella vaccine should be considered for nonimmune HIV-infected children with a $CD4^+$ T-lymphocyte percentage of 15 percent or greater, especially if they are receiving antiretroviral therapy.[419] Eligible children should receive two doses of monovalent varicella vaccine with a 3-month interval between doses and return for evaluation if they experience a postimmunization varicella-like rash. Hundreds of such HIV-infected children have now been safely immunized in the United States, and the vaccine is tolerated much as it is

*References 31, 36, 42, 176, 390, 391.

TABLE 244-15 **Combination Vaccines Licensed in the United States***

Vaccine†	Trade Name (year licensed)	Age Range	Routinely Recommended Ages
DTaP-IPV/Hib	Pentacel (2008)	6 wk-4 yr	Four-dose schedule at 2, 4, 6, and 15-18 mo
DTaP-IPV	Kinrix (2008)	4-6 yr	Fifth dose of DTaP and fourth dose of IPV
MMRV	ProQuad (2005)	12 mo-12 yr	Two doses, the first at 12-15 mo, the second at 4-6 yr
DTaP-HepB-IPV	Pediarix (2002)	6 wk-6 yr	Three-dose series at 2, 4 and 6 mo
HepA-HepB	Twinrix (2001)	≥18 yr	Three doses on a schedule of 0, 1, and 6 mo
Hib-HepB	Comvax (1996)	6 wk-71 mo	Three-dose schedule at 2, 4, and 12-15 mo

*Excludes measles-mumps-rubella (MMR), DTaP, Tdap, Td, and IPV vaccines, for which individual components are not available. DTaP/Hib (TriHIBit) no longer is manufactured.
†A hyphen (-) between vaccine products indicates that products are supplied in their final form by the manufacturer and do not require mixing or reconstitution by the user. A slash (/) indicates that the products must be mixed or reconstituted by the user.
DTaP, diphtheria–tetanus–acellular pertussis; HepA, hepatitis A; HepB, hepatitis B; Hib, *Haemophilus influenzae* type b; IPV, inactivated poliovirus; MMRV, measles-mumps-rubella-varicella.
Modified from American Academy of Pediatrics. Combination vaccines. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases.* 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012.

in healthy immunized children. Varicella vaccine has been shown to protect these children not only against varicella but also against developing herpes zoster by preventing infection with wild-type VZV.

Varicella vaccine should not be administered to persons who are receiving high doses of systemic corticosteroids (2 mg/kg/day or more of prednisone or its equivalent or 20 mg/day of prednisone or its equivalent) for 14 days or more. The recommended interval between discontinuation of corticosteroid therapy and immunization with varicella vaccine is at least 1 month. Varicella vaccine may be administered to persons receiving inhaled, nasal, and topical corticosteroids. The results of one small study indicate that two doses of the varicella vaccine in 29 children with nephrotic syndrome between 12 months and 18 years of age generally were well tolerated and immunogenic, including children receiving low-dose, alternate-day prednisone.

Household contacts of immunocompromised persons should be immunized if they have no evidence of immunity to decrease the likelihood that wild-type VZV will be introduced in the household. Transmission of vaccine-strain VZV from healthy persons has been documented in seven instances, resulting in eight secondary cases. Even in families with immunocompromised persons, including persons with HIV infection, no precautions are needed after immunization of healthy children in whom a rash does not develop. Immunized persons in whom a rash develops should avoid direct contact with immunocompromised hosts without evidence of immunity for the duration of the rash.

Receipt of antibody-containing blood products (whole blood, plasma, or parenteral immune globulin) may interfere with seroconversion to varicella vaccine. The length of time that such passively acquired antibody persists depends on the concentration and quantity of the blood product received[359] (see Table 244-12).

Although no direct evidence demonstrates that varicella vaccine is harmful to a pregnant female or her fetus, the vaccine should not be administered to women known to be pregnant or considering becoming pregnant within the month because of the theoretical risk for fetal infection associated with a live virus vaccine. Vaccinated women should avoid conception for 1 month after receiving vaccination. The manufacturer has established a Varicella Vaccination in Pregnancy Registry to monitor the maternal-fetal outcomes of pregnant women inadvertently given varicella vaccine.*

A study of nursing mothers and their infants showed no evidence of excretion of vaccine strain in human milk or of transmission to infants who are breast-feeding. Varicella vaccine should be administered to nursing mothers who lack evidence of immunity.

Reye syndrome has occurred in children infected with varicella who receive salicylates. Whether varicella vaccine may induce Reye syndrome is not known, but the vaccine manufacturer recommends that salicylates not be given within at least 6 weeks after administration of varicella vaccine.

DISEASES FOR WHICH COMBINATION VACCINES ARE AVAILABLE

A number of new and improved vaccines have been introduced over the past 20 years. Incorporation of these vaccines into already complex childhood immunization schedules has posed a challenge. In the 2012 Recommended Immunization Schedule in the United States, a minimum of 22 separate injections are needed to immunize a child from birth to 2 years of age.[46,159] At some visits, the administration of three to five separate injections can be indicated.

Combination vaccines represent one solution to the problem of increased numbers of injections. These vaccines incorporate, into a single product, antigens that prevent several diseases. Combinations licensed in recent years in the United States are shown in Table 244-15.

Licensed combination vaccines can be used whenever any components of the combination are indicated and its

*Varicella Vaccination Pregnancy Registry: reporting may be done online at www.merckpregnancyregistries.com/varivax.html or by telephone at 800-986-8999.

other components are not contraindicated and if licensed by the FDA for that dose in the series.[358] Use of combination vaccines can reduce the number of injections patients receive and alleviate parental concern associated with the number of injections. Potential advantages of combination vaccines include (1) improved vaccine coverage rates, (2) timely vaccination coverage for children who need catch-up vaccination, (3) reduced shipping and stocking costs, (4) reduced costs for extra health care visits necessitated by deferral of vaccination, and (5) facilitation of adding new vaccines into vaccination programs.

Potential disadvantages of combination vaccines include (1) adverse events that might occur more frequently after administration of a combination vaccine compared with administration of separate antigens at the same visit, such as those that occur with the MMRV vaccine and combination DTaP-hepatitis B-IPV vaccine[390,528]; (2) confusion and uncertainty about selection of vaccine combinations and schedules for subsequent doses, especially when vaccinations are given by multiple providers who might be using different products; (3) reduced immunogenicity of one or more components[232]; (4) extra doses of certain antigens in the fixed product (e.g., a provider who uses DTaP-hepatitis B-IPV vaccine will give an extra dose of hepatitis B component); and (5) a shorter shelf-life than the individual component vaccines. The economic impact of the use of combination vaccines is unclear because combination products have the potential for either increased or decreased costs compared with single-antigen component vaccines.

The minimum age for administration of a combination vaccine is the oldest minimum age for any of the individual components; the minimum interval between doses is equal to the greatest minimum interval of any of the individual components. When patients have received the recommended immunizations for some of the components in a combination vaccine, administering the extra antigen(s) in the combination vaccine is permissible if they are not contraindicated and doing so will reduce the number of injections required. However, excessive doses of toxoid vaccines (diphtheria and tetanus) may result in extensive local reactions.

Only combination vaccines licensed by the FDA should be used. Separate vaccines should not be combined into the same syringe for administration together unless such mixing is indicated for the patient's age and is explicitly specified on the FDA-approved product label inserts. Only one combination vaccine (DTaP-IPV/Hib vaccine, marketed as Pentacel) contains separate antigen components for which FDA approves mixing by the user. The safety, immunogenicity, and efficacy of unlicensed combinations are unknown.

In general, licensure of a combination vaccine by the FDA is based on studies demonstrating that the product's immunogenicity (or efficacy) and safety are comparable or equivalent to monovalent or combination products licensed previously. FDA licensure also generally indicates that a combination vaccine may be used interchangeably with monovalent formulations and other combination products with similar component antigens produced by the same manufacturer to continue the vaccination series. For example, DTaP, DTaP/Hib, and future DTaP vaccines that contain similar acellular pertussis antigens from the same manufacturer may be used interchangeably if licensed and indicated for the patient's age.[129] Licensure of a vaccine by the FDA does not necessarily indicate that the vaccine is interchangeable with products from other manufacturers. Such data are more readily available for diseases with known serologic correlates of protective immunity. For diseases without such surrogate laboratory markers, pre-licensure field vaccine efficacy (phase III) trials or post-licensure surveillance generally are required to determine protection.[413] The ACIP prefers that doses of vaccine in a series come from the same manufacturer; however, if this is not possible or if the manufacturer of doses of vaccine given previously is unknown, providers should administer the vaccine that they have available.

VACCINES WITH SELECTIVE INDICATIONS FOR CHILDREN AND ADOLESCENTS

Bacille Calmette-Guérin Vaccine

Effective control of tuberculosis in the United States has been achieved by the early identification and treatment of cases, followed by surveillance of household and other close contacts and institution of appropriate preventive measures for those at high risk for development of disease. In the United States, the mainstay of preventive therapy is isoniazid chemoprophylaxis, which is used in asymptomatically infected persons to prevent the progression of infection to disease. In selected instances, however, the potential for acquiring disease, poor compliance in contacts instructed to take chemoprophylaxis, or failure of chemoprophylaxis may justify the use of immunoprophylaxis.[163] Elsewhere in the world, bacille Calmette-Guérin (BCG) vaccine is used in more than 100 countries and is recommended routinely at birth by the WHO (see Table 244-5).[577] Of primary concern to pediatricians is the risk to an infant born to a tuberculous mother or living within a household with other identified tuberculous individuals.[30]

Preparations

BCG is the only approved vaccine against tuberculosis. BCG is a live attenuated strain derived from *Mycobacterium bovis*. All currently available BCG vaccines are derived from the original strain at the Pasteur Institute in Paris, but they have been propagated by different methods in many laboratories and therefore vary in their immunogenic and reactogenic properties. BCG (Organon Teknika Corporation) is the only vaccine licensed in the United States. Comparative evaluations of this preparation and other BCG vaccines have not been performed. BCG is administered either percutaneously or intradermally. At least 10 new potential vaccines to prevent tuberculosis are in clinical development. Nevertheless, BCG will remain in use in the foreseeable future and may continue to be used as a prime vaccine in a prime-boost immunization schedule in conjunction with

new TB vaccines.[340] BCG preparations instilled in treatment of bladder cancer are not intended to be used as vaccines.[163]

Immunogenicity and Efficacy

BCG is used primarily in young infants in an attempt to prevent disseminated and other life-threatening manifestations of *Mycobacterium tuberculosis* disease. However, BCG does not prevent infection with *M. tuberculosis*. The efficacy of different BCG vaccines seems to be highly variable. Two meta-analyses of published clinical trials and case-control studies concerning the efficacy of BCG vaccines concluded that BCG has relatively high protective efficacy (approximately 80 percent) against meningeal and miliary tuberculosis in children.[206,479] The protective efficacy against pulmonary tuberculosis, however, differed significantly among the studies, thus precluding a specific conclusion. Protection afforded by BCG in one meta-analysis was estimated to be 50 percent.[206]

Adverse Events

Four billion doses of BCG have been administered with a proven safety record.[340] BCG vaccination usually results in scarring at the site of injection. However, the vaccine has been associated uncommonly (1 to 2 percent of vaccinations) with local adverse reactions such as subcutaneous abscess and lymphadenopathy, which are not generally serious. Osteitis affecting the epiphyses of long bones is a rare complication that develops in one per million vaccinees and may occur as long as several years after receiving BCG immunization. The rate may be higher in neonates. Disseminated fatal disease occurs rarely (0.1 to 1 case per million vaccinees), primarily in persons with severely impaired immune systems.[163,360,380,517] Antituberculosis therapy, except for pyrazinamide, is recommended to treat osteitis and disseminated disease caused by BCG. Some experts also recommend treatment of chronic suppurative lymphadenitis caused by BCG. Persons with complications caused by BCG should be referred, if possible, to a tuberculosis expert for management.

Indications

In the United States, administration of BCG should be considered only in limited and select circumstances, such as unavoidable risk for exposure to *M. tuberculosis* and failure or unfeasibility of other methods of control of tuberculosis. The ACIP and the AAP have published recommendations for the use of BCG to control tuberculosis in children.[30,163]

Healthy infants from birth to 2 months of age may be given BCG without tuberculin skin testing; thereafter, BCG is given only to children with a negative tuberculin skin test. In infants and children, BCG immunization should be considered for those who are not infected with HIV in the following circumstances:

- The child is exposed continually to a person or persons with contagious pulmonary tuberculosis resistant to isoniazid and rifampin and the child cannot be removed from this exposure.
- The child is exposed continually to a person or persons with untreated or ineffectively treated contagious pulmonary tuberculosis, and the child cannot be removed from such exposure or given antituberculosis therapy.

Careful assessment of the potential risks and benefits of BCG vaccine and consultation with personnel in local area control programs for tuberculosis are strongly recommended before the use of BCG. When BCG vaccine is given, care should be taken to observe the precautions and directions for administration on the product label.

Skin Test Reactivity

Recipients of BCG should have repeat tuberculin skin tests 2 to 3 months after immunization to establish that tuberculin cellular reactivity has developed. Failure to react dictates the need for repeat BCG vaccination followed by repeat tuberculin testing.[163] The tuberculin reaction to the BCG vaccine available in the United States generally results in 7 to 15 mm of induration after vaccination and diminishes gradually during subsequent years; without revaccination or repeated exposure to *M. tuberculosis*, reactivity usually disappears within 10 years.[163] The size of the area of induration may be correlated with the number of doses of BCG.[317] However, tuberculin skin test sensitivity does not correlate with BCG efficacy.[302] In BCG recipients, differentiating between a tuberculin reaction representing acquired tuberculous infection and persisting postvaccination reactivity is difficult. Because the degree and duration of protection against tuberculous disease afforded by BCG are uncertain, a positive tuberculin reaction always must be suspected to be indicative of disease.

Precautions and Contraindications

BCG vaccine should not be administered to individuals with burns, skin infections, and certain primary or secondary immunodeficiencies, including HIV infection, because of the risk for disseminated BCG disease. The use of BCG also is contraindicated in persons receiving immunosuppressive medications, including high-dose corticosteroids.

In the United States, where the risk for acquiring tuberculosis is low, BCG vaccine should not be administered to children with known or suspected asymptomatic HIV infection.[163,441] However, in populations where the risk for contracting tuberculosis is high, the WHO has recommended that asymptomatic HIV-infected children receive BCG vaccine at birth or shortly thereafter.[577,581] Although no harmful effects of BCG vaccine on the fetus have been documented, women should avoid receiving vaccination during pregnancy.

Cholera Vaccine

In 2009, 45 countries reported 221,226 cholera cases with 4,946 deaths to the WHO, with 98 percent of cases and 99 percent of deaths reported from Africa.[223] In, 2010, an outbreak of cholera occurred in Haiti with over half a million cases and over 7,000 confirmed deaths, the first

epidemic in the Western Hemisphere since the 1991 epidemic in Peru.[4] In contrast, fewer than 300 cases of cholera have been recognized in the United States since the late 1980s.[111] Most cases have occurred in travelers to cholera-affected areas or persons who have eaten contaminated food brought or imported from these areas.[111] Although cholera remains a significant public health concern in African, Asian, and South American countries, even in these countries the risk to U.S. travelers is low. Persons following the usual tourist itinerary that use standard accommodations in countries reporting cholera are at virtually no risk for acquiring infection.[223]

Preparations

As of August 2000, the only cholera vaccine approved for use in the United States was no longer being manufactured or sold. [110] There are two major types of oral vaccines against cholera that have been licensed for commercial use in other countries: killed whole cell-based and genetically attenuated live vaccines. One killed whole-cell vaccine consists of *Vibrio cholerae* O1 with purified recombinant B-subunit of cholera toxoid (WC/rBS; Dukoral; SBL Vaccin AB, Stockholm). Another whole killed cell-based vaccine lacking the B-subunit (WC-only; ORC-Vax; Hue, Vietnam) was manufactured by the government of Vietnam; and although inexpensive to produce, it did not elicit antitoxic immunity. As such, in 2007 it was modified with international efforts to create a low-cost vaccine (Modified WC-only; mORC-Vax; VaBiotech, Vietnam) and to enable its purchase by the United Nations it is also produced in India (Sanchol; Shantha Biotechnics).[503] The other type of vaccine, an attenuated live oral genetically modified *V. cholerae* O1 strain (CVD 103-HgR; Orochol; Berna Biotech, Bern; known as Mutachol in Canada), is not currently being produced.[309]

Immunogenicity and Efficacy

WC/rBS vaccine confers protection specific to *V. cholerae* serogroup O1.[309] Immunization does not protect against *V. cholerae* serogroup O139 or other species of *Vibrio*. Field trials in Bangladesh, Peru, and Sweden have shown that the WC/rBS vaccine confers 85 to 90 percent protection for 6 months in all age groups after administration of two doses, 1 week apart. In Bangladesh, protection declined rapidly after 6 months in young children, but it was still approximately 60 percent in older children and adults after 2 years. The modified killed whole cell–only vaccine had been studied in several trials and found to be both safe and highly effective with seroconversion rates of vibriocidal antibodies ranging from 53 to 91 percent.[53,488,503,520]

Adverse Events

More than 5 million doses of WC/rBS vaccine have been supplied worldwide.[309] According to manufacturer information from clinical trials and postmarketing surveillance, mild gastrointestinal symptoms (abdominal pain, cramping, diarrhea, nausea) are most commonly reported, occurring at a frequency of 0.1 to 1 percent. Serious adverse events, including a flulike syndrome, rash, arthralgia, and paresthesias, are rare, occurring in less than 1 in 10,000 doses distributed. In several randomized controlled trials Sanchol also demonstrate low frequency of mild symptoms and no major adverse events.[53,488,520]

Indications

Routine immunization is not recommended for travelers.[223] Oral cholera vaccines may be considered for those at higher than average risk, such as health care professionals in endemic areas, aid workers in refugee camps, and those traveling to remote areas where cholera epidemics are occurring and access to medical care is limited. At present, no country requires proof of cholera immunization as a condition for entry, and the WHO recommends against such a requirement. Some local authorities, however, may require immunization (to determine local requirements, travelers may consult the embassies of the countries to which they will be traveling).

Limited data on the safety and immunogenicity of the modified whole-cell–based vaccine in children down to 1 year of age appear promising.[488] However, the WC/rBS vaccine is not recommended to be used in children younger than 2 years of age.

Precautions and Contraindications

Oral cholera vaccine should not be administered to persons with a hypersensitivity reaction to any of the vaccine components. Administration of oral cholera vaccine should be postponed for subjects suffering from acute gastrointestinal illness or acute febrile illness.

Japanese Encephalitis Virus Vaccine

Japanese encephalitis (JE) virus, the most important cause of epidemic arboviral encephalitis in Asia, has a wide clinical spectrum, ranging from asymptomatic infection to permanent neurologic sequelae, and a high case-fatality rate of 25 to 70 percent.[423,536] An inactivated mouse brain–derived vaccine has controlled JE virus infection successfully among human populations in Japan, Korea, and Taiwan since 1968.[447] Two other JE virus vaccines have been used widely, primarily in China, an inactivated primary hamster kidney cell–derived vaccine and a live attenuated vaccine.[389]

Preparations

Two JE vaccines are licensed in the United States; an inactivated mouse brain–derived vaccine (JE-VAX [JE-MB]) and an inactivated Vero cell culture-derived vaccine (IXIARO [JE-VC]). JE-MB was licensed in the United States in 1992 for use in travelers aged 1 year and older. In 2006, production of JE-MB was discontinued and all remaining doses expired in 2011.

A new inactivated cell culture–derived vaccine, JE-VC (IXIARO, Intercell Biomedical, UK; distributed by Novartis Vaccines and Diagnostics, Inc., US) was licensed in the United States in 2009 for persons 17 years of age

and older. JE-VC is an inactivated vaccine derived from the attenuated SA14-14-2 JEV strain propagated in Vero cells. Each 0.5-mL dose contains approximately 6 μg of purified, inactivated JEV proteins and 0.1 percent aluminum hydroxide as an adjuvant. The finished product does not include gelatin stabilizers, antibiotics, or thimerosal. The vaccine should be stored at 2°C to 8°C (35°F to 46°F) and should be protected from light.

The primary vaccination series for JE-VC is two doses administered 28 days apart. Each 0.5-mL dose is given by the intramuscular route; this route is different from that of JE-MB, which is administered subcutaneously. JE-VC is supplied in 0.5-mL single-dose syringes. The dose is the same for all persons aged 17 years and older. The vaccine is not licensed for use in persons aged younger than 17 years.

There currently is no JE vaccine that is licensed and available for use in children in the United States. Other JE vaccines are manufactured and routinely used for children in Asia but are not licensed in the United States. Information regarding options for obtaining JE vaccine for U.S. children is available.[168] A partial list of international travelers' health clinics in Asia that administer JE vaccines to children is available online.*

Immunogenicity and Efficacy

No efficacy data exist for JE-VC. The vaccine was licensed on the basis of its ability to induce JE virus–neutralizing antibodies as a surrogate for protection and safety evaluations in fewer than 5000 adults.[495]

Pediatric clinical trials with JE-VC are ongoing in the Philippines, Australia, Europe, and the United States. More information regarding the U.S. clinical trial is available online at the clinical trials website.†

Adverse Reactions

The most common local reactions to JE-VC are pain (33%), tenderness (36%), and erythema (10%), but severe reactions occur in fewer than 1 percent of recipients.[239] Reported systemic adverse events in the 7 days after vaccination usually are mild but include headache (26%), myalgia (21%), influenza-like illness (13%), and fatigue (13%). No serious hypersensitivity reactions or neurologic adverse events were identified among recipients enrolled in the clinical trials. However, because JE-VC was studied in fewer than 5000 adults, the possibility of rare serious adverse events cannot be excluded. Postlicensure studies and monitoring of surveillance data are ongoing to evaluate the safety of JE-VC in a larger population.

Indications

The risk for JE for most travelers to Asia is low but varies on the basis of destination, duration, season, and activities. All travelers to countries with endemic JE should be informed of the risks of JE and use personal protective measures to reduce the risk for mosquito bites. For some travelers who will be in high-risk settings, JE vaccine can further reduce the risk for infection. The CDC recommends JE vaccine for travelers who plan to spend a month or longer in areas with endemic infection during the JE virus transmission season.[10,256] JE vaccine also should be considered for shorter-term travelers if they plan to travel away from an urban area and have an itinerary or activities that will increase their risk for JE virus exposure. Information on the location of JE virus transmission and detailed information on vaccine recommendations and adverse events can be obtained from the CDC.*

Primary immunization with JE-VC consists of two doses administered 28 days apart. The immunization series should be completed at least 1 week before potential exposure to JE virus. If the primary series was administered more than 1 year previously, a booster dose may be given prior to potential reexposure.[238] Data on the response to a booster dose administered more than 2 years after the primary series are not available. Data on the need for and timing of additional booster doses also are not available.

Precautions and Contraindications

A previous severe allergic reaction after a previous dose of JE-VC is a contraindication to administration of subsequent doses.[256] JE-VC contains protamine sulfate, a compound known to cause hypersensitivity reactions in some persons.

Practitioners should use caution when considering the use of JE vaccine in pregnant women. Vaccination with JE vaccines usually should be deferred because of a theoretic risk to the developing fetus. However, pregnant women who must travel to an area in which risk for JE is high should be vaccinated if the benefits outweigh the risks of vaccination to the mother and developing fetus.

Breast-feeding is not a contraindication to vaccination. However, whether JE-VC or JE-MB is excreted in human milk is not known. Because many drugs are excreted in human milk, practitioners should use caution when considering the use of JE vaccine in breast-feeding women.

No data exist on the use of JE-VC in immunocompromised persons or patients receiving immunosuppressive therapies.

Rabies Vaccine

Rabies is a viral zoonosis transmitted in saliva and other tissue of infected mammals. The virus enters the central nervous system of the host and causes an acute, progressive encephalomyelitis that almost always is fatal. Postexposure prophylaxis is possible because of the long incubation period, usually weeks to months, of this infection.

*Centers from Disease Control and Prevention: www.cdc.gov/ncidod/dvbid/jencephalitis/children.htm.

†http://clinicaltrials.gov/ct2/show/NCT01047839?term=Japanese+encephalitis&rank=26.

*Centers for Disease Control and Prevention: wwwnc.cdc.gov/travel/.

Wild animals are the most important source of infection for both humans and domestic animals. Rabid, insectivorous bats carrying variants of the rabies virus have been responsible for nearly all human cases of rabies in the continental United States in recent years. Transmission has been shown to occur from even minor and unrecognized bites from infected bats. Of the 36 human cases of rabies diagnosed in the United States between 1980 and 1997, 21 (58 percent) were associated with bat variants.[123] Wild carnivores, especially skunks, foxes, coyotes, and raccoons, are the terrestrial animals most often infected with rabies. Wildlife rabies occurs throughout the continental United States; only Hawaii remains consistently free of rabies. Domestic animals including dogs, cats, and ferrets also may be infected, with three times as many reports of rabies annually in cats compared with dogs.[113] The likelihood of human exposure to rabies from a domestic animal in the United States is a rare occurrence, a result of effective national rabies control programs. In most other countries (e.g., Asia, Africa, and Latin America), dogs remain the most important potential source of infection. Therefore, international travelers to areas where canine rabies is still endemic have an increased risk for exposure to rabies. Twelve of the 36 human rabies deaths reported to the CDC from 1980 through 1997 appear to have been related to rabid animals outside the United States.[122,438]

Preparations

Three rabies vaccines are licensed commercially for preexposure and postexposure prophylaxis in the United States: human diploid cell vaccine (HDCV), rabies vaccine adsorbed (RVA), and purified chicken embryo cell (PCEC), but only HDCV and PCEC are being produced and available for use. The HDCV and PCEC vaccines are licensed for intramuscular administration. The HDCV product for intradermal use is no longer available. HDCV (Imovax Rabies, Sanofi Pasteur) is derived from the Pitman-Moore strain grown in human diploid cell culture. PCEC (RabAvert, Chiron Corporation-Novartis) is prepared from the fixed rabies virus strain Flury LEP grown in primary culture of chicken fibroblasts.[237] The HDCV and PCEC vaccines each contain the WHO-recommended standard of at least 2.5 IU of rabies virus antigen per 1.0-mL intramuscular dose. Although not licensed for use in the United States, two intradermal rabies vaccines are recommended by the WHO and used in countries where the two tissue culture vaccines (HDCV and PCEC) are prohibitively expensive.[8]

Immunogenicity and Efficacy

Viral neutralizing antibodies are produced within 7 to 10 days, and protective immunity usually persists for 2 years or longer. Although a definitive protective titer has not been identified, the WHO[584] and the CDC adhere to working guidelines for an acceptable response to immunization.[153] The CDC currently specifies complete viral neutralization at a 1:5 or greater titer by the rapid fluorescent-focus inhibition test as acceptable; the WHO specifies 0.5 IU/mL or more as acceptable.

No randomized, placebo-controlled human trials have documented the efficacy of vaccine for preexposure or postexposure prophylaxis. However, substantial field experience, as well as direct evidence from controlled animal studies, demonstrates a protective effect. The paucity of human cases attests to the efficacy of postexposure prophylaxis with the currently recommended vaccine and immune globulin preparations. To date, rabies has not been reported in the United States in any patient who received the currently recommended postexposure measures. Cases of human rabies occurring after postexposure administration of prophylaxis have resulted from failure to adhere to established guidelines, such as those of the CDC or the WHO.[100,241,258,502,562]

Adverse Events

Reactions occurring after administration of HDCV or PCEC vaccine are less serious and less common than those associated with the previously available vaccines.[123] Local reactions at the injection site occur in 30 to 74 percent of injections, and mild systemic reactions such as fever, headache, nausea, abdominal pain, muscle aches, and dizziness occur in 5 to 40 percent of vaccine recipients. In one report, approximately 6 percent of persons had an immune complex–like reaction 2 to 21 days after receipt of the booster dose of HDCV.[103] This systemic hypersensitivity reaction occurred less frequently in persons receiving primary vaccination. The reactions have been associated with the presence of β-propiolactone–altered human albumin in HDCV and the development of IgE antibodies to this allergen.[259]

Indications and Precautions

When used as indicated, both types of rabies vaccine are considered equally safe and effective for both preexposure and postexposure prophylaxis (see the ACIP recommendations).[387,487] Usually, an immunization series is initiated and completed with one vaccine product. No clinical studies have been conducted that documented a change in efficacy or the frequency of adverse reactions when the series is completed with a second vaccine product. Ideally, an immunization series should be completed with the same product unless serious allergic reactions occur. Corticosteroids, immunosuppressive conditions, and concurrent administration of antimalarial agents can interfere with the adequate development of an active immune response. Postvaccination titers should be checked in these circumstances.

For adults, rabies vaccination always should be administered in the deltoid area. For children, the anterolateral aspect of the thigh also is acceptable. The gluteal area never should be used for HDCV or PCEC injections because administration of HDCV in this area results in lower neutralizing antibody titers and decreased immunogenicity.[258]

Postexposure Prophylaxis. The essential components of rabies postexposure prophylaxis are wound treatment and, for previously unvaccinated persons, concurrent administration of human rabies immune globulin (HRIG)

TABLE 244-16 **Rabies Postexposure Prophylaxis for Individuals Not Previously Immunized**

Animal Type	Evaluation and Disposition of the Animal	Postexposure Prophylaxis Recommendations
Wild		
Skunk Fox Raccoon Most other carnivores Bat	Regard as rabid unless animal proven negative by laboratory tests*	Consider immediate vaccination and if not previously vaccinated, use rabies immune globulin.
Domestic		
Dog	Healthy and available for 10 days observation	Persons should not begin prophylaxis unless animal develops clinical signs of rabies†
Cat	Rabid or suspected rabid	Immediately vaccinate
Ferret	Escaped (unknown)	Consult public health officials
Other		
Livestock Small rodents Large rodents (woodchucks and beavers) Lagomorphs (rabbits and hares) Other mammals	Consider individually	Consult public health officials. Bites of squirrels, hamsters, guinea pigs, gerbils, chipmunks, rats, mice, other small rodents, rabbits, and hares almost never require antirabies prophylaxis.

*The animal should be euthanized and tested as soon as possible. Holding for observation is not recommended. Discontinue vaccine if immunofluorescence test results of the animal are negative.

†During the 10-day observation period, begin postexposure prophylaxis at the first sign of rabies in a dog, cat, or ferret that has bitten someone. If the animal exhibits clinical signs of rabies, it should be euthanized immediately and tested.

Modified from Manning SE, Rupprecht CE, Fishbein D, et al. Human rabies prevention—United States, 2008: recommendations of the Advisory Committee on Immunization Practices. MMWR Recomm Rep 2008;57(RR-3):1-28.

and vaccine (Table 244-16).[387,487] The combination of active and passive immunization is indicated for the treatment of all bite and all nonbite exposures inflicted by animals suspected or proven to be rabid. Recommendations for the management of persons with possible exposure to rabies include giving meticulous attention to thorough cleansing of the wound with soap and water. The decision to give rabies immunoprophylaxis depends on the circumstances precipitating the exposure, the species and condition of the animal inflicting the wound, and the prevalence of rabies in local animal populations. Bite and nonbite exposures, including scratches, abrasions, open wounds, or mucous membranes contaminated with saliva, are considered significant. Because the need for preventive measures is based on these specific circumstances, the local department of health should be consulted promptly concerning the necessity for initiating postexposure prophylaxis.

When possible, the brains of wild animals (skunks, foxes, coyotes, raccoons, and bats), stray dogs or cats, or symptomatic animals implicated in an exposure should be examined in certified laboratories for evidence of rabies. Immunization always should be initiated promptly and discontinued only if laboratory results are negative. Individuals exposed to healthy dogs or cats that are available for observation do not require immediate prophylactic treatment. Implicated healthy domestic dogs or cats should be quarantined and observed for at least 10 days. If symptoms develop that suggest rabies, the exposed individual should begin postexposure prophylaxis, and the brain of the animal should be examined. An unknown or unavailable animal must be regarded as potentially rabid.

Studies conducted in the United States by the CDC have documented that a regimen of one dose of HRIG and five doses of rabies vaccine (1.0 mL for each dose) given on days 0, 3, 7, 14 and 28 was safe and induced an excellent antibody response in all recipients.[387] In March 2010, ACIP changed recommendations to one dose of HRIG with four doses of rabies vaccine (still 1.0 mL for each dose) given on days 0, 3, 7, and 14. The number of doses recommended for persons with altered immunocompetence has not changed; such persons should be administered a 5-dose vaccination regimen (on days 0, 3, 7, 14, and 28) along with one dose of HRIG.[487] If anatomically feasible, the full dose of HRIG (20 IU/kg) should be infiltrated thoroughly in the area around and into the wounds. Any remaining volume should be injected intramuscularly at a site distant from that of vaccine administration. The dose of HRIG should not exceed that recommended and should not be given beyond the seventh day because HRIG can partially suppress active antibody production.

Persons previously fully vaccinated do not require HRIG and should receive only vaccine (two doses 3 days apart) because an amnestic response will occur after the administration of a booster regardless of the antibody titer.[387] Serum for antibody testing should be obtained from persons whose prophylaxis history or immune status is uncertain, and the course of postexposure active and passive immunoprophylaxis as described for nonimmune individuals should be initiated immediately. If serologic testing demonstrates adequate anti-rabies antibody, postexposure prophylaxis may be discontinued.

Once initiated, rabies prophylaxis should not be interrupted or discontinued because of local or mild systemic

adverse reactions to rabies vaccine. Usually, such reactions can be successfully managed with anti-inflammatory and antipyretic agents such as ibuprofen or acetaminophen. When a person with a history of having had serious hypersensitivity to rabies vaccine must be revaccinated, antihistamines can be administered. Epinephrine should be readily available to counteract any anaphylactic reactions, and the person should be observed carefully immediately after receiving vaccination.

Preexposure Prophylaxis. Active immunization should be considered for high-risk groups (i.e., veterinarians, animal handlers and control officers, selected laboratory workers, persons visiting countries where rabies is hyperendemic, and persons whose pursuits may involve frequent contact with rabid animals, such as spelunkers). Persons whose risk for exposure is less but whose access to immediate competent medical care is restricted also should be considered for preexposure prophylaxis. The primary series consists of three doses (1.0 mL per dose) administered on days 0, 7, and 21 or 28, given intramuscularly in the deltoid area. This three-dose series provides long-term protective immunity, and routine serologic testing for rabies antibody after primary immunization is not necessary. For those individuals who may be immunosuppressed, measurement of anti-rabies antibodies should be performed. In the case of continued or frequent exposure, serum samples should be tested every 2 years and a booster dose of rabies vaccine provided when the antibody titer falls below the minimum accepted level.[387]

Precautions and Contraindications

Because of the potential consequences of inadequately treated rabies exposure and because no indication exists that fetal abnormalities have been associated with rabies vaccination, pregnancy is not considered a contraindication to postexposure prophylaxis.[387] If the risk for exposure to rabies is substantial, preexposure prophylaxis also may be indicated during pregnancy.

Persons who have a history of serious hypersensitivity to rabies vaccine should be revaccinated with caution. Although serious systemic, anaphylactic, or neuroparalytic reactions are rare events during and after the administration of rabies vaccines, such reactions pose a serious dilemma for the patient and the attending physician. A patient's risk for acquiring rabies must be carefully considered before deciding to discontinue vaccination. Advice and assistance on the management of serious adverse reactions for persons receiving rabies vaccines may be sought from the state health department or the CDC.

Typhoid Vaccine

Typhoid fever, an acute, life-threatening febrile illness caused by the bacterium *Salmonella enterica* serovar *typhi (S. typhi)*, remains a serious public health problem throughout the developing world. Although disease prevalence varies greatly depending on the specific population studied, current global estimates suggest that 21.6 million cases of typhoid and 200,000 deaths occur annually.[214] In the United States and much of the developed world, typhoid fever has virtually disappeared.[214] Approximately 400 cases of typhoid fever, mostly among travelers, are reported to the CDC each year. Most reported cases in the United States are acquired during travel to developing countries.[335] Therefore, the primary indication for typhoid vaccination in the United States is international travel to an endemic area.[334] In developing countries without safe water and sanitation, mass immunization is a potentially effective strategy to limit both the severity and the impact of typhoid fever.[2]

The changing epidemiology of typhoid fever underscores the importance of vaccination in both international travelers and high-risk populations in endemic regions. Current studies report that the highest incidence rate is now found in children younger than 5 years of age and results in more severe disease than previously.[507,510] *S. typhi* is increasingly resistant to ampicillin, chloramphenicol, and co-trimoxazole, and quinolone resistance is now being reported.[214] Case-fatality rates, which had decreased from 10 percent to 1 percent with appropriate antibiotic therapy,[583] could rise again without safe, effective, and affordable vaccination strategies.

Preparations

The three types of typhoid vaccines are as follows: inactivated, whole-cell vaccines; live attenuated bacterial vaccines; and subunit vaccines.[77] The parenteral, heat-phenol-inactivated, whole-cell vaccine widely used for many years was highly immunogenic but also was highly reactogenic and no longer is available in the United States. It is the only vaccine currently approved for use in children as young as 6 months and is still available in several developing countries because of its affordability, although manufacturing of this vaccine may not be up to international standards.

Two vaccines currently are licensed for use in the United States, an oral live, attenuated vaccine (TY21a, Vivotif Berna) and a purified Vi (virulence) capsular polysaccharide of *S. typhi* vaccine (ViCPS) (Typhim Vi, Aventis Pasteur) for intramuscular use. Table 244-17 provides information on vaccine dosage and administration. The time required for primary vaccination differs for the two vaccines, as do the lower age limits for use in children.

Ty21a is an oral, live attenuated vaccine consisting of a stable mutant, Ty21a, developed by chemical mutagenesis of a pathogenic *S. typhi* strain. This vaccine was licensed in the United States in 1989 for use in adults and children 6 years of age and older and is available as an enteric-coated capsule. The four-dose primary vaccine series (in the United States and Canada, three-dose series in other parts of the world) is administered as one capsule every other day, taken 1 hour before a meal, for a total of four capsules. The capsules should be kept refrigerated (not frozen), and all four doses must be taken to achieve maximum efficacy. Each capsule should be taken with cool liquid no warmer than 37° C (98.6° F), approximately 1 hour before a meal. This regimen should be completed 1 week before potential exposure. The vaccine

TABLE 244-17 **Dosage and Schedule for Typhoid Fever Vaccination**

Vaccination	Age (yr)	Dose/Route of Administration	No. of Doses	Dosing Interval	Boosting Interval
Oral Live Attenuated TY21a Vaccine					
Primary series	≥6	1 capsule*/oral	4	48 hr	N/A
Booster	≥6	1 capsule*/oral	4	48 hr	Every 5 yr
Vi Capsular Polysaccharide Vaccine					
Primary series	≥2	0.50 mL/IM	1	N/A	N/A
Booster	≥2	0.50 mL/IM	1	N/A	Every 2 yr

*Administer with cool liquid no warmer than 37° C (98.6° F).
N/A, Not applicable.
Modified from Bhattarai A, Mintz E. Typhoid & paratyphoid fever. In: Brunette G, editor. *CDC health information for international travel 2012*. New York: Oxford University Press; 2012, p. 323.

manufacturer recommends that Ty21a not be administered to infants or children younger than 6 years of age.

A parenteral subunit vaccine, ViCPS, was licensed in 1994 for use in adults and children as young as 2 years of age. Primary vaccination with ViCPS consists of one 0.5-mL (25 μg) dose administered intramuscularly and should be given at least 2 weeks before potential exposure. The manufacturer does not recommend the vaccine for children younger than 2 years of age.

In circumstances of continued or repeated exposure to *S.* serotype *typhi*, booster doses are recommended to maintain immunity after primary immunization. The optimal booster schedule for either vaccine has not been determined. Current recommendations for revaccination with either vaccine are provided in Table 244-17. No data have been reported concerning the use of one vaccine as a booster after primary immunization with the other.

Efficacy

Although field trials have demonstrated the efficacy of each vaccine, no comparative studies have been performed.[167] For the heat-phenol-inactivated, whole-cell vaccine, efficacy ranged from 51 to 88 percent and lasted for as long as 7 years. The oral, live attenuated Ty21a vaccine stimulates humoral (both serum IgG and mucosal IgA antibodies) as well as cell-mediated immune responses. In trials of the Ty21a vaccine, efficacy ranged from 42 to 96 percent after administration of the initial series of three doses, with the lower efficacies seen in trials from areas with highly endemic disease.[374,508] The optimum schedule for booster immunizations has not been determined for this vaccine. Protective immunity has been shown to persist for at least 7 years in field trials in endemic countries where herd immunity may have an effect.[291] Revaccination is recommended with the same four-capsule regimen every 5 years for as long as continued exposure is likely to occur.

Immunization with ViCPS results in seroconversion, defined as a fourfold rise in serum anti-Vi antibody, in 80 percent of vaccinees within 2 weeks in endemic as well as nonendemic areas.[291,307,351] Because the vaccine is administered parenterally, mucosal immunity does not develop. The efficacy of ViCPS vaccine in clinical trials was 72 percent at 17 months[1] and 64 percent at 21 months[351] after administration of a single dose. A cluster-randomized trial in India, of 37,673 children 2 years of age and older, demonstrated 61 percent effectiveness.[521] Polysaccharide vaccines do not stimulate T cells and cannot establish immunologic memory. Additional doses of ViCPS therefore do not elicit a booster effect. Revaccination with a single dose is recommended every 2 years for persons who remain at risk.

None of the typhoid vaccines approach 100 percent efficacy, and a large inoculum of *S. typhi* can overcome vaccine-induced immunity. Vaccines do not substitute for proper hygiene and appropriate food handling practices.

Adverse Events

Reactions to the oral Ty21a vaccine generally are mild and consist of transient gastrointestinal upset, fever, headache, and occasionally rash; these reactions occur in less than 5 percent of recipients.[167] Adverse systemic effects are reported in less than 1 percent of vaccines, and neither the development of bacteremia nor person-to-person transmission has been reported.[291]

Reactions to the ViCPS vaccine also occur infrequently, being reported in approximately 7 percent of recipients.[167] These reactions include tenderness at the injection site, erythema and induration, fever, and, very rarely, rashes; systemic complaints occur in less than 2 percent.[291] In contrast, reactions to the inactivated, whole-cell vaccine occur more commonly and are more severe; they include fever in as many as 24 percent of recipients, headache, and severe local pain or swelling in as many as 35 percent of vaccinees. Thirteen to 24 percent of vaccinees subsequently have missed school or work.[375]

Indications

Typhoid vaccination in the United States is recommended only for the following groups[77,167]:

- Travelers to areas where typhoid fever is endemic and in whom a risk for exposure is recognized; risk for exposure is greatest with travel to the Indian subcontinent, Latin America, Asia, the Middle East, and Africa*

*For more information, consult the CDC website: www.cdc.gov/travel.

- Persons with intimate exposure to a documented *S. typhi* carrier, such as occurs with continuing household contact
- Laboratory workers who have frequent contact with *S. typhi* and persons living in areas outside the United States with endemic typhoid infection

Vaccination is not recommended for persons attending summer camp or for those in areas of natural disaster or for control of common-source outbreaks. Doses and schedules for the different typhoid vaccines are given in recommendations of the CDC.[77,167]

Contraindications

Ty21a is a live attenuated vaccine and should not be given to immunocompromised patients, including those receiving high doses of corticosteroids and persons with HIV infection.[77,167] Ty21a vaccine also should not be administered during a gastrointestinal illness or concurrent with certain antibiotics. The *2012 Red Book* recommends avoiding antimicrobial therapy for at least 24 hours before the first dose of oral typhoid vaccine through 7 days after the last dose.[28] Reports are conflicting regarding the use of the antimalarial agents atovaquone and proguanil; however, chloroquine and mefloquine do not appear to interfere with the immune response to oral Ty21a. Neither of the available typhoid vaccines should be given to anyone with a history of having severe local or systemic reactions after receiving a previous dose of vaccine.

Ty21a is not approved for children younger than 6 years of age, and ViCPS is not approved for children younger than the age of 2 years. The whole-cell, inactivated vaccine available outside of the United States is approved for use in children between 6 months and 2 years of age.

Information is not available on the safety of these vaccines when they are used during pregnancy. It is prudent on theoretical grounds to avoid vaccinating pregnant women.

Yellow Fever Vaccine

Yellow fever occurs only in sub-Saharan Africa and tropical South America, where it causes an estimated 200,000 cases annually.[481] As a result, yellow fever is an important vaccine-preventable disease among travelers to areas where yellow fever occurs. The yellow fever epidemic zones include Bolivia, Brazil, Colombia, Ecuador, French Guiana, Peru, Venezuela, and much of sub-Saharan Africa. From 1970 to 2009, nine cases of yellow fever were reported in unvaccinated travelers from the United States and Europe who traveled to West Africa or South America.[515] The risk to unvaccinated travelers of acquiring yellow fever probably is increasing because potential zones of transmission of yellow fever are expanding to include urban areas with large populations of susceptible humans and abundant competent mosquito vectors. Vaccination is the most effective preventive measure against yellow fever, a disease that has no specific treatment and may cause death in 20 to 50 percent of patients.[422]

Preparations

Yellow fever vaccines are derived from the original 17D yellow fever vaccine strain. The live attenuated 17D-204 and 17DD yellow fever strains are the yellow fever vaccines most commonly used.[422] The 17D-204 yellow fever vaccine, which is prepared in chick embryos, is licensed in the United States (YF-VAX, Sanofi Pasteur).[515] Primary immunization consists of a single, subcutaneous injection of 0.5 mL of reconstituted, freeze-dried vaccine for both adults and children. The vaccine should be stored at 2°C to 8°C. Because the vaccine does not contain a preservative, reconstituted vaccine that remains unused after 1 hour must be properly disposed.

Immunogenicity and Efficacy

Seroconversion rates of 93 percent have been documented in young children receiving yellow fever vaccine.[588] Immunity develops by the 10th day after primary vaccination. The titer of virus neutralizing antibodies in sera of vaccinees is used as a surrogate for vaccine effectiveness. Immunity acquired from immunization with the 17D strain virus has been demonstrated to persist for more than 10 years.[482,576] Revaccination is required no more frequently than every 10 years.[482]

No human efficacy studies have been performed with yellow fever vaccine. Worldwide, only five cases of yellow fever have been reported in vaccine recipients since yellow fever vaccine has been in use; whether any or all of them were vaccinated properly with appropriately handled yellow fever vaccine is uncertain.[422]

Adverse Events

The 17D-204 and 17DD yellow fever vaccines are among the safest and most effective viral vaccines.[422] Since 1965, approximately 8 million doses of 17D-derived yellow fever vaccine have been administered to U.S. travelers, and approximately 300 million doses have been administered to persons in areas where yellow fever is endemic. Although 2 to 5 percent of persons who receive vaccine report headaches, myalgia, and low-grade fever 5 to 10 days after receiving vaccination, less than 1 percent will report curtailing their usual activities.

Historically, yellow fever vaccine–associated adverse events were seen primarily among infants and presented as encephalitis. However, with the current yellow fever vaccine, serious adverse events associated with vaccine rarely occur, especially in children. A systematic review found that no serious adverse events were reported in four randomized controlled trials of infants and children (n = 1866).[527]

Three well-characterized serious adverse events occur after yellow fever vaccine administration: (1) immediate hypersensitivity or anaphylactic reactions, (2) yellow fever vaccine–associated neurologic disease (YEL-AND), and (3) yellow fever vaccine–associated viscerotropic disease (YEL-AVD). In general, immediate hypersensitivity reactions, characterized by rash, urticaria, or asthma, or a combination of these, are uncommon (incidence < one case/131,000 vaccinees). Unrecognized

allergy to eggs or chicken or to the hydrolyzed gelatin used to stabilize the vaccine may be responsible for hypersensitivity reactions.

YEL-AND is a serious but rarely fatal adverse event. YEL-AND manifests as several distinct clinical syndromes, including meningoencephalitis (neurotropic disease), Guillain-Barré syndrome, acute disseminated encephalomyelitis (ADEM), and bulbar palsy.[107] Meningoencephalitis occurs as a result of direct yellow fever vaccine viral invasion of the central nervous system (CNS) with infection of the meninges and/or the brain. The other neurologic syndromes (e.g., Guillain-Barré syndrome and ADEM) represent autoimmune manifestations in which antibodies and/or T cells produced in response to the vaccine cross react with neuronal epitopes and lead to central or peripheral nerve damage.

Data regarding the specific age and illness onset for the different neurologic presentations can be derived from VAERS reports.[408] Of six reported encephalitis cases, the median age was 54 years (range, 16 to 78 years), and median time to onset after vaccination was 14 days (range, 5 to 24 days). For the six Guillain-Barré syndrome cases, the median age was 53 years (range, 17 to 68 years) and median time to onset after vaccination was 13 days (range, 7 to 27 days). Finally, for the three ADEM cases, the median age was 19 years (range, 18 to 61 years), and median time to onset after vaccination was 15 days (range, 7 to 20 days). Crude estimates in the United States of the reported frequency range from four to eight cases per 1 million doses distributed.

In 2001, a previously unrecognized serious adverse reaction among recipients of YF vaccine was first described. This syndrome previously was reported as febrile multiple organ system failure and now is called yellow fever vaccine–associated viscerotropic disease. YEL-AVD mimics naturally acquired yellow fever disease, with the vaccine virus proliferating and disseminating throughout the host's tissues. As of February 2010, a total of 57 cases of YEL-AVD have been reported to the CDC from 14 countries (Australia, Belgium, Brazil, China, Colombia, Ecuador, France, Germany, Japan, Peru, Spain, Switzerland, the United Kingdom, and the United States). The cases have occurred in association with the different substrains of yellow fever vaccine produced by several manufacturers. On the basis of an analysis of cases for which information is available, YEL-AVD has occurred only after a recipient's first yellow fever F vaccination; no cases of YEL-AVD occurring in persons receiving booster doses of the vaccine have been reported.

Several risk estimates for YEL-AVD have been published. On the basis of VAERS data, the reporting rate of YEL-AVD is three to four cases per 1 million doses distributed.[378,397]

The recognition of these adverse events may be challenging because the neurologic syndromes (encephalitis, myelitis) can be difficult to distinguish from bacterial meningitis, encephalitis, or malaria and the viscerotropic syndrome can be difficult to distinguish from hemorrhagic fevers, viral hepatitis, and many other causes of multisystem failure.

The CDC initiated the YF Vaccine Safety Working Group to guide ongoing investigations of the potential underlying causes of severe adverse events associated with yellow fever vaccines.*

Indications

Yellow fever vaccine is recommended for persons 9 months of age or older traveling to or residing in areas where yellow fever is endemic.[515] Because of the increased risk for development of neurologic complications, infants aged 4 to 8 months should be considered for vaccination only when travel to high-risk areas is required and high-level protection against mosquito exposure is not feasible. Vaccination for international travel to certain destinations is required. To obtain an international certificate of vaccination, a yellow fever vaccine approved by the WHO and administered at a designated yellow fever vaccine center is required. Yellow fever vaccine centers in the United States can be identified by contacting state or local health departments. The International Health Regulations require revaccination at intervals of 10 years. Revaccination may boost antibody titers; however, evidence from several studies suggests that yellow fever immunity persists for at least 30 to 35 years and probably for life.

Precautions and Contraindications

The risk for having adverse reactions appears to be age related.[515] Infants younger than 9 months should not receive yellow fever vaccine because of the increased risk for vaccine-associated neurotropic disease developing in this age group.[10] Immunization should be delayed until an infant is at least 9 months of age. In unusual circumstances, physicians considering vaccinating infants younger than 9 months of age should contact the applicable division of the CDC for advice.†

No adverse effects of yellow fever vaccine on the developing fetus have been demonstrated. Vaccine administration to pregnant women, however, generally is not indicated because the vaccine is a live virus. Pregnant women should be considered for vaccination only when travel to high-risk areas is required and protection against mosquito exposure is not feasible.

Whether this vaccine is excreted in breast milk is not known. No reports exist of adverse events or transmission of the vaccine viruses from nursing mother to infant. As a precautionary measure, vaccination of nursing mothers should be avoided because of the theoretical risk for the transmission of virus to the breast-fed infant. When travel of nursing mothers to high-risk yellow fever endemic areas cannot be avoided or postponed, these women may be vaccinated.

Yellow fever vaccine, which is a live virus vaccine, poses a risk for encephalitis or other serious adverse events to patients with illnesses that commonly result in

*Centers for Disease Control and Prevention, Yellow Fever Vaccine Safety Working Group: http://www.cdc.gov/ncidod/dvbid/yellowfever/vaccine/special-testing.htm. Instructions for handling sera, body fluids and tissue of suspected cases are included.

†Division of Vector-Borne Infectious Diseases (970-221-6400) or the Division of Global Migration and Quarantine (404-498-1600).

immunosuppression (e.g., acquired immunodeficiency syndrome or HIV infection, leukemia, lymphoma, generalized malignancy), or patients whose immunologic responses are suppressed by drug therapy (e.g., corticosteroids, alkylating drugs, or antimetabolites) or radiation. These patients should not be vaccinated. Family members of immunosuppressed persons, who themselves have no contraindications, may receive yellow fever vaccine. If travel to an epidemic or endemic area is necessary, the patient should be instructed in ways to avoid mosquitoes and given a vaccine waiver letter.

A history of thymus disease has been identified as a contraindication to receiving yellow fever vaccine. Four of the 26 vaccine recipients with YEL-AVD worldwide have had a history of diseases involving the thymus, all of which are extremely rare, a finding suggesting that compromised thymic function (myasthenia gravis, thymoma, previous thymectomy) may be another independent risk factor for viscerotropic disease.[67] Health care providers should ask about a history of thymus disorder when they are screening a patient before administering yellow fever vaccine. For persons with such a history, alternative means of prevention should be recommended, if travel plans cannot be altered to avoid yellow fever–endemic areas.

Persons with a history of systemic anaphylaxis to eggs should not be vaccinated because the vaccines contain egg proteins and on rare occasion may induce immediate allergic reactions. Less severe or local manifestations of allergy to eggs or to feathers are not contraindications to yellow fever vaccine administration and do not warrant performing vaccine skin testing.[18] If international quarantine regulations are the only reason to immunize a patient known to be hypersensitive to eggs, attempts should be made to obtain a waiver. If immunization of an individual with a questionable history of egg hypersensitivity is considered essential because of a high risk for exposure, an intradermal skin test may be given as directed in the vaccine package insert.[515]

Vaccines Related to Bioterrorism

Two vaccines, anthrax and smallpox, are potentially available to protect children against bioterrorist attacks with *Bacillus anthracis* and variola. Neither vaccine is available commercially at this time and does not have indications for children and adolescents in the absence of a bioterrorist attack.[484] The vaccines are reviewed in the relevant disease-specific chapters.

Investigational Vaccines

Routine immunizations for children have virtually eliminated many infectious diseases from the United States. These successes have encouraged research to develop vaccines to prevent other serious viral and bacterial diseases affecting children. A 1985 report by the IOM of the National Academy of Sciences reviewed the benefits that would be associated with the development and use of new and improved vaccines in the United States.[208] The report listed 14 diseases for which vaccines were desirable. A 1999 study by the IOM noted that considerable progress had been made since the 1985 study.[209] Seven of 14 vaccines listed in the 1985 study as domestic priorities for development are now licensed. They include acellular pertussis vaccine, LAIV, and vaccines against hepatitis A and B, *H. influenzae* type b, varicella, and rotavirus.

The 1999 IOM report used a new quantitative model to compare the cost and health benefits of developing candidate vaccines.[209] This model can be used to evaluate the potential impact of a new vaccine on public health. In the 1999 report, the model was used to evaluate diseases for which candidate vaccines were being developed. The report divided 26 candidate vaccines into four groups, from most to least favorable for development. The four vaccines in the top tier include a cytomegalovirus vaccine given to adolescents, a universal influenza vaccine, a group B streptococcus vaccine for high-risk adults and pregnant women, and an *S. pneumoniae* vaccine for infants and seniors. Other diseases for which vaccines would be desirable included *Chlamydia trachomatis*, enterotoxigenic *Escherichia coli*, Epstein-Barr virus, *Helicobacter pylori*, hepatitis C virus, herpes simplex virus, HPV, *M. tuberculosis*, *Neisseria gonorrhoeae*, respiratory syncytial virus, parainfluenza virus, *Shigella*, and groups A and B streptococcus.

Two vaccines listed in the 1999 study are now licensed: pneumococcal conjugate vaccine for infants and HPV vaccine. Candidate vaccines are in human trials for many of the other pathogens listed in the 1999 IOM report. The status of licensure and recommendations for new vaccines is available from the American Academy of Pediatrics *Red Book*, which is available on the organization's website.*

Advances in biotechnology, increased understanding of the virulence factors of infectious agents, and knowledge of the host immune response have led to new approaches for vaccine development.[246] The three general categories of approaches include the following: live vaccines; killed, inactivated, or subunit vaccines; and, most recently, DNA-based vaccines. In addition, newer technologies such as adjuvants or delivery systems and vectors can be applied to improve immunogenicity. Vaccines are being developed by the application of these newer technologies to numerous infectious agents for which vaccines are not currently available, especially those pathogens listed in the 1999 IOM report.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Advisory Committee on Immunization Practices. *Update on influenza vaccine safety monitoring*. Atlanta, Ga.: Centers for Disease Control, Advisory Committee on Immunization Practices; 2012. Available at http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-jun-2012/03-influenza-Shimabukuro.pdf.
4. Ag Ayoya M, Higgins-Steele A, Massai D, et al. Health authorities' leadership reduces cholera deaths in Haiti. *Lancet* 2012; **380**:473.
10. American Academy of Pediatrics. Arboviruses. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 232.
11. American Academy of Pediatrics. Combination vaccines. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book:*

*American Academy of Pediatrics *Red Book:* http://aapredbook.aappublications.org/news/vaccstatus.shtml.

2012 report of the Committee on Infectious Diseases. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 34.
12. American Academy of Pediatrics. Diphtheria. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 307.
13. American Academy of Pediatrics. *Haemophilus influenzae* Infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al., editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 345.
14. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 361.
16. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 369.
17. American Academy of Pediatrics. Human papilloma viruses. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 524.
18. American Academy of Pediatrics. Immunizations in special clinical circumstances. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 69.
19. American Academy of Pediatrics. Influenza. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 439.
20. American Academy of Pediatrics. Measles. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 489.
22. American Academy of Pediatrics. Mumps. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 514.
23. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 553.
25. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 571.
26. American Academy of Pediatrics. Poliovirus infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 588.
27. American Academy of Pediatrics. Rubella. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 629.
28. American Academy of Pediatrics. *Salmonella* infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 635.
29. American Academy of Pediatrics. Tetanus (lockjaw). In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 707.
30. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 736.
31. American Academy of Pediatrics. Varicella-zoster. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 774.
35. American Academy of Pediatrics Committee on Infectious Diseases. Meningococcal conjugate vaccines policy update: booster dose recommendations. *Pediatrics* 2011;**128**:1213.
36. American Academy of Pediatrics Committee on Infectious Diseases. Policy statement—Prevention of varicella: update of recommendations for use of quadrivalent and monovalent varicella vaccines in children. *Pediatrics* 2011;**128**:630.
41. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of rotavirus disease: updated guidelines for use of rotavirus vaccine. *Pediatrics* 2009;**123**:1412.
43. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803.
44. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for prevention and control of influenza in children, 2012-2013. *Pediatrics* 2012;**130**:780–92.
45. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for the prevention of *Streptococcus pneumoniae* infections in infants and children: use of 13-valent pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). *Pediatrics* 2010;**126**:186.
46. American Academy of Pediatrics Committee on Infectious Diseases. Recommended childhood and adolescent immunization schedules—United States, 2012. *Pediatrics* 2012;**129**:385.
50. Ampofo K. The changing epidemiology of invasive pneumococcal disease at a tertiary children's hospital through the 7-valent pneumococcal conjugate vaccine era: a case for continuous surveillance. *Pediatr Infect Dis J* 2012;**31**:228.
55. Arguedas A, Soley C, Lindert K. Responses to 2009 H1N1 vaccine in children 3 to 17 years of age. *N Engl J Med* 2010;**362**: 370.
60. Aylward B, Yamada T. The polio endgame. *N Engl J Med* 2011;**364**:2273.
61. Bakare N, Menschik D, Tiernan R, et al. Severe combined immunodeficiency (SCID) and rotavirus vaccination: reports to the Vaccine Adverse Events Reporting System (VAERS). *Vaccine* 2010;**28**:6609.
72. Belshe R, Coelingh K, Ambrose CS, et al. Efficacy of live attenuated influenza vaccine in children against influenza B viruses by lineage and antigenic similarity. *Vaccine* 2010;**28**:2149.
73. Belshe R, Walker RI, Stoddard J, et al. Influenza vaccine—live. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 291.
75. Bernstein HH, Starke JR. Policy statement—recommendation for mandatory influenza immunization of all health care personnel. *Pediatrics* 2010;**126**:809.
77. Bhattarai A, Mintz E. Typhoid & paratyphoid fever. In: Brunette G., editor. *CDC health information for international travel 2012*. New York: Oxford University Press; 2012. p. 323.
91. Buttery JP, Danchin MH, Lee KJ, et al. Intussusception following rotavirus vaccine administration: post-marketing surveillance in the National Immunization Program in Australia. *Vaccine* 2011; **29**:3061.
106. Centers for Disease Control and Prevention. Addition of severe combined immunodeficiency as a contraindication for administration of rotavirus vaccine. *MMWR Morb Mortal Wkly Rep* 2010; **59**:687.
113. Centers for Disease Control and Prevention. Compendium of animal rabies prevention and control, 2008: National Association of State Public Health Veterinarians, Inc. (NASPHV). *MMWR Recomm Rep* 2008;**57**(RR-2):1–9.
115. Centers for Disease Control and Prevention. Delayed onset and diminished magnitude of rotavirus activity—United States, November 2007–May 2008. *MMWR Morb Mortal Wkly Rep* 2008; **57**:697.
118. Centers for Disease Control and Prevention. FDA licensure of bivalent human papillomavirus vaccine (HPV2, Cervarix) for use in females and updated HPV vaccination recommendations from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;**59**:626.
119. Centers for Disease Control and Prevention. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;**59**:630.
128. Centers for Disease Control and Prevention. Invasive pneumococcal disease in young children before licensure of 13-valent pneumococcal conjugate vaccine—United States, 2007. *MMWR Morb Mortal Wkly Rep* 2010;**59**:253.

129. Centers for Disease Control and Prevention. Licensure of a diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus, and haemophilus B conjugate vaccine and guidance for use in infants and children. *MMWR Morb Mortal Wkly Rep* 2008;**57**:1079.
130. Centers for Disease Control and Prevention. Licensure of a meningococcal conjugate vaccine for children aged 2 through 10 years and updated booster dose guidance for adolescents and other persons at increased risk for meningococcal disease—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1018.
131. Centers for Disease Control and Prevention. Measles—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:253.
134. Centers for Disease Control and Prevention. National, state, and local area vaccination coverage among children aged 19-35 months—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:689.
144. Centers for Disease Control and Prevention. Prevention and Control of Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2012–13 Influenza Season. *MMWR Morb Mortal Wkly Rep* 2012;**61**:613.
145. Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1128.
151. Centers for Disease Control and Prevention. Progress toward interruption of wild poliovirus transmission—worldwide, January 2011–March 2012. *MMWR Morb Mortal Wkly Rep* 2012; **61**:353.
154. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391.
157. Centers for Disease Control and Prevention. Recommendations on the use of quadrivalent human papillomavirus vaccine in males—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1705.
159. Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 0 through 18 Years—United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:1.
160. Centers for Disease Control and Prevention. Reduction in rotavirus after vaccine introduction—United States, 2000-2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1146.
161. Centers for Disease Control and Prevention. Report from the Advisory Committee on Immunization Practices (ACIP): decision not to recommend routine vaccination of all children aged 2-10 years with quadrivalent meningococcal conjugate vaccine (MCV4). *MMWR Morb Mortal Wkly Rep* 2008;**57**:462.
166. Centers for Disease Control and Prevention. Summary of notifiable diseases—United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;**59**:1–111. Erratum in: MMWR Morb Mortal Wkly Rep. 2012;61:562.
168. Centers for Disease Control and Prevention. Update on Japanese encephalitis vaccine for children: United States, May 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:664.
169. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, July 2009–March 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:846.
170. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, April 2011–June 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:741.
174. Centers for Disease Control and Prevention. Update: mumps outbreak—New York and New Jersey, June 2009–January 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:125.
176. Centers for Disease Control and Prevention. Update: recommendations from the Advisory Committee on Immunization Practices (ACIP) regarding administration of combination MMRV vaccine. *MMWR Morb Mortal Wkly Rep* 2008;**57**:258.
177. Centers for Disease Control and Prevention. Update: Recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL seasonal influenza vaccine (Afluria) in the United States during 2010-11. *MMWR Morb Mortal Wkly Rep* 2010;**59**:989.
181. Centers for Disease Control and Prevention. Updated recommendations for use of meningococcal conjugate vaccines—Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:72.
182. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:13.
183. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged <12 months—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**: 1424.
184. Centers for Disease Control and Prevention. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1006.
186. Centers for Disease Control and Prevention. Use of hepatitis B vaccination for adults with diabetes mellitus: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2011;**60**:1709.
204. Cohen R. Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal nasopharyngeal carriage in children with acute otitis media. *Pediatr Infect Dis J* 2012;**31**:297.
212. Cortese MM, Parashar UD. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;**58**:1.
223. Date K, Mintz E. Cholera. In: Brunette G, editor. *CDC health information for international travel 2012*. New York: Oxford University Press; 2012. p. 146.
229. Dennehy PH, Bertrand HR, Silas PE, et al. Coadministration of RIX4414 oral human rotavirus vaccine does not impact the immune response to antigens contained in routine infant vaccines in the United States. *Pediatrics* 2008;**122**:e1062.
238. Dubischar-Kastner K, Eder S, Buerger V, et al. Long-term immunity and immune response to a booster dose following vaccination with the inactivated Japanese encephalitis vaccine IXIARO, IC51. *Vaccine* 2010;**28**:5197.
239. Dubischar-Kastner K, Kaltenboeck A, Klingler A, et al. Safety analysis of a Vero-cell culture derived Japanese encephalitis vaccine, IXIARO (IC51), in 6 months of follow-up. *Vaccine* 2010; **28**:6463.
245. Eisenberg KW, Szilagyi PG, Fairbrother G, et al. Vaccine effectiveness against laboratory-confirmed influenza in children 6 to 59 months of age during the 2003-2004 and 2004-2005 influenza seasons. *Pediatrics* 2008;**122**:911.
246. Ellis R. New technologies for making vaccines. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 1335.
256. Fischer M, Lindsey N, Staples JE, et al. Japanese encephalitis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**:1.
265. Gershon A, Takahashi M, Seward J. Live attenuated varicella vaccine. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 915.
271. Gilliland SM, Forrest L, Carre H, et al. Investigation of porcine circovirus contamination in human vaccines. *Biologicals* 2012;**40**: 270.
284. Greene SK, Kulldorff M, Lewis EM, et al. Near real-time surveillance for influenza vaccine safety: proof-of-concept in the Vaccine Safety Datalink Project. *Am J Epidemiol* 2010;**171**:177.
289. Guris D, Jumaan AO, Mascola L, et al. Changing varicella epidemiology in active surveillance sites—United States, 1995-2005. *J Infect Dis* 2008;**197**(Suppl. 2):S71.
294. Halperin SA, Gupta A, Jeanfreau R, et al. Comparison of the safety and immunogenicity of an investigational and a licensed quadrivalent meningococcal conjugate vaccine in children 2-10 years of age. *Vaccine* 2010;**28**:7865.
311. Hinman AR, Orenstein WA, Schuchat A. Vaccine-preventable diseases, immunizations, and MMWR—1961-2011. *MMWR Surveill Summ* 2011;**60**(Suppl. 4):49.

327. Isaacman DJ. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *Int J Infect Dis* 2010;**14**:e197.
331. Jefferson T, Rivetti A, Harnden A, et al. Vaccines for preventing influenza in healthy children. *Cochrane Database Syst Rev* 2008;(2): CD004879.
332. Jhung MA, Swerdlow D, Olsen SJ, et al. Epidemiology of 2009 pandemic influenza A (H1N1) in the United States. *Clin Infect Dis* 2011;**52**(Suppl. 1):S13.
334. Johnson KJ. From the CDC: new country-specific recommendations for pre-travel typhoid vaccination. *J Travel Med* 2011; **18**:430.
335. Johnson KJ. Crossing borders: one world, global health: CDC updates recommendations for typhoid vaccination. *Clin Infect Dis* 2012;**54**:v.
340. Kaufmann SH. Fact and fiction in tuberculosis vaccine research: 10 years later. *Lancet Infect Dis* 2011;**11**:633.
342. Kelso JM, Greenhawt MJ, Li JT, et al. Adverse reactions to vaccines practice parameter 2012 update. *J Allergy Clin Immunol* 2012;**130**:25.
349. Klein NP, Fireman B, Yih WK, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;**126**:e1.
350. Klein NP, Lewis E, Baxter R, et al. Measles-containing vaccines and febrile seizures in children age 4 to 6 years. *Pediatrics* 2012; **129**:809.
358. Kroger A, Sumaya C, Pickering L, et al. General recommendations on immunization— recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**:1.
363. Kumar D, Beach NM, Meng XJ, et al. Use of PCR-based assays for the detection of the adventitious agent porcine circovirus type 1 (PCV1) in vaccines, and for confirming the identity of cell substrates and viruses used in vaccine production. *J Virol Methods* 2012;**179**:201.
372. Leroy Z, Broder K, Menschik D, et al. Febrile seizures after 2010-1 influenza vaccine in young children, United States: a vaccine safety signal from the vaccine adverse event reporting system. *Vaccine* 2012;**30**:2020.
378. Lindsey NP, Schroeder BA, Miller ER, et al. Adverse event reports following yellow fever vaccination. *Vaccine* 2008;**26**:6077.
383. Mackell S. Vaccine recommendations for infants & children. In: Brunette G, editor. *CDC Health information for international travel 2012*. New York: Oxford University Press; 2012. p. 490.
387. Manning SE, Rupprecht CE, Fishbein D, et al. Human rabies prevention—United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**:1.
390. Marin M, Broder KR, Temte JL, et al. Use of combination measles, mumps, rubella, and varicella vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**:1.
404. McClenahan SD, Krause PR, Uhlenhaut C. Molecular and infectivity studies of porcine circovirus in vaccines. *Vaccine* 2011; **29**:4745.
412. Michalik DE, Steinberg SP, Larussa PS, et al. Primary vaccine failure after 1 dose of varicella vaccine in healthy children. *J Infect Dis* 2008;**197**:944.
419. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**:1.
422. Monath T, Cetron M, Teuwen D. Yellow fever vaccine. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 959.
426. Morris SK, Moss WJ, Halsey N. *Haemophilus influenzae* type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008; **8**:435.
439. Nolan T, McVernon J, Skeljo M, et al. Immunogenicity of a monovalent 2009 influenza A(H1N1) vaccine in infants and children: a randomized trial. *JAMA* 2010;**303**:37.
440. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children—use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;**59**:1.
442. Ong BA, Forester J, Fallot A. Does influenza vaccination improve pediatric asthma outcomes? *J Asthma* 2009;**46**:477.
451. Parker Fiebelkorn A, Redd SB, Gallagher K, et al. Measles in the United States during the postelimination era. *J Infect Dis* 2010;**202**:1520.
453. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med* 2011;**364**:2283.
464. Plennevaux E, Sheldon E, Blatter M, et al. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. *Lancet* 2010;**375**:41.
466. Plotkin S, Rubin S. Mumps vaccines. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed: Saunders Elsevier; 2008. p. 435.
487. Rupprecht CE, Briggs D, Brown CM, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. *MMWR Recomm Rep* 2010;**59**:1.
488. Saha A. Safety and immunogenicity study of a killed bivalent (O1 and O139) whole-cell oral cholera vaccine Shanchol, in Bangladeshi adults and children as young as 1 year of age. *Vaccine* 2011;**29**:8285.
495. Schuller E, Klingler A, Dubischar-Kastner K, et al. Safety profile of the Vero cell-derived Japanese encephalitis virus (JEV) vaccine IXIARO. *Vaccine* 2011;**29**:8669.
497. Seward JF, Marin M, Vazquez M. Varicella vaccine effectiveness in the US vaccination program: a review. *J Infect Dis* 2008; **197**(Suppl. 2):S82.
500. Shapiro ED, Vazquez M, Esposito D, et al. Effectiveness of 2 doses of varicella vaccine in children. *J Infect Dis* 2011;**203**:312.
503. Shin S, Desai SN, Sah BK, et al. Oral vaccines against cholera. *Clin Infect Dis* 2011;**52**:1343.
511. Song J-H. The relationship between pneumococcal serotypes and antibiotic resistance. *Vaccine* 2012;**30**:2728.
515. Staples JE, Gershman M, Fischer M. Yellow fever vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**:1.
520. Sur D, Kanungo S, Sah B, et al. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. *PLoS Negl Trop Dis* 2011; **5**:e1289.
521. Sur D, Ochiai RL, Bhattacharya SK, et al. A cluster-randomized effectiveness trial of Vi typhoid vaccine in India. *N Engl J Med* 2009;**361**:335.
522. Sutter RW, John TJ, Jain H, et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. *Lancet* 2010;**376**:1682.
527. Thomas RE, Lorenzetti DL, Spragins W, et al. The safety of yellow fever vaccine 17D or 17DD in children, pregnant women, HIV+ individuals, and older persons: systematic review. *Am J Trop Med Hyg* 2012;**86**:359.
533. Tse A, Tseng HF, Greene SK, et al. Signal identification and evaluation for risk of febrile seizures in children following trivalent inactivated influenza vaccine in the Vaccine Safety Datalink Project, 2010-2011. *Vaccine* 2012;**30**:2024.
542. Velázquez FR, Colindres RE, Grajales C, et al. Postmarketing surveillance of intussusception following mass introduction of the attenuated human rotavirus vaccine in Mexico. *Pediatr Infect Dis J* 2012;**31**:736.
561. Watt JP, Wolfson LJ, O'Brien KL, et al. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet* 2009;**374**:903.
587. Yeh SH, Gurtman A, Hurley DC, et al. Immunogenicity and safety of 13-valent pneumococcal conjugate vaccine in infants and toddlers. *Pediatrics* 2010;**126**:e493.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Acharya IL, Lowe CU, Thapa R, et al. Prevention of typhoid fever in Nepal with the Vi capsular polysaccharide of *Salmonella typhi:* a preliminary report. *N Engl J Med* 1987;**317**:1101.
2. Acosta CJ, Galindo CM, Ali M, et al. A multi-country cluster randomized controlled effectiveness evaluation to accelerate the introduction of Vi polysaccharide typhoid vaccine in developing countries in Asia: rationale and design. *Trop Med Int Health* 2005;**10**:1219.
3. Advisory Committee on Immunization Practices. *Update on influenza vaccine safety monitoring*. Atlanta, Ga.: Centers for Disease Control, Advisory Committee on Immunization Practices; 2012. Available at http://www.cdc.gov/vaccines/acip/meetings/downloads/slides-jun-2012/03-influenza-Shimabukuro.pdf.
4. Ag Ayoya M, Higgins-Steele A, Massai D, et al. Health authorities' leadership reduces cholera deaths in Haiti. *Lancet* 2012; **380**:473.
5. Allison MA, Daley MF, Crane LA, et al. Influenza vaccine effectiveness in healthy 6- to 21-month-old children during the 2003-2004 season. *J Pediatr* 2006;**149**:755.
6. Alter MJ, Hadler SC, Margolis HS, et al. The changing epidemiology of hepatitis B in the United States. *JAMA* 1990;**263**: 1218.
7. Ambrosch F, Wiedermann G, Crooy P, et al. Immunogenicity and side-effects of a new tetravalent meningococcal polysaccharide vaccine. *Bull WHO* 1983;**61**:317.
8. Ambrozaitis A, Laiskonis A, Balciuniene L, et al. Rabies post-exposure prophylaxis vaccination with purified chick embryo cell vaccine (PCECV) and purified Vero cell rabies vaccine (PVRV) in a four-site intradermal schedule (4-0-2-0-1-1): an immunogenic, cost-effective and practical regimen. *Vaccine* 2006;**24**:4116.
9. American Academy of Pediatrics. Active immunization. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 11.
10. American Academy of Pediatrics. Arboviruses. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 232.
11. American Academy of Pediatrics. Combination vaccines. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 34.
12. American Academy of Pediatrics. Diphtheria. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 307.
13. American Academy of Pediatrics. *Haemophilus influenzae* Infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 345.
14. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 361.
15. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2006. p. 335.
16. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 369.
17. American Academy of Pediatrics. Human papilloma viruses. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 524.
18. American Academy of Pediatrics. Immunizations in special clinical circumstances. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 69.
19. American Academy of Pediatrics. Influenza. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 439.
20. American Academy of Pediatrics. Measles. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 489.
21. American Academy of Pediatrics. Measles. In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2006. p. 441.
22. American Academy of Pediatrics. Mumps. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 514.
23. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 553.
24. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering LK, Baker CJ, Long SS, et al, editors. *Red book: 2006 report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2006. p. 498.
25. American Academy of Pediatrics. Pneumococcal infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 571.
26. American Academy of Pediatrics. Poliovirus infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill. American Academy of Pediatrics; 2012. p. 588.
27. American Academy of Pediatrics. Rubella. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 629.
28. American Academy of Pediatrics. *Salmonella* infections. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 635.
29. American Academy of Pediatrics. Tetanus (lockjaw). In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 707.
30. American Academy of Pediatrics. Tuberculosis. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 736.
31. American Academy of Pediatrics. Varicella-zoster. In: Pickering LK, Baker CJ, Kimberlin DW, et al, editors. *Red book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill.: American Academy of Pediatrics; 2012. p. 774.
32. American Academy of Pediatrics Committee on Infectious Diseases. *Haemophilus influenzae* type B conjugate vaccines: recommendations for immunization with recently and previously licensed vaccines. *Pediatrics* 1993;**92**:480.
33. American Academy of Pediatrics Committee on Infectious Diseases. Hepatitis A vaccine recommendations. *Pediatrics* 2007;**120**: 189.
34. American Academy of Pediatrics Committee on Infectious Diseases. Measles: reassessment of the current immunization policy. *Pediatrics* 1989;**84**:1110.
35. American Academy of Pediatrics Committee on Infectious Diseases. Meningococcal conjugate vaccines policy update: booster dose recommendations. *Pediatrics* 2011;**128**:1213.
36. American Academy of Pediatrics Committee on Infectious Diseases. Policy statement—Prevention of varicella: update of recommendations for use of quadrivalent and monovalent varicella vaccines in children. *Pediatrics* 2011;**128**:630.
37. American Academy of Pediatrics Committee on Infectious Diseases. Poliomyelitis prevention: recommendations for use of inactivated poliovirus vaccine and live oral poliovirus vaccine. *Pediatrics* 1997;**99**:300.
38. American Academy of Pediatrics Committee on Infectious Diseases. Poliomyelitis prevention: revised recommendations for use of inactivated and live oral poliovirus vaccines. *Pediatrics* 1999; **103**:171.

39. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of pertussis among adolescents: recommendations for use of tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis (Tdap) vaccine. *Pediatrics* 2006;**117**:965.
40. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of poliomyelitis: recommendations for use of only inactivated poliovirus vaccine for routine immunization. *Pediatrics* 1999;**104**:1404.
41. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of rotavirus disease: updated guidelines for use of rotavirus vaccine. *Pediatrics* 2009;**123**:1412.
42. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of varicella: recommendations for use of varicella vaccines in children, including a recommendation for a routine 2-dose varicella immunization schedule. *Pediatrics* 2007; **120**:221.
43. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803.
44. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for Prevention and Control of Influenza in Children, 2012-2013. *Pediatrics* 2012;**130**:780–92.
45. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for the prevention of *Streptococcus pneumoniae* infections in infants and children: use of 13-valent pneumococcal conjugate vaccine (PCV13) and pneumococcal polysaccharide vaccine (PPSV23). *Pediatrics* 2010;**126**:186.
46. American Academy of Pediatrics Committee on Infectious Diseases. Recommended childhood and adolescent immunization schedules—United States, 2012. *Pediatrics* 2012;**129**:385.
47. American Academy of Pediatrics Committee on Infectious Diseases. The relationship between pertussis vaccine and central nervous system sequelae: continuing assessment. *Pediatrics* 1996; **97**:279.
48. American Academy of Pediatrics Committee on Infectious Diseases and Committee on Pediatric AIDS. Measles immunization in HIV-infected children. American Academy of Pediatrics. *Pediatrics* 1999;**103**:1057.
49. Ammann AJ, Addiego J, Wara DW, et al. Polyvalent pneumococcal-polysaccharide immunization of patients with sickle-cell anemia and patients with splenectomy. *N Engl J Med* 1977;**297**:897.
50. Ampofo K. The changing epidemiology of invasive pneumococcal disease at a tertiary children's hospital through the 7-valent pneumococcal conjugate vaccine era: a case for continuous surveillance. *Pediatr Infect Dis J* 2012;**31**:228.
51. Anderson EL, Decker MD, Englund JA, et al. Interchangeability of conjugated *Haemophilus influenzae* type b vaccines in infants. *JAMA* 1995;**273**:849.
52. Andrews N, Miller E, Grant A, et al. Thimerosal exposure in infants and developmental disorders: a retrospective cohort study in the United Kingdom does not support a causal association. *Pediatrics* 2004;**114**:584.
53. Anh DD. Safety and immunogenicity of a reformulated Vietnamese bivalent killed, whole-cell, oral cholera vaccine in adults. *Vaccine* 2007;**25**:1149.
54. Arbeter AM, Starr SE, Plotkin SA. Varicella vaccine studies in healthy children and adults. *Pediatrics* 1986;**78**(Suppl):748.
55. Arguedas A, Soley C, Lindert K. Responses to 2009 H1N1 vaccine in children 3 to 17 years of age. *N Engl J Med* 2010;**362**:370.
56. Armand J, Arminjon F, Mynard MC, et al. Tetravalent meningococcal polysaccharide vaccien groups A,C,Y,W135: clinical and serological evaluation. *J Biol Stand* 1982;**10**:335.
57. Arpadi SM, Markowitz LE, Baughman AL, et al. Measles antibody in vaccinated human immunodeficiency virus type 1-infected children. *Pediatrics* 1996;**97**:653.
58. Asano Y, Nagai T, Miyata T, et al. Long-term protective immunity of recipients of the OKA strain of live varicella vaccine. *Pediatrics* 1985;**75**:667.
59. Ascherio A, Zhang S, Hernan M, et al. Hepatitis B vaccination and the risk of multiple sclerosis. *N Engl J Med* 2001;**344**:327.
60. Aylward B, Yamada T. The polio endgame. *N Engl J Med* 2011;**364**:2273.
61. Bakare N, Menschik D, Tiernan R, et al. Severe combined immunodeficiency (SCID) and rotavirus vaccination: reports to the Vaccine Adverse Events Reporting System (VAERS). *Vaccine* 2010;**28**:6609.
62. Ball R, Braun MM, Mootrey GT. Safety data on meningococcal polysaccharide vaccine from the Vaccine Adverse Event Reporting System. *Clin Infect Dis* 2001;**32**:1273.
63. Barbour ML. Conjugate vaccines and the carriage of *Haemophilus influenzae* type b. *Emerg Infect Dis* 1996;**2**:176.
64. Barker WH. Excess pneumonia and influenza associated hospitalization during influenza epidemics in the United States, 1970-78. *Am J Public Health* 1986;**76**:761.
65. Barker WH, Mullooly JP. Impact of epidemic type A influenza in a defined adult population. *Am J Epidemiol* 1980;**112**:798.
66. Barlow WE, Davis RL, Glasser JW, et al. The risk of seizures after receipt of whole-cell pertussis or measles, mumps, and rubella vaccine. *N Engl J Med* 2001;**345**:656.
67. Barwick R. History of thymoma and yellow fever vaccination. *Lancet* 2004;**364**:936.
68. Beeler J, Varricchio F, Wise R. Thrombocytopenia after immunization with measles vaccines: review of the vaccine adverse events reporting system (1990 to 1994). *Pediatr Infect Dis J* 1996;**15**:88.
69. Bell BP, Negus S, Fiore AE, et al. Immunogenicity of an inactivated hepatitis A vaccine in infants and young children. *Pediatr Infect Dis J* 2007;**26**:116.
70. Bell TD, Chai H, Berlow B, et al. Immunization with killed influenza virus in children with chronic asthma. *Chest* 1978;**73**:140.
71. Bellini W, Rota J, Greer P, et al. Measles vaccination death in a child with severe combined immunodeficiency: report of a case. *Lab Invest* **66**:91A, 1992.
72. Belshe R, Coelingh K, Ambrose CS, et al. Efficacy of live attenuated influenza vaccine in children against influenza B viruses by lineage and antigenic similarity. *Vaccine* 2010;**28**:2149.
73. Belshe R, Walker RI, Stoddard J, et al. Influenza vaccine—live. In: Plotkin SA, Orenstein WA, Offit PA, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 291.
74. Bernstein HH, Rothstein EP, Watson BM, et al. Clinical survey of natural varicella compared with breakthrough varicella after immunization with live attenuated Oka/Merck varicella vaccine. *Pediatrics* 1993;**92**:833.
75. Bernstein HH, Starke JR. Policy statement—recommendation for mandatory influenza immunization of all health care personnel. *Pediatrics* 2010;**126**:809.
76. Bewley K, Schwab J, Ballanco G, et al. Interchangeability of *Haemophilus influenzae* type b vaccines in the primary series: evaluation of a two-dose mixed regimen. *Pediatrics* 1996;**98**:898.
77. Bhattarai A, Mintz E. Typhoid & paratyphoid fever. In: Brunette G, editor. *CDC health information for international travel 2012*. New York: Oxford University Press; 2012. p. 323.
78. Bilukha OO, Rosenstein N. Prevention and control of meningococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2005; **54**:1.
79. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. Northern California Kaiser Permanente Vaccine Study Center Group. *Pediatr Infect Dis J* 2000;**19**:187.
80. Bloch AB, Orenstein WA, Stetler HC, et al. Health impact of measles vaccination in the United States. *Pediatrics* 1985;**76**:524.
81. Block SL, Vesikari T, Goveia MG, et al. Efficacy, immunogenicity, and safety of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine at the end of shelf life. *Pediatrics* 2007;**119**:11.
82. Blom L, Nystrom L, Dahlquist G. Vaccinations and infections as risk determinants for diabetes in childhood. The Swedish Childhood Diabetes Study. *Diabetologia* 1991;**34**:176.
83. Booy R, Moxon ER, Macfarlane JA, et al. Efficacy of *Haemophilus influenzae* type b conjugate vaccine in Oxford region. *Lancet* 1992;**340**:847.
84. Breiman RF, Butler JC, Tenover FC, et al. Emergence of drug-resistant pneumococcal infections in the United States. *JAMA* 1994;**271**:1831.
85. Briss PA, Fehrs LJ, Parker RA, et al. Sustained transmission of mumps in a highly vaccinated population: assessment of primary vaccine failure and waning vaccine-induced immunity. *J Infect Dis* 1994;**169**:77.
86. Broder KR, Cortese MM, Iskander JK, et al. Preventing tetanus, diphtheria, and pertussis among adolescents: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;**55**:1.

87. Brokstad KA, Cox RJ, Olofsson J, et al. Parenteral influenza vaccination induces a rapid systemic and local immune response. *J Infect Dis* 1995;**171**:198.
88. Brunell P, Argaw T. Chickenpox attributable to a vaccine virus contracted from a vaccinee with zoster. *Pediatrics* 2000;**106**:E28.
89. Bryan J, Henry C, Hoffman A, et al. Randomized, cross-over, controlled comparison of two inactivated hepatitis A vaccines. *Vaccine* 2000;**19**:743.
90. Butler JC, Breiman RF, Campbell JF, et al. Pneumococcal polysaccharide vaccine efficacy: an evaluation of current recommendations. *JAMA* 1993;**270**:1826.
91. Buttery JP, Danchin MH, Lee KJ, et al. Intussusception following rotavirus vaccine administration: post-marketing surveillance in the National Immunization Program in Australia. *Vaccine* 2011; **29**:3061.
92. Byington CL, Samore MH, Stoddard GJ, et al. Temporal trends of invasive disease due to *Streptococcus pneumoniae* among children in the intermountain west: emergence of nonvaccine serogroups. *Clin Infect Dis* 2005;**41**:21.
93. Caceres VM, Sutter RW. Sabin monovalent oral polio vaccines: review of past experiences and their potential use after polio eradication. *Clin Infect Dis* 2001;**33**:531.
94. Cates W Jr. Estimates of the incidence and prevalence of sexually transmitted diseases in the United States. American Social Health Association Panel. *Sex Transm Dis* 1999;**26**:S2.
95. Centers for Disease Control. Diphtheria, tetanus, and pertussis: recommendations for vaccine use and other preventive measures: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR MMWR Recomm Rep* 1991;**40**(RR-10): 1–28.
96. Centers for Disease Control. Hepatitis B virus: a comprehensive strategy for eliminating transmission in the United States through universal childhood vaccination: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991;**40**(RR-13):1–25.
97. Centers for Disease Control. Measles prevention: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Morb Mortal Wkly Rep* 1989;**38**(Suppl. 9):1–18.
98. Centers for Disease Control. Pneumococcal polysaccharide vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1989; **38**:64.
99. Centers for Disease Control. Poliomyelitis prevention: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Morb Mortal Wkly Rep* 1982;**31**:22.
100. Centers for Disease Control. Rabies prevention—United States, 1991: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1991;**40**(RR-3):1–19.
101. Centers for Disease Control. Rubella vaccination during pregnancy—United States, 1971–1988. *MMWR Morb Mortal Wkly Rep* 1989;**38**:289.
102. Centers for Disease Control. Subacute sclerosing panencephalitis surveillance—United States. *MMWR Morb Mortal Wkly Rep* 1982; **31**:585.
103. Centers for Disease Control. Systemic allergic reactions following immunization with human diploid cell rabies vaccine. *MMWR Morb Mortal Wkly Rep* 1984;**33**:185.
104. Centers for Disease Control. Tetanus—United States, 1987 and 1988. *MMWR Morb Mortal Wkly Rep* 1990;**39**:37.
105. Centers for Disease Control and Prevention. Acute flaccid paralysis associated with circulating vaccine-derived poliovirus—Philippines, 2001. *MMWR Morb Mortal Wkly Rep* 2001; **50**:874.
106. Centers for Disease Control and Prevention. Addition of severe combined immunodeficiency as a contraindication for administration of rotavirus vaccine. *MMWR Morb Mortal Wkly Rep* 2010; **59**:687.
107. Centers for Disease Control and Prevention. Adverse events associated with 17D-derived yellow fever vaccination—United States, 2001–2002. *MMWR Morb Mortal Wkly Rep* 2002;**51**:989.
108. Centers for Disease Control and Prevention. Brief report: update: mumps activity—United States, January 1–October 7, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1152.
109. Centers for Disease Control and Prevention. Certification of poliomyelitis eradication—the Americas. *MMWR Morb Mortal Wkly Rep* 1994;**43**:720.
110. Centers for Disease Control and Prevention. Prevention of specific infectious diseases. In: Arguin PM, Kozarsky PE, Navin AW, editors. *Health information for international travel 2005-2006*. Atlanta, Ga.: U.S. Department of Health and Human Services, Public Health Service; 2005.
111. Centers for Disease Control and Prevention. Cholera associated with food transported from El Salvador—Indiana, 1994. *MMWR Morb Mortal Wkly Rep* 1995;**44**:385.
112. Centers for Disease Control and Prevention. Circulation of a type 2 vaccine-derived poliovirus—Egypt, 1982-1993. *MMWR Morb Mortal Wkly Rep* 2001;**50**:41.
113. Centers for Disease Control and Prevention. Compendium of animal rabies prevention and control, 2008: National Association of State Public Health Veterinarians, Inc. (NASPHV). *MMWR Recomm Rep* 2008;**57**(RR-2):1–9.
114. Centers for Disease Control and Prevention. Control and prevention of rubella: evaluation and management of suspected outbreaks, rubella in pregnant women, and sureillance for congential rubella syndrome. *MMWR Recomm Rep* 2001;**50**(RR-12):1–23.
115. Centers for Disease Control and Prevention. Delayed onset and diminished magnitude of rotavirus activity—United States, November 2007–May 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:697.
116. Centers for Disease Control and Prevention. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease—United States, 1998-2003. *MMWR Morb Mortal Wkly Rep* 2005;**54**:893.
117. Centers for Disease Control and Prevention. Elimination of rubella and congenital rubella syndrome—United States, 1969-2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:279.
118. Centers for Disease Control and Prevention. FDA licensure of bivalent human papillomavirus vaccine (HPV2, Cervarix) for use in females and updated HPV vaccination recommendations from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;**59**:626.
119. Centers for Disease Control and Prevention. FDA licensure of quadrivalent human papillomavirus vaccine (HPV4, Gardasil) for use in males and guidance from the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2010;**59**:630.
120. Centers for Disease Control and Prevention. General recommendations on immunization. Recommendations of the Advisory Committee on Immunization Practices (ACIP) and the American Academy of Family Physicians (AAFP). *MMWR Recomm Rep* 2002;**51**(RR-2):1–35.
121. Centers for Disease Control and Prevention. *Haemophilus* b conjugate vaccines for prevention of *Haemophilus influenzae* type b disease among infants and children two months of age and older: recommendations of the immunization practices advisory committee (ACIP). *MMWR Recomm Rep* 1991;**40**:1.
122. Centers for Disease Control and Prevention. Human rabies—Texas and New Jersey, 1997. *MMWR Morb Mortal Wkly Rep* 1998;**47**:1.
123. Centers for Disease Control and Prevention. Human rabies prevention—United States, 1999: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999;**48**(RR-1):1–21.
124. Centers for Disease Control and Prevention. Immunization of adolescents: recommendations of the Advisory Committee on Immunization Practices, the American Academy of Pediatrics, the American Academy of Family Physicians and the American Medical Association. *MMWR Recomm Rep* 1996;**45**(RR-13):1–16.
125. Centers for Disease Control and Prevention. Immunization of adolescents: recommendations of the Advisory Committee on Immunization Practices, the American Academy of Pediatrics, the American Academy of Family Physicians, and the American Medical Association. American Academy of Pediatrics Committee on Infectious Diseases. *Pediatrics* 1997;**99**:479.
126. Centers for Disease Control and Prevention. Impact of the sequential IPV/OPV schedule on vaccination coverage levels—United States, 1997. *MMWR Morb Mortal Wkly Rep* 1998; **47**:1017.
127. Centers for Disease Control and Prevention. Inadvertent misadministration of meningococcal conjugate vaccine—United States, June–August 2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1016.

128. Centers for Disease Control and Prevention. Invasive pneumococcal disease in young children before licensure of 13-valent pneumococcal conjugate vaccine—United States, 2007. *MMWR Morb Mortal Wkly Rep* 2010;**59**:253.
129. Centers for Disease Control and Prevention. Licensure of a diphtheria and tetanus toxoids and acellular pertussis adsorbed, inactivated poliovirus, and *Haemophilus* B conjugate vaccine and guidance for use in infants and children. *MMWR Morb Mortal Wkly Rep* 2008;**57**:1079.
130. Centers for Disease Control and Prevention. Licensure of a meningococcal conjugate vaccine for children aged 2 through 10 years and updated booster dose guidance for adolescents and other persons at increased risk for meningococcal disease. Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1018.
131. Centers for Disease Control and Prevention. Measles—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:253.
132. Centers for Disease Control and Prevention. Measles pneumonitis following measles-mumps-rubella vaccination of a patient with HIV infection, 1993. *MMWR Morb Mortal Wkly Rep* 1996;**45**: 603.
133. Centers for Disease Control and Prevention. Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Immunization Practices Advisory Committee (ACIP). *MMWR Recomm Rep* 1998;**47**(RR-8):1–57.
134. Centers for Disease Control and Prevention. National, state, and local area vaccination coverage among children aged 19–35 months—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:689.
135. Centers for Disease Control and Prevention. National, state, and urban area vaccination coverage among children aged 19–35 months—United States, 2004. *MMWR Morb Mortal Wkly Rep* 2005;**54**:717.
136. Centers for Disease Control and Prevention. Notice to readers: alternate two-dose hepatitis b vaccination schedule for adolescents aged 11–5 Years. *MMWR Morb Mortal Wkly Rep* 2000;**49**:261.
137. Centers for Disease Control and Prevention. Notice to readers: licensure of a combined measles, mumps, rubella and varicella vaccine. *MMWR Morb Mortal Wkly Rep* 2005;**54**:1212.
138. Centers for Disease Control and Prevention. Notice to readers: revised ACIP recommendation for avoiding pregnancy after receiving a rubella-containing vaccine. *MMWR Morb Mortal Wkly Rep* 2001;**50**:1117.
139. Centers for Disease Control and Prevention. Notice to readers: updated recommendations of the Advisory Committee on Immunization Practices (ACIP) for the control and elimination of mumps. *MMWR Morb Mortal Wkly Rep* 2006;**55**:629.
140. Centers for Disease Control and Prevention. Outbreak of poliomyelitis—Dominican Republic and Haiti, 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:1094.
141. Centers for Disease Control and Prevention. Pertussis—United States, 1997-2000. *MMWR* 51, 2002;
142. Centers for Disease Control and Prevention. Pertussis—United States, January 1992–June 1995. *MMWR Morb Mortal Wkly Rep* 1995;**44**:525.
143. Centers for Disease Control and Prevention. Pneumococcal vaccination for cochlear implant candidates and recipients: updated recommendations of the Advisory Committee on Immunization Practices. *MMWR Morb Mortal Wkly Rep* 2003;**52**:739.
144. Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP)—United States, 2012-13 influenza season. *MMWR Morb Mortal Wkly Rep* 2012;**61**:613.
145. Centers for Disease Control and Prevention. Prevention and control of influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1128.
146. Centers for Disease Control and Prevention. Prevention and control of meningococcal disease. recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2000;**49**:1.
147. Centers for Disease Control and Prevention. Prevention of pneumococcal disease: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* **46**:1, 1997.
148. Centers for Disease Control and Prevention. Prevention of varicella: updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1999; **48**(RR-6):1–5.
149. Centers for Disease Control and Prevention. Progress toward elimination of *Haemophilus influenzae* type b invasive disease among infants and children—United States, 1998-2000. *MMWR Morb Mortal Wkly Rep* 2002;**51**:234.
150. Centers for Disease Control and Prevention. Progress toward interruption of wild poliovirus transmission—worldwide, January 2004–March 2005. *MMWR Morb Mortal Wkly Rep* 2005;**54**: 408.
151. Centers for Disease Control and Prevention. Progress toward interruption of wild poliovirus transmission—worldwide, January 2011–March 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**: 353.
152. Centers for Disease Control and Prevention. Progress toward the global elimination of neonatal tetanus, 1989-1993. *MMWR Morb Mortal Wkly Rep* 1994;**43**:885.
153. Centers for Disease Control and Prevention. Rabies prevention—United States, 1984. *MMWR Morb Mortal Wkly Rep* 1984;**33**:393.
154. Centers for Disease Control and Prevention. Recommendation of the Advisory Committee on Immunization Practices (ACIP) for use of quadrivalent meningococcal conjugate vaccine (MenACWY-D) among children aged 9 through 23 months at increased risk for invasive meningococcal disease. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1391.
155. Centers for Disease Control and Prevention. Recommendations for use of *Haemophilus influenzae* b conjugate vaccines and a combined diphtheria, tetanus, pertussis, and *Haemophilus* b vaccine. Recommendations fo the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1993;**42**(RR-13):1–15.
156. Centers for Disease Control and Prevention. Recommendations of the Advisory Committee on Immunization Practices: revised recommendations for routine poliomyelitis vaccination. *MMWR Morb Mortal Wkly Rep* 1999;**48**:590.
157. Centers for Disease Control and Prevention. Recommendations on the use of quadrivalent human papillomavirus vaccine in males—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1705.
158. Centers for Disease Control and Prevention. Recommended childhood immunization schedule—United States, 2000. *MMWR Morb Mortal Wkly Rep* 2000;**49**:35.
159. Centers for Disease Control and Prevention. Recommended immunization schedules for persons aged 0 through 18 Years—United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:1.
160. Centers for Disease Control and Prevention. Reduction in rotavirus after vaccine introduction—United States, 2000-2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1146.
161. Centers for Disease Control and Prevention. Report from the Advisory Committee on Immunization Practices (ACIP): decision not to recommend routine vaccination of all children aged 2-10 years with quadrivalent meningococcal conjugate vaccine (MCV4). *MMWR Morb Mortal Wkly Rep* 2008;**57**:462.
162. Centers for Disease Control and Prevention. Revised ACIP recommendation for avoiding pregnancy after receiving a rubella-containing vaccine. *MMWR Morb Mortal Wkly Rep* 2001;**50**:1117.
163. Centers for Disease Control and Prevention. The role of BCG vaccine in the prevention and control of tuberculosis in the United States: a joint statement by the Advisory Council for the Elimination of Tuberculosis and the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 1996;**45**(RR-4):1–18.
164. Centers for Disease Control and Prevention. Simultaneous administration of varicella vaccine and other recommended childhood vaccines—United States, 1995-1999. *MMWR Morb Mortal Wkly Rep* 2001;**50**:1058.
165. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 1999. *MMWR Morb Mortal Wkly Rep* **48**:1–91, 2001.
166. Centers for Disease Control and Prevention. Summary of notifiable diseases: United States, 2010. *MMWR Morb Mortal Wkly Rep* 2012;59:1–111.
167. Centers for Disease Control and Prevention. Typhoid immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR* 1994;**43**(RR-14):1–8.

168. Centers for Disease Control and Prevention. Update on Japanese encephalitis vaccine for children: United States, May 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:664.
169. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, July 2009–March 2011. *MMWR Morb Mortal Wkly Rep* 2011;**60**:846.
170. Centers for Disease Control and Prevention. Update on vaccine-derived polioviruses—worldwide, April 2011–June 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:741.
171. Centers for Disease Control and Prevention. Update: eradication of paralytic poliomyelitis in the Americas. *MMWR Morb Mortal Wkly Rep* 1992;**41**:681.
172. Centers for Disease Control and Prevention. Update: recommendations to prevent hepatitis B virus transmission—United States. *MMWR Morb Mortal Wkly Rep* 1995;**44**:574.
173. Centers for Disease Control and Prevention. Update: expanded availability of thimerosal preservative-free hepatitis B vaccine. *MMWR Morb Mortal Wkly Rep* 2000;**49**:642.
174. Centers for Disease Control and Prevention. Update: mumps outbreak—New York and New Jersey, June 2009–January 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**:125.
175. Centers for Disease Control and Prevention. Update: prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007;**56**:1080.
176. Centers for Disease Control and Prevention. Update: recommendations from the Advisory Committee on Immunization Practices (ACIP) regarding administration of combination MMRV vaccine. *MMWR Morb Mortal Wkly Rep* 2008;**57**:258.
177. Centers for Disease Control and Prevention. Update: Recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding use of CSL seasonal influenza vaccine (Afluria) in the United States during 2010-11. *MMWR Morb Mortal Wkly Rep* 2010;**59**:989.
178. Centers for Disease Control and Prevention. Update: recommendations to prevent hepatitis B virus transmission—United States. *MMWR Morb Mortal Wkly Rep* 1999;**48**:33.
179. Centers for Disease Control and Prevention. Update: vaccine side effects, adverse reactions, contraindications, and precautions. Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1996;**45**(RR-12):1–35. Erratum in: MMWR Morb Mortal Wkly Rep 1997;46(10): 227.
180. Centers for Disease Control and Prevention. Update: vaccine side effects, adverse reactions, contraindications, and precautions: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1996;**45**:1.
181. Centers for Disease Control and Prevention. Updated recommendations for use of meningococcal conjugate vaccines—Advisory Committee on Immunization Practices (ACIP), 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:72.
182. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:13.
183. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine (Tdap) in pregnant women and persons who have or anticipate having close contact with an infant aged <12 months—Advisory Committee on Immunization Practices (ACIP), 2011. *MMWR Morb Mortal Wkly Rep* 2011; **60**:1424.
184. Centers for Disease Control and Prevention. Updated recommendations from the Advisory Committee on Immunization Practices (ACIP) for use of hepatitis A vaccine in close contacts of newly arriving international adoptees. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1006.
185. Centers for Disease Control and Prevention. Use of diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine as a five-dose series: supplemental recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2000;**49**(RR-13):1–8.
186. Centers for Disease Control and Prevention. Use of hepatitis B vaccination for adults with diabetes mellitus: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2011;**60**:1709.
187. Centers for Disease Control and Prevention. Vaccine preventable deaths and the Global Immunization Vision and Strategy, 2006-2015. *MMWR Morb Mortal Wkly Rep* 2006;**55**:511.
188. Chang MH, Chen CJ, Lai MS, et al. Universal hepatitis B vaccination in Taiwan and the incidence of hepatocellular carcinoma in children. Taiwan Childhood Hepatoma Study Group. *N Engl J Med* 1997;**336**:1855.
189. Chen RT, Glasser JW, Rhodes PH, et al. Vaccine Safety Datalink project: a new tool for improving vaccine safety monitoring in the United States. The Vaccine Safety Datalink Team. *Pediatrics* 1997; **99**:765.
190. Chen RT, Moses JM, Markowitz LE, et al. Adverse events following measles-mumps-rubella and measles vaccinations in college students. *Vaccine* 1991;**9**:297.
191. Chen RT, Rastogi SC, Mullen JR, et al. The Vaccine Adverse Event Reporting System (VAERS). *Vaccine* 1994;**12**:542.
192. Cherry JD, Brunell PA, Golden GS, et al. Report of the Task Force on Pertussis and Pertussis Immunization—1988. *Pediatrics* 1988;**81**:939.
193. Christenson B, Bottiger M. Measles antibody: comparison of long-term vaccination titres, early vaccination titres and naturally acquired immunity to and booster effects on the measles virus. *Vaccine* 1994;**12**:129.
194. Chu SY, Bernier RH, Stewart JA, et al. Rubella antibody persistence after immunization. *JAMA* 1988;**259**:3133.
195. Ciofi degli Atti ML, Olin P. Severe adverse events in the Italian and Stockholm I pertussis vaccine clinical trials. *Dev Biol Stand* 1997;**89**:77.
196. Classen D, Classen J. The timing of pediatric immunization and the risk of insulin-dependent diabetes mellitus. *Infect Dis Clin Pract* 1997;**6**:449.
197. Clements DA, Langdon L, Bland C, et al. Influenza A vaccine decreases the incidence of otitis media in 6- to 30-month-old children in day care. *Arch Pediatr Adolesc Med* 1995;**149**: 1113.
198. Clements DA, Moreira SP, Coplan PM, et al. Postlicensure study of varicella vaccine effectiveness in a day-care setting. *Pediatr Infect Dis J* 1999;**18**:1047.
199. Clifford GM, Smith JS, Plummer M, et al. Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. *Br J Cancer* 2003;**88**:63.
200. Clover RD, Crawford S, Glezen WP, et al. Comparison of heterotypic protection against influenza A/Taiwan/86 (H1N1) by attenuated and inactivated vaccines to A/Chile/83-like viruses. *J Infect Dis* 1991;**163**:300.
201. Cochi SL, Broome CV, Hightower MS. Immunization of US children with *Hemophilus influenzae* type b polysaccharide vaccine. *JAMA* 1985;**253**:521.
202. Cody CL, Baraff LJ, Cherry JD, et al. Nature and rates of adverse reactions associated with DTP and DT immunizations in infants and children. *Pediatrics* 1981;**68**:650.
203. Cohen C, White JM, Savage EJ, et al. Vaccine effectiveness estimates, 2004-2005 mumps outbreak, England. *Emerg Infect Dis* 2007;**13**:12.
204. Cohen R. Impact of 13-valent pneumococcal conjugate vaccine on pneumococcal nasopharyngeal carriage in children with acute otitis media. *Pediatr Infect Dis J* 2012;**31**:297.
205. Cohn ML, Robinson ED, Faerber M, et al. Measles vaccine failures: lack of sustained measles-specific immunoglobulin G responses in revaccinated adolescents and young adults. *Pediatr Infect Dis J* 1994;**13**:34.
206. Colditz GA, Berkey CS, Mosteller F, et al. The efficacy of bacillus Calmette-Guérin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. *Pediatrics* 1995;**96**:29.
207. Collins S, Mazloomzadeh S, Winter H, et al. High incidence of cervical human papillomavirus infection in women during their first sexual relationship. *BJOG* 2002;**109**:96.
208. Committee on Issues and Priorities for New Vaccine Development. *New vaccine development: establishing priorities. Vol I. Diseases of importance in the United States*. Washington, D.C.: National Academy Press; 1985.
209. Committee on Issues and Priorities for New Vaccine Development. *New vaccine develoment: establishing priorities. Vol II. Diseases*

of importance in developing countries. Washington, D.C.: National Academy Press; 1986.
210. Committee on Issues and Priorities for New Vaccine Development. *Vaccines for the 21st century: a tool for decisionmaking*. Washington, D.C.: National Academy Press; 1999.
211. Confavreux C, Suissa S, Saddier P, et al. Vaccinations and the risk of relapse in mltiple sclerosis. *N Engl J Med* 2001;**344**:319.
212. Cortese MM, Parashar UD. Prevention of rotavirus gastroenteritis among infants and children: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2009;**58**(RR-2):1–25.
213. Crossley K, Irving P, Warren JB, et al. Tetanus and diphtheria immunization in urban Minnesota adults. *JAMA* 1979;**242**:2298.
214. Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bull World Health Organ* 2004;**82**:346.
215. Cutts FT, Zaman SM, Enwere G, et al. Efficacy of nine-valent pneumococcal conjugate vaccine against pneumonia and invasive pneumococcal disease in The Gambia: randomised, double-blind, placebo-controlled trial. *Lancet* 2005;**365**:1139.
216. D'Hondt E. Possible approaches to develop vaccines against hepatitis A. *Vaccine* 1992;**10**:S48.
217. Dagan R, Amir J, Mijalovsky A, et al. Immunization against hepatitis A in the first year of life: priming despite the presence of maternal antibody. *Pediatr Infect Dis J* 2000;**19**:1045.
218. Dagan R, Givon-Lavi N, Fraser D, et al. Serum serotype-specific pneumococcal anticapsular immunoglobulin G concentrations after immunization with a 9-valent conjugate pneumococcal vaccine correlate with nasopharyngeal acquisition of pneumococcus. *J Infect Dis* 2005;**192**:367.
219. Dagan R, Leventhal A, Anis E, et al. Incidence of hepatitis A in Israel following universal immunization of toddlers. *JAMA* 2005;**294**:202.
220. Dagan R, Melamed R, Muallem M, et al. Reduction of nasopharyngeal carriage of pneumococci during the second year of life by a heptavalent conjugate pneumococcal vaccine. *J Infect Dis* 1996;**174**:1271.
221. Dagan R, Muallem M, Melamed R, et al. Reduction of pneumococcal nasopharyngeal carriage in early infancy after immunization with tetravalent pneumococcal vaccines conjugated to either tetanus toxoid or diphtheria toxoid. *Pediatr Infect Dis J* 1997; **16**:1060.
222. Danovaro-Holliday M, LeBaron C, Allensworth C, et al. A large rubella outbreak with spread from the workplace to the community. *JAMA* 2000;**284**:2733.
223. Date K, Mintz E. Cholera. In: Brunette G, editor. *CDC Health information for international travel 2012*. New York: Oxford University Press; 2012. p. 146.
224. Daubeney P, Taylor CJ, McGaw J, et al. Immunogenicity and tolerability of a trivalent influenza subunit vaccine (Influvac) in high-risk children aged 6 months to 4 years. *Br J Clin Pract* 1997;**51**:87.
225. Davidson M, Schraer CD, Parkinson AJ, et al. Invasive pneumococcal disease in an Alaska native population, 1980 through 1986. *JAMA* 1989;**261**:715.
226. Davis R, Kramarz P, Bohlke K, et al. Measles-mumps-rubella and other measles-containing vaccines do not increase the risk for inflammatory bowel disease: a case-control study from the vaccine safety datalink project. *Arch Pediatr Adolesc Med* 2001;**155**:354.
227. Decker MD, Edwards KM. Report of the nationwide multicenter acellular pertussis trial. *Pediatrics* 1995;**96**:547.
228. Decker MD, Edwards KM, Bradley R, et al. Comparative trial in infants of four conjugate *Haemophilus influenzae* type b vaccines. *J Pediatr* 1992;**1992**:184.
229. Dennehy PH, Bertrand HR, Silas PE, et al. Coadministration of RIX4414 oral human rotavirus vaccine does not impact the immune response to antigens contained in routine infant vaccines in the United States. *Pediatrics* 2008;**122**:e1062.
230. Dennehy PH, Brady RC, Halperin SA, et al. Comparative evaluation of safety and immunogenicity of two dosages of an oral live attenuated human rotavirus vaccine. *Pediatr Infect Dis J* 2005; **24**:481.
231. Dennehy PH, Goveia MG, Dallas MJ, et al. The integrated phase III safety profile of the pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *Int J Infect Dis* 2007;**11**(Suppl. 2):S36.
232. Denoel PA, Goldblatt D, de Vleeschauwer I, et al. Quality of the *Haemophilus influenzae* type b (Hib) antibody response induced by diphtheria-tetanus-acellular pertussis/Hib combination vaccines. *Clin Vaccine Immunol* 2007;**14**:1362.
233. DeStefano F. MMR vaccine and autism: a review of the evidence for a causal association. *Mol Psychiatry* 2002;**7**(Suppl. 2):S51.
234. DeStefano F, Thompson WW. MMR vaccine and autism: an update of the scientific evidence. *Expert Rev Vaccines* 2004;**3**:19.
235. Dominguez A, Salleras L, Carmona G, et al. Effectiveness of a mass hepatitis A vaccination program in preadolescents. *Vaccine* 2003;**21**:698.
236. Donahue J, Choo P, Manson J, et al. The incidence of herpes zoster. *Arch Intern Med* 1995;**155**:1605.
237. Dreesen D, Fishbein D, Kemp D, et al. Two-year comparative trial on the immunogenicity and adverse effects of purified chick embryo cell rabies vaccine for pre-exposure immunization. *Vaccine* 1989;**7**:397.
238. Dubischar-Kastner K, Eder S, Buerger V, et al. Long-term immunity and immune response to a booster dose following vaccination with the inactivated Japanese encephalitis vaccine IXIARO, IC51. *Vaccine* 2010;**28**:5197.
239. Dubischar-Kastner K, Kaltenboeck A, Klingler A, et al. Safety analysis of a Vero-cell culture derived Japanese encephalitis vaccine, IXIARO (IC51), in 6 months of follow-up. *Vaccine* 2010; **28**:6463.
240. Dunne EF, Markowitz LE. Genital human papillomavirus infection. *Clin Infect Dis* 2006;**43**:624.
241. Duvriendt J, Staroukine MM, Costy F, et al. Fatal encephalitis apparently due to rabies: occurrence after treatment with human diploid cell vaccine but not rabies immune globulin. *JAMA* 1982;**248**:2304.
242. Dworkin MS, Jennings CE, Roth-Thomas J, et al. An outbreak of varicella among children attending preschool and elementary school in Illinois. *Clin Infect Dis* 2002;**35**:102.
243. Edwards KM, Decker MD. Acellular pertussis vaccines for infants. *N Engl J Med* 1996;**334**:391.
244. Edwards KM, Meade BD, Decker MD, et al. Comparison of 13 acellular pertussis vaccines: overview and serologic response. *Pediatrics* 1995;**96**:548.
245. Eisenberg KW, Szilagyi PG, Fairbrother G, et al. Vaccine effectiveness against laboratory-confirmed influenza in children 6 to 59 months of age during the 2003-2004 and 2004–5 influenza seasons. *Pediatrics* 2008;**122**:911.
246. Ellis R. New technologies for making vaccines. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 1335.
247. Englund JA, Walter EB, Fairchok MP, et al. A comparison of 2 influenza vaccine schedules in 6- to 23-month-old children. *Pediatrics* 2005;**115**:1039.
248. Englund JA, Walter EB, Gbadebo A, et al. Immunization with trivalent inactivated influenza vaccine in partially immunized toddlers. *Pediatrics* 2006;**118**:e579.
249. Eskola J, Anttila M. Pneumococcal conjugate vaccines. *Pediatr Infect Dis J* 1999;**18**:543.
250. Eskola J, Kilpi T, Palmli A, et al. Efficacy of a pneumococcal conjugate vaccine against otitis media. *New Engl J Med* 2001; **344**:430.
251. Euler GL, Wooten KG, Baughman AL, et al. Hepatitis B surface antigen prevalence among pregnant women in urban areas: implications for testing, reporting, and preventing perinatal transmission. *Pediatrics* 2003;**111**:1192.
252. Evans G. National Childhood Vaccine Injury Act: revision of the vaccine injury table. *Pediatrics* 1996;**98**:1179.
253. Fine PEM, Clarkson JA. Reflections on the efficacy of pertussis vaccine. *Rev Infect Dis* 1987;**9**:866.
254. Fiore AE, Shapiro CN, Sabin K, et al. Hepatitis A vaccination of infants: effect of maternal antibody status on antibody persistence and response to a booster dose. *Pediatr Infect Dis J* 2003;**22**:354.
255. Fiore AE, Wasley A, Bell BP. Prevention of hepatitis A through active or passive immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;**55**(RR-7):1–23.
256. Fischer M, Lindsey N, Staples JE, et al. Japanese encephalitis vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-1): 1–27.
257. Fischer TK, Viboud C, Parashar U, et al. Hospitalizations and deaths from diarrhea and rotavirus among children <5 years

of age in the United States, 1993-2003. *J Infect Dis* 2007;**195**: 1117–25.
258. Fishbein DB, Sawyer LA, Reid-Sanden FL, et al. Administration of human diploid-cell rabies vaccine in the gluteal area [letter]. *N Engl J Med* 1988;**318**:124.
259. Fishbein DB, Yenne KM, Dreesen DW, et al. Risk factors for systemic hypersensitivity reactions after booster vaccinations with human diploid cell rabies vaccine: a nationwide prospective study. *Vaccine* 1993;**11**:1390.
260. Fombonne E, Zakarian R, Bennett A, et al. Pervasive developmental disorders in Montreal, Quebec, Canada: prevalence and links with immunizations. *Pediatrics* 2006;**118**:e139.
261. Freed GL, Katz SL, Clark SJ. Safety of vaccinations. Miss America, the media, and public health. *JAMA* 1996;**276**:1869.
262. Galazka AM, Robertson SE. Diphtheria: changing patterns in the developing world and the industrial world. *Eur J Epidemiol* 1995;**11**:107.
263. Galil K, Fair E, Mountcastle N, et al. Younger age at vaccination may increase risk of varicella vaccine failure. *J Infect Dis* 2002; **186**:102.
264. Gergen PJ, McQuillan GM, Kiely M, et al. A population-based serologic survey of immunity to tetanus in the United States. *N Engl J Med* 1995;**332**:761.
265. Gershon A, Takahashi M, Seward J. Live attenuated varicella vaccine. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*, 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 915.
266. Gershon AA, LaRussa PS. Varicella vaccine. *Pediatr Infect Dis J* 1998;**17**:248.
267. Gershon AA, Steinberg SP, LaRussa P, et al. Immunization of healthy adults with live attenuated varicella vaccine. *J Infect Dis* 1988;**158**:132.
268. Ghaffar F, Barton T, Lozano J, et al. Effect of the 7-valent pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* in the first 2 years of life. *Clin Infect Dis* 2004;**39**:930.
269. Giebink GS. Preventing pneumococcal disease in children: recommendations for using pneumococcal vaccine. *Pediatr Infect Dis* 1985;**4**:343.
270. Giebink GS, Le CT, Schiffman G. Decline of serum antibody in splenectomized children after vaccination with pneumococcal capsular polysaccharides. *J Pediatr* 1984;**105**:576.
271. Gilliland SM, Forrest L, Carre H, et al. Investigation of porcine circovirus contamination in human vaccines. *Biologicals* 2012;**40**: 270.
272. Glass RI, Kilgore PE, Holman RC, et al. The epidemiology of rotavirus diarrhea in the United States: surveillance and estimates of disease burden. *J Infect Dis* 1996;**174**(Suppl. 1):S5.
273. Glezen WP. Serious morbidity and mortality associated with influenza epidemics. *Epidemiol Rev* 1982;**4**:25.
274. Glezen WP, Couch RB. Interpandemic influenza in the Houston area, 1974-76. *N Engl J Med* 1978;**298**:587.
275. Glezen WP, Greenberg SB, Atmar RL, et al. Impact of respiratory virus infections on persons with chronic underlying conditions. *JAMA* 2000;**283**:499.
276. Glezen WP, Six H, Frank A, et al. Impact of epidemics upon communities and families. In Kendal A, Patriaca P, editors. *Options for the control of influenza*. New York: Alan R. Liss, Inc.; 1986. p. 63
277. Global Advisory Group Expanded Program on Immunization World Health Organization. Achieving the major disease control goals. *Wkly Epidemiol Rec* 1994;**69**:29.
278. Gold R, Lepow ML, Goldschneider I, et al. Kinetics of antibody production to Group A and Group C meningococcal polysaccharide vaccines administered during the first six years of life: prospects for routine immunization of infants and children. *J Infect Dis* 1979;**140**:690.
279. Gonzalez M, Pirez MC, Ward E, et al. Safety and immunogenicity of a paediatric presentation of an influenza vaccine. *Arch Dis Child* 2000;**83**:488.
280. Greco D, Salmaso S, Mastrantonio P, et al. A controlled trial of two acellular vaccines and one whole-cell vaccine against pertussis. Progetto Pertosse Working Group. *N Engl J Med* 1996; **334**:341.
281. Greenberg D, Lieberman J, Marcy S, et al. Enhanced antibody responses in infants given different sequences of heterogenous *Haemophilus influenzae* type b conjugate vaccines. *J Pediatr* 1995; **126**:206.
282. Greenberg DP. Pediatric experience with recombinant hepatitis B vaccines and relevant safety and immunogenicity studies. *Pediatr Infect Dis J* 1993;**12**:438.
283. Greenberg MA, Birx DL. Safe administration of mumps-measles-rubella vaccine in egg-allergic children. *J Pediatr* 1988; **113**:504.
284. Greene SK, Kulldorff M, Lewis EM, et al. Near real-time surveillance for influenza vaccine safety: proof-of-concept in the Vaccine Safety Datalink Project. *Am J Epidemiol* 2010;**171**:177.
285. Groothuis JR, Lehr MV, Levin MJ. Safety and immunogenicity of a purified haemagglutinin antigen in very young high-risk children. *Vaccine* 1994;**12**:139.
286. Gross PA, Russo C, Dran S, et al. Time to earliest peak serum antibody response to influenza vaccine in the elderly. *Clin Diagn Lab Immunol* 1997;**4**:491.
287. Guess HA, Broughton DD, Melton LJ 3rd, et al. Population-based studies of varicella complications. *Pediatrics* 1986;**78**:723.
288. Gupta RK, Griffin PJ, Xu J, et al. Diphtheria antitoxin levels in US blood and plasma donors. *J Infect Dis* 1996;**173**:1493.
289. Guris D, Jumaan AO, Mascola L, et al. Changing varicella epidemiology in active surveillance sites—United States, 1995-2005. *J Infect Dis* 2008;**197**(Suppl. 2):S71.
290. Gustafsson L, Hallander HO, Olin P, et al. A controlled trial of a two-component acellular, a five-component acellular, and a whole-cell pertussis vaccine. *N Engl J Med* 1996;**334**:349.
291. Guzman CA, Borsutzky S, Griot-Wenk M, et al. Vaccines against typhoid fever. *Vaccine* 2006;**24**:3804.
292. Hadler SC, McFarland L. Hepatitis in day care centers: epidemiology and prevention. *Rev Infect Dis* 1986;**8**:548.
293. Hall AJ, Greenwood BM, Whittle H. Modern vaccines: practice in developing countries. *Lancet* 1990;**1**:774.
294. Halperin SA, Gupta A, Jeanfreau R, et al. Comparison of the safety and immunogenicity of an investigational and a licensed quadrivalent meningococcal conjugate vaccine in children 2-10 years of age. *Vaccine* 2010;**28**:7865.
295. Halsey NA, Hyman SL. Measles-mumps-rubella vaccine and autistic spectrum disorder: report from the New Challenges in Childhood Immunizations Conference convened in Oak Brook, Illinois, June 12-3, 2000. *Pediatrics* 2001;**107**:E84.
296. Hambidge SJ, Glanz JM, France EK, et al. Safety of trivalent inactivated influenza vaccine in children 6 to 23 months old. *JAMA* 2006;**296**:1990.
297. Hammerschlag M, Gershon A, Steinberg S, et al. Herpes zoster in an adult recipient of live attenuated varicella vaccine. *J Infect Dis* 1989;**160**:535.
298. Hansen J, Black S, Shinefield H, et al. Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than 5 years of age for prevention of pneumonia: updated analysis using World Health Organization standardized interpretation of chest radiographs. *Pediatr Infect Dis J* 2006;**25**:779.
299. Hardy IRB, Dittmann S, Sutter RW. Current situation and control strategies for resurgence of diphtheria in newly independent states of the former Soviet Union. *Lancet* 1996;**347**:1739.
300. Harling R, White JM, Ramsay ME, et al. The effectiveness of the mumps component of the MMR vaccine: a case control study. *Vaccine* 2005;**23**:4070.
301. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet* 2004;**364**:1757.
302. Hart PDA, Sutherland I, Thomas J. The immunity conferred by effective BCG and vole bacillus vaccines, in relation to individual variations in induced tuberculin sensitivity and to technical variations in the vaccines. *Tubercle* 1967;**48**:201.
303. Heath PT, McVernon J. The UK Hib vaccine experience. *Arch Dis Child* 2002;**86**:396.
304. Heikkinen T, Ruuskanen O, Waris M, et al. Influenza vaccination in the prevention of acute otitis media in children. *Am J Dis Child* 1991;**145**:445.
305. Herman JJ, Radin R, Schneiderman R. Allergic reactions to measles (rubeola) vaccine in patients hypersensitive to egg protein. *J Pediatr* 1983;**102**:196.
306. Heron J, Golding J. Thimerosal exposure in infants and developmental disorders: a prospective cohort study in the United kingdom does not support a causal association. *Pediatrics* 2004; **114**:577.

307. Hessel L, Debois H, Fletcher M, et al. Experience with *Salmonella typhi* Vi capsular polysaccharide vaccine. *Eur J Clin Microbiol Infect Dis* 1999;**18**:609.
308. Hetherington S, Lepow ML. Epidemiology and immunology of *Hemophilus influenzae* type b infections in childhood: Implications for chemoprophylaxis and immunization. *Adv Pediatr Infect Dis* 1987;**2**:1.
309. Hill DR, Ford L, Lalloo DG. Oral cholera vaccines: use in clinical practice. *Lancet Infect Dis* 2006;**6**:361.
310. Hinman AR, Koplan JP. Pertussis and pertussis vaccine: reanalysis of benefits, risks and costs. *JAMA* 1984;**251**:3109.
311. Hinman AR, Orenstein WA, Schuchat A. Vaccine-preventable diseases, immunizations, and MMWR—1961-2011. *MMWR Surveill Summ* 2011;**60**(Suppl. 4):49.
312. Ho GY, Burk RD, Klein S, et al. Persistent genital human papillomavirus infection as a risk factor for persistent cervical dysplasia. *J Natl Cancer Inst* 1995;**87**:1365.
313. Hoberman A, Greenberg DP, Paradise JL, et al. Effectiveness of inactivated influenza vaccine in preventing acute otitis media in young children: a randomized controlled trial. *JAMA* 2003;**290**: 1608.
314. Howson CP, Katz M, Johnston RB Jr, et al. Chronic arthritis after rubella vaccination. *Clin Infect Dis* 1992;**15**:307.
315. Hsu HY, Chang MH, Liaw SH, et al. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999;**30**:1312.
316. Hviid A, Stellfeld M, Wohlfahrt J, et al. Association between thimerosal-containing vaccine and autism. *JAMA* 2003;**290**: 1763.
317. Ildirim I, Hacimustafaoglu M, Ediz B. Correlation of tuberculin induration with the number of Bacillus Calmette-Guérin vaccines. *Pediatr Infect Dis J* 1995;**14**:1060.
318. Innis BL, Snitbhan R, Kunasol P, et al. Protection against hepatitis A by an inactivated vaccine. *JAMA* 1994;**271**:1328.
319. Institute for Vaccine Safety Diabetes Workshop Panel. Childhood immunization and type 1 diabetes: summary of an Institute for Vaccine Safety workshop. *Pediatr Infect Dis J* 1999;**18**:217.
320. Institute of Medicine. Diphtheria and tetanus toxoids. In: Stratton KR, Howe CJ, Johnston RB Jr, editors. *Adverse events associated with childhood vaccines*. Washington, D.C.: National Academy Press; 1994. p. 67.
321. Institute of Medicine. Measles and mumps vaccine. In: Stratton KR, Howe CJ, Johnston RB Jr, editors. *Adverse events associated with childhood vaccines*. Washington, D.C.: National Academy Press; 1994. p. 118.
322. Institute of Medicine. In: Stratton K, Gable A, Shetty P, et al, editors. *Immunization safety review: measles-mumps-rubella vaccine and autism*. Washington, D.C.: National Academy Press; 2001.
323. Institute of Medicine. In: Stratton K, Gable A, McCormick M, editors. *Immunization safety review: thimerosal-containing vaccines and neurodevelopmental disorders*. Washington, D.C.: National Academy Press; 2001.
324. Institute of Medicine. *Immunization safety review: multiple immunizations and immune dysfunction*. Washington, D.C.: National Academy Press; 2002.
325. Institute of Medicine. *Immunization Safety Review: Vaccines and autism*. Washington, D.C.: National Academy Press; 2004.
326. Ipp MM, Gold R, Goldbach M, et al. Adverse reactions to diphtheria, tetanus, pertussis-polio vaccination at 18 months of age: effect of injection site and needle length. *Pediatrics* 1989;**83**:679.
327. Isaacman DJ. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. *Int J Infect Dis* 2010;**14**:e197.
328. Izurieta HS, Strebel PM, Blake PA. Postlicensure effectiveness of varicella vaccine during an outbreak in a childcare center. *JAMA* 1997;**278**:1495.
329. Jacobs RL, Lowe RS, Lanier BQ. Adverse reactions to tetanus toxoid. *JAMA* 1982;**247**:40.
330. James JM, Burks AW, Roberson PK, et al. Safe administration of the measles vaccine to children allergic to eggs. *N Engl J Med* 1995;**332**:1262.
331. Jefferson T, Rivetti A, Harnden A, et al. Vaccines for preventing influenza in healthy children. *Cochrane Database Syst Rev* 2008;(2): CD004879.
332. Jhung MA, Swerdlow D, Olsen SJ, et al. Epidemiology of 2009 pandemic influenza A (H1N1) in the United States. *Clin Infect Dis* 2011;**52**(Suppl. 1):S13.
333. Johnson CE, Stancin T, Fattlar D, et al. A long-term prospective study of varicella vaccine in healthy children. *Pediatrics* 1997; **100**:761.
334. Johnson KJ. From the CDC: new country-specific recommendations for pre-travel typhoid vaccination. *J Travel Med* 2011; **18**:430.
335. Johnson KJ. Crossing borders: one world, global health: CDC updates recommendations for typhoid vaccination. *Clin Infect Dis* 2012;**54**:v.
336. Kanra G, Yalcin SS, Kara A, et al. Hepatitis A booster vaccine in children after infant immunization. *Pediatr Infect Dis J* 2002; **21**:727.
337. Kaplan SL. *Streptococcus pneumoniae:* impact of antibiotic resistance in pediatrics. *Curr Probl Pediatr* 1997;**27**:187.
338. Karvonen M, Cepaitis Z, Tuomilehto J. Association between type 1 diabetes and *Haemophilus influenzae* type b vaccination: birth cohort study. *BMJ* 1999;**318**:1169.
339. Katz SL, Hinman AR. Summary and conclusions: measles elimination meeting, 16-17 March 2000. *J Infect Dis* 2004; **189**(Suppl. 1):S43.
340. Kaufmann SH. Fact and fiction in tuberculosis vaccine research: 10 years later. *Lancet Infect Dis* 2011;**11**:633.
341. Käyhty H, Karanko V, Peltolta H, et al. Serum antibodies to capsular polysaccharide vaccine of group A *Neisseria meningitidis* followed for three years in infants and children. *J Infect Dis* 1980;**142**:861.
342. Kelso JM, Greenhawt MJ, Li JT, et al. Adverse reactions to vaccines practice parameter 2012 update. *J Allergy Clin Immunol* 2012;**130**:25.
343. Kelso JM, Jones RT, Yunginger JW. Anaphylaxis to measles, mumps, and rubella vaccine mediated by IgE to gelatin. *J Allergy Clin Immunol* 1993;**91**:867.
344. Kemp A, Van Asperen P, Mukhi A. Measles immunization in children with clinical reactions to egg protein. *Am J Dis Child* 1990;**144**:33.
345. Kew O, Morris-Glasgow V, Landaverde M, et al. Outbreak of poliomyelitis in Hispaniola associated with circulating type 1 vaccine-derived poliovirus. *Science* 2002;**296**:356.
346. Kew OM, Wright PF, Agol VI, et al. Circulating vaccine-derived polioviruses: current state of knowledge. *Bull World Health Organ* 2004;**82**:16.
347. Kim-Farley RJ, Rutherford G, Lichfield P, et al. Outbreak of paralytic poliomyelitis, Taiwan. *Lancet* 1984;**2**:1322.
348. King GE, Hadler SC. Simultaneous administration of childhood vaccines: an important public health policy that is safe and efficacious. *Pediatr Infect Dis J* 1994;**13**:394.
349. Klein NP, Fireman B, Yih WK, et al. Measles-mumps-rubella-varicella combination vaccine and the risk of febrile seizures. *Pediatrics* 2010;**126**:e1.
350. Klein NP, Lewis E, Baxter R, et al. Measles-containing vaccines and febrile seizures in children age 4 to 6 years. *Pediatrics* 2012; **129**:809.
351. Klugman KP, Gilbertson IT, Koornhof HJ, et al. Protective activity of Vi capsular polysaccharide vaccine against typhoid fever. *Lancet* 1987;**2**:1165.
352. Klugman KP, Madhi SA, Huebner RE, et al. A trial of a 9-valent pneumococcal conjugate vaccine in children with and those without HIV infection. *N Engl J Med* 2003;**349**:1341.
353. Koff RS. Seroepidemiology of hepatitis A in the United States. *J Infect Dis* 1995;**171**:S19.
354. Konradsen HB, Pedersen FK, Henrichsen J. Pneumococcal revaccination of splenectomized children. *Pediatr Infect Dis J* 1990;**9**:258.
355. Koutsky LA, Harper DM. Chapter 13: Current findings from prophylactic HPV vaccine trials. *Vaccine* 2006;**24**(Suppl. 3):S114.
356. Kramarz P, Destefano F, Gargiullo PM, et al. Does influenza vaccination prevent asthma exacerbations in children? *J Pediatr* 2001;**138**:306.
357. Kretsinger K, Broder KR, Cortese MM, et al. Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP) and recommendation of ACIP, supported by the Healthcare Infection Control Practices Advisory Committee

(HICPAC), for use of Tdap among health-care personnel. *MMWR Recomm Rep* 2006;**55**(RR-17):1–37.
358. Kroger A, Sumaya C, Pickering L, et al. General recommendations on immunization—recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**:1.
359. Kroger AT, Atkinson WL, Marcuse EK, et al. General recommendations on immunization: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2006;**55**:1.
360. Kröger L, Korppi M, Brander E, et al. Osteitis caused by Bacille Calmette-Guérin vaccination: a retrospective analysis of 222 cases. *J Infect Dis* 1995;**172**:574.
361. Krugman RD, Hardy GE Jr, Sellers C, et al. Antibody persistence after primary immunization with trivalent oral poliovirus vaccine. *Pediatrics* 1977;**60**:80.
362. Krugman S. Further attenuated measles vaccine: characteristics and use. *Rev Infect Dis* 1983;**5**:477.
363. Kumar D, Beach NM, Meng XJ, et al. Use of PCR-based assays for the detection of the adventitious agent porcine circovirus type 1 (PCV1) in vaccines, and for confirming the identity of cell substrates and viruses used in vaccine production. *J Virol Methods* 2012;**179**:201.
364. Kuter BJ, Brown ML, Hartzel J, et al. Safety and immunogenicity of a combination measles, mumps, rubella and varicella vaccine (ProQuad). *Hum Vaccin* 2006;**2**:205.
365. Kuter BJ, Matthews H, Shinefield H, et al. Ten year follow-up of healthy children who received one or two injections of varicella vaccine. *Pediatr Infect Dis J* 2004;**23**:132.
366. Kuter BJ, Weibel RE, Guess HA, et al. Oka/Merck varicella vaccine in healthy children: final report of a 2-year efficacy study and 7-year follow-up studies. *Vaccine* 1991;**9**:643.
367. La Montagne JR, Noble GR, Quinnan GV, et al. Summary of clinical trials of inactivated influenza vaccine—1978. *Rev Infect Dis* 1983;**5**:723.
368. LaRussa P, Steinberg S, Meurice F, et al. Transmission of vaccine strain varicella-zoster virus from a healthy adult with vaccine-associated rash to susceptible household contacts. *J Infect Dis* 1997;**176**:1072.
369. Lavi S, Zimmerman B, Koren G, et al. Administration of measles, mumps and rubella virus vaccine (live) to egg-allergic children. *JAMA* 1990;**263**:269.
370. LeBaron CW, Bi D, Sullivan BJ, et al. Evaluation of potentially common adverse events associated with the first and second doses of measles-mumps-rubella vaccine. *Pediatrics* 2006;**118**:1422.
371. Lerman SJ, Bollinger M, Brunken JM. Clinical and serological evaluation of measles, mumps, and rubella (HPV-77:DE-5 and RA27/3) virus vaccines, singly and in combination. *Pediatrics* 1981;**68**:18.
372. Leroy Z, Broder K, Menschik D, et al. Febrile seizures after 2010-2011 influenza vaccine in young children, United States: a vaccine safety signal from the vaccine adverse event reporting system. *Vaccine* 2012;**30**:2020.
373. Letson GW, Shapiro CN, Kuehn D, et al. Effect of maternal antibody on immunogenicity of hepatitis A vaccine in infants. *J Pediatr* 2004;**144**:327.
374. Levine MM. Modern vaccines: enteric vaccines. *Lancet* 1990; **335**:958.
375. Levine MM, Noriega F. Vaccines to prevent bacterial enteric infections in children. *Pediatr Ann* 1993;**22**:719.
376. Lieberman J, Marcy S, Partridge S, et al: Hepatitis A vaccine in infants: effect of maternal antibodies on the antibody response [abstract]. Presented before the 36th annual meeting of the Infectious Disease Society of America. Denver, CO, 1998.
377. Lieberman JM, Chang SJ, Partridge S, et al. Kinetics of maternal hepatitis A antibody decay in infants: implications for vaccine use. *Pediatr Infect Dis J* 2002;**21**:347.
378. Lindsey NP, Schroeder BA, Miller ER, et al. Adverse event reports following yellow fever vaccination. *Vaccine* 2008;**26**:6077.
379. Long SS. Toddler-to-mother transmission of varicella-vaccine virus: how bad is that? *J Pediatrics* 1997;**131**:10.
380. Lotte A, Wasz-Hockert O, Poisson N, et al. BCG complications: estimates of the risks among vaccinated subjects and statistical analysis of their main characteristics. *Adv Tuberc Res* 1984;**21**:107.
381. Lowy DR, Schiller JT. Prophylactic human papillomavirus vaccines. *J Clin Invest* 2006;**116**:1167.
382. Luman ET, Fiore AE, Strine TW, et al. Impact of thimerosal-related changes in hepatitis B vaccine birth-dose recommendations on childhood vaccination coverage. *JAMA* 2004;**291**:2351.
383. Mackell S. Vaccine recommendations for infants & children. In: Brunette G, editor. *CDC health information for international travel 2012*. New York: Oxford University Press; 2012. p. 490.
384. MacKellar DA, Valleroy LA, Secura GM, et al. Two decades after vaccine license: hepatitis B immunization and infection among young men who have sex with men. *Am J Public Health* 2001; **91**:965.
385. Maldonado YA, Lawrence EC, DeHovitz R, et al. Early loss of passive measles antibody in infants of mothers with vaccine-induced immunity. *Pediatrics* 1995;**96**:447.
386. Malek MA, Curns AT, Holman RC, et al. Diarrhea- and rotavirus-associated hospitalizations among children less than 5 years of age: United States, 1997 and 2000. *Pediatrics* 2006;**117**:1887.
387. Manning SE, Rupprecht CE, Fishbein D, et al. Human rabies prevention—United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008;**57**(RR-3):1–28.
388. Maple PA, Efstratiou A, George RC, et al. Diphtheria immunity in UK blood donors. *Lancet* 1995;**345**:963.
389. Marfin AA, Eidex RS, Kozarsky PE, et al. Yellow fever and Japanese encephalitis vaccines: indications and complications. *Infect Dis Clin North Am* 2005;**19**:151.
390. Marin M, Broder KR, Temte JL, et al. Use of combination measles, mumps, rubella, and varicella vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-3):1–12.
391. Marin M, Guris D, Chaves SS, et al. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007;**56**(RR-4):1–40.
392. Markowitz L, Albrecht P, Orenstein W, et al. Persistence of measles antibody after revaccination. *J Infect Dis* 1992;**166**:205.
393. Markowitz LE, Albrecht P, Rhodes P, et al. Changing levels of measles antibody titers in women and children in the United States: impact on response to vaccination. *Pediatrics* 1996;**97**:53.
394. Markowitz LE, Dunne EF, Saraiya M, et al. Quadrivalent human papillomavirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007;**56**:1.
395. Markowitz LE, Preblud SR, Fine PE, et al. Duration of live measles vaccine-induced immunity. *Pediatr Infect Dis J* 1990;**9**:101.
396. Markowitz LE, Preblud SR, Orenstein WA, et al. Patterns of transmission in measles outbreaks in the United States, 1985-1986. *N Engl J Med* 1989;**320**:75.
397. Martin M, Tsai T, Cropp B, et al. Fever and multi-system organ failure associated with 17D-204 yellow fever vaccination: a report of four cases. *Lancet* 2001;**358**:98.
398. Marwick C. Guarded endorsement for Lyme disease vaccine [news]. *JAMA* 1998;**279**:1937.
399. Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005;**54**(RR-16):1–31.
400. Mast EE, Weinbaum CM, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP), part II: immunization of adults. *MMWR Recomm Rep* 2006;**55**(RR-16): 1–33.
401. Mathias RG, Meekison WG, Arcand TA, et al. The role of secondary vaccine failures in measles outbreaks. *Am J Public Health* 1989;**79**:475.
402. Mawhinney H, Allen I, Beare J, et al. Dysgammaglobulinaemia complicated by disseminated measles. *BMJ* 1971;**2**:380.
403. McBean AM, Thoms ML, Albrecht P, et al. Serologic response to oral polio vaccine and enhanced-potency inactivated polio vaccine. *Am J Epidemiol* 1988;**128**:615.
404. McClenahan SD, Krause PR, Uhlenhaut C. Molecular and infectivity studies of porcine circovirus in vaccines. *Vaccine* 2011; **29**:4745.
405. McCormick JB, Gusmao HH, Nakamura S, et al. Antibody response to serogroup A and C meningococcal polysaccharide

vaccines in infants born of mothers vaccinated during pregnancy. *J Clin Invest* 1980;**65**:1141.
406. McCullers JA, Saito T, Iverson AR. Multiple genotypes of influenza B virus circulated between 1979 and 2003. *J Virol* 2004; **78**:12817.
407. McLaughlin M, Thomas P, Onorato I, et al. Live virus vaccines in human immunodeficiency virus-infected children: a retrospective survey. *Pediatrics* 1988;**82**:229.
408. McMahon AW, Eidex RB, Marfin AA, et al. Neurologic disease associated with 17D-204 yellow fever vaccination: a report of 15 cases. *Vaccine* 2007;**25**:1727.
409. McQuillan GM, Kruszon-Moran D, Deforest A, et al. Serologic immunity to diphtheria and tetanus in the United States. *Ann Intern Med* 2002;**136**:660.
410. McVernon J, Andrews N, Slack MP, et al. Risk of vaccine failure after *Haemophilus influenzae* type b (Hib) combination vaccines with acellular pertussis. *Lancet* 2003;**361**:1521.
411. Merck & Co. Inc. Package Insert, GARDASIL® [Quadrivalent Human Papillomavirus (Types 6, 11, 16, 18) Recombinant Vaccine]. Whitehouse Station, NJ; 2006.
412. Michalik DE, Steinberg SP, Larussa PS, et al. Primary vaccine failure after 1 dose of varicella vaccine in healthy children. *J Infect Dis* 2008;**197**:944.
413. Midthun K, Horne AD, Goldenthal KL. Clinical safety evaluation of combination vaccines. *Dev Biol Stand* 1998;**95**:245.
414. Millar EV, O'Brien KL, Watt JP, et al. Effect of community-wide conjugate pneumococcal vaccine use in infancy on nasopharyngeal carriage through 3 years of age: a cross-sectional study in a high-risk population. *Clin Infect Dis* 2006;**43**:8.
415. Miller D, Madge N, Diamond J, et al. Pertussis immunisation and serious acute neurological illnesses in children. *BMJ* 1993; **307**:1171.
416. Miller D, Wadsworth J, Diamond J, et al. Pertussis vaccine and whooping cough and risk factors in acute neurological illness and death in young children. *Dev Biol Stand* 1985;**61**:389.
417. Miller E, Hill A, Morgan-Capner P, et al. Antibodies to measles, mumps and rubella in UK children 4 years after vaccination with different MMR vaccines. *Vaccine* 1995;**13**:799.
418. Mitus A, Holloway A, Evans A, et al. Attenuated measles vaccine in children with acute leukemia. *Am J Dis Child* 1962;**103**:413.
419. Mofenson LM, Brady MT, Danner SP, et al. Guidelines for the prevention and treatment of opportunistic infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. *MMWR Recomm Rep* 2009;**58**(RR-11):1–166.
420. Molano M, Van den Brule A, Plummer M, et al. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. *Am J Epidemiol* 2003;**158**:486.
421. Monafo W, Haslam D, Roberts R, et al. Disseminated measles infection following vaccination in a child with a congenital immune deficiency. *J Pediatr* 1994;**124**:273.
422. Monath T, Cetron M, Teuwen D. Yellow fever vaccine. In Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 959.
423. Monath TP. Flaviviruses. In: Fields BN, Knipe DM, editors. *Virology*. New York: Raven Press; 1990. p. 763.
424. Monto AS, Kioumehr F. The Tecumseh Study of Respiratory Illness, part IX: occurence of influenza in the community, 1966–1971. *Am J Epidemiol* 1975;**102**:553.
425. Morris D, Montgomery S, Thompson N, et al. Measles vaccine and inflammatory bowel disease: a national British cohort study. *Am J Gastroenterol* 2000;**95**:3507.
426. Morris SK, Moss WJ, Halsey N. *Haemophilus influenzae* type b conjugate vaccine use and effectiveness. *Lancet Infect Dis* 2008; **8**:435.
427. Mortimer EA Jr. Pertussis and pertussis vaccine. In: Aronoff SC, editor. Advances in pediatric infectious diseases, **vol. 5**. Chicago: Year Book Medical Publisher; 1990. p. 1.
428. Moxon ER, Rappuoli R. Modern vaccines: *Haemophilus influenzae* infections and whooping cough. *Lancet* 1990;**335**:1324.
429. Munoz N, Bosch FX, de Sanjose S, et al. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003;**348**:518.
430. Murphy TV, Gargiullo PM, Massoudi MS, et al. Intussusception among infants given an oral rotavirus vaccine. *N Engl J Med* 2001;**344**:564.
431. Myaux JA, Unicomb L, Besser RE, et al. Effect of diarrhea on the humoral response to oral polio vaccination. *Pediatr Infect Dis J* 1996;**15**:204.
432. National Vaccine Advisory Committee. Standards for child and adolescent immunization practices. National Vaccine Advisory Committee. *Pediatrics* 2003;**112**:958.
433. Negri E, Colombo C, Giordano L, et al. Influenza vaccine in healthy children: a meta-analysis. *Vaccine* 2005;**23**:2851.
434. Neuzil KM, Dupont WD, Wright PF, et al. Efficacy of inactivated and cold-adapted vaccines against influenza A infection, 1985 to 1990: the pediatric experience. *Pediatr Infect Dis J* 2001; **20**:733.
435. Neuzil KM, Jackson LA, Nelson J, et al. Immunogenicity and reactogenicity of 1 versus 2 doses of trivalent inactivated influenza vaccine in vaccine-naive 5-8-year-old children. *J Infect Dis* 2006; **194**:1032.
436. Nguyen HQ, Jumaan AO, Seward JF. Decline in mortality due to varicella after implementation of varicella vaccination in the United States. *N Engl J Med* 2005;**352**:450.
437. Nkowane BM, Bart KJ, Orenstein WA, et al. Measles outbreak in a vaccinated school population: epidemiology, strains of transmission and the role of vaccine failures. *Am J Public Health* 1987; **77**:434.
438. Noah D, Drenzek C, Smith J, et al. Epidemiology of human rabies in the United States, 1980 to 1996. *Ann Intern Med* 1998;**128**: 922–30.
439. Nolan T, McVernon J, Skeljo M, et al. Immunogenicity of a monovalent 2009 influenza A(H1N1) vaccine in infants and children: a randomized trial. *JAMA* 2010;**303**:37.
440. Nuorti JP, Whitney CG. Prevention of pneumococcal disease among infants and children—use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-11): 1–18.
441. O'Brien K, Ruff A, Louis M, et al. Bacillus Calmette-Guérin complications in children born to HIV-1-infected women with a review of the literature. *Pediatrics* 1995;**95**:414.
442. Ong BA, Forester J, Fallot A. Does influenza vaccination improve pediatric asthma outcomes? *J Asthma* 2009;**46**:477.
443. Onorato IM, Orenstein WA, Hinman AR, et al. Immunization of asymptomatic HIV-infected children with measles vaccine: assessment of risks and benefits. *Med Decis Making* 1989; **9**:76.
444. Onorato IM, Wassilak SG, Meade B. Efficacy of whole-cell pertussis vaccine in preschool children in the United States. *JAMA* 1992;**267**:2745.
445. Orenstein WA, Albrecht P, Herrmann KL, et al. The plaque-neutralization test as a maesure of prior exposure to measles virus. *J Infect Dis* 1987;**155**:146.
446. Orenstein WA, Bart KJ, Hinman HR, et al. The opportunity and obligation to eliminate rubella from the United States. *JAMA* 1984;**251**:1988.
447. Oya A. Japanese encephalitis vaccine. *Acta Pediatr Jpn* 1988; **30**:175.
448. Ozanne G, d'Halewyn M. Secondary immune response in a vaccinated population during a large measles epidemic. *Clin Microbiol* 1992;**30**:1778–82.
449. Palumbo P, Hoyt L, Demasio K, et al. Population-based study of measles and measles immunization in human immunodeficiency virus-infected children. *Pediatr Infect Dis J* 1992;**11**:1008.
450. Park CL, Frank AL, Sullivan M, et al. Influenza vaccination of children during acute asthma exacerbation and concurrent prednisone therapy. *Pediatrics* 1996;**98**:196.
451. Parker Fiebelkorn A, Redd SB, Gallagher K, et al. Measles in the United States during the postelimination era. *J Infect Dis* 2010; **202**:1520.
452. Pascual FB, McGinley EL, Zanardi LR, et al. Tetanus surveillance—United States, 1998–2000. *MMWR Surveill Summ* 2003;**52**:1–8.
453. Patel MM, Lopez-Collada VR, Bulhoes MM, et al. Intussusception risk and health benefits of rotavirus vaccination in Mexico and Brazil. *N Engl J Med* 2011;**364**:2283.

454. Patja A, Davidkin I, Kurki T, et al. Serious adverse events after measles-mumps-rubella vaccination during a fourteen-year prospective follow-up. *Pediatr Infect Dis J* 2000;**19**:1127.
455. Patriarca PA, Wright PF, John TJ. Factors affecting the immunogenicity of oral poliovirus vaccine in developing countries: review. *Rev Infect Dis* 1991;**13**:926.
456. Peebles TC, Levine L, Eldred MC, et al. Tetanus toxoid emergency bosters: a reappraisal. *N Engl J Med* 1969;**280**:575.
457. Peltola H, Heinonen O. Frequency of true adverse reactions to measles-mumps-rubella vaccine. *Lancet* 1986;**1**:939.
458. Peltola H, Heinonen OP, Valle M, et al. The elimination of indigenous measles, mumps, and rubella from Finland by a 12-year, two-dose vaccination program. *N Engl J Med* 1994; **331**:1397.
459. Peltola H, Mäkelä PH, Käyhty H, et al. Clinical efficacy of meningococcus group A capsular polysaccharide vaccine in children three months to five years of age. *N Engl J Med* 1977;**297**:686.
460. Peter G. Vaccine crisis: an emerging societal problem. *J Infect Dis* 1985;**151**:981.
461. Phua KB, Quak SH, Lee BW, et al. Evaluation of RIX4414, a live, attenuated rotavirus vaccine, in a randomized, double-blind, placebo-controlled phase 2 trial involving 2464 Singaporean infants. *J Infect Dis* 2005;**192**(Suppl. 1):S6.
462. Piazza M, Safary A, Vegnente A, et al. Safety and immunogenicity of hepatitis A vaccine in infants: a candidate for inclusion in the childhood vaccination programme. *Vaccine* 1999;**17**:585.
463. Pichichero ME, Edwards KM, Anderson EL, et al. Safety and immunogenicity of six acellular pertussis vaccines and one whole-cell vaccine given as a fifth dose in four- to six-year-old children. *Pediatrics* 2000;**105**:e11.
464. Plennevaux E, Sheldon E, Blatter M, et al. Immune response after a single vaccination against 2009 influenza A H1N1 in USA: a preliminary report of two randomised controlled phase 2 trials. *Lancet* 2010;**375**:41.
465. Plotkin S, Cadoz M. The acellular pertussis vaccine trials: an interpretation. *Pediatr Infect Dis J* 1997;**16**:508.
466. Plotkin S, Rubin S. Mumps vaccines. In: Plotkin S, Orenstein W, Offit P, editors. *Vaccines*. 5th ed. Philadelphia: Saunders Elsevier; 2008. p. 435.
467. Poehling KA, Edwards KM, Weinberg GA, et al. The underrecognized burden of influenza in young children. *N Engl J Med* 2006;**355**:31.
468. Poehling KA, Talbot TR, Griffin MR, et al. Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. *JAMA* 2006;**295**:1668.
469. Reef SE, Redd SB, Abernathy E, et al. The epidemiological profile of rubella and congenital rubella syndrome in the United States, 1998-2004: the evidence for absence of endemic transmission. *Clin Infect Dis* 2006;**43**(Suppl. 3):S126.
470. Reefhuis J, Honein MA, Whitney CG, et al. Risk of bacterial meningitis in children with cochlear implants. *N Engl J Med* 2003;**349**:435.
471. Reingold AL, Hightower AW, Bolan GA, et al. Age-specific differences in duration of clinical protection after vaccination with meningococcal polysaccharide A vaccine. *Lancet* 1985;**2**:114.
472. Rennels MB, Deloria MA, Pichichero ME, et al. Extensive swelling after booster doses of acellular pertussis-tetanus-diphtheria vaccines. *Pediatrics* 2000;**105**:e12.
473. Rigau-Perez JG, Overturf GD, Chan LS, et al. Reactions to booster pneumococcal vaccination in patients with sickle cell disease. *J Pediatr Infect Dis* 1983;**2**:199.
474. Ritzwoller DP, Bridges CB, Shetterly S, et al. Effectiveness of the 2003-4 influenza vaccine among children 6 months to 8 years of age, with 1 vs 2 doses. *Pediatrics* 2005;**116**:153.
475. Roberts L. Polio endgame. Polio: the final assault? *Science* 2004;**303**:1960.
476. Robertson SE, Traverso HP, Drucker JA, et al. Clinical efficacy of a new, enhanced-potency, inactivated poliovirus vaccine. *Lancet* 1988;**1**:897.
477. Robinson J, Lemay M, Vaudry W. Congenital rubella after anticipated maternal immunity: two cases and a review of the literature. *Pediatr Infect Dis J* 1994;**13**:812.
478. Rodier PM. The early origins of autism. *Sci Am* 2000;**282**:56.
479. Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. *Int J Epidemiol* 1993;**22**:1154.
480. Rodriguez ZM, Goveia MG, Stek JE, et al. Concomitant use of an oral live pentavalent human-bovine reassortant rotavirus vaccine with licensed parenteral pediatric vaccines in the United States. *Pediatr Infect Dis J* 2007;**26**:221.
481. Rogers DJ, Wilson AJ, Hay SI, et al. The global distribution of yellow fever and dengue. *Adv Parasitol* 2006;**62**:181.
482. Rosenzweig EC, Babione RW, Wisseman CL Jr. Immunological studies with group B arthropod-borne viruses, part IV: persistence of yellow fever antibodies following vaccination with 17D strain yellow fever vaccine. *Am J Trop Med Hyg* 1963;**12**:230.
483. Ross SC, Densen P. Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. *Medicine* 1984;**63**:243.
484. Rotz LD, Dotson DA, Damon IK, et al. Vaccinia (smallpox) vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2001. *MMWR Recomm Rep* 2001; **50**:1.
485. Ruben FL, Hankins WA, Zeigler Z, et al. Antibody responses to meningococcal polysaccharide vaccine in adults without a spleen. *Am J Med* 1984;**76**:115.
486. Ruiz-Palacios GM, Perez-Schael I, Velazquez FR, et al. Safety and efficacy of an attenuated vaccine against severe rotavirus gastroenteritis. *N Engl J Med* 2006;**354**:11.
487. Rupprecht CE, Briggs D, Brown CM, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies: recommendations of the advisory committee on immunization practices. *MMWR Recomm Rep* 2010;**59**(RR-2): 1–9.
488. Saha A. Safety and immunogenicity study of a killed bivalent (O1 and O139) whole-cell oral cholera vaccine Shanchol, in Bangladeshi adults and children as young as 1 year of age. *Vaccine* 2011; **29**:8285.
489. Salzman M, Garcia C. Postexposure varicella vaccination in siblings of children with active varicella. *Pediatr Infect Dis J* 1998;**17**:256.
490. Salzman MB, Sharrar RG, Steinberg S, et al. Transmission of varicella-vaccine virus from a healthy 12-month-old child to his pregnant mother. *J Pediatr* 1997;**131**:151.
491. Santosham M, Wolff M, Reid R, et al. The efficacy in Navajo infants of a conjugate vaccine consisting of *Haemophilus influenzae* type b polysaccharide and *Neisseria meningitidis* outer-membrane protein complex. *N Engl J Med* 1991;**324**:1767.
492. Schaffner W, Fleet WF, Kilroy AW, et al. Polyneuropathy following rubella immunization: a follow-up and review of the problem. *Am J Dis Child* 1974;**127**:684.
493. Schmitt-Grohe S, Stehr K, Cherry JD, et al. Minor adverse events in a comparative efficacy trial in Germany in infants receiving either the Lederle/Takeda acellular pertussis component DTP (DTaP) vaccine, the Lederle whole-cell component DTP (DTP) or DT vaccine. The Pertussis Vaccine Study Group. *Dev Biol Stand* 1997;**89**:113.
494. Schuchat A, Robinson K, Wenger JD, et al. Bacterial meningitis in the United States in 1995. Active Surveillance Team. *N Engl J Med* 1997;**337**:970.
495. Schuller E, Klingler A, Dubischar-Kastner K, et al. Safety profile of the Vero cell–derived Japanese encephalitis virus (JEV) vaccine IXIARO. *Vaccine* 2011;**29**:8669.
496. Seager C, Moriarity J, Ngai A, et al. Low incidence of adverse experiences after measles or measles-rubella mass revaccination at a college campus. *Vaccine* 1994;**12**:1018.
497. Seward JF, Marin M, Vazquez M. Varicella vaccine effectiveness in the US vaccination program: a review. *J Infect Dis* 2008;**197**(Suppl. 2):S82.
498. Seward JF, Zhang JX, Maupin TJ, et al. Contagiousness of varicella in vaccinated cases: a household contact study. *JAMA* 2004;**292**:704.
499. Shapiro ED, Berg AT, Austrian R, et al. The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *N Engl J Med* 1991;**325**:1453.
500. Shapiro ED, Vazquez M, Esposito D, et al. Effectiveness of 2 doses of varicella vaccine in children. *J Infect Dis* 2011;**203**:312.
501. Sharrar R, LaRussa P, Galea S, et al. The postmarketing safety profile of varicella vaccine. *Vaccine* 2000;**19**:916.
502. Shill M, Baynes RD, Miller SD. Fatal rabies encephalitis despite appropriate post-exposure prophylaxis: a case report. *N Engl J Med* 1987;**316**:1257.

503. Shin S, Desai SN, Sah BK, et al. Oral vaccines against cholera. *Clin Infect Dis* 2011;**52**:1343.
504. Shinefield HR, Black S. Efficacy of pneumococcal conjugate vaccines in large scale field trials. *Pediatr Infect Dis J* 2000;**19**:394.
505. Shuler CM, Iwamoto M, Bridges CB, et al. Vaccine effectiveness against medically attended, laboratory-confirmed influenza among children aged 6 to 59 months, 2003-2004. *Pediatrics* 2007;**119**:e587.
506. Siber GR, Werner BG, Halsey NA, et al. Interference of immune globulin with measles and rubella immunization. *J Pediatr* 1993; **122**:204.
507. Siddiqui FJ, Rabbani F, Hasan R, et al. Typhoid fever in children: some epidemiological considerations from Karachi, Pakistan. *Int J Infect Dis* 2006;**10**:215.
508. Simanjuntak CH, Paleologo FP, Punjabi NH, et al. Oral immunisation against typhoid fever in Indonesia with Ty21a vaccine. *Lancet* 1991;**338**:1055.
509. Simoes EA, John TJ. The antibody response of seronegative infants to inactivated poliovirus vaccine of enhanced potency. *J Biol Stand* 1986;**14**:127.
510. Sinha A, Sazawal S, Kumar R, et al. Typhoid fever in children aged less than 5 years. *Lancet* 1999;**354**:734.
511. Song J-H. The relationship between pneumococcal serotypes and antibiotic resistance. *Vaccine* 2012;**30**:2728.
512. Sprauer MA, Markowitz LE, Nicholson JK, et al. Response of human immunodeficiency virus–infected adults to measles-rubella vaccination. *J Acquir Immune Defic Syndr* 1993;**6**:1013.
513. Srivastava P, Brown K, Chen J, et al: Trends in tetanus epidemiology in the US, 1972-2001. Presented before the 39th National Immunization Conference, Washington D.C., 2005.
514. Stanley M, Lowy DR, Frazer I. Prophylactic HPV vaccines: underlying mechanisms. *Vaccine* 2006;**24**(Suppl. 3):S106.
515. Staples JE, Gershman M, Fischer M. Yellow fever vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2010;**59**(RR-7):1–27.
516. Steffen R, Kane MA, Shapiro CN, et al. Epidemiology and prevention of hepatitis A in travelers. *JAMA* 1994;**272**:885.
517. Stone M, Vannier A, Storch S, et al. Brief report: meningitis due to iatrogenic BCG infection in two immunocompromised children. *N Engl J Med* 1995;**333**:561.
518. Stratton KR, Howe CJ, Johnston RB, Jr, editors. *DPT vaccine and chronic nervous system dysfunction: a new analysis*. Washington, D.C.: National Academy Press; 1994.
519. Sugaya N, Nerome K, Ishida M, et al. Efficacy of inactivated vaccine in preventing antigenically drifted influenza type A and well-matched type B. *JAMA* 1994;**272**:1122.
520. Sur D, Kanungo S, Sah B, et al. Efficacy of a low-cost, inactivated whole-cell oral cholera vaccine: results from 3 years of follow-up of a randomized, controlled trial. *PLoS Negl Trop Dis* 2011;**5**: e1289.
521. Sur D, Ochiai RL, Bhattacharya SK, et al. A cluster-randomized effectiveness trial of Vi typhoid vaccine in India. *N Engl J Med* 2009;**361**:335.
522. Sutter RW, John TJ, Jain H, et al. Immunogenicity of bivalent types 1 and 3 oral poliovirus vaccine: a randomised, double-blind, controlled trial. *Lancet* 2010;**376**:1682.
523. Swartz TA, Handsher R, Stoeckel P, et al. Immunologic memory induced at birth by immunization with inactivated polio vaccine in a reduced schedule. *Eur J Epidemiol* 1989;**5**:143.
524. Taunay AdE, Galvao PA, deMorais JS, et al. Disease prevention by meningococcal serogroup C polysaccharide vaccine in preschool children: results after eleven months in Sao Paulo, Brazil. *Pediatr Res* 1974;**8**:429A.
525. The National Vaccine Advisory Committee. the measles epidemic: the problems, barriers and recommendations. *JAMA* 1991;**266**: 1547.
526. Thomas AR, Fiore AE, Corwith HL, et al. Hepatitis B vaccine coverage among infants born to women without prenatal screening for hepatitis B virus infection: effects of the Joint Statement on Thimerosal in Vaccines. *Pediatr Infect Dis J* 2004;**23**:313.
527. Thomas RE, Lorenzetti DL, Spragins W, et al. The safety of yellow fever vaccine 17D or 17DD in children, pregnant women, HIV+ individuals, and older persons: systematic review. *Am J Trop Med Hyg* 2012;**86**:359.
528. Thompson LA, Irigoyen M, Matiz LA, et al. The impact of DTaP-IPV-HB vaccine on use of health services for young infants. *Pediatr Infect Dis J* 2006;**25**:826.
529. Thompson WW, Shay DK, Weintraub E, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;**289**:179.
530. Tozzi A, Anemona A, Stefanelli P, et al. Reactogenicity and immunogenicity at preschool age of a booster dose of two three-component diphtheria-tetanus-acellular pertussis vaccines in children primed in infancy with acellular vaccines. *Pediatrics* 2001; **107**:e25.
531. Troisi CL, Hollinger FB, Krause DS, et al. Immunization of seronegative infants with hepatitis A vaccine (HAVRIX; SKB): a comparative study of two dosing schedules. *Vaccine* 1997; **15**:1613.
532. Trollfors B, Taranger J, Lagergard T, et al. A placebo-controlled trial of a pertussis-toxoid vaccine. *N Engl J Med* 1995;**333**:1045.
533. Tse A, Tseng HF, Greene SK, et al. Signal identification and evaluation for risk of febrile seizures in children following trivalent inactivated influenza vaccine in the Vaccine Safety Datalink Project, 2010-2011. *Vaccine* 2012;**30**:2024.
534. Tucker AW, Haddix AC, Bresee JS, et al. Cost-effectiveness analysis of a rotavirus immunization program for the United States. *JAMA* 1998;**279**:1371.
535. Tugwell BD, Lee LE, Gillette H, et al. Chickenpox outbreak in a highly vaccinated school population. *Pediatrics* 2004;**113**:455.
536. Umenai T, Krzysko R, Bektimirov TA, et al. Japanese encephalitis: current worldwide status. *Bull WHO* 1985;**63**:625.
537. Vadheim CM, Greenberg DP, Partridge S, et al. Effectiveness and safety of an *Haemophilus influenzae* type b conjugate vaccine (PRP-T) in young infants. *Pediatrics* 1993;**92**:272.
538. Van Damme P, Banatvala J, Fay O, et al. Hepatitis A booster vaccination: is there a need? *Lancet* 2003;**362**:1065.
539. Van Effelterre TP, Zink TK, Hoet BJ, et al. A mathematical model of hepatitis a transmission in the United States indicates value of universal childhood immunization. *Clin Infect Dis* 2006;**43**:158.
540. Van Herck K, Van Damme P. Inactivated hepatitis A vaccine–induced antibodies: follow-up and estimates of long-term persistence. *J Med Virol* 2001;**63**:1.
541. Vasquez M, LaRussa P, Gershon A, et al. The effectiveness of varicella vaccine in clinical practice. *N Engl J Med* 2001;**344**:1007.
542. Velázquez FR, Colindres RE, Grajales C, et al. Postmarketing surveillance of intussusception following mass introduction of the attenuated human rotavirus vaccine in Mexico. *Pediatr Infect Dis J* 2012;**31**:736.
543. Verstraeten T, Davis RL, DeStefano F, et al. Safety of thimerosal-containing vaccines: a two-phased study of computerized health maintenance organization databases. *Pediatrics* 2003;**112**:1039.
544. Verstraeten T, Jumaan AO, Mullooly JP, et al. A retrospective cohort study of the association of varicella vaccine failure with asthma, steroid use, age at vaccination, and measles-mumps-rubella vaccination. *Pediatrics* 2003;**112**:e98.
545. Vesikari T, Karvonen A, Korhonen T, et al. Safety and immunogenicity of RIX4414 live attenuated human rotavirus vaccine in adults, toddlers and previously uninfected infants. *Vaccine* 2004;**22**: 2836.
546. Vesikari T, Karvonen A, Prymula R, et al. Efficacy of human rotavirus vaccine against rotavirus gastroenteritis during the first 2 years of life in European infants: randomised, double-blind controlled study. *Lancet* 2007;**370**:1757.
547. Vesikari T, Karvonen A, Puustinen L, et al. Efficacy of RIX4414 live attenuated human rotavirus vaccine in Finnish infants. *Pediatr Infect Dis J* 2004;**23**:937.
548. Vesikari T, Matson DO, Dennehy P, et al. Safety and efficacy of a pentavalent human-bovine (WC3) reassortant rotavirus vaccine. *N Engl J Med* 2006;**354**:23.
549. Vessey S, Chan C, Kuter B, et al. Childhood vaccination against varicella: persistence of antibody, duration of protection, and vaccine efficacy. *J Pediatr* 2001;**139**:297.
550. Vestergaard M, Hviid A, Madsen KM, et al. MMR vaccination and febrile seizures: evaluation of susceptible subgroups and long-term prognosis. *JAMA* 2004;**292**:351.
551. Villa LL, Costa RL, Petta CA, et al. Prophylactic quadrivalent human papillomavirus (types 6, 11, 16, and 18) L1 virus-like particle vaccine in young women: a randomised double-blind placebo-controlled multicentre phase II efficacy trial. *Lancet Oncol* 2005; **6**:271.
552. Vodopija I, Baklaic Z, Hauser P, et al. Reactivity and immunogenicity of bivalent (AC) and tetravalent (ACW135Y)

meningococcal vaccines containing O-acetyl-negative or O-acetyl-positive group C polysaccharide. *Infect Immun* 1983;**42**:599.
553. Wagner G, Lavanchy D, Darioli R, et al. Simultaneous active and passive immunization against hepatitis A studied in a population of travelers. *Vaccine* 1993;**11**:1027.
554. Wahdan MH, Rizk F, El-Akkad AM, et al. A controlled field trial of a serogroup A meningococcal polysaccharide vaccine. *Bull WHO* 1973;**48**:667.
555. Wakefield AJ, Murch SH, Anthony A, et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet* 1998;**351**:637.
556. Walter EB, Neuzil KM, Zhu Y, et al. Influenza vaccine immunogenicity in 6- to 23-month-old children: are identical antigens necessary for priming? *Pediatrics* 2006;**118**:e570.
557. Ward B, Boulinanne N, Ratnam S, et al. Cellular immunity in measles vaccine failure: demonstration of measles antigen-specific lymphoproliferative responses despite limited serum antibody production after revaccination. *J Infect Dis* 1995;**172**:1591.
558. Watson B, Seward J, Yang A, et al. Postexposure effectiveness of varicella vaccine. *Pediatrics* 2000;**105**:84.
559. Watson BM, Piercy SA, Plotkin SA, et al. Modified chickenpox in children immunized with the Oka/Merck varicella vaccine. *Pediatrics* 1993;**91**:17.
560. Watson JC, Hadler SC, Dykewicz CA, et al. Measles, mumps, and rubella—vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1998;**47**(RR-8):1–57.
561. Watt JP, Wolfson LJ, O'Brien KL, et al. Burden of disease caused by *Haemophilus influenzae* type b in children younger than 5 years: global estimates. *Lancet* 2009;**374**:903.
562. Wattanasri S, Boonthai P, Prasert T. Human rabies after late administration of human diploid cell vaccine without hyperimmune serum. *Lancet* 1982;**2**:870.
563. Weibel RE, Buynak EB, McLean AA, et al. Persistence of antibody in human subjects for 7 to 10 years following the administration of combined live attenuated measles, mumps and rubella virus vaccines. Proc Soc Exp Biol Med 1980;**165**:260.
564. Weibel RE, Neff BJ, Kuter BJ, et al. Live attenuated varicella vaccine: efficacy trial in healthy children. *N Engl J Med* 1984; **310**:1409.
565. Weibel RE, Stokes J Jr, Buynak EB, et al. Live attenuated mumps vaccine, part III: clinical and serologic aspects in a field evaluation. *N Engl J Med* 1967;**276**:245.
566. Weinbaum C, Lyerla R, Margolis HS. Prevention and control of infections with hepatitis viruses in correctional settings. Centers for Disease Control and Prevention. *MMWR Recomm Rep* 2003; **52**(RR-1):1–36.
567. Wenger JD, Ward JI, Broome CV. Prevention of *Haemophilus influenzae* type b disease: vaccines and passive prophylaxis. *Curr Clin Top Infect Dis* 1989;**10**:306–39.
568. Werzberger A, Kuter B, Nalin D. Six years' follow-up after hepatitis A vaccination. *N Engl J Med* 1998;**338**:1160.
569. Werzberger A, Mensch B, Kuter B, et al. A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. *N Engl J Med* 1992;**327**:453.
570. Werzberger A, Mensch B, Nalin DR, et al. Effectiveness of hepatitis A vaccine in a former frequently affected community: 9 years' followup after the Monroe field trial of VAQTA. *Vaccine* 2002;**20**:1699.
571. White CJ, Kuter BJ, Hildebrand CS, et al. Varicella vaccine (VARIVAX) in healthy children and adolescents: results from 1987-9 clinical trials. *Pediatrics* 1991;**87**:604.
572. Whitney CG, Farley MM, Hadler J, et al. Decline in invasive pneumococcal disease after the introduction of protein-polysaccharide conjugate vaccine. *N Engl J Med* 2003;**348**: 1737.
573. Winer RL, Lee SK, Hughes JP, et al. Genital human papillomavirus infection: incidence and risk factors in a cohort of female university students. *Am J Epidemiol* 2003;**157**:218.
574. Wise R, Salive M, Braun M, et al. Postlicensure safety surveillance for varicella vaccine. *JAMA* 2000;**284**:1271.
575. Wise RP, Iskander J, Pratt RD, et al. Postlicensure safety surveillance for 7-valent pneumococcal conjugate vaccine. *JAMA* 2004; **292**:1702.
576. Wisseman CL Jr, Sweet BH. Immunological studies with group B arthropod-borne viruses, part III: response of human subjects to revaccination with 17D strain yellow fever vaccine. *Am J Trop Med Hyg* 1962;**11**:570.
577. World Health Organization. BCG vaccine. *Wkly Epidemiol Rec* 2004;**79**:33.
578. World Health Organization. Challenges in global immunization and the Global Immunization Vision and Strategy 2006-2015. *Wkly Epidemiol Rec* 2006;**81**:190.
579. World Health Organization. Expanded programme on immunization: statement on poliomyelitis eradication. *Wkly Epidemiol Rec* 1995;**70**:345.
580. World Health Organization. Paralytic poliomyelitis in Madagascar, 2002. *Wkly Epidemiol Rec* 2002;**77**:241.
581. World Health Organization. Special programme on AIDS and expanded programme on immunization—joint statement: consultation on human immunodeficiency virus (HIV) and routine childhood immunization. *Wkly Epidemiol Rec* 1987;**62**:297.
582. World Health Organization. WHO position paper on *Haemophilus influenzae* type b conjugate vaccines. *Wkly Epidemiol Rec* 2006; **81**:445.
583. World Health Organization. Initiative for vaccine research (IVR): typhoid fever. Global Vaccine Research Forum 2006 and Parallel Satellite Symposia, Bangkok, Thailand. 3-6 December, 2006.
584. World Health Organization Expert Committee on Biological Standardization. *Forty-third report. Technical Report Series No. 840*. Geneva: World Health Organization; 1994.
585. Wright PF, Cherry JD, Foy HM, et al. Antigenicity and reactogenicity of influenza A/USSR/77 virus vaccine in children—a multicentered evaluation of dosage and safety. *Rev Infect Dis* 1983;**5**:758.
586. Wright PF, Thompson J, Vaughn WK, et al. Trials of influenza A/New Jersey/76 virus vaccine in normal children: an overview of age-related antigenicity and reactogenicity. *J Infect Dis* 1977; **136**(Suppl):S731.
587. Yeh SH, Gurtman A, Hurley DC, et al. Immunogenicity and safety of 13-valent pneumococcal conjugate vaccine in infants and toddlers. *Pediatrics* 2010;**126**:e493.
588. Yvonnet B, Coursaget P, Deubel V, et al. Simultaneous administration of hepatitis B and yellow fever vaccines. *J Med Virol* 1986; **19**:307.
589. Zangwill KM, Belshe RB. Safety and efficacy of trivalent inactivated influenza vaccine in young children: a summary for the new era of routine vaccination. *Pediatr Infect Dis J* 2004;**23**:189.
590. Zangwill KM, Stout RW, Carlone GM, et al. Duration of antibody response after meningococcal polysaccharide vaccination in U.S. Air Force personnel. *J Infect Dis* 1994;**169**:847.
591. Zell ER, Dieta V, Stevenson J, et al. Low vaccination levels of US preschool and school-age children: retrospective assessments of vaccination coverage, 1991-1992. *JAMA* 1994;**271**:833.
592. Zerboni L, Nader S, Aoki K, et al. Analysis of the persistence of humoral and cellular immunity in children and adults immunized with varicella vaccine. *J Infect Dis* 1998;**177**:1701.
593. Zhou F, Harpaz R, Jumaan AO, et al. Impact of varicella vaccination on health care utilization. *JAMA* 2005;**294**:797.
594. Zhuji Measles Vaccine Study Group. Epidemiologic examination of immunity period of measles vaccine. *Chin Med J* 1987;**67**:19.
595. Zuckerman AJ. Effect of hepatitis B virus mutants on efficacy of vaccination. *Lancet* 2000;**355**:1382.

CHAPTER 245

Passive Immunization

Deborah Lehman • Danica J Schulte

GENERAL PRINCIPLES OF PASSIVE IMMUNITY

DEFINITION

Passive immunization is the administration of antibodies to provide immediate protection against a microbial agent, or toxin. In general, passive immunization is used to provide temporary immunity in an unimmunized subject exposed to an infectious disease when active immunization is unavailable (e.g., respiratory syncytial virus [RSV] infection), is contraindicated (e.g., varicella in an immunocompromised child), or has not been given before exposure (e.g., tetanus or rabies).

Passive immunization also is used in the management of certain disorders associated with toxins (e.g., diphtheria), in certain bites (e.g., snake and spider), in drug overdose (e.g., digoxin), as a specific (e.g., Rh_0 [D] immunoglobulin [IG]) or nonspecific (e.g., antithymocyte globulin) immunosuppressant, and in the treatment of certain infectious diseases.

The following several types of preparations are used in passive immunization (Table 245-1):

1. Standard human immune serum globulin (HISG) for general use, which is available in three forms: immunoglobulin (IG) for intramuscular use (IGIM), intravenous use (IGIV), and subcutaneous use (IGSC)
2. Special high-titer IGs with a known antibody content for specific illnesses
3. Animal sera and antitoxins
4. Monoclonal antibodies (see Table 245-2 for nomenclature)

Most of the licensed special IGs are for intramuscular use only. Plasma, serum, and even breast milk also can be used in passive immunization.

Passive immunization is not always effective; the duration is short and variable (1 to 6 weeks), and undesirable reactions may occur, especially if the antibody is of nonhuman origin. High-titer special IGs and IGIVs are identical to regular IGs and IGIVs, except that they are derived from patients hyperimmunized or convalescing from a specific infection or selected from donors with high titers to a specific antigen; they are useful in several disorders in which regular IG and IGIV are of little or no value.

ANIMAL SERA AND ANTITOXINS

Animal sera and antitoxins are derived from the serum of immunized animals, usually horses (equine). Because these sera are foreign proteins, they carry a significant risk for sensitization. Thus, they should be administered only when specifically indicated, after sensitivity tests, and by a physician prepared to deal with a hypersensitivity reaction.

A careful history must be taken before an animal serum is injected. Inquiry must be made about asthma, hay fever, urticaria, and previous injections of animal serum. Patients with a history of asthma, allergic rhinitis, or other allergic symptoms on exposure to horses may be dangerously sensitive to the corresponding serum, and the serum should be given only with the utmost caution.

Sensitivity Tests for Animal Serum

A skin prick test, followed by an intradermal skin test, always should be performed before any injection of animal serum, regardless of whether the patient has had the serum previously.[19] A skin prick test is performed by applying a drop of a 1:100 dilution of the serum in saline to the site of a superficial scratch, prick, or puncture on the volar aspect of the forearm and observing it for 20 minutes. A positive control (histamine phosphate, 0.1%) and negative control (saline) should also be applied. A positive reaction consists of erythema with wheal formation 3 mm greater than the negative control. (*Note:* Previous use of antihistamines may render results of these tests negative.)

If the skin prick test result is negative, an intradermal test is performed by injecting 0.02 mL of a 1:1000 saline dilution; again, positive (histamine phosphate, 0.1%) and negative control tests should be performed. The reaction is read 10 to 30 minutes after the injection and is positive if a wheal appears that is 3 mm greater than the negative control. If the test result is negative, it should be repeated with 0.02 mL of a 1:100 dilution. For persons with a negative history of animal allergy and no previous exposure to animal sera, the 1:100 dilution may be used initially if the skin prick test result is negative.

Although intradermal skin tests have resulted in fatalities, skin prick tests have not, but can still be associated with immediate reactions. Therefore, a skin test never should be performed (nor serum injected) unless a syringe containing 1 mL of 1:1000 epinephrine is within immediate reach and supportive care available.

Skin tests can indicate the probability of sensitivity. However, a negative skin test result is not an absolute guarantee of the absence of sensitivity. Therefore, either a specific history of allergy or a positive skin test reaction

TABLE 245-1 **Selected Antibody Preparations Available for Passive Immunity in the United States**

Product	Abbreviations and Brand Names	Principal Use
Standard Human Immune Serum Globulins for Intramuscular or Subcutaneous use		
Immunoglobulin, intravenous	IGIV, IVIG	Treatment of antibody deficiency, immune thrombocytopenic purpura, Kawasaki disease, other immunoregulatory and inflammatory diseases
Immunoglobulin, intramuscular	IGIM, IG, ISG	Treatment of antibody deficiency; prevention of measles, hepatitis A
Immunoglobulin, subcutaneous	IGSC	Treatment of antibody deficiency
Special Human Immune Globulins for Intramuscular or Subcutaneous Use		
Hepatitis B immunoglobulin	HBIG, HyperHEP B, Nabi-HB, HepaGamB	Prevention of hepatitis B
Varicella-zoster immunoglobulin	VariZIG	Prevention or modification of chickenpox
Rabies immunoglobulin	RIG	Prevention of rabies
Tetanus immunoglobulin	TIG	Prevention or treatment of tetanus
Vaccinia immunoglobulin	VIG, VIGIM	Prevention or treatment of vaccinia, prevention of smallpox
Rh_0(D) immunoglobulin	RhoGAM, MICRhoGAM, HyperRHO S/D, BayRho-D	Prevention of Rh hemolytic disease
Special Human Immunoglobulins for Intravenous Use		
Cytomegalovirus immunoglobulin	CMV-IGIV, CMVIG, CytoGam	Prevention or treatment of cytomegalovirus infection
Hepatitis B immunoglobulin, intravenous	HBIGIV, HepaGam B, Nabi-HB NovaPlus	Prevention of hepatitis B (including liver transplantation)
Vaccinia immunoglobulin	VIGIV	Prevention or treatment of vaccinia, prevention of smallpox
Rh_0(D) immunoglobulin, intravenous	WinRho SDF, Rhophylac	Treatment of immune thrombocytopenic purpura
Botulinum immunoglobulin	BIG, BabyBIG	Treatment of newborn botulism
Animal Sera and Globulins		
Tetanus antitoxin (equine)	TAT	Prevention or treatment of tetanus (when TIG is unavailable)
Diphtheria antitoxin (equine)	DAT	Treatment of diphtheria
Botulinum antitoxins (equine)		Treatment of botulism
Latrodectus mactans antivenin (equine)	Antivenin	Treatment of black widow spider bites
Crotalidae polyvalent antivenin (equine)		Treatment of most snake bites
Crotalidae polyvalent immune Fab (ovine)		Treatment of most snake bites
Micrurus fulvius antivenin (equine)		Treatment of coral snake bites

with horse serum is sufficient reason for special caution. A positive history of sensitivity to horse dander is an indication of the need for extreme caution.

Administration of Animal Serum

If the history and sensitivity test reactions are negative, the indicated dose of serum may be given intramuscularly, with epinephrine at hand.[19] The patient should be watched closely for an hour for adverse reactions.

Intravenous injection may be indicated if a high concentration of circulating antibody is required rapidly, as in botulism or diphtheria. The manufacturer's instructions should be consulted.

If the skin test reaction is positive or a history of allergy to animal serum is present and the need for the serum is unquestioned (and epinephrine is at hand), a procedure commonly referred to as *desensitization* can be undertaken, but any significant desensitization is unlikely to occur. This procedure merely results in establishing temporary tolerance to the serum. Desensitization should be performed by trained personnel with the necessary emergency equipment and drugs immediately available.

Desensitization consists of periodic (at 15-minute intervals) injections or infusions of progressively larger doses of the serum, starting at a very low dose, until tolerance is achieved. Schedules for intravenous and intradermal, subcutaneous, or intramuscular desensitization are given in the *2012 Red Book*.[19] Administration of sera after desensitization has been achieved must be continuous or protection from desensitization is lost rapidly.

TABLE 245-2 **US Adopted Nomenclature Nomenclature for Monoclonal Antibodies***

The suffix -mab is used for all monoclonal antibodies and fragments.	
The following letters preceding -mab identify the product source:	
u	human
e	hamster
o	mouse
i	primate
a	rat
xi	chimeric
zu	humanized
The general disease state subclass for which the monoclonal antibody is used is encoded by the syllables preceding the above:	
Disease or Target Class	
-vir	viral
-bac	bacterial
-lim	immune
-les	infectious lesions
-circ	cardiovascular
Tumors	
-col	colon
-mel	melanoma
-mar	mammary
-got	testis
-gov	ovary
-pr(o)	prostate
-tum	miscellaneous

*United States Adopted Name.

Hypersensitivity Reactions to Animal Serum

Hypersensitivity reactions to animal serum may be of the following four general types:

1. Anaphylactic reactions consisting of urticaria or other rashes; respiratory distress with wheezing, dyspnea, cough, hoarseness, or stridor; cardiovascular reactions, including tachycardia, hypotension, arrhythmia, cyanosis, shock, and unconsciousness; and gastrointestinal reactions, such as cramps, diarrhea, and vomiting, all occurring seconds to minutes after an injection has been administered.
2. Acute febrile reactions consisting of moderate or severe hyperpyrexia within 2 hours after an injection has been given.
3. Serum sickness reactions consisting of urticaria, arthritis, adenopathy, and fever occurring hours to days after an injection has been administered, depending on the dose and the presence or degree of previous sensitization (i.e., serum sickness occurs within hours or a few days after the second injection and within 7 to 12 days after the first injection);
4. Delayed reactions of varying nature, including peripheral neuritis, nephritis, Guillain-Barré syndrome, and myocarditis.

Treatment of Hypersensitivity Reactions to Animal Serum

For anaphylactic reactions, 1:1000 epinephrine at a dose of 0.01 mL/kg (maximum dose, 0.5 mL) is given subcutaneously or intramuscularly immediately. If improvement is not achieved immediately, 1:10,000 epinephrine at a dose of 0.01 mL/kg is given intravenously. This dose can be repeated every 10 minutes for up to three doses. The 1-mL 1:1000 epinephrine vials must be diluted 1:10 in physiologic saline (now 1:10,000) and injected slowly at a dose of 0.01 mL/kg. (A 1:10,000 dilution in a 10-mL vial also is available.)

Administration of epinephrine may be repeated in 5 to 15 minutes if the response is not satisfactory. Vasopressors and positive-pressure oxygen are helpful. For severe urticaria or edema, particularly edema of the larynx, intramuscular injection of antihistamines and corticosteroids is indicated. Administration of serum therapy (if necessary) should be resumed 6 to 8 hours later or after all visible signs of reaction have subsided.

Mild febrile reactions (temperatures <39° C [<102.8° F]) are treated with ibuprofen or acetaminophen. Severe febrile reactions can cause seizures and death and should be treated rigorously with sponge baths or other cooling means to reduce the temperature promptly. Serum sickness and serum neuritis generally are treated with corticosteroids.

HUMAN IMMUNE SERUM GLOBULIN

HISG, or gamma globulin, is available in three forms for general use: IGIM, IGIV and IVIG, and IGSC. IGIM is used primarily for the prevention of certain infectious disorders and less commonly for the treatment of antibody immunodeficiencies. IGIV is used in the treatment of primary and secondary antibody deficiencies, many immunoregulatory disorders (e.g., immune thrombocytopenic purpura and Kawasaki disease), and neurologic disorders (e.g., Guillain-Barré syndrome and peripheral neuritis). IGSC is used exclusively for treatment of antibody deficiency.

Intramuscular Immunoglobulin

Pharmacology

IGIM (IMIG) is prepared from pooled human serum by the Cohn alcohol fractionation procedure (thus deriving its alternative name of Cohn fraction II). This procedure and viral inactivation steps remove most other serum proteins, hepatitis viruses, and human immunodeficiency viruses (HIV-1 and HIV-2), thereby providing a safe product for intramuscular injection. It is reconstituted as a sterile approximate 16 percent solution (160 mg/mL) with or without preservative. It contains a wide spectrum of antibodies to viral and bacterial antigens.

IGIM is greater than 95 percent IgG, but trace quantities of IgM and IgA and other serum proteins are present. IgM and IgA are therapeutically insignificant because of their rapid half-lives (~7 days) and low concentrations in IG. IG contains all IgG allotypes (Gm and Km types).

IGIM is approved only for intramuscular or subcutaneous use, and intravenous injection of IGIM generally is contraindicated. IGIM aggregates in vitro to high-molecular-weight complexes (9.5S to 40S) that are strongly anticomplementary. These aggregates probably are responsible for the occasional systemic reactions to IGIM. The incidence of these reactions is increased if the patient has received IGIM previously or if it is inadvertently given intravenously. Agammaglobulinemic boys with affected male relatives (suggesting X-linked inheritance) may have a lower incidence of reactions.[455] Small intradermal injections of IGIM are not of value (except as a placebo), and they are contraindicated.

Intramuscular Immunoglobulin in Antibody Immunodeficiency

IGIM is used infrequently now for IgG replacement therapy in immunodeficiency. The usual dosage is 100 mg/kg/month, about equivalent to 0.7 mL/kg/month of the 16 percent (160 mg/mL) product. Two or three such injections are given at the onset of therapy as a loading dose, often over a 3- to 5-day period. The maximal maintenance dosage should not exceed 20 or 30 mL per week.

Few studies on optimal dosage are available; however, the Medical Research Council Working Party[455] found that 25 mg/kg/week (100 mg/kg/month) was therapeutically equivalent to 50 mg/kg/week but that 10 mg/kg/week was inadequate. Use of IGIM by this route has been supplanted largely by IGIV administration.

IGIM should be given at multiple sites to avoid giving more than 5 mL at any one site (10 mL in a large adult). The buttocks are the preferred site, but the anterior of the thighs also can be used. Tenderness, sterile abscesses, fibrosis, and sciatic nerve injury may result from these injections. The danger of sciatic nerve injury is especially great in a small malnourished infant with inadequate muscle and fat in the gluteal regions. Large doses of IGIM should not be given to patients with severe thrombocytopenia because of the risk for development of hematoma and infection.

The injections are given initially at monthly intervals. If the patient continues to have infection or if a characteristic symptom (e.g., cough, conjunctivitis, diarrhea, arthralgia, or purulent nasal discharge) recurs at the end of the injection period, the interval between doses is decreased to 3 or 2 weeks. Older patients often report that they can tell when their IgG level is low and they need another injection. During acute infections, IgG catabolism increases; so extra injections of IGIM often are given.

Because high serum levels of IgG cannot be maintained, serial IG assays are unnecessary in assessing the effectiveness of treatment. The maximal increase in serum IgG level after a standard IGIM injection will vary from patient to patient and from dose to dose because of different rates of absorption, local proteolysis at the injection site, and distribution within tissues.

An intramuscular injection of 100 mg/kg of IGIM usually raises the IgG serum level by 100 mg/dL after 2 to 4 days.[646] Thus, a recent IGIM injection usually does not obscure the diagnosis of hypogammaglobulinemia.

Intramuscular Immunoglobulin and Special Intramuscular Immunoglobulins for Prevention of Infectious Diseases

IGIM is recommended for prophylaxis of hepatitis A and measles. The recommended doses are found in the sections on these disorders. Special high-titer IGIMs are available for several disorders, such as hepatitis B and varicella. These products are identical to IGIM except for the high titers of specific antibodies. These products also are discussed in the sections on these disorders.

Adverse Effects of Intramuscular Immunoglobulin

Although IGIM is one of the safest biologic products available, rare anaphylactic reactions to intramuscular injections have been reported, particularly in patients requiring repeated injections.[201] The Medical Research Council Working Party[455] noted such reactions in 33/175 patients (19%) treated during a 10-year period. After approximately 40,000 injections, 85 reactions occurred; in eight patients, the injections were stopped as a result of these adverse effects and one death was recorded. Such reactions occurred at any stage of treatment and were unrelated to any particular lot number of IGIM or its anticomplementary activity. Symptoms include anxiety, nausea, vomiting, malaise, flushing, facial swelling, cyanosis, and loss of consciousness. Immediate treatment with epinephrine and antihistamines is indicated.

Persons who experience such reactions should be evaluated before receiving a repeated injection. Skin testing should be performed with several lots of IGIM.[201] A skin test result that is positive for an old but not a new lot of IG may indicate a particular idiosyncratic reaction to a particular lot. Under these circumstances, incremental doses of IG from a new lot are recommended. In other patients, IgE antibodies to IgG develop and result in positive immediate skin test reactions to all IGIM lots. In many other patients, no cause of the reactions can be found. Some of these patients will tolerate gradually increasing doses of IG, particularly if they are premedicated with a nonsteroidal anti-inflammatory agent, diphenhydramine, or corticosteroids. Finally, in a few patients, antibodies have developed to the IgA present in minute quantities in Ig and these IgA antibodies can be detected by serology.[715] This topic is discussed further in the section on adverse effects of IGIV.

Administration of exogenous gamma globulin may inhibit the endogenous synthesis of gamma globulin. In a few patients given IGIM or IGIV from early infancy, we have noted depressed IgG levels that return to normal when the injections are stopped. Amer and colleagues[18] reported decreased IgG levels in premature infants given monthly IGIM injections since birth.

IGIM injections or infusions may inhibit antibody responses to live virus vaccines such as measles or varicella. Siber and associates[618] recommend an interval of 3 months between IVIG or IGIM therapy and administration of live virus vaccines after IG doses of less than 40 mg/kg, an interval of 6 months after doses of 40 to 80 mg/kg, an interval of 8 months after doses of 80 to 400 mg/kg, and an interval of 12 months after large doses (1 to 2 g/kg).

Late side effects after IGIM injections are uncommon occurrences; however, fibrosis of the buttocks or localized subcutaneous atrophy may develop at the site of repeated injections in some patients. Repeated injections of IGIM may result in high levels of mercury from the thimerosal preservative. Although symptoms of acrodynia (mercury toxicity) developed in one patient as a result of such therapy,[441] most remain asymptomatic.

Intravenous Immunoglobulin

Intravenous IG (IGIV, IVIG) is further-treated Cohn fractionated human IG that has been rendered free of complexes and is thus safe for intravenous infusion. These products can be given in large quantities for antibody deficiencies[119]; for several autoimmune, inflammatory, and neurologic disorders[63,193]; and for prevention of transplant rejection.[336,337]

Pharmacology

The first IGIV produced in the United States, in 1981, was Gamimune, a reduced and alkylated 5 percent solution containing 10 percent maltose.[42] The second IVIG produced was prepared by acidification and treatment with pepsin; the lyophilized powder could be reconstituted as a 3, 6, or 12 percent solution (Sandoglobulin). Since then, several other IGIVs have been introduced by different manufacturers.[511]

Several methods of treating Cohn fraction II, including treatment with proteolytic enzymes, ultracentrifugation, chromatography, reduction of sulfhydryl bonds by alkylation, and incubation at low pH, have been used to eliminate high-molecular-weight complexes. Solvent and detergent treatment, pasteurization, and addition of the fatty acid caprylate are used to ensure that viral inactivation occurs,[297] and various stabilizers, such as maltose, albumin, or sucrose, are then added. These variations in manufacturing techniques result in unique characteristics among the different IG products.[511]

Although these products vary somewhat across brands and batches,[628,645] they are for the most part therapeutically equivalent and usually are selected on the basis of cost and convenience. IgG subclass differences exist.[45,628] Antibody titers to specific pathogens also may vary across lots and among different IGIVs.[226] Products differ as to their IgA content, which can be a consideration for patients with anti-IgA antibodies.[45] Premixed liquids have the advantage of convenience because the reconstitution step is not required; however, most solutions must be kept refrigerated.

The IGIV products currently available have all IgG subclasses, adequate serum half-lives (15 to 25 days), wide spectrum of antibody activity, and minimal anticomplementary activity, and they are free of bacterial and viral contamination.[315] Individual donors are checked for viral pathogens, and the final product is checked for sterility. The ability of the fractionation process to remove viral and prion particles is validated by spiking test pools with model pathogens and subjecting them to the same fractionation process used by the plasma pools for human use. Some of them are 5 to 10 percent solutions, and others are lyophilized powders that are reconstituted as 3 to 12 percent solutions.[511,647]

IGIV has several advantages over intramuscular IG, including (1) larger quantities of IgG can be given, (2) high levels of serum IgG can be achieved rapidly, (3) painful intramuscular injections are avoided, (4) tissue pooling and local proteolysis are avoided, and (5) home administration and self-administration are easier to perform.

Administration of Intravenous Immunoglobulin

Administration of IGIV requires venous access, which is sometimes a problem in small children or obese patients. It also requires close monitoring during the infusion, which usually takes 2 to 5 hours.[644] The initial rate is 0.01 mL/kg/min, and if no side effects occur, it can be doubled at 15- to 30-minute intervals to a maximal rate of 0.08 mL/kg/min. Rates above 5 mL/kg/hr (0.08 mL/kg/min) are not recommended. Vital signs should be monitored frequently, and trained personnel should be available to treat adverse reactions. Adverse effects tend to be associated with rapid rates of infusion, in patients with concurrent acute infections, previously untreated patients, or significant time elapse between infusions (>6-week intervals).

IGIV is contraindicated in patients who have had an anaphylactic reaction to IGIV or other blood products. It should be administered with great caution to patients who have IgG subclass deficiencies along with IgA deficiency, anti-IgA antibodies or both.[175]

In responsible, older patients receiving infusions without adverse effects, infusion by home self-administration can be accomplished with considerable cost savings.[51,376,636] However, in most cases, IGIV infusions are performed in the clinic setting or by a home infusion service.

A few investigators have given high concentrations (9% to 12% solutions) infused rapidly during a period of 20 to 40 minutes, which may be tolerated by some patients[594]; however, it should not be done except by experienced personnel equipped to manage adverse reactions.

The brand, lot number, dose, premedication, and any adverse reactions should be recorded. Cold solutions should be warmed to room temperature before administration.

Premedication, such as acetaminophen or diphenhydramine, is often given 30 minutes before infusions are started, particularly for the first infusion or for patients who have had prior reactions. Intravenous hydrocortisone (5 to 6 mg/kg children and 100 to

150 mg/kg in adults) or oral prednisone can be used for prevention of anticipated severe reactions.

Side Effects of Intravenous Immunoglobulins

Certain brands of IVIG may be more reactogenic. In one study involving patients with Kawasaki disease, the two IGIVs used were equivalent therapeutically, but one had a 12-fold (2% vs. 25%) increase in side effects.[569]

Immediate reactions, such as headaches, shaking chills, nausea and vomiting, myalgias, or arthralgias that occur during or shortly after the infusions, are common occurrences.[190,644,646] They may occur in as many as 10 percent of recipients, especially with a first infusion or in individuals with prior reactions.[645] They can be treated by slowing or stopping the infusions and giving nonsteroidal anti-inflammatory drugs, diphenhydramine (1 mg/kg), hydrocortisone, or other steroids. On occasion, switching to a different product (generally one available as a solution) may alleviate the reactions.

Severe Immediate Reactions. Anaphylaxis, shortness of breath, hypotension, hypertension, and other such immediate reactions are uncommon events. Because they rarely involve IgE antibodies, they are termed *anaphylactoid* reactions, and they may be associated with cytokine release, complement activation, or presence of vasoactive substance in the preparations.

Immediate Reactions in IgA-Deficient Patients. An occasional IgA-deficient patient exposed to blood, plasma, or any form of IG may develop IgE anti-IgA antibodies, which may cause a reaction to the small amount of IgA present in Ig.[120] IgG anti-IgA in high titers may cause a complement-mediated anaphylactic reaction.[715] These reactions are extremely rare, and testing patients for IgA deficiency before administration of IGIV is unnecessary. Further, patients with profound antibody deficiency do not develop anti-IgA antibodies. For the sensitized patient, the use of a low-content IgA product or premedication is indicated. Although IgA deficiency is rare, patients with this condition should not be given IGIV unless it is absolutely indicated.

Aseptic Meningitis. Headache is a common occurrence during or after IGIV infusions; however, some patients develop severe headaches 6 to 24 hours after the infusion, sometimes associated with photophobia, stiff neck, and nausea.[351,705] Several of these patients have had spinal taps that show cerebrospinal fluid (CSF) pleocytosis but negative viral and bacterial cultures. The symptoms usually resolve within 96 hours. This complication may recur despite changing brands of IGIV. Most occur when IGIV is used in large doses (e.g., 2 g/kg) for neurologic diseases. Individuals with a past history of migraine may be more susceptible to aseptic meningitis.[600] These patients may tolerate slow infusions of subcutaneous IG.[554,648] Headaches and aseptic meningitis often can be avoided by premedication with steroids or with antimigraine medications.

Renal Complications. Renal failure, usually temporary, has been reported with IGIV, particularly among patients with a history of renal disease.[554,645] When such a problem is anticipated, IGIV should be given slowly at no more than 0.5 g/kg/day.[645]

Thrombotic Complications. Several thrombotic events, including stroke, myocardial infarction, transient ischemic events, and central vein thrombosis, have occurred after IGIV infusions.[645] These events may be associated with high doses and rapid infusion, particularly among patients with preexisting cardiovascular illness, obesity, or immobility.[645,749]

Rare Side Effects. Transient neutropenia, hemolytic anemia, acidosis, and symptoms of hyperviscosity occur rarely.[111,645]

Transmission of Pathogens, Including Hepatitis C, by Intravenous Immunoglobulin

Because IGIV is derived from human donors, a remote chance of transmission of viral or other pathogens exists. Tests of donors and the final products are performed, and newer testing for prions is in place but is not of proved reliability. No instance of HIV infection or Jakob-Creutzfeldt or new-variant (mad cow) Jakob-Creutzfeldt disease have been linked to the use of IGIV. Nonetheless, some patients with immunodeficiency receiving long-term IGIV therapy have developed progressive central nervous system problems.[766]

In the early 1990s, hepatitis C was transmitted by experimental IGIV lots,[408,499] some European preparations,[83,84,406,738] and some commercially available U.S. lots.[141,591] Until the report of an outbreak of hepatitis C in 1994,[141] no U.S. cases of hepatitis associated with a commercially available IVIG had been reported. This transmission occurred shortly after hepatitis C–seropositive donors were excluded from the donor pools used in the manufacture of IGIV.

A plausible explanation for the lack of transmission before this U.S. Food and Drug Administration FDA policy was instituted, is that the hepatitis C antibodies neutralized trace amounts of hepatitis C virus not eliminated during the fractionation. As of October 1994, 137 suspected cases were reported, 88 of which were confirmed.[141,593] Of the 88 patients, 51 (58%) had primary immunodeficiencies and 63 percent eventually became symptomatic.

Bjoro and associates[84] reported that immunocompromised patients had a severe and rapidly progressive course of hepatitis C infection and that responses to interferon (IFN) were poor. Razvi and colleagues[555] have not confirmed this finding in U.S. patients.

Newer manufacturing processes and more rigorous testing seemingly have eliminated the risk for occurrence of hepatitis C transmission.

Intravenous Immunoglobulin and Intramuscular Immunoglobulin Inhibition of Vaccine Antibody Responses

Live-virus vaccines may have diminished immunogenicity when given shortly before or during the several

months after receipt of IG. Specifically, IG administration will inhibit response to measles vaccine for a prolonged period, and inhibition of rubella vaccine also has been demonstrated. The interval between measles vaccine and IG depends on the dose of IG. The inhibition of response to varicella vaccine is not known, and the recommendation is to follow that for measles vaccine administration. IG does not impair responses to oral polio vaccine, oral rotavirus vaccine, or yellow fever vaccine. In contrast to live-viral vaccines, administration of IG has not been demonstrated to cause significant inhibition of immune responses to inactivated vaccines and toxoids. Vaccines should be administered at sites different from those of IGIM. IGIV recipients should be immunized according to the recommended schedule for routine childhood immunization. The pediatric *Red Book* gives detailed recommendations.[19]

Intravenous Immunoglobulin in Primary Immunodeficiencies

Administration of IGIV is indicated for patients with profound primary antibody deficiency (quantitative and qualitative), for patients with combined immunodeficiencies, and for those with secondary immunodeficiency along with significant antibody deficiency (Table 245-3). Regular infusions of IGIV can keep patients with primary antibody immunodeficiencies free of infections for long periods or lessen the severity and frequency of chronic infections.[561]

Patients with hereditary agammaglobulinemias, common variable immunodeficiency, and immunodeficiencies with hyper-IgM clearly benefit from replacement therapy. In combined antibody and cellular defects and in secondary antibody immunodeficiencies, administration of IVIG serves as an important ancillary treatment, but it does not correct the associated T-cell defect or underlying cause of the secondary immunodeficiency.

The recommended dose of IGIV is 400 to 600 mg/kg, usually given every 3 to 4 weeks.[511,644] It should be given every 3 weeks if symptoms develop late in the period between monthly infusions. In general, the trough IgG level should be maintained at approximately 400 mg/dL higher than the pretreatment level, which will normalize the IgG level in most patients (e.g., a level >600 to 1000 mg/dL).

Certain patients who experience continued infections may receive even higher doses (i.e., 600 to 800 mg/kg every 3 to 4 weeks).[199,344] Patients receiving these larger doses may have less frequent sinopulmonary infections, improved pulmonary function, and decreased number of days of illness and hospitalization in contrast to when they received lower doses.[644] Some investigators recommend giving even higher doses (i.e., keeping trough levels above 800 mg/dL) in an effort to prevent pulmonary complications.[199,344]

Some patients with severe disease do not respond to higher doses or more frequent infusions because of permanent tissue damage or deep-seated chronic infection.

IGIV also is used in patients with antibody deficiency but with normal or nearly normal IG levels. Often, these patients need higher or more frequent doses of IGIV because their IgG catabolism is increased as a result of their high serum IgG levels. On occasion, IGIV is indicated in infants with transient hypogammaglobulinemia and persistent infection.

IGIV also has been used in patients with IgG subclass deficiencies, but controlled studies demonstrating efficacy are lacking.[82,376,621]

TABLE 245-3 **Some Immunodeficiencies in Which Human Immune Globulin May Be Beneficial**

Antibody Deficiencies
Congenital agammaglobulinemias
Common variable immunodeficiency
Immunodeficiencies with hyper-IgM
Transient hypogammaglobulinemia of infancy (sometimes)
IgG subclass deficiency with or without IgA deficiency (sometimes)
Antibody deficiency with normal immunoglobulins
Combined Deficiencies
Severe combined immunodeficiencies (all types)
Wiskott-Aldrich syndrome
Ataxia-telangiectasia
Short-limbed dwarfism
X-linked lymphoproliferative syndrome
Secondary Immunodeficiencies
Malignant neoplasms with antibody deficiencies; multiple myeloma, chronic lymphocytic leukemia, other cancers
Protein-losing enteropathy with hypogammaglobulinemia
Nephrotic syndrome with hypogammaglobulinemia
Pediatric acquired immunodeficiency syndrome
Intensive care patients: trauma, surgery, or shock
Posttransplantation
Burns
Prematurity

Special Uses of Intravenous Immunoglobulin in Antibody Deficiencies

A syndrome of polymyositis or chronic encephalitis, or both, caused by persistent enteroviral infection in patients with agammaglobulinemia has been treated successfully with very high doses of IGIV containing specific antibody to the virus.[454,547] However, some patients do not respond.[173]

A very high dose of IGIV (up to 2 g/kg/day) is used occasionally in patients with primary immunodeficiency who develop parvovirus B19 infection, autoimmune disease such as immune thrombocytopenic purpura, or persistent respiratory viral infection.[644]

Subcutaneous Human Immunoglobulin

An alternative to intramuscular injection of IG or infusion of IGIV is slow subcutaneous infusion of IGIM or IGIV.[4,78,79,235] This route is used extensively in Europe, with a 16 percent preservative-free IG. A preservative-free 16 percent product (Vivaglobin) was licensed in 2006 in the United States for subcutaneous use[498]; however, 10 to 12 percent solutions for IGIV also can be used safely.[648]

These products usually are infused into the abdominal wall or thigh with a battery-operated portable infusion

pump.[78,235] The usual dose is 100 to 150 mg/kg/week, the same monthly dose as recommended for IGIV, given at a rate of 0.05 to 0.20 mL/kg/hr. Larger doses and more rapid infusions can be accomplished by using multiple injection sites. If a patient is beginning IGSC therapy and an immediate therapeutic level is sought, loading doses of IGIV at double the monthly maintenance dose can be given 1 week before the start of IGSC. Peak serum IgG levels after IGSC occur 48 to 96 hours after the infusion.

Subcutaneous IG infusions, which can be self-administered, are well tolerated, safe, and preferred by some patients because the adverse reactions are considerably less.[78,235,281] Premedication is rarely needed. Thus, they are more suitable than IGIV for home self-administration.[234,235]

Stiehm and coworkers[648] used this route successfully in patients with poor intravenous access, aseptic meningitis, anaphylactic reactions after receiving IGIV infusions, or rapid gastrointestinal protein loss. Subcutaneous IG has been used safely in IgA-deficient adults with frequent respiratory infections,[281] during pregnancy,[80] and in children.[4]

Rapid administration of subcutaneous infusions of IG has been achieved by using two pumps at four infusion sites and increasing the rate to 20 mL/hr per pump, thereby allowing the entire infusion to be completed in little more than an hour.[235]

Immunoglobulin Administration by Other Routes (Oral, Intrathecal, Aerosol, and Local)

Multiple types of antibody have been given by unusual routes with varying degrees of success. None except intravitreous injection of monoclonal antibody is standard care.

Oral Immunoglobulin

Breast milk is the ultimate oral passive immune agent with preventive benefit for newborns. Oral IG administered to provide antimicrobial activity to the gastrointestinal tract mimics the action of antibody-rich colostrum and breast milk. In humans, little or no ingested IG is absorbed intact into the systemic circulation.[41] Some oral IG traverses the entire gastrointestinal tract undigested, particularly in premature infants.[73] Oral IG may neutralize microorganisms, inhibit colonization, and prevent microbial attachment to the gastrointestinal mucosa.

Rotavirus Infection. Barnes and colleagues[68] fed human IG or placebo for 7 days to premature infants in a nursery in which rotavirus was endemic. Rotavirus-associated diarrhea developed in 6/11 babies given placebo and in 1/14 given oral IG.

Losonsky and coworkers[417] administered oral human IG to two children with severe combined immunodeficiency and chronic rotavirus infection and demonstrated a decrease in the amount of free rotavirus excretion and survival of the IG through the gastrointestinal tract. Kanfer and associates[347] used oral human IG to treat rotavirus infection that occurred after bone marrow transplantation.

Bovine hyperimmune colostral IG enriched in rotavirus antibodies (from immunized cows) also has been used in the prevention of rotavirus infection. Davidson and colleagues[178] prevented rotavirus infection in 55 children admitted to an Australian hospital by administering a 10-day course of bovine colostrum; 9/65 control children became infected.

Turner and Kelsey[690] added at least 360 mL of cow colostrum to the formula of 31 term infants 3 to 7 months of age for an average of 101 days (range, 16 to 202 days). Rotavirus infections occurred in 11 (35%) of the colostrum-fed group and 14 (42%) of the control group, an insignificant difference, but symptomatic infection occurred in 1 (3%) of the infants receiving colostrum and 6 (18%) of the controls.

This product also has been used in two studies of the treatment of rotavirus diarrhea in Bangladesh.[464,588] In both studies, less diarrhea, fewer days of illness, less stool output, and more rapid clearance of rotavirus from the stool were noted. Guarino and coworkers[275] used oral human IG (300 mg/kg) as a single dose and observed more rapid recovery from rotavirus diarrhea.

In sum, oral IG is a promising but unproved agent for the prevention and treatment of rotavirus infection.

Necrotizing Enterocolitis. Eibl and colleagues[198] were able to prevent necrotizing enterocolitis in all 90 infants given oral IG rich in serum IgA. Six cases occurred in 91 control infants. Rubaltelli and colleagues[571] in Italy achieved similar results with oral monomeric IgG.

Cryptosporidial Infection. Bovine colostrum was used successfully to treat cryptosporidial diarrhea in HIV infection.[612] Borowitz and Saulsbury[98] used oral IG successfully to treat cryptosporidial infection in a child with acute leukemia.

Other Diarrheas. Oral human IG has been used in the post–bone marrow transplantation period to prevent the development of viral gastroenteritis[347] and to treat nonspecific diarrhea in immunodeficient patients.[457] Commercial products from bovine colostrum are available in Sweden and India.[227,530]

Considerable experimental study findings in veterinarian medicine are available on other oral IGs.

Aerosolized and Intratracheal Immunoglobulin

Aerosolized and intratracheal IG has been used with encouraging results in experimental models of pneumonia caused by RSV, pneumococci, staphylococci, *Yersinia pestis*, and parainfluenza and influenza viruses.* Aerosolized monoclonal antibodies also are being studied for specific infections, bioterrorist toxins (e.g., anthrax, ricin), and lung immunomodulation.[210,400,541,711]

*References 260, 302, 514, 550, 552, 564.

Rimensberger and colleagues[562] conducted a placebo-controlled trial of aerosolized human IG (not RSV-IVIG) in the treatment of RSV infection. No significant benefit was found, but the treatment was well tolerated.

Heikkinen and coworkers[294] used an IgA-enriched human IG as a nasal spray twice daily for 8 weeks to reduce respiratory infections in children between 1 and 4 years of age attending daycare. A 42 percent reduction was achieved in the 19 children receiving the IG versus the 20 children in the placebo group. The authors suggested that this treatment also may decrease the incidence of otitis media.

Intrathecal Immunoglobulin

Human IG has been used intrathecally for the treatment of viral encephalomyelitis in patients with antibody deficiency.[207] Monoclonal antibodies occasionally are given intrathecally in cancer therapy.[642] The use of antitoxin intrathecally in tetanus is discussed in the section on tetanus.

Other Routes

Local application of antibody has been used in the prevention and treatment of surgical and traumatic wounds.[362,540] Injections of the monoclonal antibody bevacizumab (Lucentis) into the vitrea to treat macular degeneration are under way.[170] Inner ear injection of infliximab, a tumor necrosis factor (TNF) monoclonal antibody, has been used in autoimmune neurosensory hearing loss.[703]

Intra-articular IG was of no clinical benefit to patients with rheumatoid arthritis.[56]

IMMUNOGLOBULIN IN SECONDARY IMMUNODEFICIENCIES

Many chronically ill patients have a primary illness that results in immunosuppression and increased susceptibility to infection. These secondary immunodeficiencies are considerably more common than primary immunodeficiency and are particularly common developments in hospitalized patients. Although most such patients have cellular (T-cell) deficiencies, some have antibody deficiencies either isolated or combined with other immune defects. These patients may have low IG levels, poor antibody responses to antigenic challenge, low levels of natural antibodies that may result from loss of IG, loss of immune cells, or the toxic effect of therapy or infection on the immune system. Table 245-2 lists diseases and conditions in which secondary antibody immunodeficiency can occur.

Laboratory criteria that support the use of IGIV include significant hypogammaglobulinemia (serum IgG <200 mg/dL or total IG [IgG + IgM + IgA] <400 mg/dL), absent or unexpectedly low titers of natural antibodies, absent or poor response to antigenic challenge (e.g., tetanus or pneumococcal vaccines), and lack of an antibody response to the infecting organism.[646] Some of the more common secondary immunodeficiencies with antibody defects are discussed.

Hematologic and Oncologic Diseases

Antibody deficiencies can occur with multiple myeloma, chronic lymphocytic leukemia, lymphoma, and advanced cancer, aggravated by the immunosuppressive agents used in their therapy. A double-blind multicenter study concluded that the prophylactic infusion of 400 mg/kg of IGIV every 3 weeks reduced the incidence of bacterial infections in patients with chronic lymphocytic leukemia.[166] The treatment group had fewer infections with *Streptococcus pneumoniae* and *Haemophilus influenzae*, but no difference was noted in infections caused by other gram-negative bacteria, fungi, or viruses.

This beneficial effect was confirmed in a subsequent study,[231] and although concern has been raised about its cost-effectiveness,[724] IGIV also has been shown to reduce the incidence of infections in patients with multiple myeloma[151] and in those receiving chemotherapy for lung cancer.[596] However, Blombery and colleagues[87] found no benefit in the routine use of peritransplant IVIG to reduce infectious complications in patients with multiple myeloma undergoing autologous hematopoietic stem cell transplant.

Protein-Losing States: Enteropathy, Nephrotic Syndrome, and Plastic Bronchitis

In some pediatric patients, antibody deficiency results from massive proteinuria (nephrosis), diarrhea (protein-losing enteropathy), or loss into the lung (plastic bronchitis) associated with accelerated IgG loss. Most of these patients have minimal trouble with recurrent infection, probably because antibody synthesis is intact and most likely accelerated; however, if the loss of IgG greatly exceeds synthetic capacity, symptomatic severe hypogammaglobulinemia may result. IGIV infusions can be used diagnostically in such cases; a large intravenous infusion, followed by serial measurements of serum IgG levels, can document an accelerated IgG half-life (i.e., <10 days).

These patients are candidates for IGIV therapy if they have recurrent infections or very low IgG levels (e.g., <200 mg/dL). Administration of large and repeated doses of IGIV is necessary. On occasion, antibody infusions will help control the severe diarrhea of protein-losing enteropathy.[128] Subcutaneous IG has been used in this situation because its delayed release into the circulation may result in higher long-term IgG levels than can be achieved with an equivalent IGIV monthly dose.[648]

Intensive Care Patients: Trauma, Surgery, and Septic Shock

Patients undergoing severe stress associated with trauma or extensive surgery have profound exposure and susceptibility to infection and a spectrum of immune deficiencies, including cutaneous anergy, leukocyte dysfunction,

hypogammaglobulinemia, and transiently impaired antibody synthesis.[255,475,741] Bowel stasis and hypotension may promote gram-negative sepsis, endotoxemia, or both, along with the development of severe and often irreversible shock.

Early studies by Ziegler and associates[765] and Baumgartner and colleagues[72] suggested that when antisera to a mutant J5 *Escherichia coli* endotoxin with anti–lipid A activity is used in bacteremic or surgical intensive care unit (ICU) patients, the incidence and severity of severe shock could be reduced. However, Calandra and colleagues,[125] used a human IGIV to J5 *E. coli* in 71 patients with gram-negative infections and shock and could not confirm their results. No differences were found in mortality rates, onset of time to shock, and complications.

Just and coworkers[341] administered IGIV and antibiotics to 50 patients in the ICU thought to have infection and compared their outcomes with 54 control patients who received antibiotics alone. Although no difference in survival occurred, they noted a trend indicating that the IGIV-antibiotic group had a shortened stay in the ICU, a shorter period in which respiratory therapy was required, improved renal function, and a favorable effect on infection. Additionally, Ferrara and colleagues[219] reported that IGIV represents a viable adjunct treatment option for certain cases of drug-resistant bacterial infections, and there was a clear survival advantage with the use of IGIV in adult sepsis syndromes by suppressing inflammation.

Rodriquez and colleagues[565] gave an IgM-enriched IGIV or albumin to 56 patients with sepsis undergoing abdominal surgery and found a reduction of sepsis from 55 to 25 percent. Another multicenter study of 352 postsurgical patients confirmed the observation that standard IGIV (400 mg/kg at weekly intervals) reduced the incidence of infections and shortened the stay in the ICU in contrast to patients treated with placebo or hyperimmune core-lipopolysaccharide IG.[318]

Werdan[730] reviewed the use of IGIV in sepsis and suggested certain specific subgroups may benefit (e.g., postoperative sepsis, septic shock with endotoxinemia, and sepsis with neutropenia). A single-institution study from Egypt suggested that IGIV was of benefit in children younger than 2 years in the ICU who had sepsis.[200]

Studies of IGIV in trauma patients requiring surgery[180,189,253] and in patients with head trauma[741] have shown questionable efficacy.

Monoclonal antibodies to endotoxin have been tested in clinical trials of patients in septic shock, but none was of proved efficacy.[483,731,764] Monoclonal antibody to TNF–α also has shown no efficacy in the treatment of adults with septic shock.[3,163] Monoclonal antibodies to interleukin (IL)-6 or IL-1 receptor likewise have not been effective in the treatment of shock.[237]

Prematurity

All premature infants have low levels of maternally derived IgG at birth, and levels approaching 100 mg/dL will develop in the first months of life in most.[64] These IgG levels may be further depressed by pulmonary disease (with transudation into the lungs), fever and stress (with increased IgG catabolism), and multiple blood sampling.[504] In addition, their sluggish antibody responses, their concurrent IgM and IgA deficiency, and their immature complement, phagocytic, and T-cell systems render low-birth-weight infants extraordinarily susceptible to development of infection.[410]

Attempts to decrease the incidence of infections in premature infants by using periodic IG injections date to the 1960s. Four controlled studies enrolling a total of 363 infants used IG doses of 80 to 240 mg/kg/month during their stay in the nursery (two studies), for 4 months, and for 1 year.[18,165,186,640] In two studies, a slight decrease in the number and severity of infections was noted but no difference in survival. These mixed results suggest that IG at the doses used was not of prophylactic value in these patients.

In the last 2 decades, the increasing rate of survival of premature infants and the availability of IGIV have reawakened interest in the use of antibody to prevent infections in premature infants.

An initial double-blind clinical trial by Baker and colleagues[57] suggested that IGIV at a dose of 0.5 g/kg at frequent intervals in the first weeks of life significantly reduced the frequency of sepsis, particularly that caused by *Staphylococcus epidermidis*. Subsequent studies, however, have not shown a clear benefit.

Meta-analyses of prospective, randomized, placebo-controlled prevention studies (representing 5000 infants) were performed by Jenson and Pollock[328,329] and Ohlsson and Lacy.[505] These studies differed in entry criteria, dose, and brand of IGIV, but most infants weighed less than 2000 g, were given IGIV therapy within the first week of life, and received doses of at least 400 mg/kg/month; culture-proved sepsis was used as an end point. Overall, a slight reduction (3%) was seen in the incidence of sepsis but no difference in mortality rates, length of stay, or other complications of prematurity, such as necrotizing enterocolitis, bronchopulmonary dysplasia, and intraventricular hemorrhage.

Shamin and coworkers[606] gave sufficient IGIV for 6 months to 15 of 30 matched premature infants with severe bronchopulmonary dysplasia to maintain their IgG levels above 400 mg/dL, whereas the untreated infants had levels below 200 mg/dL. The number of infections, notably pneumonia, was significantly reduced in the IGIV group (5 episodes of pneumonia and 4 other infections) in contrast to the control group (15 episodes of pneumonia and 12 other infections). In 2011, a large collaborative study of 113 hospitals in nine countries supported this conclusion. They evaluated the use of IGIV in neonatal sepsis and found no effect on the outcome of suspected or proven neonatal sepsis.[274]

Thus, the evidence to date still supports the 1990 National Institutes of Health (NIH) consensus statement that IGIV should not be given routinely to infants of low birth weight[485] but that it may be of value in selected premature infants at high risk for acquiring infection.

IGIV is sometimes of benefit in premature infants with a history of serious infections and underlying lung disease; most such infants will have hypogammaglobulinemia and

should be regarded as having transient hypogammaglobulinemia of infancy.

Hyperimmune IGIV or monoclonal antibodies enriched in antibodies against specific microbial antigens (e.g., *Staphylococcus aureus*) may be of benefit to certain high-risk newborn and premature infants. Two large multicenter double-blind clinical trials of *S. aureus* hyperimmune human IGs were completed in infants of very low birth weight. One used IG from donors immunized with a staphylococcal vaccine against serotypes 5 and 8 (Altastaph).[76,434] The other used IG from donors with a high titer to C1fA, a staphylococcal fibrinogen-binding protein (Veronate).[88,130,181,353] Neither trial showed a significantly decreased severity or incidence of staphylococcal infections.

Transplantation

Conditioning regimens to eliminate or to reduce the host's hematopoietic and immune systems before transplantation (bone marrow and solid organ) render these patients extremely susceptible to infection, particularly cytomegalovirus (CMV) infections.[691] Thus, IGIV and CMV-IGIV have been used to prevent infection, as well as to modify graft-versus-host disease and prevent solid organ rejection.

Early studies of IGIV therapy after allogeneic bone marrow transplantation were modestly successful in preventing infections, decreasing graft-versus-host reaction, and increasing survival rates.[6,71,167,261,661-663] This effect was less dramatic when CMV infection was controlled by ganciclovir and screening of blood products.[277] Two more multicenter studies showed only a modest or no effect on modifying acute graft-versus-host reactions or mortality rates.[169,743] In single-center studies, CMV-IGIV was of no benefit in CMV-positive lung transplant recipients[384] or liver transplant recipients.[265]

IGIV is of benefit in adult kidney transplant recipients with high titers of human leukocyte antigen (HLA) antibodies resulting in a shorter time to transplantation, reduced HLA antibody titers, and improved allograft survival rates.[336,337]

Burns

Bacterial sepsis, particularly *Pseudomonas* spp. and *E. coli* sepsis, is the leading cause of death in the 300,000 patients hospitalized annually in the United States for burns.[474,475] The protein loss in these patients induces hypogammaglobulinemia in proportion to the severity of the burn. High-dose IGIV prolongs survival in experimentally burned mice infected with *Pseudomonas* spp. and preliminary studies of hyperimmune IG and plasma in human burn patients were encouraging, but proof of efficacy is lacking[359,542,658] (see later discussion on burn infections).

Human Immunodeficiency Virus Infection

Refer to Chapter 191B for discussion of HIV.

INTRAVENOUS IMMUNE GLOBULIN IN IMMUNOREGULATORY AND NEUROLOGIC DISORDERS

High-dose IGIV has immunosuppressive and anti-inflammatory effects that render it a valuable agent in the treatment of several autoimmune, inflammatory, and neurologic disorders (Table 245-4).[193,511] A consensus committee has evaluated these uses in terms of likely efficacy.[511]

High-dose IGIV (1 to 2 g/kg) may work by one or more of the following mechanisms[63,193,623,672]:

1. IGIV may decrease the high levels for a pathogenic antibody (such as may be present in myasthenia gravis or idiopathic thrombocytopenic purpura) by accelerating IgG catabolism. IGIV increases IgG levels acutely and increases its catabolism, thus decreasing pathogenic antibodies present in high concentrations. It also saturates the FcRn receptor, thus inhibiting the recirculation of IgG molecules.[756] IGIV also inhibits antibody synthesis by having a direct effect on proliferating B cells, possibly through activation of the FcRII receptor.[584]
2. IGIV contains anti-idiotypic antibodies that can combine with autoimmune antibodies and remove them rapidly from the circulation, which may be a mechanism by which IGIV reduces anti-HLA antibodies in transplant recipients.
3. IGIV binds to Fc receptors on reticuloendothelial cells and causes an Fc receptor blockade, thus reducing the destruction of antibody-coated cells in the spleen or liver. It is a mechanism for the rapid response to IGIV noted in patients with immune thrombocytopenic purpura. Dimeric IGIV may be particularly effective in causing FcR blockage.
4. IGIV combines with other surface receptors to inhibit cellular activation; for example, in toxic epidermal necrolysis, IGIV prevents Fas-mediated cell death of keratinocytes by blocking the Fas receptor.[707]
5. IGIV may promote solubilization and clearance of pathologic immune complexes, such as may be present in lupus nephritis.
6. IGIV downregulates T-cell immune activation by decreasing inflammatory cytokine release or action. In Kawasaki disease, an immediate damping of the cytokine "storm" occurs after IGIV with this illness.
7. IGIV antibodies neutralize bacterial superantigens and prevent them from activating T cells. It is the probable mechanism for its beneficial effect in toxic shock syndrome.[639,672]
8. IGIV combines with complement components to prevent complement-mediated tissue injury. An example is inhibition of complement-mediated myocyte damage in dermatomyositis.
9. IGIV neutralizes virus or bacterial antigens or toxins that may trigger or cause the disease. An

TABLE 245-4 **Likelihood of Benefit of Intravenous Immunoglobulin in Autoimmune, Inflammatory, and Neurologic Diseases**

Proved Benefit*
Kawasaki disease
Graves ophthalmopathy
Immune thrombocytopenic purpura (e.g., idiopathic)
Dermatomyositis
Post–bone marrow transplantation
Neurologic diseases
  Guillain-Barré syndrome
  Chronic inflammatory demyelinating polyneuropathy
  Multifocal motor neuropathy
  Demyelinating neuropathy with IgM gammopathy

Probable Benefit†
Toxic shock syndrome
Postinfectious thrombocytopenic purpura
Neonatal isoimmune or autoimmune thrombocytopenic purpura
Neonatal isoimmune or autoimmune hemolytic anemia
Autoimmune or isoimmune neutropenia
Autoimmune hemolytic anemia
Toxic epidermal necrolysis
Prevention of antibody-mediated kidney transplant rejection
Autoimmune uveitis
Neurologic diseases
  Polymyositis
  Lambert-Eaton myasthenia syndrome
  Myasthenia gravis
  Stiff man syndrome
  Relapsing-remitting multiple sclerosis

Possible Benefit‡
Anticardiolipin antibody syndrome
Coagulopathy with factor VIII inhibitor
Bullous pemphigoid and other blistering skin diseases
Churg-Strauss vasculitis
Vasculitis with antineutrophil cytoplasmic antibodies
Other vasculitides
Stevens-Johnson syndrome
Severe rheumatoid arthritis
Systemic lupus erythematosus
Autoimmune diabetes mellitus
Posttransfusion purpura
Prevention of allograft rejection in high-risk patients with solid organ transplants
Recurrent spontaneous abortion
Cardiomyopathy
Severe persistent high-dose steroid-dependent asthma
Chronic urticaria
Autoimmune liver disease
Delayed pressure urticaria
Neurologic diseases
  Multiple sclerosis
  Rasmussen syndrome
  Intractable childhood epilepsy
  Demyelinating neuropathy with anti-GAM antibodies
  Acute disseminated encephalomyelitis
  Cerebral infarction with antiphospholipid antibodies
  HTLV-1–associated myelopathy
  Lumbosacral or brachial plexitis
  Paraproteinemic neuropathy
  Opsoclonus-myoclonus
  Postinfectious cerebellar ataxia
  Acute idiopathic dysautonomia

Unproven or No Benefit§
Non–steroid-dependent asthma
Atopic dermatitis
Epidermolysis bullosa acquisita
Hemolytic-uremic syndrome
Thrombotic thrombocytopenic purpura
Pure red cell or white cell aplasia
Acquired von Willebrand disease
Chronic fatigue syndrome
Infantile autism
Acute myocarditis
Chronic graft-versus-host disease
Inflammatory bowel disease
Neurologic diseases
  Inclusion body myositis
  Amyotrophic lateral sclerosis
  Paraneoplastic cerebellar degeneration, sensory neuropathy, encephalitis syndrome (POEMS)
  Sensory neuropathy
  Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS)
  Alzheimer's disease

Levels of benefits:
*Controlled studies demonstrate efficacy.
†Several case reports or uncontrolled series are convincing.
‡Preliminary studies are encouraging but incomplete.
§Preliminary studies are limited, equivocal, or negative.
HTLV, Human T-lymphotropic virus; *POEMS,* polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes.

example may be the neutralization of bacterial products in hemolytic-uremic syndrome.
10. IGIV contains substances such as soluble HLA antigens and soluble cytokine receptors that may inhibit immune responses.

The illnesses for which IGIV may be of benefit often are of unknown cause, refractory to standard treatment, somewhat responsive to steroids, and associated with local or generalized immune activation with fever, inflammatory cells, and cytokine release.[501] Large doses of IGIV (e.g., usually 1 to 2 g/kg/day) are necessary and may need to be repeated. IGIV preparations may not always be equivalent in action. Tsai and associates[688] found that certain brands of IGIV were less effective in Kawasaki disease than were others.

If a favorable response does not occur immediately or within a few weeks, it probably will not occur later. Proof of efficacy requires a double-blind study (e.g., as conducted for Kawasaki disease) or dramatic reversal of progressive severe disease (e.g., as noted in some cases of toxic shock syndrome). The use of IGIV in Kawasaki disease is covered in Chapter 77.

MONOCLONAL ANTIBODIES

Monoclonal antibodies specific for a single antigenic epitope have been available for laboratory use for several decades and are used increasingly as therapeutic agents. The first monoclonal antibody, licensed in 1984, was a

murine antilymphocyte antibody (anti-CD3 [OKT3]) used for immunosuppression in transplant recipients. Since then, numerous other monoclonal antibodies have been licensed for treatment, and many others are being studied in the United States and abroad.[124]

Nomenclature and Structure

The generic names of therapeutic monoclonal antibodies have been codified, as summarized in Table 245-2.[124] All end in *mab* or *nab*. Preceding this ending is a one- or two-letter prefix that denotes the animal from which it has been produced (e.g., *o* for mouse, as in muromonab; *u* for human, as in adalimumab).

Two types of monoclonal antibodies contain both human and murine components. Chimeric monoclonal antibodies, designated *xi*, contain the Fab portion of a murine IG and the Fc portion of human IG, usually an IgG1 molecule (e.g., rituximab). Humanized monoclonal antibodies, designated *zu*, have only the antibody-combining site of a murine IG (the complementary determinant region), and the rest of the molecule is a human IgG1 molecule (e.g., omalizumab). Chimeric and humanized antibodies are less antigenic and have a longer half-life than do mouse monoclonal antibodies and thus usually are preferred.

One completely human monoclonal antibody is derived from isolated and recombined human L and H chains (adalimumab).

The final prefix before the source prefix designates the disease for which the antibody is used (e.g., *vir* for viral disease, *lim* for immune modulation, and *tu* for cancer). Examples include palivizumab, omalizumab, and transtuzumab. Sometimes the last consonant is dropped for the sake of pronunciation. The beginning of the generic name is left to the manufacturer, as is, of course, the trade name.

Uses of Monoclonal Antibodies

Therapeutic monoclonal antibodies developed to date are licensed for graft rejection, cancer, autoimmunity (e.g., rheumatoid arthritis, inflammatory bowel disease, and immune cytopenias), thrombosis, infectious disease, multiple sclerosis, asthma and allergy, and macular degeneration. Like many new drugs, monoclonal antibodies also are used for disorders other than those for which they are licensed.

Only one of these antibodies, palivizumab (Synagis), directed against RSV, is used in infectious diseases for the prevention of RSV infection in high-risk infants (see the discussion of RSV).[32] However, monoclonal antibodies to microbial antigens and inflammatory mediators will play an important role in tomorrow's battle with infectious pathogens. For example, monoclonal antibodies to anthrax toxin, staphylococcal antigens, and HIV antigens and coreceptors are being studied.

PASSIVE IMMUNITY IN BACTERIAL DISEASES

Antibodies have been used for a century for the prevention and treatment of infectious diseases (Table 245-5). In bacterial disease, antibodies neutralize toxins, facilitate opsonization, and, with complement, promote bacteriolysis; in viral disease, antibodies block viral entry into uninfected cells, promote antibody-directed cell-mediated cytotoxicity by natural killer cells, and neutralize virus alone or with the participation of complement.

Before the use of antibiotics, antibodies were the only specific agents for the treatment of certain infections. Although this role has been supplanted largely by antibiotics, antibody still has a crucial role in the treatment of certain infectious diseases (see Table 245-4). The remainder of this chapter discusses the roles of antibody in prevention and treatment of bacterial and viral diseases. Other reviews are available.[132,133,405]

ANTHRAX

Anthrax is a rare but serious infectious disease, predominantly of ruminant animals, caused by an aerobic gram-positive rod, *Bacillus anthracis*.[134,423,424] Humans can be infected through the skin (cutaneous anthrax), by ingestion (gastrointestinal anthrax), or by inhaling the spores (inhalational anthrax).[424] Inhalational anthrax results from prolonged exposure to animal hides or carcasses and infected soil or, rarely, by deliberate spore exposure in the bioterrorism setting, as occurred in 2001 when anthrax spores were sent through the U.S. mail.[331,423]

After inhalation, the spores are ingested by alveolar phagocytic cells and transported to regional nodes, where they germinate and release exotoxins (lethal toxin and edema toxins), which damage cell membranes, increase capillary permeability, initiate pulmonic changes, and ultimately lead to shock and cardiovascular collapse.

A vaccine is available for individuals at high risk for exposure and the military. It is effective against cutaneous anthrax but is of unproved efficacy against inhalational anthrax.[330] Other vaccines are currently in development.[103]

Before the antibiotic era and as early as 1903, anthrax antitoxin (usually equine) was used in therapy for anthrax.[255,374] An antitoxin would be of value in a bioterrorism attack, both before exposure and after exposure. The Centers for Disease Control and Prevention (CDC) and the NIH are collecting plasma units from military recruits and volunteers immunized with the anthrax vaccine[206] to develop a human high-titer IGIV from these donors.[135,735]

Several monoclonal antibodies against anthrax antigens have been produced.* The best studied is a human monoclonal antibody 5H3 (raxibacumab).[154] Raxibacumab is a human IgG1λ monoclonal antibody directed against

*References 44, 106, 154, 313, 429, 444, 470, 661, 719.

TABLE 245-5 **Summary of the Efficacy of Antibody in the Prevention and Treatment of Infectious Diseases**

Infection	Prophylaxis	Treatment
Bacterial Infections		
Respiratory infections (streptococcal, *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*)	Proven (NR)*	Proven (NR)*
Diphtheria	Probable benefit (NR)	Proven
Pertussis	Unproven	Unproven
Tetanus	Proven	Proven
Other clostridial infections		
Clostridium botulinum	Proven	Proven
Infantile botulism	Unproven	Proven
Clostridium difficile	Unproven	Possible benefit
Staphylococcal infections		
Toxic shock syndrome	Unproven	Probable benefit
Refractory infection	Unproven	Possible benefit (NR)
Staphylococcus epidermidis in newborns	Possible benefit	Unproven
Toxic shock syndrome	Unproven	Probable benefit
Newborn sepsis	Possible benefit (NR)	Probable benefit
Shock, intensive care, and trauma	Unproven	Possible benefit (NR)
Pseudomonas infections		
Cystic fibrosis	Unproven	No benefit
Burns	Unproven	No benefit
Viral Infections		
Hepatitis A	Proven	No benefit
Hepatitis B	Proven	No benefit
Hepatitis C	Unproven	No benefit
Human immunodeficiency virus infection	Unproven	Unproven
Respiratory syncytial virus infection	Proven	Unproven
Herpesvirus infections		
Cytomegalovirus	Proven	Possible benefit
Epstein-Barr virus	Unproven	Unproven
Herpes simplex virus	Possible benefit (NR)	Unproven
Varicella-zoster virus	Proven	Unproven
Parvovirus infection	Probable benefit (NR)	Proven
Enterovirus infection in newborns	Unproven	Possible benefit
Enteroviral central nervous system infection	Proven	Proven
Ebola	Unproven	Unproven
Rabies	Proven	No benefit
Measles	Proven	No benefit
Rubella	Unproven	No benefit
Mumps	Unproven	No benefit
Tick-borne encephalitis	Possible benefit	No benefit
Vaccinia	Proven	Proven
Variola (smallpox)	Proven	Unproven

Modified from Keller MA, Stiehm ER. Passive immunity in prevention and treatment of infectious diseases. *Clin Microbiol Rev* 2000;**13**:602-14.
*Except for immunodeficient patients.
NR, Not recommended.

a component of the anthrax toxin. It was shown to be safe and well tolerated intravenously and intramuscularly in human volunteers and provided good antibody titers with a half-life of 15 to 19 days.[660] Two animal efficacy studies demonstrated that a single dose of raxibacumab, given in the absence of antibiotics, improved survival rates up to 64 percent, even when administered after animals were already showing clinical symptoms of inhalation anthrax.[461] In 2009, 20,000 doses of raxibacumab were delivered to the U.S. Strategic National Stockpile and later that year an additional 45.000 doses were made available. In 2012 the FDA approved raxibacumab as treatment in combination with antibacterial drugs, for inhalational anthrax. Raxibacumab was also approved for prevention of inhalational anthrax in instances when other therapies are not available nor appropriate. Raxibacumab is supplied by the U.S. government.

Administration of anthrax IG (AIG) to a 44-year-old man with naturally occurring inhalation anthrax demonstrated subsequent decrease in plasma and pleural fluid lethal factor and increase in antiprotective antigen IgG noted.[718]

Recommendation

In addition to raxibacumab, a limited supply of immune plasma is stockpiled by the U.S. army and is available through an emergency investigational new drug (IND) protocol. This supply would be used in conjunction with antibiotics and with vaccine, the latter to provide immunity after metabolism of the antibody. Immunotherapeutics should be considered for patients with severe systemic illness or lack of clinical response to standard therapy.[643]

BOTULISM, BOTULINUM ANTITOXIN, AND BOTULISM IMMUNOGLOBULIN

Botulism, a severe paralytic poisoning, results from ingestion or absorption of neurotoxins or spores from *Clostridium botulinum*. Several clinical variants are recognized: food poisoning from ingestion of contaminated canned food, wound botulism from a contaminated local infection, inhalational botulism among individuals working with the toxin or in bioterrorism,[47] infant botulism from ingestion of *C. botulinum* spores, and adult-type infant botulism in adults with preexisting gastrointestinal disease. In the last two types, ingested spores multiply in the gastrointestinal tract and produce toxin, which, following absorption, results in a paralytic disorder.[77,136]

Botulism resulting from cosmetic use of an unapproved botulinum toxin A has been reported.[156,637]

Seven immunologic types of *C. botulinum*, designated A through G, have been identified, each elaborating an immunologically distinct toxin. Almost all human botulism has resulted from the ingestion of toxins A, B, and E; toxin E is usually associated with fish and marine mammal products.[378,379,608]

Japanese studies, reviewed by Dolman and Iida,[187] indicate that antitoxin therapy is effective for type E botulism. The mortality rate associated with type E botulism was 49 percent in 135 untreated cases and 3.5 percent in 85 antitoxin-treated cases.

Tacket and associates[668] noted that 46 patients who had type A foodborne botulism and who received trivalent antitoxin had a lower mortality rate (27%) than 13 patients who did not receive antitoxin (46% mortality). Early antitoxin administration resulted in a shorter clinical course.

The presence of toxin in the blood long after the appearance of clinical symptoms or the ingestion of toxin gives theoretical support to the use of antitoxin to prevent further binding of toxin to tissue. Some antitoxin remains in the circulation for more than 30 days, thus indicating that a single initial dose is adequate therapy.

Equine antitoxins to types A, B, and E are the primary antitoxins available in the United States. The decision to use antitoxin is complicated by its unknown efficacy and its side effects, but approximately 80 percent of patients with ingestion-type botulism receive antitoxin.[608]

For information on infant botulism, see Chapter 146.

Recommendations

All forms of botulism, except for infant botulism, are treated with a new heptavalent botulinum antitoxin (HBAT) that now replaces the previously available bivalent antitoxin AB and monovalent antitoxin E. HBAT contains equine-derived antibody to the seven known botulinum toxin types (A to G). The administration of HBAT requires an FDA IND treatment protocol. Suspected cases should be immediately reported to local and state health authorities, and further guidance is available from the CDC's 24-hour hotline.*[,145] Additional information regarding the CDC botulism treatment program is available on the CDC website.† Administration of HBAT should occur only after testing for sensitivity to horse serum because of the risk for hypersensitivity reactions, including anaphylaxis and serum sickness.[19,20,136,608]

Antitoxin also can be given prophylactically to individuals known to have ingested contaminated food. The risk for development of serum reactions (~10%) must be weighed against the risk for contracting the disease.

A 5-year, placebo-controlled, double-blind trial of human botulinum intravenous IG (BIG) for the treatment of infant botulism showed a significant reduction in hospital days, mechanical ventilation, tube feedings, and days of hospitalization in the BIG recipients, with considerable cost savings and minimal adverse events.[48] Three retrospective studies demonstrated similar decreases in the listed interventions.[682,689,693] BIG should be started as soon as possible after the illness is suspected and is available through the California Infant Botulism Treatment and Prevention Program.‡

OTHER CLOSTRIDIAL INFECTIONS

Clostridium difficile Infections

Clostridium difficile is a ubiquitous spore-forming anaerobe often involved in antibiotic-associated diarrhea and pseudomembranous colitis.[20,21] Severity of the disease can range from an asymptomatic carrier state to fulminant colitis with toxic megacolon. Toxic strains of *C. difficile* release two antigenically distinct toxins, both of which have potent cytotoxic and inflammatory properties.[162] Infection generally leads to an antibody response to the toxins, and most individuals older than 2 years of age have the antibodies; thus, *C. difficile* antibodies are present in IGIV preparations.[581]

High levels of IgG antibodies acquired after colonization are associated with the asymptomatic carrier state; individuals who have persistent diarrhea have

*Centers for Disease Control and Prevention 24-hour hotline: 770-488-7100.

†Centers for Disease Control and Prevention botulism treatment website: http://www.bt.cdc.gov/agent/botulism.

‡Instructions for obtaining botulinum intravenous immunoglobulin from the California Infant Botulism Treatment and Prevention Program can be found at http://www.infantbotulism.org/.

significantly lower serum IgG but not IgA to toxin A.[398] Several patients have been identified with relapsing symptomatic disease who have low IgG antibody levels to toxin A.[290,407] Some of these patients had low levels of IgG[407] or IgG1 subclass deficiency.[290] and were treated successfully with IGIV. Since the initial use of IGIV to treat patients with recurrent disease, multiple studies have demonstrated that IGIV contains neutralizing antibodies to toxin A and toxin B.

IGIV treatment of individuals with severe disease, including sepsis, abdominal distention with ileus, and toxic megacolon, also has been reported.[339] The development of an IG preparation derived from volunteers immunized with *C. difficile* toxoid vaccine has the potential to provide higher titer-specific antibodies for treatment of recurrent or refractory *C. difficile* infection. The mechanism thought to be responsible for the protective effect of IGIV is the binding and neutralization of toxin A by IgG anti-toxin A antibodies.[2]

Oral administration of a bovine IgG concentrate was shown effective in preventing diarrhea and death in hamsters when challenged with *C. difficile* infection.[361] However, further investigation into such an approach in humans has not been successful.[360]

Recombinant monoclonal therapeutics have been developed and shown in a randomized, double-blind, placebo-controlled study done at 30 sites in the United States and Canada to significantly reduce the recurrence rate of *C. difficile* disease (25% in the placebo group vs. 7% in the intervention group) There was no demonstrable difference in symptom severity or duration of the initial episode of diarrhea.[420]

Recommendations

Patients with refractory *C. difficile* infections (with or without a global antibody deficiency) can be treated with 300 to 500 mg/kg of IGIV every 1 to 3 weeks as adjunct therapy.[339,453,733,734] Such therapy has been shown to increase antitoxin levels, to control diarrhea, and to prevent relapses during the treatment period.[407,581] However, controlled trials have not been conducted and therefore this use of IGIV is unproved.[333,339]

Studies with an oral anti–*C. difficile* bovine IG for both treatment and prevention have been shown to provide anti–toxin A neutralizing activity, and further development may provide a therapeutic alternative to IV therapy.[723]

Gas Gangrene *(Clostridium perfringens)*

Treatment of gas gangrene with equine antitoxin to *Clostridium perfringens* was of unproved efficacy and no longer is available. Food poisoning caused by *C. perfringens* is self-limited and is not treated with antitoxin.

Tetanus *(Clostridium tetani)*

See the later discussion on tetanus, tetanus antitoxin, and tetanus IG.

DIPHTHERIA AND DIPHTHERIA ANTITOXIN

Diphtheria is caused by toxigenic strains of *Corynebacterium diphtheriae* and occasionally *Corynebacterium ulcerans*. Diphtheria is exceedingly uncommon now because of widespread immunization, but a massive epidemic in the countries of the former Soviet Union occurred in the 1990s, with many fatalities.[343,712]

The adverse consequences of diphtheria result from the toxin elaborated and absorbed from the diphtheritic membrane. This toxin acts locally by perpetuating membrane formation and is distributed via the bloodstream to the heart, nervous system, kidney, and other organs. The larger the membrane, the more toxin that is elaborated; more toxin is elaborated from membranes coating the pharynx and tonsils than from one involving the larynx and trachea.

Diphtheria toxin is present in three forms: (1) circulating and unbound, (2) loosely bound to tissues, and (3) firmly bound to tissues. Antitoxin neutralizes circulating toxin and competes with and partially neutralizes loosely bound toxin but has no effect on tissue-bound toxin. Thus, optimal passive immunity must be initiated at an early stage of the disease by the intravenous route so that toxin can be intercepted before it becomes tissue bound.

In the 1890s, diphtheria antitoxin was used successfully to prevent spread to newly admitted pediatric patients and nurses.[209]

Antitoxin of animal origin remains the mainstay of treatment, as it was in the preantibiotic era. Diphtheria was the first illness in which antiserum was used as standard therapy. In 1901, Emil von Behring was awarded the first Nobel Prize in Medicine for this achievement. Fibiger[220] proved the efficacy of diphtheria antitoxin in 1898 when he showed that 5/204 patients given horse antitoxin died, whereas 14/201 not given antiserum died. Paschlau[527] demonstrated the importance of early administration of antitoxin in 1949; the fatality rate of 197 cases treated within 48 hours was 1.96 percent versus 8.9 percent when treatment was delayed until the fourth or fifth day. Other authors have noted similar findings.[675,676]

Tasman and associates[675,676] emphasized the importance of intravenous administration of antitoxin because rapid achievement of high blood levels results in rapid neutralization of antitoxin and the appearance, within 30 minutes, of antitoxin in saliva. They showed that the mortality rate and the severity of the myocarditis and neuritis in experimental diphtheria in guinea pigs could be reduced by giving antitoxin intravenously rather than intramuscularly.

Paul Ehrlich standardized the strength of diphtheria antitoxoin and defined 1 unit as the amount of toxin needed to kill a 250-g guinea pig in 4 days.[197]

McCloskey and Smilack[446] determined the antitoxin content of standard human IG. None of the lots tested contained diphtheria antitoxin in sufficient titer to allow its use for antitoxin therapy. They suggested that an IGIV with higher titer antitoxin be developed to eliminate the

risk for giving horse serum intravenously. Indeed, such a product is produced and used in the Ukraine.[214]

Recommendations

Diphtheria antitoxin of equine origin is indicated for all suspected or proved cases of diphtheria.[22] It is available in vials containing 20,000 U from the CDC. Before administration, skin tests must be performed to determine sensitivity. If the patient has a previous history of serum reactions or these test results are positive, a schedule of desensitization as outlined earlier must be followed.

The amount of antitoxin given depends on the location and the extensiveness of the membrane, the degree of systemic toxicity, and the duration of illness. The preferred route is intravenous, although intramuscular administration has been used historically in milder cases.

In all cases, diphtheria antitoxin should be given promptly rather than be delayed while awaiting bacterial confirmation of the diagnosis.[22]

In cutaneous diphtheria, antitoxin is of uncertain value. When used, the dose is 20,000 to 40,000 U to prevent toxic sequelae. In pharyngeal or laryngeal disease, 20,000 to 40,000 U is given; in nasopharyngeal disease, 40,000 to 60,000 U is given; and in extensive disease with neck edema or disease of more than 3 days' duration, 80,000 to 120,000 U is given.[22] Although antimicrobial therapy is a valuable aid in the treatment of diphtheria, it is not a substitute for antitoxin therapy.

Routine use of antitoxin in an asymptomatic, exposed susceptible patient is not recommended. With heavy exposure or an extremely susceptible host, antitoxin 5000 to 10,000 U intramuscularly can be given in addition to antibiotics and diphtheria immunization. Proof of efficacy is lacking, however.

In 1936, Wade Hampton Frost demonstrated that diphtheria toxoid given to school children was effective in preventing disease and resultant mortality.[229] Diphtheria antitoxin is used to prevent diphtheria and is contained in the currently used diphtheria-pertussis-tetanus (DTaP) and tetanus-diphtheria (Tdap) vaccines.

Human IGIV has variable amounts of diphtheria antitoxin, so it cannot be used as a replacement for animal antitoxin.[22]

PERTUSSIS

Pertussis antiserum was used in the 1930s for the treatment of pertussis.[100] Human pertussis IG was developed in the 1960s but was shown to have no additive benefit to antibiotics in the treatment of pertussis[60,471] and no longer is available commercially.

Granström and coworkers[262] used an experimental human hyperimmune serum from subjects immunized with a two-component acellular vaccine to treat 33 children, and an equal number received an albumin placebo. Both groups received the same antibiotic and supportive treatment. The treated children had decreased coughing and whooping, particularly if treatment was started early in the course of disease. There was no significant difference in the duration of hospitalization.

Ichimaru and colleagues[314] successfully used a high-titer human IGIV preparation to treat a severely ill 1-year-old child.

Bruss and associates[116] studied a 4 percent high-titer human pertussis IG in 26 children with pertussis and found that the product was safe at three dose levels (250, 750, and 1500 mg/kg) and provided good serum pertussis IgG levels with a half-life of 38 days. High titers of anti-PT antibodies were produced, and other studies have correlated such titers with protection against disease in animal models after aerosolized challenge.[115,590] Little antibody appeared in nasal secretions. Efficacy was not evaluated in this study, but the suggestion was made that treated infants had a shorter duration of paroxysms and the product was effective in the treatment of aerosol-induced pertussis in mice.[115]

A subsequent randomized, double-blind, placebo-controlled phase 3 trial that enrolled 25 infants was terminated because of unavailability of study product and poor recruitment. Preliminary analysis did not suggest a clinical benefit in symptoms such as cough, apnea, and oxygen desaturation in contrast to placebo.[284]

Recommendation

Because of the lack of well-designed, placebo-controlled, double-blind trials of immunomodulating agents, currently no recommendation exists for the role of passive immunity for pertussis.

RESPIRATORY AND OTHER BACTERIAL INFECTIONS

Respiratory tract infections caused by group A streptococci, *S. pneumoniae*, *H. influenzae* type b, and, to a lesser extent, *Neisseria meningitidis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae* are well recognized as occurring more frequently in patients with primary antibody deficiencies, and these infections can be reduced markedly by the regular administration of IGIV or IGIM.[219,405,499] IGIV represents a viable adjunct treatment option for certain cases of drug-resistant bacterial infections, and there is a clear survival advantage with the use of IGIV in adult sepsis syndromes by suppressing inflammation.[219] Furthermore, specific animal antisera to some of these organisms were used in the early 1930s for the treatment of severe infections (e.g., meningitis), even after the introduction of sulfonamides.[15] Efficacy varied but was clearly better than no treatment at all, and a combination of sulfonamides and antibody seemed to be synergistic.[15]

Small doses of IGIM (100 mg/kg/month) did not prevent or improve the course of respiratory infections in normal children.[69,223] However, Nydahl-Persson and colleagues[495] gave 24 children with repeated bacterial respiratory infection (i.e., pneumonia or otitis media) either trimethoprim-sulfamethoxazole or IGIV 400 mg/kg/month; both agents were effective in reducing the number of infections in comparison with a control group.

Santoshan and associates[587] administered a human intramuscular IGIM prepared from the sera of donors immunized with pneumococcal, meningococcal, and *H. influenzae* type b polysaccharide vaccines (bacterial polysaccharide IG [BPIG]) to Apache Indian infants living on reservations in Arizona. Of the 703 infants studied, 222 infants in the study group received 3 doses of BPIG, 0.5 mL/kg, at 2, 6, and 10 months of age, and the 218 infants in the control group received saline injections at the same ages. During the study period, seven cases of invasive *H. influenzae* type b disease and four cases of invasive pneumococcal disease occurred in the control group in contrast to one and two cases, respectively, in the BPIG-treated group, a significant difference ($p < .05$).

Otitis Media

Immunologically normal otitis media–prone children seem to derive little preventive or therapeutic benefit from low-dose IG given intramuscularly (100 mg/kg/month)[338] or intravenously (200 mg/kg/month).[346]

In patients with primary immunodeficiency, the frequency and severity of episodes of otitis media are diminished dramatically by the use of IG in large doses. In children with secondary antibody deficiency associated with HIV infection, IGIV in large doses (400 mg/kg/month) reduced the frequency of otitis media by 60 percent.[468,467] Patients with subtle immunologic abnormalities such as IgG subclass deficiencies, IgA deficiency, and polysaccharide antibody deficiencies may have recurrent episodes of otitis media that can be reduced with a large dose of IGIV (e.g., 400 mg/kg/month).[346,375,495,616,621]

BPIG also was shown to reduce the number of episodes of pneumococcal otitis media in high-risk Native American infants.[616] It did not, however, decrease the total number of episodes of otitis media. Large doses of RSV-IVIG (750 mg/kg/month) reduced the frequency of non-RSV otitis in young infants.[624] This product may have high antibody titers to bacteria and other viruses.[204]

Ishizaka and coworkers[321] successfully treated seven children with recurrent pneumococcal otitis media with IGIV.

The studies discussed here indicate that low-dose IGIM is ineffective in the prevention or treatment of otitis media, but high doses of IGIV (e.g., >400 mg/kg/month) may reduce the frequency and severity of otitis media in immunodeficient and otitis-prone normal children, probably by reducing both virally and bacterially mediated disease.[289] Englund and Glezen[203] suggested that passive immunization transplacentally from a recently immunized pregnant mother with *H. influenzae* or pneumococcal vaccine also should be considered.

IGIV should not be used routinely for otitis media–prone normal children but in extreme cases can be considered after failure of prophylactic antibiotics and pneumococcal immunization.

Sinusitis

Sinusitis is a common occurrence in immunodeficient patients and is difficult to prevent or eradicate, even with optimal treatment. Mofenson and colleagues[467] found that neither IGIV 400 mg/kg/month nor three times weekly sulfonamide prophylaxis prevented sinusitis from developing in children with HIV infection. Prospective data demonstrated that IGIV replacement treatment does not reduce or resolve chronic sinusitis,[219] however Desrosiers and Kilty[184] suggest IGIV may be an option in very refractory patients after endoscopic sinus surgery.

In patients with no or subtle immune defects (e.g., IgG subclass deficiencies and impaired polysaccharide antibody deficiencies), high-dose IGIV sometimes may decrease the frequency and severity of sinusitis.[104,155,549,621]

Lower Respiratory Tract Infections

Although high-dose IGIV is of proved benefit in preventing pneumonia in immunodeficient patients[122]; however, no evidence exists that it will prevent pneumonia in normal patients. Anecdotal cases of IGIV used as adjunctive therapy for refractory viral pneumonia have been reported.[579,654]

Cystic Fibrosis

Winnie and associates[742] suggested that IGIV may improve pulmonary function in pulmonary exacerbations of cystic fibrosis. All patients received antibiotics, were older than 12 years, and had no long-term benefit from the IGIV. Van Wye and coworkers[704] had similar results with the use of hyperimmune *Pseudomonas* IGIV in patients with cystic fibrosis.

A controlled trial of *Pseudomonas* hyperimmune IGIV in 116 patients with cystic fibrosis was discontinued because a 6-month interim analysis showed no reduction in acute pulmonary exacerbations.[544]

Balfour-Lynn and colleagues[62] suggested that high-dose IGIV was of short-term benefit, in both improving pulmonary function and in decreasing the need for steroids, in selected patients with cystic fibrosis and respiratory obstruction receiving inhaled and oral steroids.

In one study of 186 infants with cystic fibrosis, 92 receiving palivizumab and 94 receiving placebo over a single RSV season, the outcomes were similar between the groups and more research is required to recommend palivizumab prophylaxis in children with cystic fibrosis.[563]

Burn Infections

Kefalides and associates[359] reduced the mortality rate of severely burned children by administering plasma (1 mL/kg for each 1% of surface area burned) or IGIM (1 mL/kg on days 1, 3, and 5) from 40 to 20 percent. They concluded that solutions containing antibodies (plasma or IG) were more effective in reducing the complications of infections than were other colloids. However, Stone and colleagues[658] could not achieve any clinical benefit from IGIM therapy (0.4 mL/kg every third day until skin coverage) in 60 burned subjects versus 40 controls.

Convalescent plasma, special IG with high antibody titer to *Pseudomonas*, and *Pseudomonas* vaccines also have

been used in burn patients in an attempt to reduce infections, but without proof of efficacy.[172,188,335,482,615]

An observational study suggested that the combined use of IGIV and polymyxin B reduced the number of septic episodes and shortened the length of hospital stay in severely burned children.[428]

Firoz and colleagues[225] summarized 5 years of experience with 82 patients with toxic epidermal necrolysis in a burn unit in San Antonio and found no mortality benefit to the use of IGIV versus supportive care.

Gram-Negative Infections

The most extensive use of IG for infections in adults has involved trauma, shock, and postoperative patients thought to have gram-negative infections, as reviewed earlier under secondary immunodeficiency.* A meta-analysis involving 492 patients receiving polyclonal IGIV for sepsis and septic shock suggested a benefit, particularly among adults.[14] Monoclonal antibodies to endotoxin or cytokines were not of benefit. We conclude that this is a promising yet unproved use for IGIV.

Newborn Sepsis

As noted earlier, newborns and premature infants in particular are highly susceptible to bacterial sepsis and its sequelae. In addition to antibiotics, leukocytes, granulocyte colony-stimulating factor, and IGIV have been used as adjunctive therapy. As discussed earlier under secondary immunodeficiencies, numerous studies on the prevention and treatment of newborn sepsis are available and some suggest only slight benefit in prevention of sepsis. Ohlsson and Lacy[506] reviewed the literature regarding the use of IGIV for suspected or subsequently proved infection in neonates and found insufficient evidence exists to support the routine administration of IGIV for neonatal sepsis. In 2011, a large collaborative study of 113 hospitals in nine countries supported this conclusion. They evaluated the use of IGIV in neonatal sepsis and found no effect on the outcome of suspected or proven neonatal sepsis.[274]

IGIV may be considered for septic premature infants not responding well to conventional therapy; however, the evidence to date does not support its use. It may be particularly valuable for neutropenic septic infants because it may help mobilize leukocytes from the storage pool.

STAPHYLOCOCCAL INFECTIONS

Staphylococcal infections are ubiquitous and of varying severity. They include superficial skin infections, acute infections such as abscesses and wound infections, deep-seated indolent cellulitis, and three toxin-mediated diseases: scalded skin syndrome, toxic shock, and acute food poisoning. Antibiotics usually are effective in controlling the infections, but in some instances, the organism is antibiotic resistant or the disease is rapidly progressive. IGIV may be of adjunctive benefit in some of these situations.[362,716]

In addition to the use of IGIV, novel antistaphylococcal antibodies are being developed and are currently in clinical trials. Antibody products are directed against a variety of targets on the staphylococcal organism (i.e., capsule, lipoteichoic acid, and toxins). These products are in phase I and II trials. These antibodies are being developed for both prevention and treatment of staphylococcal disease.[504]

Altastaph is an example of a hyperimmune polyclonal IG preparation that targets CP5 and CP8 capsular polysaccharides. It was shown to prevent *S. aureus* bacteremia in mice but two phase II clinical trials failed to demonstrate efficacy.[76,233,575]

Staphylococcal Toxic Shock Syndrome

Patients with staphylococcal toxic shock syndrome, often associated with tampon use by menstruating women, have a rapid onset of fever, shock, macular desquamating rash, and multisystem organ failure.[37,458,607] The pathogenesis of the disorder results from infection with a strain that releases toxic shock syndrome toxin (TSST-1).[37] Approximately 20 percent of all staphylococcal isolates carry the gene for this toxin.[419] TSST-1 is a potent superantigen that directly activates the 5 percent of T cells that have a Vβ2 T-cell receptor[419]; such activation results in the rapid release of multiple cytokines and a clinical picture of rapidly progressive illness. Recent reports have implicated methicillin-resistant *S. aureus* (MRSA) in severe toxic shock syndrome.[327]

IGIV contains neutralizing antitoxins to the staphylococcal (and streptococcal) superantigens[674] and has been used successfully in both animal models of toxic shock syndrome[37] and several patients.[501,524,595] Although no controlled clinical trials have been performed for staphylococcal toxic shock syndrome, most authorities recommend large IGIV doses, at least 400 mg/kg, in addition to antibiotic treatment and circulatory support.[37] IGIV also downregulates cytokine synthesis and action and inhibits immune activation, thereby providing additional benefits.[37]

Higuchi and coauthors[301] reported a large family that had recurrent episodes of staphylococcal toxic shock syndrome associated with normal IG levels but low serum antibody titers to staphylococcal superantigens; two boys in the family achieved successful prophylaxis with regular IGIV infusions.

Neonatal Staphylococcal Infections

A second situation in which the administration of passive immunity may be of value is neonatal staphylococcal infection. Infection with *S. epidermidis*, is the most common cause of sepsis in premature infants and is aggravated in part by the use of catheters and parenteral lipid infusions.[57,226] Infections with *S. aureus* are also problems in neonatal intensive care units. Use of passive immunity directed at reducing *S. aureus* infections has been tested.

*References 72, 125, 180, 219, 251, 341, 764, 765.

One controlled study showed that IGIV was of value in decreasing the incidence of infections but not eliminating them,[57] but this benefit has not been found in all trials of IGIV in newborns. Although the administration of IGIV increased IgG levels in newborns, the overall morbidity and death as a result of nosocomially acquired sepsis were unchanged.

This variability may be explained by differing amounts of staphylococcal antibodies present in different IGIV brands and lots, particularly opsonic antibodies, which have been shown in animal models to be the most important correlate of clinical protection.[226] Because antibody variability exists in lots of IGIV made from pools of thousands of donors, it is not surprising that Krediet and colleagues[383] could not reliably increase the opsonic titers of premature infants by administering single-donor fresh-frozen plasma. Given that pooled IGIV has not been shown to decrease mortality in this high-risk population, the development of organism-specific IG has been explored. INH-A21 (Veronate) is a plasma-derived, donor-selected polyclonal antistaphylococcal human IG. A phase II, multicenter, double-blind clinical trial of this antibody was conducted on 512 low-birth-weight infants (50 to 1250 g), but no clinically significant differences were seen in the incidence of either *S. aureus* or coagulase-negative staphylococcus (CoNS).[88]

In a recent phase III trial with a larger enrollment, 1983 infants received either placebo or the study drug (INH-A21). The investigators were unable to demonstrate benefit in protection against late-onset sepsis resulting from either *S. aureus* or CoNS. The incidence of sepsis in the placebo group was 5 percent, in contrast to 6 percent in the treatment group.[181]

Pagibaximab, a humanized mouse chimeric monoclonal antibody directed against lipoeichoic acid, a cell wall component of gram-positive bacteria, is currently in development for prevention of gram-positive infections. A phase I and II double-blind, placebo-controlled, dose-excaltion, safety and pharmacokinetic study in infants with very low birth weight demonstrated safety and tolerability.[728] A follow-up phase II multicenter study of 2- to 5-day-old neonates, weighing 700 to 1300 g, demonstrated safety and at a dose of 90 mg/kg produced potentially protective antibody levels (>500 μg/mL).[729]

Severe Staphylococcal Infections

The last decade has seen an emergence of highly virulent and antibiotic-resistant strains of *S. aureus*. These strains are responsible for aggressive infections, including necrotizing fasciitis, and pulmonary necrosis. The virulence factors are currently being elucidated, and the Panton Valentine Leukocidin *(pvl)* gene product has been correlated with higher morbidity and mortality in MRSA infections[90] The precise role that *pvl* plays in the pathogenesis of severe *S. aureus* infections is unclear; however, it may provide a target for antibody neutralization in a severe toxin-mediated disease process.[108] Children with PVL-positive MRSA infections have been shown to mount a robust antibody response to PVL, but this antibody does not seem to be protective against future infections with the same organism.[299]

Neutralization of *S. aureus* PVL by IGIV in vitro has been demonstrated and shown to neutralize pore formation and the cytopathic effect of *S. aureus*.[236] Given this in vitro data, as well as the use in other diseases with toxin production such as toxic shock syndrome, the use of IGIV in aggressive infections caused by PVL-producing *S. aureus* could be considered as adjuvant therapy along with early débridment and active antimicrobial therapy.[288,753]

Refractory Staphylococcal Infections

A final use of IG is for the treatment of antibiotic-resistant chronic staphylococcal infection, along with intravenous antibiotics. Waisbren[716] treated 16 such patients with an antibiotic and gammaglobulin combination, with recovery noted in 13 of them. Indeed, hyperimmune plasma and IG from patients immunized with staphylococcal toxoids are widely used in Russia with apparent success.[362]

Recommendations

Large doses of IGIV are indicated for the treatment of staphylococcal toxic shock syndrome.[36] The recommended regimen is a single dose of 1 to 2 g/kg or 400 mg/kg daily for 5 days. A repeated dose may be necessary because of rapid clearance of IGIV in this illness.[36]

The value of IGIV in refractory chronic staphylococcal infections is unproven, although anecdotal reports of efficacy exist.[716]

IGIV is not recommended routinely for the prevention of infection in premature newborn infants. As noted earlier, IGIV may be of adjunctive benefit in the treatment of neonates with sepsis, particularly those with neutropenia.[330] Development of specific antistaphylococcal IGs to prevent staphylococcal infection in infants with very low birth weight continues to be an active area of investigation.[604]

STREPTOCOCCAL INFECTIONS

Circulating antibody may play a role in the prevention and treatment of group A streptococcal infection.[348] Newborns rarely develop invasive streptococcal illness, in part because of protective transplacental antibodies.[348] Equine antitoxin was used with some success in the treatment of erysipelas and scarlet fever in the 1920s and 1930s.[422,666]

Invasive Group A Infections

Invasive group A streptococcal infections, including septicemia, necrotizing fasciitis or myositis, and toxic shock syndrome, are of increasing severity and frequency.[23] Streptococcal pyrogenic exotoxins, including types A, B, and C, and mitogenic factor elaborated by certain strains of streptococci may be responsible for these serious complications of infection. Streptococcal pyrogenic exotoxins

are potent superantigens that activate certain T lymphocytes directly and lead to the massive production of multiple cytokines, with resultant shock, fever, and organ failure. IGIV contains neutralizing antibodies to these antigens,[492,493] but at varying titers across batches.[597]

Invasive soft tissue infections with group A streptococcus may benefit from high-dose IGIV.[174,249,492] In contrast to these reports, Mehta and colleagues[456] could not document a benefit for IGIV in patients admitted to the ICU with invasive group A infections from any cause.

Streptococcal Toxic Shock Syndrome

Many case reports have attested to the value of IGIV in streptococcal toxic shock syndrome since the report by Barry and coauthors in 1992.[13,70,177,402,535]

Kaul and colleagues[354] reported a study of 21 consecutive patients at several Canadian medical centers in 1994 and 1995 treated with IGIV and compared them with 32 similar patients not given IGIV at the same centers for the 3 preceding years. Both groups received appropriate antibiotics and were similar with regard to demographics, severity score, and timing of intervention. The median IGIV dose was 2 g/kg. The survival rate for the IGIV-treated group was significantly greater at 7 and 30 days (90% and 67%, respectively) than that in the untreated controls (50% and 34%, respectively; $p = .02$), and the days of hospitalization were insignificantly shortened (29 vs. 39). Serum from the IGIV-treated patients caused a marked inhibition of lymphocyte mitogenic activity to their own bacterial isolates after a single IGIV dose. This study excluded children from the analysis because of ethical considerations and the overall lower mortality seen in younger age groups.

In a large multicenter retrospective trial of children with streptococcal toxic shock syndrome[605] IGIV was administered to 84 of 192 children with streptococcal TSS and overall mortality, as well as overall hospital and drug costs, were evaluated. The investigators found no added benefit of IGIV infusion and noted the increased costs of IGIV administration.

PANDAS Syndrome and Sydenham Chorea

Murphy and colleagues[476] and Swedo and coauthors[665] reported a syndrome of tics, obsessive-compulsive behavior, or both, occurring in prepubertal children soon after the onset of a streptococcal infection. They termed this disorder *pediatric autoimmune neuropsychiatric disorder associated with streptococcal disease syndrome* (PANDAS syndrome). They reported suggestive benefit of IGIV.[536]

Treatment of Sydenham chorea, a movement disorder following streptococcal infection, with IGIV has also been anecdotally reported.[702]

The National Institute of Mental Health has an ongoing trial examining the benefits of patients with PANDAS with IGIV.*

*National Institutes of Health PANDAS clinical trial: http://clinicaltrials.gov/ct2/show/NCT01281969.

Recommendations

The use of IGIV can be considered as adjunctive therapy of toxic shock syndrome or necrotizing fasciitis in severely ill patients. Various regimens of IGIV, including 150 to 400 mg/kg/day for 5 days or a single dose of 1 to 2 g/kg, have been used, but the optimal regimen is unknown.[23] Repeated administration of IGIV may be necessary after a few days because of rapid IGIV catabolism.

IGIV may be of benefit in severe streptococcal soft tissue infections such as necrotizing fasciitis. IGIV is of unproved benefit in PANDAS syndrome.[511]

TETANUS, TETANUS ANTITOXIN, AND TETANUS IMMUNOGLOBULIN

Antitoxin for the treatment of tetanus was introduced into medicine by Behring and Kitasato in 1890; large doses (50 to 100 mL) of serum from horses immunized with tetanus toxin were used. The dose was increased gradually to 300 to 500 mL, equivalent to 300 to 500 U of antitoxin. As the means to increase the production and concentration of antitoxin developed and a high mortality rate persisted, the dosage of antitoxin was increased until doses as high as 200,000 U, repeated at weekly intervals, were recommended.[531] Despite such heroic therapy, no solid proof of efficacy was obtained.

However, in 1960, Brown and colleagues,[107] using sequential analysis, found that the mortality rate was 49 percent in 41 patients with tetanus who received 200,000 U of antitoxin, a statistically significant difference that established therapeutic efficacy. Extensive controlled studies established that mortality rates did not improve when larger doses (100,000 to 500,000 U) were used.[421,695-697]

Similarly, Patel and associates[529] could find no difference in the mortality rates of patients with tetanus treated with antitoxin doses of 5000 to 60,000 U. Adequate blood levels of antitoxin were noted in all cases, even in fatal cases, with a dose of 10,000 U. In mild cases, no antitoxin was necessary. They and others have reported considerable differences in mortality rates, ranging from 0 to 98 percent; the rate depends primarily on the severity of illness rather than on the dose of antitoxin.

Athavale and colleagues[53] established that antitoxin was of benefit in tetanus neonatorum and in tetanus in children up to 12 years of age. Antitoxin affected the mortality rate in mild and moderate cases but not in severe ones. A dose of 10,000 U was as effective as one of 30,000 U.

The mechanism of action of antitoxin is to neutralize toxin before it is transported to the nervous system through the circulation. Antitoxin also can neutralize toxin locally and prevent its systemic absorption. Thus, antitoxin can be given locally, at the site of production of toxin (e.g., at the site of a wound), intravenously (in severe cases), or intramuscularly (in less severe cases).

In 1962, an estimated 750,000 annual doses of equine tetanus antitoxin were needed in the United Kingdom,

which is equivalent to more than a million doses in the United States.[573] Serum sickness occurs in 6 to 14 percent and fatal anaphylaxis in 1/100,000 injections of equine-derived antitoxin. Thus, hyperimmune human tetanus IG (TIG), first available in the early 1960s, gradually has replaced equine tetanus antitoxin.

Rubbo and Suri[572] and Rubinstein[573] showed that TIG given intramuscularly (5 to 10 U/kg) provides adequate circulating antitoxin levels and is maintained in the circulation for considerably longer than is equine tetanus antitoxin.

The efficacy of TIG is equivalent to that of equine tetanus antitoxin. McCracken and associates[448] compared the results of 550 U of TIG with 10,000 U of tetanus antitoxin in the treatment of tetanus neonatorum. Among the 65 infants in each treatment group, no difference was noted in severity, length of hospitalization, need for sedation or gavage feeding, or mortality rate. Blake and colleagues[85] analyzed 545 tetanus cases reported to the CDC from 1965 to 1971 and found no difference in the outcome of patients treated with equine tetanus antitoxin versus TIG.

The first use of intrathecal antitoxin was recorded in 1898 by Charles Church and Dr. Rambord from the Pasteur Institute and by Cushing in 1899.[531] In 1902, a French surgeon reviewed 27 cases of tetanus treated with intrathecal antitoxin and concluded that there was no additional benefit.[739]

Gupta and associates[280] gave TIG intrathecally to alternate patients with early tetanus. Among 49 patients given intrathecal TIG (250 U), 3 deteriorated and 1 died; among 48 patients given intramuscular TIG (1000 U), 15 deteriorated and 10 died. No side effects occurred. Herrero and coworkers[300] could not prove a benefit of administering intrathecal antitoxin for tetanus neonatorum. Although an early meta-analysis of intrathecal therapy cast doubt on its efficacy,[5] studies from India[238] and Brazil[462] and a larger meta-analysis[342] suggested beneficial effect of intrathecal antibody.

TIG can be given along with tetanus toxoid for passive–active immunization. A dose of 250 U of TIG given intramuscularly at a site different from that of the toxoid does not interfere with the active antibody response.[447]

Lee and Lederman[404] measured the tetanus antitoxin titers in 29 lots of IGIV and noted considerable variability, although all lots had titers greater than 4 U/mL (mean, 21 U/mL). All lots would provide sufficient anti–tetanus toxin antibody if used at doses of 100 mg/kg as an alternative to TIG or tetanus antitoxin.

Recommendations

Prophylaxis

If a nonimmunized person sustains a serious injury or a bite, 250 to 500 U of TIG should be given intramuscularly as soon as possible.[35] The larger dose is used in the event of an extensive wound or delay in treatment. TIG is available for intramuscular administration in individual vials containing 250 U. Alum-precipitated toxoid to initiate active immunity is given at a different site with a separate syringe.

Human IGIV also can be used if TIG is not available and should be administered at a dose of 200 to 400 mg/kg.[35] This use of IGIV is not FDA approved and the antitetanus antibody levels in IGIV are not routinely assessed.

If TIG or IGIV is unavailable, tetanus antitoxin (equine) can be administered (after screening and testing for serum sensitivity have been performed).[35] Equine antitoxin for human use is available in several countries, but not in the United States, where TIG is available.

Treatment

In addition to administration of antibiotics and management of the wound, TIG should be given, but the optimal dose has not been established. At least 500 U is recommended, but doses as high as 3000 to 6000 U have been used. Part of the TIG is infiltrated near the wound, and the remainder is administered intramuscularly.[35] If the wound is extensive, TIG can be diluted with saline to infiltrate the entire area. TIG also is indicated for the treatment of tetanus neonatorum. Recent meta-analyses suggest that intrathecal use is of benefit in both adults and infants.[342] A study of infants with neonatal tetanus showed mild improvement in outcome in infants who received intrathecal TIG.[11]

Equine tetanus antitoxin has been used when TIG is unavailable, however this product is not available in the United States. If given it should be given in a single dose of 100,000 U, with 50,000 U given intramuscularly and 50,000 intravenously (after appropriate testing for sensitivity). On recovery, the patient should undergo primary immunization.

In tetanus neonatorum, McCracken and associates[448] found that 500 U of TIG given intramuscularly and 10,000 U of equine antitoxin were equally efficacious. Intrathecal TIG has been recommended[280,342,453] but is not always of benefit.[75,300] However, TIG is not licensed for this use.

PASSIVE IMMUNITY IN VIRAL INFECTION

ENTEROVIRUSES

Poliovirus

Before the development of poliomyelitis vaccines in the mid-1950s, IGIM was used extensively for the prevention of poliomyelitis. Bodian[91,92] showed that Red Cross IGIM had neutralizing antibody to all three strains of poliovirus in approximately equal titer and that rhesus monkeys given intramuscular poliovirus could be protected against disease by the subcutaneous administration of IGIM.

Bloxsom,[89] in an uncontrolled study performed during a 1948 Texas epidemic, gave 841 contacts an average dose of 2 mL of IGIM and noted only four cases at 1, 2, 3,

and 42 days after administration of the IGIM injection. His interpretation was that IGIM had been given too late to prevent the first three cases and that protection had worn off in the fourth case.

A committee on immunization of the National Foundation for Infantile Paralysis recommended in March 1951 that a controlled study be conducted on the efficacy of IGIM for the prevention of poliomyelitis during epidemics. Hammon and associates[287] subsequently undertook a massive field study in communities in three states during poliomyelitis epidemics.

Fifty-five thousand children aged 1 to 11 years received either IGIM (average dose, 0.14 mL/lb) or gelatin in a double-blind fashion. During the first week after injection was administered, 12 cases occurred in the IGIM recipients and 16 cases in the gelatin recipients. In the two groups, 3 and 23 cases occurred in the second week and 6 and 38 cases occurred in the third to fifth weeks, respectively. When protection was incomplete, decreased severity was noted. Protection waned by 6 weeks and disappeared by 8 weeks. These clinical results were confirmed by isolation of the virus or a rise in antibody titer in affected patients.

IGIM was an inefficient method of poliomyelitis prophylaxis in that it prevented only one case for every 500 to 2000 injections, and only for a brief time. Its chief value was in close family contacts of affected children and in aborting severe local epidemics.

Recommendations

The use of IGIM rarely is indicated for the prevention of poliomyelitis. An exposed unimmunized subject can be given 0.31 mg/kg of IGIM. An unimmunized patient who is traveling to an endemic or epidemic area and who cannot have vaccine can be given this dose of IGIM for temporary protection.

An immunodeficient patient inadvertently exposed to live attenuated polio vaccine who is excreting poliovirus in stool may be a candidate for receiving IGIV and oral IG in an effort to rid the gastrointestinal tract of the virus.

Other Enteroviruses

Enteroviruses, particularly echoviruses and coxsackieviruses, can cause severe disease in neonates[320,414] and in immunodeficient patients, particularly those with X-linked agammaglobulinemia or common variable immunodeficiency.[559] IGIM and IGIV have been used in both groups for the prevention and treatment of these infections.[559]

Meningoencephalitis

Chronic enteroviral meningoencephalitis with severe neurologic findings may develop in agammaglobulinemic patients; however, the incidence has decreased since routine treatment with IGIV has been instituted for these patients. IGIV does not prevent all cases, likely as a result of differing titers of antibodies to different enteroviral serotypes. McKinney and coworkers[451] summarized the results of treatment of 42 patients with chronic enterovirus meningoencephalitis: 10 received IGIM or plasma (1 survived), 10 received IGIV (7 survived), and 12 received both intraventricular IGIV and systemic IGIV (10 survived).

Misbah and colleagues[463] reviewed 15 patients with chronic enteroviral meningoencephalitis not included in the McKinney report, some of whom, however, had been described by others.[194,380] Of the 5 patients treated with IGIV, 3 survived, and of 10 treated with IGIV and intraventricular or intrathecal IGIV, 5 survived. Mellouli and colleagues[459] successfully treated an agammaglobulinemic boy who developed echovirus meningoencephalitis while receiving IGIV with intraventricular IG through an Omaya reservoir in addition to aggressive intravenous therapy.

Quartier and associates[547] reported complete clinical and virologic remission in 2 patients with X-linked agammaglobulinemia, as determined by both culture and polymerase chain reaction (PCR) assay. The first patient was treated with IGIV and a brief course of pleconaril. The second patient was treated with IGIV and pleconaril, but intraventricular IG was added when CSF pleocytosis persisted after 11 months of therapy. Complete clinical and virologic remission persisted at the 20-month follow-up. This study indicated that CSF PCR assay can be used, in addition to viral culture, as a guide to successful therapy.

Neonatal Enteroviral Infection

Severe and sometimes fatal disseminated enterovirus infection can develop in neonates.[548] Case reports have suggested benefit with the use of IGIV.[320,334,699] Kimura and coworkers[370] treated coxsackievirus B3 infection in four term infants with IGIV. The three who were treated early survived, but the infant treated 6 days after the onset of infection died. Abzug and associates[6] treated nine infants with echovirus and coxsackievirus B infection with IGIV but without apparent benefit; however, three of five infants who received high-titer IGIV had a shortened period of viremia. Rentz and colleagues[560] suggested that the maternal plasma might be used in lieu of IGIV as a source of high-titer antibody in newborns with a severe enteroviral infection. In summary, IGIV is of uncertain value, particularly if its titer against the particular virus is not known.

Nagington and colleagues[477] used IGIM successfully in an echovirus 11 outbreak in a special care nursery, but Kinney and coworkers[371] could not demonstrate a benefit of using IGIM in a similar nursery with the same virus. Pasic and associates[528] administered IGIV at 400 mg/kg prophylactically during a nursery echovirus 6 outbreak and thought that the severity of illness was decreased, although viral transmission continued.

Recommendations

IGIV may be used for critically ill neonates with disseminated enterovirus infection, although its benefit is unproved. In an outbreak situation with a known serotype and the availability of IGIV with significant titer to this serotype, IGIV should be considered.[678] Multiple

doses of 500 mg/kg as well as single doses of 750 to 1000 mg/kg have been used.[6,334,370,699]

For chronic enteroviral meningoencephalitis, McKinney and colleagues[451] recommend that sufficient IGIV be administered to maintain an IgG serum trough level of 900 to 1000 mg/dL. Even higher doses may be needed if the IGIV titer for the infecting virus is low. Quartier and coworkers[547] used 500 mg/kg of IGIV every 24 to 48 hours for 2 weeks, followed by 500 mg/kg two to three times per week for 6 weeks and then gradual reductions, but with maintenance of the trough IgG level at greater than 800 mg/dL.

Administration of IGIV by intraventricular catheter should be considered for patients who do not improve despite receiving aggressive therapy with IGIV. IGIV for intraventricular administration should have a neutral pH, be given slowly, and be at room temperature.[451] Dwyer and Erlendsson[194] used 6 percent IGIV intraventricularly, starting at doses of 120 mg/day and increasing to 600 mg/day during the first week of treatment, followed by doses of 300 mg/day for 1 to 4 weeks. Quartier and colleagues[547] administered 300 mg of IGIV daily for 15 days and then 300 mg three times per week to a patient whose intravenous IGIV therapy had failed. The duration and frequency of both intraventricular and intravenous IGIV must be individualized and determined by clinical and virologic response and normalization of CSF and ventricular fluid. An IGIV with a high titer to the infecting serotype should be used for both systemic and intraventricular therapy.

HEPATITIS A

The widest use for human IGIM has been for the prevention of hepatitis A, but this indication has been decreased by the widespread use of hepatitis A vaccine.[24] The efficacy of IGIM was demonstrated in 1945 by Stokes and Neefe[656] in aborting an epidemic in a children's summer camp, by Havens and Paul[292] in controlling an institutional epidemic, and by Gellis and associates[239] in preventing hepatitis A in the Mediterranean theater of operations at the close of World War II. The administration of combined IGIM and hepatitis A vaccine and scrupulous cleanliness can be used to interrupt the intestinal–oral circuit of transmission to abort an incipient epidemic.

IGIM is efficacious in preventing hepatitis A if it is given within 2 weeks of the last exposure.[9,24] Protection typically persists for 6 to 8 weeks. Stokes and associates[655] note that a single dose of IGIM (0.02 mL/kg) provided a degree of protection for up to 9 months in individuals residing at an institution in which hepatitis A was endemic.

The effectiveness of IGIM in hepatitis A varies from 80 to 95 percent, depending on how soon it is administered after exposure and the severity of the exposure.[721] IGIM suppresses clinical manifestations of the disease, but anicteric hepatitis is not prevented and the ratio of anicteric to icteric hepatitis may be as high as 12 : 1.[390] The period of protection exceeds the expected duration of the IGIM, supporting the concept of passive–active immunity; the hypothesis is that as a result of continuous exposure, a mild illness ensues and in turn confers long-lasting immunity.[390,653,719]

In initial studies, Stokes and Neefe[654] used an IGIM dose of 0.15 mL/lb, whereas others used doses of 0.06 to 0.12 mL/lb.[241,292] In 1951 Stokes and associates[655] showed that doses as low as 0.01 mL/lb were effective in limiting spread but did not completely prevent hepatitis. Hsia and colleagues[310] in 1954 also noted that a dose of 0.01 mL/lb was effective in preventing hepatitis among family contacts. However, Ward and associates,[722] in 1958, were able to reduce the case incidence of hepatitis in institutionalized patients from 19.5 to 7.4/1000 with 0.01 mL/lb and 1.7/1000 with 0.06 mL/lb. The larger dose may be particularly important in adults because they are subject to more severe disease.

The use of serologic tests for hepatitis A provides a way to determine the immunity of a subject, the presence of inapparent infection, the titer of hepatitis A virus (HAV) in lots of IGIM, and the validity of the passive–active immunity concept.[655,656] In other earlier studies, Krugman[385] showed that an IGIM preparation with a titer of 1 : 3200 was effective in neutralizing the infectivity of MS-1 serum, a substance known to contain HAV. Farcet and colleagues evaluated the HAV antibody titer in plasma pools from March 2003 through September 2008 and determined the HAV titers adequate for HAV protection for those receiving antibody replacement for primary immune deficiency.[211]

Most current lots of IGIM have anti-HAV antibodies when they are assayed by a competitive-inhibition radioimmunoassay. Titers greater than 1 : 100 are protective.[629]

Recommendations

Household and Sexual Contacts

All previously unimmunized people with close personal contact to a person with HAV should be given a single IGIM dose of 0.02 mL/kg or single-antigen hepatitis A vaccine as soon as possible after being exposed.[9,24,708] For healthy persons aged 12 months to 40 years of age, single-antigen hepatitis A vaccine at the age-appropriate dose is preferred. For persons older than 40 years, those younger than 12 months, immunocompromised persons, persons who have had chronic liver disease, and persons for whom vaccine is contraindicated, IGIM is preferred.[488] Serologic testing for hepatitis A is unnecessary and may delay the administration of IGIM. The use of IGIM longer than 2 weeks after exposure has occurred is not indicated.[24,224]

School Exposure

IGIM usually is not necessary for children and their teachers exposed to a single case of hepatitis A in the classroom of a day school. However, if several children are infected or if transmission in the school is documented, IGIM (0.02 mL/kg) or vaccination is indicated for those unimmunized students and teachers who have close contact with the index case. IGIM prophylaxis is

recommended for children and staff exposed at a boarding school or in a school for developmentally disabled children, where opportunities for transmission by the fecal-oral route are increased.

Institutional Outbreaks

Hepatitis A outbreaks in institutions such as boarding schools, daycare centers, custodial care centers, and prisons require aggressive action; however, the frequency of these outbreaks have decreased with increasing vaccination rates. Vaccine or IGIM should be administered to all previously unimmunized staff members and attendees if one or more cases of hepatitis A are recognized in children or staff or two or more cases among households with attendees at the centers. Vaccine or IGIM should be given to all household members with persons in diapers at the centers if two or more families are affected.[9,24] If an outbreak of hepatitis A is traced to a food handler, IGIM or vaccine should be given to close contacts and other restaurant employees.[131]

Hospital and Clinic Exposure

When an outbreak occurs, vaccine or IGIM is recommended for people in close contact with the infected patient.[9]

Common-Source Exposure

Common source exposure cases generally are identified too long after the exposure occurs for IGIM to be effective. If a person has been exposed within the previous 2 weeks, IGIM or vaccine can be given (0.02 mL/kg).[9]

Community Outbreaks

Unless a source of the infection is identified, mass use of IGIM is ineffective and not recommended because it will not interfere with transmission.[52] Immunization is recommended.

Foreign Travel

Individuals going to developing countries should receive vaccine 1 month before departure. Travelers who are administered vaccine can be assumed to be protected within 4 weeks after receiving the first vaccine dose.[146,224] If the departure date is less than 2 weeks away, 0.02 mL/kg of IGIM and vaccine can be used.[24] The IGIM will provide immediate protection and not interfere with efficacy of the vaccine. If exposure to hepatitis A will continue beyond 3 months, the IGIM dose should be 0.06 mL/kg and repeated every 5 months if exposure to hepatitis A continues and the patient cannot be immunized.[9]

Primate Exposure

Certain subhuman primates such as chimpanzees may carry HAV. Animal handlers should observe scrupulous hygiene and be given hepatitis A vaccine. If bitten and unimmunized, they should receive 0.02 mL/kg of IGIM and vaccine.[146]

Needle Exposure

IGIM is indicated for susceptible persons accidentally inoculated with blood or serum from a patient with hepatitis A. The recommended dose is 0.02 mL/kg; pregnancy is not a contraindication to the administration of IGIM.

Newborn Infants of Infected Mothers

Perinatal transmission of HAV is rare. If the mother becomes symptomatic with acute hepatitis A between 2 weeks before and 1 week after delivery, the infant can be given 0.02 mL/kg of IGIM. Efficacy has not been established.[146]

HEPATITIS B AND HEPATITIS B IMMUNOGLOBULIN

Hepatitis B virus causes a wide spectrum of illness ranging from asymptomatic seroconversion to fulminant hepatitis. Although most healthy patients recover completely, the carrier state occurs commonly in exposed immunocompromised patients, such as newborns, patients taking immunosuppressive medications, and patients with primary or secondary immunodeficiencies.

Transmission occurs after exposure to blood or other body fluids, through inoculation or sexual contact, and by close personal contact such as may occur in daycare centers. The main route of transmission used to be by blood transfusion, but with donor testing, this route is no longer common in developed countries. An important route of transmission is perinatally from the mother to the newborn infant, who is likely to remain chronically infected for a lifetime. This route usually can be prevented by active and passive immunization.

Immune Globulin Use in Hepatitis B

The initial study of IGIM in hepatitis B was conducted in 1945 by Grossman and associates,[273] who treated alternate battle casualties given whole blood or plasma with two 10-mL injections of IGIM 1 month apart. The incidence of icteric hepatitis was 1.3 percent in 384 IGIM-treated patients and 9.9 percent in 384 control patients, a highly significant difference that suggested a beneficial effect of IGIM.

In 1947, Duncan and associates[192] reported the results from a similar study, although they gave only one 10-mL injection; hepatitis occurred in 1.2 percent of 2406 patients in the IGIM-treated group and in 0.9 percent of patients in the control group, an insignificant difference. However, the mean incubation period was significantly prolonged in the IGIM-treated group (to 103 days) in contrast to the control group (87 days).

Holland and associates[305] could not alter the incidence or severity of posttransfusion hepatitis in 84 open heart surgery patients given two 10-mL doses of IGIM 1

month apart in contrast to 83 non–IGIM-treated controls. These findings were confirmed in a large cooperative study of 5189 transfused cardiovascular patients given 10 mL of IGIM during the first, fourth, and seventh postoperative weeks.[258] Redeker and associates[556] could not demonstrate that IGIM protected spouses of individuals with type B hepatitis. Similarly, Kuhns and colleagues[392] could not reduce the incidence of posttransfusion hepatitis B with 20 mL of IGIM. Both of these latter studies used IGIM with low titers of antibody to hepatitis B surface antigen (anti-HBs).

Several factors probably are responsible for the variation in effectiveness of IGIM in hepatitis B. One is the variable degree of exposure to hepatitis B virus, which can be massive (as with a blood transfusion) or minimal (e.g., a household contact). A second factor is the variable levels of anti-HBs antibody in different lots of IGIM. Because IGIM does not always contain high titers of anti-HBs, it is not recommended for hepatitis B prophylaxis. Standard IG is not effective for postexposure prophylaxis against HBV infection because the concentration of anti-HBs are too low.[26]

Hepatitis B Immunoglobulin

Soon after hepatitis B surface antigen (HBsAg) and its antibody (anti-HBs) were identified, researchers could measure and select lots (or donors) with high titers of anti-HBs of at least 1:100,000 by radioimmunoassay. This product, hepatitis B IG (HBIG), has been licensed since 1978 for prevention of hepatitis B.[24,137,139]

In 1971, Krugman and associates[386,387,390] evaluated high-titer HBIG in institutionalized children injected with the infective serum MS-2. Hepatitis developed in all 11 children exposed to MS-2 serum, and 5 remained HBsAg carriers after 320 days. Among 5 children given MS-2 serum and standard IG, hepatitis developed in 3, but none became a carrier. Of 10 children exposed to MS-2 serum and HBIG, 6 were completely protected, 1 had a transient infection, and 3 developed classic hepatitis. They concluded that HBIG was 70 percent effective under these circumstances. Their later studies confirmed that HBIG could reduce the incidence, severity, and carrier rate of HBsAg significantly after prenatal exposure to hepatitis B virus.[388]

Szmuness and associates[667] tested the efficacy of HBIG versus IGIM by giving either standard IGIM or HBIG to institutionalized children at admission and at 4-month intervals for 1.5 to 2 years and compared the incidence of hepatitis with that in untreated patients. Both globulin-treated groups had a lower attack rate (11% vs. 25% in the untreated patients) and a lower incidence of persistent hepatitis B antigen (none in the globulin-treated group vs. 13.5% in the untreated group). Thus, both IGIM and HBIG were effective in preventing or modifying nonparenterally transmitted hepatitis B in an endemic setting. Anti-HBs developed in 55 percent of the patients treated with standard IGIM, whereas antibody developed in only 23 percent of the patients treated with HBIG, thus suggesting that passive–active immunity occurred more frequently in the group who received standard IGIM than in the group who received HBIG.

Seeff and associates[599] gave either HBIG or standard IGIM to 302 individuals definitively exposed orally or parenterally to material that was infectious for hepatitis B. The incidence of both clinical and subclinical hepatitis during the first 6 months was 0.7 percent in the HBIG-treated group and 6.1 percent in the IGIM-treated group. At 6 months, 32 percent of the IGIM recipients and 6 percent of the HBIG recipients had antibody, a finding indicative of minimal passive–active immunity in the HBIG-treated group.

Grady[259] reported similar results with HBIG after accidental exposure. The incidence of hepatitis at 6 months was 7 percent (of 251 patients) with standard IGIM, 5 percent (of 208 patients) with intermediate-titer HBIG, and 2 percent (of 253 patients) with high-titer HBIG. This protection waned after 6 months, and differences in the groups became less apparent after 9 months, possibly because of reexposure, delayed onset of infection, or failure of passive–active immunity

Prevention of Vertical Transmission

Beasley and associates[73,74] studied the efficacy of HBIG in preventing perinatal transmission of the hepatitis B virus carrier state from a mother to her newborn infant. HBIG or placebo was given at birth to the infants of hepatitis B early antigen (HBeAg)-positive, HBsAg-positive carrier mothers, and the infants were monitored for at least 15 months. Of 61 placebo recipients, 92 percent became carriers; of 67 infants who received 1 mL of HBIG at birth, 54 percent became carriers; and of 57 infants who received 0.5 mL of HBIG at birth and at 3 and 6 months, 26 percent became carriers. Passive–active immunization, indicated by the presence of anti-HBs, occurred in 27 percent of the single-dose group and in 61 percent in the three-dose group.

On the basis of these and other studies[332,559] suggesting that multiple HBIG doses were more effective in interrupting vertical transmission of the HBsAg carrier state than a single HBIG dose, advisory committees in the early 1980s recommended that all infants of HBsAg-positive mothers be given HBIG (0.5 mL) immediately after birth and again at 3 and 6 months.[137,139] However, many of these infants became infected sometime after their last HBIG dose (i.e., in the second or third year of life), thus indicating a need for more durable active immunity. Shi and colleagues[611] systematically reviewed the effect of HBIG given in pregnancy to interrupt mother-to-child transmission of hepatitis B. They evaluated 37 randomized control trials between January 1990 to December 2008 and concluded that there may be some benefit in HBIG use in pregnancy to prevent intrauterine transmission.

Wong and associates[748] studied the efficacy of hepatitis B vaccine given in conjunction with HBIG in the prevention of vertical transmission of the carrier state from mother to infant. They gave hepatitis B vaccine (36 infants), hepatitis B vaccine plus one dose of HBIG (35 infants), hepatitis B vaccine plus seven monthly HBIG doses (35 infants), or placebo (35 infants) to infants of HBsAg-positive mothers. In all vaccine groups, development of a persistent carrier state was reduced significantly

in contrast to the placebo group (21% with hepatitis B vaccine alone, 2.9% with hepatitis B vaccine plus one dose of HBIG, and 6.8% with hepatitis B vaccine plus seven doses of HBIG vs. 73.2% for placebo groups). Anti-HBs developed in all infants of the treatment groups, indicating that HBIG did not interfere with active immunization.

This and other studies[669] indicate that HBIG given at the same time as hepatitis B vaccine provides optimal passive–active immunity for long-lasting prevention of the carrier state, and this dose is the current recommendation. Studies in adults also confirm that HBIG given before or simultaneously with the first dose of hepatitis B vaccine does not interfere with the antibody response to hepatitis B vaccine.[758] This approach will protect 85 to 95 percent of infants from perinatally acquired HBV infection.[150]

HBIG is not of value in the treatment of either acute[7] or chronic[558] hepatitis B infection.

Hepatitis B Immunoglobulin in Liver Transplantation

Liver transplantation in a patient infected with hepatitis B is associated with a high rate of its recurrence in the new liver and subsequent mortality (50% in 2 years). Intravenous HBIG has proved efficacy and dose-dependent response in the prevention of HBV recurrence after liver transplantation[164] and has been the mainstay of posttransplant prophylaxis of recurrent HBV since the early 1990s.[118] The HBIG doses needed to prevent reinfection are very high, and thus HBIG primarily is given intravenously.[680]

HBIG given intravenously or by the usual intramuscular route will reduce the rate of recurrence of hepatitis B.[582] In a retrospective analysis of 359 such transplants, hepatitis B recurrence was 74 ± 6 percent in 67 patients given no HBIG, 74 ± 5 percent in 83 patients given HBIG for 2 months, and 36 ± 4 percent in the 209 patients given HBIG for 6 months or longer.

Grazi and coworkers[264] reduced significantly the recurrence rate in HBsAg-positive, hepatitis B virus DNA–negative cirrhotic individuals undergoing liver transplantation with the use of HBIG for 1 year after transplantation; recurrence was noted in 8/10 controls (80%) and 4/25 HBIG-treated patients (16%). Several other reports are available.[677,679]

In addition to HBIG therapy, pretransplantation and posttransplantation antiviral drugs such as lamivudine are used. When administered in the immediate pretransplantation period to patients with a high viral burden, these drugs enhance the effectiveness of HBIG because less virus must be neutralized.[352,677] Long-term maintenance of antivirals with discontinuation of HBIG after several months may be feasible.[158,692,747]

Preliminary studies of monoclonal antibodies to hepatitis B antigens are under way.[763]

Recommendations

Prophylaxis. HBIG is recommended after a person's parenteral or mucous membrane (oral, sexual, ophthalmic) has contact with blood or body fluids from individuals with hepatitis B or with HBsAg-positive materials (e.g., blood, plasma) and for neonates born to HBsAg-positive mothers (see Table 245-5).[25]

Exposure to Blood That Contains (or May Contain) Hepatitis B Surface Antigen. No prospective studies have tested the efficacy of a combination of HBIG and hepatitis B vaccine in preventing hepatitis B after accidental exposure, including exposure by the percutaneous, ocular, and mucous membrane routes, or by human bites that penetrate the skin. Health care workers at risk for incurring such accidents should receive hepatitis B vaccine as preexposure immunization as the most effective means to prevent HBV transmission.[25] For unimmunized people the combination of HBIG and hepatitis B vaccine is recommended after significant accidental exposure (Table 245-6).

If the exposed patient is unimmunized and the blood or secretions come from an individual known to be HBsAg positive or if the infection status of the source is unknown but the source is at high risk, immediate prophylaxis is indicated. A single dose of HBIG 0.06 mL/kg, or 5 mL for adults, should be given intramuscularly as soon as possible, preferably within 24 hours of exposure.[25] Hepatitis B vaccine should be given simultaneously at a different site and repeated after 1 and 6 months (see Table 245-6). After massive exposure (i.e., by blood transfusion), much larger doses of HBIG may be indicated.

If the exposed patient has been immunized but the serologic response is not known, anti-HBs titers should be determined and the individual managed according to the results; if nonresponsive (anti-HBs <10 mIU/mL by radioimmunoassay), HBIG and a full series of vaccine should be given. In patients with a contraindication to vaccine, two doses of HBIG 0.06 mL/kg should be used, the second administered 1 month after the first. Two doses of HBIG also should be given to an exposed health care worker who is known to have no response to two series of hepatitis B vaccine.[25] Current recommendations from the CDC[146] for nonoccupational exposures are to administer vaccine alone to previously immunized individuals as a booster dose without the need to document serologic response if the exposed person has written documentation of a completed hepatitis B vaccine series. The *Red Book*[25] also agrees with this booster vaccine approach. We recommend a more individualized approach for assessing risks of the addition of HBIG.

Perinatal Exposure. Among mothers who are HBsAg positive and HBeAg positive, 85 percent of their untreated infants will become infected and be chronic carriers, and chronic hepatitis, cirrhosis, or hepatic cancer will develop in some of them. If the mother is HBsAg positive only, the risk for her offspring becoming a carrier is less but still significant. More than 90 percent of infants infected perinatally will develop chronic HBV infection.[25] Accordingly, these infants should receive HBIG prophylaxis and hepatitis B vaccine.

Infants Born to Mothers Who Are HbsAg Positive. For optimal passive–active immunity, HBIG 0.5 mL is given to the newborn at birth (preferably in the delivery room but within 12 hours at the latest). Hepatitis B vaccine (at

TABLE 245-6 **Hepatitis B Postexposure Recommendations for Hepatitis B Immunoglobulin and Hepatitis B Vaccine**

	HBIG		*Hepatitis B Vaccine*	
Type of Exposure	**Dose**	**Recommended Timing**	**Dose**	**Recommended Timing**
Perinatal				
Infant of HBsAg-positive mother	0.5 mL IM	Within 12 hr of birth	0.5 mL	First dose within 12 hr, repeat 2 times
Infant of mother whose HBsAg status is unknown	0.5 mL IM	Within 7 days of birth if mother is found to be HBsAg positive	0.5 mL	First dose within 12 hr, repeat 2 times
Premature infant (<2000 g) whose mother's HBsAg status is unknown	0.5 mL IM	Within 12 hr of birth (unless maternal HBsAg test result is negative by this time)	0.5 mL	First dose within 12 hr, repeat 3 times
Mucous Membrane or Percutaneous with Known Nonoccupational Exposure				
Nonvaccinated	0.06 mL/kg IM	Immediately	0.5 mL*	First dose immediately, repeat 2 times
Previously vaccinated, known responder	None	None	None	
Previously vaccinated, unknown response	0.06 mL/kg IM if HBs	Test patient for antiHBs; omit HBIG† if >10 mIU/mL	0.5	Give booster†
Previously vaccinated, known nonresponder	0.06 mL/kg IM	Immediately, may repeat in 1 month if <10 mIU/mL‡	0.5 mL*	Give booster immediately, repeat 2 times if necessary
Sexual: Known Exposure				
Nonvaccinated	0.06 mL/kg IM	Immediately, but no later than 14 days	0.5 mL*	First dose immediately, repeat 2 times
Vaccinated†	None	None	0.5 mL	Give booster if unknown response

*Dose for individuals younger than 20 years. The adult vaccine dose is 1 mL. The dose for immunosuppressed patients and patients undergoing dialysis varies with the vaccine used. Vaccine doses given are for Recombivax HB or Engerix-B.
†Recent CDC recommendations for exposure (sexual, sexual assault, percutaneous, or mucosal) from a source with unknown hepatitis B status are to forgo vaccine and HBIG if the exposed patient is already immunized and has written documentation of complete vaccine series and did not receive serologic testing for response. Individual assessments of potential risk must be made. See text.
‡See text for discussion of persons who have not responded to two vaccine series.
HBsAg, hepatitis B surface antigen; *HBIG,* hepatitis B immunoglobulin.

a dose of half the adult dose) is begun simultaneously and repeated at 1 and 6 months (see Table 245-5). This combination is only approximately 90 percent effective in preventing the carrier state because intrauterine infection will not be prevented.

Term Infants Born to Mothers Not Tested for HBsAG. If the HBsAg status of the mother is unknown, hepatitis B vaccine should be given to the term infant within 12 hours, and HBIG should be administered as soon as the mother is shown to be a carrier. Its effectiveness is diminished markedly if administration is delayed beyond 48 hours after birth but is recommended to be given up to 7 days after birth.[25]

The mother should be tested immediately. If she is HBsAg negative, the vaccine series is continued as recommended for other infants. If the mother is found to be HBsAg positive, HBIG and hepatitis B vaccine should be given to her infant, even if a significant delay has occurred. After the final dose of the vaccine series, the infant can be tested for HBsAg and anti-HBs at 9 to 18 months to determine the success of the HBIG and vaccine regimen. Testing should not be performed before 9 months of age to avoid detection of anti-HBs from HBIG administered during infancy and to maximize the likelihood of detecting late HBV infections.[25] If HBsAg is present, the infant is a carrier. If anti-HBs is present, the child was successfully immunized. Administration of HBIG at birth should not interfere with polio or DTaP vaccines starting at 2 months of age.

Premature Infants. Premature infants with birth weights less than 2000 g who are born to women not tested for HBsAg should be given HBIG 0.5 mL within 12 hours of birth unless the mother's HBsAg test result can be available within 12 hours and is negative. Immunization should be started immediately and repeated for a total of four doses (rather than the usual three doses) because of the poorer response of preterm infants to the vaccine.[25]

Sexual Exposure to Hepatitis B or a Carrier of Hepatitis B. Sexual exposure (including rape) to an individual who has hepatitis B or is a carrier is an indication for administration of HBIG 0.06 mL/kg intramuscularly (maximum, 5 mL) and initiation of hepatitis B vaccination. HBIG should be given as soon as possible but not after 14 days after exposure. If only HBIG is given, a second dose of HBIG is recommended after 30 days. The

CDC[146] also recommends booster vaccine alone in this situation if written documentation is available for a completed hepatitis B vaccine series.

Possible Exposure. After possible exposure (percutaneous, ingestion, sexual) to an unidentified person or body fluid in which the HBsAg status is unknown, the decision to treat with HBIG must be made individually on the basis of the likelihood that the source is HBsAg positive and the seriousness of the exposure. Hepatitis B vaccine should be initiated immediately if the exposed person is not immunized. Current *Red Book* recommendations and CDC recommendations[25] suggest that with an unknown source, vaccine alone is acceptable for an unvaccinated exposed patient and no treatment is required if the exposed person has written documentation of having previous immunization. We recommend a more individual approach to assess risks.

Ideally, the source subject should be tested for HBsAg positivity; if the results are available within 7 days for a percutaneous exposure and 14 days for a sexual exposure,[25] HBIG 0.06 mL/kg (maximum, 5 mL) can be given immediately and again at 1 month if the source is HBsAg positive and the patient did not or could not receive vaccine. When the source subject cannot be tested or when the source is likely to be HBsAg positive, HBIG is administered immediately.

If the exposed individual is a high-risk patient (e.g., immunodeficient, immunosuppressed, institutionalized, or undergoing hemodialysis) or is in an environment or health care unit for which past environmental control measures have been ineffective, HBIG should be given in addition to hepatitis B vaccine.

HBIG is not indicated on a routine basis after administration of blood transfusions. School or hospital exposure or usual household exposure is not an indication for HBIG, but household exposure is an indication for vaccine for unimmunized exposed persons.[25]

LIVER TRANSPLANTATION

For patients who are HBsAg positive at the time they undergo liver transplantation or in the rare situation in which the donor of the liver is HBsAg positive, administration of HBIG is recommended in the preoperative period and after transplantation to prevent recurrence in the transplanted liver.*

The exact dose, frequency, route, and duration of HBIG in this situation have not been established, but the dose is large and adds significantly to the cost of the transplant procedure.

The typical regimen is to use very high HBIG doses (i.e., 2000 IU [10 mL]/week) before transplantation and immediately after transplantation, along with antiviral medications.[677] The HBIG is continued for several months to years, and the dose is determined by monitoring of the hepatitis B viral burden by PCR. After several months, the HBIG may be reduced or discontinued, but the antivirals are continued.[747] (See earlier discussion.)

*References 264, 473, 582, 583, 675, 677, 682.

HEPATITIS C

Early studies suggested that lots of polyvalent IGIM or HBIG not screened for hepatitis C antibodies provided some protection against the acquisition of non-A, non-B hepatitis (presumably hepatitis C) after heart surgery or hemodialysis.[373,586,626]

Two studies provide some evidence for a prophylactic effect of IGIM. Piazza and colleagues[538] gave polyvalent IGIM (from pools containing antibodies to hepatitis C virus [HCV]) or placebo monthly for 4 to 20 months to the seronegative sexual partners of 884 patients who were seropositive for HCV. One of the 450 (representing 560 subject-years) in the IGIM group became infected versus 6/449 (500 subject-years) in the placebo group ($p = .03$; relative risk, 10.7). The authors concluded that sexual transmission of hepatitis C occurs and that IG has a protective effect.

Féray and associates[217] reviewed the records of 218 patients with hepatitis C coinfection undergoing liver transplantation who received an HBIG product that contained antibody to HCV. The incidence of HCV viremia 1 year after transplantation was significantly lower (25/46 [54%]) in patients receiving HBIG than in those not receiving it (162/172 [94%]; $p < .001$). They also reviewed 210 transplanted HCV-seronegative patients and found that hepatitis C developed within 1 year in 18/68 patients (26%) who received the HBIG product containing anti-HCV antibody in contrast to 40/86 patients (47%) who did not receive HBIG, a significant difference ($p < .001$) suggesting a preventive effect of hepatitis C antibodies.

Also supporting this concept was the observation that HCV was transmitted by pooled human IG in the early 1990s, shortly after HCV antibody–positive donors were excluded from the donor pool.[144,593] These antibodies presumably neutralized the small amounts of HCV present in the unscreened lots. Indeed, Yu and colleagues[757] demonstrated that HCV antibodies in unscreened IG lots containing HCV antibodies prevented low-dose infection in chimpanzees. Screened IGIM lots after 1991 lacking HCV antibodies did not prevent infection.

Because HBIG can prevent recurrence of hepatitis B among patients with hepatitis B undergoing liver transplantation,[264,583] researchers have postulated that hepatitis C antibodies might prevent recurrence in hepatitis C–positive liver transplant recipients. Current studies are ongoing to develop monoclonal antibodies, immunomodulators, and vaccines to effectively neutralize HCV.* Bavituximab is a monoclonal antibody that targets phosphatidylserine, a specific phospholipid component of cell membranes that become exposed on the outer membrane of cells infected with HCV. A phase I study demonstrated mild adverse events in six patients, and phase II trials are ongoing. It is also being studied for therapeutic use in patients with advanced solid tumors.[12]

*References 12, 97, 244, 355, 356, 515, 720.

Experimental Hepatitis C Immunoglobulin

Experimental HCV-IGIV is derived from human plasma units rejected for HBIG or IGIV manufacture because of the presence of hepatitis C antibodies: it does not contain hepatitis C, as tested by PCR.

Krawczynski and colleagues[382] showed that HCV-IGIV delayed but did not prevent experimental hepatitis C infection in chimpanzees in contrast to regular IGIV without hepatitis C antibodies.

Willems and colleagues[737] gave HCV-IGIV to 26 hepatitis C–positive liver-transplant recipients during the ahepatic and postoperative periods, without decreasing the rate of recurrence of hepatitis C. Davis and colleagues[179] reported a multicenter double-blind study of HCV-IGIV (Civacir, Nabi) in hepatitis C–positive liver-transplant recipients. No effect occurred on HCV RNA levels, but some decrease in liver enzyme activities in contrast to the control patients was noted. Thus, the use of antibody to prevent the development of hepatitis C after liver transplantation has not been successful and was attributed to limitations of dosing and the inability of HCV-IGIV to neutralize the HCV RNA in phase II trials.[179]

Recommendation

No passive immune product is available for hepatitis C prophylaxis.

HERPESVIRUSES

The common herpesviruses that cause human infection are CMV, Epstein-Barr virus (EBV), herpes simplex virus types 1 and 2, varicella-zoster virus, and human herpesviruses 6, 7, and 8. Although these DNA viruses produce latent infection that can reactivate, particularly in immunodeficient patients, the major uses of IGs have been in the prevention of primary varicella-zoster virus infection and prevention and treatment of CMV infection in transplant recipients and more recently in pregnant women and their fetuses with primary infection.

Cytomegalovirus

Use of Cytomegalovirus Intravenous Immunoglobulin or Intravenous Immunoglobulin in Transplantation

CMV infection remains a major complication after hematopoietic cell transplantation and solid organ transplantation. Seronegative individuals who receive an allograft from a seropositive donor are at the highest risk for acquiring CMV infection. Both polyvalent (IGIV) and hyperimmune CMV intravenous immunoglobulin (CMV-IGIV [CMVIG]) have been used since the 1980s to prevent development of CMV infection in bone marrow and solid organ transplant recipients.[744] Clinical trials in the 1990s demonstrated the efficacy of prophylaxis with IGIV in preventing CMV infection and pneumonia. CMVIG is prepared from the plasma of donors with high anti-CMV titers, but polyvalent IGIV also contains anti-CMV antibodies at lower titer. Early studies used CMVIG or IGIV alone, but later studies have combined IG with antiviral agents.

Early studies in renal and liver transplant recipients showed the efficacy of CMVIG or IGIV.[632] Two meta-analyses demonstrated decreased CMV mortality rates with the use of CMVIG or IGIV in bone marrow and solid organ transplant recipients.[746] Others have shown a similar prophylactic benefit of IGIV or CMVIG.[460,617] Ruutu and coworkers[578] showed no prophylactic benefit of CMVIG in CMV-seronegative bone marrow transplant recipients. A multicenter retrospective review[553] of CMVIG prophylaxis in addition to intravenous gancyclovir in pediatric lung transplant recipients did not demonstrate a decrease in either posttransplant CMV infection or morbidities.

Several factors that have reduced the need for CMVIG or IGIV in transplant recipients include the realization of a markedly reduced risk for development of CMV if both the donor and recipient are CMV antibody negative (D–/R–), the use of CMV-seronegative or filtered blood products, the early detection of CMV by PCR, and the use of ganciclovir-valganciclovir prophylaxis or early treatment.[285]

Snydman and colleagues,[631] in an open-label, multicenter study in liver transplantation, found that the combination of CMVIG and ganciclovir was superior to different immunosuppressive regimens in historical controls. Campbell and colleagues[127] recommended universal prophylaxis for the patients at highest risk, children receiving liver transplants (D+/R–), but the role of immunoglobulin was uncertain.

In heart and lung transplants, the use of CMVIG for CMV infection prophylaxis has been recommended by some groups if either the donor or the recipient is CMV seropositive. Weill and associates[725] showed that combined ganciclovir and CMVIG reduced the incidence of CMV infection in high-risk lung transplant recipients in contrast to historical controls. Ruttmann and colleagues[577] showed, in a retrospective study, decreased morbidity resulting from CMV infection in lung transplant recipients given both CMVIG and ganciclovir in contrast to the use of ganciclovir alone. A meta-analysis examining the use of CMV hyperimmunglobulin (CMVIG) for the prevention of CMV infection and disease in solid organ transplant recipients demonstrated that prophylactic administration of CMVIG was associated with improved total survival and a reduced frequency of CMV-related disease and deaths.[96]

Zamora and associates[761] recommended intravenous ganciclovir and CMVIG for all lung transplant recipients if either the donor or recipient is CMV seropositive; and in the situation of D+/R–, they gave seven infusions of CMVIG in the first 90 days after transplantation. Other investigators[96,176,698] and consensus statements[43,543] permit the use of combined therapy with CMVIG in high-risk heart and lung transplant recipients and other solid organ recipients, despite the absence of definitive controlled studies.

The use of IGIV for the treatment of hemophagocytic lymphohistiocytosis associated with CMV infection also has been reported.[308]

Use of Cytomegalovirus Intravenous Immunoglobulin in Perinatal Cytomegalovirus Infection

Another use of CMVIG may be the treatment of in utero CMV infection.[487] Two infusions of CMVIG were given intraperitoneally at 28 and 29 weeks' gestation to a CMV-infected fetus. The therapeutic benefit was unclear because the infant had intracranial calcifications at 2 weeks of age but was free of neurologic symptoms at 1 year of age. Matusuda and colleagues[442] and Sato and associates[589] used serial anti-CMV immunoglobulin injections into the fetal abdominal cavity to treat acute CMV infection in a fetus with a good fetal outcome.

Nigro and associates[491] treated a pregnant woman with primary CMV infection and in utero infection of one twin fetus with intravenous CMVIG at 30 weeks' gestation. CMVIG also was injected into the amniotic sac. The affected twin had better growth, decreased placental thickening, and lessened cord edema. Although born with CMV infection and hepatosplenomegaly, the child was normal at the age of 2 years.

Subsequently, Nigro and colleagues[489] gave 31 pregnant women with primary CMV infection at least one dose of CMVIG 200 U/kg during pregnancy. Ultrasonic evidence of CMV infection was seen in 15 fetuses. Nine women received additional CMVIG into their amniotic sac or umbilical cord. Only one woman gave birth to an infant with CMV infection in contrast to 7/14 women who did not receive CMVIG during pregnancy. All of these women had in utero infection confirmed by CMV-positive (culture or PCR) amniotic fluid. The infants of mothers who received CMVIG during the first half of the pregnancy were followed in a case-control study evaluating their outcome in contrast to that of 32 congenitally infected infants whose mothers did not receive CMVIG during pregnancy.[490] The risk for having a CMV-affected child with psychomotor retardation and hearing deficit was significantly higher in the untreated group, leading the authors to conclude that IG administered during the first half of pregnancy to women with primary CMV infection decreases neurologic sequelae associated with in utero CMV infection. Another group[710] compared infected infants born to women with primary CMV infection before 17 weeks' gestation who were treated with 200 U/kg of Cytotect (CMVIG product available in Europe) to those who were untreated and participants in a natural history study. At 1 year of age, 4/31 infants of Cytotect-treated mothers had poor outcome in contrast to that of 16 infants from 37 untreated mothers. Result from a double-blinded, randomized, placebo-controlled trial of Cytotect prophylaxis was recently presented; and review of the 122 subjects enrolled suggested protection against in utero fetal infection (44% in the placebo group vs. 30% in the treated group). Data also suggest that administration of IG may have an immunomodulatory effect on the placenta in addition to the neutralization of virus.[430]

Recommendations

Patients undergoing allogeneic human stem cell transplantation who are at risk for acquiring CMV disease (e.g., a CMV-seropositive recipient or a CMV-seronegative recipient and a CMV-seropositive donor) should receive either prophylactic antiviral therapy or preemptive antiviral therapy after early detection of infection by CMV PCR. Routine use of CMVIG no longer is recommended for prevention of CMV disease.[93]

Similarly, for solid organ recipients, the highest risk is a CMV-seropositive donor and a CMV-seronegative recipient, in which case antiviral prophylaxis is used. Antiviral prophylaxis rather than CMVIG or IGIV is used currently in kidney and liver transplantation.[328,602] In high-risk situations such as heart and lung transplants with either the donor or the recipient CMV seropositive, some centers use CMVIG in addition to antiviral therapy, despite lack of proof by multicenter, randomized studies.[96,543,698]

CMVIG or IGIV in combination with antiviral agents is used to treat severe CMV disease, such as pneumonia, in transplant recipients.[232,516,532,557,759] For CMVIG, 400 mg/kg on days 1, 2, and 7 and 200 mg/kg on days 14 and 21 have been used in addition to antiviral therapy,[557] although others have used a lower dose (100 mg/kg every other day for 2 weeks).[240] IGIV 500 mg/kg every other day for 10 to 59 days, followed by 500 mg/kg once or twice weekly for an extended period, also has been reported.[514] Garcia-Gallo and colleagues[234] reported the use of CMVIG as an adjunct to antiviral therapy to prevent development of pneumonitis in lung transplant recipients with invasive CMV disease and as preemptive therapy (with ganciclovir) in posttransplant recipients with persistent CMV viral load.

CMVIG for in utero CMV infection is not recommended routinely but several studies have demonstrated its safety and efficacy, and its use may be of value in selected situations with confirmed primary maternal infection or fetal infection.

Epstein-Barr Virus

EBV, the etiologic agent of infectious mononucleosis, causes severe infection in immunocompromised patients and males with X-linked lymphoproliferative syndrome. IGIV has been given to EBV-seronegative males with X-linked lymphoproliferative syndrome in an attempt to prevent EBV infection, but EBV infection developed in some of them despite IGIV adminstration.[222]

Transplant recipients with EBV-induced posttransplantation lymphoproliferative syndrome or hepatitis have been treated successfully with a combination of IGIV or CMVIG, antiviral therapy, and IFN-α.[182,500,670]

A similar approach was not successful in X-linked lymphoproliferative syndrome.[508] CMVIG was used in one case because it contains higher titers of anti-EBV antibody than IGIV.[182] One case of fulminant EBV hepatitis was treated successfully with liver transplantation, antiviral therapy, and CMVIG.[216]

A recent multicenter trial indicated that CMVIG given to liver transplant recipients did not prevent posttransplantation lymphoproliferative disease or other EBV disease.[265] However, the EBV serology was unavailable in 50 of the 82 donors, hampering interpretation of the results.

Ohta and colleagues[507] reported marked improvement of a patient with chronic EBV hepatitis after kidney

transplantation by treatment with the anti-CD20 monoclonal antibody rituximab after failure of reduction of immunosuppression. Rituximab has been used successfully in the treatment of EBV-induced posttransplantation lymphoproliferative disease in stem cell[213,614] or solid organ transplants.[159,291,298,673,754]

Rituximab also has been used in preemptive therapy in stem cell transplantation based on EBV viral load.[161,701] Boesch and colleagues[94] reported the successful use of rituximab to treat an adolescent without immunodeficiency who developed EBV-associated lymphoproliferative disease that involved massive submucosal infiltration of B cells in the lingual tonsils, trachea, and bronchi and resultant near-complete airway obstruction unresponsive to steroids. A 28-year-old recipient of an orthotopic liver transplant was successfully treated for EBV-related posttransplantation lymphoproliferative disease refractory to rituximab with antiviral therapy and IGIV.[686] Some centers use IG (IGIV or CMVIG) in addition to antiviral therapy as prophylaxis in high-risk transplant recipients.[266]

Recommendations

Current approaches to EBV-induced posttransplantation lymphoproliferative disease include reduction of immunosuppression and consideration of preemptive therapy or treatment with rituximab,[634] although antiviral medication, anticytokine therapy, chemotherapy, and immunotherapy all have been used. In a recent literature review, Giulino and colleagues[248] found evidence of a better response to rituximab in children in contrast to adults and suggested that rituximab may be appropriate as first-line therapy for posttransplantation lymphoproliferative disease in children. The role of IGIV or CMVIG in addition to the use of anti-CD20 monoclonal antibodies has not been clearly defined.

Herpes Simplex Infections

Neutralizing antibody has a protective effect in herpes simplex virus (HSV) infection in the newborn period. Mothers with a reactivated herpes infection during delivery are 10-fold less likely to transmit HSV to their newborn infants during vaginal delivery than are mothers with primary HSV infection at delivery, presumably because of the transplacental transfer of antiherpes antibody.[27,546] IGIV-containing HSV antibodies has not been used in the prevention of neonatal herpes infection, although Whitley[732] has proposed that HSV monoclonal antibody or hyperimmune IGIV be evaluated for treatment of disseminated neonatal disease.

Masci and colleagues[440] compared monthly IGIV 400 mg/kg with intermittent acyclovir treatment (800 mg twice daily, 1 week each month) in patients with recurrent genital HSV infection for 6 months and noted fewer recurrences in the IGIV-treated group.

Recommendation

IGIV is not currently recommended for the prevention or treatment of HSV infections.

Varicella-Zoster Virus and Varicella-Zoster Immunoglobulin

Immunoglobulin in Varicella

After success was achieved with IGIM in the prevention of measles and hepatitis, IGIM was evaluated for the prevention of varicella. Although Funkhouser[230] in 1948 showed some beneficial effects of standard IGIM in the prevention of varicella in an uncontrolled study, Greenberg and colleagues[268] and Schaeffer and Toomey[591] were unable to prevent chickenpox in exposed children with IGIM doses of 2.5 to 20 mL.

Others have reported anecdotal evidence for the efficacy of IGIM in large doses during the early stages of chickenpox and herpes zoster. These claims included prompt relief of pain in zoster[727] and rapid resolution of skin lesions.[403,564] However, even high-titer IG (zoster IG) does not prevent dissemination of herpes zoster.[644]

Ross[570] in 1962 gave 242 children IGIM in doses of 0.1 to 0.6 mL/lb within 3 days of exposure to chickenpox; 209 similarly exposed, uninjected children were used as controls. The attack rate was the same (97%) in both groups, thus indicating that IGIM does not prevent varicella under these conditions. However, with doses of IGIM above 0.2 mL/lb, the severity of the disease was reduced, as indicated by a decreased number of pox and reduced temperature. Children receiving the largest dose of IGIM (0.6 mL/lb) had maximal temperatures of 38.9° C (102° F) versus 41.1° C (106° F) for controls and 40 pox versus 207 for controls. Other investigators also have reported similar but uncontrolled observations that IG modifies the severity of chickenpox.[319,687]

Varicella-Zoster Immunoglobulin in Varicella

The prophylactic value of large IG doses in decreasing the incidence and severity of varicella led to a trial of high-titer plasma or IG preparations to prevent varicella.[61] These preparations include zoster IG (ZIG) and plasma (ZIP) from convalescing zoster patients and varicella-zoster IG (VZIG) prepared from high-titer plasma from normal adults.

Brunell and associates[114] in 1969 selected convalescing patients with zoster whose complement-fixing antibody titers were 1:256 or greater and prepared ZIG from their plasma; this material had titers considerably higher than did standard IG. Exposed children from six families in which chickenpox was occurring were given ZIG or IG at doses of 2 mL. In none of 6 children receiving ZIG did chickenpox develop, whereas it developed in all 6 children given IG. No antibody developed in the ZIG-treated group, thus indicating that the disease was prevented.

Because this dose did not prevent varicella in leukemic children or other high-risk patients, a larger dose (5 mL) was used in a later study to successfully modify or to prevent varicella in 8/9 high-risk children.[112,113] Severe varicella developed in 1 child given a less potent preparation of ZIG. These observations were confirmed in two later studies. Judelsohn and associates[340] gave ZIG to 56 exposed high-risk children; mild varicella occurred in 7 patients and was prevented in the others, most of whom

were susceptible, as determined by absence of serum antibody. Gershon and colleagues[242] gave ZIG to 15 seronegative high-risk exposed children; varicella was severe in 1, mild in 9, and subclinical in 5. Subclinical infection was determined by the acquisition of membrane antibody, as detected by fluorescent microscopy.

Orenstein and associates[513] studied 553 exposed, high-risk patients who received ZIG of two different titers (≥1:1280 vs. 1:2560). They found that the clinical attack rate after receipt of ZIG correlated with the type of exposure (varicella developed in 36% with household exposure, 7.7% with hospital exposure, and 0% with school exposure), the rise in antibody titer (45% of patients without a fourfold titer increase became ill in contrast to 22% of patients with a fourfold or greater rise in titer), and the titer of the administered ZIG (significantly more complications and deaths occurred in recipients of the lower titer ZIG).

Because of the limited supply of ZIG and ZIP, VZIG from normal adults became the commercially available product in the United States. Zaia and associates[760] compared the efficacy of ZIG and VZIG in immunocompromised children exposed to varicella. Varicella attack rates and the clinical severity in recipients of VZIG and ZIG did not differ significantly. A higher incidence of subclinical infection was indicated by the rise in antibody titer in ZIG recipients (31.3%) versus that in VZIG recipients (16%). A larger dose of VZIG (2.5 mL/10 kg vs. 1.25 mL/10 kg) reduced the frequency of subclinical infection (from 20% to 4.3%). Varicella developed in several high-risk patients with demonstrable serum antibody at exposure, thus indicating that history-negative, seropositive patients are at risk for development of clinical varicella-zoster infection and should be given VZIG regardless of antibody titer.

In 2006, VZIG became unavailable and was replaced by VariZIG,* which is similar to VZIG and is an immunoglobulin product made from human plasma and contains high levels of anti-varicella immunoglobulin, is approved for administration to high risk individuals following exposure.[149] IGIV and acyclovir prophylaxis are reasonable alternatives for postexposure prophylaxis if VariZIG is not available.

Intravenous Immunoglobulin in Varicella Prophylaxis

Paryani and colleagues[525,526] in 1984 observed that IGIV at doses of 4 mL/kg to 6 mL/kg resulted in serum varicella-zoster virus antibody titers comparable to those in patients receiving VZIG at standard doses. Subsequently, Chen and Liang[153] demonstrated that varicella was prevented by a single dose of IGIV at 200 mg/kg in five children with leukemia.

Immunocompromised patients who are receiving IGIV at 400 mg/kg are thought to be protected for 3 weeks after infusion and do not require additional VZIG on exposure.[38] However, Ferdman and Church[218] reported that varicella occurred in two patients (one was a 21-year-old woman) receiving 500 mg/kg of IGIV after exposure to varicella 7 and 11 days after infusion. Another child receiving 400 mg/kg IGIV developed varicella 9 days after infusion. Both had mild disease and responded well to acyclovir. They suggest that for patients with profound immunodeficiency, VZIG can be given in addition to IGIV. Kavaliotis and associates[355] found that an IGIV dose of 1 g/kg given within 6 to 24 hours of exposure was 90 percent effective in preventing varicella in an oncology unit, with no additional advantage noted with administration of VZIG. All of the children who contacted VZV received prophylactic treatment consisting of a single infusion of either 1) varicella-zoster immunoglobulin (VZIG) at a dose of 1 ml/kg (group A) or 2) intravenous immunoglobulin (IVIG) at a dose of 1 g/kg (group B) or 3) both VZIG + IVIG at the above-mentioned doses (group C).

Tokat and colleagues[683] described a 32-year-old man with severe varicella pneumonia that progressed to acute respiratory distress syndrome. IGIV 400 mg/kg for 5 days was given with a successful outcome.

Recommendations

The decision to administer VariZIG or IGIV or acyclovir to prevent chickenpox is based on the patient's susceptibility, the type of exposure, and the patient's immune competence.

Determination of Susceptibility. With the exception of bone marrow transplant recipients, most immunocompromised individuals with previous varicella infection are considered immune; however, their immune status should be confirmed with varicella antibody titers. Healthy adults reared in the United States are considered immune if they have a history of varicella, zoster, or vaccination. Immunocompromised children and adults without a history of varicella are considered susceptible because of their higher risk for development of severe infection. Bone marrow transplant recipients are considered susceptible regardless of the varicella history of the donor or recipient. If varicella or herpes zoster[437] develop after transplantation, the patient then is considered to be immune.[142,143]

An immunocompromised, unimmunized child without a history of chickenpox is considered susceptible even if antibody titers are present because receipt of blood products containing IG may result in transient seropositivity. Alternatively, such patients who received VariZIG may have had asymptomatic varicella with the subsequent development of varicella antibodies, yet they may not be protected on reexposure.[39] The value of antibody titer to determine susceptibility in these patients is controversial. However, oncology patients who have been vaccinated for varicella before illness and have been seropositive for varicella antibodies before receipt of blood products are considered immune and do not routinely need VariZIG. The severity of immune compromise is also a factor, and bone marrow transplant recipients are considered susceptible despite prior history of immunization or disease.

VariZIG is not recommended for immunodeficient patients who have been vaccinated in the past, provided

*VariZIG is available through FFF Enterprises: 800-843-7477, http://www.fffenterprises.com.

they previously were shown to have varicella antibody, even if they are seronegative at the time of exposure. These individuals are expected to have mild disease.[142] Verifying varicella immunity with antibody before forgoing VariZIG administration in this situation is a reasonable approach because administration of VariZIG can be delayed up to 10 days and remain efficacious.

Type of Exposure. Individuals residing in the same household as a patient with varicella, persons who have had face-to-face contact with a patient considered infectious, and patients sharing the same hospital room are considered to have significant exposure. Because the duration of exposure that results in transmission is not known, each exposure must be evaluated individually. Contact with a varicella vaccine recipient with a varicella rash must be considered a significant exposure for an immunodeficient patient because the vaccinee may have wild-type virus infection; vaccine strain virus rarely has been transmitted.[143]

Candidates for Varicella-Zoster Immunoglobulin or Intravenous Immunoglobulin

Normal Adults, Children, and Adolescents. Normal children, adolescents, and adults exposed to chickenpox or zoster usually are not candidates for receiving VariZIG or IGIV (Table 245-7). Because of the prolonged incubation period for varicella the chickenpox vaccine may be given if the exposed individual is older than 12 months.

Immunocompromised Children and Adults. The principal use of VariZIG is to prevent chickenpox in susceptible immunocompromised children exposed to zoster or chickenpox (see Table 245-7). These children include those with primary immunodeficiency and those with secondary immunodeficiency, including HIV infection and neoplastic disease, and those receiving immunosuppressive therapy (e.g., systemic steroids and chemotherapy).[50,502,513]

Susceptible pregnant women are also candidates for VariZIG because of their increased risk for development of severe varicella pneumonia.[401,630]

TABLE 245-7 **Candidates for Varicella-Zoster Immune Globulin (VariZIG) After Significant Exposure**

Immunocompromised children without a history of varicella and without documented response to immunization*
Susceptible, pregnant women
Newborn infants whose mother had an onset of varicella within 5 days before delivery or within 48 hours after delivery
All hospitalized premature infants born at <28 weeks' gestation or <1000 g at birth, regardless of maternal history
Hospitalized premature infants of >28 weeks' gestation whose mother has no history of chickenpox or who is seronegative; may consider its use for other premature infants. See text.

*Severity of immune compromise is a factor in the decision to give VariZIG despite immunization and disease history. See text.

Term and Premature Newborns. Newborns who acquire varicella after birth are not at high risk for development of severe disease and are not candidates for receiving VZIG, with the possible exception of those whose mothers have severe skin disease.[401] Newborn infants born to mothers in whom varicella developed within 5 days before delivery or within 2 days after delivery are at high risk for having severe varicella because they have not received transplacental protective antibodies.[142] Others, questioning the validity of these limits, have treated a small number of infants born to mothers in a window ranging from 7 days before delivery to 5 days after delivery with IGIV 500 mg/kg and a prophylactic acyclovir regimen.[312]

Exposed hospitalized premature infants born at less than 28 weeks' gestation or less than 1000 g should receive VariZIG. These infants are susceptible, regardless of the maternal history of varicella, because of incomplete transplacental transfer of maternal antibody. Hospitalized premature infants with significant exposure who were born at more than 28 weeks' gestation and whose mothers do not have a history of varicella or are seronegative also are considered candidates for receiving VariZIG because transplacental antibody would not be present.[142,401,437]

Gold and colleagues[254] showed that gestational age of more than 28 weeks and birth weight above 1000 g do not always accurately predict the presence of maternal varicella antibody. Ogilvie[502] suggested that infants born before 30 weeks' gestation be given VariZIG and that some infants born after 28 weeks will lose their maternal antibody by 60 days of age; she recommends antibody testing on exposure if it can be performed quickly to assess the need for passive immune prophylaxis. Linder and colleagues[415] also noted that only 24 percent of premature neonates born at 29 to 35 weeks had positive fluorescent antibody to membrane antigen (FAMA) titers at 2 months of age.

Gold and associates[254] recommended the routine use of VariZIG in the neonatal ICU if the FAMA assay is not available to determine susceptibility. Previous administration of blood products in the neonatal ICU may render these results uninterpretable.

Dosage. The VariZIG brand of VZIG is supplied in lyophilized vials of 125 U (reconstituted to a 5% solution for intramuscular use). The dose of VariZIG is 125 U/10 kg intramuscularly. The minimal dose for infants is 125 U (one vial), and the maximal dose is 625 U for adults (five vials).[38,147]

Although VariZIG should be given to susceptible candidates as soon as possible after exposure for maximal protection, it can be given up to 10 days after exposure.[133,149] A second dose is not needed for subsequent exposure unless it occurs beyond 3 weeks after administration of the VariZIG dose.[39] IGIV 400 mg/kg is an acceptable alternative if the child has a bleeding diathesis and cannot receive intramuscular injections or if VariZIG is not available.[142]

VariZIG is not recommended for the treatment of chickenpox, zoster, disseminated varicella, or postherpetic

neuralgia; zoster IG did not prevent dissemination of zoster in immunocompromised patients.[644]

HUMAN IMMUNODEFICIENCY VIRUS INFECTION

The rationale for use of IGIV in patients with advanced HIV disease is their increased susceptibility to common bacterial and viral infections, poor primary antibody responses to vaccine antigens despite hypergammaglobulinemia, and, in young children, a limited antibody spectrum to common bacterial pathogens. Although the central immune defect in acquired immunodeficiency syndrome (AIDS) is a loss of helper T-cell ($CD4^+$) number and function, the polyclonal B-cell activation and defective helper T-cell function result in a significant B-cell deficiency.[81,496]

Thus, in children with AIDS, bacterial infections occur more commonly than opportunistic infections. Immune-mediated thrombocytopenic purpura and viral diseases (e.g., RSV and parvovirus and other disorders amenable to IGIV prophylaxis or therapy) also may develop in patients with AIDS.

After preliminary uncontrolled studies suggested a benefit of IGIV in children with advanced HIV infection,[126,619] two large multicenter controlled studies undertaken by the National Institutes of Health–supported Pediatric AIDS Clinical Trial Group determined the efficacy of IGIV in decreasing infections and improving survival in AIDS patients.[484,638]

In the first study, 372 children received 400 mg/kg of IGIV every 4 weeks or an albumin control for 2 years.[484] Thirty percent of all children in the IGIV-treated group had serious infections in contrast to 42 percent in the placebo group. Children who were less ill (those with $CD4^+$ lymphocyte counts >200/mL) benefited in particular from IGIV. Children in this category had fewer serious infections and were hospitalized less frequently than those in the placebo group, and their $CD4^+$ counts dropped less rapidly than those of the placebo group.[466] The mortality in both groups, however, was identical. Children with $CD4^+$ counts less than 200 cells/mL (i.e., those with severely impaired immunity) who were given IGIV had no significant decrease in infections, days of hospitalization, or mortality in contrast to their counterparts in the placebo group.

The authors concluded that IGIV significantly reduced the risk for development of serious infection in some children with symptomatic HIV infection, primarily those with $CD4^+$ counts between 200 and 500 cells/mL. In a follow-up study, the placebo group was allowed to cross over to receive IGIV, with a drop in the rate of serious infections and hospitalizations.[469]

In a second trial of IGIV in which all the children received zidovudine, a significant decrease in serious bacterial infections was found again, but this benefit was limited to children who did not receive trimethoprim-sulfamethoxazole prophylaxis for *Pneumocystis jiroveci* pneumonia.[638] A consensus of an HIV Working Group[750] was that HIV-infected children with significant hypogammaglobulinemia or documented poor antibody formation may be candidates for receiving IGIV therapy. IGIV also may be of benefit to HIV-infected children who have recurrent infections not controlled by antibiotics or chronic nonspecific diarrhea with failure to thrive.

IGIV also was shown to prevent serious infections in adults with advanced HIV infection.[369]

In addition to preventing serious bacterial infections, IGIV given to children with HIV infection reduced the number of nonserious bacterial infections (by 60% for ear infections, 13% for skin infections, and 10% for other upper respiratory tract infections) and viral infections by approximately a third in both trials.[463,465,467,638] In an additional study, 135 children with symptomatic and asymptomatic perinatally acquired HIV infection in addition to uninfected infants were given monthly IVIG infusions over 12 months. The authors found significant reductions in the frequency of common bacterial infections in the HIV-symptomatic group in contrast to both the asymptomatic and the control groups.[509]

A recent study showed that monthly IGIV could be safely discontinued in children who are clinically stable and receiving highly active antiretroviral therapy (HAART).[270]

Regular IGIV infusion also improved left ventricular function in HIV-infected children with dilated cardiomyopathy.[416] It is also effective in treating certain other complications of HIV infection, including thrombocytopenia,[123,395] red cell aplasia after associated with parvovirus B19 infection,[377] Guillain-Barré syndrome,[247] and certain drug reactions.[221] IGIV, even in high doses, provides no antiviral activity to HIV.[511]

Attempts to use HIV-specific antibody to prevent HIV infection or to provide an antiviral effect have met with very limited success.

These studies have used human immune plasma or hyperimmune HIV IG (HIVIG) from asymptomatic HIV-seropositive patients, porcine antisera, or monoclonal antibodies.[651] In two of three controlled clinical trials of immune plasma in adult patients with AIDS, a modest clinical benefit was achieved, as judged by decreased opportunistic infections, a slight increase in $CD4^+$ cell counts, and improved survival; however, no striking decreases in viral burden were noted.[323,409,713]

In a double-blind, placebo-controlled multicenter study, HIV-seropositive pregnant women receiving zidovudine were given HIVIG monthly during the last trimester of pregnancy, and their newborns were given one HIVIG dose at birth; no effect on the rate of maternal-fetal HIV transmission versus that in a similar group given regular IGIV was found.[650] The rate of transmission in both groups was unexpectedly low (<5%), so the study was discontinued after 800 patients had been enrolled because the study was not statistically powered to detect a slight reduction in transmission rates.

Another study examined the antiviral effect of large doses of HIVIG in 30 children with moderately advanced HIV infection who were receiving stable antiviral treatment and showed a measurable viral burden.[649] No striking beneficial effect was observed as indicated by plasma HIV RNA levels, cellular viral culture titers, or immunologic assays.

Despite these failures, efforts to develop antibody preparations that will neutralize the virus continue; such preparations would be particularly valuable after needlestick, perinatal, or sexual exposure.[649] Under study are monoclonal antibodies to neutralizing regions of the virus, antibodies to coreceptors that may interfere with HIV attachment, and combinations of antibodies with different specificities, including combinations of HIVIG with monoclonal antibodies.[46,121,394,412-413,521] An effective neutralizing antibody also would provide valuable information toward an effective vaccine.

Recommendations

IGIV may be beneficial in symptomatic children with HIV infection to prevent bacterial and viral infections and treat certain complications (e.g., thrombocytopenia, anemia of parvovirus infection, and autoimmune hemolytic anemia [Duncan syndrome]) but has no direct antiviral activity. Because the administration of HAART plays a critical role in the reconstitution of the immune system, specifically the cellular immune system and resultant increase in humoral immunity, additional passive immunity administered with IGIV has not been shown to be of substantial benefit in children with well-controlled HIV infection. HIVIG or plasma derived from infected patients has little or no antiviral activity or clinical benefit for HIV-infected adults or children, and the administration of HIVIG during pregnancy was not shown to provide added reduction in maternal-fetal transmission of HIV.

Monoclonal antibodies directed against the virus or its cellular receptors show considerable promise but are not yet available.

MEASLES

The first successful prophylaxis of measles with convalescent serum was reported by Cenci in 1907.[113] Convalescent serum was used in the United States first in 1916 by Park and Freeman[522] and Zingher,[767] who gave either 4 or 8 mL of serum to 41 recently exposed children at New York Metropolitan Hospital. Measles did not develop in any of the 20 children who received the 8-mL dose and developed in only 3 of the 20 children who received the 4-mL dose. In 1926, Park and Freeman[522] found that 6 to 10 mL of convalescent serum was 92 percent efficacious in preventing measles in recently exposed individuals, a finding confirmed and extended by Stillerman and associates.[652] Placental extracts containing serum antibodies also were used in the prevention and modification of measles.[450]

Stokes and colleagues[654] and Ordman and associates[512] both confirmed in 1944 that IG given immediately after exposure could prevent or modify measles and given in the early stages of clinical illness could lessen its severity. In 1955, Greenberg and coworkers[269] noted a lower incidence of measles encephalitis in IGIM-modified measles.

A major use of IGIM in the 1960s was to diminish the side effects of the Edmonston strain of measles vaccine. In 1962, Krugman and associates[389] noted that the simultaneous administration of 0.02 mL/lb of IG at the time of Edmonston measles vaccination reduced the incidence of high fever from 40 to 14 percent and the incidence of rash from 10 to 2 percent. The mean titer of measles antibody achieved was somewhat reduced by the IGIM, and a slight decrease in the rate of seroconversion was noted; nonetheless, the vaccine–IGIM combination was 95 percent effective.[657] The current measles vaccine is less reactogenic and does not require concomitant IGIM injections.

Audet and colleagues[54] report that the measles neutralizing antibody titer in recent IGIV lots is decreasing because of the lower titer of vaccine-induced antibodies in contrast to natural infection among donors. Although not tested, IGIM probably has lower titers of measles antibodies, so that the recommended prophylactic doses may be inadequate.

Recommendations

IGIM or IGIV can be given to susceptible contacts within 6 days of exposure to prevent or to modify measles.[28] Infants younger than 1 year, immunocompromised patients, and pregnant women are at risk for development of severe disease and should receive immunoprophylaxis. Because HIV-infected patients with significant immunodeficiency may contract measles after exposure despite receiving immunization, they should also receive passive immunoprophylaxis.[241] IGIM is not recommended for healthy household or other close contacts if they have received at least one measles-containing vaccine unless they are immunocompromised. Measles vaccine has some efficacy if it is given within 72 hours of exposure and is preferred for the management of outbreaks in healthy individuals older than 1 year of age.[28]

The standard dose of IGIM is 0.25 mL/kg for healthy patients and 0.5 mL/kg for immunocompromised patients. The maximal dose is 15 mL. IGIV at comparable doses also can be used, but titers may be diminished in recent years.[54]

Immunodeficient children receiving regular IGIV treatments at doses of at least 400 mg/kg should be protected for up to 3 weeks after administration.[28]

Administration of IGIM to a healthy infant precludes the use of measles vaccine for 5 months if the dose was 0.25 mL/kg and 6 months if the dose was 0.5 mL/kg. For larger doses, longer intervals are needed (e.g., 8 to 11 months).[28]

IGIV in addition to other immunomodulators also has been used for treatment of acute measles infection[478] and in subacute sclerosing panencephalitis (SSPE), a delayed, rare degenerative neurologic disease complicating wild-type measles infection.[256]

MUMPS

Mumps IG was shown to have limited efficacy as postexposure prophylaxis, and this product no longer is manufactured in the United States.[29,168]

PARVOVIRUS

Human parvovirus B19, also termed *erythrovirus*, the cause of the benign childhood exanthem termed *fifth disease*, may also cause polyarthropathy, transient aplastic crises in patients with hemolytic anemia, chronic red cell aplasia in immunocompromised patients, and hemophagocytic syndrome.[109,317,755] Patients susceptible to chronic parvovirus infection include those with congenital[674] or acquired immunodeficiencies,[160,377] those receiving immunosuppressive therapy,[212,755] and organ transplant recipients.[349,435] In utero parvovirus infection can result in hydrops fetalis.[685] The use of parvovirus DNA PCR has facilitated establishment of the diagnosis in immunodeficient patients unable to make antibodies or in patients who have passively acquired parvovirus antibodies.

Parvovirus B19 has tropism for marrow erythroid progenitor cells because these cells express the blood group P antigen, the cellular receptor for the virus. Most adults have encountered and made antibodies to parvovirus, and thus IGIV is an excellent source of anti–parvovirus B19 antibodies.[598] IGIV has been used successfully to treat parvovirus-induced red cell aplasia.[396] Successfully treated patients have decreased viremia, reticulocytosis, and resolution of the anemia within 2 weeks. Sometimes relapse or persistent infection occurs, requiring maintenance IGIV therapy, particularly in patients with HIV infection and low $CD4^+$ cell counts.[376]

For treatment of chronic infection in immunodeficient patients, IGIV therapy often is effective and should be considered.[30,219] IGIV has been used successfully to treat parvovirus B19 anemia resulting from chemotherapy,[452] rituximab therapy,[609] or both.[322,635] However, IGIV was not of value in four children with persistent parvovirus infection undergoing chemotherapy for acute lymphocytic leukemia.[219] IGIV also has been used successfully for treatment of parvovirus B19 anemia in renal transplant recipients.[157,436,517,633]

Viguier and coauthors[709] administered 1 g/kg of IGIV for 2 days to treat successfully a 33-year-old woman with parvovirus B19–associated polyarteritis nodosa and fever, palpable purpura, intense myalgia, paresthesias, and polyarthritis of the hand joints. IGIV has been used with success in parvovirus B19–associated chronic fatigue syndrome in three adults[366] and one teenage boy.[449]

IGIV has been used as a supplement to transfusions in severe in utero parvovirus infection with hydrops fetalis. Selbing and coworkers[601] described a 24-week pregnant woman with severe preeclampsia whose fetus had ascites and pericardial effusion. She was given 25 g of IGIV, with resolution of the ascites, effusion, and anemia. Matsuda and colleagues[443] treated a parvovirus B19–infected hydropic fetus with two injections of IG into the fetal peritoneum, with resolution of the anemia and the hydrops.

Rugolotto and colleagues[574] treated an anemic newborn secondary to intrauterine parvovirus infection with IGIV 1 g/kg every 3 weeks for 8 months. Heegaard and coauthors[293] reported similar success of IGIV, in addition to in utero transfusions. Earlier attempts to treat older infants with persistent parvovirus-induced congenital anemia with IGIV were unsuccessful.[110]

Dennert and colleagues[183] performed a pilot study on the use of IGIV for 17 patients with chronic dilated cardiomyopathy and a significant parvovirus B19 viral load in endomyocardial biopsies demonstrating significant reduction in viral load and improvement in cardiac function. IGIV has also been used to successfully treat fulminant hepatitis resulting from parvovirus B19 in a 2-year-old Chinese boy.[129]

Human neutralizing monoclonal antibodies have been generated from infected individuals[245] for possible future therapeutic use.

Recommendations

Red cell aplasia caused by parvovirus infection in immunodeficient patients, including those with HIV infection, should be treated with IGIV 400 mg/kg/day for 5 days or 1 g/kg per day for 2 days.[109,377,472] If relapse is likely to occur because of the patient's severe immunocompromised status, IGIV 400 mg/kg can be given every 4 weeks.

Neonates with persistent congenital anemia caused by parvovirus B19 should receive IGIV 400 mg/kg for 5 days, with additional doses given if the anemia does not resolve.

Pregnant women carrying fetuses with in utero hydrops fetalis caused by parvovirus B19 infection also may be candidates for IGIV therapy.

RABIES AND RABIES IMMUNOGLOBULIN

Rabies is the ideal disease for passive immunization because the exact moment, the exact source, and the exact location of exposure usually are known. Furthermore, the long incubation period and the fact that the virus remains localized to the wound for several days enhance the effectiveness of passive immunization. Although a rabies serum was prepared initially in 1889 by Babes and Lepp,[55] experiments of Habel[282] in mice, guinea pigs, and monkeys involving the use of rabbit hyperimmune serum demonstrated that antibody worked by two mechanisms: (1) neutralizing the virus while it is still in tissues and (2) retarding the spread of virus within the nervous system, thereby prolonging the incubation period and permitting active immunity by vaccine to become established.

On the basis of these studies, a World Health Organization (WHO) Expert Committee in 1950 recommended that a field trial of the efficacy of hyperimmune rabies serum in conjunction with vaccine be conducted.[208] It was undertaken in Iran because multiple bites by a single rabid wolf coming into isolated villages were not uncommon occurrences, and this severe exposure had an associated 40 to 50 percent mortality. In 1954, a single rabid wolf bit 27 individuals, 17 of whom were bitten on the head. These 17 were divided into three groups: 5 received vaccine alone, 7 received vaccine and one dose

of antirabies serum, and 5 received vaccine and two doses of antirabies serum.[65] Of 5 persons treated with vaccine alone, 3 died of rabies; 1/7 in the one-dose antiserum group died, and 0/5 in the two-dose antiserum group died.

Antibody studies conducted on these patients indicated that a single or double dose of antiserum, followed by 14 to 21 daily doses of vaccine, results in significant levels of circulating antibody for as long as 50 days.[283] The antibody found early is supplied passively; after the 10th day, the antibody present is a result of the vaccine. Thus, optimal treatment requires both passive and active immunization.

Before 1971, the only available antiserum was of equine origin. It is still the only product available in some countries. Since 1971, human rabies IG (RIG) has been available in the United States and many other countries and is preferred because of the lessened risk for serum reactions.[620] In addition, human antibody has a half-life in the circulation twice that of equine antibody, with the result that higher levels of passive antibody are maintained. However, the antibody response to the vaccine given concomitantly is suppressed more effectively.[417]

A case of fatal rabies was reported in a 19-year-old man bitten on the finger by a rabid mongoose despite the recommended postexposure prophylaxis (RIG and five doses of human diploid cell rabies vaccine).[613] Possible reasons for failure included (1) inadequacy of the recommended dose of RIG; (2) vaccine injection into the gluteal region, where more fat is found than in the recommended deltoid region; and (3) decreased antibody response to the vaccine as a result of a possible immunodeficiency state.

Prevention of rabies consists of three essential components: thorough washing of the wound with a 20 percent soap solution followed by irrigation with povidone-iodine,[86,576] passive immunization with rabies antibody, and active immunization. Although most cases of failure are caused by lack of adherence to these three strategies,[539] isolated cases of rabies occurring despite appropriate management have been reported.

Wilde and associates[736] reported failure of prophylaxis in five Thai children who received multiple bites on the face and head; they suggested that failure to infiltrate all wounds with RIG and surgical closure before the wound was infiltrated might have been factors. Hemachudha and coauthors[295] reported failure of prophylaxis in a child with minor scratches on the nose and in a woman with a deep wound on the cheek; they suggested that direct inoculation of virus into nerve endings may have occurred.

Goudsmit and associates[257] found that a human monoclonal antibody combination was equivalent to RIG when it was used with vaccine in a hamster model of rabies. Bakker and colleagues[58,59] reported clinical data from two phase I studies evaluating monoclonal antibody cocktail CL184 against rabies for the use in resource limited areas where there is no access to human or equine rabies immunoglobulin.

Two phase 2 trials have been completed in the United States and the Philippines, and a third is currently being conducted in India.[99,252]

Recommendations

Human RIG is recommended in nonimmunized individuals for all bites by animals in which rabies cannot be ruled out and for nonbite exposure to animals proved to be or suspected of being rabid.[28,138] Such treatment should be given as early as possible after exposure and followed by vaccine administration. Decision to administer postexposure prophylaxis should be based on guidelines.[31] Two human RIG products are available in the United States—BayRab and Imogam Rabies-HT; both are prepared from the plasma of hyperimmunized donors. The recommended dose of 20 IU/kg of RIG must not be exceeded because higher doses can suppress immunologic response of the simultaneously administered vaccine.

The entire dose of RIG should be infiltrated around the wounds as soon as possible after the bite.[482]

If this is not anatomically feasible, any remaining RIG should be given intramuscularly.[31]

Wilde and colleagues,[736] recognizing the difficulty in infiltrating multiple wounds with a small volume of RIG, suggested dilution of the RIG. The WHO[751] recommends dilution of the RIG twofold to threefold in saline to ensure an adequate volume for infiltration. RIG should not be frozen, and if it is frozen, it should not be used. In addition to passive immunization with RIG on the first day of treatment, exposed patients should receive active immunization with rabies vaccine in four doses over 2 weeks.

Purified equine RIG–containing rabies antibodies may be available outside the United States and is associated by a low rate of serum sickness (<1%). Equine RIG is administered at a dose of 40 IU/kg, and desensitization before administration may be required.

Administration of one of the three vaccines licensed in the United States should be started at the same time as RIG; however, the vaccine and RIG should not be administered in the same syringe or with the same syringe or needle. Only two of the licensed vaccines are currently produced and available in the United States, human diploid cell rabies vaccine and purified chicken embryo cell vaccine. The vaccines are equally effective and administered in multiple separate doses. Vaccine should be given in the deltoid muscle or, for an infant, in the anterolateral aspect of the thigh and at an anatomic site removed from the injury or any injection sites of RIG. The vaccine should be administered on days 0, 3, 7, and 14 after exposure, with day 0 as the day of initial presentation. This new schedule replaces the previously recommended schedule that included a fifth dose administered on day 28.[28]

If RIG is not available immediately, immunization should be started and RIG given if it is available within 7 days.[31,138,482]

If vaccine is not available immediately, RIG should be administered and the vaccine given as soon as possible. If both RIG and vaccine are delayed, both should be given whenever available, regardless of the interval between exposure and treatment.[31,138,482]

The administration of passive antibody can inhibit the response to rabies vaccines; therefore, the recommended dose should not be exceeded. Vaccine never should be

administered in the same parts of the body or with the same syringe used to give RIG. Hypersensitivity reactions to RIG are rare.

RIG is not recommended for patients who previously received a full postexposure vaccine course of human diploid cell rabies vaccine, rabies virus adsorbed, or purified chicken embryo cell vaccine; who have received a three-dose preexposure intramuscular series of these vaccines; who have received a three-dose preexposure intradermal human diploid cell rabies vaccine used in the United States; or who have been immunized with any vaccine and have a documented rabies titer.[31,138,482]

Postexposure prophylaxis of immunodeficient patients is problematic. Jaijaroensup and coauthors[326] reported poor or nondetectable neutralizing antibody in five HIV-infected patients who received intradermal vaccine and proposed that higher doses of vaccine or additional boosters be considered. Serologic failure of both preexposure and postexposure rabies immunizations in children with HIV infection[519,681] has been reported. The number of doses of rabies vaccine recommended for people with altered immunocompetence has not changed; for such people, postexposure prophylaxis should be continued to comprise a five-dose vaccination regimen with one dose of RIG.

RIG is not indicated for the treatment of rabies. Hemachudha and colleagues[296] described a patient with rabies who developed total limb and facial paralysis after receiving RIG.

RESPIRATORY SYNCYTIAL VIRUS

Acute RSV infection of the respiratory tract is the most common cause of hospitalization of infants and young children and thus a significant public health expense. For high-risk patients such as premature infants and infants with chronic lung disease, RSV infection can be severe and life-threatening. The observation that passively transferred maternal antibody provided some protection from RSV infection[503] led to the development of passive immunity products to prevent and to modify the severity of infection. No effective vaccine exists.

Respiratory Syncytial Virus–Intravenous Immunoglobulin

The first immune modulator used for the prevention of RSV infection was RSV-IGIV (RespiGam), a 5 percent polyclonal human intravenous IG prepared from healthy donors with high RSV-neutralizing antibody titers. RSV-IGIV was licensed in 1996 after studies in high-risk infants demonstrated its efficacy. Groothuis and colleagues[271] conducted a prospective, blinded, randomized trial at five centers and showed that 58 premature infants who received RSV-IGIV at 750 mg/kg/month during the winter months had a significantly lower incidence of RSV lower respiratory tract disease than that observed in 58 premature control infants who did not receive RSV-IGIV (6.9% vs. 24.1%; $p = .01$). The RSV-IGIV–treated patients also had a lower incidence of moderate-to-severe RSV lower respiratory tract disease ($p = .006$), fewer days of hospitalization ($p = .020$), and fewer days in the ICU ($p = .05$).

Another large RSV-IGIV multicenter, double-blind, placebo-controlled study[545] of 510 infants with either prematurity or bronchopulmonary dysplasia also demonstrated a decreased number of hospitalizations (13.5% vs. 8.0%; $p = .047$), decreased total number of RSV hospital days ($p = .045$), and decreased days with oxygen use ($p = .007$). RSV-IGIV also decreased days of hospitalization for any respiratory illness by 38 percent and total respiratory illness hospital days by 46 percent, presumably because of antibodies to other respiratory pathogens.

An added benefit of RSV-IGIV prophylaxis was a decreased incidence of otitis media (number of episodes, 0.15 per patient vs. 0.78 per control; $p = .003$).[289,622]

In 1997, the American Academy of Pediatrics recommended RSV-IGIV for high-risk children with prematurity or bronchopulmonary dysplasia[40] but not for infants with cyanotic congenital heart disease[272,625] because of the risk for development of serious side effects. RSV-IGIV no longer is available.

Palivizumab

In 1996, palivizumab (Synagis), a humanized IgG1 monoclonal antibody against the fusion (F) glycoprotein of RSV,[250,659] entered clinical trials. Palivizumab neutralizes both type A and type B RSV and is 50 to 100 times more potent than RSV-IGIV.[585] A multicenter, randomized, double-blind, placebo-controlled study of 1502 high-risk infants (premature infants and infants younger than 2 years with bronchopulmonary dysplasia) demonstrated the efficacy of 15 mg/kg of palivizumab given intramuscularly every 30 days.[316] It resulted in significantly decreased number of RSV hospitalizations (55%; $p < .001$), RSV hospital days ($p < .001$), and days of oxygen therapy ($p < .001$).

Palivizumab was licensed in 1998, the first monoclonal antibody approved for the prevention of an infectious disease. Postlicensure studies also have supported its effectiveness.[533,568,627] Although debate continues about the cost-effectiveness of palivizumab in some countries,[358,510,664] palivizumab prophylaxis for high-risk infants is now the standard of practice in the United States.[20]

In 2003, Feltes and colleagues[215] reported the results of an international, randomized, double-blind, placebo-controlled trial of palivizumab prophylaxis in 1287 children with congenital heart disease. This study showed a 45 percent reduction in RSV hospitalizations, a 56 percent reduction in total number of hospital days, and a 73 percent reduction in total number of hospital days with oxygen with the use of palivizumab. European cardiologists and the American Academy of Pediatrics recommend use of palivizumab for infants with hemodynamically significant heart disease (uncorrected, partially corrected, or with residual disease), pulmonary hypertension or chronic lung disease, and hemodynamically significant dilated or hypertrophic cardiomyopathy causing cyanosis or requiring cardiac medication.[35,684]

The use of palivizumab for more than one season has been a concern because of the theoretical risk for side

effects resulting from the development of an antibody to the murine component.[399,494] Null and colleagues[494] found that only one child of 55 receiving palivizumab for a second season developed an antipalivizumab titer of greater than 1:40. This child had a titer of 1:160 on day 30 that fell to 1:10 on day 120 (30 days after the fourth injection), suggesting a nonspecific reactivity. No associated adverse events occurred, and the palivizumab trough concentrations were as expected.

Palivizumab or other RSV antibodies are not indicated for the treatment of RSV infection, although some have used palivizumab for treatment of adult and pediatric patients in high-risk settings.[152,368,603] Saez-Llorens and colleagues[580] found in a small pharmacokinetic study that although palivizumab was safe, it was of no clinical benefit for previously healthy children with acute RSV infection. Banna and colleagues[66] successfully used palivizumab and steroid therapy to treat an adult woman with RSV-related pneumonia after stem cell transplantation. An experimental RSV monoclonal antibody (Medi-493), given intravenously, was not of benefit for intubated patients with RSV infection and respiratory failure.[433] Studies of high-titer monoclonal antibodies may yield different results.[228,592,603]

Passive Immunity in Respiratory Syncytial Virus Treatment

Although a single dose of 1500 mg/kg of RSV-IGIV was of no benefit in normal and high-risk children with RSV infection,[568,566] DeVincenzo and associates[185] gave a single dose of RSV-IGIV (1500 mg/kg) to 11 bone marrow transplant recipients after the onset of RSV infection. All but one patient also received ribavirin. Only one patient died in this group, which compares favorably with historical controls given ribavirin therapy alone.

Ghosh and coworkers[243] gave IGIV 500 mg/kg every other day and aerosolized ribavirin to 14 adult bone marrow transplant recipients with RSV upper respiratory tract infection to prevent pneumonia and death. Ten patients resolved their infection, pneumonia developed in four, and two died. Aerosolized human IGIV was of no clinical benefit in the treatment of patients with RSV infection.[562]

Recommendations

Palivizumab is indicated for prevention of RSV infection during the RSV infection season for high-risk premature infants or infants with chronic lung disease.[32] The period of prophylaxis should be individualized, depending on the duration of the local RSV infection season.

Candidates for Prophylaxis. Candidates for prophylaxis are as follows[32]:

1. Infants younger than 24 months with chronic lung disease requiring medical treatment in the 6 months before the start of the RSV infection season. Two seasons of treatment may be required for some infants who require ongoing medical treatment.
2. Premature infants without chronic lung disease who were born before 32 weeks' gestation. For these infants, the major risk factors to consider are gestational age and the chronologic age at the start of RSV season.
3. Infants born at 28 weeks, 6 days' gestation or earlier may benefit from prophylaxis during RSV season whenever that occurs in the first 12 months of life, whereas infants born at 29 to 32 weeks' gestation may benefit most from prophylaxis up to 6 months of age,[32] although decisions should be individualized. Once an infant qualifies for initiation of prophylaxis at the start of RSV season, administration of prophylaxis should continue throughout the season and not stop on the basis of the chronologic age.[32]
4. Premature infants of 32 to 35 weeks' gestation who are 3 months or younger at the start of RSV season, including those born during the RSV season, and have one of the following two risk factors: (1) attends child care in a setting with multiple infants or young toddlers or (2) has one or more older siblings (or other child) younger than 5 years of age living in the household. This prophylaxis should continue until the infant is 3 months of age.[32]
5. Infants under 12 months of age during RSV season with congenital abnormalities of the airway or neuromuscular disease may be considered for immunoprophylaxis if their condition compromises their handling of respiratory secretions.[32]
6. Children younger than 24 months with hemodynamically significant cyanotic or acyanotic heart disease. These criteria include children requiring medication for congestive heart failure, and children with moderate-to-severe pulmonary hypertension. Children with hemodynamically insignificant heart disease are not candidates for palivizumab. This category includes atrial septal defect, small ventricular septal defect, pulmonic stenosis, mild coarctation of the aorta, and patent ductus arteriosus. Children who have had surgical correction and do not require medications for congestive heart failure also are not candidates for prophylaxis. After cardiopulmonary bypass, a postoperative dose of palivizumab can be given to maintain protection because decreased levels of antibody have resulted from cardiopulmonary bypass.[216]
7. Infants who are severely immunocompromised can be considered for prophylaxis, although there are no studies on which to base a recommendation.

Possible Indications. Possible indications for use are as follows:

1. Nosocomial outbreaks. No data exist to support palivizumab use in controlling an RSV outbreak in a hospital setting. However, palivizumab, in addition to implementation of strict isolation practices, has been used in this setting and may be of value in aborting a nosocomial outbreak of RSV infection.[1,171,350,397,622] However, current dosing recommendations for outpatients may not be adequate for some hospitalized premature infants.[752]
2. Patients with cystic fibrosis who are younger than 2 years of age also may be candidates for RSV

prophylaxis, but no data exist on which to base a recommendation.

3. Older high-risk infants who received palivizumab the previous season but remain unwell, are immunocompromised, or have recently undergone a procedure such as transplantation or operation may benefit from a second season of palivizumab. This includes immunodeficient infants receiving IGIV because IGIV has only low titers of RSV antibodies.

Dosage and Administration. Prophylaxis is given monthly from just before the start of the local RSV infection season until the end of the season. Usually, five injections are given from November to March, but starting earlier or extending into April may be required. Thus, it is important to be aware of local outbreak data. If an infant develops RSV infection while receiving prophylaxis, palivizumab should be continued because more than one RSV strain could infect the patient in a single season.[32]

The dose of palivizumab is 15 mg/kg/month given intramuscularly. It is supplied in 50- and 100-mg vials.

As discussed earlier, palivizumab is not indicated for the treatment of RSV infections.

ROTAVIRUS

Several reviews have summarized the role of oral IG in the prevention and treatment of rotavirus infection.[95,178,286,518,726] Its effectiveness varies, in part because of differences in antibody titer in the various products.* Human IG has been used to prevent rotavirus infection; Barnes and colleagues[68] and Pammi and colleagues[518] gave human gammaglobulin orally to low-birth-weight infants in a nursery in which rotavirus was endemic. Patients given human IG had a more attenuated disease than the placebo controls.

Human IG has been infused into the duodenum to treat two children with prolonged rotavirus infection.[275] One patient responded rapidly, but the other had a prolonged course. Losonsky and coworkers[418] gave human IG to three immunodeficient patients and demonstrated that it survived passage of the gastrointestinal tract in an immunologically active form.

Pooled colostrum concentrate from hyperimmunized pregnant cows with high titers to several rotavirus serotypes has been used in several studies.[117] In a controlled study, bovine immune colostrum did not prevent development of symptomatic rotavirus infection in infants, but treated patients had a decrease in the duration of diarrhea.[694] Immune bovine colostrum also was used prophylactically for 2 weeks in 10 infants in a baby care center, with evidence of a protective effect.[196]

A double-blind, placebo-controlled trial of oral lyophilized rotavirus bovine colostrum in Bangladesh showed that a 4-day course decreased diarrhea, the need for oral rehydration, and viral shedding.[588] Mitra and associates[464] also showed that cow colostrum[690] significantly shortened the duration of diarrhea and decreased stool output. Hilpert and colleagues[303] demonstrated that rotavirus colostrum decreased virus excretion in infants with rotavirus diarrhea.

A lactobacillus engineered to express a neutralizing antibody fragment of a llama immunoglobulin (lactobodies) was given orally to experimental animals with rotavirus diarrhea and markedly shortened disease duration, severity, and viral load.[520,700]

A recently licensed vaccine for rotavirus seems safe and effective.[33]

Recommendation

Because rotavirus infection usually is self-limited, no commercial passive antibody product has been developed, although it would be of use in developing countries. The neutralizing titer of human IG probably is not high enough to be consistently beneficial. Oral vaccines may decrease the need for antibody-based therapies.

RUBELLA

IG rarely is used for prevention of rubella because of its uneven efficacy. Early studies suggested some prophylactic benefit. Korns[381] in 1952 showed that one lot of IGIM at a dose of 0.1 mL/lb provided partial protection against the development of epidemic rubella. However, the titers of rubella antibodies varied significantly between lots and did not provided consistent protection.

Studies in epidemic situations have shown that 5 mL of IGIM can prevent development of clinical rubella,[263] but Brody and coworkers[105] showed that an IGIM dose of 0.55 mg/kg resulted in a decreased clinical attack rate but also a high incidence of subclinical infection. Military recruits were protected by a 15-mL dose of IGIM administered before exposure.[309]

Extensive studies on IGIM prophylaxis of rubella conducted by Green[267] and Krugman and Ward[386] did not demonstrate a protective effect of IGIM 0.12 to 0.2 mL/lb. Lundström and coworkers[426] gave 251 exposed pregnant women 4 mL of convalescent rubella IGIM and 28 exposed pregnant women 24 mL of standard IGIM. Rubella developed in 6 of the 251 (2.4%); 3 of these 6 women aborted, and 1 had an infant with probable congenital rubella. None of the 28 women given 24 mL of standard IGIM contracted rubella. Their subsequent study demonstrated that convalescent rubella IGIM given to pregnant women with clinically manifested rubella did not protect against congenital rubella or lessen the probability of fetal damage.[427]

Recommendations

IGIM prophylaxis is not indicated for children and nonpregnant adults exposed to an infected contact because of the mildness of the infection.

IGIM is not recommended for most exposed pregnant women because the clinical syndrome may be masked and congenital rubella not prevented. For exposed pregnant

*References 68, 196, 275, 276, 303, 464, 588.

women who will not consider abortion if rubella develops, IGIM may be of some value in reducing the chance for fetal infection. A dose of 0.55 mL/kg of IGIM given intramuscularly is the recommended dose. However, mothers who have received such prophylaxis after exposure have delivered infants with congenital rubella.[34] Furthermore, the administration of immunoglobulin eliminates the value of IgG antibody testing to detect maternal infection.

VACCINIA, VARIOLA, AND VACCINIA IMMUNOGLOBULIN

Vaccinia virus is closely related antigenically to smallpox (variola) virus, and thus immunity to vaccinia virus through vaccination prevents smallpox.[486] Although smallpox has been eradicated from the world since 1977, the virus nonetheless exists in a few research laboratories and is a potential weapon in biologic warfare.

Vaccination is still given to members of the military, to some health care workers, and to laboratory workers who handle cultures or animals contaminated with vaccinia virus, some recombinant vaccinia viruses, and other orthopoxviruses (e.g., monkeypox or cowpox). Passive immunization occasionally is necessary after laboratory accidents, inadvertent vaccination of high-risk individuals, and exposure of high-risk individuals to a recently vaccinated individual.

Passive immunization against variola was known as early as 1895, when Hlava and Honl[304] showed that schoolchildren could be protected from variola by the injection of 3 to 10 mL of serum from a cowpox-immune calf. At the same time, protective human antibodies developed after vaccination, and passive transmission of these antibodies from mothers to infants was demonstrated.[641] Thereafter, effective prevention of smallpox by well-organized mass vaccination campaigns diminished interest in passive immunization.

Janeway, quoted by Enders,[202] found neutralizing vaccinia antibody in IG in 1944, and Verlinde and Spaander[706] in 1949 found high titers of such antibodies in convalescent IG from recently vaccinated individuals. Gispen and associates[246] in 1956 developed a human vaccinia IG (VIG) that did not interfere with active immunity, and they proposed its use with vaccine as prophylaxis against vaccinia encephalitis. Human VIG is derived from the plasma of vaccinated donors.[140]

The value of human VIG in treating smallpox or disseminated vaccinia is based on the presence of viremia, which leads to secondary dissemination. Administration of neutralizing antibody will prevent or limit the spread of infection and thus modify clinical expression of the disease.

Studies on the efficacy of VIG in treating smallpox and vaccinia complications were initiated by Kempe and colleagues in 1955.[61,364] They used a VIG that was prepared from recently vaccinated donors with a neutralizing titer of 1:256 to 1:512, in contrast to titers of 1:16 or 1:32 in standard IGIM. The households of new admissions to the Madras (India) Smallpox Hospital were visited, and alternate family contacts received VIG 0.2 mL/lb adults and 0.05 to 0.2 mL/lb in children.[364] After 25 days, 8 cases of smallpox developed in 75 contacts not given VIG, and two cases developed in 56 contacts given VIG, a significant difference. A similar, more extensive study disclosed 21 cases of smallpox (4 severe) among 379 contacts serving as controls and 5 cases of smallpox (none severe) among 326 contacts given VIG.[365]

Kempe[363] also reported the results of 300 cases of smallpox vaccination (vaccinia) complications treated with VIGIM, including 62 cases of generalized vaccinia, 132 cases of eczema vaccinatum, 23 cases of vaccinia necrosum, 12 cases of vaccinia encephalitis, and 28 cases of autoinoculation. In addition, VIGIM was given prophylactically to 44 eczematoid children requiring smallpox vaccine (0.6 to 1.2 mL/kg). VIGIM did not affect the course of vaccinia encephalitis. Of 28 patients with autoinoculation who received VIGIM, 27 did well. In the 132 patients with eczema vaccinatum given VIGIM, nine deaths occurred; this 7 percent mortality compares favorably with the usual mortality of 30 to 40 percent with supportive care only. All 62 patients with generalized vaccinia who were given VIGIM did well, although 4 children required a second course.

Of the 23 patients with vaccinia necrosum who received VIGIM, 7 died (30%); however, this disease generally is fatal and immune defects were present in most of these patients.[67] These results strongly supported the efficacy of VIGIM and subsequently were confirmed by studies in Sweden[425] and the United Kingdom.[610]

In 1997, Kesson and coauthors[367] reported a severely immunocompromised patient with progressive vaccinia who had inadvertently received a vaccinia melanoma oncolysate vaccination but was successfully treated with VIGIM and ribavirin. Nanning[480] studied the effect of VIGIM on postvaccinia encephalitis. He gave a placebo or 2 mL of VIG to Dutch military recruits at the time of primary vaccination; three cases of encephalitis occurred in 43,630 vaccinated recruits given VIG, in contrast to 13 cases in 53,044 recruits in the control group. This 77 percent reduction is statistically significant.

Intramuscular Vaccinia Immunoglobulin and Intravenous Vaccinia Immunoglobulin

Two types of VIG are now available, VIGIM (for intramuscular use) and IV-VIG (for intravenous use). IV-VIG was approved in the United States in 2005 for specific indications—treatment of infections that involve accidental implantation in the eye (except for isolated keratitis), mouth, or other hazardous areas; eczema vaccinatum; progressive vaccinia; severe generalized vaccinia; and vaccinia in patients with burns, impetigo, varicella-zoster, poison ivy, or severe eczema. Both VIG preparations are prepared from the plasma of vaccinated donors.

IV-VIG used at a dose of 100 mg/kg results in a more rapid and higher antibody peak than does an injection of VIGIM.[306] The first civilian treated with VIGIV was a woman with vaccinia blepharoconjunctivitis; she also received topical antiviral medication.[311] Another laboratory worker with ocular vaccinia also was treated with a

single dose of VIGIV.[411] IV-VIG is available from CDC under an IND protocol that has guidelines for dosage and administration. The CDC Clinical Consultation Team can be contacted through the Clinicians Information Hotline for VIG requests to treat vaccine-related adverse events.*

Recommendations

Complications of vaccination are the usual indications for VIG. They include accidental or intentional vaccination of a patient with a contraindication to the vaccine, autoinoculation of the eye, eczema vaccinatum, severe generalized vaccinia, and vaccinia necrosum.

The initial dose for VIGIM is 0.6 mL/kg. In adults, the dose must be divided and administered over 24 to 36 hours. Repeated doses can be given at 2- to 3-day intervals for vaccine complications until recovery begins, as evidenced by no new lesions. VIG should be given as soon as possible after diagnosis is established.[8,144]

For unimmunized patients exposed to smallpox, VIGIM in combination with vaccine can be given. A dose of 0.6 mL/kg should be given as soon as possible, preferably within 1 to 3 days.[8,445,694] Current recommendations from the U.S. Army Medical Research Institute of Infectious Diseases suggest that VIGIM may be of value in postexposure prophylaxis of smallpox when it is given within the first week after exposure and concurrently with vaccination. Vaccination alone is recommended for those without contraindications. Both vaccine and VIGIM should be given if more than 1 week has elapsed since exposure occurred.

The current supplies of VIGIM would not permit its routine use in combination with vaccine for bioterrorism exposure,[144] but if additional supplies become available, a VIGIM dose of 0.3 mL/kg in combination with vaccine could be used.

Cidofovir also has been used.[101,102] VIGIM is not indicated for established smallpox, postvaccinial encephalitis, or hypersensitivity and toxic rashes that occur after vaccination. However, the lack of efficacy of VIGIM in the past does not exclude a potential beneficial effect for IV-VIG. The pathogenesis of vaccinial encephalitis is poorly understood and may involve both infection and immune response,[307] and thus VIGIV may be beneficial.

Inadvertent vaccination of a pregnant woman occasionally results in fetal complications, with an estimated fetal vaccinia case rate of 1/10,000 to 100,000 vaccinated pregnant women. Although IV-VIG has not been licensed for this purpose, it can be offered for use as an investigational product. Some investigators suggest use of IV-VIG if it is given within 10 days of vaccination of a pregnant woman.[138,142,481]

In ocular vaccinia, VIGIM is contraindicated for isolated vaccinial keratitis because it may increase corneal scarring,[411] as suggested by animal studies. If additional indications for VIGIM exist, such as conjunctivitis, blepharitis, and eczema vaccinatum, the presence of keratitis is not a contraindication for use of VIGIM. Indeed, scarring can occur from vaccinial keratitis in the absence of VIGIM treatment. The use of IV-VIG has been used in an experimental rabbit model of vaccinia virus keratitis.[16] In this study, the use of IV-VIG neither exacerbated nor ameliorated the keratitis but did not cause the corneal scarring seen with the use of VIGIM. Topical medications such as trifluridine also are recommended.[534]

In summary, VIG products appear to be generally safe with few instances of allergic or adverse reactions. Both the intramuscular and intravenous products are well tolerated, and given that the intravenous administration results in a more favorable pharmacokinetic profile than the intramuscular preparation, this may be the preferred product for significant vaccinia exposures. The recommended dosage of VIGIV (Cangene) for the management of severe complications of smallpox (vaccinia) vaccination (indications listed above) is 6000 U/kg, to be given as soon as symptoms appear.[41] Furthermore, the intravenous product has clinical advantages over the intramuscular product that include absence of mercury, rapid absorption, and more favorable pharmacokinetics.[745]

IV-VIG at a very high dose was used in the treatment of a smallpox vaccinee with undiagnosed acute myelogenous leukemia who developed progressive vaccinia. The total dose given to this patient was 16,740,000 U over 4 days with good resolution.[148] The CDC has developed clinical evaluation tools for smallpox vaccine adverse reactions, which are available on their website.*

REGIONAL VIRUSES

Argentine Hemorrhagic Fever

Junin virus, the etiologic agent of Argentine hemorrhagic fever (AHF), causes a febrile illness with high mortality rates from vascular or neurologic complications.[537] Patients may either improve or have severe hemorrhagic or neurologic manifestations (or both) within the first 2 weeks of illness.[431] Maiztegui and colleagues[431] found that immune plasma given before the ninth day of illness to 91 patients reduced the mortality rate to 1.1 percent in contrast to a mortality rate of 16.5 percent in the 97 patients given normal plasma ($p < .01$). Ten of the patients who received immune plasma relapsed with fever and cerebellar signs after several weeks, as did eight others who were not part of the study. The neurologic relapse was self-limited in all but one patient, who died.

With a case-fatality rate of the illness without treatment between 15 and 30 percent, the use of a live attenuated vaccine has markedly reduced the incidence of AHF.[205] Present specific therapy involves the transfusion of immune plasma with defined doses of neutralizing antibodies during the prodromal phase of illness.[17] However, alternative forms of treatment are called for

*Centers for Disease Control and Prevention Clinical Consultation Team Clinicians Information Hotline: 877-554-4625, http://www.bt.cdc.gov/agent/smallpox/index.asp.

*Centers for Disease Control and Prevention clinical evaluation tools: http://emergency.cdc.gov/agent/smallpox/vaccination/clineval.

because of the current difficulties in early detection of AHF, related to its decrease in incidence, troubles in maintaining adequate stocks of immune plasma, and the absence of effective therapies for severely ill patients that progress to a neurologic-hemorrhagic phase.[205]

Difficulty in obtaining immune plasma, possible neurologic complications of plasma treatment, and lack of efficacy late in the course of the disease have led to the use of ribavirin, despite its limitation late in the disease.[204] An effective, live attenuated viral vaccine currently is in use, with a protective efficacy of 95 percent against AHF.[17,432]

Ebola Infection

Ebola virus, a filovirus, causes severe and often fatal hemorrhagic fever; it has no effective treatment. Kudoyarova-Zubavichene and coworkers[391] reviewed the use of hyperimmune goat or equine serum in the prevention and treatment of Ebola infection. Goat hyperimmune serum protects guinea pigs from experimental infection if it is given less than 24 hours before exposure and provides some benefit if it is given within 72 hours after exposure. This product was used for emergency prophylaxis in four patients exposed by laboratory accidents. Mild infection developed in one definitely exposed patient, but Ebola disease did not develop in the other three patients with questionable exposure. All four patients also received recombinant human INF-α2. Equine serum has protected baboons from low-dose virus challenge but not cynomolgus monkeys from a high-dose virus challenge.[325] Equine serum given to monkeys on the day of infection and 5 days later did not prevent death.[324]

Gupta and coauthors[279] reported that a murine polyclonal immune serum protected 100 percent of normal mice and mice with severe combined immunodeficiency after a lethal challenge. Wilson and colleagues[740] were able to protect mice with monoclonal antibodies to Ebola glycoprotein for up to 2 days after infection. Parren and associates[523] were able to protect guinea pigs by giving a human neutralizing antibody up to 1 hour after infection with Ebola virus. Protection of mice by neutralizing monoclonal antibody before and up to 2 days after virus was also shown.[671] Thus, antibody may be a useful agent in the prevention of Ebola disease. Other human monoclonal antibodies are being developed.[195,438,439,762]

Recommendation

The use of antibody in prevention and treatment of Ebola infection is unproved, but monoclonal antibodies are being developed. Animal studies support a role for antibody in the prevention of Ebola infection.

Tick-Borne Encephalitis

Tick-borne encephalitis is caused by a flavivirus that is endemic in Russia and eastern and central Europe. Several neurologic syndromes are associated with this infection—febrile headache, aseptic meningitis, meningoencephalitis, meningomyeloencephalitis, and postencephalitic syndrome.[191] Meningoencephalitis is the most severe form and can result in death or permanent paresis. Effective vaccines are available[191,278] and have been successful in dramatically lowering the incidence of this disease.[393]

Passive immunization with a hyperimmune human globulin has been used for postexposure prophylaxis in endemic countries, but it may result in antibody-dependent enhancement of infection.[372,717] Arras and colleagues[49] question these case reports in contrast to the large number of doses of passive antibody given. Günther and Hagland[278] contend that hyperimmunoglobulin is not indicated for children younger than 14 years because of concerns that antibody may worsen the disease. The lack of efficacy trials also has led to questioning of the use of this product.[10]

In 1995, von Hedenström and colleagues[714] recommended hyperimmunoglobulin for postexposure prophylaxis in addition to immunization. In a review of 656 patients with tick-borne encephalitis in southern Germany, Kaiser[345] concluded that postexposure prophylaxis with hyperimmunoglobulin is less effective than with vaccine and should be used only when necessary. It is currently believed that vaccine is the best method for disease prevention.

Acknowledgment

We are grateful to Drs. E. Richard Stiehm and Margaret A. Keller, the authors of this chapter in the sixth edition of this book.

NEW REFERENCES SINCE THE SIXTH EDITION

2. Abougergi MS, Kwon JH. Intravenous immunoglobulin for the treatment of *Clostridium difficile* infection: a review. *Dig Dis Sci* 2011;**56**:19–26.
11. Ahmad A, Qaisar I, Naeem M, et al. Intrathecal anti-tetanus human immunoglobulin in the treatment of neonatal tetanus. *J Coll Physicians Surg Pak* 2011;**21**:539–41.
12. Ahn J, Flamm SL. Hepatitis C therapy: other players in the game. *Clin Liver Dis* 2011;**15**:641–56.
13. Al-Rawi S, Woodward LJ, Knight J. Puerperal streptococcal toxic shock syndrome treated with recombinant human activated protein C and intravenous immunoglobulin. *Int J Obstet Anesth* 2009;**18**:169–72.
16. Altmann S, Brandt CR, Murphy CJ, et al. Evaluation of therapeutic interventions for vaccinia virus keratitis. *J Infect Dis* 2011;**203**:683–90.
17. Ambrosio A, Saavedra M, Mariani M, et al. Argentine hemorrhagic fever vaccines. *Hum Vaccin* 2011;**7**:694–700.
19. American Academy of Pediatrics. Active and passive immunization. In: Pickering LK, editor: *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 1–69.
22. American Academy of Pediatrics. Botulism and infant botulism. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 281–4.
21. American Academy of Pediatrics. Clostridium difficile. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 285–9.
22. American Academy of Pediatrics. Diptheria. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 307–11.
23. American Academy of Pediatrics. Group A Streptococcal Infections. In: Pickering LK, editor. *2012 red book: report of the*

Committee on Infectious Diseases. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 668–80.
26. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, editor: *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. 337–56.
27. American Academy of Pediatrics. Herpes Simplex. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 398–408.
28. American Academy of Pediatrics. Measels. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 489–99.
29. American Academy of Pediatrics. Mumps. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 514–18.
30. American Academy of Pediatrics. Pavrovirus. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 539–41.
31. American Academy of Pediatrics. Rabies. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 600–7.
34. American Academy of Pediatrics. Rubella. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 629–34.
35. American Academy of Pediatrics. Tetanus. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 707–12.
36. American Academy of Pediatrics. Toxic shock syndrome. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 653–4.
38. Reference deleted in proofs.
59. Bakker AB, Python C, Kissling CJ, et al. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine* 2008;**26**: 5922–7.
87. Blombery P, Prince HM, Worth LJ, et al. Prophylactic intravenous immunoglobulin during autologous haemopoietic stem cell transplantation for multiple myeloma is not associated with reduced infectious complications. *Ann Hematol* 2011;**90**:1167–72.
99. Both L, Banyard AC, van Dolleweerd C, et al. Passive immunity in the prevention of rabies. *Lancet Infect Dis* 2012;**12**:397–407.
108. Brown EL, Bowden MG, Bryson RS, et al. Pediatric antibody response to community-acquired *Staphylococcus aureus* infection is directed to Panton-Valentine leukocidin. *Clin Vaccine Immunol* 2009;**16**:139–41.
118. Buchanan C, Tran TT. Current status of liver transplantation for hepatitis B virus. *Clin Liver Dis* 2011;**15**:753–64.
129. Cao YH, Zhang GY, Zhang GC. Successful treatment with high-dose intravenous immunoglobulin for parvovirus B19 infection associated with acute fulminant hepatitis in a chinese child. *Clin Pediatr (Phila)* 2009;**48**:674–6.
148. Centers for Disease Control and Prevention. Progressive vaccinia in a military smallpox vaccinee: United States, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:532–6.
149. Centers for Disease Control and Prevention. FDA approval of an extended period for administering VariZIG for postexposure prophylaxis of varicella. *MMWR Morb Mortal Wkly Rep* 2012; **61**:212.
150. Centers for Disease Control and Prevention. Postvaccination serologic testing results for infants aged </=24 months exposed to hepatitis B virus at birth: United States, 2008-2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:768–71.
158. Cholongitas E, Vasiliadis T, Antoniadis N, et al. Hepatitis B prophylaxis post liver transplantation with newer nucleos(t)ide analogues after hepatitis B immunoglobulin discontinuation. *Transpl Infect Dis* 2012;**14**:479–87.
163. Congly SE, Burak KW, Coffin CS. Hepatitis B immunoglobulin for prevention of hepatitis B virus infection and recurrence after liver transplantation. *Expert Rev Clin Immunol* 2011;**7**:429–36.
176. Czer LS, Ruzza A, Vespignani R, et al. Prophylaxis of cytomegalovirus disease in mismatched patients after heart transplantation using combined antiviral and immunoglobulin therapy. *Transplant Proc* 2011;**43**:1887–92.
183. Dennert R, Velthuis S, Schalla S, et al. Intravenous immunoglobulin therapy for patients with idiopathic cardiomyopathy and endomyocardial biopsy-proven high PVB19 viral load. *Antivir Ther* 2010;**15**:193–201.
184. Desrosiers MY, Kilty SJ. Treatment alternatives for chronic rhinosinusitis persisting after ESS: what to do when antibiotics, steroids and surgery fail. *Rhinology* 2008;**46**:3–14.
195. Dye JM, Herbert AS, Kuehne AI, et al. Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. *Proc Natl Acad Sci U S A* 2012;**109**:5034–9.
205. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. *Antiviral Res* 2008;**78**:132–9.
211. Farcet MR, Planitzer CB, Stein O, et al. Hepatitis A virus antibodies in immunoglobulin preparations. *J Allergy Clin Immunol* 2010; **125**:198–202.
219. Ferrara G, Zumla A, Maeurer M. Intravenous immunoglobulin (IVIg) for refractory and difficult-to-treat infections. *Am J Med* 2012;**125**:1036 e1031–8.
225. Firoz BF, Henning JS, Zarzabal LA, et al. Toxic epidermal necrolysis: five years of treatment experience from a burn unit. *J Am Acad Dermatol* 2012;**67**:630–5.
233. Garcia-Lara J, Foster SJ. Anti-*Staphylococcus aureus* immunotherapy: current status and prospects. *Curr Opin Pharmacol* 2009;**9**: 552–7.
244. Giang E, Dorner M, Prentoe JC, et al. Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. *Proc Natl Acad Sci U S A* 2012;**109**:6205–10.
253. Control GA f. R. *Human monoclonal antibodies for PEP*. Available at: <http://www.rabiescontrol.net/news/news-archive/humanmonoclonals.html>.
256. Goraya J, Marks H, Khurana D, et al. Subacute sclerosing panencephalitis (SSPE) presenting as acute disseminated encephalomyelitis in a child. *J Child Neurol* 2009;**24**:899–903.
274. Group IC, Brocklehurst P, Farrell B, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011; **365**:1201–11.
291. Hatton O, Martinez OM, Esquivel CO. Emerging therapeutic strategies for Epstein-Barr virus+ post-transplant lymphoproliferative disorder. *Pediatr Transplant* 2012;**16**:220–9.
299. Hermos CR, Yoong P, Pier GB. High levels of antibody to Panton-Valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clin Infect Dis* 2010;**51**:1138–46.
307. Hot A, Madoux MH, Viard JP, et al. Successful treatment of cytomegalovirus-associated hemophagocytic syndrome by intravenous immunoglobulins. *Am J Hematol* 2008;**83**:159–62.
349. Kassis C, Champlin RE, Hachem RY, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. *Biol Blood Marrow Transplant* 2010;**16**:1265–71.
351. Katz LH, Paul M, Guy DG, et al. Prevention of recurrent hepatitis B virus infection after liver transplantation: hepatitis B immunoglobulin, antiviral drugs, or both? Systematic review and meta-analysis. *Transpl Infect Dis* 2010;**12**:292–308.
356. Keck ZY, Xia J, Wang Y, et al. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. *PLoS Pathog* 2012;**8**:e1002653.
368. Khanna N, Widmer AF, Decker M, et al. Respiratory syncytial virus infection in patients with hematological diseases: single-center study and review of the literature. *Clin Infect Dis* 2008; **46**:402–12.
396. Kurz H, Herbich K, Janata O, et al. Experience with the use of palivizumab together with infection control measures to prevent respiratory syncytial virus outbreaks in neonatal intensive care units. *J Hosp Infect* 2008;**70**:246–52.
402. Lamont RF, Sobel JD, Carrington D, et al. Varicella-zoster virus (chickenpox) infection in pregnancy. *BJOG* 2011;**118**:1155–62.

414. Lindamood KE, Fleck P, Narla A, et al. Neonatal enteroviral sepsis/meningoencephalitis and hemophagocytic lymphohistiocytosis: diagnostic challenges. *Am J Perinatol* 2011;**28**:337–46.
420. Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N Engl J Med* 2010; **362**:197–205.
430. Maidji E, Nigro G, Tabata T, et al. Antibody treatment promotes compensation for human cytomegalovirus-induced pathogenesis and a hypoxia-like condition in placentas with congenital infection. *Am J Pathol* 2010;**177**:1298–310.
439. Marzi A, Yoshida R, Miyamoto H, et al. Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever. *PLoS One* 2012;**7**:e36192.
461. Migone TS, Subramanian GM, Zhong J, et al. Raxibacumab for the treatment of inhalational anthrax. *N Engl J Med* 2009;**361**: 135–44.
476. Murphy TK, Storch EA, Lewin AB, et al. Clinical factors associated with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *J Pediatr* 2012;**160**:314–19.
478. Nakajima M, Sakuishi K, Fukuda S, et al. Recovery from adult measles encephalitis immediately after early immunomodulation. *Clin Infect Dis* 2008;**47**:148–9.
481. National Association of State Public Health Veterinarians, I. Compendium of animal rabies prevention and control, 2011. *MMWR Recomm Rep* 2011;**60**:1–17.
488. Nguyen CT, Tran TT. Hepatitis vaccination and prophylaxis. *Clin Liver Dis* 2009;**13**:317–29.
490. Nigro G, Adler SP, Parruti G, et al. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy: a case-control study of the outcome in children. *J Infect Dis* 2012;**205**:215–27.
504. Ohlsen K, Lorenz U. Immunotherapeutic strategies to combat staphylococcal infections. *Int J Med Microbiol* 2010;**300**:402–10.
518. Pammi M, Haque KN. Oral immunoglobulin for the prevention of rotavirus infection in low birth weight infants. *Cochrane Database Syst Rev* 2011;(11):CD003740.
531. Patel NB, Pendleton C, Quinones-Hinojosa A, et al. A unique experiment in neurological surgery: intracerebral injection of antitoxin for tetanus. *J Neurosurg* 2010;**112**:1318–21.
543. Ranganathan K, Worley S, Michaels MG, et al. Cytomegalovirus immunoglobulin decreases the risk of cytomegalovirus infection but not disease after pediatric lung transplantation. *J Heart Lung Transplant* 2009;**28**:1050–6.
561. Rezaei N, Hedayat M, Aghamohammadi A, et al. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol* 2011; **127**:1329–41, e1322; quiz 1342–3.
563. Robinson KA, Odelola OA, Saldanha IJ, et al. Palivizumab for prophylaxis against respiratory syncytial virus infection in children with cystic fibrosis. *Cochrane Database Syst Rev* 2012;(2):CD007743.
588. Schepens B, Ibanez LI, De Baets S, et al. Nanobodies(R) specific for respiratory syncytial virus fusion protein protect against infection by inhibition of fusion. *J Infect Dis* 2011;**204**:1692–701.
592. Shah JN, Chemaly RF. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011;**117**:2755–63.
600. Shah PS, Kaufman DA. Antistaphylococcal immunoglobulins to prevent staphylococcal infection in very low birth weight infants. *Cochrane Database Syst Rev* 2009;(2):CD006449.
604. Shah SS, Hall M, Srivastava R, et al. Intravenous immunoglobulin in children with streptococcal toxic shock syndrome. *Clin Infect Dis* 2009;**49**:1369–76.
611. Shi Z, Li X, Ma L, et al. Hepatitis B immunoglobulin injection in pregnancy to interrupt hepatitis B virus mother-to-child transmission-a meta-analysis. *Int J Infect Dis* 2010;**14**:e622–34.
622. Silva Cde A, Dias L, Baltieri SR, et al. Respiratory syncytial virus outbreak in neonatal intensive care unit: impact of infection control measures plus palivizumab use. *Antimicrob Resist Infect Control* 2012;**1**:16.
643. Stern EJ, Uhde KB, Shadomy SV, et al. Conference report on public health and clinical guidelines for anthrax. *Emerg Infect Dis* 2008;**14**.
678. Tebruegge M, Curtis N. Enterovirus infections in neonates. *Semin Fetal Neonatal Med* 2009;**14**:222–7.
686. Trappe R, Riess H, Anagnostopoulos I, et al. Efficiency of antiviral therapy plus IVIG in a case of primary EBV infection associated PTLD refractory to rituximab, chemotherapy, and antiviral therapy alone. *Ann Hematol* 2009;**88**:167–72.
692. Ueda Y, Marusawa H, Kaido T, et al. Efficacy and safety of prophylaxis with entecavir and hepatitis B immunoglobulin in preventing hepatitis B recurrence after living-donor liver transplantation. *Hepatol Res* 2012.
702. van Immerzeel TD, van Gilst RM, Hartwig NG. Beneficial use of immunoglobulins in the treatment of Sydenham chorea. *Eur J Pediatr* 2010;**169**:1151–4.
710. Visentin S, Manara R, Milanese L, et al. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. *Clin Infect Dis* 2012;**55**:497–503.
720. Wang Y, Keck ZY, Saha A, et al. Affinity maturation to improve human monoclonal antibody neutralization potency and breadth against hepatitis C virus. *J Biol Chem* 2011;**286**:44218–33.
728. Weisman LE, Thackray HM, Garcia-Prats JA, et al. Phase 1/2 double-blind, placebo-controlled, dose escalation, safety, and pharmacokinetic study of pagibaximab (BSYX-A110), an antistaphylococcal monoclonal antibody for the prevention of staphylococcal bloodstream infections, in very-low-birth-weight neonates. *Antimicrob Agents Chemother* 2009;**53**:2879–86.
729. Weisman LE, Thackray HM, Steinhorn RH, et al. A randomized study of a monoclonal antibody (pagibaximab) to prevent staphylococcal sepsis. *Pediatrics* 2011;**128**:271–9.
762. Zeitlin L, Pettitt J, Scully C, et al. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc Natl Acad Sci U S A* 2011;**108**:20690–4.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abadesso C, Almeida HI, Virella D, et al. Use of palivizumab to control an outbreak of syncytial respiratory virus in a neonatal intensive care unit. *J Hosp Infect* 2004;**58**:38–41.
2. Abougergi MS, Kwon JH. Intravenous immunoglobulin for the treatment of *Clostridium difficile* infection: a review. *Dig Dis Sci* 2011;**56**:19–26.
3. Abraham E, Wunderink R, Silverman H, et al. Efficacy and safety of monoclonal antibody to human tumor necrosis factor alpha in patients with sepsis syndrome: a randomized, controlled, double-blind, multicenter clinical trial. TNF-alpha MAb Sepsis Study Group. *JAMA* 1995;**273**:934–41.
4. Abrahamsen TG, Sandersen H, Bustnes A. Home therapy with subcutaneous immunoglobulin infusions in children with congenital immunodeficiencies. *Pediatrics* 1996;**98**:1127–31.
5. Abrutyn E, Berlin JA. Intrathecal therapy in tetanus: a meta-analysis. *JAMA* 1991;**266**:2262–7.
6. Abzug MJ, Keyserling HL, Lee ML, et al. Neonatal enterovirus infection: virology, serology, and effects of intravenous immune globulin. *Clin Infect Dis* 1995;**20**:1201–6.
7. Acute Hepatic Failure Study Group. Failure of specific immunotherapy in fulminant type B hepatitis. *Ann Intern Med* 1977;**86**:272–7.
8. Advisory Committee on Immunization Practices (ACIP). Vaccinia (smallpox). *Vaccine* 2001;**50**:1–25.
9. Advisory Committee on Immunization Practices, Centers for Disease, Control and Prevention. Update: Prevention of hepatitis A after exposure to hepatitis A virus and in international travelers. Updated recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007;**56**:1080–4.
10. Aebi C, Schaad UB. [TBE-immunoglobulins: a critical assessment of efficacy]. *Schweiz Med Wochenschr* 1994;**124**:1837–40.
11. Ahmad A, Qaisar I, Naeem M, et al. Intrathecal anti-tetanus human immunoglobulin in the treatment of neonatal tetanus. *J Coll Physicians Surg Pak* 2011;**21**:539–41.
12. Ahn J, Flamm SL. Hepatitis C therapy: other players in the game. *Clin Liver Dis* 2011;**15**:641–56.
13. Al-Rawi S, Woodward LJ, Knight J. Puerperal streptococcal toxic shock syndrome treated with recombinant human activated protein C and intravenous immunoglobulin. *Int J Obstet Anesth* 2009;**18**:169–72.
14. Alejandria MM, Lansang MA, Dans LF, et al. Intravenous immunoglobulin for treating sepsis and septic shock. *Cochrane Database Syst Rev* 2002;(1):CD001090.
15. Alexander HE. Treatment of Haemophilus influenzae infection and of meningococcic and pneumococcic meningitis. *Am J Dis Child* 1943;**66**:172–87.
16. Altmann S, Brandt CR, Murphy CJ, et al. Evaluation of therapeutic interventions for vaccinia virus keratitis. *J Infect Dis* 2011;**203**:683–90.
17. Ambrosio A, Saavedra M, Mariani M, et al. Argentine hemorrhagic fever vaccines. *Hum Vaccin* 2011;**7**:694–700.
18. Amer J, Ott E, Ibbott FA, et al. The effect of monthly gammaglobulin administration on morbidity and mortality from infection in premature infants during the first year of life. *Pediatrics* 1963;**32**:4–9.
19. American Academy of Pediatrics. Active and passive immunization. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 1–69.
20. American Academy of Pediatrics. Botulism and infant botulism. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 281–4.
21. American Academy of Pediatrics. Clostridium difficile. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 285–9.
22. American Academy of Pediatrics. Diptheria. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 307–11.
23. American Academy of Pediatrics. Group A Streptococcal Infections. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 668–80.
24. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2000. p. 326–35.
25. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 335–55.
26. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009. p. 337–56.
27. American Academy of Pediatrics. Herpes Simplex. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 398–408.
28. American Academy of Pediatrics. Measels. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 489–99.
29. American Academy of Pediatrics. Mumps. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 514–18.
30. American Academy of Pediatrics. Pavrovirus. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill, 29th ed. Elk Grove Village, IL: American Academy of Pediatrics; 2012. p. 539–41.
31. American Academy of Pediatrics. Rabies. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 600–7.
32. American Academy of Pediatrics. Respiratory syncytial virus. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 560–6.
33. American Academy of Pediatrics. Rotavirus. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 572–4.
34. American Academy of Pediatrics. Rubella. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 629–34.
35. American Academy of Pediatrics. Tetanus. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 707–12.
36. American Academy of Pediatrics. Toxic shock syndrome. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 653–4.
37. American Academy of Pediatrics. Toxic shock syndrome. In: Pickering LK, editor. *2012 red book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006. p. 660–5.
38. Reference deleted in proofs.
39. Reference deleted in proofs.
40. American Academy of Pediatrics Committee on Infectious Diseases, Committee on Fetus and Newborn. Respiratory syncytial virus immune globulin intravenous: indications for use. *Pediatrics* 1997;**99**:645–50.
41. Ammann AJ, Stiehm ER. Immune globulin levels in colostrum and breast milk, and serum from formula- and breast-fed newborns. *Proc Soc Exp Biol Med* 1966;**122**:1098–100.
42. Ammann AJ, Ashman RF, Buckley RH, et al. Use of intravenous gamma-globulin in antibody immunodeficiency: results of a multicenter controlled trial. *Clin Immunol Immunopathol* 1982;**22**:60–7.
43. Anonymous. Cytomeglovirus. *Am J Transplant* 2004;**4**:51–8.
44. Reference deleted in proofs.
45. Apfelzweig R, Piszkiewicz D, Hooper JA. Immunoglobulin A concentrations in commercial immune globulins. *J Clin Immunol* 1987;**7**:46–50.

46. Armbruster C, Stiegler GM, Vcelar BA, et al. Passive immunization with the anti-HIV-1 human monoclonal antibody (hMAb) 4E10 and the hMAb combination 4E10/2F5/2G12. *J Antimicrob Chemother* 2004;**54**:915–20.
47. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon: medical and public health management. *JAMA* 2001;**285**:1059–70.
48. Arnon SS, Schechter R, Maslanka SE, et al. Human botulism immune globulin for the treatment of infant botulism. *N Engl J Med* 2006;**354**:462–71.
49. Arras C, Fescharek R, Gregersen JP. Do specific hyperimmunoglobulins aggravate clinical course of tick-borne encephalitis? *Lancet* 1996;**347**:1331.
50. Arvin AM. Varicella-zoster virus. *Clin Microbiol Rev* 1996;**9**: 361–81.
51. Ashida ER, Saxon A. Home intravenous immunoglobulin therapy by self-administration. *J Clin Immunol* 1986;**6**:306–9.
52. Askenasy OM. A community outbreak of hepatitis A in a religious community in Indiana: failure of immune serum globulin to prevent the spread of the infection. *Epidemiol Infect* 2000;**124**: 309–13.
53. Athavale VB, Pai PN. Role of tetanus antitoxin in the treatment of tetanus in children. *J Pediatr* 1966;**68**:289–93.
54. Audet S, Virata-Theimer ML, Beeler JA, et al. Measles-virus-neutralizing antibodies in intravenous immunoglobulins. *J Infect Dis* 2006;**194**:781–9.
55. Babes V, Lepp M. Recherches sur la vaccination antirabique. *Ann Inst Pasteur* 1889;**3**:385–90.
56. Bagge E, Geijer M, Tarkowski A. Intra-articular administration of polyclonal immunoglobulin G in rheumatoid arthritis: a double-blind, placebo-controlled pilot study. *Scand J Rheumatol* 1996;**25**: 174–6.
57. Baker CJ, Melish ME, Hall RT, et al. Intravenous immune globulin for the prevention of nosocomial infection in low-birth-weight neonates. The Multicenter Group for the Study of Immune Globulin in Neonates. *N Engl J Med* 1992;**327**:213–19.
58. Bakker AB, Marissen WE, Kramer RA, et al. Novel human monoclonal antibody combination effectively neutralizing natural rabies virus variants and individual in vitro escape mutants. *J Virol* 2005;**79**:9062–8.
59. Bakker AB, Python C, Kissling CJ, et al. First administration to humans of a monoclonal antibody cocktail against rabies virus: safety, tolerability, and neutralizing activity. *Vaccine* 2008;**26**: 5922–7.
60. Balagtas RC, Nelson KE, Levin S, et al. Treatment of pertussis with pertussis immune globulin. *J Pediatr* 1971;**79**: 203–8.
61. Balfour HH Jr, Groth KE, McCullough J, et al. Prevention or modification of varicella using zoster immune plasma. *Am J Dis Child* 1977;**131**:693–6.
62. Balfour-Lynn IM, Mohan U, Bush A, et al. Intravenous immunoglobulin for cystic fibrosis lung disease: a case series of 16 children. *Arch Dis Child* 2004;**89**:315–19.
63. Ballow M. Mechanisms of action of intravenous immune serum globulin in autoimmune and inflammatory diseases. *J Allergy Clin Immunol* 1997;**100**:151–7.
64. Ballow M, Cates KL, Rowe JC, et al. Development of the immune system in very low birth weight (less than 1500 g) premature infants: concentrations of plasma immunoglobulins and patterns of infections. *Pediatr Res* 1986;**20**:899–904.
65. Baltazard M, Bahmanyar M. [Field trials with rabies vaccine on persons bitten by rabid wolves]. *Bull World Health Organ* 1955; **13**:747–72.
66. Banna GL, Aversa SM, Cattelan AM, et al. Respiratory syncytial virus-related pneumonia after stem cell transplantation successfully treated with palivizumab and steroid therapy. *Scand J Infect Dis* 2004;**36**:155–7.
67. Barbero GJ, Gray A, Scott TF, et al. Vaccinia gangrenosa treated with hyperimmune vaccinal gamma globulin. *Pediatrics* 1955;**16**: 609–18.
68. Barnes GL, Doyle LW, Hewson PH, et al. A randomised trial of oral gammaglobulin in low-birth-weight infants infected with rotavirus. *Lancet* 1982;**1**:1371–3.
69. Baron S, Barnet EV, Goldsmith RS, et al. Prophylaxis of Infections by gamma globulin. *Am J Hyg* 1964;**79**:186–95.
70. Barry W, Hudgins L, Donta ST, et al. Intravenous immunoglobulin therapy for toxic shock syndrome. *JAMA* 1992;**267**: 3315–16.
71. Bass EB, Powe NR, Goodman SN, et al. Efficacy of immune globulin in preventing complications of bone marrow transplantation: a meta-analysis. *Bone Marrow Transplant* 1993;**12**:273–82.
72. Baumgartner JD, Glauser MP, McCutchan JA, et al. Prevention of gram-negative shock and death in surgical patients by antibody to endotoxin core glycolipid. *Lancet* 1985;**2**:59–63.
73. Beasley RP, Hwang LY, Lin CC, et al. Hepatitis B immune globulin (HBIG) efficacy in the interruption of perinatal transmission of hepatitis B virus carrier state. Initial report of a randomised double-blind placebo-controlled trial. *Lancet* 1981;**2**:388–93.
74. Beasley RP, Hwang LY, Stevens CE, et al. Efficacy of hepatitis B immune globulin for prevention of perinatal transmission of the hepatitis B virus carrier state: final report of a randomized double-blind, placebo-controlled trial. *Hepatology* 1983;**3**:135–41.
75. Begue RE, Lindo-Soriano I. Failure of intrathecal tetanus antitoxin in the treatment of tetanus neonatorum. *J Infect Dis* 1991; **164**:619–20.
76. Benjamin DK, Schelonka R, White R, et al. A blinded, randomized, multicenter study of an intravenous *Staphylococcus aureus* immune globulin. *J Perinatol* 2006;**26**:290–5.
77. Berg BO. Syndrome of infant botulism. *Pediatrics* 1977;**59**: 321–3.
78. Berger M. Subcutaneous immunoglobulin replacement in primary immunodeficiencies. *Clin Immunol* 2004;**112**:1–7.
79. Berger M, Cupps TR, Fauci AS. Immunoglobulin replacement therapy by slow subcutaneous infusion. *Ann Intern Med* 1980;**93**: 55–6.
80. Berger M, Cupps TR, Fauci AS. High-dose immunoglobulin replacement therapy by slow subcutaneous infusion during pregnancy. *JAMA* 1982;**247**:2824–5.
81. Bernstein LJ, Krieger BZ, Novick B, et al. Bacterial infection in the acquired immunodeficiency syndrome of children. *Pediatr Infect Dis* 1985;**4**:472–5.
82. Björkander J, Bengtsson U, Oxelius VA, et al. Symptoms and efficacy of intravenous immunoglobulin prophylaxis in patients with low serum levels of IgG subclasses. *J Allergy Clin Immunol* 1986;**77**:124–7.
83. Björkander J, Cunningham-Rundles C, Ludon P. Intravenous immunoglobulin prophylaxis causing liver damage in 16 of 77 patients with hypogammaglobulinemia or IgG subclass deficiency. *Am J Med* 1988;**84**:107–11.
84. Bjoro K, Froland SS, Yun Z, et al. Hepatitis C infection in patients with primary hypogammaglobulinemia after treatment with contaminated immune globulin. *N Engl J Med* 1994;**331**:1607–11.
85. Blake PA, Feldman RA, Buchanan TM, et al. Serologic therapy of tetanus in the United States, 1965-1971. *JAMA* 1976;**235**:42–4.
86. Bleck TP, Rupprecht CE. Rhabdoviruses. In: Mandell GL, Douglas RG, Bennett JE, et al, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 6th ed. Philadelphia: Churchill Livingstone; 2005. p. 2047–56.
87. Blombery P, Prince HM, Worth LJ, et al. Prophylactic intravenous immunoglobulin during autologous haemopoietic stem cell transplantation for multiple myeloma is not associated with reduced infectious complications. *Ann Hematol* 2011;**90**:1167–72.
88. Bloom B, Schelonka R, Kueser T, et al. Multicenter study to assess safety and efficacy of INH-A21, a donor-selected human staphylococcal immunoglobulin, for prevention of nosocomial infections in very low birth weight infants. *Pediatr Infect Dis J* 2005;**24**:858–66.
89. Bloxsom A. Use of immune serum globulin (human) as prophylaxis against poliomyelitis. *Tex State J Med* 1949;**45**:468–70.
90. Bocchini CE, Hulten KG, Mason EO Jr, et al. Panton-Valentine leukocidin genes are associated with enhanced inflammatory response and local disease in acute hematogenous *Staphylococcus aureus* osteomyelitis in children. *Pediatrics* 2006;**117**:433–40.
91. Bodian D. Neutralization of three immunological types of poliomyelitis virus by human gamma globulin. *Proc Soc Exp Biol Med* 1949;**72**:259–61.
92. Bodian D. Experimental studies on passive immunization against poliomyelitis. I. Protection with human gamma globulin against intramuscular inoculation, and combined passive and active immunization. *Am J Hyg* 1951;**54**:132–43.

93. Boeckh M, Nichols WG, Papanicolaou G, et al. Cytomegalovirus in hematopoietic stem cell transplant recipients: current status, known challenges, and future strategies. *Biol Blood Marrow Transplant* 2003;**9**:543–58.
94. Boesch RP, Daines M, Kaul A, et al. Lymphoproliferative disorder of the airway of an adolescent without immunodeficiency. *Int J Pediatr Otorhinolaryngol* 2005;**69**:1591–4.
95. Bogstedt AK, Johansen K, Hatta H, et al. Passive immunity against diarrhoea. *Acta Paediatr* 1996;**85**:125–8.
96. Bonaros NE, Kocher A, Dunkler D, et al. Comparison of combined prophylaxis of cytomegalovirus hyperimmune globulin plus ganciclovir versus cytomegalovirus hyperimmune globulin alone in high-risk heart transplant recipients. *Transplantation* 2004;**77**:890–7.
97. Borgia G. HepeX-C (XTL Biopharmaceuticals). *Curr Opin Investig Drugs* 2004;**5**:892–7.
98. Borowitz SM, Saulsbury FT. Treatment of chronic cryptosporidial infection with orally administered human serum immune globulin. *J Pediatr* 1991;**119**:593–5.
99. Both L, Banyard AC, van Dolleweerd C, et al. Passive immunity in the prevention of rabies. *Lancet Infect Dis* 2012;**12**: 397–407.
100. Bradford WF. Use of convalescent blood in whooping cough. *Am J Dis Child* 1935;**50**:918–28.
101. Bray M, Buller M. Looking back at smallpox. *Clin Infect Dis* 2004;**38**:882–9.
102. Bray M, Wright ME. Progressive vaccinia. *Clin Infect Dis* 2003;**36**:766–74.
103. Brey RN. Molecular basis for improved anthrax vaccines. *Adv Drug Deliv Rev* 2005;**57**:1266–92.
104. Brodsky L, Afshani E, Pizzuto M, et al. Open trial of intravenous immune serum globulin for chronic sinusitis in children. *Ann Allergy Asthma Immunol* 1997;**79**:119–24.
105. Brody JA, Sever JL, Schiff GM. Prevention of rubella by gamma globulin during an epidemic in Barrow, Alaska, in 1964. *N Engl J Med* 1965;**272**:127–9.
106. Brossier F, Levy M, Landier A, et al. Functional analysis of *Bacillus anthracis* protective antigen by using neutralizing monoclonal antibodies. *Infect Immun* 2004;**72**:6313–17.
107. Brown A, Mohamed SD, Montgomery RD, et al. Value of a large dose of antitoxin in clinical tetanus. *Lancet* 1960;**2**: 227–30.
108. Brown EL, Bowden MG, Bryson RS, et al. Pediatric antibody response to community-acquired *Staphylococcus aureus* infection is directed to Panton-Valentine leukocidin. *Clin Vaccine Immunol* 2009;**16**:139–41.
109. Brown KE. Parvovirus B19. In: Mandell GL, Douglas RG, Bennett JE, et al, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. Philadelphia: Churchill Livingstone; 2005. p. 1891–9.
110. Brown KE, Green SW, Antunez de Mayolo J, et al. Congenital anaemia after transplacental B19 parvovirus infection. *Lancet* 1994;**343**:895–6.
111. Brox AG, Cournoyer D, Sternbach M, et al. Hemolytic anemia following intravenous gamma globulin administration. *Am J Med* 1987;**82**:633–5.
112. Brunell PA, Gershon AA. Passive immunization against varicella-zoster infections and other modes of therapy. *J Infect Dis* 1973; **127**:415–23.
113. Brunell PA, Gershon AA, Hughes WT, et al. Prevention of varicella in high risk children: a collaborative study. *Pediatrics* 1972; **50**:718–22.
114. Brunell PA, Ross A, Miller LH, et al. Prevention of varicella by zoster immune globulin. *N Engl J Med* 1969;**280**:1191–4.
115. Bruss JB, Siber GR. Protective effects of pertussis immunoglobulin (P-IGIV) in the aerosol challenge model. *Clin Diagn Lab Immunol* 1999;**6**:464–70.
116. Bruss JB, Malley R, Halperin S, et al. Treatment of severe pertussis: a study of the safety and pharmacology of intravenous pertussis immunoglobulin. *Pediatr Infect Dis J* 1999;**18**:505–11.
117. Brussow H, Hilpert H, Walther I, et al. Bovine milk immunoglobulins for passive immunity to infantile rotavirus gastroenteritis. *J Clin Microbiol* 1987;**25**:982–6.
118. Buchanan C, Tran TT. Current status of liver transplantation for hepatitis B virus. *Clin Liver Dis* 2011;**15**:753–64.
119. Buckley RH, Schiff RI. The use of intravenous immune globulin in immunodeficiency diseases. *N Engl J Med* 1991;**325**:110–17.
120. Burks AW, Sampson HA, Buckley RH. Anaphylactic reactions after gamma globulin administration in patients with hypogammaglobulinemia: detection of IgE antibodies to IgA. *N Engl J Med* 1986;**314**:560–4.
121. Burton DR, Pyati J, Koduri R, et al. Efficient neutralization of primary isolates of HIV-1 by a recombinant human monoclonal antibody. *Science* 1994;**266**:1024–7.
122. Busse PJ, Razvi S, Cunningham-Rundles C. Efficacy of intravenous immunoglobulin in the prevention of pneumonia in patients with common variable immunodeficiency. *J Allergy Clin Immunol* 2002;**109**:1001–4.
123. Bussel JB, Haimi JS. Isolated thrombocytopenia in patients infected with HIV: treatment with intravenous gammaglobulin. *Am J Hematol* 1988;**28**:79–84.
124. Bussel JB, Giulino L, Lee S, et al. Update on therapeutic monoclonal antibodies. *Curr Probl Pediatr Adolesc Health Care* 2007;**37**: 113–44.
125. Calandra T, Glauser MP, Schellekens J, et al. Treatment of gram-negative septic shock with human IgG antibody to *Escherichia coli* J5: a prospective, double-blind, randomized trial. *J Infect Dis* 1988;**158**:312–19.
126. Calvelli TA, Rubinstein A. Intravenous gamma globulin in infant acquired immunodeficiency syndrome. *Pediatr Infect Dis* 1986;**5**: 207–10.
127. Campbell AL, Herold BC. Strategies for the prevention of cytomegalovirus infection and disease in pediatric liver transplantation recipients. *Pediatr Transplant* 2004;**8**:619–27.
128. Cannon RA, Blum PM, Ament ME, et al. Reversal of enterocolitis-associated combined immunodeficiency by plasma therapy. *J Pediatr* 1982;**101**:711–17.
129. Cao YH, Zhang GY, Zhang GC. Successful treatment with high-dose intravenous immunoglobulin for parvovirus B19 infection associated with acute fulminant hepatitis in a chinese child. *Clin Pediatr (Phila)* 2009;**48**:674–6.
130. Capparelli EV, Bloom BT, Kueser TJ, et al. Multicenter study to determine antibody concentrations and assess the safety of administration of INH-A21, a donor-selected human staphylococcal immune globulin, in low-birth-weight infants. *Antimicrob Agents Chemother* 2005;**49**:4121–7.
131. Carl M, Francis DP, Maynard JE. Food-borne hepatitis A: recommendations for control. *J Infect Dis* 1983;**148**:1133–5.
132. Casadevall A. Passive antibody therapies: progress and continuing challenges. *Clin Immunol* 1999;**93**:5–15.
133. Casadevall A, Scharff MD. Return to the past: the case for antibody-based therapies in infectious diseases. *Clin Infect Dis* 1995;**21**:150–61.
134. Center for Infectious Disease Research and Policy. *Anthrax: current, comprehensive information on pathogenesis, microbiology, epidemiology, diagnosis, treatment, and prophylaxis*. Minneapolis, Minn: The Center; n.d.
135. Center for Infectious Disease Research and Policy. *HHS to buy 20,000 courses of anthrax antitoxin*. Minneapolis, Minn: The Center; n.d.
136. Centers for Disease Control. *Botulism in the United States, 1899-1977:* handbook for epidemiologists, clinicians and laboratory workers. Atlanta: Centers for Disease Control; 1979.
137. Centers for Disease Control and Prevention. Postexposure prophylaxis of hepatitis B. *MMWR Morb Mortal Wkly Rep* 1984;**33**: 285–90.
138. Centers for Disease Control and Prevention. Rabies prevention: United States, 1984. *MMWR Morb Mortal Wkly Rep* 1984;**33**:393–402, 407–8.
139. Centers for Disease Control and Prevention. Recommendations for protection against viral hepatitis. *MMWR Morb Mortal Wkly Rep* 1985;**34**:313–24, 329–35.
140. Centers for Disease Control and Prevention. Vaccinia (smallpox) vaccine; recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1991;**40**:1–10.
141. Centers for Disease Control and Prevention. Outbreak of hepatitis C associated with intravenous immunoglobulin administration: United States, October 1993–June 1994. *MMWR Morb Mortal Wkly Rep* 1994;**43**:505–8.

142. Updated Recommendations for Use of VariZig-United States, 2013. *MMWR* 2013;**62**:574–6.
143. Centers for Disease Control and Prevention. Guidelines for preventing opportunistic infections among hematopoietic stem cell transplant recipients. *MMWR Recomm Rep* 2000;**49**:1–125.
144. Centers for Disease Control and Prevention. Vaccinia (smallpox) vaccine, Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2001;**50**:1–25.
145. Centers for Disease Control and Prevention. Smallpox vaccination and adverse reactions: guidance for clinicians. *MMWR Recomm Rep* 2003;**52**:1–28.
146. Centers for Disease Control and Prevention. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendation of the Advisory Committee on Immunization Practices (ACIP). II. Immunization of adults. Appendix B: postexposure prophylaxis to prevent hepatitis B virus infection. *MMWR Recomm Rep* 2006;**55**:1–33.
147. Deleted in proofs.
148. Centers for Disease Control and Prevention. Progressive vaccinia in a military smallpox vaccinee: United States, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:532–6.
149. Centers for Disease Control and Prevention. FDA approval of an extended period for administering VariZIG for postexposure prophylaxis of varicella. *MMWR Morb Mortal Wkly Rep* 2012; **61**:212.
150. Centers for Disease Control and Prevention. Postvaccination serologic testing results for infants aged </=24 months exposed to hepatitis B virus at birth: United States, 2008-2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:768–71.
151. Chapel HM, Lee M, Hargreaves R, et al. Randomised trial of intravenous immunoglobulin as prophylaxis against infection in plateau-phase multiple myeloma. The UK Group for Immunoglobulin Replacement Therapy in Multiple Myeloma. *Lancet* 1994;**343**:1059–63.
152. Chavez-Bueno S, Mejias A, Merryman RA, et al. Intravenous palivizumab and ribavirin combination for respiratory syncytial virus disease in high-risk pediatric patients. *Pediatr Infect Dis J* 2007;**26**:1089–93.
153. Chen SH, Liang DC. Intravenous immunoglobulin prophylaxis in children with acute leukemia following exposure to varicella. *Pediatr Hematol Oncol* 1992;**9**:347–51.
154. Chen Z, Moayeri M, Zhou YH, et al. Efficient neutralization of anthrax toxin by chimpanzee monoclonal antibodies against protective antigen. *J Infect Dis* 2006;**193**:625–33.
155. Cheng YK, Decker PA, O'Byrne MM, et al. Clinical and laboratory characteristics of 75 patients with specific polysaccharide antibody deficiency syndrome. *Ann Allergy Asthma Immunol* 2006;**97**:306–11.
156. Chertow DS, Tan ET, Maslanka SE, et al. Botulism in 4 adults following cosmetic injections with an unlicensed, highly concentrated botulinum preparation. *JAMA* 2006;**296**:2476–9.
157. Choi SH, Chang SP, Won JC, et al. A case of persistent anemia in a renal transplant recipient: association with parvovirus B19 infection. *Scand J Infect Dis* 2002;**34**:71–5.
158. Cholongitas E, Vasiliadis T, Antoniadis N, et al. Hepatitis B prophylaxis post liver transplantation with newer nucleos(t)ide analogues after hepatitis B immunoglobulin discontinuation. *Transpl Infect Dis* 2012;**14**:479–87.
159. Choquet S, Leblond V, Herbrecht R, et al. Efficacy and safety of rituximab in B-cell post-transplantation lymphoproliferative disorders: results of a prospective multicenter phase 2 study. *Blood* 2006;**107**:3053–7.
160. Chuhjo T, Nakao S, Matsuda T. Successful treatment of persistent erythroid aplasia caused by parvovirus B19 infection in a patient with common variable immunodeficiency with low-dose immunoglobulin. *Am J Hematol* 1999;**60**:222–4.
161. Clave E, Agbalika F, Bajzik V, et al. Epstein-Barr virus (EBV) reactivation in allogeneic stem-cell transplantation: relationship between viral load, EBV-specific T-cell reconstitution and rituximab therapy. *Transplantation* 2004;**77**:76–84.
162. Cleary RK. Clostridium difficile-associated diarrhea and colitis: clinical manifestations, diagnosis, and treatment. *Dis Colon Rectum* 1998;**41**:1435–49.
163. Cohen J, Carlet J. INTERSEPT: an international, multicenter, placebo-controlled trial of monoclonal antibody to human tumor necrosis factor-alpha in patients with sepsis. International Sepsis Trial Study Group. *Crit Care Med* 1996;**24**:1431–40.
164. Congly SE, Burak KW, Coffin CS. Hepatitis B immunoglobulin for prevention of hepatitis B virus infection and recurrence after liver transplantation. *Expert Rev Clin Immunol* 2011;**7**: 429–36.
165. Conway SP, Gillies DR, Docherty A. Neonatal infection in premature infants and use of human immunoglobulin. *Arch Dis Child* 1987;**62**:1252–6.
166. Cooperative Group for the Study of Immunoglobulin in Chronic Lymphocytic Leukemia. Intravenous immunoglobulin for the prevention of infection in chronic lymphocytic leukemia: a randomized, controlled clinical trial. *N Engl J Med* 1988;**319**: 902–7.
167. Copelon EA, Tutschka PJ. Immunoglobulin in bone marrow transplantation. In: Morell A, Nydegger UE, editors. *Clinical use of intravenous immunoglobulins*. New York: Academic Press; 1986. p. 117–21.
168. Copelovici Y, Strulovici D, Cristea AL, et al. Data on the efficiency of specific antimumps immunoglobulins in the prevention of mumps and of its complications. *Virologie* 1979;**30**: 171–7.
169. Cordonnier C, Chevret S, Legrand M, et al. Should immunoglobulin therapy be used in allogeneic stem-cell transplantation? A randomized, double-blind, dose effect, placebo-controlled, multicenter trial. *Ann Intern Med* 2003;**139**:8–18.
170. Costa RA, Jorge R, Calucci D, et al. Intravitreal bevacizumab for choroidal neovascularization caused by AMD (IBeNA Study): results of a phase 1 dose-escalation study. *Invest Ophthalmol Vis Sci* 2006;**47**:4569–78.
171. Cox RA, Rao P, Brandon-Cox C. The use of palivizumab monoclonal antibody to control an outbreak of respiratory syncytial virus infection in a special care baby unit. *J Hosp Infect* 2001;**48**: 186–92.
172. Craig RD. Immunotherapy for severe burns in children. *Plast Reconstr Surg* 1965;**35**:263–70.
173. Crennan JM, Van Scoy RE, McKenna CH, et al. Echovirus polymyositis in patients with hypogammaglobulinemia: failure of high-dose intravenous gammaglobulin therapy and review of the literature. *Am J Med* 1986;**81**:35–42.
174. Crum NF, Wallace MR. Group B streptococcal necrotizing fasciitis and toxic shock-like syndrome: a case report and review of the literature. *Scand J Infect Dis* 2003;**35**:878–81.
175. Cunningham-Rundles C, Zhou Z, Mankarious S, et al. Long-term use of IgA-depleted intravenous immunoglobulin in immunodeficient subjects with anti-IgA antibodies. *J Clin Immunol* 1993;**13**: 272–8.
176. Czer LS, Ruzza A, Vespignani R, et al. Prophylaxis of cytomegalovirus disease in mismatched patients after heart transplantation using combined antiviral and immunoglobulin therapy. *Transplant Proc* 2011;**43**:1887–92.
177. Darenberg J, Ihendyane N, Sjolin J, et al. Intravenous immunoglobulin G therapy in streptococcal toxic shock syndrome: a European randomized, double-blind, placebo-controlled trial. *Clin Infect Dis* 2003;**37**:333–40.
178. Davidson GP, Whyte PB, Daniels E, et al. Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. *Lancet* 1989;**2**:709–12.
179. Davis GL, Nelson DR, Terrault N, et al. A randomized, open-label study to evaluate the safety and pharmacokinetics of human hepatitis C immune globulin (Civacir) in liver transplant recipients. *Liver Transpl* 2005;**11**:941–9.
180. De Simone C, Delogu G, Corbetta G. Intravenous immunoglobulins in association with antibiotics: a therapeutic trial in septic intensive care unit patients. *Crit Care Med* 1988;**16**:23–6.
181. DeJonge M, Burchfield D, Bloom B, et al. *A phase III, randomized, double-blind, multi-center clinical trial comparing the safety and efficacy of Veronate versus placebo for the prevention of nosocomial staphylococcal sepsis in premature infants*. Poster, Pediatric Academic Societies; 2006.
182. Delone P, Corkill J, Jordan M, et al. Successful treatment of Epstein-Barr virus infection with ganciclovir and cytomegalovirus hyperimmune globulin following kidney transplantation. *Transplant Proc* 1995;**27**:58–9.
183. Dennert R, Velthuis S, Schalla S, et al. Intravenous immunoglobulin therapy for patients with idiopathic cardiomyopathy and

endomyocardial biopsy-proven high PVB19 viral load. *Antivir Ther* 2010;**15**:193–201.
184. Desrosiers MY, Kilty SJ. Treatment alternatives for chronic rhinosinusitis persisting after ESS: what to do when antibiotics, steroids and surgery fail. *Rhinology* 2008;**46**:3–14.
185. DeVincenzo JP, Hirsch RL, Fuentes RJ, et al. Respiratory syncytial virus immune globulin treatment of lower respiratory tract infection in pediatric patients undergoing bone marrow transplantation: a compassionate use experience. *Bone Marrow Transplant* 2000;**25**:161–5.
186. Diamond EF, Purugganan HB, Choi HJ. Effect of prophylactic gamma globulin administration on infection morbidity in premature infants. *IMJ Ill Med J* 1966;**130**:668–70.
187. Dolman CE, Iida H. Type E botulism: its epidemiology, prevention and specific treatment. *Can J Public Health* 1963;**54**: 293–308.
188. Donta ST, Peduzzi P, Cross AS, et al. Immunoprophylaxis against klebsiella and *Pseudomonas aeruginosa* infections. The Federal Hyperimmune Immunoglobulin Trial Study Group. *J Infect Dis* 1996;**174**:537–43.
189. Douzinas EE, Pitaridis MT, Louris G, et al. Prevention of infection in multiple trauma patients by high-dose intravenous immunoglobulins. *Crit Care Med* 2000;**28**:8–15.
190. Duhem C, Dicato MA, Ries F. Side-effects of intravenous immune globulins. *Clin Exp Immunol* 1994;**97**(Suppl. 1):79–83.
191. Dumpis U, Crook D, Oksi J. Tick-borne encephalitis. *Clin Infect Dis* 1999;**28**:882–90.
192. Duncan GG, Christian HA, et al. An evaluation of immune serum globulin as a prophylactic agent against homologous serum hepatitis. *Am J Med Sci* 1947;**213**:53–7.
193. Dwyer JM. Manipulating the immune system with immune globulin. *N Engl J Med* 1992;**326**:107–16.
194. Dwyer JM, Erlendsson K. Intraventricular gamma-globulin for the management of enterovirus encephalitis. *Pediatr Infect Dis J* 1988;**7**:30–3.
195. Dye JM, Herbert AS, Kuehne AI, et al. Postexposure antibody prophylaxis protects nonhuman primates from filovirus disease. *Proc Natl Acad Sci U S A* 2012;**109**:5034–9.
196. Ebina T. Prophylaxis of rotavirus gastroenteritis using immunoglobulin. *Arch Virol Suppl* 1996;**12**:217–23.
197. Ehrlich DP. *Dictionary of Scientific Biography* 1971;**4**:295–305.
198. Eibl MM, Wolf HM, Furnkranz H, et al. Prevention of necrotizing enterocolitis in low-birth-weight infants by IgA-IgG feeding. *N Engl J Med* 1988;**319**:1–7.
199. Eijkhout HW, van Der Meer JW, Kallenberg CG, et al. The effect of two different dosages of intravenous immunoglobulin on the incidence of recurrent infections in patients with primary hypogammaglobulinemia: a randomized, double-blind, multicenter crossover trial. *Ann Intern Med* 2001;**135**:165–74.
200. El-Nawawy A, El-Kinany H, Hamdy El-Sayed M, et al. Intravenous polyclonal immunoglobulin administration to sepsis syndrome patients: a prospective study in a pediatric intensive care unit. *J Trop Pediatr* 2005;**51**:271–8.
201. Ellis EF, Henney CS. Adverse reactions following administration of human gamma globulin. *J Allergy* 1969;**43**:45–54.
202. Enders JF. Chemical, clinical, and immunological studies on the products of human plasma fractionation. X. the concentrations of certain antibodies in globulin fractions derived from human blood plasma. *J Clin Invest* 1944;**23**:510–30.
203. Englund JA, Glezen WP. Passive immunization for the prevention of otitis media. *Vaccine* 2001;**19**:S116–21.
204. Enria DA, Maiztegui JI. Antiviral treatment of Argentine hemorrhagic fever. *Antiviral Res* 1994;**23**:23–31.
205. Enria DA, Briggiler AM, Sanchez Z. Treatment of Argentine hemorrhagic fever. *Antiviral Res* 2008;**78**:132–9.
206. Enserink M. Anthrax: 'borrowed immunity' may save future victims. *Science* 2002;**295**:777.
207. Erlendsson K, Swartz T, Dwyer JM. Successful reversal of echovirus encephalitis in X-linked hypogammaglobulinemia by intraventricular administration of immunoglobulin. *N Engl J Med* 1985;**312**:351–3.
208. Expert Committee on Rabies. Hyperimmune antirabies serum. *WHO Tech Rep Ser* 1950;**28**:23–5.
209. Faber HK, McIntosh R. *History of the American Pediatric Society 1887-1965*. New York: McGraw-Hill; 1966.
210. Fahy JV, Cockcroft DW, Boulet LP, et al. Effect of aerosolized anti-IgE (E25) on airway responses to inhaled allergen in asthmatic subjects. *Am J Respir Crit Care Med* 1999;**160**:1023–7.
211. Farcet MR, Planitzer CB, Stein O, et al. Hepatitis A virus antibodies in immunoglobulin preparations. *J Allergy Clin Immunol* 2010;**125**:198–202.
212. Fattet S, Cassinotti P, Popovic MB. Persistent human parvovirus B19 infection in children under maintenance chemotherapy for acute lymphocytic leukemia. *J Pediatr Hematol Oncol* 2004;**26**: 497–503.
213. Faye A, Quartier P, Reguerre Y, et al. Chimaeric anti-CD20 monoclonal antibody (rituximab) in post-transplant B-lymphoproliferative disorder following stem cell transplantation in children. *Br J Haematol* 2001;**115**:112–18.
214. Fedorovskaia EA, Mel'nik EA, Lyspak SA, et al. [Donor human antidiphtheritic immunoglobulin is an alternative to xenogeneic antidiphtheritic serum]. *Lik Sprava* 2005:74–6.
215. Feltes TF, Cabalka AK, Meissner HC, et al. Palivizumab prophylaxis reduces hospitalization due to respiratory syncytial virus in young children with hemodynamically significant congenital heart disease. *J Pediatr* 2003;**143**:532–40.
216. Feranchak AP, Tyson RW, Narkewicz MR, et al. Fulminant Epstein-Barr viral hepatitis: orthotopic liver transplantation and review of the literature. *Liver Transpl Surg* 1998;**4**:469–76.
217. Féray C, Gigou M, Samuel D, et al. Incidence of hepatitis C in patients receiving different preparations of hepatitis B immunoglobulins after liver transplantation. *Ann Intern Med* 1998;**128**: 810–16.
218. Ferdman RM, Church JA. Failure of intravenous immunoglobulin to prevent varicella-zoster infection. *Pediatr Infect Dis J* 2000;**19**: 1219–20.
219. Ferrara G, Zumla A, Maeurer M. Intravenous immunoglobulin (IVIg) for refractory and difficult-to-treat infections. *Am J Med* 2012;**125**:1036 e1031–8.
220. Fibiger J. Om serum behandling af difteri. *Hospitalstidende* 1898; **6**:337–9.
221. Fields KS, Petersen MJ, Chiao E, et al. Case reports: treatment of nevirapine-associated dress syndrome with intravenous immune globulin (IVIG). *J Drugs Dermatol* 2005;**4**:510–13.
222. Filipovich AH, Gross T, Jyonouchi H, et al. Immune-mediated hematologic and oncologic disorders, including Epstein-Barr virus infection. In: Stiehm ER, editor. *Immunologic disorders in infants and children*. 4th ed. Philadelphia: W. B. Saunders; 1996. p. 855–88.
223. Finkel KC, Haworth JC. Clinical trial to assess the effectiveness of gamma-globulin in acute infections in young children. *Pediatrics* 1960;**25**:798–806.
224. Fiore L, Dunn SJ, Ridolfi B, et al. Antigenicity, immunogenicity and passive protection induced by immunization of mice with baculovirus-expressed VP7 protein from rhesus rotavirus. *J Gen Virol* 1995;**76**(Pt 8):1981–8.
225. Firoz BF, Henning JS, Zarzabal LA, et al. Toxic epidermal necrolysis: five years of treatment experience from a burn unit. *J Am Acad Dermatol* 2012;**67**:630–5.
226. Fischer GW, Cieslak TJ, Wilson SR, et al. Opsonic antibodies to *Staphylococcus epidermidis:* in vitro and in vivo studies using human intravenous immune globulin. *J Infect Dis* 1994;**169**:324–9.
227. Floren CH, Chinenye S, Elfstrand L, et al. ColoPlus, a new product based on bovine colostrum, alleviates HIV-associated diarrhoea. *Scand J Gastroenterol* 2006;**41**:682–6.
228. Fuller H, Del Mar C. Immunoglobulin treatment for respiratory syncytial virus infection. *Cochrane Database Syst Rev* 2006;(4) CD004883.
229. Frost WH, Frobisher M Jr, van Volkenburgh VA, et al. *Diphtheria in Baltimore: a comparative study of morbidity, carrier prevalence and antitoxic immunity in 1921-24 and 1933-36*. New York: Commonwealth Fund; 1941.
230. Funkhouser WL. The use of serum gamma globulin antibodies to control chicken pox in a convalescent hospital for children. *J Pediatr* 1948;**32**:257–9.
231. Gamm H, Huber C, Chapel H, et al. Intravenous immune globulin in chronic lymphocytic leukaemia. *Clin Exp Immunol* 1994; **97**(Suppl. 1):17–20.
232. Garcia-Gallo CL, Gil PU, Laporta R, et al. Is gammaglobulin anti-CMV warranted in lung transplantation? *Transplant Proc* 2005;**37**:4043–5.

233. Garcia-Lara J, Foster SJ. Anti-*Staphylococcus aureus* immunotherapy: current status and prospects. *Curr Opin Pharmacol* 2009;**9**:552–7.
234. Gardulf A, Hammarstrom L, Smith CI. Home treatment of hypogammaglobulinaemia with subcutaneous gammaglobulin by rapid infusion. *Lancet* 1991;**338**:162–6.
235. Gardulf A, Nicolay U, Asensio O, et al. Rapid subcutaneous IgG replacement therapy is effective and safe in children and adults with primary immunodeficiencies: a prospective, multi-national study. *J Clin Immunol* 2006;**26**:177–85.
236. Gauduchon V, Cozon G, Vandenesch F, et al. Neutralization of *Staphylococcus aureus* Panton Valentine leukocidin by intravenous immunoglobulin in vitro. *J Infect Dis* 2004;**189**:346–53.
237. Gaur S, Kesarwala H, Gavai M, et al. Clinical immunology and infectious diseases. *Pediatr Clin North Am* 1994;**41**:745–82.
238. Geeta MG, Krishnakumar P, Mathews L. Intrathecal tetanus immunoglobulins in the management of tetanus. *Indian J Pediatr* 2007;**74**:43–5.
239. Gellis SS, Stokes JJ, Brother GM, et al. The use of human immune serum globulin (gamma globulin) in infectious (epidemic) hepatitis in the Mediterranean theater of operations. I. Studies on prophylaxis in two epidemics of infectious hepatitis. *J A M A* 1945;**128**:1062–3.
240. George MJ, Snydman DR, Werner BG, et al. Use of ganciclovir plus cytomegalovirus immune globulin to treat CMV pneumonia in orthotopic liver transplant recipients. The Boston Center for Liver Transplantation CMVIG-Study Group. *Transplant Proc* 1993;**25**:22–4.
241. Gershon AA. Measles virus (rubeola). In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 1801–9.
242. Gershon AA, Steinberg S, Brunell PA. Zoster immune globulin: a further assessment. *N Engl J Med* 1974;**290**:243–5.
243. Ghosh S, Champlin RE, Englund J, et al. Respiratory syncytial virus upper respiratory tract illnesses in adult blood and marrow transplant recipients: combination therapy with aerosolized ribavirin and intravenous immunoglobulin. *Bone Marrow Transplant* 2000;**25**:751–5.
244. Giang E, Dorner M, Prentoe JC, et al. Human broadly neutralizing antibodies to the envelope glycoprotein complex of hepatitis C virus. *Proc Natl Acad Sci U S A* 2012;**109**:6205–10.
245. Gigler A, Dorsch S, Hemauer A, et al. Generation of neutralizing human monoclonal antibodies against parvovirus B19 proteins. *J Virol* 1999;**73**:1974–9.
246. Gispen R, Lansberg HP, Nanning W. The effect of antivaccinia gamma globulin on smallpox vaccination in view of a proposed attempt to prevent postvaccinal encephalitis. *Antonie Van Leeuwenhoek* 1956;**22**:89–102.
247. Gisslen M, Fredman P, Fuchs D, et al. Temporarily controlled HIV-1 replication after intravenous immunoglobulin treatment of Guillain-Barré syndrome. *Scand J Infect Dis* 2005;**37**:877–81.
248. Giulino LB, Bussel JB, Neufeld EJ. Treatment with rituximab in benign and malignant hematologic disorders in children. *J Pediatr* 2007;**150**:338–44, 344 e331.
249. Giuly E, Velly L, Gouin F. [Strategies of management of necrotizing soft tissue infections]. *Ann Fr Anesth Reanim* 2006;**25**:978–81.
250. Givner LB. Monoclonal antibodies against respiratory syncytial virus. *Pediatr Infect Dis J* 1999;**18**:541–2.
251. Glinz W, Grob PJ, Nydegger UE, et al. Polyvalent immunoglobulins for prophylaxis of bacterial infections in patients following multiple trauma: a randomized, placebo-controlled study. *Intensive Care Med* 1985;**11**:288–94.
252. Global Alliance for Rabies Control. *Human monoclonal antibodies for PEP*. Available at: <http://www.rabiescontrol.net/news/news-archive/humanmonoclonals.html>.
253. Gold H, Chester PA. Studies on anthrax: clinical report of ten human cases. *J Lab Clin Med* 1935;**21**:134–52.
254. Gold WL, Boulton JE, Goldman C, et al. Management of varicella exposures in the neonatal intensive care unit. *Pediatr Infect Dis J* 1993;**12**:954–5.
255. Gooding AM, Bastian JF, Peterson BM, et al. Safety and efficacy of intravenous immunoglobulin prophylaxis in pediatric head trauma patients: a double-blind controlled trial. *J Crit Care* 1993;**8**:212–16.
256. Goraya J, Marks H, Khurana D, et al. Subacute sclerosing panencephalitis (SSPE) presenting as acute disseminated encephalomyelitis in a child. *J Child Neurol* 2009;**24**:899–903.
257. Goudsmit J, Marissen WE, Weldon WC, et al. Comparison of an anti-rabies human monoclonal antibody combination with human polyclonal anti-rabies immune globulin. *J Infect Dis* 2006;**193**:796–801.
258. Grady GF. Prevention of post transfusion hepatitis by gamma globulin: preliminary report—a cooperative study. *JAMA* 1970;**214**:140–2.
259. Grady GF, Lee VA. Hepatitis B immune globulin: prevention of hepatitis from accidental exposure among medical personnel. *N Engl J Med* 1975;**293**:1067–70.
260. Graham BS, Tang Y, Gruber WC. Topical immunoprophylaxis of respiratory syncytial virus (RSV)–challenged mice with RSV-specific immune globulin. *J Infect Dis* 1995;**171**:1468–74.
261. Graham-Pole J, Camitta B, Casper J, et al. Intravenous immunoglobulin may lessen all forms of infection in patients receiving allogeneic bone marrow transplantation for acute lymphoblastic leukemia: a pediatric oncology group study. *Bone Marrow Transplant* 1988;**3**:559–66.
262. Granström M, Olinder-Nielsen AM, Holmblad P, et al. Specific immunoglobulin for treatment of whooping cough. *Lancet* 1991;**338**:1230–3.
263. Grayston JT, Watten RH. Epidemic rubella in Taiwan, 1957-1958. III. Gamma globulin in the prevention of rubella. *N Engl J Med* 1959;**261**:1145–50.
264. Grazi GL, Mazziotti A, Sama C, et al. Liver transplantation in HBsAg-positive HBV-DNA–negative cirrhotics: immunoprophylaxis and long-term outcome. *Liver Transpl Surg* 1996;**2**:418–25.
265. Green M, Michaels MG, Katz BZ, et al. CMV-IVIG for prevention of Epstein Barr virus disease and posttransplant lymphoproliferative disease in pediatric liver transplant recipients. *Am J Transplant* 2006;**6**:1906–12.
266. Green M, Reyes J, Webber S, et al. The role of antiviral and immunoglobulin therapy in the prevention of Epstein-Barr virus infection and post-transplant lymphoproliferative disease following solid organ transplantation. *Transpl Infect Dis* 2001;**3**:97–103.
267. Green RH, Balsamo MR, Giles JP, et al. Studies of the natural history and prevention of rubella. *Am J Dis Child* 1965;**110**:348–65.
268. Greenberg M. Gamma globulin in pediatrics. *Med Clin North Am* 1947;**31**:602–8.
269. Greenberg M, Pellitteri O, Eisenstein DT. Measles encephalitis. I. Prophylactic effect of gamma globulin. *J Pediatr* 1955;**46**:642–7.
270. Grisaru-Soen G, Lau W, Arneson C, et al. Randomized controlled trial of short-term withdrawal of i.v. immunoglobulin therapy for selected children with human immunodeficiency virus infection. *Pediatr Int* 2007;**49**:972–7.
271. Groothuis JR, Simoes EA, Hemming VG. Respiratory syncytial virus (RSV) infection in preterm infants and the protective effects of RSV immune globulin (RSVIG). Respiratory Syncytial Virus Immune Globulin Study Group. *Pediatrics* 1995;**95**:463–7.
272. Groothuis JR, Simoes EA, Levin MJ, et al. Prophylactic administration of respiratory syncytial virus immune globulin to high-risk infants and young children. The Respiratory Syncytial Virus Immune Globulin Study Group. *N Engl J Med* 1993;**329**:1524–30.
273. Grossman EB, Stewart SG, Stokes J Jr. Post-transfusion hepatitis in battle casualties and a study of its prophylaxis by means of human immune serum globulin. *J Am Med Assoc* 1945;**129**:991–4.
274. Group IC, Brocklehurst P, Farrell B, et al. Treatment of neonatal sepsis with intravenous immune globulin. *N Engl J Med* 2011;**365**:1201–11.
275. Guarino A, Canani RB, Russo S, et al. Oral immunoglobulins for treatment of acute rotaviral gastroenteritis. *Pediatrics* 1994;**93**:12–16.
276. Guarino A, Guandalini S, Albano F, et al. Enteral immunoglobulins for treatment of protracted rotaviral diarrhea. *Pediatr Infect Dis J* 1991;**10**:612–14.
277. Guglielmo BJ, Wong-Beringer A, Linker CA. Immune globulin therapy in allogeneic bone marrow transplant: a critical review. *Bone Marrow Transplant* 1994;**13**:499–510.

278. Günther G, Haglund M. Tick-borne encephalopathies: epidemiology, diagnosis, treatment and prevention. *CNS Drugs* 2005;**19**: 1009–32.
279. Gupta M, Mahanty S, Bray M, et al. Passive transfer of antibodies protects immunocompetent and imunodeficient mice against lethal Ebola virus infection without complete inhibition of viral replication. *J Virol* 2001;**75**:4649–54.
280. Gupta PS, Kapoor R, Goyal S, et al. Intrathecal human tetanus immunoglobulin in early tetanus. *Lancet* 1980;**2**:439–40.
281. Gustafson R, Gardulf A, Granert C, et al. Prophylactic therapy for selective IgA deficiency. *Lancet* 1997;**350**:865.
282. Habel K. Seroprophylaxis in experimental rabies. *Public Health Rep* 1945;**60**:545–60.
283. Habel K, Koprowski H. Laboratory data supporting the clinical trial of anti-rabies serum in persons bitten by a rabid wolf. *Bull World Health Organ* 1955;**13**:773–9.
284. Halperin SA, Vaudry W, Boucher FD, et al. Is pertussis immune globulin efficacious for the treatment of hospitalized infants with pertussis? No answer yet. *Pediatr Infect Dis J* 2007;**26**: 79–81.
285. Ham JM, Shelden SL, Godkin RR, et al. Cytomegalovirus prophylaxis with ganciclovir, acyclovir, and CMV hyperimmune globulin in liver transplant patients receiving OKT3 induction. *Transplant Proc* 1995;**27**:31–3.
286. Hammarstrom L. Passive immunity against rotavirus in infants. *Acta Paediatr Suppl* 1999;**88**:127–32.
287. Hammon WM, Coriell LL, Ludwig EH, et al. Evaluation of Red Cross gamma globulin as a prophylactic agent for poliomyelitis. V. Reanalysis of results based on laboratory-confirmed cases. *J Am Med Assoc* 1954;**156**:21–7.
288. Hampson FG, Hancock SW, Primhak RA. Disseminated sepsis due to a Panton-Valentine leukocidin producing strain of community acquired meticillin resistant *Staphylococcus aureus* and use of intravenous immunoglobulin therapy. *Arch Dis Child* 2006; **91**:201.
289. Harrison CJ. The other effect of intravenously administered respiratory syncytial virus-enriched immune globulin for prophylaxis: less acute otitis media. *J Pediatr* 1996;**129**:193–6.
290. Hassett J, Meyers S, McFarland L, et al. Recurrent *Clostridium difficile* infection in a patient with selective IgG1 deficiency treated with intravenous immune globulin and *Saccharomyces boulardii*. *Clin Infect Dis* 1995;**20**:266–8.
291. Hatton O, Martinez OM, Esquivel CO. Emerging therapeutic strategies for Epstein-Barr virus+ post-transplant lymphoproliferative disorder. *Pediatr Transplant* 2012;**16**:220–9.
292. Havens WPJ, Paul JR. Prevention of infectious hepatitis with gamma globulin. *J A M A* 1945;**129**:270–1.
293. Heegaard ED, Hasle H, Skibsted L, et al. Congenital anemia caused by parvovirus B19 infection. *Pediatr Infect Dis J* 2000;**19**: 1216–18.
294. Heikkinen T, Ruohola A, Ruuskanen O, et al. Intranasally administered immunoglobulin for the prevention of rhinitis in children. *Pediatr Infect Dis J* 1998;**17**:367–72.
295. Hemachudha T, Mitrabhakdi E, Wilde H, et al. Additional reports of failure to respond to treatment after rabies exposure in Thailand. *Clin Infect Dis* 1999;**28**:143–4.
296. Hemachudha T, Sunsaneewitayakul B, Mitrabhakdi E, et al. Paralytic complications following intravenous rabies immune globulin treatment in a patient with furious rabies. *Int J Infect Dis* 2003;**7**:76–7.
297. Henin Y, Marechal V, Barre-Sinoussi F, et al. Inactivation and partition of human immunodeficiency virus during Kistler and Nitschmann fractionation of human blood plasma. *Vox Sang* 1988;**54**:78–83.
298. Herman J, Vandenberghe P, van den Heuvel I, et al. Successful treatment with rituximab of lymphoproliferative disorder in a child after cardiac transplantation. *J Heart Lung Transplant* 2002;**21**:1304–9.
299. Hermos CR, Yoong P, Pier GB. High levels of antibody to Panton-Valentine leukocidin are not associated with resistance to *Staphylococcus aureus*-associated skin and soft-tissue infection. *Clin Infect Dis* 2010;**51**:1138–46.
300. Herrero JIH, Beltrán RR, Sánchez AMM. Failure of intrathecal tetanus antitoxin in the treatment of tetanus neonatorum. *J Infect Dis* 1991;**164**:619–20.
301. Higuchi S, Awata H, Nunoi H, et al. A family of selective immunodeficiency with normal immunoglobulins: possible autosomal dominant inheritance. *Eur J Pediatr* 1994;**153**:328–32.
302. Hill J, Eyles JE, Elvin SJ, et al. Administration of antibody to the lung protects mice against pneumonic plague. *Infect Immun* 2006; **74**:3068–70.
303. Hilpert H, Brussow H, Mietens C, et al. Use of bovine milk concentrate containing antibody to rotavirus to treat rotavirus gastroenteritis in infants. *J Infect Dis* 1987;**156**:158–66.
304. Hlava J, Honl I. Serum Vaccinium und seine Wirkungen. *Wien Klin Rundschau* 1895;**9**:625–7.
305. Holland PV, Rubinson RM, Morrow AG, et al. Gamma-globulin in the prophylaxis of posttransfusion hepatitis. *JAMA* 1966;**196**: 471–4.
306. Hopkins RJ, Lane JM. Clinical efficacy of intramuscular vaccinia immune globulin: a literature review. *Clin Infect Dis* 2004;**39**: 819–26.
307. Hopkins RJ, Kramer WG, Blackwelder WC, et al. Safety and pharmacokinetic evaluation of intravenous vaccinia immune globulin in healthy volunteers. *Clin Infect Dis* 2004;**39**:759–66.
308. Hot A, Madoux MH, Viard JP, et al. Successful treatment of cytomegalovirus-associated hemophagocytic syndrome by intravenous immunoglobulins. *Am J Hematol* 2008;**83**:159–62.
309. Houser HB, Schalet N. Prevention of rubella with gamma globulin. *Clin Res* 1958;**6**:281–2.
310. Hsia DY, Lonsway M Jr, Gellis SS. Gamma globulin in the prevention of infectious hepatitis; studies on the use of small doses in family outbreaks. *N Engl J Med* 1954;**250**:417–19.
311. Hu G, Wang MJ, Miller MJ, et al. Ocular vaccinia following exposure to a smallpox vaccinee. *Am J Ophthalmol* 2004;**137**: 554–6.
312. Huang YC, Lin TY, Lin YJ, et al. Prophylaxis of intravenous immunoglobulin and acyclovir in perinatal varicella. *Eur J Pediatr* 2001;**160**:91–4.
313. Hull AK, Criscuolo CJ, Mett V, et al. Human-derived, plant-produced monoclonal antibody for the treatment of anthrax. *Vaccine* 2005;**23**:2082–6.
314. Ichimaru T, Ohara Y, Hojo M, et al. Treatment of severe pertussis by administration of specific gamma globulin with high titers antitoxin antibody. *Acta Paediatr* 1993;**82**:1076–8.
315. Imbach P, Perret B, Babington R, et al. Safety of intravenous immunoglobulin preparations. *Vox Sang* 1991;**61**:1–4.
316. Impact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. *Pediatrics* 1998;**102**:531–7.
317. Inoue S, Kinra NK, Mukkamala SR, et al. Parvovirus B-19 infection: aplastic crisis, erythema infectiosum and idiopathic thrombocytopenic purpura. *Pediatr Infect Dis J* 1991;**10**:251–3.
318. Intravenous Immunoglobulin Collaborative Study Group. Prophylactic intravenous administration of standard immune globulin as compared with core-lipopolysaccharide immune globulin in patients at high risk of postsurgical infection. *N Engl J Med* 1991;**327**:234–40.
319. Iriarte PV, Tangco A, Jagasia KH, et al. Effect of gamma globulin on modification of chickenpox in children with malignant disease. *Cancer* 1965;**18**:112–16.
320. Isacsohn M, Eidelman AI, Kaplan M, et al. Neonatal coxsackievirus group B infections: experience of a single department of neonatology. *Isr J Med Sci* 1994;**30**:371–4.
321. Ishizaka A, Sakiyama Y, Otsu M, et al. Successful intravenous immunoglobulin therapy for recurrent pneumococcal otitis media in young children. *Eur J Pediatr* 1994;**153**:174–8.
322. Isobe Y, Sugimoto K, Shiraki Y, et al. Successful high-titer immunoglobulin therapy for persistent parvovirus B19 infection in a lymphoma patient treated with rituximab-combined chemotherapy. *Am J Hematol* 2004;**77**:370–3.
323. Jacobson JM, Colman N, Ostrow NA, et al. Passive immunotherapy in the treatment of advanced human immunodeficiency virus infection. *J Infect Dis* 1993;**168**:298–305.
324. Jahrling PB, Geisbert TW, Geisbert JB, et al. Evaluation of immune globulin and recombinant interferon-a2b for treatment of experimental Ebola virus infections. *J Infect Dis* 1999;**179**:224–34.
325. Jahrling PB, Geisbert J, Swearengen JR, et al. Passive immunization of Ebola virus-infected cynomolgus monkeys with

immunoglobulin from hyperimmune horses. *Arch Virol Suppl* 1996;**11**:135–40.
326. Jaijaroensup W, Tantawichien T, Khawplod P, et al. Postexposure rabies vaccination in patients infected with human immunodeficiency virus. *Clin Infect Dis* 1999;**28**:913–14.
327. Jamart S, Denis O, Deplano A, et al. Methicillin-resistant *Staphylococcus aureus* toxic shock syndrome. *Emerg Infect Dis* 2005; **11**:636–7.
328. Jassal SV, Roscoe JM, Zaltzman JS, et al. Clinical practice guidelines: prevention of cytomegalovirus disease after renal transplantation. *J Am Soc Nephrol* 1998;**9**:1697–708.
329. Jenson HB, Pollock BH. Meta-analyses of the effectiveness of intravenous immune globulin for prevention and treatment of neonatal sepsis. *Pediatrics* 1997;**99**:E2.
330. Jenson HB, Pollock BH. The role of intravenous immunoglobulin for the prevention and treatment of neonatal sepsis. *Semin Perinatol* 1998;**22**:50–63.
331. Jernigan JA, Stephens DS, Ashford DA, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg Infect Dis* 2001;**7**:933–44.
332. Jhaveri R, Rosenfeld W, Salazar JD, et al. High titer multiple dose therapy with HBIG in newborn infants of HBsAg positive mothers. *J Pediatr* 1980;**97**:305–8.
333. Jodlowski TZ, Oehler R, Kam LW, et al. Emerging therapies in the treatment of *Clostridium difficile*-associated disease. *Ann Pharmacother* 2006;**40**:2164–9.
334. Johnston JM, Overall JC Jr. Intravenous immunoglobulin in disseminated neonatal echovirus 11 infection. *Pediatr Infect Dis J* 1989;**8**:254–6.
335. Jones RJ, Roe EA, Gupta JL. Controlled trial of *Pseudomonas* immunoglobulin and vaccine in burn patients. *Lancet* 1980;**2**: 1263–5.
336. Jordan SC, Tyan D, Stablein D, et al. Evaluation of intravenous immunoglobulin as an agent to lower allosensitization and improve transplantation in highly sensitized adult patients with end-stage renal disease: report of the NIH IG02 trial. *J Am Soc Nephrol* 2004;**15**:3256–62.
337. Jordan SC, Vo AA, Peng A, et al. Intravenous gammaglobulin (IVIG): a novel approach to improve transplant rates and outcomes in highly HLA-sensitized patients. *Am J Transplant* 2006;**6**: 459–66.
338. Jorgensen F, Andersson B, Hanson LA, et al. Gamma-globulin treatment of recurrent acute otitis media in children. *Pediatr Infect Dis J* 1990;**9**:389–94.
339. Juang P, Skledar SJ, Zgheib NK, et al. Clinical outcomes of intravenous immune globulin in severe *Clostridium difficile*-associated diarrhea. *Am J Infect Control* 2007;**35**:131–7.
340. Judelsohn RG, Meyers JD, Ellis RJ, et al. Efficacy of zoster immune globulin. *Pediatrics* 1974;**53**:476–80.
341. Just HM, Voge W, Metzger M, et al. Treatment of intensive care unit patients with severe nosocomial infections. In: Morell A, Nydegger UE, editors. *Clinical use of intravenous immunoglobulins*. New York: Academic Press; 1986. p. 346–52.
342. Kabura L, Ilibagiza D, Menten J, et al. Intrathecal vs. intramuscular administration of human antitetanus immunoglobulin or equine tetanus antitoxin in the treatment of tetanus: a meta-analysis. *Trop Med Int Health* 2006;**11**:1075–81.
343. Kadirova R, Kartoglu HU, Strebel PM. Clinical characteristics and management of 676 hospitalized diphtheria cases, Kyrgyz Republic, 1995. *J Infect Dis* 2000;**181**(Suppl. 1):S110–115.
344. Kainulainen L, Varpula M, Liippo K, et al. Pulmonary abnormalities in patients with primary hypogammaglobulinemia. *J Allergy Clin Immunol* 1999;**104**:1031–6.
345. Kaiser R. The clinical and epidemiological profile of tick-borne encephalitis in southern Germany 1994-98: a prospective study of 656 patients. *Brain* 1999;**122**(Pt 11):2067–78.
346. Kalm O, Prellner K, Christensen P. The effect of intravenous immunoglobulin treatment in recurrent acute otitis media. *Int J Pediatr Otorhinolaryngol* 1986;**11**:237–46.
347. Kanfer EJ, Abrahamson G, Taylor J, et al. Severe rotavirus-associated diarrhoea following bone marrow transplantation: treatment with oral immunoglobulin. *Bone Marrow Transplant* 1994;**14**:651–2.
348. Kaplan EL, Gerber MA. Group A, group C, and group G beta-hemolytic streptococcal infections. In: Feigin RD, Cherry JA, editors. *Textbook of pediatric infectious diseases*. 4th ed. Philadelphia: W. B. Saunders; 1998. p. 1106–16.
349. Kariyawasam HH, Gyi KM, Hodson ME, et al. Anaemia in lung transplant patient caused by parvovirus B19. *Thorax* 2000;**55**: 619–20.
350. Kassis C, Champlin RE, Hachem RY, et al. Detection and control of a nosocomial respiratory syncytial virus outbreak in a stem cell transplantation unit: the role of palivizumab. *Biol Blood Marrow Transplant* 2010;**16**:1265–71.
351. Kato E, Shindo S, Eto Y, et al. Administration of immune globulin associated with aseptic meningitis. *J A M A* 1988;**259**:3267–71.
352. Katz LH, Paul M, Guy DG, et al. Prevention of recurrent hepatitis B virus infection after liver transplantation: hepatitis B immunoglobulin, antiviral drugs, or both? Systematic review and meta-analysis. *Transpl Infect Dis* 2010;**12**:292–308.
353. Kaufman D. Veronate (Inhibitex). *Curr Opin Investig Drugs* 2006;**7**:172–9.
354. Kaul R, McGeer A, Norrby-Teglund A, et al. Intravenous immunoglobulin therapy for streptococcal toxic shock syndrome: a comparative observational study. The Canadian Streptococcal Study Group. *Clin Infect Dis* 1999;**28**:800–7.
355. Kavaliotis J, Loukou I, Trachana M, et al. Outbreak of varicella in a pediatric oncology unit. *Med Pediatr Oncol* 1998;**31**:166–9.
356. Keck ZY, Sung VM, Perkins S, et al. Human monoclonal antibody to hepatitis C virus E1 glycoprotein that blocks virus attachment and viral infectivity. *J Virol* 2004;**78**:7257–63.
357. Keck ZY, Xia J, Wang Y, et al. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. *PLoS Pathog* 2012;**8**:e1002653.
358. Keeton BR, Tulloh R, Marsh MJ, et al. Recommendation for the use of palivizumab as prophylaxis against respiratory syncytial virus in infants with congenital cardiac disease [author reply]. *Cardiol Young* 2004;**14**:467–9.
359. Kefalides NA, Arana JA, Bazan A, et al. Role of infection in mortality from severe burns: evaluation of plasma, gamma-globulin, albumin and saline-solution therapy in a group of Peruvian children. *N Engl J Med* 1962;**267**:317–23.
360. Kelly CP, Chetham S, Keates S, et al. Survival of anti-*Clostridium difficile* bovine immunoglobulin concentrate in the human gastrointestinal tract. *Antimicrob Agents Chemother* 1997;**41**:236–41.
361. Kelly CP, Pothoulakis C, Vavva F, et al. Anti-*Clostridium difficile* bovine immunoglobulin concentrate inhibits cytotoxicity and enterotoxicity of C. difficile toxins. *Antimicrob Agents Chemother* 1996;**40**:373–9.
362. Kelly J. Immunotherapy against antibiotic-resistant bacteria: the Russian experience with an antistaphylococcal hyperimmune plasmaand immunoglobulin. *Microbes Infect* 2000;**2**:1383–92.
363. Kempe CH. Studies smallpox and complications of smallpox vaccination. *Pediatrics* 1960;**26**:176–89.
364. Kempe CH, Berge TO, England B. Hyperimmune vaccinal gamma globulin; source, evaluation, and use in prophylaxis and therapy. *Pediatrics* 1956;**18**:177–88.
365. Kempe CH, Bowles C, Meiklejohn G, et al. The use of vaccinia hyperimmune gamma-globulin in the prophylaxis of smallpox. *Bull World Health Organ* 1961;**25**:41–8.
366. Kerr JR, Cunniffe VS, Kelleher P, et al. Successful intravenous immunoglobulin therapy in 3 cases of parvovirus B19-associated chronic fatigue syndrome. *Clin Infect Dis* 2003;**36**:e100–6.
367. Kesson AM, Ferguson JK, Rawlinson WD, et al. Progressive vaccinia treated with ribavirin and vaccinia immune globulin. *Clin Infect Dis* 1997;**25**:911–14.
368. Khanna N, Widmer AF, Decker M, et al. Respiratory syncytial virus infection in patients with hematological diseases: single-center study and review of the literature. *Clin Infect Dis* 2008;**46**: 402–12.
369. Kiehl MG, Stoll R, Broder M, et al. A controlled trial of intravenous immune globulin for the prevention of serious infections in adults with advanced human immunodeficiency virus infection. *Arch Intern Med* 1996;**156**:2545–50.
370. Kimura H, Minakami H, Harigaya A, et al. Treatment of neonatal infection caused by coxsackievirus B3. *J Perinatol* 1999;**19**: 388–90.
371. Kinney JS, McCray E, Kaplan JE, et al. Risk factors associated with echovirus 11' infection in a hospital nursery. *Pediatr Infect Dis* 1986;**5**:192–7.

372. Kluger G, Schottler A, Waldvogel K, et al. Tickborne encephalitis despite specific immunoglobulin prophylaxis. *Lancet* 1995;**346**: 1502.
373. Knodell RG, Conrad ME, Ginsberg AL, et al. Efficacy of prophylactic gamma-globulin in preventing non-A, non-B post-transfusion hepatitis. *Lancet* 1976;**1**:557–61.
374. Knudson GB. Treatment of anthrax in man: history and current concepts. *Mil Med* 1986;**151**:71–7.
375. Knutsen AP. Patients with Igg subclass and/or selective antibody deficiency to polysaccharide antigens: initiation of a controlled clinical trial of intravenous immune globulin. *J Allergy Clin Immunol* 1989;**84**:640–5; discussion 645–7.
376. Kobayashi RH, Kobayashi AD, Lee N, et al. Home self-administration of intravenous immunoglobulin therapy in children. *Pediatrics* 1990;**85**:705–9.
377. Koduri PR, Kumapley R, Valladares J, et al. Chronic pure red cell aplasia caused by parvovirus B19 in AIDS: use of intravenous immunoglobulin—a report of eight patients. *Am J Hematol* 1991; **61**:16–20.
378. Koenig MG, Drutz DJ, Mushlin AI, et al. Type B botulism in man. *Am J Med* 1967;**42**:208–19.
379. Koenig MG, Spickard A, Cardella MA, et al. Clinical and laboratory observations on type E botulism in man. *Medicine (Baltimore)* 1964;**43**:517–45.
380. Kondoh H, Kobayashi K, Sugio Y, et al. Successful treatment of echovirus meningoencephalitis in sex-linked agammaglobulinaemia by intrathecal and intravenous injection of high titre gammaglobulin. *Eur J Pediatr* 1987;**146**:610–12.
381. Korns RF. Prophylaxis of German measles with immune serum globulin. *J Infect Dis* 1952;**90**:183–9.
382. Krawczynski K, Alter MJ, Tankersley DL, et al. Effect of immune globulin on the prevention of experimental hepatitis C virus infection. *J Infect Dis* 1996;**173**:822–8.
383. Krediet TG, Beurskens FJ, van Dijk H, et al. Antibody responses and opsonic activity in sera of preterm neonates with coagulase-negative staphylococcal septicemia and the effect of the administration of fresh frozen plasma. *Pediatr Res* 1998;**43**:645–51.
384. Kruger RM, Paranjothi S, Storch GA, et al. Impact of prophylaxis with cytogam alone on the incidence of CMV viremia in CMV-seropositive lung transplant recipients. *J Heart Lung Transplant* 2003;**22**:754–63.
385. Krugman S. Effect of human immune serum globulin on infectivity of hepatitis A virus. *J Infect Dis* 1976;**134**:70–4.
386. Krugman S, Ward R. Rubella: demonstration of neutralizing antibody in gamma globulin and re-evaluation of the rubella problem. *N Engl J Med* 1958;**259**:16–19.
387. Krugman S, Giles JP. Viral hepatitis, type B (MS-2-strain): further observations on natural history and prevention. *N Engl J Med* 1973;**288**:755–60.
388. Krugman S, Giles JP, Hammond J. Viral hepatitis, type B (MS-2 strain) prevention with specific hepatitis B immune serum globulin. *JAMA* 1971;**218**:1665–70.
389. Krugman S, Giles JP, Jacobs AM, et al. Studies with live attenuated measles-virus vaccine: comparative clinical, antigenic, and prophylactic effects after inoculation with and without gammaglobulin. *Am J Dis Child* 1962;**103**:353–63.
390. Krugman S, Ward R, Giles JP, et al. Infectious hepatitis: studies on the effect of gamma globulin and on the incidence of inapparent infection. *JAMA* 1960;**174**:823–30.
391. Kudoyarova-Zubavichene NM, Sergeyev NN, Chepurnov AA, et al. Preparation and use of hyperimmune serum for prophylaxis and therapy of Ebola virus infections. *J Infect Dis* 1999;**179**: 218–23.
392. Kuhns WJ, Prince AM, Brotman B, et al. A clinical and laboratory evaluation of immune serum globulin from donors with a history of hepatitis: attempted prevention of post-transfusion hepatitis. *Am J Med Sci* 1976;**272**:255–61.
393. Kunz C. Tick-borne encephalitis vaccination and the Austrian experience. *Vaccine* 2003;**21**:S1/50–S51/55.
394. Kuritzkes DR, Jacobson J, Powderly WG, et al. Antiretroviral activity of the anti-CD4 monoclonal antibody TNX-355 in patients infected with HIV type 1. *J Infect Dis* 2004;**189**:286–91.
395. Kurtzberg J, Friedman HS, Kinney TR, et al. Management of human immunodeficiency virus-associated thrombocytopenia with intravenous gamma globulin. *Am J Pediatr Hematol Oncol* 1987;**9**:299–301.
396. Kurtzman G, Frickhofen N, Kimball J, et al. Pure red-cell aplasia of 10 years' duration due to persistent parvovirus B19 infection and its cure with immunoglobulin therapy. *N Engl J Med* 1989; **321**:519–23.
397. Kurz H, Herbich K, Janata O, et al. Experience with the use of palivizumab together with infection control measures to prevent respiratory syncytial virus outbreaks in neonatal intensive care units. *J Hosp Infect* 2008;**70**:246–52.
398. Kyne L, Warny M, Qamar A, et al. Asymptomatic carriage of *Clostridium difficile* and serum levels of IgG antibody against toxin A. *N Engl J Med* 2000;**342**:390–7.
399. Lacaze-Masmonteil T, Seidenberg J, Mitchell I, et al. Evaluation of the safety of palivizumab in the second season of exposure in young children at risk for severe respiratory syncytial virus infection. *Drug Saf* 2003;**26**:283–91.
400. Lahn M, Kanehiro A, Hahn YS, et al. Aerosolized anti-T-cell-receptor antibodies are effective against airway inflammation and hyperreactivity. *Int Arch Allergy Immunol* 2004;**134**:49–55.
401. Lamont RF, Sobel JD, Carrington D, et al. Varicella-zoster virus (chickenpox) infection in pregnancy. *BJOG* 2011;**118**:1155–62.
402. Lamothe F, D'Amico P, Ghosn P, et al. Clinical usefulness of intravenous human immunoglobulins in invasive group A streptococcal infections: case report and review. *Clin Infect Dis* 1995; **21**:1469–70.
403. Lea WA Jr, Taylor WB. Gamma globulin in the treatment of herpes zoster. *Tex State J Med* 1958;**54**:594–6.
404. Lee DC, Lederman HM. Anti-tetanus toxoid antibodies in intravenous gamma globulin: an alternative to tetanus immune globulin. *J Infect Dis* 1992;**166**:642–5.
405. Lee ML, Gale RP, Yap PL. Use of intravenous immunoglobulin to prevent or treat infections in persons with immune deficiency. *Annu Rev Med* 1997;**48**:93–102.
406. Lehner PS, Webster AD. Hepatitis C from immunoglobin infusions. *BMJ* 1993;**306**:1541–2.
407. Leung DY, Kelly CP, Boguniewicz M, et al. Treatment with intravenously administered gamma globulin of chronic relapsing colitis induced by *Clostridium difficile* toxin. *J Pediatr* 1991;**118**: 633–7.
408. Lever AM, Webster AD, Brown D, et al. Non-A, non-B hepatitis occurring in agammaglobulinaemic patients after intravenous immunoglobulin. *Lancet* 1984;**2**:1062–4.
409. Levy J, Youvan T, Lee ML. Passive hyperimmune plasma therapy in the treatment of acquired immunodeficiency syndrome: results of a 12-month multicenter double-blind controlled trial. The Passive Hyperimmune Therapy Study Group. *Blood* 1994;**84**: 2130–5.
410. Lewis DB, Tu W. The physiologic immunodeficiency of immaturity. In: Stiehm ER, Ochs HD, Winkelstein JA, editors. *Immunologic disorders in infants and children*. 5th ed. Philadelphia: Saunders; 2004. p. 687–760.
411. Lewis FM, Chernak E, Goldman E, et al. Ocular vaccinia infection in laboratory worker, Philadelphia, 2004. *Emerg Infect Dis* 2006;**12**:134–7.
412. Li A, Baba TW, Sodroski J, et al. Synergistic neutralization of a chimeric SIV/HIV type 1 virus with combinations of human anti-HIV type 1 envelope monoclonal antibodies or hyperimmune globulins. *AIDS Res Hum Retroviruses* 1997;**13**:647–56.
413. Li A, Katinger H, Posner MR, et al. Synergistic neutralization of simian-human immunodeficiency virus SHIV-vpu+ by triple and quadruple combinations of human monoclonal antibodies and high-titer anti-human immunodeficiency virus type 1 immunoglobulins. *J Virol* 1998;**72**:3235–40.
414. Lindamood KE, Fleck P, Narla A, et al. Neonatal enteroviral sepsis/meningoencephalitis and hemophagocytic lymphohistiocytosis: diagnostic challenges. *Am J Perinatol* 2011;**28**:337–46.
415. Linder N, Waintraub I, Smetana Z, et al. Placental transfer and decay of varicella-zoster virus antibodies in preterm infants. *J Pediatr* 2000;**137**:85–9.
416. Lipshultz SE, Orav EJ, Sanders SP, et al. Immunoglobulins and left ventricular structure and function in pediatric HIV infection. *Circulation* 1995;**92**:2220–5.
417. Loofbourow JC, Cabasso VJ, Roby RE, et al. Rabies immune globulin (human): clinical trials and dose determination. *JAMA* 1971;**217**:1825–31.
418. Losonsky GA, Johnson JP, Winkelstein JA, et al. Oral administration of human serum immunoglobulin in immunodeficient

patients with viral gastroenteritis: a pharmacokinetic and functional analysis. *J Clin Invest* 1985;**76**:2362–7.
419. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med* 1998; **339**:520–32.
420. Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against *Clostridium difficile* toxins. *N Engl J Med* 2010; **362**:197–205.
421. Lucas AO, Willis AJ, Mohamed SD, et al. A comparison of the value of 500,000 I.U. tetanus antitoxin (horse) with 200,000 I.U. in the treatment of tetanus. *Clin Pharmacol Ther* 1965;**6**:592–7.
422. Lucchesi PF, Bowman JE. Antitoxin versus no antitoxin in scarlet fever. *JAMA* 1930;**103**:1049–51.
423. Lucey D. Anthrax. In: Mandell GL, Douglas RG, Bennett JE, et al, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 6th ed. Philadelphia: Churchill Livingstone; 2005. p. 3618–24.
424. Lucey D. Bacillus anthracis (anthrax). In: Mandell GL, Douglas RG, Bennett JE, et al, editors. *Mandell, Douglas, and Bennett's principles and practice of infectious diseases*. 6th ed. Philadelphia: Churchill Livingstone; 2005. p. 2485–91.
425. Lundström R. Complications of small-pox vaccination and their treatment with vaccinia immune gamma globulin. *J Pediatr* 1956; **49**:129–40.
426. Lundström R, Thoren C, Blomquist B. Gamma globulin against rubella in pregnancy. I. Prevention of maternal rubella by gamma globulin and convalescent gamma globulin: a follow-up study. *Acta Paediatr* 1961;**50**:444–52.
427. Lundström R, Thoren C, Blomquist B. Gamma globulin against rubella in pregnancy. II. Manifest maternal rubella in early pregnancy treated with convalescent gamma globlin: a follow-up study. *Acta Paediatr* 1961;**50**:453–6.
428. Lyons JM, Davis C, Rieman MT, et al. Prophylactic intravenous immune globulin and polymixin B decrease the incidence of septic episodes and hospital length of stay in severely burned children. *J Burn Care Res* 2006;**27**:813–18.
429. Mabry R, Rani M, Geiger R, et al. Passive protection against anthrax by using a high-affinity antitoxin antibody fragment lacking an Fc region. *Infect Immun* 2005;**73**:8362–8.
430. Maidji E, Nigro G, Tabata T, et al. Antibody treatment promotes compensation for human cytomegalovirus-induced pathogenesis and a hypoxia-like condition in placentas with congenital infection. *Am J Pathol* 2010;**177**:1298–310.
431. Maiztegui JI, Fernandez NJ, de Damilano AJ. Efficacy of immune plasma in treatment of Argentine haemorrhagic fever and association between treatment and a late neurological syndrome. *Lancet* 1979;**2**:1216–17.
432. Maiztegui JI, McKee KT Jr, Barrera Oro JG, et al. Protective efficacy of a live attenuated vaccine against Argentine hemorrhagic fever. AHF Study Group. *J Infect Dis* 1998;**177**:277–83.
433. Malley R, DeVincenzo J, Ramilo O, et al. Reduction of respiratory syncytial virus (RSV) in tracheal aspirates in intubated infants by use of humanized monoclonal antibody to RSV F protein. *J Infect Dis* 1998;**178**:1555–61.
434. Mandy GT, Weisman LE, Horwith G, et al. Safety of a *Staphylococcus aureus* (SA) intravenous immune globulin (AltaStaph) in very-low-birth-weight (VLBW) infants. *Abstract Pediatr Res* 1999; **47**:343.
435. Marchand S, Tchernia G, Hiesse C, et al. Human parvovirus B19 infection in organ transplant recipients. *Clin Transplant* 1999;**13**: 17–24.
436. Mareschal-Desandes R, Andre JL, Lesesve JF, et al. Successful treatment of chronic parvovirus B19 infection by high-dose immunoglobulin. *Clin Nephrol* 2003;**59**:311–12.
437. Marin M, Guris D, Chaves SS, et al. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2007;**56**:1–40.
438. Maruyama T, Parren PW, Sanchez A, et al. Recombinant human monoclonal antibodies to Ebola virus. *J Infect Dis* 1999;**179**: 235–9.
439. Marzi A, Yoshida R, Miyamoto H, et al. Protective efficacy of neutralizing monoclonal antibodies in a nonhuman primate model of Ebola hemorrhagic fever. *PLoS One* 2012;**7**:e36192.
440. Masci S, De Simone C, Famularo G, et al. Intravenous immunoglobulins suppress the recurrences of genital herpes simplex virus: a clinical and immunological study. *Immunopharmacol Immunotoxicol* 1995;**17**:33–47.
441. Matheson DS, Clarkson TW, Gelfand EW. Mercury toxicity (acrodynia) induced by long-term injection of gammaglobulin. *J Pediatr* 1980;**97**:153–5.
442. Matsuda H, Kawakami Y, Furuya K, et al. Intrauterine therapy for a cytomegalovirus-infected symptomatic fetus. *BJOG* 2004;**111**: 756–7.
443. Matsuda H, Sakaguchi K, Shibasaki T, et al. Intrauterine therapy for parvovirus B19 infected symptomatic fetus using B19 IgG-rich high titer gammaglobulin. *J Perinat Med* 2005;**33**:561–3.
444. Maynard JA, Maassen CB, Leppla SH, et al. Protection against anthrax toxin by recombinant antibody fragments correlates with antigen affinity. *Nat Biotechnol* 2002;**20**:597–601.
445. McClain DJ. Smallpox. In: Sidell FR, Takafuji ET, Franz DR, editors. *Medical aspects of chemical and biological warfare*. Washington, D. C., Bethesda, Md: Borden Institute, Walter Reed Army Medical Center, Office of the Surgeon General, U. S. Army, U. S. Army Medical Department Center and School, U. S. Army Medical Research and Material Command, Uniformed Services University of the Health Sciences; 1997. p. 539–59.
446. McCloskey RV, Smilack J. Diphtheria antitoxin content of human immune serum globulins. *Ann Intern Med* 1972;**77**:757–8.
447. McComb JA, Dwyer RC. Passive-active immunization with tetanus immune globulin (human). *N Engl J Med* 1963;**268**: 857–62.
448. McCracken GH Jr, Dowell DL, Marshall FN. Double-blind trial of equine antitoxin and human immune globulin in tetanus neonatorum. *Lancet* 1971;**1**:1146–9.
449. McGhee SA, Kaska B, Liebhaber M, et al. Persistent parvovirus-associated chronic fatigue treated with high dose intravenous immunoglobulin. *Pediatr Infect Dis J* 2005;**24**:272–4.
450. McKhann CF, Green AA, Coady H. Factors influencing the effectiveness of placental extract in the prevention and modification of measles. *J Pediatr* 1935;**6**:603–14.
451. McKinney RE Jr, Katz SL, Wilfert CM. Chronic enteroviral meningoencephalitis in agammaglobulinemic patients. *Rev Infect Dis* 1987;**9**:334–56.
452. McNall RY, Head DR, Pui CH, et al. Parvovirus B19 infection in a child with acute lymphoblastic leukemia during induction therapy. *J Pediatr Hematol Oncol* 2001;**23**:309–11.
453. McPherson S, Rees CJ, Ellis R, et al. Intravenous immunoglobulin for the treatment of severe, refractory, and recurrent *Clostridium difficile* diarrhea. *Dis Colon Rectum* 2006;**49**:640–5.
454. Mease PJ, Ochs HD, Wedgwood RJ. Successful treatment of echovirus meningoencephalitis and myositis-fasciitis with intravenous immune globulin therapy in a patient with X-linked agammaglobulinemia. *N Engl J Med* 1981;**304**:1278–81.
455. Medical Research Council Working Party. Summary report: hypogammaglobulinemia in the United Kingdom. *Lancet* 1969;**1**: 163–9.
456. Mehta S, McGeer A, Low DE, et al. Morbidity and mortality of patients with invasive group A streptococcal infections admitted to the ICU. *Chest* 2006;**130**:1679–86.
457. Melamed I, Griffiths AM, Roifman CM. Benefit of oral immune globulin therapy in patients with immunodeficiency and chronic diarrhea. *J Pediatr* 1991;**119**:486–9.
458. Melish ME, Murata S, Fukunaga C, et al. Vaginal tampon model for toxic shock syndrome. *Rev Infect Dis* 1989;**11**:238–46, 219–28.
459. Mellouli F, Arrouji Z, Debre M, et al. [Successful treatment of Echovirus 27 meningoencephalitis in agammaglobulinaemia with intraventricular injection of gammaglobulin: a case report]. *Arch Pediatr* 2003;**10**:130–3.
460. Messori A, Rampazzo R, Scroccaro G, et al. Efficacy of hyperimmune anti-cytomegalovirus immunoglobulins for the prevention of cytomegalovirus infection in recipients of allogeneic bone marrow transplantation: a meta-analysis. *Bone Marrow Transplant* 1994;**13**:163–7.
461. Migone TS, Subramanian GM, Zhong J, et al. Raxibacumab for the treatment of inhalational anthrax. *N Engl J Med* 2009;**361**: 135–44.
462. Miranda-Filho Dde B, Ximenes RA, Barone AA, et al. Randomised controlled trial of tetanus treatment with antitetanus immunoglobulin by the intrathecal or intramuscular route. *BMJ* 2004;**328**:615.
463. Misbah SA, Spickett GP, Ryba PC, et al. Chronic enteroviral meningoencephalitis in agammaglobulinemia: case report and literature review. *J Clin Immunol* 1992;**12**:266–70.

464. Mitra AK, Mahalanabis D, Ashraf H, et al. Hyperimmune cow colostrum reduces diarrhoea due to rotavirus: a double-blind, controlled clinical trial. *Acta Paediatr* 1995;**84**:996–1001.
465. Mofenson LM, Moye JJ. Intravenous immune globulin for the prevention of infections in children with symptomatic human immunodeficiency virus infection. *Pediatr Res* 1992;**33**:80–9.
466. Mofenson LM, Bethel J, Moye J Jr, et al. Effect of intravenous immunoglobulin (IVIG) on CD4+ lymphocyte decline in HIV-infected children in a clinical trial of IVIG infection prophylaxis. The National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *J Acquir Immune Defic Syndr* 1993;**6**:1103–13.
467. Mofenson LM, Korelitz J, Pelton S, et al. Sinusitis in children infected with human immunodeficiency virus: clinical characteristics, risk factors, and prophylaxis. National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *Clin Infect Dis* 1995;**21**:1175–81.
468. Mofenson LM, Moye J Jr, Bethel J, et al. Prophylactic intravenous immunoglobulin in HIV-infected children with CD4+ counts of $0.20 \times 10(9)$/L or more: effect on viral, opportunistic, and bacterial infections. The National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *JAMA* 1992;**268**:483–8.
469. Mofenson LM, Moye J Jr, Korelitz J, et al. Crossover of placebo patients to intravenous immunoglobulin confirms efficacy for prophylaxis of bacterial infections and reduction of hospitalizations in human immunodeficiency virus-infected children. The National Institute of Child Health and Human Development Intravenous Immunoglobulin Clinical Trial Study Group. *Pediatr Infect Dis J* 1994;**13**:477–84.
470. Mohamed N, Clagett M, Li J, et al. A high-affinity monoclonal antibody to anthrax protective antigen passively protects rabbits before and after aerosolized *Bacillus anthracis* spore challenge. *Infect Immun* 2004;**73**:795–802.
471. Morris D, McDonald JC. Failure of hyperimmune gamma globulin to prevent whooping cough. *Arch Dis Child* 1957;**32**:236–9.
472. Mouthon L, Guillevin L, Tellier Z. Intravenous immunoglobulins in autoimmune- or parvovirus B19-mediated pure red-cell aplasia. *Autoimmun Rev* 2005;**4**:264–9.
473. Muller R, Gubernatis G, Farle M, et al. Liver transplantation in HBs antigen (HBsAg) carriers: Prevention of hepatitis B virus (HBV) recurrence by passive immunization. *J Hepatol* 1991;**13**:90–6.
474. Munster AM. Immunologic response of trauma and burns: an overview. *Am J Med* 1984;**76**:142–5.
475. Munster AM. Infections in burns. In: Morell A, Nydegger UE, editors. *Clinical use of intravenous immunoglobulins*. New York: Academic Press; 1986. p. 339–44.
476. Murphy TK, Storch EA, Lewin AB, et al. Clinical factors associated with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *J Pediatr* 2012;**160**:314–19.
477. Nagington J, Gandy G, Walker J, et al. Use of normal immunoglobulin in an echovirus 11 outbreak in a special-care baby unit. *Lancet* 1983;**2**:443–6.
478. Nakajima M, Sakuishi K, Fukuda S, et al. Recovery from adult measles encephalitis immediately after early immunomodulation. *Clin Infect Dis* 2008;**47**:148–9.
479. Nance FC, Hines JL, Fulton RE, et al. Treatment of experimental burn wound sepsis by postburn immunization with polyvalent *Pseudomonas* antigen. *Surgery* 1970;**68**:248–53.
480. Nanning W. Prophylactic effect of anti-vaccinial gammaglobulin against post-vaccinial encephalitis. *Bull World Health Organ* 1962;**27**:317–24.
481. Napolitano PG, Ryan MA, Grabenstein JD. Pregnancy discovered after smallpox vaccination: is vaccinia immune globulin appropriate? *Am J Obstet Gynecol* 2004;**191**:1863–7.
482. National Association of State Public Health Veterinarians, I. Compendium of animal rabies prevention and control, 2011. *MMWR Recomm Rep* 2011;**60**:1–17.
483. National Committee for the Evaluation of Centoxin. The French National Registry of HA-1A (Centoxin) in septic shock: a cohort study of 600 patients. *Arch Intern Med* 1994;**154**:2484–91.
484. National Institute of Child Health and Development Intravenous Immunoglobulin Study Group. Intravenous immune globulin for the prevention of bacterial infections in children with symptomatic human immunodeficiency virus infection. *N Engl J Med* 1991;**325**:73–80.
485. National Institutes of Health Consensus Development Conference. Diseases, doses, recommendations for intravenous immunoglobulin. HLB Newsletter. *Natl Inst Heart Lung Blood Dis* 1990;**6**:73–8.
486. Neff JM. Introduction to Poxviridae. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 1552.
487. Negishi H, Yamada H, Hirayama E, et al. Intraperitoneal administration of cytomegalovirus hyperimmunoglobulin to the cytomegalovirus-infected fetus. *J Perinatol* 1998;**18**:466–9.
488. Nguyen CT, Tran TT. Hepatitis vaccination and prophylaxis. *Clin Liver Dis* 2009;**13**:317–29.
489. Nigro G, Adler SP, La Torre R, et al. Passive immunization during pregnancy for congenital cytomegalovirus infection. *N Engl J Med* 2005;**353**:1350–62.
490. Nigro G, Adler SP, Parruti G, et al. Immunoglobulin therapy of fetal cytomegalovirus infection occurring in the first half of pregnancy: a case-control study of the outcome in children. *J Infect Dis* 2012;**205**:215–27.
491. Nigro G, La Torre R, Anceschi MM, et al. Hyperimmunoglobulin therapy for a twin fetus with cytomegalovirus infection and growth restriction. *Am J Obstet Gynecol* 1999;**180**:1222–6.
492. Norrby-Teglund A, Basma H, Andersson J, et al. Varying titers of neutralizing antibodies to streptococcal superantigens in different preparations of normal polyspecific immunoglobulin G: implications for therapeutic efficacy. *Clin Infect Dis* 1998;**26**:631–8.
493. Norrby-Teglund A, Muller MP, McGeer A, et al. Successful management of severe group A streptococcal soft tissue infections using an aggressive medical regimen including intravenous polyspecific immunoglobulin together with a conservative surgical approach. *Scand J Infect Dis* 2005;**37**:166–72.
494. Null D Jr, Pollara B, Dennehy PH, et al. Safety and immunogenicity of palivizumab (Synagis) administered for two seasons. *Pediatr Infect Dis J* 2005;**24**:1021–3.
495. Nydahl-Persson K, Petterson A, Fasth A. A prospective, double-blind, placebo-controlled trial of i.v. immunoglobulin and trimethoprim-sulfamethoxazole in children with recurrent respiratory tract infections. *Acta Paediatr* 1995;**84**:1007–9.
496. Ochs HD. Intravenous immunoglobulin in the treatment and prevention of acute infections in pediatric acquired immunodeficiency syndrome patients. *Pediatr Infect Dis J* 1987;**6**:509–11.
497. Reference deleted in proofs.
498. Ochs HD, Gupta S, Kiessling P, et al. Safety and efficacy of self-administered subcutaneous immunoglobulin in patients with primary immunodeficiency diseases. *J Clin Immunol* 2006;**26**:265–73.
499. Ochs HD, Stiehm ER, Winkelstein J. Antibody deficiencies. In: Stiehm ER, Ochs HD, Winkelstein JA, editors. *Immunologic disorders in infants and children*. 5th ed. Philadelphia: Saunders; 2004. p. 356–426.
500. Oettle H, Wilborn F, Schmidt CA, et al. Treatment with ganciclovir and Ig for acute Epstein-Barr virus infection after allogeneic bone marrow transplantation. *Blood* 1993;**82**:2257–8.
501. Ogawa M, Ueda S, Anzai N, et al. Toxic shock syndrome after staphylococcal pneumonia treated with intravenous immunoglobulin. *Vox Sang* 1995;**68**:59–60.
502. Ogilvie MM. Antiviral prophylaxis and treatment in chickenpox: a review prepared for the UK Advisory Group on Chickenpox on behalf of the British Society for the Study of Infection. *J Infect* 1998;**36**(Suppl. 1):31–8.
503. Ogilvie MM, Vathenen AS, Radford M, et al. Maternal antibody and respiratory syncytial virus infection in infancy. *J Med Virol* 1981;**7**:263–71.
504. Ohlsen K, Lorenz U. Immunotherapeutic strategies to combat staphylococcal infections. *Int J Med Microbiol* 2010;**300**:402–10.
505. Ohlsson A, Lacy JB. Intravenous immunoglobulin for preventing infection in preterm and/or low-birth-weight infants. *Cochrane Database Syst Rev* 2001;(1):CD000361.
506. Ohlsson A, Lacy JB. Intravenous immunoglobulin for suspected or subsequently proven infection in neonates. *Cochrane Database Syst Rev* 2004;(1)CD001239.

507. Ohta K, Shimizu M, Nakai A, et al. Rituximab therapy for Epstein-Barr virus-related chronic hepatitis following living donor kidney transplantation. *Am J Kidney Dis* 2006;**48**:986–9.
508. Okano M, Pirruccello SJ, Grierson HL, et al. Immunovirological studies of fatal infectious mononucleosis in a patient with X-linked lymphoproliferative syndrome treated with intravenous immunoglobulin and interferon-alpha. *Clin Immunol Immunopathol* 1990; **54**:410–18.
509. Olopoenia L, Young M, White D, et al. Intravenous immunoglobulin in symptomatic and asymptomatic children with perinatal HIV infection. *J Natl Med Assoc* 1997;**89**:543–7.
510. Onuzo OC. Re: recommendation for the use of palivizumab as prophylaxis against respiratory syncytial virus in infants with congenital cardiac disease. *Cardiol Young* 2004;**14**:466–7; author reply 467–9.
511. Orange JS, Hossny EM, Weiler CR, et al. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. *J Allergy Clin Immunol* 2006;**117**:525–53.
512. Ordman CW, Jennings CG, Janeway CA. Chemical, clinical, and immunological studies on the products of human plasma fractionation. Xii. The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prevention and attenuation of measles. *J Clin Invest* 1944;**23**: 541–9.
513. Orenstein WA, Heymann DL, Ellis RJ, et al. Prophylaxis of varicella in high-risk children: dose-response effect of zoster immune globulin. *J Pediatr* 1981;**98**:368–73.
514. Ottolini MG, Hemming VG, Piazza FM, et al. Topical immunoglobulin is an effective therapy for parainfluenza type 3 in a cotton rat model. *J Infect Dis* 1995;**172**:243–5.
515. Owsianka A, Tarr AW, Juttla VS, et al. Monoclonal antibody AP33 defines a broadly neutralizing epitope on the hepatitis C virus E2 envelope glycoprotein. *J Virol* 2005;**79**:11095–104.
516. Paar DP, Pollard RB. Immunotherapy of CMV infections. *Adv Exp Med Biol* 1996;**394**:145–51.
517. Pamidi S, Friedman K, Kampalath B, et al. Human parvovirus B19 infection presenting as persistent anemia in renal transplant recipients. *Transplantation* 2000;**69**:2666–9.
518. Pammi M, Haque KN. Oral immunoglobulin for the prevention of rotavirus infection in low birth weight infants. *Cochrane Database Syst Rev* 2011:CD003740.
519. Pancharoen C, Thisyakorn U, Tantawichien T, et al. Failure of pre- and postexposure rabies vaccinations in a child infected with HIV. *Scand J Infect Dis* 2001;**33**:390–1.
520. Pant N, Hultberg A, Zhao Y, et al. Lactobacilli expressing variable domain of llama heavy-chain antibody fragments (lactobodies) confer protection against rotavirus-induced diarrhea. *J Infect Dis* 2006;**194**:1580–8.
521. Pantophlet R, Burton DR. GP120: target for neutralizing HIV-1 antibodies. *Annu Rev Immunol* 2006;**24**:739–69.
522. Park WH, Freeman RGJ. The prophylactic use of measles convalescent serum. *JAMA* 1926;**87**:556–8.
523. Parren PW, Geisbert TW, Maruyama T, et al. Pre- and postexposure prophylaxis of Ebola virus infection in an animal model by passive transfer of a neutralizing human antibody. *J Virol* 2002; **76**:6408–12.
524. Parsonnet J. Nonmenstrual toxic shock syndrome: new insights into diagnosis, pathogenesis, and treatment. *Curr Clin Top Infect Dis* 1996;**16**:1–20.
525. Paryani SG, Arvin AM, Koropchak CM, et al. Comparison of varicella zoster antibody titers in patients given intravenous immune serum globulin or varicella zoster immune globulin. *J Pediatr* 1984;**105**:200–5.
526. Paryani SG, Arvin AM, Koropchak CM, et al. Varicella zoster antibody titers after the administration of intravenous immune serum globulin or varicella zoster immune globulin. *Am J Med* 1984;**76**:124–7.
527. Paschlau VA. Zur umstrittenen Wirksamkeit des Diphtherie Heilserums. *Dtsch Med Wchenschr* 1949;**74**:1569–73.
528. Pasic S, Jankovic B, Abinun M, et al. Intravenous immunoglobulin prophylaxis in an echovirus 6 and echovirus 4 nursery outbreak. *Pediatr Infect Dis J* 1997;**16**:718–20.
529. Patel JC, Mehta BC, Nanavati BH, et al. Role of serum therapy in tetanus. *Lancet* 1963;**1**:740–3.
530. Patel K, Rana R. Pedimune in recurrent respiratory infection and diarrhoea: the Indian experience. The Pride Study. *Indian J Pediatr* 2006;**73**:585–91.
531. Patel NB, Pendleton C, Quinones-Hinojosa A, et al. A unique experiment in neurological surgery: intracerebral injection of antitoxin for tetanus. *J Neurosurg* 2010;**112**:1318–21.
532. Paya CV. Role of immunoglobulins and new antivirals in treatment of cytomegalovirus infection. *Transplant Proc* 1995;**27**: 28–30.
533. Pedraz C, Carbonell-Estrany X, Figueras-Aloy J, et al. Effect of palivizumab prophylaxis in decreasing respiratory syncytial virus hospitalizations in premature infants. *Pediatr Infect Dis J* 2003; **22**:823–7.
534. Pepose JS, Margolis TP, LaRussa P, et al. Ocular complications of smallpox vaccination. *Am J Ophthalmol* 2003;**136**:342–53.
535. Perez CM, Kubak BM, Cryer HG, et al. Adjunctive treatment of streptococcal toxic shock syndrome using intravenous immunoglobulin: case report and review. *Am J Med* 1997;**102**:111–13.
536. Perlmutter SJ, Leitman SF, Garvey MA, et al. Therapeutic plasma exchange and intravenous immunoglobulin for obsessive-compulsive disorder and tic disorders in childhood. *Lancet* 1999; **354**:1153–8.
537. Peters CJ. Lymphocytic choriomeningitis virus, Lassa virus and the South American hemorrhagic fevers. In: Mandell GL, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 5th ed. Philadelphia: Churchill Livingstone; 2000. p. 1855–62.
538. Piazza M, Sagliocca L, Tosone G, et al. Sexual transmission of the hepatitis C virus and efficacy of prophylaxis with intramuscular immune serum globulin: a randomized controlled trial. *Arch Intern Med* 1997;**157**:1537–44.
539. Plotkin SA. Rabies. *Clin Infect Dis* 2000;**30**:4–12.
540. Poelstra KA, Barekzi NA, Slunt JB, et al. Surgical irrigation with pooled human immunoglobulin G to reduce post-operative spinal implant infection. *Tissue Eng* 2000;**6**:401–11.
541. Poli MA, Rivera VR, Pitt ML, et al. Aerosolized specific antibody protects mice from lung injury associated with aerosolized ricin exposure. *Toxicon* 1996;**34**:1037–44.
542. Pollack M. Antibody activity against *Pseudomonas aeruginosa* in immune globulins prepared for intravenous use in humans. *J Infect Dis* 1983;**147**:1090–8.
543. Preiksaitis JK, Brennan DC, Fishman J, et al. Canadian society of transplantation consensus workshop on cytomegalovirus management in solid organ transplantation final report. *Am J Transplant* 2005;**5**:218–27.
544. Press release, Nabi. Discontinuation of phase II clinical trial of HyperGAM+CF. Boca Raton, Fla, July 1996.
545. PREVENT Study Group. Reduction of respiratory syncytial virus hospitalization among premature infants and infants with bronchopulmonary dysplasia using respiratory syncytial virus immune globulin prophylaxis. *Pediatrics* 1997;**99**:93–9.
546. Prober CG, Sullender WM, Yasukawa LL, et al. Low risk of herpes simplex virus infections in neonates exposed to the virus at the time of vaginal delivery to mothers with recurrent genital herpes simplex virus infections. *N Engl J Med* 1987;**316**:240–4.
547. Quartier P, Foray S, Casanova JL, et al. Enteroviral meningoencephalitis in X-linked agammaglobulinemia: intensive immunoglobulin therapy and sequential viral detection in cerebrospinal fluid by polymerase chain reaction. *Pediatr Infect Dis J* 2000;**19**: 1106–8.
548. Rabkin CS, Telzak EE, Ho MS, et al. Outbreak of echovirus 11 infection in hospitalized neonates. *Pediatr Infect Dis J* 1988;**7**: 186–90.
549. Ramesh S, Brodsky L, Afshani E, et al. Open trial of intravenous immune serum globulin for chronic sinusitis in children. *Ann Allergy Asthma Immunol* 1997;**79**:119–24.
550. Ramisse F, Binder P, Szatanik M, et al. Passive and active immunotherapy for experimental pneumococcal pneumonia by polyvalent human immunoglobulin or F(ab′)2 fragments administered intranasally. *J Infect Dis* 1996;**173**:1123–8.
551. Ramisse F, Deramoudt FX, Szatanik M, et al. Effective prophylaxis of influenza A virus pneumonia in mice by topical passive immunotherapy with polyvalent human immunoglobulins or F(ab′)2 fragments. *Clin Exp Immunol* 1998;**111**:583–7.
552. Ramisse F, Szatanik M, Binder P, et al. Passive local immunotherapy of experimental staphylococcal pneumonia with human intravenous immunoglobulin. *J Infect Dis* 1993;**168**:1030–3.

553. Ranganathan K, Worley S, Michaels MG, et al. Cytomegalovirus immunoglobulin decreases the risk of cytomegalovirus infection but not disease after pediatric lung transplantation. *J Heart Lung Transplant* 2009;**28**:1050–6.
554. Rault R, Piraino B, Johnston JR, et al. Pulmonary and renal toxicity of intravenous immunoglobulin. *Clin Nephrol* 1991;**36**:83–6.
555. Razvi S, Schneider L, Jonas MM, et al. Outcome of intravenous immunoglobulin-transmitted hepatitis C virus infection in primary immunodeficiency. *Clin Immunol* 2001;**101**:284–8.
556. Redeker AG, Mosley JW, Gocke DJ, et al. Hepatitis B immune globulin as a prophylactic measure for spouses exposed to acute type B hepatitis. *N Engl J Med* 1975;**293**:1055–9.
557. Reed EC, Bowden RA, Dandliker PS, et al. Treatment of cytomegalovirus pneumonia with ganciclovir and intravenous cytomegalovirus immunoglobulin in patients with bone marrow transplants. *Ann Intern Med* 1988;**109**:783–8.
558. Reed WD, Eddleston AL, Cullens H, et al. Infusion of hepatitis-B antibody in antigen-positive active chronic hepatitis. *Lancet* 1973;**2**:1347–51.
559. Reesink HW, Reerink-Brongers EE, Lafeber-Schut BJ, et al. Prevention of chronic HBsAg carrier state in infants of HBsAg-positive mothers by hepatitis B immunoglobulin. *Lancet* 1979;**2**: 436–8.
560. Rentz AC, Libbey JE, Fujinami RS, et al. Investigation of treatment failure in neonatal echovirus 7 infection. *Pediatr Infect Dis J* 2006;**25**:259–62.
561. Rezaei N, Hedayat M, Aghamohammadi A, et al. Primary immunodeficiency diseases associated with increased susceptibility to viral infections and malignancies. *J Allergy Clin Immunol* 2011;**127**:1329–41, e1322; quiz 1342–23.
562. Rimensberger PC, Burek-Kozlowska A, Morell A, et al. Aerosolized immunoglobulin treatment of respiratory syncytial virus infection in infants. *Pediatr Infect Dis J* 1996;**15**:2009–16.
563. Robinson KA, Odelola OA, Saldanha IJ, et al. Palivizumab for prophylaxis against respiratory syncytial virus infection in children with cystic fibrosis. *Cochrane Database Syst Rev* 2012;(2): CD007743.
564. Rodarte JG, Williams BH. Treatment of herpes zoster and chickenpox with immune globulin. *Arch Dermatol* 1956;**73**:553–6.
565. Rodriguez A, Rello J, Neira J, et al. Effects of high-dose of intravenous immunoglobulin and antibiotics on survival for severe sepsis undergoing surgery. *Shock* 2005;**23**:298–304.
566. Rodriguez WJ, Gruber WC, Groothuis JR, et al. Respiratory syncytial virus immune globulin treatment of RSV lower respiratory tract infection in previously healthy children. *Pediatrics* 1997;**100**:937–42.
567. Rodriguez WJ, Gruber WC, Welliver RC, et al. Respiratory syncytial virus (RSV) immune globulin intravenous therapy for RSV lower respiratory tract infection in infants and young children at high risk for severe RSV infections. Respiratory Syncytial Virus Immune Globulin Study Group. *Pediatrics* 1997;**99**: 454–61.
568. Romero JR. Palivizumab prophylaxis of respiratory syncytial virus disease from 1998 to 2002: results from four years of palivizumab usage. *Pediatr Infect Dis J* 2003;**22**:S46–54.
569. Rosenfeld EA, Shulman ST, Corydon KE, et al. Comparative safety and efficacy of two immune globulin products in Kawasaki disease. *J Pediatr* 1995;**126**:1000–3.
570. Ross AH. Modification of chicken pox in family contacts by administration of gamma globulin. *N Engl J Med* 1962;**267**: 369–76.
571. Rubaltelli FF, Benini F, Sala M. Prevention of necrotizing enterocolitis in neonates at risk by oral administration of monomeric IgG. *Dev Pharmacol Ther* 1991;**17**:138–43.
572. Rubbo SD, Suri JC. Passive immunization against tetanus with human immune globulin. *Br Med J* 1962;**2**:79–81.
573. Rubinstein HM. Studies on human tetanus antitoxin. *Am J Hyg* 1962;**76**:276–92.
574. Rugolotto S, Padovani EM, Sanna A, et al. Intrauterine anemia due to parvovirus B19: successful treatment with intravenous immunoglobulins. *Haematologica* 1999;**84**:668–9.
575. Rupp ME, Holley HP Jr, Lutz J, et al. Phase II, randomized, multicenter, double-blind, placebo-controlled trial of a polyclonal anti-Staphylococcus aureus capsular polysaccharide immune globulin in treatment of *Staphylococcus aureus* bacteremia. *Antimicrob Agents Chemother* 2007;**51**:4249–54.
576. Rupprecht CE, Gibbons RV. Clinical practice. Prophylaxis against rabies. *N Engl J Med* 2004;**351**:2626–35.
577. Ruttmann E, Geltner C, Bucher B, et al. Combined CMV prophylaxis improves outcome and reduces the risk for bronchiolitis obliterans syndrome (BOS) after lung transplantation. *Transplantation* 2006;**81**:1415–20.
578. Ruutu T, Ljungman P, Brinch L, et al. No prevention of cytomegalovirus infection by anti-cytomegalovirus hyperimmune globulin in seronegative bone marrow transplant recipients. The Nordic BMT Group. *Bone Marrow Transplant* 1997;**19**:233–6.
579. Sabroe I, McHale J, Tait DR, et al. Treatment of adenoviral pneumonitis with intravenous ribavirin and immunoglobulin. *Thorax* 1995;**50**:1219–20.
580. Saez-Llorens X, Moreno MT, Ramilo O, et al. Safety and pharmacokinetics of palivizumab therapy in children hospitalized with respiratory syncytial virus infection. *Pediatr Infect Dis J* 2004;**23**: 707–12.
581. Salcedo J, Keates S, Pothoulakis C, et al. Intravenous immunoglobulin therapy for severe *Clostridium difficile* colitis. *Gut* 1997; **41**:366–70.
582. Samuel D, Bismuth A, Serres C, et al. HBV infection after liver transplantation in HBsAg positive patients: experience with long-term immunoprophylaxis. *Transplant Proc* 1991;**23**:1492–4.
583. Samuel D, Muller R, Alexander G, et al. Liver transplantation in European patients with the hepatitis B surface antigen. *N Engl J Med* 1993;**329**:1842–7.
584. Samuelsson A, Towers TL, Ravetch JV. Anti-inflammatory activity of IVIG mediated through the inhibitory Fc receptor. *Science* 2001;**291**:484–6.
585. Sanchez PJ. Immunoprophylaxis of respiratory syncytial virus disease. *Pediatr Infect Dis J* 2000;**19**:791–801; discussion 802–4.
586. Sanchez-Quijano A, Pineda JA, Lissen E, et al. Prevention of post-transfusion non-A, non-B hepatitis by non-specific immunoglobulin in heart surgery patients. *Lancet* 1988;**1**:1245–9.
587. Santosham M, Reid R, Ambrosino DM, et al. Prevention of *Haemophilus influenzae* type b infections in high-risk infants treated with bacterial polysaccharide immune globulin. *N Engl J Med* 1987;**317**:923–9.
588. Sarker SA, Casswall TH, Mahalanabis D, et al. Successful treatment of rotavirus diarrhea in children with immunoglobulin from immunized bovine colostrum. *Pediatr Infect Dis J* 1998;**17**: 1149–54.
589. Sato A, Hirano H, Miura H, et al. Intrauterine therapy with cytomegalovirus hyperimmunoglobulin for a fetus congenitally infected with cytomegalovirus. *J Obstet Gynaecol Res* 2007;**33**: 718–21.
590. Sato H, Sato Y. Protective activities in mice of monoclonal antibodies against pertussis toxin. *Infect Immun* 1990;**58**:3369–74.
591. Schaeffer M, Toomey JA. Failure of gamma globulin to prevent varicella. *J Pediatr* 1948;**33**:749–52.
592. Schepens B, Ibanez LI, De Baets S, et al. Nanobodies(R) specific for respiratory syncytial virus fusion protein protect against infection by inhibition of fusion. *J Infect Dis* 2011;**204**:1692–701.
593. Schiff RI. Transmission of viral infections through intravenous immune globulin. *N Engl J Med* 1994;**331**:1649–50.
594. Schiff RI, Sedlak D, Buckley RH. Rapid infusion of Sandoglobulin in patients with primary humoral immunodeficiency. *J Allergy Clin Immunol* 1991;**88**:61–7.
595. Schlievert PM. Use of intravenous immunoglobulin in the treatment of staphylococcal and streptococcal toxic shock syndromes and related illnesses. *J Allergy Clin Immunol* 2001;**108**:S107–110.
596. Schmidt RE, Hartlapp JH, Niese D, et al. Reduction of infection frequency by intravenous gammaglobulins during intensive induction therapy for small cell carcinoma of the lung. *Infection* 1984;**12**:167–70.
597. Schrage B, Duan G, Yang LP, et al. Different preparations of intravenous immunoglobulin vary in their efficacy to neutralize streptococcal superantigens: implications for treatment of streptococcal toxic shock syndrome. *Clin Infect Dis* 2006;**43**:743–6.
598. Schwarz TF, Roggendorf M, Hottentrager B, et al. Immunoglobulins in the prophylaxis of parvovirus B19 infection. *J Infect Dis* 1990;**162**:1214.
599. Seeff LB, Wright EC, Finkelstein JD, et al. Efficacy of hepatitis B immune serum globulin after accidental exposure. Preliminary report of the Veterans Administration Cooperative Study. *Lancet* 1975;**2**:939–41.

600. Sekul EA, Cupler EJ, Dalakas MC. Aseptic meningitis associated with high-dose intravenous immunoglobulin therapy: frequency and risk factors. *Ann Intern Med* 1994;**121**:259–62.
601. Selbing A, Josefsson A, Dahle LO, et al. Parvovirus B19 infection during pregnancy treated with high-dose intravenous gammaglobulin. *Lancet* 1995;**345**:660–1.
602. Seu P, Winston DJ, Holt CD, et al. Long-term ganciclovir prophylaxis for successful prevention of primary cytomegalovirus (CMV) disease in CMV-seronegative liver transplant recipients with CMV-seropositive donors. *Transplantation* 1997;**64**:1614–17.
603. Shah JN, Chemaly RF. Management of RSV infections in adult recipients of hematopoietic stem cell transplantation. *Blood* 2011; **117**:2755–63.
604. Shah PS, Kaufman DA. Antistaphylococcal immunoglobulins to prevent staphylococcal infection in very low birth weight infants. *Cochrane Database Syst Rev* 2009:CD006449.
605. Shah SS, Hall M, Srivastava R, et al. Intravenous immunoglobulin in children with streptococcal toxic shock syndrome. *Clin Infect Dis* 2009;**49**:1369–76.
606. Shamin M, Giacola GP, West K. The use of intravenous immunoglobulin (IVIG) to prevent infection in bronchopulmonary dysplasia: report of a pilot study. *J Perinatol* 1991;**40**:239–44.
607. Shands KN, Schmid GP, Dan BB, et al. Toxic-shock syndrome in menstruating women: association with tampon use and *Staphylococcus aureus* and clinical features in 52 cases. *N Engl J Med* 1980;**303**:1436–42.
608. Shapiro RL, Hatheway C, Swerdlow DL. Botulism in the United States: a clinical and epidemiologic review. *Ann Intern Med* 1998; **129**:221–8.
609. Sharma VR, Fleming DR, Slone SP. Pure red cell aplasia due to parvovirus B19 in a patient treated with rituximab. *Blood* 2000; **96**:1184–6.
610. Sharp JC, Fletcher WB. Experience of anti-vaccinia immunoglobulin in the United Kingdom. *Lancet* 1973;**1**:656–9.
611. Shi Z, Li X, Ma L, et al. Hepatitis B immunoglobulin injection in pregnancy to interrupt hepatitis B virus mother-to-child transmission-a meta-analysis. *Int J Infect Dis* 2010;**14**:e622–34.
612. Shield J, Melville C, Novelli V, et al. Bovine colostrum immunoglobulin concentrate for cryptosporidiosis in AIDS. *Arch Dis Child* 1993;**69**:451–3.
613. Shill M, Baynes RD, Miller SD. Fatal rabies encephalitis despite appropriate post-exposure prophylaxis: a case report. *N Engl J Med* 1987;**316**:1257–8.
614. Shimasaki N, Mori T, Shimada H, et al. Epstein-Barr virus-associated posttransplant lymphoproliferative disorder after a cord blood stem cell transplantation presenting with pulmonary nodules. *J Pediatr Hematol Oncol* 2004;**26**:124–7.
615. Shirani KZ, Vaughan GM, McManus AT, et al. Replacement therapy with modified immunoglobulin G in burn patients: preliminary kinetic studies. *Am J Med* 1984;**76**:175–80.
616. Shurin PA, Rehmus JM, Johnson CE, et al. Bacterial polysaccharide immune globulin for prophylaxis of acute otitis media in high-risk children. *J Pediatr* 1993;**123**:801–10.
617. Siadak MF, Kopecky K, Sullivan KM. Reduction in transplant-related complications in patients given intravenous immuno globulin after allogeneic marrow transplantation. *Clin Exp Immunol* 1994;**97**(Suppl. 1):53–7.
618. Siber GR, Werner BG, Halsey NA, et al. Interference of immune globulin with measles and rubella immunization. *J Pediatr* 1993; **122**:204–11.
619. Siegel FP, Oleske J. Management of the acquired immune deficiency syndrome: Is there a role for immune globulins? In: Morell A, Nydegger UE, editors. *Clinical use of intravenous immunoglobulins*. New York: Academic Press; 1986. p. 373–84.
620. Sikes RK. Human rabies immune globulin. *Public Health Rep* 1969;**84**:797–801.
621. Silk HJ, Ambrosino D, Geha RS. Effect of intravenous gammaglobulin therapy in IgG2 deficient and IgG2 sufficient children with recurrent infections and poor response to immunization with *Hemophilus influenzae* type b capsular polysaccharide antigen. *Ann Allergy* 1990;**64**:21–5.
622. Silva Cde A, Dias L, Baltieri SR, et al. Respiratory syncytial virus outbreak in neonatal intensive care unit: impact of infection control measures plus palivizumab use. *Antimicrob Resist Infect Control* 2012;**1**:16.
623. Silvestris F, Cafforio P, Dammacco F. Pathogenic anti-DNA idiotype-reactive IgG in intravenous immunoglobulin preparations. *Clin Exp Immunol* 1994;**97**:19–25.
624. Simoes EA, Groothuis JR, Tristram DA, et al. Respiratory syncytial virus-enriched globulin for the prevention of acute otitis media in high risk children. *J Pediatr* 1996;**129**:214–19.
625. Simoes EA, Sondheimer HM, Top FH Jr, et al. Respiratory syncytial virus immune globulin for prophylaxis against respiratory syncytial virus disease in infants and children with congenital heart disease. The Cardiac Study Group. *J Pediatr* 1998;**133**:492–9.
626. Simon N. Prevention of non-A, non-B hepatitis in haemodialysis patients by hepatitis B immunoglobulin. *Lancet* 1984;**2**:1047.
627. Singleton R, Dooley L, Bruden D, et al. Impact of palivizumab prophylaxis on respiratory syncytial virus hospitalizations in high risk Alaska Native infants. *Pediatr Infect Dis J* 2003;**22**:540–5.
628. Skavaril F, Gardi A. Differences among available immunoglobulin preparations for intravenous use. *Pediatr Infect Dis* 1988;**7**:43–8.
629. Smallwood LA, Tabor E, Finlayson JS, et al. Antibodies to hepatitis A virus in immune serum globulin. *J Med Virol* 1981;**7**: 21–7.
630. Smego RA Jr, Asperilla MO. Use of acyclovir for varicella pneumonia during pregnancy. *Obstet Gynecol* 1991;**78**:1112–16.
631. Snydman DR, Falagas ME, Avery R, et al. Use of combination cytomegalovirus immune globulin plus ganciclovir for prophylaxis in CMV-seronegative liver transplant recipients of a CMV-seropositive donor organ: a multicenter, open-label study. *Transplant Proc* 2001;**33**:2571–5.
632. Snydman DR, Werner BG, Heinze-Lacey B, et al. Use of cytomegalovirus immune globulin to prevent cytomegalovirus disease in renal-transplant recipients. *N Engl J Med* 1987;**317**:1049–54.
633. So BJ, Chae KM, Lee KK, et al. Pure red cell aplasia due to parvovirus B19 infection in a renal transplant patient: a case report. *Transplant Proc* 2000;**32**:1954–6.
634. Soltys K, Green M. Posttransplant lymphoproliferative disease. *Pediatr Infect Dis J* 2005;**24**:1107–8.
635. Song KW, Mollee P, Patterson B, et al. Pure red cell aplasia due to parvovirus following treatment with CHOP and rituximab for B-cell lymphoma. *Br J Haematol* 2002;**119**:125–7.
636. Sorensen RU, Kallick MD, Berger M. Home treatment of antibody-deficiency syndromes with intravenous immune globulin. *J Allergy Clin Immunol* 1987;**80**:810–15.
637. Souayah N, Karim H, Kamin SS, et al. Severe botulism after focal injection of botulinum toxin. *Neurology* 2006;**67**:1855–6.
638. Spector SA, Gelber RD, McGrath N, et al. A controlled trial of intravenous immune globulin for the prevention of serious bacterial infections in children receiving zidovudine for advanced human immunodeficiency virus infection. Pediatric AIDS Clinical Trials Group. *N Engl J Med* 1994;**331**:1181–7.
639. Sriskandan S, Ferguson M, Elliot V, et al. Human intravenous immunoglobulin for experimental streptococcal toxic shock: bacterial clearance and modulation of inflammation. *J Antimicrob Chemother* 2006;**58**:117–24.
640. Steen JA. Gamma globulin in preventing infections in prematures. *Arch Pediatr* 1960;**77**:291–4.
641. Steinberg GM. Wissenschaftliche Untersuchungen über das spezifische Infektionsagens der Blattern und die erzeugung kunstlicher Immunität gegen diese Krankheit. *Zentralbl Bakteriol* 1896; **19**:857–68.
642. Stemmler HJ, Schmitt M, Harbeck N, et al. Application of intrathecal trastuzumab (Herceptintrade mark) for treatment of meningeal carcinomatosis in HER2-overexpressing metastatic breast cancer. *Oncol Rep* 2006;**15**:1373–7.
643. Stern EJ, Uhde KB, Shadomy SV, et al. Conference report on public health and clinical guidelines for anthrax. *Emerg Infect Dis* 2008;**14**.
644. Stevens DA, Merigan TC. Zoster immune globulin prophylaxis of disseminated zoster in compromised hosts. A randomized trial. *Arch Intern Med* 1980;**140**:52–4.
645. Stiehm ER. Recent progress in the use of intravenous immunoglobulin. *Curr Probl Pediatr* 1992;**22**:335–48.
646. Stiehm ER. Human intravenous immunoglobulin in primary and secondary antibody deficiencies. *Pediatr Infect Dis J* 1997;**16**: 696–707.
647. Stiehm ER. Immune globulin therapy. In: Mintz PD, editor. *Transfusion therapy: clinical principles and practice*. 2nd ed. Bethesda, MD: AABB Press; 2005. p. 391–422.

648. Stiehm ER, Casillas AM, Finkelstein JZ, et al. Slow subcutaneous human intravenous immunoglobulin in the treatment of antibody immunodeficiency: use of an old method with a new product. *J Allergy Clin Immunol* 1998;**101**:848–9.
649. Stiehm ER, Fletcher CV, Mofenson LM, et al. Use of human immunodeficiency virus (HIV) human hyperimmune immunoglobulin in HIV type 1-infected children (Pediatric AIDS clinical trials group protocol 273). *J Infect Dis* 2000;**181**:548–54.
650. Stiehm ER, Lambert JS, Mofenson LM, et al. Efficacy of zidovudine and human immunodeficiency virus (HIV) hyperimmune immunoglobulin for reducing perinatal HIV transmission from HIV-infected women with advanced disease: results of Pediatric AIDS Clinical Trials Group protocol 185. *J Infect Dis* 1999;**179**: 567–75.
651. Stiehm ER, Mofenson L, Zolla-Pazner S, et al. Summary of the workshop on passive immunotherapy in the prevention and treatment of HIV infection. The Passive Antibody Workshop Participants. *Clin Immunol Immunopathol* 1995;**75**:84–93.
652. Stillerman M, Marks HH, Thalhimer W. Prophylaxis of measles with convalescent serum. *Am J Dis Child* 1944;**67**:1–14.
653. Stogner SW, King JW, Black-Payne C, et al. Ribavirin and intravenous immune globulin therapy for measles pneumonia in HIV infection. *South Med J* 1993;**86**:1415–18.
654. Stokes J, Maris EP, Gellis SS. Chemical, clinical, and immunological studies on the products of human plasma fractionation. Xi. The use of concentrated normal human serum gamma globulin (human immune serum globulin) in the prophylaxis and treatment of measles. *J Clin Invest* 1944;**23**:531–40.
655. Stokes J Jr, Farquhar JA, Drake ME, et al. Infectious hepatitis; length of protection by immune serum globulin (gamma globulin) during epidemics. *JAMA* 1951;**147**:714–19.
656. Stokes JJ, Neefe JR. The prevention and attenuation of infectious hepatitis by gamma globulin. *JAMA* 1945;**127**:144–5.
657. Stokes JJ, Hilleman MR, Weibel RE, et al. Efficacy of live, attenuated measles virus vaccine given with human immune globulin. *N Engl J Med* 1961;**265**:507–13.
658. Stone HH, Graber CD, Martin JD Jr, et al. Evaluation of gamma globulin for prophylaxis against burn sepsis. *Surgery* 1965;**58**: 810–14.
659. Storch GA. Humanized monoclonal antibody for prevention of respiratory syncytial virus infection. *Pediatrics* 1998;**102**: 648–51.
660. Subramanian GM, Cronin PW, Poley G, et al. A phase 1 study of PAmAb, a fully human monoclonal antibody against *Bacillus anthracis* protective antigen, in healthy volunteers. *Clin Infect Dis* 2005;**41**:12–20.
661. Sullivan KM. Immunoglobulin therapy in bone marrow transplantation. *Am J Med* 1987;**83**:34–45.
662. Sullivan KM. Intravenous immune globulin prophylaxis in recipients of a marrow transplant. *J Allergy Clin Immunol* 1989;**84**:632–8; discussion 638–9.
663. Sullivan KM, Kopecky KJ, Jocom J, et al. Immunomodulatory and antimicrobial efficacy of intravenous immunoglobulin in bone marrow transplantation. *N Engl J Med* 1990;**323**:705–12.
664. Suresh S, Schilling S, Dakin C, et al. Palivizumab prophylaxis of respiratory syncytial virus infection in high-risk infants: a note of caution. *J Paediatr Child Health* 2003;**39**:637.
665. Swedo SE, Leonard HL, Garvey M, et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *Am J Psychiatry* 1998;**155**:264–71.
666. Symmers D, Lewis K. The antitoxin treatment of erysipelas. *JAMA* 1932;**99**:1082–4.
667. Szmuness W, Prince AM, Goodman M, et al. Hepatitis B immune serum globulin in prevention of nonparenterally transmitted hepatitis B. *N Engl J Med* 1974;**290**:701–6.
668. Tacket CO, Shandera WX, Mann JM, et al. Equine antitoxin use and other factors that predict outcome in type A foodborne botulism. *Am J Med* 1984;**76**:794–8.
669. Tada H, Yanagida M, Mishina J, et al. Combined passive and active immunization for preventing perinatal transmission of hepatitis B virus carrier state. *Pediatrics* 1982;**70**:613–19.
670. Taguchi Y, Purtilo DT, Okano M. The effect of intravenous immunoglobulin and interferon-alpha on Epstein-Barr virus-induced lymphoproliferative disorder in a liver transplant recipient. *Transplantation* 1994;**57**:1813–15.
671. Takada A, Feldmann H, Stroeher U, et al. Identification of protective epitopes on Ebola virus glycoprotein at the single amino acid level by using recombinant vesicular stomatitis viruses. *J Virol* 2003;**77**:1069–74.
672. Takei S, Arora YK, Walker SM. Intravenous immunoglobulin contains specific antibodies inhibitory to activation of T cells by staphylococcal toxin superantigens [comment]. *J Clin Invest* 1993; **91**:602–7.
673. Tamai K, Koyama T, Saga T, et al. Posttransplant lymphoproliferative disorder in a lung transplant recipient. *J Thorac Imaging* 2005;**20**:280–3.
674. Tang ML, Kemp AS, Moaven LD. Parvovirus B19-associated red blood cell aplasia in combined immunodeficiency with normal immunoglobulins. *Pediatr Infect Dis J* 1994;**13**:539–42.
675. Tasman A, Lansberg HP. Problems concerning the prophylaxis, pathogenesis and therapy of diphtheria. *Bull World Health Organ* 1957;**16**:939–73.
676. Tasman A, Minkenhof JE, Vink HH, et al. Importance of intravenous injection of diphtheria antiserum. *Lancet* 1958;**1**:1299–304.
677. Tchervenkov JI, Tector AJ, Barkun JS, et al. Recurrence-free long-term survival after liver transplantation for hepatitis B using interferon-alpha pretransplant and hepatitis B immune globulin posttransplant. *Ann Surg* 1997;**226**:356–65; discussion 365–8.
678. Tebruegge M, Curtis N. Enterovirus infections in neonates. *Semin Fetal Neonatal Med* 2009;**14**:222–7.
679. Terrault NA, Vyas G. Hepatitis B immune globulin preparations and use in liver transplantation. *Clin Liver Dis* 2003;**7**:537–50.
680. Terrault NA, Zhou S, Combs C, et al. Prophylaxis in liver transplant recipients using a fixed dosing schedule of hepatitis B immunoglobulin. *Hepatology* 1996;**24**:1327–33.
681. Thisyakorn U, Pancharoen C, Ruxrungtham K, et al. Safety and immunogenicity of preexposure rabies vaccination in children infected with human immunodeficiency virus type 1. *Clin Infect Dis* 2000;**30**:218.
682. Thompson JA, Filloux FM, Van Orman CB, et al. Infant botulism in the age of botulism immune globulin. *Neurology* 2005;**64**: 2029–32.
683. Tokat O, Kelebek N, Turker G, et al. Intravenous immunoglobulin in adult varicella pneumonia complicated by acute respiratory distress syndrome. *J Int Med Res* 2001;**29**:252–5.
684. Tolluh RM, Marsh M, Blackburn M, et al. Working Group of the British Paediatric Cardiac Association: Recommendations for the use of palivizumab as prophylaxis against respiratory syncytial virus in infants with congenital cardiac disease. *Cardiol Young* 2005;**13**:420–3.
685. Torok TJ. Human parvovirus B19. In: Remington JS, Klein JO, editors. *Infectious diseases of the fetus and newborn infant*. 4th ed. Philadelphia: W. B. Saunders; 1995. p. 668–702.
686. Trappe R, Riess H, Anagnostopoulos I, et al. Efficiency of antiviral therapy plus IVIG in a case of primary EBV infection associated PTLD refractory to rituximab, chemotherapy, and antiviral therapy alone. *Ann Hematol* 2009;**88**:167–72.
687. Trimble GX. Attenuation of chickenpox with gamma globulin. *Can Med Assoc J* 1957;**77**:697–9.
688. Tsai MH, Huang YC, Yen MH, et al. Clinical responses of patients with Kawasaki disease to different brands of intravenous immunoglobulin. *J Pediatr* 2006;**148**:38–43.
689. Tseng-Ong L, Mitchell WG. Infant botulism: 20 years' experience at a single institution. *J Child Neurol* 2007;**22**:1333–7.
690. Turner RB, Kelsey DK. Passive immunization for prevention of rotavirus illness in healthy infants. *Pediatr Infect Dis J* 1993;**12**: 718–22.
691. Tutscha PJ. Diminishing morbidity and mortality of bone marrow transplantation. *Vox Sang* 1986;**51**:87–94.
692. Ueda Y, Marusawa H, Kaido T, et al. Efficacy and safety of prophylaxis with entecavir and hepatitis B immunoglobulin in preventing hepatitis B recurrence after living-donor liver transplantation. *Hepatol Res* 2012.
693. Underwood K, Rubin S, Deakers T, et al. Infant botulism: a 30-year experience spanning the introduction of botulism immune globulin intravenous in the intensive care unit at Childrens Hospital Los Angeles. *Pediatrics* 2007;**120**:e1380–1385.
694. U.S. Army Medical Research Institute of Infectious Diseases. *Smallpox: medical management of biological casualties handbook*. 4th ed. Fort Detrick, Md: The Institute; 2001.

695. Vakil BJ, Tulpule TH, Armitage P, et al. A comparison of the value of 200,000 I.U. tetanus antitoxin with 50,000 I.U. in the treatment of tetanus. *Clin Pharmacol Ther* 1963;**4**:182–7.
696. Vakil BJ, Tulpule TH, Armitage P, et al. A comparison of the value of 200,000 I. U. tetanus antitoxin (horse) with 20,000 I. U. in the treatment of tetanus. *Clin Pharmacol Ther* 1964;**5**:695–8.
697. Vakil BJ, Tulpule TH, Armitage P, et al. A comparison of the value of 200,000 I.U. of tetanus antitoxin (horse) with 10,000 I.U. in the treatment of tetanus. *Clin Pharmacol Ther* 1968;**9**:465–71.
698. Valantine HA, Luikart H, Doyle R, et al. Impact of cytomegalovirus hyperimmune globulin on outcome after cardiothoracic transplantation: a comparative study of combined prophylaxis with CMV hyperimmune globulin plus ganciclovir versus ganciclovir alone. *Transplantation* 2001;**72**:1647–52.
699. Valduss D, Murray DL, Karna P, et al. Use of intravenous immunoglobulin in twin neonates with disseminated coxsackie B1 infection. *Clin Pediatr (Phila)* 1993;**32**:561–3.
700. van der Vaart JM, Pant N, Wolvers D, et al. Reduction in morbidity of rotavirus induced diarrhoea in mice by yeast produced monovalent llama-derived antibody fragments. *Vaccine* 2006;**24**:4130–7.
701. van Esser JW, Niesters HG, van der Holt B, et al. Prevention of Epstein-Barr virus-lymphoproliferative disease by molecular monitoring and preemptive rituximab in high-risk patients after allogeneic stem cell transplantation. *Blood* 2002;**99**:4364–9.
702. van Immerzeel TD, van Gilst RM, Hartwig NG. Beneficial use of immunoglobulins in the treatment of Sydenham chorea. *Eur J Pediatr* 2010;**169**:1151–4.
703. Van Wijk F, Staecker H, Keithley E, et al. Local perfusion of the tumor necrosis factor alpha blocker infliximab to the inner ear improves autoimmune neurosensory hearing loss. *Audiol Neurootol* 2006;**11**:357–65.
704. Van Wye JE, Collins MS, Baylor M, et al. Pseudomonas hyperimmune globulin passive immunotherapy for pulmonary exacerbations in cystic fibrosis. *Pediatr Pulmonol* 1990;**9**:7–18.
705. Vera-Ramirez M, Charlet M, Parry GJ. Recurrent aseptic meningitis complicating intravenous immunoglobulin therapy for chronic inflammatory demyelinating polyradiculoneuropathy. *Neurology* 1992;**42**:1636–7.
706. Verlinde JD, Spaander J. [Neutralization of vaccine virus by gamma globulin]. *Ned Tijdschr Geneeskd* 1949;**93**:2958–62.
707. Viard I, Wehrli P, Bullani R, et al. Inhibition of toxic epidermal necrolysis by blockade of CD95 with human intravenous immunoglobulin. *Science* 1998;**282**:490–3.
708. Victor JC, Monto AS, Surdina TY, et al. Hepatitis A vaccine versus immune globulin for postexposure prophylaxis. *N Engl J Med* 2007;**357**:1685–94.
709. Viguier M, Guillevin L, Laroche L. Treatment of parvovirus B19-associated polyarteritis nodosa with intravenous immune globulin. *N Engl J Med* 2001;**344**:1481–2.
710. Visentin S, Manara R, Milanese L, et al. Early primary cytomegalovirus infection in pregnancy: maternal hyperimmunoglobulin therapy improves outcomes among infants at 1 year of age. *Clin Infect Dis* 2012;**55**:497–503.
711. Vitale L, Blanset D, Lowy I, et al. Prophylaxis and therapy of inhalational anthrax by a novel monoclonal antibody to protective antigen that mimics vaccine-induced immunity. *Infect Immun* 2006;**74**:5840–7.
712. Vitek CR. Diphtheria. *Curr Top Microbiol Immunol* 2006;**304**: 71–94.
713. Vittecoq D, Chevret S, Morand-Joubert L, et al. Passive immunotherapy in AIDS: a double-blind randomized study based on transfusions of plasma rich in anti-human immunodeficiency virus 1 antibodies vs. transfusions of seronegative plasma. *Proc Natl Acad Sci U S A* 1995;**92**:1195–9.
714. von Hedenström M, Heberle U, Theobald K. Vaccination against tick-borne encephalitis (TBE): influence of simultaneous application of TBE immunoglobulin on seroconversion and rate of adverse events. *Vaccine* 1995;**13**:759–62.
715. Vyas GN, Perkins HA, Fudenberg HH. Anaphylactoid transfusion reactions associated with anti-IgA. *Lancet* 1968;**2**:312–15.
716. Waisbren BA. The treatment of bacterial infections with the combination of antibiotics and gamma globulin. *Antibiot Chemother* 1957;**7**:322–32.
717. Waldvogel K, Bossart W, Huisman T, et al. Severe tick-borne encephalitis following passive immunization. *Eur J Pediatr* 1996; **155**:775–9.
718. Walsh JJ, Pesik N, Quinn CP, et al. A case of naturally acquired inhalation anthrax: clinical care and analyses of anti-protective antigen immunoglobulin G and lethal factor. *Clin Infect Dis* 2007;**44**:968–71.
719. Wang F, Ruther P, Jiang I, et al. Human monoclonal antibodies that neutralize anthrax toxin by inhibiting heptamer assembly. *Hum Antibodies* 2004;**13**:105–10.
720. Wang Y, Keck ZY, Saha A, et al. Affinity maturation to improve human monoclonal antibody neutralization potency and breadth against hepatitis C virus. *J Biol Chem* 2011;**286**:44218–33.
721. Ward R, Krugman S. Etiology, epidemiology and prevention of viral hepatitis. *Prog Med Virol* 1962;**4**:87–118.
722. Ward R, Krugman S, Giles JP, et al. Infectious hepatitis; studies of its natural history and prevention. *N Engl J Med* 1958; **258**:407–16.
723. Warny M, Fatimi A, Bostwick EF, et al. Bovine immunoglobulin concentrate-clostridium difficile retains *C difficile* toxin neutralising activity after passage through the human stomach and small intestine. *Gut* 1999;**44**:212–17.
724. Weeks JC, Tierney MR, Weinstein MC. Cost effectiveness of prophylactic intravenous immune globulin in chronic lymphocytic leukemia. *N Engl J Med* 1991;**325**:81–6.
725. Weill D, Lock BJ, Wewers DL, et al. Combination prophylaxis with ganciclovir and cytomegalovirus (CMV) immune globulin after lung transplantation: effective CMV prevention following daclizumab induction. *Am J Transplant* 2003;**3**:492–6.
726. Weiner C, Pan Q, Hurtig M, et al. Passive immunity against human pathogens using bovine antibodies. *Clin Exp Immunol* 1999;**116**:193–205.
727. Weintraub II. Treatment of herpes zoster with gamma globulin. *J Am Med Assoc* 1955;**157**:1611.
728. Weisman LE, Thackray HM, Garcia-Prats JA, et al. Phase 1/2 double-blind, placebo-controlled, dose escalation, safety, and pharmacokinetic study of pagibaximab (BSYX-A110), an anti-staphylococcal monoclonal antibody for the prevention of staphylococcal bloodstream infections, in very-low-birth-weight neonates. *Antimicrob Agents Chemother* 2009;**53**:2879–86.
729. Weisman LE, Thackray HM, Steinhorn RH, et al. A randomized study of a monoclonal antibody (pagibaximab) to prevent staphylococcal sepsis. *Pediatrics* 2011;**128**:271–9.
730. Werdan K. Supplemental immune globulins in sepsis. *Clin Chem Lab Med* 1999;**37**:341–9.
731. Wheeler AP, Bernard GR. Treating patients with severe sepsis. *N Engl J Med* 1999;**340**:207–14.
732. Whitley RJ. Neonatal herpes simplex virus infections: is there a role for immunoglobulin in disease prevention and therapy? *Pediatr Infect Dis J* 1994;**13**:432–8; discussion 438–9.
733. Wilcox MH. Treatment of *Clostridium difficile* infection. *J Antimicrob Chemother* 1998;**41**(Suppl. C):41–6.
734. Wilcox MH. Descriptive study of intravenous immunoglobulin for the treatment of recurrent *Clostridium difficile* diarrhoea. *J Antimicrob Chemother* 2004;**53**:882–4.
735. Wild MA, Xin H, Maruyama T, et al. Human antibodies from immunized donors are protective against anthrax toxin in vivo. *Nat Biotechnol* 2003;**21**:1305–6.
736. Wilde H, Sirikawin S, Sabcharoen A, et al. Failure of postexposure treatment of rabies in children. *Clin Infect Dis* 1996;**22**:228–32.
737. Willems B, Ede M, Marotta P, et al. Anti-HCV human immunoglobulins for the prevention of graft infection in HCV-related liver transplantation, a pilot study. *J Hepatol* 2002;**36**:S32.
738. Williams PE, Yap PL, Gillon J, et al. Transmission of non-A, non-B hepatitis by pH4-treated intravenous immunoglobulin. *Vox Sang* 1989;**57**:15–18.
739. Wilson E. Neurosurgical treatment for tetanus. *J Hist Neurosci* 1997;**6**:82–5.
740. Wilson JA, Hevey M, Bakken R, et al. Epitopes involved in antibody-mediated protection from Ebola virus. *Science* 2000; **287**:1664–6.
741. Wilson NW, Ochs HD, Peterson B, et al. Abnormal primary antibody responses in pediatric trauma patients. *J Pediatr* 1989; **115**:424–7.
742. Winnie GB, Cowan RG, Wade NA. Intravenous immune globulin treatment of pulmonary exacerbations in cystic fibrosis. *J Pediatr* 1989;**114**:309–14.
743. Winston DJ, Antin JH, Wolff SN, et al. A multicenter, randomized, double-blind comparison of different doses of intravenous

immunoglobulin for prevention of graft-versus-host disease and infection after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2001;**28**:187–96.
744. Winston DJ, Pollard RB, Ho WG, et al. Cytomegalovirus immune plasma in bone marrow transplant recipients. *Ann Intern Med* 1982;**97**:11–18.
745. Wittek R. Vaccinia immune globulin: current policies, preparedness, and product safety and efficacy. *Int J Infect Dis* 2006;**10**: 193–201.
746. Wittes JT, Kelly A, Plante KM. Meta-analysis of CMVIG studies for the prevention and treatment of CMV infection in transplant patients. *Transplant Proc* 1996;**28**:17–24.
747. Wong SN, Chu CJ, Wai CT, et al. Low risk of hepatitis B virus recurrence after withdrawal of long-term hepatitis B immunoglobulin in patients receiving maintenance nucleos(t)ide analogue therapy. *Liver Transpl* 2007;**13**:374–81.
748. Wong VC, Ip HMH, Reesink HW, et al. Prevention of the HBsAg carrier state in newborn infants of mothers who are chronic carriers of HBsAg and HBeAg by administration of hepatitis B vaccine and hepatitis B immunoglobulin. *Lancet* 1983;**1**: 921–6.
749. Woodruff RK, Grigg AP, Firkin FC, et al. Fatal thrombotic events during treatment of autoimmune thrombocytopenia with intravenous immunoglobulin in elderly patients. *Lancet* 1986;**2**: 217–18.
750. Working Group on Antiretroviral Therapy, National Pediatric HIV Resource Center. Antiretroviral therapy and medical management of the human immunodeficiency virus–infected child. *Pediatr Infect Dis J* 1993;**12**:513–22.
751. World Health Organization. WHO recommendations on rabies postexposure treatment and the correct technique. I. In: *Guide for rabies post-exposure treatment*. Geneva: World Health Organization; 1997. p. 1–10.
752. Wu S-Y, Bonaparte J, Pyati S. Palivizumab use in premature infants in the neonatal intensive care unit. *Pediatrics* 2004;**114**: 554–6.
753. Yanagisawa C, Hanaki H, Natae T, et al. Neutralization of staphylococcal exotoxins in vitro by human-origin intravenous immunoglobulin. *J Infect Chemother* 2007;**13**:368–72.
754. Yedibela S, Reck T, Niedobitek G, et al. Anti-CD20 monoclonal antibody treatment of Epstein-Barr virus-induced intrahepatic lymphoproliferative disorder following liver transplantation. *Transpl Int* 2003;**16**:197–201.
755. Young NS. Parvovirus infection and its treatment. *Clin Exp Immunol* 1996;**104**(Suppl. 1):26–30.
756. Yu MY, Bartosch B, Zhang P, et al. Neutralizing antibodies to hepatitis C virus (HCV) in immune globulins derived from anti-HCV-positive plasma. *Proc Natl Acad Sci U S A* 2004;**101**: 7705–10.
757. Yu Z, Lennon VA. Mechanism of intravenous immune globulin therapy in antibody-mediated autoimmune diseases. *N Engl J Med* 1999;**340**:227–8.
758. Zachoval R, Jilg W, Lorbeer B, et al. Passive/active immunization against hepatitis B. *J Infect Dis* 1984;**150**:112–17.
759. Zaia JA. Prevention and treatment of cytomegalovirus pneumonia in transplant recipients. *Clin Infect Dis* 1993;**17**:392–9.
760. Zaia JA, Levin MJ, Preblud SR, et al. Evaluation of varicella-zoster immune globulin: protection of immunosuppressed children after household exposure to varicella. *J Infect Dis* 1983;**147**: 737–43.
761. Zamora MR, Nicolls MR, Hodges TN, et al. Following universal prophylaxis with intravenous ganciclovir and cytomegalovirus immune globulin, valganciclovir is safe and effective for prevention of CMV infection following lung transplantation. *Am J Transplant* 2004;**4**:1635–42.
762. Zeitlin L, Pettitt J, Scully C, et al. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. *Proc Natl Acad Sci U S A* 2011;**108**:20690–4.
763. Zhang JL, Gou JJ, Zhang ZY, et al. Screening and evaluation of human single-chain fragment variable antibody against hepatitis B virus surface antigen. *Hepatobiliary Pancreat Dis Int* 2006;**5**: 237–41.
764. Ziegler EJ, Fisher CJ Jr, Sprung CL, et al. Treatment of gram-negative bacteremia and septic shock with HA-1A human monoclonal antibody against endotoxin: a randomized, double-blind, placebo-controlled trial. The HA-1A Sepsis Study Group. *N Engl J Med* 1991;**324**:429–36.
765. Ziegler EJ, McCutchan JA, Fierer J, et al. Treatment of gram-negative bacteremia and shock with human antiserum to a mutant *Escherichia coli*. *N Engl J Med* 1982;**307**:1225–30.
766. Ziegner UHM, Kobayashi RH, Cunningham-Rundles C, et al. Progressive neurodegeneration in patients with primary immunodeficiency disease on IVIG treatment. *J Clin Immunol* 2002;**102**: 12–18.
767. Zingher A. Convalescent whole blood, plasma, and serum in prophylaxis of measles. *JAMA* 1924;**82**:1180–7.

SECTION **XXIV**

OTHER PREVENTIVE CONSIDERATIONS

CHAPTER 246

Public Health Aspects of Infectious Disease Control

Kathleen H. Harriman • Laurene Mascola

PUBLIC HEALTH AND INFECTIOUS DISEASE CLINICIANS

Infectious disease physicians and other clinicians who provide care for patients with known or suspected infectious diseases play an essential role in infectious disease surveillance and are important public health partners. Systematic infectious disease surveillance is critical to inform disease prevention and control efforts and public health practitioners rely on clinicians to report cases of infectious disease.

In addition to the routine reporting of reportable infectious diseases, which is essential for public health surveillance, often the first notification to public health authorities of an unusual case or cluster of disease cases is made by an astute clinician. Only after public health authorities are alerted to an unusual case or cluster of disease cases via reporting by a clinician or by analysis of surveillance data may an investigation take place and any necessary control measures be implemented.

Frontline clinicians play a crucial role in recognizing patients with an unusual constellation of symptoms, symptoms of a disease of public health importance that is rarely seen in the United States, a severe presentation of what is typically a less severe infectious disease, or a severe illness with the hallmarks of infectious disease for which a diagnosis cannot be found. Such cases take on even more importance when the patient has a history of international travel, exposure to exotic animals, or other unusual exposures. When such cases are reported to public health, often they can be evaluated with diagnostic methods that may not be available commercially.

This chapter describes the mutually beneficial collaboration between public health and infectious disease clinicians and includes information on prophylaxis recommendations for some pediatric infectious diseases (Table 246-1).

PUBLIC HEALTH

> *"The success or failure of any government in the final analysis must be measured by the well-being of its citizens. Nothing can be more important to a state than its public health; the state's paramount concern should be the health of its people."*
>
> FRANKLIN DELANO ROOSEVELT

In 1988, the Institute of Medicine in its landmark report *The Future of Public Health*, defined public health as "what we as a society do collectively to assure the conditions in which people can be healthy."[116]

This definition of public health and its overarching core functions (i.e., assessment, policy development, and assurance), which were identified in that report and continue to be used, have led to further descriptions of the essential services of public health (Fig. 246-1).[43]

The scope of public health covers many human health areas, including infectious diseases, chronic diseases, vaccines and immunizations, travelers' health, emergency preparedness and response, environmental health, congenital defects, disabilities, genetics and genomics, health promotion, injury and violence, and workplace safety and health. This chapter will focus on the prevention and control of infectious diseases, although the same principles can be applied to many other health problems.

INFECTIOUS DISEASE SURVEILLANCE

One of the essential services of public health is monitoring the health of the population. Public health surveillance is one method used to monitor the incidence of infectious diseases. The Centers for Disease Control and Prevention (CDC) Working Group on Public Health Surveillance Systems defines public health surveillance as "the ongoing, systematic collection, analysis, interpretation, and dissemination of data about a health-related event for use in public health action to reduce morbidity and mortality and to improve health" and notes that outbreak identification requires "an increase in frequency of disease above the background occurrence of the disease."[40]

The Institute of Medicine's 1988 report highlighted the role and goals of surveillance by recognizing that one of the three core functions of public health—assessment—relies on public health surveillance to identify and describe problems, guide decisions about appropriate actions, and monitor progress.[116]

In 2011, the Council of State and Territorial Epidemiologists stated that the overarching goal of surveillance is "To provide actionable health information to public health staff, government leaders, and the public to guide public health policy and programs."[163]

Uses of infectious disease surveillance data include identifying outbreaks, informing and evaluating disease

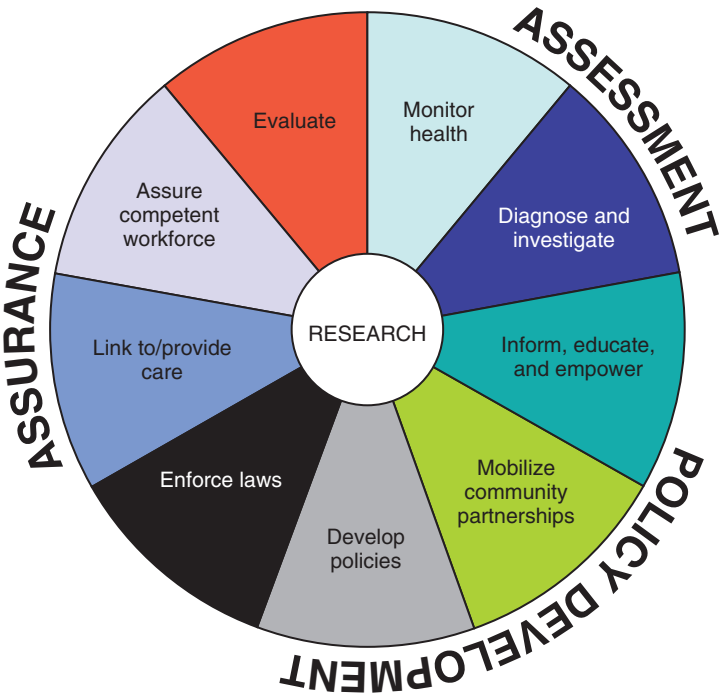

FIGURE 246-1 ■ Core functions of public health and how they relate to the 10 essential services of public health. (From Centers for Disease Control and Prevention. Core functions of public health and how they relate to the 10 essential services. (See companion Expert Consult site for color version.) Available at: http://www.cdc.gov/nceh/ehs/ephli/core_ess.htm.)

prevention and control efforts, determining vaccination strategies and assessing vaccine safety, monitoring antimicrobial resistance, monitoring health care–associated infections, and guiding allocation of resources for disease prevention, control, and treatment programs.

In the United States, the CDC, state, local, and territorial health departments carry out public health functions, including infectious disease surveillance. Public health authorities at the CDC, state, and territorial health departments collaborate in determining which diseases should be nationally notifiable to CDC. Additionally, reporting of specified infectious diseases is required by law in each state. Although health care providers and laboratories are typically designated reporters, state laws vary with regard to the persons who are responsible for reporting, the diseases or conditions that are reportable, and the circumstances under which reporting is to occur.[35,116] Specific information is generally available online or on request from state or local public health agencies.

Although public health is responsible for evaluating and taking action on infectious diseases identified via disease surveillance, unless a report of disease is initiated—by a health care provider, infection preventionist, or laboratory—no information is generated. Without baseline data on the incidence of infectious diseases, an increase or decrease in cases might not be recognized. Timely and accurate public health surveillance data can lead to earlier outbreak detection and more rapid implementation of prevention and control measures. In addition, the impact of intervention activities and other public health activities can be evaluated more accurately, leading to a more rational establishment of priorities.

However, the collection of data without analysis, interpretation, and dissemination of the data is of no value. Public health surveillance system data, obtained by both traditional and nontraditional means, such as syndromic surveillance, can be used for immediate public health action, program planning and evaluation, and formulating research hypotheses.[59,61] When used properly, surveillance data help in directing disease control activities and evaluating their impact.

Incentives to primary reporting sources to maintain good reporting, such as ongoing feedback, are essential for maintaining a useful surveillance system. After surveillance information is analyzed, the results are fed back to the primary reporting source and to others interested in the data. Such feedback serves to sustain interest in the reporting system and thereby maintains the quality of data obtained and continuously updates persons at each level of the surveillance system about the changing patterns of disease occurrence and new strategies for disease prevention and control.

One vehicle for the feedback of national surveillance information is the *Morbidity and Mortality Weekly Report*, which is published by CDC.[47] CDC also publishes periodic summaries of surveillance activities relating to specific diseases. In addition, regular summaries of surveillance activities are published by many state and local health departments as well as the World Health Organization (WHO).[172] Today, most public health agencies have publically accessible websites and most of these summaries are easily available online.

Increased use of automated reporting systems using electronic health records and laboratory results have streamlined the reporting process and improved the completeness and timeliness of surveillance data.[81] In addition, social media is increasingly playing a role in infectious disease surveillance. Public health practitioners use social media to monitor trends and collect data and the public also uses social media to monitor and report

Text continued on p. 3545

TABLE 246-1 **Prophylaxis Against Infectious Diseases in Children**

Disease	Target Group	Value of Prophylaxis	Recommended Drug for Postexposure Prophylaxis	Vaccine for Target Group for Preexposure Prophylaxis	References
Acute glomerulonephritis	Those with group A streptococcal infection in nephritis outbreaks	Proposed	Benzathine penicillin G IM, penicillin V PO, amoxicillin PO *For those allergic to penicillin:* Clindamycin PO, erythromycin PO, clarithromycin, PO azithromycin PO	No	7, 94, 154, 169, 170
Acute rheumatic fever (ARF)	Those with group A streptococcal infection other than impetigo	Shown	Benzathine penicillin G IM, penicillin V PO, amoxicillin PO *For those allergic to penicillin:* Clindamycin PO, erythromycin PO, clarithromycin PO, azithromycin PO The American Heart Association (AHA) no longer recommends prophylaxis for bacterial endocarditis for patients with rheumatic heart disease. For the few patients with rheumatic heart disease in whom prophylaxis is still recommended (e.g., people with prosthetic valves), current AHA recommendations should be followed using an agent other than a penicillin.	No	7, 155, 175
	Those with well-documented history of ARF (including cases that manifested only with Sydenham chorea) and those who have documented evidence of rheumatic heart disease	Shown	Benzathine penicillin G IM, Penicillin V PO, Sulfadiazine/sulfisoxazole PO *For those allergic to penicillin:* Erythromycin PO, azithromycin PO, continuous long-term prophylaxis should be initiated as soon as the diagnosis of ARF or rheumatic heart disease is made.		
Poststreptococcal–reactive arthritis (PSRA)	Those with previous PSRA	Proposed	Patients should be observed carefully for 1 to 2 years for carditis. Some experts recommend secondary prophylaxis for these patients during the observation period. If carditis occurs, the patient should be considered to have had ARF, and secondary ARF prophylaxis should be continued.	No	7, 167
Anthrax	Those exposed in an intentional release	Proposed	When organism susceptibility is unknown, ciprofloxacin PO and doxycycline PO are equivalent first-line antimicrobial agents. Levofloxacin PO is a second-line antimicrobial agent for people ≥6 months of age. When susceptibility to penicillin has been confirmed, amoxicillin PO should be used.	Yes (adults) Anthrax Vaccine Adsorbed is not licensed for use in pediatric populations and has not been studied in children.	3, 57, 58, 177

Chlamydia trachomatis	Infants born to infected women		Prophylactic antimicrobial treatment is not indicated, because the efficacy of such treatment is unknown. Infants should be monitored to ensure treatment if infection develops. If adequate follow-up cannot be ensured, some experts recommend that preemptive therapy be considered. Topical antibiotic therapy alone is inadequate.	No	4, 176
	Sexual contacts of confirmed cases	Shown	Azithromycin PO, doxycycline PO *Alternatives:* Erythromycin PO, erythromycin ethylsuccinate PO, ofloxacin PO, levofloxacin PO		
Cholera	Close contacts	Rarely indicated	Tetracycline PO, doxycycline PO, trimethoprim-sulfamethoxazole PO, furazolidone PO, erythromycin PO	Yes	108, 174
Diphtheria	Close contacts	Shown	Erythromycin PO, Benzathine, penicillin G IM	Yes	5, 56, 83, 93, 141
Gonorrhea	Infants born to infected women	Shown	Erythromycin ophthalmic ointment *and* ceftriaxone IV or IM	No	6
	Sexual contacts of confirmed cases	Shown	Combination therapy with ceftriaxone IM and either azithromycin PO as a single dose or doxycycline PO twice daily for 7 days		68, 86, 176
Haemophilus influenzae type b	Index case Household contacts when at least 1 contact <48 months of age is partially or unimmunized or is immunocompromised child of any age or vaccination status Childcare and preschool contacts when ≥2 cases have occurred <60 days when partially or unimmunized children are present	Shown	Cefotaxime or ceftriaxone for treatment and eradication of carriage (index case). If other antimicrobial used for treatment, rifampin should be used to eradicate carriage. Rifampin (contacts)	Yes	1, 9, 133
Hepatitis A infection	Close contacts	Shown	Hepatitis A vaccine immunoglobulin IM	Yes	10, 100
Hepatitis B (HBV) infection	Infants born to infected women Those who have been exposed to HBV-containing blood or other potentially infectious materials	Shown	Hepatitis B vaccine, hepatitis B immunoglobulin IM	Yes	11, 140

Continued on following page

TABLE 246-1 **Prophylaxis Against Infectious Diseases in Children** (Continued)

Disease	Target Group	Value of Prophylaxis	Recommended Drug for Postexposure Prophylaxis	Vaccine for Target Group for Preexposure Prophylaxis	References
Human Immunodeficiency Virus (HIV) infection	Infants born to infected women Those who have been exposed to HIV-containing blood or other potentially infectious materials	Shown	Consult a specialist or contact the National HIV/AIDS Clinician's Perinatal HIV Hotline at: 888-448-8765 Consult a specialist or contact the National HIV/AIDS Clinician's Post-Exposure Prophylaxis Hotline at: 888-448-4911	No	12, 145, 162
Influenza	Those at high risk for influenza complications and institutional residents	Shown	Oseltamivir PO, zanamivir PO	Yes	13, 99
Leprosy	Household contacts (not in United States)			Yes (bacilli Calmette-Guérin) BCG vaccine and heat killed leprosy vaccine Neither vaccine is recommended for household contacts of people with leprosy in the United States	14, 156
Malaria	Travelers to malarious areas	Shown	Specifics are highly detailed. Consult CDC at http://www.cdc.gov/malaria/ or the CDC Malaria Hotline at 770-488-7788)	No	15, 31, 134, 142
Measles	Susceptible close contacts	Shown	MMR vaccine immunoglobulin IM	Yes	16, 87, 95, 122, 160, 164
Meningococcal disease	Close contacts, index case	Shown	Rifampin* PO, ceftriaxone IM Ciprofloxacin* PO, azithromycin PO If ceftriaxone or cefotaxime not used for treatment, should receive prophylaxis as earlier to eradicate carriage. *Not recommended for pregnant women.	Yes for serogroups A, C, Y, and W135 in the United States	17, 34, 70, 84, 110, 112, 179
Methicillin-resistant *Staphylococcus aureus* infection	Those with recurrent skin or soft tissue infections despite optimizing wound care and hygiene measures Close contacts in settings with ongoing transmission despite optimizing wound care and hygiene measures	Proposed	Mupirocin (topical) with skin antiseptic solution (e.g., chlorhexidine) or dilute bleach baths. An oral antimicrobial to which the strain is susceptible may be used in combination with rifampin if infections recur despite use of the measures described earlier.		23, 130

Ophthalmia neonatorum (chlamydia and gonorrhea)			Three agents are licensed for neonatal ocular prophylaxis in the United States: 1% silver nitrate solution, 0.5% erythromycin ointment, and 1% tetracycline ophthalmic ointment or suspension. However, only 0.5% erythromycin ointment is available commercially in the United States. Although all 3 agents are effective against gonococcus, none prevents transmission of *C. trachomatis* from mother to infant.		20, 176
Pertussis	Household and other close contacts, child care contacts, irrespective of vaccination status Some states recommend prioritizing prophylaxis to high risk close contacts, i.e., infants <1 yr of age, pregnant women, and those with infant contact	Shown	Azithromycin PO, Erythromycin PO, Clarithromycin PO, Trimethoprim-sulfamethoxazole PO	Yes	2, 18, 56, 83, 105, 107
Plague	Those exposed to a known or suspected plague source, such as *Yersinia pestis*–infected fleas or infectious tissues Close contacts of pneumonic cases People exposed via an intentional release	Proposed	Doxycycline PO, ciprofloxacin PO *For children <8 years of age:* Tetracycline PO, chloramphenicol PO, and trimethoprim-sulfamethoxazole PO are also alternative drugs. Ciprofloxacin PO, doxycycline PO, amoxicillin PO	Not in United States	19, 115
Rabies	Those potentially exposed to rabies virus via a break in the skin caused by the teeth of a rabid animal or by contamination of scratches, abrasions, or mucous membranes with saliva or other potentially infectious material, such as neural tissue, from a rabid animal		Rabies vaccine, Rabies immunoglobulin IM	Yes	21, 136, 157
Streptococcal infection, invasive Group A disease	Household contacts		A clearly effective regimen has not been identified to justify routine chemoprophylaxis of all household contacts. Clinicians may choose to offer targeted prophylaxis to household contacts who are ≥65 years of age or who are members of other high-risk populations (e.g., people with HIV infection, varicella, or diabetes mellitus).		7, 166

Continued on following page

TABLE 246-1 **Prophylaxis Against Infectious Diseases in Children** (Continued)

Disease	Target Group	Value of Prophylaxis	Recommended Drug for Postexposure Prophylaxis	Vaccine for Target Group for Preexposure Prophylaxis	References
Streptococcal infection, group B (GBS)	Women with group B streptococci isolated from urine during current pregnancy or who have had a previous infant with GBS disease* If GBS status is not known at onset of labor or rupture of membranes, intrapartum prophylaxis should be administered to all women with gestation <37 wk, duration of membrane rupture ≥18 hours, or intrapartum temperature of ≥38.0°C (100.4°F) *Not recommended for cesarean deliveries performed before labor onset in women with intact amniotic membranes.	Shown	*Intrapartum:* Penicillin G IV (ampicillin) IV is an acceptable alternative). *For those allergic to penicillin:* Without a history of anaphylaxis: Cefazolin IV With a history of anaphylaxis: Clindamycin IV if organism susceptible, Vancomycin IV if organism not susceptible or unknown. Prophylaxis for infants born to mothers who have received adequate intrapartum prophylaxis is not recommended.		8, 28, 85
Syphilis	Infants born to infected women Those exposed <90 days before the diagnosis of primary, secondary, or early latent syphilis in a sex partner Those exposed >90 days before the diagnosis of primary, secondary, or early latent syphilis in a sex partner if serologic test results are not available immediately and the opportunity for follow-up is uncertain	Shown	Consult an expert. See CDC recommendations[116] Benzathine penicillin G IM	No	24, 176
Tuberculosis	Close contacts with impaired immunity (e.g., HIV infection) and all contacts <4 years of age even if tuberculin skin test or interferon-γ release assay is negative	Shown	Isoniazid PO	Yes. BCG indications in the United States	25, 51, 54, 63
Varicella	Susceptible close contacts at high risk for severe disease	Shown	Varicella vaccine, varicella-zoster, immunoglobulin (VariZIG)	Yes	26, 137
Yaws	Close contacts	Shown	Azithromycin PO Benzathine penicillin G IM (use when azithromycin is not available or when the patient cannot be treated with azithromycin)	No	143, 173, 142a

IM, Intramuscularly; *IV*, intravenously; *PO*, orally.

disease. The use of social media and online resources will continue to enhance public health surveillance.

HOW PUBLIC HEALTH CAN ASSIST CLINICIANS

Clinicians can contact public health agencies for consultation when they encounter patients who may have infectious diseases with known, unknown, or unclear etiologies. When a clinician encounters a patient who has an unusual or severe presentation of what appears to be an infectious disease or becomes aware of a cluster of patients with similar symptoms, public health may be able to assist with determining a diagnosis.

Point-of-care bi-directional communication between public health practitioners and clinicians can facilitate clinical decision making based on the most current public health information. For example, public health practitioners can inform clinicians about infectious diseases known to be circulating in the area of the patient's residence and about infectious diseases patients may have been exposed to during travel. Public health practitioners can also provide recommendations for treatment and isolation of infected patients and postexposure prophylaxis for their contacts, including first responders such as emergency medical services personnel.

In addition, public health laboratories can be extremely helpful to clinicians. Although clinical laboratories provide excellent service for routine testing, they may not be able to test for more obscure pathogens. If an unusual disease is suspected, public health laboratories can perform testing for an array of possible agents, including biologic threats. Public health practitioners and laboratories also may be able to assist clinicians in establishing a diagnosis when the differential diagnosis contains several possible infectious disease entities with similar clinical presentations. New infectious disease diagnostic technologies will continue to be developed and are likely to be initially available only at public health laboratories.

Public health laboratories can often also detect more common pathogens such as *Neisseria meningitidis* or *Streptococcus pneumoniae* in clinical specimens that have not grown in culture in clinical laboratories by using nucleic acid–based assays such as polymerase chain reaction (PCR) because the sensitivity of the PCR test is not affected by prior antibiotic treatment.[148] Public health laboratories may also perform PCR testing for other diseases such as measles, which can be diagnostically challenging in an era of low incidence.

In addition to identifying disease causing pathogens, public health laboratories are able to use molecular methods to perform strain typing that further characterizes pathogens and may identify novel strain types. Such testing may also detect new agents and identify outbreaks. A public health laboratory first identified 2009 pandemic H1N1 influenza A.

Advancement in laboratory methods has also enhanced the usefulness of surveillance data in outbreak detection by linking bacterial isolates obtained from geographically scattered cases. CDC coordinates PulseNet,[42] a national network of public health and food regulatory agency laboratories that perform standardized molecular subtyping (genetic fingerprinting) on foodborne bacteria by pulsed-field gel electrophoresis (PFGE).

PFGE testing can be used to distinguish strains of organisms such as *Escherichia coli* O157:H7, *Salmonella*, *Shigella*, *Listeria*, or *Campylobacter* at the DNA level. DNA "fingerprints," or patterns, are submitted electronically to a dynamic database at CDC. This database is available on demand to participants, which allows for rapid comparison of the patterns and identification of clusters, which can lead to identification and removal of contaminated food products from the homes of consumers thereby preventing further disease.

Testing methods such as such as antigen-based tests and nucleic acid–based assays that do not depend on culture and produce no isolate have become increasingly common, particularly for enteric bacterial pathogens. The use of these tests has implications for public health surveillance. Although such tests may identify more cases, they may also produce large numbers of false positive results if their specificity is low and they are used in low-prevalence populations. In addition, such tests do not allow public health practitioners to monitor microbiologic characteristics such as antimicrobial resistance or to use other isolate characteristics such as subtype and susceptibility profile that are used in addition to other data to attribute illness to specific sources. Therefore, some isolate-based monitoring will remain necessary, although it may be possible to establish sentinel sites to obtain isolates.[89]

Although the increasing availability of home tests for reportable infectious diseases such as human immunodeficiency virus, hepatitis B, and hepatitis C will make such tests more accessible to the public, surveillance activities for diseases that can be diagnosed in this manner will be hampered and the impact of this is difficult to predict.

OUTBREAK INVESTIGATION

When faced with an unusual case or cluster of cases that may be of public health concern, public health authorities conduct an investigation in an attempt to determine or confirm a diagnosis, describe the scope of the problem, and, if unknown, identify a cause. Front-line clinicians may be called upon to assist in such investigations.

When investigating a possible outbreak, a useful approach is for investigators to address the inquiry systematically using CDC's 10-step method[51]:

Prepare for Fieldwork

The following are the steps in preparing for fieldwork:

1. Establish the existence of an outbreak
2. Verify the diagnosis
3. Define and identify cases
4. Describe and orient the data in terms of time, place, and person
5. Develop hypotheses
6. Evaluate hypotheses
7. Refine hypotheses and carry out additional studies

8. Implement control and prevention measures
9. Communicate findings

Several exercises illustrating these principles, including communicable and noncommunicable disease outbreaks, can be found at the CDC's training site for epidemic intelligence service officers.[44]

PUBLIC HEALTH PREVENTION AND CONTROL MEASURES

Once an unusual case or cluster of cases is recognized and an investigation has occurred, many tools are available for public health intervention, if it is indicated. Two important interventions, immunization and the treatment of individual patients, are not considered in this chapter because they are addressed at length elsewhere in this book. Public health tools that are discussed here include isolation, quarantine, and preexposure and postexposure prophylaxis.

Other public health tools that are not discussed here but are also relevant to the control of infectious diseases include modifications to human behaviors that affect exposure to infectious diseases such as sexual behavior and injection drug use, water and food sanitation, food preparation methods, and so on.

Additionally, various prevention and control measures can be applied to animals, such as vaccination and treatment or prophylaxis, that decrease their risk for spreading diseases to humans.[121] Finally, prevention and control measures can be applied to the environment, such as proper disposal of waste, vector control, and appropriate design and proper maintenance of buildings and or other equipment that can reduce opportunities for disease transmission. An example of such a control measure would include proper maintenance of spas or cooling towers to prevent *Legionella* transmission.

Isolation

Isolation is the separation of persons who have a specific infectious illness from those who are healthy and the restriction of their movement to stop the spread of that illness during the period of communicability.[49] Indications for isolation of patients with infectious diseases have changed greatly in this century with increasing knowledge about modes of transmission and with antimicrobial therapy, which rapidly renders patients with many diseases noninfectious. The American Academy of Pediatrics (AAP) *Red Book: Report of the Committee on Infectious Diseases* is an excellent resource for information about isolation recommendations.[22]

Quarantine

In addition to containing the spread of communicable illnesses through isolation, another less commonly used control measure is quarantine. Quarantine is the separation and restriction of movement of persons who, while not yet ill, have been exposed to an infectious agent and therefore may become infectious.[45,46] When quarantine is used it is imposed for a period equal to the longest usual incubation period of the disease.

The history and practice of quarantine began as early as the 14th century in Italy to control plague by requiring ships arriving in Venice from infected ports to anchor for 40 days before landing.[46] The Italian word, *quaranta*, meaning 40, turned into the commonly used word *quarantine*.

In recent years, health departments in the United States have rarely implemented quarantine. However, it was used during the 2003 severe acute respiratory syndrome (SARS) outbreak and is occasionally used for susceptible persons who have been exposed to measles and did not receive postexposure prophylaxis.

Preexposure and Postexposure Prophylaxis

The word *prophylaxis* is used in this section to describe the use of antimicrobial or immunologic agents to prevent infection before or after exposure to an infectious disease. The decision to initiate prophylaxis and the choice and dosage of agents require careful consideration of both the child's clinical status and the epidemiologic factors that may place the child at risk for acquiring the disease. Public health authorities should be notified of cases and exposures of reportable infectious diseases; they may need to conduct an investigation to ensure that all exposed persons have been identified and, when necessary, treated or placed under observation. In addition, they often can provide useful additional information on recommended prophylaxis regimens.

A summary of several diseases for which prophylaxis may be administered to children is given in Table 246-1. Some special situations, such as prophylaxis for immunodeficient children and perioperative prophylaxis, are omitted from the discussion. For a more detailed discussion of the use of prophylaxis in prevention of infectious disease, the regularly updated AAP *Red Book: Report of the Committee on Infectious Diseases* should be consulted.[22]

COMMON PATTERNS OF DISEASE SPREAD

Animal to Person

Many infectious diseases are transmitted from animals to humans (zoonoses). Of all emerging diseases in the 20th and 21st century, over 75 percent are zoonotic in origin.[104] Clinicians who routinely inquire about animal contacts as part of the clinical and epidemiologic history of an ill patient frequently find valuable clues to an obscure diagnosis.

Details of the clinical manifestations, differential diagnosis, and treatment of zoonoses are found in the appropriate chapters elsewhere in this book. For a complete description of the circumstances under which each agent is spread from animal to person, consult a textbook of zoonoses. Often emerging infectious diseases in humans, such as SARS, are found to have an animal reservoir.[124]

Person-to-Person

As members of groups—including families, school, camp, childcare center, and religious organizations—children are at risk for acquiring or spreading infectious agents that are transmitted from person-to-person. Knowledge of the typical patterns of spread can be useful in planning surveillance or in designing control measures. For example, recognition that diarrhea commonly results from person-to-person spread in childcare centers and that it can indicate inadequate hygienic practices may justify specific surveillance for diarrhea in that setting. Documentation of secondary cases in various categories of contacts of cases of meningococcal disease permits focusing the delivery of chemoprophylaxis on high-risk groups.

In this section, disease transmission in the household, childcare centers and schools is briefly discussed. The important areas of disease transmission in childcare centers and hospitals are discussed in separate chapters in more depth. Maternal transmission of specific infections to the fetus or neonate is also reviewed elsewhere in this book. Spread of enteric diseases within institutions for the developmentally disabled is recognized widely as an important problem, but it does not pertain directly to the practice of most clinicians and is not discussed in this chapter.

EXAMPLES OF EXPOSURE SOURCES

Contaminated Food

Many foods have been shown to be the vehicle of transmission in outbreaks caused by a wide range of bacteria, viruses, and parasites, in addition to many naturally occurring toxins and human-made chemicals. Inherent contamination of meat, poultry, and eggs with *Salmonella*, *Yersinia*, shiga toxin–producing *E. coli*, listeria, and other pathogens, as well as deficiencies in food storage and preparation and ingestion of raw or improperly cooked animal products in both eating establishments and the home still contribute to outbreaks and sporadic cases of gastrointestinal infectious diseases.[80,102,145,150] In addition, practices with known risks such as drinking unpasteurized milk can result in infection.[64,75]

Outbreaks of infection and secondary hemolytic-uremic syndrome resulting from infection with shiga toxin–producing *E. coli* strains such as O157:H7 and O104:H4 have been related to juice,[117,168] lettuce,[103] spinach,[66] sprouts,[39,171] and unpasteurized milk[64,106] and other food items. In addition, new problems can result from changes in the food industry and in patterns of food consumption.[33,147] In recent years, several large outbreaks of salmonellosis have been associated with the widespread distribution of fresh produce, a previously rare vehicle of salmonella transmission.[33]

CDC publishes a "Foodborne Outbreak Online Database"[78] and although the number of outbreaks reported to public health authorities represents only a small fraction of those that occur, these reports can provide important insight into the epidemiology of foodborne disease.

Clinicians must work in conjunction with public health authorities by confirming diagnoses through appropriate diagnostic methods and promptly reporting findings or suspicions so that foodborne outbreaks can be recognized in a timely fashion to control and prevent these illnesses most effectively.[49]

Recreational Water

An increasingly important problem is the occurrence of outbreaks of waterborne disease associated with recreational water use, such as for swimming or wading,[32,82,153] hot tubs and spas,[114,123] water parks,[72] and decorative fountains.[109] Diseases transmitted in this manner include shigellosis, cryptosporidiosis, norovirus gastroenteritis, viral conjunctivitis, giardiasis, legionellosis, enterohemorrhagic *E. coli* O157:H7, and *Pseudomonas* dermatitis ("hot tub folliculitis").[69,178]

Deaths may occur, especially with cases of primary amebic meningoencephalitis caused by any of several free-living amebae in persons who had recently been swimming, typically in warm, stagnant water.[74,131] Of note, a case of primary amebic meningoencephalitis caused by *Naegleria fowleri* was recently reported in an area at much higher latitude than previous cases.[119]

Unpasteurized Milk

Unpasteurized milk has been demonstrated as the vehicle of transmission in outbreaks of brucellosis, tuberculosis, and diphtheria, as well as outbreaks of diseases caused by group A streptococci, *Salmonella*, *Shigella*, *Campylobacter*, listeria, *Yersinia enterocolitica*, and staphylococcal toxin.[118,126] Exposure to unpasteurized milk should be rare in the United States. Unfortunately, the use of raw milk as a "health food" continues to account for outbreaks of disease, particularly outbreaks caused by *E. coli* O157:H7, *Salmonella*, and *Campylobacter*.[64,106,126,149] In addition, children sometimes drink raw milk while visiting dairy farms on school field trips, during other youth activities, or during travel to other countries, which puts them at risk for infectious diarrhea and shiga toxin–producing *E. coli*–related renal failure.

Bioterrorism and Natural Disasters

Unfortunately, an important responsibility of public health authorities is to help prepare the nation and clinicians for the possibility of biologic, radiologic, or chemical terrorism and natural disasters. Children have special vulnerabilities to biologic terrorism and chemical agents because of their unique physiology and the limited availability of age-appropriate and weight-appropriate antidotes and treatment.[138,151] Also, clinicians' unfamiliarity with presenting clinical syndromes from either biologic or chemical exposures can delay appropriate diagnosis and treatment.[88,151,159]

Another issue is that the Strategic National Stockpile, a national repository of antimicrobial agents, chemical antidotes, antitoxins, and other life-support medications and equipment, can only stockpile U.S. Food and Drug Administration (FDA)-licensed items and only for their

FDA-approved indications.[139] Often FDA indications for antimicrobial and other therapeutic agents for children after biologic terrorist agent release are lacking, causing the stockpile to be deficient in certain therapeutic agents for children.

Terrorism preparedness is a unique and hopefully rare component of general emergency and disaster preparedness. However, natural disasters are more common and infections that are typically rare in the United States can result from compromised personal hygiene, wounds and injuries, contaminated food and water, and exposure to insect vectors. Prompt recognition and appropriate management by clinicians of unusual diseases is critical during and after disaster situations.

COMMON DISEASE TRANSMISSION SETTINGS

Households

Spread of disease agents in the family can be considered in two stages: introduction of the agent into the family, usually by a single member, and spread to other family members. Spread to other members can be described in terms of rapidity and intensity, the latter commonly measured by the secondary infection rate (or secondary attack rate), which is defined as the proportion of the contacts of the index carrier or index case who become infected (or ill) in a given interval after exposure. The secondary infection rate varies from agent to agent and may be affected by the age and sex of the introducer; the size of the family; crowding; the age of household contacts; previous immunity; and interventions attempted, including vaccination, isolation, treatment, and prophylaxis. The ratio of the secondary attack rate to the incidence of disease in the community is a measure of the importance of the family as a focus of disease.[41,101]

Childcare Centers

It is well known that infectious disease agents are easily transmitted among children in childcare centers and that outbreaks occur in these settings.[37,111,125] Interventions that have proved valuable for reducing infections within childcare centers include formal written policies for infection prevention and control within the childcare center, formal education of childcare center staff concerning infection prevention practices, good hand hygiene by both staff and children, appropriate cleaning of contaminated surfaces, separation of food preparation and diaper changing areas, exclusion of certain ill children, cohorting ill children when exclusion is not possible, ensuring adequate age-appropriate immunization of childcare attendees and staff, and optimal ratios of children to staff.[36]

Schools

Typically, infectious disease spread in schools is less than that in families and childcare centers. However, schools may serve to spread infection from family to family. Settings that result in a greater level of person-to-person contact than found in typical classroom settings, such as participation in school sports[29,76] or attendance at boarding schools, summer camps, and college dormitories also can result in more efficient transmission of disease.[113,158,161] In recent years, measles, mumps, influenza, meningococcal disease, norovirus, and methicillin-resistant *Staphylococcus aureus* outbreaks have occurred in these settings.*

HEALTH INFORMATION FOR INTERNATIONAL TRAVEL

International travel, whether by child or adult, necessitates preparation beforehand to obtain the proper immunizations and contingency plans for the possibility of encountering unsanitary food or water during the travel.[98,120,135,146] Illnesses acquired abroad may not be manifested until after returning home, with consequent diagnostic difficulty. Clinicians should query parents regarding travel when children have unusual symptoms or febrile rash illnesses.

Active and passive immunization are covered elsewhere in this book, and updated travel-related immunization information is available at CDC's traveler's health website.[52,98,135,146] Disease-specific information for travelers and clinicians is available in CDC's "Yellow Book" at that website.[52]

In addition, travel notices are available that can alert travelers and clinicians to infectious disease outbreaks occurring throughout the world. For example, during the period 2003 to 2009, substantial progress was made toward the goal of measles elimination in Europe. However, in late 2009 through 2011, measles virus transmission increased as a result of declining immunization rates and outbreaks in Europe became widespread and cases are still occurring. Infants traveling to parts of the world where measles is circulating should be vaccinated as young as 6 months of age.†

Safe water may be found in many hotels in large cities throughout the world, but only water from adequately chlorinated sources can be considered truly safe. Where chlorinated water is not available, canned or bottled carbonated beverages and beverages made from boiled water or that contain alcohol may be safe; however, an outbreak caused by commercially bottled water contaminated by non-O1 *Vibrio cholerae* has been described.[53] Ice made from untreated water may contaminate an otherwise safe beverage, either directly or by leaving contaminated water on the outside of the container. The CDC has prepared guidance on preparation and storage of safe water that can be found online.[48]

Food should also be selected carefully. In areas of the world where hygiene and sanitation are poor, travelers should avoid intake of salads, uncooked vegetables, unpasteurized milk, and milk products such as cheese and should eat only fruits and vegetables that can be peeled by the traveler or have been cooked and are still hot. In addition to a recommendation that all children receive hepatitis A vaccine, persons who are traveling to

*References 67, 71, 73, 77, 84, 127.
†References 55, 79, 98, 135, 146, 165.

countries with high or intermediate levels of endemic hepatitis A infection are also recommended to receive hepatitis A vaccine before departure.[100]

Children younger than 2 years of age have a high risk for acquiring traveler's diarrhea.[30,132,152] Traveler's diarrhea is caused by several bacterial pathogens, including *E. coli*, *Shigella*, *Campylobacter*, *Salmonella*, *Aeromonas*, *Plesiomonas*, and noncholera vibrios. Noroviruses are also an important cause of morbidity among travelers. Recently, genetic risk factors associated with susceptibility to specific pathogens have been identified.[92] Immediate medical attention should be sought for an infant or child with blood or mucus in the stool, fever with rigors, or persistent vomiting or diarrhea with dehydration.

Because infants and small children are especially at risk for becoming dehydrated, parents need to be aware of the signs of dehydration and be prepared to use oral rehydration solutions containing appropriate concentrations or electrolytes as a preventive measure while medical attention is being obtained. WHO oral rehydration solutions are widely available at stores and pharmacies in most developing countries.

For more information on traveler's diarrhea, including antimicrobial treatment and oral rehydration therapy, please see the CDC Yellow Book.[52]

VECTORBORNE DISEASE

Vectors such as mosquitos and ticks can transmit infectious diseases. Examples of tick-borne diseases in the United States include anaplasmosis, babesiosis, ehrlichiosis, Lyme disease, Rocky Mountain spotted fever, tick-borne relapsing fever, tick paralysis, and tularemia. Mosquito-borne diseases that have been found in the United States include West Nile virus infection, eastern equine encephalitis, La Crosse encephalitis, St. Louis encephalitis, and western equine encephalitis. Malaria has rarely been transmitted in the United States,[65] but several dengue outbreaks have been detected in the United States since 1980, including seven outbreaks in southern Texas along the U.S.-Mexico border, one outbreak in Hawaii in 2001, and two outbreaks in southern Florida in 2009 and 2010.[38] Other vectorborne infections that can be carried back from foreign travel include chikungunya, Rift Valley fever, yellow fever, and many others. In addition, fleas can spread endemic or murine typhus, plague, and cat-scratch fever, and body lice can spread epidemic typhus, relapsing fever, and trench fever.[90,91,97,128]

The CDC has excellent online resources for vectorborne diseases,[87] and local and state public health practitioners can provide clinicians with information on vectorborne diseases in the geographic regions they serve and other regions where patients may have traveled. In addition, public health laboratories may be able to assist in establishing a diagnosis.

VECTOR PROTECTION

Personal protection against vectors such as mosquitos and ticks includes avoiding vector-infested areas, wearing protective clothing, using insect repellents applied to skin or clothing, and frequently inspecting for and promptly removing attached ticks. These individual preventive measures can be practiced in any circumstance where exposure to infected mosquitos or ticks may occur.[129]

Of the active ingredients currently registered with the U.S. Environmental Protection Agency (EPA), the CDC reports that DEET (N,N-diethyl-m-toluamide) and picaridin (KBR 3023) demonstrated a higher degree of efficacy and that products containing these active ingredients typically provide longer-lasting protection than others.[50] Another effective product is IR3535 (3-[N-butyl-N-acetyl]-aminoproprionic acid, ethyl ester).

Oil of lemon eucalyptus and its synthetic equivalent *p*-menthane 3,8-diol, a plant-based repellent, also is registered with the EPA. When oil of lemon eucalyptus was tested against mosquitoes in two recent scientific publications in the United States, it provided protection similar to that of repellents with low concentrations of DEET. Oil of lemon eucalyptus products should NOT be used in children younger than 3 years of age.

The EPA characterizes the active ingredients DEET and picaridin as "conventional repellents" and oil of lemon eucalyptus as a "biopesticide repellent," which is derived from natural materials. More information on repellent active ingredients is available online at the EPA website.[96]

Although no definitive studies exist in the scientific literature about what concentration of DEET is safe for children, no serious illness has been linked to the use of DEET in children when used according to manufacturer's recommendations.

The American Academy of Pediatrics has supported the 2001 EPA recommendation that appropriate use of DEET at concentrations of up to 30 percent pose no significant risk to children or adults but that DEET should not be used in children younger than 2 months of age because of increased skin permeability.[27] The CDC currently recommends DEET at concentrations up to 50 percent for both adults and children older than 2 months of age.

Certain products containing permethrin are recommended for use on clothing, shoes, bed nets, and camping gear and are registered with EPA for this use. Permethrin is highly effective as an insecticide and a repellent. Permethrin-treated clothing repels and kills ticks, mosquitoes, and other arthropods and retains this effect after repeated laundering.

PROPHYLAXIS AGAINST INFECTIOUS DISEASES IN CHILDREN

Many infectious diseases can be prevented by preexposure or postexposure prophylaxis. Table 246-1 describes prophylaxis recommendations for selected infectious diseases in children.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Agrawal A, Murphy TF. *Haemophilus influenzae* infections in the *H. influenzae* type b conjugate vaccine era. *J Clin Microbiol* 2011; **49**:3728–32.
2. Altunaiji S, Kukuruzovic R, Curtis N, et al. Antibiotics for whooping cough (pertussis). *Cochrane Database Syst Rev* 2007; **18**(3):CD004404.
3. American Academy of Pediatrics. Anthrax. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 228–32.
4. American Academy of Pediatrics. Chlamydia trachomatis. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 276–81.
5. American Academy of Pediatrics. Diphtheria. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 307–11.
6. American Academy of Pediatrics. Gonococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 336–44.
7. American Academy of Pediatrics. Group A streptococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 668–80.
8. American Academy of Pediatrics. Group B streptococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 680–5.
9. American Academy of Pediatrics. *Haemophilus influenzae* infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 345–52.
10. American Academy of Pediatrics. Hepatitis A. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 361–9.
11. American Academy of Pediatrics. Hepatitis B. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 369–90.
12. American Academy of Pediatrics. Human immunodeficiency virus Infection. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 418–39.
13. American Academy of Pediatrics. Influenza. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 439–53.
14. American Academy of Pediatrics. Leprosy. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 466–9.
15. American Academy of Pediatrics. Malaria. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 483–9.
16. American Academy of Pediatrics. Measles. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 489–99.
17. American Academy of Pediatrics. Meningococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 500–9.
18. American Academy of Pediatrics. Pertussis (whooping cough). In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 553–66.
19. American Academy of Pediatrics. Plague. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 569–71.
20. American Academy of Pediatrics. Prevention of neonatal ophthalmia. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 880–1.
21. American Academy of Pediatrics. Rabies. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 607.
22. American Academy of Pediatrics. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
23. American Academy of Pediatrics. Staphylococcal infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 653–68.
24. American Academy of Pediatrics. Syphilis. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 690–703.
25. American Academy of Pediatrics. Tuberculosis In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 736–59.
26. American Academy of Pediatrics. Varicella-zoster infections. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 774–89.
27. American Academy of Pediatrics, Committee on Environmental Health. Pesticides. In: Pickering LK, editor. *Red book: 2012 report of the Committee on Infectious Diseases*. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 515–48.
28. American Academy of Pediatrics, Committee on Infectious Diseases. Recommendations for the prevention of perinatal group B streptococcal (GBS) disease. *Pediatrics* 2011;**128**:611–6.
29. Anderson BJ. Managing herpes gladiatorum outbreaks in competitive wrestling: the 2007 Minnesota experience. *Curr Sports Med Rep* 2008;**7**:323–7.
30. Ang JY, Mathur A. Traveler's diarrhea: updates for pediatricians. *Pediatr Ann* 2008;**37**:814–20.
31. Arguin PM, Mali S. *Malaria*. CDC yellow book. 2012. Available at: http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/malaria.htm.
32. Artieda J, Pineiro L, Gonzalez M, et al. A swimming pool-related outbreak of pharyngoconjunctival fever in children due to adenovirus type 4, Gipuzkoa, Spain, 2008. *Euro Surveill* 2009;**14**: 19125.
33. Barton Behravesh C, Mody RK, Jungk J, et al. 2008 outbreak of *Salmonella* Saintpaul infections associated with raw produce. *N Engl J Med* 2011;**364**:918–27.
34. Bilukha OO, Rosenstein NC. Prevention and control of meningococcal disease. *MMWR Recomm Rep* 2005;**54**(RR-07):1–21.
35. Birkhead GS, Maylahn CM. State and local public health surveillance in the United States. In: Lee LM, Teutsch SM, Thacker SB, St. Louis ME, editors. *Principles and practice of Public health surveillance*. 3th ed. New York, NY: Oxford University Press; 2012. p. 381–98.
36. Brady MT. The familial aggregation of infectious diseases. *Am J Public Health* 2005;**33**:276–85.
37. Brown JA, Hite DS, Maguire HF, et al. Outbreak of a shiga toxin-producing *Escherichia coli* serotype O26:H11 infection at a child care center in Colorado. *Pediatr Infect Dis J* 2012;**31**:379–83.
38. Tomashek KM. Dengue fever and dengue hemorrhagic fever. In: *CDC yellow book*. 2012. Available at: http://wwwnc.cdc.gov/travel/yellowbook/2012/chapter-3-infectious-diseases-related-to-travel/dengue-fever-and-dengue-hemorrhagic-fever.htm.
39. Buchholz U, Bernard H, Werber D, et al. German outbreak of *Escherichia coli* O104:H4 associated with sprouts. *N Engl J Med* 2012;**365**:1763–70.
40. Buehler JW, Hopkins RS, Overhage JM, et al. Framework for evaluating public health surveillance systems for early detection of outbreaks: recommendations from the CDC Working Group. *MMWR Recomm Rep* 2004;**53**:1–11.
41. Cauchemez S, Donnelly CA, Reed C, et al. Household transmission of 2009 pandemic influenza A (H1N1) virus in the United States. *N Engl J Med* 2009;**361**:2619–27.
42. Centers for Disease Control and Prevention. *CDC PulseNet*. n.d. Available at: http://www.cdc.gov/pulsenet/.

43. Centers for Disease Control and Prevention. *Core functions of public health and how they relate to the 10 essential services*. Available at: http://www.cdc.gov/nceh/ehs/ephli/core_ess.htm. n.d.
44. Centers for Disease Control and Prevention. *EIS case studies*. n.d. Available at: http://www.cdc.gov/eis/casestudies/casestudies.htm. n.d.
45. Centers for Disease Control and Prevention. *Fact sheet on isolation and quarantine*. n.d. Available at: http://www.cdc.gov/sars/quarantine/fs-isolation.html.
46. Centers for Disease Control and Prevention. *History of quarantine*. n.d. Available at: http://www.cdc.gov/quarantine/HistoryQuarantine.html.
47. Centers for Disease Control and Prevention. *MMWR Morb Mortal Wkly Rep* n.d. Available at: http://www.cdc.gov/mmwr/.
48. Centers for Disease Control and Prevention. *Personal preparation and storage of safe water*. n.d. Available at: http://www.cdc.gov/healthywater/emergency/safe_water/personal.html.
49. Centers for Disease Control and Prevention. *Quarantine and isolation*. n.d. Available at: http://www.cdc.gov/quarantine.
50. Centers for Disease Control and Prevention. *Questions & answers: Insect repellent use and safety*. n.d. Available at: http://www.cdc.gov/ncidod/dvbid/westnile/qa/insect_repellent.htm.
51. Centers for Disease Control and Prevention. *Steps of an outbreak investigation*. n.d. Available at: http://www.cdc.gov/excite/classroom/outbreak/steps.htm.
52. Centers for Disease Control and Prevention. *Travelers' health*. n.d. Available at: http://wwnc.cdc.gov/travel.
53. Centers for Disease Control and Prevention. Surveillance for waterborne-disease outbreaks: United States, 1993-1994. *MMWR Surveill Summ* 1996;**45**:1–33.
54. Centers for Disease Control and Prevention. The role of BCG vaccine in the prevention and control of tuberculosis in the United States: a joint statement by the Advisory Committee for the Elimination of Tuberculosis and the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 1996;**45**(RR-4):1–18.
55. Centers for Disease Control and Prevention. Measles, mumps, and rubella: vaccine use and strategies for elimination of measles, rubella, and congenital rubella syndrome and control of mumps: Recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 1998;**47**(RR-8):1–57.
56. Centers for Disease Control and Prevention. Use of diphtheria toxoid-tetanus toxoid-acellular pertussis vaccine as a five-dose series. *MMWR Morb Mortal Wkly Rep* 2000;**49**(RR-13):1–8.
57. Centers for Disease Control and Prevention. Interim recommendations for antimicrobial prophylaxis for children and breastfeeding mothers and treatment of children with anthrax. *MMWR Morb Mortal Wkly Rep* 2001;**50**:1014–6.
58. Centers for Disease Control and Prevention. Update: Investigation of bioterrorism-related anthrax and interim guidelines for clinical evaluation of persons with possible anthrax. *MMWR Morb Mortal Wkly Rep* 2001;**50**:941–8.
59. Centers for Disease Control and Prevention. Updated guidelines for evaluating public health surveillance systems. *MMWR Morb Mortal Wkly Rep* 2001;**50**(Suppl. 13):1–35.
60. Centers for Disease Control and Prevention. Diagnosis and management of foodborne illnesses: a primer for physicians and other health care professionals. *MMWR Morb Mortal Wkly Rep* 2004;**53**(RR-4):1–33.
61. Centers for Disease Control and Prevention. Syndrome and outbreak detection using chief-complaint data: experience of the real-time outbreak and disease surveillance project. *MMWR Morb Mortal Wkly Rep* 2004;**53**(suppl):28–31.
62. Centers for Disease Control and Prevention. Controlling tuberculosis in the United States: recommendations from the American Thoracic Society, CDC, and the Infectious Diseases Society *of America. MMWR* 2005;**54**(RR-12):1–81.
63. Centers for Disease Control and Prevention. Guidelines for the investigation of contacts of persons with infectious tuberculosis. Recommendations from the National Tuberculosis Controllers Association and CDC. *MMWR Recomm Rep* 2005;**54**(RR-15):1–47.
64. Centers for Disease Control and Prevention. *Escherichia coli* 0157:H7 infections in children associated with raw milk and raw colostrum from cows: California, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**57**:625–628.
65. Centers for Disease Control and Prevention. Locally acquired mosquito-transmitted malaria: a guide for investigations in the United States: September 6, 2006. *MMWR Recomm Rep* 2006;**55**(RR-13):1–9.
66. Centers for Disease Control and Prevention. Ongoing multistate outbreak of *Escherichia coli* serotype O157:H7 infections associated with consumption of fresh spinach: United States, September 2006. *Morb Mortal Wkly Rep* 2006;**55**:1045–6.
67. Centers for Disease Control and Prevention. Update: multistate outbreak of mumps—United States, January 1–May 2, 2006. *Morb Mortal Wkly Rep* 2006;**55**:1–5.
68. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted disease treatment guidelines, 2006: fluoroquinolones no longer recommended for treatment of gonococcal infections. *Morb Mortal Wkly Rep* 2007;**56**:332–6.
69. Centers for Disease Control and Prevention. Communitywide cryptosporidiosis outbreak: Utah, 2007. *Morb Mortal Wkly Rep* 2008;**57**:989–93.
70. Centers for Disease Control and Prevention. Emergence of fluoroquinolone-resistant *Neisseria meningitidis:* Minnesota and North Dakota, 2007–2008. *Morb Mortal Wkly Rep* 2008;**57**:173–5.
71. Centers for Disease Control and Prevention. Norovirus outbreak in an elementary school: District of Columbia, February 2007. *MMWR Morb Mortal Wkly Rep* 2008;**56**:1340–3.
72. Centers for Disease Control and Prevention. Outbreak of cryptosporidiosis associated with a splash park: Idaho, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**58**:615–8.
73. Centers for Disease Control and Prevention. Outbreak of measles: San Diego, California, January–February 2008. *MMWR Morb Mortal Wkly Rep* 2008;**57**:1–4.
74. Centers for Disease Control and Prevention. Primary amebic meningoencephalitis: Arizona, Florida, and Texas, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**7**:573–7.
75. Centers for Disease Control and Prevention. *Campylobacter jejuni i*nfection associated with unpasteurized milk and cheese: Kansas, 2007. *MMWR Morbid Mortal Wkly Rep* 2009;**57**:1377–9.
76. Centers for Disease Control and Prevention. Methicillin-resistant *Staphylococcus aureus* among players on a high school football team: New York City, 2007. *MMWR Morbid Mortal Wkly Rep* 2009;**58**:52–5.
77 Centers for Disease Control and Prevention. Update: mumps outbreak: New York and New Jersey, June 2009-January 2010. *MMWR Morbid Mortal Wkly Rep* 2010;**59**:129.
78. Centers for Disease Control and Prevention. *Foodborne outbreak online database (FOOD)*. Available at: http://wwwn.cdc.gov/foodborneoutbreaks/.
79. Centers for Disease Control and Prevention. Increased transmission and outbreaks of measles: European Region, 2011. *MMWR Morbid Mortal Wkly Rep* 2011;**60**:1605–10.
80. Centers for Disease Control and Prevention. Multistate outbreak of listeriosis associated with Jensen Farms cantaloupe: United States, August-September 2011. *MMWR Morbid Mortal Wkly Rep* 2011;**60**:1357–8.
81. Centers for Disease Control and Prevention. State electronic disease surveillance systems: United States, 2007 and 2010. *MMWR Morbid Mortal Wkly Rep* 2011;**60**:1421–3.
82. Centers for Disease Control and Prevention. Surveillance for waterborne disease outbreaks and other health events associated with recreational water: United States, 2007–2008. *MMWR Surveill Summ* 2011;**60**:1–32.
83. Centers for Disease Control and Prevention. Updated recommendations for use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine from the Advisory Committee on Immunization Practices, 2010. *MMWR Morbid Mortal Wkly Rep* 2011;**60**:13–5.
84. Centers for Disease Control and Prevention. Outbreak of meningococcal disease associated with an elementary school: Oklahoma, March 2010. *MMWR Morbid Mortal Wkly Rep* 2012;**61**:217–21.
85. Centers for Disease Control and Prevention. Prevention of perinatal group B streptococcal disease. Revised guidelines from CDC, 2010. *MMWR Recomm Rep* 2012;**59**(RR-10):1–36.
86. Centers for Disease Control and Prevention. Update to CDC's sexually transmitted diseases treatment guidelines, 2010: oral cephalosporins no longer recommended treatment for gonococcal infections. *MMWR Morbid Mortal Wkly Rep* 2012;**61**:590–4.

87. Centers for Disease Control and Prevention. *Division of Vector-Borne Diseases*. n.d. Available at: http://www.cdc.gov/ncezid/dvbd/.
88. Committee on Environmental Health and Committee on Infectious Diseases. Chemical-biological terrorism and its impact on children. *Pediatrics* 2006;**118**:1267–78.
89. Cronquist AB, Mody RK, Atkinson R, et al. Impacts of culture-independent diagnostic practices on public health surveillance for bacterial enteric pathogens. *Clin Infect Dis* 2012;**54**(Suppl. 5): S432–9.
90. Dahlgren FS, Holman RC, Paddock CD, et al. Fatal rocky mountain spotted fever in the United States, 1999-2007. *Am J Trop Med Hyg* 2012;**86**:713–9.
91. Dahlgren FS, Mandel EJ, Krebs JW, et al. Increasing incidence of *Ehrlichia chaffeensis* and *Anaplasma phagocytophilum* in the United States, 2000-2007. *Am J Trop Med Hyg* 2011;**85**:124–31.
92. de la Cabada Bauche J, Dupont HL. New developments in traveler's diarrhea. *Gastroenterol Hepatol (NY)* 2011;**7**:88–95.
93. Doull JA. Factors influencing selective distribution in diphtheria. *Prev Med* 1930;**4**:371–404.
94. Eison TM, Ault BH, Jones DP, et al. Post-streptococcal acute glomerulonephritis in children: clinical features and pathogenesis. *Pediatr Nephrol* 2011;**26**:165–80.
95. Endo A, Izumi H, Miyashita M, et al. Current efficacy of postexposure prophylaxis against measles with immunoglobulin. *J Pediatr* 2001;**138**:926–8.
96. Environmental Protection Agency. *Active ingredients found in insect repellents*. Available at: http://www.epa.gov/pesticides/health/mosquitoes/ai_insectrp.htm.
97. Feder HM Jr. Lyme disease in children. *Infect Dis Clin North Am* 2008;**22**:315–26.
98. Ferguson LE, Yetman RJ. International travel with children: tips for physicians and families. *Pediatr Rev* 2006;**27**:e16–e22.
99. Fiore AE, Fry A, Shay D, et al. Antiviral agents for the treatment and chemoprophylaxis of influenza recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 2011;**60**(RR01):1–24.
100. Fiore AE, Wasley A, Bell BP, et al. Prevention of hepatitis A through active or passive immunization. *MMWR Recomm Rep* 2006;**55**(RR07):1–23.
101. Frost WH. The familial aggregation of infectious diseases. *Am J Public Health* 1938;**28**:7–13.
102. Gaffga NH, Barton Behravesh C, Ettestad PJ, et al. Outbreak of salmonellosis linked to live poultry from a mail-order hatchery. *N Engl J Med* 2012;**366**:2065–73.
103. Gajraj R, Pooransingh S, Hawker JI, et al. Multiple outbreaks of *Salmonella braenderup* associated with consumption of iceberg lettuce. *Int J Environ Health Res* 2012;**22**:150–5.
104. Gibbs EP. Emerging zoonotic epidemics in the interconnected global community. *Vet Rec* 2005;**157**:673–9.
105. Grob PR. Prophylactic erythromycin for whooping-cough contacts. *Lancet* 1981;**1**:772–3.
106. Guh A, Phan Q, Nelson R, et al. Outbreak of *Escherichia coli* O157:H7 associated with raw milk, Connecticut, 2008. *Clin Infect Dis* 2010;**15**:1411–7.
107. Halperin SA, Bortolussi R, Langley JM, et al. A randomized, placebo-controlled trial of erythromycin estolate chemoprophylaxis for household contacts of children with culture positive *Bordetella pertussis* infection. *Pediatrics* 1999;**104**:e42.
108. Harris JB, LaRocque RC, Qadri F, et al. Cholera. *Lancet* 2012;**379**:2466–76.
109. Haupt TE, Heffernan RT, Nehls-Lowe H, et al. An outbreak of legionnaires disease associated with a decorative water wall fountain in a hospital. *Infect Control Hosp Epidemiol* 2012;**33**(2): 185–91.
110. Hellenbrand W, Hanquet G, Heuberger S, et al. What is the evidence for giving chemoprophylaxis to children or students attending the same preschool, school or college as a case of meningococcal disease? *Epidemiol Infect* 2011;**139**:1645–55.
111. Hennepin County Minnesota HSaPHD. *Infectious diseases in childcare settings and schools*. Available at: http://hennepin.us/childcaremanual.
112. Hoek MR, Christensen H, Hellenbrand W, et al. Effectiveness of vaccinating household contacts in addition to chemoprophylaxis after a case of meningococcal disease: a systematic review. *Epidemiol Infect* 2008;**136**:1441–7.
113. Huang AS, Cortese MM, Curns AT, et al. Risk factors for mumps at a university with a large mumps outbreak. *Public Health Rep* 2009;**124**:419–26.
114. Huhulescu S, Simon M, Lubnow M, et al. Fatal *Pseudomonas aeruginosa* pneumonia in a previously healthy woman was most likely associated with a contaminated hot tub. *Infection* 2011;**39**: 265–9.
115. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon: medical and public health management. Working Group on Civilian Biodefense. *JAMA* 2000;**283**:2281–90.
116. Institute of Medicine. *The future of public health*. Washington, DC: National Academy Press; 1998.
117. Jain S, Bidol SA, Austin JL, et al. Multistate outbreak of *Salmonella typhimurium* and *Saintpaul* infections associated with unpasteurized orange juice-United States, 2005. *Clin Infect Dis* 2009;**48**: 1065–71.
118. Jay-Russell MT. Raw (unpasteurized) milk: are health-conscious consumers making an unhealthy choice? *Clin Infect Dis* 2010;**51**: 1418–9.
119. Kemble SK, DeVries AS, Drehner DM, et al. Fatal *Naegleria fowleri* infection acquired in Minnesota: possible expanded range of a deadly thermophilic organism. *Clin Infect Dis* 2012;**54**: 805–9.
120. Kendall ME, Crim S, Fullterton K, et al. Travel-associated enteric infections diagnosed after return to the United States, Foodborne Diseases Active Surveillance Network (FoodNet), 2004-2009. *Clin Infect Dis* 2012;**54**(Suppl. 5):S480–7.
121. Kim Farley RJ. Principles of infectious disease control. In: Detels R, Beaglehole R, Lansang MA, Gulliford M, editors. *Oxford textbook of public health*. New York, NY: Oxford University Press; 2011.
122. King GE, Patriarca PA, Dales LG, et al. Clinical efficacy of measles vaccine during the 1990 measles epidemic. *Pediatr Infect Dis J* 1991;**10**:883–8.
123. Kura F, Amemura-Maekawa J, Yagita K, et al. Outbreak of Legionnaires' disease on a cruise ship linked to spa-bath filter stones contaminated with *Legionella pneumophila* serogroup 5. *Epidemiol Infect* 2006;**134**:385–91.
124. Lau SK, Woo PC, Li KS, et al. Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats. *Proc Natl Acad Sci USA* 2005;**102**:14040–5.
125. Lee MB, Greig JD. A review of enteric outbreaks in child care centers: effective infection control recommendations. *J Environ Health* 2008;**71**:24–32.
126. Leedum JM. Milk of nonhuman origin and infectious diseases in humans. *Clin Infect Dis* 2006;**43**:610–5.
127. Lessler J, Reiche NG, Cummings DA, et al. Outbreak of 2009 pandemic influenza A (H1N1) at a New York City school. *N Engl J Med* 2009;**361**:2628–36.
128. Lindsey NP, Hayes EB, Staples JE, et al. West Nile virus disease in children, United States, 1999-2007. *Pediatrics* 2009;**123**:e1084–e1089.
129. Lindsey NP, Staples JE, Lehman JA, et al. Surveillance for human West Nile virus disease: United States, 1999-2008. *MMWR Surveill Summ* 2010;**59**:1–17.
130. Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the infectious diseases society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children. *Clin Infect Dis* 2011;**52**:e18–55.
131. Lopez C, Budge P, Chen J, et al. Primary amebic meningoencephalitis: a case report and literature review. *Pediatr Emerg Care* 2012;**28**(3):272–6.
132. Mackell S. Traveler's diarrhea in the pediatric population: etiology and impact. *Clin Infect Dis* 2005;**41**(Suppl. 8):S547–52.
133. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* disease: United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.
134. Mali S, Kachur SP, Arguin PM, et al. Malaria surveillance: United States, 2010. *MMWR Surveill Summ* 2012;**61**:1–17.
135. Maloney SA, Weinberg M. Prevention of infectious diseases among international pediatric travelers: considerations for clinicians. *Semin Pediatr Infect Dis* 2004;**15**:137–49.
136. Manning SE, Rupprecht CE, Fishbein D, et al. Human rabies prevention: United States, 2008. *MMWR Recomm Rep* 2008; **57**(RR03):1–28.
137. Marin M, Güris D, Chaves SS, et al. Prevention of varicella. *MMWR Recomm Rep* 2007;**56**(RR-04):1–40.

138. Markenson D. The treatment of children exposed to pathogens linked to bioterrorism. *Infect Dis Clin North Am* 2005;**19**: 731–45.
139. Markenson D, Reynolds S, Committee on Pediatric Emergency Medicine and Task Force on Terrorism. The pediatrician and disaster preparedness. *Pediatrics* 2006;**17**:362.
140. Mast EE, Margolis HS, Fiore AE, et al. A comprehensive immunization strategy to eliminate transmission of hepatitis B virus infection in the United States: recommendations of the Advisory Committee on Immunization Practices (ACIP) Part 1: immunization of infants, children, and adolescents. *MMWR Recomm Rep* 2005;**54**(RR16):1–23.
141. McCloskey RV, Green MJ, Eller J, et al. Treatment of diphtheria carriers: benzathine penicillin, erythromycin, and clindamycin. *Ann Intern Med* 1974;**81**:788–91.
142. Meremikwu MM, Donegan S, Sinclair D, et al. Intermittent preventive treatment for malaria in children living in areas with seasonal transmission. *Cochrane Database Syst Rev* 2012;(2): CD003756.
142a. Mitjà O, Hays R, Ipai A, et al. Single-dose azithromycin versus benzathine benzylpenicillin for treatment of yaws in children in Papua New Guinea: an open-label, non-inferiority, randomised trial. *Lancet* 2012;**379**:342–7.
143. Mitjà O, Hays R, Rinaldi AC, et al. New treatment schemes for yaws: the path toward eradication. *Clin Infect Dis* 2012;**55**: 406–12.
144. National Institutes of Health Panel on Treatment of HIV-Infected Pregnant Women and Prevention of Perinatal Transmission. *Recommendations for use of antiretroviral drugs in pregnant HIV-1-infected women for maternal health and interventions to reduce perinatal HIV transmission in the United States*. Available at: http://aidsinfo.nih.gov/contentfiles/lvguidelines/PerinatalGL.pdf.
145. Neil KP, Biggerstaff G, MacDonald JK, et al., A novel vehicle for transmission of *Escherichia coli* O157:H7 to humans: multistate outbreak of *E. coli* O157:H7 infections associated with consumption of ready-to-bake commercial prepackaged cookie dough–United States, 2009. *Clin Infect Dis* 2012;**54**:511–8.
146. Neumann K. Family travel: an overview. *Travel Med Infect Dis* 2006;**4**:202–17.
147. Newell DG, Koopmans M, Verhoef L, et al. Food-borne diseases: the challenges of 20 years ago still persist while new ones continue to emerge. *Int J Food Microbiol* 2010;**139**(Suppl. 1): S3–S15.
148. Ni H, Knight AI, Cartwright K, et al. Polymerase chain reaction for diagnosis of meningococcal meningitis. *Lancet* 1992;**340**: 1432–4.
149. Oliver SP, Boor KJ, Murphy SC, et al. Food safety hazards associated with consumption of raw milk. *Foodborne Pathog Dis* 2009;**6**: 793–806.
150. Ong KL, Gould LH, Chen DL, et al. Changing epidemiology of *Yersinia enterocolitica* infections: markedly decreased rates in young black children, Foodborne Diseases Active Surveillance Network (FoodNet), 1996-2009. *Clin Infect Dis* 2012;**54**(Suppl. 5): S385–90.
151. Patel MM, Schier JG, Belson MG. Recognition of illness associated with covert chemical releases. *Pediatr Emerg Care* 2006;**22**: 592–601.
152. Pitzinger B, Steffen R, Tschopp A. Incidence and clinical features of traveler's diarrhea in infants and children. *Pediatr Infect Dis J* 1991;**10**:719–23.
153. Polgreen PM, Sparks JD, Polgreen LA, et al. A statewide outbreak of *Cryptosporidium* and its association with the distribution of public swimming pools. *Epidemiol Infect* 2012;**140**: 1439–45.
154. Rammelkamp CH, et al. Epidemic nephritis. *Trans Assoc Am Physicians* 1954;**67**:276–82.
155. Rémond MG, Wheaton GR, Walsh WF, et al. Acute rheumatic fever and rheumatic heart disease-priorities in prevention, diagnosis and management: a report of the CSANZ indigenous cardiovascular health conference, Alice Springs 2011. *Heart Lung Circ* 2012;**21**:632–8.
156. Rodrigues LC, Lockwood DNJ. Leprosy now: epidemiology, progress, challenges, and research gaps. *Lancet Infect Dis* 2011;**11**: 464–70.
157. Rupprecht CE, Briggs D, Brown CM, et al. Use of a reduced (4-dose) vaccine schedule for postexposure prophylaxis to prevent human rabies. *MMWR Recomm Rep* 2010;**59**(RR02):1–9.
158. Schaffzin JK, Pollock L, Schulte C, et al. Effectiveness of previous mumps vaccination during a summer camp outbreak. *Pediatrics* 2007;**120**:e862–e868.
159. Schobitz EP, Schmidt JM, Poirier MP. Biologic and chemical terrorism in children: an assessment of residents' knowledge. *Clin Pediatr (Phila)* 2008;**47**:267–70.
160. Sheppeard V, Forssman B, Ferson MJ, et al. The effectiveness of prophylaxis for measles contacts in NSW. *N S W Public Health Bull* 2009;**20**:81–5.
161. Smith A, Coles S, Johnson S, et al. An outbreak of influenza A(H1N1)v in a boarding school in South East England, May-June 2009. *Euro Surveill* 2009;**14**:19263.
162. Smith DK, Grohskopf LA, Black RJ, et al. Antiretroviral postexposure prophylaxis after sexual, injection-drug use, or other nonoccupational exposure to HIV in the United States. *MMWR Recomm Rep* 2005;**54**(RR-02):1–20.
163. Smith PF, Hadler JL, Stanbury M, et al. Blueprint version 2.0: Updating Public Health Surveillance for the 21st Century. *J Public Health Manage Pract* 2013;**19**:231–9.
164. Subbarao EK, Andrews-Mann L, Amin S, et al. Post-exposure prohylaxis for measles in a neonatal intensive care unit. *Pediatrics* 1990;**117**:782–5.
165. Tanne JH. US travellers to the London Olympics are warned about contracting measles. *BMJ* 2012;**344**:e2357.
166. The Prevention of Invasive Group A Streptococcal Infections Workshop Participants. Prevention of invasive group A streptococcal disease among household contacts of case patients and among postpartum and postsurgical patients: recommendations from the Centers for Disease Control and Prevention. *Clin Infect Dis* 2002;**35**:950–9.
167. van der Helm-van Mil AH. Acute rheumatic fever and poststreptococcal reactive arthritis reconsidered. *Curr Opin Rheumatol* 2010;**22**:437–42.
168. Vojdani JD, Beuchat LR, Tauxe RV. Juice-associated outbreaks of human illness in the United States, 1995 through 2005. *J Food Prot* 2008;**71**:356–64.
169. Weinstein L, LeFrock J. Does antimicrobial therapy of streptococcal pharyngitis or pyoderma alter the risk of glomerulonephritis? *J Infect Dis* 1971;**124**:229–31.
170. Welch TR. An approach to the child with acute glomerulonephritis. *Int J Pediatr* 2012;**2012**:1–3.
171. World Health Organization. *EHEC outbreak: update 13*. n.d. Available at: http://www.euro.who.int/en/what-we-do/health-topics/emergencies/international-health-regulations/news/news/2011/06/ehec-outbreak-update-13.
172. World Health Organization. *The weekly epidemiological record*. n.d. Available at: http://www.who.int/wer/en/.
173. World Health Organization. *Yaws: Key Facts*. n.d. Available at http://www.who.int/mediacentre/factsheets/fs316/en
174. World Health Organization. Cholera vaccines: WHO position paper. *Wkly Epidemiol Rec* 2010;**85**:117–28.
175. Wilson W, Taubert KA, Gewitz M, et al. Prevention of infective endocarditis: Recommendations by the American Heart Association—guideline from the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. *Circulation* 2007;**116**: 1736–54.
176. Workowski KA, Berman S, CDC. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**(RR-12): 110.
177. Wright JG, Quinn CP, Shadomy S, et al. Use of anthrax vaccine in the United States. *MMWR Recomm Rep* 2010;**59**(RR06):1–30.
178. Yu Y, Cheng AS, Wang L, et al. Hot tub folliculitis or hot hand-foot syndrome caused by *Pseudomonas aeruginosa*. *J Am Acad Dermatol* 2007;**57**:596–600.
179. Zalmanovici Trestioreanu A, Fraser A, Graffer-Gvili A, et al. Antibiotics for preventing meningococcal infections. *Cochrane Database Syst Rev* 2011:CD004785.

CHAPTER 247

Infections in Child Care Environments

Sheryl L. Henderson • Ellen R. Wald

Of the estimated 20 million children younger than 5 years identified in the 2010 U.S. Census, approximately 12 million (60%) were in some type of regular child care arrangement at least once a week. Approximately half of these received care provided by parents or relatives. Of the remainder, nearly 4.5 million (23%) preschool-age children were cared for in an out-of-home organized child care facility (such as a daycare, nursery, or preschool), and 2.4 million (12%) had care provided by a nonrelative either in their own home or a family daycare setting.[229]

The term *child care* is used to describe a variety of different options for the supervision of children in the absence of a parent or legal guardian. Types of child care include large child care centers housed in nonresidential settings that provide care for at least seven children in one location (included here are situations in which the parent's employer is the sponsor of the child care center, frequently at the site of employment, and also preschool and nursery schools in which child care is provided in a structured, primarily educational setting); child care homes in which care is provided in a residential setting for six or fewer children; before- and after-school care designed to bridge the gap between organized schools (e.g., preschool or public or private school) and parental departure for or return from work; and cooperatives in which child care is rendered by parents who alternate in caring for their own children as well as other enrolled children.

The quality of care provided to children attending various child care facilities varies widely. Although child care centers are licensed in most states, only 23 percent of children with regular child care arrangements attend licensed facilities.[229] State regulations vary from state to state; some are lenient in setting suggested ratios for staff to child, and some have no regulations governing group size. Even if impeccable regulations and well-trained caregivers were available, the regulations would not protect the majority of children who are in unlicensed facilities.

FACTORS AFFECTING TRANSMISSION OF INFECTIOUS AGENTS

Several studies have demonstrated increased numbers of infections in children attending out-of-home group child care versus those cared for at home.[60,165-167,234]

Multiple factors contribute to the transmission of infectious agents in the group child care setting (Table 247-1), the most important of which are host factors relating to the immunologic susceptibility of young children. Young infants are experiencing decreased immunity from maternal antibody and thus have increased immune susceptibility. Once infection is established in a child, it can be transmitted easily because of the natural tendency for intimacy in an age group that has not established acceptable toileting practices and is ignorant of basic hygienic practices. Respiratory and gastrointestinal pathogens frequently contaminate toys. Caregivers may have received insufficient training in infection control practices. A lack of policy regarding immunization and health care screening of employees may contribute to the spread of infection. In addition, overcrowding, understaffing, and poorly designed physical environments foster transmission of infectious agents in child care facilities. A lack of or inadequate numbers of hand washing facilities or the availability of sanitizing solutions for both children and providers creates an almost insurmountable barrier to infection control. Major risk factors for spread of infection are related to the age of the participants, the number of children, and the ratio of staff to children. The last is determined in part by the age of the children; younger children require higher staff-to-child ratios. However, in overcrowded situations with inadequate staff, infection can be spread easily because of inappropriate hand hygiene or inattention to other facets of infection control.

Unique factors identified as particularly important in the spread of gastrointestinal organisms are large numbers of diaper-aged children in centers where staff members who diaper children are also responsible for food preparation.[22,105] Separating diaper-aged children from those who are older is important to limit spread of enteric illness. If meals are prepared in the daycare setting, separating this activity from toilet areas is essential. If food is brought from home, it should be transported properly and stored to avoid spoilage. Lack of attention to this issue may result in additional illness. Infants and toddlers are indiscriminate in mouthing objects and fingers, thus contributing to the potential organisms that naturally populate the saliva and the respiratory tract to be transmitted readily from child to child.

Child care centers that operate for profit also are at higher risk for having infectious disease outbreaks, probably as a result of lower staff-to-children ratios.[219] Finally, parents may choose not to withdraw ill children from

TABLE 247-1 Risk Factors for Infectious Disease in Child Care Facilities

Children

Immunologic susceptibility to infectious agents
Lack of toilet training
Natural tendency to intimacy
Frequent oral contact with environment
Lack of awareness and practice of good hygiene

Caregivers

Insufficient training in infection control
Lack of policy regarding immunization
Inadequate screening for infectious diseases

Environmental and Economic Problems

Inappropriate staff-to-child ratios
Overcrowding
Failure to separate age groups
Poorly designed physical layout
  Inadequate or poorly placed sinks
  Failure to separate toilet areas from areas of food preparation
Parental pressure to admit sick children to daycare

attendance at child care voluntarily because of the expense and inconvenience of creating alternative child care arrangements.

MODES OF TRANSMISSION OF INFECTIOUS DISEASES IN OUT-OF-HOME CHILD CARE

The major modes of transmission include respiratory, gastrointestinal (primarily fecal-oral), person-to-person skin contact, and contact with infected body fluids—blood, urine, saliva, or other fluids. Some infections may use more than one mode of transmission (e.g., herpes simplex virus [HSV] can be spread through direct skin contact [if the skin is not intact] or exposure to infected body fluids). The most important pathogens and their modes of transmission of infection in child care are shown in Table 247-2. Respiratory and gastrointestinal infections are the most common causes of illness in infants, toddlers, and preschoolers, regardless of whether these children attend out-of-home child care.[125]

TABLE 247-2 Pathogens and Modes of Transmission of Infection in Child Care

Mode of Transmission	Bacteria	Viruses	Parasites
Respiratory	*Haemophilus influenzae* type b	Adenovirus	
	Streptococcus pneumoniae	Coronavirus	
	Neisseria meningitidis	Human metapneumovirus	
	Streptococcus pyogenes	Influenza A and B	
	Bordetella pertussis	Measles	
	Mycobacterium tuberculosis	Mumps	
	Kingella kingae	Parainfluenza	
		Parvovirus B19	
		Respiratory syncytial	
		Rhinovirus	
		Rubella	
		Varicella	
Fecal-oral	*Campylobacter* organisms	Enteroviruses	*Cryptosporidium parvum*
	Clostridium difficile	Hepatitis A	*Enterobius vermicularis*
	Escherichia coli O157:H7	Adenovirus	*Giardia lamblia*
	Salmonella organisms	Astrovirus	
	Shigella organisms	Calicivirus	
		Enterovirus	
		Rotavirus	
		Astrovirus	
Person-to-person by skin contact	*Streptococcus pyogenes*	Herpes simplex	*Pediculus capitis*
	Staphylococcus aureus		*Sarcoptes scabiei*
			Trichophyton species
			Microsporum species
Contact with blood, urine, or saliva		Cytomegalovirus	
		Hepatitis B	
		Herpes simplex	
		Human immunodeficiency virus	

Modified from American Academy of Pediatrics. Children in out-of-home child care. In: Pickering L, editor. *2006 Red Book: report of the Committee on Infectious Diseases*. 27th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2006, p. 132.

Respiratory

For some microbiologic species causing infection, the mode of transmission is the airborne route. The organism is aerosolized and remains in the air, like cigarette smoke. The microorganisms are present in small droplet nuclei (<5 μm) and are capable of traveling distances on air currents. Direct contact with the infected individual is not necessary for spread of the infection. Agents known to be transmitted by this means include the measles and varicella viruses and *Mycobacterium tuberculosis*.

Most commonly, respiratory organisms are spread by the production of droplets laden with infective particles. When an infected person talks, sneezes, or coughs, droplets containing these microorganisms are formed and expelled, and they may land on the mucosa of another individual. These droplets may also be transmitted directly from mucosa to mucosa when close physical contact occurs. More often, droplets land on nonporous surfaces (e.g., cribs, tables, toys, chairs) or on clothes and paper (fomites) and remain infective for minutes to hours. Hand contact with contaminated surfaces and fomites can result in infection if the hands touch the nasal or conjunctival mucosa.[107,125] Agents that can be transmitted by droplet spread (mucosa to mucosa), from finger to mucosa, or by fomites include most respiratory viruses (respiratory syncytial virus [RSV], rhinovirus, human metapneumovirus, coronavirus, human herpes virus 6 [HHV-6], influenza virus, parainfluenza virus, adenovirus, parvovirus B19, measles virus, mumps virus, rubella virus, and varicella-zoster virus), *Bordetella pertussis*, *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Streptococcus pyogenes*.* Finger-to-mucosa spread of respiratory pathogens is the most important and common mechanism for transmission of viral and bacterial infections.[115] Consequently, hand hygiene that includes hand washing and the use of alcohol-based sanitizers is an essential element of preventing spread of infection.[193,125]

Gastrointestinal

Spread of gastrointestinal organisms is by fecal-oral transmission. The number of organisms required to produce infection will determine whether infection occurs by person-to-person spread or whether a food or fluid intermediary is required. For example, rotavirus, *Giardia lamblia*, and *Shigella* spp. are transmitted readily in small numbers of organisms on the hands (without obvious gross contamination) after person-to-person contact or by touching infected surfaces. In contrast, *Salmonella*, rarely a cause of diarrheal outbreaks in the child care setting, requires large numbers of organisms to produce infection. Accordingly, an intermediary step of food or beverage contamination is required to allow organisms to replicate up to the necessary inocula.

Numerous studies have demonstrated fecal organisms on child care center environmental surfaces with which infants and toddlers have had contact as well as on the hands of care providers.[80,136,230,236] Contamination of the environment is highest when the children are younger than 3 years. This age predilection correlates with the number of children still wearing diapers.[231] Important pathogens, including fecal bacteria, rotavirus, hepatitis A virus (HAV), and *G. lamblia* cysts, are able to survive on environmental surfaces for periods ranging from hours to weeks.[56,144]

Skin

Bacterial, viral, and parasitic infections of the skin can be transmitted by person-to-person spread by direct contact. Bacterial pathogens such as *S. pyogenes* and *Staphylococcus aureus* usually are not primarily invasive unless a break in the integument occurs, such as after minor trauma (e.g., insect bites). HSV may be transmitted from skin or mucosa to skin by direct contact, again only if the skin is broken. Infestations such as scabies and lice are transmitted by person-to-person spread by mobile parasites. The superficial dermatophytes responsible for tinea infections (*Trichophyton*, *Microsporum*, and *Epidermophyton*) are transmitted by person-to-person spread or by contact with infected fomites such as combs, hairbrushes, and hats.

Blood, Urine, and Saliva

Hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), and cytomegalovirus (CMV) can be transmitted by blood and sexual activity (presumably resulting in microtrauma, thereby leading to blood exposure). However, transmission of HCV by sexual activity is an uncommon occurrence. Although both HBV and HIV can be demonstrated in urine and saliva, exchange of these body fluids is unlikely to transmit infection. At child care centers, transmission of CMV or HSV probably occurs between attendees by contamination of toys with saliva. Mothers and care providers can become infected with CMV by the finger-to-mucosa route after contamination of hands by urine or saliva.

PATTERNS OF INFECTION IN OUT-OF-HOME CHILD CARE

There are four general patterns of disease manifestations associated with children and adults in the child care setting that are important to recognize (Table 247-3).[101] Most of the increased infectious disease burden found in child care centers is experienced by the children themselves. Certain specific infections, such as those caused by RSV, cause significant illness almost exclusively in the children. However, for several illnesses, adult personnel may experience some of the same respiratory and gastrointestinal symptoms as the children do. Examples of infections for which child care attendees and staff share an increased burden of disease include shigellosis, giardiasis, and invasive meningococcal disease. There are also infections that cause mild or no symptoms in children but have significant repercussions for infected adults, whether parents or child care providers. The most notable of these

*References 82, 90, 91, 104, 107, 108.

TABLE 247-3 **Patterns of Occurrence of Infections Experienced in Daycare**

Patterns of Occurrence	Examples
Clinical manifestation of infection primarily in children	Respiratory syncytial virus and rotavirus infections
Infection affecting children, daycare staff, and close family members	Shigellosis, giardiasis, *Neisseria meningitidis* infection
Inapparent infection in children with clinically important infection in adult contacts	Hepatitis A
Inapparent or mild infection in children and adults but with possibly serious consequences for fetus in pregnant contact	Cytomegalovirus infection, rubella, parvovirus B19 infection, coxsackievirus A16 infection

Modified from Goodman RA, Osterholm MT, Granoff DM, et al. Infectious diseases and child day care. *Pediatrics* 1984;**74**:134-9. Copyright 1984, American Academy of Pediatrics.

diseases is hepatitis A, in which adults have an illness of moderate severity that often leads to extended absences from work. The incidence of hepatitis A disease has declined in the United States since introduction of routine hepatitis A vaccination in children.[41] Finally, with some infections, clinical manifestations in adults are mild, but the infection may have potential effects on the fetus if the child care provider or mother of a child care attendee is pregnant. CMV and rubella virus are associated with severe birth defects if infection occurs in the first trimester. Infection with parvovirus B19 during pregnancy poses a risk for the development of nonimmune hydrops in the fetus.

INFECTIOUS AGENTS IN CHILD CARE

Infections Spread by the Respiratory Route

Upper Respiratory Tract Infections

Respiratory viruses (rhinovirus, coronavirus, RSV, human metapneumovirus, parainfluenza virus, influenza virus, adenoviruses, HHV-6, and Epstein-Barr virus) are the most common causes of infection in preschool-age children.[24,82,91,238] The range of clinical manifestations includes asymptomatic infection, simple upper respiratory tract infection (rhinitis), acute otitis media, pharyngitis, croup, tracheitis, bronchiolitis, and pneumonitis. Disease may be mild or severe and involve a single level or more than one level of the respiratory tree. Children may experience multiple infections with each agent because of antigenic diversity within virus subtypes (e.g., influenza A virus), existence of multiple subtypes (e.g., rhinoviruses, coronaviruses), and failure of immunity to develop after a single exposure (e.g., RSV). Viruses are shed from the site of infection (conjunctiva, nose, throat) even before clinical symptoms develop, thereby rendering control of the spread of these infections in child care difficult. Documentation that children in out-of-home child care (family or large child care centers) experience more respiratory tract infections than those noted in children in home care is ample.* The risk of acquiring infection for children in group or family care is intermediate, between that for large child care centers and home care.[234]

The most common bacterial complication of viral upper respiratory tract infection is acute otitis media. The peak age incidence of otitis media is between 6 and 18 months, similar to that of viral upper respiratory tract infections. Most children have had at least one episode of acute otitis media by the time they reach their second birthday, and approximately one third will have had at least three episodes by that time.[223] Not surprisingly, because the frequency of episodes of viral respiratory tract infections is increased in children attending out-of-home child care, these children experience a notable increase in the frequency of episodes of otitis media.[186,212,215,217,234] Two reviews strongly supported the notion that attendance at child care is a major risk factor for acquiring acute otitis media (Table 247-4)[191,228] Wald and colleagues[234] provided data indicating that the risk of hospitalization for performance of myringotomy with tube placement is highest for children in large child care environments. Similarly, the risk of contracting recurrent acute otitis media and persistent middle ear effusion also is higher for children in child care than for those who receive care at home.[67,86,213,225] Researchers do not recommend that children with mild respiratory infections be excluded from child care or be separated from the group of well children because this strategy has not been shown to achieve an overall reduction in infections for children in child care facilities.

Systemic Viral Infections

Cytomegalovirus. CMV is a common cause of infection in preschool-age children. Most often, the infection is completely asymptomatic.[94] Rarely, the child may experience a febrile illness with lymphadenopathy and hepatosplenomegaly. In the United States, approximately 0.7 percent of newborns are infected with CMV in utero and shed virus at birth.[135] Of these, approximately 90 percent are asymptomatic.[32,214] Sources of CMV in the child care setting are infants and children who have been infected by their mothers by vertical transmission in utero, perinatally (2% to 3%), or post partum through breastfeeding (~6%). Potential sources of transmission in group child care are saliva and urine. Strangert and coworkers[216] reported the isolation of CMV from 7 of 10 children between 21 and 30 months of age in one child care center. Strom[218] found that 13 of 18 (72%) children between 24 and 36 months of age in a single nursery excreted CMV. Children younger than 3 years who acquire CMV after birth excrete CMV in urine and saliva for 6 to 42 months (mean, 18 months).[6] Other investigators demonstrated that peak rates of infection occur in children between 1 and 3 years of age, when viral excretion may be

*References 75, 89, 123, 150, 151, 165, 166, 212, 215, 234.

TABLE 247-4 **Comparisons of Annualized Rates of Acute Respiratory Tract Infections, Otitis Media, and Antibiotic Treatment among Children in Various Modes of Childcare**

Study	Length of Time Observed	Annual Rate of Respiratory Infection (per child-year)	Annual Rate of Otitis Media (per child-year)	Annual Rate of Antibiotic Treatment (per child-year)
Wald et al.[234]*				
DCC (n = 33)	12-18 months	6.3		
DCH (n = 23)	12-18 months	5.1		
HC (n = 97)	12-18 months	3.9		
Strangert[217]*†				
DCC (n = 108)	8 months	7.5	1.2	1.9
DCH (n = 42)	8 months	7.5	1.3	1.6
HC (n = 57)	8 months	3.0	0.5	0.8
Stahlberg [212]‡				
DCC (n = 23)	8 weeks	13.8	2.3	4.8
DCH (n = 23)	8 weeks	12.0	0.9	2.8
HC (n = 23)	8 weeks	9.9	1.1	1.4
Reves and Jones[186]§				
DCC (n = 42)	8 weeks	8.1	6.0	9.1
DCH (n = 72)	8 weeks	2.6	1.6	2.7
HC (n = 156)	8 weeks	3.0	1.6	2.5

*Telephone interview every 2 weeks.
†Active surveillance in DCC; telephone call every 4 months for children in DCH and HC.
‡Daily diary in all groups.
§Health maintenance organization abstraction covering an 8-week period for each child.
DCC, daycare center; DCH, daycare home; HC, home care.
Modified from Reves RR, Jones JA. Antibiotic use and resistance patterns in day care centers. *Semin Pediatr Infect Dis* 1990;**1**:212-21.

documented in as many as 70 percent of children in child care.*

Household contact is a common source of CMV in young children. A population-based study evaluating risk factors for CMV among 4- to 10-year-olds in the United States participating in the Third National Health and Nutrition Examination Survey identified CMV status of household members (mother and siblings) to be a strong predictor of CMV seroprevalence in the child. In this study, previous child care attendance was not shown to be a significant predictor.[214]

Infection in preschool-age children leads to viral shedding documented by positive CMV cultures obtained from swabs of the throat (thereby contaminating saliva) and urine. Transmission probably occurs through fomites (toys and blankets) contaminated with saliva rather than by respiratory droplets.[211] Shedding of CMV is as chronic among infected toddlers in child care centers as it is in children with perinatal or congenital infection.[6,175,176]

The greatest risk for CMV acquisition is during early pregnancy in child care providers or mothers of children attending daycare.[2-5] Such acquisition may lead to clinically evident congenital CMV infection (microcephaly, hepatosplenomegaly, chorioretinitis, psychomotor retardation, and deafness) in approximately 5 to 10 percent of infected children. Another 10 to 15 percent of infants experience occult but potentially damaging infection that results in milder degrees of hearing loss and learning disabilities.[211] Because of the high rates of asymptomatic excretion by children, it is important for child care personnel to be aware of the risks of transmission and to routinely practice appropriate hand hygiene.[15]

Parvovirus B19. Parvovirus B19, the etiologic agent of erythema infectiosum or fifth disease, is spread by the respiratory route. Viral replication, viremia, and nasopharyngeal shedding occur approximately 1 week before the development of clinical symptoms. This benign disease of childhood (in a normal host) may occur in preschool- and school-age children. The classic clinical illness is characterized by an erythematous rash on the face that gives a "slapped-cheek" appearance. This rash may follow a mild, nonspecific illness with fever, myalgias, and headache. The rash progresses after the first day and spreads as a maculopapular eruption beginning on the proximal ends of the extremities and extending to the distal portions and trunk. After several days, these lesions develop into a lacy reticular pattern on the proximal parts of the extremities and then fade.

Less typical presentations include the papulopurpuric gloves-and-socks syndrome, rubelliform rash, and petechial rash. A community outbreak of parvovirus with petechial rash among children in Wisconsin was recently described.[79] Unrecognized in immunocompetent hosts is an infection of the erythrocyte precursor that leads to transient red blood cell aplasia.

In adults, parvovirus B19 infection frequently causes arthralgia and arthritis. Its clinical importance in the context of daycare infections is transmission from an infected preschool-age child to a pregnant mother or

*References 2, 126, 134, 164, 174, 175.

child care provider.[97] Infection of the fetus may lead to nonimmune hydrops secondary to infection of erythrocyte progenitor cells. Estimates of fetal loss when a pregnant woman of unknown antibody status is exposed to parvovirus are 2.5 percent after household exposure and 1.5 percent after occupational exposure in a school or child care facility.[20]

Children are not excluded from group child care once rash is present as they are no longer contagious.[13] Routine exclusion of pregnant women from a child care center where erythema infectiosum is identified is not recommended.[15,110]

Hand, Foot, and Mouth Disease. Hand, foot, and mouth disease (HFMD) is a clinical syndrome characterized by a vesicular exanthem on the distal parts of the extremities and mild stomatitis. Infections are often asymptomatic or mild, but they can be associated with fever and significant pain with the vesicles. Severe stomatitis can lead to dehydration. HFMD is most commonly caused by the enteroviruses coxsackievirus A16 and enterovirus 71, although other enteroviruses have been identified. Enterovirus infections are common among preschool children as shown in seroepidemiologic studies. The disease is mild in children but has a high attack rate.

Transmission is person to person through contact with infected saliva, vesicles, respiratory secretions, or stool. Outbreaks usually occur during summer or autumn months in the United States. Two outbreaks of coxsackievirus A16 HFMD have been reported in children attending child care.[84,85] In recent years, outbreaks of HFMD associated with coxsackievirus A6 have been described worldwide; one outbreak in four geographically distinct states in the United States related to coxsackievirus A6 occurred during winter months. Of the 63 cases, 34 occurred in children younger than 2 years. The symptoms were more severe than those usually associated with coxsackievirus A16.[34]

The most significant clinical concern is the possibility of the development of infection during pregnancy, which may lead to spontaneous abortion.[84,172] Exclusion from group child care is not routinely recommended as symptomatic and asymptomatic children may shed virus in stool for several weeks.[15]

Local Bacterial Infections

***Streptococcus pyogenes* or Group A Streptococci.** Group A streptococci (GAS) are a frequent cause of respiratory and skin infections. The most common expression of infection with GAS is the development of pharyngitis and fever in an elementary school–age child. Usually, the throat infection is accompanied by tender anterior cervical nodes. In classic infection, none of the usual signs of viral upper respiratory disease—coryza, cough, and conjunctivitis—are present. Illness peaks in the late winter and spring. In contrast, streptococcal infection of a preschool-age child or child in group care takes the form of a protracted upper respiratory infection. Specifically, these patients have low-grade fever, persistent nasal discharge, anorexia, and cervical adenopathy.

Although exposure of child care attendees to an index case of streptococcal pharyngitis can be assumed to result in secondary cases, relatively few epidemics of GAS infection have been reported in child care facilities.[83,122,207] An outbreak occurred in a child care center in which preschool children shared facilities with kindergarten children in an after-school program. During a 3-month period, 47 percent of the child care population had positive pharyngeal cultures for GAS or positive results on a rapid antigen detection test.[207]

GAS can also be transmitted person to person by skin-to-skin contact and colonize healthy skin. Superficial infection (impetigo) may then occur at sites of traumatized skin (e.g., insect bites or scratches). Often, GAS and *S. aureus* are both isolated from sites of impetigo. Erysipelas and cellulitis due to GAS can result in an abrupt onset of fever and dramatic cutaneous erythema and tenderness often accompanied by regional adenopathy. Outbreaks of perianal cellulitis have been reported from child care centers.[163,195] A cluster of 12 cases of perianal cellulitis, characterized by a well-demarcated rash around the anus with itching, rectal pain, blood-streaked stools, and occasionally a purulent discharge, was reported from a kindergarten setting in Denmark.[178]

The diagnosis of streptococcal skin infection can be made by careful performance of wound or surface cultures (obtained after careful cleansing of the periwound area) or examination of tissue aspirates. Spread of typical impetigo occurs commonly within families and presumably also would occur within child care centers. Children with GAS infections of the throat or skin should be excluded from group child care for 24 hours after the initiation of appropriate antibiotic treatment.

Invasive Bacterial Diseases

***Kingella kingae*.** *K. kingae* is a fastidious gram-negative coccobacillus that colonizes the oropharynx and is becoming increasingly recognized as an important cause of osteoarticular infections in young children (younger than 3 years); it has also been identified in cases of bacteremia and endocarditis.[78,241] Colonization rates are highest in children younger than 2 years. Preceding stomatitis and upper respiratory viral infections have been recognized as risk factors for invasive disease.[18,78] Outbreaks of osteomyelitis and septic arthritis associated with *K. kingae* have been reported in child care settings in Minnesota[138] and Israel.[242] In 2007, a cluster of cases in a daycare center in North Carolina was reported in association with a case of endocarditis. Genetically similar *Kingella* isolates were obtained from asymptomatic children in the same daycare.[200] Colonization rates in these child care centers ranged from 7 to 42 percent.

***Neisseria meningitidis*.** *N. meningitidis* is a gram-negative, polysaccharide-encapsulated diplococcus found colonizing the nasopharynx. With the reduction of invasive *H. influenzae* type b and *S. pneumoniae* disease due to effective immunization strategies, *N. meningitidis* is the leading cause of bacterial meningitis in the United States. Meningococcemia is a major manifestation with significant morbidity. Less commonly, pericarditis, pneumonia,

arthritis, otitis media, or epiglottitis can occur. Active surveillance (1998-2007) in the United States observed the highest rates of invasive meningococcal disease among infants younger than 1 year, although the case-fatality rate was the lowest in this age group.[57]

The pathogenesis is hematogenous dissemination from the nasopharynx as a site of colonization. Transmission is person to person through infected respiratory droplets. Household contacts exposed to patients with meningococcal disease have a significantly higher risk of acquiring infection than does the general population.[160,162]

Secondary spread of infection with *N. meningitidis* has been recognized as a potential risk, especially in situations of crowding, such as in military barracks and college dormitories. In addition, reports suggest that the risk of acquiring secondary disease caused by *N. meningitidis* is increased in the child care setting.[131,147,169,192] In a Belgian report,[71] exposure in a daycare nursery during a prolonged meningococcal epidemic conferred a risk for infection that was 76 times greater than that in children of similar age who received care at home.

Prompt institution of chemoprophylaxis is the recommended strategy for management of all intimate contacts of a person with invasive *N. meningitidis* disease. Rifampin for 2 days (5 mg/kg/dose in infants <1 mo or 10 mg/kg every 12 hours to a maximum 600 mg) is the preferred regimen for children. Alternative medications in children and adults include single doses of ceftriaxone, azithromycin, or ciprofloxacin. Throat culture to identify those who require prophylaxis is not recommended. In an outbreak, meningococcal conjugate vaccine may also be used for prevention of secondary cases if the outbreak serotype is one that is contained in the vaccine.[15] Children with meningococcal disease should be excluded from child care until they have received antimicrobial treatment for 24 hours.

Two quadrivalent conjugate vaccines are licensed in the United States. Both contain components against the serogroups A, C, Y, and W135 and are recommended for routine administration to children at the age of 11 years or as young as 9 months for those at high risk for meningococcal disease (e.g., children with functional or anatomic asplenia, complement deficiencies, or travel to areas of the world where meningococcal disease is hyperendemic or endemic[15]). Serogroup B is present in the United States and accounts for 50 to 60 percent of disease in infants,[57] but it is not yet included in the vaccine.

***Mycobacterium tuberculosis*.** Infection caused by *M. tuberculosis* is one in which the direction of transmission is from an adult or adolescent to a child. Young children rarely are contagious; therefore, the identification of tuberculosis in a young child is a sentinel event and should prompt public health investigation to identify the index case. Cases of tuberculosis in children have been traced to attendance at daycare.[98,148,168] Screening of personnel who work in child care settings, both staff and volunteers at high risk for tuberculosis exposure, is essential to prevent childhood tuberculosis disease. Children identified with active tuberculosis are excluded from group child care until effective therapy is initiated with good adherence and they are determined to be noninfectious to others.[13]

Infections of the Gastrointestinal Tract

Parasitic Infections

***Giardia lamblia*.** *G. lamblia* is the most common intestinal parasite of humans and a major cause of diarrhea in child care centers.[27,185,199] Infection can be caused by the ingestion of as few as 10 cysts. Transmission is fecal-oral and most commonly results from person-to-person spread, although waterborne outbreaks have been documented.[137] Parents of daycare attendees are at risk for acquiring infection.[179] Asymptomatic excretion does occur and is most common in children younger than 3 years. Demonstration of *G. lamblia* cysts on environmental surfaces indicates additional potential for transmission.[56] Infection with this parasite causes infestation of the duodenum and proximal jejunum and results in asymptomatic carriage more often than clinical disease.[128] After an incubation period of approximately 2 weeks, symptomatic patients experience diarrhea, intermittent abdominal pain, anorexia, and flatulence. The most notable feature of the infection is its tendency to become protracted, thereby leading to weight loss, failure to thrive, and anemia. The diagnosis is made by identifying organisms by sensitive immunoassays or direct fluorescent antibody assays in stool samples or occasionally by examination of duodenal aspirates or intestinal biopsy specimens. After resolution of symptoms, either by virtue of treatment or by spontaneous cure, cysts may be shed for a long time.[128] Treatment is not recommended for asymptomatic individuals shedding *G. lamblia* cysts. For symptomatic individuals, treatment may be undertaken with metronidazole, tinidazole, or nitazoxanide. Relapses occur in approximately 15 percent of patients, often requiring a second course of treatment.

***Cryptosporidium*.** *Cryptosporidium* parasites can cause diarrhea in immunocompromised hosts, but infection usually results in self-limited illness in immunocompetent children and adults.[59] The usual clinical symptoms are watery diarrhea and low-grade fever with abdominal pain and weight loss. Vomiting occurs in approximately 30 percent of patients.

Cryptosporidium oocysts can be found in the soil, in water, in food, or on surfaces contaminated by feces. Transmission is fecal-oral. The most common source of transmission and outbreaks has been the swallowing of contaminated water (either drinking or recreational).[153] Increased incidence of cryptosporidiosis occurs during summer and early fall months, which corresponds to months with increased recreational water activities.[45] Like *G. lamblia*, *Cryptosporidium* has a low infectious dose for humans. Infection can occur with as few as 10 to 30 oocysts.[52] Spread of infection is facilitated in daycare centers because oocysts shed in the feces of infected persons are highly resistant to common disinfectants, including chlorine. Many outbreaks of diarrhea caused by *Cryptosporidium* have been reported from daycare centers.* Asymptomatic children and adults shed oocysts for weeks after infection.

*References 10, 11, 58, 62, 113, 222.

The diagnosis is made by examination of stool for oocysts with special stains such as the Kinyoun acid-fast stain. Alternative methods include direct fluorescent antigen detection and enzyme immunoassays specific for *Cryptosporidium* antigen. Routine stool examinations for ova and parasites can miss the cryptosporidium oocysts. Recommended treatment is a 3-day course of nitazoxanide. Treatment can reduce the duration of diarrhea and shedding of oocysts in stool.[12] Exclusion from child care is recommended until the child is asymptomatic.

Bacterial Pathogens

Shigella. Infection with *Shigella* organisms causes illness of variable severity but easy transmissibility. Accordingly, *Shigella* is one of the most common causes of diarrhea outbreaks in the daycare population,[35,21,96] and *Shigella* infection was among the first enteric diseases recognized to be spread in daycare centers. Infection can be caused by as few as 10 to 100 organisms. Although a low inoculum is sufficient to cause infection, the organism does not survive well outside the human host. Fecal-oral transmission by person-to-person contact is the primary mode of transmission. However, contaminated environmental objects or food can be the agents of transmission. Infection with *Shigella* may be caused by one of four species (*S. sonnei*, *S. flexneri*, *S. dysenteriae*, and *S. boydii*).

Shigellosis is primarily a disease of young children.[15] In outbreaks, younger children have the highest attack rate and are the most effective transmitters of infection.[224] Daycare personnel and family contacts also experience high attack rates.[121,221] After an incubation period of several days, fever and watery diarrhea, followed by crampy abdominal pain, tenesmus, and mucoid bloody stools, occur in patients with classic disease. In many cases, the illness is mild and self-limited and is indistinguishable from other causes of gastroenteritis. The presence of fecal leukocytes on examination of stool provides supportive information; stool culture is diagnostic.

Treatment of shigellosis with an appropriate antimicrobial effectively terminates the illness. Antimicrobial treatment decreases the duration of symptoms and fecal shedding. Untreated persons continue to excrete organisms for several weeks. Susceptibility varies both within and outside the United States, and therefore testing of stool isolates is indicated. Isolates with increased resistance to ampicillin and trimethoprim-sulfamethoxazole are being reported.[21,132]

Children should be excluded from child care if there is frequent stooling and difficulty containing stool in a diaper, frequent accidents in toilet-trained children, or blood or mucus in stool. They can return to child care once treatment is completed and two stool cultures collected at least a day apart are negative.

Campylobacter jejuni. *C. jejuni* is a common cause of bacterial diarrhea in children in the United States, but it is less likely than *Shigella* to cause outbreaks of diarrheal disease in daycare centers.[129,145,173] Most often, a high inoculum of *Campylobacter* is required to produce infection, thereby necessitating a food or water vehicle, such as contaminated foods, undercooked poultry, or unpasteurized milk.[81] Less often, transmission can occur by person-to-person spread of smaller numbers of organisms. The clinical findings are variable, but the usual onset includes fever, abdominal pain, and diarrhea. Watery diarrhea and low-grade fever with only modest constitutional signs of illness also may be observed. Abdominal pain may be present and, if severe, may mimic appendicitis or intussusception. The illness usually lasts between 3 and 7 days. The diagnosis can be made by culture of stool; examination of stool by darkfield microscopy or direct smear may be informative if it is performed by an experienced observer.

Treatment with erythromycin and azithromycin is effective in terminating excretion of the infective organism, which may be important in controlling epidemics or outbreaks. The impact of macrolides on the clinical course of disease is variable. Without treatment, an individual may shed bacteria up to 3 weeks. Antibiotic susceptibility patterns of the organism should be used to guide treatment.

Salmonella. *Salmonella* is the most common cause of bacterial diarrhea in many parts of the United States, but it is an uncommon cause of gastroenteritis outbreaks in daycare centers.[149] Because the organism is widespread in nature and has 2200 different serotypes, it has been difficult to control. Infection usually occurs after the ingestion of contaminated food or beverages. The most common serotypes in the United States are *Salmonella* serotype *typhimurium* and *Salmonella* serotype *enteritidis*.

Numerous outbreaks have been associated with ingestion of contaminated food or contact with colonized reptiles such as turtles.[111] Person-to-person spread seldom occurs, except in infancy, when the infective dose is low.[239] Children younger than 4 years have the highest incidence of symptomatic *Salmonella* infection.

The most common expression of infection with *Salmonella* is uncomplicated gastroenteritis. The illness begins approximately 12 to 36 hours after exposure and often is characterized by initial vomiting followed by diarrhea. Abdominal pain and fever are frequent accompaniments; occasionally, stools contain mucus and blood. Although most cases are self-limited, *Salmonella* infection can be distinguished from other causes of gastroenteritis by its occasionally protracted course.

Children younger than 5 years may excrete *Salmonella* organisms up to 3 months after infection. This highlights the great need for attention to hygiene in a daycare setting, particularly the separation of the activities of food handling and diaper changing. Exposure to reptiles and rodents should be limited.[15]

The diagnosis is made by culture of stool. In general, antimicrobials are not recommended unless bacteremia is documented or the episode becomes protracted and severe. Antimicrobial therapy can prolong the duration of fecal shedding of organisms. Management of special hosts, such as neonates and immunocompromised patients, is controversial. Although some authors recommend antimicrobial therapy for these groups, few data support this recommendation. For nontyphoidal *Salmonella* infection, exclusion from daycare is recommended

until the diarrhea resolves and the child is able to participate fully in care. Negative stool cultures are not required. Obtaining stool cultures from daycare contacts or the index case in convalescence is not necessary.[224] A high rate of asymptomatic colonization with *Salmonella* of multiple serotypes has been documented in daycare centers in Mexico.[171]

If *S. typhi* is isolated, temporary exclusion is recommended. Return to care is dependent on three negative stool cultures and clearance by a health professional for children younger than 5 years.[13]

Clostridium difficile. *C. difficile* is the classic cause of pseudomembranous colitis in patients who have received or are receiving antimicrobial agents, and it rarely causes disease unassociated with antimicrobial use. The organism, a gram-positive, spore-forming rod, is distributed widely in soil and in the gastrointestinal tract of humans. It frequently is found colonizing the gastrointestinal tract in up to 50 percent of asymptomatic infants. Infection is acquired by ingestion of spores from the environment or an infected person. Disease is a consequence of the elaboration of toxin or toxins by vegetative organisms. Hospital environments and daycare facilities are reservoirs for the organism.[139,140] During outbreaks of *C. difficile* diarrhea in daycare centers, environmental contamination is increased, as is recovery of *C. difficile* from the hands of children and staff.[140]

Clinical symptoms include fever, diarrhea, and abdominal cramps. Stools may contain blood and mucus. The illness varies in severity from mild to life-threatening. The diagnosis of *C. difficile* gastroenteritis is made by recovery of the toxin from a stool sample analyzed by enzyme-linked immunosorbent assay or nucleic acid techniques. Treatment includes cessation of antibiotics and supportive care. Specific antimicrobial therapy may be necessary to achieve clinical cure. Metronidazole is recommended for initial treatment in mild or moderate disease. Oral vancomycin is used initially in severe disease. There are newer agents being studied for the treatment of *C. difficile* in children. Children and personnel should be excluded from group child care until they are asymptomatic.

Escherichia coli. Infection with Shiga toxin–producing strains of *E. coli* (STEC, the most common of which is *E. coli* O157:H7) is characterized by bouts of often bloody diarrhea and abdominal pain. Significant morbidity may occur, with approximately 5 to 10 percent of infected children developing hemolytic-uremic syndrome.

Ruminants such as cattle are the main reservoir for STEC. Transmission occurs by the fecal-oral route through contaminated food or water, fomites, or direct contact with an infected person or carrier animal. Numerous outbreaks have occurred in association with raw meat, unpasteurized milk, contaminated foods, and recreational water.

Outbreaks have been reported in child care settings. In 1986, an outbreak of diarrhea caused by *E. coli* O157:H7 was reported from a daycare attended by 107 children.[210] Thirty-four percent of attendees became ill, with a significant increase in risk for younger children. Approximately one third of the children with diarrhea had bloody stools, and hemolytic-uremic syndrome developed in three children. Although infection with *E. coli* O157:H7 usually occurs after the ingestion of contaminated foods, person-to-person spread of infection most likely occurred in this epidemic. The diarrheal illness also was documented in family members of ill children.

Subsequent epidemiologic studies in Minnesota confirmed the mode of transmission for *E. coli* O157:H7 to be person to person in the daycare setting.[26] Accordingly, symptomatic children should be excluded from daycare until stools are formed and two stool specimens obtained 24 hours apart are culture negative for *E. coli* O157:H7 (obtained 48 hours after any antibiotic treatment).[15] Unfortunately, fecal shedding of *E. coli* O157:H7 may be prolonged in young children.[220]

A systematic analysis of 90 outbreaks occurring worldwide (1982-2006) identified approximately 20 percent of cases arising from secondary spread. The highest proportion of secondary cases was among children younger than 6 years.[208]

E. coli O157:H7 is recovered from stool samples after being plated on MacConkey-sorbitol agar. Sorbitol-negative colonies of *E. coli* are picked and screened with O157 antisera by tube agglutination. Several sensitive, specific, and rapid immunologic assays for detection of Shiga toxin are available and should be used along with culture for identification.[44] Antibiotic treatment is not recommended for persons with diarrhea caused by *E. coli* O157:H7.[240]

Viral Pathogens

Norovirus. The calciviruses norovirus and saporovirus are nonenveloped RNA viruses that have a worldwide distribution and are a cause of human gastroenteritis. Saporovirus is much less frequently an agent of gastroenteritis than is norovirus. Transmission is by person-to-person spread through the fecal-oral route or through contaminated food or water. Noroviral particles found in vomitus are potentially infectious through the airborne route during forceful vomiting. Norovirus is antigenically diverse; therefore, repeated bouts of diarrhea may occur with exposure to different viral variants. Norovirus is highly contagious; a very small inoculum is required to establish infection. Outbreaks frequently are observed in closed populations, including child care centers and on cruise ships. The attack rate usually is high. Two reports highlighted the transmissibility of this agent in the child care setting.[102,127]

Astroviruses. Astroviruses are common causes of diarrheal outbreaks in daycare[9,206] and other closed populations. Children younger than 4 years are infected most commonly, and the illness has a winter predominance. Transmission usually is by person-to-person spread by the fecal-oral route. This illness is indistinguishable from the other viral causes of gastroenteritis, presenting with watery diarrhea that may be accompanied by vomiting, fever, or abdominal pain. Healthy, asymptomatic individuals can shed virus for several weeks.

Infections Spread by Skin Contact

Methicillin-Resistant Staphylococcus aureus *(MRSA)*

S. aureus is part of the endogenous flora of humans; the nose is the primary site of carriage in adults and children. At any one time, approximately 30 percent of people carry *S. aureus* in their anterior nares.[155] Transmission is primarily through person-to-person skin contact but also through direct contact with contaminated environmental surfaces.

During the 1990s, there was an increasing prevalence of disease caused by methicillin-resistant *S. aureus* (MRSA) in children without predisposing risk factors, such as contact with the health care system.[38,116] Studies of colonization in daycare centers demonstrated likely transmission within the child care setting.[1,117,201]

This community-acquired MRSA (CA-MRSA) has a genetic predisposition for causing skin and soft tissue infections (e.g., abscesses, carbuncles, and furuncles). More rarely, CA-MRSA may cause severe invasive infections, such as bacteremia, endocarditis, osteomyelitis, pyomyositis, or necrotizing pneumonia. Methicillin-susceptible strains of *S. aureus* cause similar clinical findings, although to a lesser extent. Recent surveys of the incidence of CA-MRSA disease in hospital and community settings (including child care) have demonstrated the most rapid increase among young children.[63,92,161]

A survey of MRSA isolates from national databases derived from hospital discharges during a 10-year period from 1998 to 2007 demonstrated a 16-fold increase in hospitalization rates for CA-MRSA infections in children. The proportion of *S. aureus* abscesses caused by the CA-MRSA phenotype increased from 8.7 to 60.3 percent in this same period.[161] An epidemiologic survey (2001-2002) using data from the Active Bacterial Core surveillance program demonstrated that disease incidence related to CA-MRSA was highest among individuals younger than 2 years. Of the 1647 patients with CA-MRSA isolates, 77% had skin and soft tissue infection.[92]

Children with documented MRSA should not be excluded from child care for colonization or for skin or soft tissue infections. Any open and draining wounds should be covered.

Scabies

Scabies is an infection of the skin caused by infestation with the female mite *Sarcoptes scabiei*. It is an obligate parasite that undergoes its complete life cycle in humans. Transmitted by person-to-person spread from an infested individual, the mite buries itself beneath the stratum corneum and burrows along for its 30-day life span while laying two or three eggs per day. The larval and nymphal mites scatter after hatching to embed themselves in skin at distant sites. Some 3 to 6 weeks later, a pruritic eruption (worse at night) develops and leads to excoriation, bleeding, and crusting. The distribution of lesions varies with age. In adults and older children, the eruption, which consists of papules, vesicles, and nodules, occurs commonly in the interdigital spaces of the hands, on the extensor surface of the elbows, and around the umbilicus, waist, axillary lines, and genital area. In infants and young children, vesicular and eczematous lesions can be found on the hands and feet as well as on the face and head.

The diagnosis is made by scraping the lesions and demonstrating the mite, ova, or mite feces. Treatment of the index case should be undertaken with a scabicide, preferably 5 percent permethrin in children older than 2 months, applied to the entire body. It is applied from the neck down in older children and adults. Lindane is no longer recommended. Asymptomatic contacts should be treated simultaneously because they may be infected unknowingly and may be capable of transmitting the mite during this asymptomatic period. Clothing and bed linens that had contact with the skin of infected persons during the 3 days before treatment should be laundered in hot water. Alternatively, items can be stored in plastic for at least 4 days.[13]

Although scabies is spread primarily by intimate personal contact, skin-to-skin transmission can result from prolonged casual contact, as occurs in institutional settings, nursing homes, and daycare centers.[194] Mites can survive on inanimate surfaces for 2 to 3 days, a period that permits transmission by fomites such as clothes, bed linen, and furniture. An outbreak of scabies was reported in a hospital-affiliated daycare facility. Elimination of the problem required a coordinated effort with simultaneous treatment of all potentially infected individuals.[194] An infected child should be excluded from daycare until treatment has occurred, which is usually overnight.

Head Lice

Head lice infestation occurs commonly in children attending daycare, preschool, and elementary school as a consequence of infection with *Pediculus capitis*. It affects children of every socioeconomic status. The insect, a hemophagocytic ectoparasite, obtains nourishment by sucking capillary blood from the scalp. Female lice attach egg cases (nits) to the hair shafts at or very near the scalp. The eggs hatch 8 to 11 days later, and the louse nymphs are released.

The diagnosis is often made when the symptoms of scalp pruritus, excoriation, pyoderma, and regional lymphadenopathy cause the caregiver to inspect the scalp closely. Nits are observed readily, although live lice may be difficult to see, especially in light infestations. Transmission is by direct head-to-head contact and, less commonly, spread by fomites or live lice.

Treatment is with topical pediculicides, used as directed with care. These include permethrin lotions and pyrethrin-based products. Resistance has been identified with these products. In addition, products such as malathion, benzyl alcohol lotion, spinosad suspension, and ivermectin lotion may be used. Lindane shampoo is no longer recommended because of safety concerns.[15] Nits can be removed mechanically with spiral combs. Clothing and infested bedding can be washed on high temperatures (130° F [54.4° C]). Children should be excluded from the group child care setting at the end of the day until treatment has occurred.[13]

Infections Spread by Contact with Blood, Urine, and Saliva

Cytomegalovirus

CMV can be transmitted by blood, urine, and saliva as described earlier.

Herpes Simplex Virus

HSV is a DNA virus that commonly infects mucosal surfaces. Inoculation and replication at the site of infection are followed by the establishment of latency in sensory ganglia. Primary infection with HSV type 1 most often occurs in the oral mucosa. Approximately 15 percent of children 3 years of age and younger will have gingivostomatitis as the manifestation of primary HSV-1 infection.[19,50,196] Symptoms of primary infection range from asymptomatic to severe, painful ulcerations of the oral mucosa and perioral regions, resulting in dehydration because of inability or reluctance to swallow. Stomatitis may be accompanied by signs of upper respiratory infection, fever, pharyngitis, and regional lymphadenopathy. Reactivation of virus is manifested most often by asymptomatic shedding of virus in the saliva or the presence of labial ulcerations (e.g., cold sores). Other sites of recurrent lesions may be seen (e.g., herpetic whitlow resulting from a child's autoinoculating his or her fingers through sucking). Children who have perinatally acquired HSV may have recurrent infections at sites related to the original infection.

Transmission in a group child care setting is through direct contact with herpetic lesions or infected saliva (e.g., through sharing of mouthed objects). Studies have demonstrated high attack rates of HSV infection and stomatitis in orphanages[50,106] and daycare settings.[196,142] One study of 90 children in a daycare center (aged 0 to 37 months) in Japan identified four clusters of infection during the 4 years of observation. Horizontal transmission was confirmed by genetic analysis of the isolated viruses.[142]

Children with herpetic gingivostomatitis do not need to be excluded from group care unless there is difficulty in controlling drooling. Children and staff with recurrent infection do not need to be excluded.[13] Covering of active lesions with clothing or bandages during child care is sufficient.[15]

Human Immunodeficiency Virus

The potential transmission of HIV by blood or body fluid exposure can cause great concern. However, the risk of nonsexual, nonpercutaneous transmission is low. The risk for health care workers with percutaneous exposure to known HIV-infected blood by needle stick has been estimated to be fewer than 3 in 1000 exposures.[25] To date, HIV infection has not been reported to be transmitted in a child care center.

There are rare case reports of transmission of HIV within households with an infected member.[36,88,95,233] In most cases, transmission occurred in a setting with ongoing exposure of nonintact skin (e.g., eczema) or mucosal surfaces to infected blood or body fluids. Recent reports identified three children who were likely infected through the practice of food premastication by an HIV-infected caregiver. In two cases, the premastication occurred at a time the caregiver had bleeding gums or mouth sores.[95] In light of its rare horizontal transmission to nonsexual contacts within households with an infected member, HIV would not be expected to spread in child care.[93,133]

One potential high-risk situation may involve HIV-positive children who are persistent biters or who have extensive weeping skin lesions. However, several reports indicate a lack of transmission to individuals bitten or scratched by a person infected with HIV.[77,190,205,226]

Since the institution of the guidelines to identify HIV-positive women during pregnancy and to intervene with peripartum antiretroviral medication use,[37] the incidence of perinatal HIV infection in the United States has declined dramatically.[40] Recent estimates are that fewer than 200 infants are born in the United States with HIV infection. Children who are born in countries with high rates of HIV infection and immigrate or are adopted into the United States should be screened for HIV infection.

Parents and guardians need not disclose their child's HIV status to child care staff. Personnel should be trained in the use of standard precautions and use gloves for potential exposures to blood or body fluids. Children who have weeping skin lesions that cannot be covered or bleeding problems should be excluded from child care until the condition is resolved.[13]

VACCINE-PREVENTABLE DISEASES

Since the beginning of the vaccine era in the early 20th century, many of the infectious illnesses that were easily spread among children and household contacts have now been significantly decreased in number. A comparison of annual morbidity of 10 infectious diseases for which universal vaccination is recommended for U.S. children showed approximately 95 to 100 percent decrease in morbidity for all infections (2007 vs. baseline 20th century annual morbidity data).[181] There are currently 14 vaccines that are recommended for children in the United States before entrance into elementary school. These provide protection to young infants and children through the development of individual and herd immunity. However, there continues to be a need for awareness of the clinical presentations of vaccine-preventable diseases as unimmunized or underimmunized children (by family choice or due to immunocompromised status) may be exposed in group child care settings. Many infections are still present in high rates in other countries, and children born in these countries who immigrate may be asymptomatic carriers of infectious organisms (e.g., HAV).

Diphtheria

At the time of this writing, there has not been a case of diphtheria in the United States since 2003, although it is

still present and endemic in countries with low rates of vaccination. Diphtheria is caused by production of toxin by the bacterium *Corynebacterium diphtheriae*. Clinical presentation may include nasal symptoms, membranous pharyngitis, obstructive laryngotracheitis, or skin manifestations. The prominent symptoms are usually a severe sore throat and croup accompanied by toxemia.

Corynebacterium diphtheriae is spread by respiratory droplets from people with infection or who are carriers after intimate contact. Individuals are susceptible if they have not been immunized or have been immunized only partially. Treatment is with antitoxin and antibiotics (erythromycin or penicillin). Prophylaxis can be accomplished with erythromycin or penicillin if an individual is found to be a carrier. Immunization with a diphtheria toxoid–containing vaccine is effective in preventing disease and spread of infection.

Haemophilus influenzae Type b Infection

Haemophilus influenzae type b (Hib) used to be the major bacterial pathogen of childhood and was responsible for meningitis, epiglottitis, pneumonia, facial cellulitis, and septic arthritis. These infections occurred in children between 2 months and 5 years of age, with a peak occurring in those between 6 and 18 months of age. The organism colonizes the nasopharynx before becoming bloodborne; hematogenous dissemination results in a distant focus of infection. Infected children usually have a several-day history of mild upper respiratory symptoms followed by an abrupt onset of high fever and localized symptoms indicating the site of infection (e.g., irritability or seizures in central nervous system infection, cutaneous findings in cellulitis). Colonized individuals who lack anticapsular antibody are susceptible to the development of invasive disease.[103]

The availability and universal use (in infancy) of effective vaccines for the prevention of Hib infection have changed the epidemiology of infections caused by this pathogen dramatically.[204] Conjugate vaccines were introduced in 1987. Although there has been a dramatic decline in invasive disease since then, cases of invasive Hib disease are still reported sporadically. The incidence of Hib disease between 1999 and 2008 remained highest among children younger than 1 year.[154] Schulte and coworkers[197] reviewed the pattern of invasive Hib infection in New York State. These investigators demonstrated a dramatic decline in the incidence of Hib disease in children younger than 5 years, both those in daycare and those in home care. The decrease in cases of infection attributable to Hib occurred in children attending daycare centers before it was observed in non-daycare attendees, perhaps because of the requirement for Hib vaccine before enrollment in daycare. Rarely, cases are reported among fully vaccinated children in a daycare center.[159] In 2007, a voluntary recall of Hib vaccine by one supplier led to underimmunization of children younger than 15 months as immunization schedules were adjusted. Minnesota reported an increase in invasive disease (five children 3 years of age or younger) in 2008, higher than the previous 16 years. The majority of children were underimmunized because of parental refusal. A simultaneous survey of nasopharyngeal Hib carriage showed low rates of carriage in this age group.[152]

The virtual disappearance of this once common and serious problem and the high immunization rates with Hib vaccine obviate a lengthy discussion about the need for rifampin prophylaxis after exposure to cases of invasive disease. In the rare instance of invasive Hib disease occurring in a child care center housing unimmunized or partially immunized children (younger than 4 years), all daycare contacts (adults and children) should receive rifampin 20 mg/kg/day once daily for 4 days to a maximum dose of 600 mg.

Streptococcus pneumoniae Disease

Pneumococci are a common cause of bacterial infections of the upper and lower respiratory tracts, including otitis media, sinusitis, and pneumonia.[198] As respiratory pathogens, they also cause other upper respiratory tract infections, such as conjunctivitis, and important systemic illnesses, including occult bacteremia, bacteremic periorbital cellulitis, and meningitis. Multiply antibiotic–resistant *S. pneumoniae*, a problem that began in the 1960s, is now widespread in South Africa, in large parts of Europe, and, since 1990, in the United States.

S. pneumoniae is spread easily in the child care setting related to the high rates of nasopharyngeal carriage in young children. The ease of transmission of penicillin-resistant *S. pneumoniae* (tracked by antibiotic resistance patterns, capsular types, and genetic patterns) in the child care setting has been demonstrated amply.[76,114,183,184] In addition, although the precise risk of the acquisition by children of secondary cases of pneumococcal disease in daycare versus home care is unknown, several outbreaks of invasive *S. pneumoniae* disease in daycare centers have brought greater attention to this issue.[53,61,184]

A pneumococcal conjugate vaccine containing seven common serotypes of *S. pneumoniae* (PCV7) was licensed in the United States in March 2000. The use of PCV7 and other pneumococcal conjugate vaccines has been shown to reduce both nasopharyngeal colonization with the serotypes of *S. pneumoniae* contained in the vaccine (including the carriage of antibiotic-nonsusceptible *S. pneumoniae*)[64,65,109] and rates of invasive disease due to vaccine serotypes.[143] This secondary effect of the conjugate vaccines has resulted in a decrease of both colonization and disease among the contacts of vaccinees.[100]

In a large field trial conducted at the Kaiser Permanente Vaccine Study Center with the heptavalent vaccine, the vaccine performed very well. The trial achieved a 93 percent reduction in invasive disease (meningitis and bacteremia), a 73 percent reduction in consolidative pneumonia, and a 7 percent reduction in episodes of otitis media.[29] The implementation of PCV7 also had an impact on the rates of carriage and disease caused by penicillin-nonsusceptible strains. Active surveillance data from 1996 to 2004 demonstrated an 81 percent decrease in invasive disease caused by penicillin-nonsusceptible pneumococci in children younger than 2 years.[143]

However, soon after the introduction of PCV7, replacement pneumococcal strains were identified in the nasopharynx of healthy, asymptomatic children and were

also the cause of invasive disease (e.g., meningitis or bacteremia).[66,118,177] A 13-valent pneumococcal conjugate vaccine (PVC13) that incorporated antigens of the more invasive pneumococcal serotypes (e.g., serotype 19A) was licensed in 2010 and is now recommended for the universal vaccination of infants.

Influenza

Influenza is a common cause of upper respiratory infection in preschool-age children. The severity of infection is variable, from mild nasal congestion to a severe sepsis syndrome. Influenza can cause significant morbidity in people of all ages; children younger than 5 years carry a large burden of disease. Secondary lower respiratory tract infections with *S. aureus* or *S. pneumoniae* among other pathogens contributes to much of the morbidity in severe cases.

Influenza is transmitted by person-to-person spread through infected droplets with a cough or sneeze or indirectly from contaminated objects. Secondary transmission to household and child care contacts is a common occurrence.

Epidemics occur seasonally during the colder months of the year. However, in 2009-2012, a pandemic of influenza A (H1N1) strain was seen globally that began in the spring. During this pandemic, there was an observed fourfold increase in mortality among children compared with previous years.[49]

Because of variation in influenza A antigens, components of influenza vaccines are adjusted annually. The antigens for H1N1 have subsequently been included in the seasonal vaccine. There is a trivalent inactivated influenza vaccine and a live attenuated viral vaccine for seasonal immunization of persons older than 6 months.[15]

Children with influenza should not be excluded from child care unless they are unable to participate or if caring for the child would compromise the available staff.[13]

Varicella

Varicella, or chickenpox, is a highly contagious infection that is spread easily by the airborne route and by respiratory droplets. It is characterized by a pruritic, generalized vesicular rash that occurs in one to six crops, each crop separated by 24 to 36 hours. Fever usually is mild. The most common complication is secondary bacterial pyoderma, and although it is uncommon, pneumonia and secondary invasive infection by *S. pyogenes* can cause significant morbidity. Varicella-zoster virus remains latent in sensory ganglia after primary infection but can be reactivated as herpes zoster or "shingles," in which case it is manifested as a vesicular eruption involving one to three sensory dermatomes. If infection is acquired in early pregnancy, varicella embryopathy (limb bands or amputation) has been noted in a small fraction of children.

In 1995, live attenuated varicella vaccine became available for universal use in the United States in children between 12 and 18 months of age. The availability of this vaccine altered the epidemiology of varicella in the United States dramatically. The vaccine has been demonstrated to be highly effective during most outbreaks of varicella in child care[55,130] and in clinical practice.[232] The general effectiveness of the vaccine is 83 to 86 percent against all forms of the disease and 97 to 100 percent against moderate to severe disease. However, through active surveillance, cases of breakthrough disease in children who had received one dose of varicella vaccine were reported. Outbreaks were reported in elementary schools with high rates of vaccine coverage.[39,227] Breakthrough disease was seen in a daycare in Connecticut, in which 25 children had varicella (68% were vaccinated). Disease was milder among vaccinated children.[94] This led to the recommendation by the Advisory Committee on Immunization Practices (ACIP) in 2006 for a universal two-dose varicella vaccine schedule. The first dose is given when the child is 12 to 15 months of age, and a second dose is given at 4 to 6 years of age.[42]

Approximately 10 to 15 percent of the time, a child may have a vesicular rash at the site of vaccination, 5 to 26 days after receiving the immunization. If this does occur without further spread of rash or systemic symptoms, the child may attend child care with the lesions covered.[13] A child with chickenpox disease should be excluded from the child care setting until all lesions are scabbed over and the child is able to participate in activities.

Measles

Measles (rubeola) is a highly contagious respiratory infection. After many years of decline, it again reached epidemic proportions in impoverished and medically underserved inner-city areas in the United States between 1989 and 1992. Although susceptibility to measles virus infection occurs when maternal antibody wanes, the peak age group for measles in the preimmunization era was children between 5 and 9 years old. More recently, outbreaks have involved preschool-age children, thus setting the stage for outbreaks in daycare facilities.

Measles virus is spread by droplets, hand transmission, and the airborne route. The illness usually is moderate to severe; high fever and prominent respiratory symptoms (cough and coryza) and conjunctivitis are present for several days before onset of the rash. Diarrhea occasionally may be a prominent feature. After the rash erupts, the fever and respiratory symptoms persist for several more days. Pneumonia and encephalitis are complications of measles virus infection; each occurs at an incidence of 1 per 1000 cases.

Since the year 2000, measles has no longer been considered endemic in the United States. However, measles cases continue to be reported each year; there were 222 measles cases reported in 2011. Of these cases, 200 were linked to importation by travelers or to one of 17 outbreaks. Of the infected youth (younger than 19 years) who were eligible for vaccination, 50 percent were unvaccinated because of religious, philosophical, or personal objection.[48]

Measles is prevented effectively by immunization. Current recommendations are to immunize twice, once when the child is between 12 and 15 months of age and

again at 4 to 6 years of age. For exposed, susceptible children, vaccination is recommended with MMR vaccine within 72 hours of exposure. During an outbreak, vaccine may be given to children 6 to 11 months of age. However, a child who receives measles vaccine before 1 year of age must still receive the vaccine at 12 to 15 months of age; the first dose is not counted toward the two required for full protection. Immune globulin can be given within 6 days to an exposed, susceptible individual for whom the live viral vaccine is contraindicated. This includes infants younger than 12 months and immunocompromised individuals.[15] Children with measles and any exposed unimmunized children should be excluded from the group care setting until at least 2 weeks after the onset of rash in the last case of measles.[13]

Rubella

Rubella, another exanthematous disease of childhood, is much milder than rubeola but attacks a similar age group. Like measles, until recently, rubella had been nearly eradicated by routine universal immunization with MMR vaccine.

Rubella is characterized clinically by mild fever, lymphadenopathy (postauricular and occipital), and rash. The illness is difficult to distinguish from other viral exanthems. Infection may be completely asymptomatic. Children with documented rubella should be excluded from daycare until 7 days after the onset of illness.

If rubella is contracted during the first or early second trimester of pregnancy, a severe fetal infection resulting in microcephaly, deafness, congenital heart defects, eye disorders, and psychomotor retardation may result. Acquisition of infection during pregnancy may be a potential hazard for daycare personnel or mothers of children who attend daycare if an outbreak of rubella occurs in the daycare setting. In a large outbreak of rubella in Nebraska, 14 children (9 of whom were younger than 12 months) and 2 parents acquired their infection in a daycare center.[68]

The incidence of congenital rubella syndrome can be minimized by appropriate immunization of preschoolers and personnel. Any child or personnel with rubella infection (not congenital) should be excluded from the group child care setting until 7 days after the onset of rash. Any exposed, unimmunized or underimmunized children should be excluded from a group setting until they are immunized or determined by public health officials to be noninfectious if they do not receive immunization. A child with congenital rubella syndrome is considered contagious until at least 1 year of age unless two cultures of clinical specimens obtained 1 month apart are negative for rubella virus after 3 months of age.[15]

Pertussis

Pertussis, or whooping cough, is a highly communicable respiratory disease caused by *Bordetella pertussis*. The attack rate and severity of disease are highest in the first year of life. The illness classically is divided into three phases: catarrhal, paroxysmal, and convalescent. In the first stage, the child has no fever but has symptoms, primarily rhinorrhea and a cough. When the nasal symptoms resolve, however, the cough becomes and remains prominent for many weeks. The cough is characterized by paroxysms that are followed by an inspiratory effort (whoop) or leave the child exhausted and occasionally apneic. The convalescent stage is the many weeks necessary for complete recovery. Infected children younger than 1 month are at greatest risk for significant morbidity and mortality. Treatment with a macrolide is effective in decreasing the shedding of organisms, but it does not alter the course of the disease.

Complete immunization with a five-dose series of acellular pertussis vaccine is effective in preventing most cases of pertussis.[54] However, pertussis continues to cause disease in vaccinated and unvaccinated persons, with peaks in incidence occurring every few years. In 2004, there was a significant national increase in pertussis cases nationwide. In 2012, a pertussis epidemic occurred in the state of Washington, with highest incidence among infants younger than 1 year and children aged 10 to 14 years.[47] Similar age trends were seen nationwide in the many geographic areas affected by the epidemic.

After a person has been exposed to a case of pertussis, erythromycin, clarithromycin, and azithromycin provide effective prophylaxis and are first-line drugs, despite their negligible clinical effect after the paroxysmal stage begins. Trimethoprim-sulfamethoxazole is an alternative in a person older than 2 months who is unable to tolerate a macrolide or has macrolide-resistant pertussis documented. Prophylaxis is recommended for exposure in child care. If appropriate, booster doses of acellular diphtheria-tetanus-pertussis vaccine should be given to exposed children in a daycare or household setting. A child who has pertussis or has been exposed to a case of pertussis and is symptomatic should be excluded from daycare until 5 days of an appropriate course of a macrolide (azithromycin or erythromycin) has been received.[15] If no treatment is received, exclusion is for 21 days after cough onset. Immunization status should be updated if a person is underimmunized. Child care staff are an appropriate target group for receipt of a tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine licensed as a single booster vaccine for persons older than 10 years with no upper age limit.[46]

Poliomyelitis

Poliovirus is an enterovirus that now rarely causes disease in the United States. Although most infections with poliovirus are subclinical, in the preimmunization era, polio was the major cause of acquired paralytic disease, especially in older children and young adults. In some developing countries, it remains a major health problem.

Poliomyelitis is prevented effectively by use of either the live oral poliovirus vaccine or the inactivated, enhanced-potency parenteral vaccine (IPV).[182] The ACIP of the Centers for Disease Control and Prevention (CDC) and the American Academy of Pediatrics recommend a four-dose all-IPV vaccine schedule for routine immunization of all infants and children in the United States.[15]

Mumps

Mumps is a relatively benign infection of childhood that often is asymptomatic. The most prominent clinical feature is parotitis. On occasion, a clinically significant central nervous system infection causes unilateral sensorineural deafness. This infection had been dramatically reduced by widespread use of the MMR vaccine. However, in the past decade, large outbreaks have occurred in the United States. In 2006, an outbreak occurred primarily among vaccinated young adults in the Midwest,[70] and in 2009, an outbreak occurred primarily among vaccinated school-age children in a religious community in New York and New Jersey.[43] Children with mumps should be excluded from the group child care setting until 5 days after the onset of parotid swelling.[15]

Rotavirus Infection

Before the introduction of rotavirus vaccine for the immunization of infants in 2006, rotavirus was the most common identifiable infectious cause of gastroenteritis leading to hospitalization in young children.[30,72,120,156,235] It accounted for more than 25 percent of cases of diarrhea in the United States, and it is an important cause of diarrhea in developing countries as well, contributing substantially to the worldwide mortality figures for gastroenteritis.

A low inoculum of rotavirus is needed to cause infection. The virus can be recovered from asymptomatic hosts, especially neonates and infants.[51] It is shed in large numbers in the stool of symptomatic patients, thereby contributing to the high prevalence of the virus on environmental surfaces during outbreaks.[237] Viral shedding occurs both before and after symptoms have appeared.[180] Most illnesses caused by rotavirus occur in the cooler months in temperate climates, but they occur year-round in tropical areas. The peak attack rate is in the 6- to 24-month-old age group. After a brief incubation period of 2 to 3 days, patients may have prodromal respiratory symptoms (coryza and cough) and then varying combinations of vomiting, diarrhea, and low-grade fever that last 3 to 5 days. The range of severity is broad, and dehydration occasionally may be profound. Before the introduction of vaccine, many outbreaks were reported in group child care settings.[23,170,180,189] In prospective studies of diarrheal illness in children attending daycare, rotavirus was implicated in 6 to 24 percent of cases of gastroenteritis[22,23,120,219] as well as in 20 to 40 percent of outbreaks.[23]

Rotavirus cannot be cultivated easily in tissue culture in a viral diagnostic laboratory. The diagnosis is made with an antigen detection method on a stool specimen. Treatment is supportive, and exclusion from daycare is recommended until the diarrhea resolves.

In early 2006, a bovine rotavirus–based pentavalent vaccine was licensed for use in U.S. infants. Three doses of the vaccine are given orally at 2, 4, and 6 months of age.[16] In 2008, a monovalent attenuated human rotavirus was licensed for a two-dose schedule given orally at 2 and 4 months. Both have been shown to be effective in preventing rotavirus infection of any severity.[31,33,73,209]

Hepatitis A Virus

Before the institution of universal immunization of young children, HAV, an enterovirus, was a common cause of acute hepatitis in children. Like other enteric pathogens, this organism is transmitted by the fecal-oral route. Manifestations of infection vary remarkably according to age. In young children (younger than 6 years), infection with HAV may be entirely asymptomatic or associated with relatively mild and nonspecific symptoms, such as low-grade fever, anorexia, nausea, vomiting, and diarrhea in 30 percent of patients. Jaundice, a more specific marker of liver disease, occurs in less than 10 percent of children younger than 6 years.[99] In contrast, adults with hepatitis A often are icteric in conjunction with other gastrointestinal symptoms. Infection, when symptomatic, usually lasts several weeks but occasionally can become protracted.

Transmission is primarily by person-to-person spread, but fomites may play an important role because the organism can persist and remain infective in the dried state for months.[157] HAV is shed in high density in the stool of infected persons from 2 weeks before until 1 week after the onset of clinical symptoms. The diagnosis usually is made by detecting serologic evidence of marker antibodies against HAV (immunoglobulin M anti-HAV). Treatment is symptomatic. Prevention of illness in contacts can be accomplished with intramuscular immune serum globulin and with hepatitis A vaccine.[15] Children and adults (especially food handlers) with hepatitis A disease should be excluded from the group child care setting until 1 week after the onset of illness.[15]

Hepatitis A vaccine was initially recommended for individuals in high-risk situations (e.g., travel, immunocompromised status) in 1996. Since the recommendation for universal vaccination of children 12 to 23 months of age, there has been a significant reduction in the incidence of acute hepatitis A disease.[41] Recent clusters of infection have been reported among close contacts of asymptomatic, newly arrived international adoptees from countries with high rates of hepatitis A.[87] The American Academy of Pediatrics, the ACIP, and the CDC have recently recommended vaccination of close contacts of internationally adopted children at risk for hepatitis A, including parents and babysitters.[17]

Hepatitis B Virus

HBV is a DNA-containing virus that causes infections with a wide range of clinical manifestations from asymptomatic seroconversion to fatal hepatitis. Infection is more likely to be asymptomatic in children than in adults.[158] Common symptoms include fever, fatigue, anorexia, malaise, and jaundice. Other gastrointestinal symptoms, such as nausea, vomiting, and diarrhea, may be prominent. Joint symptoms (arthritis and arthralgias) and cutaneous lesions (papular acrodermatitis) may be noted early in the course of the illness.

The most common modes of transmission of HBV in adults are contact with blood and sexual activity. Young children usually acquire infection with HBV by vertical transmission from their mother at delivery. The maternal

infection may be acute or chronic. Infection acquired vertically by an infant usually is asymptomatic, but it leads to chronic carriage of hepatitis B surface antigen in most cases. With increasing immigration and adoption of infants from HBV-endemic areas, more HBV carrier children will be identified.[112] It is important to screen children from areas where the prevalence of hepatitis B is more than 2% as they arrive to the United States.

Transmission of HBV within the daycare setting is rare but has been reported.[69,74,202] In one case, the probable source was a bite by a child who was a carrier of HBV.[202] In another, a child care worker with chapped hands was exposed to the blood of a child who was an HBV carrier.[74] The third case involved an aggressive 21-month-old child with weeping dermatitis and a history of biting other children. Other investigations failed to demonstrate transmission in daycare facilities despite long-term contact, including one situation with a high potential for blood exposure.[203]

Implementation of the current recommendation to screen all pregnant women for HBV and to undertake universal immunization against hepatitis B in infancy is the most effective way to prevent the spread of this infection. In the United States, there has been a steady increase during the past decade in the uptake of vaccination of infants at birth, regardless of maternal hepatitis B status. Approximately 70 percent of infants received a birth dose in 2007.[243] Infants born to mothers positive for hepatitis B surface antigen are recommended to receive hepatitis B immune globulin in addition to the vaccine. The incidence of acute hepatitis B disease in youth younger than 19 years has decreased by 98 percent.[15] Because of the low risk of transmission within the group child care setting, the American Academy of Pediatrics, the American Public Health Association, and the CDC do not recommend exclusion of HBV-infected children from child care or HBV screening of children as a criterion for entry. Exclusion of any child is appropriate if the child has weeping sores that cannot be covered or uncontrolled bleeding problems.

CONTROL OF INFECTIONS IN OUT-OF-HOME CHILD CARE

The American Academy of Pediatrics, the American Public Health Association, and the National Resource Center for Health and Safety in Child Care and Early Education have developed consensus guidelines on planning and practices in child care that promote the health of children, including practices that prevent and control the spread of infection. There is information on collecting and maintaining health records of children and staff, planning the physical space of the facility, cleaning and disinfecting spaces, educating staff and families on recognizing potentially infectious illnesses, and establishing exclusion policies for specific infections.[14]

Written Policies

Each child care facility should have written policies for preventing and managing child and staff illness. This includes having written procedures for hand washing and hand hygiene; personal hygiene policies; environmental sanitation policies and procedures; and policies for collecting, filing, and updating health and immunization records of children and staff.

Environment and Physical Plant Characteristics

In the planning of child care facilities, space and equipment needs that minimize the risk for transmission of infectious agents must be considered. Because fecal contamination is related strongly and inversely to age, having physical premises large enough to separate children younger than 3 years from older children is important. The food handling and food storage areas should be separated from the toileting and diapering space. When tables in the play area are used for food, they must be sanitized before food service. Because contamination of hands is the most critical factor in transmission of infection, hand washing facilities must be easily available to staff and children. Such facilities are especially important in the diaper-changing/toileting and food preparation areas, and separate sinks should be used in each of these areas. The hand washing sinks should be made of nonporous materials and preferably be hands free and in easy reach of soap and towel dispensers.

Because fecal-contaminated surfaces are major vehicles for the spread of enteric infections, particular care must be taken in planning the space and selecting equipment for diaper-changing and toileting activities. When materials are selected, the choice should be based on durability and ease of cleaning and disinfecting. Diaper-changing tables should be made of nonporous materials, without cracks or crevices, that can be cleaned and disinfected easily. They should be cleaned and disinfected after each use. A hands-free, plastic-lined container should be used for disposal of soiled diapers and placed within easy reach of the changing table. The toddler area should be equipped with training toilets and junior-sized toilets with appropriate steps. The use of potty chairs is strongly discouraged. If they are used, they should be constructed of easily cleaned nonporous materials, used only in the bathroom area, cleaned in a utility sink designated for potty chairs, and disinfected after each use (both chair and sink).[14]

Immunization and Screening of Children and Staff

Primary immunizations in infants and toddlers have greatly reduced the number of many of the communicable illnesses that were easily transmitted among children in closed settings decades ago. Currently, the 14 vaccine-preventable diseases for a preschool child are diphtheria, tetanus, pertussis, poliomyelitis, influenza, measles, mumps, rubella, varicella, rotavirus infection, *H. influenzae* infection, *S. pneumoniae* disease, hepatitis A, and hepatitis B. Studies have shown that children who attend registered child care facilities are more likely to be up-to-date in their immunizations than are children cared for at home.[119] Laws requiring age-appropriate

vaccination of children attending licensed child daycare programs exist in almost all states.

Employees and volunteers should have a health assessment before initiating work with children. This includes updating immunizations or documenting immunity to infections such as hepatitis A and B, measles, mumps, rubella, varicella, diphtheria, tetanus, polio, pertussis (to include one dose of acellular pertussis), and influenza. Recommendations for vaccination of children and adults are updated regularly by the ACIP.[7] Staff should receive annual influenza vaccinations, especially as young infants are a most vulnerable population for significant morbidity with influenza illness.

Before initiating work in a child care setting, adult and adolescent staff should be screened for tuberculosis by skin testing or a blood interferon-γ release assay. If the initial screening result is negative, those at risk should have annual tuberculosis testing. If the result is positive, a chest radiograph should be performed, and the staff member should be evaluated and cleared by a health care provider or public health official before beginning child care activities.[14]

Education

An integral part of the control of infection within a daycare center is education of staff and families. The staff must understand the general principles of transmission and control of infections. Education before job placement and frequent in-service trainings reinforce the importance of some basic techniques, especially hand hygiene. Supervision is essential to ensure compliance with policies.[141]

Parents should be educated about recognition of illness, especially illnesses for which the child receives the best care at home. The rationale and importance of compliance with daycare center rules should be emphasized. The child care program should inform parents of the need to share information about communicable illnesses in the child or a family member.

Hygienic Standards and Interventions

The key factor in prevention of disease in the child care setting is maintenance of optimal hygienic standards based on recognized mechanisms of transmission of infection. Hand hygiene is considered the single most important preventive measure[28,101,141,179,193] in recognition of the finding that both respiratory and enteric pathogens are spread by contaminated hands. Hand hygiene includes hand washing and the use of alcohol-based sanitizers.

Two studies showed a modest benefit of increased attention to hand washing in preventing respiratory tract infections in children younger than 2 years and in preventing episodes of diarrhea in children older than 2 years.[187,188] Two other studies have evaluated the role of alcohol-based sanitizers in the homes of children in out-of-home child care in reducing the secondary spread of illness. One demonstrated a reduction in gastrointestinal illness among family members,[193] and the other showed a reduction in respiratory illnesses.[146] Applicability to the child care environment also has been evaluated as part of several multipronged interventional programs.[125] A meta-analysis of studies evaluating the effect of hand hygiene interventions on reducing respiratory and gastrointestinal illness in community settings showed interventions that involved hand hygiene education plus the use of ordinary soaps had the greatest decrease in gastrointestinal and respiratory illnesses. The majority of studies evaluated were performed in child care centers and involved children younger than 5 years. No significant differences in benefits were noted with the use of alcohol-based sanitizers or antibacterial soaps.[8]

Exclusion Policy

The use of exclusion policies for specific infectious illnesses reflects the understanding that when children have moderate to severe illnesses, they may require more individualized care than available staff can provide, and their continued presence in the child care setting may expose more children and staff to infection. Children with enteric illnesses shed infectious agents in their stool. When stool is not easily contained in a diaper or toilet-trained children have accidental stooling due to diarrhea, transmissibility of infection increases significantly. Similarly, respiratory agents shed in secretions are readily transmissible through coughing, sneezing, or contamination of hands or environmental surfaces.

The indications for exclusion of children from child care and return to care vary on the basis of the infection. In some cases, children known to have highly infectious illnesses should not be allowed to attend daycare until treatment is initiated (e.g., group A streptococcal infections) or the symptoms have resolved (e.g., diarrhea caused by *Shigella*, rotavirus, or *Giardia*) or until transmissibility has waned, as in pertussis, varicella, measles, and mumps. In addition, there are certain symptom complexes for which children should be excluded if the specific pathogen or the contagiousness of their illness is unknown (e.g., if a child has a high fever and a rash or diarrhea of unknown etiology). A complete list of recommendations for exclusion is presented in Table 247-5.

There are some children attending group child care who remain unimmunized or underimmunized because of either contraindications for health reasons (e.g., immunocompromised state) or exemptions for a family's philosophical, religious, or personal beliefs. In the event of an outbreak of a vaccine-preventable disease (such as measles), these children should be excluded from child care until return is indicated by disease-specific guidelines.[13]

There are conditions that do not necessarily require exclusion from child care. These include asymptomatic excretion of an enteropathogen, purulent or nonpurulent conjunctivitis, a rash without a fever or behavioral change, CMV infection, the carrier state of HBV infection, HIV infection, and parvovirus B19 infection in an immunocompetent host. For many infections, the highest risk of transmission of disease occurs before the appearance of recognizable symptoms. Once illness occurs, other children already have been exposed, and exclusion is a less effective strategy. For this reason, exclusion has not been shown to be successful in reducing the frequency of viral upper respiratory tract infections.

TABLE 247-5 **Recommendations for Temporary Exclusion from Child Care**

Symptom Complexes	Specific Etiology	Temporarily Exclude?	If Excluded, Readmit When
Illness that prevents child from participating comfortably in activities		Yes	Exclusion criteria are resolved and child is able to participate without compromising staff's ability to care for other children
Illness that results in a greater need for care than the program staff can provide without compromising their ability to care for other children		Yes	
Other health conditions that may indicate severe illness (e.g., fever, lethargy, persistent crying, difficulty breathing)		Yes	
Cold symptoms: runny nose, scratchy throat, coughing, sneezing, watery eyes, fever	Adenovirus Coxsackievirus Enterovirus Parainfluenza virus Respiratory syncytial virus Rhinovirus Coronavirus Influenza	No, unless: Fever accompanied by behavior change Looks or acts very ill Difficulty breathing Blood red or purple rash not associated with injury	
Conjunctivitis (pinkeye)	Bacterial Viral	No, unless child meets other exclusion criteria	For bacterial conjunctivitis: once parent has discussed with health professional Antibiotics may or may not be prescribed Exclusion criteria are resolved
Fever		No, unless: Behavior change Unable to participate Care would compromise staff's ability to care for other children	Able to participate Exclusion criteria are resolved
Diarrhea Frequent loose or watery stools compared with normal pattern Abdominal cramps Fever Sometimes accompanied by vomiting		Yes, if: Stool is not contained in the diaper Diarrhea is causing "accidents for toilet-trained children" Stool frequency exceeds 2 or more stools above normal for that child Blood or mucus in stool, abnormal color of stool Dry mouth, no tears or no urine output in 8 hours Jaundice	Diapered children have their stool contained by the diaper Toilet-trained children do not have toileting accidents Able to participate without compromising staff's ability to care for them
	E. coli O157:H7	As above	As above Test results from 2 consecutive stool cultures taken 24 hours apart are negative for *E. coli* O157:H7

Continued on following page

TABLE 247-5 **Recommendations for Temporary Exclusion from Child Care** (Continued)

Symptom Complexes	Specific Etiology	Temporarily Exclude?	If Excluded, Readmit When
	Salmonella	As above	As above For *S. typhi*, health professional must clear child for readmission and children (<5 years) must have 3 negative stool cultures
	Shigella	As above	As above When infected child is treated and 2 stool cultures taken 24 hours apart are negative Health professional must clear child
Mouth sores	Herpes simplex virus Enterovirus (e.g., coxsackievirus)	No, unless drooling steadily related to mouth sores	Able to participate Exclusion criteria are resolved
	Hepatitis A	Yes, children and staff, especially food handlers	One week after illness onset and contacts have received vaccine or immune globulin as recommended
	Impetigo (*S. pyogenes, S. aureus*)	Yes, wash and cover sores Exclude at end of day	When topical, oral, or systemic antibiotics are started If sores can be covered and kept dry
	Head lice	Yes, at end of day	After receiving recommended treatment
	Measles	Yes, ill child and unimmunized contacts not immunized within 72 hours of exposure	Until 4 days after rash onset Unimmunized contacts at least 2 weeks after onset of rash in last case of measles
	Mumps	Yes, ill child and unimmunized contacts during outbreak	Until 5 days after onset of swelling Unimmunized contacts—until health department determines safe return
	Pertussis	Yes	After 5 days of appropriate antibiotic treatment
	Scabies	Yes	Until treatment is completed
	Staphylococcal skin infection (MRSA)	No; open or draining boils or abscesses should be covered	
	Streptococcal pharyngitis	Yes	After 24 hours of antibiotic treatment
	Tuberculosis	Yes	When local health officials determine child to be noninfectious to others
	Varicella	Yes	When all blisters have scabs

Modified from American Academy of Pediatrics. *Managing infectious diseases in child care and schools: a quick reference guide.* 2nd ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009.

Prophylaxis of Close Contacts

Prophylaxis is a strategy that may be useful in the management of some infections that occur in child care centers. For example, if a child has had invasive disease caused by *N. meningitidis*, rifampin prophylaxis for all child care contacts may prevent secondary or associated cases. If a child has pertussis, exposed children may be protected by a booster immunization, if appropriate, and administration of erythromycin or azithromycin for prophylaxis.

During community epidemics, vaccination can be used as a strategy to prevent secondary infections. For example, immunization with MMR vaccine is successful in terminating epidemics of measles in elementary or high schools. Varicella vaccine also can be used throughout a community and up to 3 days after exposure to curtail the spread of disease. Hepatitis A vaccine is an appropriate intervention for controlling an outbreak of hepatitis A in child care centers and other settings.[124]

NEW REFERENCES SINCE THE SIXTH EDITION

7. Advisory Committee on Immunization Practices. Available from http://www.cdc.gov/vaccines/schedules/hcp/index.html.
8. Aiello AE, Coulborn RM, Perez V, et al. Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. *Am J Public Health* 2008;**98**:1372–81.
13. American Academy of Pediatrics. *Managing infectious diseases in child care and schools: a quick reference guide*. 2nd ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009.
14. American Academy of Pediatrics, American Public Health Association, and National Resource Center for Health and Safety in Child Care and Early Education. *Caring for our children: national health and safety performance standards; guidelines for early care and education programs*. 3rd ed (CFOC3). Elk Grove, Ill: American Academy of Pediatrics; Washington DC: American Public Health Association; 2011. Available from http://nrckids.org/CFOC3/.
15. American Academy of Pediatrics; Pickering LK, editor. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
21. Arvelo W, Hinkle CJ, Nguyen TA, et al. Transmission risk factors and treatment of pediatric shigellosis during a large daycare center–associated outbreak of multidrug resistant *Shigella sonnei*: implications for the management of shigellosis outbreaks among children. *Pediatr Infect Dis J* 2009;**28**:976–80.
33. Castilla J, Beristain X, Martinez-Artola V, et al. Effectiveness of rotavirus vaccines in preventing cases and hospitalizations due to rotavirus gastroenteritis in Navarre, Spain. *Vaccine* 2012;**30**:539–43.
34. Centers for Disease Control and Prevention. Severe hand, foot, and mouth disease associated with coxsackievirus A6—Alabama, Connecticut, California, and Nevada, November 2011–February 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:213–14.
43. Centers for Disease Control and Prevention. Mumps outbreak—New York, New Jersey, Quebec, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1270–4.
44. Centers for Disease Control and Prevention. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Morb Mortal Wkly Rep* 2009;**58**(RR-12):1–20.
45. Centers for Disease Control and Prevention. Cryptosporidiosis and giardiasis surveillance 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**(SS-6):1–14.
47. Centers for Disease Control and Prevention. Pertussis epidemic—Washington, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:517–22.
48. Centers for Disease Control and Prevention. Measles—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:253–7.
49. Centers for Disease Control and Prevention. Update: influenza activity—United States, 2009-2010 season. *MMWR Morb Mortal Wkly Rep* 2012;**59**:901–8.
57. Cohn AC, MacNeil JR, Harrison LH, et al. Changes in *Neisseria meningitidis* disease epidemiology in the United States, 1998-2007: implications for prevention of meningococcal disease. *Clin Infect Dis* 2010;**50**:184–91.
60. Cote SM, Petitclerc A, Raynault MF, et al. Short- and long-term risk of infections as a function of group child care attendance: an 8-year population-based study. *Arch Pediatr Adolesc Med* 2010;**164**:1132–7.
70. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. *N Engl J Med* 2008;**358**:1580–9.
73. Desai SN, Esposito DB, Shapiro ED, et al. Effectiveness of rotavirus vaccine in preventing hospitalization due to rotavirus gastroenteritis in young children in Connecticut, USA. *Vaccine* 2010;**28**:7501–6.
78. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
79. Edmonson MB, Riedesel EL, Williams GP, et al. Generalized petechial rashes in children during a parvovirus B19 outbreak. *Pediatrics* 2010;**125**:e787–92.
82. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
87. Fischer GE, Teshale EH, Miller C, et al. Hepatitis A among international adoptees and their contacts. *Clin Infect Dis* 2008;**47**:812–14.
95. Gaur AH, Freimanis-Hance L, Dominguez K, et al. Knowledge and practice of prechewing/prewarming food by HIV-infected women. *Pediatrics* 2011;**127**:e1206–11.
98. Gillman A, Berggren I, Bergstrom SE, et al. Primary tuberculosis infection in 35 children at a Swedish day care center. *Pediatr Infect Dis J* 2008;**27**:1078–82.
109. Hampton LM, Farley MM, Schaffner W, et al. Prevention of antibiotic-nonsusceptible *Streptococcus pneumoniae* with conjugate vaccines. *J Infect Dis* 2012;**205**:401–11.
111. Harris JR, Bergmire-Sweat D, Schlegel JH, et al. Multistate outbreak of *Salmonella* infections associated with small turtle exposure, 2007-2008. *Pediatrics* 2009;**124**:1388–94.
117. Hewlett AL, Falk PS, Hughes KS, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* in a university medical center day care facility. *Infect Control Hosp Epidemiol* 2009;**30**:985–92.
152. Lowther SA, Shinoda N, Juni BA, et al. *Haemophilus influenzae* type b infection, vaccination, and *H. influenzae* carriage in children in Minnesota, 2008-2009. *Epidemiol Infect* 2012;**140**:566–74.
154. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* disease—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.
161. Mera RM, Suaya JA, Amrine-Madsen H, et al. Increasing role of *Staphylococcus aureus* and community-acquired methicillin-*resistant Staphylococcus aureus* infections in the United States: a 10-year trend of replacement and expansion. *Microb Drug Resist* 2011;**17**:321–8.
200. Sena AC, Seed P, Nicholson B, et al. *Kingella kingae* endocarditis and a cluster investigation among daycare attendees. *Pediatr Infect Dis J* 2010;**29**:86–8.
208. Snedeker KG, Shaw DJ, Locking ME, et al. Primary and secondary cases in *Escherichia coli* O157 outbreaks: a statistical analysis. *BMC Infect Dis* 2009;**9**:144.
209. Soares-Weiser K, Maclehose H, Bergman H, et al. Vaccines for preventing rotavirus diarrhoea: vaccines in use. *Cochrane Database Syst Rev* 2012;(2):CD008521.
214. Staras SA, Flanders WD, Dollard SC, et al. Cytomegalovirus seroprevalence and childhood sources of infection: a population-based study among pre-adolescents in the United States. *J Clin Virol* 2008;**43**:266–71.
229. United States Census Bureau. *Who's minding the kids? Child care arrangements: Spring 2010 (SIPP 2008)*. Updated December 5, 2011; cited June 28, 2012. Available from http://www.census.gov/hhes/childcare/.
243. Zhao Z, Murphy TV, Jacques-Carroll L. Progress in newborn hepatitis B vaccination by birth year cohorts—1998-2007, USA. *Vaccine* 2011;**30**:14–20.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Adcock PM, Pastor P, Medley F, et al. Methicillin-resistant *Staphylococcus aureus* in two child care centers. *J Infect Dis* 1998;**178**:577–80.
2. Adler SP. The molecular epidemiology of cytomegalovirus transmission among children attending a day care center. *J Infect Dis* 1985;**152**:760–8.
3. Adler SP. Molecular epidemiology of cytomegalovirus: evidence for viral transmission to parents from children infected at a day care center. *Pediatr Infect Dis* 1986;**5**:315–18.
4. Adler SP. Cytomegalovirus transmission among children in day care, their mothers and caretakers. *Pediatr Infect Dis J* 1988;**7**:279–85.
5. Adler SP. Molecular epidemiology of cytomegalovirus: viral transmission among children attending a day care center, their parents, and caretakers. *J Pediatr* 1988;**112**:366–72.
6. Adler SP. Molecular epidemiology of cytomegalovirus: a study of factors affecting transmission among children at three day-care centers. *Pediatr Infect Dis J* 1991;**10**:584–90.
7. Advisory Committee on Immunization Practices. *Immunization schedules*. Available from http://www.cdc.gov/vaccines/schedules/hcp/index.html.
8. Aiello AE, Coulborn RM, Perez V, et al. Effect of hand hygiene on infectious disease risk in the community setting: a meta-analysis. *Am J Public Health* 2008;**98**:1372–81.
9. Akihara S, Phan TG, Nguyen TA, et al. Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan. *Arch Virol* 2005;**150**:2061–75.
10. Alpert G, Bell LM, Kirkpatrick CE, et al. Cryptosporidiosis in a day-care center. *N Engl J Med* 1984;**311**:860–1.
11. Alpert G, Bell LM, Kirkpatrick CE, et al. Outbreak of cryptosporidiosis in a day-care-center. *Pediatrics* 1986;**77**:152–7.
12. Amadi B, Mwiya M, Musuku J, et al. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. *Lancet* 2002;**360**:1375–80.
13. American Academy of Pediatrics. *Managing infectious diseases in child care and schools: a quick reference guide*. 2nd ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2009.
14. American Academy of Pediatrics, American Public Health Association, and National Resource Center for Health and Safety in Child Care and Early Education. *Caring for our children: national health and safety performance standards; guidelines for early care and education programs*. 3rd ed (CFOC3). Elk Grove, Ill: American Academy of Pediatrics; Washington DC: American Public Health Association; 2011. Available from http://nrckids.org/CFOC3/.
15. American Academy of Pediatrics; Pickering LK, editor. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012.
16. American Academy of Pediatrics Committee on Infectious Diseases. Prevention of rotavirus gastroenteritis: guides for use of rotavirus vaccine. *Pediatrics* 2007;**119**:171–82.
17. American Academy of Pediatrics Committee on Infectious Diseases. Recommendations for administering hepatitis A vaccine to contacts of international adoptees. *Pediatrics* 2011;**128**:803–4.
18. Amir J, Yagupsky P. Invasive *Kingella kingae* infection associated with stomatitis in children. *Pediatr Infect Dis J* 1998;**17**:757–8.
19. Amir J, Harel L, Smetana Z, et al. The natural history of primary herpes simplex type 1 gingivostomatitis in children. *Pediatr Dermatol* 1999;**16**:259–63.
20. Anderson LJ. Human parvoviruses. *J Infect Dis* 1990;**161**:603–8.
21. Arvelo W, Hinkle CJ, Nguyen TA, et al. Transmission risk factors and treatment of pediatric shigellosis during a large daycare center–associated outbreak of multidrug resistant *Shigella sonnei*: implications for the management of shigellosis outbreaks among children. *Pediatr Infect Dis J* 2009;**28**:976–80.
22. Bartlett AV, Moore M, Gary GW, et al. Diarrheal illness among infants and toddlers in day care centers. I. Epidemiology and pathogens. *J Pediatr* 1985;**107**:495–502.
23. Bartlett AV 3rd, Reves RR, Pickering LK. Rotavirus in infant-toddler day care centers: epidemiology relevant to disease control strategies. *J Pediatr* 1988;**113**:435–41.
24. Beem MO. Acute respiratory illness in nursery school children: a longitudinal study of the occurrence of illness and respiratory viruses. *Am J Epidemiol* 1969;**90**:30–44.
25. Bell DM. Occupational risk of human immunodeficiency virus infection in healthcare workers: an overview. *Am J Med* 1997;**102**:9–15.
26. Belongia EA, Osterholm MT, Soler JT, et al. Transmission of *Escherichia coli* O157:H7 infection in Minnesota child day-care facilities. *JAMA* 1993;**269**:883–8.
27. Black RE, Dykes AC, Sinclair SP, et al. Giardiasis in day-care centers: evidence of person-to-person transmission. *Pediatrics* 1977;**60**:486–91.
28. Black RE, Dykes AC, Anderson KE, et al. Handwashing to prevent diarrhea in day-care centers. *Am J Epidemiol* 1981;**113**:445–51.
29. Black S, Shinefield H, Fireman B, et al. Efficacy, safety and immunogenicity of heptavalent pneumococcal conjugate vaccine in children. *Pediatr Infect Dis J* 2000;**19**:187–95.
30. Blacklow NR, Greenberg HB. Medical progress—viral gastroenteritis. *N Engl J Med* 1991;**325**:252–64.
31. Boom JA, Tate JE, Sahni LC, et al. Effectiveness of pentavalent rotavirus vaccine in a large urban population in the United States. *Pediatrics* 2010;**125**:e199–207.
32. Cannon MJ. Congenital cytomegalovirus (CMV) epidemiology and awareness. *J Clin Virol* 2009;**46**(Suppl 4):S6–10.
33. Castilla J, Beristain X, Martinez-Artola V, et al. Effectiveness of rotavirus vaccines in preventing cases and hospitalizations due to rotavirus gastroenteritis in Navarre, Spain. *Vaccine* 2012;**30**:539–43.
34. Centers for Disease Control and Prevention. Severe hand, foot, and mouth disease associated with coxsackievirus A6—Alabama, Connecticut, California, and Nevada, November 2011–February 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:213–14.
35. Centers for Disease Control and Prevention. Multiply resistant shigellosis in a day-care center—Texas. *MMWR Morb Mortal Wkly Rep* 1986;**35**:753–5.
36. Centers for Disease Control and Prevention. Human immunodeficiency virus transmission in household settings—United States. *MMWR Morb Mortal Wkly Rep* 1994;**43**:347, 353–6.
37. Centers for Disease Control and Prevention. Recommendations of the U.S. Public Health Service Task Force on the use of zidovudine to reduce perinatal transmission of human immunodeficiency virus. *MMWR Morb Mortal Wkly Rep* 1994;**43**(RR-11):1–15.
38. Centers for Disease Control and Prevention. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*—Minnesota and North Dakota, 1997-1999. *JAMA* 1999;**282**:1123–5.
39. Centers for Disease Control and Prevention. Outbreak of varicella among vaccinated children—Michigan, 2003. *MMWR Morb Mortal Wkly Rep* 2004;**53**:389–92.
40. Centers for Disease Control and Prevention. Achievements in public health: reduction in perinatal transmission of HIV infection—United States, 1985-2005. *MMWR Morb Mortal Wkly Rep* 2006;**55**:592–7.
41. Centers for Disease Control and Prevention. Prevention of hepatitis A through active or passive immunization. *MMWR Morb Mortal Wkly Rep* 2006;**55**(RR-7):1–23.
42. Centers for Disease Control and Prevention. Prevention of varicella—recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Morb Mortal Wkly Rep* 2007;**56**(RR-04):1–40.
43. Centers for Disease Control and Prevention. Mumps outbreak—New York, New Jersey, Quebec, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:1270–4.
44. Centers for Disease Control and Prevention. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Morb Mortal Wkly Rep* 2009;**58**(RR-12):1–20.
45. Centers for Disease Control and Prevention. Cryptosporidiosis and giardiasis surveillance 2010. *MMWR Morb Mortal Wkly Rep* 2010;**59**(SS-6):1–14.
46. Centers for Disease Control and Prevention. FDA approval of expanded age indication for a tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1279–80.
47. Centers for Disease Control and Prevention. Pertussis epidemic—Washington, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:517–22.

48. Centers for Disease Control and Prevention. Measles—United States, 2011. *MMWR Morb Mortal Wkly Rep* 2012;**61**:253–7.
49. Centers for Disease Control and Prevention. Update: influenza activity—United States, 2009-2010 season. *MMWR Morb Mortal Wkly Rep* 2012;**59**:901–8.
50. Cesario TC, Poland JD, Wulff H, et al. Six years experience with herpes simplex virus in a children's home. *Am J Epidemiol* 1969;**90**:416–22.
51. Champsaur H, Questiaux E, Prevot J, et al. Rotavirus carriage, asymptomatic infection, and disease in the first two years of life. I. Virus shedding. *J Infect Dis* 1984;**149**:667–74.
52. Chappell CL, Okhuysen PC, Sterling CR, et al. *Cryptosporidium parvum:* intensity of infection and oocyst excretion patterns in healthy volunteers. *J Infect Dis* 1996;**173**:232–6.
53. Cherian T, Steinhoff MC, Harrison LH, et al. A cluster of invasive pneumococcal disease in young children in child care. *JAMA* 1994;**271**:695–7.
54. Christie CD, Marx ML, Daniels JA, et al. Pertussis containment in schools and day care centers during the Cincinnati epidemic of 1993. *Am J Public Health* 1997;**87**:460–2.
55. Clements DA, Moreira SP, Coplan PM, et al. Postlicensure study of varicella vaccine effectiveness in a day-care setting. *Pediatr Infect Dis J* 1999;**18**:1047–50.
56. Cody MM, Sottnek HM, O'Leary VS. Recovery of *Giardia lamblia* cysts from chairs and tables in child day-care centers. *Pediatrics* 1994;**94**(Pt 2):1006–8.
57. Cohn AC, MacNeil JR, Harrison LH, et al. Changes in *Neisseria meningitidis* disease epidemiology in the United States, 1998-2007: implications for prevention of meningococcal disease. *Clin Infect Dis* 2010;**50**:184–91.
58. Combee CL, Collinge ML, Britt EM. Cryptosporidiosis in a hospital-associated day-care-center. *Pediatr Infect Dis J* 1986;**5**: 528–32.
59. Cordell RL, Addiss DG. Cryptosporidiosis in child-care settings—a review of the literature and recommendations for prevention and control. *Pediatr Infect Dis J* 1994;**13**:310–17.
60. Cote SM, Petitclerc A, Raynault MF, et al. Short- and long-term risk of infections as a function of group child care attendance: an 8-year population-based study. *Arch Pediatr Adolesc Med* 2010;**164**: 1132–7.
61. Craig AS, Erwin PC, Schaffner W, et al. Carriage of multidrug-resistant *Streptococcus pneumoniae* and impact of chemoprophylaxis during an outbreak of meningitis at a day care center. *Clin Infect Dis* 1999;**29**:1257–64.
62. Crawford FG, Vermund SH, Ma JY, et al. Asymptomatic cryptosporidiosis in a New York City day care center. *Pediatr Infect Dis J* 1988;**7**:806–7.
63. Creech CB, Kernodle DS, Alsentzer A, et al. Increasing rates of nasal carriage of methicillin-resistant *Staphylococcus aureus* in healthy children. *Pediatr Infect Dis J* 2005;**24**:617–21.
64. Dagan R, Givon-Lavi N, Zamir O, et al. Reduction of nasopharyngeal carriage of *Streptococcus pneumoniae* after administration of a 9-valent pneumococcal conjugate vaccine to toddlers attending day care centers. *J Infect Dis* 2002;**185**:927–36.
65. Dagan R, Givon-Lavi N, Zamir O, et al. Effect of a nonavalent conjugate vaccine on carriage of antibiotic-resistant *Streptococcus pneumoniae* in day-care centers. *Pediatr Infect Dis J* 2003;**22**: 532–9.
66. Dagan R. Impact of pneumococcal conjugate vaccine on infections caused by antibiotic-resistant *Streptococcus pneumoniae*. *Clin Microbiol Infect* 2009;**15**(Suppl 3):16–20.
67. Daly K, Giebink GS, Le CT, et al. Determining risk for chronic otitis media with effusion. *Pediatr Infect Dis J* 1988;**7**:471–5.
68. Danovaro-Holliday MC, LeBaron CW, Allensworth C, et al. A large rubella outbreak with spread from the workplace to the community. *JAMA* 2000;**284**:2733–9.
69. David E, McIntosh G, Bek MD, et al. Molecular evidence of transmission of hepatitis B in a day-care centre. *Lancet* 1996;**347**: 118–19.
70. Dayan GH, Quinlisk MP, Parker AA, et al. Recent resurgence of mumps in the United States. *N Engl J Med* 2008;**358**:1580–9.
71. De Wals P, Hertoghe L, Borlee-Grimee I, et al. Meningococcal disease in Belgium. Secondary attack rate among household, day-care nursery and pre-elementary school contacts. *J Infect* 1981;**3**(Suppl):53–61.
72. Dennehy PH, Nelson SM, Spangenberger S, et al. A prospective case-control study of the role of astrovirus in acute diarrhea among hospitalized young children. *J Infect Dis* 2001;**184**:10–15.
73. Desai SN, Esposito DB, Shapiro ED, et al. Effectiveness of rotavirus vaccine in preventing hospitalization due to rotavirus gastroenteritis in young children in Connecticut, USA. *Vaccine* 2010;**28**: 7501–6.
74. Deseda CC, Shapiro CN, Carroll K, et al. Hepatitis B virus transmission between a child and staff member at a day-care center. *Pediatr Infect Dis J* 1994;**13**:828–30.
75. Doyle AB. Incidence of illness in early group and family day-care. *Pediatrics* 1976;**58**:607–13.
76. Doyle MG, Morrow AL, Van R, et al. Intermediate resistance of *Streptococcus pneumoniae* to penicillin in children in day-care centers. *Pediatr Infect Dis J* 1992;**11**:831–5.
77. Drummond JA. Seronegative 18 months after being bitten by a patient with AIDS. *JAMA* 1986;**256**:2342–3.
78. Dubnov-Raz G, Ephros M, Garty BZ, et al. Invasive pediatric *Kingella kingae* infections: a nationwide collaborative study. *Pediatr Infect Dis J* 2010;**29**:639–43.
79. Edmonson MB, Riedesel EL, Williams GP, et al. Generalized petechial rashes in children during a parvovirus B19 outbreak. *Pediatrics* 2010;**125**:e787–92.
80. Ekanem EE, Dupont HL, Pickering LK, et al. Transmission dynamics of enteric bacteria in day-care-centers. *Am J Epidemiol* 1983;**118**:562–72.
81. Evans MR, Roberts RJ, Ribeiro CD, et al. A milk-borne *Campylobacter* outbreak following an educational farm visit. *Epidemiol Infect* 1996;**117**:457–62.
82. Fairchok MP, Martin ET, Chambers S, et al. Epidemiology of viral respiratory tract infections in a prospective cohort of infants and toddlers attending daycare. *J Clin Virol* 2010;**49**:16–20.
83. Falck G, Kjellander J. Outbreak of group A streptococcal infection in a day-care center. *Pediatr Infect Dis J* 1992;**11**:914–19.
84. Ferson MJ, Bell SM. Outbreak of coxsackievirus A16 hand, foot, and mouth disease in a child day-care center. *Am J Public Health* 1991;**81**:1675–6.
85. Ferson MJ. Infections in day care. *Curr Opin Pediatr* 1993;**5**: 35–40.
86. Fiellau-Nikolajsen M. Tympanometry in 3-year-old children. Type of care as an epidemiological factor in secretory otitis media and tubal dysfunction in unselected populations of 3-year-old children. *ORL J Otorhinolaryngol Relat Spec* 1979;**41**:193–205.
87. Fischer GE, Teshale EH, Miller C, et al. Hepatitis A among international adoptees and their contacts. *Clin Infect Dis* 2008; **47**:812–14.
88. Fitzgibbon JE, Gaur S, Frenkel LD, et al. Transmission from one child to another of human immunodeficiency virus type 1 with a zidovudine-resistance mutation. *N Engl J Med* 1993;**329**:1835–41.
89. Fleming DW, Leibenhaut MH, Albanes D, et al. Secondary *Haemophilus influenzae* type b in day-care facilities. Risk factors and prevention. *JAMA* 1985;**254**:509–14.
90. Freitas RB, Monteiro TA, Linhares AC. Outbreaks of human-herpes virus 6 (HHV-6) infection in day-care centers in Belem, Para, Brazil. *Rev Inst Med Trop Sao Paulo* 2000;**42**:305–11.
91. Frenck RW, Glezen WP. Respiratory tracti infections in children in day care. *Semin Pediatr Infect Dis* 1990;**1**:234–44.
92. Fridkin SK, Hageman JC, Morrison M, et al. Methicillin-resistant *Staphylococcus aureus* disease in three communities. *N Engl J Med* 2005;**352**:1436–44.
93. Friedland GH, Saltzman BR, Rogers MF, et al. Lack of transmission of HTLV-III/LAV infection to household contacts of patients with AIDS or AIDS-related complex with oral candidiasis. *N Engl J Med* 1986;**314**:344–9.
94. Galil K, Lee B, Strine T, et al. Outbreak of varicella at a day-care center despite vaccination. *N Engl J Med* 2002;**347**:1909–15.
95. Gaur AH, Freimanis-Hance L, Dominguez K, et al. Knowledge and practice of prechewing/prewarming food by HIV-infected women. *Pediatrics* 2011;**127**:e1206–11.
96. Genobile D, Gaston J, Tallis GF, et al. An outbreak of shigellosis in a child care centre. *Commun Dis Intell* 2004;**28**:225–9.
97. Gillespie SM, Cartter ML, Asch S, et al. Occupational risk of human parvovirus B19 infection for school and day-care personnel during an outbreak of erythema infectiosum. *JAMA* 1990;**263**: 2061–5.

98. Gillman A, Berggren I, Bergstrom SE, et al. Primary tuberculosis infection in 35 children at a Swedish day care center. *Pediatr Infect Dis J* 2008;**27**:1078–82.
99. Gingrich GA, Hadler SC, Elder HA, et al. Serologic investigation of an outbreak of hepatitis A in a rural day-care center. *Am J Public Health* 1983;**73**:1190–3.
100. Givon-Lavi N, Fraser D, Dagan R. Vaccination of day-care center attendees reduces carriage of *Streptococcus pneumoniae* among their younger siblings. *Pediatr Infect Dis J* 2003;**22**:524–32.
101. Goodman RA, Osterholm MT, Granoff DM, et al. Infectious diseases and child day care. *Pediatrics* 1984;**74**:134–9.
102. Gotz H, de Jong B, Lindback J, et al. Epidemiological investigation of a food-borne gastroenteritis outbreak caused by Norwalk-like virus in 30 day-care centres. *Scand J Infect Dis* 2002;**34**: 115–21.
103. Granoff DM, Gilsdorf J, Gessert CE, et al. *Haemophilus influenzae* type b in a day care center: relationship of nasopharyngeal carriage to development of anticapsular antibody. *Pediatrics* 1980;**65**: 65–8.
104. Gwaltney JM Jr, Moskalski PB, Hendley JO. Hand-to-hand transmission of rhinovirus colds. *Ann Intern Med* 1978;**88**:463–7.
105. Hadler SC, Erben JJ, Francis DP, et al. Risk factors for hepatitis A in day-care centers. *J Infect Dis* 1982;**145**:255–61.
106. Hale BD, Rendtorff RC, Walker LC, et al. Epidemic herpetic stomatitis in an orphanage nursery. *JAMA* 1963;**183**:1068–72.
107. Hall CB, Douglas RG Jr, Geiman JM. Possible transmission by fomites of respiratory syncytial virus. *J Infect Dis* 1980;**141**: 98–102.
108. Hall CB, Douglas RG Jr. Modes of transmission of respiratory syncytial virus. *J Pediatr* 1981;**99**:100–3.
109. Hampton LM, Farley MM, Schaffner W, et al. Prevention of antibiotic-nonsusceptible *Streptococcus pneumoniae* with conjugate vaccines. *J Infect Dis* 2012;**205**:401–11.
110. Harger JH, Adler SP, Koch WC, et al. Prospective evaluation of 618 pregnant women exposed to parvovirus B19: risks and symptoms. *Obstet Gynecol* 1998;**91**:413–20.
111. Harris JR, Bergmire-Sweat D, Schlegel JH, et al. Multistate outbreak of *Salmonella* infections associated with small turtle exposure, 2007-2008. *Pediatrics* 2009;**124**:1388–94.
112. Hayani KC, Pickering LK. Screening of immigrant children for infectious diseases. *Adv Pediatr Infect Dis* 1991;**6**:91–110.
113. Heijbel H, Slaine K, Seigel B, et al. Outbreak of diarrhea in a day-care-center with spread to household members—the role of *Cryptosporidium*. *Pediatr Infect Dis J* 1987;**6**:532–5.
114. Henderson FW, Gilligan PH, Wait K, et al. Nasopharyngeal carriage of antibiotic-resistant pneumococci by children in group day-care. *J Infect Dis* 1988;**157**:256–63.
115. Hendley JO, Wenzel RP, Gwaltney JM. Transmission of rhinovirus colds by self-inoculation. *N Engl J Med* 1973;**288**: 1361–4.
116. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA* 1998;**279**:593–8.
117. Hewlett AL, Falk PS, Hughes KS, et al. Epidemiology of methicillin-resistant *Staphylococcus aureus* in a university medical center day care facility. *Infect Control Hosp Epidemiol* 2009;**30**: 985–92.
118. Hicks LA, Harrison LH, Flannery B, et al. Incidence of pneumococcal disease due to non-pneumococcal conjugate vaccine (PCV7) serotypes in the United States during the era of widespread PCV7 vaccination, 1998-2004. *J Infect Dis* 2007;**196**: 1346–54.
119. Hinman AR. Vaccine-preventable diseases and child day care. *Rev Infect Dis* 1986;**8**:573–83.
120. Hjelt K, Nielson OH, Paerregaard A, et al. Acute gastroenteritis in children attending day-care centres with special reference to rotavirus infections. II. Clinical manifestations. *Acta Paediatr Scand* 1987;**76**:763–8.
121. Hoffman RE, Shillam PJ. The use of hygiene, cohorting, and antimicrobial therapy to control an outbreak of shigellosis. *Am J Dis Child* 1990;**144**:219–21.
122. Hsueh PR, Teng LJ, Lee PI, et al. Outbreak of scarlet fever at a hospital day care centre: analysis of strain relatedness with phenotypic and genotypic characteristics. *J Hosp Infect* 1997;**36**: 191–200.
123. Hurwitz ES, Gunn WJ, Pinsky PF, et al. Risk of respiratory illness associated with day-care attendance: a nationwide study. *Pediatrics* 1991;**87**:62–9.
124. Hurwitz ES, Deseda CC, Shapiro CN, et al. Hepatitis infections in the day-care setting. *Pediatrics* 1994;**94**(Pt 2):1023–4.
125. Huskins WC. Transmission and control of infections in out-of-home child care. *Pediatr Infect Dis J* 2000;**19**(Suppl):S106–10.
126. Hutto C, Ricks R, Garvie M, et al. Epidemiology of cytomegalovirus infections in young children: day care vs. home care. *Pediatr Infect Dis* 1985;**4**:149–52.
127. Isakbaeva ET, Bulens SN, Beard RS, et al. Norovirus and child care: challenges in outbreak control. *Pediatr Infect Dis J* 2005; **24**:561–3.
128. Ish-Horowicz M, Korman SH, Shapiro M, et al. Asymptomatic giardiasis in children. *Pediatr Infect Dis J* 1989;**8**:773–9.
129. Itoh T, Saito K, Maruyama T, et al. An outbreak of acute enteritis due to *Campylobacter fetus* subspecies *jejuni* at a nursery school of Tokyo. *Microbiol Immunol* 1980;**24**:371–9.
130. Izurieta HS, Strebel PM, Blake PA. Postlicensure effectiveness of varicella vaccine during an outbreak in a child care center. *JAMA* 1997;**278**:1495–9.
131. Jacobson JA, Filice GA, Holloway JT. Meningococcal disease in day-care centers. *Pediatrics* 1977;**59**:299–300.
132. Jain SK, Gupta A, Glanz B, et al. Antimicrobial-resistant *Shigella sonnei:* limited antimicrobial treatment options for children and challenges of interpreting in vitro azithromycin susceptibility. *Pediatr Infect Dis J* 2005;**24**:494–7.
133. Jones DS, Rogers MF. Human immunodeficiency virus infection in children in day care. *Semin Pediatr Infect Dis* 1990;**1**:280–6.
134. Jones LA, Duke-Duncan PM, Yeager AS. Cytomegaloviral infections in infant-toddler centers: centers for the developmentally delayed versus regular day care. *J Infect Dis* 1985;**151**:953–5.
135. Kenneson A, Cannon MJ. Review and meta-analysis of the epidemiology of congenital cytomegalovirus (CMV) infection. *Rev Med Virol* 2007;**17**:253–76.
136. Keswick BH, Pickering LK, DuPont HL, et al. Survival and detection of rotaviruses on environmental surfaces in day care centers. *Appl Environ Microbiol* 1983;**46**:813–16.
137. Keystone JS, Krajden S, Warren MR. Person-to-person transmission of *Giardia lamblia* in day-care nurseries. *Can Med Assoc J* 1978;**119**:241–2, 247–8.
138. Kiang KM, Ogunmodede F, Juni BA, et al. Outbreak of osteomyelitis/septic arthritis caused by *Kingella kingae* among child care center attendees. *Pediatrics* 2005;**116**:e206–13.
139. Kim K, DuPont HL, Pickering LK. Outbreaks of diarrhea associated with *Clostridium difficile* and its toxin in day-care centers: evidence of person-to-person spread. *J Pediatr* 1983;**102**:376–82.
140. Kim KH, Fekety R, Batts DH, et al. Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. *J Infect Dis* 1981;**143**:42–50.
141. Kotch JB, Weigle KA, Weber DJ, et al. Evaluation of an hygienic intervention in child day-care centers. *Pediatrics* 1994;**94**(Pt 2): 991–4.
142. Kuzushima K, Kimura H, Kino Y, et al. Clinical manifestations of primary herpes simplex virus type 1 infection in a closed community. *Pediatrics* 1991;**87**:152–8.
143. Kyaw MH, Lynfield R, Schaffner W, et al. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. *N Engl J Med* 2006;**354**:1455–63.
144. Laborde DJ, Weigle KA, Weber DJ, et al. The frequency, level, and distribution of fecal contamination in day-care-center classrooms. *Pediatrics* 1994;**94**:1008–11.
145. Lauwers S, Deboeck M, Butzler JP. *Campylobacter* enteritis in Brussels. *Lancet* 1978;**1**:604–5.
146. Lee GM, Salomon JA, Friedman JF, et al. Illness transmission in the home: a possible role for alcohol-based hand gels. *Pediatrics* 2005;**115**:852–60.
147. Leggiadro RJ, Baddour LM, Frasch CE, et al. Invasive meningococcal disease: secondary spread in a day-care-center. *South Med J* 1989;**82**:511–13.
148. Leggiadro RJ, Callery B, Dowdy S, et al. An outbreak of tuberculosis in a family day-care home. *Pediatr Infect Dis J* 1989;**8**: 52–4.
149. Lieb S, Gunn RA, Taylor DN. Salmonellosis in a day-care-center. *J Pediatr* 1982;**100**:1004–5.

150. Loda FA, Glezen WP, Clyde WA Jr. Respiratory disease in group day care. *Pediatrics* 1972;**49**:428–37.
151. Louhiala PJ, Jaakkola N, Ruotsalainen R, et al. Form of day care and respiratory infections among Finnish children. *Am J Public Health* 1995;**85**(Pt 1):1109–12.
152. Lowther SA, Shinoda N, Juni BA, et al. *Haemophilus influenzae* type b infection, vaccination, and *H. influenzae* carriage in children in Minnesota, 2008-2009. *Epidemiol Infect* 2012;**140**:566–74.
153. Mac Kenzie WR, Hoxie NJ, Proctor ME, et al. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. *N Engl J Med* 1994;**331**(3): 161–7.
154. MacNeil JR, Cohn AC, Farley M, et al. Current epidemiology and trends in invasive *Haemophilus influenzae* disease—United States, 1989-2008. *Clin Infect Dis* 2011;**53**:1230–6.
155. Mainous AG 3rd, Hueston WJ, Everett CJ, et al. Nasal carriage of *Staphylococcus aureus* and methicillin-resistant *S. aureus* in the United States, 2001-2002. *Ann Fam Med* 2006;**4**:132–7.
156. Matson DO, Estes MK. Impact of rotavirus infection at a large pediatric hospital. *J Infect Dis* 1990;**162**:598–604.
157. McCaustland KA, Bond WW, Bradley DW, et al. Survival of hepatitis A virus in feces after drying and storage for 1 month. *J Clin Microbiol* 1982;**16**:957–8.
158. McMahon BJ, Alward WL, Hall DB, et al. Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985;**151**:599–603.
159. McVernon J, Morgan P, Mallaghan C, et al. Outbreak of *Haemophilus influenzae* type b disease among fully vaccinated children in a day-care center. *Pediatr Infect Dis J* 2004;**23**:38–41.
160. Meningococcal Disease Surveillance Group. Meningococcal disease. Secondary attack rate and chemoprophylaxis in the United States, 1974. *JAMA* 1976;**235**:261–5.
161. Mera RM, Suaya JA, Amrine-Madsen H, et al. Increasing role of *Staphylococcus aureus* and community-acquired methicillin-resistant *Staphylococcus aureus* infections in the United States: a 10-year trend of replacement and expansion. *Microb Drug Resist* 2011; **17**:321–8.
162. Munford RS, Taunay Ade E, de Morais JS, et al. Spread of meningococcal infection within households. *Lancet* 1974;**1**:1275–8.
163. Muotiala A, Saxen H, Vuopio-Varkila J. Group A streptococcal outbreak of perianal infection in a day-care centre. *Adv Exp Med Biol* 1997;**418**:211–15.
164. Murph JR, Bale JF Jr, Murray JC, et al. Cytomegalovirus transmission in a Midwest day care center: possible relationship to child care practices. *J Pediatr* 1986;**109**:35–9.
165. National Institutes of Child Health and Human Development Early Child Care Research Network. Child care and common communicable illnesses. *Arch Pediatr Adolesc Med* 2001;**155**:481–8.
166. National Institutes of Child Health and Human Development Early Child Care Research Network. Child care and common communicable illnesses. *Arch Pediatr Adolesc Med* 2003;**157**: 196–200.
167. Nesti MM, Goldbaum M. Infectious diseases and daycare and preschool education. *J Pediatr (Rio J)* 2007;**83**:299–312.
168. Nolan CM, Barr H, Elarth AM, et al. Tuberculosis in a day-care home. *Pediatrics* 1987;**79**:630–2.
169. O'Donovan D, Iversen A, Trounce J, et al. Outbreak of group C meningococcal infection affecting two preschool nurseries. *Commun Dis Public Health* 2000;**3**:177–80.
170. O'Ryan ML, Matson DO, Estes MK, et al. Molecular epidemiology of rotavirus in children attending day care centers in Houston. *J Infect Dis* 1990;**162**:810–16.
171. Oberhelman RA, Flores-Abuxapqui J, Suarez-Hoil G, et al. Asymptomatic salmonellosis among children in day-care centers in Merida, Yucatan, Mexico. *Pediatr Infect Dis J* 2001;**20**:792–7.
172. Ogilvie MM, Tearne CF. Spontaneous abortion after hand-foot-and-mouth disease caused by Coxsackie virus A16. *Br Med J* 1980;**281**:1527–8.
173. Pai CH, Sorger S, Lackman L, et al. Campylobacter gastroenteritis in children. *J Pediatr* 1979;**94**:589–91.
174. Pass RF, August AM, Dworsky M, et al. Cytomegalovirus infection in day-care center. *N Engl J Med* 1982;**307**:477–9.
175. Pass RF, Hutto SC, Reynolds DW, et al. Increased frequency of cytomegalovirus-infection in children in group day-care. *Pediatrics* 1984;**74**:121–6.
176. Pass RF, Hutto C. Group day-care and cytomegaloviral infections of mothers and children. *Rev Infect Dis* 1986;**8**:599–605.
177. Pelton SI, Loughlin AM, Marchant CD. Seven valent pneumococcal conjugate vaccine immunization in two Boston communities. *Pediatr Infect Dis J* 2004;**23**:1015–22.
178. Petersen JP, Kaltoft MS, Misfeldt JC, et al. Community outbreak of perianal group A streptococcal infection in Denmark. *Pediatr Infect Dis J* 2003;**22**:105–9.
179. Pickering LK, Woodward WE. Diarrhea in day-care-centers. *Pediatr Infect Dis J* 1982;**1**:47–52.
180. Pickering LK, Bartlett AV, Reves RR, et al. Asymptomatic excretion of rotavirus before and after rotavirus diarrhea in children in day-care centers. *J Pediatr* 1988;**112**:361–5.
181. Pickering LK, Baker CJ, Freed GL, et al. Immunization programs for infants, children, adolescents, and adults: clinical practice guidelines by the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**49**:817–40.
182. Plotkin SA. Inactivated polio vaccine for the United States—a missed vaccination opportunity. *Pediatr Infect Dis J* 1995;**14**: 835–9.
183. Radetsky MS, Istre GR, Johansen TL, et al. Multiply resistant pneumococcus causing meningitis: its epidemiology within a day-care centre. *Lancet* 1981;**2**:771–3.
184. Rauch AM, O'Ryan M, Van R, et al. Invasive disease due to multiply resistant *Streptococcus pneumoniae* in a Houston, Tex, day-care center. *Am J Dis Child* 1990;**144**:923–7.
185. Rauch AM, Van R, Bartlett AV, et al. Longitudinal study of *Giardia lamblia* infection in a day care center population. *Pediatr Infect Dis J* 1990;**9**:186–9.
186. Reves RR, Jones JA. Antibiotic use and resistance patterns in day care centers. *Semin Pediatr Infect Dis* 1990;**1**:212–21.
187. Roberts L, Jorm L, Patel M, et al. Effect of infection control measures on the frequency of diarrheal episodes in child care: a randomized, controlled trial. *Pediatrics* 2000;**105**(Pt 1):743–6.
188. Roberts L, Smith W, Jorm L, et al. Effect of infection control measures on the frequency of upper respiratory infection in child care: a randomized, controlled trial. *Pediatrics* 2000;**105**(Pt 1): 738–42.
189. Rodriguez WJ, Kim HW, Brandt CD, et al. Common exposure outbreak of gastroenteritis due to type 2 rotavirus with high secondary attack rate within families. *J Infect Dis* 1979;**140**: 353–7.
190. Rogers MF, White CR, Sanders R, et al. Lack of evidence of transmission of human immunodeficiency virus from infected children to their household contacts. *Pediatrics* 1990;**85**:210–14.
191. Rovers MM, Zielhuis GA, Ingels K, et al. Day-care and otitis media in young children: a critical overview. *Eur J Pediatr* 1999; **158**:1–6.
192. Saez-Nieto JA, Perucha M, Casamayor H, et al. Outbreak of infection caused by *Neisseria meningitidis* group C type 2 in a nursery. *J Infect* 1984;**8**:49–55.
193. Sandora TJ, Taveras EM, Shih MC, et al. A randomized, controlled trial of a multifaceted intervention including alcohol-based hand sanitizer and hand-hygiene education to reduce illness transmission in the home. *Pediatrics* 2005;**116**:587–94.
194. Sargent SJ, Martin JT. Scabies outbreak in a day-care center. *Pediatrics* 1994;**94**(Pt 2):1012–13.
195. Saxen H, Muotiala A, Rostila T, et al. Outbreak of perianal *Streptococcus pyogenes* infection in a day-care center. *Pediatr Infect Dis J* 1997;**16**:247–9.
196. Schmitt DL, Johnson DW, Henderson FW. Herpes simplex type 1 infections in group day care. *Pediatr Infect Dis J* 1991;**10**: 729–34.
197. Schulte EE, Birkhead GS, Kondracki SF, et al. Patterns of *Haemophilus influenzae* type b invasive disease in New York State, 1987 to 1991: the role of vaccination requirements for day-care attendance. *Pediatrics* 1994;**94**(Pt 2):1014–16.
198. Schwartz B, Giebink GS, Henderson FW, et al. Respiratory infections in day care. *Pediatrics* 1994;**94**(Pt 2):1018–20.
199. Sealy DP, Schuman SH. Endemic giardiasis and day care. *Pediatrics* 1983;**72**:154–8.
200. Sena AC, Seed P, Nicholson B, et al. *Kingella kingae* endocarditis and a cluster investigation among daycare attendees. *Pediatr Infect Dis J* 2010;**29**:86–8.
201. Shahin R, Johnson IL, Jamieson F, et al. Methicillin-resistant *Staphylococcus aureus* carriage in a child care center following a case

of disease. Toronto Child Care Center Study Group. *Arch Pediatr Adolesc Med* 1999;**153**:854–8.
202. Shapiro CN, McCaig LF, Gensheimer KF, et al. Hepatitis B virus transmission between children in day care. *Pediatr Infect Dis J* 1989;**8**:870–5.
203. Shapiro ED. Lack of transmission of hepatitis B in a day care center. *J Pediatr* 1987;**110**:90–2.
204. Shapiro ED, Murphy TV, Wald ER, et al. The protective efficacy of *Haemophilus* b polysaccharide vaccine. *JAMA* 1988;**260**(10): 1419–22.
205. Shirley LR, Ross SA. Risk of transmission of human immunodeficiency virus by bite of an infected toddler. *J Pediatr* 1989; **114**:425–7.
206. Silva AM, Leite EG, Assis RM, et al. An outbreak of gastroenteritis associated with astrovirus serotype 1 in a day care center, in Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 2001;**96**:1069–73.
207. Smith TD, Wilkinson V, Kaplan EL. Group A streptococcus–associated upper respiratory tract infections in a day-care center. *Pediatrics* 1989;**83**:380–4.
208. Snedeker KG, Shaw DJ, Locking ME, et al. Primary and secondary cases in *Escherichia coli* O157 outbreaks: a statistical analysis. *BMC Infect Dis* 2009;**9**:144.
209. Soares-Weiser K, Maclehose H, Bergman H, et al. Vaccines for preventing rotavirus diarrhoea: vaccines in use. *Cochrane Database Syst Rev* 2012;(2):CD008521.
210. Spika JS, Parsons JE, Nordenberg D, et al. Hemolytic uremic syndrome and diarrhea associated with *Escherichia coli* O157:H7 in a day care center. *J Pediatr* 1986;**109**:287–91.
211. Stagno S, Pass RF, Cloud G, et al. Primary cytomegalovirus infection in pregnancy. Incidence, transmission to fetus, and clinical outcome. *JAMA* 1986;**256**:1904–8.
212. Stahlberg MR. The influence of form of day-care on occurrence of acute respiratory-tract infections among young-children. *Acta Paediatr Scand* 1980;**282**:5–87.
213. Stahlberg MR, Ruuskanen O, Virolainen E. Risk-factors for recurrent otitis-media. *Pediatr Infect Dis J* 1986;**5**:30–2.
214. Staras SA, Flanders WD, Dollard SC, et al. Cytomegalovirus seroprevalence and childhood sources of infection: a population-based study among pre-adolescents in the United States. *J Clin Virol* 2008;**43**:266–71.
215. Strangert K. Respiratory illness in preschool-children with different forms of day-care. *Pediatrics* 1976;**57**:191–6.
216. Strangert K, Carlstrom G, Jeansson S, et al. Infections in preschool-children in group day-care. *Acta Paediatr Scand* 1976; **65**:455–63.
217. Strangert K. Otitis media in young children in different types of day-care. *Scand J Infect Dis* 1977;**9**:119–23.
218. Strom J. Study of infections and illnesses in a day nursery based on inclusion-bearing cells in the urine and infectious agent in feces, urine and nasal secretion. *Scand J Infect Dis* 1979;**11**: 265–9.
219. Sullivan P, Woodward WE, Pickering LK, et al. Longitudinal study of occurrence of diarrheal disease in day care centers. *Am J Public Health* 1984;**74**:987–91.
220. Swerdlow DL, Griffin PM. Duration of faecal shedding of *Escherichia coli* O157:H7 among children in day-care centres. *Lancet* 1997;**349**:745–6.
221. Tauxe RV, Johnson KE, Boase JC, et al. Control of day-care shigellosis—a trial of convalescent day-care in isolation. *Am J Public Health* 1986;**76**:627–30.
222. Taylor JP, Perdue JN, Dingley D, et al. Cryptosporidiosis outbreak in a day-care-center. *Am J Dis Child* 1985;1391023–5.
223. Teele DW, Klein JO, Rosner B. Epidemiology of otitis media during the first 7 years of life in children in greater Boston—a prospective, cohort study. *J Infect Dis* 1989;**160**:83–94.
224. Thompson SC. Infectious diarrhea in children—controlling transmission in the child-care setting. *J Paediatr Child Health* 1994; **30**:210–19.
225. Tos M, Poulsen G, Borch J. Tympanometry in 2-year-old children. *ORL J Otorhinolaryngol Relat Spec* 1978;**40**:77–85.
226. Tsoukas CM, Hadjis T, Shuster J, et al. Lack of transmission of HIV through human bites and scratches. *J Acquir Immune Defic Syndr* 1988;**1**:505–7.
227. Tugwell BD, Lee LE, Gillette H, et al. Chickenpox outbreak in a highly vaccinated school population. *Pediatrics* 2004;**113**(Pt 1): 455–9.
228. Uhari M, Mantysaari K, Niemela M. A meta-analytic review of the risk factors for acute otitis media. *Clin Infect Dis* 1996;**22**: 1079–83.
229. United States Census Bureau. *Who's minding the kids? Child care arrangements: Spring 2010 (SIPP 2008)*. Updated December 5, 2011; cited June 28, 2012. Available from http://www.census.gov/hhes/childcare/.
230. Van R, Morrow AL, Reves RR, et al. Environmental contamination in child day-care centers. *Am J Epidemiol* 1991;**133**:460–70.
231. Van R, Wun CC, Morrow AL, et al. The effect of diaper type and overclothing on fecal contamination in day-care centers. *JAMA* 1991;**265**:1840–4.
232. Vazquez M, LaRussa PS, Gershon AA, et al. The effectiveness of the varicella vaccine in clinical practice. *N Engl J Med* 2001; **344**:955–60.
233. Wahn V, Kramer HH, Voit T, et al. Horizontal transmission of HIV infection between two siblings. *Lancet* 1986;**2**:694.
234. Wald ER, Dashefsky B, Byers C, et al. Frequency and severity of infections in day care. *J Pediatr* 1988;**112**:540–6.
235. Waters V, Ford-Jones EL, Petric M, et al. Etiology of community-acquired pediatric viral diarrhea: a prospective longitudinal study in hospitals, emergency departments, pediatric practices and child care centers during the winter rotavirus outbreak, 1997 to 1998. The Pediatric Rotavirus Epidemiology Study for Immunization Study Group. *Pediatr Infect Dis J* 2000;**19**:843–8.
236. Weniger BG, Ruttenber AJ, Goodman RA, et al. Fecal coliforms on environmental surfaces in two day care centers. *Appl Environ Microbiol* 1983;**45**:733–5.
237. Wilde J, Van R, Pickering L, et al. Detection of rotaviruses in the day care environment by reverse transcriptase polymerase chain reaction. *J Infect Dis* 1992;**166**:507–11.
238. Williams JV, Harris PA, Tollefson SJ, et al. Human metapneumovirus and lower respiratory tract disease in otherwise healthy infants and children. *N Engl J Med* 2004;**350**:443–50.
239. Wilson R, Feldman RA, Davis J, et al. Salmonellosis in infants: the importance of intrafamilial transmission. *Pediatrics* 1982;**69**: 436–8.
240. Wong CS, Jelacic S, Habeeb RL, et al. The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. *N Engl J Med* 2000;**342**:1930–6.
241. Yagupsky P. *Kingella kingae:* from medical rarity to an emerging paediatric pathogen. *Lancet Infect Dis* 2004;**4**:358–67.
242. Yagupsky P, Erlich Y, Ariela S, et al. Outbreak of *Kingella kingae* skeletal system infections in children in daycare. *Pediatr Infect Dis J* 2006;**25**:526–32.
243. Zhao Z, Murphy TV, Jacques-Carroll L. Progress in newborn hepatitis B vaccination by birth year cohorts—1998-2007, USA. *Vaccine* 2011;**30**:14–20.

CHAPTER 248

Animal and Human Bites

Morven S. Edwards

Many children delight in teasing dogs, and without caution go too near them, by which they get miserably torn and mangled. … What these boys had been doing to enrage the dog we cannot tell, but suspect they had been tormenting him in some way, thinking that as he was chained he could not injure them. But they were mistaken in this, and one of them is likely to be bitten very severely.[6]

AUTHOR UNKNOWN, 1830

HISTORICAL ASPECTS

The consequences to children of bites resulting from provoking dogs, as noted in the opening quotation, were of concern in the 19th century just as they are today.[23] In early reports, infected bite wounds were noted to contain fusiform bacilli and spirochetal organisms.[95] We now appreciate that a vast number of aerobic and anaerobic organisms composing the normal flora of the biting animal's mouth are potential bite wound pathogens. Human bites have been recorded since the biblical era and currently are a common cause of serious medical and surgical diseases. They are the third most frequent type of bite after dog and cat bites. Reports of secondary infection occurring after a human bite in children have been noted in the United States since at least 1910.[64]

The importance of débridement and drainage in treatment of infected bite wounds was understood in the preantibiotic era, but these infections carried a high morbidity rate.[93] In a report from 1936, amputation was required in one third of cases in which treatment was delayed for 24 hours or longer.[118] Adjuncts to cleansing, such as electrocauterization and even radiation therapy, were used in efforts to prevent or to treat infection, but not until the introduction of penicillin was the outcome of bite wound infections improved over that achieved by symptomatic therapy alone.[93]

EPIDEMIOLOGY

More than 100 million cats and dogs are kept as pets in the United States.[47] The estimated annual incidence is 1 to 2 million dog bites, 400,000 cat bites, 250,000 human bites, and 45,000 snake bites.[48] Rabbits, skunks, squirrels, horses, rats, hogs, and monkeys cause at least 1 percent of bite injuries. Unprovoked pet ferret attacks have caused severe facial injuries.[34] Taken together, however, bites from nondomestic animals, generally thought to pose a higher risk for transmission of rabies, constitute less than 1 percent of reported bite wounds. The right arm is bitten most frequently, presumably because of defensive attempts by victims using their dominant arm. At least three quarters of bites are located on extremities. Facial bites represent only 10 percent of the total, but two thirds of them are sustained by children younger than 10 years.

Children are the most common victims of animal bites. From one half to three fourths of dog bites are reported in persons younger than 20 years. Dog bites cost more than $1 billion per year in the United States.[89] The peak incidence is in children 5 to 9 years of age.[97] Many bites do not require a visit to the physician and are not reported. Up to 1 percent of all pediatric emergency department visits during the summer months are for treatment of animal bites.[28,66] Approximately 5 percent of children requiring medical care for dog bite require hospital admission.[25] Most bites occur in late afternoon and early evening hours. Boys sustain dog bites twice as often as do girls, but girls are bitten more frequently by cats.

Large dogs account for most animal bites and are implicated most frequently in bites with a fatal outcome. Ten to 20 fatal human attacks occur yearly in the United States. At least 25 breeds of dogs were implicated in 238 fatalities from dog bites during the past 25 years.[104] The most common breeds included pit bull terriers (51%) and Rottweilers (9%) in one series of 551 pediatric dog bite injuries.[69] These animals can exert a biting force of 1500 psi, several times that of a German shepherd. The severity of wounds inflicted by pit bull breeds also is due to their tendency to inflict multiple bites and to bite and grind their molars into tissue. In one study, 94 percent of injuries caused by pit bulls were the consequence of unprovoked attacks.[9] In most instances (75%), the dog's owner is known by the victim, although only a small percentage of bites are caused by a family-owned dog. Stray dogs account for 10 percent of bites inflicted. When the circumstances are known, most mammalian bites are provoked, although the victim may not have agitated the animal intentionally.[109]

Infection is a common complication of animal bites. Between 3 and 20 percent of dog bites and 20 to 50 percent of cat bites for which medical care is sought become infected.[26,68] With the exception of monkey bites,

which have a high infection rate, infection seldom develops after bites by other mammals. Factors influencing the risk for development of infection include the patient's age, type and location of the wound, and length of time that transpires between the bite and initiation of treatment. Wounds of the hand are more likely to become infected than are those of the arm, leg, or face. Puncture wounds more often become infected than do lacerations, superficial wounds, or wounds with skin and soft tissue defects. Infection is likely to develop when wounds are repaired surgically or when care is delayed more than 24 hours after injury.

The incidence of infection after human bites is estimated to be 10 to 30 percent. Because of the typically superficial nature of human bite wound injuries in children, the infection rate in children is estimated to be approximately 10 percent. Human bites have their peak incidence in the spring and early summer[80] and on Saturdays, especially for the adolescent age group. Alcohol and drug abuse may play roles in some of these instances. Bites occur more commonly in males than in females except for the 15- to 20-year-old age groups, in which females are bitten more frequently.[21,80] Human bites occur most often in men 20 to 25 years of age. Most bites in teenagers are associated with aggressive behavior. Among 322 children who suffered human bites, 21 percent were younger than 5 years, 21 percent were 5 to 10 years of age, and 58 percent were older than 10 years.

Biting is a common occurrence in the child care center setting, for which it is the third most frequently reported injury. The incidence peaks in midmorning and during the early school year in September. Toddlers from 13 to 30 months of age are bitten more often than are infants and other preschoolers,[110] and boys are bitten more frequently than are girls.[44,111] Bites account for 3 and 6 percent of reported injuries in boys and girls, respectively,[39] and may be self-inflicted in many cases.[21] Approximately 50 percent of all children enrolled in daycare suffer bite wounds, with a rate of 3.9 bites per child per year in general but 9.4 bites per male child per year for full-time enrollees.[44,108] Most of these bites are minor and do not break the skin. A higher proportion of bites in younger children (such as preschoolers) are to the face, whereas most bites in adolescents are on the upper extremities and hands.

Paronychial infections are not actual bites, but when infection occurs, it is related to contamination by oral flora when children bite or suck their fingers. Brook[17] noted children from 2 to 9 years of age (mean age, 5 years 8 months) who were treated at a children's hospital for paronychial infection and reported the bacteriologic and clinical findings of these infections.

Bites as harbingers or signs of child abuse are noted more commonly in the 0- to 4-year-old age group[93]; the age of the abusing parents was younger than 20 years for mothers and younger than 22 years for fathers. An attempt should be made to identify the biter and measure distances between circular bite marks to ascertain the spread. In addition, biting children may have learned this behavior from abusive adults and may themselves be victims of human bites.

MICROBIOLOGY

The oral flora of the biting animal, rather than skin flora of the victim, is the source of most isolates from bite wounds, but both can be sources for infection.[1] Infections usually are polymicrobial and contain mixed aerobic-anaerobic isolates. Table 248-1 enumerates bacterial isolates from the wounds of 50 patients with dog bites and 57 patients with cat bites that were infected at the time of arrival at an emergency department for care.[113] A median of five bacterial isolates were found per culture. Slightly more than half of the wounds yielded both aerobes and anaerobes, and anaerobes alone were isolated from slightly more than one third of the wounds. *Pasteurella* spp. were the isolates most frequently found in dog and cat bites (50% and 75%, respectively), with *Pasteurella canis* being isolated most commonly from dog bites and *Pasteurella multocida* subspecies *multocida* and *septica* the most common isolates from cat bites. *Pasteurella*

TABLE 248-1 **Bacteria Isolated from 50 Infected Dog Bites and 57 Infected Cat Bites**

	Number of Patients (%)	
Bacteria	**Dog Bite**	**Cat Bite**
Aerobes		
Pasteurella	25 (50)	43 (75)
Streptococci	23 (46)	26 (46)
Staphylococcus aureus	10 (20)	2 (4)
Other staphylococci	13 (23)	18 (31)
Neisseria	8 (16)	11 (19)
Corynebacterium	6 (12)	16 (28)
EF-4b	5 (10)	9 (16)
Moraxella	5 (10)	20 (35)
Enterococcus	5 (10)	7 (12)
Bacillus	4 (8)	6 (11)
Pseudomonas	3 (6)	3 (5)
Actinomyces	3 (6)	2 (4)
Brevibacterium	3 (6)	2 (4)
Weeksella	2 (4)	4 (7)
Eikenella corrodens	1 (2)	1 (2)
Capnocytophaga	1 (2)	4 (7)
Acinetobacter	0	4 (7)
Other	19 (38)	19 (33)
Anaerobes		
Fusobacterium	16 (32)	19 (33)
Bacteroides	15 (30)	16 (28)
Porphyromonas	14 (28)	17 (30)
Prevotella	14 (28)	11 (19)
Propionibacterium	10 (20)	10 (18)
Peptostreptococcus	8 (16)	3 (5)
Eubacterium	2 (4)	1 (2)
Others	1 (2)	5 (9)

Modified from Talan DA, Citron DM, Abrahamia FM, et al. Bacteriologic analysis of infected dog and cat bites. *N Engl J Med* 1999;**340**:85-92.

carrier rates are as high as 66 percent for dogs and 90 percent for cats, so one is not surprised that this organism is associated so commonly with bite wound infections. Infection has occurred as a consequence of bites by large cats, including lions,[18] cougars,[71] and tigers,[20] as well as by house cats. The type of wound commonly inflicted (i.e., puncture by cats versus laceration by dogs) might explain the species-specific disparity in infection rates.

Other aerobic agents commonly isolated from infected dog or cat bites include streptococci, staphylococci, *Moraxella*, *Neisseria*, and corynebacteria. Although streptococci often are β-hemolytic, *Streptococcus pyogenes* was isolated from only 12 percent of dog bites and none of the infected cat bites in one large series.[113] *Staphylococcus aureus* was isolated frequently from infected dog bites but infrequently from cat bite wounds. Species such as *Capnocytophaga canimorsus* and *Neisseria weaveri*, previously classified under the Centers for Disease Control and Prevention alphanumeric system, are notable because infections in association with these species have occurred in splenectomized persons or those with immunocompromising conditions.[4,76]

Animal bites are implicated as a vehicle for transmission of a number of systemic infectious diseases caused by viruses, fungi, and mycobacteria in addition to bacteria (Table 248-2).[96] Transmission of such unusual pathogens as orf virus, *Pseudallescheria boydii*, or *Mycobacterium chelonae* by bites of a goat, dog, or ferret, respectively, has been described.[65,74,101] Cats can transmit such unusual infections as diphtheria, coccidioidomycosis, and tularemia.[13,42,116] Tularemia can also be transmitted by the bite of such diverse species as wild boar, coyote, hog, lamb, muskrat, opossum, raccoon, rat, skunk, squirrel, snapping turtle, and weasel.[7,19,81]

The bacteriology of human bite wounds also reflects the human oral flora of the biter.[16,112] Human saliva and dental plaque can contain more than 42 different species of bacteria in concentrations of 10^8 colony-forming units per milliliter. Table 248-3 lists common human bite wound isolates and their relative frequency of isolation.[12,16,57,86,112] α-Hemolytic streptococci, especially *Streptococcus anginosus*, are the most frequent isolates. Other common bacterial isolates include *Streptococcus pyogenes*, *Staphylococcus aureus*, *Haemophilus* spp., *Eikenella corrodens*, and oral anaerobes, especially *Fusobacterium*, *Prevotella*, *Veillonella*, and *Peptostreptococcus* spp. Cultures of virtually all infected bite wounds grow bacterial pathogens; wound cultures of patients who initially are seen less than 8 hours after incurring an injury, before the development of clinical infection, yield potential bacterial pathogens in 85 percent of cases.[47] The bacteriology of early-treated, colonized wounds is remarkably similar to the bacteria isolated from wounds evaluated later with established infection. The average bite wound yields between 3.4 and 5.4 bacterial isolates per wound, including 1.7 to 2.4 aerobes and 1.7 to 3 anaerobes. Anaerobes are isolated from more than 50 percent of human bite wounds, almost always in mixed culture with aerobes, and they are associated more often with more serious infections, amputation, and the presence of abscesses.[52,112] Many of the anaerobes isolated from human bite wounds, especially *Prevotella* and *Porphyromonas* spp., are β-lactamase producers.[17,112]

E. corrodens, a capnophilic, gram-negative rod that is part of the normal human oral flora, is an important pathogen in clenched-fist injuries.[49] It has an unusual antimicrobial susceptibility pattern in that it is susceptible to penicillin but resistant to first-generation cephalosporins, β-lactamase–stable penicillins (such as oxacillin), and erythromycin.[51] When it is unrecognized or treated with the incorrect antibiotic, *E. corrodens* has been associated with therapeutic failure. Usually isolated in mixed culture, it may be missed by microbiologists because of its slow growth characteristics and overgrowth of other bacterial colonies. *E. corrodens* produces a small colony that "pits" or "corrodes" the agar surface and has a light yellow pigment and an odor like that of hypochlorite bleach.

The bacteriology of paronychial infections is similar to that of other human bite infections, except that aerobes and anaerobes each have been isolated in pure culture in some cases. Brook[17] found a total of 3.6 isolates per specimen: 1.4 aerobes, 2 anaerobes, and 0.2 *Candida albicans;* β-lactamase–producing organisms, including isolates of *Prevotella melaninogenica* and *Prevotella oralis*, were found in 45 percent of the wounds.

In addition to oral bacterial infection, human bites have been associated with viral infections, such as those caused by herpes simplex virus,[40,83] cytomegalovirus,[83] hepatitis B virus,[26,63] hepatitis C virus,[32] and possibly human immunodeficiency virus (HIV),[5,70,106] as well as syphilis,[35] tuberculosis,[47] actinomycosis,[14] and tetanus[85] (Table 248-4).

TABLE 248-2 Diseases Transmitted by Animal Bite Wounds

B-virus
Blastomycosis
Brucellosis
Cat-scratch disease
Coccidioidomycosis
Diphtheria
Hemorrhagic fever
Leptospirosis
Monkeypox
Mycobacterium chelonae
Mycobacterium marinum
Mycobacterium fortuitum
Orf
Plague
Rabies
Rat-bite fever*
Sporotrichosis
Tularemia

*Both infections caused by *Streptobacillus moniliformis* and caused by *Spirillum minus*.

CLINICAL MANIFESTATIONS

Signs of infection after animal bites develop within hours to several days after injury. Infection is the usual reason that patients seek medical attention more than 8 to 12 hours after injury, whereas those seen earlier are more

TABLE 248-3 Approximate Prevalence and Bacteriology of Isolates from Human Bite Wound Infections

Isolate	Prevalence (%)	Present in OB	Present in CFI
Aerobes			
Streptococci			
α-Hemolytic	28-90	+	+
Streptococcus anginosus	52	+	+
β-Hemolytic			
Group A	14-26	+	+
Other	12	+	+
γ-Hemolytic	3-33	+	–
Enterococci	6-11	–	+
Staphylococcus aureus	13-55	+	+
Coagulase-negative staphylococci	6-53	+	+
Haemophilus influenzae	2-6	+	+
Haemophilus species (other)	11-20	+	+
Eikenella corrodens	10-30	+	+
Micrococcus species	3-5	–	+
Moraxella species	3	+	–
Neisseria species	11-15	+	+
Corynebacterium species	28-41	+	+
Acinetobacter calcoaceticus	2-6	–	+
Campylobacter species	16	+	+
Escherichia coli	6	+	–
Klebsiella pneumoniae	3-6	+	+
Enterobacter species	3-4	+	–
Nocardia species	3	–	+
Anaerobes			
Acidaminococcus species	2	+	+
Actinomyces species	4-8	+	+
Bacteroides ovatus	6	–	+
Bacteroides ureolyticus	3-11	+	–
Bacteroides species (unspeciated)	12-33	+	+
Bifidobacterium species	11	–	+
Clostridium perfringens	4	+	–
Prevotella (Bacteroides) melaninogenica	15-22	+	+
Prevotella (Bacteroides) intermedia	11-26	+	+
Prevotella (Bacteroides) oralis	6-17	+	+
Prevotella buccae	15	+	+
Prevotella disiens	4	–	+
Prevotella loescheii	3	+	+
Porphyromonas species	2	+	+
Eubacterium species	3-11	+	+
Fusobacterium nucleatum	12-33	+	+
Fusobacterium necrophorum	4-6	–	+
Fusobacterium species (other)	17	–	+
Peptostreptococcus anaerobius	3	+	–
Peptostreptococcus asaccharolyticus	22	+	+
Peptostreptococcus intermedius	3	+	–
Peptostreptococcus (Finegoldia) magnus	2-17	+	+
Peptostreptococcus micros	12-20	+	+
Gemella (Peptostreptococcus) morbillorum	3	+	–
Peptostreptococcus prevotii	3	+	–
Peptostreptococcus species (other)	3-22	+	+
Veillonella parvula	9-11	+	+
Veillonella species (other)	6-18	+	+
Spirochetes	5-30	+	+

CFI, clenched-fist injuries; OB, occlusional bite wounds.
+, present; –, absent.
Based on a compilation of data from references 12, 16, 57, 86, and 112.

TABLE 248-4 Diseases Transmitted by Human Bite Wounds

Actinomycosis
Cytomegalovirus infection
Hepatitis B virus infection
Hepatitis C virus infection
Herpesvirus infection
HIV infection
Invasive group A streptococcal infection
Syphilis
Tetanus
Toxic shock syndrome
Tuberculosis
Whitlow

concerned with prophylaxis or surgical repair. Findings suggestive of wound infection include localized swelling, erythema, and pain with or without serosanguineous or purulent drainage (Fig. 248-1). The clinical findings vary with the infecting organism, site of injury, and type of bite. The jaws and teeth of dogs are likely to produce multiple puncture wounds as well as jagged lacerations with devitalized tissue. These lesions can be associated with depressed skull fractures, sometimes in more than one cranial region. Puncture wounds, particularly those inflicted by cat bites, often are deceptively innocuous. Inoculation of organisms deep into poorly vascularized areas, such as tendon sheaths, fascia, joints, and bones, is likely to result in an infected wound.

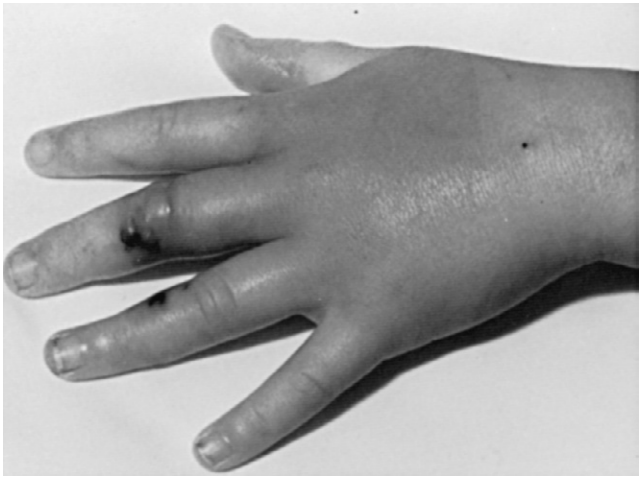

FIGURE 248-1 ■ Dog bite wound–associated tenosynovitis in a 4-year-old girl. *Enterococcus* was isolated from the wound culture.

TABLE 248-5 Potential Complications of Animal Bite Wounds

Arthritis
Brain abscess
Endocarditis
Endophthalmitis
Meningitis
Mycotic aneurysm
Orbital cellulitis
Osteomyelitis
Pneumonia
Sepsis
Synovitis
Tendinitis, tenosynovitis

The characteristic findings of *P. multocida* infection include a rapid onset of intense pain, swelling, and erythema, often within hours after injury. Cellulitis is usually evident within 24 to 36 hours after the bite. Despite these intense local symptoms, patients generally are afebrile, and less than 20 percent have lymphangitis and regional adenitis. Patients with wound infection caused by staphylococci or streptococci usually experience less intense pain, have a delay between injury and the onset of symptoms of days rather than hours, and may have a more diffuse, less fiery cellulitis. Gas in tissues of the forearm clinically suggestive of clostridial gas gangrene has occurred in infections caused by *Streptococcus anginosus* and *Streptococcus mutans* after horse bite lacerations.[82] Wound infection clinically resembling that caused by *P. multocida* from which the related but more unusual gram-negative rod *Actinobacillus lignieresii* was isolated has been reported in a child who sustained a facial bite by a horse.[27]

Some of the complications resulting from direct extension or generalized spread of infection caused by animal bites are summarized in Table 248-5. Tenosynovitis caused by *P. multocida* can be apparent within hours or can be manifested days to weeks after injury, when persistence of swelling, tenderness, or pain and a mass overlying the involved tendon sheath suggest the diagnosis. *Pasteurella* osteomyelitis that develops after a cat bite is characterized by pain, swelling, and tenderness over the involved bone.[67] When the periosteum has been entered, osteomyelitis can develop despite early administration of local and antibiotic treatment. Bites to the cranium occur with relative frequency in small children because their heads are at the level of the mouth of medium- to large-sized dogs. Complications of perforating cranial bites in children include compound depressed skull fractures, brain abscess, dural lacerations, and extensive intracerebral injuries, which can prove fatal.[2,18] Brain abscess and meningitis can occur as complications of these injuries.

In patients with systemic infections transmitted by animal bites, the incubation periods and clinical manifestations vary with the causative agent. For example, streptobacillary rat-bite fever occurs after an incubation period of less than 1 week, whereas spirillary rat-bite fever, or sodoku, has a 2-week asymptomatic interval after the bite. For many of the infections listed in Table 248-2,* the bite wound serves as the site of inoculation and has healed completely during the incubation period. For example, fatal encephalitis has resulted from the bite or scratch of a monkey that is actively shedding B virus *(Herpesvirus simiae)*. Institution of acyclovir treatment intravenously at the time of injury may abort progression of the disease.[90] Isolation of *Capnocytophaga canimorsus* is common among patients with splenectomy, alcoholism, and chronic lung disease.[94] The symptoms heralding the onset of systemic infections caused by animal bites are discussed in their respective chapters.

A high index of suspicion can be required to trace the infection to the animal source. For example, most cases of human plague in the United States result from bites by infected fleas, but contact with a domestic cat infected with *Yersinia pestis* can be the source of infection.[41] With tularemia, an ulcerative or pustular lesion develops at the bite site 4 to 7 days after injury and is associated with fever, chills, and painful regional adenopathy. In a case of *Mycobacterium marinum* infection that developed after a dolphin bite, one of several discrete fluctuant masses containing the isolate developed in an area just proximal to the original wound.[37] *Mycoplasma* has been implicated in "seal finger" from a seal skin, tooth, or claw injury, an infection characterized by severe pain and massive swelling and erythema 4 to 8 days after injury.[10] With cat-scratch disease caused by *Bartonella henselae*, a papule or pustule may be present at the original bite site when systemic signs develop. An ulceration at the site of a rodent bite has been described with sporotrichosis.[62]

Human bites can be categorized as those resulting in paronychial infection, occlusional bites, and clenched-fist injuries. Potential complications of human bites are noted in Table 248-6. Paronychia is an infection (inflammation) of the structures of the distal phalanx, either those surrounding the nail or the bone itself. Most are caused by finger sucking in younger children but may be caused by accidental biting. The area is red, tender, and swollen and may have some underlying purulence.

*References 8, 30, 33, 45, 46, 75, 79, 87, 99, 105, 120.

TABLE 248-6 **Potential Complications of Human Bite Wounds**

Abscess
Cellulitis
Compartment syndrome
Fracture
Necrotizing fasciitis
Nerve severance/injury
Osteomyelitis
Scarlet fever
Sepsis
Septic arthritis
Tendon severance/injury
Tenosynovitis
Toxic shock syndrome

Most occlusional wounds are minor and require routine care with cleansing and bandaging. The infection rate after occlusional bite wounds is 10 to 30 percent, 87 percent of which may require hospitalization.[11] Wounds to the hand and with any edema or crush injury and those that involve a bone or a joint have a greater potential to become infected. Infections usually are manifested as cellulitis. If the child is treated early, these injuries rarely result in serious complications. These infections generally spread proximally and in less than 5 percent of cases have associated fever, lymphangitis, or lymphadenopathy. A malodorous discharge or abscess can be present if anaerobes are involved. Complications may be limited to skin defects when skin avulsion has occurred, but septic arthritis and osteomyelitis may develop if the joint or bone is involved.[58] In immunocompromised hosts, such as those with hematologic malignant disease or neutropenia, sepsis may occur. Amputation can be necessary as a result of the initial bite or as a result of serious chronic infection.[77] Tendon injury, primary nerve injury and tenosynovitis, compartment syndrome, and resultant secondary nerve damage can occur.[88]

Clenched-fist injuries, or "fight bites," are the most serious of human bite wounds and occur when the closed fist of one person strikes another in the teeth and a break in the skin occurs.[60] The most common cause is a fight, although cases can occur during contact sports. The metacarpophalangeal joint of the middle finger of the dominant hand is most often involved.[49] The break in the skin may be only 2 to 5 mm in length but often results in penetration into the joint or even into bone. Patients and physicians may underestimate the potentially serious nature of these wounds. Bacteria penetrate the knuckle area during the initial contact and, on relaxation of the hand, are carried by the tendons proximally into the potential spaces of the hand. Infection can spread laterally, between the collateral and accessory ligaments, or dorsally into the thin-walled bursa overlying the metacarpal head or into the palmar deep spaces of the hand. The swelling usually spreads proximally and not distally from the site of injury. Patients often avoid seeking attention initially because of the circumstances surrounding the injury and 6 to 8 hours later seek medical attention with a painful, throbbing, swollen, infected hand. Purulent exudate can be foul-smelling if *P. melaninogenica* or other anaerobes are present.

The range of motion of the hand usually is limited by swelling and edema but also can be limited as a result of tendon or nerve injury or severance. Osteomyelitis, which usually involves the small bones of the hand, often is manifested as continued pain, swelling, and erythema with or without drainage at 10 days to 3 weeks after the injury has occurred. Osteomyelitis and septic arthritis caused by *E. corrodens*, often in association with α-hemolytic streptococci, frequently are insidious and persistent and may lead to amputation, especially if treated with the wrong antibiotics.[49] Permanent limitation of range of motion and joint stiffness can occur. Other complications include fracture, collar-button abscess, and muscle atrophy.

Reports[5,70,106] have noted the transmission of HIV from biter to victim. HIV is isolated uncommonly from the saliva of infected persons, and when it has been recovered, it has been in low number. Consequently, the possibility of HIV transmission is considered unlikely, although it is not negligible. One report reviewed instances of HIV transmission by human bites and concluded that "no well documented case of HIV transmission through bites exists"; the risk of HIV transmission is possible biologically but appears to be unlikely.[98] A review and commentary of professional sports-related injuries also noted the unlikely possibility for HIV to be transmitted by sporting events and outlined similar commonsense control measures.

DIAGNOSIS AND TREATMENT

The most important aid in establishing the diagnosis of infection after bite wounds is the proper use of wound cultures. A culture specimen need not be obtained from children evaluated in the immediate postinjury period (the first 8 hours) unless the bite is located on the face or hand or signs of infection are present. Isolates from an uninfected but contaminated wound do not predict the future development of infection. Routine determination of the microorganisms colonizing bites on the face or hands is useful because of the potentially devastating consequences of infection at these locations. After the wound has been cleansed, culture should be performed routinely when evaluation is at an interval exceeding 8 hours from the time of injury, except for wounds evaluated more than 24 hours after injury with no signs of infection.

When material is obtained for wound culture, the microbiology laboratory should be informed that the source of the specimen is a bite wound. This information should optimize accuracy because *P. multocida* can be mistaken morphologically for *Neisseria* or *Haemophilus influenzae*. Appropriate media must be used for the isolation of anaerobes, and gram-negative rods should be considered pathogens. A blood culture should be performed if fever or systemic toxicity is evident, although associated bacteremia occurs rarely. Diagnostic imaging is indicated when the periosteum has been penetrated. Computed tomographic or magnetic resonance imaging can detect periosteal defects, particularly in children with cranial

bite wounds. For patients in whom wound infection has extended locally, sutures, if present, should be removed and material from the involved tissue compartment harvested for study. Material should be aspirated from areas of cellulitis or drained from areas of frank abscess formation. If osteomyelitis is suspected, a bone biopsy specimen should be submitted for Gram stain, culture, and histopathologic evaluation. If the course of the wound infection is indolent, acid-fast stains and mycobacterial cultures should be performed. Serum for serologic testing should be obtained from patients with symptoms suggesting a systemic infection. The details of the indicated diagnostic evaluation for systemic infections transmissible by animal and human bites are specified in the appropriate chapters.

The basic elements of management are outlined in Table 248-7. The wound should be cleansed with normal saline, and any foreign body or debris and necrotic tissue should be removed. Cautious débridement is indicated for some wounds, with care being taken to not create a potential skin defect. The wound then is irrigated, with a syringe and needle/catheter tip used as a high-pressure jet to diminish the bacterial inoculum. Puncture wounds should be cleansed but not irrigated because irrigation may damage tissues further.

The value and risks associated with primary closure in patients who are initially seen less than 8 hours after injury and without any symptoms or signs of established infection have not been studied in a prospective or randomized manner. Early primary closure has been successful for wounds to the face and neck.[117] The blood supply to the head and face area is superior to that of most other anatomic areas, and these areas are rarely dependent; therefore, edema and swelling resolve more rapidly or do not develop. In one prospective study, primary closure in patients presenting less than 8 hours after injury was associated with only slightly higher development of wound infection than that occurring after non–bite wound lacerations.[22] Most nonpuncture animal bite wounds except those involving the hands or feet can be treated by primary closure without substantially increasing the risk for development of infection.[43] Wounds to the hands should be observed and left open for either delayed primary or secondary closure. Infected wounds should not be closed.

Some clinicians prefer to approximate wound edges with adhesive strips or tissue adhesive rather than to suture wounds. In cases of clenched-fist injury, a hand surgeon should examine the patient to determine whether the joint capsule or bone has been compromised. With many hand infections, especially after exploration of clenched-fist injuries, immobilization in a plaster splint is recommended. Elevation of a swollen or inflamed part is crucial to healing. The elevation should be maintained above the level of the heart. Legs and hands also can be elevated with pillows.

Definitive data on which to base the use of antibiotic prophylaxis after animal bites are lacking. Smith and colleagues[107] summarized prospective clinical trials that have addressed the issue. Significantly lower infection rates were observed for prophylaxis of wounds in patients presenting at 9 to 24 hours after injury but not for those presenting less than 9 hours later in another study.[15] Prophylactic dicloxacillin, cephalexin, or erythromycin was not beneficial therapy for low-risk dog bite wounds in another prospective study because infection rates for wounds in both the antibiotic and control groups did not exceed 5 percent.[28] A meta-analysis of eight randomized trials totaling 783 patients with dog bite wounds found that prophylactic antibiotics did reduce the incidence of infection.[24] The estimated cumulative incidence of infection was 16 percent in controls, and the relative risk was 0.56 (95% CI, 0.38-0.82) in patients given antibiotics. Treatment of approximately 14 patients was required to prevent one infection. Some researchers consider antibiotic use therapeutic rather than prophylactic in this setting and suggest that antibiotic therapy be given in all cases of dog bites except those initially evaluated more than 24 hours after injury with no clinical signs of infection.[119]

Until additional data are available, a reasonable approach appears to be administration of prophylactic antibiotics in the following circumstances: dog bites more than 8 hours old; moderate to severe bites less than 8 hours old, especially if edema or crush injury is present; puncture wounds, particularly if bone or joint penetration may have occurred; facial wounds; all hand or foot bites; wounds in the genital area; and wounds in immunocompromised persons.[47,115]

Antimicrobial agents should be selected empirically according to the most likely pathogens and their usual

TABLE 248-7 Management Procedures for Bite Wounds

Obtain history from patient
Situation leading to injury
Place of occurrence
Patient allergies
Other medications (potential interactions)
Perform evaluation of patient
Nerve function
Tendon function
Vascular integrity
Range of motion
Potential bone and joint involvement
Diagram or photograph wound
Mark leading edge of cellulitis
Culture wound (if infected)
Irrigate wound (do not irrigate puncture wounds)
Débride wound cautiously
Obtain diagnostic imaging for penetrating injuries overlying bones or joints, if fracture suspected or foreign body suspected
Drain abscesses
Operative débridement (for removal of devitalized tissue and for severe bites, as indicated)
Administer antimicrobial agents (prophylactic therapy, 3 to 5 days; longer duration for established infection)
Elevate injured area
Close the wound (see text)
Immobilize wound area (3 days for hands)
Have the patient exercise the injured area (if previously immobilized)
Administer tetanus toxoid
Submit health department report (if required)

TABLE 248-8 **Comparative in Vitro Antimicrobial Activity of Selected Oral Antimicrobial Agents Against Common Bite Wound Pathogens**

	*Staphylococcus aureus**	*Eikenella corrodens*	Streptococci	*Haemophilus*	*Pasteurella*	Anaerobes
Amoxicillin	–	+	v	v	v	v
Amoxicillin–clavulanic acid	v	+	+	+	+	+
Cephalexin	v	–	v	–	v	–
Cefaclor	v	–	v	v	v	–
Cefuroxime	v	v	v	v	v	–
Cefprozil	v	–	v	v	v	–
Dicloxacillin	v	–	v	–	–	–
Erythromycin	v	–	v	–	v	–
Azithromycin	v	v	v	–	v	v
Clarithromycin	v	v	v	–	v	–
Trimethoprim-sulfamethoxazole	+	+	+	+	+	–
Moxifloxacin	+	+	+	+	+	+
Clindamycin	+	–	+	–	–	+

*Community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has emerged as a significant problem in some regions.
Note: Because of contraindications in pediatric patients, tetracyclines and fluoroquinolones are not included.
+, active; –, poorly active or inactive; v, variable.

susceptibility patterns to antimicrobial agents.[50,57] If cultures are performed, therapy should be adjusted according to the organisms isolated and their specific susceptibility to antimicrobial agents. The activity of antibiotics commonly used against the usual bite wound pathogens is outlined in Table 248-8. Common pathogens to be considered in the selection of antimicrobial therapy include streptococci, *S. aureus*, *Haemophilus* spp., *E. corrodens*, *P. multocida*, and β-lactamase–producing oral anaerobes. The oral agent of choice is amoxicillin–clavulanic acid; for penicillin-allergic children, a combination such as an extended-spectrum cephalosporin or trimethoprim-sulfamethoxazole plus clindamycin can be substituted.[3] Many other combination regimens, including cefuroxime plus clindamycin or metronidazole, may be used. The intravenous agents of choice are ampicillin-sulbactam and ticarcillin-clavulanate.[3] For penicillin-allergic children, combination therapy with an extended-spectrum cephalosporin or trimethoprim-sulfamethoxazole plus clindamycin or meropenem should be administered.[3] Consideration should be given, in the era of methicillin-resistant *S. aureus*, to empirical addition of vancomycin to children with serious bite wounds, pending the results of cultures. Additional potentially useful intravenous agents include cefoxitin, meropenem or ertapenem, and combinations of such agents as cefuroxime or cefotaxime plus metronidazole.

First-generation cephalosporins are of limited utility because of poor activity against *E. corrodens* and some anaerobes[51] and should not be used as empirical monotherapy. Erythromycin also has poor activity against *P. multocida*, *E. corrodens*, and *Fusobacterium nucleatum*,[51] and its use as monotherapy can lead to therapeutic failure. Newer macrolides, including azithromycin, show improved activity compared with erythromycin against the spectrum of bite wound pathogens, but *Haemophilus* spp. and *F. nucleatum* remain relatively resistant.[57]

The quinolones, particularly moxifloxacin, exhibit broad activity against the pathogens implicated in bite wounds.[55,56,112] Linezolid, an oxazolidinone, appears to be more active than the macrolides against the gram-positive organisms and fusobacteria isolated from human bite wounds, but some aerobic gram-negative organisms are at the susceptibility breakpoint.[54] Once patient-specific cultures return, antimicrobial therapy should be adjusted in accordance with the patient's individual isolates and susceptibility pattern. The duration of antimicrobial therapy is determined by the type and severity of infection. Prophylactic antimicrobial agents typically are given for 3 to 5 days. If any question exists about the prompt filling of a prescription because of financial or other concerns, a dose of intramuscular or intravenous antibiotics should be administered and hospitalization considered.

Patients' wounds should be re-examined within 24 to 48 hours. If outpatient therapy is initiated and the cellulitis advances, hospitalization is indicated. Indications for hospitalization also include patient noncompliance and virtually all clenched-fist injuries.

As a general guideline, a suggested duration of therapy, assuming that proper drainage has been established, is 10 days for cellulitis or localized abscess, 2 to 3 weeks for tenosynovitis, and 3 to 6 weeks for osteomyelitis. When improvement is evident, treatment can be completed orally. At least 4 weeks of intravenous therapy should be administered to patients with endocarditis. Although the number of patients is too small for the required dosage to be established with certainty, ampicillin (400 mg/kg/day) has been used successfully for the treatment of *P. multocida* meningitis.[59]

In every child sustaining a bite wound, immunization status should be determined to assess the need for tetanus prophylaxis and whether rabies prophylaxis should be undertaken (see the appropriate chapters).

REPTILE BITES

Approximately 8000 people in the United States are bitten yearly by poisonous snakes. Approximately half occur in people younger than 20 years. The curiosity of children can contribute to this exposure because many are bitten while handling poisonous snakes.[91] Boys are more likely than girls to incur snake bites requiring antivenom therapy.[84] An average of five persons die of snake bites each year.[73]

Venomous snakes are divided into four families, two of which, the Crotalidae (rattlesnakes, copperheads, and water moccasins) and the Elapidae (coral snakes), are found in the United States. Snake venoms are among the most complex of proteins. The local effects of the venom of pit vipers (Crotalidae), which is rich in proteolytic activity, include swelling, pain, edema, ecchymosis, and tissue necrosis with the formation of bullae.[103] Systemic effects result from increased blood vessel permeability and hemolysis and may include hematuria, hematemesis, and disseminated intravascular coagulopathy. The venom of the eastern coral snake causes minimal local tissue destruction. It is a neurotoxin that produces paresthesia of the involved extremity, followed by involvement of the cranial nerves and bulbar paralysis.

Goldstein and associates[53] isolated 58 aerobic and 28 anaerobic organisms after culturing the venom from 15 rattlesnakes. The aerobes most commonly isolated were *Pseudomonas aeruginosa*, *Proteus* spp., and *Staphylococcus epidermidis*. Among the anaerobes, *Clostridium* spp. were the isolates found most frequently; *Bacteroides fragilis* also was recovered. Defecation of prey during ingestion contributes to this preponderance of gastrointestinal flora. When the fangs are cleansed carefully before collection, the venom itself is sterile, thus indicating that bacterial isolates potentially contaminating snake bite wounds are a reflection of the oral flora.

Alligators are generally not aggressive toward humans.[72] In persons sustaining alligator bite wounds, infection is caused most commonly by *Aeromonas hydrophila*. A report of mixed infection with *A. hydrophila*, *Enterobacter agglomerans*, and *Citrobacter diversus* and the frequency with which *Proteus vulgaris* and *Pseudomonas* spp. are isolated from alligator mouths suggest that treatment of alligator bites should be directed at gram-negative species.[36] *Vibrio* infection should be suspected in victims of shark bites as well as in all wounds exposed to salt water.[92] *Aeromonas* spp. and other gram-negative bacilli as well as *S. aureus* and anaerobes can infect shark bites.[100]

The exotic pet industry is growing in the United States, and the common green iguana is a popular pet. Few cases of infections from iguana bites have been reported and most bites do not have serious effects because these creatures are generally nonaggressive, but *Serratia marcescens* cellulitis has been observed.[61,38]

The inflammatory changes of envenomation can be difficult to differentiate from those of infection. At least one instance of osteomyelitis as a complication of an infected snake bite has been documented.[102] Some experts suggest use of a broad-spectrum antibiotic for injuries with severe tissue involvement but not for bites with minor or minimal envenomation.[102] Others suggest that prophylactic antibiotics are not required in snake bite victims.[78,114] Amoxicillin-clavulanate is an appropriate oral antimicrobial and ampicillin-sulbactam plus gentamicin is an appropriate parenteral regimen for empirical treatment of reptile bite wounds.[3] The oral alternatives for penicillin-allergic children are the same as those given for other animal bite wounds; one parenteral regimen for a penicillin-allergic child is clindamycin plus gentamicin. Treatment should be guided by the results of Gram stain and susceptibility testing.

PREVENTION

Bite wounds are, at least in part, preventable injuries. Prevention strategies include appropriate infection control measures in the sports setting and education of young athletes as well as their coaches and trainers. Bites can occur in the daycare setting, even when caregivers are vigilant. A cross-sectional survey of 5- to 15-year-olds found that dog bite prevention knowledge was poor, particularly among younger children.[29] There is as yet no direct evidence that educational programs actually lead to a reduction in dog bite rates in children and adolescents. Educating young children could improve their attitude and behavior toward dogs, and data are needed to evaluate dog bite rates as outcomes of such interventions.[31]

Acknowledgment

The author appreciates the contributions to prior editions by Dr. Ellie J. C. Goldstein in his chapter "Human Bites." Many parts of his excellent chapter have been retained unchanged in this revision.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abrahamian FM, Goldstein EJC. Microbiology of animal bite wound infections. *Clin Microbiol Rev* 2011;**24**:231–46.
3. American Academy of Pediatrics. Bite wounds. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 203.
13. Berger A, Schmidt-Wieland T, Huber I, et al. Human diphtheria infection from a cat bite. *Ann Intern Med* 2011;**155**:646–8.
25. Daniels DM, Ritzi RB, O'Neil J, et al. Analysis of nonfatal dog bites in children. *J Trauma* 2009;**66**:S17–22.
29. Dixon CA, Mahabee-Gittens EM, Hart KW, et al. Dog bite prevention: an assessment of child knowledge. *J Pediatr* 2012;**160**:337–41.
31. Duperrex O, Blackhall K, Burri M, et al. Education of children and adolescents for the prevention of dog bite injuries. *Cochrane Database Syst Rev* 2009;(2):CD004726.
34. Ferrant O, Papin F, Dupont C Jr, et al. Injuries by a pet ferret on a child: morphological aspects and comparison with other mammalian pet bite marks. *J Forensic Legal Med* 2008;**15**:193–7.
38. Forrester MB. Iguana bites reported to Texas poison centers. *Am J Emerg Med* 2010;**28**:817–19.
42. Gaidici A, Saubolle MA. Transmission of coccidioidomycosis to a human via a cat bite. *J Clin Microbiol* 2009;**47**:505–6.
61. Grim KD, Doherty C, Rosen T. *Serratia marcescens* bullous cellulitis after iguana bites. *J Am Acad Dermatol* 2010;**62**:1075–6.
62. Hayfron K, Wiedeman JA. A 7-year-old girl with ulcerative lesion after a rodent bite. *Pediatr Infect Dis J* 2010;**29**:185, 193.

65. Iyengar KP, Nadkarni JB, Gupta R, et al. *Mycobacterium chelonae* hand infection following ferret bite. *Infection* 2013;**41**:237–41.
68. Kannikeswaran N, Kamat D. Mammalian bites. *Clin Pediatr* 2009;**48**:145–8.
69. Kaye AE, Belz JM, Kirschner RE. Pediatric dog bite injuries: a 5-year review of the experience at the Children's Hospital of Philadelphia. *Plast Reconstr Surg* 2009;**124**:551–8.
72. Langley RL. Adverse encounters with alligators in the United States: an update. *Wild Environ Med* 2010;**21**:156–63.
73. Langley RL. Deaths from reptile bites in the United States, 1979-2004. *Clin Toxicol (Phila)* 2009;**47**:44–7.
74. Lederman ER, Austin C, Trevino I, et al. Orf virus infection in children: clinical characteristics, transmission, diagnostic methods, and future therapeutics. *Pediatr Infect Dis J* 2007;**26**:740–4.
84. Matteucci MJ, Hannum JE, Riffenburgh RH, et al. Pediatric sex group differences in location of snakebite injuries requiring antivenom therapy. *J Med Toxicol* 2007;**3**:103–6.
89. Oehler RL, Velez AP, Mizrachi M, et al. Bite-related and septic syndromes caused by cats and dogs. *Lancet Infect Dis* 2009;**9**:439–47.
96. Pickering LK, Marano N, Bocchini JA, et al. Exposure to nontraditional pets at home and to animals in public settings: risks to children. *Pediatrics* 2008;**122**:876–86.
97. Quirk JT. Non-fatal dog bite injuries in the USA, 2005-2009. *Public Health* 2012;**126**:300–2.
101. Ruinemans GM, Haagsma CJ, Hendrix R. Tenosynovitis caused by a *Pseudallescheria boydii* infection and symptoms of reflex sympathetic dystrophy after a dog bite. *J Clin Rheumatol* 2011;**17**:363–4.
116. Weinberg AN, Branda JA. Case 31-2010. A 29-year-old woman with fever after a cat bite. *N Engl J Med* 2010;**363**:1560–8.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 249

BIOTERRORISM

Robert J. Leggiadro

The intentional delivery of *Bacillus anthracis* spores through mailed letters or packages established the clinical reality of bioterrorism in the United States in the autumn of 2001.[8,43] An understanding of the epidemiology, clinical manifestations, and management of the more credible biologic agents is critical to limiting morbidity and mortality from a bioterrorism attack.[15,30,38,49]

Implementation of an effective response to deliberate release of biologic agents by terrorists requires detection and reporting of cases as soon as possible.[6,12] Prompt recognition of unusual clinical syndromes and increases above seasonal levels in the incidence of common syndromes or deaths from infectious agents is critical to an effective response.[6,22,38,47]

HISTORY

The concept of biologic warfare or terrorism is not a new one. In 1346, the Tatar force attacking Kaffa (also Caffa, now Feodossia, Ukraine) was struck by a plague epidemic.[13,66] To take advantage of this development, the Tatars catapulted the corpses of their plague victims into Kaffa. An outbreak of plague did occur in the city, representing one of the first biologic attacks recorded.[13,66]

Smallpox first was used as a biologic weapon during the French and Indian War of 1754 to 1767.[13] Apparently, British troops deliberately infected Native Americans with smallpox by giving them blankets from infected patients.

A secret branch of the Japanese army, Unit 731, reportedly caused outbreaks of plague by dropping plague-infected fleas over populated areas of China in World War II.[37] Bomb experiments of weaponized anthrax spores were conducted by the Allies on uninhabited Gruinard Island near the coast of Scotland and resulted in heavy contamination in 1942.[57] Viable anthrax spores persisted until the island was decontaminated with formaldehyde and seawater in 1986.

In response to a perceived German biologic warfare threat, the United States began research into the offensive use of biologic agents in 1943 at Camp Detrick (renamed Fort Detrick in 1956) in Frederick, Maryland.[13] President Nixon stopped all offensive biologic and toxin weapon research and production by executive order in 1969. Included among the agents destroyed as a result of this action were *B. anthracis*, botulinum toxin, *Francisella tularensis*, *Coxiella burnetii*, Venezuelan equine encephalitis virus, *Brucella suis*, and staphylococcal enterotoxin B. Begun in 1953, the U.S. defensive program at Fort Detrick continues today as the U.S. Army Medical Research Institute of Infectious Diseases (USAMRIID).[13]

The Biological and Toxin Weapons Convention was ratified in 1972 and went into effect in 1975.[67] It prohibits the development, production, stockpiling, or transfer of biologic weapons agents (microbial pathogens and toxins)

REFERENCES

1. Abrahamian FM, Goldstein EJC. Microbiology of animal bite wound infections. *Clin Microbiol Rev* 2011;**24**:231–46.
2. Alpert G, Sutton LN. Brain abscess following cranial dog bite. *Clin Pediatr (Phila)* 1984;**23**:580.
3. American Academy of Pediatrics. Bite wounds. In: Pickering LK, Baker CJ, Kimberlin DW, Long SS, editors. *Red Book: 2012 report of the Committee on Infectious Diseases*. 29th ed. Elk Grove Village, Ill: American Academy of Pediatrics; 2012. p. 203.
4. Andersen BM, Steigerwalt AG, O'Conner SP, et al. *Neisseria weaveri* sp. nov., formerly CDC group M-5, a gram-negative bacterium associated with dog bite wounds. *J Clin Microbiol* 1993; **31**:2456–66.
5. Andreo SM, Barra LA, Costa LJ, et al. HIV type 1 transmission by human bite. *AIDS Res Hum Retroviruses* 2004;**20**:349–50.
6. Anonymous. *The book of accidents: designed for young children*. New Haven, Conn: Sidney's Press; 1830.
7. Arav-Boger R. Cat-bite tularemia in a seventeen-year-old girl treated with ciprofloxacin. *Pediatr Infect Dis J* 2000;**19**:583–4.
8. Ariel H, Haas H, Weinberg H, et al. *Mycobacterium fortuitum* granulomatous synovitis caused by a dog bite. *J Hand Surg* 1983; **8**:342–3.
9. Avner JR, Baker MD. Dog bites in urban children. *Pediatrics* 1991;**88**:55–7.
10. Baker AS, Ruoff KL, Madoff S. Isolation of *Mycoplasma* species from a patient with seal finger. *Clin Infect Dis* 1998;**27**:1168–70.
11. Baker MD, Moore SE. Human bites in children. *Am J Dis Child* 1987;**41**:1285–90.
12. Barnham I. Once bitten, twice shy: microbiology of bites. *Rev Med Microbiol* 1991;**2**:31–6.
13. Berger A, Schmidt-Wieland T, Huber I, et al. Human diphtheria infection from a cat bite. *Ann Intern Med* 2011;**155**:646–8.
14. Blinkhorn RJ, Strimbu V, Effron D, et al. "Punch" actinomycosis causing osteomyelitis of the hand. *Arch Intern Med* 1988;**148**: 2668–70.
15. Brackenbury PH, Muwanga C. A comparative double blind study of amoxicillin/clavulanate vs placebo in the prevention of infection after animal bites. *Arch Emerg Med* 1989;**6**:251–6.
16. Brook I. Microbiology of human and animal bite wounds in children. *Pediatr Infect Dis J* 1987;**6**:29–32.
17. Brook I. Bacteriologic study of paronychia in children. *Am J Surg* 1981;**141**:703–5.
18. Burdge DR, Scheifele D, Speert DP. Serious *Pasteurella multocida* infections from lion and tiger bites. *JAMA* 1985;**253**:3296–7.
19. Capellan J, Fong IW. Tularemia from a cat bite: case report and review of feline-associated tularemia. *Clin Infect Dis* 1993;**16**:472–5.
20. Capitini CM, Herrero IA, Patel R, et al. Wound infection with *Neisseria weaveri* and a novel subspecies of *Pasteurella multocida* in a child who sustained a tiger bite. *Clin Infect Dis* 2002;**34**:e74–6.
21. Chang A, Lugg MM, Nebedum A. Injuries among preschool children enrolled in day-care centers. *Pediatrics* 1989;**83**:272–7.
22. Chen E, Horning S, Shepherd S, et al. Primary closure of mammalian bites. *Acad Emerg Med* 2000;**7**:157–61.
23. Cone TE Jr. Book of accidents. Excerpt VIII. Worrying dogs. *Pediatrics* 1971;**47**:460.
24. Cummings P. Antibiotics to prevent infection in patients with dog bite wounds: a meta-analysis of randomized trials. *Ann Emerg Med* 1994;**23**:535–40.
25. Daniels DM, Ritzi RB, O'Neil J, et al. Analysis of nonfatal dog bites in children. *J Trauma* 2009;**66**:S17–22.
26. Davis LG, Weber DJ, Kemon SM. Horizontal transmission of hepatitis B virus. *Lancet* 1989;**1**:889–93.
27. Dibb WL, Digranes A, Tønjum S. *Actinobacillus lignieresii* infection after a horse bite. *BMJ* 1981;**283**:583–4.
28. Dire DJ, Hogan DE, Walker JS. Prophylactic oral antibiotics for low-risk dog bite wounds. *Pediatr Emerg Care* 1992;**8**:194–9.
29. Dixon CA, Mahabee-Gittens EM, Hart KW, et al. Dog bite prevention: an assessment of child knowledge. *J Pediatr* 2012;**160**: 337–41.
30. Dournon E, Moriniere B, Matheron S, et al. HFRS after a wild rodent bite in the Haute-Savoie: and risk of exposure to Hantaan-like virus in a Paris laboratory. *Lancet* 1984;**1**:676–7.
31. Duperrex O, Blackhall K, Burri M, et al. Education of children and adolescents for the prevention of dog bite injuries. *Cochrane Database Syst Rev* 2009;(2):CD004726.
32. Dusheik GM, Smith M, Schever PF. Hepatitis C virus transmitted by human bite. *Lancet* 1990;**336**:503–4.
33. Edlich RF, Spengler MD, Rodeheaver GT. Mammalian bites. *Compr Ther* 1983;**9**:41–7.
34. Ferrant O, Papin F, Dupont C Jr, et al. Injuries by a pet ferret on a child: morphological aspects and comparison with other mammalian pet bite marks. *J Forensic Legal Med* 2008;**15**:193–7.
35. Fiumara NJ, Exnor JH. Primary syphilis following a human bite. *Sex Transm Dis* 1981;**8**:21–82.
36. Flandry F, Lisecki EJ, Domingue GJ, et al. Initial antibiotic therapy for alligator bites: characterization of the oral flora of *Alligator mississippiensis*. *South Med J* 1989;**82**:262–6.
37. Flowers DJ. Human infection due to *Mycobacterium marinum* after a dolphin bite. *J Clin Pathol* 1970;**23**:475–7.
38 . Forrester MB. Iguana bites reported to Texas poison centers. *Am J Emerg Med* 2010;**28**:817–19.
39. Fuller EM. Injury-prone children. *Am J Orthopsychiatry* 1948; **18**:708–23.
40. Fuortes L, Melson E. Brief report: primary and recurrent herpes simplex infection in a pediatric nurse resulting from a human bite. *Infect Control Hosp Epidemiol* 1989;**10**:120.
41. Gage KL, Dennis DT, Orloski KA, et al. Cases of cat-associated human plague in the western US, 1977-1998. *Clin Infect Dis* 2000;**30**:893–900.
42. Gaidici A, Saubolle MA. Transmission of coccidioidomycosis to a human via a cat bite. *J Clin Microbiol* 2009;**47**:505–6.
43. Garbutt F, Jenner R. Best evidence topic report. Wound closure in animal bites. *Emerg Med J* 2004;**21**:589–90.
44. Garrard J, Leland N, Smith DK. Epidemiology of human bites to children in a day-care center. *Am J Dis Child* 1988;**142**: 643–50.
45. Glaser C, Lewis P, Wong S. Pet-, animal-, and vector-borne infections. *Pediatr Rev* 2000;**21**:219–32.
46. Gnann JW Jr, Bressler GS, Bodet CA III, et al. Human blastomycosis after a dog bite. *Ann Intern Med* 1983;**98**:48–9.
47. Goldstein EJC. Bite wounds and infection. *Clin Infect Dis* 1991; **14**:633–40.
48. Goldstein EJC. Management of human and animal bite wounds. *J Am Acad Dermatol* 1989;**21**:1275–9.
49. Goldstein EJC, Barones MF, Miller TA. *Eikenella corrodens* in hand infections. *J Hand Surg* 1983;**8**:563–6.
50. Goldstein EJC, Citron DM. Comparative activities of cefuroxime, amoxicillin–clavulanic acid, ciprofloxacin, enoxacin, and ofloxacin against aerobic and anaerobic bacteria isolated from bite wounds. *Antimicrob Agents Chemother* 1988;**32**:1143–8.
51. Goldstein EJC, Citron DM. Susceptibility of *Eikenella corrodens* to penicillin, apalcillin, and twelve cephalosporins. *Antimicrob Agents Chemother* 1984;**26**:947–8.
52. Goldstein EJC, Citron DM, Finegold SM. Role of anaerobic bacteria in bite wound infections. *Rev Infect Dis* 1984;**6**(Suppl): 177–83.
53. Goldstein EJC, Citron DM, Gonzalez H, et al. Bacteriology of rattlesnake venom and implications for therapy. *J Infect Dis* 1979;**140**:818–21.
54. Goldstein EJC, Citron DM, Merriam CV. Linezolid activity compared to those of selected macrolides and other agents against aerobic and anaerobic pathogens isolated from soft tissue bite infections in humans. *Antimicrob Agents Chemother* 1999;**43**: 1469–74.
55. Goldstein EJC, Citron DM, Merriam CV, et al. Activity of gatifloxacin compared to those of five other quinolones versus aerobic and anaerobic isolates from skin and soft tissue samples of human and animal bite wound infections. *Antimicrob Agents Chemother* 1999;**43**:1475–9.
56. Goldstein EJC, Citron DM, Merriam CV, et al. In vitro activities of the des-fluoro(6) quinolone BMS-284756 against aerobic and anaerobic pathogens isolated from skin and soft tissue animal and human bite wound infections. *Antimicrob Agents Chemother* 2002; **46**:866–70.
57. Goldstein EJC, Nesbit CA, Citron DM. Comparative in vitro activities of azithromycin, Bay y 3118, levofloxacin, sparfloxacin, and 11 other oral antimicrobial agents against 194 aerobic and anaerobic bite wound isolates. *Antimicrob Agents Chemother* 1995; **39**:1097–100.
58. Gonzalez MN, Papierski P, Hal R Jr. Osteomyelitis of the hand after a human bite. *J Hand Surg Am* 1993;**18**:520–2.

59. Goytia VK, Demmler GJ, Pannaraj PS, et al. An unusual cause of sepsis and meningitis in a neonate. *Semin Pediatr Infect Dis* 2006;**187**:225–7.
60. Griego RD, Rosen T, Orengo IF, et al. Dog, cat and human bites: a review. *J Am Acad Dermatol* 1995;**33**:1019–29.
61. Grim KD, Doherty C, Rosen T. *Serratia marcescens* bullous cellulitis after iguana bites. *J Am Acad Dermatol* 2010;**62**:1075–6.
62. Hayfron K, Wiedeman JA. A 7-year-old girl with ulcerative lesion after a rodent bite. *Pediatr Infect Dis J* 2010;**29**:185, 193.
63. Hui AY, Hung LC, Tse PC, et al. Transmission of hepatitis B by human bite—confirmation by detection of virus in saliva and full genome sequencing. *J Clin Virol* 2005;**33**:254–6.
64. Hultgen JD. Partial gangrene of the left index finger caused by symbiosis of the fusiform *Bacillus* and the *Spirochaeta denticola*. *JAMA* 1910;**10**:887–90.
65. Iyengar KP, Nadkarni JB, Gupta R, et al. *Mycobacterium chelonae* hand infection following ferret bite. *Infection* 2013;**41**:237–41.
66. Jaffe AC. Animal bites. *Pediatr Clin North Am* 1983;**30**:405–13.
67. Jarvis WR, Banko S, Snyder E, et al. *Pasteurella multocida* osteomyelitis following dog bites. *Am J Dis Child* 1981;**135**:625–7.
68. Kannikeswaran N, Kamat D. Mammalian bites. *Clin Pediatr* 2009;**48**:145–8.
69. Kaye AE, Belz JM, Kirschner RE. Pediatric dog bite injuries: a 5-year review of the experience at the Children's Hospital of Philadelphia. *Plast Reconstr Surg* 2009;**124**:551–8.
70. Khajotia RR, Lee E. Transmission of human immunodeficiency virus through saliva after a lip bite. *Arch Intern Med* 1997;**157**: 1901.
71. Kizer KW. *Pasteurella multocida* infection from a cougar bite: a review of cougar attacks. *West J Med* 1989;**150**:87–90.
72. Langley RL. Adverse encounters with alligators in the United States: an update. *Wild Environ Med* 2010;**21**:156–63.
73. Langley RL. Deaths from reptile bites in the United States, 1979-2004. *Clin Toxicol (Phila)* 2009;**47**:44–7.
74. Lederman ER, Austin C, Trevino I, et al. Orf virus infection in children: clinical characteristics, transmission, diagnostic methods, and future therapeutics. *Pediatr Infect Dis J* 2007;**26**:740–4.
75. Lehtinen VA, Kaukonen T, Ikäheimo I, et al. *Mycobacterium fortuitum* infection after a brown bear bite. *J Clin Microbiol* 2005; **43**:1009.
76. Le Moal G, Landron C, Grollier G, et al. Meningitis due to *Capnocytophaga canimorsus* after receipt of a dog bite: case report and review of the literature. *Clin Infect Dis* 2003;**36**:e42–6.
77. Lindsey D, Christopher M, Hollenbach J, et al. Natural course of human bite wound: incidence of infection and complications in 434 bites and 803 lacerations in the same group of patients. *J Trauma* 1987;**27**:45–8.
78. LoVecchio F, Klemens J, Welch S, et al. Antibiotics after rattlesnake envenomation. *J Emerg Med* 2002;**23**:327–8.
79. Luzzi GA, Milne LM, Waitkins SA. Rat-bite acquired leptospirosis. *J Infect* 1987;**15**:57–60.
80. MacBean CE, Taylor DMcD, Ashby K. Animal and human bite injuries in Victoria, 1998-2004. *Med J Austr* 2007;**186**:38–40.
81. Magee JS, Steele RW, Kelly NR, et al. Tularemia transmitted by a squirrel bite. *Pediatr Infect Dis* 1989;**8**:123–5.
82. Marrie TJ, Bent JM, West AB, et al. Extensive gas in tissues of the forearm after horse bite. *South Med J* 1979;**72**:1473–4.
83. Mast EE, Goodman RA, Bond WW, et al. Transmission of bloodborne pathogens during sports: risk and prevention. *Ann Intern Med* 1995;**122**:283–5.
84. Matteucci MJ, Hannum JE, Riffenburgh RH, et al. Pediatric sex group differences in location of snakebite injuries requiring antivenom therapy. *J Med Toxicol* 2007;**3**:103–6.
85. Muguti GI, Dixon MS. Tetanus following human bite. *Br J Plast Surg* 1992;**45**:614–15.
86. Muguti GI, Zvomuya-Ncube M, Bvuma ET. Experiences with human bites in Zimbabwe. *Cent Afr J Med* 1991;**37**:294–8.
87. Mutombo MW, Arita I, Jezek Z. Human monkey pox transmitted by a chimpanzee in a tropical rain forest area of Zaire. *Lancet* 1983;**1**:735–7.
88. Nunley DL, Sasaki T, Atkins A, et al. Hand infections in hospitalized patients. *Am J Surg* 1980;**140**:374–6.
89. Oehler RL, Velez AP, Mizrachi M, et al. Bite-related and septic syndromes caused by cats and dogs. *Lancet Infect Dis* 2009;**9**: 439–47.
90. Ostrowski SR, Leslie MJ, Parrott T, et al. B-virus from pet macaque monkeys: an emerging threat in the United States? *Emerg Infect Dis* 1998;**4**:117–20.
91. Parrish HM. Analysis of 460 fatalities from venomous animals in the United States. *Am J Med Sci* 1963;**245**:129–41.
92. Pavia AT, Bryan JA, Maher KL. *Vibrio carchariae* infection after a shark bite. *Ann Intern Med* 1989;**111**:85–6.
93. Peeples E, Boswick JA Jr, Scott FA. Wounds of the hand contaminated by human and animal saliva. *J Trauma* 1980;**20**:383–9.
94. Pers C, Gahrn-Hansen B, Frederiksen W. *Capnocytophaga canimorsus* septicemia in Denmark, 1982-1995: Review of 39 cases. *Clin Infect Dis* 1996;**23**:71–5.
95. Peters WH. Hand infection apparently due to *Bacillus fusiformis*. *J Infect Dis* 1911;**8**:455–62.
96. Pickering LK, Marano N, Bocchini JA, et al. Exposure to nontraditional pets at home and to animals in public settings: risks to children. *Pediatrics* 2008;**122**:876–86.
97. Quirk JT. Non-fatal dog bite injuries in the USA, 2005-2009. *Public Health* 2012;**126**:300–2.
98. Rickman KM, Richman LS. The potential for transmission of human immunodeficiency virus through human bites. *J Acquir Immun Defic Syndr* 1993;**6**:402–6.
99. Robertson MG. *Brucella* infection transmitted by dog bite. *JAMA* 1973;**225**:750–1.
100. Royle JA, Isaacs D, Eagles G, et al. Infections after shark attacks in Australia. *Pediatr Infect Dis J* 1997;**16**:531–2.
101. Ruinemans GM, Haagsma CJ, Hendrix R. Tenosynovitis caused by a *Pseudallescheria boydii* infection and symptoms of reflex sympathetic dystrophy after a dog bite. *J Clin Rheumatol* 2011;**17**: 363–4.
102. Russell FE. Snake venom poisoning in the United States. *Annu Rev Med* 1980;**31**:247–59.
103. Russell FE. Venomous animal injuries. *Curr Probl Pediatr* 1973; **3**:1–47.
104. Sacks JJ, Sinclair L, Gilchrist J, et al. Breeds of dogs involved in fatal human attacks in the United States between 1979 and 1998. *Am J Vet Med Assoc* 2000;**217**:836–40.
105. Schubach A, Schubach TMP, Bastos de Lima Barros M, et al. Cat-transmitted sporotrichosis, Rio de Janeiro, Brazil. *Emerg Infect Dis* 2005;**11**:1952–4.
106. Shirley LR, Ross SA. Risk of transmission of human immunodeficiency virus by bite of an infected toddler. *J Pediatr* 1989; **114**:425–7.
107. Smith PF, Meadowcroft AM, May DB. Treating mammalian bite wounds. *J Clin Pharm Ther* 2000;**25**:85–99.
108. Solomons HC, Elardo R. Bite injuries at a day care center. *Early Child Res Q* 1989;**4**:89–96.
109. Spence G. A review of animal bites in Delaware, 1989 to 1990. *Del Med J* 1990;**62**:1425–9.
110. Sperber ND. Bite marks, oral and facial injuries: harbingers of severe child abuse. *Pediatrician* 1989;**16**:207–11.
111. Stockheim J, Wilkinson N, Ramos-Bonan C. Human bites and blood exposures in New York City schools. *Clin Pediatr* 2005;**44**: 699–703.
112. Talan DA, Abrahamian FM, Moran GJ, et al. Clinical presentation and bacteriologic analysis in patients presenting to emergency departments. *Clin Infect Dis* 2003;**37**:1481–9.
113. Talan DA, Citron DM, Abrahamia FM, et al. Bacteriologic analysis of infected dog and cat bites. *N Engl J Med* 1999;**340**:85–92.
114. Terry P. The use of antibiotics in venomous snake bite. Best Evidence Topic Report. *Emerg Med J* 2002;**19**:48–9.
115. Weber DJ, Hansen AR. Infections resulting from animal bites. *Infect Dis Clin North Am* 1991;**5**:663–80.
116. Weinberg AN, Branda JA. Case 31-2010. A 29-year-old woman with fever after a cat bite. *N Engl J Med* 2010;**363**:1560–8.
117. Weinstein RA, Stephen RJ, Morof A, et al. Human bites: review of the literature and report of a case. *J Oral Surg* 1973;**31**: 792–4.
118. Welch CE. Human bite infections of the hand. *N Engl J Med* 1936;**215**:901–8.
119. Wiggins ME, Akelman E, Weiss A-PC. The management of dog bites and dog bite infections to the hand. *Orthopedics* 1994;**17**: 617–23.
120. Wilkins EGL, Millar JGB, Cockcroft PM, et al. Rat-bite fever in a gerbil breeder. *J Infect* 1988;**16**:177–80.

for other than peaceful purposes and any devices used to deliver these agents. One hundred forty-three states are parties to the convention, with an additional 18 signatories. Unfortunately, the treaty was not accompanied by effective provisions for verification.

Although the Soviet Union signed the convention at its inception in 1972, it formed and funded an organization known most recently as Biopreparat (Chief Directorate for Biological Preparations) 2 years later.[18] This organization was designed to carry out offensive biologic weapons research, development, and production concealed behind legal and civil biotechnology research. Its capacity for production of biologic weapons included plague, tularemia, glanders, brucellosis, anthrax, smallpox, and Venezuelan equine encephalomyelitis.[1]

An incident at a military microbiology facility in Sverdlovsk (now Yekaterinburg) in the former Soviet Union in 1979 proved to be a grim warning of the dangers of biologic weapons science.[51] An accidental aerosolized release of anthrax spores resulted in at least 79 cases of anthrax, including 68 deaths. This largest reported outbreak of inhalational anthrax occurred in people living or working within a 4-km zone downwind of the facility.

Iraq's biologic warfare program commenced in earnest in 1985 after initial explorations in the late 1970s.[69] By the end of the Persian Gulf War in 1991, Iraqi scientists had investigated the biologic warfare potential of five bacterial strains, one fungal strain, five viruses, and four toxins. The United Nations Special Commission suspects that from 1991 to 1995, Iraq actively preserved biologic weapons capability, including botulinum toxin, anthrax, and *Clostridium perfringens* spores.[18]

The Japanese cult Aum Shinrikyo made several unsuccessful attempts at disseminating anthrax from rooftops and trucks in central Tokyo in the early 1990s before successfully releasing sarin nerve gas into Tokyo's subways and killing a dozen people in 1995.[68] A large community outbreak of salmonellosis was caused by the intentional contamination of restaurant salad bars for political reasons by members of a religious commune in The Dalles, Oregon, in 1984.[61]

EPIDEMIOLOGY

Potential biologic weapons share several characteristics. Ease of acquisition and production is a primary consideration. Other ideal properties include the potential to be aerosolized (particle size, 1-10 μm) and dispersed over a wide geographic area as well as resistance to sunlight, desiccation, and heat. The potential to cause lethal or debilitating disease and person-to-person transmission are important features, as is lack of effective therapy or prophylaxis.[23,30,37]

Any small or large outbreak of disease merits evaluation as a potential biologic event.[55] Unusually high rates of disease (e.g., a cluster of life-threatening pneumonia cases in previously healthy adults) as well as unusual clinical syndromes should signal a warning. Once definition of the cause and rate of attack have been determined, an epidemic curve based on the number of cases during a specific time can be calculated. The epidemic curve in a biologic event triggered by a point-source exposure most likely would be compressed, with a peak occurring in a matter of hours or days. The occurrence of a second curve peak would be possible with contagious agents as a result of person-to-person transmission. The steep epidemic curve expected in a bioterrorism attack is similar to what would be seen with other point-source exposures, such as food-borne outbreaks.

Several epidemiologic clues may be helpful in determining whether further investigation into an outbreak as a potential biologic attack is warranted. Major indicators are a large epidemic, especially in a discrete population; more severe disease than expected for a given pathogen; and a disease unusual for a given geographic area (e.g., pulmonic tularemia in an urban setting). Multiple simultaneous epidemics of different diseases, outbreaks with both human and zoonotic consequences, and unusual strains or susceptibility profiles are additional helpful parameters. Variable attack rates as a function of agent release relative to the interior or exterior of a building also would be useful.[55] Although most bioterrorism attacks will be covert,[6,12] intelligence revealing plans for an attack, terrorist claims of deliberate release, or direct physical evidence of an attack obviously would point to a biologic event.

The emergence of mosquito-borne West Nile virus encephalitis in New York City in the summer of 1999 is an example of a naturally occurring outbreak that had elements of a potential bioterrorist attack.[6,22,59] This outbreak represented a disease occurring in an unusual (previously nonendemic) area as well as one with zoonotic (birds) in addition to human consequences. It marked the first documented appearance of West Nile virus in the Western Hemisphere and the first arboviral outbreak in New York City since the yellow fever epidemics of the 19th century.[22] A large avian die-off, affecting primarily crows, preceded the outbreak in humans by at least several weeks.

CRITICAL BIOLOGIC AGENTS

In addition to anthrax, critical biologic agents include brucellosis, plague, Q fever, and tularemia; smallpox, viral encephalitis, and viral hemorrhagic fever; and illnesses related to botulinum and staphylococcal enterotoxin B toxins (Table 249-1).[6,44,47] The five more credible biologic agents are discussed further.

Anthrax

Bacillus anthracis is a large sporulatory, gram-positive rod with three distinct life cycles featuring multiplication of spores in soil, animal (herbivore) infection, and human infection.[45] Anthrax continues to occur in developing countries where the organism is highly endemic and the use of animal anthrax vaccine is not comprehensive (e.g., Iran, Iraq, Turkey, Pakistan, and sub-Saharan Africa). Human cases may be classified as either agricultural or industrial. Herders, butchers, and slaughterhouse workers in direct contact with infected animals are susceptible to acquiring agricultural infection, and workers in mills that

TABLE 249-1 Critical Biologic Agents

Bacteria
Anthrax
Plague
Q fever
Tularemia
Brucellosis
Viruses
Smallpox
Viral encephalitis
Viral hemorrhagic fevers
Toxins
Botulinum toxin
Staphylococcal enterotoxin B

process animal hair and those handling bone meal may acquire industrial infection.[56]

The three forms of human anthrax are cutaneous, inhalational, and gastrointestinal. The most common form is cutaneous, which is acquired through contact with an infected animal or animal products. The much less common inhalational form results from the deposition of spores in the lungs, and gastrointestinal anthrax occurs after the ingestion of infected meat. Because human-to-human transmission of anthrax has not been reported, standard precautions are recommended for hospitalized patients with all forms of anthrax infection.[39,60]

In the United States, 224 cases of cutaneous anthrax were reported between 1944 and 1994.[39] Most cases in recent decades were a result of exposure to wool or animal hair.[56] Cases of cutaneous, inhalational, and gastrointestinal anthrax have been reported in persons who made or handled imported animal-hide drums or participated in drumming events where imported animal-hide drums contaminated with *B. anthracis* spores were used.[17,25,40]

The clinical features and course of the first 10 confirmed cases of inhalational anthrax associated with bioterrorism in the United States in the fall of 2001 have been reported.[43] Epidemiologic investigation indicated that the outbreak was a result of intentional delivery of *B. anthracis* spores through mailed letters or packages. The median incubation period was 4 days and ranged from 4 to 6 days. Several clinical features of these patients were not emphasized in earlier reports of inhalational anthrax, a previously rare disease. Drenching sweating, nausea, and vomiting were common manifestations of the initial phase of illness in this outbreak. Pleural effusions were a remarkably consistent clinical feature. No predominant underlying diseases or conditions were noted.

None of the 10 patients had an initially normal chest radiograph. In addition to characteristic mediastinal widening (Fig. 249-1), paratracheal or hilar fullness, pleural effusions, and parenchymal infiltrates were noted. Computed tomography of the chest was more sensitive than chest radiography in revealing mediastinal lymphadenopathy, and an elevation in the proportion of neutrophils or band forms represented an early diagnostic clue.

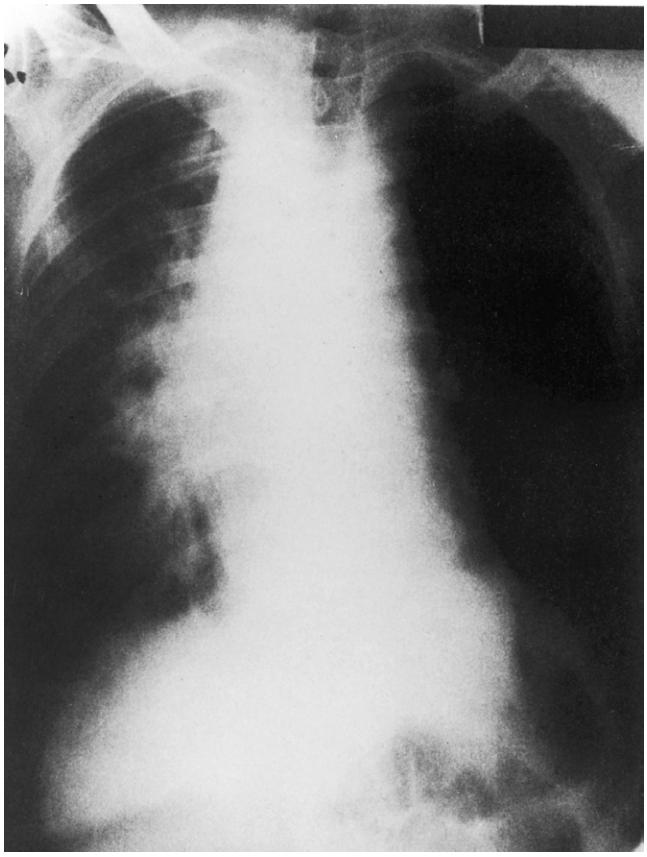

FIGURE 249-1 ■ Chest radiograph showing a widened mediastinum secondary to hemorrhagic mediastinitis in a patient with a fatal case of inhalational anthrax. (From LaForce FM, Bumford FH, Feeley JC, et al. Epidemiologic study of a fatal case of inhalation anthrax. *Arch Environ Health* 1969;**18**:798-805. Reprinted with permission of Helen Dwight Reid Educational Foundation. Published by Heldref Publications, 1319 Eighteenth St., NW, Washington, DC 20036-1802. Copyright 2000.)

Inhalational anthrax previously was reported to be a biphasic illness with influenza-like symptoms such as fever, cough, malaise, fatigue, and chest discomfort in the first phase, followed briefly by 1 to 2 days of improvement before development of the acute phase 2 to 5 days later.[14] However, this brief period of improvement between the initial and fulminant phases of illness was not observed in the first intentional outbreak associated with mail.[36]

The 55 percent survival rate in these patients was higher than previously reported (<15%). Limited data on treatment of survivors suggest that early treatment with a fluoroquinolone and at least one other active drug (e.g., rifampin, clindamycin, or vancomycin) may improve survival.[8,60] A systematic review of 82 cases of inhalational anthrax from 1900 to 2005 revealed several findings. Initiation of antibiotic or anthrax antiserum therapy during the prodromal phase of disease was associated with a substantial improvement in short-term survival. In addition, both multidrug antibiotic regimens and pleural fluid drainage were associated with decreased mortality.[31]

Nasal congestion, rhinorrhea, and sore throat, infrequently seen in this series, might help distinguish

influenza-like illness from inhalational anthrax.[16] Newer diagnostic methods for *B. anthracis* infection include polymerase chain reaction assay, immunohistochemistry, and sensitive serologic tests. Recommendations for antibiotic treatment of inhalational anthrax include use of either ciprofloxacin or doxycycline plus one or two additional antibiotics known to be active in vitro against *B. anthracis*. Additional antibiotics include clindamycin, vancomycin, imipenem, meropenem, chloramphenicol, penicillin, ampicillin, rifampin, and clarithromycin. Corticosteroids should be considered for meningitis or significant mediastinal edema.[3,36] Passive immunization with a polyclonal or monoclonal antibody product may offer adjunctive value to antibiotic therapy, especially if the organisms are resistant to multiple antibiotics.[26] Raxibacumab is a human IgG1 monoclonal antibody directed against protective antigen, a component of anthrax toxin. A single dose of raxibacumab improved survival in rabbits and monkeys with symptomatic inhalational anthrax.[52]

Cutaneous anthrax is characterized by a skin lesion evolving from a papule, through a vesicular stage, to a depressed black eschar, often surrounded by significant edema and erythema.[20,58,60] The lesion, which may mimic a spider bite, is usually painless and located on exposed parts of the body (i.e., face, neck, and arms). The incubation period ranges from 1 to 12 days but commonly is less than 7 days. With effective antimicrobial therapy, fatalities are rare occurrences (<1%). Cutaneous anthrax occurred in a 7-month-old infant who was exposed at his mother's workplace as a result of the fall 2001 attack. This infant displayed severe microangiopathic hemolytic anemia with renal involvement, coagulopathy, and hyponatremia, unusual findings with cutaneous anthrax.[24]

The organism grows readily on sheep blood agar and forms rough, gray-white colonies 4 to 5 mm in size with characteristic comma-shaped or "comet tail" protrusions.[45] *B. anthracis* is differentiated from other *Bacillus* spp. by an absence of the following: hemolysis, motility, growth on phenylethyl alcohol blood agar, gelatin hydrolysis, and salicin fermentation. Biosafety level 2 conditions for safe specimen processing in the microbiology laboratory and prompt confirmation of suspected isolates at the Centers for Disease Control and Prevention (CDC) or the USAMRIID in Fort Detrick are warranted.[39,56]

Postexposure vaccination with an inactivated, cell-free anthrax vaccine is indicated along with ciprofloxacin, doxycycline, or amoxicillin chemoprophylaxis after a proven biologic event has occurred.[60,64] Recommendations for postexposure prophylaxis (and prolonged therapy after initial therapy for inhalational or cutaneous anthrax) include a 60-day course of antibiotics followed by careful clinical observation. Preexposure vaccination may be indicated for the military and other select populations or for groups for which a calculable risk can be assessed.[64]

Smallpox

After a worldwide eradication program, the last known endemic case of smallpox occurred in Somalia in 1977, and the World Health Organization declared smallpox eradicated in 1980.[28] No animal reservoir exists. Current recognized stocks of variola virus are authorized only at the CDC in Atlanta and a Russian state laboratory in Koltsovo. However, additional variola isolates, either long held unreported or acquired through security breaches, also may exist. The World Health Assembly agreed to postpone setting a date for destruction of the world's remaining smallpox virus stocks until 2014, when the 67th annual World Health Assembly will once again take up the question of a destruction date.[32] The decision on a time line for destruction has been postponed four other times so that crucial research may be completed. Routine vaccination against smallpox ceased in the United States in 1972 and for military personnel in 1990. In December 2002, in response to the possible threat of an intentional release of smallpox virus, the U.S. government implemented a program to immunize select military and public health personnel against smallpox.[65] Release of an aerosol would be the most likely route of transmission during an act of bioterrorism.

Smallpox is transmitted by respiratory secretions and requires close person-to-person contact. The incubation period generally is 12 to 14 days, with a range of 7 to 17 days. The prodromal illness of classic variola major features an acute onset of malaise, fever, rigors, vomiting, headache, and backache. Two to 3 days later, a discrete rash appears on the face, hands, forearms, and mucous membranes; it spreads to the legs and then centrally to the trunk during the second week of illness. Lesions progress from macules to papules to pustular vesicles during the course of 1 to 2 days. Umbilicate scabs form 8 to 14 days after onset and leave depressions and depigmented scars.[29]

In contrast to varicella (chickenpox), the rash of smallpox is centrifugal, with a concentration of lesions on the face and extremities, including the palms and soles, versus the trunk in varicella (Fig. 249-2). Smallpox lesions also are synchronous in stage of development, whereas the lesions of chickenpox appear in crops every few days, which results in lesions at very different stages of maturation in different areas of the skin. Any confirmed case of smallpox represents an international emergency and must be reported to national authorities through local and state health departments.[28]

Historically, the mortality rate associated with smallpox was 30 percent for unvaccinated contacts, and currently no antiviral therapy of proven efficacy has been developed. Supplies of vaccinia vaccine and vaccinia immune globulin intravenous (VIGIV) are available only through the CDC.[28] Postexposure vaccination and strict quarantine are indicated for all household and other face-to-face contacts of suspected smallpox cases.[28]

In a limited outbreak with few cases, hospitalized patients should receive care in negative-pressure rooms with high-efficiency particulate air filtration. In addition, precautions using gloves, gowns, and masks are indicated. Home isolation and care are appropriate for most patients in larger outbreaks.[28]

Between January 24 and October 31, 2003, 37,901 volunteers in 55 jurisdictions received 38,885 smallpox vaccinations through the U.S. Department of Health and Human Services program, with a take rate of 92 percent.[11]

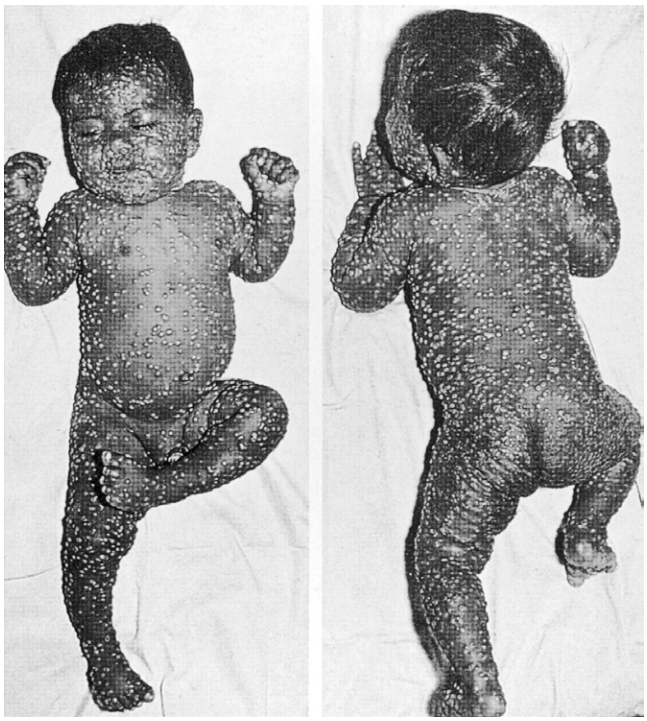

FIGURE 249-2 ■ The lesions of smallpox are at the same stage of development on each area of the body, are deeply embedded in the skin, and are more densely concentrated on the face and extremities. (From Henderson DA. Smallpox: clinical and epidemiologic features. *Emerg Infect Dis* 1999;**5**:537-9.)

The Vaccine Adverse Event Reporting System (VAERS) received 822 adverse event reports related to these vaccines, an overall reporting rate of 217 per 10,000 vaccinees; 722 nonserious reports to VAERS included multiple signs and symptoms of mild systemic and self-limited local reactions, including fever, rash, pain, and headache.[11]

No cases of preventable life-threatening adverse reactions, such as eczema vaccinatum, progressive vaccinia, or fetal vaccinia, were reported. No cases of vaccinia contact transmission occurred. No vaccinee or contact of this program received VIGIV. Rigorous smallpox vaccine safety screening, educational programs, and older vaccinees may have contributed to low rates of preventable life-threatening adverse reactions.[11]

Of the adverse events, 100 were designated serious, resulting in 85 hospitalizations, 2 permanent disabilities, 10 life-threatening illnesses, and 3 deaths. Among the serious adverse events, 21 cases were classified as myocarditis or pericarditis and 10 as ischemic cardiac events that were not anticipated on historical data. Two cases of generalized vaccinia and one case of postvaccinial encephalitis also were detected.[11]

The first confirmed case of eczema vaccinatum in the United States related to smallpox vaccination since routine vaccination was discontinued in 1972 was reported in 2007.[65] A 28-month-old child with refractory atopic dermatitis developed eczema vaccinatum after exposure to his father, a member of the U.S. military who had recently received smallpox vaccine. The child's mother also developed vaccinia infection.[65]

Treatment of the child included VIGIV, used for the first time in a pediatric patient; cidofovir, never previously used for human vaccinia infection; and ST-246, an investigational agent being studied for the treatment of orthopoxvirus infection.[65]

The child recovered without apparent sequelae or significant scarring. Although it is difficult to assess and differentiate the effects of specific therapeutic interventions, the child's immune system almost certainly played a role in the eventual control of the infection. This case illustrates the need for meticulous prevaccination screening and the potential hazards of widespread vaccination.[65]

ACAM2000, a second-generation smallpox vaccine produced in cell culture from a clonally purified master seed stock of vaccinia derived from the New York City Board of Health strain of vaccinia, has replaced the original Dryvax vaccine in the Strategic National Stockpile.[27] Replication-deficient smallpox vaccines, such as the modified vaccinia Ankara (MVA), and antivirals may offer a safer, more inclusive method of addressing smallpox vaccine–associated adverse reactions, including in individuals at risk for complications.[27]

Plague

Plague, a zoonotic illness caused by the gram-negative bacillus *Yersinia pestis*, is primarily a disease of rodents, with transmission occurring through infected fleas. Human disease is acquired through rodent flea vectors as well as respiratory droplets from animals to humans and humans to humans. Transmission of plague to humans in the United States primarily is by the bites of fleas from infected rodents. During 1990-2005, a total of 107 cases of plague were reported in the United States, a median of 7 cases per year, most commonly in the southwestern United States.[34] Indications of a deliberate release of plague bacilli would include the occurrence of cases in locations not known to have enzootic infection, in persons without known risk factors, and in the absence of previous rodent deaths.[37] Travelers can acquire plague in one area and become ill in another area where plague is not endemic (i.e., peripatetic plague).[35]

The three clinical forms of human plague are bubonic, primary septicemic, and pneumonic. Bubonic plague, characterized by the development of an acute regional lymphadenopathy, is the most frequent clinical form and accounts for 76 percent of U.S. cases.[34] However, the pneumonic form would be the most likely manifestation as a result of release of an aerosol during a biologic attack.[23,37] This clinical form is the least common, but it has the highest mortality rate; it is almost always fatal if antibiotics are not begun within 24 hours of the onset of symptoms. Septicemic plague without obvious lymphadenopathy may be more difficult to diagnose than is bubonic plague because of nonspecific manifestations (i.e., fever, chills, abdominal pain, nausea, vomiting, diarrhea, tachycardia, tachypnea, and hypotension).[37] Delay in establishing the diagnosis and initiating appropriate therapy may lead to death.[34,37,48]

The incubation period for primary pneumonic plague is 1 to 3 days. Fever, chills, headache, and rapidly progressive weakness are characteristic of all clinical forms of plague. Cough, dyspnea, and hemoptysis are distinctive of primary pneumonic plague. The sudden appearance of a large number of previously healthy patients with fever, cough, shortness of breath, chest pain, and a fulminant course leading to death should suggest the possibility of pneumonic plague or inhalational anthrax immediately. The presence of hemoptysis would strongly suggest plague.[4,37]

Y. pestis may be identified in clinical specimens by Gram, Wright-Giemsa, Wayson, and immunofluorescence staining methods in addition to standard bacterial culture. Appropriate clinical specimens include lymph node aspirates and blood as well as tracheal washes or sputum smears if pneumonic plague is suspected. Tests used to confirm a suspected diagnosis include antigen detection, immunoglobulin M (IgM) enzyme immunoassay, and polymerase chain reaction assay.[9,37] When plague is suspected, both the state and public health laboratory and public health authorities should be notified promptly.[63]

Effective therapy is available in the form of streptomycin, gentamicin, chloramphenicol, doxycycline, and ciprofloxacin.[37] Parenteral aminoglycoside therapy is recommended in a contained casualty setting (modest number of patients requiring treatment); oral therapy is recommended in a mass casualty scenario.[37] The potential benefits of doxycycline and ciprofloxacin in the treatment of pneumonic plague infection in children substantially outweigh the risks.[12,37] An inactivated, whole-cell *Y. pestis* vaccine was discontinued by its manufacturers in 1999 and no longer is available.[37]

In addition to standard precautions, droplet precautions are indicated for all patients with suspected plague until pneumonia is excluded and appropriate therapy has been initiated. Droplet precautions should be continued in patients with confirmed pneumonic plague for 48 hours after the initiation of appropriate therapy.[37] Only standard precautions are recommended for bubonic plague.

Tularemia

The etiologic agent of this zoonotic illness is *Francisella tularensis*, a gram-negative coccobacillus. The disease may be acquired from ticks and deer flies, contact with animals such as rabbits and rodents, ingestion of contaminated water, or inhalation of aerosols.[62] In a bioterrorist event, inhalation of an aerosol would be the most likely route of infection.[4,19] Human-to-human transmission of tularemia has never been reported. The annual incidence of tularemia in the United States is fewer than 200 cases; approximately 40% of all tularemia cases reported to the CDC each year occur in Arkansas, Oklahoma, and Missouri. All suspected or confirmed cases must be reported to health authorities.[62]

Clinical forms of the disease include ulceroglandular, glandular, oculoglandular, oropharyngeal, pneumonic, and typhoidal, the type reflecting the organism's portal of entry.[21,42] Either pneumonic alone or typhoidal, with or without a pneumonic component, would be the most likely clinical manifestation of tularemia as a result of aerosol release during a biologic attack.[4,19,62] A novel intraerythrocytic phase during *F. tularensis* infection was shown in a murine model of pulmonary tularemia.[33] The incubation period for tularemia is 3 to 6 days, with a range of 1 to 21 days. Typhoidal tularemia may be manifested as fever of unknown origin. Standard precautions are indicated for hospitalized patients with all forms of tularemia.[19]

The diagnosis is established most often by serologic testing, and isolation of *F. tularensis* from clinical specimens requires cysteine-enriched media or inoculation of laboratory mice. In addition to a need for special media, the laboratory should always be informed when tularemia is suspected because of the potential hazard to laboratory personnel. Suspected isolates should be confirmed by the CDC or USAMRIID through local or state health departments.[19]

Effective therapeutic agents include streptomycin, gentamicin, tetracycline, ciprofloxacin, and chloramphenicol; postexposure prophylaxis with doxycycline or ciprofloxacin may be considered.[19] The benefits of tetracycline or ciprofloxacin therapy outweigh the risks for children younger than 8 years in select clinical situations, including tularemia.[19,46] A live attenuated vaccine for preexposure use is not generally available in the United States.

Botulism

Seven distinct but related neurotoxins, A through G, are produced by different strains of *Clostridium botulinum*, an anaerobic gram-positive rod. The most common types in U.S. food-borne outbreaks are A, B, and E; outbreaks with unusual botulinum toxin types (i.e., C, D, F, and G or E not acquired from an aquatic food) would suggest deliberate release.[2] Classic neuroparalytic disease is acquired through the ingestion of preformed neurotoxin. Other forms include localized infection (wound botulism) and *C. botulinum* intestinal colonization in infants with in vivo toxin production (infant botulism). Botulism in the United States is seen most often in small clusters or single cases associated with home-canned foods. Although airborne transmission of botulinum neurotoxin does not occur naturally, aerosolization of preformed toxin would be the most likely route of transmission in a bioterrorism event.[2,23] Sabotage of food supplies is also possible. Botulism is not transmitted from human to human; standard precautions are recommended for hospitalized patients.

The incubation period for food-borne botulism generally is 12 to 36 hours (range, 6 hours to 8 days). The clinical manifestations of disease acquired by inhalation would be the same as those for food-borne botulism. Early manifestations include blurred vision, diplopia, and dry mouth. Patients are afebrile with a clear sensorium.

Later clinical features indicative of more severe disease include dysphonia, dysarthria, dysphagia, ptosis, and a symmetric, descending, progressive muscular weakness with respiratory failure.[2] Clinical suspicion is critical

because a recognized source of exposure may be absent in a biologic attack. Botulism is a reportable disease.

A toxin neutralization bioassay in mice is used to identify botulinum toxin in serum, stool, or food. *C. botulinum* also may be cultured from stool and food. Electromyography can be helpful diagnostically. Heptavalent (A-G) botulinum antitoxin (HBAT) of equine origin, available through a CDC-sponsored Food and Drug Administration Investigational Drug protocol, should be administered as soon as possible to patients symptomatic with botulism.[2,41] A pentavalent toxoid (PBT) of *C. botulinum* toxin types A, B, C, D, and E is no longer available for use as a vaccine.[53]

Viral Hemorrhagic Fever

The term *viral hemorrhagic fever* (VHF) refers to a clinical illness associated with fever and a bleeding diathesis caused by a virus belonging to one of four distinct families: Filoviridae (e.g., Ebola, Marburg), Arenaviridae (e.g., Lassa fever), Bunyaviridae (e.g., Rift Valley fever), and Flaviviridae (e.g., yellow fever). VHF agents are RNA viruses normally transmitted to humans from animal reservoirs or arthropod vectors, although the natural reservoirs and vectors of the Ebola and Marburg agents are unknown. Geographic distribution of these agents is as follows: Ebola and Marburg, Africa; Lassa, West Africa; New World arenaviruses, the Americas; Rift Valley fever, Africa and the Middle East; and yellow fever, Africa and the Americas. Most of these agents are considered serious, potential biologic threats because of their potential to be aerosolized and their high morbidity and mortality rates. Clinical features vary with the specific virus, but all are capable of causing fever, myalgia, prostration, petechiae, hemorrhage, and shock.[7]

Treatment generally is supportive, although ribavirin has some in vitro and in vivo activity against arenaviruses and bunyaviruses but not against filoviruses or flaviviruses. VHF-specific barrier precautions as well as airborne precautions are recommended for any patient with suspected or documented VHF. Effective prophylaxis after exposure to a VHF agent is not available, although guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever have been proposed on the basis of an extensive review of the literature and experience in the field.[5,7]

PREPAREDNESS AND RESPONSE

Being prepared for a bioterrorist attack or any other large-scale infectious disease outbreak relies on similar critical elements.[22,38,50,54,59] Clinician awareness and education are paramount. Because practicing physicians most likely will be the first group to encounter diseases caused by biologic weapons, they must be familiar with the signs and symptoms of the more credible biologic agents (e.g., smallpox, anthrax).[38,50,54] Recognition of an unusual case or cluster of illnesses should prompt a report to the local public health authorities.[10,22] Improved communication between human and animal health authorities is warranted because many potential bioterrorist agents, such as anthrax, brucellosis, Q fever, plague, and tularemia, are zoonotic diseases.[22] Knowledge of the processes by which the hospital laboratory, the local or state health department, or the CDC performs diagnostic studies to implicate or to exclude biologic agents also is important.[6,22,38] Local, state, and federal public health agencies must coordinate plans for dealing with a large-scale outbreak caused by a biologic event.[6,10,22,59] They should address rapid investigation of the outbreak, public education, mass distribution of antibiotics and vaccines, the capacity to care for mass casualties, and proper, expeditious treatment of the dead.[6,22,38,59]

NEW REFERENCES SINCE THE SIXTH EDITION

5. Bausch DG, Hadi CM, Khan SH, et al. Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever. *Clin Infect Dis* 2010;**51**:1435–41.
9. Butler T. Plague into the 21st century. *Clin Infect Dis* 2009;**49**:736–42.
17. Cutaneous anthrax associated with drum making using goat hides from West Africa—Connecticut, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:628–31.
25. Gastrointestinal anthrax after an animal-hide drumming event—New Hampshire and Massachusetts. *MMWR Morb Mortal Wkly Rep* 2010;**59**:872–7.
26. Grabenstein JD. Countering anthrax: vaccines and immunoglobulins. *Clin Infect Dis* 2008;**46**:129–36.
27. Handley L, Buller RM, Frey SE, et al. The new ACAM2000 vaccine and other therapies to control orthopoxvirus outbreaks and bioterror attacks. *Expert Rev Vaccines* 2009;**8**:841–50.
31. Holty J-EC, Bravata DM, Liu H, et al. Systematic review: a century of inhalational anthrax cases from 1900 to 2005. *Ann Intern Med* 2006;**144**:270–80.
32. Homelandsecuritynewswire.com. Decision on smallpox virus destruction delayed for three years, May 27, 2011. Accessed September 7, 2011.
33. Horzempa J, O'Dee DM, Stolz DB, et al. Invasion of erythrocytes by *Francisella tularensis*. *J Infect Dis* 2011;**204**:51–9.
34. Human plague—four states, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1–3.
35. Imported plague—New York City, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:725–8.
40. Inhalation anthrax associated with dried animal hides—Pennsylvania and New York City, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:280–2.
41. Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigational botulinum antitoxin E. *MMWR Morb Mortal Wkly Rep* 2010;**59**:299.
52. Migone T-S, Subramanian GM, Zhong J. Raxibacumab for the treatment of inhalational anthrax. *N Engl J Med* 2009;**361**:135–44.
53. Notice of CDC's discontinuation of investigational pentavalent (ABCDE) botulinum toxoid vaccine for workers at risk for occupational exposure to botulinum toxins. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1454–5.
62. Tularemia—Missouri, 2000-2007. *MMWR Morb Mortal Wkly Rep* 2009;**58**:744–8.
63. Two cases of human plague—Oregon, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:214–15.
64. Use of anthrax vaccine in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 2010;**59**(RR-6):1–30.
65. Vora S, Damon I, Fulginiti V, et al. Severe eczema vaccinatum in a household contact of a smallpox vaccinee. *Clin Infect Dis* 2008;**46**:1555–61.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Alibek K. *Biohazard*. New York: Random House; 1998. p. 298–300.
2. Arnon SS, Schechter R, Inglesby TV, et al. Botulinum toxin as a biological weapon. Medical and public health management. *JAMA* 2001;**285**:1059–70.
3. Bartlett JG, Ingelsby TV, Borio L. Management of anthrax. *Clin Infect Dis* 2002;**35**:851–8.
4. Bartlett JG, Dowell SF, Mandell LA, et al. Practice guidelines for the management of community-acquired pneumonia in adults. *Clin Infect Dis* 2000;**31**:347–82.
5. Bausch DG, Hadi CM, Khan SH, et al. Review of the literature and proposed guidelines for the use of oral ribavirin as postexposure prophylaxis for Lassa fever. *Clin Infect Dis* 2010;**51**:1435–41.
6. Biological and chemical terrorism: strategic plan for preparedness and response. Recommendations of the CDC Strategic Planning Workshop. *MMWR Recomm Rep* 2000;**49**(RR-4):1–14.
7. Borio L, Inglesby T, Peters CJ, et al. Hemorrhagic fever viruses as biological weapons. Medical and public health management. *JAMA* 2002;**287**:2391–405.
8. Bush LM, Abrams BH, Beall A, et al. Index case of fatal inhalational anthrax due to bioterrorism in the United States. *N Engl J Med* 2001;**345**:1607–10.
9. Butler T. Plague into the 21st century. *Clin Infect Dis* 2009;**49**: 736–42.
10. Campbell GL, Hughes JM. Plague in India: a new warning from an old nemesis. *Ann Intern Med* 1995;**122**:151–3.
11. Casey CG, Iskander JK, Raper MH, et al. Adverse events associated with smallpox vaccination in the United States, January-October 2003. *JAMA* 2005;**294**:2734–43.
12. Chemical-biological terrorism and its impact on children: a subject review. American Academy of Pediatrics. Committee on Environmental Health and Committee on Infectious Diseases. *Pediatrics* 2000;**105**:662–70.
13. Christopher GW, Cieslak TJ, Pavlin, JA, et al. Biological warfare. A historical perspective. *JAMA* 1997;**278**:412–17.
14. Cieslak TJ, Eitzen EM. Clinical and epidemiologic principles of anthrax. *Emerg Infect Dis* 1999;**5**:552–5.
15. Cole LA. The specter of biological weapons. *Sci Am* 1996;**275**: 60–5.
16. Considerations for distinguishing influenza-like illness from inhalational anthrax. *MMWR Morb Mortal Wkly Rep* 2001;**50**:984–6.
17. Cutaneous anthrax associated with drum making using goat hides from West Africa—Connecticut, 2007. *MMWR Morb Mortal Wkly Rep* 2008;**57**:628–31.
18. Davis C. Nuclear blindness: an overview of the biological weapons programs of the former Soviet Union and Iraq. *Emerg Infect Dis* 1999;**5**:509–12.
19. Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon. Medical and public health management. *JAMA* 2001;**285**:2763–73.
20. Dixon TC, Meselson M, Guilliemin J, et al. Anthrax. *N Engl J Med* 1999;**341**:815–26.
21. Evans ME, Gregory DW, Schaffner W, et al. Tularemia: a 30-year experience with 88 cases. *Medicine (Baltimore)* 1985;**64**:251–69.
22. Fine A, Layton M. Lessons from the West Nile viral encephalitis outbreak in New York City, 1999: implications for bioterrorism preparedness. *Clin Infect Dis* 2001;**32**:277–82.
23. Franz DR, Jahrling PB, Friedlander AM, et al. Clinical recognition and management of patients exposed to biological warfare agents. *JAMA* 1997;**278**:399–411.
24. Freedman A, Afonja O, Chang MW, et al. Cutaneous anthrax associated with microangiopathic hemolytic anemia and coagulopathy in a seven-month-old infant. *JAMA* 2002;**287**:869–74.
25. Gastrointestinal anthrax after an animal-hide drumming event—New Hampshire and Massachusetts. *MMWR Morb Mortal Wkly Rep* 2010;**59**:872–7.
26. Grabenstein JD. Countering anthrax: vaccines and immunoglobulins. *Clin Infect Dis* 2008;**46**:129–36.
27. Handley L, Buller RM, Frey SE, et al. The new ACAM2000 vaccine and other therapies to control orthopoxvirus outbreaks and bioterror attacks. *Expert Rev Vaccines* 2009;**8**:841–50.
28. Henderson DA, Inglesby TV, Bartlett JG, et al. Smallpox as a biological weapon. Medical and public health management. *JAMA* 1999;**281**:2127–37.
29. Henderson DA. Smallpox: clinical and epidemiologic features. *Emerg Infect Dis* 1999;**5**:537–9.
30. Henderson DA. Bioterrorism as a public health threat. *Emerg Infect Dis* 1998;**4**:488–92.
31. Holty J-EC, Bravata DM, Liu H, et al. Systematic review: a century of inhalational anthrax cases from 1900 to 2005. *Ann Intern Med* 2006;**144**:270–80.
32. Homelandsecuritynewswire.com. Decision on smallpox virus destruction delayed for three years, May 27, 2011. Accessed September 7, 2011.
33. Horzempa J, O'Dee DM, Stolz DB, et al. Invasion of erythrocytes by *Francisella tularensis*. *J Infect Dis* 2011;**204**:51–9.
34. Human plague—four states, 2006. *MMWR Morb Mortal Wkly Rep* 2006;**55**:1–3.
35. Imported plague—New York City, 2002. *MMWR Morb Mortal Wkly Rep* 2003;**52**:725–8.
36. Inglesby TV, O'Toole T, Henderson DA, et al. Anthrax as a biological weapon, 2002. Updated recommendations for management. *JAMA* 2002;**287**:2236–52.
37. Inglesby TV, Dennis DT, Henderson DA, et al. Plague as a biological weapon. Medical and public health management. *JAMA* 2000; **283**:2281–90.
38. Inglesby TV, O'Toole T, Henderson DA. Preventing the use of biological weapons: improving response should prevention fail. *Clin Infect Dis* 2000;**30**:926–9.
39. Inglesby TV, Henderson DA, Bartlett JG, et al. Anthrax as a biological weapon. Medical and public health management. *JAMA* 1999;**281**:1735–45.
40. Inhalation anthrax associated with dried animal hides—Pennsylvania and New York City, 2006. *MMWR Morb Mortal Wkly Rep* 2006; **55**:280–2.
41. Investigational heptavalent botulinum antitoxin (HBAT) to replace licensed botulinum antitoxin AB and investigational botulinum antitoxin E. *MMWR Morb Mortal Wkly Rep* 2010;**59**:299.
42. Jacobs RF, Narain JP. Tularemia in children. *Pediatr Infect Dis* 1983;**2**:487–91.
43. Jernigan JA, Stephens DS, Ashford DA, et al. Bioterrorism-related inhalational anthrax: the first 10 cases reported in the United States. *Emerg Infect Dis* 2001;**7**:933–44.
44. Kortepeter MG, Parker GW. Potential biological weapons threats. *Emerg Infect Dis* 1999;**5**:523–7.
45. LaForce FM. Anthrax. *Clin Infect Dis* 1994;**19**:1009–14.
46. Leggiadro RJ. Tetracycline for Rocky Mountain spotted fever. *Pediatrics* 1991;**87**:124–5.
47. Leggiadro RJ. The threat of biological terrorism: a public health and infection control reality. *Infect Control Hosp Epidemiol* 2000;**21**: 53–6.
48. Mann JM, Shandler L, Cushing AH. Pediatric plague. *Pediatrics* 1982;**69**:762–7.
49. McDade JE, Franz D. Bioterrorism as a public health threat. *Emerg Infect Dis* 1998;**4**:493–4.
50. Medical examiners, coroners, and biologic terrorism. A guidebook for surveillance and case management. *MMWR Morb Mortal Wkly Rep* 2004;**53**(RR-8):4–12.
51. Meselson M, Guillemin J, Hugh-Jones M, et al. The Sverdlovsk anthrax outbreak of 1979. *Science* 1994;**266**:1202–8.
52. Migone T-S, Subramanian GM, Zhong J. Raxibacumab for the treatment of inhalational anthrax. *N Engl J Med* 2009;**361**: 135–44.
53. Notice of CDC's discontinuation of investigational pentavalent (ABCDE) botulinum toxoid vaccine for workers at risk for occupational exposure to botulinum toxins. *MMWR Morb Mortal Wkly Rep* 2011;**60**:1454–5.
54. Patt HA, Feigin RD. Diagnosis and management of suspected cases of bioterrorism: a pediatric perspective. *Pediatrics* 2002;**109**: 685–92.
55. Pavlin JA. Epidemiology of bioterrorism. *Emerg Infect Dis* 1999; **5**:528–30.
56. Pile JC, Malone JD, Eitzen EM, et al. Anthrax as a potential biological warfare agent. *Arch Intern Med* 1998;**158**:429–34.
57. Regis E. *The biology of doom. The history of America's secret germ warfare project*. New York: Henry Holt; 1999. p. 27–31.
58. Roche KR, Chang MW, Lazarus H. Cutaneous anthrax infection. *N Engl J Med* 2001;**345**:1611.
59. Schoch-Spana M. Implications of pandemic influenza for bioterrorism response. *Clin Infect Dis* 2000;**31**:409–13.

60. Swartz MN. Recognition and management of anthrax—an update. *N Engl J Med* 2001;**345**:1621–6.
61. Torok TJ, Tauxe RV, Wise RP, et al. A large community outbreak of salmonellosis caused by intentional contamination of restaurant salad bars. *JAMA* 1997;**278**:389–95.
62. Tularemia—Missouri, 2000-2007. *MMWR Morb Mortal Wkly Rep* 2009;**58**:744–8.
63. Two cases of human plague—Oregon, 2010. *MMWR Morb Mortal Wkly Rep* 2011;**60**:214–15.
64. Use of anthrax vaccine in the United States. Recommendations of the Advisory Committee on Immunization Practices (ACIP), 2009. *MMWR Recomm Rep* 2010;**59**(RR-6):1–30.
65. Vora S, Damon I, Fulginiti V, et al. Severe eczema vaccinatum in a household contact of a smallpox vaccinee. *Clin Infect Dis* 2008;**46**:1555–61.
66. Wheelis M. Biological warfare at the 1346 siege of Caffa. *Emerg Infect Dis* 2002;**8**:971–5.
67. Wheelis M. Investigating disease outbreaks under a protocol to the biological and toxin weapons convention. *Emerg Infect Dis* 2000;**6**:595–600.
68. WuDunn S, Miller J, Broad WJ. How Japan germ terror alerted world. *New York Times* 1998:1 and 6.
69. Zilinskas RA. Iraq's biological weapons. *JAMA* 1997;**278**:418–24.

This page intentionally left blank

PART **VI**

APPROACH TO THE LABORATORY DIAGNOSIS OF INFECTIOUS DISEASES

This page intentionally left blank

CHAPTER 250

Bacterial Laboratory Diagnosis

Paula A. Revell • Lakshmi Chandramohan

Laboratory tests can lead to or confirm diagnoses as well as aid in choosing appropriate therapy. The challenge of the clinical diagnostic laboratory is to provide clinically relevant information in a timely manner by reporting accurate and significant results. However, laboratory diagnosis of bacterial infections begins at the bedside, not in the laboratory, with the appropriate collection and transport of specimens. This initial step is one of the most critical steps in the process of making an accurate laboratory diagnosis as no level of technical expertise or amount of specialized technology can correct for an improperly collected or transported specimen. Most bacteriology testing depends on the cultivation and identification of living organisms, and as such, more than any other area of the clinical laboratory, microbiology is affected by inappropriate specimen management practices. Examples of common problems include incorrect or insufficient specimen labeling, insufficient volume of specimen collected, incorrect transport media, and delays in transport of the specimen to the laboratory. Some common principles for quality specimen collection and transport are listed in Table 250-1.

In addition to culture-based methods of bacterial identification, non–culture-based techniques for several bacterial pathogens are described in this chapter.

CULTURE-BASED DETECTION

Whereas technologic advancements have been made in the area of direct pathogen identification by use of molecular methods as well as mass spectrometry, the isolation of live organisms is still critical, in particular because of the need to determine antimicrobial susceptibility results. Bacterial culture depends on the growth of organisms on various different types of culture media, including selective, differential, and enriched. Enriched media will support growth of the majority of bacterial strains, including fastidious organisms; examples include sheep blood agar and chocolate agar. Selective media inhibit the growth of certain bacteria while allowing the growth of others. The most common method of selecting against certain organisms is with the addition of antibiotics to the media; an example of this type of medium is oxacillin agar to select for methicillin-resistant *Staphylococcus aureus*. Differential media aid in distinguishing similar types of organisms on the basis of their phenotype when they are grown on the media. Examples of differential media include chromogenic agars that change color when a certain organism grows on the media and MacConkey agar, which permits the distinction between lactose-fermenting and non–lactose-fermenting gram-negative bacilli.

Each particular type of specimen has distinct requirements for optimal collection and transport as well as recommended media types to support growth of suspected organisms. The individual specimen-specific strategies for identification and diagnosis of various bacterial infections are described in the following sections.

Specimen-Specific Collection and Cultivation Requirements

Bloodstream Infection

Sepsis has long been associated with significant morbidity and mortality; according to the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics, sepsis was the eleventh leading cause of death in the United States in 2009.[25] Given the significant impact of sepsis, the detection of microorganisms circulating in the bloodstream of a patient is among the most critical roles of the clinical microbiology laboratory. Detection of bacterial pathogens is typically accomplished through the incubation of a blood sample in a bottle that contains a specialized liquid medium on a continuously monitored blood culture system. A positive blood culture can confirm a cause of the septic event as well as allow appropriate antimicrobial susceptibility testing and thus optimal antibiotic therapy. The automated blood culture systems routinely used in microbiology laboratories are described as continuously monitored because the system takes a reading every 10 to 20 minutes to evaluate possible growth of microorganisms. Several systems are currently available with differences in detection methods, but all perform comparably. This constant monitoring is accomplished without entering the bottle, and thus the contamination rate for these systems is lower than that of the lysis-centrifugation system (Isolator, Wampole Laboratories, Cranbury, NJ).

The single variable with the greatest impact on the sensitivity of detection of bacterial pathogens in blood is the volume of blood that is cultured.[19,46] Whereas this observation is based primarily on data from studies of adult patients, several studies show that increasing the volume of blood cultured increases the sensitivity of detection in a pediatric population as well.[20] In addition, multiple studies have demonstrated that low-level bacteremia in pediatric patients is common, and thus by increasing the volume of blood cultured, the likelihood

TABLE 250-1 Principles for Quality Specimen Collection and Transport

Use sterile technique to minimize contamination.
Collect as close to the site of infection as possible.
Collect at optimal times to recover the pathogens of interest.
Obtain a sufficient quantity of the specimen to perform the requested tests.
Use appropriate collection devices and specimen containers to ensure recovery of all organisms.
Collect all microbiology specimens before the institution of antibiotics.
Properly label and seal the specimen container before transport.
Minimize transport time or maximize transport media. There is always some loss of viability during transport.

of detecting low-level bacteremia increases.[41] Because pediatric patients have a wide range of blood volumes, there is not one recommended volume for optimal blood culture collection. The most common approach to guiding appropriate volume of blood cultured from pediatric patients is the implementation of a weight-based guideline.[32]

As is the case with any specimen, blood cultures should be transported to the laboratory in a timely manner. Several studies have shown that delays in entry of blood culture bottles to the blood culture system can result in falsely negative cultures (decreasing sensitivity of detection) and increases in time to detection (delaying the reporting of positive results).[67] Optimally, the blood culture bottle should be loaded onto the blood culture system within 4 hours of collection. If delay in entry to the blood culture system is required, during the delay time, the sample should not be incubated at 37° C (98.6° F) as preincubation may significantly compromise results.[79] Optimal temperature for transport of blood culture bottles is room temperature.

Before the introduction of automated, continuously monitored blood culture detection systems, the detection of certain fastidious organisms required incubation times up to 2 weeks. However, with use of the automated systems, both routine and fastidious organisms will be detected within a 5-day incubation time. This 5-day incubation time is adequate for detection of fastidious organisms such as *Brucella*, *Francisella*, *Haemophilus*, *Actinobacillus*, *Cardiobacterium*, *Eikenella*, *Kingella*, *Abiotrophia*, and *Granulicatella*.[18,80] Importantly, there are limitations to the routine blood culture; for example, *Streptobacillus moniliformis* is sensitive to even low levels of the anticoagulant SPS, which is added to aerobic blood culture bottles. In cases with suspected rat-bite fever, the recommendation would be to include an anaerobic blood culture bottle as the anaerobic bottles contain a lower level of SPS.[70] Certain organisms are so slow growing that they will likely be missed with the routine 5-day incubation time of a standard blood culture; examples of these pathogens are filamentous fungi, *Nocardia* spp., and slow-growing mycobacterial species. Because these microorganisms do require longer incubation times and different media than the routine blood culture provides, it is best to send separate specimens for specialized fungal or mycobacterial testing if these organisms are suspected.

Respiratory Tract Infections

Although the majority of respiratory tract infections have viral causes, the focus of this chapter is on bacterial pathogens. The laboratory diagnosis of respiratory tract infections is complicated by the fact that the respiratory tract is normally colonized with a complex and robust microbiologic community. Importantly, many of the organisms that are normal colonizers of the respiratory tract can also be pathogenic when they are overgrown or out of balance with the normal ecology of the respiratory tract. Common components of normal respiratory flora that are also respiratory tract pathogens include but are not limited to *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Neisseria meningitidis*, and *Moraxella catarrhalis*.

For the purposes of laboratory testing, the respiratory tract is divided in two on the basis of anatomy. The upper respiratory tract includes the areas from the anterior nasal passages to the larynx, and the lower respiratory tract includes the structures beyond the larynx. Typically, samples collected from the upper respiratory tract include swabs and washes and are not considered sterile, whereas samples collected from the lower respiratory tract typically require more invasive collection techniques, such as bronchoalveolar lavage or lung biopsy, and may be considered sterile. Because of the difficult and invasive nature of true lower respiratory specimen collection, laboratories often accept specimens that require less invasive techniques, such as expectorated sputa or tracheal aspirates, in an effort to diagnose a lower respiratory tract infection. However, these less invasive specimens are not sterile and must be considered in the context of normal respiratory flora.

Upper respiratory tract infections account for more physicians' office visits than any other type of infection,[13] and although the majority of these infections are the result of viral infections, bacterial causes are also important. Examples of infections that fall into the "upper respiratory" category include pharyngitis, otitis media, and otitis externa.

Diagnosis of pharyngitis can be made by both culture and antigen-based methods. Typically, the specimen is collected on a throat or oropharyngeal swab; swabs made of synthetic fibers are optimal, and there may be an advantage to use of a flocked swab for optimal sample collection.[35] In the pediatric patient population, agents other than *Streptococcus pyogenes* (group A streptococcus) can cause pharyngitis and must be considered. Not all laboratories routinely identify organisms other than group A streptococci, and thus special requests should be made to ensure appropriate testing. In addition to group A streptococci, large colony-forming, β-hemolytic organisms from the Lancefield groups C and G as well as *Arcanobacterium hemolyticum* may cause pharyngitis with clinical symptoms similar to those of group A streptococcal pharyngitis.[78]

Otitis externa and otitis media are usually diagnosed clinically and managed empirically; however, in cases of spontaneous perforation of the tympanic membrane or when tympanocentesis is performed, fluid can be sent for culture and microbiologic evaluation. Fluid is the optimal specimen for bacterial culture. Nasopharyngeal specimens are not representative of the etiologic agents of otitis media.[37]

Laboratory diagnosis of lower respiratory tract infections, primarily pneumonia, is complicated by the difficulty of collecting appropriate lower respiratory tract samples. In addition, delays in transport of specimens to the laboratory may result in overgrowth of significant pathogens by nonpathogenic colonizing flora or the loss of significant pathogens.[14,55] Whereas organisms isolated from specimens such as pleural fluid or tissue from lung biopsy must always be considered significant, these highly invasive procedures are relatively rarely performed. The most common specimens collected to aid in diagnosis of pneumonia include sputa and tracheal washes or aspirates. Results from cultures of these specimen sources in any scenario are complicated by the presence of normal upper airway flora, and the determination of significance for any given isolated organism is difficult.[14] To aid in the determination of the quality of either sputa or bronchial aspirate specimens, laboratories perform microscopy-based screening of specimens and reject samples that are not adequate. Specimen quality is evaluated on the basis of the presence and quantity of organisms, squamous epithelial cells, and polymorphonuclear leukocytes. A large number of squamous epithelial cells represents oropharyngeal contamination, and these specimens should be rejected with a recommendation for recollection (with the exception of cases in which *Legionella* or mycobacterial infections are suspected).[71] The presence of large numbers of polymorphonuclear leukocytes can indicate a quality specimen; however, there are exceptions to this observation. In the case of tracheal secretions or aspirates from ventilated, low-birth-weight infants, large numbers of polymorphonuclear leukocytes were associated with the length of intubation and not the development of respiratory symptoms.[52] Although bronchoalveolar lavage is not as commonly performed because of the more invasive nature of the procedure, bronchoalveolar lavage specimens provide quality lower respiratory tract samples. The procedure includes the wedging of the bronchoscope channel into an airway lumen followed by the injection of saline through the lumen. The injected saline is aspirated out in several aliquots. The initial aliquot removed will be contaminated with upper airway secretions and should be discarded before bacterial culture. In an effort to aid in establishing significance of various pathogens isolated from bronchoalveolar lavage cultures, some laboratories perform quantitative cultures and establish cutoffs for significance.[10]

Central Nervous System Infections

Because of the significant morbidity and mortality associated with bacterial meningitis, the rapid and timely identification of the causative agent is critical. Cerebrospinal fluid (CSF) specimens are collected by lumbar puncture and must be transported to the laboratory at room temperature and processed without delay. Owing to the rapid changes in the pH of the CSF on removal from the patient and the hypotonic nature of CSF, the viability of microorganisms is reduced as time passes, potentially increasing the likelihood of false-negative culture results[24]; in addition, host cells may lyse, resulting in an altered cell count. If sequential tubes of CSF are collected, the first tube is not ideal for microbiologic studies because of the possibility of contamination with skin flora or blood from the initial lumbar puncture, which can alter culture results. The second tube should be sent for microbiologic studies whenever possible. Although bacterial stain and culture can be performed on small amounts of fluid, it is recommended to send larger amounts of CSF because of the typically low number of microorganisms present in the specimen. For isolation of mycobacteria from CSF, a minimum of 5.0 mL of specimen is recommended.[3]

On receipt into the laboratory, CSF specimens should be routinely Gram stained and set up for bacterial culture. Sensitivity of the Gram stain can be improved by concentrating the specimen via cytocentrifugation before staining.[69] Positive Gram stain results can offer useful information about organism morphology and Gram stain phenotype; but large numbers of organisms are needed for visualization, and a negative Gram stain result does not indicate a lack of pathogenic organisms in the CSF.[44] Staining with acridine orange (a fluorescent stain) increases the sensitivity of detection of microorganisms, but this stain does not provide information about the Gram stain phenotype (e.g., gram positive or gram negative).[43,45] CSF specimens should be inoculated onto both chocolate agar and sheep blood agar and incubated for 72 hours. Routine inoculation of broth-based media is not necessary and may result in isolation of contaminants.[54,74] Inoculation of broth media may add value in cases in which anaerobic or other unusual organisms are suspected or when hardware is in place. Direct bacterial antigen testing of CSF by latex agglutination is not recommended.[58]

Urinary Tract Infections

Pediatric urinary tract infections are most commonly caused by gram-negative enteric bacteria. *Escherichia coli* causes 80% of infections. Other common pathogens include but are not limited to *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., group B streptococcus and *Enterococcus* spp., and occasionally *Pseudomonas* spp.[7,82] As is the case for respiratory tract pathogens, urinary tract pathogens must be isolated and identified in and among a background of other normal bacterial flora. To distinguish organisms that colonize the distal urethra from true urinary tract pathogens, quantitative bacterial culture is performed on urine specimens. To prevent misleading results, it is important that urine specimens be kept refrigerated if they are not inoculated in a timely manner (less than 2 hours); in cases of prolonged storage of urine at ambient temperature, false-positive culture results can be obtained. A midstream, clean-catch, voided urine specimen is ideal; however, these specimens are difficult

to obtain in many pediatric age groups. Catheterized specimens are the ideal choice for patients in whom the clean-catch method is not possible. Soiled diapers and urine collected by affixing a bag to the perineum are not acceptable specimens. Regardless of the specimen, a known volume of urine, 0.001 mL for catheter samples or clean-catch samples, is inoculated onto plate media. This plating technique allows the number of colony-forming units (CFU) per milliliter of urine to be quantified. Current recommendations based on previous studies state that 50,000 CFU/mL of a single uropathogenic organism in a properly collected urine specimen in the presence of pyuria is indicative of a urinary tract infection.[75] Any quantity of bacterial growth from urine collected through a suprapubic aspiration is considered significant.

Gastrointestinal Infections

The most common manifestation of gastrointestinal infection in pediatrics is acute diarrhea, and as is the case with pharyngitis, the most common cause is not bacterial. Viral, parasitic, or immune-related issues most commonly cause diarrhea. However, the ability to rule out a bacterial cause is critical, and thus the clinical microbiology laboratory must have appropriate processes in place to isolate and to identify bacterial stool pathogens. Feces should be collected in a sterile container and transported to the laboratory in a timely fashion to avoid overgrowth with normal flora and the potential loss of pathogenic organisms. If rapid transport to the laboratory is not possible, transport media such as Cary-Blair can be used to stabilize the organisms during transport to the laboratory. Because of the presence of high quantities of commensal flora in feces, stool cultures require a combination of both selective and differential media to identify potential pathogens. Most standard stool cultures will include at least two selective and differential media types; commonly used media formulations for the isolation of pathogens such as *Salmonella* and *Shigella* include but are not limited to MacConkey agar, xylose-lysine-deoxycholate agar, and Hektoen enteric agar. Other examples of media formulated for the detection of specific stool pathogens are cefsulodin-Irgasan-novobiocin (CIN) agar for isolation of *Yersinia* species, thiosulfate–citrate–bile salts–sucrose (TCBS) agar for the isolation of *Vibrio* species, and Campylobacter agar for the isolation of *Campylobacter jejuni* and *Campylobacter coli*. Another important pediatric enteric pathogen is Shiga toxin–producing *E. coli* (STEC); most reported STEC infections in the United States are caused by *E. coli* O157:H7, with an estimated 73,000 cases occurring each year.[51] Non-O157 STEC organisms can also cause diarrheal illness and have been associated with outbreaks and sporadic illness.[40] Direct plating methods for the detection of *E. coli* O157:H7 should be performed on a selective and differential agar, such as sorbitol–MacConkey agar (SMAC), cefixime–tellurite–sorbitol–MacConkey agar (CT-SMAC), or CHROMagar O157. The toxin genes can be detected by molecular methods, or toxin can be detected by enzyme immunoassay after broth enrichment.[36] There is no single definition of what pathogens are routinely screened for in the stool culture. Owing to the variability in the stool culture process, the clinician must inquire as to what pathogens are routinely screened for and which pathogens may require a request for specialized culture methods in the testing laboratory.

NON–CULTURE-BASED DETECTION METHODS

Several non–culture-based approaches are used to detect bacterial infections, including microscopy, detection of bacterial antigens or antibodies against the organism, and detection of nucleic acids by molecular methods. A detailed overview of these detection methods for bacteria is provided in this section.

Microscopy

Direct microscopic observation of the specimen is a simple yet reliable technique for demonstrating the presence of microorganisms as well as inflammatory cells in the specimen. Differential (Gram, Kinyoun, Giemsa) and fluorescent (acridine orange, auramine-rhodamine) stains are most commonly used. The Gram stain is a rapid, inexpensive method for assessing the presence of an etiologic agent in the specimen. Preliminary assessments from a stained specimen can indicate the Gram phenotype (positive or negative), the morphology (cocci vs. bacilli), and the spatial arrangement (clusters, chains, or diploid formation). Other differential stains routinely used for bacterial identification are the Kinyoun stain for *Mycobacterium* spp., the modified Kinyoun stain for *Nocardia* spp. and *Streptomyces* spp., and the Wright-Giemsa stain for bacteria *(Borrelia)* or bacterial inclusions *(Anaplasma*, *Ehrlichia)* in clinical specimens. Fluorescent stains are generally more sensitive than differential stains; however, the Gram phenotype is not available by this type of stain. Although useful information may be collected through a positive direct stain, a negative stain result does not rule out the presence of microorganisms, and thus all direct bacterial stains should be followed by culture.

Molecular Diagnostic Techniques

Molecular techniques have become increasingly important tools for the detection and identification of infectious diseases. The introduction of nucleic acid–based testing has provided increased sensitivity of detection and decreased time to detection for many bacterial pathogens. Molecular tests that are widely used in clinical microbiology can be subdivided into nonamplified and amplified methods.

Nonamplified methods consist of DNA- or RNA-labeled probes that bind to the target nucleic acids and generate a signal from the attached reporter molecule. The probe-based technology is useful for the rapid identification of organisms that already have been enriched

for by culture; the distinct advantage of this method over other amplification-based methods is that there is no risk of contamination of subsequent samples with amplified products. Commercially available examples of probe-based testing include the AccuProbe culture identification test, a rapid DNA probe test that uses the technique of nucleic acid hybridization for the identification of *Mycobacterium tuberculosis* isolated from culture,[2] and peptide nucleic acid probes for the detection of *S. aureus* and *Pseudomonas aeruginosa* from blood culture bottles.[61,63]

Nucleic acid amplification–based tests have become the "gold standard" for the detection of many infectious diseases. Amplification-based methods like polymerase chain reaction (PCR), including variations such as real-time, reverse transcriptase, multiplex, and nested PCR, have the principal advantage of increased sensitivity derived from amplifying the target nucleic acid sequence. The number of pathogens that can be detected by PCR is rapidly increasing. Although nucleic acid amplification–based tests have evolved to include highly complex testing methods, there are also simplified, cartridge-based testing systems available for use in clinical practice, such as the GeneXpert system (Cepheid, Sunnyvale, Calif) for methicillin-resistant *S. aureus* and the Verigene gram-positive blood culture test (Nanosphere, Northbrook, Ill) for identification of organisms from positive blood cultures. Other commercially available, Food and Drug Administration (FDA)–cleared assays use various nucleic acid detection technologies, such as transcription-mediated amplification, strand displacement amplification, ligase chain reaction, nucleic acid sequence–based amplification, and hybrid capture.

Molecular techniques have been particularly useful for the detection and identification of fastidious organisms (those that have specialized growth conditions or are slow or noncultivable under laboratory conditions). There are numerous molecular tests to aid in the identification of poorly cultivable or atypical organisms, including *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Bordetella pertussis*, *Bartonella henselae*, *Mycoplasma pneumoniae*, and many others. The following paragraphs summarize some of the clinically relevant advances in molecular testing for pediatric patients.

Bordetella pertussis

Although infection with *B. pertussis* can occur at any age, babies younger than 6 months, infants, and young children are at utmost risk.[57] *B. pertussis* infection mostly remains a clinical diagnosis; however, laboratory detection is often sought for confirmation. Isolation of the organism by culture has been the historical standard test for confirmation of diagnosis and is essential if evaluation of antimicrobial resistance is required. However, successful isolation of the organism declines significantly beyond the first 2 weeks of illness and with prior antibiotic therapy.[28,76] Furthermore, *B. pertussis* is fastidious and requires 3 to 6 days of incubation to form detectable colonies by culture. *B. pertussis* often will not grow if cultures are not initiated within 48 hours of sample collection.[48] As per CDC recommendation, detection of *B. pertussis* DNA by PCR has become increasingly available for the timely diagnosis of pertussis.[6] Optimal specimens for PCR testing include nasopharyngeal swabs and aspirates. Throat swabs and anterior nasal swabs have low rates of DNA recovery and should be avoided for *B. pertussis* testing.[6] The increased specificity and rapidity of PCR testing allow the results to be available within 24 to 48 hours. There are currently no FDA-approved *B. pertussis* PCR assays, and there are no standardized protocols, reagents, or reporting formats for *B. pertussis* PCR testing. Consequently, PCR assays vary widely among laboratories. Most *B. pertussis* PCR assays use a single DNA target, the insertion sequence IS481 that is present in multiple copies in the *B. pertussis* chromosome and in fewer copies on the chromosomes of *Bordetella holmesii* and *Bordetella bronchiseptica*.[66] The presence of IS481 in the chromosomes of nonpertussis strains can lead to false positivity.[31] Therefore, interpretation of PCR results should be done carefully in conjunction with evaluation of clinical symptoms.

Tuberculosis

The poor sensitivity of standard laboratory tests for the detection of *M. tuberculosis* is a major obstacle to diagnosis of tuberculosis in children. In addition, the nonspecific clinical presentation, paucibacillary nature of pediatric disease, and inability to expectorate sputum in children further complicate the laboratory diagnosis of tuberculosis.[42] Adequate clinical specimens are often difficult to obtain from children younger than 8 years because of lack of sputum production, and first morning gastric aspirates are an important specimen for pediatric patients.[72] Even with best practice, *M. tuberculosis* is isolated in less than 50% of clinically suspected pediatric cases.[1] Therefore, most cases are still diagnosed on the basis of clinical and radiographic findings. However, rapid laboratory confirmation of tuberculosis can lead to earlier treatment initiation, targeted antimicrobial therapy, and better clinical outcomes.[9] Tests based on nucleic acid amplification have become an important tool for the diagnosis of tuberculosis and, when results are positive, can detect *M. tuberculosis* directly from a specimen with a rapid turnaround time of hours or days in contrast to the weeks required with culture. Whereas the currently available molecular methods are rapid and have excellent specificities, their sensitivities do not equal those of culture-based methods, particularly in cases of smear-negative specimens.[47,59] Unfortunately, most pediatric tuberculosis cases are smear negative.

Several molecular methods for detection of tuberculosis are FDA cleared or approved. The enhanced amplified *M. tuberculosis* direct test (E-MTD; Gen-Probe, San Diego, Calif) and the Amplicor *M. tuberculosis* test (Roche Diagnostic Systems, Somerville, NJ) are FDA approved for detection of *M. tuberculosis* complex in smear-positive respiratory specimens.[47,59] The Gen-Probe test is also approved for use with smear-negative respiratory specimens from patients thought to have tuberculosis. The E-MTD test (isothermal transcription mediated) and the

Amplicor test (PCR based) rely on amplification of a portion of the 16S rRNA followed by hybridization and detection of a probe specific for *M. tuberculosis* complex bacteria. None of these tests has been approved for direct detection from extrapulmonary specimens. There are no specific recommendations made on the use of molecular testing in the diagnosis of tuberculosis in children who cannot produce sputum and in the diagnosis of extrapulmonary tuberculosis.

Clostridium difficile

Laboratory methods for detection of *Clostridium difficile* have undergone considerable evolution since the organism's etiologic association with antibiotic-associated infectious diarrhea and pseudomembranous colitis or *C. difficile*–associated disease in hospitalized patients was established. The diagnostic gold standards for *C. difficile* detection included anaerobic bacterial culture coupled with toxin detection or tissue culture cytotoxic assay, both of which are time-consuming, expensive, and technically challenging.[11] Although rapid detection kits (i.e., immunoassay) are commercially available, they lack sufficient sensitivity and specificity without serial testing.[15,22,27] The emergence of community-acquired *C. difficile* cases without antecedent antibiotic exposure in addition to the emergence of a quinolone-resistant "hypervirulent" strain of *C. difficile* (designated BI/NAP1/027) in adult and pediatric patients led to significant technical improvements in diagnostic laboratory testing.[50,60] One diagnostic approach to the identification of *C. difficile* is to use a rapid, real-time PCR–based molecular assay that targets the disease-producing *C. difficile* toxin genes. Factors that decrease the viability of organisms (e.g., treatment with antibiotics) lower the sensitivity of detection for culture and rapid immunoassays, but loss of viability does not affect molecular assays. There are currently at least six FDA-cleared assays available for detection of *C. difficile*. Most methods are based on real-time PCR with the following exceptions: illumigene *C. difficile* assay (Meridian Bioscience, Cincinnati, Ohio), a loop amplification–based method; and Portrait Toxigenic assay (Great Basin Corp., West Valley City, Utah), an isothermal amplification and chip-based method. All these tests yield results in fewer than 24 hours, with sensitivities reported at greater than 95 percent and negative predictive values of 99 percent.[5,77] However, positive *C. difficile* PCR assays on stool specimens in infants younger than 12 months usually represent colonization, and testing should be performed in infants only when other possible causes of diarrhea have been ruled out.[4]

Neisseria gonorrhoeae *and* Chlamydia trachomatis

Adolescents and young adults have higher rates of infection with *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (GC) than any other age group does,[62] and nucleic acid amplification testing has greatly facilitated sensitive and specific screening in this population. These assays are extremely sensitive with a limit of detection as low as a single gene copy.[16,33] In addition, testing of urine specimens offers noninvasive sample collection. These tests provide the ability to test for both GC and CT in the same specimen. The most commonly used FDA-approved assays are for testing on cervical swabs from women and urethral swabs and urine from men and women. Importantly, none of these assays is approved for testing of specimens from children. The identification of sexually transmitted diseases like *C. trachomatis* and *N. gonorrhoeae* infections in children should raise suspicion for sexual assault, and there are both medical and legal implications to the diagnosis of sexually transmitted diseases in infants and children.[38] Thus, testing requires the use of a highly specific method; the gold standard for detection of GC/CT in children and infants has been culture from appropriate specimens.[81]

Sequence-Based Technologies

Sequence-based technologies have been used to improve the speed and accuracy of identification of organisms that are difficult to identify by conventional methods or to detect and identify uncultivable organisms. Currently, Sanger sequencing is the most commonly used sequencing method. For bacterial identification, 16S ribosomal genes are common targets of interest.[17] There are several open access ribosomal sequence databases that can be used to produce alignments of the 16S ribosomal sequences and subsequent species assignment. By comparing the organism's 16S rRNA gene sequence with those found in databases of known organisms, the identity of etiologic agents from clinical specimens can be successfully determined.[17,29,39,56] Other technologies, such as pyrosequencing and single-cell and whole genome sequencing methods, are also promising and powerful tools in clinical microbiology.[64,65] Examples of commercially available whole genome sequencing platforms include the SOLiD system (Life Technologies, Carlsbad, Calif) and Genome Sequencer FLX system (Roche Life Sciences, Branford, Ct).[49] These rapid, accurate, and user-friendly methods are able to generate megabases of sequence information about the whole organism. To date, routine application of sequence-based technologies is not widely available in most clinical diagnostic laboratories.

Mass Spectroscopy

Matrix-assisted laser desorption/ionization time-of-flight mass spectroscopy (MALDI-TOF-MS) is a technology that provides rapid identification of a variety of pathogens in clinical specimens within hours.[12,23] This technology uses unique protein spectral profiles of the microorganisms to rapidly identify organisms in clinical specimens from known catalogued databases of these profiles.[21,26,34] In contrast to the culture-based methods of detection and identification of microorganisms, MALDI-TOF-MS allows the detection of bacterial macromolecules in complex mixtures without isolation.[8,53] Although MALDI-TOF-MS is being used by large diagnostic centers to identify various bacterial species from clinical

specimens, it is not currently FDA approved for routine diagnostic testing.[11,30,68,73]

NEW REFERENCES SINCE THE SIXTH EDITION

4. Bartlett JG. *Clostridium difficile:* progress and challenges. *Ann N Y Acad Sci* 2010;**1213**:62–9.
5. Bartlett JG. Detection of *Clostridium difficile* infection. *Infect Control Hosp Epidemiol* 2010;**31**(Suppl 1):S35–7.
6. Best practices for health care professionals on the use of polymerase chain reaction (PCR) for diagnosing pertussis. *Centers for Diseases Control and Prevention, 2011.* Available at http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html. Accessed November 9, 2011.
7. Bhat RG, Katy TA, Place FC. Pediatric urinary tract infections. *Emerg Med Clin North Am* 2011;**29**:637–53.
8. Bizzini A, Durussel C, Bille J, et al. Performance of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol* 2010;**48**:1549–54.
9. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010; **363**:1005–15.
10. Cantral DE, Tape TG, Reed EC, et al. Quantitative culture of bronchoalveolar lavage fluid for the diagnosis of bacterial pneumonia. *Am J Med* 1993;**95**:601–7.
12. Carbonnelle E, Mesquita C, Bille E, et al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2011;**44**:104–9.
15. Carroll KC. Tests for the diagnosis of *Clostridium difficile* infection: the next generation. *Anaerobe* 2011;**17**:170–4.
16. Cheng A, Qian Q, Kirby JE. Evaluation of the Abbott RealTime CT/NG assay in comparison to the Roche Cobas Amplicor CT/NG assay. *J Clin Microbiol* 2011;**49**:1294–300.
22. Crobach MJ, Dekkers OM, Wilcox MH, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile* infection (CDI). *Clin Microbiol Infect* 2009;**15**:1053–66.
23. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol Rev* 2012;**36**:380–407.
25. Deaths and mortality. *Centers for Disease Control and Prevention*. Available at http://www.cdc.gov/nchs/fastats/deaths.htm. Accessed November 9, 2012.
27. Eastwood K, Else P, Charlett A, et al. Comparison of nine commercially available *Clostridium difficile* toxin detection assays, a real-time PCR assay for *C. difficile* tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. *J Clin Microbiol* 2009;**47**:3211–17.
29. Edwards KJ, Logan JM, Langham S, et al. Utility of real-time amplification of selected 16S rRNA gene sequences as a tool for detection and identification of microbial signatures directly from clinical samples. *J Med Microbiol* 2012;**61**:645–52.
33. Gaydos CA, Cartwright CP, Colaninno P, et al. Performance of the Abbott RealTime CT/NG for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 2010;**48**:3236–43.
35. Goldfarb DM, Slinger R, Tam RK, et al. Assessment of flocked swabs for use in identification of streptococcal pharyngitis. *J Clin Microbiol* 2009;**47**:3029–30.
36. Gould LH, Bopp C, Strockbine N, et al. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;**58**:1–14.
47. Ling DI, Flores LL, Riley LW, et al. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. *PLoS One* 2008;**3**:e1536.
48. Loeffelholz M. Towards improved accuracy of *Bordetella pertussis* nucleic acid amplification tests. *J Clin Microbiol* 2012;**50**:2186–90.
49. Loman NJ, Misra RV, Dallman TJ, et al. Performance comparison of benchtop high-throughput sequencing platforms. *Nat Biotechnol* 2012;**30**:434–9.
53. Mellmann A, Cloud J, Maier T, et al. Evaluation of matrix-assisted laser desorption ionization–time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. *J Clin Microbiol* 2008;**46**:1946–54.
59. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. *Curr Opin Infect Dis* 2009;**22**:174–82.
60. O'Connor JR, Johnson S, Gerding DN. *Clostridium difficile* infection caused by the epidemic BI/NAP1/027 strain. *Gastroenterology* 2009;**136**:1913–24.
63. Peleg AY, Tilahun Y, Fiandaca MJ, et al. Utility of peptide nucleic acid fluorescence in situ hybridization for rapid detection of *Acinetobacter* spp. and *Pseudomonas aeruginosa*. *J Clin Microbiol* 2009;**47**: 830–2.
64. Petrosino JF, Highlander S, Luna RA, et al. Metagenomic pyrosequencing and microbial identification. *Clin Chem* 2009;**55**:856–66.
68. Seng P, Drancourt M, Gouriet F, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009;**49**:543–51.
73. Steensels D, Verhaegen J, Lagrou K. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for the identification of bacteria and yeasts in a clinical microbiological laboratory: a review. *Acta Clin Belg* 2011;**66**:267–73.
75. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.
77. Viala C, Le Monnier A, Maataoui N, et al. Comparison of commercial molecular assays for toxigenic *Clostridium difficile* detection in stools: BD GeneOhm Cdiff, XPert *C. difficile* and illumigene *C. difficile*. *J Microbiol Methods* 2012;**90**:83–5.
79. Wilms MC, Stanzel S, Reinert RR, et al. Effects of preincubation temperature on the detection of fastidious organisms in delayed-entry samples in the BacT/ALERT 3D blood culture system. *J Microbiol Methods* 2009;**79**:194–8.
81. Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1–110.

The full reference list for this chapter is available at expertconsult.com.

REFERENCES

1. Abadco DL, Steiner P. Gastric lavage is better than bronchoalveolar lavage for isolation of *Mycobacterium tuberculosis* in childhood pulmonary tuberculosis. *Pediatr Infect Dis J* 1992;**11**:735–8.
2. Badak FZ, Goksel S, Sertoz R, et al. Use of nucleic acid probes for identification of *Mycobacterium tuberculosis* directly from MB/BacT bottles. *J Clin Microbiol* 1999;**37**:1602–5.
3. Baron EJ, Carroll KC. Rapid diagnosis of tuberculous meningitis: what is the optimal method? *Infections Med* 2005;**22**:633–7.
4. Bartlett JG. *Clostridium difficile:* progress and challenges. *Ann N Y Acad Sci* 2010;**1213**:62–9.
5. Bartlett JG. Detection of *Clostridium difficile* infection. *Infect Control Hosp Epidemiol* 2010;**31**(Suppl 1):S35–7.
6. Best practices for health care professionals on the use of polymerase chain reaction (PCR) for diagnosing pertussis. *Centers for Diseases Control and Prevention, 2011*. Available at http://www.cdc.gov/pertussis/clinical/diagnostic-testing/diagnosis-pcr-bestpractices.html. Accessed November 9, 2011.
7. Bhat RG, Katy TA, Place FC. Pediatric urinary tract infections. *Emerg Med Clin North Am* 2011;**29**:637–53.
8. Bizzini A, Durussel C, Bille J, et al. Performance of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. *J Clin Microbiol* 2010;**48**:1549–54.
9. Boehme CC, Nabeta P, Hillemann D, et al. Rapid molecular detection of tuberculosis and rifampin resistance. *N Engl J Med* 2010;**363**:1005–15.
10. Cantral DE, Tape TG, Reed EC, et al. Quantitative culture of bronchoalveolar lavage fluid for the diagnosis of bacterial pneumonia. *Am J Med* 1993;**95**:601–7.
11. Carbonnelle E, Beretti JL, Cottyn S, et al. Rapid identification of staphylococci isolated in clinical microbiology laboratories by matrix-assisted laser desorption ionization–time of flight mass spectrometry. *J Clin Microbiol* 2007;**45**:2156–61.
12. Carbonnelle E, Mesquita C, Bille E, et al. MALDI-TOF mass spectrometry tools for bacterial identification in clinical microbiology laboratory. *Clin Biochem* 2011;**44**:104–9.
13. Carroll K, Reimer L. Microbiology and laboratory diagnosis of upper respiratory tract infections. *Clin Infect Dis* 1996;**23**:442–8.
14. Carroll KC. Laboratory diagnosis of lower respiratory tract infections: controversy and conundrums. *J Clin Microbiol* 2002;**40**:3115–20.
15. Carroll KC. Tests for the diagnosis of *Clostridium difficile* infection: the next generation. *Anaerobe* 2011;**17**:170–4.
16. Cheng A, Qian Q, Kirby JE. Evaluation of the Abbott RealTime CT/NG assay in comparison to the Roche Cobas Amplicor CT/NG assay. *J Clin Microbiol* 2011;**49**:1294–300.
17. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev* 2004;**17**:840–62.
18. Clinical and Laboratory Standards Institute. *Principles and procedures for blood cultures; approved guideline*. CLSI document M47-A. Wayne, Pa: Clinical and Laboratory Standards Institute; 2007.
19. Cockerill FR 3rd, Wilson JW, Vetter EA, et al. Optimal testing parameters for blood cultures. *Clin Infect Dis* 2004;**38**:1724–30.
20. Connell TG, Rele M, Cowley D, et al. How reliable is a negative blood culture result? Volume of blood submitted for culture in routine practice in a children's hospital. *Pediatrics* 2007;**119**:891–6.
21. Conway GC, Smole SC, Sarracino DA, et al. Phyloproteomics: species identification of Enterobacteriaceae using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *J Mol Microbiol Biotechnol* 2001;**3**:103–12.
22. Crobach MJ, Dekkers OM, Wilcox MH, et al. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing *Clostridium difficile* infection (CDI). *Clin Microbiol Infect* 2009;**15**:1053–66.
23. Croxatto A, Prod'hom G, Greub G. Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology. *FEMS Microbiol Rev* 2012;**36**:380–407.
24. Cunniffe JG, Whitby-Strevens S, Wilcox MH. Effect of pH changes in cerebrospinal fluid specimens on bacterial survival and antigen test results. *J Clin Pathol* 1996;**49**:249–53.
25. Deaths and mortality. *Centers for Disease Control and Prevention*. Available at http://www.cdc.gov/nchs/fastats/deaths.htm. Accessed November 9, 2012.
26. Demirev PA, Ho YP, Ryzhov V, et al. Microorganism identification by mass spectrometry and protein database searches. *Anal Chem* 1999;**71**:2732–8.
27. Eastwood K, Else P, Charlett A, et al. Comparison of nine commercially available *Clostridium difficile* toxin detection assays, a real-time PCR assay for *C. difficile* tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. *J Clin Microbiol* 2009;**47**:3211–17.
28. Edelman K, Nikkari S, Ruuskanen O, et al. Detection of *Bordetella pertussis* by polymerase chain reaction and culture in the nasopharynx of erythromycin-treated infants with pertussis. *Pediatr Infect Dis J* 1996;**15**:54–7.
29. Edwards KJ, Logan JM, Langham S, et al. Utility of real-time amplification of selected 16S rRNA gene sequences as a tool for detection and identification of microbial signatures directly from clinical samples. *J Med Microbiol* 2012;**61**:645–52.
30. Friedrichs C, Rodloff AC, Chhatwal GS, et al. Rapid identification of viridans streptococci by mass spectrometric discrimination. *J Clin Microbiol* 2007;**45**:2392–7.
31. Fry NK, Tzivra O, Li YT, et al. Laboratory diagnosis of pertussis infections: the role of PCR and serology. *J Med Microbiol* 2004;**53**:519–25.
32. Gaur AH, Giannini MA, Flynn PM, et al. Optimizing blood culture practices in pediatric immunocompromised patients: evaluation of media types and blood culture volume. *Pediatr Infect Dis J* 2003;**22**:545–52.
33. Gaydos CA, Cartwright CP, Colaninno P, et al. Performance of the Abbott RealTime CT/NG for detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. *J Clin Microbiol* 2010;**48**:3236–43.
34. Girault S, Chassaing G, Blais JC, et al. Coupling of MALDI-TOF mass analysis to the separation of biotinylated peptides by magnetic streptavidin beads. *Anal Chem* 1996;**68**:2122–6.
35. Goldfarb DM, Slinger R, Tam RK, et al. Assessment of flocked swabs for use in identification of streptococcal pharyngitis. *J Clin Microbiol* 2009;**47**:3029–30.
36. Gould LH, Bopp C, Strockbine N, et al. Recommendations for diagnosis of Shiga toxin–producing *Escherichia coli* infections by clinical laboratories. *MMWR Recomm Rep* 2009;**58**:1–14.
37. Groothuis JR, Thompson J, Wright PF. Correlation of nasopharyngeal and conjunctival cultures with middle ear fluid cultures in otitis media. A prospective study. *Clin Pediatr (Phila)* 1986;**25**:85–8.
38. Hammerschlag MR. Appropriate use of nonculture tests for the detection of sexually transmitted diseases in children and adolescents. *Semin Pediatr Infect Dis* 2003;**14**:54–9.
39. Janda JM, Abbott SL. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J Clin Microbiol* 2007;**45**:2761–4.
40. Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin–producing *Escherichia coli*. *Clin Infect Dis* 2006;**43**:1587–95.
41. Kellogg JA, Manzella JP, Bankert DA. Frequency of low-level bacteremia in children from birth to fifteen years of age. *J Clin Microbiol* 2000;**38**:2181–5.
42. Khan EA, Starke JR. Diagnosis of tuberculosis in children: increased need for better methods. *Emerg Infect Dis* 1995;**1**:115–23.
43. Kleiman MB, Reynolds JK, Watts NH, et al. Superiority of acridine orange stain versus Gram stain in partially treated bacterial meningitis. *J Pediatr* 1984;**104**:401–4.
44. La Scolea LJ Jr, Dryja D. Quantitation of bacteria in cerebrospinal fluid and blood of children with meningitis and its diagnostic significance. *J Clin Microbiol* 1984;**19**:187–90.
45. Lauer BA, Reller LB, Mirrett S. Comparison of acridine orange and Gram stains for detection of microorganisms in cerebrospinal fluid and other clinical specimens. *J Clin Microbiol* 1981;**14**:201–5.
46. Lee A, Mirrett S, Reller LB, et al. Detection of bloodstream infections in adults: how many blood cultures are needed? *J Clin Microbiol* 2007;**45**:3546–8.
47. Ling DI, Flores LL, Riley LW, et al. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. *PLoS One* 2008;**3**:e1536.

48. Loeffelholz M. Towards improved accuracy of *Bordetella pertussis* nucleic acid amplification tests. *J Clin Microbiol* 2012;**50**:2186–90.
49. Loman NJ, Misra RV, Dallman TJ, et al. Performance comparison of benchtop high-throughput sequencing platforms. *Nat Biotechnol* 2012;**30**:434–9.
50. McDonald LC, Killgore GE, Thompson A, et al. An epidemic, toxin gene–variant strain of *Clostridium difficile*. *N Engl J Med* 2005; **353**:2433–41.
51. Mead PS, Slutsker L, Dietz V, et al. Food-related illness and death in the United States. *Emerg Infect Dis* 1999;**5**:607–25.
52. Meduri GU. Diagnosis of ventilator-associated pneumonia. *Infect Dis Clin North Am* 1993;**7**:295–329.
53. Mellmann A, Cloud J, Maier T, et al. Evaluation of matrix-assisted laser desorption ionization–time-of-flight mass spectrometry in comparison to 16S rRNA gene sequencing for species identification of nonfermenting bacteria. *J Clin Microbiol* 2008;**46**:1946–54.
54. Meredith FT, Phillips HK, Reller LB. Clinical utility of broth cultures of cerebrospinal fluid from patients at risk for shunt infections. *J Clin Microbiol* 1997;**35**:3109–11.
55. Monroe PW, Muchmore HG, Felton FG, et al. Quantitation of microorganisms in sputum. *Appl Microbiol* 1969;**18**:214–20.
56. Muldrew KL, Miller RR, Kressin M, et al. Necrotizing fasciitis from *Vibrio vulnificus* in a patient with undiagnosed hepatitis and cirrhosis. *J Clin Microbiol* 2007;**45**:1058–62.
57. Munoz FM. Pertussis in infants, children, and adolescents: diagnosis, treatment, and prevention. *Semin Pediatr Infect Dis* 2006;**17**: 14–19.
58. Nigrovic LE, Kuppermann N, McAdam AJ, et al. Cerebrospinal latex agglutination fails to contribute to the microbiologic diagnosis of pretreated children with meningitis. *Pediatr Infect Dis J* 2004;**23**: 786–8.
59. Nyendak MR, Lewinsohn DA, Lewinsohn DM. New diagnostic methods for tuberculosis. *Curr Opin Infect Dis* 2009;**22**:174–82.
60. O'Connor JR, Johnson S, Gerding DN. *Clostridium difficile* infection caused by the epidemic BI/NAP1/027 strain. *Gastroenterology* 2009;**136**:1913–24.
61. Oliveira K, Procop GW, Wilson D, et al. Rapid identification of *Staphylococcus aureus* directly from blood cultures by fluorescence in situ hybridization with peptide nucleic acid probes. *J Clin Microbiol* 2002;**40**:247–51.
62. Olshen E, Shrier LA. Diagnostic tests for chlamydial and gonorrheal infections. *Semin Pediatr Infect Dis* 2005;**16**:192–8.
63. Peleg AY, Tilahun Y, Fiandaca MJ, et al. Utility of peptide nucleic acid fluorescence in situ hybridization for rapid detection of *Acinetobacter* spp. and *Pseudomonas aeruginosa*. *J Clin Microbiol* 2009;**47**:830–2.
64. Petrosino JF, Highlander S, Luna RA, et al. Metagenomic pyrosequencing and microbial identification. *Clin Chem* 2009;**55**:856–66.
65. Pinard R, de Winter A, Sarkis GJ, et al. Assessment of whole genome amplification–induced bias through high-throughput, massively parallel whole genome sequencing. *BMC Genomics* 2006; **7**:216–37.
66. Reischl U, Lehn N, Sanden GN, et al. Real-time PCR assay targeting IS481 of *Bordetella pertussis* and molecular basis for detecting *Bordetella holmesii*. *J Clin Microbiol* 2001;**39**:1963–6.
67. Sautter RL, Bills AR, Lang DL, et al. Effects of delayed-entry conditions on the recovery and detection of microorganisms from BacT/ALERT and BACTEC blood culture bottles. *J Clin Microbiol* 2006;**44**:1245–9.
68. Seng P, Drancourt M, Gouriet F, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Infect Dis* 2009;**49**:543–51.
69. Shanholtzer CJ, Schaper PJ, Peterson LR. Concentrated Gram stain smears prepared with a cytospin centrifuge. *J Clin Microbiol* 1982;**16**:1052–6.
70. Shanson DC, Pratt J, Greene P. Comparison of media with and without "Panmede" for the isolation of *Streptobacillus moniliformis* from blood cultures and observations on the inhibitory effect of sodium polyanethol sulphonate. *J Med Microbiol* 1985;**19**:181–6.
71. Sharp SE, Robinson A, Saubolle M, et al; Sharp SE, coordinating editor. *Lower respiratory tract infections*. Washington, DC: ASM Press; 2004. Cumitec 7B.
72. Starke JR. Tuberculosis in children. *Curr Opin Pediatr* 1995;**7**: 268–77.
73. Steensels D, Verhaegen J, Lagrou K. Matrix-assisted laser desorption ionization–time of flight mass spectrometry for the identification of bacteria and yeasts in a clinical microbiological laboratory: a review. *Acta Clin Belg* 2011;**66**:267–73.
74. Sturgis CD, Peterson LR, Warren JR. Cerebrospinal fluid broth culture isolates: their significance for antibiotic treatment. *Am J Clin Pathol* 1997;**108**:217–21.
75. Subcommittee on Urinary Tract Infection, Steering Committee on Quality Improvement and Management. Urinary tract infection: clinical practice guideline for the diagnosis and management of the initial UTI in febrile infants and children 2 to 24 months. *Pediatrics* 2011;**128**:595–610.
76. van der Zee A, Agterberg C, Peeters M, et al. A clinical validation of *Bordetella pertussis* and *Bordetella parapertussis* polymerase chain reaction: comparison with culture and serology using samples from patients with suspected whooping cough from a highly immunized population. *J Infect Dis* 1996;**174**:89–96.
77. Viala C, Le Monnier A, Maataoui N, et al. Comparison of commercial molecular assays for toxigenic *Clostridium difficile* detection in stools: BD GeneOhm Cdiff, XPert *C. difficile* and illumigene *C. difficile*. *J Microbiol Methods* 2012;**90**:83–5.
78. Waites KB, Saubolle MA, Talkington DF, et al; Sharp SE, coordinating editor. *Laboratory diagnosis of upper respiratory tract infections*. Washington, DC: ASM Press; 2006. Cumitech 10A.
79. Wilms MC, Stanzel S, Reinert RR, et al. Effects of preincubation temperature on the detection of fastidious organisms in delayed-entry samples in the BacT/ALERT 3D blood culture system. *J Microbiol Methods* 2009;**79**:194–8.
80. Wilson ML, Mirrett S, Reller LB, et al. Recovery of clinically important microorganisms from the BacT/Alert blood culture system does not require testing for seven days. *Diagn Microbiol Infect Dis* 1993;**16**:31–4.
81. Workowski KA, Berman S. Sexually transmitted diseases treatment guidelines, 2010. *MMWR Recomm Rep* 2010;**59**:1–110.
82. Zorc JJ, Kiddoo DA, Shaw KN. Diagnosis and management of pediatric urinary tract infections. *Clin Microbiol Rev* 2005;**18**: 417–22.

CHAPTER 251

Fungal Laboratory Analysis: Specimen Collection, Direct Detection, and Culture

David A. Bruckner

Fungi were regarded as insignificant causes of infection historically. The most important factor responsible for the increase in the number of fungal infections has been an alteration in the immune response of humans. Organ transplant recipients, particularly during the posttransplantation period, and patients with malignant neoplasms and debilitating immunologic or metabolic disorders, such as systemic lupus erythematosus, diabetes mellitus, and alcohol or injection drug abuse, are particularly susceptible to fungal infections. Opportunistic fungi of low or limited virulence are detected with increasing frequency in subcutaneous or disseminated infections.

SPECIMEN COLLECTION

When an infectious process is suspected, the most appropriate tests directed toward the detection of the etiologic agent must be ordered by the physician. Knowledge of the pathophysiology of the disease process is important in determining the optimal time for specimen collection. These collections include specimens for culture, direct detection of the etiologic agent with antigen tests, nucleic acid detection, stains, and antibody methods.[1,3,4,6,8-11] All specimens should be considered hazardous, and universal precautions must be used for all specimen collection procedures. A sufficient quantity of a specimen must be obtained for the techniques required to detect the fungal agent directly, for culture, for antigen detection, or for serology. Procedures used for specimen collection for culture of bacteria also can be used for culture of fungi (Table 251-1). Microbiologists and pathologists are available to guide physicians in selecting the most appropriate specimen for culture and other tests to achieve rapid detection and recovery of the microorganism causing the infection. Most clinical laboratories have established guidelines for specimen collection with minimal volumes of specimen to be collected. In addition, specimens should be collected in sterile containers before the initiation of therapy to ensure that optimal recovery of organisms is achieved. An important factor is that adequate specimen volumes, which include up to 5 mL of cerebrospinal fluid (CSF) and 200 mL of urine for fungal culture, be submitted to the laboratory. Specimens collected for 24 hours, such as urine and sputa, are unsuitable for fungal culture because of bacterial overgrowth. Swab specimens are considered inadequate for the recovery of fungi, particularly when multiple culture tests (bacteriology, mycology, mycobacteriology, and virology) from the same swab specimen are requested. In many instances, surgeons send tissue for a histologic diagnosis and neglect to submit a request for culture. If an infection caused by a dimorphic fungus such as *Coccidioides immitis* is suspected, the physician should notify the laboratory because of hazards associated with culture of this organism. A poorly collected specimen may result in the failure to recover the organism, or it may lead to therapy directed at a contaminating or commensal organism.

Specimens should be transported to the laboratory as soon as possible under optimal conditions, which usually are advised in the laboratory specimen collection manual that should be available to all physicians. Specimen transport and storage at room temperature is recommended. Ideally, specimens should be received in the laboratory within 2 hours of being collected. If a delay occurs in setting up cultures, the specimen should be held at room temperature or 4°C (39.2°F) to prevent bacterial overgrowth. Unacceptable specimens that should not be processed include specimens in containers that leak, specimens received many days after collection, and specimens held under inappropriate temperature conditions. Some fungal isolates, such as *Cryptococcus neoformans* and *Histoplasma capsulatum*, do not survive well at refrigerated or freezing temperatures.

DIRECT DETECTION

In the laboratory, direct wet mounts or smears can be prepared and a portion of the specimen can be used for culture (Table 251-2). Blood, CSF, and urine also can be used for the detection of antigens and nucleic acids by enzyme immunoassay, latex agglutination, polymerase chain reaction, and radioimmunoassay.[2,6-8] These assays have proved to be useful for the detection of infections caused by *Aspergillus*, *C. neoformans*, and *H. capsulatum*.[2] Although polymerase chain reaction techniques may be used for the direct detection of fungal infections, they are used in research or have been validated in specific laboratories for clinical use but are not readily available in most clinical laboratories.[5] Direct microscopic examination, including frozen sections of tissue to provide an

TABLE 251-1 **Specimen Collection Guidelines**

Specimen	Recommendations	Transport Device/Comments
Abscess/wounds	Collect as per bacterial culture.	Collect in sterile container with as much material as possible. If swab is used, use exclusively for mycology. RT
Blood	Collect as per bacterial culture.	BacT/Alert, BACTEC, VersaTREK, or Septi-Chek (biphasic) bottles. Isolator (lysis-centrifugation). RT, never refrigerate
Bone marrow	Collect as per bacterial culture.	Collect in sterile container, Isolator, or heparin (green top) tube. If clotted, may have to homogenize or cut into pieces to place on mycology media. RT
CSF	Collect as per bacterial culture. Fluid volumes greater than 1 mL should be centrifuged or filtered.	Collect in sterile container. RT, never refrigerate
Eye	Corneal scrapings should be inoculated directly onto mycology media. Vitreous fluid should be collected by needle aspiration.	Direct inoculation onto mycology media without cycloheximide. Vitreous fluid can be placed in a sterile container, or the syringe with needle removed can be sent to the laboratory. RT
Fluids other than CSF and urine	Collect as per bacterial culture. Fluid volumes greater than 1 mL should be centrifuged or filtered.	Collect in sterile container. RT
Hair	Can use Wood lamp to help select infected hairs. Also scrape border of infected area if lesions are present.	Place 10-12 hair roots in sterile container. RT
Nails	Swab surface with 70% alcohol. Collect softened material from beneath nail bed.	Use sterile container or directly inoculate mycology media. RT
Prostatic secretions	Empty bladder before giving prostate massage. For detection of *Blastomyces* and less commonly *Coccidioides, Cryptococcus,* and *Histoplasma.*	Place in sterile container or directly inoculate mycology plates. RT
Respiratory	Collect as per bacterial culture using same rejection criteria for sputum. Early morning sputum is the preferred specimen.	Use sterile container. RT
Skin	Disinfect surface area with 70% alcohol to remove bacterial contaminants. Scrape skin surface at the edge of the lesion.	Use sterile container or directly inoculate mycology media. RT
Tissue/biopsy	Collect specimen per surgical technique.	Sterile container to which a small amount of sterile saline or water may be added to keep tissue moist. RT
Urine	Collect as per bacterial culture. Early morning specimen is preferred. Use midstream collection technique.	Use sterile wide-mouthed container. RT
Vaginal	Collect specimen as per bacterial culture. Primarily for *Candida* infections.	Swab transport device. RT

CSF, cerebrospinal fluid; RT, room temperature.

immediate presumptive diagnosis, should be performed on most specimens. A definitive diagnosis may be made when specific fungal elements pathognomonic for fungal infections are detected in direct specimen stains. Examples include *Blastomyces dermatitidis*, *Cryptococcus neoformans*, *Coccidioides immitis*, and *Pneumocystis jiroveci*. Detection of wide and narrow, wavy, or ribbon-like nonseptate hyphae is consistent with a mucormycosis. Direct specimen examinations always must be followed with culture because the direct examination can never be used to rule out infection, and recovery of the organism may be necessary for speciation.

Serology may be used to diagnose fungal infections either because other direct detection methods were negative or the organism failed to grow in culture. In general, serology is not as sensitive as are other direct methods, such as antigen detection, particularly in immunocompromised patients. Serology has been useful in the diagnosis of *C. immitis* and *H. capsulatum* infections and can be used to follow successful therapy in coccidioidomycosis.[7] Serologic methods include complement fixation, enzyme immunoassay, immunodiffusion, and latex agglutination.

During infections, fungal cell wall antigens are released into surrounding tissue and body fluids (blood, CSF, respiratory secretions, and urine).[7] The results from antigen tests are much more rapid than culture. Their detection can be useful in supporting a clinical diagnosis when culture and histopathology are negative. Detection of glucuronoxylomannan has been successfully used to diagnose and follow *C. neoformans* and *Cryptococcus gattii* infections. This test is highly sensitive, and circulating

TABLE 251-2 **Methods for Detection of Fungi in Human Specimens**

Stain/Method	Use	Comments
Aspergillus galactomannan EIA, β-D-glucan EIA	To detect galactomannan or polysaccharide cell wall component in serum or fluid	An EIA test is used as an adjunct to culture for the detection of *Aspergillus* or *Candida* in high-risk patients. The test has low sensitivity and specificity. β-D-Glucan is found in most fungi, whereas the galactomannan test is used to detect aspergillosis.
Acid-fast, modified	To detect *Nocardia*	Most *Nocardia* will stain pink.
Calcofluor white	Binds to chitin and cellulose of fungal cell wall	Rapid detection of fungi and *Pneumocystis*. Staining time is 1 minute; requires fluorescent microscope. Organisms fluoresce blue-white. May be used with Evans blue to suppress background fluorescence.
Cryptococcal antigen test (EIA)	Detects antigen in CSF and blood	A reliable and sensitive method for the detection of cryptococcal antigen. Can be used to monitor therapy.
Fontana-Masson	Detects *Cryptococcus* and other dematiaceous fungi	Stains melanin black or dark brown.
Giemsa	Stains most bacteria and fungi	Used for the detection of *Histoplasma* and *Penicillium marneffei* in blood and bone marrow. *Pneumocystis* trophozoites and intracystic sporozoites can be detected. Organisms stain blue-purple.
Gridley	Stains fungal cell wall	Cell wall stains purple-red with yellow background.
Gomori methenamine silver	Stains most organisms in tissue	Fungi including *Pneumocystis* cysts stain gray-black. Difficult to control staining reaction and may be difficult to interpret because of background staining.
Gram	Used primarily for detection of bacteria	Yeast cells and pseudohyphae stain gram-positive; *Cryptococcus* stains gram-positive surrounded by a capsular halo; hyphae will stain gram-negative.
Hematoxylin and eosin	Used to demonstrate patient's tissue reactions	Fungi stain violet to blue-purple. Can see fungal pigments.
Histoplasma urine antigen (EIA)	To identify infections of *H. capsulatum*	More sensitive and faster than culture. Test can cross-react with *Coccidioides immitis* and other dimorphic fungi.
India ink	To detect *Cryptococcus* in CSF specimens	To help detect capsule of *Cryptococcus neoformans*. Not very sensitive, and capsule may be difficult to detect.
Mucin (Mayer or Southgate mucicarmine)	Stains mucopolysaccharide or fungal capsule	Cryptococcal capsule stains red.
Papanicolaou	Used to detect the presence of malignant cells	Fungal elements stain pink. Some fungi may not stain.
Periodic acid–Schiff	Stains glycogen, mucin, mucoproteins, and glycoprotein	A mucin stain used to stain the capsular mucopolysaccharide of fungal organisms. Capsule will have a pink-magenta or purple color.
Pneumocystis DFA	Detects *Pneumocystis* cysts and trophozoites	Rapid staining method specific for *Pneumocystis* in respiratory specimens.
PCR	Can be used for direct detection of fungal pathogens	Not commercially available but has been developed in specific diagnostic laboratories for the detection of selected fungal pathogens.
Potassium hydroxide	To detect yeast and hyphal elements in wet mounts	Used as a clearing agent to make fungi more visible in tissue, hair, nails, and body fluids. Digests proteinaceous material of host cells, leaving fungi intact.
Wright	Stains most bacteria and fungi	Used for the detection of *Histoplasma* and *Penicillium marneffei* in blood and bone marrow. *Pneumocystis* trophozoites and intracystic sporozoites can be detected. Organisms stain blue-purple.

CSF, cerebrospinal fluid; DFA, direct fluorescent antibody; EIA, enzyme immunoassay; PCR, polymerase chain reaction.

capsular polysaccharide can be quantitated to determine a patient's therapeutic response. Invasive aspergillosis is a diagnostic challenge, and the development of a galactomannan test (polysaccharide cell wall component of *Aspergillus*) had been helpful in certain patient populations. The test results have better predictability among patients with hematologic malignant disease than among those receiving a solid-organ transplant. False-positive test results are common in lung transplant patients. Urine antigen tests for the diagnosis of histoplasmosis and blastomycosis are sensitive and more rapid than culture or serologic results. Other fluids can be used with these tests for detection of blastomycosis and histoplasmosis, but the test may have to be validated by the laboratory before the results can be used clinically. β-D-Glucan is a major cell wall component of most fungi with the exception of *Mucorales* and low amounts in *Cryptococcus* and *Blastomyces.* This serum antigen has been used as a diagnostic marker for invasive fungal infections. False-positive test results for β-D-glucan and galactomannan have occurred in patients exposed to products containing these polysaccharides.

Nucleic acid tests for the detection of fungal pathogens may be available in specialized reference centers, but none is approved by the Food and Drug Administration for routine clinical diagnosis.[8]

PRIMARY ISOLATION MEDIA

The most reliable and sensitive means for diagnosis of a fungal infection is isolation of the organism on culture media.[1,3,9-11] A variety of media are commercially available for primary isolation of fungal organisms (Table 251-3). The medium chosen should support the growth of fungal isolates while inhibiting bacterial organisms from specimens collected from nonsterile sites. Traditional antibiotics used include chloramphenicol, gentamicin, penicillin, and streptomycin. Antibacterial agents can inhibit actinomycetes; therefore, media without antibiotics should be chosen if this agent is suspected, or a combination of media with and without antibiotics could be used.

Media may use cycloheximide, a protein synthesis inhibitor, to suppress the growth of saprophytic fungi. It also inhibits *Aspergillus*, *Candida*, *Cryptococcus*, *Trichophyton*, and most *Mucorales*. Cycloheximide can be used in combination with various antibacterial agents listed earlier.

Chromogenic media are useful for the recovery and identification of *Candida* spp.[5] Dermatophyte test medium (DTM) was designed specifically for the culture and isolation of dermatophytes. Other media used can be those

TABLE 251-3 **Primary Isolation Media**

Media	Supplements	Use
CHROMagar, Candida or Albicans ID agar	Chromogenic substrates and antibiotics	Selective and differential medium for recovery of clinically important yeasts. Assimilation of chromogenic substrates and colony color development allow presumptive identification of common *Candida* species. *Candida* species detected is manufacturer dependent.
Dermatophyte test medium	Chloramphenicol, cycloheximide, gentamicin	Selective and differential medium for recovery of dermatophytes *(Epidermophyton, Microsporum, Trichophyton).* Medium turns from yellow to red with growth of dermatophytes.
Inhibitory mold agar (IMA)	Chloramphenicol; gentamicin may be added	Selective and enriched medium used to isolate cycloheximide-sensitive fungi *(Candida, Cryptococcus, Histoplasma, Zygomycetes,* some *Aspergillus)* and inhibit bacterial growth.
Mycosel	Chloramphenicol and cycloheximide	Selective medium for isolation of dimorphic fungi and dermatophytes. Chloramphenicol inhibits bacterial growth and cycloheximide inhibits rapidly growing molds and yeasts.
Potato flake agar	Can be made selective by addition of chloramphenicol and cycloheximide	Useful for production of conidia and pigment.
Sabouraud brain-heart infusion agar (SABHI)	Used with (selective) or without antibiotics. Antibiotics include chloramphenicol, ciprofloxacin, cycloheximide, penicillin, and streptomycin. Could add sheep blood for recovery of *Histoplasma.*	General purpose medium for growth of dimorphic and other fungi.
Sabouraud dextrose agar (SDA)	None	General purpose medium supporting growth of aerobic actinomycetes and all fungi.
	Addition of olive oil on surface	Selective for cultivation of *Malassezia furfur.*
Yeast extract phosphate medium	Ammonium hydroxide and chloramphenicol	Selective medium promotes recovery of slow-growing dimorphic fungi. Chloramphenicol inhibits bacterial growth and ammonium hydroxide inhibits growth of other fungi.

containing caffeic acid for the detection of *C. neoformans* by its phenol oxidase activity or media overlaid with olive oil (long-chain fatty acid source) for the recovery of *Malassezia furfur*.

When processing tissue, avoid the use of a mortar and pestle or tissue grinder. Hyphal elements, particularly aseptate hyphae, are easily destroyed in this process, rendering culture recovery impossible. Recommendations are to mince the tissue with a scissors or scalpel blade into 1-mm cubes and to place these fragments onto the agar surface.

Nonselective media will permit the recovery of rapidly growing yeast and fungi as well as of fastidious, slow-growing fungi. Selective media will enhance the recovery of slower-growing dimorphic fungi while inhibiting the faster-growing yeast and fungi that may contaminate the medium and render isolation of the slower-growing organisms impossible.

In general, a battery of media, including media containing sheep blood (BHI and Sabouraud brain-heart infusion agar [SABHI]), is used for the recovery of fungal isolates. Although sheep blood inhibits conidiation, it does improve the recovery of fungal isolates. Agar plates or screw-capped tubes can be used for isolating fungal isolates. Most mycologists prefer agar plates because of the ease of handling the cultures, and the larger surface area provides for better isolation of colonies. Plates usually are sealed with air-permeable tape or shrink-wrap to prevent inadvertent opening of the plate and also to slow down or to retard dehydration of the media because many plates are held for 4 weeks in the laboratory before the specimen is signed out as negative. Examination of culture plates or agar tubes should be performed in a certified biologic safety cabinet. Most fungi will grow on routine bacteriology plates; however, the incubation time usually is short and may not allow sufficient time for the fungi to grow and be visually noticed on the plates.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Larone DH. *Medically important fungi. A guide to identification*. 5th ed. Washington, DC: ASM Press; 2011.
4. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev* 2011;**24**:247–80.
8. Smith JA, Kauffman CA. Pulmonary fungal infections. *Respirology* 2012;**17**:913–26.
10. Versalovic J, editor. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 252

Viral Laboratory Diagnosis

Romney M. Humphries • Marjorie J. Miller

In the past, diagnostic testing for viral agents consisted primarily of isolation of virus in tissue culture and serologic studies. These tests were often perceived as too impractical, nonproductive, and slow to warrant implementation in the hospital laboratory. Fortunately, in recent years technologic developments and an expanding commercial availability of reagents and test kits have enabled laboratories to provide effective virologic diagnostic services with turnaround time to results and charges similar to those for other areas of the microbiology laboratory.[84,85,125,153] Although an increasing number of these reagents are being approved or licensed as in vitro diagnostic devices, a major hurdle to adding these technologies to laboratory test menus is the requirement that the laboratory verify manufacturer-established performance characteristics for each test, before implementation. Such verification studies often are extensive and taxing to laboratory resources, although manufacturers may offer educational and technical support to assist the laboratory, if permitted by local regulations. Practical guidance for verification studies has been published elsewhere.[20,60,163]

Presently, virologic services offered by clinical laboratories may range from referral of specimens to a reference laboratory to limited detection of viral antigens (e.g., respiratory syncytial virus, influenza, adenovirus, rotavirus) or expansion to include culture and qualitative and quantitative molecular assays. This chapter presents the general aspects of diagnosis of viral infections, including specimen selection, collection, and transport; conventional and modified culture for virus isolation and identification; and rapid detection of viral antigens and nucleic acids directly in specimens. Specific aspects of diagnosis are presented in the respective chapters covering the viral agents.

REFERENCES

1. Anaissie EJ, McGinnis MR, Pfaller MAS. *Clinical mycology*. Philadelphia: Churchill Livingstone; 2003.
2. Foy PC, van Burik AH, Weisdorf DJ. Galactomannan antigen enzyme-linked immunosorbent assay for diagnosis of invasive aspergillosis after hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2007;**13**:440–3.
3. Larone DH. *Medically important fungi. A guide to identification*. 5th ed. Washington, DC: ASM Press; 2011.
4. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. *Clin Microbiol Rev* 2011;**24**:247–80.
5. Odds FC, Bernaerts R. CHROMagar *Candida*, a new differential isolation medium for presumptive identification of clinically important *Candida* species. *J Clin Microbiol* 1994;**32**:1923–9.
6. Rickerts V, Mousset S, Lambrechy E, et al. Comparison of histopathological analysis, culture and polymerase chain reaction assays to detect invasive mold infections from biopsy specimens. *Clin Infect Dis* 2007;**44**:1078–83.
7. Saubolle M. Laboratory aspects in the diagnosis of coccidioidomycosis. *Ann N Y Acad Sci* 2007;**1111**:301–14.
8. Smith JA, Kauffman CA. Pulmonary fungal infections. *Respirology* 2012;**17**:913–26.
9. Sutton DA. Specimen collection, transport and processing: mycology. In: Murray PR, editor. *Manual of clinical microbiology*. 8th ed. Washington, DC: ASM Press; 2003. p. 1659–67.
10. Versalovic J, editor. *Manual of clinical microbiology*. 10th ed. Washington, DC: ASM Press; 2011.
11. Winn WC Jr, Allen SD, Janda WM, et al. *Koneman's color atlas and textbook of diagnostic microbiology*. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.

SPECIMEN COLLECTION AND TRANSPORT

Laboratory diagnosis of viral infections depends primarily on the collection of appropriate specimens, in sufficient volume, at the correct time during the course of the illness using optimal transport and storage conditions. Improvements in the sensitivity and accuracy of laboratory assays will be rendered insignificant if preanalytical considerations are overlooked.

Specimen Collection Sites

The selection of appropriate specimens as early in the acute phase of the illness as possible is critical to the recovery of viruses. A guide to recommended collection sites for specific viral syndromes is presented in Table 252-1. In general, the extent of the diagnostic investigation should be dictated by the characteristics of the illness being studied. For example, in common respiratory illnesses such as pharyngitis or croup, collection of a single specimen from the throat usually is sufficient. In other situations in which an illness is severe or unique, collection of specimens from multiple sites is important. In addition, in unusual cases, serum should be obtained for frozen storage in case subsequent serologic studies are required.

In severe illnesses, invasive procedures (e.g., brain, cardiac, liver, lung biopsies, and needle aspiration of body fluids) frequently must be performed to obtain material for laboratory study. Many medical specialists argue against using these invasive procedures because little can be done to treat viral illnesses. However, in our experience, the knowledge gained from a positive viral identification justifies some risk incurred in collecting specimens. At present, antiviral drugs are similar to antibacterial and antifungal agents in that they generally can be used effectively only after definitive identification of the etiologic agent. Demonstration of a viral etiology in diseases such as encephalitis, pneumonia, or cardiac disease can prevent the unnecessary administration of antibiotics and steroids. In many cases, overuse of services, patient trauma, and overall cost can be reduced when a viral agent is confirmed. Finally, the prognosis of a particular illness is more accurate when the specific etiology is known.

Collection of Specimens

Specimens for virus culture or direct examination are generally obtained as they are for other microbiologic studies. The primary purpose of a transport medium is to provide a protective protein, neutral pH, and antibiotics for control of microbial contamination and, most importantly, prevention of desiccation. Many viral transport and storage media are available commercially; their utility has been reviewed elsewhere.[52,97] Saline or holding media that contain serum should be avoided. Useful liquid transport media (i.e., 2-mL aliquots in screw-capped vials) consist of tryptose phosphate broth with 0.5 percent bovine albumin; Hanks balanced salt solution with 5 percent gelatin or 10 percent bovine albumin; or buffered sucrose phosphate (0.2 mol/L, 2-SP),[87,97] which has been used as a combined transport medium for viral, chlamydial, and mycoplasmal culture requests[184] and is appropriate for long-term frozen storage of specimens and isolates.[87] Many of these transport media also have been evaluated and found acceptable for use in rapid methods (e.g., enzyme-linked immunosorbent assay [ELISA][144,202] and nucleic acid amplification tests [NAATs]).[131]

Convenient and practical collection devices, such as UTM Kits (Copan), or Universal Viral Transport Kits (Becton Dickenson), consist of a swab, usually a nylon flocked swab on a plastic shaft, and a self-contained transport medium. Calcium alginate swabs, which are toxic to herpes simplex virus (HSV), and wooden shafts, which may be toxic to viruses and the cell culture system itself, should not be used. Nylon flocked swabs should be used over traditional swabs whenever possible, because these are associated with both enhanced absorbance to, and subsequent release of, specimen from the swab.

Throat

Specimens from the throat should be obtained with a swab in a manner similar to that used for bacterial culture. The posterior pharyngeal wall and tonsillar surfaces, any inflamed or erythematous areas, and any visible lesions are swabbed firmly without contact with the tongue and anterior oral cavity.

Nose and Nasopharynx

Specimens should be collected with nasopharyngeal swabs that have thin, flexible shafts by inserting the swab into the nasopharynx and rotating it to obtain the maximal number of ciliated, columnar epithelial cells and then placing the swab in transport medium. Alternatively, nasopharyngeal secretions can be obtained from infants by nasal washing.[82] In this method, a small amount of sterile phosphate-buffered saline (3 to 7 mL) is squeezed into the nose with a nasal bulb aspirator (1 oz, tapered) and then immediately withdrawn and placed in a sterile, screw-capped container. Nasopharyngeal aspirates also may be obtained with a mucus collection device. An appropriately sized catheter is inserted nasally into the posterior of the nasopharynx; intermittent suction is applied as the catheter is withdrawn. Aspirate is washed through the tubing with 5 to 8 mL of transport medium or sterile phosphate-buffered saline. Nasopharyngeal washes and aspirates may be placed into a viral-specific transport medium, which may enhance sensitivity of both direct antigen tests and molecular methods.[26] Washes and aspirates (vs. traditional swabs) are preferred for direct antigen detection because more epithelial cells are obtained by this method.[46] However, the enhanced absorbent capacity of flocked swabs over traditional swabs for exfoliated virus-infected epithelial cells, when combined with the relative ease of collection of a nasopharyngeal swab in contrast to an aspirate or wash, make flocked swabs an attractive option, particularly for very young patients. Nasopharyngeal specimens collected using

TABLE 252-1 **Specimen Collection Guide for the Diagnosis of Viral Infections Based on the Viral Syndrome and Etiologic Agent Suspected**

Main Location or Category of Illness	Clinical Diagnosis	*Specimen Collection Source*			Etiologic Agent Suspected*
		Most Practical	Most Definitive	Other Sources	
Upper respiratory tract	Common cold, nasopharyngitis	Nasopharynx/nose	Nasopharynx/nose	Nasopharyngeal/nasal wash, stool, blood	Rhinoviruses, coronaviruses, parainfluenza viruses, RSV, enteroviruses, adenoviruses
	Pharyngitis	Throat	Throat	Stool, blood	Adenoviruses, enteroviruses, Epstein-Barr virus, influenza viruses, parainfluenza viruses
	Herpangina, other enanthems	Throat	Lesions	Stool, blood	Enteroviruses, HSV
	Laryngitis, laryngotracheitis	Throat	Larynx or trachea	Nasopharyngeal/nasal wash	Parainfluenza viruses, influenza viruses
	Parotitis, other salivary gland enlargement	Throat	Stensen duct	Urine, blood, cerebrospinal fluid	Mumps virus, enteroviruses
Lower respiratory tract	Bronchitis, bronchiolitis, pneumonia	Throat	Bronchoalveolar lavage, bronchial washing biopsy	Stool, blood, sputum, nasopharyngeal/nasal wash	RSV, human metapneumovirus, parainfluenza viruses, adenoviruses, influenza viruses, cytomegalovirus
	Pleurodynia	Throat	Throat	Stool	Enteroviruses
	Pleural effusion	Pleural fluid	Pleural fluid	Throat, stool, blood	Enteroviruses, adenoviruses
Heart	Myocarditis, pericarditis, conduction defects	Throat	Pericardial fluid, biopsy	Stool, blood, urine	Enteroviruses, influenza viruses, adenoviruses
Central nervous system	Meningitis	Throat	Cerebrospinal fluid	Stool, blood, urine	Enteroviruses, mumps virus, arboviruses, HSV-2, lymphocytic choriomeningitis virus
	Encephalitis	Throat	Brain biopsy	Cerebrospinal fluid, stool, blood, urine	Arboviruses, mumps virus, enteroviruses, HSV-1, influenza viruses
	Guillain-Barré syndrome, cerebellar ataxia, transverse myelitis, poliomyelitis	Throat	Throat	Stool, blood, cerebrospinal fluid	Influenza viruses, arboviruses, enteroviruses, Epstein-Barr virus
Genital tract	Orchitis, epididymitis	Throat	Testicular biopsy	Stool, blood, urine	Mumps, enteroviruses, lymphocytic choriomeningitis virus
	Herpes genitalis	Lesions	Lesions	Vagina, cervix, urethra	HSV
Urinary tract	Cytomegalovirus infection	Urine	Urine	Throat, blood	Cytomegalovirus
	Hematuria and/or pyuria	Throat, urine	Urine	Blood, stool	Arboviruses, enteroviruses, mumps virus, adenoviruses
Gastrointestinal tract	Nausea and/or vomiting	Throat	Throat	Stool, blood	Enteroviruses, influenza viruses, noroviruses
	Diarrhea	Stool	Stool	Throat	Rotaviruses, enteroviruses, adenoviruses, noroviruses
	Abdominal pain	Throat	Throat	Stool, blood	Enteroviruses, adenoviruses

	Acute abdomen mesenteric adenitis	Throat	Mesenteric lymph node biopsy, peritoneal fluid	Stool, blood	Enteroviruses, adenoviruses
	Hepatitis	Throat	Liver biopsy	Stool, blood	Hepatitis A, B, C, E, and G viruses, Epstein-Barr virus, adenoviruses, enteroviruses, cytomegalovirus
	Pancreatitis	Throat	Duodenal fluid	Stool, blood	Enteroviruses
	Reye syndrome	Throat	Liver biopsy	Blood	Influenza viruses, varicella virus
	Hepatosplenomegaly	Throat	Blood, liver biopsy	Stool, urine	Adenoviruses, enteroviruses, Epstein-Barr virus, cytomegalovirus
Reticuloendothelial system	Generalized lymphadenopathy	Throat	Blood, lymph node biopsy	Stool, urine	Adenoviruses, enteroviruses, Epstein-Barr virus, cytomegalovirus
	Immunodeficiency	Blood	Blood	Lymph nodes	HIV
Bone or joints	Osteomyelitis	Bone	Bone	Throat, blood, urine, skin lesion	Rubella virus, vaccinia virus
	Arthritis	Joint fluid	Joint fluid	Throat, blood, urine	Rubella virus, arboviruses
Muscle	Myositis	Throat	Muscle biopsy	Stool, blood	Influenza virus, enteroviruses
Skin	Exanthematous disease	Throat	fluid, skin biopsy Vesicular	Stool, blood, urine, eye	Measles virus, rubella virus, varicella virus, enteroviruses, HSV
Eye	Conjunctivitis, including pharyngoconjunctival fever	Conjunctiva	Conjunctiva	Throat, stool	Adenoviruses, enteroviruses
Eye	Keratoconjunctivitis	Conjunctiva, cornea	Conjunctiva, cornea	Throat, stool	Adenoviruses, herpes simplex virus, varicella virus
	Retinitis	Vitreous, aqueous fluid	Vitreous, aqueous fluid		Cytomegalovirus, herpes simplex virus, varicella virus
Fever	Nonspecific febrile illness (human-to-human transmission)	Throat	Blood	Stool, urine, cerebrospinal fluid	Enteroviruses, influenza viruses, adenoviruses, cytomegalovirus
	Nonspecific febrile illness (arthropod vector)	Blood	Blood	Throat, urine, cerebrospinal fluid	Arboviruses
	Fever of unknown origin	Blood	Blood	Urine, stool, throat	Hepatitis A, B, C, E, and G viruses, cytomegalovirus, herpes simplex virus, Epstein-Barr virus, adenoviruses
Congenital infection	Rubella virus, cytomegalovirus infections	Throat	Blood	Urine, nasopharynx, biopsy material, cerebrospinal fluid	Rubella virus, cytomegalovirus
	HIV infection	Blood	Blood	Lymph node	HIV
Perinatal and neonatal infections	Herpes simplex virus, cytomegalovirus, enterovirus infections	Throat	Blood	Urine, nasopharynx, stool, skin lesions, cerebrospinal fluid	Enteroviruses, RSV, influenza viruses, herpes simplex virus, cytomegalovirus

*This list includes only the more commonly associated agents. It is likely that with more general use of viral diagnostic services, new virus–disease associations will be made.
HIV, Human immunodeficiency virus; *RSV,* respiratory syncytial virus, *HSV,* herpes simplex virus.

flocked swabs yield sensitivity equivalent to that of aspirates for direct antigen detection[3,26] and in many cases, better sensitivity for polymerase chain reaction (PCR)-based methods.[134,207]

Other Respiratory Specimens (Sputum, Tracheal Aspirates, Bronchial Washings, Bronchoalveolar Lavage)

Collection depends on the volume obtained. Volumes of 0.5 mL or larger should be placed in a sterile container and sealed tightly. If the volume is less than 0.5 mL, the specimen is placed in 2 mL of transport medium.

Eye

Exudate or pus should be removed first with a sterile swab. Conjunctival specimens may be obtained by pressing a swab premoistened with sterile saline firmly against the inflamed areas. The swab is returned to the self-contained transport device. Corneal scrapings should be obtained by an ophthalmologist or other trained person and placed in transport medium immediately.

Body Fluids Other Than Blood

Body fluids such as urine (clean voided, 10 to 20 mL), cerebrospinal fluid (CSF) (2 to 5 mL), pleural effusion, and peritoneal, pericardial, or joint fluid should be collected under sterile conditions and placed in securely sealed, sterile containers. For small volumes (≤0.5 mL), the specimen may be placed in transport medium.

Lesions

For lesions, fresh vesicles should be selected because recovery of virus from older lesions decreases significantly. They should be gently swabbed with sterile saline. The vesicle is ruptured and both fluid and cells collected from the base of the lesion. Material obtained from several lesions may be pooled. Desiccation must not be allowed to occur; swabs should be submitted moistened with their self-contained transport medium or, if that is unavailable, placed in 2 mL of transport medium. Specimens may be collected with a swab or aspirated with a 26-gauge needle attached to a tuberculin syringe. Aspirated fluid should be rinsed into 1 to 2 mL of transport medium.

For direct antigen detection by immunofluorescence (direct fluorescent antibody [DFA]), the vesicle is ruptured as previously described and epithelial cells are collected by firmly swabbing the base of the lesion. Cells are transferred to a clean glass slide by firmly rolling the swab back and forth over a 5- to 10-mm area (dime size) or, if a ringed slide is used, into the center of the ring. For differentiation of HSV types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV), three slides should be prepared.

Stool and Rectal Specimens

Fecal specimens should be collected only when the specific primary diagnosis is diarrhea or when concomitant serologic study with paired sera can be performed. Both enteroviruses and adenoviruses can be recovered readily from stool, but because these agents are carried in the lower gastrointestinal tract for considerable periods of time after acute infection, use of this source for establishing the diagnosis of specific illnesses is limited.

For recovery of enteroviruses, fresh stool specimens are better than rectal swabs; 2 to 5 g (2-3 tsp) of formed or liquid specimen should be transferred to a sterile, leakproof container. However, from a practical point of view, a rectal swab is the simplest method of specimen collection. A rectal swab can be obtained immediately, whereas collection of a stool specimen usually entails considerable delay. Of importance is that the swab contain visible stool; the swab should be inserted 3 to 5 cm into the rectum, rolled against the mucosa, and then transported in transport medium.

Blood

Some viruses can be recovered from serum or red blood cells (RBCs), but in general, leukocytes are a better source of virus. Fresh blood (optimally 5 mL) is collected in a suitable anticoagulant (i.e., heparin, ethylenediaminetetraacetic acid [EDTA], or acid citrate dextrose) and transported to the laboratory for processing by density gradient [132] centrifugation or other methods to enrich for leukocytes. EDTA is the anticoagulant of choice because it is an acceptable transport for culture, as well as for molecular methods such as hybridization and PCR.

Bone Marrow

Approximately 2 mL should be aspirated into a tube or syringe containing heparin anticoagulant, mixed thoroughly to prevent clotting, and transported without further additives or diluents.

Biopsy Specimens

For biopsy specimens, fresh tissue obtained from the affected site should be placed in 2 mL of transport medium to prevent desiccation.

Autopsy Specimens

Most postmortem specimens are almost useless for viral cultivation because of the manner in which autopsies usually are performed. If labile agents are to be recovered, the autopsy should be performed within 4 hours of death. All tissues (as 1- to 2.5-cm cubes) for study should be obtained aseptically, and individual tissue should be collected with sterile instruments and placed in separate sterile containers. Because the usual autopsy routine entails fixation of specimens, of vital importance is that specimens for viral isolation not be placed in containers with fixatives such as formalin or other preservatives.

Transport to the Laboratory

The method of handling specimens from the time of collection to laboratory processing is critical not only for

preservation of virus infectivity for recovery in culture but also for optimal preservation of nucleic acid integrity before molecular detection methods. In general, the less time that transpires between collection and processing by the laboratory, the greater is the chance of detecting the virus. Specimens should not be frozen or exposed to temperatures higher than 22° C (71.6° F). For short-term storage (<5 days) of most viruses, the specimen should be held at 4° C (39.2° F) until it can be processed in the laboratory. For transport to the laboratory, this temperature can be achieved with the use of cold packs or ice. Transport of specimens at 4° C is less critical for testing by molecular-based assays, provided an appropriate transport medium is used and the delay is not greater than one day.[50]

When considerable delay between collection and culture will elapse (e.g., transport to other laboratories, holiday schedules, and so on), special preparation is necessary. Such preparation needs to be individualized and based on the most likely viral cause of a particular illness. For example, when an enteroviral etiologic agent is suspected, freezing the specimen (–70° C [–94° F]) and shipping it with dry ice are satisfactory. In contrast, a urine specimen from a patient with possible cytomegalovirus (CMV) infection should not be frozen. It should be shipped under wet ice at 4° C (39.2° F) because CMV in urine is stable for several days at this temperature. In addition, media that protect viruses against the deleterious effects of temperature fluctuations, particularly freeze-thawing, are available for transport and long-term storage.[87,97] For specific characteristics of individual viruses, see the respective chapters in this book.

LABORATORY DIAGNOSIS OF VIRAL INFECTIONS

In recent years, rapid technologic advances have provided powerful and achievable methods for the detection of viruses in clinical specimens. Direct detection of viral antigens such as the herpes group viruses (HSV, VZV, CMV); respiratory syncytial virus (RSV); influenza viruses; adenoviruses; and rotaviruses, usually by DFA testing, has become routine and has made obtaining same-day or next-day results a reality. In addition, detection of viral antigens in cell culture by the application of immunofluorescence or immunoperoxidase staining, before the appearance of cytopathic effect, has greatly reduced the time needed to report a positive result. More recently, widespread availability of commercial reagents and kits for molecular detection and quantitation of viral nucleic acids in specimens has made these methods accessible for many hospitals. Laboratories may combine both culture and nonculture approaches to optimize viral disease diagnosis, although many are eliminating culture in deference to the superior sensitivity, specificity, and turnaround times achievable by molecular methods. Table 252-2 summarizes optimal methods for the laboratory identification of specific viruses, and more in depth information is presented in the individual chapters of this book.

Virus Isolation

Culture remains the cornerstone to viral diagnostic testing and although not performed in all hospitals, is available in many reference and public health laboratories. Unlike rapid methods, which are limited to the detection of specific viral antigens or nucleic acids, culture is open-ended and permits the detection of unexpected or new viruses. In addition, a broad range of specimens can be evaluated, whereas rapid methods usually are approved or licensed for use with a limited number of specific specimens. Viruses that can be isolated in culture and identified readily include adenoviruses, HSV, VZV, CMV, enteroviruses, rhinoviruses, influenza and parainfluenza viruses, RSV, rubella virus, mumps virus, and measles virus. The number and type of isolates encountered depend on the patient population, season, and type of specimens submitted. For optimal laboratory diagnosis, indicating the name of the suspected virus on the requisition is important because some viruses require specific cell lines, procedures for identification, or both. Cultures are maintained for different time frames before finalizing a report of negative results, depending on the virus being sought; for example, HSV cultures usually are observed for 5 to 7 days, CMV for 1 month, and all others for 2 to 4 weeks. Improvements and modifications in culture methods have resulted in more rapid identification of isolates, with greater than 70 percent being reported within 5 days.[48,91]

Traditional Culture

For traditional culture, clinical samples are inoculated onto one or more cell lines, selected based on the submitted specimen and suspected virus. Cultures are observed at regular intervals for evidence of viral infection characterized by the appearance of cytopathic effect or the ability to hemadsorb or hemagglutinate RBCs. Adenoviruses, CMV, HSV, VZV, rhinoviruses, and enteroviruses can be identified and reported by their characteristic cytopathic effect. The mean time between inoculation of a specimen and the appearance of cytopathic effect usually is 1 week or less for most of this group, except CMV and some adenoviruses, which may require longer incubation. Cultures containing HSV and enteroviruses often are positive within 1 to 2 and 3 to 5 days, respectively. In the event of atypical or questionable cytopathic effect, immunofluorescence with monoclonal antibodies can be used to identify CMV, VZV, HSV-1, HSV-2, adenoviruses, enteroviruses, and some select coxsackieviruses and echoviruses in infected cell cultures. Although it rarely is performed, definitive identification of adenovirus and enterovirus serotypes by the neutralization test may be used if requested for epidemiologic or other reasons. More recently, molecular-based serotyping methods have been developed for both adenoviruses and enteroviruses with specificities equivalent to the neutralization test.[115,140]

Respiratory viruses, including parainfluenza and influenza viruses, which may or may not produce cytopathic effect, generally can be detected within 5 to 7 days of inoculation of the specimen with the traditional

TABLE 252-2 **Common Laboratory Methods for Diagnosis of Specific Viral Illnesses***

Virus	Culture	Antigen Detection	Serology	Nucleic Acid Amplification Test (NAAT)	Most Useful Method for Diagnosis
Adenoviruses	Routine	DFA ELISA	Reference or public health laboratory	Detected by some multiplexed respiratory virus assays Single-analyte commercial NAAT available	Culture or NAAT
Arboviruses of North America	Reference or public health laboratory	Not routine	Reference or public health laboratory	Reference or public health laboratory	Serology or NAAT
Coronaviruses	Not practical by routine methods (except SARS-CoV, which should not be cultured outside public health laboratories)	Not routine	Not routine	Detected by some multiplexed respiratory virus PCR assays	PCR
Rhinoviruses	Routine, must be differentiated from enteroviruses by acid lability	Not routine	Not routine	Detected by some multiplexed respiratory virus PCR assays	Culture or NAAT
Enteroviruses	Routine, some coxsackieviruses are difficult to culture by routine methods	Not routine	Not routine	NAAT from CSF routine Detected by some multiplexed respiratory virus NAATs	NAAT (especially from CSF) Culture
Cytomegalovirus	Routine	Not routine	Routine	Both qualitative and quantitative NAATs routine	NAAT Culture
Epstein-Barr virus (EBV)	Not practical by routine methods	Not routinely performed	Routine	Quantitative NAATs routine	NAAT
Herpes simplex virus	Routine	DFA	Routine	Single-analyte commercial NAAT available for lesions Routine from CSF	NAAT (especially from CSF) Culture
HIV	Not routine	p24 antigen assay	Routine	Quantitative NAATs routine	Serology NAAT
Human metapneumovirus (hMPV)	Routine by shell vial assay, detect by hMPV-specific IF	DFA	Not routine	Detected by some multiplexed respiratory virus NAATs Single-analyte commercial NAAT available	DFA NAAT
Influenza viruses	Routine	DFA Rapid antigen tests (ELISA)	Not routine	Multiple NAATs available, including those that subtype virus Detected by some multiplexed respiratory virus NAATs	Culture DFA NAAT

Measles virus	Routine (in reference laboratories)	Not routine	Routine	Reference or public health laboratory	Serology NAAT
Mumps virus	Routine (in reference laboratories)	Not routine	Routine	Reference or public health laboratory	Serology NAAT
Parainfluenza viruses	Routine	DFA	Not routine	Detected by some multiplexed respiratory virus NAATs	Culture NAAT
Respiratory syncytial virus	Routine	DFA Rapid antigen tests (ELISA)	Not routine	Detected by some multiplexed respiratory virus NAATs	DFA Culture NAAT
Rabies virus (animal infections)	Not routinely performed	DFA at public health laboratory	Public health laboratory	Public health laboratory	Submit to public health laboratory
Rubella virus	Reference laboratory, by interference of enterovirus replication	Not routine	Routine	Not routine	Serology
Poxviruses	Not routine	Not routine	Not useful for diagnosis	Vaccinia virus PCR at public health laboratory	Clinical diagnosis (molluscum) Submit to public health laboratory
Varicella-zoster virus	Routine, but virus does not survive transport well	DFA	Not routine	Routine, as laboratory developed tests	DFA Culture NAAT
Hepatitis A virus	Not routine	Not routine	Routine	Not routine	Serology
Hepatitis B virus	Not practical by routine methods	Not routine	Routine	Routine	Serology (diagnosis) NAAT (to monitor treatment)
Hepatitis C virus	Not practical by routine methods	Practical method not available	Routine	Both qualitative and quantitative NAATs routine	Serology (diagnosis) NAAT (diagnosis and to monitor treatment)
Norovirus	Not practical by routine methods	ELISA	Not routine	Public Health or Reference laboratory	Antigen testing NAAT
Rotavirus	Not practical by routine methods	ELISA	Not routine	Not routine	ELISA
Adenovirus 40 and 41	Not practical by routine methods	ELISA	Not routine	Not routine	ELISA

*For details, see chapters for the specific viral agents in this textbook.
CSF, Cerebrospinal fluid; *DFA,* direct fluorescent antibody; *ELISA,* enzyme-linked immunosorbent assay; *PCR,* polymerase chain reaction; *SARS-CoV,* severe acute respiratory syndrome–corona virus.

hemadsorption method, in which a suspension of guinea pig RBCs is added to the cell culture. The RBCs are observed to adhere to tissue culture cells infected with these viruses, usually 3 to 5 days after the inoculation of specimens for most isolates.[133] An adaptation of this method uses cell culture medium and guinea pig RBCs in a microtiter plate format to detect viral hemagglutinins in suspension. The results are similar to those observed with hemadsorption, but the adapted method is more rapid and simple to perform when screening numerous specimens.[98] RSV is identified presumptively, usually within 3 to 5 days of inoculation of the specimen, by its typical cytopathic effect (syncytium formation) and inability to hemadsorb and hemagglutinate guinea pig RBCs. This group of viruses is identified definitively by immunofluorescence with monoclonal antibodies.[64,176] The traditional method for isolation and identification of rubella virus uses primary African green monkey kidney cells in conjunction with the interference assay. After specified intervals following inoculation have transpired, cultures are challenged with a second virus, usually echovirus 11, incubated for an additional 3 to 4 days, and examined for cytopathic effect. The presence of rubella virus interferes with growth of the challenge virus, and no cytopathic effect is observed; on the other hand, if the challenge virus grows and produces cytopathic effect, the specimen is considered negative for rubella virus. Final identification of the isolate requires neutralization of the interference with specific rubella antibody.[176,204]

NAATs, serologic tests, or a combination of the two are recommended for viruses that are difficult or impossible to isolate in commonly employed cell lines or that require special techniques for detection of their presence. For instance, both NAAT and serology are useful for establishing the diagnosis of mumps virus. Although mumps virus can be isolated in the same cell lines used for other paramyxoviruses and detected by hemadsorption or hemagglutination, as previously described, such requests are rare. Buccal or oral swabs collected early in disease may be tested for mumps by a reverse transcription PCR assay. If negative, acute, and convalescent serum specimens may reveal diagnosis in patients with signs and symptoms of mumps. Similarly, most cases of arbovirus infection require the study of paired sera for antibody development for diagnosis. Human immunodeficiency virus (HIV) infection generally is identified best by the demonstration of serum antibody or antigen with the use of ELISA. Measles virus is relatively difficult to grow, and identification can take considerable time; when measles is suspected, the diagnosis is confirmed most easily by serologic study. Paired sera collected 1 week apart usually reveal a fourfold antibody titer rise. Alternatively, demonstration of specific IgM antibody allows the diagnosis to be established with the use of a single serum sample.

Modified Culture

A variety of physical and chemical methods have been evaluated for their ability to enhance the rapid detection of virus in culture.[91] These methods include the effect of low- and high-speed rolling of cultures, centrifugation, chemicals such as dimethyl sulfoxide, hormones such as dexamethasone, and enzymes such as trypsin on the isolation of viruses. A technique that entails the physical method of centrifugation with early antigen detection in cell culture and is termed variously the shell vial assay (SVA), the spin-amplified technique, or culture-amplified antigen detection is used routinely in most laboratories to decrease the time to detection of a positive culture. Cell lines are propagated on 12-mm round coverslips in 1-dram shell vials. An aliquot of specimen is inoculated into the vial, centrifuged at 700 × *g* for 1 hour, and incubated 1 to 3 days, depending on the virus being sought. Antigen typically is detected by immunofluorescence or occasionally by immunoperoxidase staining with monoclonal antibodies directed against a specific virus, before the appearance of cytopathic effect.

SVA was described initially and evaluated extensively for HSV* and CMV,† and its use since then has been extended to the rapid identification of VZV, adenovirus, RSV, influenza virus, parainfluenza virus, measles virus, and human metapneumovirus antigens in culture.[64] In addition, SVA has been applied to the detection of enteroviruses,[103,112] human herpesvirus type 6,[129] arboviruses (including dengue,[95] yellow fever, St. Louis encephalitis, West Nile,[95] and Venezuelan encephalitis equine virus).[24]

Pooled antibodies directed against seven commonly isolated respiratory viruses (i.e., adenovirus; influenza viruses A and B; parainfluenza virus types 1, 2, and 3; and RSV) also have been evaluated, and are commercially available.[123,146,161] To be adequately sensitive for each of these respiratory viruses, either two or more single cell lines in shell vial are used or mixed cell lines are applied to shell vial cultures. Mixed cell lines are particularly useful, in that they broaden the range of cultivable viruses, reduce the requirement for multiple single cell lines, and decrease the time to detection of a positive result and have sensitivity similar to that of conventional culture. The commercially available R-Mix (Diagnostic Hybrids) mixed cell line detects commonly isolated respiratory viruses. R-Mix consists of one cell line to support replication of RSV, adenovirus, and parainfluenza (e.g., A549) and another to support replication of influenza (e.g., Mv1Lu). R-Mix Too was developed after recognition that SARS-coronavirus (CoV) readily infected Mv1Lu cell lines[68]; R-Mix Too mitigates the possibility that SARS-CoV be inadvertently cultured in a routine clinical laboratory without appropriate biosafety precautions, by replacing Mv1Lu with MDCK cell lines.[68] R-Mix cells are available cryopreserved, with a shelf life of 6 months from date of manufacture, thereby enabling shipment to regions outside North America. Mixed cell lines are also useful for rapid isolation of human metapneumovirus[183] enteroviruses,[11,63,89] and herpes group viruses.[90] The sensitivity of mixed cell versus conventional single-cell lines for virus detection ranges from 92 to 100 percent for respiratory viruses[12,59,88,90,217] to 85 to

*References 23, 76, 117, 152, 160, 171.
†References 57, 72, 74, 75, 109, 151, 149, 162.

97 percent for enteroviruses,[63,89] with time to detection of a positive result reduced from an average of 5 to 10 days to 1 to 2 days for respiratory viruses and 1 to 3 days for enteroviruses.

Although rapid, sensitive, and specific, SVA is an adjunct to, and not a substitute for, traditional culture. In contrast to traditional culture, the sensitivity of SVA averages approximately 85 percent (range, 70% to 100%) and depends on numerous variables, including the cell line(s),[221] the age of cells,[57] the concentration of virus,[221] the type of specimen,[109,151] the number of vials inoculated,[151] and the type of reagents used.[55,76,130,152,171]

Other culture modifications include hybridization[55,61] and ELISA[9,47,127,203,209] for rapid detection of viruses. A modification of cell culture for the rapid detection of HSV is the enzyme-linked virus-inducible system (ELVIS).[186,187] This system consists of a mixture of modified baby hamster kidney cells and MRC-5 cells. The modified baby hamster kidney cells contain an HSV-inducible promoter and an *Escherichia coli* LacZ reporter gene that produces β-galactosidase only when cells are infected with HSV. After the addition of a substrate for the β-galactosidase, infected cells stain blue, whereas uninfected cells remain colorless. ELVIS appears to be comparable to culture for the detection of HSV-positive specimens (95% and 100% sensitivity) in a similar time frame, 1.4 to 1.7 days.[31,122]

Direct Detection

Cytology

Historically, rapid detection of viral infection relied on light microscopy and evaluation of tissues and exfoliated cells for viral inclusions or other cytopathic effects. Cytologic identification of virus is most useful in illnesses with vesicular exanthems such as HSV or VZV infection. A scraping of the base of an HSV or VZV lesion (Tzanck smear) reveals multinucleated giant and balloon cells when stained with hematoxylin and eosin, Wright, or Giemsa stain. In contrast, vesicular lesions caused by enteroviral infections do not contain giant or balloon cells, and allergy-related lesions contain eosinophils. Cytologic study of urine in congenital CMV infection reveals cells with the characteristic "owl's eye" intranuclear inclusions in 25 to 50 percent of cases, and nasal or pharyngeal smears in measles may reveal typical giant cells. These types of stains are no longer routinely performed in most diagnostic virology laboratories because they have been replaced by immunofluorescence, which is more sensitive and specific.[33,34] Whereas the Tzanck smear cannot differentiate HSV from VZV and the presence of multinucleated giant cells is necessary to make the diagnosis, immunofluorescence can identify the causative agent specifically and the presence of viral antigen in balloon or giant cells is diagnostic.

Antigen Detection

Antigen-detection methods are available for most commonly isolated viruses. Advantages of these methods include speed, standardized reagents and kits, the ability to detect nonviable, nonculturable, or difficult-to-grow viruses, and the ability to detect antigen when culture may be negative late in the course of infection. Generally, these methods are not as sensitive as NAAT or an optimized culture for viruses that can be cultured; thus, culture backup is recommended. These tests also are approved for specific specimen types. DFA, indirect immunofluorescent assay (IFA), and ELISA, are used most commonly for antigen detection in the clinical laboratory.

Immunofluorescence

Antigen detection by immunofluorescence requires collection of the appropriate cell types (i.e., those in which the virus propagates) in adequate numbers for evaluation. In some cases, the specimen is placed on the slide directly (e.g., HSV or VZV detection), but usually the sample is transported to the laboratory for processing and slide preparation (e.g., for respiratory virus detection). As is the case for all methods discussed in this chapter, the importance of adequate specimen collection cannot be overemphasized. When stringent specimen quality criteria are applied (e.g., ≥60 columnar epithelial cells per sample for influenza testing), DFA sensitivity approaches that of PCR.[156] However, such adequate specimens are infrequently collected, even when staff are trained in proper collection techniques.[110]

DFA methods use virus-specific monoclonal antibody labeled with fluorescein dye to detect antigen and usually require approximately 30 to 45 minutes to complete. If processing of the specimen is required and slides are prepared in the laboratory, additional time is necessary. For IFA, unlabeled monoclonal antibody is added to the sample and incubated for 30 minutes, followed by a wash and the addition of fluorescein-labeled anti-mouse immunoglobulin. After an additional 30 minutes of incubation has transpired and a wash step performed, the slide can be examined for typical fluorescence (for excellent photographic examples, see Rossier and colleagues[169]). IFA can be completed in 2 to 3 hours, depending on specimen-processing requirements and the number of viral antigens being sought. The advantage of using DFA or IFA is the ability to determine specimen adequacy and perform small-batch and demand testing. A high-quality microscope and skilled, experienced personnel are required for optimal preparation and evaluation of the specimen.

The first DFA reagent available for use in the diagnostic laboratory was for the detection of HSV[77] in genital, dermal, oral, and anal lesions. The test has not been validated for the detection of HSV in CSF, asymptomatic genital specimens, tracheal aspirates, or bronchoalveolar lavage (BAL) specimens. The sensitivity of DFA versus culture for HSV detection ranges from 72 to 92 percent[105,159] and, like culture, depends on the stage of the lesion.[33,34] DFA or culture detected HSV in 96.7 percent of vesicles, 79.2 percent of pustules, 44.7 percent of ulcers, and 16.7 percent of crusts.[33] In contrast, detection of HSV by NAAT is less influenced by lesion stage[58] and

may increase yield of a positive result in 13 percent of vesicles, 28 percent of ulcers, and 20 percent of crusts.[177] Nonetheless, lesion duration and morphology remain strongly associated with the likelihood of HSV detection by NAAT and negative results were obtained in 41.4 percent of vesicles, 48.4 percent of ulcers, and 76.9 percent of crusts.[177]

DFA is an excellent method for detecting VZV in vesicular lesion material. Because the virus is labile, the sensitivity of DFA is superior to that of culture,[30,49,73,175] and culture backup is not necessary. However, the utility of DFA in the post–varicella vaccine era is diminished by the fact that breakthrough disease in previously vaccinated patients presents as a macular or papular rash, from which virus-infected cells for DFA testing cannot be readily collected. In such instances, NAAT performed on a swab or scraping of the rash is associated with 95 to 100 percent sensitivity.[110,211]

Rapid detection of CMV antigen in lung tissue or BAL specimens[81,120,205,212] and in liver tissue[150] for the diagnosis of CMV pneumonia or hepatitis has been evaluated. The sensitivity is less than that of culture or SVA and ranges from 56 to 100 percent; results were best when a mixture of antibodies directed against early and late viral antigens was used.[205] Sensitivity may be reduced further because BAL specimens have virtually replaced lung biopsy for the diagnosis of CMV pneumonia. A comparison of the cellular portion, supernate, and whole BAL for isolation of CMV in culture suggested that in most BAL specimens, CMV is associated with the cell-free rather than the cellular component.[29] The antigenemia assay[199,200] is a sensitive, specific, and rapid method for detecting CMV viremia, but has widely been replaced in clinical practice by CMV DNA qualitative and quantitative PCRs, which are described below. The antigenemia assay consists of separation of leukocytes from whole blood, spotting or cytocentrifuging cells onto microscope slides, fixation and permeabilization, staining with monoclonal antibodies, and detection by fluorescein-labeled or peroxidase-labeled secondary antibodies. Antigen-positive leukocytes are quantitated.

Finally, DFA and IFA are useful for the rapid detection of common respiratory virus* in nasopharyngeal washes, aspirates, and suctions. DFA for RSV has been evaluated extensively,† and its sensitivity in contrast to that of optimized culture is 80 to 97 percent. As is the case for VZV, RSV is relatively labile, and antigen detection actually may be more sensitive than culture; thus, culture backup usually is not required. However, culture or multiplex NAATs may still be advisable because up to 30 percent of specimens submitted for establishing the diagnosis of RSV contain other viruses.[16,17,43] The sensitivity of DFA and IFA for detection of influenza virus A and B, parainfluenza virus 1, 2, and 3, and adenovirus antigens varies in contrast to that of culture (range, 29% to 100%‡), so culture or NAATs must also be performed for optimal diagnosis.

*References 124, 126, 165, 185, 190, 206, 208, 215.
†References 27, 101, 124, 132, 165, 190, 196.
‡References 9, 93, 106, 123, 124, 185, 190.

Enzyme-Linked Immunosorbent Assays

Standardized, well-characterized commercial kits for the detection of viral antigen by ELISA are available for HSV, RSV, influenza virus A and B, rotavirus, norovirus, adenovirus, HIV (p24), HTLV I and II, and hepatitis B virus (HBV). Intracellular and extracellular antigen can be detected; the methods are generally simple technically and are less affected by suboptimal specimen handling than are viral cultures. ELISAs come in two basic formats, either a classic microtiter or tube system or self-contained membrane assays. The microtiter or tube format uses antibodies lining the surface of the solid phase to capture viral antigen, which in turn is reacted with an enzyme-labeled secondary antibody, followed by detection with the addition of substrate and color development. Results are read spectrophotometrically and thus are objective, thereby eliminating some of the difficulties inherent in microscopic techniques, which require technical skill and expertise for evaluation of specimens. These tests are relatively inexpensive, can be automated, are suitable for processing large numbers of specimens, and generally can be completed within 2 to 4 hours. Specimens normally are batched and cannot be tested on demand, as previously described for immunofluorescence. In addition, the adequacy of the specimen cannot be ascertained. Membrane assays use the membrane as the solid support to retain viral antigen, to which enzyme-labeled antibodies are added to react with trapped antigen. Antigen-antibody complexes are detected via a chromogenic substrate, which is read visually. Although they are rapid (15 to 20 minutes) and simple to perform, these tests are more expensive, require more hands-on time, and do not result in objective end points. False-positive results and interpretation can be a problem. Detection of influenza and RSV antigens in nasopharyngeal aspirates, washes, suctions, and swabs has been evaluated extensively in standard and membrane ELISA formats.

Many of the membrane-based ELISA tests are Clinical Laboratory Improvement Amendments (CLIA)-waived; however, they may be performed in emergency departments, outpatient clinics, and physician offices for point-of-care diagnosis of influenza A and B and RSV in patients with compatible respiratory symptoms. However, the performance of these rapid ELISAs may vary significantly from the specifications listed in the package insert and year-to-year based on circulating virus type and subtype. The sensitivity of the BinaxNOW Influenza A&B was 75 percent for influenza A virus and 72 percent for influenza B during one influenza season, but only 48.9 and 36 percent, respectively, for a subsequent season, because of antigenic drift in the seasonal influenza virus.[69] Similarly, the reported sensitivity of various ELISAs to detect novel influenza viruses, such as the 2009 (H1N1) pandemic and the H3N2v 2012 influenza strains was 0 to 70 percent,* depending on test manufacturer. Because of these concerns, negative results always should be backed up by culture, immunofluorescence, or NAAT. The performance of the ELISAs also depends on the

*References 1, 2, 22, 56, 71, 92, 201.

prevalence of the virus among patients with febrile respiratory tract infections. When the prevalence of influenza in children with compatible symptoms was 5 percent, the predictive value of a positive rapid test was found to be 50 percent in contrast to reverse transcription polymerase PCR (RT-PCR) and the predictive value of a negative test to be 98 percent.[79] Conversely, when the prevalence of influenza was 21 percent, the positive predictive value was 85 percent and the negative predictive value was 91 percent.[79] Thus, for optimal diagnosis, these devices should not be used when disease prevalence is low or in the off-season. Contemporary performance of the rapid influenza ELISAs commercially available in the United States is summarized in Table 252-3.

Children shed high titers of RSV; therefore, membrane-based ELISA for RSV performs significantly better in the pediatric population than do the influenza tests. However, optimal performance is based on patient age and timing of specimen collection relative to onset of symptoms. The BinaxNow RSV was positive in 79 to 91 percent of children younger than 1 year of age in contrast to NAAT-confirmed or culture-confirmed RSV infection, but in only 52 percent of those 1 year of age and older[128] to 25 percent of adults.[145] The assay was positive in 92 percent of patients with 0 to 1 day of symptoms, 78 percent with 2 to 4 days, and 65 percent with 5 or more days of symptoms.[128] Similar to influenza, performance of RSV rapid antigen tests varies significantly year-to-year and with virus prevalence.[37] Commercially available RSV rapid antigen assays evaluated between 2007 and 2012 are summarized in Table 252-3.

For the diagnosis of rotavirus and adenovirus 40 and 41 infection, ELISA is the only rapid test available in the clinical laboratory. Rotavirus antigen detection by standard ELISA has been studied thoroughly.[53,56,162] Many of the assays have excellent sensitivity (93% to 100%) and specificity (98% to 100%) in contrast to that of electron microscopy, although the best results were obtained with monoclonal (vs. polyclonal) antibody-based kits (see elsewhere[57,195] for a review of available tests). Some assays can be read either visually or spectrophotometrically, so instrumentation is not always necessary. Several membrane enzyme immunoassays also are available for rotavirus antigen detection (e.g., TestPack and ImmunoCard) and, as is typical of this format, are somewhat less sensitive than standard ELISA, with approximately 10^7 virions/mL required for detection of a positive specimen. The reported sensitivity and specificity versus electron microscopy are 94 to 100 percent and 90 to 100 percent, respectively.[19,28,44] An excellent ELISA for detection of

TABLE 252-3 **Summary of Select Rapid Influenza and Respiratory Syncytial Virus Assays**

Rapid Test (Manufacturer)	Acceptable Specimens	Sensitivity* (%)	Specificity* (%)	Reference
Influenza				
BinaxNOW Influenza A&B	Nasal wash, swab, and aspirate NP swab	Influenza A: 9.6-78 Influenza B: 35-63	Influenza A: 98-100 Influenza B: 98-100	38, 39, 41, 46, 69, 71, 108, 178
Directigen EZ Flu A+B (Becton-Dickinson)	NP wash, swab, aspirate Throat swab	Influenza A: 54-67 Influenza B: 53	Influenza A: 100 Influenza B: 100	13, 108, 111
QuickVue Influenza A+B Test (Quidel)	NP Swab Nasal wash, swab, and aspirate	Influenza A: 26-73 Influenza B: ND	Influenza A: 98-100 Influenza B: ND	41, 155
Sofia Influenza A+B (Quidel)	Nasal wash NP wash, swab, and aspirate	Influenza A: 82-83 Influenza B: 78	Influenza A: 100 Influenza B: 100	108, 166
BD Veritor System for Rapid Detection of Flu A+B (Becton Dickinson)	NP swab Nasal swab	Influenza A: 70-85 Influenza B: 47-93	Influenza A: 96-99 Influenza B: 99-100	13, 78, 166
Xpect Flu A&B (Remel)	Nasal wash and swab Throat swab	Influenza A: 48 Influenza B: 20	Influenza A: 98 Influenza B: 100	38
Respiratory Syncytial Virus				
BinaxNOW RSV (Alere)	Nasal wash, swab	67-90	93-100	37, 70, 111, 118, 128, 167, 181, 191
Quickvue RSV (Quidel)	NP wash, swab, and aspirate	83-91	88-92	40, 100
Directigen EZ RSV (BD)	NP wash, swab, and aspirate, tracheal aspirates	80-90	90-94	7, 181
3M Rapid Detection RSV (3M)	NP swab, aspirate, or nasal wash	64-85	93-100	70, 167, 192, 213
TRU RSV (Meridian)	NP aspirate, swab, nasal swab, or wash	60-90.7	91.7-100	18, 86, 118, 191, 193
Xpect RSV (Remel)	NP swab, aspirate, or wash	87	100	86

*Performance characteristics were documented in publications between 2007 and 2012, for pediatric patients where data were available.
ND, Not done.

adenovirus 40 and 41 in stool specimens is available and yields a reported sensitivity, specificity, positive predictive value, and negative predictive value of 96, 96, 95, and 96 percent, respectively.[84,96,216]

Nucleic Acid Detection

Molecular methods for the detection, identification, and characterization of microorganisms are being adapted increasingly for use in the clinical laboratory. These assays provide a timely and specific diagnosis, although the cost of reagents can range from $30 to more than $200 per test. Nucleic acids may be detected either directly in clinical specimens via labeled DNA or RNA probes and hybridization or after amplification of selected nucleic acid targets and detection of the amplified product. Direct hybridization formats are no longer commonly employed in clinical laboratories and are not discussed herein. In contrast, NAATs are now widely used in virology laboratories of academic centers for routine diagnosis of viral infections. In these settings, NAATs were first developed in-house primarily for the identification of viruses that are not readily cultured (e.g., HSV from CSF) and for those in which time to detection by traditional means is too long to affect patient care (e.g., CMV). The past 5 years have witnessed an explosion in the number of NAATs for the rapid detection of all viruses commonly encountered in clinical practice. However, at the time of writing, the majority of laboratories continue to use suboptimal antigen tests for rapid viral diagnosis, in large part because of the cost of reagents and lack of expertise for molecular methods. However, contrary to this belief, when all costs are accounted for, including length of stay, frequency of diagnostic testing, and antibiotic use, diagnosis of respiratory viruses in children by a multiplex molecular method has been shown to be associated with cost savings equaling the cost of the assay or higher.[51,119]

Increased use of molecular testing for the diagnosis of viral infections has stimulated several recent developments, including automation of nucleic acid extraction, amplification, detection, and reporting and interpretation of results. Automation of specimen processing significantly improves extraction by rapidly and efficiently providing standardized, reproducible nucleic acids for amplification, comparable to those obtained with more labor-intensive manual methods.[52,135] Amplification and detection are integrated in real-time PCR and occur simultaneously, in contrast to conventional PCR, in which these processes are separate. Such systems also significantly reduce the opportunity for amplicon contamination. Finally, increased availability of commercial, mid- to low-complexity cartridge-based NAATs may facilitate molecular testing in smaller laboratories situated in community hospitals, emergency departments, or clinics, for rapid, near point-of-care testing.

In most cases, NAATs offer enhanced sensitivity and specificity over the other assays described in this chapter. However, clinical specimens sometimes contain inhibiting substances that interfere with the performance of NAATs. Internal control nucleic acids, generally in the form of bacteriophages or murine viruses, may be spiked into clinical specimens to monitor for inhibition. Additional limitations of NAATs are the potential for sample to sample or amplicon cross contamination, leading to false-positive results and the inability to distinguish between actively replicating virus and residual viral nucleic acids.

NAATs approved or cleared by the U.S. Food and Drug Administration (FDA) at the time of writing are discussed in the following sections with respect to their application for the diagnosis and monitoring of certain viral infections. The analytical and clinical performance characteristics of these tests are well defined, and these assays undergo routine quality assurance monitoring. Because the list of FDA-approved/cleared molecular assays is ever expanding, readers are directed to the Association of Molecular Pathology website* for a comprehensive up-to-date list of FDA-approved/cleared tests. In addition, laboratories may develop NAATs through use of analyte-specific reagents that are widely available for a variety of viral targets. These laboratory-developed tests can then be validated for clinical use in accordance with CLIA requirements, which must include assessment of accuracy or bias, precision, linearity, limit of detection, interfering substances, and stability. The sensitivity and specificities of these laboratory developed assays will vary across laboratories, and thus results from different laboratories cannot be used for comparison.

Single-Analyte Nucleic Acid Amplification Tests

Many single-analyte NAATs are available for detection of viruses in clinical specimens; these are listed in Table 252-4. NAATs are particularly useful for establishing the diagnosis when culture is too insensitive, lengthy, difficult, impractical, or unavailable. For example, NAATs on CSF are now the procedure of choice for diagnosis of HSV encephalitis,[194,197] despite the fact that no FDA-approved assays for the detection of HSV in CSF are currently available. Regardless, laboratory-developed CSF PCRs are commonly used for diagnosing HSV encephalitis, with reported sensitivity and specificity greater than 97 percent. These PCR-based methods are compared to a combination of traditional methods along with clinical observation and radiodiagnostic studies for case definition, because HSV rarely is recovered from CSF in culture. Further, PCR may be used for diagnosis even after start of acyclovir treatment. HSV can be detected by PCR of CSF in 98 percent of cases after 7 days therapy, 47 percent after 14 days, and 21 percent after more than 14 days.[167] Commercial HSV NAATs (Table 252-4) are also commonly used for detection of HSV DNA in skin and genital lesions and are associated with enhanced sensitivity in contrast to culture. NAATs are able to identify asymptomatic HSV, detect the presence of HSV for a longer period (in older lesions and even fixed tissue), and have greater diagnostic value than culture and clinical observations for assessing genital

*Association of Molecular Pathology website: http:www.amp.org/FDATable/FDATable.doc.

TABLE 252-4 **Comparison of Select Single-Analyte and Multiplexed Nucleic Acid Amplification Tests the Qualitative Detection of Viral Targets**

Assay (Manufacturer)	Targets	Extraction	Method*	Approved Specimens	Time to Results
Single-Analyte NAATs					
ProAdenoTM+ Assay (Gen-Probe)	Adenovirus	Offline	RT-PCR and Taqman chemistry	Nasopharyngeal swab	4 hr
NucliSens EasyQ Enterovirus (bioMerieux)	Enterovirus	Offline	Nucleic acid sequence–based amplification (NASBA)	Cerebrospinal fluid	4 hr
Xpert EV (Cepheid)	Enterovirus	Online	RT-PCR and Taqman chemistry	Cerebrospinal fluid	2.5 hr
APTIMA HCV RNA (Gen-Probe)	HCV	N/A	Transcription-mediated amplification (TMA) and hybridization protection assay	Plasma Serum	3 hr
AMPLICOR HCV (Roche)	HCV	Online	RT-PCR	Plasma Serum	6 hr
BD ProbeTec Herpes Simplex Virus	HSV 1 & 2	Online	Strand displacement amplification (SDA)	Anogenital lesions on patients >17 years	3 hr
IsoAMP HSV (BioHelix)	HSV 1 & 2	N/A	Helicase-dependent amplification (HDA)	Genital lesions Oral lesions	1.5 hr
MultiCode-RTx Herpes Simplex 1 & 2 (Luminex)	HSV 1 & 2	Offline	RT-PCR and Multicode chemistry	Vaginal lesions on patients >17 years	4 hr
Pro hMPV (Gen-Probe)	Human Metapneumovirus	Offline	RT-PCR and Taqman chemistry	Nasopharyngeal swab	4 hr
hMPV Assay (Quidel)	Human Metapneumovirus	Offline	RT-PCR and Taqman chemistry	Nasopharyngeal swab Nasal swab	3 hr
Multiplexed NAATs					
ProFlu+ Assay (Gen-Probe)	Influenza A Influenza B RSV	Offline	RT-PCR and Taqman chemistry	Nasopharyngeal swab	4 hr
ProFAST+ (Gen-Probe)	Influenza A H1 Influenza A/H3 2009 H1N1 Influenza	Offline	RT-PCR and Taqman chemistry	Nasopharyngeal swab	4 hr
Quidel Molecular Influenza A+B	Influenza A Influenza B	Offline	RT-PCR and Taqman chemistry	Nasopharyngeal swab Nasal swab	3 hr
Simplexa FluA/B & RSV (Focus Diagnostics)	Influenza A Influenza B RSV	Offline	RT-PCR and Scorpion Probes	Nasopharyngeal swab	3 hr
Simplexa FluA/B & RSV Direct (Focus Diagnostics)	Influenza A Influenza B RSV	Online	RT-PCR and Scorpion Probes	Nasopharyngeal sab	1.5 hr
Simplexa Influenza A H1N1 (2009) (Focus Diagnostics)	Influenza A Influenza A/H1N1	Offline	RT-PCR and Scorpion Probes	Nasopharyngeal swab	3 hr
Verigene Respiratory Virus Plus (Nanosphere)	Influenza A Influenza A/H1 Influenza A/H3 2009 H1N1 Influenza B RSV A RSV B	Online	Proprietary gold nanoparticle hybridization	Nasopharyngeal swab	3.5 hr
Xpert Flu (Cepheid)	Influenza A Influenza A 2009 Influenza B	Online	RT-PCR with Molecular Beacons	Nasal aspirate, wash, Nasopharyngeal swab	1.5 h

*For details on test method, refer to the text.

HSV and treatment efficacy in patients with recurrent genital HSV.[32,45,83,136,137] NAATs also are useful for diagnosing ocular infections caused by specific viruses and supporting information in the clinical diagnosis of specific ocular disease syndromes.[94,219]

Similar to HSV, enterovirus is now commonly detected from CSF by NAAT because of slow growth or failure of certain serotypes to grow in culture. The sensitivity of the commercial enterovirus NAATs is 83 to 100 percent, whereas culture of CSF yields an isolate in only 36

percent of patients with enteroviral meningitis.[141] Xpert EV is a cartridge-based assay designed to be run on demand, in emergency departments, yielding results in a time frame suitable for making medical management decisions. Such assays are particularly helpful as CSF evaluation for pleocytosis is not always reliable for diagnosis of enteroviral meningitis because specimens with no pleocytosis may be culture-positive or PCR-positive.[168,172] The Xpert EV is associated with a sensitivity of 82 to 100 percent in contrast to laboratory developed assays.[42,139,141] A second commercial enterovirus NAAT licensed for use with CSF is the NucliSENS EasyQ Enterovirus assay (bioMeriuex), which is associated with similar sensitivity of 88 to 89 percent.[25] In contrast to the Xpert assay, which is an RT-PCR assay with real-time detection using Taqman probes, the EasyQ assay amplifies target enteroviral RNA by nucleic acid sequence–based amplification (NASBA). NASBA amplifies RNA under isothermal conditions, via simultaneous reverse transcription and T7 RNA polymerase to generate RNA copies of the enterovirus target. As the EasyQ enterovirus assay requires offline extraction of viral RNA from CSF and requires at least 5 hours to results, it is more suited to a central laboratory that is capable of performing high-complexity testing.

Single-analyte NAATs have been used for the qualitative detection of CMV, VZV, EBV, HBV, and human metapneumovirus from a variety of specimens. See Espy and colleagues[54] for a complete review.

Multiplexed Assays

Multiplexed assays for the detection of influenza A, influenza B, and RSV are listed in Table 252-4. These assays have the potential to replace DFA and rapid antigen tests as a sensitive and specific means by which to rapidly detect these viruses. The ProFAST, Simplexa Influenza A H1N1 (2009), Xpert Flu, and Verigene Respiratory virus Plus assays have the added benefit of providing a differentiation between the influenza A virus subtypes; this feature may aid in the recognition of new influenza A virus. All of the assays described in this section have excellent performance, with published sensitivities and specificities of more than 95 percent for their specific targets,* when performed per manufacturer instructions. The ProFAST (Gen-Probe) assays are multiplex real-time RT-PCR for the qualitative detection and discrimination of seasonal influenza A/H1, A/H3, and 2009 H1N1 viral nucleic acids. The assay targets the hemagglutinin gene and does not detect influenza B or C. In contrast, the ProFlu+ (Gen-Probe) assay targets the matrix gene for influenza A, the nonstructural genes NS-1 and NS-2 from influenza B, and the polymerase gene of RSV A and RSV B. In both assays, specimen is processed on an off-line nucleic acid extractor, and NAAT is performed on a Cepheid SmartCycler II instrument. The assay is based on Taqman chemistry, in which a target-specific probe with a 5′ fluorophore and a 3′ fluorescence resonance energy transfer (FRET) quencher anneals to the viral cDNA. While the probe is intact, fluorescence is quenched by FRET by the quencher molecule. However, during DNA amplification, the 5′ to 3′ exonuclease activity of Taq DNA polymerase cleaves the fluorophore from the probe, displacing it from the quencher, and allowing fluorescence. Assays for the detection of human metapneumovirus (Pro hMPV+), adenovirus (ProAdeno+), and parainfluenza (ProParaflu+) are also available from the Gen-Probe Prodesse line of assays. Like the Prodesse assays, the Quidel Molecular Influenza A+B uses Taqman chemistry in a real-time RT-PCR assay for the detection of influenza A and B.

The Verigene system is based on RT-PCR amplification of viral target nucleic acid, followed by detection via Nanosphere's proprietary gold nanoparticle hybridization technology. Amplicons from RT-PCR are hybridized to a microarray followed by silver-coated gold nanoparticle probe hybridization. Product is detected by light-scattering off the nanoprobes. Advantages of the test include minimal number of manipulations required by the technologist, for example, pipetting of specimens to the test cartridge at the start of the assay and removal to the reader at the end of the assay. However, throughput is low, with only one sample per processer. A second assay available as a sample-in, result-out format is the Simplexa Flu A/B & RSV Direct, a real-time RT-PCR assay performed on the 3M Integrated Cycler. This assay can be run in batches of eight samples per run. Also available is the Simplexa Flu A/B & RSV, in which extraction must be performed off-line, but which has a throughput of up to 96 samples. The Simplexa assays are based on scorpion probe chemistry. Scorpion probes consist of a 5′ fluorophore and 3′ quencher, which are held in close proximity by complementary sequences on the 5′ and 3′-end of the probe, resulting in a hairpin loop configuration. After PCR amplification, the probe sequence binds to its complement on the amplicon and this hybridization opens the hairpin loop such that fluorescence is no longer quenched. Sensitivity of the Simplexa Flu A/B & RSV Direct assay was reported for influenza A as 82 to 96 percent, influenza B, 76 to 99 percent, and RSV 95 to 98 percent.[5,179] Specificity is greater than 99 percent for all three targets.

Finally, the Cepheid Xpert Flu is a cartridge-based assay that uses real-time PCR with molecular beacon detection and is performed on the GeneXpert instrument. The assay is low-complexity, with less than 15 minutes hands-on time required to pipette the sample into the cartridge and start the assay; results are available in under 1 hour.

Highly Multiplexed Assays

Until recently, NAATs for infectious diseases testing were relatively simple, detecting at most eight targets because of limitations in specificity and detection of multiple analytes. However, in recent years, highly multiplexed NAATs have been developed for the detection of a wide variety of viruses that might be present in a single clinical sample, providing the key advantage of traditional culture. These assays also can detect multiple viral agents in a single specimen. These assays are best suited to sample

*References 5, 14, 46, 143, 157, 179, 180, 188.

types in situations in which a wide range of viruses may cause similar symptoms, such as with respiratory tract infections or gastroenteritis. To date, the highly multiplexed assays have been approved for use with respiratory specimens. The first diagnostic virus panel approved for use by the FDA is the xTAG RVP. The following virus types and subtypes are identified by the RVP: influenza A, influenza A subtype H1, influenza A subtype H3, influenza B, RSV A, RSV B, parainfluenza 1, parainfluenza 2, and parainfluenza 3 virus, hMPV, rhinovirus, and adenovirus. Viral nucleic acids are coextracted from nasopharyngeal swab specimens spiked with an internal control (MS2 phage), using an off-line automated extractor. Multiplex RT-PCR is then performed using primers specific to the viral targets and the control; these primers contain a tag sequence for target-specific primer extension (TSPE). The target-specific primers are then extended through addition of biotinylated deoxycytidine triphosphate to the reaction mixture, which is incorporated into the TSPE products. Following TSPE, products are mixed with color-coded microspheres with anti-tags. The anti-tag binds the tags present on the TSPE products, hybridizing the amplicons to the beads. The beads are read via solution-based microarray on the Luminex xMAP system by two lasers—the first identifies the virus-specific color-coded bead, the second determines whether amplicon is hybridized to the bead. The clinical performance of the xTAG RVP is well established (Table 252-5), but the assay has several limitations. Sensitivity for certain adenoviruses (species C, serotypes 7a and 41) is poor, and primers for detection of rhinovirus cross react with enterovirus. Furthermore, sensitivity for novel influenza A viruses is unknown. A second key limitation of this test is the risk for false-positive findings as a result of cross contamination by amplified nucleic acids, which are pipetted manually between stages of the assay. Additional limitations include time-to-results, which is 8 to 10 hours, and technical hands-on time. A second generation of the RVP was released in 2011, RVP FAST, which detects fewer analytes than the xTAG RVP, but has a

TABLE 252-5 **Comparison of the Highly Multiplexed Assays for the Detection of Respiratory Viruses**

Test	Method	Specimen	Time to Result	Targets	Sensitivity	Specificity	Reference
xTAG RVP (Luminex)	End-point RT-PCR with microsphere-based detection	NP swab	8-10 hr	Adenovirus Influenza A Influenza A H1 Influenza A H3 Influenza B PIV-1 PIV-2 PIV-3 hMPV Rhinovirus RSV A RSV B	58-100 98-100 94-100 71 100 71 86-97 91-94 82-86 98	99-100 100 100 99-100 99-100 99-100 99-100 96-100 100 100	67, 147, 164
xTAG RVP FAST (Luminex)	Same as xTAG RVPv1	NP swab	3-5 hr	Adenovirus Influenza A Influenza A H1 Influenza A H3 Influenza B RSV hMPV Rhinovirus	62-100 69-95 50 100 41-90 60-95 89-100 89-98	100 99-100 100 100 100 99-100 100 92-100	10, 62, 67, 99, 148
FilmArray Respiratory Panel (BioFire Diagnostics)	Onboard nucleic acid extraction followed by RT-PCR and detection	NP swab	1 hour	Adenovirus Coronavirus NL63 Coronavirus 229E Coronavirus OC43 Coronavirus HKU1 hMPV Rhino/enterovirus Influenza A Influenza A/H1 Influenza A/H3 Influenza A/H1-2009 Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 RSV	45-100 100 100 100 100 80-100 91.6 97-100 100 88-100 94-100 88-100 94-100 88-100 94-100 33 100	100 99 99 99 100 100 96-98 100 100 100 100 100 99 100 100 96	10, 114, 164

streamlined protocol with fewer manual steps and a turnaround time of 3 to 4 hours. The RVP FAST detects influenza A, influenza A subtype H1, influenza A subtype H3, influenza B, RSV, hMPV, rhinovirus, and adenovirus. The RVP FAST assay has reduced sensitivity for the detection of influenza B, possibly as a result of sequence variation in the primer binding region of the hemagglutinin gene among various strains.[148] The RVP FAST is also less sensitive for the detection of parainfluenza, and for this reason the target is not included in the FDA-approved version of the assay.

The FilmArray respiratory viral panel (Idaho Technology) is a fully closed system with a specimen-in, result-out format.[158] This sealed system minimizes the contamination concerns associated with the xTAG RVP assays. Viral nucleic acids are detected by a nested multiplex PCR; product is detected using end-point melting curve data on the FilmArray instrument. The assay requires little hands-on time, and results are available in just over 1 hour. A significant drawback of the system is the throughput, because only one sample can be processed on the instrument at a time. Thus, larger laboratories may need multiple instruments to meet test volume. The FilmArray is cleared for the detection of 15 viruses and subtypes, including those in the xTAG RVP in addition to human coronaviruses NL63, HKU1, 229E, OC43, and parainfluenza 4. Similar to the xTAG RVP assays, detection of adenovirus is problematic,[35,113,154] particularly species C; serotypes 2 and 6 are poorly detected (see Table 252-4).

Viral Nucleic Acid Quantitation Assays

Quantitative NAATs (qNAATs) play an important role in the diagnosis and management of viral disease. Viral load assays are used to predict disease progression, distinguish symptomatic from asymptomatic infection, monitor the development of antiviral resistance, and assess the efficacy of antiviral therapy. Table 252-6 lists the FDA-approved quantitative molecular assays currently available.

qNAAT assays for the management of HIV were the first to be used routinely for the early detection of infection, resolution of indeterminate Western blot results, evaluation of suspected prenatal and intrapartum infection, and monitoring of the viral load for prognosis and evaluation of therapeutic efficacy. The goal of antiretroviral therapy is a reduction of HIV-1 RNA below the limits of detection of the more sensitive assays (<20 to 75 cp/mL).[149,198,214] Detection and quantitation of serum HBV DNA can assist in diagnosis of early acute infection, distinguish active from inactive infection, and monitor response to antiviral therapy. In early, acute infection in which HBsAg is not detectable or yields equivocal results, detection of HBV DNA may be used to make the diagnosis. Viral load can predict the course of chronic HBV infection as well. Viral loads are high in chronic active, replicative infection in contrast to low or undetectable in chronic inactive infection. The aim of antiviral therapy is long-term suppression of viral replication; increasing HBV DNA levels may signal emergence of drug-resistant

TABLE 252-6 **U.S. Food and Drug Administration–Approved Quantitative Molecular Assays**

Target	Specimen	Linear Range	Method	Test Name	Manufacturer
HBV	Plasma, serum	10-10^9 IU/mL	Real-time PCR	Abbott Real-time HBV	Abbott Molecular, Inc., Des Plaines, Ill
	Plasma, serum	20-1.7 × 10^8 IU/mL	Real-time PCR	COBAS AmpliPrep/ COBAS TaqMan HBV Test v2.0	Roche Molecular Diagnostics Pleasanton, Calif
HCV	Plasma, serum	12-1.0 × 10^8 IU/mL	Real-time RT-PCR	Abbott Real-time HCV	Abbott Molecular, Inc., Des Plaines, Ill
	Serum	615-7.69 × 10^6 IU/mL	bDNA	VERSANT HCV RNA 3.0 Assay	Siemens Healthcare Diagnostics, Deerfield, Ill
	Plasma, serum	43-6.9 × 10^7 IU/mL	Real-time RT-PCR	COBAS AmpliPrep/ COBAS TaqMan HCV Test v2.0	Roche Molecular Diagnostics Pleasanton, Calif
HIV	Plasma	40-1.0 × 10^7 cp/mL	Real-time RT-PCR	Abbott Real-time HIV-1	Abbott Molecular, Inc., Des Plaines, Ill
	Plasma	176-3.47 × 10^6 cp/mL	NASBA	NucliSens HIV-1 QT	bioMerieux, Inc., Durham, NC
	Plasma	20-1.0 × 10^7 cp/mL	Real-time RT-PCR	COBAS AmpliPrep/ COBAS TaqMan HIV-1 Test v2.0	Roche Molecular Diagnostics, Pleasanton, Calif
	Plasma	75-5.0 × 10^5 cp/mL	bDNA	VERSANT HIV-1 RNA 3.0 Assay	Siemens Healthcare Diagnostics, Deerfield, Ill
CMV	Plasma	150-1.0 × 10^7 cp/mL	Real-time PCR	COBAS AmpliPrep/ COBAS TaqMan CMV Test	Roche Molecular Diagnostics, Pleasanton, Calif

CMV, Cytomegalovirus; *HBV,* hepatitis B virus; *HCV,* hepatitis C virus; *HIV,* human immunodeficiency virus; *PCR,* polymerase chain reaction; *RT-PCR,* reverse-transcriptase PCR.

strains.[173] Detection and quantitation of hepatitis C virus (HCV) RNA is useful for establishing the diagnosis of acute hepatitis before seroconversion, for detection of chronic HCV in seronegative patients, for resolution of indeterminate serologic results, and to monitor patients for disease progression and response to therapy.* qNAATs are essential for management of chronic HCV. Viral load and genotype determine the likelihood of sustained viral response (SVR) and potential duration of treatment. Response to treatment is measured by achievement of SVR, defined as the absence of demonstrable viral replication 6 months after completion of a full course of treatment.

As is typical of other herpes group viruses, CMV possesses a high prevalence rate, establishes latency, and although usually asymptomatic in the normal host, causes disease in immunologically compromised hosts. Transplant recipients, patients with HIV or acquired immunodeficiency syndrome (AIDS), patients with cancer, and newborns are usually at highest risk for severe disease. Optimal patient management relies on laboratory testing. Quantitative assays are useful for early detection of CMV infection, differentiating latent infection from active disease, predicting disease progression, and monitoring response to antiviral therapy.[53,66,210,222] In general, higher viral loads correlate with risk for disease development and lower levels are associated with asymptomatic infection. Recommendations for serial monitoring using qNAATs have been published.[6,8,104] Generally, threefold to fivefold changes in viral load may represent a biologically significant change in viral replication. Viral load cutoffs correlate with change in patient management across transplant groups and laboratories because of variations in assay design and a former lack of a reference standard. The availability of an FDA-approved assay calibrated against the WHO international standard should clarify interpretation of results and aid in the establishment of significant viral load cutoffs within different immunosuppressed populations.

FDA-approved qNAAT assays do not exist for Epstein-Barr virus (EBV), BKV, and adenoviruses; however, quantitation of these viruses is routinely performed. As mentioned earlier, laboratory developed tests vary in assay design and thus yield results that cannot be compared across laboratories. No standardized cutoff values have been determined for the purpose of clinical management. Differences include specimen type and volume, nucleic acid extraction method, PCR primers and probes, amplification chemistry, calibrators used to create standard curves, and result reporting (copies per milliliter, copies per PCR, and copies per DNA concentration).

Conventional diagnostic methods generally are not useful in the evaluation of EBV-related disorders in immunosuppressed patients. Culture is not practical or useful, and serology is difficult to interpret in the setting of immunosuppression. PCR offers the possibility of rapid detection of EBV in a variety of clinical specimens and allows a semiquantitative or quantitative estimate of viral load. PCR has been used for establishing the diagnosis of AIDS-related central nervous system lymphoma,[66] EBV-related lymphoproliferative disorders,* and other EBV-associated diseases (e.g., infectious mononucleosis, fatal infectious mononucleosis, chronic active EBV infection, and EBV-associated hemophagocytic syndromes) in which increased DNA concentrations are associated with more severe clinical categories.[218] PCR is particularly useful for the early identification and diagnosis of lymphoproliferative disorders (e.g., posttransplant lymphoproliferative disorder [PTLD]) in pediatric patients after they have undergone liver transplantation, for monitoring EBV levels so that immunosuppression can be adjusted, and as a prognostic marker.† Viral load measurement of EBV is routinely used to diagnose or predict impending PTLD. High levels are nearly always associated with PTLD and increasing DNAemia predicts impending PTLD before appearance of signs and symptoms. Significant cutoffs range from 10^2 to 10^4 for serum and plasma and 10^3 to 10^4 for whole blood.[80]

Primary infection with BKV typically occurs in childhood and usually results in chronic, asymptomatic infection in immunocompetent individuals. Whereas clinical disease is rare in immunocompetent adults, BKV infections are a significant cause of morbidity and mortality in immunosuppressed patients, particularly in renal transplant recipients and those with hematologic malignancies. Disease manifestations in renal transplants include BKV nephropathy (BKVN), which may result in renal dysfunction and graft loss. Asymptomatic hematuria, hemorrhagic cystitis, and renal impairment may occur in bone marrow transplant recipients and patients with hematologic malignancy. BKVN affects 1 to 10 percent of renal transplant recipients with frequencies of graft loss between 10 to 80 percent within 5 years. BKV quantitative PCR can facilitate early diagnosis of BKVN, guide management of immunosuppressive therapy, and monitor response to intervention. Prospective studies have shown that high level BKV viruria (1 to 3 months after transplant) precedes sustained viremia (1 to 12 weeks before PVAN [polyomavirus-associated nephropathy]), which in turn precedes allograft dysfunction. Reduction of immunosuppression or treatment with cidofovir when the viral load reaches a specific level has been associated with improved clinical outcomes. BKV quantitative cutoffs that indicated the need for renal biopsy were DNA loads greater than 10^7 copies/mL in urine or plasma loads greater than 10^4 that persist for longer than 3 weeks.[15,107]

Human adenoviruses are associated with a variety of diseases affecting all organ systems. Most disease in immunocompetent individuals is self-limiting; however, severe or fatal disseminated disease may occur in bone marrow, stem cell, or solid organ transplant recipients. Treatment includes administration of cidofovir and reduction of immunosuppression. Viral load testing is increasingly being used to predict disease dissemination (>10^4 copies/mL) and monitor antiviral therapy, although viral load thresholds are still to be determined.[65]

*References 21, 116, 142, 170, 174, 182, 220.

*References 36, 102, 121, 138, 189, 195.
†References 36, 80, 102, 121, 138, 189, 195.

SUMMARY

Methods for the laboratory diagnosis of viral infections, including culture, SVA, antigen detection, and molecular diagnostic methods, have been reviewed. The use of these tests in some combination is most practical because a single test may not yield a diagnosis. For many viruses, culture has been the gold standard or reference method for evaluation of the new, rapid tests. However, culture is neither 100 percent sensitive nor always diagnostic of symptomatic infection. With the newer methods, particularly molecular diagnostic methods, the gold standard is changing, and evaluation of new techniques must be compared against a spectrum of laboratory and clinical data. Standards for performance and guidelines for the use and interpretation of molecular techniques are becoming available. Quantitative (vs. qualitative) viral results may be useful for interpreting tests, particularly with regard to viruses causing latent infection or for monitoring therapy or disease progression. Finally, interpretation of any result requires integration of the clinical history, laboratory data, treatment records, and observation of trends over time.

NEW REFERENCES SINCE THE SIXTH EDITION

1. Abu-Diab A, Azzeh M, Ghneim R, et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. *J Clin Microbiol* 2008;**46**:2414–17.
3. Alby K, Popowitch EB, Miller MB. Analytical performance of the nanosphere verigene RV+ assay and the Focus Simplexa Flu A/B & RSV Kit. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
5. Aslanzadeh J, Zheng X, Li H, et al. Prospective evaluation of rapid antigen tests for diagnosis of respiratory syncytial virus and human metapneumovirus infections. *J Clin Microbiol* 2008;**46**:1682–5.
6. Atkinson C, Emery VC. Cytomegalovirus quantification: where to next in optimising patient management? *J Clin Virol* 2011; **51**:223–8.
8. Babady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast Assay and the Idaho Technology FilmArray RP Assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J Clin Microbiol* 2012;**50**:2282–8.
11. Barger M, Vestal D, Nye M, et al. Evaluation of the BD Veritor (TM) Flu A/B assay compared to R-Mix/Mink Lung (ML) Cell culture, BD Directigen (TM) EZ Flu A/B and PCR for the detection of influenza A and B viruses. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
12. Barney T, Poppe H, Western M, et al. Performance of a novel multiplexed molecular assay for the detection of influenza A and influenza B on two real-time amplification Platforms. 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
13. Bechert CJ, Schnadig VJ, Payne DA, et al. Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. *Am J Clin Pathol* 2010;**133**:242–50.
15. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
16. Brandt K, Beck J, Shchyschuk L, et al. Respiratory syncytial virus detection by Tru RSV, Binax NOW RSV, direct immunofluorescent staining and tissue culture. Paper presented at the Canadian Academy of Clinical Microbiology and Infectious Diseases (CACMID), Toronto, On, 2009.
18. Burd EM. Validation of laboratory-developed molecular assays for infectious diseases. *Clin Microbiol Rev* 2010;**23**:550–76.
19. Butt AA, Kanwal F. Boceprivir and telaprevir in the management of hepatitis C virus-infected patients. *Clin Infect Dis* 2012;**54**: 96–104.
20. Caceda ER, Kochel TJ. Application of modified shell vial culture procedure for arbovirus detection. *PLoS One* 2007;**2**:e1034.
21. Capaul SE, Gorgievski-Hrisoho M. Detection of enterovirus RNA in cerebrospinal fluid (CSF) using NucliSens EasyQ Enterovirus assay. *J Clin Virol* 2005;**32**:236–40.
22. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) Virus: United States, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:826–9.
23. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for influenza A (H3N2) virus and updated case count: United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:619–21.
24. Chan KH, Peiris JSM, Lim W, et al. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. *J Clin Virol* 2008;**42**:65–9.
33. Couturier MR. Validation of the Idaho Technology Inc FilmArray Respiratory Panel for clinical use at Primary Children's Medical Center, Salt Lake City Utah. Paper presented at the 27th Annual Clinical Virology Symposium. Daytona, Fla; 2011.
35. Cruz AT, Cazacu AC, Greer JM, et al. Performance of a rapid assay (Binax NOW) for detection of respiratory syncytial virus at a children's hospital over a 3-year period. *J Clin Microbiol* 2007; **45**:1993–5.
36. Cruz AT, Cazacu AC, Greer JM, et al. Rapid assays for the diagnosis of influenza A and B viruses in patients evaluated at a large tertiary care children's hospital during two consecutive winter seasons. *J Clin Virol* 2008;**41**:143–7.
37. Cruz AT, Demmler-Harrison GJ, Caviness AC, et al. Performance of a rapid influenza test in children during the H1N1 2009 influenza A outbreak. *Pediatrics* 2010;**125**:e645–50.
38. Dale SE, Mayer C, Mayer MC, et al. The performance characteristics of the Quidel QuickVue® RSV Test for nasal wash specimens collected from three different clinical sites. Paper presented at the 23rd Annual Clinical Virology Symposium and Annual Meeting Pan American Society for Clinical Virology. Clearwater, Fla; 2007.
39. Dale SE, Mayer C, Mayer MC, et al. Analytical and clinical sensitivity of the 3M rapid detection influenza A+B assay. *J Clin Microbiol* 2008;**46**:3804–7.
40. de Crom SC, Obihara CC, van Loon AM, et al. Detection of enterovirus RNA in cerebrospinal fluid: comparison of two molecular assays. *J Virol Methods* 2012;**179**:104–7.
41. De Paulis M, Gilio AE, Ferraro AA, et al. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011;**87**:307–13.
44. Dimaio MA, Sahoo MK, Waggoner J, et al. Comparison of Xpert Flu rapid nucleic acid testing with rapid antigen testing for the diagnosis of Influenza A and B. *J Virol Methods* 2012;**186**: 137–40.
48. Druce J, Garcia K, Tran T, et al. Evaluation of swabs, transport media, and specimen transport conditions for optimal detection of viruses by PCR. *J Clin Microbiol* 2012;**50**:1064–5.
49. Dundas NE, Ziadie MS, Revell PA, et al. A lean laboratory: operational simplicity and cost effectiveness of the Luminex xTAG respiratory viral panel. *J Mol Diagn* 2011;**13**:175–9.
50. Dunn JJ, Billetdeaux E, Skodack-Jones L, et al. Evaluation of three Copan viral transport systems for the recovery of cultivatable, clinical virus isolates. *Diagn Microbiol Infect Dis* 2002;**45**: 191–7.
56. Faix DJ, Sherman SS, Waterman SH. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;**361**:728–9.
58. Filen F, Strand A, Allard A, et al. Duplex real-time polymerase chain reaction assay for detection and quantification of herpes simplex virus type 1 and herpes simplex virus type 2 in genital and cutaneous lesions. *Sex Transm Dis* 2004;**31**:331–6.
60. Forbes BA. Introducing a molecular test into the clinical microbiology laboratory: development, evaluation, and validation. *Arch Path Lab Med* 2003;**127**:1106–11.
62. Gadsby NJ, Hardie A, Claas EC, et al. Comparison of the Luminex Respiratory Virus Panel fast assay with in-house real-time PCR for respiratory viral infection diagnosis. *J Clin Microbiol* 2010; **48**:2213–16.
64. Garcia LS, editor. *Clinical microbiology procedure handbook*. Washington, D.C.: American Society for Microbiology; 2007.

65. Gazenmueller T, Heim A. Adenoviral load diagnostics by quantitative polymerase chain reaction: techniques and application. *Rev Med Virol* 2012;**22**:194–208.
67. Gharabaghi F, Hawan A, Drews SJ, et al. Evaluation of multiple commercial molecular and conventional diagnostic assays for the detection of respiratory viruses in children. *Clin Microbiol Infect* 2011;**17**:1900–6.
68. Gillim-Ross L, Taylor J, Scholl DR, et al. Discovery of novel human and animal cells infected by the severe acute respiratory syndrome coronavirus by replication-specific multiplex reverse transcription-PCR. *J Clin Microbiol* 2004;**42**:3196–206.
69. Ginocchio CC, Lotlikar M, Falk L, et al. Clinical performance of the 3M Rapid Detection Flu A+B Test compared to R-Mix culture, DFA and BinaxNOW Influenza A&B Test. *J Clin Virol* 2009;**45**:146–9.
70. Ginocchio CC, Swierkosz E, McAdam AJ, et al. Multicenter study of clinical performance of the 3M Rapid Detection RSV test. *J Clin Microbiol* 2010;**48**:2337–43.
71. Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J Clin Virol* 2009; **45**:191–5.
78. Griego-Fullbright C, Henderson K, Kindig S, et al. Performance of the BD Veritor (TM) assay for the simultaneous detection of influenza A and influenza B in respiratory specimens using an immunochromatrographic assay with an automated reader. Presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
79. Grijalva CG, Poehling KA, Edwards KM, et al. Accuracy and interpretation of rapid influenza tests in children. *Pediatrics* 2007; **119**:e6–11.
80. Gulley ML, Tang W. Using Epstein-Barr viral load assays to diagnose, monitor, and prevent posttransplant lymphoproliferative disorder. *Clin Microbiol Rev* 2010;**23**:350–66.
86. Hoffman J, Matthews E, Beckman K. Comparison of two rapid antigen tests for respiratory syncytial virus (RSV) in a community hospital laboratory. Paper presented 24th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology. Clearwater, Fla; 2009.
92. Hurt AC, Baas C, Deng YM, et al. Performance of influenza rapid point-of-care tests in the detection of swine lineage A(H1N1) influenza viruses. *Influenza Other Respi Viruses* 2009;**3**:171–6.
95. Jayakeerthi RS, Potula RV, Srinivasan S, et al. Shell vial culture assay for the rapid diagnosis of Japanese encephalitis, West Nile and dengue-2 viral encephalitis. *Virol J* 2006;**3**:2.
99. Jokela P, Piiparinen H, Mannonen L, et al. Performance of the Luminex xTAG Respiratory Viral Panel Fast in a clinical laboratory setting. *J Virol Methods* 2012;**182**:82–6.
100. Karnauchow TM, Milk R, Sullivan L. Performance of QuickVue(R) RSV for the detection of RSV in pediatric patients. Paper presented at 23rd Annual Clinical Virology Symposium. Clearwater, Fla; 2007.
104. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010;**89**:779–95.
107. Laskin BL, Goebel J. Cost-efficient screening for BK virus in pediatric kidney transplantation: a single-center experience and review of the literature. *Pediatr Transplant* 2010;**14**:589–95.
108. Lee CK, Cho CH, Woo MK, et al. Evaluation of Sofia fluorescent immunoassay analyzer for influenza A/B virus. *J Clin Virol*: 2012; **55**:239–43.
110. Leung J, Harpaz R, Baughman AL, et al. Evaluation of laboratory methods for diagnosis of varicella. *Clin Infect Dis* 2010;**51**:23–32.
111. Liao RS, Tomalty LL, Majury A, et al. Comparison of viral isolation and multiplex real-time reverse transcription-PCR for confirmation of respiratory syncytial virus and influenza virus detection by antigen immunoassays. *J Clin Microbiol* 2009;**47**: 527–32.
113. Loeffelholz M. Comparison of the FilmArray Respiratory Panel and Prodesse assays for the detection of respiratory pathogens. Paper presented at 27th Annual Clinical Virology Symposium. Daytona, Fla; 2011.
114. Loeffelholz MJ, Pong DL, Pyles RB, et al. Comparison of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory pathogens. *J Clin Microbiol* 2011; **49**:4083–8.
115. Lu X, Erdman DD. Molecular typing of human adenoviruses by PCR and sequencing of partial region of the hexon gene. *Arch Virol* 2006;**151**:1587–602.
118. Mack K, Salamon D, Marcon M. Evaluation of the TRU™ RSV Test for rapid detection of respiratory syncytial virus in pediatric patients. Paper presented at 24th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology. Clearwater, Fla; 2008.
119. Mahony JB, Blackhouse G, Babwah J, et al. Cost analysis of multiplex PCR testing for diagnosing respiratory virus infections. *J Clin Microbiol* 2009;**47**:2812–17.
128. Miernyk K, Bulkow L, DeByle C, et al. Performance of a rapid antigen test (Binax NOW(R) RSV) for diagnosis of respiratory syncytial virus compared with real-time polymerase chain reaction in a pediatric population. *J Clin Virol* 2011;**50**:240–3.
134. Munywoki PK, Hamid F, Mutunga M, et al. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real-time PCR using nasopharyngeal flocked swabs. *J Clin Microbiol* 2011;**49**:3365–7.
139. Ninove L, Nougairede A, Gazin C, et al. Comparative detection of enterovirus RNA in cerebrospinal fluid: GeneXpert system vs. real-time RT-PCR assay. *Clin Microbiol Infect* 2011;**17**:1890–4.
140. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J Clin Microbiol* 2006;**44**:2698–704.
141. Nolte FS, Rogers BB, Tang YW, et al. Evaluation of a rapid and completely automated real-time reverse transcriptase PCR assay for diagnosis of enteroviral meningitis. *J Clin Microbiol* 2011;**49**: 528–33.
143. Novak-Weekley SM, Marlowe EM, Poulter M, et al. Evaluation of the Cepheid Xpert Flu Assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses. *J Clin Microbiol* 2012;**50**:1704–10.
145. Ohm-Smith MJ, Nassos PS, Haller BL. Evaluation of the Binax NOW, BD Directigen, and BD Directigen EZ assays for detection of respiratory syncytial virus. *J Clin Microbiol* 2004;**42**:2996–9.
147. Pabbaraju K, Tokaryk KL, Wong S, et al. Comparison of the Luminex xTAG respiratory viral panel with in-house nucleic acid amplification tests for diagnosis of respiratory virus infections. *J Clin Microbiol* 2008;**46**:3056–62.
148. Pabbaraju K, Wong S, Tokaryk KL, et al. Comparison of the Luminex xTAG respiratory viral panel with xTAG respiratory viral panel fast for diagnosis of respiratory virus infections. *J Clin Microbiol* 2011;**49**:1738–44.
149. U.S. Department of Health and Human Services, Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services*. January 10, 2011; 1-166. Available at: http://www.aidsinfo.nih.gov/contentfiles/adultandadolescentGL.pdf
154. Pierce VM, Elkan M, Leet M, et al. Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. *J Clin Microbiol* 2012;**50**: 364–71.
155. Poeppl W, Herkner H, Burgmann H, et al. Performance of the QuickVue Influenza A+B rapid test for pandemic H1N1 (2009) virus infection in adults. *PLoS One* 2011;**6**:e28089.
156. Pollock NR, Duong S, Cheng A, et al. Ruling out novel H1N1 influenza virus infection with direct fluorescent antigen testing. *Clin Infect Dis* 2009;**49**:e66–8.
157. Popowitch EB, Rogers E, Miller MB. Retrospective and prospective verification of the Cepheid Xpert influenza virus assay. *J Clin Microbiol* 2011;**49**:3368–9.
158. Poritz MA, Blaschke AJ, Byington CL, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One* 2011;**6**:e26047.
163. Rabenau HF, Kessler HH, Kortenbusch M, et al. Verification and validation of diagnostic laboratory tests in clinical virology. *J Clin Virol* 2007;**40**:93–8.
164. Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. *J Clin Microbiol* 2011;**49**:2449–53.
166. Relich RF, Snyder C, Orwig K, et al. Comparison of four rapid lateral flow assays for detection of influenza A and B antigens in

stored samples. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona Fla; 2012.
167. Riley AM, Wong K, Williams L, et al. Evaluation of the 3M™ Rapid Detection RSV test and the BinaxNOW® RSV test for detection of respiratory syncytial virus in pediatric patients. Paper presented at the 25th Annual Clinical Virology Symposium. Daytona, Fla; 2009.
173. Schiff ER, editor. Optimizing management strategies in patients with chronic hepatitis B. *Am J Med* 2008;**121**:S1–2.
174. Schiff ER. Diagnosing and treating hepatitis C virus infection. *Am J Manag Care* 2011;**17**(suppl 4):S108–15.
177. Scoular A, Gillespie G, Carman WF. Polymerase chain reaction for diagnosis of genital herpes in a genitourinary medicine clinic. *Sex Transm Infect* 2002;**78**:21–5.
178. Self WH, McNaughton CD, Grijalva CG, et al. Diagnostic performance of the BinaxNow Influenza A&B rapid antigen test in ED patients. *Am J Emerg Med* 2012;**30**:1955–61.
179. Selvaraju S, Earley A, Graf P, et al. Evaluation of a novel Simplexa (TM) Influenza A/B & RSV Direct Assay for Laboratory Diagnosis of Influenza and RSV Viral Infections. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
180. Selvaraju SB, Selvarangan R. Evaluation of three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses. *J Clin Microbiol* 2010;**48**:3870–5.
181. Selvarangan R, Abel D, Hamilton M. Comparison of BD Directigen EZ RSV and Binax NOW RSV tests for rapid detection of respiratory syncytial virus from nasopharyngeal aspirates in a pediatric population. *Diagn Microbiol Infect Dis* 2008;**62**:157–61.
188. Stellrecht KA, Butt SA, Maceira VP, et al. Comparison of three commercial real-time PCR systems for the detection of respiratory viruses. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
191. Sullivan L, Kern C, Milk R, et al. Performance of Meridian TRU and Binax NOW assays for the detection of RSV in pediatric and adult patients. Paper presented at the 25th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology. Daytona, Fla; 2009.
192. Swierkosz E, Bonnot TJ. Clinical evaluation of the 3M(TM) Rapid Detection RSV Test in nasopharyngeal aspirate specimens. Paper presented at the 25th Annual Clinical Virology Symposium. Dayton, Fla; 2009.
193. Taggart EW, Petti CA, She RC. Comparison of direct fluorescent antibody to two rapid antigen tests for RSV and Influenza A and B. Paper presented at the 25th Annual Clinical Virology Symposium. Clearwater, Fla; 2009.
194. Tebas P, Nease RF, Storch GA. Use of the polymerase chain reaction in the diagnosis of herpes simplex encephalitis: a decision analysis model. *Am J Med* 1998;**105**:287–95.
197. Tyler KL. Update on herpes simplex encephalitis. *Rev Neurol Dis* 2004;**1**:169–78.
201. Vasoo S, Stevens J, Singh K. Rapid antigen tests for diagnosis of pandemic (Swine) influenza A/H1N1. *Clin Infect Dis* 2009;**49**:1090–3.
204. Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: American Society for Microbiology; 2011.
207. Walsh P, Overmyer CL, Pham K, et al. Comparison of respiratory virus detection rates for infants and toddlers by use of flocked swabs, saline aspirates, and saline aspirates mixed in universal transport medium for room temperature storage and shipping. *J Clin Microbiol* 2008;**46**:2374–6.
211. Weinmann S, Chun C, Mullooly JP, et al. Laboratory diagnosis and characteristics of breakthrough varicella in children. *J Infect Dis* 2008;**197**(suppl 2):S132–8.
213. Wilson DA, Mulherin P, Quimson L, et al. Clinical evaluation of the 3M(TM) Rapid Detection RSV Test. Paper presented at the 25th Annual Clinical Virology Symposium. Daytona, Fla; 2009.
214. Wilson LE, Gallant JE. HIV/AIDS: the management of treatment-experienced HIV-infected patients: new drugs and drug combinations. *Clin Infect Dis* 2009;**48**:214–21.

The full reference list for this chapter is available at expertconsult.com.

CHAPTER 253

Parasitic Laboratory Diagnosis

David A. Bruckner • Robyn Shimizu-Cohen

Parasitic infections usually are associated with tropical areas; however, many infections (*Dientamoeba fragilis*, *Entamoeba histolytica*, *Enterobius vermicularis*, *Giardia lamblia*, and *Toxoplasma gondii*) have worldwide distribution. With the ability of travelers to move freely throughout the world in a short time span, clinical laboratories must be able to recommend specimen collection and detection/identification methods for all types of parasitic infections. It is helpful if the physician informs the laboratory of the parasitic infection that is suspected or the travel history of the patient because some organisms are detected more easily using specific methods (e.g., *Giardia* and *Cryptosporidium*—enzyme immunoassay [EIA] or direct fluorescent antibody [DFA] techniques) or alternative methods (e.g., microfilaria—Nuclepore filtration or Knott concentration procedure).[9,11,12] The life cycle of the parasite and the time of infection are important factors in determining the type of specimens to be collected and the techniques used to detect the infection (Table 253-1). Multiple specimens might have to be

REFERENCES

1. Abu-Diab A, Azzeh M, Ghneim R, et al. Comparison between pernasal flocked swabs and nasopharyngeal aspirates for detection of common respiratory viruses in samples from children. *J Clin Microbiol* 2008;**46**:2414–17.
2. Ahluwalia G, Embree J, McNicol P, et al. Comparison of nasopharyngeal aspirate and nasopharyngeal swab specimens for respiratory syncytial virus diagnosis by cell culture, indirect immunofluorescence assay, and enzyme-linked immunosorbent assay. *J Clin Microbiol* 1987;**25**:763–7.
3. Alby K, Popowitch EB, Miller MB. Analytical performance of the nanosphere verigene RV+ assay and the Focus Simplexa Flu A/B & RSV Kit. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
4. Reference deleted in proofs.
5. Aslanzadeh J, Zheng X, Li H, et al. Prospective evaluation of rapid antigen tests for diagnosis of respiratory syncytial virus and human metapneumovirus infections. *J Clin Microbiol* 2008;**46**:1682–5.
6. Atkinson C, Emery VC. Cytomegalovirus quantification: where to next in optimising patient management? *J Clin Virol* 2011;**51**: 223–8.
7. August MJ, Warford AL. Evaluation of a commercial monoclonal antibody for detection of adenovirus antigen. *J Clin Microbiol* 1987;**25**:2233–5.
8. Babady NE, Mead P, Stiles J, et al. Comparison of the Luminex xTAG RVP Fast Assay and the Idaho Technology FilmArray RP Assay for detection of respiratory viruses in pediatric patients at a cancer hospital. *J Clin Microbiol* 2012;**50**:2282–8.
9. Barenfanger J, Drake C, Mueller T, et al. Combination of mixed cell lines E-Mix A and E-Mix B supports growth of all clinical enterovirus isolates tested. Abstract M21. Paper presented at the 15th Annual Clinical Virology Symposium. Clearwater, Fla; 2000.
10. Barenfanger J, Drake C, Mueller T, et al. R-Mix cells are faster, at least as sensitive and marginally more costly than conventional cell lines for the detection of respiratory viruses. *J Clin Virol* 2001;**22**:101–10.
11. Barger M, Vestal D, Nye M, et al. Evaluation of the BD Veritor (TM) Flu A/B assay compared to R-Mix/Mink Lung (ML) Cell culture, BD Directigen (TM) EZ Flu A/B and PCR for the detection of influenza A and B viruses. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
12. Barney T, Poppe H, Western M, et al. Performance of a novel multiplexed molecular assay for the detection of influenza A and influenza B on two real-time amplification Platforms. 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
13. Bechert CJ, Schnadig VJ, Payne DA, et al. Monitoring of BK viral load in renal allograft recipients by real-time PCR assays. *Am J Clin Pathol* 2010;**133**:242–50.
14. Blanding JG, Hoshiko MG, Stutman HR. Routine viral culture for pediatric respiratory specimens submitted for direct immunofluorescence testing. *J Clin Microbiol* 1989;**27**:1438–40.
15. Bonzel L, Tenenbaum T, Schroten H, et al. Frequent detection of viral coinfection in children hospitalized with acute respiratory tract infection using a real-time polymerase chain reaction. *Pediatr Infect Dis J* 2008;**27**:589–94.
16. Brandt K, Beck J, Shchyschuk L, et al. Respiratory syncytial virus detection by Tru RSV, Binax NOW RSV, direct immunofluorescent staining and tissue culture. Paper presented at the Canadian Academy of Clinical Microbiology and Infectious Diseases (CACMID), Toronto, On, 2009.
17. Brooks RG, Brown L, Franklin RB. Comparison of a new rapid test (TestPack Rotavirus) with standard enzyme immunoassay and electron microscopy for the detection of rotavirus in symptomatic hospitalized children. *J Clin Microbiol* 1989;**27**:775–7.
18. Burd EM. Validation of laboratory-developed molecular assays for infectious diseases. *Clin Microbiol Rev* 2010;**23**:550–76.
19. Butt AA, Kanwal F. Boceprivir and Telaprevir in the management of hepatitis C virus-infected patients. *Clin Infect Dis* 2012;**54**: 96–104.
20. Caceda ER, Kochel TJ. Application of modified shell vial culture procedure for arbovirus detection. *PLoS One* 2007;**2**:e1034.
21. Capaul SE, Gorgievski-Hrisoho M. Detection of enterovirus RNA in cerebrospinal fluid (CSF) using NucliSens EasyQ Enterovirus assay. *J Clin Virol* 2005;**32**:236–40.
22. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for detection of novel influenza A (H1N1) Virus: United States, 2009. *MMWR Morb Mortal Wkly Rep* 2009;**58**:826–9.
23. Centers for Disease Control and Prevention. Evaluation of rapid influenza diagnostic tests for influenza A (H3N2) virus and updated case count: United States, 2012. *MMWR Morb Mortal Wkly Rep* 2012;**61**:619–21.
24. Chan KH, Peiris JSM, Lim W, et al. Comparison of nasopharyngeal flocked swabs and aspirates for rapid diagnosis of respiratory viruses in children. *J Clin Virol* 2008;**42**:65–9.
25. Cheeseman SH, Pierik LT, Leombruno D, et al. Evaluation of a commercially available direct immunofluorescent staining reagent for the detection of respiratory syncytial virus in respiratory secretions. *J Clin Microbiol* 1986;**24**:155–6.
26. Chernesky M, Castriciano S, Mahony J, et al. Ability of TESTPACK ROTAVIRUS enzyme immunoassay to diagnose rotavirus gastroenteritis. *J Clin Microbiol* 1988;**26**:2459–61.
27. Clarke LM, Daidone BJ, Inghida R, et al. Differential recovery of cytomegalovirus from cellular and supernatant components of bronchoalveolar lavage specimens. *Am J Clin Pathol* 1992;**97**: 313–17.
28. Coffin SE, Hodinka RL. Utility of direct immunofluorescence and virus culture for detection of varicella-zoster virus in skin lesions. *J Clin Microbiol* 1995;**33**:2792–5.
29. Cole LJ, Campbell MB, Gleaves CA. Detection of HSV from clinical specimens using the ELVIS™ tube culture test as compared to standard cell culture. Abstract M20. Paper presented at the 12th Annual Clinical Virology Symposium. Clearwater, Fla; 1996.
30. Cone RW, Hobson AC, Palmer J, et al. Extended duration of herpes simplex virus DNA in genital lesions detected by the polymerase chain reaction. *J Infect Dis* 1991;**164**:757–60.
31. Corey L. Laboratory diagnosis of herpes simplex virus infections. Principles guiding the development of rapid diagnostic tests. *Diagn Microbiol Infect Dis* 1986;**4**:111S–19S.
32. Corey L, Spear PG. Infections with herpes simplex viruses. II. *N Engl J Med* 1986;**314**:749–57.
33. Couturier MR. Validation of the Idaho Technology Inc FilmArray Respiratory Panel for clinical use at Primary Children's Medical Center, Salt Lake City UT. Paper presented at the 27th Annual Clinical Virology Symposium. Daytona, Fla; 2011.
34. Cox KL, Lawrence-Miyasaki LS, Garcia-Kennedy R, et al. An increased incidence of Epstein-Barr virus infection and lymphoproliferative disorder in young children on FK506 after liver transplantation. *Transplantation* 1995;**59**:524–9.
35. Cruz AT, Cazacu AC, Greer JM, et al. Performance of a rapid assay (Binax NOW) for detection of respiratory syncytial virus at a children's hospital over a 3-year period. *J Clin Microbiol* 2007; **45**:1993–5.
36. Cruz AT, Cazacu AC, Greer JM, et al. Rapid assays for the diagnosis of influenza A and B viruses in patients evaluated at a large tertiary care children's hospital during two consecutive winter seasons. *J Clin Virol* 2008;**41**:143–7.
37. Cruz AT, Demmler-Harrison GJ, Caviness AC, et al. Performance of a rapid influenza test in children during the H1N1 2009 influenza A outbreak. *Pediatrics* 2010;**125**:e645–50.
38. Dale SE, Mayer C, Mayer MC, et al. The Performance characteristics of the Quidel QuickVue® RSV Test for nasal wash specimens collected from three different clinical sites. Paper presented at the 23rd Annual Clinical Virology Symposium and Annual Meeting Pan American Society for Clinical Virology. Clearwater, Fla; 2007.
39. Dale SE, Mayer C, Mayer MC, et al. Analytical and clinical sensitivity of the 3M rapid detection influenza A+B assay. *J Clin Microbiol* 2008;**46**:3804–7.
40. de Crom SC, Obihara CC, van Loon AM, et al. Detection of enterovirus RNA in cerebrospinal fluid: comparison of two molecular assays. *J Virol Methods* 2012;**179**:104–7.
41. De Paulis M, Gilio AE, Ferraro AA, et al. Severity of viral coinfection in hospitalized infants with respiratory syncytial virus infection. *J Pediatr (Rio J)* 2011;**87**:307–13.
42. Dennehy PH, Hartin M, Nelson SM, et al. Evaluation of the ImmunoCardSTAT! rotavirus assay for detection of group A rotavirus in fecal specimens. *J Clin Microbiol* 1999;**37**:1977–9.

43. Diaz-Mitoma F, Ruben M, Sacks S, et al. Detection of viral DNA to evaluate outcome of antiviral treatment of patients with recurrent genital herpes. *J Clin Microbiol* 1996;**34**:657–63.
44. Dimaio MA, Sahoo MK, Waggoner J, et al. Comparison of Xpert Flu Rapid Nucleic Acid Testing with rapid antigen testing for the diagnosis of influenza A and B. *J Virol Methods*. 2012; in press.
45. Doller G, Schuy W, Tjhen KY, et al. Direct detection of influenza virus antigen in nasopharyngeal specimens by direct enzyme immunoassay in comparison with quantitating virus shedding. *J Clin Microbiol* 1992;**30**:866–9.
46. Drew WL. Controversies in viral diagnosis. *Rev Infect Dis* 1986; **8**:814–24.
47. Drew WL, Mintz L. Rapid diagnosis of varicella-zoster virus infection by direct immunofluorescence. *Am J Clin Pathol* 1980; **73**:699–701.
48. Druce J, Garcia K, Tran T, et al. Evaluation of swabs, transport media, and specimen transport conditions for optimal detection of viruses by PCR. *J Clin Microbiol* 2012;**50**:1064–5.
49. Dundas NE, Ziadie MS, Revell PA, et al. A lean laboratory: operational simplicity and cost effectiveness of the Luminex xTAG respiratory viral panel. *J Mol Diagn* 2011;**13**:175–9.
50. Dunn JJ, Billetdeaux E, Skodack-Jones L, et al. Evaluation of three Copan viral transport systems for the recovery of cultivatable, clinical virus isolates. *Diagn Microbiol Infect Dis* 2002;**45**: 191–7.
51. Einsele H, Ehninger G, Steidle M, et al. Polymerase chain reaction to evaluate antiviral therapy for cytomegalovirus disease. *Lancet* 1991;**338**:1170–2.
52. Espy MJ, Smith TF. Detection of herpes simplex virus in conventional tube cell cultures and in shell vials with a DNA probe kit and monoclonal antibodies. *J Clin Microbiol* 1988;**26**:22–4.
53. Espy MJ, Rys PN, Wold AD, et al. Detection of herpes simplex virus DNA in genital and dermal specimens by LightCycler PCR after extraction using the IsoQuick, MagNA Pure, and BioRobot 9604 methods. *J Clin Microbiol* 2001;**39**:2233–6.
54. Espy MJ, Uhl JR, Sloan LM, et al. Real-time PCR in clinical microbiology: applications for routine laboratory testing. *Clin Microbiol Rev* 2006;**19**:165–256.
55. Espy MJ, Wold AD, Jespersen DJ, et al. Comparison of shell vials and conventional tubes seeded with rhabdomyosarcoma and MRC-5 cells for the rapid detection of herpes simplex virus. *J Clin Microbiol* 1991;**29**:2701–3.
56. Faix DJ, Sherman SS, Waterman SH. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 2009;**361**:728–9.
57. Fedorko DP, Ilstrup DM, Smith TF. Effect of age of shell vial monolayers on detection of cytomegalovirus from urine specimens. *J Clin Microbiol* 1989;**27**:2107–9.
58. Filen F, Strand A, Allard A, et al. Duplex real-time polymerase chain reaction assay for detection and quantification of herpes simplex virus type 1 and herpes simplex virus type 2 in genital and cutaneous lesions. *Sex Transm Dis* 2004;**31**:331–6.
59. Fong CKY, Mi Kyung L, Griffith BP. Evaluation of R-mix fresh cells in shell vials for detection of respiratory viruses. *J Clin Microbiol* 2000;**38**:4660–2.
60. Forbes BA. Introducing a molecular test into the clinical microbiology laboratory: development, evaluation, and validation. *Arch Path Lab Med* 2003;**127**:1106–11.
61. Forman MS, Merz CS, Charache P. Detection of herpes simplex virus by a nonradiometric spin-amplified in situ hybridization assay. *J Clin Microbiol* 1992;**30**:581–4.
62. Gadsby NJ, Hardie A, Claas EC, et al. Comparison of the Luminex Respiratory Virus Panel fast assay with in-house real-time PCR for respiratory viral infection diagnosis. *J Clin Microbiol* 2010;**48**: 2213–16.
63. Garber M, Vestal D, Body BA. Comparison of E-Mix A and E-Mix B fresh cells and conventional tissue tube culture for the detection of enteroviruses. Abstract S32. Paper presented at the 17th Annual Clinical Virology Symposium. Clearwater, Fla; 2001.
64. Garcia LS, editor. *Clinical Microbiology Procedures Handbook*, ed 3. Washington, D.C.: American Society for Microbiology; 2007.
65. Gazenmueller T, Heim A. Adenoviral load diagnostics by quantitative polymerase chain reaction: techniques and application. *Rev Med Virol* 2012;**22**:194–208.
66. Gerna G, Zipeto D, Parea M, et al. Monitoring of human cytomegalovirus infections and ganciclovir treatment in heart transplant recipients by determination of viremia, antigenemia, and DNAemia. *J Infect Dis* 1991;**164**:488–98.
67. Gharabaghi F, Hawan A, Drews SJ, et al. Evaluation of multiple commercial molecular and conventional diagnostic assays for the detection of respiratory viruses in children. *Clin Microbiol Infect* 2011;**17**:1900–6.
68. Gillim-Ross L, Taylor J, Scholl DR, et al. Discovery of Novel Human and Animal Cells Infected by the Severe Acute Respiratory Syndrome Coronavirus by Replication-Specific Multiplex Reverse Transcription-PCR. *J Clin Microbiol* 2004;**42**: 3196–206.
69. Ginocchio CC, Lotlikar M, Falk L, et al. Clinical performance of the 3M Rapid Detection Flu A+B Test compared to R-Mix culture, DFA and BinaxNOW Influenza A&B Test. *J Clin Virol* 2009;**45**:146–9.
70. Ginocchio CC, Swierkosz E, McAdam AJ, et al. Multicenter study of clinical performance of the 3M Rapid Detection RSV test. *J Clin Microbiol* 2010;**48**:2337–43.
71. Ginocchio CC, Zhang F, Manji R, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. *J Clin Virol* 2009;**45**: 191–5.
72. Gleaves CA, Hursh DA, Meyers JD. Detection of human cytomegalovirus in clinical specimens by centrifugation culture with a nonhuman cell line. *J Clin Microbiol* 1992;**30**:1045–8.
73. Gleaves CA, Lee CF, Bustamante CI, et al. Use of murine monoclonal antibodies for laboratory diagnosis of varicella-zoster virus infection. *J Clin Microbiol* 1988;**26**:1623–5.
74. Gleaves CA, Smith TF, Shuster EA, et al. Rapid detection of cytomegalovirus in MRC-5 cells inoculated with urine specimens by using low-speed centrifugation and monoclonal antibody to an early antigen. *J Clin Microbiol* 1984;**19**:917–19.
75. Gleaves CA, Smith TF, Shuster EA, et al. Comparison of standard tube and shell vial cell culture techniques for the detection of cytomegalovirus in clinical specimens. *J Clin Microbiol* 1985;**21**:217–21.
76. Gleaves CA, Wilson DJ, Wold AD, et al. Detection and serotyping of herpes simplex virus in MRC-5 cells by use of centrifugation and monoclonal antibodies 16 h postinoculation. *J Clin Microbiol* 1985;**21**:29–32.
77. Goldstein LC, Corey L, McDougall JK. Monoclonal antibodies to herpes simplex viruses: use in antigenic typing and rapid diagnosis. *J Infect Dis* 1983;**147**:829–37.
78. Griego-Fullbright C, Henderson K, Kindig S, et al. Performance of the BD Veritor (TM) assay for the simultaneous detection of influenza A and influenza B in respiratory specimens using an immunochromatrographic assay with an automated reader. Presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
79. Grijalva CG, Poehling KA, Edwards KM, et al. Accuracy and interpretation of rapid influenza tests in children. *Pediatrics* 2007;**119**:e6–11.
80. Gulley ML, Tang W. Using Epstein-Barr viral load assays to diagnose, monitor, and prevent posttransplant lymphoproliferative disorder. *Clin Microbiol Rev* 2010;**23**:350–66.
81. Hackman RC, Myerson D, Meyers JD. Rapid diagnosis of cytomegaloviral pneumonia by tissue immunofluorescence with a murine monoclonal antibody. *J Infect Dis* 1985;**151**:325–9.
82. Hall CB, Douglas RG, Jr. Clinically useful method for the isolation of respiratory syncytial virus. *J Infect Dis* 1975;**131**: 1–5.
83. Hardy DA, Arvin AM, Yasukawa LL, et al. Use of polymerase chain reaction for successful identification of asymptomatic genital infection with herpes simplex virus in pregnant women at delivery. *J Infect Dis* 1990;**162**:1031–5.
84. Herrmann EC Jr. Experience in providing a viral diagnostic laboratory compatible with medical practice. *Mayo Clinic Proc* 1967; **42**:112–23.
85. Herrmann JE, Perron-Henry DM, Blacklow NR. Antigen detection with monoclonal antibodies for the diagnosis of adenovirus gastroenteritis. *J Infect Dis* 1987;**155**:1167–71.
86. Hoffman J, Matthews E, Beckman K. Comparison of two rapid antigen tests for respiratory syncitial virus (RSV) in a community hospital laboratory. Paper presented 24th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology. Clearwater, Fla; 2009.

87. Howell CL, Miller MJ. Effect of sucrose phosphate and sorbitol on infectivity of enveloped viruses during storage. *J Clin Microbiol* 1983;**18**:658–62.
88. Huang YT, Turchek BM. Mink lung cells and mixed mink lung and A549 cells for rapid detection of influenza virus and other respiratory viruses. *J Clin Microbiol* 2000;**38**:422–3.
89. Huang YT, Yam P, Jollick JA. CaCO2 and engineered BGMK mixed cells for the detection of enteroviruses. Abstract S31. Paper presented at the 17th Annual Clinical Virology Symposium. Clearwater, Fla; 2001.
90. Huang YT, Hite S, Duane V, et al. Application of mixed cell lines for the detection of viruses from clinical specimens. *Clin Microbiol Newsl* 2000;**22**:89–92.
91. Hughes JH. Physical and chemical methods for enhancing rapid detection of viruses and other agents. *Clin Microbiol Rev* 1993;**6**:150–75.
92. Hurt AC, Baas C, Deng YM, et al. Performance of influenza rapid point-of-care tests in the detection of swine lineage A(H1N1) influenza viruses. *Influenza Other Respi Viruses* 2009;**3**:171–6.
93. Irmen KE, Kelleher JJ. Use of monoclonal antibodies for rapid diagnosis of respiratory viruses in a community hospital. *Clin Diagn Lab Immunol* 2000;**7**:396–403.
94. Jackson R, Morris DJ, Cooper RJ, et al. Multiplex polymerase chain reaction for adenovirus and herpes simplex virus in eye swabs. *J Virol Methods* 1996;**56**:41–8.
95. Jayakeerthi RS, Potula RV, Srinivasan S, et al. Shell vial culture assay for the rapid diagnosis of Japanese encephalitis: West Nile and dengue-2 viral encephalitis. *Virol J* 2006;**3**:2.
96. Johansson ME, Uhnoo I, Svensson L, et al. Enzyme-linked immunosorbent assay for detection of enteric adenovirus 41. *J Med Virol* 1985;**17**:19–27.
97. Johnson FB. Transport of viral specimens. *Clin Microbiol Rev* 1990;**3**:120–31.
98. Johnston SL, Wellens K, Siegel C. Comparison of hemagglutination and hemadsorption tests for influenza detection. *Diagn Microbiol Infect Dis* 1992;**15**:363–5.
99. Jokela P, Piiparinen H, Mannonen L, et al. Performance of the Luminex xTAG Respiratory Viral Panel Fast in a clinical laboratory setting. *J Virol Methods* 2012;**182**:82–6.
100. Karnauchow TM, Milk R, Sullivan L. Performance of QuickVue(R) RSV for the detection of RSV in pediatric patients. Paper presented at 23rd Annual Clinical Virology Symposium. Clearwater, Fla; 2007.
101. Kellogg JA. Culture vs direct antigen assays for detection of microbial pathogens from lower respiratory tract specimens suspected of containing the respiratory syncytial virus. *Arch Pathol Lab Med* 1991;**115**:451–8.
102. Kimura H, Morita M, Yabuta Y, et al. Quantitative analysis of Epstein-Barr virus load by using a real-time PCR assay. *J Clin Microbiol* 1999;**37**:132–6.
103. Klespies SL, Cebula DE, Kelley CL, et al. Detection of enteroviruses from clinical specimens by spin amplification shell vial culture and monoclonal antibody assay. *J Clin Microbiol* 1996;**34**:1465–7.
104. Kotton CN, Kumar D, Caliendo AM, et al. International consensus guidelines on the management of cytomegalovirus in solid organ transplantation. *Transplantation* 2010;**89**:779–95.
105. Lafferty WE, Krofft S, Remington M, et al. Diagnosis of herpes simplex virus by direct immunofluorescence and viral isolation from samples of external genital lesions in a high-prevalence population. *J Clin Microbiol* 1987;**25**:323–6.
106. Landry ML, Ferguson D. SimulFluor respiratory screen for rapid detection of multiple respiratory viruses in clinical specimens by immunofluorescence staining. *J Clin Microbiol* 2000;**38**:708–11.
107. Laskin BL, Goebel J. Cost-efficient screening for BK virus in pediatric kidney transplantation: a single-center experience and review of the literature. *Pediatr Transplant* 2010;**14**:589–95.
108. Lee CK, Cho CH, Woo MK, et al. Evaluation of Sofia fluorescent immunoassay analyzer for influenza A/B virus. *J Clin Virol* 2012;**55**:239–43.
109. Leland DS, Hansing RL, French ML. Clinical experience with cytomegalovirus isolation using conventional cell cultures and early antigen detection in centrifugation-enhanced shell vial cultures. *J Clin Microbiol* 1989;**27**:1159–62.
110. Leung J, Harpaz R, Baughman AL, et al. Evaluation of laboratory methods for diagnosis of varicella. *Clin Infect Dis* 2010;**51**:23–32.
111. Liao RS, Tomalty LL, Majury A, et al. Comparison of viral isolation and multiplex real-time reverse transcription-PCR for Confirmation of Respiratory Syncytial Virus and Influenza Virus Detection by Antigen Immunoassays. *J Clin Microbiol* 2009;**47**:527–32.
112. Lipson SM, David K, Shaikh F, et al. Detection of precytopathic effect of enteroviruses in clinical specimens by centrifugation-enhanced antigen detection. *J Clin Microbiol* 2001;**39**:2755–9.
113. Loeffelholz M. Comparison of the FilmArray Respiratory Panel and Prodesse assays for the detection of respiratory pathogens. Paper presented at 27th Annual Clinical Virology Symposium. Daytona, Fla; 2011.
114. Loeffelholz MJ, Pong DL, Pyles RB, et al. Comparison of the FilmArray Respiratory Panel and Prodesse real-time PCR assays for detection of respiratory pathogens. *J Clin Microbiol* 2011;**49**:4083–8.
115. Lu X, Erdman DD. Molecular typing of human adenoviruses by PCR and sequencing of partial region of the hexon gene. *Arch Virol* 2006;**151**:1587–602.
116. Lunel F, Mariotti M, Cresta P, et al. Comparative study of conventional and novel strategies for the detection of hepatitis C virus RNA in serum: amplicor, branched-DNA, NASBA and in-house PCR. *J Virol Methods* 1995;**54**:159–71.
117. MacDonald RL, Hughes BL, Aarnaes SL, et al. Evaluation of a shell vial centrifugation method for the detection of herpes simplex virus. *Diagn Microbiol Infect Dis* 1988;**9**:51–4.
118. Mack K, Salamon D, Marcon M. Evaluation of the TRU™ RSV Test for rapid detection of respiratory syncytial virus in pediatric patients. Paper presented at 24th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology. Clearwater, Fla; 2008.
119. Mahony JB, Blackhouse G, Babwah J, et al. Cost analysis of multiplex PCR testing for diagnosing respiratory virus infections. *J Clin Microbiol* 2009;**47**:2812–17.
120. Martin WJ, 2nd, Smith TF. Rapid detection of cytomegalovirus in bronchoalveolar lavage specimens by a monoclonal antibody method. *J Clin Microbiol* 1986;**23**:1006–8.
121. Martinez OM, Villanueva JC, Lawrence-Miyasaki L, et al. Viral and immunologic aspects of Epstein-Barr virus infection in pediatric liver transplant recipients. *Transplantation* 1995;**59**:519–24.
122. Mason T, Bloom G, Leland D. Evaluation of ELVIS HSV Gold, a commercially available tube culture system featuring a genetically engineered cell line and a histochemical assay for detection of herpes simplex virus (HSV). Abstract M24. Paper presented at the 12th Annual Clinical Virology Symposium. Clearwater, Fla; 1996.
123. Matthey S, Nicholson D, Ruhs S, et al. Rapid detection of respiratory viruses by shell vial culture and direct staining by using pooled and individual monoclonal antibodies. *J Clin Microbiol* 1992;**30**:540–4.
124. McDonald JC, Quennec P. Utility of a respiratory virus panel containing a monoclonal antibody pool for screening of respiratory specimens in nonpeak respiratory syncytial virus season. *J Clin Microbiol* 1993;**31**:2809–11.
125. McIntosh K. Recent advances in viral diagnosis. *Arch Pathol Lab Med* 1980;**104**:3–6.
126. McQuillin J, Madeley CR, Kendal AP. Monoclonal antibodies for the rapid diagnosis of influenza A and B virus infections by immunofluorescence. *Lancet* 1985;**2**:911–14.
127. Michalski FJ, Shaikh M, Sahraie F, et al. Enzyme-linked immunosorbent assay spin amplification technique for herpes simplex virus antigen detection. *J Clin Microbiol* 1986;**24**:310–11.
128. Miernyk K, Bulkow L, DeByle C, et al. Performance of a rapid antigen test (Binax NOW(R) RSV) for diagnosis of respiratory syncytial virus compared with real-time polymerase chain reaction in a pediatric population. *J Clin Virol* 2011;**50**:240–3.
129. Milburn GL, Carrigan D, Dienglewicz R, et al. Diagnosis of active human herpesvirus six (HHV-6) infection in immunocompromised patients with a rapid shell vial assay. Abstract C136. Paper presented at the 96th General Meeting of the American Society of Microbiology. New Orleans; 1996.
130. Miller MJ, Howell CL. Rapid detection and identification of herpes simplex virus in cell culture by a direct immunoperoxidase staining procedure. *J Clin Microbiol* 1983;**18**:550–3.
131. Miller MJ, Wagar EA, Moe AA, et al. Comparison of nucleic acid hybridization, culture, and shell vial assay for detection of CMV

in blood. Abstract C89. Paper presented at the 96th General Meeting of the American Society of Microbiology. New Orleans; 1996.
132. Minnich LL, Ray CG. Comparison of direct and indirect immunofluorescence staining of clinical specimens for detection of respiratory syncytial virus antigen. *J Clin Microbiol* 1982;**15**: 969–70.
133. Minnich LL, Ray CG. Early testing of cell cultures for detection of hemadsorbing viruses. *J Clin Microbiol* 1987;**25**:421–2.
134. Munywoki PK, Hamid F, Mutunga M, et al. Improved detection of respiratory viruses in pediatric outpatients with acute respiratory illness by real-time PCR using nasopharyngeal flocked swabs. *J Clin Microbiol* 2011;**49**:3365–7.
135. Murphy DG, Cote L, Fauvel M, et al. Multicenter comparison of Roche COBAS AMPLICOR MONITOR version 1.5, Organon Teknika NucliSens QT with Extractor, and Bayer Quantiplex version 3.0 for quantification of human immunodeficiency virus type 1 RNA in plasma. *J Clin Microbiol* 2000;**38**:4034–41.
136. Nahass GT, Goldstein BA, Zhu WY, et al. Comparison of Tzanck smear, viral culture, and DNA diagnostic methods in detection of herpes simplex and varicella-zoster infection. *JAMA* 1992;**268**:2541–4.
137. Nahass GT, Mandel MJ, Cook S, et al. Detection of herpes simplex and varicella-zoster infection from cutaneous lesions in different clinical stages with the polymerase chain reaction. *J Am Acad Dermatol* 1995;**32**:730–3.
138. Niesters HG, van Esser J, Fries E, et al. Development of a real-time quantitative assay for detection of Epstein-Barr virus. *J Clin Microbiol* 2000;**38**:712–15.
139. Ninove L, Nougairede A, Gazin C, et al. Comparative detection of enterovirus RNA in cerebrospinal fluid: GeneXpert system vs. real-time RT-PCR assay. *Clin Microbiol Infect* 2011;**17**:1890–4.
140. Nix WA, Oberste MS, Pallansch MA. Sensitive, seminested PCR amplification of VP1 sequences for direct identification of all enterovirus serotypes from original clinical specimens. *J Clin Microbiol* 2006;**44**:2698–704.
141. Nolte FS, Rogers BB, Tang YW, et al. Evaluation of a rapid and completely automated real-time reverse transcriptase PCR assay for diagnosis of enteroviral meningitis. *J Clin Microbiol* 2011;**49**: 528–33.
142. Nolte FS, Thurmond C, Fried MW. Preclinical evaluation of AMPLICOR hepatitis C virus test for detection of hepatitis C virus RNA. *J Clin Microbiol* 1995;**33**:1775–8.
143. Novak-Weekley SM, Marlowe EM, Poulter M, et al. Evaluation of the Cepheid Xpert Flu Assay for rapid identification and differentiation of influenza A, influenza A 2009 H1N1, and influenza B viruses. *J Clin Microbiol* 2012;**50**:1704–10.
144. Ogburn JR, Hoffpauir JT, Cole E, et al. Evaluation of new transport medium for detection of herpes simplex virus by culture and direct enzyme-linked immunosorbent assay. *J Clin Microbiol* 1994; **32**:3082–4.
145. Ohm-Smith MJ, Nassos PS, Haller BL. Evaluation of the Binax NOW, BD Directigen, and BD Directigen EZ assays for detection of respiratory syncytial virus. *J Clin Microbiol* 2004;**42**: 2996–9.
146. Olsen MA, Shuck KM, Sambol AR, et al. Isolation of seven respiratory viruses in shell vials: a practical and highly sensitive method. *J Clin Microbiol* 1993;**31**:422–5.
147. Pabbaraju K, Tokaryk KL, Wong S, et al. Comparison of the Luminex xTAG respiratory viral panel with in-house nucleic acid amplification tests for diagnosis of respiratory virus infections. *J Clin Microbiol* 2008;**46**:3056–62.
148. Pabbaraju K, Wong S, Tokaryk KL, et al. Comparison of the Luminex xTAG respiratory viral panel with xTAG respiratory viral panel fast for diagnosis of respiratory virus infections. *J Clin Microbiol* 2011;**49**:1738–44.
149. Paya CV, Wold AD, Smith TF. Detection of cytomegalovirus infections in specimens other than urine by the shell vial assay and conventional tube cell cultures. *J Clin Microbiol* 1987;**25**:755–7.
150. Paya CV, Holley KE, Wiesner RH, et al. Early diagnosis of cytomegalovirus hepatitis in liver transplant recipients: role of immunostaining, DNA hybridization and culture of hepatic tissue. *Hepatology* 1990;**12**:119–26.
151. Paya CV, Wold AD, Ilstrup DM, et al. Evaluation of number of shell vial cell cultures per clinical specimen for rapid diagnosis of cytomegalovirus infection. *J Clin Microbiol* 1988;**26**:198–200.
152. Peterson EM, Hughes BL, Aarnaes SL, et al. Comparison of primary rabbit kidney and MRC-5 cells and two stain procedures for herpes simplex virus detection by a shell vial centrifugation method. *J Clin Microbiol* 1988;**26**:222–4.
153. Peterson LR, Moore BM, Edelman CK, et al. Primary virus isolation by a satellite laboratory. *Arch Pathol Lab Med* 1980;**104**: 9–10.
154. Pierce VM, Elkan M, Leet M, et al. Comparison of the Idaho Technology FilmArray system to real-time PCR for detection of respiratory pathogens in children. *J Clin Microbiol* 2012;**50**: 364–71.
155. Poeppl W, Herkner H, Burgmann H, et al. Performance of the QuickVue Influenza A+B rapid test for pandemic H1N1 (2009) virus infection in adults. *PLoS One* 2011;**6**:e28089.
156. Pollock NR, Duong S, Cheng A, et al. Ruling out novel H1N1 influenza virus infection with direct fluorescent antigen testing. *Clin Infect Dis* 2009;**49**:e66–8.
157. Popowitch EB, Rogers E, Miller MB. Retrospective and prospective verification of the Cepheid Xpert influenza virus assay. *J Clin Microbiol* 2011;**49**:3368–9.
158. Poritz MA, Blaschke AJ, Byington CL, et al. FilmArray, an automated nested multiplex PCR system for multi-pathogen detection: development and application to respiratory tract infection. *PLoS One* 2011;**6**:e26047.
159. Pouletty P, Chomel JJ, Thouvenot D. Detection of herpes simplex virus in direct specimens by immunofluorescence assay using a monoclonal antibody. *J Clin Microbiol* 1987;**25**:958–9.
160. Pruneda RC, Almanza I. Centrifugation-shell vial technique for rapid detection of herpes simplex virus cytopathic effect in Vero cells. *J Clin Microbiol* 1987;**25**:423–4.
161. Rabalais GP, Stout GG, Ladd KL, et al. Rapid diagnosis of respiratory viral infections by using a shell vial assay and monoclonal antibody pool. *J Clin Microbiol* 1992;**30**:1505–8.
162. Rabella N, Drew WL. Comparison of conventional and shell vial cultures for detecting cytomegalovirus infection. *J Clin Microbiol* 1990;**28**:806–7.
163. Rabenau HF, Kessler HH, Kortenbusch M, et al. Verification and validation of diagnostic laboratory tests in clinical virology. *J Clin Virol* 2007;**40**:93–8.
164. Rand KH, Rampersaud H, Houck HJ. Comparison of two multiplex methods for detection of respiratory viruses: FilmArray RP and xTAG RVP. *J Clin Microbiol* 2011;**49**:2449–53.
165. Ray CG, Minnich LL. Efficiency of immunofluorescence for rapid detection of common respiratory viruses. *J Clin Microbiol* 1987;**25**:355–7.
166. Relich RF, Snyder C, Orwig K, et al. Comparison of four rapid lateral flow assays for detection of influenza A and B antigens in stored samples. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona Fla; 2012.
167. Riley AM, Wong K, Williams L, et al. Evaluation of the 3M™ Rapid Detection RSV test and the BinaxNOW® RSV test for detection of respiratory syncytial virus in pediatric patients. Paper presented at the 25th Annual Clinical Virology Symposium. Daytona, Fla; 2009.
168. Romero JR, Hinrichs SH, Cavalieri SJ, et al. Potential health care cost saving from PCR-based rapid diagnosis of enteroviral meningitis. Abstract. Paper presented at the Society of Pediatric Research Annual Meeting. Washington, D.C; May 6-10, 1996.
169. Rossier E, Miller HR, Phipps PH. *Rapid viral diagnosis by immunofluorescence: an atlas and practical guide*. Ottawa: University of Ottawa Press; 1989.
170. Roth WK, Lee JH, Ruster B, et al. Comparison of two quantitative hepatitis C virus reverse transcriptase PCR assays. *J Clin Microbiol* 1996;**34**:261–4.
171. Salmon VC, Turner RB, Speranza MJ, et al. Rapid detection of herpes simplex virus in clinical specimens by centrifugation and immunoperoxidase staining. *J Clin Microbiol* 1986;**23**: 683–6.
172. Sawyer MH, Holland D, Aintablian N, et al. Diagnosis of enteroviral central nervous system infection by polymerase chain reaction during a large community outbreak. *Pediatr Infect Dis J* 1994;**13**:177–82.
173. Schiff ER, editor. Optimizing management strategies in patients with chronic hepatitis B. *Am J Med* 2008;**121**:S1–2.
174. Schiff ER. Diagnosing and treating hepatitis C virus infection. *Am J Manag Care* 2011;**17**(suppl 4):S108–15.

175. Schirm J, Meulenberg JJM, Pastoor GW, et al. Rapid detection of varicella-zoster virus in clinical specimens using monoclonal antibodies on shell vials and smears. *J Med Virol* 1989;**28**:1–6.
176. Schmidt NJ, Emmons RW, editors. *Diagnostic procedures for viral, rickettsial, and chlamydial infections*. 6th ed. Washington, D.C.: American Public Health Association; 1989.
177. Scoular A, Gillespie G, Carman WF. Polymerase chain reaction for diagnosis of genital herpes in a genitourinary medicine clinic. *Sex Transm Infect* 2002;**78**:21–5.
178. Self WH, McNaughton CD, Grijalva CG, et al. Diagnostic performance of the BinaxNow Influenza A&B rapid antigen test in ED patients. *Am J Emerg Med* 2012;**30**:1955–61.
179. Selvaraju S, Earley A, Graf P, et al. Evaluation of a novel Simplexa (TM) Influenza A/B & RSV Direct Assay for laboratory diagnosis of influenza and RSV viral infections. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
180. Selvaraju SB, Selvarangan R. Evaluation of three influenza A and B real-time reverse transcription-PCR assays and a new 2009 H1N1 assay for detection of influenza viruses. *J Clin Microbiol* 2010;**48**:3870–5.
181. Selvarangan R, Abel D, Hamilton M. Comparison of BD Directigen EZ RSV and Binax NOW RSV tests for rapid detection of respiratory syncytial virus from nasopharyngeal aspirates in a pediatric population. *Diagn Microbiol Infect Dis* 2008;**62**: 157–61.
182. Seme K, Poljak M. Use of a commercial PCR kit for detection of hepatitis C virus. *Eur J Clin Microbiol Infect Dis* 1995;**14**: 549–52.
183. Shaw CJ, Page J, Jollick JA, et al. Directly labeled fluorescent monoclonal antibodies for the detection of metapneumovirus. Paper presented at the 22nd Annual Clinical Virology Symposium. Clearwater Beach, Fla; 2006.
184. Smith TF, Weed LA, Pettersen GR, et al. Recovery of chlamydia and genital *Mycoplasma* transported in sucrose phosphate buffer and urease color test medium. *Health Lab Sci* 1977;**14**:30–4.
185. Spada B, Biehler K, Chegas P, et al. Comparison of rapid immunofluorescence assay to cell culture isolation for the detection of influenza A and B viruses in nasopharyngeal secretions from infants and children. *J Virol Methods* 1991;**33**:305–10.
186. Stabell EC, Olivo PD. Isolation of a cell line for rapid and sensitive histochemical assay for the detection of herpes simplex virus. *J Virol Methods* 1992;**38**:195–204.
187. Stabell EC, O'Rourke SR, Storch GA, et al. Evaluation of a genetically engineered cell line and a histochemical β-galactosidase assay to detect herpes simplex virus in clinical specimens. *J Clin Microbiol* 1993;**31**:2796–8.
188. Stellrecht KA, Butt SA, Maceira VP, et al. Comparison of three commercial real-time PCR systems for the detection of respiratory viruses. Paper presented at the 28th Annual Clinical Virology Symposium. Daytona, Fla; 2012.
189. Stevens SJC, Pronk I, Middeldorp JM. Toward standardization of Epstein-Barr virus DNA load monitoring: unfractionated whole blood as preferred clinical specimen. *J Clin Microbiol* 2001;**39**: 1211–16.
190. Stout C, Murphy MD, Lawrence S, et al. Evaluation of a monoclonal antibody pool for rapid diagnosis of respiratory viral infections. *J Clin Microbiol* 1989;**27**:448–52.
191. Sullivan L, Kern C, Milk R, et al. Performance of Meridian TRU and Binax NOW assays for the detection of RSV in pediatric and adult patients. Paper presented at the 25th Annual Clinical Virology Symposium and Annual Meeting of the Pan American Society for Clinical Virology. Daytona, Fla; 2009.
192. Swierkosz E, Bonnot TJ. Clinical Evaluation of The 3M(TM) Rapid Detection RSV Test in Nasopharyngeal Aspirate Specimens. Paper presented at the 25th Annual Clinical Virology Symposium. Dayton, Fla; 2009.
193. Taggart EW, Petti CA, She RC. Comparison of direct fluorescent antibody to two rapid antigen tests for RSV and Influenza A and B. Paper presented at the 25th Annual Clinical Virology Symposium. Clearwater, Fla; 2009.
194. Tebas P, Nease RF, Storch GA. Use of the polymerase chain reaction in the diagnosis of herpes simplex encephalitis: a decision analysis model. *Am J Med* 1998;**105**:287–95.
195. Telenti A, Marshall WF, Smith TF. Detection of Epstein-Barr virus by polymerase chain reaction. *J Clin Microbiol* 1990;**28**: 2187–90.
196. Thomas EE, Book LE. Comparison of two rapid methods for detection of respiratory syncytial virus (RSV) (Testpack RSV and ortho RSV ELISA) with direct immunofluorescence and virus isolation for the diagnosis of pediatric RSV infection. *J Clin Microbiol* 1991;**29**:632–5.
197. Tyler KL. Update on herpes simplex encephalitis. *Rev Neurol Dis* 2004;**1**:169–78.
198. U.S. Department of Health and Human Services, Panel on Antiretroviral Guidelines for Adults and Adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. Department of Health and Human Services. January 10, 2011; 1-166. Available at: http://www.aidsinfo.nih.gov/contentfiles/adultandadolescentGL.pdf.
199. Van der Bij W, Torensma R, Van Son WJ, et al. Rapid immunodiagnosis of active cytomegalovirus infection by monoclonal antibody staining of blood leucocytes. *J Med Virol* 1988;**25**:179–88.
200. Vand der Bij W, Schirm J, Torensma R, et al. Comparison between viremia and antigenemia for detection of cytomegalovirus in blood. *J Clin Microbiol* 1988;**26**:2531–5.
201. Vasoo S, Stevens J, Singh K. Rapid antigen tests for diagnosis of pandemic (Swine) influenza A/H1N1. *Clin Infect Dis* 2009;**49**: 1090–3.
202. Verano L, Michalski FJ. Herpes simplex virus antigen direct detection in standard virus transport medium by Du Pont Herpchek enzyme-linked immunosorbent assay. *J Clin Microbiol* 1990; **28**:2555–8.
203. Verano L, Michalski FJ. Spin-amplified culture followed by enzyme immunoassay for detection of herpes simplex virus in patient specimens: a comparative study. *Clin Diagn Virol* 1993;**1**: 23–8.
204. Versalovic J, Carroll KC, Funke G, et al, editors. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: American Society for Microbiology; 2011.
205. Volpi A, Whitley RJ, Ceballos R, et al. Rapid diagnosis of pneumonia due to cytomegalovirus with specific monoclonal antibodies. *J Infect Dis* 1983;**147**:1119–20.
206. Walls HH, Harmon MW, Slagle JJ, et al. Characterization and evaluation of monoclonal antibodies developed for typing influenza A and influenza B viruses. *J Clin Microbiol* 1986;**23**:240–5.
207. Walsh P, Overmyer CL, Pham K, et al. Comparison of respiratory virus detection rates for infants and toddlers by use of flocked swabs, saline aspirates, and saline aspirates mixed in universal transport medium for room temperature storage and shipping. *J Clin Microbiol* 2008;**46**:2374–6.
208. Waner JL, Whitehurst NJ, Downs T, et al. Production of monoclonal antibodies against parainfluenza 3 virus and their use in diagnosis by immunofluorescence. *J Clin Microbiol* 1985;**22**: 535–8.
209. Warford AL, Chung JW, Drill AE, et al. Amplification techniques for detection of herpes simplex virus in neonatal and maternal genital specimens obtained at delivery. *J Clin Microbiol* 1989;**27**: 1324–8.
210. Warford AL, Kao SY, Valantine H, et al. Evaluation of CMV plasma PCR for monitoring of heart transplant recipients receiving both ganciclovir and Cytogam prophylaxis. Abstract S18. Paper presented at the 12th Annual Clinical Virology Symposium. Clearwater, Fla; 1996.
211. Weinmann S, Chun C, Mullooly JP, et al. Laboratory diagnosis and characteristics of breakthrough varicella in children. *J Infect Dis* 2008;**197**(suppl 2):S132–8.
212. Weiss RL, Snow GW, Schumann GB, et al. Diagnosis of cytomegalovirus pneumonitis on bronchoalveolar lavage fluid: comparison of cytology, immunofluorescence, and in situ hybridization with viral isolation. *Diagn Cytopathol* 1991;**7**:243–7.
213. Wilson DA, Mulherin P, Quimson L, et al. Clinical evaluation of the 3M(TM) Rapid Detection RSV Test. Paper presented at the 25th Annual Clinical Virology Symposium. Daytona, Fla; 2009.
214. Wilson LE, Gallant JE. HIV/AIDS: the management of treatment-experienced HIV-infected patients: new drugs and drug combinations. *Clin Infect Dis* 2009;**48**:214–21.
215. Wong DT, Welliver RC, Riddlesberger KR, et al. Rapid diagnosis of parainfluenza virus infection in children. *J Clin Microbiol* 1982; **16**:164–7.
216. Wood DJ, Bijlsma K, de Jong JC, et al. Evaluation of a commercial monoclonal antibody-based enzyme immunoassay for detection of

adenovirus types 40 and 41 in stool specimens. *J Clin Microbiol* 1989;**27**:1155–8.
217. Yam P, Cornish N, Kruger R. The use of mixed shell vials for respiratory virus detection from clinical specimens. Abstract S17. Paper presented at the 15th Annual Clinical Virology Symposium. Clearwater, Fla; 1999.
218. Yamamoto M, Kimura H, Hironaka T, et al. Detection and quantification of virus DNA in plasma of patients with Epstein-Barr virus-associated diseases. *J Clin Microbiol* 1995;**33**:1765–8.
219. Yamamoto S, Pavan-Langston D, Kinoshita S, et al. Detecting herpesvirus DNA in uveitis using the polymerase chain reaction. *Brit J Ophthal* 1996;**80**:465–8.
220. Zeuzem S, Ruster B, Roth WK. Clinical evaluation of a new polymerase chain reaction assay (Amplicor HCV) for detection of hepatitis C virus. *Gastroenterol* 1994;**32**:342–7.
221. Zhao LS, Landry ML, Balkovic ES, et al. Impact of cell culture sensitivity and virus concentration on rapid detection of herpes simplex virus by cytopathic effects and immunoperoxidase staining. *J Clin Microbiol* 1987;**25**:1401–5.
222. Zipeto D, Revello MG, Silini E, et al. Development and clinical significance of a diagnostic assay based on the polymerase chain reaction for detection of human cytomegalovirus DNA in blood samples from immunocompromised patients. *J Clin Microbiol* 1992;**30**:527–30.

TABLE 253-1 **Diagnostic Stages and Anatomic Sites of Parasites**

	Blood	CNS	Eye	GI	G/U	Li/Sp	Lung	Lymph Node	Muscle	Skin	Other
Protozoa											
Acanthamoeba spp.		t	c/t							c/t	
Babesia spp.	**RBC**										Serology
Balantidium coli				**c/t**							
Cryptosporidium spp.				**oocyst**							
Cyclospora cayetanensis				**oocyst**							
Dientamoeba fragilis				**t**							
Entamoeba histolytica		t		**c/t**		t	t				Serology
Giardia lamblia				**c/t**							
Isospora belli				**oocyst**							
Leishmania donovani	A(**WBC**)					**A**					Serology
Leishmania spp.										**A**	Serology
Naegleria spp.		**t**								c/t	
Plasmodium spp.	**RBC**										Serology
Toxoplasma gondii	t(WBC)	c	c			c/t	c/t	c/t	c		Serology
Trichomonas vaginalis					t						
Trypanosoma cruzi	T(plasma)								**A**		Serology
Trypanosoma gambiense	**T(plasma)**	**T**									
Trypanosoma rhodesiense	**T(plasma)**	**T**									
Helminths											
Ancylostoma duodenale				**a/e**							
Ascaris lumbricoides				**a/e**			l				
Brugia malayi	**mf**							a			
Clonorchis sinensis				**e**		a					
Diphyllobothrium latum				**a/e**							
Dipylidium caninum				**a/e**							
Echinococcus spp.		l				l	l				Serology
Enterobius vermicularis				**a/e**							
Fasciola hepatica				**e**		a					
Fasciolopsis buski				**a/e**							
Heterophyes spp.				**a/e**							
Hymenolepis spp.				**a/e**							
Loa loa	**mf**		mf								
Metagonimus spp.				**a/e**							
Necator americanus				**a/e**							
Onchocerca volvulus			mf							**a/mf**	
Opisthorchis spp.				**e**		a					
Paragonimus spp.				e			**a/e**				
Schistosoma spp.	a			**e**	**e**						Serology
Strongyloides stercoralis				**a/l**							Serology
Taenia spp.		l	l	**a/e**						l	
Trichinella spiralis				a/l					**a**		Serology
Trichuris trichiura				**a/e**							
Wuchereria bancrofti	**mf**							a			

Note: Bold type indicates parasites most frequently detected at that source.
a, adult; *A,* amastigote; *CNS,* central nervous system; *c,* cyst; *e,* eggs; *GI,* gastrointestinal tract; *GU,* genitourinary tract; *l,* larvae; *Li/Sp,* liver/spleen; *mf,* microfilaria; *RBC,* red blood cell; *t,* trophozoite; *T,* trypomastigote; *WBC,* white blood cell.

examined before the etiologic agent can be detected (e.g., colon aspirates or biopsy tissue for *E. histolytica*, blood for malaria). All specimens must be considered infectious and must be handled with universal safety precautions using proper protective equipment.

The diagnosis of parasitic infections is dependent on macroscopic and microscopic examination of blood, feces, respiratory, tissue, and urine specimens. Specimens must be collected using methods that allow for the preservation of parasites when they are transported to the laboratory. Most stool specimens are collected at home or in a physician's office that may be a considerable distance from the laboratory performing the stool examination. Trophozoites disintegrate rapidly and do not form cysts after passage; therefore, stool specimens should be collected in containers from stool collection kits that have preservatives. The preservatives fix the specimens so that the trophozoites can be recognized easily on permanent stained slides and prevent the continued development of larvae and eggs.[3,11,13] Many laboratories have limited or no experience in detecting unusual infections such as malaria or filaria. It is recommended these specimens be sent to referral laboratories with greater experience.

If questions of when and how to collect specimens to maximize the recovery of parasites occur, the specific laboratory to be used should be consulted about the collection methods of choice.

STOOL SPECIMENS FOR DETECTION OF INTESTINAL PARASITES

Liquid or semisolid stool specimens should be examined within 30 to 60 minutes, respectively, from the time of collection for protozoan trophozoites. Because most clinical laboratories cannot perform the examination in this time frame, stool specimens should be placed in preservative immediately after passage (Table 253-2). Most stool collection kits contain a combination of vials such as sodium acetate–acetic acid–formalin (SAF) and polyvinyl alcohol (PVA); however, one-vial kits are becoming the system of choice.[3,11,13] Specimens should be collected before radiologic examination with barium is performed, which may prevent the detection of intestinal parasites for up to 10 days after administration. Other substances or medications that also can interfere with the detection of intestinal parasites for a prolonged period of time include antibiotics (aminoglycosides, metronidazole, and tetracycline), antimalarial agents, bismuth, mineral oil, and nonabsorbable antidiarrheal agents. The specimen should be collected in a clean, wide-mouthed container and must not be contaminated with toilet water or urine. Once collected, the specimen should be transferred to a container with preservative. After being mixed with the preservative, the specimen can be stored at room temperature until it is transported to the laboratory.

Physicians recommend that three specimens be collected on alternate days within 10 days to rule out most intestinal infections. The probability of detecting stool parasites with a single stool specimen is as low as 50 percent. Multiple specimens collected from the same patient on the same day should not be accepted. Many intestinal protozoa such as *Giardia* have a cyclical population cycle, and the concentration of trophozoites and cysts in the stool can vary considerably from day to day.

OTHER SPECIMENS FOR DETECTION OF INTESTINAL PARASITES

Sigmoidoscopy samples may be submitted for the detection of *E. histolytica*. No fewer than six samples (slides) should be submitted to the laboratory. Aspirates or scrapings can be collected and sent to the laboratory for direct wet mount observations, immunoassay, or polymerase chain reaction (PCR) (not commercially available). Preservatives such as Schaudinn fluid or PVA can be used to fix the samples to microscopic slides. Biopsy specimens should be submitted to the pathology laboratory for histologic preparation.

Duodenal aspirates and biopsy specimens can be collected for the detection of *Cryptosporidium*, *Giardia*, and *Strongyloides*. Collection techniques similar to those mentioned for the detection of *E. histolytica* can be used. In addition, duodenal contents may be sampled using the EnteroTest. The nylon string can be sent directly to the laboratory for processing and microscopic examination for parasitic organisms.

Strongyloides larvae might be detected in respiratory specimens during autoinfection or hyperinfection cycles. Serologic testing can be used to detect *Strongyloides* infections with low worm burdens.

E. vermicularis infections are detected best using the cellulose tape method. Specimens should be collected in the early morning before bathing or having a bowel movement. The adhesive side of the cellulose tape is pressed against the anal folds; and after completion of sampling, the tape is placed with the adhesive side down onto a microscope slide. *Enterobius* eggs and adults may be trapped on the tape (commercial collection kits are available).[3] Multiple specimens (four to six) may be collected on consecutive days to rule out the presence of infection. Eggs from infections with *Taenia* adult worms also may be detected using this method.

STOOL PROCESSING AND EXAMINATION FOR PARASITES

All specimens should be examined using a concentration technique and performing a permanently stained slide (Fig. 253-1). If fresh, unpreserved specimens are received, a direct wet mount should be performed for the detection of motile protozoan trophozoites and protozoan cysts. Helminth eggs and larvae also may be seen. If necessary, a few drops of saline may be used to emulsify the stool and a drop placed on a microscopic slide and covered with a 22 × 22-mm coverslip. The wet mount should be examined using 100× and 400× magnification. A drop of iodine solution may be added to highlight parasite internal structures; however, parasite motility will be lost. A concentrate and a permanent stained slide also should be prepared from this fresh specimen.

TABLE 253-2 **Common Intestinal Tract Specimen Preservatives**

Stool Preservative	Concentrate	Permanent Stain	Immunoassay	Advantages	Disadvantages
Formalin	Yes	No	Yes	Easy to prepare and has a long shelf life; preserves helminth eggs and larvae and protozoan cysts well; can be used with modified acid-fast and trichome stains; can be used with immunoassays.	Does not preserve trophozoite morphology adequately; not suitable for permanent stains; specimen does not adhere to slide.
MIF	Yes	No	No	Has a long shelf life; fixes and stains parasite forms simultaneously.	Two solutions must be mixed immediately before use; trophozoite morphology is poorly preserved; not suitable for modified acid-fast or trichrome stains; iodine interferes with immunoassays.
PVA	Yes	Yes	No	Excellent preservation of trophozoite morphology; suitable for PCR assays	Contains Hg making disposal quite costly; morphology of larvae and eggs makes identification difficult; unsuitable for modified stains; PVA interferes with immunoassays
PVA, modified	Yes	Yes	No	Does not contain mercury; disposal is less expensive	Copper or zinc (substitute for mercury) does not preserve trophozoite and cyst morphology as well as mercury, making identification difficult; PVA interferes with immunoassays.
SAF	Yes	Yes	Yes	Does not contain mercury; disposal is less expensive; has a long shelf life; permanent stain of choice is iron hematoxylin; preserves helminth eggs and larvae and protozoan cysts well; can be used with most immunoassays and special stains	Must use albumin-coated slides for permanent stains; contains formalin.
Schaudinn	No	Yes	No	Excellent preservation of trophozoite morphology	Contains Hg, making disposal quite costly; not recommended for concentrates and has poor adhesive properties for liquid and mucoid specimens.
Schaudinn, modified	Yes	Yes	Yes	Does not contain Hg, PVA, or formalin	Uses Zn as substitute for Hg. May need to coat slide with albumin for better specimen adhesion.

MIF, merthiolate-iodine-formalin; *PCR,* polymerase chain reaction; *PVA,* polyvinyl alcohol; *SAF,* sodium acetate–acetic acid–formalin.

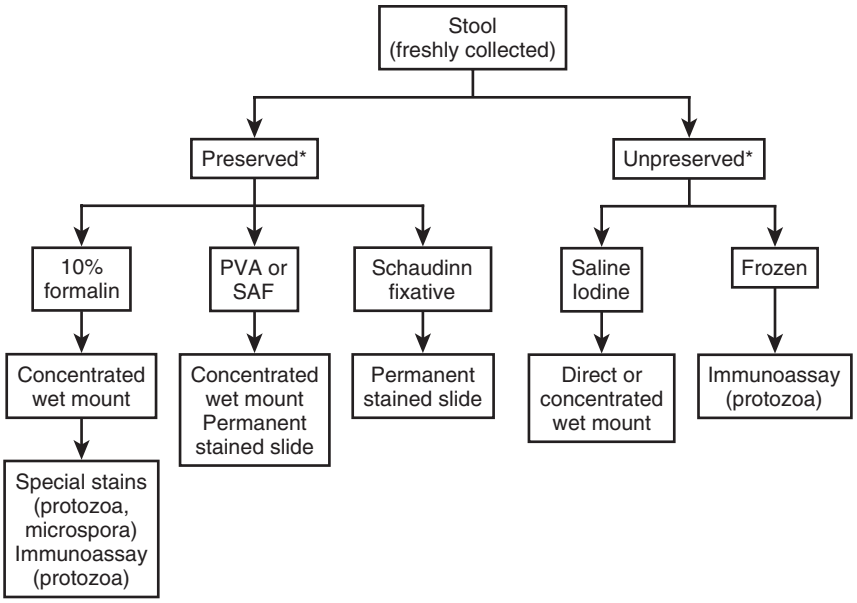

FIGURE 253-1 ■ Parasite stool examination. *Worms (cestodes, nematodes, trematodes)—fix in 70 percent ethanol. PVA, polyvinyl alcohol; SAF, sodium acetate–acetic acid–formalin.

Common concentration techniques include the flotation method using zinc sulfate ($ZnSO_4$) and the sedimentation method. Specimens fixed in formalin, merthiolate-iodine-formalin (MIF), PVA, SAF, or Schaudinn can be used. The flotation method gives a cleaner preparation to observe cysts and eggs, but the high specific gravity tends to distort cysts and eggs, rendering identification difficult. Heavier eggs (e.g., *Ascaris* or operculated eggs) will not float and will be found in the sediment; therefore, one should examine both top and bottom layers for a complete examination. The sedimentation method is used most widely in laboratories. Parasites and fecal debris are pelleted to the bottom of the tube by centrifugation. After the supernatant is discarded, the sediment is examined for parasites at 100× and 400× magnification.

Permanent stained slides should be prepared on every stool specimen that is examined for ova and parasites.[3,11,13] The stained slide is used primarily to detect and identify protozoa, trophozoites, and cysts. Gomori trichrome (Wheatley modification) and iron hematoxylin stains frequently are used in laboratories. Both stains have advantages and disadvantages that should be weighed, as should the expertise of laboratory personnel, before selecting the stain of choice for the laboratory.

Certain parasites are not detected easily by wet mount or routine permanent stained slides stained with Gomori trichrome (Wheatley modification) and iron hematoxylin. They include *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Isospora belli*, and microsporidia. The oocysts of *Cryptosporidium*, *Cyclospora*, and *Isospora* can be detected easily by the modified acid-fast stain. Microsporidia can be detected using a modified trichrome stain.[8,13]

Culture of fresh stool or contents of the large intestine have been used to detect *E. histolytica;* however, this methodology is not practical for most clinical laboratories.

EIA kits are commercially available for the detection of *E. histolytica/Entamoeba dispar* group, *G. lamblia*, and *Cryptosporidium*. For the *E. histolytica/E. dispar* group, only fresh or frozen stool can be used in the assay. Formalin interferes with reagents to detect *E. histolytica* and cannot be used. EIA kits are available that will discriminate between these organisms using fresh or frozen stool. EIA kits for the detection of *G. lamblia* and *Cryptosporidium* can be used with fresh, frozen, or formalin fixed stool. DFA tests have been developed to detect *G. lamblia* and *Cryptosporidium.* Depending on the manufacturer, either fresh or formalinized specimens can be used. PCR analysis also has been used to detect *E. histolytica*, *G. lamblia*, and *Cryptosporidium.* PCR test kits are not commercially available, whereas DFA and EIA kits are readily available. The DFA and EIA kits have excellent sensitivity and specificity compared with microscopy. The disadvantage of using DFA or EIA kits is that they detect only one or two pathogens at one time so other pathogens might be missed. Additional parasite examinations would have to be performed to detect other parasitic pathogens.

Detection of *Strongyloides stercoralis* is enhanced by culturing a small amount of fresh stool on an agar plate and looking for larval tracks across the plate, as evidenced by lines of bacterial growth.[4] Other methods include the Baermann concentrate, Harada-Mori, or Petri dish filter paper slant.[3,11,13] It is possible that hookworms eggs could hatch and the larvae would have to be differentiated from *Strongyloides* larvae.

BLOOD PARASITES

Numerous important parasites can be detected in blood, although most laboratories will see these organisms only during proficiency testing (Fig. 253-2).[6,7,10] These parasites include *Babesia*, *Leishmania donovani*, microfilaria,

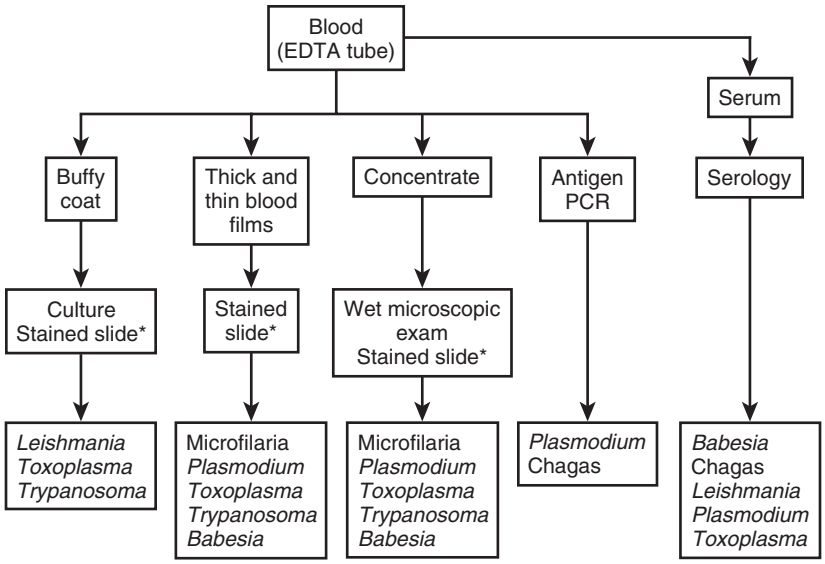

FIGURE 253-2 ■ Blood parasite examination. *Preferred stain is Giemsa; however, Wright stain is also acceptable. PCR, polymerase chain reaction.

Plasmodium, and *Trypanosoma*. They may be found in CSF, macrophages, plasma, tissue, or red blood cells depending on the parasite. Maintaining proficiency to speciate *Plasmodium* or to recognize the amastigote stages of *Leishmania* is difficult for many laboratory technicians because these infections are seen so infrequently in the United States. A very important factor is that the physician inform the laboratory personnel about the travel and medication history of the patient. If the patient took partial prophylaxis for malaria, a longer time should be spent examining the blood film owing to low parasitemia. Similarly, differences in periodicity of microfilaria in the blood exist and the timing of collection of specimens becomes more important. Alternative concentration techniques not normally used in most laboratories, such as Nuclepore filtration or the Knott concentration procedure, can be used to detect microfilaria in low numbers in the blood, and Nuclepore filtration also may be used with urine.[3,11,13] Without the knowledge of what infection the physician may be considering, these concentration procedures are not routinely performed on blood or other specimens and the diagnosis of infections could be delayed or missed. Requests for the diagnosis of malaria must be considered to be STAT requests owing to the morbidity and mortality mostly associated with *Plasmodium falciparum* infections.

Although some parasites (trypanosomes and microfilaria) can be detected by their motility in whole blood, identification is dependent on morphology as seen with a permanent stain. The blood film stain of choice is Giemsa, although Wright stain can also be used, or Delafield hematoxylin stain can be used for the microfilarial sheath. Because Giemsa stains may not differentiate the microfilaria nuclei adequately, Delafield hematoxylin stain may be necessary to speciate the microfilaria.

Although blood films can be prepared in clinics or at the bedside (finger or ear lobe sticks), the recommendation is that blood be collected in ethylenediaminetetraacetic acid (EDTA, lavender-topped tube) so that multiple slides (thick and thin blood films) may be prepared in the laboratory and concentration methods be used. EDTA-collected blood provides the best overall morphology of parasites, and the tubes should be filled to the proper level to reduce distortion of the blood cells and organisms by the anticoagulant. Heparin tubes (green-topped tubes) also can be used; however, these blood-filled tubes cannot be used for PCR testing. The blood should be examined for parasites within 4 to 6 hours of being collected.

Blood should be drawn immediately when parasitemia is suspected. For malaria, blood should be drawn every 6 to 8 hours for 36 hours to rule out infection. Pediatric patients may have blood samples drawn at 10-hour intervals. One set of negative blood films does not rule out the presence of malaria. When the parasitemia level is low, procedures other than thick films, such as antigen detection, serology, or PCR analysis may be the more sensitive method to detect *Babesia*, Chagas disease, malaria, or microfilaria.[7] Serology has not been shown to be an effective method to detect active infections of African trypanosomiasis in nonendemic areas.

Blood Processing and Examination for Parasites

If the physician does not indicate the specific blood parasite suspected, fresh blood should be used as a wet mount to look for the presence of microfilaria or trypanosomes by their characteristic shape and motility. If these parasites are detected, a permanent stained slide will have to be performed to identify the organisms to species. When thick and thin blood films are examined for the presence of parasites, no fewer than 300 microscopic oil fields should be examined before finalizing a result for the presence of *Babesia* or malaria. One may use lower magnification when looking for the presence of trypanosomes or microfilaria, but the entire film should be observed before finalizing a result. Thin films are used for the detection

of blood parasites, for enumeration of infected cells, and also to determine the species of parasite. Once the films are air dried, the film can be fixed with 100 percent methanol and stained with Giemsa stain or Wright stain without the methanol fixation process. If *Babesia* or malarial parasites (*P. falciparum* and *Plasmodium vivax*) are present, one should quantitate the number of infected red blood cells. This quantification can be expressed as the number of infected cells/100 red blood cells observed in the blood film. Serial specimens collected at various times during therapy can be used to monitor the success or failure of therapy. Continued presence of parasites in blood films without a marked decrease in parasitemia within the first 24 hours is an indication of therapeutic failure.

Thick films are prepared with one or two drops of blood that should be stirred in a small circle for 10 to 20 seconds at various intervals as it dries on the slide to prevent the blood from sloughing from the slide during the staining process. The thicker the film, the more likely the blood will slough. Detecting and identifying the presence of parasites on thick films is extremely difficult, particularly with inexperienced laboratory personnel. Once the thick film has dried, the blood film is laked with water or buffer before staining if Wright stain is used, or the films can be placed directly into Giemsa stain without prior laking. Laking disrupts the red cell membrane, allowing the hemoglobin to be removed and leaving parasites, white blood cells, and platelets intact. The search for microfilaria can be performed at a lower magnification, whereas the search for *Babesia*, malaria, and trypanosomes must be done with oil magnification looking at 200 to 300 oil microscopic fields.

Concentration techniques also can be used to detect *Babesia*, *Leishmania*, malaria, microfilaria, and trypanosomes. Microhematocrit capillary tubes can be centrifuged, and the parasitized red blood cells and trypanosomes can be detected near the buffy coat interface. The tube can be scored and snapped, and the buffy coat can be used to prepare a thin film. Alternatively, the tube can be observed under a microscope at the buffy coat interface for the presence of motile trypanosomes.

Microfilaria can be concentrated using the Knott procedure or Nuclepore membrane filtration technique. Normally, a 5-μm filter is used, but if *Mansonella perstans* microfilaria is suspected, a 3-μm filter is recommended. Most laboratories would not be familiar with or normally use concentration techniques unless specifically asked to do so.

Antigen tests for malaria have been developed and approved for clinical use in the United States.[5,7] These tests are extremely useful for laboratories that have limited expertise and exposure to specimens containing malaria or *Babesia*. It is difficult to differentiate malaria from babesiosis in patients with infections with very young trophozoites. In this situation, the malarial antigen test can be very helpful. The antigens detected in these tests include histidine-rich protein-2, lactate dehydrogenase, and aldolase. False-negative antigen test results are known to occur owing to mutations of histidine-rich protein-2 in *P. falciparum* infection. The sensitivity of the antigen tests to detect infections cause by *P. vivax*, *Plasmodium malariae*, or *Plasmodium ovale* is considerably reduced compared with *P. falciparum*. The histidine-rich protein-2 remains in the blood for a considerable period of time after therapy so it cannot be used to follow therapy.

UROGENITAL SPECIMENS

Trichomonas vaginalis is the most common pathogen; however, schistosome eggs, microfilaria, and microsporidium also may be recovered from urogenital specimens. Many *T. vaginalis* infections are diagnosed on clinical signs and symptoms without a specimen being collected. The detection of *T. vaginalis* usually is based on direct observation in wet mounts prepared from vaginal and urethral discharges, prostatic secretions, and urine sediment. Urine specimens should be obtained from first-voided urine in the morning. Specimens collected for the detection of *T. vaginalis* should not be refrigerated because *T. vaginalis* is susceptible to cold temperatures. Specimens can be diluted with a drop of saline and observed under low power in reduced illumination for the presence of motile flagellates. Stained slides, such as Giemsa or Papanicolaou smears, also can be used, but the majority of the infections with *T. vaginalis* are identified by the direct wet mount.

Culture (InPouch TV) can be used and is recommended by the Centers for Disease Control and Prevention (CDC) for patients suspected of harboring *T. vaginalis* infections when no organisms were detected by the direct wet mount method.[1] Specimens can be collected with a brush, swab, or sponge. Specimens should be inoculated as soon as possible into the media. Cultures showing no organisms after incubation for 4 days can be reported as negative. Commercially available DFA, EIA, and DNA probe kits have been approved by the U.S. Food and Drug Administration for the detection of *T. vaginalis*.[2,3,11,13] Alternative detection methods can be quite expensive owing to limited test utilization depending on the prevalence of infections in the area or community.

The Knott concentration procedure and the Nuclepore membrane filtration technique have been used for the detection of microfilaria in the urine. Microfilaria most frequently detected using these methods are *Wuchereria bancrofti* and *Onchocerca volvulus*.

Schistosoma hematobium eggs found in the urine can be concentrated by sedimentation, centrifugation, or the Nuclepore membrane filtration procedure. The preferred specimen is urine collected at midday, when the patient is most active and most likely to have eggs in the urine.

Serology

Definitive diagnosis of parasitic infections is usually made by detection of diagnostic parasite forms in blood, urine, other body fluids, tissue, or feces. A serologic diagnosis may be required when the parasites reside in tissues or other body sites and it is difficult to acquire a diagnostic specimen (e.g., in a patient with chronic Chagas disease or neural cysticercosis). Serology testing may also be

useful when parasites are in very low numbers in specimens and are difficult to detect (e.g., in a patient with strongyloidiasis, malaria, or babesiosis).

A positive serologic test in an individual who resides in an endemic area must be interpreted with caution because the result may reflect past exposure and be unrelated to current clinical condition. A positive test in an individual without any past exposure may indicate a recent infection; however, this result must be correlated with the patient's clinical picture.

The detection of IgA, IgE, IgG, and IgM antibodies to specific parasite antigens may or may not be helpful in establishing a diagnosis. With the exception of serology tests for the diagnosis of toxoplasmosis and the screening of the blood supply for antibodies to *Trypanosoma cruzi*, most of the other parasitic serology tests have limited laboratory availability. Although these tests will have been validated through in-house testing, except for *Toxoplasma* and *T. cruzi*, there are no established external proficiency testing programs such as those offered through the College of American Pathologists. Even though the test methodology may be the same between different laboratories, reagents used in the tests have not been standardized between laboratories if they do not use the same kit manufacturer. This can lead to varying results.

In the United States, most serologic tests for the diagnosis of a parasitic infection are limited to the most commonly acquired extraintestinal infections. These are available at a few referral laboratories, specialized testing centers and the CDC. Information on availability of parasite serology tests can be acquired from referral laboratories or from CDC.* The CDC offers the most comprehensive services for tests available and will also provide some information on unusual serologic testing not available in the United States.

OTHER SPECIMENS

Other important pathogenic parasites, such as ocular infections with *Acanthamoeba* and *Toxoplasma gondii*, can be detected in other anatomic sites and are addressed in specific chapters.

NEW REFERENCES SINCE THE SIXTH EDITION

3. Garcia LS. *Diagnostic medical parasitology*. 5th ed. Washington, D.C.: ASM Press; 2007.
5. Murray CK, Gasser RA Jr, Magill AL, et al. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev* 2008;**21**:97–110.
6. National Committee for Clinical Laboratory Standards/Clinical and Laboratory Standards Institute. *Laboratory diagnosis of bloodborne diseases: approved guidelines M15-A*. Wayne, Pa.: Clinical and Laboratory Standards Institute; 2000.
7. Rosenblatt JE. Laboratory diagnosis of infections due to blood and tissue parasites. *Clin Infect Dis* 2009;**49**:1103–8.
10. Vannier E, Krause PJ. Human babesiosis. *N Engl J Med* 2012;**366**:2397–407.
11. Versalovic J. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press; 2011.

The full reference list for this chapter is available at expertconsult.com.

*Inquiries can be made to DPDX@CDC.gov or by calling the CDC division of Parasitic Diseases at (404)718-4100.

REFERENCES

1. Borchardt KA, Smith RF. An evaluation of an InPouch TV culture method for diagnosing *Trichomonas vaginalis* infections. *Genitourin Med* 1991;**67**:149–52.
2. Briselden AM, Hillier SL. Evaluation of Affirm VP microbial identification test for *Gardnerella vaginalis* and *Trichomonas vaginalis*. *J Clin Microbiol* 1994;**32**:148–52.
3. Garcia LS. *Diagnostic medical parasitology*. 5th ed. Washington, D.C.: ASM Press; 2007.
4. Koga KS, Kasuya C, Khamboonruang K, et al. A modified agar plate method for detection of *Strongyloides stercoralis*. *Am J Trop Med Hyg* 1991;**45**:518–21.
5. Murray CK, Gasser RA Jr, Magill AL, et al. Update on rapid diagnostic testing for malaria. *Clin Microbiol Rev* 2008;**21**:97–110.
6. National Committee for Clinical Laboratory Standards/Clinical and Laboratory Standards Institute. *Laboratory diagnosis of blood-borne diseases: approved guidelines M15-A*. Wayne, Pa.: Clinical and Laboratory Standards Institute; 2000.
7. Rosenblatt JE. Laboratory diagnosis of infections due to blood and tissue parasites. *Clin Infect Dis* 2009;**49**:1103–8.
8. Ryan NJ, Sutherland G, Coughlan K, et al. A new trichome-blue stain for detection of microsporidial species in urine, stool and nasopharyngeal specimens. *J Clin Microbiol* 1993;**31**:3264–9.
9. Scheffler EH, Van Etta LL. Evaluation of rapid commercial enzyme immunoassay for detection of *Giardia lamblia* in formalin-preserved stool specimens. *J Clin Microbiol* 1994;**32**:1807–8.
10. Vannier E, Krause PJ. Human babesiosis. *N Engl J Med* 2012; **366**:2397–407.
11. Versalovic J. *Manual of clinical microbiology*. 10th ed. Washington, D.C.: ASM Press; 2011.
12. Weber R, Bryan RT, Schwartz DA, Owen RL. Human microsporidial infections. *Clin Microbiol Rev* 1994;**7**:426–61.
13. Winn WC Jr, Allen SD, Janda WM, et al.: *Koneman's color atlas and textbook of diagnostic microbiology*. 6th ed. Baltimore: Lippincott Williams & Wilkins; 2006.

This page intentionally left blank

INDEX

A

Abacavir, 2613
Abdominal abscess, **704-705**
  anaerobic bacteria in, 1829
  clinical manifestations of, 704-705
  complications of, 705
  diagnosis of, 705
  Eikenella corrodens, 1577
  Escherichia coli, 1445
  fever of unknown origin in, 845
  treatment of, 705
Abdominal pain
  in appendicitis, 680-681
  in cholecystitis, 669
  in *Dientamoeba fragilis* infection, 2885
  differential diagnosis of, 681, 682t
  in enteroviral infection, 2070, 2072t
  Helicobacter pylori infection and, 1694
  in hepatitis, 648-649
  in pancreatitis, 689
  in peritonitis, 701
  in pyogenic liver abscess, 674
  in pyomyositis, 736-737
  in Whipple disease, 643
Abdominal wall, *Neisseria gonorrhoeae* infection of, 1288
Abortion
  enterovirus infection and, 2091-2092
  poliomyelitis and, 2100
  septic, 1797
Abscess
  abdominal. *See* Abdominal abscess
  abdominal wall, *Neisseria gonorrhoeae*, 1288
  adrenal, 706
  Bartholin duct, 579, 579f
  Bezold, 236, 239
  brain. *See* Brain abscess
  breast, **890**
  Brodie, 719
  epidural
    vs. acute transverse myelitis, 523
    cerebral, 443t, **470**
    spinal, 443t, **470-472**, 472f
  gallbladder, 670
  hair follicle, **776-777**
  head and neck. *See* Head and neck infection
  hepatic. *See* Liver abscess
  iliopsoas, 706-707, 707f
  intra-abdominal. *See* Abdominal abscess
  intraperitoneal, 704
  kidney. *See* Renal abscess
  liver. *See* Liver abscess
  lung. *See* Lung abscess
  muscle, **735-739**, 736t, 737f-738f. *See also* Myositis; Pyomyositis
Abscess *(Continued)*
  orbital, 800-801, 800f
  palatal, 141, 143f
  pancreatic, 696, 704-708
  parapharyngeal, 170f, **172-175**
  pelvic, **687**
  periapical, 141, 142f-143f, 144, **146**
  perinephric, 706
  peritoneal, 700-701. *See also* Peritonitis
  peritonsillar, **168-170**, 168f, 170f, 173-175
  psoas, 706
  renal, **553-558**, 555f-557f
  retroperitoneal, **706-709**, 707f
  retropharyngeal, **170-175**, 170f-171f
  scalp, 891, 1282-1283, 1295
  splenic, 673, 704-705
    in infective endocarditis, 355, 357
  submandibular, 149, 150f
  submental space, 142, 144f
  subperiosteal, mastoiditis and, 236, 239
  subphrenic, 704-705
  in toxic shock syndrome, 851t, 852
  tubo-ovarian, **590**
  visceral, 704
Absidia spp. infection
  after hematopoietic stem cell transplantation, 938
  after kidney transplantation, 1013
  neonatal, 899
ACAID (anterior chamber–associated immune deviation), 810
Acanthamoeba polyphaga mimivirus infection, **2048-2050**, 2049f-2050f
Acanthamoeba spp., 2977-2978, 2978f
  as pathogen reservoir, 2985
Acanthamoeba spp. infection (acanthamebiasis), **2975-2986**
  clinical manifestations of, 2979-2980
  CNS, 494t-498t, 501, 2975, 2979, 2981, 2984-2985
  contact lens–related, 1019, 2979-2980
  corneal, 808-809, 2977, 2979-2980, 2980f, 2985
  cutaneous, 2979, 2981, 2984
  diagnosis of, 2981-2983
  epidemiology of, 2975-2977
  neuroimaging in, 2980
  pathogenesis of, 2981
  pathology of, 2981
  prevention of, 2985-2986
  treatment of, 2983-2985
Acetaminophen
  in acute bronchitis, 264
  in common cold, 137
  in fever, 87
  vs. ibuprofen, 87-88
  ibuprofen with, 87
  in otitis media, 228
  in pharyngitis, 164
  in roseola infantum, 771
  in varicella, 85
Achromobacter spp., 1573
Achromobacter spp. infection, **1573-1575**
  clinical manifestations of, 1574-1575
  diagnosis of, 1575
  epidemiology of, 1573-1574
  pathophysiology of, 1574
  treatment of, 1575
Achromobacter xylosoxidans infection, in cystic fibrosis, 336, 341
Acid-base balance, 75-76
Acidosis
  lactic, in septic shock, 829, 832
  metabolic, 75-76
    in malaria, 2928
    in septic shock, 832
  respiratory, 75
Acinetobacter spp., 1568-1569
Acinetobacter spp. infection, **1568-1572**
  bloodstream, 1570
  burn wound, 1052-1053
  clinical manifestations of, 1569-1571
  diagnosis of, 1571
  epidemiology of, 1569, 1570t
  intracranial, 1570
  after lung transplantation, 962
  nosocomial, 1569
  pathogenesis of, 1569
  prevention of, 1572
  prognosis for, 1572
  respiratory tract, 1570-1571
  treatment of, 1571-1572
  urinary tract, 1571
Acoustic reflectometry, 218
Acquired immunodeficiency syndrome (AIDS). *See* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS)
Acremonium spp. infection, 479, **2857-2858**
Acrodermatitis, papular (Gianotti-Crosti syndrome), 649, 749, **766**, **783**, 783f
  in HAV infection, 2140
Acrodermatitis chronica atrophicans, in Lyme disease, 1734
ActA protein, 16
Acticoat, in burn injury, 1059
Actin, neutrophil, dysfunction of, 54
Actin tail
  of *Listeria monocytogenes*, 16
  of *Shigella flexneri*, 16
Actinomyces spp. infection (actinomycosis), **1820-1824**
  abdominal, 1821-1822
  bone, 725
  cervicofacial, 184, 1821, 1821f
  clinical manifestations of, 1821-1823, 1821f-1822f
  diagnosis of, 1823-1824
  hepatic, 1821-1822
  in immunocompromised host, 1823
  laryngeal, 1822-1823
  meningeal, 1822-1823

Page numbers followed by *f* indicate figures; t, tables; *b*, boxes. Page numbers in boldface refer to main discussions.

Actinomyces spp. infection (actinomycosis) *(Continued)*
parotid gland, 191-192
pathogenesis of, 1820-1821
pathology of, 1821, 1821f
pelvic, 1822
pulmonary, 1822, 1822f
retroperitoneal, 706-707, 709
treatment of, 1824
Acute disseminated encephalomyelitis (ADEM), 503, **513-520**
clinical manifestations of, 514
diagnosis of, 513-514, 514t, 515f
epidemiology of, 513
evaluation of, 518-519
immunologic factors in, 517-518
infection and, 515-516, 516t
measles virus, 2392
pathogenesis of, 518
prognosis for, 520
recurrence of, 514-515, 520
treatment of, 519-520
vaccine-associated, 516-517, 516t
variants of, 514-515
Acute hemorrhagic leukoencephalitis, 515
Acute lobar nephropathy, 539, 554, 555f, 556
Acute lung injury (ALI), 867. *See also* Acute respiratory distress syndrome (ARDS)
Acute lymphoblastic leukemia, human bocavirus infection in, 1861
Acute mountain sickness, 3118
Acute necrotizing ulcerative gingivitis, 147, 147f
Acute-phase reactants, 70
Acute-phase reaction, 65, 66f, 79-80. *See also* Metabolism
Acute respiratory distress syndrome (ARDS), **866-875**
clinical course of, 867t, 868-869
definition of, 867, 867t
etiology of, 867-868, 867t
exudative stage of, 869
fibrotic stage of, 870
immunomodulators in, 870
incidence of, 868
in influenza virus infection, 2352
mortality with, 869
pathology of, 869-870
pathophysiology of, 869-870
proliferative stage of, 869-870
treatment of, 870-872
corticosteroids in, 872-873
EMCO in, 873-874
fluid therapy in, 873
isolation in, 873
mechanical ventilation in, 870-872
nitric oxide in, 872
nutrition in, 873
oxygenation goals in, 871
prone positioning in, 873
rescue therapies in, 873-874
sedation and analgesia in, 873
surfactant replacement in, 872-873
Acute retroviral syndrome, 161
Acute transverse myelitis, **520-528**
clinical presentation of, 521-522
CSF examination in, 523
diagnosis of, 520, 521t
differential diagnosis of, 523-525, 523t, 524f
disability after, 528
disease-associated, 523-525, 525t-526t
epidemiology of, 521
idiopathic, 523, 527
Acute transverse myelitis *(Continued)*
imaging in, 522, 522f
immune system in, 527
infection and, 526-527, 526t
postinfectious, 526-527, 526t-527t
prognosis for, 528
radiation-induced, 524
recurrence of, 528
treatment of, 527-528
Acute urethral syndrome, 531
Acyclovir, 3277-3280, 3277f
adverse effects of, 3280
after bone marrow transplantation, 2032
in bone marrow transplantation, 1966
in CMV infection, 1984-1987, 1985f
in CMV infection prevention, 1988
dosage for, 3278t, 3280
in erythema multiforme, 1951
in heart transplantation, 949
in hematopoietic stem cell transplantation, 940-941
in herpes labialis, 155
in herpes zoster ophthalmicus, 804
in HSV infection, 1958-1964
indications for, 3278t, 3279-3280
in infectious mononucleosis, 2012
in kidney transplant–related VZV infection, 1009
in lung transplantation, 975
mechanism of action of, 3277-3278
in neonatal HSV infection, 819, 1962-1963
pharmacokinetics of, 3278-3279
during pregnancy, 1967
resistance to, 3277-3278
spectrum of activity of, 3277
structure of, 1985f
in VZV infection, 2028-2029
Adefovir dipivoxil, in HBV infection, 1922
Adenitis, *Streptococcus agalactiae*, 1161
Adeno-associated viruses, 1891
Adenoidectomy
in otitis media, 232
in otitis media with effusion, 229
in rhinosinusitis, 202
Adenosine deaminase
deficiency of, **48-49**
in severe combined immunodeficiency, 915
Adenotonsillectomy, 164
Adenovirus(es), 1888-1891
animal susceptibility of, 1891
antigenic composition of, 1890
classification of, 1888-1889, 1889t
multiplication of, 1890-1891
physical properties of, 1889-1890
tissue culture growth of, 1890
Adenovirus infection, **1888-1911**
age and, 1891
appendiceal, 1896t, 1902
bladder, 1901
bronchial, 262, 263t, 1896t, 1897
bronchiolitis obliterans in, 1896t, 1898
clinical manifestations of, 1895-1905, 1896t
CNS, 494t-498t, 1894, 1896t, 1903
coinfection with, 1893-1894
congenital, 1905
conjunctival, 802-803, 1896t, 1898-1899
cytokines in, 1895
diagnosis of, 1906-1907, 3611-3612, 3617
differential diagnosis of, 1905-1906
in dilated cardiomyopathy, 1902
Adenovirus infection *(Continued)*
encephalitic, 494t-498t, 1896t, 1903
vs. St. Louis encephalitis virus infection, 2259
epidemiology of, 1891-1893
exanthem in, 741, 742t-748t, 760, 1896t, 1900-1901
fecal virus excretion after, 1893
gastrointestinal tract, 607t, 608, 618-619, 1896t, 1901-1902
genitourinary tract, 1896t, 1901
hearing loss in, 1904
after heart transplantation, 955
after hematopoietic stem cell transplantation, 941-942
hemolytic-uremic syndrome in, 1901
hemorrhagic fever in, 1901
hepatic, 655, 1894, 1902
historical perspective on, 1888
host factors in, 1892
hyperlucent lung in, 1898
immune response to, 1894-1895
immunization against, 1908
in immunocompromised host, 1903-1904
after intestinal transplantation, 997t, 999
in intussusception, 1902
joint, 1904
keratoconjunctival, 1899-1900
after kidney transplantation, 1007, 1010-1011
laryngotracheal, 1897
after liver transplantation, 997t, 999
after lung transplantation, 981-982
meningeal, 488, 1896t, 1903
in mesenteric lymphadenitis, 1902
in military recruits, 1892, 1896-1898, 1896t
myocardial, 1896t, 1902
nasopharyngeal, 156, 157t, 158, 1895-1896, 1896t
neonatal, 1905
nosocomial, 1893, 3382
obesity and, 1905
ocular, 1896t, 1898-1900
oculogenital, 1901
pancreatic, 694
pathogenesis of, 1893-1894
pathology of, 1894-1895, 1894f
pericardial, 1902-1903
pertussis-like illness with, 1617, 1898
pharyngeal, 158-161, 159t-160t, 1895-1896, 1896t
pharyngoconjunctival fever in, 1893-1894, 1899, 1899t-1900t
in preseptal cellulitis, 799
prevalence of, 1891
prevention of, 1908
pulmonary, 284, 295-296, 1894, 1894f, 1896t, 1897-1898
renal, 1901
respiratory tract, 1895-1898, 1896t
roseola with, 769
seasonal patterns of, 1892
social factors in, 1892
testicular, 1901
thyroid, 1904
tonsillar, 1895-1896
transmission of, 1892-1893
treatment of, 1907-1908
Adenylate cyclase toxin, 17
of *Bordetella pertussis*, 1618t, 1619, 1624
Adhesins
nonpilus, 6-9, 7f-9f
pilus, 3-6, 4f-5f
Adhesions, peritoneal, 687-688

Adoptees. *See* International adoptees and refugees
Adrenal gland
  abscess of, 706
  in *Corynebacterium diphtheriae* infection, 1305
  hormones of, 81-82
  in *Leptospira* spp. infection, 1748
Adult respiratory distress syndrome. *See also* Acute respiratory distress syndrome (ARDS)
  in septic shock, 830, 832, 835
  in toxic shock syndrome, 858
Adult T-cell leukemia, HTLV-1 and, 2565-2569, 2566t-2567t
Aedes aegypti, 2243-2244
  in dengue, 2279-2280
  eradication and control of, 2289-2290
  in yellow fever, 2274, 2278
Aedes albopictus, 2242-2243
Aedes vigilax, 2248-2249
Aerobactin, 538
Aerococcus spp., 1248-1249
Aerococcus spp. infection, **1248-1250**
  clinical manifestations of, 1249
  diagnosis of, 1249
  epidemiology of, 1249
  pathophysiology of, 1249
  treatment of, 1249-1250
Aerolysin, 1540
Aeromonas caviae infection, 1542
Aeromonas hydrophila infection
  diagnosis of, 618, 1539-1540
  ecthyma gangrenosum with, 1543
  epidemiology of, 1538-1539
  gastrointestinal, 607t, 612, 616t, 623t, 1541-1542
  pathogenesis of, 1540-1541
  pulmonary, 1543
  urinary tract, 1543
  wound, 1542
Aeromonas spp., 1539-1540
  virulence factors of, 1540-1541
Aeromonas spp. infection, **1538-1545**
  clinical manifestations of, 1541-1543
  diagnosis of, 1539-1540, 1543-1544
  epidemiology of, 1538-1539
  pathogenesis of, 1540-1541
  treatment of, 1540, 1544
Aflatoxin, 2705
African sleeping sickness. *See Trypanosoma brucei gambiense* infection (African sleeping sickness); *Trypanosoma brucei rhodesiense* infection (African sleeping sickness)
Agammaglobulinemia, 907t
  X-linked, 46-47, **908-909**
    vs. chronic bronchitis, 267
    diarrhea in, 600
Age
  adenovirus infection and, 1891
  Babesia spp. infection and, 2918
  chronic fatigue syndrome and, 1087
  coronavirus infection and, 2489-2490
  Eastern equine encephalitis and, 2227
  in epidemiologic study, 108
  Escherichia coli infection and, 1450-1451, 1450t
  Haemophilus influenzae type b infection and, 1674
  HAV infection and, 2132-2133
  HSV infection and, 1936
  infant botulism and, 1803-1804, 1803f
  intestinal transplantation and, 990
Age *(Continued)*
  Kawasaki disease and, 1066-1067
  liver transplantation and, 990
  measles and, 2376, 2376t
  PANDAS and, 1145
  Paracoccidioides spp. infection and, 2781-2782, 2782t
  parainfluenza virus infection and, 2363-2364, 2363f
  rotavirus infection and, 2182
  RSV infection and, 2412-2413, 2413t, 2417f
  rubella virus infection and, 2200-2201, 2200t-2201t, 2201f
  Salmonella spp. infection and, 1493-1494
  St. Louis encephalitis virus infection and, 2257, 2257f, 2260
  Streptococcus pneumoniae infection and, 1199-1200, 1199f-1200f, 1202-1203, 1203f
  toxic shock syndrome and, 850, 850f
  tuberculosis and, 1342
Aggregatibacter actinomycetemcomitans infection, **1610-1611**
  in actinomycosis, 1820, 1823-1824
  periodontal, 140, 147, 1823
  treatment of, 1824
Aggregatibacter (Haemophilus) aphrophilus infection, **1685-1686**
Agranulocytosis, infantile (Kostmann syndrome), 917
AICDA, 909
AIDS (acquired immunodeficiency syndrome). *See* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS)
Air crescent sign, in *Aspergillus* spp. infection, 2712
Air filtration, in lung transplant–related *Aspergillus* infection prevention, 970
Air pollution, cough and, 268
AIRE, 915-916
Airplane travel, rhinosinusitis and, 195
Airway
  diameter of, bronchiolitis and, 276
  inhalation injury of, 1048-1049
  management of
    in epiglottitis, 257-258
    in laryngotracheitis, 257
    in septic shock, 835
  obstruction of
    in EBV infection, 2004
    in Ludwig angina, 149-150
Alanine aminotransferase (ALT)
  in HAV infection, 2130f, 2138
  in hepatitis, 650
Albaconazole, in *Scedosporium* spp. infection, 2850
Albendazole, 3347
  in *Echinococcus granulosus* infection, 3046
  in *Enterobius vermicularis* infection, 567, 3019
  in eosinophilic meningitis, 483-484
  in *Toxocara canis* infection, 3023
Albumin
  serum, in pyogenic liver abscess, 674
  urinary, in yellow fever, 2276
Alcohol use/abuse
  diphtheria and, 1303
  pancreatitis with, 691
Aldosterone, 81
Aleppo button. *See Leishmania* spp. infection (leishmaniasis), Old World
Alfuy virus infection, 2316t
Alice in Wonderland syndrome, 505, 2004
Alkalosis, respiratory, 75
Allergic bronchopulmonary aspergillosis, 2708-2709
  in cystic fibrosis, 341-342
  treatment of, 2721
Allergic rhinitis, vs. common cold, 136
Allergy/allergic reaction. *See also* Anaphylaxis
  cephalosporin, 1296, 3199
  croup and, 247
  egg, 3426, 3451
  vs. infection-related rash, 760
  influenza vaccine, 2352
  mumps vaccine, 2405-2406
  otitis media and, 211
  penicillin, 3189-3191, 3189t
  probiotics in, 3371
  rhinosinusitis and, 195, 201
  vs. roseola infantum, 771
Alligator bite, 3578
Alloiococcus otitidis infection, 214
Allopurinol, in visceral leishmaniasis, 2958
Alopecia areata, vs. tinea capitis, 785
Alveolar macrophages, in *Streptococcus pneumoniae* infection, 1225
Amantadine, 3268-3270, 3268f, 3268t
  in laryngotracheitis, 257
Amebiasis. *See Entamoeba histolytica* infection (amebiasis)
Ameboma, 2869-2870
Amikacin, 3208-3211, 3208t, 3231t-3234t
Amino acids
  metabolism of, 70-72
  requirements for, 71-72
Aminoglycosides, 3208-3211, 3208t, 3231t-3233t
  adverse effects of, 3210-3211
  indications for, 3210
  mechanisms of action of, 3208
  pharmacokinetics of, 3209-3210
  resistance to, 3149-3150, 3150f, 3150t, 3208-3209
  in vitro activity of, 3209
Aminopenicillins, 3183t-3184t, 3186-3187, 3231t-3233t
p-Aminosalicylic acid, in tuberculosis, 1367t, 1369
Aminotransferase, serum, in Kawasaki disease, 1076
Amodiaquine, in malaria, 2942
Amoxicillin, 3183t-3184t, 3186-3187, 3231t-3233t
  in chronic bronchitis, 269-270
  in odontogenic infection, 145
  in otitis media, 227, 228t, 231
  in rhinosinusitis, 200
Amoxicillin-clavulanate, 3187-3188
  Clostridium difficile–related infection and, 633-634
  in otitis media, 227-228, 228t
  in rhinosinusitis, 200
Amphotericin B, 3292f
  in *Aspergillus* spp. infection, 2717-2718
  in *Blastomyces dermatitidis* infection, 2732t, 2733
  in blastomycosis, 478
  in cancer patient, 928
  in *Candida* esophagitis, 597
  in *Candida* meningitis, 474-475
  in *Coccidioides* spp. infection, 477-478, 2777-2778
  in *Cryptococcus neoformans* infection, 475-476, 2804-2805
  in fungal endocarditis, 368
  in *Fusarium* spp. infection, 2848

Amphotericin B *(Continued)*
in *Histoplasma capsulatum* infection, 476-477
lipid formulations of, 3294-3298, 3296t, 3347
ABCD, 3296-3297, 3296t
ABLC, 3296t, 3297
in candidemia, 2747
in cardiac candidiasis, 2748
in chronic disseminated candidiasis, 2749
in CNS candidiasis, 2749
efficacy of, 3296
in endovascular candidiasis, 2748
indications for, 3298
L-AmB, 3296t, 3297-3298
in *Leishmania* spp. infection, 2951-2952, 2958
in neonatal *Candida* spp. infection, 2758
pharmacokinetics of, 3294-3296
safety of, 3296
in lung transplantation, 969
in *Mucor* spp. infection, 479, 2843-2844
in *Naegleria fowleri* infection, 2984
in neonatal zygomycosis, 899
in *Paecilomyces* spp. infection, 2857
in *Paracoccidioides* spp. infection, 2792-2793
in *Penicillium marneffei* infection, 2855
in *Rhizopus* spp. infection, 479, 2843-2844
in *Sporothrix schenckii* infection, 479, 2835
Amphotericin B deoxycholate, 3291-3294, 3347
adverse effects of, 3292-3293
drug interactions with, 3293-3294
in endovascular candidiasis, 2748
in *Histoplasma capsulatum* infection, 2825
indications for, 3294, 3294t-3295t
in lung transplant–related *Aspergillus* infection, 968
mechanism of action of, 3291
monitoring of, 3293
in neonatal *Candida* spp. infection, 2758
nephrotoxicity of, 3292-3293
in ocular candidiasis, 2749
in oropharyngeal candidiasis, 2747
in pericardial candidiasis, 2748
in peritoneal candidiasis, 2748
pharmacodynamics of, 3291-3292
pharmacokinetics of, 3292, 3293t
spectrum of activity of, 3291
Ampicillin, 3183t-3184t, 3186-3187, 3231t-3233t
in community-acquired pneumonia, 291, 291t
exanthematous reaction to, 2002, 2002f
in infectious mononucleosis, 2002, 2002f
in meningitis, 446, 450t
in odontogenic infection, 146t
Ampicillin-sulbactam, 3188, 3231t-3233t
Amylase
in mediastinitis, 420
in pancreatitis, 690, 692
Amyloidosis, in infective endocarditis, 355
Anal cancer, HPV and, 1873t, 1875, 1880-1881
Analgesia, in ARDS, 873
Anaphylaxis. *See also* Allergy/allergic reaction
animal serum and, 3493
HBV vaccine and, 1926
in IgA deficiency, 911
IGIM and, 3494
IVIG and, 3496
Anaphylaxis *(Continued)*
penicillin and, 3189-3190
vaccine and, 3426
Anaplasma spp. infection (anaplasmosis), 494t-498t, 751t-752t, **2661-2663**
Lyme disease and, 1734, 2663
Ancylostoma braziliense infection, 757t-758t, 3024
Ancylostoma duodenale infection, **3016-3018**, 3327t-3346t
Anemia, 77t, 78
aplastic, in infectious mononucleosis, 2002
in *Bartonella bacilliformis* infection, 1726
in congenital syphilis, 1770
hemolytic
in human erythrovirus B19 (human parvovirus B19) infection, 1855
in infectious mononucleosis, 2002
in *Leptospira* spp. infection, 1746-1747
in *Mycoplasma pneumoniae* infection, 2682
in rubella virus infection, 2212
in HIV infection, 2607-2608
in infective endocarditis, 358, 358t
in Kawasaki disease, 1075-1076
in malaria, 76, 78, 2926-2927
sickle-cell. *See* Sickle-cell disease
in Whipple disease, 644
Aneurysm, in Kawasaki disease, 1071, 1080-1084. *See also* Kawasaki disease
Angiography
in Kawasaki disease, 1083
in pyogenic liver abscess, 674
Angioneurotic edema, acute, 250t-251t, 253
Angiostrongylus cantonensis infection, **481-484**, 494t-498t, 3024. *See also* Meningitis, eosinophilic
Anidulafungin, 3308t, 3313f, 3317-3318, 3317t
in *Aspergillus* spp. infection, 968, 2718
Animal bite. *See* Bites, animal
Anisakis spp. infection, 3024
Anorectum, gonococcal infection of, 1287
Anoxia, hepatic injury with, 657
Ant bite, 758t
Anterior chamber–associated immune deviation (ACAID), 810
Anthrax, 602t-603t, **1311-1316**
bioterrorist, 3580-3582
clinical manifestations of, 753t-756t
cutaneous, 753t-756t, 1313-1314, 1313f, 3581-3582
vs. ecthyma, 776, 1315
diagnosis of, 1314-1315
differential diagnosis of, 1314-1315
epidemiology of, 1312
gastrointestinal, 1313-1314
historical perspective on, 1311-1312
inhalational, 1312-1314, 3581-3582, 3581f
meningeal, 1314
pathogenesis of, 1312-1313
postexposure treatment in, 3540t-3544t, 3582
prevention of, 1315-1316
prognosis for, 1315
transmission of, 1312
treatment of, 1315, 3503-3505
Anti-NMDAR (anti-*N*-methyl-D-aspartate receptor) encephalitis, 509
Antibiotic(s), **3182-3241.** *See also* Antibiotic prophylaxis; Antibiotic resistance *and specific antibiotic classes and agents*
in *Achromobacter* spp. infection, 1575
in *Acinetobacter* spp. infection, 1571-1572
Antibiotic(s) *(Continued)*
in *Actinomyces* spp. infection, 1824
administration of, 3230-3239
diffusion properties and, 3235
dosage schedules for, 3230, 3231t-3234t
drug-drug interactions with, 3239
duration of, 3235-3236
food-drug interactions in, 3234
in hepatic insufficiency, 3236
in home setting, 3239
intramuscular, 3234-3235
intravenous, 3234-3235, 3239, **3257-3265**, 3258t-3259t. *See also* Outpatient intravenous antimicrobial therapy
neonatal dosage schedules for, 3230, 3233t
oral vs. parenteral preparations in, 3234
pharmacokinetic-pharmacodynamic interface in, 3160-3163, 3166t. *See also at* Drug(s)
in renal insufficiency, 3236
restrictions on, 3238
in school/daycare setting, 3238
summary guidelines for, 3239-3240, 3240t
topical, 3236-3237
in *Aerococcus* spp. infection, 1249-1250
in *Aeromonas* spp. infection, 1540, 1544
in *Aggregatibacter actinomycetemcomitans* infection, 1610, 1824
in *Aggregatibacter (Haemophilus) aphrophilus* infection, 1686
in anthrax, 1315
in appendicitis, 686
in *Arcanobacterium haemolyticum* infection, 1322-1323, 1326
in *Babesia* spp. infection, 2919-2920
in *Bacillus cereus* infection, 1321
in bacterial meningitis, 446-449, 450t-451t, 1263-1266
in bacterial vaginosis, 578
in *Bacteroides* spp. infection, 1831-1833, 1831t
in *Bartonella bacilliformis* infection, 1728
in *Bartonella henselae* infection, 1727-1728
in *Bartonella quintana* infection, 1728
in *Bartonella* spp. endocarditis, 1728
in bite injury, 3575-3577, 3577t
in *Bordetella pertussis* infection, 1629-1630
in brain abscess, 465t, 468
in Brazilian purpuric fever, 1689-1690
in *Brucella* spp. infection, 1614-1615
in *Burkholderia cepacia* infection, 1601
in *Burkholderia pseudomallei* infection, 1601
in burn wound infection, 1060
in *Campylobacter jejuni* infection, 1655-1656
in catheter-related infection, 3389-3390
in cervical lymphadenitis, 185-188
in cervicitis, 585-586
in chemotherapy-associated infection, 925-928
in *Chlamydia pneumoniae* infection, 2644
in *Chlamydia psittaci* infection, 2639
in *Chlamydia trachomatis* infection, 2637-2638
in cholangitis, 663t
in cholera, 1553, 1554t
cholestasis with, 662
in *Chromobacterium violaceum* infection, 1564
in chronic bronchitis, 269-270
in chronic osteomyelitis, 726-727

Antibiotic(s) *(Continued)*
in *Chryseobacterium* spp. infection, 1580-1581
in *Citrobacter* spp. infection, 1431-1433
in *Clostridium difficile* infection, 637
in coagulase-negative staphylococcal infection, 1138-1139, 1138t
colitis with. *See* Colitis, antibiotic-associated
in community-acquired pneumonia, 289-292, 291t
in conjunctivitis, 802
in *Corynebacterium diphtheriae* infection, 1309-1310
in *Coxiella burnetii* infection, 2665
in craniofacial surgery–related infection, 1046
in croup syndromes, 256
in *Cryptosporidium* spp. infection, 2903
in CSF shunt infection, 1033-1036, 1033t, 1035f, 1037t
in cystic fibrosis–related infection, 333, 337-344
in cystitis, 546-548, 547t
in dacryoadenitis, 797-798
in dacryocystitis, 798
diarrhea with, 599. *See also* Colitis, antibiotic-associated
in diarrheogenic *Escherichia coli* infection, 1462-1463
in *Dientamoeba fragilis* infection, 2886, 2886t
in *Edwardsiella tarda* infection, 1533
in *Eikenella corrodens* infection, 1577-1578
in *Elizabethkingia* spp. infection, 1580-1581
in *Enterobacter* spp. infection, 1437-1438
in enterovirus infection, 2103
in epiglottitis, 259
in erysipelas, 776
in *Erysipelothrix rhusiopathiae* infection, 1328-1329
in *Escherichia coli* UTI, 1444t, 1446-1447
in fever without source, 839
in *Francisella tularensis* infection, 1664
in furunculosis, 777
in *Fusobacterium* spp. infection, 1831-1833, 1831t
in gas gangrene, 1799
in gastrointestinal tract infection, 620t, 622, 623t
in gonorrhea, 571-574
in group C streptococcal infection, 1152
in group G streptococcal infection, 1152
in *Haemophilus aegyptius* infection, 1689-1690
in *Haemophilus ducreyi* infection, 1688
in *Haemophilus influenzae* infection, 1678-1679, 1678t
in *Haemophilus parainfluenzae* infection, 1690
in *Hafnia alvei* infection, 1535
hearing loss and, 1664
in *Helicobacter pylori* infection, 1697t
in hordeolum, 796
in impetigo, 774-775
in inclusion conjunctivitis, 804
in infective endocarditis, 363-368, 364t-367t
in intra-abdominal abscess, 705
in intracranial pressure monitor–related infection, 1040
in intrathecal pump infusion device–related infection, 1041-1042
in keratitis, 807, 808t

Antibiotic(s) *(Continued)*
in *Kingella kingae* infection, 1703-1704
in *Klebsiella granulomatis (Calymmatobacterium granulomatis)* infection, 1643
in *Klebsiella* spp. infection, 1468
in *Legionella pneumophila* infection, 1712-1713
in *Leptospira* spp. infection, 1756-1757
in *Leuconostoc* spp. infection, 1247
in *Listeria monocytogenes* infection, 1333
in lung abscess, 319-321
in Lyme disease, 1735-1738, 1736t
in lymphogranuloma venereum, 584
in mandibular/maxillary osteomyelitis, 153-154
in mastoiditis, 238-240
in methicillin-resistant *Staphylococcus aureus* infection, 1125-1128, 1127f
in *Moraxella catarrhalis* infection, 1253
in *Morganella morganii* infection, 1470-1471
in *Mycobacterium ulcerans* infection, 1416
in *Mycoplasma pneumoniae* infection, 2686-2687
in *Mycoplasma* spp. infection, 2696-2697
in myositis, 739
in nasopharyngitis, 163-164
in necrotizing fasciitis, 779
in *Neisseria gonorrhoeae* infection, 1293-1297, 1293t-1294t
in *Neisseria meningitidis* infection, 1263-1266
in neonatal conjunctivitis, 806
in neonatal meningitis, 886
in neonatal musculoskeletal infection, 890
in neonatal parotitis, 891
in neonatal pneumonia, 892
in neonatal septic arthritis, 734
in neonatal urinary tract infection, 888-889
in *Nocardia* spp. infection, 1422-1423
in nontuberculous mycobacterial infection, 1387-1390, 1388t
normal flora effects of, 89-90
in oral infections, 144-145, 146t
in orbital cellulitis, 800
in *Orientia tsutsugamushi* infection (scrub typhus), 2661
in osteomyelitis, 717-719, 718t
in otitis externa, 206-207
in otitis media, 225-229, 225t, 227t-228t
pancreatitis with, 691-692, 691t
in *Pantoea agglomerans* infection, 1537
in parapharyngeal abscess, 173
in parotitis, 192
in *Pasteurella multocida* infection, 1548
in *Pediococcus* spp. infection, 1248
in pelvic inflammatory disease, 588-589, 589t, 1296
in pericarditis, 378-379, 378t
in peritonitis, 702-704, 703t
in peritonsillar abscess, 169, 174-175
pharmacokinetic-pharmacodynamic interface of. *See at* Drug(s)
in pharyngitis, 163-164
in *Plesiomonas shigelloides* infection, 1566
in pleural effusion, 313-314
in pneumococcal pneumonia, 1233, 1234t
in *Porphyromonas* spp. infection, 1831-1833, 1831t
in post–lung transplantation β-lactamase–producing multidrug-resistant infection, 962

Antibiotic(s) *(Continued)*
in post–lung transplantation *Pseudomonas aeruginosa* infection, 961-962
in preseptal cellulitis, 799
in *Prevotella* spp. infection, 1831-1833, 1831t
in prostatitis, 558-559
in *Proteus* spp. infection, 1473-1474
in *Providencia* spp. infection, 1475-1476
in *Pseudomonas* spp. infection, 1597-1601, 1598t
in pyogenic liver abscess, 675
in pyomyositis, 737-738
in relapsing fever, 1742
in renal abscess, 556-557
in retroperitoneal infection, 708-709
in retropharyngeal abscess, 171-172
in rheumatic fever, 414, 414f, 414t
in rhinosinusitis, 200-201
in *Rhodococcus equi* infection, 1427-1428
in *Rickettsia akari* infection, 2657
in *Rickettsia conorii* infection, 2656
in *Rickettsia prowazekii* infection, 2658-2659
in *Rickettsia typhi* infection, 2659
in Rocky Mountain spotted fever, 2654
in *Salmonella* ser. Typhi infection, 1505-1506, 1505t
in *Salmonella* spp. infection, 1504-1507, 1504t
in sepsis neonatorum, 883-884
in septic arthritis, 732-733
in septic shock, 833-836, 833t
in *Serratia* spp. infection, 1490-1491
in *Shigella* spp. infection, 1483-1485, 1484t
in spinal epidural abscess, 472
in *Spirillum minus* infection, 1760
in *Staphylococcus aureus* infection, 1123-1128, 1124t-1125t
in *Stenotrophomonas maltophilia* infection, 1608-1609
in *Streptobacillus moniliformis* infection, 1719
in *Streptococcus agalactiae* infection, 1154, 1163, 1164t
in *Streptococcus pneumoniae* infection, 1218-1219
in *Streptococcus pyogenes* infection, 1147-1149, 1148t
in *Streptococcus* viridans infection, 1194-1195
in subdural empyema, 470
subeschar, in burn wound infection, 1060
in syphilis, 1776t-1777t, 1777-1780, 1780f
topical, 3236-3237
in burn injury, 1059
in nasolacrimal duct obstruction, 797
in otitis externa, 206
in staphylococcal blepharitis, 794
in toxic shock syndrome, 856-857
in trachoma, 805
in traveler's diarrhea, 3115
in *Ureaplasma* spp. infection, 2696-2697
in urethritis, 533-534, 534t
in urinary catheter–related infection, 3394
in urinary tract infection, 546-548, 547t
in uvulitis, 166-167
in ventilator-associated pneumonia, 3392
in *Vibrio parahaemolyticus* infection, 1559
in *Vibrio vulnificus* infection, 1562
in Whipple disease, 645-646, 646t
in *Yersinia enterocolitica* infection, 1530-1531

Antibiotic(s) *(Continued)*
in *Yersinia pestis* infection, 1513
in *Yersinia pseudotuberculosis* infection, 1530-1531
Antibiotic prophylaxis, **3242-3257.** *See also* Postexposure prophylaxis
in asplenia, 3251-3252
in bacterial meningitis prevention, 1266-1267, 1266t, 3249
in bacterial meningitis prevention, 458-461
after bites, 781, 3251
in *Bordetella pertussis* infection prevention, 1636, 3249
in burn patient, 1060-1061
in cancer patient, 928-929
in cardiovascular surgery, 3255-3256
in catheter insertion, 3244
in cholangitis, 665
in chronic granulomatous disease, 918
in craniofacial surgery, 1045
in CSF leak, 3252
in CSF shunt infection prevention, 1038
in endocarditis prevention, 368-369, 369t, 417-418, 1195-1196, 3246-3247, 3246t-3248t
in epiglottitis prevention, 259
in *Escherichia coli* UTI prevention, 1446
in extracorporeal membrane oxygenation, 1023
in *Haemophilus influenzae* type b infection prevention, 1682, 3249-3250
in heart transplantation, 947
in hematopoietic stem cell transplantation, 935
in hemoglobinopathy, 3252
in IgA deficiency, 911
in IgG subclass deficiency, 911-912
in immunodeficiency disorders, 64
indications for, 3237-3238, 3237t
in intracranial pressure monitor–related infection prevention, 1040
in intrathecal pump infusion device–related infection prevention, 1042
in *Kingella kingae* infection prevention, 1704
in measles prevention, 2392-2393
in necrotizing enterocolitis, 3243-3244
in *Neisseria meningitidis* infection prevention, 1266-1267, 1266t, 3249
in neonate, 3242-3244
in neurosurgical procedures, 3253-3255
in nosocomial infection prevention, 3393
in otitis media prevention, 231, 3247-3248
in pancreatitis, 696
in *Pneumocystis* infection prevention, 3011t, 3012
principles of, 3242, 3243t
in rheumatic fever prevention, 3244-3246, 3245t, 3540t-3544t
in sickle-cell disease, 60, 3252
in *Streptococcus agalactiae* infection prevention, 1165-1168, 1166t, 1168f, 3243
in *Streptococcus pneumoniae* infection prevention, 1236
surgical, 3252-3256, 3253t-3254t
in cardiovascular procedures, 3255-3256
in general procedures, 3253
in neurosurgical procedures, 3253-3255
in type 1 leukocyte adhesion deficiency, 53
in urinary tract infection prevention, 548, 3248
Antibiotic prophylaxis *(Continued)*
in vesicoureteral reflux, 549-551, 549t, 550f-551f
in Wiskott-Aldrich syndrome, 913
in *Yersinia pestis* infection prevention, 1514
Antibiotic resistance, **3137-3159**
in *Achromobacter* spp. infection, 1575
in *Aeromonas* spp. infection, 1540
aminoglycoside, 3149-3150, 3150f, 3150t, 3208-3209
in *Arcanobacterium haemolyticum* infection, 1323
in *Bacteroides fragilis* infection, 1825-1826
in *Campylobacter jejuni* infection, 1655-1656, 1655t, 3155
carbapenem, 3139-3145
ceftaroline, 3153-3154
cellular mechanisms of, 3137-3139, 3138t
cephalosporin, 3139-3145
chloramphenicol, 3157-3158, 3215
in cholera, 1553
colistin, 3207
in cystic fibrosis, 3154
daptomycin, 3153
in *Enterobacter* spp. infection, 1435, 1437
in *Enterococcus* spp. infection, 1177-1182, 1179t. *See also Enterococcus* spp. infection, treatment of, resistance in
fluoroquinolone, 3226
glycopeptide, 3151-3154, 3152t
in *Haemophilus influenzae* infection, 1668
in *Hafnia alvei* infection, 1535
in *Klebsiella* spp. infection, 1468
β-lactam, 3139-3145, 3140f-3141f, 3140t, 3202-3203
lincosamide, 3145-3147, 3220
linezolid, 3153, 3223
macrolide, 3145-3147, 3211-3212
methicillin. *See Staphylococcus aureus* infection, methicillin-resistant
monobactam, 3139-3145
in *Neisseria gonorrhoeae* infection, 1291-1293, 1293f
in otitis media, 3147
penicillin, 3139-3145. *See also* Penicillin, resistance to
in pneumonia, 3147
in *Providencia* spp. infection, 1475-1476
in *Pseudomonas aeruginosa* infection, 1601-1603, 3154
quinolone, 3154-3157
in *Salmonella* ser. Typhi infection, 1495
in *Salmonella* spp. infection, 624, 1494
in *Serratia marcescens* infection, 1489
in *Shigella* spp. infection, 1484-1485, 3147-3148
in *Staphylococcus aureus* infection. *See Staphylococcus aureus* infection, methicillin-resistant
in *Stenotrophomonas maltophilia* infection, 1608-1609
in *Streptococcus pneumoniae* infection, 1219-1223, 1220t-1221t, 1222f, 3144-3145, 3155. *See also Streptococcus pneumoniae* infection, antibiotic resistance in
in *Streptococcus* viridans infection, 1194
streptogramin, 3145-3147
tetracycline, 3217-3218
trimethoprim-sulfamethoxazole, 3147-3149, 3148f, 3228
in urinary tract infection, 3148
vancomycin, 3151-3154, 3152t, 3204. *See also* Vancomycin, resistance to
in *Yersinia enterocolitica* infection, 1531
Antibody (antibodies). *See also specific immunoglobulins*
anti-GQib, 505
anti-*Toxoplasma*, 817
anti–streptolysin O, 1141, 1147
in common colds, 135-136
deficiencies of, 46-48
in IgA deficiency, 47
in IgG subclass deficiency, 47
IVIG in, 3497
laboratory evaluation of, 906
normal/elevated immunoglobulin levels and, 47-48
in transient hypogammaglobulinemia of infancy, **47**
in X-linked agammaglobulinemia, 46-47
in newborn, 42-44, 43f, 43t
TSST-I, 852, 856-857, 863-864
Anticancer agents. *See* Chemotherapy
Anticholinesterase insecticide poisoning, pancreatitis with, 692
Anticonvulsants
in brain abscess, 469
in meningitis-related seizures, 455
Antidiuretic hormone (ADH), inappropriate secretion of, 76, 81
in bacterial meningitis, 438, 453-454
Antiendotoxin therapy, in *Neisseria meningitidis* infection, 1264-1265, 1265t
Antifungal agents, **3291-3327**, 3292f. *See also specific antifungal agents*
in *Acremonium* spp. infection, 2858
in *Aspergillus* spp. infection, 2717-2721
in *Blastomyces dermatitidis* infection, 2732-2734, 2732t
in cancer patient, 928
in *Candida* spp. infection, 2746-2749, 2746t
in *Coccidioides* spp. infection, 2777-2778
in *Cryptococcus neoformans* infection, 2804-2805
in Entomophthorales infection, 2845
in *Fusarium* spp. infection, 2848
in *Histoplasma capsulatum* infection, 2824-2829, 2824t
in *Mucor* spp. infection, 2843-2844
in neonatal *Candida* spp. infection, 2757-2760
in *Paecilomyces* spp. infection, 2857
in *Paracoccidioides* spp. infection, 2792-2793
prophylactic, 2745-2746
in cancer patient, 929
in neonatal *Candida* spp. infection prevention, 2760-2765, 2764t
in *Rhizopus* spp. infection, 2843-2844
in *Scedosporium* spp. infection, 2850
in *Sporothrix schenckii* infection, 2835
topical, 3321t, 3324
in *Trichoderma longibrachiatum* infection, 2857
Antifungal azoles, 3301-3312, 3301f
mechanism of action of, 3302, 3302f
resistance to, 3302-3303
spectrum of activity of, 3302
Antigen
for delayed hypersensitivity skin test, 907
impaired response to. *See* Antibody (antibodies), deficiencies of
Antihistamines
in common cold, 137
in rhinosinusitis, 201
in rhinovirus infection, 2126

Antilymphocyte antibody therapy (OKT3), in kidney transplantation, 1007
Antimalarial agents, 2936-2943, 2937t-2940t
  prophylactic, 2943-2945, 2944t
Antimicrobial stewardship programs, 3414, 3414t
Antimonials, in visceral leishmaniasis, 2957
Antimotility agents
  in diarrheogenic *Escherichia coli* infection, 1463
  in traveler's diarrhea, 3114
Antioxidants, 77
Antiparasitic agents, **3327-3354**, 3327t-3346t, 3351t-3353t. *See also specific antiparasitic agents*
  adverse effects of, 3347
  in *Ancylostoma duodenale* infection, 3018
  in *Ascaris lumbricoides* infection, 3014-3015
  in *Babesia* spp. infection, 2919-2920
  in *Balantidium coli* infection, 2896
  in *Blastocystis hominis* infection, 2876
  in *Clonorchis sinensis* infection, 3052
  in *Cryptosporidium* spp. infection, 2903
  in *Dientamoeba fragilis* infection, 2886, 2886t
  in *Diphyllobothrium* spp. (fish tapeworm) infection, 3041
  in *Dipylidium caninum* (dog tapeworm) infection, 3041
  in *Echinococcus granulosus* infection, 3046
  in *Entamoeba coli* infection, 2877-2878
  in *Entamoeba histolytica* infection, 2873-2874, 2873t
  in *Enterobius vermicularis* infection, 3019
  in *Fasciola buski* infection, 3057
  in *Fasciola* spp. infection, 3053-3054
  in *Giardia lamblia* infection, 2882-2883, 2883t
  in *Hymenolepis nana* (dwarf tapeworm) infection, 3042
  manufacturers of, 3354
  in microsporidial infection, 2913
  in *Necator americanus* infection, 3018
  in *Onchocerca volvulus* infection, 3027
  in *Opisthorchis* spp. infection, 3052
  in *Paragonimus* spp. infection, 3056
  in pregnancy, 3351, 3351t-3353t
  in *Schistosoma* spp. infection, 3068
  in *Strongyloides fuelleborni kellyi* infection, 3021
  in *Strongyloides stercoralis* infection, 3021
  in *Taenia saginata* infection, 3032
  in *Taenia solium* infection, 3037-3039
  in *Toxocara canis* infection, 3023
  in *Toxoplasma gondii* infection, 817, 3000-3002, 3001t
  in *Trichomonas vaginalis* infection, 2892-2893, 2893t
  in *Trichuris trichiura* infection, 3016
  in *Trypanosoma brucei* infection, 2971-2973
  in *Trypanosoma cruzi* infection, 2966-2967
  in *Wuchereria bancrofti* infection, 3026
Antipyretics, **86-88**
  adverse effects of, 85
  indications for, 86
Antiretroviral agents, 600
  in adult T-cell leukemia, 2569
  in HIV-1 infection, 2593-2594, 2609-2617, 2610t, 2611f-2612f
  in HIV-2 infection, 2586-2588
  in HTLV-1–associated myelopathy, 2573
Anti–streptolysin O (ASO) antibodies/titer, 1141, 1147
  in rheumatic fever, 411
Antitoxin, 3491-3493
  in botulism, 1794, 3505
  in *Corynebacterium diphtheriae* infection, 1309-1310, 3506-3507
  in tetanus, 1815-1816, 3511-3512
Antituberculosis drugs, 1366-1369, 1367t
Antitussives, in common cold, 137
Antiviral agents, **3265-3290**, 3266t. *See also specific antiviral agents*
  in adenovirus infection, 1907-1908
  in arenavirus infection, 2475
  in BK virus infection, 1869
  in CMV infection, 1984-1987, 1985f, 2601-2602
  in Crimean-Congo hemorrhagic fever, 2533-2534
  in erythema multiforme, 1951
  in genital HSV infection, 1960-1961, 1960t
  in hantavirus infection, 2510-2511
  in HBV infection, 1920-1923, 1923t
  in HCV infection, 2323-2324
  in HHV-6 infection, 2019
  in HHV-8 infection, 2021
  historical perspective on, 3265-3268
  in hMPV infection, 2440
  in HPV infection, 1884
  in HSV infection, 1958-1964, 1961t
  in HSV infection prevention, 1966-1967
  in infectious mononucleosis, 2012
  in influenza virus infection, 2348-2351, 2349t
  in La Crosse virus encephalitis, 2524-2525
  mechanisms of action of, 3267, 3267f
  in monkeypox, 2044
  in post-transplant lymphoproliferative disease, 2012
  in rhinovirus infection, 2126
  in RSV infection, 2426
  stockpiles of, 2358
  in subacute sclerosing panencephalitis, 2390
  in VZV infection, 2028-2029
  in West Nile virus infection, 2272
Aortic regurgitation, in Kawasaki disease, 1081
Aphthous stomatitis, recurrent, 159t-160t, 161
Aplastic crisis
  diagnosis of, 1855
  in human erythrovirus B19 (human parvovirus B19) infection, 1849-1850
Apnea, in RSV infection, 2419
ApoA-I, 74
Apocrine glands, infection of, **777**
Apoi virus infection, 2316t
Appendectomy, 685-686
  malignancy and, 687-688
  in nonperforated appendicitis, 685
  in perforated appendicitis, 685-686
Appendicitis, **679-689**
  adenovirus, 1902
  bacteria in, 683t, 684
  clinical manifestations of, 680-681
  complications of
    early, 686-687
    late, 687-688
  coxsackievirus, 2070
  diagnosis of, 681-683
  differential diagnosis of, 682t
  epidemiology of, 679-680
  fungi in, 683t, 685
  historical perspective on, 679
  measles virus, 2392
Appendicitis *(Continued)*
  microbiology of, 683-685, 683t
  mortality with, 686
  neonatal, 681
  nonperforated, 685
  parasites in, 683t, 684-685
  Pasteurella multocida, 1547
  pathophysiology of, 680
  pelvic abscess and, **687**
  perforated, 701
    epidemiology of, 680
    treatment of, 685-686, 703, 703t
  in preschool-aged children, 681
  prognosis for, 686-687
  treatment of, 685-686
  viruses in, 683t, 685
  Yersinia enterocolitica, 1520, 1527
  Yersinia pseudotuberculosis, 1521, 1527
APTIMA test, in *Trichomonas vaginalis* infection, 2892
Aquacel, in burn injury, 1059
Arcanobacterium haemolyticum, 1322, 1323t
  toxin of, 1322
Arcanobacterium haemolyticum infection, **1322-1327**
  adult, 1325
  clinical manifestations of, 1324-1326, 1324f, 1324t
  CNS, 1325
  cutaneous, 753t-756t, 1325
  differential diagnosis of, 1326
  EBV infection and, 1324
  endocardial, 1325-1326
  epidemiology of, 1323
  exanthem in, 1324-1325, 1324f
  historical perspective on, 1322
  in Lemierre's disease, 1326
  pathogenesis of, 1323-1324
  pharyngeal, 1324-1325
  prognosis for, 1326
  thoracic, 1325
  treatment of, 1326
    antimicrobial susceptibility in, 1322-1323
  vancomycin-resistant, 1323
ARDS. *See* Acute respiratory distress syndrome (ARDS)
Area under the curve (AUC), 3161, 3161f
Arenavirus(es), 2467, 2468t, 2469f
Arenavirus infection, **2466-2477.** *See also* Lassa virus infection; Lymphocytic choriomeningitis virus (LCMV) infection
  bioterrorist, 3585
  clinical manifestations of, 2471-2472, 2471t
  diagnosis of, 2473-2475
  differential diagnosis of, 2475
  epidemiology of, 2467-2470, 2470t
  historical perspective on, 2466-2467
  nosocomial, 3401-3402
  pathogenesis of, 2472-2473
  pathology of, 2472-2473
  prevention of, 2476-2477
  prognosis for, 2475
  transmission of, 2470-2471
  treatment of, 2475, 3533-3534
  vaccine against, 2476-2477
Argentine hemorrhagic fever. *See* Junín virus infection
Argyll Robertson pupil, 812
Arlt lines, 804-805
Arrhythmias
  in myocarditis, 389, 397
  in pericarditis, 376

Artemether, 3347
Artemether-lumefantrine, 3347
  in malaria, 2937t-2940t, 2941
Arteriovenous fistula, endocarditis and, 352
Artesunate, 3347
  in malaria, 2942
Arthralgia
  in bacterial meningitis, 441
  in human erythrovirus B19 (human parvovirus B19) infection, 1851
  in Whipple disease, 643
Arthritis. *See also* Septic arthritis
  in adenovirus infection, 1904
  in bacterial meningitis, 441, 1261-1262
  Campylobacter jejuni, 1654
  Candida, 2742, 2749
  chikungunya virus, 2244, 2247
  Coccidioides spp., 2773-2774
  enterovirus, 2078
  in erythema infectiosum, 1849
  fungal, 730, **734**
  gonococcal, 729t, 730, 734
  HTLV-1, 2575-2576
  human erythrovirus B19 (human parvovirus B19), 1851
  in Kawasaki disease, 1074, 1082
  Lyme, 730, 812, 1731-1733, 1736
  Mycoplasma pneumoniae, 2683
  poststreptococcal, 412. *See also* Rheumatic fever
  reactive, 734
    with *Campylobacter jejuni* infection, 1654
    with *Shigella* spp. infection, 1481
    with *Yersinia enterocolitica* infection, 1528-1529
  Ross River virus, 2248-2251. *See also* Ross River virus arthritis
  rubella virus, 2208, 2211, 2219
  Sporothrix schenckii, 2834
  Streptobacillus moniliformis, 1717
  tuberculous, 1355-1356
Arthritis-dermatitis syndrome, in gonococcal infection, 1287, 1288f
Arthrocentesis, in Whipple disease, 643
Arthropod bites, 2862t-2863t, **3070-3079.** *See also specific arthropods*
  cutaneous manifestations of, 758t, 763t-764t
  vs. virus-induced exanthem, 762-763
Arthus reaction, penicillin-related, 3190
Ascaris lumbricoides infection (ascariasis), **3013-3015**
  appendiceal, 684
  biliary, 660, 3014
  clinical manifestations of, 757t-758t, 3014
  diagnosis of, 3014
  epidemiology of, 3013-3014
  neonatal, 3014
  ocular, 815
  pancreatic, 694, 694f, 707-708, 3014
  pathophysiology of, 3014
  prevention of, 3015
  prognosis for, 3015
  treatment of, 3014-3015, 3327t-3346t
Aschoff body, 408
Aspartate aminotransferase (AST), in hepatitis, 650, 2138
Aspergilloma, 2708
Aspergillus flavus, 2705
Aspergillus fumigatus, 2705
Aspergillus fumigatus infection, 2708-2709
  after heart transplantation, 953
  after lung transplantation, 965-970
Aspergillus spp., 2704-2705
Aspergillus spp. infection (aspergillosis), **2704-2722**
  bone, 726
  bronchopulmonary, allergic, 2708-2709
    in cystic fibrosis, 341-342
    treatment of, 2721
  burn wound, 1053
  in cancer patient, 922, 926-927, 927t
  cerebral, 2707, 2713-2714, 2714f
  chest radiography in, 2712
  chronic, 2708
  in chronic granulomatous disease, 56-57, 917, 918f, 2706, 2713f
  clinical manifestations of, 2705-2709
  corneal, 807-808
  CT in, 2712-2714, 2713f
  cutaneous, 791f, 2707-2708
  in cystic fibrosis, 336, 341-342, 2713f
  diagnosis of, 2711-2717
    antibody testing in, 2714
    bronchoalveolar lavage in, 2715-2716
    culture in, 2711-2712, 2712f
    G test in, 2716
    galactomannan antigen in, 926, 927t, 2714-2715
    imaging in, 2712-2714, 2713f-2714f
    PCR in, 2716-2717
  endocardial, 363
  epidemiology of, 2709-2711
  exanthem in, 757t
  after hematopoietic stem cell transplantation, 937-938, 2707-2711, 2713
  immune response to, 23, 2705
  after intestinal transplantation, 994-995, 995t
  after liver transplantation, 994, 995t
  after lung transplantation, 959-960, 965-970
  meningeal, 478-479
  neonatal, 899
  orbital, 801
  pancreatic, 695
  paranasal sinus, 196-197, 196t, 201, 2706, 2707f-2708f
  pulmonary, 2706
    CT in, 2712-2713, 2713f
    treatment of, 2717-2721, 3295t
  uveal, 813
Aspiration, prevention of, 3393
Aspiration (diagnostic/therapeutic)
  in brain abscess, 468
  in cervical lymphadenitis, 184
  in craniofacial surgery–related infection, 1046
  in CSF shunt–related infection, 1032
  in lung abscess, 319, 321
  in mastoiditis, 237-238
  in middle ear effusion, 218-220
  in osteomyelitis, 714-715
  in pericarditis, 378, 378t
  in peritonitis, 702
  in peritonsillar abscess, 169
  in prosthetic joint infection, 1028
  in septic arthritis, 731, 732t
Aspirin
  adverse effects of, 85
  in Kawasaki disease, 1078-1079, 1078t
  Reye syndrome and, 86-87, 657, **676-677**, 2351
Asplenia, 59
  antibiotic prophylaxis in, 3251-3252
  Streptococcus pneumoniae infection and, 1224, 1232
Asthma
  acetaminophen and, 87
  Chlamydia pneumoniae infection and, 2641-2642
  vs. chronic bronchitis, 266
  coronavirus infection and, 2491
  HIV-related, 2604
  hMPV infection and, 2439
  infectious, **271-282.** *See also* Bronchiolitis
    definition of, 271
    diagnosis of, 278
    differential diagnosis of, 278, 278t
    enterovirus in, 2066
    historical perspective on, 273
  vs. interstitial lung disease, 297
  Moraxella catarrhalis colonization and, 1252
  Mycoplasma pneumoniae infection and, 2679
  rhinosinusitis and, 197, 202
  rhinovirus infection and, 2123
  RSV infection and, 2421
  Schistosoma spp. infection and, 3063
  T lymphocytes in, 277-278
Astrovirus infection, 607t, 608
  in daycare facilities, 3558
Asunaprevir, in HCV infection, 2324
Asymmetric periflexural viral exanthem, **784**, 784f
Ataxia-telangiectasia, 50, 904-905, 907t, **914**
  vs. chronic bronchitis, 267
  clinical features of, 905f, 914
Atazanavir, 2614-2615
Atherosclerosis, CMV and, 1980
ATM gene, 50
Atopic disease, probiotics in, 3371
Atovaquone, 3347
  in hematopoietic stem cell transplantation, 938-939
Atovaquone-proguanil
  in malaria, 2937t-2940t, 2941
  in malaria prevention, 2944t, 2945
Atrial ectopic tachycardia, in myocarditis, 389
Attachment invasin locus (Ail) protein, 13, 1523-1524
Audiometry
  in mastoiditis, 239
  in otitis media, 218
Autism, vaccine and, 3429
Autoimmune polyendocrinopathy ectodermal dystrophy (APECED), 915-916
Autolysin, of *Streptococcus pneumoniae*, 1214
Autotransporter proteins, 6
  conventional, 6-7, 8f-9f
  trimeric, 6, 8f-9f
Axilla, temperature measurement by, 86
Azathioprine, in myocarditis, 396-397
Azithromycin, 3211-3215, 3213t, 3231t-3233t, 3347
  drug interactions with, 3215
  in heart transplant–related *Legionella pneumophila* infection, 949
  in *Naegleria fowleri* infection, 2984
  in nontuberculous mycobacterial lymphadenitis, 187
  in odontogenic infection, 146t
  topical, in neonatal conjunctivitis prevention, 1281
  in trachoma, 805
Aztreonam, 3200, 3231t-3233t
  in meningitis, 448

B

B lymphocytes, 40-42. *See also* Immunoglobulin(s)
  in burn injury, 1050
  in common variable immunodeficiency, 49
  in EBV infection, 1995
  fetal, 42
  in Kawasaki disease, 1077
  in RSV infection, 2415-2416
  in Wiskott-Aldrich syndrome, 50
B19 virus. *See* Human erythrovirus B19 (human parvovirus B19) infection
Babesia spp. infection (babesiosis), **2915-2920**
  clinical manifestations of, 2918
  diagnosis of, 2916f, 2918-2919
  epidemiology of, 2915-2917, 2916f
  immune response in, 2917-2918
  in immunocompromised host, 2918
  Lyme disease and, 1734
  pathogenesis of, 2917-2918
  prevention of, 2920
  transfusion-related transmission of, 2917
  treatment of, 2919-2920, 3327t-3346t
Bacillary angiomatosis, **1726**
  diagnosis of, 1727
  treatment of, 1728
Bacillary peliosis hepatis, **1726**
  diagnosis of, 1727
  treatment of, 1728
Bacille Calmette-Guérin (BCG), 3477-3478
  in HIV-infected patient, 1377
  in Kawasaki disease, 1074, 1077
  in *Mycobacterium leprae* infection, 1409-1410
  in *Mycobacterium tuberculosis* infection, 1376-1378, 1377t
  in *Mycobacterium ulcerans* infection, 1416-1417
Bacillus anthracis, 1312. *See also* Anthrax
Bacillus cereus, 1317
Bacillus cereus infection, **1316-1321**
  bloodstream, 1319
  clinical manifestations of, 1319-1320
  CNS, 1320
  complications of, 1320, 1321t
  diagnosis of, 618, 1320
  encephalopathy with, 677
  epidemiology of, 1317-1318
  gastrointestinal, 602t-603t, 607t, 613, 1319
  hepatic, 1320
  ocular, 820, 1319
  pathogenesis of, 1318-1319
  prevention of, 1321
  pseudoinfections with, 1320
  pulmonary, 1320
  skeletal, 1319
  soft tissue, 1319
  treatment of, 1320-1321, 1321t
Bacillus spp. infection
  burn wound, 1052
  endocardial, 362-363
Baclofen, in tetanus, 1817
Bacteremia, **824-836.** *See also* Septic shock
  Achromobacter xylosoxidans, 1574
  Acinetobacter spp., 1570
  Aerococcus spp., 1249
  Aeromonas spp., 1543
  Aggregatibacter (Haemophilus) aphrophilus, 1685-1686
  in appendicitis, 684
  Bacillus cereus, 1319
  Bacteroides fragilis, 1828
  Bordetella holmesii, 1637
Bacteremia *(Continued)*
  in burns, 1055
  Campylobacter spp., 1652-1653
  catheter-related, 3385-3391, 3386t-3388t, 3387f
  Chryseobacterium spp., 1580
  Citrobacter spp., 1431
  clinical presentation of, 831-833
  Clostridium perfringens, 1797
  Clostridium septicum, 1798
  Clostridium spp., 1798
  coagulase-negative staphylococci, 1133-1136, 1134t
  diagnosis of, 831-833
  Elizabethkingia meningosepticum, 1580
  Enterobacter spp., 1436
  Enterococcus spp., 1174-1177
  Escherichia coli, 1441
    clinical manifestations of, 1444
    epidemiology of, 1441
    pathogenesis of, 1443-1444
    treatment of, 1447
  Haemophilus influenzae, 1669-1670
  Haemophilus influenzae type b, 1675, 1678-1679
  Haemophilus parainfluenzae, 1690
  Hafnia alvei, 1534-1535
  after heart transplantation, 947-948
  after hematopoietic stem cell transplantation, 935-936, 1136
  incidence of, 824
  in infective endocarditis, 353-354, 353t
  after kidney transplantation, 1006
  Kingella kingae, 1700, 1702-1703
  Klebsiella pneumoniae, 1467-1468
  Leuconostoc spp., 1247
  after lung transplantation, 960
  in meningitis, 431-432, 432t
  Moraxella catarrhalis, 1252
  Morganella morganii, 1470
  in myositis, 739
  Neisseria gonorrhoeae, 1287
  Neisseria meningitidis, 1257
  Nocardia spp., 1421
  occult, 837-839, 840t, 1226-1228
  in osteomyelitis, 712
  Pantoea agglomerans, 1536
  Pasteurella multocida, 1547
  pathophysiology of, 825-831
  Pediococcus spp., 1248
  in pelvic abscess, 687
  in peritonitis, 702
  Proteus spp., 1473
  Providencia spp., 1475
  in pyogenic liver abscess, 672-673
  in pyomyositis, 735
  renal abscess and, 553
  Salmonella spp., 1500-1501, 1504-1505
  in septic arthritis, 728
  Serratia spp., 1490
  Shigella spp., 1481
  Staphylococcus aureus, 361-362, 824, 833-834, 1121
  Staphylococcus epidermidis, **1134-1136**, 1135t
  Stenotrophomonas maltophilia, 1607-1609
  Streptococcus agalactiae, 1160
  Streptococcus pneumoniae, 1226-1228, 1227t, 1235
  Streptococcus viridans, 1190
  treatment of, 833-836
  Vibrio parahaemolyticus, 1558
  in Whipple disease, 642-643
  without focus, 1227-1228
  Yersinia enterocolitica, 1528
Bacterial infection. *See also specific infections, infectious organisms, and sites of infection*
  adhesins in, 3-9, 4f-5f, 7f-9f
  antiphagocytic factors in, 18
  biofilms in, 11-12, 12f. *See also* Biofilm
  cell invasion in, 13-14, 14f
  cell-to-cell spread in, 16
  cold-like symptoms in, 132, 133t
  colonization in, 3-12. *See also* Colonization
  complement evasion in, 18
  encapsulation in, 19-20
  host response to, 23. *See also* Complement; Immunity; Metabolism
  immune system evasion in, 18-20
  intracellular pathogen survival in, 14-15
  laboratory diagnosis of, **3589-3595**
    culture in, 3589-3592, 3590t. *See also* Culture, bacterial
    mass spectroscopy in, 3594-3595
    microscopy in, 3592
    molecular techniques in, 3592-3594. *See also* Polymerase chain reaction (PCR)
    sequence-based technologies in, 3594
  nomenclature in, **1096-1112**, 1097t-1112t
  toxins in, 16-18. *See also* Toxin(s)
Bactericidal/permeability increasing (BPI) protein, 3365-3366
  recombinant, in *Neisseria meningitidis* infection, 1264-1265, 1265t, 3365-3366
Bacteriocins, of *Pediococcus* spp., 1248
Bacteriuria. *See also* Urinary tract infection (UTI)
  asymptomatic, 538-539
    Escherichia coli, 1441, 1443
    during pregnancy, 1154
  definition of, 542, 542t
  neonatal, 888-889
Bacteroides fragilis infection
  antibiotic resistance in, 1825-1826
  appendiceal, 684. *See also* Appendicitis
  bloodstream, 1828
  bone, 1829-1830
  CNS, 1828-1829
  diagnosis of, 1830
  endocardial, 367
  epidemiology of, 1827
  intra-abdominal, 1829
  joint, 1829-1830
  peritoneal, 700
  pleuropulmonary, 1830
  retroperitoneal, 706-707
  soft tissue, 1829
  treatment of, 1831-1833, 1831t
Bacteroides spp., 1825-1826
Bacteroides spp. infection, **1825-1834**
  bloodstream, 1827-1828
  bone, 1829-1830
  clinical manifestations of, 1827-1830
  CNS, 1828-1829
  cutaneous, 1829
  diagnosis of, 1830
  epidemiology of, 1826-1827
  head and neck, 1828
  intra-abdominal, 1829
  joint, 1829-1830
  pathogenesis of, 1827
  pleuropulmonary, 1830
  prevention of, 1833
  soft tissue, 1829
  treatment of, 1831-1833, 1831t
BAL. *See* Bronchoalveolar lavage (BAL)
Balamuthia mandrillaris, 2978-2979, 2978f

Balamuthia mandrillaris infection (balamuthiasis), **2975-2986**
clinical manifestations of, 2980
CNS, 494t-498t, 501, 511, 2975, 2980-2981, 2984-2985
diagnosis of, 2981-2983
epidemiology of, 2975-2977
neuroimaging in, 2980
pathology of, 2981
prevention of, 2985-2986
treatment of, 2983-2985
Balantidium coli infection (balantidiasis), **2894-2896**, 3327t-3346t
Balkan endemic nephropathy, 1867
Balloon catheter sinuplasty, 202
Bam H1A rightward transcript (BART), 1993-1994
Banna virus, 2175
Bannwarth syndrome, 1733
Banzi virus infection, 2316t
Barium x-ray
esophageal, 594-595, 595f
in *Shigella* spp. infection, 1483
in Whipple disease, 645
Barmah Forest fever, 2251-2252
Bartholin gland/duct
abscess of, 579, 579f
gonococcal infection of, 1284, 1285f
inflammation of, 579
Bartonella bacilliformis infection (bartonellosis, Carrión disease, Oroya fever), **1725-1726**
diagnosis of, 1727
exanthem in, 753t-756t, 1725-1726
prognosis for, 1729
treatment of, 1728
Bartonella henselae, 1721, 1721t
Bartonella henselae infection (cat-scratch disease), **1721-1725**
bone, 1723-1724
cervical lymphadenitis in, 178, 181
clinical manifestations of, 753t-756t, 1722-1725, 1723f-1724f
cutaneous lesions in, 753t-756t, 1724-1725
diagnosis of, 185, 1726-1727
differential diagnosis of, 1727
encephalitic, 494t-498t
endocardial, 363
epidemiology of, 1722
fever of unknown origin in, 844
hepatosplenic, 1722-1723, 1724f, 1728
lymphadenopathy in, 1722, 1723f
neurologic complications of, 1723
ocular manifestations of, 813
oculoglandular syndrome of Parinaud in, 1722, 1724f
pathobiology of, 1722
vs. plague, 1513
prevention of, 1729
prognosis for, 1729
vs. St. Louis encephalitis virus infection, 2259
transmission of, 1721-1722
treatment of, 187-188, 1727-1728
typical, 1722
vasculoproliferative, **1726-1728**
Bartonella quintana infection (trench fever), **1726**
diagnosis of, 1727
endocardial, 363
exanthem in, 753t-756t
ocular, 815
treatment of, 1728
vasculoproliferative, **1726-1728**
Bartonella spp., 1720-1721, 1721t
Bartonella spp. infection, **1720-1729.** *See also specific species infections*
Basic fibroblast growth factor, in *Streptococcus pneumoniae* infection, 1225
Basidiobolus spp., 2837
Basidiobolus spp. infection (entomophthoromycosis), **2844-2845**
Bat bite. *See* Rabies
Baylisascaris procyonis infection
encephalitic, 494t-498t
meningeal, **481-484**, 3023-3024. *See also* Meningitis, eosinophilic
ocular, 815
Beau lines, in Kawasaki disease, 1073-1074
Bed bugs, 758t, **3076-3077**
Bed rest
in *Corynebacterium diphtheriae* infection, 1310
in rheumatic fever, 413
Beef tapeworm *(Taenia saginata)* infection, **3030-3032**, 3327t-3346t
Behavior/behavioral disorders
congenital rubella and, 2217
infection and, 109-110
otitis media and, 222
urinary tract infection and, 536, 548
Behçet syndrome, 159t-160t, 161
Bejel, 1783t, **1787-1788**
Benznidazole, 3347
in *Trypanosoma cruzi* infection, 399, 2967
Benzodiazepines, in tetanus, 1816-1817
Benzyl alcohol, 3347
in lice, 3075
Bevacizumab, in West Nile virus infection, 2272
Bezold abscess, 236, 239
Bias, 102, 124-125
Bifidobacteria, in gastrointestinal tract infection, 626
Bile duct, common
infection of. *See* Cholangitis
obstruction of, 670
pancreatitis with, 692
Biliary atresia, 657
biloma in, 661-662
complications of, 664
hepatic portoenterostomy and, 658-659, 664-665
reovirus and, 2170-2171
Biliary stents, 667
Biliary system
infection of. *See* Cholangitis
obstruction of, 659. *See also* Cholangitis
surgical treatment of, 664
Bilirubin, serum
in HAV infection, 2138
in hepatitis, 650
in leptospirosis, 656
Biloma, 661-662
Biofilm, 11-12, 12f
in burn-related infection, 1050-1051
Citrobacter spp., 1430
of coagulase-negative staphylococci, 1132-1133, 1133f
in CSF shunt infection, 1030-1031
in implant-related infection, 1017
Neisseria gonorrhoeae, 1277
Pseudomonas aeruginosa, 11, 14f, 1588
Bipolaris spp. infection, **2855-2858**
Biopsy
in acute disseminated encephalomyelitis, 519
in *Aspergillus* spp. infection, 2711, 2712f
Biopsy *(Continued)*
in burn wound infection, 1056-1057, 1056f
in cervical lymphadenopathy, 185
in cholangitis, 661
in CMV infection, 1974, 1974f, 1984
in *Cryptosporidium* spp. infection, 2902
in *Entamoeba histolytica* infection, 2872
in HBV infection, 1919-1920, 1919f
in *Helicobacter pylori* infection, 1695
in HPV infection, 1878-1879, 1879f
in HSV encephalitis, 1948
in hypersensitivity pneumonitis, 304
in interstitial lung disease, 299-300
in *Leishmania* spp. infection, 2951
in *Leptospira* spp. infection, 1749
in *Mycoplasma pneumoniae* infection, 2673
in myocarditis, 393
in *Naegleria fowleri* infection, 2982, 2982f
in *Nocardia* spp. infection, 1421
in *Pneumocystis* infection, 3010
in Rocky Mountain spotted fever, 2653
Biostatistics, **120-125**
bias in, 102
categorical variables in, 101-102, 122-123, 123t
confidence intervals in, 123
confounding variables in, 123-124, 124t
continuous variables in, 100-101, 122
of diagnostic tests, 125-127, 126f, 126t-127t
frequency distribution in, 100-101, 100f
hypothesis testing in, 120-121
meta-analysis in, 124-125
multiple comparisons in, 121-122
statistical power in, 121
statistical significance in, 120, 122
summary statistics in, 99-102
type I error in, 121
type II error in, 121
of vaccine efficacy, 127-128, 127t
Bioterrorism, **3579-3585**, 3581t
anthrax, 3580-3582, 3581f. *See also* Anthrax
botulism, 1793, 3584-3585
epidemiology of, 3580
historical perspective on, 3579-3580
monkeypox, 2044
plague, 3583-3584
preparedness for, 3547-3548, 3585
smallpox, 2034, 3582-3583, 3583f
tularemia, 3584
viral hemorrhagic fever, 3585
Bird fancier's lung, 301, 302t-303t, 303f-305f
Bismuth subsalicylate
in gastrointestinal tract infection, 626
in rotavirus infection, 2186
in traveler's diarrhea, 3114
Bites, **3570-3579**
animal, 780-781, 780t. *See also* Cat bite; Dog bite; Rabies
complications of, 3572-3575, 3574f, 3574t
diagnosis of, 3575-3577
epidemiology of, 3570-3571
Erysipelothrix rhusiopathiae infection with, 1328
in *Francisella tularensis* infection, 1659-1660
microbiology of, 3571-3572, 3571t-3573t
polymicrobial, 1829
prevention of, 3578
treatment of, 3251, 3575-3577, 3576t-3577t

Bites *(Continued)*
arthropod, 2862t-2863t, **3070-3079**
cutaneous manifestations of, 758t, 763t-764t
vs. virus-induced exanthem, 762-763
in child abuse, 3571
in daycare facilities, 3571
historical perspective on, 3570
human, 780, 780t, 3573t
clinical manifestations of, 3574-3575
diagnosis of, 3575-3577
epidemiology of, 3571
microbiology of, 3572, 3573t
treatment of, 3251, 3575-3577, 3576t
reptile, 3578
tick. *See* Tick bite
Bithionol, 3348
BK virus, 1864-1865, 1864t
tumors and, 1868
BK virus infection, **1863-1870**
clinical manifestations of, 1866-1869
diagnosis of, 1868-1869, 3617
epidemiology of, 1865-1866
after hematopoietic stem cell transplantation, 942
after kidney transplantation, 1010
pulmonary, 1868
treatment of, 1869
urinary tract, 1867, 1867f
Black widow spider bite, **3077**
Bladder
infection of, 538. *See also* Urinary tract infection (UTI)
Schistosoma spp. infection of, 3064
suprapubic aspiration of, 541, 541f, 541t
Blastocystis hominis infection, **2875-2876**, 3327t-3346t
Blastomyces dermatitidis, 2723-2724, 2724f
Blastomyces dermatitidis infection (blastomycosis), **2723-2734**
bone, 726, 2728, 2729f-2730f
clinical manifestations of, 757t, 2727-2731, 2727f-2730f
CNS, 478, 480t, 494t-498t, 2729, 2730f
cutaneous, 757t, 2728, 2728f
diagnosis of, 2731-2732, 2731f
disseminated, 2727-2729, 2728f
epidemiology of, 2724-2726, 2724f, 2725t
historical perspective on, 2723
in immunocompromised host, 2729-2730
after kidney transplantation, 1013
after lung transplantation, 974
meningeal, 478, 480t
neonatal, 2730-2731
pathogenesis of, 2726-2727
pleural effusion in, 309-310
in pregnancy, 2730-2731, 2733
pulmonary, 2727, 2727f
treatment of, 2732-2734, 2732t, 3295t
Blau syndrome, 847-848
Bleach, in *Staphylococcus aureus* infection prevention, 1128-1129
Blepharitis. *See also* Eyelid infection
HSV, 1945
staphylococcal, 794
Blepharoconjunctivitis, *Pseudomonas aeruginosa*, 1591
Blistering distal dactylitis, **775**
Blood-brain barrier
in bacterial meningitis, 433-435, 434f
bacterial traversal of, 432-433, 433t
Blood group MSN, epiglottitis and, 249
Blood group O, cholera and, 1551
Blood group P, human erythrovirus B19 (human parvovirus B19) infection and, 1844-1845, 1854
Blood smear examination
in *Anaplasma* infection, 2662-2663
in *Babesia* spp. infection, 2918-2919
in *Ehrlichia* infection, 2662-2663
in immunodeficiency evaluation, 62
in malaria, 2930, 2931f-2934f
in parasite identification, 3625-3626
in *Trypanosoma cruzi* infection, 2959, 2960f, 2965-2966
Blood transfusion
CMV transmission with, 1972-1973
EBV transmission with, 1998, 2014-2015
HAV transmission with, 2135
HEV transmission with, 2163
HIV transmission with, 2592-2593
malaria transmission with, 2923
prion transmission with, 2622-2623
Trypanosoma cruzi transmission with, 2961
Yersinia enterocolitica transmission with, 1518
Blow dryer, in lice, 3075-3076
Boceprevir, in HCV infection, 2324
Body temperature, 83-84. *See also* Fever
Boerhaave syndrome, 419
Bolivian hemorrhagic fever. *See* Arenavirus infection
Bone
infarction of, 724
infection of. *See* Osteomyelitis
pancreatitis-related lesions of, 696
tuberculosis of, 1355-1356
Bone marrow
chloramphenicol-related suppression of, 3216-3217
examination of, in fever of unknown origin, 842-843
in *Paracoccidioides* spp. infection, 2784
Bone marrow transplantation (BMT). *See also* Hematopoietic stem cell transplantation (HSCT)
acyclovir in, 1966, 2032
in chronic granulomatous disease, 57
infection after
adenovirus, 1903-1904
Aspergillus spp., 2711
Hafnia alvei, 1534-1535
HSV, 1952, 1952f, 1954
IVIG prevention of, 3501
parainfluenza virus, 2368
Streptococcus pneumoniae, 1232-1233
in type 1 leukocyte adhesion deficiency, 53
Bone scan
in *Kingella kingae* infection, 1703
in osteomyelitis, 716-717, 716f
Bordetella avium, 1616
Bordetella bronchiseptica, 1616-1620, 1617t
Bordetella bronchiseptica infection, 1617, 1637
historical perspective on, 1616
after lung transplantation, 983
Bordetella hinzii, 1616-1617
Bordetella hinzii infection, 1637
Bordetella holmesii, 1616-1620, 1617t
Bordetella holmesii infection, 1617, 1637
historical perspective on, 1616
Bordetella parapertussis, 17, 1616-1620, 1617t
Bordetella parapertussis infection, 1617, 1626, 1636-1637
historical perspective on, 1616
immunization against, 1625
Bordetella pertussis, 1616-1620, 1617t
adenylate cyclase toxin of, 1618t, 1619, 1624
Bordetella pertussis (Continued)
dermonecrotic toxin of, 1618t, 1619, 1624
filamentous hemagglutinin of, 6, 7f, 1618t, 1619, 1623
fimbriae of, 1618-1619, 1618t
lipopolysaccharide of, 1618t, 1620
pertactin of, 1618t, 1619, 1623
pertussis toxin of, 1618t, 1619, 1624
toxins of, 17, 1618-1620, 1618t, 1623-1625
tracheal cytotoxin of, 1618t, 1620
type III secretion system of, 1618t, 1620
Bordetella pertussis infection (pertussis, whooping cough), **1616-1639**
adolescent/adult, 1622-1623, 1627
asymptomatic, 1626
clinical manifestations of, 1625-1627
coinfection with, 1617, 1626-1627
complications of, 1625-1626
cough of, 1624-1625
in daycare facilities, 3563
diagnosis of, 1627-1629, 3593
differential diagnosis of, 1627
epidemiology of, 1620-1623, 1620f
historical perspective on, 1616
immune response to, 1624-1625
immunization against, 1630-1636, 3457-3461
acellular vaccines for, 1630-1633, 1631t-1633t, 3458, 3458t
adverse events with, 1634-1635, 1635t
adverse events with, 1633-1635, 3459
contraindications to, 1636, 3460-3461
disease incidence and, 1620-1621, 1620f
efficacy of, 3459
encephalitis after, 502
failure of, 1620-1621
indications for, 3460
neurologic illness with, 3459
precautions for, 3460-3461
schedules for, 1635-1636
whole-cell vaccines for, 1630, 3458
adverse events with, 1633-1634
incidence of, 1620-1621, 1620f
in infant, 1626-1627, 1626t
isolation precautions in, 1636
mild, 1626
morbidity with, 1621-1622
mortality with, 1621-1622
neonatal, 891-892, 1626-1627, 1626t
neurologic, 502
nosocomial, 3382
pathogenesis of, 17, 1623-1625
pathology of, 1623
prevention of, 1630-1636, 3249
prognosis for, 1630
rate of, 1622-1623
transmission of, 1622
treatment of, 1629-1630, 1636, 3507
Bordetella petrii, 1616-1617
Bordetella petrii infection, 1637
Bordetella spp., 1616-1620, 1617t. *See also specific species infections*
Bordetella trematum, 1616-1617
Bordetella trematum infection, 1637
Bornholm disease, 739, 2067-2068
Borrelia burgdorferi, 1730, 1730t
antibodies to, 1734-1735
Borrelia burgdorferi infection. *See* Lyme disease
Borrelia spp., 1730t, 1739-1740
Borrelia spp. infection (relapsing fever), **1739-1742**
clinical manifestations of, 753t-756t, 1741-1742, 1741t

Borrelia spp. infection (relapsing fever) *(Continued)*
diagnosis of, 1742
differential diagnosis of, 1742
epidemiology of, 1730t, 1740
pathogenesis of, 1740-1741
transmission of, 1740
treatment of, 1742
Botryomycosis, 773, 1823
Botulism, 602t-603t
foodborne, **1791-1795**
bioterrorist, 1793, 3584-3585
CDC contact for, 1793-1794
clinical manifestations of, 1792-1793, 1792t
diagnosis of, 1793-1794
differential diagnosis of, 1793
epidemiology of, 1791-1792
pathophysiology of, 1792
prevention of, 1795
prognosis for, 1794-1795
reporting requirements for, 1794
treatment of, 1794, 3505
vaccine against, 1795
infant, **1801-1809**
age and, 1803-1804, 1803f
clinical manifestations of, 1805-1807, 1806f, 1806t-1807t
diagnosis of, 1807-1808, 1807t
epidemiology of, 1803-1805, 1803f, 1803t
historical perspective on, 1801
pathogenesis of, 1802-1803
prevention of, 1809
prognosis for, 1808-1809
risk factors for, 1802-1803
treatment of, 1808
wound, 1792
clinical manifestations of, 1793
treatment of, 1794, 3505
Botulism immune globulin, 1808, 3505
Bovine papular stomatitis virus infection, 2046
Bovine spongiform encephalopathy, 2621, 2623-2624
Bowman membrane, 806
Brain
Aspergillus spp. infection of, 2707, 2713-2714, 2714f
cryptococcoma of, 2803
lymphoma of, 2011, 2013
Naegleria fowleri–related pathology of, 2979f
tetanospasmin effects on, 1813
tuberculoma of, 1354
tumor of, 467
Brain abscess, **462-468**
Aggregatibacter (Haemophilus) aphrophilus, 1686
amebic, 2871
anticonvulsants in, 469
Arcanobacterium haemolyticum, 1325
Blastomyces dermatitidis, 2729, 2730f
Brucella spp., 1613
Candida, 2742-2743, 2757
Citrobacter spp., 1430-1431
clinical manifestations of, 463t, 464-466, 466t
corticosteroids in, 468-469
CSF examination in, 443t, 466
CT in, 466-467, 467f
culture in, 466
diagnosis of, 466-468, 467f
electroencephalography in, 467-468
gas within, 467
Brain abscess *(Continued)*
infective endocarditis and, 356-357
laboratory tests in, 466
mastoiditis and, 239
meningitis and, 457, 466
microorganisms in, 463-464, 465t
mortality with, 462
MRI in, 466-467
in neonatal meningitis, 885-887
Nocardia spp., 1421
odontogenic origin of, 151
otitis media and, 224-225, 225f
Pantoea agglomerans, 1536
pathogenesis of, 462-464, 463t
pathology of, 462-464, 463f-465f
Proteus spp., 1472-1473
Pseudomonas aeruginosa, 1598-1599
rhinosinusitis and, 202
rupture of, 463, 466
treatment of, 465t, 468
tuberculous, 1354
vs. tumor, 467
Brain stem encephalitis, 505
Brainerd diarrhea, 602
Branchial cleft cyst, 182
Brazilian hemorrhagic fever. *See* Arenavirus infection
Brazilian purpuric fever, **1688-1690**
BRCA1 gene, 50
Breast
abscess of, **890**
cancer of, 50
Breast-feeding
appendicitis and, 680
gastrointestinal tract infection and, 622, 626-627
Helicobacter pylori infection and, 1693
HEV infection and, 2163
infant botulism and, 1804
influenza virus infection and, 2356-2357
neonatal intestinal flora and, 89
otitis media and, 211
rotavirus infection and, 2182
RSV infection and, 2427
Salmonella spp. infection and, 1507
Shigella spp. infection and, 1486
Streptococcus pneumoniae infection and, 1204, 1236
West Nile virus infection and, 2264
Brodie abscess, 719
Bronchiectasis
in cystic fibrosis, 325, 325f
HTLV-1, 2576
Pasteurella multocida, 1547
Bronchiolitis, **271-282**
adenovirus, 1897
airway diameter and, 276
bacteria in, 272-273, 280
clinical presentation of, 274-275
in community-acquired pneumonia, 286
complications of, 281-282
definition of, 271
diagnosis of, 278
differential diagnosis of, 278, 278t
environmental factors in, 276, 276t
eosinophils in, 277-278
epidemiology of, 273-274, 273t
etiology of, 272-273, 272t-273t, 2066, 2122-2123
gender and, 274
historical perspective on, 272
hospitalization for, 274-275
human bocavirus, 1860
immunologic factors in, 277, 282
mortality with, 274, 281
Bronchiolitis *(Continued)*
Mycoplasma pneumoniae, 2679
necrotizing, 286
pathophysiology of, 275-278, 276t
patient positioning in, 280
prevention of, 280-281
prognosis for, 281-282
treatment of, 278-280
vitamin deficiency and, 280
wheezing with, 272, 273t, 274, 281-282
Bronchiolitis obliterans, 295-296
in adenovirus infection, 1898
in lung transplant recipient, 982
Bronchitis
acute, **262-265**
clinical presentation of, 263-264
diagnosis of, 264
differential diagnosis of, 264
epidemiology of, 262
etiology of, 262, 263t
pathology of, 262-263
pathophysiology of, 262-263
phases of, 264
prevention of, 265
prognosis for, 264-265
treatment of, 264
chronic, **265-271**
differential diagnosis of, 265-269, 266f
epidemiology of, 266t, 269
etiology of, 269, 269t
treatment of, 269-270, 270f
enterovirus, 2066
Mycoplasma pneumoniae, 2679
Bronchoalveolar lavage (BAL)
in *Aspergillus* spp. infection, 2715-2716
in *Blastomyces dermatitidis* infection, 2731
in burn patient, 1057
in cystic fibrosis, 325
galactomannan in, 967, 2715-2716
in *Histoplasma capsulatum* infection, 2820-2821
in HIV infection, 2599
in hypersensitivity pneumonitis, 304
in interstitial lung disease, 299
in interstitial pneumonitis, 948
in *Nocardia* spp. infection, 1421
in *Pneumocystis jiroveci* infection, 972-973
Bronchodilators
in bronchiolitis, 279
in chronic bronchitis, 269
in RSV infection, 2425-2426
Broncholithiasis, *Histoplasma capsulatum*, 2813
Bronchoscopy, in lung abscess, 318-319
Bronchus(i)
abscess rupture into, 318
tuberculosis-related obstruction of, 1345-1347, 1346f
Brown fat, 75, 84
Brown recluse spider bite, 758t, **3077**
Brucella abortus infection, 1612, 1612t
Brucella melitensis infection, 1612-1613, 1612t
pancreatic, 695
Brucella spp., 1612, 1612t
Brucella spp. infection (brucellosis), **1611-1615**
chronic, 1615
clinical manifestations of, 1613-1614
CNS, 1613-1615
cutaneous, 1613
diagnosis of, 1614
endocardial, 362, 1613-1615
epidemiology of, 1612
exanthem in, 753t-756t

Brucella spp. infection (brucellosis) *(Continued)*
fever of unknown origin in, 844
gastrointestinal tract, 602t-603t, 1613
genitourinary tract, 1613
hematologic abnormalities with, 1613-1614
historical perspective on, 1611
ocular, 813, 1613
osteoarticular, 725, 1613
pancreatic, 695
pathogenesis of, 1612-1613
in pregnancy, 1612
relapsing, 1615
respiratory tract, 1613
transmission of, 1612
treatment of, 1614-1615
Brudzinski sign, 439
Brugia spp. infection (filariasis), **3025-3026**, 3327t-3346t
Bruton disease. *See* Agammaglobulinemia, X-linked
BTK, 908-909
Bubonic plague. *See Yersinia pestis* infection (plague)
Buccal cellulitis, 151
Buckley syndrome (hyper-IgE syndrome), **55**, 919
Buffy coat test, in malaria, 2930-2935
Bulbar palsy, in infant botulism, 1806, 1806f, 1806t
Bulking agent injection therapy, in vesicoureteral reflux, 551
Burkholderia cepacia, 1583-1584
vs. *Stenotrophomonas maltophilia*, 1608
virulence factors of, 1589
Burkholderia cepacia infection
clinical manifestations of, 1592
in cystic fibrosis, 335-336, 335t, 341, 345t, 1593-1594
diagnosis of, 1597
epidemiology of, 1585-1586
after lung transplantation, 960-962
nosocomial, 1595
treatment of, 1601
vaccine against, 1604
Burkholderia gladioli infection, 1586
Burkholderia mallei infection, 753t-756t
Burkholderia pseudomallei, 1583-1584
virulence factors of, 1589
Burkholderia pseudomallei infection (melioidosis)
clinical manifestations of, 1592-1593
diagnosis of, 1596-1597
epidemiology of, 1586
treatment of, 1601
Burkholderia thailandensis infection, 1586
Burkitt lymphoma
EBV and, 1996, 2005, 2005f, 2013
reovirus and, 2171
treatment of, 2013
Burn(s), **1047-1062**
B lymphocytes in, 1050
bronchoalveolar lavage in, 1057
cell-mediated immunity in, 1050
circulatory impairment with, 1048
complement in, 1049-1050
cytokine response in, 1049
cytologic findings in, 1047
depth of, 1047, 1048f
dressing for, 1057-1059
excision and grafting in, 1061
first-degree, 1047, 1048f
fourth-degree, 1047, 1048f
gastrointestinal decontamination in, 1061
Burn(s) *(Continued)*
humoral immunity in, 1050
immune response in, 1049-1050
infection of, 3405
Acinetobacter spp., 1052-1053
Aspergillus spp., 1053
Bacillus spp., 1052
biomarkers in, 1055
biopsy of, 1056-1057, 1056f
bronchoalveolar lavage in, 1057
Candida spp., 1053, 1053t
clinical manifestations of, 1054-1056, 1055t
Clostridium tetani, 1052
complications of, 1055-1056
culture in, 1056-1057
Curvularia spp., 1053
Enterococcus spp., 1052, 1052t
fungal, 1053, 1053t
Fusarium spp., 1053
gram-negative bacteria in, 1052-1053, 1052t
gram-positive bacteria in, 1051-1052, 1052t, 1056f
histology of, 1057, 1057f, 1057t
HSV, 1053-1054, 1944, 1944f, 1953
IVIG use and, 1061, 3501, 3508-3509
local signs of, 1054, 1055t
microbiology of, 1050-1054, 1051t
Mucor spp., 1053
parasite, 1054
Penicillium spp., 1053
prevention of, 1057-1061
antibiotics in, 1060-1061
decontamination in, 1061
immunomodulators in, 1061
infection control in, 1061-1062
surgery in, 1061
Pseudomonas aeruginosa, 1052-1053, 1057f, 1593
Rhizomucor spp., 1053
Rhizopus spp., 1053
Staphylococcus aureus, 1051-1052, 1051t
Staphylococcus epidermidis, 1051-1052, 1051t
Streptococcus pyogenes, 1051-1052, 1051t
systemic signs of, 1054-1055, 1055t
treatment of, 1057-1061
antibiotics in, 1059-1060
cerium nitrate silver sulfadiazine in, 1058
chlorhexidine in, 1059
citric acid in, 1059
mafenide acetate in, 1058-1059
membrane dressings in, 1059
nystatin in, 1059
povidone-iodine in, 1059
silver nitrate in, 1058
silver sulfadiazine in, 1058
sodium hypochlorite in, 1059
subeschar antibiotic infusion in, 1060
topical antimicrobial agents in, 1058-1060
viral, 1053-1054, 1057, 1060
inflammation in, 1048-1050
inhalation injury with, 1048-1049
macrophages in, 1050
neutrophils in, 1049
PMN chemotaxis in, 56
second-degree, 1047, 1048f
T lymphocytes in, 1050
third-degree, 1047, 1048f
tissue changes in, 1048
toxic shock syndrome with, 860
Bursitis, septic, 732
Buruli ulcer, **1410-1417.** *See also Mycobacterium ulcerans* infection
clinical manifestations of, 1411f, 1413-1415, 1413f-1414f
diagnosis of, 1415
epidemiology of, 1411-1412
HIV infection and, 1415
pathology of, 1412-1413, 1412f
prevention of, 1416-1417
prognosis for, 1416
treatment of, 1416
Bussuquara virus infection, 2316t

C

C1, deficiency of, 51
C2, deficiency of, 51, 1225
C3, 29-31, 29f
deficiency of, 51, 55-56, 907
C3a, 31
C4, deficiency of, 51, 907
C4a, 31
C5, 29f
deficiency of, 51-52, 55-56
C5a, 29f, 31
C5a peptidase, 18
C5a-ase, of *Streptococcus agalactiae*, 1154, 1158
C6, deficiency of, 51-52
C7, deficiency of, 51-52
C8, deficiency of, 51-52
C9, 31
deficiency of, 429
C protein, of *Streptococcus agalactiae*, 1153-1154
C-reactive protein (CRP), 74
in burn wound infection, 1055
in craniofacial surgery–related infection, 1046
in CSF shunt–related infection, 1033
in fever of unknown origin, 842
in infective endocarditis, 358, 358t
in Kawasaki disease, 1075-1076
in meningitis, 444
in occult bacteremia, 838
in osteomyelitis, 719
in prosthetic joint infection, 1027
in sepsis neonatorum, 883
in *Streptococcus agalactiae* infection, 1162
in *Streptococcus pneumoniae* infection, 1224
in urinary tract infection, 543
Calcaneus, osteomyelitis of, 719-720
Calcification
in neurocysticercosis, 3034, 3038
in tuberculosis, 1347, 1348f-1349f
Calcitonin, 82
in toxic shock syndrome, 829-830
Calcium
intracellular, in cystic fibrosis, 331
loss of, 69
Calculi, gallbladder. *See* Gallstones
Calicivirus(es), 2147-2151, 2148f
antigenic properties of, 2150-2151
classification of, 2149, 2149t
genome of, 2149-2150, 2149f
recombination of, 2150, 2150f
structure of, 2148-2149
Calicivirus infection, **2147-2158.** *See also Norovirus* infection; *Sapovirus* infection
exanthem in, 749
historical perspective on, 2147-2148
Calymmatobacterium granulomatis. *See Klebsiella granulomatis*
CAM. *See* Complementary and alternative medicine (CAM)

Camelpox, **2046**
Campylobacter concisus infection, 1646t, 1650, 1652
Campylobacter curvus infection, 1653
Campylobacter fetus infection, 1646t, 1649-1650, 1653, 1653t
Campylobacter hyointestinalis infection, 1646t, 1650, 1652
Campylobacter jejuni, 1645-1646, 1645f
Campylobacter jejuni infection, 602t-603t, 609, **1644-1657**
  antibiotic resistance in, 1655-1656, 1655t, 3155
  clinical manifestations of, 1651-1654
  in daycare facilities, 3557
  diagnosis of, 618, 1654-1655
  differential diagnosis of, 1654
  epidemiology of, 1646-1650, 1646f, 1647t-1648t, 1653t
  extraintestinal, 1652-1653, 1653t
    treatment of, 1655-1656
  Guillain-Barré syndrome after, 1653-1654
  historical perspective on, 1644-1645
  immune response to, 1651
  immunoreactive complications of, 1653-1654
  neonatal, 888, 1653, 1656
  pathogenesis of, 1650-1651
  pathology of, 1650
  perinatal, 1653
  prevention of, 1656
  transmission of, 1649
  treatment of, 1655-1656, 1655t
Campylobacter lari infection, 1646t, 1650, 1652
Campylobacter spp., 1646t-1647t
Campylobacter spp. infection, 607t, 609, 753t-756t. *See also specific species infections*
  antibiotic resistance in, 3155
  treatment, 623-624, 623t
Campylobacter upsaliensis infection, 1646t, 1650, 1652
Campylobacter ureolyticus infection, 1652
Cancer. *See also* Leukemia; Lymphoma
  bacterial meningitis and, 437
  Blastomyces dermatitidis infection in, 2730
  breast, 50
  chemotherapy-associated infection in, **921-933**
    bacterial, 921-922, 922t
    chest radiography in, 925
    CMV, 1973
    fungal, 922, 2730
      evaluation of, 926-927, 927t
      risk stratification for, 924
      treatment of, 928
    hospital-acquired, 929
    laboratory evaluation of, 924-925
    occult, 923
    patient history in, 924
    physical examination in, 924
    prevention of, 928-929
    Pseudomonas spp., 1594-1596
    risk stratification for, 923-924
    treatment of, 925-928
    viral, 922-923, 923t
  EBV-related, 2005-2006, 2005f
    histopathology of, 1996-1997
    immunopathogenesis of, 1995-1996
    treatment of, 2012-2013
  fever without origin in, 840
  Helicobacter pylori infection and, 1696-1698, 1697f
  intestinal flora in, 94
  intravenous immunoglobulin in, 3499
Cancer *(Continued)*
  liver abscess with, 673
  vs. lung abscess, 319
  Mycoplasma spp. and, 2696
  nasopharyngeal, 1995-1996
  pleural effusion with, 313
  polyomaviruses and, 1868
  Pseudomonas spp. infection and, 1594-1596
  reovirus effect on, 2171
  Schistosoma spp. infection and, 3064
  septic arthritis with, 730
Candida albicans
  adhesins of, 9
  biofilm formation by, 11
  for delayed hypersensitivity skin test, 907
  Hwp1 of, 9
  INT1 of, 9
Candida albicans infection. *See also Candida* spp. infection (candidiasis)
  appendiceal, 685
  biliary, 660
  cutaneous, **787-789**, 788f
  endocardial, 354, 357-358, 363
  epidemiology of, 2736
  epiglottitis, 245
  exanthem in, 757t
  hepatic, 673-675
  in HIV infection, 148-149, 2602
  muscle, 739
  myocardial, 383f, 2740-2741, 2748
  neonatal, 892-893, 896, 2750. *See also Candida* spp. infection (candidiasis), neonatal
  ocular, 820
  oral, **787-788**, 787f
  pathogenesis of, 2737-2739
  treatment of, 788
  uvular, 167
  vulvovaginal, 569-570, 570f, 788
Candida glabrata infection, 2736. *See also Candida* spp. infection (candidiasis)
Candida krusei infection, 2736. *See also Candida* spp. infection (candidiasis)
Candida parapsilosis infection, 2736. *See also Candida* spp. infection (candidiasis)
Candida spp., 2735
  colonization by, 2751-2753
Candida spp. infection (candidiasis), 893f, **2735-2769**
  bloodstream, 2740
    epidemiology of, 2735-2737
    neonatal, 2754, 2759
    portal of entry in, 2738-2739
    treatment of, 2747-2748
  burn wound, 1053, 1053t
  in cancer patient, 922
  cardiac, 2740-2741
    treatment of, 2748
  clinical manifestations of, 2739-2744
  CNS, 2742-2743
    neonatal, 2757
    treatment of, 2749
  corneal, 807-808
    contact lens–related, 1019
  cutaneous, 2740
    neonatal, 893-894, 893f, 2753, 2754t, 2755f
    treatment of, 2747
  diagnosis of, 2735, 2744-2745
  disseminated, 2736-2744
    chronic, 2743-2744, 2749
    neonatal, 2755-2756, 2756f
  drug resistance of, 474
  endocardial, 357-358, 363, 2740, 2748
Candida spp. infection (candidiasis) *(Continued)*
  endovascular, 2740
    treatment of, 2748
  epidemiology of, 2735-2737
  esophageal, 593-597, 595f-596f, 2739
    treatment of, 2747
  exanthem in, 757t
  gallbladder, 2741
  gastrointestinal tract, 2741
  after heart transplantation, 948
  after hematopoietic stem cell transplantation, 936-937
  hepatic, 673-675
  hepatosplenic, 2743-2744
    treatment of, 2749
  host response to, 23
  immune response to, 2738-2739
  after intestinal transplantation, 994, 995t
  after kidney transplantation, 1013
  after liver transplantation, 993-994, 995t
  after lung transplantation, 970-971
  meningeal, 474-475, 480, 480t, 488, 2742-2743, 2749
    neonatal, 894-895, 897, 2754, 2757
  mucocutaneous, **787-789**, 788f
    chronic, 915-916, 2740
    neonatal, 893-894, 893f, 2753-2754
  mycetoma with, 2749
  neonatal, **892-897**, 893f, **2749-2766**
    acquired, 894
    bloodstream, 2754
    clinical manifestations of, 2753-2757
    CNS, 894, 2757
    congenital, 788-789, 894, 896
    cutaneous, 893-894, 893f, 2740, 2753-2754, 2754t, 2755f
    diagnosis of, 896, 2757
    disseminated, 894, 896-897, 2755-2756, 2756f
    drug-related risk factors in, 2765
    endocardial, 895, 897, 2756
    endophthalmic, 2756
    epidemiology of, 2749-2751, 2750f
    horizontal transmission in, 2752
    intra-abdominal, 895-897
    joint, 895, 897
    meningeal, 894-895, 897, 2754, 2757
    mortality with, 2766
    mucocutaneous, 893-894, 893f, 2753-2754
    neurodevelopmental impairment with, 2766, 2766f
    ocular, 895, 897, 2756
    oral, 896
    pathogenesis of, 2753
    peritoneal, 2754-2755
    prevention of, 897, 2760-2765
      fluconazole in, 2760-2761, 2764t
      lactoferrin in, 2765
      nystatin in, 2762-2763, 2764t
      recommendations for, 2763-2765, 2764t-2765t
    prognosis for, 2765-2766, 2766f
    pulmonary, 896
    renal, 894, 897, 2757
    risk factors for, 892, 2751-2753, 2752f
    treatment of, 896-897, 2757-2760
      adjunctive therapy in, 2759-2760
      amphotericin B in, 2758
      caspofungin in, 2758-2759
      central venous catheter and, 2759, 2765
      combination, 2759

Candida spp. infection (candidiasis) *(Continued)*
fluconazole in, 2758
voriconazole in, 2758
urinary tract, 2754
vertical transmission in, 2751
ocular, 2743
neonatal, 2756
treatment of, 2749
oral, **787-788**, 787f
in HIV infection, 148-149
in neonate, 892-893, 896
oropharyngeal, 159t-160t, 161, **2739**
immune response to, 2738
treatment of, 2746-2747
osteoarticular, 2742
treatment of, 2749
pathogenesis of, 2737-2739
peritoneal, 2741
treatment of, 2748
prevention of, 2745-2746
pseudomembranous, 2739
pulmonary, 2741
splenic, 673
treatment of, 2746-2749, 2746t, 3294t, 3321t
urinary tract, 540, 2741-2742
neonatal, 2754, 2757
treatment of, 2748-2749
uveal, 813, 820
vulvovaginal, 569-570, 570f, 788, 2739-2740
in pregnancy, 2765
treatment of, 2747
Candida tropicalis infection, 2736. *See also* *Candida* spp. infection (candidiasis)
Capillaria spp. infection, 3024, 3327t-3346t
Capillaritis, pulmonary, 297
Capnocytophaga, overgrowth of, 56
Capreomycin, in tuberculosis, 1367t
Capsule
Cryptococcus neoformans, 20
Haemophilus influenzae, 19-20
Staphylococcus aureus, 1113-1114, 1115f
Streptococcus agalactiae, 1154, 1158
Streptococcus pneumoniae, 1206t, 1211-1212, 1211f
Captopril, in murine myocarditis, 396
Carbacephems, 3199-3200
Carbamazepine, in chorea, 414
Carbapenem(s), 3200-3202
dosage schedule for, 3231t-3233t
resistance to, 3139-3145
Carbapenemases, 3142
Carbohydrates
hormone regulation of, 82
intestinal microflora effects on, 93-94
metabolism of, 72-73
Carbuncle, **776-777**
Cardiac tamponade, in pericarditis, 374
Cardiomyopathy
Chagas, 2963
dilated
adenovirus infection and, 1902
in enteroviral infection, 2075-2077
in myocarditis, 382, 387, 393
Cardiorespiratory arrest, in epiglottitis, 254
Cardioverter-defibrillator, implantable (ICD), infection with, 1025-1026
management of, 1026
microbiology of, 1025
Cardiovirus, 2051-2052. *See also* Saffold virus(es)
Carditis. *See also* Myocarditis
Lyme, 1733, 1735
Caries. *See also* Odontogenic infection
antifungal preparations and, 148-149
Garré sclerosing osteomyelitis with, 154, 154f-155f
in HIV infection, 149
nursing bottle, 146
pathogens in, 140, 1193
periapical abscess with, 141, 142f
Caroli disease, 666-667
Caroli syndrome, 666-667
Carpet cleaning, Kawasaki disease and, 1068
Carrión disease, **1725-1726**
diagnosis of, 1727
prognosis for, 1729
treatment of, 1728
Case-fatality rate, in epidemiologic study, 105
Caspofungin, 3308t, 3313f, 3315-3317
in *Aspergillus* spp. infection, 2718
in cancer patient, 928
dosing for, 3316-3317, 3316t
efficacy of, 3315-3316
in neonatal *Candida* spp. infection, 2758-2759
in oropharyngeal candidiasis, 2747
in transplant-related aspergillosis, 938, 968
Cassava poisoning, pancreatitis with, 692
Cat bite, 780-781, 780t
clinical manifestations of, 3572-3575
diagnosis of, 3575-3577
epidemiology of, 3570-3571
Erysipelothrix rhusiopathiae infection with, 1328
Francisella tularensis infection with, 1660
microbiology of, 3571-3572, 3571t-3572t
Pasteurella multocida infection with, 1546-1547, 1567
polymicrobial, 1829
prevention of, 3578
treatment of, 3251, 3575-3577, 3576t
Cat-scratch disease. *See Bartonella henselae* infection (cat-scratch disease)
Catalase
in chronic granulomatous disease, **56-58**
of *Helicobacter pylori*, 1693
Cataracts
in measles, 811
in rubella, 811, 818, 2213, 2214t-2216t, 2220-2221
surgery for, endophthalmitis after, 820
Catecholamines, plasma, 81-82
Cathelicidin, 24
Catheter-associated infection, 3385-3391, 3386t-3388t, 3387f
endocardial, 352
intestinal transplantation and, 991, 993
kidney transplantation and, 1006
Klebsiella spp., 1467-1468
liver transplantation and, 991, 993
Malassezia furfur, 2853
prevention of, 1139, 3244, 3390-3391
Proteus spp., 1472-1473
Providencia spp., 1475
Stenotrophomonas maltophilia, 1607-1609
treatment of, 3389-3390
Cavernous sinus thrombosis, vs. orbital cellulitis, 151
C4b binding protein, 18
CCAAT/enhancer binding protein epsilon (C/EBPe), 54
CD1 proteins, 37-38
CD11/CD18, in type 1 leukocyte adhesion deficiency, 53
CD18, in *Salmonella typhimurium* infection, 15
CD40L, 909-910
CD81, in hepatitis C virus infection, 11
Cdc42 protein, 14
Cefaclor, 3192t, 3193, 3231t-3233t
adverse effects of, 3199
Cefadroxil, 3191t-3192t, 3192-3193
Cefamandole, 3193
Cefazolin, 3192-3193, 3192t, 3231t-3233t
Cefdinir, 3192t, 3197-3198, 3231t-3233t
in rhinosinusitis, 200
Cefditoren, 3192t, 3198, 3231t-3233t
Cefepime, 3192t, 3198, 3231t-3233t
in meningitis, 448
Cefixime, 3192t, 3196, 3231t-3233t
Cefoperazone, in meningitis, 448
Cefotaxime, 3192t, 3195, 3231t-3233t
in meningitis, 447, 450t
in septic shock, 833-834, 833t
Cefoxitin, 3192t, 3193-3194, 3231t-3233t
in meningitis, 448
Cefpodoxime proxetil, 3192t, 3196-3197, 3231t-3233t
in rhinosinusitis, 200
Cefprozil, 3192t, 3194, 3231t-3233t
Ceftaroline, 3198-3199
resistance to, 3153-3154
Ceftaroline fosamil, 3238
Ceftazidime, 3192t, 3196, 3231t-3233t
in meningitis, 448, 450t
Ceftibuten, 3197, 3231t-3233t
Ceftizoxime, 3192t, 3195
Ceftriaxone, 3192t, 3195-3196, 3231t-3233t
biliary effects of, 662
in cervical lymphadenitis, 186
in meningitis, 447, 450t
pancreatitis with, 692
in septic shock, 833-834, 833t
Cefuroxime, 3192t, 3194, 3231t-3233t
in meningitis, 448
Cefuroxime axetil, 3194, 3231t-3233t
Cell cytotoxicity neutralization assay (CCNA), in *Clostridium difficile* infection, 635, 636t
Cell-mediated immunity. *See* Immunity, adaptive
Cellulitis, **777-778**
buccal, 151
Clostridium perfringens, 1796-1799
cochlear implant and, 1018
crepitant, 1829
facial, 440-441, 1231-1232
Haemophilus influenzae type b, 778, 778f, 1676
orbital. *See* Orbital cellulitis
periorbital (preseptal), 151, 798-801
peritonsillar, vs. abscess, 168-169
Streptococcus agalactiae, 1161, 1162f
Streptococcus pneumoniae, 1231-1232
supraglottic, 248
tissue expander and, 1017
Yersinia enterocolitica, 1528
Central line–associated bloodstream infection (CLABSI), 3385-3386, 3386t
Central nervous system (CNS). *See also* Brain; Spinal cord
dysfunction of, fever of unknown origin in, 846
immune activation in, 433-435, 434f
infection of. *See* Brain abscess; Encephalitis; Meningitis
in infective endocarditis, 356-357
progenitor cells of, 436
tuberculosis of, 1352-1354
in Whipple disease, 643, 644t, 646

Central venous catheter. *See also* Catheter-associated infection
endocarditis and, 352
infection with, 1136, 3385-3391, 3386t-3388t, 3387f
protocols for, 2765, 2765t
removal of, in neonatal *Candida* spp. infection, 2759
Centruroides spp. bite, **3077-3078**
Cephalexin, 3192-3193, 3192t, 3231t-3233t
in cervical lymphadenitis, 186
Cephalic pustulosis, 789, 789f
Cephalosporins, 3191-3199, 3191t
adverse effects of, 3199
allergy to, 1296
dosage schedules for, 3231t-3233t
fifth-generation, 3198-3199
first-generation, 3191t-3192t, 3192-3193, 3231t-3233t
fourth-generation, 3191t-3192t, 3198, 3231t-3233t
in neonatal meningitis, 886
in odontogenic infection, 145, 146t
pharmacokinetics of, 3191-3192, 3192t
resistance to, 3139-3145
second-generation, 3191t-3192t, 3193-3194, 3231t-3233t
in *Staphylococcus aureus* infection, 1124t, 1125
third-generation, 3191t-3192t, 3194-3198, 3231t-3233t
Cephalothin, 3192-3193, 3192t
Cephapirin, 3192-3193, 3192t
Cephradine, 3192-3193, 3192t
Cercopithecine herpesvirus 1 (herpes B virus) infection, 655
Cerebellar ataxia, acute, 505
Cerebellitis, EBV, 2004
Cerebral edema
at high-altitude illness, 3118
in meningitis, 439
Cerebral embolism, in infective endocarditis, 355
Cerebral infarction
in infective endocarditis, 356-357
in *Leptospira* spp. infection, 1753
in *Neisseria meningitidis* infection, 1260
Cerebrospinal fluid (CSF)
leakage of, 3252
peritoneal, 701
shunt for. *See* Cerebrospinal fluid (CSF) shunt
Cerebrospinal fluid (CSF) examination, 443t
in *Acanthamoeba* spp. infection, 2979
in acute disseminated encephalomyelitis, 519
in acute hemorrhagic encephalitis, 443t
in acute transverse myelitis, 523
in African sleeping sickness, 2970
in aseptic meningitis, 444-445, 490
in aspergillosis, 478-479
in *Bacillus anthracis* meningitis, 1314
in *Bacillus cereus* infection, 1320
in bacterial meningitis, 441-446, 443t-444t, 449, 451t, 455, 1262
in blastomycosis, 478, 480t
in brain abscess, 443t, 466
in *Brucella* spp. meningitis, 1613
in *Candida* spp. infection, 2743
in *Citrobacter* meningitis, 1430
in CMV infection, 1982
in *Coccidioides* spp. infection, 477, 480t, 2774, 2778
in congenital syphilis, 1777
in Creutzfeldt-Jakob disease, 2628
Cerebrospinal fluid (CSF) examination *(Continued)*
in cryptococcal meningitis, 443t, 475
in cryptococcal meningoencephalitis, 2801-2802
in Eastern equine encephalitis, 2228
in encephalitis, 506, 508-509
in enterovirus meningitis, 2089
in eosinophilic meningitis, 483
in *Escherichia coli* meningitis, 1446
in fungal meningitis, 479-480, 480t
Gram stain in, 442
in *Haemophilus influenzae* type b meningitis, 1675
in histoplasmosis, 476, 480t
in HSV encephalitis, 1946-1947
in HTLV-1 infection, 2571
in human monocytic ehrlichiosis meningitis, 2662
immunochromatographic membrane test in, in pneumococcal pneumonia, 1217
in intracranial pressure monitor–related infection, 1039-1040
in Japanese encephalitis virus infection, 2297
in La Crosse virus infection, 2522, 2522t
in *Leptospira* spp. infection, 1751
in Lyme disease, 1735
in mastoiditis, 238
in measles virus encephalitis, 2386
in mumps meningitis, 2401
in *Naegleria fowleri* infection, 2979
in *Neisseria meningitidis* infection, 1262
in neonatal *Candida* spp. infection, 894-895, 897
in neonatal HSV infection, 1955
in neonatal meningitis, 885-887, 1135
in orbital cellulitis, 800
in Oropouche fever virus meningitis, 2543
in *Pasteurella multocida* meningitis, 1547
in pneumococcal meningitis, 1228
in poliovirus infection, 2101
in rabies, 2450
in roseola infantum, 771
in shunt-related infection, 1032-1033, 1036, 1137
in spinal epidural abscess, 472
in St. Louis encephalitis virus infection, 2257
in *Streptococcus agalactiae* meningitis, 1162
in subacute sclerosing panencephalitis, 2389-2390
in subdural empyema, 443t, 469
in syphilis, 1770
in tick-borne encephalitis virus infection, 2309
transferrin band in, 441
in tuberculous meningitis, 1353
in *Ureaplasma* spp. infection, 2691-2692
VDRL test in, 819-820
in Venezuelan equine encephalitis, 2240
in viral meningitis, 489
in West Nile virus infection, 2270
in Western equine encephalitis, 2234-2235
Cerebrospinal fluid (CSF) shunt
endocarditis and, 352
infection with, 698, 700t, 701-703, **1029-1038**
clinical manifestations of, 1031-1032
coagulase-negative staphylococci in, 1136-1137
complications of, 1036
Cerebrospinal fluid (CSF) shunt *(Continued)*
CSF examination in, 1032-1033, 1036, 1137
diagnosis of, 1032-1033, 1137
epidemiology of, 1029-1030
etiology of, 1030, 1030t
Gram stain in, 1035, 1035f
hepatic, 673
pathogenesis of, 1030-1031
prevention of, 1038
prognosis for, 1037
third ventriculostomy in, 1034
treatment of, 1033-1036, 1033t, 1035f, 1037t
Cereolysin, 1318-1319
Cereulide, 1318
Cerium nitrate silver sulfadiazine, in burn injury, 1058
Ceruloplasmin, plasma, 79
Cerumen, 204
Cervical cancer, HPV and, 1873t, 1874-1875, 1877-1878
Cervical lymphadenitis, **175-189**
anatomy of, 176, 176f
biopsy in, 185
clinical presentation of, 179-181, 179f-180f, 183f
diagnosis of, 184-185
differential diagnosis of, 181-184, 182t-183t
epidemiology of, 176-178, 177t
etiology of, 176-178, 177t, 183, 183t
noninfectious, 182-183, 182t
pathophysiology of, 178-179
prevention of, 188
prognosis for, 188
treatment of, 185-188
Cervical lymphadenopathy
in infectious mononucleosis, 1996, 1999, 1999f, 1999t
in Kawasaki disease, 1074
Cervicitis, 584-586
Chlamydia trachomatis, 586
Neisseria gonorrhoeae, 586, 1284-1285, 1284f
Schistosoma spp., 3065
Cervicofacial actinomycosis, 184
Cesarean delivery, in neonatal HSV infection prevention, 1964-1965
CFTR (cystic fibrosis transmembrane conductance regulator) gene, 60
CH50 assay, 907
Chagas disease. *See Trypanosoma cruzi* infection (trypanosomiasis)
Chagoma, 2961, 2962f
Chalazion, **796-797**
Chancre, 1765, 1768
Chancroid, 583t, **1686-1688**
clinical manifestations of, 753t-756t, 1687
coinfection with, 1688
diagnosis of, 1687-1688
epidemiology of, 1686-1687
pathogenesis of, 1687
prevention of, 1688
treatment of, 1688
Chandipura virus infection, 500-501
Changuinola virus, 2176
Chapare virus infection, 2470t, 2471. *See also* Arenavirus infection
Charcot triad, 660, 660t
Chédiak-Higashi syndrome, **54**, 905-906, 919
Chemokine(s), 26t, 27-28
in bacterial meningitis, 434-435, 434f
in bronchiolitis, 278, 282

Chemokine(s) *(Continued)*
modulation of, 27-28
nomenclature for, 27
in phagocyte recruitment, 31-32
Chemokine receptors, 27
in HIV infection, 10-11
Chemotaxins, impaired generation of, 55-56
Chemotherapy
appendicitis and, 681
Clostridium difficile infection and, 634
fever and neutropenia with, **921-933.** *See also* Cancer, chemotherapy-associated infection in
epidemiology of, 921-923, 922t-923t
evaluation of, 924-925
persistent, 926-927, 927t
prevention for, 928-929
risk stratification for, 923-924
treatment of, 925-926
pancreatitis and, 691
Chest pain
in infective endocarditis, 356
in pericarditis, 374
in pneumonia, 288
Chest radiography
in *Actinomyces* spp. infection, 1822f
in acute eosinophilic pneumonia, 297
in ARDS, 868-869, 868f-869f
in *Aspergillus* spp. infection, 2712
in atypical measles, 2384, 2384f, 2392
in bronchiolitis, 275
in cancer patient, 925
in *Chlamydia pneumoniae* infection, 2641, 2642f
in *Chlamydia trachomatis* infection, 2633f
in chronic granulomatous disease, 918f
in CMV pneumonitis, 1976, 1976f
in *Coccidioides* spp. infection, 2773, 2773f
in congenital syphilis, 1770-1771
in *Cryptococcus neoformans* infection, 2800
in fever of unknown origin, 842
in hantavirus pulmonary syndrome, 2506, 2506f
in *Histoplasma capsulatum* infection, 2817-2819, 2818f
in hMPV infection, 2439f
in hypersensitivity pneumonitis, 303-304, 303f
in immunodeficiency evaluation, 906, 906f
in infectious mononucleosis, 2011
in influenza virus pneumonia, 2344f
in inhalational anthrax, 3581, 3581f
in *Leptospira* spp. infection, 1751, 1753
in lung abscess, 319, 319f
in mimivirus infection, 2048, 2050f
in *Mucor* spp. infection, 2842
in *Paracoccidioides* spp. infection, 2783, 2785f
in parapneumonic pleural effusion, 310, 311f
in *Pneumocystis* infection, 3009, 3009f
in recurrent respiratory papillomatosis, 1878-1879, 1878f
in *Rhizopus* spp. infection, 2842
in RSV infection, 2417-2418, 2417f
in severe combined immunodeficiency, 906f
Chest tube thoracostomy, in lung abscess, 321
Chi-square statistic, 122-123, 123t
Chickenpox. *See* Varicella (chickenpox)
Chigger bite, 758t
Chikungunya virus, 2242
Chikungunya virus infection, 500, 742t-748t, **2241-2248**
clinical manifestations of, 2244, 2245t-2246t
vs. dengue, 2282
diagnosis of, 2245-2247
epidemiology of, 2243-2244
geographic distribution of, 2243-2244
pathogenesis of, 2245
pathology of, 2245
prevention of, 2247
prognosis for, 2247
transmission of, 2242-2243
in traveler, 3116
treatment of, 2247
zoonotic, 2243
Child abuse
bites and, 3571
sexual. *See* Sexual abuse
Child care facilities. *See* Daycare facilities
Childhood-onset acute neuropsychiatric symptoms (CANS), 1146. *See also* PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection)
Chinese restaurant syndrome, 604, 604t
Chlamydia pneumoniae, 2639-2640, 2640f
Chlamydia pneumoniae (Chlamydophila pneumoniae) infection, 161, 292, **2639-2644**
atherogenesis and, 75
clinical manifestations of, 2641-2642, 2642f
diagnosis of, 2642-2644
endocardial, 363
epidemiology of, 2640-2641, 2641t
meningeal, 488
in otitis media, 214
in rhinosinusitis, 195-196
treatment of, 2644
Chlamydia psittaci, 2638
Chlamydia psittaci infection (psittacosis), **2638-2639**
clinical manifestations of, 2638-2639
diagnosis of, 2639, 2640t
endocardial, 363
epidemiology of, 2638
exanthem in, 750, 751t-752t
vs. *Legionella pneumophila* infection, 1713
treatment of, 2639
Chlamydia trachomatis, 2640f
Chlamydia trachomatis infection, 161, **2631-2638**
in adolescents, 2635
Bartholin duct, 579
blindness with, **2636**
cervical, 586
conjunctival, 804-805
neonatal, 805-806, 805t, 2633-2634
diagnosis of, 2636-2637, 3594
epidemiology of, 2631-2632
fallopian tube, **587-588**
in infants and children, 2634-2635
lymphogranuloma venereum with, **2635-2636**
Neisseria gonorrhoeae infection and, 1297
neonatal, 900-901, 2632-2633
conjunctival, 805-806, 805t, 2633-2634
diagnosis of, 2633-2634
epidemiology of, 2632-2633, 2632t
historical perspective on, 2632
prevention of, 2634
pulmonary, 2633, 2633f
treatment of, 2634
ocular, 804-805
Chlamydia trachomatis infection *(Continued)*
in otitis media, 214
pulmonary, 292, 1617-1618
neonatal, 2633, 2633f
treatment of, 2637-2638
urethral, **530-535**, 531f, 534t, 579
vulvovaginal, 572t-573t, 574-575, **584**, 584f
Chlamydophila pneumoniae. *See Chlamydia pneumoniae (Chlamydophila pneumoniae)* infection
Chloramphenicol, 3215-3217, 3231t-3233t
adverse effects of, 3216-3217
drug interactions with, 3217
indications for, 3216
mechanisms of action of, 3215
in meningitis, 446, 448, 450t
pharmacokinetics of, 3216
resistance to, 3157-3158, 3215
in Rocky Mountain spotted fever, 2654
in vitro activity of, 3215-3216
Chlorhexidine
in burn wound infection, 1059
in hematopoietic stem cell transplantation, 935
in *Staphylococcus aureus* infection prevention, 1128-1129
Chloroquine, 3348
in malaria, 2936-2941, 2937t-2940t
in malaria prevention, 2943-2944, 2944t
Chloroquine phosphate, 3348
N-Chlorotaurine (NCT), in adenovirus infection, 1908
Cholangiography, in *Clonorchis sinensis* infection, 3052
Cholangitis, **658-667**
biliary atresia and, 664-665
in Caroli disease, 666-667
choledochal cysts and, 666-667
clinical presentation of, 660-661, 660t
complications of, 664
congenital anomalies and, 666-667
diagnosis of, 660t, 661-662
differential diagnosis of, 662-663, 662t
echinococcal, 660
after endoscopic procedures, 667
etiology of, 659-660, 659t
fungal, 660
in immunocompromised host, 666
after liver transplantation, 665-666, 993
mycobacterial, 660
parasitic, 660, 664
pathogenesis of, 659-660
prevention of, 664
stent-related, 667
treatment of, 663-664, 663t
viral, 660
Cholecystectomy, 670
Cholecystitis, **667-671**
acalculous, 670-671, 670t
Candida, 2741
clinical presentation of, 669
complications of, 670-671
Escherichia coli, 1445-1446
etiology of, 667-669, 668t
evaluation of, 669
in *Leptospira* spp. infection, 1752
management of, 669-670
Choledochal cyst, 666-667
Choledocholithiasis, 670
Cholelithiasis. *See* Gallstones
Cholera, **611-612**, **1548-1555**
clinical manifestations of, 602t-603t, 607t, 1551-1552, 1551f
diagnosis of, 602t-603t, 616t, 618, 1552

Cholera *(Continued)*
epidemiology of, 611-612, 1550-1551
historical perspective on, 1549
host factors in, 1551
immunization against, 1554-1555, 3478-3479
nitrogen balance in, 68-69
pathogenesis of, 612, 1550
prevention of, 1554-1555
rapid dipstick test for, 1552
treatment of, 623t, 625, 1552-1553, 1552t, 1553f, 1554t
Cholestasis
drug-induced, 662
gallstones with, 668
sepsis-induced, 662
Cholesteatoma, 223, 233-234, 239
Cholesterol
bacterial infection and, 74
viral infection and, 73-74
Choline-binding protein A (CbpA), of *Streptococcus pneumoniae*, 1211, 1211f, 1213
Chorea, 413
in rheumatic fever, 410, 414
Choreiform movements, in PANDAS, 1145
Chorioamnionitis
Candida, 788-789
Eikenella corrodens, 1577
Ureaplasma spp., 2690
Chorioretinitis, 809
Ascaris lumbricoides, 815
Candida, 813
CMV, 1977
Coccidioides spp., 813-814
Cryptococcus neoformans, 814
Histoplasma capsulatum, 813
LCMV, 811-812
Loa loa, 815
in Q fever, 815
rickettsial, 815
Toxoplasma gondii, 2991, 2994, 2994f, 2999-3000
West Nile virus, 2268-2269, 2272
Choroid, tuberculosis of, 1352, 1357
Choroiditis, 809
Bartonella henselae, 813
Brucella, 813
Onchocerca, 815
Schistosoma, 815
Chromium, metabolism of, 77t
Chromobacterium violaceum, 1563
Chromobacterium violaceum infection, 776, **1563-1564**
clinical manifestations of, 1564
diagnosis of, 1564
epidemiology of, 1563
pathophysiology of, 1563-1564
treatment of, 1564
Chromoblastomycosis, 479, **789-790**, 790f
Chrondritis, suppurative, in burn patient, 1055
Chronic cavitary pulmonary aspergillosis, 2708
Chronic fatigue syndrome, **1085-1091**
cardiovascular factors in, 1088-1089
clinical manifestations of, 1086, 1088-1089
definition of, 1086, 1086t
diagnosis of, 1089-1090
EBV and, 2004
endocrine factors in, 1088
enterovirus and, 2091
epidemiology of, 1087, 1087t
etiology of, 1087-1088
Chronic fatigue syndrome *(Continued)*
genetic components in, 1088
historical perspective on, 1086-1087
human erythrovirus B19 (human parvovirus B19) infection and, 1854
immune dysfunction and, 1088
infection and, 1086-1087
management of, 1090, 1090t
neurologic factors in, 1088
prognosis for, 1091
psychological factors in, 1089
sleep disturbances in, 1089
Chronic granulomatous disease, **56-58**, 58f, 917-918
Aspergillus spp. infection in, 56-57, 917, 918f, 2706, 2713f
Burkholderia cepacia infection in, 1595
clinical features of, 917-918, 918f, 1122
diagnosis of, 907, 918
interferon-γ in, 3364
liver abscess in, 673
lung abscess in, 317
osteomyelitis in, 724
pathogenesis of, 907t, 918
treatment of, 918
X-linked, 56
Chronic necrotizing pulmonary aspergillosis, 2708
Chronic nonsuppurative sclerosing osteomyelitis (Garré sclerosing osteomyelitis), 154, 154f-155f
Chronic obstructive pulmonary disease (COPD), *Mycoplasma pneumoniae* infection and, 2679
Chronic progressive panencephalitis, in rubella virus infection, 2216
Chronic recurrent multifocal osteomyelitis (CRMO), 714
Chryseobacterium spp., 1578-1579
Chryseobacterium spp. infection, **1578-1581**
clinical manifestations of, 1579-1580
diagnosis of, 1580
epidemiology of, 1579
pathophysiology of, 1579
treatment of, 1580-1581
Churg-Strauss syndrome, 297
Cidofovir, 3278t, 3287-3289, 3287f
in adenovirus infection, 1907
in BK virus infection, 1869
in CMV infection, 1984-1986
in HPV infection, 1884
in kidney transplant–related CMV infection, 1008
in lung transplant–related adenovirus infection, 982
in monkeypox, 2044
Cigarette smoke exposure
cough and, 268
normal flora and, 89-90
otitis media and, 211
Streptococcus pneumoniae infection and, 1236
Ciguatera poisoning, 604, 605t
Ciliary dyskinesia, 60
Cimetidine, in papillomas, 1883
Cimex lectularius, 758t
Cimex lectularius infestation, **3076-3077**
Cineangiography, in infective endocarditis, 359
Ciprofloxacin, 3225-3227, 3231t-3233t
in anthrax, 1315
in odontogenic infection, 145
Circumcision, urinary tract infection and, 536, 552
Citric acid, in burn wound infection, 1059
Citrobacter diversus infection, 886
Citrobacter farmeri infection, 1431
Citrobacter freundii infection
diagnosis of, 1431
gastrointestinal tract, 1431
meningeal, 1429
Shiga toxins in, 17, 1430-1431
soft tissue, 1431
treatment of, 1431-1433
Citrobacter koseri infection
diagnosis of, 1431
meningeal, 1429-1431, 1432f
treatment of, 1432-1433
treatment of, 1431-1433
Citrobacter rodentium, 7-8
Citrobacter spp., 1429
Citrobacter spp. infection, **1429-1433**
clinical manifestations of, 1430-1431
diagnosis of, 1431
epidemiology of, 1429-1430
pathophysiology of, 1430
prevention of, 1433
in septic shock, 834
treatment of, 1431-1433
Cladophialophora bantiana infection, **2855-2858**
after lung transplantation, 974
Cladophialophora carrionii infection, **789-790**
Cladosporium spp. infection, after lung transplantation, 974
Clarithromycin, 3211-3215, 3213t, 3231t-3233t, 3348
in common cold, 138
drug interactions with, 3215
in nontuberculous mycobacterial lymphadenitis, 187
Claudin-I, 16
Clavicle, in congenital syphilis, 1772
Clenched-fist injury, 3572, 3575
Climate, infection and, 110
Clindamycin, 3220-3222, 3348
in cervical lymphadenitis, 186
in methicillin-resistant *Staphylococcus aureus* infection, 1127-1128
in odontogenic infection, 145, 146t
in rhinosinusitis, 200
in *Staphylococcus aureus* infection, 1125
in *Toxoplasma gondii* infection, 3001
Clinical trial, 96-97, 97t. *See also* Epidemiology
ethics in, 97
of vaccines, 127-128
Clitoris, infection of, 578-579
Clofazimine
in *Mycobacterium leprae* infection, 1407-1408, 1407t
in tuberculosis, 1367t
Clonorchis sinensis, 3049, 3050f-3051f
Clonorchis sinensis infection (clonorchiasis), **3049-3052**
clinical manifestations of, 3051
diagnosis of, 3051-3052
epidemiology of, 3050-3051
imaging in, 3052
pathogenesis of, 3050f, 3051
prevention, 3052
transmission of, 3050-3051
treatment of, 3052, 3327t-3346t
Clostridium baratii infection, 1801, 1804. *See also* Botulism, infant
Clostridium botulinum, 1791-1792, 1801-1802
in corn syrup, 1805
in honey, 1804-1805

Clostridium botulinum infection (botulism)
foodborne, **1791-1795**
clinical manifestations of, 602t-603t, 1792-1793
diagnosis of, 1793-1794
differential diagnosis of, 1793
epidemiology of, 1791-1792
pathophysiology of, 1792
prevention of, 1795
prognosis for, 1794-1795
treatment of, 1794
infant, **1801-1809**
clinical manifestations of, 1805-1807, 1806f, 1806t-1807t
diagnosis of, 1807-1808, 1807t
epidemiology of, 1803-1805, 1803f, 1803t
historical perspective on, 1801
pathogenesis of, 1802-1803
prevention of, 1809
prognosis for, 1808-1809
treatment of, 1808
wound, 1792
clinical manifestations of, 1793
treatment of, 1794
Clostridium butyricum infection, 1801, 1804. *See also* Botulism, infant
Clostridium difficile
colonization with, 633-635
NAP1 strain of, 632-633
toxins of, 634, 634f
transmission of, 635
vaccine against, 638
Clostridium difficile infection
antibiotic-associated, 599, **632-639**
amoxicillin-clavulanic acid and, 599
clindamycin and, 145
clinical manifestations of, 635
diagnosis of, 635-636, 636t, 1799, 3594
differential diagnosis of, 636-637
epidemiology of, 632-633
historical perspective on, 632
after kidney transplantation, 1006
pathogenesis of, 632-635, 634f
prevention of, 637-638, 1800
treatment of, 637, 1799
chemotherapy-associated, 599
in daycare facilities, 3558
gastrointestinal, 607t, 613, 616t, 618
after intestinal transplantation, 994
treatment of, 623t
after hematopoietic stem cell transplantation, 935
after intestinal transplantation, 994
IVIG in, 3505-3506
in necrotizing enterocolitis, 635
nosocomial, 3401
Clostridium perfringens, 1795-1796
Clostridium perfringens infection, **1795-1800**
diagnosis of, 618, 1798-1799
epidemiology of, 1795-1796, 1796t
gastrointestinal, 602t-603t, 607t, 613, 1797-1800
myonecrotic, 1796, 1798-1800, 3506
panophthalmic, 1798
pathophysiology of, 1795-1796
pneumatosis cystoides intestinalis in, 1798
prevention of, 1800
soft tissue, 1798-1799
uterine, 1797-1800
Clostridium septicum infection, 1797
bloodstream, 1798
Clostridium sordelli infection, 1797-1798
Clostridium spp., 1795, 1796t
nonpathogenic, 1810
Clostridium spp. infection, **1795-1800.** *See also specific species infections*
bloodstream, 1797-1798
bone, 1797-1798
clinical manifestations of, 1796-1797
diagnosis of, 1798-1799
enteric, 1797
epidemiology of, 1795-1796, 1796t
joint, 1797-1798
meningeal, 1797
myonecrotic, 1796
neonatal, 1797
pathophysiology of, 1795-1796
in pneumatosis cystoides intestinalis, 1798
prevention of, 1800
prognosis for, 1799-1800
soft tissue, 1796-1797
trauma-related, 1798
treatment of, 1799
uterine, 1797
Clostridium tetani, 1810, 1812
Clostridium tetani infection (tetanus), **1809-1819**
burn wound, 1052
cephalic, 1813
cerebral effects in, 1813
clinical manifestations of, 1813-1815
diagnosis of, 1815
differential diagnosis of, 1815
epidemiology of, 1810
generalized, 1813-1815
historical perspective on, 1809-1810
immunization against, 1818, 3470-3472
incidence of, 1810-1812, 1811f
in injection drug users, 1814
local, 1813
mortality with, 1817-1818
motor end-plate effects in, 1812-1813
neonatal, 1814-1815, 1819
pathogenesis of, 1812
pathophysiology of, 1812-1813
prevention of, 1818-1819
prognosis for, 1817
seizures in, 1814, 1816-1817
spinal cord effects in, 1813
sympathetic nervous system effects in, 1813
tetanus toxoid in, 1818-1819, 1818t
treatment of, 1815-1817
trismus in, 1814
Clotrimazole
in oropharyngeal candidiasis, 2746
in vulvovaginal fungal infection, 570
Cloxacillin, 3183t-3184t, 3185-3186, 3231t-3233t
in cervical lymphadenitis, 186
Clubbing (digital), in infective endocarditis, 357
Clue cell, 577, 577f
Clumping factor, 8-9, 1113, 1115f, 1118, 1129
Clutton joints, 1772
CMV. *See* Cytomegalovirus (CMV)
CMX001 (hexadecyloxypropyl-cidofovir)
in adenovirus infection, 1907
in BK virus infection, 1869
CNS. *See* Central nervous system (CNS)
Coagulopathy, in septic shock, 829
Cobalt, metabolism of, 77t
Coccidioides immitis, 2769-2770, 2770f
Coccidioides immitis infection (coccidioidomycosis), **2769-2779**
epidemiology of, 2770-2771
exanthem in, 757t, 762
Coccidioides immitis infection (coccidioidomycosis) *(Continued)*
extrapulmonary, 725-726
after intestinal transplantation, 1000
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 973
meningeal, 477-478, 480t, 483
ocular, 813-814
retropharyngeal, 174
Coccidioides spp. infection (coccidioidomycosis), **2769-2779**
bone, 2771, 2772f, 2773-2774
clinical manifestations of, 2772-2775, 2773f
culture in, 2775-2776, 2775f
cutaneous, 2773
diagnosis of, 2775-2777, 2775f
disseminated, 2771, 2774f
clinical manifestations of, 2773-2775
in immunosuppressed host, 2772
treatment of, 2777-2778
encephalitic, 494t-498t
epidemiology of, 2770-2771
exanthem in, 757t, 762, 2772-2773
granuloma in, 2769-2770, 2770f, 2773
in immunocompromised host, 2774-2775
meningeal, 477-478, 480t, 483, 2774
treatment of, 2778
pathogenesis of, 2771-2772, 2772f
pathology of, 2770f, 2771
in pregnancy, 2772, 2774
prevention of, 2779
prognosis for, 2778-2779
skin test in, 2776
in traveler, 3116-3117
treatment of, 2777-2778, 3295t
Cochlear implant–associated infection, 1017-1018
meningeal, 224, 437, 1017-1018, 1229
Coenurosis, 815, **3039-3040**
Cognitive development, otitis media and, 222, 222f
Cold, common. *See* Common cold
Cold agglutinins
in *Mycoplasma pneumoniae* infection, 2674, 2685
in pleural effusion, 313
Cold sore (herpes labialis), 154-155, 742t-748t, 1950, 1950f, 1952-1953, 1952f
Colic, probiotics in, 3371
Colicins, in urinary tract infection, 538
Colistin, 3207-3208, 3231t-3233t
Colitis. *See also* Gastrointestinal tract infection
antibiotic-associated, **632-639**
clinical manifestations of, 635, 3222
differential diagnosis of, 636-637
etiology of, 632-634
historical perspective on, 632
in *Klebsiella oxytoca* infection, 1468
laboratory studies in, 635-636, 636t
pathogenesis of, 634-635, 634f
prevention of, 637-638
treatment of, 637
differential diagnosis of, 2872
in *Entamoeba histolytica* infection, 2870, 2873
hemorrhagic, in *Escherichia coli* O157:H7 infection, 1454-1455
in *Trichuris* infection, 3016
Collagen-binding protein, of *Staphylococcus aureus*, 8-9

Colonization, 3-12
biofilms in, 11-12, 12f
Hwp1 protein in, 9
vs. infection, 103-104
INT1 protein in, 9
microbiome-related resistance to, 93
nonpilus adhesins in, 6-9, 7f-9f
normal flora, 89
pilus adhesins in, 3-6, 4f-5f
tissue tropism in, 10-11
Colonization factor antigens, in *Escherichia coli* infection, 1451, 1455, 1456f
Colonoscopy, in *Clostridium difficile* infection, 636
Colorado tick fever virus infection, **2173-2175**
clinical manifestations of, 742t-748t, 749, 2173-2174
diagnosis of, 2174-2175
differential diagnosis of, 2174
epidemiology, 2173, 2173f
pathophysiology of, 2174
prevention of, 2175
treatment of, 2175
Colposcopy, in HPV infection, 1882
Coltiviruses, 2172. *See also* Colorado tick fever virus infection
Common cold, **132-139**
age and, 2120, 2120f
clinical manifestations of, 136, 2121-2125, 2121t
complications of, 138-139
coronavirus in, 2490-2491
coxsackievirus A21 in, 2065
diagnosis of, 136-137, 2125, 2125t
differential diagnosis of, 136
enteroviruses in, 2064-2065
environmental temperature and, 2120
epidemiology of, 133-134, 133t, 2111-2113
etiology of, 132, 133t, 1895, 2064-2065, 2064t, 2110-2111, 2110f. *See also* Rhinovirus
gender and, 2120
historical perspective on, 132, 2109-2110
immune response in, 2118-2121
in immunocompromised host, 2125
in infants, 136
mucociliary transport in, 135
Mycoplasma pneumoniae in, 2678
pathogenesis of, 2118-2121
pathophysiology of, 134-136
prevention of, 139, 2117, 2117t, 2125-2126
prognosis for, 138-139
psychosocial factors in, 2120-2121
risk factors for, 134
seasonal distribution of, 134, 2112, 2113f
socioeconomic status and, 134
transmission of, 134, 2114-2118, 2114f, 2116f, 2117t
treatment of, 137-138, 2125-2126
Common variable immunodeficiency (CVID), 49, **910**
vs. chronic bronchitis, 267
diarrhea in, 600
furunculosis and, 777
IgA deficiency and, 911
Complement, **28-31**, 3365
activation of, 29-31, 29f
alternative pathway of, 29f, 30
in burns, 1049-1050
classical pathway of, 29-30, 29f
deficiencies of, 51-52, 429, **916-917**
clinical features of, 916
diagnosis of, 907, 917
Complement *(Continued)*
Neisseria meningitidis infection and, 1256-1257
pathogenesis of, 916-917
treatment of, 917
effector functions of, 30-31
evaluation of, 63
in *Haemophilus influenzae* infection, 1672
in immune regulation, 31
in inflammatory response, 31
in Kawasaki disease, 1077-1078
mannan-binding lectin pathway of, 29f, 30, 51
microbicidal activity of, 31
in *Neisseria* spp. infection, 18, 52, 429, 1279
in neonate, 44-45, 44t
opsonic activity of, 30-31
pathogen evasion of, 18
in septic shock, 829
in *Streptococcus pneumoniae* infection, 1209-1210, 1225
Complementary and alternative medicine (CAM), in common cold, 138
Computed tomography (CT)
in *Actinomyces* spp. infection, 1822f, 1823-1824
in acute disseminated encephalomyelitis, 518-519
in acute eosinophilic pneumonia, 297
in acute lobar nephropathy, 554, 555f, 556
in amebic CNS infection, 2980
in appendicitis, 682-683
in *Aspergillus* spp. infection, 2706, 2708f, 2712-2714, 2713f
in bacterial meningitis, 445-446, 446f, 454, 458
in *Bartonella henselae* infection, 1722-1723, 1724f
in brain abscess, 466-467, 467f
in chronic fatigue syndrome, 1088
in chronic osteomyelitis, 726, 726f
in *Citrobacter* spp. infection, 1431-1432, 1432f
in *Clostridium difficile* infection, 636
in *Cryptococcus neoformans* infection, 2797f, 2800
in cystic fibrosis, 325
in dental abscess, 152f
in Eastern equine encephalitis, 2228
in encephalitis, 506-507
in fungal infection, 926-927
high-resolution, in interstitial lung disease, 298-299
in *Histoplasma capsulatum* infection, 2817, 2818f
in HSV encephalitis, 1946-1947, 1947f
in hypersensitivity pneumonitis, 303-304, 303f
in intra-abdominal abscess, 705
in La Crosse virus encephalitis, 2522, 2523f
in *Legionella pneumophila* infection, 1710
in lung abscess, 319, 320f
in lung transplant–related *Aspergillus* infection, 966
in mastoiditis, 237-238, 238f
in mediastinitis, 420
in meningeal *Streptococcus agalactiae* infection, 1160, 1160f
in *Mycoplasma pneumoniae* infection, 2677-2678
in *Nocardia* spp. infection, 1421
Computed tomography (CT) *(Continued)*
in orbital cellulitis, 800, 800f
in osteomyelitis, 717
in pancreatitis, 690
in parapharyngeal abscess, 173
in parapneumonic pleural effusion, 310
in parotid gland enlargement, 192
in pericarditis, 377
in peritonsillar abscess, 169
in pyogenic liver abscess, 674, 674f
in recurrent respiratory papillomatosis, 1879f
in renal abscess, 555f, 556, 557f
in retroperitoneal infection, 708
in retropharyngeal abscess, 171, 171f
in rhinosinusitis, 199
in subacute sclerosing panencephalitis, 2389
in subdural empyema, 469, 470f
in tuberculous meningitis, 1353
in urinary tract infection, 546
Concentration quenching, 3174-3175, 3174f
Condoms, 1299
Conduction, in heat transfer, 84
Condyloma acuminata, 580-581, 580f-581f, 1873t, 1875-1877
Condylomata lata, 1770
Confidence interval, 123
Congenital heart disease
brain abscess and, 462
Candida spp. infection and, 2751
infective endocarditis with, 351, 351t
rubella virus–induced, 2214t-2216t, 2217, 2221
Congestive heart failure
in infective endocarditis, 356
in rheumatic fever, 414
Conidiobolus spp., 2837
Conidiobolus spp. infection (entomophthoromycosis), **2844-2845**
Conjunctiva (conjunctivae), 801
bulbar, in ataxia-telangiectasia, 905f
indigenous flora of, 90, 91t
infection of. *See* Conjunctivitis
Loa loa under, 3027
papilloma of, 1879-1880
Conjunctivitis, **801-806**
Achromobacter spp., 1574
adenoviral, 802-803, 1898-1899
Bacillus cereus, 1319
bacterial, 802
in bronchiolitis, 275
chemical, neonatal, 805-806, 805t
chlamydial, 804-805
neonatal, 805-806, 805t, 900-901, 2633-2634, 3242-3243
contact lens–related, 1019
Corynebacterium diphtheriae, 1305
EBV, 810
enteroviral, 810, 2073-2074, 2074t
gonococcal, 1288, 3242-3243. *See also* Ophthalmia neonatorum
Haemophilus aegyptius, 1689
hemorrhagic, acute, **810**
HSV, 803-804, 1945
inclusion, 804
in Kawasaki disease, 1073
Leishmania spp., 814
Leptospira spp., 1750-1752
Loa loa, 815
Lyme, 812
measles virus, 811
meningococcal, 1261
Moraxella catarrhalis, 1252

Conjunctivitis *(Continued)*
neonatal, 805t, 890, 3242-3243. *See also* Ophthalmia neonatorum
chlamydial, 805-806, 805t, 900-901, 2633-2634, 3242-3243
gonococcal, 805-806, **1280-1282**
Pantoea agglomerans, 1536
in Q fever, 815
rickettsial, 815
Rift Valley fever virus, 812
rubella virus, 811, 2212
Stenotrophomonas maltophilia, 1607
Streptococcus pneumoniae, 1231
in trench fever, 815
tuberculous, 1357
viral, 802-805
VZV, 804
Yersinia enterocolitica, 1528
Conjunctivitis-otitis syndrome, 212, 217
Connective tissue disease
fever of unknown origin in, 840
pulmonary manifestations of, 297
Constipation
in enteroviral infection, 2068t, 2070
in infant botulism, 1805
probiotics in, 3371
urinary tract infection and, 536
Contact dermatitis, vs. otitis externa, 208
Contact lenses, infection with, 1019, 2979-2980, 2980f
Contact precautions. *See also* Isolation precautions
in *Clostridium difficile* infection prevention, 637
Contagion, concept of, 103
Continuous ambulatory peritoneal dialysis (CAPD), infection with, 698, 700t, 701-703
Contraception, pelvic inflammatory disease and, 587
Convection, 84
Convulsions. *See* Seizures
Cooling therapy, in fever, 88
Copper, metabolism of, 77t, 79
Cor pulmonale, in *Schistosoma* spp. infection, 3065-3066
Corn syrup, infant botulism and, 1805
Cornea, 806
inflammation of. *See* Keratitis
Coronary artery (arteries)
aneurysm of, 1071, 1080-1084
atherosclerosis of, CMV and, 1980
vasculitis of, 1071-1072, 1074-1075. *See also* Kawasaki disease
Coronavirus, 2487-2488, 2487f
isolation of, 2494
Coronavirus infection, **2486-2495**
age and, 2489-2490
asthma and, 2491
asymptomatic, 2489
clinical manifestations of, 2490-2494
CNS, 2494
diagnosis of, 2494
enteric, 2493-2494
epidemiology of, 2488-2490, 2489f
exanthem in, 749
IgE in, 136
in immunocompromised host, 2491
Kawasaki disease and, 1070
lower respiratory tract, 2491
nosocomial, 3382
pathogenesis of, 2490
pulmonary, 284, 2491
transmission of, 2490
upper respiratory tract, 2490-2491
Corticosteroids
Acanthamoeba spp. infection and, 2985
in acute disseminated encephalomyelitis, 519
in acute transverse myelitis, 527-528
in ARDS, 872-873
Aspergillus infection and, 2710-2711
in bacterial meningitis, 451-453
in *Bartonella henselae* infection, 1728
in *Bordetella pertussis* infection, 1630
in brain abscess, 468-469
in bronchiolitis, 279
in chronic bronchitis, 269
in croup syndromes, 255-256
in cystic fibrosis, 344
in encephalitis, 510
in enterovirus infection, 2103
in eosinophilic meningitis, 483-484
in hematopoietic stem cell transplantation–related *Pneumocystis jiroveci* infection, 939
in hypersensitivity pneumonitis, 305
immunization and, 3451
infectious complications of, 255-256
in influenza virus infection, 2351
injection of, in chalazion, 796-797
in interstitial lung disease, 300
after Kasai procedure, 665
in Kawasaki disease, 1079-1080
in *Leptospira* spp. infection, 1757
in Murray Valley encephalitis, 2303
in *Mycoplasma pneumoniae* infection, 2687
in myocarditis, 396
in *Neisseria meningitidis* infection, 1264
in neonatal meningitis, 886
in orbital cellulitis, 800
in otitis media with effusion, 229
in parainfluenza virus infection, 2370
in peritonsillar abscess, 169
in PFAPA syndrome, 164
in pharyngitis, 164
in *Pneumocystis* infection, 3010
in rheumatic fever, 413
in rhinosinusitis, 201
in rhinovirus infection, 2126
in RSV infection, 2426
in *Salmonella* ser. Typhi infection, 1506
in *Schistosoma* spp. infection, 3068
in septic shock, 834-835
in toxic shock syndrome, 857
in tuberculosis, 1374-1375
in urinary tract infection, 548
Cortisol, plasma, 81
Corynebacterium diphtheriae, 1301-1302
carriage of, 1302-1303, 1309-1310
colony types of, 1302
toxin of, 1302, 1304
Corynebacterium diphtheriae infection, **1301-1311**
vs. *Arcanobacterium haemolyticum* infection, 1325
aural, 1305
cervical lymphadenopathy in, 177
clinical manifestations of, 1305-1306
conjunctival, 1305
cutaneous, 1305
in daycare facilities, 3560-3561
diagnosis of, 1306-1307
differential diagnosis of, 1307
endocardial, 362-363, 1303, 1306
epidemiology of, 1302-1304
exanthem in, 753t-756t
historical perspective on, 1301
immunization against, 1308-1309, 3430-3431
Corynebacterium diphtheriae infection *(Continued)*
joint, 1306
laryngeal, 243t, 250t-251t, 253, 1305, 1307
myocardial complications of, 1306
nasal, 1305, 1307
neurologic complications of, 1306
ocular complications of, 1306
vs. parainfluenza virus infection, 2369
pathogenesis of, 1304-1305
pharyngeal, 1305, 1307
during pregnancy, 1306
prevention of, 1308-1309
prognosis for, 1310
supportive treatment in, 1310
tonsillar, 1305, 1307
treatment of, 1309-1310, 3506-3507
vulvovaginal, 569, 1305
Corynebacterium jeikeium infection, 1423
clinical manifestations of, 1424
diagnosis of, 1426
epidemiology of, 1424
pathophysiology of, 1424
treatment of, 1426
Corynebacterium minutissimum infection, 1425
Corynebacterium pseudodiphtheriticum infection, 1425
Corynebacterium pseudotuberculosis infection, 1424-1425
Corynebacterium spp., 1423-1424
Corynebacterium spp. infection, **1423-1426.** *See also specific species infections*
clinical manifestations of, 1424-1426
diagnosis of, 1426
endocardial, 362-363, 1303, 1306
epidemiology of, 1424
pathophysiology of, 1424
treatment of, 1426
Corynebacterium striatum infection, 1425
Corynebacterium ulcerans, 1301, 1303
Corynebacterium ulcerans infection, 1425-1426
Corynebacterium urealyticum infection, 1424-1426
Corynebacterium xerosis infection, 1425
Cotton-wool spots, 811
Cough
in acute laryngotracheitis, 249, 250t-251t
air pollution–related, 268
in *Chlamydia trachomatis* infection, 1617-1618
in common cold, 137
in croup syndromes, 250t-251t
differential diagnosis of, 265-269, 266f, 1627
irritant-related, 268-269
in lung abscess, 318
in *Mycoplasma pneumoniae* infection, 1617-1618
in rhinosinusitis, 197
treatment of, 269-270, 270f
whooping. *See Bordetella pertussis* infection (pertussis, whooping cough)
Cowpox, **2044-2045**
Coxiella burnetii, 2663-2664
Coxiella burnetii infection (Q fever), **2663-2665**
chronic, 2664
clinical manifestations of, 2664
diagnosis of, 2664-2665
differential diagnosis of, 2665
encephalitic, 494t-498t
endocardial, 363, 2664

Coxiella burnetii infection (Q fever) *(Continued)*
epidemiology of, 2664
exanthem in, 750, 751t-752t
hepatic, 656-657
historical perspective on, 2663
vs. *Legionella pneumophila* infection, 1713
ocular, 815
pathology of, 2664
in pregnancy, 2664
treatment of, 2665
Coxsackie-adenovirus receptor (CAR), 11
Coxsackievirus
antigenic characteristics of, 2056
classification of, 2052-2053, 2052t-2053t
historical perspective on, 2051
host range of, 2056
prevalence of, 2057-2059, 2058t
replication of, 2053-2054, 2053t
Coxsackievirus infection, **2051-2109**
asymptomatic, 2063-2064, 2063t
in bronchiolitis, 2066
cerebellar ataxia and, 2088t, 2090
clinical manifestations of, 2063-2090
congenital, 2091-2092
in croup, 2066
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
in epididymitis, 2077
exanthems in, 742t-748t, 749, 760, 762, 764, 769, 2078-2086, 2079f, 2080t-2081t, 2082f-2083f, 2083t
gastrointestinal, 2068-2073, 2068t-2069t, 2071t-2072t
Guillain-Barré syndrome and, 2088t, 2090-2097
hematologic findings in, 2078
hemolytic-uremic syndrome in, 2078
hepatic, 2070-2072
herpangina in, 2066t
in immunocompromised host, 2091
meningeal, 485-487, 2086-2090, 2089t
muscle, 2078
myocardial, 384-387, 385f, 2075-2077, 2076t, 2104. *See also* Myocarditis
neonatal, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t-2089t
nonspecific febrile illness in, 2064, 2064t
in orchitis, 2077
paralytic, 2090
pericardial, **379-380**, 2075-2077, 2076t
peritoneal, 2070
pharyngeal, 2064t, 2065
pulmonary, 2067
respiratory manifestations of, 2064-2068, 2064t
rheumatic fever and, 2077
sudden infant death and, 2091
treatment of, 2103-2104
Coxsackievirus A
classification of, 2052-2053, 2052t-2053t
replication of, 2053-2054, 2053t
Coxsackievirus A infection
congenital, 2091-2092
conjunctival, 2073-2074, 2074t
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
gastrointestinal, 2068-2073, 2069t, 2071t
hematologic findings in, 2078
hemolytic-uremic syndrome in, 2078
hepatic, 2070-2072
Coxsackievirus A infection *(Continued)*
meningeal, 2089t
muscle, 2078
myocardial, 2075-2077, 2076t
neonatal, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t
nonspecific febrile illness in, 2064, 2064t
ocular, 2074-2075
parotid gland, 2066
pericardial, 2075-2077, 2076t
pharyngeal, 2064t, 2065
photophobia in, 2074
respiratory manifestations of, 2064-2068, 2064t
sudden infant death and, 2091
treatment of, 2103-2104
Coxsackievirus A2 infection, 2079, 2080t-2081t
Coxsackievirus A3 infection, 2079
Coxsackievirus A4 infection, 2061, 2065, 2079, 2079f, 2080t-2081t
Coxsackievirus A5 infection, 2079, 2080t-2081t
Coxsackievirus A6 infection, 762, 764, 2079, 2080t-2081t
Coxsackievirus A7 infection, 2061, 2079, 2080t-2081t
paralytic, 2090
Coxsackievirus A9 infection, 760, 2079-2082, 2080t-2081t, 2082f
photophobia in, 2074
pulmonary, 2067
Coxsackievirus A10 infection, 2080t-2081t, 2082
acute lymphonodular pharyngitis in, 2066
Coxsackievirus A16 infection
abortion and, 2091-2092
chronic, 2083
epidemiology of, 2057
exanthem in, 749, 762
hand-foot-and-mouth disease in, 2066, 2080t-2081t, 2082-2083, 2082f-2083f, 2083t
pathology of, 2055f
Coxsackievirus A21 infection, 2065
Coxsackievirus A24 infection, conjunctival, 810, 2073-2074
Coxsackievirus B
classification of, 2052-2053, 2052t-2053t
replication of, 2053-2054, 2053t
Coxsackievirus B infection
bronchial, 2066
congenital, 2091-2092
conjunctival, 2073-2074, 2074t
in croup, 2066
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
in epididymitis, 2077
gastrointestinal, 2068-2073, 2068t-2069t, 2071t
hemolytic-uremic syndrome in, 2078
hepatic, 655, 2070-2072
meningeal, 2086-2090, 2088t-2089t
muscle, 739
myocardial, 2075-2077, 2076t
myocardial infarction and, 2077
neonatal, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t
nonspecific febrile illness in, 2064, 2064t
Coxsackievirus B infection *(Continued)*
ocular, 2074-2075
in orchitis, 2077
pancreatic, 693, 2072
parotid gland, 2066
pathology of, 2061, 2062f
pericardial, 2075-2077, 2076t
peritoneal, 2070
pharyngeal, 2064t, 2065
respiratory manifestations of, 2064-2068, 2064t
sudden infant death and, 2091
treatment of, 2103-2104
Coxsackievirus B1 infection, 2055f, 2080t-2081t, 2083
peritoneal, 2070
pulmonary, 2067
Coxsackievirus B2 infection, 2080t-2081t, 2083
Coxsackievirus B3 infection, 2080t-2081t, 2083-2084
neonatal, 2093
Coxsackievirus B4 infection
CNS, 2061, 2062f
diabetes mellitus and, 2072-2073
exanthem in, 2080t-2081t, 2084
myocardial, 2061, 2062f
renal, 2077-2078
Coxsackievirus B5 infection, 760, 2080t-2081t, 2084
peritoneal, 2070
Coxsackievirus B6 infection, 2080t-2081t, 2084
pulmonary, 2067
CR3 (CD11b/CD18), 6
Craniofacial anomalies, in otitis media, 217
Craniofacial surgery, 1043-1046
evaluation for, 1045
infection with, **1044-1045**
evaluation of, 1045-1046
microbiology of, 1045
needle aspiration in, 1046
treatment of, 1046
irrigation during, 1045
meningitis with, 1044-1045
perioperative antibiotics in, 1045
reconstructive materials in, 1044
staging of, 1045
in Treacher Collins syndrome, 1044
Creatinine, urinary, 69
Creutzfeldt-Jakob disease (CJD), **2619-2629**
clinical manifestations of, 811, 2627-2628, 2627t
diagnosis of, 2628
epidemiology of, 2621-2624
pathogenesis of, 2624-2627
prevention of, 2628-2629
treatment of, 2628
Crigler-Najjar syndrome, 657
Crimean-Congo hemorrhagic fever, **2531-2535**
clinical manifestations of, 2532
diagnosis of, 2533
epidemiology of, 2531-2532, 2532f
pathogenesis of, 2533, 2533f
prognosis for, 2534
treatment of, 2533-2534
Crimean-Congo hemorrhagic fever virus, 2531
Crohn disease, 2872
appendicitis and, 687-688
vs. *Clostridium difficile* infection, 637
gallstones in, 668
vs. *Shigella* spp. infection, 1483

Cronobacter sakazakii infection, 1434, 1436, 1438
neonatal, 886
Cross-agglutinin absorption test (CAAT), in *Leptospira* spp. infection, 1755
Crotamiton, 3348
Croup, **241-260**, 242t
clinical presentation of, 249-253, 250t-251t
diagnosis of, 253
differential diagnosis of, 250t-251t, 253, 2369
epidemiology of, 245-246
etiology of, 242-244, 243t-244t, 2066
historical perspective on, 241-242
in *Mycoplasma pneumoniae* infection, 2679
pathogenesis of, 247-248
pathology of, 247-248
prevention of, 259
prognosis for, 259
spasmodic, 243t, 250t-251t, 252
etiology of, 242-244, 243t-244t
vs. laryngotracheitis, 253
treatment of, 254-257
terminology for, 242, 243t
treatment of, 254-257
Crowding, *Streptococcus pneumoniae* infection and, 1201, 1204
Cryoglobulinemia, in HAV infection, 2140
Cryopyrin-associated periodic fever syndromes, 847-848
Cryptococcal capsular polysaccharide antigen test, 475, 480
Cryptococcoma, 2803
Cryptococcus gattii, 2796-2797, 2798t, 2799
Cryptococcus neoformans, 2796-2797
capsule of, 20, 2796, 2797f
ecology of, 2797-2799, 2798t
extracellular enzymes of, 2797
melanin of, 2796-2797
virulence factors of, 2796
Cryptococcus neoformans infection (cryptococcosis), **2795-2806**
biliary, 660
bone, 726
CNS, 475-476, 480t, 2801-2803
CSF examination in, 443t, 475
treatment of, 2804-2805
congenital, 2804
cutaneous, 757t, 2804
diagnosis of, 2797f, 2798t, 2800-2802
epidemiology of, 2797-2799
host response to, 23
immune reconstitution inflammatory syndrome with, 972, 2803-2804
immune response to, 2799
interferon-γ in, 2805
after intestinal transplantation, 1000
intraparenchymal, 2803
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 971-972
neonatal, 897
pathogenesis of, 2799
prevention of, 2805
prostatic, 2804
pulmonary, 2800-2801
treatment of, 2804
secondary prophylaxis in, 2805
treatment of, 2804-2805
after lung transplantation, 971-972
uveal, 814
Cryptosporidium spp., 2896-2897, 2908f
life cycle of, 2897, 2898f
Cryptosporidium spp. infection (cryptosporidiosis), **2896-2905**
biliary, 660
clinical manifestations of, 602t-603t, 607t, 614-615, 617, 2900-2901, 2901t
in daycare facilities, 3556-3557
diagnosis of, 619, 2901-2902, 2902f, 2902t
epidemiology of, 2897-2899
extraintestinal, 2901
after hematopoietic stem cell transplantation, 943-944
immune response to, 2900
in immunocompromised host, 2898-2899, 2901, 2903
oral immunoglobulin in, 3498
pancreatic, 694-695
pathogenesis of, 2899-2900
pathology of, 2900
prevention of, 2903-2904
transmission of, 2899
treatment of, 625, 2903, 3327t-3346t, 3498
waterborne, 2899, 2904
CSF. *See* Cerebrospinal fluid (CSF)
Culex annulirostris, 2249, 2292
Culex tritaeniorhynchus, 2292
Culiseta melanura, 2226, 2229
Culiseta pipiens
in St. Louis encephalitis virus infection, 2254, 2254f
in West Nile virus infection, 2263
Culiseta quinquefasciatus, in St. Louis encephalitis virus infection, 2254, 2254f
Culiseta tarsalis, 2230-2231, 2237
in St. Louis encephalitis virus infection, 2254, 2254f
Cullen sign, in pancreatitis, 689-690
Culture
amebic
in *Acanthamoeba* spp. infection, 2982-2983
in *Balamuthia mandrillaris* infection, 2983
in *Naegleria fowleri* infection, 2982-2983
bacterial, 3589-3592, 3590t
in *Acinetobacter* spp. infection, 1571
in *Actinomyces* spp. infection, 1823
in *Aggregatibacter actinomycetemcomitans* infection, 1610, 1824
in *Arcanobacterium haemolyticum* infection, 1326
in *Aspergillus* spp. infection, 2712
in bloodstream infection, 3589-3590
in *Bordetella pertussis* infection, 1627-1629
in brain abscess, 466
in *Brucella* spp. infection, 1614
in burn wound, 1056-1057
in *Campylobacter* spp. infection, 1645, 1646t
in chemotherapy-associated infection, 924-925
in *Citrobacter* spp. infection, 1430
in CNS infection, 3591
in coagulase-negative staphylococcal infection, 1133-1134
in CSF shunt–related infection, 1033
in endophthalmitis, 820
in *Enterobacter* spp. infection, 1436-1437
in *Erysipelothrix rhusiopathiae* infection, 1328
in fever of unknown origin, 842
in gastrointestinal tract infection, 3592
in genital tract infection, 561
Culture *(Continued)*
in *Haemophilus influenzae* infection, 1667
in *Haemophilus influenzae* type b infection, 1677
in *Haemophilus parainfluenzae* infection, 1690
in *Kingella kingae* infection, 1703
in *Leptospira* spp. infection, 1754
in *Leuconostoc* spp. infection, 1247
in *Listeria monocytogenes* infection, 1332-1333
in meningitis, 442, 445
in *Neisseria gonorrhoeae* infection, 1274, 1289-1291, 1289t
in *Neisseria meningitis* infection, 1262
in neonatal *Candida* spp. infection, 896
in neonatal joint infection, 889
in neonatal osteomyelitis, 889
in *Pasteurella multocida* infection, 1545, 1548
in *Pseudomonas aeruginosa* infection, 1582-1583
in respiratory tract infection, 3590-3591
in rhinosinusitis, 199-200
in *Rhodococcus equi* infection, 1427
in *Salmonella* ser. Typhi infection, 1503
in *Salmonella* spp. infection, 1503
in sepsis neonatorum, 883
sputum
in *Blastomyces dermatitidis* infection, 2731
in *Cryptococcus neoformans* infection, 2800-2801
in cystic fibrosis–related infection, 332, 333t
in *Legionella pneumophila* infection, 1711
in *Staphylococcus aureus* infection, 1113, 1114f, 1123
in *Stenotrophomonas maltophilia* infection, 1605-1606, 1608
in *Streptobacillus moniliformis* infection, 1716, 1716f, 1718-1719
in streptococcal pharyngitis, 1147
in *Streptococcus pneumoniae* infection, 1215-1216
in *Streptococcus* viridans infection, 1194
in *Trichomonas vaginalis* infection, 2891
in urinary tract infection, 3591-3592
in *Vibrio parahaemolyticus* infection, 1559
in *Vibrio vulnificus* infection, 1561
in vulvovaginitis, 561
in *Yersinia enterocolitica* infection, 1530
in *Yersinia pestis* infection, 1509-1510
in *Yersinia pseudotuberculosis* infection, 1530
in *Chlamydia pneumoniae* infection, 2642-2643
in *Chlamydia trachomatis* infection, 2636
fungal, 3596, 3597t, 3599-3600, 3599t
in *Aspergillus* spp. infection, 2711-2712, 2712f
in *Blastomyces dermatitidis* infection, 2731, 2731f
in *Candida* spp. infection, 896, 2735, 2744
in *Coccidioides* spp. infection, 2770, 2775-2776, 2775f
in cryptococcal meningoencephalitis, 2802
in dermatophyte infection, 787
in *Histoplasma capsulatum* infection, 2820-2821
in *Paracoccidioides* spp. infection, 2788

Culture *(Continued)*
in *Mycoplasma pneumoniae* infection, 2686
stool. *See* Stool culture
in tuberculosis, 1364-1365
urine. *See* Urine culture
viral, 3605-3609, 3606t-3607t
in adenovirus infection, 1890
in CMV infection, 1981-1982
in Colorado tick fever virus infection, 2174-2175
in coronavirus infection, 2494
in dengue, 2283, 2285-2286
in EBV infection, 2011
in hantavirus infection, 2498-2499
in HSV infection, 1934, 1955-1956, 1956f
in influenza virus infection, 2345
in measles, 2374-2375, 2375f
in mimivirus infection, 2049-2050
in mumps, 2396
in rhinovirus infection, 2111, 2112f, 2125
in RSV infection, 2409, 2424
in rubella virus infection, 2197-2198
in West Nile virus infection, 2270
in yellow fever, 2277
Cupriavidus spp., 1584
Cupriavidus spp. infection, 1595-1596
Curvularia spp. infection, 1053
Cutaneous larva migrans, 3024, 3327t-3346t
Cutaneous T-cell lymphoma, 2569
Cyclitis, 809
Cycloserine, in tuberculosis, 1367t
Cyclospora cayetanensis, 2906
Cyclospora cayetanensis infection (cyclosporiasis), **2905-2907**
clinical manifestations of, 602t-603t, 607t, 615, 2906-2907
diagnosis of, 617, 2907, 2907f
epidemiology of, 2906
transmission of, 2906
treatment of, 625, 2907, 3327t-3346t
Cyclosporine, in heart transplantation, 955, 955t
Cyclosporine A, in HTLV-1–associated myelopathy, 2573
Cyst(s)
Bartholin, 579, 579f
branchial cleft, 182
choledochal, 666-667
hydatid. *See Echinococcus granulosus* infection (echinococcosis, hydatid disease)
thyroglossal duct, 182
Cystic fibrosis, **323-348**
Achromobacter spp. infection in, 336, 341, 1574
anti-inflammatory therapy in, 344
Aspergillus spp. infection in, 336, 341-342, 2713f
Bordetella hinzii infection in, 1637
bronchiectasis in, 325, 325f
Burkholderia spp. infection in, 335-336, 335t, 345t, 1593-1594
treatment of, 341
vs. chronic bronchitis, 267
clinical manifestations of, 323-326, 324t
diagnosis of, 326-327, 327t
gallstones in, 668
glucose metabolism in, 324
Haemophilus influenzae infection in, 334
hepatitis in, 657
immunization in, 344-345
immunodeficiency in, 60
Cystic fibrosis *(Continued)*
infection in, 331-332
age-specific prevalence of, 332
anti-inflammatory therapy in, 344
antibiotic resistance in, 3154
antimicrobial susceptibility testing in, 333
coronavirus, 2491
diagnosis of, 332-333, 333t
environmental prevention of, 345-346, 346t
epidemiology of, 331-332
immunization against, 344-345
inhaled antibiotic therapy in, 342-343
long-term suppressive therapy in, 342-343
macrolide antibiotics in, 343
molecular diagnostics in, 333
nosocomial, 3405-3406
pathogenesis of, 327-331, 329f-330f
prevention of, 344-346
treatment of, 337-344
IVIG in, 3508
liver disease in, 324-325
lung abscess and, 317
lung transplantation in, 343-344
mycobacterial infection in, 336-337, 1385-1386
nontuberculous *Mycobacterium* infection in, 341
pathogenesis of, 327-331, 329f-330f
Pseudomonas aeruginosa infection in, 60, 335, 1593-1594
antibiotic resistance in, 3154
clinical manifestations of, 1593-1594
diagnosis of, 1596-1597
eradication of, 337-338
pathogenesis of, 328-330, 329f, 1587-1589
prevention of, 337, 1603-1604
treatment of, 339-340, 1599-1601
viral infection and, 334
Scedosporium spp. infection in, 336
Staphylococcus aureus infection in, 334
eradication of, 337-338
methicillin-resistant, 338, 340-341
prevention of, 337
treatment of, 340-341
Stenotrophomonas maltophilia infection in, 336, 341, 1607
survival with, 326
viral infection in, 333-334
Cystic fibrosis transmembrane conductance regulator *(CFTR)* gene/protein, 267, 323, 327, 692, 1587
Cystic hygroma, 182
Cysticercosis. *See* Neurocysticercosis; *Taenia solium* infection (cysticercosis)
Cystitis, 538. *See also* Urinary tract infection (UTI)
chemical, 540
granulomatous, 540
hemorrhagic, in adenovirus infection, 1901
treatment of, 546-548, 547t
Cystoisospora belli, 2907-2909, 2908f
Cystoisospora belli infection, **2907-2911**
clinical manifestations of, 607t, 615, 2909
diagnosis of, 617, 2909-2910, 2910f
epidemiology of, 2909
pathophysiology of, 2909, 2909f
transmission of, 2909
treatment of, 2910-2911, 3327t-3346t
Cytochalasin D, 16
Cytokines, 25-27, 26t, 3357-3363, 3359t-3360t
in acute disseminated encephalomyelitis, 518
in adenovirus infection, 1895
in *Babesia* spp. infection, 2917
in bacterial meningitis, 433-435, 434f
in burns, 1049
in chronic fatigue syndrome, 1088
in *Cryptosporidium* spp. infection, 2900
in cystic fibrosis, 331
in influenza virus infection, 2338
in Kawasaki disease, 1076
in *Legionella pneumophila* infection, 1709
in malaria, 2926
in measles, 2379
measurement of, 80
in mycobacterial infection, 1382
in *Neisseria meningitidis* infection, 1257-1258, 1263
in *Pneumocystis* infection, 3008
proinflammatory, 65, 79-80
in rhinovirus infection, 2119, 2123
in RSV infection, 2416
in *Salmonella* spp. infection, 1498-1499, 1499f
in *Schistosoma* spp. infection, 3063
in septic shock, 827, 828f
in *Shigella* spp. infection, 1479
in *Streptococcus agalactiae* infection, 1158-1159
in *Streptococcus pneumoniae* infection, 1209-1210, 1224
T-cell secretion of, 39-40
Toll-like receptor effects on, 25
in tuberculosis, 1342
Cytolethal distending toxin, 1650
Cytomegalovirus (CMV), 1969-1970
antibody to, 1982-1983
detection of, 1981-1982
genotypes of, 1970
pp65 antigen of, 1982
Cytomegalovirus (CMV) infection, **1968-1991**
in adolescents, 1971-1972
autoimmune disease and, 1981
biliary, 660
in burn patient, 1053
clinical manifestations of, 742t-748t, 1975-1981
CNS, 1978-1980, 1979f
congenital, 881, 1970
clinical manifestations of, 1975
CNS, 1978-1979, 1979f
cutaneous, 1981
diagnosis of, 1983-1984
differential diagnosis of, 1975
hearing loss and, 1975, 1980
hepatitis in, 1978
ocular, 818, 881
treatment of, 1986-1987
cutaneous, 742t-748t, 1981
in daycare facilities, 3553-3554
diagnosis of, 951, 1981-1984
disseminated, 1974
endocrine, 1980-1981
epidemiology of, 1970-1974
esophageal, 594-597, 596f
fetal, 1975, 1983-1984
gastrointestinal tract, 1978
genitourinary, 1981
after heart transplantation, 950-952
after hematopoietic stem cell transplantation, 939-940
hepatic, **654**, 1977-1978

Cytomegalovirus (CMV) infection *(Continued)*
historical perspective on, 1969
in HIV-infected host, 811, 1979-1980, 2601-2602
immune response to, 1974-1975
in immunocompromised host, 1973-1975, 1984
immunomodulatory effects of, 974
after intestinal transplantation, 996-997, 997t, 1001
after kidney transplantation, 1007-1008
after liver transplantation, 996-997, 997t, 1001
after lung transplantation, 974-976
mononucleosis syndrome in, 1976
murine, 74
myelitic, 526
myocardial, 1980
neonatal, 811
treatment of, 1987
nosocomial, 1972-1973, 3383
ocular, 811, 1977
otic, 1980
pancreatic, 694
pathogenesis of, 1974-1975
pathology of, 1974, 1974f
perinatal, 1971
clinical manifestations of, 1975-1976
diagnosis of, 1984
pharyngeal, 159t-160t, 161
postnatal, 1971
CNS, 1979
diagnosis of, 1984
prevention of, 1987
during pregnancy, 1970, 1972, 1983, 1986, 1988
prevention of, 1987-1989
antiviral agents in, 1988-1989
immune globulin in, 1987-1988, 3520-3521
immunization in, 1989
perinatal, 3521
transplantation-related, 1987, 3520
pulmonary, 1976-1977, 1976f, 1984
retinal, 811, 1977
transmission of
blood transfusion, 1972-1973
intrafamilial, 1972
nosocomial, 1972-1973
sexual, 1972
treatment of, 1984-1987, 1985f, 3283-3289
Cytosine, 3298f

D

D test, 1126-1127, 1127f
Daclatasvir, in HCV infection, 2324
Dacryoadenitis, **797**
noninfectious, 797
Dacryocystitis, **797-798**
Pseudomonas aeruginosa, 1591
Dacryostenosis, 806
Dactylitis
distal, blistering, **775**
tuberculous, 1355
Dakin solution, in *Vibrio vulnificus* infection, 1562
Dane particle, 1912, 1915. *See also* Hepatitis B virus (HBV) infection
Dapsone, 3348
in hematopoietic stem cell transplantation, 938-939
Dapsone *(Continued)*
in *Mycobacterium leprae* infection, 1406, 1407t
in *Pneumocystis* infection, 3010
Daptomycin, 3206, 3238
in coagulase-negative staphylococcal infection, 1139
in methicillin-resistant *Staphylococcus aureus* infection, 1127
resistance to, 3153
in vancomycin-resistant *Enterococcus* spp. infection, 1184
Darunavir, 2615
Dawson inclusion body encephalitis. *See* Subacute sclerosing panencephalitis (SSPE)
Daycare facilities, **3550-3569**
Aeromonas spp. infection in, 1542
antibiotic administration in, 3238
astrovirus infection in, 3558
bites in, 3571
Campylobacter jejuni infection in, 3557
chickenpox in, 3562
Clostridium difficile infection in, 3558
CMV infection in, 1971, 3553-3554
common cold in, 133-134
Cryptosporidium infection in, 3556-3557
diarrheal disease in, 599
diphtheria in, 3560-3561
Escherichia coli infection in, 3558
Giardia lamblia infection in, 2879, 3556
Haemophilus influenzae type b infection in, 3561
hand, foot, and mouth disease in, 3555
HAV infection in, 2134-2135, 3564
HBV infection in, 3564-3565
head lice in, 3559
HIV infection in, 3560
HSV infection in, 3560
infection control in, 3565-3569
education for, 3566
exclusion policies and, 3566, 3567t-3568t
hygiene standards for, 3566
immunization in, 3565-3566
physical plant factors in, 3565
prophylaxis in, 3569
written policies for, 3565
infection patterns in, 3552-3553, 3553t
infection transmission in, 3548, 3550-3552, 3551t
airborne, 3552
bloodborne, 3552
contact-related, 3552
feces-related, 3552
food-related, 3552
saliva-related, 3552
sexual, 3552
influenza in, 3562
Kingella kingae infection in, 1701, 3555
measles in, 3562-3563
methicillin-resistant *Staphylococcus aureus* infection in, 3559
mumps in, 3564
Mycobacterium tuberculosis infection in, 3556
Neisseria meningitidis infection in, 3555-3556
norovirus infection in, 3558
otitis media in, 211, 3553, 3554t
parvovirus B19 infection in, 3554-3555
pertussis in, 3563
poliomyelitis in, 3563
rotavirus infection in, 3564
rubella in, 3563
Daycare facilities *(Continued)*
Salmonella spp. infection in, 1493-1494, 1507, 3557-3558
scabies in, 3559
Shigella infection in, 3557
Streptococcus pneumoniae infection in, 1201, 1204, 3561-3562
Streptococcus pyogenes infection in, 3555
upper respiratory tract infection in, 3553, 3554t
vaccine-preventable diseases in, 3560-3565
varicella in, 3562
Deafness. *See* Hearing loss
Débridement, in necrotizing fasciitis, 779
Decongestants, in common cold, 137
Decontamination, gastrointestinal tract, in burn patient, 1061
DEET (*N*,*N*-diethyl-3-metylbenzamide), 1737, 2175, 2526
neurotoxicity of, 2235-2236, 2311
precautions for, 2236, 2236t
Defecation, infection and, 109
Defective virus. *See* Hepatitis D virus (HDV) infection
Defensins, 24-25, 34t, 35, 3365-3366
Deferoxamine, mucormycosis and, 2840
Dehydration
in cholera, 1551-1552, 1551f
in gastrointestinal tract infection, 76, 620-622, 620t-621t
in rotavirus infection, 2186
Delayed fluorescence immune assay, in *Corynebacterium diphtheriae* infection, 1307
Delhi boil. *See Leishmania* spp. infection (leishmaniasis), Old World
Delta agent/virus. *See* Hepatitis D virus (HDV) infection
Delta toxin, of *Staphylococcus epidermidis*, 1133
Demeclocycline, 3217-3220
Demodex brevis infection, 795-796, 796f
Demodex folliculorum infection, 795-796, 796f
Dendritic cells
natural killer cell interaction with, 28
plasmacytoid, 38
Dengue, **2278-2291**
vs. chikungunya, 2244, 2245t-2246t, 2282
clinical manifestations of, 2281-2282
diagnosis of, 2284, 2285t
encephalitic, 500
epidemiology of, 2280
exanthem in, 742t-748t, 760, 762
geographic distribution of, 2280-2281
hemorrhagic diathesis in, 2281-2282
host range of, 2280
immunization against, 2289
laboratory studies in, 2285-2286
pathogenesis of, 2282-2284
pathology of, 2283
prevention of, 2289-2290
prognosis for, 2288-2289
reporting requirements for, 2288
severe, 2285, 2285t
transmission of, 2279-2280
in travel, 3115
treatment of, 2286
Dengue hemorrhagic fever/dengue shock syndrome, **2278-2291**
clinical manifestations of, 2282
diagnosis of, 2284-2285, 2284t
pathogenesis of, 2283-2284
prevention of, 2289-2290
prognosis for, 2288-2289
treatment of, 2286-2288

Dengue virus, 2279
  identification of, 2285-2286
Dental caries. *See* Caries; Odontogenic infection
Dental procedures. *See also* Odontogenic infection
  cervical lymphadenitis and, 183, 183f
  cervicofacial actinomycosis and, 184
  prophylactic antibiotics for, 368-369, 369t, 417-418, 1195-1196, 3246-3247, 3246t-3247t
  rhinosinusitis and, 195
Depigmentation, in pinta, 1785
Depression, in chronic fatigue syndrome, 1089
Dermacentor andersoni tick, **3071-3072**
  in Colorado tick fever virus infection, 2173-2174, 2173f
  in Rocky Mountain spotted fever, 2651, 2654
Dermacentor variabilis tick, 2651, **3071-3072**
Dermatitis
  allergic, 208
  contact, 208
  diaper, 788, 788f, 2740, 2747
  infective, 742t-748t, 749-750
  molluscum, 783
  streptococcal, perianal, **775**
Dermatobia hominis infection, 757t-758t
Dermatoblepharitis, HSV, 803
Dermatophyte infection. *See at* Tinea
Dermatophytid reaction, 784-785, 785f
Dermonecrotic toxin, 1618t, 1619, 1624
Descemet's membrane, 806
Devic disease, 515
Device-related infection. *See* Prosthetic/implantable device–related infection
Dexamethasone. *See also* Corticosteroids
  in bacterial meningitis, 451-453
  in croup, 255-256
  in encephalitis, 510
  in septic arthritis, 733
Dextranomer/hyaluronic acid copolymer (Deflux), in vesicoureteral reflux, 551
Diabetes insipidus, fever of unknown origin in, 846
Diabetes mellitus
  coxsackievirus B4 and, 2072-2073
  cystic fibrosis and, 324
  furunculosis and, 777
  genetic susceptibility to, 72
  immunization and, 3428-3429
  liver abscess in, 673
  mucormycosis and, 2840
  mumps and, 2402
  viral infection and, 72
Dialysis
  in diarrheogenic *Escherichia coli* infection, 1462
  infection with, 3406-3407
  mucormycosis with, 2840
  osteomyelitis with, 723
  tuberculosis with, 1358
Diaper dermatitis, 788, 788f, 2740
  treatment of, 2747
Diarrhea, 599-606. *See also* Gastrointestinal tract infection
  acid-base disorders in, 76
  acute, 606
  antibiotic-associated, 599. *See also* Colitis, antibiotic-associated
  in *Bacillus cereus* infection, 1319
  bacterial infection and, 602t-603t, 607t, 608-613, 610t, 616t, 622, 623t
  in *Balantidium coli* infection, 2895
Diarrhea *(Continued)*
  Brainerd, 602
  chronic, 606
  in *Cryptosporidium* spp. infection, 2901
  in daycare facilities, 599
  dehydration in, 76, 620-622, 620t-621t
  diagnostic evaluation of, 616-619, 616t, 620f
  differential diagnosis of, 636-637
  dysenteric, 606, 622-624. *See also Shigella* spp. infection (shigellosis)
  in *Entamoeba histolytica* infection, 2870
  in enteroviral infection, 2069-2070
  in *Escherichia coli* infection. *See Escherichia coli* infection, diarrheogenic
  in food poisoning, 601-606, 602t-605t
  health care–related, 636-637. *See also* Colitis, antibiotic-associated
  in immunosuppressed host, 599-600, 600t
  inflammatory, 606
  neonatal, 887-888
  noninflammatory, 606
  oral immunoglobulin in, 3498
  parasitic infection and, 607t, **613-616**, 616t, 625
  probiotics in, 3370
  prolonged, 606
  in rotavirus infection, 2183-2185
  traveler's, 600-601, 1451, 1463, **3110-3115.** *See also* Enterotoxigenic *Escherichia coli* (ETEC) infection
    epidemiology of, 3110-3112
    management of, 3113-3115
    prevention of, 3112-3113
  treatment of, 619-626, 620t-621t, 623t
  viral infection and, 606-608, 607t, 608f, 616t
  in waterborne disease, 606
  watery, 606
  in Whipple disease, 643
Diazepam, in tetanus, 1816
Diazo reactants, urinary, 70
DIC. *See* Disseminated intravascular coagulation (DIC)
Dicloxacillin, 3183t-3184t, 3185-3186, 3231t-3233t
  in cervical lymphadenitis, 186
Didanosine, 2612
Dideoxyinosine, pancreatitis with, 692
Dientamoeba fragilis, 2884, 2885f
Dientamoeba fragilis infection (dientamoebiasis), **2884-2887**
  clinical manifestations of, 2885
  diagnosis of, 2885-2886
  epidemiology of, 2884-2885
  treatment of, 2886, 2886t
Diet
  infection and, 109
  intestinal flora and, 89-90, 93
  in pancreatitis, 695-696
Diethylcarbamazine citrate, 3348
Diffuse infiltrative lymphocytosis syndrome, 189, 191
Diffusely adherent *Escherichia coli* (DAEC) infection, 610t, 611, 1449
  clinical manifestations of, 1450t, 1455
  diagnosis of, 1461
  epidemiology of, 1450t, 1453
  pathogenesis of, 1460
  prevention of, 1463-1464
  treatment of, 1461-1463
DiGeorge syndrome, 48, 904-905, 907, **912-913**
  clinical features of, 905f, 912
  diagnosis of, 912-913
DiGeorge syndrome *(Continued)*
  pathogenesis of, 907t, 912
  treatment of, 913
Digital thermometer, 85
Digitalis, in myocarditis, 395
Diloxanide furoate, 3348
Diphtheria. *See Corynebacterium diphtheriae* infection
Diphyllobothrium spp. (fish tapeworm) infection, **3040-3041**, 3327t-3346t
Diplopia, in Whipple disease, 644
Dipylidium caninum (dog tapeworm) infection, **3041**, 3327t-3346t
Direct fluorescent antibody (DFA) test, 3606t-3607t, 3609-3610
  in influenza virus infection, 2345
Dirofilaria immitis infection (dirofilariasis), **3028**
Disease
  definition of, 112
  epidemiology of. *See* Epidemiology
  vs. infection, 103-104, 111
Diskitis, 720-721, 721f
Dispersin, 1460
Disseminated intravascular coagulation (DIC)
  in influenza virus infection, 2345
  in *Neisseria meningitidis* infection, 1258
  phospholipids and, 75
  in septic shock, 829
Diuretics, pancreatitis with, 691
Dobutamine, in myocarditis, 395
Dog, *Leptospira* infection of, 1744-1745, 1757-1758
Dog bite, 780-781, 780t. *See also* Rabies
  clinical manifestations of, 3572-3575, 3574f
  diagnosis of, 3575-3577
  epidemiology of, 3570-3571
  Erysipelothrix rhusiopathiae infection with, 1328
  microbiology of, 3571-3572, 3571t-3572t
  Pasteurella multocida infection with, 1547, 1567, 3574
  polymicrobial, 1829
  prevention of, 3578
  treatment of, 3251, 3575-3577, 3576t
Dog tapeworm *(Dipylidium caninum)* infection, **3041**, 3327t-3346t
Donovan bodies, 1642, 1642f
Donovanosis. *See* Granuloma inguinale
Dopamine, in myocarditis, 395
Doripenem, 3238
Doxycycline, 3217-3220, 3218t, 3231t-3233t
  in epidemic typhus, 2658-2659
  in lymphogranuloma venereum, 584
  in malaria, 2942
  in malaria prevention, 2944t, 2945
  in murine typhus, **2659-2660**
  in Rocky Mountain spotted fever, 2654
  in scrub typhus, 2661
Dracunculus medinensis infection, **3028**, 3327t-3346t
DRESS (Drug Reaction with Eosinophilia and Systemic Symptoms) syndrome, 657
Dressing, burn wound, 1057-1059
Drug(s), **3159-3181.** *See also* Antibiotic(s); Antifungal agents; Antiparasitic agents; Antiviral agents *and specific drugs and drug classes*
  absolute bioavailability (F) of, 3160-3161
  absorption of, 3161, 3161f
    ontogeny effects on, 3164, 3165t
  bioequivalence of, 3161

Drug(s) *(Continued)*
biopharmaceutics of, 3161
biotransformation of
ontogeny effects on, 3166-3167, 3168t
phase I pathways in, 3167, 3168t
phase II pathways in, 3167-3169, 3168t
cholestasis with, 662
clearance (Cl) of, 3161
compartment models of, 3161-3162
diffusion of, 3235
disposition of, 3162
distribution of, 3163
ontogeny effects on, 3164-3165, 3165f
dose-response curve of, 3162, 3162f
efficacy of, 3170-3174
combination drug regimens and, 3174, 3180
concentration above minimal inhibitory concentration and, 3171f, 3172-3173
inoculum size and, 3179-3180
minimal inhibitory concentration and, 3170, 3170f
pharmacodynamic determinants of, 3169-3180
pharmacogenetic determinants of, 3181
pharmacokinetic determinants of, 3169
subinhibitory concentrations and, 3175-3178
suprainhibitory concentrations and, 3174-3175, 3174f
time above minimal inhibitory concentration and, 3170-3172, 3171f
total-body exposure above minimal inhibitory concentration and, 3173-3174
elimination half-life of, 3162
ontogeny effects on, 3166, 3166t
fever of unknown origin with, 846
first-pass effect of, 3162
hepatitis with, 651t, 657
normal flora and, 89-90
pancreatitis with, 691-692, 691t, 696
pharmacokinetic-pharmacodynamic interface of, 3160-3163, 3166t
ontogeny effects on, 3163-3167, 3165f-3166f, 3165t-3166t, 3168t
postexposure effects of, 3178-3179
protein binding of, 3162
ontogeny effects on, 3165, 3166t
relative bioavailability of, 3162
renal excretion of, 3236
ontogeny effects on, 3165-3166, 3166f
steady state of, 3162
urethral symptoms with, 540
volume of distribution of, 3163
Drug abuse
endocarditis and, 352, 357
HAV infection and, 2135
tetanus and, 1814
Dubin-Johnson syndrome, 657
Dust, in hypersensitivity pneumonitis, 301, 302t-303t
Dust mites, Kawasaki disease and, 1068
Dwarf tapeworm *(Hymenolepis nana)* infection, **3042**, 3327t-3346t
Dysentery, 606, 622-624, 1476. *See also Shigella* spp. infection (shigellosis)
Dyspnea, in community-acquired pneumonia, 287
Dysrhythmias
in myocarditis, 389, 397
in pericarditis, 376
Dystonia, group A β-streptococcal infection and, 516
α-Dystroglycan, 2473
Dystrophin, in myocarditis, 387

E

E-test, in pneumococcal disease, 1218
Eagle effect, 3174-3175, 3174f
Ear(s)
external, 204
dermatologic disease of, 208
furunculosis of, 208
herpes zoster infection of, 208, 208f
inflammation of. *See* Otitis externa
normal flora of, 204-205
relapsing polychondritis of, 208
middle
effusion of, 209-210, 218-220. *See also* Otitis media with effusion
inflammation of. *See* Otitis media
tuberculosis of, 1357
pain in
in otitis externa, 205, 208
in otitis media, 217
in rhinosinusitis, 198
tropical. *See* Otitis externa
Eastern equine encephalitis, 494t-498t, **2226-2230**
age and, 2227
clinical manifestations of, 2227-2228
diagnosis of, 2228-2229
differential diagnosis of, 2229
ecology of, 2226
epidemiology of, 2226-2227, 2227f
pathogenesis of, 2228
pathology of, 2228
prevention of, 2229
prognosis for, 2228
sequelae of, 2228
treatment of, 2229
Eastern equine encephalitis virus, 2226
Ebola virus, 2478, 2479f
Ebola virus infection, **2479-2486**
bioterrorist, 3585
clinical manifestations of, 2483
diagnosis of, 2484
differential diagnosis of, 2483-2484
epidemiology of, 2479-2482, 2480t, 2481f
exanthem in, 742t-748t, 749
nosocomial, 3401-3402
pathology of, 2483
prevention of, 2485, 3534
treatment of, 2484-2485
EBV. *See* Epstein-Barr virus (EBV)
EBV-encoded RNA (EBER), 1993-1994
EBV nuclear antigen (EBNA), 1993-1994
Echinacea, in common cold, 138
Echinocandins, 3312-3319
adverse effects of, 3314-3315
in cancer patient, 928
drug interactions with, 3315
in hematopoietic stem cell transplantation–related aspergillosis, 938
in lung transplant–related aspergillosis, 968
in lung transplant–related candidiasis, 971
mechanism of action of, 3312-3313, 3313f
pharmacodynamics of, 3314
pharmacokinetics of, 3314, 3315t
resistance to, 3314
spectrum of activity of, 3313-3314
Echinococcus granulosus, 3044
Echinococcus granulosus infection (echinococcosis, hydatid disease), **3044-3047**
clinical manifestations of, 660, 757t-758t, 3044-3045
diagnosis of, 3045, 3046f
epidemiology of, 3044
myocardial, 400
ocular, 815
orbital, 801
PAIR treatment of, 3046-3047
pathogenesis of, 3044
prevention of, 3047
pulmonary, 3045, 3047
surgical treatment of, 3047
transmission of, 3044
treatment of, 3046-3047, 3327t-3346t
Echinococcus multilocularis infection, 660, 757t-758t, 3047
Echinococcus vogeli infection, 3047
Echocardiography
in endocarditis, 359-360
in Kawasaki disease, 1074-1075, 1080-1081
in myocarditis, 387, 388f, 392-393
in pacemaker-related infection, 1024
in pericarditis, 376-377, 376f
in rheumatic fever, 409, 411
Echovirus
antigenic characteristics of, 2056
classification of, 2052-2053, 2052t
host range of, 2056
prevalence of, 2057-2059, 2058t
Echovirus infection, **2051-2109**
asymptomatic, 2063-2064, 2063t
bronchial, 2066
in bronchiolitis, 2066
cerebellar ataxia and, 2088t, 2090
clinical manifestations of, 2063-2090
congenital, 2091-2092
conjunctival, 2073-2074, 2074t
diagnosis of, 2101-2103
encephalitic, 2088t, 2090
in epidemic pleurodynia, 2067-2068
exanthem in, 2078-2086, 2080t-2081t, 2083t
gastrointestinal, 2068-2073, 2069t, 2071t-2072t
Guillain-Barré syndrome and, 2088t, 2090-2097
hemolytic-uremic syndrome in, 2078
hepatic, 2070-2072
herpangina in, 2066t
historical perspective on, 2051
in immunocompromised host, 2091
joint, 2078
meningeal, 485-487, 2086-2090, 2088t-2089t
muscle, 2078
myocardial, 2075-2077, 2076t
neonatal, 2092-2097, 2094t. *See also* Enterovirus infection, neonatal
neurologic manifestations of, 2086-2090, 2088t
ocular, 2074-2075
oral cavity, 2066
pathology of, 2062
pericardial, 2075-2077, 2076t
peritoneal, 2070
pharyngeal, 2064t, 2065
photophobia in, 2074
pulmonary, 2067
stillbirth and, 2092
treatment of, 2103-2104
Echovirus 1 infection, 2080t-2081t, 2084

Echovirus 2 infection, 2080t-2081t, 2084
Echovirus 3 infection, 2080t-2081t, 2084
Echovirus 4 infection, 742t-748t, 749, 760, 2080t-2081t, 2084
Echovirus 5 infection, 2080t-2081t, 2084
Echovirus 6 infection, 742t-748t, 749, 2077-2078, 2080t-2081t, 2084
Echovirus 7 infection, 2080t-2081t, 2084
Echovirus 9 infection
  exanthem in, 742t-748t, 749, 760, 2080t-2081t, 2084-2085, 2085f, 2085t
  hepatic, 655
  oral cavity, 2066
  pathology of, 2062
Echovirus 11 infection
  in croup, 2066
  cytopathic effect of, 2054f
  epidemiology of, 2061
  exanthem in, 2080t-2081t, 2085
  neonatal, 2093
  oral cavity, 2066
  pathology of, 2062
Echovirus 13 infection, 2080t-2081t, 2085
Echovirus 14 infection, 2080t-2081t, 2085
Echovirus 16 infection, 760, 769, 2080t-2081t, 2085
Echovirus 17 infection, 2080t-2081t, 2085
Echovirus 18 infection, 2080t-2081t, 2085
Echovirus 19 infection, 2080t-2081t, 2085-2086
Echovirus 21 infection, 2080t-2081t, 2086
Echovirus 24 infection, 2080t-2081t, 2086
Echovirus 25 infection, 2080t-2081t, 2086
Echovirus 30 infection, 655, 2080t-2081t, 2086
Echovirus 32 infection, 2080t-2081t, 2086
Echovirus 33 infection, 2080t-2081t, 2086
ECMO. *See* Extracorporeal membrane oxygenation (ECMO)
Ecthyma contagiosum, 742t-748t
Ecthyma gangrenosum, **776**
  Aeromonas hydrophila, 1543
  Pseudomonas aeruginosa, 1590f, 1596
Eculizumab, in diarrheogenic *Escherichia coli* infection, 1463
Eczema herpeticum, 1943-1944, 1943f, 1953
Eczema vaccinatum, 2036, 2038-2039, 3532
Edema
  angioneurotic, 250t-251t, 253
  cerebral
    at high-altitude, 3118
    in meningitis, 439
  in cutaneous anthrax, 1303, 1313f
  infection-induced, 76
  palpebral
    in roseola infantum, 770
    in *Trypanosoma cruzi* infection, 2963
  pulmonary
    in epiglottitis-related intubation, 258
    at high-altitude, 3118
    in malaria, 2928
Edema factor, in anthrax, 1303
Edge Hill virus infection, 2316t
Edwardsiella tarda infection, **1532-1533**
Efavirenz, 2614
Effusion
  middle ear, 209-210, 218-220. *See also* Otitis media with effusion
  pericardial, 373-374, 376-377, 376f-377f
Eflornithine, 3348
  in *Trypanosoma brucei* infection, 2972-2973
Ehrlichia spp. infection (ehrlichiosis), 494t-498t, 751t-752t, **2661-2663**
Eicosanoids, 75
Eikenella corrodens, 1576
Eikenella corrodens infection, **1576-1578**
  clinical manifestations of, 1577
  diagnosis of, 1577
  epidemiology of, 1576
  in osteomyelitis, 725
  pathophysiology of, 1576-1577
  treatment of, 1577-1578
Electrical alternans, in pericarditis, 376
Electrocardiography (ECG)
  in endocardial fibroelastosis, 395
  in endocarditis, 359
  in infectious mononucleosis, 2002
  in Kawasaki disease, 1072, 1074-1075
  in *Leptospira* spp. infection, 1753
  in myocarditis, 389-392, 390f-392f, 394
  in pericarditis, 375-376
  in *Trypanosoma cruzi* infection, 2964-2965
  in visceral leishmaniasis, 2957
Electroencephalography (EEG)
  in brain abscess, 467-468
  in Creutzfeldt-Jakob disease, 2628
  in encephalitis, 507
  in HSV encephalitis, 1946-1947, 1947f
  in Japanese encephalitis virus infection in, 2296
  in La Crosse virus encephalitis, 2522
  in meningitis-related seizures, 455
  in St. Louis encephalitis virus infection, 2258
  in subacute sclerosing panencephalitis, 2389
  in tetanus, 1814
  in West Nile virus infection, 2270
Electrolytes, 75-76
Electromyography (EMG), in infant botulism, 1808
Electronic thermometer, 85
Electrophoresis
  HSV, 1957-1958, 1958f
  in meningitis, 442
  rotavirus, 2179, 2180f
  Staphylococcus aureus, 1119
Elek test, in *Corynebacterium diphtheriae* infection, 1307
Elizabethkingia spp., 1578-1579
Elizabethkingia spp. infection, **1578-1581**
  clinical manifestations of, 1579-1580
  diagnosis of, 1580
  epidemiology of, 1579
  pathophysiology of, 1579
  treatment of, 1580-1581
Embolism, in infective endocarditis, 355
Empyema
  anaerobic bacteria in, 1830
  in neonate, 892
  pulmonary, **306-315**
    diagnosis of, 310-313, 311f-312f
    epidemiology of, 307
    management of, 313-315
    microbiology of, 309-310, 309t
    pathophysiology of, 307-308, 308t
    prognosis for, 315
  subdural, **469-470**
    clinical manifestations of, 469
    CSF examination in, 443t, 469
    diagnosis of, 469, 470f
    treatment of, 470
Emtricitabine, 2613
Encephalitis, **492-512**
  adenovirus, 494t-498t, 1896t, 1903
  amebic, 494t-498t, 501
    Acanthamoeba spp., 494t-498t, 501, 2975, 2979, 2981, 2984-2985
    Balamuthia mandrillaris, 494t-498t, 501, 511, 2975, 2980-2981, 2984-2985
Encephalitis *(Continued)*
  anti-NMDAR, 509
  arbovirus, 494t-498t, 499-500, 510
  bacterial, 494t-498t, 501
  Banna virus, 2175
  brain stem, 505
  chronic, 503
  clinical manifestations of, 504-505
  CMV, 1979
  coxsackievirus B4, 493-499, 494t-498t, 2061, 2062f
  cysticercal, 3034, 3038
  diagnosis of, 507-509, 508t
  differential diagnosis of, 505-506
  Eastern equine, **2226-2230**, 2227f
  EBV, 2003-2004, 2011
  echovirus, 2088t, 2090
  EEG in, 507
  enterovirus, 493-499, 494t-498t
  enterovirus 71, 2062
  epidemiology of, 503
  etiology of, 493-503, 494t-498t
  evaluation of, 506-507
  fungal, 494t-498t, 501
  herpesvirus, 494t-498t, 499
  historical perspective on, 493
  hMPV, 494t-498t, 2439
  HSV, 494t-498t, 499, 505, 1945-1948, 1946t, 1949t
    acute retinal necrosis after, 1945
    clinical manifestations of, 1946, 1946t
    diagnosis of, 1946-1947, 1947f-1948f
    differential diagnosis of, 1948, 1949t
    vs. HSV meningitis, 1946
    pathogenesis of, 1939, 1946
    sequelae of, 1958
    vs. tick-borne encephalitis virus infection, 2310-2311
    treatment of, 1960
  human erythrovirus B19 (human parvovirus B19), 1853
  imaging in, 506-507
  influenza virus, 2352
  Japanese, 494t-498t, **2291-2301.** *See also* Japanese encephalitis virus infection
  La Crosse virus, 494t-498t, 500, 510, **2513-2527.** *See also* La Crosse virus (LACV) infection
  LCMV, 494t-498t, 500
  limbic, HHV-6 infection and, 2018
  measles virus, 494t-498t, 500, 2385-2386, 2391-2392
  mumps virus, 494t-498t, 500, 2401
  Murray Valley, **2301-2303**
  Mycoplasma pneumoniae, 494t-498t, 501-502, 2683-2684
  paramyxovirus, 494t-498t, 500-501
  parasite, 494t-498t, 501
  parechovirus, 493-499, 494t-498t
  pathogenesis of, 503-504
  pathology of, 504
  postimmunization, 502
  postinfectious, 493, **503**
    imaging in, 507
    pathogenesis of, 504
    pathology of, 504
  postvaccinial, 2038-2039
  prevention of, 511
  primary, 493
  prognosis for, 510-511
  reovirus, 2171
  rhabdovirus, 500-501
  Rocio virus, **2314-2315**
  Rocky Mountain spotted fever, 494t-498t, 501

Encephalitis *(Continued)*
in roseola infantum, 771
rotavirus, 2186
rubella virus, 494t-498t, 500, 2208, 2212
St. Louis encephalitis virus, **2253-2260.** *See also* St. Louis encephalitis virus infection
seizures in, 509-510
tick-borne, **2303-2313**, 2304f, 2306f. *See also* Tick-borne encephalitis virus infection
treatment of, 509-510
tuberculous, 494t-498t, 504
Venezuelan equine, **2237-2241**, 2238f, 2238t. *See also* Venezuelan equine encephalitis
viral, 493-501, 494t-498t
West Nile virus, **2261-2273.** *See also* West Nile virus infection
Western equine, **2230-2237**, 2231f-2233f, 2233t. *See also* Western equine encephalitis
Encephalitozoon bieneusi infection, 625
Encephalitozoon intestinalis infection, 625
Encephalomyelitis
disseminated, acute. *See* Acute disseminated encephalomyelitis (ADEM)
postherpetic, 1948
Encephalopathy, 493
in *Bacillus cereus* infection, 677
in *Bordetella pertussis* infection, 1623
DEET-related, 2235
evaluation of, 506-507
hepatic, probiotics in, 3371
HIV-related, 2597-2598, 2597f, 2604-2605
hMPV, 2439
influenza-associated, 502, 2343, 2352
in malaria, 2928
MMR-related, 2393-2394
pertussis-associated, 502
in Reye syndrome, 676-677
in septic shock, 830
toxic, in *Shigella* spp. infection, 1481
Endocardial fibroelastosis, vs. myocarditis, 395
Endocarditis. *See* Infective endocarditis
Endocrine disorders, CMV infection and, 1980-1981
Endometritis, **587**
Endophthalmitis, **820**
Aeromonas spp., 1543
Bacillus cereus, 1319
Candida, 813, 820, 936-937, 2743, 2749
neonatal, 895, 897, 2756
Coccidioides spp., 813-814
Cryptococcus spp., 814
Histoplasma capsulatum, 2816-2817
Morganella morganii, 1470
Plesiomonas shigelloides, 1566
Pseudomonas aeruginosa, 1591, 1599
Staphylococcus epidermidis in, 1138
Stenotrophomonas maltophilia, 1607
Endoscopic retrograde cholangiopancreatography (ERCP)
in cholangitis, 661-662
cholangitis after, 667
in pancreatitis, 690
Endoscopic sinus surgery, in rhinosinusitis, 201-202
Endoscopy
in *Aspergillus* spp. infection, 2706, 2707f
in *Candida* infection, 2739
Endoscopy *(Continued)*
in *Helicobacter pylori* infection, 1695, 1695f
in Whipple disease, 645
Endothelium, in phagocyte recruitment, 31-32
Endotoxin. *See* Lipopolysaccharide (LPS)
Enfuvirtide, 2611
Entamoeba coli infection, **2877-2878**
Entamoeba dispar, 2867
Entamoeba histolytica, 2867, 2868f
vs. *Entamoeba coli*, 2877
Entamoeba histolytica infection (amebiasis), 607t, 614, 662, **2866-2874**
ameboma in, 2869-2870
asymptomatic, 2873
biopsy in, 2872
cerebral, 2869, 2871
clinical manifestations of, 757t-758t, 2870-2871
colitis in, 2870, 2873
complications of, 2872-2873
cutaneous, 2871
diagnosis of, 2871-2872
diarrhea in, 2870
differential diagnosis of, 2870-2872
epidemiology of, 2867-2868, 2868t
extraintestinal, 2870-2871
diagnosis of, 2871-2872
treatment of, 2873-2874, 2873t
hepatic, 2869-2873
treatment of, 2873-2874
immune response to, 2869-2870
intestinal, 2870
treatment of, 2873, 2873t
metastatic, 2871
treatment of, 2873-2874, 2873t
ocular manifestations of, 814
pathogenesis of, 2868-2869, 2868f
pericardial, 2871
pleural, 309-310, 2869
prognosis for, 2874
vs. *Shigella* spp. infection, 1483
stool examination in, 2871
thoracic, 2871
treatment of, 625, 2873-2874, 2873t, 3327t-3346t
vulvovaginal, 569
Entecavir, in HBV infection, 1922-1923
Enteritis necroticans, 1797
Enteroaggregative *Escherichia coli* (EAEC) infection, 610t, 611, 1449
clinical manifestations of, 1450t, 1455
diagnosis of, 1460-1461, 1461f
epidemiology of, 1450t, 1453
pathogenesis of, 1460
prevention of, 1463-1464
prognosis for, 1461
treatment of, 1461-1463
Enterobacter spp., 1434
Enterobacter spp. infection, **1433-1439**
antibiotic-resistant, 1435, 1437
biliary, 659-660, 659t
bloodstream, 1436
clinical manifestations of, 1436
diagnosis of, 1436-1437
endocardial, 362
epidemiology of, 1434-1435
hospital-acquired, 1434
meningeal, 1435-1436
pathophysiology of, 1435-1436
risk factors for, 1434-1435
in septic shock, 834
treatment of, 1437-1438
Enterobactin, 538
Enterobius vermicularis infection (enterobiasis), 566-567, 566f, **3018-3019**
appendiceal, 684, 3019
bladder, 540
clinical manifestations of, 757t-758t, 3019
diagnosis of, 566, 566f, 3019
epidemiology of, 3018
pathophysiology of, 3019
treatment of, 3019, 3327t-3346t
vaginal, 3019
Enterococcus spp., 1170-1172, 1171f, 1172t
adherence properties of, 1173-1174
pili of, 5-6
typical vs. atypical, 1171, 1172t
virulence of, 1173-1174
Enterococcus spp. infection, **1170-1187**
bloodstream, 1174-1177
burn wound, 1052, 1052t
clinical manifestations of, 1174-1177
diagnosis of, 1177
endocardial, 361, 366, 366t-367t, 1174-1175
epidemiology of, 1172-1173
intra-abdominal, 1175
joint, 1177
meningeal, 1175-1176
neonatal, 882, 1176-1177
nosocomial, 1172, 1174, 3399-3400
prevention of, 1186
pathogenesis of, 1173-1174
peritoneal, 700
prevention of, 1185-1186
treatment of, 1182-1185
in antibiotic-resistant infection, 1183-1185
in antibiotic-susceptible infection, 1182-1183
resistance in, 1177-1182
acquired, 1178-1181, 1179t
aminoglycoside, 1178-1180
daptomycin, 1181
intrinsic, 1177-1178
β-lactam, 1177, 1179t, 1180
linezolid, 1181
prevention of, 1185-1186
quinupristin-dalfopristin, 1181
teicoplanin, 1180
testing for, 1181-1182
TMP-SMX, 1178
vancomycin, 1179t, 1180-1181
urinary tract, 1173-1174
vancomycin-resistant, 1170, 1179t, 1180-1181, 3151-3152
epidemiology of, 1172-1173
nosocomial, 3399-3400
prevention of, 1185-1186
treatment of, 1183-1185, 1185t
Enterocolitis. *See also* Gastrointestinal tract infection
necrotizing
Candida, 895-897
Clostridium difficile, 635
Cronobacter sakazakii, 1436
Klebsiella spp., 474
oral immunoglobulin in, 3498
platelet-activating factor and, 3366-3367
prevention of, 3243-3244
probiotics in, 3371-3372
Yersinia enterocolitica, 1526-1527
Enterohemorrhagic *Escherichia coli* (EHEC)
intimin of, 7-8, 1457
Shiga toxin of, 17, 1457-1459

Enterohemorrhagic *Escherichia coli* (EHEC) infection, 609-610, 610t, 616t, 619, 1449. *See also Escherichia coli* O157:H7 infection
clinical manifestations of, 1450t, 1454-1455
diagnosis of, 1460-1461
epidemiology of, 1450t, 1451-1452
food sources of, 1452
hemolytic-uremic syndrome with, 17, 1452, 1454, 1463
hemorrhagic colitis with, 1454
pathogenesis of, 1457-1459
prevention of, 1463-1464
prognosis for, 1461
vs. *Shigella* spp. infection, 1483
treatment of, 624, 1461-1463
Enteroinvasive *Escherichia coli* (EIEC) infection, 602t-603t, 610t, 611, 1449
clinical manifestations of, 1450t, 1455
diagnosis of, 1460-1461
epidemiology of, 1450t, 1452-1453
pathogenesis of, 1459-1460, 1459f
prevention of, 1463-1464
prognosis for, 1461
treatment of, 1461-1463
Enteropathogenic *Escherichia coli* (EPEC)
attaching and effacing lesion of, 7-8, 9f
atypical, 1451
intimin of, 7-8, 9f
locus of enterocyte effacement of, 7-8
pili of, 5
serotypes of, 1450t, 1451
Enteropathogenic *Escherichia coli* (EPEC) infection, 602t-603t, 610-611, 610t, 611f, 1449
clinical manifestations of, 1450t, 1453-1454
diagnosis of, 1460-1461
epidemiology of, 1450t, 1451
in neonate, 888
pathogenesis of, 1457, 1457f-1458f
prevention of, 1463-1464
prognosis for, 1461
treatment of, 1461-1463
Enterotoxigenic *Escherichia coli* (ETEC) infection, 602t-603t, 610t, 611, 1449
clinical manifestations of, 1450t, 1453
diagnosis of, 1460-1461
epidemiology of, 1449-1451, 1450t
pathogenesis of, 1455-1457, 1456f
prevention of, 1463-1464
prognosis for, 1461
treatment of, 1461-1463
vaccine against, 628, 1463-1464
Enterotoxins
Aeromonas spp., 1540
detection of, 1460-1461
enteroaggregative *Escherichia coli*, 1460
enterotoxigenic *Escherichia coli*, 1455-1457
rotavirus, 2184
Shigella spp., 1479
Staphylococcal aureus, 862-863, 1117-1118
Vibrio cholerae, 1550
Yersinia enterocolitica, 1525
Enterovirus. *See also* Coxsackievirus; Echovirus; Poliovirus; Rhinovirus
antigenic characteristics of, 2056
classification of, 2052-2053, 2053t
historical perspective on, 2051-2052
host range of, 2056
replication of, 2053-2054, 2053t
Enterovirus infection, **2051-2109.** *See also specific enterovirus infections*
acute lymphonodular pharyngitis in, 2066
asymptomatic, 2063-2064, 2063t
Enterovirus infection *(Continued)*
bronchial, 2066
cardiovascular manifestations of, 2075-2077, 2076t
chronic fatigue syndrome and, 2091
clinical manifestations of, 2063-2090, 2063t-2064t, 2066t, 2068t-2069t, 2071t-2072t, 2074t, 2076t
in common cold, 2064-2065, 2064t
congenital, 2091-2092
conjunctival, 810, 2073-2075, 2074t
in croup, 2066
cytopathologic features of, 2054-2056, 2054f
diabetes mellitus and, 2072-2073
diagnosis of, 2101-2103
clinical, 2101
laboratory, 2101-2102
differential diagnosis of, 2102-2103
encephalitic, 493-499, 494t-498t, 2088t, 2090
in epidemic pleurodynia, 2067-2068
epidemiology of, 2056-2059
in epididymitis, 2077
exanthems in, 742t-748t, 749, 760, 762, 769, 2078-2086, 2079f, 2080t-2081t, 2082f-2083f, 2083t, 2085f, 2087t
fibromyalgia and, 2091
gastrointestinal manifestations of, 2068-2073, 2068t-2069t, 2071t-2072t
genitourinary manifestations of, 2077-2078
geographic features of, 2056-2057
Guillain-Barré syndrome and, 2088t, 2090-2097
hematologic manifestations of, 2078
hemolytic-uremic syndrome in, 2078
hepatic, **655**, 2070-2072
herpangina in, 2065, 2066t
historical perspective on, 2051-2052
host factors in, 2060-2061
in immunocompromised host, 2091
intraoral manifestations of, 2065-2066, 2066t
joint manifestations of, 2078
meningeal, 485-487, 486t, 489, 2086-2090, 2088t-2089t
muscle manifestations of, 2078
myocardial, 384-387, 385f, 2075-2077, 2076t, 2104
neonatal, 2092-2097, 2094t
cardiovascular, 2096, 2096t
clinical manifestations of, 2093-2097
epidemiology of, 2092-2093
exanthem in, 2097
gastrointestinal, 2095-2096
hepatic, 2096
inapparent, 2093-2095
IVIG in, 3513
mild, 2095
neurologic, 2097
pancreatic, 2096
pathogenesis of, 2092-2093
respiratory, 2095
sepsis-like, 2095
neurologic manifestations of, 2086-2090, 2088t-2089t, 2104
nonspecific febrile illness in, 2064, 2064t, 2066t, 2068t, 2103
nosocomial, 3384
ocular, 2073-2075, 2074t
oral cavity, 2066
in orchitis, 2077
pancreatic, 2072
Enterovirus infection *(Continued)*
parotid gland, 2066
pathogenesis of, 2059-2063, 2059f-2060f
pathology of, 2054-2056, 2055f, 2061, 2062f
pericardial, 2076t
pharyngeal, 2064t, 2065
photophobia in, 2074
prevalence of, 2057-2059, 2058t
prevention of, 2105-2107
prognosis for, 2104-2105
pulmonary, 2067
renal, 2077-2078
respiratory manifestations of, 2064-2068, 2064t
vs. Rocky Mountain spotted fever, 2653
vs. St. Louis encephalitis virus infection, 2259
stillbirth and, 2092
sudden infant death and, 2091
vs. tick-borne encephalitis virus infection, 2310-2311
transmission of, 2056
treatment of, 2103-2104
Enterovirus 68 infection, 2067
Enterovirus 70 infection, 810, 2073-2074
Enterovirus 71 infection
cardiac, 2077
CNS, 2062
epidemiology of, 2057, 2061
exanthem in, 764, 2080t-2081t, 2086
paralytic, 2090
pericardial, 2076t
Entomophthorales, 2837, 2838f
Entomophthorales infection (entomophthoromycosis), **2844-2845**
Environment, in epidemiologic study, 110-111, 114-116
Enzyme immunoassay (EIA)
in *Bartonella* spp. infection, 1727
in *Clostridium difficile* infection, 635, 636t
in enterovirus infection, 2056
in HIV infection, 2608, 2608t
in *Norovirus* infection, 2153-2154
Enzyme-linked immunosorbent assay (ELISA), 3606t-3607t, 3610-3612
in *Aspergillus* spp. infection, 2714
in *Bordetella pertussis* infection, 1628-1629
in *Brucella* spp. infection, 1614
in *Campylobacter jejuni* infection, 1654-1655
in Colorado tick fever virus infection, 2174-2175
in *Corynebacterium diphtheriae* infection, 1307
in *Cryptosporidium* spp. infection, 2902
in dengue, 2285
in Ebola virus infection, 2484
in *Francisella tularensis* infection, 1663
in *Giardia lamblia* infection, 2882
in *Haemophilus ducreyi* infection, 1687-1688
in HCV infection, 2321-2323
in HEV infection, 2165
in human erythrovirus B19 (human parvovirus B19) infection, 1856
in influenza, 3610-3612, 3611t
in Japanese encephalitis virus infection, 2298
in LCMV infection, 2464-2465
in *Leptospira* spp. infection, 1755
in Lyme disease, 1735
in mumps, 2403
in *Mycoplasma pneumoniae* infection, 2685-2686

Enzyme-linked immunosorbent assay (ELISA) *(Continued)*
in *Paracoccidioides* spp. infection, 2791-2792
in relapsing fever, 1742
in Ross River virus arthritis, 2250
in rotavirus infection, 2183, 3611-3612
in RSV infection, 2424-2425, 3611, 3611t
in Toscana virus infection, 2549
in *Toxoplasma gondii* infection, 2994-2996, 2995t
in *Trypanosoma cruzi* infection, 2966
in Venezuelan equine encephalitis, 2240
in VZV infection, 2028
in West Nile virus infection, 2271
in Western equine encephalitis, 2235
Eosinophil(s)
in bronchiolitis, 277-278
CSF, 483
Eosinophilic meningitis. *See* Meningitis, eosinophilic
Eosinophilic pneumonia, acute, **297**
Ependymitis, in mumps meningitis, 2401
Epidemic pleurodynia, 739
Epidemiology, **95-102**
analytic study in, 95-96
case-control study in, 98-99, 98t-99t, 124t, 128
cohort study in, 97-99, 98t, 128
cross-sectional study in, 98-99
descriptive study in, 95
disease causation study in, 103-120. *See also* Infection, epidemiologic study of
experimental study in, 96-97
observational study in, 96-98, 128
study design in, 95-96
summary statistics in, 99-102, 100f. *See also* Biostatistics
validity in, 95
Epidermodysplasia verruciformis (Lewandowsky-Lutz dysplasia), 1871-1872, 1876
Epidermolysins, 1117
Epididymitis
enterovirus, 2077
after gonococcal urethritis, 1287
Epididymo-orchitis, 2401-2403
Epidural abscess
cerebral, 443t, **470**
spinal, **470-472**, 472f
clinical manifestations of, 471
diagnosis of, 443t, 471-472, 472f
treatment of, 472
Epiglottitis (supraglottitis), **241-260**, 243t
acute, 250t-251t
anatomy of, 248-249, 248f
clinical presentation of, 252-253
culture in, 254
diagnosis of, 253-254, 254f
epidemiology of, 246-247
etiology of, 244-245
genetic factors in, 249
Haemophilus influenzae type b, 1675-1676, 1679
vs. laryngotracheitis, 253-254
MSN erythrocyte antigens in, 249
necrotizing, 245
noninfectious, 245
pathophysiology of, 248-249
prevention of, 259
prognosis for, 259
radiography in, 254, 254f
tongue depressor examination in, 254
treatment of, 257-259
uvulitis and, 166
viral, 245
Epilepsy
fever of unknown origin in, 846
after pertussis immunization, 1634
Epinephrine
in animal serum–related anaphylaxis, 3493
in bronchiolitis, 279
in croup, 256-257
in parainfluenza virus infection, 2369-2370
plasma, 81-82
Epiphysis, osteomyelitis of, 719
Epithelium. *See also* Skin
barrier function of, 24-25
Epstein-Barr virus (EBV), 1992
antibodies against, 1997, 2007-2010, 2008t, 2009f, 2010t
cell transformation with, 1994
electron microscopy of, 2011
genome of, 1993
genotypes of, 1995
latency of, 1993-1994
nucleic acid testing for, 2010-2011
oropharyngeal shedding of, 1997-1998
replication of, 1993
structure of, 1993
tissue culture for, 2011
urinary shedding of, 1998
Epstein-Barr virus (EBV) infection, 158-161, **1992-2015.** *See also* Infectious mononucleosis
Arcanobacterium haemolyticum infection and, 1324
B-cell lymphoma and, 2013
Burkitt lymphoma and, 1996, 2005, 2005f
in burn patient, 1053
chronic, 2001, 2014
chronic fatigue syndrome and, 1086-1087
CNS, 1996, 2003-2004
corneal, 807
diagnosis of, 2007-2011, 2007f, 2008t, 3617
differential diagnosis of, 2011-2012
disseminated, 2000-2001
encephalitic, 494t-498t, 499, 505
epidemiology of, 1997-1998
exanthem in, 742t-748t, 749, 760
gastric carcinoma and, 2006
after heart transplantation, 952-953
after hematopoietic stem cell transplantation, 940-941
hemophagocytic lymphohistiocytosis and, 181, 1997, 2001, 2001f, 2014
hepatitis with, 654
histopathology of, 1996-1997
historical perspective on, 1992
HIV infection and, 2006-2007, 2014
Hodgkin disease and, 2005, 2013
immune response to, 2008-2010, 2009f, 2010t
immunopathogenesis of, 1995-1996
after intestinal transplantation, 997-998, 997t
after kidney transplantation, 1008-1009
after liver transplantation, 997-998, 997t
after lung transplantation, 976-978
lymphomatoid granulomatosis and, 2006
nasopharyngeal carcinoma and, 2005, 2013
pancreatitis with, 694
post-transplant lymphoproliferative disease and, 2005-2006, 2012-2013, 2015, 3521-3522
prevention of, 2014-2015, 3521-3522
prognosis for, 2014
Epstein-Barr virus (EBV) infection *(Continued)*
psychiatric features of, 2004
pyothorax-associated lymphoma and, 2006
rhinosinusitis with, 197
silent, 1998-1999
T-cell lymphoma and, 2006
transmission of, 1998
treatment of, 2012-2014
tumor-associated, 2005-2006, 2005f
histopathology of, 1996-1997
immunopathogenesis of, 1995-1996
treatment of, 2012-2013
uveal, 810
vaccine against, 2015
X-linked lymphoproliferative syndrome and, 2000-2001, 2013-2014
Equine antitoxin, in tetanus, 1815-1816
Errors, statistical, 121
Ertapenem, 3202, 3231t-3233t
Erwinia spp., 1535. *See also Pantoea agglomerans*
Erysipelas, 753t-756t, **776**, 1143
Erysipelothrix rhusiopathiae, 1327
Erysipelothrix rhusiopathiae infection, 753t-756t, **1327-1329**
clinical manifestations of, 1327-1328
cutaneous manifestations of, 1327-1328
diagnosis of, 1328
endocardial, 362-363
epidemiology of, 1327
pathophysiology of, 1327
treatment of, 1328-1329
Erythema chronicum migrans, 753t-756t
Erythema infectiosum, 742t-748t, 760
clinical manifestations of, 1847-1849, 1847f-1849f
diagnosis of, 1855-1856
differential diagnosis of, 1855
epidemiology of, 1845-1846
joint manifestations of, 1849, 1851
neurologic illness in, 1853
pathogenesis of, 1846-1847, 1846f
during pregnancy, 1852-1853
Erythema marginatum, in rheumatic fever, 409-410, 410f
Erythema migrans, 753t-756t, 1731f, 1732, 1734-1735
Erythema multiforme, **764**, 765t. *See also* Stevens-Johnson syndrome
in *Coccidioides* spp. infection, 2772-2773
HSV infection and, 764, 1951
urethritis and, 531
Erythema multiforme bullosum, in human erythrovirus B19 (human parvovirus B19) infection, 1849
Erythema nodosum, **764**, 765t, 1354-1355
in *Coccidioides* spp. infection, 2772-2773
after *Yersinia enterocolitica* infection, 1528-1529
Erythema nodosum leprosum, 1404-1405, 1404f
treatment of, 1409
Erythrocyte sedimentation rate (ESR)
in fever of unknown origin, 842
in infective endocarditis, 358, 358t
in Kawasaki disease, 1075-1076
in mastoiditis, 238
in *Mycoplasma pneumoniae* infection, 2678
in occult bacteremia, 838
in osteomyelitis, 719
in rheumatic fever, 410-411
in rhinosinusitis, 198
in sepsis neonatorum, 883

Erythromycin, 3211-3215, 3213t, 3231t-3233t
in chronic bronchitis, 269-270
drug interactions with, 3215
in heart transplant–related *Legionella pneumophila* infection, 949
in lymphogranuloma venereum, 584
in odontogenic infection, 145
topical
in inclusion conjunctivitis, 804
in neonatal conjunctivitis prevention, 805-806, 1281
in trachoma, 805
Erythromycin ethylsuccinate–sulfisoxazole acetyl, 3229
Erythrovirus infection. *See* Human erythrovirus B19 (human parvovirus B19) infection
Escherichia coli, 1439, 1440t
vs. *Entamoeba histolytica*, 2867
molecular tests on, 619, 620f
in toxic shock syndrome, 853
virulence factors of, 1443t, 1450t, 1460
Escherichia coli infection
appendiceal, 684. *See also* Appendicitis
biliary, 659-660, 659t, 663, 1445-1446
blood-brain barrier in, 432-433, 433t, 1443t, 1444
bloodstream, 432, 432t. *See also* Meningitis, bacterial
clinical manifestations of, 1444
epidemiology of, 1441
pathogenesis of, 1443-1444
treatment of, 1447
bone, 1441
CSF shunt, 1030, 1030t
diarrheogenic, 607t, **609-611**, 610t, 616t, **1449-1465.** *See also* Enteroaggregative *Escherichia coli* (EAEC) infection; Enterohemorrhagic *Escherichia coli* (EHEC) infection; Enteroinvasive *Escherichia coli* (EIEC) infection; Enteropathogenic *Escherichia coli* (EPEC) infection; Enterotoxigenic *Escherichia coli* (ETEC) infection
clinical manifestations of, 1453-1455
diagnosis of, 610t, 617, 619, 620f, 1460-1461, 1461f
epidemiology of, 1449-1453
pathogenesis of, 1455-1460, 1456f-1459f
prevention of, 1463-1464
prognosis for, 1461
transmission of, 1449-1453
treatment of, 623t, 624-625, 1461-1463
endocardial, 362, 1441
extraintestinal, **1439-1448**, 1440t
clinical manifestations of, 1444-1445
diagnosis of, 1445-1446
epidemiology of, 1439-1441
pathogenesis of, 1441-1444, 1442f, 1443t
prognosis for, 1447
treatment of, 1444t, 1446-1447
hepatic, 673
intra-abdominal, 1445
after lung transplantation, 962
meningeal, 435
clinical manifestations of, 1444-1445
diagnosis of, 1446
epidemiology of, 1441
pathogenesis of, 1444
peritoneal, 700, 1441
clinical manifestations of, 1445
Escherichia coli infection *(Continued)*
pulmonary, 1441
clinical manifestations of, 1445
diagnosis of, 1446
retroperitoneal, 706-709
in septic shock, 834
urinary tract, **537-538.** *See also* Urinary tract infection (UTI)
clinical manifestations of, 1444
diagnosis of, 1445-1446
epidemiology of, 1440-1441, 1440t
outcomes of, 1447
pathogenesis of, 1441-1443, 1442f
treatment of, 1444t, 1446-1447
uropathogenic. *See* Uropathogenic *Escherichia coli* (UPEC)
Escherichia coli O104:H4 infection, 1455
Escherichia coli O157:H7 infection, 624. *See also* Enterohemorrhagic *Escherichia coli* (EHEC) infection
in daycare facilities, 3558
diagnosis of, 1460
foodborne, 1452
hemolytic-uremic syndrome with, 1452, 1454, 1463
hemorrhagic colitis with, 1454-1455
Shiga toxin in, 17, 619
Esophagitis, **593-597**
bacterial, 593, 597
barium esophagography in, 594-595, 595f
Candida, 593-597, 595f-596f, 2739, 2747
clinical manifestations of, 593-594, 594t
CMV, 594-597, 596f
diagnosis of, 594-596
differential diagnosis of, 594
esophagoscopy in, 595-596, 595f-596f
etiology of, 593
fungal, 593, 597
in gastroesophageal reflux, 594
HSV, 594-597, 596f, 1948-1949, 1953
after kidney transplantation, 1006
pathophysiology of, 593
pill, 594
prognosis for, 597
treatment of, 596-597
viral, 596f, 597
Esophagography, 594-595, 595f
Esophagoscopy, 595-596, 595f-596f
Esophagus
enlargement of, in *Trypanosoma cruzi* infection, 2965
perforation of, mediastinitis with, 419-420
Estrogen, *Trichomonas vaginalis* infection and, 2889
Ethambutol, in tuberculosis, 1367t, 1368-1369
Ethics, in clinical trials, 97
Ethionamide, in tuberculosis, 1367t, 1369
Ethnicity
in epidemiologic study, 108, 113-114
in *Streptococcus pneumoniae* infection, 1203
Etravirine, 2614
European brown hare syndrome virus, 2155
Eustachian tube, in otitis media, 216, 216f
Evaporation, 84
Exanthem, **741-768.** *See also at specific infections*
blood-mediated, 750
diagnosis of, 766-767, 766t
differential diagnosis of, 766-767
epidemiology of, 750
etiology of, 741-750
arthropod, 758t, 763t-764t
bacterial, 753t-756t, 760t-766t
chlamydial, 751t-752t
Exanthem *(Continued)*
fungal, 757t
helminthic, 757t-758t, 761t, 763t
mycoplasmal, 751t-752t
protozoan, 757t-758t, 761t, 763t
rickettsial, 751t-752t, 760t-763t
viral, 742t-748t, 760t-766t, 782
historical perspective on, 741
immune-mediated, 750
in immunocompromised host, 766
macular, erythematous, 759-760, 760t
maculopapular, erythematous, 760-762, 761t
in meningitis, 765, 766t
nodular, 764, 764t
papular, 764, 764t
pathology of, 750-759
pathophysiology of, 750-759
petechial, 762-763, 763t. *See also* Petechiae
purpuric, 762-763, 763t. *See also* Purpura
in respiratory disease, 766, 766t
in toxic shock syndrome, 858
toxin-mediated, 750
ulcerative, 764, 764t. *See also* Ulcer(s)
urticarial, 763-764, 763t
vesicular, 762, 762t
viral, periflexural, **784**, 784f
Exanthem criticum. *See* Roseola infantum
Exanthem subitum. *See* Roseola infantum
Exchange transfusion, in septic shock, 836
Exfoliatins, 1117
Exophiala jeanselmei infection, after lung transplantation, 974
Exotic pet bite, 3578
External auditory canal, 204
inflammation of. *See* Otitis externa
Extracorporeal membrane oxygenation (ECMO)
antibiotic prophylaxis with, 1023
in ARDS, 873-874
Candida spp. infection and, 2751
infection with, 1022-1023
in septic shock, 836
Extrinsic allergic alveolitis. *See* Hypersensitivity pneumonitis
Eyach virus infection, vs. Colorado tick fever virus infection, 2174
Eye(s), **794-822.** *See also* Eyelid infection
Corynebacterium diphtheriae infection effects on, 1306
examination of
in infant botulism, 1806, 1806f, 1806t
in orbital cellulitis, 799-801
inflammation/infection of. *See* Endophthalmitis; Keratitis; Uveitis; Vitritis
Neisseria gonorrhoeae infection of, 1288. *See also* Ophthalmia neonatorum, gonococcal
presumed histoplasmosis of, 2816-2817
red. *See* Conjunctivitis
T cells of, 810
tuberculosis of, 1357
in Whipple disease, 644
Eyelid hygiene, in staphylococcal blepharitis, 794
Eyelid infection, **794-801**
anterior, 794-795
Demodex, 795-796, 796f
HSV, 1945
meibomian gland, **796-797**
molluscum contagiosum, 794-795, 795f
parasitic, 795-796, 795f
Phthirus pubis, 795, 795f

Eyelid infection *(Continued)*
posterior, 796-797
preseptal cellulitis with, 798-801
sebaceous gland, **796**
staphylococcal, 794

F

Face
cellulitis of. *See also* Orbital cellulitis; Periorbital cellulitis
in meningitis, 440-441
Streptococcus pneumoniae, 1231-1232
dermatophyte infection of, **785-786**, 785f
Haemophilus influenzae type b cellulitis of, 778, 778f
pustulosis of, 789, 789f
Facial nerve paralysis
mastoiditis and, 236, 239
otitis media and, 217, 224
Factitious fever, 846
Factor H, 18
Faget sign, 2276
Failure to thrive, immunodeficiency and, 904-905
Famciclovir, 3278t, 3282-3283
in HSV infection, 1959
in VZV infection, 2029
Familial dysautonomia (Riley-Day syndrome), fever of unknown origin in, 846
Familial granulocytopenia syndrome, 917
Familial Mediterranean fever, fever of unknown origin in, 847
Family, in infection transmission, 111, 3548
Fascial space infection
infraorbital space, 149
masticator space, 142, 148-149, 149f
submandibular space, 142, 149-150, 150f
Fasciitis, necrotizing. *See* Necrotizing fasciitis
Fasciola buski, 3050f, 3056
Fasciola buski infection (fasciolopsiasis), **3056-3057**
clinical manifestations of, 3057
diagnosis of, 3057
epidemiology of, 3056-3057
pathology of, 3057
prevention of, 3057
treatment of, 3057, 3327t-3346t
Fasciola gigantica, 3052
Fasciola hepatica, 3050f, 3052
Fasciola spp. infection (fascioliasis), **3052-3054**
clinical manifestations of, 3053
diagnosis of, 3051f, 3053
epidemiology of, 3052
imaging in, 3053
pathogenesis of, 3050f, 3053
prevention of, 3054
transmission of, 3052
treatment of, 3053-3054, 3327t-3346t
Fatal familial insomnia, 2620-2621, 2627t
Fatigue, chronic. *See* Chronic fatigue syndrome
Fecal leukocytes, 617
Fecal microbiota transplantation, in *Clostridium difficile* infection, 637
Feet
dermatophyte infection of, **786**
in Kawasaki disease, 1073-1074
puncture wound of, 724-725
Ferritin
CSF, in meningitis, 445
fever effects on, 85
Ferritin *(Continued)*
plasma, 78
serum, in septic shock, 829
Fertility, in cystic fibrosis, 325
Fetor hepaticus, 649
Fetus. *See also at* Newborn
antibody production by, 44
B cells in, 42
bacterial infection in, **881-892**
epidemiology of, 881-882, 881t
pathogenesis of, 881-882
CMV infection in, 1975, 1983-1984
EBV infection in, 1998
enterovirus infection in, 2091-2092
fungal infection in, **892-900**
HSV infection in, 1954-1955
human erythrovirus B19 (human parvovirus B19) infection of, 1847, 1852-1853
Mycoplasma pneumoniae infection in, 2684
poliovirus infection in, 2100-2101
rubella virus infection in, 2206-2208, 2213. *See also* Rubella virus infection, congenital
stillborn, enterovirus infection and, 2092
syphilis in, 1762, 1766. *See also* Syphilis, congenital
T cells in, 42
viral infection in, **877-881**, 878t
clinical manifestations of, 878-879
developmental malformation and, 877
diagnosis of, 878-880
laboratory diagnosis of, 880-881
pathogenesis of, 877-878, 878f
treatment of, 881
Fever, **83-88.** *See also* Fever of unknown origin (FUO); Fever without source (FWS)
adverse effects of, 85
antipyretics for, 86-88
in appendicitis, 681
in bacterial meningitis, 455, 1228-1229
beneficial effects of, 85
in brain abscess, 464
in bronchitis, 263
in croup syndromes, 250t-251t
in CSF shunt–related infection, 1032
drug, 846
effects of, 84-85
in epiglottitis, 250t-251t, 252
in *Escherichia coli* UTI, 1440-1441
external cooling for, 88
factitious, 846
in *Francisella tularensis* infection, 1661
hectic, 841
in infective endocarditis, 356
in influenza virus immunization, 2352
intermittent, 841
in Kawasaki disease, 1073, 1075
after kidney transplantation, 1007
in lung abscess, 318
in measles, 2381-2382
measurement of, 85-86
metabolic changes with. *See* Metabolism
in neutropenia, 921. *See also* Chemotherapy, fever and neutropenia with
nitrogen balance and, 67, 68f
in *Opisthorchis* spp. infection, 3051
in orbital cellulitis, 799
oxygen consumption with, 65
in parapneumonic effusion, 310
pathogenesis of, 84
periodic, 159t-160t, 162, 181
Fever *(Continued)*
pharyngoconjunctival, 1893-1894, 1899, 1899t-1900t
in pneumococcal meningitis, 1228-1229
relapsing, 841. *See also* Relapsing fever (*Borrelia* spp. infection)
remittent, 841
in renal abscess, 554
in retroperitoneal infection, 708
in rhinosinusitis, 197
in roseola infantum, 770
in rubella virus infection, 2211, 2218
seizures with, 85
after smallpox immunization, 2038
spiking, 841
in spinal epidural abscess, 471
sustained, 841
treatment of, 86-88
typhoid. *See Salmonella* ser. Typhi infection (typhoid fever)
in urinary tract infection, 536
Fever of unknown origin (FUO), **840-848**
in bacterial endocarditis, 845
in bone and joint infection, 845
in brucellosis, 844
in cancer patient, 923
in cat-scratch disease, 844
in central nervous system dysfunction, 846
in connective tissue disease, 840
definition of, 837, 840
in diabetes insipidus, 846
diagnosis of, 841-843, 843t
drug-related, 846
factitious, 846
in familial dysautonomia, 846
vs. fever without source, 837
in hemophagocytic lymphohistiocytosis, 846-847
in hepatic infection, 845
in immunodeficiency disorders, 845
in infantile cortical hyperostosis, 847
infectious causes of, 843t, 844-846
in inflammatory bowel disease, 847
in intra-abdominal abscess, 845
in juvenile idiopathic arthritis, 840, 847
laboratory evaluation in, 842-843
in leptospirosis, 844
in malaria, 844
in malignancy, 840
noninfectious causes of, 846-848
patient history in, 841
pattern of fever in, 841
in periodic fever disorders, 847-848
physical examination in, 842
pupillary constrictor response in, 842
in pyogenic liver abscess, 674
in salmonellosis, 844
in toxoplasmosis, 844
in tuberculosis, 844-845
in tularemia, 845
in upper respiratory tract infection, 845-846
in viral infection, 845
Fever therapy, 85
Fever without source (FWS), **837-840**
clinical assessment in, 839
clinical management of, 839-840
definition of, 837
vs. fever of unknown origin, 837
occult bacteremia and, 837-839, 840t
Fibromyalgia, enterovirus and, 2091
Fibronectin-binding protein A, of *Staphylococcus aureus*, 8-9
Fidaxomicin, 3225
in *Clostridium difficile* infection, 637

Fifth disease. *See* Erythema infectiosum
Filamentous hemagglutinin (FHA), 6, 7f
  of *Bordetella pertussis*, 6, 7f, 1618t, 1619, 1623
Filariasis, **3025-3028**
  Brugia spp., **3025-3026**
  Loa loa, 815, **3026-3027**
  Wuchereria bancrofti, 757t-758t, 815, **3025-3026**
FimH, 13, 14f
Finger(s)
  blistering infection of, **775**
  Candida paronychia of, 788, 788f
  desquamation of, in Kawasaki disease, 1073-1074
  herpetic whitlow of, 155, 1944-1945, 1944f-1945f
  paronychia of, 788, 788f, 3571-3572, 3574
Fingernails, dermatophyte infection of, **786**, 786f
Fire ant bite, 758t
Fish poisoning, 604, 605t
Fish tapeworm (*Diphyllobothrium* spp.) infection, **3040-3041**, 3327t-3346t
Fistula
  bronchopleural, 321-322
  tracheoesophageal, 268
Fite-Faraco stain, in *Mycobacterium leprae* infection, 1398, 1399f
Fitz-Hugh-Curtis syndrome, **589-590**
Flagella, *Pseudomonas aeruginosa*, 1586-1587
Flavobacterium meningosepticum infection, 448-449
Fleas, 758t
  in *Yersinia pestis* infection transmission, 1510-1511
Flexal virus infection, 2472. *See also* Arenavirus infection
Flora, normal, 88-89. *See also* Microbiome
  colonization with, 89
  exogenous influences on, 89-90
  external ear, 204-205
  in newborn, 89
  oral, 140, 141t
  resident vs. transient, 88
  site-specific, 90-93, 91t
Flow cytometry, in malaria, 2936
Fluconazole, 3301f, 3303-3305, 3348
  adverse effects of, 3304
  in *Blastomyces dermatitidis* infection, 478, 2733-2734
  in *Candida* esophagitis, 596-597
  in *Candida* meningitis, 475
  in candidemia, 2747
  in *Coccidioides* spp. infection, 477, 2778
  in *Cryptococcus neoformans* infection, 2804-2805
  drug interactions with, 3304
  in esophageal candidiasis, 2747
  in hematopoietic stem cell transplantation–related aspergillosis, 937
  in *Histoplasma capsulatum* infection, 2825
  indications for, 3294t-3295t, 3304-3305, 3321t
  in neonatal *Candida* spp. infection, 2758, 2760-2761, 2764t
  in oropharyngeal candidiasis, 2746-2747
  in osteoarticular candidiasis, 2749
  in *Paracoccidioides* spp. infection, 2793
  in peritoneal candidiasis, 2748
  pharmacodynamics of, 3303
  pharmacokinetics of, 3303-3304, 3304t
Fluconazole *(Continued)*
  prophylactic
    in hematopoietic stem cell transplantation, 936
    in neonatal *Candida* spp. infection, 897
  in tinea unguium, 787
  in urinary tract candidiasis, 2748-2749
  in vulvovaginal fungal infection, 570, 2747
Flucytosine, 3298-3300, 3298f, 3348
  adverse effects of, 3300
  in *Candida* meningitis, 474-475
  in *Cryptococcus neoformans* infection, 476, 2804-2805
  dosage for, 3300
  drug interactions with, 3300
  indications for, 3300
  pharmacodynamics of, 3299, 3299f
  pharmacokinetics of, 3299-3300, 3300t
  resistance to, 3298-3299
  spectrum of activity of, 3298-3299
Fluid overload, in dengue hemorrhagic fever/dengue shock syndrome, 2288
Fluid therapy
  in ARDS, 873
  in *Bacillus cereus* infection, 1320
  in cholera, 1552-1553, 1552t, 1553f
  in *Corynebacterium diphtheriae* infection, 1310
  in dengue hemorrhagic fever/dengue shock syndrome, 2286-2288
  in diarrheogenic *Escherichia coli* infection, 1461-1462
  in gastrointestinal tract infection, 620-622, 620t-621t
  in *Leptospira* spp. infection, 1757
  in neonatal diarrhea, 888
  in rotavirus infection, 2186
  in *Shigella* spp. infection, 1483
  in toxic shock syndrome, 857-858
  in *Vibrio parahaemolyticus* infection, 1559
Flukes, 3050f-3051f, 3327t-3346t
  intestinal, 2865, 3056-3058
  liver, 660, 2865-2866, 3049-3054
  lung, 2865-2866, 3054-3056
Fluorescent antibody to membrane antigen (FAMA), in VZV infection, 2028
Fluorescent treponemal antibody–antibody absorption (FTA-ABS) test, 819-820, 1773-1774, 1773t
Fluoridation, 1195
5-Fluorocytosine, in fungal endocarditis, 368
Fluoroquinolones, 3225-3227, 3231t-3233t
  adverse effects of, 3227
  indications for, 3226-3227
  mechanisms of action of, 3226
  pharmacokinetics of, 3226
  resistance to, 3226
  in vitro activity of, 3226
Fluorouracil, 3298-3300, 3298f
  in genital HPV infection, 581
Fly infestation (myiasis), 816, **3072-3074**
Focal neurologic signs
  in bacterial meningitis, 439, 440t
  in brain abscess, 463t, 464-466, 466t
Foix-Alajouanine syndrome, 523
Folinic acid, in *Toxoplasma gondii* infection, 3001t
Folliculitis, **776-777**
  Pseudomonas aeruginosa, 1599
Fomites, 107
Fonsecaea pedrosoi infection, **789-790**, 790f
Food(s)
  antimicrobial agent interaction with, 3234
  Clostridium difficile in, 635
Food(s) *(Continued)*
  contaminated, 109, 602-604, 3547. *See also* Food poisoning
  weaning, 627
Food poisoning, 601-606. *See also* Gastrointestinal tract infection *and specific infections*
  Aeromonas hydrophila, 602t-603t, 607t, 612, 616t
  Bacillus cereus, 602t-603t, 613, 1317-1320
  bacterial, 602-604, 602t-603t
  Campylobacter jejuni, 602t-603t, 609. *See also Campylobacter jejuni* infection
  chemical, 604, 604t
  clinical manifestations of, 601-602
  Clostridium botulinum, 602t-603t, **1791-1795.** *See also* Botulism
  Clostridium perfringens, 602t-603t, 613, 1797-1800
  Cryptosporidium spp., 602t-603t, 614-615, 2899. *See also Cryptosporidium* spp. infection (cryptosporidiosis)
  diagnosis of, 616-619, 616t. *See also* Stool culture; Stool specimen examination
  differential diagnosis of, 1793
  epidemiology of, 601
  Escherichia coli. *See Escherichia coli*, diarrheogenic
  HAV, 2134-2136
  HEV, 2163
  inorganic toxin, 604, 604t
  Listeria monocytogenes, 607t, 613, 618. *See also Listeria monocytogenes* infection (listeriosis)
  marine toxin, 604, 605t
  mushroom, 604, 604t
  parasite, 602-604, 602t-603t, 607t, **613-616**, 616t. *See also specific parasitic infections*
  Plesiomonas shigelloides, 607t, 612-613, 616t
  prevention of, 604-606, 626-628, 1321
  Salmonella spp., 602t-603t, 609, 1493. *See also Salmonella* spp. infection (salmonellosis)
  Shigella spp., 608-609. *See also Shigella* spp. infection (shigellosis)
  Staphylococcus aureus, 613. *See also Staphylococcus aureus* infection
  treatment of, 619-626, 620t-621t
  Vibrio cholerae, 602t-603t, **611-612.** *See also* Cholera
  Vibrio parahaemolyticus, 602t-603t, 612. *See also Vibrio parahaemolyticus* infection
  Vibrio vulnificus, 1560
  viral, 602-604, 602t-603t
  Yersinia enterocolitica, 602t-603t, 612. *See also Yersinia enterocolitica* infection (yersiniosis)
Foot (feet)
  dermatophyte infection of, **786**
  in Kawasaki disease, 1073-1074
  puncture wound of, 724-725
Foreign body
  esophageal, 419
  laryngeal, 250t-251t, 253
  pulmonary, 316-317
  in toxic shock syndrome, 852-853
  vaginal, 567-570, 567f
Foreign body reaction, implant-related, 1016
Fosamprenavir, 2615
Foscarnet, 3278t, 3286-3287, 3286f
  in acyclovir-resistant HSV infection, 1964
  in CMV infection, 1984-1987, 1985f
  in hematopoietic stem cell transplantation, 940

Foscarnet *(Continued)*
in kidney transplant–related CMV infection, 1008
structure of, 1985f
in VZV infection, 2029
FOXP3, 915-916
Fracture, osteomyelitis after, 719
Francisella tularensis, 1658
subspecies of, 1658, 1660
Francisella tularensis infection (tularemia), **1657-1665**
bioterrorist, 3584
clinical manifestations of, 753t-756t, 1661-1663
diagnosis of, 1663-1664
differential diagnosis of, 1663-1664
epidemiology of, 1658-1660
fever of unknown origin in, 845
glandular, 1662
historical perspective on, 1657-1658
host factors in, 1660-1661
immune response to, 1660-1661
vs. *Legionella pneumophila* infection, 1713
nodular, 1662-1663
oculoglandular, 1662
oropharyngeal, 1662
pathogenesis of, 1660-1661
prevention of, 1664-1665
pulmonary, 1662-1663
seasonality of, 1659
transmission of, 1659
treatment of, 1664
vs. tuberculosis, 1661
typhoidal, 1662
ulceroglandular, 1662
Free oxygen radicals, *Streptococcus pneumoniae* release of, 1214
Frequency distribution, 100-101, 100f
Friction rub, 374
Friedländer pneumonia, 1467
FTA-ABS (fluorescent treponemal antibody–antibody absorption) test, 819-820, 1773-1774, 1773t
Fuchs heterochromic uveitis, 2213
Fumagillin, 2913
Fungal infection. *See also specific infections*
appendiceal, 683t, 685
biliary, 660
bone, 725-726
burn wound, 1053, 1053t
in cancer patient, 922, 924, 928
corneal, 807-808
contact lens–related, 1019
CT in, 926-927
ear canal, **207**
encephalitic, 494t-498t, 501
endocardial, 352, 361t, 363, 367-368
esophageal, 593, 597
exanthem in, 757t
after hematopoietic stem cell transplantation, 936-939
hepatic, 651t, 657, 673-675
in HIV-infected host, 2602
host response to, 23. *See also* Immunity
after intestinal transplantation, 991
joint, 730, **734**
after kidney transplantation, 1006, 1012-1014
laboratory diagnosis of, **3596-3600**, 3599t
β-D-glucan assay in, 926, 938, 967, 2716, 2744-2745, 3597-3599, 3598t
culture in, 3596, 3597t, 3599-3600, 3599t
direct detection methods in, 3596-3599, 3598t
Fungal infection *(Continued)*
galactomannan in, 926, 927t, 3597-3599, 3598t
PCR in, 926
left ventricular assist device, 1022
after liver transplantation, 813, 991, 993-994, 995t
after lung transplantation, 959-960, 965-974
meningeal. *See* Meningitis, fungal
muscle, 739
neonatal, **898-899**
acquired, 898-899
congenital, 898
ocular, 820
orbital, 801
pancreatic, 695
pericardial, 373, 379
peritoneal, 701, 703
in rhinosinusitis, 196-197, 196t, 201
urinary tract, 540
uveal, 813-814
vulvovaginal, 569-570, 570f
Fungus (fungi), 2702-2704. *See also* Fungal infection
Funisitis, **890**
necrotizing, 1766
Furazolidone, 3348
Furunculosis, 208, **776-777**
Fusarium spp., 2846
Fusarium spp. infection (fusariosis), **2846-2852**
burn wound, 1053
clinical manifestations of, 2846-2847, 2847f
corneal, 807-808, 2846-2847
cutaneous, 757t, 2847, 2847f
diagnosis of, 2847-2848
disseminated, 2847, 2847f
after lung transplantation, 974
pathogenesis of, 2846
treatment of, 2847-2848
Fusobacterium spp., 1826
Fusobacterium spp. infection, **1825-1834**
Arcanobacterium haemolyticum infection with, 1326
bloodstream, 1827-1828
bone, 1829-1830
clinical manifestations of, 1827-1830
CNS, 1828-1829
cutaneous, 1829
diagnosis of, 1830
epidemiology of, 1826-1827
head and neck, 174, 1828
hepatic, 673
intra-abdominal, 1829
joint, 1829-1830
oral cavity, 140-141
in otitis media, 214
pathogenesis of, 1827
pleuropulmonary, 1830
prevention of, 1833
soft tissue, 1829
treatment of, 1831-1833, 1831t

G

Galactomannan, 938, 3597-3599, 3598t
in *Aspergillus* spp. infection, 926, 927t, 2714-2715
BAL fluid, 967, 2715-2716
in *Blastomyces dermatitidis* infection, 2731-2732
in *Coccidioides* spp. infection, 2777
in *Fusarium* spp. infection, 2847-2848
urine, 2731-2732
Gallbladder
inflammation of. *See* Cholecystitis
in Kawasaki disease, 1074, 1082
in *Leptospira* spp. infection, 1750, 1751f
perforation of, 670
stones of. *See* Gallstones
Gallbladder sludge, ceftriaxone therapy and, 447
Gallium, metabolism of, 77t
Gallstones, 667-669, 668t. *See also* Cholecystitis
cholesterol, 668
in cystic fibrosis, 324-325, 668
epidemiology of, 667
formation of, 667-668
pancreatitis with, 692
pigmented, 668
in sickle-cell disease, 670
Gamma globulin. *See* Immunoglobulin(s)
Ganciclovir, 3278t, 3283-3285, 3283f
adverse effects of, 3285
in CMV infection, 1984-1987, 1985f
in CMV infection prevention, 1988
dosage for, 3278t, 3284-3285
in heart transplant–related CMV infection, 951-952
in hematopoietic stem cell transplantation, 940
in hematopoietic stem cell transplantation–related CMV infection, 940
indications for, 3284
in kidney transplant–related CMV infection, 1008
in lung transplantation, 975-976
mechanism of activity of, 3283
pharmacokinetics of, 3283-3284
resistance to, 3283
spectrum of activity of, 3283
structure of, 1985f
Gangosa, 1787
Gangrene
in Kawasaki disease, 1082
pulmonary, 1467
Gardnerella vaginalis, vaginal colonization with, 562
Gardnerella vaginalis infection, 572t-573t, 577-578, 577f
Garré sclerosing osteomyelitis, 154, 154f-155f
Gas gangrene, 1796
diagnosis of, 1798
prevention of, 1800
prognosis for, 1799-1800
treatment of, 1799, 3506
Gastric acidity, *Yersinia enterocolitica* infection and, 1526
Gastric cancer
EBV infection and, 2006
Helicobacter pylori infection and, 1696-1698, 1697f
Gastric lavage, in botulism, 1794
Gastritis, 1694. *See also Helicobacter pylori* infection
Gastroenteritis. *See* Gastrointestinal tract infection
Gastroesophageal reflux
vs. chronic bronchitis, 268
esophagitis in, 594
Gastrointestinal tract
bleeding from, in Whipple disease, 643
decontamination of, in burn patient, 1061
indigenous flora of, 91t, 92-93

Gastrointestinal tract infection, **598-632**
adenovirus, 607t, 608, 618-619, 1896t, 1901-1902
Aeromonas spp., 607t, 612, 616t, 1541-1542
astroviral, 607t, 608
Bacillus anthracis, 1314
Bacillus cereus, 607t, 613, 1317-1320
bacterial, 607t, 608-613, 616t, 628
Balantidium coli, **2894-2896**, 3327t-3346t
Basidiobolus ranarum, 2844
Blastocystis hominis, **2875-2876**, 3327t-3346t
Brucella spp., 602t-603t, 1613
Campylobacter, 607t, 609, 616t, 623-624, 623t, **1644-1657.** *See also Campylobacter jejuni* infection
Candida, 2741
Citrobacter spp., 1431
Clostridium difficile, 607t, 613, 616t. *See also Clostridium difficile* infection, antibiotic-associated
Clostridium perfringens, 602t-603t, 607t, 613, 618, 1797-1800
CMV, 1978
coronavirus, 2493-2494
coxsackievirus, 2068-2073, 2068t-2069t, 2071t
Cryptosporidium spp., 614-615, 625, **2896-2905**, 2898f, 2901t-2902t
Cyclospora, 615, 625, **2905-2907**, 2907f
Cystoisospora belli, 607t, 615, 617, **2907-2911**, 2908f
diagnosis of, 616-619, 616t, 1503
coccidia stains in, 617
fecal leukocytes in, 617
immunologic tests in, 618-619
molecular tests in, 619, 620f
ova examination in, 617
stool culture in, 618. *See also* Stool culture
stool examination in, 616-617. *See also* Stool specimen examination
diarrhea in, 599-606, 600t. *See also* Diarrhea
Dientamoeba fragilis, **2884-2887**, 2885f, 2886t
echoviral, 2068-2073, 2069t, 2071t
Edwardsiella tarda, 1533, 2072t
Entamoeba coli, **2877-2878**
Entamoeba histolytica, 614, 625, **2866-2874.** *See also Entamoeba histolytica* infection (amebiasis)
Enterococcus spp., 1175
enterovirus, 2068-2073, 2068t-2069t, 2071t
epidemiology of, 598-606
Escherichia coli, 607t, **609-611**, 610t, 611f, 616t, 623t, 624-625, **1449-1465.** *See also Escherichia coli* infection, diarrheogenic *and specific species*
foodborne, 601-606, 602t-603t. *See also* Food poisoning
Giardia intestinalis, 602t-603t, 607t, 614, 625
Giardia lamblia, **2878-2883**, 2880f, 2881t, 2883t
Hafnia alvei, 1534
Histoplasma capsulatum, 2815
human bocavirus, 1860-1861
in international adoptees and refugees, 3128-3130
Listeria monocytogenes, 607t, 613
microsporidial, 607t, 615, 617, 625, 2911t, 2912
Gastrointestinal tract infection *(Continued)*
Mucor spp., 2842
Mycobacterium tuberculosis, 1357-1358
norovirus, 607-608, 607t, 616t, **2151-2154**, 2152f. *See also Norovirus* infection
nosocomial, 3382-3383
pancreatitis with, 695
parasitic, 607t, **613-616**, 616t, 625, 3012-3021. *See also specific parasitic infections*
diagnosis of, 617
parechoviral, 2068-2073, 2068t-2069t, 2071t
Plesiomonas shigelloides, 607t, 612-613, 616t, **1565-1566**
prevention of, 626-628
Rhizopus spp., 2842
rotavirus, 606-607, 607t, 608f, 616t, 627, **2176-2195.** *See also* Rotavirus infection
respiratory symptoms with, 158
Salmonella, 607t, 609, 616t, 623t, 624, **1491-1509.** *See also Salmonella* spp. infection (salmonellosis)
Schistosoma spp., 3065. *See also Schistosoma* spp. infection (schistosomiasis)
Shigella, 607t, 608-609, 616t, 623, 623t, **1476-1487**, 1478f, 1480t. *See also Shigella* spp. infection (shigellosis)
Staphylococcus aureus, 602t-603t, 607t, 613, 618
Strongyloides stercoralis, 615-616, 625. *See also Strongyloides stercoralis* infection (strongyloidiasis)
transmission of, 598-599
treatment of, 619-626, 620t
antimicrobials in, 620t, 622, 623t
antisecretory agents in, 626
fluid therapy in, 620-622, 620t-621t
nutritional management in, 620t, 622
probiotics in, 626
zinc in, 625-626
vaccines against, 627-628
Vibrio cholerae, 607t, **611-612**, 616t, 623t, 625, **1548-1555.** *See also* Cholera
Vibrio parahaemolyticus, 607t, 612, 616t, **1556-1559.** *See also Vibrio parahaemolyticus* infection
Vibrio vulnificus, **1560-1562**
viral, 606-608, 607t, 616t
waterborne, 606, 627, 3547
in Whipple disease, 642-643
Yersinia enterocolitica, 607t, 612, 616t, **1514-1532.** *See also Yersinia enterocolitica* infection (yersiniosis)
Gatifloxacin, pancreatitis with, 692
Gemella morbillorum infection, 362-363
Gene(s)
in primary immunodeficiency, 907, 907t
in septic shock, 831
virulence-associated, 3
Gene therapy
in chronic granulomatous disease, 57-58
in immunodeficiency disorders, 64
in severe combined immunodeficiency disease, 49, 915
in type 1 leukocyte adhesion deficiency, 53
Genital tract infection, **560-591.** *See also specific infections*
CMV, 1972
EBV, 1998
evaluation of, 560-561
HPV. *See* Human papillomavirus (HPV) infection, anogenital
Genital tract infection *(Continued)*
HSV. *See* Herpes simplex virus (HSV) infection, genital
Mycobacterium tuberculosis, 1358
physical examination in, 561
ulcers with, 582-584, 583t
vaginal specimen collection in, 561, 561f
Genitourinary tract, indigenous flora of, 91t, 93
Genome
Blastomyces dermatitidis, 2723-2724
Bordetella spp., 1617
Borrelia hermsii, 1740
calicivirus, 2149-2150, 2149f
Campylobacter jejuni, 1645-1646
CMV, 1969
coronavirus, 2487
Corynebacterium diphtheriae, 1302
EBV, 1993
hantavirus, 2496-2497
HBV, 1912-1915
HCV, 2318-2319, 2318f
HDV, 1928
hepatitis A virus, 2128-2129, 2129f
HEV, 2159, 2159f
HHV-6, 2016
hMPV, 2435, 2435f
HPV, 1872
HSV, 1933
human bocavirus, 1858
human erythrovirus B19 (human parvovirus B19), 1844
influenza virus, 2327
measles virus, 2374
Mycobacterium leprae, 1393
Mycobacterium ulcerans, 1412
parainfluenza virus, 2361, 2362f
polyomavirus, 1864
rabies virus, 2445-2446
reovirus, 2168
retrovirus, 2553-2556, 2554f, 2581-2582
rotavirus, 2179, 2180f
RSV, 2408, 2409f
Staphylococcus aureus, 1120
Streptococcus pneumoniae, 1211-1212
Treponema pallidum, 1761
Trypanosoma brucei, 2968
Vibrio cholerae, 1550
Vibrio parahaemolyticus, 1556
VZV, 2022
West Nile virus, 2261, 2262f
Yersinia spp., 1515-1516
Gentamicin, 3208-3211, 3208t, 3231t-3234t
in burn wound infection, 1059
in coagulase-negative staphylococcal infection, 1138-1139
in meningitis, 450t
in neonatal conjunctivitis prevention, 1281
Germ theory of disease, 103
German measles. *See* Rubella virus infection
Gerstmann-Sträussler-Scheinker syndrome, 2620-2621, 2627t
Gianotti-Crosti syndrome (papular acrodermatitis), 649, 749, **766**, **783**, 783f
in HAV infection, 2140
Giant cells, in myocarditis, 383-384
Giardia intestinalis infection, 602t-603t, 607t, 614, 619
treatment of, 625
Giardia lamblia, 2878-2879
Giardia lamblia infection (giardiasis), **2878-2883**
clinical manifestations of, 757t-758t, 2881-2882, 2881t

Giardia lamblia infection (giardiasis) *(Continued)*
in daycare facilities, 3556
diagnosis of, 2882
epidemiology of, 2879
in immunocompromised hosts, 2881
pathogenesis of, 2879-2881, 2880f
retinal manifestations of, 814
treatment of, 2882-2883, 2883t, 3327t-3346t
Gilbert syndrome, 657
Gingiva, 146-147, 147f
inflammation of, 147, 147f
linear erythema of, 149
Gingivitis, 147, 147f
HIV (linear gingival erythema), 149
Gingivostomatitis, HSV, 154-155, 1940-1941, 1940f, 1959
Glanzmann thrombasthenia, 53-54
Glaucoma
vs. conjunctivitis, 806
in rubella, 818, 2213, 2214t-2216t, 2221
surgery for, endophthalmitis after, 820
Glioma, brain stem, 505
Global health, **3081-3091**
definition of, 3081-3082, 3082t
future directions for, 3087-3090, 3088f-3089f
goals for, 3084, 3085f, 3085t
historical perspective on, 3082-3083
neglected tropical diseases in, 3084-3087, 3085t, 3086f-3087f
social factors in, 3084, 3084t
Glomerulonephritis
in human erythrovirus B19 (human parvovirus B19) infection, 1855
infective endocarditis and, 354-355
membranoproliferative, HBV infection and, 1919
in syphilis, 1767
in Whipple disease, 644
Gloves and socks syndrome, 741, 742t-748t, 1849
Glucagon, 82
pancreatic, 72
β-D-Glucan assay, 926, 3597-3599, 3598t
in *Aspergillus* spp. infection, 938, 967, 2716
in *Candida* spp. infection, 936-937, 970, 2744-2745
in *Fusarium* spp. infection, 2847-2848
Glucocorticoids, plasma, 81
Glucose
in arthritides, 731, 732t
blood, 72
CSF, 885-886
release of, 82
Glucose-6-phosphate dehydrogenase (G6PD) deficiency, 58-59
HAV infection and, 2138
HEV infection and, 2164-2165
Glucose tolerance test, 82
Glutamate, in meningitis, 435
Glutathione peroxidase deficiency, 58-59
Glutathione synthetase deficiency, 59
Glycerol, in bacterial meningitis, 453
Glyceryl monolaurate, 864-865
Glycocalyces, 1017
Glycogen storage disease type 1B, 54-55
Glycyrrhizin, in HEV infection, 2166
Gnathostoma spinigerum infection, 3327t-3346t
encephalitic, 494t-498t
meningeal, **481-484.** *See also* Meningitis, eosinophilic
ocular, 815
Gongylonema spp. infection, 3327t-3346t
Gonococcal infection/gonorrhea. *See Neisseria gonorrhoeae* infection
Gradenigo syndrome, 224
Graft-versus-host disease
vs. chronic bronchitis, 267-268
in hematopoietic stem cell transplantation, 933
Gram-negative bacilli, 1097t-1112t. *See also specific organisms and infections*
anaerobic, 1097t-1112t
Gram-negative cocci, 1097t-1112t. *See also specific organisms and infections*
Gram-positive bacilli, 1097t-1112t. *See also specific organisms and infections*
Gram-positive cocci, 1097t-1112t. *See also specific organisms and infections*
Gram stain
in *Actinomyces* spp. infection, 1823
in *Aerococcus* spp. infection, 1249
in bacterial vaginosis, 578
in burn wound infection, 1056f
in *Clostridium perfringens* infection, 1796
CSF, 442, 1035, 1035f
in *Escherichia coli* pneumonia, 1446
in *Francisella tularensis* infection, 1663
in *Haemophilus ducreyi* infection, 1687
in *Listeria monocytogenes* infection, 1332
in meningitis, 445, 1262-1263
in *Neisseria gonorrhoeae* infection, 1290, 1290f
in *Neisseria meningitidis* infection, 1262-1263
in *Nocardia* spp. infection, 1419-1420
in staphylococcal infection, 1114f
in urethritis, 532-533, 532f
Granulocyte colony-stimulating factor (G-CSF), 3362-3363
glucose metabolism and, 73
in *Streptococcus pneumoniae* infection, 1225-1226
in type 1 leukocyte adhesion deficiency, 52-53
Granulocyte-macrophage colony-stimulating factor (GM-CSF), 3362-3363
in burns, 1049
Granuloma
in *Candida* infection, 2740
in *Coccidioides* spp. infection, 2769, 2770f, 2773
in *Histoplasma capsulatum* infection, 2811-2813, 2826-2827, 2826f
in *Paracoccidioides* spp. infection, 2785
in *Schistosoma* spp. infection, 3063
in *Toxocara* infection, 814
in Whipple disease, 642
Granuloma inguinale, 583t, **1640-1644**
clinical manifestations of, 1641-1642, 1642f
diagnosis of, 1642-1643, 1642f
epidemiology of, 1640-1641
pathogenesis of, 1641
prevention of, 1643-1644
prognosis for, 1643
treatment of, 1643
Granuloma venereum. *See* Granuloma inguinale
Granulomatous disease. *See* Chronic granulomatous disease
Granzymes, 28
Gray baby syndrome, 3217
Grey Turner sign, 689-690
Griseofulvin, 3319-3320, 3319f, 3321t
in tinea capitis, 787
Ground-glass hepatocytes, 1919, 1919f
Group A β-hemolytic streptococcal infection. *See Streptococcus pyogenes* infection
Group B β-hemolytic streptococcal infection. *See Streptococcus agalactiae* infection
Group C β-hemolytic streptococcal infection, 1150-1152
clinical manifestations of, 1151
epidemiology of, 1151
treatment of, 1152
Group G β-hemolytic streptococcal infection, 1150-1152
clinical manifestations of, 1151
epidemiology of, 1151
treatment of, 1152
Growth hormone (GH), plasma, 81
Growth restriction, in rubella virus infection, 2213, 2214t-2216t
Guanarito virus infection, 2469, 2470t. *See also* Arenavirus infection
Guillain-Barré syndrome
vs. acute transverse myelitis, 524
vs. botulism, 1793
after *Campylobacter jejuni* infection, 1653-1654
EBV infection and, 2004
enterovirus infection and, 2088t, 2090-2097
HAV infection and, 2140
HBV vaccine and, 1926
vs. infant botulism, 1808
rubella virus infection and, 2212
Guinea worm infection, **3028**
Gumma, 1765-1766, 1768, 1788
Gypsy moth rash, 758t

H

HA-1A antiendotoxin therapy, in *Neisseria meningitidis* infection, 1264-1265, 1265t
HAART. *See* Highly active antiretroviral therapy (HAART)
HACEK infection, endocardial, 362, 367, 367t, 1823
Haemophilus aegyptius, 1688-1689
Haemophilus aegyptius infection, **1688-1690**
clinical manifestations of, 1689
epidemiology of, 1689
pathogenesis of, 1689
treatment of, 1689-1690
Haemophilus ducreyi, 1686-1687, 1686t
Haemophilus ducreyi infection, **1686-1688**
clinical manifestations of, 753t-756t, 1687
coinfection with, 1688
diagnosis of, 1687-1688
epidemiology of, 1686-1687
pathogenesis of, 1687
prevention of, 1688
treatment of, 1688
Haemophilus influenzae, 1666-1668, 1686t
antibiotic resistance of, 1668
capsular polysaccharides of, 1666t, 1667
capsule of, 19-20
carriage of, 1668-1669
cell wall antigens of, 1667-1668
Hap protein of, 6-7
Hia adhesin of, 6-7, 9f
IgA proteases of, 1668
lipo-oligosaccharide of, 1667-1668
lipopolysaccharide of, 19
polysaccharides of, 1672
serotypes of, 19
typing of, 1668

Haemophilus influenzae infection, **1665-1684**
antibiotic resistance of, 1668
anticapsular antibody in, 1671
bloodstream, 1670
bronchial
acute, 262, 263t
chronic, 269, 269t
cellular immune response in, 1672
clinical manifestations of, 1675-1677
complement in, 1672
in cystic fibrosis, 334
diagnosis of, 1677-1678
endocardial, 362
epidemiology of, 1673-1675
exanthem in, 753t-756t
genetic factors in, 1672
historical perspective on, 1665-1666
IgG subclass-specific antibody in, 1671-1672
immune response to, 1670-1673
invasive, 1676-1677
pathogenesis of, 1669-1670
treatment of, 1678-1679, 1678t
mastoid, 234, 235t
meningeal, 429-430, 430f, 450t-451t, 460-461, 1670, 1675. *See also* Meningitis, bacterial
mucosal, 1669, 1677
treatment of, 1678t, 1679
nasopharyngeal, 158, 162-163
non–type b, 1674, 1676-1677
nontypeable strains in, 1674-1675, 1677
in otitis media, 212-215, 212t, 226
pathogenesis of, 1668-1670
phagocytosis in, 1672-1673
pharyngeal, 158
in rhinosinusitis, 195, 196t
treatment of, 1678-1679, 1678t
antibiotic resistance in, 1668
vulvovaginal, 568
Haemophilus influenzae type b
capsule of, 19-20
antibody against, 1666, 1671
polysaccharides of, 1667
carriage of, 1668-1669
Haemophilus influenzae type b infection, **1665-1684**
age and, 1674
bloodstream, 1675
bone, 711, 717-718
clinical manifestations of, 1675-1677
in daycare facilities, 3561
diagnosis of, 1677-1678
epidemiology of, 1673-1674, 1673t, 1678t
in epiglottitis, 244-249, 259, 1675-1676
immunization against, 1679-1682, 3431-3433, 3432t
active, 1679-1681, 1680t
disease reemergence and, 1674
in epiglottitis prevention, 259
in meningitis, 429, 461
passive, 1681-1682
in pneumonia prevention, 285, 292
results of, 1666, 1682
invasive, 1669-1670
joint, 728-730, 729t, 732-733, 1676
meningeal, 429-430, 430f, 450t-451t, 460-461, 1670, 1675. *See also* Meningitis, bacterial
mucosal, 1669
neonatal, 1676
pericardial, 372-373, 378, 1676
in preseptal cellulitis, 798
prevention of, 1679-1682, 3249-3250
pulmonary, 1675
Haemophilus influenzae type b infection *(Continued)*
secondary, 1674
soft tissue, 778, 778f, 1676
treatment of, 1678-1679, 1678t
prophylactic, 1682
uvular, 165, 167
viral infection and, 1670
Haemophilus parainfluenzae, 1686t, 1690
Haemophilus parainfluenzae infection, **1690**
Hafnia alvei infection, **1534-1535**
Hair follicles, infection of, **776-777**
Hair shaft, white piedra of, 790-791
Halo sign, in *Aspergillus* spp. infection, 2712
Halofantrine, in malaria, 2942
Hamman sign, 419-420
Hand(s)
in Kawasaki disease, 1073-1074
in sporotrichosis, 791f
tinea nigra of, 790, 790f
Hand and foot syndrome, vs. osteomyelitis, 724
Hand-foot-and-mouth disease (coxsackievirus A16 infection), 2066, 2082-2083, 2082f-2083f, 2083t
in daycare facilities, 3555
epidemiology of, 2057
exanthem in, 742t-748t, 749, 762, 764
ocular manifestations of, 2074-2075
Hand hygiene, 3411
in *Clostridium difficile* infection prevention, 638
in influenza virus infection prevention, 2355
Hansenula spp. infection. *See Pichia* spp. infection
Hantavirus, 2496-2500, 2498f, 2499t-2500t
tissue culture for, 2498-2499
Hantavirus infection, **2496-2513**
clinical manifestations of, 2503-2507
complications of, 2507-2508
diagnosis of, 2509-2510
differential diagnosis of, 2509
epidemiology of, 2497f, 2501-2503, 2501f
exanthem in, 742t-748t, 749-750
geographic distribution of, 2501-2502
hemorrhagic fever with renal syndrome in, 2496, 2503-2504, 2504t, 2510
historical perspective on, 2496
hospital infection control in, 2511-2512
immunization against, 2511
incidence of, 2502-2503
nephropathia epidemica in, 2496, 2504-2505
noncardiogenic pulmonary edema in, 2509
pathogenesis of, 2508-2509
pathology of, 2508-2509, 2508f
prevalence of, 2502
prevention of, 2511-2512
pulmonary syndrome in, 2505-2507, 2506f, 2510
seasonal patterns of, 2502
transmission of, 2500
treatment of, 2510-2511
Hantavirus pulmonary syndrome, 2505-2507, 2506f, 2510
differential diagnosis of, 2509
treatment of, 2510-2511
Haplorchis spp. infection (heterophyiasis), **3057-3058**
Harry Eagle effect, 739
HAV. *See* Hepatitis A virus (HAV)
Haverhill fever, 1715-1716, 1718. *See also Streptobacillus moniliformis* infection (rat-bite fever)
HBV. *See* Hepatitis B virus (HBV)
HDV. *See* Hepatitis D virus (HDV)
Head and neck cancer, HPV and, 1880
Head and neck infection, **167-175**
Bacteroides spp., 1828
epidemiology of, 167-168
Fusobacterium spp., 1828
in infectious mononucleosis, 2004
infraorbital space, 149
masticator space, 142, 148-149, 149f
microbiology of, 173-175
parapharyngeal, **172-173**
peritonsillar, **168-170**, 168f, 170f
Porphyromonas spp., 1828
Prevotella spp., 1828
retropharyngeal, **170-172**, 170f
submandibular space, 142, 149-150, 150f
Headache, in brain abscess, 464
Health care worker, infection in, 3412-3413
Health care–associated infection, **3377-3419**
Acinetobacter spp., 1569
adenovirus, 1893, 3382
Burkholderia cepacia, 1595
in burn injury, 1061-1062, 3405
in cancer patients, 929
Clostridium difficile, 3382, 3401
CMV, 1972-1973, 3383
coronavirus, 3382
in cystic fibrosis, 3405-3406
in dialysis, 3406-3407
Ebola virus, 3401-3402
Enterococcus spp., 1172, 1174, 1186
enterovirus, 3384
gastrointestinal tract, 3382-3383
Haemophilus influenzae, 3385
HAV, 2135, 3384
in health care worker, 3412-3413
historical perspective on, 3377-3378
HSV, 3383
human erythrovirus B19, 3384
in immunocompromised host, 3404-3405
influenza virus, 2352, 3380-3382
in intestinal transplantation, 993
intravascular catheter–associated, 3385-3391, 3386t-3388t, 3387f. *See also* Catheter-associated infection
Lassa fever virus, 2470-2471
lice, 3385
in liver transplantation, 991, 993
in long-term care facilities, 3407
measles, 3383-3384
methicillin-resistant *Staphylococcus aureus*, 3398-3399
Moraxella catarrhalis, 1252
multidrug-resistant gram-negative bacilli, 3400
mumps, 3383-3384
Neisseria meningitidis, 3385
in neonate, 3402-3404
norovirus, 3382
pertussis, 3382
plague, 3401
prevention of, 1061-1062, 3407-3414, 3409t
antimicrobial stewardship programs in, 3414, 3414t
hand hygiene in, 3411
isolation precautions in, 3411-3412
occupational health policies in, 3412-3413
outbreak investigations in, 3408, 3411t
policies and procedures for, 3408-3411, 3410t
Standard Precautions in, 3379, 3380t, 3412

Health care–associated infection *(Continued)*
surveillance in, 3408
Transmission-Based Precautions in, 3379, 3381t, 3412
visitor policies in, 3412
prion, 2622-2623
Pseudomonas aeruginosa, 1594
respiratory virus, 3379-3382
Rickettsia rickettsii, 2651
rotavirus, 3382
RSV, 2427-2428, 3379
rubella, 3383-3384
Salmonella spp., 1493, 1507
scabies, 3385
Serratia marcescens, 1488-1489
smallpox, 3401
Stenotrophomonas maltophilia, 1608
Streptococcus agalactiae, 1155
Streptococcus pneumoniae, 3385
surgery-associated, 3395-3398, 3396t
transmission of, 3378-3379
tuberculosis, 3384-3385
urinary tract, 3393-3395
vancomycin-resistant enterococci, 3399-3400
ventilator-associated, 3391-3393
VZV, 2025, 2030, 3383
Yersinia enterocolitica, 1519
Hearing loss
adenovirus infection and, 1904
antibiotics and, 1664, 3206, 3211
audiometric tests for, 218
CMV infection and, 1975, 1980
in Kawasaki disease, 1082
mastoiditis and, 237
meningitis and, 452, 457-458, 1259
mumps and, 2402
otitis media and, 217-218, 222
rubella and, 2213, 2214t-2216t, 2220-2221
syphilis and, 1772
Heart
infection of. *See* Infective endocarditis; Myocarditis; Pericarditis
rubella-related defects of, 2214t-2216t, 2217, 2221
syphilis of, 1768
tuberculosis of, 1351
in Whipple disease, 644
Heart block
in infective endocarditis, 359
in myocarditis, 389, 392f, 396
Heart disease
acquired. *See* Kawasaki disease
chagasic. *See Trypanosoma cruzi* infection (trypanosomiasis)
congenital, infective endocarditis with, 351, 351t
Heart failure
in infective endocarditis, 356
in rheumatic fever, 414
Heart murmurs
vs. friction rub, 374
in infective endocarditis, 356
in myocarditis, 389, 395
in rheumatic fever, 409
Heart sounds
in myocarditis, 389
in pericarditis, 374
Heart transplantation, **945-956**
antibiotic prophylaxis in, 947
evaluation for, 946-947, 946t
HBV infection and, 1924
immunization after, 955-956
immunosuppression in, 946, 955, 955t
Heart transplantation *(Continued)*
infection after, 946t, 947-950
adenovirus, 955, 1904
Aspergillus fumigatus, 953
CMV, 950-952
EBV, 952-953
erythrovirus B19 (parvovirus B19), 954
HSV, 949, 1952
influenza virus, 954
Legionella pneumophila, 949
mediastinal, 948-949
Nocardia asteroides, 953-954
Pneumocystic jiroveci, 954
RSV, 949-950
sternal, 948-949
Streptococcus pneumoniae, 954, 1233
Toxoplasma gondii, 953
VZV, 954-955
mortality with, 945-946
Heat energy, 84
Heat therapy, in *Sporothrix schenckii* infection, 2835
Heat transfer, 84
Heavy metal poisoning, 604, 604t
Heggers solution (0.025%), in burn wound infection, 1059
Helicobacter pylori, 1692-1693, 1692f-1693f
enzymes of, 1693
Helicobacter pylori infection, **1691-1699**
abdominal pain and, 1694
cancer and, 1696-1698, 1697f
clinical manifestations of, 1694, 1697f
diagnosis of, 1695, 1695f, 1696t
epidemiology of, 1693-1694, 1694f
historical perspective on, 1691-1692
immunization against, 1698
inflammatory response in, 1692-1693, 1693f
pancreatitis and, 695
probiotics in, 3370
recurrence of, 1694, 1698
transmission of, 1693-1694
treatment of, 1696-1698, 1697t
Helium-oxygen therapy, in RSV infection, 2425
Helminth infection, 2862t, **3012-3029**. *See also specific helminth infections*
cutaneous manifestations of, 757t-758t, 761t, 763t
encephalitic, 494t-498t
host response to, 23-24
HTLV-1 infection and, 2571
meningeal. *See* Meningitis, eosinophilic
uveal, 814-815
Helper virus. *See* Hepatitis D virus (HDV) infection
Hemangioendothelioma, 657
Hematoma, spinal, vs. acute transverse myelitis, 523
Hematopoietic stem cell transplantation (HSCT)
in adult T-cell leukemia, 2568-2569
graft-versus-host disease in, 933
infection after, **933-945**
Absidia, 938
adenovirus, 941-942, 1903-1904
Aspergillus, 937-938, 2707-2711, 2713
bacterial, 935-936
BK virus, 1867, 1867f, 1869
Candida spp., 936-937
Clostridium difficile, 935
CMV, 939-940, 3520-3521
Cryptosporidium spp., 943-944
EBV, 940-941
epidemiology of, 933-934
Hematopoietic stem cell transplantation (HSCT) *(Continued)*
fungal, 936-939
HHV-6, 941, 2017
HSV, 939
human metapneumovirus, 943
human polyomavirus type I, 942
influenza virus, 942
Mucor, 938
mycobacterial, 935-936
parainfluenza virus, 943
phase I (pre-engraftment), 934, 934f
phase II (early post-engraftment), 934, 934f
phase III (late post-engraftment), 934, 934f
Pneumocystis jiroveci, 938-939
protozoal, 943-944
rhinovirus, 943
Rhizopus, 938
RSV, 942-943
staphylococcal, 1136
Streptococcus pneumoniae, 935, 1232-1233
Strongyloides stercoralis, 943
Toxoplasma gondii, 943
viral, 939-943
VZV, 941
mortality with, 933
mumps immunization and, 2406
in severe combined immunodeficiency, 915
vaccination after, 944
in Wiskott-Aldrich syndrome, 913
Hematospermia, in *Schistosoma* spp. infection, 3065
Hemochromatosis, 657, 673
Hemolysins
Aeromonas spp., 1540
Staphylococcus aureus, 17, 1115-1117
Streptococcus agalactiae, 1154
Streptococcus pyogenes, 1141
in urinary tract infection, 538
Vibrio parahaemolyticus, 1557-1558
Hemolysis, in congenital syphilis, 1770
Hemolytic anemia
in human erythrovirus B19 (human parvovirus B19) infection, 1849-1850, 1855
in infectious mononucleosis, 2002
in *Leptospira* spp. infection, 1746-1747
in *Mycoplasma pneumoniae* infection, 2682
rubella virus infection and, 2212
Hemolytic-uremic syndrome (HUS), 17
in adenovirus infection, 1901
in enterohemorrhagic *Escherichia coli* infection, 1452, 1454, 1463
in enterovirus infection, 2078
in *Salmonella* ser. Typhi infection, 1501
in *Shigella* spp. infection, 1481
Hemophagocytic lymphohistiocytosis (hemophagocytic syndrome), 181
EBV and, 1997, 2001, 2001f, 2014
fever of unknown origin in, 846-847
HHV-8 and, 2021
treatment of, 2014
Hemorrhage, splinter, in infective endocarditis, 357
Hemorrhagic fever(s)
arenaviral. *See* Arenavirus infection
bioterrorist, 3585
Crimean-Congo, **2531-2535**, 2532f-2533f
Dengue. *See* Dengue hemorrhagic fever/dengue shock syndrome
filoviral. *See* Ebola virus infection; Marburg virus disease (Marburg fever, Marburg hemorrhagic fever)

Hemorrhagic fever(s) *(Continued)*
hepatitis with, 656
Omsk, **2316**
Hemorrhagic fever with renal syndrome
in adenovirus infection, 1901
in hantavirus infection, 2503-2504, 2504t, 2510-2511
Hendra virus infection, 494t-498t, 500-501
HEPA (high-efficiency particulate air) filtration, in lung transplant–related *Aspergillus* infection prevention, 970
Hepadnavirus type 1. *See* Hepatitis B virus (HBV) infection
Heparin, in *Neisseria meningitidis* infection, 1265
Hepatic abscess. *See* Liver abscess
Hepatic portoenterostomy, cholangitis and, 658-659, 664-665
Hepatic steatosis, 657-658
Hepatitis, **648-658**
adenovirus, 655, 1902
vs. anoxic injury, 657
autoimmune, 657
HAV infection and, 2141
bacterial, 650, 651t, 656-657
clinical manifestations of, 648-650
CMV, **654**, 1977-1978
coxsackievirus, 2070-2072
drug-related, 651t, 657
echovirus, 2070-2072
enterovirus, **655**
erythrovirus B19 (parvovirus B19), 655, 1854
fungal, 651t, 657
granulomatous, fever of unknown origin in, 845
hemorrhagic fever virus, 656
herpes B virus, 655
HHV-6, 654, 2017
HHV-7, 654
HHV-8, 654
historical perspective on, 648
HSV, 653-654, 1949, 1954
infectious, 650-657
isoniazid-related, 1366-1368
laboratory tests in, 649-650
Lassa virus, 2473
leptospiral, 656
macrolide-related, 3214-3215
measles virus, 655
noninfectious, 648, 651t, 657-658
parasite, 651t, 657
patient history in, 648-649
physical examination in, 649
rash in, 649
rickettsial, 656-657
rubella virus, 655-656, 2213
Shigella spp., 1481
syphilitic, 1771
treponemal, 656
viral, 650-656, 651f-652f, 651t. *See also specific hepatitis viruses*
laboratory tests in, 649-650, 662
pancreatitis in, 693-694, 693t
patient history in, 648-649
physical examination in, 649
serum iron in, 78
VZV, 654
Hepatitis A virus (HAV), 2128-2129
classification of, 2128
genome of, 2128-2129, 2129f
Hepatitis A virus (HAV) infection, 650, 651t, **2128-2147**
age and, 2132-2133
atypical, 2139-2141, 2139t
Hepatitis A virus (HAV) infection *(Continued)*
autoimmune hepatitis after, 2141
cholestatic, 2140-2141
clinical manifestations of, 2137-2139
community-wide epidemics of, 2134
cutaneous manifestations of, 2140
in daycare facilities, 2134-2135, 3564
diagnosis of, 2138-2139
epidemiology of, 2129-2134, 2131f-2132f
extrahepatic manifestations of, 2140
foodborne, 2134-2136
fulminant, 2139-2141
geographic patterns of, 2130-2134, 2131f
historical perspective on, 648, 2128
in illicit drug users, 2135
immune response to, 2136-2137
immunization against, 2142-2146, 2142t, 2145t, 3433-3437, 3434t, 3436t
immunoglobulin prevention of, 2141-2142
in international adoptees and refugees, 3122-3124
laboratory abnormalities in, 2138
laboratory tests in, 649-650, 651f
in MSM population, 2134-2135
neonatal, 2130
neurologic manifestations of, 2140
nosocomial, 2135, 3384
pancreatitis with, 693-694
pathogenesis of, 2136
pathology of, 2136
postexposure prophylaxis in, 2142, 2144, 3436-3437, 3436t
in pregnancy, 2130, 2140
prevention of, 2141-2146, 3514-3515
race/ethnicity and, 2133, 2133f
relapsing, 2139
in schools, 2135
transfusion-related, 2135
transmission of, 2129-2130, 2130f, 2134
travel-related, 2134-2135, 2141, 3515
treatment of, 2141
vascular manifestations of, 2140
Hepatitis B core antigen (HBcAg), 1912, 1918f-1919f, 1919
Hepatitis B e antigen (HBeAg), 1912, 1915, 1918f
Hepatitis B surface antigen (HBsAg), 1912, 1918f
Hepatitis B virus (HBV), 1911-1915
genome of, 1912-1915
genotypes of, 1913, 1916
life cycle of, 1913-1914, 1914f
particles of, 1912f, 1915, 1918f
surface antigens of, 1912, 1912f
Hepatitis B virus (HBV) infection, 650-652, **1911-1927**
acute, 1917-1918, 1918f
chronic, 1915, 1918-1919, 1918f
epidemiology of, 1917
follow-up for, 1927, 1927t
in daycare facilities, 3564-3565
epidemiology of, 1916-1917
exanthem in, 649, 742t-748t, 749
extrahepatic manifestations of, 1919
fulminant, 1916, 1920
HDV infection and, 1929-1931, 1930f-1931f
hepatocellular carcinoma and, 1916
histopathology of, 1919-1920, 1919f
historical perspective on, 648
HIV infection and, 1923-1924
immune response to, 1915-1916
Hepatitis B virus (HBV) infection *(Continued)*
immunization against, 1917, 1917t, 1925-1927, 1925t, 3437-3441, 3440t
hepatocellular carcinoma and, 1916
in international adoptees and refugees, 3124-3125
after kidney transplantation, 1007, 1014
Knodell-Ishak score in, 1919-1920
laboratory tests in, 649-650, 652f
natural history of, 1917-1919
neonatal, 1915, 1917, 1925-1926
pancreatitis with, 693-694
pathogenesis of, 1913-1916, 1914f
prevention of, 1917, 1917t, 1924-1927, 1925t, 3515-3519, 3518t
HBIG in, 1924-1925, 3516-3517
household precautions in, 1927
in liver transplantation, 3517-3519
perinatal, 3517-3518, 3518t
postexposure prophylaxis in, 3517-3519, 3518t
after sexual exposure, 3518-3519, 3518t
in solid-organ transplantation, 1924
transmission of, 1917
treatment of, 1920-1923, 1923t, 1927
adefovir dipivoxil in, 1922
entecavir in, 1922-1923
interferon-α in, 1920-1922
lamivudine in, 1921-1922
polymerase inhibitors in, 1921
tenofovir disoproxil fumarate in, 1922
Hepatitis C virus (HCV), 2318-2319, 2318f
genotypes of, 2319-2320
Hepatitis C virus (HCV) infection, 652-653, **2317-2326**
in burn patient, 1053-1054
chronic, 2320
clinical manifestations of, 2320-2321
diagnosis of, 2321-2323, 2322f
encephalitic, 494t-498t
epidemiology of, 2319
exanthem in, 742t-748t, 749
extrahepatic manifestations of, 2320-2321
genetic factors in, 2323
historical perspective on, 648, 2318
immune response to, 2321
in international adoptees and refugees, 3124-3125
after kidney transplantation, 1007, 1014-1015
laboratory tests in, 649-650
pathogenesis of, 11, 16, 20, 2319
prevention of, 2324-2325, 3519-3520
severity of, 2321-2323
sexual transmission of, 2320
transmission of, 2320
IVIG-related, 3496
treatment of, 2323-2324
vertical transmission of, 2320
Hepatitis D antigen (HDAg), 1928-1929
Hepatitis D virus (HDV), 1928
Hepatitis D virus (HDV) infection, 653, **1927-1933**
clinical manifestations of, 1929-1931
diagnosis of, 1929
epidemiology of, 1928
HBV infection with, 1929-1931, 1930f-1931f
historical perspective on, 648
immune response to, 1928-1929
laboratory tests in, 649-650
pathogenesis of, 1928-1929
prevention of, 1932
treatment of, 1931-1932

Hepatitis E virus (HEV), 2159-2160
classification of, 2160
genome of, 2159, 2159f
genotypes of, 2160
life cycle of, 2160
structure of, 2159-2160
Hepatitis E virus (HEV) infection, 653, **2158-2167**
breast-feeding and, 2163
clinical manifestations of, 2164-2165, 2165f
diagnosis of, 2165-2166
endemic, 2160-2162, 2161t
epidemic, 2162
epidemiology of, 2160-2163
extrahepatic manifestations of, 2164-2165
foodborne, 2163
historical perspective on, 648, 2158
immune response to, 2163, 2164f
immunization against, 2166-2167
laboratory tests in, 649-650
mortality in, 2164
pancreatitis with, 693-694
pathogenesis of, 2163
in pregnancy, 2163-2164
prevention of, 2166-2167
transfusion-related transmission of, 2163
treatment of, 2166
vaccine against, 2166-2167
vertical transmission of, 2163
zoonotic, 2162
Hepatobiliary scintigraphy (HIDA scan)
in cholangitis, 661
in cholecystitis, 669
Hepatoblastoma, 657
Hepatocellular carcinoma, 1916
Hepcidin, 78
Hernia, inguinal, 687-688
Herpangina, 2065, 2066t
vs. HSV gingivostomatitis, 1941
Herpes B virus infection, 494t-498t, 655, 812
Herpes gladiatorum, 1945
Herpes labialis, 154-155, 1950, 1950f, 1952-1953, 1952f
Herpes simplex virus (HSV), 1933-1934
antibodies against, 1957
detection of, 1955-1958, 1956f-1957f
electron microscopy of, 1956, 1957f
gel electrophoresis of, 1957-1958, 1958f
immediate early genes of, 20
latency of, 20, 1938
lytic replication cycle of, 20
Herpes simplex virus (HSV) infection, **1933-1968**
abrasion-related, 1944, 1944f
acyclovir-resistant, 1963-1964
anorectal, 1948-1949
burn-related, 1053-1054, 1944, 1944f, 1953
clinical manifestations of, 742t-748t, 1940-1955
CNS, 494t-498t, 499, 505, 1945-1948, 1946t, 1947f-1948f, 1949t
acute retinal necrosis after, 1945
in immunocompromised host, 1954
pathogenesis of, 1939
vs. tick-borne encephalitis virus infection, 2310-2311
treatment of, 1960
complications of, 1958
congenital, 818-819, 881, 1954
ocular manifestations of, 818-819
pathogenesis of, 1939-1940
conjunctival, 803-804
neonatal, 805-806, 805t
Herpes simplex virus (HSV) infection *(Continued)*
corneal, 803-804, 1945, 1959
in daycare facilities, 3560
diagnosis of, 1955-1958, 1956f-1957f
digital, 1944-1945, 1944f-1945f
disseminated, 1954
epidemiology of, 1935-1938
in epiglottitis, 245
erythema multiforme and, 764
esophageal, 594-597, 596f, 1948-1949, 1953
external auditory canal, 208
fetal, 1954-1955
gastrointestinal tract, 1948-1949
genital, 572t-573t, 582, 583t, 742t-748t, 1941-1943
clinical manifestations of, 1941-1943, 1942f
complications of, 1943
epidemiology of, 1936
psychological aspects of, 1943
recurrent, 1942f, 1950-1951, 1966-1967
treatment of, 1960-1961, 1960t
after heart transplantation, 949
after hematopoietic stem cell transplantation, 939
hepatic, 653-654, 1949
in HIV-infected host, 1943, 1952-1953, 1953f
immune response to, 1938-1939
in immunocompromised host, 1951-1955, 1951t, 1952f-1953f, 1961-1962, 1961t
after kidney transplantation, 1006-1007
labial, 1950, 1950f, 1952-1953, 1952f
after liver and intestinal transplantation, 998-999
after lung transplantation, 978
meningeal, 487, 1943, 1946
mucocutaneous, 1943-1945, 1943f-1944f, 1953
recurrence of, 1950
treatment of, 1961-1962
neonatal, 1935, 1954-1955
CNS, 1955
disseminated, 1954-1955
epidemiology of, 1936-1938
hepatic, 653-654
pathogenesis of, 1939-1940
prevention of, 1964-1966, 3522
recurrent, 1967
treatment of, 1962-1963
nosocomial, 3383
ocular, 810, 1945
treatment of, 1959
oral, 154-155, 742t-748t, 1940-1941, 1940f
in HIV infection, 149
recurrent, 1950, 1950f
treatment of, 1959
pathogenesis of, 1938-1940
pharyngeal, 159t-160t, 161, 1941
during pregnancy, 1936-1937, 1965, 1967
prevention of, 1964-1967, 3522
prognosis for, 1958
pulmonary, 1953-1954
recurrent, 1949-1951, 1950f
erythema multiforme with, 1951
pathogenesis of, 1938-1939
sequelae of, 1958
sports-related, 1945
transmission of, 1934-1935
treatment of, 1958-1964, 3279-3280, 3282-3283, 3288
acyclovir resistance and, 1963-1964
prophylactic, 1966-1967
Herpes simplex virus (HSV) infection *(Continued)*
renal dysfunction and, 1961, 1961t
susceptibility testing in, 1958
vaccine against, 1966
Herpes zoster, 742t-748t, **2021-2033**
clinical manifestations of, 2027-2028, 2027f
complications of, 2027-2028
diagnosis of, 2028
epidemiology of, 2023-2024
immunization against, 2023-2024
pathogenesis of, 2024-2025
prevention of, 2030-2032
prognosis for, 2029-2030
risk factors for, 2023-2024
after solid-organ transplantation, 979
transmission of, 2023
treatment of, 2028-2029
Herpes zoster ophthalmicus, 804, 810
Herpes zoster oticus, 208, 208f
Herpes zoster uveitis, 810
Herpesvirus simiae. *See* Herpes B virus infection
Herpetic whitlow, 155, 1944-1945, 1944f-1945f
Heterophyes spp. infection (heterophyiasis), **3057-3058**
Hexachlorophene, toxicity of, 1121
HHV-6 infection. *See* Human herpesvirus–6 (HHV-6) infection
HHV-7 infection. *See* Human herpesvirus–7 (HHV-7) infection
HHV-8 infection. *See* Human herpesvirus–8 (HHV-8) infection
Hia adhesin, 6-7, 9f
Hidradenitis suppurativa, **777**
High-altitude illness, 3118
Highlands J virus, 2231
Highly active antiretroviral therapy (HAART), 600, 2598
immune reconstitution inflammatory syndrome and, 1360-1361, 2601
postexposure, 2594
Higouménakis sign, 1772
Hip
dislocation of, 733
septic arthritis of, 730-732, 730t, 731f
toxic (transient) synovitis of, 732
tuberculosis of, 1355
Hippocampus, in bacterial meningitis, 435-436
Hirschsprung disease, *Clostridium difficile* infection and, 634
Histo spots, 813
Histocompatibility leukocyte antigen (HLA), in acute disseminated encephalomyelitis, 517
Histoplasma capsulatum, 2807-2808, 2808f
Histoplasma capsulatum infection (histoplasmosis), **2807-2832**
clinical manifestations of, 2811-2817, 2812t
CNS, 476-477, 480t, 494t-498t, 2811, 2816
treatment of, 2828
CT in, 2817, 2818f
cutaneous, 757t, 2813
diagnosis of, 2819-2824, 2819t-2820t
antigen test in, 2822-2823
complement fixation test in, 2821-2822
culture in, 2820-2821
histology in, 2819-2820, 2819f
immunodiffusion test in, 2822
molecular methods in, 2823-2824
serology in, 2821-2823
skin test in, 2823

Histoplasma capsulatum infection (histoplasmosis) *(Continued)*
disseminated, 2813-2817
in HIV-infected patient, 2602, 2815-2816, 2827-2828
in immunocompromised host, 2814-2815
in immunosuppressed host, 2827
in infant, 2814
treatment of, 2827
epidemiology of, 2808-2809
gastrointestinal tract, 2815
granuloma in, 2811-2813
immune response to, 23, 2810-2811
after intestinal transplantation, 1000
joint, 2828-2829
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 973-974
mediastinal, 422, 2826-2827, 2826f
mediastinal fibrosis in, 2813, 2817-2819
meningeal, 476-477, 480t, 2816, 2821, 2828
ocular, 813, 2816-2817
pathology of, 2811
pathophysiology of, 2809-2811
pericardial, 373, 377-378, 2813, 2828-2829
pleural, 309-310
prevention of, 2829-2830
prognosis for, 2829
pulmonary, 2812-2813
cavitary, 2813
treatment of, 2826
radiography in, 2817-2819, 2818f
in traveler, 3116-3117
treatment of, 2824-2829, 2824t, 3295t
surgical, 2829
Histoplasma capsulatum var. *duboisii*, 2808, 2817
HIV-1. *See* Human immunodeficiency virus type 1 (HIV-1)
HIV-2. *See* Human immunodeficiency virus type 2 (HIV-2)
HIV infection. *See* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS)
hMPV infection. *See* Human metapneumovirus (hMPV) infection
H1N1 influenza. *See* Influenza virus infection, H1N1
Hodgkin disease, 182-183, 182t
EBV and, 1997, 2005
reovirus and, 2171
Holocyclotoxin, 3071
Honey, infant botulism and, 1804-1805
Hookworm infection, **3016-3018**
clinical manifestations of, 3017-3018
diagnosis of, 3018
epidemiology of, 3017
pathophysiology of, 3017
prevention of, 3018
treatment of, 3018, 3327t-3346t
Hordeolum, **796**
Hormones
adrenal gland, 81-82
carbohydrate-regulating, 82
measurement of, 81-82
pituitary gland, 81
thyroid gland, 82
Hospitalization
infection during. *See* Health care–associated infection
normal flora effects of, 89-90
otitis media and, 212
Hot-foot syndrome, 1589-1591
Hot-tub syndrome, 776-777, 1589-1591
Howell-Jolly bodies, 906
HPV. *See* Human papillomavirus (HPV)
HSCT. *See* Hematopoietic stem cell transplantation (HSCT)
HSV. *See* Herpes simplex virus (HSV)
Human bite, 780, 780t, 3573t
clinical manifestations of, 3574-3575
diagnosis of, 3575-3577
epidemiology of, 3571
microbiology of, 3572, 3573t
treatment of, 3251, 3575-3577, 3576t
Human bocavirus (HBoV), 1858
historical perspective on, 1858
Human bocavirus (HBoV) infection, **1858-1863**
in common cold, 132
diagnosis of, 1861
epidemiology of, 1858-1859
exanthem in, 741, 742t-748t, 1861
gastrointestinal tract, 1860-1861
in immunocompromised host, 1861
Kawasaki disease and, 1861
pathogenesis of, 1859
pulmonary, 284, 1860
respiratory tract, 1859-1860
treatment of, 1861
Human erythrovirus B19 (human parvovirus B19), 1844-1845
historical perspective on, 1843-1844
Human erythrovirus B19 (human parvovirus B19) infection, **1843-1857.** *See also* Erythema infectiosum
aplastic crisis in, 1849-1850
clinical manifestations of, 1847-1855, 1847f, 1848t
CNS, 1853
in daycare facilities, 3554-3555
diagnosis of, 1855-1856
differential diagnosis of, 1855
epidemiology of, 1845-1846
erythema multiforme bullosum in, 1849
exanthem in, 741, 1847-1849, 1847f-1849f
fetal, 1847, 1852-1853, 1856
genotypes of, 1843
glove and sock syndrome in, 1849
after heart transplantation, 954
hematologic manifestations of, 1849-1850
hepatic, 655, 1854
historical perspective on, 1843-1844
immune response to, 1846
in immunocompromised host, 1847, 1851-1852, 1856
joint manifestations of, 1851
lymphadenitis in, 1854-1855
myocardial, 1853-1854, 1856
nosocomial, 3384
ocular, 811
pancytopenia in, 1850
pathogenesis of, 1846-1847, 1846f
prevention of, 1856-1857, 3527
prognosis for, 1856
red cell aplasia and, 1854
renal, 1855
thrombocytopenia in, 1850
transmission of, 1845-1846
treatment of, 1856
uveal, 811
Human fibroblast–derived interferon, in adenovirus infection, 1908
Human granulocytic anaplasmosis, **2661-2663**
Human herpesvirus–6 (HHV-6), 2016, 2017f
isolation of, 2019
Human herpesvirus–6 (HHV-6) infection, **2016-2019.** *See also* Roseola infantum
clinical manifestations of, 2016-2018
congenital, 2018
diagnosis of, 2019
encephalitic, 494t-498t, 499
epidemiology of, 2016
exanthem in, 742t-748t, 749, 765, 2017, 2017t
after hematopoietic stem cell transplantation, 941, 2018
hepatic, 654, 2017
after kidney transplantation, 1010, 2017
after liver transplantation, 999
after lung transplantation, 979
meningeal, 487, 2018
neurologic complications of, 2018
pathogenesis of, 2018-2019
posttransplantation acute limbic encephalitis and, 2018
treatment of, 2019
Human herpesvirus–7 (HHV-7) infection, **2016-2019.** *See also* Roseola infantum
clinical manifestations of, 2018
exanthem in, 742t-748t, 749
hepatic, 654
after kidney transplantation, 1010
after lung transplantation, 979
meningeal, 487, 2018
neurologic complications of, 2018
pathogenesis of, 2018-2019, 2019f
after transplantation, 999
Human herpesvirus–8 (HHV-8) infection, **2019-2021**
clinical manifestations of, 2020
diagnosis of, 2021
exanthem in, 742t-748t, 749
hemophagocytic lymphohistiocytosis and, 2021
hepatic, 654
after kidney transplantation, 1010
after lung transplantation, 979
transmission of, 2020-2021
after transplantation, 999
treatment of, 2021
Human immune globulin. *See* Immunoglobulin(s)
Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS), **2591-2619.** *See also* Retroviruses
acute retroviral syndrome in, 161
anemia in, 2607-2608
Bacille Calmette-Guérin vaccine complications in, 1377
BK virus infection in, 1867
Blastomyces dermatitidis infection in, 2729-2730
in burn patient, 1054
Campylobacter jejuni infection in, 1651
Candida infection in, 148-149, 474, 2602
caries in, 149
cervical lymphadenitis in, 177-178
cholangitis in, 666
clinical manifestations of, 2598-2603, 2598t
CMV infection in, 811, 1973, 1979-1980, 2601-2602
Coccidioides spp. infection in, 2775-2776
complications of, 2603-2608
cardiac, 2607
CNS, 2604-2605
gastrointestinal, 2605-2606
hematologic, 2607-2608
neurocognitive, 2605

Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS) *(Continued)*
oncologic, 2606-2607
psychosocial, 2605
pulmonary, 2603-2604, 2603f
renal, 2607
Cryptococcus infection in, 476, 2602, 2798
Cryptosporidium spp. infection in, 614-615, 2897-2898, 2901, 2903
in daycare facilities, 3560
definition of, 2591-2592
diagnosis of, 2608-2609, 2608t
EBV infection in, 2006-2007, 2014
epidemiology of, 2591-2595, 2592t
exanthem in, 742t-748t, 749-750
gastrointestinal tract infection in, 599-600, 600t
gonococcal infection and, 1296
Haemophilus ducreyi infection in, 1688
Hafnia alvei infection in, 1534-1535
HBV infection in, 1923-1924
Histoplasma capsulatum infection in, 476-477, 2602, 2815-2816, 2827-2828
HSV infection in, 1943, 1952-1953, 1953f, 2602
HTLV-1 infection and, 2577
immune dysfunction in, 2596-2598, 2597f
immune reconstitution inflammatory syndrome and, 1360-1361
in international adoptees and refugees, 3125
IVIG in, 3525-3526
Kaposi sarcoma in, 654, **2019-2021**, 2606
Klebsiella granulomatis (Calymmatobacterium granulomatis) infection in, 1643
leiomyoma in, 2606-2607
leiomyosarcoma in, 2606-2607
Leishmania spp. infection in, 2949, 2956-2957
linear gingival erythema in, 149
lymphocytic interstitial pneumonitis/hyperplasia in, 296, 1997, 2007
lymphoid interstitial pneumonitis in, 268
lymphoma in, 2006-2007, 2013
microsporidial infection in, 615
molluscum contagiosum in, 782-783, 795
monitoring of, 2609
mumps immunization in, 2406
mycobacterial infection in, 684
Mycobacterium avium-intracellulare complex infection in, 1384-1385, 2600-2601
Mycobacterium leprae infection in, 1403
Mycobacterium ulcerans infection in, 1415
myocarditis in, 398
neutropenia in, 2607
non-Hodgkin lymphoma in, 2606
nontuberculous mycobacterial infection in, 1384-1385
ocular manifestations of, 811
oral hairy leukoplakia in, 2007
oral manifestations of, 148-149
osteomyelitis in, 724
pancreatitis in, 692, 694
Paracoccidioides spp. infection and, 2787
parotid gland enlargement in, 191-192
pathogenesis of, 2595-2598, 2596f
chemokine receptors in, 10-11
pleural effusion in, 310
Pneumocystis infection in, 2599-2600, 2599f, 2600t, 3007-3009
postexposure prophylaxis in, 2594
Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS) *(Continued)*
probiotics in, 3372
prognosis for, 2617
progressive multifocal leukoencephalopathy in, 1869
Pseudomonas aeruginosa infection in, 1595
recalcitrant erythematous desquamating disorder and, 860
Rhodococcus equi infection and, 1427-1428
RSV infection in, 2421
selenium deficiency in, 79
Shigella spp. infection in, 1482
sinopulmonary infection in, 268
Sporothrix schenckii infection in, 2834
staging of, 2591-2592, 2592t
Streptococcus pneumoniae infection and, 1204-1205, 1232
syphilis and, 1762, 1778
Toxoplasma gondii infection in, 2993, 3003-3004
transmission of, 2592-2595
perinatal, 2593-2594
sexual, 2594
transfusion-related, 2592-2593
transplant-related, 1015
treatment of, 600, 2609-2617, 2610t, 2611f
immune-based, 2616
immune reconstitution inflammatory syndrome and, 1360-1361
integrase inhibitors in, 2615-2616
non-nucleoside reverse transcriptase inhibitors in, 2613-2614
nucleoside reverse transcriptase inhibitors in, 2612-2613, 2612f
nucleotide reverse transcriptase inhibitors in, 2613
protease inhibitors in, 2614-2615
supportive, 2616
Trichomonas vaginalis infection and, 2890
Trypanosoma cruzi infection in, 2965
tuberculosis in, 1336, 1339, 1360, 2601
vaccine against, 2616-2617
varicella immunization in, 2032
VZV infection in, 2026, 2602
Human immunodeficiency virus type 1 (HIV-1), 2581t, 2595, 2595f. *See also* Human immunodeficiency virus (HIV) infection/acquired immunodeficiency syndrome (AIDS); Retroviruses
HIV-2 infection and, 2583-2584
Human immunodeficiency virus type 2 (HIV-2), 2581-2582, 2581t. *See also* Retroviruses
Human immunodeficiency virus type 2 (HIV-2) infection, **2580-2588**
diagnosis of, 2585-2586
epidemiology of, 2582-2583
HIV-1 infection and, 2583-2584
monitoring of, 2588
natural history of, 2585-2586
perinatal transmission of, 2584-2585
sexual transmission of, 2584
transmission of, 2584-2585
treatment of, 2586-2588
Human leukocyte antigen (HLA)
in granuloma inguinale, 1641
in human erythrovirus B19 (human parvovirus B19) infection, 1845
in *Mycobacterium leprae* infection, 1395
in rheumatic fever, 406
in rubella virus infection, 2204
in Whipple disease, 641
Human metapneumovirus (hMPV), 2435, 2435f
strain variation in, 2436
Human metapneumovirus (hMPV) infection, **2434-2443**
asthma and, 2439
clinical manifestations of, 2437-2439
CNS, 494t-498t, 2439
coinfection with, 2438-2439
in common cold, 132
diagnosis of, 2440
differential diagnosis of, 2439-2440
epidemiology of, 2435-2437
exanthem in, 742t-748t, 749
geographic distribution of, 2435
after hematopoietic stem cell transplantation, 943
historical perspective on, 2434-2435
immune response to, 2437
in immunocompromised host, 2439
incidence of, 2436-2437
lower respiratory tract, 2438-2439, 2438t, 2439f
after lung transplantation, 980
otitis media and, 2438-2439
pathogenesis of, 2437
pathology of, 2437
prevalence of, 2436-2437
prevention of, 2440
prognosis for, 2440
pulmonary, 284, 285t, 2438-2439, 2438t, 2439f
seasonal pattern of, 2436
Streptococcus pneumoniae immunization and, 1230
treatment of, 2440
upper respiratory tract, 2438
vaccine against, 2440
Human monocytic ehrlichiosis, **2661-2663**
Human papillomavirus (HPV), 1871-1873, 1872t-1873t
Human papillomavirus (HPV) infection, **1871-1887**
anal cancer and, 1873t, 1875, 1880-1881
anogenital, 572t-573t, **580-581**, 580f-581f, 1873t, 1876-1877
colposcopy in, 1882
differential diagnosis of, 1877
epidemiology of, 1874-1876
female-specific, 1877-1878
male-specific, 1878
Pap smear for, 581, 1881-1882
cancer and
female-specific, 1873t, 1874-1875, 1877-1878
male-specific, 1873t, 1875-1876, 1878
clinical manifestations of, 1876-1881
conjunctival, 1879-1880
cutaneous, 742t-748t, 1873t, 1876
cytology of, 1881-1882
diagnosis of, 1881-1882
DNA assays in, 1882
epidemiology of, 1874-1876
gastrointestinal, 1880-1881
histology of, 1882
historical perspective on, 1871
immunization against, 1884-1886, 3441-3443
bivalent vaccine in, 1885-1886
quadrivalent vaccine in, 1884-1886
after kidney transplantation, 1010
molecular assay in, 1882
nasal, 1879
oropharyngeal cancer and, 1875-1876, 1880

Human papillomavirus (HPV) infection *(Continued)*
prevention of, 1884-1886
bivalent vaccine in, 1885-1886
quadrivalent vaccine in, 1884-1886
respiratory, 1878-1879, 1878f-1879f
surgical treatment of, 1882-1883
treatment of, 1882-1884
Human parvovirus B19 infection. *See* Human erythrovirus B19 (human parvovirus B19) infection
Human polyomaviruses. *See* BK virus; JC virus; Simian virus 40 (SV40)
Human T-cell lymphotropic virus type 1 (HTLV-1) infection, **2560.** *See also* Retroviruses
adult T-cell leukemia and, 2565-2569, 2566t-2567t
arthritis and, 2575-2576
bronchiectasis and, 2576
disease associations of, 2561t, 2564-2565
epidemiology of, 2560-2577
exanthem in, 742t-748t, 749-750
helminthic coinfection with, 2571
HIV-1 infection and, 2577
infective dermatitis and, 2574-2575, 2574t
lymphocytic pneumonitis and, 2576
myelopathy and, 2569-2573, 2570t
ocular, 811
parenteral transmission of, 2564
polymyositis and, 2576
sexual transmission of, 2562
Sjögren syndrome and, 2576
transmission of, 2562-2564
uveal, 2573-2574
vertical transmission of, 2562-2564
Human T-cell lymphotropic virus type 2 (HTLV-2) infection, **2577-2580.** *See also* Retroviruses
clinical manifestations of, 2579-2580
epidemiology of, 2578
pathogenesis of, 2577-2578
transmission of, 2579
Trypanosoma cruzi infection and, 388-389
Human T-cell lymphotropic virus type 3 (HTLV-3) infection, 2580
Human T-cell lymphotropic virus type 4 (HTLV-4) infection, 2580
Humerus, *Streptococcus agalactiae* infection of, 1161, 1161f
Hyaluronate lyase, of *Streptococcus pneumoniae*, 1211
Hyaluronidase, 17-18
of *Streptococcus pneumoniae*, 1214
Hydatid disease. *See Echinococcus granulosus* infection (echinococcosis, hydatid disease)
Hydrocephalus
in meningitis, 438, 445, 1260
otitic, 237
Hydrogen peroxide, in glutathione synthetase deficiency, 59
Hydrophobia, 2450
Hydrops fetalis, 1847, 1852, 1856, 2692
Hydrosalpinx, 589, 589f
Hydroxychloroquine, in interstitial lung disease, 300
17-Hydroxycorticosteroids, plasma, 81
Hygiene. *See also* Infection control practices
in *Clostridium difficile* infection prevention, 637-638
in CMV infection prevention, 1989
in HSV infection prevention, 1964-1966
infection and, 109-110
Hygiene *(Continued)*
in influenza virus infection prevention, 2355
in *Leptospira* spp. infection prevention, 1757
perineal, vulvovaginitis and, 564-566
in *Salmonella* spp. infection prevention, 1507
Hygroma, cystic, 182
Hylesia alinda (moth), 758t
Hymenolepis diminuta (rat tapeworm) infection, **3042**
Hymenolepis nana (dwarf tapeworm) infection, **3042**, 3327t-3346t
Hyper-IgE syndrome, **55**, 919
furunculosis and, 777
lung abscess and, 317
Hyper-IgM syndrome, **49-50**, 907t, **909-910**
vs. chronic bronchitis, 267
Cryptosporidium spp. infection and, 2900
Hyperbaric oxygen therapy
in gas gangrene, 1799
in mucormycosis, 2844
in necrotizing fasciitis, 150
Hyperbilirubinemia, differential diagnosis of, 662-663, 662t, 669
Hypercholesterolemia, after HIV infection treatment, 2606-2607
Hypercobaltemia, 77t
Hypercupremia, 77t
Hyperferremia, hepatitis-induced, 78
Hypergallemia, 77t
Hyperglycemia, 72
in septic shock, 829, 832
Hyperimmunoglobulinemia D with periodic fever syndrome, 847
Hyperimmunoglobulinemia E, **55**, 317, 919
Hyperinfection syndrome, *Strongyloides stercoralis*, 615-616
Hypermagnesemia, 77t
Hyperpigmentation, in Whipple disease, 644
Hyperreactive malarial syndrome, 2929
Hypersensitivity pneumonitis, 297, **301-305**
clinical presentation of, 301-303
diagnosis of, 303-304, 303f-305f
etiology of, 301, 302t-303t
forms of, 301
pathogenesis of, 301
prognosis for, 305
treatment of, 304-305
Hypersensitivity reaction
animal serum–related, 3493
cephalosporin-related, 3199
chloramphenicol-related, 3217
lincomycin-related, 3222
penicillin-related, 3189-3191, 3189t
TMP-SMX–related, 3229
Hypersideremia, 77t
Hypertriglyceridemia, 74
Hyperventilation, in septic shock, 831
Hyperzincemia, 77t
Hyphal wall protein 1 (Hwp1), 9
Hypoaminoacidemia, 70
Hypocalcemia
in septic shock, 829-830, 832
in toxic shock syndrome, 829-830
Hypochromia, 77t
Hypoferremia, 78
Hypogammaglobulinemia, transient, of infancy, **47**, **912**
Hypoglycemia, 72
in cholera, 1551
in malaria, 2928
in newborn, 72
in septic shock, 829, 832
Hypokalemia, in *Leptospira* spp. infection, 1747
Hyponatremia, 76
in *Leptospira* spp. infection, 1753
in septic shock, 832
Hypopyon, 809-810
Hyposideremia, 77t, 78
Hypotension
in chronic fatigue syndrome, 1088-1089
in *Leptospira* spp. infection, 1748
Hypothalamic-pituitary-adrenal axis, in chronic fatigue syndrome, 1088
Hypothesis testing, 120-121. *See also* Biostatistics
Hypoxemia, in community-acquired pneumonia, 287-288, 287t
Hypoxia, in *Leptospira* spp. infection, 1747
Hypozincemia, 77t, 79

I

Ibuprofen
vs. acetaminophen, 87-88
acetaminophen with, 87
in fever, 87
in otitis media, 228
Ichthyosis, PMN chemotaxis in, 56
IcsA protein, 16
IcsP protein, 16
Id reaction, 784-785, 785f
Ig. *See* Immunoglobulin(s)
Igbo-ora fever, 2251
IGIM. *See* Immunoglobulin(s), intramuscular (IGIM)
IGIV. *See* Immunoglobulin, intravenous (IVIG, IGIV)
IKBKG gene defect (NEMO defect), 916, 916f
Ilheus virus infection, 2316t
Imipenem, in meningitis, 448
Imipenem-cilastatin, 3200, 3231t-3233t
Imiquimod
in genital HPV infection, 581
in papillomas, 1883
Immotile cilia syndrome
vs. chronic bronchitis, 267
lung abscess and, 317
rhinosinusitis and, 195
Immune complexes
in infective endocarditis, 354-355, 358
in *Streptococcus agalactiae* infection, 1158
Immune globulin. *See* Immunoglobulin(s)
Immune reconstitution inflammatory syndrome (IRIS)
cryptococcosis and, 972, 2803-2804
HAART and, 1360-1361, 2601
tuberculosis and, 1360-1361
Immunity
adaptive, 35-36, 36f
antigen-presentation in, 36-38, 37f
B cells in, 40-42
in burns, 1050
CD1 antigen-presenting molecules in, 37-38
class I MHC in, 36-37, 38f
class II MHC in, 37, 38f
complement in, 31
deficiencies of, 46-48. *See also specific immunodeficiency disorders*
dendritic cells in, 38
immunoglobulins in, 40-41, 41f
pathogen evasion of, 19
T cells in, 38-40

Immunity *(Continued)*
concept of, 103
deficiencies in, **45-59.** *See also* Immunodeficiency disorders *and specific disorders*
fever effects on, 85
innate, 24-42, 35f
chemokines in, 27-28
complement in, **28-31**, 29f
cytokines in, 25-27, 26t
defensins in, 24-25
epithelium in, 24-25
natural killer cells in, 28
phagocytes in, 31-35, 32f-34f, 34t
Toll-like receptors in, 25, 25t
virus evasion of, 20
intestinal flora effects on, 93
in newborn, 42-45, 43f, 43t-44t
Immunization, **3420-3490.** *See also* Vaccine(s)
acute disseminated encephalomyelitis with, 516-517, 516t
adenovirus, 1908
anthrax, 1315-1316
arenavirus, 2476-2477
cholera, 1554-1555
Clostridium botulinum, 1795
CMV, 1989
combination vaccines for, 3476-3477, 3476t
contraindications to, 3426
Corynebacterium diphtheriae, 1308-1309
in cystic fibrosis, 344-345
dengue virus, 2289
diabetes mellitus and, 3428-3429
in DiGeorge syndrome, 913
dose for, 3422
DTaP/Tdap/DTP, 1630-1636, 3430-3431, 3457-3461
acellular vaccines for, 1630-1633, 1631t-1633t, 3458, 3458t
adverse events with, 1634-1635, 1635t
adverse events with, 1633-1635, 3430-3431, 3459
contraindications to, 1636, 3460-3461
disease incidence and, 1620f
efficacy of, 3459
encephalitis after, 502
failure of, 1620-1621
indications for, 3460
neurologic illness with, 3459
precautions for, 3460-3461
schedules for, 1635-1636
tolerance and, 44
whole-cell vaccines for, 1630, 3458
adverse events with, 1633-1634
efficacy of, 3420, 3421t
encephalitis after, 502
in endocarditis prevention, 369
group A streptococci, 406-407, 407f
Haemophilus ducreyi, 1688
Haemophilus influenzae type b, 461, 1679-1682, 3431-3433, 3432t
active, 1679-1681, 1680t
disease reemergence and, 1674
in epiglottitis prevention, 259
in meningitis, 429, 461
in meningitis prevention, 429
passive, 1681-1682
in pneumonia prevention, 285, 292
results of, 1666, 1682
hantavirus, 2511
HAV, 2142-2146, 2142t, 2145t, 3433-3437, 3434t, 3436t

Immunization *(Continued)*
HBV, 1917, 1917t, 1925-1927, 1925t, 3437-3441, 3440t
multiple sclerosis and, 3429
after heart transplantation, 955-956
Helicobacter pylori, 1698
HEV, 2166-2167
HPV, 1884-1886, 3441-3443
bivalent vaccine in, 1885-1886
quadrivalent vaccine in, 1884-1886
in immunodeficiency disorders, 63-64
in immunodeficiency evaluation, 62-63
implementation of, 3425-3426
influenza virus, 2353-2356, 3443-3448
adverse events with, 3445-3446
availability of, 2357
contraindications to, 3446-3448
economics of, 2356
efficacy of, 2355-2356, 3444-3445
encephalitis after, 502
after hematopoietic stem cell transplantation, 942
indications for, 3447f
maternal, 2356
in otitis media prevention, 231
precautions for, 3448f
precautions with, 3446-3448
preparations for, 3443-3444
production of, 2357
recommendations for, 2357, 3446
safety of, 2354-2355, 3445-3446
in international adoptees and refugees, 3130-3132, 3131t
for international traveler, 3094-3107, 3097t-3105t, 3427, 3428t
Japanese encephalitis virus, 2299-2300, 3106, 3479-3480
before kidney transplantation, 1003-1004
lapsed, 3423
Leptospira, for animals, 1757-1758
maternal, 44
measles, 2393-2394, 3448-3452
acute disseminated encephalomyelitis with, 517
adverse events with, 3449-3450
autism and, 3429
complications of, 2393-2394
contraindications to, 2393, 3450-3452
efficacy of, 3449
encephalitis after, 502
in HIV-infected host, 3429
immune globulin interaction with, 3451, 3452t
indications for, 3450
meningitis and, 488
precautions for, 2393, 3450-3452
preparations for, 3449
recommendations for, 2393
misconceptions about, 3426, 3427t
MMR/MMRV, 2031-2032, 2404-2405, 3472-3473
autism and, 3429
mumps, 2404-2405, 3455-3457
acute disseminated encephalomyelitis with, 517
adverse effects of, 2405, 3456
contraindications to, 2405-2406, 3457
in immunocompromised host, 2406
indications for, 3456
meningitis and, 488
precautions for, 3457
Mycobacterium leprae, 1409-1410
Mycobacterium tuberculosis, 1376-1378, 1377t
Mycoplasma pneumoniae, 2687
Neisseria gonorrhoeae, 1300

Immunization *(Continued)*
Neisseria meningitidis, 459-460, 460t, 1267-1270, 3452-3455
adverse events with, 3454
conjugate vaccine for, 1267-1270, 3454
contraindications to, 3455
polysaccharide vaccine for, 1267, 3453-3454
precautions for, 3455
recommendations for, 1268-1269, 1268t, 3454-3455
nitrogen balance and, 69, 69f
parainfluenza virus, 2370-2372
passive, **3491-3536.** *See also* Antitoxin; Immunoglobulin(s); Monoclonal antibodies; Passive immunization
for pediatric traveler, 3094-3107, 3097t-3105t
poliovirus, 2105-2106, 3463-3466
in premature infant, 44
protective efficacy of, 127-128, 127t
rabies, 781, 2458-2460, 2459t-2460t, 3480-3483
acute disseminated encephalomyelitis with, 516
encephalitis after, 502
meningitis and, 488
record keeping for, 3423-3424
rotavirus, 627, 2187-2191, 2187f, 3466-3468
adverse events with, 3467
contraindications to, 3468
efficacy of, 3466-3467
indications for, 3467-3468
non-live, 2191-2192
Rotarix, 2187f, 2190-2191, 2190f-2191f
RotaShield, 2187-2188, 2187f
RotaTeq, 2187f, 2188-2190, 2189f
safety of, 2191
schedule for, 2191
route of administration for, 3422, 3422t
rubella, 2221-2224, 3468-3470
acute disseminated encephalomyelitis with, 517
adverse reactions to, 2222-2223, 3469
contraindications to, 2223, 3469-3470
efficacy of, 3469
global results of, 2201-2203
inadvertent, 2223-2224
indications for, 3469
precautions for, 3469-3470
during pregnancy, 2223-2224
safety of, 3427-3429
Salmonella ser. Typhi, 1507
schedules for, 3420-3422, 3424-3425, 3424f-3425f, 3426t
for pediatric traveler, 3094-3107, 3097t-3105t
in sickle-cell disease, 60
simultaneous administration in, 3423, 3423t
smallpox, 749, 2036-2038
acute disseminated encephalomyelitis with, 517
complications of, 2037t, 2038-2039, 2039t
encephalitis after, 502
meningitis and, 488
myocarditis and, 382
Streptococcus agalactiae, 1168-1169
Streptococcus pneumoniae, 285, 292, 315, 1237-1244, 3461-3463
adverse events with, 3463
approval of, 97
conjugated vaccine for, 1238-1244, 1238f, 1238t, 1240f-1241f, 1240t

Immunization *(Continued)*
efficacy of, 3462-3463
human metapneumovirus infection and, 1230
indications for, 3463
in meningitis prevention, 458-459
in otitis media prevention, 230-231, 230t-231t
unconjugated vaccine for, 1237
tetanus, 1818-1819, 1818t, 3470-3472, 3472t
tick-borne encephalitis virus, 2311-2312, 2312f
typhoid, 1507, 3483-3485, 3484t
encephalitis after, 502
in urinary tract infection prevention, 552
vaccines for, 3476-3477, 3476t
Venezuelan equine encephalitis virus, 2240-2241
Vibrio cholerae, 1554-1555, 3478-3479
VZV, 2030-2032, 3472-3476
active, 2031-2032
adverse events with, 3473-3474
contraindications to, 3475-3476
efficacy of, 2031-2032, 3473
after hematopoietic stem cell transplantation, 941
indications for, 3474-3475
before kidney transplantation, 1009
before lung transplantation, 978-979
passive, 2030-2031
safety of, 2031
West Nile virus, 2272-2273
yellow fever, 2277-2278, 3485-3487
zoster, 2023-2024
Immunochromatographic membrane test, in pneumococcal pneumonia, 1217
Immunodeficiency disorders. *See also specific immunodeficiency disorders*
appendicitis in, 684
bacterial meningitis in, 436
cholangitis in, 666
dermatologic manifestations of, 62
diarrhea in, 599-600
evaluation for, 61-63
laboratory studies in, 62-63
patient history in, 25t, 61-62
physical examination in, 62
exanthems in, 766
fever of unknown origin in, 845
gene therapy in, 64
infection prevention in, 63-64
management of, 63-64
otitis media in, 211
pancreatitis in, 694
primary, **45-59**, **903-920.** *See also specific immunodeficiency disorders*
evaluation of, 903-908
cellular immunity in, 906-907
chest radiography in, 906, 906f
complement in, 907
delayed hypersensitivity skin tests in, 907
genetic testing in, 907, 907t
humoral immunity in, 906
laboratory tests in, 905-908, 906t
medical history in, 903-904, 904t
phagocyte function in, 907
physical examination in, 904-905, 905f
T cells in, 908
infection and, 903-904, 904t, 908, 908t
management of, 908, 908t, 3497, 3497t
X-linked, 904, 904t
secondary, 59-60, 903
Immunoglobulin(s), 40-41. *See also specific immunoglobulins*
aerosolized, 3498-3499
age-related levels of, 43t
anthrax, 3504
botulinum, 1808, 3505
CMV, 951, 1985-1986, 1988-1989, 3520-3521
in heart transplantation, 951-952
in lung transplantation, 975
in perinatal infection, 3521
in transplant recipient, 3520
HBV, 1924-1925, 3516-3517
in liver transplantation, 1924, 3517-3519
HCV, 3520
intramuscular (IGIM), 3493-3495
adverse effects of, 3494-3495
in antibody deficiency, 3494
in burn injury, 3508-3509
in HAV infection prevention, 2141-2142, 3514-3515
in HBV infection prevention, 3515-3516
in HCV infection prevention, 3519-3520
in infectious disease prevention, 3494
in measles prophylaxis, 2394-2395, 3526
pharmacology of, 3493-3494
in poliomyelitis prevention, 3512-3513
in respiratory infection, 3507-3509
in rubella prophylaxis, 2219-2220, 2224, 3531-3532
vaccine interactions with, 3496-3497
vaccinia, 3532-3533
in VZV infection, 3522
intrathecal, 3499
intratracheal, 3498-3499
intravenous (IVIG, IGIV), 3495-3497
administration of, 3495-3496
anaphylaxis with, 3496
in antibody deficiency, 3497
aseptic meningitis with, 3496
in burn injury, 1061, 3501, 3508-3509
in *Clostridium difficile* infection, 3505-3506
in CMV infection prevention, 3520-3521
in common variable immunodeficiency, 49, 910
in cystic fibrosis, 3508
cytokine effects of, 3361
in encephalitis, 510
in enteroviral meningoencephalitis, 3513
in enterovirus infection, 2103, 3513
in hematologic disease, 3499
hepatitis C transmission with, 3496
in HIV infection, 3525-3526
in HSV infection, 3522
in human erythrovirus B19 (human parvovirus B19) infection, 1852, 1854, 1856, 3527
in hyper-IgM syndrome, 50
in IgA deficiency, 47
in IgG subclass deficiency, 47
in immunoregulatory disorders, 3501-3502, 3502t
in intensive care patients, 3499-3500
in Kawasaki disease, 1073, 1078-1079, 1078t
in *Mycoplasma pneumoniae* infection, 2687
in myocarditis, 397
Immunoglobulin(s) *(Continued)*
in neonatal enteroviral infection, 3513
in neonatal staphylococcal infection, 3509-3510
in neurologic disorders, 3501-3502, 3502t
in oncologic disease, 3499
in otitis media, 3508
in PANDAS syndrome, 3511
pharmacology of, 3495
in pneumonia, 3508
in premature infant, 3500-3501
in primary immunodeficiency, 3497, 3497t
in protein-losing diseases, 3499
renal complications of, 3496
in respiratory infection, 3507-3509
in rheumatic fever, 413-414
in RSV infection, 2428-2429, 3530-3531
in secondary immunodeficiency, 3497t, 3499-3501
in sepsis, 3509
side effects of, 3496
in sinusitis, 3508
in staphylococcal infections, 3509-3510
in staphylococcal toxic shock syndrome, 835, 857, 3509
in *Staphylococcus aureus* infection, 3510
in streptococcal infections, 3510-3511
in streptococcal toxic shock syndrome, 861, 3511
in Sydenham chorea, 3511
thrombotic complications of, 3496
in transplantation patient, 3501
vaccine interactions with, 3496-3497
in VZV prophylaxis, 3523-3525
in West Nile virus infection, 2272
in X-linked agammaglobulinemia, 47, 909
isotypes of, 41-42
laboratory evaluation of, 62-63, 906
mumps, 3526
neonatal, 42-44, 43f, 43t
normal/elevated levels of, antibody deficiency with, 47-48
oral, 3498
in cryptosporidial infection, 3498
in diarrhea, 3498
in necrotizing enterocolitis, 3498
in rotavirus infection, 2186, 3498, 3531
paranasal sinus, 195
pertussis, 3507
rabies, 2454, 3527-3529
RSV, 2415, 2428-2429, 3529-3531
in lung transplantation, 980
structure of, 40-41, 41f
subcutaneous (IGSC), 3497-3498
tetanus, 1815-1816, 3511-3512
tick-borne encephalitis virus, 2312
vaccinia (VIG), 2036, 3532-3533
VZV, 2030, 3522-3525, 3524t
in hematopoietic stem cell transplantation, 941
West Nile virus, 2272
zoster, 3522-3523
Immunoglobulin A (IgA), 41
in adenovirus infection, 1894-1895
anaphylaxis with, 911
in *Bordetella pertussis* infection, 1622, 1628
in *Campylobacter jejuni* infection, 1651
deficiency of, 47, 911
vs. chronic bronchitis, 267
in *Helicobacter pylori* infection, 1695

Immunoglobulin A (IgA) *(Continued)*
in Kawasaki disease, 1070, 1072
in *Kingella kingae* infection, 1702
in *Mycobacterium leprae* infection, 1398
in *Mycoplasma pneumoniae* infection, 2673-2674, 2685-2686
in neonate, 43-44
in parainfluenza virus infection, 2366
polymeric, 1225
in rhinovirus infection, 2119
in rotavirus infection, 2180-2181
in RSV infection, 2415-2416
in rubella virus infection, 2204, 2206
secretory, 43-44
in gonococcal infection, 1280
in *Shigella* spp. infection, 1479
in *Streptococcus pneumoniae* infection, 1223
in *Toxoplasma gondii* infection, 2996
in *Trichomonas vaginalis* infection, 2890
in VZV infection, 2024
Immunoglobulin A (IgA) protease
of *Haemophilus influenzae*, 1668
of *Neisseria gonorrhoeae*, 1278
of *Streptococcus pneumoniae*, 1214
Immunoglobulin D (IgD), 41-42, 847
Immunoglobulin E (IgE), 41
in bronchiolitis, 277
in coronavirus infection, 136
increased levels of, **55**, 317, 777, 919
in Kawasaki disease, 1076-1077
in RSV infection, 2415-2416
in *Toxoplasma gondii* infection, 2996
Immunoglobulin G (IgG), 41-42
in *Acanthamoeba* spp. infection, 2983
in adenovirus infection, 1894-1895
in *Bartonella* spp. infection, 1727
in *Bordetella pertussis* infection, 1622, 1628
in *Brucella* spp. infection, 1614
in burns, 1050
in *Chlamydia pneumoniae* infection, 2642-2644
in CMV infection, 1982-1984
in *Coccidioides* spp. infection, 2776
complement activation by, 29-31
in congenital rubella, 2207, 2221
deficiency of, 62-63, 906
in common variable immunodeficiency, 910
in hyper-IgM syndrome, 909-910
in X-linked agammaglobulinemia, 909
in *Haemophilus influenzae* type b infection, 1671-1672
in HAV infection, 2130f, 2137-2138
in HEV infection, 2163, 2165
in HSV infection, 1957
in human erythrovirus B19 (human parvovirus B19) infection, 1846, 1855-1856
in hypersensitivity pneumonitis, 304, 304f
in infectious mononucleosis, 2008-2010, 2009f
in Kawasaki disease, 1076-1077
in *Kingella kingae* infection, 1702
in La Crosse virus infection, 2520-2521, 2521f
in LCMV infection, 2464
maternal, 42-44, 43f, 880
in measles, 2379
in mumps, 2400
in *Mycobacterium leprae* infection, 1398
in *Mycoplasma pneumoniae* infection, 2670, 2673-2674
in *Naegleria fowleri* infection, 2983
in pancreatitis, 692
in parainfluenza virus infection, 2366
Immunoglobulin G (IgG) *(Continued)*
in *Pseudomonas aeruginosa* infection, 1588-1589
in rhinovirus infection, 2119
in *Rickettsia prowazekii* infection, 2658
in rotavirus infection, 2180-2181
in RSV infection, 2415-2416, 2425
in rubella virus infection, 2204, 2206, 2218-2219
in *Shigella* spp. infection, 1479
in *Staphylococcus aureus* infection, 1129
in *Streptococcus pneumoniae* infection, 1223
in syphilis, 1767
in tick-borne encephalitis virus infection, 2310
in *Toxoplasma gondii* infection, 2994-2995, 2995t
in *Trichomonas vaginalis* infection, 2890
in VZV infection, 2024
in yellow fever, 2277
Immunoglobulin G1 (IgG1), deficiency of, 47, 911-912
Immunoglobulin G2 (IgG2), deficiency of, 47, 911-912
Immunoglobulin G3 (IgG3), deficiency of, 47, 911-912
Immunoglobulin G4 (IgG4), deficiency of, 47
Immunoglobulin G (IgG) subclass deficiency, 47
laboratory evaluation of, 62-63
Immunoglobulin M (IgM), 41
in *Acanthamoeba* spp. infection, 2983
in adenovirus infection, 1906-1907
anti-*Toxoplasma*, 817
in *Balamuthia mandrillaris* infection, 2983
in *Bartonella* spp. infection, 1727
in *Bordetella pertussis* infection, 1628
in *Brucella* spp. infection, 1614
in *Campylobacter jejuni* infection, 1651
in Chikungunya virus infection, 2245-2247
in *Chlamydia pneumoniae* infection, 2642-2644
in CMV infection, 1982-1983
in *Coccidioides* spp. infection, 2776
complement activation by, 29-30
in congenital rubella, 2207, 2218-2219
in dengue, 2285-2286
in Eastern equine encephalitis, 2229
in *Entamoeba histolytica* infection, 2869-2870
in enterovirus infection, 2056, 2102
in hantavirus infection, 2509-2510
in HAV infection, 2130f, 2137-2138
in *Helicobacter pylori* infection, 1695
in HEV infection, 2163, 2165
in HHV-6 infection, 2019
in human erythrovirus B19 (human parvovirus B19) infection, 1846-1847, 1855
increase in, **49-50**
vs. chronic bronchitis, 267
immunoglobulin deficiency with, 907t, **909-910**
in infectious mononucleosis, 2008-2010, 2009f
in Japanese encephalitis virus infection, 2298
in Kawasaki disease, 1076-1077
in La Crosse virus infection, 2520-2521
in *Leptospira* spp. infection, 1755
in Lyme disease, 1735
maternal, 880-881
in measles, 2379
in mumps, 2400, 2403
Immunoglobulin M (IgM) *(Continued)*
in *Mycobacterium leprae* infection, 1398
in *Mycoplasma pneumoniae* infection, 2673-2674, 2685-2686
in *Naegleria fowleri* infection, 2983
neonatal, 43-44
in parainfluenza virus infection, 2366
in Powassan virus infection, 2314
in rhinovirus infection, 2119
in Ross River virus arthritis, 2250
in rotavirus infection, 2180-2181
in RSV infection, 2425
in rubella virus infection, 2204, 2206, 2218-2219
in St. Louis encephalitis virus infection, 2258-2259
in syphilis, 1767, 1774
in tick-borne encephalitis virus infection, 2285, 2310
in *Toxoplasma gondii* infection, 2995-2996, 2995t
in *Trypanosoma brucei* infection, 2971
in Venezuelan equine encephalitis, 2240
in VZV infection, 2024, 2028
in West Nile virus infection, 2271
in Western equine encephalitis, 2235
in yellow fever, 2277
Immunoglobulin Y (IgY), in rotavirus infection, 2186
Immunoscintigraphy, in infective endocarditis, 359
Immunosuppressants. *See also* Corticosteroids
in heart transplantation, 946, 955, 955t
in intestinal/liver transplantation, 990-991
in lung transplantation, 958-959
in myocarditis, 396-397
Impetigo, 753t-756t, **772-775**
bullous, 753t-756t, 773-774, 774f-775f
nonbullous (simple), 772-773, 1143, 1149
epidemiology of, 773, 1141
transmission of, 1141
treatment of, 774-775
Implantable cardioverter-defibrillator (ICD)–associated infection, 1025-1026
management of, 1026
microbiology of, 1025
Implantable device–related infection. *See* Prosthetic/implantable device–related infection
Inappropriate secretion of antidiuretic hormone, 76, 81
in bacterial meningitis, 438, 453-454
Inclusion conjunctivitis, 804
Incontinentia pigmentosa with immunodeficiency, 916, 916f
Incubation period, 105-106, 106f
India ink staining, in cryptococcal meningoencephalitis, 2802
Indirect immunofluorescent antibody (IFA) assay
in *Anaplasma* infection, 2663
in *Babesia* spp. infection, 2918-2919
in *Ehrlichia* infection, 2663
in influenza virus infection, 2345
in La Crosse virus infection, 2520-2521, 2521f
in Mediterranean spotted fever, 2656
in *Naegleria fowleri* infection, 2982, 2982f
in Rocky Mountain spotted fever, 2653-2654
in scrub typhus, 2660
in *Toxoplasma gondii* infection, 2994-2996, 2995t
in typhus, 2658

Indium 111-labeled leukocyte scan
in appendicitis, 683
in osteomyelitis, 717
Infant. *See also* Newborn; Premature infant
Bordetella pertussis infection in, 1626-1627, 1626t
botulism in. *See* Botulism, infant
Campylobacter jejuni infection in, 888, 1653
cervical lymphadenitis in, 179
common cold in, 136
enterovirus infection of, 2092-2097, 2094t, 2096t
epidemic keratoconjunctivitis in, 1900
febrile seizures in, 85
fever without source in, 839-840
Histoplasma capsulatum infection in, 2814
Leishmania spp. infection in, 2955
Mycobacterium leprae infection in, 1394
necrotizing enterocolitis in, 635
nitrogen balance in, 69, 69f
nosocomial infection in, 3402-3404
Pasteurella multocida infection in, 1547
poliovirus infection in, 2100-2101
septic arthritis in, 730
streptococcosis syndrome of, 179
transient hypogammaglobulinemia of, **47**, **912**
tuberculosis in, 1358-1359
Infant formula, botulism and, 1804
Infantile cortical hyperostosis (Caffey disease), 847
Infantile epidemic keratoconjunctivitis, 1900
Infantile osteomyelitis, 153-154, 153f
Infantile periarteritis nodosa, 1071. *See also* Kawasaki disease
Infantile spasms, after pertussis immunization, 1634
Infarction
bone, vs. osteomyelitis, 724
cerebral
in infective endocarditis, 356-357
in *Leptospira* spp. infection, 1753
in *Neisseria meningitidis* infection, 1260
hepatic, 673
myocardial
vs. human erythrovirus B19 (human parvovirus B19) infection, 1854
in Kawasaki disease, 1081, 1083-1084
spinal cord, vs. acute transverse myelitis, 523, 524f
splenic, 357, 704, 2682
Infection. *See also specific infections, infectious agents, and sites of infection*
age patterns in, 108, 113
behavior and, 109-110
climate and, 110
cyclic variation in, 118-119
definition of, 103-104
in developing world, 112
vs. disease, 111
emerging, 119-120
endemic, 116-117
environment and, 110-111, 114-116
epidemic, 116-118
epidemiologic study of, 103-120
behavioral factors in, 109-110
biologic factors in, 108-109
environmental factors in, 110-111
historical perspective on, 103
host factors in, 107-108
infectious agents in, 104-107, 106f, 107t
population patterns in, 111-120, 116f-117f
ethnic patterns in, 108, 113-114
family episodes of, 114
Infection *(Continued)*
geographic factors and, 110-111, 114-116
geologic factors and, 110-111
global variation in, 115
health status and, 108-109
host factors in, 107-108
immunity and, 109. *See also* Immunity
in individual, 111-113
in kinships, 114
latent, 105-106, 106f
local patterns of, 115-116, 116f
long-term trends in, 119
monitoring of, 117, 117f
outbreak of, 117
pandemic, 117
persistent, 106
population occurrence of, 112-114, 116f-117f
racial patterns in, 108, 113-114
reporting requirements for, 112
seasonal variation in, 118-119
sex patterns in, 108, 113
socioeconomic patterns in, 110-111, 114
temporal patterns of, 116-120
time clusters of, 117-118
transmission of, 107, 107t
Infection control practices, 1061-1062, 3407-3414, 3409t
antimicrobial stewardship programs in, 3414, 3414t
in cancer patient care, 929
in daycare facilities, 3565-3569, 3567t-3568t
hand hygiene in, 3411
isolation precautions in, 3411-3412. *See also* Isolation precautions
occupational health policies in, 3412-3413
outbreak investigations in, 3408, 3411t
policies and procedures for, 3408-3411, 3410t
Standard Precautions in, 3379, 3380t, 3412
surveillance in, 3408
Transmission-Based Precautions in, 3379, 3381t, 3412
visitor policies in, 3412
Infectious mononucleosis, 158-161, **1999-2000**. *See also* Epstein-Barr virus (EBV)
acute phase of, 1999-2000, 1999f-2000f, 1999t
airway obstruction in, 2004
aplastic anemia in, 2002
cardiac manifestations of, 2002
complications of, 2001-2004
diagnosis of, 2007-2011, 2007f, 2008t
differential diagnosis of, 2011-2012
epidemiology of, 1997-1998
exanthem in, 742t-748t, 760, 1999-2000, 2002, 2002f
gastrointestinal manifestations in, 2003
hematologic manifestations of, 2002-2003
hemolytic anemia in, 2002
hepatic manifestations in, 2003
historical perspective on, 1992
imaging studies in, 2011
immune response in, 2008-2010, 2009f, 2010t
in immunodeficient host, 2006
immunopathogenesis of, 1995
neck abscess in, 2004
neurologic manifestations in, 2003-2004
neutropenia in, 2003
ocular involvement in, 810
pancreatitis with, 694
Infectious mononucleosis *(Continued)*
pancytopenia in, 2003
in pregnancy, 1998
prodrome in, 1999
pulmonary disease in, 2004
renal manifestations in, 2004
resolution phase of, 2000
respiratory manifestations in, 2004
splenic manifestations in, 2003
thrombocytopenia in, 2002-2003
transmission of, 1998
treatment of, 2012
in young children, 2000
Infective dermatitis, 742t-748t, 749-750, 2574-2575, 2574t
Infective endocarditis, **350-370**
Achromobacter xylosoxidans, 1574-1575
acute, 350
Aerococcus viridans, 1249
age-related incidence of, 350-351
Aggregatibacter actinomycetemcomitans, 1610, 1823
Aggregatibacter (Haemophilus) aphrophilus, 1686
Arcanobacterium haemolyticum, 1325-1326
arteriovenous fistula-related, 352
Bacillus spp., 362-363
bacteremia in, 353-354, 353t
Bacteroides fragilis, 367
Bartonella spp., 363, 1725, 1727-1729
Brucella spp., 362, 1613-1615
in burn patient, 1055
Candida, 354, 357-358, 363, 2740, 2748
neonatal, 895, 897, 2756
catheter-related, 352
Chlamydia spp., 363
clinical manifestations of, 356-357, 356t
CNS involvement in, 356-357
coagulation system in, 354
complications of, 355, 355f
congenital heart disease and, 351, 351t
Corynebacterium diphtheriae, 362-363, 1303, 1306
Coxiella burnetii, 363, 2664
CSF examination in, 443t
culture-negative, 358, 368
distant organ disease in, 355
Duke criteria for, 360, 360t-361t
echocardiography in, 359-360
Eikenella corrodens, 1577
emboli in, 355
enterococcal, 361, 366, 366t-367t
Enterococcus spp., 361, 366, 366t-367t, 1173-1175
epidemiology of, 350-352, 351t
Erysipelothrix rhusiopathiae, 362-363, 1328
Escherichia coli, 362, 1441
fever of unknown origin in, 845
fungal, 352, 361t, 363, 367-368
gram-negative, 361t, 362, 367
gram-positive bacilli, 362-363
HACEK, 362, 367, 367t, 1823
Haemophilus parainfluenzae, 1690
immunopathologic factors in, 354-355, 358
intravenous drug abuse and, 352, 357
Kingella kingae, 1703-1704
Klebsiella spp., 362, 367
laboratory evaluation in, 357-360, 358t, 360t-361t
Lactobacillus spp., 362-363
Legionella spp., 363
Listeria monocytogenes, 362-363
microbiology of, 360-363, 361t

Infective endocarditis *(Continued)*
mitral valve, 352
Neisseria gonorrhoeae, 362
neonatal, 357
pacemaker-related, 352
pathophysiology of, 352-355, 353t, 355f
pneumococcal, 361, 1232
prevention of, 368-369, 369t, 417-418, 1195-1196, 3246-3247, 3246t-3248t
prognosis for, 352
prophylactic antimicrobials against, 368-369, 369t, 417-418, 3246-3247, 3246t-3248t
prosthetic valves and, 351, 357, 365-366, 366t
coagulase-negative staphylococci in, 1137
Proteus spp., 1473
Pseudomonas aeruginosa, 362, 1595, 1597-1598
rheumatoid factor in, 355
serologic testing in, 358-359
staphylococcal, 361-362, 361t, 366-367, 367t, 1137
Stenotrophomonas maltophilia, 1607
Streptobacillus moniliformis, 1717-1719
streptococcal, 360-361, 361t, 365-366, 365t-366t, 1151-1152, 1232
Streptococcus viridans, 360-361, 361t, 365-366, 365t-366t, 1191-1192, 1195
subacute, 350, 355f
surgical treatment of, 368, 368t
treatment of, 363-368, 364t-368t
initiation of, 364
monitoring of, 364
vegetations in, 353-355, 355f
ventriculoatrial shunt–related, 352
von Reyn criteria for, 360
in Whipple disease, 644
Infectivity, 105
Infertility
appendicitis and, 687-688
cystic fibrosis and, 325
pelvic inflammatory disease and, 1286
Ureaplasma spp. infection and, 2689
Inflammation
in bacterial meningitis, 433-435, 434f
in burns, 1048
complement in, 31
cytokines in, 65, 79-80
joint. *See* Arthritis; Juvenile idiopathic (rheumatoid) arthritis; Rheumatoid arthritis
liver. *See* Hepatitis
metabolic changes with. *See* Metabolism
Inflammatory bowel disease (IBD), 2872
Campylobacter jejuni infection and, 1654
vs. *Clostridium difficile* infection, 637
fever of unknown origin in, 847
probiotics in, 3370-3371
Infliximab
adenovirus infection and, 1904
in Kawasaki disease, 1080
Influenza A virus, 2327-2338, 2327f-2328f.
See also Influenza virus infection
avian, 2329-2330, 2334-2335
canine, 2329-2330
ecology of, 2329-2330
evolution of, 2331-2332
novel, 2334-2336
surveillance for, 2330-2332
swine, 2329-2330, 2335-2336
testing for, 2346-2348
transmission of, 2330, 2337-2338
Influenza B virus, 2327-2338, 2327f-2328f.
See also Influenza virus infection
evolution of, 2331-2332
surveillance for, 2331
testing for, 2346-2348
transmission of, 2337-2338
Influenza C virus, 2327-2338
Influenza virus infection, **2326-2359**
in acute bronchitis, 262, 263t
avian subtypes in, 2334-2335
bacterial coinfection with, 2345, 2351-2352
bloodstream, 2345
cardiac complications of, 2343
chest radiography in, 2340, 2344f
clinical manifestations of, 2340-2345, 2342t-2343t
complications of, 2340
in daycare facilities, 3562
diagnosis of, 981, 2345-2358, 2348f, 3606t-3607t, 3610-3612, 3611t
disseminated intravascular coagulation with, 2345
economic impact of, 2333
encephalitic, 494t-498t, 502, 2343, 2352
epidemiology of, 2332-2334
exanthem in, 742t-748t, 749, 760-762
genetic factors in, 2338
global burden of, 2333-2334
after heart transplantation, 954
after hematopoietic stem cell transplantation, 942
hepatic complications of, 2344
in high-risk groups, 2333
historical perspective on, 2327
H1N1, 749, 981, 2330-2331, 2336-2337
H5N1, 2334-2335, 3115-3116
immune response to, 2338
immunization against, 2353-2356, 3443-3448
adverse events with, 3445-3446
availability of, 2357
contraindications to, 3446-3448
economics of, 2356
efficacy of, 2355-2356, 3444-3445
encephalitis after, 502
indications for, 3447f
maternal, 2356
precautions for, 3448f
precautions with, 3446-3448
preparations for, 3443-3444
production of, 2357
recommendations for, 2357, 3446
safety of, 2354-2355, 3445-3446
after intestinal transplantation, 997t, 999
after kidney transplantation, 1007
after liver transplantation, 997t, 999
after lung transplantation, 980-981
mortality with, 2333-2334
musculoskeletal complications of, 2344
in neonate, 2339
neurologic complications of, 2343
nonpharmaceutical prevention of, 2358
nosocomial, 2352, 3380-3382
novel viruses in, 2334-2336
ocular, 812
outbreaks of, 2352-2353
pandemic, 2330-2331, 2336-2337
preparedness for, 2358
pathogenesis of, 2338-2345
pathology of, 2338-2339, 2341f
during pregnancy, 2345
prevention of, 2352. *See also* Influenza virus infection, immunization against
breast-feeding and, 2356-2357
intrapartum, 2356-2357
Influenza virus infection *(Continued)*
pulmonary, 284, 2342-2343, 2344f
renal complications of, 2345, 2352
respiratory failure/distress with, 2352
seasonal, 2329, 2332-2334
sepsis with, 2345
Staphylococcus aureus infection and, 1122, 2338
Streptococcus pneumoniae infection and, 1215
sudden death with, 2345
surveillance for, 2331-2332
swine subtypes in, 2335-2336
transmission of, 2337-2338, 2345
in traveler, 3115-3116
treatment of, 2348-2351, 2349t
antiviral agents in, 2348-2351, 2349t, 3269-3270, 3272-3275
antiviral susceptibility testing in, 2345-2346
corticosteroids in, 2351
investigational therapies in, 2351
salicylates in, 2351
supportive care in, 2351
vertical transmission of, 2345
Infraorbital space, infection of, 149
Infrared thermometer, 85-86
Inhalation injury, 1048-1049. *See also* Burn(s)
bronchoalveolar lavage in, 1057
Inkoo virus, 2514, 2517
Innate immunity. *See* Immunity, innate
Inoculation arthritis, 728
Inoculation osteomyelitis, 724-725
Inosiplex, in subacute sclerosing panencephalitis, 2390
Insecticide poisoning, pancreatitis with, 692
Insulin, plasma/serum, 82
in septic shock, 829
INT1 protein, 9
Integrase inhibitors, 2615-2616
Integrin(s), in phagocyte recruitment, 31-32
Integrin activation deficiency defect, 53-54
Integrons, in antibiotic resistance, 3138-3139, 3138t
Interferon-alpha (INF-α), 80, 3363-3364
in congenital rubella, 2208
in HCV infection, 2323-2324
in HSV infection, 1966
pancreatitis with, 692
in papillomas, 1883
in rhinovirus infection prevention, 2125-2126
Interferon-alpha 2a (INF-α2a)
in HBV infection, 1920-1922
in HDV infection, 1931-1932
Interferon-alpha 2b (INF-α2b)
in common cold, 138
in HDV infection, 1920-1921, 1931-1932
in West Nile virus infection, 2272
Interferon-beta (INF-β), 3363-3364
in myocarditis, 397
Interferon-gamma (INF-γ), 3364
in chronic granulomatous disease, 57, 918
in *Cryptococcus neoformans* infection, 2805
defects in, 50-51
in mycobacterial infection, 1382
in *Mycobacterium leprae* infection, 1398
in *Trypanosoma cruzi* infection, 2967
in viral infection, 135
Interferon-gamma release (IGR) assay
in kidney transplantation, 1004, 1012
in *Toxoplasma gondii* infection, 2997
in tuberculosis, 1363-1364

Interleukin(s), 26t
Interleukin-1 (IL-1), 26-27, 26t, 80, 3358-3360, 3359t
  in burns, 1050
  in common cold, 135
Interleukin-1β (IL-1β), in bacterial meningitis, 434-435
Interleukin-2 (IL-2), 3359t, 3361
  in *Mycobacterium leprae* infection, 1398
  recombinant, in burns, 1050
Interleukin-4 (IL-4), 3362
Interleukin-6 (IL-6), 70, 3360t
Interleukin-8 (IL-8), 3359t, 3361-3362
  in upper respiratory tract infection, 135
Interleukin-9 (IL-9), 3361-3362
Interleukin-10 (IL-10), 3360t
  in septic shock, 827
Interleukin-12 (IL-12), 3359t, 3362
  defects in, 50-51
  in mycobacterial infection, 1382
Interleukin-17 (IL-17), 3359t, 3362
  in myocarditis, 387
Interleukin-18 (IL-18), 3362
Interleukin-1 (IL-1) receptor antagonist, 3360, 3360t
Internalins, 13
International adoptees and refugees, **3121-3133**, 3122f
  American trypanosomiasis in, 3129
  bacterial infection in, 3123t, 3126-3128
  blood lead levels in, 3130
  complete blood count in, 3130
  dermatologic diseases in, 3130
  enteric bacterial infections in, 3128
  hepatitis A in, 3122-3124, 3123t
  hepatitis B in, 3123t, 3124-3125
  hepatitis C in, 3123t, 3125
  HIV infection in, 3123t, 3125
  immunization of, 3130-3132, 3131t
  infectious disease screening in, 3122-3130, 3123t, 3128t
  intestinal parasites in, 3128-3130
  malaria in, 3129
  syphilis in, 3123t, 3126
  tissue parasites in, 3129
  tuberculosis in, 3126-3128, 3127f-3128f
International health, 3082t
International travel, **3091-3120**
  African trypanosomiasis and, 3116
  avian influenza A and, 3115-3116
  chikungunya and, 3116
  coccidioidomycosis and, 3116-3117
  dengue and, 3115
  diarrhea prevention and, **3110-3115**
  HAV infection and, 2134-2135, 2141
  health counseling for, 3117-3118, 3548-3549
  health kit for, 3118
  high-altitude destination and, 3118
  histoplasmosis and, 3116-3117
  information resources for, 3118, 3119t
  Japanese encephalitis virus and, 2294, 2295t-2296t, 3106
  leishmaniasis and, 3117
  leptospirosis and, 3117
  malaria prevention and, 2926, 3107-3110, 3109f, 3111t
  pretravel assessment for, 3092-3094
  rabies and, 3106-3107
  risks of, 3092
  schistosomiasis and, 3116
  sexually transmitted diseases and, 3117
  tick-borne encephalitis and, 3116
  tuberculosis and, 3116
  typhoid fever and, 3107
International travel *(Continued)*
  vaccination for, 3094-3107, 3097t-3105t, 3427, 3428t
Interstitial lung disease, **295-306**
  biopsy in, 299-300
  bronchoalveolar lavage in, 299
  classification of, 295-297, 296t
  clinical presentation of, 297-298
  CT in, 298-299
  diagnosis of, 298-300, 298t
  infection and, 295-296
  prognosis for, 300-301
  pulmonary function tests in, 298
  treatment of, 300
Intervertebral disk, infection of, 720-721, 721f
Intestinal transplantation, infection after, **989-1002**
  adenovirus, 997t, 999, 1903
  age and, 990
  bacterial, 994-995
  catheter and, 991, 993
  CMV, 990, 996-997, 997t, 1001
  coccidioidal, 1000
  cryptococcal, 1000
  donor-related factors in, 990
  early (0 to 30 days), 991-992, 991t
  EBV, 990, 997-998, 997t, 1001
  fungal, 991, 994-995, 995t
  HHV-6, 999
  HHV-7, 999
  Histoplasma capsulatum, 1000
  immunosuppression and, 990-991
  intermediate (31 to 180 days), 992, 992t
  intra-abdominal, 994
  intraoperative factors in, 990
  late (after 180 days), 992-993, 992t
  Mycobacterium tuberculosis, 1000
  nosocomial, 993
  Pneumocystis jiroveci, 1000
  post-transplant factors in, 990-991
  predisposing factors in, 989-991
  pretransplant evaluation in, 1000-1001
  pretransplant factors in, 989-990
  prevention of, 1001
  RSV, 997t, 999
  timing of, 991-993, 991t-992t
  viral, 995-1001, 997t
  wound, 994
Intimin, 7-8, 9f, 1457
Intracranial pressure (ICP)
  in cryptococcal meningoencephalitis, 2802-2803
  in meningitis, 438-439, 454
Intracranial pressure (ICP) monitor, infection with, 1038-1040
  clinical manifestations of, 1039
  diagnosis of, 1039-1040
  epidemiology of, 1038-1039, 1039t
  etiology of, 1039
  treatment of, 1040
Intraocular lenses, infection with, 1019
Intrathecal pump infusion device–associated infection, 1040-1042
  clinical manifestations of, 1041
  diagnosis of, 1041
  epidemiology of, 1041
  etiology of, 1041
  treatment of, 1041-1042
Intrathecal serotherapy, in neonatal tetanus, 1816
Intrauterine device (IUD), *Actinomyces* spp. infection and, 1822
Intravenous antimicrobial therapy. *See* Outpatient intravenous antimicrobial therapy
Intravenous immunoglobulin. *See* Immunoglobulin(s), intravenous (IVIG, IGIV)
Intussusception
  adenovirus and, 1902
  rotavirus infection and, 2186
Invasins, 13, 1523-1524
Iodine, metabolism of, 77t
Iodoquinol, 3348-3349
IpaB protein, 18
IPEX (immunodeficiency, polyendocrinopathy, enteropathy, X-linked) syndrome, 915-916
Ipratropium, in bronchiolitis, 279
Iquitos virus, 2543-2544
Iridocyclitis, 809
  Ascaris lumbricoides, 815
  coccidioidal, 813-814
  herpes zoster, 810
  HSV, 810
  Loa loa, 815
  rickettsial, 815
  Wuchereria bancrofti, 815
IRIS (immune reconstitution inflammatory syndrome), 972, 1360-1361
Iritis, 809
  Brucella, 813
  herpes zoster, 810
  HSV, 810
  mumps, 811
  tuberculous, 1357
Iron
  bacterial acquisition of, 538
  deficiency of, hookworm infection and, 3017
  metabolism of, 77t, 78
    in *Pseudomonas aeruginosa* infection, 1588
    in *Yersinia enterocolitica* infection, 1525
  Neisseria gonorrhoeae metabolism of, 1278
  serum, 78
    fever and, 85
    mucormycosis and, 2840
Iron transporters, of *Streptococcus pneumoniae*, 1214
Irrigation, nasal
  in common cold, 137
  in rhinosinusitis, 201
Irritable bowel syndrome
  probiotics in, 3371
  with *Shigella* spp. infection, 1481
Isocitrate lyase, 15
Isolation precautions, 3411-3412, 3546
  in ARDS, 873
  in *Bordetella pertussis* infection, 1636
  in congenital rubella, 2220
  in methicillin-resistant *Staphylococcus aureus* infection, 1126
  in monkeypox, 2044
  in *Shigella* spp. infection, 1486
  in tuberculosis, 1375
  in *Yersinia pestis* infection, 1514
Isoniazid, 1366-1369, 1367t
  before lung transplantation, 963-964
Isoproterenol, in myocarditis, 395
Isospora belli. *See Cystoisospora belli*
Itraconazole, 3301f, 3305-3308, 3349
  adverse effects of, 3307
  in *Blastomyces dermatitidis* infection, 478, 2732-2734, 2732t
  in *Candida* esophagitis, 596-597
  in *Coccidioides* spp. infection, 477
  drug interactions with, 3307
  in hematopoietic stem cell transplantation–related aspergillosis, 937

Itraconazole *(Continued)*
in *Histoplasma capsulatum* infection, 2825
indications for, 3294t-3295t, 3307-3308, 3321t
in *Paracoccidioides* spp. infection, 2793
pharmacodynamics of, 3305
pharmacokinetics of, 3305-3307, 3306t
in *Sporothrix schenckii* infection, 2835
in tinea capitis, 787
in tinea unguium, 787
Ivermectin, 3349
in lice, 3075
in onchocerciasis, 809
in scabies, 3074
in *Strongyloides stercoralis* infection, 3021
IVIG. *See* Immunoglobulin, intravenous (IVIG, IGIV)
Ixodes tick, **3071-3072**
in Lyme borreliosis, 1730, 1730t, 1737-1738
in Powassan virus infection, 2313
prevention of, 1737
in tick-borne encephalitis virus infection, 2304-2305, 2306f

J

Jamestown Canyon virus, 2514, 2520
Janeway lesion, 355, 357
Japanese encephalitis virus, 2291-2293, 2292f
Japanese encephalitis virus infection, 494t-498t, **2291-2301**
clinical manifestations of, 2294-2296, 2294f
complications of, 2297
diagnosis of, 2298
differential diagnosis of, 2298
epidemiology of, 2292f-2293f, 2293-2294
historical perspective on, 2291
immune response to, 2297
immunization against, 2299-2300, 3479-3480
pathology of, 2296-2297
pathophysiology of, 2297
in pregnancy, 2297
prevention of, 2299-2300
prognosis for, 2298-2299
relapse in, 2297
in travelers, 2294, 2295t-2296t
treatment of, 2298
Jarisch-Herxheimer reaction, 812
Jaundice
in cholecystitis, 669
in congenital rubella, 2220
differential diagnosis of, 662-663, 662t
in HAV infection, 2137, 2140-2141
in *Leptospira* spp. infection, 1746-1747, 1752
in *Paracoccidioides* spp. infection, 2786
in yellow fever, 2276
Jaw
lumpy, 184
osteomyelitis of, **152-154**, 152t
Garré, 154, 154f-155f
infantile, 153-154, 153f
suppurative, 153, 153f
JC virus, 1864-1865, 1864t
tumors and, 1868
JC virus infection, **1863-1870**
clinical manifestations of, 1866-1869
CNS, **1866-1867**, 1866f
diagnosis of, 1868-1869
epidemiology of, 1865-1866, 1865f
after kidney transplantation, 1010
treatment of, 1869
Jenner, Edward, 103
Jewelry, intraoral/perioral, 155
Job syndrome (hyper-IgE syndrome), **55**, 317, 777, 919, 3364
Joint(s)
bacterial infection of. *See* Septic arthritis
fungal infection of, **734**
inflammation of. *See* Arthritis; Juvenile idiopathic (rheumatoid) arthritis; Rheumatoid arthritis
prosthetic, infection with, **1026-1029**
clinical manifestations of, 1027
diagnosis of, 1027-1028
microbiology of, 1027
prevention of, 1029
risk factors for, 1026
treatment of, 1028-1029
synovial fluid of, 728
synovium of, 728
tuberculosis of, 1355-1356
viral infection of, 728
in Whipple disease, 643
Jugular vein, palpable "cord" at, 236
Junín virus infection, 2467-2468, 2470t, 2471, 2475. *See also* Arenavirus infection
immune plasma in, 3533-3534
vaccine against, 2476
Juvenile diabetes mellitus, 72
Juvenile idiopathic (rheumatoid) arthritis
aspirin in, 677
fever of unknown origin in, 840, 847
human parvovirus B19 and, 1851
joint fluid findings in, 732t
Juvenile-onset recurrent respiratory papillomatosis, 1878-1879
Juvenile periodontitis, 140, 147
PMN chemotaxis in, 56

K

K antigen
of *Klebsiella pneumoniae*, 1467
in urinary tract infection, 537-538
Kala-azar. *See Leishmania* spp. infection (leishmaniasis), visceral
Kanagawa phenomenon, in *Vibrio parahaemolyticus* infection, 1557-1558
Kanamycin, 3208-3211, 3208t, 3231t-3233t
in tuberculosis, 1367t
Kaposi sarcoma, 654, 742t-748t, 749, **2019-2021**, 2606
Kaposi sarcoma–associated herpesvirus infection. *See* Human herpesvirus–8 (HHV-8) infection
Kaposi varicelliform eruption, 1943-1944, 1943f, 1953
Karshi virus infection, 2316t
Kartagener syndrome, 195, 267
Kasai procedure, cholangitis and, 658-659, 664-665
Katayama fever, 3064
Kawasaki disease, 180, **1064-1085**
acute, 1072
adult, 1066
age and, 1066-1067
arthritis in, 1082
aspirin in, 677
autoantibodies in, 1077
carpet shampooing and, 1068
cervical lymphadenopathy in, 180
clinical manifestations of, 1072-1075, 1074t, 1081t
communicability of, 1068
complications of, 1081-1082, 1081t
Kawasaki disease *(Continued)*
coronary artery aneurysm in, 1071, 1080-1084
cutaneous manifestations of, 1073-1074
diagnosis of, 1065t, 1069f, 1073
epidemics of, 1067
epidemiology of, 1065-1068
ethnicity and, 1066
etiology of, 1068-1071
family cases of, 1067
gallbladder involvement in, 1074, 1082
gender ratio in, 1065-1066
genetics and, 1070-1071
geography and, 1067
hearing loss in, 1082
hemophagocytic syndrome in, 1082
hepatitis in, 657
historical perspective on, 1064-1065
human bocavirus infection and, 1861
immune response in, 1070, 1072
immunologic findings in, 1076-1078
incidence of, 1065
inclusion bodies in, 1070
incomplete, 1069f, 1075
laboratory findings in, 1075-1078, 1075t
vs. mastoiditis, 237
mitral regurgitation in, 1081
mortality in, 1066
myocardial infarction in, 1081, 1083-1084
pathology of, 1071-1072
peripheral artery aneurysm in, 1081-1082
peripheral gangrene in, 1082
pharyngeal involvement in, 159t-160t, 161
phases of, 1072-1075
prognosis for, 1082-1084
race and, 1066
rash in, 1074
recurrence of, 1067
renal pathology in, 1072
respiratory infection and, 1068
retrovirus and, 1069
seasonality of, 1067-1068
sequelae of, 1080-1081
subacute, 1072
treatment of, 1078-1081, 1078t
failure of, 1080
follow-up after, 1081, 1083-1084
rescue therapy in, 1080
risk assessment after, 1083-1084
urethritis in, 531
uvulitis in, 166
Kawasaki disease–like illness, in *Yersinia pseudotuberculosis* infection, 1529
Kemerovo virus, 2175
Keratitis, **806-809**
Acanthamoeba spp., 808-809, 2977, 2979-2980, 2980f, 2985, 3327t-3346t
Bacillus cereus, 1319
bacterial, 807, 808t
Citrobacter spp., 1431
contact lens–related, 1019
epithelial, 807
fungal, 807-808
contact lens–related, 1019
Fusarium spp., 2846-2847
HSV, 803-804, 1945, 1959
in Kawasaki disease, 1073
in leprosy, 813
microsporidia, 809, 2912-2913
vs. neonatal conjunctivitis, 806
protozoan, 808-809
Pseudomonas aeruginosa, 1591
Rhodococcus ruber, 1426
rubella virus, 2212
Stenotrophomonas maltophilia, 1607

Keratitis *(Continued)*
stromal (interstitial), 807, 812, 819
in congenital syphilis, 1771
syphilitic, 812
vaccinia, 3533
Keratoconjunctivitis
Achromobacter spp., 1574
adenovirus, 1894, 1899-1900, 1908
HSV, 803, 1945
microsporidia, 809, 2912-2913
Kernig sign, 439
Ketoconazole, 3349
in *Candida* esophagitis, 596-597
in *Histoplasma capsulatum* infection, 2825-2826
in *Paracoccidioides* spp. infection, 2793
in *Sporothrix schenckii* infection, 2835
Kidney(s)
abscess of. *See* Renal abscess
aminoglycoside toxicity to, 3210-3211
amphotericin B deoxycholate toxicity to, 3292-3293
Candida spp. infection of, 894, 897
cidofovir toxicity to, 3289
colistin toxicity to, 3208
in *Corynebacterium diphtheriae* infection, 1305
drug clearance by, 3236
in EBV infection, 2004
infection of, 706, 708. *See also* Pyelonephritis; Renal abscess; Urinary tract infection (UTI)
in influenza virus infection, 2345, 2352
radionuclide imaging of, in urinary tract infection, 544-545, 545f
scarring of, 538, 544-545, 545f, 551-552
Schistosoma spp. infection of, 3064
in toxic shock syndrome, 853, 858
transplantation of. *See* Kidney transplantation
tuberculosis of, 1358
ultrasonography of, in urinary tract infection, 544
in Whipple disease, 644
in yellow fever, 2276
Kidney failure
IVIG effects on, 3496
in *Leptospira* spp. infection, 1747-1748, 1752-1753, 1757
in malaria, 2928
in septic shock, 830, 832, 835
Kidney transplantation
antilymphocyte antibody therapy in, 1007
evaluation for, 1003-1004, 1003t
infection after, **1002-1015**
adenovirus, 1010-1011
BK virus, 1867-1869
catheter-related, 1006
CMV, 1007-1008, 3520-3521
early period, 1004-1007, 1005t
EBV, 1008-1009
fungal, 1012-1014
HBV, 1007, 1014
HCV, 1007, 1014-1015
HIV, 1015
HSV, 1006-1007, 1952
human erythrovirus, 1011
human herpesvirus, 1010, 2017
IVIG use and, 3501
late period, 1005t, 1014-1015
middle period, 1005t, 1007-1014
mycobacterial, 1011-1012
Mycobacterium tuberculosis, 1012, 1358
Mycoplasma, 1011
Nocardia spp., 1012
Kidney transplantation *(Continued)*
papillomavirus, 1010
parasitic, 1014
polyomavirus, 1010
pulmonary, 1006
respiratory, 1007, 1014
urinary tract, 1005-1006, 1014
viral, 1006-1007
VZV, 1009-1010
West Nile virus, 1011
wound, 1004-1005
rejection after, 1007
Kikuchi-Fujimoto disease, 181
Kindlin-3, 53-54
Kingella kingae, 1700
carriage of, 1700
Kingella kingae infection, **1699-1704**
bone, 711, 712t, 714, 717-718. *See also* Osteomyelitis
clinical manifestations of, 1702-1703
in daycare facilities, 1701, 3555
diagnosis of, 1703
endocardial, 362, 1703-1704
epidemiology of, 1700-1701
historical perspective on, 1699-1700
immune response to, 1702
joint, 728, 729t, 732-733
pathogenesis of, 1701-1702, 1701f
prevention of, 1704
transmission of, 1700
treatment of, 1703-1704
viral coinfection with, 1701-1702
KIPyV, 1864, 1864t, 1868
Kissing bug bite, 758t
Klebsiella granulomatis, 1640
Klebsiella granulomatis infection, **1640-1644**
clinical manifestations of, 753t-756t, 1641-1642, 1642f
diagnosis of, 1642-1643, 1642f
epidemiology of, 1640-1641
pathogenesis of, 1641
prevention of, 1643-1644
prognosis for, 1643
treatment of, 1643
Klebsiella oxytoca infection, 1468
Klebsiella planticola, 1465-1466
Klebsiella pneumoniae infection, **1465-1469**
antibiotic resistance in, 1466, 1468
biliary, 659-660, 659t, 663
bloodstream, 1467-1468
clinical manifestations of, 1467-1468
diagnosis of, 1468
epidemiology of, 1466
hepatic, 1467
after liver transplantation, 1468
after lung transplantation, 962
neonatal, 882
pathophysiology of, 1466-1467
pulmonary, 1466-1467
treatment of, 1468
vitamin C in, 77
Klebsiella spp., 1465-1466
K antigen of, 1467
Klebsiella spp. infection, **1465-1469.** *See also specific species infections*
antibiotic resistance in, 1468
clinical manifestations of, 1467-1468
CSF shunt, 1030, 1030t
diagnosis of, 1468
endocardial, 362, 367
epidemiology of, 1466
hepatic, 673
pathophysiology of, 1466-1467
peritoneal, 700
in septic shock, 834
Klebsiella spp. infection *(Continued)*
treatment of, 1468
uveitis after, 816
Knee, tuberculosis of, 1355
Koch, Robert, 103
Koilocytosis, in HPV infection, 1882
Kokobera virus infection, 2316t
Koplik spots, 161, 2378, 2381-2382, 2381f
Kostmann syndrome, 917
Koutango virus infection, 2316t
Kunjin virus infection, 742t-748t
Kuru, 2620-2622, 2627t
Kussmaul sign, 374, 374f
Kwashiorkor, 76
Kyasanur Forest disease, **2315-2316**

L

L-Selectin, in phagocyte recruitment, 31-32, 33f
La Crosse virus (LACV), 2514-2517, 2516f
La Crosse virus (LACV) infection, **2513-2527**
clinical manifestations of, 494t-498t, 500, 510, 2519-2520
diagnosis of, 2520-2522, 2521f, 2522t, 2523f
differential diagnosis of, 2522-2523
ecology of, 2516-2517, 2516f
epidemiology of, 2513-2514, 2514f, 2517-2519, 2518t
Glasgow Coma Scale in, 2524
meningeal, 487
pathogenesis of, 2519
pathology of, 2525-2526
prevention of, 2526
prognosis for, 2525
treatment of, 2523-2525
vaccine against, 2526
Laboratory tests, 125-127. *See also specific tests*
accuracy of, 125-126
in bacterial infection, **3589-3595**, 3590t. *See also* Culture, bacterial; Polymerase chain reaction (PCR)
in fungal infection, **3596-3600**, 3597t-3598t
normal vs. abnormal, 125-126, 126f
in parasitic infections, **3620-3627**, 3621t, 3623t, 3624f-3625f. *See also* Parasitic disease, laboratory diagnosis of
predictive value of, 126-127, 126t-127t
reproducibility of, 125
sensitivity of, 125-126
specificity of, 125-126
validity of, 125
in viral infection, **3600-3620.** *See also* Viral infection, laboratory diagnosis of
Labyrinthitis
mastoiditis and, 236
otitis media and, 224
Lacrimal gland, inflammation of, **797**
Lacrimal sac, infection of, **797-798**
β-Lactam antibiotics, 3182-3183. *See also* Penicillin(s)
resistance to, 3139-3145
β-lactamase production and, 3139-3143
carbapenemase production and, 3142
mechanism of, 3140-3143, 3140f-3141f, 3140t, 3202-3203
penicillin-binding protein alterations in, 3143-3145
β-Lactamase(s), 3139-3143, 3140f-3141f
extended-spectrum, 3141-3142, 3202-3203

β-Lactamase(s) *(Continued)*
group 1, 3140-3141, 3140t
group 2, 3140t, 3141-3142
group 3 (metalloenzymes), 3140t, 3142-3143
β-Lactamase inhibitors, 3187-3189
Lactate, CSF, 444
Lactate dehydrogenase, CSF, 444
Lactobacillus spp. infection
endocardial, 362-363
gastrointestinal tract, 626
Lactococcus spp., vs. *Enterococcus* spp., 1171
Lactoferrin
in neonatal *Candida* spp. infection prevention, 2765
in *Streptococcus pneumoniae* infection, 1226
Lactose intolerance, 622
Lagovirus, 2149, 2149f, 2149t
Lagovirus infection, **2154-2155**
Lamivudine, 2613
in HBV infection, 1921-1922
Lamotrigine, pancreatitis with, 691
Lampion (lantern) cells, 1846
Langat virus infection, 2316t
Language development, otitis media and, 222, 222f
Laninamivir, 3270-3273
Lantibiotics, of *Staphylococcus epidermidis*, 1133
Large intestine
enlargement of
in *Shigella* spp. infection, 1481
in *Trypanosoma cruzi* infection, 2965
indigenous flora of, 91t, 92-93
infection of. *See* Gastrointestinal tract infection
Laryngotracheitis, adenovirus, 1897
Laryngitis, 242t-243t, 250t-251t
etiology of, 242-244, 243t-244t
measles virus, 2385, 2392
treatment of, 257
Laryngotracheitis, 242t-243t, **249**, 250t-251t
vs. epiglottitis, 253-254
etiology of, 242-244
measles virus, 2385, 2392
pathogenesis of, 247-248
prognosis for, 259
vs. spasmodic croup, 253
treatment of, 254-257
Laryngotracheobronchitis, 242t-243t, 250t-251t, **252-253**
etiology of, 242-244, 243t-244t
treatment of, 257
Laryngotracheobronchopneumonitis, 242t-243t, 250t-251t, **252-253**
etiology of, 242-244, 243t-244t
treatment of, 257
Larynx, recurrent papillomatosis of, 1878-1879
Lassa virus infection, 656, 2467, 2470, 2470t. *See also* Arenavirus infection
bioterrorist, 3585
clinical manifestations of, 2471-2472, 2471t
diagnosis of, 2474
differential diagnosis of, 2475
exanthem in, 742t-748t, 749, 760
hepatitis in, 2473
immune response to, 2473
nosocomial, 2470-2471
prevention of, 2476-2477
treatment of, 2475
Latency, viral, 20
Latent membrane proteins, 1993-1994
Latent period, in infection, 105-106, 106f
Latex particle agglutination test
in *Haemophilus influenzae* type b infection, 1677-1678
in meningitis, 442, 1263
Lazy leukocyte syndrome, 56
LCMV. *See* Lymphocytic choriomeningitis virus (LCMV) infection
Lead poisoning
in international adoptees and refugees, 3130
vs. St. Louis encephalitis virus infection, 2259
Lebombo virus, 2176
Lecithin cholesterol acetyltransferase (LCAT), 74
Leech therapy, *Aeromonas* spp. infection with, 1543
Leflunomide, in hematopoietic stem cell transplantation–related CMV infection, 940
Left ventricular assist device (LVAD)–associated infection, 1020-1022, 1020f
microbiology of, 1022
Left ventricular dysfunction, in myocarditis, 387-389, 388f
Legionella longbeachae infection, 1713-1714
Legionella pneumophila, 1705-1706, 1706t
antibody to, 1707
Legionella pneumophila infection (Legionnaires' disease), **1705-1714**
clinical manifestations of, 753t-756t, 1710-1711
differential diagnosis of, 1713
dual infection in, 1713
epidemics of, 1708
epidemiology of, 1706-1708
extrapulmonary, 1709-1710, 1709t, 1712-1713
after heart transplantation, 949
historical perspective on, 1705
immune response to, 1709
in immunosuppressed host, 1707
laboratory findings in, 1710-1711
mortality with, 1713
vs. *Mycoplasma pneumoniae* infection, 2684
pathogenesis of, 1708-1710
pathology of, 1708-1709
radiography in, 1710
risk factors for, 1707-1708
treatment of, 1712-1713
urine antigen testing in, 1711
Legionella spp., 1705-1706, 1706t
Legionella spp. infection, **1705-1714.** *See also Legionella pneumophila* infection (Legionnaires' disease)
endocardial, 363
pancreatic, 695
Lemierre's disease, *Arcanobacterium haemolyticum* infection in, 1326
Leiomyoma, 2606-2607
Leiomyosarcoma, 2606-2607
Leishmania braziliensis infection, 757t-758t, 2952f, 2953
Leishmania donovani infection, 2954-2958, 2954f, 2956f
Leishmania mexicana infection, 757t-758t, 2952-2953, 2952f
Leishmania spp., 2948t, 2949
Leishmania spp. infection (leishmaniasis), **2947-2958**
cutaneous, 2948t
New World, 2948t, 2952-2954, 2953f
diagnosis of, 2953
L. braziliensis, 757t-758t, 2952f, 2953
Leishmania spp. infection (leishmaniasis) *(Continued)*
L. mexicana, 757t-758t, 2952-2953, 2952f
prevention of, 2954
treatment of, 2953-2954, 3327t-3346t
Old World, 2948t, 2950-2952
clinical manifestations of, 2950f-2951f, 2951
diagnosis of, 2951
epidemiology of, 2950
pathology of, 2950-2951
prevention of, 2952
treatment of, 2951-2952, 3327t-3346t
epidemiology of, 2949
ocular, 814
in traveler, 3117
visceral, 2948t, 2954-2958
clinical manifestations of, 2955-2956
diagnosis of, 2956-2957, 2956f
hormonal disturbances in, 2955-2956
infantile, 2955
pathology of, 2954-2955, 2954f
prevention of, 2958
prognosis for, 2957
treatment of, 2957-2958, 3327t-3346t
Leishmania tropica infection, 757t-758t, 2950-2952, 2950f-2951f, 2954f
Leishmanin skin test, 2951, 2957
Lemierre disease, 1828
Lemon eucalyptus oil repellent, 2236
Lenses
contact, infection with, 1019
intraocular, infection with, 1019
Lepromin test, 1396, 1397t, 1405-1406
Leprosy, **1392-1410**
in armadillos, 1394, 1396
Bacille Calmette-Guérin immunization in, 1409-1410
borderline, 1397t, 1399-1400, 1400f, 1402
classification of, 1397t, 1401
clinical manifestations of, 753t-756t, 1397t, 1398f-1400f, 1402f-1403f
diagnosis of, 1405-1406
differential diagnosis of, 1405-1406
elimination of, 1410
epidemiology of, 1392, 1394-1396
erythema nodosum in, 1404-1405, 1404f
treatment of, 1409
genetic factors in, 1395-1396
histoid, 1401, 1403, 1405-1406
histopathology of, 1397t, 1398-1401, 1399f-1401f
historical perspective on, 1392-1394
HIV infection and, 1403
human leukocyte antigens and, 1395
immunology of, 1396-1398, 1397t
indeterminate, 1397t, 1398, 1398f-1399f, 1401-1402
in infant, 1394
lepromatous, 1397t, 1400-1403, 1400f-1401f, 1403f-1404f
Lucio, 1403
mouse footpad assay in, 1405
multibacillary, 1401-1403
neuritic, 1403
ocular, 813, 1401
pathogenesis of, 1396-1401
pathology of, 1396-1401, 1396f
paucibacillary, 1401-1402
peripheral nerves in, 1401-1402, 1402f, 1405-1406
placental transmission of, 1393-1394
prevention of, 1409-1410
prognosis for, 1406

Leprosy *(Continued)*
reactions in, 1403-1405, 1404f
treatment of, 1408-1409
reversal reaction in, 1400f, 1404, 1404f
treatment of, 1408-1409
smear examination in, 1405
Toll-like receptors and, 1395-1396
transmission of, 1393-1394
treatment of, 1406-1409, 1407t
tuberculoid, 1397t, 1398-1399, 1399f, 1402, 1402f, 1404f
Lepto Dipstick, 1755-1756
Leptomeningitis. *See also* Meningitis
tuberculous, 1354
Leptospira spp., 1743
lipids of, 1746
virulence factors of, 1745
Leptospira spp. infection (leptospirosis), **1743-1759**
anicteric, 1750-1752, 1751f
animal immunization against, 1757-1758
animal reservoirs in, 1743-1744
cardiovascular findings in, 1748
clinical manifestations of, 753t-756t, 1749-1753, 1749f
diagnosis of, 1754-1756
epidemiology of, 1743-1745
fever of unknown origin in, 844
gallbladder in, 1750, 1751f
hemolytic anemia in, 1746-1747
hepatic, 656, 1746-1747
historical perspective on, 1743
icteric (Weil syndrome), 1749f, 1752-1753
immune response to, 1745-1746, 1750
jaundice in, 1746-1747
musculoskeletal findings in, 1749
nervous system findings in, 1748
ocular involvement in, 812-813, 1748-1749
pancreatitis in, 695
pathophysiology of, 1745-1749
prevention of, 1757-1758
prognosis for, 1753-1754
pulmonary involvement in, 1748, 1750-1751, 1753
renal injury in, 1747-1748
transmission of, 1744-1745
in traveler, 3117
treatment of, 1756-1757
Leptospiral endostatin-like proteins, 1745
Leptospiral immunoglobulin-like proteins, 1745
Lethal factor, in anthrax, 1303
Leuconostoc spp., 1246
vs. *Enterococcus* spp., 1171
Leuconostoc spp. infection, **1246-1247**
clinical manifestations of, 1247
diagnosis of, 1247
epidemiology of, 1246-1247
pathophysiology of, 1247
treatment of, 1247
Leucovorin, in *Toxoplasma gondii* infection, 3000
Leukemia
adult T-cell, 2565-2569, 2566t-2567t
Aspergillus infection and, 2711
Candida infection and, 2743-2744
coagulase-negative staphylococcal bacteremia in, 1135-1136
ecthyma in, 776
hematopoietic stem cell transplantation for. *See* Hematopoietic stem cell transplantation (HSCT)
HSV infection and, 1952
human bocavirus infection in, 1861
Leukemia *(Continued)*
intravenous immunoglobulin in, 3499
mumps immunization and, 2406
Pseudomonas spp. infection and, 1594-1596
VZV infection and, 2026, 2026f
Leukemoid reaction, in *Shigella* spp. infection, 1482
Leukocidins, of *Staphylococcus aureus*, 1115-1117
Leukocyte adhesion deficiency, 904-906, **918-919**
clinical features of, 905f, 918
diagnosis of, 919
pathogenesis of, 907t, 918-919
treatment of, 919
type 1, 52-53, 918-919
type 2, 53, 919
type 3, 53-54, 919
Leukocyte esterase, in urinary tract infection, 543
Leukocyte response integrin (LRI), filamentous hemagglutinin interaction with, 6
Leukotriene(s)
in burns, 1049
in *Streptococcus pneumoniae* infection, 1224-1225
Leukotriene B_4
in Kawasaki disease, 1078
of *Streptococcus agalactiae* infection, 1158
Leukotriene C_4, in bronchiolitis, 277
Levofloxacin
in odontogenic infection, 145
in tuberculosis, 1367t, 1369
Lice, 758t, **3074-3076**
body, 3075-3076
diagnosis of, 3075
disease transmission by, 3075
eyelid, 795, 795f
head, 3074-3076
in daycare facilities, 3559
nosocomial, 3385
pubic, 3075-3076
in relapsing fever, 1740, 1741t
treatment of, 3075-3076, 3327t-3346t
Lichtheimia spp., 2837
Light's criteria, 311-312
Lincomycin, 3220-3222
Lincosamides, 3220-3222, 3231t-3233t
adverse effect of, 3222
drug interactions with, 3222
indications for, 3221-3222
mechanisms of action of, 3220
pharmacokinetics of, 3221
resistance to, 3145-3147, 3220
in vitro activity of, 3220-3221
Lindane shampoo, 795
Linear gingival erythema, 149
Linezolid, 3222-3224, 3231t-3233t
adverse effects of, 3223-3224
in coagulase-negative staphylococcal infection, 1139
indications for, 3223
mechanisms of action of, 3223
in methicillin-resistant *Staphylococcus aureus* infection, 1127
in *Nocardia* spp. infection, 1422
pharmacokinetics of, 3223
resistance to, 3153, 3223
in tuberculosis, 1367t
in vancomycin-resistant *Enterococcus* spp. infection, 1183-1184
in vitro activity of, 3223
Lipase, serum, in pancreatitis, 690, 692
Lipids
eicosanoid, 75
energy metabolism and, 74-75
metabolism of, 73-75, 73t
plasma, in Kawasaki disease, 1076
Lipo-oligosaccharide
Haemophilus ducreyi, 1686
Haemophilus influenzae, 1667-1668
Moraxella catarrhalis, 1251
Neisseria gonorrhoeae, 1278
Neisseria meningitidis, 1254, 1257
Lipovnik virus, 2175
Lipopolysaccharide (LPS), 19, 24
in animal models, 825t, 826-827
Bordetella pertussis, 1618t, 1620
Brucella spp., 1613
components of, 826
Helicobacter pylori, 1693
in meningitis, 435
in septic shock, 827-831, 828f. *See also* Septic shock
Shigella, 1478-1479
in urinary tract infection, 537-538
Lipopolysaccharide-binding protein (LBP), 827, 828f
Lipoproteins, 73-74
high-density, 74
low-density, 74
Lipoteichoic acid, of *Streptococcus pneumoniae*, 1210, 1211f
Liquid crystal thermometer, 86
Listeria monocytogenes, 1329
ActA of, 16
carriage of, 1330
internalins of, 13
Listeria monocytogenes infection (listeriosis), **1329-1335**
clinical manifestations of, 753t-756t, 1331-1332, 1332f, 1332t
clinical manifestations of, 607t, 613
diagnosis of, 618, 1332-1333
endocardial, 362-363
epidemiology of, 1330, 1330f
exanthem in, 753t-756t, 1332, 1332f
immune response to, 1331
after kidney transplantation, 1011
neonatal, 882, 1331-1333, 1332t
pathogenesis of, 15-16, 1330-1331
prevention of, 1333-1334
prognosis for, 1333
transmission of, 1330
treatment of, 1333
Listeriolysin O, 15
Listeriopods, 16
LITH gene, 668-669
Liver. *See also at* Hepatic
abscess of. *See* Liver abscess
anoxic injury to, 657
bacterial infection of, 650, 651t, 656-657. *See also* Liver abscess
in congenital syphilis, 1771
in *Corynebacterium diphtheriae* infection, 1305
in cystic fibrosis, 324-325
drug metabolism by, 3236
Entamoeba histolytica infection of, 2869-2874
fatty infiltration of, 657-658
hydatid disease of. *See Echinococcus granulosus* infection (echinococcosis, hydatid disease)
infarction of, 673
in infectious mononucleosis, 1996, 2003
inflammation of. *See* Hepatitis
in influenza virus infection, 2344

Liver *(Continued)*
isoniazid effects on, 1366-1368
in *Leptospira* spp. infection, 1746-1747, 1752-1753
in *Mycoplasma pneumoniae* infection, 2682
Schistosoma spp. infection of, 3065
in septic shock, 830
in toxic shock syndrome, 853
transplantation of. *See* Liver transplantation
trauma to, abscess with, 673
tuberculosis of, 1357
tumors of, 657
viral infection of. *See* Hepatitis
in yellow fever, 2276
Liver abscess
Achromobacter xylosoxidans, 1574-1575
amebic, 662, 2869-2872
complications of, 2872-2873
differential diagnosis of, 2872
prognosis for, 2874
treatment of, 2873-2874, 2873t
differential diagnosis of, 2872
fever of unknown origin in, 845
Klebsiella pneumoniae, 1467
after liver transplantation, 993
pyogenic, 662, **672-675**, 674f
clinical manifestations of, 673-674
complications of, 675
cryptogenic, 673
diagnosis of, 674, 674f
drainage of, 674-675
epidemiology of, 672
microbiology of, 673
mortality with, 675
pathogenesis of, 672-673
prognosis for, 675
treatment of, 674-675
Liver failure
in *Bacillus cereus* infection, 1320
endogenous bacterial toxins in, 94
Liver flukes, 660, 2865-2866, 3049-3054, 3050f-3051f
Liver transplantation
in HAV infection, 2141
in HBV infection, 1924
in HDV infection, 1932
infection after, **989-1002**
adenovirus, 997t, 999, 1903
age and, 990
bacterial, 993-994
bile duct, 665-666
catheter and, 991, 993
CMV, 996-997, 997t, 1001, 3520-3521
coccidioidal, 1000
cryptococcal, 1000
donor-related factors in, 990
early (0 to 30 days), 991-992, 991t
EBV, 997-998, 997t, 1001
fungal, 813, 991, 993-994, 995t, 2745-2746
HBV, 1924, 3517-3519
HHV-6, 999
HHV-7, 999
Histoplasma capsulatum, 1000
immunosuppression and, 990-991
intermediate (31 to 180 days), 992, 992t
intraoperative factors in, 990
Klebsiella pneumoniae, 1468
late (after 180 days), 992-993, 992t
Mycobacterium tuberculosis, 1000
nosocomial, 991, 993
pancreatic, 692-693
Pneumocystis jiroveci, 1000
post-transplant factors in, 990-991
Liver transplantation *(Continued)*
predisposing factors in, 989-991
pretransplant evaluation in, 1000-1001
pretransplant factors in, 989-990
prevention of, 1001, 3517-3519
RSV, 997t, 999
timing of, 991-993, 991t-992t
viral, 995-1001, 997t
Ljungan virus infection, 2092
Loa loa filariasis (loiasis), 815, **3026-3027**, 3327t-3346t
Long-term care facilities, infection in, 3407
Loperamide
in diarrheogenic *Escherichia coli* infection, 1463
in gastrointestinal tract infection, 626
Lopinavir/ritonavir, 2615
Loracarbef, 3199-3200, 3231t-3233t
Louping ill virus infection, **2315-2316**
Louse. *See* Lice
Louse-borne relapsing fever, **1739-1742**, 1741t
Ludwig angina, 149-150, 150f
Lujo virus infection, 2470-2472, 2470t. *See also* Arenavirus infection
Lumbar puncture. *See* Cerebrospinal fluid (CSF) examination
Lumpy jaw, 184
Lung(s). *See also* Pneumonia
abscess of. *See* Lung abscess
acute injury to, 867. *See also* Acute respiratory distress syndrome (ARDS)
anthrax of, 1312-1314, 3581-3582, 3581f
Aspergillus spp. infection of, 2706, 2708, 2713f, 2717-2721
biopsy of
in *Aspergillus* spp. infection, 2711, 2712f
in hypersensitivity pneumonitis, 304
in interstitial lung disease, 299-300
in *Pneumocystis* infection, 3010
Blastomyces dermatitidis infection of, 2727, 2727f
Cryptococcus neoformans infection of, 2800-2801, 2804
EBV infection of, 2004
Histoplasma capsulatum infection of, 2812-2813, 2826
HIV-related disorders of, 2603-2604, 2603f
hyperlucent, unilateral, 1898
interstitial disease of. *See* Interstitial lung disease
shock, 830, 832
Sporothrix schenckii infection of, 2834
transplantation of. *See* Lung transplantation
in Whipple disease, 644
Lung abscess, **315-322**
age and, 316, 318
clinical features of, 317t, 318
diagnosis of, 319, 319f-320f
differential diagnosis of, 318-319
hematogenous seeding of, 316
immunodeficiency and, 317
Klebsiella spp., 1467
microbiology of, 317-318, 320t
mortality with, 315-316, 321
Mycoplasma pneumoniae, 2677
pathophysiology of, 316-317
primary, 316
prognosis for, 321
rupture of, 318
secondary, 316-317
surgery for, 321
tonsillectomy and, 316
Lung abscess *(Continued)*
treatment of, 319-321
viral infection and, 316
Lung flukes, 2865-2866, 3054-3056
Lung transplantation
in cystic fibrosis, 343-344
HBV infection and, 1924
historical perspective on, 957
indications for, 957
infection after, **957-988**
Acinetobacter, 962
adenovirus, 981-982
anastomotic, 959-960
Aspergillus spp., 959-960, 965-970
bacterial, 960-965
Blastomyces dermatitidis, 974
bloodstream, 960
Bordetella bronchiseptica, 983
bronchiolitis obliterans and, 982
Burkholderia cepacia, 960-962
Candida spp., 970-971
CMV, 974-976, 3520-3521
Coccidioides immitis, 973
Cryptococcus neoformans, 971-972
EBV, 976-978
Escherichia coli, 962
etiology of, 957, 958t
fungal, 959-960, 965-974
HHV-6, 979
HHV-7, 979
HHV-8, 979
Histoplasma capsulatum, 973-974
hMPV, 980
HSV, 978
immunosuppression and, 958-959
incidence of, 957
influenza virus, 980-981
Klebsiella pneumoniae, 962
LCMV, 983
Mycobacterium tuberculosis, 962-964
nontuberculous *Mycobacterium* spp., 964-965
parainfluenza virus, 980, 2368
Pneumocystis jiroveci, 972-973
Pseudomonas aeruginosa, 960-962
rabies, 983
risk factors for, 959
RSV, 979-980
sites of, 959-960
thoracic cavity, 959-960
timing of, 958-959, 958t-959t
treatment duration in, 959
viral, 957, 974-982
VZV, 978-979
West Nile virus, 983
zoonotic, 983
in interstitial lung disease, 300
mortality after, 957
Lupus pneumonitis, 297
Lupus vulgaris, 753t-756t
Lutein, 77
Lycopene, 77
Lyell syndrome, 753t-756t
Lymantria dispar. *See* Gypsy moth rash
Lyme disease, **1729-1739**
acrodermatitis chronic atrophicans in, 1734
Anaplasma spp. infection and, 1734, 2663
arthritis in, 730, 1731-1733, 1736
asymptomatic seroconversion in, 1734
Bannwarth syndrome in, 1733
cardiac manifestations of, 1733, 1735
chronic, 1736-1737
clinical manifestations of, 1731f, 1732-1734

Lyme disease *(Continued)*
coinfection in, 1734
diagnosis of, 1734-1735
epidemiology of, 1730
erythema migrans in, 753t-756t, 1731f, 1732, 1734-1735
historical perspective on, 1729
immune response to, 1730-1731
vs. infective endocarditis, 358-359
lymphocytoma in, 1731f, 1734
meningeal, 1732-1733
neurologic manifestations of, 494t-498t, 1732-1735
nonspecific illness in, 1733
ocular manifestations of, 812
pathogenesis of, 1730-1732
in pregnancy, 1734
vs. tick-borne encephalitis virus infection, 2311
vs. tick-borne relapsing fever, 1741t
treatment of, 1735-1738, 1736t
Lymph node(s)
cervical, 176, 176f. *See also* Cervical lymphadenitis
in infectious mononucleosis, 1996, 1999, 1999f, 1999t
in Kawasaki disease, 1074
in monkeypox, 2042
in *Paracoccidioides* spp. infection, 2784, 2784f-2785f, 2786
in rubella virus infection, 2210-2211, 2218
in *Toxoplasma gondii* infection, 2991
in Whipple disease, 644
Lymphadenitis
cervical. *See* Cervical lymphadenitis
in human erythrovirus B19 (human parvovirus B19) infection, 1854-1855
mesenteric
in adenovirus infection, 1902
in tuberculosis, 1357
in nontuberculous mycobacterial infection, 1383-1384
Streptococcus agalactiae, 1161
tuberculous, superficial, 1356
Lymphocyte. *See* B lymphocytes; T lymphocytes
Lymphocyte-specific protein-1 (LSP-1), 54
Lymphocyte transformation test, in *Toxoplasma gondii* infection, 2996
Lymphocytic choriomeningitis virus (LCMV) infection, 811-812, **2463-2466**
clinical manifestations of, 2464-2465
congenital, 817, 2465
diagnosis of, 2465
encephalitic, 494t-498t, 500
epidemiology of, 2463-2464
historical perspective on, 2463
after lung transplantation, 983
meningeal, 487
in transplant recipient, 2465
treatment of, 2465
Lymphocytic interstitial pneumonitis, 296, 1997, 2007
HIV-related, 2603-2604, 2603f
Lymphocytoma, borrelial, 1731f, 1734
Lymphogranuloma venereum, 582-584, 583t, 584f, **2635-2636**
vs. plague, 1513
Lymphohistiocytosis, hemophagocytic. *See* Hemophagocytic lymphohistiocytosis (hemophagocytic syndrome)
Lymphoid interstitial pneumonitis/hyperplasia, vs. chronic bronchitis, 268
Lymphokines, 3357-3362
Lymphoma
body cavity, 2007
Burkitt, 1996, 2005, 2005f, 2013
cerebral, 2011, 2013
coagulase-negative staphylococcal bacteremia in, 1135-1136
Hodgkin, 182-183, 182t, 1997, 2005, 2013
HTLV-1–associated, 2569
non-Hodgkin, 182-183, 182t, 2006-2007
pancreatic, 692
plasmablastic, 2006
pyothorax-associated, 2006
T-cell, 2006
in Whipple disease, 644
Lymphomatoid granulomatosis, 2006
Lymphoproliferative disease
post-transplant. *See* Post-transplant lymphoproliferative disease (PTLD)
X-linked, 915
Lysogeny, of *Streptococcus pneumoniae*, 1215
Lysosomal trafficking regulator (LYST), **54**
Lysozyme, 24, 34t, 35
Lyssavirus, 2445-2447, 2445f-2446f. *See also* Rabies

M

M protein, 10, 18
in rheumatic fever, 405-406, 405t, 407f
Streptococcus pyogenes, 1140
Machupo virus infection, 2466, 2468-2469, 2470t, 2471. *See also* Arenavirus infection
Macrolides, 3211-3215
adverse effects of, 3214-3215
dosage schedules for, 3231t-3233t
drug interactions with, 3215
indications for, 3213-3214
mechanisms of action of, 3211
pharmacokinetics of, 3212-3213, 3213t
resistance to, 3145-3147, 3211-3212
in vitro activity of, 3212
Macrophage(s)
in burns, 1050
in Whipple disease, 641-642, 642f, 645
Macrophage activation syndrome, in Kawasaki disease, 1082
Mafenide acetate, in burn injury, 1058-1059
Maggots, ocular, 816
Magnesium sulfate, in tetanus, 1816-1817
Magnetic resonance cholangiopancreatography (MRCP), 690
Magnetic resonance imaging (MRI)
in acute disseminated encephalomyelitis, 515f, 518-519
in acute transverse myelitis, 522, 522f
in amebic CNS infection, 2980
in *Aspergillus* spp. infection, 2707, 2713-2714, 2714f
in bacterial meningitis, 445-446, 454
in *Blastomyces dermatitidis* infection, 2730f
in brain abscess, 466-467
in chronic fatigue syndrome, 1088
in chronic osteomyelitis, 726, 726f
in Creutzfeldt-Jakob disease, 2628
in cryptococcal meningoencephalitis, 2801-2802
in Eastern equine encephalitis, 2228
in encephalitis, 506-507
in HSV encephalitis, 1948f
in HTLV-1 infection, 2571
in intra-abdominal abscess, 705
in Japanese encephalitis virus infection in, 2296
in myocarditis, 392-393
Magnetic resonance imaging (MRI) *(Continued)*
in necrotizing fasciitis, 779
in neurocysticercosis, 3035-3036, 3036f
in osteomyelitis, 715-716, 716f
in pericarditis, 377
in progressive multifocal leukoencephalopathy, 1866-1867, 1866f
in pyogenic liver abscess, 674
in pyomyositis, 737, 738f
in retroperitoneal infection, 707f, 708
in rhinosinusitis, 199
in *Schistosoma* spp. infection, 3067-3068
in spinal cord infarction, 524f
in spinal epidural abscess, 471-472, 472f
in subdural empyema, 469
in tick-borne encephalitis virus infection, 2285, 2308f
in urinary tract infection, 545-546
in vertebral osteomyelitis, 722
in West Nile virus infection, 2270
Magnetic resonance sialography, 192
Majeed syndrome, 714
Major histocompatibility complex (MHC)
class I, 36-37, 38f
class II, 37, 38f
Malaria, **2921-2947**
acid-base changes in, 2928
anemia in, 76, 78, 2926-2927
autochthonous, 2923
bloodborne, 2923
cerebral, 2928
chemoprophylaxis in, 2943, 2944t
in children, 2929
clinical manifestations of, 757t-758t, 2926-2929
congenital, 2924, 2929
cryptic, 2925
diagnosis of, 2930-2936
antibody detection in, 2936
antigen detection in, 2935
Buffy coat test in, 2930-2935
dipstick tests in, 2935
DNA probes in, 2935
flow cytometry in, 2936
microscopy in, 2930, 2931f-2934f
PCR in, 2935
EBV and, 1996
endemic, 2923
epidemiology of, 2923, 2924f-2925f, 3107-3108
etiology of, 2922-2923, 2922f, 2927t
fever of unknown origin in, 844
genetic factors in, 2926
glucose metabolism in, 72-73
historical perspective on, 2921-2922
host-parasite interaction in, 2925-2926
hyperactive malarial syndrome in, 2929
hyperlactatemia in, 75-76
hypoglycemia in, 2928
in international adoptees and refugees, 3129
metabolic acidosis in, 75-76
mortality from, 2922
mosquito bite in, 2922f, 2923, 2925-2926
myocarditis in, 400
nitrogen balance in, 68
ocular manifestations of, 814
pancreatitis in, 694-695
pathophysiology of, 2926, 2927t
personal protective measures in, 2943
in pregnancy, 2929-2930
prevention of, 2943-2946, 2944t
in travelers, 3108-3110, 3109f, 3111t

Malaria *(Continued)*
recurrent, 2926
renal complications of, 2928
respiratory complications of, 2928
Salmonella spp. infection and, 1498
Schistosoma spp. infection and, 3063-3064
serum iron in, 78
severe, 2927, 2928t
thyroid hormone levels in, 82
transmission of, 2923-2925
in travelers, 2926, 3108-3110, 3109f, 3111t
treatment of, 2936-2943, 2937t-2940t, 3327t-3346t
adjunctive, 2943
emergency, 2945-2946
supportive, 2942-2943
vaccine against, 2946
Malassezia spp. infection, **789**, 789f, **2852-2854**
neonatal, 789, 789f, 897-898
Malathion, 3349
in lice, 3075
Malignant otitis externa, 207-208
Malnutrition
bacterial meningitis and, 437
Cryptosporidium spp. infection and, 2901
hookworm infection and, 3017
murine tuberculosis and, 70
protein, 108-109
Mandible, osteomyelitis of. *See* Osteomyelitis, mandibular/maxillary
Manganese, metabolism of, 77t
Mannitol, in encephalitis, 510
Mannose-binding lectin (MBL)
deficiency/dysfunction of
furunculosis and, 777
Neisseria meningitidis infection and, 1257
in *Streptococcus pneumoniae* infection, 1205, 1226
Mansonella ozzardi infection, **3028**, 3327t-3346t
Mansonella perstans infection, **3028**, 3327t-3346t
Mantoux test. *See* Tuberculin skin test
Maraviroc, 2611
Marburg virus, 2478-2479
Marburg virus disease (Marburg fever, Marburg hemorrhagic fever), **2478-2486**
bioterrorist, 3585
clinical manifestations of, 2483
diagnosis of, 2484
differential diagnosis of, 2483-2484
epidemiology of, 2478-2479, 2479f, 2480t, 2482-2483
exanthem in, 742t-748t, 749, 760
pathology of, 2483
prevention of, 2485
treatment of, 2484-2485
Maribavir, in CMV infection, 1986
Marine toxins, 604, 605t
Mass spectroscopy, 3594-3595
Masticator space, infection of, 142, 148-149, 149f
Mastoid process, 224, 233-234
Mastoidectomy, 238-239
Mastoiditis, **233-240**
anatomy of, 233-234
chronic, 234, 236, 239
clinical presentation of, 234-236, 236f
coalescent, 233
complications of, 236-237
diagnosis of, 237-238, 238f
differential diagnosis of, 237
Mastoiditis *(Continued)*
historical perspective on, 233
microbiology of, 234, 235t
otitis media–related, 223-224
pathophysiology of, 233-234
prevention of, 240
prognosis for, 240
Streptococcus pneumoniae, 1231
treatment of, 238-240
Matrix-assisted laser desorption ionization–time-of-flight mass spectrometry, in *Streptococcus pneumoniae* infection, 1216
Matrix metalloproteinase–9, in ARDS, 870
Maxilla, osteomyelitis of. *See* Osteomyelitis, mandibular/maxillary
Mayaro fever, 2252-2253
MCPyV, 1864, 1864t, 1867-1868
Measles, **2373-2395**
acute disseminated encephalomyelitis with, 516
appendiceal, 2392
aspirin in, 85
atypical, 2383-2384
clinical manifestations of, 2383-2384, 2384f
diagnosis of, 2391
exanthem in, 2384
historical aspects of, 2383
pathogenesis of, 2384
vs. Rocky Mountain spotted fever, 765
treatment of, 2392
cardiac involvement in, 2385
clinical manifestations of, 2381-2391
CNS, 494t-498t, 500, 2385-2386, 2391-2392
complications of, 2384-2386, 2392
corneal, 807
in daycare facilities, 3562-3563
in developing countries, 2390
diagnosis of, 2382, 2391
differential diagnosis of, 2391
epidemiology of, 2375-2377, 2375f, 2376t
eradication of, 2394
exanthem in, 742t-748t, 749, 2378, 2382, 2382f
fulminant, 2390
geographic distribution of, 2376
hepatitis with, 655
historical perspective on, 2373-2374
host factors in, 2376-2377
immune response to, 2379-2380, 2380t
immunization against, 2393-2395, 3448-3452
acute disseminated encephalomyelitis with, 517
adverse events with, 3449-3450
autism and, 3429
complications of, 2393-2394
contraindications to, 2393, 3450-3452
efficacy of, 3449
encephalitis after, 502
in HIV-infected host, 3429
immune globulin interaction with, 3451, 3452t
indications for, 2393, 3450
meningitis and, 488
precautions for, 2393, 3450-3452
preparations for, 3449
in immunocompromised host, 2390-2391
incidence of, 2376, 2376t
incubation period in, 2381
Koplik spots in, 2378, 2381-2382, 2381f
laboratory findings in, 2382
laryngitis in, 2385, 2392
modified, 2382-2383
Measles *(Continued)*
mutant, 2388-2389
nosocomial, 3383-3384
ocular manifestations of, 811
otitis media in, 2385, 2392
pathogenesis of, 2377-2378, 2378t
pathology of, 2378, 2378f
pharyngeal, 159t-160t, 161
prevalence of, 2375-2376, 2375f
prevention of, 2381
antibiotics in, 2392-2393
immunization in, 2393-2395
immunoglobulin in, 2394-2395, 3526
quarantine in, 2394
prodromal period in, 2381-2382, 2381f
pulmonary involvement in, 2384-2385, 2384f, 2391-2392
recovery from, 2380-2381
recurrent, 2383
reporting requirement for, 2394
respiratory tract involvement in, 2378, 2392
seasonal patterns of, 2376
subacute sclerosing panencephalitis after, **2386-2390**
thrombocytopenic purpura in, 2386
tracheitis in, 2385, 2392
transmission of, 2377
treatment of, 2391-2393
vitamin A deficiency and, 807, 2391-2392
Measles virus, 2374-2375
animal susceptibility of, 2375
antigenic composition of, 2374
classification of, 2374
isolation of, 2374-2375, 2375f
physical properties of, 2374, 2374f
Mebendazole, 3349
in *Echinococcus granulosus* infection, 3046
in *Enterobius vermicularis* infection, 567, 3019
Mechanical ventilation, 870-872
low tidal volume, 871
permissive hypercapnia with, 871-872
permissive hypoxemia with, 872
pneumonia with, 892, 3391-3393
Meconium ileus, in cystic fibrosis, 324-325
Mediastinitis, **419-423**
acute, 419-422, 420t
esophageal perforation and, 419-420
infection extension and, 420-421, 421f
postoperative, 421-422, 421t
chronic, 420t, 422-423
fibrosing, 422
in *Histoplasma capsulatum* infection, 2813, 2817-2819, 2828-2829
after heart transplantation, 948-949
postoperative, 421-422, 421t
retropharyngeal abscess and, 172
Mediastinum, 419
Mediterranean spotted fever, 2648t-2649t, **2655-2656**
Mefloquine, 3349
in malaria, 2937t-2940t, 2941
in malaria prevention, 2944t, 2945
Megacolon
toxic, with *Shigella* spp. infection, 1481
in *Trypanosoma cruzi* infection, 2965
Meglumine antimoniate, 3349
in visceral leishmaniasis, 2957
Meibomian gland
granulomatous inflammation of, **796-797**
infection of, **796**
Melanin, *Cryptococcus neoformans* production of, 2796-2797

Melarsoprol, 3349
in *Trypanosoma brucei* infection, 2972
Melioidosis. *See Burkholderia pseudomallei* infection (melioidosis)
Meningitest, 445
Meningitis
adrenal function in, 81
aseptic, **484-492**
adenovirus, 488, 1889t, 1903
arbovirus, 486t, 487
bacterial, 486t, 488-489
vs. bacterial meningitis, 444-445
C-reactive protein in, 444
Chlamydia pneumoniae, 488
clinical manifestations of, 489-490
diagnosis of, 490-491
differential diagnosis of, 490
drug-related, 488
EBV, 2003-2004
enterovirus, 485-487, 489, 2086-2090, 2088t-2089t
epidemiology of, 488-489
etiology of, 485-488, 486t
exanthem in, 765, 766t
fungal, 486t, 488
HHV-6, 487, 2018
HHV-7, 487, 2018
historical perspective on, 485
HIV-1, 487, 2605
HSV, 487, 1943
vs. HSV encephalitis, 1946
human erythrovirus B19 (human parvovirus B19), 1853
IVIG-related, 3496
La Crosse virus, 487
LCMV, 487
mumps virus, 488, 2401
vs. tick-borne encephalitis virus infection, 2310-2311
Mycobacterium tuberculosis, 488-489, 1352-1354, 1373
Oropouche fever virus, 2542-2543
parasite, 486t, 488
PCR in, 444
poliovirus, 2098. *See also* Poliovirus infection (poliomyelitis)
prognosis for, 491
recurrent, 489-490, 1946
reovirus, 2171
rotavirus, 2186
Toscana virus, 2548
treatment of, 491
VZV, 487, 2031
West Nile virus, 487, 489, **2261-2273.** *See also* West Nile virus infection
bacterial, **425-461**
Achromobacter spp., 1574
Acinetobacter spp., 1570
Actinomyces spp., 1822-1823
adjunctive therapy in, 449-453
Aerococcus viridans, 1249
Aeromonas spp., 1541
anti-inflammatory therapy in, 451
antidiuretic hormone in, 438, 453-454
arthralgia in, 441
arthritis in, 441
Bacillus anthracis, 1314
Bacillus cereus, 1320
bacteremia in, 431-432, 432t
Bacteroides spp., 1828-1829
blood-brain barrier in, 432-433, 433t
Bordetella holmesii, 1637
Borrelia spp., 1732-1733
brain abscess and, 466
Brucella spp., 1613-1615
Meningitis *(Continued)*
C-reactive protein in, 444
cancer and, 437
cellulitis in, 440-441
cerebral edema in, 439
chronic systemic disease and, 437
Citrobacter spp., 1429-1430, 1432-1433, 1432f
clinical manifestations of, 438-441, 439t-440t
clinical score in, 445
Clostridium spp., 1797
cochlear implant and, 437, 1017-1018, 1229
complications of, 1228-1229
corticosteroids in, 451-453
countercurrent immunoelectrophoresis in, 442
cranial nerve involvement in, 439
Cronobacter sakazakii, 1436, 1438
CSF examination in, 441-446, 443t-444t, 449, 451t, 455
vs. CSF shunt infection, 1030
CT in, 445-446, 446f, 454, 458
culture in, 442, 445
diagnosis of, 441-446, 443t-444t, 446f
differential diagnosis of, 441
Ehrlichia, 2662
Elizabethkingia meningosepticum, 1579-1581
Enterococcus spp., 1175-1176
epidemiology of, 425-430
Erysipelothrix rhusiopathiae, 1328
Escherichia coli, 1444-1445
epidemiology of, 1441
pathogenesis of, 1443t, 1444
postoperative, 1445
treatment of, 1447
exanthem in, 765, 766t
ferritin in, 445
focal neurologic signs in, 439, 440t
Fusobacterium spp., 1828-1829
genetic factors in, 436
glycerol in, 453
Gram stain in, 445
Haemophilus influenzae, 426t, 429-430, 430f, 450t-451t, 460-461, 1670, 1675, 1678-1679
Haemophilus parainfluenzae, 1690
Hafnia alvei, 1535
hearing loss and, 452, 457-458, 1259
humidified environment and, 431
hydrocephalus in, 438, 445
impaired consciousness in, 438
incidence of, 425-430, 426t
increased intracranial pressure in, 438-439, 454
inflammation in, 433-435, 434f
lactate dehydrogenase in, 444
lactate in, 444
latex particle agglutination test in, 442
Leptospira spp., 1748, 1751
Leuconostoc spp., 1247
malnutrition and, 437
mastoiditis and, 239
Meningitest in, 445
Moraxella catarrhalis, 1252-1253
Morganella morganii, 1470-1471
MRI in, 445-446, 454
mucosal colonization in, 431-432
Neisseria gonorrhoeae, 1288
Neisseria meningitidis, 426t, 428-429, 428f, 450t-451t, 459-460, 460t, 1258-1260. *See also Neisseria meningitidis* infection
Meningitis *(Continued)*
neonatal, 431, 436, 881-882, 885-887
neurologic sequelae of, 455-457, 456t-457t
neuronal injury in, 434f, 435-436
Nocardia spp., 1421
optic nerve involvement in, 440
otitis media and, 224
outpatient therapy in, 449
Pantoea agglomerans, 1536
Pasteurella multocida, 1547
pathogenesis of, 431-437, 446t
pathology of, 437-438, 437f
pathophysiology of, 431
pericardial effusion with, 441
persistent fever in, 455
Plesiomonas shigelloides, 1565-1566
polymerase chain reaction test in, 442-444
prevention of, 458-461, 3249
PRISM score in, 458
procalcitonin in, 444
prognosis for, 455-458
Proteus mirabilis, 1472-1474
Providencia spp., 1475
Pseudomonas aeruginosa, 1598-1599
radionuclide imaging in, 445
rapid antigen detection test in, 442
recurrent, 430-431, 441, 449
risk factors for, 436-437
route of infection in, 431
Salmonella spp., 1500-1501, 1505
seizures in, 439-440, 440t, 455
sequelae of, 455-458, 456t-457t
Serratia spp., 1490
shock in, 440, 454
vs. St. Louis encephalitis virus infection, 2259
Stenotrophomonas maltophilia, 1607
Streptococcus agalactiae, 1160, 1160f, 1162
Streptococcus pneumoniae, 426-427, 426t, 437, 450t-451t, 458-459, 1228-1229, 1235. *See also Streptococcus pneumoniae* infection
Streptococcus viridans, 1193
subdural effusion in, 438, 440, 454-455
supportive care in, 453-455
vs. tick-borne encephalitis virus infection, 2311
trauma-related, 432
treatment of, 446-455, 446t
anti-inflammatory therapy in, 451
antibiotics in, 446-449, 450t-451t
chemoprophylactic, 458-461
corticosteroids in, 451-453
CSF examination and, 442
fluid restriction in, 453-454
glycerol in, 453
immunoprophylactic, 458-460
supportive care in, 453-455
venous thrombosis in, 438
ventriculitis in, 437-438
vs. viral meningitis, 444-445
Yersinia pestis, 1512-1513
in burn patient, 1056
chemical, 431
cochlear implant and, 224, 437, 1017-1018, 1229
craniofacial surgery and, 1044-1045
eosinophilic, **481-484**
clinical manifestations of, 482-483
diagnosis of, 483
differential diagnosis of, 483
epidemiology of, 482

Meningitis *(Continued)*
etiology of, 481-482
pathogenesis of, 482
prevention of, 484
prognosis for, 484
treatment of, 483-484
fungal, **473-481**, 486t, 488
Acremonium spp., 479
Aspergillus spp., 478-479
Blastomyces dermatitidis, 478, 480t
Candida, 474-475, 480t, 2742-2743, 2749
neonatal, 894-895, 897, 2754, 2757
clinical manifestations of, 473-479
Coccidioides spp., 477-478, 480t, 483, 2774, 2778
Cryptococcus neoformans, 475-476, 480t
diagnosis of, 474, 479-480, 480t
epidemiology of, 473
Histoplasma capsulatum, 476-477, 480t, 2816, 2821, 2828
Mucor spp., 479
Sporothrix schenckii, 479
Mollaret, 489-490, 1946
Mycoplasma pneumoniae, 2683-2684
neonatal, 882
Candida, 894-895, 897
Clostridium spp., 1797
coagulase-negative staphylococci, 1135
Klebsiella pneumoniae, 882
Listeria monocytogenes, 882
Neisseria meningitidis, 1260
Streptococcus agalactiae, 881-882
Streptococcus viridans, 1192
noninfectious, 441
Meningococcal meningitis, 426t, 428-429, 428f, 450t-451t, 459-460, 460t, 1258-1260. *See also Neisseria meningitidis* infection
Meningococcemia, 432, 432t, 824, 829, 831, 1258-1260
chronic, 1260
cutaneous manifestations of, 1259, 1259f
differential diagnosis of, 1258t
treatment of, 833-834, 833t
Meningoencephalitis, **492-512.** *See also* Encephalitis
amebic, 501, 3327t-3346t
CMV, 1978-1980, 1979f
cryptococcal, 2801-2803
clinical manifestations of, 2801
complications of, 2802-2803
diagnosis of, 2801-2802
treatment of, 2804-2805
enteroviral, 2104, 3513
historical perspective on, 493
HSV, 1954
neonatal, 805
Morganella morganii, 1470
mumps, 2401, 2403
Naegleria fowleri, 2979, 2979f, 2981, 2984-2985, 3327t-3346t
rubella virus, 2213-2216, 2214t-2216t
Menstruation, *Staphylococcus aureus* infection and. *See* Toxic shock syndrome (TSS)
Mepitel, in burn injury, 1059
Merkel cell carcinoma, 1867-1868
Meropenem, 3201-3202, 3231t-3233t
in meningitis, 448, 450t
Mesenteric adenitis
Yersinia enterocolitica, 1520-1521, 1527
Yersinia pseudotuberculosis, 1521
Mesenteric lymphadenopathy, in *Yersinia enterocolitica* infection, 1527, 1527f
Meta-analysis, 124-125
Metabolic acidosis, in malaria, 2928
Metabolic disease
hepatitis with, 657
pancreatitis with, 691t, 692
vs. Reye syndrome, 677
Metabolism, **65-83**, 66f
acid-base, 75-76
amino acid, 70-72
carbohydrate, 72-73
copper, 79
electrolyte, 75-76
iron, 78
lipid, 73-75, 73t
nitrogen, 67-70, 68f-69f
selenium, 79
trace element, 77-79, 77t
vitamin, 76-77
zinc, 78-79
Metagonimus spp. infection (heterophyiasis), **3057-3058**
Metamorphopsia (Alice in Wonderland syndrome), 505, 2004
Metapneumoviruses. *See* Human metapneumovirus (hMPV)
Methicillin, 3183t-3184t, 3185-3186, 3231t-3233t
Methicillin-resistant *Staphylococcus aureus*. *See Staphylococcus aureus*, methicillin-resistant (MRSA)
3-Methylhistidine, 71
urinary, 69-70
Metorchis spp. infection (metorchiasis), **3058**, 3327t-3346t
Metronidazole, 3229-3230, 3231t-3233t, 3349
adverse effects of, 3230
in bacterial meningitis, 450t
in bacterial vaginosis, 578
in *Clostridium difficile* infection, 637
in odontogenic infection, 145, 146t
Mezlocillin, 3183t-3184t, 3187, 3231t-3233t
MHA-TP (microhemagglutination assay–*Treponema pallidum*), 1773-1774, 1773t
Micafungin, 3308t, 3313f, 3318-3319, 3319t
in *Aspergillus* spp. infection, 2718
in hematopoietic stem cell transplantation–related aspergillosis, 938
in lung transplant–related aspergillosis, 968
Miconazole, 3349
in *Naegleria fowleri* infection, 2984
Microbacterium spp. infection, in otitis externa, 206
Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), 8-9
Staphylococcus aureus, 1118
Microbiome, **88-94**
adverse effects of, 94
beneficial effects of, 93-94
exogenous influences on, 89-90
site-specific, 90-93, 91t
Microhemagglutination assay–*Treponema pallidum* (MHA-TP), 1773-1774, 1773t
Microscopic agglutination test (MAT), in *Leptospira* spp. infection, 1755
Microsporidia, 2864, 2911-2912, 2911t
Microsporidia infection, **2911-2913**
biliary tract, 2912
clinical manifestations of, 2912-2913
diagnosis of, 2913
disseminated, 2913
epidemiology of, 2912
Microsporidia infection *(Continued)*
gastrointestinal, 607t, 615, 2912
stains for, 617
treatment of, 625, 2913
ocular, 809, 2912-2913
treatment of, 2913, 3327t-3346t
Microsporum canis infection, 784
Midazolam, in tetanus, 1816
Military recruits
adenovirus infection in, 1892, 1896-1898, 1896t
viral pneumonia in, 1897-1898, 2026
Milk
infection and, 109, 3547
raw, 602
in tick-borne encephalitis virus infection, 2306-2307
Yersinia enterocolitica contamination of, 1518
Milker's nodules, 742t-748t
Miller-Fisher syndrome, after *Campylobacter jejuni* infection, 1654
Miltefosine, 3349
in *Leishmania* spp. infection, 2952, 2958
Mimivirus infection, **2048-2050**, 2049f-2050f
Minocycline, 3217-3220, 3218t
in *Nocardia* spp. infection, 1422
Mirizzi syndrome, 670
Mist therapy
in acute bronchitis, 264
in croup, 255
Mites, 758t, 795-796, 796f, **3073**
Mitoxantrone, in monkeypox, 2044
Mitral regurgitation, in Kawasaki disease, 1081
Modoc virus infection, 2316t
Mollaret meningitis, 489-490, 1946
Molluscum contagiosum, **782-783**
clinical manifestations of, 742t-748t, 782-783, 783f
epidemiology of, 782
eyelid, 794-795, 795f
genital, **581-582**
Molluscum contagiosum virus, 2047
Molluscum dermatitis, 783
Monkeypox, **2040-2044**
bioterrorist, 2044
clinical features of, 742t-748t, 749, 2042
congenital, 2042
diagnosis of, 2042-2043, 2043t
epidemiology of, 2040-2042
exanthem of, 742t-748t, 749, 2042
prevention of, 2043-2044
vs. smallpox, 2036
treatment of, 2043-2044
Monobactams, 3200, 3231t-3233t
resistance to, 3139-3145
Monoclonal antibodies, **3355-3357**, 3493t, **3502-3503**
adenovirus infection and, 1904
in diarrheogenic *Escherichia coli* infection, 1463
in heart transplant–related RSV infection, 950
in hematopoietic stem cell transplantation–related EBV infection, 940-941
in hematopoietic stem cell transplantation–related RSV infection, 943
in Kawasaki disease, 1080
in lung transplant–related RSV infection, 980

Monoclonal antibodies *(Continued)*
in post-transplant lymphoproliferative disease, 977-978, 2012-2013
in RSV infection, 2426-2427, 2429, 3503, 3529-3530
in septic shock, 835
in toxic shock syndrome, 857
in West Nile virus infection, 2272
Monokines, 3357-3362
Mononucleosis
CMV-induced, 1976, 1981
EPV-induced. *See* Infectious mononucleosis
Monosodium glutamate, 604, 604t
Montelukast, in RSV infection, 2426
Moon molars, 1771
Moraxella catarrhalis, 1251
lipo-oligosaccharide of, 1251
Moraxella catarrhalis infection, **1250-1253**
clinical manifestations of, 753t-756t, 1252-1253
epidemiology of, 1251-1252
immune response to, 1251
in mastoiditis, 234
nosocomial, 1252
in otitis media, 212-215, 212t
pathogenesis of, 1251
treatment of, 1253
vulvovaginal, 567
Moraxella spp. infection, conjunctival, 802
Morganella morganii infection, **1469-1471**
Morphine sulfate, in tetanus, 1817
Mosquito bite, 758t
in malaria, 2922f, 2923, 2925-2926
Motavizumab, in RSV infection, 2429
Moth-associated dermatitis, 758t
Motor neuron disease, enteroviral infection and, 2090-2091
Mouth. *See* Oral cavity
Moxifloxacin
in odontogenic infection, 145
in tuberculosis, 1369
MRSA. *See Staphylococcus aureus*, methicillin-resistant (MRSA)
MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), 8-9
of *Staphylococcus epidermidis*, 1132-1133
Mucocutaneous lymph node syndrome. *See* Kawasaki disease
Mucor spp., 2837, 2838f
Mucor spp. infection (mucormycosis), **2839-2844**
burn wound, 1053
clinical manifestations of, 2840-2842, 2840t-2841t
cutaneous, 791f, 2841, 2841f
diagnosis of, 2842-2843
disseminated, 2842
epidemiology of, 2837-2839
gastrointestinal, 2842
after hematopoietic stem cell transplantation, 938
after kidney transplantation, 1013
meningeal, 479
neonatal, 899
pathogenesis of, 2839-2840
pathology of, 2840
prevention of, 2844
prognosis for, 2844
pulmonary, 2841-2842
rhinocerebral, 2840
risk factors for, 2839, 2839t
treatment of, 2843-2844
Mucous membranes, bacterial colonization of, 431-432
Mucous patches, in congenital syphilis, 1765, 1770
Mulberry molars, 1771
Multiceps multiceps infection. *See Taenia multiceps* infection (coenurosis)
Multiple sclerosis, 515, 518, 520
HBV vaccine and, 1926, 3429
Mumps, 190-191, **2395-2407**
clinical manifestations of, 742t-748t, 2400-2403
containment of, 2406
in daycare facilities, 3564
deafness with, 2402
diabetes mellitus and, 2402
diagnosis of, 2403
differential diagnosis of, 2403
encephalitic, 494t-498t, 500, 2401
epidemiology of, 2396-2399, 2397f-2399f, 2397t
gonadal, 2401-2402
historical perspective on, 2395-2396
immune response to, 2400
immunization against, 2404-2405, 3455-3457
acute disseminated encephalomyelitis with, 517
adverse effects of, 2405, 3456
contraindications to, 2405-2406, 3457
in immunocompromised host, 2406
indications for, 3456
meningitis and, 488
precautions for, 3457
incidence of, 2396-2398, 2397f-2398f, 2397t
vs. mastoiditis, 237
meningeal, 488, 2401
vs. tick-borne encephalitis virus infection, 2310-2311
meningoencephalitic, 2401
morbidity with, 2398-2399
mortality with, 2398-2399
nephritis with, 2402
nosocomial, 3383-3384
ocular manifestations of, 811
pancreatic, 693, 2402
pathogenesis of, 2399-2400
pathology of, 2400
in pregnancy, 2402-2403, 2405
prevention of, 2404-2406, 3526
prognosis for, 2404
transmission of, 2399
treatment of, 2403
typical, 2400-2401, 2401f
Mumps virus, 2396
antigenic composition of, 2396
physical properties of, 2396
tissue culture of, 2396
Mupirocin
in burn wound infection, 1059
in impetigo, 774-775
in *Staphylococcus aureus* infection prevention, 1128-1129
Murphy sign, 669
Murray Valley encephalitis, 494t-498t, **2301-2303**
Muscles
abscess of, **735-739**, 736t. *See also* Myositis; Pyomyositis
catabolism of, 71-72
Mushroom poisoning, 604, 604t
Myalgia, in *Leptospira* spp. infection, 1749-1750
Myasthenia gravis, vs. botulism, 1793
Mycetoma
actinomycotic, 1393
Candida, 2749
Mycobacterial infection, **1381-1391.** *See also specific mycobacterial infections*
cervical lymphadenitis in, 177-178, 177t, 180, 183t, 187-188
cutaneous, 1386-1387
in cystic fibrosis, 336-337, 341
cytokines and, 1382
epidemiology of, 1381-1382
after hematopoietic stem cell transplantation, 935-936
HIV infection and, 1384-1385
immunodeficiency and, 916
interferon-γ receptor defects and, 50-51
interleukin-12 defects and, 50
after kidney transplantation, 1012
after lung transplantation, 964-965
lymphadenitis in, 1383-1384
microbiology of, 1382-1383, 1382t-1383t, 1389-1390
pulmonary, 1385-1386. *See also* Tuberculosis
treatment of, 1387-1390, 1388t
Mycobacterium abscessus infection, 1382, 1383t, 1389-1390
in cystic fibrosis, 1385-1386
after lung transplantation, 964-965
Mycobacterium avium-intracellulare complex infection, 1382t, 1387-1389
disseminated, 1388-1389
HIV infection and, 1384-1385, 2600-2601
after lung transplantation, 964-965
lymphadenitis in, 178-179, 1382
parotitis in, 191
peritoneal, 1389
pulmonary, 1385-1386, 1388
treatment of
in HIV-infected host, 1385
after lung transplantation, 965
Mycobacterium bolletii infection, 1387
Mycobacterium bovis infection, 700, 1382t
Mycobacterium chelonae infection, 1383t, 1390
in cervical lymphadenitis, 178
cutaneous, 1387
after lung transplantation, 964-965
Mycobacterium fortuitum infection, 1383t, 1390
cutaneous, 1387
hair follicle, 777
after lung transplantation, 964-965
Mycobacterium genavense infection, 1384-1385
Mycobacterium gordonae infection, 1382t
Mycobacterium haemophilum infection, 1387
Mycobacterium interjectum infection, 178
Mycobacterium kansasii infection, 1382, 1382t, 1389
HIV infection and, 1384
after lung transplantation, 964-965
pulmonary, 1385-1386
Mycobacterium lentiflavum infection, 178
Mycobacterium leprae, 1393, 1399-1400, 1399f-1401f. *See also* Leprosy
vaccines against, 1409-1410
Mycobacterium malmoense infection, 1389
Mycobacterium marinum infection, 1382, 1382t
cutaneous, 1386
after lung transplantation, 964-965
Mycobacterium massiliense infection, 1387, 1390
Mycobacterium mucogenicum infection, 1390
Mycobacterium neoaurum infection, 1390

Mycobacterium scrofulaceum infection, 178, 187, 1382, 1382t, 1389
HIV infection and, 1384
Mycobacterium septicum infection, 1390
Mycobacterium simiae infection, pulmonary, 1386
Mycobacterium smegmatis infection, 1390
Mycobacterium szulgai infection, 1382t, 1386, 1390
Mycobacterium tuberculosis, 1341. *See also* Tuberculosis
Mycobacterium ulcerans, 1381
Mycobacterium ulcerans infection, 1382t, 1386-1387
animal models of, 1413
bite-related, 780
clinical manifestations of, 1411f, 1413-1415, 1413f
complications of, 1415, 1415f
cutaneous, 1386-1387
diagnosis of, 1415
differential diagnosis of, 1415
edematous, 1413-1414, 1414f
epidemiology of, 1411-1412
HIV infection and, 1415
after lung transplantation, 964-965
nodular, 1412-1413
osseous, 1414-1416, 1414f-1415f
papular, 1413
pathogenesis of, 1412-1413
pathology of, 1412-1413, 1412f
plaque, 1413
prevention of, 1416-1417
prognosis for, 1416
scarring with, 1415, 1415f
surgical treatment of, 1416
transmission of, 1411-1412, 1411f
treatment of, 1416
ulcerative, 1411f, 1414
Mycobacterium wolinskyi infection, 1390
Mycobacterium xenopi infection, 1382t, 1386
Mycolactone, 1410, 1412
Mycoplasma fermentans, 2694
Mycoplasma genitalium, 2693-2694
Mycoplasma genitalium infection, **2693-2694**
clinical manifestations of, 532-533, 2694
differential diagnosis of, 2694
epidemiology of, 2694
treatment of, 2697
Mycoplasma hominis, 2692
Mycoplasma hominis infection, **2692-2693**
clinical manifestations of, 2693
differential diagnosis of, 2693
epidemiology of, 2692
after kidney transplantation, 1011
neonatal, 901, 2693
pharyngeal, 159t-160t, 161
treatment of, 2696-2697
Mycoplasma penetrans infection, **2694**
Mycoplasma pirum, 2695
Mycoplasma pneumoniae, 2669-2671
animal susceptibility to, 2671
antigenic composition of, 2670, 2674
Mycoplasma pneumoniae-associated mucositis, 2680, 2680t
Mycoplasma pneumoniae infection, **2669-2687**
vs. *Arcanobacterium haemolyticum* infection, 1326
asthma and, 2679
in bronchitis, 2679
in bullous hemorrhagic myringitis, 2678-2679
cardiac manifestations of, 2681-2682
clinical manifestations of, 2675-2685, 2676t
Mycoplasma pneumoniae infection *(Continued)*
CNS, 494t-498t, 501-502, 2683-2684
cold agglutinin antibodies in, 2674, 2685
cough with, 1617-1618
croup in, 2679
diagnosis of, 2685-2686
differential diagnosis of, 2685
enanthem in, 2679-2681
epidemiology of, 2671-2672
exanthem in, 750, 751t-752t, 764, 2679-2681, 2680t-2681t
fetal, 2684
gastrointestinal manifestations of, 2682-2683
hematologic manifestations of, 2682
hepatic manifestations of, 2682
immune response to, 2673-2674
immunization against, 2687
incubation period in, 2672
joint manifestations of, 2683
after kidney transplantation, 1011
meningeal, 488-489
mixed infections with, 2684
muscular manifestations of, 2683
nasopharyngeal, 2678
neonatal, 2685
in otitis media, 2678-2679
pancreatic, 695, 2682-2683
pathogenesis of, 2672-2675
pathology of, 2673
pertussis-like illness with, 1617
pharyngeal, 159t-160t, 161, 2678
prevention of, 2687
pulmonary, 285t, 287-289, 292, 2675-2678, 2676t. *See also* Pneumonia, community-acquired
vs. *Legionella pneumophila* infection, 1713
recurrent, 2684
in rhinosinusitis, 195
in sinusitis, 2679
splenic manifestations of, 2682
tonsillectomy and, 2684
treatment of, 2686-2687
upper respiratory tract, 2678
Mycoplasma salivarium, 2695
Mycoplasma spp., 1097t-1112t, 2668-2669, 2669t
as culture contaminant, 2696
Mycoplasma spp. infection, **2668-2700.** *See also specific species infections*
cancer and, 2696
historical perspective on, 2668
in HIV-infected host, 2696
in immunocompromised host, 2696
Neisseria meningitidis infection and, 1256
prevention of, 2697-2698
treatment of, 2696-2697
zoonotic, 2695-2696
MyD88 protein, in murine myocarditis, 386
Myelitis
CMV-associated, 526
transverse, acute. *See* Acute transverse myelitis
Myelopathy
HTLV-1– associated, 2569-2573, 2570t
HTLV-2– associated, 2580
Myeloperoxidase (MPO) deficiency, 59
Myiasis, 816, **3072-3074**
Myocardial infarction (MI)
coxsackievirus B infection and, 2077
vs. human erythrovirus B19 (human parvovirus B19) infection, 1854
in Kawasaki disease, 1081, 1083-1084
smallpox immunization and, 2039
Myocarditis, **381-400**
adenovirus, 1896t, 1902
autoantibodies in, 387
bacterial, 383
borderline, 393
Candida, 383f, 2740-2741, 2748
CMV, 1980
in congenital rubella, 2214t-2216t, 2217
after *Corynebacterium diphtheriae* infection, 1306
diagnosis of, 389-394
biomarkers in, 394
biopsy in, 393
electrocardiography in, 389-392, 390f-392f
PCR in, 382, 393-394
radiography in, 389, 389f
troponin T in, 394
virologic study in, 394
differential diagnosis of, 394-395
diphtheritic, 397
enterovirus, 384-387, 385f, 2075-2077, 2076t, 2104
epidemiology of, 381
etiology of, 381-382, 382t
giant cell, 383-384
in HIV infection, 398
human erythrovirus B19 (human parvovirus B19), 1853-1854, 1856
idiopathic dilated cardiomyopathy in, 382, 387, 393
immune factors in, 386-387
influenza virus, 2343
Leptospira spp., 1748
measles virus, 2385
Neisseria meningitidis, 1262
parasitic, 398-400
pathogenesis of, 384-387, 385f
pathology of, 383-384, 383f-385f
phases of, 384-386
prognosis for, 397
rubella virus, 2212
Shigella spp., 1481
smallpox vaccination and, 382
treatment of, 395-397
immune modulating agents in, 396-397
ventricular dysfunction in, 387-389, 388f
viral, **381-400**, 384f-385f
in Whipple disease, 644
Myocardium, in toxic shock syndrome, 853
Myonecrosis
Clostridium perfringens, 1796, 1798-1800
spontaneous, 1796
streptococcal, 1798
Myopathy, in Whipple disease, 644
Myopericarditis, smallpox immunization and, 2039
Myositis
Aeromonas spp., 1543
bacterial, **738-739.** *See also* Pyomyositis
clinical presentation of, 738-739
diagnosis of, 739
treatment of, 739
enterovirus, 2078
fungal, 736t, 739
in influenza virus infection, 2344
parasitic, 736t, 739
viral, 736t, 739
Myringitis, hemorrhagic, bullous, 2678-2679
Myringotomy, 218-220
in mastoiditis, 238
in otitis media, 232
in otitis media with effusion, 229

N

Naegleria fowleri, 2977, 2977f
Naegleria fowleri infection, **2975-2986**
  clinical manifestations of, 2979, 2979f
  CNS, 493-499, 494t-498t, 501-502
  diagnosis of, 2981-2983, 2982f
  epidemiology of, 2975-2977
  myocardial, 400
  neuroimaging in, 2980
  pathogenesis of, 2981
  pathology of, 2981
  prevention of, 2985-2986
  treatment of, 2983-2985
Nafcillin, 3183t-3184t, 3185-3186, 3231t-3233t
  in bacterial meningitis, 450t
  in septic shock, 833-834, 833t
Nagayama spots, 2017
Nanophyetus salmincola infection, 3327t-3346t
Naproxen, in rheumatic fever, 413
Narcolepsy, after influenza virus immunization, 2352-2353
Nasal polyps, rhinosinusitis and, 195
Nasal spray, in cold prevention, 139
Nasal tissues, virucidal, in cold prevention, 139, 2117, 2117t
Nasoantral window, in rhinosinusitis, 201
Nasolacrimal duct, obstruction of, 797
Nasopharyngeal carcinoma
  EBV infection and, 1995-1997, 2005
  treatment of, 2013
Nasopharyngitis, **156-158**
  adenovirus, 156, 157t, 158, 1895-1896, 1896t
  clinical presentation of, 158
  vs. common cold, 132
  diagnosis of, 163
  differential diagnosis of, 157t, 162-163
  epidemiology of, 156-158
  etiology of, 156, 157t
  Mycoplasma pneumoniae, 2678
  pathophysiology of, 158
  prevention of, 164
  prognosis for, 164
  treatment of, 163-164
Nasopharynx, indigenous flora of, 91t, 92
Nasotracheal intubation
  in epiglottitis, 257-258
  otitis media and, 211
Native Americans, diphtheria in, 1303
Natural killer cells, 28
  granzyme release by, 28
  in HIV infection, 2597-2598
  perforin release by, 28
  regulation of, 28
Nausea. *See also* Vomiting
  in appendicitis, 680-681
NBT dye test, in chronic granulomatous disease, 57, 58f
Nebovirus, 2148-2149, 2149t
Nebovirus infection, **2155**
Necator americanus infection, 757t-758t, **3016-3018**, 3327t-3346t
Neck abscess, **167-175.** *See also* Head and neck infection
  epidemiology of, 167-168
  microbiology of, 173-175
  parapharyngeal, **172-173**
  peritonsillar, **168-170**, 168f, 170f
  retropharyngeal, **170-172**, 170f
Necrotizing enteritis, *Clostridium perfringens*, 1797-1800
Necrotizing enterocolitis (NEC)
  Candida, 895-897
  Clostridium difficile, 635
Necrotizing enterocolitis (NEC) *(Continued)*
  Cronobacter sakazakii, 1436
  Klebsiella spp., 474
  oral immunoglobulin in, 3498
  platelet-activating factor and, 3366-3367
  prevention of, 3243-3244
  probiotics in, 3371-3372
Necrotizing epiglottitis, 245
Necrotizing fasciitis, **778-780**
  Bacteroides spp., 1829
  cervicofacial, 150, 151f
  Chryseobacterium spp., 1580
  clinical manifestations of, 778-779, 779f
  Clostridium perfringens, 1796-1797
  diagnosis of, 779
  Pseudomonas aeruginosa, 1599
  treatment of, 779-780
Necrotizing funisitis, 1766
Necrotizing lymphadenitis, 181
Necrotizing otitis externa, 207-208
Necrotizing ulcerative gingivitis (trench mouth), 147, 147f
Nedocromil sodium, in common cold, 138
Negishi virus infection, 2316t
Negri bodies, 504, 2453
Neisseria gonorrhoeae, 1273-1275, 1274t
  biofilm of, 1277
  DNA transformation of, 1274
  epithelial cell invasion by, 1276-1277, 1276f
  genetic characteristics of, 1274-1275
  IgA protease of, 1278
  iron metabolism by, 1278
  lipo-oligosaccharide of, 1278
  opacity-associated proteins of, 1277-1278
  peptidoglycan of, 1278-1279
  phagocytosis resistance of, 1276
  pili of, 5, 19, 1277
  porin protein of, 1278
  reduction modifiable protein of, 1279
  ribosomal protein L12 of, 1279
  strain typing of, 1275
  transglycosylases of, 1278-1279
  type IV secretion system of, 1279
  virulence factors of, 1277-1279
Neisseria gonorrhoeae infection, **1271-1301**
  abdominal wall, 1288
  anal, 586
  anorectal, 1287
  Bartholin gland/duct, 579, 1284, 1285f
  cervical, 586
  in child sexual abuse, 1297-1299, 1298t
  complement evasion in, 18
  conjunctival, 802
    neonatal, 805-806, 805t, **1280-1282**, 3242-3243
  cutaneous manifestations of, 1287, 1288f
  diagnosis of, 1274, 1288-1291, 1289t, 1290f, 3594
    medicolegal aspects of, 1291
    specimen collection for, 1291
  disseminated, 1279, 1287, 1288f
    neonatal, 1283, 1283t, 1295
  endocardial, 362
  endocervical, 1284-1285, 1284f
  epidemiology of, 1271-1273, 1272f-1273f, 1286t
  exanthem in, 753t-756t
  fallopian tube, **587-588**
  immune response to, 1279-1280
  joint, 729t, 730, 734
  meningeal, 1288
  ocular, 1288
  pathogenesis of, 18-19, 1275-1280, 1276f
Neisseria gonorrhoeae infection *(Continued)*
  pelvic, 1285-1286, 1286f
    treatment of, 1296
  perinatal, 1280-1283, 1280t, 1282f, 1283t. *See also* Ophthalmia neonatorum
    prevention of, 1299-1300
    treatment of, 1293-1295, 1293t
  pharyngeal, 1288
    treatment of, 1296
  prevention of, 1299-1300
  scalp wound, 1282-1283, 1295
  shunt-associated, 1288
  Skene's gland, 1284
  treatment of, 1293-1297, 1293t-1294t
    antibiotic resistance and, 1291-1293, 1293f
    cephalosporin allergy and, 1296
    in children and adolescents, 1294t, 1295-1297
    Chlamydia trachomatis coinfection and, 1297
    follow-up for, 1297
    in HIV-infected children, 1296
    in neonate, 1293t, 1294-1295
    during pregnancy, 1297
    syphilis infection and, 1297
  urethral, **530-535**, 532f, 534t, 1286-1287, 1287f, 1293f
  urinalysis in, 1285, 1291
  vaccine against, 1300
  vulvovaginal, 571-574, 572t-573t, 1283-1284, 1284f
Neisseria meningitidis, 1254-1255, 1274t
  DNA transformation of, 1254
  lipo-oligosaccharide of, 1254, 1257
  nasopharyngeal carriage of, 428, 432, 1256-1257
  phase variation in, 1254
  pili of, 1254
  porins of, 1254-1255
Neisseria meningitidis infection, **1253-1271**
  blood-brain barrier in, 432-433, 433t
  bloodstream, 432, 432t, 824, 829, 831, 1258-1260
    chronic, 1260
    cutaneous manifestations of, 1259, 1259f
    differential diagnosis of, 1258t
    treatment of, 833-834, 833t
  cerebral infarction with, 1260
  clinical manifestations of, 1258-1262
  complement deficiency and, 52, 429, 1256-1257
  conjunctival, 802, 1261
  cutaneous manifestations of, 750, 753t-756t, 762-763, 1258-1259, 1259f
  in daycare facilities, 3555-3556
  diagnosis of, 1262-1263
  empyema with, 1260
  epidemiology of, 1255-1257
  hearing loss after, 1259
  host genetic factors in, 1256-1257, 1263
  hydrocephalus with, 1260
  immunization against, 459-460, 460t, 1267-1270, 3452-3455
    adverse events with, 3454
    conjugate vaccine for, 1267-1270, 3454
    contraindications to, 3455
    polysaccharide vaccine for, 1267, 3453-3454
    precautions for, 3455
    recommendations for, 1268-1269, 1268t, 3454-3455

Neisseria meningitidis infection *(Continued)*
joint, 729t, 1261-1262
mannose binding lectin deficit and, 1257
meningeal, 428-429, 428f, 450t-451t, 459-460, 460t, 1258-1260. *See also* Meningitis, bacterial
mortality with, 1258, 1263
Mycoplasma infection and, 1256
myocardial, 1262
nasopharyngeal, 158, 162-163
neonatal, 1260
neurologic complications of, 1259
pathogenesis of, 1257-1258
pathology of, 1257-1258
pericardial, 373, 378-379, 1262
pharyngeal, 158, 1261
postexposure prophylaxis for, 3455
prevention of, 1266-1267, 1266t, 3249
prognosis for, 1263
properdin deficiency and, 1257
pulmonary, 1260-1261
vs. Rocky Mountain spotted fever, 2653
subdural effusion with, 1260
thrombosis with, 1260
treatment of, 446-455, 450t-451t, 1263-1266, 3365-3366
adjunctive/experimental, 446-455, 1264-1266, 1265t
viral infection and, 1256, 1260
vulvovaginal, 567
Neisseria sicca infection, 567, 1274t
Nelfinavir, 2615
Nematode infection, **3012-3029.** *See also specific nematode infections*
classification and nomenclature in, 2864-2865
hepatic, 673
NEMO defect, 916, 916f
Neomycin, 3208-3211, 3208t
Neonatal cephalic pustulosis, 789, 789f
Neonatal toxic shock syndrome–like exanthematous disease, 860-861
Neonate. *See* Newborn
Nephritis
adenovirus, 1901
CSF shunt–related, 1032
enterovirus, 2077-2078
interstitial
BK virus, 1867
Leptospira spp., 1747
mumps, 2402
postinfectious, in impetigo, 773
Nephronia, 706, 708
Nephropathia epidemica, 2496, 2504-2505
Nephropathy
HIV-associated, 2607
lobar, 539, 554, 555f, 556
Nephrotic syndrome
in malaria, 2928
in *Streptococcus pneumoniae* infection and, 1232
in syphilis, 1767
Netilmicin, 3208-3211, 3208t, 3231t-3233t
Neuraminidase, of *Streptococcus pneumoniae*, 1212
Neuraminidase inhibitors, 3270-3273, 3270f
adverse effects of, 3273
dosage for, 3272-3273
indications for, 3272
mechanism of action of, 3271
pharmacokinetics of, 3271-3272
resistance to, 3271
spectrum of activity of, 3270
Neuritis, rubella virus, 2212
Neuroblastoma, 182-183, 182t
Neurocysticercosis
calcification in, 3034, 3038
diagnosis of, 3035-3037, 3036f
epidemiology of, 3033
giant lesions in, 3035, 3039
MRI in, 3035-3036, 3036f
multiple lesions in, 3034, 3038
parenchymal, 3034, 3036f
pathogenesis of, 3033-3034
prevention of, 3039
prognosis for, 3039
single lesion in, 3034, 3037-3038
spinal, 3035
subarachnoid, 3035, 3036f, 3038
treatment of, 3037-3039
ventricular, 3034-3035, 3036f, 3037-3038
Neurodevelopment, neonatal *Candida* spp. infection and, 2766, 2766f
Neurologic disorders
in bacterial meningitis, 439, 440t, 455-457, 456t-457t
in *Bartonella henselae* infection, 1723
in *Bordetella pertussis* infection, 502
colistin-related, 3208
in coronavirus infection, 2494
in *Corynebacterium diphtheriae* infection, 1306
in coxsackievirus infection, 2086-2090, 2088t-2089t
DTP vaccine and, 3459
in echovirus infection, 2086-2090, 2088t
in enterovirus infection, 2086-2090, 2088t, 2097, 2104
in erythema infectiosum, 1853
in HHV-6 infection, 2018
in HHV-7 infection, 2018
in infectious mononucleosis, 2003-2004
in infective endocarditis, 356-357
in influenza virus infection, 2343
IVIG in, 3501-3502, 3502t
in Lyme disease, 1732-1735
metronidazole-related, 3230
in Murray Valley encephalitis, 2303
in *Neisseria meningitidis* infection, 1259
after pertussis immunization, 1633-1634
rabies, 2450
in rubella virus infection, 2212-2216, 2214t-2216t, 2221
in St. Louis encephalitis virus infection, 2257
in tick-borne encephalitis virus infection, 2311
Neurologic examination
in chronic fatigue syndrome, 1088
in St. Louis encephalitis virus infection, 2257
Neuromuscular blocking agents, in tetanus, 1817
Neuromyelitis optica, 515, 519, 525, 526t
Neuropathy
CMV, 1979-1980
Corynebacterium diphtheriae infection and, 1304-1306
Neuroretinitis
Baylisascaris procyonis, 815
in cat-scratch disease, 813
Neurosurgery, antibiotic prophylaxis in, 3253-3255
Neurosyphilis, 483, 812, 1768. *See also* Syphilis
congenital, 1770, 1772
treatment of, 1778
Neutropenia
burn-related, 1049
chemotherapy-associated, **921-933**
bacterial infection and, 921-922, 922t
epidemiology of, 921-923
evaluation of, 924-925
fungal infection and, 922, 924, 926, 927t, 928
occult infection and, 923
patient history in, 924
persistent, 926-927
physical examination in, 924
prevention of, 928-929
risk stratification for, 923-924
treatment of, 921, 925-928
viral infection and, 922-923, 923t
cyclic, 917
in infectious mononucleosis, 2003
in neonate, 45
Streptococcus pneumoniae infection and, 1205
Neutrophil(s), 3365
actin of, dysfunction of, 54
defective adhesion of, 52-55
defective chemotaxis of, 55
in hyper-IgE syndrome, 55
dysfunction of, *Streptococcus pneumoniae* infection and, 1205
in *Francisella tularensis* infection, 1661
glutathione synthetase deficiency in, 59
in glycogen storage disease type 1B, 54-55
granules of, 33-35, 34t
in Chédiak-Higashi syndrome, **54**
deficiency of, 54
in Kawasaki disease, 1077
in meningitis, 435
myeloperoxidase deficiency in, 59
Nevirapine, 2613-2614
Newborn. *See also* Infant; Premature infant
adenovirus infection in, 1905
antibiotic dosage schedule for, 3230, 3233t
antibody in, 42-44, 43f, 43t
appendicitis in, 681
Ascaris lumbricoides infection in, 3014
B cells in, 42
bacterial infection in, **881-892**
bloodstream, **882-885**, 1134-1135, 1135t
bone, **889-890**
breast, **890**
conjunctival, 890
epidemiology of, 881-882
gastrointestinal, 887-888
joint, **889-890**
meningeal, 885-887
orbital, 890
otic, 887
parotid gland, 890-891
pathogenesis of, 881-882, 881t
pulmonary, 891-892
scalp, 891
umbilical cord, **890**
umbilicus, **890**
urinary tract, 888-889
Blastomyces dermatitidis infection in, 2730-2731
Bordetella pertussis infection in, 1626-1627, 1626t
Burkholderia pseudomallei infection in, 1592
Campylobacter jejuni infection in, 888, 1653
Campylobacter spp. infection in, 1653
Candida spp. infection in, **892-897**, **2749-2766.** *See also Candida* spp. infection (candidiasis)
cell-mediated immunity in, 42

Newborn *(Continued)*
Chlamydia trachomatis infection in, 900-901, 2632-2633, 2633f
Clostridium difficile colonization in, 633-635
Clostridium spp. infection in, 1797
CMV hepatitis in, **654**
complement in, 44-45, 44t
conjunctivitis in, **805-806**, 805t
coronavirus infection in, 2491
Cryptococcus neoformans infection in, 2804
diarrhea in, 887-888
Elizabethkingia meningosepticum infection in, 1579
Enterococcus spp. infection in, 1176-1177
enterovirus infection in, 2092-2097, 2094t, 2096t. *See also* Enterovirus infection, neonatal
Escherichia coli bacteremia in, 1441
fungal infection in, **898-899**
acquired, 898-899
Aspergillus, 899
congenital, 898
Zygomycete, 899
group B streptococcal infection in. *See* *Streptococcus agalactiae* infection
Haemophilus influenzae infection in, 1676
HAV infection in, 2130
HBV infection in, 1915, 1917, 1925-1926
HHV-6 infection in, 2018
host response of, 42-45
HSV infection in, 1954-1955. *See also* Herpes simplex virus (HSV), neonatal
hypoglycemia in, 72
indigenous flora of, 89
cutaneous, 90
intestinal, 93
respiratory, 92
infective endocarditis in, 357
influenza virus infection in, 2339
lincomycin-related gasping syndrome in, 3222
Listeria monocytogenes infection in, 1331-1333, 1332t
malaria in, 2924, 2929
monkeypox in, 2042
Mycobacterium leprae infection in, 1394
Mycoplasma hominis infection in, 901, 2693
Mycoplasma pneumoniae infection in, 2685
myocarditis in, 387-389
Neisseria gonorrhoeae infection in, 1280-1283, 1283t. *See also* Ophthalmia neonatorum
neutropenia in, 45
nosocomial infection in, 3402-3404
osteomyelitis in, 713, 713f, 723
otitis media in, 215
phagocytes in, 45
Pseudomonas aeruginosa infection in, 1592
Ritter disease in, 774, 775f
RSV infection in, 2411-2412, 2413t, 2418-2419
rubella virus infection in. *See* Rubella virus infection, congenital
sepsis in, **882-885**, 3509
septic arthritis in, 733-734
Shigella spp. infection in, 1481-1482
Staphylococcus epidermidis infection in, 882, 3509-3510
Streptococcus agalactiae infection in, 881-882, **1153-1169.** *See also* *Streptococcus agalactiae* infection
Streptococcus viridans infection in, 1192
T cells in, 42
tetanus in, 1814-1815, 1819, 3512
Newborn *(Continued)*
toxic shock syndrome–like exanthematous disease in, 860-861
Toxoplasma gondii infection in, **900**, 900t
Trypanosoma cruzi infection in, 2965
tuberculosis in, 1358-1359
Ureaplasma spp. infection in, 901, 2689-2690
vaginal discharge in, 563, 563f
viral infection in, **877-881**, 878t
clinical manifestations of, 879-880, 879t
developmental malformation and, 877
diagnosis of, 878-880
differential diagnosis of, 880
hospital-associated, 878
laboratory diagnosis of, 880-881
maternal evaluation in, 879
maternal infection and, 877
natal/perinatal, 877-878
pathogenesis of, 877-878, 878f
postnatal, 878
treatment of, 881
yeast infection in, **892-900.** *See also* Newborn, *Candida* spp. infection in
Cryptococcus neoformans, 897
Malassezia spp., 897-898
Pichia spp., 898
Rhodotorula spp., 898
Trichosporon spp., 898
Newbury-1 virus, 2155
Niclosamide, 3349
Nicotinamide adenine dinucleotide (NADH) oxidase, of *Streptococcus pneumoniae*, 1214
Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, 33-35, 34f
deficiency of, 58-59
Nifurtimox, in *Trypanosoma cruzi* infection, 399, 2966-2967
Nipah virus infection, 494t-498t, 500-501
Nitazoxanide, 3349
Nitrates, urinary, 70
Nitric oxide (NO), 3367
in ARDS, 872
biologic effects of, 70-71
in septic shock, 826
Nitrites, urinary, 543
Nitroblue tetrazolium (NBT) dye test, 907, 918
Nitrofurantoin, in burn wound infection, 1059
Nitrogen, 67-70
balance studies of, 67-69, 68f-69f
fecal, 69
urinary, 69-70
Nocardia farcinica infection, 1420
Nocardia spp., 1419-1420, 1421f
Nocardia spp. infection, **1418-1423**
bloodstream, 1421
clinical manifestations of, 1420-1422
CNS, 1421
cutaneous, 1419-1420
diagnosis of, 1421-1422
after heart transplantation, 953-954
immune response to, 1420
after kidney transplantation, 1012
pathogenesis of, 1419-1420
pathology of, 1420, 1421f
prognosis for, 1422-1423
pulmonary, 1419-1421
in rhinosinusitis, 197
risk of, 1420
traumatic, 1420-1421
treatment of, 1422-1423
Nodule(s), 764, 764t
Milker's, 742t-748t
Mycobacterium ulcerans, 1412-1413
in rheumatic fever, 410
Nomenclature
bacterial, 1097t-1112t
fungal, 2702-2704
parasite, 2861-2866, 2862t-2863t
viral, 1836-1843, 1837f, 1838t-1840t, 1841f-1842f
Non-Hodgkin lymphoma, 182-183, 182t
Non-nucleoside reverse transcriptase inhibitors, 2613-2614
Nonshivering thermogenesis, 84
Nonsteroidal anti-inflammatory drugs (NSAIDs)
pancreatitis with, 691
in rheumatic fever, 413
Norepinephrine, plasma, 81-82
Norovirus, 2149, 2149f, 2149t
Norovirus infection, 607-608, 607t, 616t, **2151-2154**
clinical manifestations of, 2153
in daycare facilities, 3558
diagnosis of, 2153-2154
differential diagnosis of, 2153
epidemiology of, 2151
immune response to, 2152-2153
nosocomial, 3382
pathogenesis of, 2152
pathology of, 2152
prognosis for, 2156
seasonal patterns of, 2151, 2152f
treatment of, 2155-2156
vaccine against, 2156
Norwalk agent/virus. *See Norovirus*
Nose
indigenous flora of, 91t, 92
infection of. *See* Rhinosinusitis
polyps of, 195
saline irrigation of
in common cold, 137
in rhinosinusitis, 201
surgery on, toxic shock syndrome after, 860
Nuchal rigidity, 439
Nuclear factor κB
in *Cryptosporidium* spp. infection, 2900
in gonococcal infection, 1277, 1279
Nuclear factor κB–enhancing modulator (NEMO) deficiency, 916, 916f
Nucleic acid amplification test (NAAT). *See also* Polymerase chain reaction (PCR)
in *Chlamydia trachomatis* infection, 2636-2637
in *Clostridium difficile* infection, 636, 636t
highly multiplexed, 3614-3616, 3615t
multiplexed, 3613t, 3614
in *Neisseria gonorrhoeae* infection, 1290-1291
quantitative, 3616-3617, 3616t
single-analyte, 3612-3614, 3613t
in viral infection, 3606t-3607t, 3608, 3612, 3613t, 3615t-3616t
Nucleic acid hybridization test (NAHT), in *Neisseria gonorrhoeae* infection, 1290-1291
Nucleoside reverse transcriptase inhibitors, 2612-2613, 2612f
pancreatitis with, 692
Nucleotide reverse transcriptase inhibitors, 2613
Nursing bottle caries, 146

Nutrition
in ARDS, 873
in gastrointestinal tract infection, 620t, 622
in *Shigella* spp. infection, 1485
Nystatin
in burn wound infection, 1059
in localized *Candida* spp. infection, 788
in neonatal *Candida* spp. infection, 2762-2763, 2764t
in vulvovaginal fungal infection, 570

O

Obesity, adenovirus infection and, 1905
Obsessive-compulsive disorder, in PANDAS, 1144-1145
Obturator sign, 730, 731f
Occludin-1, 16
Occupation, *Paracoccidioides* spp. infection and, 2782
Occupational health, 3412-3413
Ocular larva migrans, 3022-3023
Oculoglandular syndrome of Parinaud, 1722, 1724f
Oculorespiratory syndrome, after influenza virus immunization, 2352-2353
Odontogenic infection
cervical lymphadenitis and, 183, 183f
cervicofacial actinomycosis and, 184
complications of, 149-151, 149f-151f
Garré sclerosing osteomyelitis with, 154, 154f-155f
nursing bottle–related, 146
osteomyelitis with. *See* Osteomyelitis, mandibular/maxillary
pathogens in, 140-141, 141t
periapical, 141, 142f-143f, **146**
pericoronal, 141, 147-148, 148f
periodontal, 141, 146-147, 147f
vs. rhinosinusitis, 199
spread of, 141-142, 142f-144f
surgical treatment of, 144
treatment of, 142-149, 146t
Oesophagostomum spp. infection, 3024
Oestrus ovis infection, ocular (ophthalmomyiasis), 816
Omenn syndrome, 904-905
Omphalitis, **890**, 904
Omsk hemorrhagic fever, **2316**
Onchocerca volvulus infection, 757t-758t, 809, 815, **3027-3028**, 3327t-3346t
Onychomycosis, dermatophyte, **786-787**, 786f
O'nyong-nyong virus infection, 742t-748t, 2251
Oophoritis, **590**
mumps, 2401-2402
Opacity-associated proteins, of *Neisseria gonorrhoeae*, 1277-1278
Ophthalmia neonatorum, **805-806**, 805t
chlamydial, 805-806, 805t, 2633-2634
treatment of, 2634
gonococcal, **1280-1282**
clinical features of, 1282, 1282f
epidemiology of, 1280-1281
prevention of, 1281, 3242-3243
treatment of, 1293-1295, 1293t
Ophthalmomyiasis, 816
Ophthalmoplegia, in Whipple disease, 644
Opisthorchis spp., 3049, 3050f
Opisthorchis spp. infection, **3049-3052**
clinical manifestations of, 660, 3051
complications of, 3051
diagnosis of, 3051-3052
Opisthorchis spp. infection *(Continued)*
epidemiology of, 3050-3051
imaging in, 3052
pathogenesis of, 3051
prevention, 3052
transmission of, 3050-3051
treatment of, 3052, 3327t-3346t
Opsonins, 30-31
in phagocytosis, **32-33**, 33f
Optic nerve, in bacterial meningitis, 440
Optic neuritis
in acute disseminated encephalomyelitis, 514-515
CMV, 811
Oral cavity
cancer of, HPV infection and, 1873t, 1880
Candida spp. infection of, **787-788**, 787f
in HIV infection, 148-149
in neonate, 892-893, 896
treatment of, 788
flora of, 91t, 92, 94, 140, 141t, 1189-1190
HSV infection of, 154-155, 742t-748t, 1940-1941, 1940f
in HIV infection, 149
recurrent, 1950, 1950f
treatment of, 1959
infection of, **140-155**
anatomic considerations in, 141-142, 142f-144f
complications of, 149-151
enteroviral, 2066
etiology of, 140-141, 141t
examination for, 144
fascial space spread of, 149-150, 149f-150f
in HIV infection, 148-149
intracranial complications of, 145, 151
mediastinitis and, 420-421
necrotizing fasciitis with, 150, 151f
nursing bottle–related, 146
orbital complications of, 151
osteomyelitis with. *See* Osteomyelitis, mandibular/maxillary
periapical, 141, 142f-143f, **146**
pericoronal, 141, 147-148, 148f
periodontal, 141, 146-147, 147f
sinusitis with, 150-151, 152f
treatment of, 142-149, 146t
piercings of, 155
temperature measurement by, 86
trismus of, 144, 148-149, 168
tuberculosis of, 1357
Oral contraception
pelvic inflammatory disease and, 587
toxic shock syndrome and, 853
Oral hairy leukoplakia, 1997, 2007
Oral rehydration therapy
in cholera, 1552t, 1553
in gastrointestinal tract infection, 620t-621t, 621-622
in rotavirus infection, 2186
in *Shigella* spp. infection, 1483
in *Vibrio parahaemolyticus* infection, 1559
Orbit
hydatid cyst of, 801
infection of
implant-related, 1018-1019
odontogenic origin of, 151
Orbital cellulitis, **799-801**, 800f
vs. dacryoadenitis, 797
differential diagnosis of, 800
fungal, 801
Haemophilus influenzae, 1676
in infantile osteomyelitis, 153-154, 153f
neonatal, 890
Orbital cellulitis *(Continued)*
odontogenic origin of, 151
Streptococcus pneumoniae, 1231-1232
Orbiviruses, 2172, 2175-2176
Orchitis
adenovirus, 1901
enterovirus, 2077
mumps, 2401-2403
Orf virus infection, 742t-748t, **2046**
vs. ecthyma, 776
Organic acids, in cold prevention, 139
Oriental sore. *See Leishmania* spp. infection (leishmaniasis), Old World
Orientia tsutsugamushi infection (scrub typhus), 751t-752t, 2648t-2649t, **2660-2661**
Ornidazole, 3349
Ornithodoros tick, 1740
Oropharyngeal cancer, HPV and, 1875-1876, 1880
Oropharyngeal candidiasis, **2739**
immune response to, 2738
treatment of, 2746-2747
Oropharynx
flora of, 91t, 92
infection of. *See* Oral cavity, infection of; Pharyngitis
Oropouche fever, **2537-2544**
clinical manifestations of, 2542
diagnosis of, 2543
differential diagnosis of, 2543-2544
epidemiology of, 2539-2541
meningeal, 2542-2543
pathogenesis of, 2542-2543
prevention of, 2544
transmission of, 2541
treatment of, 2544
vs. Venezuelan equine encephalitis, 2240
Oropouche fever virus, 2538-2539, 2538f-2539f
Oroya fever, **1725-1726**
diagnosis of, 1727
prognosis for, 1729
treatment of, 1728
Orthopedic fixator devices, infection with, 725
Orungo virus, 2175-2176
Oseltamivir, 3268t, 3270-3273, 3270f
in community-acquired pneumonia, 290
in influenza virus infection, 2348-2351, 2349t
in laryngotracheitis, 257
Osler nodes, 355, 357
Ossicles, otitis media-related effects on, 223
Osteoarthritis, *Candida* spp., 895, 897
Osteochondritis
in congenital syphilis, 1769
of foot, 724-725
Osteomyelitis, **711-727**
Achromobacter xylosoxidans, 1574-1575
Actinomyces spp., 725
Aeromonas spp., 1543
anaerobic bacteria in, 711, 1829-1830
Bacillus cereus, 1319
Bartonella henselae, 1723-1724
Blastomyces dermatitidis, 2728, 2729f-2730f
Brucella spp., 725, 1613
C-reactive protein in, 719
Candida, 2742, 2749
cervical, retropharyngeal abscess and, 174
chronic, 726-727, 726f
Clostridium spp., 1797-1798
Coccidioides spp., 2771, 2772f, 2773-2774, 2774f
culture-negative, 714

Osteomyelitis *(Continued)*
epidemiology of, 711
Escherichia coli, 1441
etiology of, 711, 712t
fever of unknown origin in, 845
fungal, 725-726
Haemophilus influenzae type b, 1676
hematogenous, **711-724**
Brodie abscess in, 719
in chronic granulomatous disease, 56-57, 724
clinical manifestations of, 712-714, 713f, 713t
closed fracture and, 719
CT in, 717
diagnosis of, 714-717
differential diagnosis of, 714
epiphyseal, 719
in hemodialysis patient, 723
in hemoglobinopathy, 723-724
in HIV infection, 724
microbiology of, 714-715
MRI in, 715-716, 716f
neonatal, 713, 713f, 723
in nontubular bones, 719-720
pathogenesis of, 711-712, 712f
radiography in, 713, 715-717, 715f
radionuclide imaging in, 716-717, 716f
spinal, 720-723, 721f-722f
treatment of, 717-719, 718t
Kingella kingae, 1700, 1702
mandibular/maxillary, **152-154**, 152t
clinical findings in, 152-153
Garré, 154, 154f-155f
infantile, 153-154, 153f, 889
microbiology of, 152
predisposing factors in, 152
suppurative, 153, 153f
mastoiditis and, 237
multifocal, recurrent, chronic (CRMO), 714
Mycobacterium ulcerans, 1414-1416, 1414f
neonatal, 713, 713f, 723, **889-890**
mandibular/maxillary, 153-154, 153f, 889
nonhematogenous, **724-725**
contiguous sources of, 725
orthopedic devices and, 725
puncture wound, 724-725
Pantoea agglomerans, 1536
Proteus spp., 1473
Pseudomonas aeruginosa, 1591, 1599
rhinosinusitis and, 198
Salmonella spp., 1505
Sporothrix schenckii, 2834
Staphylococcus epidermidis, 1137
Streptococcus agalactiae, 1161
Streptococcus pneumoniae, 711, 712t
Streptococcus viridans, 1193
therapeutic drug monitoring in, 718-719
Otalgia, 208
differential diagnosis of, 220
in otitis externa, 205
in otitis media, 217
Otitic hydrocephalus, 237
Otitis externa, **203-209**
acute, 205-207
diagnosis of, 205, 205f
etiology of, 205-206, 206t
management of, 206-207
anatomy in, 204
chronic, 207
differential diagnosis of, 208, 208f
epidemiology of, 204
vs. mastoiditis, 237
Otitis externa *(Continued)*
necrotizing, 207-208
predisposing factors in, 204, 204t
prevention of, 208
protective mechanisms in, 204
Pseudomonas aeruginosa, 1591, 1599
Otitis media, **209-232**
acoustic reflectometry in, 218
adhesive, 223
anatomic abnormalities and, 211
antibiotic resistance in, 3147
audiometric test in, 218
behavioral disturbances with, 222
chemoprophylaxis in, 231
Chlamydia pneumoniae, 2642
cholesteatoma with, 223
clinical course of, 228
clinical presentation of, 217
complications of, 221-225, 221f
conjunctivitis with, 217
cost-related aspects of, 212
craniofacial anomalies and, 217
criteria for, 216-217
in daycare facilities, 3553, 3554t
developmental disorders and, 222, 222f
diagnosis of, 216-217
differential diagnosis of, 220
drug-resistant, 226
environmental risk factors for, 211-212
epidemiology of, 210-212
etiology of, 212-215, 212t-213t
eustachian tube in, 216, 216f
facial paralysis with, 217, 224
genetic factors in, 211
Haemophilus influenzae, 1677
hearing loss with, 217, 222
hMPV infection and, 2438-2439
immunodeficiency and, 211
intracranial complications of, 224-225, 225f
labyrinthitis with, 224
management of, 225-229, 225t, 227t-228t, 3508
mastoiditis with, 223-224
in measles, 2385, 2392
meningitis with, 224
Moraxella catarrhalis, 1252
Mycoplasma pneumoniae, 2678-2679
myringotomy in, 218-220
neonatal, 215, 887
observation for, 226-227, 227t
ossicular chain abnormalities with, 223
otalgia in, 217
otorrhea in, 217
otoscopy in, 217-218, 217f, 219f
parainfluenza virus infection and, 2367
pathogenesis of, 215
pathophysiology of, 215-216
petrositis with, 224
pneumococcal vaccine in, 230-231, 230t-231t
poverty and, 211
prevention of, 230-232, 230t-231t, 3247-3248
Proteus spp., 1473
Pseudomonas aeruginosa, 1591, 1599
radiography in, 220
recurrence of, 212, 3247-3248
risk factors for, 210-211, 210t
seasonal variation in, 211-212
Streptococcus pneumoniae, 1231, 1233-1236
suppurative, chronic, 212-213, 223
symptomatic therapy in, 228
tinnitus in, 217
tympanic membrane in, 215-216
tympanic membrane perforation with, 223
Otitis media *(Continued)*
tympanocentesis in, 218-220
tympanometry in, 218, 220f
tympanosclerosis with, 223
tympanostomy tubes in, 229, 232
vertigo in, 217, 222
viral infection and, 214, 2123-2124, 2169-2170
Otitis media with effusion, 209-210, **221**
etiology of, 212-213
management of, 229
pathogenesis of, 215, 215f
viral infection and, 2124
Otomycosis, **207**
Otorrhea, in otitis media, 217
Otoscopy, in otitis media, 217-218, 217f, 219f
Outpatient intravenous antimicrobial therapy, **3257-3265**
administration of, 3264
agent selection for, 3261-3263, 3262t-3263t
complications of, 3264-3265
evaluation of, 3257-3258, 3258t
indications for, 3261, 3262t
monitoring in, 3263
outcome analysis of, 3264-3265
program for, 3258-3261, 3259t, 3260f
Over-the-counter medications, in common cold, 137
Oxacillin, 3184t, 3185-3186, 3231t-3233t
in bacterial meningitis, 450t
Oxamniquine, 3350
Oxygen therapy
in bronchiolitis, 278-280
in croup, 255
hyperbaric
in gas gangrene, 1799
in mucormycosis, 2844
in necrotizing fasciitis, 150
in RSV infection, 2425
in septic shock, 835
Oxymetazoline, in rhinosinusitis, 200
Oxytetracycline, 3217-3220

P

P-Selectin, in phagocyte recruitment, 31-32, 33f
p value, 120
Pacemaker
infection with, 352, 1023-1025
clinical manifestations of, 1024
laboratory findings in, 1024
management of, 1024-1025
microbiology of, 1024
in myocarditis, 396
Pacifier thermometer, 86
Pacifier use, otitis media and, 211
Paecilomyces spp. infection, **2856-2857**
Pain
abdominal. *See* Abdominal pain
chest. *See* Chest pain
ear. *See* Otalgia
in herpes zoster, 2027-2028
in spinal epidural abscess, 471
Palivizumab
in heart transplant–related RSV infection, 950
in hematopoietic stem cell transplantation–related RSV infection, 943
in lung transplant–related RSV infection, 980
in RSV infection, 2426-2427, 2429, 3503, 3529-3530

Palpebral edema
in roseola infantum, 770
in *Trypanosoma cruzi* infection, 2963
Pancreas, abscess of, 696, 704-708. *See also* Pancreatitis
Pancreatic duct, obstruction of, 692
Pancreatic pseudocyst, 696
Pancreatitis, **689-697**
autoimmune disease-related, 691t, 692
bacterial, 693t, 695
chronic, 690, 692
clinical manifestations of, 689-690, 708
complications of, 696
coxsackievirus B, 2072
diagnosis of, 690, 707-708
drug-related, 691-692, 691t, 696
fibrosing, chronic, 692
fungal, 695
hereditary, 692
infectious, 693-695, 693t
metabolic disease-related, 691t, 692
mumps, 2402
mycoplasmal, 695, 2682-2683
noninfectious, 690-693, 691t
obstruction-related, 691t, 692, 707-708
osteolytic lesions after, 696
parasitic, 694-695, 694f
pathogenesis of, 695
pseudocyst in, 696
scoring system for, 690
trauma-related, 691, 691t
treatment of, 695-696, 709
vasculitis-related, 691t, 692
viral, 693-694, 693t
Pancytopenia
in human erythrovirus B19 (human parvovirus B19) infection, 1850
in infectious mononucleosis, 2003
PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection), 410, **1143-1146**
diagnosis of, 1144-1145
evidence for, 1146
pathogenesis of, 1144, 1144f
prevention of, 1145-1146
treatment of, 1145, 3511
Pandoraea spp., 1584
Pandoraea spp. infection, 1595-1596
Panophthalmitis
Bacillus cereus, 1319
Clostridium spp., 1798
Morganella morganii, 1470
Yersinia enterocolitica, 1528
Pantoea agglomerans, 1535
identification of, 1537
Pantoea agglomerans infection, **1535-1537**
clinical manifestations of, 1536
diagnosis of, 1537
epidemiology of, 1535-1536
pathophysiology of, 1536
treatment of, 1537
Panton-Valentine leukocidin, 1116-1117
Papanicolaou smear
in HPV infection, 581, 1881-1882
in HSV infection, 1956
in *Trichomonas vaginalis* infection, 2892
PapG adhesin, 537
Papillitis, 810
Papilloma. *See also* Human papillomavirus (HPV) infection; Warts
in yaws, 1786
Papillomatosis, respiratory, recurrent, 1878-1879, 1878f-1879f
Papillon-Lefèvre syndrome, 673, 1610
Papular acrodermatitis (Gianotti-Crosti syndrome), 649, 749, **766**, **783**, 783f
in HAV infection, 2140
Paracoccidioides spp., 2780-2781, 2783
identification of, 2787-2792, 2787f, 2789t-2790t
Paracoccidioides spp. infection (paracoccidioidomycosis), **2780-2795**
age and, 2781-2782, 2782t
bone involvement in, 2784, 2785f, 2787
bone marrow involvement in, 2784
clinical manifestations of, 2784f-2785f, 2786-2787, 2786t
diagnosis of, 2787-2792, 2787f, 2789t-2790t
epidemiology of, 2781-2783
gender and, 2782, 2782t
geographic distribution of, 2782-2783
after kidney transplantation, 1013
lymph node involvement in, 2784, 2784f-2785f, 2786
occupation and, 2782
pathogenesis of, 2783-2785
prognosis for, 2793-2794
race and, 2782
transmission of, 2781
treatment of, 2792-2793, 3295t
Paradoxical pulse, in pericarditis, 374, 375f
Paradoxical zone phenomenon, 3174-3175, 3174f
Paragonimus spp., 3050f-3051f, 3054
Paragonimus spp. infection (paragonimiasis), **3054-3055**
clinical manifestations of, 3055
CNS, 3055-3056
cutaneous, 3055
diagnosis of, 3051f, 3055-3056
eosinophilia in, 3056
epidemiology of, 3054-3055
pathogenesis of, 3050f, 3055
pleuropulmonary, 3055-3056
prevention of, 3056
transmission of, 3054-3055
treatment of, 3056, 3327t-3346t
Parainfluenza virus(es), 2361-2363
classification of, 2361-2363, 2361t, 2362f
isolation of, 2363
structure of, 2361-2363, 2362f
Parainfluenza virus (PIV) infection, **2360-2372**
in acute bronchitis, 262, 263t
age and, 2363-2364, 2363f
animal models of, 2365-2366
after bone marrow transplantation, 2368
clinical manifestations of, 2366-2368, 2367f
in common cold, 132
complications of, 2370
in croup syndromes, 242, 247-248
diagnosis of, 2368-2369
differential diagnosis of, 2369
encephalitic, 494t-498t
epidemiology of, 2363-2364
exanthem in, 742t-748t, 749, 760-762
after heart transplantation, 954
after hematopoietic stem cell transplantation, 943
historical perspective on, 2360-2361
immune response to, 2365-2366
in immunocompromised host, 2368
after intestinal transplantation, 997t, 999
after kidney transplantation, 1007
after liver transplantation, 997t, 999
after lung transplantation, 980, 2368
in otitis media, 2367
Parainfluenza virus (PIV) infection *(Continued)*
pathogenesis of, 2364-2366
pathology of, 2365
in pneumonia, 284
prevention of, 2370-2372
prognosis for, 2370
reinfection with, 2367-2368
seasonality of, 2364
transmission of, 2364
treatment of, 2369-2370
Paralysis
in enteroviral infection, 2090. *See also* Poliovirus infection (poliomyelitis)
in spinal epidural abscess, 471
Paramomycin, 3231t-3233t
Paranasal sinuses, 193-194
inflammation of. *See* Rhinosinusitis
Parapharyngeal abscess, 170f, **172-173**
clinical manifestations of, 172-173
microbiology of, 173-175
treatment of, 173
Parapoxviruses, 2041t, 2046
Parasitic disease. *See also specific parasite infections*
appendiceal, 683t, 684-685
biliary, 660, 664
in burn patient, 1054
classification and nomenclature in, 2861-2866, 2862t-2863t
encephalitic, 494t-498t, 501
eyelid, 795-796
gastrointestinal, 602-604, 602t-603t, **613-616**, 616t
stool examination in, 617
treatment of, 625
hepatic, 651t, 657
host response to, 23-24. *See also* Immunity
after kidney transplantation, 1014
laboratory diagnosis of, **3620-3627**, 3621t, 3624f-3625f
blood smear examination in, 3625-3626, 3625f
blood specimen for, 3624-3626, 3625f
duodenal specimen for, 3622
respiratory specimen for, 3622
serology in, 3626-3627
sigmoidoscopy specimen for, 3622
stool specimen for, 3622, 3623t. *See also* Stool specimen examination
tape-collected specimen for, 3622
urogenital specimen for, 3626-3627
meningeal, 488
muscle, 739
myocardial, 398-400
pancreatic, 694-695, 694f, 707-708
Paravaccinia virus infection (milker's nodule), 742t-748t
Parechovirus(es)
genome of, 2053
geographic distribution of, 2056-2057
host interaction with, 2054-2056
replication of, 2054
transmission of, 2056
Parechovirus infection, **2051-2109**
asymptomatic, 2063-2064
clinical manifestations of, 2063-2090
diagnosis of, 2101-2103
encephalitic, 493-499, 494t-498t
in epidemic pleurodynia, 2067-2068
exanthem in, 2080t-2081t, 2086
hemolytic-uremic syndrome in, 2078
herpangina in, 2065, 2066t
in immunocompromised host, 2091

Parechovirus infection *(Continued)*
myocardial, 2076t
neonatal, 2092-2097, 2094t
nonspecific febrile illness in, 2064t
respiratory manifestations of, 2064-2068, 2064t
treatment of, 2103-2104
uveal, 2074-2075
Parkinson disease, Western equine encephalitis virus infection and, 2235
Paromomycin, 3208-3211, 3208t, 3350
Paronychia, 3571-3572, 3574
Candida, 788, 788f
Parotid glands
in cat-scratch disease, 1722, 1724f
in HIV-infected host, 149, 191-192
infectious enlargement of. *See* Parotitis
noninfectious enlargement of, 192
Parotidectomy, 192
Parotitis, **189-193**
chronic, 189
clinical presentation of, 190-191
complications of, 192
diagnosis of, 190-191
differential diagnosis of, 192, 2403
enterovirus, 2066
epidemic, 189. *See also* Mumps
etiology of, 189-190, 190t
granulomatous, 189-191
in HIV infection, 191-192
neonatal, 890-891
pathophysiology of, 189, 190t
prevention of, 192-193
recurrent, 189, 191
suppurative, 189-190, 192-193
treatment of, 192
viral, 189-191, 190t. *See also* Mumps
Paroxysmal atrial tachycardia, in myocarditis, 389
Parrot, pseudoparalysis of, 1769-1770
PARV4 virus, 1843
Parvoviridae, 1838t-1840t. *See also* Human bocavirus (HBoV); Human erythrovirus B19 (human parvovirus B19) infection
genetic history of, 1845
Parvovirus B19. *See* Human erythrovirus B19 (human parvovirus B19) infection
Passive immunization, **3491-3536**
animal sera for, 3491-3493
antitoxin for, 3491-3493. *See also* Antitoxin
definition of, 3491, 3492t
human immune serum globulin, 3493-3499. *See also* Immunoglobulin(s)
monoclonal antibodies in, 3493. *See also* Monoclonal antibodies
Passive microcapsule agglutination test, in *Leptospira* spp. infection, 1756
Pasteur, Louis, 103
Pasteurella multocida, 1545-1546
Pasteurella multocida infection, **1545-1548**
clinical manifestations of, 1547, 3574
diagnosis of, 1548
epidemiology of, 1546
immune response to, 1546
pathogenesis of, 1546-1547
vs. plague, 1513
prevention of, 1548
transmission of, 1546
treatment of, 1548
Pasteurella spp., 1566-1567
Pasteurella spp. infection, **1566-1567**
Patella, osteomyelitis of, 725
Patent ductus arteriosus, in congenital rubella, 2214t-2216t, 2217
Pathogen, 3, 89. *See also* Microbiome *and specific organisms and infections*
in epidemiologic study, 104-107, 106f, 107t
facultative, 89, 91t
immunogenicity of, 105
infectivity of, 105
opportunistic, 89, 91t
pathogenicity of, 105
reservoirs for, 106-107
strict, 89
virulence of, 105
Pathogenicity, 105
Pathogenicity islands, 3
Helicobacter pylori, 1693
Salmonella spp., 1496-1497, 1496f
SPI-1, 13-14
SPI-2, 14-15
SPI-3, 15
Staphylococcus aureus, 1115, 1115t
Vibrio cholerae, 1550
Pattern recognition receptors, 433, 434f, 436
Pediatric acute-onset neuropsychiatric syndrome (PANS), 1146. *See also* PANDAS (pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection)
Pediatric autoimmune neuropsychiatric disorder associated with streptococcal infection. *See* PANDAS (autoimmune neuropsychiatric disorder associated with streptococcal infection)
Pediatric Risk for Mortality (PRISM) score, 458
Pediculus humanus. *See* Lice
Pediococcus spp., 1247-1248
vs. *Enterococcus* spp., 1171
Pediococcus spp. infection, **1247-1248**
clinical manifestations of, 1248
diagnosis of, 1248
epidemiology of, 1248
pathophysiology of, 1248
treatment of, 1248
Pelvic inflammatory disease (PID), **586-589**, 588t-589t, 1285-1286, 1829
chlamydial infection and, 534-535
complications of, 589, 589f, 1286, 1286f
diagnosis of, 1285
risk factors for, 1286
treatment of, 1296
Trichomonas vaginalis infection and, 2891
Pelvis, osteomyelitis of, 720
Pemphigus syphiliticus, 1770
Penciclovir, 3278t, 3281-3282, 3281f
Penicillin(s), 3183-3191, 3183t
in acquired syphilis, 1777-1778
adverse effects of, 3189-3191, 3189t
in bacterial meningitis, 450t, 1263-1266
in cervical lymphadenitis, 185-186
in community-acquired pneumonia, 291
in congenital syphilis, 1776t-1777t, 1779-1780, 1780f
dosage schedules for, 3231t-3233t
extended-spectrum, 3183t-3184t, 3187, 3231t-3233t
Harry Eagle effect with, 739
in myositis, 739
in *Neisseria meningitidis* infection, 1263-1266
in odontogenic infection, 145, 146t
penicillinase-resistant, 3183t-3184t, 3185-3186, 3231t-3233t
in peritonsillar abscess, 174
Penicillin(s) *(Continued)*
resistance to, 3139-3145
in *Staphylococcus aureus* meningitis, 448-449
in *Streptococcus pneumoniae* mastoiditis, 234
in *Streptococcus pneumoniae* meningitis, 427, 447
in *Streptococcus* viridans infection, 1194
in rheumatic fever, 414, 414f, 414t
in rheumatic fever prevention, 415-418
in *Streptococcus pyogenes* infection, 1147-1149, 1148t
in syphilis, 812
in tetanus, 1816
in Whipple disease, 645-646, 646t
Penicillin-binding proteins, in antibiotic resistance, 3143-3145
Penicillin G, 3183-3185, 3184t, 3231t-3233t
Penicillin V, 3183-3185, 3184t, 3231t-3233t
Penicillium marneffei infection, **2855**, 2855f
Penicillium spp. infection, burn wound, 1053
Penis
cancer of, HPV and, 1873t, 1875, 1878
HSV infection of, 1942f. *See also* Herpes simplex virus (HSV) infection, genital
Pentamidine, 3350
in hematopoietic stem cell transplantation, 938-939
pancreatitis with, 691-692
in *Pneumocystis* infection, 691-692
in *Trypanosoma brucei* infection, 2971-2972
in visceral leishmaniasis, 2958
Pentoxifylline, 3360-3361
Peptic ulcer disease, 1691, 1694. *See also* *Helicobacter pylori* infection
Peptidoglycan
of *Neisseria gonorrhoeae*, 1278-1279
of *Streptococcus pneumoniae*, 1210, 1211f, 1214
Peptidoglycan *N*-acetylglucosamine deacetylase A, of *Streptococcus pneumoniae*, 1215
Peptostreptococcus spp. infection
peritoneal, 700
retroperitoneal, 706-707
Peramivir, 3270-3273
Perforin, 28
Perianal streptococcal dermatitis, **775**
Periarteritis nodosa, infantile, 1071. *See also* Kawasaki disease
Pericardial effusion
in meningitis, 441
in pericarditis, 373-374, 376-377, 376f-377f
Pericardial fluid, 371
blood in, 377-378
in pericarditis diagnosis, 377-378
Pericardial window, 378
Pericardiocentesis, 378, 378t
Pericarditis
adenovirus, 1902-1903
bacterial, **371-379**
clinical manifestations of, 372t, 374
diagnosis of, 375-378, 376f-377f
differential diagnosis of, 378
epidemiology of, 371, 372t
etiology of, 371-373, 372t
pathogenesis of, 373-374
prognosis for, 379
treatment of, 378-379, 378t
Candida, 2740-2741, 2748
Entamoeba histolytica, 2871
enterovirus, 2075-2077, 2076t
fungal, 373, 379

Pericarditis *(Continued)*
Haemophilus influenzae type b, 372-373, 378-379, 1676
Histoplasma capsulatum, 373, 377-378, 2813
influenza virus, 2343
measles virus, 2385
Morganella morganii, 1470
mortality with, 379
vs. myocarditis, 395
Neisseria meningitidis, 373, 378-379, 1262
noninfectious, 372t
pneumococcal, 373
Pseudomonas aeruginosa, 373
rubella virus, 2212
staphylococcal, 372, 379
streptococcal, 371-373, 379, 1232
surgical treatment of, 378
tuberculous, 373, 379, 1351
viral, **379-380**
clinical manifestations of, 379
diagnosis of, 380, 380f
etiology of, 372t, 379
prognosis for, 380
in Whipple disease, 644
Pericardium, 371
infection of. *See* Pericarditis
Pericoronitis, 147-148, 148f
Perihepatitis, **589-590**
Perineum, hygiene of, 564-566
Periodic fever, 159t-160t, 162, 181
Periodic fever disorders, 847-848
Periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome, 159t-160t, 162-164, 181, 848
Periodontitis, 146-147
Aggregatibacter actinomycetemcomitans, 140, 147, 1610, 1823
Eikenella corrodens, 1577
juvenile, 140, 147
PMN chemotaxis in, 56
in leukocyte adhesion deficiency, 904-905, 905f
pathogens in, 140
Periorbital cellulitis, 151, 798-801
Periostitis, in congenital syphilis, 1769, 1772
Peripheral neuropathy
CMV, 1979-1980
in *Corynebacterium diphtheriae* infection, 1304-1305
Peripherally inserted central catheter (PICC), infection with, 1136. *See also* Catheter-associated infection
Peritoneal cavity, 698, 699f
Peritoneal dialysis, 698, 700t, 701-703
catheter-related infection with, 1137
Peritoneal pseudocyst, in ventriculoperitoneal shunt, 1031-1032
Peritonitis, **698-704**
Aeromonas spp., 1542
anatomy of, 698, 699f
Candida, 2741, 2748, 2754-2755
CAPD-associated, 698, 700t, 701-703
chemical, 698
clinical manifestations of, 701
vs. *Clostridium difficile* colitis, 635
complications of, 704
coxsackievirus B, 2070
diagnosis of, 702
dialysis-related, 3406-3407
differential diagnosis of, 702
Escherichia coli, 1441, 1445
fungal, 701, 703
Mycobacterium avium-intracellulare complex, 1389
Peritonitis *(Continued)*
noninfectious, 698
vs. noninfectious diseases, 702
Pasteurella multocida, 1547
pathogenesis of, 698, 700t
Plesiomonas shigelloides, 1566
primary (spontaneous), 698-700, 700t, 702
secondary, 700-703, 700t
Stenotrophomonas maltophilia, 1607-1608
Streptococcus pneumoniae, 1232
tertiary (recurrent), 704
treatment of, 702-704, 703t
tuberculous, 700-701, 1357-1358
VP shunt-associated, 698, 700t, 701-703
Peritonsillar abscess, **168-170**, 170f
clinical manifestations of, 168-169, 168f
microbiology of, 173-175
treatment of, 169-170, 175
Perityphlitis, 679
Permethrin, 1737, 2175, 2236, 2526, 3350
in lice, 3075
in scabies, 3074
Pertactin, of *Bordetella pertussis*, 1618t, 1619, 1623
Pertussis. *See Bordetella pertussis* infection (pertussis, whooping cough)
Pertussis-like illness
in adenovirus infection, 1617, 1898
in *Mycoplasma pneumoniae* infection, 1617
Pertussis toxin, 17, 1618t, 1619, 1624
Petechiae, 762-763, 763t
in arenaviral hemorrhagic fevers, 2471
in infective endocarditis, 357
in meningococcal infection, 750, 753t-756t, 762-763, 1258-1259, 1259f
in septic shock, 831
Petrolatum jelly, in eyelid lice, 795
Petrositis, 224
mastoiditis and, 237
PFAPA (periodic fever, aphthous stomatitis, pharyngitis, and adenitis) syndrome, 159t-160t, 162-164, 181
pH
airway, in rhinovirus infection, 135
blood, in diarrheal disease, 76
CSF, in bacterial meningitis, 444
ear canal, in otitis externa, 207
fecal, in diarrheal disease, 76
Phaeoannellomyces werneckii infection, **790**, 790f
Phaeohyphomycosis, **2855-2858**, 2856f
Phagocyte(s), 31-35
disorders of, 52-59, 917-919, 917t
qualitative, 917t, **918-919**
quantitative, 917, 917t
evaluation of, 63, 907
microbicidal activity of, 33-35, 34f, 34t
defects of, 56-59
in newborn, 45
recruitment of, 31-32, 32f-33f
Phagocytosis, **32-33**, 33f
in *Haemophilus influenzae* infection, 1672-1673
Neisseria gonorrhoeae resistance to, 1276
pathogen evasion of, 18
in *Staphylococcus aureus* infection, 1122
in *Streptococcus pneumoniae* infection, 1223
Pharyngitis, **158-164**
adenovirus, 158-161, 159t-160t, 1895-1896
Arcanobacterium haemolyticum, 1324-1326, 1324f, 1324t
in bronchiolitis, 275
Citrobacter freundii, 1431
Pharyngitis *(Continued)*
clinical presentation of, 159t-160t, 161-162
complications of, 164
Corynebacterium diphtheriae, 1305, 1307
coxsackievirus A10, 2066
diagnosis of, 163
differential diagnosis of, 159t-160t, 162-163
enterovirus, 2064t, 2065
epidemiology of, 161
etiology of, 158-161, 159t-160t
fever in, 162
gonococcal, 1288, 1296
historical perspective on, 156
HSV, 1941
meningococcal, 1261
Mycoplasma pneumoniae, 2678
pathophysiology of, 161
prevention of, 164
prognosis for, 164
reovirus, 2169-2170
Saffold virus, 2065
streptococcal, 156, 159t-160t
clinical manifestations of, 161-162, 1142-1143
diagnosis of, 163, 1146-1147
epidemiology of, 1141
non–group A, 1151
pathogenesis of, 1142
prevention of, 1141-1142, 1150
rheumatic fever and, 408, 416. *See also* Rheumatic fever
tonsillectomy in, 1150
transmission of, 1141
treatment of, 163, 1147-1149, 1148t
treatment of, 163-164
uvulitis and, 166
Yersinia enterocolitica, 1528
Pharyngoconjunctival fever, 1893-1894, 1899, 1899t-1900t
Pharynx
hyperemia of, 842
infection of. *See* Pharyngitis
Phenol-soluble modulins, of *Staphylococcus epidermidis*, 1133
Phenylalanine, 71
serum, 830
urinary, 69
Phenylalanine-to-tyrosine ratio, 71
Phenylephrine hydrochloride, in rhinosinusitis, 200
Phialophora verrucosa infection, **789-790**
Phlebotomus fever, **2535-2537**, 2536f
Phlebovirus, 2535
Phosphoglucomutase, of *Streptococcus pneumoniae*, 1214
Phospholipase D, 1322
Phosphorylcholine esterase, of *Streptococcus pneumoniae*, 1213
Photophobia, in enterovirus infection, 2074
Phthirus pubis (crabs). *See* Lice
Physostigmine ointment, in eyelid lice, 795
Pichia spp. infection, 898
Piercings
intraoral, 155
perioral, 155
Pigbel, 1797-1800
Pilin protein, of *Streptococcus pneumoniae*, 1211
Pill esophagitis, 594
Pilosebaceous unit, of external ear, 204
Pilus (pili), 3-6
Aeromonas spp., 1541
antigenic variation in, 19

Pilus (pili) *(Continued)*
assembly of, 4-5, 4f-5f
chaperone-usher pathway of, 4-5, 4f-5f
donor-strand complementation in, 4-5, 5f
donor-strand exchange in, 4-5, 5f
enterotoxigenic *Escherichia coli*, 1455
Kingella kingae, 1701-1702, 1701f
Neisseria gonorrhoeae, 1277
Neisseria meningitidis, 1254
Pseudomonas aeruginosa, 1586-1587
Streptococcus agalactiae, 5-6, 1154
structure of, 3-4, 4f
type 4, 5
uropathogenic *Escherichia coli*, 3-4, 4f, 10
Pinchindé virus infection, 2472. *See also* Arenavirus infection
Pinta, 753t-756t, 1783t, **1784-1785**
diagnosis of, 1789
treatment of, 1789-1790
Pintids, 1785
Pinworms, 566-567, 566f, **3018-3019**
Scotch tape collection of, 566, 566f
Piperacillin, 3183t-3184t, 3187, 3231t-3233t
in meningitis, 450t
Piperacillin-tazobactam, 3188-3189
Pituitary gland, hormones of, 81
PIV infection. *See* Parainfluenza virus (PIV) infection
Placenta
in syphilis, 1766
in *Ureaplasma* spp. infection, 2690
Plague. *See Yersinia pestis* infection (plague)
Plaque reduction neutralization test
in Toscana virus infection, 2549
in yellow fever, 2277
Plasmapheresis
in acute disseminated encephalomyelitis, 519
in acute transverse myelitis, 527-528
in encephalitis, 510
in *Mycoplasma pneumoniae* infection, 2687
in septic shock, 836
Plasmid(s), in antibiotic resistance, 3137-3138, 3138t
Plasmid-encoded toxin, 1460
Plasminogen, *Streptococcus pneumoniae* binding of, 1214
Plasmodium spp., 2922-2923, 2927t. *See also* Malaria
antigen detection assays for, 2935
vs. *Babesia* spp, 2918-2919
DNA probes for, 2935
life cycle of, 2922-2923, 2922f
microscopy for, 2930, 2931f-2934f
PCR for, 2935
Platelet-activating factor, 3366-3367
Platelet-activating factor receptor, in *Streptococcus pneumoniae* infection, 1224
Platelet count, in Kawasaki disease, 1076
Platelet disorders, in Wiskott-Aldrich syndrome, 913
Pleconaril, 3276-3277, 3276f
in common cold, 138
in enterovirus infection, 2103
in rhinovirus infection, 2126
Plesiomonas shigelloides, 1565
Plesiomonas shigelloides infection, 607t, 612-613, 616t, **1565-1566**
Pleura, in Whipple disease, 644
Pleural effusion
bloody, 315
etiology of, 308, 308t
exudative, 308, 311
in *Francisella tularensis* infection, 1662
Light's criteria for, 311-312
Pleural effusion *(Continued)*
malignant, 313
parapneumonic, **306-315**
chest radiography in, 310, 311f
clinical presentation of, 310
CT in, 310
diagnosis of, 310-313
drainage of, 314-315
epidemiology of, 307
fluid analysis in, 310-313
historical perspective on, 307
management of, 312f, 313-315
microbiology of, 309-310, 309t
pathophysiology of, 307-308, 308t
prevention of, 315
prognosis for, 315
radiologist-directed thoracentesis for, 314
streptokinase for, 314-315
ultrasonography in, 310, 311f
video-assisted thoracoscopic surgery for, 314
pathophysiology of, 307-308, 308t
purulent, 306-308, 308t
transudative, 306-308, 308t, 311
in tuberculosis, 309, 1343f, 1347-1348, 1348f-1349f
Pleural fluid examination
in mediastinitis, 420
in parapneumonic effusion, 310-313
Pleurodynia, epidemic, 739, 2067-2068
Pneumatocele, pneumonia and, 322
Pneumatosis cystoides intestinalis, 1798
Pneumatosis oculi, 1431
Pneumococcal histidine-containing protein A (PhpA), of *Streptococcus pneumoniae*, 1213
Pneumococcal infection. *See Streptococcus pneumoniae* infection
Pneumococcal surface protein A (PspA), of *Streptococcus pneumoniae*, 1210-1211, 1211f, 1213
Pneumocystis pneumonia, **3005-3012**
clinical manifestations of, 3008-3009, 3009f
congenital immunodeficiency and, 3007
diagnosis of, 3009-3010
after heart transplantation, 954
after hematopoietic stem cell transplantation, 938-939
HIV infection and, 2599-2600, 2599f, 2600t, 3007
immunopathogenesis of, 3007-3008
after intestinal transplantation, 1000
after kidney transplantation, 1013
after liver transplantation, 1000
after lung transplantation, 972-973
pathology of, 3008
pentamidine isethionate in, 691-692
prevention of, 3010-3012
prognosis for, 3010
transmission of, 3006-3007
treatment of, 3010, 3011t, 3327t-3346t
Pneumocystis spp., 3006, 3006f, 3009f
Pneumolysin, *Streptococcus pneumoniae*, 1209-1210, 1212-1213, 1223-1224
Pneumonia
Acinetobacter spp., 1570-1571
adenovirus, 284, 295-296, 1894, 1894f, 1897-1898, 1900
Aeromonas hydrophila, 1543
antibiotic resistance in, 3147
atypical measles, 2384, 2384f
Bacillus cereus, 1320
Blastomyces dermatitidis, 2727, 2727f
Brucella spp., 1613
Pneumonia *(Continued)*
in burn patient, 1055
Candida, 2741
Chlamydia pneumoniae, 292, 1617-1618, **2639-2644**, 2641t, 2642f
Chlamydia trachomatis, 2633, 2633f
CMV, 1976-1977
community-acquired, **283-294**
abscess with, **315-322**, 317t, 319f-320f, 320t
Acinetobacter baumannii, 1571
auscultation in, 288
bacterial, 284-285, 285t
clinical manifestations of, 287-289, 287t
diagnosis of, 289
pathogenesis of, 286-287
prevention of, 292
treatment of, 289-292, 291t
clinical manifestations of, 287-289, 287t
clinical scores in, 290
comorbid conditions with, 290
complications of, **306-322**, 307t. *See also* Lung abscess; Pleural effusion, parapneumonic
diagnosis of, 289
empyema with, **306-315**, 308t-309t, 311f-312f
vs. eosinophilic pneumonia, **297**
epidemiology of, 285
etiology of, 283-285, 285t
hospitalization for, 290
management of, 289-292, 291t
mimivirus antibodies in, 2048-2049
pathogenesis of, 285-287
pleural effusion in. *See* Pleural effusion, parapneumonic
prevention of, 285, 292
rhinovirus infection and, 2123
vs. vasculitis, 297
viral, 284, 285t
clinical manifestations of, 288
diagnosis of, 289
pathogenesis of, 285-287
prevention of, 292
treatment of, 290
coronavirus, 284, 2491
coxsackievirus, 2067
echovirus, 2067
Eikenella corrodens, 1577
enterovirus, 2067
eosinophilic, **297**
Escherichia coli, 1441, 1445-1446
Francisella tularensis, 1662-1663
Haemophilus influenzae type b, 1675
health care–associated, 3391-3393
in neonate, 892
after heart transplantation, 947-948
hMPV, 284, 285t, 2438-2439, 2439f
HSV, 1953-1954
human bocavirus, 284, 1860
influenza virus, 284, 2340, 2344f
IVIG in, 3508
after kidney transplantation, 1005-1006
Klebsiella pneumoniae, 1466-1467
Legionella spp., **1705-1714.** *See also Legionella pneumophila* infection (Legionnaires' disease)
differential diagnosis of, 1713
measles virus, 2384-2385, 2384f, 2391-2392
in military recruits, 1898
Moraxella catarrhalis, 1252
Mycoplasma pneumoniae, 285t, 287-289, 292, 2675-2678, 2676t

Pneumonia *(Continued)*
necrotizing, 321
Neisseria meningitidis, 1260-1261
neonatal, 891-892
Plesiomonas shigelloides, 1566
pneumococcal, 284-285, 1229-1231, 1230f, 1230t. *See also Streptococcus pneumoniae* infection
diagnosis of, 1217-1218
treatment of, 1233, 1234t-1235t
Pneumocystis, **3005-3012**, 3006f, 3009f. *See also Pneumocystis* pneumonia
Pseudomonas aeruginosa, 1599
reovirus, 2170
Rhodococcus equi, 1427
RSV, 284, 290. *See also* Respiratory syncytial virus (RSV) infection
Stenotrophomonas maltophilia, 1606-1609
Streptococcus viridans, 1193
Treponema pallidum, 1770-1771
Ureaplasma spp., 296, 2690
ventilator-associated, 3391-3393
in neonate, 892
VZV, 2026
in Whipple disease, 643-644
Yersinia pestis, 1512-1513
Pneumonia alba, 1770-1771
Pneumonitis
CMV, 1974, 1976-1977, 1976f, 1984
after heart transplantation, 950-951
coxsackievirus, 2061-2062
hemorrhagic, in *Leptospira* spp. infection, 1748
hookworm, 3018
HSV, after kidney transplantation, 1006
hypersensitivity, 297, **301-305**, 302t-303t. *See also* Hypersensitivity pneumonitis
influenza virus, 2340
interstitial
after heart transplantation, 948
lymphocytic, 296
lupus, 297
Schistosoma spp., 3065-3066
Strongyloides stercoralis, 3020
Pneumothorax, pneumonia and, 322
Podophyllin, in genital HPV infection, 581
Poison ivy, vs. enteroviral infection, 762
Poisoning
food. *See* Food poisoning
heavy metal, 604, 604t
insecticide, 692
Poliovirus, 2052-2056, 2052t-2053t, 2059
Poliovirus infection (poliomyelitis), **2097-2101**
abortion and, 2100
congenital, 2100
in daycare facilities, 3563
epidemics of, 2057
epidemiology of, 119
extraneural, 2063
fetal, 2100-2101
global eradication of, 2106-2107
historical perspective on, 2051-2052
immunization against, 2105-2106, 3463-3466
meningeal, 488
minor, 2098
neonatal, 2100-2101
nonparalytic (aseptic), 2098
paralytic, 2098-2101, 2099t
passive immunization against, 3512-3513
pathogenesis of, 2060, 2060f
pathology of, 2062-2063
postnatal, 2101
Poliovirus infection (poliomyelitis) *(Continued)*
prevention of, 2105-2106
treatment of, 2104
Polyangiitis, microscopic, 297
Polychondritis, relapsing, vs. otitis externa, 208
Polymerase chain reaction (PCR)
in *Acanthamoeba* spp. infection, 2983
in *Achromobacter* spp. infection, 1575
in adenovirus infection, 982, 1906
in *Anaplasma* spp. infection, 2662-2663
in arenavirus infection, 2474
in *Aspergillus* spp. infection, 2716-2717
in bacterial meningitis, 442-444, 1263
in *Balamuthia mandrillaris* infection, 2983
in *Bartonella* spp. infection, 1727
in BK virus infection, 1868-1869
in *Bordetella* spp. infection, 1628, 3593
in *Burkholderia cepacia* infection, 1597
in *Campylobacter jejuni* infection, 1654-1655
in *Candida* spp. infection, 2757
in cervical lymphadenitis, 184
in *Chlamydia pneumoniae* infection, 2643-2644
in *Chlamydia psittaci* infection, 2639
in *Clostridium difficile* infection, 3594
in CMV infection, 1982
in Colorado tick fever virus infection, 2174-2175
in congenital rubella, 2219
in coronavirus infection, 2494
in *Corynebacterium diphtheriae* infection, 1307
in *Cryptosporidium* spp. infection, 2902
in Ebola virus infection, 2484
in EBV infection, 2010-2011
in *Ehrlichia* spp. infection, 2662-2663
in encephalitis, 507-508
in *Enterobacter* spp. infection, 1436-1437
in enterovirus infection, 2102
in *Fasciola* spp. infection, 3053
in *Francisella tularensis* infection, 1663
in fungal infection, 926
in gastroenteritis, 619, 620f
in *Giardia lamblia* infection, 2882
in *Haemophilus ducreyi* infection, 1688
in HAV infection, 2130, 2139
in HCV infection, 2321-2323
in HEV infection, 2165
in HHV-6 infection, 2019
in HIV infection, 2609
in hMPV infection, 2440
in HSV encephalitis, 1948
in HSV infection, 1935, 1956-1957
in human bocavirus infection, 1861
in influenza virus infection, 981, 2345-2346
in JC virus infection, 1869
in *Kingella kingae* infection, 1703
in *Klebsiella granulomatis* (*Calymmatobacterium granulomatis*) infection, 1642
in LCMV infection, 2465
in *Legionella pneumophila* infection, 1711
in *Leptospira* spp. infection, 1755-1756
in lung transplant–related *Aspergillus* infection, 967
in malaria, 2935
in microsporidial infection, 2913
in mycobacterial infection, 1383
in *Mycobacterium leprae* infection, 1405
in *Mycobacterium ulcerans* infection, 1415
in *Mycoplasma pneumoniae* infection, 2686
Polymerase chain reaction (PCR) *(Continued)*
in myocarditis, 382, 393-394
in *Naegleria fowleri* infection, 2983
in *Neisseria meningitidis* infection, 1263
in neonatal viral infection, 880
in *Norovirus* infection, 2153
in parainfluenza virus infection, 2368-2369
in parapneumonic pleural effusion, 313
in pneumococcal disease, 1217-1218
in *Pneumocystis* infection, 3010
in *Pseudomonas aeruginosa* infection, 1597
in rhinovirus infection, 2125
in Rift Valley fever, 2530
in rotavirus infection, 2182-2183
in RSV infection, 2425
in rubella virus infection, 2218
in *Shigella* spp. infection, 1482-1483
in smallpox, 2036
in *Streptobacillus moniliformis* infection, 1718
in *Streptococcus agalactiae* infection, 1162
in syphilis, 1774
in *Taenia saginata* infection, 3031-3032
in Toscana virus infection, 2550
in *Toxoplasma gondii* infection, 2996-2997
in *Trichomonas vaginalis* infection, 2892
in *Trypanosoma brucei* infection, 2971
in *Trypanosoma cruzi* infection, 2966
in tuberculosis, 1365-1366
in urethritis, 533
in *Vibrio vulnificus* infection, 1561
in viral infection, 3616-3617, 3616t
in viral meningitis, 444, 490
in VZV infection, 2028
in West Nile virus infection, 2270
in Whipple disease, 645
in yellow fever, 2277
in *Yersinia enterocolitica* infection, 1530
in *Yersinia pseudotuberculosis* infection, 1530
Polymethyl methacrylate (PMMA)
intraocular lenses, infection with, 1019
Polymorphonuclear leukocytes (PMNs), 31-35. *See also* Neutrophil(s)
defects in, 52-59
intrinsic, 52-55
secondary, 55-56
granules of, 34t
impaired adhesion of, 45
microbicidal activity of, 33-35, 34f, 34t
neonatal, 45
recruitment of, 31-32, 32f-33f
Polymyositis, HTLV-1, 2576
Polyomavirus infection, **1863-1870**. *See also* BK virus infection; JC virus infection; Simian virus 40 (SV40)
clinical manifestations of, 1866-1869
CNS, **1866-1867**, 1866f
cutaneous, 1864, 1864t, 1867-1868
diagnosis of, 1868-1869
historical perspective on, 1863-1864
malignancy and, 1868
pulmonary, 1868
retinal, 1868
treatment of, 1869
urinary tract, 1867, 1867f
Polyradiculopathy, CMV, 1979-1980
Polysaccharide intercellular adhesin (PIA), 1132-1133
Pontiac fever, **1714**
outbreaks of, 1708
pathogenesis of, 1709-1710
Population. *See also* Epidemiology
disease in, 111-120
household unit of, 111
Por1 protein, 18
Porcine circovirus, 2191

Porins
Neisseria gonorrhoeae, 1278
Neisseria meningitidis, 1254-1255
Pork, *Yersinia enterocolitica* contamination of, 1517-1518
Porphyromonas spp., 1826
Porphyromonas spp. infection, **1825-1834**
bloodstream, 1827-1828
bone, 1829-1830
CNS, 1828-1829
cutaneous, 1829
diagnosis of, 1830
epidemiology of, 1826-1827
head and neck, 1828
intra-abdominal, 1829
joint, 1829-1830
pathogenesis of, 1827
pleuropulmonary, 1830
prevention of, 1833
soft tissue, 1829
treatment of, 1831-1833, 1831t
Portosystemic shunting, in Caroli disease, 667
Posaconazole, 3301f, 3308-3310
adverse effects of, 3308
in *Aspergillus* spp. infection, 2718
in *Blastomyces dermatitidis* infection, 2733-2734
dosing for, 3309-3310
drug interactions with, 3309
efficacy of, 3309
in *Histoplasma capsulatum* infection, 2826
pharmacodynamics of, 3308
pharmacokinetics of, 3308, 3308t
in transplant-related aspergillosis, 937-938, 968
Positive end-expiratory pressure (PEEP), in ARDS, 871
Post-transplant lymphoproliferative disease (PTLD), 654, 976-978, 997-998, 1008
diagnosis of, 976-977
EBV and, 1996, 2005-2006, 2012
after heart transplantation, 952-953
management of, 998, 1009, 2012-2013, 3521-3522
prevention of, 1001, 2015
Postauricular lymphadenopathy, vs. mastoiditis, 237
Postexposure prophylaxis, 3248-3251, 3540t-3544t, 3546
in anthrax exposure, 3540t-3544t, 3582
in *Haemophilus influenzae* type b exposure, 3249-3250, 3540t-3544t
in HAV exposure, 2142, 2144, 3436-3437, 3436t, 3540t-3544t
in HBV exposure, 3517-3519, 3518t, 3540t-3544t
in HIV exposure, 2594
in *Neisseria meningitidis* exposure, 3249, 3455, 3540t-3544t
in pertussis exposure, 3249, 3540t-3544t
in rabies exposure, 2453-2458, 2456t-2457t, 3481-3483, 3482t, 3527-3529, 3540t-3544t
in tetanus-related wound management, 3471, 3472t
in tuberculosis exposure, 3250-3251, 3540t-3544t
in VZV exposure, 3475, 3540t-3544t
Postherpetic encephalomyelitis, 1948
Postherpetic neuralgia, 2027-2028
Postinfectious encephalitis, 493, **503**
imaging in, 507
pathogenesis of, 504
pathology of, 504
Postinfectious encephalomyelitis. *See* Acute disseminated encephalomyelitis (ADEM)
Post–kala-azar dermal leishmaniasis, 2956
Postpolio syndrome, 2104-2105
Posttransplantation acute limbic encephalitis (PALE), 2018
Postural orthostatic tachycardia syndrome (POTS), 1088-1089
Potassium, fecal, 76
Potassium hydroxide examination
in tinea nigra, 790, 790f
in white piedra, 791
Pott abscess, 1355
Povidone-iodine
in burn wound infection, 1059
in neonatal conjunctivitis prevention, 805-806, 1281
Powassan virus infection, 494t-498t, **2313-2314**
Power (statistical), 121
Poxviruses, 749, 2040, 2041t. *See also* Molluscum contagiosum; Monkeypox; Smallpox
identification of, 2047
Praziquantel, 3350
Prebiotics, 3369-3370
safety of, 3372
Prednisolone, in PFAPA syndrome, 164
Pregnancy. *See also* Newborn
antiparasitic agents in, 3351, 3351t-3353t
bacteruria in, 1154
Blastomyces dermatitidis infection during, 2730-2731, 2733
Brucella spp. infection during, 1612
CMV infection during, 1970, 1972, 1983, 1986, 1988
prevention of, 1989
Coccidioides spp. infection during, 2772, 2774
Colorado tick fever virus infection during, 2174
Corynebacterium diphtheriae infection during, 1306
Coxiella burnetii infection during, 2664
Eastern equine encephalitis virus infection during, 2228
EBV infection during, 1998
Edwardsiella tarda infection during, 1533
Eikenella corrodens infection during, 1577
gonococcal infection during, 1297
HAV infection during, 2130, 2140
HBV infection during, 1917, 1917t
HEV infection during, 2163-2164
HHV-6 infection during, 2018
HSV infection during, 653-654, 1936-1937, 1965, 1967. *See also* Herpes simplex virus (HSV) infection, neonatal
human erythrovirus B19 (human parvovirus B19) infection during, 1852-1853, 1856
inadvertent rubella virus immunization during, 2223-2224
inadvertent smallpox immunization during, 3533
influenza virus infection during, 2345, 2356-2357
Japanese encephalitis virus infection during, 2260, 2297
Lyme disease during, 1734
malaria during, 2929-2930
mumps during, 2402-2403, 2405
Neisseria gonorrhoeae infection during, 1280, 1280t, 1299
Pregnancy *(Continued)*
Pasteurella multocida infection during, 1547
rubella virus infection during, 2203, 2206-2207, 2219-2220. *See also* Rubella virus infection, congenital
Salmonella ser. Typhi infection during, 1502
Salmonella spp. infection during, 608
Streptococcus agalactiae infection during, 1154-1155, 1157-1158
syphilis during, 1769, 1778
Toxoplasma gondii infection during, 2990, 2992-2993, 2992t, 2997, 3002, 3004, 3004t
tuberculosis during, 1360
Ureaplasma spp. infection during, 2689
VZV infection during, 2026-2027
West Nile virus infection during, 2264
Western equine encephalitis virus infection during, 2234
Premature infant. *See also* Newborn
bacteremia in, 1134-1135, 1135t
enterovirus infection and, 2100
immunization in, 44
indigenous flora acquisition by, 89, 93
IVIG for, 3500-3501
maternal poliomyelitis and, 2100
sinopulmonary infection in, 268
Preseptal (periorbital) cellulitis, 151, 798-801
nontraumatic, 798-799
posttraumatic, 798
Presumed ocular histoplasmosis syndrome, 2816-2817
Prevotella spp., 1826
Prevotella spp. infection, **1825-1834**
bloodstream, 1827-1828
bone, 1829-1830
clinical manifestations of, 1827-1830
CNS, 1828-1829
cutaneous, 1829
diagnosis of, 1830
epidemiology of, 1826-1827
head and neck, 1828
intra-abdominal, 1829
joint, 1829-1830
pathogenesis of, 1827
pleuropulmonary, 1830
prevention of, 1833
soft tissue, 1829
treatment of, 1831-1833, 1831t
Primaquine, 3350
in malaria, 2937t-2940t, 2941-2942
in malaria prevention, 2945
Primary ciliary dyskinesia. *See* Immotile cilia syndrome; Kartagener syndrome
Primary sclerosing cholangitis, 662-663
Prion diseases. *See* Transmissible spongiform encephalopathies
PRISM score, 458
Probiotics, **3369-3373**
in allergic disease, 3371
in antibiotic-associated diarrhea, 3370
in atopic disease, 3371
in *Clostridium difficile* infection, 637
in cold prevention, 139
in constipation, 3371
definition of, 3369-3370, 3370t
in gastrointestinal tract infection, 626
in *Helicobacter pylori* infection, 3370
in hepatic encephalopathy, 3371
for HIV-infected host, 3372
in infant colic, 3371
in infectious diarrhea, 3370, 3371t

Probiotics *(Continued)*
in inflammatory bowel disease, 3370-3371
in irritable bowel syndrome, 3371
in necrotizing enterocolitis, 3371-3372
in neonatal gastroenteritis, 888
in neonatal sepsis, 3371-3372
in respiratory tract infections, 3371
in rotavirus infection, 2186
safety of, 3372
uses of, 3370-3372, 3371t
Procalcitonin, 82
in bacteremia, 832
in burn wound infection, 1055
in meningitis, 444
in pancreatitis, 690
in septic shock, 830, 832
in urinary tract infection, 543
Proctitis, HSV, 1948-1949, 1952-1953, 1953f
Progressive multifocal leukoencephalopathy (PML), 1010, **1866-1867**, 1866f
diagnosis of, 1869
historical perspective on, 1863
treatment of, 1869
Progressive outer retinal necrosis, 810
Proguanil, 3350
in malaria, 2945
Project Bioshield Act, 2036-2037
Prone positioning, in ARDS, 873
Properdin, deficiency of, 51-52
Neisseria meningitidis infection and, 1257
Propionibacterium propionica infection (actinomycosis), **1820-1824**
clinical manifestations of, 1821-1823
diagnosis of, 1823-1824
pathogenesis of, 1820-1821
pathology of, 1821
treatment of, 1824
Prostatitis, **558-560**
chronic, 559
organisms in, 558
treatment of, 558-559
Prosthetic heart valves, infective endocarditis with, 351, 357, 365-366, 366t, 1137
Prosthetic/implantable device–related infection, **1015-1043**, 1016t
cardioverter-defibrillator, 1025-1026
coagulase-negative staphylococci in, **1136-1137**
cochlear implant, 1017-1018
contact lens, 1019
CSF shunt, **1029-1038**, 1030t, 1033t, 1035f
extracorporeal membrane oxygenation circuit, 1022-1023
host factors in, 1016
intracranial pressure monitor, 1038-1040, 1039t
intraocular lens, 1019
joint prosthesis, **1026-1029**
left ventricular assist device, 1020-1022, 1020f
microbial factors in, 1016-1017
orbital implant, 1018-1019
pacemaker, 1023-1025
tissue expander, 1017
Protease, in *Pseudomonas aeruginosa* infection, 1587
Protease inhibitors, 2614-2615
Protein, CSF, in neonatal meningitis, 885
Protein A, of *Staphylococcus aureus*, 1114
Protein C
recombinant, in *Neisseria meningitidis* infection, 1265, 1265t
in septic shock, 829
Protein-calorie malnutrition, PMN chemotaxis in, 56
Protein F, 10
Protein-losing diseases, IVIG in, 3499
Proteus mirabilis infection
meningeal, 1472-1474
urinary tract, 1472-1474
Proteus morganii. *See Morganella morganii* infection
Proteus spp., 1471
Proteus spp. infection, **1471-1474**
clinical manifestations of, 1473
CSF shunt, 1030, 1030t
diagnosis of, 1473
epidemiology of, 1472
pathophysiology of, 1472-1473
treatment of, 1473-1474
Protozoan disease. *See also specific protozoan diseases*
appendiceal, 684-685
classification and nomenclature in, 2862-2864, 2862t
corneal, 808-809
cutaneous manifestations of, 757t-758t, 761t, 763t
encephalitic, 494t-498t, 501
after hematopoietic stem cell transplantation, 943-944
host response to, 23-24
uveal, 814
Providencia alcalifaciens infection, 1475
Providencia rettgeri infection, 1475
Providencia spp., 1474
Providencia spp. infection, **1474-1476**
Providencia stuartii infection, 1475
Pseudoappendicitis
in *Yersinia enterocolitica* infection, 1527
in *Yersinia pseudotuberculosis* infection, 1529
Pseudocowpox virus infection, 2046
Pseudocyst
pancreatic, 696
peritoneal, 701
Pseudohypoparathyroidism, HTLV-1 virus and, 2571
Pseudoinfection, *Bacillus cereus*, 1320
Pseudomonas aeruginosa
endotoxins of, 1587
exotoxins of, 1587
siderophores of, 1588
type III secretion system of, 1587
typing of, 1585
virulence factors of, 1586-1587
Pseudomonas aeruginosa infection, **1582-1605**
antibiotic resistance in, 1601-1603, 3149
biofilm formation in, 11, 14f, 1588
bloodstream, 1594-1595
bone, 1591, 1599
burn wound, 1052-1053, 1057f
clinical manifestations of, 1589-1596, 1590f
cutaneous manifestations of, 1589-1591, 1590f
in cystic fibrosis, 1593-1594. *See also* Cystic fibrosis, *Pseudomonas aeruginosa* infection in
diagnosis of, 1582-1583, 1596-1597
endocardial, 1595, 1597-1598
epidemiology of, 1585
exanthem in, 753t-756t, 1589-1591, 1590f
hair follicle, 776-777
joint, 730, 1591-1592
after lung transplantation, 960-962
meningeal, 1598-1599
neonatal, 1592
nosocomial, 1594
Pseudomonas aeruginosa infection *(Continued)*
ocular, 1591, 1598-1599
in osteochondritis, 724-725
in otitis externa, 1591, 1599
in otitis media, 1591, 1599
pathogenesis of, 1586-1589
pericardial, 373
peritoneal, 700
pili in, 5
prevention of, 1603-1604
in previously healthy children, 1589-1593
prognosis for, 1604
pulmonary, 1599
skin lesions in, 1589-1591, 1590f, 1596
treatment of, 1597-1603, 1598t
antibiotic resistance in, 1601-1603, 3149
susceptibility testing in, 1601-1603
urinary tract, 1591, 1599
vaccine against, 1604
Pseudomonas alcaligenes, 1583
Pseudomonas fluorescens infection, 1585, 1595
Pseudomonas luteola, 1583
Pseudomonas mendocina infection, 1595
Pseudomonas oryzihabitans, 1583
Pseudomonas pseudoalcaligenes infection, 1583, 1585
Pseudomonas putida infection, 1595
Pseudomonas spp., 1582-1584, 1583t
Pseudomonas spp. infection, **1582-1605.** *See also specific species infections*
antibiotic resistance in, 1601-1603
burn wound, 1593
clinical manifestations of, 1589-1596, 1590f
diagnosis of, 1596-1597
endocardial, 362, 1595, 1597-1598
epidemiology of, 1585-1586
in immunosuppressed patient, 1594-1596
malignancy-related, 1594-1596
in otitis externa, 205
pathogenesis of, 1586-1589
prevention of, 1603-1604
in previously healthy children, 1589-1593, 1590f
prognosis for, 1604
retroperitoneal, 706-709
treatment of, 1597-1603, 1598t
Pseudomonas stutzeri infection, 1583, 1595
Pseudoparalysis of Parrot, 1769-1770
Pseudorubella. *See* Roseola infantum
Psittacosis. *See Chlamydia psittaci* infection (psittacosis)
Psychological disorders/factors
in chronic fatigue syndrome, 1089
in common cold, 2120-2121
in congenital rubella, 2217
in EBV infection, 2004
in genital HSV infection, 1943
in HIV infection, 2605
Pterygomandibular space, infection of, 148-149, 149f
Public health, 3082t, **3538-3549**
consultation function of, 3545
definition of, 3538, 3539f
disease exposure analysis in, 3547-3548
disease spread analysis in, 3546-3547
disease transmission settings analysis in, 3548
disease vector analysis in, 3549
in international travel, 3548-3549
outbreak investigation function of, 3545-3546
prevention and control function of, 3546
surveillance function of, 3538-3545

Publication bias, 124-125
Puffer fish poisoning, 604, 605t
Pulmonary artery stenosis, in congenital rubella, 2214t-2216t, 2217
Pulmonary capillaritis, 297
Pulmonary edema
  in epiglottitis-related intubation, 258
  in malaria, 2928
Pulmonary function tests
  in bronchiolitis obliterans, 295
  in hypersensitivity pneumonitis, 303-304
  in interstitial lung disease, 298, 298t
Pulse oximetry
  in bronchiolitis, 275
  in community-acquired pneumonia, 288
Puncture wound, osteomyelitis with, 724-725
Pupil
  Argyll Robertson, 812
  constrictor response of, 842
Purple urine bag syndrome, 1475
Purpura, 762-763, 763t
  in meningococcal infection, 1258-1259, 1259f
  in septic shock, 831
Purpura fulminans
  in meningococcal infection, 1259
  in *Streptococcus pneumoniae* sepsis, 1228, 1228f
Pyelonephritis. *See also* Urinary tract infection (UTI)
  acute, 538, 544, 545f
    treatment of, 547-548, 547t
  Candida, 2748-2749
  xanthogranulomatous, 539-540, 554
Pyemotes herfsi infection, 3073
Pyomyositis, **735-738**
  clinical presentation of, 736-737
  diagnosis of, 737, 737f-738f
  pathophysiology of, 735-736, 736t
  Stenotrophomonas maltophilia, 1607
  treatment of, 737-738
Pyopneumothorax, in neonate, 892
Pyothorax, *Arcanobacterium haemolyticum*, 1325
Pyrantel pamoate, 3350
  in *Enterobius vermicularis* infection, 567, 3019
Pyrazinamide, in tuberculosis, 1367t, 1368
Pyrenochaeta romeroi infection, after lung transplantation, 974
Pyrimethamine, 3350
  in heart transplant–related toxoplasmosis, 953
  in *Toxoplasma gondii* infection, 3000, 3001t
Pyrimethamine-sulfadoxine, in malaria, 2942, 2945
Pyrogens, 84. *See also* Fever
Pyruvate oxidase, of *Streptococcus pneumoniae*, 1214
Pyuria
  in gonococcal urethritis, 1287
  in Kawasaki disease, 1074, 1076
  in urinary tract infection, 542-543

Q

Q fever. *See Coxiella burnetii* infection (Q fever)
Quarantine, 3546
Quinacrine, 3350
Quinidine, in malaria, 2937t-2940t, 2941
Quinine, 3350
  in malaria, 2937t-2940t, 2941
Quinolones, 1600
  in heart transplant–related *Legionella pneumophila* infection, 949
  resistance to, 3154-3157
    mechanisms of, 3155-3157
Quinsy, **168-170**, 168f
Quinupristin-dalfopristin, 3231t-3233t, 3238
  in coagulase-negative staphylococcal infection, 1139
  in methicillin-resistant *Staphylococcus aureus* infection, 1127
  in vancomycin-resistant *Enterococcus* spp. infection, 1184

R

Rabbit hemorrhagic disease virus, 2148, **2154-2155**
Rabbit infectivity test, 1772
Rabies, **2444-2462**
  in animals, 2449-2450
  clinical manifestations of, 494t-498t, 502, 504-505, 2450-2452, 2451t-2452t
  diagnosis of, 509, 2452-2453, 2453t
  differential diagnosis of, 2452
  epidemiology of, 2448-2449, 2448t
  furious, 2450
  historical perspective on, 2444-2445
  immunization against, 2458-2460, 2459t-2460t, 3480-3483
    acute disseminated encephalomyelitis with, 516
    in animal bite, 781
    meningitis and, 488
  after lung transplantation, 983
  pathogenesis of, 2448-2449
  postexposure prophylaxis for, 2453-2458, 2456t, 3481-3483, 3482t, 3527-3529
    cell culture vaccine in, 2455-2457
    duck embryo vaccine in, 2455
    indications for, 2457-2458, 2457t
    nerve tissue vaccine in, 2454-2455
  transmission of, 2447-2448
Rabies virus, 2445-2447, 2445f-2446f
Rac protein, 14
Race
  in epidemiologic study, 108, 113-114
  HAV infection and, 2133, 2133f
  Kawasaki disease and, 1066
  Paracoccidioides spp. infection and, 2782
  Streptococcus pneumoniae infection and, 1203
Racecadotril (acetorphan)
  in gastrointestinal tract infection, 626
  in rotavirus infection, 2186-2187
Radiation (heat transfer), 84
Radiation therapy, acute transverse myelitis after, 524
Radiography. *See also* Chest radiography
  in appendicitis, 681-682
  in cervical lymphadenitis, 184
  in *Clostridium difficile* infection, 636
  in croup syndromes, 250t-251t
  in diskitis, 720, 721f
  in epiglottitis, 254, 254f
  in esophageal perforation, 420
  in Garré osteomyelitis, 154, 154f-155f
  in idiopathic spontaneous focal intestinal perforation, 895-896
  in immunodeficiency evaluation, 62
  in infective endocarditis, 359
  in intra-abdominal abscess, 705
  in *Kingella kingae* infection, 1703
  in *Legionella pneumophila* infection, 1710
  in *Leptospira* spp. infection, 1751f
Radiography *(Continued)*
  in leukocyte adhesion deficiency, 905f
  in mandibular osteomyelitis, 153, 153f
  in mediastinitis, 420
  in *Mycobacterium ulcerans* infection, 1414f
  in *Mycoplasma pneumoniae* infection, 2677-2678
  in myocarditis, 389, 389f
  in neonatal bone infection, 889
  in neonatal joint infection, 889
  in neonatal pneumonia, 891
  in *Nocardia* spp. infection, 1421
  in osteomyelitis, 713, 715-717, 715f
  in otitis media, 220
  in pancreatitis, 690
  in *Paracoccidioides* spp. infection, 2786-2787
  in *Paragonimus* spp. infection, 3055
  in pericarditis, 375, 376f
  in peritonitis, 702
  in pneumococcal pneumonia, 1230, 1230f
  in prosthetic joint infection, 1027-1028
  in pyogenic liver abscess, 674
  in pyomyositis, 737
  in retropharyngeal abscess, 171
  in rheumatic fever, 411
  in rhinosinusitis, 198t, 199
  in septic arthritis, 730, 731f
  in septic shock, 832
  in *Streptococcus agalactiae* infection, 1160, 1161f
  in tuberculosis, 1345, 1346f, 1348f-1350f, 1352f
  in vertebral osteomyelitis, 722, 722f
  in Whipple disease, 645
  in *Yersinia enterocolitica* infection, 1527
Radionuclide imaging
  in bacterial meningitis, 445
  in osteomyelitis, 716-717, 716f
  in prosthetic joint infection, 1027-1028
  in pyomyositis, 737, 737f
  in urinary tract infection, 544-545, 545f
  in vertebral osteomyelitis, 722
Ralstonia pickettii infection, 1595
Ralstonia spp., 1584
Ralstonia spp. infection, 1595
Raltegravir, 2616
Ramichloridium mackenziei infection, 2856, 2856f
Ramsay Hunt syndrome, 208
Rapid antigen detection test
  in meningitis, 442
  in streptococcal pharyngitis, 1147
Rapid enzyme immunoassay, in *Corynebacterium diphtheriae* infection, 1307
Rapid immunochromatographic assay, in HEV infection, 2165
Rapid influenza diagnostic test (RIDT), 2345-2346
Rapid plasma reagin (RPR) test, 1772-1773, 1773t
Rash. *See* Exanthem *and at specific infections*
Rat bite, septic arthritis with, 730
Rat-bite fever
  vs. Rocky Mountain spotted fever, 765
  Spirillum minus, 753t-756t, **1759-1760**
  Streptobacillus moniliformis, **1715-1720**
    clinical manifestations of, 1717-1718, 1717f
    diagnosis of, 1718-1719
    differential diagnosis of, 1719
    epidemiology of, 1715-1716
    exanthem in, 753t-756t, 765, 1717, 1717f

Rat-bite fever *(Continued)*
historical perspective on, 1715
joint, 730
pathophysiology of, 1718
prevention of, 1719
treatment of, 1719
Rat tapeworm *(Hymenolepis diminuta)* infection, **3042**
Raxibacumab, 3503-3504
Reactive arthritis, 734
Reactive oxygen species, in meningitis, 435
Recalcitrant erythematous desquamating disorder, 860
Receptor(s)
chemokine, in HIV infection, 10-11
intimin, 7-8, 9f
microbial tropism for, 10-11
pattern recognition, 433, 434f, 436
Toll-like, 25, 25t, 3364-3365
in influenza virus infection, 2338
in *Leptospira* spp. infection, 1747-1748
Mycobacterium leprae infection and, 1395-1396
in myocarditis, 386
in parainfluenza virus infection, 2365
in rhinovirus infection, 2118
in RSV infection, 2415
Rectum
examination of
in appendicitis, 681
in genital tract infection, 561
temperature measurement by, 86
Recurrent aphthous stomatitis, 159t-160t, 161
Recurrent respiratory papillomatosis, 1878-1879, 1878f-1879f
retinoic acid in, 1883-1884
Red blood cells, CSF, 442
Red diaper syndrome, 1490
Red eye. *See* Conjunctivitis
Reduction modifiable protein, of *Neisseria gonorrhoeae*, 1279
Reduviid bugs, in trypanosomiasis, 2959-2961
Reed-Sternberg cells, 1997
Refugees. *See* International adoptees and refugees
Rehydration therapy. *See* Oral rehydration therapy
Reiter syndrome, 531
Relapsing fever (*Borrelia* spp. infection), **1739-1742**
clinical manifestations of, 753t-756t, 1741-1742, 1741t
diagnosis of, 1742
differential diagnosis of, 1742
epidemiology of, 1730t, 1740
pathogenesis of, 1740-1741
transmission of, 1740
treatment of, 1742
Renal abscess, **553-558**
bacteremia and, 553
clinical findings in, 554
diagnosis of, 554-556, 555f-557f
drainage of, 557
fungal, 554, 2757
organisms in, 553-554
treatment of, 556-557
tuberculous, 553
Reovirus, 2168-2169
anti-cancer activity of, 2171
Reovirus infection, **2168-2172**
biliary atresia and, 2170-2171
clinical manifestations of, 2169-2171
CNS, 2171
Reovirus infection *(Continued)*
in common cold, 132
diagnosis of, 2171-2172
epidemiology of, 2169, 2169t
exanthem in, 742t-748t, 749, 2171
gastrointestinal manifestations of, 2170-2171
historical perspective on, 2168
lymphoma and, 2171
pulmonary, 2170
upper respiratory tract, 2169-2170
Reptile bite, 3578
Reservoirs, of infectious agents, 106-107
Respiratory distress. *See also* Acute respiratory distress syndrome (ARDS)
in community-acquired pneumonia, 287, 287t
in congenital rubella, 2220
in septic shock, 830, 832, 835
Respiratory failure, in influenza virus infection, 2352
Respiratory rate
in bronchiolitis, 275
in community-acquired pneumonia, 287-288
Respiratory syncytial virus (RSV), 2408-2410
animal susceptibility to, 2409-2410
antigenic properties of, 2408-2409
classification of, 2408
isolation of, 2409, 2424
structure of, 2408-2409, 2409f
Respiratory syncytial virus (RSV) infection, **2407-2434**
in acute bronchitis, 262, 263t
in adults, 2422-2423, 2422f-2423f
antibody response to, 277
in ARDS, 867-868
asthma and, 2421
breast-feeding and, 2427
in bronchiolitis, 272t-273t, 273-274. *See also* Bronchiolitis
in burn patient, 1054
clinical manifestations of, 2416-2419, 2417f-2418f, 2417t
coinfection with, 2419
in common cold, 132
complications of, 2419
diagnosis of, 278, 2424-2425, 2424f, 3611, 3611t
epidemiology of, 2410-2413, 2410t, 2411f
exanthem in, 742t-748t, 749, 760-762
geographic distribution of, 2410
after heart transplantation, 949-950
after hematopoietic stem cell transplantation, 942-943
historical perspective on, 2408
in HIV-infected host, 2421
in hospitalized children, 2122, 2122t
immune response to, 2414-2416, 2414f
in immunocompromised host, 2420-2421
after intestinal transplantation, 997t, 999
after kidney transplantation, 1007
after liver transplantation, 997t, 999
after lung transplantation, 979-980
mortality with, 2412
neonatal, 2411-2412, 2413t, 2418-2419
nosocomial, 2427-2428, 2428t, 3379
pathogenesis of, 2413-2414
pathology of, 2413-2414, 2413f
prevention of, 280-281, 2427-2431
prognosis for, 2419-2423
prophylactic treatment for, 2428-2431, 3529-3531
pulmonary, 284, 290
Respiratory syncytial virus (RSV) infection *(Continued)*
pulmonary sequelae of, 2421
radiography in, 2417f, 2418
reactive airways disease in, 2419
recurrent, 2421-2423, 2422f
seasonal patterns of, 2410-2411, 2410t, 2411f
severe/fatal, 2420
strain variation in, 2411
transmission of, 2412-2413, 2413t
treatment of, 278-280, 2425-2427
palivizumab in, 2426-2427, 2429, 3503, 3529-3530
prophylactic, 2428-2431, 3529-3531
ribavirin in, 2426, 3274-3276
vaccine against, 277, 280, 2430-2431
Respiratory tract
indigenous flora of, 91t, 92
lower, infection of. *See* Bronchitis; Influenza virus infection; Pneumonia
upper, infection of. *See* Common cold; Epiglottitis (supraglottitis); Laryngitis; Laryngotracheitis; Rhinosinusitis; Tracheitis
Retina
BKV-associated necrosis of, 1868
cotton-wool spots of, 811
in giardiasis, 814
histo spots of, 813
HSV-related necrosis of, 1945
infection of, 816-820
CMV, 811, 818, 2601
in HIV infection, 811
HSV, 818-819
LCMV, 817
rubella virus, 817-818
Toxocara canis, 814
Toxoplasma gondii, 816-817
Treponema pallidum, 819-820
VZV, 810, 819
Retinal necrosis syndrome, 810, 812
Retinitis, 809
CMV, 1973
congenital, 818
in HIV infection, 811, 2601
herpes B virus, 812
HSV, 1945
congenital, 818-819
influenza A virus, 812
mumps virus, 811
necrotizing, 810, 812
in toxoplasmosis, 816-817
Onchocerca, 815
Rift Valley fever virus, 812
rubella virus, 811
in subacute sclerosing panencephalitis, 811
in trench fever, 815
VZV, 810
Retinochoroiditis, *Toxoplasma*, 817
Retinoic acid, in recurrent respiratory papillomatosis, 1883-1884
Retinopathy
in congenital rubella, 2213, 2214t-2216t, 2220-2221
pigmentary
in congenital rubella, 817
in congenital syphilis, 819
Retinopathy of prematurity, 2756
Retroperitoneal infection, **706-709**
anatomy of, 706, 707f
clinical manifestations of, 708
complications of, 706
diagnosis of, 708
differential diagnosis of, 708

Retroperitoneal infection *(Continued)*
drainage of, 708
microbiology of, 706-708
pathogenesis of, 706
prognosis for, 709
secondary, 706
treatment of, 708-709
Retropharyngeal abscess, **170-172**, 170f
clinical manifestations of, 171, 171f
microbiology of, 173-175
treatment of, 171-172
Retroviruses, **2552-2619.** *See also* Human immunodeficiency virus type 1 (HIV-1); Human immunodeficiency virus type 2 (HIV-2); Human T-cell lymphotropic virus type 1 (HTLV-1) infection; Human T-cell lymphotropic virus type 2 (HTLV-2) infection
accessory genes of, 2554-2556
classification of, 2552-2553
genome of, 2553-2556, 2554f
life cycle of, 2556-2559, 2557f
regulatory genes of, 2554-2556
replication of, 2556-2559
structure of, 2553-2556, 2553f
testing for, 2559-2560
Reversal reaction, in *Mycobacterium leprae* infection, 1400f, 1404, 1404f, 1408-1409
Reye syndrome, 86-87, **676-677**, 2351
clinical manifestations of, 676-677
diagnosis of, 676-677
epidemiology of, 676
hepatitis in, 657
vs. influenza-associated encephalopathy, 2340
pancreatitis with, 692
prevention of, 677
vs. St. Louis encephalitis virus infection, 2259
treatment of, 677
Rhabdomyolysis, in *Mycoplasma pneumoniae* infection, 2683
Rhabdovirus infection, 500-501
Rhagades, in congenital syphilis, 1770
Rheumatic fever, **401-418**, 1143. *See also* Pharyngitis, streptococcal
ASO titer in, 411
carditis in, 409, 415
clinical manifestations of, 404t, 408-410, 409t, 410f
coxsackievirus infection and, 2077
cutaneous manifestations of, 355, 357
in developing countries, 407-408, 407f
diagnosis of, 412-413
differential diagnosis of, 412-413
epidemiology of, 401-405, 402f-403f, 403t-404t
genetic factors in, 406
immunization against, 406-407, 407f
joint fluid findings in, 732t
Jones criteria for, 409, 409t, 411-412
laboratory findings in, 410-412
pathogenesis of, 405-406, 405t
pathology of, 408
prevention of, 415-418, 1147-1148, 1148t, 1150, 3244-3246, 3245t, 3540t-3544t
prognosis for, 415
recurrence of, 414f, 414t, 417
surgery in, 414-415
treatment of, 413-414, 414f, 414t
Rheumatoid arthritis. *See also* Juvenile idiopathic (rheumatoid) arthritis
human parvovirus B19 and, 1851
Proteus spp. in, 1473
Rheumatoid arthritis *(Continued)*
vs. rheumatic fever, 413
rubella virus and, 2211
septic arthritis with, 734
Rheumatoid factor, in infective endocarditis, 355, 358, 358t
Rhinitis, in congenital syphilis, 1770, 1772
Rhinopharyngitis mutilans, 1787
Rhinorrhea, in rhinosinusitis, 197-198
Rhinosinusitis, **193-203**
acute, 200
age-related symptoms of, 197
anatomy of, 193-194
Aspergillus spp., 2706, 2707f
in burn patient, 1055
chronic, 195, 198, 201-202
clinical presentation of, 197-198, 198t
complications of, 198
culture in, 199-200
diagnosis of, 199-200
differential diagnosis of, 199
epidemiology of, 197
etiology of, 195-197, 196t, 2124
Haemophilus influenzae, 1677
historical perspective on, 193
immunity in, 195
Moraxella catarrhalis, 1252
Mycoplasma pneumoniae, 2679
odontogenic, 150-151, 152f
orbital cellulitis and, 799
pathophysiology of, 194-195
prevention of, 202
prognosis for, 202
puncture and drainage in, 199-200
recurrent, 195, 201-202
rhinovirus, 2124
sphenoidal, 198
Streptococcus pneumoniae, 1231, 1233
subacute, 200
surgical treatment of, 201-202
treatment of, 200-202, 3508
Rhinovirus, 2110-2111, 2110f
in animals, 2111
antigenic properties of, 2111
host range of, 2111
life cycle of, 2110-2111
serotypes of, 2111-2112, 2112f
structure of, 2110
in tissue culture, 2111, 2112f
Rhinovirus infection, **2109-2127.** *See also* Common cold
age and, 2120, 2120f
in asthma, 2123
clinical manifestations of, 2121-2125, 2121t
diagnosis of, 2125, 2125t
environmental temperature and, 2120
epidemiology of, 2111-2113
exanthem in, 742t-748t, 749, 760-762
after hematopoietic stem cell transplantation, 943
historical perspective on, 2109-2110
in hospitalized children, 2122-2123, 2122t
immune response to, 2118-2121
in immunocompromised host, 2125
interleukin-1 in, 135
median infectious dose in, 2113
mortality with, 2121-2122
in otitis media, 2123-2124
pathogenesis of, 2118-2121
prevention of, 2125-2126
psychosocial factors and, 2120-2121
pulmonary, 284
replication of, 2111
resistance to, 2119-2120
Rhinovirus infection *(Continued)*
seasonal distribution of, 2112, 2113f
serotype circulation in, 2112
serotype predominance in, 2112-2113
in sinusitis, 2124
T cells in, 135
transmission of, 2114-2118, 2114f, 2116f, 2117t
treatment of, 2125-2126
Rhizomucor spp., 2837
Rhizomucor spp. infection. *See also Rhizopus* spp. infection (mucormycosis)
burn wound, 1053
after kidney transplantation, 1013
neonatal, 899
Rhizopus spp., 2837
Rhizopus spp. infection (mucormycosis), **2839-2844**
bone, 726
burn wound, 1053
clinical manifestations of, 2840-2842, 2840t-2841t
cutaneous, 2841, 2841f
diagnosis of, 2842-2843
disseminated, 2842
epidemiology of, 2837-2839
gastrointestinal, 2842
after hematopoietic stem cell transplantation, 938
after kidney transplantation, 1013
neonatal, 899
pathogenesis of, 2839-2840
pathology of, 2840, 2840f
prevention of, 2844
prognosis for, 2844
pulmonary, 2841-2842
rhinocerebral, 2840
treatment of, 2843-2844
Rhodococcus equi infection, **1426-1428**
clinical manifestations of, 1427
diagnosis of, 1427
epidemiology of, 1426
extrapulmonary, 1427
pathophysiology of, 1427
treatment of, 1427-1428
Rhodococcus ruber infection, 1426
Rhodococcus spp. infection, **1426-1428**
clinical manifestations of, 1427
diagnosis of, 1427
epidemiology of, 1426-1427
pathophysiology of, 1427
treatment of, 1427-1428
Rhodotorula spp. infection, 898, **2854-2855**
Ribavirin, 3268t, 3273, 3273f
in adenovirus infection, 1907
adverse effects of, 3275-3276
in bronchiolitis, 279-280
in community-acquired pneumonia, 290
in Crimean-Congo hemorrhagic fever, 2533-2534
dosage for, 3275
in hantavirus infection, 2510-2511
in HCV infection, 2323-2324
in heart transplant–related RSV infection, 950
in hematopoietic stem cell transplantation–related RSV infection, 942-943
in hepatitis E virus infection, 2166
in hMPV infection, 2440
indications for, 3274-3275
in La Crosse virus encephalitis, 2524-2525
in laryngotracheitis, 257
in LCMV infection, 2465
mechanism of action of, 3273-3274

Ribavirin *(Continued)*
pharmacokinetics of, 3274-3276
resistance to, 3273-3274
in RSV infection, 2426, 3274-3276
spectrum of activity of, 3273
in West Nile virus infection, 2272
Ribosomal protein L12, of *Neisseria gonorrhoeae*, 1279
Rickettsia africae infection, 751t-752t, 2657
Rickettsia akari infection (rickettsialpox), 751t-752t, 2648t-2649t, **2656-2657**
Rickettsia australis infection, 751t-752t, 2657
Rickettsia conorii infection (Mediterranean spotted fever), 751t-752t, 2648t-2649t, **2655-2656**
ocular, 815
Rickettsia felis infection, 2648t-2649t, 2657, **3078**
Rickettsia japonica infection, 2657
Rickettsia mooseri infection, 815
Rickettsia parkeri infection, 2657
Rickettsia prowazekii infection (epidemic typhus), 751t-752t, 2648t-2649t, **2657-2659**
Rickettsia rickettsii, 2648t-2649t, 2650-2651. *See also* Rocky Mountain spotted fever
Rickettsia sibirica infection, 751t-752t, 2657
Rickettsia slovaca infection, 2657
Rickettsia tsutsugamushi infection (scrub typhus), 751t-752t, 2648t-2649t, **2660-2661**
Rickettsia typhi infection (endemic typhus, murine typhus), 751t-752t, 2648t-2649t, **2659-2660**, **3078**
epidemiology of, 115-116, 116f
ocular, 815
Rickettsiae infection, **2647-2666**, 2648t-2649t. *See also specific rickettsial infections*
exanthem in, 750, 751t-752t, 760t-763t, 762
in hepatitis, 656-657
Rifabutin, 3224-3225
adverse effects of, 3225
indications for, 3225
in nontuberculous mycobacterial lymphadenitis, 187
Rifampin, 3224-3225, 3231t-3233t
adverse effects of, 3225
in bacterial meningitis, 449, 450t
in coagulase-negative staphylococcal infection, 1138-1139
in epiglottitis prevention, 259
indications for, 3224-3225
before lung transplantation, 963-964
in *Mycobacterium leprae* infection, 1406-1407, 1407t, 1409
in *Mycobacterium ulcerans* infection, 1416
in tuberculosis, 1367t, 1368
Rifamycins, 3224-3225
Rifaximin, 3224-3225
Rift Valley fever, **2528-2531**
clinical manifestations of, 2529-2530
diagnosis of, 2530
epidemiology of, 2529, 2529f
historical perspective on, 2528
ocular manifestations of, 812
prevention of, 2530
treatment of, 2530
Rift Valley fever virus, 2528-2529
Rimantadine, 3268-3270, 3268f, 3268t
in laryngotracheitis, 257
Ringworm, scalp (tinea capitis), **784-785**, 785f
Rio Bravo virus infection, 2316t
Ritonavir, 2614
Ritter disease, 753t-756t, 774, 775f
Rituximab
in hematopoietic stem cell transplantation–related EBV infection, 940-941
in post-transplant lymphoproliferative disease, 977-978, 2012-2013, 3521-3522
River blindness, 757t-758t, 809, 815, **3027-3028**
Rochalimaea quintana infection. *See Bartonella quintana* infection (trench fever)
Rocio encephalitis, **2314-2315**
Rocky Mountain spotted fever, 765, **2647-2654**, 2648t-2649t
vs. *Anaplasma* spp. infection, 2663
clinical manifestations of, 2652-2653
vs. Colorado tick fever virus infection, 2174
diagnosis of, 2653
differential diagnosis of, 2653-2654
vs. *Ehrlichia* spp. infection, 2663
encephalitic, 494t-498t, 501
epidemiology of, 2650-2651, 2650f
exanthem in, 751t-752t, 762, 2652-2653
historical perspective on, 2647-2650
immune response in, 2652
pathogenesis of, 2651-2652
prevention of, 2654
prognosis for, 2654
transmission of, 2651
treatment of, 2654
Romaña's sign, 2963
Rosacea, *Demodex* infection and, 795-796
Rose spots, 760, 1501
Rosenbach, erysipeloid of, 1327-1328
Roseola infantum, **768-771**
clinical presentation of, 742t-748t, 770-771, 2017, 2017t
complications of, 771
diagnosis of, 771
epidemiology of, 768-769
etiology of, 769-770
exanthem in, 742t-748t, 749, 765, 770
historical perspective on, 768
pathophysiology of, 770
treatment of, 771
white blood cell count in, 770-771
Roseola vaccinatum, 742t-748t
Ross River virus, 2249
Ross River virus arthritis, 2248-2251
clinical manifestations of, 2249
diagnosis of, 2250
epidemiology of, 2249
exanthem in, 742t-748t, 2249
pathogenesis of, 2250
pathology of, 2250
prevention of, 2251
transmission of, 2249
treatment of, 2250-2251
Rotavirus, 2177-2178
gel electrophoresis of, 2179, 2180f
genome of, 2179, 2180f
genotypes of, 2177-2178, 2179t
non–group A strains of, 2178
NSP4 toxin of, 2184
properties of, 2178-2182, 2179f-2180f
replication of, 2179-2180
Rotavirus infection, 602t-603t, 606-607, 607t, 608f, 616t, **2176-2195**
age and, 2182
breast-feeding and, 2182
clinical manifestations of, 2185-2186
CNS, 494t-498t, 2186
in daycare facilities, 3564
Rotavirus infection *(Continued)*
diagnosis of, 2182-2183, 3611-3612
diarrhea in, 2183-2185
epidemiology of, 2184-2185
exanthem in, 742t-748t, 749
historical perspective on, 2177
hospitalization for, 2185
immune response to, 2180-2182
immunization against, 627, 2187-2191, 2187f, 3466-3468
adverse events with, 3467
contraindications to, 3468
efficacy of, 3466-3467
indications for, 3467-3468
non-live, 2191-2192
Rotarix, 2187f, 2190-2191, 2190f-2191f
RotaShield, 2187-2188, 2187f
RotaTeq, 2187f, 2188-2190, 2189f
safety of, 2191
schedule for, 2191
intestinal villi changes in, 2183
intussusception and, 2186
after kidney transplantation, 1007
nosocomial, 3382
oral immunoglobulin in, 3498, 3531
pathology of, 2183-2184
respiratory symptoms with, 158
roseola with, 769
seasonality of, 2184-2185
transmission of, 2184
treatment of, 2186-2187, 3498
Roth spots, 357
Rotor syndrome, 657
RPR (rapid plasma reagin) test, 1772-1773, 1773t
RSV infection. *See* Respiratory syncytial virus (RSV) infection
Rubella virus, 2196-2198
animal susceptibility to, 2198
antibody response to, 2205-2207
antigenic composition of, 2197
classification of, 2196
E1 glycoprotein of, 2197
physical properties of, 2196-2197
tissue culture of, 2197-2198
Rubella virus infection, **2195-2225**
acute disseminated encephalomyelitis with, 516
age and, 2200-2201, 2200t-2201t, 2201f
antibody response in, 2205-2207
asymptomatic recurrence of, 2203
clinical manifestations of, 742t-748t, 749, 2208-2217, 2210f
complications of, 2211-2213, 2219
congenital, 817-818
agency resources in, 2221
auditory manifestations of, 2213, 2214t-2216t, 2220-2221
cardiovascular manifestations of, 2214t-2216t, 2217, 2220-2221
cataracts in, 2213, 2220-2221
clinical manifestations of, 2213-2217
cutaneous manifestations of, 2214t-2216t
delayed manifestations of, 2217
diagnosis of, 2218-2219
endocrine manifestations of, 2214t-2216t, 2217
epidemiology of, 2203
expanded (persistent), 2217
gastrointestinal manifestations of, 2214t-2216t
genetic manifestations of, 2214t-2216t
genitourinary manifestations of, 2214t-2216t

Rubella virus infection *(Continued)*
glaucoma in, 2213, 2221
growth restriction with, 2213
hematologic manifestations of, 2214t-2216t
hyperbilirubinemia in, 2220
immunologic manifestations of, 2207-2208, 2214t-2216t, 2221
isolation precautions in, 2220
malformations with, 2214t-2216t, 2217
musculoskeletal manifestations of, 2221
neurologic manifestations of, 2213-2216, 2214t-2216t, 2221
ocular manifestations of, 811, 817-818, 2213, 2214t-2216t, 2220-2221
orthopedic manifestations of, 2214t-2216t
pancreatitis with, 694
pathology of, 2208, 2209t
psychiatric manifestations of, 2217
pulmonary manifestations of, 2214t-2216t
respiratory distress in, 2220
retinopathy in, 2213, 2220-2221
treatment of, 2220-2221
in daycare facilities, 3563
diagnosis of, 2217-2219
differential diagnosis of, 2217-2218
encephalitic, 494t-498t, 500, 2208, 2212
epidemic, 2198-2200, 2198f-2199f
epidemiology of, 2198-2204, 2198f-2199f
vs. erythema infectiosum, 1855
exanthem in, 742t-748t, 749, 2210-2211, 2210f
fetal, 2206-2208
fever in, 2211, 2218
gender and, 2204
genetic factors in, 2204
geographic distribution of, 2204
hepatic, 655-656, 2213
historical perspective on, 2195-2196
immune response to, 2205-2206
immunization against, 2221-2224, 3468-3470
acute disseminated encephalomyelitis with, 517
adverse reactions to, 2222-2223, 3469
contraindications to, 2223, 3469-3470
efficacy of, 3469
global results of, 2201-2203
inadvertent, 2223-2224
indications for, 3469
precautions for, 3469-3470
during pregnancy, 2223-2224
incubation period in, 2208-2209, 2218
joint complications of, 2211
lymphadenopathy in, 2210-2211, 2218
myocarditis in, 2212
neurologic complications of, 2212
nosocomial, 3383-3384
ocular manifestations of, 811, 2212
pathogenesis of, 2204-2205, 2205t
pericarditis in, 2212
in pregnancy, 2203, 2206-2207, 2219-2220
prevention of, 2221-2224, 3531-3532
prodromal period in, 2209-2210
quarantine in, 2224
reporting requirements for, 2224
seasonality of, 2204
thrombocytopenic purpura in, 2212
transmission of, 2203
treatment of, 2219-2221
uncomplicated, 2219
uveitis in, 2213
Rubella virus infection *(Continued)*
white blood cell count in, 2211
without rash, 2210
Rug cleaning, Kawasaki disease and, 1068
Rupintrivir, in rhinovirus infection, 2126

S

Sabiá virus infection, 2467, 2470t. *See also* Arenavirus infection
Saccharomyces boulardii infection, 626
Saccharomyces cerevisiae, INT1 introduction into, 9
Sacroiliitis, 720, 731
Saffold virus(es), 2051-2052
geographic distribution of, 2056-2057
replication of, 2054
transmission of, 2056
Saffold virus infection, **2051-2109**
asymptomatic, 2063-2064
bronchial, 2066
cardiac, 2077
clinical manifestations of, 2063-2090
herpangina in, 2065, 2066t
pharyngeal, 2065
St. Anthony's fire, **776**
St. Louis encephalitis virus, 2254
ecology of, 2254, 2254f
St. Louis encephalitis virus infection, 494t-498t, 510-511, **2253-2260**
clinical manifestations of, 2257-2258, 2257f, 2257t
diagnosis of, 2258-2259
differential diagnosis of, 2259
enteroviral infection and, 2090
epidemiology of, 2255-2257, 2255f-2256f
immune response to, 2258
pathogenesis of, 2258
pathology of, 2258
prevention of, 2260
prognosis for, 2260
treatment of, 2259-2260
Salbutamol, in *Bordetella pertussis* infection, 1630
Salicylates
Reye syndrome and, 86-87, 657, **676-677**, 2351
in rheumatic fever, 413
Saline solution
in bronchiolitis, 280
inhalation therapy with, in RSV infection, 2425
nasal irrigation with
in common cold, 137
in rhinosinusitis, 201
Salivary glands
in HIV infection, 149, 191-192
infectious enlargement of. *See* Parotitis
noninfectious enlargement of, 192
Salmonella ser. Typhi infection (typhoid fever), 602t-603t, **1491-1509**
antibiotic resistance in, 1495, 1502
carrier state after, 1502, 1506-1507
clinical manifestations of, 1501-1502, 1502t
complications of, 1501, 1502t
diagnosis of, 1502-1503
differential diagnosis of, 1503-1504
epidemiology of, 1494-1495
exanthem in, 753t-756t, 760, 1501
hemolytic-uremic syndrome with, 1501
host factors in, 1497-1498
immune response to, 1498-1499
immunization against, 1507, 3483-3485, 3484t
encephalitis and, 502
Salmonella ser. Typhi infection (typhoid fever) *(Continued)*
pathophysiology of, 1495-1499, 1495t
in pregnancy, 1502
relapse of, 1501
reservoir for, 1494-1495
treatment of, 1505-1506, 1505t
Salmonella ser. Typhimurium
cell invasion by, 13-14
needle complex of, 13-14, 14f
SipB protein of, 18
SPI-1 of, 13-14
SPI-2 of, 14-15
SPI-3 of, 15
Salmonella ser. Typhimurium infection, 1492t, 1493. *See also Salmonella* spp. infection (salmonellosis)
CD18 in, 15
complications of, 1499
hypertriglyceridemia and, 74
pathogenicity islands in, 13-15
serum cholesterol and, 74
Salmonella spp., 1491-1492, 1492t
pathogenicity islands of, 1496-1497, 1496f
viability of, 1493
Salmonella spp. infection (salmonellosis), **1491-1509**
bloodstream, 1500-1501
treatment of, 1504-1505
bone, 711, 712t, 1500-1501, 1500t
in daycare facilities, 3557-3558
endocardial, 362
fever of unknown origin in, 844
focal, 1500-1501, 1500t
gastrointestinal, 602t-603t, 607t, 609, 616t, 618
age and, 1493-1494
animal reservoirs in, 1492-1493
antibiotic resistance in, 624, 1494
asymptomatic, 1499-1500
breast-feeding and, 1507
carrier state after, 1502, 1506-1507
clinical manifestations of, 1500
diagnosis of, 1502-1503
differential diagnosis of, 1503-1504
epidemiology of, 1492-1494
food sources in, 1492-1493
host factors in, 1497-1498, 1498t
human reservoir in, 1493
immune response to, 1498-1499
inoculum size in, 1494
nosocomial, 1493, 1507
pathophysiology of, 1495-1499, 1495t, 1496f
prevention of, 1507
prognosis for, 1507-1508
treatment of, 623t, 624, 1504, 1504t
interleukin-12 defects and, 50
joint, 728, 729t, 730
meningeal, 1500-1501, 1505
murine, 1499, 1499f
peritoneal, 700
Schistosoma spp. infection and, 1498, 1505, 3066
uveitis after, 816
Salpingitis, **587-588**, 1284, 1286, 1286f
Salt retention, 76
San Miguel sea lion virus infection, 749, 2154
Sandfly, in leishmaniasis, 2947, 2949, 2953f
Sandfly fever, **2535-2537**, 2536f
Sapovirus, 2148f-2149f, 2149, 2149t
Sapovirus infection, **2151-2154**
clinical manifestations of, 2153
diagnosis of, 2153-2154

Sapovirus infection *(Continued)*
differential diagnosis of, 2153
epidemiology of, 2151
immune response to, 2152-2153
pathogenesis of, 2152
pathology of, 2152
prognosis for, 2156
seasonal patterns of, 2151
treatment of, 2155-2156
Sappinia diploidea, 2975, 2979
Sarcoidosis, 443t
Sarcoptes scabiei infection, 758t, **3073**, 3327t-3346t
in daycare facilities, 3559
in health care facilities, 3385
Sashimi, 602-604
Saturated solution of potassium iodide (SSKI), in *Sporothrix schenckii* infection, 2835
Sauna baths, common cold and, 138
Scabies, 758t, **3073**, 3327t-3346t
in daycare facilities, 3559
nosocomial, 3385
Scalded skin syndrome, 753t-756t
Scalp
abscess of, 891, 1282-1283, 1295
pustulosis of, 789, 789f
ringworm of (tinea capitis), **784-785**, 785f
Scarlet fever, 1142-1143. *See also* Pharyngitis, streptococcal
vs. erythema infectiosum, 1855
exanthem in, 753t-756t, 760, 1142-1143
Scarring, in *Mycobacterium ulcerans* infection, 1415, 1415f
Scedosporium spp., 2848-2849
Scedosporium spp. infection, **2848-2850**
clinical manifestations of, 2849-2850
in cystic fibrosis, 336
diagnosis of, 2850
exanthem in, 757t
after lung transplantation, 974
pathogenesis of, 2848-2849
pathology of, 2849, 2849f
treatment of, 2850
Schick test, in *Corynebacterium diphtheriae* infection, 1307
Schistosoma spp. infection (schistosomiasis), **3060-3070**
acute (Katayama fever), 3064
chronic, 3063-3064
clinical manifestations of, 757t-758t, 3064-3066
CNS, 3066
diagnosis of, 3066-3068, 3067f
epidemiology of, 3061-3062, 3061f-3062f
genital, 3065
granuloma in, 3063
hepatosplenic, 3065-3066
imaging in, 3067-3068
immune response in, 3063
intestinal, 3065
ocular, 815
pathogenesis of, 74, 3062-3064, 3062f
prevention of, 3068-3069
pulmonary, 3065-3066
rash in, 3064, 3068
Salmonella spp. infection and, 1498, 1505, 3066
in traveler, 3116
treatment of, 3068, 3327t-3346t
urinary tract, 3064-3065
vaccine against, 3069
Schools, disease in, 3548. *See also at* Daycare facilities
Scintigraphy. *See* Radionuclide imaging
Scleritis, *Stenotrophomonas maltophilia*, 1607
Scombroid fish poisoning, 604, 605t
Scorpion sting, 692, **3077-3078**
Scotch tape, for specimen collection, 566, 566f, 3622
Scrapie, 2620-2621, 2624
Screening tests
in cystic fibrosis, 326-327
for international adoptees and refugees, 3122-3130
in severe combined immunodeficiency disease, 49
in syphilis, 1768
Scrofula, 177, 1356
Scrofuloderma, 1354
Scrum pox, 1945
Seadornaviruses, 2172, 2175
Seafood poisoning, 602t-603t, 604
Sealpox virus infection, 2046
Sebaceous glands, eyelid, infection of, **796**
Sedation, in ARDS, 873
Seizures
in bacterial meningitis, 439-440, 440t, 455
in brain abscess, 464-466, 469
in encephalitis, 509-510
febrile, 85
in HHV-6 infection, 2018
in HHV-7 infection, 2018
after influenza virus immunization, 2352
after MMR/MMRV immunization, 2031, 2393-2394
in neurocysticercosis, 3037
after pertussis immunization, 1634
in roseola infantum, 771
in *Shigella* spp. infection, 1480-1481
in tetanus, 1814, 1816-1817
in tick-borne encephalitis virus infection, 2309
Selectins, in phagocyte recruitment, 31-32, 33f
Selenium
deficiency of, 79
metabolism of, 79
Semliki Forest virus infection, 2253
Sepik virus infection, 2316t
Sepsis. *See also* Bacteremia; Septic shock
Achromobacter spp., 1574
Aeromonas spp., 1541, 1543
ARDS in, 868
Bacillus cereus, 1319
Clostridium spp., 1797
definition of, 825t
Edwardsiella tarda, 1533
Elizabethkingia meningosepticum, 1579
Escherichia coli, 1441
clinical manifestations of, 1444
epidemiology of, 1441
neonatal, 881-882
hepatic dysfunction in, 656
in influenza virus infection, 2345
IVIG administration in, 3500, 3509
jaundice in, 662
after kidney transplantation, 1006
Malassezia furfur, 789, 2853
Morganella morganii, 1470
neonatal, **882-885**, 3509
Clostridium spp., 1797
Klebsiella pneumoniae, 882
Neisseria gonorrhoeae, 1283
probiotics in, 3371-3372
Staphylococcus aureus, 882
Plesiomonas shigelloides, 1565-1566
procalcitonin in, 82
Serratia spp., 1490
severe, 825t
Sepsis *(Continued)*
Shigella spp., 1481
Streptococcus pneumoniae, 1228, 1228f
Streptococcus viridans, 1191-1192
Vibrio parahaemolyticus, 1558
Vibrio vulnificus, 1560-1561
Yersinia enterocolitica, 1518
Yersinia pestis, 1512
Yersinia pseudotuberculosis, 1529
Sepsis-like illness, enteroviral, 2095
Sepsis neonatorum, **882-885**
prevention of, 885
treatment of, 883-884
Septic arthritis, **727-734**
Aeromonas spp., 1543
anaerobic bacteria, 1829-1830
β-hemolytic streptococci in, 1151
clinical manifestations of, 730, 730t
Clostridium spp., 1797-1798
Corynebacterium diphtheriae, 1306
diagnosis of, 730-731, 730t
differential diagnosis of, 732
Enterococcus spp., 1177
epidemiology of, 727
Erysipelothrix rhusiopathiae, 1328
etiology of, 728-730, 729t
Haemophilus influenzae type b, 1676, 1679
inoculation in, 728
Kingella kingae, 1700, 1702
laboratory evaluation of, 731, 732t
Neisseria gonorrhoeae, 1283
neonatal, 733-734, **889-890**, 1283
Pantoea agglomerans, 1536
pathophysiology of, 728
postoperative, 728
prognosis for, 733
Pseudomonas aeruginosa, 1591
radiography in, 730-731, 731f
vs. reactive arthritis, 734
vs. retroperitoneal infection, 708
vs. rheumatic fever, 413
in rheumatoid arthritis, 734
Streptococcus agalactiae, 1161
Streptococcus pneumoniae, 1231
Streptococcus viridans, 1193
treatment of, 732-733
antibiotic, 732-733
surgical, 732
Septic bursitis, vs. septic arthritis, 732
Septic shock, **824-836**
central nervous system effects of, 830
clinical presentation of, 831-833
cutaneous manifestations of, 831
definition of, 825t
diagnosis of, 831-833
disseminated intravascular coagulation in, 829
genes in, 831
hematologic changes in, 829
in immunocompromised host, 824, 831, 833t, 834
interleukin-1 in, 27
in Kawasaki disease, 1075
liver function in, 830
metabolic alterations in, 829-830
mortality in, 825t, 836
myocardial function in, 828-829
nitric oxide in, 3367
organisms in, 824-825, 825t, 832-833
pathophysiology of, 826-831
prognosis for, 836
renal dysfunction in, 830, 832, 835
respiratory distress syndrome in, 830, 832, 835
Streptococcus viridans, 1192

Septic shock *(Continued)*
treatment of, 833-836, 833t, 3367
airway management in, 835
corticosteroids in, 834-835
fluid therapy in, 831
immunomodulating agents in, 835-836, 835t
parenteral alimentation in, 835
tumor necrosis factor-α in, 27
vascular resistance in, 827-828
Septicemia. *See also* Sepsis; Septic shock
in chronic granulomatous disease, 57
endophthalmitis in, 820
exanthem in, 753t-756t
Serratia marcescens infection
antibiotic resistance in, 1489
clinical manifestations of, 1489-1490
epidemiology of, 1488
nosocomial, 1488-1489
pathophysiology of, 1489
in septic shock, 834
treatment of, 1490-1491
Serratia spp., 1488
biotyping of, 1489
isolation of, 1490
Serratia spp. infection, **1488-1491**
clinical manifestations of, 1489-1490
diagnosis of, 1490
endocardial, 362
epidemiology of, 1488-1489
pathophysiology of, 1489
treatment of, 1490-1491
Serum amyloid A (SAA) protein, in Kawasaki disease, 1076
Serum sickness
cefaclor and, 3199
penicillin and, 3190
Severe acute respiratory infection (SARI), 2332
Severe acute respiratory syndrome (SARS), 2486-2487, 2491-2492
Severe combined immunodeficiency (SCID), **48-49**, **914-915**
chest radiography in, 906f
clinical features of, 914
diagnosis of, 915
gene defects in, 907, 907t
screening for, 908
treatment of, 49, 915
types of, 914, 915t
Sex, in epidemiologic study, 108
Sexual abuse
Chlamydia trachomatis infection with, 572t-573t, 574
evaluation for, 561
HPV infection with, 572t-573t, 580
HSV infection with, 582
Neisseria gonorrhoeae infection with, 571, 572t-573t, 1297-1299, 1298t
nonvenereal gonococcal infection and, 530, 534
syphilis screening in, 1768
Trichomonas vaginalis infection with, 572t-573t, 575-576
Sexual activity
EBV infection and, 1998
Escherichia coli UTI and, 1442
pelvic inflammatory disease and, 587
urinary tract infection and, 536-537
Sexually transmitted diseases (STDs), 570-579, 572t-573t. *See also specific infections*
sexual abuse and, 1297-1299, 1298t
in traveler, 3117
ulcers with, 582-584, 583t
Sézary syndrome, 2569
Shell vial assay, 3608-3609
Shellfish poisoning, 602-604, 605t. *See also Norovirus* infection
Shenton line, 731, 731f
Shiga toxin, 17, 619
Citrobacter freundii, 17, 1430-1431
detection of, 1460
enterohemorrhagic *Escherichia coli*, 17, 1457-1459
Shigella spp., 1479
Shigella boydii, 1476-1477
Shigella boydii infection, 1477
Shigella dysenteriae, 1476-1477
Shigella dysenteriae infection, 1477, 1479
Shigella flexneri, 16, 1476-1477
Shigella flexneri infection, 1477, 1479
vulvovaginal, 568-569
Shigella sonnei, 1476-1477
Shigella sonnei infection, 753t-756t, 1477
vulvovaginal, 568-569
Shigella spp., 1476-1477
invasiveness of, 1478-1479, 1478f
isolation of, 1482
toxins of, 1479
type III secretion system of, 1478
Shigella spp. infection (shigellosis), 602t-603t, 607t, 608-609, 616t, 618, **1476-1487**
clinical manifestations of, 1480-1482
complications of, 1480-1481, 1480t
in daycare facilities, 3557
diagnosis of, 1482-1483
differential diagnosis of, 1483
epidemiology of, 1477
extraintestinal manifestations of, 1480-1481
historical perspective on, 1476
in HIV-infected patient, 1482
immune response to, 1479-1480
inflammatory response in, 1479-1480
laboratory findings in, 1482
neonatal, 888, 1481-1482
pathogenesis of, 18, 1478-1480, 1478f
pathology of, 1480
prevention of, 1485-1486
prognosis for, 1485
transmission of, 1477, 1485-1486
treatment of, 623, 623t, 1483-1485
antibiotic resistance in, 1484-1485, 3147-3148
undernutrition and, 1480
vaccine against, 628, 1486
Shingles. *See* Herpes zoster
Shock. *See also* Septic shock
in bacterial meningitis, 440, 454
cardiogenic, 832
in dengue hemorrhagic fever, **2278-2291**, 2284t-2285t
distributive, 832
endotoxic
in animals, 826-827, 827t
in humans, 830-831
hypovolemic, 832
in myocarditis, 395
Shock lung, 830
Short stature, 904-905
Shunt. *See* Cerebrospinal fluid (CSF) shunt
Shwachman-Diamond syndrome, 56
Sialectasis, 191
Sialography, 192
Sic (streptococcal inhibitor of complement-mediated lysis) protein, 18
Sickle-cell disease
acute chest syndrome in, 2641
antibiotic prophylaxis in, 3252
Sickle-cell disease *(Continued)*
bone infarction in, 724
Edwardsiella tarda infection in, 1533
gallstones in, 670
human erythrovirus B19 (human parvovirus B19) infection in, 1849-1850
immunodeficiency in, 59-60
liver abscess in, 673
malaria and, 2926
osteomyelitis in, 723-724
pancreatitis in, 692
Salmonella spp. infection and, 1498, 1500
Streptococcus pneumoniae infection and, 1203-1204, 1205f, 1232
Sigmoidoscopy
in *Clostridium difficile* infection, 636
in *Shigella* spp. infection, 1483
Significance (statistical), 120
tests of, 122
Silver nitrate
in burn injury, 1058
prophylactic, 805-806, 805t, 1281-1282, 3242-3243
Silver sulfadiazine, in burn injury, 1058
Simian virus 40 (SV40), 1863-1866, 1864t
renal disease and, 1867
tumors and, 1868
Sin Nombre virus, 2496-2500, 2498f, 2499t-2500t. *See also* Hantavirus infection
Sindbis fever, 742t-748t, 2252
Single-photon emission computed tomography (SPECT)
in chronic fatigue syndrome, 1088
in encephalitis, 507
Sinusitis. *See* Rhinosinusitis
SipB protein, 18
Sitz bath, in nonspecific vulvovaginitis, 564-566
Sixth disease. *See* Roseola infantum
Sjögren syndrome, 192, 2576
Skene's gland, gonococcal infection of, 1284
Skin, 772
disorders of. *See also* Exanthem *and at specific infections*
of external ear, 208
in hepatitis, 649, 2140
in immunodeficiency disorders, 62
in Kawasaki disease, 1073-1074
in pinta, 1785
in rheumatic fever, 355, 357
in sepsis neonatorum, 883
in septic shock, 831
in systemic disease, 772, 773t
in toxic shock syndrome, 854, 855f, 858, 858f
in Whipple disease, 644
in yaws, 1786-1787
flora of, 90, 91t, 772
hexachlorophene absorption by, 1121
infection of. *See also specific infections*
bacterial, **772-781**, 773t
in Chédiak-Higashi syndrome, **54**
fungal, **784-792**
in glycogen storage disease type 1B, 54-55
in hyper-immunoglobulin E syndrome, **55**
in type 1 leukocyte adhesion deficiency, 52-53
viral, **782-784**
Skin test
in *Coccidioides* spp. infection, 2776
in *Histoplasma capsulatum* infection, 2823
in immunodeficiency evaluation, 907

Skin test *(Continued)*
in *Leishmania* spp. infection, 2951, 2957
in *Paracoccidioides* spp. infection, 2792
in penicillin allergy, 3190
in *Sporothrix schenckii* infection, 2835
in tuberculosis. *See* Tuberculin skin test
Skull, osteomyelitis of, 723
SLAM-associated protein, 915
Slapped cheek appearance, 1847-1848, 1847f
Sleep
disturbances of, in chronic fatigue syndrome, 1089
position for, otitis media and, 211
Sleeping sickness. *See Trypanosoma brucei gambiense* infection (African sleeping sickness); *Trypanosoma brucei rhodesiense* infection (African sleeping sickness)
Slit-lamp examination, in uveitis, 809-810
Small intestine, flora of, 91t, 92-94
Smallpox, **2033-2039**
bioterrorist, 2034, 3401, 3582-3583, 3583f
clinical manifestations of, 742t-748t, 749, 2035-2036
diagnosis of, 2036
differential diagnosis of, 2036
epidemiology of, 2034-2035
exanthem in, 2035
flat, 2035
hemorrhagic, 2035
historical perspective on, 2033-2034
immunization against, 2036-2038
acute disseminated encephalomyelitis with, 517
complications of, 2037t, 2038-2039, 2039t, 3533
encephalitis after, 502
meningitis and, 488
pre-event, 2038
mild (variola minor), 2035
pathology of, 2035
prevention of, 2036-2039, 2037t, 2039t, 3532-3533
reporting requirements for, 2037
risk for, 2037-2038
treatment of, 2036
Snake bite, 3578
Snow, John, 103
Snowshoe hare virus, 2514, 2517, 2520
Socioeconomic conditions
acute otitis media and, 211
infection and, 110-111
Sodium hypochlorite, in burn wound infection, 1059
Sodium stibogluconate, 3350
Soil-contaminated wound, 781, 781t
Solenopsis saevissima (fire ant), 758t
SopE protein, 14
Sore throat. *See* Pharyngitis
South American blastomycosis. *See Paracoccidioides* spp. infection (paracoccidioidomycosis)
spa typing, of *Staphylococcus aureus*, 1119
Specific granule deficiency, 54
Sphincter of Oddi, obstruction of, pancreatitis with, 692
Spider bite, 758t, **3077**
Spina ventosa, 1355
Spinal cord
CMV-associated inflammation of, 526
compression of, 523
infarction of, 523, 524f
inflammation of. *See* Acute transverse myelitis
tetanospasmin effects on, 1813
tumor of, 524
Spinal epidural abscess, 523
Spinal epidural hematoma, 523
Spine
Aerococcus urinae infection of, 1249
cysticercosis of, 3035
infection of, 720-723, 722f, 725
Kingella kingae infection of, 1702
tuberculosis of, 1355
in Whipple disease, 643
Spiramycin, 3350
in *Toxoplasma gondii* infection, 817, 3000, 3001t
Spirillum minus, 1759
Spirillum minus infection (rat-bite fever), 753t-756t, **1759-1760**
clinical manifestations of, 1759-1760
diagnosis of, 1760
epidemiology of, 1759
pathology of, 1759
treatment of, 1760
Spirometra spp. infection (sparganosis), **3043**
Spleen
abscess of, 673, 704-705
in infective endocarditis, 355, 357
absence of, 59
antibiotic prophylaxis in, 3251-3252
Streptococcus pneumoniae infection and, 1204, 1224, 1232
in congenital syphilis, 1771
dysfunction of, *Streptococcus pneumoniae* infection and, 1204
infarction of, 704
in infective endocarditis, 357
in *Mycoplasma pneumoniae* infection, 2682
rupture of, in infectious mononucleosis, 2003
Schistosoma spp. infection of, 3065
in *Streptococcus pneumoniae* infection, 1224
tuberculosis of, 1357
in Whipple disease, 644
Splenectomy, 59, 3251-3252
infection after, 436-437
in Wiskott-Aldrich syndrome, 913
Splenomegaly
in infectious mononucleosis, 1996, 1999-2000, 1999t, 2003
in infective endocarditis, 357
in malaria, 2929
Spondweni virus infection, 2316t
Spondylitis
tuberculous, 1355
in Whipple disease, 643
Spondylodiscitis
Aerococcus urinae, 1249
Kingella kingae, 1702
Spontaneous focal intestinal perforation, vs. necrotizing enterocolitis, 895-897
Sporadic fatal insomnia, 2620-2621, 2627t
Sporothrix schenckii, 2832
Sporothrix schenckii infection (sporotrichosis), **2832-2836**
asteroid body in, 2833
clinical manifestations of, 2833-2834
cutaneous, 757t, 791f, 2833-2834, 2833t-2834t, 2834f
diagnosis of, 2834-2835
epidemiology of, 2832-2833
extracutaneous, 2833t, 2834
granuloma in, 2833
in HIV-infected patient, 2834
immune response to, 2833
joint, 2834
meningeal, 479
pathogenesis of, 2833
Sporothrix schenckii infection (sporotrichosis) *(Continued)*
prevention of, 2836
prognosis for, 2835-2836
pulmonary, 2834
treatment of, 2835-2836, 3295t
uveal, 814
Sports, HSV infection and, 1945
SptP protein, 14
Sputum culture
in *Blastomyces dermatitidis* infection, 2731
in *Cryptococcus neoformans* infection, 2800-2801
in cystic fibrosis–related infection, 332, 333t
in *Legionella pneumophila* infection, 1711
ST-246, 2036
Stachybotrys chartarum infection, 296
Standard Precautions, 3379, 3380t, 3412
Staphylococcal cassette chromosome *mec* (SCC*mec*), 1119
Staphylococcus aureus, **1113-1130**
bacteriophage typing of, 1119
bound coagulase test of, 1113, 1114f
capsule of, 1113-1114, 1115f
carriage of, 1120-1121
clumping factor A of, 8-9
collagen-binding protein of, 8-9
culture of, 1113, 1114f
enzymes of, 1117
fibronectin-binding protein A of, 8-9
gene regulation of, 1118
genome of, 1120
Gram stain of, 1114f
hemolysins of, 17, 1115-1117
identification of, 1113, 1114f, 1114t
L-forms of, 1118
leukocidins of, 1115-1117
methicillin-resistant (MRSA), 1119-1120. *See also Staphylococcus aureus* infection, methicillin-resistant
genome sequencing of, 1120
identification of, 1119-1120
nasal carriage of, 1120
penicillin-binding protein–2 of, 1125
MSCRAMMs of, 1118
multilocus sequence typing of, 1119
opsonization of, 1122
pathogenicity islands of, 1115, 1115t
protein A of, 1114
pulsed-field gel electrophoresis of, 1119
small colony variants of, 1118
spa typing of, 1119
staphylococcal cassette chromosome *mec* of, 1119
structure of, 1113-1119
toxins of, 862, 1114-1115, 1115f. *See also* Toxic shock syndrome (TSS); Toxic shock syndrome toxin–I (TSST-I)
enterotoxin, 862-863, 1117-1118
epidermolytic, 1117
hemolytic, 1115-1117
leukocidin, 1115-1117
typing of, 1119-1120
Staphylococcus aureus infection, **1113-1130**
bloodstream, 361-362, 824, 831, 833-834, 1121
bone, 711-712, 712t, 718. *See also* Osteomyelitis
bronchial, 269, 269t
in bullous impetigo, 773-774, 774f
burn wound, 1051-1052, 1051t
in cervical lymphadenitis, 176-179, 179f, 185-186
in community-acquired pneumonia, 291

Staphylococcus aureus infection *(Continued)*
conjunctival, 802
neonatal, 805-806, 805t
CSF shunt, 1030-1031, 1030t
cutaneous, 750, 773-774. *See also* Impetigo
in cystic fibrosis, 334, 337-338
deep neck, 173-175
diagnosis of, 1123
endocardial, 354, 361-362, 361t, 366-367, 367t
epidemiology of, 1120-1121
eyelid, 794
gastrointestinal, 602t-603t, 607t, 613, 618
hair follicle, **776-777**
hepatic, 673
host defenses against, 1121-1123
immune response to, 1122
joint, 728, 729t, 732
after kidney transplantation, 1014
menstrual. *See* Toxic shock syndrome (TSS)
methicillin-resistant, 3144
antibiotic resistance in, 3144-3145
in bullous impetigo, 774-775
in cervical lymphadenitis, 177, 186
community-acquired, 1126-1127
in cystic fibrosis, 338, 340-341
D-test of, 1126-1127, 1127f
in daycare facilities, 3559
in deep neck abscess, 175
in furunculosis, 777
genome sequencing in, 1120
isolation in, 1126
neonatal, 882
nosocomial, 3398-3399
in osteomyelitis, 712, 714, 717-718
in otitis externa, 206
in pleural effusion, 310, 313-314
in pneumonia, 291
in preseptal cellulitis, 799
in retroperitoneal abscess, 709
in septic arthritis, 732
in septic shock, 824, 833-834
strain identification in, 1119-1120, 3144
TMP-SMX in, 3148
in toxic shock syndrome, 857
treatment of, 1125-1128
vancomycin-resistant, 1128
muscle, **735-738**
neonatal
conjunctival, 805-806, 805t
epidemiology of, 1121
methicillin-resistant, 882
prevention of, 1121
treatment of, 1125t
in otitis externa, 206
pathogenesis of, 1123
accessory gene regulator in, 1118
enterotoxins in, 1117-1118
enzymes in, 1117
hemolysins in, 1115-1117
leukocidins in, 1115-1117
MSCRAMMs in, 1118
protein A in, 1114
small colony variants in, 1118
staphylococcal cassette chromosome *mec* in, 1119
toxins in, 1114-1115, 1123
pelvic, 687
pericardial, 372, 379
prevention of, 1121, 1128-1129
renal, 556
retroperitoneal, 706-707, 709
in rhinosinusitis, 195, 196t
transmission of, 1120-1121
Staphylococcus aureus infection *(Continued)*
treatment of, 1123-1128, 1124t-1125t
antibiotics in, 1124-1125, 1124t
drainage in, 1123-1124
intravenous antibiotics in, 1124, 1124t
IVIG in, 3510
vancomycin-intermediate, 3151
vancomycin-resistant, 1128, 3151-3152
viral infection and, 1122, 2338
vulvovaginal, 568
Staphylococcus auricularis, 1132t
Staphylococcus capitis, 1132t
Staphylococcus capitis infection, 1030, 1030t
Staphylococcus caprae, 1132t
Staphylococcus caprae infection, 1138
Staphylococcus cohnii, 1132t
Staphylococcus epidermidis, 1131-1132, 1132t
enzymes of, 1133
ica operon of, 1133
identification of, 1114t
MSCRAMMs of, 1132-1133
neonatal colonization with, 1131-1132
phenol-soluble modulins of, 1133
polysaccharide intercellular adhesin of, 1132-1133
toxins of, 1133
Staphylococcus epidermidis infection
bloodstream, **1134-1136**, 1135t
bone, 1137
burn wound, 1051-1052, 1051t
catheter-related, 1136
in cervical lymphadenitis, 176-177
CSF shunt, 1030, 1030t
endocardial, 1137
eyelid, 794
neonatal, 882, 3509-3510
ocular, 820, 1138
in otitis externa, 206
peritoneal dialysis catheter–related, 1137
shunt-related, 1136-1137
surgical site, 1137
urinary tract, 1137-1138
Staphylococcus haemolyticus, 1131, 1132t
Staphylococcus hominis, 1132t
Staphylococcus hominis infection, 1030, 1030t
Staphylococcus lugdunensis, 1131-1132, 1132t
Staphylococcus lugdunensis infection
bone and joint, 1138
CSF shunt, 1030, 1030t
endocardial, 1137
Staphylococcus saccharolyticus, 1132t
Staphylococcus saprophyticus, 1131-1132, 1132t
genome of, 1133
identification of, 1114t
Staphylococcus saprophyticus infection, urinary tract, 1133, 1137-1138
Staphylococcus schleiferi, 1132t
Staphylococcus simulans, 1132t
Staphylococcus spp. infection. *See also specific species infections*
coagulase-negative, **1131-1139**
antibiotic-resistant, 1138-1139, 1138t
bloodstream, **1134-1136**, 1135t
bone, 1138
clinical manifestations of, 1133-1138, 1134t
device-related, **1136-1137**
endocardial, 362, 1137
epidemiology of, 1131-1132, 1132t
historical perspective on, 1131
microbiology of, 1131, 1132t
ocular, 1138
pathogenesis of, 1132-1133, 1133f
prevention of, 1139
surgical site, 1137
Staphylococcus spp. infection *(Continued)*
treatment of, 1138-1139, 1138t
urinary tract, 1137-1138
cutaneous, **772-775.** *See also* Impetigo
endocardial, 361-362, 361t, 366-367, 367t
soft tissue, **777-778**
Staphylococcus warneri, 1132t
Staphylococcus xylosus, 1132t
Statistics. *See* Biostatistics
Stavudine, 2613
STDs. *See* Sexually transmitted diseases (STDs)
Stenotrophomonas maltophilia, 1605-1606
Stenotrophomonas maltophilia infection, **1605-1609**
clinical manifestations of, 1606-1608
in cystic fibrosis, 336, 341
diagnosis of, 1608
epidemiology of, 1606
pathophysiology of, 1606
treatment of, 1608-1609
Sternotomy, mediastinitis with, 421-422, 421t, 948-949
Stevens-Johnson syndrome, 764
Stillbirth
bacterial infection and, 881
enterovirus infection and, 2092, 2100
listeriosis and, 1332, 1332f
Stomach. *See also at* Gastro-
flora of, 91t, 92-93
Stones, gallbladder. *See* Gallstones
Stool culture, 618, 3622-3624, 3623t, 3624f
in botulism, 1794
in *Campylobacter* spp. infection, 1645, 1655
in *Hafnia alvei* infection, 1535
in *Salmonella* spp. infection, 1502-1503
in *Shigella* spp. infection, 1482
in *Vibrio parahaemolyticus* infection, 1558-1559
in *Yersinia enterocolitica* infection, 1530
in *Yersinia pseudotuberculosis* infection, 1530
Stool specimen examination, 3622-3624, 3623t, 3624f. *See also* Stool culture
in *Ancylostoma duodenale* infection, 3018
in *Ascaris lumbricoides* infection, 3014
in *Balantidium coli* infection, 2895-2896
in *Blastocystis hominis* infection, 2876
in *Campylobacter jejuni* infection, 1655
in *Clostridium difficile* infection, 636
coccidia stains for, 617
in *Cryptosporidium* spp. infection, 2901-2902, 2902f, 2902t
in *Dientamoeba fragilis* infection, 2885-2886
in *Diphyllobothrium* spp. infection, 3041
in *Dipylidium caninum* infection, 3041
in *Entamoeba histolytica* infection, 2871
in *Fasciola buski* infection, 3057
in *Fasciola* spp. infection, 3053
in *Giardia lamblia* infection, 2881t, 2882
in *Hymenolepis nana* infection, 3042
immunologic tests on, 618-619
in infant botulism, 1806-1808
leukocytes in, 617
macroscopic, 616-617
microscopic, 617
in microsporidial infection, 2913
molecular tests on, 619
in *Necator americanus* infection, 3018
in *Opisthorchis* spp. infection, 3052
ova in, 617
parasites in, 617
in *Schistosoma* spp. infection, 3066-3067, 3067f
in *Strongyloides stercoralis* infection, 3021

Stool specimen examination *(Continued)*
in *Taenia saginata* infection, 3031
techniques of, 3622-3624, 3624f
in *Trichuris trichiura* infection, 3016
Strawberry tongue, 1073-1074
Streptobacillus moniliformis, 1716-1717, 1716f
Streptobacillus moniliformis infection (rat-bite fever), **1715-1720**
clinical manifestations of, 1717-1718, 1717f
diagnosis of, 1718-1719
differential diagnosis of, 1719
epidemiology of, 1715-1716
exanthem in, 753t-756t, 765, 1717, 1717f
historical perspective on, 1715
joint, 730
pathophysiology of, 1718
prevention of, 1719
treatment of, 1719
Streptococcal toxic shock syndrome, 779, 861, 1141, 3511
non–group A, 1151
Streptococcosis syndrome of infancy, 179
Streptococcus agalactiae, 1153-1154
adherence properties of, 1158
antibodies to, 1157-1158
antigenic structure of, 1153-1154
antimicrobial susceptibility of, 1154
C protein of, 1153-1154
C5a-ase of, 1154, 1158
extracellular products of, 1154
β-hemolysin of, 1154
identification of, 1153
isolation of, 1153
nontypeable, 1153
pili of, 5-6, 1154
serologic classification of, 1153-1154, 1156f
type-specific capsular polysaccharide of, 1154, 1158
Streptococcus agalactiae infection, **1153-1169**
capsule polysaccharide types in, 1156-1157, 1156f
in cellulitis, 778
in cervical lymphadenitis, 176-177, 187
clinical manifestations of, 1159-1162, 1159t, 1163t
community transmission of, 1155
diagnosis of, 1162-1163
differential diagnosis of, 1162-1163
dystonic extrapyramidal syndrome with, 516
early-onset, 1156-1157, 1156f-1157f, 1159-1160, 1159t, 1160f
incidence of, 1155
epidemiology of, 1154-1157
group B–specific antigen in, 1153, 1162
historical perspective on, 1153
immune complexes in, 1158
immune response in, 1158-1159
incidence of, 1155
infant colonization and, 1155
joint, 1161
laboratory studies in, 1162
late, late-onset, 1156-1157, 1156f, 1159t, 1161
late-onset, 1156-1157, 1156f-1157f, 1159t, 1160-1161
incidence of, 1155
maternal, 1155
maternal antibody levels and, 1157-1158
maternal colonization and, 1154-1155, 1157
meningeal, 1160, 1160f, 1162
neonatal, 881-882
Streptococcus agalactiae infection *(Continued)*
nosocomial, 1155
osseous, 1161, 1161f
pathogenesis of, 1157-1159, 1157f
infant host factors in, 1158-1159
maternal factors in, 1157-1158
organism factors in, 1158
pharyngeal. *See* Pharyngitis, streptococcal
prevention of, 1165-1169, 3243
infant prophylaxis in, 1167-1168, 1168f
maternal immunization in, 1168-1169
maternal prophylaxis in, 1165-1167, 1166t
prognosis for, 1165
recurrent, 1161-1162
treatment of, 1165
risk factors for, 1155-1156
soft tissue, 1161, 1162f
treatment of, 1154, 1163-1165
adjunctive, 1164-1165
empirical, 1163, 1164t
supportive, 1164
vertical transmission of, 1155-1157, 1157f
Streptococcus dysgalactiae subsp. *equisimilis* infection, 1150-1152
Streptococcus milleri infection. *See Streptococcus* viridans infection
Streptococcus mitis infection. *See Streptococcus* viridans infection
Streptococcus mutans infection. *See Streptococcus* viridans infection
Streptococcus parasanguinis, pili of, 5-6
Streptococcus pneumoniae, 1210-1223
N-acetylglucosamine deacetylase A of, 1215
antibiotic tolerance of, 1215, 1220-1221
antibodies to, 1223-1224
autolysin of, 1214
capsule of, 1211-1212
carriage of, 1200-1201, 1201f-1202f, 1242-1243, 1243t
cell wall of, 1210-1211, 1211f
choline-binding proteins of, 1213
clonality of, 1216
colonization with, 92, 431-432, 1200-1201
free oxygen radical release by, 1214
genome of, 1211-1212
histidine triad proteins of, 1213
hyaluronidase of, 1214
identification of, 1215-1216
IgA protease of, 1214
invasive capacity of, 1206-1209, 1208f
iron transport in, 1214
isolation of, 1215
lipoteichoic acid of, 1210, 1211f
lysogeny of, 1215
mucosal colonization by, 431-432
NADH oxidase of, 1214
nasopharyngeal colonization by, 92
neuraminidases of, 1212
peptidoglycan of, 1210
phase variation of, 1213
phosphoglucomutase of, 1214
phosphorylcholine esterase of, 1213
plasminogen binding of, 1214
pneumolysin of, 1212-1213
pyruvate oxidase of, 1214
serotypes of, 1205-1209, 1206t, 1207f-1208f, 1216
stem peptides of, 1214
structure of, 1210-1212, 1211f
surface proteins of, 1210-1211, 1211f, 1213
teichoic acid of, 1210, 1211f
virulence factors of, 1212-1215
Streptococcus pneumoniae infection, **1198-1246**
age and, 1199-1200, 1199f-1200f, 1202-1203, 1203f
antibiotic resistance in, 1202, 1219-1223, 1220t-1221t, 3143-3145, 3155
evolution of, 1202, 1202f, 1221-1223, 1222f
in mastoiditis, 234
in meningitis, 427
asplenia and, 1204, 1224, 1232
blood-brain barrier in, 432-433, 433t
bloodstream, 432, 432t, 832-833, 1226-1228, 1227t, 1235
bone, 711, 712t, 718, 1231
breast-feeding and, 1204
bronchial, 269, 269t
in cellulitis, 778
in cervical lymphadenitis, 176-177
conjunctival, 802, 1231
crowding and, 1201, 1204
in daycare facilities, 3561-3562
defective antibody formation and, 1204-1205
diagnosis of, 1217-1218
endocardial, 361, 1232
epidemiology of, 1198-1209, 1199f-1202f
in epiglottitis, 245
ethnicity and, 1203
exanthem in, 750, 753t-756t
gender and, 1203
after heart transplantation, 954
after hematopoietic stem cell transplantation, 935, 1232-1233
historical perspective on, 1198-1199
host defenses against, 1223-1226
alveolar macrophages in, 1225
anticapsular serum IgA in, 1223
anticapsular serum IgG in, 1223
basic fibroblast growth factor in, 1225
C-reactive protein in, 1224
complement in, 1225
cytokines in, 1224
granulocyte colony-stimulating factor in, 1225-1226
intracellular killing in, 1225
intracellular signaling pathways in, 1226
lactoferrin in, 1226
leukocyte IgG receptors in, 1223
leukotrienes in, 1224-1225
mannose-binding lectin in, 1226
phagocytosis in, 1223
platelet-activating factor receptors in, 1224
pneumolysin antibody in, 1223-1224
polymeric immunoglobulin receptor in, 1225
spleen in, 1224
surface protein antibody in, 1223-1224
vitamin A in, 1224
vitamin C in, 1224
immunization against, 285, 292, 315, 1237-1244, 3461-3463
adverse events with, 3463
conjugate vaccine for, 1238-1244, 1238f, 1238t, 1240f-1241f, 1240t, 1243t, 3462
efficacy of, 3462-3463
indications for, 3463
unconjugate vaccine for, 1237
in immunocompromised host, 1204-1205, 1232-1233
joint, 728-730, 729t, 1231
after kidney transplantation, 1014

Streptococcus pneumoniae infection *(Continued)*
mannose-binding lectin and, 1205
mastoid, 234, 235t, 1231
medical conditions and, 1205, 1206t, 1244
meningeal, 426-427, 426t, 450t-451t, 458-459, 1228-1229, 1235. *See also* Meningitis, bacterial
animal model of, 1214
mortality with, 1198, 1199f, 1210
neutropenia and, 1205
in otitis media, 212-215, 212t-213t, 1231, 1233-1235
pathogenesis of, 1209-1210
pericardial, 371-373, 379, 1232
peritoneal, 1232
in preseptal cellulitis, 798-799
prevention of, 1236-1244
immunoprophylactic, 1237-1244
nonimmunologic, 1236
pulmonary, 284-286, 285t, 1229-1231, 1230f, 1230t. *See also* Pneumonia, community-acquired
treatment of, 1233
race and, 1203
in rhinosinusitis, 195, 196t, 1231, 1233
risk factors for, 1202-1204, 1203f
seasonality and, 1203-1204, 1204f
septic, 1228, 1228f
serotype and, 1201, 1201f, 1205-1209, 1206t, 1207f-1208f
serum cholesterol and, 74
in sickle-cell disease, 1203-1204, 1205f, 1232
soft tissue, 778, 1231-1232
splenic dysfunction and, 1204
treatment of, 1233-1235, 1234t-1235t
antibiotic resistance in, 1219-1223, 1220t
antibiotic tolerance in, 1215, 1220-1221
susceptibility testing in, 1218-1219, 1220t-1221t
viral infection and, 1215
vulvovaginal, 568
Streptococcus pyogenes, 1140-1141
adhesins of, 10
C5a peptidase of, 18
capsule of, 1140
carriage of, 1143
enzymes of, 1141
exotoxins of, 1141
hemolysins of, 1141
hyaluronidase of, 17-18
L forms of, 1140
M protein of, 10, 18, 1140
protein F of, 10
Sic protein of, 18
transmission of, 1141-1142
vaccine against, 1150
Streptococcus pyogenes infection, **1140-1152**
vs. *Arcanobacterium haemolyticum* infection, 1326
burn wound, 1051-1052, 1051t
in cervical lymphadenitis, 177, 179, 183t, 184-186
clinical manifestations of, 1142-1143
cutaneous, 753t-756t, 772-773, **776**, 1141, 1143. *See also* Erysipelas; Impetigo
in daycare facilities, 3555
deep neck, 173-174
diagnosis of, 1146-1147
differential diagnosis of, 1146-1147
epidemiology of, 1141-1142
in erysipelas, **776**
Streptococcus pyogenes infection *(Continued)*
finger, **775**
mastoid, 234, 235t
in myositis, **738-739**
osteoarticular, 711. *See also* Osteomyelitis
in otitis media, 212t, 214
PANDAS with, 410, **1143-1146**, 1144f. *See also* PANDAS (pediatric autoimmune neuropsychiatric disorder with streptococcal infection)
pathogenesis of, 1142
perianal, **775**
pharyngeal, 156, 158, 159t-160t
clinical manifestations of, 161-162, 1142-1143
diagnosis of, 163, 1146-1147
epidemiology of, 1141
pathogenesis of, 1142
prevention of, 1141-1142, 1150
tonsillectomy in, 1150
transmission of, 1141
treatment of, 163, 1147-1149, 1148t
prevention of, 1141-1142, 1150
rheumatogenic, 405-406, 405t. *See also* Rheumatic fever
soft tissue, **778-780.** *See also* Necrotizing fasciitis
toxic shock syndrome with, 779, 861, 1141, 1151
treatment of, 1147-1149, 1148t
uvular, 165
vulvovaginal, 568
Streptococcus salivarius infection. *See Streptococcus* viridans infection
Streptococcus sanguinis, 354
Streptococcus sanguinis infection, in aphthous stomatitis, 161
Streptococcus sanguis infection. *See Streptococcus* viridans infection
Streptococcus spp. infection. *See also Streptococcus pneumoniae* infection; *Streptococcus* viridans infection
group A. *See Streptococcus pyogenes* infection
group B. *See Streptococcus agalactiae* infection
group C, 1150-1152
group G, 1150-1152
Streptococcus viridans, 1187-1189, 1188f, 1189t
Streptococcus viridans infection, **1187-1197**
bone, 1193
clinical manifestations of, 1191-1193
dental, 1193, 1195
diagnosis of, 1194
endocardial, 360-361, 361t, 365-366, 365t-366t, 1191-1192, 1195-1196
epidemiology of, 1189-1190
in immunocompromised host, 1191-1192
joint, 1193
neonatal, 1192
pathogenesis of, 1190-1191
pharyngeal, 1193
prevention of, 1195-1196
pulmonary, 1193
treatment of, 1194-1195
antibiotic resistance in, 1194
Streptogramins, resistance to, 3145-3147
Streptokinase, in parapneumonic pleural effusion, 314-315
Streptolysin O, 1141
Streptolysin S, 1141
Streptomycin, 3208-3211, 3208t, 3231t-3233t
in tuberculosis, 1367t, 1369
Streptozyme agglutination test, 1147
Stress
infection and, 109
otitis media and, 222
rhinovirus infection and, 2120-2121
Strongyloides fuelleborni kellyi infection, **3019-3021**
Strongyloides stercoralis infection (strongyloidiasis), **3019-3021**
adult T-cell leukemia and, 2575
biliary, 660
clinical manifestations of, 607t, 615-616, 757t-758t, 3020
CNS, 3020
diagnosis of, 3020-3021
disseminated, 3020
epidemiology of, 3020
after hematopoietic stem cell transplantation, 943
hyperinfection with, 3020
after kidney transplantation, 1014
pathophysiology of, 3020
prevention of, 3021
prognosis for, 3021
treatment of, 625, 3021, 3327t-3346t
Stye, **796**
Subacute necrotizing lymphadenitis, 181
Subacute sclerosing panencephalitis (SSPE), **2386-2390**
clinical manifestations of, 2389
epidemiology of, 2386-2387
laboratory findings in, 2389-2390
ocular manifestations of, 811
pathogenesis of, 2387-2389
pathology of, 2387-2389
treatment of, 2390
Subarachnoid neurocysticercosis, 3035, 3036f, 3038
Subdural effusion, in bacterial meningitis, 438, 440, 454-455
Subdural empyema, **469-470**
clinical manifestations of, 469
CSF examination in, 443t, 469
diagnosis of, 469, 470f
treatment of, 470
Sublingual space, infection of (Ludwig angina), 149-150, 150f
Submandibular space, infection of, 142, 149-150, 150f
Sudden infant death syndrome (SIDS)
Bordetella pertussis and, 1627, 1634
enterovirus and, 2091
Sulfadiazine
in heart transplant–related toxoplasmosis, 953
in *Paracoccidioides* spp. infection, 2792
in *Toxoplasma gondii* infection, 3000-3001, 3001t
Sulfamerazine, in *Toxoplasma gondii* infection, 3000-3001
Sulfamethazine, in *Toxoplasma gondii* infection, 3000-3001
Sulfonamides, 3227-3230, 3231t-3233t, 3351
Sulfur granules, in *Actinomyces* spp. infection, 1821, 1821f, 1823
Superantigens
Kawasaki disease and, 1069-1070
T-cell activation by, 40
in toxic shock syndrome, 849, 865-866, 1141
Supraglottitis. *See* Epiglottitis (supraglottitis)
Suprapubic aspiration, 541, 541f, 541t
Suramin, 3351
in *Trypanosoma brucei* infection, 2972

Surfactant
in ARDS, 872-873
in bronchiolitis, 280
in *Pneumocystis* infection, 3008
Surgery
antibiotic prophylaxis in, 3252-3256, 3253t-3256t
infection after, 3395-3398, 3396t
Sushi, 602-604
Sweat test, in cystic fibrosis, 326
Swimmer's ear. *See* Otitis externa
Swimmer's itch, 3062. *See also Schistosoma* spp. infection (schistosomiasis)
Swimming, rhinosinusitis and, 195, 197
Swollen belly syndrome, in *Strongyloides fulleborni kellyi* infection, 3021
Sydenham, Thomas, 103
Sydenham chorea, 410, 413-414, 3511
Sympathetic nervous system, tetanospasmin effects on, 1813
Synbiotics, 3370
Synovitis, toxic (transient, reactive), 732
Syphilis, 572t-573t, 582, 583t, **1761-1782**, 1783t
acquired, 1762
clinical manifestations of, 1767-1768
treatment of, 1777-1778
cardiovascular, 1768
chancre of, 1765, 1768
clinical manifestations of, 1767-1772
CNS, 494t-498t, 1768
congenital, 812, 819-820, 1762
case definition of, 1763, 1764t
clinical manifestations of, 1769-1772, 1769t, 1771t
CNS involvement in, 1770, 1772
CSF examination in, 1777
cutaneous lesions in, 1772
dental changes in, 1771
diagnosis of, 1775-1777, 1775t
early, 1769-1771, 1769t
ectodermal changes in, 1765, 1771
epidemiology of, 1763-1764
exanthem in, 1770
fissures in, 1770
follow-up for, 1780-1781, 1781t
gastrointestinal involvement in, 1771
hearing loss in, 1772
hematologic findings in, 1770
hepatosplenomegaly in, 1771
interstitial keratitis in, 1771
late, 1771-1772, 1771t
mucous patches in, 1765, 1770
ocular, 1771
pathology of, 1766
pneumonia in, 1770-1771
prevention of, 1764-1765, 1765f, 1765t
rhinitis in, 1770
skeletal involvement in, 1769-1770, 1772
subtherapeutic maternal therapy and, 1763, 1764t
treatment of, 1776t-1777t, 1779-1780, 1780f
diagnosis of, 819-820, 1772-1777
CSF examination in, 443t, 1770, 1777
darkfield examination in, 1772
false-positive, 1774
IgM tests in, 1774
nontreponemal tests in, 1772-1773, 1773t
PCR in, 1774
rabbit infectivity test in, 1772
treponemal tests in, 1773-1774, 1773t
endemic, 1783t, **1787-1788**
Syphilis *(Continued)*
epidemiology of, 1763-1765
exanthem in, 753t-756t, 1770
glomerulonephritis in, 1767
gonococcal infection and, 1297
granuloma inguinale and, 1641
gummas in, 1765-1766, 1768
hepatitis in, 656
historical perspective on, 1761
HIV infection and, 1762, 1778
immune response to, 1767
in international adoptees and refugees, 3126
latent, 1766f, 1768
nephrotic syndrome in, 1767
ocular, 807, 812
pathogenesis of, 1766-1767
pathology of, 1765-1766
in pregnancy, 1769, 1778
prevention of, 1781-1782
primary, 583t, 1765, 1766f, 1768
reporting requirement for, 1782
screening for, 1768
secondary, 1765, 1766f, 1768
stages of, 819, 1765-1766, 1766f
tertiary, 1765-1766, 1766f, 1768
transmission of, 1762
of unknown duration, 1768
Syringomyelia, 524
Systemic inflammatory response syndrome (SIRS), 825t. *See also* Acute-phase reaction
Systemic lupus erythematosus (SLE), pulmonary manifestations of, 297

T

T lymphocytes, 38-40
activation of, 39-40
in acute disseminated encephalomyelitis, 517-518
in adenovirus infection, 1895, 1903-1904, 1907-1908
antigen specificity of, 39
in asthma, 277-278
in *Blastomyces dermatitidis* infection, 2726-2727
in burns, 1050
$CD4^+$, 39
in HIV-1 infection, 2598
in HIV-2 infection, 2585-2586, 2592-2595
$CD8^+$, 39
in bronchiolitis, 275-276
$CD4^+/CD8^+$ ratio
in HIV infection, 2596-2597
in hypersensitivity pneumonitis, 304
CMV-specific, in hematopoietic stem cell transplantation–related CMV infection, 940
in *Cryptococcus neoformans* infection, 2799
in *Cryptosporidium* spp. infection, 2900
development of, 38-39
in DiGeorge syndrome, 48
in EBV infection, 1995
evaluation of, 63, 908
fetal, 42
in *Francisella tularensis* infection, 1661
in granuloma inguinale, 1641
in *Haemophilus influenzae* type b infection, 1672
in hantavirus infection, 2509
in HAV infection, 2136-2137
in HBV infection, 1915
helper, 39
T lymphocytes *(Continued)*
in *Histoplasma capsulatum* infection, 2810
in infectious mononucleosis, 2007, 2007f
in influenza virus infection, 2338
in Kawasaki disease, 1077
in measles, 2379
memory, 40
MHC interaction with, 36, 37f
in *Mycobacterium leprae* infection, 1396-1398
in myocarditis, 386-387
ocular, 810
in parainfluenza virus infection, 2365-2366
in *Pneumocystis* infection, 3007-3008
receptors of, 39
regulatory (suppressor), 39-40
in rhinovirus infection, 135, 2119
in RSV infection, 2416
in rubella virus infection, 2206
superantigen activation of, 40
in syphilis, 1767
in toxic shock syndrome, 865-866
in tuberculosis, 1342
in VZV infection, 2024-2025
in Whipple disease, 641
in Wiskott-Aldrich syndrome, 50
Tache noire, 2655
Tachypnea
in community-acquired pneumonia, 287-288, 287t
in parapneumonic effusion, 310
Tacrolimus
in heart transplantation, 955, 955t
in lung transplantation, 968
Taenia asiatica (pork tapeworm) infection, **3032**
Taenia crassiceps infection (cysticercosis), **3039-3040**
Taenia multiceps infection (coenurosis), 815, **3039-3040**
Taenia saginata, 3030
Taenia saginata (beef tapeworm) infection, **3030-3032**
clinical manifestations of, 3031
diagnosis of, 3031-3032
epidemiology of, 3031
pathogenesis of, 3031
prevention of, 3032
prognosis for, 3032
transmission of, 3030-3031
treatment of, 3032, 3327t-3346t
Taenia solium, 3033
Taenia solium infection (cysticercosis), 739, **3032-3039**. *See also* Neurocysticercosis
clinical manifestations of, 3034-3035
diagnosis of, 3035-3037
epidemiology of, 3033
ocular, 815
pathogenesis of, 3033-3034
prevention of, 3039
prognosis for, 3039
transmission of, 3033
treatment of, 3037-3039, 3327t-3346t
Tafenoquine, in malaria, 2945
Tahyna virus, 2514, 2517
Tampon, in toxic shock syndrome, 852-853, 861
Tanapox, 742t-748t, 749
Tanapox virus infection, **2046-2047**
Tapeworm infection
Diphyllobothrium spp. (fish tapeworm), **3040-3041**
Dipylidium caninum (dog tapeworm), **3041**

Tapeworm infection *(Continued)*
Hymenolepis diminuta (rat tapeworm), **3042**
Hymenolepis nana (dwarf tapeworm), **3042**
Taenia saginata (beef tapeworm), **3030-3032**
TBX1 gene, 48
TC-83 vaccine, 2240-2241
Teeth. *See* Tooth (teeth)
Tefibazumab, in septic shock, 835
Teichoic acid, of *Streptococcus pneumoniae*, 1210, 1211f
Teicoplanin, resistance to, 3151-3154, 3152t
Telaprevir, in HCV infection, 2324
Telavancin, 3206-3207
Telogen effluvium, in toxic shock syndrome, 858
Temperature, body, 83-84. *See also* Fever
Temporal artery thermometer, 86
Tenofovir disoproxil fumarate, in HBV infection, 1922
Terbinafine, 3320-3324, 3321t, 3322f
adverse effects of, 3322-3323
drug interactions with, 3323
indications for, 3323-3324
pharmacokinetics of, 3322, 3323t
spectrum of activity of, 3322
in tinea unguium, 787
Tetanolysin, 1812
Tetanospasmin, 1814
Tetanospasmin, 1812-1813
Tetanus, **1809-1819**
cephalic, 1813
cerebral effects in, 1813
clinical manifestations of, 1813-1815
diagnosis of, 1815
differential diagnosis of, 1815
epidemiology of, 1810, 1811f
generalized, 1813-1815
historical perspective on, 1809-1810
immunization against, 1818, 3470-3472, 3472t
in injection drug users, 1814
local, 1813
mortality with, 1817-1818
motor end-plate effects in, 1812-1813
neonatal, 1814-1815, 1819
pathogenesis of, 1812
pathophysiology of, 1812-1813
prevention of, 1818-1819
prognosis for, 1817
seizures in, 1814, 1816-1817
spinal cord effects in, 1813
sympathetic nervous system effects in, 1813
tetanus toxoid in, 1818-1819, 1818t, 3470-3472
treatment of, 1815-1817, 3511-3512
trismus in, 1814
Tetanus toxoid, 1818-1819, 1818t, 3470-3472
for delayed hypersensitivity skin test, 907
Tetracycline(s), 3217-3220, 3231t-3233t
adverse effects of, 3219-3220, 3351
in *Balantidium coli* infection, 2896
in chronic bronchitis, 269-270
contraindications to, 3219-3220
drug interactions with, 3220
indications for, 3219
in malaria, 2942
mechanisms of action of, 3217
in odontogenic infection, 145
pancreatitis with, 692
pharmacokinetics of, 3218-3219, 3218t
resistance to, 3217-3218
Tetracycline(s) *(Continued)*
topical
in inclusion conjunctivitis, 804
in neonatal conjunctivitis, 806, 1281
in trachoma, 805
in vitro activity of, 3218
Thermogenesis, nonshivering, 84
Thermometers, 85-86
Thermoregulation, 84
Thermostable direct hemolysin, 1557-1558
Thigh, dermatophyte infection of, **786**, 786f
Thimerosal, 1927, 3429
Thoracentesis
in parapneumonic effusion, 310-313
in tuberculosis, 1347-1348
Thoracostomy, in lung abscess, 321
Three-day fever. *See* Roseola infantum
Thrombocytopenia
in *Brucella* spp. infection, 1613-1614
in human erythrovirus B19 (human parvovirus B19) infection, 1850
in infectious mononucleosis, 2002-2003
in measles virus infection, 2380
in rubella virus infection, 2212, 2219
Thrombocytopenic purpura
in measles, 2386
in roseola infantum, 771
in rubella virus infection, 2212
Thrombocytosis, in Kawasaki disease, 1076
Thrombophlebitis
CSF examination in, 443t
venous sinus, 236, 239-240
Thrombosis
in bacterial meningitis, 438
in CMV infection, 1980
IVIG-related, 3496
in *Neisseria meningitidis* infection, 1260
Thrush, **787-788**, 787f
in HIV infection, 148-149
in neonate, 892-893, 896
treatment of, 788
Thumb sign, in epiglottitis, 254f
Thymus, evaluation of, 906, 906f
Thyroglossal duct cysts, 182
Thyroid gland, hormones of, 82
Thyroid-stimulating hormone (TSH), 81
Thyroiditis, adenovirus, 1904
Thyroxine (T_4), 82
Ticarcillin, 3183t-3184t, 3187, 3231t-3233t
in meningitis, 450t
Ticarcillin–potassium clavulanate, 3188, 3231t-3233t
Tick bite, 3071-3072, 3071t, 3549
Aeromonas spp. recovery from, 1543
in *Babesia* spp. infection, 2915-2916, 2916f
in Colorado tick fever virus infection, 2173, 2173f
cutaneous manifestations of, 758t
in *Francisella tularensis* infection, 1659-1660
in Kemerovo-related virus infection, 2175
in Lyme borreliosis, 1730, 1730t, 1737-1738
prevention of, 1737, 2175, 2654, 2920, 3549
in relapsing fever, 1740
in Rocky Mountain spotted fever, 2651
tick removal after, 1737, 2654
Tick-borne encephalitis complex viruses, 2303-2304, 2304f
epidemiology of, 2305-2307, 2306f
Tick-borne encephalitis virus infection, 494t-498t, 500, **2303-2313**
clinical manifestations of, 2307-2309, 2307f, 2308t
Tick-borne encephalitis virus infection *(Continued)*
diagnosis of, 2310
differential diagnosis of, 2310-2311
ecology of, 2304-2305
historical perspective on, 2303
immunization against, 2311-2312, 2312f
meningeal, 487
milk-borne, 2306-2307
MRI in, 2308f, 2309
neurologic sequelae of, 2311
paralysis in, 2308-2309
passive immunization against, 2312
pathogenesis of, 2310
pathology of, 2309
prevention of, 2311-2312, 3534
prognosis for, 2311
seizures in, 2309
in traveler, 3116
treatment of, 2311
Tick-borne relapsing fever, 1731f, **1739-1742**, 1741t
Tick paralysis, **3071-3072**
clinical manifestations of, 3072
diagnosis of, 3072
pathogenesis of, 3071-3072
treatment of, 3072
Tigecycline, 3238
in *Acinetobacter* spp. infection, 1572
in coagulase-negative staphylococcal infection, 1139
in vancomycin-resistant *Enterococcus* spp. infection, 1184
Tilt-table testing, in chronic fatigue syndrome, 1088-1089
Tinea capitis, **784-785**, 785f, 787, 3321t
Tinea corporis, **785**, 787, 3321t
Tinea cruris, **786-787**, 786f
Tinea faciei, **785-787**, 785f, 3321t
Tinea incognita, 785
Tinea nigra, **790**, 790f
Tinea pedis, **786-787**, 3321t
Tinea unguium, **786-787**, 786f, 3321t
Tinea versicolor, 789, 789f
Tinidazole, 3351
Tinnitus, in otitis media, 217
Tipranavir, 2615
Tissue expander, infection with, 1017
TMP-SMX. *See* Trimethoprim-sulfamethoxazole (TMP-SMX)
Tobramycin, 3208-3211, 3208t, 3231t-3234t
Toe(s), desquamation of, in Kawasaki disease, 1073
Toenails, dermatophyte infection of, **786**, 786f
Tokyo guidelines, in cholangitis, 660, 660t
Tolerance
antibiotic, in *Streptococcus pneumoniae* infection, 1215, 1220-1221
immunologic, newborn immunization and, 44
Tonsillectomy
lung abscess and, 316
in peritonsillar abscess, 169
prophylactic, 164
in streptococcal pharyngitis, 1150
Tonsillitis, **158-164.** *See also* Pharyngitis
adenovirus, 1895-1896
Francisella tularensis, 1662
tuberculous, 1357
Tonsillopharyngitis, **158-164.** *See also* Pharyngitis
Tooth (teeth)
caries of. *See* Caries
in congenital syphilis, 1771

Tooth (teeth) *(Continued)*
conical, 916, 916f
extraction of, 146
infection of. *See* Odontogenic infection
Torovirus, 2488
Torulopsis glabrata infection, 569-570
Toscana virus, 2545, 2545f
genetic diversity of, 2550
isolation of, 2549-2550
Toscana virus infection, **2544-2551**
clinical manifestations of, 2548
CNS, 2548
diagnosis of, 2549-2550
ecology of, 2547-2548
epidemiology of, 2545-2547
historical perspective on, 2544
Total parenteral nutrition (TPN), gallstones and, 668
Toxic epidermal necrolysis, 753t-756t
Toxic shock syndrome (TSS), **848-866**
acute phase of, 854-857, 854f-855f
in AIDS, 860
antibody titer in, 852, 856, 863-864
in burn patient, 1055
cervicovaginal ulcerations in, 853
clinical presentation of, 753t-756t, 831, 854-861, 854f
definition of, 848, 849t
dermatologic features of, 854, 855f, 858, 858f
diagnosis of, 849t, 856
differential diagnosis of, 861, 862t
Enterobacteriaceae in, 853
enterotoxins in, 862-863
epidemiology of, 849-853, 850f
foreign body in, 852-853
hematologic features of, 858-859
hepatic findings in, 657, 853
histopathology of, 853-854
hypocalcemia in, 829-830
incidence of, 849-850
vs. Kawasaki disease, 1069-1070
laboratory tests in, 855-856
laryngotracheobronchopneumonitis and, 252
mild episodes of, 859-860
mortality with, 859
MRSA strains in, 856-857
mucosal disruption in, 852
murine, 853
myocardial features of, 853, 858
neonatal, 860-861
nonmenstrual, 851-852, 851t, 855f, 860
oral contraceptives and, 853
physical examination in, 854-855, 855f
postoperative, 860-862
prevention of, 861-862
pulmonary features of, 858
recurrence of, 859-861
renal features of, 853, 858
risk factors for, 850-851, 850f
sequelae of, 859
skin disruption in, 852
Staphylococcus aureus in, 852-853, 862-864. *See also* Toxic shock syndrome toxin–I (TSST-I)
subacute phase of, 854f, 857-859, 858f
surveillance for, 849-850, 850f
tampon in, 852-853, 861
treatment of, 856-857, 856t
antimicrobial agents in, 856-857
corticosteroids in, 857
drainage in, 856
fluid therapy in, 857-858
IVIG in, 835, 857, 3509
Toxic shock syndrome (TSS) *(Continued)*
TSST-I in, 852, 862. *See also* Toxic shock syndrome toxin–I (TSST-I)
Toxic shock syndrome toxin–I (TSST-I), 1115f, 1117
antibody to, 852, 857, 863-864
biologic functions of, 865-866
cellular interactions of, 865
detection of, 856
distributional kinetics of, 865
physiochemical properties of, 864
prevalence of, 863
regulation of, 864-865
in toxic shock syndrome, 852, 862
Toxic (transient) synovitis, 732
Toxin(s), 16-18
Aeromonas hydrophilia, 1540
in animals, 826-827, 827t
Arcanobacterium haemolyticum, 1322
Bacillus anthracis, 1303
Bacillus cereus, 1318-1319
Bordetella pertussis, 17, 1618t, 1619-1620, 1623-1625
Campylobacter spp., 1650
Citrobacter freundii, 17, 1430-1431
Clostridium botulinum, 1791-1792, 1801-1802
Clostridium perfringens, 1796
Clostridium tetani, 1812
Corynebacterium diphtheriae, 1302, 1304
enteroaggregative *Escherichia coli*, 1460
Enterobacter, 1435-1436
enterohemorrhagic *Escherichia coli*, 1457-1459
enteroinvasive *Escherichia coli*, 1459-1460
enterotoxigenic *Escherichia coli*, 1455-1457
epidermolytic, 1117
Haemophilus ducreyi, 1686
Helicobacter pylori, 1693
hemolytic, 1115-1117
in hepatic failure, 94
in humans, 827-831. *See also* Septic shock
Kingella kingae, 1701-1702
leukocidin, 1115-1117
marine, 604, 605t
mushroom, 604, 604t
Mycobacterium ulcerans, 1386-1387
Neisseria meningitidis, 1258
Plesiomonas shigelloides, 1565
Pseudomonas aeruginosa, 1587
Shiga, 17, 1457-1459
Shigella spp., 1479
Staphylococcus aureus, 862-863, 1114-1115, 1117-1118. *See also* Toxic shock syndrome toxin–I (TSST-I)
Staphylococcus epidermidis, 1133
Streptococcus pyogenes, 1141
T-cell activation by, 40
tick, 3071
tissue-degrading, 17-18
Vibrio cholerae, 1550
Vibrio parahaemolyticus, 1557-1558
Yersinia enterocolitica, 1525
Yersinia pestis, 1511-1512
Toxocara canis infection (toxocariasis), **3021-3023**
clinical manifestations of, 3022-3023
covert, 3022
diagnosis of, 3023
epidemiology of, 3022
hepatic, 673
myocardial, 400
ocular, 814, 3022-3023
pathophysiology of, 3022
prevention of, 3023
Toxocara canis infection (toxocariasis) *(Continued)*
prognosis for, 3023
treatment of, 3023
Toxocara cati infection, **3021-3023**
Toxoplasma gondii, 2986-2989, 2987f-2988f
isolation of, 2996
Toxoplasma gondii infection (toxoplasmosis), **2986-3005**
acquired, 757t-758t, 2991
diagnosis of, 2997
epidemiology of, 2989-2990
treatment of, 3002
acute, 2991, 2997
cervical lymphadenitis in, 181
CNS, 494t-498t, 2990-2991, 3327t-3346t
vs. EBV-associated lymphoma, 2012
congenital, 757t-758t, **900**, 900t, 2991-2993, 2992t
diagnosis of, 2997-2999, 2999t
differential diagnosis of, 2993
epidemiology of, 2990
ocular, 816-817, 2994, 2994f
prenatal diagnosis of, 2997-2999
treatment of, 3002-3004
diagnosis of, 2994-3000, 2995t
epidemiology of, 2989-2990
fever of unknown origin in, 844
after heart transplantation, 953
after hematopoietic stem cell transplantation, 943
HIV infection and, 2993, 3003-3004
immune response in, 2991
in immunocompromised host, 2991, 2997
after kidney transplantation, 1014
muscle, 739
myocardial, 400
ocular, 816-817, 2991, 2994, 2994f
diagnosis of, 2999-3000
treatment of, 3000-3004, 3001t
pancreatic, 694-695
pathology of, 2990-2991
in pregnancy, 2990, 2992-2993, 2992t, 2997, 3002, 3004, 3004t
prevention of, 3004, 3004t
Trace elements, metabolism of, 77-79, 77t
Tracheal cytotoxin, 17
of *Bordetella pertussis*, 1618t, 1620, 1624
Tracheal intubation
in epiglottitis, 257-258
otitis media and, 211
Tracheitis
bacterial, 242t, 250t-251t, **252**
treatment of, 257
measles virus, 2385, 2392
Moraxella catarrhalis, 1252
Tracheobronchitis, HSV, after kidney transplantation, 1006
Tracheoesophageal fistula, vs. chronic bronchitis, 268
Tracheostomy, in epiglottitis, 257-258
Trachoma, 804-805, **2636**
Transferrin
CSF, 441
serum, 78
Transglycosylases, of *Neisseria gonorrhoeae*, 1278-1279
Transient hypogammaglobulinemia of infancy, **47**, **912**
Translocated intimin receptor (Tir), 7-8, 9f, 1457
Transmissible spongiform encephalopathies, 503, **2619-2629**, 2620t
clinical manifestations of, 2627-2628, 2627t

Transmissible spongiform encephalopathies *(Continued)*
diagnosis of, 2628
epidemiology of, 2621-2624
pathogenesis of, 2624-2627
pathology of, 2624-2627
treatment of, 2628
Transmission-Based Precautions, 3379, 3381t, 3412
Transplantation. *See* Heart transplantation; Hematopoietic stem cell transplantation (HSCT); Intestinal transplantation; Kidney transplantation; Liver transplantation; Lung transplantation
Transposons, in antibiotic resistance, 3138, 3138t
Transverse myelitis. *See* Acute transverse myelitis
Trauma
HSV infection with, 1944, 1944f, 1953
IVIG in, 3499-3500, 3509
liver abscess with, 673
pancreatitis with, 691, 691t
pyomyositis with, 735-736, 736t
Travel. *See* International travel
Traveler's diarrhea, 600-601, 1451, **3110-3115**. *See also* Enterotoxigenic *Escherichia coli* (ETEC) infection
epidemiology of, 3110-3112
management of, 3113-3115
prevention of, 1463, 3112-3113
Treacher Collins syndrome, 1044
Tremacamra, in common cold, 138
Trematodes, 2865-2866. *See also* Flukes
Trench fever, 363, 753t-756t, **1726**, 3075
diagnosis of, 1727
treatment of, 1728
Trench mouth (acute necrotizing ulcerative gingivitis), 147, 147f
Treponema carateum infection (pinta), 753t-756t, 1783t, **1784-1785**
diagnosis of, 1789
treatment of, 1789-1790
Treponema pallidum, 1761-1762. *See also* Syphilis
identification of, 1772
virulence factors of, 1766-1767
Treponema pallidum subsp. *endemicum* infection (endemic syphilis), 1783t, **1787-1788**
diagnosis of, 1789
treatment of, 1789-1790
Treponema pallidum subsp. *pertenue* infection (yaws), 753t-756t, 1783t, **1785-1787**
diagnosis of, 1789
treatment of, 1789-1790
Treponemataceae, 1097t-1112t
Treponematoses
nonvenereal, **1782-1790**, 1783t
diagnosis of, 1789
treatment of, 1789-1790
venereal. *See* Syphilis
Triatoma infestans, 2959
Triatoma sanguisuga, 758t
Tribec virus, 2175
Trichinella spiralis (trichinosis), 3327t-3346t
cutaneous, 757t-758t
gastrointestinal, 602t-603t
muscle, 739
myocardial, 399
ocular, 815
Trichloroacetic acid, in genital HPV infection, 581
Trichobilharzia ocellata infection, 757t-758t
Trichobilharzia physellae infection, 757t-758t
Trichobilharzia stagnicolae infection, 757t-758t
Trichoderma longibachiatum infection, **2857**
Trichodysplasia spinulosa, 1867-1868
Trichomonas vaginalis, 2887
Trichomonas vaginalis infection (trichomoniasis), 572t-573t, 575-577, **2887-2894**
bacterial infection with, 2889-2891
clinical manifestations of, 2890-2891
cutaneous manifestations of, 757t-758t
diagnosis of, 576-577, 2891-2892, 3626-3627
epidemiology of, 2887-2888
gender and, 2888
immune response to, 2890
partner treatment in, 2893
pathogenesis of, 2889-2890
prepubertal, 575-576, 2888-2889
prognosis for, 2893
risk factors for, 2888
severity of, 2890
transmission of, 2888-2889
treatment of, 2892-2893, 2893t, 3327t-3346t
wet-mount preparation in, 575f, 2891-2892
Trichophyton tonsurans infection (tinea capitis), **784-785**, 785f
Trichosporon spp. infection (trichosporonosis), **790-791**, **2853-2854**
neonatal, 898
Trichostrongylus spp. infection, 3024, 3327t-3346t
Trichuris dysentery syndrome, 3016
Trichuris trichiura infection (trichuriasis), **3015-3016**
clinical manifestations of, 3016
diagnosis of, 3016
epidemiology of, 3015
pathophysiology of, 3015-3016
prevention of, 3016
treatment of, 3016, 3327t-3346t
Triiodothyronine (T_3), 82
Trimethoprim-sulfamethoxazole (TMP-SMX), 3227-3229, 3231t-3233t
adverse effects of, 3229, 3351
in cervical lymphadenitis, 186
in cholangitis, 665
in *Cyclospora cayetanensis* infection, 2907
in *Cystoisospora belli infection*, 2911
drug interactions with, 3229
in heart transplantation, 954
in hematopoietic stem cell transplantation, 938-939
indications for, 3228-3229
mechanisms of action of, 3228
in meningitis, 450t
in methicillin-resistant *Staphylococcus aureus* infection, 3148
in *Nocardia* spp. infection, 1422
pancreatitis with, 692
in *Paracoccidioides* spp. infection, 2792
pharmacokinetics of, 3228
in *Pneumocystis* infection, 3010, 3011t
in *Pneumocystis jiroveci* infection, 973, 2599-2600
resistance to, 3147-3149, 3148f, 3228
in *Staphylococcus aureus* infection, 1125
in *Toxoplasma gondii* infection, 3002
in vitro activity of, 3228
in Whipple disease, 646, 646t
Trismus
in masticator space infection, 149
in odontogenic infection, 144
Trismus *(Continued)*
in pericoronitis, 148
in peritonsillar abscess, 168
in tetanus, 1814
Trivittatus virus, 2514, 2517
Trombicula irritans, 758t
Tropheryma whipplei. *See* Whipple disease
Tropical ear. *See* Otitis externa
Tropical pulmonary eosinophilia, 3327t-3346t
Tropical pyomyositis. *See* Pyomyositis
Tropical splenomegaly syndrome, 2929
Troponin T, in myocarditis, 394
Trypanosoma brucei gambiense, 2968
variable surface glycoproteins of, 19
Trypanosoma brucei gambiense infection (African sleeping sickness), **2967-2973**
clinical manifestations of, 757t-758t, 2969-2970
diagnosis of, 2970-2971
differential diagnosis of, 2971
epidemiology of, 2968-2969
ocular manifestations of, 814
pathogenesis of, 19, 2969
prevention of, 2973
prognosis for, 2971-2973
second stage of, 2970
in traveler, 3116
treatment of, 2971-2972, 3327t-3346t
Trypanosoma brucei rhodesiense, 2968
Trypanosoma brucei rhodesiense infection (African sleeping sickness), **2967-2973**
clinical manifestations of, 2969-2970
diagnosis of, 2970-2971
differential diagnosis of, 2971
epidemiology of, 2968-2969
pathogenesis of, 2969
prevention of, 2973
prognosis for, 2971
in traveler, 3116
treatment of, 2971-2973, 3327t-3346t
Trypanosoma cruzi, 2959-2960, 2960f
Trypanosoma cruzi infection (trypanosomiasis), 398-399, **2959-2967**
acute stage of, 2963-2964
chagoma in, 2961, 2962f
chronic stage of, 2964-2965
clinical manifestations of, 757t-758t, 2962f, 2963-2965
CNS manifestations of, 2963-2964
colonic manifestations of, 387
congenital, 2965
diagnosis of, 2965-2966
early, 2963
epidemiology of, 2960-2961
esophageal manifestations of, 387
immune response to, 2962
in immunocompromised host, 2965
indeterminate stage of, 2964
in international adoptees and refugees, 3129
myocardial manifestations of, 398-399
acute stage, 2963-2964
chronic stage, 2964-2965
pathology of, 2961-2963, 2962f
ocular manifestations of, 814
pathogenesis of, 2961-2963, 2961f
pathology of, 2961-2963, 2962f
prevention of, 2967
in transplantation patient, 388
treatment of, 2966-2967, 3327t-3346t
Tryptophan, 71-72
TSPyV, 1864, 1864t, 1867-1868
TSS. *See* Toxic shock syndrome (TSS)
TSST-I. *See* Toxic shock syndrome toxin–I (TSST-I)

Tuberculids, papulonecrotic, 1354
Tuberculin skin test, 180, 184-185, 313, 1361-1363, 1362f, 1363t
  in fever of unknown origin, 842
  in kidney transplantation, 1004, 1012
  in nontuberculous mycobacterial infection, 1384
Tuberculoma, 1354
Tuberculosis, **1335-1380**, 1382t. *See also Mycobacterium tuberculosis*
  abdominal, 707, 709, 1357-1358
  in adolescents, 1359
  age and, 1342, 1343t, 1347t
  antiapoptotic mechanisms in, 15
  appendiceal, 684
  asymptomatic, 1344
  biliary, 660
  bronchial obstruction in, 1345-1347, 1346f
  care reporting of, 1376
  CNS, 443t, 1352-1354
  congenital, 1358-1359
  cutaneous, 753t-756t, 1354-1355
  in daycare facilities, 3556
  diagnosis of, 1361-1366, 3593-3594
    culture in, 1364-1365
    interferon-γ release assay in, 1363-1364
    nucleic acid amplification in, 1365-1366, 3593-3594
    tuberculin skin test in, 1361-1363, 1362f, 1363t
  dialysis-associated, 1358
  encephalitic, 494t-498t, 504
  epidemiology of, 1336-1341, 1337f-1338f, 1338t, 1340f
  exposure to, 1336
  extrathoracic, 1339, 1340f, 1352-1361
    treatment of, 1373
  fever of unknown origin in, 844-845
  gastrointestinal, 1357-1358
    vs. amebic colitis, 2872
  genetic factors in, 1341-1342
  genital, 1358
  after hematopoietic stem cell transplantation, 935-936
  historical perspective on, 1335-1336
  HIV infection and, 1336, 1339, 1360, 2601
  immune reconstitution inflammatory syndrome and, 1360-1361
  immune response in, 1342
  immunization against, 1376-1378, 1377t
  incidence of, 1336-1339
  incubation period in, 1342
  inoculation, 1358
  in international adoptees and refugees, 3126-3128, 3127f-3128f
  after intestinal transplantation, 1000
  isocitrate lyase in, 15
  after kidney transplantation, 1012, 1358
  before kidney transplantation, 1004
  latent, 1336
  after liver transplantation, 1000
  after lung transplantation, 962-964
  lymphadenitis in, 177, 177t, 179-180, 180f, 187, 1344-1347, 1345f-1346f, 1345t
  lymphohematogenous spread of, 1351-1352, 1356
  mastoid, 234, 237, 239
  meningeal, 488-489, 1352-1354, 1373
  miliary, 1352, 1352f, 1357
    pancreatitis with, 695
    pericarditis with, 373
  murine, 70
Tuberculosis *(Continued)*
  myocardial, 1351
  nitrogen balance in, 68
  nosocomial, 3384-3385
  obstructive hyperaeration in, 1345, 1346f
  ocular, 813, 1357
  otic, 214, 1357
  pancreatitis with, 695
  parotid, 191
  pathogenesis of, 15, 1342-1343, 1342t-1343t, 1343f
  pericardial, 379, 1351
  perinatal, 1358-1359
  peritoneal, 700-701, 1357-1358
  pleural effusion in, 309, 1343f, 1347-1348, 1348f-1349f
  portal of entry in, 1342, 1342t
  postnatal, 1358-1359
  in pregnancy, 1360
  prevalence of, 1336-1339
  prevention of, 1376, 3250-3251
  primary complex of, 1343f, 1344-1347, 1344t-1345t, 1346f, 1347t, 1349f
  pulmonary, 1344-1352
    asymptomatic, 1344
    atelectasis in, 1345
    calcification in, 1347, 1348f-1349f
    chronic, 1350-1351
    collapse-consolidation lesion in, 1345-1346, 1346f, 1347t
    primary complex in, 1343f, 1344-1347, 1344t-1345t, 1346f, 1347t, 1349f
    progressive, 1348-1350, 1350f
  radiography in, 1345, 1346f, 1348f-1350f, 1352f
  renal, 553, 1358
  resistance to, 1341-1342
  retroperitoneal, 707, 709
  skeletal, 1355-1356
  superficial lymph node, 1356
  syringe-transmitted, 1358
  timetable of, 1342-1343, 1343f
  transmission of, 1339-1341
  in traveler, 3116
  treatment of, 1366-1378, 1369t
    activity level and, 1375
    adherence to, 1374
    in adults, 1372
    in children, 1372-1373
    corticosteroids in, 1374-1375
    directly observed, 1374
    drug resistance in, 1337, 1367t, 1369, 1373-1374
    exposure stage, 1370-1371
    in extrapulmonary disease, 1373
    follow-up in, 1375-1376
    infection stage, 1371-1372
    isolation in, 1375
    after lung transplantation, 964
    microbiologic basis for, 1369-1370, 1369t
    pharmacologic, 1366-1369, 1367t
    summary of, 1374-1378
    susceptibility testing for, 1365
  vs. tularemia, 1661
  untreated, 1336
  urinary tract, 540
Tuberculosis verrucosa cutis, 1354
Tubo-ovarian abscess, **590**
Tularemia. *See Francisella tularensis* infection (tularemia)
Tumor. *See also* Cancer; Lymphoma
  brain, 467
  liver, 657
  spinal cord, 524
Tumor necrosis factor (TNF)
  in acute-phase reaction, 80
  in ARDS, 870
  hypertriglyceridemia and, 74
  in malaria, 2926
  in myocarditis, 386
  in septic shock, 826-829, 828f
Tumor necrosis factor-α (TNF-α), 26-27, 26t, 3358-3360, 3359t
  in bacterial meningitis, 434-435
  in burns, 1049
  in *Streptococcus agalactiae* infection, 1158
  in *Streptococcus pneumoniae* infection, 1224
Tumor necrosis factor receptor–associated periodic syndrome, 847-848
Tympanic membrane
  compliance of, 218, 220f
  examination of, 217-218, 217f, 219f
  perforation of, 215-216, 223
  scarring of, 223
  in upper respiratory tract infection, 220
Tympanic neurectomy, 192
Tympanic thermometer, 85-86
Tympanocentesis, 218-220
Tympanometry, 218, 220f
Tympanosclerosis, 223
Tympanostomy tubes
  in mastoiditis, 239
  in otitis media, 229, 232
  in otitis media with effusion, 229
Typhlitis, 679
Typhoid fever. *See Salmonella* ser. Typhi infection (typhoid fever)
Tzanck test, 1956

U

UGT1A1 gene, 668
Ulcer(s)
  Buruli. *See* Buruli ulcer; *Mycobacterium ulcerans* infection
  colonic
    in *Entamoeba histolytic* infection, 2869, 2872
    in *Yersinia enterocolitica* infection, 1527, 1527f
  cutaneous, 764, 764t
    in CMV infection, 1981
    in *Francisella tularensis* infection, 1662
    in *Leishmania* spp. infection, 2950-2951, 2951f
  genital, 582-584, 583t
    in granuloma inguinale, 583t, 1641-1642, 1642f
    in *Haemophilus ducreyi* infection (chancroid), 583t, 1687
    in HSV infection, 561f, 1941, 1942f
    in lymphogranuloma venereum, 583t, 584, 584f
    in syphilis, 583t, 1765, 1768
    in toxic shock syndrome, 853
  oral/labial, in HISV infection, 1940-1941, 1940f, 1950, 1950f
  peptic, 1694. *See also Helicobacter pylori* infection
Ulcerative colitis
  vs. *Clostridium difficile* infection, 637
  fever of unknown origin in, 847
  vs. *Shigella* spp. infection, 1483
Ultrasonography
  in acute lobar nephropathy, 555f
  in appendicitis, 682
  in cervical lymphadenitis, 184
  in cholangitis, 661
  in cholecystitis, 669

Ultrasonography *(Continued)*
in *Clonorchis sinensis* infection, 3052
in echinococcosis, 3045, 3046f
in *Fasciola* spp. infection, 3053
in HAV infection, 2137-2138
in intra-abdominal abscess, 705
in neonatal urinary tract infection, 889
in pancreatitis, 690
in parapneumonic pleural effusion, 310, 311f
in pelvic inflammatory disease, 588
in prostatitis, 560
in pyogenic liver abscess, 674
in pyomyositis, 737, 737f
in renal abscess, 554-556, 556f-557f
in retroperitoneal infection, 708
in rhinosinusitis, 199
in *Schistosoma* spp. infection, 3067
in sickle-cell disease, 670
in urinary tract infection, 544
Umbilical cord
infection of, **890**
in syphilis, 1766
Umbilicus, infection of, **890**
Uncinaria stenocephala infection, 3024
UNG, 909
Upper respiratory tract infection (URI). *See also* Common cold; Epiglottitis (supraglottitis); Laryngitis; Laryngotracheitis; Rhinosinusitis; Tracheitis *and specific infectious agents*
bronchitis and, 263
in cancer patient, 922-923, 923t
fever of unknown origin in, 845-846
Kawasaki disease and, 1068
septic arthritis and, 728
tympanic membrane in, 220
vulvovaginitis and, 568
Urea breath test, in *Helicobacter pylori* infection, 1695, 1696t
Ureaplasma spp., 2668-2669, 2669t, 2688
Ureaplasma spp. infection, **2688-2692**
clinical manifestations of, 2688-2690
differential diagnosis of, 2692
epidemiology of, 2688
in immunocompromised host, 2696
neonatal, 901, 2689-2690
chronic, 2691
clinical manifestations of, 2690-2692
CNS, 2691-2692
pulmonary, 2690
in pregnancy, 2689
prevention of, 2697-2698
pulmonary, 296, 2690
urethral, 532-533, 2689
Urease
Cryptococcus neoformans, 2797
Helicobacter pylori, 1693, 1695
Ureter
bacterial colonization of, 538
ectopic, 559, 563
Urethra
flora of, 91t, 93
inflammation of. *See* Urethritis
noninfectious irritants of, 530-531, 532t, 540
Urethritis, **530-535**
Chlamydia trachomatis, 531
diagnosis of, 533
pathophysiology of, 530, 531f
prognosis for, 534-535
screening for, 535
treatment of, 533-534, 534t
chronic prostatitis with, 559
clinical presentation of, 530-531
Urethritis *(Continued)*
culture in, 532
diagnosis of, 532-533, 532f
epidemiology of, 530
idiopathic, 530-531
infectious, 532, 532t
sexually transmitted, 579
treatment of, 533-534, 534t
Moraxella catarrhalis, 1252
Neisseria gonorrhoeae, 1286-1287, 1293f
carriage and, 534
clinical presentation of, 530-531, 1286, 1287f
complications of, 1287
diagnosis of, 532-533, 532f
female, 1284
pathophysiology of, 530
prevention of, 535
prognosis for, 534
sexual abuse and, 530, 534
treatment of, 533-534, 534t
nongonococcal, 2689, 2692, 2698
noninfectious, 531, 532t
clinical presentation of, 531
pathophysiology of, 530
nonspecific, 531, 559
pathophysiology of, 530, 531f
prevention of, 535
prognosis for, 534-535
treatment of, 533-534, 534t
vs. urinary tract infection, 540
Uric acid, urinary, 70
Urinalysis. *See also* Urine
in fever of unknown origin, 842
in gonococcal infection, 1285, 1291
in renal abscess, 554
in urinary tract infection, 542-543
Urinary catheter, infection with, 537, 540-541, 3393-3395
Urinary incontinence, in St. Louis encephalitis virus infection, 2258
Urinary tract infection (UTI), **535-553**
Achromobacter spp., 1574
Acinetobacter spp., 1571
Aerococcus urinae, 1249
Aeromonas spp., 1543
asymptomatic bacteriuria and, 538-539
bacteriology of, 537, 539
BK virus, 1867, 1867f
in burn patient, 1055-1056
C-reactive protein in, 543
in cancer patient, 925
Candida, 2741-2742, 2748-2749, 2754, 2757
catheter-related, 537, 540-541, 3393-3395
Citrobacter spp., 1431
clinical presentation of, 538-539
CMV, 1981
constipation and, 536
diagnosis of, 540-546
bacteriuria definition in, 542, 542t
CT in, 546
culture in, 542-543
dipsticks in, 543
imaging in, 544-546, 544t
microscopy in, 542-543
MRI in, 545-546
scintigraphy in, 544-545, 545f
site of infection in, 543
ultrasonography in, 544
urine collection for, 540-542, 541f, 541t
voiding cystourethrography in, 546, 546f
differential diagnosis of, 539-540
dysfunctional voiding and, 536
Enterococcus spp., 1173-1174
Urinary tract infection (UTI) *(Continued)*
epidemiology of, 535-537
Escherichia coli, **537-538**
clinical manifestations of, 1444
diagnosis of, 1445-1446
epidemiology of, 1440-1441, 1440t
pathogenesis of, 1441-1443, 1442f, 1443t
prognosis for, 1447
treatment of, 1444t, 1446
fever in, 536
fungal, 540
after heart transplantation, 948
after kidney transplantation, 1005-1006, 1014
Klebsiella spp., 1467-1468
leukocyte esterase in, 543
Morganella morganii, 1470-1471
neonatal, 888-889
nitrite in, 543
nosocomial, 3393-3395
pathogenesis of, **537-538**
physical examination in, 538, 539t
prevention of, 552, 3248
procalcitonin in, 543
prognosis for, 551-552
Proteus mirabilis, 1472-1474
Providencia spp., 1475
Pseudomonas aeruginosa, 1591, 1599
recurrence of, 551-552, 3248
risk factors for, 535-536
Schistosoma spp., 3064-3065
Serratia marcescens, 1490
sexual activity and, 536-537
Shigella spp., 1481
Staphylococcus saprophyticus, 1133, 1137-1138
Stenotrophomonas maltophilia, 1607
treatment of, 546-551
antibiotic resistance in, 3148
antibiotics in, 546-548, 547t
behavioral modification therapy in, 548
corticosteroids in, 548
prophylactic antibiotics in, 548
in uncircumcised boys, 536
vesicoureteral reflux and, 546, 546f, **548-551**
viral, 540
virulence factors in, 537-538
Urine
albumin in, in yellow fever, 2276
bacteria in, 538-539, 542, 542t. *See also* Urinary tract infection (UTI)
Candida in, 2741-2742
cattle, 1744
collection of, 540-542
bag technique for, 541-542
clean-catch, 540
in neonatal infection, 888
suprapubic aspiration for, 541, 541f
urethral catheterization for, 540-541
culture of, 542-543
in chemotherapy-associated infection, 925
in *Escherichia coli* UTI, 1445-1446
in *Leptospira* spp. infection, 1754
in neonatal *Candida* spp. infection, 896
in neonatal infection, 888-889
dipsticks for, 543
immunochromatographic membrane test of, 1217
Leptospira in, 1745, 1752
osmolality of, in septic shock, 832
Schistosoma spp. eggs in, 3066-3067, 3067f
white blood cells in, 542-543

Urine culture
in *Candida* spp. infection, 896
in chemotherapy-associated infection, 925
in *Escherichia coli* UTI, 1445-1446
in *Leptospira* spp. infection, 1754
in neonatal infection, 888-889, 896
in urinary tract infection, 542-543
Uropathogenic *Escherichia coli* (UPEC)
Fim H of, 13, 14f
immunization against, 552
P pilus of, 3-4, 4f, 10, 537
PapG adhesin of, 537
pili of, 537
toxins of, 17, 538
Uropathogenic *Escherichia coli* (UPEC) infection, **537-538.** *See also* Urinary tract infection (UTI)
clinical manifestations of, 1444
diagnosis of, 1445-1446
epidemiology of, 1440-1441, 1440t
outcomes of, 1447
pathogenesis of, 1441-1443, 1442f
treatment of, 1444t, 1446-1447
Urticaria, 763-764, 763t
Usutu virus infection, 2316t
Uterus, *Clostridium perfringens* infection of, 1797-1800
UTI. *See* Urinary tract infection (UTI)
Uveitis, **809-816**
anterior, 809-810
Ascaris lumbricoides, 815
Aspergillus spp., 813
bacterial, 812-813
Bartonella henselae, 813
Baylisascaris procyonis, 815
Borrelia burgdorferi, 812
Brucella spp., 813, 1613
Candida spp., 813
in neonate, 895, 897
CMV, 811
Coccidioides spp., 813-814
Coxiella burnetii, 815
in Creutzfeldt-Jakob disease, 811
Cryptococcus neoformans, 814
EBV, 810
Echinococcus granulosus, 815
echovirus, 2074-2075
Entamoeba histolytica, 814
enterovirus, **810**
epidemiology of, 810
fungal, 813-814
Giardia lamblia, 814
Gnathostoma spinigerum, 815
helminthic, 814-815
herpes B virus, 812
herpes zoster, 804
Histoplasma capsulatum, 813
in HIV infection, 811
HSV, 810
HTLV-1, 2573-2574
human T-cell lymphotropic virus, 811
hypopyon in, 809-810
influenza A virus, 812
insect-induced, 816
intermediate, 809
in Kawasaki disease, 1073
keratic precipitates in, 809-810
LCMV, 811-812
Leishmania, 814
Leptospira spp., 812-813, 1748-1749, 1751-1752
Loa loa, 815
measles virus, 811
Multiceps multiceps, 815
mumps virus, 811
Uveitis *(Continued)*
Mycobacterium leprae, 813
Mycobacterium tuberculosis, 813
Onchocerca volvulus, 815
parvovirus, 811
Plasmodium falciparum, 814
posterior, 809-810
posterior synechiae in, 809-810
postinfectious, 816
protozoal, 814
Rickettsia, 815
Rift Valley fever virus, 812
Rochalimaea quintana, 815
rubella virus, 811, 2213
Schistosoma spp., 815
slit-lamp examination in, 809-810
Sporothrix schenckii, 814
in subacute sclerosing panencephalitis, 811
Taenia solium, 815
Toxocara canis, 814
Treponema pallidum, 812
Trypanosoma spp., 814
viral, 810-812
VZV, 810
Wuchereria bancrofti, 815
Uvulitis, **165-167**
clinical manifestations of, 166, 166f
diagnosis of, 166
differential diagnosis of, 166
epidemiology of, 165-166
etiology of, 165
noninfectious, 166
pathogenesis of, 166
treatment of, 166-167

V

Vaccine(s), 3420-3430, 3421t. *See also* Immunization
anthrax, 1315-1316
arenavirus, 2476-2477
Burkholderia cepacia, 1604
Clostridium botulinum, 1795
Clostridium difficile, 638
dengue virus, 2289
DTaP/DTP, 1630-1636, 1631t-1633t, 1635t
Ebola virus, 2485
EBV, 2015
enterotoxigenic *Escherichia coli*, 628, 1463-1464
Haemophilus ducreyi, 1688
hantavirus, 2511
Helicobacter pylori, 1698
HIV, 2616-2617
hMPV, 2440
HSV, 1966
investigational, 3487
IVIG interactions with, 3496-3497
La Crosse virus, 2526
Norovirus, 2156
Plasmodium spp., 2946
Pseudomonas aeruginosa, 1604
reference sources on, 3429-3430
Rift Valley fever, 2530
Rift Valley fever virus, 2530
RSV, 277, 280, 2430-2431
Schistosoma, 3069
Shigella, 628, 1486
Streptococcus pyogenes, 1150
TC-83, 2240-2241
thimerosal in, 1927, 3429
vaccinia virus, 69, 69f, 749
Yersinia pestis, 1514
Vaccinia immune globulin (VIG), 2036, 3532-3533
Vaccinia necrosum, 2038-2039, 3532
Vaccinia virus, 2045-2046
recombinant, 2045-2046
Vaccinia virus infection, 494t-498t, 742t-748t, 749, 2036, 2038-2039, 2039t, 3532-3533. *See also* Smallpox
transmission of, 2039
Vagina
cancer of, HPV and, 1873t, 1874-1875, 1878
Enterobius vermicularis infection of, 3019
examination of, 561
flora of, 91t, 93, 561-562, 562t
foreign body in, 567, 567f
infection of. *See* Vulvovaginitis
specimen collection from, 561, 561f
Trichomonas vaginalis infection of. *See Trichomonas vaginalis* infection (trichomoniasis)
Vaginal discharge, 561
infectious, 563-564
neonatal, 563, 563f
premenarcheal, 563, 563f
in trichomoniasis, 576, 2891-2892
Vaginitis. *See also* Vulvovaginitis
gonococcal
postmenarcheal, 1284-1285
prepubertal, 1283-1284, 1284f
Shigella spp., 1481
Vaginosis, bacterial, 572t-573t, 577-578, 577f
Valacyclovir, 3278t, 3280-3281
in HSV infection, 1959, 1960t, 1961
in VZV infection, 2029
Valganciclovir, 3278t, 3285-3286
in CMV infection, 1984-1986, 1988
in kidney transplantation, 1008
in lung transplantation, 975-976
Validity, study, 95
Valproic acid
in chorea, 414
pancreatitis with, 691
Vancomycin, 3203-3204, 3231t-3234t
adverse effects of, 3206
in bacterial meningitis, 447-449, 450t, 452
in cervical lymphadenitis, 186
in *Clostridium difficile* infection, 637
in coagulase-negative staphylococcal infection, 1138
guidelines for, 1185, 1185t
in heart transplant–related bacteremia, 947
indications for, 3205-3206
mechanisms of action of, 3204
in methicillin-resistant *Staphylococcus aureus* infection, 1127-1128
monitoring of, 3206
pharmacokinetics of, 3205
resistance to, 3151-3154, 3152t, 3204
in *Arcanobacterium haemolyticum* infection, 1323
in *Enterococcus* spp. infection, 1170, 1179t, 1180-1181, 1183-1186, 1185t, 3399-3400
in methicillin-resistant *Staphylococcus aureus* infection, 1128
in rhinosinusitis, 200
in septic shock, 833-834, 833t
tolerance to, in *Streptococcus pneumoniae* infection, 1220-1221
in vitro activity of, 3204-3205

Variable(s). *See also* Biostatistics
  categorical, 101-102
  confounding, 123-124, 124t
  continuous, 100-101, 122
Variable surface glycoproteins, of trypanosomes, 19
Varicella (chickenpox), **2021-2033**
  acetaminophen in, 85
  in burn patient, 1054
  clinical manifestations of, 2025-2027, 2025f
  complications of, 2026, 2026f
  congenital, 819, 2026-2027
    ocular manifestations of, 819
  conjunctival, 804
  in daycare facilities, 3562
  diagnosis of, 1009, 2028
  differential diagnosis of, 2028
  epidemiology of, 2023-2024
  exanthem in, 742t-748t, 762, 2024-2025, 2025f
  external auditory canal, 208, 208f
  after heart transplantation, 954-955
  after hematopoietic stem cell transplantation, 941
  hepatic, 654
  immune response in, 2024-2025
  immunization against, 2030-2032, 3472-3476
  in immunocompromised host, 2026, 2026f
  after kidney transplantation, 1009-1010
  after lung transplantation, 978-979
  myositis with, 739
  ocular, 810
  pathogenesis of, 2024-2025
  prevention of, 2030-2032, 3522-3525
  prognosis for, 2029-2030
  prophylactic acyclovir in, 2032
  secondary, 2023
  subclinical, 2023
  transmission of, 2022-2023
  treatment of, 2028-2029
Varicella-zoster virus (VZV), 2022
  isolation of, 2028
Varicella-zoster virus (VZV) infection, **2021-2033.** *See also* Herpes zoster; Varicella (chickenpox)
  acute disseminated encephalomyelitis with, 516
  breakthrough, 2031-2032
  clinical manifestations of, 2025-2028
  diagnosis of, 2028
  encephalitic, 494t-498t, 499, 505
  epidemiology of, 2023-2024
  immunization against, 2030-2032, 3472-3476
    active, 2031-2032
    adverse events with, 3473-3474
    contraindications to, 3475-3476
    efficacy of, 2031-2032, 3473
    after hematopoietic stem cell transplantation, 941
    indications for, 3474-3475
    before kidney transplantation, 1009
    before lung transplantation, 978-979
    passive, 2030-2031
    safety of, 2031
  meningeal, 487
  nosocomial, 2025, 2030, 3383
  pathogenesis of, 2024-2025
  postexposure prophylaxis for, 3475
  prevention of, 2030-2032, 3522-3525
  prognosis for, 2029-2030
  prophylactic acyclovir in, 2032
  vs. smallpox, 2036
Varicella-zoster virus (VZV) infection *(Continued)*
  transmission of, 2022-2023
  treatment of, 2028-2029, 3279-3280
Variola virus, 2034. *See also* Smallpox
VariZIG, 2030-2031, 3523-3525, 3524t
Vas deferens, absence of, in cystic fibrosis, 325
Vasculitis
  CNS, vs. acute disseminated encephalomyelitis, 519
  coronary artery. *See* Kawasaki disease
  pancreatitis with, 691t, 692
  pulmonary, 297
  small-vessel (leukocytoclastic), 750. *See also* Exanthem
VDRL (Venereal Disease Research Laboratory) test, 819-820, 1770, 1772-1773, 1773t
Venezuelan equine encephalitis, 494t-498t, **2237-2241**
  clinical manifestations of, 2239-2240
  diagnosis of, 2240
  differential diagnosis of, 2240
  ecology of, 2238-2239
  epidemiology of, 2238-2239, 2238f
  pathogenesis of, 2240
  pathology of, 2240
  prevention of, 2240-2241
  treatment of, 2240
Venezuelan equine encephalitis virus, 2237-2238, 2238t
Venezuelan hemorrhagic fever. *See* Arenavirus infection
Venom, spider, 3077-3078
Venous sinus thrombophlebitis, mastoiditis and, 236, 239-240
Ventilator-associated pneumonia, 3391-3393
  in neonate, 892
Ventricle, brain abscess rupture into, 463, 466
Ventricular neurocysticercosis, 3034-3035, 3036f, 3037-3038
Ventriculitis, in meningitis, 437-438, 886-887
Ventriculoatrial shunt. *See* Cerebrospinal fluid (CSF) shunt
Ventriculography, in myocarditis, 387-389, 388f
Ventriculoperitoneal shunt. *See* Cerebrospinal fluid (CSF) shunt
Verruga peruana, **1725-1726**
  treatment of, 1728
Vertigo, in otitis media, 217, 222
Vesicoureteral reflux, **548-551**
  endoscopic bulking agent injection therapy in, 551
  epidemiology of, 548
  management of, 549
  natural history of, 548-549
  procalcitonin in, 543
  prognosis for, 551-552
  prophylactic antibiotics in, 549-551, 549t, 550f-551f
  surgical treatment of, 551
  voiding cystourethrography in, 546, 546f
Vesicular exanthema of swine virus infection, 2147-2148
Vesivirus, 2149, 2149f, 2149t, 2151
Vesivirus infection, **2154**
Vestibular neuritis, enteroviral infection and, 2090
Vibrio cholerae, 1549-1550. *See also* Cholera
  pili of, 5
  toxins of, 1550
Vibrio parahaemolyticus, 1556
Vibrio parahaemolyticus infection, 602t-603t, 607t, 612, 616t, **1556-1559**
  clinical manifestations of, 1558
  complications of, 1558
  diagnosis of, 618, 1558-1559
  epidemiology of, 1556-1557, 1557t
  extraintestinal, 1558
  pathogenesis of, 1557-1558
  prevention of, 1559
  treatment of, 1559
Vibrio vulnificus, 1560
  virulence factors of, 1561
Vibrio vulnificus infection, **1560-1562**
  clinical manifestations of, 1561
  diagnosis of, 1561
  epidemiology of, 1560
  pathophysiology of, 1560-1561
  prevention of, 1562
  treatment of, 1562
Vicks VapoRub, 138
Video-assisted thoracoscopic surgery (VATS), in parapneumonic pleural effusion, 314
Vincent infection (trench mouth, acute necrotizing ulcerative gingivitis), 147, 147f
Viral infection. *See also specific infections*
  cell invasion in, 16
  fever of unknown origin in, 845
  after hematopoietic stem cell transplantation, 939-943
  host response to, 22-23. *See also* Complement; Immunity
  immune suppression with, 20
  after intestinal transplantation, 995-1000, 997t
  after kidney transplantation, 1006-1007
  laboratory diagnosis of, **3600-3620**
    antigen detection in, 3606t-3607t, 3609
    autopsy specimen for, 3604
    blood specimen for, 3602t-3603t, 3604
    bone marrow specimen for, 3602t-3603t, 3604
    culture in, 3605-3609, 3606t-3607t
    cytology in, 3609
    direct fluorescent antibody test in, 3609-3610
    enzyme-linked immunosorbent assay in, 3610-3612, 3611t
    fecal specimen for, 3602t-3603t, 3604
    highly multiplexed assays in, 3614-3616, 3615t
    multiplexed assays in, 3614
    nasal specimen for, 3601-3604, 3602t-3603t
    nasopharyngeal specimen for, 3601-3604, 3602t-3603t
    nucleic acid amplification test in, 3606t-3607t, 3608, 3612, 3613t
    ocular specimen for, 3602t-3603t, 3604
    quantitative nucleic acid amplification test in, 3616-3617, 3616t
    serology in, 3606t-3607t, 3608
    shell vial assay in, 3608-3609
    single-analyte nucleic acid amplification test in, 3612-3614, 3613t
    specimen collection for, 3601-3604, 3602t-3603t
    specimen transport for, 3604-3605
    throat specimen for, 3601, 3602t-3603t
  latency in, 20
  after liver transplantation, 995-1000, 997t
  after lung transplantation, 957, 974-982

Viral infection *(Continued)*
neonatal, **877-881**, 878t. *See also* Newborn, viral infection in
normal flora and, 89-90
pulmonary. *See* Influenza virus infection; Pneumonia, community-acquired
serum cholesterol and, 73-74
Viridans streptococcal infection. *See* *Streptococcus* viridans infection
Virucidal nasal tissues, in cold prevention, 139, 2117, 2117t
Virulence, 105
Virulence-associated genes, 3
Viruses. *See also* Viral infection *ans specific viral infections*
classification of, 1836-1843, 1837f, 1838t-1840t, 1841f-1842f
DNA-containing, 1841f
RNA-containing, 1842f
Visceral larva migrans, 3022-3023. *See also* *Toxocara canis* infection (toxocariasis)
Visceral leishmaniasis. *See Leishmania* spp. infection (leishmaniasis), visceral
Vision loss
in *Chlamydia trachomatis* infection, **2636**
in Creutzfeldt-Jakob disease, 811
in histoplasmosis, 813
in LCMV infection, 811-812
in onchocerosis, 757t-758t, 809, 815
in toxocariasis, 814
Visitor policy, in infection prevention, 3412
Vitamin A
antioxidant activity of, 77
deficiency of
bronchiolitis and, 280
keratitis and, 807
measles and, 2391-2392
metabolism of, 76
Streptococcus pneumoniae infection and, 1224
Vitamin B_{12}, metabolism of, 77
Vitamin C
antioxidant activity of, 77
in common cold, 137-138
metabolism of, 77
in *Streptococcus pneumoniae* infection, 1224
Vitamin D
deficiency of, bronchiolitis and, 280
metabolism of, 77
Vitamin E
antioxidant activity of, 77
in glutathione synthetase deficiency, 59
metabolism of, 77
Vitritis, 809
Candida, 2743
Loa loa, 815
Voiding cystography, in vesicourethral reflux, 555f
Voiding cystourethrography, in vesicoureteral reflux, 546, 546f
Voiding dysfunction, urinary tract infection and, 536, 548
Vomiting
in appendicitis, 680-681
in *Bacillus cereus* infection, 1319
in enteroviral infection, 2068, 2069t
induced, in botulism, 1794
in pancreatitis, 689
in Reye syndrome, 676
in rotavirus infection, 2185
Voriconazole, 3301f, 3310-3312
in *Aspergillus* spp. infection, 2717-2719
in *Blastomyces dermatitidis* infection, 478
in cancer patient, 928
in *Candida* meningitis, 475
Voriconazole *(Continued)*
in *Coccidioides* spp. infection, 478
dosing for, 3311-3312, 3312t
drug interactions with, 3311
efficacy of, 3311
in *Fusarium* spp. infection, 2848
in heart transplant–related *Aspergillus* infection, 953
in hematopoietic stem cell transplantation–related *Aspergillus* infection, 937
in *Histoplasma capsulatum* infection, 2826
in lung transplant–related *Aspergillus* infection, 967-968
in neonatal *Candida* spp. infection, 2758
pharmacodynamics of, 3310
pharmacokinetics of, 3308t, 3310
prophylactic, 3311
safety of, 3310-3311
in *Scedosporium* spp. infection, 2850
Vulvar cancer, HPV and, 1873t, 1874-1875, 1877
Vulvovaginitis, **563-570**
Candida, 569-570, 570f, 788, 2739-2740
in pregnancy, 2765
treatment of, 2747
Chlamydia trachomatis, 572t-573t, 574-575
Corynebacterium diphtheriae, 569, 1305
diphtheritic, 569
Entamoeba histolytica, 569
fungal, 569-570, 570f
group A streptococcal, 568
HSV, 1941-1943, 1942f. *See also* Herpes simplex virus (HSV) infection, genital
Neisseria gonorrhoeae, 571-574, 572t-573t, 1283-1284, 1284f
nongonococcal *Neisseria* spp., 567
nonspecific, 564-567. *See also* Vaginosis, bacterial
foreign bodies and, 567, 567f
intestinal pinworms and, 566-567, 566f
perineal hygiene deficit and, 564-566
prepubertal, 564, 565t
prepubertal, 563-564, 563f, 566f-567f
sexually transmitted, 570-579
Shigella, 568-569
skin infection and, 568
Trichomonas vaginalis, 572t-573t, 575-577, 575f
upper respiratory tract infection and, 568
Yersinia enterocolitica, 569
VZV. *See* Varicella-zoster virus (VZV)

W

Warthin-Finkeldey giant cells, 2378, 2378f
Warts, 742t-748t. *See also* Human papillomavirus (HPV) infection
anogenital, 572t-573t, **580-581**, 580f-581f, 1873t, 1876-1877
chemical ablation of, 1882-1883
conjunctival, 1879-1880
cutaneous, 1873t, 1876
historical perspective on, 1871
immunomodulatory therapy in, 1883
nasal, 1879
surgical treatment of, 1882-1883
WAS, 913
Wasting syndrome, 79-80
Water-contaminated wound, 781, 781t
Waterborne disease, 606, 627, 3547. *See also* *specific infections*
prevention of, 1486
Waterhouse-Friderichsen syndrome, 1264
Wegener granulomatosis, pulmonary vasculitis in, 297
Wegner sign, 1769
Weight loss, in Whipple disease, 643
Weil syndrome, 1749f, 1752-1753
Wesselsbron virus infection, 2316t
West Nile virus, 2261-2262, 2262f
West Nile virus infection, **2261-2273**
breast-feeding and, 2264
clinical manifestations of, 494t-498t, 500, 510, 2267-2269
culture in, 2270
diagnosis of, 2271
electrodiagnostic studies in, 2270
epidemiology of, 2262-2266, 2265t-2266t, 2266f
exanthem in, 742t-748t, 749, 2268
flaccid paralysis in, 2268-2272
historical perspective on, 2261
immune response to, 2267
immunization against, 2272-2273
after kidney transplantation, 1011
laboratory findings in, 2270
after lung transplantation, 983
meningeal, 487, 489
neuroimaging in, 2270
neuropathology of, 2271-2272
ocular manifestations of, 2268-2269, 2272
pathogenesis of, 2266-2267
in pregnancy, 2264
prevention of, 2272-2273
prognosis for, 2269-2270
vs. Sindbis fever, 2252
vs. St. Louis encephalitis virus infection, 2259
transfusion-associated, 2264
transplantation-associated, 2264
treatment of, 2272
vertical transmission of, 2264
Western equine encephalitis, **2230-2237**
clinical manifestations of, 494t-498t, 2233-2234
diagnosis of, 2235
differential diagnosis of, 2235-2236
ecology of, 2230-2231
epidemiology of, 2231-2233, 2231f-2233f
pathogenesis of, 2234
pathology of, 2234
prevention of, 2236-2237, 2236t
prognosis for, 2234-2235
risk factors for, 2232-2233, 2233t
treatment of, 2236
Western equine encephalitis virus, 2230
Wet-mount preparation
in *Cyclospora cayetanensis* infection, 2907, 2907f
in genital tract infection, 561
in *Naegleria fowleri* infection, 2982
in *Trichomonas vaginalis* infection, 575f, 2891-2892
Wheezing. *See also* Asthma
bronchiolitis and, 272, 273t, 274, 281-282
rhinovirus infection and, 2123
Whipple disease, **639-646**
bacteremia in, 642-643
blood involvement in, 644
cardiac involvement in, 644
clinical manifestations of, 642-644, 642t-644t
CNS, 494t-498t, 643, 644t, 646
cutaneous involvement in, 644
diagnosis of, 645
electron microscopy in, 641-642, 641f-642f
epidemiology of, 640-642

Whipple disease *(Continued)*
gastrointestinal tract involvement in, 642-643, 643t
granulomas in, 642
historical perspective on, 639-640
joint involvement in, 643
light microscopy in, 641, 642f
lymph node involvement in, 644
muscle involvement in, 644
ocular involvement in, 644
PAS-positive macrophage in, 641-642, 642f, 645
pathogenesis of, 640-642, 641f-642f
pneumonia in, 643
pulmonary involvement in, 643-644
renal involvement in, 644
spleen involvement in, 644
transmission of, 641
treatment of, 645-646, 646t
White blood cell (WBC) count
in acute bronchitis, 264
in acute laryngotracheitis, 249
in adenovirus infection, 1895
in arthritides, 731, 732t
in bacteremia, 832-833
in bronchiolitis, 275
in *Clostridium difficile* infection, 636
in Colorado tick fever virus infection, 2174
in croup syndromes, 250t-251t
CSF, 441
in HSV encephalitis, 1946-1947
in intracranial pressure monitor–related infection, 1039-1040
in neonatal meningitis, 885, 1135
in shunt-related infection, 1032-1033
in viral meningitis, 489
in Eastern equine encephalitis, 2228
in fever of unknown origin, 842
in fever without source, 839-840
in hepatitis, 649-650
in infectious mononucleosis, 2007
in infective endocarditis, 358
in Kawasaki disease, 1075-1076
in *Kingella kingae* bacteremia, 1702-1703
in *Leptospira* spp. infection, 1752
in *Mycoplasma pneumoniae* infection, 2678
in neonatal *Bordetella pertussis* infection, 1626, 1629-1630
in occult bacteremia, 838
in parapneumonic pleural effusion, 312-313
in *Pasteurella multocida* meningitis, 1547
in pelvic inflammatory disease, 588
in peritonitis, 702
in pneumococcal meningitis, 1228
in prosthetic joint infection, 1028
in rheumatic fever, 412
in roseola infantum, 770-771
in rubella virus infection, 2211
in sandfly fever, 2536
in sepsis neonatorum, 883
in *Streptobacillus moniliformis* infection, 1718
in *Streptococcus agalactiae* infection, 1162
in tick-borne encephalitis virus infection, 2309
in type 1 leukocyte adhesion deficiency, 52-53
urinary, 542-543
in Venezuelan equine encephalitis, 2240
in visceral leishmaniasis, 2956
in *Yersinia enterocolitica* infection, 1526-1527
White piedra, 790-791
Whitewater Arroyo virus infection, 2467, 2469-2471, 2470t, 2473. *See also* Arenavirus infection
Whitlow, herpetic, 155, 1944-1945, 1944f-1945f
Whooping cough. *See Bordetella pertussis* infection (pertussis, whooping cough)
Widal test, 1503
Wilson disease, 657
Wimberger sign, 1769-1770
Wiskott-Aldrich syndrome, 50, 905-906, 907t, 913
Wolbachia, in *Aedes aegypti* control, 2278, 2289
Wound infection
Aeromonas hydrophila, 1542
Bacillus cereus, 1319
bite-related, 780-781, 780t
Clostridium botulinum, 1792-1794
Clostridium perfringens, 1796
Edwardsiella tarda, 1533
after intestinal transplantation, 994
after kidney transplantation, 1004-1005
Pseudomonas aeruginosa, 1591
soil-related, 781, 781t
Staphylococcus epidermidis, 1137
Stenotrophomonas maltophilia, 1607
Streptococcus aureus, 1121
surgical site, 3395-3398, 3396t
tetanus, 3471, 3472t
Vibrio parahaemolyticus, 1558
Vibrio vulnificus, 1560-1562
water-related, 781, 781t
Wuchereria bancrofti infection (filariasis), 757t-758t, 815, **3025-3026**, 3327t-3346t
WUPyV, 1864, 1864t, 1868

X

X-linked agammaglobulinemia, 46-47, **908-909**
vs. chronic bronchitis, 267
diarrhea in, 600
X-linked lymphoproliferative syndrome, 915, 2000-2001
treatment of, 2013-2014, 3521
X protein, 1913
Xanthogranulomatous pyelonephritis, 539-540
Xanthomonas maltophilia. *See Stenotrophomonas maltophilia*
Xenopsylla cheopis (Oriental rat flea), 1510-1511
Xylitol
in otitis media prevention, 231
in *Streptococcus pneumoniae* infection prevention, 1236
Xylohypha bantiana infection, 479

Y

Yaba-like disease virus infection, **2046-2047**
Yaba monkey tumor virus infection, **2046-2047**
YadA protein, 13
Yale Observation Scale, in occult bacteremia, 839
Yatapoxvirus infection, **2046-2047**
Yaws, 753t-756t, 1783t, **1785-1787**
diagnosis of, 1789
treatment of, 1789-1790
Yellow fever, 656, **2274-2278**
clinical manifestations of, 2275-2276
differential diagnosis of, 2276
Yellow fever *(Continued)*
epidemiology of, 2274f, 2275
historical perspective on, 2274
immunization against, 2277-2278, 3485-3487
laboratory diagnosis of, 2276-2277
pathology of, 2276
prognosis for, 2277
treatment of, 2277
Yellow fever virus, 2274-2275
Yersinia aldovae, 1531
Yersinia alecksiciae, 1531
Yersinia bercovieri, 1531
Yersinia enterocolitica, 1515-1516
adhesin A of, 1524
Ail protein of, 13, 1523-1524
animal reservoirs of, 1516-1517
antibodies against, 1529-1530
carriage of, 1518
enterotoxin of, 1525
injectisomes of, 1524
invasin of, 13, 1523-1524
iron metabolism of, 1525
plasmid-encoded factors of, 1524
type III secretion system of, 1524
virulence factors of, 1521-1526, 1522t-1523t
water contamination with, 1518
yersiniabactin of, 1525
Yops of, 1522t-1523t, 1524-1525
Yersinia enterocolitica infection (yersiniosis), **1514-1532**
adhesin A in, 1524
Ail protein in, 13, 1523-1524
asymptomatic, 1527-1528
bloodstream, 1528
clinical manifestations of, 607t, 612, 616t, 753t-756t, 1526-1528, 1526t, 1527f
community outbreaks of, 1518-1519
diagnosis of, 618, 1530
differential diagnosis of, 1529-1530
enterocolitis in, 1526-1527
epidemiology of, 1516-1519, 1517f, 1517t
extraintestinal, 1528
family epidemics of, 1519
food contamination and, 1517-1518
gastric acidity and, 1526
hepatic, 673
immune response to, 1521
incubation period in, 1518
invasin in, 13, 1523-1524
iron metabolism and, 1525
mesenteric adenitis in, 1520-1521, 1527
nosocomial, 1519
outbreaks of, 1518-1519
pathogenesis of, 13, 18, 1521-1526, 1522t-1523t
pathology of, 1519-1521
plasmid pYV in, 1524
postinfectious syndromes in, 1528-1529
prevention of, 1519
pseudoappendicitis in, 1527
reactive arthritis after, 1528
transfusion-related, 1518
transmission of, 1518
treatment of, 1530-1531
vulvovaginal, 569
water contamination and, 1517-1518
YadA protein in, 13
Yops proteins in, 18
Yersinia frederiksenii, 1531
Yersinia intermedia, 1531
Yersinia kristensenii, 1531
Yersinia massiliensis, 1531
Yersinia mollaretii, 1531

Yersinia pestis, 1509-1510
toxin of, 1511-1512
Yersinia pestis infection (plague), **1509-1514**
bioterrorist, 3583-3584
Caf1 in, 5
cervical lymphadenopathy in, 177, 180
clinical manifestations of, 753t-756t, 1512-1513
diagnosis of, 1513
differential diagnosis of, 1513
epidemiology of, 1509, 1511
historical perspective on, 1509
localized (pestis minor), 1511
nosocomial, 3401
pathogenesis of, 1511-1512
prevention of, 1513-1514
prognosis for, 1513
septicemic, 1512
transmission of, 1510-1511
treatment of, 1513
Yersinia pseudotuberculosis, 1515-1516
invasins of, 13
type III secretion system of, 1524
virulence factors of, 1521, 1522t-1523t
YadA of, 13
Yersinia pseudotuberculosis infection (yersiniosis), 1068, **1514-1532**
clinical manifestations of, 753t-756t, 1529
diagnosis of, 1530
differential diagnosis of, 1529-1530
Yersinia pseudotuberculosis infection (yersiniosis) *(Continued)*
epidemiology of, 1519, 1520f
immune response to, 1521
Kawasaki disease–like illness in, 1529
mesenteric adenitis in, 1521
pathogenesis of, 1521-1526, 1522t-1523t
pathology of, 1521
septicemic, 1529
treatment of, 1530-1531
Yersinia rohdei, 1531
Yersinia ruckeri, 1531
Yersinia spp., 1515-1516
identification of, 1530
typing of, 1515-1516
virulence factors of, 1521-1526, 1522t-1523t
Yersinia spp. infection, **1514-1532.** *See also specific species infections*
epidemiology of, 1516-1519
historical perspective on, 1514-1515
uveitis after, 816
Yops proteins, 18, 1522t-1523t, 1524-1525

Z

Zanamivir, 3268t, 3270-3273, 3270f
in community-acquired pneumonia, 290
in influenza virus infection, 2348-2351, 2349t
in laryngotracheitis, 257
Zeaxanthin, 77
Zidovudine, 2612
Zika fever, 749, **2316-2317**
Zinc
deficiency of, 77t, 79
in gastrointestinal tract infection, 625-626
metabolism of, 77t, 78-79
Zinc lozenges, in common cold, 138
Zonisamide, pancreatitis with, 691
Zoster, **2021-2033**
clinical manifestations of, 2027-2028, 2027f
complications of, 2027-2028
diagnosis of, 2028
epidemiology of, 2023-2024
immunization against, 2023-2024
pathogenesis of, 2024-2025
prevention of, 2030-2032
prognosis for, 2029-2030
risk factors for, 2023-2024
after solid-organ transplantation, 979
transmission of, 2023
treatment of, 2028-2029
Zygomycetes, 2837, 2838f. *See also* Entomophthorales infection (entomophthoromycosis); *Mucor* spp. infection (mucormycosis); Rhizopus spp. infection (mucormycosis)
epidemiology of, 2837-2839, 2839f
neonatal infection with, 899

This page intentionally left blank

CLINICAL KEY™

Smarter search. Faster answers.

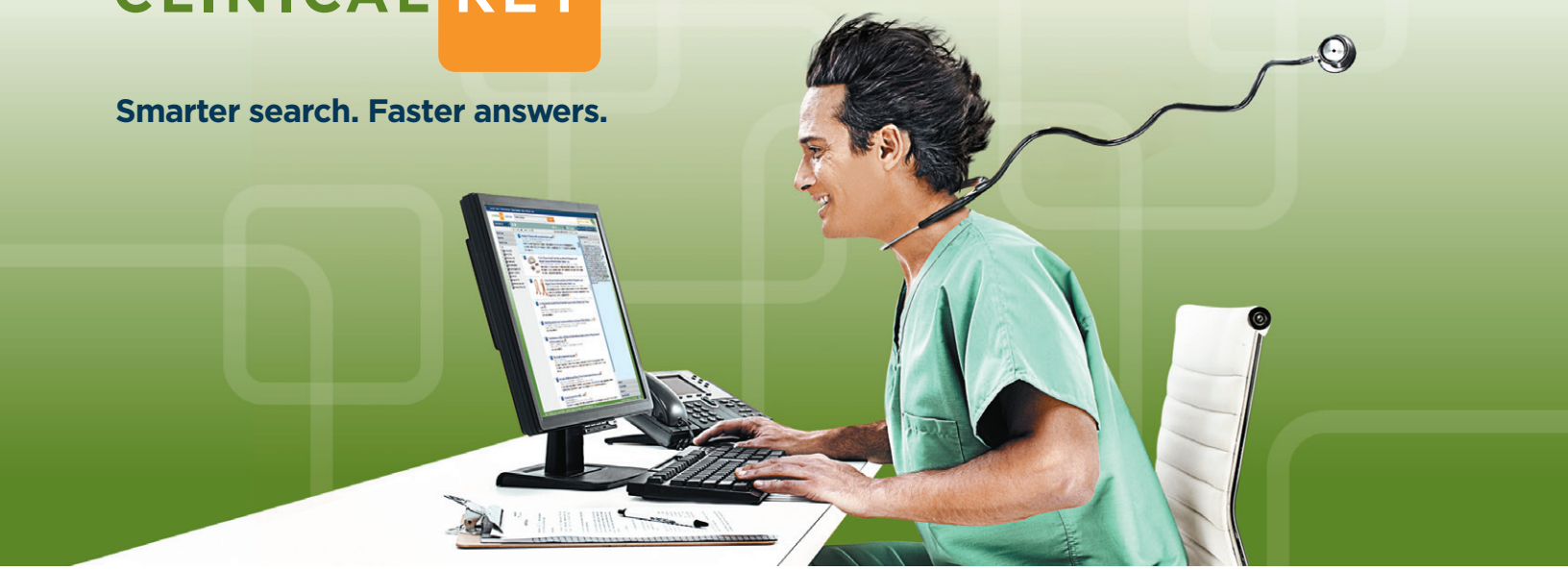

Smarter, Faster Search for Better Patient Care

Unlike a conventional search engine, ClinicalKey is specifically designed to serve doctors by providing three core components:

1 Comprehensive Content

The most current, evidence-based answers available for every medical and surgical specialty.

2 Trusted Answers

Content supplied by Elsevier, the world's leading provider of health and science information.

3 Unrivaled Speed to Answer

Faster, more relevant clinical answers, so you can spend less time searching and more time caring for patients.

Start searching with ClinicalKey today!

Visit ***ClinicalKey.com*** for more information and subscription options.

ELSEVIER